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Age Estimation from Second & Third Molar by Modified Gleiser and Hunt Method : A Retrospective Study

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Abstract

The aim of the present investigation, which is a modification of previous study done by Gleiser and Hunt(1955). The study was conducted to determine juvenile and adolescent ages of age group 10 to 19.9yrs, in South Indian Population. The evaluated materials consisted of 200 OPG'S. In this Mandibular Left 37 & 38 is the most variable tooth in the permanent dentition, which was the only data used for age estimation. The Standard deviation for Male 37 staging is ± 2.15years and for 38 staging is ± 1.29 years. And, the Standard deviation for Female 37 staging is ± 2.58 years and in 38 staging is ±2.24 years. The combination of Male tooth number 37 & 38 staging is revealing the good results with the standard deviation of ± 1.23 years and the combination of Female tooth number 37 & 38 revealing the average results of ±2.18 years. While comparing to both Male and Female tooth number staging Male is showing more accurate than females in root maturation.

Key Words: *2nd and 3rd (Left Side), Apical Closure, Gleiser and Hunt staging.*

Introduction

The last decade number of unidentified cadavers and human remains as well as number of cases lacking age documentation and therefore requiring age determination has increased. This requires age calculation, not only for differentiating the juvenile from the adult status in criminal law cases, but also for chronological age[1,2,3]. The determination of the chronological age within the age span of 10-20 years of age still remains a problem[4,5,6,7,8,9].

Similarly, dental age can be assessed among young children with greater accuracy because many teeth are undergoing development and mineralization simultaneously [10, 11, 12, 13,14]. The age span of 10 to 16 years and 17 to 19.9 years of age the second and third molar are the only teeth still in development and thereby very important for dental age calculation[3,15]. In addition, the use of wisdom teeth in age estimation has been questioned in literature [3,15,20]. Before drawing this conclusion, one has take into consideration multiple misleading factors such as small sample sized studies [3,16,17,19] case reports [10,21], improper statistics [3,18] and mixed samples of different ethnic origin [17,22,23]. Recently, Schmeling et al. published most suitable procedures that currently available for age estimation in criminal procedures, being physical examination with anthropometric measurements, radiographic examination of left hand in case of dexterity and radiographic study of dentition [24].

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Materials and Method

The study sample consisted of 200 OPG's (100 males and 100 females) of age ranging from 10 to 19.9 years with known date of birth divided into ten groups accordingly (Table 1). The radiographs were collected from archive of Department OMR, JSS Dental College and Hospital, Mysore, Karnataka, which were taken with PROMAX digital Planmeca Machine.

DURATION OF THE STUDY: MARCH 2019 TO MAY 2019

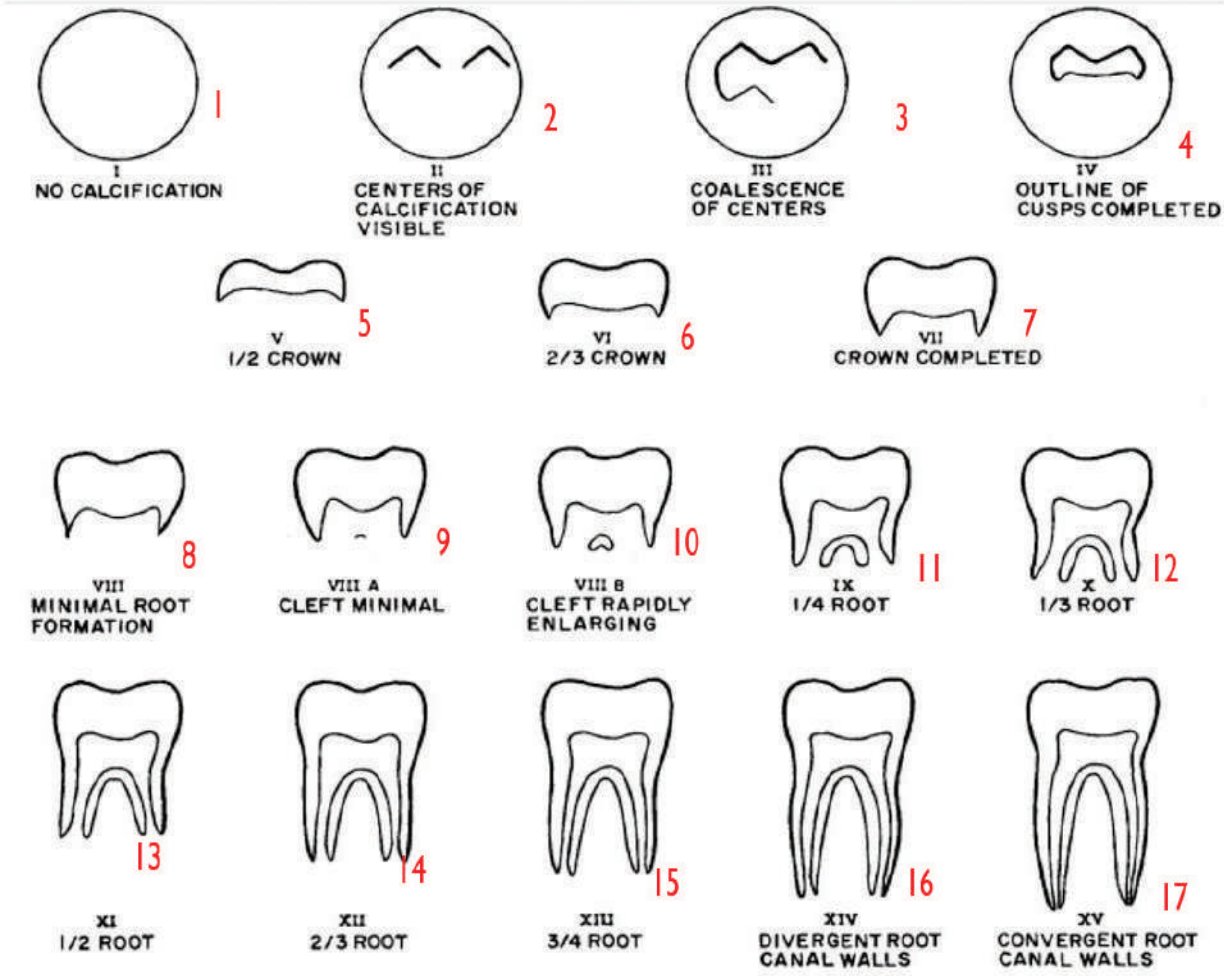
ASSESMENT OF DENTAL AGE USING GLEISER AND HUNT METHOD:

Chronological age (actual age) of individual was calculated by subtracting the birth date from date on which the radiographs were exposed for that particular individual. Digital panoramic radiographs {OPG's} of all individuals were used to assess the status of maturation on basis of calcification of permanent teeth (left side mandibular arch), (37 and 38). This present study Staging were modified and given according to Gleiser and Hunt method of 17 staging system in which the labeling nomenclature were changed from Roman (stages I to xv) to Numericals (Stages 1 to 17) [28] for easy identification.(Fig: 1)

TABLE 1: Sample Distribution

Groups	Age (years)	Male	Female
1	10 to 10.9	10	10
2	11 to 11.9	10	10
3	12 to 12.9	10	10
4	13 to 13.9	10	10
5	14 to 14.9	10	10
6	15 to 15.9	10	10
7	16 to 16.9	10	10
8	17 to 17.9	10	10
9	18 to 18.9	10	10
10	19 to 19.9	10	10
TOTAL		100	100

Figure: Modified the 17 stages illustrated below



All the mean dental age values from 37 and 38 obtained were averaged was taken to obtain overall mean age separately for 37 and 38 was done shown (Table 2). Multiple regression analysis where obtain for dental age calculation which shown (Table 3, 4, 5).

Results

Table 2: GROUP STATISTICS- T test

AGE GROUP (years)	TOOTH NUMBER STAGING	SEX	N	MEAN	SD	SDE	P VALUE
10 – 10.9	Tooth no 37 staging	Male	10	11.7000	4.62000	1.46097	
		Female	10	12.8000	3.45768	1.09341	0.554
	Tooth no 38 staging	Male	10	6.3000	2.11082	.66750	0.033*
		Female	10	3.4000	3.37310	1.06667	
11 – 11.9	Tooth no 37 staging	Male	10	10.6000	1.71270	.54160	
		Female	10	12.8000	1.54919	.48990	0.007*
	Tooth no 38 staging	Male	10	3.3000	1.49443	.47258	
		Female	10	4.1000	3.69534	1.16857	0.534
12 – 12.9	Tooth no 37 staging	Male	10	14.4000	1.26491	.40000	
		Female	10	14.6000	1.89737	.60000	0.785
	Tooth no 38 staging	Male	10	7.0000	.66667	.21082	1.000
		Female	10	7.0000	1.88562	.59628	
13 – 13.9	Tooth no 37 staging	Male	10	14.8000	1.54919	.48990	
		Female	10	.96609	.96609	.30551	0.013*
	Tooth no 38 staging	Male	10	.51640	.51640	.16330	0.242
		Female	10	2.83039	2.83039	.89505	
14 – 14.9	Tooth no 37 staging	Male	10	16.4000	.51640	.16330	
		Female	10	16.8000	.42164	.13333	0.074
	Tooth no 38 staging	Male	10	7.6000	2.54733	.80554	0.223
		Female	10	8.7000	1.05935	.33500	
15 – 15.9	Tooth no 37 staging	Male	10	16.8000	.42164	.13333	
		Female	10	16.8000	.42164	.13333	1.000
	Tooth no 38 staging	Male	10	11.0000	2.44949	.77460	0.436
		Female	10	10.1000	2.60128	.82260	
16 – 16.9	Tooth no 37 staging	Male	10	16.8000	.42164	.13333	
		Female	10	16.4000	.69921	.22111	0.139
	Tooth no 38 staging	Male	10	14.5000	2.22361	.70317	0.133
		Female	10	12.2000	4.04969	1.28062	
17 – 17.9	Tooth no 37 staging	Male	10	17.0000	.00000a	.00000	
		Female	10	17.0000	.00000a	.00000	
	Tooth no 38 staging	Male	10	16.2000	.78881	.24944	0.077
		Female	10	14.5000	2.75882	.87242	
18 – 18.9	Tooth no 37 staging	Male	10	16.9000	.31623	.10000	
		Female	10	16.9000	.31623	.10000	1.000
	Tooth no 38 staging	Male	10	15.7000	1.63639	.51747	0.641
		Female	10	16.0000	1.15470	.36515	
19 – 19.9	Tooth no 37 staging	Male	10	16.9000	.31623	.10000	
		Female	10	17.0000	.00000	00000	0.331
	Tooth no 38 staging	Male	10	16.5000	.00000	.16667	0.176
		Female	10	14.1000	5.36346	1.69607	

The group statistics reveals that statistically significant results are seen in the age groups:

· 11-11.9 years (P value = 0.007) and 13-13.9 years (P value = 0.013) for tooth number 37.

· 10-10.9 years (P value = 0.033) for tooth number 38.

The standard deviation for tooth number 37 is the lowest (0.00) in the age groups:

· 17-17.9 years for males

· 17-17.9 and 19-19.9 for females.

The standard deviation for tooth number 38 is the lowest in the age groups:

· 19-19.9 years for males (0.00)

· 14-14.9 years for females (1.05 years).

Table 3: The univariate regression Analysis 37 and 38 is as follows: (Male)

Univariate Regression (Male)	Intercept	Regression Coefficient	F- Ratio	P value	t value	P value	R2
Tooth_37_staging	3.777	0.731	87.471	.000c	9.353	.000	0.353
Tooth 38_staging	9.191	0.542	413.906	.000c	29.717	.000	0.809

Note: If R^2 value is nearer to 1, then that regression equation is good.

***Formula 1 (37)*: Age = 3.777 + 0.731 (37 stage); SD ± 2.15years**

***Formula 2 (38)*: Age = 9.191 + 0.542 (38 stage); SD ± 1.29 years**

· Therefore, in males Formula 1 ($R^2 = 0.353$) is preferred to estimate the age.

Table 4: The univariate regression Analysis 37 and 38 is as follows: (Female)

Univariate Regression (Female)	Intercept	Regression Coefficient	F- Ratio	P value	t value	P value	R2
Tooth_37_staging	.590	.899	53.466	.000c	7.312	.000	0.472
Tooth 38 staging	10.517	.439	102.508	.000c	10.125	.000	0.511

Note: If R^2 value is nearer to 1, then that regression equation is good.

***Formula 1 (37)*: Age = 0.590 + 0.899 (37 stage); SD ± 2.58 years**

***Formula 2 (38)*: Age = 10.517 + 0.439 (38 stage); SD ± 2.24 years**

· Therefore, in females Formula 1 ($R^2 = 0.472$) is preferred to estimate the age.

Table 5: Multivariate Regression Analysis (Female & Male)

Multivariate Regression (Female)	Regression Coefficients	F-ratio	P value	t-value	P values	R2
Intercept = 5.944 Tooth 37 staging Tooth 38 staging	 0.346 0.348	 37.344 37.344	 0.000 0.000	 2.543 6.302	 0.013 0.000	0.542
Multivariate Regression (Male)	Regression Coefficients	F-ratio	P value	t-value	P values	R2
Intercept= 6.953 (Constant) Tooth37 staging Tooth38 staging	 0.197 0.470	 234.400 37.344	 0.000 0.000	 3.764 14.211	 0.001 0.000	0.829

***Formula Female*: Age = 5.944 + 0.346 (37 stage) + 0.348 (38 stage); SD ±2.18 years**

***Formula Male*: Age = 6.953 + 0.197 (37 stage) + 0.470 (38 stage); SD ± 1.23 years**

Discussion

In this present study it was observed that there was a difference between Male and Female root maturation in relation to 38 and combination of 37 & 38 (nearly 1.2yrs variation). So, males are showing more accurate than females in root maturation. There is significant correlation b/w chronological age (actual age) and dental age(estimated age) in males R^2 value is 0.829 by using Multivariate Regression(37 & 38). 38 Univariate Regression of R^2 value for male is nearer to 0.809.

The Standard deviation for Male 37 staging is ± 2.15years and for 38 staging is ± 1.29 years. And, the Standard deviation for Female 37 staging is ± 2.58 years and in 38 staging is ±2.24 years. The combination of Male tooth number 37 & 38 staging is revealing the good results with the standard deviation of ± 1.23 years and the combination of Female tooth number 37 & 38 revealing the average results of ±2.18 years. While comparing to both Male and Female tooth number staging Male is showing more accurate than females in root maturation.

Age estimation by means of tooth development has been used over, long period of time, after all tooth development is an accurate measure of chronological age that seems to be independent of exogenic factor[10,17,25]. However, age estimation based on dental methods has shortcomings, especially during juvenile and adolescence when the second and third molar is the only variable dental indicator left. Indeed a great variation in position, morphology and time of formation exists.

The literature studies dealing with age estimation by using third molars as age predictors are scarce. In one study, Hispanic origin were evaluated [11], but in most of the studies subjects from different racial origin were screened [15,22].

Kohler et al. (1994), This method is based on evaluation of development and maturation of all permanent third molars. It has modified the Gleiser and Hunt (1955) method of grading first molar. It consists of ten stage of grading i.e. 3 stages of crown formation and 7 stages of root formation. It has given more priority to age of about 20 years & method enables good accuracy of juvenile or adult (</>18 years) using logistic regression formulas. It can also be used for the age estimation in 14-22 years old subjects.[26]

Mincer et al. (1993), Studied third molar development radiographically to use it as an estimator of chronological age in children and adolescents (age range 14-24 years). Development of maxillary third molar was found to be more foremost than mandibular third molars and also root formation was earlier than females used for predicting an individual is </>18 years using regression formulas.[15]

Balaraj and Nithin (2010), conducted radiological study of closure of apical foramen of both permanent mandibular second molars for determination of adolescents ages 14 – 16 years. Their study makes use of Demirjian method utilizing the description of dental formation stages of complete root formation and apical closure.[27]

Conclusion:

To obtain multiple regression formula of dental age calculation with the chronological age as the independent variable and second and third molar developmental stages as dependent variables and to calculate probability for an individual to be older than 18 years in case of full second and third molars development. Gleiser and Hunt method found much more reliable method of dental and chronological age determination of an juvenile and adolescent ages 10 to 20years using radiological method of permanent mandibular second and third molar (left side). The purpose behind the second and third molar tooth eruption and completion is its development during the age of 10 to 15years, which is lying within period of adolescent age. So, by using these two parameters (radiographic view of root maturation), the Forensic Odontologist can determine the importants behind the medicolegal cases.

Ethical Clearance: Taken From Jssdch

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Knowledge Attitude and Practice of Usage of Anti-Anxiety Drugs in Paediatric Patients among General Dental Practitioners

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Abstract

Aim : To assess the knowledge attitude and practice of usage of anti-anxiety drugs among the general dental practitioners.

Background: Anti-anxiety drugs can act as effective way to manage patients with anxiety and dental fear mainly children. Adequate knowledge is necessary for using anti-anxiety drugs. Usage of anti-anxiety drugs can help to treat the patients in an effective way.

Material and Methods: Questionnaires consisting of 15 questions was distributed to 80 general dental practitioners practicing in private dental hospitals and clinics in Chennai city in June 2017. The questionnaire was designed to enquire about the knowledge and usage of anti anxiety drug in children

Results: 83.75% of dentists are aware of the pharmacological means of anxiety reduction. 51.25% are aware of the side effects and over dosage reaction of using anti anxiety drugs. 12% of dentists are aware of the method of managing side effects and over dosage reaction of using anti anxiety drugs

Conclusion : Anti-anxiety drugs can act as a better alternative and also an adjunct to the conventional behaviour management. Proper knowledge about its mechanism of action, dosage, adverse effects are essential for the dentist to use anti-anxiety drugs in an effective way. Hence the usage of anti-anxiety drugs can be recommended and encouraged among dentists provided they have a sound knowledge about the drug

Key words: *Anti anxiety drug, dentist, behaviour management*

Introduction

Dental anxiety is one of the reason for behaviour management problem in pediatric dental patients. The common reason for with fear and anxiety for the dental treatment could be due to dentophobia , stranger anxiety or negative past dental experience. The prevalence of dental anxiety and fear which causes behaviour management problems in children undergoing dental treatments was reported to be 5% to 20%(1). The three pathways which leads to the acquisition of child fears are direct conditioning through some traumatic experience, vicarious experiences or threatening information(2).

Dental anxiety is cumulative over time and starts from childhood(3). Increased heart rate**,** altered respiratory rate**,** sweating, trembling**,** weakness are the

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physiologic responses of anxiety. Feeling of impeding danger, powerlessness, tremors are the psychological responses of dental anxiety.

Dental anxiety of children with behaviour management problems and children with special health care needs can be managed by non pharmacological and pharmacological means of behaviour management. Non pharmacological behaviour management includes Tell-show-do, modelling, contingency management, systematic desensitisation, retraining, protective stabilisation, HOME. Pharmacological behaviour management comprise of premedication with sedatives and hypnotics, anti-anxiety drugs, anti histaminics, conscious sedation , and general anaesthesia[4].

According to Girdler and Hill 2009, Dental anxiety can act as a major source of stress for general dental practitioners who treat the anxious patient[5]. A proper behaviour-guidance technique is essential to do a proper dental management in an anxious child which improves the quality of the dental treatment. In children with severe anxiety, pharmacological methods as an adjunct therapy can help a dentist to communicate more effectively with the child[1].

Pharmacological behaviour management techniques and agents used by the dentists should provide sufficient safety and efficacy. These agents should produce a mild state of depression in which the child can respond to physical and verbal stimulations and retain the ability to maintain a patent airway[6].

The aim of the present study was to identify the knowledge, attitude and practice of usage of anti-anxiety drugs in pediatric patients among general dental practitioners.

Materials and Method

The present descriptive study is questionnaires based cross sectional survey carried out among 80 general dental practitioners in chennai city, Tamil Nadu, India in June 2019. The study was carried out in private dental hospitals and clinics in chennai. The samples were collected by simple random sampling for the study. The questionnaires distributed to the dental practitioners focused on knowledge based questions, attitude based questions to assess the level of usage of anti anxiety drugs as a treatment modality for behavior management

Results

Among the 80 general dental practitioners, 45 were male and 35 were female. The mean age of the participants was 36+_3.472. The results of the present study shows that 82.5% of dentists has encountered patients with dental anxiety. 75% of dentists were aware of different means of anxiety reduction. 43.75% of dentists find the pediatric patients to be more anxious, 7.5% of the dentists find adult patients are more anxious and 48.75% finds both adult and paediatric patient to be equally anxious

86.25% of dentists uses non pharmacological means of management, 6.25% of dentists uses pharmacological means of management and 7.5% of dentists uses both pharmacological and non pharmacological means of anxiety reduction for paediatric patients (Figure 1). 65% of dentists uses non pharmacological means of management, 23.75% uses pharmacological means of management and 11.25% uses both pharmacological and non pharmacological means of anxiety reduction in adult patients

83.75% of dentists are aware of the pharmacological means of anxiety reduction and 80.26% of dentists prefer benzodiazepines, 3.42% uses barbiturates, 16.32 % are not aware of the medications used for anxiety reduction in children (Figure 2 and Figure 3)

65% of dentists uses oral route of administration of anti anxiety drugs, 15% uses nasal inhalation route, 1.25% uses intravenous route of drug administration (Figure 4). 48.75% of dentists modify drug dosage for paediatric patient, 15% of dentists does not modify drug dosage for paediatric patients (Figure 5) and 36.25% of dentists has no idea on dosage modification for anti-anxiety drugs for paediatric patients.

51.25% are aware of the side effects and over dosage reaction of using anti-anxiety drugs and 12% of dentists are aware of the method of managing side effects and over dosage reaction (figure 6). 55% of dentists prefer referring the child with dental anxiety to a pediatric dentist.

80% of dentists feels that non pharmacological means of anxiety reduction is better than pharmacological

methods. The most common reason for preferring non pharmacological means of behavior management was to avoid the complications of side effects and adverse effects of the anti-anxiety drugs. 70% of dentists prefer to get medical opinion from general practitioner before using anti-anxiety drugs.

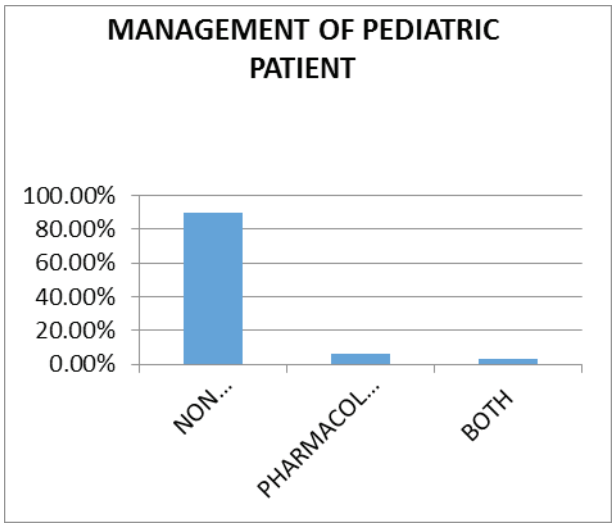


Figure 1

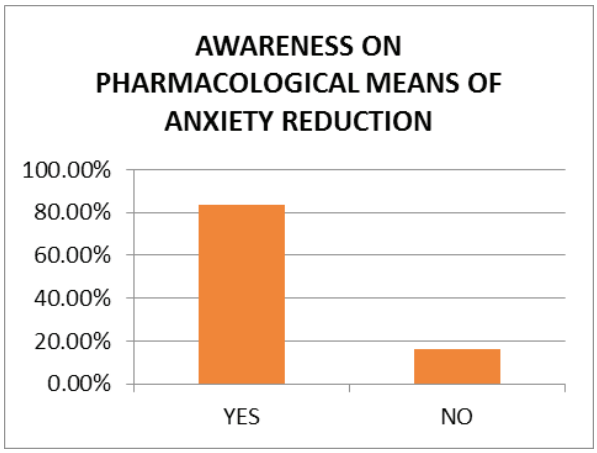


Figure 2

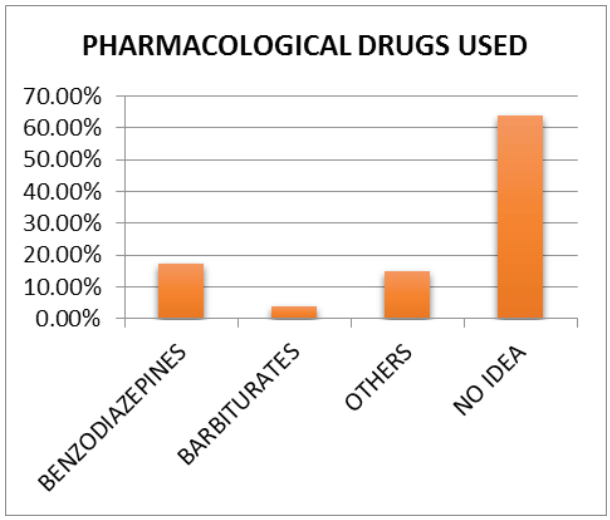


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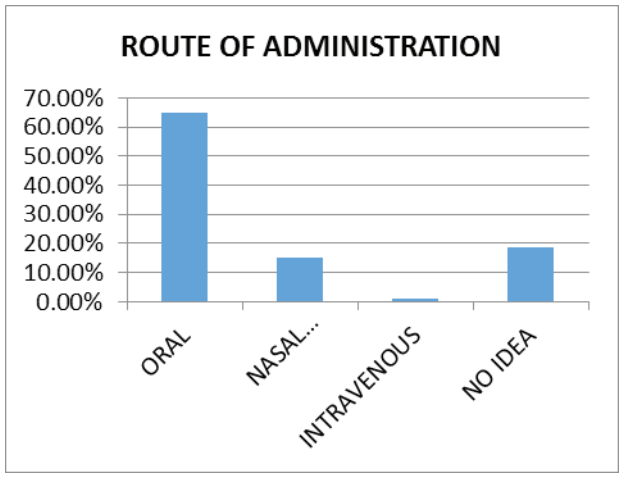


Figure 4

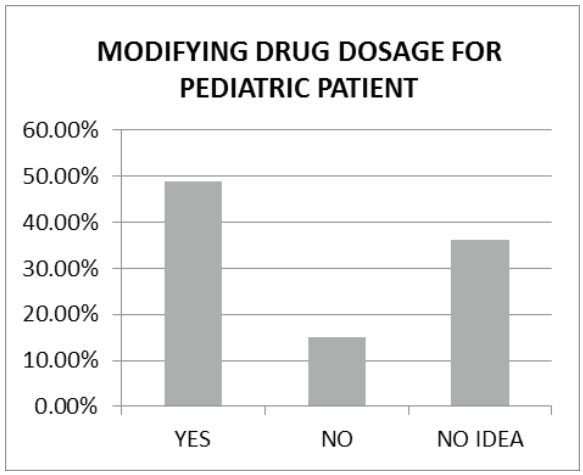


Figure 5

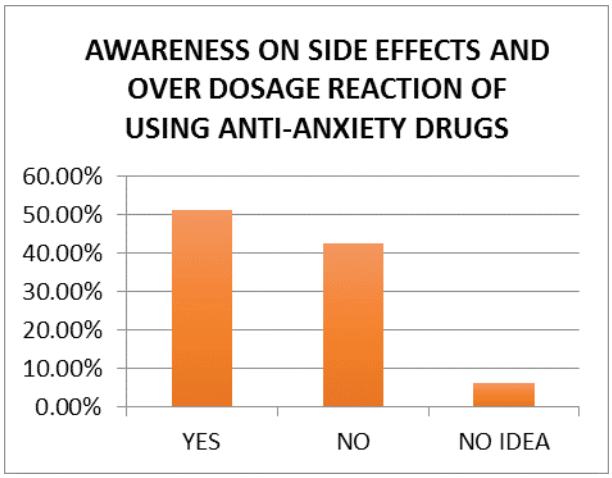


Figure 6

Discussion

The results of the present study show that most of the dentist frequently encounter patients with dental anxiety. There are different scales to assess the level of dental anxiety in children. This includes Facial Image Scale[7], Venham's Picture Test[8], Venham's Clinical Rating Scale[9], Dental Subscale of the Children's Fear Survey Schedule[10]. Other dental anxiety rating scales includes Corah's Dental Anxiety, Modified Dental Anxiety Scale[11].

Many dentists are aware of the pharmacological means of anxiety reduction. But only very few applies it to their practice. Benzodiazepines, Azapirones, anti histaminics, beta-blockers are the anti anxiety drugs used for behavior management (KD TRIPATHI). Benzodiazepines is the most commonly used anti anxiety drug[12]. Use of barbiturates as anti anxiety drug has been reduced and it's been replaced by benzodiazepines because the latter is relatively safe in higher dosage and is less likely to cause dependence when compared to barbiturates[13].

The adverse effects of benzodiazepines includes light headedness, psychomotor and cognitive impairment, vertigo. Long term usage can lead to dependence[14]. 12% of the dentists are aware of the means of managing the overdosage and side effects from using anti anxiety drugs. Despite the fact that many dentists are aware of the side effects, the number of dentists who can manage the side effects of these drugs are relatively very low.

Psychological understanding and care for paediatric patients, effective behaviour management techniques, empathic attitude and atmosphere forms the basis for successful dental treatment. There are many techniques which can be used but to achieve an optimal result for both patient and dentist. The indication for pre-medication should be first made clear, as this will affect the choice of substance and route of administration[15]

Conclusion

Anti anxiety drugs can act as a better alternative and also an adjunct to the conventional behaviour management. More number of CDE programs, workshops, can be conducted to increase the awareness, knowledge and practice of usage of anti anxiety drugs in dentistry among the dental practitioners.

Source of Funding: Nil

Conflicts of Interest: Nil

Ethical Clearance: IHEC/SDC-PEDO-1703/19/006

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Effect of Silver Diamine Fluoride Treatment on the Quality of Life of Children

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Abstract

Background: Early childhood caries is known to affect the quality of life of children. Silver diamine fluoride is a desensitizing agent used for preventing and arresting dental caries. It is inexpensive and can be easily applied.

Aim: The aim of the present study was to find the effect of Silver Diamine Fluoride (SDF) treatment on the Oral Health-Related Quality of Life

Method: ECHOIS questionnaire designed to evaluate the child's behavior, physical abilities, pain, temperament, and how well the child gets along with other people was distributed to the parents of children aged 1-5 with ECC with reversible pulpitis. The questionnaire has two components ie Family Impact Score and Child Impact Score. The parents were asked to fill the questionnaire at baseline and one month after SDF application.

Results : Significant difference was observed in the mean Child Impact Score(CIS) and Family Impact Score(FIS) at baseline and one month follow up. SDF application significantly improved the quality of life of children($P<0.005$). Wilcoxon Signed Rank Test was used to check the level of significance in the mean difference of the CIS and FIS at the base line and one month follow up

Conclusion: Children with ECC after the application of SDF showed improvement in the quality of life at one-month follow up compared with the baseline as measured by ECHOIS questionnaire

Keywords: *Dental caries, Silver diamine fluoride, Children, Quality of life*

Introduction

Early Childhood Caries (ECC) has been defined as "the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries) or filled tooth surfaces in any primary tooth in a child 71 months of age or younger[(1)]. ECC can affect children's lives, such as physical development, emotional growth, qualify of life, and learning at school. Those children seek dental care due to pain and discomfort. They often need extensive dental treatment under conscious sedation or general anesthesia, due to their inability to cooperate in a clinical setting[(2,3).]

Silver Diamine Fluoride (SDF) is an FDA approved desensitizing agent. 38% Ag (NH3)2F) is a colorless solution which contains 24–29% silver and 5–6% fluoride with pH of 10. This high alkalinity provides an unfavorable environment for dentine collagen enzyme activation[(4).] SDF is inexpensive and the treatment is very simple and non-invasive. It kills cariogenic bacteria and provides instant caries arrest thereby reducing pain and infection in children[(5)]. SDF is proven to be

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effective in preventing and arresting dental caries both for the primary teeth[6,7] and the permanent teeth in older children[8]. It is also effective in the prevention of root caries in the elderly[9].

Oral health related quality of life (OHRQoL) is a multidimensional construct that reflects people's comfort when eating, sleeping, and engaging in social interaction, their self-esteem, and their satisfaction with respect to their oral health[1]. Pain in the teeth, mouth or jaws, irritation or frustration, difficulty eating and trouble sleeping are the most common impacts of ECC on OHRQoL as reported by parents[10,11].

The Early Childhood Caries Oral Health Impact Scale (ECOHIS) is a standardized instrument that has been used to measure OHRQoL. ECOHIS consists of two subscales: CIS (Childhood Impact Scores) and FIS (Family Impact Scores). CIS show the impact of dental caries on the child and FIS demonstrate the impact of dental caries on the family[12]

The objective of the present study was to investigate the Oral Health-Related Quality of Life (OHRQoL) in preschool children with dental caries who had an application of SDF. The hypothesis of the present study was that silver diamine fluoride treatment cannot improve the quality of life of children with Early Childhood Caries.

Materials and Method

The current short terms study was approved by Institutional Review Board(IHEC/SDC-PEDO1703/19/023). A total of 30 healthy participants (ASA I and II), aged between 2-5 years with ECC who visited the Department of Pediatric Dentistry were included in the present study.

Inclusion criteria:

· Children who were difficult to treat due to behavior or medical management

· Involvement of multiple teeth that may not be treated in one visit

· Who can be benefitted from caries arrest and monitored until the patient is old enough able to cooperative for the treatment or until their appointment for treatment under GA

· High risk for developing more caries

Exclusion criteria:

· Children who are cooperative enough to have definitive treatment in clinical set up

· Medically compromised children

· silver allergy

· Children with ulcerative gingivitis or stomatitis.

The participants were recruited after explaining the study design, risks and benefits of SDF application, and the study forms were explained thoroughly to the parents and informed consent was obtained from parents prior to application of SDF. After obtaining consent from the parents, 38%SDF (Tedequim SRL., Argentina) was applied to the cavitated tooth surface after proper isolation with cotton rolls.

ECHOIS questionnaire consisting of 13 oral health-related behaviors and various questions related to the child's behavior, physical abilities, growth/development, bodily pain/discomfort, temperament and moods, and how well the child gets along with others was given to the parents. Parents were asked to fill the questionnaire on their first visit and after one month either in person for those who returned for the one-month follow-up or over the phone for those who did not return for the one-month follow-up or those who had treatment completed with conscious sedation or under general anesthesia.

Statistical Analysis

Statistical analysis was performed using SPSS Software version 17.0. (Chicago, SPSS Inc). Wilcoxon Signed Rank Test was used to determine the difference in the mean value of CIS and FIS at the base line and one month follow up. P value <0.05 was considered to be significant.

Results

A total of 30 individuals were enrolled and completed questionnaires at both time points. Among the 30 participants, 20 were female and 10 were male. The mean age the participants was 34.362+_2.664. Table 1 shows the Child Impact Score and Family Impact Score. The difference in the baseline mean value and follow up

mean value of CIS and FIS was statistically significant (P<0.0001).

Table 2 shows changes in ECOHIS items and subscales between baseline and after SDF application. The results show that there is a statistically significant difference in the quality of life in terms of improvement in the sleeping, talking and the children has less pain and was less irritable before and after SDF application. Only one parent reported that there was difficulty in sleeping after the SDF application, but it was not statistically significant. 49% percent of the parents reported that there was improvement in the Family Impact Score after SDF application.

Table 1 - Mean CIS and FIS score at baseline and follow up.

	BASELINE MEAN, SD	FOLLOW UP MEAN, SD	AVERAGE PAIRED DIFFERENCE,SD	P VALUE
Child Impact Score	5.7, 5.64	0.44, 1.12	5.1, 5.66	<0.0001
Family Impact Score	2.6, 3.56	0.47, 1.23	2.1, 3.74	0.0004

Table 2 - Changes in ECOHIS Items and Subscales between Baseline and After SDF Application

CIS	IMPROVED	SAME	WORSE	P VALUE
Missed preschool, daycare or school	13(43%)	17(57%)	0,0	0.0001
Had difficulty eating some food	12(40%)	18(60%)	0,0	0.0005
Been irritable or frustrated	13(43%)	17(57%)	0,0	0.0001
Pain in the teeth, mouth or jaws?	14(40%)	16(53%)	0,0	<0.0001
Had trouble sleeping	13(45%)	16(53%)	1,3%	0.001
Avoided smiling or laughing	9(47%)	21(70%)	0,0	0.0391
Avoided talking	10(33%)	20(67%)	0,0	0.002
Had difficulty drinking hot or cold beverages	13(43%)	17(57%)	0,0	0.0045
Had difficulty pronouncing any words.	16(53%)	14(47%)	0,0	<0.0001
Total CIS Score	20(73%)	9(24%)	1,3%	<0.0001
Financial Impact on family from child's dental problems or treatments	**11(37%)**	**18(60%)**	**1, 3%**	**0.0244**
Felt guilty	12(40%)	16(53%)	2(7%)	0.0085
Been upset	12(40%)	16(53%)	2(7%)	0.0256
Taken time off from work	0(0%)	30(100%)	0(0%)	0.0001
Total FIS Score	16, 49%	12, 46%	2, 5%	0.0004

Discussion

The preschool period is a very crucial period during the development of the child[13]. Early Childhood Caries can have serious impacts on children's lives, in terms of physical development, emotional growth; qualify of life, and learning at school. Untreated ECC can result in significant destruction of the child's teeth, which in turn affect the child's overall health[14].

The present study is a questionnaire based study where, a parent-filled "The Early Childhood Oral Health Impact Scale (ECOHIS)" questionnaire was used to analyze the association between SDF application and child's Oral Health-Related Quality of Life. The results of the present study showed that there was a statistically significant improvement in the quality of life of children and their families after SDF application.

Acs et al reported that 8.7% of children with ECC had significant decrease in the weight as a result of caries[15]. Low et al reported that 43% of children had difficulty in eating[16]. Acharya et al reported 29.2% % of children had difficulty in eating. Filstrup et al reported that 53% of children, Low et al reported 35% of children and Acharya et al reported 44% of children presented with difficulty in sleeping[2].

There was decreased pain in the teeth, improved pronunciation of words, fewer missed school days and being less irritable and frustrated. Families of the children with ECC reported less financial impact feeling less guilty than before the treatment. Thus, the results of this study show a strong association between SDF application and quality of life of children with ECC.

ECC is a major health problem in found among children in developing nations, mainly in those living in socially backward communities[14]. This could be due to low socioeconomic status, social exclusion, and sociocultural differences in oral health beliefs and practices[17]. Treatment with SDF is simple and cost-effective. Therefore, SDF can be used in community based health centers.

Decreased sample size is the major limitation of the present study. Most of the parents were not willing for SDF treatment because of its potential staining and discoloration of the tooth. Also, there could be bias in the responses of parents regarding their child's symptoms to avoid being perceived as a careless parent. Follow-up questionnaires which were completed over the phone could result in a less accurate response than those completed in person. Further studies with a large sample size and a longer follow up should be considered to support the result of the present study.

Conclusion

It can be concluded that SDF can improve the oral health related quality of life of children with early childhood caries and hence can be used in community level to arrest dental caries in preschool children.

Source of Funding: Self

Conflicts of Interest: Nil

Ethical Clearance: Taken from Institutional Review Board, Saveetha Dental College and Hospitals, Chennai, India (IHEC/SDC-PEDO1703/19/023)

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A Pilot Study on Snake Bite Deaths in the District of Malda, West Bengal, India

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Abstract

The alarming number of deaths due to snake bite are often under-reported and missed due to several interrelated socioeconomic factors. Epidemiology of fatal snake bite have been reported sparsely from West Bengal in recent past .The present investigation was devised to explore the profile of the fatal cases of envenomation in snake bite from a district of northern part of West Bengal. Deaths due to snake bite constituted 02.067% of all unnatural deaths from mortuary data. The mean age of the cases of fatal snake bite was 34.18 with SD 2.67. It was seen that the mean interval between bite and treatment was 3.35 hours with SD 0.48. The present work was a short regional research to supplement epidemiology and add to the database of deaths from snake bite in a district of West Bengal, India.

Key words *Epidemiology, Snake Bite Deaths, District, West Bengal, India*

Introduction

Mortality due to envenomation in snake bite has remained as an underrated and less studied public health issue. Primarily in developing agrarian countries like India, the alarming number of deaths is often under-reported and missed due to several interrelated socioeconomic factors. It mainly affects the rural population who has poor access to health care facilities. The World Health Organization (WHO) have designated snakebite as a neglected tropical disease and urged its member states to formulate measures to reduce mortality and morbidity from poisonous snake bite.

The victims of snake bite envenomation also suffer economic loss and the burden has to be shared by poor families. Most often the economically productive member is lost due to snake bite. WHO reported that major brunt of the problem of snake bite has to be borne by the South Asian countries including India and Sri Lanka. Snakebite remains an underestimated cause of death in rural India. Some authors indicate that because a large proportion of global totals of snakebites arise from India, global snakebite totals might also be underestimated [1]

Epidemiology of fatal snake bite has been reported sparsely from West Bengal in recent past. Earlier study from south 24-Pargana District in West Bengal showed that only 07.23% snake bite deaths were officially reported. Only 22.19% of the snake bite victims attended the hospitals. Nearly 65.7% of the snake bite deaths were due to Common Krait bite, most of them occurring in the months of June to September. Studies from a tertiary level Medical College in West Bengal [2] reveal significant number of snake bite admissions and deaths. Total number of snakebite deaths in Midnapore Medical College and Hospital (MMCH) was 222 from the period 2012–2016.The case fatality rate was 03.5%. The number of snakebite cases that attended the hospital

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during this period was 6343[3] .

An earlier Study from South India [4] was conducted on 198 snakebite victims and analyzed their socio-demographic and epidemiological details. Their study period was five years with average 40 cases per year. Their case fatality rate was observed as 03.0%. The results revealed that male to female ratio of the study participants was 2.1:1. Similar findings have been reported from the studies conducted in different parts of India[5,6,7].

Clinical and autopsy studies are well documented in fatal snake bite from published works Previous reports from Burdwan district of West Bengal have reported the incidence of severe nephrotoxicity in Cobra [8] Krait[9] and Viperbites[10] . Proven autopsy methods for diagnosis of snake bite and its classification has been devised from semi quantitative methods [11] . Regional variations in the species of poisonous snakes and the composition of venom are to be reckoned with. Earlier autopsy based research [8] reveals renal involvement in 64.28 % of fatal bites by Indian cobra (Naja naja) primarily considered neurotoxic. The major renal changes were tubular necrosis 01(7.14%), cortical necrosis 03 (21.42%) and interstitial nephritis 03(21.42%).

The present investigation was devised to explore the profile of the fatal cases of envenomation in snake bite from a district of northern part of West Bengal. Special emphasis was accorded to the determinants of fatality, the socio-demographic profile, economic burden, and medico legal aspect. Besides the public health aspect of the problem was analyzed in the light of treatment seeking behavior of the nearest of kin of the deceased.

Materials and Method

The descriptive epidemiological study was conducted at the Department of Forensic Medicine & Toxicology, Malda Medical College, Malda, West Bengal after obtaining clearance from the Ethics committee of the institution. The sample consisted of all the cases of fatal snake bite that were brought to the mortuary for medico legal autopsy. As per statutory rules all deaths due to snake bite envenomation are subjected to autopsy to ascertain the cause and manner of death. For the purpose of the study all unknown bites were excluded as they did not conform to the inclusion criterion. The study period was May 2018 to June 2019. The source of information was police inquest, hospital records (for hospital deaths), information from nearest of kin, post- mortem reports and departmental records. Major socio-demographic details, autopsy findings, history and treatment protocol was in a pretested and predesigned proforma. Descriptive statistics and study of association was performed using SPSS version 19 for windows (IBM). P value of less than .05 was considered significant.

Obseervation/Results

There was 1839 unnatural deaths during the study period of which 38 were due to snake bite envenomation (2.067%). Descriptive statistics for age, time interval between bite and admission and time interval between admission and death are seen from Table 1.

Table 1

Descriptive Statistics							
	N	Range	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic
AGE [years]	38	60.00	10.00	70.00	34.1842	2.67884	16.51348
Bite admit gap [hours]	27	13.00	1.00	14.00	3.3556	0.48871	2.53943
Admit death gap[hours]	28	71.50	.50	72.00	8.3946	2.73588	14.47692

The mean age of the cases of fatal snake bite was. 34.18 with SD 2.67. The number of males was 18 and female were 20. The mean age of male and female victims were 41.16 and 27.90 years respectively .There was significant difference in the mean age of the two sexes ; males being higher than females. (t test 2.67,df 36, p=0.011).

Majority of deaths occurred in the months of May to July, June recording highest of 12 deaths. The time of bite is seen from Figure1. Majority of the bites occurred during night between midnight and 2am.

29 (76.3%) cases in the present investigation were hospital indoor deaths while 09 were declared brought dead. The socioeconomic status , monthwise distribution are described in figures 2 and 3.

20 (52.6%) cases were bitten in their lower extremity followed by 15(39.5%) in upper extremity. For the upper extremity group, 11 (28.9%) were in the hand while only 04(10.5%) were in the forearm.

Regarding the toxicity type and presence of fang mark the present series showed no statistically significant association as from Chi square test (chi square 6.86 df 6, p value 0.333).

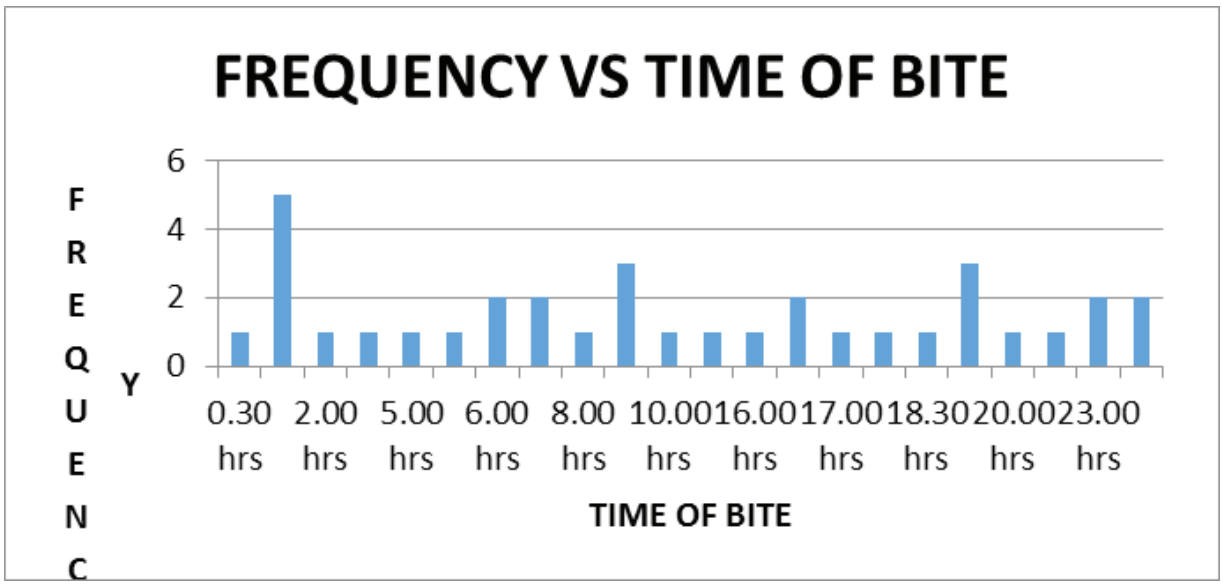


FIGURE 1

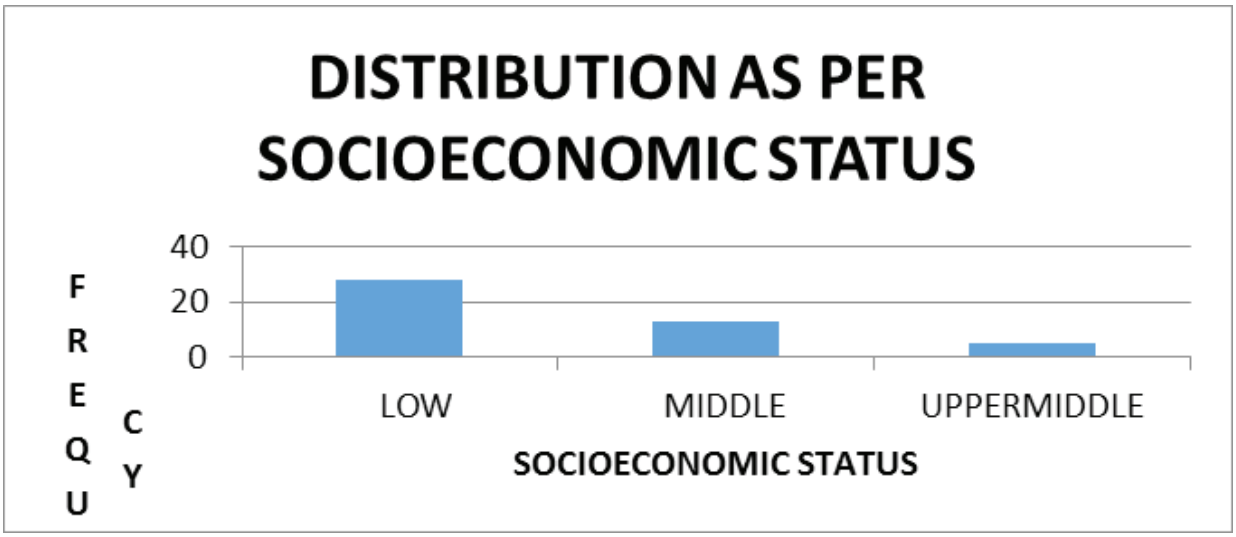


FIGURE 2

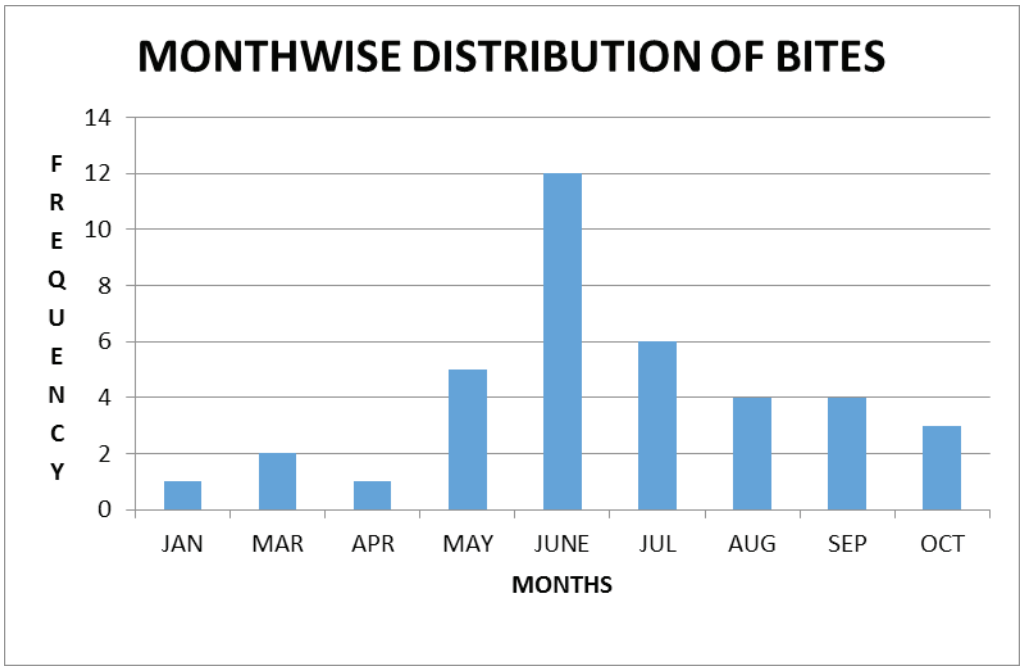


FIGURE 3

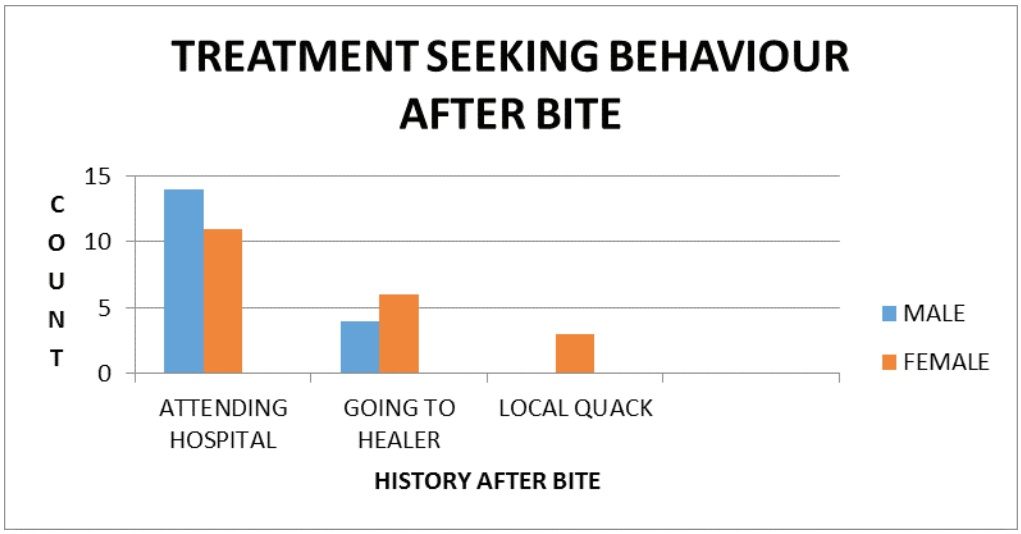


Figure 4

The detail of treatment seeking behavior was seen from figure 4. 11 females (28.95 % of total sample) were directly taken to the hospital for treatment. 04 males (10.53%) and 06 females (15.79%) were taken to some magic /traditional healer after the incident of bite. There after they were shifted to hospital or were brought dead at emergency.

It was seen that the time interval between bite and hospital admission was almost same for both type of snake bites. It was 3.31 hours for neurotoxic bites and 3.39 hours for hemotoxicbites. The interval between admission and death differed in the two types of bites being 9.31 hours for hemotoxic bites and 7.12 hours for neurotoxic bites.

Discussion

Snake bite is an important public health issue that needs more attention and in-depth analysis. The policy perspectives need to be aimed at reduction of mortality and morbidity from this natural accidental hazard. The crux of the problem still remains much underrated,

especially in our country as a whole. Review of recent research clearly indicate that the epidemiology remains unstandardized, erratic. The great variance in the data regarding snake bite related deaths (from 20000 to 50000 per annum) are alarming as many cases are not brought within the purview of official records. There is no uniform system of registering the deaths across so diverse a country. Similar has been the global concern, especially in South Asian and African Countries[1,2]. The present investigation was conducted to capture basic data on a regional basis to supplement the epidemiology of snake bite related mortality. Malda Medical college is the only regional tertiary center for treatment and medico legal cases of the district. This work can be regarded as a district level investigation of snake bite mortality from West Bengal.

In the present series of 38 cases of fatal snake bites, 18 were male and 20 were female. This is strikingly different from earlier reported studies from Indian several states [4,5,6] wherein the percentage ranged from 30 to 35 in females. The mean age in our study was 34.18 year , SD 2.67 . The minimum age was 10 years. The major age group that was affected was 25 to 40 years. This is comparable with previous reports[2,3]. This clearly indicated that the most active and productive age group is the worst victims in rural areas of our country. The findings of age wise distribution was similar to works from South India[4] where the highest proportion of snakebite was observed in the age-group of 21–30 years (*n* = 50, 25.2%), followed by 41–50 years .

This is in agreement with studies from neighboring state of Bihar[6] and reporting from South India[12,13] . Rural population far outnumber the urban counterparts in incidence and mortality of snake bites. It still remains a rural health problem that leads to loss of productivity. This compounds the socioeconomic burden of weaker section of our society.

Education wise distribution in our series reveals that majority were illiterate and primary level learners with their occupation being manual agricultural laborer or poor marginal farmer. This is an agreement with all previous researches from developing countries. Access to healthcare, logistics and awareness is poor in the rural population in major parts of our country. Besides, faith in traditional healers and other superstitions associated with snake bite lead to the gamut of the problem. Poor living conditions, improper clean homes, Kuchcha huts with numerous holes and crevices are ideal for snakes to attack human habitats readily. Sleeping habits on floor, careless about surroundings and growth of vegetation around human dwellings are the contributing factors for the high incidence of snake bite.

In the present work it was seen that the frequent biting time was around 9am during day, 4pm to 7 pm in the evening and mainly at night. Majority were bitten between 6pm and 6 am with 12 cases bitten at around 1-2 pm. This indicated the night habit of biting poisonous snakes and the unsafe sleeping conditions making people vulnerable to bites. This is different from other works where majority were day bites. Since substantial number of the victims were engaged in outdoor works during bite it can easily be inferred that snake bite still remains as an occupational hazard for agricultural and farmland workers. Similar results are reported from several regional studies from South India[12,13,] North India [14] and western India[15] .

Regarding the site of bite and activity after the incidence of bite the present series reveals some interesting facts. It was observed that 20 victims were bitten in the lower extremity, followed by 15 in upper extremity and 03 in other parts like trunk and neck. Interestingly17 (94.44%) of the lower extreme bites ranges from 2 inches above ankle to the toes. This remains the most vulnerable body parts where bites are common place. Several advocacies emphasize wearing of boots as a preventive aspect during field work. Bites on upper parts and trunk are mainly associated with nocturnal bites where the victims were either sleeping on lying in bed.

The present investigation was designed to explore the time lapse between bite and institution of treatment as a pilot study. It was seen that the mean interval between bite and treatment was 3.35 hours (SD0.48). It was noted that 25 reported directly to hospital 03 received primary care by some local medic and remaining 10 were first seen by some magic healer / traditional healer. Undue delay in reporting and initiating treatment with ASV leads to the increased mortality in snake bites. These findings are comparable with earlier works from different parts of our country. Early initiation of supportive care and ASV

is the mainstay in the therapy and reduces mortality. Herein lays the primary focus of all preventive programs in developing countries.

From the autopsy finding in the present series it was seen that bite mark was present in only 24(63.2%) cases. Prominent local changes including edema and blistering swelling and sloughing of tissue was seen in17(44.7%). Kidneys showed pinpoint hemorrhages and cortical hemorrhages in 14(36.8%) cases each. Renal changes are evident hemotoxic (Viper) bites as well as neurotoxic bites (Cobra and Krait). This has been emphasized and elucidated in earlier autopsy centered researches [8, 9, 10,11].

Awareness program and training of medical officers in rural services need to be regularized. Snakebite related fatality has truly remained as a neglected tropical disease. Advocacy by groups along with improved healthcare need to be focused on mitigating the scourge.

Conclusions

The present work was a short regional research to supplement epidemiology and add to the database of deaths from snake bite in a district of West Bengal. It is a pilot study to assess the problem. It reflects the profile of deaths due to snake bite of the entire district. Snake bite comprises 2.067% of all unnatural deaths.

Our present study emphasizes that fatal snake bite is a public health concern and need to be addressed with priority. Policy should be focused on the reduction of incidence and mortality from envenomation in snake bite. More serious large scale research on the problem of snake bite needs to be designed for assessing the socioeconomic burden and policy framework for developing management of snake bite.

Ethics Clearance : The work was approved by IEC of Malda Medical College

Conflict of Interest: No conflict of interest financial or otherwise

Funding : No financial assistance from any source was availed

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Profile of Dowry Death Cases Reported for Autopsy in Thoothukudi Medical College - A Retrospective Study

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Abstract

Violence against women inside the family is a worldwide phenomenon. Violence against women traverses every geographical area, economic layers, education level, age, and employment status. It is seen that in spite of its high education status and all around acclaimed social method of advancement, India is at top position in Dowry Death. United Nation General Assembly in 1993 embraced the Declaration on the Elimination of Violence against Women. All the medicolegal cases brought to an autopsy that is registered under section 304 B IPC are included in this study. Inclusion criteria: Death of married women within 7 years of marriage. A total of 80 cases were analyzed retrospectively. 71(88.75%) cases belong to Hindu religion. 45(56.25%) cases are from urban regions, 40(50%) cases completed high school education, 44 (55%) of the deaths occurred in 2-4 years of marriage, 63(78.75%) cases belong to arranged marriage, 32(40%) cases belonged to nuclear family, 65 (75%)cases belong to the age group 18 to 25 years, 3(3.75%) cases were homicide, Hanging is the most common form of death in this study 44(55%) cases. The government of India has attempted to battle the developing rate of dowry death by instituting different laws to keep such deaths from happening and to punish those responsible when they do happen. Notwithstanding lawful forward leaps, stunning news on dowry deaths kept on appearing in Indian papers.

Keywords: *Dowry deaths, Autopsy, Suicide.*

Introduction

Violence against women inside the family is a worldwide phenomenon. Anyway its consequences are progressively intricate and its power a lot more noteworthy in India. The most wretched part of such monstrosities is violence at home. Violence against women traverses every geographical area, economic layers, education level, age, and employment status. It is seen that in spite of its high education status and all around acclaimed social method of advancement, India is at top position in Dowry Death. United Nation General Assembly in 1993 embraced the Declaration on the Elimination of Violence against Women.

Announcement on the Elimination of Violence against Women characterizes violence against women as “any demonstration of gender-based violence that outcomes in, or is probably going to bring about, physical, sexual or mental damage, or enduring to women including dangers of such acts, pressure or self-assertive hardship of freedom; in the case of happening out in the public or private life”. customarily dowry is a onetime payment of ‘streedhan’ in lieu of her share of the family wealth at the time of her marriage (1). As far as access, participation, and prize the status of women in India isn’t equivalent to the status of men.

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Materials and Methods

All the medicolegal cases brought to an autopsy that is registered under section 304 B IPC in a period between January 2018 to August 2018 included in this study. Inclusion criteria: Death of married women within 7 years of marriage (Section 304 B IPC). Information about the deceased regarding marriage life, occupation, education, was collected from the inquest report, interview with the relatives, cause of death from postmortem certificates. The collected information was filled in the perfoma and analyzed.

Results and discussion

A total of 80 cases were analyzed retrospectively. With reference to the religion 71(88.75%) cases belong to Hindu religion, this is consistent with other studies[3],[4] 5(6.25%) cases to the Muslim religion and 4(5%) cases belong to Christianity. **Fig: 1.**

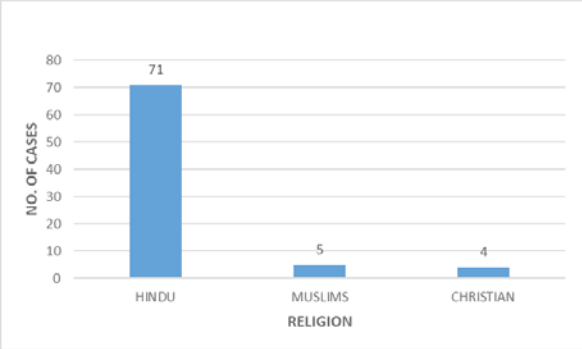


Fig:1. Distribution of religion in the study group

Out of which 45(56.25%) cases are from urban regions, 25(31.25%) from rural areas and 10(12.5%) cases belong to semi-urban areas.

In analyzing educational status among the deceased study population are, 40(50%) cases completed high school education and 25(31.25%) cases completed their degree education., 5(6.25%) cases masters degree and 10(12.50%) cases were illitrate.

Most of the deaths in this study occurred within 2- 4 years of marriage ie- 44 (55%) cases. This is consistent with the study conducted by Rao[2] In 1- 2 years after marriage 18(22.5%) cases were noted . This is in contrast with the study conducted by Pankaj verma *et all*[6], were 45% of cases occurred in this age group. In 4- 7 years after marriage 18 (22.5%) cases were seen. **Fig:2**

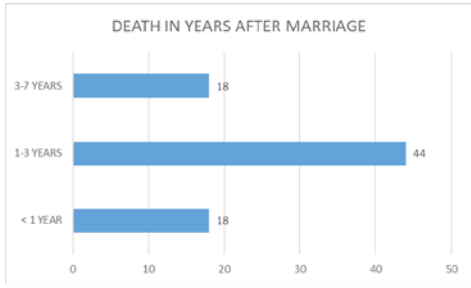


Fig:2: Death occurred –in study group number in years after marriage

In type of marriage 63(78.75%) cases belong to arranged marriage and 17(21.25%) cases love marriage.

Among the deceased 32(40%) cases belonged to nuclear and 48(60%) cases belonged to combined families. 47(58.75%) cases were working and 49 (61.25) cases were housewives and 4(5%) were students.

With reference to the age of the deceased - 65 (75%) cases belong to the age group 18 to 25 years this is consistent with study conducted by Rao[2] were 75% of cases were seen in this age group and 17 (21.25%) cases belonged to the age group between 26- 30 years. 3 (3.75%) cases belong to 30 35 years.

In the analysis of the cause of death in the study group, 3(3.75%) cases were homicide. This is in contrast with the study conducted by naresh et all[3] in which 16% of cases were homicide - 2 (2.5%)cases died of stab injury to the chest, 1(1.25%) case from ligature strangulation. Hanging is the most common form of death in this study 44(55%) cases. Which is consistent with the study conducted by Rao[2] . and in contrast with the study conducted by Kulshrestha[4] and Zine[5] . where burns is the leading cause of death. Next to that burns was seen in 17 (21.25%) cases, poisoning in 15 (18.75%) cases, one case train traffic accident. **Fig:3**

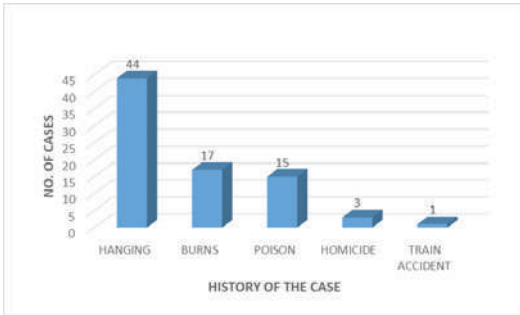


Fig:3. Distribution of cases according to the history of the case

Conclusion

The government of India has attempted to battle the developing rate of dowry death by instituting different laws to keep such deaths from happening and to punish those responsible when they do happen. The government's first effort came out with the Dowry Prohibition Act of 1961. To expand its adequacy, the government has twice revised the Act, in 1984 and 1986. The 1986 revisions require the police and a judicial magistrate to explore each unnatural death of a lady wedded under seven years. At present, the Act forbids the "giving, taking, or demanding of dowry." The Act characterizes "dowry" as property that is given or consented to be given to a newlywed by the other newlywed or either set of guardians "regarding the marriage." Violations of the Act are "punishable with a term of detainment of between a half year and two years, in addition to a fine of up to ten thousand rupees or the estimation of the dowry, whichever is higher. Notwithstanding lawful forward leaps, stunning news on dowry deaths kept on appearing in Indian papers.

Conflict of Interest: Nil

Ethical Clearance: Nil

Funding: Nil

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Closed Mitral Valvotomy (as a Surgical Treatment Option) – Does it has any Medico Legal Bearings in the Present Scenario

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Abstract

Introduction- A prospective study of closed Mitral Valvotomy in 104 patients

Method and Result- From December 2012 to December 2019, 104 patients underwent CMV. 94 Patients become symptom free after surgery and only 7 patients required Mitral valve replacement surgery on follow-up

Conclusion- Closed Mitral Valvotomy represents a satisfactory technique in terms of lower cost, high efficacy, simplicity and reproducibility

Key words: *closed mitral Valvotomy (CMV), Mitral stenosis(MS)*

Introduction

The mitral valve in essence a funnel shaped structure with the apex within the left ventricle is the valve most frequently damaged by rheumatic carditis. Rheumatic heart disease remains a major cardiac problem in developing countries like India as the consequence of rheumatic fever[1]. Mitral valve stenosis is the most common complication of valvular heart disease and it results in increase in pulmonary hypertension, heart failure, pulmonary vascular disease over a period of time and if untreated death ensues. Treatment of rheumatic mitral stenosis ranges from conservative medical management to closed mitral Valvotomy and the more recent ballon mitral valvoplasty[2-4.] Cardiac Surgery started in Department of CVTS from December 2012. Closed Mitral Valvotomy was the first surgical procedure to be performed in our CVTS department.

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Aim: The aim of this study was to evaluate the clinical status of patients with mitral stenosis following closed mitral Valvotomy

Material and Methods: All Patients with the Diagnosis of Severe Mitral Stenosis admitted in the department of CardioVascular & Thoracic Surgery , at Uttar Pradesh University of Medical Sciences , Saifai , Etawah , UP. The suitable patients were selected according to echocardiographic criteria: (1) pliable anterior Mitral leaflet, (2) Absence of significant Mitral Subvalvular disease, (3) Absence of significant calcification and (4) the Mitral valve orifice area less han (1.1 cm2). Excluded patient were those not giving consent for closed Mitral Valvotomy

Surgical Technique : All patients had general anaesthesia. Patients were positioned in right lateral recumbent position, the thoracic cavity was approached through Left Antero-Lateral Thoracotomy, through 4th Intercostal space. Purse string at LA Appendage , finger guidance through it to the Mitral Valve & Dilatation was done gradually using Hegar's dilator (through LV Apex) while monitoring the systemic pressure that was noted to dramatically increase and improve in waveform .

Results

104 CMV Surgeries performed over period of 7 years, mostly in 3rd decade of life, table 1, female outnumber males, table 2

Table -1 Age distribution of patients

Age in years	Number of patients	Percentage
11-20	06	5.7
21-30	45	43.2
31-40	31	29.8
41-50	14	13.4
51-60	08	7.6

Table 2 Sex distribution of the patients

Sex of patients	Number of patients	Percentage
Females	73	70.1
Males	31	29.8

Breathlessness was the most common symptom, table 3, and diastolic murmur the common clinical sign,table 4.

Table -3 Clinical presentation of patients with mitral stenosis*

Symptomatology	Number of patients	Percentage
Palpitations	79	75.9
Breathlessness	82	78.8
Easy fatigability	64	61.5
Edema	56	53.8
Decreased urinary output	34	32.6

*More than one symptom was present in a patient

Table -4 Clinical signs in patients with MS

Clinical signs	Number of patients	percentage
Diastolic murmur	92	88.4
Anaemia	81	77.8
Atrial fibrillation	59	56.7
Hepatomegly	45	43.2
Lost first heart sound	38	36.5

Transesophageal echocardiography (TTE) was done in all and all had features of MS

CMV was done in 104 patients. Only **7** patients required Mitral Valve Replacement surgery on follow up because of symptoms became severe (2 patients after 4 months , 5 patients after 1 Yr)

94 patient became symptom free after surgery and can perform more than ordinary daily activities, most of them on regular follow up. Post procedure mortality in closed mitral Valvotomy was one due to severe icterus and renal failure. This patient had pre-operative icterus. After 17th day of procedure she developed anuria and could not be saved inspite of vigorous try. Two patients lost for follow up

Discussion

Rheumatic fever is the most common cause of mitral stenosis. Although the attack rate for rheumatic fever is equal among genders, MS is 2 to 3 times more common in women[5], and similar observations have been made in present study. Elevated C- protein levels, indicative of ongoing generalized inflammation, were found in many patients before Valvotomy, which supports an inflammatory origin of MS[6]. Symptomatology in the present study is in accordance to other reports[5]

95 patient became symptom free after surgery in our study. CMV should be the procedure of choice for MS in patients with non- calcified pliable valves, the result and cost of operation comparing favourable with those of open heart procedures[7]

Only **7** patients required Mitral Valve Replacement surgery on follow up because of symptoms became severe

Definitive treatment is mitral valve replacement, either by Biological or Mechanical prostheses. Biological Prosthesis is not recommended in young age as it undergoes degeneration fast due to ongoing rheumatic activities. Mechanical prosthesis will require life-long oral anticoagulation and therefore close monitoring for International Normalizing Ratio (INR) is required[8]. Though the operative mortality has to be around 1% our figures of 0.96% are in similarities to others[7,9]

Palliative Surgical treatment has been recommended in developing countries (if Valve replacemnt / Repair is not feasible due to Economical reasons or expertise) as a simple, economical and safe procedure with a good outcome as this does not require life time anticoagulation[7](provided patient has given consent specifically for this procedure)

Conclusion

CMV should always be done in patients who have only pure mitral stenosis, non calcified pliable valve, expert hands, and in such patients in such centres, where facilities for cardiopulmonary bypass are not available. Closed Mitral Valvotomy represents a satisfactory technique in terms of lower cost, high efficacy, simplicity and reproducibility. **We conclude that CMV does not pose any Medico legal bearing as an surgical treatment option for pure**

non calcified severe Mitral Stenosis provided that :

*All patients and patient party should be clearly counselled regarding all the treatment option available (Valve replacement , Valve repair , Closed Mitral / open mitral valvotomy) as well as cost for the different procedures ,major intra and postoperative risk factors , as it is the patient who should choose and decide regarding the operative procedure so as to prevent and medico legal bearings later on.

Conflict of Interest: Nil

Source of Funding : Self

Ethical Clearance: Clearance was taken from ethical committee

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Chest Injury Outcomes due to Road Traffic Accidents – an Institutional Experience

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Abstract

Background -Thoracic trauma is one of the leading causes of mortality and morbidity in developing world countries. Complications in chest trauma develop secondary to rib fractures and subsequently leading to pain, inadequate ventilation as well as direct injury to lung parenchyma due to fractured rib segments.

Methods - In this study, we present our 2 year experience (August 2017 to August 2019) in the management and clinical outcome of 236 chest trauma cases as a result of road traffic accidents. Patients were divided into three groups based on the presence of rib fractures. The groups were evaluated to demonstrate the relationship between the numbers of rib fractured and associated injuries.

Results – Mean hospitalization time was 7.5 days. Mortality rate was 1 % for the patients with only blunt chest trauma, 4 % in patients with more than two rib fractures and 20 % for those with flail chest. We found that there is significant association between the mortality rate and the number of ribs fractured, the patient's age as well time since the accident.

Conclusion – Risk of increased morbidity and mortality was associated with presence of more than two rib fractures, age over 60 years and delayed hospitalization after the injury.

Keywords – *Rib Fractures, Chest Injury, Blunt trauma*

Background

Blunt chest trauma is associated with a high risk of morbidity and mortality [1]. Rib fracture constitutes a major part of blunt chest trauma and each additional rib fracture is associated with an increasing likelihood of developing complications [2, 3]. Each additional rib fracture in the elderly population increases the chances of mortality by 19% and developing pneumonia by 27% [3, 4]. Respiratory complications develop more with the rib fractures as due to severe pain there is inadequate ventilation [5]. Even a single rib fracture is associated with significant morbidity and consequences particularly in the elderly population [6, 7]. This causes decreased lung volume, atelectasis and can even progress to pneumonia, prolonged ventilation and mortality [8].

Patients and methods – This is a retrospective analysis of all blunt chest trauma patients secondary to road traffic accident. Over a 2 year period, between august 2017 to august 2019, 236 patients with chest injuries following road traffic accidents were admitted to emergency and trauma centre , Uttar Pradesh University of Medical Sciences , Saifai (UPUMS). Patients who died at the scene or were declared dead on arrival were excluded because of incomplete data.

This trauma centre caters both adult as well paediatric population , since it is an superspeciality

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institution and various department work together for the management of trauma patients. Many patients with road traffic accidents are referred to our centre after first management at nearby hospitals.

Patient's physical condition on presentation, number of ribs fractured, patient's age were the main factors affecting our decision for hospitalization. All patients not requiring hospitalization were given symptomatic treatment and called back for follow up visits at 24 hour, at the end of the first week of injury and on regular intervals till 2 months if there were no complications.

Patients were divided into three major groups based on the presence of rib fractures. Group I consisted of patients without rib fractures; Group II consisted of patients with one or two rib fractures; Group III consisted of patients with more than two rib fractures. The groups were evaluated to demonstrate the relationship between the number of rib fractures and associated injuries.

Results - There were 192 male (81.4%), and 44 female (18.6%) patients. Mean age was 45 years, ranging from 6 to 87 years. Of 236 patients, 154 (65.3%) were hospitalized and the remaining 82 (34.7%) patients were followed without hospitalization. Mean hospitalization time was 7.5 days, ranging from 3 to 22 days.

Chest pain and dyspnea were the most common symptoms at presentation whereas sensitivity over the chest wall, bone crepitation and subcutaneous emphysema were the most common findings on physical examination. All patients with chest discomfort with minimal findings on physical examination were followed up without hospitalization. Soft tissue trauma and rib fractures were the most common problems observed following blunt thoracic traumas. Rib fractures were detected in 146 patients, and all were hospitalized.

Rib fractures were associated with subcutaneous emphysema in 37 patients (25.3%). Total 122 patients presented with Hemo/pneumothorax , in which 112 patients were with rib fractures(76.7%) and in 10 patients without rib fractures.

Extremity fractures were found to be the most commonly associated extrathoracic injuries in patients with rib fractures (Table 1)

Table 1: Associated injuries in patients with chest injuries

	Group I(Patients without rib fractures) n= 90		**Group II (Patients with one or two rib fractures) n=135**		**Group III (Patients with more than two rib fractures) n=11**	
Extremity/pelvis	4	4.4	5	3.7	-	
Intra-abdominal / retroperitoneal	1	1.1	1	0.7	2	18.1
Facial trauma	1	1.1	2	1.5	2	18.1
Cranial trauma	2	2.2	2	1.5	2	18.1
Spinal/vertebral trauma	-		1	0.7	3	27.3
Clavicular fracture	3	3.3	2	1.5	1	9.1
Diaphragmatic injury	-		-		3	27.3
Hemothorax/ Pneumothorax	10	11.1	104	77	8	72.7

Chest tube drainage was performed in 116 patients with pneumothorax, hemothorax or hemopneumothorax. Tube thoracostomy was performed successfully in all, but 5 of these patients required thoracotomy as they were having ongoing blood loss. 11 patients presented with flail chest. All patients with flail chest were followed in the Intensive Care Unit. The management of these patients were based on the trend of serial measurements for arterial blood gases. In all patients with flail chest, analgesia was provided through administration of parenteral analgesics and epidural analgesics. In two of these patients, tracheotomy was performed aiming suctioning of secretions and giving oxygen to the patients more effectively. Of all patients with flail chest, three patients died. Mean hospitalization time for these patients was 17.8 days. Primary bronchial anastomosis was performed in two patients, removal of intrathoracic hematoma and control of bleeding in two patients, removal of empyema and decortication in one patient, and thoracic wall stabilization and control of bleeding in one patient. Three patients underwent thoracotomy with laparatomy due to diaphragmatic rupture. One patient undergoing diaphragmatic repairing died postoperatively.

In total, no deaths in Group I, one death in Group II and 3 deaths in Group III were observed (Table 2).

Age (years)	Group I (n=90)		Group II (n=135)			Group III (n=11)			Total		
	Patients	Mortality	Patients	Mortality	%	Patients	Mortality	%	Patients	Mortality	%
0-14	07	-	6	-	-	1	-		14	-	-
14-59	78	-	118	-	-	8	2	25	204	2	0.1
>60	05	-	11	1	9.1	2	1	50	18	2	11..1
Total	90	Nil	135	1	0.7	11	3	27.2	236	4	1.7

The difference was statistically significant for Group II versus Group III , but mortality was not significantly greater in Group II compared to Group I . Mortality rate was 1.7% for all patients with rib fractures.

Discussion

Non-penetrating chest injuries are seen very frequently in civil populations. The major reasons for blunt chest injuries are traffic accidents with an incidence of 70–80% [9]. Rib fractures are reported as the most common pathologies associated with chest trauma (35–40%) [10]. In our series, the incidence of rib fractures was 57.2% (patient with one or 2 rib fracture) and 4.6% (patients with more than 2 rib fractures) and all were hospitalized. Although some authors have suggested that patients with rib fractures require hospitalization not only for their associated injuries but also for pain control and pulmonary complications [11], there is not an immediate indication for hospitalization for the patients with rib fractures. According our opinion, the majority of patients with 'simple' thoracic injuries can safely be treated at the level of the primary health care center or as outpatients at the district hospitals as previously reported [12]. It is recommended that all rib fractures should be

re-evaluated at 48 to 72 h after injury because of the frequent late appearance of pulmonary complications [13].

The presence of more than two rib fractures is a marker of severe injury. Eighty-one percent of our patients had hemothorax and/or pneumothorax and most of them presented with associated extra thoracic injuries. Lee reported that mortality doubles (1.8 versus 3.9%) for patients with three or more rib fractures and those with no rib fractures [14]. The presence of fractures of the first or second ribs has also been reported to be indicative of severe trauma. Poole reviewed all series of fractures of first and second ribs and found a 3% risk for aortic injury and a 4.5% risk for injury to a brachiocephalic vessel [15]. In our series, we did not observe any major vascular injury. Subcutaneous emphysema is a clear indication of injury to the respiratory tract. Seventy-six percent of cases were associated with hemothorax and/or pneumothorax in this study. All of the patients with subcutaneous emphysema had fractured ribs, which also led to lung injury. Kalyanaraman et al. reported that lung injury seems to be associated with rib fractures in 74% of cases with subcutaneous emphysema [16]. In most of the cases, subcutaneous emphysema is self-limited and it is essential to identify and treat the underlying cause. Some authors have reported that acute respiratory failure caused by massive subcutaneous emphysema requires decompression of the anterior mediastinum [17–19]. Flail chest is a serious problem in blunt chest trauma practice because of the risk of respiratory insufficiency where stabilization may be required. Although recently surgical procedures have been mentioned to decrease the mortality and morbidity rate by some authors [20, 21], operative fixation has not yet been widely accepted [13]. We prefer performing surgical procedures only when thoracotomy is required for another indication and fixation by mechanical ventilation only in case of respiratory insufficiency. In flail chest, mortality rate is reported between 11 and 40% [22–25]. Our mortality rate for flail chest was 27.2%, comparable to studies by clark GC, Freeland M and Shacford SR et al.

The maintenance of pulmonary and tracheal hygiene, effective eradication of pleural fluid and air played a significant role in minimizing pulmonary complications. If the conservative treatment is not sufficient and intrathoracic organ injuries are detected, early or late thoracotomy should be performed. In the literature there has been a significant decline in the number of operations over recent years [26]. Richardson states that less than 5% of patients sustaining blunt chest trauma have indications for a thoracotomy [27]. In our series, only 05 patients required thoracotomy and the operation rate was 3.4% in hospitalized patients. Rising mortality rates depend on the severity and complexity of the wounds and older age. Hospital mortality rates for isolated chest injuries were reported to range from 4 to 8%, and increased to 13–15% when another organ system was involved and to 30–35% when more than one organ system was involved [28]. Lee et al reported the mortality rate as 1.8% in all patients with blunt chest trauma [14]. This rate was 1% in our series. Most traumas occur between 14 and 60 years of age. Mortality rate significantly increased in overall patients with more than two rib fractures. However, we did not found any correlation between the mortality rate and the number of rib fractures for the elderly patients. It takes a less severe injury to be lethal in this age group. Osteopenic changes and co-existent underlying disease may also play a significant role in this process [21]. The clinical state of the patient, severity of the trauma, age, presence of more than two rib fractures, presence of flail chest, presence of subcutaneous emphysema and possible intrathoracic injury help in making the decision for hospitalization.

Conclusion

Chest trauma is an important public health problem accounting for a substantial proportion of all trauma admission at UPUMS, Saifai. The risk of mortality in chest trauma has been associated with the presence of more than two rib fractures, age older than 60 years and delayed hospitalization after the injury. The ability to identify those patients having significantly higher risk for morbidity and mortality ensures the establishment of treatment priorities and efficient management of existing injuries.

Conflict of Interest- Nil

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Ethical Clearance: Institutional Ethical Committee Clearance was taken.

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Assessment of Factors Influencing Academic Performance of Nursing Students

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Abstract

Objectives: this study is intended to assess factors influencing academic performance of nursing students in selected institution.

Methods: This is a descriptive cross sectional design conducted at Ambala, Haryana, India. 420 participants (308 females and 112 males) consisting of age group between 17 to 25 years were included. Responses were collected by structured scale consisting of 22 items in 3 point scale.

Results: Maximum students (88.6%) agreed that good and supportive relationship between teacher and students fosters better academic performance of students. A significant number felt that different teaching strategies used by the nurse educators help to improve academic performance. More than half of the students (53.8%) disagree that students from low socioeconomic status have low scores in their academic performance compared to their classmates with high socioeconomic status. From all of the academic factors, highest mean is of teacher related factors. There is a significant association found with mother's education.

Conclusion: These findings to be helpful for nursing college teachers and managers to advance academic performance of students.

Key words: *factors, academic performance, nursing students*

Introduction

Education is considered most imperative aspect for individual and country development. It not inculcates knowledge, essential abilities, skills but also for escalation and progress. An erudite person accomplishes desired target and renders services to community as learning plays pivotal role in enhancing academic achievement. Education also plays an important role in augmentation of human wealth and personality building[1]. Educational institutions have no value without learners. Quality graduates produced by excellent educational institutions took part in nation development and become leaders of country[2, 3].

There is an increased call for convalesce the students learning environment although nursing education is combination of theoretical and practical learning experiences. Skill and competency not only requisite for nursing students, knowledge in subjects also required. Nursing students need to supported and guided in institutional environment to strengthen knowledge, skills and attitude[4, 5]. There is a range of factors that affects quality of student performance. The determinants of academic performance are student attitude, institution resources, classroom environment, class assignments, daily study hour, accommodation, social circle and event participation as well. Institution resources and classroom environment are key aspects

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influencing academic performance. Parental pressure teacher motivation has also impact on enhancement of academic performance[1, 6].

Kapur study concluded that attitude of the student, resources, classroom environment, leadership and skill ability of principal and faculty members, health status of students and role of parents are common factors affecting academic performance. In addition to role of parents also elaborated on positive and negative factors of parents. Pleasant home environment, encouraging attitude of family members, and provision of resources, well & professionally educated parents, small family size and focus on child need are parental positive factors. Negative factors are discouraging parental perspective, large family size, poverty, lack of provision of resources and extra classes. Social interaction is imperative for students in an institution although social circle influences academic performance of students[1].

Persistent problems in student quality are weak performance and failure rate. Failure rate enhances pressure on students as well as on parents. Recognition of these factors could lessen the failure rate[7]. Students stress, anxiety, fear and depression confirm impinge on performance. These problems hamper the psychological approach of students. Teachers and parent's motivation enable the student to enhance performance. Counselling services should be involved within college premises as posse's adequate expression of emotions including anger.

This study intended to assess factors affecting academic performance which helps the organization to advance the performance of students. On the basis of literature review this study assesses factors such as: Study environment, communication and teacher related factors, parent related factors and student related factors.

Methodology

This was a descriptive cross sectional design using quantitative methodology. The study participants comprised of all four year students of B.Sc Nursing conveniently selected from colleges of Ambala, Haryana. Setting of the study was Maharishi Markandeshwar deemed to be university, Mullana, Haryana. All students of B.Sc Nursing batch who were willing to participate incorporated in the present study. Total enumerative sampling technique was adopted to collect data. Out of total population (560), 420 questionnaires were completed. All the students were explained about the nature and purpose of the study and consent was obtained. Authorized approval was gotten from Institutional Ethics Committee.

Researcher developed structured academic factors influencing scale with maximum score of 66 and 22 minimum. This inquiry form contains two parts: Part I sample characteristics comprises 15 items and part II comprises 22 items addressed on the factors affecting academic performance. The respondents were asked to agree or not to a list of statements to measure their perceptions about factors, on a three-point Likert scale.

Results

Responses of questionairrre were analysed using SPSS Version 20 to determine frequency, percentage, mean, standard deviation, t-test and F-test.

Frequency and percentage distribution of nursing students according to their sample characteristics display that majority of subjects were female 308 (73.3%) and half of the subjects were in age group of 20-22 years i.e. 215(51.2%). Maximum of sample's father's and mother's education was matriculate 122 (29%), 134 (31.9%). More than half of sample's father guides them in their study i.e. 251 (59.8%) followed by mother 74 (17.6%). More than half of samples gave 2-4 hours for self study 234 (55.7%), 1-2 hours for house hold work i.e.272 (64.8%) and 6-8 hours for sleep i.e. 340(81%). In previous years academic performance 223(53.1%) subject took 61-74% marks followed by 95(22.6%) took marks between 51-60%. Maximum of the subjects participate in cultural activities 204(48.6%), 89(21.2%) participate in sports activities and 127(30.2) have not participate in any kind of co-curricular activities.

In addition, result pointed that mean score of factors affecting academic performance is 55.7 and standard deviation is 4.7 with range 41-66.

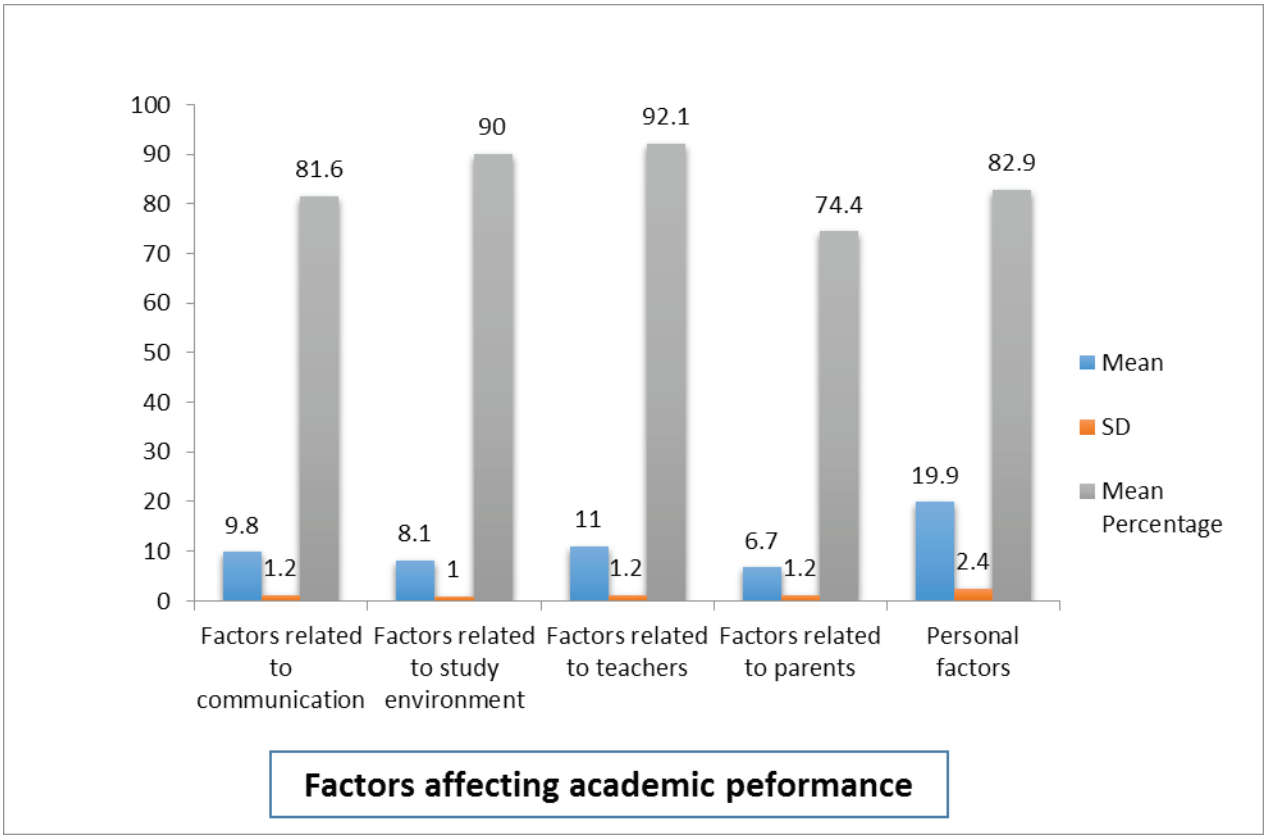


Figure 1 presented area wise mean, SD and mean percentage that factors related to teachers and factors related to study environment has highest mean percentage i.e. 92.1 and 90 and mean + SD 11 + 1.2 and 8.1 + 1. Whereas factors related to parents have lowest mean percentage i.e. 74.4 and mean + SD 6.7 + 1.2.

Table-1: Frequency and percentage distribution of communication factors affecting academic performance

N=420

Sr. No	Items	Agree f(%)	Neutral f(%)	Disagree f(%)
I believe that				
1.	Communication through smart phones (calls and messages), emails facilitates easy transmission of information between the teacher and the student.	296 (70.5)	110 (26.2)	14 (3.3)
2.	I spend time in studying along with friends.	210 (50)	154 (36.7)	56 (13.3)
3.	A good and supportive relationship between teacher and students fosters better academic performance of students.	372 (88.6)	44 (10.5)	4 (1)
4.	Spending more time with friends has negative effect on academic performance.	112 (26.7)	186 (44.3)	122(29)

From Table 1 results, it must be noted that a significant number 372(88.6%) agreed in good and supportive relationship between teacher and students fosters better academic performance. Nearly two third, 296 (70.5%) students agreed that communication through smart facilitates easy transmission of information between teacher and student. Above one fourth, 122(29%) students disagreed in spending more time with friends has negative effect on academic performance.

Table-2: Frequency and percentage distribution of Study environment factors affecting academic performance

Sr. No	Items	Agree f(%)	Neutral f(%)	Disagree f(%)
I believe that				
1.	Classroom computer technological gadgets are important tools to engage student nurses in meaningful learning.	337(80.2)	73(17.4)	10(2.4)
2.	Institution internet and my phone internet service favour my academic performance.	291(69.3)	112(26.7)	17(4)
3.	Availability of study space (classroom and accommodation) supports my academic achievement.	309(73.6)	100(23.8)	11(2.6)

From Table 2, responses showed that a significant number 337(80.2%) Classroom computer technological gadgets are important tools to engage student nurses in meaningful learning. Followed by, 309 (73.6%) students agreed that Availability of study space (classroom and accommodation) supports my academic achievement.

Table-3: Frequency and percentage distribution of teacher related factors affecting academic performance

N=420

Sr. No	Items	Agree f(%)	Neutral f(%)	Disagree f(%)
I believe that				
1.	Availability of qualified nurse educators ensures positive learning outcomes in terms of student achievements.	356 (84.8)	62 (14.8)	2 (0.5)
2.	Different teaching strategies used by the nurse educators help me to understand the topic(s) which also improve my academic performance.	366 (87.1)	50 (11.9)	4 (1)
3.	Academic support services offered by teachers help improve my academic performance.	343 (81.7)	71 (16.9)	6 (1.4)
4.	Problems discussed with mentors in mentor's meeting helps students to improve their academic performance.	267 (63.6)	119 (28.3)	34 (8.1)

Data shown in Table 3 revealed that a significant number 366(87.1%) different teaching strategies used by the nurse educators help me to understand the topics. Maximum number of students agreed availability of qualified nurse educators ensures positive learning outcomes in terms of student achievements. Majority students reported 343(81.7%) academic support services offered by teachers followed by 267(63.6%) problems discussed with mentors in mentor's meeting helps students to improve their academic performance.

Table-4: Frequency and percentage distribution of Parent related factors affecting academic performance

N=420

Sr. No	Items	Agree f(%)	Neutral f(%)	Disagree f(%)
I believe that				
1.	Parents must be involved in the education of their children as good predictor of better academic performance of students.	330 (78.6)	82 (19.5)	8 (1.9)
2.	Students whose parents are literate have greater advantage in their academic performance than those whose parents are non literate.	193 (46)	164 (39)	63 (15)
3.	Students from low socioeconomic status have low scores in their academic performance compared to their classmates with high socioeconomic status.	78 (18.6)	116 (27.6)	226 (53.8)

Results shown in Table 4 revealed that a majority students 330 (78.6%) agreed that parents must be involved in the education of their children. More than half students 226(53.8%) disagreed in students from low socioeconomic status have low scores in their academic performance compared to their classmates with high socioeconomic status. Less than half, 193 (46%) students agreed that Students whose parents are literate have greater advantage in their academic performance than those whose parents are non literate.

Table-5: Frequency and percentage distribution of Personal factors affecting academic performance

N=420

Sr. No	Items	Agree f(%)	Neutral f(%)	Disagree f(%)
I believe that				
1.	Difficulty in understanding English language affects student's academic performance.	237(56.4)	115(27.4)	68(16.2)
2.	Staying at far distance to institution affects the student's academic performance.	189(45)	161(38.3)	70(16.7)
3.	Female students perform better than male students do academically.	92(21.9)	153(36.4)	175(41.7)

Cont... **Table-5: Frequency and percentage distribution of Personal factors affecting academic performance**

4.	Gap in study period has impact on student academic achievement.	257(61.2)	127(30.2)	36(8.6)
5.	Personal/Social issues influence academic performance.	310(73.8)	100(23.8)	10(2.4)
6.	Deterioration of health halts academic performance.	306(72.9)	107(25.5)	7(1.7)
7.	Poor Concentration in class influence academic performance.	328(78.1)	81(19.3)	11(2.6)
8.	Class absenteeism affects academic performance	315(75)	88(21)	17(4)

Table 5 showed that a significant number 328(78.1%) agreed that poor Concentration in class influence academic performance followed by 315(75%) Class absenteeism affects academic performance. Many students237 (56.4%) agreed that difficulty in understanding English language affects student's academic performance. Less than half 175 (41.7%) students disagreed that female students perform better than male students do academically.

To further deepen the academic factors finding, ANOVA association findings suggest that only mother's education was statistically significant at a level of 0.05significance, where F value is 3.19 and p value is 0.05 and other sample characteristics are not significant at 0.05 level of significance. Age, gender, class, residence, study hours, previous year marks and co curricular activities found to be not significant.

Discussion

Education is considered as first step in human era in present globalisation world. It plays vital role in career opportunities for better living wage[8]. To advance the student progress in the present study, researcher assessed factors affecting academic performance. Researcher assessed respondents in terms of age, parent's education, economic status, study hours, guidance and previous year academic performance. Researcher measured their perceptions about factors, on a three-point Likert scale. Further findings concluded that a significant number agreed that different teaching strategies used by the nurse educators, good student teacher relationship, availability of qualified nurse educators, academic support services offered by teachers, classroom computer gadgets and involvement of parents accelerate academic performance. Nearly half students disagreed that students from low socioeconomic status and female students perform academically. In ANOVA/t association findings analysed that only mother's education was statistically significant with factors.

Dube & Mlotshwa conducted survey on perceptions of enrolled student nurses on factors influencing their academic performance. Results of study suggested that use of information communication technology, phone calls, emails and electronic discussion boards classroom computer technological gadgets and internet services gave positive influence on the academic performance. English language considered barrier. A great impact was found in family related factors[6]. In the present study findings revealed that involvement of family members enhance academic performance.

Farooq and fellow mates assessed academic factors and compared with annual examination marks. Results revealed that parent's education and socioeconomic status have significant effect on academic performance. In the present study mothers education found to significant with factors[8].

Learning services are not difficult to measure and not tangible, so studies investigated that student behaviour, social media, online resources, background affects the academic performance. Phone calls and text messages found to be strongest tie in student interaction.

Personality and social environment have considerable dependence on academic performance[9].

Conclusion

The result of the study revealed the factors responsible for academic performance. There is an utmost most to recognize factors and do needful change in teaching strategies, organization modalities to enhance academic performance. Parents and teachers must adhere in student guidance for their bright future.

Conflict of Interest: Nil

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Author's Contributions: All author contributed equally to this work and all authors read and approved final manuscript.

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A Descriptive Correlation Study on Stress and Psychological Wellbeing among Nursing Students in Selected Nursing Institutes of Ambala, Haryana

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Abstract

Introduction-There is widespread recognition that stress can have reflective negative impacts on nurses' psychological well-being. Stressful environment was repeatedly causing a negative effect on the academic performances and psychological well-being of the nursing student

Method- A correlation study was selected. Total 120 nursing students were included by disproportionate stratified random sampling. Data was collected by using DASS-42 (14 items of stress) and psychological general wellbeing index

Result- Finding of the study showed that there was significant moderate negative correlation between stress and psychological wellbeing.

Conclusion-The study concluded there was a significant correlation between stress and psychological wellbeing scores among nursing students. Hence, it infers that if stress decreases psychological well being increases and vice versa.

Keywords: *Stress, Psychological wellbeing, Nursing student*

Introduction

Life events and stress contributed significantly to psychological distress. The pattern of psychological distress differed between the nursing students. Stress and psychological distress may have negative outcomes for the retention of nursing students in program of study. Student nurses experience significant stress and psychological distress during their training and this may contribute to sickness, absence and attrition.[1]

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Student nurses experience significant stress and psychological distress during their training and this may contribute to sickness, absence and attrition. Stress is a well-recognized feature of the life of undergraduate nursing students. Increasing body of evidence examining the impact of stress on health and psychological well-being in student nurses, as they are known to carry higher levels of stress than other student's population. Excessive levels of stress may lead to physical and mental health problems and may affect student's academic performance.[2]

A huge body of research exist which suggests that medical practitioner and medical students may be at greater than before risk of a number of personal and psychological problem. The negative impact of

psychological well-being is well characterized in the literature outcome.[3]

The consequence of stress on psychological well-being affect the student ability as stress has been known as barrier to concentrate on study, decrease problem solving ability, and show adverse symptoms and illness in the students such as anxiety and depression. It is important to learn stress management technique from which students able to manage their as well as patient stress.[4]

Nursing Students perceived that their education is highly stressful and continue score above average on stress scale in many studies, it associated with decrease psychological well being, poor academic performance, and impaired cognitive function and some student think to quit their studies in between.[5]

Material and Methods

The study was conducted during the period from October 2018 to April 2019 in Haryana, India. Total 364 students were screened by DASS 42 from two nursing institutes, among them 169 students were had stress. One hundred twenty students were selected by using disproportionate stratified random sampling, total 120 subjects were taken. Data was collected by using DASS-42 (14 items of stress) and psychological general well being index.

Description of Tool

DASS 42 (14items for stress) Scale to assess the stress: A standardized tool was used to assess the stress among nursing students. It consists of 42 items with a 4-point rating scale in which only 14 items were used to check the stress. Which were collected from participants with self-report technique (Paper and pencil). This tool is incorporated in the study after getting prior permission from the tool developers. The calculated Cronbach Alpha Internal consistency was 0.79 (Acceptable range is 0.7-0.9).

Psychological General Well Being Index

A standardized tool was used to assess the psychological wellbeing index among Nursing students. It consists of 22 items (With 6-point rating scale) in relevant to anxiety, depressed mood, positive wellbeing, self-control, general health, and vitality. This tool was incorporated in the study after getting prior permission from the tool developer. The calculated Cronbach Alpha Internal consistency was 0.79 (Acceptable range is 0.7-0.9).

Data Analysis

Descriptive statistics

Frequency and Percentage distribution was used to describe the level of stress among nursing students

Inferential statistics-

Pearson's correlation was used to check the correlation between stress and psychological well being

Result

Data were entered in Microsoft Excel, and analysis was done with SPSSversion20. More than half of the Nursing Students (56.9%) were having mild stress. Nearly one third (31%) and were having moderate stress, least (10.3%) were having severe stress and (1.7%) were having extremely severe stress shown in figure 1

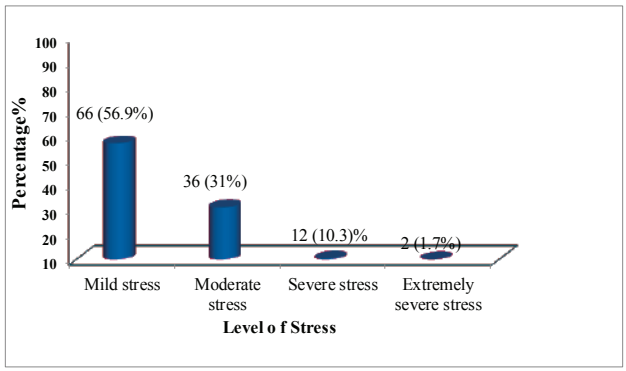


Figure 1- Cylindrical bar diagram showing frequency and Percentage Distribution in terms of Level of Stress among nursing students

Table 1-depicts the Range, Mean, Median, Standard deviation of stress and psychological well being among nursing students The mean score of stress was 19.53 ± 5.02 and median 18.00 with range of 15-41. Further it revealed that the mean score of psychological well being was 62.15±11.77 and median was 63.00 with range 31-85.

Table 1-Range, Mean, Standard deviation, Median of stress and psychological well being among nursing students **N=116**

Variable	Range	Mean ± SD	Median
Stress	15-41	19.53 ± 5.02	18.00
Psychological well being	31-85	62.15±11.77	63.00

Minimum score-0 Maximum score – 42(stress)

Minimum score- 0 Maximum score -110(psychological well being)

Table 2 depicts Area wise Mean, Standard deviation and median of Psychological well

Table 2: Area wise Mean, Standard deviation of difference, Median of Psychological well being among nursing students

N=116

Variable	Mean ± S.D	Median
Anxiety	12.3± 3.97	12.00
Depressed mood	9.24± 2.70	9.00
Positive well being	10.65±2.79	11.00
Self control	8.65 ± 2.66	8.00
General health	9.36±2.56	9.00
Vitality	11.94 ± 2.6	12.00

Table 3- depicts the correlation between stress and psychological wellbeing scores among nursing students. It shows that there was a statistically significant moderate negative correlation(r= -0.38, p=0.001) between stress and psychological wellbeing among Nursing students. Hence, it infers that if stress decreases psychological wellbeing increases and vice versa.

Hence it inferred that there was a significant correlation between stress and psychological well-being scores among Nursing students.

Table 3-Correlation between stress and psychological well being scores among nursing students

N=116

Correlation(pretest)	Stress r (P value)	Psychological well being r(P value)
Stress	XX	-0.38(0.001) **

**-Significant ($p<0.01$) $r(114)= 0.19$

Discussion

In the present study, 2(1.7%) students were having extremely severe stress, 12(10.3%) students were having severe stress, 36(31%) were having moderate stress, 66(56.9%)students were having mild stress. These findings are contrast with the study conducted by Dr. Indira. A, Kalavath B (2012)[6] where 15(25%) students had mild stress, 22(36.7%) students had moderate stress, 23(38.3%) students had severe stress. Further these finding were consistent with the findings of Ribeiro Feitosa Cestari,Islene Victor Barbosa (2014)[7] where they found that majority (64%)of the nursing student had stress.

In present study, there was a significant moderate correlation between stress score and psychological wellbeing score(r=0.31, p=0.05) in experimental group and a significant moderate positive correlation between pre stress and psychological wellbeing score in comparison group among nursing students (r=0.43, p=0.00). These finding were in contrast with the finding of Kimarie Brown, Pauline Anderson- Johnson and Anderson Norman McPherson(2016)[8] where it was found that there was no significant relationship between stress and psychological well-being. Further this finding were consistent with the finding of GuiyuanQiao ,Sijian Li, where it was found that there were negative correlation between stress and psychological well-being.

Conclusion

The study concluded that there was a significant correlation between stress and psychological wellbeing scores among Nursing students. If stress decreases psychological wellbeing increases and vice versa.

Conflict of Interest: NIL

Funding Sources: NIL

Ethical approval: The ethical clearance was obtained from university research ethics committee of Maharishi Markandeshwar(Deemed to be University), Mullana, Ambala(MMDU/IEC/1167) and the study were carried out in accordance with the guidelines laid by Indian Council of Medical Research ICMR(2017). Prior permission was taken from the principle of nursing institutes to conduct the study. The written consent was collected from the nursing students prior to the study. The purpose of carrying out a research project was explained and assurance of confidentiality was given to the participants.

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Observations on Inter-Rater Agreement in Assessing Fusion Activities at Elbow, Wrist and Pelvis by Conventional and Digital X-Ray Films

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Abstract

The present study was designed to measure the inter-rater reliability between two observers in opining and grading the degree of fusion of the secondary centres of ossification using digital and conventional X ray. Attempt was made to examine whether the digital x-ray is superior to conventional X-ray as regards the interrater reliability. It was found that The Kappa score ranged from 0.560 to 0.761 for conventional x-ray films; whereas it ranged from 0.840 to 0.946 in case of digital x-rays. Interrater agreement was noted to be near perfect using digital x ray for all the centres of ossification applied in the study .The accuracy of digital radiography in age determination by observing the fusion activity at elbow, wrist and pelvis (Using Schmeling's staging) will be superior than conventional radiography

Keywords*: Inter-rater agreement, Cohens Kappa, Digital-Conventional X-ray elbow-wrist-pelvis, ossification*

Introduction

The human skeleton undergoes age related morphological and structural changes. Of these changes, the secondary centre of ossification and their fusion activity follow a definite chronological pattern. This phenomenon is utilized in estimating age from radiological examination of bones .

Radiography is the commonest modality used to determine bone age. Radiological methods of assessing fusion activity may be by conventional or digital x ray. Non-radiological techniques of visualizing bone maturity (i.e. Ultrasonography,) have also been reported, but are not as accurate and reliable as radiography. Forensic Age Estimation constitutes a field of expertise in clinical forensic medicine. It aims at defining as accurately as possible the chronological age of individuals by radiological examination for bone development. This is often applied in legal proceedings, immigration cases and competitive sports to determine age of the subject

Examining the X ray film is it conventional or digital, requires ample amount of experience and expertise. The interpretation of the degree of fusion activity is often wrought with subjectivity and can depend on several factors including the type and quality of the film. Therefore, the repeatability, standardization and inter-observer agreement are import factors to reckon with.

The concept of inter-rater reliability or agreement pervades many areas of modern research and it becomes an important facet of accuracy of observation. Inter-rater reliability can be measured by various statistical methods. Cohen's Kappa is a commonly employed method to explore the agreement between two raters.

G. Galstaun [(1)] is considered as the pioneer of

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Radiological age estimation of Indian subjects. He prepared a chart containing the ages of appearance and fusion of several secondary centres based on conventional radiological examination on specific regional Indian population. It is widely used as reference and accepted in judicial proceedings.

Hjren et al. (2011) [2] conducted a study to show the urgent need of better legal procedures for age determination. In an earlier research conducted on a population of 114 immigrant Moroccan males in 2003, Garamendi et al. [3], found Carpus X-ray (Greulich and Pyle method) to be the most useful method to predict chronological age of over or under 18.

In 2000, Schmeling et al [4] in their study showed the importance of developing a standardized method of estimation of age. They established that combination of physical and secondary sexual characteristics, dental X-ray, X-ray of left hand along with X-ray and/or CT Scan of clavicles would increase the diagnostic accuracy for estimation of age.

Numerous researches have established that x ray remains the modality of choice in estimating age in almost all populations [5-14]. With the advent of digital X-ray its gradual increase in clinical application was noteworthy. It was followed that digital images would far out match the conventional ones in quality and clarity. Likewise it raises several questions: What would be the rational choice? Given the cost involved, what would be the option in a setting with fewer resources? Besides, how far did the observers agree on the findings using digital images compared to conventional X-ray? These pertinent questions prompted us to examine the interrater agreement in assessing the fusion activity in several bones using conventional and digital X-ray.

The present study was designed to measure the inter-rater reliability between two observers in opining and grading the degree of fusion of the secondary centres of ossification using digital and conventional x-ray. Also attempt was made to examine whether the digital x-ray is superior to conventional X-ray as regards the interrater reliability.

Materials and Methods

The present research was conducted in Department of F.S.M. of Burdwan Medical College, a tertiary Medical Teaching Institution of West Bengal, India. Thirty sets of conventional X-rays (each set consisting of 3 radiographs one each of elbow, wrist and pelvis in AP view), and 30 similar sets of digital X-rays (each set consisting of 3 radiographs) were used in the study. All the X-ray plates from the conventional group were examined using standard view box by first two authors of this study separately after blinding. The raters gave their scoring on the degree of fusion using Schmelings score for all the centres separately. Then the scores were examined for inter-rater agreement by calculating Cohens Kappa for each centre. Similar scoring and calculations were done for the digital X-ray group on all those 8 centres. Finally the ratings were compared and designated according to the degree of agreement. Prior to starting the experiments a series of workshops on the detection of fusion activity and scoring system was held by the authors under the guidance of the senior-most author. Thereafter two were randomly chosen to conduct the final scoring. This was done to exclude the bias that might arise out of difference in experience and expertise of the observers.

Analysis and calculation for Cohen's Kappa statistics was done by SPSS software for windows version 19. P value of less than 0.05 was considered significant when applicable.

Inclusion Criteria- All 30 sets of digital X-rays of elbow joint in the departmental archive, irrespective of sex, age and side.

Exclusion Criteria- All the X-ray films which were very old, worn out and inconspicuous (with improper exposure)

The identity of the subjects was not disclosed.

Schmeling's Score [15] :-

Schmeling differentiated five stages of bony ossification:

- Stage 1: ossification centre is NOT ossified
- Stage 2: ossification centre is ossified, BUT epiphyseal plate is not ossified
- Stage 3: epiphyseal plate is partly ossified

• Stage 4: epiphyseal plate is fully ossified and epiphyseal scar is visible

• Stage 5: epiphyseal plate is fully ossified and epiphseal scar is NO longer visible'

Kappa Scoring [16,17,18] :- The Kappa values vary from 0 to 1, where.

0 = agreement equivalent to chance.

0.1 – 0.20 = slight agreement.

0.21 – 0.40 = fair agreement.

0.41 – 0.60 = moderate agreement.

0.61 – 0.80 = substantial agreement.

0.81 – 0.99 = near perfect agreement

1 = perfect agreement

X-rays were taken from the departmental archive. Each set comprised the following:--

1) AP view of the wrist joint

2) AP and Lateral views of Elbow joint (in the same plate)

3) AP view of Pelvis.

Centres of ossification used in the study

1.	Lateral epicondyle (LE)	2.	Lower end of radius (LR)
3.	Medial epicondyle (ME)	4.	Lower end of ulna (LU)
5.	Tip of the olecranon (OL)	6.	Iliac crest (IC)
7.	Head of radius (HR)	8.	Ischial tuberosity (IT)

Results

Table 1- :- Symmetric Measures of Lateral Epicondyle (Digital X-ray)

LATERAL EPICONDYLE DIGITAL		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.847	.083	7.498	.000
N of Valid Cases		30			

Table- 2 :- Symmetric Measures of Lateral Epicondyle (Conventional X-ray)

LATERAL EPICONDYLE CONVENTIONAL		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.560	.127	4.378	.000

Table- 3 :- Symmetric Measures of Ischial Tuberosity (Digital X-ray)

ISCHIAL TUBEROSITY DIGITAL		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.840	.089	6.187	.000
N of Valid Cases		30			

Table- 4:- Symmetric Measures of Ischial Tuberosity (Conventional X-ray)

ISCHIAL TUBEROSITY CONVENTIONAL		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.761	.107	4.984	.000
N of Valid Cases		30			

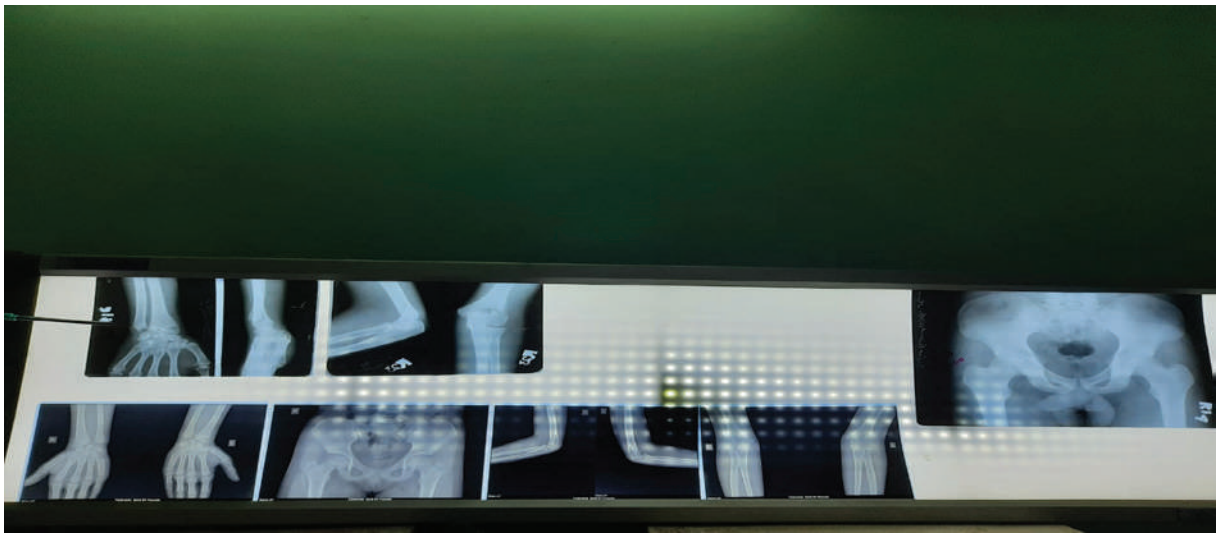
TABLE 5:- SHOWING INTER-RATER AGREEMENT IN THE FUSIONAL ACTIVITIES OF THE 8 BONY PARAMETERS WHILE STUDYING CONVENTIONAL AND DIGITAL X-RAYS

SL. NO.	BONY PARAMETERS	OBSERVED KAPPA SCORING	
		CONVENTIONAL X-RAY	DIGITAL X-RAY
1	LE	0.560	0.847
2	ME	0.670	0.946
3	OL	0.733	0.891
4	HR	0.747	0.870
5	LR	0.691	0.861
6	LU	0.670	0.857
7	IC	0.760	0.844
8	IT	0.761	0.840

1.	Lateral epicondyle=LE	2.	Lower end of radius= LR
3.	Medial epicondyle=ME	4.	Lower end of ulna=LU
5.	Tip of the olecranon=OL	6.	Iliac crest= IC
7.	Head of radius=HR	8.	Ischial tuberosity= IT

Table 6 Cross tabulation of scores for lateral epicondyle using digital x ray						
Count						
		DLE2				Total
		2.00	3.00	4.00	5.00	
DLE1	2.00	4	0	0	0	4
	3.00	0	14	3	0	17
	4.00	0	0	2	0	2
	5.00	0	0	0	7	7
Total		4	14	5	7	30

Photograph 1:- Digital & Conventional X-rays in 4 in 1 view box in Dept .of FSM, BMCH



Discussions

The present investigation, perhaps for the first time, was contemplated to examine the interrater agreement in assessing the fusion activity of centres of ossification of different bones of upper limbs and pelvis using conventional and digital X-ray.

The degrees of fusion activity of 8 ossification centres were assessed by using Schmeling's Score. The commonly assessed centres around elbow, wrist and pelvis were used in the study. The inter-rater agreement was calculated by estimating Cohen's Kappas.

The Kappa score was found to range from 0.560 to 0.761 for conventional x-ray films; whereas it ranged from 0.840 to 0.946 in case of digital x-rays.

The highest inter-observer agreement using conventional X-ray was noticed in the fusion activities of Ischial Tuberosity with value of K= 0.761. The lowest inter-rater reliability was noted in scoring the fusion activity of Lateral Epicondyle using conventional x-ray (K=0.560)

For digital X ray the highest agreement was noted in medial epicondyle (K=.946) while the lowest agreement was found in the ischial tuberosity (K=0.840)

For the lateral epicondyle the maximum inter observer agreement was noticed in the fusion activities graded as 4 and 5 as per Schmeling's stage. However, the observers agreed less when the Schmeling's stage was 3 while using digital X ray films. (as ascertained from the cross-tabulation Table 6).

The results with conventional X-ray for the same centre showed that the agreement was far less. This may be due to difference in opinion regarding presence / disappearance of the conjoint epiphyseal Cross tabulation of all other centres similarly showed more agreement at stage 3 and 4. Moreover, the epiphyseal scar also leads to difference in opinion and level of agreement.

There was difference in level of agreement on the fusion activities of the above noted 8 bony parameters between digital and conventional x-ray. The former showed near perfect agreement for all the centres while the latter showed moderate to significant agreement. This study clearly indicates that the digital x-rays produce much more precision than conventional x-rays. Earlier works have also showed that measurements using digital x ray can be successfully used for age assessment in adolescents.[17]

The secondary ossification centres play an important role in assessment of age from x ray so their activity needs to be assessed correctly. This is the basis of scientific evidence for determination of age [4, 5-7, 11]. Though several interrelated factors influence the ossification, estimates based on chronology help arrive at the age of the subject. This has been agreed upon by several researchers [9-12, 14].

Agreement between observers serves as the essential perquisite of the efficacy and utility of a method.

Assessment of fusion from films or comparing with known atlases for given population has been the age-old practice of determining age. With newer techniques, more research and suitable charts need to be evaluated for estimating age.

In the Indian context, especially for the Bengali population, the Galstaun [(1)] chart needs to be re assessed and refined using digital X- ray. More studies are required prior to formulating database for a given population. Herein lays the importance of the present investigation where agreement was noted to be near perfect using digital X-ray.

Cohen's Kappa was run to test the inter-rater agreement between two observers. Kappa statistics measures the agreement over and above that by chance. However, the correctness of the observation in this context cannot be tested. Both observers agreeing to large extent does not however mean that they were both correct in their interpretation. This is the methodological issue that needs to be considered. Later there is a scope to design the study with two or more raters who are randomly selected and applying robust technique like Fleiss18 kappa

Conclusions

Interrater agreement was noted to be near perfect using digital X-ray for all the centres of ossification applied in the study.

The accuracy of digital radiography in age determination by observing the fusion activity at elbow, wrist and pelvis (Using Schmeling's staging) will be superior to conventional radiography.

The advantages of digital x-ray are higher resolution, low exposure, data storage, processing, and retrieval and near perfect agreement between raters.

To the best of our knowledge this is perhaps the first reported study to assess and compare interrater agreement on fusion activity of ossification centres of upper extremity and pelvis using digital and conventional x ray.

Ethical Clearance:- Taken from Institutional Ethics Committee (IEC) of Burdwan Medical College, Purba Bardhaman.

Source of Funding :- Nil

Conflict of Interest :- Nil

Declaration

A shortened version of this study was presented by Second Author as oral presentation in Forensic Medicon-2020 at Hyderabad, India , 41st Annual National Conference Of the Indian Academy of Forensic Medicine.

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Contriving an Opinion of Cause of Death in Autopsies

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Abstract

Contriving an opinion of cause of death is something that requires a good expertise in the subject knowledge and the right set of discriminative skills. The immediate and basic causes of death, circumstances surrounding the death, and the investigation findings of police officers are all the necessary prerequisites to be gathered, before formulating an opinion. Decisions on the cause of death most often de facto will decide the manner of death.

A forensic pathologist can give causes of death in a logical sequential manner. Hume's and Mill's philosophy is something to be always borne in the mind of a forensic pathologist. Istanbul Protocol is the only literature mentioning as to how to opine an effect, with respect to the causes or circumstances that led to the effect. This can be extrapolated to have five different compartmentalised categories of opinions. Unless there is certain uniformity in opining, the more are the chances of confusion among our fraternity and the judiciary.

Keywords: *Cause of Death, Forensic Pathology, Manner of Death, Opinion*

Introduction

Practicing the subject by doing a complete observation, as well as dissection during medico-legal autopsy is one thing; and application of all the information gathered during medico-legal autopsy to conclude the cause of death is quite another thing. It is a further gifted talent to compare such findings obtained, with the circumstances surrounding death to come up with the right conclusion as to the manner of the death. An opinion as to the cause of death is framed based upon the judgements and or discriminations of the forensic pathologists. Such discriminative skills are acquired on experience and from a priori reasoning.[1]

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Certification of death is a very important source of statistical data to get information regarding the health status of a population. Hence, the cause of death section in a medico-legal report should contain both the unnatural and natural diseases that led to the death of an individual. Either of the two would have caused death ultimately, but they both need not be mutually exclusive. One event could have led to the other, or might have contributed for the other event to occur.[2]

Decisions of cause of the death will most often de facto will decide the manner of death.[1] As far as the doctors are concerned, a forensic pathologist is considered to be the most knowledgeable person regarding proper death certification.[2]

What is 'Cause of Death'?

Cause of death is the injury, disease, or a combination of the two that initiates a train of physiological disturbances that result in termination of an individual's life. Immediate cause of death is the disease or injury present at the time of the death which caused death. Proximate cause of death is the original disease process or injury or event that led to a string of unbroken train of

events eventually leading to death.[2]

This review paper is intended to guide any doctor as to how to frame a proper opinion of the cause of death, at the end of a medico-legal autopsy. Many a time, in natural diseases the best cause of death is listed. This may not necessarily be the actual cause of death. For example in case of a less severe coronary artery occlusion, since the pathologist does not find any other obvious lesions incompatible with life, s/he opines coronary artery disease to be the cause of death; especially when they are sufficient to cause death beyond all reasonable doubts. This will be supported by medical history and other circumstantial evidences. It is intriguing that the decedent was surviving all these years with the existing occlusion of the coronary artery. To some extent this fashion of opining is a subjective phenomenon, but this is the most practical way of practicing the subject. Hence it is always advisable to discover all the possible pathological/physiological processes in the decedents, before concluding an opinion.[1] Further, in order to determine the cause of death, the mechanism of death has to be understood. In many natural diseases due to the absence of pathognomonic autopsy findings and blurred ancillary investigations, the mechanism of the death cannot be determined.[2] The cause of death thus opined will not be disclosed in any official document provided to the next of kin of the deceased. Death certificate issued from local authorities do not mention the 'medical cause' of death, since it is only available from the death register of the authorities.[3,4]

Why is it Scientific or Logical to Opine?

In a medico-legal report, the cause of death section represents a medical opinion that may vary from pathologist to pathologist.[5] Unlike clinicians, completion of the cause of death section in a medico-legal case requires careful judgment and interpretation of interacting causes of death. The opinion thus formulated is the sum total of all the autopsy findings, investigation results and the interpretations of circumstances surrounding death.[6]

For a forensic surgeon, diagnosis as to the cause of death is always an opinion. Such an opinion should be a reasonable conclusion, correlating the autopsy findings with the history and the circumstances surrounding the death.[7] It is both unethical and unproductive to provide a mere speculative cause of death, without sufficient facts or evidence.[8]

How to Opine Cause of Death?

Twentieth World Health Assembly (1967) defined the cause of death as "all those diseases, morbid conditions or injuries which either resulted in or contributed to death and the circumstances of the accident or violence which produced any such injuries" [9]. Later in 1990, an additional line (d) was added, under Part I of the M.C.C.D.[10] The 'circumstances' as mentioned in the definition indicate the manner of the death.

Consider the situation where a diabetic male with chronic ischemic heart disease dies due to pneumonia and the body is sent for a post-mortem examination. A clinician who would be treating such a patient in his/her various stages of illnesses will have a proper sequential knowledge of all the events that ultimately lead to death. Whereas a forensic expert, while exploring the cadaver, so as to reconstruct the events prior to death, will be handicapped when it comes to the task of arranging the events in a sequential order, for opining a cause of death.[5] A forensic pathologist, instead of giving the causes of death in separate columns/boxes, can describe them as a list of logical chain of events. S/he can also avoid as far as possible using complex medical terminologies, for benefit of the judiciary. For example, an opinion can be framed as – The death was due to massive gastrointestinal bleeding as a result of the ruptured veins of the food pipe (esophageal varices), a complication of hepatic cirrhosis as a result of chronic alcoholism.[2] The end of the chain of list (basic cause of death) actually determines the manner of death too. For example if bronchopneumonia is the immediate cause of death and if hip fracture is the basic cause (the actual triggering event), then the manner of death becomes accidental; provided the fracture had occurred accidentally.[2]

The causation of death philosophy: David Hume had quoted that for a cause "Y" to occur, there must be an effect "X", unless otherwise "Y" doesn't occur. But John Stuart Mill disagreed to Hume and said that a cause is a result of sum total of conditions/effects that occurred, from which one exclusive condition cannot be chosen out. For Hume, a statement such as "the rising sun causes daylight" would have been reasonable. But according to Mill, he would include 'atmosphere' too.

The presence or absence of the atmosphere is also a condition necessary for the daylight to occur.[1] Hume's approach is suitable to situations where there is only one fatally potential condition incompatible with life, when opining a cause of death. This restrictiveness can be seen in case of coronary atherosclerosis as a cause of death. Development of coronary atherosclerosis is not always followed by death and death does not always occur when coronary atherosclerosis has only developed. Yet, coronary atherosclerosis has been widely accepted as a reasonable proposition for pathological basis of an enormous number of deaths.[1]

An elderly male, a smoker with history of chronic bronchitis and emphysema was stabbed at the back aspect of the chest. During treatment he had an episode of cardiovascular collapse. Following resuscitation he developed cerebral infarction and was discharged from the hospital. But few weeks later he collapsed and died at home. The autopsy revealed massive pulmonary thromboembolism, deep vein thrombosis of calf veins and adenocarcinoma of the rectum with metastasis. This case can be opined in two different ways – either Hume's or Mill's theory of causation. If the opinion is based on the Hume's approach, where the stab wound initiated a chain of events leading to pulmonary thromboembolism further leading to death; then during trial, an intelligent lawyer can question the reason for completely excluding possibility of the complications of cancer leading to the death. In such tricky situations Mill's approach of multiple causes is always sensible to choose. As per Mill, there is no scientific and precise way of apportioning weight to each causes of death. As a corollary no cause of death can be discarded. In other words the responsibility of death should be shared by both the event of stabbing and the pre-existing carcinoma. Hence, both these principles should be borne in mind, and whenever required Mill's theory should be applied.[1]

Sometimes discrepancies can occur between treatment records and the opinion as to the cause of the death in the autopsy report. This has resulted in lawsuits alleging negligence against the clinicians.[11] Such problems can be avoided when the opinion as to the cause of death is done carefully and only upon facts derived from autopsy findings, as well as circumstances surrounding death. It is desirable for a forensic surgeon to wait until all the additional data are obtained after an autopsy, before commenting on the cause of death.

When opinions are pending, they must be followed up regularly, so as to submit at the earliest the final cause and manner as to the death.[2,5] Opinions can be amended by the pathologist once any additional information are obtained. Even the manner alone can be amended leaving the cause of death to be the same.[2]

Should Manner of Death Find Place in Opinion?

It is not imperative that the manner of death must be spelled out in each case while framing cause of death.[6] This is because in some cases it is implied from the cause of death itself. Moreover there is a misconception that the forensic pathologist must be only looking into the cause of death. The real skill of such an expert is when s/he contributes to the reconstruction of events surrounding the death.[1] In situations where the death is especially due to an external cause, the forensic pathologist has to specify the manner of death.[5] Further, if the expert is not able to comment upon the manner of the death, then it can be opined as "Could not be determined". When the opinion as to the manner of death is from corroboration of the autopsy findings with the inputs of the investigating officer, then it must be mentioned in the opinion of the pathologist.[12] The pathologist need not hesitate to correlate the investigations findings with his/her autopsy findings, so as to comment upon the manner of death. Literature also mentions that it is desirable to opine manner first, followed by the cause of the death![13]

Different Types of Opinions

While writing the opinion as to the cause of death, the major findings have to be listed in the order of the importance. It is not necessary to list most of the minute extraneous findings. The speculations regarding circumstances should be kept to a minimal level.

A popular, yet wrong belief is that doctors should never be definite while stating their opinions.[12] For the same reason they use words such as *probably* etc while stating their opinion as to the cause of death. This unnecessarily creates a bad impression for the judiciary because of the doubtful opinion. A forensic expert should never hesitate to give a definite opinion whenever they can reasonably do so.[12] A medico-legal opinion should be said in reconciliation with the history provided by

investigating officers, the autopsy findings and from the results of the ancillary investigations.

Istanbul protocol has provided guidelines as to how to opine the injuries present in a body in relationship with the history of the causation of those injuries.[14] This is the only literature mentioning as to *how to opine an effect with respect or in relationship with the causes or circumstances* that led directly or indirectly to it. It seems to be both scientific and logical to extrapolate this for opining causes of death in all medico-legal cases. If so, there can be five different types of medico-legal opinions as to the cause of death, the last one being an addition of the authors themselves while the rest four is being adopted from the protocol. Unless there is certain uniformity in opining cause and manner of the death, the more are the chances for confusion/s amongst the fraternity as well as the judiciary.

The five different types of medico-legal opinions can be – Diagnostic of, Typical of, Consistent with, Not consistent with and by Exclusion. They are elaborated as follows:

1. Diagnostic – The death *could not have been* caused in any other way

2. Typical – The death is *usually due to* this cause but *there are other* possible causes

3. Consistent – The death *could have been* produced by the cause but *there are many other* possible causes

4. Not consistent – The death was *not as per* the history/circumstances provided by the investigating officer or from the treatment records, but *due to some other* cause

5. Exclusion – There should be *no injuries / signs of violence* on the body to account for death. Poisoning and common natural diseases *should be ruled out* by ancillary investigations. But death due to *other uncommon natural causes could not* be ruled out.

Consider the example of a case of hanging to be written in these five different ways:

1. The autopsy findings and circumstances were diagnostic of death due to hanging.

2. The autopsy findings and circumstances were typical of death due to hanging.

3. The autopsy findings and circumstances were consistent with death due to hanging.

4. The autopsy findings or the circumstances were not consistent with hanging. The autopsy findings were consistent with death due to strangulation.

5. There were no injuries / signs of violence on the body to account for death. Poisoning and common natural diseases has been ruled out by ancillary investigations. However death due to other uncommon natural causes could not be ruled out.

Sometimes no absolute statements can be made regarding the autopsy findings. This can lead to uncertainties in the opining of cause and manner of death. In such scenarios the forensic pathologist should elaborate on his uncertainties with the impression s/he has, rather than leaving them open for debate by another expert or the judiciary.[6] This is because the pathologist who examines a body for the first time will be the most qualified person to express his/her opinion in relation to the findings and interpretations/conclusions.

Conclusion

The cause of death section of a medico-legal report includes both natural and unnatural causes, along with the manner of the death. A cause of death is an opinion always, which should be supported by facts and circumstances surrounding the death. This section of the medico-legal report should include the entire sequences of causes of deaths concluding at the event which triggered the chain. Mill's theory should be considered whenever required. Opinions can be kept pending or can be amended later depending upon the new information/s obtained at later stages of investigation. There can be five different categories of opining a cause of death, along with the manner.

Conflict of Interest: Nil

Source of funding: Nil

Ethical Clearance: Not applicable since meta-analysis of existing literature

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A Study of Patterns of Thoracic Injuries in the Victims of Vehicular Incidents Brought to the GMCH Mortuary for Medicolegal Autopsies

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Abstract

The present study aims to highlight the pattern of thoracic injuries sustained by the victims of fatal road traffic accidents (RTA) brought to the GMCH Mortuary for medico legal autopsies. The study is an autopsy based observation during 1st April 2019 to 31st March 2020. In this study, incidence of thoracic injury among all cases of vehicular accident is 23.83%. A male preponderance (89%) was observed with a male-female ratio of 7.7:1. Peak incidence was observed in the age group of 21-30 years (27.24%). In all cases, thoracic injuries are associated with external or internal injuries on other parts of the body. Pleura are the most commonly involved organs in the thoracic regions. Majority of the victims were pedestrians ((36.6 %) followed by two-wheelers (31.06%). The study indicates the pattern of thoracic injuries sustained along with the trend of road traffic accidents in the region.

Keywords: *pattern, injuries, autopsy, incidence, accidents*

Introduction

As per definition, an accident is that "occurrence in a sequence of events which usually produces an unintended injury, death or property damage".[1]

During 2017 there were almost 1.19 million deaths from road accidents in the world. Persons injured in road traffic accidents occupy nearly 10-30% of beds in hospitals.[1] The outcome of a fatal road traffic accident is a severe economic loss to the community as it entails wastage of productive years of life through both disability and death and not merely a tragic loss to the bereaved family. According to study conducted by the National Transportation Planning and Research Centre, in every 4 minutes a person is killed or injured in road accidents in India.[2]

Thoracic injury directly accounts for 20-25% of deaths due to trauma and act as a contributory factor in another 25% of trauma deaths. However approximately 85% of thoracic trauma can be managed without major surgical intervention if diagnosed properly at time. From 1st April 2018 to 31st March 2019 total postmortem examination carried out in our Department was 2476 and out of that road traffic accident was 759. From 1st April 2019 to 31st March 2020 total postmortem examination carried out in our Department is 2625 and out of that road traffic accident is 986. So an increasing trend can be observed.

Materials and Method

Present study has been carried out in the Department of Forensic Medicine, Gauhati Medical College and Hospital. Autopsies that are carried out on the cases brought by the police from within the district administrative area of Kamrup of the state Assam and few referral cases from the neighbouring districts. The study period extended from 1st April 2019 to 31st

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March 2020. During this period a total number of 2625 autopsies were carried out in the department, out of these in 986 numbers of cases the death was associated with road traffic accidents. Out of these roads traffic accident victims a total of 235 cases had some kind of injury on the chest wall or the organs inside the thoracic cage, which were selected for evaluation.

Autopsies where the nature of injury could not be ascertained were excluded.

All decomposed bodies are omitted from the study.

Data were obtained regarding age and gender of the victims, time and place of accident, type of vehicle involved in the accident, the type of injuries sustained, outcome of the accident, etc., in a self-designed proforma (questionnaire). Data derived for each parameter were tabulated using the Microsoft Excel for further analysis.

Results

A total of 2625 medico-legal autopsies were performed during the one year study period of 1st April, 2019 to 31st March 2020. Out of these vehicular accident cases were 986, i.e. 37.56% of total number of cases.

Fraction of Chest injury:

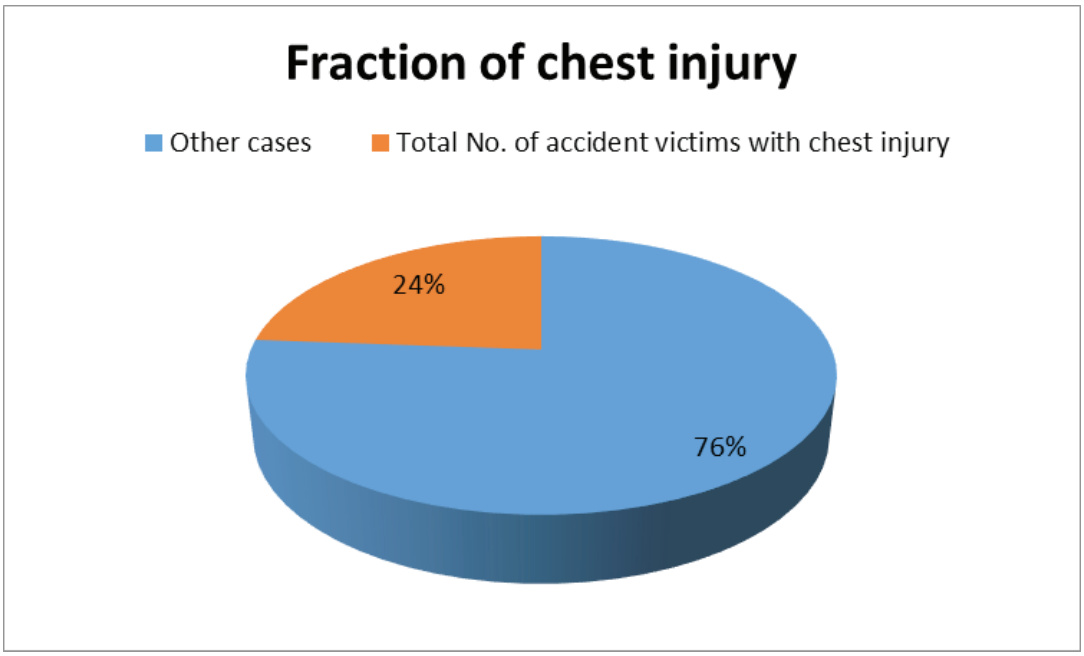


Fig no. 1: Pie-diagram showing the fraction of victims with thoracic injury among all cases of vehicular accident

Age Distribution:

In the present study, the age of the victims ranged from 5 years to 78 years. The victims were divided according to their age into 7 groups.

The peak incidence was observed in the age group of 21-30 years comprising of 64 cases (27.24%) followed by 31-40 years age group with 57 cases (24.26%).

Sex Incidence:

It is observed that among the 235 cases studied, 208 were male comprising 89% and 27 were female comprising 11%. The male to female ratio in the present study is 7.7:1.

Site of injury

In the present study most of the victims (103 or 43.83%) had injury on all regions of the body, followed by Chest+Head+Limbs (81 or 34.47%). None of the victims had injury only on chest. Along with other regions limbs are involved in most of the cases (222 or 94.47%). The incidence of injuries on different sites of

body was also analyzed on the basis of different types of victims. This is shown in the table.

Table no. 1: Distribution of injuries on different categories of victim

Place of injury	Pedestrian	2-wh rider	occupant	Driver	Bicyclist	Pillion rider	Total	Percen-tage
Only chest	0	0	0	0	0	0	0	0
Chest+Head	5	2	3	0	1	0	11	4.68
Chest+Abdomen	0	0	0	0	0	0	0	0
Chest+Limbs	3	1	2	2	2	0	9	4.6
Chest+Head+Abdomen	0	2	0	0	0	0	2	0.85
Chest+Head+Limbs	27	26	12	3	7	6	81	34.47
Chest+Abdomen+Limbs	15	10	3	1	0	0	29	12.34
All regions	36	32	16	9	3	6	102	43.4
Total	86	73	36	15	13	12	235	100

Most of the victims had injuries in all regions of the body. Most common associated injury was limb injury, in 222 cases (94.47%), followed by head injury, in 197 cases (83.83%).

Pleural injury:

In the present study laceration is the most common among the different types of injury.

Lung injury:

Lungs were injured in 122 cases (51.91%) among the total 235 cases in the present study.

Laceration is the most common injury in lung (43.41%). In most of the cases lungs are injured bilaterally.

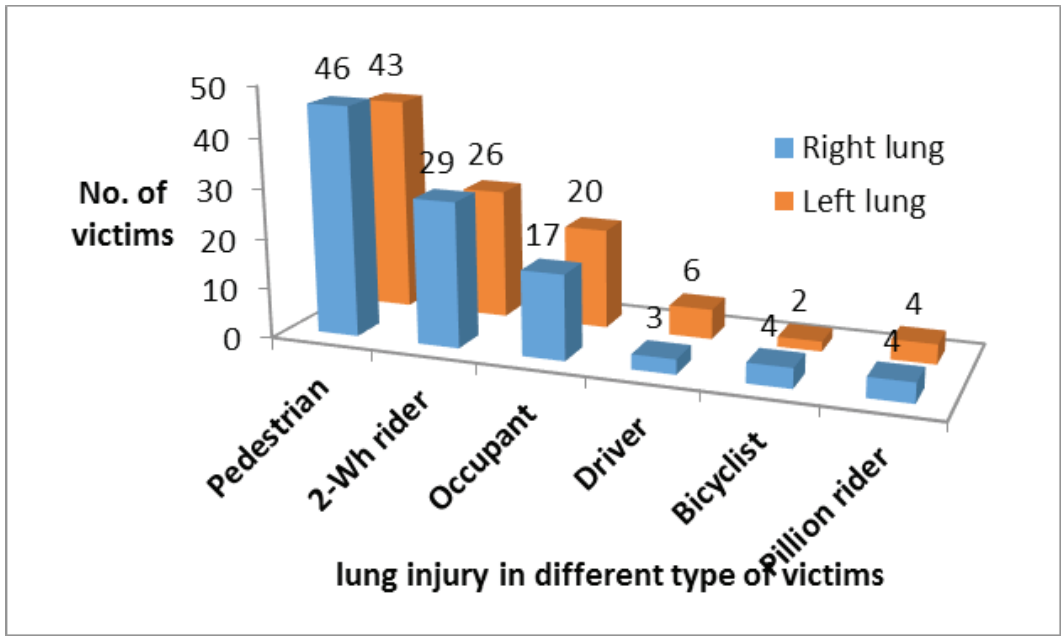


Fig no. 2: Lung injury in various categories of victims

Blood in pleural cavity:

A total of 107 cases (45.53%) had liquid and clotted blood in the pleural cavity. In four cases, cavity is empty even after presence of injury on the organs because of draining of blood in presence of laceration or crush injury.

Pericardial injury:

A total of 29 cases (12.34%) had some kind of pericardial injury. Laceration is the most common injury.

Heart:

It is observed that heart was injured in a total number of 14 cases (5.96%), on the basis of naked eye examination and histopathological examination.

From the below figure it is seen that right atrium is injured in 5.96%, right ventricle in 4.68%, Left atrium in 5.53% and left ventricle in 6.38% of all cases of thoracic injuries.

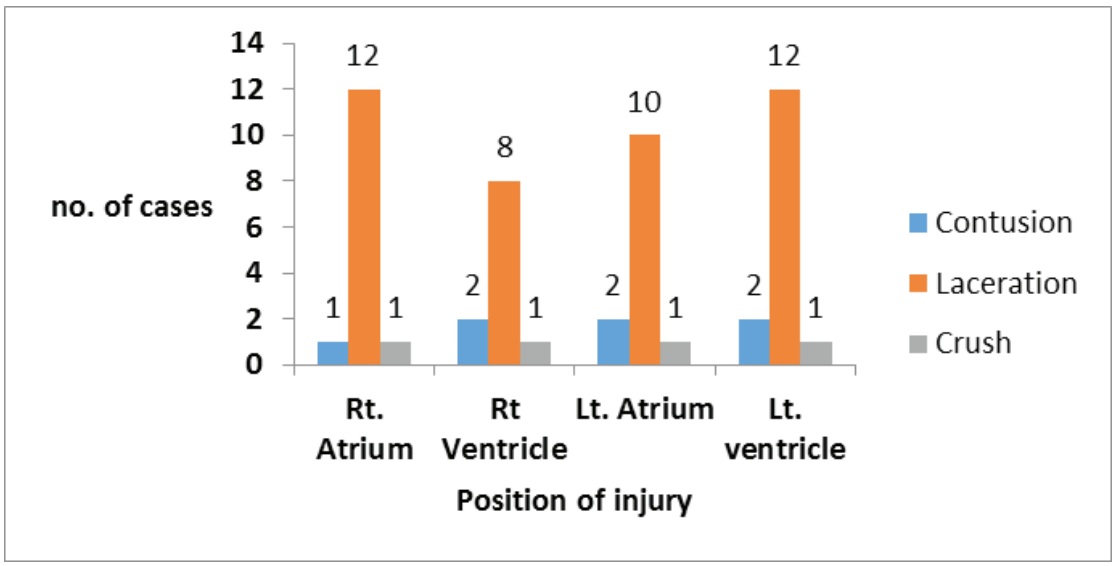


Fig no. 3: Position of cardiac injuries

Injury on ribs:

Rib fracture was present in total 139 cases (2 were crushed) comprising 59.15% of all 235 cases or 14.1% of all fatal vehicular accident cases.

Injury on vertebrae:

Fracture of vertebrae was found in five cases and crush injury was found in four cases. All of these were found to be associated with some sort of spinal cord injury as well as other visceral injury in chest.

Cause of death:

The incidence of different causes of death among the victims is given in the following table.

Table no. 2: Causes of death among the victims

Cause of death	No. of victims	Percentage
Coma	97	41.28%
Haemorrhage and shock	78	33.19%
Coma+haemorrhage and shock	22	9.36%
Instantaneous	33	14.04%
Exhaustion	2	0.85%
Spinal shock	2	0.85%
Syncope	1	0.43%
Total	235	100.00%

Coma without haemorrhage and shock is the most common cause of death (41.28%) followed by haemorrhage and shock without coma (33.19%). Death was instantaneous in 14.04% cases.

Discussion

A total of 2625 medico legal autopsies were performed during my study period of 1st April 2019 to 31st March 2020. Out of these 986 (37.56%) cases died in vehicular accidents.

This finding is almost consistent with NCRB report of 2018.[3]

Out of total 986 cases of vehicular accidents, percentage of cases having thoracic injury is 23.83%.

Epidemiological Factors

Age:

In the present study, peak incidence was observed in the age group of 21-30 years comprising of 64 (27.24%) cases. This is closely followed by age group 31-40 years witsh 57 (24.26%) cases and 41-50 years with 43 (18.31%) cases. This means that 164 (69.77 % of total) people lost their in prime of their life. Age groups 0-10 years (9 cases), 51-60 years (23 cases) and >61 years (13 cases) have less number of cases.

These findings are consistent with Bairagi K. (2005)[4], Das T.K. (2007)[5], Husain M. et al (2009)[6], Malik Y. (2010)[7], Goyal A. et al. (2014)[8], Reddy N. B. (2014)[9], Shrivastava S. R. (2014)[10], Seid M. et al. (2015)[11], Sharma S. M. (2016)[12], Singh S.K. (2017)[13],

Smith et al. (2017)[14].

The reason for the above is that young adults are the prime bread earners of the family and remain outdoors during most of the day, while persons in extremes of the age usually remain indoors whereas children are confined to the outskirts of the residential premises only.

Sex:

In the present study males comprises a majority (89%) compared to female who were only 11%. Male to female ratio is 7.7:1.

This finding is consistent with Sinha YN, Bairagi K.K. and Das K.C. (2005)[4], Das T.K. (2007)[5], Goyal A. et al. (2014)[8], Reddy N. B. (2014)[9], Shrivastava S. R. (2014)[10], Seid M. et al. (2015)[11], Sharma S. M. (2016)[12], Singh S.K. (2017)[13], Smith et al. (2017)[14].

The reason for the male majority is that men everywhere are more up and about and exposed to outdoor activities, traveling between the home and place of work, while women remain mainly indoors at the home involved in household work.

Injury

Site of injury:

In this study, incidence of chest injury among all cases of vehicular accident is 23.83%. In all the cases chest injury is associated with one or more other region injury. In majority of cases (43.83%) all regions of the body are involved. Head injury is associated with total 83.83% cases.

The incidence of chest injury is highest among the pedestrians, (in 86 cases, 36.6%). This fact tallies with the observation made by Reddy et al. (2014).[9]

Pleural injury:

In this study it is seen that laceration of pleura is more common than contusion and bilateral involvement is more common than unilateral injury. Left side involvement is more common than right side.

Lung injury:

Laceration of lung is more common than contusion and bilateral involvement is more common than unilateral injury. Right side involvement is more common than left side. In two case lungs are crushed.

Again lung involvement is most common in pillion riders.

Heart injury:

Heart was injured in a total number of 14 cases (5.96%), on the basis of naked eye examination and histopathological examination. Laceration is more common than contusion. In one case heart is lacerated. Among the chambers right atrium is most commonly involved (5.96%).

Among the different types of victims, heart is most commonly involved in pillion rider (16.67%).

These finding are almost consistent with Reddy et al. (2014)[9] and El-Menyar (2016)[15].

In all case of laceration of heart death was instantaneous.

Trachea was involved in 7 (2.98%) cases. It is one of the organ which are less likely to be injured in vehicular accident cases.

Ribs:

Rib fracture was present in total 139 cases (2 were crushed) comprising 59.15% of all 235 cases or 14.1% of all fatal vehicular accident cases.

In majority of cases (48.2%) ribs are fractured bilaterally.

This finding is almost consistent with Reddy et al. (2014)[9].

Vertebrae:

Fracture of vertebrae was found in five cases and crush injury was found in four cases. All of these were found to be associated with some sort of spinal cord injury as well as other visceral injury in chest.

Crush injury:

Crush injury is found to be most common on head (15.74%).

Cause of death:

In this study coma without haemorrhage and shock is the most common cause of death (41.28%) followed by haemorrhage and shock without coma (33.19%). Death was instantaneous in 14.04% cases.

Fatal chest injury leads to haemorrhage and shock and thereby death in majority of cases which are consistent with finding of Gushinge and Kadu (2017)[16].

Among pedestrian Haemorrhage and shock without coma is the most common cause of death and in 2 wheeler rider coma without haemorrhage and shock is the most common cause of death

Conclusion

Vehicular accidents continue to be a growing menace, incurring heavy loss of valuable life and man-power along with a corresponding drain of precious resources.

Primarily three factors comprise road safety - infrastructure (roadways), vehicle design and human behaviour. So it is necessary to improve the condition of traffic by improving road and by proper implementation of traffic laws. To reduce the harm done when crashes occur, seat belt for all vehicular occupants and use of helmet for both riders of two wheeler should be enforced. Children should sit in rear seats only. Air bag should be mandatory in all vehicles.

To prevent the loss of life due to injuries to the vital organs (to reduce post crash harm) it is necessary to have good emergency communication and transportation.

Stress and strains of busy life of modern days has a deleterious effect on the power of mental concentration which is very much essential in driving. Banning of mobile phone while driving should be strictly implemented. Since the heavy moving vehicles are responsible for majority of deaths, it is of immense value to periodically screen the drivers to check their physical and psychological efficiency.

The use of alcohol measuring gadgets may decrease the use of alcohol during driving.

So, it is clear that multidisciplinary approach is required to prevent deaths and disabilities resulting from road traffic accidents.

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Assessment of Knowledge and Attitude Towards Medical Ethics among Medical Students

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Abstract

Background: A sense of discontent with services of medical practitioners and growing reliance on alternative medicine with increased public awareness, negative publicity leads to increased lawsuits against medical professionals. Medical ethics training provides a basis for balancing one's obligation to patient and practice, as well as responsibility to those seeking medical care. **Aims and Objectives:** To assess medical students' knowledge and attitude towards medical ethics. **Material and Method:** A cross-sectional questionnaire-based study among 246 medical undergraduates was conducted. Data was analyzed for percentage, mean and 't' test. **Results:** Majority knew importance of medical ethics in medical profession and existence of institutional ethics committee. 84.9% agreed that written informed consent should be mandatory. Response was statistically significant regarding confidentiality (p=0.001883), utmost respect for human life (p=0.00542) and respect for dignity and privacy of patient (p=0.001408). 80.4% disagreed that ethical conduct is important only to avoid legal action. 82.5% agreed strongly to integrate medical ethics in medical curriculum. **Conclusion:** Though students had adequate knowledge about medical ethics, reinforcement at all levels of medical education is necessary.

Keywords*: Medical ethics, Medical students, Knowledge, Attitude.*

Introduction

As per Medical Council of India, primary objective of medical professionals is to render services to humanity[1]. A sense of dissatisfaction towards medical professionals' services and increasing reliance on alternative medicine along with increased public awareness, negative publicity leads to increased litigation against healthcare professionals[2-3]. Medical ethics concerns the moral principles governing the application of medical knowledge while dealing with patients[4-6] and serves as basis for decision-making in cases of conflicts, confusion and dilemmas resulting from biomedical technologies[1-2].

Medical practitioners are expected to be competent, compassionate and knowledgeable. Current system of medical education offers only an outline of ethical problems, but doesn't educate medical students in addressing ethical dilemmas they face during clinical practice[3,6]. This issue can be curbed by improving medical curriculum and creating educational opportunities for potential healthcare practitioners[5,7]. If sensitization is done at early stage of medical education, it will help litigations against medical professionals to reduce. This will provide a basis for balancing one's duty to their patient and profession, as well as the obligation to those seeking medical care[8]. Under this context, the present study was undertaken to assess medical students' knowledge and attitude towards medical ethics.

Material and Methods

A cross-sectional questionnaire-based study among medical undergraduates (in two groups) of different

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batches was conducted between April 2019 and December 2019, after obtaining permission from the Institutional Ethics Committee. Group I comprised of students studying in the 3rd semester who recently had attended medical ethics lectures and Group II of students studying in the 6th to 9th semesters and had attended medical ethics lectures in their 3rd semester. First year students were excluded from the study because they had no formal medical ethics education. The study was conducted in Forensic Medicine Department, N.K.P. Salve Institute of Medical Sciences & Research Center and Lata Mangeshkar Hospital, Nagpur. The students who were willing to participate were considered for the study. A pilot test on 25 students was undertaken. Prior to administrating questionnaire, participants were addressed regarding purpose and nature of the study and were informed that data collected would be anonymous and their participation would be voluntary. After obtaining written informed consent, the questionnaires were distributed to the students. A pre-validated questionnaire was consisted of two sections for knowledge and attitude assessment. First section included questions to assess knowledge and second to assess attitude. Questions were graded on a scale from 1 to 5 according to the Likert scale (1-strongly disagree, 2-disagree, 3-not sure, 4-agree and 5-strongly agree). Scores 1 and 2 were put together to show disagreement, scores 4 and 5 were put together for agreement and score 3 showed that student was uncertain of statements' response. For comparative as well as overall analysis, a mean Likert score was calculated for each element among participants from different semesters. Data was entered in excel sheet and analyzed by using Epi-Info 7 statistical software for number, percentage, mean, standard deviation, 't' test. A p-value of <0.05 was considered to be significant.

Results

Out of 270 students, 246 (91.1%) consented to participate in study. Regarding importance of medical ethics in medical profession, 240 (97.5%) strongly agreed. 232(94.3%) medical students had knowledge of the Hippocratic Oath and its contents and 193(78.4%) students were aware of existence of institutional ethics committee and its specific role.

Predominant source of knowledge was lectures on medical ethics followed by clinical postings (Figure 1). 208(84.9%) agreed that written informed consent should be obtained for routine investigations, general and genital examination, operations or procedures. 207(84.1%) students agreed that children should never be treated without parents' consent except in emergency. 205(83.3%) participants knew that spousal consent should be granted for sterilization, which was statistically significant (p=0.008851). Response was statistically significant regarding confidentiality in medical practice (p=0.001883), physician must maintain utmost respect for human life (p=0.00542), dignity and privacy of the patient (p=0.001408), importance to follow guidelines for intimate examination of patients even for anaesthetized or sedated patients like informed consent, maintain confidentiality (p=0.00289) (Table 1). 197(80.1%) participants stated that patient's spouse should be informed of venereal diseases, hepatitis or HIV status or any other sexually transmitted diseases.

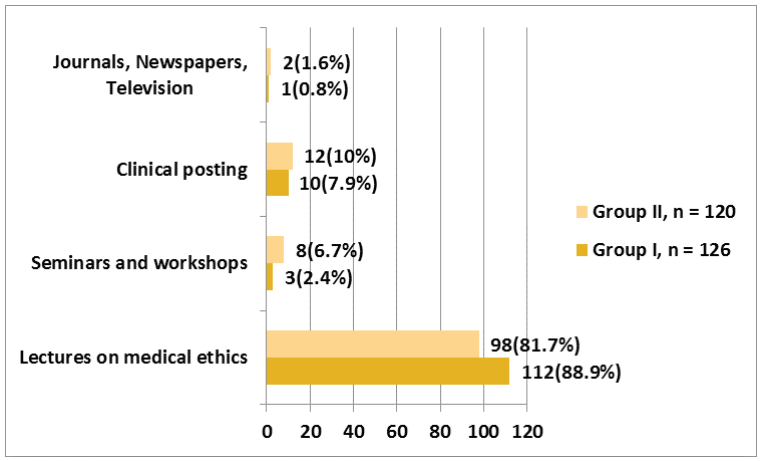


Figure 1: Source of knowledge of medical ethics among medical students

A good number of participants 198(80.4%) disagreed that ethical conduct is important only to avoid legal action. Significant responses were observed when inquired whether to discuss ethical, social and legal issues of patient during clinical rounds along with clinical aspects of a patient's care (p=0.00289). 197(80.1%) participants opined to disclose information to patients regarding their treatment and 203(82.5%) opined to involve patients in decision making of their management. They should always be told about wrong doing 205(83.3%) and close relatives must always be told about patient's condition 207(84.1%). Statistically significant response was obtained when inquired whether during treatment (in non-emergency situations), patient's wishes should be adhered to (p=0.002004) and regarding right of a patient to refuse life supporting treatment (p=0.001867) (Table 1). 209(84.9%) respondents opined that a doctor should not refuse treatment if patient is unable to bear fees. 197(80.1%) participants agreed that clinically confirmed cases should be subjected to routine laboratory investigations.

It was agreed by 200(81.3%) participants that physicians cannot print photograph, qualification and specialty on signboard. 185(75.2%) were acquainted with fact that a physician cannot run an open shop for dispensing drugs and appliances prescribed by other doctors (p=0.00451) (Table 1). 195(79.3%) students concurred that a doctor should not criticize another physician in presence of patient or health personnel and 193(78.5%) opined that doctor should prescribe brand name rather than generic name of a drug in medical practice. Maximum participants agreed strongly that doctor should do what is best irrespective of the patient's opinion (p=0.00124). About 197(80.1%) participants were of opinion that doctor should keep records of certificates issued. When questioned whether medical ethics teaching should be integrated within medical curriculum, maximum 203(82.5%) students agreed strongly.

Table 1: Statistically significant responses regarding various issues of medical ethics

Sr. No	Questions	Group I (n=126) Group II (n=120)	Strongly agree	Agree	Don't know	Disagree	Strongly disagree	P value
1	Consent of spouse is must for sterilization	I	60(47.6%)	40(31.7%)	15(11.9%)	6(4.7%)	5(3.9%)	0.008851
		II	50(41.7%)	55(45.8%)	10(8.3%)	3(2.5%)	2(1.7%)	
2	Physician should protect patient's confidentiality	I	60(47.6%)	45(35.7%)	10(7.9%)	5(3.9%)	60(47.6%)	0.001883
		II	52(43.3%)	50(41.7%)	12(10%)	5(4.2%)	52(43.3%)	
3	Doctor must respect patient's dignity and privacy	I	50(39.7%)	49(38.9%)	17(13.5%)	5(3.9%)	5(3.9%)	0.001408
		II	48(40%)	50(41.7%)	19(15.8%)	2(1.7%)	1(0.8%)	
4	Physician must maintain utmost respect for human life	I	55(43.6%)	44(34.9%)	15(11.9%)	8(6.3%)	4(3.2%)	0.00542
		II	55(45.8%)	49(40.8%)	12(10%)	3(2.5%)	1(0.8%)	

Cont... **Table 1: Statistically significant responses regarding various issues of medical ethics**

5	During clinical teaching, it is important to follow guidelines for intimate examinations of patients	I	56(44.4%)	45(35.7%)	16(12.6%)	5(3.9%)	4(3.2%)	0.0074
		II	55(45.8%)	50(41.7%)	12(10%)	2(1.7%)	1(0.8%)	
5	During clinical rounds along with clinical aspects of a patient's care, it is also essential to discuss ethical, social and legal issues	I	50(39.7%)	49(38.8%)	16(12.7%)	7(5.5%)	4(3.2%)	0.00289
		II	52(43.3%)	51(42.5%)	15(12.5%)	1(0.8%)	1(0.8%)	
6	During treatment, patient's wishes must always be adhered to	I	50(39.7%)	48(38.1%)	15(11.9%)	8(6.3%)	5(3.9%)	0.002004
		II	52(41.2%)	49(38.8%)	13(10.3%)	5(4.2%)	1(0.8%)	
7	Doctor should do what is best irrespective of patient's opinion	I	55(43.6%)	40(31.7%)	20(15.8%)	6(4.7%)	5(3.9%)	0.00124
		II	56(46.6%)	50(41.7%)	10(8.3%)	3(2.5%)	1(0.8%)	
8	Patient has right to refuse life supporting treatment	I	57(45.2%)	49(38.8%)	12(9.5%)	4(3.2%)	4(3.2%)	0.001867
		II	53(44.2%)	50(41.2%)	15(12.5%)	1(0.8%)	1(0.8%)	
9	A physician cannot run an open shop for dispensing drugs and appliances prescribed by other doctors	I	50(39.7%)	45(35.7%)	15(11.9%)	6(4.7%)	10(7.9%)	0.004513
		II	47(39.1%)	43(35.8%)	25(20.8%)	3(2.5%)	2(1.7%)	

Discussion

Ethical conflicts are common during initial years of career of a medical professional that make inculcating a sound foundation in medical ethics important. In present study, response rate was 91.1%[2- 3, 6- 7, 9-11]. 97.5% participants agreed that medical ethics is very important in medical profession, which is analogous to earlier studies[1-3, 6, 10-12]. Maximum students were aware about the Hippocratic Oath and its contents[2-3,10] and of existence and specific functions of the institutional ethics committee which is similar to prior research[2-3,6, 11,13]. However, very few students were aware of same. [1,9,12]

Lectures on medical ethics were predominant source of knowledge [1-2, 6, 9-11, 14], however in studies by Suja Purushothaman et al[3] and Janakiram C et al[7], newspaper, internet and television and in studies by Angadi MM et al[12] and Mohammad M et al[13], it was textbooks and journals. Previous studies[1, 5, 9-10, 14] suggested that written informed consent should be obtained in case of routine investigations, general and genital examination, operations or procedures, which was in agreement with our study. Maximum students agreed that children should never be treated without parents' consent except in emergency, which is comparable with preceding studies[1-5, 9], however 48% agreed in study by Imran N et al[15]. 84.1% agreed that confidentiality is most important in ethical practice of medicine which should be protected by physician[2,6,9-10,14]. Physician must maintain utmost respect for human life and respect dignity and privacy of patients[6]. Most of the participants felt that during intimate examination of the patient, certain guidelines

like informed consent, maintain confidentiality, even for anaesthetized or sedated patients should be followed which is similar to findings by Chatterjee B[9] and Nepal S et al[14].

Earlier researchers observed that most of the participants in their studies disagreed that ethical conduct is important only to avoid legal action[2, 4-5, 11-12], which was comparable with our findings, however participants were not very clear about that in a study conducted by Chatterjee B et al[9]. 85.4% participants were of opinion that during clinical rounds along with clinical aspects of a patient's care, it is also essential to discuss ethical, social and legal issues of that patient, which is analogous with prior studies[5, 9]. Most of the respondents were of opinion that doctor should disclose information to patients regarding their treatment[6]. In a study conducted by Jatana SK et al[6], most of the participants strongly disagreed to involve patients in case of decision making of their management. Respondents in maximum number supported that the patient should always be told about wrong doing[1-2, 4-5, 9-12, 14] and close relatives must always be told about the patient's condition[2, 9]. Positive response for during treatment, patient's wishes in non-emergency situations should be adhered to was in agreement with Rajput CL et al[1], Iswarya S et al[5], Chatterjee B et al[9], Nepal S et al[14], however Suja Purushothaman et al[3] noted that majority participants were not aware of that. Regarding right of a patient to refuse life supporting treatment, statistically significant response was noted, however when participants in lesser number showed agreement in a study by Nepal S et al[14]. Students in a study by Nepal S et al[14] opined that a doctor should not refuse treatment if patient is unable to bear fees, which is in concordance with our study.

In present study, most students felt that clinically confirmed cases should also undergo laboratory investigations as a routine which is comparable to previous studies[1- 2, 5, 9]. 81.3% students disagreed that it was appropriate for doctors to print photograph, qualification and specialty on the signboard. In a study by Rajput CL et al[1] only 21.4% students felt that a doctor may do so, in a study by Chaterjee B et al[9], 52.2% participants disagreed that it was appropriate for doctors and in a study by Angadi et al[12] 31.6% students felt so. 75.2% were acquainted with the fact that a physician cannot run an open shop for dispensing drugs and appliances prescribed by other doctors[1, 9, 12]. 79.3% students agreed that a doctor should not criticize another physician in presence of patients or health personnel[6].

Also, majority of the students opined that it is better to use brand name rather than generic name for prescription to patients, which is in agreement with findings of Iswarya S et al[5] and Angadi MM et al[12]. In a study by Rajput CL et al[1] around 39.2% students feel that it's better to use brand name than generic name of drug whereas in the study by Chatterjee B et al[9], 59.9% students said it is better to prescribe generic drug than brand name in accordance with the principle of ethical conduct. The doctor should do what is best irrespective of the patient's opinion. Similar findings were noted in previously conducted studies[1, 4-5, 9-11], however in a study by Walrond ER et al[2], 85.5% participants disagreed for the same. Most of the students had a knowledge that it is always necessary for physicians to keep a copy of the certificate issued by them. This is comparable with findings of Rajput CL et al[1]. In a study by Chatterjee B et al[9], only 38.8% disagreed that it was necessary for a doctor to retain a copy of the certificate issued by him or her. Regarding integration of medical ethics in the medical curriculum, findings were comparable with earlier studies[4, 6].

Conclusion

For modern day medical practice, good understanding and comprehension of healthcare ethics is crucial. The present study has attempted to assess medical students' knowledge and attitude about ethical issues and their importance in medicine practice with clear needs to integrate realistic elements of ethical instruction into the curriculum of the undergraduate program.

When compared between students studying in different years exploring various issues, it indicates that there is no proportionate increase in knowledge of ethical issues along with an increase in the years of medical education; the mean score of second year students was better than that of their seniors in most of the cases. Thus though students had adequate knowledge about medical ethics, reinforcement at all levels of medical education is necessary and gives emphasis to the importance of continuing education in ethics throughout the undergraduate, internship, and postgraduate periods.

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Different Types of Diatoms Seen in the River Narmada in India

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Abstract

Aim: To determine the different types of diatoms present in the waters of River Narmada flowing through 5 distinct cities of India and ending into the Arabian Sea. Different types of Diatoms were observed with change in the course of water. **Objective:** The study shall assist to determine the different types of diatoms present in the river Narmada. **Materials and Method:** The study does not require any special instruments and the technique used to extract the diatoms from the waters is also very simple to perform. In this study the acid digestion technique is not used but the technique is slightly modified in order to prevent the damage to the walls of the diatoms. **Result:** Various types of diatoms were seen and the diatoms present in the water collected from different cities of India are found to be different including the species unique to the area. Conclusion: The study describes the type of diatom present in the water of river Narmada collected from different places.

Keywords: *Diatoms, Drowning, Forensic, Narmada*

Introduction

Diatoms are unicellular, photosynthetic, eukaryotic organisms that are present in almost all the water bodies including springs, rivers, ponds, lakes ditches and also in freshwaters, brackish and marine waters (Smol and Stomer 2010), and also terrestrial habitats like wet rocks, mosses and soil (Falasco et al 2014;Tofilovska et al, 2014). Diatoms are large group consisting of 200 genera and approximately 13000 species, out of which 92 genera and about 569 species are reported in India. The diatoms are distinguished from other types of algae due to their structure and sculpturing of their walls.

The cell of the diatom consists of cell wall and protoplast. The cells are covered by a siliceous wall, called **frustule.** It consists of two overlapping halves called the theca. The upper one is **epitheca** and lower one is called **hypotheca**

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The theca consist of 2 portions

a. Valve: the upper flattened top

b. Connecting band or cingulum- incurved region

The common region of the connecting bands, where both the theca are fitted together, is called the **girdle.** Depending on symmetry, the cells are divided into 2 orders:

1. Pennales (bilaterally symmetrical)
2. Centrales (radially symmetrical)

In some pinnate diatoms, an elongated slit is present on their valves, called **raphe.** The raphe is interrupted at its midpoint by thickening of the walled called the **central nodule** as seen in figure 1.2.1 a and b**.** Similar thickening is also present at the ends called **polar nodules.** Some members of the order Pennales, do not have raphe, called pseudoraphe.

Based on electron microscopic studies, Hendey (1971) observed 4 basic secondary structures:

1. Punctae (small perforations on valve surface)

2. Canaliculi (tubelike narrow channels which run through the valve surface)

3. Areolae (large box like depressions)

4. Costae (riblike structures on the valve structures)

The cell wall of diatom is mainly made up of substances impregnated with silica. The content of silica varies from 1% to about 50% on the basis of dry weight of the cell.

Range of Diatoms

Different estimates of diatom diversity have been offered. Mann and Droop (1996) suggested there may be in excess of 200,000 species of diatoms (extent and fossil), but more recently Guiry (2012) gave the much reduced estimate of 20,000, with 12,000 already described (and accepted) 8000 species yet under discovery. Using modern diatom species concepts, and with the increased use of fine ultrastructural features and molecular analysis for species discrimination, a more realistic total number of species could be 30,000 to 100,000 (Mann and Vanormelingen 2013). It is probably agreed that around 15000 are currently accepted and known.

Drowning and Diatoms

Diatoms analysis is a valuable tool in forensic science and is useful in diagnosis of drowning cases. The basic principle with diatom test in drowning is based on correlation between diatoms that are present in medium where the possible drowning took place. As described in the morphology, the diatoms have silica-based skeletons that do not readily decay and can also be detected in heavily decomposed bodies. Diatoms do not occur naturally in the body hence if laboratory tests show their presence in the body, then it is a good evidence of drowning as the cause of death.

This study introduces a new technique for extracting of diatoms from the water samples collected from different cities of India which include:

The 5 different places used for collection of samples include:

1. Amarkantak (0 km)- origin
2. Jabalpur (227 km)
3. Ahmedabad (1127 km)
4. Bharuch (1327 km)
5. Khambhat (1467 km)- sea (end)

Materials:

- Water samples from 5 different sites
- Micropipette- 1000 µl
- Micro-tips- 1000 µl
- Distilled water- diatom free
- Hot plate
- Centrifuge tubes 15 ml
- Centrifuge machine
- Microscopic slide
- Crystalline violet stain
- Light microscope
- Fluorescence microscope
- Immersion oil

Method:

Technique For Water Sampling:

Waters were collected from the following cities of India

1. Amarkantak plateau, Amarkantak
2. Gwari ghat, Jabalpur
3. Narmada Canal, Ahmedabad
4. Bhigurishi Temple, Bharuch
5. Sea Shore of Gulf of Khambhat

Extraction of diatoms from the water samples

The method used for extraction of diatoms from water samples requires no special treatment as well as no use of chemicals is done in the method mentioned below. The earlier used acid digestion technique uses nitric acid and hydrochloric acid which can damage the structure of diatom is not advised for the extraction of diatoms from the water sample.

Following steps were used to extract the diatoms

- Total 10 ml water sample was taken in 15 ml centrifuge tube and centrifuged at 4000 rpm for 5

minutes

· The resulting supernatant was removed carefully using a micropipette and by not disturbing the pellet

· The pellet was suspended in 1.0 ml sterilized distilled water

· From this prepared solution the slides were prepared for microscopic analysis

Microscopic Analysis:

· Slide preparation:

· A clean microscopic slide was taken and a drop of dissolved pallet was put on it. The slide was then heat fixed on the hotplate at 100^0C until the evaporation of water. After 1 to 2 minutes of heat fixing the slide it was stained with crystalline violet.

Diatom Staining Procedure

Simple staining: After heat fixing the slide either crystalline violet or safranin stain solution was used placed over the heat fixed slide using a dropper. The slide was then left for 1 minute and washed with distilled water. The slide was then observed under 100X oil immersion lens microscope.

Results

Various types of diatoms which were observed in the study are:

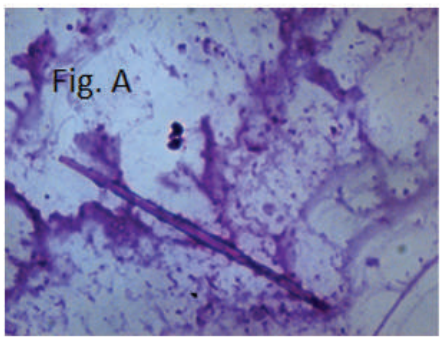
1. Nitzschia
2. Fragillaria
3. Navicula
4. Synedra ulna
5. Synedra Tabulate
6. Caloneis Bacillum
7. Fregilaria capunica
8. Fragellaria intermedia
9. Diatoma vulgaris
10. Nitzchia acidoclinata
11. Hyalosynedra lacvigata
12. Grammataphora subtilissima

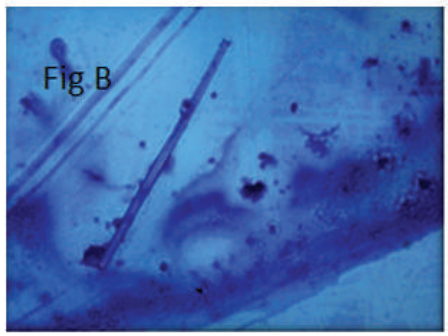
Microscopic Analysis

The analysis of samples showed different species of diatoms in different waters of river Narmada. The variation could not be observed in the form of species for all the diatoms among different cities. The photographs were taken with the help of a digital camera at 100X oil immersion magnification in light and fluorescence microscope.

Water sampling 1: Amarkantak Waters

The diatoms species present were:



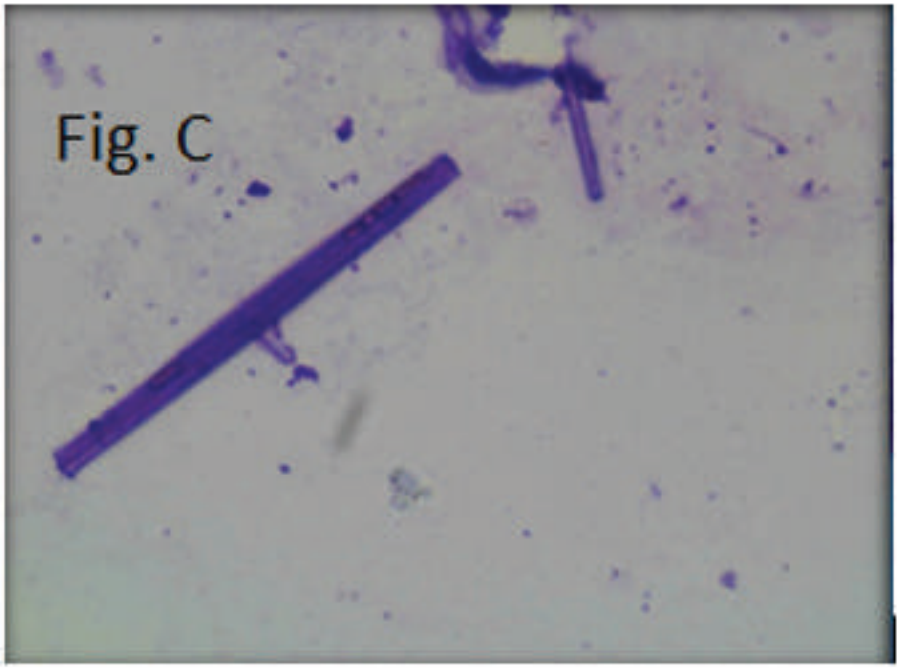


The above images show the following types of diatom

Figure A: Hyalsynedra Lacvigata

Figure B: Synedra Ulna

The different types of diatoms seen in Jabalpur waters of Narmada river are:



The different types of diatoms observed in the Ahmedabad water of Narmada river are:

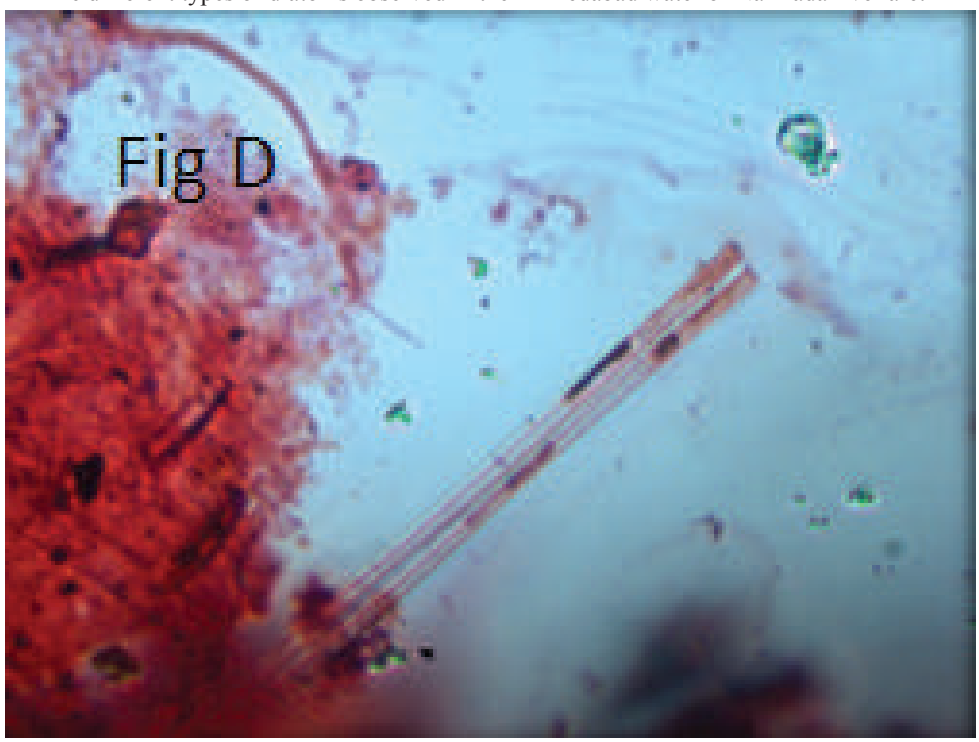


Figure B: Synedra Ulna

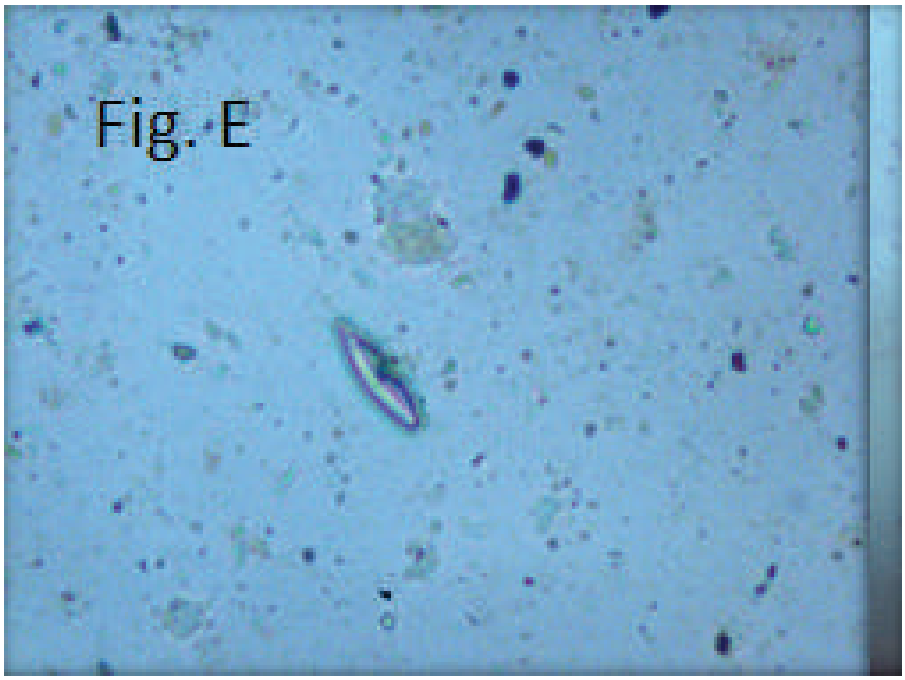
The different types of diatoms observed in the Ahmedabad water of Narmada river are:

The above images show the following types of diatom

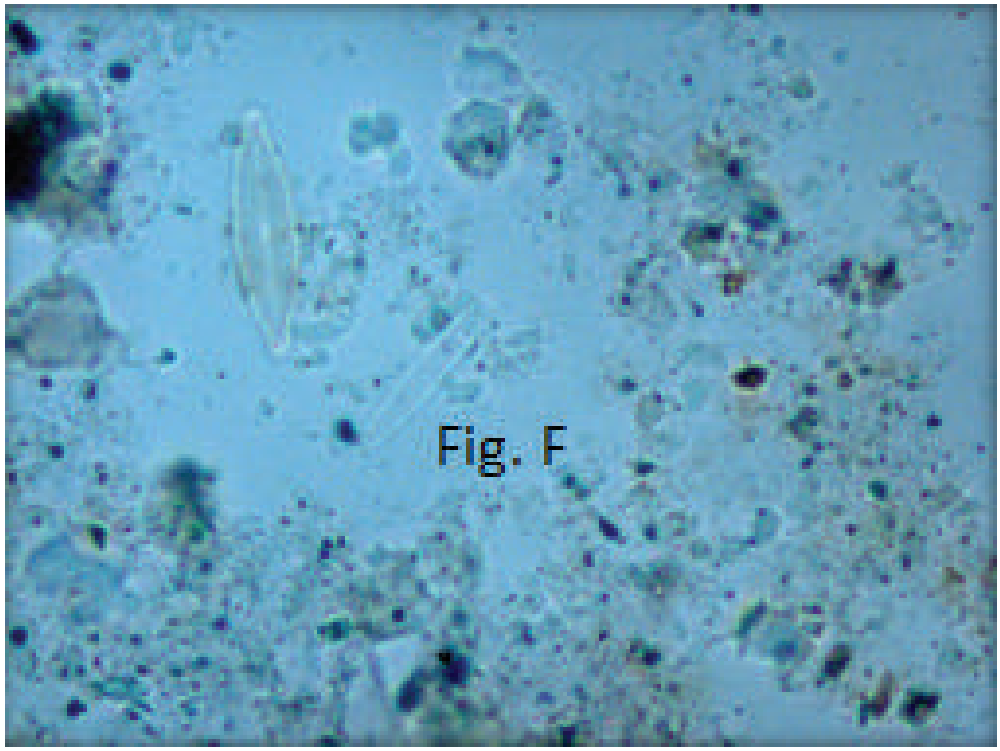
Figure C: Fregilaria apunica

Figure D:Fragillaria Intermedia

The different types of diatoms observed in the Bharuch water of Narmada river are:



The different types of diatoms observed in the Gulf of Khambhat sea where the Narmada river meets the sea are:



The above images show the following types of diatom

Figure E: Navicula

Figure F: Caloneis

Due to reduced distance between Bharuch and Gulf of Khambhat the navicular species of Diatoms were found to be dominant in the gulf of Khambhat, as similar environmental conditions persists.

Discussion

The results of the study mentioned in the above section were done using a limited number of teeth samples and thus the results obtained did not have an appropriate relevancy. The types of diatoms observed from the same river that is Narmada varied from city to city. Hence the environmental conditions play a very important role in the type of diatom found in the waters of the same river:

The variations seen in the type included:

1. Narmada river at origin (Amarkantak): Hyalosynedra lacvigata, Synedra Ulna, Synedra tabulata, Nitzschia,

2. Narmada river flowing through the city of Jabalpur: Navicula, Fregilaria capunica, Fragillaria intermedia, Grammatophora,

3. Narmada flowing in Ahmedabad: Nitzschia, Nitzchia Sicula, Nitzchia Acidonata, Fragillaria intermedia, Navicula

4. Narmada flowing in Bharuch: Navicula Hamiltoni, Navicula, Caloneis, Nitzchia

5. Narmada meeting the Arabian sea in the Gulf of Khambhat: Navicula, Caloneis, Nitzschia.

Diatom was first described in lung fluid by Hoffman in the year 1986[1]. However, Rovenstorff had successfully utilized it to solve a case of a drowning mystery in 1904. Incze successfully detected the diatoms in blood and parenchymal organs in 1942. Tamaska detected diatom in bone marrow in 1949. In the 1960s and 1970s, Timperman using large series of drowning cases, provided evidence for the validity of diatom test[2]. Detection of diatoms has been applied as an important sign of drowning in the beginning of 20th century and the use of diatoms for the diagnosis of drowning cases was debated soon after they were first debate. Over the past decades diatoms are screened for high lipid content. Geologists claim that much of crude oil comes from diatoms. Sanjay K.R, Nagendra Prasad M isolated diatom Navicula cryptocephala, from fresh water source was grown on suitable median for extracting and characterizing the oil for biodiesel production[3]. T.A. Nadia Fucci developed a simple and economical procedure for the destruction of human organic material for the diatom examination is presented. The author tested a minimal amount of H_2SO_4 diluted solution to detect diatoms in several tissues from human corpses under crime investigation, immersed in sea and river water. The method was compared with a traditional method that includes digestion with large amount of strong mixture of sulphuric and nitric acid (90%). The new procedure showed that all siliceous frustules of sea and river diatoms are more resistant to H_2SO_4 diluted treatment and are still recognizable after digestion, and observation under the microscope is better than the other procedure. Moreover, the microscopic observation of ameboid protozoa was also possible[4].

Conclusion

This study determines that the type and species of diatoms change, with the changes in the environmental conditions. The study serves to reduce the time to extract the diatoms and in order to come to a conclusion of cause of death. The extraction diatoms through this technique requires 3-4 hours to come to a conclusion which is very less when compared to other techniques used in forensic science. The technique used does not involve any damage to the evidence which is important as the diatoms are brushed from the surface of the diatom and no invasive techniques are used.

Ethical Clearance: N/A

Source of Funding: None

Conflict of Interest: None

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A Study of Age Estimation by Radiological Assessment of Ossification Centers at Hand Wrist and Elbow Joint at a Tribal Teaching Medical College of Adilabad

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Abstract

Background: Radiological assessment of the skeletal ages is an important tool for the estimation of age in the living. Such data can be very useful for forensic practitioners especially in developing countries some of the population is uneducated where the birth records are not present.

Methods: This retrospective study was conducted in the Department of Forensic Medicine and Toxicology, Rajiv Gandhi Institute of Medical Sciences [RIMS], Adilabad. A total of n=26 males and n=46 females cases were examined for the ossification at 4 areas which include left-Hand epiphyses (the distal end of radius and ulna), Base of the first metacarpal and Conjoint Epiphyses. The assessment of radiographic images of hand and elbow joints was based on the revised method for epiphyseal union assessment.

Results: The mean ages at ossification for stage I in males were found to be 12.90 years. At stage II we found the mean age of 15.18 years. In stage III we found the mean age of 16.15 years and stage IV the mean age value at all ossification centers was 19.18 years. Similarly for females the mean values of all ossification centers at the stage I estimated the age of 14.12 years. In stage II the mean age estimated was 15.27 years and stage III the mean age estimated was 16.29 years and stage IV mean age estimated was 17.96 years.

Conclusion: Within the constraints of the present study we found that hand wrist and elbow joint radiographs are very useful for estimation of bone age in cases up to 18 years of age. We used digital radiographs along stages for determining the degree of ossification which has a greater degree of reproducibility and reliability.

***Keywords**: Age Estimation, Radiological Assessment, Ossification Centers, distal end of radius ulna*

Introduction

Age determination for the individual has considerable importance in the medicolegal field. It is well known that accurate estimation of a living person can prove to be important for humanitarian, administrative, and legal purposes especially in cases when the subject is considered as minor (Child). The UN Convention on rights of child defines a 'child' as every human below the age of 18 years. [1] The signs of progress of ossification and changes during various periods of development can be studied by means of X-rays in living individuals and these changes are age-related. [2] Therefore it is possible to determine the approximate age of an individual by radiological examination of the bone till ossification is complete. [3] the most commonly used regions for the radiographic evaluation of skeletal age in the left hand and wrist, the elbow joint, and the medial epiphysis of right clavicle all are considered useful when the subject in question is less than 21 years of age. There are a number

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of factors that affect the stage of epiphyseal union which includes geographic distribution, socioeconomic status, climate, nutrition, and genetics. [4, 5] Ethnicity is also very important when this kind of study done in a neglected population. With this background, we in the present study tried to evaluate the age as determined by the stages of ossification at centers in our predominantly tribal population. There is hardly any data of such a study done in the tribal region of Adilabad.

Material and Methods

This retrospective study was conducted in the Department of Forensic Medicine and Toxicology, Rajiv Gandhi Institute of Medical Sciences [RIMS], Adilabad. The radiographs were taken as a part of the medicolegal examination for age determination requested by law enforcement agencies in cases where there were no official birth certificates of the individual or where there was a dispute of age. We examined the digital radiographs of all the cases from Jan 2018 to December 2019. Institutional Ethical committee permission was obtained for the study. The inclusion criteria were medicolegal cases of both sexes reporting for age determination to our institute, availability of digital x-ray records of the cases. Exclusion criteria were cases with fractures of the epiphysis, congenital anomalies of bones, or cases with bone disorders. A total of n=26 males and n=46 females cases were examined for the ossification at 3 areas which include left-Hand epiphyses (the distal end of radius and ulna), Base of the first metacarpal and Conjoint Epiphyses. The assessment of radiographic images of hand and elbow joints was based on the revised method for epiphyseal union assessment by S. S. Bhise et al; [2]. Stage 0 is a radiolucent area observed between epiphysis and diaphysis. In stage I there is a continuous radiolucent gap between epiphysis and diaphysis separable at extremes. Stage 2 is the presence of a radiopaque line between epiphysis and diaphysis. In stage 3 there a continuous radiopaque line called union scar. In stage 4 there is the disappearance of both epiphysis and diaphysis and bone is fused as one. All the x-rays were examined by a single observer and the available data was entered in MS Excel spreadsheet the mean of age at various stages of ossification was determined and graphs were plotted. Finally, the mean of ossification at 3 centers with stage 1, stage 2 stage 3 and stage 4 were calculated and plotted as shown in Graph 4.

Results

In the present study a total of n=26 male and n=46 female cases. We assessed in all the cases radiographic ossification stages at 3 places which includes hand epiphyses (distal end of radius and ulna), the base of the first metacarpal, Conjoint Epiphyses, and details given in table 1.

The minimum age based on the stage of ossification at the hand epiphyses (the distal end of radius and ulna) in males was 11.1-12 years and the maximum age based on the ossification at hand epiphyses was found to be 22.1 – 23 years. We did not have any case of stage 0 in this study. Out of n=26 males, n=9 cases were found in stage I ossification the mean age was found to be 13.52 years. In stage II there was a single case with a mean age of 15.5 years. In stage III n=4 cases with a mean age were 16.45 years and in stage IV n=12 cases the mean age was 19.7 years. The mean age of all the patients was plotted in graph 1.

In females for hand epiphyses (the distal end of radius and ulna) minimum and maximum age based on ossification stages were estimated at 13.1 – 14 years and 19.1 – 20 years respectively. Out of n=46 cases, n=8 cases were found in stage I with a mean age of 14 years. Stage II n=22 cases were present with a mean age of 15.17 years. Stage III there were n=9 cases with a mean age of 16.32 years and in stage IV n= 7 cases with a mean age of 17.86 years graph 1.

The age estimation as per the base of first metacarpal ossification in radiographs in males found the minimum age was 11.1-12 years and the maximum age was estimated at 22.1 to 23 years. In stage I ossification we found n=7 cases with the mean age estimated at 12.94 years. In stage II n=3 cases with the mean age of 15.16 years. In stage III there were n=2 cases with a mean age of 16.5 years and in stage IV n=14 cases with the mean age of 19.06 years total of n=26 cases. Graph 2 shows the mean age at various stages of ossification.

Radiographic age estimation by assessing the base of the first metacarpal in females (n=46) found the minimum age of 13.1-14 years and the maximum age was 19.1-20 years. There was no case in stage 0

ossification in stage I ossification there were n=4 cases with mean age estimated to be 14.56 years. In stage II n=24 cases with a mean age of 15.14 years. Stage III n=4 cases with a mean age of 16.25 years. In stage IV n=14 cases with a mean age of 17.94 years given in graph 2.

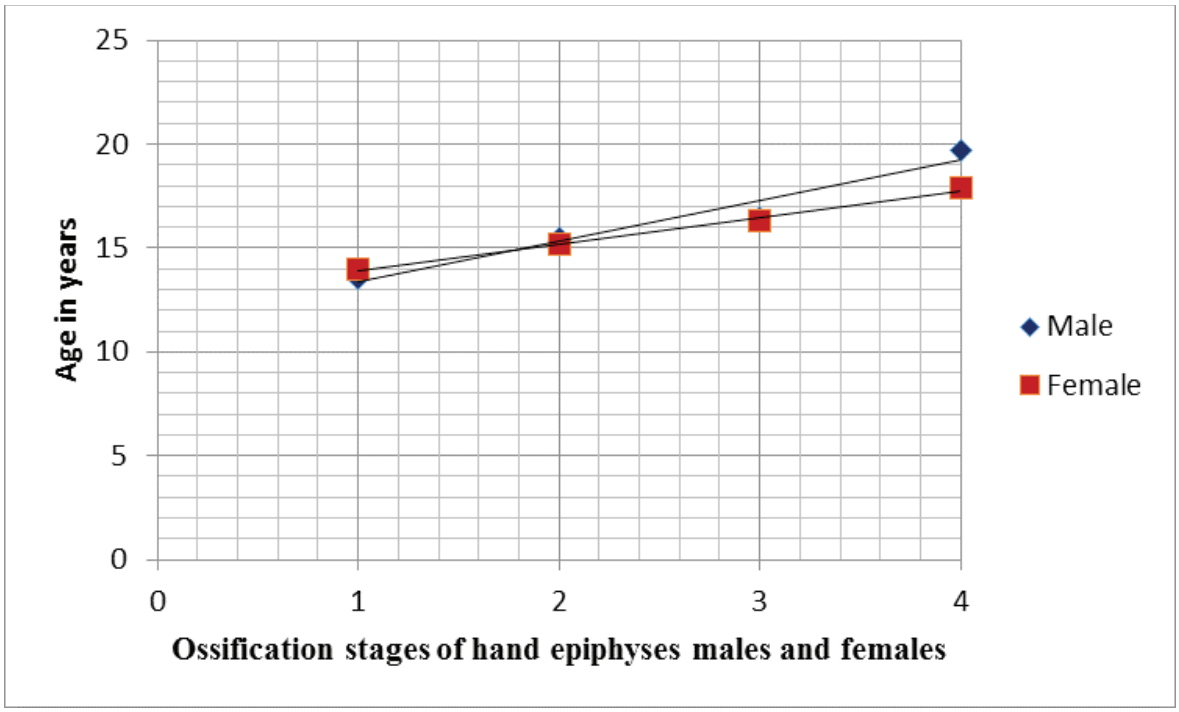
The ossification of Conjoint Epiphyses in the humerus for males estimated the minimum age of 11.1-12 years and the maximum age of 22.1 to 23 years. There were n=2 cases in stage I with a mean age of 12.25 years and n=6 cases in stage II with a mean age of 14.88 years and stage III n=2 cases mean age of 15.5 years and stage IV n=16 cases with a mean age of 18.8 years given in graph 3.

The ossification stages of Conjoint Epiphyses in females revealed the age ranges from 13.1-14.0 years minimum to 19.1-20 years maximum. In stage I ossifications there were n=3 cases mean age 13.8 years. In stage II n=4 cases with mean age 15.5 years. In stage III there were n=4 cases with mean age 16.3 years and in stage IV there were n=35 cases with a mean age of 18.1 years shown in graph 3.

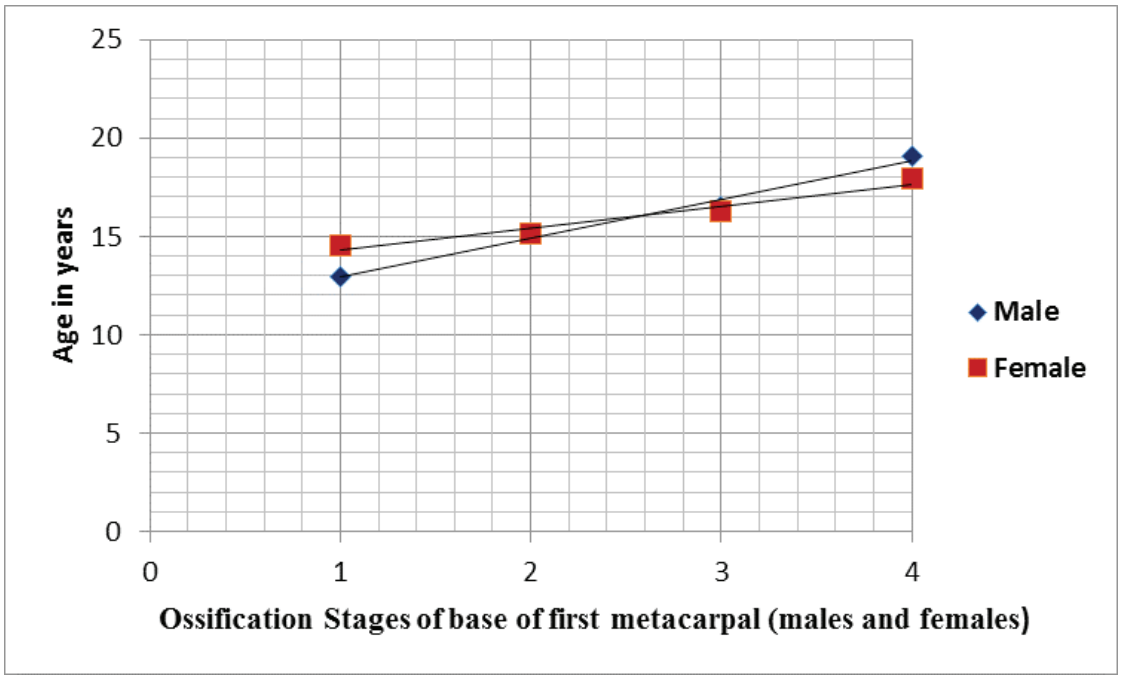
The mean ages at ossification Stage I at various centers was plotted in male and the mean value obtained for stage I ossification was found to be 12.90 years. At stage II we found the mean age of 15.18 years. In stage III we found the mean age of 16.15 years and stage IV the mean age value at all ossification centers was 19.18 years given in graph 7. Similarly for females the mean values of all ossification centers at the stage I estimated the age of 14.12 years. In stage II the mean age estimated was 15.27 years and stage III the mean age estimated was 16.29 years and stage IV mean age estimated was 17.96 years shown in graph 4.

Table 1: Showing the number of cases and stages of ossification based on radiographic evaluation at various centers

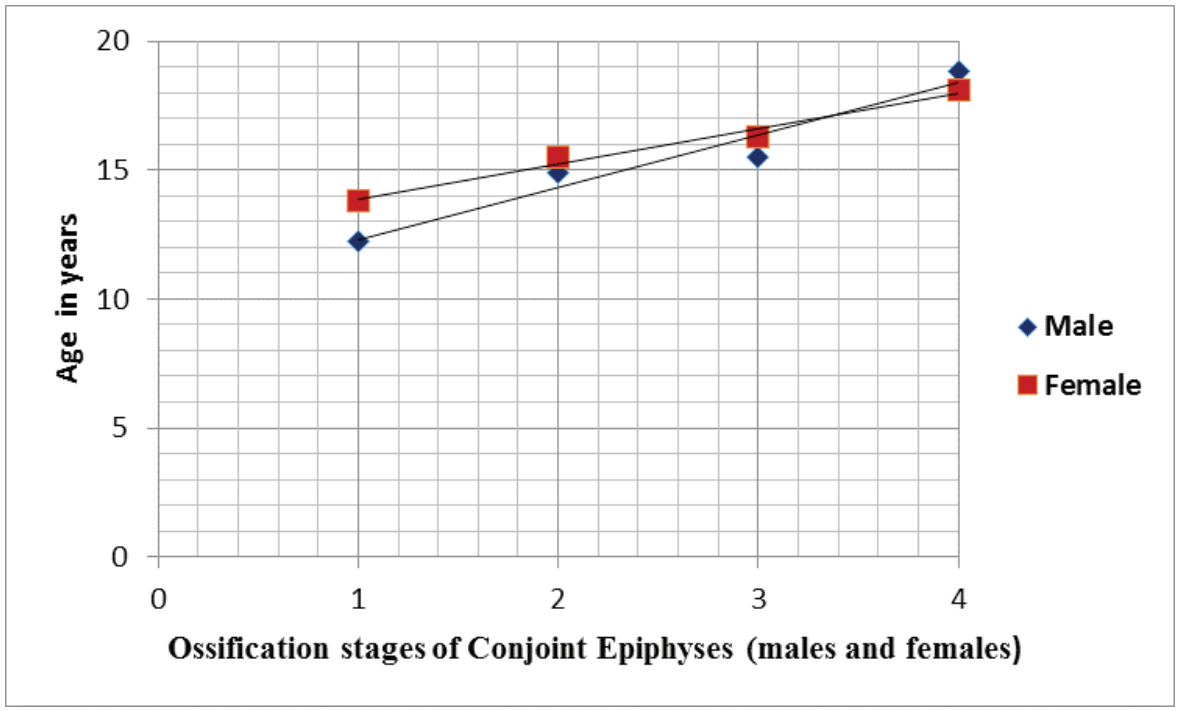
No of Patients (N)	Stages of Ossification					No of Patients (N)	Stages of Ossification				
	0	**1**	**2**	**3**	**4**		**0**	**1**	**2**	**3**	**4**
	Staging of Hand epiphyses (Male)						Staging of Hand epiphyses (Female)				
26	0	9	1	4	12	46	0	8	22	9	7
	Staging of Base of first Metacarpal (Male)						Staging of Base of first Metacarpal (Female)				
26	0	7	3	2	14	46	0	4	24	4	14
	Staging of Conjoint Epiphyses (Male)						Staging of Conjoint Epiphyses (Female)				
26	0	2	6	2	16	46	0	3	4	4	35



Graph 1: Showing the mean age at different ossification stages of hand epiphyses (males and females)



Graph 2: Showing the mean age at different ossification stages of base of first metacarpal (males and females)



Graph 3: Showing the mean age at different ossification stages of Conjoint Epiphyses (males and females)

Discussion

The present study was done in n=26 male and n=46 females the male to female ratio was 1:1.76. This study consisted of a predominantly tribal population of Adilabad District in Telangana State with different ethnicity, race, socioeconomic status and residency, and these factors do impact the epiphyseal union. In a country as diverse as India, it is difficult to follow a simple standard for determination of age across the entire country. We in the present study estimated the ossifications at 3 centers and mean values were considered this will negate the small variations between the different stages at different centers. Previous studies have shown that union age for each epiphyseal plate at the knee joint and hand joint has similar union stages. [2, 6] A study by Majed O. Aljuaid et al; [7] showed a stronger correlation of the hand joint bones both males and females as compared to knee joint for estimation of age accurately. In the present study, we found in males the average age at stage I (beginning of union) was 12.90 years and stage IV (complete union) was 19.18 years. For females Stage, I (beginning of union) was 14.12 and stage IV (complete union) was 17.96 years. It shows that the average age of fusion is higher in males as compared to females. The female subjects commence fusion at the age of 13 - 14 years of age and complete it by 17-18 years while the male subjects commence fusion at 14-15 years and complete it at 19- 20 years. A study by S Krishnamoorthy et al; [8] found that complete fusion of distal end of the ulna was found in 18-19 years in males and 17 – 18 years in females. In the current study, we found the distal end of ulna fused with shaft completely at 19.7 years in males and 17.86 years in females agreeing with the results of the above study. Other studies in India by Hepworth in Punjab [9], Kothari in Marwar [10], and Sunil et al; [11] all have found similar results across India. S. Shanmugasundaram et al; [12] found the base of the metacarpal fusion was completed from 15 to 19 years. The average age range of fusion of 4th and 5th metacarpals ranges from 15 – 17 years in girls in boys' fusion occurs between 17 to 18 years. In our study, we found the base of the first metacarpal in boys ossified completely by 19 years, and in females, it occurred at 17.94 years. In our study, we found in boys the Conjoint Epiphyses was in stage 3 unions at 15.5 years and stage IV was at 18.8 years and in females, Conjoint Epiphyses was in stage 3 unions in 16.3 and stage IV in 18.1 years. Similar observations have been found by Lal et al; [13] and Jain S [14]. Although we found that in several studies they have used different criteria

for evaluation of ossifications hence there may be some variations between the results of the present study compared to the other studies.

Conclusion

Within the constraints of the present study, we found that hand wrist and elbow joint radiographs are very useful for estimation of bone age in cases up to 18 years of age. We used digital radiographs along stages for determining the degree of ossification which has a greater degree of reproducibility and reliability. Although several studies have been conducted for age estimation using radiographs there was no uniform method of assessment of age. We hope that in future a standardized method could be adopted for wider application across the country.

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Admission, Transfer and Discharge Procedure in Hospital: Review Article

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Abstract

Background: Admission to hospital can be traumatic experience with anxiety and fear for anyone. The person loses his identity, independence and control of daily living. The duration and severity of illness influence his/her reaction to admission procedure. This study aim to determine the role of nurse in admission discharge and transfer procedure in hospital

Methods: A PubMed, Google scholar and textbooks (2000-2018) literature review was undertaken to define the admission, discharge and transfer procedure in hospital.

Results: We identified 10 relevant articles and books. The most frequently noted form of admission discharge and transfer procedure in hospital.

Conclusions: This article bring to light the procedure of admission, points to be remember during hospitalization of patient because admission to hospital can be traumatic experience with anxiety and fear for anyone

Keywords: *Admission, transfer, discharge, hospital*

Introduction

Admission is defined as allowing a patient to stay in hospital for observation, investigation, treatment and care.[1] Admission is the entry of a patient into a hospital / ward for therapeutic / diagnostic purposes.[2]

Purposes of Admission:

— To receive the patients in the ward for admission according to his condition

— To provide comfort and safety to the patient

— To provide immediate care

— To be ready for any emergency.[2]

Principles/ important points involved in the admission of the patient:

1. Sudden change in the environment produces fear and anxiety

2. Entering hospital is a threat to one's personal identity.

3. People have diversity of habits and modes of behavior.

4. Illness can be a novel experience for the patient and bring stress on his physical and mental health.[3]

Actions:

- Personnel in admitting dept. should be instructed to give respect and establish healthy

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relationship to gain confidence and cooperation of pt and his family.

- Nurses should make every effort to be friendly and courteous and make him feel that he will receive sympathetic as well as efficient care.
- Talking with the patient and clarifying their doubts, listening to their problems; introducing to hospital dept, the hospital personnel and other patients; explaining the hospital routine; taking care of the valuables; giving explanations for treatments; allowing family members and friends to spend some time with the patient and above all establishing effective Nurse patient relationship can reduce fear and anxiety to a greater extent.
- The nurse should address them by their name and proper title.
- Allow the patient to use his own articles as far as hospital policy permits. (every pt loves to use his own articles)
- Find out the likes and dislikes of patient and include the pt in his plan of care. He should feel that he is considered and cared for.
- The hospital routines should be not be too rigid. E.g. some pts may not wish to be dressed in hospital gowns.
- Every nurse should understand the behavioral pattern of patients according to his age, sex, race, caste and socioeconomic factors.
- Nurse should recognize the various needs of the patient and meet them without delay.
- Nurse should give proper explanations about the disease, its prognosis and its complications in the language the patient can understand.[3'4]

Types of admission:

Emergency Admission

Patients are admitted in acute conditions requiring immediate treatment E.g. patients with heart attack, accidents, acute appendicitis, poisonings, labor pains, diarrhea, dysentery, hemetemesis, shock etc.

Routine Admission:

It means that the patients are admitted for investigations and planned treatment and surgeries. Eg the patients with hypertension, diabetes, jaundice, hernia, chronic renal failure.[4,5]

Procedure of Emergency Admission:

- · As patient come to emergency, according to his/her condition provide strecher or wheel chair to patient.
- Provide bed and privacy to the patient
- Take brief history and complaints of patient, if patient is not able to give history then obtain from relatives.
- Check vital signs and cal doctors on duty.
- Carry out the doctors prescriptions accurately.
- Allow only one caretaker to be near the patient and send to medical record department to prepare the patient folder. See that all the details are entered or not.
- If admission is required ask the doctor to give admission form and ask caretaker to sign the consent.
- Patient is shifted toward after giving initial care. If the emergency is busy then request the particular ward to come and take the patient.
- No patient should be sent out without any treatment.[5,6]

Routine Admission Procedure:

Articles Required:

- Bed
- Vital signs tray
- Weighing machine and measuring tape
- Physical examination tray
- Admission slip

- Patient's case sheet and progress notes
- Investigation forms
- Intake output chart, Admission consent form
- Bed bath tray
- Bed pan or urinal[5]

Activities carried out in outpatient department:

Reception of the patient

The personnel in the admitting department should greet the patient and make him at ease. In emergency condition, no time should be lost to initiate the treatment.

Recording of social and medical data

The clerk in the records section is responsible for recording certain data that are essential:

- Identification data
- Patients too ill to answer questions should be admitted immediately and the necessary data is supplied by the family or friends.

Medical examination:

- medical history
- TPR &BP
- Investigations such as X Ray
- The patients who are suffering from mild ailments are sent home with necessary treatment. Others are admitted to the hospital for further investigations and treatment.

Activities carried out in inpatient department

Transporting the patients from OPD to IPD:

- Patients who are not very ill and are allowed to walk are escorted to clinical division by a nurse or an attendee.
- A seriously ill patient should never be left in the hands of untrained personnel.
- A female patient should never be left with a male attender.

Reception of the patient by the ward sister:

- The ward sister or nurse admitting the pt introduces her and greets the pt and his relatives. Makes effective nurse patient relationship.
- If the pt is very sick, she should put him to bed immediately. The patient who is not very ill can be allowed to take rounds in the ward. Introduce other pts to him and vice versa, and so with the other nursing personnel working in the ward.
- Orient the pt to the whole ward.
- Explain hospital policies, procedures and routines to the pt and his relatives. Helping the patient to occupy his bed
- His temp, pulse and respirations are recorded at the time of admission and later on at regular basis.
- Record patient data in admission register.
- Special equipments such as oxygen cylinder, suction machine, cardiac monitor, emergency trolley should be there for any emergency.
- Help him to change the dress (acc to hospital policy)
- Bed Bath (bed ridden pt) or bathroom bath (ambulatory pt).
- The nurse must make the arrangements to carry out the investigations if ordered by the doctor.

Care of valuable and clothing:

- Dirty clothing should be sent home with the relatives for washing.
- The clean clothing should be kept safely for the daily use.
- In the absence of the relatives, they are numbered, labeled and kept in store until such time that is handed over to the relatives.
- Never throw anything that belongs to the patient. It may be something he treasures. Negligence in handling patient's belongings brings criticism upon the hospital and makes the personnel and other patients liable to suspicion.

• Encourage the pt to send the jewellery, money and other valuable such as radio, watch etc to home with the responsible person.

• On discharge or death or transfer of the pt, the nurse should see that the clothes and other valuables are returned to the pt or his relatives. Get the receipt from the pt or nearest relative and keep it safe.6,7,8

Nurse's responsibilities in admission:

■ Preparation of the unit (Unit is the place where the pt is kept during hospital stay)

• Keeping the bed ready

• Position the bed

■ Ambulatory pt (normal position of bed)

■ Pt on stretcher (bed in lowest position)

• Entry of patient in admission registers with identification data, DOA, Diagnosis and IP number.

• Assemble necessary articles and supplies (eg hospital gown, bed bath articles,etc)

• Assemble special equipment and supplies

(oxygen cylinder, cardiac monitors etc)

• Nurse should make every effort to be friendly and courteous with the pt

• Make proper observation of pt's condition

• Orient pt and relatives to hospital policies

• Observe policies in medico legal cases

• Recognize the various needs of the pts and meet them without delay

• Nurse should find out likes and dislikes of pt and include pt in his plan of care

• Care of pt's valuables and clothes

• Record patients medicine in medicine book if available.4,7

Transferring the Patient

A patient may be transferred from one room to another within the healthcare facility for several reasons. Sometimes the transfer is made at the patient's request

a. A different type of room (such as a private room)

b. A transfer for personal reasons, such as to find a more compatible roommate medical staff may request it.

• The physician may request the patient be transferred from one level of nursing care to another because of a change in the patient's condition that might require more or less specialized care.

• the patient may be moved into intensive care when his/her condition becomes more critical

• Transferred onto a regular medical floor when his/her condition improves.

• Sometimes the nursing staff will transfer a patient closer to the nursing station where the patient's condition can be supervised more closely.

• The patient may also be transferred if the room location or equipment in the room is needed for a more critically ill patient.

• If the patient did not ask to be transferred, he/she may be upset, especially if the patient does not understand the reason for the transfer.8,9

Responsibilities

• make sure all the patient's belongings are transferred with him/her

• Collect the belongings and any equipment that will be moved

• Check with the nursing supervisor before moving any equipment to another floor

• Check drawers, closets, tables, windowsills, the bathroom, and the bed covers for articles that might be forgotten

• The nurse will collect the patient's chart and medicines.

• The ward clerk will make the necessary changes in the patient's records, billing charges, and other forms.

• Nurse will post the transfer on the patient's chart include the time, room numbers transferred from and to, the reason for the transfer and the patient's attitude toward the move should also be charted.7

Moving the Patient

• Before moving the patient, make sure the new room or floor is ready to receive the patient.

• If the patient is moved in the bed, personal belongings can be placed on the bed.

• The patient should be in a comfortable position with the side rails raised.

• If the patient is moved by stretcher or wheelchair, move the patient first. Then move the patient's belongings on a cart.

• To prevent falls, never leave the patient alone in the hallway when you are transferring him/her to another floor.10

When the patient arrives at the new room

• Introduce the patient to the personnel who will be caring for them and their new roommates

• Orient the patient to the new room

• Assist the patient into the bed or a comfortable chair, attach the signal cord within easy reach, and make sure the patient is comfortable before leaving.

After transferring the patient in the new unit

• Return any wheelchair or stretcher used to transport the patient to the proper place

• Clean the patient's room

• Report to the nursing supervisor when the room is ready for another patient.9,10

Discharge or dismissal from the hospital means the departure of pt from the hospital.2

Purposes of discharge:

• To be certain that the pt has information about his/ her condition, follow up visits or referral to other health care agencies

Discharge:

• To provide for a safe, efficient return of all the patients clothing, valuables and to check all the hospital equipment clothing stay in the hospital

• To assist the pt to manage successfully the change from the hospital environment to home environment

• Provide for continuity of care at home.2,3

Types of discharge:

Pt is discharged from the ward as follow

1. Cured and discharged when the treatment of the pt is over from the hospital

2. LAMA: Leave against medical advice,(due to any personal reason of the pt)

3. DOR: Discharge on request

4. Absconded: Leaving hospital without any prior information

5. Transferred to another hospital i.e refer the patient to other hospital for further treatment

6. Death: Pt may expire during hospitalization.3

Discharge planning:

• Discharge preparation

• Physical Consideration

• Psycho-cognitive consideration

• Family Consideration

Steps for discharging a patient:

• Make sure that there is written instruction for discharge and follow up description

• Make sure that the family and the patient understand the instruction for care.(diet, medication, exercise)

• If the pt or relatives decide to leave the hospital against advise of his doctor, leave him against LAMA

• Assist the pt to dress.

- Collect the discharge slip and prescriptions that the pt is to take with him for follow up care
- Complete the pt's record and discharge summary
- Transport the pt's record and his belongings via a wheel chair.
- Care of the unit after discharge.6

Role of Nurse in Discharge Procedure:

- No pt should be discharged without the doctor's written order. The physician writes on the pt's chart when the pt is to be discharged.
- Instructions regarding further care, medication, treatment, follow up etc should be clearly written and interpreted to the pt and his family members.
- Provide the pt with the medications or direct him to purchase what is needed for him.
- Entry of the patient should be there in discharge register with identification data, DOD and remarks.
- Any of the hospital property that was given to the patient for his use in the hospital should be checked and received back before he leaves. The articles in the patient's unit should be checked and see that they are complete, including the bed linens.
- Before the patient leaves the hospital, the nurse should confirm whether he has paid all the hospital bills.
- If the patient is not able to walk or not allowed to walk, the nurse should see that he is safely transferred either on wheel chair or on stretcher.
- The dietary department should be informed of the patient discharge.
- If the patient leaves the hospital against the medical advice he should be ask to sign release form which stated that neither hospital nor the doctors are responsible for any ill effect happening after his departure. This form will be filled with patients records.1,2

Medico-Legal Case (MLC):

A patient who is admitted to the hospital with some unnatural pathology and has to be taken care of in concurrence with the police and/or court like1

- Road traffic accidents
- Injuries inflicted during fights
- Suicide
- Burns
- Poisoning
- Rape victim1,2

Role of Nurse in an MLC during admission:

- Obtain complete history from patient or significant others
- Inform the police on duty in the hospital and the CMO
- When it is made an MLC, record it on the patient's file with red ink on the right hand top corner
- Do not give any statement about patient's condition to police, magistrate or media
- Only a doctor is authorised to give information3

Role of Nurse in an MLC during discharge:

- When a patient has to be discharged, inform the police on duty in the hospital and the CMO
- Discharge only after their clearance
- If an MLC patient absconds, inform the CMO immediately and the treating doctor
- No MLC patient can go with LAMA
- Document the care given to patients timely, accurately and duly sign the nurses' notes
- Records and documents pertaining to the treatment of the patient has to be stored safely and should be handed over to the authorized person as designated by the hospital authority
- In case of death of an MLC, the body is not to be handed over to the relatives
- It needs to be accurately labelled and sent to the mortuary

• CMO and/or police officer should be informed simultaneously.4

Conclusion

Standardization of admission and discharge processes are largely in our control. There is a significant opportunity to create important benefits for increasing bed capacity and hospital throughput.

Conflict of Interest: NIL

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Descriptive Study on Emotional Intelligence Amidst Nursing Undergraduates of Choosed Nursing Institute

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Abstract

Background: Emotional intelligence is that portion of the human soul which persuades people to perform. Emotional intelligence may accommodate student nurses while managing their personal and patients' emotions, manifesting genuine emotional reaction, while being empathetic and effective communicator undergraduate nurse determine effective way to visualize emotions without introducing conflict of interest. It also helps in dealing with instinctive feelings, such as anger and dissatisfaction, in a nurse–patient relationship.

Objectives: Researchers have set the objectives of Computing the level of emotional intelligence among nursing undergraduates, and to find out the Correlation among different components of emotional intelligence tool.

Methodology: Researchers have used Descriptive research design, 179 Nursing Undergraduates of one nursing Institute, have been recruited as a sample with Consecutive non probability technique. Researchers have used standardized schutte self reported emotional intelligence tool in the form of 5 point likert scale,

Results: Researchers have identify that 130 sample (72.63%) have average emotional intelligence, 25 sample (13.97%) have good emotional intelligence, 24 sample (13.41%) have poor emotional intelligence. Mean distribution suggests that perception of emotions has mean of 35.13, managing own emotions mean is 35.21, managing others emotions mean is 31.41, Utilization of emotions mean is 24.55.

Conclusion: It also reciprocates that each of the components of emotional intelligence tool have positive correlation with each other components. Thus Researchers have concluded that to boost the emotional stability an individual should emphasize their own self. This may also lead in positive social change, reducing emotional conflicts, resulting in high productivity.

***Key words**: Descriptive Study; Emotional Intelligence; Nursing Undergraduates; Selected Nursing Institute.*

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Introduction

Nursing is defined as a most kind profession full of knowledge, compassion, and skill. Nurses are the heart of the hospital, where they have to deal with copious obstacles in terms of physical, mental, spiritual challenges[1]. Emotion is a great component to be followed and managed by a nurse and nursing students as well, during all type of challenges nursing students who is about to enter in a nursing professional

field need to acquire skill to manage all sort of situation being emotionally stable. Nursing students have to evolve Intrapersonal relationship, nursing students are competent enough with technical and critical thinking skills, however it is as salient to nurture one's self where they need to burgeon soft people skills to communicate with people, this soft people skill is known as emotional intelligence.[2]

Nursing profession is highly engaged with individual's health, as a result of rapidly growing demands and need of a patient, it leads in high expectation by patients to be met during the hospital care. Nursing freshman seek to manage plethora of situation where they have to take many critical decision while maintaining several emotional crises, scholars are expected to not only improvised their IQ, however EI (Emotional Intelligence) also plays an important role[3]. With this motto researchers have taken this initiations to discover the level of "Emotional Intelligence" of undergraduate nursing students studying in *one nursing Institute* . This is the first phase followed by the creating crash course for EI and implementation of crash course in order to compute the effectiveness of crash course on emotional intelligence.

Objectives:

1. Compute the level of emotional intelligence among nursing undergraduates.

2. Correlation among various components of "Emotional Intelligence" tool.

Assumptions:

Researchers assume that student nurse may have different level of emotional intelligence.

Research Questions:

Are there any contrasts of emotional intelligence among various nursing undergraduates of different years?

Methodology

Research Design **:** Descriptive research design

Setting **:** One Institute of Nursing, Gujarat

Subject : Nursing Undergraduates

Sampling techniques : Consecutive non probability technique

Sample size : 179 sample

Variables : Demographic variable: Age in Years, Gender, Religion, Frequency of backlog, Program in which student study, Father's Education, Mother's Education.

Research variable : Emotional Intelligence

Explanation of the tool: Tool comprise of 2 section:

Section 1: Deals with 7 demographic variables. It is in the form of Semi structured

Section 2: This is a standardized schutte self reported emotional intelligence tool in the form of 5 point likert scale deals with 33 items to determine emotional intelligence categorized in 4 components "Perception of emotions"; "Managing own emotions"; "Managing others emotions"; "Utilization of emotions". Researchers have taken written permission from the original auther to use this tool.

Statistics: Descriptive statistics: mean, median, SD, frequency distribution

Inferential statistics: correlation

Result

The collected statistics were edited, tabulated, analyzed, interpreted furthermore finding were presented in the shape of tables and graphs representing in the following areas.

Segment I: Plotting data Frequency and Percentage data distribution of Population characteristics Variable

Segment II : Data Frequency and percentage data distribution of Emotional Intelligence score

Segment III: Correlation between various components of emotional intelligence

SEGMENT I: PLOTTING DATA FREQUENCY AND PERCENTAGE DATA DISTRIBUTION OF POPULATION CHARACTERISTICS VARIABLE

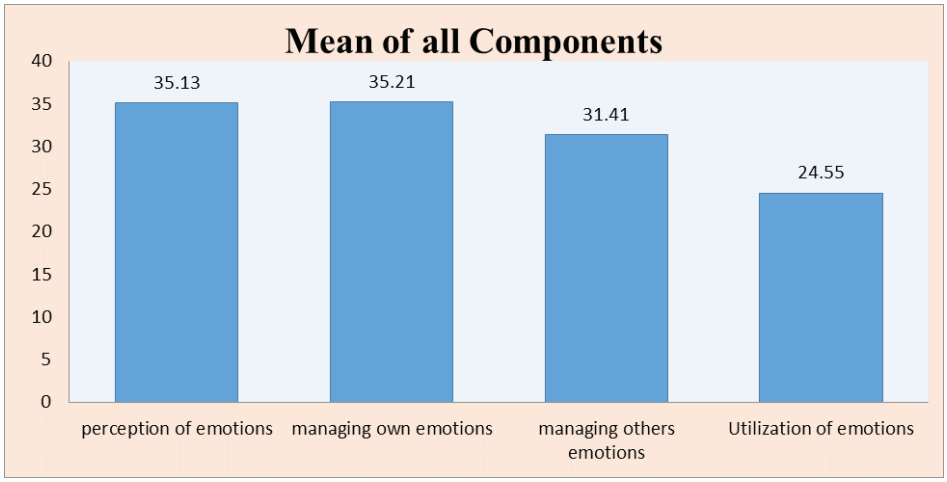
This section reveals data that 98 sample (54.75%) are having age group between 19 to 20 years, 41 (22.91%) sample are having age group of 21 to 22 years, 39 (21.79) sample are having age group of 17 to 18, only 1 (0.56%) is in age group of 23 to 24 years, non of them are in age group 25 and more than that. For gender 162 (90.5%)sample are female, where as 17 sample (9.50%) are Male. For religion 170 sample (162%) are Hindu, 8 sample(4.47%) are Christian, only 1 sample (0.56%) is Muslim. For the frequency of Backlog 167 sample (93.30%) are not having any backlog, 8 sample (4.47%) are having 1 backlog, 3 sample (1.68%) are having 2 backlog, 1 sample (0.56%) is having 3 backlog. For the year of a study 50 students (27.93% are in the first year B.Sc. Nursing, 48 sample (26.82%) are in second year B.Sc. Nursing, 46 sample (25.70%) are in third year B.Sc. Nursing, 35 sample (19.55%) are in the fourth year B.Sc. Nursing. For father's education 91 sample's (50.84%) fathers have bachelor degree, 55 sample's (30.73%) have studied till high secondary school, 33 sample's (18.44%) fathers have studied till primary school. For mother's education 77 sample's (43.02%) mothers have bachelor degree, 61 sample's (34.08%) mothers have studied till high secondary school, 41 sample's (22.91%) mothers have studied till primary school. For diet 143 sample (79.89%) have vegetarian diet, 36 sample (20.11%) have non vegetarian diet. For no. of birth order 125 samples (69.83%) are first child, 45 sample (25.14%) are second child, 9 sample (5.03%) are having birth order of third and above. 164 sample (91.62%) sample live with their both the parents, 9 sample (5.03%) sample stays with only mother, 5 sample (2.79%) live with their guardian, only 1 sample(0.56%) lives with father only.

SEGMENT II : "DATA FREQUENCY AND PERCENTAGE DATA DISTRIBUTION OF EMOTIONAL INTELLIGENCE SCORE"

Table 1 : Data Frequency and Percentagedata distribution of Emotional Intelligence score

Class Interval		Frequency	Percentage
Poor emotional	33 −118	24	13.41%
Average	119−139	130	72.63%
Good	140 −165	25	13.97%

Inference: Above table suggests that 130 sample (72.63%) have average emotional intelligence, 25 sample (13.97%) have good emotional intelligence, 24 sample (13.41%) have poor emotional intelligence



Graph 1: Mean of al components of Emotional Intelligence

Inference: Above mentioned graph suggests that perception of emotions has mean of 35.13, managing own emotions mean is 35.21, managing others emotions mean is 31.41, Utilization of emotions mean is 24.55.

SEGMENT III: CORRELATION AMONGST VARIOUS COMPONENTS OF EMOTIONAL INTELLIGENCE

Table 2: Correlation between various components of emotional intelligence

Components		r value	Significance
"Perception of emotions"	"Managing own emotions"	0.59	Strong Positive correlation
"Perception of emotions"	"Managing others emotions"	0.46	Strong Positive correlation
"Perception of emotions"	"Utilization of emotions"	0.35	Weak Positive correlation
"Managing own emotions"	"Managing others emotions"	0.59	Strong Positive correlation
"Managing own emotions"	"Utilization of emotions"	0.49	Strong Positive correlation
'Managing others motions"	"Utilization of emotions"	0.5	Strong Positive correlation

Inference: Above mentioned table reciprocates that each of the components of emotional intelligence have positive correlation with each other components

Discussion

There is a significant footprint of review manifest that private sector nurse indulge in High Emotional intelligence rather than government sector.[4] This implicates that there may be a good exposure and freedom regarding occupational, a qualitative work experience may also resemble significant improvement in emotional intelligence, thus it would be a life saving areas of interest if Emotional intelligence will be reckoned in a nursing curriculum. With this computation a nurse will develop an exemplary skill, communication and stability in personal as well as occupational life by upbringing self esteem and outreaching to a grand success. Researchers have identify that table suggests that 130 sample (72.63%) have average emotional intelligence, 25 sample (13.97%) have good emotional intelligence, 24 sample (13.41%) have poor emotional intelligence. Mean distribution suggests that perception of emotions has mean of 35.13, managing own emotions mean is 35.21, managing others emotions mean is 31.41, Utilization of emotions mean is 24.55. It also reciprocates that each of those components of emotional intelligence have positive correlation with each other components.

Conclusion

To the best of our comprehension, this literature review implicates the relationship amongst emotional intelligence and nursing fraternities. With specific association to all above mentioned studies we researchers have come up with the serious note of discussion. These Review divulge plethora of facts, these facts are fascinating and interesting. This study review that there is a different score of emotional intelligence among nursing undergraduates of one nursing institute. Researchers believe that to boost the emotional stability an individual should emphasize their own self. This may also lead in positive social change, reducing emotional conflicts, resulting in high productivity.

Recommendations:

The researchers endorse that there must be a crash course on emotional intelligence for nursing students in Nursing Syllabus, with this aim researchers have undergone with certified course on EI and have become life time coach of EI, very soon researchers will introduce this course among Nursing students of their institute during a second phase of a study.

Limitations:

· This research study is limited to one nursing Institute's Nursing Student's only.

· This study doesn't show any relationship between demographic variables and score of emotional intelligence.

Ethical Consideration: Inform consent was obtained from the sample who have participated in research study in electronic form. Confidentiality of all sample's information is maintained properly.

Conflict of Interest: There is no any means of conflict of interest within the researchers

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Role of Cardiac Troponin I in Case of Death Time Estimation- An in Vitro Study

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Abstract

Background- Beta blockers are used for treating hypertension but few of them when taken in overdose can be fatal. Acebutolol is one such beta-adrenoreceptor blocking agent that is considered one of the most toxic beta blockers when taking overdose. Determination of postmortem interval is a common practice in medicolegal investigation.

Objective: The present study was conducted to investigate the degradation pattern of cTnI with respect to time.

Materials And Methods: Group 1 were anesthetized with Sodium pentobarbital (40 IP). The chest cavity was then quickly opened, and the heart was excised out. Group 2 (Asphxial Death) were sacrificed by clamping a tracheostomy tube. Group 3 (Acebutolol Death) were sacrificed by giving an oral lethal dose of Acebutolol(6620mg/kg). Incubation at 28º C for different time interval (0 hrs, 6 hrs, 12 hrs, 24hrs, 48 hrs, 72 hrs, 96 hrs and 100 hrs). The heart was then isolated and freezed at-80ºC.The extraction of protein from tissue was optimized so as to stop or minimize the degradation of protein during the extraction process. The conventional method was used for extraction with slight modifications in the extraction buffer and an added step of carrying every step of the extraction process at low temperature (1-4ºC)

Results- The data clearly established that the degradation pattern of cTnI is different for all the three groups- Control, Acebutolol and Asphyxia. In case of Acebutolol the degradation of cTnI into its molecular fragments is at a much faster rate when compared to the Control and Asphyxia groups, The difference in degradation of cTnI is because Acebutolol is highly cardiotoxic, and its effect on cardiac tissues is high and immediate.

Conclusion- The degradation of intact cTnI into smaller molecular fragments is also dependent upon temperature. In order to know more about the measurement of cTnI fragmentation for the determination of the postmortem interval, further investigations are necessary to understand more about the cTnI degradation pattern.

Keywords- *Acebutolol, Cardiotoxicity, Asphyxia, Beta-Adrenoreceptor Blocking, Cardiac Troponin (CTn)*

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Introduction

In developing countries, population explosion, poverty, increasing stress and strain in daily life, often lead to cases of suicides, homicides, and accidents. With urbanization, rural areas are also not left aloof and this can be seen from the increasing incidence of suicides as

well as of homicides.[1] A large number of suicides are encountered due to asphyxia death. The word Asphyxia was derived from the Greek and means 'pulseless'. It is a condition of the severely deficient supply of oxygen to the body that arises from being unable to breathe normally. When a person is subjected to the asphyxia, unconsciousness generally occurs in 2 - 3 mins, and death in 4 5 mins.[2,3] However, it is now recognized that these are in no ways sensitive or specific for asphyxia. Thus, as there are no diagnostic pathological features of acute asphyxia, autopsies may have negative or non-specific findings that might only suggest the possibility.

Beta blockers are used for treating hypertension but few of them when taken in overdose can be fatal. Acebutolol is one such beta-adrenoreceptor blocking agent that is considered one of the most toxic beta blockers when taking overdose.[2,3]

The cardiac troponin (cTn) has been known as a marker of heart damage and myocardial cell death for more than 10 years.[3] In toxicological studies Troponin has been established as a biomarker for drug-induced cardiac injury. Previous studies have suggested the possible application of cTnI in the post-mortem diagnosis of acute myocardial infarction, cardiac contusions and for the estimation of post-mortem interval.[4-9] TnI is present both in skeletal as well as cardiac muscles. But, since heart is a well-protected organ, the effect of external conditions is less as compared to the skeletal muscles. Therefore, cTnI was chosen as the material of study. It undergoes degradation with time which proceeds in an orderly manner leading to the appearance of a wide diversity of short fragments of it. But due to the lack of data and standardized procedures a general agreement has not been established to use cardiac troponin in forensic case work. Techniques based on biochemical changes offers advantage over physical methods since they are neither altered nor contaminated easily and rapidly.

So, the present study was conducted to investigate the degradation pattern of cTnI with respect to time. An analysis of cardiac troponin I with regard to the cause of death (Cardio toxicity due to Acebutolol, Asphyxia, and control) was studied.

Materials and Methods

This was a comparative analytical trial conducted on total 33 cases, in the time period of 4 months from August 2019 to December 2019.

Subjects were divided into three groups, Group 1 (Control, n = 11), Group 2 (Asphyxia, n=11), Group 3 (Acebutolol, n=11). The technique involved in the study for the detection was Western blot, which could be a technique to detect the expression of a selected protein in tissue homogenates.

Chemicals used in the study for sample buffer- 0.125 MTrisHCl (pH6.8), 4% (w/v) SDS, 20% (v/v) Glycerol, 10 % (v/v) β Mercaptoethanol, 0.01% (w/v) acid-base indicator, for running buffer- 250 mMTris, 1.92M Glycine, 1% SDS, for polyacrylamide gel Solution- 30% Acrylamide, 10% SDS, 10% APS, TEMED, 1.5MTris (pH 8.8), H2O. Armamentarium used in the study included Homogenizer: RQ127 (Remi Motors, India), Ultra High Speed Centrifugation machine: Remi Motors, India, Blotting Kit: C.B.S. Scientific Company, 300VPowerPac: C.B.S. Scientific Company.

Group 1 were anesthetized with Sodium pentobarbital (40 IP). The chest cavity was then quickly opened, and the heart was excised out. Group 2 (Asphyxial Death) were sacrificed by clamping a tracheostomy tube. Group 3 (Acebutolol Death) were sacrificed by giving an oral lethal dose of Acebutolol (6620 mg/kg). Incubation at 28º C for different time interval (0 hrs, 6 hrs, 12 hrs, 24hrs, 48 hrs, 72 hrs, 96 hrs and 100 hrs). The heart was then isolated and freezed at-80ºC.The extraction of protein from tissue was optimized so as to stop or minimize the degradation of protein during the extraction process. The conventional method was used for extraction with slight modifications in the extraction buffer and an added step of carrying every step of the extraction process at low temperature (1-4ºC). Frozen myocardial samples were cut into small pieces in sample trays placed on dry ice. 1 g of cardiac tissue was homogenized with 5 ml of ice-cold extraction buffer (10 mm sodium phosphate, 100 mm Tries, 200 mm Sodium chloride, 0.1% sodium azide, 1 tablet in 50 ml of Complete Protease Inhibitor cocktail, ph8.0). Samples are then centrifuged at 5000 g for 10 min. The supernatant was collected for analysis. The proteins migrate in response to an electrical field through pores in the gel matrix. The combination of gel

pore size and protein charge, size and shape determines the migration rate of a protein. Proteins separated using SDS-PAGE before are transferred to a membrane where they're probed by antibodies specific to the target protein Following a blocking step to stop any nonspecific binding of antibodies to the surface of the membrane, the membrane is probed with a primary antibody that attaches to the antigen of interest. After a washing step, the membrane is incubated with a secondary antibody that's reactive toward the first antibody. After probing with secondary antibody, the membrane is washed again and incubated with an appropriate enzyme substrate. one amongst the substrates used for protein detection is luminol-based and produce a chemiluminescent signal. Chemiluminescence could be a reaction that produces energy released within the kind of light within the presence of peroxidase and a peroxide buffer. The intensity of the signal after development should correlate with the abundance of the antigen on the membrane. Ethical clearance was obtained from the moral committee of the institute. Statistical analysis was done by Oneway ANOVA on the three groups.

Results

The data clearly established that the degradation pattern of cTnI is different for all the three groups- Control, Acebutolol and Asphyxia. In case of Acebutolol the degradation of cTnI into its molecular fragments is at a much faster rate when compared to the Control and Asphyxia groups, The difference in degradation of cTnI is because Acebutolol is highly cardiotoxic, and its effect on cardiac tissues is high and immediate. The myocardial damage is maximum in case of Acebutolol poisoning and then in case of Asphyxia and then in Control group. The two groups were compared with the control group. It was observed that in case of death due to Acebutolol poisoning the rate at which intact cTnI fragmented into lower molecular weight fragments was high as compared to that of control and asphyxial group (TABLE-1)

A one-way ANOVA was used to test for preference differences among the three groups. The null hypothesis was that no difference exists between the populations. The molecular weights differed significantly across the three. The F value is greater than the F critical value and thus the null hypothesis (H0) is rejected (TABLE-2)

TABLE 1: COMPARISON OF DECREASE IN MOLECULAR WEIGHTS OF cTnI WITH RESPECT TO TIME IN VARIOUS GROUPS OF STUDY

HOURS	CONTROL	ACEBUTOLOL	ASPHYXIA
0	23	24	23
6	23	15.4	21.4
12	22.9	13.4	19.1
24	21.8	12.8	17.1
48	16.9	9.8	15.4
72	14.8	7.5	12.4
96	7.16	6.2	7.11

TABLE 2: STATISTICAL ANALYSIS OF CHANGE IN MOLECULAR WEIGHTS WITH RESPECT TO POSTMORTEM INTERVAL IN THE THREE GROUPS OF STUDY

GROUPS	COUNT	SUM	AVERAGE	VARIANCE
Control	8	132	18.57122868	38.62914
Acebutolol	8	90.8	12.82837152	40.04904
Asphyxia	8	116.6	16.6	35.14

Cont... **TABLE 2: STATISTICAL ANALYSIS OF CHANGE IN MOLECULAR WEIGHTS WITH RESPECT TO POSTMORTEM INTERVAL IN THE THREE GROUPS OF STUDY**

		ANOVA			
Source of Variation	SS	do	F	P-value	F crit
Between Groups	122.0781953	260.5380464	1.49473	0.220320	3.454456
Within Groups	683.168471 6	16	37.95834506		
Total	804.345665 5	22			

Discussion

A qualitative approach was followed to determine whether the degradation of cTnI relies upon the rationale for death. Rate of degradation of cTnI into lower mass fragments with time within the three different cases and was observed that just just in case of death thanks to Acebutolol poisoning, the intact cTnI fragmented at a far faster rate than up to hurry and Asphyxia group. Thus, the speed of fragmentation of intact cTnI into lower mass fragments depended upon the rationale for death. An increasing death rate as results of violence constitutes an oversized group in medico legal autopsies. During the 21-year period, there have been 134 asphyxial deaths autopsied by the Department of medication which comprise 15.7% of all forensic autopsies; with 20.8% of the cases are aged between 30 and 39 years. Although it absolutely was varying per the methods of asphyxiation, suicide was found to be the design of death within the bulk of the cases.[10] during a study, the peripheral levels of cardiac troponin T (cTnT) in serial medico legal autopsy cases with a survival time <24 h and within 48 h post-mortem to assess the validity of investigating myocardial damage with special relevance traumatic causes of death. These included blunt and sharp instrument injury (n=122 and 21, respectively), asphyxiation (n=35), drowning (n=27), fire fatalities (n=94), hyperthermia (n=13), hypothermia (n=6), fatal methamphetamine (MA) abuse (n=12) and monoxide gas (CO) poisoning (n=5) as compared with infarction (MI, n=57) and cerebrovascular diseases (n=13). Cases within 12 h post-mortem usually showed lower cardiac and pericardial cTnT levels than did those of longer post-mortem time of 12–48 h. within the first post-mortem period of <12 h, significantly elevated serum cTnT levels were observed for hyperthermia.7 just in case of Control group the foremost bands of size 23, 23, 22.9, 21.8, 16.9, 14.8 and 7.16 kDa were observed at 0, 6, 12, 24, 48, 72 and 96 hrs respectively. just in case of Acebutolol group the foremost bands of size 24, 15.9, 13.4, 12.8, 9.8, 7.5 and 6.2 kDa were observed at 0, 6, 12, 24, 48, 72 and 96 hrs respectively. Just in case of Asphyxia group the foremost bands were of size 23, 21.4, 19.1, 17.1, 15.4, 12.4 and 7.11 kDa were observed at 0, 6, 12, 24, 48, 72 and 96 hrs respectively. Cardiac troponin T (cTnT) and troponin I (cTnI) became acknowledged as useful biochemical markers of drug-induced cardio toxicity. During this study we examined the discharge kinetics of cTnT and cTnI using an in vitro model of isolated rat neonatal ventricular cardiomyocytes (NVCM, 72h treatment with 0.1-3 microM of daunorubicin) and compared it with data from a rabbit model of chronic anthracycline-induced cardiomyopathy in vivo (3mg/kg of daunorubicin weekly, 10 weeks). In cell-culture media, the cTnI and cTnT concentrations were concentration- and time-dependently increasing in response to daunorubicin exposure and were negatively exponentially related to cardiomyocyte viability. With 3microM daunorubicin, the relative increase of AUC of cTnT and cTnI was 2.4- and 5.3-fold over the increase of LDH activity, respectively. In rabbits, the daunorubicin-induced cardiomyopathy was associated with progressive increase of both cTnT and cTnI. Although the correlation between cTnT and cTnI cumulative release (AUCs) was found (R=0.81; P<0.01) and both cardiac troponins corresponded well with the echocardiographically-assessed systolic dysfunction (R=0.83 and 0.81 for cTnT and cTnI, respectively; P<0.001), the first significant increase in cTnI levels was observed earlier (at a cumulative daunorubicin dose of 200mg/m(2)) than with

cTnT (350mg/m(2)). finally, our study has confirmed cTnT and cTnI as very sensitive and specific markers of anthracycline-induced cardiotoxicity.[8] during a study we examined the discharge kinetics of cTnT and cTnI using an in vitro model of isolated rat neonatal ventricular cardiomyocytes (NVCM, 72h treatment with 0.1-3 microM of daunorubicin) and compared it with data from a rabbit model of chronic anthracycline-induced cardiomyopathy in vivo (3mg/kg of daunorubicin weekly, 10 weeks). In cell-culture media, the cTnI and cTnT concentrations were concentration- and time-dependently increasing in response to daunorubicin exposure and were negatively exponentially related to cardiomyocyte viability. With 3microM daunorubicin, the relative increase of AUC of cTnT and cTnI was 2.4- and 5.3- fold over the increase of LDH activity; respectively.[6] The info clearly established that the degradation pattern of cTnI is different for all the three groups- Control, Acebutolol and the Asphyxia. Just in case of Acebutolol the degradation of cTnI into its molecular fragments is at a far faster rate when put next to the Control and Asphyxia groups. The difference in degradation of cTnI is because Acebutolol is extremely cardiotoxic, and its effect on cardiac tissues is high and immediate. The myocardial damage is maximum {in case just in case} of Acebutolol poisoning then in case of Asphyxia then up to hurry group. The degradation of cTnI relies upon the severity of myocardial damage at the time of death. The 2 groups were compared with the control group. it absolutely was observed that just in case of death thanks to Acebutolol poisoning the speed at which intact cTnI fragmented into lower mass fragments was high as compared thereto of control and asphyxial group. A one-way ANOVA was accustomed test for preference differences among the three groups. The null hypothesis was that no difference exists between the populations.

Conclusion

The degradation pattern of cardiac troponin I can be used for determining post-mortem interval and its rate of degradation is dependent upon the cause of death which can be used for establishing the cause of death. It was also evident in our study that the degradation of intact cTnI into smaller molecular fragments is also dependent upon temperature. In order to know more about the measurement of cTnI fragmentation for the determination of the post-mortem interval, further investigations are necessary to understand more about the cTnI degradation pattern.

Conflicts of Interest: The author declare that there is no conflict of interest regarding the publication of this paper.

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Ethical Clearance: Ethical clearance has been taken from Institutional Ethical Committee

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Estimation of Stature from Head Length in Medical Students

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Abstract

Background: Identification of an individual, living or dead is of paramount importance in forensic practice, especially when dismembered body parts or skeletal remains are received for examination. Estimation of stature is one of the important criteria in terms to determine the identity of individual. Many studies have revealed the relationship between stature and some parts of the skeleton and the body parts in consideration. This study will helpful when only skull is available for medico-legal examination. **Aim:** To estimate stature of person from head length. **Material and Methods:** A cross sectional study was conducted in Krishna Institute of Medical Sciences for a period of 2 months from April to May 2018. Data was collected by measuring stature using stadiometer and head length using spreading calliper in 200 medical undergraduate students (100 males and 100 females). **Results:** There is low statistically significant correlation between head length and stature (r=0.25 for males and r=0.22 for females). **Conclusion:** Accurate stature estimation is not possible from percutaneous head length.

Keywords: *Stature, head length, correlation, regression, identification.*

Introduction

Identification of an individual, living or dead is of paramount importance in forensic practice, especially when dismembered body parts or skeletal remains are received in mass disaster situation like plane crash, earthquake, tsunami and road traffic accidents for examination.[1] In such situations, determining stature, age, sex and race are important to identify the individual.[2] Stature is one of the most important features in identifying an individual.[3] It is determined by a combination of environmental and genetic factors.[4]

Stature is defined as "natural height of a person in standing position". It varies at different times of the day by one and half to centimetres. It is less in evening and afternoon due to reduced elasticity of the inter-vertebral disc and longitudinal vertebral muscle.[5] It increases progressively and becomes maximum at the age between 21-25 years. Later, for every 25 years, it is shortened by 2.5 cm due to thinning of intervertebral discs and some stooping posture as a result of decreased tone of muscles.[6]

There is biological relationship between stature and every part of human body which helps to estimate the stature from mutilated and dismembered body parts.[7, 8] There is no derived formula which can be applied universally to estimate stature from different body parts because the relationship between stature and different body parts vary according to age, sex, ethnicity and race.[9]

Estimation of stature from head length helps to identify an individual especially when only head/skull of the deceased is available.[10] The present study was attempted to find the correlation between head length and stature and estimate stature from head length among medical students.

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Materials and Methods

A cross sectional study was conducted for a period of 2 months from April 2018 to May 2018 in Department of Forensic Medicine and Toxicology, on 100 male and 100 female medical undergraduate students who are born and bought up in different parts of India and of the age group of 18 to 24years. Medical student with history of accident, head injury, congenital deformity, hormonal imbalance, kyphosis, scoliosis were excluded from this study. Sample size was calculated by assuming correlation between stature and head length , r = 0.4, the minimum number of subjects to be included (n) calculated as 84 with 95% confidence and 95% power, thus, 100 males and 100 females were assessed in present study. After obtaining informed consent from students, the height of an individual was measured with help of stadiometer as a distance between vertex and standing platform.[11] Student was standing in anatomical position, with palm facing medially and head in Frankfurt plane. All measurements were taken between 9am to 11am by single observer, repeated thrice and the mean was taken to eliminate diurnal as well as inter-observer variations. All measurements were taken in centimeters up to two decimal points. The head length was measured using spreading caliper from behind in an anatomical standing position, head being in Frankfurt plane.

Data was collected in pre-structured proforma, and analysed using descriptive statistics: correlation coefficient was calculated. Regression equation was formulated to estimate the stature by using software Statistical Package for the Social Science (SPSS) 20**.**

Results

The study consists of 200 healthy subjects consisting of 100 males and 100 females. The most common age was 20 years (males 38% and females 40%) followed by 21 years (males 28% and females 23%) as shown in Table 1.

Table1. Frequency of males and females according to age.

Age (years)	Male		Female	
	Frequency	Percent	Frequency	Percent
18.00	8	8.0	5	5.0
19.00	18	18.0	27	27.0
20.00	38	38.0	40	40.0
21.00	28	28.0	23	23.0
22.00	7	7.0	4	4.0
23.00	1	1.0	1	1.0
Total	100	100	100	100.0

The mean age of males was found to be 20.11±1.072 years, ranging between 18 years and 23 years and mean age of females was found to be 19.97±0.979 years, ranging between 18 years and 23 years (Table 2). The mean length of head of males was found to be 18.34±0.667, ranging between 16.7cm and 20 cm and mean length of head of females was found to be 17.22±0.726, ranging between 15.6 cm and 18.6 cm. The mean stature of males was found to be 173.71±6.259 cm, ranging between 157.5cm and 189 cm and mean stature of females was found to be 158.75±6.578 cm, ranging between 141 cm and 175 cm as shown in Table 2.

Table 2. Descriptive statistics for age, head length and stature.

Parameter		Minimum	Maximum	Mean± Std. Deviation
Age(yr)	Male	18	23	20.11±1.072
	Female	18	23	19.97±0.979
Head length (cm)	Male	16.7	20	18.34±0.667
	Female	15.6	18.6	17.22±0.726
Stature(cm)	Male	157.5	189	173.71±6.259
	Female	141	175	158.75±6.578

Table 3. Regression equations for estimation of stature from head length

	Correlation Coefficient(r)	p value (2-tailed)	Regression Equation	SEE
Male	0.255	0.010	Stature=129.848+2.391x head length	16.811
Female	0.222	0.026	Stature=124.106+2.011x head length	15.380

The present study showed low significant positive correlation between stature and head length for males with correlation coefficient (r) 0.255 which is statistically significant at 0.01 level (p value 0.01) as shown in table 3.

Regression equation for males is calculated as follows –

Stature (in cm) = 129.848+2.391x head length (in cm).

The present study showed low significant positive correlation between stature and head length for females with correlation coefficient (r) 0.229 which is statistically significant at 0.05 level (p value 0.026) as shown in table 3.

The regression equation for females is calculated as follow –

Stature (in cm) = 124.106+2.011x head length (in cm).

Figure 1: Scatter diagram showing correlation between stature and head length in males.

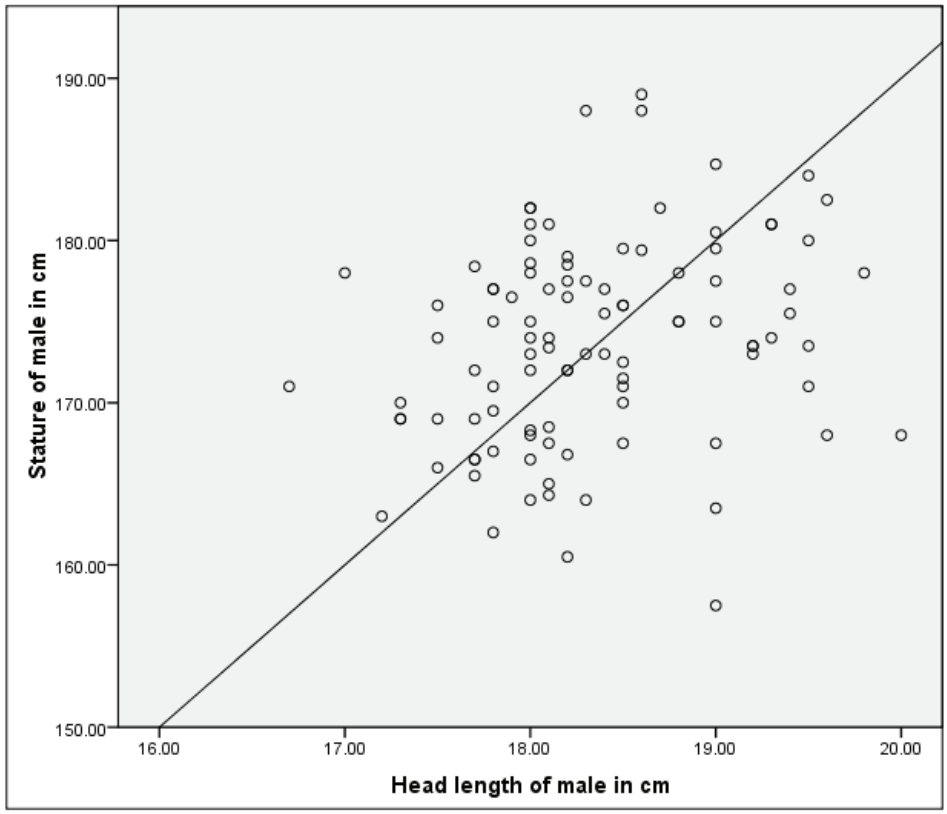
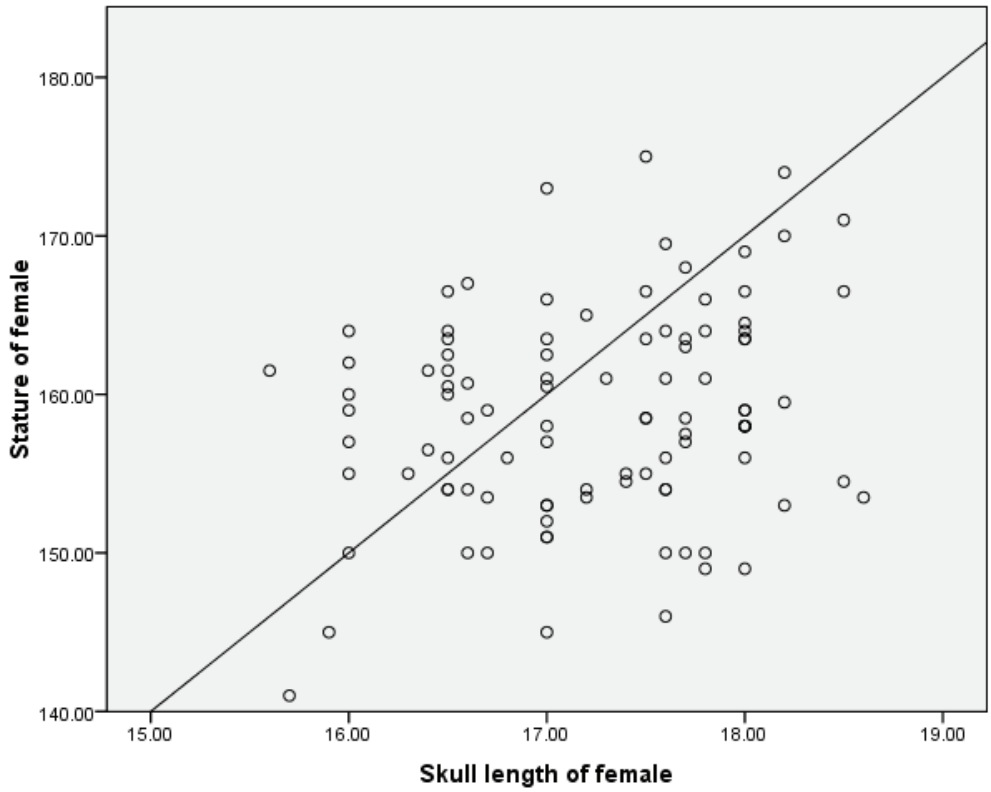


Figure 2: Scatter diagram showing correlation between stature and head length in females.



Discussion:

Identification of the deceased is one of the main aims in forensic practice. It becomes extremely difficult when incomplete skeletal remains or highly decomposed bodies are brought for examination. In such situations, the main aim is to determine the stature, age, sex, and race. Estimation of stature is of great importance in forensic practice especially when head/skull is only available. The regression analysis is considered as the best and the most reliable method for such estimation.[12]

In the present study, it was observed that head length is more for males than females. Similar observation was found in a studies conducted by Kamal R with mean head length 18.43±1.05 cm in males and 17.63±0.86 cm in females, Reddy M with mean head length 19.6±0.9296 cm in males and 18.926±0.644 cm in females, Mani DR with mean head length 20.41±0.68 cm in males and 17.84±0.67 cm in females, Agnihotri AK with mean head length18.66±0.79 cm in males and 18.13±0.91cm in females, Shah RK with mean head length 18.553±0.604 cm in males and 17.841±0.547 cm in females and Rajani Singh with mean head length 17.0±0.7 cm in males and 16.0±0.8 cm in females because the male cranium is bigger than the females.[2,3,4,12-17.]

It was found in the present study that mean stature for males is more compared to females because males are taller than females. This could be due to correlation of Y chromosome with stature and males attain puberty two years later resulting more growth compared to females.[18,19] Similar observations were found in studies conducted by Kamal R (164.63±6.84 cm for males and 151.01±5.4 cm for females), Reddy M (mean stature 169.9±0.571 cm in males and 158.166±0.608 cm in females), Mani R(172.23±16.55 cm for males and 159.69±8.15 cm for females), Manandhar B (169.45±7.06 cm for males and 160.27±10.38 cm for females), Agnihotri AK(173.40±7.70 cm for males and 157.36± 6.17 cm for females), Shah RK (175.94±5.91 cm for males and 161.11±5.62 cm for females), Singh R (167.2±7.8 cm for males and 157.0±6.0 cm for females), Wankhede KP (mean stature 170.97±6.80 cm in males and 156.89±5.89 cm in females).[2-4, 9, 12-14, 20.].

The present study showed that head length is not good indicator for estimation of stature of an individual both in males and females because of low significant positive correlation between stature and head length [correlation coefficient (r) for males 0.255 and females (r) 0.222] which are statistically significant at 0.01 level (p value 0.01) as shown in table 3. It could be due to the fact that the growth of skull (head length) is genetically determined.[12] Similar observations are found in studies conducted by Kamal R [male (r) 0.355 and female (r) 0.182], Agnihotri [male (r) 0.331 and female (r) 0.159], Kokatanur [male (r) 0.299 and female (r) 0.126], Chourasia R [male (r) 0.241 and female (r) 0.173] and Wube B [male 0.422 and female 0.168].[2,12, 17, 21, 22.] In contrast to this, the study conducted by Reddy M showed positive correlation between stature and head length with correlation coefficient (r) for males 0.715 and (r) 0.701 for females, by Manandhar B [(r) 0.706 for males and (r) 0.69 for females] and by Nemade P [(r) 0.745 for males and (r) 0.677 for females][3,9, 23].

The estimation of stature from long bones is better than flat bones because long bones correlate positively with the stature compared to flat bones.[24] But in forensic examination especially when head/skull is only available, head length is good predictor for estimating stature. As the correlation coefficient is considered to be significant only when it is more than 0.5, head length is not good predictor for estimating stature both in males and females because correlation coefficient for males is 0.255 and 0.222 for females which are less than 0.5 as mentioned in table 3.

The regression equations and correlation coefficients vary from one geographical location to another as stature is dependent on multiple factors such as race, nutritional, regional and geographical factors. Regression equations of one population cannot be applied to another population for stature estimation as they are population specific[25].

Conclusion

The current study showed that accurate stature estimation is not possible from percutaneous head length as low significant positive correlation between stature and head length for males as well females was found in this study. Further investigations should be carried out on large study sample.

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Compliance with Ethical standards

Ethical approval: "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethics committee of Krishna Institute of Medical Sciences, Karad."

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Comparative Evaluation of Stress Distribution Around Mandibular Overdentures Surrounded by Three and Four Implants: A Three-Dimensional Finite Element Analysis

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Abstract

Background and objectives: Placement of more than two implants in the interforaminal area may create a greater implant-to-bone contact area that allows for better stress distribution and minimizes crestal bone loss. The purpose of this study was to analyze and compare the stress distribution around implants of the three or four implant-retained mandibular overdenture.

Methodology: Two finite element models comprising an edentulous mandible with three and four implant supported mandibular overdenture with bar and clip attachment was used. A vertical load of 30 pounds was applied bilaterally to the first molar. Maximum and minimum von Mises stress values in the bone around the implants were evaluated.

Results: The highest stress value was observed in the bone around the distal implants and lowest stress value was concentrated in the middle implant in the molar region in three implant supported overdenture. The highest stress value was concentrated in the bone around the distal implants and lowest stress value was concentrated in the anterior implants in four implant supported overdenture. Stress values were more on four implant supported overdenture than three implant supported overdenture.

Conclusion: Within the limitation of the study, three implant supported overdentures demonstrated lesser stress in comparison to the four implant supported overdentures. The stresses generated were higher in the bone around the distal implants and lowest in the middle and anterior implants.

***Key words**: Finite element analysis; overdenture; implant; cantilever; edentulous mandible*

Introduction

The prosthetic management of the completely edentulous patient has long been a major concern. Mandibular implant-retained overdentures are the most effective treatment for edentulous patients. [1-4]

Mandibular overdentures are usually supported by two to five implants between the mental foramina with soft tissue support in the posterior areas[5-7]. It has been concluded that the stress distribution pattern is more uniform when the load is applied over an increasing number of implants[8]. Also, it is useful for retaining and

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stabilizing dentures and has economic benefits.[5,9] More than two implants in the inter-foraminal area may create a greater implant-to-bone contact area allowing better stress distribution and minimizes crestal bone loss.[10]

Another point to be considered is the cantilever length which influence forces transferred to implants and bone and has direct clinical effect on marginal bone loss, so it is important that its effects on stress transfer to be investigated.[11-12]Regardless of the length of the cantilever, the greatest tensions will always be located on the region of the implant closest to the load application point.[13]

Finite element analysis (FEA) has been widely used to investigate the stress and strain distribution at the peri-implant bone interface in edentulous jaws. [2,14-15]Despite limitations of this theoretical method, FEA is acceptable in stress distribution analysis in complicated structures such as human alveolar bone.[16]Studies using FEA has several benefits as against studies with real models. The experiments are repeatable, there are no ethical considerations and the study designs may be modified and changed as per the requirement.[17]

Methodology

In this in vitro study, the experimental designs of simulated three and four implant-retained overdenture models were fabricated using Materialise MIMICS software (Materialise Interactive Medical Image Control System; Materialise, Leuven, Belgium). MIMICS uses the computerized tomography (CT) images to generate a 3Dimensional (3D) point cloud model which was then transferred to CATIA (Computer Aided 3D Interactive Application) software to obtain a 3-D solid model (Figure 1).In the first model, three implants and in the second one, four implants3.75 mm × 10 mm ((Nobel Biocare),were embedded.

In the first model, one implant was placed in the midline of the arch, and the others were placed approximately 12 mm from the midline. In the other model, the implants were placed 8mm apart. In the models, the implants were vertically oriented, perpendicular to the occlusal plane, and parallel to each other. Implants were connected using dolder bar and clip attachments (RHEIN 83 Co-Cr castable type). Two clips were placed in three implant□retained models and three clips in four implant□retained models.

The cantilever bars for each model was considered with 13 mm length. The mucosa and cortical bone were reproduced as a 2 mm and 2.5 mm layer, respectively to simulate D2 type bone[18]. The models were scanned with a profile projector to obtain reverse-engineered sketches. The numerical values obtained from the sketches were put in the CATIA software to create the geometric model of the components.

The overdenture prosthesis was created using a CT image of the denture and MIMICS software. The surface model was then transferred to CATIA software to obtain a 3D solid model of the overdenture. All the geometric models i.e, implant with attachment systems, overdenture prosthesis, and the bone were combined with a Boolean operation to create two 3D working models in the ANSYS Software. The implant-bone interface was assumed to be fully osseointegrated.

Geometric working models were then converted into the finite element model using ANSYS software. Table 1 represents numbers of elements and nodes in all study models. The analysis was performed on a computer with Windows 10/Intel Pentium DProcessor3.00+/CPU: 3.00 GHz/RAM: 32GB.

All materials were assumed linear, elastic, and isotropic; their properties were taken from the literature [13, 19-22][Table 2].The boundary conditions were set as fixed between the implant and the bone. The connection between the attachment system (patrix) and denture was modelled as fixed.

A vertical static load of 30 pounds was applied bilaterally in the central fossa region of the first molar tooth, and load distribution patterns were analysed[23].

Results

Maximum and minimum von Mises stress values were obtained for each model under bilateral vertical loading conditions. Data for von Mises stresses were produced numerically, color-coded and compared among the models. Stress analysis revealed that for three implants with cantilever, highest value of the stress was seen in the bone surrounding the distal implants placed approximately in the canine region. The stress distribution pattern around the middle implant was

different from other regions. Stress distribution patterns in the distal implants were similar. The minimum stresses were concentrated in the middle implant. Maximum stresses in the bone around the implant were: 399.32 MPa and minimum stresses in the bone around the implant: 45.124 MPa.

Stress analysis revealed that for four implants with cantilever maximum stresses were concentrated at the bone surrounding the distal implants placed approximately in the premolar region. The minimum stresses were concentrated in the anterior implants placed approximately in the canine region. The stress distribution pattern around the implants placed approximately at the canine region was different from other regions. Stress distribution patterns in the distal implants were similar.Maximum stresses in the bone around the implant on bilateral vertical loading was: 495.74 MPa and minimum stresses in the bone around the implant on bilateral vertical loading was: 10.476 MPa. The stress distribution in the bone around the four implant supported overdenture with cantilever bar were higher than three implant supported overdenture with cantilever bar.

Table 1: Number of nodes and elements in all meshed components

Modelling situations	Three implants		Four implants	
	Nodes	Elements	Nodes	Elements
13mm cantilever	106235	95968	125986	98936

Table 2: Material properties used in the finite element model

Anatomic structure	Young's modulus (mpa)	Poisson's ratio	References
Cortical bone	13700	0.30	16
Cancellous bone	1370	0.30	16
Cobalt chromium alloy	218000	0.33	64
Overdenture	2700	0.35	63
Implant complex	110000	0.35	62

Discussion

The stress pattern around implants supporting overdentures is considered to be more complicated than in fixed prostheses due to the resiliency of the mucosa and movement of the prosthesis[5, 24-25]. In the present study, the load levels of 30 pounds were selected because they were within the range of normal occlusal mastication force and near the maximum load measured in implant overdenture patients.[5, 24-25]The first molar was selected as maximum bite forces are concentrated in the region where there is maximum contraction of all elevator muscles.[25-26]The vertical bilateral load was applied to simulate the masticatory forces. Also, loads were applied vertically as it was found by many studies that applying vertical loads on the overdenture generate more stresses than do oblique forces.[27]

In the models with three implant supported overdenture, applying 30 pounds load at the molar region increased the induced stresses in the bone around the distal implants and decreased the induced stresses in the bone around the middle implant. Under similar conditions, with four implant supported overdenture, induced stresses were more in the bone around the distal implants and less in the bone around the anterior implants. Cantilever is a class-1 lever, which increases the amount of stress around the implants closest to the force application and induces less stress around the farthest implants from the point of force application[10]. A study by Liu et al showed that in the three-implant-supported overdentures, no strain concentration was found in the cortical bone around the middle implant.[7]

These finding agree with findings of previously done studies[24,28] which concluded that implant retained overdenture associated with bar-clip anchor with two distally placed cantilevers displayed the greatest stress level, although cantilever extensions of bar attachments have been recommended for mandibular implant-retained overdentures to increase denture stability against non-axial loading.

When stress values were considered in implants, maximum stress concentration was seen around the neck of the implant in the present study. This is similar to the results obtained by various other studies which demonstrated that, bone loss begins around the implant neck due to higher bone stresses at the crestal region.[19]

The highest stress generation was noticed when

four implants were used to support the mandibular overdenture. This is because the magnitude of stress distribution is directly proportional to implant number and inter implant distance based on leverage principle. This finding may not be, only related to the implant number but also to the unequal load reaching the distal and anterior implants and the supporting structures as the load was applied bilaterally on the molar region. This may also be due to premature contact between the acrylic denture base with the contra lateral implant. Hence, precise relief of the acrylic denture base around the overdenture abutments can be an important issue, especially when more resilient clips are used for the bar attachment.

Some authors believe that increasing the number of implants can reduce the stresses on the supporting implants.[29] In the triangular arches adding the middle implant may serve as an indirect retainer or as a vertical stop for preventing sitting of the anterior portion of over denture.[29] Therefore, a third implant can be placed between the original two when patients rehabilitated by two-implant overdentures report constant and obvious denture rotation around the fulcrum line.[7] Additionally, the risk of screw loosening is also reduced with a third implant, and the resultant greater surface area of implant to bone allows better distribution of forces and, therefore, minimizes the loss of crestal bone.[10]

Reliable clinical conclusions cannot be drawn from finite element studies as they are theoretical in nature and have some limitations when predicting the response of the biological tissues to applied loads,[19] therefore, reliable clinical conclusions cannot be drawn. It is important to know that peri-implant tissues are complex and their simulation in FEA is approximate.[19] In the current study, it was assumed that the bone-implant interface was completely osseointegrated. This assumption may not reflect the actual clinical situation[17].In addition, it was assumed that all the structures in the model are homogeneous and isotropic and they possess linear elasticity. But in reality living tissues behave differently, they are transversely isotropic and inhomogeneous. The design of occlusal surface also can influence the stress distribution.

Conclusion

Stress values in the bone around the four implant supported overdenture were higher than three implant supported overdenture.In three implant supported overdenture, stress values were higher in the bone around the distal implants and lowest in the middle implant. In four implant supported overdenture with cantilever, stress values were higher in the bone around the distal implants and lowest in the anterior implants.

Ethical Clearance: Obtained from AJIMS ethics committee, Mangalore for the study.

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A Study on Oxygen Consumption in Freshwater Fish *Labeo Rohita* Exposed to Lethal and Sub Lethal Concentrations of Ethion 50%Ec

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Abstract

The pesticide pollution different classes like organochlorines, organophosphates, carbamates and other new generation ones affect the fish oxygen consumption in sublethal concentrations. Respiratory responses to lethal concentrations increase the ventilation volume and symptoms of pesticide intoxication suggesting that the effect on respiratory surface were high in fish. Hence, in the present study an attempt has been made to study the effect of lethal(1.2µg/l) and sub-lethal 1/10(0.12µg/l) LC_{50} and 96hrs of 50% mortality concentrations of Ethion 50% EC an Organophosphate effect on oxygen consumption for 24 h at each 2 hours interval to the Indian major carp, Labeo rohita (Hamilton). Oxygen consumption of aquatic animals is a very sensitive physiological process and therefore, alteration in the respiratory activity is considered as an indicator of stress of animals exposed to pesticides. The significant drop in the rate of oxygen consumption was observed in the present study in lethal and sub-lethal concentrations.

Keywords: *Ethion 50%EC, Labeo rohita, Lethal and sub lethal concentrations, O_2Consumption*

Introduction

Oxygen consumption plays a major role in the physiology of fish and it is also an index of its metabolic rate. Various factors like size, temperature, stocking density, seasonal variation[1] physiological factors and starvation influence oxygen consumption in fish. Besides this, various constituents of medium also affect oxygen consumption and fish absorbs oxygen directly from the water into their blood stream using their gills. Oxygen stress is a common cause of disease outbreaks the amount of oxygen in water decreases as temperature and altitude increases. As temperatures increases, fish metabolism also increase. Hence, they consume more amount of oxygen and the increase in temperature leads to increase fish metabolism causes oxygen depletion in the summer.

Fish have long been valued as an excellent indicator of water pollution and oxygen consumption is a very sensitive physiological process and the change in respiratory activity has been used as an indicator of stress in animal exposed to toxicants. Hypoxia caused significant increase in haemoglobin, haematocrit and mean corpuscular haemoglobin concentration[2,3]. The physiological and behavioral alterations occur in fishes in the absence of sufficient oxygen. The fishes were affected, either directly through uptake from the water, or indirectly through their diet vegetation, invertebrates or smaller fish[4].

The rate of oxygen consumption is considered as a reflection of the total metabolism and metabolic rate of the aquatic organisms. The water current flows around the gills; carries the toxicants directly, before that of all other internal organs. The changes in oxygen uptake is

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widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental stress factors because it reflects the energy expenditure and, ultimately, the food requirements. The changes in the respiratory activity of fish have been used by several investigators as indicators of response to pesticides[5]. The oxygen consumption is a useful measure of sub-lethal effects because energy processes being disturbed are indicators of overall physiological state and of pesticide poisoning leading to respiratory distress which is used to assess the toxic stress.

The O_2 consumption is a very sensitive physiological process and the change in respiratory activity has been used as an indicator of stress in animals exposed to toxicants[6]. The oxygen consumption is not often used as a bioindicators of pollution associated stress in biological early warning systems. Respiratory responses were found to be less sensitive, but also could be successfully used in bioassay testing of treated industrial and municipal effluents, before they are discharged into receiving waters. In the gill epithelium, the tissue will have intracellular and extracellular exchange of ions related to oxygen and chloride shift any xenobiotics chemical having the capacity to alter this structure will affect the oxygen carrying capacity of haemoglobin and reduction in oxygen uptake. The gill damage observed in fish exposed to toxicant was due to impairment in the respiratory metabolism. This is also due to the fact that any pollutant including pesticide should enter into the fish, mainly through the gills only[7].

The metallic pollution and pesticides of different classes like organochlorines, organophosphates, carbamates and other new generation ones affect the fish oxygen consumption in sublethal concentrations. Respiratory responses to lethal concentrations increase the ventilation volume and symptoms of pesticide intoxication suggesting that the effect on respiratory surface were high in fish[8]. Hence, in the present study an attempt has been made to study the effect of sub-lethal and lethal concentrations of Ethion 50% EC on oxygen consumption for 24 h at each 2 hours interval to the Indian major carp, *Labeo rohita* (Hamilton).

Material and Methods

The fish Labeo rohita were brought from local fisheries forms of Kuchipudi, Guntur (DT). The experiment on the oxygen consumption of the fish Labeo rohita was carried out in a respiratory apparatus developed [9]. The fish were acclimatized to the laboratory conditions in well aerated water for 7 days, used in the toxicity experiments (Table. 1). During this period, the fish were regularly fed with add libitum, but the feeding was stopped before two days prior to the experiment. The fish measuring 6 to 7 ± ½ cm in length and 6 to 8 ± ½ gm in weight, all the precautions laid down on recommendations of the toxicity tests to aquatic organisms are followed[10] and the method was used to estimate O_2 levels in lethal and sub lethal concentrations[11].

Results and Discussion

Present study O_2 consumption of fish Labeo rohita exposed to Ethion 50%EC. 0hr in control (0.892), in sublethal (0.880) and lethal concentration was in (0.846) and sublethal (0.952) lethal (8.18) respectively and 6hrs O_2 consumption in control (0.718) and sublethal (1.360) lethal (0.712) i.e sudden change in O_2 consumption levels were observed in the sublethal concentrations to compare with lethal concentration after other hrs the O_2 consumption levels were gradually decreased in all exposure of the fish Labeo rohita.

The whole animal oxygen consumption of control and experimental fish, calculated per gram wet body weight in sub-lethal and lethal concentrations of Pesticide Ethion 50% EC commercial grade for Labeo rohita. The results of the experiments and values are graphically represented by taking time on X-axis and the amount of oxygen consumed per gram body weight on Y-axis, in the fig.

Oxygen consumption measurements provide a robust indicator of whole animal stress and concomitant water quality. Respiratory rate is the basic parameter, and serves as one of the indicators of environmental stress[12]. Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress[13,14]. Oxygen uptake rate would rapidly decreased[15].

In the present study sub-lethal concentrations of Ethion 50% EC; it was observed that fish Labeo rohita showed tendency of increase in oxygen consumption during the initial time of exposures i.e. 1 to 6 hours and a gradual decrease was observed during the subsequent period of study. The sub-lethal concentration of toxicants is inevitable. In such a case, the fish Labeo rohita was more sensitive to toxicant. The toxicant stress in oxygen consumption along with depletion in oxygen in aquaculture practices make them less fit and reduction in growth due to lack of proper metabolism. The fish was in more stress during first hour and later they are showing signs of recovery. That recovery is evident as the toxicant exposure is increased in time, during 24 hrs experiment.

A comparison is made between the effects of lethal concentrations of Ethion 50% EC on Labeo rohita decreased in oxygen consumption was observed. Hence, it has sensitive on toxic stress as a result of more oxygen consumption and the results were compared with other researchers. In controls also, the rate of oxygen consumption was gradually decreased and this can be attributed to the starved conditions and the reduced metabolic rates of the starved fish. In exposed fish, the reduction in oxygen uptake can be correlated to the extent of damage of gill epithelium, throughout the experimental period, the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake, increased mucus secretion, higher ventilation volume and decrease in oxygen uptake efficiency, labored breathing and engulfing of air through the mouth fish Labeo rohita observed exposed to Ethion 50% EC.

The increased oxygen consumption at initial stages of exposure i.e. 6 h This present study is in agreement with[16] dimethoate and carbaryl in which an elevation in oxygen uptake is observed during initial stages of exposure i.e., 1-4 hours followed by decrease in subsequent hours. Oxygen consumption was first stimulated and then inhibited in Labeo rohita exposed to lethal and sublethal concentrations of Ethion 50% EC.

The amount of oxygen consumed in mg/g body weight/hrs of the fish *Labeo rohita* exposed to sub-lethal and lethal concentration of Ethion 50%EC:

Hours	Control	50%ECSub-lethal	% Change	50% EC Lethal	% Change
0	0.892 ±0.006	0.880 ±0.006	1.34	0.874 ±0.005	2.01
2	0.846 ±0.004	0.952 ±0.003	12.52	0.818 ±0.006	3.30
4	0.793 ±0.004	1.118 ±0.006	40.98	0.752 ±0.006	5.17
6	0.778 ±0.006	1.360 ±0.003	74.80	0.712 ±0.005	8.48
8	0.769 ±0.005	0.742 ±0.005	4.56	0.654 ±0.005	14.95
10	0.736 ±0.006	0.698 ±0.004	5.16	0.618 ±0.005	16.03
12	0.729 ±0.003	0.682 ±0.007	6.44	0.595 ±0.003	18.38
14	0.715 ±0.004	0.623 ±0.003	12.86	0.570 ±0.003	20.27
16	0.708 ±0.004	0.547 ±0.003	22.74	0.535 ±0.004	24.43
18	0.789 ±0.003	0.538 ±0.006	31.28	0.496 ±0.003	36.65
20	0.692 ±0.003	0.450 ±0.003	34.97	0.390 ±0.005	43.64
22	0.687 ±0.003	0.415 ±0.004	39.59	0.324 ±0.004	52.83

Values are the mean of five observations: Standard Deviation is indicated as (±)., ***Value are significant at $p < 0.05$***

Gills are the major respiratory organs and all metabolic pathways depend upon the efficiency of the gills for their energy supply and damage to these vital organs causes a chain of destructive events, which ultimately lead to respiratory distress[13]. Pronounced secretion of mucus layer over the gill lamellae has been observed during pesticide stress. Secretion of mucus over the gill curtails the diffusion of oxygen[17], which may ultimately reduce the oxygen uptake by the animal. In the present study, the reduction in oxygen uptake can be correlated to the extent of damage of gill epithelium[18].

The metabolic rate (in relation to respiration) of fish could be increased under chemical stress. [15]the dimethoate is efficiently absorbed across the gill and diffused into the blood stream resulting toxic stress to fish. Similar trend was reported in Channa punctatus[19]. Labeo rohita exposed to permethrin[16]. Similarly earlier works revealed a significant reduction in oxygen consumption by many fish species under the toxic stress of aquatic pollutants such as insecticides[5,20].

Oxygen consumption of aquatic animals is a very sensitive physiological process and therefore, alteration in the respiratory activity is considered as an indicator of stress of animals exposed to pesticides. The significant drop in the rate of oxygen consumption was observed in the present study. That gill architecture was severely affected in the fish Labeo rohita exposed to pesticide. The respiratory metabolism was impaired and damage in the gills of fish exposed to the pesticides[16] . An initial increase in oxygen consumption in sublethal and lethal concentrations followed by a decline in subsequent hours and lethal dosed fish[21]. The decreased rate of oxygen consumption with the increase in the test concentrations of pesticide also consequently decreased metabolic rate.

[21]Observed enhancement in the oxygen consumption rate initially in the fishes of sub-lethal and median lethal exposures to endosulfan might be due to a sudden response of the fish to the impending toxicity of endosulfan However, the declining respiratory rates recorded in the subsequent periods in sub-lethal and median lethal exposures suggest that the fish could not succeed in their attempts of boosting oxidative metabolism. The analysis of data from the present investigation indicates a considerable effect of Ethion 50% EC. The present study revealed alterations in the oxygen consumption of Labeo rohita exposed to sublethal and lethal concentrations probably due to impaired oxidative metabolism and pesticide induced stress.

The rate of oxygen consumption is influenced by several factors such as activity, temperature, body size and stage in life cycle, season, time of the day and genetic background [23,22]. Since most fish breathe water in which they live, changes in the chemical properties might reflected in the animal's ventilator activity, particularly the environment affects respiratory gas exchange. Oxygen consumption is an important parameter to assess the toxicological stress, since it serves as index of energy expanded and speaks of physiological and metabolic state of an organism. Generally toxicants gain entry through food chain or respiratory surfaces, the physiological function affected on oxygen consumption.

The metabolic rate has been measured by determining the oxygen consumption, which provides information on the ability of the fish to extract oxygen from pesticide polluted water. An early symptom of acute pesticide toxicity is respiratory distress[17,24]. Respiratory rate has been recognized as an important indicator of stress in organisms exposed to toxic substances. Toxicants from the environment mainly enter fish by means of their respiratory systems[25]. A mechanism of toxicant uptake through gills probably occurs by simple diffusion through pores and is then absorbed through cell membranes.

Oxygen consumption in lethal and sub lethal concentration indicate the sequence type of compensatory mechanism, which operates within the animal to overcome the load of toxic stress. In the present study the variation in oxygen consumption is an indicator of stress, which frequently used to evaluate the changes in metabolism under environmental deterioration. It is evident from the studies that the Ethion 50% EC fresh water fish L. rohita affected. Oxygen consumption of L. rohita under lethal and sublethal concentrations of pesticide to compared with control. Since most fish breathe in the water in which they live, changes in the chemical properties in it might be reflected in the animal's ventilator activity;

particularly the environment factors affect respiratory gas exchange[26]. The fluctuated response in respiration might attribute to respiratory distress because of the impairment of oxidative metabolism. Disturbance in the oxidative metabolism under cypermethrin toxicity in Cyprinus carpio[25].

Oxygen is essential for many metabolic processes that are vital to aerobic life. This dependence on oxygen, forces aerobic life to with stand considerable toxicity[27]. The oxygen paradox derives from the chemical nature of oxygen, which in its atomic form (0) is a free radical and in its molecular form (O_2) is a free bi-radical[28]. [29] Which have speculated that during oxidative stress, catalase activity decreases, hydrogen peroxide accumulates and thereby more peroxidation of lipids is favored. Contaminated water may contain a wide range of organic and metallic pollutants, including polynuclear aromatic hydrocarbons, organochlorine pesticides (DOT, dieldrin), polychlorinated dibenzo-p-dioxins, dibenzofurans and other heterocyclic, organophosphate fertilizers, estrogenic compounds and many metals. Most of these are powerful oxidants[30].

Conclusion

Oxygen consumption in lethal and sub lethal concentration indicate the sequence type of compensatory mechanism, which operates within the animal to overcome the load of toxic stress. In the present study the variation in oxygen consumption is an indicator of stress, which frequently used to evaluate the changes in metabolism under environmental deterioration. It is evident from the studies that the Ethion 50% EC fresh water fish *L. rohita* affected.

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Conflict of Interest: I declare that, there is no conflict in research.

Source of Funding: Self

Ethical Clearance: In the present study says that the Ethion (50% EC) is effect the aquatic life.

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Effectiveness of Faradism Under Pressure Versus Complex Decongestive Therapy in Subjects with Peripheral Oedema

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Abstract

Background: Oedema is defined as excessive accumulation of free fluid in interstitial tissue spaces and serous cavities. The oedema may be of 2 main types: Localized when a particular organ or limb is involved e.g. lymphatic oedema, inflammatory oedema, allergic oedema and generalized when it is systemic in distribution, particularly seen in the subcutaneous tissues. The gold standard therapy for lymphedema is complex decongestive therapy (CDT). The first stage of CDT includes manual lymphatic drainage (MLD), compression therapy, exercise, and good skin care. The second phase, consisting of self-managing lymphatic massage, daily use of compression garments, and self-directed continuation of the exercises, should be implemented only after the completion of the first phase. Also, Faradism under pressure (FUP) is beneficial in oedematous conditions. In FUP, the pumping action of the alternate muscle contraction and relaxation, brings about increased venous and lymphatic return. The fluid is propelled towards the heart by an inward pressure on the tissue spaces and veins caused by the contraction of the muscle.

Objectives: The objectives of the study were as follows: To determine effect of faradism under pressure in peripheral oedema. To determine effect of complex decongestive therapy. To compare the effect of faradism under pressure and complex decongestive therapy in peripheral oedematous conditions.

Methods: Ethical clearance was obtained from the institutional ethical committee. A total of 20 subjects were assessed and all were included in the study based on inclusion criteria. Oedema was diagnosed using volumetric and girth assessment. **Results**: Intra-group statistical analysis of intervention group, pre-post volumetric measurement score was 33.6 ±9.192 and was found to be extremely significant and pre-post girth assessment score was 2.540 ± 0.9513 which was extremely significant. **Conclusion**: We found that both FUP and CDT were significantly effective in reducing peripheral oedema but CDT was more effective compared to FUP. This treatment was effective by reducing the volumetric and girth measurements which improved quality of life of patients.

Keywords: *Oedema, Faradism under pressure, Complex decongestive therapy*

Introduction

Oedema is defined as excessive accumulation of free fluid in interstitial tissue spaces and serous cavities. When oedema is in the subcutaneous tissues, momentary pressure of finger produces depression which indicates pitting oedema. The other type is non-pitting oedema in which no pitting is produced on pressure e.g. in myxoedema, elephantiasis. The oedema may be of 2 main types: Localized when a particular organ or limb is involved e.g. lymphatic oedema, inflammatory oedema, allergic oedema and generalized when it is systemic in distribution, particularly seen in the subcutaneous tissues.[1,2] Chronic accumulation of oedema in both or single limb indicates venous insufficiency in case of

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dependent oedema and hemosiderin deposition. Skin care is important to prevent skin breakdown and ulcers.[3] Pathogenesis of oedema is based on Starling's rule on capillary fluid dynamics. Starling's rule states that net filtration through capillary membrane is proportional to the hydrostatic pressure difference across the membrane minus the oncotic pressure difference. These pressures are called Starling's forces. These forces are maintained by enhanced sodium reabsorption from renal tubules.[4] Oedema is classified in 4 types depending upon mechanism causing edema : Increased capillary hydrostatic pressure, decreased plasma oncotic pressure, enhanced hydraulic permeability of capillary walls, lymphatic obstruction.[4,5] In increased capillary permeability, capillary endothelium is injured by capillary poisons such as toxins and their products, histamine, anoxia, venoms, certain drugs and chemicals and capillary permeability of plasma proteins is enhanced due to development of gaps in endothelial cells which leads to leakage of plasma proteins into interstitial fluid. So, it causes decrease in plasma oncotic pressure and increase in oncotic pressure of interstitial fluid which leads to formation of oedema.[5.6]

The gold standard therapy for lymphedema is CDT.[7] The quantity of physical pressure plays a vital role in CDT. Microcirculation takes place between blood vessels and tissue, as a function of pressure. In the context of the total lymphatic system, the lymphatic load is transported from the interstitial space to the blood stream through lymphatic vessels. The lymphatic vessel system, distinguished by particular anatomical conditions like initial lymphatic vessels, precollectors, collectors, and lymphatic strains, is the beneficial target of CDT.[8] The first stage of CDT includes MLD, compression therapy, exercise, and good skin care.[7,8] MLD is executed in an upward direction to support the lymphatic flow. It particularly involves the use of circular or spiral movements using small pressures directed towards the nearest groups of lymph nodes, proximal to the drainage area. It is important to start the drainage from the central part and then move to distal parts of the body. The MLD is carried out for 45–60 minutes. Compression therapy rises tissue pressure by encouraging external compressive pressure using bandaging or wearing compression garments. Multi-layer compression bandaging (MCB) uses bandages of alternating degree of compression, additional materials, and support materials, e.g. cotton-wool bandages. The second phase, consisting of self-managing lymphatic massage, daily use of compression garments, and self-directed continuation of the exercises, should be implemented only after the completion of the first phase.[7,9,10]

In FUP, the pumping action of the alternate muscle contraction and relaxation, brings about increased venous and lymphatic return. The fluid is propelled towards the heart by an inward pressure on the tissue spaces and veins caused by the contraction of the muscle.[11]

But there is no evidence regarding comparison of the effectiveness of faradism under pressure and complex decongestive therapy in subjects with peripheral oedema.

Materials and Methods

It is a conventional study which was carried out in Krishna hospital, Karad. The duration of the study was 6 months. A total of 20 subjects were included in the study and the sample size was calculated using formula. All the subjects were included in this study based on inclusion criteria. Inclusion criteria includes subjects with chronic oedema in feet, subjects with duration of oedema more than 6 weeks, age 25-50 years and both males and females are included in this study. Exclusion criteria includes subjects with acute oedema, active infection, peripheral artery disease and cardiac oedema. Subjects were assessed for oedema using volumetric and girth measurement.

Procedure

After getting ethical approval from institutional ethical committee, this study was carried out in Krishna hospital, Karad. Informed consent was taken from the subjects who fulfilled inclusion criteria. Subjects underwent oedema assessment which included volumetric and girth assessment. Assessment was carried out on both legs and their difference was noted.[12]

Group A was given CDT for 5 days/week for 3 weeks. Patients underwent CDT that included MLD, compression therapy with a short stretch bandage for 23 hours per day, MCB, exercises, and skin care. The CDT was performed for 1-hour a day. The MLD was performed in proximal to distal direction with light skin massage. 17 Nonelastic multilayer compression bandages were

applied and changed daily. All patients were educated on appropriate skin care, such as skin hygiene, applying moisturizer daily, and avoiding mechanical, thermal, and barotrauma. The patients also performed the exercise program, such as breathing exercise, flexibility exercises for helping facilitate lymphatic flow, gentle resistance exercises, isometric exercises.[7,8]

Group B was given faradism under pressure for 5days/week for 3 weeks. Pre and Post Volumetric and girth assessment tests were done and treatment scores were taken for statsitical analysis.

Statistical Analysis

- Within the groups, the data was analysed by **paired t** test.
- Between the groups, the data was analysed by **unpaired t** test.
- The p and t values were calculated.

Effectiveness of faradism under pressure versus complex decongestive therapy in subjects with peripheral oedema was analysed.

Results

Data Presentation

Table 1: Paired t-test between Pre and Post Volumetric Assessment of Group A and Group B

GROUP A								
Paired Samples Test								
Volumetric Assessment	**Paired Differences**					**T**	**Df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Pre volumetric – Post volumetric	33.600	9.192	2.907	27.025	40.175	11.560	9	<0.0001
GROUP B								
Paired Samples Test								
Volumetric Assessment	**Paired Differences**					**T**	**Df**	**Sig. (2-tailed)**
	Mean	**Std. Deviation**	**Std. Error Mean**	**95% Confidence Interval of the Difference**				
				Lower	**Upper**			
Pre - Post Volumetric	29.200	7.584	2.398	23.775	34.625	12.176	9	<0.0001

In Table No.1, the paired difference was calculated, the mean paired difference was 33.6 ±9.192 with standard error mean was 2.907 with 27.025 to 40.175 (95% CI), t-value was 11.56 and p< 0.0001. And there was statistical significance between Pre and Post Volumetric Assessment of Group A and the mean paired difference was 29.20 ± 7.584 with standard error mean was 2.398 with 23.775 to 34.625 (95% CI), t-value was 12.176 and p < 0.0001. And there was statistical significance between Pre and Post Volumetric Assessment of GroupB.

Table 2: Paired t-test between Pre and Post Girth Assessment of Group A and Group B

GROUP A								
Paired Samples Test								
	Paired Differences							
				95% Confidence Interval of the Difference				
Girth Assessment	**Mean**	**Std. Deviation**	**Std. Error Mean**	**Lower**	**Upper**	**T**	**Df**	**Sig. (2-tailed)**
Pre - Post Girth	2.540	0.9513	0.3008	1.860	3.220	8.444	9	<0.0001
GROUP B								
Paired Samples Test								
	Paired Differences							
				95% Confidence Interval of the Difference				
Girth Assessment	**Mean**	**Std. Deviation**	**Std. Error Mean**	**Lower**	**Upper**	**T**	**Df**	**Sig. (2-tailed)**
Pre - Post Girth	2.770	1.101	0.3480	1.983	3.557	7.959	9	<0.0001

In the above tables, the paired difference was calculated, the mean paired difference was 2.540 ± 0.9513 with standard error mean was 0.3008 with 1.860 to 3.220 (95% CI), t-value was 8.444 and p< 0.0001. And there was statistical significance between Pre and Post Girth Assessment of Group A and the mean paired difference was 2.770 ± 1.101with standard error mean was 0.3480 with 1.983 to 3.557 (95% CI), t-value was 7.959 and p < 0.0001. And there was statistical significance between Pre and Post Girth Assessment of Group B.

Table 3: Comparison between Pre-Volumetric Assessment of Group A and Group B

Volumetric Assessment	Mean	SD	P-value	Unpaired t-test Value	degree of freedom
Pre-Volumetric Assessment Group A	45.800	10.369	0.4232	0.8196	18
Pre-Volumetric Assessment Group B	42.7	5.964			

In the above table, mean pre volumetric assessment of Group A was 45.800, SD was 10.369 and mean pre volumetric assessment of Group B was 42.7, SD was 5.964. Unpaired t-test value was 0.8196 and P-value was 0.04232. Also, the Mean Difference was -3.1 with 95% CI -11.047-4.847. Hence on basis of p-value we can state there was no statistical significance between Pre volumetric assessment of Group A and Group B.

Table 4: Comparison between Pre-Girth Assessment of Group A and Group B

Girth Assessment	Mean	SD	P-value	Unpaired t-test Value	degree of freedom
Pre-Girth Assessment Group A	3.46	1.166	0.6281	0.4929	18
Pre- Girth Assessment Group B	3.2	1.193			

In above table, mean pre girth assessment of Group A was 3.46 and SD was 1.166 and mean pre girth assessment of Group B was 3.2 and SD was 1.193. Unpaired t-test value was 0.4929 and P-value was 0.6281. Also, the Mean Difference was -0.2600 with 95% CI -1.368-0.8482. Hence on basis of p-value we can state there was no statistical significance between Pre girth assessment of Group A and Group B.

Table 5: Comparison between Post Volumetric Assessment of Group A and Group B

Volumetric Assessment	Mean	SD	P -value	Unpaired t-test value	degree of freedom
Post Volumetric Assessment Group A	12.2	5.554	0.5339	0.6343	18
Post Volumetric Assessment Group B	13.5	3.342			

In above table, mean post volumetric assessment of Group A was 12.2, SD was 5.554 and mean post volumetric

assessment of Group B was 13.5, SD was 3.342. Unpaired t-test value was 0.6343 and P-value was 0.5339. Also, the Mean Difference was 1.300 with 95% CI -3.006-5.606. Hence on basis of p-value we can state there was no statistical significance between Post Volumetric assessment of Group A and Group B.

Table 6: Comparison between Post Girth Assessment of Group A and Group B.

Girth Assessment	Mean	SD	P -value	Unpaired t-test value	degree of freedom
Post Girth Assessment Group A	0.92	0.7376	0.0681	1.941	18
Post Girth Assessment Group B	0.43	0.3057			

In above table, mean post volumetric assessment of Group A was 0.92, SD was 0.7376 and mean post volumetric assessment of Group B was 0.43, SD was 0.3057. Unpaired t-test value was 1.941 and P-value was 0.0681. Also, the Mean Difference was -0.0681 with 95% CI -1.020-0.04043. Hence on basis of p-value we can state there was no statistical significance between Post Girth assessment of Group A and Group B.

Discussion

In this study effectiveness of faradism under pressure and complex decongestive therapy in peripheral oedema was compared and primary goal was to evaluate effect of complex decongestive therapy on peripheral oedematous conditions. We found significant effect of CDT and FUP on peripheral oedematous conditions but no significant improvement in volumetric measurements in inter-group comparison. Various studies revealed positive effects of CDT on lymphedema volume and HRQoL in BCRL. The effect of 2–4 week of CDT on quality of life (QoL) and severity of pain by Mondry et al in the same population demonstrated decrease in girth had a significant correlation with the reduction in pain VAS scores, but not with the increase in QoL. Kim et al. found that during the maintenance phase of CDT, QoL significantly improved and in contrast with the study of Mondry et al., it was correlated with the reduction in limb volume. Buragadda et al. compared patients with BCRL receiving CDT and a home program or conventional treatment. The conventional treatment group performed MLD, a low elastic compression garment, glenohumeral mobilization, and deep breathing exercise. And the 1-hour home program included self-lymphatic drainage, skin care, and remedial exercises. The results of this study indicated significant improvements in the arm function, pain, and lymphedema volume in both groups but greater improvements were seen in the group receiving the home program. The effects of compression garments were compared with compression bandaging within the initial treatment phase of a decongestive lymphatic therapy for a total of 10 treatment sessions in BCRL. The results of this study revealed greater volume reduction, but worse upper extremity functions in compression bandaging group compared to compression garment group.[10]

Application of CDT in patients with early lymphedema would provide better recovery for upper extremity functions, as well as better volume reduction.

Pre and post treatment volumetric and girth measurements were compared. Within the group comparison, post treatment there was significant improvement noted in volumetric and girth measurements in both the groups. Between the group comparison, post treatment there was no significant improvement noted in volumetric measurements but significant improvement was noted in girth measurement inter group comparison.

Previous studies did not signify any comparision in the effect of FUP and CDT in subjects with peripheral oedema.

Thus, present study was undertaken to find effectiveness of FUP versus CDT in subjects with peripheral oedema which obtained significant results. And thus, concluded with significant decrease in oedema.

Conclusion

We found that both FUP and CDT were significantly effective in reducing peripheral oedema but CDT was more effective compared to FUP. This treatment was effective by reducing the volumetric and girth measurements which improved quality of life of patients.

Conflicts of Interest: The authors declare that there are no conflicts of interest concerning the content of the present study.

Funding source: This study was funded by Krishna Institute of Medical Sciences Deemed to Be University Karad, Maharashtra.

Ethical Approval: From institutional ethical committee of Krishna Institute of Medical Sciences Deemed to Be University Karad, Maharashtra.

Abbreviations:

CDT: Complex decongestive therapy, FUP: Faradism under pressure, MLD: Manual lymphatic drainage, MCB: Multi-layer compression bandaging.

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The Effectiveness of Hot Fomentation with Epsom Salt Versus Common Salt on Knee Joint Pain among Elderly in the Selected Old Age Home of Pune City

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Abstract

Background & Objective: Aging is a multidimensional process of change in the physical, psychological and social. In India Osteoarthritis 2nd most cause of knee joint pain with the prevalence of 22 to 39%. Our aim was to assess the effectiveness of hot fomentation with Epsom salt versus Common salt on knee joint pain among elderly

Methods: A Quasi Experimental, Non- randomized one group pre-test post-test control group design was adopted involving 40 elderly who have knee joint pain. After obtaining informed consent elderly were allocated into two groups of 20 each by using the Non-probability purposive sampling technique. Hot fomentation (100 ml of water at the temperature of 30-40 degree Celsius) with 2gm of Epsom salt for the experimental group and common salt for the attention control group for 5 days once a day. Global pain scale was used to assess the level of pain.

Results: The significant change was observed in Pain level as evident that the t-value is 26.29, p-value of Epsom is 0.00001 that is less than 0.05 and the t-value is -21.03, p-value of Common salt is 0.0001 that is less than 0.05, both the interventions were effective but Epsom salt is more effective than common salt on knee joint pain. Hence, null hypothesis (H_0) was rejected. None of the demographic variables are found to have significant association with the level of knee joint pain.

Conclusion: The effectiveness of hot fomentation with Epsom salt is higher than the common salt so null hypothesis is rejected.

Key words: *Hot fomentation, Epsom Salt, common Salt, knee joint pain, elderly.*

Introduction or Background

Each multicellular organism is able to develop and maintain its identity using energy from the sun for so long. That's why deterioration prevails over synthesis, and the age of organism. Aging is a natural process. In elderly age blurred vision, joint pain, back pain, skin changes, low immunity are the common problems.

Knee joint pain is the very common in elderly person. Knee joint pain is usually present with structural changes morning stiffness, instability, unable to walk, bony enlargement, unable to use stairs and restricted movement.[1] WHO report (2010) worldwide, Osteoarthritis affect 9.6% men and 18% of women the age of 60 or above and the condition will be 4th leading cause of disability by 2020.

Majority of the cases the condition can be prevented by a proper lifestyle and food habits.[2]Arthritis knee pain relief with Epsom salt baths is a popular topic that is often brought up amongst people with pain in their knees.[3] There are other homes remedies for knee joint pain is soaking in warm water with Common salt are one of the oldest forms of alternative therapy, and there's good reason why this practice has stood the test of time.

The findings shows that it makes the joints looser. It reduces pain and prolonged effect that goes beyond the period of immersion. Not only does heat relax muscles, it also stimulates blood flow and improves circulation, helps increases range of motion, and reduces stiffness in

painful joints. [4]

Applying the heat by general or local which produce the physiological change in the body causing vasodilatation and relaxation of muscles which relieves the pain.

Material and Methods

A Quantitative approached with Quasi Experimental Non- randomized one group pre-test post-test control group design was found to be the most appropriate. This study was conducted in the selected old age home of Pune city. Total 40 elderly who met the inclusion criteria were selected by using the Non-probability purposive sampling technique. The samples were allocated into two groups with 20 each for the experimental and attention control group. Hot fomentation (100 ml of water at the temperature of 30-40 degree Celsius) with 2gm of Epsom salt for the experimental group and common salt for the attention control group for 5 days once a day. Global pain scale was used to assess the level of pain.

Results

The analysis and interpretation of the data collected to determine the effectiveness of hot fomentation with Epsom salt versus Common salt on knee joint pain is carried out based on objectives set by the researcher taking the level of significance as 0.05.

Table 1: Descriptive statistics of effectiveness of hot fomentation with Epsom salt on knee joint pain among elderly.

n=20

Pain level		Frequency		Percentage		Calculated t- value	p-value
		Pre-test	Post-test	Pre-test	Post-test		
Mild (0-70)	n=20	1	9	5	45	26.29	0.00001
Moderate (71-140)		19	11	95	55		
Severe (141-210)		0	0	0	0		
		Pre-test		Post-test			
Mean		119		99.15			
SD		1.41		4.95			

The above table provides data about the effectiveness of application of hot water with Epsom salt on knee joint pain among the elderly. The mean pain level was from 119(pre-test) to 99.15(post-test) with SD from 1.41(pre-test) to 4.95(post-test). As the calculated t-value is 26.29 and p-value= 0.00001, where $p<0.05$, Significant at 0.05 level of significance. Hence, statistically there is effectiveness of hot fomentation with Epsom salt on knee joint pain.

Table 2: Descriptive statistics of effectiveness of hot fomentation with common salt on knee joint pain among elderly. **n=20**

Pain level		Frequency		Percentage		Calculated t-value	p-value
		Pre-test	Post-test	Pre-test	Post-test		
Mild (0-70)		0	0	0	0		
Moderate (71-140)		11	20	55	100		
Severe (141-210)		9	0	45	0		
	n=20	Pre-test		Post-test			
Mean		137.4		115		-21.03	0.0001
SD		9.90		2.83			

The above table provides data about the effectiveness of application of hot water with common salt on knee joint pain among the elderly. The mean pain level was from 137.4(pre-test) to 115(post-test) with SD from 9.90(pre-test) to 2.83(post-test). As the calculated t-value is -21.03 and p-value= 0.00001, where $p<0.05$, Significant at 0.05 level of significance. Hence, statistically there is effectiveness of hot fomentation with common salt on knee joint pain.

Figure 1: Comparison of Effectiveness of Hot Fomentation with Epsom Salt And Common Salt On Knee Joint Pain. **n=40**

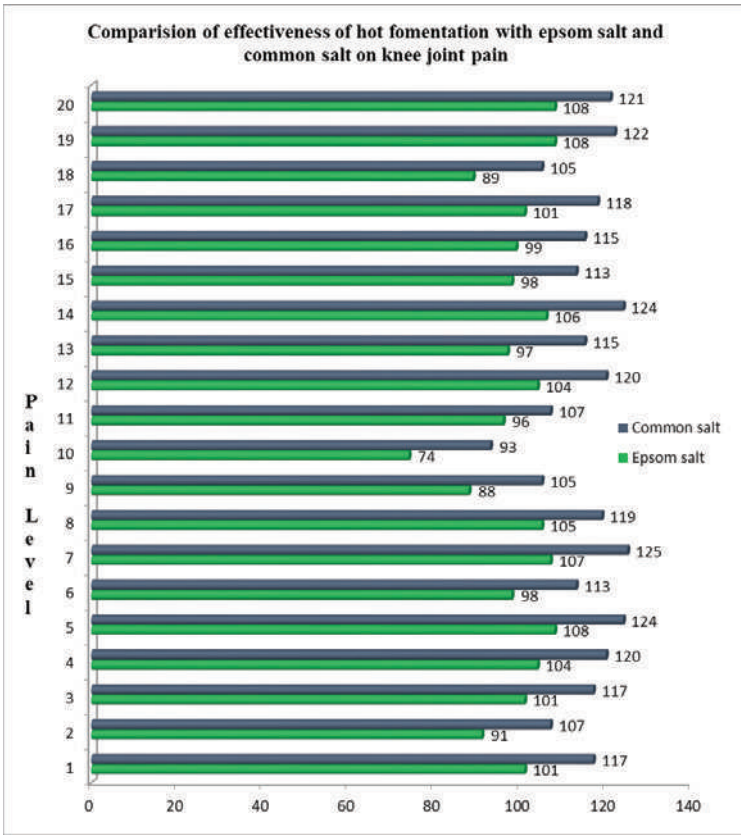


Figure 1: Bar diagram showing comparison of effectiveness of hot fomentation with Epsom salt and Common salt on knee joint pain

The figure above represents the comparison between the effectiveness of hot fomentation with Epsom salt and common salt. Figure shows that pre-test son the level of knee joint pain.

The significant change was observed in Pain level as evident that the t-value is 26.29, p-value of Epsom is 0.00001 that is less than 0.05 and the t-value is -21.03, p-value of Common salt is 0.0001 that is less than 0.05, both the interventions were effective but Epsom salt is more effective than common salt on knee joint pain. Hence H_0 was rejected.

Conclusion

The findings of the study shows that there significant difference between the score of the experimental attention control group. Therefore, hot fomentation with Epsom salt brought out to be significant.

Conflict of Interest – Nil

Source Of Funding – Self

Code of Conduct – No ethical issue was there in the study

Ethical Clearance – Obtained from Institutional Research Committee

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Effectiveness of Postural Drainage and Forced Expiratory Technique Along with Segmental Relaxation Technique on Airway Clearance in Chronic Obstructive Pulmonary Disease Patients

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Abstract

Background: Chronic Obstructive Pulmonary Disease is the major leading cause of death. In COPD, mostly death occurred due to inappropriate airway clearance. So if these condition left untreated then this inflammation leads to repeated cycles of injury and repair of the airway wall. Few studies have shown the effect of postural drainage and forced expiratory technique on COPD patients for airway clearance. In this study postural drainage and forced expiratory technique along with segmental relaxation from Laura Mitchell Relaxation Technique use for mucus clearance as soon as possible in COPD patients

Aim: To study the effect of segmental relaxation followed by postural drainage and forced expiratory technique on airway clearance in COPD patients.

Material and Method: In this pre-post intervention study 28 individuals with COPD were included. They were randomly divided into 2 groups with 14 individuals in each group. Group A were treated with segmental relaxation from Laura Mitchell Relaxation Technique along with postural drainage and forced expiratory technique and Group B were treated with postural drainage and forced expiratory technique. After pre-post assessment, data was analysed with the help of appropriate statistical methods.

Conclusion: In that postural drainage and forced expiratory technique has less effect than these techniques that is postural drainage and forced expiratory technique along with segmental relaxation from Laura Mitchell Relaxation Technique in COPD patients.

Keywords: *segmental relaxation from Laura Mitchell Relaxation Technique, postural drainage, forced expiratory technique, mucus clearance in COPD patients.*

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Introduction

Chronic Obstructive Pulmonary Disease is the major leading cause of death.[1] In recent years, mucociliary dysfunction due to COPD is one of the leading cause of death. Globally 11.7% (14.3% male, 7.6% female) people suffering from COPD[2] and 2 to 22% among the men and 1.2 to 19% among women in different population-based studies through India.[3] According to

WHO, COPD is defined that a lung disease characterized by chronic obstruction of lung airflow that interferes that with normal breathing and is not fully reversible.[1]

The diagnosis of COPD are chronic bronchitis and emphysema. As chronic bronchitis and emphysema are different conditions but they relate to each other. In chronic bronchitis, there is inflammation of the bronchial tubes while emphysema is the atypical everlasting enlargement of the air sacs.[1]And the classification of COPD is based on the GOLD criteria; as they are mild, moderate and severe. In mild exacerbation, there is mild airflow limitation ($FEV_1/FVC<70\%$ but $FEV_1 \geq 80\%$), in moderate stage, there is deteriorating of airflow limitation ($30\% \leq FEV_1 \geq 80\%$ projected). In severe stage, there is severe airflow limitation ($FEV_1 <30\%$ projected).[4]

Respiratory tract infection is a precipitating factor in 70% to 80% of COPD, however about third of severe exacerbation of COPD cause can't be recognized.[5,6]The major cause of COPD is smoking; followed by places where air is dusty, smoky, polluted like coal mines and industries such as cement, textiles, chemicals and electro plating jewelry.[1]In India, 3-5 times more people suffering from COPD due to smoking than non-smoking.[3]

The main signs of COPD are infrequent shortness of breath particularly after exercise, long lasting periodic cough, mucus production. The indications of COPD are wheezing, weight loss, chronic dyspnea, cough and sputum, chest tightness, anxiety secondary to dyspnea. [7]

The airflow limitation due to narrowing of upper and lower respiratory track is an inflammatory response of the lungsto noxious particles or gases. This progressive and rentless loss of muscle function is the result of emphysema due to destruction of lung parenchyma and narrowing of small airways as a result of chronic inflammation.[8]

The risk factors of COPD are Smoking and air pollution in that COPD is more common in males than in females nevertheless there is an increase in tobacco smoking among females. Some studies have demonstrated that air pollution suggestively proliferations the risk of exacerbations.[9,10,11] Normal drainage of secretions hampered due to effect of smoking, air pollution, micro-organisms and many more factors which causes COPD.[12] previous study showed that presence of green sputum as opposed to white sputum indicates that there is high bacterial load in respiratory tract secretion.[13]

To cure this condition treatment is necessary. In this study to assess the lung capacity O2 saturation, peak flow meter and MMRC grades were used. From these outcome measures we can see the lung capacity and can also interpreted the severity of the condition. So if these condition left untreated then this inflammation leads to repeated cycles of injury and repair of the airway wall.[14] in this study treatment protocol included segmental relaxation, postural drainage and forced expiratory technique. This segmental relaxation from Laura Mitchell's Relaxation technique, principles of this technique are 1. Tightening or contracting muscles results in movement 2. Movement causes reposition of the joints and limbs 3. when we are awake, the brain will register a change in body position through muscle, joint and skin sensation. 4. The brain is only aware of the movement it causes. Movements are controlled by the nervous system; if one group of muscles is instructed to tighten, the opposite group of muscles for that action receives an instruction to relax.[15] followed by postural drainage, in that position and chest manipulation (percussion, vibration and shaking) are required to dislodge the secretions and to remove the secretions throw-out the body coughing and huffing combining both termed as forced expiratory technique.[16]

Methods

An experimental study was carried out using pre and post study design. The place of the study was Krishna institute of medical sciences, Physiotherapy department, Karad. There were 28 participants in the study with COPD. The samples were chosen using the simple random sampling method. Participants was included as per inclusion and exclusion criteria.

Results

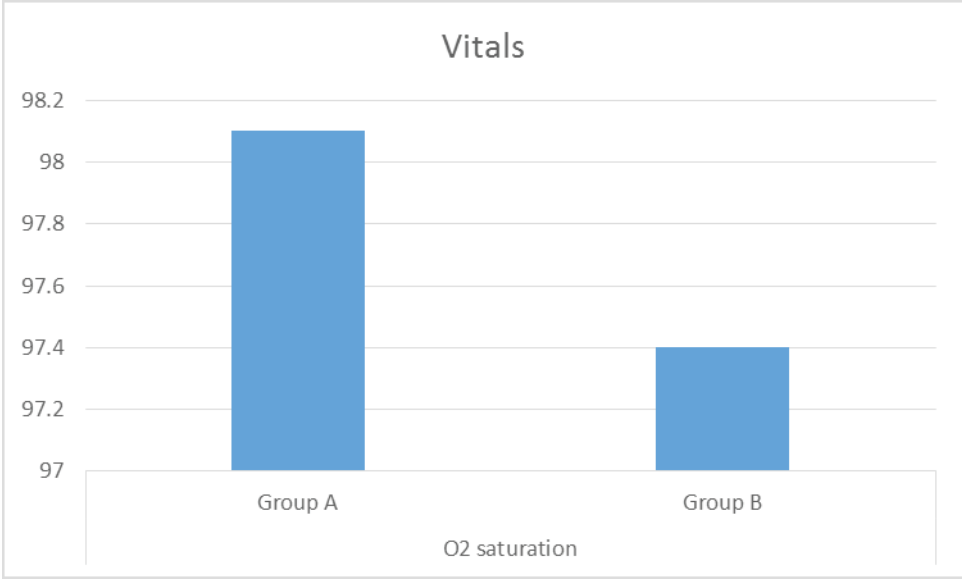
There were 28 patients enrolled, among which 14 were randomly place in experimental and controlled group respectively.

The percentage of age group 40-60 years of group A and group B is 14.28% and 35.71% respectivrly. Then age group 61-80 years of group A and group B is 50% and 35.71%. And last age group 81-100 years of group A and group B is 35.71% and 28.57% respectively. And

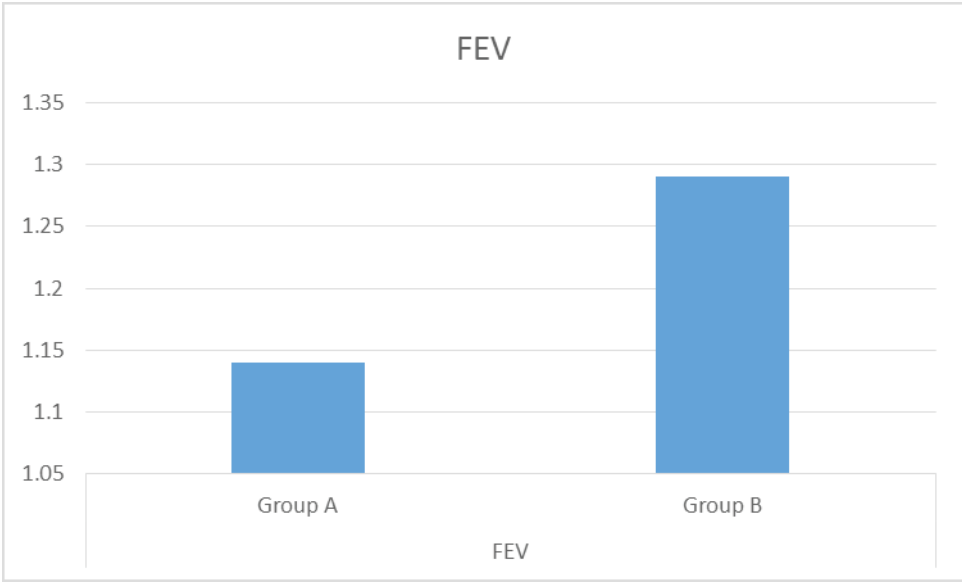
the frequency of males and females in group A is 10 and 4 respectively while in group B is 9 males and 5 females were there.

Table 1 shows that mean and standard deviation of both groups as well as paired t value and p values of vitals, peak flow meter and MMRC grades. In that the values are mentioned in this table is not only for morning sessions but also afternoon session and this values were taken before and after the treatment.

Table 1 shows that mean and standard deviation of both groups as well as paired t value and p values of vitals, peak flow meter and MMRC grades. In that the values are mentioned in this table is not only for morning sessions but also afternoon session and this values were taken before and after the treatment.



Graph 1. Post comparison of vitals of morning sessions of Group A vs Group B

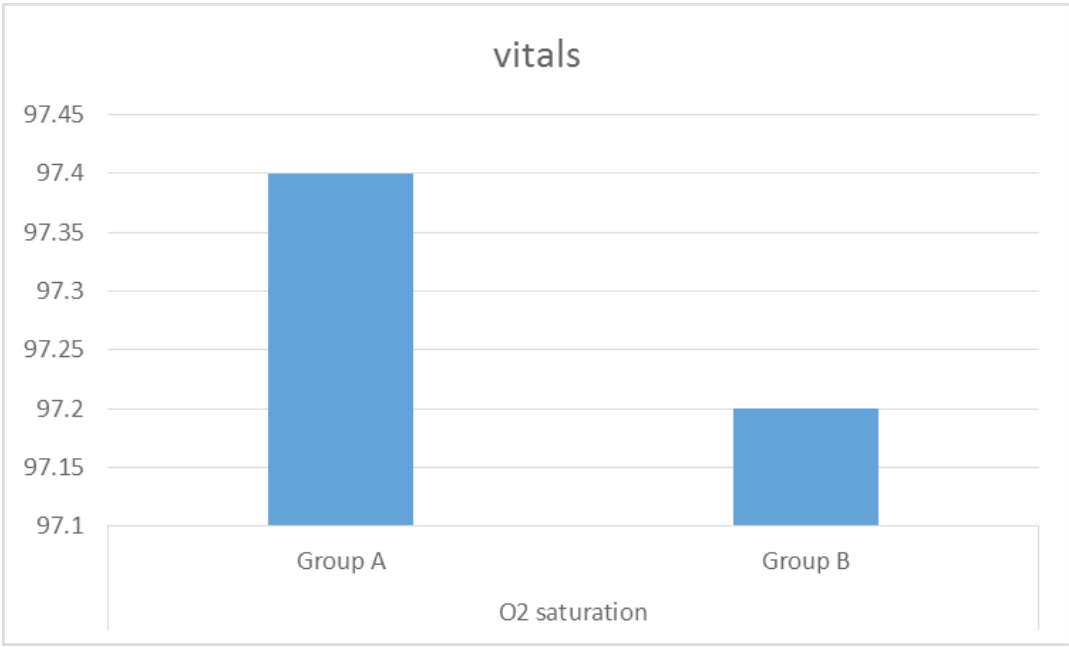


Graph 2. Post comparison of FEV of morning sessions of Group A vs Group B

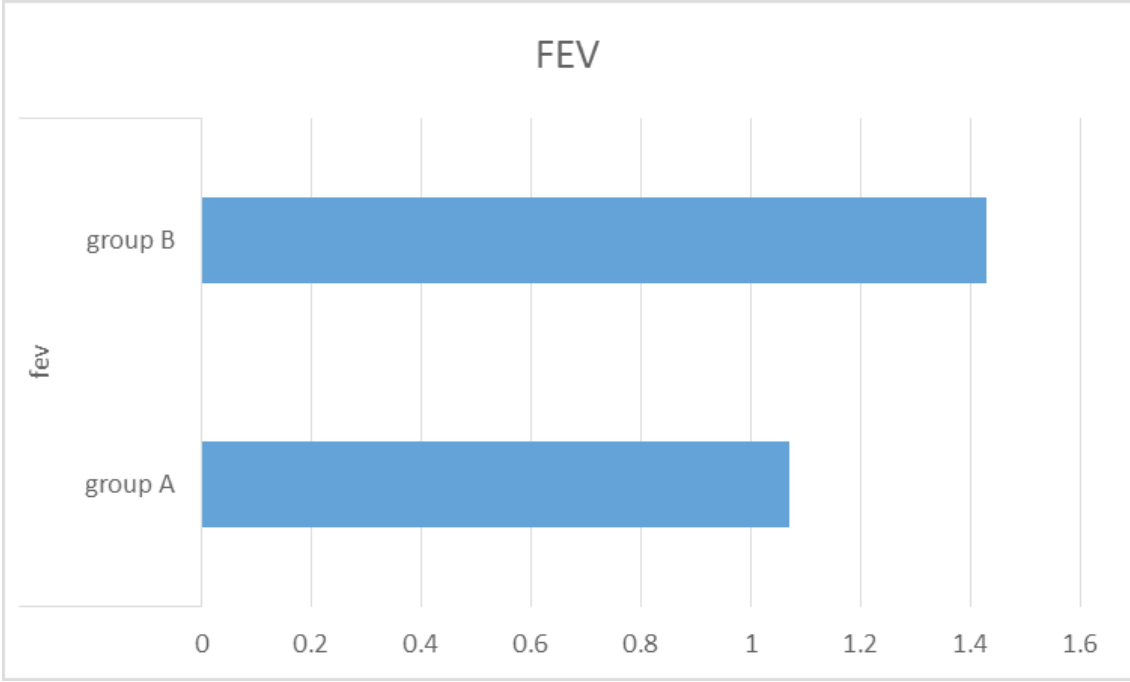
Table 2 shows comparision between the post treatments of morning sessions as well as afternoon sessions respectively.

It also shows that there is significant difference is present between two groups for O2 saturation and in peak flow meter measurement but there is equal for MMRC grades.

So overall it shows that there group A have more effect than group B.



Graph 3. Post comparison of vitals of afternoon sessions of Group A vs Group B



Graph 4. Post comparison of FEV of afternoon sessions of Group A vs Group B

Table 1. Group A (segmental relaxation + postural drainage + forced expiratory technique) and Group B (postural drainage + forced expiratory technique)

			Mean ± SD		Paired 't' value	P value
			Pre	Post		
	Vitals O2 saturation	Morning	96.5 ± 1.225	98.1 ± 0.917	6.904	< 0.0001
		Afternoon	96.7 ± 1.139	97.4 ± 0.829	6.817	< 0.0001
Group A	Peak flow meter FEV	Morning	2.07 ± 0.27	1.14 ± 0.363	13.000	< 0.0001
		Afternoon	2.00 ± 0.39	1.07 ± 0.27	7.320	< 0.0001
	MMRC grades	Morning	3.71 ± 0.47	2.93 ± 0.475	6.904	< 0.0001
		Afternoon	3.43 ± 0.51	2.43 ± 0.51	9.539	< 0.0001
	Vitals O2 saturation	Morning	96.6 ± 1.555	97.4 ± 1.151	3.667	0.0028
		Afternoon	96.6 ± 1.136	97.2 ± 1.122	3.309	0.0057
Group B	Peak flow meter FEV	Morning	2.14 ± 0.363	1.29 ± 0.469	8.832	< 0.0001
		Afternoon	2 ± 0.392	1.43 ± 0.514	4.163	0.0011
	MMRC grades	Morning	3.71 ± 0.469	2.71 ± 0.611	9.539	< 0.0001
		Afternoon	3.64 ± 0.497	2.57 ± 0.514	15.000	< 0.0001

Table 2. Post comparison of morning and afternoon sessions of Group A vs Group B

sessions			Mean	SD	Unpaired t test	P value
	O2 saturation	Group A	98.1	0.917	1.816	0.0404
		Group B	97.4	1.151		
Morning	FEV	Group A	1.14	0.363	0.9014	0.1878
		Group B	1.29	0.469		
	MMRC	Group A	2.71	0.475	1.036	0.1549
		Group B	2.93	0.611		
	O2 saturation	Group A	97.4	0.829	2.300	0.0149
		Group B	97.2	1.122		

Cont... **Table 2. Post comparison of morning and afternoon sessions of Group A vs Group B**

Afternoon	FEV	Group A	1.07	0.267	2.308	0.0146
		Group B	1.43	0.514		
	MMRC	Group A	2.43	0.514	0.7360	0.2342
		Group B	2.57	0.514		

Discussion

This research aimed to study the effectiveness of postural drainage and forced expiratory technique along with segmental relaxation technique on airway clearance in chronic obstructive pulmonary disease patients Evidences suggests that postural drainage and forced expiratory technique is effective treatment for the airway clearance.

The signs and symptoms of COPD infrequent shortness of breath particularly after exercise, long lasting periodic cough, mucus production and are wheezing, weight loss, chronic dyspnea, cough and sputum, chest tightness, anxiety secondary to dyspnea.

To cure this condition treatment is necessary. In this study to assess the lung capacity O2 saturation, peak flow meter and MMRC grades were used. From these outcome measures we can see the lung capacity and can also interpreted the severity of the condition. So if these condition left untreated then this inflammation leads to repeated cycles of injury and repair of the airway wall[14].

Subjects were selected for the study according to the selection criteria. Demographic data and consent form was taken from them. Included participants was divided into two groups by random sampling method. Pre and post assessment was taken before and after the treatment respectively with the help of outcome measures.

This is a study of segmental relaxation from Laura Mitchell Relaxation Techniques followed by postural drainage and forced expiratory techniques in COPD patients. The study was conducting in and around Karad.

The subjects were divided in 2 groups: group 1 is experimental group while group 2 is control group. In experimental group we would give segmental relaxation followed by postural drainage and forced expiratory technique. And in control group we should give segmental relaxation along with forced expiratory technique.

First, therapist should tell all procedure which are performed on them and then they should have to follow their commands for segmental relaxation technique.

Patient should be in supine position. Asked them to concentrate on their breathing then give orders for shoulders that pull their shoulders towards feet and feel that position and slowly relax; followed by give orders to body that press your body into the support and again feel the pressure of your body on the support and slowly relax. And last order for the breathing i.e. take a deep breath; feel your tummy swell outs your breath out then breath out easily repeat this twice.

After this relaxation technique, postural drainage should be given by therapist. Followed by postural drainage, forced expiratory technique should be given to remove those secretions which were loosen and dislodge by segmental relaxation along with postural drainage.

The experimental results were statistically analysed. The significant difference between the two groups was investigated with the unpaired t test and within the group with paired t test.

In this study treatment protocol included segmental relaxation, postural drainage and forced expiratory technique. This segmental relaxation from Laura Mitchell's Relaxation technique, principles of this technique are 1. Tightening or contracting muscles results in movement 2. Movement causes reposition of the joints and limbs 3. when we are awake, the brain will register a change in body position through muscle,

joint and skin sensation. 4. The brain is only aware of the movement it causes. Movements are controlled by the nervous system; if one group of muscles is instructed to tighten, the opposite group of muscles for that action receives an instruction to relax.[15]

Followed by postural drainage, in that position and chest manipulation (percussion, vibration and shaking) are required to dislodge the secretions and pass on to central airways and to remove the secretions throw-out the body. Coughing and huffing combining both termed as forced expiratory technique.[16] previous study suggest that postural drainage is most effective treatment for hypersecretion. And for eliminating these secretion coughing is most helpful treatment as shown in previous study.[16]

As compared to previous study and as shown in graph treatment with segmental relaxation gives speedy recovery for mucus clearance.

Conclusion

In that postural drainage and forced expiratory technique has less effect than these techniques that is postural drainage and forced expiratory technique along with segmental relaxation from Laura Mitchell Relaxation Technique in COPD patients.

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Conflict o Interest - Nil

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Ethical Clearance: The Institutional Ethics committee has hereby given permission to initiate the research project titled, "Effectiveness of postural drainage and forced expiratory technique along with segmental relaxation technique on airway clearance in chronic obstructive pulmonary disease patients".

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The Perturbation Encompassing Dental Amalgam Toxicity: A Review

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Abstract

Amalgam in dentistry has been in use for over 150 years and still been widely used because of its low cost, easy application, durability, bacteriostatic effect, and strength. Whenever aesthetics isn't the prime concern it is possibly used with no age bar, in areas of stress, as a foundation for cast-metals, ceramic restorations, and below-par oral hygiene conditions. Its advantages such as long-lasting and being less technique sensitive among all restorative materials, there has been growing concern about amalgam that it causes mercury toxicity. Mercury (Hg) has been historically popular as one of the extremely toxic nonradioactive materials. Therefore, the present article aims to discuss and assess a brief historical review of literature on its adverse effects, toxicological aspects that have been noted, potential risks relating to the dental personnel's and its safe and effective way of manipulating and handling concerning the use of mercury in dentistry.

Keywords: *mercury; dental amalgam; dentistry; toxicity;*

Introduction

Dental amalgam was used first by the Chinese. Su Kung (659 AD) mentioned the use of a mixture in the Material Medica[1]. A municipal physician in Germany, Europe named Johannes stokers in 1528 recommended amalgam as a restorative material[2]. Dr.Wilmer Eames in 1959 recommended mercury to alloy ratio of1:1, thus lessening the ratio of 8:5 mercury to an alloy that others had previously been recommending[3]. Mercury is present in the environment and thus humans are regularly exposed to it via water, food, and air[4]. Mercury exposure from restoration depends on the size and number of restorations, its composition, patients chewing habits, texture of the food, brushing, grinding of teeth, and a lot of other physiological factors[5]. Amalgam's universal acceptance as a restorative material came after the investigations of Black in 1895, 1896, 1908[6]. Dental amalgam as defined by the American Dental Association (ADA) is an alloy that is composed of mercury, tin, copper, and silver along with some other metallic compounds added to enhance its physical and mechanical properties[7]. The purpose of this paper is to review the literature regarding the toxicology and adverse effects of mercury from dental amalgam, to evaluate and ensure safety on dental amalgam use.

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Mercury exposure from dental amalgam restorations

Can occur through several ways: (1) Elemental mercury released from dental amalgam which is present in the aerosol can be inhaled; (2) Particles of dental amalgam abrading from restored surfaces during mechanical wear of restorations or can even be produced

while placing or replacing the restorations, and the abrading particles from the surface restorations could be ingested; (3) Swallowing of the dissolved corrosion produced inorganic and elemental mercury products from saliva can take place; (4)"tattooing"[8].

Adverse effects of amalgam restorations: 1. Fluoride and mercury are widespread in the natural environment. 2. When high levels of exposure occur, adverse health effects have been observed. Fluoride and mercury are widespread in the natural environment. 3. When high levels of exposure occur, adverse health effects have been observed.

Fluoride and mercury are widely spread in our natural environment. Adverse health effects have been observed earlier whenever high-level exposure has occured[9]. There has been a reasonable understanding of the pharmacokinetics of mercury, its absorption, distribution, and elimination[10]. Several studies have reported that mercury levels in the urine and blood are indicative of amalgam exposure by the dental filling in the general population and by the occupational practice in dental practitioners[11][12][13]. Mercury vapor exposure is usually linked with occupational and accidental exposures[14][15].

Some of the local adverse effects include:

a) Corrosion -While amalgam has known to be a strong and durable restorative material, different phases in amalgam have different corrosion potential[16].

b) Amalgam tattoos- have been generally diagnosed clinically to beas dark grey to blue, flat macules rendering a sub-mucosal cluster of embedded amalgam that remains unchanged for several years. They have been found very commonly in the alveolar mucosa and the gingivae[17]and present in barely 1% of adults[18].

c) oral lichenoid reactions and erythematous lesions on the tongue and mucosa, are mainly due to abrasion of the restoration by the rough surfaces. Any restoration that is not appropriately placed, finished and polished can induce such an effect. These types of adverse mucosal reactions can usually resolve on taking out of the restorative material and will not require additional treatment[19].

Systemic Inflammation and Autoimmunity Related Outcomes

In the inclusion of allergic mechanisms, some studies on dental amalgam have examined the possible relevance to systemic inflammations and autoimmunity[20]. The primary effects on the human body include a change in the cell membrane, morphology of mitochondria, and physiologic differences in enzymatic activity. The central nervous system (CNS) is the most affected, which can display higher-significant deleterious results, involving tremors, abnormal reflexes, paraesthesia, nerve-conduction disturbances, headaches, balance disorders, changes in the pupillary reflex, language disorders, changes in the ability to spell, and disturbances of memory, concentration, and motor coordination[21][22]. Renal - Kidney Dysfunction multiple studies have inspected the outcome of the existence of amalgam restorations on the extent of mercury present in urine and bodily fluids[23]. Episodes of a true allergy to mercury have been rare and attempts to link its usage with conditions like multiple sclerosis and Alzheimer's disease has not been proven significantly, however, there may be some union between amalgam restorations and oral lichenoid lesions[24].

Risks to Dental Personnel

For long it has been recognized that in dentistry chronic exposure to mercury vapor owing to inadequate manipulating of dental amalgam can pose a potential health hazard in the workplace [25]. The use of dental amalgam has the potential for continuous occupational exposure of a dental practitioner to mercurial vapor that may be absorbed via the skin and the lungs [26][27]. Mercury poisoning could be characterized by tumours of arms, legs, or face and could also be incorporated with progressive, tremulous illegible handwriting, and slurred speech. It is been published by literature research that Dentists manipulating with amalgam have chances of increased mercury exposure [28][29][30]. One study has revealed that dental female assistants exposed to amalgam elicit a high rate of infertility [31]. Some Studies have witnessed that workers exposed to mercury also experience problems with eyesight, digestion, and urinary system [32][33][34]. The American Dental Association alarmed that amalgam filling material is hazardous to the dental office personnel but could be

safe in patients' mouths [35].

Handling mercury is the most critical and dangerous exposure channel. The exposure limit at workplaces is regulated by each country, as per its occupational exposure limit.

Mercury exposure risk can be minimized by ensuring careful handling procedures. Instantaneously steps should be taken for shielding the health of dentists and dental assistants through relevant preventive measures.

Testing for mercury toxicity: The average daily dose of inhaled mercury vapor was 1.7 μg (range from 0.4 to 4.4 μg), which is approximately 1% of the threshold limit value of 300 to 500 μg/day established by WHO, based on a maximum allowable environmental level of 50 μg/day in the workplace. Numerous diagnostic methods to detect the level of mercury in the body exist, which include tests for urine, saliva, stool, blood, hair analysis, and others. These tests determine if mercury is in the body and/or if it is being excreted. A study was conducted measuring intraoral vapor levels over a 24-h period in patients having at least nine amalgam restorations exhibited that the average dose of daily inhaled mercury vapor was about 1.7 μg (range from 0.4 to 4.4 μg), which approximates 1% of the threshold limit value of 300 to 500 μg/day indicated by WHO, based on a maximum permitted environmental level of 50 μg/day in the workplace[36]. Electrolyte assays, Complete blood cell count, renal, and hepatic function tests are some of the general laboratory tests available to detect mercury intoxication. Cardiovascular monitoring, Electrocardiography (ECG), electroneuromyography, pulmonary function test (PFT), and neuropsychological tests are also sometimes used for the evaluation[37].

The most precise way to calibrate the level of mercury in the body would be by detecting concentration levels of mercury in each organ tissue. However, as this can turn to be a strenuous task, using samples of hair, blood, urine, or fingernail remains the most common method of mercury measurement. As per some studies on occupational mercury exposure, recent exposure to mercury is supposedly reflected in blood and urine mercury concentration levels[38][39]. To date, there are no universal criteria available for the diagnosis of mercury overload.

The safe and effective use of dental amalgam: Effective management of amalgam includes placement, removal, storage, elimination, and disposal of amalgam waste as well as amalgam spills/mercury spills 'management during the phase-down period:

1. Amalgam Placement

a. Instrument or technology modification;

b. Modifying process during placement;

c. Operative changes e.g. Enhancement of house-keeping and inventory control;

d. Reforming or re-designing of amalgam product; and

e. Establishment of Standard guidelines for mercury spills management.

2. Removal of Amalgam Restorations: Standard guidelines must be established for use by dentists. These guidelines will include:

a. Using a rubber dam;

b. Using a special suction tube;

c. fasters cutting burs for reducing mercury vapor;

d. Using the copious amount of water during cutting;

e. Cutting, breaking amalgam into chunks;

f. Ensure using safety goggles;

g. Advocate clean filtered air in the dental surgery; and

h. To avoid any contact with mercury and amalgam particles or scraps cover skin completely.

3. Amalgam waste storage: Before dispose of appropriately storing amalgam waste in a sealed container containing water, as a part of the medical waste to avoid vaporization of any spills.

4. Amalgam waste disposal: Modern methods should be employed for disposing amalgam waste on contrary to the traditional methods of solid waste disposal[40].

Conclusion

Globally the consumption and production of mercury has been declining, although the advocacy in discontinuing and banning dental amalgam as a dental restorative material is been considered to be unwarranted and unsubstantiated, as its direct relation to systemic adverse effects has not been scientifically proven. As a principle of toxicology, we have to accept that there might be certain groups in the community who can be sensitive to mercury exposure and require minimization. By taking safe and effective measures it is viable to help reduce the environmental freight of mercury from the dental clinics. Amalgam restorations, therefore, endure to be safe and effective. Although dental amalgam use is trending towards minimization, still there continues to be a wide-spread belief that in certain clinical situations dental amalgam is a material of choice. Dentists need to educate their patients and even the health care professionals who might have been overly concerned regarding amalgam safety. However, amalgam due to its durability, reliability, and technique insensitivity runs to be one of the best bargains in the operative and restorative armamentarium.

Conflict of Interest – Nil

Ethical Clearance- Not applicable

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A Study to Evaluate The Influence of Thread Designs Affecting Stability of Immediately Loaded Implant at Maxillary Posterior Region

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Abstract

Purpose: For successful osseointegration of an immediately loaded implant, the fundamental element is primary stability. Establishment of primary stability is influenced by combination of various factors one of them being implant geometry. Evaluation of primary stability and the factors influencing it is essential for successful treatment outcome.

Methods: A total of 20 patients were divided into two groups. the implant placed in group I was with square thread designs (Ankylos® implant ,DENTSPLY, Sirona) and group 2 was V shaped thread design MIS seven implant(MIS implant Ltd, Israel) in the pre molar-molar region. The stability was assessed with the aid of resonance frequency analysis (Osstell™, Integration Diagnostics, Sweden) at the time of implant placement, at three months and at six months. A repeated measure ANOVA and Bonferroni Pairwise comparison was used to compare the ISQ within each group.

Results: There was a statistically significant difference in the stability at baseline to six months ($p=0.006$), from baseline to three months ($p=0.006$) and from three months to six months ($p<0.001$) in both the groups. Correlation could be found between implant geometry and stability observing improved stability in group 1 compared to group 2.

Conclusion: The result from this material showed that the all implants were successfully osseointegrated with a mean ISQ of 80 and 71 at six months in group I and group II respectively. Implants with square threads showed a higher stability at baseline, at three months and at six months in comparison to the v shaped thread.

Keywords: *Isq,Ostell,V Shaped Impalnts,Implant Stability*

Introduction

Tooth loss can result in functional as well as esthetic debilitation. With advance in implant design and surface texture combined with a popular need for reduced treatment time immediate loading in implant supported prosthesis is a predictable treatment option. However the success of immediately loaded implants depends upon the integrity of the bone to implant interface that is essentially determined by primary stability.[1]

Primary stability is the lack of motion of an implant after its installation into the surgical site influenced by quality of the bone and density, surgical bed and implant body design. Initial bone to implant contact is identified in prevention of interfacial micro movement, a crucial

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factor in attaining increased primary stability which is a pre requisite for immediate loading

Therefore the aim of this study was to assess if the level of implant stability is affected by thread design in the maxillary posterior region.

Aims and Objectives

· To evaluate the stability of immediately loaded implants of V shapedtapered thread design and square shaped tapered threaddesign.

· To compare and monitor the changes in implant stability of V shaped and square shaped thread designs at the time of placement, three and sixmonths followingplacement

Materials & Methods

The study was conducted on a total of twenty patients in a University set up Pre- operative assessment to evaluate adequate or optimal bone height was considered a pre requisite.

Inclusion Criteria:

ž Maxillary posteriorregions

ž adequate bone height and width for implantplacement

ž Sufficient bone volume without the need for bone augmentationprocedures.

ž Healed maxillary edentulousregion.

Exclusion Criteria

· Presence of systemicdiseases:

1. Uncontrolled diabetesmellitus
2. Hypertension
3. Hard and soft tissuepathology

ž Use of medications detrimental to the process ofhealing.

ž Harmful oral habits such as smoking andbruxism

ž Presence of any activeinfection

ž Limited mouthopening

Group I received the square shaped thread design implants. (ANKYLOS,Dentsply Sirona) and the group II received the V shaped thread design (MIS Implant Technologies Ltd, Israel)

Osstell-Implant Stability:

The resonance frequency analysis is determined by the Osstell™ (Integration Diagnostics, Sweden) device in assessing implant stability and osseointegration. The instrument is attached to a probe via a cable and the measurements are displayed on the backlit display. The instrument comes attached with a power supply, a smart peg mount and an ISQprobe.

SmartPeg:

The smartpeg resonates and measurements are made only when attached to the implant. The pegs are of single use and soft in material hence cannot be reused.

According to the SmartPeg reference guide, the smartpeg used for this study was: MIS SEVEN IMPLANT: Type 32 Article10040

ANKYLOS IMPLANT: Type 16 Article100388

Micromotion and Implant Stability Quotient:

There is a co-relation between lateral load and micro motion. Increase in the lateral load leads to resultant increase in the micro motion. However, this is also influenced by the different bone quality. Osstell™ (Integration Diagnostics, Sweden) thereby acts by measuring this micro motion with the aid of resonance frequency of smart peg.[2]

Surgical Protocol:

Prior to the operation patient were subjected to a prophylactic antibiotic therapy

A mid crestal incision was given in the edentulous area using a bard parker handle. A full-thickness mucoperiosteal flap wasraised.

Sequential drills were used and osteotomies were performed following the manufacturers drilling sequence for each implant system. The implant length and diameter to be placed were based on the pre- assessed availability of bone height and width at a speed of 25Ncm assessed by a physio dispenser. Immediately after implant placement,

the implant stability quotients were assessed with the aid of Osstell™ (Integration Diagnostics,Sweden)device. Followed by the measurement of the ISQ, an abutment were inserted and manually torqued. A provisional prosthesis made with temporary acrylic resin (3M ESPE,Protemp 4) was cemented

Measurement of Implant Stability:

After implant insertion, the resonance frequency analysis was assessed using the Osstell™ (Integration Diagnostics, Sweden) for establishment of implant stability quotient. The SmartPeg is tightened with 4-5Ncm finger tight pressure using the mount. The SmartPeg acts as a sensor for the vibration induced by the transducer probe held at a distance of 3-4 mm which is attached to the instrument via a cable and measurements are displayed on the back-lit display. In the current study measurement are obtained in one direction, buccolingually.

At each follow up sessions, the abutments were untorqued and the Smartpeg was re-inserted and stability was assessed followed by radiographic assessment.

Prosthetic Procedure:

Fabrication of Provisional Prosthesis:

Diagnostic impression was made prior to the implant surgery and a mock up template of the tooth being replaced were custom made. The silicone putty index of the prepared mock up guide was carried into the patients' mouth with a high fracture resistance temporization material (3M ESPE PROTEMP 4). The prosthesis was then analyzed and evaluated for no occlusal contacts in centric and excursive movements

Patient was recalled six months after surgery for final prosthesis.

Results

Descriptive statistics mean, standard deviation were calculated. Unpaired t test was used to calculate ISQ values between the Square shaped and V shaped design. Repeated measures ANOVA was used to compare ISQ values within the square shaped and also within V shaped design. $P<0.05$ is considered to be statistically significant. Microsoft Excel and IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp was used for statistical analysis.

Table 1: Implant Stability between two groups at baseline, three months and six months-Buccal

	Group	N	Mean	Std. Deviation	t	P	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	Square shaped thread design	10	78.20	5.245	3.726	0.002	3.838	13.762
	V shaped thread design	10	69.40	5.317				
3 months	Square shaped thread design	10	75.20	5.712	2.821	0.011	1.864	12.736
	V shaped thread design	10	67.90	5.859				
6months	Square shaped thread design	10	80.00	4.807	3.473	0.003	3.279	13.321
	V shaped thread design	10	71.70	5.832				

Buccal

The mean and standard deviations of the implant stability at baseline, three months and six months was compared. The mean ISQ increased in square shaped thread design and it differed significantly at baseline (P=0.002), three months (0.0110) and at six months (0.003)

Table 2: implant stability between two groups at baseline, three months and six months-lingual

	Group	N	Mean	Std. Deviation	t	P	95% Confidence Interval of the Difference	
							Lower	Upper
Baseline	Square shaped thread design	10	78.00	5.375	3.535	0.002	3.448	13.552
	V shaped thread design	10	69.50	5.380				
3 months	Square shaped thread design	10	75.30	5.908	2.627	0.017	1.501	13.499
	V shaped thread design	10	67.80	6.828				
6months	Square shaped thread design	10	80.00	4.807	3.713	0.002	3.777	13.623
	V shaped thread design	10	71.30	5.638				

Lingual

The mean and standard deviation of the implant stability at baseline, three months and six months was evaluated. The mean ISQ increased in square shaped thread designs compared to vshaped thread designs and it differed significantly between the groups at baseline (P=0.002), three months

(P=0.017) and at six months (P=0.002)

Table 3: implant stability in square shaped thread design from baseline to six months- Buccal

	Mean	Std. Deviation	N
Baseline	78.20	5.245	10
3 months buccal	75.20	5.712	10
6months buccal	80.00	4.807	10

F=47.250 P<0.001

The data obtained from the group 1 so revealed that ISQ in square shaped thread design differed significantly from baseline to six months.

Table 4: Implant stability in square shaped thread design from baseline to six months- Lingual

	Mean	Std. Deviation	N
Baseline	78.00	5.375	10
3months lingual	75.30	5.908	10
6 months lingual	80.00	4.807	10

F=41.609 P<0.001

The data obtained in table 4 so revealed that the mean ISQ in square shaped thread design lingually differed significantly with a P value of 0.001.

Table 5: Implant stability in V shaped thread design from baseline to 6mths-Buccal

	Mean	Std. Deviation	N
Baseline	69.40	5.317	10
3 months buccal	67.90	5.859	10
6months buccal	71.70	5.832	10

F=11.101 P=0.001

The data obtained from table 5 revealed that ISQ in v shaped thread design from baseline to six months differed significantly

Table 6: Implant stability in V shaped thread design from baseline to 6 months- Lingual

Lingual

	Mean	Std. Deviation	N
Baseline	69.50	5.380	10
3months lingual	67.80	6.828	10
6 months lingual	71.30	5.638	10

F=6.457 P=0.008

The data obtained in table 6 so revealed that the ISQ lingually differed significantly with a P value of 0.008 in group II.

Discussion

The field of implant dentistry has undergone rapid evolution in the last two decades. The original machined surface implant advocated by Branemark required a healing period of three to six months prior to placement of a definitive prosthesis. Advances in surface texture and implant geometry coupled with patient's demand for a faster treatment, have resulted in shorter healing periods and predictable osseointegration.[3] Thus loading protocols have also been modified. It is a well-known fact that dense bone provides better stabilization of the implant fixture leading to a more predictable osseointegration. In cases of immediate loading any occlusal or muscular forces applied to the prosthesis is transmitted to the surrounding bone through its implant fixture. Thus in case of immediate loading with areas of dense bone such as the maxilla factors such as implant design assumes a greater role.[45]

The angle formed between the lines drawn from the long axis of the implant to the thread face is termed the face angle.[15] It has been reported that with increase in the face angle, shear stress is increased. According to various studies, V shaped thread design has an angle of 30 degree. Thus Greater forces are generated by the V shaped thread design and due to the decreased face angle of square shaped threadstheyminimizethe stressactingon animplant.[4,6,7]

Interspace between the major and minor thread diameter is the thread depth.it is agreed that shallow thread depth aids in easier implant placement. Enhanced bone contact can be achieved due to the increase in the functional surface area of the threads frequently seen in square shaped threads.[4] Deeper threads also proved beneficial for the placement of implant in soft bone and regions of high occlusal load.

The current study included a total of 20 patients divided into two groups of 10 each. Implant stability was later assessed at baseline, three months and at six months. An initial ISQ of 78.20±5.245 for group 1 and a mean ISQ of 69.40±5.317 was achieved respectively which was appreciable for immediate loading as suggested by studies done previously. Several studies have proved the influence high primary stability on the success rate of implants thus providing information in detecting failing implants.[8]

It was also observed that the stability at baseline was considerably higher for group I when measured buccally and lingually with a mean ISQ of 78.20±5.245. the result obtained was in level with the study performed by Torrella et al which achieved a higher primary stability with tapered square shaped thread in comparison to the cylindrical implants with v shaped thread design. Thus it can concluded that implant design plays a crucial role in implant stabilization the reason being deeper thread patterns that allows for a large surface area for bone to contact. Thus thread design has an influence on osseointegration.[46]

The mean ISQ for group I and group II comparatively decreased at 3 months 75.20±5.712 and 67.90±5.859 respectively when measured buccally and 75.30±5.908 and 67.80±6.828 when measured lingually. The results obtained werein relation to the study conducted by Glauser et al where the implant stability quotient dropped at three months due to the extensive remodelling that takes place.[8]

However all implants were clinically stable at six months with a mean ISQ of 80±4.807 and 71.70±5.832 when measured buccally with no difference in the lingual measurement ISQ of 80±4.807 and 71.3±5.638

The stability of implant fixture in the bone was assessed using resonance frequency analysis. The results obtained revealed a significant rise in stability of group I and group II implants from baseline to six months i.e. the mean ISQ for group I was observed to increase from 78.2 to 80 and 69.4 to 71.7 for group 2.`

The results reported were in accordance to the study conducted by Sennerby et al where after the drop in ISQ at three months there was a gradual increase in between 3-12 months which is the possible time for bone remodelling cycle to occur. Glauser et al have reported that this change was due to the interfacial stiffness of the bone and the bone to implant interface. The probable reason given by the authors for low implant stability at three months was due to the relaxation of the stress between the bone and implant that was created during implant insertion.[8]Sennerby et al claim that this can be also due to the regular healing pattern of the cortical bone in response to the surgical wound created. According to a study the entire time frame for bone remodelling takes about 4 months. [28]

Other than the mechanical determinants required for stabilizing the implant bone density also plays an essential role in immediate loading.in the present study both the group of implant were placed in the maxillary posterior region and the bone is said to be softer in this region. Thus it was found that the fixtures showed a higher initial stability contradicting various other studies. With regards to the assessment of stability no errors were detected in the resonance frequency analysis. The stability quotients were consistent at initial, third and six months post implant insertion. Thus in accordance to the study conducted by Huang et al it can be conclude that magnitude of load in regions with boor density can be affectively estimated by resonance frequency analysis.

Conclusion

In conclusion the data obtained from the present study reveals establishment of a good primary stability is essential for the success of immediate loading and thereby is influenced by various other factors such as the quality of bone and implant geometry.it can also be concluded that in regions of unpredictable initial stability increasing the surface area of an implant with deeper thread design with a smaller pitch or face angle can aid in a predictable outcome. The stability assessed by resonance frequency was reliable and reproduced the implant stability with accuracy. The device showed a decrease in ISQ in region of compromised implant positions thereby reporting that it was greatly influenced by bone density and implantposition.

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Ethical Clearance: Taken from the instituitional committee

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Modes, Manner of deaths and types of injuries in Warangal Municipal Limits

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Abstract

Accident is an unexpected, unplanned occurrence which may involve injury or it may be defined as an unpremeditated event resulting in recognizable damage. Railway related injuries are not those uncommon occurrences in forensic practice. Among the varied presentation of injuries, superficial injuries along with fractures were commonly observed. Over the last 15 years many railway accidents have happened in Andhra Pradesh and in India. Following these train accidents, there has been a large amount of public debate about safety management on the Indian railways. These accidents have raised issues regarding the effectiveness of the safety management of the railway system. This paper presents a summary of the results of a preliminary systemic analysis of several rail accidents in and around Warangal City. The present study was conducted in the Department of Forensic Medicine, Warangal Medical College, Warangal from January 2013 to June 2014 i.e., 12 months, during which the total postmortem cases were 74 occurred in the jurisdiction of the Govt. Railway Police Station, Warangal. The factors taken to enumerate the study are cause of death, manner of death, relationship between scene of offense and cause of death, relation of intoxication status to the manner of death, age versus manner of death and period of survival.

Key words: *analysis, damage, factors, management, superficial injuries, preliminary*

Introduction

Evolution is never ending process. Mankind has evolved from primates' eras back. The same Human is trying to develop over the yesteryears by inventing things, which have mechanical advantages. One of them are the Locomotives. Every comfort has some impending dangers within them. Similarly, the moving trains are also proving fatal.[1]

The structure and designing of the train is made so safe that it is not easily possible to sustain injuries by the train, unless there is gross negligence or willful act is made to have such injuries. The areas of the movement of train are also made safe by providing several protective measures but still deaths are occurring on the railway tracks. Several commissions are made by the Governments to look in to these factors which are unsafe for public use. In spite the people living near and around the tracks are so negligent and erratic in using the track, it is always unsafe for these people.[2]

The injuries and deaths due to accidents are inescapable in the modern way of living. The accidental deaths are mostly due to the road traffic accidents but the deaths due to railway fatalities are also not negligible, especially in the areas where railway traffic is higher. A train accident is defined as a "collision, derailment, or any other event involving the operation of on-track equipments." Train accidents can cause devastating damages and personal injuries including the death of the person. Trains are frequently involved in accidents that critically injured passengers and innocent bystanders. These accidents are indeed disastrous and catastrophic due to the speed that trains travel.[3]

Materials and Methods

This study has been carried out from January 2013 to June 2014 from the ethical committee of Kakatiya

Medical College, Warangal, Andhra Pradesh. The present study was conducted in the Department of Forensic Medicine, Warangal Medical College, Warangal from January 2013 to June 2014 i.e., 18 months, during which the total postmortem cases were 74 occurred in the jurisdiction of the Govt. Railway Police Station, Warangal, Andhra Pradesh. The records maintained for each case in this department are post mortem requisition given by Investigating Officer in their inquests, treatment records from hospital if treated, history from blood relatives and friends, observation of the circumstances at the scene by visiting the scene of offence, photographs taken from the scene of offence and findings in the Post mortem Examination certificates. The materials used are inquest Reports, inpatient Case sheets, per usual of police papers, data from district crime records bureau (DCRB), Warangal. Records from Medical Record Section of Kakatiya Medical College, Warangal, Andhra Pradesh. Post mortem Reports of all cases, Information collected from the Investigation Officer, Relatives and friends of the deceased accompanying dead bodies.

Observations and Results

Warangal is one city which is well connected to all parts of the country by railway line. Kazipet is one of the biggest Railway Junctions. Govt. Railway Police Station is situated in Warangal Railway station and the outpost of this Police Station is located in Kazipet. The number of accidents in the jurisdiction of these stations is also increasing day by day. The same amount of load will be reflected on to the mortuary staff working in this jurisdiction. There are 74 deaths occurred in the jurisdiction of the Govt. Railway Police Station, Warangal. All of them are subjected to Post mortem examination in the mortuary of Kakatiya Medical College, Warangal from January 2013 to June 2014.

In this study from Figure No. 1 it can be cleared that Suicides are common in younger age groups and accidents are common in elderly people. More suicides occurred in the age group of 21 to 50 years and more accidents occurred in 51 to 90 years of age groups. "Multiple Injuries" is the leading Cause of Death including in "suicides". This is in contrast to the decapitation observed as a method of suicide in the western countries. Isolated Head injury is the next leading Cause of Death in suicides. Surprisingly there is one "Accidental Decapitation" found in the present study. As many as 51 "suicides" occurred during the study period because of railway injuries. Among them "Multiple Injuries" are leading over "Head Injury" and "Decapitation" and Traumatic amputation of Trunk". Among the 23 "Accidental" deaths 14 are because of "Multiple Injuries" and 7 are due to "Head Injury" and one decapitation. There is no homicidal death seen in the present study occurring on railway track.

In this study from Figure No. 2, out of 74 cases, 26 persons of the total 44 identified deceased are from rural areas. This needs a special concern that, the track needs fencing in the rural areas

62 persons died in deserted areas, and 36 persons died in the night, which shows that, the railway track needs a vigilance to reduce these deaths.

As many as 71 dead bodies are first seen by the railway employees. This is because the deaths are witnessed by the drivers, are the gang-men or key-men making rounds on the track.

In figure 3, out of 74 cases, intoxication status could not be made properly, however it is "Zero" in the present study. This may be because of subjecting the dead bodies for Post mortem Examination after 3 days of death, as they are kept for identification during this period. As many as 51 suicides occurred during the study period because of railway injuries. Among the 23 accidental deaths 14 are because of multiple Injuries and 7 are due to head Injury and one decapitation. There is no homicidal death seen in the present study occurring on railway track.

In Table 1, age versus manner of death is showed. Out of 74, suicides are common in younger age groups and accidents are common in elderly people. More suicides occurred in the age group of 21 to 50 years and more accidents occurred in 51 to 90 years of age groups. More number of deaths is occurring in young aged persons i.e. from 21 to 40 years of age, with the mean age 34.5 year. This is less than the age group observed in America which is 39 years. This shows the economic loss to the nation because of the person losing their lives in their productive age group.

In Table 2, the period of survival is discussed. Except for one person, who survived of accidental fall and sustained multiple Injuries, and died after 12 hours of admission. All other died on the spot on the track. Many of them are unattended. This indicates the severity of the railway injuries.

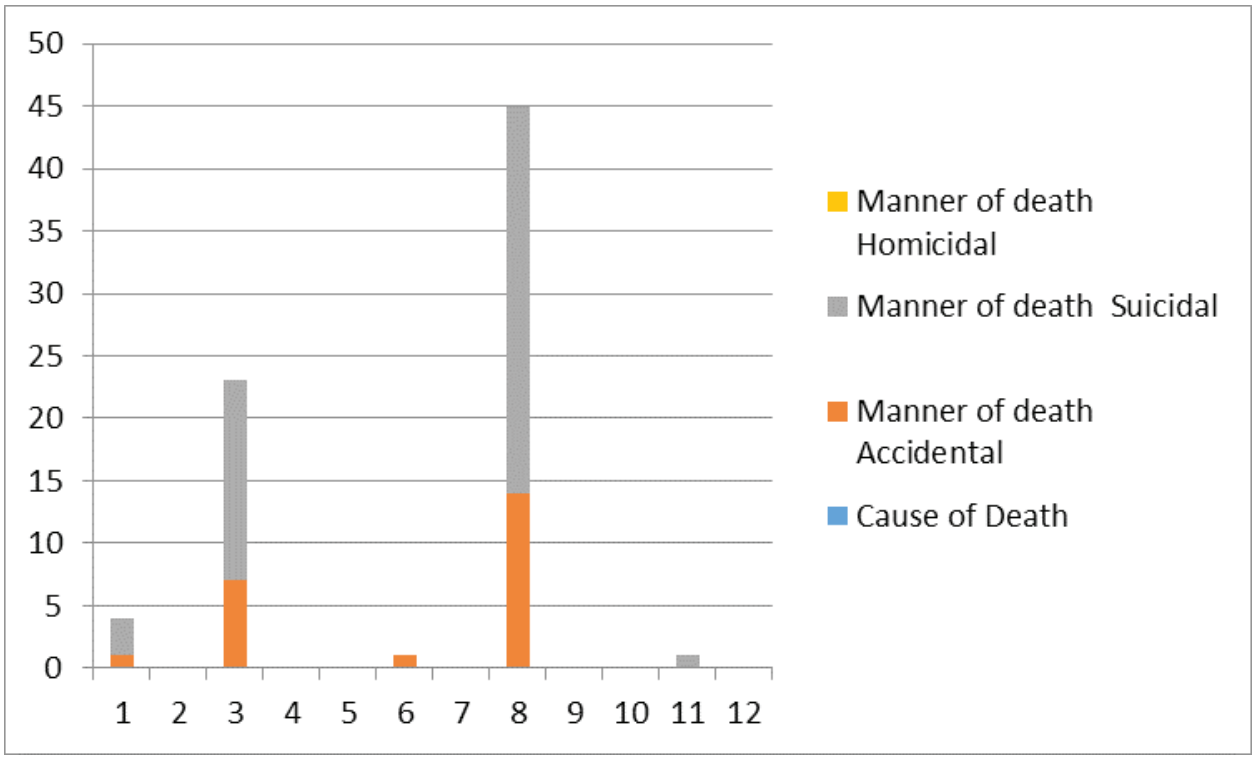


Figure 1: Causes of death and manner of death

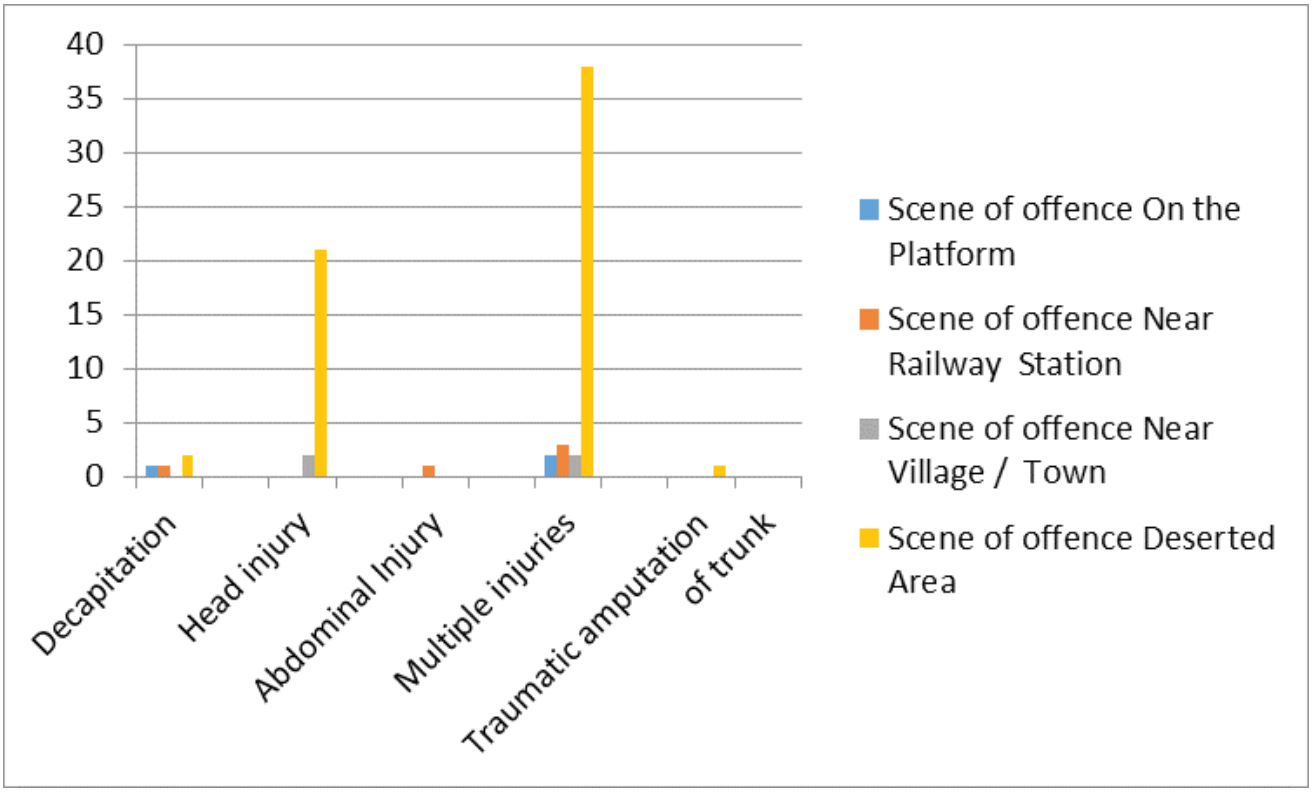


Figure 2: Relation between scene of offense and cause of death

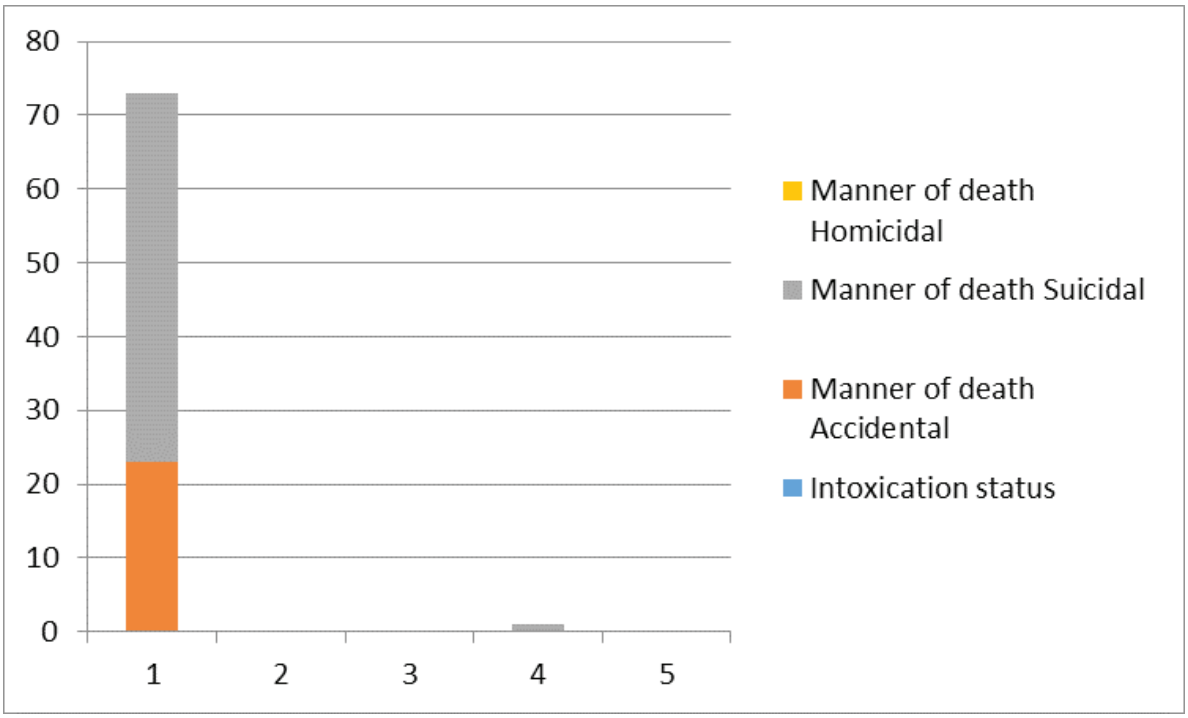


Figure 3: Relation of intoxication status to the manner of death

Table 1

Age	Manner of death			Total
	Accident	Suicide	Homicide	
0 to 11 yr	0	0	0	0
11 to 20 yr	0	2 (3.92%)	0	2
21 to 30 yr	5 (21.74%)	17 (33.33%)	0	22
31 to 40 yr	4 (17.39%)	19 (37.25%)	0	23
41 to 50 yr	5 (21.74%)	5 (9.8%)	0	10
51 to 60 yr	2 (8.70%)	4 (7.84%)	0	6
61 to 70 yr	2 (8.70%)	3 (5.88%)	0	5
71 to 80 yr	4 (17.39%)	0	0	4
81 to 90 yr	1 (4.35%)	0	0	1
91 to 100 yr	0	1 (1.96%)	0	1
Total	23 (100%)	51 (100%)	0	74

Age versus manner of death

Table 2

Cause of Death	Period of survival					Total n (%)
	Immediate death	< 6 hours	6 to 24 hours	1 to 3 days	>3 Days	
Decapitation	4	0	0	0	0	4 (5.4)
Head injury	23	0	0	0	0	23 (31.08)
Abdominal Injury	1	0	0	0	0	1 (1.35)
Multiple injuries	44	0	1	0	0	45 (60.81)
Traumatic amputation of trunk	1	0	0	0	0	1 (1.35)
Total	73 (98.65)	0	1 (1.35)	0	0	74 (100)

Period of survival in relation to cause of death

Discussions and Suggestion

Death is one inevitable event the human life, can result from natural or unnatural means. It can be result of injuries sustained. Injuries caused by a fast-moving object can result in death. Especially trivial injuries produced by fast moving trains can result in fatality.

According to Bernoulli's Principle, when an object moves a greater velocity, it produces a low pressure in its path. Trains moving greater speed also produce a low pressure in their path and suck the objects towards them because they are placed in high pressure area and result in fatal injuries.[9] That is why a red painted area is made on the margins of the platform of Railway stations, so that people should not enter in to it when a train comes on to the platform.[4]

In spite of the precautions and the preventive measures adopted by the Railway authorities, still many deaths are occurring on the track because of the railway related injuries.

The evidence is clearly shown in the present study by getting 74 deaths in whom railways (Trains) Produced injuries resulting in the death of persons. The annual incidence of railway related deaths is coming to 2.65% (44 deaths in the year 2013 to the total of 1661 Post mortem Examinations done), this is almost equal to the study made in Germany and much less in comparison to the rest of south east Asian countries. [3,4,5]This is because the magnitude of railway related accidents may be same throughout the world, if the track is accessible to the people, but in Warangal geographical area, the suicidal methods adopted differ from rest the habitat. In this area people are committing suicides by poisoning more than other methods.

When we compare the Cause of Death with the manner of death, it is found that, in 31 (41.89%) cases of suicides, there are multiple injuries. In 16 (21.62%) cases of suicides, there are head injuries. In 3 (4.05%) cases of suicides, there are decapitations. In one case (1.35%) of suicide there is traumatic amputation of trunk.[7]

As many as 71 dead bodies are first seen by the railway employees. This is because the deaths are witnessed by the drivers, are the gang-men or key-men making rounds on the track. Suicides are common in younger age groups and accidents are common in elderly people. More suicides occurred in the age group of 21 to 50 years and more accidents occurred in 51 to 90 years of age groups.

Suggestions

· All railway related deaths are invariably fatal in nature and are preventable by strict vigilance of railway staff on the track.

· There should be proper vigilance on the railway tracks about the suspiciously moving persons on the track, by the gang men.

· Level crossing should be properly maintained round the clock.

· Impatient driving of the vehicle across the track should be watched out by CCTVs footages and corrected time to time

· Trespassing should be penalized with heavy penalties, to discourage the movements on the track

· Proper fencing should be made in the areas of movement of people

· Grazing domestic animals on the railway tracks should be discouraged

· suicidal spots on the track should identified and properly protected

· Infrared beams should be provided to identify the movement of the people on the railway track

· Platforms should be provided with proper height to boarding and alighting.

Conclusion

Most of the railway fatalities were accidental in nature and in the bread earning age group particularly among the males. The increasing number of population, overcrowding in the trains, reckless and careless behavior of the passengers, pedestrians and the train drivers towards safety norms are the constant causes of railway fatalities. The high levels of the railway fatalities make a strong case for the necessary accident control interventions. Public as well as the railway authorities must take some measures to bring down these fatalities. People must follow some easy set of laws like do not travel on footboard, do not enter or get down from running trains, do not try to cross the level crossing gate when it is closed, be alert and reduce your speed while approaching railway unmanned level crossing, never guess the speed of the train and adhere to the set norms of railway safety to curb this menace. By comparing the data of the present study of 2013 with that of the previous studies, the incidence of railway accident cases are gradually increasing in number day by day.

Ethical Clearance: This study has been carried out in the year 2013, after from the ethical committee of Kakatiya Medical College/General Hospital, Warangal.

Source of Funding: Self

Conflict of Interest: Nil

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Pattern of Suicidal Deaths in the First Month of Lockdown at A Tertiary Care Hospital: a Time Trend Analysis

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Abstract

Lockdowns and quarantines were set up in various countries around the world to combat the Corona virus pandemic. The pandemic has its mental health consequences which have been aggravated by the lockdown measures. The resulting psychological distress and depression has ultimately led to suicides. This study explores the trends of suicide in the month of the lockdown and compared it with those to the prior months. The study was conducted between 25th January 2020 to 24th April 2020 and March 25th 2019 to April 24th 2019 at Cooch Behar Government Medical College and Hospital, Cooch Behar. 52.1% cases of suicide were recorded in the month of lockdown which increased from 34.1% in February 2020 and 43.1% in March 2020. Most cases of suicide in the period of lockdown were in the second and third decade of life with a male predominance. For the period of lockdown, housewives, businessmen and labourers were found out to be the vulnerable population. A social and public health response in addition to a mental health response is crucial to prevent suicidal behaviour in lockdown period.

Keywords: *COVID-19 suicide, Lockdown, COVID-19 mental health, Economic recession.*

Introduction

With the turn of the New Year, the entire world was slowly engulfed by the pandemic of the novel corona virus. To combat this highly contagious virus, different countries came up with lockdowns and quarantines for various spans of time, India not being an exception. Lockdown is a situation in which people are not allowed to enter or leave an area freely because of an emergency, in this case the corona virus pandemic. The first case of COVID 19 in India was reported in the state of Kerala on 30th January 2020 and in West Bengal on 17th March 2020.[1,2] A nationwide lockdown was imposed in India on the 25th March 2020.

As evident in some of the previous pandemics, COVID 19 is also having some grave psychological consequences. People are being riddled with fear, anxiety and chaos. The impending isolation from near and dear ones petrify people. They become panic stricken. And this panic contagion demoralizes people who are overwhelmed by emotions leading to hopelessness and desperation. Incidentally, these psychological changes often spread its ugly wings with suicides being the fatal outcome.

Every year, almost one million people die from suicide.[3] According to WHO, every 40 seconds a person dies by committing suicide somewhere in the world.[4] And there have been evidences that suggest deaths by suicide had increased during pandemics.

In the Indian context, the NCRB data suggests that overall a total of 1,34,516 suicides were reported in 2018, which showed an increase of 3.6% in comparison to 2017.[5] Our state of West Bengal accounted for 13,255 of these suicides in 2018.[5] No specific data were obtained from our place of study i.e. Cooch Behar. In our present study we have tried to find out the number of suicides in the month of the lockdown and its prevalence as compared to the previous two months and the same

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time frame from the previous year.

Methodology

This observational, descriptive, comparative and analytical study was conducted in the department of Forensic Medicine and Toxicology, Cooch Behar Government Medical College and Hospital. The period of study was from 25th January 2020 to 24th April 2020 and March 25th 2019 to April 24th 2019. All autopsies conducted during this period of time except the unknown decomposed bodies and the obscure autopsies were considered in this study.

The cases for the year of 2020 were divided into three months viz. March 25th 2020 to April 24th 2020 (the first month of the lockdown – April 2020), February 25th 2020 to March 24th 2020 (the month prior to the lockdown – March 2020) and January 25th 2020 to February 24th 2020 (couple of months prior to the lockdown – February 2019). The cases from the month of lockdown was also compared to the same time period from last year i.e., March 25th 2019 to April 24th 2019 (April 2019), to decipher if there is any variations owing to seasonal changes or climatic changes.

Detailed history of the case was initially noted from the inquest reports, bed head tickets, injury report and other relevant documents provided. Informations were gathered from the deceased's close relatives, friends, police and other available persons who were present at the time of the incidence and those accompanying the victims, with special reference to general information like Name, Age, Sex and Occupation. The suspected causes of death and modes of death were preliminary noted from the investigating report submitted by the police i.e., the inquest paper. These were further corroborated with the autopsy findings, the information shared by the deceased's relatives and other available persons and the place of occurrence visit in some cases.

The data collected during this study was tabulated in a pre determined proforma. It was further analyzed in tabular form along with its representation in form of diagrams and charts.

Results

A total of 335 cases which fulfilled the inclusion and exclusion criteria's were recorded.

There were 85 recorded cases in February 2020 out of which 45 (52.9%) of them had an accidental manner of death while 29 (34.1%) of them were suicidal (Figure 1). During March 2020, most of the cases were suicidal (43.1%) which was followed by accidental deaths (40.3%). During the month of lockdown (April 2020), 96 cases were recorded considering the inclusion and exclusion criteria's. Out of these, there were 50 (52.1%) suicidal cases, 27 (28.1%) accidental cases and 14.6% natural cases. During the same period last year (April 2019), 82 cases were reported, out of which 40.2% was suicidal and accidental each.

Of the number of people committing suicide in the period of February 2020, 33.3% were between the age group of 21-30 years followed by 23.3% in the group 11-20 years (Table 1). But in the next period March 2020, 25.8% of people in the age group of 51-60 years committed suicide while 22.6% of people were in the 11-20 and 21-30 years bracket. In the lockdown period (April 2020), 26% of those committing suicide were between the age group of 21- 30 years and 31- 40 years each followed by 18% in the age group of 41-50 years. While in April 2019, about one third of those committing suicide were between 21 -30 years.

A female predominance among the number of suicides was seen only in year of 2019 where 51.5% females committed suicide (Table 2). An increasing trend of suicide among the males was seen in 2020 .In the first period, i.e. February 2020, 51.7% males committed suicide. In the next period 58.1% males committed suicide and 54% males succumbed in April 2020.

In the Table 3, it was observed that, housewives account for most of the persons committing suicide. Number of housewives committing suicide increased from 31% in February 2020 and 25.7% in March 2020 to 17 (34%) in the month of lockdown. 16% of business men and 14% of labourers committed suicide in April 2020.

Hanging was the common method to commit suicide in all the months, 80% during the month of lockdown, 72.7% in April 2019 and 67.7% and 65.5% in the months of March and February of 2020 respectively. The other notable methods in the month of lockdown were self-ingestion of poison 10% cases and self-immolation 4% cases. (Table 4)

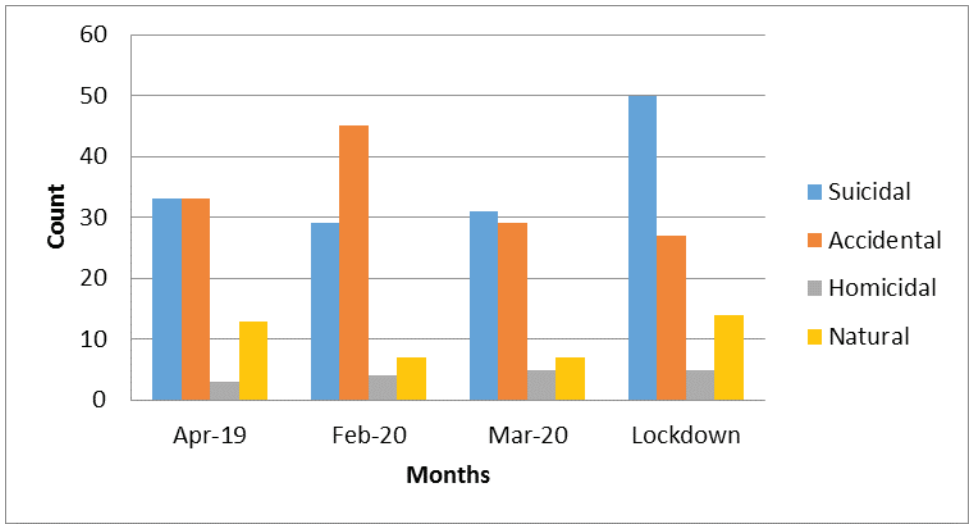


Figure 1: Manner of death in relation to months.

Table 1: Age wise distribution of suicidal cases.

Age group	April 2019	February 2020	March 2020	April 2020
Below 10 years	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
11 to 20 years	7 (21.2%)	7 (24.1%)	7 (22.6%)	6 (12.0%)
21 to 30 years	10 (30.3%)	10 (34.5%)	7 (22.6%)	13 (26.0%)
31 to 40 years	3 (09.1%)	4 (13.8%)	4 (12.9%)	13 (26.0%)
41to 50 years	3 (09.1%)	0 (0.0%)	3 (09.7%)	9 (18.0%)
51 to 60 years	4 (12.1%)	4 (13.8%)	8 (25.8%)	5 (10.0%)
61 to 70 years	4 (12.1%)	3 (10.3%)	1 (03.2%)	3 (06.0%)
Above 70 years	2 (06.1%)	1 (03.4%)	1 (03.2%)	1 (02.0%)

Table 2: Sex wise distribution of suicidal cases.

Sex	April 2019	February 2020	March 2020	April 2020
Female	17 (51.5%)	14 (48.3%)	13 (41.9%)	23 (46.0%)
Male	16 (48.5%)	15 (51.7%)	18 (58.1%)	27 (54.0%)

Table 3: Occupation wise distribution of cases of suicidal cases.

Occupation	April 2019	February 2020	March 2020	April 2020
Farmer	6 (18.2%)	4 (13.8%)	5 (16.1%)	3 (06.0%)
Labourer	3 (09.1%)	4 (13.8%)	5 (16.1%)	7 (14.0%)
Service men	1(03.0%)	2 (06.9%)	2 (06.5%)	1 (02.0%)
Business men	2(06.0%)	2 (06.9%)	2 (06.5%)	8 (16.0%)
Students	4(12.1%)	3 (10.3%)	2 (06.5%)	5 (10.0%)
Housewife	10 (30.3%)	9 (31.0%)	8 (25.7%)	17 (34.0%)
Unemployed	5(15.2%)	4 (03.4%)	5 (16.1%)	7 (14.0%)
Unknown	2(06.0%)	1 (03.4%)	2 (06.5%)	2 (04.0%)

Table 4: Distribution of cases according to methods of suicide.

Cause of death	April 2019	February 2020	March 2020	April 2020
Burn	3 (09.1%)	5 (17.2%)	3 (09.7%)	2 (04.0%)
Hanging	24 (72.7%)	19 (65.5%)	21 (67.7%)	40 (80.0%)
Poisoning	4 (12.1%)	4 (13.8%)	6 (19.4%)	5 (10.0%)
Others	2 (06.0%)	1 (03.4%)	1 (03.2%)	3 (06.0%)

Discussion

The mental health effects of any pandemic is bound to be profound, the corona virus disease COVID 19 not being an exception. The lockdown following this pandemic has even adverse effect on the general population. They feel as if they have been sentenced for an unknown crime to an indeterminate period of punishment. And this feeling of impending doom has led to a surge in the suicide rates.

The number of cases of suicide has drastically increased from 34.1% and 43.1% in the couple of months prior to suicide to 52.1% during the first one month of the lockdown following the pandemic. This is in concurrence to studies in Hong Kong where cases of suicides did show an alarming rise during the 2003 SARS pandemic and the USA during the 1918-19 influenza pandemic.[6,7]

Most of the subjects committing suicides were in the middle age group with 52% in the age bracket of 21-40 years (26% in each decade) and another 18% within 41-50 years of age. These age groups denote the working class or those people on whom the family burden in the utmost. The thought of how they are going to support

their families in such a period of crisis has made people anxious. Panic attacks have increased, depression has surged and people in such a stressful situation are opting for suicide as the only way out. This is in concurrence to Srivatsava et al, who identified presence of a stressful life event as definite risk factors for attempting suicide.[8]

The other notable group who have been particularly affected are those who are addicted to consumption of alcohol or other substances. Most of them belong to the age group of 21- 50 years. Due to the closure of most shops, it has been difficult for them to procure these with ease. It inevitably leads to withdrawal symptoms like insomnia, tremors, palpitations, restlessness and suicidal ideation. These symptoms look identical to those in depressive episodes, often presenting with similar levels of hopelessness and ultimately compel people to commit suicide.[9]

Lockdown has also seen a surge in the number of domestic abuse cases, worst affected are the children and women who are now trapped inside their homes with their abusers. Men who are either unemployed or frustrated being home often take to abusing their wife and children. The Women commission of West Bengal have also stated that cases of domestic violence have increased since the imposition of lockdown.[10] And domestic violence is one of the risk factors for suicide. In a study conducted at Bengaluru, it was found that in more than one-third of females, the major risk factor for suicide was domestic abuse.[11] Our study shows that about 46% of those who committed suicide during the lockdown period were females. There was also a rise among the housewives committing suicide (34%) during the period of lockdown. A study by Banerjee et al also supported the above findings and stated that the commonest cause of suicide in women was quarrel with husband.[12]

Apart from housewives, those involved in business and the labourers comprise the next major groups in which the suicidal rates have increased. It goes without saying that the lockdown has led to unemployment especially among the labourers and daily wage earners. People have had their business touching a slump. They don't know how they will get back on their feet, even when the isolation measures are lifted. And loss of employment and financial stressors are perceived as risk factors for suicide.[13] In the United States, during the period of recession from 2007 -2009, the bleak job market and economic crisis lead to an estimated 4750 excess suicide deaths.[14] Our study is also in trend with those in Europe which indicates a significant rise in suicides from the economic recession, totalling more than 1000 excess deaths in UK alone.[15,16]

There is no denying the fact that suicide is likely to become a more pressing concern as the pandemic spreads. It is bound to have long term negative effects on the economy, the vulnerable groups and the general population as whole. Preventing suicides therefore needs urgent action and serious consideration. Mental health initiatives focussing on educating the public on how to best deal with immense pressure and anxiety should be established immediately. Targeted mental health surveillance of population at risk, including patients with prior mental health diagnosis and elderly should be implemented and followed by effective interventions to minimize suicidal thoughts. Governments should consider providing financial grants for food and unemployment support. The media has an immensely vital role to play. They should refrain from irresponsible media reporting of suicide and highlight on the precise facts about the causes and circumstances of suicide with due consideration to mental health problems. [17] It is vital that they adhere to the existing and COVID 19 specific guidelines regarding reporting of such suicidal deaths.[18] Further well designed and large scale studies are required to look deeply in to the risk factors for suicide and formulate proper preventive measures.

Conclusion

The COVID 19 pandemic and the nationwide lockdown have no doubt caused distress and have left thousands susceptible to mental health problems and suicidal behaviour. Our study is only a harbinger of what's to come. The suicidal surge may even persist for longer and actually peak later than the actual pandemic. Thus, the need of the hour is a well-coordinated multi departmental and multi phased approach to curb this surge.

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Ethical Clearance: Taken from institutional ethics committee.

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Radiological Study of Ossification Centres Around Pelvis to Determine Their Age of Appearance and Fusion among Bengali Population – A Retrospective Study

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Abstract

Introduction and background: Radiological data of appearance and fusion of ossification centres are mostly used to determine age of individuals and compare with chronological age for medicolegal purposes. X-Ray of pelvis is very important for elderly adolescent age group. Objectives: This study has been conducted to study the age of appearance and fusion of ossification centres around pelvis for known chronological aged subjects from Bengali population and compare them with known standards. Methods: In this retrospective study x-ray films of 74 females and 220 males of 8 to 21 years age group from the period of 1st January 2017 to 31st December 2019 have been observed. Data analyzed in Microsoft office excel 10 and presented in the form of table and diagram. Conclusion: The study reveals age of appearance and fusion of ossification centres around pelvis in Bengali population which corroborate with standard values in some cases and in some do not match. It will help to estimate age of individuals for medicolegal purpose.

Key words *– Radiological, medicolegal, adolescent, pelvis, tri-radiate cartilage, iliac crest*

Introduction

Estimation of age is relevant from both civil and criminal point of view in medicolegal practice. Identification of an individual, establishing criminal responsibility, juvenile crime, participation in sports are some major fields where age estimation is required to be done. For this purpose we have to depend mostly on dental and radiological data. The appearance of ossification centres is completed around 5 years and after this fusion acts as calendar upto the age of 25[15]. Where X- ray of wrist and elbow helps to determine age in children and early adolescents, x-ray of pelvis is mostly done for older adolescents. For this purpose still we are dependent upon age old data. Moreover as we know that skeletal development is influenced by nutritional status, environmental condition and other factors, studies on different geographical area are necessary in present scenario. The present study has been carried out among 220 males and 74 females of Bengali subjects between the age group of 8 to 21 years. Our objectives are to observe status of appearance and fusion of ossification centres around pelvis for a known chronological age, to determine range of their appearance and fusion in males and females and to compare them with known standards.

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Materials and Methods

It was a cross sectional study. The study was carried out in Dept. of FSM, Medical College Kolkata over a period of six months. State of appearance and fusion of ossification centres of iliac crest, ischial tuberosity, head of femur, greater trochanter, lesser trochanter and tri-radiate cartilage were examined. X-Ray plates of all 220 males and 74 females who attended the department for age estimation between 1st January 2017 and 31st December 2019 were studied in a Census sampling except those cases who have fallen under exclusion criteria. Stated age of the subjects were confirmed from age proof documents. Epiphysis of said bony structures

were examined to observe

1. Whether appeared
2. Whether fusion started
3. Whether fusion is in process
4. Whether fusion completed

Epiphyseal scar at the junction of epiphysis and metaphysis has been considered as indicator of complete fusion [14[.

Results and Observation

220 males (8 to 21 years) and 74 females (8 to 16 years) were divided into 13 and 9 groups respectively with 1 year interval between each group. Number of male and female subjects in different age groups is respectively as follows:

8 yrs – 3 and 6 (total 9), 9 yrs – 22 and 10 (total 32), 10 years – 20 and 9 (total 29), 11 years – 18 and 11 (total 29), 12 years – 36 and 21 (total 57), 13 years – 29 and 6 (total 35), 14 years – 29 and 5 (total 34), 15 years – 20 and 2 (total 22), 16 years – 21 and 4 (total 25), 17 years – 18 and 0 (total 18), 18 years – 1 and 0 (total 1), 19 years – 1 and 0 (total 1), 21 years – 2 and 0 (total 2)

Appearance of iliac crest:

Among females, radiological evidence of appearance have been detected between 11 to 16 years of age. Iliac crest appeared in 36.49% of total sample. 100% subjects showed evidence of appearance at the age of 13, 15 and 16. In males, 43.18% showed evidence of appearance at and beyond 12 years of age. 100% subjects showed evidence of appearance at the age of 15 years and above. **(Table 1)**

Fusion of Iliac crest:

Among females, none showed evidence of complete fusion. Fusion did not start in any subject before 12 years. Fusion started among 100% subjects at 15 and 16 years of age whereas process of fusion has been noticed between 12 to 14 years. Among males, fusion completed in only 2.27% subject (at the age of 19 and 21). Fusion did not start upto 11 years of age whereas evidence of fusion process has been noticed between 12 to 18 years of age. **(Table 1)**

Appearance of Ischial tuberosity:

It did not show radiological evidence of appearance upto 11 years of age in both genders. In 100% cases it appeared at 15 and 16 years of age in females and at 18, 19 and 21 years of age in males. **(Table 2)**

Fusion of Ischial tuberosity:

No evidence of complete fusion in females and 2.25% of complete fusion in males among whom the data was 100% at the age of 21. Fusion did not start upto 11 years of age in females and 12 years of age in males. In females, process of fusion has been noticed between 12 to 16 years of age and in males its 13 to 19 years.. **(Table 2)**

Disappearance of tri-radiate cartilage:

In females, upto the age of 10 years, tri-radiate cartilage was present in all cases. It was disappeared in 100% cases at the age of 14, 15 and 16. Among males, it was detected upto 11 years of age in 100% subjects whereas disappeared in 100% cases at the age of 17, 19 and 21 **(Table 3)**

Appearance of head of radius:

It appeared in all subjects earliest at the age of 8 years. **(Table 4)**

Fusion of head of femur:

Fusion started in all subjects except one 10 yrs old female. Complete fusion took place in 35.14% of female population (100% at the age of 15 and 16 years). Process of fusion noticed between 8 to 14 years age. In males, fusion was complete in 100% subject of 18, 19 and 21 years of age whereas process of fusion has been detected in 61.36% males between the ages of 8 to 17. **(Table 4)**

Appearance of Greater Trochanter:

All subjects have shown evidence of appearance of greater trochanter. **(Table 5)**

Fusion of Greater Trochanter:

In females fusion did not start in only one and completed in 18.92% individuals between the age group of 12 to 16. Process of fusion has been noticed between 8 to 15 years of age group. Among males 24.55% of

sample showed evidence of complete fusion between the age of 13 to 21 and it was 100% at 18, 19 and 21 years age. Process of fusion has been observed in 150 males between the age of 8 to 17. **(Table 5)**

Appearance of lesser trochanter:

Evidence of appearance has been observed in 78.38% females (mostly 12 to 16 years) and 77.27% males (mostly 14 to 21 years) **(Table 6)**

Fusion of lesser trochanter:

In females 100% showed complete fusion at 15 and 16 years of age. 47.30% males showed process of fusion between 8 to 14 years. Among males, complete fusion was detected in 100% population at 18, 19 and 21 years of age. Process of fusion was detected in 42.73% subjects between 8 to 17 years age. **(Table 6)**

Table 1: Age (in years) of appearance and fusion of Iliac Crest (n = 294)

Age (yrs)	Not appeared		Appeared		Fusion not started		Fusion in process		Fusion completed	
	F	M	F	M	F	M	F	M	F	M
8	6	4			6	3				
9	10	22			10	22				
10	9	20			9	20				
11	9	18	2		11	18				
12	11	31	10	5	17	33	4	3		
13		20	6	9	5	27	1	2		
14	2	9	3	20	2	24	3	5		
15		1	2	19		10	2	10		
16			4	21		8	4	13		
17		1		17		3		13		2
18				1				1		
19				1						1
21				2						2

Table 2: Age (in years) of appearance and fusion of Ischial Tuberosity (n = 294)

Age (yrs)	Not appeared		Appeared		Fusion not started		Fusion in process		Fusion completed	
	F	M	F	M	F	M	F	M	F	M
8	6	3			6	3				
9	10	22			10	22				
10	9	20			9	20				
11	11	18			11	18				
12	15	34	6	2	19	36	2			
13	4	27	2	2	6	27	3	2		
14	2	17	3	12	2	25		4		
15		8	2	12	1	14	1	6		
16		4	4	17		9	4	11		1
17		3		15		4		13		1
18				1				1		
19				1				1		
21				2						2

Table 3: Age (in years) of disappearance of tri-radiate Cartilage (n = 294)

Age (yrs)	Present		Absent	
	F	M	F	M
8	6	3		
9	10	22		
10	9	20		
11	8	18	3	
12	10	33	11	3
13	2	22	4	7
14		12	5	17
15		2	2	18
16		1	4	20
17				18
18		1		
19				1
21				2

Table 4: Age (in years) of appearance and fusion of Head of Femur (n = 294)

Age (yrs)	Not appeared		Appeared		Fusion not started		Fusion in process		Fusion completed	
	F	M	F	M	F	M	F	M	F	M
8			6	3			6	3		
9			10	22		3	10	19		
10			9	20	1	3	7	17	1	
11			11	18		3	8	13	3	2
12			21	36		1	12	34	9	1
13			6	29		2	3	24	3	3
14			5	29			1	15	4	14
15			2	20				6	2	14
16			4	21				3	4	18
17				18				1		17
18				1						1
19				1						1
21				2						2

Table 5: Age (in years) of appearance and fusion of Greater Trochanter (n = 294)

Age (yrs)	Not appeared		Appeared		Fusion not started		Fusion in process		Fusion completed	
	F	M	F	M	F	M	F	M	F	M
8			6	3			6	3		
9			10	22		2	10	20		
10			9	20	1	5	8	15		
11			11	18		3	11	15		
12			21	36		4	18	32	3	
13			6	29		2	4	26	2	1
14			5	29			1	22	4	7
15			2	20			1	10	1	10
16			4	21				3	4	18
17				18				4		14
18				1						1
19				1						1
21				2						2

Table 6: Age (in years) of appearance and fusion of Lesser Trochanter (n = 294)

Age (yrs)	Not appeared		Appeared		Fusion not started		Fusion in process		Fusion completed	
	F	M	F	M	F	M	F	M	F	M
8	4	2	2	1	4	2	2	1		
9	8	18	2	4	8	19	2	3		
10	3	16	6	4	4	19	5	1		
11	1	10	10	8	5	14	6	4		
12		3	21	33	1	4	14	32	6	
13		1	6	28		6	4	21	2	2
14			5	29			2	18	3	11
15			2	20				9	2	11
16			4	21				3	4	18
17				18				2		16
18				1						1
19				1						1
21				2						2

Discussion

Age old Galstaun chart is followed till now for estimation of skeletal age. In this study we tried to produce a standard reference range of age for appearance and fusion of ossification centres around pelvis so that it can help to corroborate skeletal age and chronological age of Bengali population in present scenario. Such studies among males and females have been conducted in many parts of India but it's not a common one among Bengali population in recent years.

As of conventional data, in our study ossification centres appeared early in females compared to males[15[. Though earliest age of appearance of Ischial Tuberosity is same (12 years) in males and females according to present study 100% appearance occurred earlier in females. Similarly fusion also completed earlier in females except Ischial Tuberosity.

In our study appearance of iliac crest started by 11 years in females and 12 years in males and completed by 15 years and 17 to 18 years in females and males respectively. Starting age of appearance corroborates with finding of Srinivasa Naik M et al[10] but completion age is less. Findings of our study also nearly matched with finding of Bhise S.S. et al [2]. We could not draw any conclusion regarding fusion of iliac crest in females, in males it completed by 19 to 21 years which corresponds with result of Patel G et al[9].

Considering Ischial Tuberosity, appearance completed by 15 to 16 years in females and 18 to 19 years in males. It corresponds with findings of Srinavasa Nik M et al [10] for females and corroborates with finding of Bhise S.S et al [2] for males. Here also we could not draw any conclusion for age of fusion for females but in males, fusion completes by 21 years which is less than the finding of Sing P et al [3] but corroborates nearly with study of Joshi A et al[4].

As per present study, tri-radiate cartilage completely disappeared by 14 years in females and 17 years in males which corroborates with finding of Shilajiya et al [8] and Galstaun G[13] for females. It does not correspond with the findings of Bangal RS et al [5].

Fusion of head of femur completes by 15 years in females and 18 years in males. The value corroborates with Galstaun G [13] for females. Value corresponds with findings of Srinivasa Naik M et al [13] and Sharma Y et al [1].

Greater trochanter fused by the age of 16 in females and 18 in males. Value does not corroborate with findings of Galstaun G [13]. Value corroborates with finding of Sharma Y et al [1].

In present study lesser trochanter fuses by 15 years in females which corroborates with Galstaun G but its 18 years for males which does not corroborates with Sharma Y et al [1] but corroborates with Srinivasa Naik M et al [10].

Conclusion

Such a study among both males and females on this large sample size was required in West Bengal. By this study we reached a conclusion about age of appearance of ossification centres around pelvis which vary in many cases from age old data which is considered till date. The data recommended by us can be taken as reference in future for the purpose of age estimation among elderly adolescent group.

Conflict of Interest: Nil

Ethical Clearance: Obtained from Institutional Ethics Committee, Medical College Kolkata

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A Quasi Experimental Study to Assess the Effectiveness of Assertiveness Training Program on Self Esteem and Interpersonal Communication Satisfaction among Nursing Students in Selected Nursing Institutes of Ambala, Haryana

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Abstract

Background: Assertiveness is an important behavior for today's professional nurse. As nurses move away from traditional roles and perceived stereotypes, it is increasingly being recognized that a nurse needs to behave in an Assertive manner. Assertiveness is necessary for effective nurse/patient communication, it is suggested that its development may also aid the confidence and self esteem. So aim of the study to evaluate effectiveness of assertiveness training program on self esteem and interpersonal communication satisfaction among nursing students of selected institutes of Ambala Haryana.

Methodology: Quasi-Experimental research design "Non equivalent control group pre test-post test design was used. Data was collected from 60 nursing students (30 in experimental and 30 in comparison) by convenience sampling technique. Standardized Rosenberg self esteem Scale and Interpersonal Communication Satisfaction Inventory were used to collect data.

Conclusion: The 't' test analysis showed a significant difference before and after the administration of Assertiveness training program. in terms of self esteem(t=11.78 and p=0.001) and Interpersonal communication satisfaction (t=12.78 and p=0.001) at 0.05 level of significance. Repeated measures ANOVA shows that there is a significant difference within the group in terms of self Esteem (F=4.45, p value=0.002) and Interpersonal Communication Satisfaction (F=56.60, p value=0.001) in experimental group.

Key Words: *Effectiveness, Assertiveness training program, Self Esteem, Interpersonal Communication Satisfaction, Nursing students.*

Introduction

Today`s nursing students are the nucleus of the professional nurses of tomorrow, who will interact with their colleagues and other health care professionals on a daily basis and provide the care for patients, their families and society in the area of health and education in the future. Nursing students need more Assertive because they interact in different setting like they work in multidisciplinary team in which he/she play important role.[1]

Assertive behavior may be encouraged through educational methods or training programs. It is preferable that nurses receive this educational preparation during undergraduate programs. Assertiveness is important for a healthy self-esteem and for your overall wellbeing. By developing assertiveness skills and improving confidence and self-esteem it can be easier to navigate through the systems and to have better outcomes both for nurse and patient.[2]

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Most of the research evidences found that Assertiveness is closely associated with self-confidence, self-esteem, anxiety and especially school and colleges anxiety, low level of self esteem and poor interpersonal communication. Poor assertiveness can raise the academic anxiety and academic failure. It also leads to learning disabilities and decreased efficiency in students. Assertiveness is a learnable skill and mode of communication. Assertive students work in a confident manner. It is important for a students to be assertive, so that he or she may express his thoughts and feelings effectively. Increasing your assertiveness can help you both personally and professionally. This article focuses the importance of being assertive.[3]

Student nurses on a daily basis interact with other patients, colleagues, doctors and other staff, because the nature of nursing work require a high degree of personal and group interaction. It is crucial that nurses and students develop assertiveness skills so that they can provide safe and effective care for patients.[4] Expressing positive and negative feelings honestly and straightforwardly, without anxiety or intimidation. This can also allow the nurses to improve their care to their clients, peers, and even themselves. Nurse educators are the leaders of the next generation of nurses, and it is vital that they motivate their students to express their opinion and personal rights.[5]

The nature of nursing demands that nursing students face various stressful situations, and the relationship between self-esteem and the quality of nursing care is undeniable. In nursing, it is suggested that self-esteem is one of the most influential factors on occupational behavior of nursing students. Accordingly, there is no doubt in the significance of self-esteem among nursing students.[6]

According to our review of the literature, it seems that the majority of studies on nursing students' self-esteem have focused on determining the level of self-esteem or strategies to improve it and most of the studies suggest that in initial year of the course nursing students may have chances of low self esteem and problem in interpersonal communication because of changing environment from home to hostels or school to colleges.[7]

Nursing practice should have a commitment to caring directed to oneself, caring directed to others and caring directed to life itself and to well being. This kind of perspective is one of the bases of human existence and is inherent to all human beings. This process of caring cannot exist without the sharing of information and feelings and a very close relationship between the nurse and his/her patient. The main aim of this study is to assess the effectiveness of assertiveness training program on self esteem and interpersonal communication among nursing students.[8]

Material and Method

The study was conducted during the period from October 2018 to April 2019 in Haryana, India. Total 60 students were selected from two institutes (30 in experimental and 30 in comparison group) by using convenience sampling technique. Assertiveness training was provided to experimental group (Assertiveness training program has a set of 7 sessions. This include several teaching Methods i.e lecture cum discussion with the help of power point presentation, brain Storming, and examples from real life, Modeling ,Role playing, getting Participants feedback, providing feedback, and Assigning Homework).Data was collected by using standardized Rosenberg self esteem scale and interpersonal communication satisfaction inventory.

Description of Tool

Part- A – (Performa for socio demographic variables).

It consisted of items regarding socio demographic variables. The selected variables included the age , gender ,type of family, monthly income of family, education status of father, , education status of mother, occupation of father, occupation of mother, current year of the study, percentages of marks obtained in higher secondary, choice of course selection**,** hobbies, socioeconomic status.

Part-B (The Rosenberg Self –Esteem Scale).

It is a standardized tool that contains -10 items and developed by sociologist Dr. Morris Rosenberg to assess the self-esteem of the nursing students .Each statement had five responses it was rated on 4 point Likert scale.

Part (C) Interpersonal Communication Satisfaction Inventory

Interpersonal communication satisfaction inventory is a self-report which included 20 items used to Interpersonal communication satisfaction measures in nursing students. The maximum score of Interpersonal communication satisfaction inventory was 100 and minimum was 20.

Data Analysis

(a) Descriptive statistics:

· Frequency and percentage distribution to describe selectedvariables.

· Frequency , percentage , mean, mean percentage , Standard Deviation and range of score to describe the self esteem , Interpersonal communication satisfaction.

(b) Inferential statistics:

· Karl Pearson's correlation was used to check the relationship between self esteem and interpersonal communication satisfaction among nursing students.

· Anova and T-test was used to check the association of selected sample characteristics with self esteem and interpersonal communication satisfaction among nursing students.

Result

Data were entered in Microsoft excel, and analysis was done with SPSS version 20. Result of the study shows that Assertiveness training program is effective in improving the self esteem and interpersonal communication satisfaction among nursing students.

Table 1: Mean, mean difference, standard deviation of difference, Standard Error of Mean Difference and 't' value of Self Esteem among Nursing students after Assertiveness training in experimental and comparison group.

N=60

Variable	Group	Mean ± S.D.	MD	SEMD	't' value	df	P Value
Self Esteem post-test1	Experimental group(n=30) Comparison group(n=30)	16.10±1.85 10.30±1.93	5.76	0.48p	11.78	58	0.001*
Self Esteem post-test2	Experimental group(n=30) Comparison group(n=30)	15.10±1.82 10.50±2.25	4.60	0.53	8.68	58	0.001*

$^{t}(58)= 1.67$ *- significant ($p \leq 0.05$)

Table.1 depicts the level of self esteem among nursing students after administration of assertiveness training program. Independent t test was applied and the mean difference between experimental group and comparison was found to be statistically significant (t=11.78,p=0.001) at 0.5 level of significance and in post test II Independent t test score was (t=8.68,p=0.001).

Hence it inferred that assertiveness training program is effective in improving the self esteem of the nursing students

Table 2: Mean, mean difference, standard deviation of difference, Standard Error of Mean Difference and 't' value of Interpersonal communication satisfaction among Nursing students after Assertiveness training in experimental and comparison group.

N=60

Variable	Group	Mean ± S.D.	MD	SEMD	't' value	df	P Value
Interpersonal Communication Satisfaction post-test I	Experimental group(n=30) Comparison group(n=30)	69.63±6.40 48.60±6.33	21.03	1.64	12.78	58	0.001*
Interpersonal Communication Satisfaction post-test II	Experimental group(n=30) Comparison group(n=30)	70.90±6.21 48.47±7.38	22.43	1.76	12.92	58	0.001*

*- significant ($p \leq 0.05$)

Table.2 depicts the level of Interpersonal Communication Satisfaction among nursing students after administration of assertiveness training program. Independent t test was applied and the mean difference between experimental group and comparison was found to be statistically significant (t=12.78,p=0.001) at 0.5 level of significance and in post test II Independent t test score was (t=12.92,p=0.001).Hence it inferred that assertiveness training program is effective in improving the Interpersonal Communication Satisfaction of the nursing students**.**

Table-3: Repeated measures ANOVA showing the significant difference within groups in term of Self Esteem in the experimental group and comparison

N=60

Group	Test	Mean	F value	P value
Experimental group(n=30) Pre test Post test	Pre test Post test-1 post test-2	14.57 16.10 15.07	4.45	0.002*
Comparison group(n=30) Pre test Post test	Pre test Post test-1 post test-2	10.30 10.50 11.47	1.97	0.15NS

NS -Not significant (p>0.05) *- significant ($p \leq 0.05$)

Table 3 depicts repeated measures ANOVA showing the significant difference within the group in terms of self esteem. In experimental group computed F value was 4.45,p=0.002 which was statistically significant at 0.05 level of significance, where as in comparison computed F value was 1.97, p=0.15 which was statistically non significant at 0.05 level of significance.

Table 4: Repeated measures ANOVA showing the significant difference within groups in term of Interpersonal communication satisfaction in the experimental group and comparison.

N=60

Group	Test	Mean	F value	P value
Experimental group(n=30) Pre test Post test	Pre test Post test-1 post test-2	52.57 69.63 70.90	56.60	0.001*
Comparison group(n=30) Pre test Post test	Pre test Post test-1 post test-2	48.07 48.60 48.47	0.06	0.63NS

NS -Not significant (p>0.05) *- significant (p ≤ 0.05)

Table 4 depicts repeated measures ANOVA showing the significant difference within the group in terms of Interpersonal communication satisfaction. In experimental group computed F value was 56.60,p=0.001 which was statistically significant at 0.05 level of significance, where as in comparison computed F value was 0.06, p=0.63 which was statistically non significant at 0.05 level of significance.

Table 5: **Correlation between Self esteem and Interpersonal communication satisfaction scores among Nursing students** **N=60**

Correlation(pre test)	Self Esteem r (p value)	Interpersonal communication Satisfaction(r and p value)
Self esteem and Interpersonal communication Satisfaction	XX	0.09 (0.48 NS)

NS -Not significant (p>0.05) r(58)=0.27

Discussion

The findings of the present study stated that In experimental group mean post-test-1 Self esteem score (16.10±1.85) was higher than that of mean pre-test score (14.57±2.09), where as in comparison group, mean post-test-1 Self esteem score 10.30 ±1.93. It is also observed that mean post- test scores of experimental group was higher than mean post-test scores of comparison group .The findings of the present study is consistent with the study conducted by Nisha et al on effectiveness of Assertiveness training program in improving self esteem among nursing students which shows that in experimental group mean post-test self esteem score (20.97±3.07) was higher than that of mean pre-test score (11.47±2.58), where as in control group , mean post-test self esteem score (14.57±2.09) was almost similar to the mean pre- test score (11.47±2.58). It is also observed that mean post-test scores of experimental group was higher than mean post-test scores of comparison group.

Conclusion

The study concluded that Assertiveness training program is effective in improving the self esteem and interpersonal communication satisfaction among nursing students.

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Awareness of MBBS Students on Effects of Workplace Violence and Conflict Management in Hospital Scenario

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Abstract

According to World Health Organisation, healthcare workers are four times more prone to injuries due to workplace violence compared to other professions. An ongoing study by the Indian Medical Association inferred that about 75% of doctors have faced violence at work, where many of the cases were reported from Emergency wards and Intensive Care Units and the patient's relatives were the prime offenders. Poor doctor-patient communication and weak conflict resolution skills are some of the preventable reasons for violence on doctors. A questionnaire-based study was conducted on MBBS students to assess the effects of violence on doctors and their level of preparedness in dealing with violent situations. Majority of the students were not taught precautionary measures or methods of managing conflicts and thus are not properly equipped in tackling workplace violence. By improving their manner of conduct towards the patients in likely incidents of violence and training them in managing conflicts, such acts shall be curbed effectively.

Keywords: *Awareness, Violence, MBBS Students, Protective Laws, Effects of Violence, Conflict Management*

Introduction

Workplace violence against healthcare workers is known as violence in healthcare settings, it refers to the violent acts that are directed towards doctors, nurses or other healthcare staff at work or on duty.[1] Rise in violence against doctors is a global phenomenon. Doctors in both developing as well as developed countries are plagued by this issue alike. In Australia, a survey showed that in the last three years, there has been a 48% increase in assaults whereas, in China about 85% of doctors had experienced violence in their workplace and in America a 2018 survey showed that out of, 47% of the emergency physicians had been assaulted.[2] 60% of General Physicians in England and about 50% of doctors in Germany were confronted with aggressive behaviour, with 10% of them experiencing critical to violent attacks, such as criminal damage to property and/or physical assault. Nowadays, it has become a fashion for patient attenders to resort to unruly forms of violence against doctors and it has become a common phenomenon across all the States of India. Herd mentality along with political influence are main causes behind mobs attacking doctors with some time lapse after the inciting incident, indicating that these may be planned strikes and not merely random acts committed in the heat of the moment.[3] Exorbitant costs of treatment at private hospitals combined with poorly maintained government hospitals are causing more dissatisfaction among people and the constant portrayal of doctors in bad light by media have added to the discontent.

According to a study conducted in China, every additional article on violence against doctors lead to 0.6% decrease in students enrolling in medical courses.[4] Studies show that doctors exposed to violent

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incidents suffered from depression, reliving experience or flashbacks and insomnia.[5,6] Poor doctor-patient communication and weak conflict resolution skills are some of the preventable reasons for violence on doctors. Hence, there is a need to improve communication between the patient and doctor and impart required training skills to the medical students to handle workplace violence. The main objective of this study is to assess MBBS students on the effects of violence on doctors and their level of preparedness in dealing with violent situations.

Methodology

This was a cross sectional prospective study done over a period of 2 months from May to June 2020. Questionnaire (dichotomous and multiple-choice questionnaire) was distributed through online survey administration application by using google forms which were forwarded to the students from all medical colleges across the states of Telangana and Andhra Pradesh. Depending upon the effective response in completing the forms within the given time period of two months, 405 MBBS undergraduate students belonging to three academic years i.e 2nd, 3rd and final year MBBS, were included in this study. The data was analysed in *IBM SPSS Statistics* software and expressed as numbers and percentages and presented in the form of text, tables and figures.

Results

Table -1: Awareness on violence

Variables		Frequency	%
About rise in violent incidents on doctors	Yes	404	99.8
	No	1	0.2
Awareness prior to joining MBBS course	Yes	198	48.9
	No	207	51.1

In spite of the continuous rise in violence on doctors, 282 (69.6%) of the participants would recommend others to join MBBS while, 123 (30.4%) of them would not. 326 (80.4%) of the students consider that specialities dealing with emergencies are more prone to violence, 23 (5.7%) consider all clinical branches to be at risk and 52 (12.9%) of them consider all doctors are at risk irrespective of their speciality. 1 % of them did not know the answer to the question posed. Considering certain specialities to be more vulnerable to violence, 323 (79.8%) would not avoid choosing these specialities in their future career but, 82 (20.2%) would avoid them. With the incidence of violence more in government hospitals, 133 (32.8%) participants would not opt for government service in future but, 272 (67.2%) would not be deterred by this.

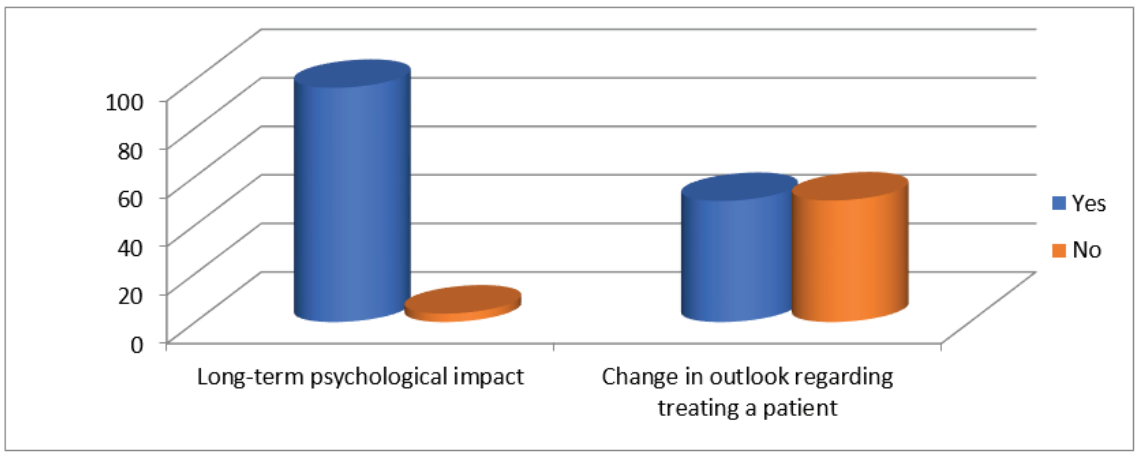


Fig 1: Effects of Violence on Doctors (in %)

Table 2: Level of Preparedness

Variables		Frequency	%
Training in managing violent incidents	Yes	80	19.8
	No	325	80.2
Precautionary Measures	Yes	96	23.7
	No	309	76.3
Audio-visual recordings in patient counselling rooms as evidence of violence	Yes	346	85.4
	No	5	1.2
	Don't Know	54	13.3
Awareness of any National and State Laws protecting doctors	Yes	166	41
	No	239	59

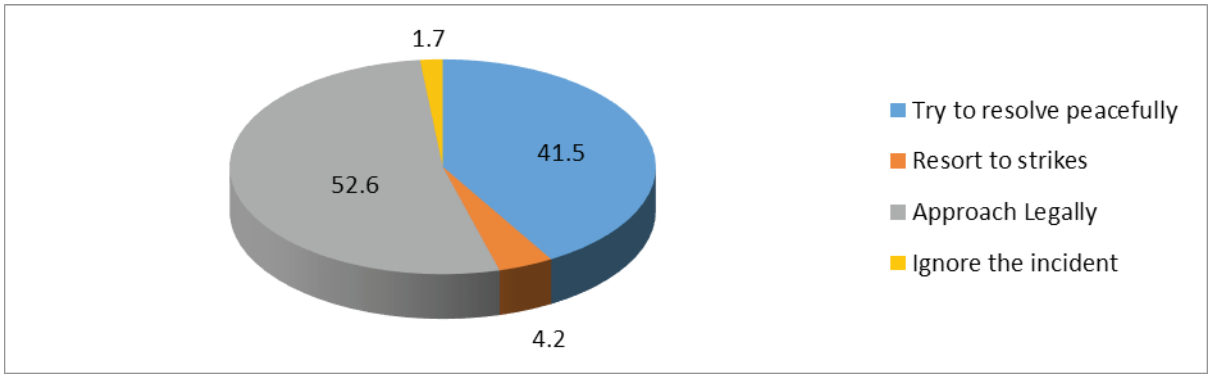


Fig 2: Reaction to violent incidents (in %)

Discussion

A survey conducted by the Indian Medical Association showed that about 75% of doctors have faced violence at work in the last 5 years. 48.8% of such incidents were reported from Emergency wards and Intensive Care Units. In 68.33% of these cases; patient's relatives were the prime offenders.[7] 99.8% students were aware of the rise in incidence of violence on doctors. But, only 51.1% of them were aware of these incidents before joining MBBS. This is due to lack of awareness on the issue in public despite the continuous rise in violent incidents against doctors in the last decade. 30.4% of them would not advice others to consider MBBS as a career option. According to The Economic Survey 2019-2020, as of January 2020, there is one doctor for every 1456 people in the country which is less than the WHO recommendation of 1 doctor for every 1000 population.[8] To meet this goal, India would need 5 lakh more doctors. If the growing violence on doctors deters young generation to not consider medicine as their profession, this would lead to severe shortage of doctors.

80.4% consider doctors specialised in Emergency Medicine, General Medicine, Surgery, Obstetrics & Gynaecology and Anaesthesia (specialities dealing with emergencies) to be most vulnerable for attacks. 5.7% of

the participants consider all clinical specialities are at risk of violence. 12.9% say irrespective of their speciality, all doctors including junior residents are at high risk of facing violence. A study of occurrence of violence in various departments, in a Tertiary Care Hospital of South Delhi revealed that, 59.6% of respondents experienced violence in Department of Obstetrics and Gynaecology followed by the medical departments (including General Medicine, Psychiatry, Paediatrics, Dermatology, Physical medicine and Rehabilitation) and surgical departments. Other departments like Casualty, Radiology, Forensic medicine and Pathology reported incidents of violence too. [9] 20.2% of the participants in the current study said they would not choose specialities which are more prone to violent attacks.

The average cost of treatment at private hospitals is almost five times more than in government hospitals and as about 85% of Indians from rural areas and 82% of urban residents are not insured under any public health scheme, due to which the government institutes are overburdened.[10] Moreover, anxiety due to long waiting periods, poor infrastructure, unhygienic and crowded conditions are some of the reasons for increased incidents of violence in the primary health centres and government hospitals.[11] Considering this, 67.2% of the students were not deterred by this issue but, 32.8% of them responded that they do not want to join government service. In the current scenario, majority of Indian doctors, about 9 lakh are involved in private hospitals as opposed to just about 1 lakh doctors employed in the government hospitals.[12] If students do not join government service in future, this would add to the burden of already understaffed government setups.

In study conducted by M.A. Zaid et al, in Kuwait, 86% of 87 doctors exposed to violent incidents reported one or more of the symptoms consisting of stress, depression, insomnia, reliving violent experiences. The duration of symptoms was longer in doctors exposed to verbal insults or threats of imminent violence coupled with single act of violence.[5] In a study conducted by Gohil RK et al, 50% of participants who faced violence felt fearful, frustrated and 25% developed recurrent headaches following the events.[6] 96.5% of the participants think that such incidents will have a long-term psychological impact. 49.9% of the students consider that increasing violence on doctors will change their outlook while treating a patient. Doctors are becoming cautious and reluctant to take up serious and grave cases, which in turn compromises healthcare defeating the purpose of the healthcare delivery system.[13] If the doctors are exposed to continuous violence, many of them might start practicing defensive medicine, thus focusing on saving themselves rather than treating a patient.[14] The survey conducted by Indian Medical Association found that, fear of violence was the main source of stress for as many as 46.3% of the doctors surveyed, followed by fear of being sued which was 24.2%. About 62.8% of the doctors were not able to treat their patients without any fear of violence.[13] In the study by Kumar M et al, 75.8% of the doctors interviewed admitted that any kind of workplace violence affected their state of mind which in turn had an impact on their studies, duties and also personal life.[9]

Majority of the students i.e. 80.2% were not aware of any methods to tackle workplace violence. In Kumar et al study, majority of participants believed that the violent incidents could have been prevented and they were not aware of any violence prevention policy at their workplace.[9] Only 6.4% of the respondents had received some kind of training for recognizing and preventing workplace violence of which all of them were from the Department of Psychiatry where conflict management is a part of their curriculum and denied attending any special training program.[9] Medicos should be taught to identify early indicators of violent behaviour such as **s**taring and eye contact, **t**one and volume of voice, **a**nxiety, **m**umbling and **p**acing (STAMP) and methods that help can resolve conflicts.[14] 76.3% of the students say that they were not taught measures that can be taken to prevent violent incidents. The legal importance of communication, consent and proper medical documentation are discussed in forensic medicine, but only in theory. It would be beneficial to include these topics in their practical curriculum and assessing them by conducting live simulations as a recent study in the British Journal of Medicine (BMJ) showed that Indian doctors of public sector spend less than two minutes seeing a patient. In contrast, Swedish doctors spend as much as 22.5 minutes per patient and in the USA, each patient gets 21 minutes of a doctor's time.[10]

When faced with a violent situation, 52.6% of the participants said they would approach legally whereas, 41.5% said they would try to resolve the issue peacefully. Study by Kumar M et al, only 14.6% agreed that the head of the unit took notice of the incidence and approcahed the concerned authorities regarding the matter and in none of the cases police enquiry was initiated.[9] Punishments are not uniform all over India. Without any separate provision on violence against doctors in the Indian Penal Code (IPC) or Criminal Procedural Code (Cr.PC), filing a complaint becomes a hassle, as the police are unaware of the section under which the accused should be booked. So, students themselves should always be well-versed with all the protective laws to approach legally and maintain a copy of it. When approaching legally, proper evidences must be presented in the court to prove that violence has occurred. Photographs of the incident, Audio-Video recordings, copy of medical records, written and signed statements from witnesses can be used as evidences. 85.4% of the participants consider Audio-Visual recordings as proofs of violence, but 13.3% do not know if these can be used as evidence of violence in courts. Protection of Medicare Service Persons (MPA) and Medicare Service Institutions Acts were passed in 19 states across India. This act was passed in Telangana and Andhra Pradesh in 2008.[15] Even so, 59% of the participants were unaware of them.

Conclusion

With a continuing rise in workplace violence against healthcare professionals, a doctor must be well prepared in taking protective measures personally and be able to tackle these untoward incidents. Poor doctor-patient relationship is due to lack of emphasis on communication skills in medical education. The current study shows that medicos are grossly underprepared in handling patient discontent and aggression which may turn chaotic leading to violent incidents. MBBS students should be taught in college to maintain their calm and be responsible during such times without compromising the quality of patient care. Enhancing communication skills and developing strategies to resolve conflicts are crucial to avert unpleasant scenarios. By taking adequate precautionary measures in such likely incidents of violence, improving their manner of conduct towards the patients and substantially training them in managing conflicts, such acts shall be curbed effectively. This can be achieved by introducing this topic in Forensic Medicine curriculum, so that students may have a better knowledge and awareness on various protective laws for doctors. Clinical aspects of Forensic Medicine should be included in the Compulsory Rotatory Internship so that students can practice handling medicolegal cases and also get trained in tackling such violent incidents efficiently.

Ethical Clearance: Taken

Conflict of Interest: Nil

Source of Funding: Nil

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The Effectiveness of Information Education Communication (IEC) on Knowledge and Practices Regarding Patient Safety Measure among Nursing Officers in Selected Hospital of Pune City

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Abstract

Background & Objective: Patient safety is very important in the provision of quality care. Our aim was to assess the Information Education Communication (IEC) on knowledge and practices regarding patient safety measure among nursing officers.

Methods: A Pre-Experimental one group pre-test post-test design was adopted involving 60 nursing officers. After obtaining informed consent nursing officers were allocated by using the Non-probability Convenience sampling technique. IEC module was given to the nursing officers after the Pre-test was taken. An interval of 7 days was given then post-test was obtained. Tool used in the study are Self-Structured Questionnaire on assessment of knowledge and practice.

Results: The average change in the level of knowledge was significantly increase in post-test as compared to pre-test. In pre-test 70% of nursing officers have average knowledge, 28.33% have poor knowledge and 1.67% have good knowledge. The mean knowledge of the nursing officers was 6.37 and standard deviations was 0.71. While in the post-test 78.33% of nursing officers have good post-test knowledge, 21.67% have average knowledge and no nursing officers have poor knowledge. The mean knowledge of the nursing officers was 11.7 and standard deviations 1.75.

The change in the level of practice was also significantly increase in post-test as compared to pre-test. In pre-test 60% of the nursing officers have average practice score, while 36.67% have poor practice and 3.33% have good practice. The mean practice of the nursing officers was 6.35 and standard deviations was 2.37 while in the post-test 53.33% of the nursing officers have good practice, 46.67% have average practice and no nursing officers have poor practice. The mean practice of the nursing officers is 13.5 and standard deviations from to 0.71.

Conclusion: The findings of the study shows that there is highly significant difference between the score of pre-test and post-test. Therefore, and null hypothesis (H_0) is rejected and IEC module is effective.

Key words: *Information Education Communication (IEC), knowledge, practices, patient safety measure*

Introduction or Background

The care givers attempt caring the sick, they inadvertently make mistakes that either worsen the situation of the patient or result in death. These errors are inevitable but with acknowledgement of the possibility of the incidence of errors in the care process, it could be reduced to the minimum.[(1)]

Patient safety is one of the vital component in treating patient and is affected by various factors such hospital environment, the complexity of the quality and quantity of healthcare workforce, medical processes, technology and team work. The nurse is responsible

for all decisions and performance associated with the delivery of a safe level of care. To avoid the errors the staff must be competent in the use of devices and update their knowledge and skills through regular review and assessment of competence. In working to improve patient safety and quality care, the goal is to change the system that is the right thing prevent individuals from committing errors and build high reliability organizations

Safe nursing practice includes an understanding of the legal boundaries in which nurses must function. In 1986 the Government of India passed the Consumer Protection Act (CPA) to protect the consumer against unreasonable practices which create risk or harm. One of the common sources of negligence that have resulted in lawsuits against hospital and nurses is medication error.[2] Even though much care is taken by nurses for administration of medication still medication errors challenge patient's safety during their stay in the hospital.

Medication errors are associated with increasing number of medications, childhood and older age, and specific medications and medications for certain disease states (e.g., musculoskeletal, oncology and immunosuppression, dermatology, ophthalmology, otolaryngology conditions, infections and cardiovascular. [3]

The Joint Commission on Accreditation of Healthcare Organizations (JCAHO) and the World Health Organization (WHO) has estimated that medical errors happen in 1 out of 10 patients every year globally but according to Harvard Study conducted by Professor Jha revealed that 5.2 medical errors happen annually in India and among that, medication and hospital-acquired infection errors are the major ones.

In India, the statistical figure shows 400,000 deaths due to adverse drug reactions and 720,000 adverse events per annum.[4]

Many studies reveal that education programmes can help the nurses to increase their awareness regarding mediation error.

Material and Methods

A Pre-Experimental one group pre-test post-test design was adopted. Total 60 nursing officers who met the inclusion criteria were selected by using Non-probability Convenience sampling technique. IEC module was given to the nursing officers after the Pre-test was taken. An interval of 7 days was given then post-test was obtained. The study was conducted in the selected hospitals of Pune city. Tool used in the study are Self-Structured Questionnaire on assessment of knowledge and practice.

Results

The analysis and interpretation of the data collected to determine the effectiveness of IEC module on Knowledge and practices among nursing officers is carried out based on objectives set by the researcher taking the level of significance as 0.05.

Table 1: Descriptive statistics on effectiveness of Information Education Communication (IEC) on Knowledge regarding patient safety measure among nursing officers.

n=60

Knowledge level		Frequency		Percentage	
		Pre-test	Post-test	Pre-test	Post-test
Poor	n=60	17	0	28.33	0.00
Average		42	13	70.00	21.67
Good		1	47	1.67	78.33
		Pre-test		Post-test	
Mean		6.37		11.7	
SD		0.71		1.75	

The above table provides data about the effectiveness of Information Education Communication (IEC) on Knowledge regarding patient safety measure among nursing officers. In pre-test 70% of nursing officers have average knowledge, 28.33% have poor knowledge and 1.67% have good knowledge. The mean knowledge of the nursing officers was 6.37 and standard deviations was 0.71. While in the post-test 78.33% of nursing officers have good post-test knowledge, 21.67% have average knowledge and no nursing officers have poor knowledge.

Table 2: Descriptive statistics on effectiveness of Information Education Communication (IEC) on practice regarding patient safety measure among nursing officers.

n=60

Practice level		**Frequency**		**Percentage**	
		Pre-test	Post-test	Pre-test	Post-test
Poor	n=60	22	0	36.67	0.00
Average		36	28	60.00	46.67
Good		2	32	3.33	53.33
		Pre-test		Post-test	
Mean		6.35		13.5	
SD		2.37		0.71	

The above table provides data about the effectiveness of Information Education Communication (IEC) on practice regarding patient safety measure among nursing officers. . In pre-test 60% of the nursing officers have average practice score, while 36.67% have poor practice and 3.33% have good practice. The mean practice of the nursing officers was 6.35 and standard deviations was 2.37 while in the post-test 53.33% of the nursing officers have good practice, 46.67% have average practice and no nursing officers have poor practice. The mean practice of the nursing officers is 13.5 and standard deviations from to 0.71.

Discussion

The above findings of the study is supported by a study conducted by a Systematic Literature Review by on patient safety culture among nurses which was published in December 2014. The main purpose of the study was to investigate nurse's perceptions about patient safety culture and factors that need to be emphasized in order to develop the culture of safety among nurses. A descriptive and cross-sectional design was used. The sample size was 414 registered nurses who are working in government hospital. Survey method was used to collect data. Results shown that the nurses perceived that their supervisor should have more expectation, feedback and communication regarding errors, teamwork across hospital units and hospital handoffs. Nurses who had more years of experience and were working in teaching hospitals had more perception of patient safety culture. The researcher concluded that Learning and continuous improvement, hospital management support, supervisor/

manager expectations, feedback and communications about error, teamwork, hospital handoffs and transitions were found to be major patient safety culture predictors[5]

Conclusion

The findings of the study shows that there is highly significant difference between the score of pre-test and post-test. Therefore, and null hypothesis (H_0) is rejected and IEC module is effective.

Conflict of Interest – Nil

Source of Funding – Self

Code of Conduct – No ethical issue was there in the study

Ethical Clearance – Obtained from Institutional Research Committee

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Study of Profile of Homicidal Deaths in South Chennai- A Retrospective Study

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Abstract

Killing of an individual is the highest level of aggression found in all cultures. The overall risk of suffering a violent death as a result of intentional homicide has been declining steadily for a quarter of a century. In 2017, there were 6.1 homicide victims per 100,000 population worldwide, compared with a rate of 7.4 in 1993. The present study is a retrospective study conducted in Department of Forensic Medicine, Government Kilpauk Medical college in the period Jan 2017 to march 2019. A total of 70 homicidal death cases were analysed retrospectively. In analysing religion 61(87.14%) cases belong to Hindu religion, 33 (47.14%) cases were labourers, 68 (97.14%) belong to nuclear family, Some sort of quarrel or oral arguments was seen in 19 (27.14%) cases, place of death 28 (40%) cases seen in the victim own house. Cause of death is Shock and haemorrhage due to multiple cut injuries was seen in 22 (31.42%) cases. Head is the most common part of the body with injuries seen in 37 (52.85%) cases. Defence wounds seen in 20 (28%) of cases.

Strengthening the rule of law is central to reducing homicide levels. Policies aimed at tackling homicide should address drivers of homicide both at the individual level (such as the age and sex of a person) and at the macro level (such as unemployment, inequality, absence of the rule of law, the prevalence of gender stereotypes in society and the presence of organized crime). Targeted and efficient interventions require a comprehensive understanding of the scale of homicide and its various drivers, which is essential for ensuring that Sustainable Development Goal targets related to violence can be met by 2030

***Key words**: Homicide, Autopsy, Injuries*

Introduction

The overall risk of suffering a violent death as a result of intentional homicide has been declining steadily for a quarter of a century. In 2017, there were 6.1 homicide victims per 100,000 population worldwide, compared with a rate of 7.4 in 1993. While gaps still remain in terms of the quality and availability of national data, these estimates are based on the latest and most comprehensive data submitted by Member States to UNODC up to 2017. According 2018 data of National Crime Record Bureau , 29017 homicidal deaths reported in India. In Tamilnadu 1569 cases reported. Maximum number of cases noted in Uttar Pradesh 4018(1)

The International Classification of Crime for Statistical Purposes (ICCS), developed by the United Nations Office on Drugs and Crime (UNODC), provides a framework for the definition and classification of unlawful killings, both in conflict and non-conflict situations. Homicide is defined in ICCS as “unlawful death inflicted upon a person with the intent to cause death or serious injury”. This statistical definition

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contains three elements that characterize the killing of a person as "intentional homicide": 1. The killing of a person by another person (objective element) 2. The intent of the perpetrator to kill or seriously injure the victim (subjective element) 3. The unlawfulness of the killing (legal element) [2]

Materials and Methods

The present study is a retrospective study conducted in Department of Forensic Medicine, Kilpauk Medical college in the period Jan 2017 to march 2019. All cases brought by police with alleged history of homicide included in the study. All the documents pertaining to the deceased such as history of the case, accident register, inquest report, First Information Report, post-mortem certificate, viscera report, Histopathology reports.

Inclusion criteria:

1. All cases brought for autopsy under section 302 IPC.

2. Cases brought under 174 CrPC, but turned to be homicide after autopsy.

Exclusion criteria:

1. Custodial deaths were excluded from the study.

Results and Discussion

Total of 70 cases were analysed, 47(67.14%) cases deceased was males and in 23 (32.85%) cases females. This is in consistent with the most of the studies conducted in India[3],[4],[7] . In analysing religion 61(87.14%) cases belong to Hindu religion, 5 (7.14%)cases belong to Muslim religion and 3(4.28%) cases belong to Christian religion. In one (1.42%) case religion not known.

In analysing occupation of the deceased, 33 (47.14%) cases were labourers, 16 (%) were self employed, 9(12.85%) were house wife. 7(10%) cases were students, 3(4.28%) were professional. One (1.42%) was unemployed, and occupation was not known in one (1.42%) case

In analysing age of the deceased 25 (35.71%) cases was seen in age group of 19- 30 years. This is consistent with the study conducted by Hugar *et. all* [3] [10] [11]. The high incidence in this age group, the victim may be due to person in this age group are more aggressive, short tempered, and least tolerant. There is a male predominance in most of the age groups [4]. In age group of 31- 40 years 15 (21.42%) cases was seen, In age group of 41- 50 years 12 (17.14%) cases was seen, In age group of 51- 60 years 9 (12.85%) cases was seen. In age group of 61- 70 years 4 (5.71%) cases was seen. Fig:1.

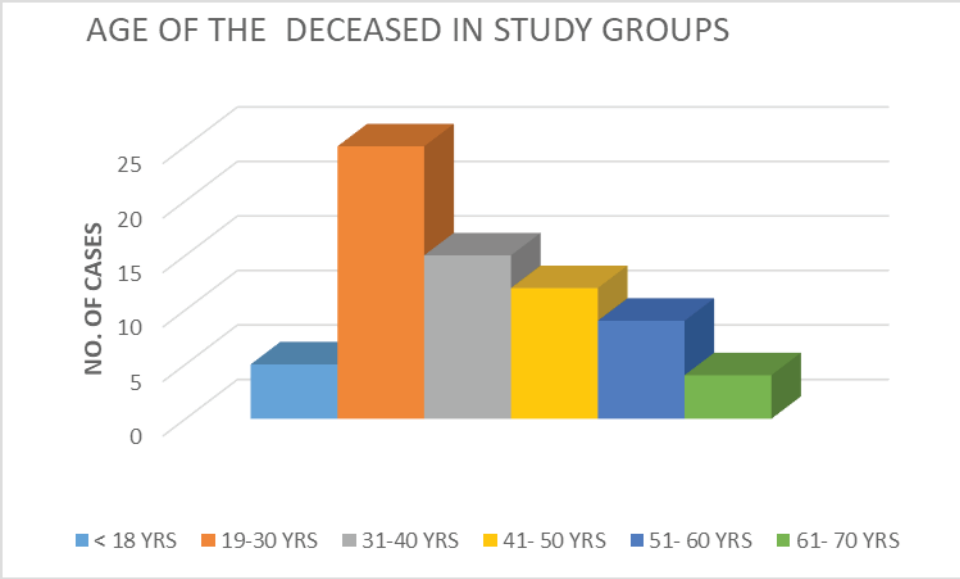


Fig: 1. Distribution of cases among the age groups.

In analysing type of family of the deceased 68(97.14%) belong to nuclear family, one(1.42%) case belong to joined family and one (1.42%) case not known.

In analysing the precipitating factors, dispute related to cash was seen in 14 (20%) cases, previous enemity was seen in 19 (27.14%) cases. Infedility was seen in 14 (20%) cases. Some sort of quarrel or oral arguments was seen in 19 (%) cases. And in 4 (5.714%) cases precipitating factors was not seen. Fig: 2.

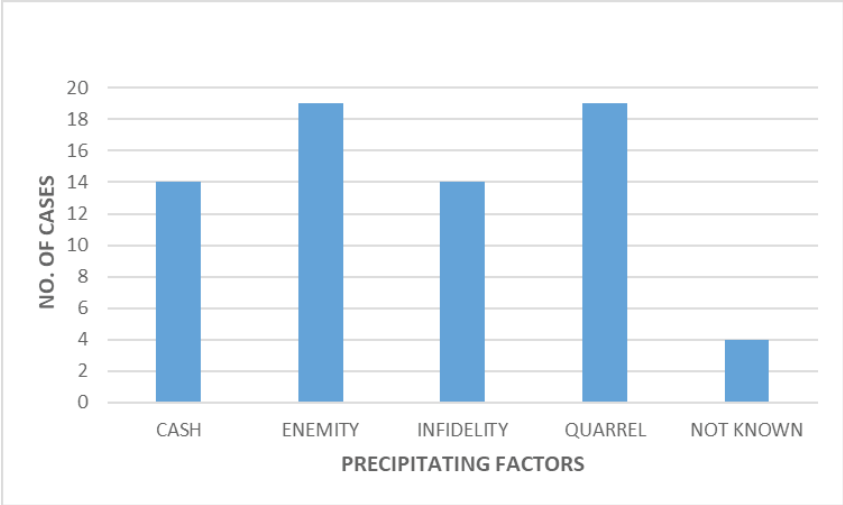


Fig: 2 Distribution of precipitating factors among the cases

In analysing place of death 28 (40%) cases seen in the victim own house and this is in consistent with the study conducted by Lee-Germon *et.al* [6]. However in studies conducted in other parts of India most of the homicide occurred in open place[7][8] . In this study 31 (44.28%) cases in street. This in consistent with study conducted by Mevarak, M[5] in which (33.2%)cases were in public place. In 4(5.71%) cases seen in bush and 3(4.28%) cases in work place . Inside commercial shop 4 (5.71%) cases was seen. Fig:3.

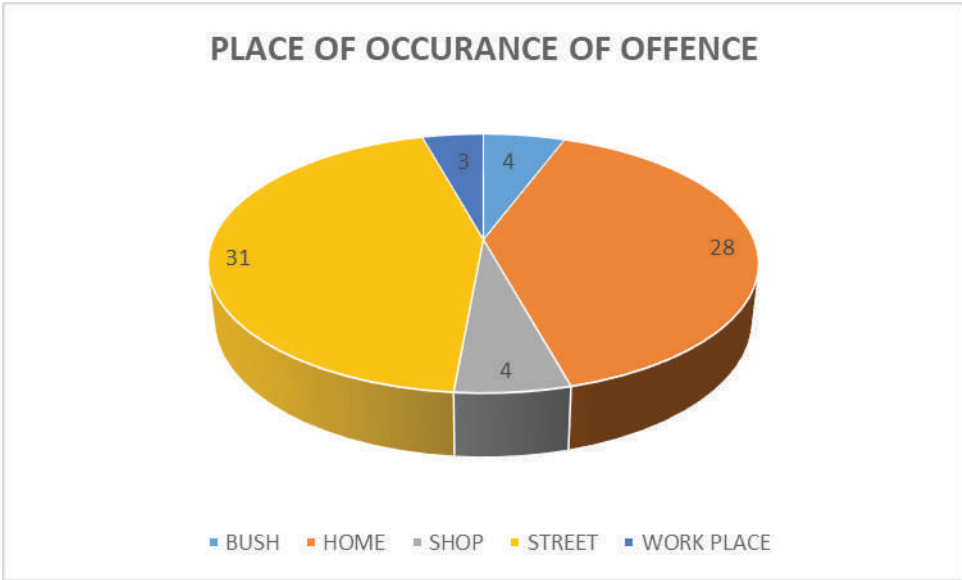


Fig: 3. Place of occurrence of offence

In analysing addiction status of the deceased alcohol consumption was seen in 12 (17.13%) cases. Both alcohol and tobacco was seen in 22(31.42 %) cases. No addiction was seen in 35 (50%) of cases and not known in 1 (1.42%) case.

In analysing number of accused persons involved, one person is involved in 28 (40%) of cases, two persons were involved in 8 (11.42%) cases, three persons were involved in 4 (5.71%) cases, four persons were involved in 9 (12.85%) cases five persons were involved in 6 (8.57%) cases, six persons were involved in 2 (2.85 %) cases seven persons were involved in 3 (4.28%) cases and in 10 (14.28%) cases no of assailants not known.

In analysing sex of the assailants male sex was seen in 55 (78.57%) of cases, female sex was seen in 3 (4.28%) cases and both the sexes were seen in 4 (5.71%) cases. Fig: 4

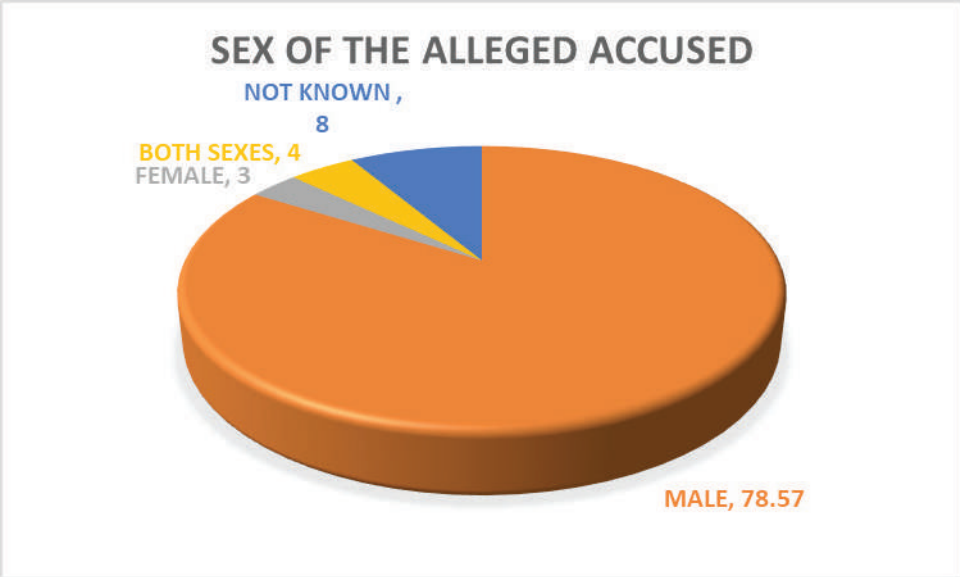


Fig: 4. Sex of the alleged accused

In analysing time of occurrence of the incidence 31(44.28%) cases was seen in 6.00 pm to 12.00 AM this is consistent with the study conducted by Mada et. al[11] whereas this is contrast with the study conducted by Sakulsaengprapha *et al*[12]. where majority of homicide occurred (33.6%) between 12.00 AM to 6.00 PM. In 19 (27.14%) cases was seen in 12.00 PM to 6.00 AM. In time interval 6.00 AM to 12.00 PM, 8 (11.42%) cases was seen. In time interval of 12.00 AM to 6.00 PM, 5 (7.14%)cases seen. In 7(10%) cases time of offence not mentioned in the reports. Fig: 5.

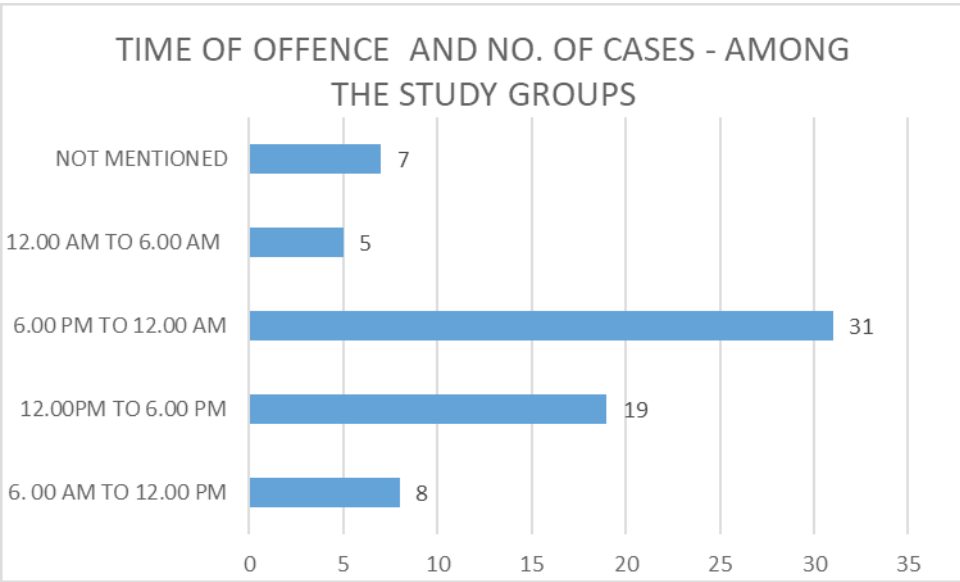


Fig: 5. Time of occurrence of the offence among the study group

In analysing cause of death 23(32.85%) cases were females. In this 23 (32.85%) female cases stab injury to the neck is seen in 3 (4.28%) cases, asphyxia due to throttling is seen in 4 (5.71%) cases. Asphyxia due to strangulation seen in 4 (5.71%) cases. Smothering in one (1.42%) case. Two female cases of drowning were seen, In which the deceased age was four and five years. Blunt injury to the head seen in 3(4.28%) cases.

In analysing cause of death in both sexes, 15 (21.42%) cases died of blunt injury to the head. Shock and haemorrhage due to multiple cut injuries was seen in 22 (31.42%) cases. Shock and haemorrhage due to multiple stab injuries as cause of death was seen in 6 (8.57%) cases. Shock and haemorrhage due to cut injury to head and neck as a cause of death was seen in 10 (14.28%) cases. Asphyxia due to throttling as cause of death seen in 5 (7.14%) cases. Asphyxia due to drowning was seen in 2 (2.85 %) cases **Table:** 1

Table:1 Cause of death among study group

S.NO	CAUSE OF DEATH	NO. OF CASES		TOTAL
		Male	Female	
1	Blunt injury to the head	12(17.14%)	3(4.28 %)	15 (21.42%)
2	Shock and haemorrhage due to multiple cut injuries	18(25.71%)	4(5.71 %)	22 (31.42%)
3	Shock and haemorrhage due to multiple Stab injury	3(4.28%)	3(4.28 %)	6 (8.57%)
4	Shock and haemorrhage due to cut injury to head and neck	9(12.85%)	1(1.42%)	10 (14.28%)
5	Asphyxia due to throttling	1(1.42%)	4(5.71%)	5 (7.14 %)
6	Asphyxia due to strangulation	1(1.42%)	4(5.71%)	5 (7.14%)
7	Shock and haemorrhage due to gunshot	1(1.42 %)	Nil	1 (1.42%)
8	Poison ingestion	1(1.42 %)	Nil	1 (1.42%)
9	Combined asphyxia due to external compression of the neck and head injury	1(1.42%)	1(1.42%)	2(2.85 %)
10	Asphyxia due to Drowning	Nil	2(2.85 %)	2(2.85%)
11	Smothering	Nil	1(1.42%)	1(1.42%)
TOTAL		47	23	70

Defence wounds seen in 20 (28.57%) of cases. This is consistent with the study conducted by Sachidananda Mohanty et al [9]. Mostly situated in outer aspects of forearm, wrist and back of fingers. None of the defence injuries seen in lower limbs. In 50 (71.42%) cases defence injury was not seen.

Head is the most common part of the body with injuries seen in 37 (52.85%) cases. This in contrast to the study conducted by Jhaveri S *et.al* [3] where neck (16.8%) is the common part of the body were injuries seen followed by head (15.9%)

The drivers of homicide are manifold, and socioeconomic factors such as inequality, unemployment and political instability can create environments that are conducive to lethal violence. Demographic characteristics, such as the sex and age of an individual, may act as either drivers of, or protective factors against, homicide. The rapid growth of cities and the consequent mass movement of people from rural to urban areas have frequently been cited as possible drivers of homicide.

However, UNODC analysis of data[2] from 68 cities suggests that there is no positive correlation between urban growth and increases in homicide rates. In fact, the population in the sample cities grew by 9 per cent between 2005 and 2016, but the number of homicides decreased by 26 per cent. This relationship holds for all regions, with the strongest diverging trends in Asia, followed by Europe. On the basis of this analysis it can be concluded that cities and rapid urban growth are not risk factors for rising levels of homicide

Conclusion

ongterm investment in education policies is associated with a drop in the homicide rate. Income inequality is more strongly linked to homicide than other aspects of development. Long term investment in educational policies is associated with drop in homicide rates. Strengthening the rule of law is central to reducing homicide levels. Policies aimed at tackling homicide should address drivers of homicide both at the individual level (such as the age and sex of a person) and at the macro level (such as unemployment, inequality, absence of the rule of law, the prevalence of gender stereotypes in society and the presence of organized crime). Targeted and efficient interventions require a comprehensive understanding of the scale of homicide and its various drivers, which is essential for ensuring that Sustainable Development Goal targets related to violence can be met by 2030. Comprehensive data and information about mechanisms that facilitate homicide perpetration can guide policymakers in devising more effective, targeted interventions.

Conflict of Intrest: Nil

Ethical Clearance: Nil

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YouTube TM Videos as a Source of Information on Clear Retainers

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Abstract

Background: Internet is one of the most important information sources in dental field to obtain information regarding treatment procedures. The content and quality of information about clear retainers available on YouTube is not known. The aim of the study was to analyze the source and quality of information about clear retainers on YouTube.

Methods: The search keywords were "clear retainers". The first 100 results were arranged in decreasing order of "relevance" using default YouTube algorithm. Two orthodontists independently viewed and analyzed the videos for video content using customized 10-point Likert scale and video information and quality index (VIQI) to determine the quality of the videos.

Results: Out of 100 videos screened, 48 videos met the inclusion criteria. The inter investigator results were similar in evaluating video content and the findings was not significant except for comparison by investigator II (p=0.006). For VIQI, internal consistency showed good reliability of Cronbach's Alpha .855. Most of the videos were uploaded by layperson (64.6%) rather than dental professionals (29.2%). Instructions on retainer hygiene was the most commonly covered topic (64.6%), followed by handling the retainer (60.4%). The least mentioned content was the procedure of making the clear retainer (19.8%).

Conclusion: The results of the study sheds light on the inadequacies in the content of the YouTube videos on clear retainers and also points out the underrepresentation of videos uploaded by dental professionals and orthodontists. The study suggests that YouTube is currently not an appropriate source of information on clear retainers. Dental professionals and academic institutions hold a responsibility for improving the content of YouTube about clear retainers and directing the patients to professional online sites for reliable information.

Keywords: *YouTube; orthodontic retainers; social media; internet*

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Introduction

The esthetic paradigm shift in orthodontics has led to the evolution of orthodontic appliances to incorporate esthetics to the functional goals and requirements of orthodontics treatment.[1] Clear retainer is one of the most common types of retainer preferred by the patients due to its excellent esthetic characteristics, simplicity of use, superior formability, maintenance of good oral

hygiene, and lesser discomfort compared to the Hawley retainers.[2-4]

Healthcare information is usually provided by healthcare professionals.However, the Internet's ease and accessibility, privacy, the patient's willingness to be more knowledgeable and the reduced cost of consultation compared to traditional healthcare consultation have contributed to the rise of social media from being used for entertainment to being used for education and information.[5] YouTube, a free-to-access video sharing-site, is the third most frequently visited website after google and Facebook, with more than 1 billion unique viewers every month and approximately 360 hours of video being uploaded every 24 hours.[6-8] The relative ease of uploading, searching, watching and sharing videos through different media, such as smartphones, personal computers and televisions has been attributed to the popularity of YouTube. However, the nature of the content of the uploaded videos are not peer-reviewed, which raises concern about the reliability, accuracy and quality of information, particularly because of freedom of expression.[9,10]

YouTube has been evaluated to investigate the video content and quality of information on dental topics such as oral cancer,[11] dental education,[12] root canal treatment,[13] lingual orthodontics[14] and orthognathic surgery.[15] However, the content and quality of information on clear retainers available on YouTube is not known. The aim of the study was to analyze the source and quality of information on clear retainers on YouTube.

Materials and Methods

The study included publicly available YouTube videos and was deemed exempt from the institutional ethical committee. There has been no interaction or attempt to contact any YouTube users and their names have not been identified or disclosed.

YouTube video search strategy

YouTube website (http://www.youtube.com) was searched on 18th April, 2018 for videos related to clear orthodontic retainers. The search keywords used were "clear retainers" with the search filter as "sort by relevance" as the default YouTube algorithm. The first 100 videos were selected for the study based on the previous research which showed 95% of users clicked only on the initial 3 pages of output to obtain the intended information.[16] For the purpose of the study, a new YouTube account was created and a playlist of the identified videos was created. Retrieved video source locators (URLs) were saved.

The inclusion criteria were:

1) English language

2) Clear retainer as the primary content

3) Acceptable audio-visual quality

Multi part videos were considered as one for analysis

The exclusion criteria were:

1) No audio

2) Irrelevant material (videos about other types of retainers, parodies, comics)

3) Duplication

4) Duration more than 15 minutes

5) Non English.

Video analysis

Two orthodontists independently viewed and analyzed the videos. The following general parameters were extracted for each included videos: 1) title 2) number of views 3) likes 4) dislikes 5) number of comments 6) duration and 7) date of upload. The videos were categorized based on their ownership as dentist/ professional, commercial and layperson. The target population of each video was categorized as layperson, professional and layperson/professional.

The videos were assessed for video content using customized 10-point Likert scale and video information and quality index (VIQI) to determine the quality of the videos.

The video content scale was used to assess the following components of the information regarding clear retainers in the videos: 1) Definition of retainer 2) Procedure of fabricating clear retainer 3) Instructions on inserting and removing the retainer 4) Comparison with other retainers 5) Durability 6) Hygiene 7) Speech performance 8) Wear time 9) Psychological impact 10)

Pain/ discomfort.

The VIQI scale uses a 5-point Likert scale ranging from 1 (poor quality) to 5 (high quality) to evaluate the following video characteristics: flow of information, information accuracy, quality (one point each for use of still images, animation, interview with individuals in the community, video captions, and a report summary), and precision (level of coherence between video title and content).[17]

Viewers' interaction was calculated based on

$$\text{Interaction index} = \frac{\text{number of likes} - \text{number of dislikes}}{\text{total number of views}} \times 100$$

Statistical Analysis

Data was entered independently by two assessors on Microsoft excel spread sheet. Statistical analysis was performed using SPSS software program (IBM SPSS Statistics 20). Descriptive statistics was generated. For internal consistency reliability test was conducted. Chi square test was conducted to find the significance of evaluation done by the investigators. Correlations were determined by using Pearson's test. A p value of <0.05 was considered statistically significant.

Results

Screening of videos

Out of the initial 100 videos screened, 52 videos were eliminated as per the inclusion criteria. And the final sample size was 48. **(Table 1)**

Contents of videos

Two orthodontists independently viewed the Contents to determine the quality of the videos with 10 selected parameters. A customized 10 point Likert scale was used to evaluate the information content of the clear retainer videos. Result showed **(Graph 1)** instructions on cleaning the retainer was the most commonly covered topic (64.6%), followed by instructions on handling the retainer (60.4%), definition (52.1%), speech (45.8%), wear-time (41.7%), pain (31.3%), comparison with other types of retainer (30.2%) and psychological impact (29.2%). The least mentioned content was durability and the procedure of making the clear retainer (19.8%). Among the included videos 15 videos were considered high content videos (≥5 total content score) and 33 videos as low content videos (<5 total content score).

Results showed the evaluation done by both the investigators and the findings were not significant for the contents of the videos except for "comparison" by investigator II which showed significance at .01 level.

Ownership of videos

More than half of the videos were uploaded by layperson (64.6%) rather than dental professionals (29.2%). **(Graph 2)**

Viewership of videos

Majority of the viewers of the analyzed YouTube videos were laypersons (87.4%). **(Graph 3)**

The mean viewing rate was 3800.42 with (range: 1-446794 views). The overall mean number of 'likes' was 146 (range: 0-956) and mean number of dislikes was 15 (range: 0-234). The mean length of the videos on clear retainers was 4.8464 minutes (range:0.20-12.45 minutes). The mean of days since upload was 1167.17 (range: 4-3586). Each parameter was found significant at .01 level. **(Table 2)**

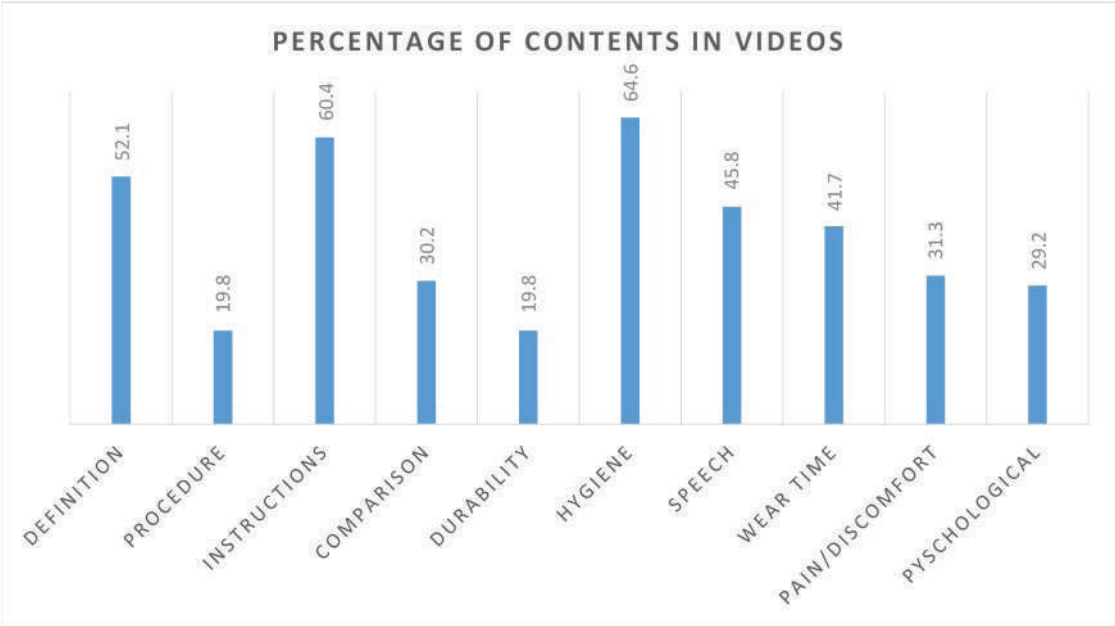
The mean interaction index was calculated using the formula and was found to be 10.57

The highest viewed YouTube video on clear retainer (44,6794 views) was uploaded by a layperson and had a total content score of 6 and total VIQI sore of 12. It was also the most disliked video with 234 dislikes. The most liked video (956 likes) was also uploaded by a layperson and had a total content score of 4 and total VIQI score of 8.

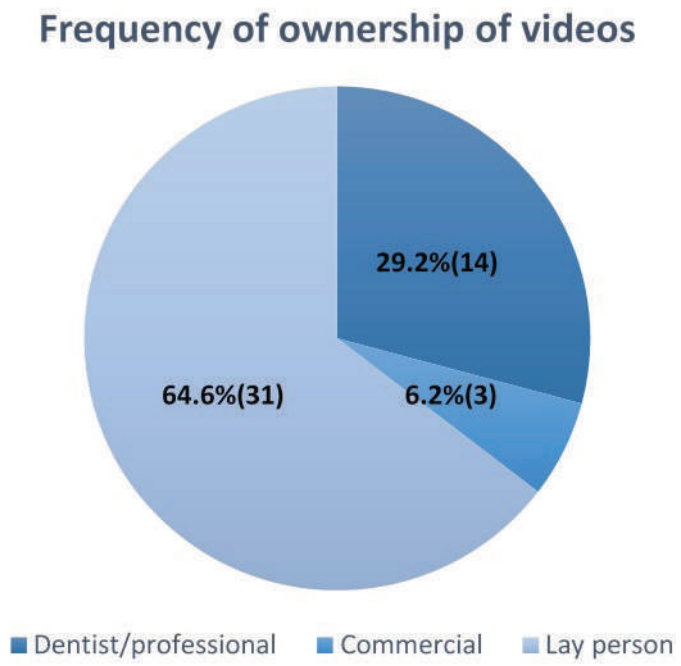
For VIQI, internal consistency was calculated and result showed good reliability with Cronbach's Alpha .855.

Pearson correlation **(Table 3)** between VIQI score and Total content score was highly significant at p=0.001 level. There was highly significant correlation between total content score and length of the video and number of dislikes at p=0.001. Correlation between total content score and number of views, number of likes, number of comments and interaction index was significant at

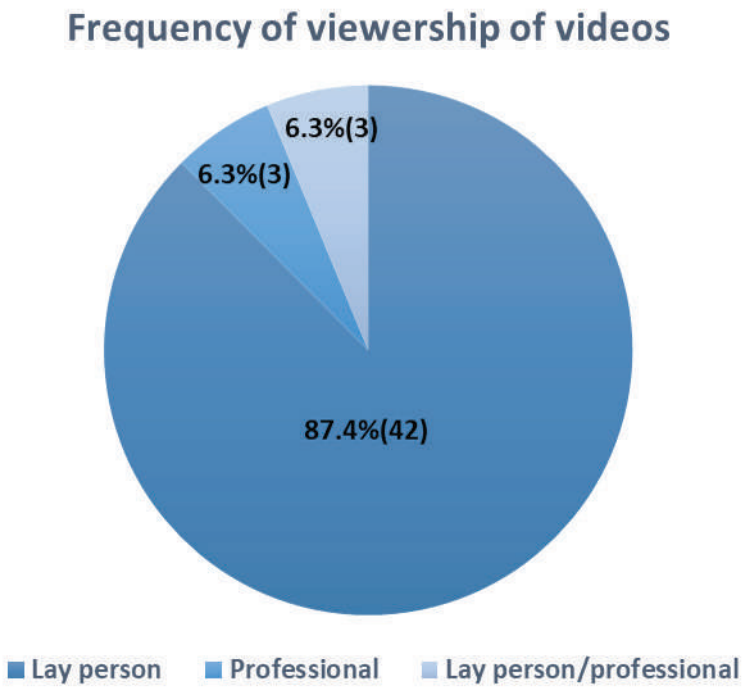
p=0.05 level.



Graph 1: Contents in videos



Graph 2: Ownership of videos



Graph 3: Viewership of videos

Table 1: Descriptive statistics of excluded videos

Sl. No.	Criteria	Frequency	Percentage
1	No audio	19	36.5%
2	Irrelevant	23	44.2%
3	Duplicate	5	9.6%
4	Duration more than 15 minutes	4	7.7%
5	Not English	1	1.9%
Total		52	100%

Table 2: Significance of each parameter of video content

Investigator 1						
Parameter		**Mean**	**SD**	**t**	**F**	**Sig. (p)**
1	Definition	1.75	.438	27.707	1.093	.362
2	Procedure	1.88	.334	38.868	1.049	.380
3	Instructions	1.50	.505	20.567	.416	.743
4	Comparison	1.77	.425	28.885	1.013	.396
5	Durability	1.79	.410	30.245	1.692	.183
6	Hygiene	1.38	.489	19.471	.531	.664

Cont... **Table 2: Significance of each parameter of video content**

7	Speech	1.60	.494	22.489	.840	.479
8	Wear-time	1.58	.498	22.018	.855	.471
9	Psychological	1.69	.468	24.959	1.237	.308
10	Pain/discomfort	1.71	.459	25.767	.144	.933
Investigator 2						
1	Definition	1.21	.410	20.398	.506	.680
2	Procedure	1.73	.449	26.676	.905	.446
3	Instructions	1.29	.459	19.482	.702	.556
4	Comparison	1.63	.489	23.012	1.515	.224
5	Durability	1.81	.394	31.836	4.748	.006**
6	Hygiene	1.33	.476	19.391	.594	.622
7	Speech	1.48	.505	20.299	.731	.539
8	Wear-time	1.58	.498	22.018	1.461	.238
9	Psychological	1.69	.468	24.959	.905	.446
10	Pain/discomfort	1.67	.519	22.243	.348	.790

**significant at .01 level.

Table 3: Descriptive statistics of YouTube video demographics

parameter	minimum	maximum	mean	SD	'p'
Views	1	446794	3800.42	75386.052	.001**
Likes	0	956	146	261.561	.001**
dislikes	0	234	15	38.592	.001**
No. of comments	0	394	46.81	92.620	.001**
Length (minutes)	0.20	12.45	4.8464	3.42346	.001**
Days uploaded	4	3586	1167.17	813.169	.001**

** significant at .01level

Discussion

The aim of our study was to provide a detailed analysis of the information available on YouTube about clear retainers. The use of social media to gather information has infiltrated every field including dentistry. In an effort to make informed dental care decisions, patients are increasingly turning to the Internet to better understand their conditions and treatments. The internet has become a linchpin of information in healthcare system. Patients prefer visual content in contrast to other scientific platforms to gain better information regarding their orthodontic treatment. YouTube is the third most visited website in the world, however the patient usage of YouTube video to gain health information is unknown. Several studies have analyzed the YouTube as source of information regarding dental issues such as oral cancer,[11] dental education,[12] root canal treatment,[13] lingual orthodontics[14] and orthognathic surgery.[15] No studies have analyzed the YouTube resources on clear retainers.

Previous studies have used different systems to evaluate the quality of information on YouTube website. Since there is no validated scoring system to evaluate videos on YouTube a subjective evaluation scoring system was used. The scoring system used in this study is simple and can be used to evaluate both the content and the quality of YouTube videos easily. It has been tested in a previous study analyzing YouTube video on Lingual orthodontic treatment.[14] Internal consistency showed good reliability between the investigators (Cronbach's Alpha .855), implying that the technique was valid.

According to previous studies the YouTube video database is dynamic and rapidly changes over a short period of time. This study evaluated only 100 videos generated by the search engine, which represents only a small proportion of videos related to clear retainers. However prior statistics suggests that the 90% of the viewers do not go beyond the first three pages of YouTube search results. Also the mean views of the evaluated videos were high (33689.62 views) suggestive of the videos routinely accessed by the viewers, making the results applicable in wider setting.

We observed that amongst the 100 top videos on Clear retainers only 48 videos were found to be eligible for inclusion in the study. Among the included videos only 15 videos were high content videos. When the video content was analyzed none of the videos mentioned all the contents on the content scoring scale. Most of the videos gave information on only one or two content which led to majority of low content videos. The procedure of making the clear retainer and the durability was the least covered topic. Instructions on cleaning the retainer was the most covered topic. This could be because majority of the videos were patient-uploaded videos conveying their experience with clear retainers. Layperson shared more videos compared to dental professions due to the ease of sharing the videos and lack of standardization of the uploaded videos on YouTube. The source of information of clear retainers from dental professionals, orthodontists and commercial sources were under represented. This correlated with systematic review conducted by Madathil KC et al that found patients were the main source of healthcare information on uploaded videos.[17] We believe that the dental academic institutions and dental professionals can provide reliable information on clear retainers, particularly orthodontists. Orthodontists should take initiative in delivering reliable and accurate and information on clear retainers for the benefit of patients via social media, such as YouTube

There was a positive correlation between total content score and the VIQI. The evolving technology enables YouTube users to produce high quality videos. There was highly significant correlation between total content score, length of the video and number of dislikes. The most viewed video was also the most disliked video with a high content score. However, the most liked video was a low content video. The videos were analyzed by experts in the field and it was felt that they would judge videos differently when compared to patients. One must also be aware that the evaluated YouTube variables can be manipulated. As YouTube Ranks its videos based on viewership scores, the high content videos may not be ranked in the beginning of search list and can be missed by the viewers.

This study possesses several limitations. First, this study used only one search term 'clear retainers'. The use of other related search term may yield variation in the searched results. The search term used in this study was decided by the investigating orthodontists, however a patient might use other search term and get different results. Secondly, the study was a cross sectional

study and represents 'snapshot' data collection. Since YouTube database is not static, several videos are being added or deleted over a short period of time, making the search results inevitable for change. Third, the videos assessed were only in English language. Further studies including videos with other languages can be performed to know the influence of cultural variables on patients and orthodontic treatment.

Considering the increasing number of patients using YouTube as the source of information on clear retainers, dental professionals should be aware of the quality of information available to them. It is important to recognize YouTube as a media of educating the patients on clear retainers. Dental professionals who are keen to create information videos on clear retainers should consider planning and defining the contents on clear retainers. The content scoring system used in this study is simple and easy which can be used by orthodontist to create and upload their videos.

Conclusion

The quality and content of information about clear retainers on YouTube is inadequate. The study suggests that YouTube is currently not an appropriate source of information on clear retainers.

Most of the videos were uploaded by Layperson rather than dental professionals.

Majority of the videos discuss the hygiene of the retainer, but only few videos mention about the durability and procedure of making clear retainers. Hence, high content videos are very less and dispersed in the search history, making it difficult for patients to find useful videos which are reliable and accurate.

Orthodontists should be aware of the source and content of information available to the patients through social media. Dental professionals and academic institutions should take an initiative in improving the content of YouTube about clear retainers and directing the patients to professional online sites for reliable information.

Ethical Clearance: The study included publicly available YouTube videos and was deemed exempt from the institutional ethical committee. There has been no interaction or attempt to contact any YouTube users and their names have not been identified or disclosed

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Effect of Various Mesh Designs on Shear Bond Strength of New and Recycled Brackets –A Comparative, Invitro Study

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Abstract

Introduction: Various chemical and mechanical methods are used to enhance the retention of adhesive to the metal bases of the brackets.Inspite of several efforts,bracket failure is one of the commonest problems faced during treatment.Debonded brackets are generally recycled and reused.This study was designed to compare the shear bond strength(SBS)of five commonly available mesh designs and to determine which mesh design offers least reduction of SBS following recycling.

Method: Sample consisted of 5 types of brackets with different mesh designs, bonded to 70 extracted human premolars.The SBS was checked using Instron universal testing machine.The brackets were recycled using sandblasting procedure and were rebonded on the sample teeth.Bond strength testing was again carried out using the same procedure.

Results: Mean SBS of Discovery smart bracket with laser structured base was highest followed by 3M Unitek brackets.The brackets by American Orthodontics had lowest bond strength.After recycling with sandblasting procedure,an insignificant reduction in SBS was noticed in all five groups.

Conclusion: Laser structured base design was superior to other bracket base designs.All five bracket types had optimal bond strength required for successful bonding both before and after recycling (>7.8 MPa).

Key words – *Shear bond strength, recycled brackets*

Introduction

Good bond strength and retention of orthodontic brackets to the tooth surface is critical for a favorable outcome of orthodontic treatment.An orthodontic bracket must be able to resist a force of 6-8 MPa for clinical success.[1]

Various chemical and mechanical methods such as milling undercuts in the bracket bases, welding different diameter mesh wires to the bases, brazing, chemical etching or sintering with porous metal powder & perforating the bracket bases are used to enhance the retention of the adhesive to the metal bases of the brackets.Recent advancements include laser structured bracket bases and metal plasma coated bases to further improve retention.Inspite of several efforts to prevent bracket failure,it is one of the commonest problems clinicians face during treatment.This is usually the result either of the patient's accidentally applying inappropriate forces to the bracket or of a poor bonding technique. Debonded brackets are generally recycled and reused in the orthodontic office as the advantages are both economic and environmental.[6]There are various methods to recycle brackets such as direct flaming[2], Buchman method[3],sandblasting[4],green stone,and tungsten carbide bur[5].

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Previous studies have reported that recycling of brackets can lead to reduction in bracket quality,loss of identification marks,lack of sterility, increased risk of cross infection and lower bond strength.[6,7]

Since there are varieties of bracket bases that are available to the orthodontists, there have been various studies evaluating the success and advantage of one bracket base to another.

Several studies have compared the SBS of orthodontic brackets with different base surface areas and base designs. However,a literature search revealed that a comparison of SBS of different mesh designs after recycling,has not been previously studied.

Therefore,the present study was undertaken to determine which among the various commonly available mesh designs offer least reduction in bond strength following recycling.

Methodology

A total of 70 extracted human premolars were collected and stored in distilled water.

Inclusion criteria:Intact premolars extracted for orthodontic purpose.

Exclusion criteria-

Teeth with history of any pre-treatment with chemical agents like hydrogen peroxide restorations,cracks due to the extraction forceps and caries.

The teeth were cleansed and polished with pumice and rubber prophylactic cups for 10 seconds and washed with water.Transbond XT adhesive system(3M Unitek,Calif)was used to bond all brackets to teeth.

Teeth were then embedded in cold cure acrylic blocks which were fitted into the jig of the universal testing machine(Instron-3366, load capacity 7kN, Instron Corp, UK)to determine the SBS(fig 1).

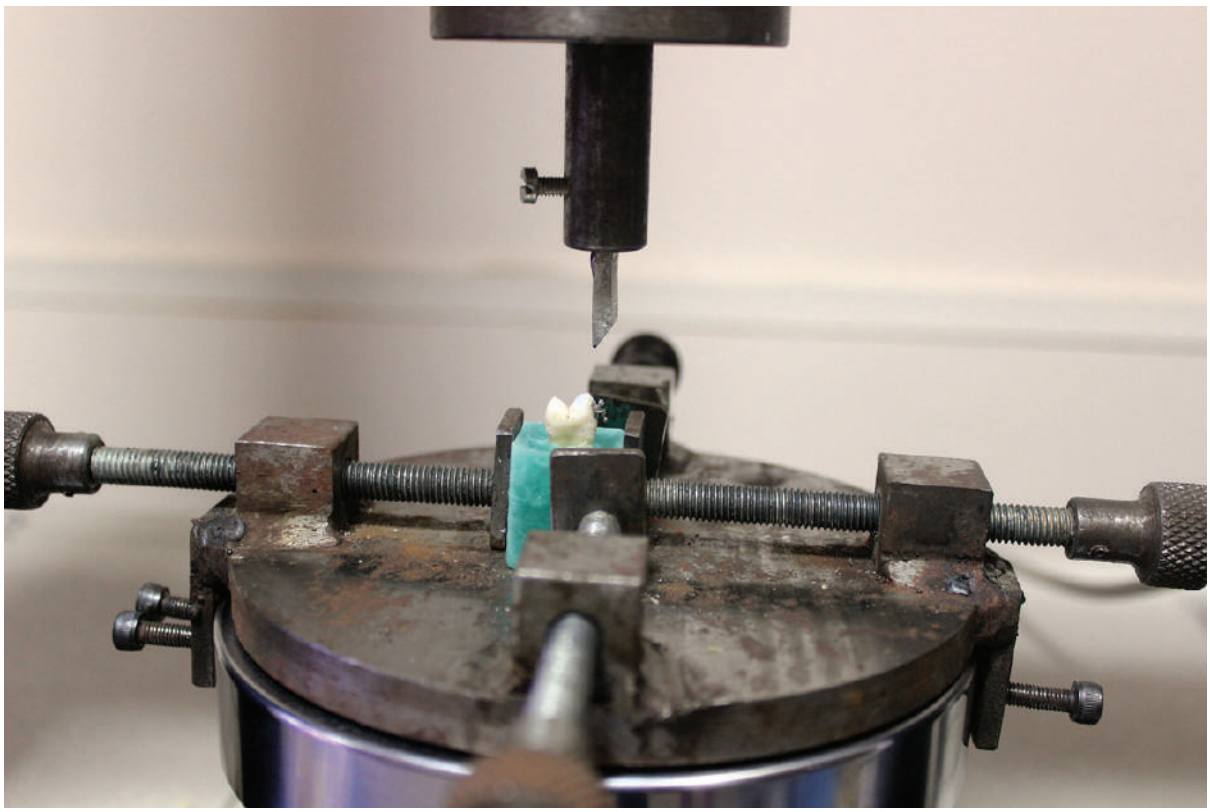


Fig 1:Instron universal testing machine

The sample blocks were coded in 5 different colours corresponding to five brackets used,

Group 1:American Orthodontics(AO),dual mechanical retention, 80 gauge mesh over a photo-chemically etched base(fig 2a)

Group 2:RMO(mini taurus contur-lok bases) laminate of stainless steel foil to an 80x80 mesh, oriented at 45^0 (fig 2b)

Group 3: Discovery smart (Dentaurum,Germany) laser structured base (fig 2c)

Group 4: Twin torque bracket(3M-Unitek,calif.) Dyna-Bond II mesh base(fig 2d)

Group 5: Mini diagonal Roth(Leone,Italy)(fig 2e)

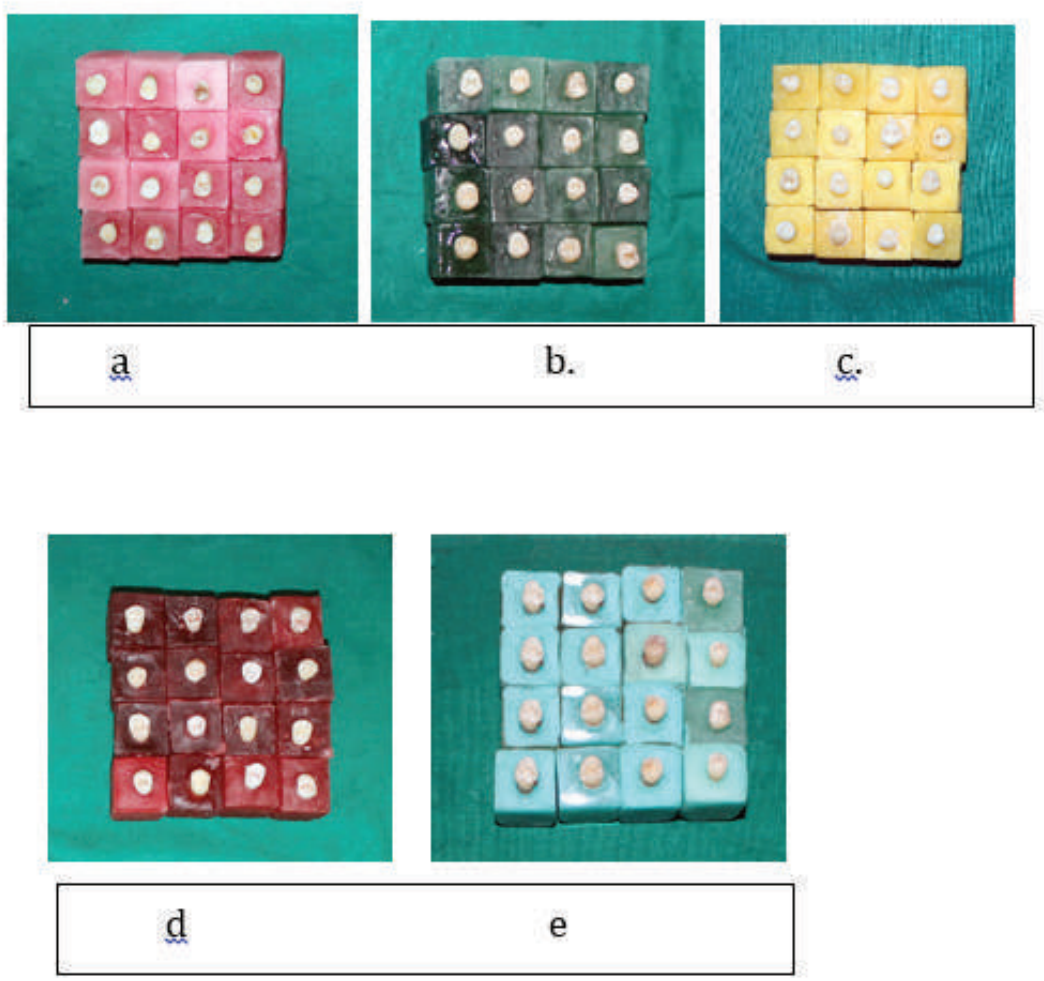


Fig 2: Teeth embedded in colour coded acryclic blocks

SBS testing was done using the universal testing machine-Instron.An occlusogingival load was applied to the brackets using SS metal blades producing shear force at the bracket base tooth interface,at a crosshead speed of 1mm/min until rupture of bracket occured.

After debonding the entire sample of brackets was subjected to sandblasting using 50micron alumina abrasive particles for 15 sec at a distance of 10mm. Brackets were rebonded to the tooth surface and bond strength testing was carried out on Instron machine.

Statistical Analysis

Descriptive statistics,including the mean,standard deviation were calculated for each group.ANOVA was used to determine whether significant differences existed

in the overall SBS of the 5 groups.Posthoc tukey test was done to compare the five groups.Significance for these statistical tests was predetermined at $P < .001$.Student's paired *t*-test was used to compare the SBS before and after recycling ($P < .05$).

Results

The results of ANOVA test showed that there was a significant difference in the SBS of the five groups. The Discovery brackets with laser structured base had highest mean SBS of 22.44MPa followed by 3M Unitek with mean SBS of 19MPa.The brackets by AO had lowest SBS of 10.75Mpa.

Recycled brackets of all the five groups showed a reduction in bond strength when compared to the new brackets(Table I)

Post recycling ,bond strength of 3M Unitek was found to be the highest (18.46MPa),followed by Discovery smart brackets (17.414MPa).Leone brackets had lowest SBS of 9.89MPa.

Table II shows the results of Posthoc tukey test that was used to compare SBS of each group with the other 4 groups.

The comparison of 3M Unitek brackets with other groups show that SBS of these brackets was significantly higher than brackets by RMO,AO and Leone and were comparable to Discovery brackets.

Similarly when the brackets by RMO,AO and Leone were compared,difference between their SBS was insignificant but compared to the Discovery brackets their SBS was significantly lower.

Posthoc tukey test for recycled brackets also gave similar results with bond strength of 3M Unitek and Dentaurum being significantly higher compared to the other three.

Paired t test showed that there was significant difference in SBS for Leone and Dentaurum before and after recycling.However for the other three groups,reduction in bond strength following recycling was not significant.(Table III)

TABLE I- ONE WAY ANOVA TEST TO EVALUATE THE SBS OF THE GROUPS ON BONDING, REBONDING AND THE DIFFERENCE

		N	Mean	Std. Deviation	F/ statistics	Mean square/df2	Ps value
SBS(N/m2)	3M UNITEK	14	19.057	2.9011	48.776	386.043	<0.001
	AO	14	10.75	2.127			
	LEONE	14	12.071	2.0934			
	DENTAURUM	14	22.443	3.8872			
	RMO	14	11.543	2.672			
	Total	70	15.173	5.4621			
SBS(N/m2) (Recycled brackets)	3M UNITEK	14	18.464	5.1244	22.684	29.44	<0.001
	AO	14	9.907	0.9499			
	LEONE	14	9.893	2.1073			
	DENTAURUM	14	17.414	3.4402			
	RMO	14	10.593	3.0786			
	Total	70	13.254	4.9991			

Cont... **TABLE I- ONE WAY ANOVA TEST TO EVALUATE THE SBS OF THE GROUPS ON BONDING, REBONDING AND THE DIFFERENCE**

Difference in the SBS	3M UNITEK	14	0.607	6.698	2.041	47.79	0.099
	AO	14	0.864	2.5485			
	LEONE	14	2.179	3.4235			
	DENTAURUM	14	5.043	5.4895			
	RMO	14	0.921	4.8866			
	Total	70	1.923	4.9831			

TABLE II- POSTHOC TUKEY TEST

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	P VALUE
SBS MPa (N/m2)	3M UNITEK	AO	8.3071*	<0.001
		LEONE	6.9857*	<0.001
		DENTAURUM	-3.3857*	0.018
		RMO	7.5143*	<0.001
	AO	LEONE	-1.3214	0.727
		DENTAURUM	-11.6929*	<0.001
		RMO	-0.7929	0.945
	LEONE	DENTAURUM	-10.3714*	<0.001
		RMO	0.5286	0.987
	RMO	DENTAURUM	-10.9000*	<0.001
SBS (N/m2) (Recycled brackets)	3M UNITEK	AO	8.5571*	<0.001
		LEONE	8.5714*	<0.001
		DENTAURUM	1.05	0.912
		RMO	7.8714*	<0.001
	AO	LEONE	0.0143	1
		DENTAURUM	-7.5071*	<0.001
		RMO	-0.6857	0.981
	LEONE	DENTAURUM	-7.5214*	<0.001
		RMO	-0.7	0.979
	RMO	DENTAURUM	-6.8214*	<0.001
DIFFERENCE IN THE SBS	3M UNITEK	AO	-0.2571	1
		LEONE	-1.5714	0.911
		DENTAURUM	-4.4357	0.122
		RMO	-0.3143	1
	AO	LEONE	-1.3143	0.951
		DENTAURUM	-4.1786	0.163
		RMO	-0.0571	1
	LEONE	DENTAURUM	-2.8643	0.524
		RMO	1.2571	0.959
	RMO	DENTAURUM	-4.1214	0.174

TABLE III- PAIRED T TEST TO COMPARE THE 5 GROUPS INDIVIDUALLY

Group		Mean	N	Std. Deviation	Paired Differences		P VALUE
					Mean Difference	Std. Deviation	
3M UNITEK	SBS BEFORE	19.057	14	2.9011	0.5929	6.7068	0.746
	SBS AFTER	18.464	14	5.1244			
AO	SBS BEFORE	10.75	14	2.127	0.8429	2.5437	0.237
	SBS AFTER	9.907	14	0.9499			
LEONE	SBS BEFORE	12.071	14	2.0934	2.1786	3.426	0.033
	SBS AFTER	9.893	14	2.1073			
DENTAURUM	SBS BEFORE	22.443	14	3.8872	5.0286	5.4878	0.004
	SBS AFTER	17.414	14	3.4402			
RMO	SBS BEFORE	11.543	14	2.672	0.95	4.8807	0.479
	SBS AFTER	10.593	14	3.0786			

Discussion

Bond failure during orthodontic treatment is frequent and undesirable.As a result,SBS of new and recycled brackets has been a subject of great interest in orthodontic research.Some studies have shown that recycling of bonded and rebonded orthodontic attachments adversely affects SBS.

Early metal bases were perforated with 12 to 16 holes or fabricated with lipped-edges,to create undercuts for the adhesive to flow into.[21] Significantly improved bond strengths were seen when perforated or lipped-edge bases were replaced with stainless steel mesh pads,due to exponentially increased areas for the adhesive to flow into and lock-in the bracket base-cement interface.[10,21,22] Reynolds and Von Fraunhofer found mesh bases to be 2.8 times as retentive as perforated bases.[18] Mesh pads were made by laminating stainless steel wires of differing diameters and configurations to stainless steel foil.The potential exists for numerous variations in mesh size,mesh number/gauge and aperture size.These variables can significantly change bond strengths especially when coupled with the many different available adhesives filler particle sizes.[23] In the present study five commonly used types of bracket base designs were studied.The base surface area of the brackets was determined using a vernier caliper and it ranged from 9-11 mm^2.Only one type of adhesive i.e.Transbond XT was used to ensure that any variations in SBS were clearly attributable to variations in bracket base design.

The SBS was highest for Discovery Smart brackets with laser structured base(22.44MPa). Olivier Sorel introduced this base design in 2002.[30] The smooth surface of injection molded single piece bracket base is treated by a powerful Nd: YAG laser,melting and evaporating the metal and burning hole-shaped retentions.This base design offers a combination of macro and micro retention ensuring superior bond strength.

The brackets by 3M Unitek ranked second in SBS. These brackets have an 80 gauge mesh bonding pad that matches the curvature of the tooth for maximum contact and strong bond strength.Mesh based brackets with larger mesh spaces provide a greater SBS than

those with smaller mesh apertures.[23] Matasa claimed that the mesh number and the wire diameter of the mesh are the most important influencing factors.[23]

RMO brackets ranked third with the SBS of 11.54 MPa.These brackets had a bonding base made of a laminate of stainless steel foil to an 80x80 mesh which is oriented at 45 degree.

The brackets by Leone had mean SBS of 12.07 MPa and ranked fourth among the tested groups.We found that the bond strength of these brackets was inferior when compared to the mesh type base design. Similar to our findings,some studies have reported higher bond strengths with mesh bases than integral undercut bases.[8,18,24] Lopez et al[25] found mesh brackets vs. integral bases debonded at 21.5MPa ± 3.5 and 13.83MPa ± 2.78, respectively. Willems et al[26] found mesh brackets vs. integral bases debonded at 13.0MPa ± 2.1 and 3.9MPa ± 0.8, respectively.

Few studies found integral undercut bases to have higher bond strengths.[11,14,15] Sharma-Sayal et al[14] found integral undercut bases vs. mesh brackets debonded at 9.73MPa ±1.64 and 8.05MPa ± 2.75,respectively. Wang et al[16] found integral undercut bases vs. mesh brackets debonded at 9.32MPa ± 1.77 and 8.04MPa ± 2.46, respectively.

The brackets by AO,with a 80 gauge mesh over a photochemically etched base were found to have the least SBS.This can be attributed to poor penetration of resin which results in air inclusion inhibiting polymerization of uncured resin.[27]

Similar to this study,Tavarez also noticed that brackets recycled by aluminum oxide blasting had similar SBS when compared with new ones,and the bond strength values obtained after 90-µm particle aluminum oxide blasting were consistently higher than those obtained by an industrial process or by silicon carbide stone grinding.[17]

Bishara showed that rebonded teeth have significantly lower SBS due to residual adhesive on the enamel surface.[30]Therefore,the current findings suggest that this problem could be compensated by mechanical retention created by recycling procedures.

The mean SBS of the groups ranged from 10.75-22.44 MPa before recycling and 9.89-18.46 MPa after recycling.Reynolds suggested 5.9 to 7.8 MPa as the optimal bond strength required for bonding of brackets to enamel.[9] The results of our study show that all the bracket types had optimal bond strength required for successful bonding both before and after recycling.

Conclusions

On the basis of the results of the study the following conclusions were drawn-

1. Laser structured bracket base design(Discovery brackets) was found to be superior to other bracket base designs with the highest mean SBS.
2. Reconditioning of brackets by sandblasting led to a statistically insignificant decrease in SBS of all five groups.
3. SBS of brackets with DynaBond II mesh base(3M Unitek)showed least reduction following recycling.
4. All the five bracket types had optimal bond strength required for successful bonding both before and after recycling(>7.8 MPa).

Conflict of Interest: None

Source of Funding: None.

Ethical Clearance:This research work was approved by Institutional Ethics committee,Manipal College of Dental Sciences,Mangalore,India.

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Medicolegal Aspects of COVID-19: Microbial Terrorism

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Abstract

The entire World is going through hard times because of the emergence of new pandemic named Coronavirus or COVID-19. The virus originated from Wuhan and is spreading Worldwide. There are many questions arising regarding the origin of this fatal virus; whether it is natural or man-made. In the past years, many microorganisms were used as a bio-warfare agents for causing destruction. In 2001, anthrax attack took place in US by the use of bacteria, *Bacillus anthracis* which acted as a bioweapon and lead to number of deaths in Texas, US. This review summarises the origin, epidemiology, medico-legal aspectsand the recent researches of the virus indicating its nature of being manmade or a natural virus.

Keywords*:COVID-19, Identification, Epidemiology, Diagnosis, bioweapon.*

Introduction

A newly emerged virus in the year 2019, is named Corona Virus (2019-nCoV) by World Health Organisation (WHO). It was discovered in 1960 and firstly reported in December 2019 when tremendous number of pneumonia cases were reported in Wuhan, China. As a result, etiological research was carried out by the government of China for taking preventive measures to control this epidemic[1]. It is genomically analysed and suggested that a strain of CoV(2019 n-CoV) has been named as Severe Acute Respiratory Syndrome CoV-2 (SARS- CoV-2) recently. This viral infection was suspected to be zoonotic in origin[2]. Coronavirus causes infection in respiratory and intestinal tracts in humans and non-humans.

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In 2002-2003 serious upsurge of Severe Acute Respiratory disorder in Guandong province of China made it highly pathogenic to humans. Familial mass of pneumonia upsurge append to the evidence of pandemic COVID-19. The graph of this disease is reaching heights because of human to human transmission of this viral disease[3].

Infectious Bronchitis Virus (IBV) was the firstly discovered CoV in chickens and human CoVs causing respiratory diseases. Common cold in humans was caused by CoV-OC43 (HCoV-OC43). SARS CoV in 2002 was discovered after the arrival of HCoV-229E and HCoV-OC43. [6]

Diversity of Coronavirus:

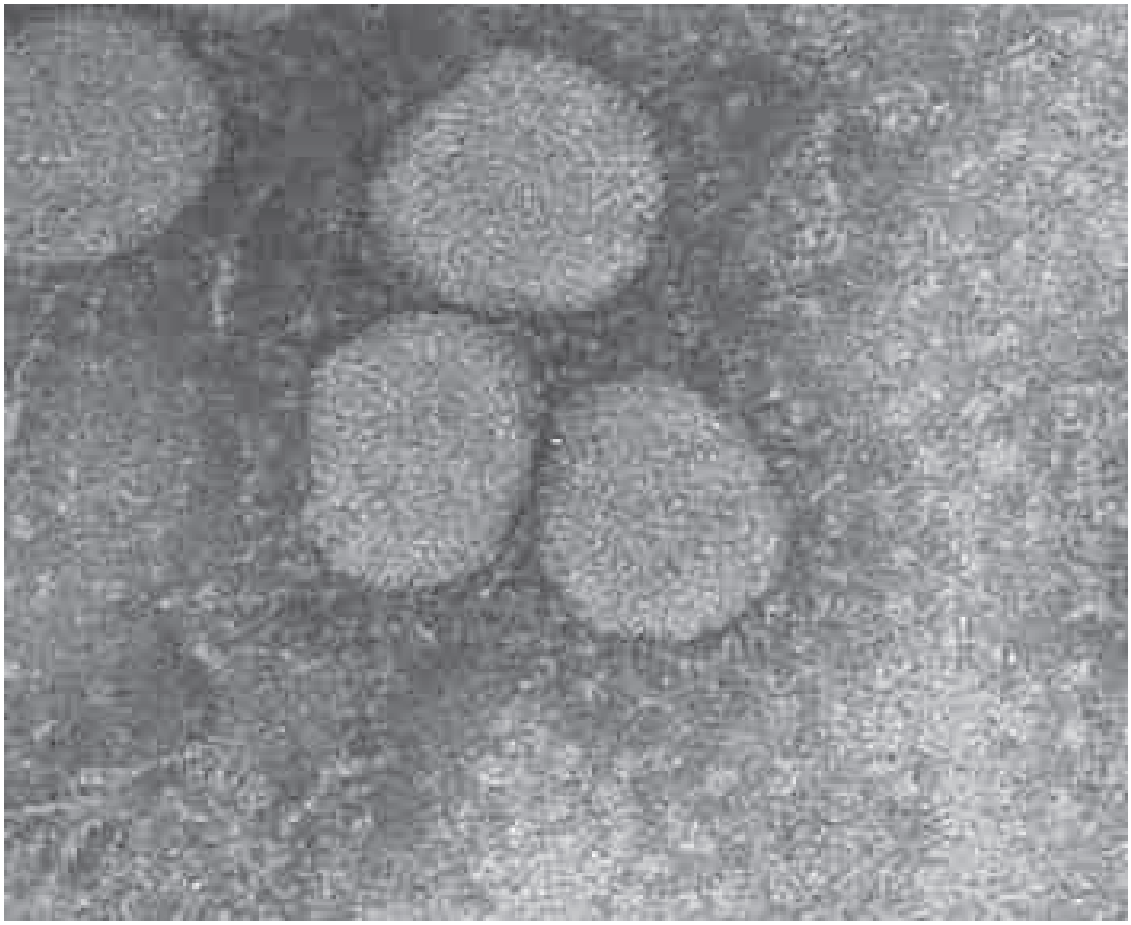
Coronavirus belongs to subfamily *coronavirinae* and member of *coronaviridae* family and order *Nidovirales* as given by the International Committee on Taxonomy of Viruses. According to the studies, it is found that four genera of this subfamily are there namely; Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus. These subfamilies are designated on the basis of their genomic structure along with the

phylogenetic relationship[4].

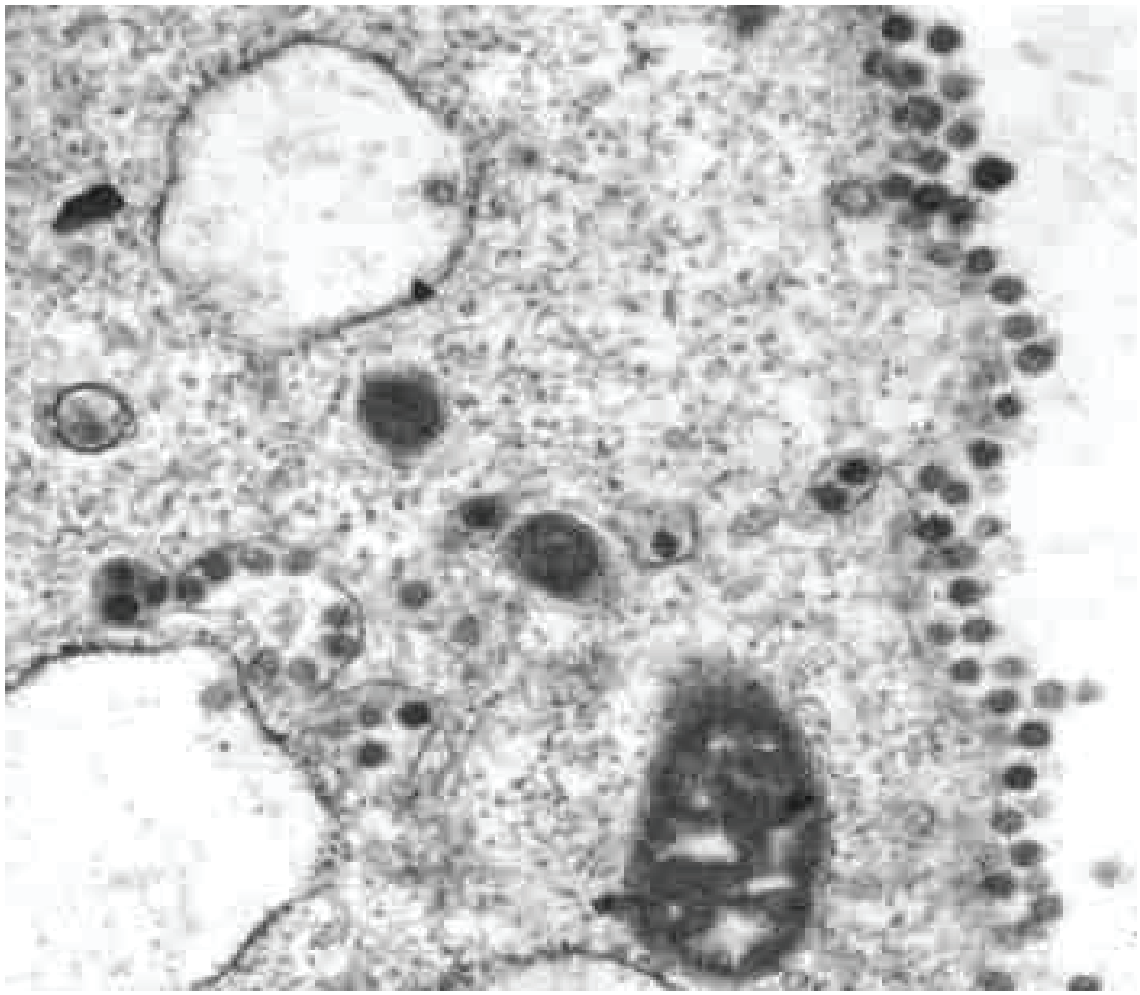
Respiratory illness and gastroenteritis are caused by alpha and betacoronaviruses in humans and animals. A study claimed that Betacononavirus infects only mammals. Gamma and Delta coronaviruses affects the bird species but some of them are reported fatal for mammals also. These two genera of coronavirus induce mild upper respiratory diseases in immuno-competent host.[5]

Structure of Coronavirus:

This newly popped SARS-CoV-2 is a Single-stranded RNA having spherical structure consisting of spike proteins. Coronavirus is alleged so because word corona is derived from *latin* word *corona* which means 'crown'. The virus has a royal crown like appearance when it is observed under an electron microscope. CoV is protected by a lipid bilayer and consist of certain proteins namely, spike (S) protein, Nucleocapsid (N) protein, Membrane (M) protein, Envelope (E) protein and haemagglutinin esterase (HE).[7]This haemagglutinin esterase protein increases the admittance and pathogenicity of the coronavirus. SARS – CoV- 2 has identical structure as that of coronavirus including other constituents like poly-proteins, nucleoproteins,membrane proteins like RNA polymerase. Other enzymes present in this virus include helicase, glycoprotein, accessory proteins, 3-chymotrypsin – like protease. It is studied that SARS – CoV-2 having spike protein consist of 3-D structure. [6]



(a)



(b)

Figure1. (a) and (b) Microscopic view of Coronavirus.

Source: Department of Microbiology, The University of Hong Kong and the Government Virus Unit, Department of Health, Hong Kong SAR China.

Identification of COVID-19:

Coronavirus is a contagious disease, it is imperative to find the root cause of this epidemic. Dr. Zhengli Shi also known as "Bat Women" from the Wuhan Institute of Virology and Biosafety was one big suspect in this matter. She was suspected of being a creator of this virus. She was working on an experiment with her colleagues in which she did mutation of amino acid so that it get compatible to SARS virus. Moreover, she had published many articles related to SARS virus.[7]

On 3rd February, 2020,Dr. Zhengli Shi claimed that Wuhan virus is of bat origin.

Liu et al detected SARS-CoV from Pangolin lung samples (2020). According to their study, dead Malayan Pangolins were carrying genomic as well as Darwinism evidence of presence of identical CoV like that of SARS-CoV-2 namely Pangolin (Co-V). Pangolin CoV showed most identical features with SARS-CoV-2 beside RaTG13.[8]

RNA – Sequencing was performed on the lung samples of Pangolins. Furthermore, genomic characteristics of Pangolins were studied to find out the origin. Last but not the least, the researchers made a phylogenetic relationship in between the suspected animal species like Pangolin – CoV (from pangolin) RaTG13(from rodents), SARS – CoV-2.[8]

Secondly, bats are also considered as a probable species for the origin of this COVID – 19. The study

depicted that 96% of whole genome sequencing was identical between SARS – CoV- 2 and CoV of bats. Hence, bats species also came in the list of hosts of zoonotic viruses such as Nipah and Hendra virus. These hosts seldom show any clinical symptoms. The overall nucleotide sequence identity between SL – CoV Rp3 (bat) and SARS CoV Tor 2 was 92%. [9]

The current database sequence suggests that all human coronavirus have zoonotic origin. SARS – CoV, HCoV – NL63, HCoV – 229E, MERS - CoV originated from bat species. [10]

Physiochemical Properties:

Virus particle is an oval shaped and diameter varying from 60 to 100 nm (approx.) SARS – CoV and MERS – CoV together give information regarding physiochemical properties of CoVs. Ultraviolet light or heating at 56 degrees for 30 minutes inactivates SARS – CoV- 2. This virus is also sensitive to chemicals like peracetic acid, chloroform, 75% ethanol, diethyl ether. This virus remains for 72 hours on surface like plastic and stainless steel[11].

Genomic Variation

Earlier, a study depicted that largest RNA among all viruses is of coronavirus containing approx. 27-30 kb. Six to seven regions are present in a genome of virus and all of them are well organised. Each region consist of one or more open reading fragment and they are separated by junction sequence containing signals. These signals aid in transcription of multiple sub-genomic mRNA's. [12]

A recent study has described that RNA genome of CoV is second largest in RNA viruses, largest genome is of Planarian Secretory Cell Nidovirus (PSCNV). It consists of 41.1 kilo base genome size. Structural along with the Non-structural proteins are coded by viral RNA. [13] There is no interferences of recombinant events. RNA virus is unstable and hence continuous scrutiny of SARS–CoV-2 spreading human to non-humans is very imperative for controlling the disease.

In the entire genome of Wuhan-Hu-1 coronavirus (WHCV), single strain of SARS – CoV-2 is of 29.9 kb. Moreover, the studies have suggested that genome of CoVs contain fluctuating numbers of open reading frames (ORFs) ranging from 6-11. The positive sense RNA of SARS-CoV and MERS-CoV shows genomes of 27.9 kb and 30.1 kb respectively. [13]

High mutation ratses characterise all RNA viruses, evolution of CoVs and transmission from one species to another. Results and analysis by various researchers have proved that SARS and MERS- CoVs have emerges from ancestral CoVs nurtured by bats. It has been reported that animals are considered as intermediate hosts and humans are terminal hosts.[5]

Epidemic to Pandemic

On December 29,2019 four patients of an acute respiratory syndrome were reported in Wuhan city of China. Wuhan Health Commission (WHC) reported that there is linkage of this virus with that of local seafood market. The Wuhan Institute of Virology of China claimed that out of 33, 585 environmental samples contained coronavirus nucleic acid depicting that this virus have emerged from the wild animals from that local market[15].

In China, there were 11791 confirmed cases of corona virus and 17988 suspected cases in 34 cities of China (as on 31st January,2020). 213 deaths were reported globally since then.This epidemic was converted into pandemic when the virulentdiseaseaffected 19 more countries all over the world.

COVID-19 was regarded as contagious disease. The identification, diagnosis, clinical course, management of this viral disease was done when this disease affected the US province. Globally, the positive cases as on February 16, 2020 were 51,857 in 25 countries as claimed by World Health Organization[14].

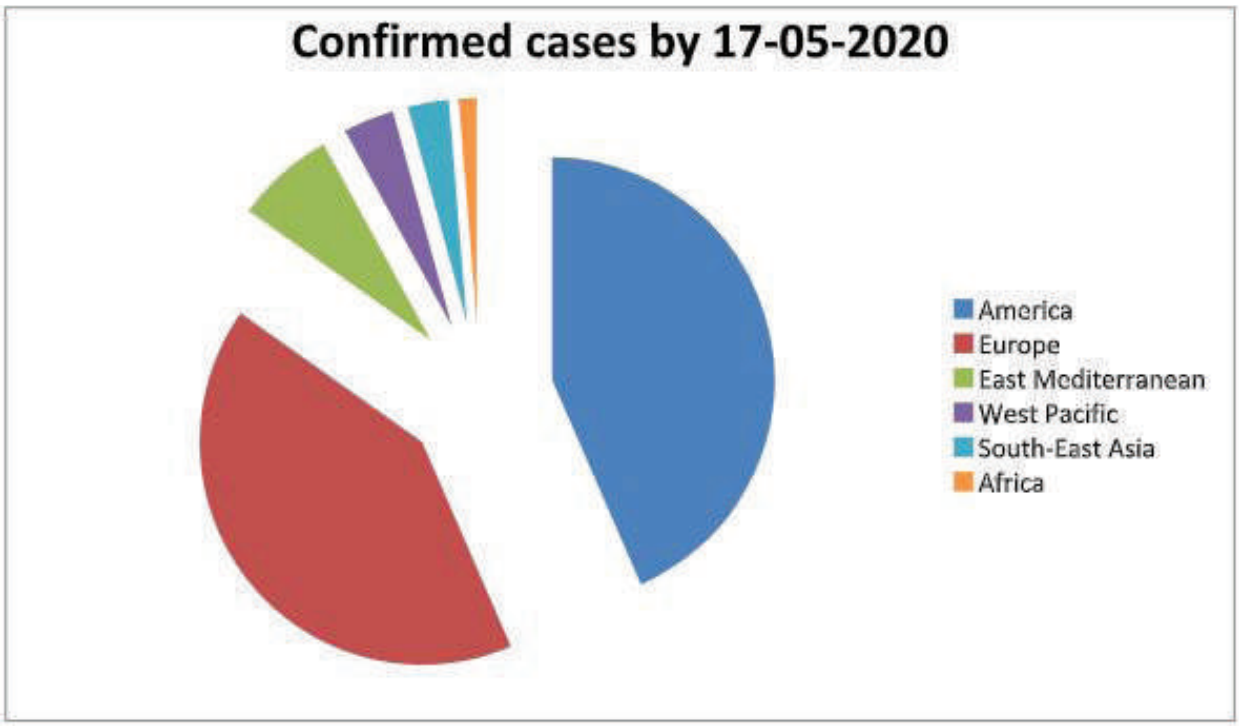


Figure 2. Confirmed cases of COVID-19 till May 17, 2020

Source: WORLD HEALTH ORGANIZATION (WHO)

IS COVID-19 A BIOLOGICAL WEAPON?

In the past years, many microorganisms have been reported which have caused pandemic in the world. These microbes include; Plague, Salmonella, Anthrax, H5N1.

COVID-19 is just one more epidemic which is spreading worldwide like a forest fire. The main reason behind this is that it spread by human to human transmission hence, it is regarded as contagious in nature. Recent researches suggested that this virus is contagious even when the person do not show any symptom.

The similarity between all the epidemics is that they are global and cause widespread destruction all around. One cannot deny the fact that these epidemics go hand in hand with globalization and has barbarous effect on the economy, trade and tourism.

This pandemic has been originated from Wuhan and affected the entire world. People are looking for the reasons for the outbreak of these epidemics.

It is sceptical that this deadly coronavirus affected the world in short time span. It can be opined that SARS-Cov-2 is a man-made virus. *Virola* virus was not a man-made, it undergone certain mutations and then got transferred into humans. So, it might be possible that COVID-19 might be a genetically modified virus or it may be a recombinant virus. [18]

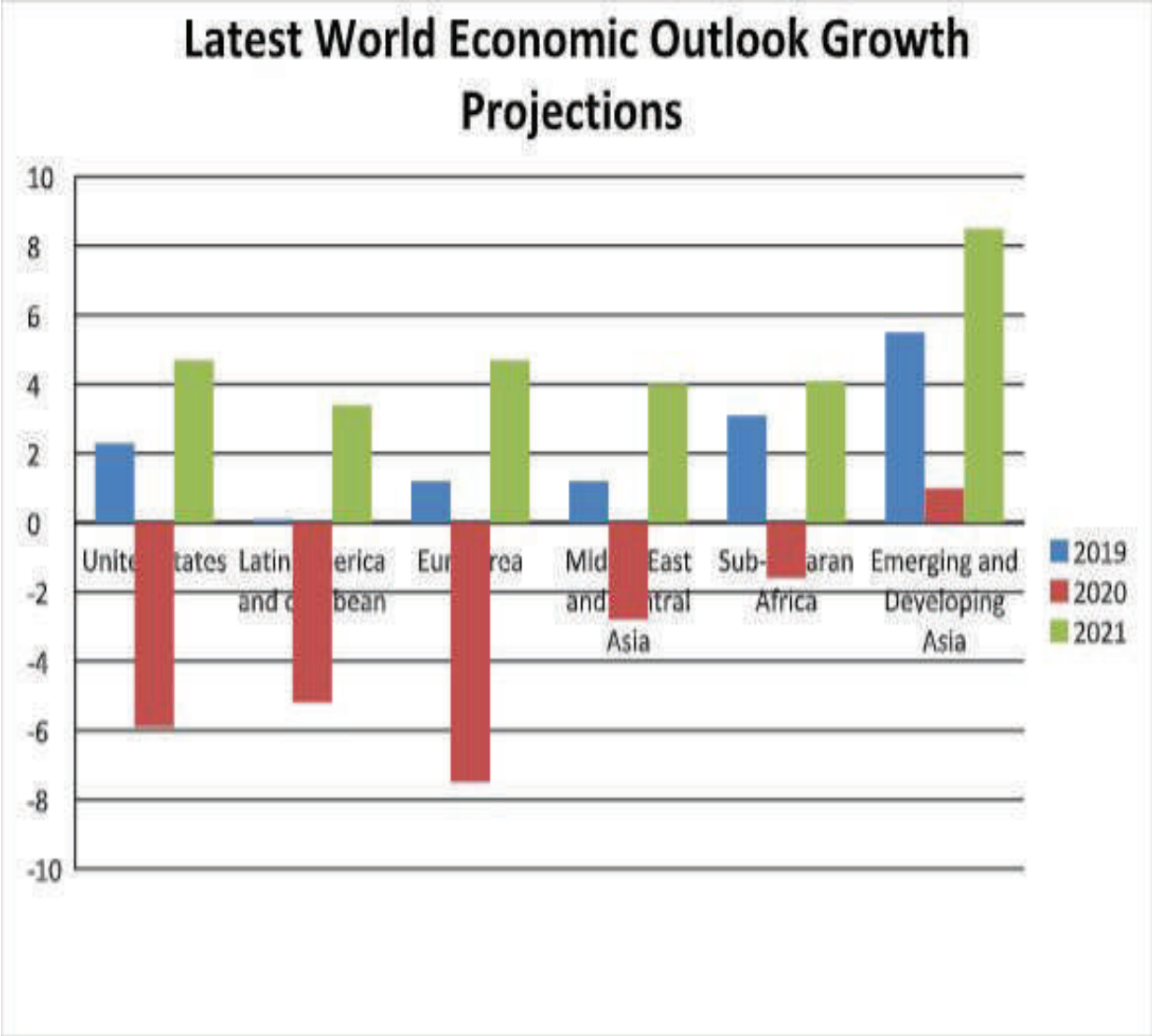


Figure 3. Graph depicting GDP of various countries.

Source: International Monetary Fund (IMF)

	2019	2020	2021
United States	2.3	-5.9	4.7
Euro Area	1.2	-7.4	4.7
Latin America and The Caribbean	0.1	-5.2	3.4
Sub-Saharan Africa	3.1	-1.6	4.1
Middle East and Central Asia	1.2	-2.8	4.0
Emerging and Developing Asia	5.5	1.0	8.5
WORLD	2.1	-3.0	5.8

Source: International Monetary Fund (IMF)

Clinical Analysis:

Yelin et al., used pooling approach for standard RT-qPCR for evaluation of COVID-19. The researchers used swabs from nostrils, throat for the analysis. Pooling works in conjugation with RT-qPCR but it gives 10% false negative results. [16].

Muhammad Farooq along with his co-worker Abdul Hafeez used radiographs for the analysis and claimed that chest X-rays of patients infected by COVID-19 depicted certain abnormalities in radiography. [17]

Hence, Laboratory detection of this virus included certain clinical findings like genome sequencing, RT-PCR technique and Serological methods like Enzyme Linked Immunosorbent Assay (ELISA). This method was based on SARSr - CoVRp3 nucleocapsid protein and was created to ascertain the immunoglobulins IgM and IgG. But the drawback came out when this test gave false positive results among human beta-coronavirus genus.[18]

Vaccines And Drugs:

The viruses which are affecting the world severely with devastating effects include Ebola virus, Nipah virus, Zika virus have started a race of exploring and designing of new vaccines, drugs, therapeutics to get cure of these diseases.

Many drugs and agents are detected under clinical trial by following adequate protocols but efficacy has not yet established for any drug therapy.

Remdesivir:

It is an antiviral drug manufactured by Gilead sciences. It inhibits the replication of virus and this drug is proved commendable for MERS infected rats and monkeys.

Favilavir:

Favilavir is the first drug approved for coronavirus in China. It is licensed as an experimental drug and research is still going on for the effectiveness of this drug.

Liponavir; Ritonavir:

This drug is HIV Protease inhibitor. It supresses the coronavirus activity by binding with an enzyme which help in coronavirus replication.

COVID-19 convalescent Plasma:

The blood Plasma samples accumulated from patients retrieved from the viral disease which mightconsist of antibodies against SARS-CoV-2.

Hydrochloroquine:

Hydrochloroquine is an anti-malarial drug. Clinical trials performed in China showed potency and pertinent protection by this drugs countering COVID-19 linked pneumonia.

Immune System Booster:

Vitamin C and B6 are recommended for enhancing the immunity. Vitamin B6 is important to sustain biochemical reactions. Vitamin E also act as an antioxidant and helps in fighting against infections.

Currently, no such drug, vaccine has been made to cure COVID-19. [19]

Medicolegal Repercussion:

The people infected by the coronavirus were kept under check to ascertain the symptoms caused by this disease. A study done in Wuhan, China revealed that the major symptoms at the inception of illness was fever. 98% of the sufferers were suffering from acute fever. 76% of affected people were having cough. Fatigue was also a parameter which was proved as a major symptom. More than half of the patients were suffering from dyspnea.[20] Other Symptoms which were rarely present included headache, diarrhoea and haemoptysis.[21]

In a study performed in hospitals of Wuhan proposed that hospital related transmission of s2019-nCoV was doubtful in 41% of patients and mortality rate came out to be 4.3%. Adults and infants were subjected more to this viral disease and required ICU care.[22] Currently no vaccine has been made to cure this contagious disease, the safest way to get rid of this disease is to avoid viral exposure.[5]

Wuhan Coronavirus: Manmade or Recombinant

Wuhan coronavirus is strangely identical to 2 bat; ZC45 and ZXC21 as revealed by a study. Recombination

has to take place twice during evolution of Wuhan coronavirus. The ancestor bat corona virus had to acquire through recombination with SARS like coronavirus. [20]

On 2nd January,2020 Director General of Wuhan Institute of Virology released a notice regarding the strict prohibition and disclosure of any information regarding this disease. There were number of news and articles published against Wuhan Institute of Virology regarding origin of coronavirus from this lab. [7]

Through all the news and instances COVID-19 doesn't seem to be a natural virus. There are lots of queries and conjectures on its mutation and manmade interventions.

Conclusion

COVID-19 has proved to be the threatening virus. It is a pandemic which is spreading devastation all over the world. There are many controversies regarding the origin and spreading of this disease. The world is facing severe crisis due to fall in economic GDP and globalisation along with high fatality rate.

COVID-19 might be a Bio-warfare agent which has caused gigantic demolition in the world.

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Effectiveness of Module on Knowledge and Practice Regarding Prevention of Medication Errors among Critical Care Nurses in Selected Hospital

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Abstract

Introduction and objective: Medication administration is the major tasks of each nurses in their clinical practice. Medication administration errors are preventable if the nurses have adequate knowledge and practices regarding the medications. Hence the main objective of this study was to determine the effectiveness of module on knowledge and practices regarding prevention of medication errors among critical care nurses**.**

Methodology: Pre experimental one group pretest and posttest research method used for the study. 50 critical care nurses were selected using convenient sampling method and given questionnaire to assess their knowledge in pretest and posttest done after 1week of intervention. Checklist used to assess their practices in pretest and posttest**.**

Result: The mean score of knowledge in pre-test was 13.2 and in post-test was 21.5 and the t-value was 27.7 The p value was less than 0.05. The mean score of practice in pre-test was 35.3 and in post-test was 48.0, and the t value was 55.3 The p value was less than 0.05. This shows that the module is effective. Hence the null hypothesis is rejected.

Conclusion: The improvement found in the post test proved that the module is effective in improving the knowledge and practices regarding prevention of medication errors among critical care nurses.

Keywords: *Effectiveness, Module, knowledge and practices, medication errors, critical care nurses*

Introduction

During recent years, health care demand is greatly increased. Due to technological advancements and increased globalization the need for patient safety is in high demand. An error in the health care supply can put the organization and also the patients' health in jeopardy. Nurses play a key role in any health care services. Thus, medication administration safety is mainly depending upon the nurses' knowledge and practices. Medication errors have been assumed to count for over one fourth of causes of adverse drug event. Such errors can occur anytime in the process of medication handling by the health care workers or even by the consumers and they are preventable [(1)]. Medication errors are a frequent reason for iatrogenic adverse events. They can lead to severe morbidity, prolonged hospitals stay, unnecessary diagnostic tests, unnecessary treatments, distrust in the healthcare system, severe injury or even patient death [(2)].

Presently in world there are more than 20 thousand variety of drugs available. In spite of their therapeutic effects they all come with their own side effects and complications too. So, it is essential for nurses and nursing students to have the updated pharmacological knowledge to hamper any potential harms [(3)]. Research

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has shown that the rate of medication errors by nurses and nursing students is high; however, the report of these errors by them is low [4]. Medication errors are multifaceted problems and to avoid them we need to discover proper standardised solutions. Many standard protocols provided by WHO, NABH etc will be helpful in preventing medication errors to a greater extent if they are properly followed. In day to day life the major function of nurses involves implementing the medication orders by doctors which is the essential part of the patient care and treatment, thus, in turn leads to patient safety [5].

Medication errors are a grave problem in the world and any one medication error can threaten the patient safety and may lead to even death of them. Medication errors are a constant problem in every hospital. Studies shows that medication errors and adverse drug reactions (ADRs) are one of the important elements for unfavourable events in hospitals which prone to disability and death in up to 6.5% of hospital admissions. Medication error is recognized as the eighth leading cause of death. A medication error is any preventable action which in return lead to inappropriate medication use or patient harm while the medication is in the control of healthcare professional, patient or consumer.

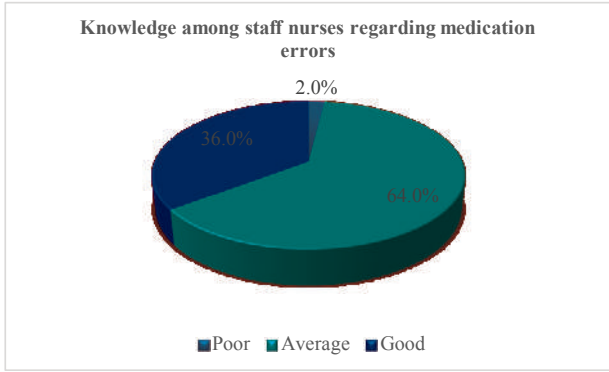
Material and Methods

A Pre-Experimental one group pretest posttest design with quantitative approach was used, as this study was aimed, the approach was found to be most appropriate. The group received module regarding the prevention of medication error (contains basic rights, common conversions, drug and fluid calculation formulas, LASA drugs, medication storage, common emergency drugs, common abbreviations, and instructions for prevention of medication errors) This study was conducted in selected hospital of Pune city. The selection was based on easy accessibility, cooperation and availability of samples. Total 50 Critical care unit staff nurses of selected hospital, Pune city who met the inclusion criteria were selected. Tool used for the collection of data by demographic data and self-structured questionnaire on assessment of knowledge and Checklist to assess on practice.

Findings: The analysis and interpretation of the data collected to determine the Effectiveness of module on knowledge and practices among critical care nurses which is carried out based on objectives set by the researcher taking the level of significance as 0.05

Figure No . 1: Pie diagram on assessment of knowledge regarding medication errors among staff nurses

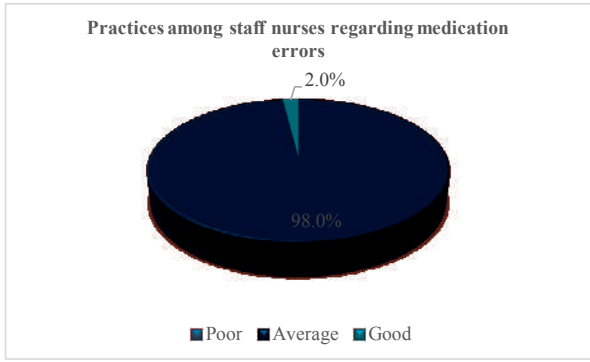
N=50



the pie diagrams state 2% of the nurses had poor knowledge (score 0-7), 64% of the staff nurses had average knowledge (score 11-20) and 36% of them had good knowledge (score 21-30) regarding medication errors.

Figure No . 2: Pie diagram on assessment of practice regarding medication errors among staff nurses

N= 50



The pie diagram show that 98% of the staff nurses had average practices (score 28-39) and 2% of them had good practices (score 40-51) regarding medication errors

Table No 1: Effectiveness of hand module on knowledge regarding medication errors among staff nurses

N=50

Knowledge	Pretest		Posttest	
	Freq	%	Freq	%
Poor (score 0-7)	1	2.0%	0	0.0%
Average (score 8-14)	32	64.0%	0	0.0%
Good (score 15-22)	18	36.0%	50	100.0%

In pretest, 2% of the staff nurses had poor knowledge (score 0-7), 64% of the staff nurses had average knowledge (score 11-20) and 36% of them had good knowledge (score 21-30) regarding medication errors. In posttest, all of them had good knowledge (score 21-30) regarding medication errors. This indicates that the knowledge of staff nurses improved remarkably after hand module regarding medication errors.

Table No 2: Paired t-test for effectiveness of hand module on knowledge among staff nurses regarding medication errors

N=50

	Mean	SD	t	df	p-value
Pretest	13.2	2.2	27.7	49	0.000
Posttest	21.5	0.7			

Researcher applied paired t-test for effectiveness of hand module on knowledge among staff nurses regarding medication errors. Average knowledge score in pretest was 13.2 which was 21.5 in posttest. T-value for this test was 27.7 with 49 degrees of freedom. Corresponding p-value was small (less than 0.05), the null hypothesis is rejected. It is evident that the hand module is significantly effective in improving the knowledge of staff nurses regarding medication errors.

Table No.3: Effectiveness of hand module on practices among staff nurses regarding medication errors

N=50

Practices	Pretest		Posttest	
	Freq	%	Freq	%
Poor (Score 17-27)	0	0.0%	0	0.0%
Average (Score 28-39)	49	98.0%	0	0.0%
Good (Score 40-51)	1	2.0%	50	100.0%

In pretest, 98% of the staff nurses had average practices (score 28-39) and 2% of them had good practices (score 40-51) regarding medication errors. In posttest, all of them had good practices (score 40-51) regarding medication errors. This indicates that the practices of staff nurses improved remarkably after hand module regarding medication errors.

Table No IV.4: Paired t-test for effectiveness of hand module on practices among staff nurses regarding medication errors

N=50

	Mean	SD	t	df	p-value
Pretest	35.3	2.7	55.3	49	0.000
Posttest	48.0	1.1			

Average practices score in pretest was 35.3 which was 48 in posttest. T-value for this test was 55.3 with 49 degrees of freedom. Corresponding p-value was small (less than 0.05), the null hypothesis is rejected. It is evident that the hand module is significantly effective in improving the practices of staff nurses regarding medication errors.

Fisher's exact test was used for the association of knowledge of the nurses with selected demographic variables. All the p-values are large (greater than 0.05), none of the demographic variables was found to have significant association with the knowledge among staff nurses working in critical care unit regarding medical errors.

Fisher's exact test was used for the association of practices of the nurses with selected demographic variables. All the p-values are large (greater than 0.05), none of the demographic variables was found to have significant association with the practice among staff nurses working in critical care unit regarding medical errors.

Discussion

In the present study, 50 staff nurses working in critical care unit were selected to participate in the study. Pre experimental, one group pre-test, post-test method has been used for the study design. Among the 50 nurses participated in the study 68% of the staff nurses were of 21-25 years of age, thus, proving majority of the critical care nurses are freshly completed their degrees, 76% of nurses were females and 24% of them were males, this clearly says that nursing in India is still a female dominated profession. When asked about barriers of practicing safe medication administration 34% nurses replied that time and work pressure is always the barrier followed by 22% nurses felt interruption during medication process is always the barrier. Multiple medication due at time was 8% and personal family issues 4%.Pre-test knowledge and practice of staff nurses regarding prevention of medication errors was assessed using questionnaire and checklist. Module which has information regarding safe medication administration given to every participants and instructions given about the usage of the module. Post-test assessment done after 1 week using the same questionnaire and checklist. The mean score of knowledge in pre-test was 13.2 and in post-test was 21.5 and the t-value was 27.7 with the degree of freedom 49. The p value was less than 0.05. the mean score of practice in pre-test was 35.3 and in post-test was 48.0, and the t value was 55.3 with the degree of freedom 49. The p value was less than 0.05. Hence the null hypothesis is rejected. This shows that the module is effective in improving the knowledge and practice of staff nurses in preventing medication errors.

Conclusion

Medication errors are a major concept of debate during the recent years in the health care system. Nurses are a key member in this debate as they handle the majority of medication administration and spend much of their times near the patients. Many research studies states that nurses commit majority of the medication errors when compared with other health care staffs. The factors causing medication errors among nurses can be due to administration factors, or maybe personal factors. Proper guidelines and protocol are required to minimize these errors. This study used a module with some necessary information regarding safe medication practices to find its effectiveness in preventing medication errors. The findings of the study proved that there is a highly significant effectiveness of the module on knowledge and practices regarding preventing medication errors among staff nurses working in critical

care unit.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Obtained from Institutional Research Committee of Symbiosis College of Nursing

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Non-Compressive Mechanical Injuries to the Neck: An Autopsy based Study

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Abstract

Study of non-compressive mechanical injury to neck determines the causes of deaths by these injuries and preventive measures to be taken. The study was conducted in the department of forensic medicine, Government Medical College Srikakulam. Postmortem examination of each case was carried out various types of non-compressive mechanical neck injuries were recorded, and analyzed over a period of 6 months. Total number of 354 postmortems were conducted among these 41cases had non-compressive mechanical injuries to neck. Injuries were most common in male gender (82.92%) and in age group between 20-40 years. Improving proper traffic rules may reduce mortality due to these injuries.

Key Words: *Non-Compressive Mechanical Injury; Neck; Autopsy.*

Introduction

An injury is any harm, whatever illegally caused to any person in body, mind, reputation or property. Mechanical injuries are injuries produced by physical violence[1]. The neck is protected by head above and chest below respectively. Usually head and chest are involved in injury, but with extensive force for a considerable duration, neck is also involved.[2]Many vital structures are placed in the neck to carry blood, impulses, and air in both directions and food downwards to the rest of gastro-intestinal tract. In cases where there are multiple injuries not confined to the neck, more obvious lesions thus overlooking or delaying the diagnosis of significant injuries to this region.[3]

The incidence of neck injuries has in the past been generally underestimated[4]. One injury that frequently overlooked at autopsy in Road traffic accident cases is the atlanto-occipital dislocation.[5]Blunt carotid artery injuries commonly occur in road traffic accidents, direct blow and fall from height.[6] Blunt impact injuries to the posterior compartment of neck involve injury to cervical vertebrae, spinal cord, vertebral arteries and other structures behind the pre vertebral fascia[1]. In the middle cervical injury, the common carotid artery is the most vulnerable vessel, although other large vessels such as the external and internal carotid arteries as well as the internal jugular vein may be involved.[7]

Stab injuries can damage one or more of the major organ systems of the neck including great vessels, nerves, larynx, trachea, esophagus and spinal column. Most reported cases of spinal cord injury following a stab in the neck are homicidal, and injury site is either back or lateral side of the neck[1].

Choking is produced as a result of mechanical obstruction to the airways. It is commonly accidental in nature.[1] The neck region is frequently subjected to a number of procedures by healthcare management, including airway, esophageal instrumentation, invasive vascular maneuvers such as angiography, intravenous cannulation, tracheostomy etc.[3] Nasogastric tubes insertion may cause trophic ulcers in the larynx followed by perichondritis of the cricoids leading to chondritis, collapse and stenosis of larynx.[8] The introduction of I.V. cannulae into veins in the neck may cause large

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hematoma and more diffuse bleeding into the tissues along-side the larynx. These resuscitation injuries may be mistaken for those due to assault or from steering wheel impact injuries.[9]

A compressive injury to neck includes manual strangulation, ligature strangulation, and hanging. A Non-compressive mechanical injury to neck defined as injuries which are produced by physical violence like blunt/sharp/firearm/thermal/chemical/physical to neck.[1] Study of non-compressive mechanical injury to neck determines the causes of deaths by these injuries and preventive measures to be taken.

Materials and Methods

The present study was conducted for a period of 6 months from October 2019 to March 2020, in the mortuary, department of Forensic Medicine, Government Medical College Srikakulam. During the study period, 354 autopsies were conducted of which 41 (11.58%) cases have non-compressive mechanical injuries to the neck. Age, Gender, Manner, Types of major external injuries to the neck, Internal structures involved, Type of force involved, Severity of these injuries to cause death, Number of cases with only cervical vertebra and spinal cord involved are examined, data gathered from police and relatives were recorded in this study. The non-compressive mechanical injuries to neck in persons who admitted to casualty or ward and survived were not included in this study.

Observations and Discussion

A total number of 354 postmortems were conducted for a period of 6 months in the department of Forensic medicine, Government Medical College, Srikakulam. During the study period majority of cases were Road Traffic Accidents, Railway injuries and fall from height. Out of 354 cases 41cases had non-compressive mechanical injuries to neck. The involvement of neck with non-Compressive mechanical injuries were common in all cases of Drowning (100%) followed by Thermal injuries (40.00%), Assault (28.57%), Railway injuries (28.20%), Road traffic accidents (11.02%). This can be explained by Striking the head on the bottom of the water after miscalculation of the water depth may be the cause for more number of neck injuries in drowning. This study correlates with Sahoo et al.[10] least number of neck injuries in Road traffic accidents can be explained by head and body relatively broad areas and may have direct contact with road comparatively neck.

More number of non-compressive mechanical injuries to neck observed in age group between 20-40 years (68.29%) followed by 41-59 years (19.51%) and Males(82.92%) were more in number than females(17.07%). The incidence is high among Males and 20-40 years age groups may be because they are more actively involved in outdoor occupation, driving etc. more preventive measures to be taken to prevent these types of deaths in these age group males. This study correlates with Jani CB et al. [2]and Bener et al.[11]

The highest number of these type of cases were accidental (73.17%) followed by suicides (19.51%). This can be explained by most accidental cases were due to Road traffic accidents, Railway injuries ,Fall from height, Drowning, and in suicides are due to Railway injuries, Thermal injuries etc, where neck is more prone to be involved. Neck involvement was least in homicidal cases (7.5%) as the head, chest and abdomen are commonly targeted. This study correlates with Sahoo et al.[10] Mohanthy et al.[12]Contrasts with Hugar BS.[13]

Lacerations (39.02%) were the commonest type of mechanical injury observed in non Compressive mechanical injury to the neck followed by abrasions. It can be explained on basis of more number of accidental cases are involved in neck injuries, where lacerations and abrasions are more common. Stab and chop wounds were the least observed injuries because these types of injuries may be common in homicidal deaths where head, chest and abdomen are commonly targeted. This study correlates with Hu et al.[14]

Most commonly involved Internal Structure was trachea (53.65%) followed by vertebra and spinal cord (43.90%). This may be due to trachea is situated superficially and in decapitation injuries, drowning, and whiplash injuries in road traffic accidents, vertebra and spinal cord are more prone to be involved. In 31.7% cases major vessels like carotid arteries are involved. This study correlates with Moar's study.[6]

Highest number of these type of injuries were due to blunt force (65.85%) followed by Obstructive force (31.70%). This may be due to the majority of these cases

were road traffic accidents and railway injuries, which had resulted injuries in the form of lacerations, contusions and abrasions due to blunt force and in drowning, burns, trachea was obstructed due to Obstructive force. Sharp force was the least (2.43%) among these types of injuries because sharp injuries are common in homicides which are very less cases (7.31%) in this study. This study correlates with Bener et al.[11] and contrasts with Hugar BS.[13]

More number (68.29%) of non-compressive mechanical injuries to the neck were non-fatal and in (7.31%) of deaths these injuries contributed to the death. This may be because these non-compressive mechanical injuries were mostly lacerations and abrasions which are nonfatal. In (24.39 %) cases injuries to structures of the neck were solely sufficient to cause death where trachea, vertebra may be involved which causes respiratory failure and death.

In 19.51% of deaths by Non-compressive mechanical injuries no obvious external injuries on the neck were observed but internal neck structures like cervical Vertebra and spinal cord were damaged. This can be explained by an indirect force transmitted along the spine as in cases of fall from height and due to acceleration or deceleration force in cases of road traffic accidents. This study correlates with Tonge et al.[4]and contrasts with N T Satish.[15]

Table No. 1: Total Number of cases and various causes for Non-Compressive Mechanical Injury to the Neck.

S. no	Cause Of Death	Total no of cases	No of cases with Non-Compressive Mechanical Injury To The Neck	Percentage
1	Road Traffic Accidents	127	14	11.02%
2	Railway injuries	39	11	28.20%
3	Fall from Height	16	02	12.50%
4	Thermal injuries	10	04	40.00%
5	Assault	07	02	28.57%
6	Drowning	06	06	100.00%
7	Others	149	02	1.34%
8	Total	354	41	11.58%

Table No. 2: Age distribution In Non-Compressive Mechanical Injury to the Neck

S.No	Age in years	No of cases	Percentage
1	<20	04	9.75%
2	20-40	28	68.29%
3	41-59	08	19.51%
4	>60	01	2.43%

Table No. 3: Types of major external injuries In Non-Compressive Mechanical Injury to the Neck

S. No	Type of injury	No of cases	Percentage
1	Laceration	16	39.02%
2	Abrasion	11	26.82%
3	Contusion	6	14.63%
4	Thermal	5	12.19%
5	Incised wound	2	04.87%
6	Stab/Chop wound	1	2.43%

Table No. 4: Internal structures involved in Non-Compressive Mechanical Injury to the Neck

S. No	Internal Structures Involved	No of cases	Percentage
1	Trachea	22	53.65%
2	Vertebra& Spinal cord	18	43.90%
3	Strap muscles	17	41.46%
4	Major vessels	13	31.70%
5	Thyroid gland and cartilage	12	29.26%
6	Esophagus	11	26.82%
7	Hyoid bone	10	24.39%

Table No. 5: Severity of death due to Non-Compressive Mechanical Injury to the Neck

S. No	Severity	No of cases	Percentage
1	Fatal	10	24.39%
2	Contributory	03	07.31%
3	Non-fatal	28	68.29%

Table No. 6: Number of Cases with Only Cervical Vertebra and Spinal Cord Involved

S. No	Type of Cases	No of cases	Percentage
1	RTA	5	12.19%
2	Railway Injuries	1	2.43%
3	Fall from Height	1	2.43%
4	Others	1	2.43%
5	Total	8	19.51%

Conclusions

1. Males aged between 20-40 years were more vulnerable for non compressive mechanical injuries to neck.

2. Road traffic accidents and railway injuries were the major causes of these injuries.

3. This type of injuries to neck was involved in all deaths due to drowning.

4. Blunt and obstructive force was the commonest type for these types of injuries.

5. The common types of injuries in non-compressive mechanical injuries to neck were lacerations, abrasions and contusions.

6. Most of these injuries were nonfatal.

7. In 19.51% of cases most commonly injured internal neck structures like trachea, cervical vertebra and spinal cord were only injured with no external injuries.

8. Commonest manner of death is accidental.

Suggestions And Recommendations

1. Higher mortality among males aged 20-40, will retard economic growth rates. So there should be strong health policy for preventive as well as curative health services.

2. Government should initiate better health awareness campaigns for healthy life styles, environment modifications, and safety measures to avoid accidental deaths.

3. Proper punishments should be implemented to maintain Personal responsibility when driving and avoid alcohol before driving will reduce many road traffic accidents.

4. Headrests which are properly fitted and intelligent seat design play a major role in preventing or reducing the severity of whiplash injuries.

5. In suspected cases of neck injuries, before shifting the patient to hospital immobilize the head, neck and shoulder area. Cervical collar should be applied as soon as possible.

6. In all suspected cases of blunt injury to the neck where there were no obvious external neck injuries, examination of deep structures of the neck like vertebra, spinal cord and vertebral vessels should be carried out carefully.

7. During autopsy in road traffic accident cases atlanto-occipital dislocation should be observed carefully to avoid wrong opinion of post mortem report.

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Evaluate the Effectiveness of Recreational Therapy on Level of Wellbeing and Hope among Psychiatric Inpatients

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Abstract

Background: In India at least 13.7 per cent of general population has various mental disorders, 10.6 per cent of them require immediate interventions. While nearly 10 per cent of the population has common mental disorders, 1.9 per cent of the population suffers from severe mental disorders.

Objective: To evaluate the effectiveness of recreational therapy on level of wellbeing and hope among psychiatric inpatients

Method: The present study adopted a Quantitative research approach. The sample comprised of 33 psychiatric in patients with psychotic and neurotic disorder admitted in psychiatry ward of MMIMSR hospital, Haryana on the basis of purposive sampling technique. The data were collected before and after the administration of recreational therapy, and only two components of recreational therapy (Physical exercise and Mandala Art therapy) were administered to the patients for 8 consecutive days with 16 sessions. The level of wellbeing and hope were assess by using Wardwick Edenburg Mental Wellbeing scale and Herth Hope Index.

Conclusion: The results of the study show a significant mean difference in the level of hope and level of wellbeing. The value of p=0.00 was also statically significant at 0.05 level of significance. From the study, it can be concluded that recreational therapy was effective in increasing the level of wellbeing and hope among the psychiatric inpatients.

Key Words: *Psychiatric inpatients, level of wellbeing, hope, recreational therapy, physical exercise, mandala art therapy.*

Introduction

World Health Organization (WHO) states that mental health is: ... a state of well-being in which the individual realizes his or her own abilities, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community. Maintaining a positive, mental health is just as important as staying physically healthy. Being active, having a sense of belonging and having a purpose in life all contribute to happiness, good mental health[1]. Although mental health care represents a substantial portion of health services, it has been one of the slowest settings to develop and to implement outcome measurement. In acute mental health condition, intervention has been found to be effective for symptoms like depression, anxiety and self-efficacy. Whereas in severe condition and persistent mental illness, documented outcomes have been demonstrated related to symptoms of psychosis, cognitive, social functioning and self-efficacy. However, there is a less outcomes studies conducted which have documented a relationship between leisure functioning and wellbeing[2].

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Recreation helps in enhancing physical, cognitive, emotional, social and leisure development so that individuals participate fully and independently in chosen life pursuits. Recreational Therapeutic means a treatment service design to restore, remediate and rehabilitate a person's level of function and independence in life activities, to promote health and wellness as well as reduce or eliminate the activity limitations and restrictions to participate in life situations caused by an illness and disabling conditions[3].

Materials and Methodology

Design of the study:

The present study adopted a Quantitative research approach. A formal administrative approval was obtained from the ethical committee of selected hospital Mullana, Ambala to conduct the final study. The research participants were enrolled in the study after written informed consent and they were assured about the confidentiality of their response.

Samples of the study:

The participants in the study were 33 psychiatric inpatients with psychotic and neurotic disorder admitted in psychiatry ward of selected hospital. The participants had been provided their informed consent prior to their inclusion in the study and consent to participate. The participants in the study were patients who were receiving treatment in psychiatric ward of selected hospital, Haryana, India and divided into two criteria i.e. inclusion and exclusion criteria. The inclusion criteria were patients who i) are psychotic and neurotic patients, ii) are willing to participate in the study, iii) understand Hindi, iv) attended minimum 6 sessions of recreational therapy. The exclusion criteria included who i) are not available at the time of data collection ii) are mentally incapable and cannot follow the instructions.

A total of 36 patients have participated in the study but due to the early discharge of the patients 3 of them have been dropped out from the study. All the patient was stable at the time of their participation in the study. Written consent was taken from the participant before proceeding to data collection. On 1st day, pre-assessment was taken by administering the tool i.e., Sample characteristics, Wardwick Edenburg Mental wellbeing scale, Herth's Hope Index to assess the level of wellbeing and hope. And then recreational therapy was given daily for 8 days and observational checklist was also assessed for group involvement of the participants during the mandala art session. On the 8th day post-test was taken. The application of recreational therapy was given for a total of 16 sessions that mainly includes physical exercise for 15 minutes & Mandala Art Therapy for 20 minutes which was given 8 sessions each for 8 consecutive days.

Statistical Analysis

The results of the present study were analyzed by using SPPS version 20.0. Sample characteristics of the participants were analyzed by using frequencies, percentage. Range, mean, median, standard deviation, and mean difference were used to describe level of wellbeing and hope before and after the administration of recreational therapy among psychiatric inpatients. Paired' test was used to check the pre-test and post test score differences. ANOVA and independent 't' test were used to check the association between sample characteristics and level of wellbeing and hope.

Kolmogorov-Smirnov test were also applied to checked the normality of wellbeing and hope where it was found that wellbeing score in pretest ($p= 0.12$), post-test ($p= 0.09$) were normally distributed as calculated value is not significant at 0.05 level and also the hope score in pre-test ($p=0.17$), post-test ($p= 0.11$) has been distributed normally as calculated value is not significant at 0.05 level and therefore parametric test was applied to assess the level of wellbeing and hope.

Results and Discussion

Sample characteristics:

The sample characteristics included more than half (54.5%) of age group between 20-30 years. Majority of the participants i.e., (75.8%) belongs to Hindu religion, one third (45.5%) of the participants were illiterate. All the participants (100%) were unemployed and also more than half, (57.5%) of the participants were earning between (18,953- 18949) rupees per month. Large portions of the participants (57.6%) were married and one third of them (39.4%) were having more than two children**.** More than half (57.6%) of the participants were having the hobby for watching TV and also Majority

(57.6%) of the participants belonged to lower middle class. One third of the participants were diagnosed with schizophrenia disorder, followed by conversion disorder with (27.3%), and depression (15.2%) and only (3%) of the participants were having Obsessive Compulsive Disorder. The duration of illness of majority of the participants was at the range of 1-3 years. Only (45%) of the participants were having 3 times of episode of illness. More than half (54.5%) of the participants were having insight of grade 3 and (45.4%) of the participants is followed by insight grade of 4. All the participants have not received any regular divisional or recreational activities during the intervention and also majority of the participants did not have any associated medical illness.

All participants were receiving medications, out of which half of the participants were using antipsychotic drugs like Tab. Serenace 5mg (Haloperidol), Tab. Clozaril 25mg (Clozapine), Tab. Zyprexa 5mg (olanzapine). Less number of participants was using antidepressants like Tab. Zoloft (sertraline) 25mg, and minimum of the participants were using antianxiety drugs like Petril 0.5mg (Clonazepam), Tab. Consern 20mg (Betapro-ET).

Frequency and percentage distribution of psychiatric inpatients in terms of level of wellbeing and hope:

Percentage distribution of psychiatric inpatients in terms of level of wellbeing and hope was shown in figure 1 and 2. Almost 90.9% of the participants were having low level of mental well being and after administration of recreational therapy 93.9% of the participants were having average level of mental wellbeing. Whereas in terms of level of hope 80% of the participants were having low level of hope but after receiving recreational therapy 97% was having high level of hope.

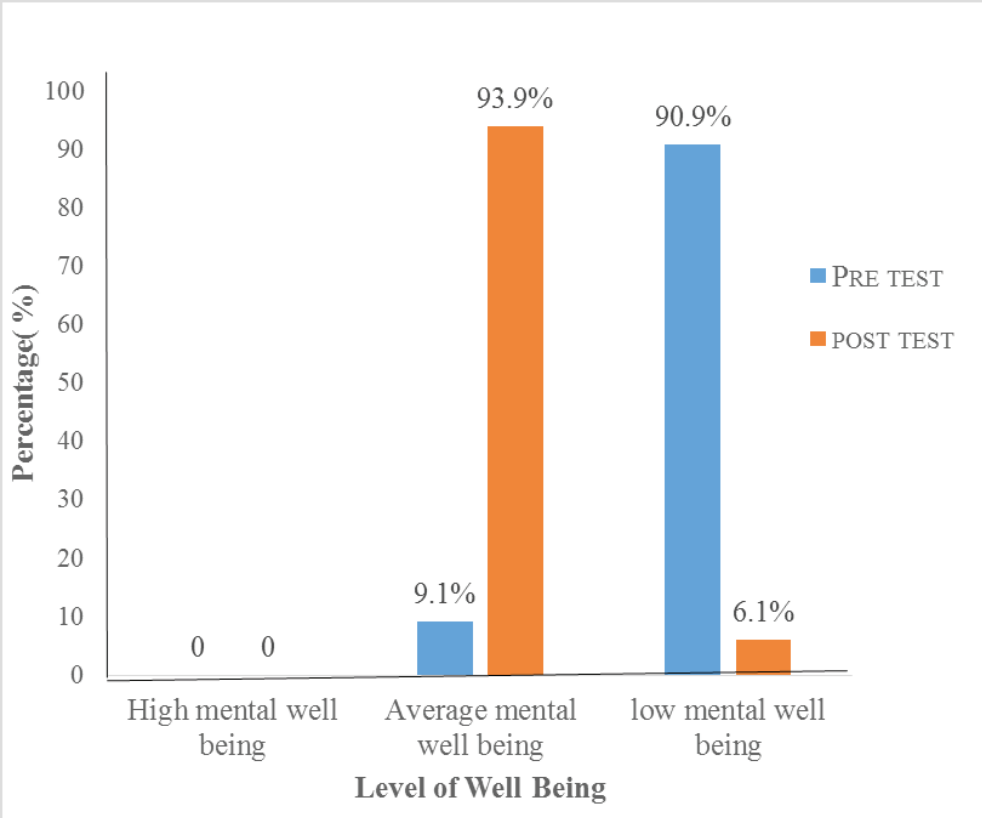


Figure 1: Level of wellbeing among Psychiatric inpatients

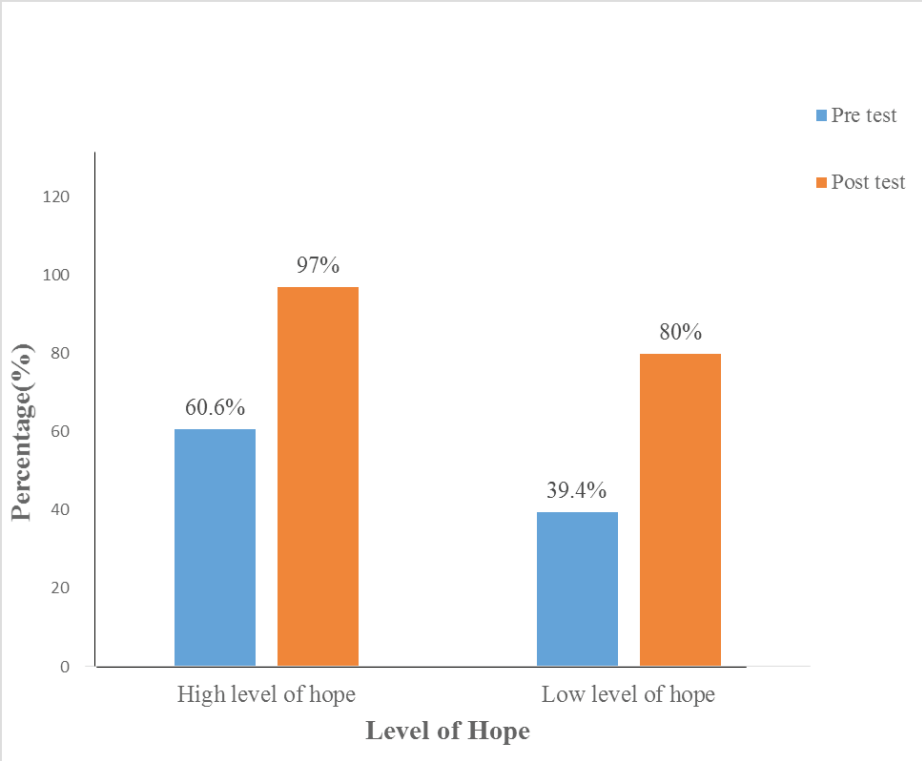


Figure 2 : Level of hope among psychiatric inpatients.

Evaluate the effectiveness of recreational therapy on level of wellbeing and hope among psychiatric inpatients:

In level of wellbeing, the mean pre-test score and the mean post-test score shows a significant difference among the psychiatric inpatients as the calculated 't' value was found to be 13.92 and p=0.00 which highly significant. In the level of hope also, there was a significant difference in the mean pretest post-test hope among the psychiatric inpatients as the calculated 't' value was found to be 8.20 and p=0.00 which was highly significant at the level of 0.005 level of significance. This concludes that, recreational therapy was effective in increasing the level of wellbeing and hope among the psychiatric inpatients**.** The level of wellbeing and hope before and after the intervention of recreational therapy was shown in table 1.

Table 1: Mean, Mean difference, Standard deviation of difference, Standard error of mean difference and 't' value of Wellbeing and Hope before & after administration of recreational therapy.

N=33

Variable	Test	Mean±SD	MD	SDD	SEMD	't'value	df	p value
wellbeing	Pre-test Post test	39.57±2.42 49.84±3.6	10.27	1.28	0.73	13.92	32	0.00**
Hope	Pre-test Post test	30.12±1.62 33.03±1.79	2.91	0.16	0.35	8.20	32	0.00**

*Significant (p≤0.05) t(32)=2.16NS - Not significant

Correlation between wellbeing and hope score among psychiatric inpatients:

There was a negative co-relation between level of wellbeing and hope which was found to be not significant at 0.05 level of significance. This concludes that there is no significant relationship between wellbeing and hope score among psychiatric inpatients. The Correlation between wellbeing and hope score among psychiatric inpatients is shown in table 2.

Table 2: Correlation of level of wellbeing and hope among psychiatric inpatients before and after administration of recreational therapy. N=33

Correlation	Level of wellbeing (r and p value)	Hope (r and p value)
Level of well being	XX	0.275(0.121)NS
Level of Hope	0.237(0.184)NS	XX

NS - Not significant r(31)=0.811 *Significant (p=≤0.05)

Association of wellbeing and hope scores with sample characteristics among psychiatric inpatients with sample characteristics was tested by using one-way ANOVA and Independent't' test. There was no significant association found among the wellbeing and hope scores with sample characteristics.

Discussion

In the present study, recreational therapy was found to be effective intervention in increasing the level of wellbeing and hope among psychiatric inpatient as there was significant increase in level of wellbeing score (t=13.92, p=0.00) and hope score (t= 8.20, p= 0.00). The finding was consistent with the study conducted by **Kim H et al**[4] where the results revealed that there was an overall increase in the level of wellbeing and hope among the psychiatric inpatients after giving recreational therapy (mean score of 9.5 and 15.3 at pre- and post-test, respectively). These results suggest that recreational therapy can have a positive effect on the level of wellbeing and hope of psychiatric inpatients.

In this study, physical activity as one of the components of recreational therapy was found to be an effective intervention in increasing the level of wellbeing among the psychiatric inpatients as there was a significant increase in level of wellbeing score (t=13.92, p=0.00). This infers that physical activity has a positive result in enhancing the wellbeing of the patient. The findings are similar to the study conducted by **Babyak M et al**[5] where they found that exercise therapy was a good alternative to improve the wellbeing among depressive patients.

In the present study, physical exercise was found to be effective intervention in increasing the level of wellbeing among psychiatric inpatient as the level of wellbeing score (t=13.92, p=0.00) was significant at the level of 0.05 level. The findings were consistent with the study conducted by **Abrantes et al**[6] to examined the impact of physical exercise as an adjuvant intervention to regular care in reducing Obsessive Compulsive disorder symptoms. The result revealed that there was a reduction in OCD symptoms and lastly there was an improvement in overall sense of wellbeing after the intervention of physical exercise.

In this present study, there was no association found between hope among psychiatric inpatients with sample characteristics. These findings are similar to the study conducted by **Rani RA et al**[7] where it was found that there was no association of age, gender, education, marital status and occupation with the level of hope.

In this study, mandala art therapy which was also another component of recreational therapy which was found to be effective intervention in increasing the

level of hope among psychiatric inpatients as there was significant increase in level of hope score (t= 8.20, p= 0.00). The findings were consistent with the study conducted by **Babouchkina A et al**[8] where the results revealed that creation of mandala art has a specific efficacy for reducing negative moods. There was a significantly greater mood improvement which results in the enhancement in the hope level as mandala serves as an active ingredient was an overall increase in the level of wellbeing and hope among the psychiatric inpatients after giving recreational therapy (mean score of 9.5 and 15.3 at pre- and post-test, respectively). These results suggest that recreational therapy can have a positive effect on the level of wellbeing and hope of psychiatric inpatients.

In the present study, there was a significant difference in terms of wellbeing after administration of physical exercise among the psychiatric inpatients as the calculated value was significant at the level of 0.05. The findings of the study were similar with the study conducted by **Zschucke E et al**[9], where the findings of the study reveal that there was a relationship between physical exercise and wellbeing. Hence the study concludes physical exercise as one of the associated factors which improve health measures and wellbeing of patients.

In the present study there was no significant correlation found between level of wellbeing and hope among psychiatric inpatients before and after administration of recreational therapy. The calculated 'p' value was found to be 0.121 and 0.184 which was not significant at 0.05 level of significance. These findings are contradictory to the study conducted by **Kato T et al**[10] where they found that there was a correlation between Hope and wellbeing.

In this study, the level of hope was found to be low among psychiatric inpatients. The finding of the study was contradictory to study conducted by **Coskun E et al**[11] where they found that the hope levels of psychiatric inpatients was found to be moderate.

Conclusion

In the conclusion, the findings of the present study provide that Recreational Therapy was effective in improving level of wellbeing and hope among psychiatric inpatients as there was a significant difference between pre-test and post-test group. The findings also show that the components of recreational therapy that is physical exercise and mandala art therapy helps in improving the wellbeing and hope among the psychiatric inpatients. Further, no correlation was found between wellbeing and hope.

Ethical Clearance- Taken

Source of Funding- Self

Conflict of Interest- No

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A Study of Autopsy Findings in Cases of Sudden Cardiac Death at Kakatiya Medical College and Hospital Warangal

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Abstract

Background and Objectives: The incidence of sudden cardiac death is increasing in developing countries due to changes in lifestyle and food habits. We in the present study tried to evaluate the autopsy findings of cases of sudden cardiac death reported to Kakatiya Medical College and MGM Hospital Warangal.

Methods: The present study was carried in the Department of Forensic Medicine and Toxicology, Kakatiya Medical College and MGM Hospital Warangal from January 2019 to Jan 2020. All the cases including both sexes those who died suddenly and unexpectedly brought to the institute for post-mortem examination were studied.

Results: In 10% of cases major blockage was noted in both right and left main coronary arteries. Left main trunk blockage was noted in 31% of cases 5% of cases the blockage was seen in only right coronary arteries. In 32% of cases, the blockage was severe >90%. The highest incidence of major blocks was seen in the age group 60-70 years. The younger age group showed the tendency of blockage of the left coronary arteries. The less commonly affected was the circumflex branch and right coronary arteries. MI was the cause of death in 44% of cases and 5% of cases there was evidence of old and fresh infarcts.

Conclusion: Within the limitations of the current study it can be concluded that most of the cases of sudden cardiac death in this study were from 51-70 years. The most commonly affected victims were males by a ratio of 17:3. Autopsy plays an important role in the accurate diagnosis of sudden cardiac death. Therefore meticulous postmortem examination and histopathological examination are needed to minimize the risk of negative reporting.

***Keywords**: Sudden Cardiac Death (SCD), Autopsy Findings, Myocardial Infarction (MI), Coronary Artery Disease (CAD).*

Introduction

Sudden cardiac death as per WHO includes the death of a person usually seen within 24 hours of the onset of symptoms [1]. The widely accepted definition of sudden death is a death that is not caused due to trauma, poisoning, violent asphyxia which occurs within twenty-four hours of the onset of symptoms [2]. There is a rising trend in cases of sudden cardiac death across the world particularly in the urban population during the last five decades [3,4]. The majority of cases of sudden cardiac death in the older population are attributed to atherosclerotic coronary artery diseases whereas cardiomyopathies are the cause of death in younger (<35 years) age [5]. Studies in India have shown the incidence of ischemic heart disease has increased to 10% [6]. The most common cause of death recorded in autopsy in cases of sudden cardiac death is myocardial infarction due to coronary insufficiency [7]. In some of the cases of SCDs, the cardiac abnormality cannot be identified despite histopathologic

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examination and toxicology screening in such cases they will be classified as sudden arrhythmic death syndrome [SADS] [5]. The incident is associated with the development of ventricular tachycardia progressing to ventricular fibrillation and at lastly Asystole. Identifying patients at risk of developing sudden cardiac death is an important challenge for clinicians but unfortunately, the majority of sudden cardiac death victims who suffer from cardiac diseases are discovered through the postmortem examination. We in the present study tried to evaluate the causes of sudden cardiac death in cases reported to Kakatiya Medical College and MGM Hospital Warangal.

Material and Methods

The present study was carried in the Department of Forensic Medicine and Toxicology, Kakatiya Medical College and MGM Hospital Warangal from January 2019 to Jan 2020. All the cases including both sexes those who died suddenly and unexpectedly brought to the institute for post-mortem examination were studied based on the inclusion and exclusion criteria

Inclusion criteria

1. Cases in which autopsy results showed deaths due to cardiovascular system involvement.

2. Cases where the autopsy was done within the 24 hours of the death of the subject.

3. Cases that were brought in an unconscious state to casualty and subsequently died due to cardiovascular causes.

Exclusion criteria

1. Cases of unnatural deaths were not included in the study.

2. Cases where the cause of death remained obscure despite detailed post-mortem examination including histopathological, toxicological and microbiological examinations.

3. Cases of death which reported after 24 hours for post mortem examination

4. Deaths due to non-cardiac origin.

The dissection of the heart was done from the subjects as per the description by Davidson's technique [8]. The ventricles were cut transversely to view the endocardium including its colors, scars, and fibrosis if present. The autopsy findings were focused on details of the gross appearance of heart its surfaces, walls, coronary arteries, valves, great vessels, the weight of heart was measured and recorded in all cases, condition of papillary muscles was noted. The myocardial infarction was considered recent if hemorrhage paleness and softening found in the myocardium. The specimens were preserved in 10% formol saline [is prepared by dissolving 8.5 g of sodium chloride in 900 ml of distilled water and mixing with 100 ml of 40 percent formaldehyde] and Hematoxylin and Eosin staining for microscopic examination. The microscopic examinations in such cases revealed cytoplasmic hypereosinophilia, granularity, edema, and hemorrhages. The signs of old infarct were the presence of triangular-shaped scars of 0.5cms or more and directed towards the apex. All the data was recorded in MS Excel Spreadsheet and statistical analysis was done using SPSS version 17 on windows format.

Results

A total of n=100 patients were autopsied and studied during the study period from Jan 2019 to Jan 2020. The ratio of male to female was 17: 3 for sudden cardiac death (85% male and 15% female). Table 1 shows the demographic profile of the subjects included in the study. It is evident from the study that most of the patients were belonging to 51 to 70 age groups with a total of 58% of subjects.

The 20% of cases were found dead without any complaints of illness before admission in casualty and 21% of cases the history of illness was unknown. 39% of patients had a history of ischemic heart disease and were on various medications during the time of incidence. In 6% of patients, the existence of cardiac disease was not known by the subjects. 59% of patients were showing signs and symptoms of illness and 20% had ignored the signs and symptoms and 39% were in casualty before the event. Most of the patients died within 24 to 48 hours of the appearance of symptoms. Most of the signs and symptoms by the patients in the study were sweating in 30% followed by dyspnea in 59% cases, pericardial pain in 50% of cases other symptoms were vomiting and cyanosis in 30% cases.

The Middle-income group was mostly involved in sudden cardiac death 41% of cases followed by the higher income group in 30%. The most common cardiac pathology in these cases was coronary artery disease [CAD]. The majority of cases 57% of deaths were reported outside the hospital. The anatomical location of block and percentage of atherosclerotic lesions was studied in the autopsy.

In 10% of cases, a major blockage was noted in both right and left main coronary arteries. Left main trunk blockage was noted in 31% of cases 5% of cases the blockage was seen in only right coronary arteries. In 32% of cases, the blockage was severe >90%. In most of the cases, the type of occlusion observed was in the form of concentric central pinhole type to crescentic with the lumen of the vessel pushed to one side. The highest incidence of major blocks was seen in the age group 60-70 years. The younger age group showed a tendency of blockage of the left coronary arteries. The less commonly affected was the circumflex branch and right coronary arteries. MI was the cause of death in 44% of cases and 5% of cases there was evidence of old and fresh infarcts.

Statistical analysis was done by the following method: All of the available data was entered in MS Excel spreadsheet and analyzed by SPSS version 17 on windows format

Table 1: Showing the demographic profile of cases included in the study

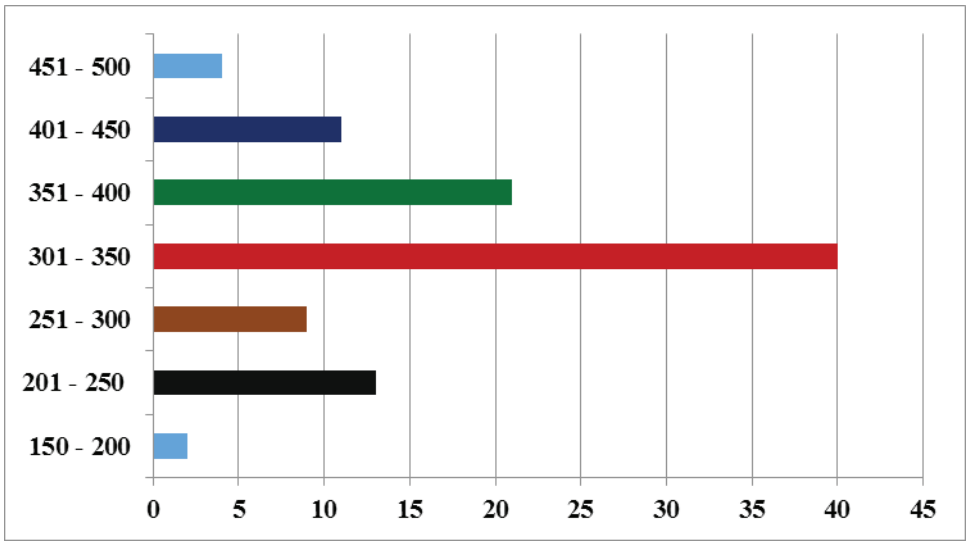
Age group	Male	Female	Total (%)
21 – 30	04	00	04
31 – 40	09	01	10
41 – 50	10	03	13
51 – 60	15	07	22
61 – 70	34	02	36
> 70	13	02	15
Total	85	15	100

Table 2: duration of onset of symptoms and death

Duration of symptoms	Male	Female	Total (%)
< 30 minutes	08	07	15
1 – 2 hours	04	03	07
12 – 24 hours	33	04	37
unknown	40	01	41

Table 3: showing the socioeconomic status of the cases in the study

Socioeconomic status	Male	Female	Total (%)
Low Income Group	07	05	12
Middle Income Group	39	02	41
High Income Group	29	01	30
Unspecified	10	07	17



Graph 1: Showing the Weight of the heart in grams after an autopsy

Table 4: Types of cardiac pathology found in the present study

Pathology	Male	Female	Frequency (%)
Myocardial Infarction	35	09	44
Coronary Artery Disease	26	03	29
Rheumatic Heart Disease	05	02	07
Hypertrophic cardiomyopathy	03	00	03
Hypertensive heart disease	07	01	08
Alcoholic cardiomyopathy	06	00	06
Aneurysm of Heart	03	00	03
Total	85	15	100

Table 5: Showing the involved artery with the percentage of blockage in cases of the study

Involved artery	Frequency	Blockage percentage		
		70 – 100%	40 – 70%	< 30 %
Left Main trunk	41	29	10	2
Left Anterior Descending branch	44	35	5	4
Left circumflex artery	20	8	4	8
Right coronary artery	15	10	2	3

tes)

Discussion

The postmortem examination of the case of SCD includes macroscopic and histological studies of the heart apart from the heart, examinations of other organs is done to rule out possible non-cardiac causes

of death [9]. The macroscopic examinations include the abnormalities of pulmonary arteries for embolus and patterns and origins of coronary arteries and distributions and examination of luminal obstruction. The size of chambers of heart, conditions of valves, the thickness of walls and presence of zones of old or new infarcts [10]. The intactness of papillary muscles and chordae tendineae should be done [11]. In the present study, we found Myocardial infarction as the major contributory cause of sudden cardiac death in 44% of patients. The overall male to female ratio in the study was 17:3. Framingham's study has found the cases of SCD are 10 times more common in males as compared to females [12]. Other similar studies have reported the male to female ratio of coronary heart disease from 3:2 to 8:1 [13-15]. The highest number of cases of sudden cardiac death was between 60 - 70 years of age group. Chugh SS has found a higher prevalence of SCD in the geriatric age group 75-85 years [13]. It has been found that the age-related trends of SCD increase with age with no predilection to race or sex [16]. Derya AA et al; have shown that a large number of sudden natural death was in the age group 50-59 years [17]. DS Rao et al; [18] found the largest number of cases of sudden natural deaths were between age group 45 - 65 years. Although cases of sudden death in this study were aged above 60 years we also found 22% of cases in the age of 51-60 years. The incidence of SCD is increasing due to changes in lifestyles, use of refined carbohydrates, stress and strain in life and lack of regular medical check-ups. Kuller et al; have shown that cases of sudden non-traumatic death are due to arteriosclerotic diseases [19]. In the present study, the timing of death was in early morning hours from 5 AM to 12 PM in 47% of cases. According to Chaudhari VA et al; higher frequency in the morning may be related to the stress of life which includes the fulfillment of duties, transportation and other work-related activities [20]. The hemodynamic stress to CVS appears to be greater in the morning and any vulnerable atherosclerotic plaque causes sudden cardiac death. The situation appears to be compounded by higher levels of catecholamines in the morning hours. Studies have also found that there is a greater tendency of platelet agreeability in morning hours [21, 22]. In this study, we found the left anterior descending branch involvement in 40% of cases. Most of the coronary arteries were blocked within 2cms of origin of the artery and in 32% of cases, the blockage was severe >90%. The left anterior descending branch supplies over half of the heart muscle hence most of the time blockage is found at the beginning of the artery. In most of the cases, the type of occlusion observed was in the form of concentric central pinhole type to crescentic with the lumen of the vessel pushed to one side. The involvement of the left anterior descending artery was higher with severe narrowing as compared to cases where the right coronary artery is involved. Bohrod MG has found the ranges of the left anterior descending branch were 45-64% while the right coronary artery was involved in 24-46% and the left circumflex artery is involved in 3 -10% of cases [23].

Conclusions

Within the limitations of the current study, it can be concluded that most of the cases of sudden cardiac death in this study were from 51-70 years. The most commonly affected victims were males by a ratio of 17:3. Autopsy plays an important role in the accurate diagnosis of sudden cardiac death. Therefore meticulous postmortem examination and histopathological examination are needed to minimize the risk of negative reporting. The use of newer and more sophisticated techniques like 3T-MRI, S100 Calcium Binding Protein A1 (S100A1), and quantitative myoglobin essay may be done wherever feasible.

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Radiographic Evaluation of Third Molar Development in Relation to Chronological Age among South Indian Population

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Abstract

Background: The aim of the study was to correlate the radiologic development status of third molars with that of chronologic age specifically among individuals belonging to Dakshina Kannada and Udupi district in the age group of 16-22 years.

Method: A total of 152 orthopantomograms (OPG) were evaluated to assess the development status and scored using the evaluation method suggested my Gleiser and Hunt. Intra- and Inter-observer reliability was evaluated using the k statistics. Statistical test used were multiple regression analysis and Pearson correlation coefficients.

Results and Conclusion: The formula to determine chronological age among females is Age= 13.416 + (-0.873 Upper Right (UR)) + 0.803 Upper left (UL) + 0.536 Lower left (LL) + 0.362 Lower Right (LR) with standard deviation of 1.61, and for males is Age = 12.766 (-0.187 UR) + 0.748 UL + 0.873 LL + (-0.541 LR) with SD of 1.57.

Key Words: *Third molars, Age estimation, Orthopantamogram,*

Introduction

Age estimation in forensic medicine has a wide application and is a crucial area of research. Precision in forensic age estimation techniques can be challenging. It is known that the age estimation methods in living individuals need to have higher standards of accuracy compared to age estimation for the decedent. [1] There are also limited methods available for age estimation in the living. The Forensic Anthropology Society of Europe recommend three methods of analysis for age estimation which include, physical examination, radiographic assessment of left hand and dental examination (including OPG); including radiographic assessment of the clavicle's medial epiphysis to determine if the individual is over or below 21 years of age.[2] Radiological analysis in forensic is extremely feasible and most economical, and thus is central to age estimation methods. Although there are variety of skeletal maturity indicators for age estimation in radiology, examination of teeth is increasingly gaining popularity in determining chronological age because of the reported low variability of dental indicators.[3] Also, imaging of teeth are less expensive and involve less exposure to radiation in comparison to other radiological indicators of skeletal maturity.[4] There is extensive research published which deal with age estimation techniques among adults and non-adults separately. Especially among non-adults the teeth are effective indicators of chronological age. However, there is limited research that study tooth related age estimation techniques among individuals in

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the transition zone of juvenile to adulthood.

Determining the chronological age of an individual in the transition period as juvenile or adult has enormous legal implications in civil/criminal cases. It is crucial in cases of child labour, child marriage inheritance disputes etc. Accurate age estimation is critical in criminal proceedings, wherein the age of a suspect without valid identification documents might have to be assessed to determine the applicability of general criminal law in force for adults or criminal law for juveniles. Increase in incidence of migration and asylum seekers have elevated the importance of determining the age of the undocumented.[1,3] In India the legally relevant age limit for reaching adult status is 18 years.

Third molars are among the few indicators in forensic radiology that can be used to differentiate the age of an individual in the transition zone from juvenile to adult. Generally, all permanent teeth would have completed development around 18 years of age, except for the third molars. Thus, during these transition years, i.e from 16 to 22 years the third molars are the only developing teeth that are viable for age estimation.[3,5,6] The higher incidence of congenital abscess of third molar and its variability in morphology and eruption pattern has led to questions regarding its significance as developmental marker has been questioned. Nevertheless, third molar development is an option to be explored as an age estimation indicator for medicolegal purposes, particularly in absence of other biological indicators during the transition phase between adolescence and adulthood.[7,8]

Ethnicity, genetics and various environmental factors affect tooth development. The variations in tooth development based on these factors have been well documented. Therefore, availability of a population specific reference data set for tooth developmental is essential for forensic age estimation of living people.[3,9,10] Thus, this observational study was carried out to correlate the radiologic development status of third molars with that of chronologic age specifically among individuals belonging to Dakshina Kannada and Udupi district in the age group of 16-22 years.

Material and Methods

A prospective study was carried out in which the patients above the age 16 years and below the age of 23 years, visiting the radiology section of the department for an OPG were included. The digital radiographs were taken generated on a Kodak (Gendex, IL, USA). They were stored as JPEG files. The exact date of birth of the subjects and the date on which the OPG was taken were noted. A written informed consent was taken from the patients included in the study.

Inclusion criteria

1. Patients between the age groups of above 16 years and below 23 years

2. Patients in whom OPG is indicated for diagnosis of condition for which they have visited the institution

3. Patients who were resident of Dakshina Kannada or Udupi district

4. Presence of atleast one third molar as evaluated in the OPG

Exclusion Criteria

1. Patients with systemic disease that could affect development of dentition

2. Dental pathology related to third molars

3. History of trauma to jaw bones

4. Panoramic radiographs in which the visualization of the third molars is have errors obscured due to error or improper inclination of the molar

A total of 200 OPGs were assembled for evaluation. The chronological age of the patient on the day the OPG was taken was calculated using an online age calculator available at http://www.calculator.net/.

The OPGs were evaluated by two trained observers. The observer was blinded to the chronological age of the subjects included in the study.

Developmental status of the third molar was determined based on its radiographic appearance and a score was allocated to each tooth based on the method framed by Kohler et al[11], which is a modification of the method given by Gleiser and Hunt.[12] This method of scoring divides the development of teeth into 10 stages and a score is given corresponding to a particular stage of tooth development. The least score was noted in case

the tooth had multiple roots. The scoring was carried out by two specialized observers and inter-observer reliability was calculated. Also, at the beginning of the study, the observers repeated measurements of 20 OPGs with an interval of 2 months in order to evaluate intra-observer reliability.

Statistical analysis: All the analysis was done using SPSS 19 version. Pearson correlation coefficient was calculated to evaluate the correlations among the 4 molar stages. Multiple Linear regression was used to calculate the regression equation to predict the actual age from the 3rd molar eruption stage. Different combinations of equations were generated separately for males and females.

Results

A total of 200 OPGs were included in the study. Among these OPGs a total of 152 OPGs had all four third molars. The rest had atleast one molar missing. As there was insufficient sample size to calculate a multiple regression formula for groups with one or more missing molars they were excluded from the study. Thus only 152 OPG s which had all four third molars were included to get the results. Out of the 152 OPGs 84 belonged to females and 68 belonged to males.

The k statistics for inter and intraobserver reliability was 0.74 and 0.78 respectively. For males, statistical analysis revealed a high Pearson correlation coefficient of 0.93 between the developmental stage of Upper right molar (UR) and upper left molar(UL) and a similar correlation coefficient of 0.98 was found between Lower right molar (LR) and Lower left molar (LL). For females, correlation coefficients of 0.93 and 0.95, respectively, were found. High correlation coefficients of 0.9 were found between upper and lower third molar combinations both for males and females (Table 1).None of the individuals in both male and female group younger than 18 years were given score of 10 i. e none of the individuals younger than 18 years showed complete root formation. The formulas derived from multiple regression analysis for all third molar scores for females is Age= 13.416 + (-0.873 UR) + 0.803 UL + 0.536 LL + 0.362 LR with standard deviation of 1.61and for males it was found to be Age = 12.766 (-0.187 UR) + 0.748 UL + 0.873 LL + (-0.541 LR) with a standard deviation of 1.57.

Discussion:

Accurate age estimation in living has considerable significance from the ethical, legal and criminal perspective. Verification becomes essential when credible proof of chronological age is unavailable. Accurate age estimation depends on calculations derived from reference studies that have included population with similar genetic–geographic origin and the concomitant socio-economic status. Apart from scientifically sound research, accurate age estimation also required contemporarily prescribed legal requirements.[9,10]

Age estimation methods have been traditionally studied in two categories of adults and non-adults. However, the transition zone between adults and non-adults has not been sufficiently explored. This is surprising since determination of chronological age of an individual in the transition zone from juvenile to adult has varied legal implications, with far reaching consequences in consideration to criminal law.[3,4,9] In India the legally relevant age limit for reaching adult status is 18 years and considering the chronology of tooth development third molars are the only permanent tooth that can be assessed for dental age estimation.[3,5,6]

Various studies have utilized third molars for predicting chronological age. In these studies, the most common method used to assess the third molars have been Demirjian's method or Kohler et al[11] modification of the method of Gleiser and Hunt.[12] The modified method of Gleiser and Hunt that has been followed in the present study has been utilized by Mesotten et al.[3], K. Gunst [13] and M. Van Vlierberghe et al.[10] However, methodology to obtain the formula for predicting the chronological has been different in the above mentioned studies. M. Van Vlierberghe et al [10] applied single quadratic and support vector regression whereas Mesotten et al[3], K. Gunst [13] Thevissen et al[14] and Lee et al[15] have utilized a multiple regression analysis. Analysis through Bayesian approach was considered in a separate study in which the authors concluded that there was no drastic advantage of this method in comparison to traditional regression analysis. Recently, Arge et al[16] have utilized transitional analysis for third molar development assessment. This method may not accurately predict the age of an individual, however it is a good indicator of the probability of the age at a given tooth development status. In the present

study, the methodology followed was according to Mesotten et al.[3]

In the above mentioned studies it was found that the completion of third molar formation occurred earlier in males than females. No such comparisons could be made in the present study as there was variability in the number of subjects in the male and female group. In the study by Mesotten et al[1] and Grunt et al, [13] it was found some individuals below the age of 18 years showed full molar development, which enabled them to calculate the probability for an individual to be older than 18 years in case of full third molar development. However, in the present study none of the individuals below 18 years of age showed complete root formation. In fact, among the individuals above 18 years only 5.2% of the upper molars and 6.3% of the lower molars showed completion of third molar development. This clearly indicated that there is a lag in the development of third molars in the South Indian population from Dakshina Kannada and Udupi district in comparison to the Caucasian population from Belgium. Also, completion of the third molar development can be a good indicator in the population group of the present study to predict the chronological age of being above 18 years, i. e of being above the legal age limit. Previous findings have also shown that the mineralization of the wisdom teeth is a population specific process and does not occur in every ethnic group at the same age.[5]

In the study by Mesotten et al[3] which was later furthered by Grunt et al,[13] formulae were obtained not only when all third molars were present, but also for situations with one or more missing molars. In the present study due to the small sample size, formulae could not be derived in case of one or more missing molars. The main advantage of this study is the selection of radiographs of individuals in a narrow age range. This will help to get a more accurate predication of age owing to narrow mean age. The main limitation of this study is the small sample size, due to which formulae could not be derived in case of one or more missing third molar.

Table 1: Pearson correlation coefficients between upper and lower third molars for males and females

Sex		UR	UL	LL	LR
Females (n=84)	UR	1	.993**	.939**	.940**
	UL	.993**	1	.940**	.944**
	LL	.939**	.940**	1	.995**
	LR	.940**	.944**	.995**	1
Males	UR	1	.993**	.933**	.937**
	UL	.993**	1	.941**	.939**
	LL	.933**	.941**	1	.988**
	LR	.937**	.939**	.988**	1

Conclusion

The study revealed that chronological age can be determined using developmental stages of all four third molars with standard deviation of 1.61 among females and, 1.57 for males. Despite certain limitations, third molars can be used to determine the chronological age of individual in the transition period from juvenile to adulthood.

Conflict of Interest: There are no conflicts of interest in this research work **Source of Funding:** None

Acknowledgement: Nil

Ethical Clearance: Institutional Ethical Committee Clearance was obtained prior to conducting the study

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Soft Tissue Cephalometric Norms for Orthognathic Surgery in South Indian Population

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Abstract

Background and Aim: Orthodontic treatment is aimed at achieving good facial aesthetic and occlusion. Proportioned soft tissue contours form an integral part of orthodontic treatment. It is important to establish standard cephalometric norms for various population groups. The present study was aimed to establish soft tissue norms (for Orthognathic surgery) for South Indian population and to compare the values with that of Caucasian standards.

Materials and methods: 50 South Indian subjects comprising of 25 males and 25 females in the age group of 18-30 years were selected based on inclusion and exclusion criteria who were subjected to lateral cephalometric radiographic analysis. Soft tissue parameters were analysed in the study group and compared with the Caucasian group based on Burstone and Legan analysis.

Results: South Indian population showed increased lower face protrusion, lower lip protrusion, upper lip protrusion and deep mentolabial sulcus in both males and females when compared to Caucasian population. An increased lower face-throat angle was seen in South Indian males. Diverse results were also seen in some other soft tissue parameters.

Conclusion and clinical significance: The current study shows that certain soft tissue cephalometric parameters in South Indian population are significantly different from the Caucasian population in both males and females. The differences in both racial groups are clearly evident in the study and can thus be of utmost clinical importance while planning out orthognathic surgery.

Keywords*: cephalometric norms, orthognathic surgery, soft tissue analysis*

Introduction

Facial esthetics has been studied by artists and philosophers. A good facial harmony requires proportioned soft tissues contours with underlying balanced skeletal and dental structures. Facial characteristic of an individual also depends upon racial, ethnic background, heredity and domain location. Aim of an orthodontic treatment is to achieve good facial esthetics and occlusion. Fixed orthodontic treatment along with orthognathic surgery is one method of treatment used to correct severe skeletal dysplasia. Such cases require accurate diagnosis and treatment planning.[1-2]

Cephalometric norms are used as a guide by a clinician during orthodontic diagnosis and treatment planning.[3] In the past, studies have mainly concentrated on the hard tissue relationship and these studies have identified several linear and angular norms. In recent years, orthodontics has seen a shift in paradigm where soft tissue esthetics is being primarily considered.[4] Soft

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tissue facial features of an individual are mainly studied clinically by taking morphometric measurement and also by cephalometry. Several authors noticed the variation in the facial morphology in different ethnic groups.[4] Most of the studies are done in Caucasian population and these norms may be inappropriate to apply for the different ethnic and racial groups.[5]

Burstone and Legan developed soft tissue cephalometric analysis for orthognathic sugery (COGS) at the University of Connecticut in the year 1989 and it is most commonly used by orthodontists and maxillofacial surgeons. [2, 6, 7] This analysis was done on white subjects of European American ancestry. Different ethnic and racial groups have been previously studied for cephalometric norms which have been established in many studies.[8-12] Therefore, it is important to develop norms for various population groups with a standard method. Thus the present study was conducted with following objectives:

1. To establish Soft tissue norms (for Orthognathic surgery) for South Indian population

2. To compare the values with that of Caucasian standards.

Methodology

The study was conducted on 50 South Indian individuals comprising of 25 males and 25 females with age ranging from 18 to 30 years who were visiting the outpatient department of the institution.

The subjects included in the study were with class I molar relationship with minimal or no crowding of teeth, straight facial profile and all teeth anterior to second molar being present. The subjects with gross facial asymmetry, history of trauma or previous orthodontic treatment, history of previous maxillofacial or plastic surgery and significant medical history were excluded from the study. Ethical clearance was obtained from the institutional ethical committee (Cert.no:ABSM/EC/27/2009). Informed consent was obtained from all the subjects.

All subjects were subjected to Lateral cephalometric radiographic analyses with natural head position; teeth in maximum intercuspation and lip at rest using standardized technique.

Standardization of the cephalometric technique armamentarium:

Standardized 8" x 10" Kodak lateral radiographic head films with intensifying screen were used for each subject on PLANMECA 2002 CC PROLINE advanced panoramic and cephalometric equipment. The X-ray source to subject distance was kept at a constant distance of 5 feet. The film was kept at a constant distance of 16 cms away from the midsagittal plane of the subject oriented in Natural Head Position. A rectangular mirror was hung on the wall facing the cephalostat at a distance of 5 feet from subject and at a height of 6 feet from the ground.

Lateral cephalograms of both male and female groups were analysed. Landmarks used for the soft tissue analysis have been described in Fig. 1. The paramaters used in the analysis have been shown in Fig. 2, 3, 4, 5.

Statistical Analysis

The data obtained was subjected to statistical analysis using Statistical Package for Social Sciences (Windows version 17.0, SPSS Inc., Chicago, Illinois, USA). Mean and standard deviation were determined for male and female group. The mean and standard deviation of each group were subjected to Student's t-test for comparison with the normal values obtained by Burstone and Legan [2] where p value ≤ 0.05 was considered to be statistically significant.

Result

The mean and standard deviation obtained in both male and female groups was tabulated (table 1). Soft tissue parameters in male and female groups of South Indian population were compared with Caucasian male and female groups.

Statistically highly significant difference was found on comparing South Indian male subjects with Caucasian males with respect to the following parameters; lower face protrusion [12.14 ± 4.00], lower face-throat angle [105.92 ± 7.03], upper lip protrusion [3.98 ± 1.08], lower lip protrusion and mentolabial sulcus [5.26 ± 1.65] (table 2). On comparing South Indian female subjects with Caucasian females, the soft tissue parameters which were found to have statistically highly significant difference were lower face protrusion [3.54 ± 3.782],

upper lip protrusion [3.74 ± 1.370], lower lip protrusion [3.62 ± 1.494], lower lip length [42.72 ± 3.323], mento labial sulcus [4.5400 ± 1.567] and inter labial gap [1.7600 ± 1.164] (table 3).

Table 1: Orthognathnic Cephalometric Analysis Measurement Of Soft Tissue For South Indian Male And Female

Measurement	Mean (Male)	Standard deviation	Mean (Female)	Standard deviation
Facial form				
Facial Convexity angle (Gn-Sn-Pg')	12.1400	4.00916	11.2200	3.73073
Mid face protrusion (G-Sn)	7.0600	2.74363	7.1200	2.37294
Lower face protrusion (G-Pg')	3.3600	3.41113	3.5400	3.78297
Lower face – Throat Angle (Sn-Gn'-C)	105.9200	7.03515	102.7800	8.90234
Lower Vertical Height – DepthRatio (Sn-Gn'/C-Gn')	1.2984	.16237	1.2616	.21195
Soft tissue chin tickness (Pg-Pg')	11.7240	3.68220	12.4200	2.48613
Lip Position and Form				
Naso – Labial Angle (Cm-Sn-Ls)	104.9200	9.35824	104.0000	7.28583
Upper lip protrusion (Ls to{Sn-Pg'})	3.9800	1.08474	3.7400	1.37022
Upper Incisor disply (stms-I)	2.3200	1.26557	2.6400	1.03602
Lower lip protrusion (Li to{Sn-Pg'})	3.4600	1.44280	3.6200	1.49499
Upper lip length (Sn-Stms)	21.0000	3.84057	20.5600	4.78783
Lower lip length(Stms-Me')	45.4800	3.51331	42.7200	3.32315
Mento labial sulcus (Si to{Li-Pg'})	5.2600	1.65278	4.5400	1.56738
Inter Labial Gap (Stms-Stmi)	1.9000	1.08012	1.7600	1.16476

Table 2: Comparison Of Orthognathic Cephalometric Analysis Measurement Of Soft Tissue Between South Indian Male And Caucasian Male

Measurement	SOUTH INDIAN MALE		CAUCASIAN MALE		t-value	p-value
	Mean (Male)	Standard deviation	Mean (Male)	Standard deviation		
Facial form						
Facial Convexity angle(G-Sn-Pg')	12.1400	4.00916	12.0000	4.00000	.9850	0.912
Mid face protrusion (G-Sn)	7.0600	2.74363	6.0000	4.00000	1.093	0.279
Lower face protrusion(G-Pg')	3.3600	3.41113	.0000	4.00000	3.196	0.0025h.sig
Lower face – Throat Angle(Sn-Gn'-C)	105.920	7.03515	100.0000	7.00000	2.982	0.004 h-sig
Lower Vertical Height – DepthRatio (Sn-Gn'/C-Gn')	1.2984	.16237	1.2000	.21195	1.841	0.071
Soft tissue chin tickness (Pg-Pg')	11.7240	3.68220	12.0000	2.00000	.3290	0.746
Lip Position and Form						
Naso – Labial Angle (Cm-Sn-Ls)	104.920	9.35824	102.0000	8.00000	1.187	0.241
Upper lip protrusion (Ls to{Sn-Pg'})	3.9800	1.08474	3.0000	1.00000	3.322	0.0017 h sig
Upper Incisor disply (stms-I)	2.3200	1.26557	2.0000	2.00000	.6750	0.502
Lower lip protrusion (Li to{Sn-Pg'})	3.4600	1.44280	2.0000	2.00000	2.960	0.0048 h sig
Upper lip length (Sn-Stms)	21.0000	3.84057	22.0000	2.00000	1.154	0.254
Lower lip length (Stms-Me')	45.4800	3.51331	44.0000	2.00000	1.832	0.074
Mento labial sulcus (Si to{Li-Pg'})	5.2600	1.65278	4.0000	2.00000	2.428	0.019 sig
Inter Labial Gap (Stms-Stmi)	1.9000	1.08012	2.0000	2.00000	.2190	0.828

TABLE 3: Comparison of Orthognathic Cephalometric Analysis Measurement Of Soft Tissue Between South Indian Female And Caucasian Female

Measurement	SOUTH INDIAN FEMALE		CAUCASIAN FEMALE		t-value	p-value
	Mean (Female)	Standard deviation	Mean (Female)	Standard deviation		
Facial form						
Facial Convexity angle (G-Sn-Pg')	11.2200	3.73073	12.0000	4.00000	.784	.512
Mid face protrusion (G-Sn)	7.1200	2.37294	6.0000	3.00000	1.54	.129
Lower face protrusion (G-Pg')	3.5400	3.78297	.0000	4.00000	5.34	0.001 vhs
Lower face – Throat Angle(Sn-Gn'-C)	102.7800	8.90234	100.0000	7.00000	.437	.676
Lower Vertical Height – Depth Ratio (Sn-Gn'/C-Gn')	1.2616	.21195	1.2	.16237	0.067	.912
Soft tissue chin tickness (Pg-Pg')	12.4200	2.48613	11.7240	3.68220	0.128	.786
Lip Position and Form						
Naso – Labial Angle (Cm-Sn-Ls)	104.0000	7.28583	102.0	8.0	1.453	.135
Upper lip protrusion (Ls to{Sn-Pg'})	3.7400	1.37022	3.00	1.00	.118	.834
Upper Incisor disply (stms-I)	2.6400	1.03602	2. 00	2.00	1.422	.142
Lower lip protrusion (Li to{Sn-Pg'})	3.6200	1.49499	2.00	1.00	4.512	0.001 vhs
Upper lip length (Sn-Stms)	20.5600	4.78783	20.00	2.00	1.122	.267
Lower lip length (Stms-Me')	42.7200	3.32315	40.00	2.00	3.6	0.001 vhs
Mento labial sulcus (Si to{Li-Pg'})	4.5400	1.56738	4.00	2.0	1.582	.123
Inter Labial Gap (Stms-Stmi)	1.7600	1.16476	2.00	2.0	.442	.728

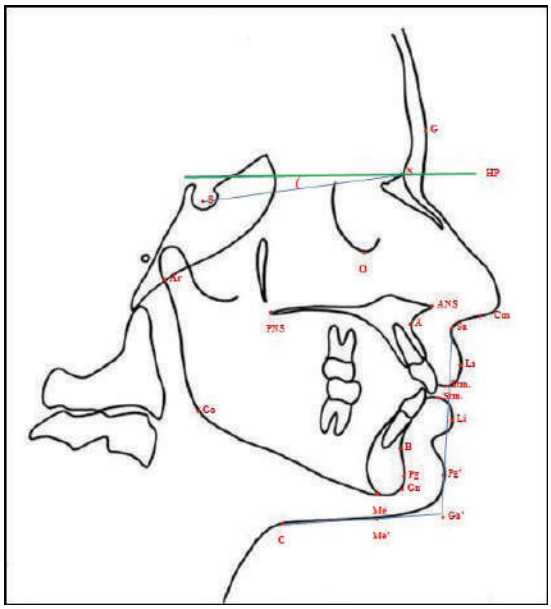


Fig 1: Soft tissue Landmarks: Glabella (G), Columella point (Cm), Subnasale (Sn), Labrale superius (Ls), Stomion superius (Stm_s), Stomion inferius (Stm_i), Labrale inferius (Li), Mentolabial sulcus (Si), Soft tissue pogonion (Pg'), Soft tissue gnathion (Gn'), Soft tissue menton (Ms), Subnasale (Sn), Cervical point (C)

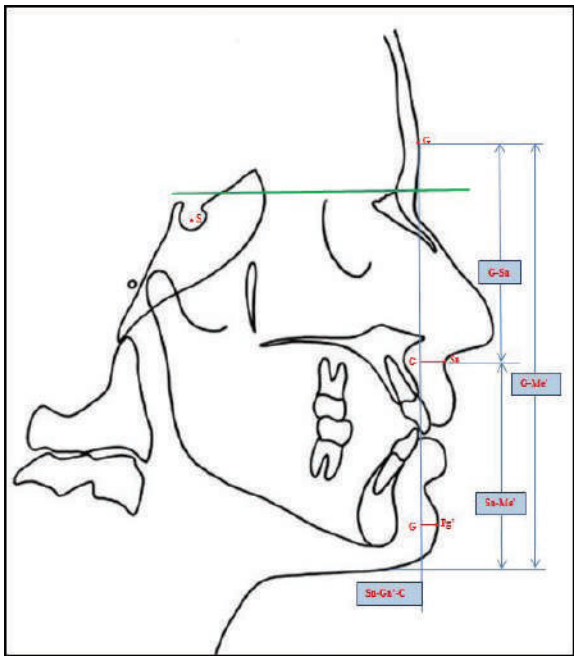


Fig. 2: Facial form linear and angular measurements

FACIAL FORM: Mid face protrusion (G-Sn), Lower face protrusion(G-Pg')

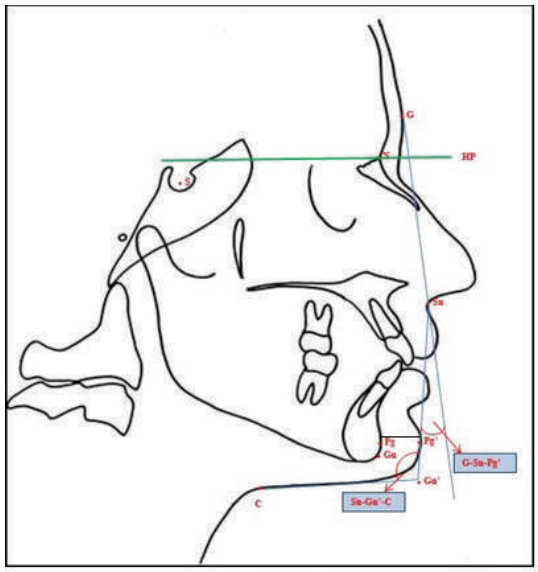


Fig. 3: Facial convexity angle (G-Sn-Pg) and Lower face throat angle (Sn-Gn'-C)

Soft tissue Chin thickness (Pg-Pg') and Lower face height depth ratio (Sn-Gn'/C-Gn')

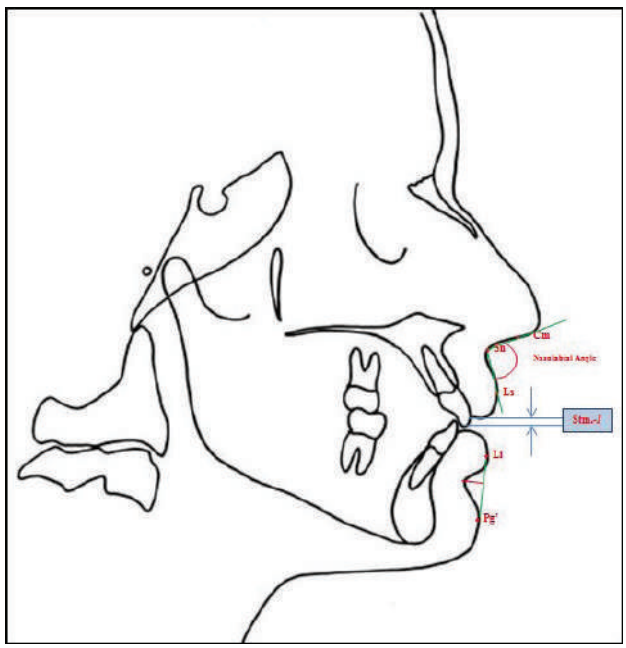


Fig.4: Lip position and Form linear and angular measurements

LIP POSITION AND FORM: Nasiolabial angle (Cm-Sn-Ls), Upper incisor display (Stm_s-*I*), Inter labial gap (Stm_s-Stm_i), Mento labial sulcus(Si to{Li-Pg'})

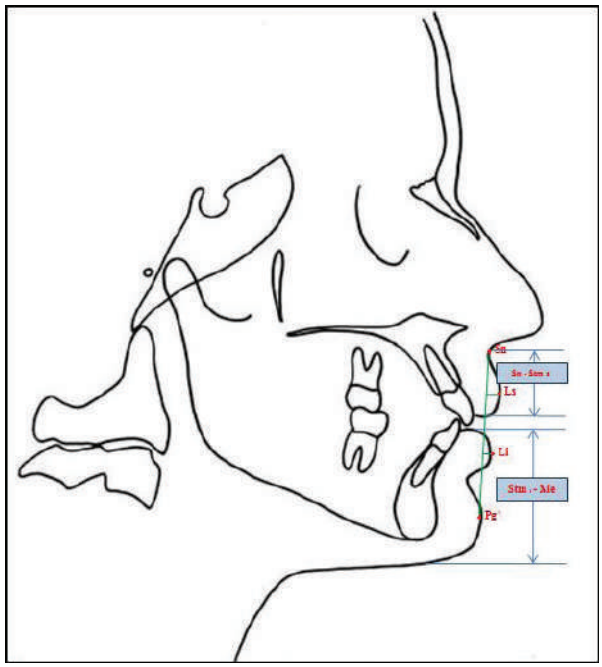


Fig.5: Lip position and Form - Linear Measurements

LIP POSITION AND FORM: Upper lip length(Sn-Stm_s), Lower lip length (Stm_s-Me'), Upper lip protrusion (Ls to{Sn-Pg'}), Lower lip protrusion (Li to{Sn-Pg'})

Discussion

Achieving an ideal soft tissue facial profile is the primary goal of the orthodontic treatment. Soft tissue profile of an individual depends on underlying maxillary and mandibular jaw position. It is achieved by only orthodontic treatment or growth modulation procedures or surgical orthodontic treatment in severe skeletal deformity cases. It involves execution of proper diagnosis and treatment planning. [13]

Soft tissue cephalometric norms play an important role in diagnosis and treatment planning of orthognathic surgical cases. Legan and Burstones' soft tissue analysis is most commonly used for orthognathic and cosmetic surgical cases. These norms are established on European-American population. Various studies have been conducted on different ethnic groups like Ukrainian, Iraqi, Iranian and Japanese population.[14-17] Also studies done with various other cephalometric analysis on the Indian population have shown varied results when compared with that of the Caucasian population.[18-20] In India, Caucasian standards are widely used for orthognathic surgery as cephalometric norms for different ethnic groups are not available. The present cephalometric study presents norms developed for the soft tissue cephalometric analysis for orthognathic surgery for South Indian population.

Most of the patients presenting for orthognathic surgery are young adults. In this study the sample was limited to young adults with a mean age of 21 years as cephalometric norms of children and adults with advanced age may undergo changes. [17,19, 21, 22]

Facial form describes the overall horizontal soft tissue profile. In our study Facial Convexity angle (Gn-Sn-Pg') was found to be more in South Indian males than Caucasian males which indicates a convex profile in South Indian male group. This was in accordance with studies conducted by Jain P et al [19], Al-Gunaid T et al [23] and Hamid M et al. [24] Facial Convexity angle (Gn-Sn-Pg') was found to be less in South Indian females than the Caucasian females.

Mid face protrusion (G-Sn) was found to be more in both South Indian males and females than Caucasians with the reading statistically not significant. This was similar to study conducted by Flynn TR et al. [25] Studies were conducted by Flynn TR et al [25] and Alcaide RE et al [17] where Lower face protrusion (G-Pg') was found to be more in study groups than Caucasians with the values being statistically highly significant. Similar results were also obtained in our study.

Lower face – Throat Angle (Sn-Gn'-C) was found to be more in both South Indian males and females than Caucasians which was statistically significant in males. This is similar to the study done by Jain P et al [19] and Hamid M et al. [24] The results signify that this parameter should be considered during the correction of anteroposterior dysplasia especially chin reduction procedures.

Variations in results were obtained in relation to Lower Vertical Height – Depth Ratio (Sn-Gn'/C-Gn') and soft tissue chin thickness between the study group and Caucasian group but were statistically not significant.

In lip position and form relations; Upper lip protrusion (Ls to {Sn-Pg'})) and Lower lip protrusion (Li to {Sn-Pg'}) both were found to be more in South Indian males and females than Caucasians. This was

similar to the study done by Flynn TR et al, [25] Uysal et al, [26] and Erbay et al. [10] Statistically highly significant results were obtained with respect to Mento labial sulcus (Si to {Li-Pg'}) which was found to be more in South Indian males and females than Caucasians. This was similar to the studies done by Flynn TR et al [25], Rafael E Alcaide et al [17], Hamid M et al [24] and Celebi AA et al. [27]

Varied results were obtained in relation to Upper Incisor disply (stm_s-*I*), Upper lip length (Sn-Stm_s), Lower lip length (Stm_s-Me') and Inter Labial Gap (Stm_s-Stm_i) which were statistically non-significant when South Indian group was compared with Caucasian group.

Conclusion

The present study has derived normal soft tissue cephalometric (COGS) values for South Indian male and female population. There was increased Facial convexity, Mid-face and Lower face protrusion with more obtuse Lower face throat angle in South Indian subjects. Upper and Lower lip protrusion, Upper and Lower lip length was found to be more along with deep Mento-labial sulcus in South Indian subjects. Differences in both racial groups was clearly evident in the study and hence the soft tissue cephalometric parameters studied can be used as a standard and be helpful in planning out orthognathic surgery.

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Assessment of Extraction Technique Using Physics Forceps and the Conventional Method : A Comparative Study

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Abstract

The subsequent bone loss that occurs after extraction is inevitable.The morphologic changes seen following tooth extraction is due to the body deactivating the system caused by disuse atrophy.[1]Extraction using conventional elevators and forceps involves the separation of the periodontal ligament attachments, expansion of the alveolus and lifting of the tooth out of the socket with the forcep beaks which invites unnecessary trauma including broken roots and bone, inflammation and post operative pain, loss of tissue, and stress for the patient and dental team.[2] Nevertheless, These can be easily reduced through current site preservation techniques such as using the 'Physics Forceps™' that can reduce the degree of damage and extent of resorption that physiologically occurs following tooth extraction by utilising just two opposing forces. These two forces eliminates the need for the third force (the clinician's arm) and thereby the risk of fracturing the dental structures would be dramatically reduced.In addition to this the extraction procedure can be much more comfortable to the patient.[3]

The following study aimed at assessing the amount of extraction defect caused by the conventional extraction technique and the physics forceps design which employs a new extraction technique.In this study ,100 patients indicated for extraction were categorised in two equal groups using the two techniques.

Both the extraction defects in these groups was assessed with the use of an Atraumatic Safe Extraction Score, Extraction Defect Sounding Classification System, Visual Analog Scale and the Amount Of Strain experienced by the operator.

The results showed that there was significant success with tooth extraction using the Physics forceps ™ as compared with the conventional technique. Moreover the results indicated that the Physics Forceps™ causes less bone defect , reduced operator strain and minimal discomfort to the patient than the conventional technique.

Key Words: *Physics Forceps, Atraumatic Extraction, EDS , VAS*

Introduction

Tooth extraction is a traumatic and unnatural procedure which evokes a cascade of biodynamic processes of cellular changes leading to physiologic alterations to alveolar bone and soft tissue architecture. Due to the loss of bundle bone there is inevitable loss of bone after extraction. However we can reduce the extent of the damage by following atraumatic extraction procedures.

One such atraumatic extraction technique using the 'PHYSICS FORCEPS™' can reduce the degree of damage and extent of resorption that physiologically occurs following tooth extraction. The Physics forceps™was developed by Dr. Richard Golden in 2004 is an innovative design that provides a simple mechanical

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advantage by employing first-class lever mechanics. Utilizing the patented "beak and bumper" technique, one can simply and predictably extract virtually any tooth in any condition, while preserving the buccal bone and socket. The beak of the Physics Forceps^{TM} is designed to apply controlled pressure parallel to the long axis of the root. The bumper acts as a simple fulcrum or pivot point.(figure 1)

The extraction socket with an undamaged alveolus and well preserved soft tissues can be successfully treated with post extraction procedures like immediate implant placement ,FPD.

The evaluation of clinical presentation of alveolar defects can only be accurately made immediately following extraction,since damage often occurs during the process of tooth removal and the periodontal attachment shrouds hard tissue architecture.

A classification of the extraction defect as it presents immediately following tooth removal would be beneficial for the clinician in establishing the most appropriate plan for treatment.

The purpose of this study was to evaluate and compare the efficacy of the conventional forceps with the Physics Forceps^{TM}and to assess the amount of bone defect caused by them which will help to simplify decision making process when planning for dental implant therapy following tooth extraction.

Methodology

100 patients were categorized into two groups of 50 patients each:

GROUP I : Extraction using the conventional extraction technique which was considered as control group for the study.

GROUP II : Extraction in 50 patients using the Physics Forceps.^{Tm}

Every patient underwent an adequate pre-surgical preparation consisting of adequate case history, gross scaling, blood test and radiographic examination wherever indicated . Following the standard surgical protocol, the extraction procedure was carried out under local anesthesia.

Inclusion Criteria: Healthy patients (17-60 yrs) who are indicated for extraction with Severely decayed tooth with a minimum of 3mm tooth structure above the crestal bone with 1/4th surface intact.Failed root canal treated tooth,Orthodontic extractions & immediate implant placements.

Exclusion Criteria

1. Subjects with oro-facial cancer or under chemotherapy or head and neck radiotherapy.

2. Tooth associated with periodontitis / periapical pathology.

3. Any subject who is contraindicated for radiograph.

4. Pregnant and lactating mothers.

5. All conditions which are contraindicated for tooth extraction.

6. Primary tooth, 3rd molar and teeth with greater than grade 1 mobility.

7. Root stumps with soft caries at the coronal third.

1 .Atraumatic Safe Extraction Score The success of extraction will be based on the atraumatic safe extraction score which is based on the following criteria:

I. Complete success: extraction without crown and root fracture

II. Limited success with root tip fracture: extraction involving partial root tip fracture.

III. Limited success with osteotomy: fracture-free extraction but associated with buccal or interdental fracture.

IV. Failure to extract.[4]

2. A Verbal Rating Scale :Visual analog scale was used to assess the pain experienced by the patient . Patient was told to rate his/her pain experience on a scale of 1 to 10 at time of extraction. 1-3(Mild)4-6(Moderate)7-10(Severe).

3.The Extraction Defect Sounding classification system (EDS): Following tooth extraction, EDS was

performed using the tip of a conventional periodontal probe.Initially, the crest of the extraction defect was evaluated, noting the position of the crestal bone in relationship to the gingival margin, as well as to the future prosthetic gingival margin using the prefabricated surgical template. Any discrepancies between these two relationships was noted. Sounding of the bony crest includes the buccal and palatal plates as well as the interproximal bone peaks.Further examination of the buccal plate was then performed. This evaluation was done to uncover any fenestration or dehiscence-type defects. In addition, when sounding the inner aspect of the socket with a probe, any vibrations felt digitally will indicate a thin alveolar plate. A similar evaluation was also performed on the palatal plate.A thin buccal alveolar plate often leads to partial or complete buccal plate loss following healing. When inadequate socket bleeding is present, perforations of the cribriform plate with a periodontal curette or rotary instrument was performed to facilitate wound healing.

Assessment Of Defect: The EDS, extraction defect sounding, classification describes the condition of the hard as well as soft tissues immediately following tooth removal, prior to healing and remodeling of the extraction socket and provides basic treatment guidelines to achieve predictable implant integration and esthetics. This classification only applies after the treatment decision has been made to remove a tooth and an objective evaluation of the extraction defect is made.[5](figure 2)

4.**The amount of wrist discomfort** experienced by the operator during extraction using the forceps was also assessed similar to the Visual Analog Scale.

Statistical Analysis

Chi Square test was applied to find out the difference in the amount of defect caused by the physics forceps and the conventional forceps.

Results

A total of 100 patients (51 males and 49 females) had undergone extraction.

1.The EDS score is depicted below (Table 1)

2. In the ASE score , the results analyzed showed that in the conventional group showed poorer scores of ASE THAN physics forceps driven extraction.(Table 2).There was a significant difference on statistical analysis.

3. While assessing the amount of strain to the wrist ,Physics forcep users showed lower values of strain levels.(figure 3)

4. While assessing the visual pain analog scale ,physics forceps user showed lower values of VAS scores. (Figure 4)

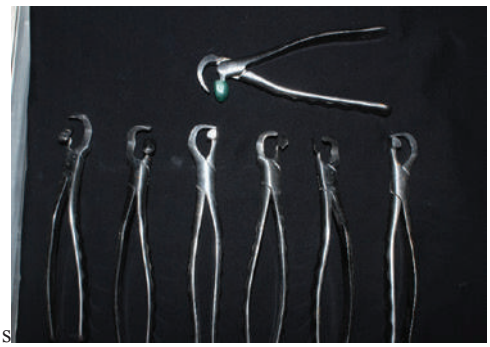


FIGURE 1: Physics Forceps Instruments

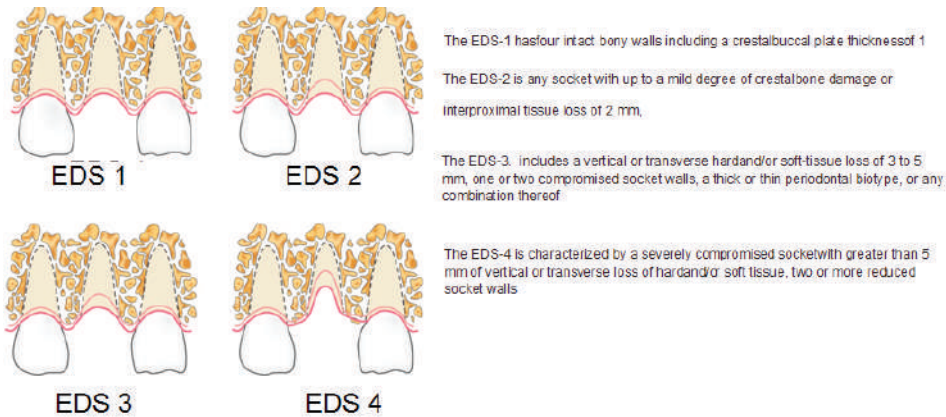


FIGURE 2:EDS classification

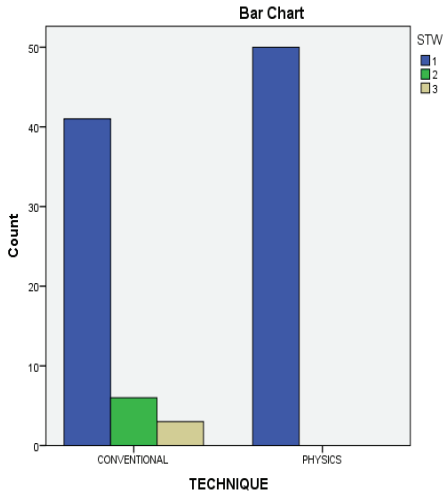


Figure 3:bar chart showing readings of strain to wrist

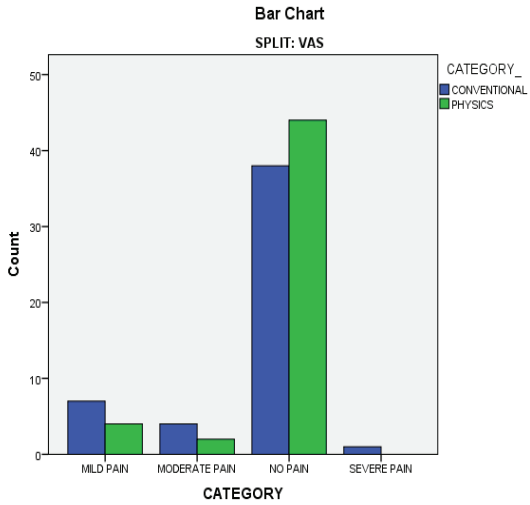


Figure 4: Visual analog scale for conventional and physics forceps extraction

Table 1:Comparison of EDS SCORE

			TECHNIQUE		Total
			CONVENTIONAL	PHYSICS	
EDS SCORE	1	Count	6	31	37
		% within EDS SCORE	16.2%	83.8%	100.0%
		% within TECHNIQUE	12.0%	62.0%	37.0%
	2	Count	12	15	27
		% within EDS SCORE	44.4%	55.6%	100.0%
		% within TECHNIQUE	24.0%	30.0%	27.0%
	3	Count	18	4	22
		% within EDS SCORE	81.8%	18.2%	100.0%
		% within TECHNIQUE	36.0%	8.0%	22.0%
	4	Count	14	0	14
		% within EDS SCORE	100.0%	0.0%	100.0%
		% within TECHNIQUE	28.0%	0.0%	14.0%
Total		Count	50	50	100
		% within EDS SCORE	50.0%	50.0%	100.0%
		% within TECHNIQUE	100.0%	100.0%	100.0%
		Value	df	Asymp. Sig. (2-sided)	
Pearson Chi-Square		40.134a	3	<0.001	
N of Valid Cases		100			
0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.00. There is a significant difference found.					

Table 2: comparison ASE technique

			ASE				Total
			1	2	3	4	
TECHNIQUE	CONVENTIONAL	Count	39	6	3	2	50
		% within TECHNIQUE	78.0%	12.0%	6.0%	4.0%	100.0%
		% within ASE	45.3%	85.7%	75.0%	66.7%	50.0%
	PHYSICS	Count	47	1	1	1	50
		% within TECHNIQUE	94.0%	2.0%	2.0%	2.0%	100.0%
		% within ASE	54.7%	14.3%	25.0%	33.3%	50.0%
Total		Count	86	7	4	3	100
		% within TECHNIQUE	86.0%	7.0%	4.0%	3.0%	100.0%
		% within ASE	100.0%	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests		
	Value	Exact Sig. (2-sided)
Fisher's Exact Test	5.498	.099
N of Valid Cases	100	

Discussion

Ideal tooth `extraction is defined as 'Painless removal of whole tooth or tooth root with minimal trauma to investing tissues',so that wound heals uneventfully and postoperative prosthetic problems are minimal. A tooth can be atraumatically and successfully extracted out of the socket depending upon the normality of technique used,morphology of the tooth,its position in the dental arch and condition of the surrounding bone.However simply removing the entire tooth is no longer the only goal of exodontia;in the present scenario of implant dentistry the ultimate goal is presentation of alveolar bone following tooth removal.

Scull p Et al[1]reviewed the Physics Forceps ™ describing the method of extraction using these forceps. The article also highlights the method of extraction using the physics forceps and also describes the disadvantages of the conventional method of extraction.

Harry Dym Et al[2]reviewed and highlighted exodontia tips as well as new techniques to make simple and complex exodontia more predictable and with improved patient outcomes. This article has a brief discussion on physics forceps, a highlights the uses of class 1 lever mechanics to extract teeth without having to use excessive force or squeezing motion, on basic and complex exodontias.

Carl E. Misch et al [3]presented a paper about biomechanical rational for atraumatic tooth extraction. An extraction device physics forceps has been developed to apply a biomechanical rational to the extraction process of a tooth using a class 1 lever, creep and shear component of force. The physics forceps is really a dental extractor than a forceps and uses first-class lever mechanics. Creep is a phenomenon whereby a material continues to change shape over time under a constant load. In tooth extraction, creep may occur in bone and periodontal ligament

Andersen KL Et al[7] conducted a study which was undertaken to investigate the stress-strain levels and distribution within the periodontal ligament for various types of physiological and orthodontic force systems, assuming that the bone resorption process, leading to tooth movements, is partly controlled by those conditions.

During extraction procedures using conventional forceps,the beaks were seated as far apically as possible without compression of the soft tissues. This is expedited by adequately reflecting the cervical gingiva. This also contributes to the efficient use of force and its proper direction,thereby reducing the possibility of root fracture.

While using the Physics Forceps,first class lever mechanism is used. One handle of device is connected to bumper which acts as a fulcrum, during extraction. The beak of the forceps is positioned lingual or palatal root of the teeth,and to the gingival sulcus,the bumper is most often placed in the facial aspect, typically the mucogingival junction ,making sure that there is no squeezing of the handle or teeth.The handles were rotated as one unit for a few degrees and then the action is stopped for one minute,the torque generated on the tooth,periodontal ligament ,and bone is related to length of the handle to the bumper(8cm),divided by the distance from the bumper to the forcep beak.(1cm)

As a result the a force on the handle connected to the bumper will increase the force on the tooth ,PDl ,and bone by 8 times. No force was required to be placed on the beak which was only on the lingual aspect of the tooth root, therefore,the tooth doesn't split crush or fracture.

Riley established the creep curve of bone whereby a constant load of 60 MPa, the bone over time changes shape in different stages,majority occurs in frst minute,,whereby strain of bone (change of length / original length)is modified. The higher the force that's applied the greater the deformation of the bone. This allows the tooth socket to expand and the tooth is permitted to exit the socket.[8]

A secondary creep occurs over time ,allows the bone to further deform when the force is applied during a 1-5 min period .The longer the time ,the greater the deformation, however it expresses only 10-20 pc difference compared to initial 1 min strain,,eventually the third phase of the curve causes the bone to fracture if the load is applied over a long time frame ,representing creep rupture. A similar phenomenon ,occurs in PDL complex. Mechanical forces shift lateral forces to tooth ,causing primary movement to PDL . A greater force over time causes a slight additional tooth movement and therefore the creep of the periodontal complex is similar to creep of the bone. Whereby constant load weakens PDL thus a constant load on tooth ,over time increase the tooth socket dimension and decreases the strength of PDL complex. Once creep has expanded and weakened the PDl and bone the handle of the extraction device may be slowly rotated another few degrees for 10-30 seconds ,this action contributes to the creep rupture of the ligament and usually elevates the tooth a few mm from the socket.

This randomized study obviously implies an increased awareness of a new surgical technique that may be used to render the aspect of exodontia more predictable, less trauma to the patient and less anxiety producing for the treating surgeon. In this prospective study,the results obtained from 100 patients(50 patients for each technique),showed that the Physics Forceps^{TM} had a 94% success rate in extraction ,caused less amount of discomfort to the operator as well as the patient and caused minimal amount of alveolar bone defect, whereas the conventional method had a 78% success rate in extraction and caused mild amount of discomfort to the operator and the patient

A surgeon should avoid any traumatic extraction leading to further bone remodelling and ultimately bone loss. No matter what technique is used the surgeon should approach all exodontia procedures prepared and confident,having both the necessary clinical skill,appropriate surgical equipment and knowledge to make exodontia procedures less traumatic for both the patient and the surgeon.

Conclusion

From,the results it can be concluded that the Physics Forceps ^{TM} has certain advantages over the conventional method of extraction .The significant advantages of the physics forceps over the conventional technique are:

1. It causes less trauma to the periodontal ligament and surrounding tissues.

2. It causes less amount of alveolar bone loss there by preserving bone necessary for implant therapy.

3. The amount of effort needed by the operator to extract the tooth is decreased.

4. Causes less discomfort to the patient.

However, the Physics Forceps ™ is also associated with a few limitations :

1. In cases of decreased alveolar ridge height ,placing the bumper in the lingual sulcus becomes cumbersome making it difficult in extracting the tooth.

2. The bumper which is placed in the buccal sulcus has caused ulcer in certain patients postoperatively.

3. The Physics Forceps ™ works better in cases where sufficient anatomical tooth structure is present.

Conflicts of Interests: Nil

Ethical Clearance: Taken By Instituitional Clearance Committee

Source of Funding: Self

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Correlation between Gestational Age and Head Circumference in Third Trimester

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Abstract

Introduction: In today's advanced medical world maternal child health care the important part of the health care system and import components is estimation fetal gestational age.

Gestational age is age of unborn baby, defined in weeks as beginning from first day of last menstrual period prior to conception. Trimester is period of three calendar months during a pregnancy. Clinically, the gestational period is divided into three trimesters. Estimation of gestational age and thereby forecasting expected date of delivery (EDD) is not only concern of the Individual but it is invaluable in the diagnosis of intrauterine growth retardation of fetus and obstetric planning other fetal parameters are useful Hence; we planned the present study to estimate fetal HC length for measuring the gestational age and compare it with other parameters.

Material and methods: The study Correlation between gestational age and head circumference in Third trimester was carried out at Govt. Medical College and Hospital, Nanded, between July 2011 to July 2013 period . The study included 132 pregnant women the data so collected was then subjected to statistical analysis by expert statistician with the help of SYSTAT Crainsoft version 12 software. Standard statistical methods, parametric methods were used for the evaluation and significance.

Results: As the value of R is 0.8612, the variation in fetal growth on the basis of Head Circumference during third trimester can be explained to the extent of 86.12%. The value of R is highly significant (Student's't' test value = 228.92, $p<0.0001$, very highly significant) showing that there is statistically highly Positive or strong positive association between gestational age and head Circumference

Conclusion: From the present study Head Circumference found to be statistically highly significant. The regression equations derived for growth parameter for estimating gestational age in a normally developing fetus, increase with gestational age, showed good correlation with gestational age. In Present study, Head circumference is the sensitive parameter and a result of present study was comparable with previous studies.

Keywords: *Fetal growth, Gestational age, head circumference, Third Trimester, Regression equation*

Introduction

Evaluation of age of an individual using various methods is required for medico legal purposes in both civil and criminal matters.[1, 2]

Human development is continuous process that begins when an oocyte from female is fertilized by a sperm. Cell division, programmed cell death, differentiation, growth and cell rearrangement transform the fertilized oocyte into a multicellular adult human being.[3]

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The most developmental changes occur during the embryonic and early fetal periods. The human development is divided into prenatal and postnatal period. There are many changes that occur from the 3rd to 8th week (calculated from the date of fertilization) called as embryonic development. Changes occur from 9th week to birth are meaningful because they signify that the embryo has developed into a recognizable human being called a fetus.4

Gestational age is age of unborn baby, defined in weeks as beginning from first day of last menstrual period prior to conception. Trimester is period of three calendar months during a pregnancy.[5] Clinically, the gestational period is divided into three trimesters. Estimation of gestational age and thereby forecasting expected date of delivery (EDD) is not only concern of the Individual but it is invaluable in the diagnosis of intrauterine growth retardation of fetus and obstetric planning.

The parameters either singly or in combination useful in predicting the gestational age with fair degree of accuracy are Naegeles formula, Date of quickening, Palpation of fetal parts and Auscultation of fetal heart sound. [6]

The methods like physical examination, menstrual history, and laboratory methods have limitations in assessing fetal maturity, development and well being. At the same time Roentgenography likes procedures having hazards of invasive procedure or radiation compelled the research of safer, non-invasive and reliably predictive investigation modality, it was brought forth in the form of Ultrasonography. Added advantage of it being evaluation of multiple parameters in the same readings. Ultrasonography is non-ionising, non-invasive, safe and accurate method of objectively evaluating the fetal growth in utero.

Correct assessment of gestational age is a cornerstone of management of any obstetric case. Various calculation methods are used in the field of forensic medicine to determine age[7], measurement and external characteristics are useful for estimating fetal age. Crown rump length is the method of choice for estimating fetal age until the end of the first trimester because there is very little variability in fetal size during this period. In second and third trimester, fetus grows sufficiently in size; several structures can be identified and measured ultrasonographically.

Accurate knowledge of gestational age is a keystone in the obstetrical ability to successfully manage the antepartum care of the patient and is critically important in the interpretation of antenatal test and successful planning of appropriate therapy and interventions.

Materials and Methods

The study Correlation between gestational age and head circumference in Third trimester was carried out at Govt. Medical College and Hospital, Nanded, between July 2011 to July 2013 period . The study included 132 pregnant women. Who were selected on the following basis.

The subjects were females attending ANC clinic for Ultrasonography screening at Medical College and Hospital. Subjects of the study mainly include urban as well as rural areas in the vicinity.

Inclusion criteria:-

1. Women with known LMP
2. Women with regular menstrual cycle
3. Women with singleton &uncomplicated pregnancy
4. Women having age between 18-34 yrs

Exclusion criteria:-

1. Women with multiple pregnancies
2. Women with irregular menstrual cycles
3. Women having diabetes mellitus
4. Women with diseases like hypertension, chronic renal disease, heart diseases, iron deficiency anemia. Women having Fetus with congenital anomalies.

For collection of the data proper permission was obtained from ethical committee and radiology department.

1. In this study various particulars of the subjects like age, menstrual and obstetric history had been recorded in the Proforma.
2. The American Institute of Ultrasound in

Medicine recommendations were used for measurements of all the fetal parameters.[8]

3. The fetal Head Circumference (HC) was measured at the same level as that of the Biparietal Diameter (BPD) around the outer perimeter of the calvarium. This measurement is not affected by head shape. Interpretation of the measurements of fetal Head circumference was done with the help of computer assembled along with the Ultrasound machine. Date of Ultrasonography of subject is recorded and Gestational age of the fetus in terms of weeks was calculated from last menstrual period in the Proforma.

4. The data so collected was then subjected to statistical analysis by expert statistician with the help of SYSTAT Crainsoft version 12 software. Standard statistical methods, parametric methods were used for the evaluation and significance.

Results

The study of Correlation between gestational age and head circumference in Third trimester by Ultrasonography was carried out at Govt. Medical College & Hospital Nanded.

The data collected was formulated according to the menstrual weeks. All the observations of the fetal growth parameters were taken in centimeters.

Standard deviation of parameter for each week was calculated. Similarly the statistical mean of each parameter for each week was calculated. The weeks of gestation were defined as completed week. For e.g. 13th week refers to 13.00 to 13.86 weeks of menstrual age. 7 days = 1week, hence 1day = 0.14 weeks. Like this subsequently for each day.

Ultrasonographic Head Circumference was measured in a total of 132 subjects. The observations of week wise mean values and standard deviation of fetal Head Circumference are shown in table no.1

Table No. 1: Mean and Standard Deviations of Fetal Head Circumference (Week wise)

Menstrual age in weeks	No. of cases	Mean	Standard deviation
29	10	26.57	1.12
30	9	26.79	0.72
31	11	27.93	0.99
32	12	28.76	1.01
33	14	28.74	1.66
34	13	29.51	1.87
35	8	30.31	0.87
36	13	30.70	1.29
37	10	31.38	0.64
38	14	31.83	0.59
39	8	32.63	0.49
40	6	33.03	1.42
41	2	31.92	0.07
42	2	32.85	1.20
Total	132		

Regression output for 3rd trimester (29 to 42 weeks)

Constant = 3.82

Standard error of Y ests = 1.034

Coefficient Of determination (R) = 0.8612

No. of observations = 132

Degree of freedom = 130

X coefficients = 1.299

Regression equation:

G.A = 3.82 + 1.299 x HC

From the above equation it is clear that during the third trimester, for every 1cm increase in HC, the gestational age (G.A) increases by 1.299 weeks.

As the value of R is 0.8612, the variation in fetal growth on the basis of Head Circumference during third trimester can be explained to the extent of 86.12%. The value of R is highly significant (Student's't' test value = 228.92, $p<0.0001$, very highly significant) showing that there is statistically highly Positive or strong positive association between gestational age and head Circumference.

Discussion

Ultrasonography is primary imaging modality in the evaluation of fetus because of its wide availability, low cost, and lack of adverse effects. The chorionic sac and its contents may be visualized by Ultrasonography during the embryonic and fetal periods. Placental and fetal size, multiple fetuses, abnormalities of placental shape, and abnormal presentations can also be determined. Ultrasound examinations are also helpful for diagnosing abnormal pregnancies at a very early stage. Rapid advances in Ultrasonography have made this technique a major tool for prenatal diagnosis of fetal abnormalities. Thus Ultrasonography is a most reliable check on the growth of the fetus.[4]

By the end of 12 weeks, the upper limbs have almost reached their final relative lengths, but the lower limbs are still not so well developed and are slightly shorter than their final relative lengths. By the end of 12 weeks, primary ossification centers appear in the skeleton, especially in the cranium (skull) and long bones. By the 16 weeks the head is relatively small compared with that of the 12-week fetus and the lower limbs have lengthened. Ossification of the fetal skeleton is active during this period, and the bones are clearly visible on ultrasound images and determination age is also important medico legally because In India, the Consumer Protection Act (CPA) came into existence in 1986, which was enacted for better protection of the interests of consumers.[9]

Fetal head, body and extremity measurements have been widely reported and found to be used in second and third trimester[4].

The differentiation between hard and soft tissues of the embryo is possible after about 10th week of gestation when other parameters like Head circumference, Measurements of fetal parts during routine Ultrasonography screening have been recommended.[10]

Studies by Indian authors Vaidya[11] (1986), Khandeparkar[12] (1986), Ghamande[13] (1989), Rajan R[14] (1991) were reflection of the fetal growth parameters in a particular region of India. India being a multi racial country, regional differences in the growth pattern of fetal parameters is expected.

The present study is a cross sectional analysis of fetal growth parameters in 132 subjects was conducted considering the above views. Transabdominal sonography of these subjects was performed and the measurements of fetal growth parameters were recorded in a proforma and subjected to statistical analysis.

The table no. 2 Shows week wise averages of the measurements of the Head Circumference as studied by Rajan et al[14] (1991) compared with those calculated in the present study. The present study correlates with the study**.**

From the table no.3 it is clear that the measurements of Head Circumference in present study are comparable with the findings of Scammon & Calkins and Kesari, Vare (HC measured from aborted fetuses) and Bhusari (HC measured ultrasonographically) shows that actual Head Circumference value for human fetuses lie very close to sonar value derived in the study. [15]

Table no. 2: Table showing comparison between week wise mean of the measurements of head circumference

Gestational Age	Rajan(1991)	Present Study
29	26.1	26.57
30	27.1	26.79
31	28.2	27.93
32	28.5	28.76
33	28.8	28.74
34	29.2	29.51
35	30	30.31
36	30.9	30.7
37	31.3	31.38
38	31.5	31.83
39	32.1	32.63
40	32.9	33.03
41	-	31.92
42	-	32.85

Table no. 3: Table showing comparison between week wise mean of the measurements of Head circumference:

Gestational age in weeks	Scammon and Calkins (1929)	Vare (1976)	Kesari GV (1979)	Bhusari PA(2010)	Present study
32	28.59	29.50	28.98	29.42	28.76
36	31.99	31.80	30.31	31.67	30.70
40	35.19	35	32.98	32.76	33.85

The regression equations for the Head Circumference derived in the present study are as follows:

HC	3rd TRIMESTER
	G.A = 3.82 + 1.299 x HC

Summary and Conclusions

The present study by taking into consideration of Head circumference recorded. This cross-sectional study was carried out on ANC women with age group 18-34 yrs. This study was carried out during the period July 2011 to July 2013 in Govt. Medical College and Hospital Nanded. All the subjects had sound knowledge about their menstrual dates. Data was collected from these subjects with regards to fetal growth parameters and recorded in the proforma.

The data so collected was tabulated according to the menstrual weeks The statistical mean and standard deviation of each parameter for each week was calculated with statistical software SYSTAT version. Comparison of week wise mean value was done with studies done previously and represented in a tabular form & graphical representation of the results was done.

Finally, sonographically measured parameters during Third trimesters of pregnancy were subjected to statistical analysis by simple linear regression. The regression was done separately for each parameter and for each week.

1. Head Circumference is found to be statistically highly significant.

2. The regression equations derived for growth parameter for estimating gestational age in a normally developing fetus, increase with gestational age, showed good correlation with gestational age.

3. Assessment of gestational age helped in calculating the EDD (expected date of delivery) in all patients, thus improving the antepartum management. Gestational ages are fairly accurate predictors of fetal growth.

4. In Present study, In Third trimester Head circumference is the sensitive parameter and results of present study was comparable with previous studies

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Conflict of Interest: Author declares that there is no conflict of interest

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Assessment of Knowledge, Attitude and Practices of Allied Health Care Professional Students Towards Universal Precaution

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Abstract

A universal precaution is an approach to infection control to treat all human blood and certain human body fluids. History and examination cannot reliably identify all patients infected. Universal precautions are framed to prevent health care professionals who are exposed to various infections and by practicing various basic principles of infection control. A cross sectional survey was conducted to assess knowledge, attitude amongst allied health care professional about universal precautions and to investigate their practice towards universal precaution. First year Students of School allied health sciences, who were willing to participate included in the study. Study showed overall moderate knowledge among the students. Hence training sessions of universal precaution may need to be conducted regularly for these students. Awareness amongst students can be achieved to maximum level by conducting awareness programs every year by the Infection control committee in the medical college.

Keywords: *Universal precaution, Allied health sciences, Infection control, knowledge.*

Introduction

Universal precautions is an approach for an infection control to treat all human blood and certain human body fluids, History and examination cannot reliably identify all patients infected with human immunodeficiency virus and other blood borne pathogens. The United States Centers for Disease Control has proposed a series of procedures that health care workers should use with all patients.[1]

Universal precautions formulate to prevent health care professionals who are exposed to various infections and by practicing various basic principle of infection control through hand washing, use of protective barriers, like mask, gloves, gown and eyewear and safe handling of various instruments. Implementation of these universal precautions is important, as health care organization has a responsibility to protect staff from potential danger and loss of man power if suffers from any injuries or illnesses due to direct or indirect exposure.[2]

Due lack of training about safe handling of blood or body fluids , may leads to increase chances of occupational hazards related to students who are in direct contact with patients [3]

Health care professional, who are working in hospitals are directly in contact with various patients of unknown serological status. The risk of infection after known occupational exposure is about 25%, there is a approximate 0.4 % risk of infection with HIV after percutaneous exposure to HIV contaminated blood to health care worker[4], although the collective risk may increase as high as 2% amongst hospital staff [5]

Exposure to Sero positive patient is common in the developing countries. It was observed that accidental

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exposure of health care staff as high as 13% while providing care for Sero positive patients.[6]

The majority of reported cases of occupational blood exposure were among nurses, paramedical staff and minorities were among physicians[7]. Similarly, nurses and health care staff are most frequently involved in occupationally acquired HIV infection[8]. Among midwives frequency of body fluid exposure showed that around 65% of them had experienced exposure to amniotic fluids or blood at least once in the past 6 months [9].

Use of universal precautions significantly decreases the number of incidents of occupation exposure to blood[10,11] Nevertheless, the level of compliance with universal precautions is generally low, and the weakest aspects reported are not practicing hand decontamination[12]

The objectives of the present study were to assess knowledge, attitude amongst allied health care professional about universal precautions and to investigate their practices towards universal precaution, so that the information would be useful in identifying specific areas that may need further attention in the continuing education of Allied health care students and in providing feedback to these groups about improving safe practices.

Material and Methods

This study was conducted under School of allied health sciences, DMIMS, Wardha. It was a cross sectional study and first year students of School allied health sciences who were willing to participate included in the study. After taking their informed consent total of 150 students, brief introduction about the scope of our study, general demographic data was collected.

Complete procedures involved in the study was explained to them and the assurance of confidentiality of the data collected was given. Structured questionnaire on knowledge, attitude and practice was sent by internet to every participant.

Results

All 150 students had replied positively, out of these 35% were female and 64% male as depicted in Table 1.

Table1:Gender wise distribution of Allied Health Care Professional students

Gender	Number (n=150)	Percentage
Male	96	64%
Female	54	35%

Most of the students answered correctly the knowledge based questions on universal precaution related to disease and infective status of patient 63.8% and 84.5% respectively. Response for isolation of infected patient was 91.5%, for decontamination of devices was 65%. Universal practices and vaccine answered correctly by 67.1% and 58 % respectively. Cleaning and procedure of discarding of waste correctly answered by 87.2% and 88.8% students respectively in Table No.2

Table 2 Distribution of Allied Health Science students as per knowledge of universal precautions

Sr.No	Knowledge about of universal precautions	Response % Answering correctly
1	Have you ever heard of universal precautions?	83%
2	Universal precautions are applied to patients with HIV and HBV only	63.8
3	Universal Precautions should be applied to all persons regardless of their infection status	84.5
4	Isolation is necessary for patients with droplet and blood borne infections.	91.5

Cont... **Table 2 Distribution of Allied Health Science students as per knowledge of universal precautions**

5	For decontamination of devices or instruments washing with usual detergent is enough	65
6	Used needles can be recapped after giving an injection	56.4
7	There is effective vaccine against all type of flu illness	57
8	Infected area should be cleaned promptly with sodium hypochlorite	87.2
9	Used mask is rap in a tissue paper or polythene bag and immediately discard it in the yellow waste bag	88.8

Table 3 - Distribution of Allied Health students as per practices they followed for universal precautions

Sr No.	Practice of universal precautions	Percentage (%)
1.	I assume that blood and all body fluids of patients are infectious	75.2
2.	I wear mask, gown and eye wear if procedures and patient care activities are likely to cause splashing of blood and body fluids	89.7
3.	I dispose of used needles into a sharp box after injection	81.2
4.	I wear gloves as the first step for cleaning contaminated surfaces	96.5
5.	Washing with soap and water for 5 minutes is my first step after contact with infective material	86.3
6.	I apply universal precautions in situations that might lead to contact with sweat	73.5
7.	If I have a wound, I wear gloves before caring for patients	94

Table 4: Distribution of Allied Health students as per their attitude towards universal precautions

Sr No.	Attitude towards universal precaution practices	Strongly Agree (%)	Agree (%)	Not sure (%)	Disagree (%)	Strongly Disagree (%)
1.	Using frequent hand washing can prevent you from getting flu like disease	42.7	41	12	1.7	2.6
2.	I generally avoid to wear face during routine procedure	11.2	22.2	18.8	34.2	12.7
3.	Wearing face mask during patient care is effective in preventing infectious disease	12.8	0.9	3.4	35	47.9
4.	Reusing face mask can increase chances of getting infectious disease	10.2	4.2	6.8	41.5	37.3

Attitude and Practice of universal precautions Concerning the use of protective devices, almost all respondents agreed on the practice of wearing protective material when exposed to deep body fluids or blood products (Table 3 & 4).

The use of gloves and wearing facemask when exposed to sweat, practices were poor, as only 33.9% of Allied health care professional students answered question correctly. The respondents' practices toward disposal of sharps into a sharp box were good, regarding wearing gloves as the first step in cleaning surfaces, Also 86.3% of Allied health care professional students agreed that washing with soap and water for 5 minutes is the first step after contact with infective materials by 5 point likert scale[13].

Discussion

The study showed an overall moderate understanding of universal precautions among Allied health care professional, despite the fact that the majority of occupational blood exposures involved health care professionals. Furthermore, the knowledge score in our study was average; this may be related to the lack of regular training of universal precautions, especially in hospital.

Although the efficacy of universal precautions is contended, they remain a valuable way to minimize or prevent accidental exposure of staff to pathogens.[14]

It is necessary to strengthen and clarify the concept of universal precautions and infection control guidelines among hospital staff, especially the staff of the allied health care professionals who are in direct contact with Patient care. Universal precautions are usually integrated in the current student training curriculum of health care workers. However, there is a lack of regular integration of universal precautions guidelines as part of the job training in our hospitals.

Although infection control committees in hospitals have programmes of regular training and examination, proper implementation is necessary from better outcome. It should be more regular and comprehensive.

Ryan et al[14] recommend that there should be a systematic evaluation, knowledge pertaining to universal precautions. A more comprehensive ongoing educational program on universal precautions should be organized and this should be considered as a mandatory refresher course for all health care professionals in the hospitals.

Van Wissen[16] suggested that one way to achieve this is to select tarn get groups on the basis of prior knowledge. The content of the programme should be tailornmade according to the recommendations of the target group, and there could be a sympathetic forum in which more personal issues can be confidentially discussed.

Most of the respondents in present study agreed that universal precautions should be used for all patients irrespective of their blood borne infection status. Many of health care staff readily admits their lack of knowledge. Support sessions should be provided for all staff in which feelings and fears can be openly discussed.[17]

Young et al, revealed that health care professional did not always used adequate protection if they would not be aware of patient's infective status.[18]

Denic LM suggested that regular education up gradation is necessary at various level of knowledge in prevention of accidents, which will be helpful in reduction of occupation risk [19,20]

Conclusion

In this study, out of total 20 questions, students of allied health care professional had better knowledge in about 9 questions, Moderate knowledge in questions 7 and Poor knowledge in 4 questions. Hence study showed overall moderate knowledge among the students. Hence training sessions of universal precaution may need to be conducted frequently for the students. Awareness amongst students can be achieved to maximum level by conducting awareness programs every year by the Infection control committee in the medical college

It seems that Allied health care professional students have lack of systematic program of education about universal precautions during clinical practice.

Occupational safety requires for reducing risks. Use of protective barrier is a major element of universal precautions, so we should encourage their use. Protective barriers must be readily available; infection committee members should ensure safe practices and

resolve related issues. Also the education of students during the years of clinical practice is very important. Post educational surveys or observational studies about universal precautions as practiced in clinical settings need further attention.

Further studies should include physicians as well as support staff in order to gain a broader picture of the practice of universal precautions in hospitals.

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An analysis of the Inventory Level of Consumables in the Wards of a Tertiary Care Teaching Hospital in South India

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Abstract

Material management is important and crucial for all healthcare industries because it not only deals with the financial aspects but also deals with the well-being of the hospital and quality care of the patient. Reducing inventory cost is a critical responsibility of healthcare managers in hospitals. Inventory management should be done with the help of proper value analysis. This study attempts to assess the inventory techniques and monthly maintenance figure which have contributed to efficient inventory management in a tertiary care teaching hospital.

Keywords: *Monthly Maintenance Figure, Inventory management, Material management, hospitals*

Introduction

Inventory management is a discipline primarily about specifying the shape and placement of stocked goods. It is required at different locations within a facility or within many locations of a supply network to precede the regular and planned course of production and stock of materials.

The motive behind materials administration is to achieve control over the procurement, stockpiling, retrievability, circulation, utilization and transfer of provisions and gear so as to complete the essential obligations of the association in a proficient, successful and sparing way. Materials administration looks to guarantee access to the correct materials, at the opportune time, to the perfect place, in any event at the lowest cost.[1] This study endeavors to survey two methods: Monthly maintenance figures and stock records which have added to effective administration of stock at a two thousand bedded tertiary care teaching hospital. In an investigation, it was discovered that around 33% of the annual hospital budget is spent on purchasing materials and supplies, including medicines.[2] In any tertiary care doctor's facility, roughly 33% of the annual budget is spent on purchasing materials and supplies, with prescriptions constituting a significant majority of the same.[3]

There is a requirement for sensible arranging, planning, sorting out and maintenance of the drug store and general consumables in a way that produces productive clinical services.[4] Health administrators should utilize logical low-cost strategies to boost their profits. The medicinal store is a standout amongst the most widely utilized offices of the doctor's facility and one of only a handful of zones where a lot of cash is spent on repeat purchases. These underline the requirement for arranging, planning and sorting out the medicinal stores in a way that brings efficiency in clinical and pharmaceutical services.[5] Inventory management tries

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to accomplish maximal yield with minimal venture input. Everything might be viewed as essential and there is an apparent need to supply increased services.[6] Hospital supervisors must set up proficient stock framework approaches for ordinary working conditions that additionally guarantee the clinics' capacities to take care of crisis demand conditions.[7,8] The word stock refers to any sort of asset having financial value and is maintained to satisfy the present and future needs of an organization. The hospitals have a centralized supply department where equipment and supplies are stored and from which they are distributed throughout the hospital to different units and departments. The supply department ensures the timelines, availability and safety of the supplies and equipment delivered throughout the organization directly. Store network costs devour as much as 40 % of the aggregate working spending plan, the second-biggest cost for healthcare organisations. Indeed, even little changes in production network execution can enormously affect a healthcare organisation's cot structure, lessening store network costs by as much as 10 to 12 %. This impacts the quality of patient care and the revenue costs associated with that care. Inventory optimization can account for 10% of the overall savings.[9]

This study depicts one such strategy to have better stock control over consumables at ward level. It is called Monthly Maintenance Figures, which was initially utilized in Military Clinics. Another zone of enhancing effectiveness in material administration is through enhancing turnover of stock. The general stores in the hospital under examination followed the utilization of stock and advanced another technique called stock file. The hospital has grown over the years from 950 beds to 2050 beds as of 2014, with an average bed occupancy of 1750 and an Outpatient average of 2500 patients for each day. It is indeed a test to oversee materials particularly consumables in the wards.

Methodology

Phase 1: Observation of the indenting method by the wards to the stores and collection of the data from the general stores of issued consumables materials.

Phase 2: Verifying the issued items from the wards and consumption of those consumables by the patients.

Phase 3: Verifying the bed consumption data from the Medical Records Department and analyzing the data with bed occupancy of those 3 months.

MMF (Monthly Maintenance Figure), is an inventory management technique implemented in our hospital which predicts the usage of consumables based on the consumption pattern of the previous records of the consumables at the ward level.

Previous year consumption pattern was analyzed which contained the information related to consumables issued to the wards with average bed occupancy rate of the hospital. The main objective was to analyze the inventory stock days and their holding cost, revising the MMF (monthly maintenance figure) based on the above study for **A** and **B** items in the wards.

The list of consumables with ABC analysis was analyzed and segregated. **A** and **B** items from the list were compared against the data with inventory records from the wards and stores. The data was compared retrospectively with previous two years data to obtain an average consumption for all the respective wards.

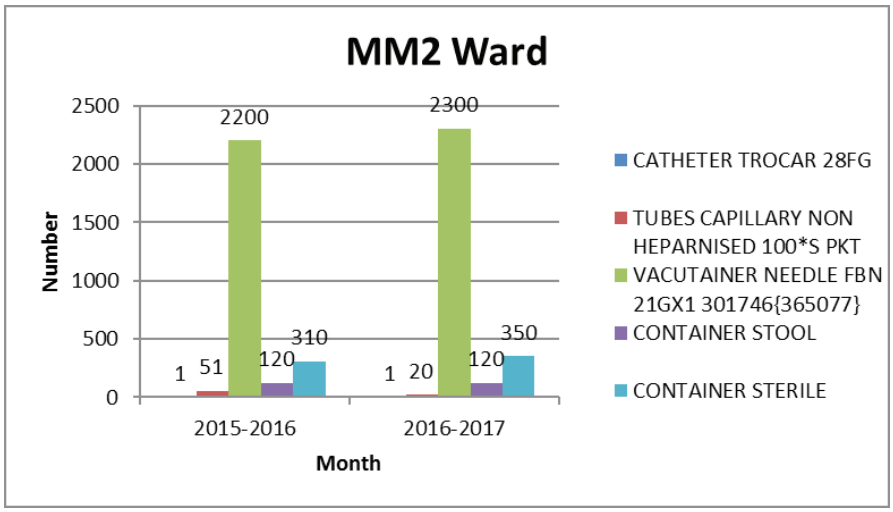
The list of consumables specifically for the month of July to September 2016-17 and July to September 2017-18 was compared. These three months were opted for because of the high purchase pattern in these three months for the respective years and also the increased bed occupancy. After taking an average, the consumption quantity of the particular item of the respective three months for both the years was given a fixed quantity, stating that the particular item is consumed not beyond the average value, and a standard deviation was fixed to the average quantity. By fixing the average consumption quantity with standard deviation, it can be assured that the consumption pattern will not cross beyond the standard deviation. After providing the consumption quantity, the cost per unit for particular item was calculated using the average quantity and the same was compared with the purchase cost of the consumables. The control measure was first implemented in the surgery and medicine wards. Based on the feedback received from these wards, the technique was later applied to rest of the wards in the hospital.

It was possible to estimate the financial resource that could be saved. For example, if the consumption of

'Airway plastic size 03' before taking average consumption and the standard deviation is 140 pieces and after taking average rounding up to 100 and S.D of 20, it can be said that the consumption of airway plastic cannot exceed 160 and go below 80 pieces. The holding stock, average consumption and standard deviation with a final value of the particular consumables was calculated for all the issued consumables to their respective wards.

Results

Comparing the stock consumption for three months (July, August, September) of consumables for 2015-16 and 2016-17, Graph no 1 shows the consumption pattern of July to September of 2015-16, consumption pattern of July to September of 2016-17 of Medicine Male 2 Ward.



Graph 1: Represents the sample of the MM 2 ward consumption pattern of the consumables of the respective 3 months (July, August, September) of 2015-16 and 2016-17.

The consumption pattern was deviating in both the years. 2200 pieces of VACUTAINER NEEDLE was consumed in the year 2015-16 and 2300 pieces in the year 2016-17, which happened to be the highest. Whereas the least consumption was the CATHETER TROCAR 28F. Similarly, there was a 12.9% increase in the consumption of sterile containers. On the other hand, stool container consumption remained constant.

Table 1 represents a sample taken from the ward consumption pattern along with mean and standard deviation. Average consumption quantity was obtained from the respective two years data and the quantity was frozen. It can be stated that the stock maintenance should not be more than or less than the standard deviation.

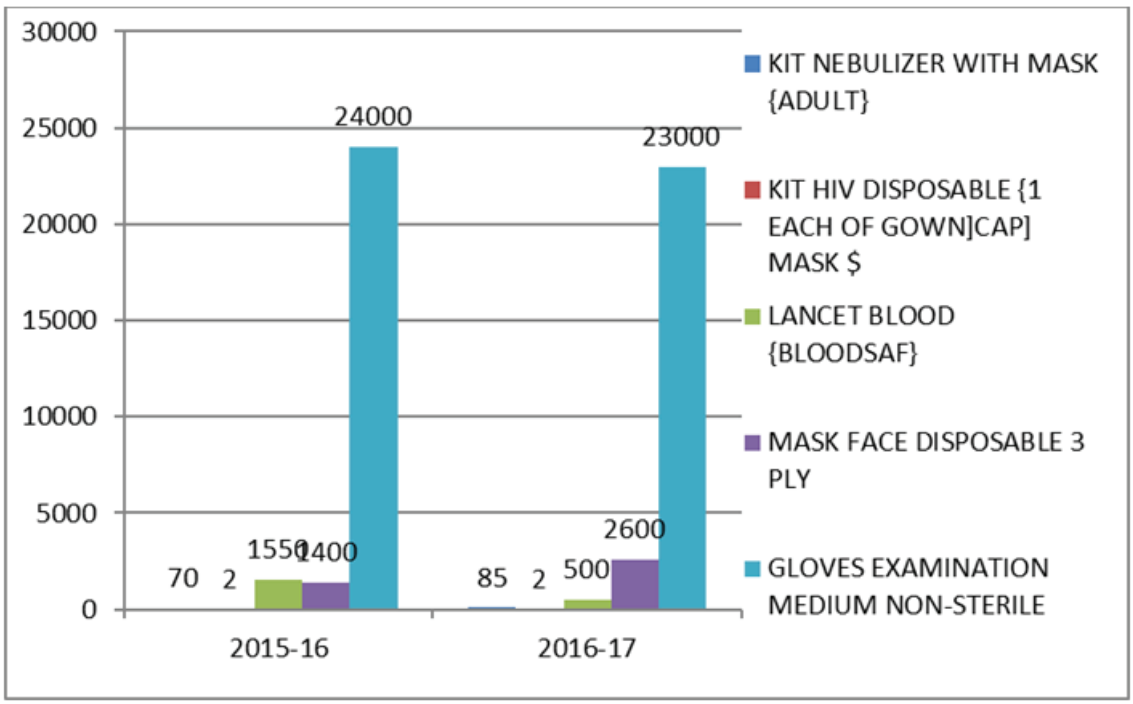
Table 1

CODE	LIST OF ITEMS	AVERAGE OF BOTH	STANDARD DEVIATION
GS CAT531	CATHETER TROCAR 28FG	1	0
GS TUB163	TUBES CAPILLARY NON-HEPARNISED 100*S PKT	35.5	±21.9
GS VAC049	VACUTAINER NEEDLE FBN 21GX1301746(365077)	883.3	430.8
GS CON206	CONTAINER STOOL	48	±37.1
GS CON309	CONTAINER STERILE	110	±20

Table 2: Calculated mean, standard deviation of a sample of consumables along with their unit cost stating the final value after revising MMF

CODE	LIST OF ITEMS	AVERAGE OF BOTH	STANDARD DEVIATION	COST/UNIT	FINAL COST
GS CAT531	CATHETER TROCAR 28FG	1	0	282.6	282.6
GS TUB163	TUBES CAPILLARY NON-HEPARNISED 100*S PKT	35.5	±21.9	1.18	41.89
GS VAC049	VACUTAINER NEEDLE FBN 21GX1301746(365077)	883.3	±430.8	4.62	4081
GS CON206	CONTAINER STOOL	48	±37.1	5.34	256.32
GS CON309	CONTAINER STERILE	110	±20	4.14	455.4

The above table shows the sample of selected consumables of Medicine Male 2 wards, where the consumption rate of Vacutainer needle FBN was highest and Catheter was the lowest among the consumables after taking the average quantity from the respective 3 months. The final value of the vacutainer needle after taking the mean was Rs. 4081/-



Graph 2

The above graph shows sample of the consumables in MF 1 WARD. Variation can be observed in consumption pattern of the respective items and holding stock. The consumption of gloves reduced by 1000 for the year 2016-17 from 24000 in 2015-16. Similarly, the use of lancets also dropped to 500 from 1550. The consumption of face mask had increased by a significant 85.71% for the year 2016-17.

Table 3

CODE	LIST OF ITEMS	AVERAGE	STANDARD DEVIATION	FINAL VALUE
GS KIT064	KIT NEBULIZER WITH MASK {ADULT}	25.83	±8.61	1714.3
GS KIT188	KIT HIV DISPOSABLE {1 EACH OF GOWN] CAP] MASK $	1.33	±0	115.42
GS LAN001	LANCET BLOOD {BLOODSAF}	512.5	±85.39	246
GS MAS018	MASK FACE DISPOSABLE 3 PLY	666.66	±258.1989	824.7
GS GLO084	GLOVES EXAMINATION MEDIUM NON-STERILE	7833.33	±1169.045	12220

Table 3 shows the sample of selected consumables of MF 1 WARD, where the consumption rate of gloves was the highest and the consumption rate of HIV disposable kit was the lowest among the consumables. After taking the average quantity from the respective 3 months, the final value of the Gloves was Rs. 12,220/-

Table 4

WARDS	BEFORE/ RUPEES	AFTER/RUPEES	SAVINGS/RUPEES
MM WARD	224795.8	78862.42	145933
MM 2 WARD	227319.13	80335.5	146983.6
MM 3 WARD	237358.07	94603.22	142754.8
MF 1 WARD	244990.24	80127.4	164862.8
MF 2 WARD	73971.6	19245.6	54726
MF 3 WARD	232311.1	75306.1	157005
SM 1 WARD	104158.2	28572.2	75586
SM 2 WARD	63222	33813.7	29405
SM 3 WARD	47495.5	14658.3	32837
SM 4 WARD	160667.5	46106.5	114561
SF 1 WARD	79496.5	25727	53768.9
SF 2 WARD	81361.8	22738.9	58622.9
SF 3 WARD	45261.8	12560.3	32701.5

Table 4 shows that after revising the MMF (monthly maintenance figure), the control measure was implemented in the surgery and medicine wards. A projected saving of Rs. 11,52,681/- per month and Rs. 45,02,724/- annually was achieved.

Discussion

Materials management is an important issue for healthcare systems because it influences clinical and financial outcomes. Reducing costs in hospital inventory management is always critical for administrators at healthcare institutions. This study attempted to assess two techniques: Monthly maintenance figures and Inventory Index which had contributed to efficient management of inventory at this medical college teaching hospital. A retrospective study was carried out in the general store of this medical college teaching hospital. The study involved perusal of records maintained in the general stores and interviews of staff in general stores, nursing staff and inventory control team. After implementing MMF, it was observed that the stock kept at the store level reduced thereby the holding value of the stock also reduced. Using similar inventory control techniques, considerable savings can be made at the ward level. In today's dynamic environment, it is imperative to manage materials efficiently. It enhances profitability and productivity. The study focuses on improving organizational efficiency and productivity by using this inventory management technique MMF (monthly maintenance figure). It suggests that administrators can use MMF as a control method to have a better control over stock outs or reduce the holding stock while perpetuating sustainability through this inventory management technique.

Ethical Clearance- Taken from Institutional ethics committee

Source of Funding- Self

Conflict of Interest - None

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COVID -19 Pandemic: Immediate Psycological Impact During The 3TD Stage of Lockdown among General Population of Selected Areas of Vadodara City

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Abstract

The Covid -19 pandemic & lockdown has cause deliberate effect to human's physical & mental health & trigger many psychological problems like anxiety, loneliness, stress, depression & other panic disorder. The investigators interested to find out the psychological impact of lockdown among general population & correlation with different components of modified psychosocial impact event scale. Researchers have used non – experimental descriptive research deign with Non – randomized convenient sampling & 500sample recruited for the study. Researchers have used standardized modified impact event scale in the form of 5 point likert scale, they have identified that 366 sample(73.2%)have moderate stress,110 sample (22%) identified with severe stress & remaining 24 sample (4.8%) have mild stress. Another finding revel that each component having positive correlation with each other. The significant association of psychological impacts & selected demographic variables were found with use of modified impact event scale. Researcher have concluded that this type of event or diseases outbreak affect the mental health of the person , increased stress among individual & also affect the daily life of the person.

Key words: *Covid -19 pandemic, immediate psychological impact, 3rd stage of lockdown, general population*

Introduction

People are advanced to be social animals & are wired to live in groups like family & friends. Being detached from the society can affect our health & it can be caused mental as well as physical health problems. Some possible psychological effects like increased anxiety, tension, loneliness, depression, stress may cause due to isolation. Society always help us to face this type of situation but if we lose the personal connection so it gives huge emotional impact on average person & difficult to deal[1].

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The first case of corona virus in India was reported on 30th January, 2020. At the point of time when Covid-19 was declared pandemic by WHO, it also promoted worldwide approaches like isolation, travel limitation, shut down of schools & colleges, no gate to gathers help to diminishing viral spread. This pandemic has sparked worldwide fear, anxiety, post traumatic stress disorder & other long term mental health problems[2]. On 22nd march, our Prime Minister Narendra Modi declared voluntary public curfew after that government has lined up with it lockdown in 75 areas where Covid cases had happened. Furthermore, the leader requested an across country lockdown for 21 days influencing the whole 1.3 billion populace off India .On April 14th the lockdown has increased till 3rd may by our Prime Minister[3].

Lockdown has lead to unemployment & business loss which has driven individual to experience ill effect of post traumatic stress disorder (PTSD).Due to so many issues the government of Gujarat propelled out one helpline number -1100 to provide mental guidance

during lockdown. However similar findings given by the city, based psychologist that 30% increases cases of anxiety & stress disorder. In the present study researcher want to find out the stress among general population of the selected area of Vadodara city.

Objectives

1. To Determine the Immediate Psychological impact; **intrusion** during the 3rd Stage of lockdown COVID-19, pandemic among general population.

2. To Assess the Immediate Psychological impact; **Avoidance** during the 3rd Stage of lockdown COVID-19, pandemic among general population.

3. To Ascertain the Immediate Psychological impact; **hyperarousal** during the 3rd Stage of lockdown COVID-19, pandemic among general population.

4. To find out the Correlation between components of impact event scale.

5. To Find out association between psychological impact with selected demographic variables among general population.

Assumption:

1. Social isolation, loneliness & health related anxiety may affect human psychological health.

2. Increasing unemployment & financial insecurity also increased impact on mental health.

Methodology

Research design: Non – experimental descriptive research design

Research setting: Selected areas of Vadodara city. Following are the setting of my study:

1. Waghodia road, Vadodara

2. Karelibag, Vadodara

3. Old Padra road, Vadodara

Subject: The samples in this study are general population residing in selected areas of vadodara city. Who has fulfilled in to inclusion criteria.

Sampling technique: Non – randomized convenient sampling

sample size:500sample

Variables: Demographic variables: age in years, gender, family monthly income, marital status, type of family, area of living, zone of your area, any family belongs to medical profession, major source of update during lockdown

Research variable: Immediate psychological impact during 3rd stage of lockdown

Explanation of tool:

Section 1: It includes 13 demographic variables.

Section 2: This is the modified impact event scale in the form of likert scale deals with 25 items to determine the stress after any traumatic event.

This scale is categorized in 3 components "Intrusion" , Avoidance", "Hyperarosal"

Pilot study: Pilot study was conducted in Ajwa Road of Vadodara city. After administering tool among 50 sample the reliability score was checked by using Spearman Brown Prophecy formula (r=0.86) reliability test. so it reveals that the tool is highly reliable.

Statistics: Descriptive statistics : Mean , Median , Standard Deviation & Frequency Distribution, Inferential statistics : Correlation & Chi-square .

Result

Analysis has been done in different phases which are given in sections.

Section: I Findings related demographic variables of general population.

Section: II Findings related modified impact event scale score

Section: III Correlations between various component of modified impact event scale

Section IV: Findings of association between impact event scale with selected demographic variables among general population.

SECTION: I FINDINGS RELATED DEMOGRAPHIC VARIABLES OF GENERAL POPULATION

Section 1: Frequency & Percentage Distribution of Demographic Variables

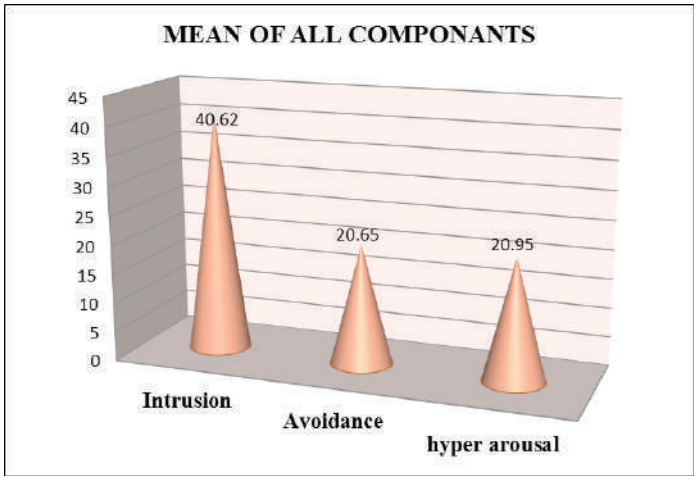
This section reveals data that 275 sample (55%) were having age while another 225 sample (45%) were female.215 (43%) sample are having age group of 18 to 28 years, 98 (19.6%) sample are having age group of 29 to 39, 64 (24.6%) is in age group of 40 to 50 years, remaining 64 samples are in age group of 50-60. For marital status 190 sample (38%) are single , 305 sample(61%%) are married, only 5 sample (1%) are widowed. For the family size 307 (61.4%) from joint family & remaining 184 (36.8%) from nuclear family & 9 samples (1.8%) from extended family. For employment status 55(11%) are no having any job & majority of samples 365(73%) are employed. For monthly income of family 283 samples are having more than 30,000 income for month, another 146 samples are having salary between 20,000 to 30,000 per month. For area of living majority of samples 391(78%) come from urban area & remaining 109 (21.8%) come from rural area. for zone of areas majority of 186 samples from orange zone, another 169 from green zone & remaining 145 (29%) from red zone. 120 (24%) samples are living in hotspot area & remaining 380(76%) samples are living in non-hotspot area.204 (40.8%) samples family member is going for work during lockdown &160 (32%) samples family member belongs to medical profession. majority of the samples 267(53.4%) are using television for update during lockdown. none of them having experienced any pandemic & epidemic outbreak .

SECTION: II FINDINGS RELATED MODIFIED IMPACT EVENT SCALE SCORE

Table 1: Data frequency & percentage distribution of impact event scale score

Class interval		Frequency	Percentage
No stress	25	0	0
Mild stress	26-59	24	4.8%
Moderate stress	60-93	366	73.2%
Severe stress	94-125	110	22%

Interpretation: Above table shows that 366 sample(73.2%)have moderate stress,110 sample (22%) identified with severe stress & remaining 24 sample (4.8%) have mild stress



Graph 1: Mean of all component of modified impact event scale

Interpretation: Above graph revels that that among all the components intrusion means score is (40.62), Avoidance mean is (20.65) & hyperarousal mean is (20.95).

SECTION –III CORRELEATION AMONG THE VARIOUS COMPONANTS OF IMPACT EVENT SCALE

Table 2: **Correlation among the various Components of impact event scale**

Components		r value	significance
Intrusion	Avoidance	0.29	Weak positive correlation
Avoidance	Hyper arousal	0.17	Weak positive correlation
Hyperarousal	intrusion	0.77	Strong positive correlation

Interpretation: Above table revels that each component is having positive correlation with each other.

SECTION IV: FINDINGS OF ASSOCIATION BETWEEN IMPACT EVENT SCALE WITH SELECTED DEMOGRAPHIC VARIABLES.

Above section revels that selected demographic variables are associate with impact event scale gender of the general population with x2 value 10.41(1df=3.84),age of the population x2 value 51.69 (3df=7.82),marital status x2 value is 9.84 (3df=7.82), area of living x2 value is 10.16(1df=3.84), zone of area x2 value is 23.44 (2df=5.99) , living in hotspot area x2 value is 26.88 (1df=3.84), family going out for work during lockdown period x2 value is 8.4 (1df=3.84) , any family member belongs to medical profession 23.5 (1df =3.84), Major source of update during lockdown related to Covid -19 30.60 (3df=7.82).

Discussion

The study revealed that lockdown during Covid -19 causing physical & mental stress. Moreover work from home, restriction on travelling that factors may cause individual crisis. Fear of infection is increasing panic situation among all. Researcher have identify that 366 sample (73.2%)have moderate stress,110 sample (22%) identified with severe stress & remaining 24 sample (4.8%) have mild stress & all the components of modified impact event scale have a positive correlation with each other. all the selected demographic variables are associated with modified impact event scale.

Conclusion

With specific review with all other studies, we conclude that we have to give more attention towards women, youngsters, peoples with responsibilities & concern for others. According to this study researcher find out that this type of event or diseases outbreak affect the mental health of the person. Majority of the samples are identified with moderate & severe stress.

Recommendation

The researcher recommends that government should plan for tele-counseling, encouraging people for meditation, conducting research on psychological consequences & develop helpful information for public.

Ethical Consideration:

Inform consent was obtained from the sample who has participated in study by electronic form. All the data of the sample kept confidential.

Conflict of Interest: There is no any means of conflict of interest within the researchers.

Funding Agency: Investigators have not received any fund from agency.

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Study of Pattern of Fusion of Coronal Suture Using Skull Radiography and its Association with Documented Age in Males

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Abstract

Cases of age estimation are seen by Forensic Practitioners. Identification is the establishment of the individuality of a person. A medico-legal dispute can arise at any age. Age estimation is needed to apply for government posts or pension or in cases of amnesia. Legal enforcement agencies often bring cases for estimation of age, a few examples are that for employment, superannuation, pension settlements and benefits. Use of fusion of sutures on the skull with other factors has been found reliable for age estimation. In the present study we studied skull roentgenogram of patients. These patients were advised roentgenogram for diagnostic and therapeutic purpose from other clinical departments. We studied the stage of fusion of coronal sutures in those roentgenograms where age of the patient was documented after verification against a standard identity proof. The suture was divided into upper half and lower half and stage of fusion was documented. We followed the Key stage of fusion of sutures. The earliest age of commencement of suture fusion was at 34 years and 30 years in its upper half and lower half respectively in our study. The earliest age of completion of suture fusion was 56 years in upper half and 50 years at lower half.

Keywords*: Coronal Suture; Roentgenogram; Skull; Male; Fusion; stage*

Introduction

Law enforcement agencies often refer cases of dispute of age to Forensic Practitioners [1,2]. Identification is the establishment of the individuality of a person. It is either Complete/ absolute or Partial / Incomplete. Complete identification is where all factors are known. Incomplete identification is where certain facts are ascertained and others are unknown[3,4,5]. Race, age, sex and stature are considered the four parameters in forensic identification [4,5]. Age of an individual is determined from teeth, bones, secondary sexual characters, general development and other features [2, 4, 5].

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The aim of the study is to study the pattern of fusion of coronal suture using skull radiographs and its association with documented age in male population in Western India. The objective is to study the stages of progression of closure of coronal sutures and compare with the existing literature.

Materials and Methods

The present study was conducted in Department of Forensic Medicine and Toxicology at Tertiary set up in Western India from November 2015 to November 2017. The study was a descriptive cross-sectional study.

Cases included were those who had been advised skull roentgenograms from clinical departments for therapeutic and diagnostic purposes. After informing the patients that on their approval their approval will be used for the study. An informed consent form was given

to the patients who were advised roentgenograms. Only those who consented and whose age was verified with a documented identity card were included in the study. Cases with fracture skull or no age proof document were not included in the study. Antero-posterior view or Postero-anterior view or lateral view of skull were included in the study.

The study was approved by the institutional ethics committee.

Methodology

1. Roentgenograms of cases where skull radiograph were taken were used for the study. The roentgenograms were studied in the computer where digital roentgenograms were taken. The digital roentgenograms were stored on a Compact disc. The roentgenogram of the patient where documented age (with reference to the stated Date of Birth in Dependent Card/ Aadhar card/Birth certificate/ Driving license/ Voter's ID) was recorded were studied. Coronal suture was divided into upper and lower parts[6] as shown in **Table 1**. Stage of fusion of the sutures was studied on the roentgenogram as 1- not commenced, 2- in process of fusion and 3-fused [7] as illustrated in Figure 1,2,3 Respectively).

Results

A total of 221 male individuals were included in the study for fusion of coronal suture in males. Table 1 shows the distribution of the stage of fusion in coronal suture in males in our study.

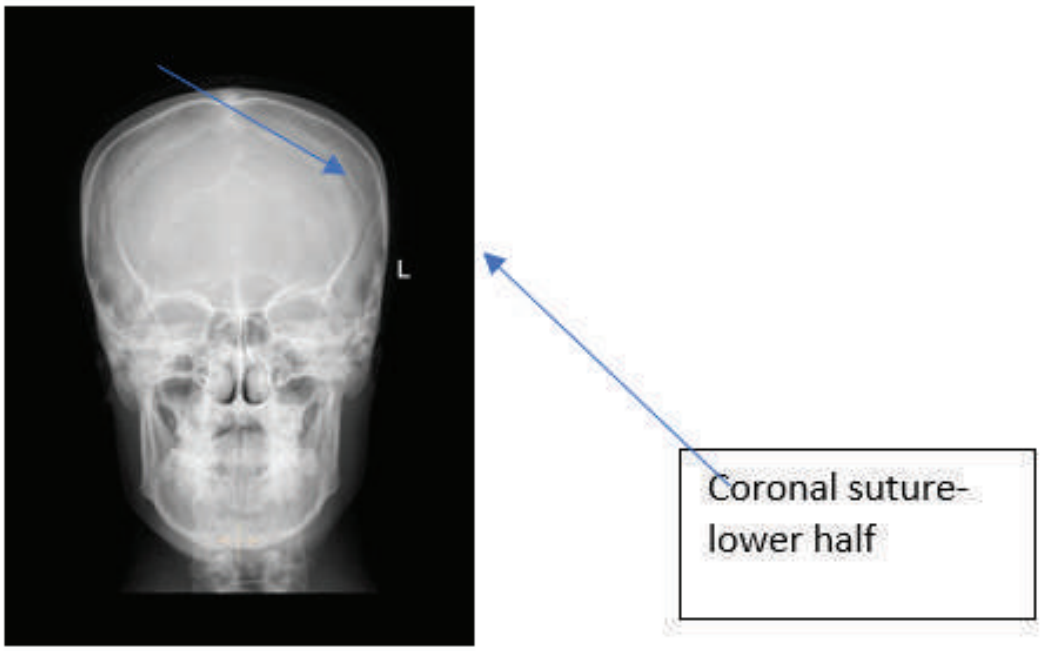


Figure 1:21 years/ Male(stage 1)

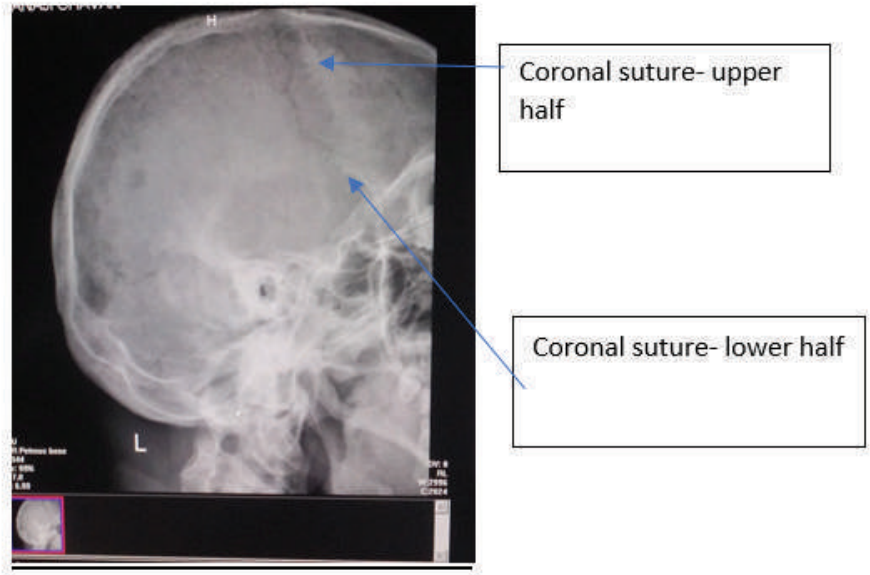


Figure 2: 48/ Male showing coronal suture in the process of fusion in male.

Legend to Table Number 1

Upper half: upper half of coronal suture in male

Lower half: lower half of coronal suture in male

Stage of fusion of suture

Not commenced: fusion not commenced in coronal suture

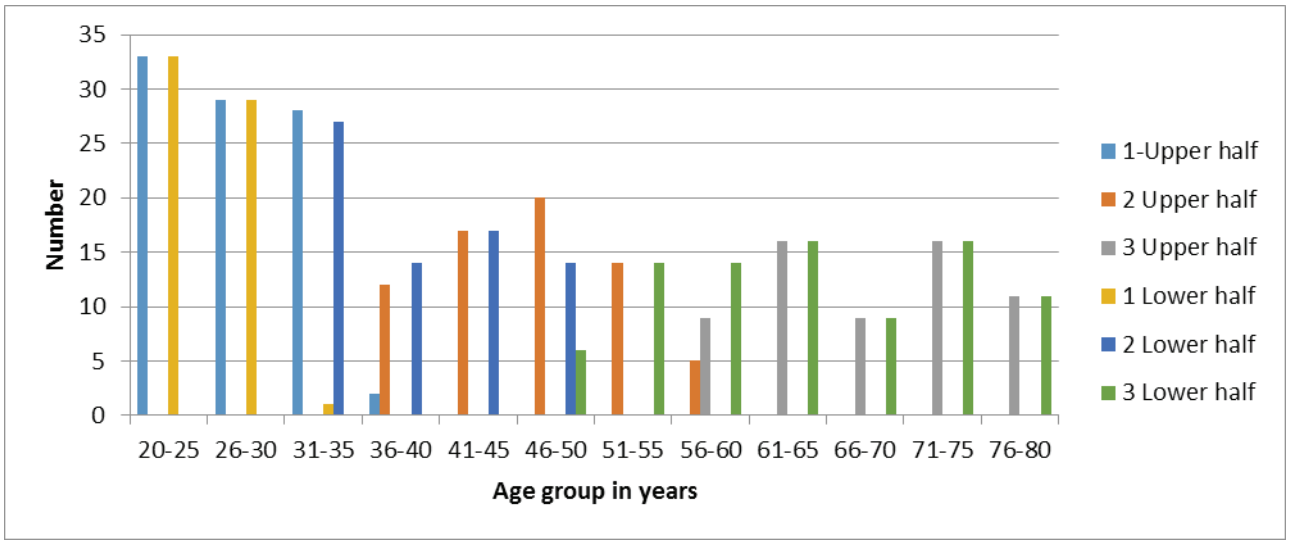
In process : in process of fusion in coronal suture

Fused: fused sutures in coronal suture

AGE in years	NUMBER OF CASES		UPPER HALF						LOWER HALF					
	NUMBER	PERCENTAGE	NOT COMMENCED		IN PROCESS		FUSED		NOT COMMENCED		IN PROCESS		FUSED	
			NO	%	NO	%	NO	%	NO	%	NO	%	NO	%
20-25	33	16%	33	100%	0	-	0	-	33	100%	0	-	0	-
26-30	29	13%	29	100%	0	-	0	-	29	100%	0	-	0	-
31-35	28	13%	28	100%	0	-	0	-	1	4%	27	96%	0	-
36-40	14	6%	2	12%	12	88 %	0	-	0	-	14	100 %	-	-
41-45	17	8%	0	-	17	100 %		-	0	-	17	100 %	0	-
46-50	20	9%	0	-	20	%	0	-	0	-	14	70%	6	30%
51-55	14	6%	0	-	14	100 %	0	-	0	-	0	-	14	100%
56-60	14	6%	0	-	5	36 %	9	64 %	0	-	0	-	14	100%
61-65	16	7%	0	-	0	-	16	10 0%	0	-	0	-	16	100%
66-70	9	4%	0	-	0	-	9	10 0%	0	-	0	-	9	100%
71-75	16	7%	0	-	0	-	16	10 0%	0	-	0	-	16	100%
76-80	11	5%	0	-	0	-	11	10 0%	0	-	0	-	11	100%
TOTAL	221	100	175		31		28		138		56		40	

Figure 3 : 76 years/Male with fused coronal suture.

Fusion of Coronal Suture in Male



Graph 1 showing the number of samples in different stages of suture fusion in upper and lower half of coronal suture in male .

In our study, earliest age of commencement of fusion was 34 and 31 years in the upper and lower half respectively.

In the lower half earliest age of completion of fusion was 56 and 50 years in upper and lower half respectively.

P value for age versus upper half of coronal suture is < 0.001as the age increases the trend is not commenced to fused.

P value for age versus lower half of coronal suture is < 0.001as the age increases the trend is not commenced to fused.

Test used is Fisher's exact test.

Discussion

Due to faulty documentation of birth of an individual, many a dispute arise in the civil and criminal courts. Instances of it are documentation in refugee asylums, cases of amnesia or dementia, pension benefits, state benefits in the case of senior citizenship. Need for scientific confirmation of age is required even when birth documents are available [1,2].

Chronological age is the time passed since birth. Bone age is the degree of maturation and fusion of the parts of bones. Bone age depends up on genetic, dietary and environmental factors[8].

Skull roentgenograms are advised by Oto-rhino-laryngologist, dental surgeons, surgeons and physicians for common complaints in clinics. The same roentgenograms were taken if the patient consented for the study.

Sutures are like epiphyseo-diaphyseal plane. Edges of the sutures are the loci of growth [6]. In skull roentgenograms, the suture is divided into upper and lower halves. An antero-posterior view and postero-anterior view of the radiograph of the skull shows the Sagittal, Coronal and Lambdoid sutures [7,9].

On roentgenogram evaluation, where lucency of line is seen to be less than 2 cm, known age of the patient helps in deciding if it is a fracture or a part of a suture. Persistent lucency beyond the age of fusion indicates towards a fracture [10]. Plain film Radiography is the most cost effective method in evaluating skull fractures, major sutures and common vascular grooves [11,12].

Interobserver variation is common in roentgenogram assessment [13]. Film is affected by the shadows of the damaged tissue and subcutaneous tissue. It can be reduced by taking into account a second factor like documented age in this study.

Description of Sutures where Fusion has not commenced

Interdigitations are present in the anatomical location of sutures. They are radiolucent in appearance with a characteristic serrated appearance in the Outer Table [14, 15].

Description of Sutures in Process of Fusion

Beginning of fusion in sutures is identified by irregular radio-opacity on each side of the suture. With progress in fusion, radiolucent appearance of fusion and margins decreases [14, 15].

Description of Fused Sutures

Fused sutures are described as disappearance of all or part of the affected suture lines with loss of the normal inter-digitations.They appear straight rather than a serrated radiolucent line [14, 15].

In Legal Medicine[16], Anthropology [17], Neurosurgery [18] a lot of research has been done in the correlation between the fusion of cranial sutures and age of the individual. The incidence of Roentgenogram or Computed tomography for age correlation has been variable [19].

The age range of our study is 20 years to 80 years which is similar to other studies. [7, 16, 17, 20, 21, 22, 23.]

Sutures can be seen in a majority of roentgenograms [20, 24]. Antero-posterior view of the skull showed lambdoid sutures and a portion of the sagittal suture, sometimes coronal suture was also seen. The lateral view will showed the lambdoid and coronal sutures [1,2, 25]

Obliteration of cranial suture on roentgenograms has been studied with with greying of hair, wrinkles, arcus senilis and menopause [7] or with documented age [21, 22, 23.]

In our study the fusion of sutures started at 32 years and were fused by 60 years in all the cases similar to many studies in India [21, 22, 23] and in the west [16, 20, 26,27, 28].

It has been opined if male skull is seen with serration of Coronal suture, age is assumed to be less than thirty years in [16, 17, 25, 29, 30.] Similar to our study, fusion was observed to begin in the upper part of the suture [21,22,23,24, 31, 32, 33, 34]. A comparative account is given in **Table 2**.

Table Number 2: Comparative account of Coronal suture fusion

Author	Method	Time of closure of Coronal Suture
Krogman6	Gross skeleton	24 years to 38 years
Parikh 35	Roentgen ray	35 years to 40 years
Vij 3	Roentgen ray	Lower half – 40 years to 50 years Upper half – 50years to 60 years
Ramachandran 36	Roentgen ray	Lower half – 40 years to 60 years Upper half – 50 years to 60 years
Dikshit40	Roentgen ray	Start – 25 years to 30 years Complete – 40 years
Mukherjee 37	Roentgen ray	Lower half – 25 years to 30 years Upper half – 40 years to 45 years
Karmakar38	Roentgen ray	Start – 25 years to 30 years Complete – 30 years to 40 years
Pillay 39	Roentgen ray	40 years to 50 years
Gaur et al.22	Roentgen ray	31 years -50 years

Table Number 2: Comparative account of Coronal suture closure

Conclusion

We studied the radiological stages of fusion of Coronal suture of the skull in association with the documented age and found a significant correlation between the two using a Scoring method. Coronal suture was found to start fusion by 31 years and earliest completion was at 50 years. Ectocranial suture closure can be used for age estimation with other associated factors. In our study the trend of correlation is increasing with age which strengthen the view that there is a significant relationship between suture closure and age. It is important to refine the methods of scoring or quantifying these structures to make it an unbiased observation.

Ethical clearance : a prior approval was obtained from the institutional ethical committee :IEC/DEC/ AFMC/FM&T

Conflict of Interest: None to Declare

Source of Funding: None to Declare

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Pattern of Firearm Injuries: A Five Year Retrospective Study at a Tertiary Care Centre in Central India

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Abstract

Firearm injuries are reported all over the world. From very basic thermodynamic structures to the most sophisticated semi-automatic and automatic guns of the present times, firearms are one of the common causes of significant mortality. In our country there is rampant use of unlicensed country - made firearms, particularly in rural areas. The present study was conducted at a teaching cum tertiary care hospital in Gwalior region of Central India, a region which is notoriously famous for the use of illicit firearms.

The study was retrospectively done analysing the firearm injury cases brought to the mortuary of G.R. Medical College, Gwalior from January 2015 - December 2019 for post-mortem examination. All relevant information regarding autopsy cases were gathered from relatives, police paper; inquest reports, ballistic reports, crime scene findings, autopsy reports. A scientific tabulation of all relevant information was done to arrive at various epidemiological markers relating to firearm injury cases.

In the present study it was concluded that young males were more vulnerable for firearm related deaths with 21-30 years age range showing maximal preponderance. While country made weapons and shotguns accounted for greater majority of cases, rifled weapons were involved either. A plethora of underlying social and psychological factors accounted for such death though region specific familial rivalries stood first in order. Maximum observed diurnal frequency of firearm deaths were reported in relatively calm hours of night, though no time segment was spared. While in most cases single shots were reported, few other had multiple inflictions. Head (including face) was the commonest site involved followed by thoraco-abdominal injuries. Homicide was the most common manner of firearm deaths.

Keywords: *Firearms, shotguns, autopsy reports*

Introduction

With the progression of human civilization, the quest for weapons have seen an unprecedented high in the present times.[1] Right from periods of early and medieval history to present day, firearms of one type or the other have resulted in great mortalities. As on date there is a sharp increase in demand/usage of firearms by commoners of society. In our country, particularly in rural settings firearms are often associated with pride. With the easy availability of country made weapons in country like ours mingled with increase in day to day interpersonal conflicts, familial issues, social stress, infidelity, thinning familial and moral fabric, firearm mortalities have reached an all time high.[2] The disastrous effect of projectile fired from firearm emanates out from the tissue injuries caused by reciprocal interaction of body tissues and projectile and transient cavitatory

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effects of the vibrant release of energy.[3,4]

In Gwalior region of Central India firearm culture is very old with known in famous dacoities and kidnaps including social stigmata's and pride. However, no scientifically assembled datas exists in this regard.

The present study was done to study various epidemiological parameters related to firearm injuries to quantity the magnitude of problem as existing in the society.

Aims and Objectives

The present study was undertaken to find various epidemiological markers related to firearm injuries as follows:

- To study the sex wise distribution of firearm cases.
- To study age wise distribution of firearm cases.
- To find out the location of firearm injury cases (rural settings/urban happenings).
- To study the type of weapon used.
- To study the motive behind the firearm injury cases.
- To study the intra-day distribution of firearm cases.
- To study the site of firearm injury cases.
- To know the number of shots associated with firearm cases.

Methodology

This study was done retrospectively analysing the medico-legal post-mortem reports of firearm injury cases in last five years from January 2015 to December 2019 brought for autopsy at mortuary of Gajra Raja Medical College, Gwalior (M.P.). All firearm injury cases which witnessed spot death and were brought for examination with virgin firearm wounds and without any exploration were incorporated in the present study. Maximal para-death information was collected based on history (if available), spot examination reports, police information, panchnamas, requisition papers, final reports produced by police, weapon reports and personal information of the deceased as received from the relatives. Decision as regard to type of firearm was based upon the detailed examination of wounds, status of burning, blackening, tattooing around the wound, opinion of the ballistic experts. Classification of injury as homicidal, suicidal or accidental was based upon close and detailed analysis of all available documents. In few cases were no opinion could be arrived at were labelled as uncertain.

Results

A total number of 70 cases of death due to firearm injuries were analysed. Out of 70 cases only 59 cases were of males and 11 cases were of females. Maximum number of cases (31 cases) were reported to be in the age group 21-30 years, in the age group 11-20 years there were 14 cases, 11 cases in group 31-40 years while 10 cases were reported in 41-50 years age group. No cases were reported in age range 61-90 years and one case was reported between 91-100 years of age. Of all the cases studied, 37 injury cases (52.86%) were from distant range, 24 (34.29%) from close range and 9 cases (12.86%) were from contact range.

Table 1 : Distribution of cases according to gender

Gender	No. of cases	Percentage
Male	59	84.29
Female	11	15.71
Total	70	100

Table 2 : Distribution of cases according to age

Age group (yrs)	No. of cases	Percentage
1-10	1	1.43
11-20	14	20
21-30	31	44.29
31-40	11	15.71
41-50	10	14.29
51-60	2	2.86
61-70	0	0
71-80	0	0
81-90	0	0
91-100	1	1.43
Total	70	100

A total of 26 cases were from country made weapons while 24 cases were from rifled firearms and remaining 15 cases were by shot guns, in 5 cases the type of weapon remained unidentified. In the greater majority of cases i.e. 54 (77.14%) there was single injury mark present while in 16 cases (22.86%) multiple injuries marks were present. A total of 37 cases (52.86%) were from rural background while 33 cases (47.14%) were reported from urban areas.

Table 3 : Distribution of cases according to types of weapon

Nature of weapon	No. of cases	Percentage
Rifled	24	34.29
Shot gun	15	21.43
Country gun	26	37.14
Unidentified	5	7.14
Total	70	100

Head including face was by far the most important site of injury with 26 cases (it included both distant range shots and contact blasts) followed by thorax including 17 cases. Neck was the site of injury in 8 cases. Seven cases of death due to firearm injuries over extremities were recorded. Spinal column turned out to be the least common site involved with 3 cases in the present study.

Table 4 : Distribution of cases according to location of site

Location of site	No. of cases	Percentage
Abdomen	9	12.86
Thorax	17	24.29
Head and face	26	37.14
Neck	8	11.43
Limbs	7	10
Spine	3	4.29
Total	70	100

Property dispute in families was by far the most commonest reason for reported firearm injuries (37 cases). Personal revenge and extra-marital affairs amounted to second most commonest cause of firearm injuries (12 each). Impulse and harsh fire respectively were there in 4 and 2 cases. Three cases were reported whereby robbery was the underlying intent.

Table 5 : Distribution of cases according to motive

Motive	**No. of cases**	**Percentage**
Extra marital affair	12	17.14
Disputed ancestral property	37	52.86
Revenge	12	17.14
Impulse rage	4	5.71
Harsh fire	2	2.86
Robbery	3	4.29
Total	70	100

A maximum number of 24 cases of firearm injuries were observed from 6 p.m. - 12 midnight followed by 22 cases from 12 midnight to 6 in the morning. From 12 noon to 6 p.m. a total of 13 cases were observed and least number of cases in the study were recorded from 6 a.m. to 12.00 noon.

Table 6 : Distribution of cases according to time

Time	**No. of cases**	**Percentage**
12 mid night to 6 am	22	31.43
6 am to 12 noon	11	15.71
12 noon to 6 pm	13	18.57
6 pm to 12 mid night	24	34.29
Total	70	100

On overall analysis of the data a total of 40 cases (57.14%) turnout to be homicidal, 15 cases (21.43%) were of suicidal nature while in 15 cases (21.43%) the dilemma remained and they were categorized uncertain.

Discussion

Gun shot injuries due to firearms is a world wide phenomena. Globally such injuries have been reported in almost each and every part of the world. From Sub-Saharan Africa[5,6] to United States[7] from United Kingdom[8] to Pakistan[9] there are reports of firearm injuries. Homicidal and suicidal firearm injuries are commonly reported from developed countries.

As per latest data, India ranks third all over the world in firearm related deaths, next only to Brazil and United States. Nearly 9 in 10 killed in India were men and most commonly involved people aged 20-24 years.[10]

According to the United Nations Organizations, young males were more vulnerable to the victims of such violent crimes due to their high risk taking behaviours ranging from street quarrels to drugs, from possession of weapons to gang memberships.[11]

In our study, males out numbered females with male:female ratio 5.36:1. More so, relatively young people in the age range of 11-30 years were victims in the largest proportions. Similar results were obtained in the study conducted by Fazle Ras Malik et al.[12]

Country-made weapons were the most common type used to produce gun shot wounds. Similar results were obtained in the study conducted by Fazle Ras Malik et al[12] and Sangeeta Kumari et al[2]. In developed countries most common firearm weapon are rifled guns. A possible reason for such difference is because there is easy availability of local manufacturers of country made weapons and shot guns in developing countries like ours as against extra technically sound rifled weapons. Also, it is rather easier to get license of shot guns. Sangeeta Kumari et al[2] reports that 55% of cases were from rural background. In our study also a total of 37 of 70 cases were from rural background while 33 cases were from urban areas among to a total of 52.8%.

In our study, mostly shots were aimed from distant range in majority of cases. In study by Sachan R et al[13] close range was found to be the commonest. However, in our study close range was the next after distant range. 7 cases of contact blast were also observed in our study.

Majority of cases in our study were homicidal deaths. These included deaths from distant range and close range combined together. A total of 15 cases could not be labelled as homicidal/suicidal. In the study by Fazle Ras Malik et al[12] too the homicidal cases outnumbered other manners of death. Sangeeta Kumari et al[2] reports 'homicidal' as the most commonest manner of death. Kohli et al[14] and Singh BP et al[15] reports similar observations. In most of such homicidal cases single site entry wound/injury marks were present. However, in 16 cases there were multiple injury marks which were due to firearm injuries associated with assault. In the study by Brain Guetsclow et al[16] suicidal deaths outnumbered homicidal deaths in past 30 years experience in United States.

According to Hagras et al[3] most firearm injury cases happened during night. Second in line were reported in afternoon while others were reported in morning. In our study also most of the cases were observed at night. According to Sangeeta Kumari et al[13] 50% of the cases were observed in night.

Sachan R et al[13] reported that property dispute were the underlying cause in most of the cases to be followed by incidences like dacoities and personal animity. This is in alignment with the present study were disputed ancestral property issues were the commonest of all reasons for firearm fatalities in our regions. Also, most of these cases were reported from relatively rural background and familial rivalries were seen transcending through generations. This in turn is also attributable to easily availability of country made weapons.

Revenge and extra-marital affairs was yet another of an important reason underlying firearm injury cases in present study. However, the trend here was more often observed from relatively urban areas and more often then not were seen caused by rifled weapons. Impulse rage and harsh fire were also observed in the present study.

Similar findings were observed by Iram Khan et al[17] whereby they observed family feud to be the commonest of all reasons for firearm related deaths. Conflict and robberies were amongst the next few causes in this study.

In the present study head including face turned out to be the commonest involved site in firearm injuries cases. An equal number of cases of injury to thorax and abdomen when combined together were observed in the study. Regional injuries over neck, limbs and spine were amongst other bodily location observed. Head and face were more commonly involved in gun shot injury cases with suicidal manners while thoracoabdominal involvement was more commoner in distant range shots with homicidal manner. This is in accordance with the principle of common knowledge that head (face and temple) are the sites of easiest access for suicidal attempts. Also, due to greater surface area available thoracoabdominal injuries are more often involved in distant range shots.

In the study by Iram Khan et al[17] similar results were obtained as they mention head and neck region to be the most preferred location for execution of suicidal ideations while relatively broad areas of trunk (chest + back + abdomen) providing greater available surface for aiming to be most commonest location in homicidal infections.

In a study in Saudi Arabia[18] head and chest was found to be a common site for firearm entrance wounds. According to Hussain et al[9] head and neck injuries accounted for most deaths attributable to firearms.

Conclusion

The present study highlights the facts that in the developing country like ours homicidal firearm injuries are more than suicidal ones as against the trends in developed countries. Male gender, young age alongwith easy availability of country made firearm weapons account for most mortality. Underlying cause of such inflictions includes trivial fights with minimal provocation to more sinister family feuds, continuing generations, mingled with elevated sense of pride in a society, full up of social/familial stigmatas. Though, cases were found distributed all throughout the day, night were more suited for silent and easy execution of such intends which, at least, can be partly attributable to intoxication. Involvement of vital cavities of body including cranium, chest, abdomen and visceral organs therein make it easier for the perpetrator to materialize their motives with ease.

Given the ever increasing trends of firearm injuries, it is of utmost important that government authorities put a strict vigilance and control over assemblage, production, distribution, circulation and licensing of such deadly weapons coupled with stringent measures to curb such menace with commitment. On the other hand, strengthening the social fabric and reducing interpersonal conflicts are to be attempted as social levels to change the mindset of the people in general.

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Ethical Clearance: From Institutional ethical committee

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Accuracy of Mesiodistal Crown Measurements using CBCT Images: A Comparison with Plaster Models

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Abstract

Aim: Comparison of the mesiodistal crown measurements made on plaster models and CBCT-images.

Materials & Methods: Thirty pre-existing CBCT records of the maxillary arch and type-III stone orthodontic models, soaped and polished were used in this study. A digital vernier caliper was used to make measurements on each cast. The largest mesiodistal crown dimension of each tooth was measured from central incisor to the second premolar of one quadrant of the maxillary arch. The OnDemand 3D program (Cybermed Co, Seoul, Korea) was used to convert CT DICOM images into 3D reconstructed images. The mesiodistal crown width measured using the OnDemand 3D program.

Results: The precision of the measurements on the plaster models and on the CBCT 3D reconstructed images were the same. There was no significant difference between them as evaluated by paired t test.

Conclusion: The distance and the precision of the measurements on the plaster models and on the CBCT 3D reconstructed images are equivalent. CBCT 3D reconstructed images can replace the process of making plaster models.

Keywords: *CBCT, study models, orthodontics.*

Introduction

The CBCT, specifically developed for the maxillofacial region, provides numerous clinical applications, from the positioning of dental implants to the study of the respiratory system. Furthermore, it provides greater spatial resolution of high-quality images, through shorter scans and with less radiation than conventional computed tomography.

In the field of orthodontics, CBCT allows us to undertake a more precise diagnosis, by providing information on the three-dimensions of the orofacial structures and to replace two-dimensional (2D) radiographs. Moreover, we can obtain three-dimensional images of teeth that can then be measured; measurements that traditionally were done by hand on plaster study models.[1] CBCT scans have the potential to be as acceptable to patients and practitioners as other forms of dental models.

Plaster models of the dentition have been used traditionally for orthodontic evaluation and are considered the "gold-standard" for arch space analysis. Some of the advantages of plaster dental models include accuracy, a high level of physical permanence over time and a relatively low production cost.[2] However, plaster models have disadvantages, including breakage, storage costs and weight. It has been evident from experience that plaster casts present precise and reliable information about patients' dental arches, position of teeth and their dimensions.[3] Patients' orthodontic data is usually obtained from measurements of dental casts by a digital caliper and are further stored for future use. Currently

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it is necessary to store the patients' plaster casts for legislative and further clinical workups, however with the use of stored patients' data in the digital form we not only retain the possibility of reproducing the dental casts when the necessities arise we also eliminate the unnecessary storage of plaster casts which are not readily required. The disadvantage of using the plaster casts include the burden of their storage, risk of damage or breakage, their heavy weight and difficulties in sharing their data with other professionals involved in the patients' care[4].

The purpose of this study was to compare measurements made on traditional plaster models with measurements made from digital renderings of CBCT scans of the same patients.

Materials & Methods

A retrospective, hospital-based study was conducted using the following pre-existing records of 30 consecutive patients who attended the Department of Orthodontics & Dentofacial Orthopaedics, School of Dental Sciences, Sharda University, Greater Noida:

® CBCT records of the maxillary arch.

® Type-III stone orthodontic models.

In this study, a Digital Vernier Caliper with a measuring range of 0–150 mm/ 0–6 inch, resolution: 0.01mm/ 0.0005 in, repeatability: 0.01mm/ 0.0005 in, accuracy: + 0.02 mm/ 0.001 in. (<100 mm), + 0.03 mm/ 0.001 in. (>100 – 150 mm), maximum measurement speed: 1m/s, was used to make measurements on each cast to the nearest 0.01 millimeter. The largest mesiodistal crown dimension was measured of each tooth from central incisor to the second premolar of one quadrant of the maxillary arch. As described by Hunter and Priest in 1960, the greatest mesiodistal diameter was taken from the anatomic mesial contact point to the anatomical distal contact point of each tooth measured parallel to the occlusal plane.[5]

The CBCT images of these subjects were obtained from the archives of the Department of Oral Medicine & Radiology using CareStream 9300, USA at a slice thickness of 0.75 mm.

The CT DICOM (digital imaging and communication in medicine) images were converted into 3D reconstructed images using the OnDemand 3D program (Cybermed Co, Seoul, Korea). Radiodensity was set between +1000 and +3000 on the Hounsfield unit (HU) scale so that all the hard tissue of a tooth could be expressed.

With these 3D reconstructed images, the 3D tooth images were acquired by eliminating the adjacent anatomic structures and the mesiodistal crown width measured using the OnDemand 3D program.



Fig. 1. Digital Vernier Caliper



Fig. 2. Orthodontic Study Models



Fig. 3. Measurement of mesio-distal width of orthodontic study models

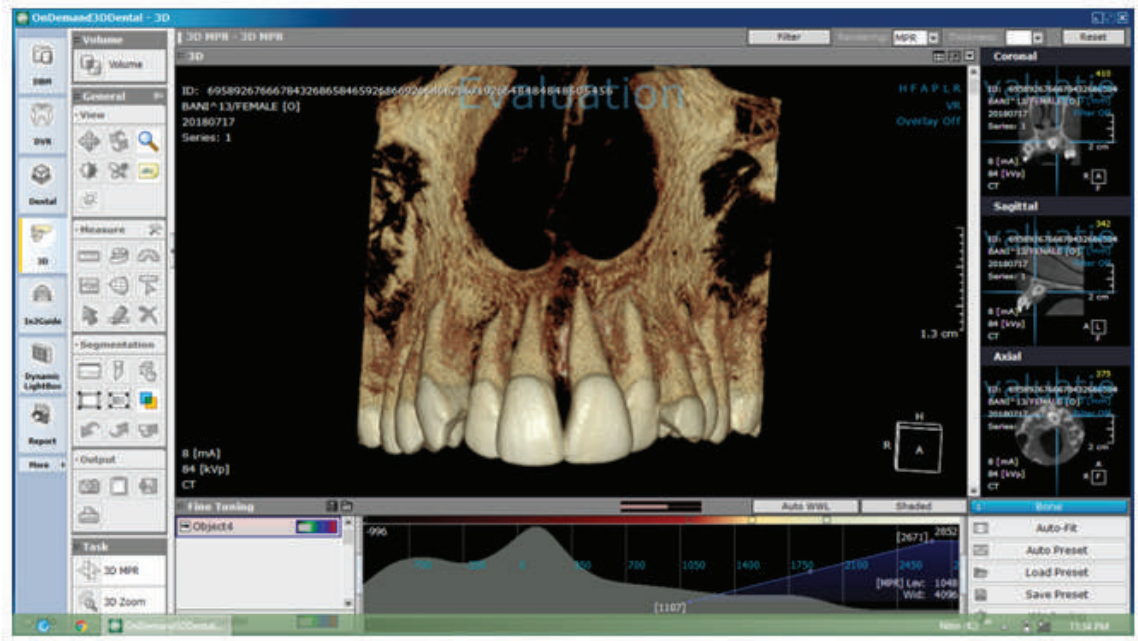


Fig. 4. 3D reconstructed CBCT image of maxilla

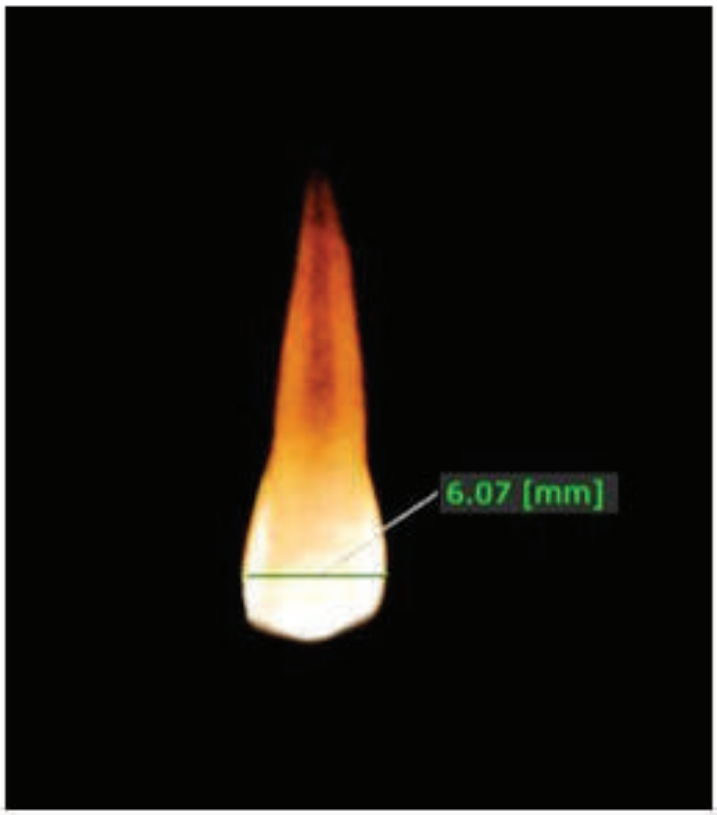


Fig. 5. Measurement of mesio-distal width using OnDemand 3D software

Study Design

The mesiodistal width of the crown of five permanent teeth in a maxillary quadrant was measured. The teeth included were the central incisor to the second premolar of one quadrant of the maxillary arch on the plaster model and in the CBCT image for the same subject. The data was collected, compiled and analyzed statistically using software for statistical analysis. After testing for normality, the paired student's t-test was applied for comparison between the groups. Level of significance (p-value) was set at 5%. This data was compared using statistical analysis to verify if the digital images from CBCT are an acceptable alternative to plaster models.

Inclusion Criteria

- A complete permanent dentition of ten permanent from the second premolar of one quadrant to the second premolar of the other quadrant of the maxillary arch .
- Absence of anomalies in the number, size, and dental shape.
- Good quality of study models.
- Absence of large occlusal restorations or the presence of prostheses

Exclusion Criteria

- Blebs or voids on the plaster models
- Fractures on the teeth of the plaster models
- Missing teeth
- Definitive obstructions (e.g., odontoma or supernumerary teeth);
- Impacted teeth.

Results

The precision of the distance measurements on the plaster models and on the CBCT 3D reconstructed images were the same. This was confirmed by the estimated characteristics including confidence intervals.

There was no significant difference between them as evaluated by paired t test. The accuracy of the CBCT 3D reconstructed images for measurements with respect to the plaster models was estimated using standard deviation of differences between the plaster models and the CBCT images. The standard deviation values did not significantly differ between the plaster models and the CBCT 3D reconstructed images.

Table 1: Mesiodistal crown measurements on the plaster models and CBCT 3D reconstruction images

Parameters	Group	N	Mean	S.D.	Std. Error Mean	df	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference		Cohen's d	t	p Value
									Lower	Upper			
CENTRAL INCISOR	CBCT	30	10.5427	0.5487	0.1002	29	-0.1043	0.051	-0.2096	0.0009	0.17349726	2.0277	0.0519
	CAST	30	10.6470	0.6494	0.1186								
LATERAL INCISOR	CBCT	30	7.8187	0.4140	0.0756	29	-0.0923	0.048	-0.1912	0.0065	0.20883077	1.9103	0.0660
	CAST	30	7.9110	0.4683	0.0855								
CANINE	CBCT	30	8.0103	0.6505	0.1188	29	-0.0127	0.052	-0.1187	0.0934	0.02188272	0.2443	0.8087
	CAST	30	8.0230	0.5005	0.0914								
FIRST PREMOLAR	CBCT	30	7.0273	0.5235	0.0956	29	-0.0140	0.007	-0.0289	0.0009	0.02667165	1.9187	0.0649
	CAST	30	7.0413	0.5263	0.0961								
SECOND PREMOLAR	CBCT	30	6.5677	0.4149	0.0757	29	-0.0360	0.029	-0.0947	0.0227	0.08532681	1.2550	0.2195
	CAST	30	6.6037	0.4288	0.0783								

Discussion

The goal of the present study was to compare the accuracy of measurements taken on a CBCT image with manual measurement data of a dental cast. There were no significant differences observed in between the measurement methods. Of the two methods, the smallest mean error (0.075 mm) was observed when registering the CBCT dental image.

Although producing a dental stone cast model can be laborious, this is the preferred method due to requirement of less skill than in CBCT image processing and measurements.

There has not been any study comparing directly the linear measurements taken on a 3D reconstructed CBCT image and plaster model. Kasparova et al. (2013) in a study compared traditional plaster casts, digital models and 3D printed copies of dental plaster casts based on various criteria in order to determine whether 3D printed copies obtained using an open source system can replace traditional plaster casts in dental practice. They concluded that the scans can replace traditional plaster casts primarily due to their accuracy and price.[3]

Disadvantages include high cost of CBCT imaging and the exposure to radiation. It was noted by Signorelli et al. (2016) that although a single CBCT image can replace most of the conventional set of orthodontic radiographs, one set of conventional orthodontic radiographs still entails 2-4 times less radiation than one CBCT. In CBCT, reducing the height of the field of view and shielding the thyroid are advisable methods and must be implemented to lower the exposure dose.[6] The advantages of the CBCTs is that they do not require production of a dental stone model and that registration can be non-invasive. Keeping patients' data in digital form can help solve the storage problem, as all of the study models must be kept for certain period of time, which requires a large storage space. On the other hand, the CBCT files take up no physical space. This is an environmentally sensible choice as opposed to the traditional gypsum casts.[7] Digitalization of the data also offers a solution for retrieval and transfer of the patients' data as and when required by the team of various physicians responsible in the care of the patient. However, from the legal point of view one must be vigilant when sharing patients' data as there are potential confidentiality and privacy issues that could be prone to misuse.

Further investigations are required in this field and there is a need to focus on simplifying the process of CBCT imaging. There is also a scope for reconstructing data for 3D printers directly from the CBCT scans to eliminate the intermediary step of manufacturing traditional plaster casts.

Conclusions

The precision of the measurements on the plaster models and on the CBCT 3D reconstructed images are equivalent. CBCT 3D reconstructed images can replace the process of making plaster models. The CBCT imaging has the advantage of being faster, easy to store and environmentally sustainable option when compared to taking impressions and making plaster models.

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The Personal Identification by Means of Cheiloscopy among People of North India

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Abstract

Introduction: Personal identification is becoming increasingly important not only in legal medicine but also in criminal investigation, identification and in Genetic Research. The grooves present on the lips are unique for every person. Cheiloscopy is a forensic investigation technique that deals with the identification based on lip traces.The aim of this study was to assess the specific lip print pattern for personal identification, its uniqueness among males and females and the reliability of the technique in suspect identification at the crime site.

Materials and Methods: The study sample comprised 200 individuals (100 males and 100 females) aged between 10 and 50 years; dark-colored lipstick was applied uniformly on the lips and clear impressions of the lips were taken on the bond paper. The scanned lip-print image studied using Microsoft office picture manager. Fingerprints were analyzed by following the classification given by Suzuki and Tsuchihashi. Chi-square test and p-value of the samples were calculated.

Results: The study revealed that the lip print patterns for each individual were unique and there was no peculiar pattern distribution among male and female subjects.

Conclusion: Cheiloscopy is analogous to fingerprint analysis and can be used as a tool for individual identification along with dermatoglyphics. It can also be used as an aid in forensic sex determination.

Keywords: *Cheiloscopy, Lip print, sex determination, Lip pattern*

Introduction

Personal identity of human is most challenging and is a fundamental process in Legal Medicine, both in civil and criminal terms.[1]Article 6 of Universal Declaration of Human Rights states that everyone has a right to recognition everywhere as a person before the law.[2] There are many well-known methods of human identification, commonly as fingerprints, palm-prints and foot-prints by recognizing the pattern of furrows and ridges. Similarly Lip-print is also a known method (Cheiloscopy) for identification of an individual. Lip prints are identifiable as early as the 6th week of intrauterine life, and from then on, their pattern rarely changes, resisting many afflictions such as herpetic lesions.[3]The creases on the vermillion border of the lips, which appear as white area in the lip prints, and the raised reddish areas outlined by these creases appear as dark areas are analogous to furrows and ridges present on finger, foot and palm.[4] Japanese scientists, Tsuchihashi and Suzuki have carried out the most extensive research in this field and established that lip print patterns are unique for each human being. They also proposed a classification of lip prints, that is the most widely used classification in literature to date.[5] Awareness of the modern techniques of crime detection has alerted the criminals for taking sufficient precautions like the use of gloves. In such circumstances, the identification of criminals using accurate methods like fingerprint analysis fails to establish a positive identity.

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The investigators can rely on cheiloscopy as supportive evidence in specific investigations[6]and study on variations in the patterns of grooves among males and females could also help in sex determination.[7]

The objective of this study was to assess the specific lip print pattern for personal identification, their uniqueness among males and females and the reliability of technique in suspect identification at the crime site.

Material and Method

The present prospective study was conducted in the Department of Forensic Medicine, CMC, Ludhiana. The study sample comprised of 200 individuals (100 males and 100 females) from general population in the age group of 10-50 years over a period of 6 months following inclusion and exclusion criteria. The subjects willing to participate in the study and having healthy upper and lower lips along with well defined vermilion border, vermilion zone and labial tubercle were included in the study. Subjects with pathology like deformity, inflammation, trauma, malformation and surgical scars of lips and allergy to lipstick or having generalized skin allergy were excluded.

The study protocol was approved by an institutional ethical committee before commencing the study. All the subjects were grouped separately in four sets of 10 males and 10 females each, according to the age groups like A-10 to 20 years, B- 21 to 30 years, C- 31 to 40 years, D- 41 to 50 years. Each subject identity was hidden from the analyzer. Informed written consent was taken before obtaining the lip prints.

Procedure of collection of sample

The subject was asked to clean his/her lip thoroughly with water and dry it with tissue paper. A non glossy dark colored lipstick (Lakme, Hindustan Lever Ltd., India) was applied on the lips starting from the mid line and moving up to the vermillion border. The lipstick was allowed to dry for about 2 minutes. To ensure the lipstick spreads evenly over lips, the subject was asked to rub the upper and lower lips together prior to taking the print. The bond paper bearing serial number and date was provided to subject. The subject was asked to press his/her lips onto the paper by holding it between the lips, so as to leave a clear impression of lips on the bond paper. The lip-print was scanned and image obtained was magnified and was studied using Microsoft office picture manager. Samples analysis were calculated by Suzuki and Tsuchihashi classification[8]as,

Type I - A clear-cut groove running vertically across the lip,

Type I' - Partial-length groove of Type I,

Type II - A branched groove,

Type III - An intersected groove,

Type IV - A reticular pattern and

Type V - Other patterns.

The data was statistically analysis. Chi-square test was applied to see the significant differences in lip print patterns and demographic parameters and p-value less than 0.05 was considered significant.

Results

Lip prints of each individual showed unique pattern. All four quadrants of upper and lower lips in each individual did not have only one pattern but appeared to have a mixture of different patterns. The most common pattern of upper lips in males were that of the Type I' (30.75%) followed by Type II(27.25%), Type I(21.5%), Type IV (8.25%), Type V (6.5%) and Type III (5.75%). Where as in females the most predominant pattern of upper lips was that of Type II (35.25%) followed by Type I (33.5%), Type I' (15%), Type IV (8.5%), Type III (4.5%) and Type V (3.25%)(Table 1).

The most predominant pattern of lower lips in males were that of the Type I (42.75%) followed by Type II (32.75%), Type I' (13%), Type IV and Type V (4 %) each and Type III (3.5%).Where as in females the most predominant pattern of lower lips were that of Type I (40.25%) followed by

Type II (31.25%), Type I (10.25%), Type IV (9.75%), Type III and Type V (4.25%) each (Table 2). The percentage of lower lip print patterns (Type I and Type II) in both sexes was almost similar in different quadrant (Table 2) . The p- value between both sexes was insignificant among lower lip pattern.

Among males and females, the upper lip showed a predominance of Type II (31.25%) of all the patterns followed by Type I (27.5%), Type I'(22.88%), Type IV (8.37%), Type III (5.13%) and Type V (4.87%). The lower lip showed a predominance of Type I (41.5%) among all patterns. This was followed by Type II (32%), Type I' (11.62%), Type IV (6.88%), Type V (4.12%) and Type III (3.88%).(table 3)(p-value significant(0.00))

Comparison of Pattern of lateral and middle segments of upper lip and lower lips in males and females separately is shown in Table-4 and Table- 5. p-value significant in the lateral segment of both lip patterns of males(0.00)(Table 4) and p-value significant among middle segment of lower lip pattern in males and females, 0.00 and 0.01 respectively.(Table 5)While comparing the lip patterns of males and females(Table 6) the Chi-square test (30.569) and p-value (0.00) is significant.

Table- 1: Lip print patterns in different segments of the upper lip of study group population

Types	Gender	ULL	ULM	URM	URL	TOTAL	%age	Chi-Square test	p-value
I	Males Females	27 33	17 28	26 33	16 40	86 134	21.5 33.5	4.129	0.247
I'	Males Females	21 10	36 19	31 15	35 16	123 60	30.75 15	22.564	0.000
II	Males Females	30 44	29 29	24 37	26 31	109 141	27.25 35.25	1.791	0.618
III	Males Females	5 4	3 5	8 6	7 3	23 18	5.75 4.5	1.961	0.590
IV	Males Females	7 4	10 15	7 7	9 8	33 34	8.25 8.5	-	0.601
V	Males Females	10 5	5 4	4 2	7 2	26 13	6.5 3.25	1	0.801
Total	Males females	100 100	100 100	100 100	100 100	400 400	100 100	-	-

ULL- upper left lateral, ULM- Upper left middle, URM – Upper right middle, URL – Upper right lateral

Table 2: Lip print patterns in different segments of the lower lip of 100 males and 100 females

Types	Gender	LLL	LLM	LRM	LRL	TOTAL	%age
I	Males Females	44 36	50 49	46 44	31 32	171 161	42.75 40.25
I’	Males Females	13 14	9 13	14 5	16 9	52 41	13 10.25
II	Males Females	33 28	28 24	32 29	38 44	131 125	32.75 31.25
III	Males Females	5 5	4 1	1 7	4 4	14 17	3.5 4.25
IV	Males Females	3 10	5 9	4 12	4 8	16 39	4 9.75
V	Males Females	2 7	4 4	3 3	7 3	16 17	4 4.25
Total	Males females	100 100	100 100	100 100	100 100	400 400	100 100

LLL- Lower left lateral, LLM- Lower left middle, LRM –Lower right middle, LRL – Lower right lateral

Table- 3: Showing lip patterns on both lips of the study group and its percentage

Types	**Type I**	**Type I’**	**Type II**	**Type III**	**Type IV**	**Type V**	**Total**
Upper lip%	220(27.5)	183(22.88)	250(31.25)	41(5.13)	67(8.37)	39(4.87)	800
Lower lip %	332(41.5)	93(11.62)	256(32)	31(3.88)	55(6.88)	33(4.12)	800
Total	552(34.5)	276(17.25)	506(31.63)	72(4.5)	122(7.62)	72(4.5)	1600

Table 4: Comparison of Lateral Lip print patterns of both lips in males and females separately

Types	Type I	Type I'	Type II	Type III	Type IV	Type V	Total
Upper lip % (ULL) Male	43(21.5)	56(28)	56(28)	12(6)	16(6)	17(6.5)	200
Lower lip % (LLL) Male	75(37.5%)	29(14.5%)	71(35.5)	9(4.5)	7(3.5)	9(4.5)	200
Upper lip % (ULL) Female	73(36.5)	26(13)	75(37.5)	7(3.5)	12(6)	7(3.5)	200
Lower lip % (LLL) Female	68(34)	23(16.5)	72(36)	9(4.5)	18(9)	10(5)	200

ULL (Upper Lateral Lip), LLL (Lower Lateral Lip)

Table 5: Comparison of Lip print patterns of Middle segment both lips in males and females separately

Types	Type I	Type I'	Type II	Type III	Type IV	Type V	Total
Upper lip% (UML) Male	43(21.5)	67(33.5)	53(26.5)	11(5.5)	17(8.5)	9(4.5)	200
Lower lip % (LML) Male	96(48)	23(11.5)	60(30)	5(2.5)	9(4.5)	7(3.5)	200
Upper lip %(UML) Female	61(30.5)	34(17)	66(33)	11(5.5)	22(11)	6(3)	200
Lower lip% (LML) Female	93(46.5)	18(9)	53(26.5)	8(4)	21(10.5)	7(3.5)	200

UML (Upper Middle Lip), LML (Lower Middle Lip)

Table - 6: Comparison of lip patterns of males and Females

Type	I	I'	II	III	IV	V	Total
Male (%)	257(32.13)	175(21.87)	240(30)	37(4.62)	49(6.13)	42(5.25)	800
Female (%)	295(36.87)	101(12.63)	266(33.25)	35(4.37)	73(9.13)	30(3.75)	800

Discussion

When there is lacking evidence like finger prints the valuable lip grooves can be used successfully.[3] The edges of the lips have sebaceous glands with sweat glands in between therefore, secretions of oil and moisture enable development of 'latent' or persistent lip prints, analogous to finger prints.[9] Few studies on lip morphology and patterns have shown that chelioscopy has a potential to become an additional weapon for personal identification.[10]

In the present study lip prints were recorded by closed lips method. In closed mouth position, the lip exhibits well-defined grooves, where as in the open position the grooves are relatively ill defined and difficult to interpret.[11]

In the present study, no two lip print patterns matched each other, thus establishing the uniqueness of lip prints, which is in agreement with the report of Tsuchihashi *et al*.[8]The most predominant lip pattern in our study group was Type I (34.75%) followed by Type II (31.63%), Type I' (17.25%), Type IV (7.62%) and Type III and Type V (4.5%) each(Table 3) which is in accordance with study conducted by Multani et al.[12]. They also reported the most common lip pattern as Type I(27.5%) in the entire population of their study and the least common was Type V (7%). While Verghese *et al*., in their study on Kerala population, found that Type IV pattern was predominant.[13]

In our study we observed almost same lip print patterns in both sexes except in male Type V was 5.25% where as in females it was 3.75 %.(Table 6). We observed Type I lip pattern most commonly prevalent in them, where as other workers reported Type II as the predominant pattern in both sexes (males (42.2%), females (43.63%) respectively.[14]

In the present study most common lip print pattern in males was Type I (32.13%) but some studies reported Type IV [15]and Type III[16]as the predominant pattern in males.

We observed Type I most predominant lip print pattern in females (36.87%) which was in-

accordance with many studies,[15]in contrary, some studies revealed Type II as the most common pattern.[16,17]

In males Type I' and Type II lip pattern was common in lateral quadrant of upper lip and Type I was common in lower lip lateral quadrant. Where as in females Type II was predominant in both upper and lower lateral quadrants of lips.(Table 4) Many studies were documented for sex determination by other workers and they documented certain pattern trends prevalent in either sex. According to them Type I and I' are dominant in Females in third and fourth quadrants of lower lip and Type II is common in Males in the second quadrant of upper lip, left side. Individuals with all quadrants having different pattern were common in males whereas having same pattern in all quadrants were seen in females.[18]

Cheiloscopy is a simple, easy to access, non-invasive technique and does not require a very complex instrument so it can be used as a tool for identification of an individual along with finger print analysis. Its utilization at a scene of crime depends on a high degree of the skill of members of law enforcement agencies.

Limitations of the Study Subjects with lip pathologies and deformities could not be established as they were not assessed. Forensic identification of such individual remains questionable.

Conclusion

Cheiloscopy is analogous to fingerprint analysis. Lip prints can add additional evidence to a crime scene, and this is valuable, especially in cases of lacking other evidence, like fingerprints.[19] It can also be used as an aid in forensic sex determination and for personal identification.

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An Autopsy Study of Medicolegal Aspects of Burn Death among Married Females

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Abstract

Background - Burn injuries are very common cause of morbidity and mortality among Indian populations specially the bride burning cases which are related to dowry deaths. The study was aimed to analyze the epidemiology, pattern, cause and manner of deaths to formulate burn the possible preventive strategies.

Methods - In present study we analyzed the autopsy done in the department of forensic science & toxicology, Institute of Medical Science, BHU, Varanasi, U.P., India from the period of March 2014 to August 2015. We reported total 155 unnatural deaths of women who died within seven years of their marriage and out of them total 96(62%) cases were due to burn.

Conclusion- The most of the cases (51%) reported within 2 to 5 years after marriage and majority (70%) of them happened in kitchen where cooking gas was most common (64%) source of burn. Maximum (35%) case were having total body surface area(TBSA) 51-60% and 92% of victim died in hospital and septicemia was major (60%) cause of death. Most of the deaths (56.25%) were suicidal in nature. Such bridal deaths are major concern toward the society, health agencies and law enforcing agencies.

Key words – *Autopsy, Bridal burning, Dowry death.*

Introduction

Burn injuries are commonly caused by flame, cylinder and stove blast, electricity, radiations, hot water and other liquids and chemicals. Which can involve any part of body from the most superficial layer to deepest part i.e. muscles, visceral organs and even bones of the body. The burn cases are reported from all over the world and pose a serious global public health issue with approx 195000 death annually from fire related burn only.

Fire related cause are 15th leading cause of death for children and young adults aged 5-29 yrs. Highest morality is observed among children less than 5 yrs and among older people over 70yrs of age. Many of survivors suffer lifelong disability and disfigurement after resulting in stigma and rejection[1].

The important issue is that most of female deaths within 7 years of their marriage found to be due to burn injuries. The common causes of such unnatural deaths among females within 7 years of their marriage are dowry, dissatisfaction in married life either due to drunkness of husband or financial issues, unnecessary interference by in-laws and marital infidelity[2]. Many of such deaths can be prevented by timely medical invention and strict implication of law. Dowry deaths are punishable under 304B & 498A IPC[3].

Aim & Objectives

To study the burn source, pattern, extent of burn, cause and manner of death to formulate the possible

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social, medical and legal preventive strategies.

Material & Methods

The study was carried out on female deaths who died due to burn within seven years of their marriage and brought for autopsy in the dept. of Forensic Medicine & Toxicology, Institute of Medical Science, BHU, Varanasi, U.P., India from period of March 2014 to August 2015.

We analyzed the autopsy findings, panchnama papers and bed head tickets. We also have taken history from the family members, relatives and neighbors of deceased as well as police personals.

Data has been analyzed in respect of age, religion, habitat, duration since marriage, Dowry demand, torture, socioeconomic status, place of incidence, delay in treatment, type of hospital, source of burn, area of burn, cause of death, nature of death etc. Finally the data was compiled, tabulated, analyzed and concluded.

Result and Discussion-

The present study demonstrate the pattern of burn death in females within seven years of their marriage were total 96 burn deaths were reported out of total 154 death due to all cause.

Table-1 Duration since marriage-

Duration in years	Number	%
<1 yr	14	14.58
1-2 yrs	15	15.62
2-3 yrs	18	18.75
3-4 yrs	14	14.58
4-5 yrs	17	17.70
5-6 yrs	6	6.25
6-7 yrs	12	12.5
Total	96	100

Table-1 shows that in present study maximum 49(51%) deaths were reported between 2 to 5 years after marriage. least number of deaths 6(%) were reported between 5 to 6 years after marriage. These findings are contradictory to Radhika R.H. et al[4] where maximum deaths 44(73.33%) were reported within 3 years after marriage.

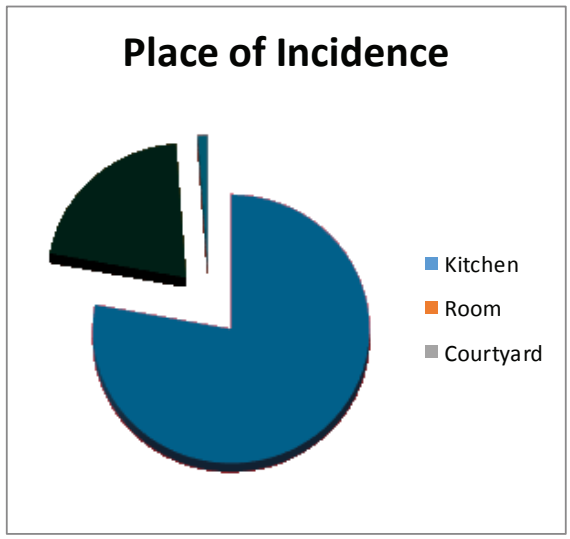


Figure-1 Place of Incidence-

Figure 1 shows that maximum cases happened in the kitchen i.e. 67(70%) cases followed by 18(19%) cases in the room. This could be due to either accidental burn or suicidal burn. Some time homicidal is also done to simulate accidental burn.

Table-2 Source of Burn-

Source	Number	%
Cooking Gas	64	66.66
Kerosene oil & Matches	25	26.04
Stove Blast	5	5.20
Cylinder Blast	2	2.08
Total	96	100

Table 2 describes that in 64 (66%) cases the source of burn was cooking gas followed by 25(26%) cases due to of kerosene oil & matches. The cases due to stove blast and cylinder blast were 5(5%) and 2(2%) respectively.

These findings are similar to the 40 females burn case study by Shankar Gowri et al[5] where in most of 18(45%) cases were due to cooking gas followed by

14 (35%) cases due to kerosene oil & matches. These finding are contradictory to 32 females case study of Rahul Chawla et al[6] where maximum 13(40%) cases were due to stove blast and only 2(6%) cases were due to clothes caught fire from gas while working.

Table-3 Burnt body Surface area-

Area of burn	Number
<30%	0
31-40%	5
41-50%	11
51-60%	34
61-70%	23
71-80%	10
81-90%	10
91-100%	3
Total	96

Table 3 describes that majority of cases 34(35%) having TBSA 51-60% followed by 23 cases (23%) with TBSA 61-70%. These findings are contradictory to the 38 case study by Manjit Nayak et al[7] where majority of cases 21% having TBSA 30-40% followed by 20% cases with TBSA 20%.

Table-4 Place of Death

Place	Number	%
On Spot	5	5.20
Way to hospital	3	3.12
Hospital	88	91.66
Total	96	100

Table 4 shows that Most of the victims 88(%) died in hospital during treatment. In 5 cases victim died on spot followed by 3 death on the way to hospital. These findings are similar to the 95 cases study by Pradeep Kumar Mishra et al[8] where 87cases (91.6%) death were reported in hospital followed by 8 (8.4%) deaths on spot.

Table-5 Cause of death-

Cause	Number	%
Septicemia	60	62.5
Neurogenic shock	6	6.25
Asphyxia	14	14.58
Other complication of Burn & Multi-organ failure	16	16.66
Total	96	100

Table 5 shows that most of deaths 60(62.5%) were due to septicemia followed by 16(17%) deaths due to multi organ failure and 14(14.58%) deaths due to asphyxia. Only 6 (6.25%) cases were due to neurogenic shock. These findings are similar to findings of Harish Dasari et al[9] who also reported maximum death 36.4% and 27.2% due to septicemia and multi organ failure respectively .

Table-6 Nature of Death-

Nature	Number	%
Accidental	39	40.62
Suicidal	54	56.25
Homicidal	3	3.12
Undetermined	0	0
Total	96	100

Table 6 shows that 54(%) victim committed suicide followed by 39(%) accidental burn only 3 cases of burn were due to homicide. These findings are contradictory to the findings by B.L.

Choudhary et al[10] where slightly more 37(41%) deaths were accidental followed by 33(36%) deaths suicidal in nature.

Conclusion and Acknowledgement -

In the present study of female deaths after 7 years of their marriage. Maximum deaths were reported up to 5 yrs. after marriage. Majority of the incidences happend while victims was working in the kitchen and cooking gas was the main source of burn. Many times in-laws and husband give false history to hide the issue of domestic violence and dowry demand.

In majority of cases TBSA was 50-70% and in some case TBSA was >80% burn most of the cases she died in hospital during treatment and septicemia was most common cause of death followed by other complication like multi organ failure.

Majority of victims committed suicide followed by accidental deaths.

Suggestions

1. Girls should be promoted to be more and professionally qualified to become economically independent.

2. Child marriage, if solemnized in any part of country must be strictly prohibited.

3. Demand of Dowry should be discouraged through various campaigns.

4. Govt. should take strict legal action if any dowry case come in notice.

5. Social boycott of such families who are found to be involved in such criminal activities.

Ethical Clearance : Taken from Ethical Committee, IMS, Banaras Hindu University, Varanasi.

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Contribution by authors

We declare that this work was done by the author(s) and all liabilities related to the content of this study will be done by the authors.

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Parental Perception on the Effect of Various Factors in a Dental Clinic Affecting Child's Behaviour

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Abstract

Background: Societal, professional views and parenting styles have evolved tremendously over the years. Thus, it is important to understand the parental perceptions on the various factors influencing child behaviour in the dental clinic.

Aim: To record and evaluate the parent's views regarding the various factors that influence child behaviour in a dental clinic.

Materials and Methods: A cross sectional study was conducted among 164 parents who were asked to complete a close-ended questionnaire divided into 4 categories- dentist, dental clinical settings, dental treatments and child related factors. This data was analyzed using descriptive statistics.

Results: According to the study results, the parents perceived that the dental treatments and dental settings played a significant role ($p< 0.05$) that affected the child's behaviour in a dental clinic. There was no significant differences between the "yes" and "no" responses for the dentist and child related factors.

Conclusion: The parental attitudes and styles are constantly changing as the society evolves. Thus it is important to understand and reassess their beliefs and update our way of managing the dental setup and treatments regularly.

Keywords*: child, behaviour, dental care, parent, perception*

Introduction

Dental fear prevails to be a major source of problem while managing and treating children in the dental setup [1].While adult patients tend to develop their own anxiety coping strategies throughout the years, this task often becomes the responsibility of the dentist in pediatric dentistry [2] .The origin of dental fear and uncooperative behaviour of children and proper understanding of it may aid in helping the pediatric dentists to plan and adopt appropriate behaviour management strategies [3].

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Dental fear and behaviour have a multi-factorial origin broadly divided into personal characteristics, environmental factors, or situational factors [4]. Dentists recommend various behaviour guidance methods based upon the child's health, special health care needs, dental needs, type of treatment required, consequences of no treatment, emotional and intellectual development, parents' preferences, dentist's preferences and skills.[5]

The impact of dental atmosphere on child is a major aspect [6]. Every child is considered unique and a major aspect of behaviour management deals with understanding parental perceptions and determining factors that influence child's behaviour. Over the years, there has been a changing trend in the parenting styles, and societal and professional perceptions [5]. Dentistry always aims to place emphasis on the human aspect of the dental professional- patient relationship [7].

Thus, it is important to understand the parental perceptions which will present an opportunity to work together and select the best method to provide a safe, effective and comfortable dental visit [5]. Studies related to environmental factors are few and most of the studies were conducted in European and South-East Asian regions [8]. Environmental and situational factors have been previously studied as potential causes of dental fear and behavioural problems but such results have been found to be inconsistent [5, 8-11]. Hence, the aim of this study was to understand parents' perception regarding the factors that influence child's behaviour in a dental set up in Chennai, India.

Materials and Methods

The present study was a cross-sectional study conducted in the Department of Paediatric Dentistry, Saveetha Dental College and Hospitals. The study design was reviewed and approved by the Institutional Review Board (SDC/PEDO-1704/19/014).

Participant selection:

A total of 170 parents who visited the Department of Pediatric Dentistry for their child's dental treatment were included. Amongst that 164 parents agreed to participate in the study. The parents of children who required special needs or have associated systemic conditions were excluded. Thus, parents of children with normal developmental milestones and with at least one prior dental visit were included in the study.

A validated questionnaire [5] was provided to the parents and asked to complete within treatment hours. The questionnaire comprised of 16 close ended questions divided into 4 categories: dentist, dental setting, dental treatment and child related factors.

Statistical Analysis

Based on the information collected, responses were recorded, processed and a descriptive analysis was followed.

Results

The results of 164 participants were tabulated and analyzed accordingly.The majority of the parents who brought their child for dental treatment belonged to the age range of 31-40 years (52%) and the least belonged to 41-50 years(17%) with a distribution of 43% males and 57% females (Table 1)

Table 1: Socio-demographic Characteristics of parents

Variables	N	%
Age(years)		
21-30	51	31
31-40	85	52
41-50	28	17
Gender		
Male	70	43
Female	94	57

The major reason for bringing the child for dental visit (Table 2) was pain (40 %) followed by decay (24%), routine check-up (15%), trauma (11%) and other reasons (10%). According to responses recorded from parents in terms of yes or no (Table 3), the probabilities of the t- test for the four parameters under evaluation were dentist (p=0.0989), dental settings (p=0.0187), dental treatment (p=0.00018), and child related factors (p= 0.17809).

Table 2: Parent's reason for their child's dental visit

Reason	N	%
Decay	40	24
Pain	65	40
Trauma	18	11
Routine check-up	24	15
Others	15	10

Table 3: Parent's perception on various factors affecting the behaviour of their child in the clinic

	YES	%	NO	%
DENTIST				
Dentist attitude(friendly/stern)	115	70	49	30
Dentist attire(with or without lab coat)	99	60	65	40
Gender	92	56	72	44
Total	306	62	186	38
DENTAL SETTINGS				
Pleasantness of dental setup (colour, decoration, etc.)	117	71	47	29
Friendliness of staff	115	70	49	30
Total	232	70.5	96	29.5
DENTAL TREATMENT				
Duration of procedure	120	73	44	27
Type of procedure (filling, scaling, extraction, etc.)	122	74	42	25
Dental appointment timing(morning/afternoon)	99	60	72	40
Sound and noise of instrument	130	79	34	21
Absence/Presence of parent's during procedure	112	63	52	31
Behaviour of other children undergoing treatment	99	60	65	40
Total	682	69	309	31
CHILD				
Age of the child	84	51	80	49
Gender of the child	79	48	85	52
Previous dental experience of the child	96	59	68	41
Diet consumed by the child	61	37	103	63
Previous dental experience of peers/ and siblings	82	50	82	50
Total	402	49	418	51

According to the results, the parent's perception in respect to two parameters such as dentists and child factors, there was no significant difference noted between the two responses [Yes or No] ($p > 0.05$), whereas there was a significant difference noted for the factors: dental settings and dental treatment ($p<0.05$).

Discussion

Getting to understand and learn about parents' perceptions regarding various factors in dental clinic and thereby making the parent understand about the influence of various factors in dental clinic play a major role in establishing a positive dental outcome [12].

As per the study results, 62% of parents agreed that the attitude, attire and gender of the dentist affect the child's behaviour in dental clinic. Amongst that, dentist's attitude (70%) and dentist attire (60%) were believed to play a role in child behaviour. There was no major difference in the results when the gender of the dentist was taken into criteria. Thus, to gain the trust of the children and parent, the dentist's attitude, body language, and communication skills play a crucial role in creating a positive dental experience for the child and parent [13].

Over the years, there has been a concern that the white coat and professional clothing worn by the dentist can increase anxiety in the children. The protective mask may also act as a stressor for the child and thus it is recommended to introduce the child to the dental environment without the mask [14]. It was found that 58% of parents preferred non-white coat attires and formal attires among dentists [15, 16].In a study conducted in India, it was noted that children preferred their dentists to wear dental scrubs and white coat but were highly anxious on seeing their dentist with any protective wear [17].

American Academy of Pediatric Dentistry (AAPD) describes that the orientation of dental environment and atmosphere play an important role influencing the child's behaviour in dental clinic [13]. According to the present study, friendliness of staff and pleasantness of the dental set up such as colour, decoration etc. affects the child's behaviour according to 70% and 71% of parents respectively. Thus a cumulative of 70.5% parents agreed that the dental settings in general played a crucial role in child's behaviour in dental clinic. The results are in accordance with another study which exhibited the effects of the atmosphere and dental settings on the child's behaviour and emotions [18]. The children exhibited positive emotions towards bright colours (e.g., pink, blue) and negative emotions towards dark colours (e.g. black, brown)[19].Thus the use of child friendly colours like blue and yellow enhances a positive dental attitude in the child's mind [20].

Dental healthcare environment is getting more competitive everyday and patients are more demanding than before [21]. The results show that the dentists should pay attention to the dental settings in addition to focussing on the treatment which in turn will motivate the children to undergo the dental procedure in a well behaved manner [5].

According to our study results, 69% of parents agreed that the dental treatment has an effect on the child's behaviour. Amongst that, 73% agreed that the treatment duration affects the child's behaviour, which is similar to previous studies [5, 22].The dental instrument's sound and noise influence the child's behaviour according to 79% of parents and the type of dental procedure was believed to have some effect on the child according to 74% parents. In a similar study, 77% of parents agreed that the type of procedures, such as drilling/ restoration and the sight of instruments cause fear and anxiety in the child; however, it is known that procedures like injection may produce a high level of anxiety [23].

63% of parents agreed that the presence of parents during treatment might influence the behaviour of the child. However, a study stated that the presence/ absence of parents did not have any effect on the child's behaviour [24].When the timing of the dental appointment was taken into criteria, 56% of the parents in our study felt that early morning or afternoon dental appointments had some impact on the child's behaviour. Early morning appointments are preferable for young children as they are more rested and thus cooperative. The children may become tired or cranky from missing nap during afternoons or later periods of the day [25]. However according to a study by Lechner, timing of the day of the dental appointment had no significant effect on child's behaviour [26].Thus, it can be stated that time scheduling for dental appointment should be

more dependent on convenience than possible behaviour effect based scheduling [27].

49% of parents in our study stated that the child related factors such as age, gender, previous dental experience and diet of a child including the previous dental experience of the child's sibling had an influence on the child's behaviour. A longitudinal behavioural study stated that the child undergoing dental care was directly influenced by the psychomotor development of the child [28]. 59% of parents agreed that previous dental experience of the child might have influenced the child's cooperation towards the dental treatment. It is said that a previous traumatic experience in a dental clinic or during hospitalization for any other reason can also provoke anxiety and fear in the child [29, 30].

Previous dental experience of the child's sibling and the gender of the child were considered to impact the child's behaviour according to 50% and 48% of the parents in this study. One study stated that girls are more willing to visit the dentist and cooperate compared to boys of the same age [23] .The diet consumed by the child is believed to affect the child's behaviour according to 37% of parents in the current study. Diet is proven to affect the cognitive ability and behaviour of children and adolescents [31].

This study is the first of its kind carried out in India for the evaluation of parental perception about various factors affecting the child's behaviour in a dental environment. One possible limitation of this study might be the small sample size. Thus, further research in a prospective intervention based method is required to ascertain whether understanding of parental perceptions can serve to be an effective tool in the prevention of child's behavioural problems; and also study the changing trends in various countries.

Conclusion

Dental treatment and dental settings play a key role affecting the child's behaviour according to the perception of the parents in our study. The success of any paediatric dental practice not only depends on the skills of the dentist but also depends on the patients and their parents. Thus gaining a positive dental experience from both the child and the parent is necessary. This relies on the understanding and constant updating of the changing parental styles and attitudes of the society.

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Effect of Music on the Vital Signs and Behaviour of Children During the Recovery Period after General Anaesthesia Procedure - A Randomized Controlled Trial

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Abstract

Background: Music has been suggested as a complementary treatment and this study evaluates the effect of music listened to on the vital signs and behaviour of children during the recovery period after day surgery.

Study design: This study followed a randomized controlled design which involved a total sample size of 62 children randomly assigned into 2 categories- experimental group (31) and control group (31).In both the groups pulse rate , diastolic blood pressure, systolic blood pressure, oxygen saturation, respiratory rate and the mean behaviour score before and after music therapy as well as the surgical procedure were evaluated. Frankl's behaviour rating scale was used to evaluate the behaviour.

Results: Children who listened to music during the recovery period demonstrated a more positive behaviour than the group who did not listen to music . The experimental group exhibited a significantly lower value in relation to pulse rate, diastolic blood pressure, systolic blood pressure and behaviour rating score and a significantly higher value in relation to oxygen saturation.($p<0.05$)

Conclusion: Music therapy can be considered as a complementary treatment method in children during the recovery period as it shows a positive effect on behaviour, pulse rate, oxygen saturation, diastolic and systolic blood pressure.

Keywords: *Music, behaviour, child, vital signs, day surgery*

Introduction

Music has been found to have health and/or wellbeing benefits across a large spectrum of practices from community choir participation to its use in waiting rooms and surgical settings as background music, both to directly influence mood and arousal levels and to distract from other unpleasant thoughts and feelings. Thus, engagement or even just exposure to music can play a role in improving the mood, offsetting or masking pain and anxiety, playing a role in enhancing cardiovascular fitness and leading to a greater social integration.[1]

Complementary treatment modalities aim to provide relaxation by the activation of sensory perception in addition to the moderating effect on some physiological indicators in patients. Besides, decreasing physiological indicators such as pulse rate, blood pressure and respiration, the relaxing effect can prove beneficial in controlling and preventing some intensive care-related complications such as sleep disorder, pain, and anxiety and behaviour related problems.[2]

Music has been regarded as one of the complementary treatment methods [3–5].Music therapy drives individuals to express their emotions such as pain, stress and fear

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thus in-turn helps in meeting their physical, emotional and psychological requirements [6,7].There has been an increasing body of evidence which suggests that music is beneficial in the moderation of pain, stress and anxiety, and may have financial as well as clinical benefits as opposed to more traditional interventions.[1]

Several studies have stated that the vital signs and anxiety level of patients who listened to music in the post-operative period were lower and well controlled when compared to the control group.[2,3] Since, anxiety directly or indirectly influences the child behaviour, the present study was conducted to evaluate the effect of music listened during the recovery period after pediatric day case surgery on the behaviour and vital signs of children.

Materials and Methods

This study was conducted in the department of Pediatric Dentistry in coordination with the Pediatric Surgery department and recovery room facilities of Saveetha Dental College and Hospitals, Chennai, India. The study design was reviewed and approved by the Institutional Review Board (SDC/PEDO-1704/19/014).

The study included 62 children who underwent day case surgery at the Pediatric Operation theatre between December 2019 and January 2020 after obtaining informed consent from the parents. This study followed a randomized controlled design which involved the effect of music listened during the recovery period after day case surgery under general anesthesia in the Pediatric Surgery department on the behaviour and vital signs of children. The sample size was arrived at 62 from a previous study [2] with 90% power and randomly assigned into 2 categories- experimental group (31) and control group (31).Randomization was based on lottery method. Blinding could not be done in this study as there was an obvious visible differentiation.

The inclusion were as follows: 1) children between the ages of 4 to 6 years, 2) parents who understood the study and voluntarily agreed for their children to participate, 3) children who had to undergo day case surgery under general anesthesia at the Pediatric Operation theatre, 4) had not undergone any surgery before, 5) had not been diagnosed with a hearing impairment .The children with mental retardation or special healthcare needs were excluded from the study.

In both groups, pulse, diastolic blood pressure, systolic blood pressure, oxygen saturation, respiratory rate and the mean behaviour score before and after music therapy as well as the surgical procedure were noted using a monitor and evaluated. Frankl's behaviour rating scale was used to evaluate behaviour.

Tables:

The classical music which was chosen to be listened to by the study group was "The Art of the Fugue" by Bach which is more than an hour and consists of 18 episodes. In that, the study group were made to listen to Contrapunctus Episode 3.This was preferred as the piece moves slowly and smoothly and has 4 sound bangs [8].All the participants of the study group listened to the music at the same volume with the same type of headphone and music player for 20 minutes during the recovery period. It was ensured that the headphones were externally covering all parts of the ear and filtering all external noise.

Procedure:

The study's protocol was approved by the Institutional Review Board of Saveetha Dental College and Hospitals. The participation was completely on a voluntary basis and it was informed that they could withdraw from the study any time before the study commencement. All queries were addressed and cleared before the commencement of the procedure.

Experimental group:

The parents and children were introduced to the music player and the headphones to be used, and informed that the patient should be wearing the headphone till they woke up completely. The pre-operative behaviour score and the vital signs were noted in the patient data form 15 minutes before the operation. In the post-operative period after the child had entered the recovery room after the operative procedure, the post-operative vital signs and behaviour score before listening to music were noted. After which, the child was made to listen to the chosen classical music using the headphones provided. They were made to listen to the music for 20 minutes until the doctors said that the child had woken up completely. The post-operative vital signs

and behaviour score after listening to music was then noted down in the same patient data form.

Control group:

The pre-operative vital signs and behaviour scores were evaluated and noted down in the patient data form 15 minutes before the commencement of the operation. The time when the patients entered back to the recovery room was noted and the post-operative vital signs and behaviour scores were recorded after the patient had completely woken up. The patients in this group did not listen to any music.

Statistical Analysis

The data were analyzed using SPSS software version 16.0.T -test was used for evaluation of the pre- and post-operative vital signs and behaviour rating scores respectively in the groups. The results were evaluated with a p value < 0.05 considered as significant.

Results

On analyzing the pre-operative group's vital signs and Frankl's scale score, there was no significant difference between the groups in relation to all the vital signs and behaviour rating score.($p<0.05$ for each value)

On comparison of the post-operative vital signs in control group and in experimental group (after music therapy), a statistically significant difference was observed in terms of pulse rate, diastolic blood pressure, systolic blood pressure, oxygen saturation and behaviour rating score ($p< 0.05$). Experimental group exhibited a significantly lower value in terms of pulse rate, diastolic blood pressure, systolic blood pressure and behaviour rating score and a significantly higher value in terms of oxygen saturation. However, no significant difference in respiratory rate was observed between the two groups. [Table 1]

On analysis of the experimental group in terms of pre- and post intervention (i.e., before and after listening to music), in the post-operative period after entering recovery room, a statistically significant difference was observed in all the vital signs and in the behaviour rating score.($p< 0.05$) After music therapy, there was a significant increase in oxygen saturation value whereas other vital signs observed in the study and Frankl's score demonstrated a significantly lower value compared to the values recorded before music therapy post-operatively. [Table 2]

Table 1: Comparison of the control and experimental groups in terms of pre-operative vital signs and behaviour rating score and post-operative vital signs and behaviour rating score (after music therapy in experimental group)

Pre-operative values	Experimental group	Control group	p value
Pulse rate	96.35 +/- 7.85	95.55 /- 8.15	0.693
Diastolic pressure	57.77 +/- 7.60	59.16 + /- 5.76	0.422
Systolic pressure	96.06 +/- 7.00	95.81+/- 8.15	0.883
Respiratory rate	22.87 +/- 2.97	23.06+/- 2.58	0.785
Oxygen saturation	99.32 +/- 0.70	99.35+/- 0.70	0.858
Frankl's scale score	3.55 +/- 0.57	3.55 +/- 0.57	1

Cont... **Table 1: Comparison of the control and experimental groups in terms of pre-operative vital signs and behaviour rating score and post-operative vital signs and behaviour rating score (after music therapy in experimental group)**

Post-operative(after music therapy)	Experimental group	Control group	p value
Pulse rate	98.77 +/- 6.57	105.81+/8.24	0.0004*
Diastolic pressure	57.16 +/- 8.49	69.65+/- 6.24	0.0011*
Systolic pressure	97.26 +/- 6.60	101.26+/- 7.38	0.028*
Respiratory rate	22.61 +/- 2.26	24.03+/- 3.50	0.063
Oxygen saturation	99.55+/- 0.50	98.87+/-0.92	0.001*
Frankl's scale score	2.87 +/- 0.499	3.74 +/- 0.44	0*

*p<0.05 statistically significant

Table 2: Comparison of the experimental group post-operatively in terms of vital signs before and after music therapy

Parameters	Before intervention	After intervention	p-value
Pulse rate	100.55 /-7.33	98.77 +/- 6.57	0.001*
Diastolic pressure	59.52 /-7.83	57.16 +/- 8.49	0*
Systolic pressure	99.03 /-6.68	97.26 +/- 6.60	0*
Respiratory rate	24.74 /-2.73	22.61 +/- 2.26	0*
Oxygen saturation	99.23 /-0.67	99.55+/- 0.50	0.001*
Frankl's scale score	3.84 /-0.37	2.87 +/- 0.499	0*

*p<0.05 statistically significant

Discussion

Audio distraction is a non-aversive technique in which patients listen to music or stories when undergoing a stressful procedure. Considering its success in medical settings and with adult dental patients, many pediatric dentists and parents believe that this technique can also be beneficial in children and in other procedure settings despite a lack of evidence to support its effectiveness in these settings [9]. Several studies have revealed that listening to music decreased the level of anxiety, pain, analgesic need and stress hormone level of the patients [5,10–16].At present, music is used in palliative care for therapeutic purposes, surgical procedures, paediatric departments, intensive care units, departments of psychiatry, oncology, gynaecology, coronary care, radiotherapy and chemotherapy procedures [5,7,17].

In the present study, no significant difference was observed in the pre-operative vital signs and behaviour rating in both groups. These findings suggest the similarity of the groups in our study. After the surgical procedure, and listening to music (experimental group), a statistically significant difference was observed in the pulse rate, diastolic blood pressure, systolic blood pressure, oxygen saturation and behaviour rating score, with the experimental group exhibiting a significantly lower value in relation to pulse rate, diastolic blood pressure, systolic blood pressure and behaviour rating score and a significantly higher value in relation to oxygen saturation. There was no significant difference in respiratory rate between the two groups. As the change between the groups was observed to be statistically significant, it was concluded that the music had a positive effect on the pulse rate, diastolic blood pressure, systolic blood pressure, oxygen saturation and behaviour. When pre and post intervention (listening to music) was compared after the surgery in the experimental group, a statistically significant difference was observed in all the vital signs and in the behaviour rating score. A significant increase was observed in the oxygen saturation value and a significant decrease in other vital signs - pulse rate, diastolic blood pressure, systolic blood pressure, respiratory rate and the Frankl's scale score after music therapy compared to before listening to music in the same group. This further emphasizes our study results and the positive effect of music.

According to previous studies done in this regard, results revealed that diastolic and systolic pressure were observed to be lower after listening to music [5,11,16,18–21] .The results of the above studies are in accordance with our study results. However, in another study by Sabzevari et al., the systolic pressure was lower in the group which listened to music but there was no difference in the diastolic pressure.[22]

Several studies have reported a positive effect on respiratoy rate, with a lower value in experimental group compared to the control groups [2,5,12,20,21]. In our study too, the respiratory rate was observed to be lower in the experimental group compared to control group but the difference was not statistically significant. Music is proven as an effective way to lower the stress of patients causing a positive behaviour outcome [6] .This was in accordance with our study results showing a significant positive behaviour outcome according to Frankl's scale score.

One of the limitations of this study was the small sample size of children. Thus, further studies are needed in this regard with a multi center based approach and a larger sample size. The music suggested by the children's parents or other relaxing music can also be evaluated for the same.

Conclusion

Music therapy produced a positive effect on the pulse rate, diastolic and systolic blood pressure and oxygen saturation values. Also, music listened during the recovery period had a positive effect on child's behaviour. Thus, it can be concluded that the music therapy can be used as a complementary treatment in children during the recovery period.

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Effectiveness of Nursing Handoff Education Programme on Competencies of Handing Over among Nursing Students Posted in Selected Hospital of Ambala, Haryana

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Abstract

Background: An effective handover supports the transition of critical information and provides continuity of care, whereas poor communication at handover can harm patient safety. **Objectives:** To assess and compare the competencies of handing over among nursing students before and after administration of Nursing Handoff Education Programme, to determine the association of competencies of handing over among nursing students with selected sample characteristics of nursing students. **Design:** Quasi-Experimental research design "Non equivalent control group pre test-post test design was used. **Participants**: Data was collected from 60 nursing students (30 in each group) by convenience sampling technique. **Setting:** Pediatric laboratory setting and Pediatric medical and surgical wards of MMIMS&R Hospital. Structured handing over checklist was used to collect data. **Results:** the mean post test competency scores of nursing students regarding of case scenario was higher in experimental group (28.3) than in the comparison group (20.93). No significant association was found in case scenario I, II, III regarding handing over among nursing students with their sample characteristics.

Key words: *Effectiveness, Nursing Handoff Education Programme, competencies, handing over, nursing students.*

Introduction

A healthy nation they say is a wealthy nation. Healthcare is important to the society because people get ill, accidents and emergencies do arise and the hospitals are needed to diagnose, treat and manage different types of ailments and diseases.[1] Health care professionals typically take great pride and exert painstaking effort to meet patient needs and provide the best possible care. A common problem regarding hand-offs, or hand-over, centers on communication: expectations can be out of balance between the sender of the information and the receiver. This misalignment is where the problem often occurs in hand-off communication.[2]

The joint commission on accreditation of health care organization (JCAHO) defines handoff as "Interactive process of exchange patient specific information from one caregiver to another for the purpose of ensuring the continuity and safety of patient care. Poor communication might lead to inaccurate sharing information about patient details and some important aspects may be missed. Staff communication should be more developed and facilitated in healthcare organizations.[3]

In handing over the methods of communication is verbal, written, electronic and sometimes combination of them. These are permanent and legal document. Written documentation is done with the help of ISBAR format,

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I- Identification

S – Situation (discussion of the current patient condition).

B – Background (discussion of the background and patient history).

A – Assessment.

R – Recommendations (and orders that need to be completed).[4]

Mindful communication as a safety practice is relatively unexplored in healthcare, and specifically nursing. Refinement of this concept is needed as is a 'peeling the onion' approach to develop and refine its theoretical and empirical foundation. As safety is a fundamental societal contract, communication is a fundamental part of the fabric of professional nursing practice.[5]

Methodology

The study was conducted during the period from March 2018 to June 2019 in the state of Haryana, India. A sample of 60 nursing students (30 in experiment and 30 in comparison group) participated in this study with the prior permission from the principals of Maharishi Markandeshwar Colleges of Nursing. Quasi-Experimental research design "Non equivalent control group pre test-post test design" was used in this study. In this study Sample comprised of Bsc Nursing 3rd year students of MM College of Nursing who had Pediatric posting at the time of data collection. 30 B.Sc 3rd year students posted in Child Health Nursing clinical area from 8th September to 8th October 2018 comprised of Comparison Group and 30 B.Sc 3rd year students posted in Child Health Nursing clinical area from 9th October to 9th of November 2018 consisted of Experimental Group. Data was collected by sample characteristic performa and structured handing over checklist.

Description of Tool

1. Sample Characteristic Performa

It consists of 7 items in which age, gender, previous year practical marks (medical surgical), percentage of marks scored in theory (medical surgical), previous knowledge of handing over, duration of handing over, clinical attendance of the present ward, interest in clinical and the technique used was self report paper and pencil.

2. Structured Handing Over Checklist

The Structured handing over Checklists were developed according to ISBAR format, identification, situation, background, assessment, recommendation. The items in the checklist were prepared by the researcher. 3 different Structured Handing over Checklist were used for 3 case scenarios and all three checklists were having separate marking. The checklist were divided into 5 parts that is I=identification, S= situation, B=background, A=assessment, R= recommendation[6]. The scoring for the checklist was 1 for the right response and 0 for the wrong response. Technique used for collecting data was audio recording and self report.

Procedure

Formal administrative permission was obtained from the Principal MM. College of Nursing, Mullana, Ambala, Haryana. Data collection was done in the month of October 2018 and November 2018.

The consent form was taken from the students, purpose of the study was told by the researcher and the students were also informed about the audio recording, that their responses will be recorded and kept confidential. 68 students were taken in the study (34 in Comparison group and 34 in Experimental group) but 8 students were drop outs. Total 60 students were in the study (30 in Comparison group and 30 in Experimental group).

In Comparison group, on day 1, pre test was taken by giving three case scenarios (2 of medical condition and one of surgical condition). The students recorded their responses and the checklist was filled by the researcher by listening to those recordings. Each recording was of 1.5 to 2.5 minutes. On day 2 and 3, the students attended the clinical postings in respective wards. On day 4th Post-test I in Pediatric Laboratory setting was conducted and the students were given three case scenarios (2 of medical condition and one of surgical condition). The students recorded their responses and the checklist was filled by the researcher by listening to those recordings. Clinical post test II was conducted on day 6. The students

were assigned patient in the medical and the surgical wards and at the shift change, students recorded their handing over according to the patient's condition.

In Experimental group, on day 1, pre-test was taken by giving three case scenarios (2 of medical condition and one of surgical condition). The students recorded their responses and the checklist was filled by the researcher by listening to those recordings. Each recording was of 1.5 to 2.5 minutes. On the same day after the pre-test, Nursing Handoff Education Programme including introduction about handing over definition, purposes, techniques, advantages and introduction to ISBAR format i.e., (Identification, Situation, Background, Assessment, Recommendation) format was taught to the students. On day 2, 3 administration and discussion of case scenarios was done, handouts of right responses given and students were sensitized with checklists for self evaluation. The case scenarios which were given for the pre test to the students were used to develop the competencies in lab settings and students recorded their response of each case scenario and students filled the checklist by their own for every single attempt until they achieve 100% of the competencies and their responses were crosschecked by the researcher and the right response of the cases was dictated by the researcher. On day 6, post test II in clinical setting was conducted, where the students were assigned patient in the medical and the surgical wards and at the shift change students recorded their handing over according to the patient's condition.

Data Analysis

Kolmogorov- Smirnov test was applied to check the normality of data distribution. Data was normally distributed in both experimental and comparison groups, hence parametric tests were applied.

Descriptive statistics:

Frequency, percentage, mean, mean percentage, Standard Deviation and range of score to describe the competencies of handing over among nursing students.

Inferential statistics:

ANOVA and T-test for association selected sample characteristics of competencies of handing over among nursing students.

Findings

Table 1: Mean, Mean difference, Standard deviation, Standard error of mean difference and't' value of Competencies of Handing Over before administration of Nursing Handoff Education Programme in Experimental and Comparison Group

N=60

Case scenario	Mean±SD	Mean%	MD	SEMD	't' value	df	P value
CS I (Medical) Exp group (n = 30) Comp group (n = 30)	12.37±2.26 12.23±2.25	51.5% 50.9%	0.13	0.584	0.22	58	0.82NS
CS II (Medical) Exp group (n = 30) Comp group (n = 30)	16.03±5.00 14.00±2.40	45.8% 40.00%	2.03	1.01	2.00	58	0.05NS
CS III (Surgical) Exp group (n = 30) Comp group (n = 30)	17.00±4.42 13.30±2.40	47.8% 35.00%	3.7	0.92	4.24	58	0.04*
Total mean score	Exp group 15.5 Comp group13.5						

NS -Not significant (p>0.05) *- significant (p <0.05)

t (58)=2.001

Table 1 It infers that at the baseline both the groups had no significant difference in competencies of handing over in case scenario I and case scenario II but the p-value for case scenario III was found to be statistically significant, which indicates that both the group were homogenous in terms of competencies of handing over in case scenario I and II.

Table 2: Mean, Mean difference, Standard deviation, Standard error of mean difference and't' value of Post-Test Competencies of Handing Over (Pediatric Laboratory Setting) among Experimental and Comparison Group

N=60

Case scenario	Mean±SD	Mean %	MD	SEMD	't' value	df	Pvalue
CS I(Medical) Exp group (n = 30) Comp group (n = 30)	20.13±2.17 17.13±2.96	83.87% 71.37%	3.00	0.67	4.46	58	0.00*
CSII (Medical) Exp group (n = 30) Comp group (n = 30)	30.40±3.04 21.3±7.52	86.85% 60.37%	9.26	1.48	6.25	58	0.00*
CSIII (Surgical) Exp group (n = 30) Comp group (n = 30)	34.3±2.63 24.53±3.83	90.44% 64.55%	9.83	0.85	11.57	58	0.00*
Total mean score	Exp group 28.3 Comp group 20.93						

NS -Not significant (p>0.05) *- significant (p < 0.05)

t (58)=2.001

Table 2. The findings of the table showed that after administering Nursing Handoff Education Programme both the groups had significant difference in case scenario I, II & III. Hence, the research hypotheses was accepted and the null hypotheses was rejected.

Table 3: Mean, Mean difference, Standard deviation, Standard error of mean difference and't' value of Post-Test Competencies of Handing Over (Clinical setting) among Experimental and Comparison Group

N=60

Clinical Post test	Mean±SD	Mean %	MD	SEMD	't' value	Df	Pvalue
Exp group (n = 30) Comp group (n = 30)	27.23±2.95 11.73±2.74	66.4% 28.6%	15.50	0.73	21.06	58	0.00*

^{NS} -Not significant (p>0.05) *- significant (p <0.05)

t(58)=2.001

Table 3. The clinical exposure of the nursing students was effective but not as much as Nursing Handoff Educational Programme.

Table 4: Mean, Mean difference, Standard deviation, Standard error mean difference, "t" value of Pre test and Post-test scores of Competencies of Handing over (Pediatric laboratory setting) among Nursing students in Experimental and Comparison Group

N=60

Case scenario	Mean	Mean%	MD	SEMD	't' value	Df	Pvalue
Experimental group							
CS I(Medical) Pre test Post test	 12.37 20.13	 51.54% 83.87%	7.76	0.52	14.7	29	0.00*
Cs II (Medical) Pre test Post test	 16.03 30.40	 45.8% 86.85%	14.3	1.08	13.2	29	0.00*
CS III (Surgical) Pre test Post test	 17.87 34.37	 47.02% 90.77%	16.5	0.85	19.0	29	0.00*
Comparison group							
CS I (Medical) Pre-test Post-test	 12.23 17.13	 50.95% 71.37%	4.9	0.63	7.69	29	0.00*
CS II (Medical) Pre-test Post-test	 14.00 21.13	 40.00% 60.37%	7.13	1.24	5.74	29	0.00*
CS III (Surgical) Pre-test Post-test	 13.30 24.53	 35.00% 64.5%	11.2	0.78	9.62	29	0.00*

Cont... **Table 4: Mean, Mean difference, Standard deviation, Standard error mean difference, "t" value of Pre test and Post-test scores of Competencies of Handing over (Pediatric laboratory setting) among Nursing students in Experimental and Comparison Group**

N=60

Total mean score	Experimental group Pre-test 15.42 Post-test 28.3 Comparison group Pre-test 13.17 Post-test 20.93						

^{NS} -Not significant ($p>0.05$) *- significant ($p<0.05$)

t (29)=2.04

Table 4. Hence, it infers that in Experimental and Comparison group there was a significant difference (p=0.00) in pre test and post test (Pediatric laboratory setting) all the case scenario at <0.05 level of significance within the group.

Discussion

In the present study more than half of the students were female (in Exp group=63.3% & Comp group=50%) and males were 36.7% in experimental group and 50% in the comparison group. These findings are contrast with the study conducted by Abeer Mohamed Seada, Somaya Ahmed Bayoumy (2017) among nursing interns where the number of male nurse intern were more than half (63.7%) age group of less 22-less than 24 but in this study most of the participant were in the age group of 21-22 ,experimental group=43% and comparison group =70% and some were in the age group of 19-20, experimental group=56.7% and comparison group=30%.[7]

In this study the highest mean score for the right response in post test II (clinical setting) was 2.00 (100%) for Identification (name, unit, dr. incharge, date of hospitalization) and for diagnosis and vital signs were also high (2.00, 100%). This finding is in contrast with the findings of the study of Bev O' Connell, Kate Macdonald & Cherene Kelly in which they had calculated the mean, percentage and SD for the items, it was a three point Likert scale they have used for taking the responses like disagree, neither agree nor disagree and agree was 94.3% of the participants had agreed to the response "I am able to clarify information that has been provided to me" and the lowest mean score was 2.15 (9.1% participants agreed for "I find handover takes too much time). [8]

Conclusion

Nurses play an important role in providing safe handing over of the patient. The nurse-to-nurse handover is not taught formally during training, yet it is one of the most important rituals of the nursing shift. Nurses are the backbone of the hospital and the health care team. The Nursing Handoff Education Programme was effective to improve the competencies of handing over among nursing students as there was a significant difference between experimental and comparison group in pediatric laboratory setting as well as clinical setting.

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Ethical approval: The ethical clearance was obtained from university research ethics committee of Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala (MMU/IEC/1167) and the study was carried out in accordance with the guidelines laid

by Indian Council of Medical Research ICMR. The permission was taken to conduct the study from the principals of the selected institutes. The written consent from the students was collected prior to the study. The purpose for carrying out research project was explained and assurance of confidentiality was given to the participant.

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Study of Spectrum of Various Pressure Abrasions Caused by Ligature Material in Violent Asphyxial Deaths

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Abstract

From since a long time, in day to day work deaths due to pressure around the neck have been found to be a common cause of death during post mortem examination. Hanging and ligature strangulation are the two forms of violent mechanical asphyxia in which ligature mark is found. The study was conducted in department of forensic medicine and toxicology at J.L.N. Medical College, Ajmer for a period of one year i.e. from June 2019 to June 2020. During this period a total number of 104 cases of violent asphyxia death caused by pressure abrasion due to ligature were observed. In the present studytypes of ligature material used and pressure abrasion produced by them in different situation and their manner of infliction is discussed. In this study chunni (soft material) was the most common ligature material used i.e. in 32.6% cases. Fixed knot pattern was the most common (94 %of total cases). Most of the cases were occurred in closed space (room)

Keywords: *pressure abrasions, hanging, ligature strangulation, Ligature materials*

Introduction

Pressure abrasion due to compression around neck by a ligature is a far old practice to commit suicide or homicide in mankind. It is a painless (or minimal pain inflicted) and sudden procedure. Ligature mark is found in two types of neck compression (1) hanging (2) ligature strangulation. Hanging is a form of death produced by suspension of the body by a ligature round the neck, constricting force being the weight of the body (or a part of the body weight) [1]. A good number of people die each year by suicide adopting various methods in the world according to WHO (world health organization) and research reports[2,3]. In India hanging is among the top 5 methods of choice for committing suicide [4]. The type and position of knot play an important role in the causation of death in hanging [11]. It is represented by an inverted "**v**" shaped mark [9].

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In strangulation cases ligature material applied around the neck or less, as homicide have been perpetrated by assaulter pulling (**U** shaped) ligature against the front [14] and side of neck or (turn around the neck) **O** shaped encircling the neck, while standing at the back. It is also well known fact that discontinuity along the course of the ligature mark is another important criterion while describing ligature mark of hanging or strangulation.

Authors have mentioned that hanging mark almost never completely encircles the neck [5-6]. In strangulation, unless the killer is pulling upwards, there will be no gap in the mark. However, there can be discontinuity along the course of ligature mark due to interposing clothing, scalp or beard hairs or fingers of victim in both hanging and strangulation [6].

In hanging cases obliquity along the course of ligature mark over the neck is common and conclusive finding. Authors have reported that hanging mark is situated obliquely across the circumference of neck [6-9]. The mark of hanging usually rises to a 'peak' pointing the junction of the noose and vertical part of the ligature, this being a distinguishing feature from ligature strangulation [5]. Thick and long beard or clothes on neck may lead to

formation of dull and ill formed ligature mark; thus, the ligature mark becomes a crucial aid in diagnosis and evaluation of dead body. It becomes dry, hard and parchmentized, after couple of hour after death, if the skin has been excoriated. The pattern of ligature may also be seen. Hence, examination of ligature material and mark becomes an indispensable part of autopsy. It is a known fact that it is difficult to differentiate the ligature marks of hanging and strangulation, in this study an attempt will be made to establish different characteristics findings of patterns of ligature marks in hanging and strangulation cases.

Material and Method

The study was conducted in department of forensic medicine and toxicology at J.L.N. Medical College, Ajmer for period of one year i.e. from 6th June 2019 to 23 June 2020. During this period a total number of 104 cases of violent asphyxial death caused by pressure abrasion due to ligature around the neckthat were received at mortuary were observed. Thorough external examination was done and approximate age, sex, position of body, stains (blood, saliva, semen) were determined. Much more emphasis was made over ligature material, appearance of ligature mark. A meticulous local external examination of neck was done with naked eye and hand lens to quantify and correlate ligature mark with its appearance in relation to material, impression, pattern, colour, course, type of knot, level of ligature, skin changes over the ligature mark etc. A standard autopsy technique was adopted.

Observations and Result

During the study period total 927 bodies were brought to J.L.N. Medical College mortuary for post-mortem examination out of which 104(11.21%)cases were of hanging and strangulation. Out of these 104 cases 98 cases were of hanging and 6 cases were of strangulation. History about the incident was taken from the police & relatives.

Table 1: shows age-wise distribution of cases

No. of Cases M/F Age Group	Male	Female	Total no. in per age group	Total
<20	9(8.65%)	5(4.80%)	14(13.46%)	104(100%)
20- 29	23(22.11%)	15(14.42%)	38(36.53%)	
30-39	25(24.03)	7(6.73%)	32(30.76%)	
40-49	8(7.69%)	2(1.92%)	10(9.61%)	
50- 59	6(5.76%)	1(0.96%)	7(9.61%)	
≥60	3(2.88%)	0(0%)	3(2.88%)	

As shown in **Table no. 1** maximum number of cases were In the age group of 20-29 yrsi.e 38 cases (36.53%) followed by age group 30-39 yrs number of cases were 32 (30.76%). Minimum agerecorded was 14 yrs and maximum was 100 yrs.

Table 2: Shows distribution of ligaturematerial

S. No.	Ligature material	No. of Cases	
1.	Chunni	36(34.61%)	
2.	Stole	21(20.19%)	
3.	Cotton Rope	17(16.34%)	
4.	Sari	10(9.61%)	
5.	Nylon Rope	9(8.65%)	104(100%)
6.	Bed Sheet	5(4.80%)	
7.	Niwar	2(1.92%)	
8.	wire	2(1.92%)	
9.	Belt	1(0.96%)	
10.	Turban	1(0.96%)	

As shown in **table no. 2**the most commonly used ligature material was chunni 36(34.61%) followed by Stole 21(20.19%) and Cotton Rope 17(16.34%) Least used material was belt and Turban 1(0.96%).

Table 3: Distribution of hanging and strangulation cases on the basis of encirclement of ligature mark

Encirclement		Hanging	strangulation	Total
Continuous	Transverse O shaped	0	4	10(9.61%)
	Oblique O shaped	6	0	
Discontinuous	Transverse U shaped	0	1	94(90.38%)
	Transverse Interrupted	0	1	
	Oblique	79	0	
	oblique V shaped	13	0	
Total		98	6	104 (100%)

Among 6 cases of strangulation, 4 cases were homicide in nature and 2 were accidental type. Horizontal **U** shaped ligature mark was seen over the neck in 1(0.96%) case and in 3 cases (2.88%) ligature was encircled the neck completely in **O** shaped manner. In accidental strangulation, ligature mark in one case was horizontal interrupted and for another case it was horizontal**O** shaped. In all 6 cases of ligature strangulation hematoma was found in the neck muscles and other structure. In 4(66.6%) out of 6 strangulation cases the

ligature mark was complete. Among 98(94.23%) cases of hanging oblique **V** shaped ligature mark was present in 13(12.5%) cases. Oblique **O** shaped ligature mark seen in 6(5.76%) cases, mostly seen in tight loop noose. Internal finding of neck in all cases of hanging was pale, white, glistening underlying soft tissue. In this study I noticed that ligature mark can seen over neck in different ways like **O** shaped**, U**shaped**, V** shaped as per ligature material run over neck. 10(9.6%) cases out of 104 cases had complete encircling of ligature mark around neck, rest of 94(90.3%) cases had interruption in continuity of ligature mark.

Table 4: pattern of ligature mark

Level			Length of ligature			Direction		Total Cases
ATC	OTC	BTC	≤NC	≈NC	≥NC	Oblique	Transverse	
94(90.3%)	7(6.73%)	3(2.88%)	93(89.42%)	7(6.73%)	4(3.84%)	98(94.23%)	6(5.76%)	104

In 94(90.3%) cases out of 104 ligature mark was found above the thyroid cartilage, in 7 cases (6.73%) ligature mark was present over the thyroid cartilage and in remaining 3 (2.88%) cases it was below the thyroid cartilage . In these reported cases there were 93(89.42%) cases in which length of ligature mark was less than neck circumference in 7(6.73%) cases length of ligature mark was equals to neck circumference and 4(3.84%) cases it was more than that. ligature mark was placed obliquely in 100(96.1%) cases out of 104 and in 4(3.84%) it was transverse. Knot impression was present on either side of neck in inverted "v" shape in 30 cases out of 104.

Table 5: colour and appearance of ligature mark

Total Cases	Not visible	Soft faint non parchmentized	Parchmentized reddish brown	Parchmentized Leathery dark brown
104	7(6.73%)	8(7.65%)	36(34.61%)	53(50.96%)

Ligature mark was parchmentized reddish brown in 36 (34.61%) cases out of 104 cases, in 53(50.96%) it was with parchmentized leathery dark brown changes of skin, skin of ligature mark in 8 (7.65%) cases were soft non parchmentized and in rest of 7(6.73%) cases skin changes not visible. As per history given by relatives there were 25 (24.03%) cases, in which suspension time was more than 6 hrs which was evident by position livor mortis, there were 68 cases (65.38%) in which suspension time was from few minutes to 6 hr, 11 (10.57%)cases were reported within few minutes of suspension. The pressure abrasion of ligature mark is found well grooved in 19 cases where in suspension period is more than 6 hrs. The longer the body is suspended the deeper and prominent will be the ligature groove[22].

The pressure abrasion was not visible in 15(14.42%) cases where in suspension time was within few minutes as well as the time elapsed after death was within 6 hrs. The time since death gradually increases, by then the consistency of pressure abrasion also increases more towards parchmentisation. In 36(34.61%) cases pressure abrasion was found fairly parchmentized and reddish brown up to the depth of epidermis in which time elapsed after death was nearly 12 hrs. in 53(50.69%) cases pressure abrasion parchmentized and leathery, in

most of cases dark brown up to the depth of dermis in which time elapsed after death was nearly 24 hrs.

Discussions & Conclusion

In our study 38 cases were observed in age group 20-29 years then 32 cases were observed in age group 30-39 years. These findings were similar to the findings of studies previously done by Kumar et al [17] 21 to 30 years of age, other previous studies by Yadav et al [18], Meera and singh[19], also reported the largest age group to be affected as 21 to 30 years.

In present study, chunni was the most common used ligature material (32.6%) which is very common cloth in wardrobe. Studies by Naik S K et al [12], Sharma B R [13], have shown variation with this but both concluded on that soft material being more commonly used than the hard one. Meera and Singh [19] in Manipur, Who were also reported that the most common ligature material to be used was sari or duptta, followed by wire/ rope. However, study done in Ranchi by Kumar et al [17]concluded that hard ligature material such as rope was most common.

In present study maximum no. of cases (90.3%) showed ligature mark above the thyroid cartilage, 7 (6.73%) had mark over thyroid cartilage level and 3(2.88%) had marks below thyroid cartilage level. This was comparable with the findings of the study done by ballur[20,] who observed that in 83% of cases. Another study did by Sudheer and Nagaraja[21], who reported 88% cases, have ligature marks above the thyroid cartilage. This coincides with a study done by Reddy KSN, which showed that in 80% cases ligature marks situated above thyroid cartilage level between chin and larynx, in 15 % cases at the level of thyroid cartilage and in 5% cases below the cartilage [9].

Among 6 cases of strangulation, in 3 cases (50%) ligature mark was situated below the thyroid cartilage, in rest 3 cases (50%) in situated over the thyroid cartilage level. Among 104 cases 98 cases(94.23%) cases were hanging, in which direction of ligature mark was oblique which was consistent with observation by Naik S K [12], in all 6 cases of ligature strangulation, the direction of the ligature mark was Transverse which was consistent with observations of Naik S K [12]. In this study the ligature mark was continuous in 10(9.61%) and non continuous 94(90.38%) study cases

Source of Funding: By self

Ethical Clearance: Taken from institutional ethics committee of J.L.N. Medical College Ajmer

Conflict of Interest: The recent episode of suicidal demise of sh. Shushant Singh Rajpoot, A celebrity prompts and inspires me to publish paper on pressure abrasions over neck in violent asphyxia deaths.

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Observational Study of Sociodemographic Profile on Female Autopsy Cases of J.L.N Medical College, Ajmer

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Abstract

Introduction:- Unnatural deaths of females in a particular geographic area is the reflection of its law and order situation. Lower incidences indicate presence of peace, harmony and security in the society. Present study is designed to determined unnatural deaths of females in Ajmer city. This study includes the various socio-etiological aspects of un-natural female death. The findings of our study is useful in planning of prevention polices.

Aims & objectives:- -To collect the statistical data of Autopsy cases of unnatural female death cases brought to J.L.N. Hospital, Ajmer mortuary

Objectives:- To study the various socio demographic data of autopsy cases of un-natural deaths of females.

Material and Method:- Population Universe:-

All Medico-legal Autopsies conducted in Department of Forensic Medicine and Toxicology at J.L.N. Medical College and Associated Group of Hospitals, Ajmer. These were total 953 medico-legal autopsies in number

Case Study:- As present study comprised 170 autopsy cases of unnatural female deaths

Study period:- 1st April 2019 to 31st March 2020

Method:- Performa for study was prepared on the basis of information obtained from Autopsy, Panchnama, questionnaire of relatives and all collected data were put into the master-chart, which was prepared and then feed into the computer in Excel worksheet and then analyzed.

Observation & Results:- More than half of victims were married (69.41%) Among the married women, maximum cases (61.86%) were of more than 7 years of marriage life. Highest number of cases (40%) was seen in Summer. Literate women comprise of about 66.47% of total cases. Highest number of cases (50.58%) was of accidental in nature. Suicidal deaths constituted more than half of deaths which occurred within in seven year of married life. Burns (30.58%) was most common cause of death. It was also same in road traffic accidental deaths (30.58%). Most common poison which causes 66.66% deaths is OPC while Second most common was ALP (13.88%).

Recommendations:-our target group for prevention of unnatural deaths should be married woman of first two year of married life of age segment of middle economic literate Hindu group

Key Words:- *woman, ruler, married, accidents, age*

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Introduction

Women have a special value in the Indian Society. Respected Dr. Baba Saheb Ambedkar had quoted as "I measure the progress of a community by the degree of

progress which women have achieved." Similarly our 1st prime Minister Jawahar Lal Nehru also stated as "You can tell the condition of a nation by looking at the status of its women." Therefore death of woman especially under un-natural condition should be thoroughly studied and investigated. Un natural death of female may be associated with several factors such as occupational problems, lack of emotional and financial support, inability to bear a child, sexual jealousy and marital infidelity, failure in love and scolding by patients of unmarried girls to disharmony among husband and wife or other family members extending up to dowry death[1]

More and more women in this part of the country are now coming out of the safe territory of their homes for education and employment and thus exposing themselves newer causes of death, like road traffic accidents and occupational deaths.

The cause of death profile is an important cornerstone of public health information. At provincial level it is needed for health planning and deciding on intervention strategies. Data on unnatural deaths in a particular geographic area can give the reflection of its law and order situation. Lower incidences reflect presence of peace, harmony and security to human life and property.

Ajmer is a district of Rajasthan in western India. Ajmer is having majority of population of middle class and lower middle class socioeconomic strata. Although Ajmer city has population of 542,321;(Census 2011)[2] out of which male and female are 278,545 and 263,776 respectively Hindu 453,013 (83.53 %), Muslims 62,825 11.58 % Jain 13,583 (2.50 %) Christian 6,813 1.26 %, Ajmer has a sex ratio of 947 females for every 1000 males and literacy rate is of 86.37%[3]

Present study is designed to determined unnatural deaths of females in Ajmer city. By this study, we can know the various socio-etiological aspects of un-natural causes of female death which will be useful for awareness of society and improve the situation.

Aims and Objectives:-

Aims:-

- To collect the statistical data of unnatural female death cases brought to J.L.N. Hospital, Ajmer mortuary

Objectives

Primary Objectives:-

1. To study the various demographic data (Age, Sex, Marital status, Rural / Urban, Religion Education status etc.) in un-natural deaths of females.

2. To understand the magnitude and pattern of different aspects of unnatural deaths of females.

Secondary Objectives:-

1. To analyze the various reason for unnatural deaths of females.

2. To study the different epidemiological and medico-legal aspects of un-natural deaths of females.

3. To suggest measures to prevent unnatural deaths in females.

Material and Method

Study Universe:-

All Medico-legal Autopsies conducted in Department of Forensic Medicine and Toxicology at J.L.N. Medical College and Associated Group of Hospitals, Ajmer. These were total 953 medico-legal autopsies in number

Case Study

Inclusion criteria:-

-All autopsy cases conducted on females with un natural deaths, those had given consent for study

Exclusion criteria:-

-Autopsy cases considered as obscure and negative autopsy

-Autopsy cases of female skeleton\ segmented body

As present study comprised 170 autopsy cases of unnatural female deaths, which were done in the mortuary of Department of Forensic Medicine and Toxicology at J.L.N. Medical College and Associated Group of Hospitals, Ajmer

Study period:- 1st April 2019 to 31st March 2020

Method

A detailed history from police and relatives regarding socio economical status, marital status, habits, illness (mental / other disease / deformity), previous attempted suicides, suicide note if any etc. were taken. Detailed history from police regarding scene of crime, position of body etc were taken. post mortem findings were analysed. The reports of the relevant samples and viscera, subjected to chemical analysis, histo-pathological examination and other investigations during autopsy were used to conclude the final cause of death. Performa for study was prepared and all collected data were put into the master-chart, which was prepared and then feed into the computer in Excel worksheet and then analysed.

Observation :-

The present study comprised 953 autopsy cases of unnatural death case out of them female deaths were 170 (17.83%) during the period from 1st April 2019 to 31st March 2020. In the present study, maximum cases (28.23%) were seen in age group of 21-30 years followed by 21.76% cases in age group of 31-40 years and 17.64% cases in age group of 11-20 years. Minimum cases (2.94%) were seen in >60 years of age. In this study high number of cases was seen in April, May, June 2019 & March 2020. Lowest number of cases is seen in September 2019 and February 2020. According to season wise distribution, there were maximum cases (40%) seen in summer, followed by winter and in monsoon (30%). There were more cases seen in ruler region (56.47%) than urban region (43.52%). Housewives (73) constituted the largest single occupation category amounting 42.94%. There were about 8.2% student, 11.76 % labourers while 11 victim (6.4%) of total had engaged with different type of employment. 5.29% were not applicable which included children < 5 years and unknown, while non-worker (old age) were comprised 8.82% c.

Single largest class was found to be those of literate women (primary Education) who comprised of about 66.47% of total cases. 29.41% were illiterate. 4.11% were not applicable due to below 5 yrs age.

Highest number of cases 48.82% were seen in medium socioeconomic group followed by 35.88% cases in low economic population i.e. BPL. Interestingly high economic group (Income Tax Payers) have only 15.29% of total unnatural deaths.

There was more number of cases seen in nuclear family (61.17%) than joint family (38.82%). Maximum number of cases 25.29% is observed in those who have 5 members in the family followed by 17.64% cases seen in those who have 4 or 6 family members. Lowest number of cases (2.34%) was seen in family of 2 (2.94%) members.

Hindus 149 cases (87.64%) comprised the single largest category followed by 18 cases of Muslims (10.58%). 3 cases (1.76%) were belonging to the Christianity religion. This is because of the India have higher population of Hindus.

Out of total number of cases, more than half of victims were married 69.41%, while 21.64% victims were unmarried. 5.88% victims were window, divorcee cases were 2.94%

Out of 118 married cases, maximum cases 61.86 % were seen above 7 years of married life while rest cases 38.13% seen within 7 years of married life.

Maximum cases 28.88% were seen within 1styear of married life. About (44.43%) cases were seen in within two years of married life. Highest number of cases 50.58% was of accidental in nature while 37.05% cases were of suicidal. Homicidal cases constituted only 2.94% cases.

Suicidal deaths 57.77% were occurred within seven years of married life. After those Accidental deaths 33.33% were common. Homicidal deaths were present only in 2.22% cases

In most of age group, accidental deaths (50.58%) were most common. Maximum accidental deaths 45.34% were present in age group of 11 to 30 years. Highest suicidal deaths 39.68% were seen in age group of 21-30 years followed by 25.39% in age group of 31-40 years. In age group of 21-30 years, both accidental and suicidal deaths were more. No suicides seen in the age group of 0-10 years.

In this study, highest number of suicidal deaths (39.68%) was seen in age group of 21-30 years followed by 25.39% in age group of 31-40 years.

Burns (30.58%) was most common cause of death in the total 170 cases. Out of burns, septicaemia due to burns and shock due to burns constitute 10.00% and 20.58% respectively. After that multiple injuries in road traffic accidents (30.58%), hanging (4.70%), poisoning (20.58%), were main cause of death. Drowning cases were seen in 2.94%. Strangulation and throttling were seen in 1.17% and 0.58% cases respectively.

In our study, the peak age of the female homicide victim was 2nd, 3rd and 4th decade. Maximum cases (34.61%) in which body surface area of burns was 80-89%, while in 13.46% in 50-59% & 70-79% surface area, Less than 30% burns found only in 3.84% cases.

Most common substance of poisoning is Organo phosphorus poison which caused 66.66% deaths. Aluminium phosphide was present in 13.88% poisoning deaths, Zinc phosphide (11.11%), 2.77% cases were of corrosive poisoning followed by snake bite poisoning & Chloroquine poisoning (2.77%) separately.

TABLE NO. 1: DISTRIBUTION OF CASES ACCORDING TO CAUSE OF DEATH IN DIFFERENT AGE GROUPS

Cause of death		Age groups (years)														Total	
		0-10		11-20		21-30		31-40		41-50		51-60		>60			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Burns	Septicemia	1	7.69	1	3.33	5	10.42	5	13.51	3	12.50	2	15.38	0	0	17	10.00
	Shock	2	15.38	5	16.67	12	25.00	8	21.62	4	16.67	3	23.08	1	20	35	20.59
Poisoning		5	38.46	8	26.67	13	27.08	5	13.51	1	4.17	3	23.08	0	0	35	20.59
Road Traffic Accident		4	30.77	12	40.00	13	27.08	12	32.43	7	29.17	2	15.38	1	20	51	30.00
Hanging		0	0.00	2	6.67	3	6.25	1	2.70	1	4.17	1	7.69	0	0	8	4.71
Drowning		1	7.69	2	6.67	1	2.08	0	0.00	0	0.00	1	7.69	0	0	5	2.94
Strangulation		0	0.00	0	0.00	1	2.08	1	2.70	0	0.00	0	0.00	0	0	2	1.18
Throttling		0	0.00	0	0.00	0	0.00	0	0.00	1	4.17	0	0.00	0	0	1	0.59
Natural		0	0.00	0	0.00	0	0.00	5	13.51	7	29.17	1	7.69	3	60	16	9.41
Total		13	100	30	100	48	100	37	100	24	100	13	100	5	100	170	100

TABLE NO. 2: DISTRIBUTION OF CASES ACCORDING TO MANNER OF DEATH IN DIFFERENT AGE GROUPS

Age group (years)	Accidental		Suicidal		Homicidal		Natural		Total	
	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%	No. of Cases	%
0-10	10	11.63	0	0.00	2	40.00	1	6.25	13	7.65
11-20	19	22.09	9	14.29	1	20.00	1	6.25	30	17.65
21-30	20	23.26	25	39.68	1	20.00	2	12.5	48	28.24
31-40	15	17.44	16	25.40	0	0.00	6	37.5	37	21.76
41-50	14	16.28	7	11.11	1	20.00	2	12.5	24	14.12
51-60	6	6.98	4	6.35	0	0.00	3	18.75	13	7.65
61-70	2	2.33	2	3.17	0	0.00	0	0	4	2.35
>70	0	0.00	0	0.00	0	0.00	1	6.25	1	0.59
Total	86	100.00	63	100.00	5	100.00	16	100	170	100.00

Summary and Conclusions

1. Maximum cases (28.23%) were seen in age group of 21-30 years while minimum cases (2.94%) were seen in age group of >60 years.

2. According to season wise distribution, there were maximum cases (40%) seen in Summer, followed by Winter and Monsoon (30%)

According to Statistics of NCBI 2018[4], majority of accidental deaths in India were occurred in summer (April to June), which was also correlated with those of, present study.

3. Highest number of cases seen in ruler region (56.47%) as compared to Urban region (43.52%).

4. Housewives constituted the largest single occupation category amounting nearly 42.94%.

5. Single largest class was found to be those of literate women (Primary Education) which comprise of about 66.47% of total cases while 29.41% are illiterate.

6. As per Socio-economic status, highest number of cases (48.82%) was seen in middle class.

7. More number of cases was seen in nuclear family (61.17%) as compared to joint family (38.82%). This might be attributable to changing socio-cultural and family patterns in metropolitan and sub-urban regions[6].

8. Hindus (87.64%) comprised the single largest category Hindus 149 cases (87.64%) comprised the single largest category followed by Muslims (10.58%).. This is because, India have higher population of Hindus[6].

9. More than half of victims were married (69.41%) while (21.64%) victims are unmarried.

10. Among the married women, maximum cases (61.86%) were seen above 7 years of marriage life while rest cases (38.13%) seen within 7 years of married life.

11. Maximum cases (28.88%) were seen in1st year of marriage life. About (68.86%) cases were present in within four year of marriage life among the considering total cases of within 7 years of married life.

12. Highest number of cases (50.58%) was of accidental in nature while 37.05% cases were of suicidal. Homicidal cases constituted only 2.94% cases.

13. Suicidal deaths (57.77%) constituted more than half of deaths which occurred within in seven year of married life. After that, Accidental deaths (33.33%) were common. Homicidal deaths were present only in 2.22% cases.

14. In most of age group, accidental deaths (50.58%) were most common. Maximum accidental deaths 45.34% were present in age group of 11 to 30 years. Highest suicidal deaths 39.68% were seen in age group of 21-30 years followed by 25.39% in age group of 31-40 years. In age group of 21-30 years, both accidental and suicidal deaths were more. No suicides seen in the age group of 0-10 years. Suicide cases were quiet less after 40 years, probably as the age advances, the girls become mature and handle the situation in much efficient manners in life[5].

According to statistics of NCBI (2018)[4], around 35.7% suicide victims were youths in the age group of 15-29 years and 34.8% were middle aged persons in the age group 30-44 years.

15 Burns (30.58%) was most common cause of death. It was also same in road traffic accidental deaths (30.%) poisoning (20.59%) and natural (9.41%). Most common poison which causes 66.66% deaths is OPC while Second most common was ALP (13.88%). According to statistics of NCBI (2018)[4], the accidental deaths due to un-natural Cause were mainly on account of Road Accidents (37.1%), Railway Accidental and Rail-Road accidents (8.3%), Drowning (8.5%), Poisoning (7.6%), Burns (6.6%), Electrocution (2.4%) and others (13.2%) in India.

Recommendation

a) Social measures

1. There is a need to educate females particularly from poor families, so That they can become independent.

2. Females should be encouraged to marry at an appropriate age.

b) Counseling

Centers shall be developed to provide free counseling to the families and newly wedded couple about their expected problems and their solutions in initial years of their interaction and formative years of new social and familial relationship so that story of their rest of the life in their new role is nicely scripted.

(c) Strong legislation and its co-ordinate implementation

1. Registration of marriages shall be made popular and compulsory with proper disclosure of list of items gifted to newlywed from either side or declaring women as having all rights over these.

2. Anti-dowry cell and women protection cell concepts shall be brought up more. Besides police personals, these cells shall also includes of social workers, psychiatrist, counsellors and other trained staff in effective dealing with the crisis and also preventing and controlling such crimes.

3. An effective coordination should be sought between the non-government voluntary and law enforcing agencies to prevent and contour crime against women.

(d) Preventive measures

1. Risk factors of suicides among women should be identified and should be minimized accordingly.

2. A change in attitude and mindset of society to make home/workplace a safer and happier place for a woman.

3. Traffic awareness regarding safety measures amongst women should be promoted.

Source of funding:- expenses beard by own

Ethical Approval:- taken appropriately

Point of conflict:- No help is taken from related beneficiaries.

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Study of Incidence and Pattern of Snake Bite Cases Brought in Tertiary Care Hospital, Rims, Ranchi, Jharkhand

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Abstract

The hospital-based cross-sectional was done to check the toxic nature of snake bite. The study was carried in 375 patients, patients were divided in the group of 46 male belong to the age group of 20 - 49 (67.39%), female (48.27%) and farmers (60%), in RIMS Ranchi from 1st Jan to 31st Dec 2019. Among farmers 63.04% was male and 55.17% was females. The most of the females shoed to be housewife among famers. The result showed maximum victims among farmers, and it was shown to be illiterate. The Maximum victims such as 81.3% were belongs to lower middle and upper lower class while 93.3% were belongs to rural region. There is a statistically significant data of occurrence in rural and urban area, the statistical analysis was done based on 5 % confidence level (p=0.05) (student t –test analysis). The result showed 60% were bitten in fields followed by 36.1% at home. Among all the groups, 37.3% bite was 12.01 pm to 6PM and 32% during 12.01 AM to 6AM. Of the 225 cases who had bites in fields, 125 bitten between 12.01 pm to 6 pm, and the number showed, 135 cases at home, 110 cases bitten during 12.01 am to 6 am. The study showed 60.1% victims had been bitten by snake in the month of June to September (monsoon season). The conclusion showed adult males were more prone to snake bites since they were more commonly involved in agricultural/outdoor activities. The specific reason not known, but it can be concluded based on sociodemographic profile of the literacy population

Keywords: *Snake bite, literacy, male, female, farmers, snake toxicity*

Introduction

Snake bite is a major public health problem throughout the world especially in tropical and subtropical countries like India, particularly in the rural areas. In Greek mythology, Snake represent Goddess Hygeia was worshipped along with Asclepius, the God of Health. The tradition is carried over to modern time and the snake has found a place in the universal symbol of medical profession. [1]

In general, about 70% of bites are due to snakes which are non-poisonous, of the rest, 15% are dry bites and only 15% cause envenomation. Venom is the saliva, proteolytic enzymes, some organ or system specific poisons i.e. neurotoxic, Vasculotoxic, or Myotoxic, ejected during the bite, from the modified parotid glands. Most of the time, mixed manifestations will be seen. Neurotoxicity is a key feature of some envenoming, and there are many unanswered questions regarding its manifestations. Venomous snakes found all over the world sparing few countries, and therefore, cases of snake bite were reported from all over the world[1]. Total number of snake bites in world is around 500,000 per year with approximately forty thousand to one lac deaths as reported by various authors. [1-3]

The incidence being relatively higher in tropical countries as compared to developed countries. [4] Approximately 330 species of snakes exist in India, of which about 70 species are poisonous. The commonest poisonous snakes are common krait, spectacled cobra,

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saw scaled viper and Russell's viper. [2] Pit viper (Hyphenate - hyphenate) is equally deadly, though not recognized by many and that could be one of the causes of increased mortality by pit viper envenomation. [5] In India, there are estimated 10,000 to 50,000 deaths per year due to poisonous snakebite. [3]

In this study, population is predominantly native tribal (rural as well as urban) in Jharkhand state, peculiar Clinical, Demographic and Social profile significantly varies from other regions of our country, that may lead to variation in many statistical data. Special attention was given to specific and significant facts in reference to our study population. Snake bite is a neglected tropical disease of global importance. [6] Data from the million deaths study in India estimates that snake bite deaths are more than 30-fold higher than recorded in official hospital returns. [7] As per various sources, there was no such study conducted in this area to know the profile of snake bites. Therefore, this study was designed to find the incidence and pattern of snake bites admitted to various departments of RIMS, believing that the findings of this study will help in control of snake bites.

Material and methods

Study place: Different indoor/outdoor/emergency departments of RIMS, Ranchi, Jharkhand

Study duration: one year from 1st January 2018 to 31st December 2019

Study design: Hospital-based cross-sectional and observational study conducted for duration of one year from 1st Jan 2018 to 31st Dec 2019. The study comprised all of 375 cases of snake bite. The data collected from following records and format.

1) In-patient case records of snake bite victims from central/paediatric emergency.

2) Performa which was designed to collect information regarding the incidence.

3) Police inquest and post mortem reports in cases where death occurred.

Inclusion criteria:

1) Snake bite of all age and sex groups.

2) History of unknown bite responding to ASV.

3) No history of snake bite but presenting with symptoms of snake bite and responding to ASV treatment.

4) Cases admitted to emergency departments and brought dead cases of snake bite directly/indirectly from different departments to Forensic Medicine and Toxicology, RIMS Ranchi.

Exclusion criteria: Unknown bites not responding to ASV were excluded from the study.

Method of collection of data: A Performa was designed to collect relevant information regarding the incident, the snake (if seen), first aid taken and socio demographic profile from the patient and/or relatives.

Statistical analysis: All the data thus collected was tabulated and statistical analysis was done using Microsoft Excel and Graph Pad Instat software.

Results

Table 1: Distribution of victims based on Age, Sex, Occupation and Education

Age and Sex distribution						
Age in years	Male		Female		Total	
	n	%	n	%	n	%
0-9	5	2.17	5	3.45	10	2.7
10-19	35	15.22	35	24.14	70	18.7
20-29	65	28.26	25	17.24	90	24

Cont.... **Table 1: Distribution of victims based on Age, Sex, Occupation and Education**

30-39	55	23.92	25	17.24	80	21.3
40-49	35	15.22	20	13.79	55	14.7
50-59	15	6.52	10	6.90	25	6.7
60-69	15	6.52	25	17.24	40	10.7
> 70	5	2.17	0	0.00	5	1.3
Total	230	100	145	100	375	100
	X2=7.62, df=2, p=0.02 (significant)					
Distribution of victims on the basis of Occupation and Sex						
Farmer/Farm wage workers	145	63.04	80	55.17	225	60
House wife	0	0.00	45	31.03	45	12
Snake charmer	5	2.17	0	0.00	5	1.3
Student	40	17.39	20	13.79	60	16
Others*	40	17.39	0	0.00	40	10.7
Total	230	100	145	100	375	100
*others-2 drivers, 1 child, 1 postman, 1 clerk, 1 Sheppard, 1 mess staff and 1 shop employee						
Distribution of victims on the basis of Education and Sex						
Illiterate	100	43.48	75	51.72	175	46.7
Primary	65	28.26	30	20.69	95	25.3
Secondary	45	19.57	30	20.69	75	20
PUC	15	6.52	10	6.90	25	6.7
Not applicable*	5	2.17	0	0.00	5	1.3
Total	230	100	145	100	375	100
* Not applicable- 1 male child of 3yrs was not eligible for schooling. Among the study group						

Table 2: Distribution of cases as per Socio-economic status

Socio-economic status	n	%
Class I -Upper class	10	2.7
Class II- Upper middle class	20	5.3
Class III- Lower middle class	110	29.3
Class IV- Upper lower class	115	52.0
Class V- Lower class	40	10.7
Total	375	100

Table 3: Region wise distribution of cases

Region	Male		Female		Total	
	n	%	n	%	n	%
Rural	225	97.83	100	86.21	350	93.3
Urban	5	2.17	20	13.79	255	6.7
Total	230	100	145	100	375	100

P= 3.86 at df= 1 p=0.04 (significant)

Table 4: Distribution based on time and place of occurrence of the incident

Place of occurrence	Time				Male		Female		Total	
	6.01am-12 noon	12.01pm-6 pm	6.01pm- 12 mid night	12.01am-6am	n	%	n	%	n	%
Home	0	5	20	110	75	32.61	60	41.38	135	36.1
Field	50	125	40	10	150	65.21	75	51.72	225	60.0
Animal shelter	5	0	0	0	0	0	5	3.45	5	1.3
Garbage pit	0	5	0	0	0	0	5	3.45	5	1.3
Road	0	5	0	0	5	2.17	0	0	5	1.3
Total	55	140	60	120	230	100	145	100	375	100

Table 5: Distribution of cases based on month of incident

Month	Male		Female		Total	
	n	%	n	%	n	%
Jan	10	4.35	0	0	10	2.7
Feb	0	0.00	5	3.45	5	1.3
March	0	0.00	5	3.45	5	1.3
April	20	8.70	5	3.45	25	6.7
May	15	6.52	10	6.90	25	6.7
June	30	13.04	10	6.90	40	10.7
July	30	13.04	40	27.59	70	18.7
August	25	10.87	30	20.69	55	14.7
September	40	17.39	20	13.79	60	16
October	35	15.22	5	3.45	40	10.7
November	5	2.17	10	6.90	15	4
December	20	8.70	10	3.45	25	6.7
Total	230	100	145	100	375	100

Discussion

Age and Sex

Maximum victims (60%) belonged to 20-49 years age group. Among 230 males and 145 females, 67.39% and 48.27% belonged to 20-49 yrs age group respectively. In both the sex groups, a statistically significant difference (p value 0.02) was found among 20-49 years age group. Of both the sex groups, males were predominant 61.3% as compared to females 38.7% (sex ratio1.6:1).

Similar observations were made in studies conducted by Kulkarni ML & Anees S (1994) [8] in Karnataka, Ganneru B & Sasidhar RB (2007) [9] in Andra Pradesh and Suchitra N et al. (2008) [10] in Kerala. This observation is not consistent with the findings recorded in the study conducted by Monterio NP et al. (2010) [11] in Manipal, where female predominance was recorded with male to female ratio of1:1.5.

The probable reason for predominance in males and 20-49 yrs age group is increased agricultural activity among these individuals. If considered demographically, Jharkhand region, result we expected would be like Monterio NP et al. (2010) [11], but results were not like that. We thought that here in Jharkhand the more working population was of female, but this effect was counterbalanced by much more outdoor as well as sports activities of male population here, which makes them vulnerable population for snake bite. Probably that's the reason of our study results with this respect.

Occupation

Maximum (60%) victims were farmers. Among the male's majority 63.04% were farmers. Similarly, among female victims 55.17% (maximum) were farmers followed by housewives 31.03%. The above observations made in the study are consistent with the studies conducted by Lal P et al. (2001) [12] in JIPMER Hospital Pondicherry, Chauhan S et al. (2005) [13] in

PGIMER Hospital Chandigarh and Shetty AK & Jirli SP (2010) [14] in Belgaum. The predominance of farmers can be attributed to increased frequency of human confrontation with snakes in agricultural fields owing to the snake habits, habitat and prey preferences.

Education and Socio-economic status

46.7% were illiterates and the rest 53.3% were literates having different levels of education. Majority 25.3% had primary education followed by 20% had secondary education and 6.7% had PUC level education. None of the victims were graduates. The above observation depicts that illiterate victims and victims with non-technical (unskilled) education are more involved in agricultural works, hence more exposed to snake bites. The low literacy level also leads to lack of knowledge regarding precautions to be taken to avoid snakebites.

Socio-economic status classification based on Modified B G Prasad classification for rural population and Kuppuswamy classification for urban population in the study group. Maximum victims 81.3% belonged to lower middle and upper lower class.

Region

Maximum victims 93.3% belonged to rural region. A statistically significant (p value 0.004) association was noted between snake bite occurrence and rural region. This finding is consistent with the observations in the studies made by Kulkarni ML & Anees S (1994) [8] in Karnataka, Ganneru B & Sasidhar RB (2007) [9] in Andra Pradesh, Shetty AK & Jirli SP (2010) [14] in Belgaum and Mohapatra et al. (2011) [4] in India.

Place of occurrence

Maximum 60% had snake bites in the fields followed by 36.1% snake bites at home. Similar finding was noted in the study conducted by Suchithra N et al. (2008) [10] in Kerela, Monterio NP et al. (2010) [11] in Manipal and Shetty AK & Jirli SP (2010) [14] in Belgaum.

Time of occurrence

Maximum 37.3% of snake bites occurred between 12.01PM to 6 PM followed by 32% between 12.01 AM to 6 AM. Similar finding was observed in the study conducted by Bawaskar HS et al. (2008) [15] in rural Maharashtra and Monterio NP et al (2010) [11] in Manipal.

Relationship between Place of occurrence and Time of occurrence of the incident

Of the 225 bites that occurred in the field, maximum 55.5% occurred during 12.01PM to 6PM. Of the 135 bites that occurred at home, maximum 81.5% bites occurred during 12.01 AM to 6 AM.

Relationship between Time of occurrence and Probable type of snake

Of the 375 victims, only 110 victims saw the snake that bit them. Of these 110 cases, 50% (55) of the snakes were identified as Saw Scalded Viper (SHERCHANDWA), of which 54.6% (30) occurred during 12.01AM to 6AM. Of the 25 cases of Russell's viper, all bites occurred during 12.01PM to 6PM.

Here, identification of the probable type of snake that bite the victims was made by us based on the description of the snake given by the victims / relatives. In our study no killed snake was brought along with the victim for identification.

Seasonal variations in the incidence of snake bite

60.1% (maximum) occurred during June to September (monsoon season) followed by 24.1% during October to January (winter season). Similar observations were made in the study conducted by Lal P et al. (2001) [12] in JIPMER Hospital Pondicherry, Bawaskar HS (2002) [16] in Mahad region of Maharashtra, Bawaskar HS et al. (2008) [15] in rural Maharashtra, Shetty AK & Jirli SP (2010) [14] in Belgaum and Mohapatra B et al (2011) [4] in India. Whitekar R (2005) [6] in his study conducted in Kerala, observed that the month of May had highest incidence of snake bite. However, in our study, the highest incidence of snake bite was in the month of July 18.7%.

Limitation of the study

Best efforts were put in to record all the cases of snake bite which were admitted to the Rajendra Institute of Medical Sciences Ranchi. However, few cases who consulted doctors practicing other medicinal systems (Homeopathy, Ayurveda) were not recorded. Our study was done with all the necessary efforts as well as

precautions.

Conclusions

Snake bite is a common medical emergency encountered all over the country. This study includes snake bite cases admitted/brought dead to RIMS, Ranchi. Rural adult males were more prone to snake bites as they are commonly involved in agricultural/ outdoor activities. Incidence and mortality due to snake bite can be prevented by educating the rural people about the snake species prevalent in the respective regions, their habits and public health education regarding the recommended first aid.

Source of Funding: Self

Conflict of Interest: None

Ethical Clearance: Taken

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Profile of Organophosphorus Poisoning Cases in a Tertiary Care Hospital in Central India

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Abstract

Introduction: Organophosphorus poisoning is an important medical emergency and its pattern and outcome varies from place to place. Given the widespread availability and use of these chemicals, OP poisoning is quite common following either accidental or intentional exposures.

Aims and Objective: This study was conducted to evaluate the spectrum of acute poisoning in a tertiary care hospital.

Materials and Methods: This is a retrospective study of organophosphorus poisoning cases brought to emergency department/casualty of a tertiary care hospital from Jan 2011 to Dec 2014. **Result:** Total 805 cases of acute poisoning were recorded over aperiod of 4 years. Acute poisoning was common in men, and age group of 20–40 years was the most affected. The most common offending population was poor class (V) and financial burden was the most common reason noted.

Conclusion: From these results we found that Organo Phosphorous compounds are commonly ingested with suicidal intent due to its easy availability by young population in rural areas, more commonly males with economical burdens.

Key words: *Organophosphorous compounds, acetylcholinesterase, pesticides. Acute Poisoning*

Introduction

This difference in the type of poisoning seen within the country may be due to the difference in the pattern in the use and availability of poisons. Ours is an agriculture-based society and these compounds are easily available and thus, it is the most common class implicated in cases of poisoning.

Due to the low cost and easy availability, they have also become an agent of choice for suicide and self-poisoning. Frequently used OP include malathion, parathion, chlorpyrifos, diazinon, dichlorvos, fenitrothion, tetrachlorvinphos, and azinphos-methyl.[1,2,3]

OP compounds inhibit acetylcholinesterase, and this result in the accumulation of acetylcholine with theoverstimulation of cholinergic synapses. Clinical signs and symptoms depend on the nature of the OP compound, the amount consumed, time lapse between exposure and admission to the hospital, and the severity.[2,3]

Organophosphorus (OP) pesticide self-poisoning is estimated to kill around 200 000 people each year, largely in the Asia-Pacific region. This predominantly occurs in rural communities and is often an impulsive act comparable to self-poisoning with medication in the west; the critical difference being the 10–20% case fatality rate (compared to 0.3% in Britain for example).[4]

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The International Program on Chemical Safety (IPCS)/EC/EAPCCT Poison Severity Score (IPCS PSS) was developed by the International Program on Chemical Safety, the European Community and the European Association of Poisons Centres and Clinical Toxicologists to create a scoring system that produces a qualitative evaluation of the morbidity caused by different forms of poisoning.[5]

Material and Methods

This study was conducted after due permission from institutional ethical committee. All cases admitted to the emergency department/ casualty of the MGIMS hospital over a period of 4 years (Jan 2011 to Dec 2014) was evaluated retrospectively. Data were obtained from the hospital information system. All patients with acute poisoning were included in the study. The cases of organophosphorus poising (OPP) were included and snake bite, food poisoning, and allergic reactions to drugs were excluded in this study.

All poisoned patients were seen on admission by study doctors. Patients were included in this study if they had a history of OP pesticide ingestion as indicated by the patient or relatives, the transferring doctor or the pesticide bottle.

Demographic and routine laboratory results were recorded for the duration of the patients' stay in the intensive care unit. All patients received standard medical treatment under the direction of the hospitals' consultant physicians. Data were documented, analysed as percentage, and final conclusion was drawn.The study involved analysis of de-identified patient data.

Results

A total of 3692 patients visited to the emergency department/ casualty of the hospital over a period of 4 years. A total of 805 patients were of OPP poisoning. Most cases of acute poisoning presented in the age group of 20-40 years (45.59%) followed by 40-60 years (38.13%), and the majority of patients were of rural background. Men dominated the study with M: F ratio of 1.4:1. Majority of the poisoning cases presented between 20 -40 years of age group.

The mean age in our study was 32.47± 12.95 years.

Majority of the patients consumed poison with suicidal intent (91.80%) as compared with accidental (6.8%) and homicidal (1.3%) cases. With regard to the route of exposure, ingestion was the most common mode of intake compared to inhalational poisoning.Table:1, 2

Table 3 showing the distribution related to socio economic status having maximum number of cases from lower middle and poor social economic status classified according to Prasad et al[6]

Table 1: Distribution of patients by their age, sex and mode of poisoning

	Male				Female		Total
Age(years)	**Suicidal**	**Accidental**	**Homicidal**	**Suicidal**	**Accidental**	**Homicidal**	
< 20	55	4	1	34	2	0	96
20-29	73	19	3	89	7	3	194
30-39	53	7	0	113	0	0	173
40-49	96	4	4	36	4	0	144
50-59	137	5	0	18	3	0	163
>60	23	0	0	12		0	35
Total	437	39	8	302	16	3	805

Table 2: Distribution of patients by their sex and cause of poisoning due to suicide and homicide

	Marital	Financial	Failure	Family	Discord with parents	Separation/death of boyfriend or girlfriend	Loss of	Total
Male	53	312	39	27	17	13	23	484
Female	63	147	21	58	8	17	7	322
Total	116	459	60	85	25	30	30	805

Table 3: Poisoning type by socio-economic (se) class based on modified Prasad classification[6]

Social class	No. of cases
Upper high (class I)	4
High (class II)	2
Upper middle (Class III)	3
Lower middle(ClassIV)	186
Poor(Class V)	531
Below poverty line(ClassVI)	79
Total	805

Discussion

Our study completed over period of four years involved 805 patients. Majority of the patients were young with a mean age of 32.47 years.

Study the Maharani B found that commonest poisoning agent was organ phosphorus compounds and Most of the cases were in the age group of 21-30 years finding is similar to present study.[7]The study conducted by Jeyaratnam found around 2 million people hospitalized for suicide attempts mostly includes serious patients.[8]

Acute poisoning was commonly seen among male laborers, farmers, house wives and students. More cases were reported during summer season and day time. The finding of present study coincides with the study conducted over the population of North Karnataka by padmanabha,[9] similarly findings coincided with study conducted by Mishra[10] and Pal et al [11] on population of Bengal.

The study conducted by Hubdekari[12] on study the population Karnataka found male predominance which is similar to the present study. Similarly study conducted by Aron R et al found predominance of young men comparable with women. Majority of the patients were from rural areas, which can be explained by the fact that in the rural areas the compounds are easily available due to their utility in farming practices.[13]

The findings are similar to the study done by Das et al the commonest route of exposure was oral and Majority of cases were in the age group of 11-30 years and maximum cases were found between 21-30 years.[14]

Conclusion

We found that Organo Phosphorous compounds are commonly ingested withsuicidal intent due to its easy availability by young population in rural areas, more commonly males with economical burdens. A large number require intensive care in tertiary centers but initial management and resuscitation in periphery maybe invaluable. The incidence of poisoning and its morbidity and mortality can be reduced by developing and implementation of effective prevention strategies.

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Conflict of Interest: Author declares that there is no conflict of interest

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Effect of Embalming Fluid on the Histological Appearance of Organs - A Cadaveric Study

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Abstract

Objective: Histopathological investigations with tissues from embalmed cadavers are generally not considered ideal for determining the cause of death at autopsy in forensic medicine. Instead fresh tissue fixed in 10% formalin is usually preferred. The aim of the present study was to compare the histological appearances of samples processed from routine formalin-embalmed cadaver with samples fixed in 10% formalin from a fresh cadaver.

Methods: The study was carried out on cadavers received as donation to the department of Anatomy from body donors. Tissue samples of four organs comprising kidney, lung, liver and spleen were obtained from a fresh cadaver (used as control) as well as from embalmed cadavers. The quality of the slides was graded 1 to 3 based on organ microscopic architecture, cell morphology and state of epithelium, as follows: 1 - high degree of cell distortion, 2 -moderately good sections and 3 - near normal.

Results: Kidney: Slight architectural distortion, with collapsed convoluted tubules were observed in embalmed tissue sections (grade 2). Lung: The architecture of the embalmed tissue showed alveolar spaces that were cystically dilated, but pneumocytes appeared normal (grade 2). Liver and spleen: The cell morphology and The cell morphology and architecture appeared almost as preserved as in fresh tissue. (grade 3).

Conclusion: Of the four organs studied, liver and spleen were well preserved and close to normal, whereas cytoarchitecture of lung and kidney specimens showed slight distortion. However this is a preliminary study and efforts are on to study more number of specimens with varying concentrations of formalin.

Key words: *histopathology, embalmed body, cytoarchitecture, cellular morphology.*

Introduction

Tissues from an embalmed cadaver are generally not preferred for histopathological investigations in forensic medicine, especially for determining the cause of death at autopsy. However, if only an embalmed cadaver is available then it is better to have some tissue than no tissue at all. The microscopic findings of various organs in the deceased can contribute in a major way to the forensic investigation which will enable the forensic pathologist to integrate the subjective (history) with the objective (lab investigation and post-mortem results) for reconstruction of circumstances and cause of death on legislative basis[1]. There is dearth of published literature regarding the use of tissues from a formalin embalmed cadaver for forensic histopathology, hence the need for this study, which was undertaken to compare the

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histological appearance of samples processed from four formalin-embalmed cadavers with histological tissue samples obtained from an un-embalmed cadaver and processed as per the standard procedure. Moreover, the study is also intended to explore the possibility of utilizing tissues from formalin-embalmed cadavers for teaching purposes, for which tissue samples are routinely taken from guinea pigs, rabbits, etc

Materials and Methods

Tissue samples were obtained from cadavers received as donation to the department of Anatomy from the period June 2018 to August 2019. The cadavers were embalmed as per the standard embalming protocol using 10% formalin. Four cadavers without any pathological findings affecting the tissues and age group between 44-89 were selected for the study. Tissue samples were obtained from four organs, i.e., kidney, spleen, liver and lung. The organs were dissected and exposed as per instructions in Cunningham's Dissection Manual[2] and the tissues were taken separately from all the four cadavers and collected in labelled containers. Fresh tissue samples from all the four above mentioned organs from an un-embalmed cadaver were used as control. These samples were fixed in 10% formalin after which they were processed as per the standard procedure and stained with haematoxylin and eosin. The sixteen tissue samples from the four embalmed cadavers, as well as the control samples from the un-embalmed cadaver, were processed directly without any additional fixation as per standard histological technique, and sections of 4 micron thickness each were obtained, which were stained using routine haematoxylin and eosin staining procedure.

The slides were examined with a light microscope and photomicrographs were taken using Carl Zeiss multiheader microscope fitted with a digital camera. The quality of stained sections was assessed as per the criteria proposed by Nlebedum et al[3].

Accordingly, the sections were assessed for

a. General organ microscopic architecture,

b. Cell morphology and state of epithelium graded on a scale of 1 to 3

Grade – 1 Refers to a high degree of distortion

Grade – 2 Moderately good sections

Grade – 3 Sections very close to normal

Observations

The sections obtained from four organs belonging to formalin-embalmed cadavers were observed and graded as per the criteria proposed by Nlebedum et al[3]. The results are depicted in Table 1. Photomicrographs of fresh samples of spleen, lung, liver, kidney are represented in Fig-1A, Fig-2A, Fig-3A, Fig-4A respectively.

With regard to spleen, cell morphology and architecture appeared almost preserved as in fresh tissue. White and red pulp, as well as the covering capsule and vascular endothelium appeared normal. All four samples were graded 3. (Fig-1B)

All four lung tissue samples presented distorted architecture, visualised as irregular distorted alveolar spaces. However at higher magnification, cell morphology appeared normal. Hence all specimens were graded 2. (Fig-2B)

Liver tissues were well preserved in three specimens. General organ microscopic architecture was mildly distorted, but cell morphology of hepatocytes and Kupffer cells was well preserved and normal. Portal triads, central veins, capsule, and sinusoids were normal. All three samples were graded 3 (Fig-3B). In one liver sample, lobular architecture, central veins and portal triads were normal, but hepatocytes and Kupffer cells were poorly preserved.

One specimen of kidney had high degree of distortion with poor cellular details and collapsed convoluted tubules and was graded 1(Fig-4B1). Three specimens had only slight architectural distortion with collapsed convoluted tubules of grade 2.The epithelial lining appeared moderately good (Fig-4B2). The capsule appeared to be well preserved (Fig-4B3).

Table 1: Histological grading of cadaveric tissues

Grade				
Organs	**Cadaver 1**	**Cadaver 2**	**Cadaver 3**	**Cadaver 4**
Kidney	2	2	1	2
Lung	2	2	2	2
Liver	3	1	3	3
Spleen	3	3	3	3

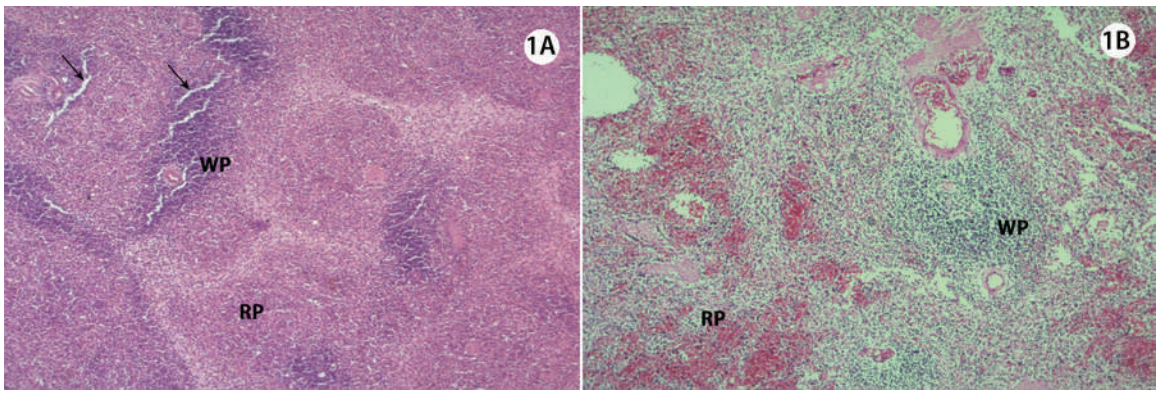


Figure 1A: Photomicrograph of spleen - Fresh sample - 100x. WP –white pulp, RP – red pulp, arrow indicates open spaces in the splenic parenchyma,1B: Photomicrograph of spleen - 100x.Sample from embalmed cadaver grade 3. WP – white pulp, RP – red pulp.

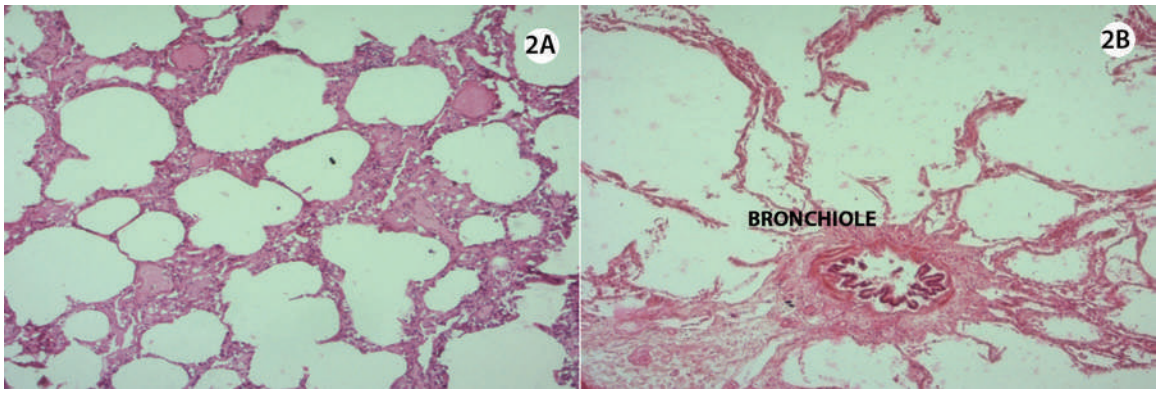


Figure 2A: Photomicrograph of lung - Fresh sample - 100x, 2B: Photomicrograph of lung -100x. Sample from embalmed cadaver grade 2.

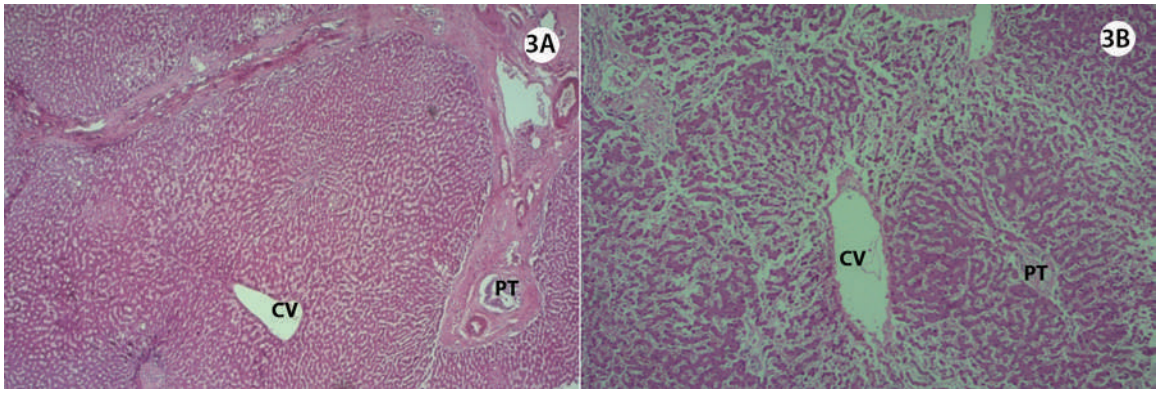


Figure 3A: Photomicrograph of liver - Fresh sample - 100x. CV - central vein, PT – portal triad, 3B: Photomicrograph of liver - 100x. Sample from embalmed cadaver grade 3.CV - central vein, PT – portal triad.

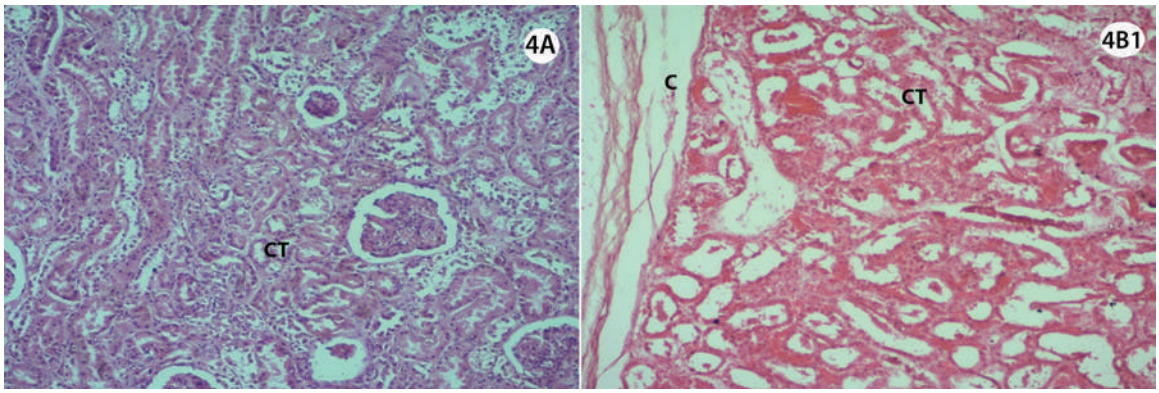


Figure 4A: Photomicrograph of kidney - Fresh sample - 100x.CT- convoluted tubules, 4B1: Photomicrograph of kidney - 100x. Sample from embalmed cadaver grade 1.C- capsule, CT- convoluted tubules.

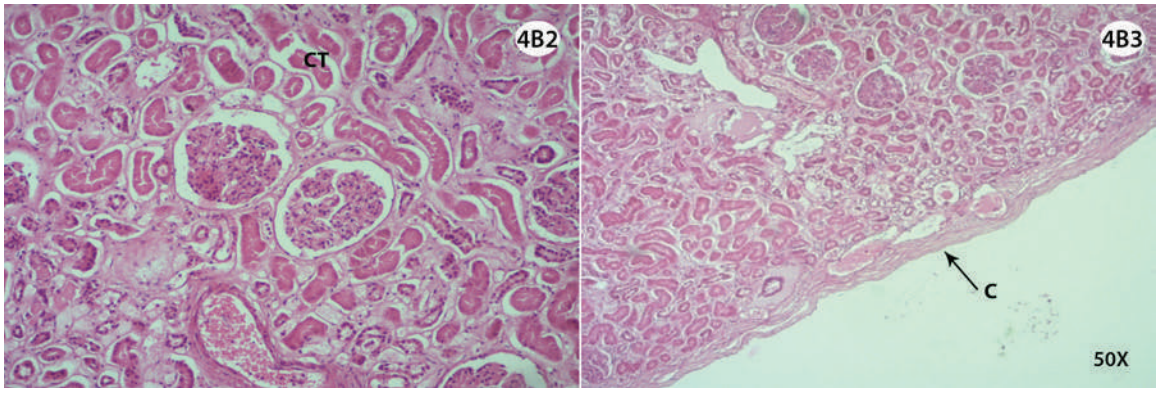


Figure 4B2: Photomicrograph of kidney - 100x. Sample from embalmed cadaver grade 2 .CT- convoluted tubules, 4B3: Photomicrograph of kidney - 50x. - Sample from embalmed cadaver grade 2. C- capsule.

Discussion

Archana Kalyankar et al[4] studied the histological samples of skeletal muscle tissue obtained from twelve embalmed human cadavers and compared them with skeletal muscle tissue obtained from guinea pig samples and processed as per standard histological technique, and their results indicated the feasibility of muscle tissue from human cadaver for teaching and research activity. They also proved that embalmed human skeletal muscle

tissue sections are better in staining and appearance than that of guinea pig slides.

Gupta and Gauba[5] conducted a study to standardize the histology of different tissue types obtained from embalmed cadavers available in the department of Anatomy. They reported that all the tissues studied including skin, muscle, cartilage, artery, spleen, brain, trachea, liver and small intestine showed excellent tissue organisation with nuclei, cell membranes and cytoplasmic details, being clear and well preserved. They concluded that human cadaver could be an ideal source of tissue for teaching and research.

In our present study, out of the four organs evaluated, kidney and lung tissues presented maximum architectural distortion. With regard to the kidney specimen, our study is in agreement with Nlebedum et al[3] with reference to collapsed convoluted tubules, but the capsule was not found detached in our specimens, and cellular morphology appeared moderately well preserved. Nicholson et al[6] compared the histological samples of heart, liver, kidney, skeletal muscle and skin obtained by embalming with four different fluids, namely formalin, traditional Dunedin mix, Michigan mix and phenoxyethanol mix. The results of the study showed satisfactory or good quality tissue, except the one embalmed with Dunedin mix.

Adequate preservation of kidney sample was also observed and mentioned as comparable to liver tissue which showed good preservation of morphology in their study.

Lung tissues presented with distorted irregular alveolar spaces, but cellular morphology was normal in all the four samples studied, and the same was observed by Nlebedum et al[3]. But unlike in his study, spleen was well preserved in all the four tissue specimens of our series, and all were graded with a score of 3. They had observed open tissue spaces in the organ parenchyma, but these could be artefacts because in certain sections of splenic tissues from fresh cadaver we did observe open spaces in the organ parenchyma. Three out of four tissue specimens of liver, presented with only mild architectural distortion, but the capsule, sinusoids, portal triads were all well preserved. Cellular morphology also appeared normal.

Nicholson et al[6] in their study mentioned good preservation of morphology in the sections of liver, which support the above. Regarding the liver samples, similar observations were reported by Nlebedum et al[3]; Gupta and Gauba[5]. Architectural distortion could be due to the lack of ability of the embalming fluid to properly fix the cells of the connective tissue stroma.

Better preservation of liver and spleen could be due to increased vascularity.

Gupta and Gauba[5] had explored the possibility of utilizing cadaveric tissue for histological studies and reported that cadaveric tissue can be considered ideal for teaching and research.

According to a study by Kalanjati et al[7], low concentration of formalin (5- 7.5%) was more suitable for processing cadavers to be used for anatomy teaching and learning than cadavers embalmed with higher concentration of formalin. Histological samples with low formalin technique should be studied so that adverse effects of formalin can be minimised. However the use of embalmed cadaveric tissue in forensic histopathology is dependent on whether the diagnosis is heavily based on cellular morphology or architectural distortion. The present study proves that the former is reliable.

Conclusion

Despite embalming, cellular morphology appears well preserved in the organs studied, and hence can be considered fairly reliable for forensic investigations. Architectural distortion is observed in tissue samples taken from cadavers embalmed using 10% formalin, and hence cannot be relied upon for forensic purposes. However, more studies are required to be undertaken with different concentrations of formalin to ascertain whether the tissue architecture can be improved upon. It is important to note that the vascularity of an organ also has a role in preserving tissue architecture. However for routine teaching purposes, the slides prepared from tissues of embalmed cadavers appear to be good enough.

Conflict of Interest - Nil

Source of Funding - Nil

Ethical Clearance - NA

Limitation of The Study

The study was carried out on cadavers meant for routine dissection by first year medical students. Hence tissues could not be taken from the same cadavers pre and post embalming as it would have interfered with their dissection protocol.

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Statistical Analysis of Rheumatoid Arthritis in Association With Demography and Biochemical Investigation

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Abstract

In this paper we discussed the basic statistical analysis of the biological data and associated with the demographic profile and biochemical profile of the samples of arthritis. Arthritis is a multi-factorial disorder consisting of more than 100 types of arthritis[9], it is really difficult to say that a particular disease is causing that disorder. For determination of Rheumatoid Arthritis (RA) we have used biochemical tests of the 70 individual affected with rheumatoid arthritis. The peripheral blood serum and plasma has been collected using vacutainers containing EDTA, the samples have gone through 5 tests for the determining the pattern of arthritis. By using this method pre-diagnostic measure for arthritis, results have shown us that most of the patient have correlative increase and decrease in their serum and plasma level when the patient is affected with RA. Various test such as Hb, Uric acid, serum glucose, serum calcium levels are used to determine the presence of arthritic profile. The results have found completely based on correlation of the demographic data with the arthritic marker, diabetic marker, lipid marker, kidney function marker, liver marker. The results have found that maximum correlation is shown among the liver marker as its maximum correlation with all the arthritic markers and demographic data.

Keywords*: Statistical analysis, Arthritis, Rheumatoid arthritis, demography, biochemical investigations.*

Introduction

Statistical modelling are widely used in science, epidemiology, and health services etc., research to analyse and interpret data,

Obtained from surveys or existing data sources such as claims files and electronic health records. The veracity of such inferences relies not only on the quality and completeness of the collected data, but also on the validity of the researcher's model[14].

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Correlation is a statistical method used to evaluate the strength of relationship between two quantitative variables. A high correlation means that two or more variables have a strong relationship with each other, while a weak correlation means that the variables are hardly related. Scatter diagram is the simplest way of graphic representation of a bivariate data, where the given set of 'n' pairs of observations on two variables X and Y say (X_1, Y_1), (X_2, Y_2) …(X_n, Y_n) may be plotted as dots by considering X-values on X-axis and Y-values on Y-axis. By scatter diagram, we can get some idea about the correlation between X and Y. In a scatter diagram, if the points are closer and show either upward or downward trend then there is high degree of correlation.

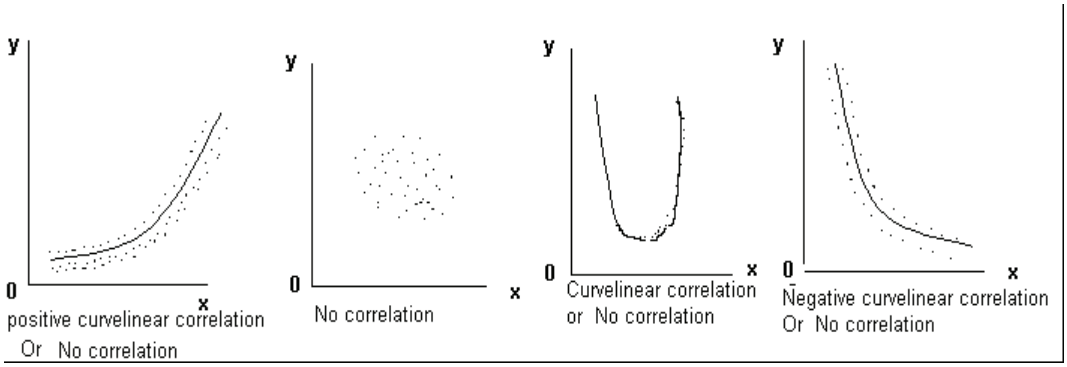


Diagram 1: Scatter diagram shows the direction of correlation

Chi Square Test of Goodness of Fit:

The goodness of fit of a statistical model describes how well it fits a set of observations. Measures of goodness of fit typically summarize the discrepancy between observed values and the values expected under the model in the data. A very powerful test for testing the significance of the discrepancy between theory and experiment was given by prof. Karl Pearson in 1900 is known as Chi-Square Goodness of Fit. It enables us to find if the deviation of the experiment from theory is just by chance or is it really due to the inadequacy of the theory to fit the observed data

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where O = observed value; E = Expected value

For a Chi-square test, a p-value that is less than or equal to significance level indicates there is sufficient evidence to conclude that the observed distribution is not the same as the expected distribution. In this paper we applied Chi square test to find the association between demographic profile and the Rheumatoid Arthritis of the samples and the results obtained is discussed in conclusions. Arthritis the term is derived from Greek arthro- meaning joint and it is meaning inflammation[10]. Arthritis is a multi-factorial disorder consisting of more than 100 types of arthritis, it is really difficult to say that a particular disease is causing that disorder[3]. Humans have been suffering from that disorder almost over a thousands of years and it's still a major concern for the society till date[14]. Majorly arthritis is divided into two parts Osteoarthritis and Rheumatoid arthritis. Rheumatoid arthritis is an autoimmune disorder that affects hands and feet, it's an inflammatory disease and often occurs with severe pain in the joints[4]. Other types include lupus, gout, fibromyalgia, and septic arthritis occurs in 3 % of people and Rheumatoid arthritis occurs in only 0.2% of people, more than 20% of USA population are suffering from rheumatoid arthritis[4]. As of till date no particular reason behind rheumatoid arthritis is found to be genuine as the symptoms vary from person to person. Someone having arthritis may or may not feel the presence of the disease as the joint inflammation is very mild and often it gets worsen as the age progresses[16]. 1 out of 5 people may show a very mild symptoms of rheumatoid arthritis. Blood tests, x-rays helps to diagnose an arthritis patient. Various biochemical tests are involved in determining arthritis. Rheumatoid arthritis contains complex pleiotropic network of inflammatory cytokines and having numerous pathway[7]. The current concept is that inflammation and tissue destruction is related to cellular interaction of rheumatoid synovium[12].

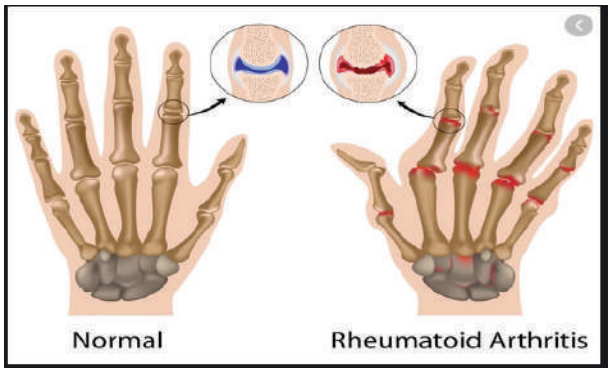


Diagram2: diagramatic representation of Rheumatoid Arthritis

The Progression of Arthritis:

Rheumatoid arthritis begins with the inflammation of the synovial membrane of the joints, especially in the small ones of the fingers and feet and is mostly bilateral. The inflammatory cells, if in inappropriate large amount, destroy body tissue. The synovial fluid accumulates and the joints swell in time and thicken into an abnormal tissue. During the course of time the abnormal tissue erodes the joint's cartilage and possibly scar tissue will be formed, connecting the bone.

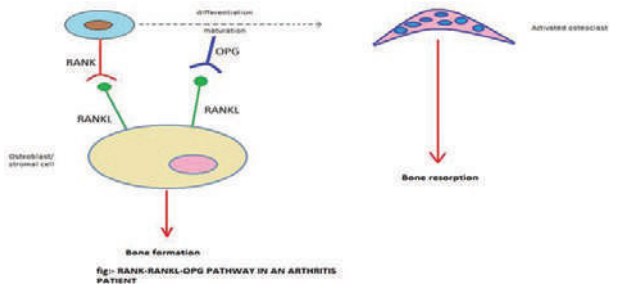


Diagram 3: Rank-Rankl-OPG pathway

Materials and Methods

Materials: For performing biochemical assays the chemical kit were ordered from laboratory and assays were performed in the university laboratory. For performing Immuno-histochemical assays were performed using chemicals such as xylene, ethanol, haematoxylin, PBS, FBS, Antibodies (primary, secondary), blocking solution, DAB substrate solution etc. other reagents Merck Pvt Ltd, Trizol solution, chloroform, DEPC water.

Methods: About 70 patients at the age range of 25-78 were taken for the study of RA. The patient tissue& blood samples were collected from Orthopaedics Dept., Tripura Medical College and Dr B.R Ambedkar memorial Teaching Hospital. The patients suffering from Rheumatoid Arthritis fulfilled the American Rheumatism Association Criteria and were suffering from RA for about 6 months. For the study of control, 60 healthy subjects are chosen. Informed consent was taken from the subjects. Various quantitative Biochemical profiles such as Uric acid levels, glucose levels, calcium levels, cholesterol levels. Qualitative assays includes Rheumatoid factor-Reactive protein. Haematological analysis includes Haemoglobin% and Erythrocyte sedimentation rate (ESR). Patients were diagnose as arthritis, serum samples are taken and biochemical assays were taken. OD values are measured and compared with the normal control patient and hence the values are observed and studied for arthritic profile.

Table 1: Reagent compositions

HAEMOGLOBIN REAGENT 1000 Ml
Potassium Phosphate 2.0 mmol/L Potassium ferricyanide 0.60 mmol/L Potassium cyanide 0.90 mmol/L Sodium chloride 1.4 mmol/L
HAEMOGLOBIN STANDARD 1 x 4 Ml
Cyanmethaemoglobin standard con. 60 mg/dL
URIC ACID:
URIC ACID (S.L) R1 2 x 30 mL / 2 x 50 mL / 2 x 100 mL
Phosphate Buffer (pH 7.0) 180 mmol/L
TOPS 1.10 mmol/L
Amino-4-antipyrine 0.25 mmol/L Uricase> 50 U/L Peroxidase> 100 U/L Preservatives and Stabilizers
URIC ACID STANDARD 1 x 4 mL Uric acid standard concentration 8 mg/dL

GLUCOSE:
REAGENT COMPOSITION
GLUCOSE (S.L)
R1 5 x 100 mL / 1 x 1000 mL
Tris Buffer, (pH 7.40) 92 mmol/L
Phenol 0.3 mmol/L
Glucose Oxidase 15000 U/L
4- Aminophenazone 2.6 mmol/L
GLUCOSE STANDARD 1 x 4 mL
Glucose standard concentration 100 mg/dL

CHOLESTEROL:
REAGENT COMPOSITION
CHOLESTEROL (S.L)
R1 5 x 25 mL / 5 x 100 mL / 4 x 250 mL
Pipes buffer (pH 6.70) 50 mmol/L
Phenol 24 mmol/L
Sodium Cholate 0.5 mmol/L
4-aminoantipyrine 0.5 mmol/L
Cholesterol Esterase > 180 U/L
Cholesterol Oxidase> 200 U/L
Peroxidase> 1000 U/L
CHOLESTEROL STANDARD 1 x 4 mL
Cholesterol std. concentration 200 mg/dL

Results

A significant increase ($p<0.0001$) in the values of biochemical levels among the patients suffering from Rheumatoid Arthritis (RA) comparing with healthy individuals (Table 1). All the results are expressed in mean +sd.

Table 2: Measures of metabolic markers in rheumatoid arthritis (RA) subjects.

Parameter	Rheumatoid arthritis (n= 70)	Rheumatoid arthritis control (n=60)
Age	47.53	46.06
	11.93	13.91
Haemoglobin (%)	9.18	13.24
	1.48	1.43
Uric Acid (mg/dl)		3.98
		0.7
	-7.84	
	1.74	
Glucose (mg/dl)	117.87	90.16
	29.45	7.84
Calcium (mg/dl)	8.15	9.88
	1.14	1.68

Cont ... **Table 2: Measures of metabolic markers in rheumatoid arthritis (RA) subjects.**

This study was carried out using 70 RA ($p<0.0001$) the demographic data for the patient are shown in Table3.

Biochemical investigations:

Table 3: Individuals demographic data

Biochemical Markers	Rheumatoid arthritis	RA control	P value
Age	46.32±11.9	46±13.76	<0.0001
Hb %	10.2±1.62	14.24±1.410	<0.0001
ESR (mm/hr)	79.04±20.72	23.2±1.22	<0.0001
RF	65%	-	<0.0001
CRP	8.67±14.28	0.58±0.33	<0.0001
Uric Acid (mg/dl)	7.036±1.047	3.98±0.68	<0.0001
DAS 28	2.18-8.29	4.21±1.51	<0.0001
GAS (0-100)	5-70	26.81±18.61	<0.0001

In this study we have seen age plays a crucial role among the RA patients as in the middle aged population (>30 years) have a higher level of CRP (p<0.0001). The BMI (p<0.0001) value gets decreased more in the middle-aged population. In other studies have seen that Disease Activity Score (DAS 28) and Global assessment score (GAS) (p<0.0001) is directly proportional with the duration of RA and also with the middle aged population it increases. Here we excluded all the patients who were under anti-rheumatic drug treatments. Biochemical investigations found that inflammatory markers i.e., Hb%, ESR, RF, CRP, Uric Acid have a correlation with age, lower level in Hb% (p<0.0001) among the middle aged population. Also found that ESR mm/hr (p<0.0001) gets increased (79.04+20.72) in rapid amount among the middle aged and old aged population. Other study include RF which was investigative found that 65% of RA patient is RF positive. CRP (p<0.0001) serum levels is much higher compared to the control subjects. The serum uric acid (p<0.0001) level is significantly increases in RA patient compared to control subjects shown in table 4. Matrix correlation for demographic data and inflammatory markers are shown in fig-1.

Figure 1: matrix correlation of demographic data and inflammatory markers

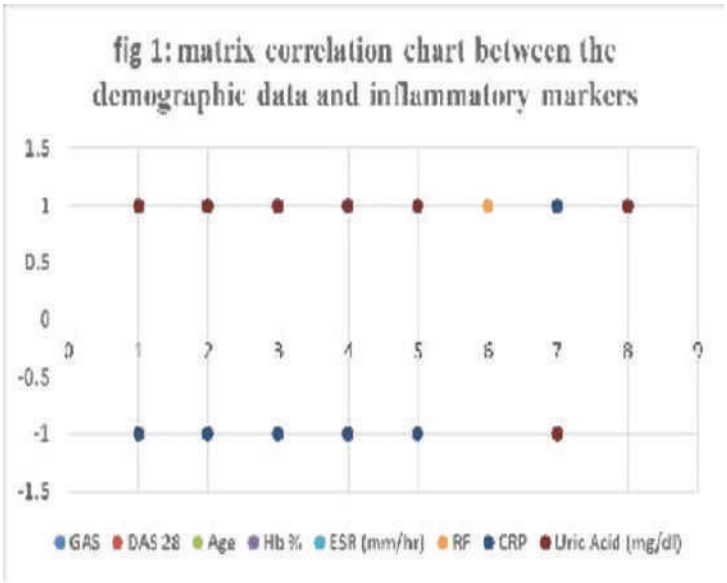


Table 4: Inflammatory markers of patients with rheumatoid arthritis (mean +SD)

Characteristic	Range	Mean ±SD
Age	27-48	47.53 ±11.90
Duration	0.8-17.5	4.01 ±3.86
BMI	17-30	22.69 ±2.79
CRP	0.99 -19.14	8.67 ±14.28
DAS 28	2.18 -8.29	4.21 ±1.51
GAS (0-100)	5-70	26.81 ±18.61

(p<0.0001) gets increased (79.04+20.72) in rapid

Diabetic marker that is glucose shows a positive correlation ($p<0.0001$) with Hb, Uric acid, GAS, DAS 28 (+1). As above mentioned data, the arthritic profile of patients by comparing all the biochemical profile we have seen a major correlation among the increase and decrease among the biochemical values with related to patient age, bmi and other factors. Some changes in the serum biochemical values varies in a particular fashion when the person is affected with arthritis rather than the control patients (subjects).The peripheral venous blood collected from patients with vacutainers with EDTA for serum collection and plasma collection using plasma sample collecting vacutainers. Dietary manipulation is a way for patients to assume some control over their own health and management of their disease. Patients should be able to expect advice from their doctors regarding dietary therapies for which there is a high level of evidence. Some parameters commonly measured in joint diseases together with others which have been claimed to represent better markers of disease activity in RA. It was not surprising that the group with RA had a high percentage of abnormal results for RF, CRP, Uric acid. Other biochemical marker include glucose levels, Cholesterol increases with age among patient suffering Arthritis.

Conclusion

The goal of statistical modelling is to summarize a test's results in such a way that, based on the samples we observed that demography variables like Age, BMI are highly correlated with the disease Rheumatoid Arthritis and also biochemical factors like glucose, uric acid shows significance with the disease present Rheumatoid arthritis plays a major concern role in the life of an affected individual, the lifestyle and livelihood is mostly traumatized and it affects the confidence level in a whole[10-12]. Definite workability in an individual is very much hampered and it affects their social life as well. RA it affects the major population nearly 25% of Indian population is affected by arthritis. To determine its severity have done clinical tests in the middle aged population[19]. The metabolic profiles in the population saw a huge changes according to their ages and severity is also seen and extremely drastic changes can be observed. After taking the correlation graph have seen a massive changes in the levels among control and arthritic patient. By going through all the sugar, Haemoglobin etc. other profiles have seen that according to age those metabolic profiles tends to change if the person has arthritis compared to control subjects. The patients suffering from arthritis have a particular pattern according to their respective ages and by understanding their profile we can clinically diagnose that patient and can determine the presence of arthritis in a patient in the early stages. The validity of these results could be questioned because individuals did not have disease of equal severity[5]. However, all patients had disease severe enough to warrant hospital attention and were selected by virtue of persistent symptoms. It was not possible to match the disease groups for age and sex because they characteristically affect different ages and have varying sex distributions. After going through all the tests and matching the correlation among all the metabolic profiles, we have seen that liver function is much correlated and matched with the arthritic profile compared with other metabolic function. Thus it can be used as a detector marker for Rheumatoid arthritis and can be used as an early diagnostic purpose for Rheumatoid Arthritis.

Ethical Clearance: Approval for the conduction of the study was obtained from the Institutional Ethics Committee of Vellore Institute of Technology

Source of Funding: Self

Conflict of Interest: Nil

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Surrogacy in India: Ethical and Legal Aspect

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Abstract

India has emerged as a surrogacy capital and destination for couples from different countries, in the past two decades. Fertility clinics operating across India, offering artificial insemination, IVF and surrogacy services to Indian citizens and foreign couples have increased dramatically. With the boom in surrogacy, there are increased instances of complex legal and ethical issues. Incidents of unethical practices such as exploitation of surrogate mothers, selective breeding, abandoning of children have increased. This paper highlights the various case scenarios in India and the timeline of various regulations brought by the government of India to regulate surrogacy services. Various guidelines and regulation bills across the period have been discussed in simple tables with the up to date surrogacy regulation bill of 2019. Issues not addressed in the recent Indian regulation have also been discussed.

Keywords: *surrogacy; artificial insemination; IVF; commercial surrogacy; surrogate mothers*

Introduction

Background and Significance

Many countries, around the world, prohibit commercial surrogacy contracts and it's extremely difficult to hire a surrogate mother. Comparatively, the procedural charges/rates for IVF and surrogacy procedure cost less than one third in India, than globally, and the easy availability of surrogacy services have triggered the global inflow of patients to India. The package for surrogacy, costs from $10,000 to $35,000 in India, whereas in United States it's around $59,000 to $80,000. The "Reproductive Tourism" industry is estimated to generate more than $400 million a year from the 3,000 fertility clinics operating across India, offering artificial insemination, IVF and surrogacy services in 2012.[1] The number of medical tourists increased from 150,000 in 2005 to 450,000 in 2008 and the number of births through surrogacy doubled with estimates ranging from 200 to 350 in the same year.[2]

Surrogate services are advertised, rampantly in India, mostly by the agents of the infertility clinic. Surrogates are selected by agents targeting uneducated, poor females. Because of thriving sarcastic terms such as womb for rent, womb outsourcing, baby farms, and baby factory have emerged. There are incidents of unethical practice of exploiting surrogate mothers, abandonment of children, and import of human embryos and gametes on the rise in India. Global criticism and reporting, on commercial surrogacy, through different media, continues to cast a grey shadow. There are increased instances of complex legal and ethical issues arising out of it. This paper will highlight the various regulations brought by the government and the medico-legal issues regarding surrogacy

Discussion

Life of a surrogate in India

According to WHO about 186 million women globally, are unable to conceive.[3] Multifactorial medical reasons of biological inability to conceive a child exists

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among couples, pushing them to restore to surrogacy services.[4] Certain unidentified endometrial factor leading to repeated IVF failures also force couples to go for surrogacy.[5] Surrogacy helps in 70% of the infertile couples to become parents.[6]

The report titled "Surrogate Motherhood- Ethical or Commercial" has studied a total of 100 surrogate mothers and 50 commissioning parents with their families from Delhi and Mumbai.[2] Most of them are uneducated, and their understanding of the clauses and information while signing the contract is disputed. The role of the government authorities while the agreement is framed and signed is nil, and the records of the signed agreement are also kept dark.

The surrogate mother receives of 3 - 4 lakh, from the contract and gets around Rs 25,000 to Rs 40,000 extra if she delivers a twin baby or undergoes caesarean section. However, the amount received in the event of termination of pregnancy, complications during or continuation of it after gestation is unclear. According to Dar et al., Duffy et al., and Parkinson's et al., 3.2 % and 10% of surrogate mothers had hypertensive disorders in pregnancy and 1.1 % and 7.9% of surrogate mothers had placenta praevia/placental abruption.[7,8,9]

Mrs Premila Vaghela, a 30-year-old surrogate, died during her 8thmonth of gestation due to a seizure attack in the hospital. Following a seizure attack, she was shifted for an emergency caesarean section and the 8-month fetus was delivered and monitored in the neonatal intensive care unit. The surrogate mother died, and no complaints were filed because her family got the amount, from the commissioned American family.[10]

Cross-border surrogacy

Surrogacy is not officially permitted in Austria, Bulgaria, Denmark, Finland, France, Germany, Italy, Spain and Sweden. Altruistic surrogacy is allowed in Belgium, Greece, Netherlands, Australia, Canada, New Zealand and the UK.[11,12] Commercial surrogacy is legal in Georgia, Israel, Ukraine, Russia, India and California, USA, while in many states of the USA only altruistic surrogacy is allowed.

The Foreign nationals, seeking surrogacy services, bring about many legal and ethical challenges regarding the status of the parent and child, immigration and citizenship.[13,14] By regularizing surrogacy, legal and immigration troubles in cross-border surrogacy arrangements can be avoided.[15] Since India became the desired destination for surrogacy with no clarity and anticipations of legal issues. The surrogacy clinics, government officials, the intending overseas couples and children born out of surrogacy had to face lots of problems regarding citizenship and immigration.[16] At this point two important cases which involved cross border surrogacy are too be discussed:

Baby Manji Yamada case[17]

A Japanese couple Dr Yuki Yamada and Dr Ikufumi Yamada visited India in 2007 for availing surrogacy services. After visiting a surrogacy clinic and choosing a surrogate mother in Anand, District of Gujarat a surrogacy agreement was made between the biological parents/intending couples and the surrogate mother. However, the couple got divorced and the biological father Dr Ifukumi Yamada left to Japan due to expiry of his visa. The child was born on 25thJuly 2008 and was nursed and cared in the clinic. The Municipality at Anand issued a birth certificate indicating the name of the genetic father. The grandmother of the baby, Ms Emiko Yamada filed a Certificate of Identification from the Regional Passport Authority to take back Baby to Japan. However; her petition was rejected because in India child to be legally adopted before leaving the country, but bars single men from adopting. Manji's father was denied travel documents for the baby and subsequently, a petition was filed in the Supreme Court. After that India issued a Certificate of Identification instead of a passport by the Regional Passport Authority, Rajasthan following Supreme Court directions just to facilitate transit out of the Indian Territory.

Jan Balaz case [17]

Mr Jan Balaz and his wife Mrs Susanne Anna Lohle, German nationals, had a twin babies 'Balaz Nikolas' and 'Balaz Leonard' by fertilizing a donated ova by the sperm of Jan Balaz through a surrogate mother M/s. Marthaben Immanuel Khristi - a citizen of India. However the birth certificate was issued for the twin babies with the name of Jan Balaz mentioned as the father and the mother's name was mentioned as Marthaben Immanuel Khristi, the name of a surrogate mother, instead of the Susanne

Anna Lohle wife of Jan Balaz. The couples applied for a passport for taking back the twin babies and passport was issued in the name of above-mentioned babies. However, the passports were asked to be surrendered through a notice served to Mr Jan Balaz. Following which, a case was filled in the High Court of Gujarat which involved the question of the nationality of twins born to an Indian surrogate mother. After exhaustive analysis and precedence with the Baby Manji case, the court directed passport Authorities to issue a certificate of identity.

These two cases highlighted the need for regulation and oversight of surrogacy; which was widely covered in Indian and global media about legal and diplomatic crisis paving way to various legislations that came into effect.

Various legislations passed in India in context to Surrogacy

ICMR guidelines, 2006 [17]

The Indian Council of Medical Research (ICMR) published guidelines for accreditation, supervision and regulation of ART clinics in India. The guideline has rolled out points to uphold the rights of a surrogate mother. However, did not restrict commercial surrogacy and Foreign Nationals availing these services. Penalties and offences also not mentioned

The Assisted Reproductive Technology (Regulation) Bill, 2008[17]

Elaborates the physical infrastructure and manpower requirement for an infertility clinic ART procedures in patient selection, surrogate mother and donor. *The confidentiality* of records should be maintained. Rights and duties of donors, surrogate mother and child were outlined.

Draft Assisted Reproductive Technology (Regulation) Bill, 2014[18]

Expatiated about the setting of national and state boards to regulate and monitor the ART clinic; along with banning foreign nationals from obtaining surrogacy services. Highlighting the issues related to complications, disabilities, and death of the surrogate and abandoning of children.

The Surrogacy (Regulation) Bill, 2016[19]

Status of the bill

- Introduced in LokSabha: Nov 21, 2016
- Referred to Standing Committee: Jan 12, 2017
- Report of the standing committee: Aug 10, 2017
- The Union Cabinet approval for moving official amendments in the "Surrogacy (Regulation) Bill, 2016".
- Bill lapsed

Key Points

- To allow ethical altruistic surrogacy to the intending infertile Indian married couple of 23-50 years and 26-55 years for female and male respectively;
- The intending couples should be legally married for at least five years and should be Indian citizens.
- The intending couples shall not abandon the child, and the child born out of surrogacy procedure shall have the same rights and privileges as of the biological child;
- A surrogate mother should be a close relative of the intending couple and an married woman having a child of her own and age of 25-35 years.
- The surrogate mother shall be allowed to act as a surrogate mother only once.
- To constitute the Surrogacy Board at the National and state level to perform functions conferred on it under the Act.
- That no person or organization of any kind shall undertake commercial surrogacy or issue advertisements, abandon the child born through surrogacy, exploit the surrogate mother, sell human embryo or import human embryo and contravention of the said provisions shall be an offence punishable with imprisonment for a term which shall not be less than ten years and with fine, which may extend to ten lakh rupees.

The comparison between the various guidelines and bills concerning the surrogate mother, intending couple are highlighted [**Tables 1 and 3]**

TABLE 1: CRITERIA AND RIGHTS OF A SURROGATE MOTHER

SURROGATE MOTHER	THE ASSISTED REPRODUCTIVE TECHNOLOGY (REGULATION) BILL, 2008	DRAFT ASSISTED REPRODUCTIVE TECHNOLOGY (REGULATION) BILL, 2014	THE SURROGACY (REGULATION) BILL, 2016
Age	21- 45 years	25-35 years	25-35 years
Marital Status	Not specified	Should be married	Should be married and a close relative
Times to be a Surrogate	Not more than 3 successful live births	Only once	Only once
Number of times to transfer the embryo	3	3	2
Mandatory testing for Sexually transmitted diseases and blood transfusion	Yes	Yes	Yes
Certificate of medical and psychological fitness	Not specified	Not specified	Yes
Right to abort	**Not specified**	**Yes, as per MTP Act**	**Yes, as per MTP Act**
Monetary benefits by the intending couples	Yes	Yes	No, except for treatment
Insurance	Yes (Not specified)	Yes (Not specified)	Yes, (for 16 months) including long term complications and disability
In the event of death	Not specified	Not specified	If due to pregnancy, amount to be given

TABLE 2: CRITERIA AND RIGHTS OF INTENDING COUPLE

INTENDING COUPLES	THE ASSISTED REPRODUCTIVE TECHNOLOGY (REGULATION) BILL, 2008	Draft Assisted Reproductive Technology (Regulation) Bill, 2014	THE SURROGACY (REGULATION) BILL, 2016
Age	21 years	Not specified	Male: 23- 50 years Female: 26-55 years
Marital Status	Married, Unmarried couples, single women	Married couples	Married couples for five years
Nationality i. Overseas Citizen of India (OCIs), People of Indian Origin (PIOs), Non Resident Indians (NRIs) and foreigner married to an Indian citizen ii. Foreign National	Permitted Permitted	Permitted Not allowed	Permitted Not allowed
Infertility registration certificate	Not specified	Not specified	Yes, from Medical board
Right to choose the surrogate	Yes	Yes	No
Right to decide on termination of pregnancy	No	No	No
Penalty in the event of abandoning the child	Not specified	Not specified	Yes

The Surrogacy (Regulation) Bill, 2019[20]

Status of the bill

- Passed in Lok Sabha: Aug 05, 2019
- Referred to Select Committee: Nov 21, 2019
- Report of Select Committee: Feb 05, 2020
- Cabinet approval of the bill: Feb 26, 2020

The changes between the surrogacy regulation bill between 2016 and 2019 are highlighted in [Table 3].

TABLE 3: DIFFERENCE BETWEEN SURROGACY REGULATION BILLS 2016 AND 2019

S. No	KEY AREAS	SURROGACY REGULATION BILL 2016	SURROGACY REGULATION BILL 2019 [THE SELECT COMMITTEE RECOMMENDATION ON THE SURROGACY (REGULATION) BILL, 2019]
1.	Type of surrogacy	Allows altruistic surrogacy and commercial surrogacy banned.	Rather than complete ban on commercial surrogacy consideration given to economic and health aspect of a surrogate mother intention which could be due to: (i) render a paid service and make money **(or)** (ii) Do it for altruistic reasons.
2.	Surrogate mother	Close relative	Any willing woman
3.	Infertility certificate	Five year waiting period	Couple with known/diagnosed infertility can opt immediately
4.	Insurance coverage for surrogate mother	16 months	Extending to 36 months
5.	Time frame to obtain certificates of eligibility and essentiality	No specified time frame	The intending couple or the surrogate mother can file an appeal with the state government within 30 days from the rejection of the surrogacy application.
6.	Persons who can avail surrogacy services	Legally married Indian couple	Include women who are windows, divorcees or intending couples of Indian origin, between the ages of 35-45 years.

Conclusion

The conception, that India's surrogacy clinics are practicing unethical methods to attract people to yield much profit is growing among people. The Government has tried to regulate the surrogacy clinics through various regulations and act. But still, many steps have to be done by the Government to strictly enforce the rules. Commercial Surrogacy, must be fully abolished in India, surrogacy clinics must be strictly monitored and duly penalized if they found guilty. A regulated and ethical means of surrogacy in the best of the patient and community interest is the need of the hour.

Recommendations

1. The proper time frame for the intending couple to get an infertility and eligibility certificate from the medical board and order on parentage with custody of the child needs to be well defined.

2. Since India has decriminalized consensual homosexuality their right to obtain surrogacy services to beget a child must be addressed.

3. Ought to address the emerging issues like children born out of ART techniques with their dead parent's gametes/cells.

4. Passing the long-pending Assisted Reproductive Technology (ART) Bill will make the surrogacy act more powerful.

Ethical Clearance- Not Applicable

Source of Funding- Self

Conflict of Interest- Nil

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Literature Review in Forensic Practitioner and COVID-19

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Abstract

In the present scenario where everyday new facts of the SARS-CoV2 virus arises, a glimpse on the existing guidelines and the role of the forensic practitioner in COVID-19 cases has been studied. In our study we found that extensive studies have been done on the droplet mode of transmission and prevention of the disease. A comparative glimpse at the guidelines showed the importance of isolation and prevention practices. Using the National guidelines, International guidelines on the disposal of body and autopsy practises a study was done. It was observed that the basis of all guidelines was infection prevention and isolation techniques. Use of disinfectants after contact has been the basis in guidelines.

Keywords: *Forensic Practitioner; COVID-19; autopsy; disinfection; national guidelines; international guidelines*

Introduction

The term Forensic Practitioner has been defined in Forensic Science Regulator as a person who collects, discovers and analyses scientific evidence from crime scene to court [1].

World Health organisation has given the official name of the disease Corona Virus Disease abbreviated as COVID-19 [2]. Royal College of Pathology in its COVID-19 guidelines has advised if death is believed to be due to confirmed case of COVID-19 infection , a post mortem is not needed and medical certification of cause of death can be given. Inclusion of autopsy workers has been included in their Yellow manual with effect from 2003 [3].

In 1965, history of Corona Virus began. Tyrell and Bynoe studied the organisms isolated from patients of human embryonic tracheal culture and found it to be infectious agent as when they introduced it into human volunteers, they were infected with common cold. 229E, B814, OC 43 were discovered in sixties with Surface (S), Membrane (M) and envelope (E) protein [4].

This was accepted as a new genus of virus, CORONA. Named so because in electron micrograph of its negatively stained appearance, the surface projections gave it a crown like appearance [4]. Corona virus develop in the cytoplasm of infected cells, budding into cytoplasmic vesicles from the endoplasmic reticulum. They are extruded from cells within the same time frame and cell is destroyed. They are capable of genetic mutation if two viruses infect a cell at the same time [4]. The virus has been named SARS-CoV2 by the institute of taxonomy after the genotype was isolated by China [2].

Methodology

We reviewed and did a literature search on the guidelines on the internet about the role of forensic practitioner in COVID-19. Our keywords were forensic, autopsy, COVID-19, biosafety level. After collecting guidelines of various health agencies and reliable associations we were able to draw a comparative glance at the guidelines issued by the authorities to have a clear picture on the role on forensic practitioner in COVID-19. We were finally able to finalise on the following guidelines: Royal College of Pathology.

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New briefing on COVID-19, CDC-COVID19 and health care departments and professionals, WHO Status update COVID-19, WHO infection Prevention control guidelines, Institute of Forensic Medicine Malaysia, precautions for handling and disposal of dead bodies, Hong Kong, COVID 19 GUIDELINES India, AIIMS New Delhi COVID -19 Guidelines.

Results

In the literature we found the basic concept of droplet transmission [2]. It is spread of infectious agents from infected source caused by dissemination of droplets. As in the guidelines, during coughing, sneezing and talking, transmission occurs when at a distance of less than one meter they are propelled. They are then deposited on the conjunctiva, eyes, mouth, nose, throat. Maximum volume travel short distance, less than 1 meter and do not remain suspended in the air. Airborne transmission is when the droplet nuclei remain infectious when suspended I the air for a long time. They are obligate and preferential type of airborne transmission. Community transmission is increasing test through sentinel samples. Local transmission is where source of infection is within the location reporting [2].

Universal precautions were introduced by Centre of Disease Control in 1985. In 1996 the CDC guideline for isolation, precaution sin hospital combined Universal precautions and body substance isolation into what is known as Standard Precautions. Irrespective of the disease state, the precautions which are applied are known as Standard Precautions. It includes hand hygiene and Personal protective equipment. Hand hygiene includes hand wash, hand rub or wash with anti-septic and surgical hand wash. Additional precautions are used in specific circumstances. Personal protective equipment are used to protect the health care workers from getting infected [5,6].

A comparative study of the various guidelines weas done. It was found that all guidelines emphasized on the principles of infection prevention and control practices. They emphasised on the use of the hand hygiene, universal precautions and appropriate waste disposal [5,6,7,8,9,10]. The guidelines were in union on the decision avoiding of embalming the body [6,8,9,10]. The confirmed cases of the COVID-19 were not to be sent for autopsy [3]. Infection prevention practices were emphasised repeatedly for health care workers, people involved in transferring the body, relatives of the deceased, mortuary staff [3,6,7,8,9,10,11]. Use of body bag was advised by all to reduce contact with body fluids [6,7,8,9,10]. Disinfectants to be used wherever the body was in contact [6,7,8,9,10]. Use of safety cabinets and negative pressure in autopsy rooms has been advised [6,7]. Use of airborne infection isolation room has also been advised [7]. Air exchange of 6 per hour and differential pressure with exhaust and closed door has been advised [6,7]. Use of such instruments which reduce aerosol generation like round ended scissors, PM 40, hand shears, oscillating saw with vacuum shrouds is advised [6,7,8,9]. Mortuary and dissection comparison is tabulated in table 1. Disposal of waste as per the national guidelines [6,7].One dissector at a time and one cavity dissection at one time has been stated in mostly all guidelines [3]. Respecting the religious sentiments of the deceased and with the use of standard precautions body can be either cremated or buried after handing over to relatives [8,9].

The difference in the guidelines were as under- while some said that autopsy could be done on probable cases of COVID-19 [9], others advise that is preferable to avoid autopsy in such cases [6,8]. Transfer of the body from ward to mortuary was advised in some [9], others said that if autopsy is not warranted, body can be handed over to the relatives [8]. Body bag was advised by all the guidelines [6,7,8,9,10]. A single layer[6,7] of impermeable body bag to double layer[9] is recommended. Some guidelines have said that a layer of white sheet outside the body [9] and then a double layer of body bag [9] can be used. Variation in the concentration of sodium hypochlorite from 0.5-3 % has been advised[6,7,8,9,10]. Table 2 enlists the differences in body bag and waste disposal. Few guidelines mention use of ethyl alcohol or disinfectants approved by their authorities [7] Use of airborne infection isolation room, safety cabinets has also been advised [6,7] and avoid water splash or spray. Specimen collection has been emphasised especially the upper respiratory tract swab and lower respiratory tract swabs. Clinical specimen and formalin fixed tissue specimen to be sent for histopathology analysis[7]. Two sample collection from the same site has been advised to test for the virus and other respiratory pathogens[7]. Unfixed organs held firm on the table and sliced with a sponge Added protection when dissecting lungs[3,8] and washing intestine under water [6]. Body can be handed over to relatives after

autopsy [6,7,8,9,10]. Sometimes, body is refrigerated before handing it over to relatives[9]. The relatives if they wish to see the body, are under supervision[9] and practise infection prevention guidelines. Ash can be collected after the cremation [8].

Discussion

The basis of the guidelines is the current knowledge of droplet transmission and travel related cases. Biosafety level 2-3 is recommend by the guidelines[3,6]. The infective aerosol, infected body fluid, nonintact skin, existing co-morbidities of the deceased and the health care workers form the basis of the guidelines [6,7]. The minimising of aerosol generation is emphasised by the use of instruments and guiding principles of not re-capping the needles and syringes and disposal in puncture proof container [6,7,8,9,10]. Removal of intravenous catheter and central lines or ports at the ward and disinfecting the surfaces is to reduce the chance of infection with the fomites [8]. Packing of the body in layers of adsorbents and body bag with disinfectant application in outer layer emphasise on the same principle[9]. Preventing aerosol in the dissection room by using negative pressure rooms, closing doors and appropriate instruments has been underlined by all guidelines[6,7,8,9,10]. One dissector at a time in one cavity is the underlying principle [3]. Tasking role before conducting the autopsy has been highlighted [9]. Emphasis on specimen collection has been duly laid [6,7]. Handing over the body to the relatives post autopsy respecting their religious sentiments should be done [6,7]. Disinfection and chemicat pre-treatment of the liquids before disposal was emphasised in all [6,7,8,9,10].

Administrative emphasis on containment approach and empowering officers to take such measures to facilitate emergency management and Government policies in managing the outbreak is resonant in all guidelines.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: taken from IEC committee in Mar 2020

Table Number 1: Differences in Mortuary and Dissection

Legend

COVID-19: Corona Virus Disease 2019

NIFM,Malaysia: National Institute of Forensic Medicine, Malayasia

MoHFW: Ministry of Health and Family Welfare

CDC: Centre of disease Control and Prevention

WHO: World Health Organisation Infection Prevention and Control during Health Care for Probable or confirmed cases MERS-CoV

Table Number 1:

Guidelines Features	NIFM, Malaysia	MoHFW	CDC	WHO
Autopsy in confirmed cases	Not needed	---	--	Not needed
Autopsy in probable cases	Done	To be avoided	Jurisdictional decision	To be avoided
Mortuary room	Respective mortuary	Negative pressure	Airborne infection isolation rooms Biosafety cabinets	Negative pressure Exhaust fans Dress in/out Autopsy room

Cont... **Table Number 1:**

Number of people conducting autopsy	3-4. role assigned before conducting the autopsy.	Minimum number of people.	Minimum number of people.	Minimum number of people.
Instruments	Prevent aerosol production	Round ended scissors PM 40	Hand shears/Oscillating saw with vacuum shrouds	Avoid use of power saw, splashes
Body cavity	one person at a time	One cavity at atime	--	One person at a time
Organ dissection	Respiratory tract specimen. Blood specimen	Unfixed organs held firm on the table. Sliced with a sponge Added protection when dissecting lungs	1. Upper respiratory tract swab 2. Lower respiratory tract swab 3.Formalin fixed autopsy tissue 4. Clinical specimens	1. Remove tissue with absorbent material 2. Washing of intestines 3. Lung : additional respiratory protection

Table Number 2

Guidelines Features	**NIFM, Malaysia**	**MoHFW**	**CDC**	**WHO**
Body bag	Body bag 1. White cotton 2. 02 Body bag Disinfect	Body bag: 1. Leak proof plastic bag 2. Mortuary sheet/bed sheet 3. Disinfect	Body bag	Single layer
Environmental cleaning	0.5% sodium hypochlorite Autopsy room: BSL 2	1% sodium hypochlorite Contact time: 30 mins Air dry	EPA approved products	1% sodium hypochlorite Contact time: 10 mins Ethyl alcohol
Linen	Disinfect	Standard precautions Biohazard bag -> yellow	Biohazard bag- disinfect	Linen
Disposal of sharps	Puncture proof container			

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Hypertrophic Cardiomyopathy as a cause of Sudden Cardiac Death—An Autopsy Study

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Abstract

Sudden cardiac death (SCD) is an unexpected death with underlying cardiac pathology. Hypertrophic cardiomyopathy is one of the causes of SCD. It comes almost second important cause after Ischemic pathology. However, it remains to be explored. It is a 10-year retrospective study done between (2002-2020) including all the postmortem cases of SCD coming to the Department of Pathology for histopathological examination. The present study highlights various histopathological features of hypertrophic cardiomyopathy.

Keywords: *Sudden cardiac death, Hypertrophic cardiomyopathy, autopsy*

Introduction

Sudden Death as per WHO guidelines is defined as unexpected natural death within one hour or less from onset of symptoms; or a non-witnessed death within 24 hours in someone without prior symptoms.[1] SCD is an enigma and presents a great challenge to a pathologists and cardiac autopsy is a main diagnostic tool to ascertain the cause. [2]

In SCD underlying pathology has a great role with Myocardial infarction leading cause followed by Hypertrophic cardiomyopathy (HCM), conduction abnormalities etc. [3]

This study highlights HCM which acts as a silent killer particularly in young individuals. It is a primary myocardial characterised by hypertrophic non dilated ventricles and this is without any obvious cause such as hypertension or aortic stenosis. In HCM there is disproportionate hypertrophy of ventricular septum, with myofiber disarray in ventricular septum and left ventricular cavity, mitral valve thickening along with coronary atherosclerosis. [4,5]

The aim of the study is to highlight that HCM is an entity where there is SCD and diagnosis is made on autopsy. So, pathologist plays a vital role in diagnosis of HCM.

Material & Methods

The present study is a retrospective study done over a period of 10 years between 2002-2020 in which total 470 heart autopsies received in Department of Pathology were analysed. The specimens were received in 10% formalin.

Inevery case important detail regarding clinical history, suspected cause of death and postmortem findings were taken from forensic expert and post-mortempapers.

The gross examination is vital in studying cardiac pathology. Weight of heart, ventricular surface is examined along with status of coronary arteries. The heart specimens were opened by modified Virchow's method following the direction of blood flow. All the chambers were washed off any blood clots and examined for any pathology of valves or endocardium. Thickness of ventricular walls and interventricular

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septum was also measured. Multiple blocks were taken from representative areas. After routine processing haematoxylin and eosin staining was done.

On microscopic examination features which were highlighted were myofiber disarray, hypertrophy, fibrosis, nuclear features, and coronary artery atherosclerosis. Myofiber disarray was classified as per criteria established by Maron and Roberts. [6,7] They are as following

Category IA:cardiac muscles are aligned obliquely or perpendicularly to one another forming a tangled mass or pinwheel configurations

Category IB: myofibers are arranged in relatively broad bundles being normally arranged

Category IIA: relatively narrow (one or two cell) longitudinally cut bundles of cells interlaced in various directions among larger groups of transversely cut cells

Category IIB: like IIA except that longitudinal bundles are more linear.

Results

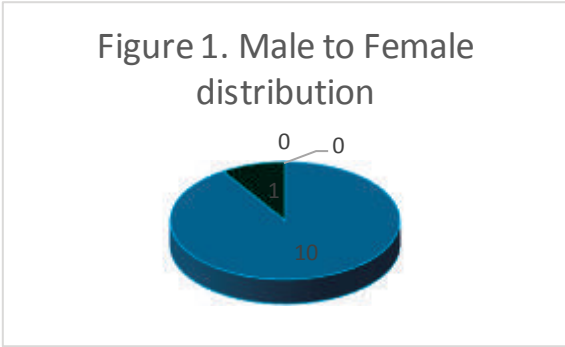
The study was conducted in Department of Pathology between period of 2002-2020 in which total 470 heart specimens were received and out of them 55(11.5%) were diagnosed hypertrophic cardiomyopathy.

The age ranged between 21-72 years (average 43.5years) with maximum cases occurring in 3rd and 4th decade (table 1).

Table No. 1 Age wise distribution of HCM

Age (years)	Total no. of cases	% of cases
0-19	01	1.8%
20-39	23	41.8%
40-59	20	36.3%
60-79	06	10.9%
80-99	05	9.2%

There is male preponderance with M: F of 10:1(Figure 1).



In all cases presentation with SCD with no previous history of any cardiac pathology. All this information was collected from detailed history provided in the police records provided as part of request letter.

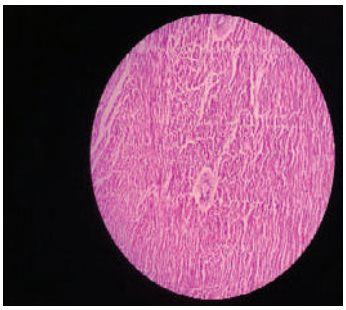
The weight of heart ranged between 300-880 gms (mean: 410 gms)(Picture1). On further grossing left ventricular thickness & interventricular septum ranged between 1.5-3.2 cms (average of 25.2 cms). Right ventricular wall thickness ranged between 0.8-2.0 cms (average of 1.3 cms).

45.5% of cases showed features of atherosclerosis in coronary arteries. In 18.1% cases degree of atherosclerosis was severe as there was foci of dystrophic calcification.

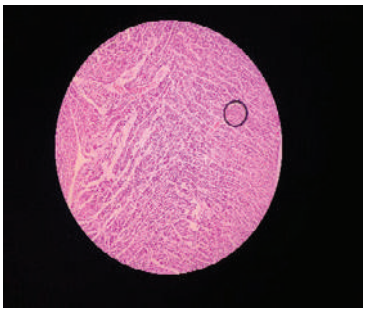
On microscopic examination of all cases myofiber hypertrophywas noted with increased nuclear size and hyperchromasia. The nuclei were cigar shaped. Focal patchy interstial fibrosis was also seen. The most important feature was myofiber disarray. Category 1A was the most common as described by Maron and Roberts. [6,7]About minimum of 20 sections were examined to describe the myofiber disarray [picture 2&3].



Picture 1: Gross examination of heart exhibiting cardiomegaly and increased left ventricular wall thickness.



Picture 2: Sections exhibiting myofiber hypertrophy (H&E X400)



Picture 3: Sections exhibiting myofiber disarray (H&E X400)

Discussion

Hypertrophic cardiomyopathy has been known by a confusing array of names, reflecting its clinical heterogeneity. It was the preferred name because it describes the disease spectrum.[8] it was an inherited autosomal dominant disorder in which proteins encoding the cardiac sarcomere are mutated.[9] These proteins play varied roles i.e. contractile, structural and regulatory. [10,11] This was a main reason of diverse HCM spectrum.

This disease affects a wide spectrum of age group and particularly younger patients i.e. less than 30 years of age.[12]Our study was in concordance with above said findings as the most common age group affected was in third and fourth decade (41.8%). But this disease spares none, as wide age group was affected from young as 20 years to 84 years.

Male preponderance was noted with male to female ratio of 10:1 which was consistent with findings of various studies which presented with 60% more male bias.[13] These findings are in contrast with genetic inheritance of disease i.e. being an autosomal dominant disorder it should be equal in both sexes. So, the underlying reason need to be explored and here autopsy plays a major role.

The clinical presentation of all individuals was sudden cardiac death, and this was the most common clinical symptom.[12] The presentation was common in all age groups thus highlighting a complex underlying pathology which needs to be explored further. But the most common precipitating factor of sudden death was arrhythmia.[6]

On gross examination of heart weight was on the higher side of normal for age and sex of the patient. In the present study heart weight ranged between 300-880 gms with average of 410 gms. This finding was same as in various studies where heart weight was increased. [4,5,6]

Microscopic examination of all specimens was done with extensive sampling of heart specimens as myofiber disarray was considered as gold standard. Maron and Roberts criteria was followed for quantification of disarray. [6,7] However as per various studies no single feature was diagnostic additional histological features like myofiber hypertrophy, boxing of nucleus with interstial fibrosis were also especially important. [6,7]

However, the main important feature was that myocardium shows asymmetric hypertrophy and extensive sampling holds the key for the correct diagnosis. This was supported by study done by Phadke et al and Davies MJ. [6,14] Thus stressing the role of autopsy pathology as biopsy can miss the area having pathology.

This study highlights that Hypertrophic cardiomyopathy was one of the causes of sudden cardiac death. Histopathological examination was the key which can be done only on extensive sampling. It was important as we can advise genetic testing of the family and thereby can diagnose the disease in the family.

Conclusion

Hypertrophic cardiomyopathy is an autosomal dominant disorder and important cause of sudden cardiac death. It is a silent killer which runs in the families. Autopsy plays an important diagnostic role with histopathological findings holding the key for diagnosis.

Source of Funding: Self

Conflict of Interest: NIL

Ethical Clearance: Taken

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Attitude and Knowledge toward Organ Donation among Arts and Science Students

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Abstract

Aim: A cross sectional study was undertaken to study the attitude and knowledge towards organ donation among, higher secondary students, graduate students and post graduate students at different arts and science colleges in Srinagar (Jammu and Kashmir).

Material and Method: 200 students participated in the study between the age group of 17 to 21 years. The students responded to self-administered questionnaires (pre validated) that comprised of seventeen questions.

Result: The students responded completely to the self-administered questionnaires that comprised of seventeen questions. Out of 200 students who participated in the study 118 (59%) were male and 82 (41%) were female. Majority of the students 164 (82%) had information about organ donation and were of the opinion that organ donation can save lives 136 (68%). Majority of the participants 111 (55.5%) believed that organs can be donated after death. The main source of information regarding organ donation was found to be newspaper and magazines 87 (43.5%), followed by television/radio 63 (31.5%) and the most common organ that can be donated was considered to be kidney 81 (40.5%) followed by eye 62 (31%). 115 (57.5%) of participants were unwilling to donate organs based on their religious belief. It was found that 64 (32%) of students were willing to receive organ and majority of students 136 (68%) were against charging of money for organs but were in favour of formulation of law regarding organ donation 183 (91.5%).

Conclusion: This study suggested that majority of students had information about organ donation and were of the opinion that organ donation can save lives but most of them were unwilling to donate organs based on their religious belief. Further study should be conducted to know the awareness and attitude towards organ donation among general public.

Key words: *Organ donation, Students, Knowledge, Attitude*

Introduction

Many organs from dead person are being removed to be transplanted into the living for giving relief for disease or for increasing life expectancy. The tissues and organs may be acquired from living persons or from cadavers[1]. In certain conditions like liver failure and end stage kidney disease, organ transplantation is considered to be the best alternative[2]. Currently there are two problems related to organ transplantation, on one hand there is dearth of organ donors and a long waiting list, on the other hand in the Third world countries like India, Pakistan, Philippines, and other countries, every year thousands of people from United States, Australia, Europe and middle east, come in search of poor donors who are ready to donate their organs for financial recompense[3]. The growing number of successful transplantations has increased the hope for life, and the technological advancements in medicine,

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awareness and acceptance among the doctors have increased the need for more donors. There is a vast gap in the number of patients requiring organ transplant and possible donors[4]. The decision to donate the organ is a crucial process prior to transplantation. It is influenced by personal or family opinions that are strongly influenced by various factors like demographic, socioeconomic, and cultural distinctiveness resulting in varying degree of willingness for organ donation among populations[5]. This study is an attempt to know the knowledge and attitude towards organ donation amongst the students in Srinagar.

Methodology

A cross sectional study was carried out among 200 higher secondary students, undergraduate students and post graduate students, to assess the knowledge and attitude towards organ donation. Questionnaire was anonymised, used to collect information on gender, age, religion and attitude of respondents toward organ donation. Information was sought on the reasons for approving or disapproving organ donation. The researcher distributed the questionnaire and collected the data after getting the clearance from the ethical committee of the university. Data was collected through pre-validated self-administered, structured questionnaire prepared by studying the literature[4, 11].

All the respondents were briefed about the study before administering the questionnaire and written consent was taken for participating in the study.

Result

A total of 200 under graduate students, graduate students and post graduate students participated in the study from different colleges in Srinagar belonging to age group of 17 to 22 years. Majority of students (33%) belonged to 20 years of age and least (7%) were of17 years.

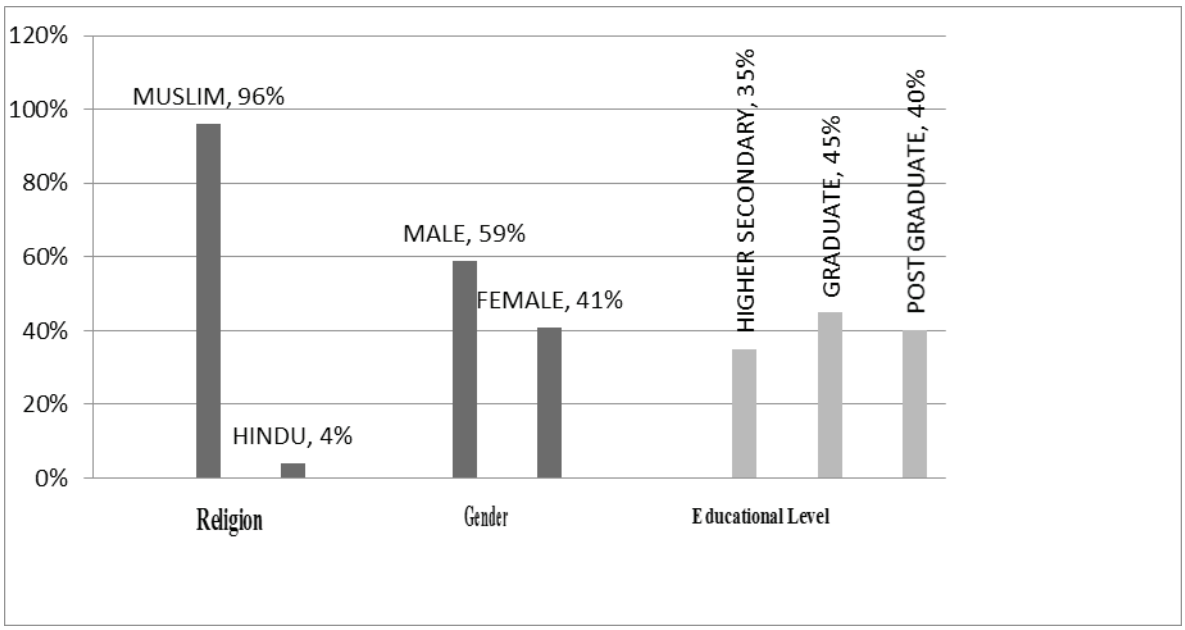


Figure 1: Graphical presentation of religion, gender and educational level

Out of 200 students who participated in the study 118 (59%) were male and 82 (41%) were female. Out of 200 students 96% were Muslims and 4% were Hindu. Majority of the participants were graduate 90 (45%), followed by higher secondary 70 (35%) and post graduate 40 (20%

Table no 1: Level of knowledge / awareness towards organ donation

Parameter	Frequency (n=200)	Percentage (%)
Do you have any information regarding the occurrence of organ donation in India?		
Yes	164	84%
No	36	18%
Not sure	---	---
Do you think organ donation can save lives?		
Yes	173	86.5%
No	7	3.5%
Not sure	20	10%
When do you think organs can be donated?		
Alive	70	35%
Brain death	19	9.5%
After death	111	55.5%
Reasons for organ donation.		
To save someone's life	136	68%
Out of compassion / sympathy	14	7%
For money	32	16%
As a responsibility	18	9%
From where did you obtain information about organ donation?		
Newspaper, magazines, books	87	43.5%
Television / radio	63	31.5%
Family and / or friends	14	7%
Internet	30	15%
Others	6	3%
Which organ do you think can be donated?		
Eye	62	31%
Liver	15	7.5%
Pancreas	---	----
Skin	9	4.5%
Bone marrow	5	2.5%
Heart	25	12.5%
Lung	3	1.5%
Kidney	81	40.5%
Do you think there is danger to the health of the donar from		
Infection	113	56.5%
Pain	36	18%
Weakness	51	25.5%
None	----	----

When asked about the knowledge regarding occurrence of organ donation in India it was found that majority of the students 164 (82%) knew about it. When asked whether organ donation can save lives, 173 (86.5%) of participants agreed, 7 (3.5%) participants disagreed whereas 20 (10%) were not sure. When asked about their views on when organs can be donated, 111 (55.5%) of participants were of the opinion that organs could be donated after death, 70 (35%) believed that organs could be donated during one's lifetime and 19 (9.5%) thought that organ donation could take place in brain death. When asked for reason supporting organ donation, 136 (68%) believed that organ donation can save someone's life, 14 (7%) were of the opinion that it was out of compassion/sympathy, 32 (16%) opined for money and 18 (9%) considered it as a responsibility. In response to a query of information regarding organ donation it was found that the main source of information

was newspaper and magazines 87 (43.5%), followed by television/radio 63 (31.5%), internet 30 (15%), family and friends 14 (7%) and others 6 (3%). It was found that regarding individual organ donation, participants were of the opinion that the most common organ that can be donated is kidney 81 (40.5%), eye 62 (31%), heart 25 (12.5%), liver 15 (7.5%), skin 9 (4.5%), bone marrow 5 (2.5%) and lung 3 (1.5%) respectively. When asked regarding danger to the health of the donar from organ donation infection 113 (56.5%) was considered to be the most common cause followed by weakness 51 (25.5%) and pain 36 (18%).

Table 2: Attitude towards organ donation

Parameters	Frequency n=200)	Percentage (%)
Will you be willing to donate your organs?		
Yes	36	18%
No	115	57.5%
Not sure	19	9.5%
If no give reasons		
Cadaveric manipulation	58	29%
Disfigurement	27	13.5%
Religion does not permit	115	57.5%
Can lead to organ trade	----	
Would you be willing to receive an organ?		
Yes	64	32%
No	49	24.5%
Not sure	87	43.5%
Have you donated any organ?		
Yes	-------	------
No	200	100%
Not sure	--------	------
Do you think it is right to charge money for organs?		
Yes	24	12%
No	136	68%
Not sure	30	15%
Should there be a law regarding organ donation?		
Yes	183	91.5%
No	12	6%
Not sure	5	2.5%

When asked whether the students were willing to donate their organs, it was found that 36 (18%) of the participants were willing to donate organs, 115 (57.5%) of participants were unwilling whereas 19 (9.5%) were not sure. Among those who were unwilling to donate organs, the most common reason was found to be religion 115 (57.5%), followed by cadaveric manipulation 58 (29%) and disfigurement 27(13.5%). It was found that 64 (32%) of students were willing to receive organ, 49 (24.5%) were unwilling and 87 (43.5%) students were not sure regarding organ donation. When asked whether there should be a law regarding organ donation it was found that majority 183 (91.5%) of students were in favour of it, 12 (6%) were against it and 5 (2.5%) were not sure. When asked whether it was right to charge money for organs, majority of the students were against it 136 (68%), 24 (12%) were in favour of it and 30 (15%) were not sure. None of the participants have ever donated any organ 200 (100%).

Discussion

This study is an attempt to know the knowledge and attitude of arts and science students towards organ donation as most of studies have been carried out on doctor/nurse and general population. This study suggested that majority of students 164 (82%) were aware of organ donation. Majority of the students were of the opinion that organ donation can save lives 136 (68%). 111 (55.5%) believed that organs can be donated after death but 115 (57.5%) of participants were unwilling to donate organs based on their religious belief. 64 (32%) of students were willing to receive organ but were against charging of money for organs 136 (68%). 183 (91.5%) were in favour of formulation of law regarding organ donation. Shoubaki H carried out a study from October 2003 to May 2004 on 1600, Qataris and non-Qataris males and females aged 17 years which showed that 67.8% male and 70.4% female participants were aware of organ donation and transplantation. 61.4% of males and 70.4% of females disagreed the idea of importing organs from out- side and 79.9% males and 87.6% female did not agree to organ donation for money[6]. In May 2004, Ashraf O et al conducted a study on 357, males and females attending the medical and surgical outpatient department and found that knowledge regarding organ donation in Pakistan and rest of the world was 65.5% and 72.8% respectively. Television 47.9% was the major source of information. According to 93.6% of participants organ donation could save life and that 84% were of the opinion that organs could be donated after death. Majority, 97.5% were in favour of formulation of law regarding organ donation[11]. In 2005, Bogh L carried out a study on 689 staff members of intensive care unit in 17 ICUs in 15 hospitals in northern Denmark and found that 11% participants were against organ donation. 49% of the ICU health care professionals were willing to donate their own organs after death. 74% of the general Danish population was willing to donate organs after death. 95% of the doctors were positive to organ donation compared to 81% of the nurses. 70% of the doctors were willing to donate their own organs after death compared to 45% of the nurses[7]. A study was conducted by Saleem T et al in 2009 on 440 adults in Karachi, Pakistan and found that 35.3% of participants were in favour of organ donation. Television was the major source of information. Only 3.5% had themselves donated an organ with only one person being an actual kidney donar[8]. A study conducted by Annadurai K between January 2012 to September 2012 on 400 college students belonging to 18 years and above at Hindustan Arts & Science College, Chennai, Tamil Nadu showed that all the participants were aware of the term organ donation but knowledge about different aspects was low. 86.1% were not aware of legislation. 75%of respondents were in favor of organ donation and only 2% were registered for organ donation[9]. Badrolhisam NI conducted a study in 2012 on 213 Malaysian population and found that majority of Malaysians were not aware of the shortage of organ donors in Malaysia. Some of the participants were willing to donate their organs even if their religious believes opposes organ donations[10]. A study was carried out by Satish KV in 2014 on 200 undergraduate students, postgraduate students, nurses, teachers and public and found that 73% were aware about organ donation, 18% had never heard about it and 9% did not respond. 66% felt organ donation is done to save someone's life, 9% believed it was out of compassion/ sympathy, 9% opined that it was for money, 10% consider it as a responsibility and 6% gave other reasons. 90% of the people felt that there is some amount of risk involved in organ donation. 28% of participants felt that their organs would be misused. 78% of the participants were willing to donate organs after their death[4].

Conclusion

Finding of the study revealed that the majority of the students were aware of organ donation and were of the opinion that organ donation can save lives, but were unwilling to donate organs based on their religious belief. Majority of the students were against charging of money for organs and were in favour of formulation of law regarding organ donation. Awareness programmes should be conducted to make the general public aware about organ donation and how it can save lives through the various mass media campaign like publishing articles in newspaper, advertisement, short films and panel discussion by experts. More extensive studies should be carried out to know the awareness and attitude towards organ donation among general public.

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The Study of Finger print Patterns among Male and Female

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Abstract

Dermatoglyphics, the study of epidermal ridges on palm, sole and digits, is considered as most effective and reliable evidence of identification and plays a major role in any crime investigation. The finger prints are unique and distinguishable for every individual. The objective of the present study was to study the finger prints of different Individuals and compare particular pattern in the different gender. This study was conducted on 150(75 males and 75 females in the age group of 18-50 years) persons randomly selected. Finger prints were recorded with the help of Camalin ink on bond paper and were analyzed. The mean age for male was 35.78 years and for female 28.52years. The predominant pattern among both Male and Female was loop, (62.8%) in Male and (58.8%) in Female respectively which was followed by whorl (24.53%) in Male and (32%) in Female respectively. Plain arch pattern in male and female was 10.14% and 7.34% respectively. Ulnar loop was commonly seen in all fingers whereas radial loop was predominantly seen on index fingers in both sexes.

***Key words**: Finger prints; Whorl; Groove; Gender; Arch; Ulnar loop; Radial loop*

Introduction

Identity is asset of physical characteristics, functional or psychic, normal or pathological that defines an individual. The term Identity is defined as whatever makes an entity definable and recognizable. The various methods by which identity of a person can be known include fingerprints foot prints, bite marks, lip prints, DNA profiling, iris imaging, etc.[1] The skin on the palmar and planter surface of the hand and foot is continuously wrinkled with narrow minute raised portion of the epidermis known as friction ridges. These ridges are also referred to as 'dermal ridges' or 'dermal papillae.' Fingerprints are impressions made by these minute ridges or patterns found on fingertips. Early, in the fetal period, proliferation of the corium (dermis) forms papillary projections into the epidermis forming papillary ridges. The pattern of the papillary ridges in the hands is completely established between 11th and 24th weeks of gestation.[2]The ridges thus, formed during the foetal period do not change their course or alignment throughout the life of an individual, until destroyed by decomposition of the skin after death.[3] The study of fingerprint is called dermatogylphics and its use as means of identification is called dactyloscopy.[4, 5,6] It is based on the principle that the individual peculiarities of the patterns formed by the arrangements and distribution of the papillary or epidermal ridges on the finger tips are absolutely constant and persistent throughout life, from infancy to old age, and that the patterns of two hands do not resemble each other. Even the finger prints of twins are not similar.[7]Sir William Janus Herschel, in 1858, first used this system in India. Later, in 1892 Sir Francis Galton systematized this method, and first Fingerprint Bureau was established in Kolkata.[8]

Fingerprint based recognition systems work in two modes: verification and identification. In verification mode, the systems verify the person's identity using a 1: 1 comparison between the person's fingerprints and those stored in the record. Verification process confirms whether the identity of the person with the fingerprint is

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the valid person. However, the process used in fingerprint identification systems is more complex than the process employed in verification especially for large database because fingerprint identification requires the input fingerprints to be compared with all the fingerprints in the database for matching. While verification uses 1:1 comparison for matching, fingerprints identification require 1:N comparison to establish if the individual is present in the database.[9] Finger prints follow the Locard's Principle of Exchange. The secretions in the fingerprints contain residues various chemicals and their metabolites which can be detected and used for the forensic purposes.[10]They can be found in the scene of occurrence from which the presence of a suspect or a victim or any other person can easily be proved. Fingerprints are now a day used in many of the offices, educational institutions to validate the presence of an individual. Galton type dermatoglyphic patterns is said to vary in different ethnic groups, and its association with diseases of genetic origin has been reported in the past.[11,12]The aim of this study was to determine fingerprint patterns of an individual. This will serve as an important aid in sex determination and help in forensic identification at the site of crime.

Material and Methods

The present prospective study was carried out on 150 voluntarily persons (75 male and 75 female persons) of 18-50 years from general population over a period of 6 months. Persons having healthy hands were included in this study. Individual with any congenital or acquired deformity or scars on fingers, suffering from any chronic skin disease, having worn finger prints or extra or bandaged fingers was excluded.

The study protocol was approved by an institutional ethical committee before commencing it. Informed consents were obtained from the subjects before taking the samples. Each individual was asked to wash his/her hands thoroughly with soap and water to remove dirt and oil and dry them using a towel. He/she was then asked to press his/her fingertips on the stamp pad of CAMLIN Company of size 157×96 mm and roll it laterally on the ink slab and then placed on a white paper with one lateral edge and roll over in opposite direction. Care was taken to avoid sliding of fingers to prevent smudging of the print. The primary fingerprint patterns were observed with the help of a magnifying lens and were identified into basic three patterns; loops, whorls and arches according to Galton□s classification.[8] Each finger in the finger print slip was assigned a number, eg: The 1st number was given to the right thumb and 10th number to left little finger. Confidentiality of the subjects was maintained. The distribution of fingerprint patterns in both hands of individuals and its relationship with sex were analyzed for percentage proportions and compared. Statistical analysis was performed using chi-square and p value < 0.05 was considered to be statistically significant.

Results

The mean age for male was 35.78 years and for female 28.52years.The predominant pattern among both males and females was loop, (62.8%) in males and (58.8%) in females which was followed by whorl (24.53%) in males and (32%) in females respectively. (Table number 1) Comparison of all fingerprint patterns between males and females using the chi-square test showed a value of 24.905 with 2 degrees of freedom and significant difference ($p < 0.001$). Amongst the loop pattern radial loop pattern was predominant in males (21.06%) and least common pattern was that of accidental loop (0.53%) where as in females the most pre-dominate pattern amongst loop was that of ulnar loop (20.26%) as shown in table number 2. Plain Whorl pattern was predominant in females (30.26%) as compared to males (23.73%).Where as plain arch pattern in male and female was 10.13% and 7.33% respectively (Table 2).(Chi-square value 109.909 with 9 degrees of freedom and p-value <0.0001).

Table - 1: Distribution of fingerprint patterns in male and females

Fingerprint pattern	Males (%)	Females (%)	Chi-square value	p-value
Loops (Plain loop + Radial loop +Ulnar loop +Double loop + Central pocket loop + Accidental loop)	471 (62.8)	441 (58.8)	109.909 with 9 degrees of freedom	< 0.0001
Whorl + Accidental whorl)	184 (24.53)	240 (32)		
Arches(Arch+ Tented arch)	95 (12.67)	69 (9.2)		

Table - 2 : Distribution of fingerprint patterns and its percentage

Fingerprint pattern	Males (%)	Females (%)	Chi-square test	p-value
Plain loop	72 (9.6)	105 (14)	109.909 with 9 degrees of freedom	< 0.0001
Radial loop	158(21.06)	147(19.6)		
Ulnar loop	151 (20.13)	151 (20.14)		
Double loop	42 (5.6)	17 (2.3)		
Central pocket loop	44(5.86)	20 (2.65)		
Accidental loop	4 (0.53)	1(0.13)		
Whorl	178 (23.73)	227 (30.26)		
Accidental whorl	6 (0.8)	13 (1.73)		
Plain Arch	76 (10.13)	55 (7.33)		
Tented arch	19 (2.53)	14 (1.86)		
Total	750(100)	750 (100)		

Table- 3: Distribution of patterns in different digits in male and females both hands

Patterns	Males					Females				
	Little finger	Ring finger	Middle finger	Index finger	Thumb	Little finger	Ring finger	Middle finger	Index finger	Thumb
Plain loop	34	16	8	-	14	28	18	10	23	26
Radial loop	14	31	37	48	28	16	30	29	57	15
Ulnar loop	68	24	29	30	-	30	34	33	30	24
Double loop	-	7	6	15	14	4	3	5	1	4
Central pocket loop	11	10	17	-	6	6	3	6	2	3
Accidental loop	-	-	-	-	4	-	-	-	-	1
Whorl	17	45	28	29	59	47	36	52	30	62
Accidental whorl	-	-	-	-	6	4	3	-	-	6
Arch	6	17	15	19	19	15	18	12	3	7
Tented arch	-	-	10	9	-	-	5	3	4	2
Total	150	150	150	150	150	150	150	150	150	150

Discussion

The pattern of the papillary ridges on the fingers remains unchanged in an individual, throughout his/her life.[13]The first ever work for dermatoglyphics was done somewhat around 3000 years back when Chinese used fingerprints to sign legal documents.[14]In the pre-independence era of India, Herschel used fingerprints for personnel identification.[15]

The present study was done to identify the gender of a person in the general population of North India and also as a tool of forensic identification at the site of crime.

In this study the predominant fingerprint pattern among both male and female was loop, 62.8% and 58.8% respectively. It is in accordance with study done by other workers who reported it to be 62.17% and 63.17% respectively.[16]The simple loops characterized by one triradius and one core are very common in most of the human populations and moreover, for some human groups they represent the most frequent patterns of fingers´ terminal phalanges.[17,18] Due to their frequent presence on fingers, fingerprints of loops are important

pieces of physical evidence in criminal investigation and forensic personal identification using fingerprinting. Assessment of loops and its size is a part of some widely used fingerprint identification systems.[19]In our study incidence of whorl pattern in male and female population was 24.53% and 32% respectively which was almost in accordance with the studies done by Koneru et. al. on a Manipur population and reported it to be 36% and 37% respectively.[20]Whereas other workers reported it to be 32% and 29% respectively.[21]The whorl is that type of pattern in which at least two deltas are present with a re-curve in front of each.[14] In the present study incidence of arches in male and female was 12.67% and 9.2% respectively whereas much higher incidence was reported by other workers, 48.26% and 51.73% respectively.[22]Arches can be classified into plain arch and tented arch. Plain arch is made up of ridges lying one above the other. Tented arch consists of one up thrusting ridge, which tends to bisect superior ridges at right angles. [14]In the present study ulnar loop pattern was predominantly seen on the little fingers of male population followed by whorl pattern on thumb, radial loop pattern on the index fingers and arch pattern less commonly seen on different fingers.(Table 3).Whereas on the female hands, radial loop pattern was predominantly seen on the index fingers followed by whorl pattern on thumbs, ulnar loop pattern was common on different fingers and arch pattern on ring fingers. (Table 3) Nithin V et al. in their study reported ulnar loop as the most frequent fingerprint pattern in the total population as well as in the sex wise distribution.[23]A study by Bansal et al. found that males showed predominance of loop pattern in all the fingers except ring finger which showed whorl pattern in both the hands. Females showed prevalence of loop pattern in all the fingers respectively.[24]Ulnar loops are highly abundant in all fingers. In contrast, radial loops occur much less frequently, mostly on the index fingers and their frequency on other fingers is much lower with virtually zero frequency on the little fingers. The highest occurrence of the radial loops on the index finger was explained by radially asymmetrical position of the embryonic pad of the index finger, which is formed in a functional position against embryonic pad of the opposing thumb.[25]

Limitations of the Study: Subjects with finger pathologies and deformities could not be established as they were not assessed. Forensic identification of such individual remains questionable.

Conclusion

Dermatoglyphics has long been documented as a scientific and valuable method for medico-legal, anthropological and genetic studies. Based on the results of this study, it is hereby concluded that the prediction of gender of a person is not possible on the basis of the person's fingerprint pattern alone but is useful forensic tool in human identification. It aids in identification of suspect in case of crime.

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Midazolam as A Sedative Agent in Paediatric Dentistry- A Literature Review

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Abstract

Behaviour management forms an integral part of managing children in the day to day paediatric dental practice. Pharmacological behaviour management strategies serve to be an effective alternative when the basic behaviour management technique fails. Pharmacological management strategies involve the administration of general anaesthesia or sedation. Recently, midazolam has gained popularity for use in conscious sedation in paediatric dentistry. Midazolam has a short half-life, which results in rapid onset and recovery in paediatric dental patients, hence encouraging its use in paediatric dentistry.

Keywords: *midazolam, conscious sedation, children, paediatric dentistry*

Introduction

Over the years, one of the most difficult tasks encountered by dentists worldwide is the control of dental fear and anxiety in paediatric patients. In a report by Klingberg and Broberg, the prevalence of dental fear and anxiety was estimated to be in a range of 5.7 % to 19.5%.Among these, around 9% of them exhibit behaviour management problems [1].It has been observed that children tend to be more anxious as well as uncooperative between the ages of 3 to 7 years [2] and this factor was found to decrease with age [3].

The worldwide prevalence of dental anxiety among children ranges from 3% to 43% [4]. The methods such as voice control, intimidation or the use of physical restraints have gained less eminence in recent years [5]. The guidelines proposed by the American Academy of Paediatric Dentistry includes both pharmacological and non-pharmacological methods for behaviour management of anxious children in dental clinics [6]. Thus, pharmacological intervention has gained popularity as it helps in reducing the child's anxiety while enabling successful completion of the dental procedures [7].

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PHARMACOLOGICAL BEHAVIOUR MANAGEMENT STRATEGIES

Pharmacological management strategies involve the use of general anaesthesia and sedation [8]. When treatment charges are taken into consideration, treatment under general anaesthesia involves higher cost when compared to sedation [9]. Thus, several factors influence the decision on the type of pharmacological intervention to be provided [8].Respiratory risk, cardiovascular risk, paradoxical reactions and morbidity are some of the post-operative complications involved [10]. Also, it requires an exclusive specialized training to perform sedation as well as general anaesthesia[11] accompanied with the burden of surplus cost. Hence, a proper judgement on the method of pharmacological behaviour management is necessary [12].

USE OF SEDATION IN PAEDIATRIC DENTISTRY

The chair side management of anxiety during dental treatment has made sedation to gain recognition in the

field of paediatric dentistry [13]. Conscious sedation can be defined as the use of a drug or drugs to produce a depressed state of central nervous system during which the patient remains conscious, retains protective reflexes, maintain a patent airway and has the ability to understand and respond to verbal commands thereby enabling the treatment to be carried out [14, 15].

The sedation guidelines require the agent to satisfy the following criteria [16]:

• The sedative agent should depress the central nervous system so that the operator can carry the procedures without physiological and psychological stress

• The patient should comprehend communication during treatment and respond to verbal commands

• There should be a margin of safety which is enough to prevent unlikely loss of consciousness and loss of protective reflex.

Oral route is considered to be one of the easiest and the most acceptable methods of drug administration. Most commonly orally administered drugs are tamazepam, midazolam, chloral hydrate, ketamine and diazepam. Nevertheless, the onset and duration of action is highly variable. Another drawback is the inability to titrate the drugs being used [17].

Intranasal route is a painless, needless approach and does not require the use of intravenous catheters. The common drugs administered through this route include midazolam, dexmedetomidine, ketamine and sufentanil. The intranasal sedatives can be administered by drops using a syringe or dropper or sprayed/atomized medication delivery system [18].

Drugs administered through the inhalation route act on various areas of the brain by passing through lungs and past the alveoli to reach the brain through the blood brain barrier[16]. The excretion of the drug occurs faster and more easily through the lungs. Thus, a continuous induction of the sedative agent is required for maintaining the adequate level of sedation[19].

Administration of drugs via the intravenous route involves direct injection of the drug into the bloodstream. Most commonly used intravenous sedative agents include benzodiazepines, diazepam, midazolam, propofol and ketamine. The primary advantage is its rapid onset of action. Nevertheless, a level of co-operation from patient is needed to establish the IV line [16].

IMPORTANCE OF MIDAZOLAM SEDATION IN PAEDIATRIC DENTISTRY

Midazolam has been long used as a pre-anaesthetic sedative drug in adults and more recently in children[20]. Midazolam HCL was first synthesized in 1976 by Fryer and Walser[21]. Because of its chemical structure, it is oxidized by the liver much more rapidly and thus has a short duration of action[22]. The National Institute of Health and Clinical Excellence have suggested the use of midazolam for dental procedures in paediatric patients[23].

INTRANASAL MIDAZOLAM

In 2015, Musani IE showed 0.1 mg/kg intranasal midazolam to cause a faster onset of action and recovery compared to 0.2mg/kg of midazolam through oral route [24]. Whereas Shashikiran, 2006[25] and Lam, 2005 [26] observed no difference in the behaviour between 0.2 mg/kg midazolam administered intranasally and intramuscularly. In 2001, al-Rakaf et al compared three different dosages of intranasal midazolam and all three dosages (0.3mg/kg, 0.4 mg/kg and 0.5mg/kg) were effective in modifying the behaviour of the child to accept the dental treatment [27]. In 2007, F. Gilchrist used intranasal midazolam in the dosage of 0.25mg/kg which provided adequate anxiolysis to complete the intended dental procedure with an average onset of sedation time as 13 minutes and average discharge time of 46 minutes [28]. In 2016, Fathima P observed that 0.5mg/kg dose was more effective than 0.3mg/kg in reduction of anxiety[29].

Some of the common side-effects associated with use of intranasal midazolam are transient desaturation, hiccups, nausea, vomiting, enuresis, headache, vertigo, hypersalivation, hallucination, dizziness, diplopia, behavioural disinhibition /paradoxical reaction, burning sensation of nasal mucosa and bitter taste sensation by children [28, 30] .The bitter taste of the drug can be minimized by the use of a highly concentrated dose which in turn reduces the quantity of dose administered [31]. Intravenous administration of Flumazenil can reverse the effect of midazolam and prevent unwanted

complications [32].

ORAL MIDAZOLAM

Midazolam has been administered orally at doses between 0.2-1.0 mg/kg and has an average onset of action of 20-30 minutes. Singh et al. observed that oral midazolam in a dose of 0.5mg/kg is a suitable premedication for paediatric patients during short dental procedures [33]. Another study compared two doses of oral midazolam (0.3 mg/kg or 0.5 mg/kg) [34].Both dosages proved to be successful, without intra-operative or postoperative complications. Fraoneet al. evaluated the effect of orally administered midazolam (0.5mg/kg) in three age groups: group I (24-35 months) group II (36-47 months) and group III (47-59 months) with no significant clinical differences [35]. Saarnivaara et al. recommended oral midazolam doses of 0.5 mg/kg for children less than five years and 0.4 mg/kg for older children [36].

Oral midazolam is commonly used in combination with nitrous oxide for sedation in paediatric dental patients. Al-Zahrani et al., reported that a combination of oral midazolam and nitrous oxide was effective and safe in young patients who required minimal restorative dental treatment [37]. Pisalchaiyong et al. observed that the midazolam/nitrous oxide combination was more efficacious than diazepam/nitrous oxide combination [38]. Ozen et al. also reported similar results with a combination of 0.5 mg/kg oral midazolam and nitrous oxide (50%).The working time might be prolonged with the use of nitrous oxide while simultaneously incorporating its own beneficial effects namely analgesia [39].

INTRAVENOUS MIDAZOLAM

It is well-established that the intravenous route has a rapid onset of action and is the administrative route of choice in emergency clinical situations. It also allows incremental titration of midazolam (usually 1mg/min) preventing under/over sedation [40]. In a study comparing nitrous oxide and intravenous midazolam in adolescents, the results showed good effectiveness and safety when intravenous midazolam protocol was accepted [41]. Another study reported that intravenous midazolam with or without premedication and/or inhalation sedation was effective and safe in patients with intellectual disability [42].

SUBLINGUAL, BUCCAL AND INTRAMUSCULAR MIDAZOLAM ROUTES

Sublingual route of midazolam has been used in a few studies in recent years. Shanmugaavel et al. evaluated anxiety outcome comparing intranasal midazolam 0.2 mg/kg with sublingual midazolam 0.2 mg/kg and found no statistically significant difference in anxiety but sublingual midazolam was better accepted than intranasal route [43] Sunbul et al., 2014 compared intranasal midazolam 0.3 mg/kg with buccal midazolam 0.3 mg/kg and found no significant difference between the two groups in relation to the overall behaviour[44]. Thus, midazolam spray can be effectively used through the buccal mucosa in children who give poor compliance with the intranasal administration [45]. This provides evidence that midazolam could be safely and successfully employed by intranasal and intramuscular routes for paediatric conscious sedation in a routine dental setup. However, whenever the clinical situation warrants a faster action, peak and recovery, the intranasal route should be the obvious choice [25].

Conclusion

Midazolam has been used for conscious sedation through various administrative routes and has proven to be an effective sedative agent for carrying out dental procedures in children. Further research is required in this regard comparing its safety and efficacy with other drugs and when used in combination.

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Study on the Appearance and Fusion of Iliac Crust Epiphyseal Center among Natives of Ajmer Region

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Abstract

Introduction:- Scientific determination of age of an individual is required for the law enforcement agencies in both civil and criminal matters.

Aims and Objects:- Statistical study on the appearance and fusion of ossification centers of iliac crust among natives of Ajmer region and comparative study between both sexes.

Material and Method:- This prospective study has been conducted on 102 subject (48 males and 54 females) of the Ajmer region in Rajasthan of known age group between 13-21 years in the Department of Forensic Medicine and Toxicology in J.L.N. Medical College and Associated Hospitals, Ajmer between 1stApril 2019 to31stMarch 2020.

Inclusion Criteria:- Cases whom parents are born at Ajmer region. Case subjects are also residing at Ajmer since birth, consented for study

Exclusion Criteria

1. They should not be with any physical disability,congenital,nutritional or endocrinal anomaly.
2. Person should have accurate record of their birth date.

Observation:-

In Males appearance of iliac crest in present study was found at the age 15yrs age in all cases. Simultaneously fusion of iliac crest was found at the age of 19.3 yrs in 100% cases.

Similarly In female's appearance of iliac crest in present study was found at the age 14.2yrs age in 100% cases. Similarly fusion of iliac crest was found in majority of cases appeared at the age of 19 yrs in all cases.

Conclusion:- iliac crest fusion is found earlier in females of Ajmer region

Key Words:- *Iliac bone, ossification center, radiological examination*

Introduction

Scientific determination of age of an individual is a necessity for the law enforcement agencies in both civil and criminal matters, determination of age became more important where illiteracy is a common feature and awareness for birth registration is too less. Various processes and methods have been evolved through decades. As a matter of fact, estimation of age is being starts from the moment of conception. During the span of life, various features of growth and development occur in a chronological order. Survey Committee (2014) on medicolegal practices in India[1] had recommended to the government to encourage Zone wise study of problem

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of determination of age. Applicability of regional data is vital importance in academic medical issues and legal proceedings as various regional factors also effects on ossification of bones[2]. Extensive work on determination of age of an individual on the basis of epiphyseal union has been carried out in different countries of world as well as in different states in India.

"Owing to variation in climate, dietary, hereditary and other factors affecting the people of different provinces of India, it cannot be reasonably expected to formulate a uniform standard for determination of age of union of epiphyses for the whole of India."(Franklin) in Modi's Jurisprudence and Toxicology, 1988)[2].

Keeping in view the above statement and also that no recent study what-so-ever has been done in Ajmer region of Rajasthan where mixed population resides, the present study has been undertaken to explore the pattern of diaphyseo-epiphyseal union in the iliac bones of pelvis,

Aims and Objects:-

Aims:-

-to collect systematic statistical data of ossification center appearance and fusion of iliac crest

Primary Objectives

1. Study on the age of appearance and fusion of ossification centers of iliac bone

2. Comparative study on appearance and fusion of ossification centers in girls and boys.

Secondary Objectives:-

1. -Study of secondary sexual characteristics with respect to appearance and fusion of ossification centers of iliac bone

Material and Method

The102 subjects were selected randomly from various schools,the cases attending the OPD of the Department of Forensic Medicine and Toxicology in J.LN. Hospital Ajmer and from various faculty members and staff.

The subjects selected for study were between the age group of 13-14 years, 14-15 years, 15-16 years, 16-17 years, 17-18 years, 18-19 years, 19— 20 years and 20 —21 years.Age, as stated by them was further confirmed by obtaining a copy of birth certificate or from school record duly verified by the head of the school.

After obtaining informed consent for their radiological and clinical examination x-ray of their pelvis, hip joint were taken.

Exclusion criteria:-

- Subjects having congenital, pathological, traumatic, Nutritional loco-motor disorders were excluded.

- Subjects having age not within study age groups are not included our study

Observation

Table No. 1: Age and Sex wise distribution of subjects

Age group (in years)	Male		Female		Total	Percentage
	Number	Percentage	Number	Percentage		
13-14	8	7.84	5	4.9	13	12/4
14-15	9	8.82	6	5.88	15	14.70
15-16	7	6.86	10	9.80	17	16.66
16-17	3	2.94	8	7.84	11	10.78
17-18	6	5.88	9	8.82	15	14.70
18-19	5	4.90	8	7.84	13	12.74
19-20	3	2.94	5	4.90	8	7.84
20-21	7	6.86	3	2.94	10	9.80
Total	48	47.04	54	52.94	102	100

Table No. 1 depicts the age and sex wise distribution of 102 cases in the present study. All the cases were distributed in eight age groups. There were 48 males and 54 females and male to female ratio was 8:9 in all the age groups.

Table No. 2: Distribution of secondary sexual characters vis-a-vis age in males

Age group	Number of Cases	Presence of Hair						Voice			
		Pubic		Axillary		Upper Lip		High pitched		Low pitched	
		No.	%	No.	%	No.	%	No.	%	No.	%
13-14	8	2	25	-	-	-	-	8	100	-	
14-15	9	8	88.8	3	33.3	-	-	9	100	-	-
15-16	7	7	100	6	85.7	3	42.8	4	57.1	3	42.8
16-17	3	3	100	3	100	2	66.6	1	33.3	2	66.6
17-18	6	6	100	6	100	6	100	-	-	6	100
18-19	5	5	100	5	100	5	100	-	-	5	100
19-20	3	3	100	3	100	3	100	-	-	3	100
20-21	7	7	100	7	100	7	100	-	-	7	100

Table No. 2 depicts the distribution of various secondary sexual characters in male subjects involving appearance of hair and character of the voice. It is clear that pubic hair appears in majority of cases (88.8%) in 14-15 yrs age

group; Axillary hair at 15-16 yrs age group in 85.7% cases and upper lip hair at 16-17 yrs age group in 66.6% cases.

The character of voice becomes low pitched in age group 16-17 years in majority of cases (66.6%) in present study and by the end of 18 years it becomes low pitched in cent percent cases. However it is low pitched in 42.8% cases in 15-16 years age group. None of the case reported of hoarseness of the voice in 14-15 years group.

Table No. 3
Distribution of secondary sexual characters vis-a-vis age in females

Age group	Number of Cases	Presence of hair				Development of Breast	
		Pubic		Axi lary			
		No.	%	No	%		No.
13-14	5	3	60	2	40	2	40
14-15	6	6	100	4	66.6	3	50
15-16	10	10	100	10	100	10	100
16-17	8	8	100	8	100	8	100
17-18	9	9	100	9	100	9	100
18-19	8	8	100	8	100	8	100
19-20	5	5	100	5	100	5	100
20-21	3	3	100	3	100	3	100

Table No. 3 depicting appearance of pubic hair in females in majority of cases (60%) at 13-14 years age group and at the completion of 15 years in 100% cases. Axillary hair appears in 66.6% cases in 14-15 years age group and present in cent percent cases in 15-16 years age group. However their presence is in 40% cases at 13-14 years age group. Development of breast is a continuous process and in this study, cases of breast development was 40% in 13-14 years age group, 50% in 14-15 years age group and found in cent percent females by the end of 16 years.

Table No. 4: Incidence of Epiphyseal appearance and fusion of ossification centers for iliac crest in males

Age group in Years	Number of Cases	sIliac crest			
		Appearance		Fusion	
		No.	%	No.	%
13-14	8			-	-
14-15	9	3	33.3	-	-
15-16	7	5	71.42	-	-
16-17	3	3	100	-	-
17-18	6	6	100	-	-
18-19	5	5	100	1	20
19-20	3	3	100	2	66.6
20-21	7	7	100	7	100

Table No. 4 shows incidence of appearance and fusion of ossification centers for iliac crest in males. Iliac crest was found appeared in 71.42% cases in 15-16 years age group and reaches to 100% in 16-17 years age group. However the incidence of appearance for iliac crest was 33.3% in 14-15 years age group. In none of the case iliac crest found appeared in 13-14 years age group.

Fusion of iliac crest was found in 100% cases in 20-21 years age group in males but in majority of cases (66.6%) it was found fused in 19-20 years age group. However incidence of fusion of iliac crest is 20% in 18-19 years age group in this study.

Table No. 5 Incidence of Epiphyseal appearance and fusion of Iliac bone vis-a-vis age in female of Ajmer region

Age group in Years	Number of Cases	Iliac crest			
		Appearance		Fusion	
		No.	%	No.	%
13-14	5	2	40	-	-
14-15	6	5	83.3	-	-
15-16	10	10	100	-	-
16-17	8	8	100	-	-
17-18	9	9	100	-	-
18-19	8	8	100	4	50
19-20	5	5	100	4	80
20-21	3	3	100	3	100

The Table No. 5reveals incidence of appearance and fusion of iliac crest in females.Iliac crest appears in 40% cases in 13-14 age group. In majority of cases, (83.3%) it appears in 14-15 age group and in 15-16 years age group it appears in 100% cases.The incidence of fusion of iliac crest was 0% in 17-18 years age groups in females. It was found fused in 50% cases in 18-19 years age group and in majority of cases (80%) it was found fused in 19-20 years age group. None the case was found unfused after 20 years of age.

Discussion

Epiphysis for Iliac crest:

Epiphyseal center for iliac crest in present study is found appeared in majority (83.3%) of females in age group 14-15 years. It is appeared as an irregular dot of about 2-3 mm diameter or a small crescent or a thin line just over medial 1/3 part of ilium on anterior side, in beginning with passage of age it spreads over brim ilium as a crest.

Iliac crest was found appeared in 71.42% cases in 15-16 years age group and reaches to 100% in 16-17 years age group. However the incidence of appearance for iliac crest was 33.3% in 14-15 years age group. In none of the case iliac crest found appeared in 13-14 years age group.

Fusion of iliac crest was found in 100% cases in 20-21 years age group in males but in majority of cases (66.6%) it was found fused in 19-20 years age group. However incidence of fusion of iliac crest is 20% in 18-19 years age group in this study.

In our study axillary hair developed 100 percent cases at 16-17 yrs in male and 15 to 16 yrs in female. Hence can be used to correlate it with radiological finding in respect to appearance of iliac crest ossification center appearance.

Table No. 6: Ages of appearance and fusion of iliac crest According to different workers

S.No.	Workers	Place of Study	Appearance		Fusion	
			Female	Male	Female	Male
In India						
1	Hepworth (1929)	Punjab	-	-	19	-
2	Pillai (1936)	Madras	14-18	-	14-18	-
3	Galastaun (1937)	Bengal	14	17	17-19	19-20
4	Agarwal&Pathak (1957)	Punjab	-		18.5-19	20
5	Bajaj et. al (1976)	Delhi	15.3	-	19	-
6	Sakxena&Vyas (1969)	M. P	-	-	18-19	20
7	I Git& Singh (1971)	Punjab	12-17.5	-	20-21	19-21
8	Das Gupta et al (1974)	U. P.	-	-	19-20	21-22
9	Kolte&Fakhrudin (1976)	Maharashtra	13-16	-	17-22	-
10	Anjana Mathur (1984)	Ajmer Rajasthan	14-16	-	17-21	-
11	Yogesh (1994)	Marwar Rajasthan	15-16	16-17	18-19	18-19
12	ApurvaNandy (1995)	Bengal			19-20	
13	Bhatia B. L. (1996)	Central Rajasthan	14-15	15.5-16.5	18-19	19-20
14.	Jain Sheetal (1996)	Jaipur Rajasthan	-	-	17-18	18-19
14	Bairwa H. L. (1998)	Jaipur Rajasthan	15-16	15-16	17-18	18-19
15	DuttaSumant (2000)	Jaipur Rajasthan	14-16	-	18-19	19-20
16	Present Study (2002)	Jaipur Rajasthan	14-15	14.5-15.5	18.5-19.5	19.5-20.5
In Foreign Countries						
17	Davis and Parson (1927)	England	18	18	23	23
18	Flecker (1932)	Australia	14	-	15-16	18

Conclusion

1. In Female subjects appearance and fusion of various epiphyseal centers occurs 6 months to $1^1/2$ years earlier than in males.

2. In both sexes appearance and fusion of various epiphyseal centers occurs earlier in Indian subjects than in subjects of European countries.

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Google Site as a Tool for Teaching Undergraduate Students in Forensic Medicine

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Abstract

Background: Today, students are well versed with use of internet and web based teaching can become important source to supplement traditional teaching. Web based teaching via google site can touch deeper aspect of learning by serving as good tool for revision.

Objectives: To develop web based teaching module via google site and evaluate students' performance before and after exposure to web based teaching module along with perceptions of students and faculties on it.

Methods: Web based teaching module in Forensic Medicine on one topic of core competency (Mechanical injury) was developed and validated. Module contained lecture notes, photographs, etc. on google sites. Consent form, feedback form and pre-test and post-test Questionnaire (Total 10 multiple choice questions) were formed and validated. All the medical undergraduate students (5^{th} semester) were subjected to pre-test questionnaire, web based teaching module, post-test questionnaire followed by feedback after obtaining their informed written consent via google site. Feedback of faculties were also obtained and analysed.

Results: Out of 138 participants, 84 were male and 54 were female. There was no statistically significant difference between perceptions of male and female participants. Comparison of pre and post test scores of students was done using Wilcoxon's sign rank test. Students scored significantly more in post-test. Both students and faculties gave positive feedback for web based teaching module.

Discussion: Classroom traditional teaching is always limited to time, place, person and mood of students while web based teaching via google site can be accessed by students on their own time, ease and pace without any limitation. Higher domain can be explored and in depth teaching is possible via web based teaching.

Conclusion: Web based teaching module via google site was found to be very useful to enhance teaching experience of undergraduate students as good tool of revision.

Key words: *Web based teaching, Google site, Forensic Medicine, Competency, Undergraduate, Students.*

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Introduction

Today, students are well versed with use of internet and web based teaching is important source to supplement traditional teaching as part of curriculum [1, 2]. Current competency based curriculum of Forensic

Medicine if supplemented by web based teaching module will be useful to enhance teaching learning process [3]. Google site is one of the platforms to create free web based teaching module which can be freely accessed by students.

Aim and Objectives

- To develop web based teaching module via google site on one core competency in Forensic Medicine (Mechanical Injury).
- To evaluate students' performance before and after exposure to web based teaching module.
- To analyze students' and faculties' perceptions regarding web based teaching module.

Materials and Methods

Step 1: Web based teaching module in Forensic Medicine was developed on one topic of core competency of Forensic Medicine (Mechanical Injury) as per the new competency based curriculum of MCI. Module contained lecture notes, photographs, etc. on google sites and validated.

Step 2: Consent form, feedback form (**Table – 1**) and pre-test and post-test Questionnaire (Total 10 multiple choice questions) were formed and validated [4-16] regarding web based teaching module and uploaded on google site.

Step 3: Approval of Institutional Ethical Committee was taken.

Step 4: All the medical undergraduate students (5th semester) were subjected to pre-test questionnaire, web based teaching module, post-test questionnaire followed by feedback after obtaining their informed written consent. Feedback of faculties was obtained on web based teaching module.

Step 5: Pre and Post-test performance of students as well as their feedback on web based teaching module were analysed. Feedback of faculties was analysed.

Results

Total 138 students of 2nd MBBS (Fifth semester) were subjected to pre-test questionnaire, web based teaching module of Mechanical injury, post-test questionnaire and feedback after obtaining their informed written consent.

Frequency and median score of various parameters of feedback given by students were calculated as per **Table - 2**.

Out of 138 participants, 84 were male and 54 were female. There was no statistically significant difference between perceptions of male and female participants.

Comparison of pre and post test scores of students was done using Wilcoxon's sign rank test. Students scored significantly more in post-test. Only 5% scored less in post-test and 9% had similar pre- and post-test scores as per **Table - 3**.

Median score of various parameters of feedback given by faculties were calculated as per **Graph - 1**.

Table – 1: Feedback form.

Sr. No.	Question	Responses				
		SD	D	NS	A	SA
1	Web based teaching module of Mechanical injury via google site is relevant					
2	Content of web based teaching module is adequate					
3	Presentation of module on google site is adequate					
4	Mechanical injury web based teaching module is useful					

Cont ... **Table – 1: Feedback form.**

5	Time duration of module is adequate					
6	Mechanical injury web based teaching module should be continued for every year					
7	Other topics of Forensic Medicine should be taught by such web based teaching module via google site					
8	Such practice of web based teaching will improve my overall performance					
9	Practice of web based teaching in other subjects will be helpful for me					
10	Web based teaching via google site is good tool for revision					

(Responses: SD-Strongly Disagree, D- Disagree, NS-Not Sure, A-Agree, SA-Strongly Agree)

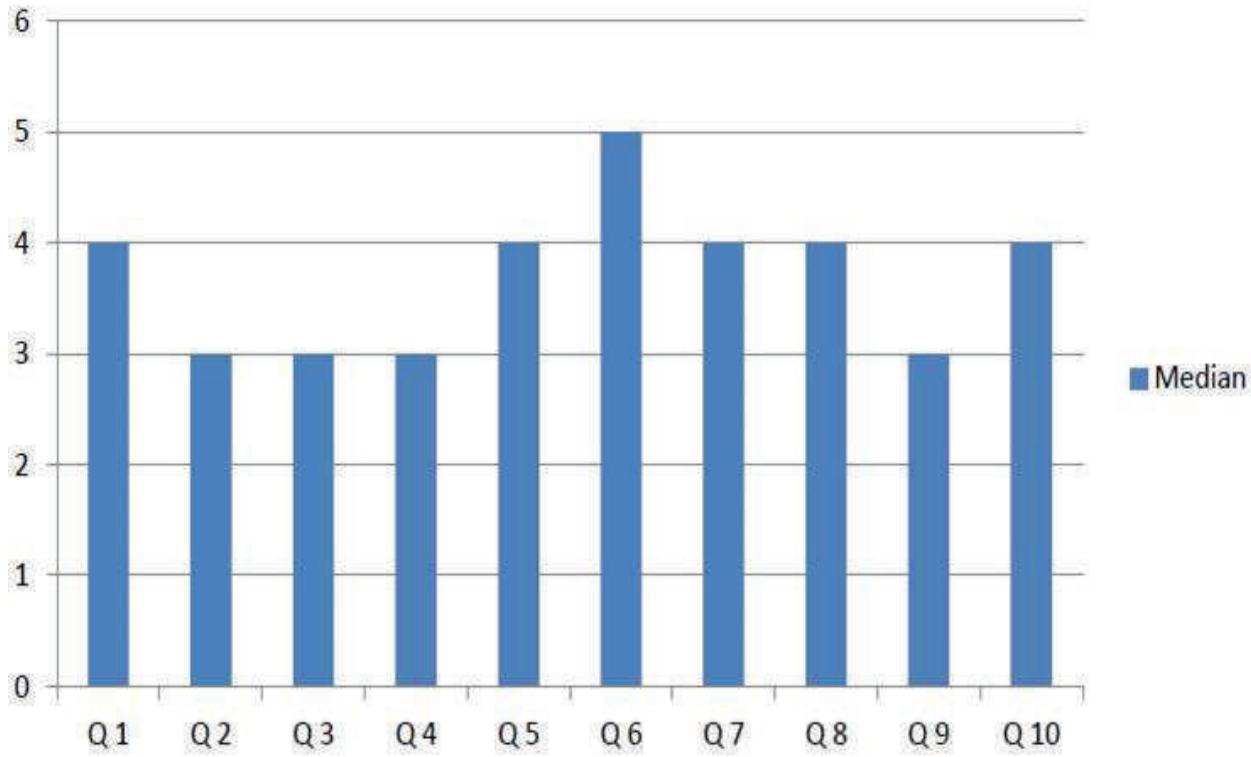
Table – 2: Frequency and median score of feedback given by students.

Question No	Frequency of Responses					Median
	SD	D	NS	A	SA	
1	10	12	17	72	27	4
2	7	10	21	76	24	4
3	6	5	10	32	85	5
4	1	4	11	32	90	5
5	3	6	22	76	31	4
6	13	16	31	58	20	4
7	16	19	28	53	22	4
8	9	7	8	30	84	5
9	3	13	84	31	7	3
10	5	13	40	60	20	4

Table – 3: Comparison of pre and post-test responses via Wilcoxon's sign rank test.

Test Statistics	
	Post-Pre
Z	-7.133a
Asymp. Sig. (2-tailed)	.001
a. Based on negative ranks.	

Graph – 1: Median score of feedback given by faculties.



Discussion

Web based or internet based learning is frequently called as online learning or e-learning because it includes online course content. Discussion forums via e mail, video conferencing, webinars or live lectures (video streaming) are all conceivable through the web. Web based courses may also provide teaching materials in form of power point presentation, useful notes, pictures, videos, static pages such as printed course materials, etc. [17-20].

A "virtual" learning environment (VLE) or managed learning environment (MLE) is an all in one teaching and learning software package. Many universities are equipped with various learning management system (LMS) also. A VLE typically combines functions such as discussion boards, chat rooms, online assessment, tracking of students' use of the web, and course administration. VLEs act as any other learning environment in that they distribute information to learners. VLEs can, for example, enable learners to collaborate on projects and share information. However, the focus of web based courses must always be on the learner—technology is not the issue, nor necessarily the answer [18].

Advantages and disadvantages of online assessment are always there like any other learning system. Advantages are like students can obtain speedy feedback on their academic performance, beneficial for self-assessments for example, multiple choice questions, suitable way for students to submit assessment from remote sites at their own time and pace, computer marking is an efficient use of staff time without bias. Disadvantages are like trained and skilled faculties are required, most online assessment is limited to objective questions, security can be an issue, difficult to authenticate students' work due to lack of direct observation, computer marked assessments tend to be knowledge based and measure surface learning, mostly lacking in direct observation of practical skills, etc. [18].

Conclusion

Web based teaching module was found to be very useful to enhance teaching experience of undergraduate students. Students and faculties gave positive feedback

regarding web based teaching module in Forensic Medicine. Web based teaching module is useful to improve students' performance.

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Clinical Profile of Snake Bite in Children at a Tertiary Care Center

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Abstract

Background: Snake bite is a common neglected emergency in children. The present research was undertaken to study clinical profile of snake bite in children below 12 years of age. **Method:** This was a 4 years retrospective (2011-2014) and 2 years prospective (2015-2016), observational study conducted in 43 children with history of snake bite admitted in paediatric Intensive care unit and ward of a tertiary care teaching hospital during a period of 6 years from 2011 to 2016. **Results:** Majority of cases were in the age groups of 9-12 years (46.5%) with male preponderance (65.1%). 79.10% children had snake bite during outdoor activity at day time (76.74%), lower limbs were the commonest site (65.1%) and 53.4% cases did not receive any appropriate first aid after snake-bite. 69.76% children were vasculotoxic. Common symptom/sign were local pain, swelling (95.3%) and cellulitis (70.02%). 46.51% children had moderate and 44.1% had severe grade of envenomation. ASV was administered in 95.3% of cases and most of the cases were treated with 11- 20 vials of ASV. Out of 3 died cases, 2 died due to respiratory failure and 1 died due to DIC with shock with AKI. **Conclusion:** The most vulnerable to snake bites were boys aged between 9-12 years. It must be emphasized that this study probably represent a biased population of sicker children, but we believe it should provide a good overview of children with snakebite present to tertiary hospitals.

Keywords: *Snake bite, Vasculotoxic, Envenomation, Respiratory failure, First aid, Swelling, Cellulitis*

Introduction

Snake-bite is a life-threatening medical emergency and major public health problem throughout the World, especially in tropical countries like India and also one of the commonest cause of morbidity and mortality, particularly in rural areas [1]. There are more than 2000 species of snakes in the world and about 300 species are found in India out of which 52 are venomous. The venomous snakes found in India belong to three families Elapidae, Viperidae and hydrophidae (Sea Snakes). The most common Indian elapids are Naja naja (Indian Cobra) and Bungarus caeruleus (Indian Krait), Daboia russalie (Russells' Viper) and Echis carinatus (Saw scaled viper) [2]. Clinical effects of envenoming by same species of snake are almost similar except a few regional variations. Kraits are active during night hours, often biting a person sleeping on floor bed. Maximum Viper and Cobra bites occur during the day or early darkness, while watering the planta-tion or walking bare foot in grown grass or soybean crops [3].

Every year about 2000 deaths occur due to snake-bite in Maharashtra. The majority of them remain unreported because many villagers go to traditional healers [4]. Children do not react to snake bites in the same way as adults. In children, the event is more severe since they are exposed to a larger amount of venom per meter

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square of body surface. Delay in seeking medical aid or ignorance among primary care physicians about the correct treatment of snake-bite is also responsible for the high morbidity and mortality. It is important to decide not only the proper regimens but also the modality of treatment in complication of snake bite cases. There are few studies focusing on snake bite on pediatric age group in India, and few across the world; with this background, present study was planned to study the clinical profile of snake bite in children at tertiary care center.

Materials and Methods

The present study was a 4 years retrospective (2011-2014) and 2 years prospective (2015-2016), observational study conducted in 43 children of age <12 years with history of snake bite admitted in paediatric Intensive care unit and ward of a tertiary care teaching hospital during a period of 6 years from 2011 to 2016. All children below 12 years admitted with unknown bite were excluded from the study.

Records of children below 12 years with history of snake bite admitted in Paediatric Intensive care unit or ward from 2011-2014 were analyzed retrospectively whereas records of 2015-2016 were analyzed prospectively. Demographic characteristics of the patients such as age, gender and the snake bite event like time of bite, site of bite, time of arrival to-hospital were recorded in a pre-structured proforma. Symptoms and signs such as local swelling, nausea, vomiting, ptosis, tachycardia, hypotension, impending respiratory failure, single breath count, bite to time of anti-snake venom (ASV), any treatment be-fore referral, total dose of anti-snake venom administered and duration of stay were documented. Complete blood count, liver function test, renal function tests, urine examination, coagulation profile (PT and INR) along with 20 minutes WBCT were also done. Outcome was defined in the form of discharge after completion of treatment, death or discharge against medical advice (DAMA). Severity of envenomation was correlated with the duration of hospital stay.

Statistical Analysis

Qualitative data was presented in the form of percentage. Correlation was studied between severity of envenomation and duration of hospital stay was estimated using Spearman's rho. Correlation of time lapse and mortality was studied using mean ± standard deviation and compared using unpaired't' test.

Observations and Results

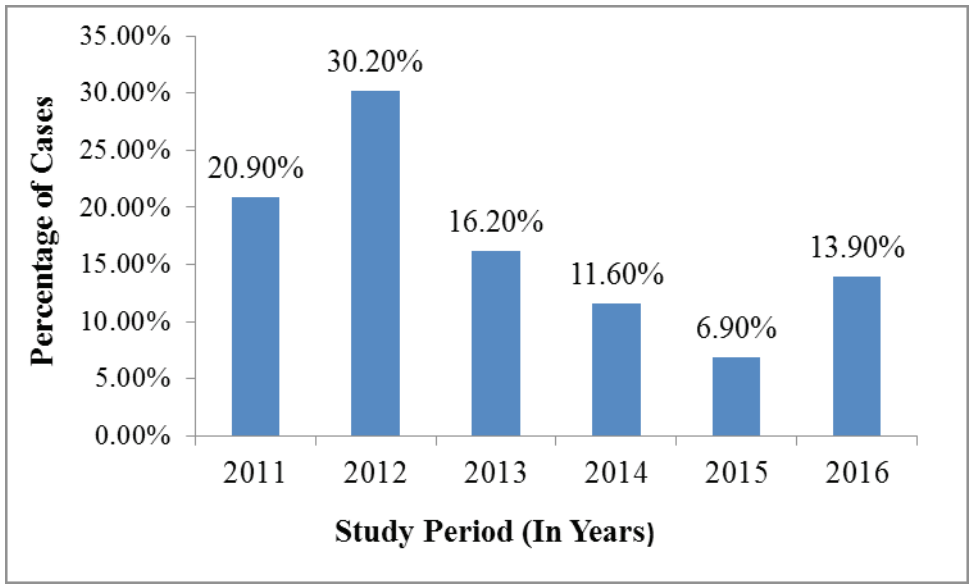
Among a total of 43 cases, 28(65.1%) were males and 15(34.8%) were females with male to female ratio of 1.8:1. The highest incidence of snake bite was observed in age group of 9 to 12 years (46.5%) with mean age of 7.1years, (Table 1). Most of the cases (20;46.5%) were from Thane district followed by Mumbai (14;32.55%) and Raigad district (9;20.93%).

Table 1: Age and sex-wise distribution of children with snake bite

Age group (in years)	Female	Male	Total
0-3	3 (20%)	3 (10.7%)	6 (14%)
4-8	6 (40%)	11 (39.3%)	17 (39.5%)
9-12	6 (40%)	14 (50%)	20 (46.5%)
Total	15 (100%)	28 (100%)	43 (100%)

During the study period maximum 13(30.2%) cases where admitted in year 2012 followed by 9(20.9%) in 2011 as depicted in figure 1.

Figure 1: Year wise distribution of snake bite cases



Most of the victim 34(79.10%) were bitten outdoors and at day-time (33;76.74%), lower limbs were the commonest site (65.1%) and 53.4% cases did not receive any appropriate first aid after snake-bite, (Table 2). 29(67.44%) cases received ASV and fourteen (32.56%) do not received any ASV before reaching tertiary care.

Table 2: Distribution of cases according to location, time, and site of snake bite, type of first aid received on spot

Parameters		No. of cases	Percentage
Location of bite	Indoor	09	20.90
	Outdoor	34	79.10
Time of bite	Day	33	76.74
	Night	10	23.25
Site of bite	Head and neck	07	16.3
	Upper limb	08	18.6
	Lower limb	28	65.1
Type of first aid received	Appropriate	14	32.55
	Inappropriate	23	53.48
	No first aid	06	13.95

26(60.4%) cases reached tertiary care within 0-12 hour, 13(30.23%) cases between 12-24 hours after snake bite. Four (9.3%) cases required more than 24 hours to reach tertiary care hospital of which one survived. Among total 43 cases, 30(69.76%) were observed to be vasculotoxic, 13(30.23%) were neurotoxic and no case of musculotoxic envenomation was observed.

Commonest symptoms/signs seen were local pain (41;95.3%) and localized cellulitis (40;93.02%). 28(65.1%) cases showed deranged >20WBCT whereas deranged LFTs (PT INR) were seen in 23(53.5%) cases. Around 8 cases (18.6%) showed hematuria as shown in Table 3.

Table 3: Distribution of cases according to Signs/Symptoms and Laboratory parameters

Signs/Symptoms	No. of cases	Percentage
Local pain and swelling	41	95.3
Anxiety and fear	35	81.39
Nausea/vomiting	27	62.8
Numbness in face and limbs	03	7.0
Local bleeding	24	55.8
Cellulitis	40	90.02
Ptosis	13	30.2
Respiratory difficulty	07	16.3
Hematuria	08	18.6
Laboratory parameters	No. of cases	Percentage
>20 min Whole Blood Clotting time	28	65.1
Deranged LFT (PT INR)	23	53.5
Haematuria	08	18.6
Metabolic acidosis	06	14.0
Anaemia	05	11.6
Deranged renal function	04	9.3

Only 4(9.3%) cases had mild envenomation, twenty (46.51%) had moderate and 19 (44.18%) had severe envenomation, which had 3 mortality. All children admitted received intravenous fluids and antibiotics. ASV was given to 41 (95.3%) cases and maximum cases required around 11-20 vials (mean-17.44 vials) of ASV, (Table 4).

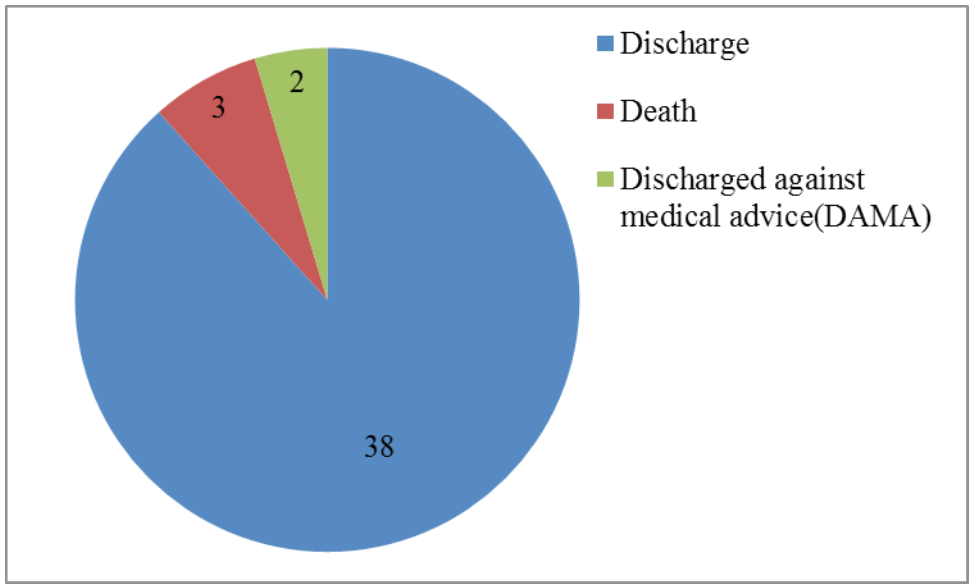
Table 4: Treatment given and no of ASV vial required

Treatment given	No. of cases	Percentage
Tetanus toxoid	39	90.7
Intravenous Fluids	43	100
Antibiotics	43	100
Anti-Snake Venom(ASV)	41	95.3
Oxygen	21	48.8
Ventilator Support	06	14
PRC/ FFP required	21	48.8
No of ASV vial required	Number of cases	Percentage
0-10 vials	09	20.9%
11-20 vials	23	53.5%
21-30 vials	11	25.6%

6(13.9%) cases developed compartment syndrome, 6(13.9%) had reaction to ASV. 4 cases were found to have respiratory failure, 1 case each with DIC and AKI. Most of the cases required around 6-10 days of stay at hospital (55.8%) followed by 0-5 days (39.5%) and 1 (2.3%) patient each required 11-15 days and 16-20 days of stay at hospital. There is no significant correlation between severity of envenomation and duration of IPCU stay (Spearman's rho = -0.032, p=0.839) or ward stay (Spearman's rho = -0.017, p=0.912) or total stay (Spearman's rho = -0.036, p=0.821).

Maximum i.e. 38 (88%) cases were successfully discharged, (Figure 2). Out of 43 cases, 3 died (2 cases due to respiratory failure and 1 case due to DIC with shock with acute renal failure). Those who died had a significantly higher time interval in reaching hospital (25.33 ± 0.577) as compared to those who did not die (10.53 ± 6.84), (P=0.001).

Figure 2: Outcome of the study



Discussion

Over the 6-year study period, 43 children of snake bite were admitted and managed at our institute. 46.5% of the snake bite occurred in the age of 9-12 years with mean age of 7.1yrs. The age distribution and highlights of high prevalence of snake bite in older children as similar to previous studies [5-8]. The probable reasons for this may be an active group more involved in outdoor activity like play, labor and farm work. Maximum (65.1%) cases were male which are in concordance with the majority of studies [5,9,10]. The higher incidence in boys could be due to their increased involvement in outdoor activities. The maximum cases of snakebite were from the peripheral areas of Thane like Wada, Shahapur, which has largest agricultural land. There were cases of snakebite in each year (2011-2016) and the year 2012 had maximum number of cases (13). Most of the cases of snake bite occurred in rainy season like in the month of June to September (44.1%). Study conducted by Lingayat et al [5] and Koirala et al [9] reported similar trends. However, the incidence varies in India due to different pattern of rainfall and agriculture activities. Majority of (79.10%) cases occurred during outdoor activity whereas 76.7% was during the day time. 65.5% of snakebites were in the lower limbs. These findings are similar to other studies [7,8].

Only 32.55% received appropriate treatment, 53.48% received inappropriate treatment, whereas 13.9% received no first aid. This shows that people are unaware about the appropriate first aid to be given to the victim of snakebite. 67.44% cases received ASV before reaching our tertiary care center. This is seen to be beneficial for the snakebite victim when they are delayed in reaching the tertiary referral center and a comparatively improved outcome is noted. 60.4% of cases were admitted in hospital after snakebite within 0-12 hours, whereas, 9.3% cases admitted after 24 hours of snakebite. We observe that delay in seeking medical aid was largely attributed to ignorance, inappropriate first aid, transportation delay, late referral from primary care centers due to unawareness of the snake-bite and its treatment. Mortality was observed in cases which required significantly higher time interval, more than 24 hours in reaching tertiary care hospital as there was no transportation facility at odd hours and unavailability of Pediatric intensive care facility with in 50kms of area. Type of envenomation vasculotoxicity was more common than neurotoxicity. Many other researchers reported a large number of neurotoxicity in their studies[5,8].

The local pain and swelling was commonest symptom (95.3%) whereas cellulitis was the commonest sign. Previous studies[5,8,11] reported swelling and pain as the commonest symptom and depend upon the type of envenomation. Neurotoxic snakebites mainly present with ptosis and respiratory difficulties while vasculotoxic presents with prolonged bleeding. In

existing study, according to the grade of severity [12], 46.5% showed moderate grade and 44.1% showed severe grade of snakebite. It was observed that cases with severe envenomation with other factors like delay in treatment had higher mortality. 65.1% cases had more than 20min whole blood clotting time deranged 53.5% cases had Liver function test with PT INR deranged, whereas 18.6% cases showed hematuria. After analysis of the laboratory report and clinical features, maximum types of snakes seen were vasculotoxic which resemble the study of Mead et al [13].

All cases received intravenous fluids and antibiotics. 95.3% (41 cases) received ASV as 2 cases went discharge against medical advice (DAMA) before receiving any ASV, 48.5% required blood transfusion in the form of FFP/PRC. 14% cases required ventilator support. Most of the cases which received ASV had moderate/severe envenomation. All the patients who received blood products had deranged coagulation profile. All cases requiring ventilator support had severe envenomation. 53.5% cases receiving ASV between 11-20 vials (mean dose-17.44 vials). Most of the cases were hemotoxic with moderate/severe envenomation hence requiring more amount of ASV as similar to the findings of Kumaravel et al [8] and Koirala et al [9]. 13.9% cases developed compartment syndrome requiring incision and release. 9.3% had respiratory failure requiring ICU and ventilatory support. 13.9%(6 cases) developed allergic reaction to ASV but no fatality. DIC and renal failure were observed in one case each. These complications were seen in cases which did not receive early ASV, increasing the rate of morbidity and mortality. Maximum cases (55.8%) had 6-10 days of stay in hospital as similar to study by Kumaravel et al [8].

Most cases (88.4%) were discharged whereas 9%(3 cases) died and 5%(2 cases) went discharge against medical advice (DAMA). DAMA was mainly due to ignorance of parents to understand the seriousness and curability of the condition. There was mortality of 3 cases, all had severe envenomation and reached hospital after 24 hours of snakebite. Two cases had neurotoxic envenomation and died due to respiratory failure whereas one case had vasculotoxic snakebite died secondary to DIC with shock and acute kidney injury. There was no significant correlation was observed between severity of envenomation and duration of hospital stay.

Conclusion

The most vulnerable to snake bites were boys aged 9-12 years because they spend considerable time outdoors. Common symptom/sign were pain, swelling and cellulitis. Delay in seeking medical aid was due to ignorance by the parents, inappropriate first aid provided, transportation delay, late referral from primary care center due to lack of awareness of snakebite and its treatment. Mortality was higher in those cases which required significantly higher time interval in reaching hospital and required Pediatric intensive care which was not available within 50 kms.

It must be emphasized that hospital based study probably represent a biased population of sicker children, but we believe it should provide a good overview of children with snakebite present to tertiary hospitals.

Ethical Clearance: Taken from Lokmanya Tilak Municipal Medical College and General Hospital, Sion, Mumbai Maharashtra, India

Source of Funding: Self-funded

Conflict of Interest: Nil

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Soft Palate Dimensions and Configuration Changes in Oral Submucous Fibrosis (OSMF)- A Radiographic Study

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Abstract

Introduction: It is a highly potent and premalignant condition affecting the oral cavity of people consuming arecanut, gutka. The clinical presentation of Oral Submucous Fibrosis depends on the stages of the diseases during detection is characterized by juxta epithelial inflammatory reaction and progressive fibrosis of submucosal tissue that affect most parts of the oral cavity, such as in the soft palate.

Aim: To assess the changes in dimensions and configuration of soft palate by using digital lateral cephalometry was considered**.**

Materials and Methods: A total of 10 subjects of both the gender, in the age group 20-60 years, were selected from the Outpatient Department of Oral Medicine and Radiology. These 10 Subjects were divided into two group, group I comprised 5 patients which is clinically diagnosed Oral Submucous Fibrosis (OSMF) and group II comprised of 5 individuals having habit with no mucosal changes, the dimensions and configuration (shapes, anteroposterior length and supero inferior width of soft palate) was evaluated using digital lateral cephalometry. The data were statistically evaluated by using the SPSS statistical software 21.0 Version, the Chi Square test was used for the comparison of categorical variables. The level of the significance for the present study was fixed at 5%.

Results: In the subjects with Oral sub mucous fibrosis (OSMF) the shape commonly observed are Type 1-leaf shaped followed by Type 2 Rat shape. The mean AP score in Oral sub mucous fibrosis (OSMF) was 26.82±4.77 whereas in the subjects without Oral sub mucous fibrosis (OSMF) the mean AP score was 33.62±2.67. The difference between the groups was statistically significant. In the subjects with Oral Submucous Fibrosis (OSMF) the mean SI score was 7.80 ±1.77 whereas in the subjects without Oral Submucous Fibrosis (OSMF) the mean SI score was 9.34± 1.42. The difference between the groups was statistically non-significant.

Conclusion: As stages of Oral sub mucous fibrosis (OSMF) increases the anteroposterior soft palate length get decreased and supero inferior soft palate width get increased.

Key words*: OSMF, Cephalometry, soft palate, anteroposterior length, superoinferior width.*

Introduction

The oral cavity is a complex form with advanced details and varied functions. It is limited by the palate, the floor of mouth, the cheek, and the lips, as well as by uvula and the palatine arches on each side of uvula. [1] Soft palate is considered to be mobile part situated posterior to hard palate which is fibromuscular in nature. The salient feature of soft palate is that it helps in velopharyngeal closure and thus aid in normal

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physiologic function like sucking, swallowing and pronunciation,phonation,respiration.[2]

Few studies in the literature dimensional changes of soft palate with increasing age, change in cleft lip and palate and in patient with sleep apnoea was noticed.[3]The lateral skull view is the most important view for assessment of oral structure including hard and soft tissue images.[1,2] Haider et al in 2000, did a cross sectional study and reached to conclusion that the soft palate is the first tissue to be affected in Oral Sub Mucous Fibrosis (OSMF) and as the disease increase in severity, are more likely to be found in anterior as well.[4]

Lateral cephalogram is good diagnostic aid to assess the soft palate and its morphology .These images showed as different configurations of soft palate noticed in various individuals, as the morphological variants and changes in angulation of soft palate i.e. 30 degree or greater between distal part of uvula ,The longitudinal axis of soft palate noticed which can lead to various condition such as obstructive sleep apnoea , difficulty in swallowing, speech and respiration.[1,3] It is less expensive , more useful, easily achieved with reduced radiation exposure [5]

Oral submucous fibrosis (OSMF) is one of the most common premalignant lesion conditions affecting the oral cavity of people consuming arecanut and gutka. It is a chronic, progressive scarring disease affecting the people of south east Asian origin, including the Indian subcontinent.[6]

The aim of the study is to assess the soft palate dimensions and configuration in Oral sub mucous fibrosis (OSMF) patient using digital lateral cephalogram with respect to different clinical stages of oral submucous fibrosis (OSMF) in clinically diagnosed patients and to compare the various shapes and size of soft palate with respect to age and gender in OSMF.

Material and Method

The present study was conducted in department oral medicine and radiology of Teerthanker Mahaveer dental college and research Centre, Moradabad, India. This study was approved from institutional authorized ethical committee. Also, written informed consent was obtained from each subject prior to the study.

A total of 10 subjects were taken in the study and divided as two groups, group I comprised 5 patients who is clinically diagnosed Oral Submucous Fibrosis (OSMF) and group II comprised of 5 individuals having habit with no mucosal changes with age group 20-60 years. With Inclusion criteria that Patients clinically diagnosed with oral submucous fibrosis (OSMF) and were considered. And Normally healthy individuals for control group with the history of habit without any mucosal changes,

Exclusion criteria Congenital anomalies i.e. congenital cleft lip and palate, systemic disease, syndrome, any Odontogenic or space infections, Patients with TMJ pathologies and traumatic conditions, Pregnant females, Patient who underwent surgeries for carcinoma of soft palate, hard palate or tongue were excluded from study.

Based on history of habit and clinical features, diagnosis and grading of the OSMF. the clinical diagnosis was made and classify the stages of OSMF by Dr. Ranganathan et al (2001).[7] they are of four group as

Group 1- only symptoms, with no demonstrable restriction of mouth opening.

Group 2- limited mouth opening 20 mm sand above.

Group 3-mouth opening less than 20 mm.

Group 4-OSMF advanced with limited mouth opening. Precancerous changes seen throughout the mucosa.

All the lateral cephalogram was taken by using Digital lateral cephalograms in radiology department using Planmeca proline XC, panoramic and cephalometric machine. A tube potential of 68kvp , a tube current of 5mA, and exposure time of 17 sec were used to develop the contrast digital image saved in operating system Microsoft Windows XP Professional.

All these radiographs were observed and categorized and evaluated by oral and maxillofacial radiologist. The length of the soft palate will be evaluated by measuring linear distance from posterior nasal spine to tip of uvula in anteroposterior length and the greatest width to be measured for the superior inferior diameter of the soft palate.

Different patterns of soft palate were recorded and classified according to Shapes reported by Supriya Rathore et al 2019 [8] as follows Type 1 (leaf shape),Type 2(rat tail), Type 3(butt shape), Type 4(straight line shape), Type 5(s shape), Type 6(crook shape), Type 7(u shape) Type8 (bifid shape).

The data was entered in the prescribed Performa and analysed using the SPSS statistical software 21.0 Version. The descriptive statistics included frequency and percentages. For the purpose of inferential statistics, the Chi Square test was used for the comparison of categorical variables. The level of the significance for the present study was fixed at 5%.

Results

Among 10 subjects four females and six male Patient was considered and after observing and statistical analysis the result obtained were

SOFT PALATE SHAPES ACCORDING TO STAGES OF OSMF

In the subjects with Grade I OSMF 100% of the subjects were having leaf pattern of soft palate whereas in subjects with Grade II OSMF 50% were having Distorted and rest 50% were having Leaf pattern. In the subjects with Grade III OSMF 50% were having Leaf and 50% were having Rat Pattern, The difference between the groups was statistically significant.

COMPARIOSN OF AP AND SI BETWEEN SUBJECTS WITH AND WITHOUT OSMF

In the subjects with OSMF the mean AP score was 26.82±4.77 whereas in the subjects without OSMF the mean AP score was 33.62±2.67. The difference between the groups was statistically significant.

In the subjects with OSMF the mean SI score was 7.80 ±1.77 whereas in the subjects without OSMF the mean SI score was 9.34± 1.42. The difference between the groups was statistically non-significant.

Shapes observed in the study



Fig.1 DISTORTED SHAPE



Fig .2 LEAF SHAPE



Fig .3 RAT TAIL



Fig. 4 BUTT SHAPE

Table- 1 : PALATE SHAPES ACCORDING TO STAGES OF OSMF

	Distorted	Leaf	Rat tail	P value
Stage I	0	1	0	0.001
	.0%	100.0%	.0%	
Stage II	1	1	0	
	50.0%	50.0%	.0%	
Stage III	0	1	1	
	.0%	50.0%	50.0%	

Chi Square test at $p \leq 0.05$ is significant

Table -2 : COMPARIOSN OF AP AND SI BETWEEN SUBJECTS WITH AND WITHOUT OSMF

	GP	Mean	Std. Deviation	Std. Error Mean	P value
AP	Without OSMF	33.62	2.67	1.19	0.030 (Significant)
	With OSMF	26.82	4.77	2.38	
SI	Without OSMF	9.34	1.42	0.637	0.190 (Non-Sign)
	With OSMF	7.80	1.77	0.886	

Disscusion

Oral Submucous fibrosis has a multifactorial etiology, areca nut and gutka considered to be most common and superior cause for the disease.it does not only influence buccal mucosa, tongue, lips but submucosa of soft palate as well. [6,1] Acquiring careful understanding concerned to the changes noticed in soft palate during the increasing stages of oral submucous fibrosis and in the individual who is having a habit with no mucosal changes will be helpful for the dentist to halt the progressive disease at that particular stage to prevent further complication and restore structural and functional outcomes.

The clinical observation of soft palate is not possible, so to evaluate the changes in soft palate lateral cephalometry is a prime radiograph to be concerned. The cephalometry helps to visualize the dimensional and configurational changes noticed in the soft palate with less radiation exposure.

In present study distorted shape is noticed in group I which is correlating with study done by khaitan t et al [1] in healthy individuals.

In the present study, group I (OSMF)shows predominantly 60%leaf shape followed by 20% distorted and 20 %rat shape which was in agreement with studies done by Ashwani et al[6], Deshmukh et al [5], chintamaneni et al [9], Shanker et al [10]. And leaf shape pattern is more predominant in group II of about 40% leaf and 40% rat shape which is in accordance to the studies done by khaitan .t .et al 1, Nagaraj et al [2], Santosh et al [3], you et al[10].

According to the stages in our study group it was found that leaf shaped, group 2-50% distorted ,50% leaf shaped ,group-3 50%leaf and 50% rat shape observed, which is similar to the study done by Patil et al[5] in group 1 only group 2 and group 3 result was dissimilar to our study.

According to anteroposterior length and superoinferior width in group I was 26.82±4.77 and SI 7.80 ±1.77 and Group II AP length 33.62±2.67 and SI width 9.34± 1.42. Corelating with Patil et al[5], Supriya et al[8] Chintamaneni et al [9],Shanker et al [10], with respect to AP length the SI width was non-significant in our study.

Conclusion

As the disease progresses the dimension and configuration of soft palate also changes. Lateral Cephalogram can be efficiently used to assess the shapes and size of soft palate which is altered in individual with or without the habits, so it can be beneficial in assessing fibroses in individual with habit at an early stage.

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Shapes observed in the study

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An Observational Analysis of Suicidal Deaths during COVID 19 Pandemic Lockdown at Lucknow, India

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Abstract

COVID 19 Pandemic has affected the global population. . In this paper we have tried to study the possible contributing factors to suicides along with the demographic data of people committing suicides. **Aim of the study-** to analyse the role and magnitude of severity of these factors contributing to suicides and devising possible strategies to help the vulnerable groups to cope with such situations in future**. Material &methods-** We had conducted an observational analysis of suicidal deaths which occurred in Lucknow during the Lockdown period in India. **Observations**- A total 59 suicides were reported in Lucknow during the lockdown period of 69 days. Majority of suicides were done by Hanging (93.2%), Economic factors accounted for 49.2%, domestic conflicts 23.7%, psychological and emotional factors (27.1%). **Conclusion-** we should try to develop strategies and appropriate and timely interventions to eliminate the contributing predisposing factors to suicide.

Keywords *–Suicides, Lockdown, Hanging*

Introduction

COVID 19 Pandemic has affected the global population. Various countries have taken different measures to deal with the situation and mostly a lockdown was implemented to prevent the community spread. Government and other institutions throughout the world are intensely focussed on saving the physical bodies from COVID 19.Along with the physical threats and the compelled containment strategies along with it has inflicted deep hurt to our emotional well being. As it was noted that many individuals worldwide started developing suicidal ideations during this Global Pandemic and they tried all sorts of suicide prevention trainings to deal with the psychological crisis during the lockdowns [1, 2]. Lockdowns did help in halting the progress of the disease in India, but at the same time it had a detrimental effect on the lives of various people leading to 300 suicides during the lockdown period [3]. In this paper we have tried to study the possible contributing factors to suicides along with the demographic data of people committing suicides. The aim of the study was to analyse the role and magnitude of severity of these factors contributing to suicides and devising possible strategies to help the vulnerable groups to cope with such situations in future, keeping the Indian scenario in mind[4].

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Material & Methods

We had conducted an observational analysis of suicidal deaths which occurred in Lucknow during the Lockdown period in India, which was taken as a preventive method to contain the spread of Corona Virus. The Lockdown period lasted for around 69 days, beginning from 24thMarch 2020, and continuing till 31st May 2020.Data was collected from all the four zones of Lucknow, with the assistance of Joint commissioner Police, Lucknow.

We used a standardized data extraction procedure to collect data on the following parameters

1. Contributing Probable stressors during the lockdown

a) Interpersonal conflicts

b) Financial losses

c) Emotional loss-death of a loved one /relationship breakups

d) Heightened anxiety/corona fear

e) Extreme poverty/starvation

2. Demographics: Age; sex; occupation & living conditions

3. Clinical variables:

a) pre existing mental health illness-depression, anxiety disorder,

b) substance abuse, including alcohol, drugs, or both

c) co morbid medical conditions.

d) COVID 19 status

4. Method of suicide

Observations

A total 59 suicides were reported in Lucknow during the lockdown period of 69 days.

Table-1:Suicides according to age and sex; age standardized suicide rates

Sno.	Age range	Male	Female	Total number
1.	<18years	0	3	3(5.08%)
2.	18-35 years	16	22	38(64.4%)
3.	36-60 years	13	1	14(23.72%)
4.	>60 years	4	0	4(6.77%)
5.	Total	33(55.9%)	26(44.4%)	59

33 suicides were by males, 26 suicides were by females

Table-2: Manner of Suicide

S.no	Manner of suicides	Male	Female	Total
1.	Hanging	32	23	55(93.2%)
2.	Poisoning	0	2	2(3.3%)
3.	Drowning	1	1	2(3.3%)

Majority of suicides were done by hanging (55 cases),only 2 cases of suspected poisoning and two cases of suicides by jumping into the river were noted.

Table-3:Probable Contributing Factors leading to suicide

S.no	Reason for suicide	Male	Female	Total
1.	Financial losses/job loss	13	3	16(27.1%)
2.	Domestic conflicts & violence	4	10	14(23.7%)
3.	Poverty and hunger	12	1	13(22.2%)
4.	Anxiety & depression	4	12	16(27.1%)

Economic factors accounted for29 cases, followed by domestic conflicts with 14 cases, psychological and emotional factors accounted for 16 cases

Table-4: Occupations linked to suicides

S.no.	Occupation	Male	Female	Total
1.	Business /self employed	9	1	10(16.9%)
2.	Daily wages worker	13	2	15(25.4%)
3.	Govt. employee	2	1	3(5.0%)
4.	Domestic help	3	6	9(15.2%)
5.	Housewife	-	10	10(16.9%)
6.	Farmer	4	-	4(6.7%)
7.	Student	1	4	5(8.4%)
8.	Health care worker	1	2	3(5.0%)

Discussion

In our study, we found 59 cases of suicides which occurred during the lockdown period of 69 days, in Lucknow, the capital of largest state of India. Among these, 55.9% suicides were seen in males and 44.4% suicides were done by females. Maximum suicides were reported in the 18 -35 year group (64%). The younger less than18 age group and above 60 age group had the least number of cases. The possible logical explanation is that the productive adults bore the maximum grunt related to hurdles and stress arising because of the lockdown, whereas the children and the elderly felt protected and secure within their family environment.

The most common method which was used for suicide commission was found to hanging (93.2%). We would again attribute this to the lockdown effect,

as people had limited accessibility to other means. There were only two cases which were suspected of self ingestion of a toxic substance, and two individuals committed suicide by jumping into the river.

The possible contributing factors which triggered these suicides were mainly financial or related to loss of income during lockdown (49.2%). There was a significant increase in the number of domestic violence cases which triggered the plug in many vulnerable females (23.7%).Though the government arranged for free distribution of essential commodities to the poor, there were some unfortunate who couldn't access these facilities and therefore ended their life because of extreme poverty and hunger. Psychological and emotional factors as a result of isolation and separation from partner, relationship break ups led to severe anxiety and depression which acted as a catalyst for triggering the suicide in 27% cases[5,6].

The daily wagers and the self employed citizens were badly stuck by the lockdown (36%). The future uncertainties and the increasing toll of COVID 19 worldwide drew the strings of unemployment, hopelessness and helplessness, and forcing them to take such drastic steps. The domestic helps which are an integral part of the working middle class families in India and the housewives ended their lives mainly because of increased domestic conflicts as most the husbands were staying at home .the subtle seeds of frustration and anger against an invisible enemy led to a volatile temper and increased episodes of domestic violence.

The forefront health care workers went through a different dilemma. They were the true corona warriors and they were expected to be on duty while the others were having a forced vacation and apparent good times during lockdown[7,8]. The ethical obligations of medical profession, along with anxiety and fear of contracting the infection while treating the COVID 19 positive patients acted as a predisposing factor and led to three suicides among health care workers[9,10].

Conclusion

The Corona Pandemic has fuelled anxiety and loneliness, without any clues related to its end point. Mental health services in India were highly inadequate in the pre Covid times, with only 4000 Psychiatrists to cater to such a large population. The government along with help of various NGOs should work on the development of mental awareness of individuals. The health care sector needs to strengthen its suicide screening services. Community based gatekeeper training programmes for early identification of suicidal ideations should be implemented. If we could work on appropriate and timely intervention for the prevention of risk factors precipitating suicides, we could save a significant number of precious and valuable lives in foreseeable lockdowns.

Conflict of Interest-None

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Forensic Odontology in India: Present Scenario, Hurdles & The Way Forward

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Abstract

The utilization of forensic odontology services in India is not proportional with the volume of research undergoing in this specialty. A lot of scientific knowledge is generated in this field, but recommendations on how to translate this knowledge into real world for the benefit of stake holders are lacking. This article presents some of the key areas where forensic odontology can be of significant help, highlighting the present scenario and hurdles associated with them along with recommendations for better utilization of forensic odontology services. A special act for the regulation of activities of a forensic odontologist would expedite the mainstreaming of forensic odontology. A credentialing body modeled on the functioning of various international organizations needs to be constituted in India.

Key Words: *Forensic Odontology, Disaster Victim Identification, Bite Mark, Age Estimation*

Background

Developing nations including India are vulnerable to many sort of disasters, spanning from natural to man made. The nation recently witnessed the wrath of nature in the form of floods hitting the Uttar Pradesh and Bihar regions causing multiple casualties[1]. Many lives are also lost because of human error in the form of structural failure, Kolkata flyover collapse being the latest example[2]. Most of the bodies of victims of such disasters remain unidentified.

With gender equality being the center of sustainable development, crime against women are on the rise in India[3]. Latest Govt. report on crimes against women states that, more than 1,28,000 rape cases were pending trial in Indian courts by 2017, the latest year for which data is available[4]. More than 100 children are sexually abused everyday in India as per National Crime Records Bureau data[5]. Many cases of sexual violence remain pending for long period in courts for the want of evidence.

With India implementing Citizenship (Amendment) Act, 2019 the country is facing issues about illegal immigrants and refugees. These illegal immigrants/ refugees have no official documents such as Govt. issued identity card, address proof or birth certificate with them. In addition to this issue, in India many births occur outside hospital setup and they are never reported to the authorities[6]. Therefore many Indians too, along with the illegal immigrants and refugees, do not have birth certificates. While deciding between illegal immigrants and Indian legal citizens depends upon specific documentary proofs, the issue of date of birth, thus age remains unanswered.

Insurance regulatory & development authority of India (IRDAI) is set to include dental treatment under the umbrella of insurance cover[7], but it has not addressed the potential problem of dental fraud.

Utilization of forensic odontology (FO) services is not proportional with the volume of research undergoing in this specialty. Many scientists are doing the novel work of improving the existing knowledge by

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continuous research in the field, but no research papers are yet published that speak of a solution to the problem of negligible real time usage of this specialty. There is a sizeable gap between the representation of FO in research and academic areas and its actual utilization for the intended purpose. With the exception of a few publicized cases, utilization of FO is almost non existent in India.

Discussion

Disaster Victim Identification (DVI)

As citizens of different nationalities can become victims of a disaster, the nation's DVI policy holds great importance. DVI is primarily the responsibility of the nation in which the disaster happens.

Present scenario:

National Disaster Management Authority is the nodal authority for disaster management[8]. But no policy on DVI exists.

Hurdles:

Lack of DVI policy has been the cause of many unidentified bodies[9] thus contributing to the persistent agony to the families of the departed.

Recommendations:

Initially, DVI policy of Interpol[10] can be adopted to constitute a core team of experts which includes forensic odontologist, forensic anthropologist, forensic geneticist and a fingerprint expert.

Expected outcome:

Efficient implementation of DVI policy will result in rapid closure of matters (financial, social, etc) associated with the departed.

Identification of human bodies & remains recovered from a crime scene

Investigation of a homicide case cannot progress until the body or recovered remains are positively identified. Dental identification is promising in cases of mutilated, charred or decomposed bodies.

Present scenario:

Unidentified bodies recovered from a crime scene are photographed and these photographs are circulated with other police stations (on official websites) for visual recognition. Unidentified bodies are stored in mortuary freezer for 72 hours, after which they are disposed[11, 12]. In very few cases only, recovered human remains are dispatched for Deoxyribose Nucleic Acid (DNA) identification.

Hurdles:

Not many efforts are put in for scientific identification[13]. An unidentified body stalls further investigation of a homicide case[12]. DNA identification, which is technique sensitive, costly and time consuming, does not guarantee results in every case[14]. Services of forensic odontologist are not sought on a regular basis.

Recommendations:

In such cases, oral autopsy should be made mandatory. Forensic odontologist will perform oral autopsy to facilitate dental identification of the unidentified body or human remain in the form of a skull[15].

Expected outcome:

Scientific identification of unidentified bodies will expedite the process of investigation and will assure accuracy of the death certificate thus issued.

Bite-mark analysis

Bite-marks not only allow for the identification of the biter, but their presence on different areas of the body is also a tell tale sign of the nature of offence committed.

Present scenario:

Medical officers are responsible for collecting evidences from a sexual offence survivor[16]. Govt. has given detailed guidelines on the components of the medicolegal examination report of sexual violence[16]. Forensic medicine experts collect such evidences in cases the victim is dead.

Hurdles:

Although the guidelines acknowledge bite mark as

one of the many physical injuries possibly found on the body of a victim, it does not comment on collection of bite mark evidence itself. Neither medical officers nor forensic medicine experts are trained in identifying, collecting & interpreting bite-mark evidence.

Recommendations:

Services of a forensic odontologist should be sought in each and every case of a sexual assault and rape for identification of bite-mark injuries and relevant evidence collection. Forensic odontologist should be appointed at each one stop center[17] dedicated to the survivors of such crimes.

Expected outcome:

Accurate interpretation of bite-mark evidence will add significant merit to the prosecution's case and lead to speedy delivery of justice[18].

Age estimation of living individuals

It is required in cases where knowing the age of the victim or accused is pivotal to the outcome of the case.

Present scenario:

As many births in India occur outside hospital setup[6], birth certificate is never issued in such cases. Indian civil & criminal courts, call for a bone age test" to solve the problem of age with such people[19]. Such cases are referred to radiologists and forensic medicine experts. Age estimation is also done for sexual violence survivors[16].

Hurdles:

Bone age test means skeletal age estimation. Overall, the accuracy of dental age estimation is better and in developing age group the accuracy is remarkable as compared to skeletal age estimation[20]. Annexure 3 of Govt. issued document[16] which defines guidelines for age estimation of the sexual violence survivor, are not in accordance with the latest research in the field of forensic age estimation, especially dental age estimation. Radiologist and forensic medicine experts are not trained in dental age estimation.

Recommendations:

Dental age estimation (DAE) should be made mandatory to those who are not in possession of birth certificate or when its authenticity is contested. Judiciary should seek opinion of forensic odontologist for dental age estimation in such cases along with bone age test. DAE also has immense applications with respect to sports and child labour (The Factories Act, 1948).

Expected outcome:

As DAE is in a narrow range, chances of a juvenile being wrongly estimated as an adult and an adult being wrongly estimated as juvenile are far too less. DAE will result in better resolution of issues that are age sensitive, so that justice is not miscarried.

Age estimation and sex determination of human remains

Age and sex are important aspects of biological profile of the dead and their assessment allows the police to narrow down the possibilities in identification efforts. The question of age and sex is also significant where human remains are recovered from archaeological sites.

Present scenario:

Opinion of anthropologists and radiologists is taken to estimate age and determine sex of the human remains recovered from a crime scene or archaeological excavation site[21].

Hurdles:

The opinion of forensic odontologists is rarely sought in such situations. In western world such expertise has given valuable insights into the case[22].

Recommendations:

Expertise of forensic odontologists in areas such as face reconstruction and opinion on the race, ethnicity, culture, dietary habits, occupation can greatly help both the law enforcement as well as archaeologists, anthropologists and historians.

Expected outcome:

Knowledge of biological profile in the form of age, sex, race, ethnicity, etc. will not only solve the mystery of identity of the victim of a crime but also help us understand human history in a better way.

Opinion on dental trauma with respect to Section 320 Indian Penal Code (IPC)

Fracture or avulsion of a tooth is defined as grievous hurt under section 320 IPC.

Present scenario:

General practicing dentists are unaware of legal implications of dental trauma. Many cases of oro-dental trauma, which are in fact medico-legal in nature go unnoticed in general dental practice.

Hurdles:

Private dentists are focused only on the treatment aspect of such cases, completely ignorant of the legal implications. Not many dentists are versed with IPC section 320 which describes grievous hurt.

Recommendations:

Practicing dentists should be trained to identify and manage medico-legal cases or they should refer such cases to forensic odontologist or appropriate authorities. Emergency care and legal aspects of oro-dental trauma should be a part of dental undergraduate training.

Expected outcome:

Proper documentation of oro-dental injuries by the treating dentist will help law enforcement authorities in investigation.

Opinion in abuse cases

Signs of physical abuse are concentrated in head, neck and face region of the victim.

Present scenario:

Many cases of abuse go unnoticed by dentists. Dental practitioners are unaware of their vantage position to detect cases of abuse, because the region where signs of abuse are mostly found is actually the expertise of a dentist.

Hurdles:

As the abuse is not detected, thus intervened at right time, the abuse may continue, resulting in deep psychological trauma apart from the physical injuries incurred[23].

Recommendations:

Dental practitioners should be made aware of their social responsibility of reporting a potential case of abuse to appropriate authorities. Dentists who are already trained to diagnose & treat oro-dental trauma should be oriented towards this issue.

Expected outcome:

Many lives can be saved by timely intervention and stoppage of this vicious cycle of abuse.

Opinion in dental fraud cases

Multinational companies give various health related perquisites to their employees such as reimbursement of expenses incurred for dental treatment and dental insurance.

Present scenario:

This dental treatment expenditure is reimbursed to the employee upon submission of relevant documents as per the company's policy. Insurance claims are also settled in a similar manner.

Hurdles:

The employees may produce fake documents in collusion with a dentist for financial gains[24]. With India on the verge of inclusion of dental treatment in health insurance, the possibility of dental insurance fraud cannot be denied. This menace has already plagued developed nations where dental insurance is already in the mainstream[25].

Recommendations:

Corporates offering dental benefits and insurance companies should hire forensic odontologists as claim scrutiny officers.

Expected outcome:

IRDAI will make necessary provisions to keep a check on fraudulent cases. Fraudulent claims can be segregated from the genuine claims, increasing the overall efficiency of claim settlement procedure.

Conclusion

Govt. in the financial budget for 2020-21 has

announced a central forensic science university, which is a welcome move as it will increase the number of forensic experts in the country. This move will also spread awareness of forensic services among the stake holders.

Section 45 of The Indian Evidence Act, 1872 bestows the crown of an Expert Witness on the forensic odontologist. Although no additional legal provision is necessary in this regard; however, a special act for the regulation of activities of a forensic odontologist would expedite the mainstreaming of FO. To achieve this goal, Govt. should formulate an expert core committee consisting of all the stake holders such as police, judicial, scientific personnel, social workers and representatives of the people. The task of the core committee is putting up the road map of FO in the form of a bill. The bill should then be passed as an Act through appropriate constitutional provisions. Thereafter, the Govt. should issue directives to concerned authorities, regarding implementations of the recommendations of core committee in compliance with the said Act.

The functioning of forensic odontology societies of developed nations such as American Board of Forensic Odontology, British Association of Forensic Odontology, Bureau Of Legal Dentistry, Australian Society of Forensic Odontology needs to be studied. A credentialing body modeled on the functioning of these organizations needs to be constituted which will maintain a list of qualified experts as well as formulate the standard operating procedures related to different aspects of forensic case work to maintain quality of the forensic reports. Govt. should make necessary amendments in relevant acts to accommodate and better utilize the services of FO.

Complete utilization of the spectrum of FO services will ensure more names assigned to the unidentified bodies, higher conviction rate in sexual violence and homicide cases and reduction in child abuse. Forensic odontology is central to the overall motive of uplifting humanity even after death by promotion of humanitarian and ethical forensics.

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Impact of Nutrient-Gene-Interaction on Periodontal Diseases- An Over View

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Abstract

The key role to maintain equilibrium between microbial challenge and host response is played by nutrition, which is also important in various chronic diseases like cardiovascular disease, type 2 diabetes & rheumatoid arthritis. An individual's baseline inflammatory position is influenced by macronutrients & micronutrients which modulate pro inflammatory and anti-inflammatory cascades. The aim of this review is to give an insight on mechanism of action of various macronutrients, micronutrients and some of the trace elements with their positive effects in resolution of various chronic diseases such as diabetes, cardiovascular, cancer and periodontal diseases. Thus dietary changes can be associated with genetic composition, this in turn may be due to changes in individual response. This highlights the significance of unravelling the role of nutrient gene interaction in progression of chronic diseases. Periodontitis is linked to a number of chronic systemic diseases which emphasize the significance of effective periodontal disease management for tooth retention as well as for overall health.

Keywords: *Genomics, Nutrition, Periodontitis, Inflammation*

Introduction

Periodontal disease is a microbial originated chronic inflammatory disease, in which impaired immune inflammatory processes are responsible for the majority of host tissue damage and ultimately leading to tooth loss

Periodontal diseases have multifactorial etiology and have a variety of risk factors Risk factors may be modifiable genetic, environmental (e.g., stress, bacterial challenge) and non –modifiable factors like lifestyle changes, nutrition and smoking. Environmental factors (including food nutrients), and genetic makeup play an important role in phenotypic appearance of a trait via central dogma of biology. The vitality of the periodontal tissues, in both health and disease, depends strongly on an adequate source of essential nutrients being available to the host[1,2]. For many years, the literature has cited the significance of nutrition in a range of chronic inflammatory diseases, all of which are linked with periodontitis, but in recent years, there has been an increased research investigating this phenomenon; Thus, this article aims to discuss the impact of nutrition in periodontitis and how can individuals nutritional status effect this complex disease processes.

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Nutrition and Its Role

Nutrient is defined as a source of nourishment, where food, which can be metabolized by an organism to give energy and build tissue whereas nutrition is the organic process by which an organism assimilates food and uses it for growth and maintenance.

Six major classes of nutrients are fats, carbohydrates, proteins, minerals, vitamins, and water; among these the first three are considered as "macronutrients" because they needed in large quantities where as other three are

called as "micronutrients" since their daily requirement is in minimal quantity to maintain an optimal health. Common dietary chemicals have direct or indirect effect on the human genome, Determination of functions is regulated by the genes, and nutrition is able to modify the grade of gene expression[1]

NUTRITION AND PERIODONTAL DISEASE:

An individual's baseline inflammatory position is influenced by nutrients which gets regulated by pro-inflammatory & anti-inflammatory cascades. Periodontitis is associated with decreased serum micronutrients level. Poor diet, lifestyle and genetic factors have an impact on distribution, absorption, synthesis of micronutrients and bioavailability.

Difference in response of individuals to dietary modifications can be credited to difference in genetic makeup that highlights the significance of exploring the role of nutrient gene interaction in development of chronic diseases. In recent years there is increasing interest in association between nutrition & periodontal disease which may be attributed to: better understanding of mechanism behind periodontal tissue damage, advent of modern genomic tools & potential protective role of nutrients. A number of systemic diseases have been linked with periodontitis which stresses the significance of successful periodontal disease management to achieve tooth retention as well as sound overall health[2].

OXIDATIVE STRESS AND ITS ROLE IN PERIODONTAL DISEASE:

The pathogenesis of a wide range of chronic inflammatory diseases such as cardiovascular disease, type 2 diabetes & metabolic syndrome have been affected by oxidative stress. Oxidative stress is a common link between periodontitis & chronic systemic disease.[3]

Intake of food rich in antioxidants (like blueberries, broccoli, dark chocolate and red beans etc.) decreases the oxidative stress. Normally there is a balance between oxidants and antioxidants but when this balance is disturbed, there is an increase in oxidants level and decrease in antioxidant levels resulting in oxidative stress which causes local tissue damage. (Figure :1)

Antioxidants have an antagonistic effect on oxidative stress and *glutathione is* considered as most essential small molecule antioxidant, which is available in both reduced (GSH) and oxidized form (GSSG) and the ratio of GSH: GSSH normally favors GSH which is a potent scavenger of free radicals, cell metabolism, DNA synthesis and repair. Higher degree of periodontal disease has been associated with depletion of glutathione. Selenium dependent glutathione enzyme is involved in decline of destructive lipids and phospholipid hydro peroxides to harmless products.[4] Smoking increases the oxidative stress by reducing Vitamin C and Glutathione levels.[5] Increased intake of dietary sugars causes generation of mitochondrial superoxide radicals during ATP synthesis that enhances the oxidative stress. In hyperglycemia, the binding of glucose to protein in the tissues & bloodstream leads to formation of advanced glycated end products (AGE).

Neutrophils have receptors for AGE called RAGE (receptors for advanced glycated end products). Interaction of AGE-RAGE activates NADPH oxidase enzyme complex to generate oxygen free radicals, this process is known as respiratory burst. Metabolism of excess saturated fat causes increased serum low density lipoprotein cholesterol (LDL) which upon oxidation forms oxidized LDL. This oxidized LDL binds to toll like receptors present on the neutrophil membrane thereby activating the NADPH oxidase enzyme to generate oxygen free radicals which further add on to the oxidative stress. These dietary cellular interactions that are increased after consumption of meal containing simple sugars and saturated fat produce inflammation which is associated with increased serum glucose and lipid levels, oxidative stress & downstream pro-inflammatory effect and is known as Meal Induced Inflammation.[6] (Figure: 2)

Coenzyme Q10 (CQ10)[7] is a natural coenzyme present in the inner membrane of mitochondria & plasma lipoproteins. It functions to produce ATP which is a bio energizer. Also, CQ10 scavenges the free radicals and reactive oxygen species thereby reducing the collagen degradation. Since CQ10 functions like a potent antioxidant, vegetables rich in CQ10 like broccoli, spinach, cauliflower and animal food like red beef should be incorporated in our diet.

NUTRITION –SYSTEMIC DISEASES AND PERIODONTAL DISEASE:

Nutrition is implicated in various inflammatory diseases and conditions like cardiovascular disease, type 2 diabetes, inflammatory bowel disease. All of these diseases have been connected with periodontal disease. Nutrition plays a key role in maintaining the balance between microbial challenge and host response. Common risk factor associated with chronic diseases include increased intake of saturated fat, sugars and decreased intake of fruits, vegetables and fiber.

Single vitamin antioxidant and supplementation fail to demonstrate the expected benefits and excess consumption of single vitamin destabilizes antioxidant network and causes generation of vitamin radicals which is more damaging to health than the deficiency of that vitamin. Physiologic impact of nutrients on host depends upon factors like bioavailability, absorption from gut into circulation, conversion to active bio-form & transport to target cells etc. Differences in response of individuals to dietary supplementation can be associated to differences in their genetic composition.

Vitamins like A, E, C and trace elements like Se, Cu, Zn are important in maintaining the epithelial integrity and structure, however they get depleted during periods of inflammation.[8] These trace elements and vitamins modulate immune cell function by regeneration of redox regulated transcription factor thereby counteracting reactive oxygen species damage eventually affecting the production of cytokines and prostaglandins. Zinc deficiency increases the susceptibility to periodontal disease progression.[9] n- PUFA intake increases tissue concentration of eicosapentaenoic acid & docosahexaenoic acid which down regulate inflammation. Elderly patients with intake of lowest level of n-PUFA fatty acids had higher incidence of periodontal disease and it was concluded that in elderly individuals there exist an inverse relationship between dietary fatty acids and progression of periodontal disease.[10]

NUTRIENT-GENE INTERACTION:

Nutrigenomics is an emerging field of science and technology unraveling the inter-relationship between nutrients & human genome using modern tools like transcriptomics, metabolomics, epigenomics and proteomics. These technologies monitor the activity of multiple genes simultaneously at the level of the proteins by proteomics, at the level of metabolites by metabolomics and finally at the level of RNA by transcriptomics.[11]

The whole set of RNA transcripts produced by the genome at any one time is termed as transcriptome. The study of the transcriptome and is thus a global way of looking at gene expression in aging, health and disease is called as transcriptomics. Proteomics deals with studies to explore protein structures and activities. The studying of the metabolite profiles in biological samples like urine, saliva, and blood plasma is called as metabolomics[11].

Use of any one among these tools will not provide all the answers. The use of a combination of these tools will undoubtedly bring about a new understanding of health and disease. Applications in nutrigenomics research relates to health and prevention of chronic diseases through diet. There is a necessity to comprehend the molecular mechanism that define homeostasis at cellular, biochemical and organ levels in relation to healthy & diseased states. This highlights the need for molecular biomarkers that can detect pre-disease state and specifically reflect delicate changes in homeostasis and the efforts of the body to preserve it.

Diversity in response of individuals to change in dietary supplementation may be attributed to difference in their genetic makeup therefore, the interactions between genotype and diet is essential in defining the risk factors of common complex diseases including periodontal diseases (Figure:3).

According to a study conducted in 2006, when compared to men who consumed lowest amount of wholegrain, men with higher intake of wholegrain were 23% less likely to develop periodontitis.[12] Data collected from third National Health and Nutrition Examination Survey states that there was statistically significant association between markers of increased body mass and periodontitis and have concluded that obesity might be a potential risk factor for development of periodontal disease, especially in younger individuals.[13,14,15,]

NUTRIGENOMICS AND SYSTEMIC DISEASES

Meta-analysis comparing Asian and Western population has estimated approximately 30% reduction in prostate cancer risk in men consuming high soy isoflavone and approximately 15% reduction in breast cancer risk for pre-menopausal women. A study [1617] was conducted on 2000 Swedish men to demonstrate interaction between dietary isoflavone intake, polymorphism within estrogen receptor β gene and the risk of prostate cancer. It was observed that individuals consuming increased amount of phytoestrogen and isoflavone who were homozygous or heterozygous for single nucleotide polymorphism in estrogen receptor gene had a significantly reduced risk of prostate cancer.

Study relating colon cancer risk and nutrigenomics revealed that individuals who consumed high amount of well-cooked red meat carrying with a variant of N-acetyl transferase 2 gene had a significantly increased risk of colon cancer, and there was no statistically significant increase in risk in individuals with genetic variants alone or dietary exposure alone.[17]

A chief modifiable risk factors for cardiovascular disease is plasma lipid concentration. Endogenous and Pexogenous ligands like fatty acids, fibrates and their derivatives activate peroxisome proliferator activator receptor α (PPAR-α). Once ligand binding occurs it translocate into the nucleus and modulates gene expression (like expression of several key proteins involved in metabolism of triglycerides-rich lipoproteins and high density lipoproteins). In several populations, a polymorphism of PPAR-α gene has been detected which appear to affect its transcriptional response to dietary fatty acid intake. In populations with low intake of PUFA, subjects who carried PPAR-α polymorphism showed increased plasma triglycerides levels whereas there was little association between PUFA and plasma triglycerides levels in individuals without this polymorphism.[12]

Cross susceptibility between periodontal disease and type II diabetes may be partly attributed to common genetic risk factors which can be modulated by diet. Study was conducted on diabetic African American subjects to look for the association between periodontal disease and interleukin I genotype and they identified that genotype IL-1B-511 was significantly associated with periodontal disease.[18]

A study showed that in patients with type II diabetes, there was significant association between variant genotypes of both Interleukin-1A, Interleukin-1B and periodontitis which was not observed in non-diabetic controls.[19] Recent genomic studies have recognized a genetic susceptibility locus for type II diabetes comprising a non-synonymous single nucleotide polymorphism in β-cell specific, zinc transporter gene which may be crucial in insulin release & insulin storage.[20]

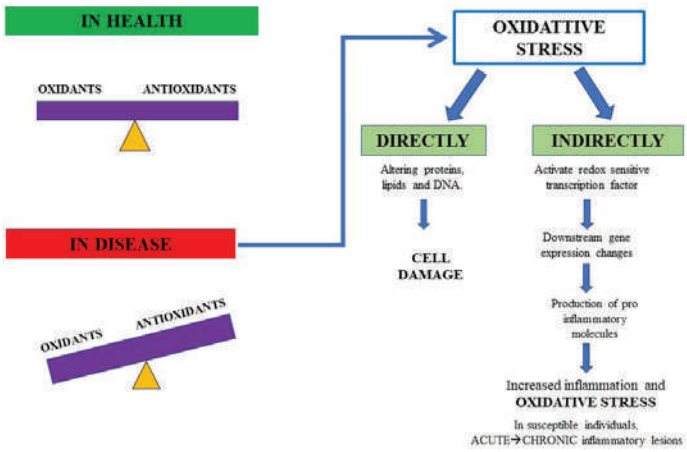


Fig: 1: Mechanism of Oxidative Stress Mediated Destruction

Single nucleotide gene polymorphism has been related to increased risk of type II diabetes concluding the key role of zinc in etiology of type II diabetes. A study was conducted in an insulin secreting β cell-line model which revealed that there was a positive effect on glucose induced insulin secretion when extracellular zinc concentration was increased implicating a potential benefit of zinc supplementation on susceptibility to type II diabetes with C allele.[21]

These studies show the importance of understanding observed differences in the response of an individual to dietary modification which can be attributed to differences in their genetic makeup thereby highlighting the significance of unravelling the role of nutrient gene interaction in progression of chronic diseases[21].

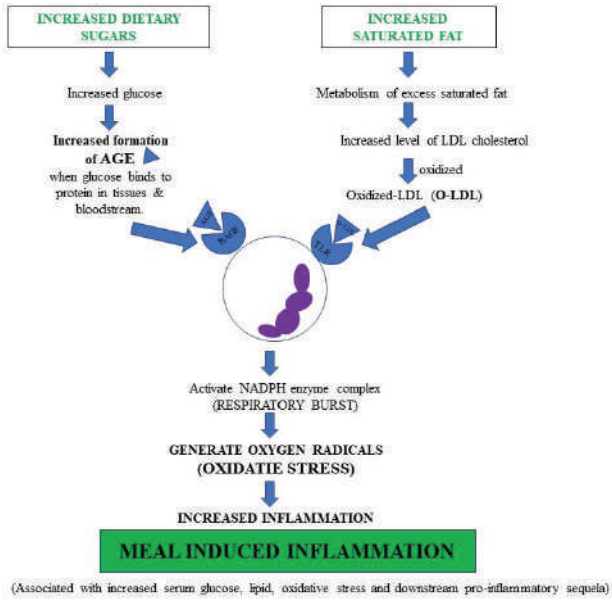


Fig: 2: Meal induced inflammation

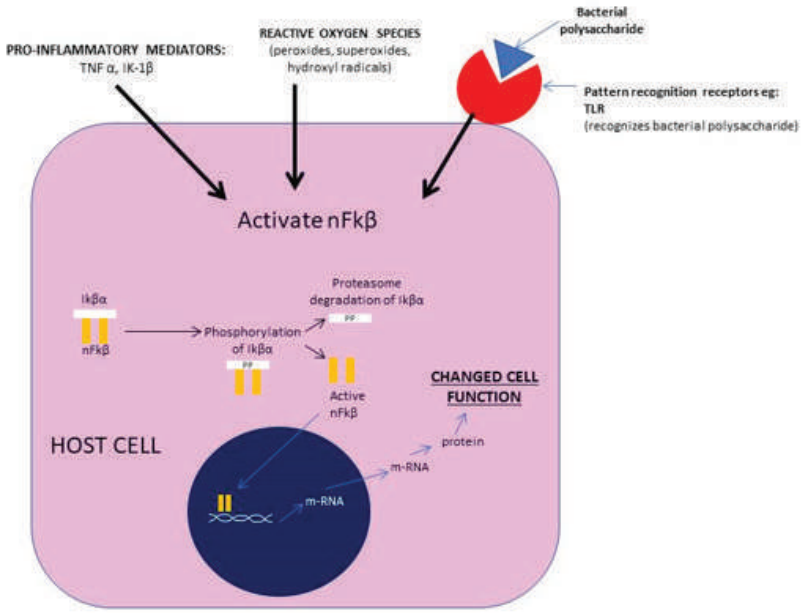


Fig: 3: Various factors that result in changes in gene expression

AGE: Advanced glycated end products

RAGE: Receptors of advanced glycated end products O LDL- Oxidized low density lipoprotein

TLR- Toll like receptors

Conflict of Interest: Nil

Source of Funding: Nil

Conclusion

Diet is a major environmental factor that has an effect on the genome to modulate disease risk. Genetic makeup of an individual is also an attributing factor for various systemic diseases that ultimately affects the natural host mechanism for the resolution of inflammation in chronic diseases like periodontitis. A clear understanding of nutrient-gene interaction is important for disease prevention through optimization of dietary recommendations. However, further studies are needed to better understand the use of nutrients to maintain good health and to prevent chronic diseases through modifiable epigenetic mechanisms.

Ethical Clearance: Approval for publication was received research and review committee of the institution

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Comparison of Bone Mineral Density in Patients with Celiac Disease and Healthy Individuals

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Abstract

Objectives: To study the effects of celiac disease on bone mineral density.

Design: Case-control study.

Setting: The study was conducted in Department of Anatomy, Sardar Patel Medical College, Bikaner, Rajasthan.

Participants: A total of 90 patients, 60 control group healthy normal and 30 celiac disease patients case group were incorporated in study with informed consent.

Material and Methods: The bone mineral density was measured in case and control group at calcaneum by peripheral dual X-ray absorptiometry along with body mass index and age.

Results: The significant difference was found between the bone mineral density of case and control group (0.87±0.49 & 0.61±0.44: p=0.0001) and 43.33% subjects had osteopenia in case group while only 5% subjects had osteopenia in healthy group. The significant difference of bone mineral density in both groups below the age of 15 years was non existing but between the age group of 16-30 years it was significant(-1.17±0.68 & 0.68±0.21: p=0.001)

Conclusion: Celiac disease patients are at higher risk of osteopenia so it is suggested that they should be investigated in early time of diagnosis mainly in the growing years because it affects most at this time.

Key Words: *osteopenia, celiac disease, Bone mineral density, Body mass index*

Introduction

Celiac disease is an autoimmune disorder resulting from genetic as well as environmental factors[1]. Approximately 1% of the world's population is affected by it[2]. The gluten protein stimulates celiac disease which mainly found in wheat, rye and barley and common symptoms which can be experienced are abdominal pain, weight loss, vomiting, dermatitis herpetiformis, hypocalcemia and constipation, although clinical indications of celiac disease differ considerably according to age group[3]. Celiac disease causes malabsorption which leads to low bone mineral density, osteoporosis, rickets[4] and out of these the more common complication of celiac disease is low bone mineral density[5]. As Celiac disease causes bone diseases the prevalence of affecting by these are much more extensive for late diagnosed patients and relatively lower in early diagnosed patients[6].

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To measure the bone mineral density dual energy x-ray absorptiometry method is greatly opted[7] because of its high accuracy and great precision as well as low radiation exposure[8]. Even though there is a common agreement that gluten free diet improves the calcium metabolism and reverse the effects of low bone mineral density[9].

In the view of this consideration, the aim of the study was to evaluate the difference between the bone mineral density of case group and control group so that the risk of osteopenia can be observed.

Materials & Methods

This cross sectional study was conducted in Sardar Patel Medical College and associated group of hospital, Bikaner, Rajasthan. This study included total 90 subjects, 60 control group and 30 case group, in which control group included the healthy subjects and in case group celiac disease patients were included. The subjects which had any surgery and other disease except celiac disease were excluded from this study. All subjects gave informed voluntary consent to participate in the study. This study was approved by ethical committee of Sardar Patel Medical College. Bone mineral density was measured at the calcaneum bone by peripheral dual energy x-ray absorptiometry.

An appropriate lifestyle questionnaire was provided to the all subjects to determine date of birth, sex, weight and height. Some questions were asked to the older subjects and few parents of young subjects. The height was measured manually by non stretchable measuring tape or measuring stick for accuracy of the data. Weight is measured by portable weighing machine. Bone mineral density was measured with a dual-energy x-ray absorptiometer. Any metal object was removed from the area to be scanned. During the measurement the subjects were made to place the heel in the foot support and the lower leg rests back against the leg support. Readings in the digital screen were recorded. Although scan time was short (15 seconds) again care must be taken to ensure that the patient keeps still and does not move as there are no immobilization devices supplied. For accessing the bone mineral density WHO osteoporosis definition, based on T-score results was considered. According to that T-score greater than -1 is normal, T-score between -1 and -2.5 is osteopenia and T-score less than -2.5 is referred as osteoporosis. To compare the bone mineral density values of control group and case group multiple regression analysis were used.

Result

In this study, bone mineral density of case group was significantly low than the control group and the mean±sd value was 0.87±0.49 of case group while mean±sd value of control group was 0.61±0.44. p value was (p=0.0001) which was highly significant. Table no. 1 showing the details mentioned above.

Table 1- Distribution of study population according to their BMD

(n=90)

	Case (n =30)	**Control (n=60)**	**P VALUE**
BMD	**MEAN ±SD**	**MEAN ±SD**	
	0.8763±0.491	0.618 ± 0.445	0.0001*

The case group showed comparatively more percentage of osteopenia than in the control group. 13 out of 30 subjects were osteopenic in case group almost 43.33% and 17 subjects out of 30 were normal but in the control group only 5% subjects showed the osteopenia which means 57 out of 60 subjects were absolutely normal. Table no. 1 showing above metioned details.

Table 2 -Distribution of study population according to presence of osteopenia (n=90)

Osteopenia	Case (n =30)		Control (n =60)	
	Frequency(n)	Percent	Frequency(n)	Percent
Present	13	43.33%	3	5.00%
Absent	17	56.67%	57	95.00%
TOTAL	30	100.00%	60	100.00%

In the age group of below 15 years mean±sd value of bone mineral density for case group was -0.54±0.41 and for the control group it was -0.57±0.51 that leads the p value to 0.84 (p=0.84) which was not significant but in the age group of 16-30 years the mean±sd value for case and control group was -1.17±0.27 & 0.68±0.21 and p value was 0.001 (p=0.001) which was significant.

The results and findings of our study correlates compared to published data (Burrows R[10] et al 1999) and which shows the difference of bone mineral density values between the celiac disease patients and the healthy controls. They included 39 celiac disease patients in case group and 39 healthy subjects in control group. The mean±sd value for case group was (-0.98±1.0) and for control group was (0.19±0.93) and the p value was lower than 0.000 which was highly significant. Another similar study by Stefano Pantaleoni[11] et al 2014 also compared with our study which reported that in 169 patients, 71 patients (42%) were normal, 62 patients (37%) were osteopenic meanwhile 36 patients (21%) were osteoporotic.

Conclusion

In our study we found that the bone mineral density levels low in the case group than the control group and comparatively more subjects were suffering from the osteopenia in case group than the control subjects. So celiac disease patients are suggested to access the bone mineral density in early time of diagnosis to escape from the risk of developing osteopenia. Although some authors suggested that bone mineral density values can be reversed by the gluten free diet. According to our study, the patients of 16-30 years of age group are at higher risk of lower bone mineral density and these years are very crucial for development of skeletal growth of an individual as bone formation and resorption activities are high during skeletal growth and because peak bone mass is gained in childhood and adolescence, the precautions must be taken. The bone mineral density test must be done and strict gluten free diet should also be followed for good bone health and to minimize the risk of osteopenia. In our study, the age group of below 15 years did not show any significant difference in bone mineral density between the case group and healthy group. Therefore the patients under the age of 15 years should also take care of their diet and should also be taking the bone mineral density test so that the risk of developing osteopenia in growing age is least.

As our study was a cross sectional study, a longitudinal and large population study is also required to get a detailed examination of calcium metabolism of celiac disease patients to get more detailed data for reference and to evaluate whether or not life style and dietary precautions help to make up for the decreased BMD in CD patients over time and avoid skeletal complications.

Ethical Clearance - taken from ethical committee of Sardar Patel Medical college, Bikaner, Rajasthan.

Source of Funding - Self

Conflict of Interest - Nil

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Study of Profile of Accused of Sexual Offences in Thoothudi Medical College- A Retrospective Study

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Abstract

Sexual violence is a major public health and human rights problem with consequences on women's physical, mental sexual and reproductive health. Data on sexual violence typically come from police, clinical settings, nongovernmental organisations and survey research. The magnitude of the problem of sexual violence may be viewed as tip of iceberg floating in water. There is limited amount of information on sexually violent men, it appears that sexual violence is found in almost all countries in all socioeconomic classes and in all age groups from childhood onwards. Data on alleged accused also show that most direct their acts at women whom they already know. A total of 70 cases were analysed, 40 (57.14%) cases belong to Hindu religion, 44 (62.85%) cases were unmarried, 32 (45.71%) cases were illiterate, 26 (37.14%) cases the offence occurred in open space, 34(48.57%) cases the incident was consensual act. In 35(50%) cases the alleged accused and victim was lovers, the age of the accused in 38(54.28%) cases were between 19-25 years, age of the victims in 55 (78.57 %) cases was in between 13 – 18 years. In examination of alleged accused there were no scars, ulcer, sinus discharge and sexually transmitted disease in any of the case. Sexual violence has generally been a neglected area of research in most parts of the world, yet the evidence suggests that it is a public health problem of substantial proportions. Much more needs to be done both to understand the phenomenon and to prevent it.

Keywords: *Sexual Assault, Alleged Accused, Accused Examination*

Introduction

Sexual violence is a major public health and human rights problem with consequences on women's physical, mental sexual and reproductive health. Sexual violence occurs in persons with intimate partnership, within large family or community or during times of war conflict, it is a deeply violating and painful experience for the survivor.(1) Available data suggest that in some countries nearly one in four women may experience sexual violence by an intimate partner and up to one third of adolescent girls report their first sexual experience as being forced(2)

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Data on sexual violence typically come from police, clinical settings, nongovernmental organisations and survey research. The magnitude of the problem of sexual violence may be viewed as tip of iceberg floating in water. Many women do not report sexual violence to police because fear being blamed, not believed or otherwise mistreated they are ashamed. Data from medicolegal clinics, on the other hand, may be biased towards the more violent incidents of sexual abuse. The proportion of sexual offence victims who seek medicolegal services for problems related to sexual violence is also relatively small. There is limited amount of information on sexually violent men, it appears that sexual violence is found in almost all countries in all socioeconomic classes and in all age groups from childhood onwards. Data on alleged accused also show that most direct their acts at women whom they already know (3).

Materials and Methods

The present study is a retrospective study conducted in Department of Forensic Medicine, Thoothukudi Medical college in the period Jan 2019 to June 2019. All the alleged accused of sexual offences brought to the department as medicolegal cases were included in the study. All the documents pertaining to the alleged accused such as history of the case, First Information Report, potency certificate, Forensic Science Lab report, were analysed in a systematic manner.

Results and Discussion

A total of 70 cases were analysed, in analysing religion 40 (57.14%) cases belong to Hindu religion, 10 (14.28 %) cases belong to Christian and 2(2.85 %) cases belong to the Muslim religion. Religion not mentioned in 18(25.71%) cases. **Fig:1.**

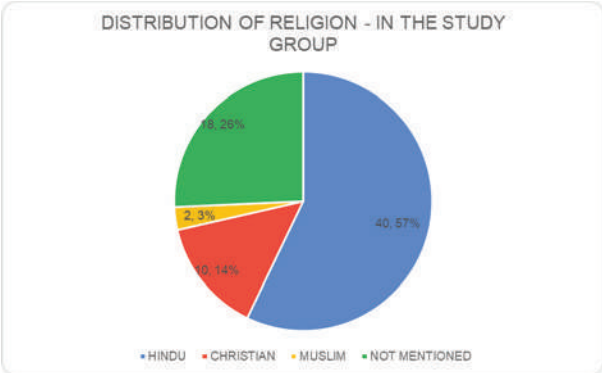


Fig:1. Distribution of religion among the study group

In analysing marital status of the accused 44 (62.85%) cases were unmarried, 26 (37.14%) cases were married. In analysing marital status of the victims 67(95.71%) cases were unmarried this is consistent with the study conducted by Surender K P[4] 97.23% victims were unmarried and 1.10% were married.

In analysing occupation of the deceased 58 (82.85%) cases were labour, 6(8.57%) cases were students and professional 4 (5.71%) cases and not mentioned in 2 (2.85%) cases

In analysing education status 32 (45.71%) cases were illiterate, 28(40%) cases completed high school, 4(5.71%) case completed degree and education status not mentioned in 6 (8.57%) cases.

In analysing the place of occurrence of the offence, 26 (37.14%) cases seen in open space, this is in contrast with the study conducted by surender[4] were 37.5% cases offence occurred in the house of the accused by 22 (31.42 %) cases were in accused house, 17 (24.28%) cases in relative house, in 5 (7.14%) cases victims house was the place of occurrence.

In analysing manner of offences 34(48.57%) cases was consensual, and in 28(40%) cases some amount of force was involved in the incident, in 8(11.42%) cases nothing was mentioned. In these cases, the age of the victim was less than 12 years. Fig:2

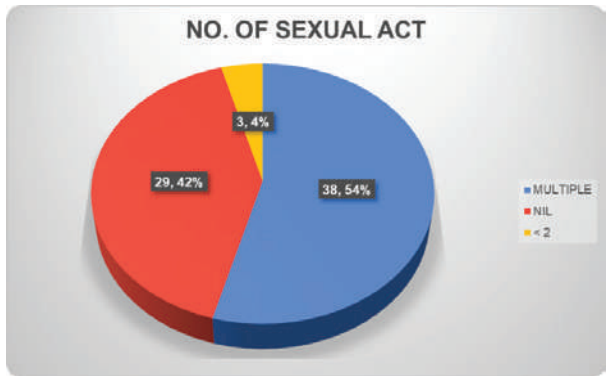


Fig:2. Number of sexual act by the alleged accused.

In analysing the relationship of the accused to the victim 35(50%) cases were lovers. This contrast to the study conducted by Lakew Z [5], were 42% were strangers. In this study 13 (18.57%) cases the alleged accused was neighbour. In 6(8.57%) cases stranger, friends and relatives in 5(7.14%) cases each. In 4(5.71%) cases student and teacher relationship existed. Fig: 3

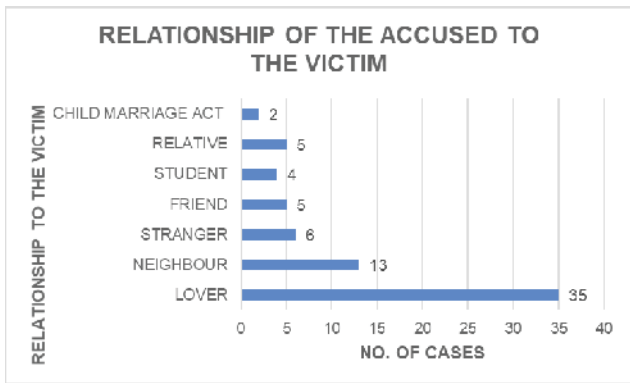


Fig:3. Relationship of the accused to the victim in the study group

In analysing age of the accused 38(54.28%) cases were seen in 19-25 years, this is consistent with the study conducted by Shrikant [6] and 73% of cases seen in 11-30 years in study conducted by Uchendu [8]. (22.85%) cases in 26 -32 years. 4 5.71(%) cases seen in 33- 40 years. 5 (7.14%) cases seen in 41 – 48 years. 4 (5.71%) cases seen in 49-64 years. The lowest age of the accused in the present study is 17 years and highest recorded age

of the accused is 68 years

In analysing age group of the victims 55 (78.57 %) cases were seen in 13 – 18 years, this is consistent with the study conducted by Rege S[7] were 65% of cases were seen in this age group. 9 (12.85%) cases 5- 12 year, in all the 9 cases in this age group, squeezing lips kissing, and or hugging. There was no history of vaginal or anal penetration in none of the case in this age group. 4 (5.71%) cases in 19-25 years, 2(2.85 %) cases in 26 – 32 years. **Fig:4**

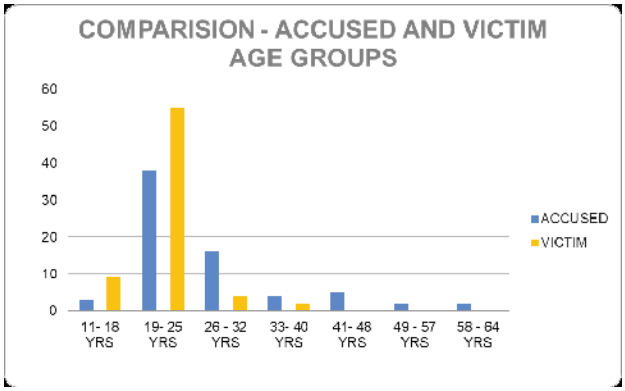
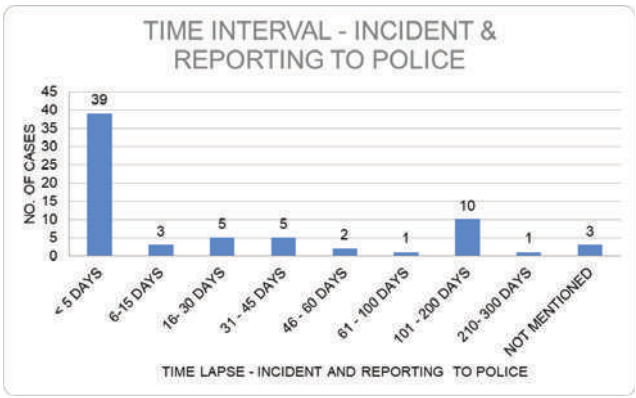


Fig: 4. comparison of age groups of accused and victim



In analysing time interval between reporting to the police station and medicolegal examination by the doctor, 18 (25.71%) cases was seen in 6-15 days this in contrast to the study conducted by Sarkar Se t al [9] were 61.11% were examined after six days. 16(22.85 %) cases was seen in less than 5 days, 14(20 %) cases was seen in 16-30 days. 8(11.42%) cases was in 31-45 days and 46-60 days each. 6 (8.57 %) cases was seen in 101-200 days. The reason for profound delay in medical examination after reporting to police station was not mentioned in the documents submitted by the concerned authority for examination. **Fig:5.**

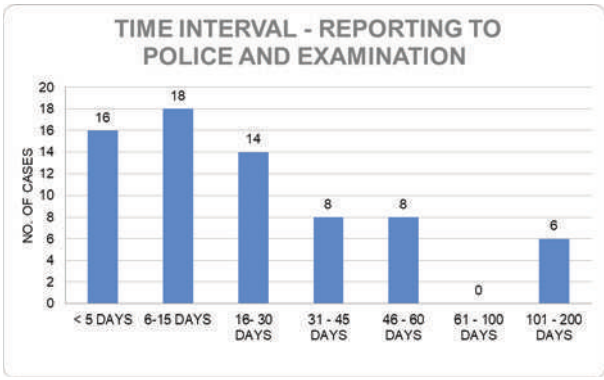


Fig:5. Time interval in reporting to police and examination

In physical examination reports of all the alleged accused there were no scars, ulcer, sinus discharge, sexually transmitted disease, was noted. Opinion to the potency in all the cases was given as *"There is nothing to suggest that the above examined individual is impotent"*. Samples to forensic science lab was not taken in any case. As most of the cases brought to department after Judicial custody and there was delay in reporting to the police station and department subsequently.

Table:1. Distribution of cases in the study group

Religion			
Hindu- 40(57.14%)	Christian- 10(14.28%)	Muslim-2(2.85%)	Not Mentioned-18(25.71%)
Education			
Illiterate-32(45.71%)	School-28(40%)	Graduate-4(5.71%)	Not Mentioned-6(8.57%)
Occupation			
Labour- 58(82.85%)	Student- 6(8.57%)	Professional -4(5.71%)	Not Mentioned- 2(2.85%)
Marital Status			

Cont.. **Table:1. Distribution of cases in the study group**

Married- 26(37.14%)	Unmarried -44(62.85%)				
Place Of Offence					
Victim House- 5(7.14%)	Accused House-22(31.42%)	Relative House-17(24.28%)	Open Space -26(37.14%)		
Manner Of Offence					
Forcible-28(40%)	Consensual-34(48.57%)	Nil-8 (11.42%)			
Relationship To The Victim					
Lover -35(50%)	Neighbour-13(18.57%)	Stranger-6(8.57%)	Friend -5(7.14%)	Student -4(5.71%)	Relative-5(7.14%)
Age Of The Accused					
11-18 Years-3(4.28%)	19-25 Years-38(54.28%)	26-32 Years-16(22.85%)	33-40 Years-4(5.71%)	41- 48 Years-5(7.14%)	49-64 Years-4(5.71%)
Age Of The Victim					
5- 12 Years-9(12.85%)	13-18 Years-55(78.57%)	19-25 Years-4(5.71%)	26-32 Years -2(2.85%)		
Time Interval Between the Incident And Reporting To Police					
< 5 Days-39(55.71%)	6- 15 Days-3(4.28%)	16-30 Days-5(7.14%)	31-45 Days-5(7.14%)	46-60 Days-2(2.85%)	61-100 Days-1(1.42%)
101-200 Days-10 (14.28%)	210-300 Days- 1(1.42%)	Not Mentioned-3(4.28%)			
Time Interval Between Reporting to The Police and Examination By Doctor					
< 5 Days -16(22.85%)	6- 15 Days -18(25.71%)	16-30 Days -14(20%)	31-45 Days-18(25.71%)	46-60 Days-8(11.42%)	61-100 Days-0
101-200 Days -6(8.57%)					
No. Of Sexual Attempts					
< 2 -3(4.28%)	Multiple -38(54.28%)	Nil – 29(41.42%)			
Ejaculation					
Present -35(50%)	Absent -29(41.42%)	Not Mentioned -6(8.57%)			
Genital Penetration					
Present -41(58.57%)	Absent -29(41.42%)				
Touching Private Parts Only					
Lips & Buttocks -9(12.85%)	Breast & Genitals -8(11.42%)				

Conclusion

Sexual violence is a common and serious public health issue affecting the people each year throughout the world. Which operates in range of social, cultural and economic contexts. Health professionals have a large role to play in supporting the victims of sexual assault – medically and psychologically and collecting evidence to assist prosecutions. The health sector is considerably more effective in countries where the protocols and guidelines for managing cases and collecting evidence,

where staff are well trained and where there is a good collaboration with the judicial system. Ultimately the strong commitment and involvement of government and civil society are required to end sexual violence. Sexual violence has generally been a neglected area of research in most parts of the world, yet the evidence suggests that it is a public health problem of substantial proportions. Much more needs to be done both to understand the phenomenon and to prevent it.

Conflict of Interest: Nil

Ethical Clearance: Nil

Funding: Nil

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Profile of Medicolegal Death in Females in Thoothudi Medical College- A Retrospective Study

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Abstract

The cause of unnatural deaths in females differ from that of males. Unnatural death is an intentional (hanging, poison ingestion) or unintentional death (road traffic accidents) as a result of external factors. The socioeconomic differences in lifestyle are associated with differences in attitudes towards life and hence accordingly the incidence of physical violence, suicides are encountered. There is an increasing incidence of traffic accidents among females in recent years, this may be a result of development in education and ocupational opportunities for female sex. A total of 70 cases were analysed, in analysing religion 60 (85.71%) cases belong to Hindu religion. 62 (88.57%) cases were married, 55 (78.57%) cases were house wife, 49 (70%) cases were illiterate, 20 (28.57%)cases was seen in 61-70 years of age group, road traffic accidents 16 (22.85%) cases were noted. Distribution and causes of unnatural female deaths in present study is not similar to the pattern found in most of the other Indian studies The present study revealed that the unintentional trauma, mainly the road traffic accidents are responsible for unnatural deaths of females i.e pedestrian and pillion riders. This highlights the importance of timely interventions of road safety measures.

Keywords: *Female Deaths, Autopsy, Road Traffic Accidents*

Introduction

The cause of unnatural deaths in females differ from that of males. Unnatural death is an intentional (hanging, poison ingestion) or unintentional death (road traffic accidents) as a result of external factors. Suicide rates in Indian females is 93878 in 2016(1) There is an increase in incidence of unnatural deaths in female especially first few years of marriage. The most obvious reason behind such deaths are unending demands of dowry (cash/ kinds) by their husbands &/or in laws, for which they sometimes kill or torture the bride in such a way that she commits suicide(2). The socioeconomic differences in lifestyle are associated with differences in attitudes towards life and hence accordingly the incidence of physical violence, suicides are encountered (3) There is an increasing incidence of traffic accidents among females in recent years, this may be a result of development in education and ocupational opportunities for female sex. This study has been carried out to access the pattern of female deaths and various factors associated with such deaths in Thoothukudi district of Tamilnadu, India

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Materials and Methods

The present study is a retrospective study conducted in Department of Forensic Medicine, Thoothukudi Medical college in the period Jan 2018 to June 2018. All the deceased females brought to the department as medicolegal cases were included in the study. All the documents pertaining to the deceased such as history of the case, accident register, inquest report, First Information Report, post-mortem certificate, viscera report, Histopathology reports were analysed in a systematic manner.

Inclusion criteria:

1. Medicolegal cases of female sex.

Exclusion criteria:

1. Transgenders.

2. Unknown bodies, whose identity was not established at the time of autopsy.

Results and Discussion

A total of 70 cases were analysed, in analysing religion 60 (85.71%) cases belong to Hindu religion, 6 (8.57%) cases belong to Christian and 2 (2.85%) cases belong to the Muslim religion. **Fig:1.**

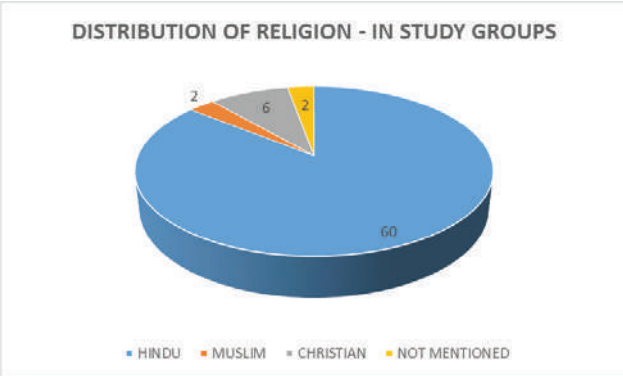


Fig:1. Distribution of religion among the study group

In analysing marital status 62 (88.57%) cases were married, 6 (8.57%) cases were unmarried. Marital status was not mentioned in 2(2.87%) cases

In analysing occupation of the deceased 55 (78.57%) cases were house wife, 7(10%) cases were labours, students and business one case each and not mentioned in 2(2.85%) cases. **Fig:2**.

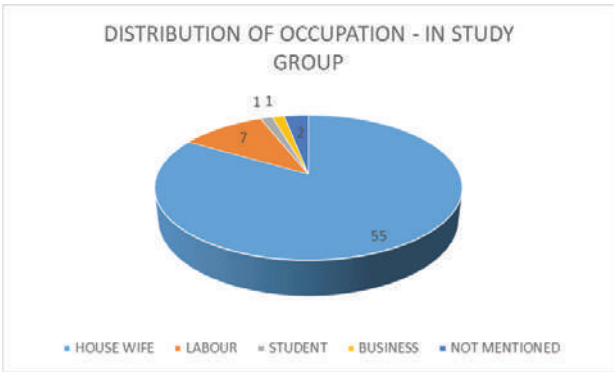


Fig:2. Distribution of occupation of the individuals in the study group

In analysing education status 49 (70%) cases were illiterate, 16 (22.85%) cases completed high school, 1(1.42%) case completed degree and education status not mentioned in 4 (5.71%) cases.

In analysing distribution of cases among age groups 20 (28.57%)cases was seen in 61-70 years of age group. In age group of 19-30 years 12(17.14%) cases were seen. In 31- 40 years 10(14.28%) cases were seen. In 81-90 years of age group 8(11.42%) cases were seen. 71- 80 years 7 (10%) cases were seen. **Fig:3.**

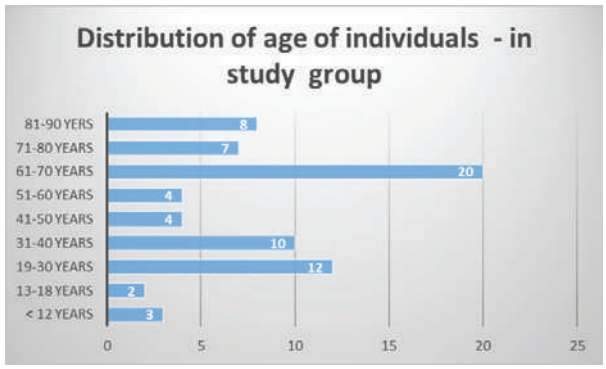


Fig:3. Distribution of age of the individuals in study group

In analysing history of the cases 16(22.85%) cases were involved in road traffic accident. This is in consistent with the study conducted by Thounaojam *et.al*[4], in which 68. % were victims of road traffic accident. This is in contrast with the study conducted by Pathak A *et.al* [5] were burns is the leading cause of death in 45% of cases followed by death due to injuries.

In this study history of burns was seen in 8(11.42%) cases. 10 cases sudden death was noted. In study conducted by Gonnade U et.al [6]reported that 73 % of burns cases were females. Out of which 88.75% were female.

In 8 (11.42%) cases hanging was seen. Drowning was seen in 8 (11.42%)cases. Poisoning was seen in 8(11.42%) cases. Homicide in 2(2.85%) cases and lightening, electrocution, insect bite one case each.

In analysing road traffic accidents 16 (22.85%) cases were noted, in which 8 (11.42%) cases were pedestrians, 6 (8.57%)cases were pillion riders and none of the pillion riders was wearing helmet. all the cases of pillion riders died of injury to the head. This consistent with the study conducted by Prasannan.k[7]. 2(2.85%) cases were two-wheeler riders and helmet were not worn in these 2 cases(2.85%).

In analysing viscera of the cases 26(65%) cases viscera was not preserved, in 40 (57.14%) cases viscera report not showed any poison or substance of abuse. In 2(2.85%) cases organophosphorus poison was detected. Carbofuran and Para phenylene Diamine was detected in one case each. No opinion to the cause death was given in 5(7.14%) cases in these five cases autopsy findings, Histopathology reports, Viscera reports, Inquest reports were within normal limits. In this 4 (5.71%) cases body was in decomposed state and in one case (1.42%) not decomposed. In 6(8.57%) cases though autopsy findings, histopathology, viscera reports within normal limits, from the history of the case and inquest reports opinion was given as "*Cause of death consistent with history of the case."* Table:1.

Table:1. Distribution of cause of death in the study group.

Injuries	Road Traffic Accident	Pedestrian		8(11.42%)
		Rider- Two Wheeler		6(8.57%)
		Pillion Rider		2(2.85%)
	Train traffic accident	Crossing the rail track		4(5.71%)
	Homicide	Blunt weapon		1(1.42%)
Asphyxia		Hanging		10(14.28%)
		Drowning		8(11.42%)
		Strangulation		2(2.85%)
Sudden Deaths		Cardiovascular System		5(7.14%)
		Renal Disease		1(1.42%)
Autopsy, Histopathology, Viscera Reports With In Normal Limits		No Definite Opinion to the cause of death	Body Decomposed	4(5.71%)
			Not decomposed	1(1.42%)
		Cause of Death Consistent with History	Insect bite	1(1.42%)
			Lightening	1(1.42%)
			Electrocution	1(1.42%)
			Poison	3(4.28%)
Thermal Deaths		Burns		8(11.42%)
Death Due to Poison Ingestion		Organophosphorus		2(2.85%)
		Carbofuran		1(1.42%)
		Paraphenylene diamine		1(1.42%)

In analysing manner of death accident was seen in 32 (45.71%) cases, suicide in 9 (12.85%) cases, natural deaths in 10 (14.28%) cases. Homicide in 3 (4.28%) cases. No definite opinion to the manner of death was given in 6 (8.57%) cases.

It was noted that in 2018 1,51,417 fatal accidents were reported by States and Union Territories. The State of Tamilnadu, India, recorded the highest number of road accidents (63, 920) in 2018 while the highest numbers reported as killed in 2018 were in the State of Uttar Pradesh (22,256). Both these States have maintained their leads in terms of number of accidents and number of persons killed since 2017 despite the several road safety initiatives taken by both the Central and State Government. In India 78,974 (16.91%) deaths was caused by vehicle hitting the pedestrian

According to the report titled "Road Accident Analysis in Tamil Nadu March 2019", out of the 978 persons killed in the accidents involving two-wheelers, 508 riders and pillion riders did not wear a helmet. "About 52 per cent of the death in two-wheelers were occurred due to non-wearing of helmets."

Conclusion

Women, especially the young are vulnerable for intentional unnatural deaths associated with family disputes, highlighting the importance of utilising available counselling service Where burns are the leading cause of death. Distribution and causes of unnatural female deaths in present study is not similar to the pattern found in most of the other Indian studies The present study revealed that the unintentional trauma, mainly the road traffic accidents are responsible for unnatural deaths of females i.e pedestrian and pillion riders. This highlights the importance of timely interventions of road safety measures.

Conflict of Interest: Nil

Ethical Clearance: Nil

Funding: Nil

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Estimation of Stature using Ulnar Length in a Population of Chennai

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Abstract

Estimation of stature plays a major role in the field of Forensic Medicine and anthropometry especially helps in establishing the identity of an individual from mutilated, decomposed and amputated body parts. In our study, stature was estimated from the percutaneous length of ulna. Study sample consisted of 96 adult males and 66 adult females between the age group of 18 - 22 years, born and brought up in Chennai. The length of the of the right and left ulna was measured with a vernier caliper from the tip of the olecranon to the styloid process and the height of the individual was measured with a calibrated stadiometer. The measurements were noted in the proforma. The data was statistically analyzed and linear regression formulae were derived.

Key Words: *Ulnar length; Stature; Linear regression.*

Introduction

Estimation of stature has a significant importance in the field of forensic medicine and anthropometry. Anthropometry is a series of systematized measuring techniques that express quantitatively the dimensions of human body and skeleton.[1] The ultimate aim of using anthropometry is to help the law enforcement agencies in achieving ''personal identity'' in case of unknown human remains.[2]

Establishing the identity of an individual from mutilated, decomposed,and amputated body fragments has become important in recent times, due to natural disasters (such as earthquakes, tsunamis, cyclones, floods) and man-made disasters (such as terror attacks, bomb blasts, wars, and plane crashes). The ulna is a long bone on the medial side of forearm. Proximally it has an olecranon process, and at its distal end is a styloid process. The whole length of the subcutaneous border of ulna is palpable down up to the styloid process. The length of ulna has been shown to be a reliable and precise means in predicting stature of an individual.

Different equations for the estimation of stature were established for whites and blacks, and for males and females.[5] The equations that were derived by Trotter and Gleser in the early 1950 for Americans and were being continuously revised using data from different sources. In 1977, they proposed new equations using radius and ulnar length. In 1961, Allbrook[14]attempted to develop standards for the estimation of stature from a British sample using ulnar length. Krogman and Iscan [7]compiled the most detailed description of stature estimation from skeletal remains.

Pearson estimated the stature from long bones by formulating the regression equations. He also noted that these formulae were population specific and should not be applied to individuals of different population groups. In 2005, Devi and Nath [6] formulated multiplication factors for stature estimation from upper extremity among male and female Tangkhul Nagas of Manipur.

Materials and Methods

The present study comprised of a total 200 Medical

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size was calculated to be 162.

N = 1.96^2x1.32^2 /0.269^2 = 96 males and N = 1.96^2x0.92^2 /0.22^2 = 66 females

About 10 % of the sample size [20] was added to take care of any refusal to participate in the study and the total sample size arrived for the study is 182. The final corrected sample size was 200. Their age ranged between 18 to 22 years and this study was done in 2016-17 for a period of 6 months. After obtaining informed consent from the participants of the study, the data was recorded in our proforma. Students with significant systemic diseases, history of old fractures, skeletal anomalies, developmental disorders, metabolic disorders which could have affected the bony growth were excluded from the study. The measurements were taken at a fixed time of a day to eliminate diurnal variation.The length of the of the right and left ulna was measured with a standard vernier caliper from the tip of the olecranon to the tip of the styloid process with the elbow in full flexion and the height of the individual was measured from crown to heel with head oriented in Frankfurt's plane with a calibrated stadiometer. The measurements were tabulated in the proforma and the data was analyzed using SPSS version 16.0 statistical package. T test was used to find out the association between right and ulna with reference to gender**.** ANOVA test was done to find out the correlation The linear regression equations were derived and a p value of <0.05 was considered significant.

Observations and Analysis

Table No. 1. Mean, SD for all the parameters - Both sexes together

Parameters (cm)	Mean	SD
Height	162.092	10.14
Length of Ulna (right)	25.787	2.89
Length of Ulna (left)	25.645	2.85

Table 2. Mean , SD for all the parameters in Male and Female Cases

Parameters (cm)	Male Cases		Female Cases	
	Mean	SD	Mean	SD
Height	168.020	9.76	156.044	6.21
Length of Ulna (right)	26.614	2.92	26.492	2.85
Length of Ulna (left)	26.492	2.85	24.780	2.58

Table 3. Comparison of length of right and left ulna

Subject	Z value	P value
Both sexes together	0.62	>0.05
Male	0.76	>0.05
Female	0.69	>0.05

*****independent T test

Table No. 3 shows Comparison of right and left ulna. From the table 3, it is found that the mean value of length of right and left ulna of study group is statistically insignificant in male, female and both together (P >0.05). So for further statistical analysis, Length of left ulna will be considered, as per recommendation of the international agreement for paired measurements at Geneva (1912).

Correlation coefficient:

Pearson's correlation coefficient was used to examine the relationship between length of ulna and height. Correlation coefficient between total height and length of ulna was found to be statistically significant and positive in both males and females.

Table No: 4 Pearson's correlation coefficient

Subjects	Correlation Coefficient (r)	Coefficient of determination (%)	P value
Both sexes together	0.75	56.4	< 0.01
Male	0.86	73.1	< 0.01
Female	0.58	33.6	< 0.01

Table No. 4 Correlation of height with length of ulna is 0.86 in males, 0.58 in females and 0.75 in both together, which are positive and statistically highly significant (P < 0.01) i.e. if length of ulna increases or decreases, the height of the subject also increases or decreases and vice versa.

Linear Regression Equation:

Regression analysis was performed for estimation of stature using the length of ulnas as independent variable.

Table 5: Regression equation for height with length of ulna in male, female and both sexes together

Subjects	Correlation Coefficient (r)	Coefficient of determination (%)	P value
Both sexes together	0.75	Y = 93.54+ 2.67X	< 0.01
Male	0.86	Y = 90.57+ 2.92X	< 0.01
Female	0.58	Y = 121.52+1.39X	< 0.01

Y = height and X= ulnar length

Table no. 5 - linear regression equation for height with length of ulna in male, female and both together, where,

Y = Height/ Stature (cm)

X = Length of ulna (cm)

93.54, 90.57, 121.52 are intercept (constant) for male, female and both together respectively.

2.67, 2.92, 1.39 are regression coefficient for male, female and both together.

From the above table it is seen that the regression formula within a region also varies between male and female

population of that region.

Results

The observations were analyzed separately for both right and left ulna in each sex on all subjects and results are tabulated. The mean ages of the study subjects (Male 21.184 ± 3.27 and Female) 21.01 ± 3.31) were not significantly different between genders. Significant (P< 0.05) Gender differences in mean height and length of ulna was found in the study. Mean right and left ulna lengths of the male (26.614±2.92 and 26.492±2.85) were significantly larger than that of the females (24.944±2.64 and 24.780±2.58) of all ages.

Discussion

Sex determination of an unknown individual and the estimation of stature is one of the most important aspects in forensic medicine and anthropological studies. Estimation of stature is essential for the calculation of body mass index, which is used for assessment of nutrition. However, its measurement is not always practical in old or frail bedridden patients who cannot stand or those who are suffering from vertebral column deformities. In such patients, formulae based on the ulna length provide an alternative stature predictor [19]. Pan worked on cadavers and derived relation between total ulnar length and total height of an individual [22].According to Trotter M et al., there is an increase in the height of 2.5 cm after death [10]. Hence prediction of height using ulna in living has definitive advantage over the cadavers. [14]. The Correlation coefficient between the total height and ulna length was found to positive indicating a strong relationship between the two parameters.

The present study deals with observations on correlation of total standing height with length of ulna. The stature of an individual mainly being genetically predetermined is an inherent characteristic that needs to be estimated for identification of an unknown individual. Therefore, formulae based on the length of ulna provide an alternative stature predictor under such circumstances. The average height of adult males within a population is significantly higher than that of adult females. The result obtained in this study is in agreement with the above statement.

Allbrook [14] Derived regression formulae for estimation of stature from length of ulna as

Stature= 88.94 + 3.06(ulna length) ± 4.4 (SE)

Athwale [11] derived a regression formulae for estimation of stature in 100 Maharashtrian males of age ranging from 25- 30 years

Stature = 56.9709 cm+ 3.9613×average length of right and left ulna (cm) ± 3.64cm. and from left radius (cm) +3.66cm

Lal and Lala [15]: worked on a population of 258 of age ranging from 12 to 21 years in north Bihar and stated that ulnar mean multiplication factor was comparable in all series. They claimed that ulnar multiplication factor is better guide for calculation of height when it is not definitely known to which part of the country the individual belongs.

Maloy Kumar [9] derived regression equation for estimation of stature from the length of ulna in males of West Bengal in age range of 20-50 years.

Estimation of height from right ulna Y1 = 50.642+ 4.1896X1 ± 7.7302

Estimation of height from left ulna Y2 = 76.289+ 3.256X2 ± 9.082

Ilayperumal [4] derived regression equations for stature estimation from length of ulna in both males and females in Srilankan population.

Thummar B et.al [13] derived regression equation for estimation of stature from length of right and left ulna in both males and females.

For males regression equation for right ulna is Y = 181.11+3.117X and for left ulna equation is Y = 65.76+3.667X

For females equation for right ulna is Y = 17.10+5.34X and for left ulna equation

is Y = 18.95+5.33X

Conclusion

From the present study, it has been concluded that the mean height and length of ulna is more in males than in females. Gender differences in mean height and length of ulna were found to be highly significant (P <0.05). There is a positive correlation between stature and length

of ulna. Simple linear regression equation so far derived can be used for estimation of height in Chennai region. If either of the measurement (length of ulna or total height) is known, the other can be calculated. The present study deals with observations on correlation of t.

Ethical Clearance: From Institutional ethical committee.

Source of Funding :None

Conflict of Interest :Nil

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Original Article

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A Study of Pattern of Thoracic Injuries in Cases of Railway Traffic Accidents Brought to the Post Mortem Centre

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Abstract

Post mortem examination has important value in case of railway accidents when the thoracic injury are incorporated. There were so many statistical data present which had defined the thoracic injury but no statistical data is observed regarding the traumatic lesion of the vital organs in thoracic region in cases of Railway traffic accident deaths. This significant oversight can lead to find out the exact cause of death in cases of railway traffic accidents.[1] It can help to improve the hospital emergency centre as far as concerned to chest injury victims in case of Railway traffic Accidents. Observations made were compared with the previous studies. The study period extended from the 1st JUNE2012 to 31st OCTOBER 2014. During this two (2) years period, a total number of 867 Autopsies were carried out in the department of Forensic Medicine, out of which, 74 cases of railway accident were selected for evaluation in the present study. Approval of local institutional ethical committee has been taken. It has been observed that chest fatalities are quite common and their correlation done with other authors.

Key Words: *Indian Railways, Contusions, Lacerations, thoracic injuries, fatalities.*

Backgrounds

Railways being one of the most comfortable means of transportation have a long history and an unprecedented contribution to the human civilization. The basic design if the Railway consists of a locomotive or a self- propelled motor- unit drawing a train of cars over a track of two parallel rails placed together on a permanent stretch of roadway or railway. The flanged wheels rolling on iron or steel ribs, causes minimal friction thereby allowing a smooth and comfortable journey with the added advantage of being cheap, safe and reliable means of transport for everyone.

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With increasing advantage of the railway it has been observed that as the day progresses the railway also causes misadventures disadvantages in terms of injury with deaths of the passengers. In India as per the report from the Railway Year book 2011-12[4], in the year of 2011-12, there were 235 passengers were killed and 358 injured and about 585.89 Lakhs Rs compensation has paid by the railway. An autopsy surgeon usually encounters isolated cases where death results from some kind of railway track incident. While in most cases, an opinion regarding the nature of death can be provided by a close observation of the injury pattern alone, but in some cases, it becomes almost impossible to form an opinion about the nature of death. A correct opinion regarding the cause and nature of death becomes important in view of the increasing number of railway injuries and fatalities, which mount to a high number of compensation cases. The correct opinion helps the Railway to verify the cases liable for compensation and also helps in formulating ways for prevention of the

same.

Aims and Objectives

1) To study the causative factors.

2) To study external and internal chest injuries peculiar to railway fatalities.

3) To study cause and manner of death

4) To assess and evaluate the difference and pattern of thoracic injury on the basis of previous studies.

Material and Methods

The present study was carried out in the post mortem center situated of a medical college situated at Central India. It is prospective study .The post mortem center conducts autopsies on following railway accident victims: 1. Cases brought dead by any of the nearby police station. 2. Cases of Railway accidents admitted to this hospital (Either directly or as a transfer from any other hospital) who succumb to the injuries. The study period extended from the 1st JUNE2012 to 31st OCTOBER 2014. During this two (2) years period, a total number of 867 Autopsies were carried out in the department of Forensic Medicine, out of which, 74 cases of railway accident were selected for evaluation in the present study. Approval of local institutional ethical committee has been taken. Criteria for selection or exclusion of cases: 1. Victims of railway accidents that took place onside the train (Running or stationary), or on the railway track. Or on the railway platform and brought to the postmortem center with history of railway accidents as per the police inquest. 2. Non- train accidents64: Accidents to people on railway premises but not connected with the movement of railway vehicles, were excluded. 3. The natural deaths which occurred in train, railway track, railway platform, railway premises and brought by railway police were not included in the current study.

Materials and Methods

Collection of data: Information that provided the primary data in each case was collected from different sources. They were as follows:

1. Examination of the inquest report and history from the relative if available.

2. In case of hospitalized victims, records of the treatment were perlustrate

3. Finding of the autopsy reports.

All data collected from different sources were recorded in specially designed proforma for each case for further collective evaluation.

The information was collected and studied mainly under the following headings.

a) Brief history

b) Causal factors

c) Post mortem examination

d) Cause of death History and causal factors.

A compendious picture about the back ground of the victims was elicited after taking history from investigating police and relatives of the victims. This included detailed particulars such as name, age, sex, address, religion, educational status, socio economic status, occupation, marital status. Similarly an idea about the circumstances of the event leading to death was made out by other epidemiological data such as place of incident, nature of incident leading to death, time of occurrence, weather conditions; and survival period, etc. very importantly Evidence of suicide notes along with body also noticed.

Post Mortem examination.

The detailed post mortem examination carried out in each case comprised of an external and an internal examination.

a). External examination included general condition of the body, including external orifices, injuries sustained, etc.

b). Internal examination is carried out systematically by opening the three principles body cavities viz. the chest, abdomen and cranium and dissecting the contents by Letulle's method. The location and the extent of the injury were specially observed to note any pattern if any.

Observation & Result

Thorax

Internal injuries to thorax are summarized in the following table no 1.

Injury to thorax	Cases	Percentage
Ribs	24	32.43%
Sternum	8	10.81%
Lungs	19	25.67%
Heart	3	4.05%
None	48	64.87%
N.B. There is overlapping as combination injuries to thorax present in the victims.		

Fracture of ribs was observed in 24 (32.43%) cases. Injuries to lungs were observed in 19 (25.67%) cases. In 8 (10.81%) cases sternum was fractured. Injuries to heart were observed in 3 (4.05%) cases.

Injuries to thorax were seen either in combination or in isolation as summarized in the following table-

Table No:2

Pattern of injuries to thorax	No of cases	Percentage
Only Ribs	5	6.76%
Only Sternum	0	0%
Only Lungs	1	1.35%
Ribs+lungs	10	13.51%
Ribs+lungs+heart	2	2.70%
Ribs+sternum+Lungs+heart	2	2.70%
Ribs+Sternum+Lungs	6	8.11%
Ribs+Sternum	0	0%
Sternum+Lungs	0	0%
Sternum+ heart	0	0%
Heart	0	0%
No of cases with Thoracic injuries	26	35.14%
No injuries to thorax	48	64.86%

Total Internal injuries to the thorax were observed in 26 (35.14%) victims. It was observed that injuries to the ribs along with lungs were the commonest pattern of thoracic injuries, seen in 10 (13.51%) victims, followed by combination of ribs, lung with sternum seen in 6 (8.11%) victims. Fracture to the ribs was observed in 5 (6.76%) victims. Combination of Ribs+ Sternum + Lung + heart Combination of Ribs+ Lungs+ heart were and combination of Ribs +sternum+ Lungs were observed in 2 (2.70%) victims respectively. Internal injuries to thorax were not observed in 48 (64.86%) victims.



Risky Behaviour of peoples while travelling



Crush Thorax injury.

Discussion

Internal injuries to thorax were observed in 35.13% cases. Fracture of ribs was observed in 32.43% cases. Injuries to lungs were observed in 25.67% cases, in these 25.67% cases 2.70% were having penetrating injury which was correlating with the external injuries. In 10.81% cases sternum were fractured. Injuries to heart were observed in 4.05% cases.

Findings are consistent with **Patil Ajay et al (2000)[2&3]** observed 46% had ribs and sternum fracture. Injury to lung was observed in 6% cases and injury to heart was observed in 3% cases.

Sabale PR and Mohite SC (2005-2008) [5], observed that ribs were observed in 42% victims, injuries to lungs and heart were noted in 33.76% and 5.35% victims each. He observed fracture of sternum in 6.64% cases.

v In our study the injuries to the ribs along with lungs were the commonest pattern of thoracic injuries, seen in 13.51% victims, followed by combination of ribs+ lung+ sternum 8.11% victims. Fracture to the ribs was observed in 6.76% victims. Internal injuries to thorax were not observed in 48 (64.86%) victims.

No other author described this type of combination except study done by **Sabale PR and Mohite SC (2005-2008) [5],** in their study it was observed that in 26.57% victims ribs+ lung shows injuries followed by fracture of the ribs only in 8.30% cases.

Summary & Conclusion

v Injuries to the ribs along with lungs were commonest pattern of thoracic injuries.

v Injuries to the ribs with fracture of sternum along with lungs were second commonest pattern of thoracic injuries.

v Thoracic injuries (37 %) are second only to Head injuries (73%) as far as commonest types of injuries associated with the Railway accidents are concerned.

v Fractures of ribs and sternum in 46% cases. Injury to lung was observed in 6% cases and injury to the heart was observed in 3% cases.

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v Conflict of Interest: nil

v Ethical Approval: Not needed.

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Awareness among Medical Students about Forensic Odontology

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Abstract

Every tooth possesses a set of unique characteristics and the way the teeth are arranged in different oral cavities are unique in every individual. Therefore, dentition is useful for individual identification and comparison, especially in mass disasters where the bodies of the victims become mutilated beyond recognition. Forensic odontology deals with the science of dentistry to aid in the administration of justice. There is a huge void in the number of forensic odontology experts required to meet the demand and those that are actually getting trained in this concern. In order to ease this, there is a need to incorporate MBBS undergraduate curriculum with the shortlisted particulars of forensic dentistry. The study was conducted to evaluate the awareness and knowledge about forensic dentistry amongst the 2nd Year MBBS under graduates in Mahavir Institute of Medical Sciences. The study showed that the level of awareness on topic was adequate in the participants but they had little knowledge about the practical application of forensic odontology in routine practice. Conducting regular seminars and workshops can be helpful to develop the practical skill required in handling forensic odontology related cases in emergency department.

Keywords: *Awareness, Forensic Odontology, 2nd Year MBBS students, Forensic Dentistry, Mass Disasters.*

Introduction

The dental patterns are unique for every individual. This uniqueness is due to the changing status of a person's teeth throughout the life & also due to variety of treatments given by the dentist. Teeth are the hardest, robust tissues and are more reliable than any other remains for identification of the individual. Therefore, dentition is useful for individual identification and comparison, if records exist for the purpose [1].

Forensic odontology deals with the science of dentistry to aid in the administration of justice.[2] Forensic odontology has importance in identification, estimation of age of a person, determination of sex, determination of race, DNA evidence, bite-mark evidence, Injury, occupation, habit, time since death and detection of poison[3]. It is very essential for identification of individuals in the event of any disaster. In recent times, natural and man-made disasters are occurring frequently, in such catastrophies the bodies of the victims become mutilated beyond recognition and also the body parts of individual are shattered where there are least chances of obtaining any piece of identity. Here the vital role of forensic odontologist comes into picture in the identification of such individuals.

Forensic odontology is still in its infancy state in India where as in other developed countries it has acquired a recognized branch of dentistry in medical forensicology [4]. Forensic odontologist plays a very important role in crime investigation of any type by assisting the legal authorities in the confirmation of identity. In India, there is a huge void in the number of forensic odontology experts required to meet the demand and those that are actually get trained in this concern. In

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order to ease this we need to incorporate our curriculum of the medical undergraduates with the shortlisted particulars of forensic dentistry. Studies show that only 12% of all dentists maintained complete dental records[5]. It states that the knowledge and awareness about Forensic Odontology is less among the dental doctors and much lesser in MBBS students. This study necessitates the exposure of medical graduates to the basic principles and techniques of the forensic odontology.

This study was undertaken to analyse and assess the awareness about forensic odontology among the MBBS graduates in Mahavir Institute of Medical Sciences. This article is an attempt to reinforce the awareness among MBBS students about various disciplines of forensic odontology and a role of an odontologist in identification of a person.

Methodology

This was a cross sectional prospective study done over a period of 3 months from 1st July to 30th September 2019. 150 MBBS under graduate students belonging to one academic year, 2nd year MBBS studying at Mahavir Institute of Medical Sciences, comprising of both male and female sex. The participants were given a questionnaire (Dichotomous Two Category Questionnaire) during their teaching sessions and were briefed about the objectives of the study and asked to choose the answer which they found appropriate. The data were analysed in SPPS and expressed as number and percentages and presented in the form of text, tables, and figures.

Results

Table 1: Awareness on forensic odontology

Statement/Fact		Frequency	Percentage
Application Of Knowledge on Dental Sciences to Legal Investigation is defined as Forensic Odontology	Agree	140	93.3%
	Disagree	7	4.7%
	Don't Know	3	2%
Dental Examination helps in identification of unknown persons	Agree	112	74.7%
	Disagree	27	18%
	Don't Know	11	7.3%
The term Forensic Dentistry is Interchangeable with Forensic Odontology	Agree	71	47.3%
	Disagree	22	14.7%
	Don't Know	57	38%
Forensic Odontology plays a key role in mass disasters	Agree	91	60.7%
	Disagree	32	21.3%
	Don't Know	27	18%
Bite marks are referred as "Dental Fingerprints"	Agree	107	71.3%
	Disagree	21	14%
	Don't Know	22	14.7%

67 (44.7%) participants were aware of the uniqueness of the tooth print and that it could be used as a valuable tool in forensic science for personal identification. 38 (25.3%) students did not consider them to be unique and 45(30%) of them did not know about tooth prints. 141 (94%) of the respondents agreed that maintaining records will be helpful for the forensic experts in identification, 2(1.3%) disagreed and 7(4.7%) did not understand its importance. 132 (88%) of the participants were aware that the effects of Drugs, Nutrition and Diseases can also be manifested on the teeth, 8 (5.3%) disagreed to this and 10 (6.7%) of them were not aware of this fact. 93 (62%) of them were aware that there are some dental characteristics which are predominant in one of the racial groups which help in the racial identification process. 14 (9.3%) disagreed to this and 43 (28.7%) of them did not know about the differences in teeth in different races. Loss of tooth due to assault is grievous Hurt, 116 (77.3%) were aware of this, 27 (18%) believed the given statement to be false and 7 (4.7%) of them did not consider it to be grievous hurt. Only 59 (39.3%) of the students were aware that it was difficult to dislodge your tooth or have it knocked completely out without affecting the surrounding soft and hard tissues. 52 (34.7%) disagreed and 39 (26%) said they did not have knowledge about this.

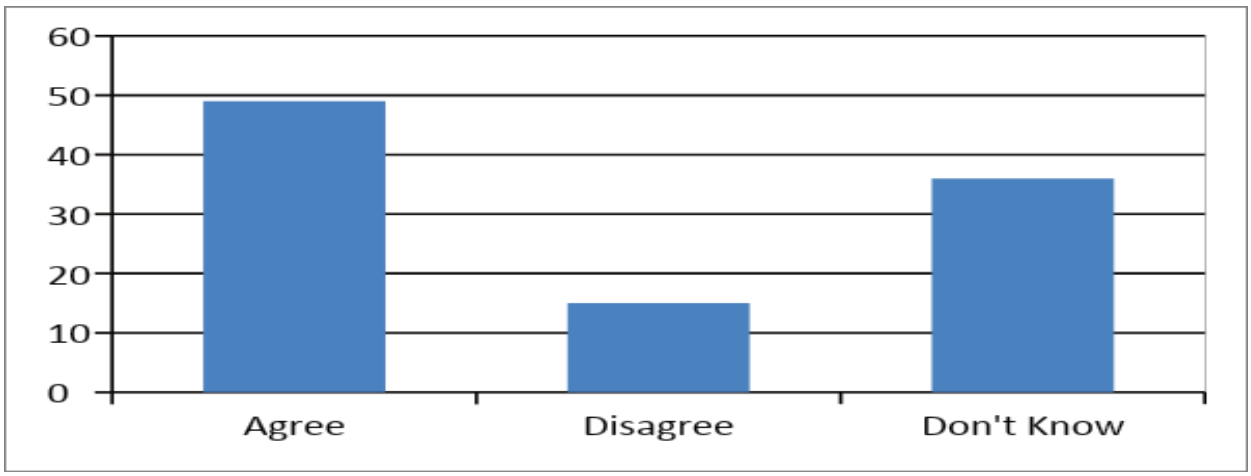


Fig 1: If Blood Group can be determined from the cells of the Dental pulp cavity

Table 2: Awareness on application of Forensic Odontology

Statement/Fact		Frequency	Percentage
A missing tooth can also help in establishing identity of a person	Agree	104	69.3%
	Disagree	25	16.7%
	Don't Know	21	14%
DNA of an individual can be obtained from teeth also	Agree	91	60.7%
	Disagree	25	16.6%
	Don't Know	34	22.7%
Criminals can be identified through Bite marks left either in human tissues or in food stuffs	Agree	108	72%
	Disagree	16	10.7%
	Don't Know	26	17.3%

Cont... **Table 2: Awareness on application of Forensic Odontology**

In mass disasters, comparison of Ante-mortem and Post-mortem Dental X-rays is done for Identification	Agree	104	69.3%
	Disagree	11	7.3%
	Don't Know	35	24.3%
Dentures are useful in identification if they have patient's name or code number included in them	Agree	75	50%
	Disagree	14	9.3%
	Don't Know	61	40.7%

In Mongolians, lower molars have three roots. 17 (11.3%) of them agreed to this fact, 17 (11.3%) disagreed and 116 (77.3%) did not know the differences in molar teeth among races. 39 (26%) of the respondents are agreed that in civilized societies, first permanent molar is larger than third molar of this fact. 21 (14%) disagreed and 90 (60%) did not know of such a distinction between civilized and primitive societies. Only 48 (32%) were aware that Y-chromosome can be demonstrated from pulp of teeth. 22 (14.7%) disagreed and 80 (53.3%) did not know.

Discussion

Many studies have been done in the last decade to know the level of the knowledge and awareness on the subject of forensic odontology among students but all have been done on dental graduates and postgraduate students. Forensic odontology has multiple disciplines which not only includes dental identifications but also has recognition in abuse of children and elderly, age assessment and bite mark analysis that have legal importance.[6] As per the curriculum established by the Medical Council of India, Forensic Odontology is taught in forensic medicine as a part of anthropology. Keeping that in the background, this study was conducted to assess the level of awareness among MBBS students on the subject.

According to the current study, 93.3% of the study group had basic knowledge regarding the definition of Forensic Odontology. 71.3% knew each individual has unique dentition and hence, they are also referred to as dental fingerprints. The term "Ameloglyphics" means the study of enamel rod end patterns.[7] The uniqueness of the tooth print could be used as a valuable tool in forensics for personal identification, but 55.3% were unaware of it. Though majority i.e. 74.7% of them were aware of the importance of dental examination in unknown and unidentified bodies, 39.3% did not know the vital role it played in identification of disfigured, mutilated or unidentifiable bodies in mass disasters. Forensic odontology is regarded as one of the most reliable and economical scientific methods for victim identification in mass disasters.[8] Mass disasters are sudden, violent and unexpected events that are associated with a large number of casualties. These casualties can be treated both on an outpatient or surgical basis and medical doctors are first respondents to such cases. Thus, they may inherently become involved in the identification of victims. In mass disasters, like fire accidents or blast injuries, visual recognition of facial features and fingerprints is often not possible due to extensive soft tissue destruction; this requires the use of hard tissues such as bones and dentition for human identification. Though 94% were aware that maintaining Dental Records can be useful for forensic Investigation, 31.6% did not know that comparison of Ante-mortem and Postmortem Dental X-rays can be done for identification. *Dentures* are prosthetic devices that replace the missing teeth. They are made to be acid resistant, to survive elevated temperatures and to be easily retrievable after an accident. All dentures are marked with codes or unique identification number.[9] 50% of the study group did not know that dentures have a crucial role in identification when they are marked with patient's name or code number on them. These criteria show that the students were aware of general principles of forensic odontology; they had minimal knowledge about the applications of forensic odontology in routine practice. These findings are similar to those found by Khare P et al in their study on dental practitioners.[10]

As per the study conducted by Preethi S et al in Chennai, 93% percent of the dental practitioners lacked

formal training in collecting, evaluating and presenting dental evidence.[11] Hence, medical graduates must not only be aware of basic techniques of identification but also about preservation of ideal samples to be sent for DNA analysis. 49.3% of the participants were not aware of the fact that teeth act as a source of DNA material. Pulp and cementum are the most valuable sources of nuclear DNA in the tooth and both these tissues along with dentine are good sources of mitochondrial DNA.

Only 51.3% of the participants were aware that the presence of ABO blood group from soft and hard dental tissues makes it possible to assist in human identification even in decomposed bodies. 68% of the respondents did not know Y-chromosome can be demonstrated from pulp of teeth and sex determination by fluorescent staining of the Y chromosome is a reliable technique in teeth with healthy pulps.[12]

Though 62% of the students were aware that each human race has its own characteristic appearance regarding dentition and there are certain dental characteristics which are predominant in some racial groups which help in the racial identification process. Majority did not know about specific characteristics of each human race like Mongolians have shovel-shaped incisors and their mandibular molars have three roots, 88.6% of the participants did not know about that. 74% of them were also unaware of distinctions between civilized and primitive societies.

Teeth mineralisation gives a better estimate of chronological age than the bone mineralisation.[13] Age of the individual can be estimated based on the type of etching patterns on the enamel.[14] Dental profile comprises of a group of specific individual characteristics related to the teeth and surrounding tissues. These characteristics help in the estimation of age, sex, socio-economic status, personal habits, oral health, occupation and dietary status of the person.[15] 69.3% agreed that a single tooth can also help in establishing identity of a person, 30.7% did not know about dental profiling. 88% of the group was aware that teeth and surrounding tissues are affected by many local and systemic diseases. Drug abuse can lead to acidic erosion of enamel and increased numbers of decayed, missing and filled teeth in the mouth.

Majority of the students were aware of the medicolegal aspects of forensic dentistry. 77.3% of the respondents were aware of that fracture or dislocation of a bone or tooth, Grievous hurt as it is included in the seventh clause of Sec.320 of the Indian Penal Code. But 60.7% did not know that it is difficult to dislodge a healthy tooth without fracturing or loosening the neighbouring teeth. Bite marks can occur either in the victim or attacker or on both. Teeth are used as a weapon by the perpetrator and also in self-defence by the victim. In addition to the location of the bite mark, the type of severity of the injury may give investigators relevant clues as to the mental state of offender. 72% said they knew about bite mark analysis and the key role it plays in identification of the perpetrator.

Conclusion

The study revealed that majority of students showed adequate awareness in the basics of forensic odontology but lacked in knowledge about the applications of the subject in routine practice. Forensic Odontology plays a major role in the identification of unknown persons, unclaimed dead bodies and mutilated body parts in cases of mass disasters and natural calamities. Bite mark analysis has also been used as evidence by the judiciary all around the world including India. Because of inadequate practical exposure and training regarding latest trends in Forensic odontology and minimal importance given to the subject at the undergraduate level, it still remains at a nascent stage in India. Similar studies should be conducted on a wider scale to include medical graduates and emergency physicians.

As a health-care provider, medical professionals should understand the implications of forensic odontology. Hence, medical students should be included in periodic conferences and seminars conducted in forensic odontology which would improve their knowledge. Workshops may be conducted so that the practical skill would be developed in handling forensic odontology related cases in emergency medicine and forensic medicine departments.

Ethical Clearance: Taken

Conflict of Interest: Nil

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Google Classroom - An Effective Tool for Online Teaching and Learning in this COVID era

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Abstract

Background: Nationwide lockdown due to corona virus pandemic compelled the medical colleges to conduct online classes to compensate for the loss of academic period. Among all the available apps, Google classroom was found to be more user friendly app with many built in features. After using one month of Google classroom app in the department of Forensic medicine, a feedback survey was done to know the effectiveness of online classes and in particular about the advantages and disadvantages of Google classroom as a tool for teaching and learning online.

Material and Methods: A Questionnaire which had both objective and open ended questions was administered to 150 phase II MBBS students who underwent online classes conducted by department of Forensic Medicine and 6 instructors who took classes.

Results: Students felt that undergoing online classes was the best way of utilizing lockdown time (54%). Both students and teachers found the app very user-friendly and easy to navigate. Students felt that, using anytime anywhere (68%), interaction through private comment section (90%), getting the constructive feedback (86%) and material available for revision all the time (84%) were the advantages of Google classroom. While teachers found, tracking of assignment, grading system and record keeping as advantages. No automated update option, network issues, No search option and No integration were few disadvantages quoted by students and teachers.

Conclusion: Online classes are the best way of utilizing the lockdown time to make up the loss of academic period. Google classroom is an effective tool for teaching and learning online classes because of its ease of use and features which are both teacher friendly and student friendly.

Key words: *Online teaching; Google classroom app; nationwide lockdown; feedback; teaching-learning interface.*

Introduction

India declared nationwide lockdown due to Corona virus pandemic. During this lockdown, universities including RGUHS (Rajiv Gandhi University of Health Sciences) Bangalore, Karnataka took steps to declare holidays for medical colleges to contain the virus. Meanwhile RGUHS also urged medical colleges to start online classes to compensate for the loss of academic period.

An online classroom is virtual classroom that allows participants to communicate with one another, view presentations or videos, interact with other participants and engage with resources in work group [(1).] An online class which utilizes electronic technologies for learning is called e- learning [(2)]. Online classes can be synchronous that is usually scheduled in real-time and students should put aside their time to attend the online session whereas in asynchronous type one utilizes Computer

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Mediation Communication (CMC) to achieve "anytime and everywhere" learning through online discussion [3].

In online classes students are not in the physical classroom. So making them connected is a great challenge to teachers. Here we are dealing with students who know technology very well [4] and are adult learners who prefer self-directed learning, exhibit readiness to learn, orientation to learning that is problem-centered rather than subject-centered and exhibit a relatively high degree of internal motivation [5,6]. While on the other hand, instructors' moves from being at the center of the interaction or the source of information to the 'guide on the side' [7].

Research indicates that individuals prefer educational programs, "that are flexible with time and location [6]. An app in mobile can improve the online learning experience by improving communication [8]. There are many apps for online teaching and learning like, Zoom, Cisco webex, Impartus etc which provide live streaming of lectures. But many of these allow fewer students in free version. One of the major hurdles for live streaming of the class is poor internet connectivity which results in audio and video being not synchronous.

Web 2.0 technologies have been integrated for many years into educational systems [9]. Google is a popular Web 2.0 tools and Google Classroom is a new tool introduced in Google Apps for Education in 2014[10]

Google classroom can be used for synchronous teaching using Google meet or asynchronous teaching using Google classroom. Asynchronous teaching and learning allows students to use material anytime and anywhere without time limit [10]. Google Classroom is a learning management system that aims to simplify creating, distributing and grading assignments [1] . By keeping, all these points in mind, we decided to go ahead with Google classroom app as a tool for online teaching and learning.

Forensic medicine has been described as a key to the past, the explanation of the present and to some extent a signpost to the future [11]. Subject like Forensic Medicine, needs concepts which are clear and properly associated. We used Google classroom as an online teaching and learning platform to teach forensic medicine for MBBS phase II students. After using this app for 1 month, we collected students feedback on online classes and both students and teachers feedback on ease of use, advantages and disadvantages of Google classroom app.

Aims and Objectives

1. To analyse the effectiveness of online teaching

2. To get the feedback of students and teachers on Google classroom app with respect to ease of access, advantages and disadvantages.

Materials and Methods

Second year MBBS students (n=150, Boys =82, Girls=68) and 6 instructors of department of Forensic medicine, S. Nijalingappa Medical College, Bagalkot, were included in the study.

Sampling: Purposive sampling

Study design: Descriptive

Methodology

We created a Forensic Medicine class on Google Classroom app and asked students to join. Then instructors were asked to upload their power point presentation with background narrations narrated in their own voice on scheduled time in app. PPT having background narrations in instructors voice, to some extent gives the feel of connectedness to students.

After the scheduled class was over, instructors were asked to upload assignments to the students covering the key concepts of the lecture. A stipulated time was given to student to complete the assignment and upload the answers. During this time, instructor was available for discussion with students to clear their doubts. Once the student submits the answers, teacher would evaluate the assignments and return the file with constructive feedback to the students. The students who have handed in the response in a given stipulate time were marked as present others considered as absent. Three late submissions (submission after the stipulated time) were marked as 1 absent (considering the network issues).

Data collection method: After one month of using Google classroom app for online teaching and learning, a feedback questionnaire was administered to the students. Questionnaire had 2 components. Questionnaire on ease

of App was adapted from Shaharanee et al[10] where students were asked to mark their response n 5- point Liekert's scale 1- strongly disagree to 5- strongly agree). Other component of questionnaire contained combination of closed ended and open ended questions advantages and disadvantages of online classes and app. Instructors feedback was also collected.

Statistical Analysis: Collected data was analysed using Microsoft excel sheet.

Results

Table 1; shows the feedback of students about the advantages of online classes. About 54 % students felt that this was the best way of utilizing the lockdown time. Online classes allow them to maintain continuity with the subject (53%), regular assignments after the class help them to remember the concept well (48.6%). There were also responses regarding no hurry to copy the notes (18%) and stress free study (6.6%).

Table 2; shows the feedback responses on disadvantages of online classes. Around 39.33% students felt that online classes were not effective as traditional classes because it lacks student teacher interaction or no classroom environment. Few students also complained about distraction by pop ups (9.33%) and headache and eyestrain due to excessive use of mobile (5.33%)

Table 3; show the mean scores of the ease of use of Google classroom app. Among student, the scores of all the components were high. The highest score was for access of material on this app (4.71) then followed by signing into the app (4.62) and then navigation of the system (4.55). Among teacher, signing into the app (4.80) and easy navigation of the system (4.89) score were high.

Table 4; shows the student responses about the advantages of the Google classroom. Around 68% students were responded saying it as they can use the app anytime and anywhere, they can view the material many times and revise (84%) which helps them to remember the subject well. Around 90 %(135) students were happy about the private comment section which enables them to communicate with teacher. About 86% (129) students felt that instant feedback by the instructor helps them to improvise themselves in the area of lacunae. Few students (45%) also commented that they can keep a track of their assignments and grading system helps them to monitor their progress.

Table 5; shows the disadvantages of Google classroom app. Around 46.6% of students responded that, app consumes lot of data and needs more storage space. Students also experienced network issues due to which they got late announcement (38%). Few students (21%) expressed the need for search option in the app, which will save them the time to find the material easily. Few students also expressed that they took time to adjust for the app (10.6%). Around 3.3% students experienced difficulty in uploading the assignment.

Table 1: Advantages of online teaching

Feedback responses	Boy (n=82)	Girl (n=68)	Total (n=150)
Good utilization of lockdown time	51.2%(42)	57% (39)	54%(81)
Syllabus can be covered	45%(37)	63.2%(43)	53%(80)
Assignments helps in remembering the concepts	34.1%(28)	66.1%(45)	48.66%(73)
Can do other activities along with learning	12,2%(10)	11.7%(8)	12%(18)
No hurry to copy material	14.6%(12)	22%(15)	18%(27)
Overcame shyness	3.6%(3)	2.9%(2)	3.3%(5)
Stress free study	4.8%(4)	8.8%(6)	6.6%(10)

Table 2: Disadvantages of online classes when compared to traditional classroom

Feedback responses	Boy (n=82)	Girl (n=68)	Total (n=150)
No classroom environment	36.5%(30)	42.6%(29)	39.33%(59)
Not interesting	6.1%(5)	11.7%(8)	8.66%(13)
Distractions by pop ups	14.6%(12)	2.9%(2)	9.33%(14)
Eye strain and headache	3.6%(3)	7.3%(5)	5.33%(8)

Table 3: Ease of Google classroom app usage (To be marked on Liekert scale, 1- strongly disagree to 5- Strongly agree). Method adapted from Shaharanee et al[10]

Questions	Student (n=150)	Teachers (n=6)
Accessible on all the devices mobile models	4.22	4.33
Signing on to the Google Classroom	4.62	4.80
Accessing course materials	4.71	---
Sending and receiving assignment	4.12	---
Submitting Assignment	4.00	---
Navigating the system	4.55	4.89
Personalised learning experience	4.09	---

Table 4: Advantages of Google classroom app

Feedback responses	Boy (n=82)	Girl (n=68)	Total (n=150)
All classes in single app	24.4%(20)	27.9%(19)	26%(39)
Use anywhere and anytime	63.4%(52)	73.5%(50)	68%(102)
Can watch many times and revise	84.1%(69)	83.8%(57)	84%(126)
Regular notifications of classes	48.7%(40)	41.1%(28)	45.33%(68)
Interaction through private comment section	36.58%(30)	72%(49)	90%(135)
The grading system help in monitoring my performance	42.6%(35)	69.1%(47)	52.66%(79)
The feedback provided by the lecturer is useful.	89%(73)	82.3%(56)	86%(129)
Good for self learning process best alternative for physical class	15.8%(13)	29.4%(20)	22%(33)
Time saver	8.5%(7)	8.8%(6)	8.66%(13)
Paperless or less paper work	10.9%(9)	27.9%(19)	18.66%(28)

Table 5: Disadvantages of Google classroom app

Feedback responses	Boy (n=82)	Girl (n=68)	Total (n=150)
No search option	24.3%(20)	17.6%(12)	21.33%(32)
Difficult to edit assignment and upload	3.6%(3)	2.9%(2)	3.33%(5)
No automated update	1.2%(1)	0%(0)	06%(1)
Took time to adjust for this app	7.3%(6)	14.7%(10)	10.66%(16)
No integration			
It consumes lot of data and storage space	50%(41)	42.6%(29)	46.66%(70)
Late announcement due to network problems	32.9%(27)	29.4%(20)	38%(57)

ABBREVIATIONS

MBBS: Bachelor of Medicine, Bachelor of Surgery

Discussion

Online teaching and its advantages

As India announced nationwide lockdown, many students got stuck at their hometown. In our study around 54% of students responded as online classes were the best way of utilizing lockdown time and regular assignments help them to remember things better than physical classroom (53%). Students felt study was stress free and there was no hurry to take notes. Few students, who were shy to ask questions in the physical classroom, were comfortable in asking questions through private comment section. The great advantage of online classes is that students become active learners. They are directed to solve problems through a self-regulated process. "This takes an approach of social constructivism in which the student must steer his/her own learning process"[12].

Effectiveness of online classes

In our study around 59% of students expressed that, online classes were not. This may be possibly due to facilitators not been effective in making the class interactive. The primary disadvantage reported by students in online courses is the absence of interaction between instructor and student [13] which makes online classes less effective. Research supports the fact that course satisfaction is contingent upon course interaction [14]. Students benefit greatly from strong feelings of a sense of community[15] and students who received consistent personalized instructor feedback exhibited higher satisfaction levels and academic gains" [16]. The goal of online classes is to create a transformative learning which is an outgrowth of self-directed learning.

Ease of usage of Google classroom app

As Google classroom app is accessible on mobile there is high potential to increase the communication and interaction among participants in online classes [8].

In our study, both teachers and students felt that, Google classroom app was easy to sign, easy access of material (4.71) and easy navigating system (4.55) and lowest score was for submission of assignment (4.0). This may be possibly due to the network issues faced by students. Google Classroom provides easy-to-use learning features with students of all categories able to cooperate. Mobile access to learning materials that are easy to interact and easy to mark is critical to online teaching and save time also [17, 18].

Even though the students of 21st century are very well versed with use of app on mobiles but social use does not translate directly into educational use [19]. In order to benefit from online courses, students must be comfortable with the technology. In our study we

found that, students were comfortable in using Google classroom app.

Advantages of Google classroom app

Google Classroom is an educational interactive tool that allows creating an informatively rich educational environment [20]. Whole process of administering assignments, grading, formative assessment, and feedback is simplified and streamlined as it integrates with other Google apps[1] . In our study, students expressed that interaction through private comment section (90%) helped them to overcome shyness while constructive feedback given by the instructors (86%) helped them to learn better. In online environment, students receive far less informal feedback thus the provision of effective assignment feedback is particularly important. In this app, students can view their grading and keep a track of their assignments and also view the assignments submitted by other students. This helps them know where they stand and get the sense of learning community. Mobile friendly app (68%) and material available for revision (84%) were other advantages quoted.

Disadvantages of Google classroom app

Though Google classroom is equipped with user friendly teaching and learning interface, it has certain disadvantages like no search option (21.33%), no automated update option, missed new announcements (38%) and app consumes lot of data and storage space.

Teachers' perspectives on Google classroom app

Google Classroom helps teachers save time, keep classes organized, and improve communication with students [1]. This app also allows teachers to welcome and associate the guardians so they can track their children's performance and get email declarations relating to the learning of their kids [21]. Teachers suggested having a feature of individual folders for students.

Conclusion

Online classes are the best way of utilizing the lockdown time to make up the loss of academic period. Google classroom is an effective tool for teaching and learning online classes because of its ease of use and features which are both teacher friendly and student friendly.

Ethical Clearance: Ethical clearance obtained from institutional ethical committee.

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A Study of Autopsy Cases - Viscera Sample Reports A Retrospective Study

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Abstract

Analysis of viscera. i.e subjecting the internal organs, body fluids and secretions to Forensic Science lab is a part of routine procedure in complete autopsy. The viscera is sent to lab for detected of any poison or substance abuse eg. alcohol, organophosphorus poisoning. This retrospective study was conducted in Government Thoothukudi Medical College, Department of Forensic Medicine, during the period of Jan to March 2018. A total of 100 cases whose viscera samples sent to the forensic science lab were analyzed by perusing inquest reports, history of the case, autopsy findings, histopathology reports, and viscera reports. The data obtained from the study was analysed. 78 (78%) cases were males and 22(22%) cases were females. The maximum number of cases 21(21%) was seen in 31- 40 years. Alleged history of poison ingestion was noted in 21(21%) cases, out of which poison was detected in 10(10%) cases only. The poison was not detected in 11 (11%)cases. with a history of sudden death in 35 cases out of which 9(9%) cases poison was detected. organophosphorus compounds were found in 11(11%) cases and ethyl alcohol was detected in 6(6%) cases. Paraphenylene diamine was detected in one case and cyanide was detected in one case(1%). Hence it is recommended to send the viscera samples to the lab in all cases of sudden deaths.

***Keywords**: Autopsy, Viscera, Forensic Science Lab*

Introduction

Analysis of viscera. i.e subjecting the internal organs, body fluids and secretions to Forensic Science lab is a part of routine procedure in complete autopsy. The viscera is sent to lab for detected of any poison or substance abuse eg. alcohol, organophosphorus poisoning. Poison is a substance that causes damage or injury to the body and endangers one's life due to its exposure by means of ingestion, inhalation, or contact by Thomas WF *et. all* [(1)]. Worldwide intentional poisoning is one of the important causes of mortality and morbidity by Eddleston M *et. all* [(2)]. Distress due to loss in the business, failure in romance or differences with the intimate partner, or examination, emotional disturbances, and chronic diseases are the common reasons for intentional poisoning by Chowdhary AN *et. all* [(3)].

The society of Forensic Toxicology and American Academy of Forensic Science combinedly recommends the following organs to be preserved for viscera analysis. Brain, Liver, Kidney 50 gms each. Heart blood 25 ml, peripheral blood 10 ml. Vitreous, bile and urine available quantity is collected [(4)]. Studies have revealed that pesticides are the commonly used poisoning agents for intentional poisoning in India. As agriculture is a major profession in the rural part of India farmers stock the pesticides to eradicate the weeds and pests. Due to the easy availability of pesticides, they are commonly used by individuals to end their life in stressful situations. In medico legal cases most of the history of the case was not clear. The body may be found dead in some remote area or the house. The history of poison consumption

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may be concealed by relatives due to want to insurance money.

Materials and Methods

This retrospective study was conducted in Government Thoothukudi Medical College, Department of Forensic Medicine, during the period of Jan to March 2018. A total of 100 cases whose viscera samples sent to the forensic science lab were analyzed by perusing inquest reports, history of the case, autopsy findings, histopathology reports, and viscera reports. The data obtained from the study was analyzed. The cases in which viscera samples were not sent to the lab were excluded from the study.

Results and Discussion

Out of 100 cases analyzed 78 (78%) cases were males and 22(22%) cases were females. In marital status 74(74%) cases were married and 19(19%) cases were unmarried and marital status was not known in 7(7%) cases. In analyzing religion 14(14%) cases belong to Christianity, 2 (2%) cases belong to Muslims and in 4 (4%) cases not mentioned as their identity of the body was not established. 80(80%) cases belong to the Hindu religion. In the age group of 31- 40 years maximum number of cases 21(21%) was seen. In the age group of 51- 60 years 20(20%) cases were seen.(Fig. 1)

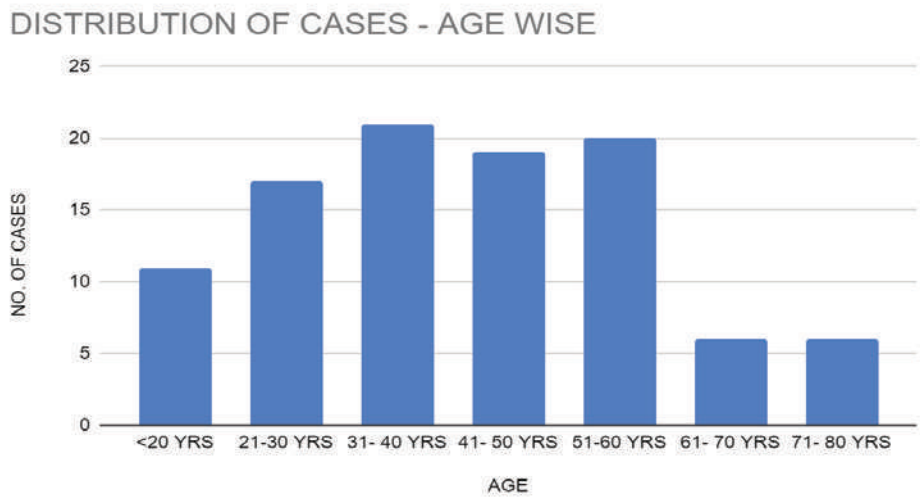


Fig:1. Distribution of cases among age group

Alleged history of poison ingestion was noted in 21(21%) cases, out of which poison was detected in 10(10%) cases only. The poison was not detected in 11 (11%)cases. (Fig:2)

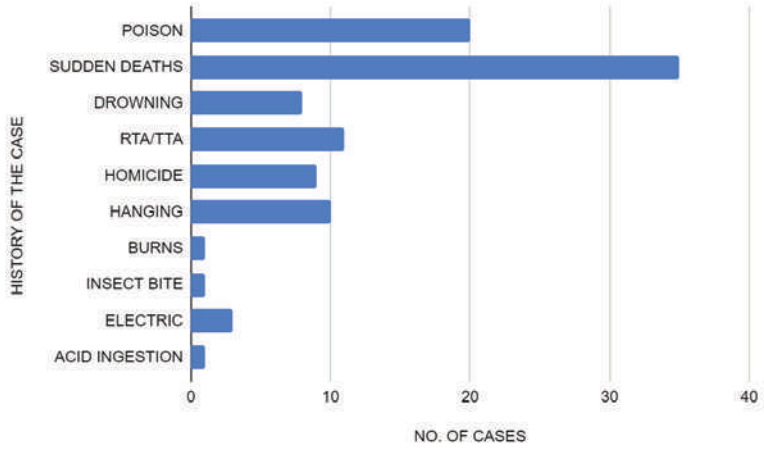


Fig:2. Distribution of cases according to the history of the case

This is similar to the study conducted by Gupta B.D *et.all* [5] 7.37% of cases poison was not detected. In these cases, the autopsy report showed a smell of kerosene/ pungent smell/change in the color of gastric contents. Hence the opinion was given as "*postmortem findings consistent with history and inquest report, clinical findings of poison, however, nature of poison was not detected in viscera report ''.*

The reason for non-detection of poison in the viscera may be due the deceased may have been unusually susceptible to the effects of the poison. The poison has been removed from the gastrointestinal tract by vomiting and purging. The poison may be detoxified, conjugated, and eliminated by the kidneys. The detection of highly potent toxic substances with low lethal dose is quite difficult. Interference of proteins, fats, and decomposition products sometimes makes the detection difficult or rather impossible. Certain vegetable poisons may not be detected in the viscera as they have no reliable tests, while some organic poisons, especially the alkaloids and glucosides, may, by oxidation during life or by putrefaction after death, be split up into other substances having no characteristic reactions sufficient to reveal identification. This is especially likely to occur in case of very volatile, for example, gaseous poisons. Lack of reliable means of extracting the poison from substances containing it or no satisfactory tests available for its identification Tyagi A *et.all*[6]. Biological toxins and venoms that may be protein in nature cannot be separated from body tissues. Want care or skill on the part of the analyst [7]

. In cases brought dead with a history of sudden death in 35 cases out of which 9(9%) cases poison was detected. A poison was detected in one case of homicide i.e.- ethyl alcohol. Out of detected cases organophosphorus compounds were found in 11(11%) cases and ethyl alcohol was detected in 6(6%) cases. Paraphenylene diamine was detected in one case and cyanide was detected in one case(1%). Alcohol/ poison not detected in none of the cases with history of road traffic accident(11%).(Fig.3)

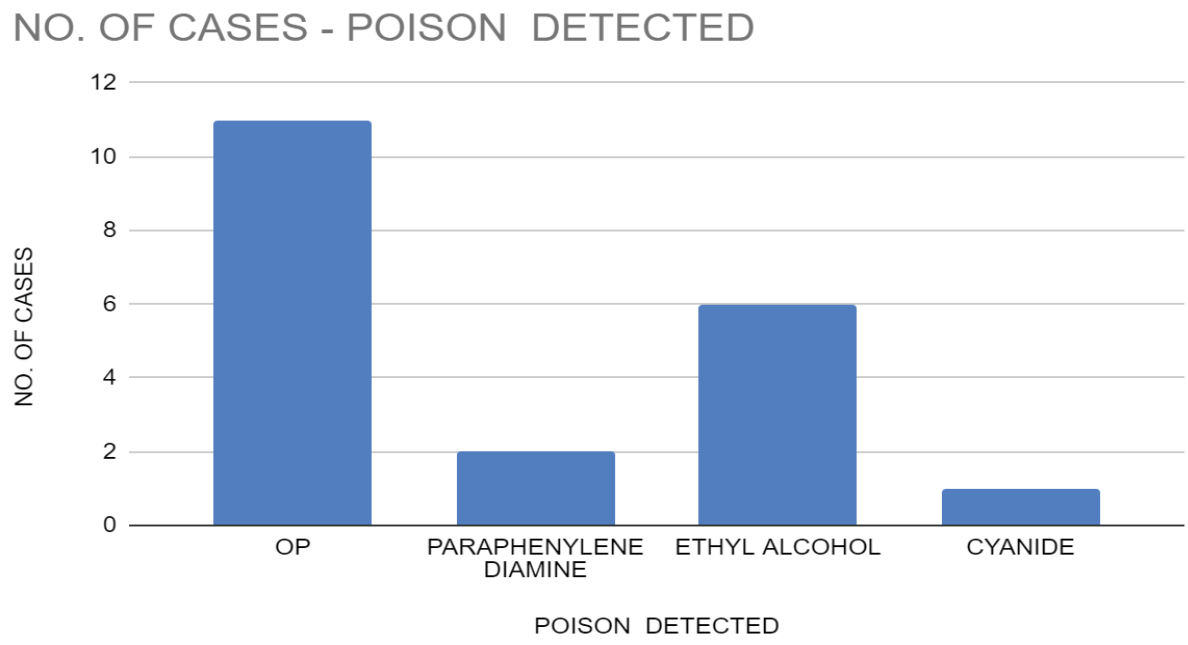


Fig:3 . Type of poison detected in the study group.

The cases reported as sudden deaths were seen in 40 to 60 years. In less than 20 years of age group 11(11%) cases were seen. Out of 35 (35%) cases with alleged history of sudden death, 25(25%) of cases of atherosclerotic coronary arterial heart disease was the cause of death. This corresponds to the study conducted by Fatma Sene *et.all* [8] and 1 (1%) due to esophageal variceal bleed. *No definite opinion on the cause of death* was given in 14(14%) cases. *"In* these 14 (14%)cases, no external or internal injuries seen anywhere on the body, gross and microscopic features of the organ within normal limits, no poison was detected in the viscera.

Another difficulty arises in concluding the cause of death is when viscera detected ethyl alcohol in internal organs but it is not at a fatal level, meanwhile autopsy findings of heart within normal limits, microscopy of heart, and other internal organs within normal limits. In this study, 3(3%) cases were fit into the above-mentioned scenario. Forensic lab quantification of ethyl alcohol only done, the rest of the common poisons such as organophosphorus poisons, aluminum phosphide, plant poisons quantification was not done.

Conclusion

Ideally all cases subjected to autopsy should be sent to viscera analysis for detection of poison. In mortuaries with excess overload of cases especially in our country not all cases brought for medico legal examination were subjected to viscera analysis. Only the cases with alleged history of poison ingestion, homicide cases, suspicious death, death in young age, viscera samples were preserved and sent to the lab. Major junk of cases was sudden death in this study 35 (35%) cases of sudden death were seen, in which no history of poison ingestion/ drug abuse was noted. Viscera analysis detected poison in these cases. Hence it is beneficial for the doctor to send the samples for viscera analysis in all cases of sudden deaths.

Conflict of Intrest: Nil.

Source of Funding: Self.

Ethical Clearance: Nil

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Digital Visualizer as an Aid-assisted Teaching in Anatomy

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Abstract

Background: Innovative medical education technologies are used as alternate mode of teaching-learning of anatomy to undergraduate students. As knowledge of anatomy helps students in their clinical area, effective innovative teaching-learning tools need to be explored to build up their competency in anatomy.

Aim: To find the outcome of application of digital visualizer in systematic teaching-learning of anatomy to undergraduate dental students.

Materials and methods: A cross sectional study was undertaken among first year dental students studying at Penang International Dental College, Salem, India. 50 students were divided in two groups. The groups were subjected to conventional and aid-assisted teaching using digital visualizer in a cross-over design on separate topics with comparable level of difficulty. Both groups were assessed by pre and post-test followed by feedback from them in reference to use of digital visualizer as aid in regular anatomy teaching-learning process.

Results: Both the conventional and aid-assisted teaching showed significant changes in students pertaining to gaining of subject knowledge and the mean post-test score was found to be higher with the use of digital visualizer. The post-test scoring using digital visualize in both groups was higher in osteology teaching (10.21±0.76 & 10.39±0.83) and cadaver specimen demonstration (8.76±0.44 & 8.80±0.41). In the feedback analysis, majority of the students considered digital visualizer as an innovative aid for teaching-learning in anatomy.

Conclusion: The use of medical educational technologies can assist as a comprehensive tool in regular teaching learning sessions. The study will be further continued for first year medical students of our institution.

Keywords – *Anatomy, Digital Visualizer, Medical education, Teaching-learning process.*

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Introduction

The use of advanced technology in medical education started gaining its importance over many years. The modern trend of use of advanced technology has been developed due to various challenges that were faced in medical education such as change in healthcare environment, new generation of learners' expectations, change in curriculum mainly focusing on competencies and milestones and finally rapidly changing technology and its advancement.[1] The prevalence of use of

technology for both undergraduates and postgraduates has become more common. The applications of advanced technology were used in training programs, continuing medical education and workshops.The technology aided teaching learning modalities facilitate the learners in all ways and means such as knowledge acquisition, improves decision making capacity, improves the necessary skills, and moreover the technology provides an educational environment that enables the learnersto learn without endangering the patient.

The advancement of computer technologies enables the teacher to assess the learner'scompetencies and milestones, and trains the student, to access the medical knowledge at any time and thus becoming a life-long learner.The use of technology in medical education should be an additional tool or method for learning process and it should not turn to be an alternative or replacement for face-to-face learning. Technologies should be one of the tools in the educational toolbox. The medical educators should focus on the use and application of new technologies to transform the learning process as a collaborative and empowering experience for the learners.[2]

Digital visualizer provides high resolution magnified sharper image of any material that is focused on its inbuilt camera. These high resolution images enable the demonstrator to display all minute details of any small objects to a large group of learners. When a computer or laptop is connected with this digital visualizer, demonstration of any structures / procedures can be done easily even to a larger group of learners or trainees in outreach sites also.[3,4] The first year students of medicine and dentistry in India are taught anatomy before they are exposed to patient care in the second year. The basic anatomy knowledge is very much essential for students to develop their clinical skills. The need of effective, innovative teaching-learning method has to be explored to enrich the competenciesof students in anatomy.[5]

During routine anatomy dissection the osteology and embryology demonstration is carried out for groups of 10-15 students (approximately). The implementation of competency based medical education (CBME) curriculum is much focused on the small teaching learning groupswhich is found to be difficult due to the paucity of faculty. In anatomy the osteology demonstration, embryology model demonstration, OSPE demonstration, basic clinical examination and basic clinical investigations interpretation (X-rays) can be managed well with lesser faculty to teach and train a large group of students through tutorials. In the conventional teaching method the students always complain that they were not able to interpret foramen in bones, parts in embryology models and so on. Some advanced innovative technological aids for better viewing and interpretation of demonstrated structures to the larger groups is very much essential in the current senario.[6] Digital visualizer was introduced in the teaching-learning process for MBBS students at All India Institute of Medical Sciences (AIMS), Jodhpur (2016).[6]In the present studydigital visualizer was introduced in the teaching-learning process for BDS students from Malaysia at Penang International Dental College, Salem, India (2017).

Aim

- To find the outcome of application of digital visualizer in systematic teaching-learning of anatomy to undergraduate dental students.

Primary objectives

- To assess the performance of students taught by digital visualizer

Secondary objectives:

- To compare the performance of students by routine conventional teaching methods and visualizer aided teaching methods.

- To compare the teaching-learning process of 2 different sections of anatomy (osteology and cadaver head & neck specimen demonstration).

Materials & Methods

The cross sectional study was approved by IEC of VMKV Medical College & Hospitals, Salem (Reference-VMKVMC/IEC/18/48). A qualitative and quantitative analysis was undertaken among the first year dental students studying at Penang International Dental College, Salem. 50 students were divided in two groups by random allocation method (Group A - 25 & Group B - 25). Groups were subjected to conventional and aid-assisted teaching using digital visualizer (Fig.1)

in a cross-over design separately for the topics from osteology and cadaveric head & neck specimen demonstration with comparable level of difficulty as two different sessions. Both groups were assessed by pre and post-test for both the sessions separately followed by feedback using validated structured questionnairefrom the students in reference to use of digital visualizer as aid in regular anatomy teaching-learning process. The mean, standard deviation and standard error mean of the assessment were calculated. The data were analyzed by paired t test to compare both the assessment using online GraphPadQuickCals t test calculator.



Figure – 1 Aid-assisted teaching using digital visualizer

Results

A cross-over design separately for the topics from osteology and cadaveric head & neck specimen demonstration was done as two different sessions for both the groups. Both groups were assessed by pre and post-test for both the sessions separately followed by feedback from the students.Both the conventional and aid-assisted teaching showed significant changes in the mean post-test score for both the sessions. The post test results of aid assisted teaching with the use of digital visualizerwas found to be statistically significant when compared to pretest (Table1). In the feedback analysis, it was noted that majority of the students considered digital visualizer as an innovative aid for teaching-learning in anatomy (Table 2).

Table – 1:Comparison of studentsperformance using conventional method and digital visualizer in osteology and cadaveric specimen demonstration

Groups	Assessment	Osteology demonstration		Cadaveric specimen demonstration	
		Conventional Teaching	Visualizer teaching	Conventional Teaching	Visualizer teaching
Group A	Pre test	3.56 ± 2.09	4.21± 1.24	3.44±0.51	3.84±0.85
	Post test	8.5 ± 2.04	10.21 ± 0.76	7.12±0.83	8.76±0.44
	P Value	0.001***	0.001***	0.001***	0.001***
Group B	Pre test	3.42 ± 1.91	3.17 ± 1.76	3.88±0.83	3.68±0.80
	Post test	9.78 ± 0.69	10.39 ± 0.83	7.01±0.87	8.80±0.41
	P Value	0.001***	0.001***	0.001***	0.001***

*Values are expressed as Mean ± SD, n = 25 in each group,# - non significant, * - significant, P value *P<0.05, **P< 0.01, ***P<0.001*. Statisticalanalysis –Paired t test.

Table – 2: Feedback of students on usage of digital visualizer in teaching process

S.no.	Parameters	Yes	No	Not sure
1.	Do you want the usage of modern teaching aids by lecturers?	50 (100%)	-	-
2.	Is digital visualizer teaching useful in osteology demonstration?	50(100%)	-	-
3.	Do you prefer this visualizer aid as support mechanism for face-to-face learning or as an individual tool?	49(98%)	-	1 (2%)
4.	Is it useful in terms of perception of minor details?	45 (90%)	1	4 (8%)
5.	Is there an ability to interact and clear doubt?	49 (98%)	-	1 (2%)
6.	In comparison of visualizer with conventional teaching at the table with bones, is this method better?	45(90%)	1 (2%)	4 (8%)
7.	Is the understanding of the topic easier with visualizer?	49 (98%)	1 (2%)	-
8.	Is identifying the structures with visualizer more clear and precise?	48 (96%)	-	2 (4%)
9.	Is it easier to memorize the details?	45 (90%)	1 (2%)	4 (8%)
10.	Is the linking of the structures with the verbal information faster and easier in thoughts with visualizer?	50 (100%)	-	-
11.	Whether we need to continue use of visualizer for demonstration?	49 (98%)	-	1 (2%)
12.	Does visualizer have a role in enhancing confidence in subject and improving knowledge?	47 (94%)	-	3 (6%)
13.	Does the use of digital visualizer make lecture interesting and interactive?	49 (98%)	-	1 (2%)
14.	Is digital visualizer good as an innovative aid for teaching-learning in anatomy?	49 (98%)	-	1 (2%)

Discussion

Today's student generations are tech-savvy and they have divergent domain of learning by visual, auditory, reading, kinesthetic modes (VARK).[6] The facilitators should train themselves in these teaching-learning modes and apply them to impart knowledge to students in their own way of interest.[7,8,9] Earlier the education system mainly focused on the educator only where topic was taught as an information by them and not on the learner, who has been just considered as receiver of the information. But now the scenario has been totally changed towards learner oriented education. For many decades the teaching-learning delivery medium was via the "chalk-and-talk" method and Overhead Projector

(OHP) transparencies were considered as popular techniques.[10] It was reported in various studies that use of traditional methods are considered to be the best for teaching-learning in biomedicine and medicine courses.[11]

Digital visualizer allows documents, books or slides, drawing, models and specimens to be displayed whenever required for demonstration and its usage is considered to be more feasible than overhead projector based presentation.[2]Various studies showed that the application of innovative teaching aids are necessary for illustrations and understanding not only in Anatomy,[12]but also in other subjects like Pharmacology,[13]Physiology,[14] Biochemistry,[15] Community medicine,[16] Obstetrics and Gynaecology[17] etc.

A study done by Ghatak et al., 2016using digital visualizer in India at AIMS, Jodhpur reported that both conventional and digital visualizer aid-assisted teaching when compared, digital visualizer teaching for osteology showed significantly higher post-test scoring(17.31±2.63) and embryology teaching also showed higher post-test scoring(15.72±1.79) whereas the post-test scoring of students using conventional methods was lower for both osteology teaching (14.46±2.76) and embryology teaching (14.08±2.30).[6] In the present study similarly the post-test scoring in both groups was higher in osteology teaching using digital visualizer(10.21±0.76 & 10.39±0.83) whereas the post-test scoring in both groups was found to be lower in conventional methods (8.5±2.04 & 9.78±0.69) (Table 1). The head and neck cadaveric specimen demonstration was also done using both methods of teaching. Similarly the post-test scoring in both groups was higher in cadaveric specimen demonstration using digital visualizer(8.76±0.44 & 8.80±0.41) whereas the post-test scoring in both groups was found to be lower in conventional methods (7.12±0.83 & 7.01±0.87) (Table 1).

Student's feedback on the use of digital visualizer in teaching-learning of anatomy:

Students' feedback is a need to evaluate any teaching-learning methodology and which helps the facilitator to identify the pros and cons of those various methods. [18] In the present study all the students preferred the usage of modern teaching aids by lecturers. The digital visualizer teaching was found to be useful in both osteology and cadaveric specimen demonstration for the students. In their feedback on the usage of visualize, students opined that their perception of minor details was good, identifying the structures was more clear and precise, understanding the topic was made easy, free to interact and clear their doubts, felt that the method was enhancing their confidence in subject and improving the knowledge. The students wanted to continue the use of visualizer for demonstration and also felt that it was good as an innovative aid for teaching-learning in anatomy (Table 2). The feedback was found to be similar to that of the study done by Ghatak et al., 2016.[6] Various studies report that any system can be considered possible in its execution but the system has to be constantly modified as per the requisites of the students.[19]

Conclusion

The use of newer medical educational technologies can assist as a comprehensive tool in regular teaching learning sessions but they cannot replace the conventional teaching methods. A thorough understanding and comprehension of a topic can be made much easier with the aid by the facilitator. The outcome of the study in application of digital visualizer in systematic teaching-learning of anatomy to undergraduate dental students was found to be more feasible and applicable. The performance of students taught using digital visualizer was found to be good than the routine conventional teaching methods. When the teaching-learning process of different sections of anatomy such as osteology, cadaveric specimen demonstration was compared with conventional method, the digital visualizer teaching method was found to be more effective for all sections. Infuture the study will be further continued for first year medical students of our institution.

Limitations of the Study: The study has to be done on more topics and in different sections of anatomy in a larger group of students. The study has to be continued over years together to show its effectiveness.

Ethical Clearance: The study was approved by IEC of VMKV Medical College & Hospitals, Salem (Reference-VMKVMC/IEC/18/48).

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Assessment of Assertiveness and Self Esteem among Nursing Students in Selected Nursing Colleges: A Systematic Review

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Abstract

Background: Assertive behaviour promotes a feeling of personal power and self-confidence. These two components are commonly lacking in individual/students. Becoming more assertive empowers individuals by empowering self-esteem, without diminishing the esteem of others. **Purpose:** To systematically review the articles to assess assertiveness among nursing students in selected Nursing colleges. **Method:** The search of significant articles including assertiveness and its correlates among nursing students was carried out in PubMed/Medline, Google Scholar and other databases. It includes both Qualitative and Quantitative studies were evaluated according to predefined quality criteria. Medline and PubMed were searched to identify peer-reviewed English-language studies published between 1985 and March 2018 reporting Assertiveness among nursing students. Total 250 studies were selected for review, out of which only 30 Studies were found appropriate to be included in the study. **Results:** Analysis was done 90 papers were retrieved by the search. Among them, 30 were evaluated to be of higher quality. These Studies included sample of nursing students and excluded Non Nursing Students. **Conclusion:** Based on the review it was concluded that the level of assertiveness among nursing students studying in advanced Semesters was increased slightly as compared to those displayed by first-semester. It was also concluded that concluded that there was significant correlation found between the assertiveness and academic achievement and with self- esteem.

Keywords: *Assertiveness, Self Esteem, Nursing Students.*

Introduction

Assertive behavior helps us feel good about ourselves and increases our self-esteem. Assertive honesty is not an outspoken declaration of everything that is on one's mind. It is instead an accurate representation of feelings, opinions, or preferences expressed in a manner that promotes self-respect and respect for others. Communication must occur in an appropriate context in ordered to be considered assertive.[1]

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Students nurses who are aggressive defend their own basic right by violent the basic right of other. Feeling are often expressed dishonestly and inappropriate .They express an air of superiority , their voice are often loud, demanding and angry and cold , without emotion They not listen anyone and showing aggression if task is not fulfilled according to their desire. When nursing students express a lack of confidence and a lack of assertiveness, patients may notice. If those patients do not feel that nursing students are confident in their work, skills and practices they will not have a strong trusting relationship.[2]

Self-esteem is directly related to assertiveness. It is used to describe a person's overall sense of self-worth or personal value. In other words, how much you appreciate and like yourself. It plays a significant role in your motivation and success throughout your life.[3]

Material and Methods

Research Design: A systematic review was conducted by electronic search to identify articles in PubMed/ Medline, and Google Scholar.

Inclusion criteria: The study includes the studies like Descriptive, cross sectional, systematic review, quasi experimental studies and qualitative which are related to assessment of assertiveness and Self Esteem among Nursing Students.

Exclusion criteria: The study excludes studies on assessment of assertiveness and self-esteem among non-Nursing students.

Data Analysis: The data was grouped and analyzed in terms of Meta-analysis. Relevant articles based on the topic assertiveness and Self Esteem among Nursing Students were identified by search of significant articles PubMed/Medline, SCOPUS, CINAHL, PsychoINFO, Elsevier and Google Scholar with the following key words: Assertiveness, Self Esteem and Nursing Students

The search generated a total 90 articles. Out of 50 articles, the full texts of the 13 articles were retrieved and reviewed.

Results

A systematic review was done to assess assertiveness and Self Esteem among nursing students in selected Nursing colleges. Total 50 studies were selected for review out of which 13 were found appropriate for systematic review. The reviews were divided into two different areas:

Ø Related to Assertiveness among the nursing students.

Ø Related to Self Esteem among Students.

Related to Assertiveness among the nursing students

Out of 8 studies, 1 Study was Comparative study done to compare the assertiveness scores of sophomore level nursing students and senior level-nursing students. The findings of the study revealed that the sophomore level students were found to be more assertive than the senior level-nursing students.[2] Among these Studies, 3 Studies were Cross- Sectional Studies assessed the level of assertiveness among the IInd year B.Sc. nursing students and To find out the association between level of assertiveness among second year B.Sc. Nursing students with their selected socio demographic variable[4], 2nd study was conducted to assess the relationship of assertive behavior and self-esteem with each other and with other demographic variables among nurses[5] and 3rd study was carried out to evaluate the relationship between assertiveness and depression as well as investigate assertiveness and depression levels in the students[6]. Among these Studies,2 studies were Descriptive research studies which assess level of assertiveness among undergraduate Nursing students in Greece[7] whereas other study was conducted assess the changing level of assertiveness during the pre-registration among nursing students[8] . 1 study was correlational study which was conducted to assess the correlation between the assertive behavior and self-esteem among adolescents[9].

Sample Review

A descriptive study was conducted to investigate the high assertiveness level i e sophomore level nursing students and senior level-nursing students. A total of195 nursing students were selected by using convenience sampling techniques. Data was collected by using demographic variables and Rathus assertiveness schedule. The data was analyzed by using the descriptive and inferential statistics. The results of the study showed that majority of the sophomore and senior level-nursing students were Caucasian. Hence, the study concluded that males were having higher assertive behavior then female students. A significant difference (p=.049) was found between assertiveness scores of students who were employed (4.69) and those who were not (9.21). The researcher suggests that the students who were not employed may not be aware about knowledge and effective communication skills essential for successful assertiveness.

A cross- sectional study was conducted to find out the association between the assertiveness with selected demographic variables among 100 B. Sc. (N) students by using purposive sampling techniques. Data was collected by using socio demographic variables and Rathus Assertiveness rating scale. Data was analyzed by using the descriptive and inferential statistics .The Chi-Square test was used to assess the association between

the level of assertiveness among B.Sc.(N) 2nd year students with their demographic variables The result of the study showed that out of 100 B.Sc.(N) students, 3(3%) of nursing students in the category of non-assertive, 6(6%) students was situational nonassertive, 27(27%) students was somewhat assertive, 41(41%) students were assertive and 23(23%) students was probably aggressive .The findings of the study showed that there is no significant association between the level of assertiveness among BSc(N) 2nd year students with the selected socio demographic variables like age, religion, place of residence and type of family.

A descriptive correlational survey was done to find out the relationship between assertiveness and self-esteem among 60 adolescents was selected by using random sampling technique. Data was collected by using Rathus assertiveness schedule and Rosenberg self- esteem Scale. The data was analyzed by using descriptive and inferential statistics. The result of the study showed that majority of the adolescents (75%) in the category of moderately assertive and high self-esteem was (53.33%).There was a positive correlation (r=0.64) found between the assertive behavior and self-esteem of the adolescents and a significant association was found between the assertive scores with the age & education level of the adolescents. Hence it was concluded that Education level of the adolescents was the only variable significantly associated between the assertive and self-esteem scores.[6]

Related to Self Esteem among Students

Out of 6studies, 1 study was Quasi-experimental study which was conducted to evaluate or enhance the self-esteem among 922 nursing students[10]. Among these studies, 2 studies were Cross sectional study which was conducted to assess the self-esteem among 300 nursing students which showed that male and female student respondents indicated positive senses of self-esteem[11] whereas other study was conducted medical universities in Tehran among 332 students by using the stratified sampling to determine the self-esteem of nursing students and compare the self-esteem of nursing students to four of medical universities in Tehran.[12] Among these studies, 1 study was exploratory study which aims to assess the level of self-esteem among adolescents and to associate the self-esteem among adolescent with their selected demographic variables and the finding of the study showed that majority of adolescents had moderate level of self-esteem[13]. Among these studies, 1 study was correlational study which was conducted to investigate the relationship between the self-esteem , assertiveness and academic achievement among 701 female students and the result of the study concluded that there was significant correlation found between assertiveness and academic achievement, and finally self- esteem with assertiveness[14].

Sample Review

A cross sectional study was conducted to assess the self-esteem among 300 nursing students selected by stratified random sampling in college of Nursing (University of Mosul, Iraq in 2015. Data was collected by using the demographic variables and RSES. The data were analyzed by using descriptive and inferential statistics. The result of the study showed that male and female student respondents indicated positive senses of self-esteem, when the perceived self-worthy of the male students was differentiate with the female. It also showed that self-esteem scores were (29.3±0.2) with range (20-40), (54.7%) of nursing students had Moderate self-esteem level, (29%) had High level self-esteem and (16.3%) had low level self-esteem. Male students had more scores (30.4±1.2) than female students (29.1±0.1)

A Correlational study was conducted to investigate the relationship between the self-esteem , assertiveness and academic achievement among 701 female students by using the stratified random sampling techniques .Data were collected by using the demographic information , Coppersmith Self-Esteem scale and Gambrills and Ritchie's assertiveness questionnaire. Data were analyzed by using the descriptive and inferential statistics. The result of the study concluded that there was a significant correlation found between assertiveness and academic achievement ($p \leq 0.001$), assertiveness and birth order was (p: 0.006), and finally self- esteem with assertiveness was ($p \leq 0.001$).

Ethical Clearance: Ethical approval was taken from the institutional ethical committee of M.M University Mullana, Ambala. , Haryana for conducting the study.

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Cryotherapy as an Adjunct to Cleaning and Shaping in Endodontics: A Review

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Abstract

Cryotherapy refers to treatments that are done at reduced temperatures. In the recent times, Cryotherapy has found use in the field of endodontics during root canal irrigation, primarily to reduce post-operative pain after non-surgical endodontic therapy. In this review, we are throwing some light over use of Cryotherapy in root canal irrigation, its clinical implications along with positive and negative side effects based on currently available literature. Vital pulp cryotherapy is a process in which sterile water ice shavings are applied on exposed pulp tissue. Cryogenic fluid, when used as an irrigant has shown efficacy in antibacterial action as it can reach the desired depth and bring about immediate freezing of bacterial cells along with their subsequent Cryodestruction. Use of cold saline solution (2.5 ^{0}C) as the final irrigant brings about reduction in external root surface temperature by more than 10^{0}C for 4 minutes, which might be produce a local anti-inflammatory effect in the periradicular tissues. In addition, Various clinical and experimental studies have highlighted the role of Cryoirrigation in controlling post-operative pain after endodontic therapy.

***Key words**: Cryotherapy, Endodontics, Root canal irrigants*

Introduction

The word "cryotherapy" comes from the term "cryos" meaning "very cold" or "ice-cold" in Greek language. Therefore, cryotherapy refers to treatments that are done at reduced temperatures.[1] The early Egyptians used low temperatures in the treatment of inflammation and lesions as early as 2500 BCE. Hippocrates, who used Cryotherapy, suggested local and/or systemic ice application for medicinal purposes.[2] The purpose of Cryotherapy is to reduce heat and as a result, benefit from a reduction in inflammation.[3] Cryotherapy has been in use in control of pain for more than 50 years.[4] The three important physiological tissue reactions following the application of low temperatures are, a reduction in the local blood flow, a decrease in metabolic activity, neural receptor activation in the subcutaneous tissues and skin.[5] This treatment is commonly used in various medical fields, like neurology,[6] orthopaedics, [7]physiotherapy,[8] traumatology,[9] plastic surgery,[10] maxillofacial surgery,[11] dental surgery,[12] and relatively recently endodontics.[13,14]

Cryotherapy has been in use for more than 50 years in oral medicine and in pathology. However, Evidence of tissue degradation by freezing dates back to 1851.

Initially, this use was limited to treating lip cancer and cancer of the oral cavities. It's uses in the area of the head and neck are currently extensive and include treatment of various benign growths in the skin, as well as malignant lesions.[15]

In the recent times, cryotherapy has found use in the field of endodontics during root canal irrigation, primarily to reduce post-operative pain after non-

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surgical endodontic therapy.[14] In this review, we are throwing some light over use of cryotherapy in root canal irrigation, its clinical implications along with positive and negative side effects based on currently available literature.

1.Vital Pulp Cryotherapy

When a carious tooth is diagnosed with reversible or irreversible pulpitis and caries excavation leads to direct or indirect pulp exposure, then vital pulp capping or partial pulpectomy is the choice of treatment. However, Pulps that have a pre-treatment diagnosis of necrosis or upon pulp chamber access are observed to have partial necrosis of the pulp; vital pulp therapy is contraindicated. It is necessary to remember that pulpal bleeding may be a clinical marker for the severity of pulpal inflammation.[16] Haemorrhaging in pulps with a pre-treatment diagnosis of mild or reversible pulpitis appears to stagnate and be easily regulated with ice application. Cryo-technique involves the use of sterile water ice shavings over the exposed pulpal tissue. The melted ice should then be removed with a high-speed suction after one minute, followed by irrigation with 17% EDTA.[17] Use of sodium hypochlorite is not advised while performing vital pulp cryotherapy procedure, since it has been shown to destroy pulpal stem cells.[18] Use of EDTA solution is encouraged since has been shown to release bioactive growth factors from dentin, thereby stimulating secretion of matrix, odontoblastic differentiation, and tertiary dentin formation along with promoting adhesion, migration, and differentiation of dental pulp stem cells.[18,19]

In case of profuse bleeding, partial pulpectomy can be performed in order to remove the inflamed pulp tissue before placing sterile ice shavings while performing vital pulp cryotherapy.[17]

2.Role in Antimicrobial Action

Various irrigating solutions have been used in endodontics to reduce the bacterial load in the root canal system. Of the available solutions, Sodium hypochlorite (NaOCl) is currently the most commonly used.[20,21]

Yamamoto and Harris[22] have explained the effects of Cryotherapy involving use of liquid nitrogen over microorganisms. The process of freezing and thawing has shown to cause disruption of the cell wall, leakage of intracellular constituents and conformational changes in protein structure. The cryogenic fluid has been experimentally used in various studies against the gold standard irrigant, sodium hypochlorite, and has shown more efficacy in antibacterial action as it can reach the desired depth and bring about immediate freezing of bacterial cells along with their subsequent cryodestruction.[23]

3. Role in Reducing periapical inflammation

Periapical Inflammation has been associated with injury to the periapical tissues before, during or after the endodontic therapy. The result of periapical extension of pulpal inflammation, excessive instrumentation beyond the root apex during cleaning and shaping of root canals or over extended obturation can lead to periapical injury and result in periapical inflammation with classic signs of increased local temperature, swelling, pain and redness. Vera *et al.*[24] have established the fact that cryotherapy helps to reduce the external root surface temperature during endodontic treatment. Use of cold saline solution (2.5 ^{0} C) as the final irrigant brings about reduction in external root surface temperature by more than 10^{0}C for 4 minutes, which might be produce a local anti-inflammatory effect in the periradicular tissues.[24] Inflamed periradicular tissues may be treated with cryotherapy by intracanal irrigation with a cold substance along with a negative pressure irrigation device. The micro cannula of the negative pressure system can be applied to the full working length (WL) and to the continuous flow of the irrigant.[24,25]

4.Role in Reducing Post-operative pain

Various clinical and experimental studies have highlighted the role of cryoirrigation in controlling post-operative pain after endodontic therapy. Keskin *et al*.[14] evaluated the efficacy of saline solution at a temperature of 2.5 °C when used as the final irrigant on postoperative pain after single-visit root canal treatment of vital teeth, and found it to be extremely effective. Similar results were found by Al Nahlawi *et al*. [13]upon evaluation of level of post endodontic pain with the usage of intracanal cryotherapy with negative pressure irrigation (EndoVac) after vital single-visit endodontic treatment. As far as pain control during treatment of necrotic pulps is concerned, Vera *et al*. [26] concluded

the results in favour of cryotherapy after conducting randomised control trials. Gundogdu *et al*.[1] found that all the cryotherapy applications i.e. intracanal, intraoral, and extra oral application resulted in lower postoperative pain levels and lower VAS scores of pain level in all the human subjects. Pain control with intracanal cryotherapy can be attributed to the fact that cold therapy results in a decrease in the flow of blood due to induced vasoconstriction at the application site followed by reduction in tissue metabolism and oxygen utilisation which in turn minimises oedema and local release of pain mediators.[6] At the same time, leukocytes play a vital role in the inflammatory response of a soft tissue lesion. Therefore, cryotherapy has been shown to be effective in decreasing the amount of leukocytes adhering to the endothelial wall of capillaries, resulting in less of these cells migrating to the affected tissues, reducing endothelial dysfunction and inflammatory response.[27] Moreover, it affects peripheral nerve endings by decreasing the threshold needed to trigger the tissue nociceptors and the pace of painful nerve impulses. Cryotherapy causes a local anaesthetic effect by lowering the threshold for activation of these tissue nociceptors and the pain signal conductivity.[26]

Conclusion

Cryotherapy has been used in other dental specialities over the years, and has recently found its use in Endodontics. Studies have been conducted to evaluate the effectiveness of cryoirrigation in reducing periapical inflammation, reducing post-operative pain and also it's antimicrobial action. In addition, cryotherapy has also been shown to be an effective adjunct in vital pulp therapy. The initial research on cryotherapy have shown promising results. Further research is required to investigate the benefits of cryoirrigation in treatment of pulpal and periradicular diseases.

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Prevalence of Pelvic Girdle Pain in Post Partum Women

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Abstract

Background: Pelvic girdle pain is frequently reported musculoskeletal disorder in pregnant women. The condition is complex and underlying mechanism is unclear. Onset of pain is usually at 14-30 weeks of gestation.

Pregnancy hormones soften and stretch the ligaments of the body allowing the pelvis to open slightly during labour thus allowing easier passage. This leads to less stable pelvis. Women experiences reduced capacity for activities such as standing, walking and sitting.PGP results in greater disability in pregnant women. Pelvic instability and pain due to increased lumbar, thoracic and pelvic mobility due to secretion of relaxin hormone lead to increased ligament laxity and there is slightly larger range of movement in the pelvic joint. [3]

PGP is diagnosed on the basis of site of pain and ability to the pain by using the pain provocation tests. PGP is managed conservatively by a multidirectional team. It include addressing the psychosocial implication for the women and family. Approach should be implemented for activity modification , pelvic support garments, manage acute exacerbations, physiotherapy and exercise program to prevent progression of symptoms.

Objective: To find out the prevalence of pelvic girdle pain in post partum women.

Method: An observational study was carried out using a cross sectional study design. The study was conducted in the city of Karad, Maharashtra. Study was conducted using a sample size of 64 post partum women for a period of 6 months. The inclusion criteria was 20-40 years of females were included who were had FTND. Exclusion criteria was obese females, pre pregnancy low back pain, women with high risk of pregnancy and any kind of trauma to pelvic during pregnancy.

Results: The results showed that 60.93% women have pelvic girdle pain during their post partum period by using FABER, P4 test and pelvic girdle questionnaire (PGQ).

Conclusion: On the basis of the result of the study, it was concluded that there is significant pelvic girdle pain in post partum period.

Keywords: *Pelvic girdle, post partum period, FABER test, P4 test, Pelvic Girdle Questionnare (PGQ).*

Introduction

Pelvic girdle pain is frequently reported musculoskeletal disorder in pregnant women. It is universal problem affecting approximately 50% of all pregnant women. The condition is complex and underlying mechanism is unclear. Pain is not limited to a particular trimester during pregnancy but it is often experienced throughout pregnancy and the postpartum period. Onset is usually at 14-30 weeks of gestation. Pregnancy hormones soften and stretch the ligaments of the body allowing the pelvis to open slightly during labour thus allowing easier passage. This leads to less stable pelvis.Some women appear to be capable to

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handle the increased range of movement by improving muscle function but the other women cannot.[1]

Pelvic girdle pain (PGP) is defined as pain between the posterior iliac crest and the gluteal fold, particularly vicinity of the sacroiliac (SI) joint, which may radiate to the thighs and hips. Pelvic girdle pain can occur with or without pain in pubic symphysis.

PGP disorder comprise a distinct subgroup with a unique clinical presentation and need for specific management. Women experiences reduced capacity for activities such as standing, walking and sitting. PGP results in greater disability specially in the pregnant women. It will lead to poorer quality of life as result of being unable to carry out normal roles and predisposes to chronic pain syndrome.

There are multiple factors are responsible for development of PGP. It is related to hormonal, biomechanical, traumatic, metabolic, degenerative factors and genetic factors.[2] For developing PGP biomechanical pathophysiology is shifting of maternal centre of gravity towards anteriorly that transfers the forces onto lumbar spine and the enlarging uterus causes stress in the lower back and pelvic girdle. Anterior shift is associated with pubic symphysis pain and that indicates the women is having pelvic girdle pain. There are compensatory postural changes occurring in the pregnant women and that are increased lumbar lordosis, rounded shoulders, gait changes.

There will be pelvic instability and pain due to increased lumbar, thoracic and pelvic mobility due to secretion of relaxin hormone lead to increased ligament laxity and there is slightly larger range of movement in the pelvic joint.[3]It is not compensated by altered neuro-motor control, therefore pain may occur lead to widening and separation of the symphysis pubis.

Women with pelvic girdle pain experiences stabbing, shooting, burning type of deep unilateral or bilateral pain in gluteal region between the iliac crest and gluteal fold that may radiate to posterior thigh.Pain while turning in the bed is may be sign of having pelvic girdle pain.PGP is reported when there are increase in weight bearing activities, walking, standing up from chair and climbing stairs. Another commonly seen are poorer quality of life, work dissatisfaction and stress[4]. Women with previous pelvic or lower back pain, trauma to the pelvis, multiparity, increase in body mass index, physically demanding work, emotional distress, addiction of smoking and drinking while pregnancy, age factor, hormonal contraceptive use increases the woman's risk for developing the pelvic girdle pain.[5]

PGP is diagnosed on the basis of site of pain and ability to the pain by using the pain provocation tests.PGP is managed conservatively by a multidirectional team. It include addressing the psychosocial implication for the women and family. Approach should be implemented for activity modification , pelvic support garments, manage acute exacerbations, physiotherapy and exercise program to prevent progression of symptoms.

Methodology

An observational study was carried out using a cross sectional study design. The study was conducted in the city of karad, Maharashtra. The samples were chosen using the simple random sampling method. Study was conducted using a sample size of 64 post partum women for a period of 6 months. The inclusion criteria was 20-40 years of females were included who were had FTND. Exclusion criteria was obese females, pre pregnancy low back pain, women with high risk of pregnancy and any kind of trauma to pelvic during pregnancy.

Procedure:

The study was conducted in Krishna hospital, Karad. Subjects of this study were selected according to inclusion and exclusion criteria and written consent was taken. Full term normal delivery women's were included in this study. They were instructed with all the details assessment that they have going to undergo.

The patient were assess for the pelvic girdle pain with the help of FABER and P4 test. To perform FABER test patient is in supine with hip flexed. Patients leg is then externally rotated and abducted, with the ipsilateral heel resting on the opposite knee. The test is positive if there is the pain in the ipsilateral joints of the pelvis.

In the P4 test patient lies in the supine position and hip is flexed to 90^0.Downward pressure is applied to the knee along the axis of the femur. The test is positive if pain in elicited in the gluteal region.

After the confirmation of pelvic girdle pain they ask to fill up the pelvic girdle questionnaire. Total score of pelvic girdle questionnaire was calculated.

Findings

Table no.1 PGQ score

PGQ score	Frequency	%
≤50	25	39.06%
≥51	39	60.94%

This table shows that percentage of PGQ score below 50 is 39.06% and above 51 is 60.94% respectively.

Table no. 2 PGQ Score in age group 20 -≤30

	Mean±SD	T value	P value	Interference
Age (20-≤30)	26.70± 2.57	60.44	<0.0001	Extremely significant
PGQ Score	48.85±16.33	17.44	<0.0001	Extremely significant

This table shows the age group wise PGQ score. The age group is 20-≤30 with mean±SD 26.70± 2.57 and PGQ score mean SD is 48.85 ±16.33

Table no.3 PGQ Score in age group 31-≤ 40

	Mean±SD	T value	P value	Interference
Age (31-≤40)	33.83±2.119	87.46	<0.0001	Extremely significant
PGQ Score	55.26±12.42	24.37	<0.0001	Extremely significant

This table shows the age group wise PGQ score. The age group is 31-≤40 with mean±SD is 33.83±2.119 and PGQ score mean±SD is 55.26±12.42.

Table no.4 FABER Test findings

	Findings	%
No. of positive findings	39	60.94%
No. of negative findings	25	39.06%

This table is used for positive and negative FABER test findings. Above table shows that there are total 39 subjects have positive FABER test and 25 subjects have negative FABER test .

Table no.5 P4 Test findings

	Findings	%
No. of positive findings	39	60.94%
No. of negative findings	25	39.06%

This table is used for positive and negative P4 test findings.

Above table shows that there are total 39 subjects have positive P4 test and 25 subjects have negative P4 test .

Discussion

Pelvic girdle pain is the frequently reported musculoskeletal disorder in pregnant women. Pain is experienced throughout pregnancy and post-partum period. There are multiple factors that will lead to pelvic girdle pain that are hormonal, genetic, biomechanical, traumatic. Due to this factors there will be reduced capacity for activities like walking , standing and sitting, later it result in greater disability if remain unnoticed. It may lead to poorer quality of life as being unable to carry out normal roles in the life. Therefore early detection and treatment of pelvic girdle pain is important factor.

The aim of the study was to find out the prevalence of pelvic girdle pain in post-partum women. The project were done in 6 months of duration with sample size of 64 females with age group of 20-40 years. Subjects were selected as per inclusion and exclusion criteria. The confirmatory diagnosis of pelvic girdle pain was done with the help of FABER test and P4 test.

After confirmation of pelvic girdle pain patient's consent form was taken and then ask to fill the pelvic girdle questionnaire, which includes 25 questions and their total score were calculated.60.34% patients have total score above 51 out of 100.

Table no 1. Shows PGQ score frequency and percentage. ≤ 50 score percentage is 39.06 % and ≥51 score percentage is 60.34 %

Table no 2. Shows PGQ score in age group 20-≤30.

Table no 3. Shows PGQ score in age group 31-≤40.

Table no 4 &5 shows FABER test and P4 test findings respectively. In that 39 subjects have positive findings and 25 subjects have negative findings.

This study showed that there is significant pelvic girdle pain in the post-partum period of the pregnant women with the help of P4 and FABER tests. Which were causative factors for the pelvic girdle pain. Therefore the findings suggest that therapist should consider the factors that impact on pelvis and around structures in the pregnancy which leads to pelvic girdle pain in post-partum women.

Conclusion

On the basis of the results of the study, it was concluded that there is significant pelvic girdle pain in the post-partum women. After analysing the pelvic girdle questionnaire almost 60.94% of patients have total score above 51 out of 100 and maximum number of positive findings on FABER and P4 test are 39 subjects.

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Practices of Documentating the Pre-Operative Informed Consent in Obstetrics and Gynaecology in a Tertiary Care Hospital – An Interventional Study

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Abstract

An informed consent form plays a major role both in giving the needed information for the patient and as a legal tool for the doctor to carry out various diagnostic and therapeutic procedures. This paper was an interventional study, conducted in Department of Forensic Medicine, Sri Manakula Vinayagar Medical College and Hospital, Madagadipet, Puducherry to audit and improve the current practices of using informed consent forms by the faculties of Department of Obstetrics and Gynecology. The practice of documenting the informed consent forms obtained during the major surgeries of the concerned department was analyzed using a validated check-list. Deficiencies were identified in the documentation and an intervention was carried out to stress on the importance of documentation in the consent forms and the post-interventional consent forms were analyzed against the same validated check-list. The results of both the pre- and post-intervention data on the documentation of consent form were analyzed and compared, which showed a significant improvement in the documentation of the informed consent forms by the faculties of the Obstetrics and Gynecology Department. This study result will emphasis the role of intervention mode used to enhance the documentation of informed consent forms.

Keywords: *Informed consent form; Deficiencies; Documentation; Intervention.*

Introduction

"Consent connotes agreement, compliance or permission given voluntarily without compulsion.[1] Indian contracts act, section 13 states that "two or more person are said to consent when they agree upon the same thing in the same sense" and section 14 states that "consent is said to be free when it is not caused by coercion, undue influence, fraud, misrepresentation and mistake".[2] Initially, the information given in informed consent forms are complex and difficult for the patient to understand. In the era of 21st century, patients are becoming more aware about this consent form, as the information is available for them at fingerprints through the wide spread access of internet and media.[3] Informed consent in medical practice is based on the decision between the doctor and the patient, where the doctor has to explain and the patient must understand about the details of consent form in the same sense. This can be done by actively involving the patient in decision making process towards the surgery.[4, 5] Failing to do the above process of obtaining informed consent form, the doctor is said to commit an assault and will be liable for the damages caused to the patient as per section 351 IPC.[6] Though there are so much of strict regulations and increasing negligence cases under the consumer protection act because of the improper method of obtaining the informed consent form, still most of

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the doctors are having a careless attitude towards the informed consent form practice. So to access the current trends of obtaining the informed consent form, this study was carried out to evaluate the same with an aim to improve it by having an intervention and also carrying out a post-interventional analysis.

Materials and Methods

The study was done at Department of Forensic Medicine, Sri Manakula Vinayagar Medical College and Hospital, after obtaining approval from Institutional Ethics Committee (IEC No: 98/2016). It is a hospital based interventional study conducted for a period of 24 months from October 2016 to October 2018. The informed consent forms of the elective major and minor surgeries conducted during the study period by the Department of Obstetrics and Gynecology were taken up for the study, whereas emergency surgeries were taken as exclusion criteria. Sampling method was by using systemized random sampling method. The total sample size of the study was calculated as 690 consent forms, using "Epi Info Software Version 7.2 2.6", taking into account the improvement in practice of documenting informed consent forms from 34% based on previous study with 95% confidence interval and 90% power. Out of this 690 consent forms, 345 consent forms were analyzed during the pre-interventional period and another 345 consent forms were analyzed during the post-interventional period. A checklist proforma of the informed consent form consisting of 18 variables was prepared after going through the guidelines of MCI, Royal College of Obstetricians and Gynecologist of London on consent, consent form of the hospital and review of literature. This checklist proforma with 18 variables was scrutinized and validated by the faculties of Forensic Medicine and Obstetrics and Gynecology Department of the hospital, also for the convenience of result analysis these 18 variables were group into 3 categories. (Table No 1) Using this checklist, the 345 informed consent forms taken during the pre-interventional period were crosschecked for any deficiencies in the documentation. These data were entered and analyzed using "Epi Info Software Version 7.2 2.6". An intervention was carried out in form of a workshop for the faculties of Obstetrics and Gynecology department, aiming to emphasis on the importance of documentation of informed consent form and addressing the deficiencies found in the analysis of the pre-interventional period consent forms. Post-intervention another set of 345 informed consent forms of the major and minor elective surgeries were crosschecked using the checklist proforma and data were analyzed. "Student t test" was used to compare the data obtained from the pre and post interventional period and to find the effect of intervention on improving the documentation of the informed consent forms.

Table No 1: Checklist with variables prepared from informed consent form

Category A: Preliminary Variables	Category B: Procedure Variables	Category C: Legal Variables
1. Serial No 2. In-patient No 3. Date of Consent 4. Name of the patient 5. Age 6. Bed No 7. Ward No	8. Nature of the disease 9. Benefits of surgery 10. Risks of surgery 11. Alternative treatments 12. Type of anesthesia 13. Complications of surgery 14. Consequences of surgery	15. Patient signature 16. Doctor signature 17. Witness signature 18. Date of signature

Results

The study results showed that, on analyzing the 345 informed consent forms obtained during the pre-intervention period under category A variables (Preliminary variables), almost all the variables have been documented completely except the variables like bed no and ward no. Fortunately the post-interventional data analysis of the 345 informed consent forms showed significant improvement in these two variables, which

indicate the awareness created among the doctors while documenting every variables in the informed consent form have fulfilled its role. (Table No 2)

Table No 2: Pre and post interventional comparison of Category A – Preliminary variables

S.No.	Category A: Preliminary variables	Pre-intervention n (%)	Post-intervention n (%)	p value
1	Serial No	345 (100%)	345 (100%)	NA
2	In-patient No	345 (100%)	345 (100%)	NA
3	Date of consent form	345 (100%)	345 (100%)	NA
4	Name	344 (99.95%)	345 (100%)	NA
5	Age	342 (99.70%)	345 (100%)	NA
6	Bed No	0 (0%)	201 (58.26%)	0.005
7	Ward No	11 (3.19%)	202 (58.55%)	0.005

It was very evident that the doctors were perfect while documenting the category C: Legal variables like patient's signature, doctor's signature, witness signature and date of signature, since the analysis of the informed consent forms showed complete documentation i.e. 345 (100%), during both the pre and post intervention period which was a very significant finding.

The study showed that on analyzing the Category B: Procedural variables in the pre-interventional informed consent forms used for the patients who underwent major and minor elective surgeries didn't have the printed information about the details of the procedural variables like nature of the disease, benefits of the surgery, risks of the surgery, alternatives treatments, type of anesthesia, complications and consequences of the surgery. Later during the intervention workshop, postgraduate students of Obstetrics and Gynecology were assigned the role of developing customized informed consent forms including the details of the procedural variables for the commonly done major and minor surgeries in their department, under the guidance of one faculty for each postgraduate. Then during the post-intervention analysis of the informed consent forms, it was fortune to see that all the informed consent forms 345 (100%) had the details of procedural variables.

Discussion

In this study, informed consent forms were analyzed in the pre and post intervention period with an intervention mode, but there was a study done by O. C. Osime et al, titled "Current practices and medico-legal aspects of pre-operative consent" at University of Benin teaching hospital, Benin city, where they have done analysis of 133 informed and consent forms and also interviewed all the patients with any intervention and post intervention alanysis.[4]

A study conducted by Mabroka Alfoghi and Mohamed Ben Ramadan, titled "Clinical record keeping survey of patients admitted to Misurata centre hospital" at Department of Pediatrics, Libiya, out to 110 case records analyzed, the patients name were written in 110 (100%), age was written in 105 (96%) and bed number was written in 101 (92%) of case records, whereas in our study during both the pre and post intervention period, except bed number and ward number rest all of the preliminary variables were documented in all the

informed consent forms.[7]

Documenting the doctor's signature in the informed consent forms were done in 344 forms and 345 forms during the pre and post interventional period respectively, but there are studies like the one done by Catherine Leng and Kavitha Sharma, titled "An audit cycle of consent form completion: A useful tool to improve junior doctor training", where the percentage of documenting the doctor's signature was only 46% and 13% during the pre and post interventional period and another study done by Carter K et al, titled "Informed consent forms for vascular intervention: completing one audit loop", showed out 99 consent forms studies only 59 consent forms were signed by doctor's.[8, 9]

In this study, date of informed consent form was written in all the 345 (100%) consent forms, during both the pre and post interventional period, but a study done by Catherine Leng and Kavitha Sharma, titled "An audit cycle of consent form completion: A useful tool to improve junior doctor training", where the percentage of documenting the date was only 46 % and 13 % during the pre and post intervention period, respectively.[8]

When it comes to documentation of complications of the surgery, in this study it was found that during the pre and post interventional period, 0 % and 100 % respectively, was the percentage of documentation, but in a study done by Jennifer Isherwood, titled "Documenting informed consent in elective hip replacement surgery: a simple change in practice", the percentage of documenting the procedure specific complications was 86 out of the 100 consent forms analysed.[10]

Study done by Mark T Siddins, Elizabeth M Klinken and Lee R Vocale, titled "Adequacy of consent documentation in a speciality surgical unit: time for community debate?", it was found that out of 1280 consent forms analyzed, the need for surgery and relevant risks of the surgery were documented only in 10.1% and 4.1% of consent forms, but in our study during the post interventional period all the 345 (100%) of informed consent forms, has documented the need and relative risks of the surgery.[11]

Conclusion

The study have recorded and analyzed the existing standards of documenting informed consent form and the deficiencies were shared during the intervention workshop, which increased the awareness of the faculties on proper documentation, as evident from the post intervention analysis. This study has improved the consent practices related to surgical procedures in Obstetrics and Gynecology department of our institute by developing a structured informed consent form considering the national and international guidelines in both English and Tamil language, which was later approved and implemented in the institute. We feel there are scopes for improving the consent practices more by having day to day awareness methods on the concepts of consent practices, having more regular intervention methods and also keeping frequent post interventional analysis of the informed consent forms. Such studies have to be done in other specialties also with regular awareness programs and analysis of the consent form documentation, which in-turn benefits the patient by making them aware on the surgery details and the doctor's by acting as a better defense against negligence suits.

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Otitis in Aerospace-Aerotitis: A Review

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Abstract

Background: Aerotitis media is a painful disorder of ear which is experienced during ascent & descent of the flight, caused by rapid change in external pressure leading to discomfort, pain, hearing loss, tinnitus and vertigo. It can also lead to permanent hearing loss.

Objective Methods: Various research articles have been reviewed from different databases such as Google Scholar to study the various preventive strategies which can be taken to avoid this condition during flight.

Result: Valsalva maneuver is helpful in decreasing the symptoms of aerotitis media and making passengers comfortable during the air travel.

Conclusion: As aerotitis media is prevalent in ascent and descent of flight passengers, Valsalva maneuver and other preventive strategies shows beneficial effects on the symptoms of the condition through incorporating its principles.

Key Words: *Aerotitis, Tinnitus, Vertigo, Valsalva maneuver.*

Introduction

Aerotitis or otic barotrauma, a painful disorder occurs during air travel, is defined as a traumatic inflammation of middle ear. It occurs because of the difference in the pressure of middle ear and external air pressure. When the equalization of this pressure disrupts leads to initiation of this disorder called AEROTITIS (Armstrong). When the ventilation of middle ear is not adequate during ascend and descend of airplane leads to AEROTITIS. [1,2]

Etiology And Pathophysiology

It is a common problem, occurring because of the failure of Eustachian tube to maintain the equilibrium of pressure of middle ear and atmospheric pressure.[1,2]

The Eustachian tube is a narrow tube which connects middle ear to the nose and throat, this tube opens and helps in equalization of pressure. The changes in the pressure of cabin is very rapid during air travel, the Eustachian tube fails to open wide enough to equalize the pressure between cabin air and middle ear air. Thus, it stretches the ear drum causing ear pain or discomfort. [6]

The pathological changes in AEROTITIS media varies from slight congestion to extensive rupture of tissues [4].

Risk Factors

Due to anatomical difference in Eustachian tube and frequent upper respiratory tube, children may also be prone to this infection [3]. Common cold, sinusitis, otitis media, allergic rhinitis, nap during rapid pressure changes in middle ear are the risk factors of this condition.[5]

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CLINICAL FEATURES AND COMPLICATIONS

Aerotitis media can occur unilaterally as well as bilaterally [5]. Clinical features of Aerotitis media are pain, discomfort, tinnitus, vertigo, hearing loss. In extreme or severe cases, bleeding can occur . Permanent hearing loss and chronic tinnitus can be major complication in this condition.[4]

PREVENTIVE STRATEGY

According to various researchers, various preventive strategies can be adopted to reduce this problem at different level of disease.

Ø Prevention at primary level – yawning , swallowing , chewing helps in activating the muscles that are anatomically responsible for maintaining Eustachian tube bilaterally[3,5].

Ø To prevent napping during ascend or descend of air travel – we can practice preventive measures during any discomfort in our ear during the period of sudden change in pressure if we are awake [5].

Ø If a person is already suffering from common cold, allergic rhinitis or any recent ear surgeries/ infection should avoid air travel[5].

Ø Use of ear plugs helps in equalization of pressure against the ear drum [5].

Ø Decongestants can be taken 30 minute to 1 hour prior to air travel [5].

Ø Use of warm or wet towels over external ear is also suggested to decrease external pressure [6].

Ø PREVENTION AT SECONDARY LEVEL – Valsalva maneuver or gentle blow maneuver. The maneuver helps in equilibration of the pressure between middle ear and external pressure of atmosphere, it can be performed when a person feels any discomfort in ear. It is done by gently closing your nostrils and blowing through mouth not allowing air to pass through nostrils or mouth.[3,5] Breastfeeding or bottle feeding can be used a preventive measure in children.

Ø Ear packing, its recommended immediately in case of bleeding [5].

Ø PREVENTION AT TERTIARY LEVEL – surgically placed tubes in ear drums is a helpful air in middle ear in case of frequent fliers prone to severe aerotits media[5].

Conclusion

Airplane ear or Aerotitis media, one of the commonest problem in people who travels via air. It is also recognized as an occupational injury in crew members but ignored [4,5]. Previously lozenges were used as a preventive measure which was quite helpful in avoiding such ear problems but are abandoned in recent times. The flight attendants should be trained for ear preventive measures so that the rate of aerotitis media can be reduced [5].

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A Morphometric Study to Find Correlations among Various Dimensions of Proximal End of Humerus of Telugu Population

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Abstract

Background: Long bones from skeletal remains are useful for identification of the deceased, estimation of stature, determination of sex etc. in case of fracture of proximal humerus, the contra lateral head dimensions are used as a template for making prosthesis in replacement surgery.

Aims and Objectives: To study and compare the morphometric data of proximal end of humerus of Telugu population with similar studies of other ethnic populations, and generate regression equations using the measured parameters.

Material and Methods: Study was conducted on 159 unpaired dry humeri (72 right and 87 left) from the collection of Department of Anatomy, Mamata Medical College, Khammam, Using slide calliper and Osteometric board.

Results: The mean of maximum length of humeri of right/ left side were 309.88 ± 20.07/ 303.97 ± 22.07, the mean of transverse diameter of humeri of head of right/ left were 38.95 ± 3.38/38.21 ± 3.68, the mean of vertical diameter of humeri of right/ left were 42.77 ± 3.86/41.28 ± 3.84,the mean of circumference of surgical neck of right/left were 81.18 ± 8.47/79.58 ± 7.30. Circumference of surgical neck had a strong positive correlation with transverse diameter & vertical diameter of head which was statistically significant ($p < 0.001$).

Conclusion: The morphometric parameters of proximal end of right humeri were found to greater than that of the left humeri. The mean humeral length was similar to that found in studies of various Indian populations.

Keywords*: Morphometry ,Correlation, Humerus, Proximal.*

Introduction

Skeletal remains are an important tool for the identification of the deceased for estimation of stature, determination of sex and general body structure of an ethnic population [1]. The skull bone and Pelvis remains are the best and most important bones for medical, legal and forensic investigation but in absence of pelvis and cranium, other long bones are also noted to be of great help in estimation of stature, sex determination and identification of a person .

Some studies have reported that lower limb bones, especially the length of femur is most closely correlated to stature , but according to Celbis et al [2] in absence of lower limb bones, the upper limb bones are also useful for estimation of stature. As per some studies the

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total length of the long bone and the stature can also be estimated even from fragments/ segments of long bone.

In case of fracture of proximal Humerus, the contra lateral Humerus head size is used as a template for making prosthesis in replacement surgery. But some studies have shown the presence of high inter-specimen and side-specific variability. Hence the restoration of normal anatomy during replacement surgery becomes difficult, so it is important to match the normal anatomy as much as possible to attain better function of shoulder joint [3].

In a cadaveric model of Harryman et al [4]use of large size humeral head component showed diminished shoulder joint movements. Likewise when a smaller head component was used, the range of movement reduced due to reduction of surface arc available at glenohumeral joint [5].

Thus one of the important factors for arthroscopic repair failure is disproportion of head of Humerus with respect to the corresponding glenoid cavity [6], it has been observed that if the surface area of contact between head of Humerus & glenoid fossa reduces, the instability of the shoulder joint increases [7]. In Indian population only the lower size prosthesis fits in replacement surgery of shoulder joint, else restricted movements and discomfort of joint were reported [8]. Osteochondral allograft transplantation technique is popular nowadays to create a congruent, mechanically stable joint without much alteration in surrounding anatomy [9].

Study of Kornberg [10] shows a significant difference in retroversion of humeral head between dominant and non-dominant side, i.e more used and less used sides. Edelson [11] also found that in both male and female right Humeri were significantly more retroverted than left humeri.

The aim of present study is to obtain and analyze the morphometric values of proximal end of humeri, to compare with the findings of other similar studies, as well as to provide data and correlations to predict the morphometric dimensions useful for prosthetic preparation.

Materials and Methods

Materials:

The study was conducted on 159 dry unpaired intact humeri (72 right and 87 left) of unknown age and sex, from the collection of the Department of Anatomy, Mamata Medical College, Khammam, Telangana. Damaged bones or bone attached with prosthesis was discarded from the study. Osteometric board and Vernier calliper were used to take the measurements.

Methods:

The maximum length of humeri was measured using a Osteometric board. The vertical diameter and transverse diameter of head of humeri and the circumference of surgical neck was measured using a Vernier calliper **Ref Fig1**.The Pearson correlation coefficients(r) between the various morphometric dimensions and significance of the correlations coeff were calculated. The regression analysis of the observed data was done using the Data Analysis tool pack of MS Excel for windows and the trend line plots were generated.



Fig: 1 Measurement of Transverse dia, vertical dia and Humeral length

Result

The mean, standard deviation, minimum & maximum length and range were calculated for all the morphometric dimensions of humeri , the same parameters were also calculated for the left and right humeri separately **Ref Table-I**. Paired test was not carried out to assess the difference between the right and left humeri, as the available bones had not been tagged for Right and Left humeri from the same individual.

Table I: Data from statistical analysis of the humeral dimensions for combined and segregated data for left and right (mm)

	Mean + SD (mm)			Minimum(mm)		Maximum(mm)	
Parameters	**Combined**	**Right Humerus**	**Left Humerus**	**right**	**Left**	**Right**	**Left**
Max Length	304.695 + 30.379	309.875 + 20.753	303.977+22.066	265	255	381	376
Tr D head	38.542+ 3.559	38.946+ 3.383	38.208+ 3.684	31	25	47	45
Vertical D head	41.955+ 3.912	42.765+ 3.860	41.284+ 3.848	33	32	51	51
Circum surgical neck	80.302+ 7.87	81.181+8.471	79.575+ 7.305	63	64	100	100

The Regression equations and the Correlation among various Humeral dimensions were calculated using MS excel data analysis pak. For the regression equations and corresponding Scatter plots are tabulated in **Table:III,** and the regression trend line is shown in **Fig-2 &3.**

A very strong positive correlation exits between Transverse dia to Vertical dia for both right and left humeral heads (0.893 / 0.905). The circum surgical neck Dia also has a strong correlation with Transverse dia & Vertical dia **ref Table:II.** The humeral length was also noted to be having a strong correlation with the proximal morphometric dimensions a shown in **Table:II**.

Table II The Regression equations and the Correlation among various Humeral dimensions

	Regression equations		Correlation coeff	
Y to x	**Right Humerus**	**Left Humerus**	**Right Humerus**	**Left Humerus**
Transverse dia to Vertical Dia	y = 0.783x + 5.4607	y = 0.8669x + 2.4204	0.893	0.905
Transverse dia to Circum Surgical dia	y = 0.9457x + 5.1493	y = 1.2693x + 31.078	0.736	0.64
Vertical dia to CircumSurgical dia	y = 1.4972x + 17.151	y = 1.2812x + 26.683	0.682	0.675
Transverse dia to Max Length	y = 0.0973x + 8.787	y = 0.1085x + 5.2253	0.597	0.65
Vertical dia to Max length	y = 0.1291x + 2.7672	y = 0.1181x + 5.3892	0.694	0.677
Max length vs Circum surgical len	y = 1.3531x + 200.03	y = 1.5922x + 177.28	0.552	0.527

DISCUSSION:

Humerus is the longest and strongest bone of upper extremity and it can withstand chemical and environmental assault for a longer duration , the durability of Humerus has made it the commonest bone for forensic and anthropometric study. Je-Hun-Lee et al, created a specific formula of regression equation to predict the stature of Korean individuals in their study of segments of long bones [12].

Study of the segments of long bones is also helpful for providing data and correlation factors which has enormous importance while making prosthesis for replacement surgery of a bony part in Osteochondral allograft transplantation.

Present study has found that measurements of humeral length for right side has higher values than left side, which is in accordance with many other studies as shown in **Table:III.** Mean value of humeral length (right and left combined data) in our study is also comparable with the results of the various ethnic populations enumerated in **Table:III.**

Table III Comparative results of right & left Humeral length of various ethnic populations

Studies	Mean Length of right Humeri	Mean Length of left Humeri
S D Desai [13]	292.3 mm ± 22.9mm	289.45mm ± 21.8mm
Vettivel et al [14]	302.0mm ± 20.0mm	301.0mm ± 20.0mm
Udhaya et al [15]	302.8 mm ± 24.4mm	289.45mm ± 21.8mm
Zarana et al. [16]	303.9mm ± 16.6mm	303.2mm ± 15.8mm
Aydin et al [17]	304.1mm ± 17.3mm	300.4mm ± 23.9mm
Anil Kumar [18]	306.4mm ± 25.8mm	304.44mm ± 23.8mm
Akman [19]	307.1 mm ± 20.8mm	304 mm ± 18.9mm
Niraj et al [20]	308.5mm ± 19.16mm	307.2mm ± 16.13mm
Somesh et al [1]	309.6 mm ± 20.6mm	299.6 mm ± 22.5mm
B V Murlimanju [21]	309.8mm ± 18.1mm	299.6mm ± 25.4mm
Salles et al [22]	313mm ± 17.71mm	300.85mm ± 18.35mm
Present study	309.87mm ± 20.7mm	303.98mm ± 22.07mm
	Humeral length Combined	
Anudeep Singh etal	305.42 mm ± 1.4mm	North Indians
Akman et al	305.95mm ± 1.6mm	Turkish
Papaloucas [23]	330.67mm ± 3.5mm	Athenian
Derya Atamturk et al [24]	324.16mm ± 32.21mm	Istanbul
Mall Gita et al [25]	320.50mm ± 19.09mm	Munich and Colonge
Sinchal Datta	304.69mm± 30.38mm	Present study

The mean of vertical diameter and transverse diameter of head of humeri were found to be 41.95 ±3.9mm and 38.54 ±3.5mm respectively, which is comparable with corresponding findings of Ajari Ashutosha et al e.g 43.11±3.73mm and 39.65±2.97mm respectively. The vertical diameter of right and left side humeral head were found to be 42.77±3.86mm and 41.28±3.85mm and the transverse diameter on right and left humeral head were found to be 38.95±3.38mm and 38.21±3.68mm respectively, both the right side dimensions are greater than left side dimensions as in many other studies, whereas in the study of Aydin Kabakci et al the left side shows little higher value than right side. In the study of Jacek Tomczyk et al [26] the vertical and transverse diameters of male were found higher than female which they have used for determination of sex.

The mean of circumference of surgical length was 80.3±7.8mm and the same on right and left side were 81.18 ±8.47mm and 79.57±7.31mm respectively, which is different from the findings of Aydin Kabakci et al where the same for right and left humeri were found to be 86.9 ± 9.9 and 93.2 ± 8.5 respectively, with a higher value on left side.

So, in our study all the parameters of humeri were found to be more on right sided than left side, which is in accordance with many other studies on different population. Many previous studies have shown a right-bias in the dimensions of upper limb bones along with contra lateral asymmetry in lower limb bones, termed as crossed symmetry [27].

The transverse diameters of head of humeri were found to be having a strong positive correlation with the vertical diameters of head of humeri. The 'r' value (Pearson correlation coefficient) was 0.898 was highly significant ($p< 0.001$). The same correlation for right/ left side were found to be having r values (Pearson correlation coefficient) 0.893/ 0.905 , and the correlations were found to be highly significant, $p< 0.001$ for both sides.

The transverse diameter of head of humeri was found to have a very good positive correlation with the circumference of surgical neck. The r value was 0.684 and $p< 0.001$. The same correlation for right / left side were found to be with r value 0.736 / 0.640 respectively, which is also very good correlation with p value < 0.001.

Very good positive correlation was also found between vertical diameters of head of humeri. and circumference of surgical neck of humeri, with r value of 0.680 and $p< 0.001$. The same correlation on right/left sides were found to be 0.682/ 0.675 respectively with $p<0.001$ for both side Humeri.

In case of bone replacement surgery minor differences may cause functional difficulty and movement restriction after surgery, if the exact size of the contra lateral uninjured side is been taken for size determination of the prosthesis. Hence variations in the humeral head dimensions for right and left side must be considered for prosthesis development. So if circumsurgical neck dia is available then the transverse and vertical dia can be calculated for the development of the prosthesis, though data of the corresponding ethnic populations is required for patients belonging to a ethnic group.

Conclusion

The morphometric dimensions of proximal end of humerus measured in the present study were found to be higher for the right humeri compared to the left humeri, which is similar with many similar studies.

The length of humeri has shown significant variations among different populations. The Athenian, Istanbul, Munich& Cologne population have more humeral length than Indian population. The present study shows similarity in the humeral length found in different studies on Indian population.

Variations in transverse and vertical diameter was noted for right and left sides in the present study.

There is a strong highly significant ($p <0.001$) positive correlation between the circumsurgical neck and the transverse and vertical dia of the humeral head. A strong highly significant ($p <0.001$) correlation was observed between the circumsurgical dia, transverse dia and vertical dia with the humeral length. The regression equations generated will help to predict the proximal humeral dimensions in an event of unavailability of a particular morphometric dimension and also in determination of the inter specific and side specific variability of the humeral head of Indian Telugu population, thus providing data and helping in

better determination of size of the prosthesis in bone replacement surgery for telugu population. The study can be expanded over other ethnic populations of India to prepare a comprehensive database and correlations for various communities.

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A Comparative Study of Facial Indices of Maharashtrian Adults with Other Ethnic Group and Test For Reliability of Sexual Dimorphism

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Abstract

Background: Variations in different craniofacial features is studied and documented using various anthropometric techniques. The same depends on gender, race, ethnicity, nutritional and socioeconomic status of a person. So the human face and its variations has always been an object of interest for anatomists, forensic experts, anthropologists, plastic surgeons, maxillofacial surgeons, security experts and artists. The aim of the present study was to identify the facial phenotypes of the Maharashtrian Males and Females. Availability of the Facial indices will augment the already available data of other studies.

Materials and Methods: The present study was done on 505 (243 males, 262 females) pure bred Maharashtrian adults (3 generations on the maternal and paternal side of Maharashtrian origin)of the age group 18-60 years. The data was analyzed using MS excel Data Analysis Pak and Two-Sample t-test for Unequal Variances was done for facial indices of male and females to check for sexual dimorphism.

Conclusion: The predominant facial type in Maharashtrian male is Mesoprosopic (37.86%) followed by Euryprosopic (29.22%) and that of female is Mesoprosopic (33.21%) and Euryprosopic (32.06%). This is similar to the results of studies on Indian males and females except Gujarati males who have Hypereuryprosopic face and Rajput males havingHyperleptoprosopic (long)face.A statistically significant difference ($p < 0.05$). was obtained between facial indices of Maharashtrian males and females, which is a key to sexual dimorphism

***Key Words**: Anthropometry, Dimorphism, Maharashtrian, Prosopic*

Introduction

The stature evaluation, age estimation, sex determination and population are the major parameters of forensic anthropology. Many studies have been conducted, with regards to sexual dimorphism and stature estimation over various ethnic populations; a majority of them have focused their attention on bony components of the body. Though few researchers among others like Ghosh and Malik [1], Jahanshahi et al [2] and Raji et al [3] have worked on the craniofacial anthropometrics. Human face has always been an interesting subject for anatomists, anthropologists, plastic surgeons, maxillofacial surgeons, forensic experts, security experts as well as artists [4]. Facial contour and other features of a person reflect the gender, race, and ethnicity, nutritional and socioeconomic status of a person.

Anthropological studies have documented differences among morphological characters and craniofacial features of various ethnic groups using anthropometric techniques. The variations in the facial morphology due to differential growth assist us in distinguishing one person from another. Craniometrical study of prehistoric skulls and facial bones has enabled

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anthropologists to trace gradual changes in size and shape of the human head also gradual changes in the facial construction reflect the increased mental alertness, activities and intelligence.

Anthropological research is based on bodily measurements, however racial and ethnic variations exist in these measurements because human body dimensions are affected by ecological, biological, geographical, racial, gender, age and nutritional factors [5]. Cephalic and Prosopic indices are important parameters, which are useful for ascertaining the variations between different sex and ethnic groups. Facial indices play a very important role in personal identification which is useful in forensic applications and for the purpose of state defense (biometrics) etc, particularly in different techniques of facial reconstruction which helps a forensic artist recreate the final face irrespective of the method used [6]. Comparison of changes in facial indices between parents, offspring and siblings give a clue to genetic transmission of inherited characteristics [7]. This analysis of facial indices is also essential for diagnosis of genetic and acquired anomalies, for the study of normal and abnormal growth and for morphometric investigations [8].

The present work was undertaken to determine facial variations within Maharashtrian adults. In addition, comparison of facial indices was made to determine possible variations (sexual dimorphism) between males and females of Maharashtrian origin.

Aims and Objectives

Limited data is available for craniofacial parameters of Maharashtrian adults, though some studies have been done on cephalic indices in Maharashtra (sample size 300) and stature estimation from cephalic dimensions (sample size 320) [9] [10]. The aim of this study is to document and analyze the facial index data to find the variations in facial indices, to identify the facial phenotypes of Maharashtrian adults and compare the facial indices for sexual dimorphism. It will help in improving the statistical data which are already available for facial indices of Maharashtrian adults.

Materials and Methods

The present study was done on 505(243 males, 262 females) subjects, who are pure bred Maharashtrian adults (who have above 3 generations on the maternal and paternal side of Maharashtrian origin) of the age group 18-60 years with normal pleasing face and with no history of plastic, maxillofacial or reconstructive surgery. All the data were collected in fore noon to avoid any diurnal variation of facial soft tissue. The data was analyzed using MS excel Data Analysis Pak for comparison of the means of the prosopic indices of males and females and Two-Sample t-test for Unequal Variances was done for facial indices of male and females to check for sexual dimorphism.

Computation of Facial index :

The facial landmarks used were Nasion(N), Gnathion(GN) and Zygion(ZY) [11] [12].

Facial height (morphological facial length) is the straight distance measured from Nasion to Gnathion.

Facial breadth (Bizygomatic breadth) – Straight distance between the most wide apart points of both sided zygomatic arches i.e. Zygion to Zygion

Facial index is a ratio of facial height and Byzygomatic width. This has been listed elaborately by **Farkas and Munroe 1985, cleft palate journal** [13], and is calculated using the formula **Facial Index**=

$$\text{Index} = \frac{\text{NASION} - \text{GNATHION}}{\text{ZYGION} - \text{ZYGION}} \times 100.$$

Banisters classification [14] is used for phenotyping the facial type into five groups as given below–

a) Hypereuryprosopic (Very broad face) –facial index below 80.

b) Euryprosopic (Broad face) –facial index 80-84.9.

c) Mesoprosopic (Round face) – facial index 85-89.9.

d) Leptoprosopic (Long face) –facial index 90-94.9

e) Hyperleptoprosopic (Very long face) –facial index above 95.

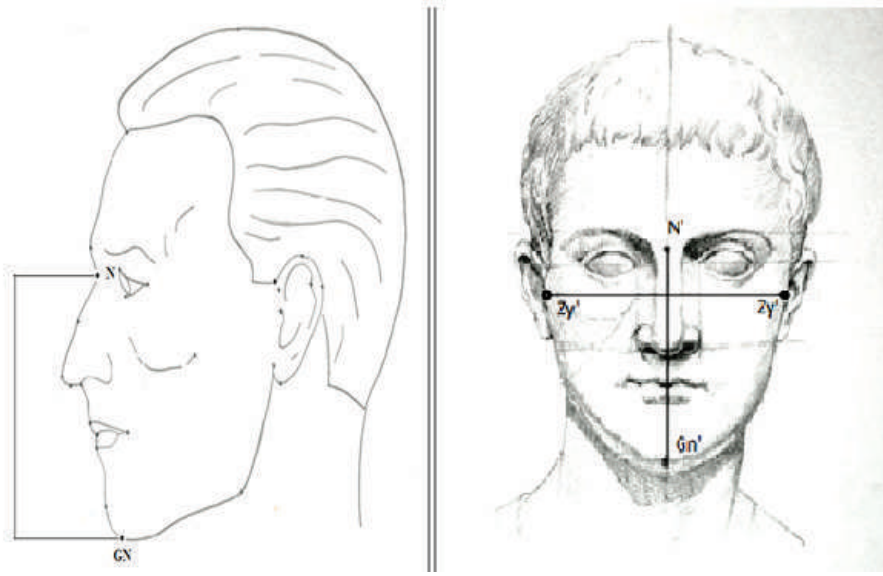


Fig: 1 Landmarks of Facial Index

Results

The mean, standard deviation, maximum &minimum the various Prosopic parameters and the Pearson's correlation coeff between Morphological facial length and Bizygomatic facial breadth was calculated, results are tabulated in **Table I**. The Mean facial index of Maharashtrian adult male is 86.61±5.97 and that of female is 85.466 ± 6.446. There exist a moderate (0.3 to 0.7) positive correlation between Bizygomatic breadth and morphological facial length of both males and females (males 0.407, females 0.37).

Table 1: Descriptive Statistics of facial indices of Maharashtrian adults

		Mean + SD	**Min**	**Max**	**Pearson's correlation of facial length to Breadth**	**Regression equation using Facial length and Bizygomatic facial breadth.**
Females	x= Facial Length (cm)	10.31+0.78	8.27	15.51	0.373 (p<0.001)	y = 0.3125x +8.8607
	y=Bizygomatic breadth (cm)	12.08+0.65	9.78	14.45		
	facial index female	85.47+6.45	70.49	115.05		
Males	x=Facial Length (cm)	11.15+0.75	9.27	14.66	0.407 p(<0.001)	y = 0.4007x + 8.431
	y=Bizygomatic breadth (cm)	12.90+0.74	9.93	14.46		
	facial index male	86.61+5.97	72.67	110.28		

The variations in prosopic index of the Maharashtrian Adults, male and female and the result of two-Sample t-test for Unequal Variances of the Facial index of Males and Females are shown in **Table 2.**

Table 2: Prosopic Indices females and males

Face shape Range of Prosopic index	Prosopic index	Females		Males	
		No	%	No	%
Hypereuryprosopic	< 79.9	46	17.56	27	11.11
Euryprosopic	80 – 84.9	84	32.06	71	29.22
Mesoprosopic	85 – 89.9	87	33.21	92	37.86
Leptoprosopic	90 – 94.9	27	10.30	33	13.58
Hyperleptoprosopic	> 95	18	6.87	20	8.23
Observations		262		243	
Variances		35.61		41.55	
Degree of freedom		503			
t value (t stat > t critical)		2.06			
Two–tailed 'p' value, Significance		0.039			
significance of Null hypothesis test		Significant (<0.05)			

The predominant facial type in Maharashtrian female is Mesoprosopic (33.21%) and Euryprosopic (32.06%), followed by Hypereuryprosopic (17.56%), Leptoprosopic (10.30%) and Hyperleptoprosopic (6.87%). The predominant facial type in Maharashtrian male is Mesoprosopic (37.86%) followed by Euryprosopic (29.22%), Leptoprosopic (13.58%), Hypereuryprosopic (11.11%) and Hyperleptoprosopic (8.23%).

A statistically significant difference (p= 0.039). was obtained between facial indices of Maharashtrian males and females.

Discussion

Anthropometry covers measurements of any aspect of human form & surface anthropometry refers to the measurement of the surface features. (Douglas, 2004). Thus Anthropometry provides means to assess facial shape. For the purpose of craniofacial measurement direct methods, Photometric measurement method and Cone Beam Computed Tomography is being used**.** In the present study direct method has been used as direct measurements are reliable and inexpensive though they are of time consuming nature.

The facial indices data provide useful parameters for evaluation of facial trauma, congenital and traumatic deformities. Craniofacial anthropometry helps in understanding the gradual evolutionary changes in human being, differences between various ethnic groups, the geographical migration and relocation of population from one climate and nutritional status to other.

The Mean facial index of Maharashtrian adult male is 86.61±5.97 and female is 85.466 ± 6.446. The predominant facial type in Maharashtrian males is Mesoprosopic followed by Euryprosopic and in Maharashtrian females it is Mesoprosopic and Euryprosopic. The mean value of facial indices of males and females observed among students population of Andhra region by V. M. Salve et al [15] were 89.50 ± 2.85 and 86.72± 3.40 respectively. The dominant type of face in males was Mesoprosopic (57.5%) and that in females was Euryprosopic (51.8%). The predominant facial types in this study are similar to the findings of the present study.

A study by V. R. Shetti et al [7] showed the mean facial index of Indian males was 87.19±5.2and that of females was 86.75±6.3respectively. The dominant facial types in both males and females were Mesoprosopic. Facial indices of Malaysian males were 85.72±5.4 which is comparable to Maharashtrian male's facial index. In

a study of Haryanvi males [16] dominant facial types in both males and females were Mesoprosopic.

Facial Indices of Turkman males and females were 87.25% ± 5.18% and 81.48% ± 5.28%, respectively. Facial Indices of Fars males and females were 88.22% ± 5.21% and 84.48% ± 5.85%, In females of both Turkmen and Fars the dominant face types Euryprosopic and for the males of both dominant face types was Mesoprosopic in study of Golalipour. [2]. Newborn of Turkmens and Fars had Hypereuryprosopic type of face, whereas the adult of same race had Mesoprosopic type of face, which suggests the developmental pattern [5].

The mean facial index of Gujarati males by U Kanan et al [17] was 81.7.Based on facial index dominant type of face shape in Gujarati males was Euryprosopic (42.96%) and similar findings were observed in Jat Sikhs (39.8%). **Ref Table:2, Fig:2, 3.**

A statistically significant difference ($p < 0.05$) was obtained between facial indices of Maharashtrian males and females. In the study by L C Prassana et al [18] Statistically significant differences ($p = 0.001$) were noticed between the facial index of north and south Indian females and a significant difference was obtained between facial indices of Indian males and females ($p = 0.003$) indicating differences in facial indices over sex and geographic regions. Study by facial indices of Twisha shah [19] indicates no statistically significant difference in facial index between Gujarati male and female while length and width of their face showed significant difference (p-value<0.05). Non-Gujarati subject results show significant difference of Facial index between sex (male and female) which is a key to sexual dimorphism.

Table 3 Comparision of Male and Female Prosopic indices with other population

	Maha, Male present study	Andhra Male	Gujrati male , Twisha Shah	Guj Male, Uttekar Kanan	south Ind male, Prassana L C	Sindhi males	Rajput males	NE Nigeria male	Turkman Race	Fars Race	Serbia Male,D Jeremic
Hypereuriprosopic	10.74	0.39	76.00	35.10	10.56	0.00	0.00	1.00	8.6	4.00	0.00
Euriprosopic	28.93	32.42	16.40	42.96	8.45	43.00	2.00	5.50	26.8	16.00	0.00
Mesoprosopic	38.02	15.63	5.30	18.20	7.04	18.22	9.00	9.50	38.4	44.00	17.78
Leptoprosopic	13.64	12.89	2.20	3.64	6.34	3.64	41.00	14.00	17.2	32.00	76.67
Hyperleptoprosopic	8.68	1.17	0.00	0.00	67.61	0.00	48.00	70.00	9.1	4.00	5.56
	Maha , female Present data	Andhra female	Guj fem, Shah	V shetti indian	South India fem, Prassana LC	Haryanvi, Female	v shetti Malay	NE Nigerian female	Turkman Race	Fars Race	Serbia Female, D Jeremic
Hypereuryprosopic	20.12	0.63	60	9.00	16.30	25	8.00	2.1	3.50	20.80	0.00
Euryprosopic	29.59	51.88	23.6	32.00	13.04	19.33	19.00	4.9	51.70	37.70	0.00
Mesoprosopic	33.14	25.00	12	32.00	10.87	35	34.00	19.6	6.90	22.20	10.59
Leptoprosopic	9.47	20.63	2.7	12.00	9.78	19	31.00	16.1	3.00	13.50	87.06
Hyperleptoprosopic	7.69	1.88	1.8	15.00	50.00	1.66	8.00	57.3	3.40	5.80	2.35

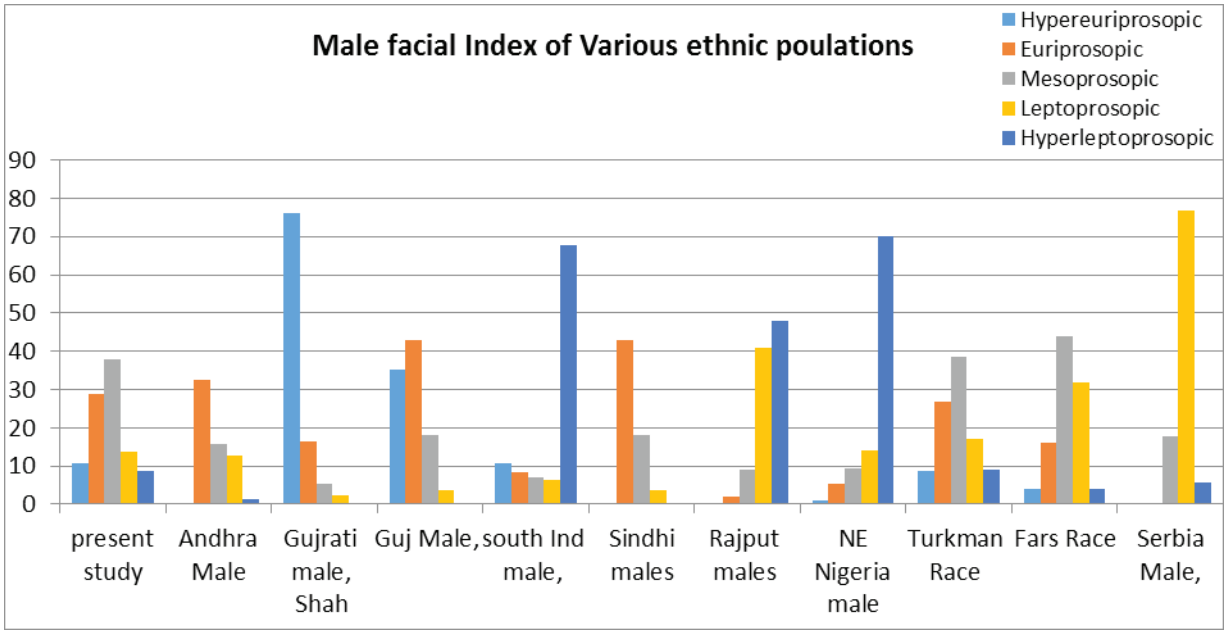


Fig: 2 Male facial indices of various ethnic groups

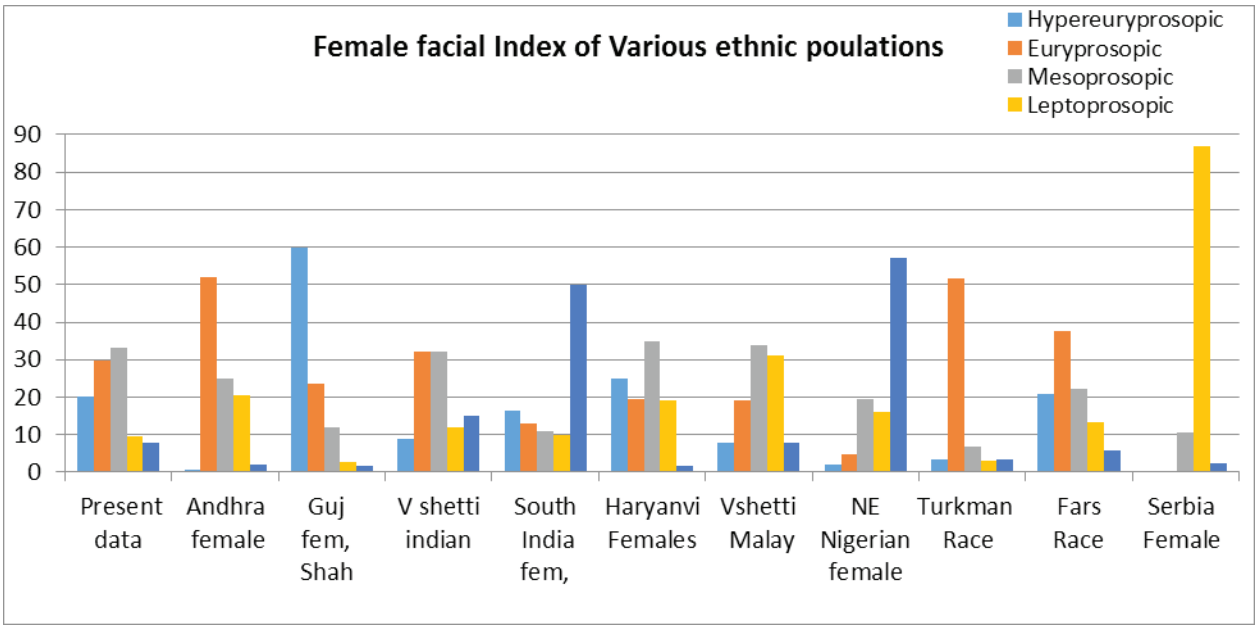


Fig: 3 Female facial indices of various ethnic groups

Conclusion

Facial measurement in the present study used direct method due to ease of collection of data. From our study, we can conclude that statistically significant difference in facial index exist for facial index for Maharashtrian males and females, the predominant facial type was noted to be Mesoprosopic and Euryprosopic, The Morphological facial length has a statistically significant, moderate (from (0.3 to 0.7) +ve correlation with Bizygomatic facial breadth for Maharashtrian adults. The facial indices of Maharashtrian males and females show a statistically significant difference ($p< 0.05$) indicating sexual dimorphism. This study has provided facial data of Maharashtrian adults which will augment the cephalic data already available. The data provided by the study can be used in medical applications such as cosmetologists, orthodontists and plastic surgeons.

Conflict of Interest: None

Sourse of Funding: None

Ethical Clearance:

The subjects involved in data collection and were explained the consent forms (in three languages English, Hindi and Marathi) approved by The Institutional Ethics Committee, Dr DY Patil Medical College Hospital and Research Centre. The subjects were intimated the risks to them and the benefits of the study to the society. They have the right to confidentiality regarding the privacy of their medical information. As per the consent form the information from the study, if published in scientific journals or presented at scientific meetings, will not reveal their identity. They also have Right to information regarding any new information obtained during this research study that may affect their decision to continue participating in the study. The data was collected at no cost to the participant.

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Awareness of Forensic Dentistry among Dental Practitioners

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Abstract

Background- Dental identification has proved its importance in identifying deceased individuals, and recently it has evolved as a new ray of hope in assisting forensic medicine, but its importance and role in forensic medicine is still in a state of infancy in India. Very less institutions offers formal training in forensic odontology, with the lack of job opportunities for qualified forensic odontologists who have obtained degrees abroad.

Materials and Methods-A cross-sectional study was conducted among 70 dental practitioners of Jaipur, Rajasthan. Data were collected with the help of a questionnaire (Based on Attitude, Knowledge and Practice criteria.) Questionnaire used in study. Informed consents were obtained.

Results-A total 70 dental practitioners responded to the questionnaire out of these 20% not maintain dental records in their clinic, 77% maintained dental records, 3% participants did not answer the question.

Conclusion- For an efficient forensic investigation, we need a dental team, comprising personnel from all branches of dentistry, working in close association with experts from other branches of forensic science. The government has a social obligation to recover, identify and hand over the remains of a deceased person to the relatives and every effort must be made to achieve this.

Key words: *Dental identification, forensic investigation, forensic dentistry.*

Introduction

Forensic dentistry a branch of forensic science is a challenging and fascinating branch that deals with the identification through the comparison of ante- and post-mortem records. Dental identification has proved its importance in identifying deceased individuals, and recently it has evolved as a new ray of hope in assisting forensic medicine, but its importance and role in forensic medicine is still in a state of infancy in India. Very less institutions offers formal training in forensic odontology, with the lack of job opportunities for qualified forensic odontologists who have obtained degrees abroad.[1] This branch can be defined as a branch of dentistry, which deals with the proper handling and examination of dental evidence and with the proper evaluation and presentation of dental findings in the interest of the justice." Assisting the legal authorities by examining dental evidence in different situations is a first job of a forensic odontologists. The three major areas of activity currently in forensic odontology are examination and evaluation of injuries to teeth, jaws, and oral tissues resulting from various causes.[2] The examination of marks with a view to subsequent elimination or possible identification of a suspect as the perpetrator.[3] The examination of dental remains from unknown persons or bodies with a view to the possible identification of the latter.[2] Dental identification takes place for number of different reasons and in number of different situation such as for the body of victim during different types of crime. In some cases body can be disfigured to such an extent that identification by a family members is not possible. Bodies of people who have been deceased for some time before discovery and those found in water also present unpleasant difficulties in identification. Through the specialty of forensic dentistry, dentist can play a vital role in this process. By identifying the victims of crime and disaster through guidelines and standards, a dentist can assist those involved in crime investigation.[2]

Materials and Methods-

A cross-sectional study was conducted among 70 dental practitioners of Jaipur, Rajasthan. Data were collected with the help of a questionnaire (Based on Attitude, Knowledge and Practice criteria.) [Table 1] Questionnaire used in study. Informed consents were obtained.

Questionnaire

1. Are you aware of the significance of maintaining dental records to identify the individuals?

· For diagnosis or ongoing treatment procedure & long-term follow-up care of the

· patient Administrative or any other purpose

· Evidence against litigation

· Case review purpose

· Compare post & ante-mortem dental data

· Legal settlements of estates where a death certificate needed

· Following details regularly maintained

2. Do you maintain dental records? (Yes/No)

3. Dental records regularly maintain? (Yes/No)

4. Duration of maintenance of dental records? (The health insurance portability & accountability act (HIPAA) require all dental records kept for at least 6 year after the patient's last visit.)

5. Patients details (Name, Age, Sex, Address)

· Medical history

· Family history

· Dental history

6. Do you maintain treatment details of the patients?

· Clinical finding

· Treatment plan

· Treatment log/payment details

7. Following methods you used for diagnosis

a. Clinical photograph

b. Study cast/diagnostic cast For treatment planning

c. Radiograph – Forage estimation

d. Investigation finding-

1. Hematological investigation

2. Biopsy

8. Are you familiar with the subject of forensic – odontology & its relation to dentistry? (Yes/No)

9. Are you aware that you can testify as an expert witness in the court to present forensic evidence? Yes/no

10. Have you ever contributed to the identification of victims in mass- disasters? (Yes/No)

11. If called on to do so would you share your patient's dental records with a government agency to help identify victims of mass disasters? (Yes/ No)

12. At undergraduate level have you studied the subject "forensic odontology"? (Yes/No)

13. Which of the following is the most accurate & sensitive method to identify an individual?

· Visual identification

· Fingerprints

· DNA comparison

· Serological comparison in individuals

· Physical anthropological examination of bones & teeth

14. How can you estimate the dental age of an individual by examining the teeth?

· Radiograph to assess Tooth calcification

· Morphological & histological changes

15. Can you identify the Races of the individuals like:-

· Mongoloid (Nepal – Assam)

- Caucasoid
- Negros

16. Are you aware of importance Bite mark?

- Human/animals teeth Orientation & locations of the mark
- Types of injury
- Color, size & shape Contour, texture & elasticity of the bite site
- Difference between upper & lower arch Bite mark patterns may be–
- Abrasion
- Bruises
- Laceration

17. Are you aware of the method of palatoscopy/ palatal rugoscopy used for sex determination in individuals? Yes/No

18. Are you aware of methods of PCR (polymerase chain reaction) used for identify the genetic relationship between individuals? Yes/No

19. Are you aware of an advanced method like ameloglyphics (tooth print)

20. Have you had any formal training in collecting, evaluating & presenting dental evidence? Yes/No

21. Would you like to do training course in forensic dentistry if given chances. YES/No

Results

A total 70 dental practitioners responded to the questionnaire out of these 20% not maintain dental records in their clinic, 77% maintained dental records, 3% participants did not answer the question [Fig.1]. 60% dental practitioners did not know to identify child abuse, while 40% said they would identify by physical injuries, scars, behavior, clothing, and nourishment. Sixty percent dental practitioners use the appropriate method for diagnosis, while rest are not [Fig 2]. 35% dental practitioner did not know the significance of bite-mark patterns of the teeth only 4% participants said that they were part of a forensic team in their respective cities, about 70% dental practitioners did not aware that they could testify as an expert witness in the court of law. Only 10% dental practitioners have formal training in collecting, evaluating and presenting dental evidence. Seventy percent of dental practitioners are not confident to deal with forensic cases.[fig. 3]

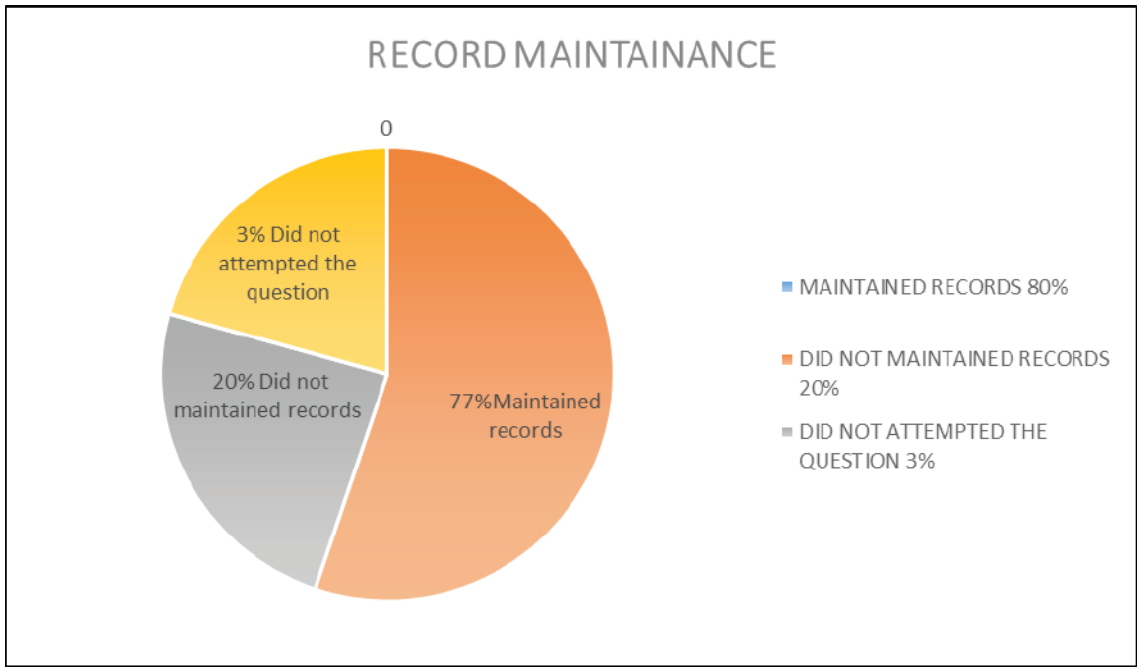


FIGURE 1: Pie chart showing percentage of subjects maintaining record

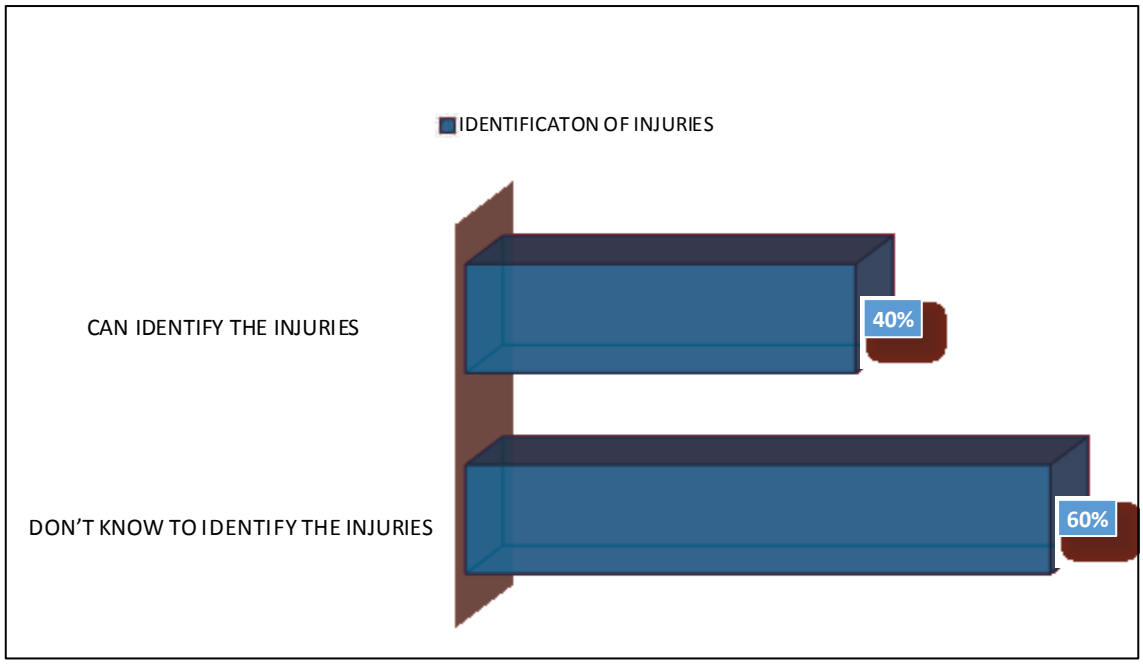


FIGURE- 2: Graph showing knowledge of individuals to identify injuries

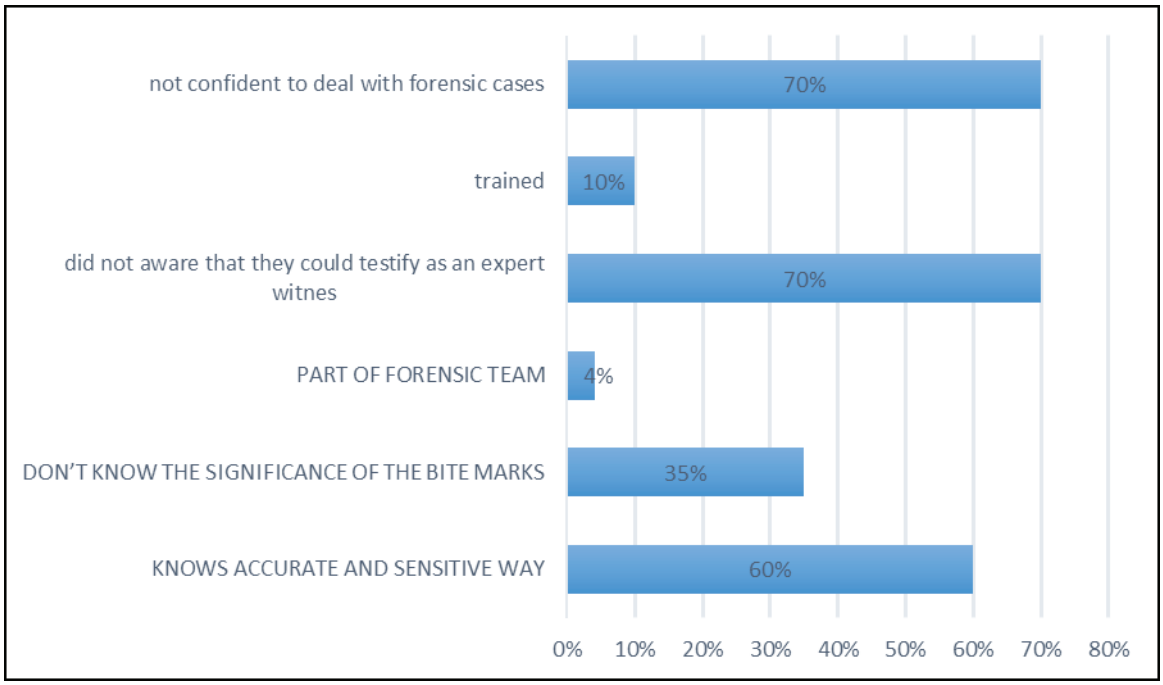


FIGURE 3: Knowledge of dental practitioners regarding forensic investigations

Discussion

Forensic odontology is an important branch of the study of dentistry that would assist in solving cases of abuses and deaths. Greater knowledge and awareness of forensic odontology among the dental practitioners would be required in the growing field of medicine. The practice of forensic odontology has gained importance in a number of developed countries across the world. However, in developing countries like India, it is yet to gain full momentum. The death toll in India due to the tsunami in 2004 was more than 15,000, but it is a question left unanswered, whether all victims were identified. This could have been made possible if there

were adequate forensic odontologist for identification of the victims. The establishment of forensic odontology as a unique discipline has been attributed to Dr. Oscar Amoeda (Father of Forensic Odontology), who identified the victims of a fire accident in Paris, France in 1898.

Today forensic odontology is considered to be a specialized and reliable method of identification of the deceased, particularly in multiple fatality incidents. Although this reputation has been gained from the application of forensic odontology in both individual identification as well as disaster situations over a number of years, the professional nature of the discipline and its practices have evolved only recently.

Therefore, success of forensic dentistry can be achieved totally only if the dental specialist and the dental institutions maintain antemortem records of their own patients with information such as name, age, sex, number of teeth present, filled teeth, dentures, and other restorations, morphological variations of teeth and mucosa with photographs and radiographs. This antemortem record will help to identify deceased persons and criminals by comparing with the postmortem records prepared by examining deceased persons during investigations, in homicide and mass disasters.[4]

This study was conducted among the dental practitioners to assess their awareness about forensic odontology [Figure 3].[1] The results show that the knowledge of forensic odontology among the dental practitioners is not adequate (Based on Questionnaires).

Teeth can be used as a weapon of attack or defense. Dentistry has much to offer to law enforcement agencies in the uncovering and solution of crime. The permanent teeth develop throughout the first two decades of life, and physiologic variations, pathologies, and effects of dental therapy may be recorded in the hard tissues of the remaining dentition throughout life and beyond. It is the role of the dentist to help extract this information and use it in the identification of the unknown body. [5] Human teeth and dental restorations have proven to remain stable during a long time as well as in extreme situations such as fire. Therefore, dentist can play an important part in the identification of severe mutilated bodies of unknown persons. The teeth may also be used as weapon and under certain circumstances, may leave information as to the identity of the biter. Analysis of bite marks is the second major responsibility of the forensic dentist. The dental practitioner has a major role to play in providing them accurate dental records on which much of forensic activity is based.[3]There is increased need for dental surgeons to have a good knowledge about forensic odontology as it is useful in identification of an individual and also discover abuse among all ages. Dentists are the health-care professionals who routinely assess the head and neck of the patients and have a great chance in identifying the signs of abuse and neglect. Every dentist has to understand the forensic implications associated with their practice. There is always a lack of involvement among dentists because of lack of training and experience, and due to their limited knowledge in this branch of dentistry. There is also a fear of litigation among dentist which commonly discourages them. "Any physician who fails to identify and report a child with historical, physical and radiological findings that indicate abuse is guilty of professional negligence."[6] There is always a lack in availability and accuracy of dental records which has a great influence in determining the success of identification.[7]

Present study showed clearly that that there is a general lack of practice of forensic odontology among dental practitioners in India.

Isher, et al also observed lack of knowledge, awareness, and practice of forensic dentistry amongst the dentists of Punjab. [8]

Baad R conducted study among dental practitioners of western Maharashtra, India and found that adequate knowledge of forensic odontology. They observed lack of formal training, inadequate forensic laboratories, poor attitude and lack of interest among dentists.[9] Another study conducted by Rahman J et al among among dental surgeons in Bhubaneswar, East India also observed that there is adequate level of knowledge and awareness regarding forensic odontology in all the groups investigated.[10]

Poor knowledge has been reported in another study conducted by Alshaqaq et al outside the India in Riyadh city of Saudi Arabia.[11]

This could be owing to multiple reasons. There are very few institutions offering formal training in forensic odontology. Most of the practitioners had no formal

training. There are no fully equipped laboratories for forensic odontology in India. Forensic odontology was not included as a part of our academic curriculum until recently. There are very few workshop conferences that have been conducted in forensic odontology per year for dental practitioners, which could kindle an interest among the students to probe deeper into the subject.

Conclusion

For an efficient forensic investigation, we need a dental team, comprising personnel from all branches of dentistry, working in close association with experts from other branches of forensic science. The government has a social obligation to recover, identify and hand over the remains of a deceased person to the relatives and every effort must be made to achieve this. Academicians, law-enforcing authorities, statutory bodies, and government have to get together and coordinate an action plan.

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Effectiveness of Ergonomic Training Program on Knowledge, Self-efficacy and Practice on Prevention of Work Related low Back Pain among Staff Nurses

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Abstract

Background: Low back pain is a major occupational problem among health care workers. Body mechanics and Ergonomic programs can prevent low back pain amongs them. The aim of the study was to assess and compare knowledge, self-efficacy and practice on prevention of work related low back pain among staff nurses before and after administration of Ergonomic Training Program in experimental and comparison group.

Methods: A Quasi Experimental Non Equivalent Control Group Pre-test and Post-test Design was used. Eighty staff nurses from MMIMS&R Hospital, Mullana were selected by convenience sampling technique. Ergonomic Training Program was administered in experimental group for two days. Standardised Nordic questionnaire for low back pain, Pain assessment scale, structured knowledge questionnaire, structured self-efficacy scale and structured observational checklist were used to collect data.

Results: The study showed that there was a significant difference in the mean post-test knowledge (p=0.001) and practice (p=0.001) scores of staff nurses in experimental and comparison group. There was a significant difference between the mean pre-test and post-test knowledge (p=0.001), self-efficacy (p=0.047) and practice scores (p=0.0001) in experimental group.

Conclusion: Ergonomic Training Program was effective in improving the knowledge, self-efficacy and practice on prevention of work related low back pain among staff nurses.

Keywords: *Effectiveness, Ergonomic Training Program, knowledge, self-efficacy, practice, staff nurses, work related low back pain*

Introduction

Worldwide, low back pain is found to be experienced for at least once in their lifetime by most of the people (estimated from 50 % to 80 %).[1] It has been estimated that the annual incidence of low back pain (LBP) in adults is found to be 15% with point prevalence of 30%.[2]In developed countries, the prevalence rate of LBP has been estimated to be 15% to 64% and more than 79% in the developing countries.[3] It is estimated to be 38% to 67% in the American nurses, 73% to 76% in nursing staffs of Germany, and 9.38% in Hong Kong nurses, and over 50% in Iranian nursing staffs.[4] One-third of the loss of work occurs due to work related accidents and diseases related to occupation.[5] Of all the LBP cases, 37% of them occur as a result of work related constructs. It changes between 12% and 38% in women, 31% and 45% in men.[6] Nursing is identified as one of the

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occupation who is at risk of developing LBP.[7] In nurses, while giving care there is repeated flexion or extension of the body, including object or patient lifting, transferring of patients which represents major bodily stress and results in back injuries.[8] The prevention and control of the musculoskeletal disorders can be achieved by using the applied science of ergonomics. Nursing involves risk activities; with high prevalence of musculoskeletal complaints related to the low back, neck and shoulder pain.1 Nurses are exposed to musculoskeletal disorders as they are mainly standing for long hours[9], maintaining wrong position for a long time during their working shifts. Low back pain among staff nurses is mainly due to repetitive motions.[10] In developing countries, the nurses have little knowledge of ergonomics principles in their workplace and they are not trained adequately to prevent and control hazards due to occupational exposure. Knowledge of ergonomics can help nurses in avoiding certain risk factors of musculoskeletal disorders and can improve health and safety in the workplace.[11] It was reported by a study that ergonomics training program can help in major improvement in lifting of patients and carrying behaviour.[12] A study also suggested the use of Ergonomics and body mechanics to prevent work related low back pain.[13]

Moreover, the researcher had personal experience that the staff nurses in clinical settings do not practice proper techniques of body mechanics and ergonomic principles in performing nursing activities during their work shifts. This has aroused the interest to conduct this study to possess the effectiveness of Ergonomic Training Program (ETP) on knowledge, self-efficacy and practice on prevention of work related low back pain among staff nurses.

Material and Methods

Research approach and design: Quantitative research approach with quasi experimental non-equivalent control group pre-test post-test research design was used.

Setting: The study was conducted in MMIMS&R Hospital, Mullana, Ambala, Haryana. It is a multispecialty tertiary care hospital.

Sampling: Convenience sampling technique was used, 80 staff nurses (40 staff nurses each in experimental and comparison group) working in various wards of MMIMS&R Hospital, Mullana, Ambala, Haryana were included in the study. Cohen's d formula was used for sample size calculation (effect size was 0.66; at power of 0.80, the recommended sample for each group was between 33-44).

Tools and techniques for data collection

Section-I: Part-A (Demographic variables of the staff nurses). Part-B (Standardised Nordic Questionnaire for low back pain among staff nurses) developed by Kuorinka I et al in 1987. Part-C (Numeric pain scale to assess the level of low back pain among staff nurses) consisting of pain score ranging from 0 (No pain) to 10 (Worst pain)

Section-II: Structured knowledge questionnaire to assess the knowledge of staff nurses regarding prevention of work related low back pain.

Section-III: Structured self-efficacy scale to assess the self-efficacy of staff nurses regarding prevention of work related low back pain.

Section-IV: Structured observational checklist to assess the practice of staff nurses regarding prevention of work related low back pain by using ergonomics and its principles.

Data collection procedure

Pilot study was conducted prior to final data collection.

On day one, pre-test was conducted. On day two after taking the pre-test, ETP (Ergonomic training program) was administered by using lecture cum discussion with the help of power point slides and videos for two hours in experimental group. On day three, demonstration and return demonstration of ergonomic principles and exercises (body stretches) to the staff nurses was carried out (the experimental group was divided into four sub-groups of ten staff nurses each with duration of one hour for each group). On day 15, after the intervention, post test was conducted for both the groups.

Ergonomic training program

It consisted of teaching regarding work related low back pain, ergonomic and its principles, and the body

stretches or exercises to reduce or prevent work related low back pain among staff nurses.

Statistical Analysis

SPSS version 20.0 was used for data analyses. For descriptive statistics, frequency, percentage, mean, median, standard deviation, range was used. For parametric tests, it included chi-square, t-test, one way ANOVA, and post hoc tests. For non-parametric tests, it included chi-square, Mann-Whitney test, Wilcoxon signed rank test and Kruskal Wallis test. K-S test was applied and parametric test was applied for knowledge score and Non-parametric tests were applied for self-efficacy score and practice score regarding prevention of work related low back pain among staff nurses.

Results

The study results showed that majority of the staff nurses were females (96.25%) and majority were from age group of 23-25 years, 81.25% of staff nurses were single, 74 (92.5%) of staff nurses were not having history of abdomen/ back/ limb surgery, 72 (90%) of them had no child, 76 (95%) were GNMs, 56 (70%) were having 0-12 months year of experience in the present working area 68 (85%) of the staff nurses were working for six hours per day, all of them had no training regarding body mechanics, 76 (95%) of them were not attending yoga class and 78 (97.5%) of them were not going to gym, 59 (73.75%) of them were having normal BMI and all of the staff nurses were not diagnosed with diseases like arthritis, prolapse inter-vertebral disc, etc.

In pre-test, 48/80(60%) had below average knowledge, 70/80 (87.5%) had good self-efficacy and 47/80(58.75%) had good practice scores regarding prevention of work related low back pain. In post-test, 23 (57.5%) had good knowledge, 21 (52.5%) had very good self-efficacy and 34 (85%) had very good practice after the administration of Ergonomic Training Program (ETP) in experimental group.

There was significant difference between pre and post test knowledge scores (t=11.45, p=0.001), self-efficacy scores (Z= -1.98, p= 0.047) and practice scores (Z= -5.17, p=0.0001) among staff nurses in experimental group.

There was a significant difference in post test knowledge scores (t=12.01, p=0.001) **[Table 1]**, and practice scores (Z= -6.84, p=0.0001) **[Table 2]** among staff nurses in experimental and comparison group. There was no significant difference in post-test self-efficacy scores (Z= -1.67, p=0.09) **[Table 3]** among staff nurses in experimental and comparison group. Therefore, it can be inferred that Ergonomic Training Program was effective in improving the knowledge and practice of staff nurses on prevention of work related low back pain.

There was a weak positive correlation between knowledge and self- efficacy score (r= 0.38, p= 0.02), and also between self-efficacy and practice score (r=0.32, p=0.047) among staff nurses in experimental group after the administration of Ergonomic training program.

There was a significant association of knowledge score with years of experience in present working area and practice score with qualification in experimental group, self-efficacy score with total year of experience. There was significant association of post-test self-efficacy score with present designation, total year of experience, hours of working/ day and work for extended duty hours. There was significant association of pre-test practice score with qualification status of the staff nurses.

It was found that most of the staff nurses i.e. 70/80 (87.5%) and 71/80 (88.75%) were not having low back pain before and after administration of Ergonomic Training Program respectively. In experimental group, 3 (7.5%) were having moderate pain (4-6 pain intensity score) in pre-test which was reduced to 1 (2.5%) in post-test.

Discussion

In the study, majority of the staff nurses were females (96.25%) and majority were from age group of 23-25 years which was consistent with the study conducted by **Sharma R** which showed that maximum number of female nurses (97.14%) was from age group of 20-30 yrs.[14] In the study, all of the staff nurses i.e. 80/80 (100%) had no training regarding body mechanics of which the result was consistent with the study conducted by **Ibrahim R et al**.[15] In the study, 76 (95%) of them were not attending yoga class and 78 (97.5%) of them

were not going to gym and 59 (73.75%) of them were having normal BMI.

All of the staff nurses were not diagnosed with diseases like arthritis, prolapse inter-vertebral disc, etc. This was inconsistent with the finding by **Sandhya RV**.[16]

It was shown that Ergonomic Training Program was effective in improving the knowledge, self-efficacy and practice scores on prevention of work related low back pain among staff nurses. The result was found to be consistent with the study conducted by **Sharma R** which showed that the mean knowledge score and practice scores after the intervention was significantly higher than that before the intervention.[14] Also similar findings were shown by the study conducted by **Ibrahim R et al** which showed significant differences of nurses' knowledge and practice scores between pre-test and post-test (p=0.0000).[15] Similar finding was shown by a study conducted by **Salah M et al** in which there was significant difference regarding total knowledge of back pain and body mechanics in pre- and post- program implementation (Z=4.43, p=0.0000 and Z=4.06, p=0.0000 respectively). Also, there was significant difference regarding practice in pre- and post-program implementation (Z= 5.85, p=0.0000 and Z=5.29, p=0.0000 respectively).[17]

In the study, there was a significant positive correlation between knowledge and self efficacy scores; self efficacy and practice scores after the administration of ergonomic training program among staff nurses in the experimental group but no correlation was found between knowledge and practice scores among the staff nurses. It was supported by the study conducted by **Khorsandi M et al** in which there was correlation between self-efficacy and practice scores (r=0.19; p=0.027).[18] The result of the study was not consistent with the finding of study conducted by **Vidya VP et al** which showed that a positive correlation was found between the knowledge and practice of body mechanics among staff nurses (r=0.611).[19]

Conclusion

The study concluded that the Ergonomic training program (ETP) was effective in improving the knowledge, self-efficacy and practice of staff nurses regarding prevention of work related low back pain. It also revealed that there was a significant positive correlation between knowledge and self-efficacy; and also between self-efficacy and practice among the staff nurses regarding prevention of work related low back pain.

Table 1: Comparison of post-test knowledge scores on prevention of work related low back pain among staff nurses in experimental and comparison group

(N=80)

Group	Mean±SD	M_D	SEM_D	t value	df	p value
Experimental group (n=40)	22.88±4.30	9.6	0.8	12.01	78	**0.001***
Comparison group (n=40)	13.25±2.67					

t (78)= 1.99

Minimum score= 0

Maximum score= 30

*=Significant (p≤ 0.05)

NS=Not significant(p>0.05)

Table 2: Comparison of post-test practice scores on prevention of work related low back pain among staff nurses in experimental and comparison group

(N=80)

Group	Mean rank	Sum of rank	Mann Whitney U test	Z value	p value
Experimental group (n=40)	58.24	2329.50	90.50	-6.84	**0.0001***
Comparison group (n=40)	22.76	910			

***=Significant (p≤ 0.05)** **NS = Not significant (p>0.05)**

Table 3: Comparison of post-test self-efficacy scores on prevention of work related low back pain among staff nurses in experimental and comparison group

(N=80)

Group	Mean rank	Sum of rank	Mann Whitney U test	Z value	p value
Experimental group (n=40)	44.83	1793	627	-1.67	0.09^{NS}
Comparison group (n=40)	36.18	1447			

***=Significant (p≤ 0.05)** **NS = Not significant (p>0.05)**

Conflict of Interest: No conflict of interest

Funding: Self

Ethical Clearance: Formal Ethical approval was obtained from the ethics committee of Maharishi

Markandeshwar (Deemed to be University), Mullana (IEC-1159). Informed consent was obtained from the respondents and they were assured about the confidentiality of their responses.

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Adolescent Behavior: Understanding in Relation to Mental Health Issues with Implication in Nursing- Review Article

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Abstract

The aim of this review article was to explore the issues and concerns of adolescents with regard to their mental health and this need for implication in nursing practice. The study materials were collected from various sources like books, journals, online data base and news paper cuttings of last ten years 2008-2018. Online search engines were such as PubMed/ Medline, SCOPUS, CINAHL, PsychoINFO, and Google Scholar by search of significant articles with the following key words: "Adolescence", "Adolescent behavior", "Adolescent AND mental health". "Aggression AND Adolescence". Further article revealed about the need of understanding adolescent behavior and its correlates, existing practices, problems and shortcomings in terms of adolescent behavioral issues, strategies to improve the situation and solve issues, nursing implication with available resources to handle the situation at current scenario and to overcome the associated mental health issues among the adolescence. Study concluded that there are various reasons due to which adolescent behavior is affected and it requires a group of strategies to handle and overcome all associated issues.

Key words: *Adolescence, Adolescent behavior, Adolescent and mental health, Adolescent and aggression, violence and substance use.*

Introduction

"The disturbed adolescent does not feel his disturbed personality. The intelligent man does not feel his intelligence or the introvert his introversion."
-B.F. SKINNER

World Health Organization (WHO) identifies Adolescence as a phase inhuman growth and development that occurs after childhood and before adulthood. It represents one of the critical transitions and is characterized by a tremendous pace in growth and developmental changes.[1]

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Adolescents are often not able to cope up with the drastic changes that take place in their physical & psychological health. The psychological problems in this period occur due to disturbed relationship with parents, teachers, seniors and peers, scholastic backwardness compared to high expectation, sexual experimentation and substance abuse. All these are often directly or indirectly associated with aggression as well as psychological morbidity.[2]

Methods

The aim of this review article was to explore the issues and concerns of adolescents with regard to their mental health and this need for implication in nursing practice. Since it is a method of concept article with evidence based narration, the study materials were collected from various sources like books, journals, online data base and news paper cuttings of last ten years 2008-2018. Online search engines were such as PubMed/ Medline, SCOPUS, CINAHL, PsychoINFO, and Google Scholar by search of significant articles with the following key words: "Adolescence", "Adolescent behavior",

"Adolescent AND mental health". "Aggression AND Adolescence". Based on the significant information found with regards to aim of the review article, concepts were formed and discussed in narrative form to yield adequate evidence based information about the need of understanding the adolescent behavior with regards to their mental health and overcoming such issues with intended nursing implications.

Findings

Findings of the review article based on the concepts are discussed as follows:

Relevance / Need for Understanding Adolescent Behavior In Mental Health

Adolescence is a time for developing independence. Typically, adolescents exercise their independence by questioning, and sometimes breaking, rules. Parents and nurses must distinguish occasional errors of judgment from a degree of misbehavior that requires professional intervention.

Adolescents and Behavioral issues

Basically the behavioral problems and issues among adolescents includes such as but not limited to Attention deficit hyperactivity disorders, Conduct disorders, Substance use disorders, Aggressive behavior, impulsiveness and violence which can lead to delinquency or delinquent behavior as it is highly associated with low self esteem.[3]

Descriptive study was conducted by **Kataria**[4] where it is found that, Emotional competent people do express emotions appropriate to the situation to their needs and to others and they attempt not to suppress appropriate emotions, reactions and communications of feelings by others. Further it is revealed that at present adolescents are facing psychological problems such as depression, anxiety and frustration, one of the increasing with rapid rate is aggression. Their aggressive attitude results into delinquent.

There are lots of reasons why an adolescent becomes aggressive and violent and end up in delinquent behavior. Especially Adolescents from low-income families often feel excluded. To raise their self-esteem and improve their status they may choose to join a juvenile delinquent group. These groups provide equal opportunities to everyone, favorably distinguishing themselves from school and family, where positions of authority are occupied by adults.[5]

Correlates of Adolescent Behavior in Relation to Mental Health

Correlates of adolescent behavior are discussed under following sub headings;

Adolescents and ADHD cum Conduct disorders

Several surveys were conducted regarding ADHD and conduct disorders among adolescents and the reviews regarding these studies are conceptualized as follows:

Study conducted by Mony EH. (2013)[6] revealed that children or teens with ADHD experience different symptoms of inattention, hyperactivity, or impulsivity either above or beyond what is developmentally appropriate. Teenagers with ADHD are seen to be at an increased risk of academic failure, dropping out of school and criminal behavior and more likely to be dismissed from employment in adulthood.

Study conducted by Du Paul GJ (2018)7 revealed that ADHD can be co-morbid with many other psychiatric problems including specific learning disabilities (SLD).

Study conducted by Raskind M. (2015)[8] revealed that along with specific learning disability, conduct disorders, aggression, poor relationship with peers, disregard of orders, low self esteem, depression, and social, emotional and cognitive problems can be also seen in the child with ADHD.

Adolescents and Aggression, Violence / Delinquency

There are number of studies which are conducted to support the evidence for aggression, violence and delinquency or delinquent behavior as one of the most concerned area among adolescence. These studies are conceptualized as follows:

Study conducted by Sharma M.K & Marimuthu P. (2014)[9] revealed that males experienced more verbal aggression, physical aggression and anger than females. The risk factors responsible for the youth aggressions were physical abuse in childhood, substance abuse such as alcohol and tobacco, negative peer influence, family violence. The study concluded that presence of correlates of risk factors pertinent to aggression among

youth implies the usages of management strategies to help them to reduce their aggression

Study conducted by Pung P.W., et al (2015)[10] revealed that there was a significant positive relationship between low self-control, peer delinquency and aggression among adolescents. The results also indicated that low self-control has an indirect effect on aggression through peer delinquency. The study concluded that Low self-control and peer delinquency are two important risk factors which are responsible for the development of aggression among adolescents.

Study conducted by Wahdan I., et al (2014)[11] revealed that overall 26.9% of adolescents at high risk and 20.2% at extremely high risk of aggression and criminal behavior. The study concluded that necessary measures must be taken to reduce the negative impacts of exposure to violence through giving special attention to changing the perception of children and adolescents to these risks.

Existing Practices / Situation/ Knowledge/ Attitude in terms of Adolescent Behavior

The existing practices, situation, knowledge and attitude regarding adolescent behavior are discussed under the following sub heading;

Reviews regarding Knowledge on Adolescents' Behavioral Issues- Parents, Teachers and Nurses perspective

Adolescence is a developmental stage marked by risk-taking and limited comprehension of dangers of risky behaviors. There are number of studies which are conducted to support the evidence for knowledge on adolescents' behavioral issues- parents, teachers and nurses' perspective. The studies are conceptualized as follows:

Study conducted by Ahern N.Ret al., (2016) [12] revealed that the survey yielded five categories of parental responses to potential risky behaviors in their child/teen including the following: talking to my children, setting up consequences, confronting the child, seeking help, and talking to others. The study findings provided important implications for developing an evidence-based education intervention to improve parents' awareness, knowledge, and identification of risk behaviors in their children/teens.

Study conducted by Albertos A., et al (2016) [13]revealed that the greater the degree of knowledge, the lower the frequency of risk behaviors among youth. The study concluded that parents' supervision criteria might be influenced by gender stereotypes, which would have a harmful effect on young males, as the lower degree of knowledge puts them at higher odds of risk behaviors.

Based on the above mentioned reviews we conclude that though we have knowledge regarding adolescent health care, there is no adequate knowledge among parents, teachers and health care workers regarding the consequences of behavioral issues and it is recommended to have proper evidence based education intervention to improve the knowledge among them.[14]

Existing Problems/Shortcomings in terms of Adolescent Behavior

The Existing problems among adolescents in terms of their behavior are discussed by understanding the following core concepts;

The Existing problems among adolescents in terms of their behavior are connected with various behavioral issues such as aggression, Delinquency, substance abuse and chronic low self esteem.

Hence it concludes the chronic low self esteem among adolescents could cause them all sort of behavioral issues and it is necessity to improve adolescents' self esteem to overcome other behavioral issues such as aggression and delinquency.

Hence the basic concepts identified through the reflective analysis of the reviews revealed the following modified conceptual ideas based on which various intervention studies need to be identified to improve self esteem among adolescents to reduce their impulsive aggression and associated use of substance, violence and delinquency which is depicted in figure 1.

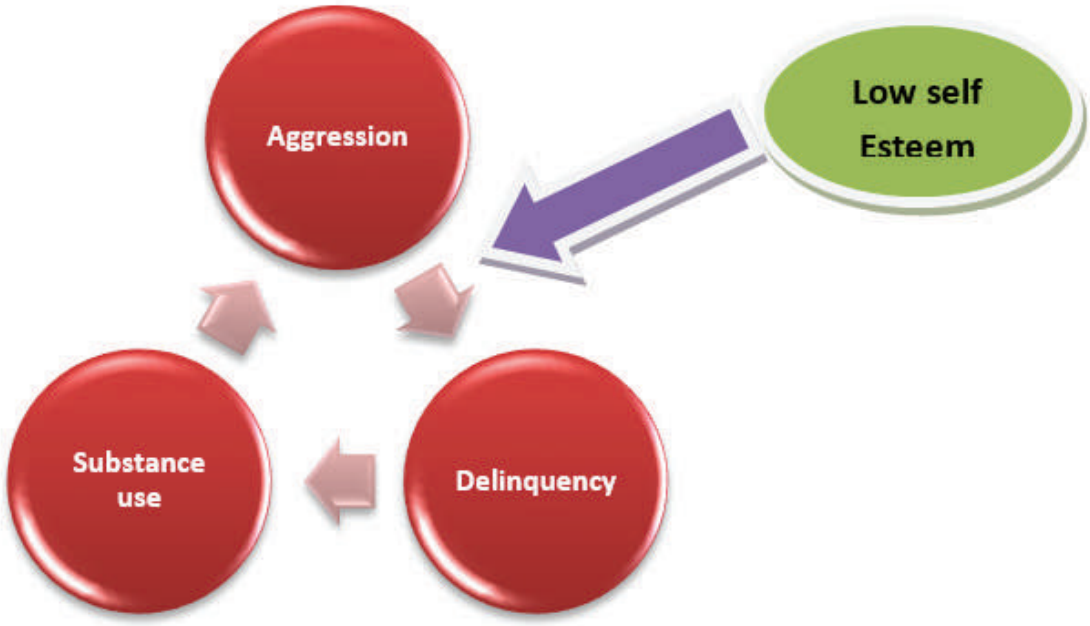


Figure 1-The Identified Core concepts of Adolescents Behavioral Issues (Modified from the concepts of Latent Trajectory Modeling-2010)[8]

Substance use Disorders, Aggression And Delinquency- A Core Concept

Violence and physical aggression among adolescents often results in unintentional injuries and homicide, two leading causes of death among adolescents. Aggressive behaviors are also associated with drug use, unsafe sex, dangerous driving, other mental health problems, across gender and ethnic groups. Differential rates of violence and physical aggression are predicted by child, familial, genetic, and environmental factors[14].

Study conducted by Shahzad S, &Yasmin S (2011) revealed that Significant differences on variables of aggression between delinquent and non-delinquent adolescents. In subscales of aggression questionnaire, delinquents were found to score high on physical aggression (t= -2.678, df= 204, p<.05), verbal aggression (t= -2.436, df= 204, p<.05) and hostility (t= -5.490, df= 204, p<.05). Similarly, those who use drugs have high level of aggression than those who do not use drugs (t= 4.921).[15]

Study conducted by Rosenfeld R., et al (2012) Findings showed on particular type of offenders (homicide offenders, gang members, drug dealers and those carrying weapons) that normally the age of start of gang groups, drug use, weapon carrying and drug dealing happened during adolescence (ages 13 to 17 years). Further study explored the order of normal age of starting offenses which found the mean age for sequence gang membership (15.9), followed by marijuana use (16.5), drug dealing (17.0), gun carrying (17.3 and hard drug use (17.5).[16]

Strategies to improve the Situation / Minimize or Solve problems in terms of Adolescent Behavior

The strategies to improve the conditions or mental health issues among adolescents are better understood by the followings;

Positive Adolescent Health Strategy

Adolescent environment is also critical in determining the behavior of youth. Thus, we need to focus on lifelong skills and healthy behaviors[17].

There are certain Recommendations these are;

Recommendation 1: Create Adolescent-Positive Societal Norms and Commitment to Adolescent Health Issues

Strategy 1: Mobilize all Sectors of Society in Supporting Adolescent Health Promotion

Strategy 2: Create a Supportive Environment for Promoting Adolescent Health

Recommendation 2: Create Opportunities For Adolescents to Engage in Healthy Behaviors

Strategy 1: Maximize Opportunities for Adolescents to Engage in Healthy Behaviors

Recommendation 3: Promote Adolescent Health

Strategy 1: Enhance the Visibility of Adolescent Health Issues and Positive Aspects of Adolescents

Strategy 2: Make Commitments to Adolescents and Adolescent Health Issues

Nursing Implications

Children and Young People's mental health nurses have a key role in providing direct support to children, young people and their families, as well as educating and supporting other professionals including teachers and school nurses to promote emotional and psychological wellbeing. Access to specialist and targeted services in a timely manner can make all the difference to a child's long term health and wellbeing.

Specialist nurses can provide a range of therapeutic interventions including cognitive behavioural therapy, dialectical therapy and family therapy, as well as the management of deliberate self-harm.[18]

Handling issues of adolescents is the responsibility of both General and school health nurse. But this must be highly implicated at schools to anticipate the issues as early as possible and to deliver the better care.

Principle of Nursing Care for Adolescents

According to Limerick 2009 there are certain nursing principles were established for achieving better mental nursing care for children and adolescents (CAMHS) those are;

- Understanding developmental theories to the core
- Mastery over therapeutic interventions
- Evaluating skills
- Professional management skills
- Professional development skills
- Communication skills[19]

Available Resources & Its use with reference to Adolescent Behavior

National Program and centers in India:

1. Youth Mental health program- SCARF (2015)
2. Youth wellness centre – 2015- SCARF (2015)
3. National Mental Health Program, 1982
4. District Mental Health Program, 1996
5. National Mental Health Policy, 2014
6. National Health Mission, 2017

Infrastructure and service in India:

Substance abuse

1. De addiction centers
2. Al-ateen services

Aggression and violence

1. Observational Homes
2. Correctional Homes/ Delinquent Homes

Conclusion

Adolescents are often not able to cope up with the drastic changes that take place in their physical & psychological health. The psychological problems in this period occur due to disturbed relationship with parents, teachers, seniors and peers, scholastic backwardness compared to high expectation, sexual experimentation and substance abuse. As discussed in the literature there are various reasons due to which adolescent behavior is affected and it requires a group of strategies to handle and overcome all associated issues.

Ethical Consideration: Since it is a review article which has been written as concept article does not require an ethical permission. Further, based on the above identified concepts an interventional study is designed to reduce aggressive behavior and impulsivity and to improve self esteem among adolescents those who are residing in delinquent homes in the state of Bihar has already been submitted for obtaining permission from Departmental Research Committee of All India Institute of Medical Sciences, Patna.

Conflict of Interest: None

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Original Research Article

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Study of Limb Injuries in Road Traffic Accident Cases-An Analytical Study in IMS And SUM Hospital, Bhubaneswar, Odisha

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Abstract

Aim and Objectives: To know: i) The age and sex wise distribution of road traffic accidents (RTA) cases. ii) Pattern of limb injuries sustained. iii) Type of vehicle involved and speed of vehicle**. Materials and Methods:** The study was carried out in the department of F.M.T in association with the Department of Accident & Emergency, Department of Orthopaedics, and Department of Surgery of IMS and SUM Hospital, Bhubaneswar. Duration of study June 2015- July 2017. Study type- cross sectional study. The number of cases were 200. **Results:** Of the 200 patients in the present study 90.5%(181) were males and only 9.5%(19) were females. Two wheelers were the most unsafe in the vehicles category involved in road traffic accidents (79.5%). Vehicle travelling between 60-80 km/hr are more susceptible (58%) to injury/accident. The most common injuries were laceration & fracture (30%) . Right lower limb(38%) was mostly involved.

Key words: *Limb injuries, road traffic accidents, vehicle type, pattern of injuries*

Introduction

Travel today is an essential requirement for the present generation. Due to increase in population in urban area and there are many reasons to travel, mobility is the primary need. Road traffic accidents are a major cause of death which is occurring in developing country now a days. In the world it is ranked in 9th position by 2020[1]. Road traffic accidents cost between 1% to 2% of their gross national product in low income and middle income countries which is more than the total development aid received by these countries[2].Road traffic accidents are multifactorial in nature which involves human, environmental factors and vehicular factors. In the world, India is one of the leading country in road traffic accident. It has been seen that there is steady rise in road traffic accidents in India and every minute there is an accident and every eight minutes a death. About eighty thousand people are killed and three lakhs forty thousand are injured every year[3]. The number of accidents caused by vehicle was four lakhs thirty thousand and six hundred and death due to vehicular accidents was one lakhs thirty three thousand nine hundred thirty eight and one lakh seventy thousand six hundred injuries according to the national crime record 2010[4]. Due to the injuries which is caused by road traffic accident, leads to increase in trauma patients admission at medical college and hospital which will take many lives and resources[5].

Aim and Objectives: To know: i) The age and sex wise distribution of road traffic accidents (RTA) cases. ii) Pattern of injuries sustained. iii) Type of vehicle involved.

Materials and Methods: The study was carried out in the department of F.M.T in association with the Department of Accident & Emergency, Department of Orthopaedics, and Department of Surgery of IMS and SUM Hospital, Bhubaneswar. Duration of study June 2015- July 2017. Study type- cross sectional study. Inclusion criteria: All patients of road traffic accident who were admitted to IMS & SUM hospital with upper & lower limb injuries. Sample was selected from all the cases of RTAs with definite history of victim either pedestrian, driver or passenger of a vehicle. Only limb

injuries were taken. Exclusion criteria: Patients with only head injury. Patient with abdomen injury, back injury without limb injury. A Total number of cases were 200 which was analysed during the study period, the details of each case were entered in a standard proforma designed for the study and the details of the vehicle types were obtained by the police and the attendants. Statistical analysis was made.

Results

Of the 200 patients in the present study 90.5%(181) were males and only 9.5%(19) were females

The present study shows that the Male: Female ratio of limb injury in road traffic accidents is 9.5:1 , i.e. the males were involved more than 9 times than females which was statistically significant. ($p< 0.001$).

The present study shows that the persons driving the vehicle are more susceptible (64%) to injury than the passengers or the pedestrians which was statistically significant. ($p< 0.001$)

The present analytical study shows that road traffic accidents occur mainly in the age group 20-39 years.

Table- 1: Frequency of distribution of vehicle involved in road traffic accident

Vehicle involved	Frequency	Percentage
Heavy vehicle	17	8.5
Four wheeler	12	6%
Two Wheeler	159	79.5%
Auto Rickshaw	4	2%
Other	2	1%
Total	200	100%

The above table shows that the two wheelers are the most unsafe in the vehicles category involved in (79.5%) followed by heavy vehicle (8.5%) .

Table-2: Frequency of distribution of speed of the vehicle in relation to road traffic accident

Speed of the vehicle	Frequency	Percentage
0-19 Km/hr	1	0.5%
20-39 Km/hr	1	0.5%
40-59 Km/hr	62	31%
60-79 Km/hr	116	58%
>80 Km/hr	20	10%

The above table shows that the .than other vehicles which was statistically significant. As the roads involved were mainly city roads there could have been less scope of travelling beyond 80 km/hr.

Table-3: Distribution of Type of Injury in road traffic accident

Type of injury	Frequency	Percentage
Abrasion	21	10.5%
Fracture	50	25%
Lacerated	12	6%
Abrasion & contusion	4	2%
Abrasion& dislocation	2	1%
Abrasion & laceration	8	4%
Abrasion & fracture	8	4%
Contusion & laceration	6	3%
Contusion & fracture	2	1%
Laceration & fracture	60	30%
Abrasion & laceration & fracture	17%	8.5
Contusion , laceration & fracture	3	1.5%
Laceration , fracture & dislocation	2	1%
Laceration , fracture & others	1	0.5%
Others	4	2%
Total	200	100%

The above table shows that the accident victims mostly suffered from laceration & fracture (30%) followed by fracture (25%). This could be due to hard surface of bitumen clad city roads and most vehicles travelling between 60-80km/hr that were involved in accidents.

Table-4: Frequency of Site of injury in road traffic accident

Site of injury	Frequency	Percentage
Right upper limb	32	16%
Right lower limb	76	38%
Left upper limb	13	6.5%
Left lower limb	38	19%
Right lower limb & left lower limb	6	3%
Right upper and lower limb	19	9.5%
Right upper limb and left upper limb	4	2%
Right lower limb and left upper limb	2	1%
Right upper limb, left upper limb and left lower limb	2	1%
Left upper limb and left lower limb	6	3%
Right upper limb and left lower limb	2	1%
Total	200	100%

The above table shows that in 38% cases right lower limb was involved. This combined with the fact that 2 wheelers were the most common vehicles involved indicates the struggle by the right handed driver to stop the vehicle at high speed resulting in fracture and laceration of the affected lower limb

Discussion

In the present study 181(90.5%) were males and 19(9.5%) were females, male to female ratio was found to be 9.5:1. The males are involved more than 9 times in road traffic accidents as than females. This is due to fact that male travel a lot more than females do, engage more in risk taking behaviour and are involved in outdoor activities of the family. This is also in accordance with the report of Rajnarayan R. Tiwari et al(2005)[6]. According to Frank et al males outnumbered females[7]. According to Nilambar et al the accident rates in males were 4.9 times higher than in females. In other study[8] male female ratio was found to be 9:1[9]. Abdul N. Batouk, et al also found that male female ratio was higher i.e 14:1[10]. M Ranjitet. al[11]also corroborates with the present study. The males outnumbered females in the ratio of 9:1[12,13,14,15,16,17,18,19,20,21,9].

The present analytical study shows that road traffic accidents occur mainly in the age group 20-39 years which resulted in the loss of expenditure in treating the patient as well as the productive age group. This is in accordance with Rajnarayan R. Tiwari et al[6]. Studies have reported that the high incident of road traffic accident in age group of 20-39years, this is due to the risk taking behaviour of the age group[22-25,26]. According to Abdul N. Batouk, the highest accidents were seen in the age group 21 – 49 years[10]. This shows that the people who are most active and productive age group are involved in road traffic accidents, which add a serious economic loss to the community[25,27]. Basu Nandy Mukhopadhyay and Majumdar et. al 1977-78. Maximum involvement (29.5%) in road accidents were seen in the age group 26-40 years, followed by age range 16-25 years (22.5%) and 41-55 years (16.5%). There were 87% male victims in road accident against 13% in females[28]. Rao D analyzed 254 cases and found that major age group between 21-30 years were affected (31.51%)[29]

G Gururaj (2008) Analyzed cases of road traffic accident in the year of 2005 and found that majority of the victims were of the age group15-44 years and belongs to the poorer section of the society[30]. Behera C et al 2009studied commonest age group involved was 21 to 30years (44.67%)[18]. Most common age group involved is 21-30 year[19]. Kuchewar SV et al (2012) analyzed 216 RTA cases and found 50% of cases are of age group 20-40 years[20]. Maximum number of victims belongs to age group between 21-30 years 28% followed by 31-40-years 20%[21]. JhaNilambar et al(2003) analysed. According to the author the maximum accidents occurs 20-29years with average age group 31.5 years[9].

The most common vehicle involved in the present study was motorized two-wheelers79.5% (179). This is because the study is carried in urban area where the two wheelers vehicle is most common mode of transport. This is in accordance with the other studies [8,22-24,26,31].

The present study shows that the vehicle travelling between 60-80 km/hr are more susceptible 58%(116) to injury/accident than other vehicles which was statistically significant. As the roads involved were mainly city roads there could have been less scope of travelling beyond 80 km/hr. Fildes B.N et al(1994) studied that the fractures depends on the velocity of the vehicle to its impact and occurred at 48m/hr or less[32]. According to Jha et al. two wheelers drivers(61.2%) were commonly involved in accidents because of their high speed driving and also due to less stability while driving[9].

The present study shows that the accident victims mostly suffered from laceration & fracture(30%) followed by fracture(25%). The present study shows that in 38% cases right lower limb was involved. This combined with the fact that the most commonly vehicles involved were two wheelers indicates the struggle by the right handed driver to stop the vehicle at high speed resulting in fracture and laceration of the affected lower limb. Tonge et al (1977) studied 76 motor cyclist victims and found that injuries to upper limb occur most frequently. Fildes B.N et al(1994) studied lower limb injuries to car occupants in Australian car accidents. There was a study in Melbourne which shows that fractures occurred 88% of crashes with lower limb injury[32]. The most common injuries were tibial fracture[19]. Patil Satish Supriya et al(2007) also studied that lower limb fracture(46.3%) were more common than upper limb(24.7%)[26]. This is in corroboration with the present study.

Summary and Conclusion

From our study it is now clear that Laceration and fracture injuries are common limb injuries being reported and two wheelers are mainly responsible for RTA limb injuries. Two wheeler male drivers outnumber female and pillion riders in sustaining injuries to limbs.

The numbers of two wheelers are likely to increase in the future along with road traffic accidents as a whole. Therefore the drivers should be educated to drive safe in the road . There should be a road safety programme for two wheelers for the prevention of accidents in the cities. To prevent the two wheeler accident the following should be done:- The roads should be designed and properly maintained . Separate roads for two wheelers should be there. Strict traffic regulation. Carrying children on two wheelers should be avoided. Road side alcohol analysis for riders should be there. Driving license should be strictly checked. Driver and pillion should wear helmet mandatory. Designing of safer vehicle. Banning unlicensed and young drivers on roads. Improvement of trauma care centres.

After analysing the data from the present study it appears to be easier to distinguish between various injury patterns and injuries sustained due to RTA coming to SUM Hospital.

Ethical Clearance- Taken from- Ethics committee, Institute of Medical Sciences (IMS) and SUM Hospital, Siksha 'O' Anusandhan University.

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Conflict of Interest: The authors declare that there is no conflict of interest.

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Reconstruction of Length of Femur from Its Fragments- A Preliminary Study in Eastern Indian Population

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Abstract

Stature being a very important data for creating the identification profile of any individual, has immense anthropological and forensic importance. Often the unavailability of intact long bones poses for difficulty to estimate the stature. So reconstruction of the total femoral length from its different fragments by applying desired statistical methods can be used as a very important tool for it. The present cross-sectional study was designed aiming for a specific formula-based model in Eastern Indian population. Fully ossified, intact, dried 60 femora of Department of F.S.M., Burdwan Medical College were measured using Hepburn's Osteometric Board and sliding digital. All the measurements were performed by multiple examiners to exclude observer biasness and the mean value was recorded. Linear regression analysis was performed using maximum Femur length as the dependent variable and the different fragments as predictors to form a linear relationship with help of SPSS Statistical software. P value of <0.05 was considered significant. Among all the measured data, the Epicondylar Breadth, Midshaft antero-posterior Diameter and Transverse Head Diameter were found as statistically reliable predictors for determination of the maximum Femoral length. This finding is thus helpful in practical scenario when either the upper end or lower end of the femur is destroyed.

***Key Words**: Stature, Identification, Eastern-Indian, Correlation, Femoral Length, Femoral fragments.*

Introduction

Estimation of stature from bones, specially the long bones has immense anthropological and forensic importance. In practical forensic application, often intact bones are not available due to mutilation or post mortem predation by animals. Therefore, reconstruction of total length of femur from its bony fragments, by applying the desired statistical methods, is necessary for building a biological profile. It has been established that femur along with tibia provide reconcilable results. Different fragmentary lengths are widely used for deriving the regression equation for estimation of Femoral maximum length which in turn can be used as a very important tool for estimating the stature.

Pearson[1] and Trotter and Glesser[2] were two pioneers of this type of anthropometric studies. Later, their researches induced several important studies like researches on Turkish population by Pelin[3] and South Africans of European descent by Chibba [4]. Bidmos [5] in 2008 used following variables measured on each femur—i) vertical neck diameter, ii) upper breadth of femur, iii) epicondylar breadth, iv) bicondylar breadth, v) lateral condyle length, and vi) medial condyle length. Stature estimation was done by using regression formulae. The said study advocated that in the absence of intact femur, regression equations could provide a reliable estimate of adult stature. Research works from India by Pan[6] on Hindus of Bengal, Bihar and Orissa not only invoked research work in India, but also it drew attention beyond Indian sub-continent. In 1999,Shrof et

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al[7] conducted a research work in India and they went for calculation of the percentile length of different segments and compared those to the total length of femur to get some regression equations to estimate total length of femur from its segments and the regression coefficient found highly significant in each case. The length of femur was estimated by the equation was almost accurate with the error of ±1 cm.

In recent past, Parmar Ajay M et al[8] worked on 50 femur bones and found correlation between maximum length of femur and its proximal & distal fragments and formulated regression equation for estimation of stature from maximum femoral length in Udaipur, Rajasthan. R Umeshbabu et al[9] in their study included 50 male femurs and 50 female femurs of South Indian origin dissected from cadavers. Linear regression equations for various morphometric parameters of proximal and distal end of femur was derived to estimate length of femur and subsequently to estimate the stature of an individual.

Pavel Timonov & Antoaneta Fusova[10] conducted a study in which they introduced a method of estimating the length of femur from partial proximal elements specific to the Bulgarian population. 140 adult femora of known age and sex were studied. In that study measuring of different variables such as vertical head diameter, transverse diameter of the head, maximum head diameter, head circumference, sagittal subtrochanteric diameter, transverse subtrochanteric diameter and supero-inferior neck diameter had been done to obtain simple and multiple linear regressions. For males, regression formula, which included head circumference measurement, yielded the best result of the data, resulting in the highest correlation and lowest standard error of estimate compared with all other variables. The vertical diameter of the head of the femora of female sex showed the better correlation with maximum length. When all the variables combined into a single equation, it was found to improve the standard error of the estimate. The regression formula revealed that the femur length can be best estimated by proximal end. The derived formulae were population and sex specific.

In a research study conducted by Shweta Solan & Roopa Kulkarni [11] over south Indian populations, total number of 150, 72 left and 78 right adult fully ossified dry and processed femora were taken. After dividing each femur bone into five segments by taking predetermined points, the segmental length along with maximum length of femur were measured in cm [mean±S.D.] and the mean total length of femora of both sides were also measured. The proportion of segments to the total length were also calculated for the stature estimation using standard regression formulae. When comparison was made between segments of right and left femora, the 'p' value of points between (i) where linea-aspera divides into medial and lateral supracondylar lines and (ii) most proximal point on the intercondylar fossa was found to be ‹0.001.

In a previous study from the same State of the current study, done by Mukhopadhyay et al [12] a correlation was made between the epicondylar breadth and the maximum femoral length. In this study the total number of samples were 65. Regression equation had been applied considering the epicondylar breadth as the independent variable and the maximum length of femur as dependant variable. Eventually the result of the regression equation had been combined with the earlier population specific method of estimation of stature to formulate a new formula for stature estimation. The method of Pan for the estimation of stature from male femur was applied to the equation from that study. Multiplying factor of 3.82 was used after suitable conversion of the units (from cm to feet) of measurement to get the stature in feet from the maximum length of femur.

The present study was designed to work out a specific regression model in Eastern Indian population as regional and racial factors are found to influence the morphometrical and morphological profile of any bone.

Materials and Methods

All the 60 femur bones (fully ossified, intact, dried and devoid of any soft tissue attachments) were taken from Department of F.S.M, Burdwan Medical College, a tertiary Medical Teaching Institution of West Bengal (Eastern India). Measurements were taken using Hepburn's Osteometric Board and sliding digital caliper with a precision of 0.01mm. with an aim for estimating the total length of femur by utilizing measurements of 10 femoral fragments.

At first, all the measurements were performed by one examiner. T repeat measurement was taken by

the same examiner after seven days. The two readings were then tested by paired sample t test to exclude intra-observer bias. Similarly the other two observers recorded and tested their measurements and finally the mean of the three set of readings were included in the study. Inter-rater agreement was tested with twenty five random samples by performing Cohens Kappa.

All the measurements were corrected to 2 decimal places and were recorded in cms (centimeters). Linear regression analysis was performed using Femur maximum length as the dependent variable and the fragments as predictors separately and in combination. Equations in the form of "y=mx +c" was obtained for the variables. The measurements obtained were analysed using SPSS Statistical software for windows version 10.0. The mean and S.D. of all the variables were calculated and a standard equation was derived for Eastern Indian subjects. P value of <0.05 was considered significant.

It was a Cross-sectional study where following study tools were used -measuring tape, flexible tape, dusting brush, Hepburn's Osteometric Board, Electronic Digital Caliper, pencil, OHP Marker, standard prepared master charts for Data recording.

Inclusion criteria- fully ossified, dried, processed femur bones (60 in number), irrespective of age, sex and race. **Exclusion criteria-** Unossified bones, bones with disease, injury or fragmentation.

The Femur maximum length was the linear distance measured between the most superior point on head of femur up to most inferior point on its condyles.

The femur bones were divided into 10 fragments basing upon the respective anatomical and morphological landmarks.



i.Epicondylar breadth(EB)-The linear distance measured between medial border of medial condyle and lateral border of lateral condyle.

ii. Vertical Neck diameter (VND)-the minimum diameter of the neck measured in supero-inferior direction.

iii. Transverse Neck diameter (TND)-the minimum diameter of the neck measured in AP direction.

iv. Neck Length (NL)-the distance measured between base of the head and intertrochanteric line at the junction of posterior part of neck with the shaft.

v. Vertical Head diameter (VHD)-the maximum vertical diameter of the head measured between its highest and lowest points.(**Fig: 1)**

vi. Transverse Head diameter (THD)-the maximum diameter of the head measured mediolaterally, perpendicular to the VHD.

Vii. Sub trochanteric transverse Diameter (STD):- The distance measured between medial and lateral surface at the proximal end of diaphysis at the point of its greatest lateral expansion, just below the lesser trochanter.

viii. Subtrochanteric AP diameter(SAPD)-the distance between anterior and posterior surface at the proximal end of diaphysis at the point of its greatest lateral expansion, measured perpendicular to STD.

ix. Mid-shaft AP diameter (MAPD)-the AP diameter measured at the midpoint of the diaphysis at the point of its highest elevation of linea aspera.

x. Mid-shaft transverse diameter (MTD)-measurement of the transverse diameter of the shaft which had been taken at right angles to MAPD.

Results

The fragments of femur in this study (i.e the variables) were divided into two groups .Those, that were proximal included the Head, Neck and Subtrochanteric measurements. The distal fragments consisted of MTD, MAPD and EB. Stepwise regression analysis was carried out with femoral length as the dependent variable and the two groups of fragments as independent variables. By stepwise adding and removing variables it was seen that for the proximal part only **THD** was the fragment that provided the best model that fit the data with R square of 0 .67. Similarly, for the lower fragments **EB** and **MAPD** produced the model with R square value of 0.75.

TABLE-1 & 2

MODEL SUMMARY OF REGRESSION WITH PROXIMAL FRAGMENTS				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	.824	.679	.674	1.54297

Coefficientsa						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	16.535	2.221		7.444	.000
	THD (CM)	6.022	.543	.824	11.083	.000

a. Predictors: (Constant), THD (CM

a. Dependent Variable: FML(cm)

Regression Equation with proximal fragment

FEMORAL LENGTH= 16.53+6.02THD

TABLE 3 & 4

MODEL SUMMARY OF REGRESSION WITH DISTAL FRAGMENTS				
Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
2	.867b	.752	.743	1.36937

a. Predictors: (Constant), EB in cm

b. Predictors: (Constant), EB in cm, MAPD CM

Coefficientsa						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
2	(Constant)	9.834	2.454		4.008	.000
	EB in cm	4.352	.335	.856	12.974	.000
	MAPD CM	-.156	.070	-.146	-2.220	.030

a. Dependent Variable: FML(cm)

Regression equation with distal fragments.

FEMORAL LENGTH=9.83+ 4.35 EB- 0.15 MAPD

TABLE -5

Descriptive Statistics Of Different Femoral Fragments					
	N	Minimum	Maximum	Mean	Std. Deviation
FML(CM)	60	34.70	46.10	41.0533	2.70131
VHD (CM)	60	3.34	4.77	4.0943	.34793
THD (CM)	60	3.30	4.79	4.0712	.36968
VND(CM)	60	2.33	3.58	2.8292	.30130
TND (CM)	60	1.83	3.12	2.4150	.30582
NL (CM)	60	2.09	3.69	2.7406	.34534
STD (CM)	60	1.90	3.40	2.8518	.32030
SAPD (CM)	60	1.90	3.18	2.3962	.32225
MAPD (CM)	60	1.90	22.09	2.9056	2.53717
MTD (CM)	60	2.00	2.79	2.3655	.19201
EB (CM)	60	6.29	8.51	7.2777	.53153

Discussions

In the present series separate regression equations were derived for the proximal and distal portion of femur to reconstruct the total femoral length from its fragments. It was seen that for the proximal end the best predictor was THD with R square of .67. This is consistent with earlier works [10,11] where the proximal fragments were useful in estimating total femoral length.

Similarly the multiple linear regression equation for the lower fragments showed consistent results with R square of 0.75. This is in agreement with previous published works [8,9,12]. The values of the various fragments are also comparable with other works on Indian population [5,7,12]

In the present study with 60 samples, the Femoral Length was considered as Dependent Variable. Correlation study was done between different segments of femur and their relationship with the FML. Among all the measured data, it was found that EB, MAPD and THD served as reliable predictors for determination of

the FML from proximal and distal parts respectively. The other 7 fragmentary lengths did not reveal statistically significant results and hence discarded for the purpose of stature determination from the femur. Few previous works have however worked out regression with similar variables.

One lacunae of the present study is that the sample size is very small, consisting of only 60 femora. The present study may be considered as a pilot project aiming to obtain a regression equation for estimating maximum length of femur from different segmental parameters of femur human. As the study is region specific, it may be applied in cases associated with the local problem of identification of human remains when bony fragments or grossly mutilated bodies are encountered in medicolegal autopsies.

Conclusions

This study highlighted that a definite and reliable regression equation can be formulated for Eastern Indian population. This will be helpful for determination of Femur Maximum Length and subsequently skeletal stature from the above noted fragmentary remains among subjects belonging to the Eastern Indian population. Another notable utility of this study is that in the given population the total femoral length can be estimated by separate equations for proximal and distal fragments. This is important and will be helpful in solving practical cases when either the upper end or lower end of the femur is destroyed by animal predators or injury.

Ethical Clearance :- Taken from Institutional Ethics Committee (IEC) of Burdwan Medical College, Purba Bardhaman.

Source of Funding :- NIL

Conflict of Interest :- Nil

Declaration

A shortened version of this study was presented by First Author as oral presentation in Forensic Medicon-2019 at Jodhpur, India , 40th Annual National Conference Of the Indian Academy of Forensic Medicine.

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Epidemiological Study of Drowning Deaths in Madurai Region

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Abstract

Background: World Health Organization described drowning as serious and neglected public health problem. Nearly about 42 people every hour and 3.7 lakh people every year die due to drowning in the world.

Method: The present study was undertaken at Government Medical College and Hospital located in Madurai on dead bodies brought for postmortem. The study was carried out during the period of January 2017 to December 2018 on 80 cases to study the various epidemiological parameters of drowning.

Results and Conclusions: We have concluded that 75% victims were males and 25% were females. Drowning deaths were commonly seen in age groups of 11 to 20 years (25%). Maximum no of drowning deaths had occurred in urban region constituting 52% followed by rural region of 48%. Study also revealed majority of the victims were students constituting males 67% and females 33%. Number of victims of drowning deaths has familial and financial problems in males 78% and females 22%, depression seen in males 71% and females 29%. Number of drowning deaths occurred in September 16.5%. Most of the drowned victims were retrieved from river 59%, Wells 25%, Pond 29%.

Keywords*: Drowning deaths, site of drowning, seasonal variation, regional variation, occupation status, personal history.*

Introduction

The well-known fact saying"water is life" reveals a fact that there is no existence without water. Water is essential for life but at the same time it can also take your life. In addition to life sustaining role,water acts as one of the most destructive forces on earth in the form of tsunami and floods which becomes the cause of loss of life of many people.So water could be said as "double edged sword".WHO has adopted new definition on drowning in 2002 as "Drowning is the process of experiencing respiratory impairment from submersion or immersion of liquid". WHO described drowning is one of the top 5 causes of death in children between 1 and 14 years and one of the ten leading causes of death between 1 and 24 years.As per the National Crime Records Bureau-Accidental Deaths and Suicides (2012), eighty persons die of drowning each day in India, which accounts for 7.4% of all unnatural deaths.

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Materials and Methods

The above study was done at GovernmentRajajiHospital,Madurai Medical college, Madurai,where nearly 4000 medico-legal autopsies are carried out each year.We have done a study on epidemiological profile of the drowning deaths. The study was carried out over the period of two years that is from January 2017 to December 2018.In this period we received 80 cases excluding bodies in advanced state of decomposition. The study includes,only the dead bodies which were retrieved from water resources,having history of drowning, brought for post mortem examination. Detailed history and relevant information was collected from police inquest and requisition regarding the age , sex, marital status, occupation, religion, etc.

Results

In our study from the period January 2017 to

December 2018 we received 80 cases.75 % of victims were males and 25 % were females.Drowning deaths were most commonly seen in the age groups of11 to 20 years[25 %].Maximum number of drowning deaths had occurred in urban region constituting 52% followed by rural region constituting 48%cases.Study also revealed that majority of the victims was students constituting males 67% and females 33%. Number of victims of drowningdeaths has familial and financial problems in males 78% and Females 22% and Depression seen in males 71% and females 29%.Number of drowning deaths occurred in the month of September 16.5 % .Most of the drowned victims was retrieved from River59%, wells 25 %, Pond9%.

Table No - 1

Sex	Number of cases	%
Male	60	75%
Female	20	25%
Total	**80**	**100%**

Table No -2

Age Wise Distribution

Age	Number of cases	%
0/10	17	21%
11-20	20	25%
21-30	10	13%
31-40	9	11%
41-50	10	13%
51-60	4	5%
61-70	4	5%
More than 70	6	7%
Total	**80**	**100%**

Table No – 3: Place of drowning

Place	Number of cases	%
River	47	59%
Well	20	25%
Pond	7	9%
Water pit	5	6%
Water tank	1	1%
Total	**80**	**100%**

Table No- 4

	Male	Female	Male%	Female %
Student	16	8	67%	33%
Farmer	15	0	100%	0%
Labourer	20	5	80%	20%
Business	7	0	100%	0%
Unknown	2	5	29%	71%
House wife	0	2	0%	100%
Total	60	20		

Table No – 5

Month wise Distribution

Month	Number of cases	%
Jan	3	3%
Feb	4	5%
Mar	9	11%
Apr	6	8%
May	3	4%
Jun	3	4%
Jul	6	8%
Aug	7	9%
Sep	13	16%
Oct	8	10%
Nov	9	11%
Dec	9	11%
Total	**80**	**100%**

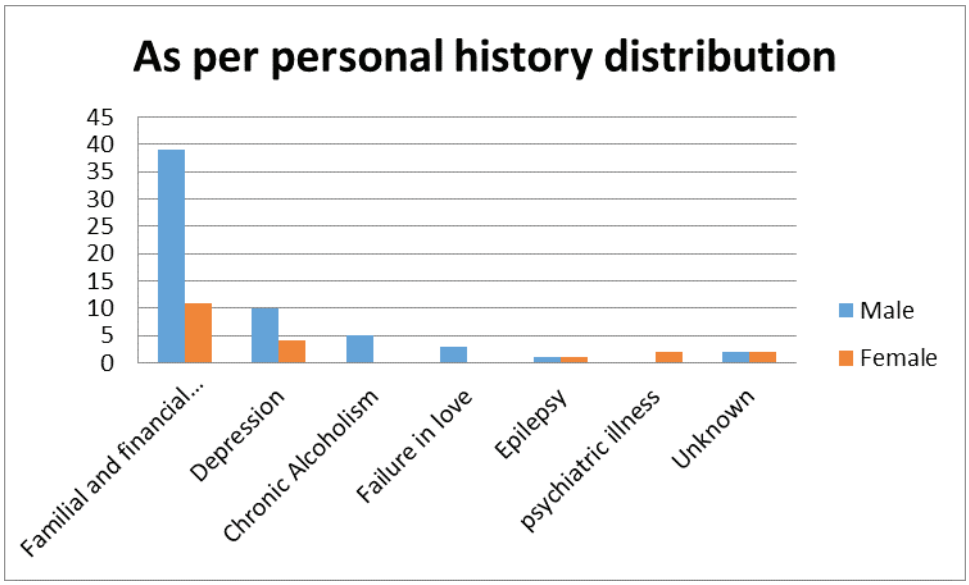


Chart No 1

Discussion

The drowning deaths were predominantly seen in male amounting to 75 % involving the age groups of 11 to 20 years followed by 0 to 10 years. These findings are consistent with that of Auer[1990][(1)],Quan[2003] [(2)],Suresh Kumar Shetty and Shetty [2007][(3)], Pathak and Mangal[2009][(4)], and SaberiAnary et al [2010][(5)]. Probable reason for drowning in 11 to 20 years is due to adventurous nature, intoxication, carelessness seen in youngsters while swimming in water. Drowning in 30 to 40 years due to familial and financial problems arising in life and their inability to deal with them. Maximum number of drowning deaths occurs in urban region constituting 52%, followed by rural region about 48%. These findings are in contrast to study carried out by Delmont and Capelozzi [2001][(6)] and Murkey et al[(7)] due to region of study.

In drowning deaths males 78% and females 22% had financial and familial problemsand depression was seen in males 71% and female 29%. The present study in accordance with Dietz and Baker [1974][(8)],Auer[1990] [(1)],Fralick et al [2013][(9)].

The maximum number of deaths occurs in September month [16.5 %].this is followed by November and December month as 11.25 %, followed by March 11.5 %. This finding is consistent with Pathak andMangal[2009] [(4)],Job[2009] [(10)],Ambade de et al[2013] [(11)] studies state maximum death in rainy season.

Most of the drowning victims were retrieved from river [58.75 %],wells[25 %],pond[8.75 %] this is in contrast with fralick et al[2013][(9)] and RangaRao et al[2014][(12)]according to the place of drowning.

Summary and Conclusion

In the above study we conclude that males show predominance to females, in drowning deaths with highest incidence seen in age group of 11 to 20 years. Drowning deaths are common in urban region followed by rural region. The study concluded that students were the prey to drowning as a result of their adventurous carefree nature and various forms of intoxication. Familial and financial problems are the most common history in drowning deaths followed by depression. Drowning deaths are most common in rainy season compared to winter season, followed by summer season. River was found to be the most common site of drowning followed by wells and ponds.

Ethical Clearance: Not needed as it is a retrospective study.

Source of Funding: Self

Conflict of Interest: Nil

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Determination of Sex and Estimation of Age by Mandibular Basal Bone Height

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Abstract

Aims and Objectives: To assess the age and sex determination through mandibular basal bone height

Materials and Methods: The study consists of 120 OPG samples, (60 males and 60 females) in the age group of 30 to 70 years, hereby the mandibular basal bone height measured as D1 and D2 on OPG's using planmeca romexis software. The distance measured between the lower border of mental foramen to the lower border of the mandible termed as D1. The distance between the lowest point of mandibular canal to the lower border of the mandible termed as D2. All these measurements were further subjected to statistical analysis like independent t-test, correlation and regression analysis to estimate age and to compare the differentiation between males and females.

Results: Overall the result shows the moderate level of significance, on multiple comparisons between age groups and sex among the population, it was observed that statistically **significant in male with relation to D1 among 61yrs to 70yrs** though other age groups show significant with less confidence interval. Female observations failed to show similar results neither with D1 nor with D2. Regression formula for age estimation and logistic regression for sex determination have been derived.

Conclusions: The present study highlighted that the male gender of the mandible can be determined by using D1 value as it gives significance, thus it can be used as an additional tool to establish the identity of a gender

Key words: *Mandibular basal bone height, mandibular canal, mental foramen orthopantomogram radiograph,*

Introduction

The mandible is a horseshoe shaped bone and the body of the mandible on either side has the basal bone component and the mandibular arch embedding teeth. Morphological changes of the mandible are thought to be influenced by the occlusal status and age [1]

The adult human mandible is a bone which exhibits a large degree of anatomical variability. This variation occurs not only between subjects or as a result of aging, but also between the right and left sides in an individual [2]

During facial growth, the maxilla and mandible translate downward and forward. Although the forward displacement of the maxilla is less than that of the mandible, the inter-arch relationship of the teeth in the sagittal view during growth remains essentially unchanged [2]. Inter-digitation is thought to provide compensatory (tooth movement) mechanism for maintaining the pattern of occlusion during growth; the maxillary teeth move anteriorly relative to the maxillary basal bone while the mandibular teeth move posteriorly relative to the basal bone of the mandible (Marshall et al., 2011).

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After growth has ceased, the single most important factor governing the gross morphological shape of the bone is related to the presence or absence of the teeth. After tooth extraction, there follows a phase of remodelling which may result in an extensive loss in the height of the jaws, particularly the mandible [3].

Mandibular body is divided into alveolar bone and basal bone. However, there is no distinct line separating basal bone from alveolar bone tissue. This is more of a physiologic than an anatomic difference [4]. Basal bone forms the dental skeletal structure, contains most of the muscle attachments, and begins to form in the foetus before the teeth develop. Classically, the mandibular basal bone is defined as that part of the mandible which is present inferior to the mandibular canal [5]. The mandibular canal and the mental foramen are the standard anatomic reference landmarks used to differentiate between the alveolar bone and the basal bone in the mandible [6].

Information is available that the aging skeleton retains a potential for growth. There is considerable evidence that continuous bone deposition takes place from adulthood to older-age and such changes have been reported on the skull and face [7]. There exists some amount of bone deposition along the lower border of the mandible as the age progresses [6]. Which contribute to increase in the height of mandibular basal bone. On the contrary, it is also proved that alveolar ridge resorption in edentulous mandibles, can extend into the base of the mandible [8], leading to decrease in the height of mandibular basal bone [9].

Panoramic radiography is the most commonly used tool in measuring the mandibular basal bone height [6, 10]. Various methods of gender determination have been established, in which the mandible plays an important role. The mandible is the strongest bone in the human body and persists in a well-preserved state longer than any other bone. Therefore, the use of morphological features of the mandible is a common approach used by anthropologists and forensic dentists in the determination of sex [11].

Aim and Objectives of the study:

1. To measure the mandibular basal bone height on OPG's

2. To compare the above measurements for age estimation and gender determination

Materials and Methods

This study consists of 120 Digital orthopantomogram radiographs, the samples were collected from the archive of Department of Oral Medicine and Radiology, JSS Dental College and Hospital, JSS Academy of Higher Education and research, Mysore, Karnataka, India. Which were taken with PROMAX digital Planmeca Machine, a total of 120 samples (60 males and 60 females) divided into 4 groups, each group consisted of 30 samples, with an equal number of gender distribution.

Inclusion criteria: panoramic radiographs (OPG) with proper diagnostic property, Radiographs with properly visible mental foramen and lower border of mandibular canal

Exclusion criteria: Presence of any pathological lesions or deformities in the mandibular region

The methodology of recording the distances (D1 andD2) followed in this study was implemented from **Xie *et al***. (1996) [6] **[Figure 1**]. A tangential line was drawn to the lower border of the mandible, on the left side and the Vertical lines were drawn from the lowest point of the mental foramen and the lowest point of the mandibular canal to the lower border of the mandible The distance measured between the lower border of mental foramen to the lower border of the mandible was termed as D1.The distance between the lowest point of mandibular canal to the lower border of the mandible was termed as D2. Similar measurements were carried out for all the radiographs in planmeca romexis viewer 2.9.2.R software, the measured values were compared and statistically analysed.

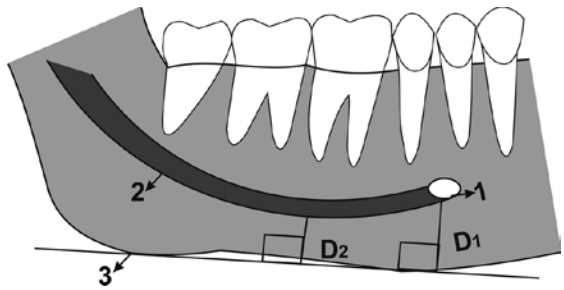


Figure 1: D1 = Distance between the lower border of the mental foramen and the lower border of the mandible, D2 = distance between the lowermost point

of the inferior alveolar canal and the lower border of the mandible, 1 = mental foramen, 2 = lower border of the inferior alveolar canal, 3 = tangential line drawn to the lower border of the mandible.



Figure 2: Measurements by planmeca romexis software

D1 and D2 measurements done on OPG in planmeca software

Results

In the present study, mandibular right side measurements were taken on OPG as D1 and D2 for all 120 samples. The statistical results are shown in the Tables 1 to 6. Further the difference of D1 and D2 values between males and females were noted and compared with other relevant studies.

In the present study, Parenthesis shows mean value,

There is significant difference between male and female in case of D1, showing the p value (0.016), There is significant difference in Male D2 as the p value shows (0.053)

Table: 1

Correlations					
SEX			**AGE**	**D1**	**D2**
MALE	AGE	Pearson Correlation	1	-.154	.361**
		Sig. (2-tailed)		.239	.005
		N	60	60	60
	D1	Pearson Correlation	-.154	1	.211
		Sig. (2-tailed)	.239		.106
		N	60	60	60
	D2	Pearson Correlation	.361**	.211	1
		Sig. (2-tailed)	.005	.106	
		N	60	60	60
FEMALE	AGE	Pearson Correlation	1	.341**	.020
		Sig. (2-tailed)		.008	.878
		N	60	60	60
	D1	Pearson Correlation	.341**	1	.300*
		Sig. (2-tailed)	.008		.020
		N	60	60	60
	D2	Pearson Correlation	.020	.300*	1
		Sig. (2-tailed)	.878	.020	
		N	60	60	60

**. Correlation is significant at the 0.01 level (2-tailed)., *. Correlation is significant at the 0.05 level (2-tailed).

Observations:

(1) In case of Males there is positive correlation(r= 0.361 with p=0.005< 0.05) between age and D2

(2) In case of Females there is positive correlation (r=0.341 with p=0.008<0.05) between age and D1

(3) In case of Females there is positive correlation (r =0.300 with p=0.02<0.05) between D1 and D2.

Among **various age groups** ranging from 30ys to 70yrs of age it was observed that overall mean value of D2 (8.368) is found to be lesser than D1(14.285), suggesting **D1 has more predominant role in determining mandibular basal bone height**. On Inter observation among the age group, statistically significant was seen in higher with D1 and age groups of 61yrs to 70yrs. On correlation analysis between the age group and D1 it was found statistically significant (Pearson correlation <0.05) whereas **No significant correlation was observed with actual age and D2**. Independent t-Test according to different age groups shows statistical significance of 0.004 (<0.05) among age groups of **61yrs to 70yrs with D1 suggesting its reliability (Table: 1).**

Among the **gender population** of various age groups it was observed that overall mean value of **D1 is higher than D2 in both male and female** showing its correlation observation. With respect to gender between actual age and **D1 it was observed male** shows significant relation with respect to age groups and D1 (0.083) than females, similarly in relation to D2 (0.029) males in **the tests for normality,** but female observation failed to show any significant correlation statistically.

Regression Analysis- stepwise

Table: 2: SEX = MALE

Coefficientsa,b						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	33.061	6.132		5.392	.000
	D2	2.065	.699	.361	2.953	.005
a. SEX = MALE						
b. Dependent Variable: AGE						

Using stepwise regression of Age on D1 and D2 we found in case of **Males**

Male Formula, Age=33.061 +2.065*D2.

This implies that Given D2 value one can predict their age (Table: 2). The P value for significance of the regression equation is 0.005< 0.05, which is significant and R^2 =0.131 (It is better if it is nearer to 1), Std. Error of the Estimate is 10.8 yrs.

Table: 3

SEX = FEMALE

Coefficientsa,b						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	24.006	9.449		2.541	.014
	D1	1.836	.665	.341	2.761	.008
a. SEX = FEMALE						
b. Dependent Variable: AGE						

Using stepwise regression of Age on D1 and D2 we found in case of **Females**

Female Formula, Age=24.006 +1.836*D1.

This implies that Given D1 value one can predict their age (Table: 3). The P value for significance of the regression equation is 0.008< 0.05, which is significant, Std. Error of the Estimate is 10.73950. Thus the age can be calculated from these regression formula according to the gender.

Table: 4: Logistic Regression to predict Sex

Variables in the Equation							
		B	**S.E.**	**Wald**	**df**	**Sig.**	**Exp(B)**
Step 1a	D1	-.087	.091	.914	1	.339	.917
	D2	-.089	.112	.629	1	.428	.915
	Constant	1.985	1.421	1.950	1	.163	7.277
a. Variable(s) entered on step 1: D1, D2.							

The formula is based on above table:

The Logistic equation to predict SEX based on D1 and D2 is given by

Log [p / (1-p)] = 1.985+ (0.087*D1- 0.089*D2).

Where p is the probability of being Male. i.e.,if P<=0.05 predict it as a male otherwise as female (Table: 4). The accuracy of predicting the male correctly is 51.7% and female as53.3% and overall percentage is 52.5.

Discussion

The mandible is the strongest bone in the human body and persists in a well-preserved state longer than any other bone. Therefore, mandibular characteristics are extremely useful or determining sex [14].On comparing the results, males had higher D1 values, **D1 has more predominant role in determining mandibular basal bone height**. On Inter observation among the age group, statistically significant was seen in higher with D1 and age

groups of 61yrs to 70yrs in males, this was similar with Xie *et al*., (1996), [6] who had achieved similar results. This can be explained on the basis of fact that sexual hormones such as androgens and oestrogen, contribute to the development of a morphologic difference in craniofacial skeletons between the genders and even to different velocity of growth in later adulthood. The speed of bone growth in adult women is lower than in mean, as observed by Enlow (1982),[4] who further stated that local factors such as masticatory muscles and bite force play an important role in craniofacial skeletal change. Tension is known to induce bone formation. Since the mandible functions as lever, contraction of jaw elevator muscles such as masseter and medial pterygoid muscle during normal chewing movements may cause a small amount of tension along lower border of mandible in the molar regions [4].

In general, men have stronger muscles and greater bite force than women, so more amount of bone deposition along the lower border of the mandible was observed in men compared to women. The sexual hormones and various strengths of force of masticatory muscles together explained the difference in height of mandibular basal bone between the genders. Further, women in postmenopausal age had accelerated rate of resorption of residual edentulous ridge due to osteoporosis, which was supported by von Wovern *et al*., (1980) [13]. In the present study both 30-40 years and 61-70 years of age group shows statistically significant mainly in the male genders, others are not significant but in contrast to Raviraj jayam study the age group of 41-50 years shows the significance.

Arati S. Panchbhai et al. (2013) conducted a study on Quantitative estimation of vertical heights of maxillary and mandibular jawbones in elderly dentate and edentulous subjects which stated that there were significant differences between elderly dentate and edentulous subjects in maxilla and mandible. The vertical measurements were significantly greater in men than in women in maxilla and the mandible. [15]

Conclusion

Human skeletal remains plays an important role in Forensic odontology and Medico-legal works to identify the individual, sometimes if a part of bone is also available, sex or age can be determined based upon different morphological and metrical parameters and this study can be applied for both living and deceased. The present study highlighted that the **male gender** of the mandible can be determined using **D1 value as it gives significance**, thus it can be used as an additional tool to establish the identity of a person, regression formulae for age estimation for male and female and logistic regression formula for sex determination have been done. Studies with larger samples may help to correlate gender determination using metric parameters.

Ethical Clearance- Taken from the JSSDCH, JSSAHER Ethical committee

Source of Funding- Self

Conflict of Interest - Nil

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A Study on the Aspiration Procedures in Syringes among Health Care Professionals

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Abstract

Aspiration during routine injection is meant to ensure that the injecting needle tip is at the anticipated location during this unsighted procedure. While injecting needle appears to be a simple procedure, it has produced a lot of controversy regarding the apparent benefits and indications. Keeping in view the huge number of injections given worldwide, it is observed that key questions regarding aspiration till now, remain unanswered. Due to very smaller number of studies and data in the literature there is no evidence of particular injecting procedure as truly beneficial or unjustified. The present integrative review was chosen to draw conclusions about the current state of knowledge and practice of aspiration and use of syringes among medical, paramedical and dental practitioners. On analyzing the questionnaire output data's, it was observed that most sound knowledge practitioners found difficulty in usage of syringes and forget to aspirate due to nervousness during injection resulting in positive aspiration ($p<0.05$) during the procedure. Henceforth a new invention in the syringe for safer and easier use or a standard injecting technique with aspiration protocol have to be established to avoid complications due to difficulty in usage of syringes during minor or major surgical procedures.

Keywords: *Syringes, Aspiration procedure, Injection, Injection technique.*

Introduction

A safe injection is one that does not harm the recipient, does not expose the provider to any avoidable risks and does not result in surplus that is dangerous for the community. Apart from these in the recent years more focus have been shifted towards the practice of aspirating for blood before injection.[1]

Injections are among the most common health care procedures, with at least 16 billion administered in developed as well as developing countries every year. 95% of injections are administered in therapeutic care, 3% are for immunization, and the rest for other purposes, such as blood transfusions. The practice of aspirating for blood before an injection has been discussed in medical journals since the early 1900s and is a tradition taught in paramedical curriculum for the past 40 years. Aspiration was performed as a protective or precautionary step by general physicians before injection to rule out intra-arterial placement of the needle tip. This precautionary technique ensures that an artery or vein hasn't been penetrated inadvertently since embolism due to accidental intra-arterial injection can lead to serious complications. Injection of either needle or the solution into the vessels may occur accidentally in all intraoral injection techniques; nonetheless, when injecting into a highly vascular area as seen in case of pterygomandibular space during inferior alveolar nerve block injection, there is always the increased risk of an intravascular injection, vascular damage and hemorrhage with hematoma formation.[2, 3]

In clinical practice of dentistry carrying out aspiration prior to any anesthesia, is necessary to prevent inadvertent intravascular injection and also to reduce the incidence of adverse reactions attributed to the overdose. For Dental students, it is very necessary to understand and prevent the complications. Local anesthetics are medicaments that induce a transitory and completely reversible loss of sensation in a confined area of the body, caused by a depression of excitation in nerve endings or an inhibition of the peripheral nerve conduction. Local

anesthetic unit contains a vasoconstrictor in addition to the local anesthetic agent. High dose or accidental intravascular injection of local anesthetic agent with vasoconstrictor may result in cardiovascular and central nervous system toxicity, as well as tachycardia and hypertension.[4, 5]

Literature and integrative reviews have specified that performing aspirating before injection has no basis in the scientific evidence. Conversely Accidental injection into the vessels may occur in all intra-oral injection techniques. The dentist often experiences the increased risk of injecting the vessels, vascular damage and hemorrhage with hematoma formation. Therefore, aspiration is necessary to avoid intravascular injection.[6]

For decades, aspiration has been added and eliminated based on narrative, hypothesis, and subjective choice. Currently, the American Academy of Pediatrics, The American Academy of Family Physicians (AAFP), the Advisory Committee on Immunizations Practices (ACIP), the United Kingdom's Department of Health, and the World Health Organization (WHO) have stated that aspiration is not necessary and serves only to prolong the injection procedure. A single use sterile syringe intended for the aspiration of fluids or for the injection of fluids (ISO 7886-1) is recommended currently for aspiration procedure apart from the routinely used disposable or auto disables syringes.[7]

In the present study an integrative review followed by questionnaire was chosen to draw conclusion about the current state of knowledge among diverse study population in the area of medical, paramedical and dentistry. Further comprehensive evidence review was obtained by combining diverse methodologies and response outcomes of targeted clinical question using a systematic search strategy and rigorous appraisal methods.

Materials and Methods

A total of 120 practitioners including 40 medical, 40 paramedical and 40 dentists were randomly chosen irrespective of their age and experience in their respective field. An integrative review in the previous literature followed by questionnaire containing 10 questions with multiple choice answers was selected. Each question was explained to individual participants and the data obtained were carefully updated and sent for statistical analysis. The 10 questions are answered with yes or no.

Q1. Have you ever experienced positive aspiration during injection?

Q2. Have you ever forgot to aspirate during injection?

Q3. Do you find difficulty while aspiration in the injection site?

Q4. Have you ever experienced needle breakage during injection?

Q5. Do you get nervous if there is positive aspiration during injection?

Q6. Will you change the needle if positive aspiration encountered?

Q7. Do you withdraw the air bubble in the syringe before giving injection?

Q8. Is your needle under tension during injection?

Q9. Method of aspiration during injection?

Q10.Are you ready to experience if there is any new invention in the syringe for easy use?

Result

The data obtained was tabulated and analyzed by SPSS statistics software Version 26.0 to obtained statistical report. Pearson Chi-Square test was performed to find the significance of the study. The level of significance was set at 0.05 as the p-value. Results of each question are tabulated below

TABLE 1: Quantitative analysis based on the above-mentioned questionnaire on syringe handling.

Q.NO	MEDICAL		PARAMEDICAL		DENTAL		TOTAL		P VALUE
	YES N (%)	NO N (%)	YES N (%)	NO N (%)	YES N (%)	NO N (%)	YES N (%)	NO N (%)	
Q1	20(50%)	20(50%)	24(60%)	16(40%)	29(72.5%)	11(27.5%)	73(60.8%)	47(39.2%)	0.1184
Q2	16(40%)	24(60%)	9(22.5%)	31(77.5%)	21(52.5%)	19(47.5%)	46(38.3%)	74(61.67%)	0.0214
Q3	18(45%)	22(55%)	24(60%)	16(40%)	22(55%)	18(45%)	64(53.3%)	56(46.6%)	0.3916
Q4	7(17.5%)	33(82.5%)	3(7.5%)	37(92.5%)	11(27.5%)	29(72.5%)	21(17.5%)	99(82.5%)	0.0562
Q5	6(15%)	34(85%)	8(20%)	32(80%)	20(50%)	20(50%)	34(28.33%)	86(71.67%)	0.00086
Q6	40(100%)	0(0%)	40(100%)	0(0%)	38(95%)	2(5%)	118(98.3%)	2(1.7%)	0.7721
Q7	40(100%)	0(0%)	40(100%)	0(0%)	40(100%)	0(0%)	120(100%)	0(0%)	NIL
Q8	11(27.5%)	29(72.5%)	11(27.5%)	29(72.5%)	6(15%)	34(85%)	28(23.34%)	92(76.67%)	0.3120
Q9	39(97.5%)	1(2.5%)	39(97.5%)	1(2.5%)	36(90%)	4(10%)	114(95%)	6(5%)	0.2061
Q10	39(97.5%)	1(2.5%)	35(87.5%)	5(12.5%)	33(82.5%)	7(17.5%)	107(89.5%)	13(10.9%)	0.0089

N= Number of responses(yes/no) for each question %= percentage of responses for each question p= statistical significance

The age wise distribution shows predominant of 20-25years of age among study population with equal participants 3-6yrs or more experience among them. All the 3groups were equally distributed to avoid calculating error. On analyzing the questionnaire output data's, it was observed that Q2 (0.0214), Q4 (0.0562), Q5 (0.00086) and Q10 (0.0089) were statistically significant and the remaining questions failed to show any significant correlation among the three groups under the study.

Discussion

However, greater concentrations (>1:50 000), or even the rapid intravascular injection of the cartridge of anesthetic solution, may have dangerous hemodynamic effects in patients with cardiovascular disease.[8, 9, 10]

Over the years various aspiration techniques for blood were followed. The most accepted being the rule of ten second that includes slow aspiration (5 to 10 seconds), slow injection (5 to 10 seconds) slow withdrawal, no rubbing. In this technique of routine injection involves the placement of the needle at its proper depth, aspirate, pulling the plunger of the syringe back slightly to determine whether the needle tip present within the lumen of a blood vessel. Followed by rotating the syringe a quarter turn and re-aspirate to ensure that

the needle tip was not laying against the wall of a vessel. If blood appears in the syringe at any time, withdraw the syringe from the tissue and prepare a different injection site.[11, 12]

Gammel et al in 1927 in his study concluded Blood not obtained upon aspiration isn't an absolute safeguard and strictly recommends careful injection technique. [13]

Peragallo-Dittko et al in 1995 conducted a Quasi-experimental cross-sectional study of adults with and without diabetes to investigate rationale for blood aspiration of the insulin syringe. 102 subjects with 204 injections aspirated yielded no blood return. He concluded that aspiration isn't a reliable indicator of correct needle placement. Individualize insulin injection technique to decrease the risk of incorrect needle placement.[14] In contrast to the above study the present study positive aspiration was observed among 15% medical, 20% paramedical and 20% dental practitioners.

Crawford et al verbally polled about 40 paramedical department staffs and found that about 50% of respondents supported the practice of aspirating for blood before injection. The final calculation of responses showed no correlation to their age, educational qualification, or other demographics included in the study. [15] However in the present study method of aspiration was not statistically significant. The responses did seem to illustrate that how they were trained to give injections during basic education strongly influenced their views. For those who'd been taught to aspirate supported the practice while those who weren't taught to do so continued to omit this step.

Kirk H. Waibel conducted a study among experienced paramedical staffs to determine whether the practice of aspiration should be continued before administration of either an allergy shot or immunization and observed the absence of blood in the syringe during aspiration of more than 36,000 allergy shots and routine immunizations. The study concluded that injection without aspiration is a safe practice.[16]

Roger and King et al in 2000 concluded that literature is consistent in recommending the following rule of aspirate for blood, draw back plunger for 5-10 seconds, if blood is present, discard and repeat.[17] Similarly in the present study no statistically significant observation was found when questioned about the method of aspiration but it was noted that all the 3 groups change the needle immediately when positive aspiration was found.

Diggle et al in 2007 observed the theory behind injection techniques to determine best practices in immunization and concluded that aspiration before injection isn't necessary.[18] Ipp et al in 2007 also observed most nurses who aspirate don't follow slow aspiration guidelines and they perform procedure too quickly.[19] In the present study 17.5% medical, 7.5% paramedical and higher range of about 27.5% experienced needle breakage at the tip during their injection procedure. In the present study a significant correlation was observed when asked about the aspiration guidelines. Most of the practitioners failed to aspirate unintentionally and get nervous during the procedure particularly when aspiration was positive had resulted in further complications.

Mohan et al in 2014 conducted a study among Interns and postgraduates who performed inferior alveolar nerve block injections using conventional technique in 250 patients undergoing minor oral surgical procedures. The study observed 20% of inferior alveolar nerve block injections were aspiration positive. It was noted that of all the observations 15.8% were on the right side and 14.8% were on the left side intravascularly. The author concluded that Aspiration of blood was significantly more common in patients aged 9–19 years than in all others irrespective of the side of injection.[20]

Trivedi et al in 2015 conducted a study on Direct Technique of the Inferior alveolar nerve block among interns posted in oral and maxillofacial surgery department. He observed that frequency of positive aspiration of the inferior alveolar nerve block by direct technique by the hands of interns was 13%. [21] The present study showed positive aspiration of 15% among medical, 20% in paramedical and higher range of about 50% in dental practitioners, this may be attributed to the fact that facial including oral and Para-oral structures are highly vascularized areas. It should be considered that about 80-90% of the study populations are prepared to experience any new invention in the syringe for easy use.

Conclusion

Very few studies confirming or rejecting current

aspiration techniques can contribute to a standardized procedure, regardless of healthcare setting. Current injection practices vary throughout healthcare organizations and practice environments. Until obtaining an established standardization, injection techniques must be individualized to the patient, the equipment, and the medication being administered to decrease the risk of incorrect needle placement. Though the present integrated review is limited to relatively narrow focus on administration of local anesthetic, routine medications and not focusing exclusively on immunizations, insulin or heparin injections it can be concluded that the need for aspiration prior to administering an injection is dependent upon multiple factors. Further studies looking at the need for aspiration during injection and introduction of newer invention in the syringe for easy use have to established to avoid difficulties in usage of syringes in order to avoid complications during the same.

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Conflict of Interest: Nil

Ethical Clearance

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A Study to Assess the Opinion of Students about Uses of Digital Education Technology during COVID-19 Lockdown in India

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Abstract

During the period of COVID-19 Lock-down the e-learning became very popular by dependency of both teachers as well as the learners to avoid interruptions in their academic progress. In a view to assess the effectiveness and evaluate the adaptation of the students towards e-learning, a survey was conducted among the nursing students in the form of feedback. The feedback was mainly focused on the student's interest, and the efforts taken by teachers in regard to class time and pace, organization and preparations of class, and fair assessment opportunities provided by teachers etc. The survey results were computed through Microsoft excel analysis based on the central tendencies, reveals that the Most of students "Agree", maximum have "Strongly Agree" and very few students have "Disagree" to the approaches and efforts of the teachers taken during e-learning. It was concluded from study that majority of the students had strongly agree that faculty organized and prepared well for every class and faculty were encouraged in discussions and responded to questions.

Keywords: *e-learning, COVID-19, Lock-down, student-feedback, Acceptance.*

Introduction

The ecosystem of the technology has widespread coverage in the field of education by means of e-learning. It is an access to educational curriculum outside of a traditional classroom with the help of electronic technologies, which is utilized for teaching and learning.

Online education or e-learning is uniquely suited to adult education as participants can access their lessons, communicate with the class or teacher, and collaborate with others wholly online.

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This is crucial due to the pandemic impact which significantly disrupted the higher education sector by affecting the process of board examinations, nursery school admissions, entrance tests of various universities and competitive examinations, which is a critical determinant of a country's economic future. The structure of schooling and learning, including teaching and assessment methodologies are the greatly affected by these closures. [2]

During the period of COVID-19 Lock-down the e-learning became very popular by dependency of both teachers as well as the learners, to avoid interruptions in their academic progress.

Learning is a lifelong process which is always considered deprived without the education, but the period of lockdown has created many challenges in the life cycle of human beings; impact of this can be reflected in rapidly adjustments and adaptations taken in the field of education.

There are many online teaching and learning platforms and learning management systems which were occasionally used as flipped classrooms, blended learning and a hybrid classroom, now-a-days used by the teachers with the aim to teach their students for better and effective learning. As the demand of e-learning is raised these days due to COVID-19 lockdown, the Government of J&K has promoted e-learning under "Samagra Shiksha'' to facilitate the students[3,4]

It is reported that learning online can be more effective in a number of ways. Some research shows that on average, students retain 25-60% more material when learning online compared to only 8-10% in a classroom. This is mostly due to the students being able to learn faster online; e-learning requires 40-60% less time to learn than in a traditional classroom setting because students can learn at their own pace, going back and re-reading, skipping, or accelerating through concepts as they choose.[5]

Teachers have adopted many pedagogical approaches to reach their students for better learning. Therefore, the feedback of the students on various e-learning approaches used by teachers is an essential to assess the effectiveness and evaluate the adaptation of the students towards e-learning.

This survey was conducted among the nursing students, in a view to assess the effectiveness and evaluate the adaptation in terms of student's feedback about e-learning.

Problem statement

A Study to assess the opinion of Students about Uses of Digital Education Technology during COVID-19 Lockdown in India

Objective of the Study

To assess the opinion of Students about Uses of Digital Education Technology during COVID-19 Lockdown in India.

Material and Method

A survey design was opted for the study in the setting of M.M. College of Nursing, MMDU, Mullana-Ambala (Haryana) with the aim to assess the effectiveness and evaluate the adaptation in terms of student's feedback about e-learning. Total 1217 responses were collected from around 500 students of nursing for the various subjects they study under all the courses. The feedback responses were collected by the Questionnaire administered through the Google forms, which consisted of a few questions related to sample characteristics i.e course and subject of feedback, other than these an opinionnaire with five point likert scale consisted of 13 questions, related to the feedback of the e-learning approach, used by teachers to teach students during the COVID-19 Lockdown . Analysis of the feedback was done using descriptive statistics based on the central tendencies, mainly the frequency and the percentages, using the microsoft excel 2007.

Results

The effectiveness and adaptation in terms of student's feedback was compiled in the form of frequency and percentage of the 13 opinions on a five point Likert Scale in cumulative and courses wise**.**

Table-1: Frequency and percentage of cumulative Feedback by all Nursing Students:

N=1217

S. No.	Contents for Instructors Feedback	Strongly Agree f (%)	Agree f (%)	Neutral f (%)	Disagree f (%)	Strongly Disagree f (%)
1.	Stimulated my interest in the subject.	268(22%)	683(56.1%)	204(16.7%)	49(4%)	13(1%)
2.	Managed classroom time and pace well.	273(22.4%)	697(57.2%)	197(16.1%)	33(2.7%)	17(1.3%)

Cont... **Table-1: Frequency and percentage of cumulative Feedback by all Nursing Students:**

N=1217

3.	Organized and prepared for every class	298(24.4%)	702(57.6%)	178(14.6%)	28(2.3%)	11(0.9%)
4.	Encouraged discussions and responded to questions.	313(25.7%)	680(55.8%)	170(13.9%)	42(3.4%)	12(0.9%)
5.	Demonstrated in-depth knowledge of the subject.	263(21.6%)	657(53.9%)	222(18.2%)	60(4.9%)	15(1.2%)
6.	Appeared enthusiastic and interested.	219(17.9%)	714(58.6%)	226(18.5%)	39(3.2%)	19(1.5%)
7.	Used a variety of instructional methods to reach the course objectives (e.g. group discussions, student presentations, etc.)	237(19.4%)	665(54.6%)	235(19.3%)	60(4.9%)	20(1.6%)
8.	Challenged students to do their best work.	259(21.2%)	710(58.3%)	203(16.6%)	31(2.5%)	14(1.1%)
9.	Actively attempt to prevent cheating in this course	249(20.4%)	645(52.9%)	237(19.4%)	51(4.1%)	35(2.8%)
10.	Provided information about the assessment was communicated clearly	235(19.3%)	717(58.9%)	207(17%)	46(3.7%)	12(0.9%)
11.	Explained the grading criteria of the course	221(18.1%)	715(58.7%)	215(17.6%)	52(4.2%)	14(1.1%)
12.	Administered the exams related to the course learning outcomes.	232(19%)	706(58%)	224(18.4%)	39(3.2%)	16(1.3%)
13.	Supported by adequate library resources.	228(18.7%)	627(51.5%)	274(22.5%)	623(51.1%)	25(2%)

The Table-1 depicts the feedback on E-Learning by all Nursing students during Covid-19 Lockdown. Maximum of the students strongly agree on item no.4 (25.7%) i.e: "Encouraged discussions and responded to questions". Maximum of the students agreed on item no. 11(58.7%) i.e: "Explained the grading criteria of the course". Maximum of the students disagree on item no. 13 (51.1%) ie: supported by adequate library resources where item no.9 (2.8%) ie: "Actively attempt to prevent cheating in this course" was strongly disagreed by maximum of students.

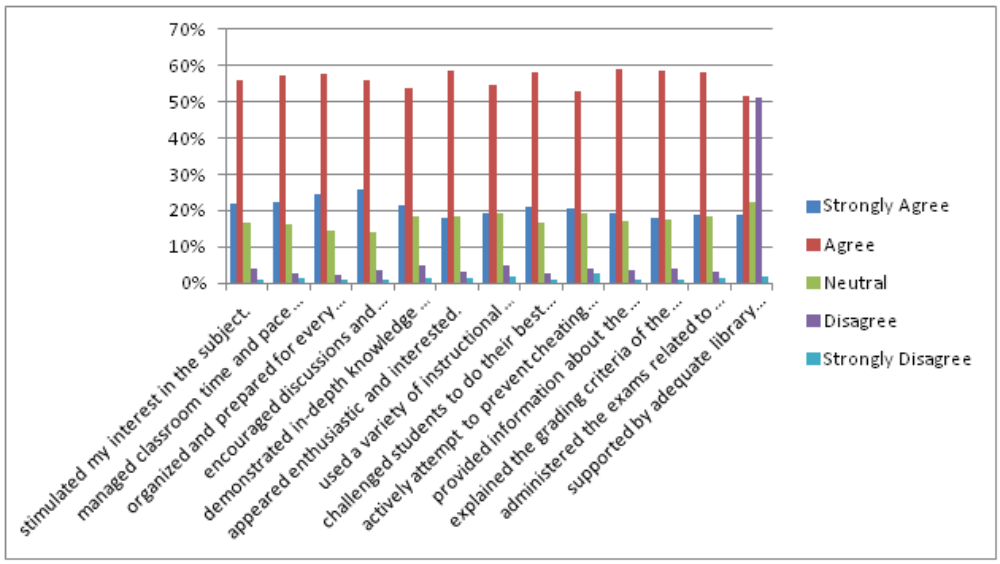


Figure-1: Bar graph of cumulative Feedback by all Nursing Students

Table-2: Frequency and percentage of Feedback components by B.Sc. Nursing students:

n=945

S. No.	Contents for Instructors Feedback	Strongly Agree f (%)	Agree f (%)	Neutral f (%)	Disagree f (%)	Strongly Disagree f (%)
1.	Stimulated my interest in the subject.	174(18.41)	521(55.13)	192(20.31)	49(5.18)	9(0.95)
2.	Managed classroom time and pace well.	180(19.04)	532(56.29)	190(20.1)	31(3.28)	12(1.26)
3.	Organized and prepared for every class	184(19.47)	565(59.78)	163(17.24)	25(2.64)	8(0.84)
4.	Encouraged discussions and responded to questions.	216(22.85)	521(55.13)	160(16.93)	40(4.23)	8(0.84)
5.	Demonstrated in-depth knowledge of the subject.	177(18.73)	497(52.59)	202(21.37)	58(6.13)	11(1.16)
6.	Appeared enthusiastic and interested.	138(14.6)	537(56.82)	218(23.06)	37(3.91)	15(1.58)
7.	Used a variety of instructional methods to reach the course objectives (e.g. group discussions, student presentations, etc.)	159(16.82)	510(53.96)	207(21.9)	56(5.92)	13(1.37)
8.	Challenged students to do their best work.	169(17.88)	545(57.67)	192(20.31)	29(3.06)	10(1.05)
9.	Actively attempt to prevent cheating in this course	170(17.98)	478(50.58)	227(24.02)	46(4.86)	24(2.53)
10.	Provided information about the assessment was communicated clearly	153(16.19)	546(57.77)	195(20.63)	43(4.55)	8(0.84)
11.	Explained the grading criteria of the course	144(15.23)	540(57.14)	202(21.37)	49(5.18)	10(1.05)
12.	Administered the exams related to the course learning outcomes.	149(15.76)	537(56.82)	212(22.43)	35(3.7)	12(1.26)
13.	Supported by adequate library resources.	150(15.87)	472(49.94)	247(26.13)	58(6.13)	18(1.9)

The Table-2 depicts the feedback on E-Learning by B.Sc Nursing students during Covid-19 Lockdown. Maximum of the students strongly agree on item no. 4 (22.85%) ie: "encouraged discussions and responded to questions". Item no. 3 (59.78) i.e: " organized and prepared for every class" was agreed by maximum of the students. Maximum of the Students give neutral response on item no 13.(26.13) i.e: "supported by adequate library resources". Disagree response was shared maximum by both Item no.5 and 13(6.13). Majority of the students strongly disagree on item no. 9 (2.53%) i.e: "Actively attempt to prevent cheating in this course"

Table-3: Frequency and percentage of Feedback components by P.B.Sc. Nursing students:

n=235

S. No.	Contents for Instructors Feedback	Strongly Agree f (%)	Agree f (%)	Neutral f (%)	Disagree f (%)	Strongly Disagree f (%)
1.	Stimulated my interest in the subject.	81(34.46)	140(59.57)	10(4.25)	0(0)	4(1.7)
2.	Managed classroom time and pace well.	73(31.06)	151(64.25)	6(2.55)	1(0.42)	4(1.7)
3.	Organized and prepared for every class	92(39.14)	123(52.34)	15(6.38)	2(0.85)	3(1.27)
4.	Encouraged discussions and responded to questions.	77(32.76)	142(60.42)	10(4.25)	2(0.85)	4(1.7)
5.	Demonstrated in-depth knowledge of the subject.	68(28.93)	142(60.42)	19(8.08)	2(0.85)	4(1.7)
6.	Appeared enthusiastic and interested.	67(28.51)	154(65.53)	8(3.4)	2(0.85)	4(1.7)
7.	Used a variety of instructional methods to reach the course objectives (e.g. group discussions, student presentations, etc.)	62(26.38)	141(60)	27(11.48)	1(0.42)	4(1.7)
8.	Challenged students to do their best work.	69(29.36)	152(64.68)	10(4.25)	0(0)	4(1.7)
9.	Actively attempt to prevent cheating in this course	61(25.95)	150(63.82)	9(3.82)	5(2.12)	10(4.25)
10.	Provided information about the assessment was communicated clearly	64(27.23)	154(65.53)	10(4.25)	3(1.27)	4(1.7)
11.	Explained the grading criteria of the course	61(25.95)	158(67.23)	9(3.82)	3(1.27)	4(1.7)
12.	Administered the exams related to the course learning outcomes.	67(28.51)	152(64.68)	8(3.4)	4(1.7)	4(1.7)
13.	Supported by adequate library resources.	64(27.23)	143(60.85)	20(8.51)	3(1.27)	5(2.12)

The Table-3 depicts the feedback on E-Learning by P.B.B.Sc Nursing students during Covid-19 Lockdown. Maximum of the students strongly Agree on item no.2(39.14%) i.e: "organized and prepared for every class" where maximum students' response agree on item no.11 (67.23%) for "Explained the grading criteria of the course". Disagree response was maximum given in item no.9 (2.12%) for "Actively attempting to prevent cheating in this course" where item no.9 (4.25%) also strongly disagree by maximum of the P.B.B.Sc. Nursing students.

Table-4: Frequency and percentage of Feedback components by M.Sc. Nursing students:

n=36

S. No.	Contents for Instructors Feedback	Strongly Agree f (%)	Agree f (%)	Neutral f (%)	Disagree f (%)	Strongly Disagree f (%)
1.	Stimulated my interest in the subject.	13(36.11)	22(61.11)	2(5.55)	0(0)	0(0)
2.	Managed classroom time and pace well.	20(55.55)	14(38.88)	1(2.77)	1(2.77)	1(2.77)
3.	Organized and prepared for every class	22(61.11)	14(38.88)	0(0)	1(2.77)	0(0)
4.	Encouraged discussions and responded to questions.	20(55.55)	17(47.22)	0(0)	0(0)	0(0)
5.	Demonstrated in-depth knowledge of the subject.	18(50)	18(50)	1(2.77)	0(0)	0(0)
6.	Appeared enthusiastic and interested.	14(38.88)	23(63.88)	0(0)	0(0)	0(0)
7.	Used a variety of instructional methods to reach the course objectives (e.g. group discussions, student presentations, etc.)	16(44.44)	14(38.88)	1(2.77)	3(8.33)	3(8.33)
8.	Challenged students to do their best work.	21(58.33)	13(36.11)	1(2.77)	2(5.55)	0(0)
9.	Actively attempt to prevent cheating in this course	18(50)	17(47.22)	1(2.77)	0(0)	1(2.77)
10.	Provided information about the assessment was communicated clearly	18(50)	17(47.22)	2(5.55)	0(0)	0(0)
11.	Explained the grading criteria of the course	16(44.44)	17(47.22)	4(11.11)	0(0)	0(0)
12.	Administered the exams related to the course learning outcomes.	16(44.44)	17(47.22)	4(11.11)	0(0)	0(0)
13.	Supported by adequate library resources.	14(38.88)	12(33.33)	7(19.44)	2(5.55)	2(5.55)

The Table-4 depicts the feedback on E-Learning by M.Sc. Nursing students during Covid-19 Lockdown. Maximum of students strongly Agree on item no. 3(61.11%) i.e: "organized and prepared for every class" where maximum students' responses agree on item no.6 (63.88%) for "appeared enthusiastic and interested.". Disagree response was maximum given in item no.8 & 13 (5.55%) for "challenged students to do their best work" and "supported by adequate library resources" where item no.7 (8.33%) was strongly disagreed by maximum of the M.Sc. Nursing students for "Used a variety of instructional methods to reach the course objectives (e.g. group discussions, student presentations, etc.)

Discussion

In the present study majority of students strongly agree (25.7%) that faculty Encouraged discussions and responded to questions. Whereas 58.7% of students agreed that faculty Explained the grading criteria of the course where students strongly disagree (2.8%) in Actively attempt to prevent cheating in this course. The findings are partially similar to the study conducted by Dr Amita [6] to assess online feedback of the higher education institution students regarding their experience about e-learning methods used by their faculty and institute students of various higher education institutions in Haryana in April 2020. The survey indicates that the faculty has done their greatest in this crisis period as the online tools have helped the students in covering the major part of their syllabus despite some problems. While another study also similar to the findings on study conducted on students pursuing bachelors in engineering in different majors at Thapar Institute of Technology[7] and questions included the most important feature for students for an e-learning platform. 70.7% of the students surveyed favored pre-recorded video lectures provided via YouTube links as the most convenient e-learning tool. Pre-recorder lectures provided via Google Drive links and Slides uploaded on course sites enjoyed a majority of 71 students and 77 students respectively. 33.5% students agreed that the e-learning tools used by professors were satisfactory and beneficial. 52.7% of students agreed on using the Zoom application to view live lectures at least 3 times a week.

Conclusion

The result of feedback on the opinion of Students about Uses of Digital Education Technology during COVID-19 Lockdown in India has brought into light a lot of facts. Majority of the students had strongly agree that faculty organized and prepared well for every class and faculty were encouraged in discussions and responded to questions. The study suggest that various alternative strategies can engaged students and keep them motivated. Hence, Students are inquiring about more liberty and academic preference.

Ethical approval: Approved by Research ethics committee of Maharishi Markandeshwar (Deemed to Be) University Mullana, Ambala.

Source of Funding: Maharishi Markandeshwar (Deemed to Be) University, Mullana.

Conflict of Interest: NIL

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Accessible India Campaign "A Silver Lining for the Persons with Disability"- An Analysis

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Abstract

As early as 1957, the then UN Rehabilitation Chief, Kurt Janson observed: "About 12 to 13 percent of the population of any given country suffers from a permanent or long term disability". So there is an urgent need to provide the person with special facilities at different levels so that they can be made to survive easily with all other individuals. There is a lack of approach on the part of the Government as well as the other officials who are liable to create a harmonious environment for the person suffering from a disability. They must make an atmosphere where they don't feel being suppressed or humiliated. Although Accessible India Campaign has not attained the desired goal it was supposed to attain but yes it has helped in acting as a guide and ray of hope amongst the Person with Disability.The objectives of the present paper are to analyze the motive and objective of the Accessible India Campaign and to critically judge its achievement and shortcomings. The researcher has adopted the Doctrinal Research Methodology for the research undertaken.

Keywords-*Accessible India Campaign, Persons with disability, Sugamya Bharat, Right to Accessibility, Fundamental Rights*

Introduction

'Accessible India Campaign' which is also known as 'Sugamya Bharat Abhiyan' is an initiative taken up by the Indian Government. 3rd December is celebrated as International Day of Person with Disability. On the same day, this campaign was launched by the central government. This campaign aims mainly at the restructuring of the total environment of the differently-abled person in a manner that the environment becomes friendly for them. The measure was also to "design of disabled-friendly buildings and Human resource policies". India has been a signatory to the UNCRPD from the year 2006 and keeping in mind Article 9 of the UNCRPD this campaign has been launched. Under this scheme, the provisions like Section 44, 45, and 46of Person with Disability Act, 1995 has also been given proper recognition. Section 44, 45 and 46 of the Act deals with protection of rights, equal opportunities, and non- discrimination in transport.[1]

The main aim and objective of this campaign are to provide accessibility to disabled persons in areas like the equal opportunity of education, employment, health care, transport, recreation, sports, and various other areas. It is framed under the Principles of the "Right Model of disability". The principles stated that the main reason for disability is the disorganized society rather than the person who bound by limitations or impairments. The barriers that have been created socially, physically, structural as well as attitudinal for the person with a disability has acted as a hindrance from participating them equally in the socio-cultural and economic activities.

To provide with Universal Accessibility to the Person with Disability, this campaign has been classified into three areas-

1. Built Environment;
2. Transport and

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3. Information & Communication Technology (ICT) Ecosystem.[2]

1. Built Environment

In Physical Environment, steps must be taken to remove the obstacles and hindrance from both the Indoor and Outdoor facilities, which include Medical facilities, Schools, and Workplaces, etc. A government building will be termed as an accessible government building only when it will be easy to get access to the building and also availing all the facilities that are present there. An accessible environment comprises of following things-

a. Services provided;

b. Ramps and Stairs;

c. Corridors and Passages;

d. Parking Areas;

e. Entry and Exit gates;

f. Emergency Exit areas.[3]

Apart from all these things, there are various others indoors and outdoors facilities that must also be made accessible. They are- Signals, Alarm System, Proper Lightening, Toilets. To determine whether a building is accessible or not and is meeting the required standards or not requires a proper and annual accessibility Audit after the building becomes fully accessible, the further annual audit is not needed. The audit will be again required to that building only when there are any proposed changes required to be done.

The Accessibility Standard is to conform to that of the standards that have been set at the international level like as set by ISO. The same standard must be complied with at the local level too. While talking about the Build Environment, the Construction of Building- Usability as well as Accessibility of the Built Environment, Delineates certain requirements as well as recommendations that are concerned with the Construction, Assembly, Fittings as well as Components. Certain targets have formed the Component Built Environment Accessibility under the Accessible India Campaign:

1. Carrying out accessibility assessment of minimum 25-50 "most important government building" of 50 cities and building them so that they are accessible by the end of the year 2016;

2. The next target was to make 50% government building of NCT and the Capital cities of all the states in India to be fully accessible by the year 2018;

3. To Complete the Audit of 50% the government buildings and offices by making all the audited building fully accessible in the other 10 cities that are most important and are not covered under the target 1&2, by the year 2019.

The department has submitted the report of its work. The accessibility audit of 1653 buildings has been done that was identified by the State Government through the panel of Access Auditors. The Audit Committee has submitted the Access report for 1469 to the State Nodal Officers so that they can submit the financial requirements for retrofitting of all these buildings. Out of all these buildings, proposals of 575 buildings have been received by the Scheme. 45.42 Crores of funds have been allotted to the states for around 242 buildings. Besides, 148 lakhs have been released so that audit can be done by conducting access-audit.[4]

2. Transport

When it comes to Economic growth, an Accessible transportation system plays a very vital role. The Inaccessible Transport system has not only denied the freedom of movement but also has restricted mobility and active participation for the majority of the population who need accessible transportation.

The new "National Urban Transport Policy (NUTP), 2014" has included within its policy universal accessibility in all the planning and also implementation inventions. The Indian Roads Congress Code 103:2012 Guidelines for the Pedestrian Facilities has provided inclusive street design standards and roads. Some important features that are linked with the Transport Infrastructure Accessibility are as follows:-

Ø Properly lit, bus shelters and streets.

Ø To move barrier-free the pedestrian crossings must be raised. They must be textured with different paving materials to make the crossing more perceivable.

Ø The footpaths must be made flat for all the

mobility aid users and the tactile pavers must also be continuous for the entire stretch for the person who is visually impaired.

Ø There must be the use of white light on footpaths that will be able to maintain a color contrast from the roads and which will also ensure that the tactile pavers are easily visible during the night.

Ø There must be an installation of Audible light signals that will make a beep sound when the light is green.

Ø Bus stops must also have barrier-free access and must have broad gates with warning tiles.

Ø The low floor buses must contain folding ramps that will help the mobility aid users.

Ø There must be a proper installation of safety belt that will secure the journey inside the buses.

Ø There must proper parking space for the wheelchairs to get parked.

Ø The Signage Panel must have audible messages and also the provision for Braille Signage.

Ø All the metro stations and the coaches of the metro must possess accessible features.[5]

The target that has been set by the Accessible India Campaign is to make 10% of the public transport that is government-owned to get fully accessible by March 2018. To fulfill the achieved target the Ministry has taken the Undertaking to ensure that 10% of the above-stated vehicles become fully accessible by March 2018.

3. Information and Communication System

Information Access has been of vital importance in society as it helps in creating opportunities for everyone. Access to information refers to all sorts of Information. The range of actions covers areas like the ability to read price tags, enter any hall physically, to take part in any sort of event, to read a brochure dealing with the vital information regarding Health-care, understanding of the train time table, or also to view a particular website or webpage.[6]

There is a need to bring all the websites that are very important to be converted to accessible friendly and it must ensure that all the public documents that have been publishing in the past and the current years get converted. The conversion must be done in the manner as set by the "International Organization for Standardization (ISO)" criteria which are found in "ISO/IEC 40500:2012, IT- W3C WEB CONTENT ACCESSIBILITY GUIDELINES (WCAG) 2.0".

'Public document' has been defined as the entire document that has been issued by the National Government and by all the State Governments and other government official documents. When it comes to this branch of accessibility, the target that has been initiated is to make at least 50% of the State as well as Central Government websites accessible by 2017 March.[7]

Till the mid-2017, already 917 State Government websites have been made accessible. Apart from this 100 Government websites of 56 Departments/ Ministries have been made accessible by the Ministry of Electronics & Information technology. On 19th January 2018, to empower Persons with Disabilities, 100 accessible websites were launched under the Accessible India Campaign. These websites deal with the working of various State governments and Union Territories. The Department has taken the help of Social media to create a footprint in the digital space. Live updates, blogs, reports, and pictures are being circulated and are updated at social networking sites to bring awareness among the people[8].

There is also a website created by the department in the name of www.accessibleindia.gov.in. The department has dedicated this website for this purpose[9]. A mobile application has also been launched by the department by which the recent happenings and updates can be brought into the notice of the general people[10].

An E-Library has also been launched by the Union Government by the name "Sugamya Pustakalaya".

'Sugamya Pustakalaya'

An online library has been launched by the Department in the name of "Sugamya Pustakalaya" for persons with print disabilities[11]. It is centered on achieving "Universal Accessibility". Their department has taken initiative by organizing workshops at various places throughout India like Mumbai, Ahmadabad,

Jaipur, Raipur, Bhubaneswar, Chennai, and Ranchi to provide information to the people regarding various contours of the Accessible India Campaign.

· It consisted of publications across various subjects that are present in the Country and also in various languages.

· It has been incorporated by the collaboration of Bookshare and the National Institute of Visually Handicapped (NIVH). The whole initiative is also powered with TCS Access. NIVH is a member organization of the daisy forum of India.

· This online library has been designed in such a manner that books that are there are also available in various accessible and desired formats for the person who is suffering from visual impairment as well as another form of print disability[12].

Udid- Unique Disability Id

The UDID project initiated by the Department of Empowerment of Persons with Disabilities aims at building a holistic end-to-end integrated system for Issuance of Universal ID & Disability Certificates for Person with Disabilities with their identification and disability details. It includes -

1. Online availability of data of Person with Disabilities across the country through a centralized web application.

2. Online filing and submission of registration application form for disability certificate/ Universal ID card;

3. The quick Assessment process for calculating the percentage of disability.

4. Non-duplication of PwDs data.

5. Online renewal and update of the information.

6. MIS reporting framework.

7. Effective management including interoperability of the benefits/schemes launched by the Government for PwD.

8. To take care of additional disabilities in the future.

9. As of 9th May 2020, 10 states have already started generating the UDID Cards. They are Madhya Pradesh, Gujarat, Odisha, Maharashtra, Kerala, Tamil Nadu, Uttar Pradesh, Chhattisgarh, Jharkhand, and Rajasthan[13].

Some of the Works Done by Various States in India

At first, for instance, when accessibility audit started taking place in India and the Buildings, as well as public places, were being accessed, there was not even one public place or building that can be stated as completely disability-friendly[14]. After the first phase of audit that has been launched in 31 cities as a part of the initiative of the Accessible India Campaign brought shocking results and it was seen that the state of toilets was appalling and various other facilities for the differently-abled person in hospitals, public buildings, railway stations, and educational institutions was not worth mentioning. It was also seen that if certain attempts were made to make the place accessible it was half-hearted. It was stated that 95% of the buildings that were audited do not have accessible toilets for the person with a disability. The rest 5% which have the toilets for the person with disability 75% out of them were in sorry states. Universal Design Centre had only found 3 out of a total of 29 buildings in Goa that possess basic facilities like ramps and all but still, none of them can be classified as fully accessible. Even the Chennai Metro Rail System was found to be good still certain areas needed improvement. Jammu and Kashmir have taken up the access audit work for around 18 cities whereas West Bengal has no taken up even a single Access Audit. Access is not done by West Bengal does not mean that they do not possess many shares of Person with Disabilities. 7.52% of the people of West Bengal are suffering from a disability. The total Indian figure is 2.21%(11).

In New Delhi, around 23 buildings have been audited out of which most were hospitals. Out of these 23 buildings, one was the Supreme Court and this audit has taken place in the year 2016. The areas that were made accessible cover the main gates, parking areas, building entrances, reception areas, ramps, lifts, the stairs, cafeteria areas, washrooms, facility of drinking water as well as disaster preparedness. However, the Supreme Court has not been made fully accessible and the areas

like toilets, cafeteria, and disastrous preparedness were missing. The Supreme Court was made accessible to the rest of the areas but these neglected areas are also of considerable importance. The Audit report has however suggested that these improvements must be done and are not too expensive.

The Access Audit Report 2016 also accessed Indira Gandhi International Airport (IGIA), in different segments for the terminals 1-C, 1-D, and 3. It was found that this airport is friendly for the person with a disability. The audit reports that were conducted at New Delhi Railway Station has been resulted in negative as the station was not found to be accessible for the person with a disability. However, various measures are being undertaken to make the station disabled-friendly. The ministry has taken efforts to install lifts and escalators so that it will be easy to travel from one platform to another[15].

The government to make the campaign successful is taking all effort even IRCTC has also made provision for booking of wheelchairs. Even to help the differently-abled person, the East Coast Railways has also introduced 'MOBILE WHEELCHAIR FOLDABLE RAMP' at the Bhubaneswar, Sambalpur as well as Visakhapatnam Railway Stations. To avail of the facility, the passenger has to inform the Wheelchair section near the Chief Ticket Inspector's office. This facility is free of cost[16].

Suggestions and Discussions

This campaign is indeed an appreciable initiative that has been taken up by the Government of India to provide a barrier-free environment for the person with disabilities. But the Campaign has restricted its scope by covering only government buildings. The ambit that has been reduced not only restricts the scope as well as the benefits that can be drawn from the Campaign. The campaign must also incorporate within its ambit the private Hospitals, schools, and all the educational institutions. Among all the problems that are faced by the differently-abled person, the most pertinent one is that of transportation. Whether it is rural areas or urban areas the problem is very much the same. Another area that needs a great concern is the accessibility of the differently-abled person to the public toilets. This has to be considered as one of the major priorities as the public toilets are in abysmal nature when it comes to accessibility. Also, the safety issue must be kept in mind and hence KIOSKs which is Disabled friendly must be installed in big shopping malls. All the signboards that are used to show various indications must be designed in such a manner that they are disabled friendly. In the future, it is considered that the Public Work Department, Land Army, and Nirmiti Kendra will be included in the implementation committee by the Government. All these departments will be taking up the civil works of the infrastructure that are to be initiated by the Government. The Disabled community and the Government must encourage the Private sector to make products as well as services that are disabled friendly. Like the news bulletin in sign language can be run by the private news channels for the person who is suffering from hearing impairment. This type of news bulletin is only shown on Doordarshan Channel. All the private taxis/cabs must introduce wheelchair accessible vehicles that will possess ramps which will ease the traveling. Even the hotels and restaurants should install a separate menu for the differently-abled person which must be written in Braille. If it is on any electronic gadgets then it must be supported by any software that will be easy for them to readout. Even the steps should be taken to make the baking facilities easier for them and it must be designed in such a manner that they do not have to be dependent while making access to the banking system. Although there is a lot more to be done by the government for the upliftment of living conditions of the persons with disability. But still, we can state that the start is good and has the potential to reach the milestone.

Ethical Clearance - Not required as the researcher has just referred to some published works. The research is doctrinally undertaken, completely by the researcher himself**.**

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Identification of Sex from Maxillary Sinus in Western Maharashtra Population

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Abstract

Background: As Maxillary Sinus are pneumatic bones delicate in cadavers, Morphological study has anatomical anthropological and medico legal important. Hence Morpho metric parameters of MXS in CT scan of both sexes were studied.

Methods: 23 male 23 female adult aged between 20-50 year healthy people were studied with CT images to measure medio-lateral, superoinferior and antero posterior dimensions and volumes of MXS in both sexes and results were analysed statistically.

Results: In ML Right mean values Males was 29-30 (SD±3.17), female 27.12 (SD±5.16 t test was 1.72 and $p<0.001$. SIR Right – Mean value of male was 40.02 (SD±3.66), female 35.11 (SD±4.40) t test 4.11 and $p<0.001$. AP (right side) Mean value of male was 44.23 (SD±3.21), female 38.03 (SD±2.30) t test 7.53 $p<0.00$. MLC (left side) mean value of male was 29.04 (SD±2.30), female 25.06 (SD±4.20) t test 3.98 $p<0.004$. SIL left side mean value of male 38.41 (SD±4.12), female 35.07 (SD±3.60) t test 2.92 $p<0.005$. APL (left side) mean value of male 42.36 (SD±2.60), female 39.05 (SD±2.45) t test value 4.44 $p<0.001$. The volumes study of MXS had VR – mean value of male 18.25 (SD±2.53), female 13.30 (SD±3.14) t test 5.88 $p<0.041$. In VL study mean value of male was 17.09 (SD±2.75), female was 12.44 (SD±3.42) $p<0.001$.

Conclusion: This pragmatic study of sexual dimorphism will be quite useful to medico-legal expert, anthropologist and anatomist

Keywords: *MXS=Maxillary Sinus, CT Scan, computerised tomographic scan, adults, Maharashtra VR = Volumes of Right MXS. Sinus, VL=volume of left MXS sinus.*

Introduction

Maxillary sinus is pneumatic bones present on either side of Norma frontals forming the floor of orbital cavity. Being pneumatic bones they are delicate and more prone to get broken. They lighten the skull and helps in the resonance of voice [(1)]. It is difficult to study the morphometric values of cadaveric Maxillary sinuses (MXS) as they break easily. Hence attempt was made to study computerised tomographic morpho-metric values in living subjects of both sexes to know the exact measurements.

The MXS is first PNS to develop and is located in the right and left maxillary bones and consists of two pyramidal shaped air filled cavities lined by mucosa. The MXS tend to appear at the end of second month of embryonic life and complete by the age between 18 to 20 years of life (2)(3), but size and shape of MXs varies amongst individuals between genders and different populations and/or ethnic groups. However the size and shape of MXS will be stabilized after second decade of life thus reliable measurements can be achieved by radiographic images after 20th year of age will be an ideal

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study [4]. Apart from this nutritional, generic hormonal environmental factors do contribute in the morphometry of MXS. Hence CT scan images of western Maharashtra were studied.

Material and Method

23 Male and 23 Female adults aged between 23-50 visiting to Vedantaa institute of Medical sciences Dhundalwadi, Dahanu, Palghar (dist)-401610, Maharashtra were studied.

Inclusive Criteria: These volunteers did not have any pathology of PNS and majority of healthy volunteers were selected for study.

Exclusion Criteria: The persons who had undergone surgery of PNS or Norma frontalis. History of fracture of Norma frontails, Immune compromised patients, and patients with pathology of PNS was excluded from study.
Methods: Non-contrast CT scan was performed to study the morphometry of Maxillary sinuses in both sexes using GECT/e dual slice CT scanner (GE health care technologies, Waukesha, WI, USA. Prior to the scan every patient was instructed to remove the metallic objects, jewellery, hairpins etc, from the head, to neck region and positioned on the CT table in prone position. The patient's neck was hyper-extended with the chin resting on pad for stabilization. Pads were inserted on both sides of head. The gantry was angulated to make it par perpendicular to the hard palate 3mm thickness were used on preliminary. Scout view extending form anterior margin frontal sinus to the posterior margin of spheroid sinus with a reconstruction matrix zone of 512X512 at 120 KV, 100MA coronal CT was performed after instructing the volunteers to remain steady during the entire procedure.

The measurements like ML and SI were made, maxillary sinus were in the widest position with the help of on screen linear on screen (fig-A). To measure the AP dimensions of the maxillary sinus, the first and last appearance of the sinus was noted in the sequential coronal CT sections and number of sections between, them were selected finally selected section were multiplied by 3 (thickness of single section to find out the AP of the sinus. Maxillary sinus volume (MSV) was calculated by using the paint on slice tool on the work station. To define a volume outline of the sinus was traced manually on each slice of the image stack using on the screen mouse pointer in the coronal plane (fig-B). Once the tracing was complete, the work station automatically segmented the entire volume of the sinus form the surrounding structure and the segmented portion could be visualized and manipulated in 3D.

At this point switching to the histogram view on the work station (fig-C) automatically reflected the volume of the sinus in the cubic centimetres (CC) of both right and left maxillary sinus. The duration of study was 2017 to 2019.

Statistical analysis: The obtained results from different parameters were studied in SPSS software computer.



Fig A – Linear measurement of medio-sinus and super inferior dimension of maxillary sinus

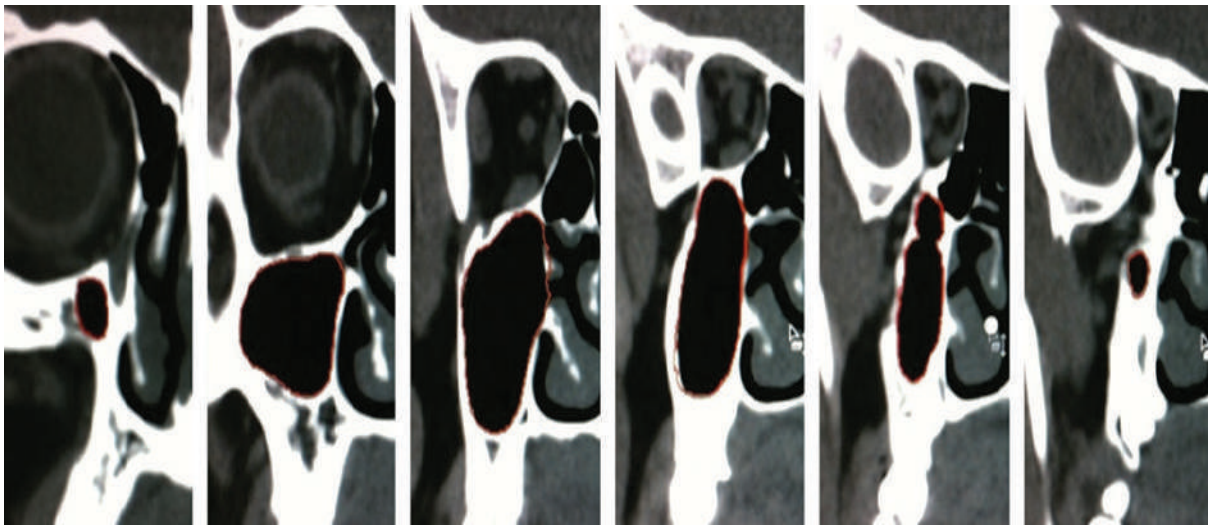


Fig B – point on slice tool



Fig C – workstation showing maxillary sinus.

Observation and Results

Table-1: Comparison of various dimensions of maxillary sinus measured on CT scan studies in both sexes. In ML Right mean values male was 29.30 (SD±3.17), female was 27.12 (SD±5.16) t test value was 1.72 and p<0.001. In the SIR (Right) mean value of male was 40.02 (SD±3.66), female was 35.11 (SD±4.40) t test value 4.11 and p value p<0.001. In AP (Right side) mean value of male was 44.23 (SD±3.21) in female 38.03 (SD±2.30) t test 7.53 p<0.001. In ML (left side) female 25.06 ((SD±4.20) t test 3.98 and p<0.01.

SIL (left side) mean value male 38.40 (SD±4.12) and female was 35.07 (SD±3.60) t test value 2.92 p<0.005.

In APL (left side) main value, male was 42.36 (SD±2.60), female 39.05 (SD±2.45) t test was 4.44 p<0.01.

Table-2: Comparison of maxillary sinus volume measured on CT in both sexes. In VR study mean value of male 18.25 (SD±2.53), female 13.30 (SD±3.14) t test value was 5.88 p<0.01. In VL study mean value of male was 17.09 (SD±2.75), female 12.49 (SD±3.42) t test 5.08 and p<0.001.

Table-1: Comparison of various dimensions of Maxillary Sinuses measured on CT scan studies in both sexes.

Sl. No	Particulars	Male (23)	Female (23)	T test value	P value
1	ML Right	29.30 (±3.17)	27.12 (±5.16)	1.72	P<0.01
2	SIR (Right)	40.02 (±3.66)	35.11 (±4.40)	4.11	P<0.01
3	AP (Right side)	44.23 (±3.21)	38.03 (±2.30)	7.53	P<0.00
4	ML (Left side)	29.04 (±2.30)	25.06 (±4.20)	3.98	P<0.04
5	SIL (Left side)	38.41 (±4.12)	35.07 (±3.60)	2.92	P<0.005
6	APL (Left side)	42.36 (±2.60)	39.05 (±2.45)	4.44	P<0.00

(MLR Right = Right Medio lateral dimension of right side. SIR (Right) = Supero inferior Dimension of right side. AP right side = Antero posterior dimension of side right. ML Left side = Medio lateral dimension of left sinus Maxillary sinus. SIL = superior-Inferior dimension of left maxillary. AP (left side)= left Antero-posterior dimension of left Maxillary sinus.

Table – 2: Comparison of Maxillary sinus volume measured an in both sexes

Sl. No	Particular	Male (23)	Female (23)	T test value	P value
1	VR	18.25 (±2.53)	13.30 (±3.14)	5.88	P<0.001
2	VL	17.09 (±2.75)	12.44 (±3.42)	5.08	P<0.001

VR = Volumes of Right Maxillary sinus

VL = Volumes of Left Maxillary sinus

Discussion

In the present study of Identification of sex from maxillary sinus in western Maharashtra of ML right study mean value of male was 29.30 (SD±3.17), female 27.12 (SD±5.16) t test was 1.72 p<0.001. AP right the mean value of male was 40.02 (SD±3.66), female 35.11 (SD±4.40) t test value was 4.11 p<0.001. In AP (Right side) mean value of male was 44.23 (SD±3.21), female 38.03 (SD±2.30) t test was 7.3 p<0.000. In ML (left side) the mean value of male was 29.04 (SD±2.30), female 25.06 (SD±4.20 t test value was 3.98 p<0.004. In SIL (left side) mean value of male was 38.41 (SD±4.12), female was 35.07 (SD±3.60) t test was 2.92 p<0.005. In APL (left side) mean value of male was 42.3 (SD±2.60), female 39.05 (SD±2.43) t test was 4.44 and p<0.00 (Table-1). In the compassion of volume of maxillary

sinus on CT in both sexes. VR mean value of male was 18.25 (SD±2.53), female 13.30 (SD±3.14) t test was 5.88 p<0.001. In VL study mean value of male was 17.09 (SD±2.75), female 12.44 (SD±3.42) t test value was 508 p<0.001 (Table-2). These findings were more or less in agreement with previous studies [5][6][7].

It was interesting to note that left MXS width was more discriminate than right MXS for sexual dimorphism in various studies of north and south Indian studies [8][9]. Because it is well established fact that, foramina or sinus/ space in the bone tends to starts at surface irregularities because strain energy tends to concentrate such points. It was also noted that right MXS was larger and wider in abroad studies [10][11]. This anatomical variability between genders could be environment or nutritional adaptations because skeleton of a particular individual is able to adapt to its owner's way of life.

It was also mentioned that till puberty sexual dimorphism is insignificant in crania but after puberty hormonal, nutritional environmental factors play vital role in the gender determination in India and abroad crania.

It can't be denied that, males needs to have correspondingly bigger lungs to support their relatively more massive muscles and body organs. Secondly males need a large air way, which begins with nose and nasopharynx. In other words, physiological changes in nasal cavity size and shape occur as direct result of respiration related needs, such as warming and humidifying inhaled air. As the MXS occupies the remaining space within the naso-maxillary complex, it also increase in size hence morpho-metric values is higher in males than females.

Summary and Conclusion

The present study of gender determination of MXS by CT scan study is an important step in identification in Medico-legal practice. It has to be borne in mind that MXS tend to stabilize morpho-metrically after second decade of life. Hence radiographic images will prove ideal in sexual dimorphism after second decade of life but this study further demands genetic, nutritional hormonal embryological study because the factors which determine the time of ossification are still obscure.

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COVID-19: Analysing the Legal Nuances of the Lockdown Order

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Abstract

COVID-19 has posed a serious challenge to countries all over the world taking lives, destroying livelihood, bringing down the economies to the ground. The question now is not how soon it will pass away but how effective will it be dealt with it. Nearly 3/4th of the countries which are affected do not have sufficient preparedness to deal with it. It may be the lacking of a Mitigation Plan, or maybe the lack of an effective health care system, or maybe the lacking of an efficient R&D, or it may also be the lack of an efficient far-sighted Leader. India has been under the shadow of a few of many problems. The government of the country relies on the Laws to bring out a Preventive Mechanism to deal with the outburst of cases and other effects. One of which is the LockDown order which has been held as one of the first, earliest, and strictest in the World. The paper seeks to analyze critically the legal backing of such Lock Down, testing the nuances of it on the Constitutional Touchstone

Key Words: *Lock Down Order, COVID-19, Mitigation, Constitution, Intelligible Differentia*

Introduction

Novel Corona popularly known as COVID-19 marked its presence in December when China informed the World Health Organisation that there is a cluster of Cases with unknown cause emerged in the City of Wuhan.[1] The disease took no time to spread in other parts of China and also in various other countries. The COVID-19 was termed as a Pandemic because Pandemic is an epidemic that spreads throughout an entire country, continent, or the whole world. As per the definition of WHO, Pandemic means "worldwide spread of a new disease". On 11th March 2020, WHO declared the COVID-19 outbreak as a pandemic, the reason was its severity and global spread.

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Most of the country invoked lockdown in their territory either fully or partially. The reason for this lockdown is that the disease spreads by coming in contact with the infected individual or anything which the individual has been in contact with. So the only possible preventive solution to this is "Social Distancing" and hence the government has ordered that every individual had to be inside their houses and follow social distancing norms.

Thus, the Government of India, as well as various State governments imposed lockdown. Initially, the lockdown was done for one-day which was also termed as '*Janta-curfew*, and then on the 24th of March, the lockdown was called for 21 days.[2] After the lockdown was brought into effect, various restrictions were placed in the Individual Rights and liberties. Apart from the essential commodities and important essential facilities, all the other goods and services were halted for 21 days i.e, till 14th April. The lockdown kept on getting further extended till 17th May as the disease started spreading exponentially, but with different relaxations and regulations.

Part III of the Indian Constitution talks about the various Fundamental Rights that have been given to the individuals by the Constitution. Individual fundamental rights can not be taken away by the legislature or the executive body without any justified reason. Neither the Constitution of India more any such law talks about the lockdown expressly. The implied power given by the laws has been given a much wider interpretation in this aspect. It can take up any justified steps to curb the situation and the pandemic. Although various rights are being curtailed during this lockdown it has to be kept in mind Fundamental Rights are not absolute and it can be curbed and curtailed in National Interest. Now the question here is, from where did the government have the right to impose lockdown? The imposition of Lockdown has infringed on various Fundamental Rights from the individuals. The paper will also analyze the various directives of the lockdown order in consonance of the Principles of Constitutional Law.

Analyzing THE LEGAL BACKING OF A LOCKDOWN ORDER:-

'LOCKDOWN' is comparatively a freshly coined term, and during the 1970s, this term was used in the United States of America. The 2nd Amendment of the US Constitution provides the right of the person to keep and bear arms. This was subsequently upheld in cases like the *District of Columbia v Heller (2008)*[3] *and M*cDonald v. Chicago (2010).[4] Keeping arms at their homes led to instances where the children and mentally disabled have misused it because of their underdeveloped mental conditions. During the 1970s, shootouts occurred near schools where the Students openly fired, and thus, considering the safety and life of other students they were locked- down in their classrooms. This was an instance that gave the impression that to control an unexpected situation against which there can be no immediate remedy, Lockdown can be called for.

In today's context, India uses two concepts which are mostly used, they are-

Section 144 of the Code of Criminal Procedure,1972[5], and Lockdown.

Section 144 of Code of Criminal Procedure, it authorizes the Executive Magistrate of any State or territory to issue a written order which may be directed against a particular individual, or a specific group of persons or on a particular. Even in very grave situations, the Magistrate can pass the order under Section 144 without giving prior notice to the individual against whom it is directed. It is generally believed that the assembly of three or more persons is prohibited under Section 144; however, it can be used to restrict even a single individual. There are various instances when this section has been invoked. Even initially Section 144 was imposed during COVID-19 situation also which was later on made much stricter.

Curfew has not been specifically mentioned in any law. The power regarding curfew is implied in a State Executive which can pass the order establishing a specific period during the people are asked to stay at their home and not to come out. It can be stated that it is an extended version of 144 which is exercised by the government. On 22nd March 2020 in the wake of COVID-19, the Government called for '*Janta- Curfew*' in which people were asked to stay at their homes from 9 am to 5 pm.

However, In India, there have been numerous instances where Section 144 and Curfew has been used, but this was for the very first time we came across the term 'Lockdown'. I delve into the legal provisions which are applicable in India we can come up which two such Laws backs up Lockdown, tacitly:-

Section 2(d) of the **Disaster Management Act, 2005[6]** has defined disaster as "catastrophe, mishap, calamity or grave occurrence in any area, arising from natural or man-made causes, or by accident or negligence which results in substantial loss of life or human suffering or damage to, and destruction of, property, or damage to, or degradation of, environment, and is of such a nature or magnitude as to be beyond the coping capacity of the community of the affected area". Section 6(1) of the Disaster Management Act, 2005 states that the National Authority shall have the responsibility for laying down the policies, plans, and guidelines for disaster management for ensuring a timely and effective response to the disaster. Section 6 (2)(i) also states that the National Authority may lay down policies on disaster management. The Indian Government has by official notification notified COVID-19 as National Disaster and Disaster Management Act, 2005 was invoked. Hence

becomes binding on the State Government.

Epidemic Diseases Act, 1897[7]provides the Union, and the State Government both have been given the power to serious epidemic disease can be regulated by various measures and can prescribe regulations.

Section 2 of the Act empowers the state government to take important measures to control the dangerous Epidemic Disease. Section 2A (as inserted in 1920) empowers the central government on its satisfaction that there is not sufficient law to meet the current situation allows it to take certain measures and impose certain regulations to control the outbreak of a particular disease. The Act has also prescribed punishment for the ones who do not obey the guidelines.

A Thread-Bare Analysis of the LockDown Order

Assessing the legal backing is not enough keeping into consideration the effect it is showing on the have-nots of the society. Therefore it is essential in analyzing the content of the LockDown order (hereafter, The Order) w.r.t the Constitutional Litmus-Test. The Ministry of Home Affairs has laid down guidelines in the regulation of various sectors. The Guidelines provide for working of essential sectors -both Governmental, Private and Semi-Governmental Organisations, with minimum essential employees, thus enforcing the Social Distancing mandate. Although the Lockdown Order is the best possible step taken by the administration for the time being to put a check on the COVID cases, how it was imposed was sudden, without giving sufficient time to be prepared for the days ahead. The Lockdown began with the Janata Curfew, which was declared by the Prime Minister himself providing only a 4-hour duration to stock essentials. For a person who can afford well, protected with a secured source of employment and a stable roof above his head, these 4 Hours might not be enough but surely is sufficient. But for a person who is a contractual laborer inhabiting temporarily at a place which is not his home, with a Pregnant wife with him or an ailing Parent/Child back at home, sustaining on daily wages, with minimal or no savings living with a fear that the Contractor won't assure him of Wage Security, this 4 hour was nothing but a preparation of Death Sentence for many. The not so well beginning of the Lockdown jolted the unorganized sector out of its existence. Any Executive order of such nature has to have a systematic Standard of Procedure. The Lockdown was laid on such a foundation that was arbitrary in itself, even if it had a legal Backup and a near-perfect content. The big schemes ensuring Right to Livelihood, a right to a well-dignified life vanishes in the thin airs when these have-nots were stranded neither with any sustenance nor with any sustenance.

The Order stands tall justifying its existence in the name of Public Interest. But assessing its contents forecasts a different narrative altogether. The Order allows for taking necessary steps for ensuring social distancing measures and puts the onus on the Central Government and State Government Authorities to ensure the same.[8] No authority can define and establish the framework within which measures are to be taken. Instead what could have been done is to gradually release a map after consulting the experts, within which the implementing authorities could work. Due to the absence of such a memorandum, the enforcing authorities who are equally scared of this Pandemic resorted to all kinds of measures starting from peaceful requests, Dramatic representations to Brute Force leading to even deaths.[9] Thus even if the direction is based on a reasonable restriction, being a vague provision exposes it to potential misuse, thus violating Article 14 (chance of Arbitrary exercise of power). Police brutality is a glaring example of it. Not only this, recently Migrants who worked in Rajasthan walked back to their villages in Uttar Pradesh but were stopped by authorities at the Uttar Pradesh border, citing safety as a reason, which is quite uncomprehending, because the alternative to walking home is to stay and die of hunger or disease. A petition[10] has been filed by Advocate Deepak Kansal, in the Supreme Court where he has highlighted the fact that the Centre's Reports which shows that "that there were no migrant workers on the roads anymore as governments had ensured that they were being housed and fed" was a false report. Along with the violation of the principles that our Constitution has laid down, all of these instances surely put a dent in our belief that we have a People-centered Governance. This is in gross distinction to the treatment meted out to the people who are stuck in other countries due to the Lockdown due to the global Covid-19. The Government of India has initiated a mission *Vande Mataram* to bring them back to the country, and where they are treated with utmost respect and dignity, whereas the migrants are being

sprayed with disinfectants. This violates Article-14 of the Indian Constitution, where the Government is undertaking Class Action without any *intelligible differentia*.

At the same time, we cannot ignore the Order made by the Ministry of Home Affairs made provisions for the rent deduction, salary payment, provision for rest, and food for stranded migrants. The Offices, Industries have been asked to work with Bare Minimum staff, thus balancing productivity and social distancing. The29th March 2020 Order has directed the private establishments for payment of full salary to all its workers, and the amount cannot be reduced due to closure of the workplace.[11] The order seems to be made for the benefit of the daily wage workers. But at the same time, consideration must be given to the Private establishments, as without any economic help, many of them will go into closure.

States like Odisha have made ensured that no migrant will have to walk by foot within its area, and the State government will make provision to drop them at the border.[12] The absence of any such provision by any other State will amount to the futility of any such exercise and calls for the necessity of cooperation from the other states as well.

The Provisions under the lockdown order works towards strengthening the fabric of Decentralisation. It provides for the Executive magistrate being the nodal officer in ensuring the enforcement of the Containment measures.[13] In various States, the Sarpanch has been made the Person in charge to ensure that any person coming to the village from outside is to stay in quarantine for a fixed period. It is very essential to understand the need to engage the Local Self Government at such times. The various actors in the Grassroots governance will be effective in creating awareness about such a Pandemic which is new to this and the previous generation. They will help dissipate any kind of Paranoia among the ignorant.

Conclusion

The Pandemic has shown us the best and the worst side of society. Now time is here when the theory of 'Welfare state'[14] will get to play its real role. For a country like India earning the distinction of successfully holding the largest lockdown, and having one of the lowest possible death rates, is no easy feat to achieve, considering the level of poverty, illiteracy, despicable state of health. This is a result of the strong belief that we have placed in our Constitutional values, the right to hold our government accountable, the Watchdog of the Indian Constitution- Supreme Court.

Conflict of Interest- Nil

Source of Funding - Self

Ethical Clearance- The author has used a doctrinal method for research. All the information and discussion in the article in the paper is based on the author's analysis is of data secured on Informed consent and available publicly.

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2. "Coronavirus | Janata curfew on March 22 between 7 am and 9 pm.The Hindu [newspaper on Internet] 2020 MARCH 19 [cited on 2020 May 26]. Available at :https://www.thehindu.com/news/national/narendra-modi-speech-live-updates-coronavirus/article31108793.ece
3. *District of Columbia v Heller [554 U.S. 570 (2008)] is* landmark case in which the Supreme Court of the United States held that the Second Amendment protects an individual's right to keep and bear arms, unconnected with service in a militia, for traditionally lawful purposes, such as self-defense within the home
4. McDonald v. City of Chicago [561 U.S. 742], case in which on June 28, 2010, the U.S. Supreme Court ruled (5–4) that the Second Amendment to the U.S. Constitution, which guarantees "the right of the people to keep and bear Arms," applies to state and local governments as well as to the federal

government

5. Section - 144, Code of Criminal Procedure,1973. [Internet][citedon2020May27] Availableathttp://legislative.gov.in/actsofparliamentfromtheyear/code-criminal-procedure-act-.1973 This provision authorises the Executive Magistrate of any state or territory to issue an order to prohibit the assembly of four or more people in an area. According to the law, every member of such 'unlawful assembly' can be booked for engaging in rioting
6. The Disaster Management Act, 2005. [Internet] [cited on 2020 May 27]Available at :https://indiacode.nic.in/handle/123456789/2045?view_type=browse&sam_handle=123456789/1362 The Government of India has declared COVID-19 as a National Disaster. The Act provides for "the effective management of disasters and for matters connected there with or incidental thereto
7. The Epidemic Diseases Act, 1897. [Internet][cited on2020May27] Available at:https://indiacode.nic.in/bitstream/123456789/10469/1/the_epidemic_diseases_act%2C_1897.pdf The Act is meant for containment of epidemics by providing special powers that are required for the implementation of containment measures to control the spread of the disease
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13. Consolidated Guidelines of MHA on Lockdown measures on containment of COVID-19. [Internet] Available at : https://www.mha.gov.in/sites/default/files/PR_ConsolidatedGuidelinesofMHA_28032020_0.pdf
14. Quadagno J.Theories of the Welfare State. Annual Review of Sociology .Vol. 13:109-128 (Volume publication date August 1987)[Internet][cited on; 2020 June1]Available at : https://doi.org/10.1146/annurev.so.13.080187.000545 . The theory of welfare state provides that;-

    "The welfare state provides that the state is the main protector and promoter of the economic and social well-being of the citizens,embodying the principles of equal opportunity, equitable distribution of wealth, and public responsibility especially for citizens unable to avail themselves."

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Pattern of Finger Prints in Raichur District Population A Prospective Studiy Conducted at Raichur Institute of Medical Sciences, Raichur

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Abstract

The present study was a prospective study conducted in LVD College, Raichur, Karnataka, and study period was December 2013 to November 2014 (One year). During study period, total of 210 subjects (40 male and 170 female) of Raichur origin were randomly selected and included in the study. The students were in the age group between 18 to 28 years. The purpose of the study was to find out the pattern of finger print patterns in Raichur district region.

Key Words: *Finger prints, Pattern, Region*

Introduction

The use of prints as a mean of personal identification is one of the common methods in forensic anthropology and the most popular prints are fingerprints. Even the fingerprints of twins are not similar[1]. Fingerprints of an individual are unique and remain unchanged from womb to tomb[2]. Dactylography also known as finger prints has been successful in field of Forensic science to identify individuals for both civil and criminal purposes. Theory of uniqueness is the scientific principle behind finger print analysis to convince the court of law.

Dactylography or the fingerprint system is based on the study of epidermal ridges and their configurations [Dermatoglyphics (derma = skin + glyphs=curves)] in the fingers, palms and soles.[3] The term dermatoglyphics was coined by Cummins.[4]

The analysis of finger prints for matching purpose generally requires the comparison of several features of the print pattern. These include patterns, which are aggregate characteristics of ridges and minutiae points which are unique features found within the patterns[6]. Galton classified the types of finger prints depending upon their primary pattern as loops, whorl, arches and composite.[5] An arch (plain and tented) is a pattern where the ridges enter from one side of the finger, rise in the centre forming an arc, and then exit the side of the finger and the loop (radial and ulna) is a pattern where the ridge enters from one side of a finger, form a curve and tend to exit from the same side they entered from. Whereas, in the whorl pattern ridges form circularly around a central point on the finger.[6] The term composite is used for combination of patterns that does not fit into any of the above classification.

Classification of Fingerprints

The most popular classification systems include the Roscher system, the Vucetich system, and the Henry

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Classification System. Of these systems, the Roscher system was developed in Germany and implemented in both Germany and Japan, the Vucetich was developed in Argentina and implemented throughout South America, and the Henry system was developed in India and implemented in most English-speaking countries.[7]

In the Henry system of classification, there are three basic fingerprint patterns: Loop, Whorl and Arch, which constitute 60–65%, 30–35% and 5% of all fingerprints respectively. There are also more complex classification systems that break down patterns even further, into plain arches or tented arches, and into loops that may be radial or ulnar, depending on the side of the hand toward which the tail points. Whorls may also have sub-group classifications including plain, accidental, double loop, peacock's eye, composites and central pocket loop.[8]

Based on the enhancing source of impression, fingerprints could be classified into various types such as Exemplar prints or known prints, which the name is given to fingerprints deliberately collected from a subject. Latent prints mean any chance or accidental impression left by friction ridge skin on a surface, regardless of whether it is visible or invisible at the time of deposition. Patent prints are chance friction ridge impressions which are obvious to the human eye and which have been caused by the transfer of foreign material from a finger onto a surface. Plastic prints are frictions ridge impression left in a material that retains the shape of the ridge detail.[9]

Prediction Potentials and Uses of Fingerprints

Sex Prediction: Females have more arches and ridges per cm², and males have more whorls. Men's prints tending to have higher levels of urea than women's.[10]

Diet and Life Style Prediction: Gelatin based tape and high-tech chemical analysis under spectroscopic microscope reveals the chemical and metabolic make-up found on a fingerprint. The study revealed that specific amino acids indicated whether the "suspect" was a vegetarian or meat-eater. Spectroscopic microscope method based on the study of chemicals and metabolic featured with a fingerprint can also reveal the use of substances, including: cigarettes, drugs, grooming products etc. [10]

Disease Risk Prediction: Patients with Alzheimer disease showed a significantly increased frequency of ulnar loops on their fingertips and a decreased frequency of whorls and arches. The positive predictive value of 6 or more digital whorls is an indicator of breast cancer.[11] It was reported dermatoglyphic correlated with diabetic's mellitus.[12] The dermatoglyphic patterns of obese patients are dependent on such inherited or genetic tendencies towards obesity.[13] Several studies have explored the relationship of dermatoglyphics alopecia[14], mental retardation, congenital heart defects and diabetes mellitus.[26] Some scientists believe that fingerprints improve grip, but others believe that it actually reduces friction between the skin and the surface of objects. One other idea is that fingerprints increase the sensitivity of touch.

Materials and Methods

The Source of data was students of L.V.D College, Raichur. The subjects were 210 persons (40 male and 170 female) of Raichur origin with known blood group and study period was one year i.e December 2013 to November 2014.

Recording of fingerprints: A self-inked pad was placed on a wooden table. The palmar aspects of the distal phalanges of a person's right hand were inked by applying firm pressure on the ink pad starting from the little finger. The unglazed white bond paper was applied firmly over a wooden pad. Then the bond paper which was divided into two (right and left), and each further into five columns marked as thumb, index, middle, ring and little. The finger prints were taken in the respective columns on the bond paper. The same procedure was done for recording the finger prints of left hand. Thus, finger prints of both hands will be obtained and recorded and classified as per Henry classification of the various finger patterns into four main types: Loops, Whorls, Arches and Composite.

Cases where there was any evidence of Permanent scar on any of the fingers, Hand deformity due to injury, Birth defect or disease of the hands, Students who were not from Raichur and Students who were below the age of 18 years or above the age of 28 years are excluded from study group. Descriptive statistical analysis comprising percentage is used to describe data and Chi-square of proportion shall be used to compare the

statistical significance of difference wherever required.

Results

During the present study a total of 210 students were included in the study group. Of which 170 (81%) were female and 40 (19%) were males. Male: Female = 1: 4.25

Table 1 shows the percentage distribution of the pattern of fingerprints on the 10 different digits on both hands the most common fingerprints pattern were loops (71.23%), followed by whorls (24.14%), arches (4.57%) and composite (0.04%).

Table 1: Fingerprint patterns in all the fingers

FP Type	Total	%
Loop	1496	71.23%
Whorl	507	24.14%
Arch	96	4.57%
Composite	1	0.04%

Table 2 shows the percentage distribution of the pattern of fingerprints on the 10 different digits on both hands, the most common fingerprints pattern were, maximum loops were seen in Little finger (16.42%), maximum whorls were seen in Ring finger(8.28%), arches were seen maximum in Index finger(2.04%) and composite was seen in Thumb(0.04%).

Table 2: Distribution of fingerprint patterns digit wise

	T	%	In	%	M	%	Ri	%	Li	%
L	314	14.95	270	12.85	328	15.61	239	11.38	345	16.42
W	88	4.19	107	5.09	70	3.33	174	8.28	68	3.23
A	17	0.81	43	2.04	22	1.04	7	0.33	7	0.33
C	1	0.04	0	0	0	0	0	0	0	0

Note: L-loops, W-whorls, A-arches and C-Composite

T-thumb, In-index finger M-middle finger, Ri-ring finger, Li-little finger

Table 3 shows the percentage distribution of the pattern of fingerprints on the 10 different digits on both hands, most loops pattern were seen in LL finger (177), maximum whorls were seen in RR (94), arches were seen maximum in LI (23) and composite was seen in RT (1).

Table 3: Distribution of fingerprint patterns in the individual fingers

	RT	RI	RM	RR	RL	LT	LI	LM	LR	LL	Total
Loop	157	131	170	112	168	157	139	158	127	**177**	1496
Whorl	43	59	31	**94**	38	45	48	39	80	30	507
Arch	9	20	9	4	4	8	**23**	13	3	3	96
Composite	**1**	0	0	0	0	0	0	0	0	0	1

RT-right thumb, RI-right index, RM-right middle, RR-right ring, RL-right little

LT-left thumb, LI- left index, LM- left middle, LR- left ring, LL- left little

Table 4 shows the percentage distribution of the pattern of fingerprints on the 10 different digits on both hands according to sex. In males (n= 400) the most common fingerprints pattern were loops (60.25%), followed by whorls (31%) and arches (8.75%). Similar patterns were seen in females (n=1700) as Loops (73.82%), Whorls (22.52%), Arches (3.59%) and Composite (0.06%). In both the sexes, loops exhibit high percentage of occurrence while composite was the least.

Table 4: Distribution of fingerprints sex wise

Finger print pattern	Male	%	Female	%	Total	%
Loop	241	60.25%	1255	73.82%	1496	71.23%
Whorl	124	31%	383	22.52%	507	24.14%
Arch	35	8.75%	61	3.59%	96	4.57%
Composite	0	0%	1	0.06%	1	0.04%
Total	400	100%	1700	100%	2100	100%

Discussion

Similar studies were conducted by various authors and their observations were as follows: in Bharadwaja et. al study the loops were the most common pattern, registering 51.87% frequency in the study; followed by whorls (35.83%) and arches (12.30%).[15]

In Bhavana D et. al study, it was observed that finger print patterns of all the fingers in both the hands. Total no of loops found in all the digits were 1178 (58.9%). Similarly numbers of whorls in all the digits of both the hands were 592(29.6%) and numbers of arches were 230(11.5%). This table clearly indicates that Loops were more common pattern followed by whorls and arches.[16] Frequency of Loops were found to be higher in females;

620 (52.63%) and Whorls were found to be higher in males; 338(57.09%). Arches were found more in females; 119(51.73%) compared to males; 111(48.26%). Loops are most commonly found fingerprint patterns and Arches are least common. The only association between gender and finger print patterns in this study is that Loops and Arches were found in higher frequency in Females compared to Males and whorls were found to be high in males compared to females.[16]

In the study conducted by Prateek Rastogi et. al, the fingerprint pattern analyses showed that, loops were the most common pattern in the study 1221(60.95%), followed by whorls 649(32.55%) while arches were present in a smaller percentage (6.5%) of the study group. Frequency of loops was found to be higher in females (52.42%) than in males (47.58%) whereas whorls were more frequent in males (55.78%) as compared to females (44.22%). 44.61% of arches were present in males and 55.38% in females.[17]

A study by Umraniya et. al it was seen that Loops were the most common pattern, registering (51.54%) frequency in the study; followed by whorls (35.79%) and arches (12.67%).[18]

In Deepa Deopa study - A total of 140 first year and second year MBBS students belonging to the age group 18- 25 year of Government Medical College, Haldwani were included in the study. Loops were the most common (58.29%) fingerprint pattern while whorls were moderate (37.00%) and arches were the least common (4.71%). Males had a higher incidence of whorls and females had a higher incidence of loops.[19]

Bharadwaja A, Saraswat PK, Agrawal SK, Banerji P, Bharadwaj S in the study, The general distribution of pattern of finger print showed high frequency (51.87%) of loops whereas whorls were moderate (35.83%) and arches were least (12.30%) in frequency.[15]

Conclusion

Identification is of paramount importance in any medico-legal investigation. Identification means the determination of the individuality of a person.[1] Personal identification is one of the most challenging processes confronted by mankind. In the living, in civil courts, identification is required in cases such as insurance, pension and inheritance claims, marriage, disputed sex and missing persons. In criminal courts, it is required in cases of absconding soldiers and criminals, persons accused of assault, rape, murder, etc., impersonation and interchange of babies in hospitals.[2] In the dead it is needed for: [3] the ethical and humanitarian need to know which individual has died, especially for the information of the surviving relatives.

Conflict of Interest: None

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Ethical Clearance: Instititutional Ethical Clearance Taken

Refernces

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Management of the Diagnostic and Therapeutic Obstacle to Successful Endodontics in C Shaped Canals: A Case Series

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Abstract

Comprehensive knowledge of the anatomy of the root canal system is of paramount importance in successful endodontic treatment. It has been stated that a single tapering canal with a single apical foramen is an exception rather than a rule. Hence, awareness of unusual morphologies in the canal system is critical for favourable outcome of treatment. Efficacious biomechanical preparation and obturation depends upon the timely identification of the root canal system. An important variation of the root canal system is the C shaped canal configuration. It presents with a fine fragile fins or webs joining the root canals causing inadequate debridement of the root canal system compromising the prognosis of treatment. This case series presents successful diagnosis, accurate radiographic visualisation of microanatomy using contrast media and management of four cases of C-shaped mandibular second molars with different morphological variations.

Key-words*: C shaped canal, contrast media, mandibular second molar, root canal treatment, root canal morphology, endodontic treatmen*t

Introduction

Accomplishing success in endodontic therapy necessitates a comprehensive and detailed expertise in the knowledge of the root canal system and its variations. [1-3] Corresponding to diagnosis and treatment planning, precision in endodontic therapy is essential for a favourable outcome. [3] Amongst the different anatomical variations in the root canal systems, the unconventional and complex configuration of the C shaped roots and root canals presents with clinical adversities and challenges, compromising the objectives of endodontic therapy.[4] Cooke and Cox in 1979 first documented the C shaped configuration in root canal anatomy, which was termed owing to the 'C' shaped cross sectional anatomy of the root and/or root canal. [5]

A high prevalence of C-shaped canal configuration has been reported in mandibular second molars ranging from 2.7% to 44.5%.[6] Various explanations have been postulated for its formation, the most comprehendible being the failure of Hertwig's epithelial root sheath to fuse on the buccal or lingual root surface. A prism shaped or conical root results when the sheath fails to fuse on both the buccal and lingual sides.[7] If the Hertwig's epithelial sheath fails to fuse on the lingual side, a buccal groove is formed and vice versa. This sort of fusion is asymmetrical with two roots joined by an attenuated inter-radicular bow, and is inversely proportional to distance between root canals i.e, smaller the distance, higher the incidence of fusion between canals.[8,9]

C shaped root canal configurations generally feature fused roots, cross-section of canal corresponding to the configurations classified by Melton or Fan, and a longitudinal groove either on the lingual or buccal surface of root showing radiographic appearance of a 'third canal' in between two roots.[1,10] Classifications given by Melton *et al*, modified by Fan *et al* are the widely accepted classification systems that characterizes the intricacies of this configuration, enabling adequate biomechanical preparation followed by a hermetic

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seal at the apex. Nonetheless, efficient pulp space therapy of root canal system remains ambiguous due to the complexities associated with the intricate canal morphology, and is highly attributed to their narrow working area and wide fins.[1,7,11]Additionally, the two-dimensional view of the radiograph professes diagnostic obstacles. Hence, conscientious debridement along with precise negotiation of root canals is fundamental in their successful treatment.[12]Shearer *et al* advocated that using a radiopaque contrast medium for evaluation of root canal morphology would be a valuable aid in detecting the exact locations of lateral canals, fins, isthmus of the anastomosis.[13]

By virtue of the morphological variations in C-shaped root and root canal cases, it is necessary to opt for a befitting system for its diagnosis and treatment. These case reports present the successful management of various C-shaped canal configurations in mandibular second molars by accurate diagnosis and strategic treatment, using iopamidol contrast media for visualising the complex canal spaces.

Case Reports

Case I

A 27-year-old female patient reported with a chief complaint of pain on her lower left back tooth since a week. Medical history was non-contributory. Intraoral examination revealed a large composite restoration on tooth #47 FDI with tenderness. Vitality test exhibited delayed response to cold and electric pulp testing. Preoperative radiograph revealed presence of secondary caries below the composite restoration approximating the pulp, with fused mesial and distal root with an invariable anatomy, raising the suspicion of a C-shaped canal [Fig 1a]. The tooth was diagnosed as a necrotic tooth with symptomatic apical periodontitis. Informed consent was obtained from the patient.

Under magnification (Zeiss Opmi Pico) of 2.5X, the tooth was anesthetized followed by isolation with rubber dam. Access to pulp chamber was achieved using Endoaccess bur #2 and refined using EZ bur (Dentsply Maillefer, Switzerland). DG16 explorer was used to locate canal orifices. After debridement of pulp chamber remnants, a C-shaped canal configuration was observed with a single canal adjacent to it i.e., semicolon shaped canal conforming to Fan's anatomic classification C2 [Figure 1b].

The working length estimation was done using apex locater (RootZX II, J.Morita, USA), and confirmed with digital radiographs [Figure 1c]. The biomechanical preparation was done using hand K files and ProTaper Gold rotary files (Dentsply Maillefer, Ballaigues, Switzerland) up to F3. To avoid strip perforation, anti□ curvature filing motion was used. The canals were flushed with 3% sodium hypochlorite (Pyrex, Prime dental products, Mumbai, India), 17% EDTA solution (Dent Wash, Prime Dental Products, Mumbai), normal saline and final rinse with 2% Chlorhexidine solution. Calcium Hydroxide intracanal medicament paste was injected in the canals and the tooth was temporized. The patient was recalled after 2 weeks.

In the following appointment, canals were flushed with 3% sodium hypochlorite and activated using endoactivator (Dentsply Maillefer, Switzerland). After a rinse of distilled water, a mixture containing 3% sodium hypochlorite, 17% aqueous EDTA and contrast medium iopromide (Manus Aktteva Biopharma LLP, Gujarat, India) was used to fill the canals from apex to orifice using a 28 guage side vented needle. A radiograph was taken to visualise canal spaces and isthmi (Fan radiographic type I)[Fig 1d]. Following this, the canals were flushed with distilled water and then with 2% chlorhexidine as the final rinse. The canals were dried using paper points (Dentsply Maillefer, Switzerland). Gutta percha cones were fitted to the working length and a radiograph was taken [Figure 1e]. The canals were obturated using continuous wave technique followed by backfill using thermoplasticized gutta percha (Elements, SybronEndo, Orange, CA, USA) after coating the canals with calcium hydroxide based sealer (Sealapex, SybronEndo, Orange, CA, USA). The access cavity was restored using flowable resin based composite as base followed by bulk fill resin (Filtek Z350 XT, 3M Espe, USA). A post-obturation radiograph revealed sealing of the isthmi. [Figure 1f]

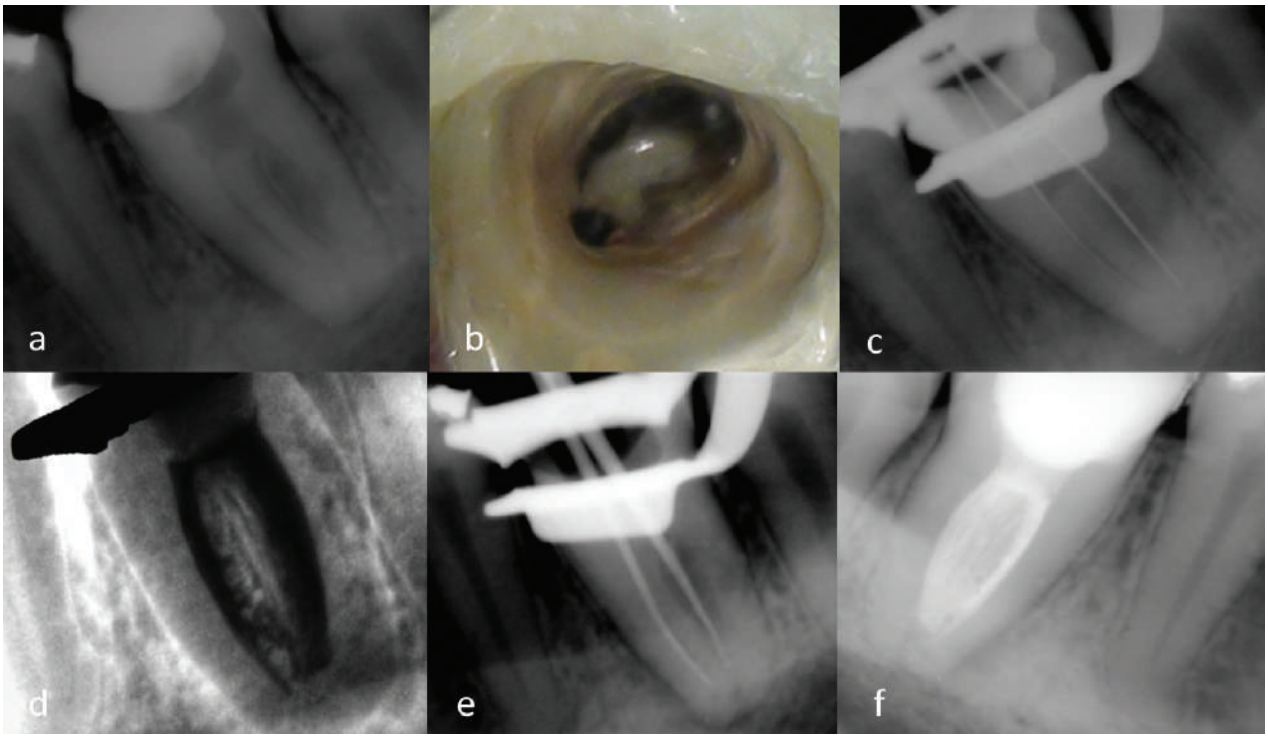


Fig 1 : a)Preoperative radiograph, b)Access cavity showing the location of the orifices of the canals in C-shape, c)Working length radiograph, d) Radiograph with contrast media in the canals, e) Master cone Radiograph, f) Post Obturation radiograph.

CASE 2

A 34-year-old male patient reported with chief complaint of pain in his lower left back tooth since two days. Clinical examination revealed occlusal caries on tooth number 37 FDI with pain on percussion. The tooth exhibited lingering response to electric and cold vitality testing. Intraoral digital radiograph revealed radiolucency involving the enamel, dentin and a pulp horn with mesial and distal roots converging, indicating possible presence of a C shaped canal configuration. [Fig 2a]. The case was diagnosed as irreversible pulpitis with symptomatic apical periodontitis.

Following obtaining informed consent from the patient, local anesthesia was administered with subsequent rubber dam isolation, after which access cavity was prepared under magnification. Two main canal orifices were located joined by a continuous fissure, indicating Fan anatomic C1 configuration at orifice level. [Fig 2b]

Working length estimation was done using apex locator locater and confirmatory digital radiographs. Cleaning and shaping was achieved by hand K files, followed by rotary instrumentation upto #30 with Hyflex CM 4% taper files (Coltene, India). 3% sodium hypochlorite was delivered using Endovac irrigation system (Sybron Endo,India) to facilitate apical cleansing while avoiding irrigant extrusion. Calcium hydroxide intracanal medicament was placed and the tooth was temporized.

On recall after 2 weeks, similar protocol of evaluation of canal anatomy by a combination of contrast media and irrigants was carried out, revealing Fan radiographic type II morphology of the canals. The canals were obturated using 4% gutta percha cone and Sealapex sealer with downpack using heated pluggers (System B, SybronEndo, Orange, CA, USA) [Fig 2c]. Access restoration was done using glass ionomer (Fuji IX, India). Post obturation digital radiograph revealed a thin margin coronally. [Fig 2d,2e]

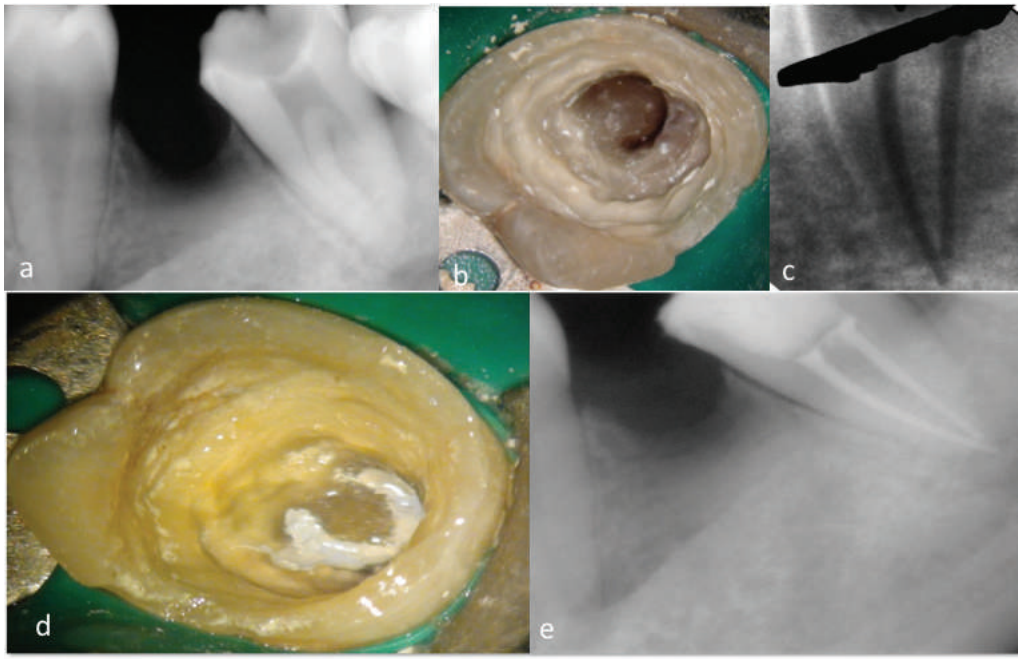


Fig 2: a) Preoperative radiograph, b)Access cavity showing the location of the orifices of the canals in C-shape, c) Radiograph with contrast media in the canals, d)Obturation at the orifice level, e) Post Obturation radiograph

CASE 3

A 26-year-old female reported with chief complaint of decay and pain in lower left back tooth since a month. Clinical examination revealed carious tooth #37 FDI with pulpal involvement and tenderness on percussion. Tooth was responsive to cold test, with pain which lingered after removal of stimulus. Radiographic examination revealed presence of deep occlusal carious lesion approaching pulp with presence of conical and fused roots with thin radiolucent line in-between, suggesting C-shaped root canal[Fig 3a]. The diagnosis was chronic irreversible pulpitis with symptomatic apical periodontitis after which informed consent was obtained from the patient prior to commencing the procedure.

Liquid dam (Oraseal,Ultradent products,Inc.,South Jordan, UT,USA) with wedgets was used for isolation for clear visibility of the pulp chamber. After gaining access into pulp chamber, semicolon shaped orifices were observed. Although a Fan anatomic C2 configuration was observed at the orifice level[Fig 3b], working length confirmatory radiograph revealed this semicolon shaped canal to be dividing into two canals. Hence, a total of three main canals were noticed, where the mesiobuccal and distal canals fused[Fig 3c]. Shaping was achieved with hand K files and protaper rotary instrumentation (upto F3). The disinfection was done using passive ultrasonic irrigation of 3% sodium hypochlorite, 17% EDTA solution and normal saline. Calcium hydroxide intracanal medicament was placed and temporized.

In the second visit, contrast medium radiograph revealed clearly visible canal spaces, showing large isthmus in coronal third, and fusion of the canals at the junction of middle and apical third[Fig 3d]. Necessary changes were done in biomechanical preparation using hand and rotary files along with targeted irrigant delivery to facilitate disinfection of these anastomoses. Master cone was placed to confirm tug back and a confirmatory radiograph was taken[Fig 3e]. The fusion point seemed to be too narrow for solid gutta percha cone to enter. Thus, Elements obturation system was used to successfully deliver plasticized gutta percha into this narrow fusion point and the canals were backfilled using extruder handpiece and condensed at a level slightly below CEJ[Fig 3f]. The access was restored by incremental build-up using flowable (base) and packable (bulk) composites.

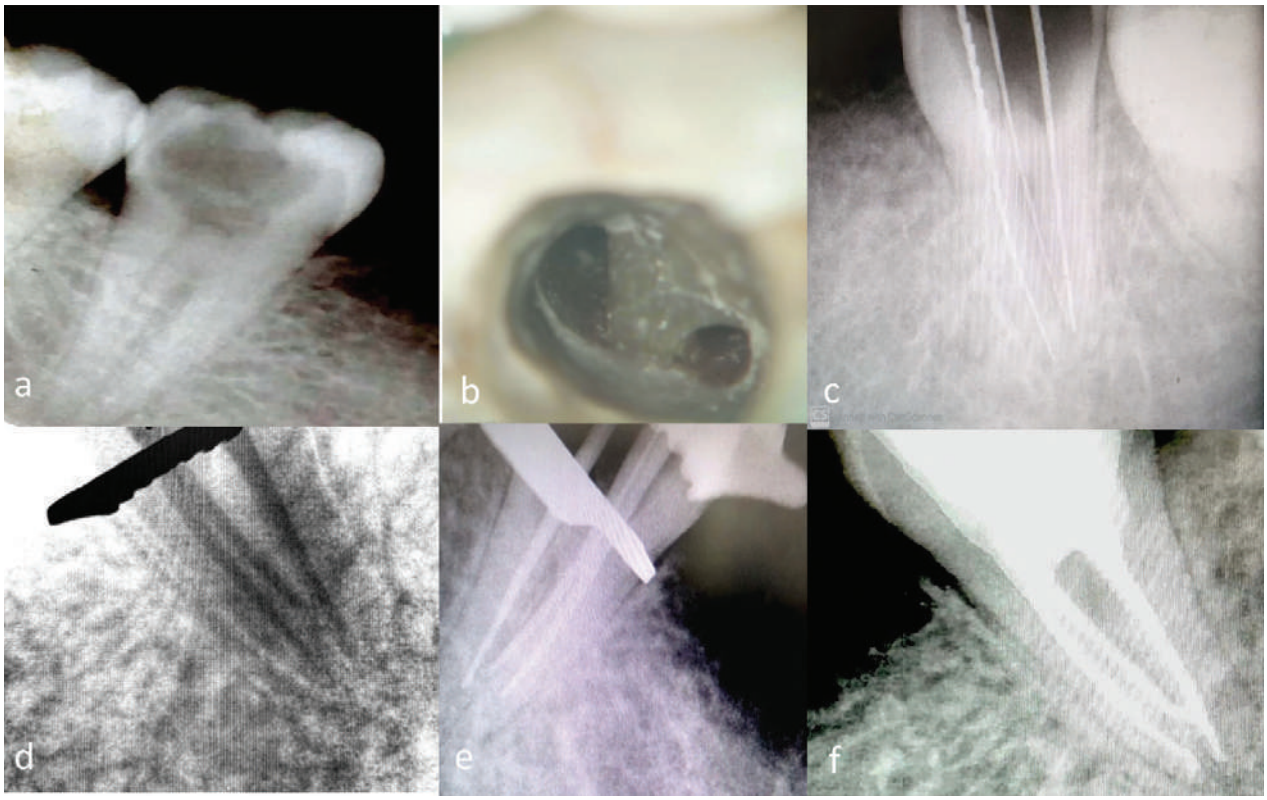


Fig 3: a) Preoperative radiograph, b)Access cavity showing the location of the orifices of the canals in C-shape, c) Working length radiograph, d)Radiograph with contrast media in the canals, e)Master cone Radiograph, f)Post Obturation radiograph

CASE 4

A female aged 22 years reported with pain in lower right back tooth since one month. Clinical examination revealed a restored tooth #47 FDI and tenderness on percussion. Tooth was responsive to cold test, with lingering pain after removal of stimulus. Radiographic examination revealed presence of a deep restoration close to pulp[Fig 4a]. It also revealed presence of dentine fusion with appearance of a 'third canal' in between, suggesting C-shaped root morphology. The case was diagnosed as chronic irreversible pulpitis with symptomatic apical periodontitis.Informed consent was obtained from the patient.

Following isolation with liquid dam, access was gained to the pulp chamber, a semicolon shaped orifice was noticed depicting Fan anatomic C2 configuration[Fig 4b]. Working length estimation was followed by a confirmatory radiograph[Fig 4c]. Cleaning and shaping was acheived by hand K files and rotary instrumentation. Similar disinfection protocols as the previous cases were followed. Intracanal medicament, calcium hydroxide was placed in the canals and recalled after 2 weeks.

On evaluating the canal anatomy with the help of contrast media, the canal spaces revealed an isthmus only in the coronal third, and two separate asymmetrical canals with separate apical exits. The distal canal seemed to be wider than the mesial due to the isthmus[Fig 4d]. A thermoplasticised gutta percha continuous wave condensation technique with System B hand piece was used for the obturation. The canals were backfilled with the extruder handpiece keeping in mind the large isthmus at the orifice level, and condensed at a level slightly below CEJ[Fig 4e,4f]. The access was restored by a sandwich technique using flowable and packable composites.

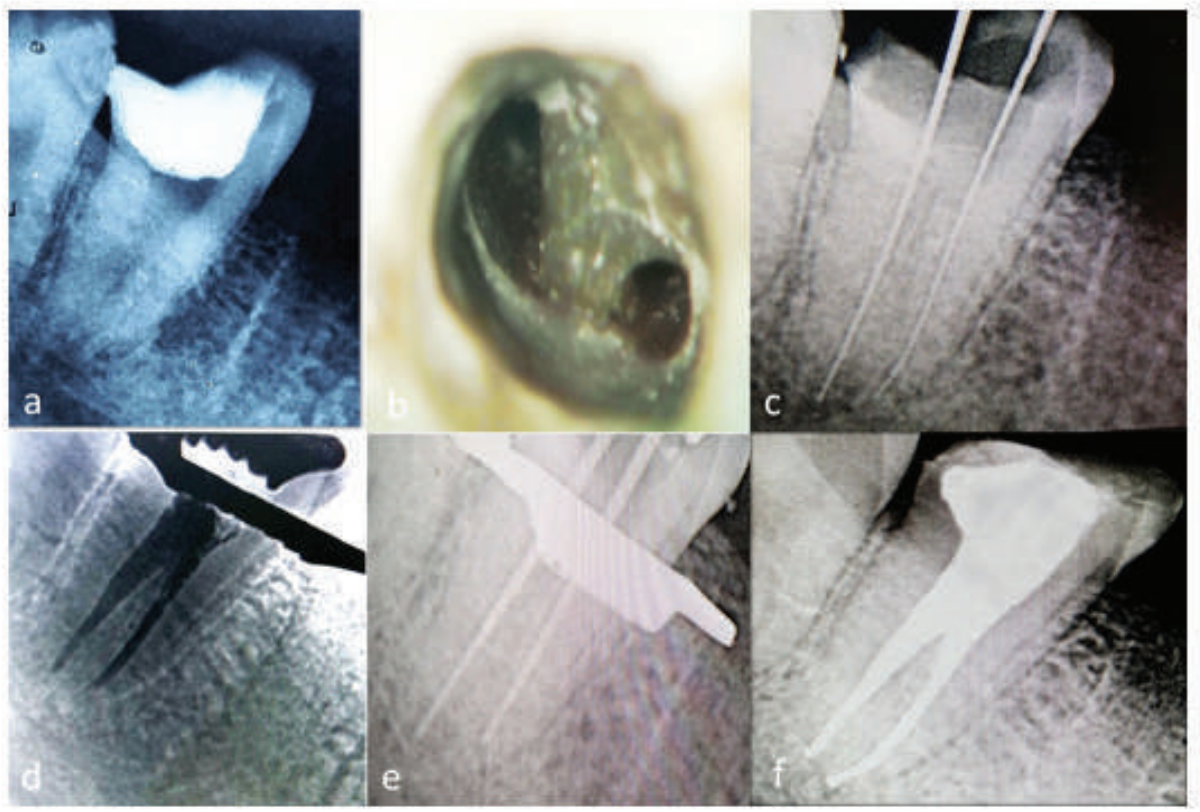


Fig 4: a) Preoperative radiograph, b)Access cavity showing the location of the orifices of the canals in C-shape, c) Working length radiograph, d)Radiograph with contrast media in the canals, e)Master cone Radiograph, f)Post Obturation radiograph

Discussion

The C shaped canal configurations has always been seen in varied forms and never affirms to any confirmed structural form.[14]The earliest classifications for the C shapes configuration were proposed by Manning and Melton *et al*.[1,7] Further, Fan *et al*. modified this classification after analysis using transverse sectioning and micro-computed tomography.[10]Al Fouzan stated that irrespective of presence of canal or orifice independently, there existed a requirement of customary outline of "C" and presence of canals in a C shaped root to be classified under this configuration.[15] These systems of classification help in interpreting anatomies of C-shaped canals and roots. The four cases reported in this case report were treated according to Fan *et al* classifications based on different preoperative radiograph angulations, clinical examination after access opening, working length estimation radiographs and contrast media radiographs.

The C shaped canal configurations have been observed to show an ethnic predilection, the anatomy being more common in Asians.[16]Other than a high incidence in mandibular second molars, the C-shaped configuration has also been reported in maxillary first molars (0.12%), maxillary third molars (4.7%), mandibular third molars (3.5%-4%), mandibular second premolars (1%) and maxillary lateral incisors with type III radicular groove. Bilateral occurrence (70-81%) has also been documented .[17]

The anatomical map of pulpal floor, orifice placed below level of CEJ, persistent bleeding or pain even after location of canal orifices and presence of a fin or web connecting the individual canals are the fundamental criteria to detect C-shaped canal configurations.[16,18] In the present case series, all cases were treated under dental operating microscope. The radiographs of working length estimation have proven to be more helpful than preoperative radiographs in detection of C-shaped canals.[20,21]The instruments converging at the

apex of root or a canal being centred in the pulp chamber and exiting through furcation are the criteria used to determine the presence of C-shaped canals.[22]

Previous studies have stated use of water soluble contrast medium during radiographic diagnosis to significantly enhance radiograph interpretation. Fan *et al* advocated that introduction of a water soluble contrast medium into a C-shaped canal system improves chances of effective canal identification even when a bone image superimposition exists, especially in cases of second molars.[23] This also gives a better understanding of the apical termination of preparation. As the deep canal spaces can be visualised pre-obturation, the preparation may be modified depending upon the contrast media radiograph, whose Visibility of contrast media in pulp spaces could be enhanced with inversion of the image digitally. Since a thermoplasticised obturation was planned for the cases, this contrast method of diagnosis helped in detecting the presence of isthmi, fins and lateral canals, which helped target those areas with adequate control. The anatomic & radiographic classifications of the four cases according to Fan's classifications are depicted in Table 1.

TABLE 1 – Categorization of the case reports presented according to Fan's classification

	Anatomic classification (based on Fan et al. 2004)	**Radiographic classification (based on Fan et al. 2007)**
Case 1	C2 at orifice level	Type I
Case 2	C1 at orifice level	Type II
Case 3	C2 at orifice level	Type I
Case 4	C2 at orifice level	Type III

The contrast media mixture containing 3% sodium hypochlorite, 17% aqueous EDTA and iopromide is similar to the Ruddle solution which contained hypaque (aqueous radiopaque solution of Iodine salts namely, ditrizoate and sodium Iodine) as the contrast dye. The mixture helps control the viscosity of the solution as iopromide alone is highly viscous. Sodium hypochlorite helps in dissolution of pulpal contents and elimination of bacteria along with their toxins from the root canal system; simultaneously disinfecting the intricate spaces and enabling the contrast medium to enter into the slender networks of C-shaped morphology. EDTA, a chelating agent, was used to reduce surface tension and remove the inorganic contents in the canal, thereby allowing unimpeded flow of the mixture. Additionally, iopromide has been shown to have high iodine content, thus diluting it with standard irrigants did not affect the radiopacity extensively. The visibility of the canal spaces on introduction of the solution mixture was excellent, especially on invertion of images digitally. This technique can also be used for detection of perforations, fractures, ledges and for other similar diagnostic measures.

Due to the presence of canal aberrations and irregularities in C-shaped canals, adequate cleaning and shaping presents an arduous challenge.[7,24] Abou-Rass *et al*. advocated anti-curvature filling to prevent strip perforation of the thinner walls.[25]This technique of filing was used in all the above cases to preserve the thin lingual wall in C shaped roots. Cheung LH *et al* suggested the ideal biomechanical preparation of C-shaped canals to be done by rotary instruments with ultrasonic irrigation as an adjunct.[26] In the present case reports, along with rotary instrumentation, the cases were irrigated with sonic and/or ultrasonic irrigation to facilitate cleansibility. Placement of calcium hydroxide as an intracanal medicament for a period of 7-14 days

has been recommended, which was followed whilst treating these cases.[27]

Attaining a three dimensional hermetic seal poses a problem due to complexities of canal anatomy. The favoured method to achieve the goals of obturation in C shaped morphology is warm vertical condensation in which the downpack is preferably done using continuous wave compaction method followed by backfill using the thermoplasticized gutta-percha technique.[16,28]In any case, achieving a three-dimensional seal of root canal is of utmost importance.

Conclusion

A fitting diagnosis and treatment plan following a precise examination plays a principle role in successful management of C-shaped canal configurations. C shaped canals present with varied configurations and has no certainties in the ways it presents. Hence, a profound awareness of the varied presentations of the C shaped canal anatomy will facilitate the management of the cases effectively and also for good long term prognosis.

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Profile of Victims of Rape in the Transkei Sub-Region of South Africa (2006-2014)

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Abstract

Background: Rape of a women is a serious public health problem in South Africa. It is a complex issue, but it become more complex when raped either elderly women or a baby. **Objective:** To study the profile of victimsof rape in the Transkei sub-region of South Africa (2006-2014).

Method: This is retrospective descriptive study over a period of 16 years (2006-2014), carried out at Sinawe Thuthuzela Center (STC), Mthatha, Eastern Cape, South Africa.

Results: Between 2006 and 2014, 6699 cases of rape were recorded in the register of Sinawe-Thuthuzela Center. Of this, 3208 (47.88%) were children of the age of 16 and below. Majority 3048 (45.5%) of rape were outside of their home. Victim was known in 3418 (51%) of cases. Only 1064 (15.9%) sustained physical injury. Alcohol has contributed in 56.2% cases. Delayed (>72 hrs) reporting to STC were 2052 (30.6%). HIV positive were found 1180 (17.6%) victims at the time of reporting to center.

Conclusion: There is a high number of rape in the Transkei sub-region of South Africa. About half of them were children under the age of 16 years. It needs urgent attention.

Keywords*: sexual assault, rape, HIV, poverty*

Introduction

Global estimates published by WHO indicate that about 1 in 3 (35%) women worldwide experienced either physical and/or sexual intimate partner violence or non-partner sexual violence in their lifetime.[1] The first sexual experience for many women was reported as forced: 17%of women in rural Tanzania, 24% in rural Peru, and 30% in rural Bangladesh.[1]South Africa has one of the highest rates of rape reported to the police in the world and the largest number of people living with HIV.[2]The 43,195 number of rapes were reported in South Africa in 2014/15, it means that for every 100,000 people in the country there were 80 rapes reported.[3]There is considerable concern about the links between rape and HIV problems.[2]There is a high number of rapes but only 14% of rapes result in convictions.[4]

A rural South African women who experienced intimate partner violence and had high gender inequity in relationships had increased incidence of HIV infection.[5]South Africa is in the midst of a catastrophic AIDS epidemic. HIV prevalence statistics in most countries indicate that up to 60% of all new infections occur among 15 to 24 year olds.[6]Alcohol dependency and binge drinking are significantly associated with transactional sex in South African women.[7] Alcohol may fuel once-off sexual encounters, and women's limited authority to negotiate sex and condom use; factors that can facilitate transmission of HIV.[8] A rural South African based study showed that 14% of sexually active young women reported engaging in transactional sex. Engagement in transactional sex was associated with an increased risk of HIV positive.[9] The trio of poverty, sexual assaults and HIV infection are complimentary to one another. [10]

A recent (2017) report on poverty showed that thirty million South African living in poverty. The number of people living in poverty has increased from 27 million

in 2011, to 30 million in 2015, with almost 14 million people living in extreme poverty. Majority (46.6%) black African's living in poverty. The poorest of the poor currently live on R531 per month and spend nearly 30 percent of this amount on food every month.[11]The purpose of this study is highlight the problem of rape in the Transkei sub-region of South Africa, and also discuss the associate related factors of rape in this region.

Subjects and Method

This is a retrospective descriptive study at Sinawe Thuthuzela Center which is attached with Mthatha Regional Hospital (MRH), Mthatha. This is one of the 30 centers in South Africa provides one stop care to the victims of rape. It is catering about a half million people in this region. It serves to 16 police stations in this region such as Ugie, Mclear, Tsolo, Qumbu, Elliot, Elliotdale, Mqanduli, Libode, Lurhasini, Bityi, Coffee Bay, Ngqeleni, Mthatha central, Madeira, Ngangelizwe, Engcobo, and Dalasile. The data were collected by the clerk with case number, age, gender, pregnancy test, HIV test results, number of perpetrators, and place of incident. These data were analyzed by ANOVA program and displayed in table and graphic forms.

Results

Between 2006 and 2014, 6699 cases of rape were recorded in the register of Sinawe-Thuthuzela center (Table 1). Of this, 3208 (47.88%) case were children of the age of 16 years and below (Table 1 and Figure 2). Majority 3048 (45.5%) of rape were taken place outside of their home (Table 1). There were 3418 (51%) victims known to the perpetrator of rape (Table 1). Only 1064 (15.9%) victims reported physical injury, and 3624 (54.1%) were genital trauma on examination (Table 1). The history of alcohol intake was found in 56.2% cases of victims of rape in this study (Table 1).

Use of condom was found in 1783 (26.6%) cases, and 3443 (51.4%) women were not washed after the rape (Table 1). The highest number 2402 (35.9%) of victims of rape were reported between 24 and 47 hours of rape, followed by more than 72 hours in cases of 2052 (30.6%) in this study (Table 1). There were 1180 (17.6%) cases were found HIV positive on screening test of HIV (Figure 3). In 3683 (55%) raped victims were complaint with HIV post exposure prophylaxis, while 911 (136%) were not complaint in this study (Table 1).

Table 1. Profile of victims of rape in the Transkei sub-region of South Africa.

Characteristics	Frequency (%)
Group of rape victims	
Adult	3491 (52.1)
child	3208(47.9)
Place of rape	
Outside home	2574(38.4)
Inside home	3048(45.5)
Not Known	1077 (16.1)
Relationship of perpetrator	
Known	3418(51.0)
Unknown	2984(44.5)
Family member	297(04.4)
Physical injury	
Yes	1064(15.9)
No	5635(84.1)
Genital injury	
Yes	3624(54.1)
No	3075(45.9)
Rape under alcohol	
Yes	3764(56.2)
No	2935(43.8)
Use of condom	
Yes	1783 (26.6)
No	4916(73.4)
Washed genitalia	
Yes	3256(48.6)
No	3443(51.4)
Delay in reporting	
<12 hours	919(13.7)
<24 hours	2402(35.9)

Discussion

Between April and December 2016, 30 069 cases of rape were reported. It has decreased from 32161 for the same period the previous year. It amounts to approximately 110 cases of rape per day. The rate decreased from 58.5 per 100 000 to 53.8 per 100 000 people.[12]There were 6699 victims (average 744 per year) of rape reported over a period of 9 years (1006-

20014) in the Sinawe Thuthuzela Center.There was approximately 41000 rapes.were recorded in 2014/15 in South Africa, which comes out 74.5 per 100 000 of population.[12] In a population of about half million in the sub-region of Transkei, the rate of rape was 148.8 per 100 000 population, which is two to three-foldhigher than South African average (Table 1). The probable causes of high rape in this region is an extreme poverty, gender inequality, patriarchal notions of masculinity, and the exposure of abuse in childhood and fractured families.[13]The additional contributory factors in perpetuating rape in this region are high unemployment, alcohol and drug abuse, family breakdown as well as the social norms and values that condone and perpetuate violence are some of the major contributing factors to violence against children in South Africa.[14]

A little less than half (45.5%) women were raped, outside of their home (Table 1). Most of them were raped either coming from their work especially little bit late in night or waiting for a taxi in town. These are young girls or women who are walking to their destination either at home or school. A little less than one third (38.4%) of womenraped at home were elderly women who are staying because of their old age or disability (Table 1). The home should be the safest place, a place of comfort and freedom from fear; yet a high number (38.4%) of women and children are attacked, raped and abused in their homes, often by people who are supposed to protect them (Table 1). It should be the responsibility of a civil society to protect these vulnerable groups from harm such as children and elderly women. The forced marriages of young children and rape for money in Xhosa community are on rife.[10]A little less than half (47.9%) in this study were children younger than 16 years of age (Table 1 and Figure 2).A recent (2017) study published by the author showed that there is a high number of rapes among elderly women in the Mthatha area of South Africa.[15] According to this study 83% cases of elderly rape taken place in the home.[15]About half (51%) women or girls were known to the victims of rape.[15]A majority of rapist were known their victims such as ex-boyfriend or some of their family members.[16]There was a recent study (2017) carried out by Jewkes et al showed that 14.3% had raped a current or ex-girlfriend or wife.[2]There were one at least one in six victims (15.9%) of rape reported physical injuries (Table 1). There were no injuries found in more than four-fifth (84.1%) of the victims of rape in this study (Table 1). Health care providers, as experts, are required to use evidence related to physical injuries in the court as a sign of resistance, and it indicate that the force has used in the sexual assault, against the will of a victim.[17]

Genital injuries are the positive sign of rape, and it was recorded in about more than half (54.1%) cases of rape (Table 1). Genital injuries especially ruptured hymen could easily identified in the young children.[18]In adult it is difficult especially among women who delivered baby through vagina. It does not mean that rape has not taken place. The interpretation of evidence of injuries in adult is much more complex.[17]This creates a problem for health care practitioners who has to testify in court especially in cases where no injuries have been found.[17]No documentation of injuries in J88 form, could be assumed by the defense lawyers that no injuries means no rape.[19]This may not be true as most of the rape occurred on the threat of gun or a knife. The major issue of concern is lack of uniformity in examining of victims of rape and the skills and experience of the examiner and discrepancies in recording of injuries.[20]

Use of alcohol was found in more than half (56.2%) of the victims of rape in this study (Table 1). Women in South Africa who consume alcohol at high rates, particularly those who are bartender, may particularly vulnerable to forced sex experiences.[21]It is alcohol played a role either perpetrator was drunk or both were drunk. It mostly happened in night hours especially in local alcohol supplying outlets like sheebeen. Men purchase alcohol for women, and in turn feel a sense of sexual entitlement that could lead to forced sex.[22]About one fourth women (26.6%) were using condom with their partners in this study (Table 1). A structured interview based study conducted in Eastern Cape Province (2014) on condom use, over all, 49.2% using condom.[23] This is higher than Transkei sub-region. The reason of this low use of condom is because of high illiteracy and misconception of use of condom in this region. There is wide spread poverty, and lack of education in the Transkei region of South Africa.[24]There is always delay in reported of these cases to Sinawe Center. About one third (30.6%) of victim has reached after 72 hours after incident of rape (Table 1). This is a serious problem because they are not qualify for 'Post Exposure Prophylaxis (PEP)' treatment, and chances of acquiring

HIV infection is high among these patients. About half (48.6%) of the patients were reported washed after rape in this study (Table 1). They washed either because of ignorance or delay in reaching to hospital. STC is only one centre catering a population of more than half million in a wide area of about 200 kms in radius (Figure 1). There is always shortage of vehicle to transport these patient from far-flung areas where is lack of roads. Every seventh patient (13.6%) in this study was found to be a defaulted PEP treatment in this study (Table 1). The prevalence of HIV infection is higher in low socio-economic class in South Africa.[25] A study carried out by the author showed that there is association between poverty, child sexual abuse and HIV in the Transkei region of South Africa.[10]Transkei is one of the poorest region in South Africa.[24] Almost every sixth (17.7%) victim of sexual assault in the Transkei sub-region was found to be HIV positive in this study (Figure 3). Similar observation was also showed by the Wabiri et al (2013) that HIV prevalence was highest among poor (20.8%) followed by those in middle (15.9%) and those in upper (4.6%) in South Africa.[25]Interventions should move beyond the individual level to be effective and target gender-based inequalities, human rights violations, including sexual violence and rape, as well as stigma and poverty reduction, both at community and tertiary educational level.[6]

Conclusion

There is a high number of rape in the Transkei sub-region of South Africa. About half of them were children under the age of 16 years. In majority of cases perpetrators were known to the victims, and were under influence of alcohol. Physical injuries were accounted only in one sixth of victims of rape, and genital injuries were found in more than half in this study. It is challenged to government as well as to law enforcement agencies to reduce this high number of rape in this region. It is fuelling HIV infection, and with it prevention, it is difficult to control HIV.

Ethical Issues

The author has ethical permission for collecting data and publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

Conflict of Interest: The facts in this report could be submitted in demand. The references were not labelled but can be produced if necessary.

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Child Homicide in the Mthatha region of South Africa of South Africa- Case Reports

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Abstract

Background: Children are the most vulnerable members of society and susceptible to being victims of crime, although the problem is hardly recognized in society. The South African Children's Act of 2005 is a fine piece of law, but its implementation at ground level is a challenge. Long-term poverty in the majority of households and high levels of crime are inherent risks to children's well-being in this region.

Objective: To highlight the problem of child homicide in the Mthatha region of South Africa.

Case report: These case reports examine three incidents of children being killed by their caregivers. In the first case the child had multiple injuries to bones, with distorted extremities and fractured ribs. The second was an infant decapitated by her mother and thrown into a nearby river. The third, a five-year-old boy, was killed by a gunshot injury to the head. The histories, postmortem findings, cause of death, medico-legal reports and the Child Protection Act of 2005 are discussed in this manuscript.

Conclusion: Crime against children takes place in the Mthatha region of South Africa, despite the existence of the Child Protection Act. This Act needs to be strictly implemented.

Keywords: *homicide, child, murder*

Introduction

In 2000, an estimated 199 000 youth homicides (9.2 per 100 000 of the population) occurred globally. In other words, an average of 565 children, adolescents and young adults between the ages of 10 and 29 years die each day as a result of interpersonal violence. Homicide rates vary considerably by region, ranging from 0.9 per 100 000 in the high-income countries of Europe and parts of Asia and the Pacific, to 17.6 per 100 000 in Africa and 36.4 per 100 000 in Latin America.[1] Thousands of children are victims of homicide annually in the world, despite the right to protection and care afforded to them under the United Nations Convention on the Rights of the Child.[2] Mortality observed in 50 countries, in all income categories over a 50-year period, clearly points to a progressive rise in violent deaths among children and young adults, especially in developing countries.[3] In 2002, an estimated 53 000 child homicides took place in the world and the highest child homicide rates were observed in sub-Saharan Africa.[3]

Little is known about child homicide in South Africa. Young children are at greater risk of being killed as a result of child abuse than adolescents, who are most commonly killed during episodes of interpersonal violence.[3] An earlier published study (2007) reported 1 449 unnatural deaths of children recorded in the Mthatha region of South Africa from 1996 to 2004. Most of these, 1 028 (70.9%), were related to trauma, of which 553 (54.4%) were caused by homicide. The homicides reported in this study[4] occurred in the form of firearm injuries 196 (19%), stab wounds 185 (18%), and blunt trauma 178 (17.3%). This represents gross violation of the rights of children under the Children's Act 38 of 2005, which is national legislation intended to protect children. The Children's Act is a fine piece of law that has the potential to bring about services that would enhance both the development and protection of children. However, like so much of our law and policy, the theory is way ahead of the capacity to deliver services on ground level.[3]The need for child protection

services far outweighs the ability of existing services to respond. The purpose of these case reports is to highlight the problem of child homicide in the Mthatha region of South Africa.

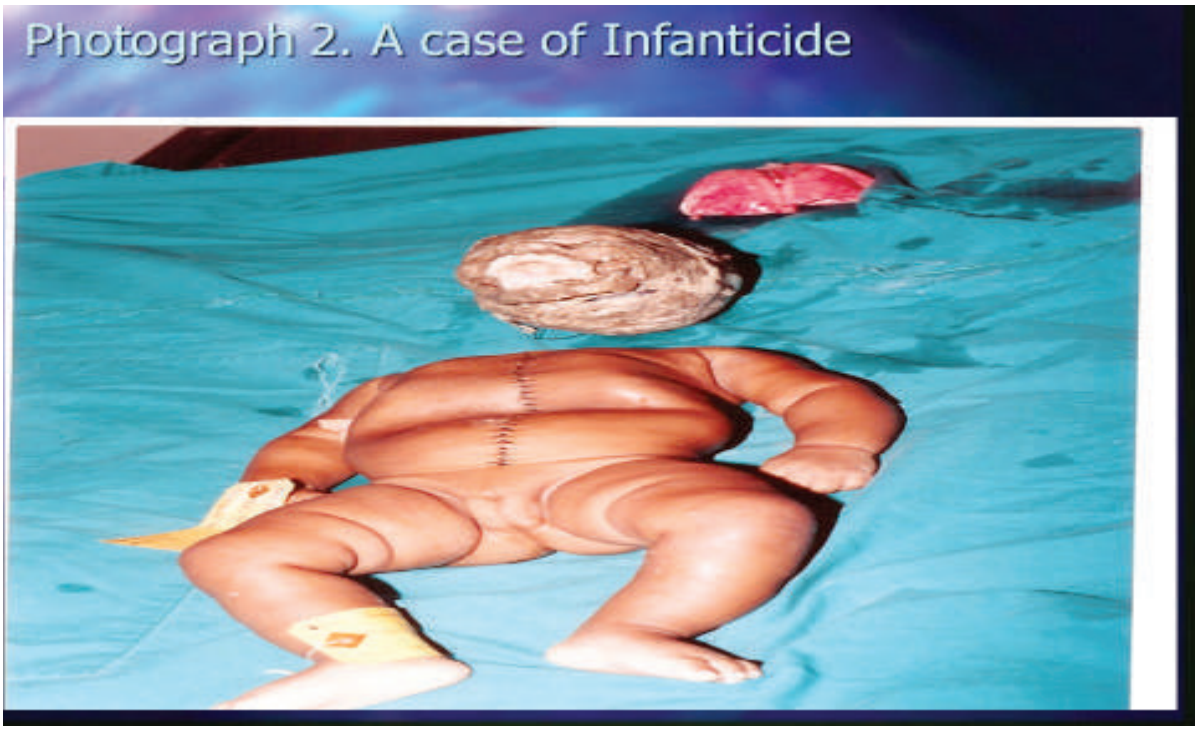
Case history 1

A two-year-old boy (called T) was autopsied in Umtata (Mthatha) General Hospital in 1998. There was a history of suspected battering by his father, as he was the only person to look after his family. The mother had died six months after the birth of this child. The father was a heavy smoker of cannabis, and had a history of alcohol abuse. The child was brought to Umtata General Hospital mortuary for an autopsy. He was severely emaciated, as bones were visible from outside the body. Multiple bony abnormalities could be palpated on the autopsy table. There were multiple old healed fractures of the left humerus, radius and ulna bones, which had distorted the upper left extremity (Photograph 1). There were also multiple nodularities on the chest, which was as a result of multiple fractures of ribs. On opening the chest and abdominal cavities, the lungs were shrunken and adhered to the chest wall. The liver and spleen were congested and edematous. The brain was edematous. Fractures of long bones and the rib cage were confirmed on autopsy.



Case history 2

An 11-month baby girl had been decapitated by her mother. There was some dispute about the legitimacy of this unfortunate girl, initiated by her father. One day the mother decapitated the baby, using a sharp object, and threw her into a river (Photograph 2). The grandmother suspected what had happened and she alerted other family members. The police were called and the mother told the story of killing the baby and throwing her into the river. The body was recovered soon after the killing, but the head was only found after a few days. On autopsy, the body was looking fresh, but the head was putrefied. The internal organs of the chest and abdominal cavities were pale and intact. The neck structures were missing. Parts of brain could not be identified, as these had liquefied.



Case history 3

A five-year-old boy was shot in the head and died on the spot. This unfortunate incident occurred in his home close to Umtata. The father claimed that it happened accidentally as they were playing with a cocked gun. On autopsy, the young boy was found to have a gunshot wound to the forehead. The wound was irregular, stellate shaped, and located on the inner and upper side of the right eyebrow (Photograph 3). There was a fracture of the frontal bone and laceration of the brain. A bullet was recovered from the skull cavity.



Discussion

The South African Children's Act of 2005 is a fine piece of law that has the potential to bring about services that would enhance both the development and protection of children. However, like so much of our law and policy, its theory is way ahead of the capacity to deliver services at ground level. Approximately 57% of individuals in South Africa were living below the poverty line in 2001.The proportion of people living in poverty in South Africa had not changed between 1996 and 2001. However, households living in poverty had sunk deeper into poverty and the gap between rich and poor had widened.[5] Limpopo and the Eastern Cape had the highest percentages of poor people, with 77% and 72% of their populations living below the poverty line, respectively.[5] Rural people of South Africa are at greater risk of being poor. Transkei is an area where people are poor, and living on meager resources. The Eastern Cape has the highest percentage of poor (24%), and this figure rises to 92% in the Transkei region.[6]Child homicide is under-investigated and therefore under-reported. Only one third of all child deaths resulting from child abuse are classified as homicides.[7] This is probably the first rural-based study in South Africa with such a large sample size and covering a period of 19 years, that addresses the problem of child homicide in the country. There is hardly any effort to stop these killing in most countries, especially in developing countries.[8] Thousands of children are victims of homicide annually, despite the right to protection and care afforded under the United Nations Convention on the Right of the Child.[8]

Homicide is the second most frequent cause of death among teenagers between 15 and 19, after unintentional injury, in the USA.[9] While the exact number of children affected is uncertain, child fatalities due to abuse and neglect remain a serious problem in the USA.[10] The South African Medical Research Council published a Research Brief in August 2012 showing that three children a day were being murdered in South Africa. South Africa has an overall child homicide rate of 5.5 per 100 000 of the population.[11] The first case discussed in this paper is that of a child of two years old who had been assaulted by a drug-addicted father. There were multiple old healed fractures of the left humerus, radius and ulna bones, which had distorted the upper left extremity. This was a typical case of battered child syndrome. There were also multiple nodularities on the chest, which were the result of multiple fractures of ribs (Photograph 1). A number of cases of child abuse occur in this region but they generally go undetected. The second case was that of an 11-month-old baby girl. There was some dispute about the legitimacy of this unfortunate girl, initiated by her father. One day the mother decapitated her, using a sharp object. It was suspected by a grandmother, and she alarmed to other family members. Police was called, and mother told the story of killing and throwing baby in river. Body was recovered soon after killing, but head was found after few days. On autopsy, the body was looking fresh, but the head was putrefied (Photograph 2). This was a case of infanticide; the mother was charged with infanticide, not murder. She was supposed to be sent to a reformatory center for rehabilitation. The third case was that of a five-year-old boy shot in head, who died on the spot. This unfortunate incident has occurred in his home in a close location to Umtata. Father has claimed that it has happened accidently as they were playing with cocked gun. On autopsy, a young boy was having a gunshot wound on the forehead. The wound is irregular, stellate shaped, and located at the inner and upper side of right eye brow. There was a fracture of frontal bone and laceration of brain. A bullet was recovered from the skull cavity (Photograph 30. Illegitimate children in this poverty-stricken region is not an uncommon problem. The children are the unprotected victims of their alcoholic or drug-addicted fathers or guardians or any other adults, who often hit them over the head. Children whose paternity is disputed are commonlyvictims of such homicide. Sexual promiscuity has not only increased the risk of HIV and other sexually transmitted infections, but also the risk of illegitimate pregnancy.[12] These illegitimate children are often either the perpetrators or the victims of homicide. Only 33% of children in South Africa live with both parents.[13] Many of them are orphans, and these are the children at greater risk of being murdered. In a normal society these children would be either in school or at home with their mothers or other caregivers.

These three cases are just the tip of the iceberg. It is difficult to understand the reason why these children were killed. An unpublished report by the author showed that the average rate of child homicide was high in the Transkei region of South Africa. It was more than 12 times higher than the world average, and also more than

five times higher than in the rest of South Africa. Males were the victims of child homicide more than twice as often as females in this region of Transkei. About four-fifths of child homicides were recorded in the age group between 11 and 18 years, and about one-eighth of the children who were victims of homicide were under the age of 10. The government will have to make concerted attempts to reduce the killing of children in the Transkei region of South Africa by improving socio-economic conditions and educating the population.Globally, across all age groups, alcohol is estimated to be responsible for 26% and 16% of years of life lost through homicide among males and females' respectively.[14] Poor people indulge in alcohol or drug use to forget their sorrow. Low socio-economic conditions are an important cause of the high level of child homicide. The Transkei region is a third world region within South Africa where people are extremely poor.[15] When there is extreme poverty, children tend to be vulnerable just by virtue of experiences such as growing up without a father, poor parenting, family violence, neglect and abuse during childhood and living in poverty and in a context of social inequality are all pathways for violence.[16]

Conclusion

Several cases of child homicide have occurred in the Transkei sub-region of South Africa. The problem needs urgent attention.

Ethical Issue: The author has ethical permission for case report publication (approved project No. 4114/1999) from the Ethical Committee of the University of Transkei, South Africa.

Conflict of Interest: None

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Poverty and non-natural deaths among former mineworkers and their families in Transkei region of South Africa

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Abstract

Background: Thousands of ex-migrant mineworkers across the former Transkei have already passed on from natural causes, are disabled, or have died due to either mining-related diseases or in non-natural ways such as accidents, suicide or homicide. Many ex-mineworkers have died prematurely, placing a strain on families.

Case History: Eighty-four family records were analyzed. Of these, 21 (25%) were found to be those of former mineworkers and their immediate family members. There were five mineworkers, and 14 were children of mineworkers. Only one was a spouse of a mineworker who had died unnaturally. Three mineworkers died as a result of firearm injuries, one was assaulted by a knobkerrie, and another one died as a result of alcoholic intoxication. Two of them had heavy drinking habits. Three mineworkers were unemployed. The causes of unnatural death were as follows: five were stabbed, two died from firearm injuries, one was killed in a motor vehicle accident, one was assaulted with a blunt object, and three committed suicide by hanging and poisoning. Most victims consumed alcohol. The history and psychosocial effects are discussed in this presentation.

Keywords*: non-natural death, poverty, mineworkers*

Introduction

South Africa ranks number one in Africa with 59935 non-natural deaths due to injury in 2000, which is overall death rate of 157.8 per 100 000 population.[1]This rate is higher than the African continental average of 139.5 per 100 000 population, and nearly twice the global average of 86.9 per 100 000 population.[2] Nearly half South Africa's deaths due to injury are caused by interpersonal violence, [3] four and half times the proportion worldwide. [4]

Approximately 57% of individuals in South Africa were living below the poverty income line in 2001.The proportion of people living in poverty in South Africa did not change between 1996 and 2018. However, households living in poverty sank deeper into poverty and the gap between rich and poor widened.[5] In South Africa 59 935 deaths due to injury occurred in 2000, which is an overall death rate of 157.8 per 100 000 of the population.[6] This rate is higher than the African continental average of 139.5 per of the 100 000 population, and nearly twice the global average of 86.9 per 100 000 of the population.[6] Data from death certificates and the National Injury Mortality Surveillance System indicate that more than a quarter of such deaths are due to road traffic injuries, with the remainder attributable to self-inflicted injuries, fires, drowning, and falls.[6]

There are about 600,000 mineworkers employed by gold mining industry alone in South Africa.[7] Majority of them are from Transkei region.About 14% of the former mineworkers who visited the Umtata 'Benefit Examination Clinic' between April and August 2000 indicated that they were given no reasons for their retrenchment.[7]There is evidence of a huge accumulation of unrecognized, therefore uncompensated, cases of pneumoconiosis and/or tuberculosis among former mineworkers living in the labor sending areas such as Transkei.[8]The purpose of this report is to describe the manner of non-natural deaths and to relate this to poverty

among mineworkers and their families in the Transkei region of South Africa.

Material and Method

This retrospective descriptive study was undertaken, reviewing 84 non-natural deaths occurring at Umtata General Hospital mortuary during the period April 2000-May 2001. Medico-legal autopsies were conducted twice a week at the hospital mortuary. A close family member who was present was interviewed before the autopsy was carried out. The histories of death were recorded, including the names, addresses, and ages of the deceased, together with the cause of death and circumstances of the death. All the autopsy histories for the specified period were reviewed, compiled and collated manually.

Results

Eighty-four families were interviewed. In 21(25%) of these the deceased were found to be former mineworkers or their children who suffered non-natural deaths: five were mineworkers and 14 children. Only one was the wife of a mineworker. Three of the mineworkers died as a result of firearm injuries, one was assaulted by a knobkerrie, and another died as a result of alcoholic intoxication. Two were alcoholics. One was employed as a security guard, another was running a business, and three were unemployed. Five children were stabbed to death, two were gunned down, one was involved in an accident, one was assaulted, two hanged themselves and one poisoned himself. One died of electrocution, and another drowned. Most of victims consumed alcohol.

Discussion

Migrant labour system has attributed very extensive socio-economic effects in the Transkei region. It was estimated that 2 million of the 5 million black workers in South Africa at the time migrant labourers.[5]Former mineworkers are sandwiched between scare resources, and there is little hope of getting re-employment. They do not have enough savings to run their families. The expenditure is at its highest when they return, as the children are grown and are in secondary or senior secondary school. Many are not re-employable because of the poor health and some are disabled.

Transkei has very high population density. Most former mineworkers reside in the far-flung and remote rural areas of the region. Their homes are scattered in the wide barren tracts of the Transkei. Poverty and unemployment levels are very high. Transkei is known for its supply of labour to the mines. Miners were respected because they earned more money than other people in this area. When the miners came home without money, the community undermined them and their families. As a result, they got frustrated, started drinking and got involved in crime. There is plenty of literature on mineworkers' health conditions, but hardly any publications on non-natural deaths. Young, adult healthy people were recruited for the mines, and when they returned home, they had been transformed into sick, unhealthy and dependent individuals. They suffered from mine-related diseases. Many died non-natural deaths caused by accidents, suicide and homicide.The children of mineworkers struggled to survive. They faced hazardous situations in their day-to-day life, since they were not educated. They usually did not have work and when they did, it was very inconsistent and risky.

LM, a 32-year-old son of a mineworker, was selling fruit. Two of his brothers had died from dehydration when young. He was stabbed to death by three drunken people in a location. Most of the children of mineworkers look after sheep and cattle. They are illiterate and are not employable. They usually drink liquor, risking their lives. XY, a 20-year-old male, was stabbed to death by his friend. He was in a tavern (place where liquor is sold). He was hired by a man to look after his livestock. He met his friend in the liquor shop. The friend became hostile and stabbed XY. His father was a mineworker and died in 1979 in a mining accident.

Taxis are the normal form of transport for poor people in this region. These are called flying coffins. The taxi industry is characterised by infighting. ENS, a 27-year-old daughter of a mineworker, was involved in an accident when a taxi driver was shot dead.

Mineworkers are known for their promiscuous behaviour at mines. One of them who was killed had four wives. ZY, a 53-year-old ex-mineworker, was shot at his home and died in hospital. He was the owner of a shop in a location. The police failed to capture the culprits. Nothing was stolen from the shop. He had four wives

and they all lived at home. He was an ex-miner who returned from the mines in 1974 and had many children. Suicides are not uncommon among mineworkers and their children. Most returning mineworkers were in a poor state of health (78.2%) and as a result found it difficult to get work[10] and ended up being unemployed. The suicide rate is higher among the unemployed. The relationship between physical health and suicide is significant. Physical illness is an important contributing factor in 11% to 51% of suicides.[9] Families of ex-mineworkers are dysfunctional. This is because they are poor. One male committed suicide when he could not buy a pair of shoes. The mother was poor and the ex-mineworker father was not supportive. LL, a 15-year-old male scholar, hanged himself in the room where he was studying. He was a grade 8 pupil. He used to go to school every day and there were no complaints from the teachers. He was a quiet boy who lived with his father and stepmother. The biological mother married someone else and lived separately from her children. The mother visited him. The boy was found hanged. He had requested a pair of shoes from the father several times. His father had not responded to his request and so he decided to take his own life.

The system of migrant labour from Transkei region created a situation where women said that their ex-mineworker husbands were not the people they had married. The men were not strong anymore and could not meet their demands. This was revealed in a case where a woman poisoned her husband. Her daughter revealed this before committing suicide. ZZN, a 27-year-old female, committed suicide by hanging. The reason was written in a suicide note she left, which was that the mother was not in close contact with her. That was because the mother had poisoned the father when he returned from the mines. The daughter had known the secret. The father's death was not reported to the police as an unnatural one. The father had been working in a platinum mine in Rustenburg for 20 years but had not saved any money for a rainy day. He was retrenched because of poor vision.Trauma is a leading cause of death in the Transkei region of South Africa. Road traffic accidents contribute substantially to the number of such deaths and more than one-third of those killed are pedestrians. NN, a seven-year-old boy, was knocked down by a car while he was crossing the road. He died instantly. The driver paid money to the family for his burial. The father of the deceased was an ex-mineworker with two wives and seven children. He was sick and coughing. The whole family was unemployed.

The migrant labour system in the Transkei region contributed very extensively to socio-economic problems. Former mineworkers now have to cope with scarce resources, and there is little hope of being re-employed. They do not have enough savings to run their families. LD, a 48-year-old ex-mineworker, was shot dead by an African man. He died instantaneously. He had been working as a guard and it appears that the motive for his killing was to steal his firearm. A bystander called the police and the perpetrator was arrested. The victim used to work in the gold mines for about 20 years and left the mines to get married. He was jobless for a year, as his health was poor, but was forced to join a security company to earn some money. The money he had saved was used to pay lobola (marriage gift). He left behind a wife and a son.

Ex-mineworkers were strong and powerful when they were taken to mines, but lost their physical strength when they retired from service. Most of them were retrenched, as they were weak and non-productive. WS, a 31-year-old ex-mineworker, was shot dead. He was working as a security guard. The criminal grabbed him and tried to take his firearm. He struggled with him until he was shot in the head. The purpose of the crime was to take the firearm. One of the culprits was arrested. The victim was not married and did not have a girlfriend either. He had worked in a gold mine for seven years in Gauteng. He was retrenched about four years previously and on returning began to work as a security guard. Mineworkers have children with their traditional wives back home but fail to support them. Although they go to work in the mines in the hope of earning substantial amounts of money, they return with meagre savings. Lack of support for the children mean that they grow up with no education and as they cannot get decent work, they start abusing alcohol or drugs and end up as victims or as perpetrators of crime. Poverty among families of mineworkers can affect the mental state of the family members seriously. Some of them decide to commit suicide, as there is no light at the end of the tunnel. There is no source of income in family, and they do not know how to generate income because of lack of education. Financial difficulties are a prominent underlying cause

of suicide.It is not uncommon in the Transkei region for drunken people to roam the roads. Sometimes they are killed by traffic. This is the reason for a large number of pedestrian deaths in South Africa. Occasionally, they are involved in interpersonal fighting. Many of them are retired mineworkers who do not have jobs. Mineworkers in South Africa are in abundance as mining is the main occupation especially in the former black homeland. These mineworkers retrenched or retire, and returned to their homeland. Transkei is a former black homeland where abundant retired mineworkers reside to their destiny. Migrancy, which for century has been such a conspicuous feature of the South African labour system, and thought to be a significant contributor to the spread of HIV/AIDS. The migrant labour system in Transkei region was attributed very extensively to contagious disease especially related with sexually transmitted diseases (STI) like HIV/AIDS.

Conclusion

Migrant ex-mineworkers have undergone serious psychosocial trauma, which is a legacy of mining work. The changing socioeconomic realities of mineworkers have had drastic effects. The journey to the mines systematically stripped them of their expectations, and made them helpless, disabled and dependent on their family members. This led to a high rate of unnatural deaths among mineworkers and their family members.

Ethical issue: Ethical permission was granted by the University of Transkei ethical committee (approved project No. 4114/1999). The names of all the cases were kept anonymous and not divulge to anyone.

Conflict of Interest: None

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A case report on Shortfall in Pension in a Dual Employment in Health and Rural University, South Africa

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Abstract

Background: Chances in dual employment are that an employee will be exploited by an employer not only in terms of salary, but also in terms of pension. The exploited an employee use to do double work, less salary and pension The question is, who will take responsibility if this happens? The primary health employer in dual employment must take responsibility for safeguarding an employee's pension in this case.

Objective: To describe and calculate pension in dual employment from 1996 to 2018.

Results: Mr BM was employed by the DOH in the government, where 5% of his salary was deducted from 1996 to 1999 to be paid into a public investment fund (GEPF). He was appointed as head of department (HOD) at the rural University in February 1996, but was kept on the payroll of the health, as he was working in the joint establishment. He was shifted from health to university payroll in 2001. This was only a shift of payroll, not the amount of work remained same before and after this. When Mr BM retired in September 2018, his pension was calculated from 2001, instead of 1996. His salary was also reduced by half by the second employer (university). This dual employment has complicated Mr BM salary as well pension and has described in this manuscript.

Conclusion: This double jeopardy of losses by first and second employer must be calculated so that the pension and salary can be paid appropriately from 1996 to 2018. It is primarily the responsibility of the Department of Health as a first entry point employer.

Keywords*: Dual employment, first and second employer, pension, health*

Introduction

The retired person is live on pension. Pension is calculated at the time of retirement based on years of service and amount of salary received during his employment. An individual came to know his/her salary short fall in the terminal part of service. It is unfair that his claim of shortfall of salary is not entertained on the basis that he is no more employed. Salary is just like debt and could be claimed at any time. Secondly, the pension is based on salary so if the pension can claim, then the salary has to be accounted after an retirement.

Dual employment in the public sector presents more difficulties than in the private sector. This is because both employers are not taking responsibility of an employee. He became a football to get kick from both sides without a refree. The question is who will take responsibility for paying an employee appropriately. In Mr BM case, the second employer (university), and they told that university pay as a university like it. Health is a government department. They handed to his appointed employee to second party (university), although they refund to second employer his 80% salary, and not to an employee directly. This became a problem as the second employer pay as they like it as first employer may not supervised properly and audited to second employer. This becomes even more difficult when the employee is not a native citizen of the country. Legal action is expensive, and few employees can afford to go to court. University authorities are not afraid of exploiting an employee, as they know that if there is compensation to be paid, it will be paid by the university, not by them

personally, an only after a long battle.

In a ground-breaking judgment, the Constitutional Court pointed out that such an employee needs greater protection, as he is vulnerable to exploitation.[1] Exploitation is more common in historically disadvantaged universities, as they were also got exploited by the apartheid regime. It has been seen that abused individual, become abuser when they come in power. These universities do not have any policy protocol in place. This is the reason after so many years of university opening; they have only few policy documents. Under dual employment, vulnerable employees must receive all possible protection.[2] In the absence of a policy protocol; these universities easily discriminate selectively against employees, as in the case of BM. The authorities do not want to develop a policy, since working without one allows them to abuse their power. A historic victory against labour brokers was celebrated by the South African Federation of Trade Unions (SAFTU) when the court dealt a deadly blow against labour brokers.[3] There is a need of such blow to university so that they will not exploit like Mr BM. The federation congratulated its affiliate, NUMSA, on the successful conclusion of its long court battle against labour brokers.[4] Mr BM needs a NUMSA at the university to deal with the unfair labour practice he suffered in dual employment.

The case revolves around the interpretation of a section of the Labour Relations Act (LRA) on the question of whether a worker was in the sole employment of the employer or in a dual employment relationship.[4] This case of Mr BM is unique and could create a history in the South African labour law. A few numbers of professors were employed in the joint establishment but needed protection if they were to speak about their problems. Workers' rights must be protected, and the law must therefore be interpreted in the context of the right to fair labour practice.[4] Labour brokers will no longer have any legal standing in the employment terms of contract workers, nor be involved any further in the equation regarding the Labour Relations Act.[4] Unions must therefore vigilant to ensure that this historic ruling is rigorously enforced and that labour brokers cannot find any way through the back door to continue exploiting workers.[2] The purpose of this case report is to highlight the problem of unfair labour law practice at disadvantaged rural universities, especially in the context of dual employment, where an employee is sandwiched between two employers.

Results

A deduction of 5% public investment commission and 7.5% pension contribution was made from 1993 to 2018. The pension was calculated from 2001 to 2018. Mr BM was a staff member of the joint establishment from 1996 to 2018, without any break. He was also acting HOD from 1996 to 2015. He was removed from the HOD position in April 2015 university but was still serving the hospital as HOD. His pension was doubly jeopardised. Firstly, his salary was not paid according to the University policy document since his appointment as acting HOD.

Secondly, Mr BM's public investment contribution was not included in his salary from 1996 to 2000. This led to a lower pension, almost half the amount he was supposed to get.

Table 1. Deduction of public investment commission in health (later merged with GEPF)

Year	Public invest comm (5%) & pension fund contribution (7.5%)	Basic salary	Category	Status
1993	291.76 (5%)	7035.75	Health	Medical officer
1994	291.76 (5%)	7035.75	Health	Medical officer
1995	291.76 (5%)	7035.75	Health	Medical officer

Cont... **Table 1. Deduction of public investment commission in health (later merged with GEPF)**

1996	291.76 (5%)	9617	Joint establishment staff	Acting HOD
1997	291.76 (5%)	8911.75	Joint establishment staff	Acting HOD
1998	291.76 (5%)	9628.25	Joint establishment staff	Acting HOD
1999	291.76 (5%)	10105.75	Joint establishment staff	Acting HOD
2000	291.76 (5%)?	10611.00	Joint establishment staff	Acting HOD
2001	1012.21 (7.5%)	13496	Joint establishment staff	Acting HOD
2002	1012.21 (7.5%)	13496	Joint establishment staff	Acting HOD
2003	1222.03 (7.5%)	16293	Joint establishment staff	Acting HOD
2004	1222.03 (7.5%)	20226	Joint establishment staff	Acting HOD
2005	1606.86 (7.5%)	21358.17	Joint establishment staff	Acting HOD
2006	1931.12 (7.5%)	24415.58	Joint establishment staff	Acting HOD
2007	1941.77 (7.5%)	24415.58	Joint establishment staff	Acting HOD
2008	2087.40(7.5%)	27832.00	Joint establishment staff	Acting HOD
2009	2882.76 (7.5%)	38436.75	Joint establishment staff	Acting HOD
2010	4926.15 (7.5%)	65682.00	Joint establishment staff	Acting HOD
2011	4926.15 (7.5%)	65682.00	Joint establishment staff	Acting HOD
2012	4926.15 (7.5%)	65682.00	Joint establishment staff	Acting HOD
2013	4926.15 (7.5%)	65682.00	Joint establishment staff	Acting HOD
2014	4926.15 (7.5%)	72298.17	Joint establishment staff	Acting HOD
2015	5812.80(7.5%)	77504.00	Joint establishment staff	HOD in Hospital
2016	6219.69(7.5%)	88734.33	Joint establishment staff	HOD in Hospital
2017	6655..07(7.5%)	95211.92	Joint establishment staff	HOD in Hospital
2018	7140.89(7.5%)	100924.67	Joint establishment staff	HOD in Hospital

Discussion

Mr. MB retired, and he got one third of the total benefit in a cash lump sum and the other two-thirds is paid out in the form of a pension over the rest of the member's life. A **provident fund member** can get the full benefit paid in a cash lump sum.[5]Little work has been carried out on the issue of dual employment in South Africa. This case of BM is probably unique in South Africa. It is consequently difficult to decide who is responsible for the shortfall in his salary and pension. If one regards the initial employer and bigger funder of the salary of Mr BM as the responsible party, then the DOH is responsible. The DOH contributed about 80% of his salary. Secondly, who was the initiator to discipline him in a case of abscondment against him? Then again, a hospital manager of the DOH instructed the faculty head to institute disciplinary enquiries against BM. Therefore, the DOH seems to be the primary or first employer.

The Pension Funds Act, promulgated in 1956, codifies the second pillar of the retirement funding system. There have been a number of ad-hoc amendments to the Act and a number of commissions have assessed

retirement funding. These include the Mouton Commission (1992), Katz Commission (1995), Smith Commission (1995), National Retirement Consultative Forum (1997) and the Taylor Committee (2002). The National Treasury (2004) issued a discussion paper on retirement fund reform stating that the government seeks to build on the strengths of the established retirement funding environment, while progressively addressing its deficiencies.[6]

The problem and complexity of dual employment have been persisting for a long time. This is because hardly any employee understands this complex relationship and it was never cleared by the employers. The DOH and the university were using their employees as they wished under an agreement on a joint establishment. This agreement was signed by the DOH and university and according to it an employee of the DOH had to work at University. The University medical school was opened in the apartheid era as a form of resistance against apartheid practitioners. At that time there was a severe shortage of medically qualified staff to teach medical students, so they had an agreement about a joint establishment. This agreement continued and was revised in 2013 without much change, when the name of University was changed to X University except the DOH employee were recognised as HOD, and they were also having full advantage of joint establishment. An employee such as Mr BM, who retired after 23 years (1996-2018) of service on the staff of the joint establishment, deserves to get the back pay and pension of 23 years due to him. The university paid him almost half the salary to which he was entitled; therefore the pension automatically came down to half. HR calculated his pension from 2001, whereas he was appointed by the university in 1996 and his salary slips show the deduction of public investment funds, which were later, merged with the GEPF contribution. Now the question is who should pay Mr BM's pension for the period between 1996 and 2000? Mr BM was a staff member of the joint establishment and acted as HOD of a department.

The initial contract was signed for three years. The benefits due at its termination were not paid despite repeated requests. Eventually a letter was received from the DOH, stating that he was not entitled to transportation back to his place of recruitment at the end of the contract. Though Mr BM had completed the three-year contract, he was not eligible for this benefit, which had not been specified in the agreement. Surprisingly several his colleagues received that benefit. Mr BM was a medical officer in the health in former government, and was a joint staff member of University as well. On joining University, the benefits of the DOH were transferred to University, such as unused leave credit. Therefore, the employment at hospital was not isolated from the appointment at University. Both (university and health) were work in hand in hand, share and transfer information to each other as an employee works in both sides. Mr BM was also appointed as acting HOD while he was a medical officer in hospital, so his accountability is on both side but only paid by the hospital. Therefore, the argument of the head is not convincing, and any unpaid money is supposed to be paid by the DOH. Additional to the fixed establishment, whether by reason of the absence of the incumbent of any post, or when it is necessary to provide staff for the performance of a class of work for which staff is not ordinarily employed on a permanent basis, or when it is necessary for any other reason to increase temporarily the staff of any department.[7]

The main problem experienced by the joint staff of Faculty developed after the implementation of the Occupation-Specific Dispensation (OSD) in 2009. The OSD scale revised every and paid by the National Government of Health through Provincial Health Department. The health paid to University, but university and did not pay to an employee. The university staff discriminated against the staff of the health, and this led to many staff members joining the health and leaving the university payroll. This move was discriminatory and selective. Mr BM retired in September 2018, and his pension as calculated was almost half of what he was entitled to. This led to serious disappointment. Pension depends upon two main factors, namely length of service and salary scale. In Mr BM's case, both were reduced. At least one employer must give protection in dual employment. Mr BM was exploited by both. He was working as a full-time employee bearing responsibility at both the hospital and the university, but in the end, he received a lower pension in his old age to live on difficult time without any funds.

The GEPF is correct in saying that payment of actuarial interest as a benefit on the exit of a member from

GEPF, consistent with the payment of minimum benefit in funds governed by the Pension Fund Act, is intended to be broadly the amount held by the GEPF on behalf of the member. Payment of a lesser amount would result in a profit to the GEPF and could be considered unfair to the member. Payment of a higher amount would result in a loss to the GEPF and might be considered enrichment to the member.[8]

Conclusion

Dual employment has had a complicated and seriously affected the life, both in terms of pension and morale of a retired individual. Pension is a pride of retired man. Pension is linked to the salary, and if salary paid half then the pension is also reduced to half. The estimation of pension will be carried out by an actuarial consultant once it becomes clear that the health must accept this responsibility.

Ethical Issue: Proper care was taken in this manuscript, as not to disclose directly to any institution or an individual, but if is knowingly or unknowingly someone find it, and then it is not author intention. It is failure of system in both health and university, and that led to victimization of Mr. BM. The author would like to high light this system failure in this report, not an individual or institution.

Conflict of Interest: None

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Complexity of Dual Employment in Department of Health and university, South Africa

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Abstract

Background: Dual employment was inherited from the Transkei government, which ruled that all doctors who worked in hospitals also had to work in the medical school of the rural University. Policies at this university are inadequate, and where they do exist their implementation is discriminatory in nature.

Case history: Mr. BM joined the Department of Health as a medical officer and was subsequently appointed as an acting head of department in the Department. He had to work in the health care system as well as at the university full-time (Joint establishment); he was working on his own in his department. He was paid as a medical officer by the health but received no remuneration from university. This was contrary to labor law and University policy and once the council realized this, a resolution was taken granting him back payment. Unfortunately, it was not implemented.

Mr. BM was working against the post of professor and acting head and carried out all the responsibilities of that post without being remunerated. Although he was paid at the occupational-specific dispensation (OSD) scale in 2009, it was not adjusted till his retirement in 2018; therefore it affected his pension as well. The case history, short payment in salary, and unfair labor practice in respect of his dual employment are discussed in this manuscript.

Conclusion: The DOH must take responsibility in his dual employment for this shortfall in salary, as it is the source of major funding.

Keywords*: dual employment, exploitation, sandwich, health*

Introduction

South Africa introduced the OSD scale as a retention policy affecting all health professionals who are registered with the Health Professions Council of South Africa. This had a significant effect in keeping specialists in South Africa. University had a policy regarding conditions of service, allowances, leave and other privileges of persons appointed at the University. The remuneration attached to the officer's appointment as an acting officer was clearly stated on page 8 of this policy document. The right of employees must be protected and promoted, according to the amended Labor Relations Act (LRA) of 2014.[1] Describing the employment relationship under the LRA between the primary and the broker employer, the employee is deemed to be employed by both, according to the LRA of 1983.Continuous employment in excess of three consecutive months by the broker employer triggered the acceptance of section 198A (1) (a) of the LRA.[1]

The occupation-specific dispensation (OSD)scale has been regulated and implemented by way of collective agreements for health professionals, including medical specialists, since July 2009 to retain the expertise of health care service providers in South Africa. The aim of the OSD was to improve the conditions of service and remuneration of public service workers, including public sector health professionals.[2] The objective of the OSD was to improve the ability of the public service to attract and retain employees, to provide differentiated remuneration dispensations for the vast number of occupations in the public service, to cater for the unique

needs of different occupations, to provide unique salary structures per occupation, to prescribe grading structures and job profiles to eliminate interprovincial variations and to provide adequate and clear salary progression and career path opportunities based on competencies, experience and performance.[3]Medical and dental practitioners, specialists, pharmacists and emergency medical services (EMS) were identified for implementation, and the OSD was implemented in these fields in July 2009.[4]

The commissioner in a NUMSA case was of the view that the dual employer interpretation would bring about several problems, such as disciplining of placed workers and deciding on which of the parties' disciplinary codes would be applicable.[5] There could be difficulties in payment and protection of an employee in a situation of dual employment. The labor court held that the rights of employees are therefore best protected by the dual employer interpretation. Primary employer in dual employment, where the employee is placed and he can be disciplined by them whenever need arise.

Results

The basic salary pattern of BM from 1996 to 2018 at the University is shown in Figure 1**.** The OSD scale was implemented in July 2009, but it was not revised till 2014; subsequently from 2015 to 2018 there was some increase, but whatever criteria Human Relations (HR) considered for this marginal increase, these did not reflect the implementation of the OSD scale (Figure 1).

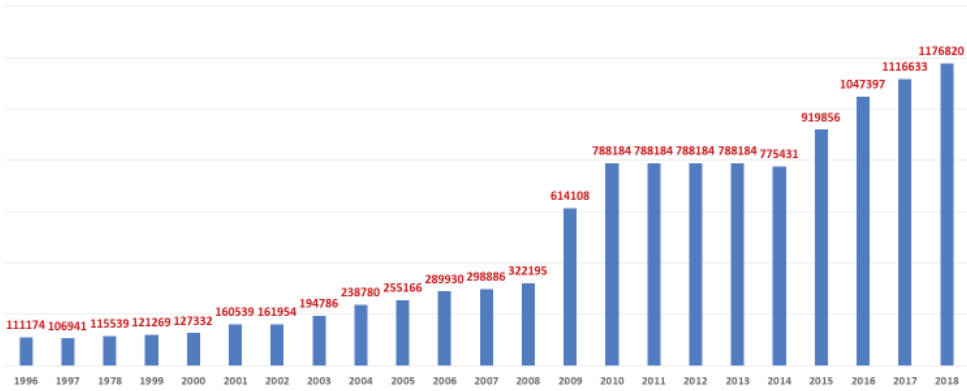


Figure 1 . Pattern of payment of salary from 1996 to 2018.

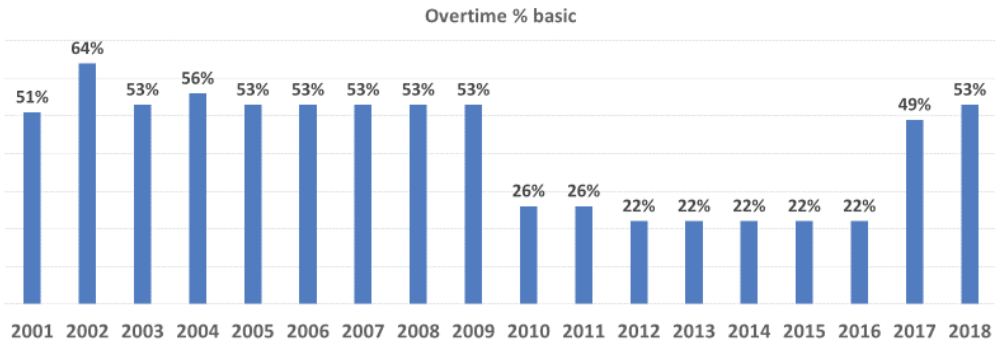


Figure 2. The pattern of Overtime paid in percentage of basic salary from 2001 to 2018.

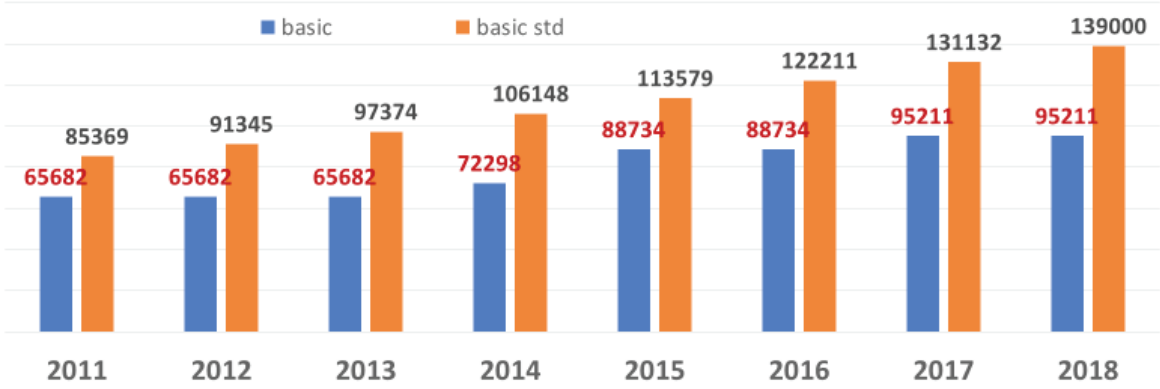


Figure 3. Comparison of a basic salary of a student vs. mine(2011-18).

The pattern of overtime payment from 2001 to 2018 is shown in Figure 2. The unilateral reduction in overtime payment from 2010 to 2016 is clearly visible in Figure 2. So, what happened was firstly a reduction in basic salary that automatically reduced over time, as it linked to basic salary. It is coming out about 53% of the basic salary (Figure 2). So, when salary has reduced, the overtime has also decrease as it is linked to it. It is 53% of a basic salary. It has not paid 53% from the year 2010 to 2016 (Figure 2).

It is very interesting to see the amounts reflected in Figure 3, indicating that a student of BM's, who qualified in 1996, was getting a basic salary significantly higher than his from 2011 to 2018.

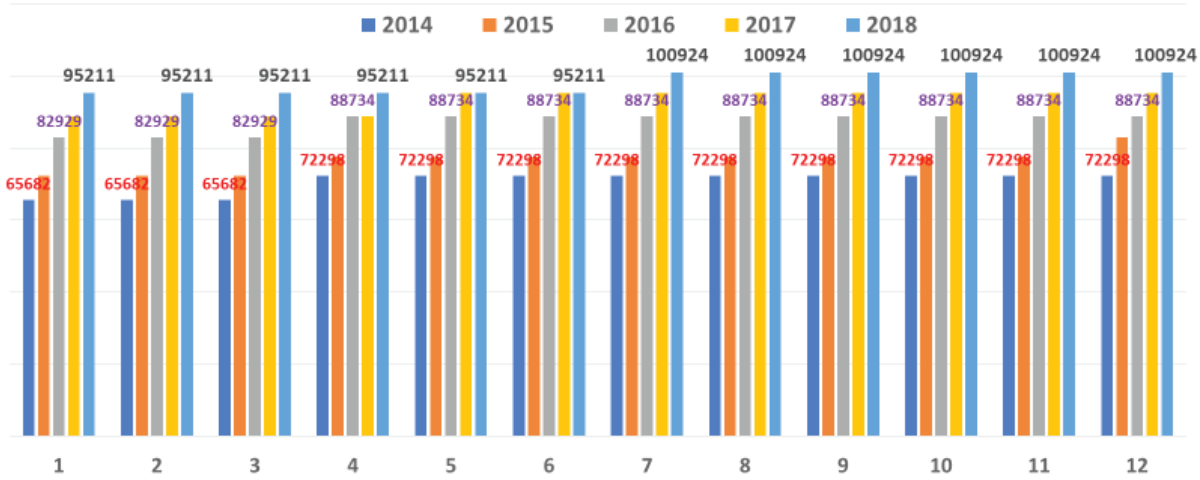


Figure 4. Monthly variability in the payment of salary from 2014 to 2018.

There was constant gross variability and lower payment in salary (Figure 1). This variability was not limited to years, but also occurred monthly (Figure 4).

Discussion

South Africa has been liberated from apartheid, but there is a tough challenge in terms of transformation into a stable, equitable, and non-racial society. Universities are regarded as power houses regarding generation of knowledge, but are also victims of racism and xenophobia.[6]Although only a few cases of racism and xenophobia have been reported; these constitute the tip of an iceberg.[7]

Some of the employees of the heath and the University were in the position of a floating ball; they keep on shifting one institution to other without any difficulties. These entities kept changing to a position where they could get more benefit. The work entailed mainly service in hospital and teaching medical students at university. The work remains the same either employed in university or in hospital especially in the case of Mr. BM. The salary is almost equal, as a professor's post is equivalent to that of a chief specialist (Level 13 and university level 5). The University receives 60% of the required funds from the DOH for the payment of their joint staff on the university payroll, according to an agreement.[9] In addition to this, the health pays an overtime allowance, which is about 53% of the basic salary, and so overall health contributes about 80% of the funds for university joint staff. Mr. BM was not paid appropriately at any time, although he was working in both the university and the health full-time. He was getting a salary that was almost that of a lecturer, whereas one of his previous students was appointed was almost getting twice higher scale (Figure 3). Mr. BM as a specialist fell in a scarce skills specialty. What criteria the University considered and how it increased the salary of staff was a mystery. Prof. D, who was assessor of the University and submitted a report, mentioned that the University was like Afghanistan, and there were indications of failure of multiple organs, including HR.[8]

The University appointed HODs in various departments, and health employees worked according to their instructions. This was an exception in the case of Mr. BM, as he was on his own in both the health and the University, so he was the head and tail of the department.[9] With the inception of the OSD scale in July 2009, most of the staff, especially the favored ones, were shifted to the health side, and other were in National Health Laboratory Services (NHLS), left few on university payroll. It was surprising fact that all the members of the clinical staff were not absorbed by the hospital. This happened without any notice by HR. Where such a change is made, there should be communication with all the staff, and they decide, to stay in university or on health payroll. But this did not happen.

When the OSD scale was instituted in 2009, a lump sum was paid with the December 2009 salary in university. University has paid less than should have been done but did pay. At that time, the faculty head wrote a letter to the HR director and head of the institution, regarding back payment on the OSD scale in 2011. BM's name was included at that time, but later the list of joint staff who supposed to get back pay of pending (2010 & 2011) OSD scale, Mr. BM name excluded in HR. When enquiries were made, the HR director replied that the faculty had decided to exclude BM's name. When HR was asked who the specialist deciding on salaries in the faculty was, he did not answer. In 2011 and 2012, most of the staff mobilized on the health side, leaving BM at the University. These facts only came to light later, as BM never suspected that this kind of thing was possible at the University. It was only at the beginning of 2012, when the medical school director showed the salary scales on his computer, that BM suspected that there was something wrong and started working on it.

This shortfall in salary occurred from 1996 to 2018, and it cannot be prescribed, as salary is like a debt. The council itself broke the prescription by its resolution in 2003 to grant back pay to Mr. BM from 1996. Secondly, there is supportive evidence of a decision in a case heard in the Constitutional Court where the employer paid after 30 years of prescription. Mr. BM has constantly been writing to the University about his back payment.[3] In 2015, National Union of Tertiary Education (NTEU) lodged a dispute on the shortfall in salary with the head of institution and demanded back pay from 1996. He replied without mentioning a word about prescription. There is a history of personal visits to offices at the University; in addition to almost 50 letters being written to the University, twice this number of personal visits occurred since 1996.

Mr. BM was sandwiched between two employers, where the health was bigger, and the University was

the agency, and pay to his employee as they like it. BM was initially (1996-2001) on the payroll of the health but worked at the University like a full-time employee. This does not need any explanation, as it is evident from a resolution by taken by the University's highest body. The University's policy on conditions of service is quite clear: to pay the difference in salary of an acting officer against the post in which that officer is working. In the case of BM, he had always worked in the post of professor and head. The health has always claimed BM as their employee and given instructions to the University. A letter written by the clinical manager gave direction to the faculty head on an enquiry about abscondment of Mr. BM. This implies that the health considered itself his first-line employer, and the University as secondary. Dual employment is always problematic, and one must report to both authorities. BM was removed from the head post after 20 years, in April 2015, but the hospital always regarded him as head and used his services accordingly.

It is difficult to know how much the health paid to the University in the case of Mr. BM. From a meeting held on September 2018 between HR of Health and university HR, it looks as if that all OSD scale funds were paid solely by the health and there was hardly any contribution from the University. The chief director who was chairing the meeting was surprised that Mr. BM was getting such a low salary, almost half of the norm, especially from 2010 to 2018. One of his students, who were working at the university and then at a hospital were getting much higher than Mr. BM.

Whatever amount of salary was paid by the health is not clear, but at the University HR was always paying a low salary to Mr. BM. The salary scales changed up to three times in a year, and at least twice in a month. This frequent fluctuation in a salary is a sign of bad management by second employer. Mr. BM has reported to the head of institution but there is no effect. Overtime was also like playing musical chairs, with payment of 53% (2001-2009) going down to 22% (2010-2016) and again being elevated to 53% (2017-2018). BM was used to working the same hours, as he was almost on his own in the department. In short, the figures indicate that there was no consistency in payment. He was paid considerably less than could be expected especially after OSD scale started in 2009. This irregularity in lower payment did not occur only yearly, but also within months, which is evident when one looks at the monthly payment record. The decrease in salary and switching on 3 consecutive disciplinary enquiries, 2 suspensions, and a forensic audit, showed that the university management has some hands, and wanted his constructive termination. Moreover, they did not allow to Mr. BM to go to Laboratory, so that he stop getting material for publication. This is called as professional death sentence. The meeting with HR health and HR University was held in the central city and matter was discussed. Mr. BM was penalized by not paying him birthday bonus, and accompanying union member, salary was decreased. Mr. BM's reduction in salary and allowances, about which he was not even informed. Since labour laws were disregarded by the HR department in the case of Mr. BM, this matter must receive urgent attention.[10]

Conclusion

The health must take responsibility for payment, as it is the primary employer, and refund the employee's salary and pension. The University has exploited Mr. BM, as an employee, by carrying out unfair labor practice. It is regrettable that university management has taken decision unilaterally, and not informed to Mr. BM. It is sad that even council resolution was not implemented.

Ethical Issues

Proper care was taken in this manuscript, as not to disclose directly to any institution or an individual, but if is knowingly or unknowingly someone find it, and then it is not author intention. It is failure of system in both health and university, and that led to victimization of Mr. BM. The author would like to high light this system failure in this report, not an individual or institution.

Conflict of Interest: The facts in this report could be submitted in demand. The references were not labeled but can be produced if necessary.

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Biometric Cardiac Profile as a Possible Identifier for sex among Sample of Egyptian Population

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Abstract

Biometric cardiac profile measured by Echocardiography is satisfying the properties of being a successful biometric technique which are universality, distinctiveness, collectability, performance, acceptability and circumvention.

Objectives: The aim of the current study is to establish biometric cardiac profile as a possible identifier for sex among Egyptian candidates.

Subjects & Methods: a prospective study involving 250 adult healthy volunteers provided equal sex distribution and age range between 18 & 70 years. The sample was divided into 5 equal groups according to age. Demographic data were collected. Cardiac parameters were measured by echocardiography. Body measurements (weight, height, body mass index 'BMI' and body surface area 'BSA') were taken from each subject to be studied with respect to the corresponding age, sex & cardiac parameters.

Results: Discriminant scoring was calculated to identify sex using echocardiography with accuracy (**65.6%**).

Conclusion and Recommendations: Presumptive categorization of persons according to sex can be achieved through cardiac profile measured by Echocardiography.

Keywords: *Egyptian, sex & biometric cardiac profile*

Introduction

Biometric identification shows great potential in bridging some of the existing security gaps. To reach a higher security level, specific characters from the human must be selected to identify a person. Biometrics use anatomical, physiological or behavioral features that are significantly different from person to other person. This is useful in security applications and authentication devices, offering an alternative to conventional methods.[1]

Echocardiography is the most widely used non-invasive imaging tool for the assessment of heart structure, functions and remains the gold standard especially with the constant evolution of new applications and technologies of various echocardiographic techniques, current guidelines propose normal reference ranges for standard echocardiography measurement for several clinical proposes. [2]

Normal values provide the background for interpretation of quantitative imaging data, to obtain reference values in normal subjects for the left ventricle

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and the left atrium. Data sets for all patients included age, gender, ethnicity, height, and weight. To ensure a normal population, subjects in these studies were excluded if any of the following criteria were met: history of drug-treated hypertension, diagnosis of diabetes and any cardiac disease. [3]

As the number and proportion of aged individuals in the population increases knowledge of normal physiologic changes associated with aging becomes more important to help in identification of sex and estimation of age. More recently we have begun to look more deeply into both the similarities and differences between both sex, with respect to the structure and function of other organ systems, one of them is the cardiovascular system. [4]

Materials and Method

Study population:

250 healthy Egyptian adults were included in the current study. The candidates were recruited -according to specific criteria-from cases coming for cardiac evaluation or conducting their routine preoperative echocardiography in Kasr Al-ainy cardiac investigation units during the period of the study. Egyptian origin and residence, from 18 years to 70 years, both sexes with no history of cardiac diseases (congenital or acquired) were included in the study. While Patients who had any congenital or acquired cardiac diseases, history of any cardiac surgery or history of systemic diseases (endocrinal, metabolic, or infectious) were excluded from the study.

Subjects were divided according to age into 5 equal age groups, each age group included 50 subjects, 25 males and 25 females as following:

- Group 1. From 18-30 years old
- Group 2. from 31-40 years old
- Group 3. from 41-50 years old
- Group 4. from 51-60 years old
- Group 5. From 60-70 years old

Equipment and instruments:

1. **Body scale:** A mechanical Chinese height and weight boy scale, China Medical Dial model

2. **Echocardiography:** Transthoracic echocardiography (TTE), PHILIPS iU22 ultrasound system for 2D image

§ Demographic data were collected from subjects through a structured questionnaire including age, gender, residence and occupation which is divided into either Intellectual workers (students, employees, teachers, secretary and doctors), Manual workers (as carpenters and mechanics) or Non-Workers (as house wives and retired persons).

Study measurements:

· **Body metric measurements**:

The parameters measured were as follows:

I. Weight in kilogram.

II. Height in meter.

III. Body mass index (BMI) (weight in kg / length in m^2). [5]

IV. Body surface area (BSA) [square root of (height in cm x weight in kg)/3600]. [6]

· Echocardiographic measurements:

1- Aortic dimension, (AO) in cm.

2- Left atrial dimension, (LA) in cm.

3- End diastolic dimension, (LVED) in cm.

4- End systolic dimension, (LVES) in cm.

5- Left ventricle fractional shortening, (FS %).

6- Left ventricle ejection fraction, (EF %).

7- Posterior wall thickness, (PWT) in cm.

8- Inter-ventricular septal thickness in cm.

9- Relative wall thickness, (2 x PWT/ LVED).

Ethical considerations:

The study approval was taken by the ethical review committee of medical research, Faculty of Medicine, Cairo University, Egypt and informed consent

forms were obtained from all participants. The study information including the purpose and details were explained to participants of both groups.

Results

Table 1 showed that there was a statistical significance between sex and **weight (**p value= 0.030). Also, there is a highly statistical significance regarding relations ***between sex and*** **height** (p value < 0.001). While, there is no statistical significant relations ***between sex and BMI and BSA.*** In addition, the table shows females were heavier and shorter than males. Also, females show higher BMI and BSA than males.

Table1; Relation between body measurements and sex

	Sex				P value
	M		F		
	Mean	SD	Mean	SD	
Weight\ kg.	66.57	8.76	69.37	11.33	0.030*
Height\ m.	1.64	0.07	1.61	0.05	< 0.001**
BMI\ kg\ m2	24.46	3.08	29.26	2.798	0.059
BSA\ m2	1.74	0.14	1.75	0.15	0.522

(Unpaired t test, *P is significant if < 0.05, **P is highly significant if < 0.01). BMI: body mass index, BSA: body surface area, M= male, F= female and SD: stander deviation.

Table 2 showed there was a highly statistical significant relations between sex and **AO, LA, LEVD, PWT & FS** with P value (0.001, 0.007, 0.001, 0.001 & 0.001) respectively. Also, there is statistical significant relations between sex and **LVES, EF & Inter-ventricular septal thickness** with P value (0.020, 0.013 & 0.034) respectively. But there was no statistical significant relations between sex and ***relative wall thickness*** also the table shows that males had higher echocardiographic diameters than females.

Table 2; Relations between echocardiographic parameters and sex

	Sex				P value
	M		F		
	Mean	SD	Mean	SD	
AO\ cm.	2.70	0.33	2.57	0.31	0.001**
LA\ cm.	3.58	0.33	3.47	0.33	0.007**
LVED\ cm.	4.86	0.37	4.72	0.34	0.001**
LVES\ cm.	3.11	0.34	3.02	0.29	0.020*
PWT\ cm.	0.90	0.11	0.85	0.10	< 0.001**
FS %	35.70	4.00	34.38	3.66	0.007**
EF %	65.46	4.52	64.06	4.43	0.013*
Inter-ventricular septal thickness\ cm.	0.88	0.12	0.85	0.11	0.034*
Relative wall thickness	0.37	0.05	0.36	0.05	0.059

(Unpaired t test, **P is highly significant if < 0.01). **AO: aortic dimension, LA: left atrial dimension, LVED: end diastolic dimension, LVES: end systolic dimension, PWT: posterior wall thickness, FS% Left ventricle fractional shortening, EF%: Left ventricle ejection fraction, SD: stander deviation, M= male and F= female.**

Discriminant function analysis for determination of sex using the most sensitive parameters in **echocardiographic** assessments shown in **table 3** which were **AO, LVED, PWT and FS.**

Table 3; Canonical Discriminant Function Coefficients for determination of sex using Echocardiographic parameters

Unstandardized coefficients	Function
AO\ cm.	1.354
LVED\ cm.	1.305
PWT\ cm.	4.957
FS%	0.125
Constant	-18.555

AO: aortic dimension, LVED: End diastolic dimension, PWT: posterior wall thickness, FS% Left ventricle fractional shortening, EF%.

Functions at group centroid

Table 4 showed that positive scores are classified as males and negative scores are classified as females. The function at group centroid of discriminant score results in males (0.400) and females (- 0.400).

Table 4; Functions at group centroid of discriminant scores for each group for determination of sex using Echocardiographic parameters only

Sex	Function
M	0.400
F	-0.400

M= male & F= female.

Calculating discriminant score and accuracy of sex determination model from measured echocardiographic parameters reveals the following results. A negative correlation was present between BMI and both **QRS interval** & **QT interval.** The stronger correlations were present with **QRS interval (r=-0.234) then with QT interval (r=-0.219) as revealed in table 4.** Also, there was a highly statistical significance between BMI and **QRS & QT intervals** with P value < 0.001.

Discriminant score I (Determination of Sex using Echocardiographic parameters only)

Where AO: aortic dimension, LVED: End diastolic dimension, PWT: posterior wall thickness, FS% Left ventricle fractional shortening.

Table 5 showed shows accuracy of sex determination model from measured Echocardiographic parameters using unstandardized canonical discriminant functions evaluated at group means in which 65.6% of original grouped cases are correctly classified.

Table 5; Unstandardized canonical discriminant functions for determination of sex using echocardiographic parameters only evaluated at group means

Classification Results					
		Sex	Predicted Group Membership		Total
			M	F	
Original	Count	M	88	37	125
		F	49	76	125
	%	M	70.4	29.6	100.0
		F	39.2	60.8	100.0
	Count	M	86	39	125
		F	49	76	125
	%	M	68.8	31.2	100.0
		F	39.2	60.8	100.0
65.6% of original grouped cases correctly classified.					

M= male & F= female.

Discussion

Regarding echocardiographic measurements relations with sex, results of the current work reported a highly statistical significant relation (P<0.01) between sex and most of measured echocardiographic parameters. Also, males had significantly larger mean values of LVED, LVES, IVSd, PWd, LA antero-posterior dimensions and aortic root dimensions which had statistical significance with sex (p= 0.001), (p= 0.020), (p= 0.034), (p < 0.001), (p= 0.007) and (p= 0.001) respectively.

Similar results was traced by [7] **who** conducted their study on normal volunteers from 14-68 years old of age of both sexes at **Sohag University Hospital.** They reported there was increase in the mean value of different cardiac dimensions in males over females with significant difference which confirms that the discussed parameters are affected by type of gender. Also, their study showed that males had significantly larger LVEDD, LVESD, IVSd, PWd, LV mass, LA antero-posterior diameter and aortic root diameter with statistical significance with sex (p<0.0001 for each). The similarity between both studies may be explained by matched ages of studied groups and also, both studies were Egyptian studies.

But in **contrast** with the current study which showed that **LV ejection fraction** was higher in males with statistical difference with sex (p=0.013), while in the study of [4] **LV ejection fraction** was higher in females with no statistical difference with sex. This explained by difference of age groups in both studies.

Sex has significant independent influences on LV end-diastolic volume (EDV) and LV end-systolic volume (ESV). After puberty sex hormones have a great effect on cardiac structures and functions that reflect on echocardiographic measurements. [3]

Regarding sex identification using echocardiographic parameters, using discriminant

function analysis for determination of sex by echocardiographic parameters, our study concluded a discriminant score = -18.555+ 1.354* (AO) +1.305* (LEVD) + 4.957* (PWT) + 0.125* (FS) where positive scores are classified as males and negative ones are classified as females.

On the other hand, [7] used simple linear logistic regression analysis to predict sex from each echocardiographic parameters and calculated equations. In which sex = 5.96-0.21* **(AO)** or 3.97-0.38***(P.W.T).** The differences between both studies may argued to differences in group's structure and statistical analysis used to interpret data in both studies.

Using discriminant function analysis for determination of sex via multiple parameters is more powerful than usage a simple logistic analysis. In which the independent variables are quantitative (cardiac parameters) started with testing the equality of means between males and females. Stepwise statistics revealed the significant predictors which were used to determine the discriminate function. [8]

Regarding relations of weight with sex, the current study found that females are heavier than males with mean value ranged from 59 to 69 kg in males versus 62 to76 kg in females which had statistical significance with sex (p =0.030). On the contrary, [4] said that; males were heavier than females 70.7± 11.17 kg versus 65.62± 10.47 kg which had statistical significance with sex. Moreover, [9] **said** that; males were heavier than females (72; 82) in males versus (55; 67) kg which had statistical significance with sex.

Regarding the relation of height with sex, the present study showed that males were taller than females with mean value ranged from 1.60 to 1.67 m in males and from 1.60 to 161m in females which had highly statistical significance with sex (p <0.001). Similar results were conducted by [4] who reported that males were taller than females 1.7± 0.07 versus 1.64± 0.08 m which had high statistical significance with sex (p <0.0001). Also, the current results came in agreement with [9] who said that males were taller than females (173;182) cm in males versus (160; 170) cm in females which had high statistical significance with sex (p <0.0001).

Conclusion and Recommendations

The Current study distinguishes itself from other studies by the selectivity of the healthy population, comprising both sexes, the broad range of ages, and the exhaustive set of measurements (body and cardiac). Presumptive categorization of persons according to sex can be achieved through cardiac profile measured by Echocardiography. Cardiac parameters shown to be affected by body metric measurements. We need to establish national data basis using the studied cardiac parameters for application in medico-legal investigations requiring individualization and positive identification of humans.

Declarations:

· **Funding:** None.

· **Acknowledgements**: None.

· **Conflict of Interest**: The authors declare that they have no competing interests.

· **Availability of data and materials:** Data will not be shared with public access.

· **Consent for publication:** Consent forms were given and signed by all subjects prior to participation

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Original article

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Expression of MRHR-CRHR1 (Type I CRH Receptor) and the Effects of Prenatal Yoga on Mother Primagravida Trimester III

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Abstract

Background: CRHR1 receptors are very important for determining the initial HPA axis response to stressful events. Changes in the CRH system and chronically elevated CRH levels are involved in stress-related affective disorders.

Methods: The design of this study is a quasi-experimental approach / quasi experimental and one group design pre and post test design with group control. The population in this study were all trimester III primigravida mothers who examined their pregnancy in PPK I ranks of the XIV / Hasanuddin Kesdam and several health centers in Makassar in 2019. The sample in this study amounted to 24 respondents

Results: the mean CRHR1 gene expression value after was greater than the mean CRHR1 gene expression before. So this means that in the control group CRHR1 gene expression in third trimester pregnant women there will be a significant increase.

Conclusion: an increase in CRHR1 gene expression means that it can be concluded that prenatal yoga decreases CRHR1 gene expression before and after treatment.

Keywords: *MRNA expression, CRHR1 gene, prenatal yoga, primigravida*

Introduction

In achieving the Sustainable Development Goals (SDGs) targets that are a continuation of the MDGs, particularly in the health sector, the participation of various community elements, including health / midwife institutions, is needed. In achieving the SDGs goals, midwives can play a role in achieving the third target of the SDGs, namely healthy and prosperous life, specifically related to maternal and infant health[1,2,3]. As a health service provider, midwives have a strategic and very unique role. Midwives as agents of reform are very close to the community and live in the midst of the community, and play a role in empowering women and the community[4,5].

Under physiological conditions, stress exposure activates the hypothalamus-pituitary-adrenal (HPA) axis. The HPA axis accepts a variety of inputs, including stressors that affect the medial parvocellular nucleus paraventricular hypothalamus (mpPVN) neurons. These neurons will synthesize corticotropin releasing hormone (CRH) and arginine vasopressin (AVP). CRH is secreted into the pituitary portal circulation and binds to the type 1 CRH receptor binding (CRH-R1) in the

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anterior pituitary and stimulates pro-opiomelanocortin (POMC) transcription through a process that involves the activation of adenylatsyclase[16,7,8,9]..

CRH is a critical upstream effector of pituitary and adrenal hormone secretion into the circulating blood. CRH and AVP bind to each receptor, CRH receptor type 1 (CRHR1) and AVP 1B binding receptor (V1bR) located in the pituitary gland. The distribution of CRH receptors in the brain is consistent with the important role of CRH as a mediator of integral stress response. Encephalic structures express abundant CRHR1 including the dorsomedial hypothalamus nucleus, dorsolateral thalamic nucleus, supramammillary nucleus, anterior paraventricular thalamic nucleus and arcuate nucleus[10,11,12].

Acute stress causes the rapid activation of parvocellular CRH neurons as shown by the rapid increase in c-fos and CRH primary transcripts, the latter showing the activation of CRH gene transcription. In this study, researchers focused on the molecular regulatory genes for the GR function, namely the FKBP5 and CRHR1 genes. FKBP5 and CRHR1 are the main proteins that regulate stress response. Where FK506 binding protein 5 encoded by genes (FKBP5) and G-protein coupled type-I CRH receptors encoded by genes (CRHR1), has been linked to several neuropsychiatric disorders. CRHR1 was chosen in this study for several reasons, where CRH is one of the main stress factors in the CNS. CRHR1 encodes the G-protein coupled type I CRH receptor (CRHR1), a protein that is essential for activation of signal transduction pathways that activate mesolimbic and HPA axis responses to various types of stress[13,14]..

CRHR1 receptors are very important for determining the initial HPA axis response to stressful events. Changes in the CRH system and elevated levels of CRH are chronically involved in affective disorders related to stress, especially anxiety and depression. Provision of central CRH in rodents raises phenotypic changes that lead to symptoms of affective disorders). Studies in animal models indicate that CRH's behavioral and hormone effects can be ascribed to CRHR-mediated actions[15,16,17]

During the hypothalamic pituitary growth hormone (HPG) stress the axis is blocked at various levels. In addition to the direct inhibitory effect of glucocorticoids on the HPG axis, increased CRH stimulates the release of somatostatin (SRIF) which is synthesized in PVN. SRIF further inhibits the secretion of growth hormone-releasing hormone (GHRH) in the arcuate nucleus (Arc) and growth hormone (GH) by the anterior pituitary somatotrope. Growth hormone exerts its anabolic action through the production of insulin-like growth factor 1 (IGF1) in the liver[18,19].

Stress besides pressing the HPA axis can also affect other neuroendocrine systems. Stress compresses the hypothalamic-pituitary-gonadal (HPG) axis on various species. The main mediator in this inhibition is stress-induced CRH stimulation. Increased CRH into the hypothalamic-pituitary portal blood flow system which successively stimulates the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary.and also interpreted as a holistic approach in the form of physical, mental and spiritual[20.21].

Materials and Methods

This study uses quantitative research with quasi experimental / quasi experimental approaches and one group design pre and post test design with group control. The population in this study were all trimester III primigravida mothers who examined their pregnancy in KDP I ranks of the XIV / Hasanuddin Kesdam and several Puskesmas in Makassar in 2019. The sample in this study amounted to 24 respondents.

CRHR1 mRNA expression check with realtime PCR

How Realtime PCR works to determine the CRHR1 gene mRNA expression profile.

1) The process of primary oligonucleotide specific genes for the CRHR1 and b-actin genes as housekeeping gene (internal control).

2) Detect CRHR1 mRNA gene using forward and Reverse specific primers

3) PCR conditions with a temperature of 95oC for 10 seconds and 60oC for 30 seconds for 45 cycles.

4) And adapted to the Tomomi Yajima protocol where the qRT PCR uses the sybrgreen qRT-PCR master mix kit, one step. This protocol is optimized for real time PCR machine CFX Connect System (USA) instruments.

5) Reference to the passive coloring is included in the reaction, diluted 1: 500. Dyes containing dyes are kept away from light. Dilute 2 x SYBR Green QRT-PCR master mix and store on ice. Following the initial melting of the master mix, the unused portion is kept at 4oC with a note, avoiding the repetitive freeze-liquid cycle.

6) The reaction of the experiment is prepared by adding the following components. Prepare a mixture of reagents for the reaction using several components as below.

7) Mixed reagents by taking a final volume of 25 µl including mRNA samples extracted according to the protocol Hatta, et al. 2017)

8) 12.5 µl of 2 x SYBR Green QRT-PCR master mix plus x µl from the initial primer (optimized concentration) plus Nuklease - free PCR - H2 level x µl final primer (optimized concentration) and also 0.375 µl reference dye solution from step 1 (optional) as well as 1.0 µl of RT / Rnase a mixture of enzyme blocks with 25 µl of the total reaction volume can also be used. The reaction is mixed slowly so as not to form bubbles (not rotated), then distribute the mixture to the test test tube by adding x µl of RNA to each test tube.

Result

Tabel 1: Test results for normality levels of

Group	Pengamatan	p-value	Distribusion
control	Before	0.087	Normal
	After	0.867	Normal
Treatment Yoga	Before	0.354	Normal
	After	0.276	Normal

In table 2, the comparison test of CRHR1 gene expression used paired sample t test.

Table 2 Results of comparison tests before CRHR1 gene expression and after treatment

Group treatment	Before Rerata ± SD	After Rerata ± SD	p-value
Control	5.79±0.51	8.58±0.6	T
Treatment yoga	6.24±0.52	5.71±0.46	0.015

Table 2 shows the mean value of CRHR1 gene expression after the greater value than the mean CRHR1 gene expression before. So this means that in the control group CRHR1 gene expression in third trimester pregnant women there will be a significant increase.

Table 3 Test results for comparing CRHR1 gene expression

Treatment	Control Rerata ± SD	Treatment Rerata ± SD	p-value
Before	5.79±0.51	6.24±0.52	0.045
After	8.58±0.6	5.7±0.45	0.000

Table 3 shows that there are significant differences (p = 0.045 <∝) on average CRHR1 gene expression. This means that the treatment of Yoga exercises in third trimester pregnant women can reduce CRHR1 gene expression**.**

Table 4 Correlation test results between variables

corelasi variable	N	coefisien korelasi (r)	p-value
Anxiety level with CRHR1	12	0.853	0.000
cortisol with gen CRHR1	12	0.956	0.000

Discussion

The mean CRHR1 gene expression results before treatment (6.24 ± 0.52) after treatment (5.71 ± 0.46). The average CRHR1 gene expression after the value was smaller than the average CRHR1 gene expression before being treated. This means that in the treatment group CRHR1 gene expression in third trimester pregnant women will be a significant decrease. In other words there is the effect of Yoga exercise treatment on CRHR1 gene expression in third trimester pregnant women,

There was a significant difference (p = 0.045 <∝) mean CRHR1 gene expression at 27-28 weeks gestation between the control group (before) (5.79 ± 0.51) and the treatment group (before) (6.24 ± 0.52). Based on the mean value of CRHR1 gene expression in the treatment group did not differ greatly with the mean value of CRHR1 gene expression in the control group. This means that it can be assumed that the mean CRHR1 gene expression initially did not differ greatly between the two groups.

Based on the mean value of CRHR1 gene expression in the treatment group the value is smaller when compared with the average value of CRHR1 gene expression in the control group. This means that third trimester pregnant women who did not receive yoga exercises showed a high CRHR1 gene expression when compared to third trimester pregnant women who received yoga exercises. In other words, the treatment of Yoga exercises in third trimester pregnant women can reduce CRHR1 gene expression. So the third minor hypothesis has been proven, that is, there is a difference in effect between non-action and prenatal Yoga exercises on CRHR1 gene expression in primigravida trimester III.

This study is in line with Four case control studies analyzing CRHR1 polymorphisms in 2336 Caucasian individuals, of which 1100 were suicidal individuals and 1236 were control subjects. analyzing Irish and Canadians with each attempted suicide, found a significant relationship between the CRHR1 gene and the severity of suicide behavior (p> 0.05). His study of 135 bipolar disorder patients was recruited for clinical assessment of childhood trauma and suicides through the childhood trauma questionnaire (CTQ) and the Columbia Suicide Severity Rating Scale (C-SSRS), investigating trauma in childhood and genetic variability on the HPA axis independently or interacting, in suicide attempts in bipolar disorder patients. Results of individuals with higher total CTQ scores, emotional abuse, sexual harassment and emotional neglect are more likely to indicate suicide[22,23].

An increase in antenatal pressure has been associated with negative outcomes in both the mother and fetus. One mechanism that is thought to underlie this relationship is the hypothalamic-pituitary-adrenal (HPA) axis. It is important to remember that the function of the HPA axis is not only modulated by environmental stimuli, but is also partly determined by genetic factors. in his research proved that the CRH-BP gene is marginally linked to suicidal behavior. The CRH-BP protein binds to CRH which limits its bioavailability and hence prevents activation of the CRH receptor and further signaling of the HPA axis. People carrying CRH-BP alleles rs7728378-C are more likely to report a history of suicidal behavior. It can be explained that abuse in childhood has been linked to permanent changes in the HPA axis, a stress management system with damaging

effects on the developing brain that can be linked to behavioral problems in life in adulthood[18,16,20].

Stress induces activation of the HPA shaft, which is under the control of several neurotransmitter systems. corticotropin-releasing hormone (CRH) is released from the hypothalamus paraventricularis (PVN) nucleus into the portal venous system, where this hormone is transported to the anterior pituitary to stimulate the secretion of adrenocorticotropic hormone (ACTH) into the blood circulation. ACTH is transported to the adrenal gland cortex, where it activates the release of the primary stress hormone cortisol into the bloodstream. Cortisol itself regulates the activation of the HPA axis through negative feedback by binding to glucocorticoid receptors (GR) in the anterior pituitary and in the hypothalamus[24,25,].

Conclusion

There was a significant difference in the mean CRHR1 gene expression in the control group before (5.79 ± 0.51) and after observation (8.58 ± 0.6), it could be interpreted that there was an increase in CRHR1 gene expression meaning it could be concluded that prenatal yoga decreased CRHR1 gene expression before and after treatment.

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Conflict of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 839/ UN4.6.4.5.31//PP36/2019), and all research subjects gave written informed consent.

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The Measurement of Cortisol Levels in the Blood Pregnant Trimester III with ELISA (Enzyme Linked Immunosorbent Assay) Method

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Abstract

Background: During pregnancy, maternal cortisol increases 2-4 times. Every type of body response in the form of stress, both physical and psychological stress can increase ACTH secretion, which in turn can increase cortisol levels.

Methods: This study uses quasi experimental / quasi experimental one group design pre and post test design with group control. The sample in this study was 24 respondents. . Cortisol level examination tool uses a microplate reader with the ELISA examination method.

Results: There was a significant difference (p = 0.000 <∝) of the average cortisol level in the control group before observation (54.43 ± 25.86) and after observation (459.15 ± 28.08Whereas in the treatment group showed a significant difference (p = 0.004 <∝) the average cortisol level before being treated (65.26 ± 28.74) and after being treated (53.65 ± 23.68) Yoga exercises.

Conclusion: There was a significant difference (p = 0,000 <∝), mean cortisol levels in the control group before observation (54.43 ± 25.86) and observations after (459.15 ± 28.08), whereas in the treatment group there were significant differences (p = 0.004 <∝) it can be concluded that prenatal yoga reduces cortisol levels before and after treatment.

Keywords: *Cortisol, blood level, ELISA, pregnant, Trimester III*

Introduction

Cortisol is the main product of the hypothalamus-pituitary-adrenal (HPA) axis, secreted both in response to threats and in a strong and consistent pattern throughout the day (diurnal cortisol rhythm). Cortisol has many effects on the body, including building up energy and shutting down systems that are not essential for dealing with threats, and also affect memory and mood[1,2].

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Under normal circumstances, stress hormones are released in small amounts throughout the day, but when faced with stress these hormone levels increase dramatically (in the range of 5-20 times). Each type of body response in the form of stress, both physical stress and psychological stress can increase secretion ACTH, which can ultimately increase cortisol levels. In conditions of anxiety, anxiety and depression, cortisol secretion increased[3,4,5] Increased cortisol affects the function of almost every cell and tissue in the body by activating glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) that modify gene expression programs through direct binding to specific DNA sequences and

recruitment of transcription complexes[6,7].

Several clinical studies have examined stressors that occur naturally and most report insignificant or low relationships. This is especially true for studies that use a single measure of cortisol from amniotic fluid, serum or saliva. Ecological momentary assessments are used to simultaneously assess mood and cortisol 5 times a day for 3 days in 83 women (6-37 weeks gestational age). possible cortisol as a biological relationship between maternal psychological stress during pregnancy and fetal development[8,9].

The results from multilevel analysis showed a strong relationship between negative mood and cortisol. For every 1.0% increase in negative moods there is an appropriate 1.9% increase in cortisol. This association was not affected by advanced gestational age cross-sectional studies at the end of pregnancy in 79 women to investigate the relationship between the factors involved in anxiety and depression[10,11,12].

Some regression analysis shows that depressed subjective feelings explain more than 50% of the variation of BDI and STAI scores. Plasma cortisol is not a significant predictor of psychometric scores and did not show a significant correlation with them in correlation analysis, and subjects with low and high cortisol levels showed the same psychometric scores. The mechanism that mediates the effects of subjective pressure remains unclear: candidates who might include monoamine neurotransmission and / or changes caused by stress in the expression or distribution of glucocorticoid receptors[13,14]..

Research to test one potential mechanism by examining the relationship between pregnancy anxiety and maternal salivary cortisol on 4 occasions during pregnancy in a sample of 448 women. Strong evidence from animal models shows that maternal pressure predicts increased fetal glucocorticoid exposure through synthesis and release of maternal glucocorticoids but similar evidence in humans that links pregnancy anxiety (or general maternal pressure) with increased levels of maternal cortisol during pregnancy is still lacking[5,9]

During pregnancy, maternal cortisol increases 2-4-fold, and crosses the placenta but is limited because the pathway is regulated by the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD2: Maternal cortisol accounts for about 30-40% of the variability in fetal cortisol concentration. Physiological increase in glucocorticoid levels during the pregnancy, facilitating fetal organ maturation by promoting cell differentiation Synthetic glucocorticoids (including dexamethasone or betamethasone) are given during the third trimester of pregnancy at risk of preterm delivery to increase the survival rate and maturation of the lungs[15,16]. Based on these references, researchers are interested in examining cortisol levels in pregnant women with prenatal yoga treatment.

Materials ad Methods

This study used quasi experimental one group design pre and post test design with group control. In this study the researchers' treatment or intervention was prenatal Yoga exercises in third trimester pregnant women (UK 28-40 weeks) ,The sample in this study was 24 respondents.

Cortisol examination procedure;

a) Prepare samples, reagents, main solutions and equipment

b) Let the blood sample freeze for 2 hours at 4 ° C. Do it

c) Centrifugation for 15 minutes, then immediately assay checks or can be divided and stored at temperatures <-20 ° C (≤ 1 month), or -80 ° C (≤ 6 months). Blood tubes must be disposible, sterile.

d) Reagents are prepared at room temperature

e) Dilute 30 ml of WBC to 750 ml of wash buffer with distilled water.

f) If crystalline deposits are found in the Wash Buffer Consentrate, warm at 40 ° C and shake gently until the crystals dissolve.

g) The solution must be cooled to room temperature before use

h) Standard Solution

1. Prepare a standard solution 15 minutes before use.

2. Standard with 1 ml Sample Diluent, allow 10 minutes to dissolve.

3. This reconstruction produces 40 ng / ml stock solution. Then make serial dilutions (40, 20, 10, 5, 2.5, 1.25, 0.63, 0 ng / mL).

i) Procedure:

1. Add 50 µl of standard or sample to each microplate hole.

2. Add 50 µl Biotinylated Detection AB. Incubation 45 minutes at 37 ° C

3. Aspiration and wash 3 times

4. Add 100 µl HRP Conjugate. Incubation 30 minutes at 37 ° C

5. Aspiration and wash 5 times

6. Add 90 µl Substrate Reagent. Incubation for 15 minutes at 37 ° C

7. Add 50 µl Stop Solution. Read at 450 nm right away.

8. Calculation of results in the form of OD values. Real values or concentrations are generated by entering OD values in standard curves.

Result

Table 1 Characteristics of education level and type of work

Variable	Group		p-value
	Control (%)	Treantment (%)	
Level education			0.590
- SMA	1 (8.3%)	3 (25%)	
- D3	11 (91.7%)	9 (75%)	
Type work			1.000
- House wife	10 (83.3%)	11 (91.7%)	
- Wiraswasta	2 (16.7%)	1 (8.3%)	

The results level education test analysis on the distribution of data on the education level of pregnant women in Table 3 showed no significant difference (p = 0.590> □) which was divided into 2 levels of education. Namely: high school and D3. Whereas there was no significant difference in the type of work (p = 1.00> ∝)

Table 2: The Comparison Of Cortisol Levels Before And After Treatment

Group observation	Before Rerata ± SD	After Rerata ± SD	p-value
Control	54.43±25.86	459.15±28.08	0.000
Treatment Yoga	65.26±28.74	53.65±23.68	0.004

Table 2 shows that there is a significant difference (p = 0,000 <∝) of the average cortisol level in the control group before observation (54.43 ± 25.86) and after observation (459.15 ± 28.08). it means Whereas in the treatment group showed a significant difference (p = 0.004 <∝) the average cortisol level before being treated (65.26 ± 28.74) and after being treated (53.65 ± 23.68) Yoga exercises

Table 3 The Correlation Test Results Between Variables In The Group

Treatment After Yoga Exercises

Correlation of variable	n	Koefisien korelasi (r)	p-value
Level anxiety and kortisol	12	0.853	0.000
Cortisol With FKBP5	12	0.937	0.000

Discussion

In the results of this study the treatment of Yoga exercises in third trimester pregnant women can significantly reduce cortisol levels. So the third minor hypothesis has been proven, that is, there is a difference in effect between non-action and prenatal Yoga exercises on cortisol levels in primigravida trimester III.

In line with previous studies where anxiety disorders were associated with increased levels of basal cortisol in saliva as well as in plasma. Stress conditions during pregnancy will affect hormonal changes and fetal growth, especially in the hormone cortisol and will result in impaired fetal growth such as low birth weight and abnormal body length. Several studies have shown that depression is associated with hyperactivity from the hypothalamic-pituitary-adrenal (HPA) axis which results in high cortisol levels[17,18,19].

During pregnancy, maternal cortisol increases 2-4 times. The physiological increase in glucocorticoid levels during pregnancy, facilitates maturation of the fetal organs by promoting cell differentiation. Synthetic glucocorticoids (including dexamethasone or betamethasone) given during the third trimester of pregnancy are at risk of preterm delivery to increase the survival rate and maturation of the lungs[8,110,114].

Research conducted on white rats (Rattus Norvegicus) that formation of cortisol and cortisol secretion increased six times in a mouse within 4 to 20 minutes after fracture of the two bones in his legs which caused the rat to be stressed. This is in line with the literature that stress can cause an increase in ACTH secretion by anterior pituitary gland followed by an increase in cortisol hormone secretion within a few minutes, prenatal yoga reduces anxiety and decreases cortisol levels. [20]

Stressors activate the hypothalamic-pituitary-adrenal (HPA) axis, which triggers the secretion of glucocorticoid hormones (cortisol for humans and corticosterone for mice) to drive systemic changes in physiological patterns, which aim to return the body to homeostasis. Exposure to high levels of glucocorticoids can cause changes in fetal programming, as well as affect the development of glucocorticoid receptors in the fetal brain. For mothers, glucocorticoids induce physiological and behavioral changes that can interfere with hereditary care[4,9,13].

Initially, stress causes an increase in diurnal cortisol, facilitating the recruitment of energy and coping resources. However, repeated or sustained increases in cortisol can cause damage to the negative feedback system of cortisol secretion, resulting in flat slopes and negative clinical and health outcomes[13,20]. Heterogeneity in the relationship of chronic stress to diurnal slope indicates that some individuals are more susceptible than others to a disturbance in the healthy relationship between stress and cortisol secretion. One factor that might be a genetic risk. [21,22]

When cortisol levels begin to fall, the inhibitory effect of cortisol on the hypothalamus and anterior pituitary decreases so that the factors that stimulate increased cortisol secretion (CRH-ACTH) will increase[9]. This system is sensitive because cortisol production or excessive administration of cortisol or other synthetic glucocorticoids can quickly inhibit the hypothalamic-pituitary axis and stop the production of ACTH[23,24]. This study contradicts previous results, in which researchers investigated whether anxiety disorders were associated with the HPA axis by using a new method of assessing cortisol in hair for a method that was considered to provide a retrospective cortisol index of secretion over a long period of time. The results showed lower hair cortisol levels in anxiety disorder patients than in healthy controls. This interpretation tentatively indicates the condition of anxiety disorders is associated with hypocortisolism[3,25].

The hypoactive HPA axis model has been used to explain adrenocortical activity in other psychiatric and psychosomatic disorders as well as in stress illustrating that participants who exhibit hypocortisolism often exhibit a series of symptoms characterized by increased sensitivity to stress, pain and fatigue[26]. which is characterized by increased sensitivity to stress, pain and fatigue. The pathway that leads to hypocortisolism is likely to result from exacerbated worries, higher tension and anxiety may lead to an initial increase in adrenocortical activity, namely hypercortisolism. With the increase in the mechanism of chronic compensation becomes activated and gradually produces a weakening of cortisol secretion, namely hypocortisolism. [16,20]

Conclusion

There was a significant difference ($p = 0.000 <\propto$), mean cortisol levels in the control group before observation (54.43 ± 25.86) and after observations (459.15 ± 28.08), whereas in the treatment group there were significant differences ($p = 0.004 <\propto$) cortisol levels before being treated (65.26 ± 28.74) and after being treated (53.65 ± 23.68), it can be concluded that prenatal yoga reduces cortisol levels before and after treatment.

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Contamination of Polynuclear Aromatic Hydrocarbons-(PNAH) in Sediments: Identification and Distribution in the River of Shatt Al-Arab-(ROSA)

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Abstract

The sediments were analyzed for total organic carbon content-(TOCC), grain size-(GS), and PNAH levels and origins. The sediments were taken from 9 stations-(ST) on ROSA. Gas chromatography-(GC) analysis showed that the PNAH level in ROSA sediments was comparatively low to medium compared to other world locations. The PNAH total levels varied from 31.86 ng/g dry weight-(DW)-(ST 9) to 88.99 ng/g DW-(ST 6). The highest PNAH levels were close to oil pollution sources at ST 2, 4, 6, and 8. The TOCC ranged from 0.32 % to 1.64 %. The data suggested that the PNAH levels in the ROSA were linked to TOCC and GS. The PNAH compounds and ratios and analysis of principal components-(AOPC) indicated that the PNAH sources in the river were biogenic-(organisms), pyrogenic-(combustion), and petrogenic-(petroleum). Ecological risk evaluation showed that PNAH did not cause any deleterious impacts on the ROSA.

***Keywords*:** Water pollution, aromatic hydrocarbons, organic carbon, Shatt Al-Arab.

Introduction

The PNAH are a widespread and stable group of pollutants in the aquatic environment. They have long transportation capacity and causes harmful environmental impacts[1]. There are many PNAH in nature but 16 compounds of them are usually studied, classified by the US-EPA as highly toxic. PNAH are generally hydrophobic and slightly soluble in water. PNAH of high-molecular-weight-(POHMW)-(≤ 4 rings) are slightly soluble in water, low evaporation and highly lipophilic than PNAH of low-molecular-weight-(POLMW)-(≥ 3 rings)[2].

Because of the hydrophobic nature of PNAH, tend to correlate with organic materials in the aquatic environment, so sediments are the ultimate PNAH stores in the water and are a good tools for monitoring PNAH[3].

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The PNAH accumulation in sediments is due to the industrial and nature releases of them[4]. PNAH industrial emissions include petrogenic and pyrogenic sources. Pyrogenic sources include fossil fuel combustion, trees and weeds burning, and volcanic eruptions, etc. Whereas, PNAH petrogenic sources are linked to the spills of oils[4]. The PNAH from petrogenic or pyrogenic sources show different behaviors and distribution. Pyrogenic PNAH are more closely associated with sediments and resistant to biodegradation than petrogenic[2].

Exposure to hydrocarbons has many health-damaging effects such as reproductive disorders, mutations in DNA, and cancer. Several guidelines for estimating PNAH damage to aquatic organisms have been documented by authors and environmental concern international organizations[1].

The employ PNAH ratios having the same molecular mass enables us to explain the PNAH formation and sources. Various criteria have been established to demonstrate the PNAH origin in aquatic sediments such as phenanthrene/anthracene-(PH/AN), fluoranthene/

pyrene-(FL/PY), anthracene/anthracene+phenanthrene-(AN/AN+PH), benzo[a]anthracene/benzo[a] anthracene+chrysene-(BA/BA+CH), and fluoranthene/ fluoranthene+pyrene-(FL/FL+PY)[6].

Despite great global interest in the PNAH effects on the ecosystem. Little is known about their concentrations and sources in some world countries. Iraq has many industries, but it has not focused much on monitoring PNAH in its environment. There is also restricted knowledge about the range to which different combustion processes attribute to PNAH releases.

This paper aims to study the PNAH existence, distribution and sources in the ROSA sediments. The ROSA is an important and unique ecosystem due to the presence of many aquatic habitats and species that live in a completely different environment (Arabian Gulf). However, it is described as having considerable inputs of various pollutants that come from urban areas and industrial locations. This ecosystem, over the years, due to its proximity to many villages, towns and various human activities such as agricultural, industrial and municipal efficacies, possesses the PNAH accumulation and other pollutants in its sediments[7].

The study area is located in the city of Basrah, between longitudes, 29°56'33.8'' N and 30°48'10.6'' N and latitudes, 47°45'03.8'' E and 48°34'37.4'' E in southern Iraq. The region consists of a number of urban, suburban, and rural settlements surrounding the ROSA. Many lands in the area are used to grow different crops and trees. Solid waste disposal and wastewater treatment sites are also part of land use. Moreover, the area is distinguished for containing some heavy and light industries, in addition to power stations and oil refineries scattered along the ROSA banks. The area also has sites for burning municipal, agricultural and household waste. The average annual temperature is 25 °C, ranging from 13 °C-(January) to 33 °C-(July). The average annual downpour is 141 mm, and its duration is from October-(1 mm) to December-(29 mm).

Materials and Methods

The used glassware has been completely cleaned out. All solvents were of an analytical degree and distilled before work. Anhydrous sodium sulfate-(Na_2SO_4) ashed in 450 °C. Silica and alumina were solvents washed and dried prior to use.

Forty-five samples of superficie sediment were taken at a depth of 10-30 cm from 9 ST located along the ROSA during 2019. The ST were Karmatt-Ali-(ST 1)-(30°48'10.6" N-47°45'03.8" E), Nahran-Amar-(ST 2)-(30°45'05.0" N-47°39'53.0" E), Al-Hurtha-(ST 3)-(30°39'48.0" N-47°44'41.0" E), Basrah-Center-(ST 4)-(30°33'00.0" N-47°47'10.0" E), Abu-Al-Khaseeib-(ST 5)-(30°27'44.5" N-48°00'06.0" E), Abadon-(ST 6)-(30°03'42.0" N-48°27'03.0" E), Al-Sibea-(ST 7)-(30°20'16.5" N-48°15'34.5" E), Al-Faw-(ST 8)-(29°58'28.6" N-48°29'09.5" E) and Ras-Al-Beshea-(ST 9)-(29°56'33.8" N-48°34'37.4" E).The samples were then transferred to the laboratory with plastic bag and stored at 3.5 °C. The sediments were exsiccated, homogenized, grinded with mortar and maul, and sifted with 400 µm sieve before analysis.

PNAH was extracted from sediment samples depending on the procedure of Jiao et al.[2]. About 30 g of sediment was placed in Soxhlet and extracted for 18 hours using hexane and methylene chloride (250 ml, 1:1). The extract was reduced to 2.5 ml by nitrogen gas. The extract was then cleaned up with a chromatographic column containing activated silica and alumina (10 g, 100-200 mesh) and Na_2SO_4 (1 g). PNAH was eluted with benzene (20 ml). Benzene was removed and the remaining sample was analyzed by GC.

To validate the sediment samples, they were spiked with a standard mixture (1 µl, 100 mg/l) of 16 PNAH to distilled water (500 ml) pre-extracted with solvent (30 ml) (blank). After that, the spiked sediments were extracted and analyzed. The PNAH standard were employed to calibrate the GC.

The PNAH were analyzed by using GC-(Agilent-USA) equipped with flame ionization detector-(FID) (Agilent-USA). The detector and injector running temperatures were 300 °C and 320 °C. The column temperature was set at 50 °C to 8 minute and then 8 °C/minute for 350°C. The rate of PNAH standards recoveries ranged of 87.6 % to 96.8 %. The limit of detection varied of 0.16 to 0.81 ng/g DW.

The sediment GS analysis was performed based on the traditional procedure of Folk[8]. The TOCC were estimated by titration technique of Walkey's and Black[9].

PNAH concentrations were statistically analyzed. The standard error-(SE) for replications was calculated. One-way analysis of variance-(ANOVA) was used to compare between samples multiple groups. Statistical significance was determined as P <0.05. The AOPC was employed to identify different PNAH sources. The data on the correlation matrix was analyzed by SPSS-(version16) of Windows-(I.B.M., Armonk, USA).

Result and Discussion

PNAH are introduced to the aquatic environment through natural and industrial operations[4]. The PNAH estimation in the ROSA sediments reflected their emission sources (pyrogenic or petrogenic). The total PNAH (naphthalene-(NA), acenaphthylene-(AC), acenaphthene-(AE), fluorene-(FU), phenanthrene-(PH), anthracene-(AN), fluoranthene-(FL), pyrene-(PY), benzo[a]anthracene-(BA), chrysene-(CH), benzo[b] fluoranthene-(BF), benzo[k]fluoranthene-(BK), perylene-(PE), benzo[a]pyrene-(BP=), indeno[1,2,3-cd]pyrene-(IP) and benzo[g,h,i]perylene-(BG)) concentrations ranged from 31.86 ng/g DW-(ST 9) to 88.99 ng/g DW-(ST 6) with high concentrations in the ROSA sediments close to the locations of high pollution sources (ST 2, 4, 6 and 7) (Fig. 1). The ROSA is habitat of numerous industries and human events, which may illustrate the marked variation in PNAH concentrations in the different ST sediments. The ROSA is exposed to PNAH pollution from various sources, including sewage waste, the waste of industrial, agricultural and household and the oil disposal from petroleum refineries and tankers, as well as the oil natural leakage from the ROSA oil production sites[7]. By comparing of the PNAH levels in the ROSA sediments with other world sites, the PNAH levels are high compared to studies of Wang et al.[10] and Zrafi et al.[6], and lower than those reported by Shilla and Routh[1] and Adeniji et al.[11].

The PNAH determination origin depends on the aromatics identification of particular sources. The FL and PY existence refers the pyrolysis importance because these components consist of condensing POLMW at elevated temperatures[6]. The CH is an ideal aromatic that indicates to petroleum origin because of its resistance to biodegradation and non-biological factors[12]. Therefore, the high CH concentration in the ROSA sediments (ST 2, 4, 6 and 7) suggest a petrogenic origin. The PE origin remains dialectical. Lin et al.[3] reported the PE origin in the marine environment sediments from terrestrial organisms. While, Wang et al.[11] showed that the PE origin is more from the aquatic environment. Shilla and Routh (2018) indicated that the PE source in the marine sediment is organic detritus. Likewise, small PE concentrations may come from industrial activities (Zrafi *et al*., 2013). In current sediments, PE is present in all ST (5.57 - 8.78 ng/g) with high concentrations at ST 1, 3, 5, 8 and 9, a large part of which is reckoned to the terrestrial origin.

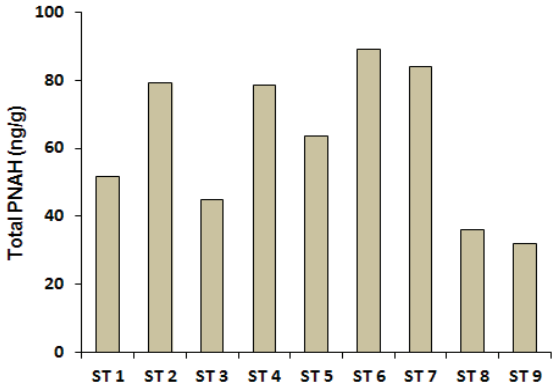


Fig. 1: PNAH spatial profiles in the ROSA sediments.

POLMW and POHMW identification is a valuable tool in determining oil pollution sources. Petroleum sources have comparatively high levels of POLMW-(2 and 3-rings), while POHMW-(≤ 4-rings) is an ideal feature of pyrolysis sources[1]. In the ROSA sediments, POHMW-(4 to 6-rings) predominated over POLMW (Fig. 2). This may be due to the high POHMW adsorption by sediments and POLMW constantly subjected to the biodegradation[10]. POHMW has been found to be environmentally stable caused carcinogenicity and genotoxicity, and these effects increase as their molecular weight increases[11].

With the geochemistry advancement, some ratios were developed to distinguish between PNAH sources. PH/AN <3 denotes pyrogenic source and >3 indicates petrogenic source[3]. FL/PY ratio of less than 1 indicates a petroleum source, while a ratio greater than 1 refers pyrolysis[6]. AN/AN+PH ratio of less than 0.1 indicates petroleum and diagenesis origin, while more than 0.1 are properties of combustion operations[4]. FL/FL+PY less than 0.4 denotes crude oil, diesel and gasoline combustion, and between 0.4 to 0.5 refers crude oil

burning and more than 0.5 indicates to pyrogenic sources[1]. BA/BA+CH <0.20 is the petroleum characteristic, from 0.20 to 0.35 indicates crude oil burning, and more than 0.35 means combustion processes[11]. The current ratios indicated that the sediments PNAH in the ST 2, 4, 6 and 7 are from petrogenic and combustion sources. While the PNAH source in the ST 1, 3, 5, 8, and 9 may be from the pyrolysis origin (Table 1), however, the last ST cannot be excluded from the petroleum inputs due to the presence of many oil effectiveness in the ROSA attributed to PNAH.

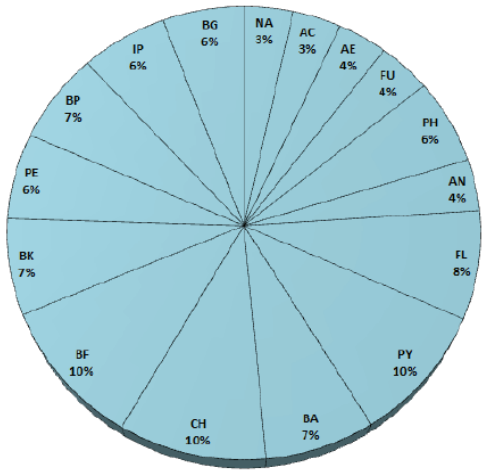


Fig. 2: PNAH distribution in the ROSA sediments.

PNAH contamination sources were identified in the ROSA sediments using AOPC. The major components in AOPC results formed 41.3%, 18.9% and 12.1% of the total variance (Table 2). PC-1 was highly loaded on POLMW, and identified as petrogenic. PC-2 was strongly loaded on parent-PNAH, and could be identified as a pyrogenic. PC-3 was heavily loaded on none of the PNAH, which was determined as an unknown source. The AOPC data showed that there are various PNAH sources in the sediments.

Several factors affecting PNAH levels have been found in sediments. Among them, TOCC and GS are examined in this study. TOCC % and GS for the sediments are shown in Table (3). TOCC % ranged from 0.33 % (ST 9) to 1.63% (ST 6). The difference in TOCC between ST sediments may be due to the variation in environments and organic matter sources. A direct relationship has been found between PNAH and TOCC % (r= 0.99). The organic matters significance for sediments in the PNAH partitioning demonstrated by[4].

Guidelines of sediment quality-(GOSQ) are a good instrument for assessing the harmful effects of pollution in aquatic sediments[11]. The range of low impacts-(ROLI), range of medium impacts-(ROMI), level of threshold impacts-(LOTI), and level of probable impacts-(LOPI) are employed to estimate the PNAH environmental toxicity in sediments (Fig, 3). In the following chemical concentrations ranges, the harmful bio-influences anticipated scarcely (<ROLI/LOTI), occasionally (≥ROLI/LOTI and <ROMI/LOPI) and repeatedly (≥ROMI/LOPI) (Olayinka *et al*., 2019). The estimations exhibited that PNAH concentrations were lower than the values of ROLI and LOPI at all investigating ST by comparing pollutant levels with GOSQ. It can be concluded that PNAH does not cause any harmful bio-effects in the ROSA sediment.

Conclusions

Sediment analysis provides worthy data on the PNAH levels and origins in the environment. This investigate demonstrated the dominance of POHMW over POLMW. Comparing with other world sediments, PNAH contamination was moderate. The most polluted ST with PNAH are close to heavy oil pollution sources (ST 2, 4, 6 and 7). The PNAH distribution in sediments shows a direct relationship with TOCC and GS. PNAH environmental risk assessment indicated that PNAH levels do not produce any instant bio-effects on the ROSA. Depending on PNAH profiles, markers and ratios and AOPC can be deduced that the PNAH origin in the ROSA was biogenic-(biosynthesis and biodegradation), pyrogenic-(combustion of fossil fuel, coal, wood, etc.), and petrogenic-(oils). Further investigates should be done on PNAH to detect their impacts and control their elevated levels harmful to mans, animals and plants in the ROSA.

Table 1: PNAH ratio in the ROSA sediments.

Ratio	ST 1	ST 2	ST 3	ST 4	ST 5	ST 6	ST 7	ST 8	ST 9
PH/AN	0.660	9.085	0.841	7.719	1.093	7.918	8.730	0.527	0.480
FL/PY	3.708	0.311	2.801	0.707	3.459	0.521	0.292	3.155	3.069
AN/AN+PH	0.468	0.080	0.374	0.208	0.355	0.130	0.087	0.514	0.624
BA/BA+CH	0.294	0.242	0.768	0.396	0.282	0.295	0.461	0.531	0.659
FL/FL+PY	0.637	0.291	0.555	0.367	0.602	0.286	0.291	0.610	0.574

Table 2. PNAH principal components-(PC) factor loading in the ROSA sediments.

PNAH	PC-1	PC-2	PC-3
NA	0.94	0.24	0.27
AC	0.92	0.43	0.17
AE	0.85	0.32	0.52
FU	0.93	0.18	0.19
PH	0.87	0.29	0.55
AN	0.74	0.26	0.21
FL	0.37	0.78	0.27
PY	0.14	0.96	0.31
BA	0.35	0.85	0.55
CH	0.37	0.94	0.06
BF	0.33	0.84	0.35
BK	0.25	0.78	0.36
PE	0.43	0.88	0.15
BP	0.24	0.83	0.24
IP	0.18	0.77	0.14
BG	0.33	0.84	0.08
Variance (%)	18.9	41.3	12.1

Table 3. Sedimentary properties of the ROSA.

ST	Sediment kind	TOCC (%)
1	Puddly-Sand	0.68
2	Sandy-Slime	0.96
3	Slime	0.61
4	Sandy-Slime	0.82
5	Sandy-Slime	0.73
6	Sandy-Slime	1.63
7	Sandy-Slime	1.01
8	Sandy-Cay	0.56
9	Sandy-Slime	0.33

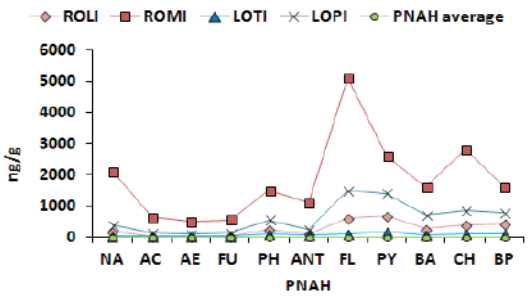


Fig. 3: PNAH average in the ROSA sediments relative to ROLI, ROMI, LOTI, and LOPI.

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Serum Androgens in Men with Rheumatoid Arthritis

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Abstract

Background: It has been hypothesised, that low serum concentrations of

androgen-anabolic hormones would play part in the aetiology of rheumatoid arthritis (RA). This study aimed to determine the role of low serum testosterone and DHEAs in men with rheumatoid arthritis.

Methods: a case control study was conducted on 73 male patients with rheumatoid arthritis and 73 healthy male age matched control at the Department of Rheumatology and medical rehabilitation, Rheumtologic outpatient, and the biologic centre, Basra teaching hospital, Basra Provence at the south of Iraq from February 2019 till March 2020.Full investigations including total serum testosterone(TST) and dehydroepiandosteron sulfate(DHEAs) levels and disease activity were measured for patients, TST and DHEAs were measured for controls. **Results**: The median total serum testosterone level of the patients and the control groups was; 2.2ng/ml and 7.8ng/ml respectively. The median DHEAs level of the patients and the control groups; 81mg/dl and 343mg/dl respectively. Men with low serum androgen levels and in particular low serum level of DHEAs and to less extent TST associated with negative rheumatoid factor and anti CCP. patients with high disease activity associated with lower androgen levels.

Conclusion: Men with rheumatoid arthritis have low levels of serum androgens, may related to the lack of the anti-inflammatory effects of androgens, lower androgen levels associated with negative serological markers and high disease activity. Androgen replacement may have associated with clinical improvement and low disease activity

Key words: *Dehehydroepiandosterone sulphate, Rheumatoid arthritis, Total serum testosterone.*

Introduction

Rheumatoid arthritis(RA) is a chronic autoimmune inflammatory disease that affects diffrent ethnic groups, Males are less likely to be affected than females. The peak incidence was at forties and fifties of age, but it will occur at any age. It is the most common form of inflammatory arthritis affects synovial joints. In early disease, the wrists, proximal interphalangeal, metacarpophalangeal, joints of the fingers, interphalangeal joints of the thumbs, and metatarsophalangeal are the most commonly affected joints in the body [(1)]. Additionally, chronic inflammation secondary to RA can lead to an increased risk of pulmonary and cardiovascular disease and changes in bone mineral density [(1)]. Rheumatoid arthritis affects 0.5–1% of the adults worldwide [(2)]. At 2010 the classification criteria of RA were revised, aiming to improve early diagnosis and management[(3)].

Auto-antibodies, like rheumatoid factor (RF) and antibodies to citrullinated protein antigens (ACPAs), are measured to diagnose RA disease [(4,5)]. RA associated with sex hormones abnormality[(6)]. The level of androgens decreases at the disease onset and may returns to the normal state after relieving the acute symptoms [(7)].

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Dehydroepiandrosterone sulphate (DHEAs) are the most abundant products of the adrenal gland secretions, but its functions yet unknown. The pathophysiology of gonad dysfunction is not clear during the illness, but the effects of cytokines and/or glucocorticoids can be noted [8]. Sexual hormones engage in immune responses, estrogen hormones intensify humoral immune responses, and progesterone and androgens considered as inhibitors of immune response [9]. The anti-inflammatory effects of androgens in physiological levels are proved [10]. The levels of serum testosterone and DHEAs in males and postmenopausal females with RA were low; hence, it is supposed that hypoandrogenism may be involved in the pathogenesis of RA [10]. Therefore it's important to study the relation of low (DHEAS) and RA and its severity. This study aimed to determine the role of low serum testosterone and DHEAs in men with rheumatoid arthritis

Patients and Methods

This case- control study was conducted on 73 male patients with rheumatoid arthritis and 73 healthy male age matched control at the Department of Rheumatology and medical rehabilitation, Rheumatologic outpatient, and biologic centre, Basra teaching hospital, Basra Provence at the south of Iraq from February 2019 till March 2020. The diagnosis of RA disease was approved by a single rheumatologist using the American College of Rheumatology/European League criteria (3). Patients with other rheumatologic, inflammatory and endocrine disorders were excluded from the study. Complete investigations including complete blood count(CBC) and erythrocyte sedimentation rate(ESR), RF, Anti CCP, total serum testosterone and DHEAs levels were measured for all participants, total serum testosterone and DHEAs levels were measured for controls, disease activity for patients measured using DAS28. A questioner about marietal status, number of children were completed by the patient and control groups. Informed permission was taken from all participants.

Statistical Analyses

statistical analysis, were performed with Chi squared and Mann-Whitney tests p-value less than 0.05, was considered significant.

Results

Table 1 shows the demographic demonstration for patients and controls, there were 73 men patients of median age, and mean disease duration; 55 and 11.97(SD= 4.5), the mean numbers of children of patients and controls were 5.2(SD= 1.3)and 8.62(SD= 1.33) respectively. There were 73 controls with median age of 55. Serum levels of TST and DHEAs for both patients and controls are shown in table 2, the median total serum testosterone level of the patients group was 2.2ng/l that is lower than that of controls 7.8ng/l which is statistically highly significant with p value of 0.0001, the median DHEAs level for patients was 81mg/dl and 343mg/dl for controls, also result is statistically highly significant with p value of 0.0001.

Table 3: shows the relationship between TST and DHEAs levels in relation to the serological status; there were direct relationship between the negative rheumatoid factor and anti CCP and low androgen levels, result more obvious for patients with low level DHEAs and negative rheumatoid factor, results that are of statistical significance with p values less than 0.05. Low androgen levels directly correlated to high disease activity which is statistically highly significant with p value of 0.001, as shown in table 4.

Table 1: The demographic characteristics of patients and controls

Characteristics	Patients	Controls	P-value
Men	73	73	
Median age (year)	55	55	0.619
Disease duration (year)	11.97 (SD= 4.5)	------	------
No. of children	5.2 (SD= 1.3)	8.62 (SD= 1.33)	0.0001

Table 2: TST and DHEAs levels in both patients and controls

Characteristics	Patient	Controls	P-value
Total No.	73	73	
Median TST (ng/l)	2.2	7.8	0.0001
Median DHEAs (mg/dl)	81	343	0.0001

Table 3: TST and DHEAs levels in relation to serological status

	RF			Anti CCP		
	Positive	Negative	P-value	Positive	Negative	P-value
No.(%)	42 (57.5%)	31 (42.5%)	-------	55 (75.3%)	18 (24.7%)	-------
TST Median (ng/l)	3.1	2.0	0.0001	2.4	2.0	0.028
DHEAs Median (mg/dl)	107.5	78	0.0001	92.0	76.5	0.04

Table 4: TST and DHEAs levels in relation to DAS28

	Disease Activity		P value
	High DAS28	Low-Moderate DAS28	
TST Median (ng/l)	2.5	7.6	0.0001
DHEAs Median (mg/dl)	95	326	0.0001

Discussion

Over the past years, considerable interest has been dedicated towards circulating steroid hormones level in researches. Much research has been undertaken in view of the possibility that certain hormone profiles may predispose people to the development of illnesses such as coronary heart disease and cancer [11-13]. Hypoandrogenism may play a role in rheumatoid arthritis and/or appear as a complication of chronic inflammatory reaction. The higher levels of testosterone in young males may somewhat play protective roles against RA [14]. Clinical improvement after a successful treatment is followed by a rise within the level of serum testosterone in RA [15]. It should be noted that patients undergoing long-term treatment with glucocorticoids may experience hypoandrogenism [16]. Results of this study showed that total serum testosterone and DHEAs levels in men with RA were significantly lower than that of the control group. Also, this study showed that the normal levels may have protective effects on RA,

and the lower levels of serum testosterone may be caused by RA or may indicate the androgen role in the pathogenesis of RA. Our results were consistent with a study done by Pikwer M. et al who found an abnormal serum testosterone level was significantly lower in patients with RA and negative RF [17].

The low level of DHEAs found in our study are in agreement with an earlier report concerning men with established disease [18]. In a study done by Dessein et al. in South Africa on 38 patients with RA, the activity of hypothalamic-pituitary-adrenal axis was reduced and DHEAs levels in females and males with RA was low [19]. An attention has been paid on sex ratios of offspring of patients with RA; it has been hypothesised that the sexes of offsprings are partially controlled by parental hormone concentrations at the time of conception [20]. There is an evidence that women with RA are more likely to conceive with daughters rather than sons [21,22]. There is indirect evidence supporting the view that low androgen concentrations are involved in the aetiopathogenesis of RA [23]. In this study we found there was direct relationship between the negative serology of RF and anti CCP and low androgen level, especially for rheumatoid factor which may indicates an independent etiological effect of low androgens in the etiopathogenesis of rheumatoid arthritis. In this study the mean number of children in the patients group was less than that in the control group, result may correlate to the burden of the disease on the socioeconomic status of the patients or it may reflect the low fertility level in these patients. We found that patients with lower androgen levels were associated with high disease activity, this result is comparable to a study done by Cutolo M. who stated that; low serum testosterone and DHEAs associated with high disease activity in patients with rheumatoid arthritis [24].

Conclusions

Men with rheumatoid arthritis associated with low levels of serum androgens; this may have related to the lack of the anti-inflammatory effects of androgens. Men with lower levels of DHEAs and to less extent TST obviously associated with negative serological markers, disease activity strongly related to low androgen levels. Androgen replacement may have associated with clinical improvement and low disease activity.

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Disposition of Policy Implementers: Study on The Hospitals Payment System Change in The Indonesian National Health Insurance Era

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Abstract

Background: Disposition of implementers to changes in hospitals payment system policies has become an important issue in the implementation of the Indonesia National Health Insurance (NHI) program. The change from fee for services to a prospective payment system in the form of the Indonesia Case Based Group (INA-CBG) have caused low acceptance from clinicians. On the other side there was potential for fraud in the hospitals claim collection scheme published by the corruption eradication commission. The objective of this study was to analyze the effect of disposition of implementers on financial pressure and fraud intention.

Methods: The study used an observational analytic approach with a survey method, where researchers conducted observations, interviews and questionnaires. The research variables consist of disposition of implementers, financial pressure, and fraud intention. The research respondents were 110 physicians in charge of patients in 12 hospitals in East Java Indonesia. Data were analyzed by path analysis.

Result: The direct causal effect of the disposition of implementers regarding the INA-CBG payment system in hospitals to financial pressure had a value of p = 0.001 and B = -0.332.

Conclussion: Disposition of implementers to changes in hospitals payment systems had a significant effect on financial pressure and fraud intention. A negative beta indicated that the disposition of implementers will have an effect on increasing financial pressure.

Keywords*: disposition of implementers, Indonesia Case Based Group, financial pressure*

Introduction

Disposition of implementers is the willingness or tendency of policy actors to implement and realize the policy seriously. Disposition of implementers is the main determinant in the success of program performance.(1),(2) According to Russell, in the neo synthesis approach to policy implementation it still puts disposition of implementers into a direct determinant of performance outcomes and feedback in addition to the local planning council and community disposition. (3) This research has had a evidence based in the implementation of National Health Insurance (NHI) in Indonesia that examines aspects of acceptance of hospitals payment systems. This is very important for success towards Universal Health Coverage (UHC) where the hospital acts as a referral health facility. (4),(5),(6)

In the context of the implementation of the national health insurance program in Indonesia, a payment system for services in hospitals has been established in the form of a prospective payment system, namely a case based payment called the Indonesia Case Based Group (INA-

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CBG). INA-CBG has been developed using a codified system of final diagnosis and actions or procedures that have become service outputs. This system has referred to the 2010 Revised ICD-10 for diagnosis and the 2010 Revised ICD-9-CM for actions or procedures. The grouping has used an information technology system in the form of the INA-CBG application to produce 1,075 case groups consisting of 786 inpatient cases and 289 outpatient cases.(7)

Every financing system has advantages and disadvantages. The advantages of INA-CBG include the speed in filing a claim, there is a risk sharing between insurance providers and health care providers.(8) Changes in the payment system for hospitals during the national health insurance program from fee for services to claims based on INA-CBG has caused mixed reactions from clinicians.

Based on interviews with the doctor in charge of the patient, information was obtained about the clinicians dissatisfaction with the payment system change. According to respondents, many INA-CBG claims were found to be lower than the prevailing hospital rates. On the other hand, the unclear time of claim payment was characterized by frequent late payment of claims from the Agency for Providing Health Insurance to hospitals. This has resulted in disruption to hospital operations and delayed service compensation for employees.

Low disposition of implementers of NHI program policies relating to service payment systems in hospitals, both in whole or in part from the policy content has an impact on program sustainability. Rejection of this policy will cause pressures for the implementers, especially for financial pressure.(9) The definition of financial pressure in this study is the impetus for fraud felt by respondents due to the INA-CBG policy as a payment system in hospitals.

At the same time the Corruption Eradication Commission (KPK) published the Public Anti-Corruption Clearing House Research.(10) According to the KPK that the amount of potential fraud claims in hospitals could reach 2 trillion rupiah. The potential for this new fraud comes from a group of health service providers, not yet from other actors such as BPJS Health staff, patients, and suppliers of medical devices and drugs.(11),(12) The biggest form of potential fraud is carried out with a billing schemes.[12] The most common scheme is by upcoding up to 50%, then the other 25% is done by unbundling, and the third is by reading with the number of findings reaching 6%. Corruption in the public health sector needs to be addressed.(13),(14)

Based on the description above, this article aims to explore the effect of the disposition of implementers on the INA-CBG payment system on financial pressure. This study is useful for improving national health insurance programs in the aspect of program implementer satisfaction.

Methods

Design Research

This research was observational analytic survey method. Researchers conducted observations, interviews and filled out questionnaires without giving treatment to respondents. Based on the time of its implementation, this study was cross sectional.

Population and Sample

The population in this study was doctor in charge of patient who was responsible for filling out medical resumes that will be the basis for filing claims at 12 hospitals in East Java, Indonesia. The sample size was 110 people taken by simple random sampling method.

Variabel and Data Collection Techniques

Exogenous variables in this research was disposition of implementers measured by two dimensions, namely: the response to policy changes and the intensity of the response of policy implementers.(15) While endogenous variables was financial pressure and fraud intention. Financial pressure was measured in four dimensions, namely: personal financial needs, external pressure, financial stability, and financial targets. (16)

Primary data collection techniques were interview respondents (face to face) using a questionnaire that aimed to explore the information needed according to the research variables through the process of verbal questioning. The questionnaire was developed using a semantic differential-type scales measurement procedure with a scale of 1-7.(17),(18) Before being used on research respondents, the questionnaire validity and reliability tests were first performed.

Data Management and Analysis

The first stage of processing was entering data into a database on a computer using software. The application used was a computer with a Statistical Product and Service Solution (SPSS) version 21 License program. The next step was to ensure completeness of data entry, data cleaning from extreme or inconsistent values, and conducted classical analysis requirements tests. Analysis of descriptive data used measures of central tendency such as: mean, median, mode, and frequency distribution. Inferential analysis used a linier regression analysis.

Results

Characteristics of Respondents

Characteristics of respondents in this study were used to determine the diversity of respondents based on the age, gender, last education, and length of work.

Table 1. Description of Research Respondent Characteristics

Characteristics	Categories	Frequency	Percentage (%)
Age	young (≤15 yars)	0	0,0
	productive (15-64 years)	107	97,3
	old (≥65 years)	3	2,7
Sex	Male	66	60,0
	Female	44	40,0
Last education	Dentist	6	5,5
	Doctor	1	0,9
	Medical Spesialist	103	93,6
Length of working	< 5 years	28	25,5
	≥ 5 years	82	74,5

The majority of respondents were of productive age, male, the last education was specialist doctors, and the length of work was ≥ 5 years. Male respondents are more open about their experiences.[19]

Disposition of Implementers

Disposition of implementers of the NHI program policies is the attitude of respondents regarding their acceptance of changes in hospital payment system policies from fee for services to INA-CBG payment systems. Description of respondents' assessment of the variable disposition of implementers obtained from the sum of all indicators categorized into 3 (three) as follows:

Table 2. Frequency Distribution Per Dimension and Composite Value of Disposition of Implementers

No	Disposition of Implementers	Categories						Total	
		rejected		hesitation		receive			
		n	%	n	%	n	%	n	%
1.	Response to payment systems in hospitals	50	45,5	54	49,1	6	5,5	110	100
2.	The response intensity of the policy implementers	67	60,9	41	37,3	2	1,8	110	100
	Composite Value	57	51,8	51	46,4	2	1,8	110	100

Based on table 2, The highest category for dimension of response to changes in payment systems in hospitals was the hesitation category (49.1%). Respondents considered the implementation of the claim system based on INA-CBG's deemed unfair and considered likely to be detrimental. In fact, there were 45.5% of respondents who tended to reject the NHI program's INA-CBG payment system in hospitals.

While the highest category for dimension of the response intensity of policy implementers was rejecting (60.9%). Respondents considered that the claims payment by the organization of social security insurance to hospitals was often late. Besides that, the tariff rates were not yet as expected. This was indicated by the difference of rates between INA-CBG and the prevailing rates at the hospital. This had an impact on respondents' dissatisfaction with the system that has run in the NHI era. The results of the most composite respondents' assessments for the construct of disposition of implementers were rejected (51.8%).

Financial Pressure: Financial pressure is an impetus for fraud felt by respondents due to the INA-CBG policy as a payment system in hospitals.

Table 3. Frequency Distribution of Per Dimension and Composite Value of Financial Pressure

No	Financial Pressure	Categories						Total	
		low		medium		high			
		n	%	n	%	n	%	n	%
1.	Personal financial needs	6	5,5	38	34,5	66	60,0	110	100
2.	External pressure	15	13,6	70	63,6	25	22,7	110	100
3.	Financial stability	12	10,9	58	52,7	40	36,4	110	100
4.	Financial targets	55	50,0	43	39,1	12	10,9	110	100
	Composite Value	7	6,4	85	77,3	18	16,4	110	100

The highest category for assessing the dimensions of personal financial needs was high (60.0%). This means that the high perceived financial needs pressure was experienced by respondents personally while providing services in the JKN era. Respondents tended to assess that the compensation in the JKN era were not in accordance with the workload, causing dissatisfaction.

The highest category for external pressure dimensions was moderate (63.6%). Respondents considered that the financial pressure felt from outside of the respondent was moderate category. This was showed by respondents' answers that there were often patient service costs that exceed INA-CBG ceiling rates. While the pressure from the hospital was considered low by the respondents. But there were 27.1% of respondents who tended to provide an assessment of high pressure from the hospital. Another indicator was the pressure from the government that was felt low by respondents.

The highest category for financial stability was medium (52.7%). Respondent's evaluation of the condition of hospital financial stability in the JKN era was in the medium category. Hospital revenue was considered to be declining and experiencing a deficit. While the most response to the dimensions of financial targets was the low category (50.0%). The low pressure felt by respondents due to whether or not a revenue target set by the hospital. However, there were 10.9% of respondents who stated the high pressure due to hospital revenue target.

The most composite assessment for financial pressure constructs was the medium category (77.3%). There were pressures felt by respondents due to changes in hospital payment system policies in the JKN era that affected financially. It should be noted that there were 16.4% of respondents assessing high financial pressure.

Analysis of the effect of the disposition of implementers on financial pressure showed significant results ($p = 0.001$; $B = -0.332$). Negative beta value indicates that increasing Disposition of Implementers affects the decrease in Perceived Pressure.

Discussion

The selection of the variable disposition of implementers in this study with the consideration that the context of the study was the implementation of national health insurance policies in Indonesia. The attitude of the agents implementing the NHI program policies is an important part of implementing a complex and interactive NHI program. Policy actors in the implementation of the NHI program involve many parties with various personal and collective agendas that will influence the course of the NHI program. One of the policies in NHI that is interesting to discuss is the policy that regulates changes in the payment system for services in hospitals from fee for services to claims based on INA-CBG's. Specific patterns of interaction and use of the health system can be identified and linked with users' expectations and opinions.[20]

Hospitals need to adapt from a retrospective payment system to a prospective payment system. Forms of payment at health providers today are intended to be able to control health costs that continue to increase due to payment of fees for services reimbursement system that is paid after the service is provided (retrospective payment system).[21] Payments made after the complete services are believed to not provide incentives for health providers to be efficient.

In line with government regulations regarding INA-CBG implementation guidelines which explain that the advantages of retrospective payment methods for providers are that the financial risk of the hospital is very small and generates unlimited income. the weakness of retrospective methods is that there is no incentive for providers to provide preventive care and will lead to suppliers induced demand. Whereas prospective payment methods have a more just advantage according to the complexity of the service and faster claim process. The disadvantage is the lack of quality coding which will cause a mismatch of the grouping process (grouping cases).[8]

Disposition of implementers is very possible considering that the program implementers are not the public policy makers. Public policy is usually top down, decision makers often do not know or are unable to touch the needs, desires or problems that must be resolved by program implementers.[1],[2] This explains why the disposition of policy implementers is important for the success and achievement of program objectives.

The respondent's intencity of payment system acceptance was shown by their assessment of the amount of the INA-CBG rate and the difference with the amount of the hospital rate. The results showed the majority of respondents refused, meaning that the majority of respondents rated the tariff rates not as expected by respondents. The difference between hospital rates and INA-CBG rates was still large. This is considered as a potential risk for hospital losses.

Late claims payment from Health Insurance Provider Body to the hospital was also a trigger for low acceptance of the INA-CBG payment system policy. The majority of respondents rate that payments were often late and this was considered disappointing by respondents. The need for quality services for NHI participants needs to be accompanied by timely payment mechanisms.[6] In addition to ensuring the satisfaction of program implementers, it is also for the sustainability of health services in hospitals that require sufficient funds to support their operations.

In the neo synthesis approach to policy implementation explains that the disposition of implementers is influenced by several factors, namely: inter-organizational communication and law enforcement activities, the characteristics of implementing agencies, and economic-socio-political conditions.[3] Communication between the Health Insurance Provider Body, the government, and health service providers in the implementation of the NHI program has been needed to build synergies in accordance with their respective roles.

The economic aspects that underlie changes in the payment system for health services in the NHI era cannot be ignored, especially the impact on the program implementers attitude.[22] large difference in tariffs between INA-CBG rates and hospital rates requires special discussion to open a compromise space from all parties concerned. In addition, the phenomenon of late payment of claims to hospitals must have a solution so that it does not affect health services.

The final conclusion is based on a composite value (according to the results of factor analysis) of the variable disposition of implementers that the majority of respondents are hesitant in accepting the claims payment system policy and almost half of the respondents give a reject response. Disposition or attitude of the executor will pose a real obstacle to the implementation of the NHI policy if the existing personnel do not implement the expected policies properly.

The implication of the results of the measurement of disposition of implementers that leads to doubt and even tends towards the rejection of the claim payment policy based on INA-CBG is the emergence of pressure due to financial risks that may occur. The test results of the influence of the disposition of implementers in the claim payment system policy based on INA-CBG on perceived pressure shows a significant value. This means that any change in the level of policy acceptance will have an effect on changes in financial pressure. The lower the acceptance of respondents to the NHI policy, it will have an impact on increasing financial pressure for respondents.

Conclusion

The conclusions are based on the results of the synthesis of the discussion which is the answer to the formulation of the problem and the purpose of the study as well as the results of the proof of the research hypothesis. Disposition of implementers of JKN program policies regarding INA-CBG's payment system had a significant effect on financial pressure. Increased disposition of implementers affected the decrease in perceived pressure.

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How to Teach Intramuscular Injection Through Virtual Learning Environment in Covid-19 Pandemic Time for Nursing Students

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Abstract

During the pandemic COVID-19 spread and the temporary lockdown of universities, there has been a global shift toward online learning. Some universities were not prepared for such circumstances, and they began attempting to meet this unprecedented task for not missing the academic year. Recently, there has been a project that is currently working on the same issue regarding teaching nursing students how to deliver intramuscular (IM) injection using online in a virtual learning environment. The objective of this study was to create a simulation in an online course using a three-dimensional (3D) learning management system (LMS) in a virtual learning environment (VLE). That can replace IM injection traditional teaching method. This LMS intended to supplement the existing lab practice to nursing undergraduate students by creating a simulated online clinic with a nurse and patient avatars. Theoretical content in the form of reading material and related videos was also provided for students' cognitive base before they start the 3D simulation training. This new course was founded on the Galvis panqueva method that resulted in a product called Online-3D-IMI-VLE. Various validation processes undertook for multiple development processes involving nursing content specialists and computer multimedia. The pilot results showed that this LMS could replace the traditional way of teaching and support online learning during the normal education environment as well as in this COVID-19 pandemic time.

Keywords: *COVID-19, Virtual Learning Environment, Intramuscular Injection, Three-Dimensional Simulation, Openlearning.com®, Learning Management System*

Introduction

Medication administration via injections is considered one of the vital skills for nursing students, which include various ways of knowledge application, problem-solving, decision making, and critical thinking. IMI sites/muscles used for administration are mentioned in literature as; ventrogluteal site, dorsogluteal site, rectus femoris muscle, deltoid muscle, and the vastus lateralis muscle [(1, 2)]. The criticisms of traditional teaching have led to an intensive search for new methods of nursing education.

Recently, the suspension of campus class is a precautionary step for pandemic COVID-19 [(3)]. The world emphasizes and is actively using online learning to replace the traditional way of teachings temporarily and to prevent students from gathering in one place and

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to decrease the spread of the virus.

For instance, universities changed their way of teaching, e.g., in Australia, some universities moved to temporary standstill online learning like [4] and [5] while other used social distance learning between the students in the class and supplement online learning [6]. Also, in Jordan during the covid-19 pandemic, many universities transferred some courses to be online courses immediately, like Al- al-Bayt University, which started to use skype, Moodle, and Google classroom to deliver live lectures by the broadcast scholar [7].

The proposed solution is to replace the traditional way of IMI teaching and to use modern technology by using the Virtual Learning Environment (VLE). The development of VLE, which incorporates innovation, interactive learning, and mimicry of the real situation, which could replace laboratory-based education, has been suggested by researchers [8].

The potential of three-dimensional virtual learning environments (3D-VLE) has intrigued many researchers recently as it allows easy replication of tools, settings, and stakeholders as virtual artefacts with minimal costs and quick turn-around time [9]. Several scientists also acknowledged the suitability of using activity theory as a framework for depicting mobile learning studies' study material and dimensions [10].

Recently, schools teachers, postgraduate lecturers, and researchers of educational technology have become increasingly interested in various options of mobile learning, by using portable instruments such as laptops and smartphones, different virtual systems of education can make learning tasks more comfortable for students in a virtually created environment, these systems also backing the execution of a self–evaluation [11, 12]. Campus lab and classroom-based simulations have been proven to be an efficient educational approach that can improve the graduates' shift from the schoolroom to practice in real-life. These simulations deliver the student an opportunity to comprehend relevant data, practice in making decisions in practical life, and record their response weather they would work around actual patients [13]. Many studies in nursing science have discussed the assistance of handling different types of simulation learning systems to increase self-confidence and performance skills [14].

Materials and Methods

Ethical Approval

The LMS development and validation were conducted as part of an ongoing study under the Research University Grant of USM. Ethical approval for this study was obtained from the Human Ethical Committee of the institution with assigned code USM/JEPeM/17020139. All participants in the study were informed regarding their participation and voluntarily gave their agreement. Participants' information confidentiality was maintained.

Participants:

This study exclusively needed a nursing content-specialist who have experience in nursing science and computer science. Five nursing contents-specialists were recruited in the study who are currently working as lecturers in the faculty of nursing, and they voluntarily participated in the study after they were informed about it, and their contribution was to ensure the contents of the LMS are valid and reliable; these issues are discussed later in validity and reliability. Also, our research team leader made a written contract with IT designers from another government university to design the simulated 3D part, and their team consisted of six personnel. Add to them the pilot group, which includes thirty students from year three undergraduate nursing program.

Materials

The WebGL by Unity® software package was used by the IT designers to design the 3D part while arranging the contents and building them into the educational platform Openlearning.com was done by the researcher. A rental server was rent for two years to upload the software on.

Procedure

The study started with data collection regarding building the LMS and how to put the lesson contents which been chosen carefully to meet the objectives of the study. Then building and designing the LMS and the simulation 3D part. At the end of the study, the validation process was employed to check whether it can replace the traditional way of IM injection teaching. A pilot study was conducted to ensure face validity; thirty students from year three nursing program were

volunteered to participate.

Design

The development of this learning management system was adopted from [15] comprised of five phases :

1) Analysis:

By analyzing the intended teaching content and measurable outcomes, the instructional objectives were defined. The analysis also evaluated the tutor related aspects, which included technological infrastructure and the work/study environment. The steps of analyzing for the creation of LMS included • Characteristics of the target students • Course selection • Determination of educational goals • Content definition

2) Design:

The steps followed in this phase include; • Instructional design • Construction of educational objectives according to Bloom's taxonomy • Determination of the evaluation approaches used in the LMS • Navigation mapping of the learning environment • Selection of learning modules on the primary interface and design of the layout.

3) Development:

It was accomplished through the rental of an internet domain for 3D practical skill module and uploaded on www.openlearning.com® for one year, development of the program layout by the IT personnel; data upload to the rented domain for didactic learning; installation and configuration of the LMS; selection of the software package management that would be utilized for the specific VLE; 3D-VLE was designed by the software development package WebGL by Unity®.

4) Evaluation:

During this phase, the entire LMS developed by IT experts was evaluated by content experts and faculty members.

5) Administration:

The administration phase consists of the process lesson selection, LMS end-user registration, proper guidance tools, learning evaluations, and post technical usage support. Frequent check-ups of the seamless functioning of the LMS were performed, and security copies were backed-up online to avoid any data loss.

Results

Results shown here are the LMS itself and the pilot group results. By using the methodology of Galvis Panqueva for designing the LMS, the Online 3D-VLE-IMI was developed. This LMS allows the undergraduate nursing students to learn and practice simulation the IMI technique in an Online 3D-VLE.

The chosen educational platform was openlearning.com®, which is an open-source software that was nominated to enable the building of the education atmosphere since it allows the integration and use of the developed applications. It provides simultaneous access to the data by multiple users, records the actions of the users, provides a questions/answers forum, allows the students to write and share information or start public or private discussions which enable interactive learning, ensures stability and security of the system, allows easy retrieval of the data.

The students can use the system anytime and anywhere to ensure them having a VLE that enables them to read and practice about IMI using the LMS. System requirements are 1) Any working desktop or laptop 2), Internet connection (only during loading the software), 3) Web browsers.

System Tools

1) Home:

It is the default screen that the user will access after clicking the provided URL https://www.openlearning.com/courses/online-3d-im-injection-vle/HomePage. It provides the lesson description and general information about the educational and 3D practical skill modules of the LMS.

2) IM Theory:

The third tab contains two slideshow presentations that provide the academic content about IMI. Three CDC videos were also added, which were produced according to the nursing procedure manual that explained step by step procedure of IMI administration.

3) 3D practice:

The fourth tab contains a link to another URL that loads the 3D skill learning module on how to deliver IMI in simulation. It includes a virtual clinic with two avatars (One nurse and one dummy patient), figure (1).



Figure 1-Virtual clinic, Nurse and Patient Avatar

After the user clicks on the patient, the user can start by choosing one of the five muscles to practice the skill figure (2).



Figure 2-Muscle Selection

When using for the first time, the user is obligated to learn each muscle to access the next muscle selection. After selecting a muscle, the users can visualize the whole muscle from different angles (anterior, posterior, and lateral views) and also a virtual representation of the deltoid muscle within.

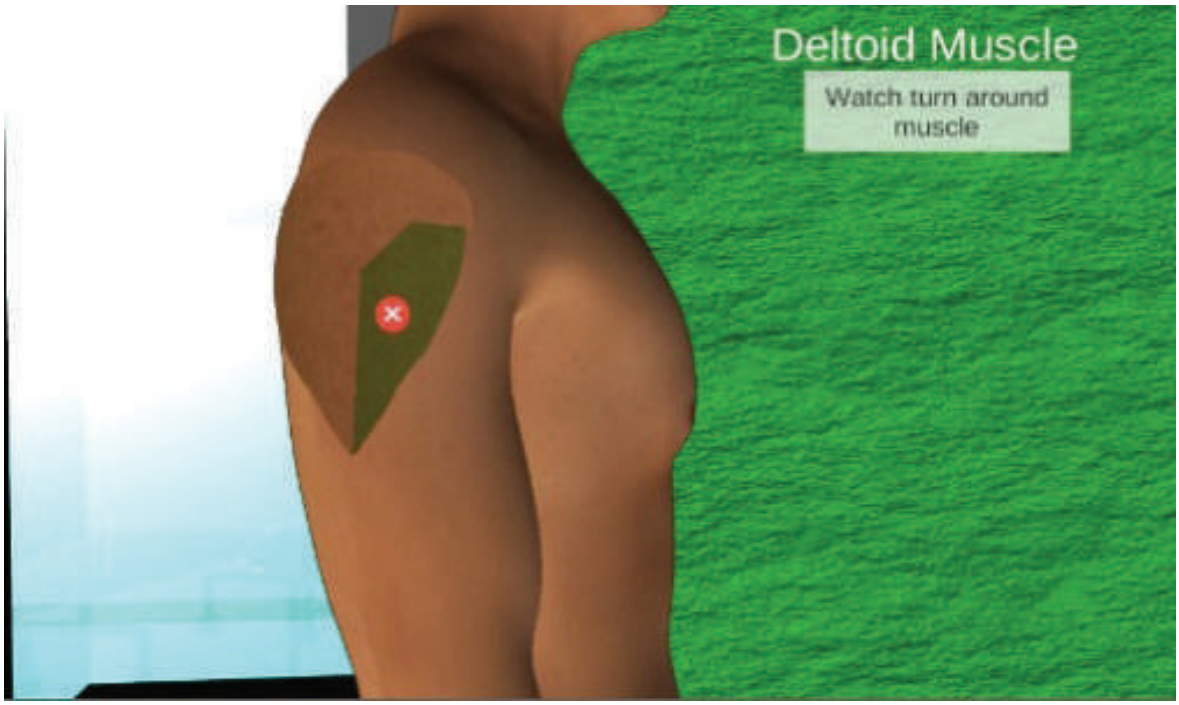


Figure 3-Wrong selection of the site of injection

At this point, the users

can notice that a virtual syringe replaces the computer mouse cursor, the user can navigate the syringe to choose the appropriate injection site. The user is allowed to administer the injection at any place within the muscle, after which the system will notify about the correct or wrong selection of the site. A red-cross mark indicates a wrong site of injection administration, and a green-tick mark indicates correct site selection. Figure (3 and 4).

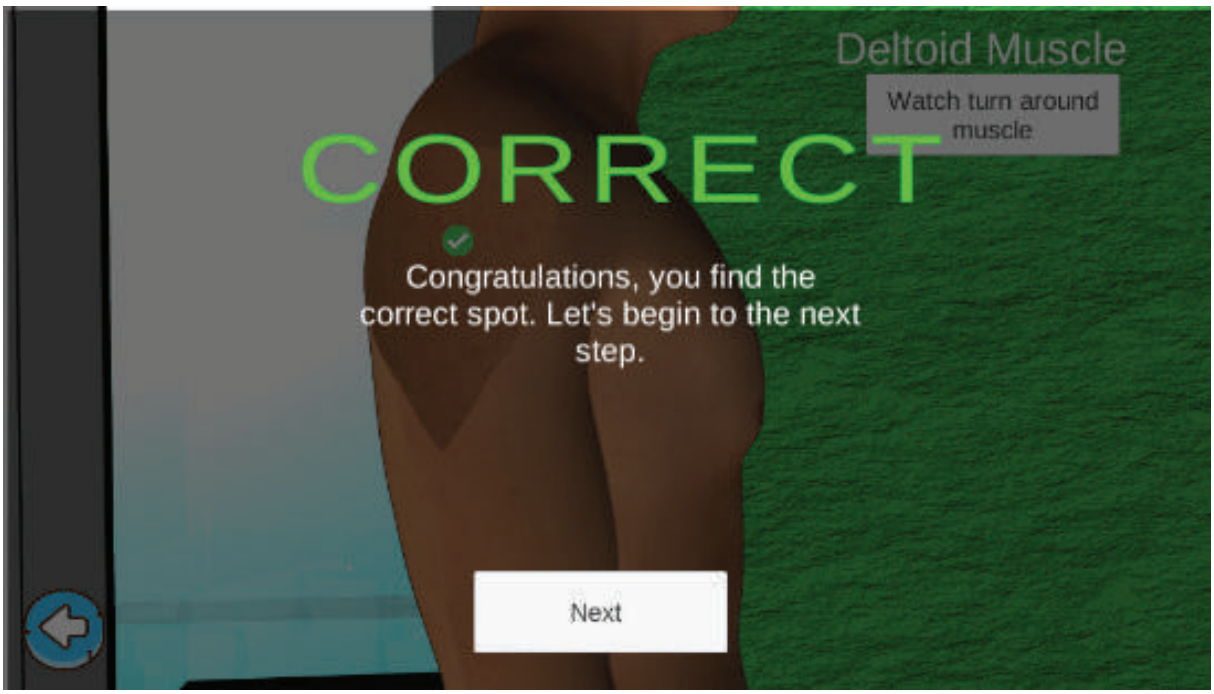


Figure 4-Correct injection site

The second stage is the syringe angle of IMI. The user can notice three different angles from which the user can choose one. Upon selecting the wrong angle, the system notifies by displaying the "WRONG" message. Upon correct selection of angle, the system automatically proceeds to the third stage to determine the depth of IMI. Figure (5)

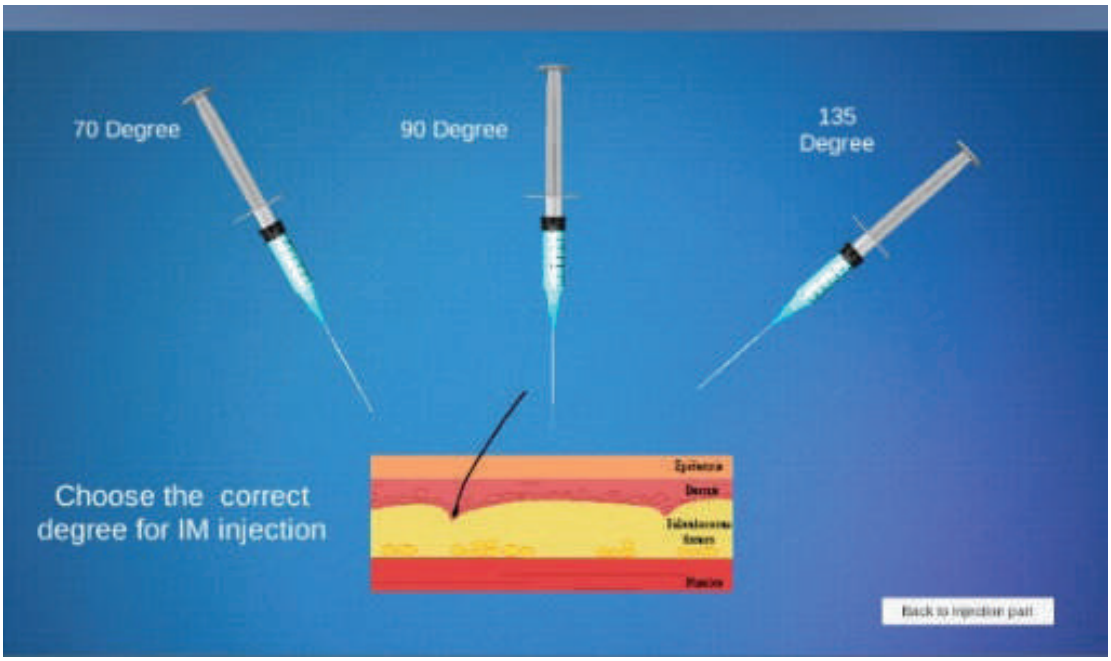


Figure 5-Syringe angle selection

The user can notice and visualize different skin layers (epidermis, dermis, subcutaneous, and muscle layers) and is allowed to choose the depth at which the injection should be delivered by clicking on the corresponding tabs next to the different layers. figure (6).

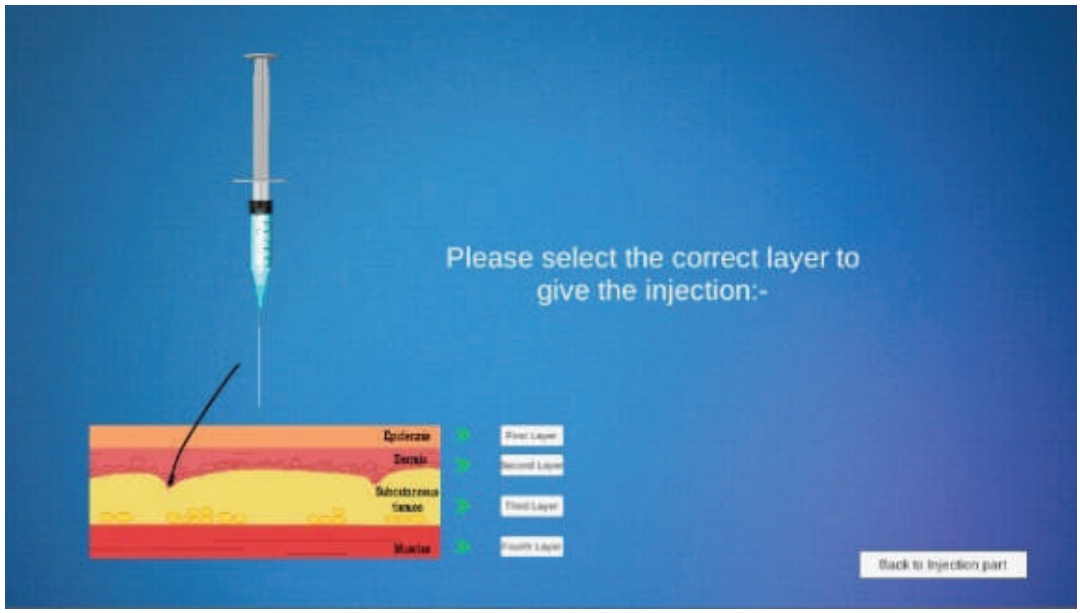


Figure 6-Layers selection/ Depth

The users can repeat the exercise as many times as they require to practice. Once the users are registered on the webpage. Some users found it an amusing way of learning and considerd it as an interactive learning-based game that facilitates them in providing the freedom to continue learning at their own pace and convenience.

Validity and Reliability

The objectives-based evaluation was performed by measuring the learning outcomes through Objective Structured Clinical Examination (OSCE) and pre-post test of knowledge IMI gain, which was primarily tested with a pilot group and then administered for both intervention and control group.

Regarding testing of knowledge gain, all participants of the pilot group were examined using Multiple Choice Questions (MCQs). This test was done before they enrolled in the program, and another one was done after the educational program finished. A pre-post test was done to measure IMI knowledge gain. Pilot group results were: clinical OSCE (mean = 90.45, SD = 11.34); this estimated the clinical performance and, an MCQ test was employed to measure IMI knowledge gained with test reliability using Kuder-Richardson 20 equal to .776 showing acceptable reliability score in 15 items MCQ.

Discussion

Meeting current challenges put the academicians and universities in confusion, how to deliver proper information and guarantee at the same time that the students understand what they are learning. In other words, how to close universities and stop face to face learning to prevent the spread of COVID-19 and be assured that the students are doing well in their academic matters.

The development of this LMS enabled the students to read and practice about IMI virtually which will help the student to expand their comprehension abilities and use their critical thinking to be able to perform IMI without the need to do real-life practice in the nursing lab, some studies like [16, 17] found an enhancement in students' capability presentation, next simulation classes. Simulation brings enhancements to increasing knowledge [18]. And they can practice virtually administering of IMI using virtual simulation available in 3D.

This LMS had gone through a series of validity and reliability tests to assure its validity, before that a conceptual theory was used to support it alongside with theoretical framework which used The Kirkpatrick Model [19] and Millers' assessment pyramid [20] to assure that this LMS was created according to the proper scientific teaching-learning methods.

On the other hand, although hundreds of colleges are providing online courses on entrepreneurship, two problems emerge. Firstly, from a macro-perspective viewpoint, very little is understood regarding the effects and efficacy of online learning [21] and much less established in the field of entrepreneurial schooling. Secondly, the capacity to teach entrepreneurship successfully digitally is likely to differ, considering the wide variety of learning goals that guide our teaching and pedagogical considerations [22]. This is why this LMS hoped to replace IMI traditional learning method and to be effective during this movement control orders.

Conclusions

It was found that this LMS can replace the traditioanl IMI teaching method which has many faults before and now with COVID-19 it become a necessity to move to online learning.

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A Virtual Learning Environment for Teaching Intramuscular Injection: A Focus Group Discussion Analysis

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Abstract

The teaching of Intramuscular Injection (IMI) is an essential topic for nursing students and consider one of the daily routines job for any nurse. However, the traditional teaching of IMI has shown some limitations to help the students acquire injection skills. Since the advancement of technology available instruments and applications available in the virtual stores, it seems employing an advanced computer technology would be more suitable in the present era, particularly with a large number of students. Content experts among nursing lecturers were invited to discuss the advantages and disadvantages of the traditional teaching method and the need to create an online program to teach IMI in a Virtual Learning Environment (VLE). A combination of structured and open-ended questions based on related literature was used in the focus group discussion to explore different opinions from the content experts. All the participants recognized the limitations of the traditional teaching of IMI and agreed to the need to create an online computer software as an alternative teaching method to teach the nursing students on IMI. Thus, since the traditional teaching method of IMI administration has the limitations, teaching and practicing IMI virtually and interactively would be more appropriate for the nursing program in producing nursing students who are competent in IMI.

Keywords: *Focus Group Discussion, Online Learning, Intramuscular Injection, Virtual Learning Environment, Nursing Education, Learning Management System*

Introduction and Literature Review

The traditional curriculum in nursing education gives attention to the teacher instead of the learner. Yet, recently there is a model change occurring now, shifting the stress from teaching to learning through using an extra student-centered program [(1)]. The changes have focused on the program layout procedure with more considerable attention to the learner, especially in skills, knowledge, and capabilities inside the program. In other words, the attention of nursing education relies on what the students will learn and creating a thriving education climate.

With regards to teaching intramuscular injection, the traditional method of teaching the nursing skills is starting with lecture-demo, return-demo, and self-practice by the nursing students, usually on non-interactive mannequins. It was found that the competencies of nursing students on injection medication administration still lacked during Objective Structured Clinical Examination (OSCE) [(2)].

The outcome of the traditional teaching method might not have been effective in ensuring satisfactory

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intramuscular skills performance among nursing students [3]. On the other hand, other studies also have not been able to provide evidence on whether the alternative teaching method could provide a better learning outcome on skills performance [4].

In view of the intramuscular injection, the procedure is compound and contains additional methods with the pinhole process itself. New multiple available technologies may assist in training future nurses to avoid dangers associated with the erroneous performance of intramuscular injection skills. The focus group discussion was conducted to obtain feedback on the traditional teaching of intramuscular injection and to obtain opinions on the proposal to create computer software to teach intramuscular injection to the nursing student in USM.

Materials and Methods

Setting

The focus group discussion was conducted in the discussion room situated in the Nursing Skills Laboratory of School of Health Sciences of University Sains Malaysia. The university is currently offering both diploma and undergraduate nursing programs.

Focus Group Participants

Participants of the focus group consisted of six senior nurse educators who were involved in teaching and supervising intramuscular injection to the diploma and undergraduate nursing students in the school of health studies, University Sains Malaysia. They were selected by using purposive sampling with criteria such as had experiences in teaching and supervising nursing students on intramuscular injection.

All the participants were aged between 35 to 60 years old. About 75% of the participants had 5 to 20 years of experience in nursing education, and the other 25% had more than 20 years. For clinical nursing experiences, 50% of the participants had 5 to 20 years, and the other 50% had more than 20 years. It shows that the participants were experts both in content and clinical.

Instrument

Focus group was used to gather qualitative information as well as to uncover possible strategies to consider for improvement. Open-ended questions were set and validated before the focus group discussion to ask and discuss the traditional teaching method of intramuscular injection and the new teaching method using computer software.

The objectives of the focus group discussion were as follows:

To understand how the nursing educators perceive the nursing students' competency in giving an intramuscular injection that is taught using the traditional teaching method.

To understand how nursing educators perceive the proposed teaching method using computer software in the virtual learning environment (VLE) to teach intramuscular injection among nursing students.

Implementation

Consent forms and demographic forms were filled and obtained by each participant after a briefing on the need's assessment using a PowerPoint presentation. The focus group discussions were facilitated by the project leader, a facilitator and a notetaker. The discussion was recorded, and discussion notes were typed using a laptop computer by the notetaker who has the skill of typing quickly and listen objectively. The discussion was held in a small meeting room, and all involved were sat in a roundtable. At the end of the discussion, refreshment was provided to all participants.

Data Analysis

Analysis of the focus group responses was performed according to the questions set for the focus group. Before the analysis, documentation of the focus group discussion was done by the facilitator, and the notetaker who tried to finalize thoughts using computer and data show by analyzing what has been agreed for, then editing the findings done through omitting repeated ideas and opinions discussed. The notetaker then summarized the discussion by writing a draft report. The draft report was then checked by the project leader and all co-researchers for the project for the agreement of the findings.

The trustworthiness of the data was ensured (Lincoln, Guba, & Pilotta, 1985). The reliability of the data was recognized until the data collected is

saturated. Different kinds of descriptions were used during the focus group discussion to discover the possible information and supplementary findings of their experiences. Credibility was determined by establishing prolonged engagement between the researcher and the focus group participants. Meanwhile, transferability is made by determining the results were suitable to be implemented in other situations [5]. Therefore, the data collected were ensured applicable to other situation and populations. To establish dependability, the preliminary categories were compared and discussed within teams. The confirmability is made by using triangulation role and the researchers' ability to understand their view to minimize bias and misunderstanding in communication.

Results

Results of the focus group discussion are presented according to the objectives as follows:

1) To understand how the educators perceive the nursing students' competency in giving an intramuscular injection that is taught using the traditional teaching method.

Generally, the participants in the focus group stated that the traditional method of teaching intramuscular injection is more of a teacher rather than student-centred. In other words, lecture-demo practices one-way communication and offers less interactive learning. According to the participants, it was also challenging to control a big group of about 40 nursing students to focus on the lecture-demo. Another issue raised by the focus group participants was the difficulty faced by the nurse educators as well as the nursing students to perform each step the same as was demonstrated during the lecture-demo.

In addition, the participants noticed that the nursing students were still lacking in mastering the techniques of handling the injection syringe correctly and firmly to give the intramuscular injection as frequently observed during OSCE and in the clinical areas. On another note, the participants observed that when using non-interactive mannequins to practice, the nursing students always faced with difficulty to feel the correct injection site and location of the sciatic nerve in the dorsogluteal site.

Regarding student's self-practice on their free time, although they were being provided with the checklist of intramuscular injection procedure and guidance from the nurse in charge of the Nursing Skills Laboratory, the nursing students often observe to come for self-practice only around the exam time. This is due to their tight teaching and learning schedule in the undergraduate nursing program. Related to this issue, it seems that there is no official record about how long they took to practice in the lab.

2) To understand how nurse educators perceive the proposed teaching method using computer software in the virtual learning environment to teach intramuscular injection among nursing students.

Participants of the focus group were triggered with questions related to using VLE for the nursing students to learn about the intramuscular injection. In this regard, general responses from the focus group participants seemed to approve that the virtual learning environment would improve the teaching method on intramuscular injection. They mentioned about the nature of using a virtual learning environment whereby the simulation is close to the reality and thus could assist the nursing students in getting a clearer picture of what is taught to them. It could also provide consistent information on the steps of giving an intramuscular injection to the nursing students compared to the lecture-demo in view of it is always ready in the online computer software. In this way, the focus group highlighted the principle of flexibility in learning which would be applied when using the software. The focus group also suggested that nursing students' participation in the practical session should be monitored via the software.

According to the focus group, rather than using a one-way communication, the software could provide a platform to allow nursing students to share information or give feedback on their performance. The focus group preferred that the software that will be produced should apply the concept of interactive learning so that it could enhance learning the intramuscular injection skills. Ideally, the graphic should have a 3-dimension so that the anatomical part of the injection site could be seen from skin layers.

The focus group suggested the computer software should allow the nursing students to virtually handle

the syringe, locate the correct site of injection. The participants in the focus group, on the other hand, argued whether the software would be able to allow the nursing students to provide privacy for the patient during giving the injection. Another recommendation by the focus group participants is to ensure the software could have two-way communication between the nursing student and the nurse educator.

Discussion

Lecture-demo has been used as a teaching methodology for psychomotor skills for many years since the program started in the institution. As frequently practiced in this institution, the lecture-demo is conducted through a PowerPoint presentation to explain about five different sites of intramuscular injection. Following this, the teaching continues with a demonstration using a dummy to identify the injection site using landmarks as well as using an orange to inject so the student feels the actual puncturing sensation of the needle. After that, the students will be divided into several groups in the nursing laboratory to practice on their own while the nurse educator passes by every group to check on the students.

According to the demographical, educational, economic and educational changes happened in the late 20 years all over the world, it becomes a necessity to add computer science and use new technology to the teaching systems. Using computers in teaching nurses had been used a long time ago, and methods of computer-assisted learnings (CAL) was mentioned in literature since the mid-1990s [6]. Paying more attention to create more cognitive nursing educational curriculums done by faculties/schools of nursing and hospital matrons was recommended by [7]. E-Learning has considered vital because its size to individualize who using it in de-centralizing the learning methodology and increase autonomy to the earners and self-guidance [8].(Gleydura, 1995 #7107)

Using computer technology in education has other valuable benefits encompasses resilience and the elevation of dynamic education [9], increase students' enthusiasm and gratification [10], fee competence and decrease in teaching time [11], continuing education [11] and information accessible using the World Wide Web [12].

The elasticity of education that permits students to engage in educational programs while using their own time and location to use a virtual environment [13] gained through mobile-communication technology. Nursing students might ease their education; it was circuitously permitting a sense of ownership. Also, it means that students be responsible for their education process rather than of the lecturers [13].

The nursing practice must be evidence-based [14-16]. Although changes happen in educational materials and new equipment or technology, nursing education programs did not cover the changes that occurred in intramuscular injection practices [17]. Administering intramuscular injections is a demise function in nursing practice [18]. Teaching medication administration techniques in nursing schools usually done through one lecture and might not revise efficiently afterwards [19]. There is no united standard intramuscular injection procedure available in nursing fundamentals textbooks, therefore different teaching methods available in different textbooks based on traditional methods rather than evidenced-based practices ones [20]. This creation of the proposed online computer software can act as a virtual classroom and nursing skills laboratory at the same time.

Fear of mis locating the injection site decreases student's ability to master intramuscular injection technique, this virtual online course intended to increase personal competence, the personal competence as we defined it in another research article is "*The ability of self-control from disruptive emotions and impulsive feelings, the ability to facilitate and guide emotional tendencies to achieve and reach intended goals, and the ability of aligning and working with others in a group or organization towards common goals*" [21].

Conclusion

The focus group agreed to the need for using the online computer software in the VLE. They hoped that the software would play 1) adding some missing features that the lecture-demo method is lacking, 2) changing the learning method to be interactive two way rather than one-way communication, 3) decreasing fear level among nursing students in learning to locate the injection landmarks and give the injection virtually using a computer rather than mannequins, 4) increasing nursing

students cognition level by using new technology, and 5) decreasing laboratory time and nurse educators required to ensure accurate delivery of the information.

Conflicts of Interest : No conflicts of interest.

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Risk Factors of Type 2 Diabetes Mellitus in Hospital of Bengkulu City, Indonesia: Case Control Study

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Abstract

Background: The prevalence of diabetes mellitus is currently increasing and is an important cause of premature death and disability. Insulin resistance in people with type 2 diabetes mellitus is caused by multiple factors such as non-modifiable factors and modifiable factors. **Objectives:** assess the relationship of factors can not be modified and factors can be modified with the incidence of type 2 diabetes mellitus. **Methods:** This is a case control study. Samples taken based on several criteria, namely: 1) patients aged ≥ 18 years, 2) patients can communicate smoothly. Risk factors were analyzed using the chi-square test, knowing the magnitude of the risk using the Odds ratio (OR). **Results:** variables related to type 2 diabetes mellitus were age (p = 0.019), OR = 2.5 (1.2-5.2), family history of diabetes (p = 0.007), OR = 2.9 (1, 3-6.1), body mass index (BMI) (p = 0.001), OR = 3.2 (1.6-6.5), high sugar diet (p = 0,000), OR = 4.2 (2.0 -8.6), low fiber diet (p = 0.018), OR = 2.5 (1.2-5.4), and physical activity (p = 0.000), OR = 7.1 (3.0- 16.6). **Conclusion:** Risk factors proven to be related to type 2 diabetes mellitus are age, family history of diabetes, BMI, high sugar diet, low fiber diet, and physical activity. While gender, central obesity and smoking cannot be proven to be risk factors for type 2 diabetes mellitus. Physical activity is a major risk factor for type 2 diabetes mellitus, with a 4 times greater risk for those who lack physical activity

Keywords: *Diabetes mellitus, risk factors, physical activity, body mass index, diet*

Introduction

Non-Communicable Diseases (NCD) is one of the leading causes of death in the world. [1] One of the NCDs that causes premature death and disability is diabetes. [2] The global prevalence of diabetes has increased almost doubled since 1980, increasing from 4.7% to 8.5% in adult populations. This reflects the associated risk factors such as obesity and overweight. Elevated blood sugar levels, passing through optimum levels, have led to an additional 2.2 million deaths, thereby increasing the risk of cardiovascular disease and neoplasia. 43% of the 3.7 million deaths occurred in patients under the age of 70 years. [3] Indonesia is the seventh-largest country in the world after China, India, the USA, Brazil, Russia, and Mexiko. [4] The prevalence of diabetes in Indonesia increases from year to year. In 1983, the prevalence of diabetes in Indonesia was 1.63%, increasing 5.7% in 2007 and estimated at 6.0% in 2030. The survey in Indonesia reported the high number of undiagnosed diabetes in Indonesia was 4.3%. Therefore, the prevalence of diabetes in Indonesia is potentially higher than the data available. [5]

Diabetes mellitus type 2 is the result of the ineffective use of insulin by the body. Risk factors for diabetes are categorized as excess weight or metabolic or physiological risk factors and hyperlipidemia (high-fat levels in the blood). Also, the factors can be modified such as an unhealthy diet, less physical activity, and smoking and alcohol. [6] With the increasing burden of diabetes, hypertension, and other risk factors, this tends to pose a major threat to the health system. [7]

Bengkulu Province, the number of new cases of diabetes diagnosed with 4,184 and 84 diabetics who passed away. The city of Bengkulu is the most contributor

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to the death of 32 deaths. Based on the recapitulation of disease data in the hospital of Bengkulu, diabetes is one of the highest contributors to the disease in the disease polyclinic during the last 3 years. Increased cases of diabetes in the disease polyclinics in the city Hospital of Bengkulu, so we want to know the risk factors of the incidence of type 2 diabetes.

Material and Methods

The design used in this study is quantitative case-control. A large sample is calculated by using a hypothetical test formula of two proportions, resulting in a sample of 70 respondents in the case. The control takes a comparison of one to one, so the number of control samples is 70 respondents. Samples taken based on several criteria are 1) patients aged ≥ 18 years, 2) patients can communicate fluently.

Data is collected in two ways, first: blood sugar, weight, height, and abdominal circumference are taken based on the record of the patient's medical records. Second: Age, gender, family history of diabetes, physical activity, high sugar diet, low fiber diet, and smoking are gathered from the results of the filling of shared questionnaires. Risk factors are analyzed using the chi-square test, to determine the risk magnitude using the Odds ratio (OR). After that, multivariate analysis is performed using multiple logistic regression.

Findings

Table.1 Risk Factors Based On Factors That Cannot Be Modified And Can Be Modified

Risk Factors	No.140	%
Age	94	67,1
≥ 40 years	46	32,9
< 40 years		
Gender		
Women	92	65,7
Men	48	34,3
Family history of Diabetes		
Yes	46	32,9
Not	94	67,1
Body Mass Index		
≥ 25	66	47,1
< 25	74	52,9
Central obesity		
Yes	58	41,4
Not	82	58,6
High sugar Diet		
Yes	76	54,3
Not	64	45,7
Low Fibre diet		
Yes	96	68,6
Not	44	31,4
Smoking		
Yes	78	55,7
Not	62	44,3
Physical Activity		
Inadequate	95	67,9
Adequate	45	32,1

The results of the study showed most patients aged ≥ 40 years (67.1%) and women (65.7%). Almost half the BMI of patients ≥ 25 (47.1%), more than one-third of patients experienced central obesity (41.4%), about 2/3 patients had less physical activity, more than half of patients consumed foods high in sugar, and about 68% of low-fiber food patterns.

Table.2 Chi-Square Test On Risk Factors Of Diabetes Mellitus Type-2

Risk Factors	Type 2 Diabetes Mellitus				P-value	OR (95% CI) Lower - Upper
	Diabetes		No Diabetes			
	No.70	%	No.70	%		
Age					0,019	2,531 1,218 - 5,261
≥ 40 years	54	77,1	40	57,1		
< 40 years	16	22,9	30	42,9		
Gender					0,593	0,776 0,385 - 1,562
Women	44	62,9	48	68,6		
Men	26	37,1	22	31,4		
Family history of Diabetes					0,007	2,915 1,390 - 6,112
Yes	31	44,3	15	21,4		
Not	39	55,7	55	78,6		
Body Mass Index					0,001	3,273 1,633 - 6,559
≥ 25	42	60,0	22	31,4		
< 25	28	40,0	48	68,6		
Central obesity					0,607	0,790 0,403 - 1,550
Yes	27	38,6	31	44,3		
Not	43	61,4	39	55,7		
High sugar Diet					0,000	4,231 2,080 - 8,604
Yes	50	71,4	26	37,1		
Not	20	62,9	44	62,9		
Low Fibre diet					0,018	2,593 1,234 - 5,452
Yes	55	78,6	41	58,6		
Not	15	21,4	29	41,4		
Smoking					0,602	1,273 0,644 - 2,518
Yes	29	41,4	25	35,7		
Not	41	58,6	45	64,3		
Physical Activity					0,000	7,176 3,091 - 16,662
Inadequate	61	87,1	34	48,6		
Adequate	9	12,9	36	51,4		

The study showed that variables that were significantly related to the incidence of diabetes mellitus type-2 were age (P = 0,019), OR = 2.5 (1, 2-5.2), Family diabetes History (P = 0,007), OR = 2.9 (1, 3-6.1), IMT (p = 0.001), OR = 3.2 (1.6-6.5), high-sugar diet (p = 0,000), OR = 4.2 (2.0-8.6), low-fibre diet (P = 0,018), OR = 2.5 (1, 2-5.4), and physical activity (P = 0,000), OR = 7.1 (3.0-16.6). .

Table.3 Multiple Logistic Regression Test Results For The Final Risk Factor Related Of Diabetes Mellitus Type-2

Risk Factors	P-Value	OR	95% THERE	
			Lower	Upper
Age	0,029	2,677	1,105	6,483
Body Mass Index	0,011	2,870	1,268	6,497
Physical Activity	0,002	4,486	1,776	11,336
High sugar Diet	0,032	2,444	1,080	5,532
Family History of Diabetes	0,106	2,024	0,860	4,765

A multivariate analysis was conducted to see the dominant variable being the risk factor of type 2 diabetes mellitus. Table 3 shows that physical activity is the dominant factor in type 2 diabetes mellitus.

Discussion

Risk Factors Cannot Be Modified

The study reported that age proved to be a risk factor of type 2 diabetes mellitus. A person who is over 40 years of age is at risk twice as much as diabetes mellitus type 2 compared to people who are easier (less than 40 years). Study in India proves the age above 45 years is the risk factor of diabetes mellitus. (6) Other studies indicate a higher prevalence occurs at the age of over 25 years, and a decrease in the prevalence of diabetes after 65 years may be due to death at an early age due to complications of diabetes. [8] The study in Malaysia proved that patients higher than 60 years significantly suffer from diabetes mellitus complications. Age ≥ 60 years is an independent risk factor for diabetic-related complications. [9] In this study, it is not proven that women are more at risk of suffering from type 2 diabetes. Studies in the Iranian state that women should be more serious about monitoring blood glucose independently and women are one of the risk factors in glycemic control and diabetic complications. [10]

The results of our study proved that the family history of diabetes is a risk factor for diabetes mellitus. People who have a history of diabetes are riskier than those who do not have a history of diabetes. Study in India proves the family history of diabetes is as a risk factor that is significantly related to diabetes mellitus. [7] The family history of diabetes with the incidence of diabetes is not directly linked to the size of the femoral adipocytes, accumulated visceral fat, or the concentration of triglycerides in the fasting plasma. However, the family history of diabetes is an independent predictor of the size of abdominal adipocytes in women. The limited peripheral adipose storage capacity is important in the pathogenesis of insulin resistance and the increased risk of diabetes mellitus in those with a family history of diabetes. [11]

Risk Factors Can Be Modified

In population studies, it proved that BMI is a risk

factor of type 2 diabetes mellitus. Their risk of having type 2 diabetes mellitus should have a BMI ≥ 25 is tripled. A study in Japan mentions most Japanese people with type 2 diabetes mellitus resistant to insulin and has a BMI > 25 is a risk factor for severe insulin resistance. [12] A recent cohort study proved a linear relationship between the initial IMT and the risk of diabetes. The risk of incidence of diabetes increased by 35% for each BMI increase of kg/m2 in the 20-30-year group and 31% in the 30-40-year-old group. [13] Obesity is a major risk factor for the development of diabetes. A meta-analysis study that detects a dose-response relationship between BMI and type 2 diabetes mellitus. This shows that per kg/m2 increase in BMI, the risk of diabetes increased by 18%. [14]

This study showed that the diet of high sugar is the risk factor of type 2 diabetes mellitus. The meta-analysis study expressed the frequent consumption of sweetened beverages associated with the incidence of type 2 diabetes mellitus. Although beverages and fruit juices that are diet supplementary sugar also show positive relationships with the incidence of type 2 diabetes. The common people who consume sweet drinks over the years may be related to the increasing number of new cases of type 2 diabetes mellitus. [15] In our study also proved that a low-fiber diet is a risk factor of type 2 diabetes mellitus. Previous meta-analysis studies reported causal relationships between fruit and vegetable consumption and the incidence of type 2 diabetes mellitus. Potential mechanistic evidence is mainly evident in fiber content, which has been shown to increase insulin sensitivity and insulin secretion to overcome insulin resistance. [16] In our study central obesity and smoking did not prove to be a risk factor for type 2 diatebes mellitus.

Our study reports that physical activity is a risk factor in type 2 diabetes mellitus. Analysis of multiple logistic regression proves that physical activity is the most dominant factor related to type 2 diabetes mellitus. Study in India proves the factors related to type 2 diabetes mellitus after adjusting for scaffolding are physical activity, intake of excessive unsaturated fats, IMT, waist-to-hip ratio, and tobacco consumption. [17] The most physical activity done by the group that suffers from type 2 diabetes mellitus is doing the homework. There are only a few respondents who do physical activities such as jogging and cycling. Meta-analysis studies show an inverse relationship between physical activity and the incidence of type 2 diabetes mellitus. The accumulated volume of physical activity that commensurates with public health recommendations currently 150 minutes per week performs moderate to strong physical activity or 75 minutes of strong physical activity per week to maintain general health. [18] But we could not prove it, we recommend a healthy lifestyle to those especially women to do Yoga gymnastics. Previous meta-analysis studies proved that yoga can relate to lipid profiles, blood pressure, BMI, waist/hip ratio, and cholesterol levels. Yoga can improve glycemic results and other risk factors to reduce the complications of type 2 diabetes mellitus in adults. [19]

Conclusion

A proven risk factor with type 2 diabetes mellitus is the age, family diabetes history, BMI, high sugar diet, low fiber diet, and physical activity. While sex, central obesity, and smoking can not be proved to be a risk factor of type 2 diabetes mellitus. This study proves that physical activity is the main risk factor of type 2 diabetes mellitus, with a 4 times greater risk magnitude in those with less physical activity.

Conflict of Interest: All authors do not have a conflict of interest to declare related to research and work.

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Validity and Reliability of the Questionnaire Assessing the Healthcare Delivery Status in Iran's Prisons

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Abstract

Considering the prisoners' statistical population in Iran and the illness-prone environment of the prisons, which necessitates the delivery of appropriate healthcare, a tool should be developed to assess the status of healthcare delivery to these prisoners. The current study has been conducted with the aim of assessing the validity and reliability of the developed questionnaire for assessing the healthcare delivery status in Iran's prisons. This research is a descriptive-analytical study. The developed questionnaire was given to the experts' panel to be assessed for content validity. Content Validity Index (CVI) and Content Validity Ratio (CVR) were calculated using the collected opinions. To assess the validity of this questionnaire, 25 people including physicians and prison authorities were asked to fill the questionnaire for another time after 15 days. Test and retest reliability were calculated using the Intraclass Correlation Coefficient (ICC) and Kappa's agreement coefficient. Data were analyzed by SPSS.26 and Excel.2019 softwares. The content validity experts verified the questionnaire based on its appropriateness, clarity, necessity, and congruity between its words and the relating culture. CVR index was higher than the least standard value (0.62) in every item. Also, all variables of the questionnaire had a CVI of higher than 0.70. Kappa's agreement coefficients of all items ranged from 0.61 to 1.00. ICC values for all items except "Time for system's admissions process" ranged from 0.71 to 1.00 in two times that tests were done. This indicates that reliability of the test and retest of the questionnaire was acceptable. The final version of the questionnaire can be used as a tool for assessment of the healthcare delivery status in prisons and planning programs to enhance that.

Keywords*: *Prison, Healthcare, Validity, Reliability

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Introduction

Prisoners' access to appropriate healthcare should be guaranteed [1]. Prisoners often come from vulnerable groups of society and are affected by most of the contagious and non-contagious diseases [2]. Public health authorities have lately paid more attention to healthcare

delivery in prisons. Higher prevalence of psychological disorders, drug abuse, infectious diseases, stress-related heart diseases, immobility, and isolation leaves no doubt that it's important to understand the existing patterns in healthcare delivery to prisoners [3]. Muller et al. 2009 believe that healthy prisons should not only be used for punishment but to protect society from felonies such as alcohol abuse and violent actions. Prisons can also prevent diseases from imposing evitable costs on society [4].

Nowadays, there is a growing belief that enhancing healthcare delivery to prisons is equal to the enhancement of public healthcare. Prison inmates have equal healthcare privileges, like other people. Prison governors should ensure that prisoners receive appropriate healthcare. The prison staff should consider inmates as patients and not prisoners. Policies enhancing prisoners' health should be included in public health policies and the healthcare managers should work in a close relationship with desmoteric medicine delivery personnel [2].

Suitable healthcare delivery to prisoners should be a top priority for authorities of every country. Because the prison itself poses serious risks for the health of prisoners. According to the international bills of rights and regulations, the determined sentence should only be limited to the prisoner's freedom. Prison sentences should not include physical harm or health care limitations for the prisoners. Also, the sentence should not subject prisoners to death or dangerous diseases due to difficulty in accessing proper healthcare. Negligence and mismanagement, resulting in absence of proper healthcare delivery, will lead to social, physiological, and physical consequences [3,5-7].

Cornfor, Sibbald et al.2007 used the prison primary health care survey questionnaire to describe the organization of health care delivery and services for the management of chronic disease in prisons and to describe systems of information transfer between organizations and types of staff to compare data between different types of prisons [8].

Campbell, Hann et al. 2001 used previously the main part of the general practice assessment survey questionnaire at the National Primary Care Research and Development Centre in a observational study to investigate variations in the quality of care across general practices in England and they measured the inter rater reliability for all items and rejected those for which the k value was < 0.6 [9].

Considering prisoners' number in Iran and the illness-prone environment of the prisons, which necessitates the delivery of appropriate healthcare, quality and quantity of these measures should be assessed. An accurate, objective, and standard assessment requires a proper tool. Therefore, authors of the current study developed this questionnaire, by examining international questionnaires and tools.

Methods

This research is a descriptive, analytical, and practical study that took place in 2019 with the aim of assessing the validity and reliability of the developed questionnaire for healthcare delivery in prisons. Reviewing the previous studies (searching the scientific databases, journals, and publications) and collecting the opinions of related experts, a questionnaire was developed. It was titled "assessing the healthcare delivery status in Iran's prisons" and was made pooling the relating questionnaires. After some modifications in wording, suggested by the experts, a few essential questions were added to the questionnaire. These measures verified the apparent validity of questions. The final questionnaire included the following subjects: Organizational structure of healthcare delivery, variety of delivered services, public services, mental healthcare, chronic diseases, drug abuse, handling of the chronic diseases, information transfer between health care facilities, staff, etc. 10 experts were asked to verify the content validity of the questionnaire based on its appropriateness, clarity, necessity, and congruity between its words and the relating culture. These experts included healthcare managers, general practitioners, a clinical specialist, an epidemiologist, and a prison nurse. Members of experts' panel were selected from expert and informed people, based on their scientific qualifications. The final list included 10 people (Table 1).

Table 1: Properties of experts' panel members

	Expertise	Education	Work experience (Year)	Quantity
1	Healthcare management	PhD	8-20	5
2	Prison general practitioner	MD	10-15	2
3	Clinical specialist	PhD	19	1
4	Epidemiology	PhD	13	1
5	Prison nurse	PhD	20	1

In this study, in order to assess the content validity by the Lawshe method, CVR and CVI coefficients were used. In order to calculate CVR, at first, the questionnaire was given to members of the experts' panel. They were asked to assess every item for its necessity on a 4 point Likert scale (necessary, necessary but needs some changes, beneficial but unnecessary, and unnecessary). After receiving the answers of the members, relating data of each member was entered into Excel software and was analyzed using mathematical and statistical formulas as Formula 1.

$$CVR = \frac{n_e - \frac{N}{2}}{\frac{N}{2}} \quad (1)$$

n_e = Number of experts approving the assessed item
N = Total number of experts
Lawshe Table is used to determine if the obtained value for CVR is proper.

The numerical value of content validity ratio is determined using the Lawshe Table. For instance, in this study that there are 10 people in the experts' panel, if CVR is bigger than 0.62, the validity of the examined topic will be significant [10]. In reports of the validity of questionnaires, the most extensive report is done by the content validity index [11-13].

Also, in order to assess CVI, criteria of appropriateness, clarity, and congruity between its words Persian culture were assessed by the members of the experts' panel on a 4 point Likert scale. Finally, CVI values for every criterion were calculated by dividing the total agreed points for every item which ranked 3rd and 4th by the total number of experts [14]. The acceptable value for CVI was considered 0.70. Obtained results for every variable of the questionnaire are shown in Table 2.

Table 2: Validity assessment of the questionnaire by CVR and CVI

	Variables of the questionnaire	CVR	CVI
1	General Medical Services	0.89	0.78
2	Pharmacy Services	0.86	0.74
3	In-patient Services	0.91	0.93
4	Specialist Services	0.92	0.79
5	Organization	0.68	0.73
6	Chronic Diseases	0.84	0.95
7	Anxiety/Depression	0.71	0.79
8	Information Transfer Between Health Care Facilities	0.69	0.75
9	Staff	0.89	0.79
10	About Prison	0.85	0.82
11	Time for system's admissions process	0.87	0.81
12	Infectious diseases	0.79	0.82
13	Health Concerns	0.84	0.89
14	Mental health screening	0.88	0.83

NOTE: CVR = Content Validity Ratio, CVI = Content Validity Index

Once the assessment of content validity was done, the reliability of the questionnaire was examined [15,16]. Reliability refers to accuracy, reproducibility, and internal consistency of the questionnaire. The reliability of this questionnaire was assessed by using the test-retest method. At first, the questionnaire was sent to 25 studied prisons and filled by the prison's authorities. After 15 days, to assess the test and retest reliability, 25 people who had filled the questionnaire were asked to do it again. Sampling was done using targeted and accessible sampling. Test and retest reliability were calculated using the Intraclass Correlation Coefficient (ICC) for quantitative variables and the Kappa's agreement coefficient for qualitative variables by SPSS.26 software. The results of reliability assessment for this questionnaire are shown in Tables 3 and 4.

Table 3: Values of agreement between nominal variables

Variables	Extent of agreement	Kappa value
-	Slight	0-0.20
-	Fair	0.21- 0.40
-	Moderate	0.41- 0.60
booking interval, out of hours care, Pharmacy Services, having formal mental health training sessions, using the care program approach, mother and baby unit, drug misuse, mental health, entering clinical information directly on to computer, having GP registrars and trainee nurses, having a lead for clinical governance, carry out a patient/prisoner satisfaction survey, formal link with the NHS complaints system, having formal meetings to discuss critical incidents, having a register of patients with chronic disease, having written guidelines for the management of patients with chronic disease, having a recall system for chronic disease, carried out a chronic disease audit in the last 2 years, a lead practitioner for diabetes in the prison, providing sessions held by a specialist nurse trained in chronic disease care, information transfer	Substantial	0.61- 0.80
have an in-patient unit on site, special chronic disease clinic, talk therapies, self-help material, type of prison, Hepatitis A, Hepatitis B, Hepatitis C, Gonorrhea, Chlamydia, Syphilis, Tuberculosis (PPD), Elevated lipids, High blood pressure, Mental health problems (excluding suicide risk), Suicide risk, Traumatic brain injury	Almost perfect	0.81- 1.00

Table 4: Values of agreement between quantitative variables

Variables	ICC
-	0- 0.60
Time for system's admissions process	0.61- 0.70
patients with IHD, patients with asthma, patients with hepatitis, patients with TB, patients with HIV, number of nurse practitioner, number of nurses – general, about the prison	0.71- 0.80
afternoon surgeries each week, evenings surgeries each week, beds, patients have registered with diabetes, number of general practitioners, number of psychiatrist – adult, number of clinical psychologists	0.81- 1

NOTE: ICC = Intraclass Correlation Coefficient

Results of content validity assessment showed that the CVR index value for every item is higher than the least standard value (0.62). The highest value for the CVR index was 0.92 that was related to "specialist services" variable and the least value was 0.68 pertaining to "organization" variable. Also, all the obtained values for the CVI index were higher than the least acceptable value (0.70). CVI value for "In-patient Services" and "Chronic Diseases" variables were 0.93 and 0.95, respectively which are the highest among others. The least value belongs to the "Organization" variable which was 0.73 (Table 2) Kappa's agreement coefficients of all items ranged from 0.61 to 1.00 which shows substantial reliability (0.61-0.80) for most of the variables and almost perfect reliability (0.81-1.00) for the remaining variables of the questionnaire as shown in Table 3 [17]. ICC values for all items except "Time for system's admissions process" ranged from 0.71 and 1.00 in the two times that tests were taken, which is higher than 0.70 (least acceptable value) and indicates acceptable reliability for test and retest of the questionnaire (Table 3, 4).

Discussion

Prisoners are among the most vulnerable groups of societies throughout the world. The least standards of healthcare that are accepted by the governments should be passed for the prisoners by the legislation bodies [18]. Prison inmates are in need of complicated medical and social care. This complexity results from a combination of infectious risk factors, drug abuse, addiction, psychological issues and detention problems [19].

Cornfor, Sibbald et al.2007 concluded that prisoners with chronic diseases are obtaining a poorer level of care compared to patients outside prison. In order to deliver an equivalent level of care for patients with chronic diseases in prisons, significant improvements in IT will need to be made and the problems concerning the recruitment and retention of general nurses will need to be addressed [8].

Barry et al.2010 assessed the basic healthcare delivery in prisons of Ireland, using semi-structured questionnaire and reviews which also involved physicians. Results showed that there was a considerable difference in standards of medical infrastructure and facilities among different prisons. In general, healthcare delivery status was not appropriate in prisons. Also, there was a vast inequality in medical care between prisons and other parts of the community, especially in the number of physicians. Most doctors mentioned that there is little political and governmental support. Also, they believed that prisoners' healthcare is not supported adequately and there is a tangible shortage in psychiatric care [20]. Prisoners' healthcare enhancement plays a crucial role in the improvement of public healthcare. Prisons' authorities should ensure that there is constant and quick access to basic medical care for every prisoner. Important components of this care include physical examination, medical consultation, enough

space with adequate equipment for desmoteric medicine, emergency care, basic and necessary medications, facilities for psychotherapy and rehabilitation, the possibility of following prescribed diets, proper hygiene, and similar items. Improvements in healthcare delivery can't be achieved without a proper tool to identify the strengths and weaknesses of desmoteric medicine. It's deduced from the results of foreign studies that cultural, social, economic, and even geographic factors affect the prevalence and incidence of diseases occurring in communities and prisons. Thus, the least expected standards of international institutions such as WHO should be considered in developing the desmoteric medicine delivery regulations [2,5-7]. The numbers of questions for each part of questionnaire are shown below in Table 5.

Table 5: Number of questions for each part of questionnaire

Number of questions	Variables
4 questions	General Medical Services
1 question	Pharmacy Services
1 question	In-patient Services
3 questions	Specialist Services
7 questions	Organization
7 questions for each disease	Chronic Diseases (including diabetes, ischemic heart disease, asthma, hepatitis, HIV infections, and TB)
2 questions	Anxiety/Depression
3 questions	Information Transfer Between Health Care Facilities
2 questions	Staff
2 questions	About Prison (type and sex of prisoners)
1 question	Time for system's admissions process
7 question	Infectious diseases
3 questions	Health Concerns
3 questions	Mental health screening of the new prisoners

Conclusion

This questionnaire, given its proper validity, reliability, and full coverage of related information on healthcare delivery in prisons, can be utilized as an efficient tool to identify the strengths and weaknesses of desmoteric medicine. It can also be used for planning programs that will enhance healthcare delivery to prisoners.

Conflicts of Interest – Nil

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Ethical Clearance- The study was approved by the ethics committee of North Tehran Branch, Islamic Azad University, Iran.

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Outcome of Anterior Cruciate Ligament Reconstruction using Femoral Intrafix for a Single Tunnel Double Bundle Anterior Cruciate Ligament Reconstruction

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Abstract

Background: Single-tunnel double-bundle anterior cruciate ligament reconstruction (ACLR) with anatomical placement of hamstring tendon graft can closely restore the anterior knee instability when compared with single bundle reconstruction.

Aim: Evaluation of the clinical and functional outcomes of ACLR using femoral intra-fix and tibial interference screw.

Methods: This is a prospective study held on 40 patients who underwent autologous hamstring graft ACLR using femoral intra-fix and tibial interference screw. After a median follow up of one year the clinical (Lysholm score), functional outcome (International Knee Documentation Committee, IKDC) and Joint laxity (assessed with KT-1000 arthrometer - MEDmetric, San Diego, CA) were evaluated.

Results: As regard IKDC 36 patients (90%) had normal or nearly normal knees postoperatively in comparison to 100% had abnormal and severely abnormal knees pre-operatively (**P value < 0.001)**. The mean Lysholm score was higher in the postoperative follow up than preoperative (91.40±7.3 Vs 53.35± 13.55) with statistically significant (**P value < 0.001)**. The mean anterior translation of tibia improved from 7.55 mm preoperatively to 2.1 mm after one year of ACLR.

Conclusion: ACLR using femoral intra-fix and tibial interference screw provide secure graft fixation and allow early rehabilitation. The clinical and functional outcome of this fixation technique is rewarding.

Keywords: *ACL, Hamstring graft, Lysholm score, IKDC, Intrafix*

Introduction

The anterior cruciate ligament (ACL) consists of 2 major functional bundles, the antero-medial bundle (AMB) and the postero-lateral bundle (PLB).(1) The goal of anatomic double bundle (DB) ACL reconstruction (ACLR) is to provide a close restoration of the normal knee kinematics, function, and decrease the risk of degenerative osteoarthritis of the knee joint.(2) In anatomic DB reconstruction two tunnels in the tibia and in the femur are necessary to reconstruct both (AM) and (PL) bundles, which is difficult to impossible in narrow notches.(3) Most of the clinical studies comparing single-bundle (SB) to DB reconstruction do not present a fair comparison since often one or both surgical techniques were not performed anatomically. In addition, associated injuries such as chondral injuries, meniscus tears and osteoarthritic changes should be taken into account when conducting comparative clinical studies.(4)

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Materials and Methods

Patient's selection

This is a prospective study held on 40 patients aged (20-40) years operated from March 2016 to October 2018 for their ACL deficient knees with autologous hamstring graft fixed by femoral intrafix on the femoral side and interference screw on the tibial side. Patients with multi-ligamentous injury, infection, inflammatory or degenerative pathologies and previous history of ACLR on the same knee were excluded from the study. Institutional ethics committee permission was obtained and informed consent was taken from all patients. Preoperative evaluation include history, clinical examination, ligamentous laxity measurement using KT-1000 arthrometer, radiographs and magnetic resonance imaging of the knee joint were done. After complete clinical and radiological evaluation, patients were arthroscopically treated with ACLR using hamstring graft.

Surgical technique

The knee joint was accessed through three standard portals anteromedial (AM), anterolateral (AL) and accessory anteromedial (AAM). Meniscal injuries were addressed with partial resection. The harvested hamstring tendons (Gracilis and Semitendinosus) were prepared as tendons were folded in half over a single strand of #2 sutures to create two bundles. Each side was whip-stitched to 30 mm from fold. On the femoral side, the AM bundle was marked while the PL bundle left unmarked. The graft is whip-stitched on the tibial side; thickness and length of the graft were measured. (Fig.1a). A 45° micro-fracture awl was inserted through the AAM portal marking the centrum of the femoral foot print, which should be 2.5 mm plus the planned tunnel radius from the posterior articular cartilage. The knee was hyper-flexed to (110°-120°) allowing guide wire to move with the rotating condyle anteriorly, avoiding posterior wall blow out, now drilling the femoral tunnel using appropriate sized reamer. The depth should be 30-35 mm (Fig. 1b, c).

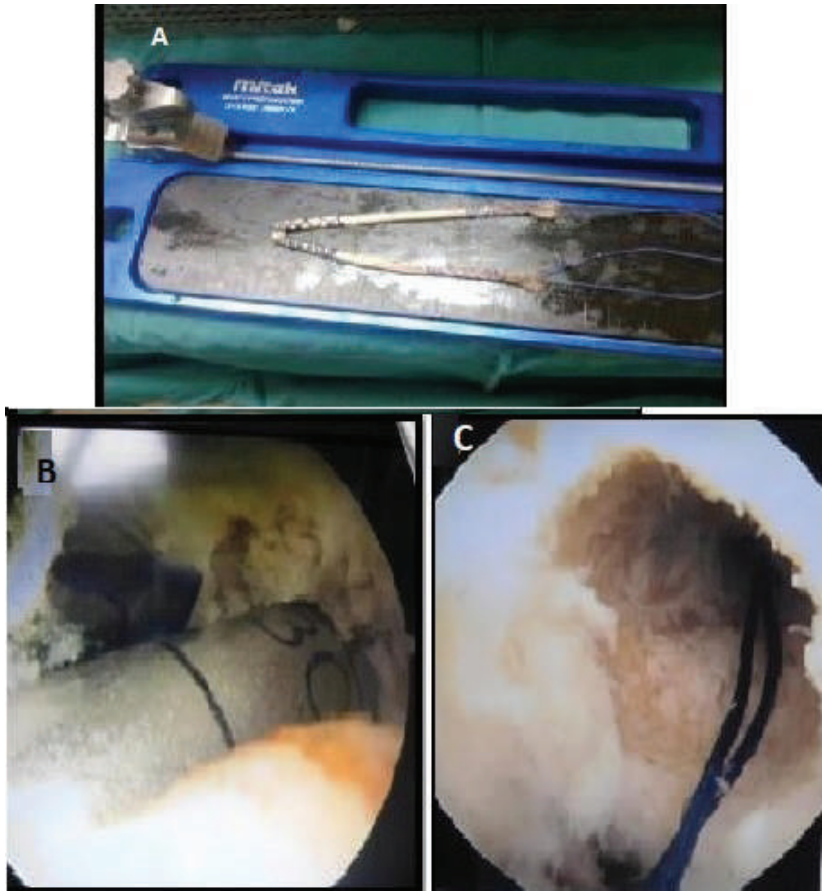


Fig 1. (a) Hamstring graft preparation.

(b) The femoral tunnel length 30mm. (c) intact posterior wall

Before advancing the graft into the femoral tunnel, the AM and PL bundles were rotated to the desired position (Fig.2a,b). A sheath trial of the same size of the graft was introduced via the AAM portal separating the AM & PL bundles (i.e. one strand posterior, the other inferior within the single tunnel), then rotate the sheath trial to orient its broader convex surface (laser marked) to an anterior distal position in the femoral tunnel and manually insert trial tip between graft bundles until snug.

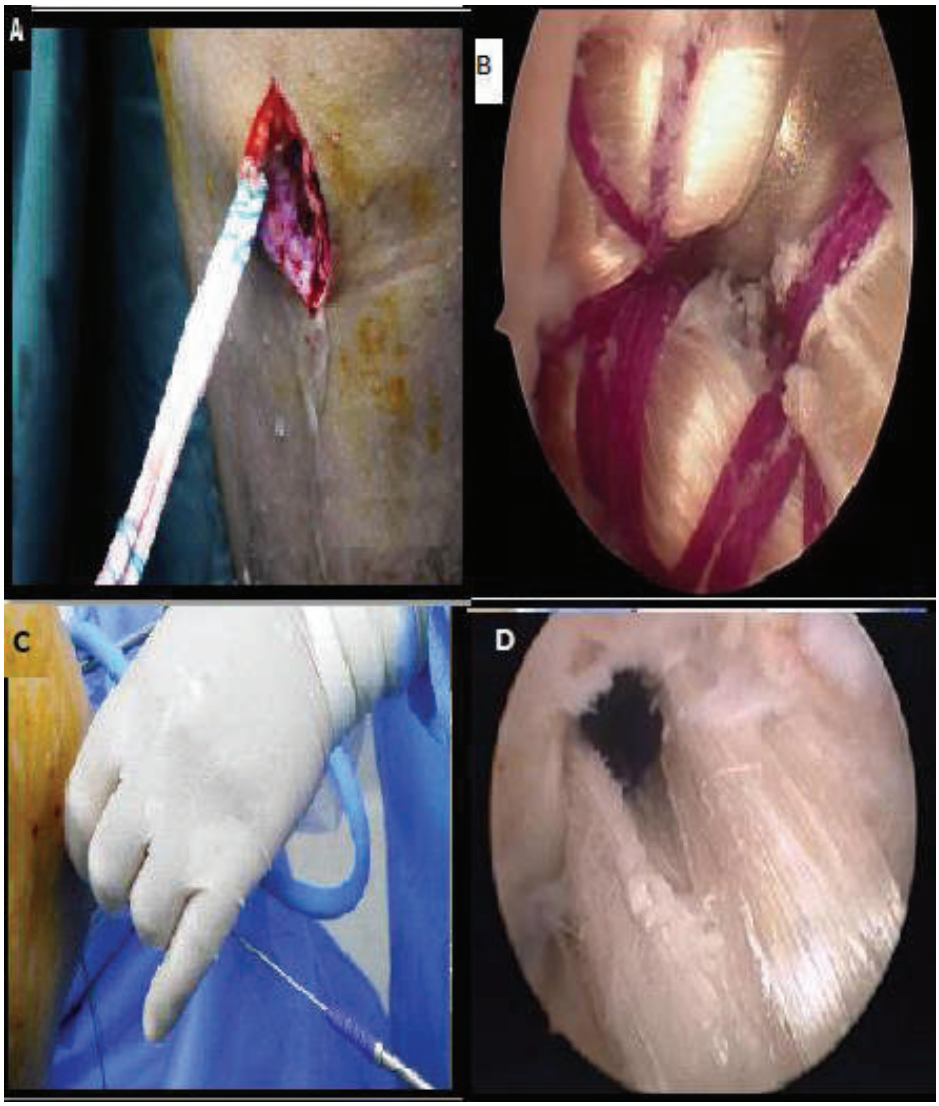


Fig2. (a) The AMB and PLB are rotated to the desired position. (b) Adjusting The graft position before inserting the guide wire, (c) Sheath Application, (d) The sheath is separating the two bundles.

Drive sheath over the guide wire between graft strands in the tunnel until it fully seated to a depth of 2mm-3mm into the tunnel as indicated by the laser marking on the rear shoulder of the inserter. The shoulder of the sheath inserter should be slightly recessed to prevent sheath extrusion during screw insertion (Fig.2c,d).The screw is next inserted over a guide wire into the sheath (Fig.3). Tension should be applied to both ends of the graft during screw insertion to prevent the graft from wrapping around the screw. If any material from the sheath has extruded during screw insertion, it is now removed. [8]

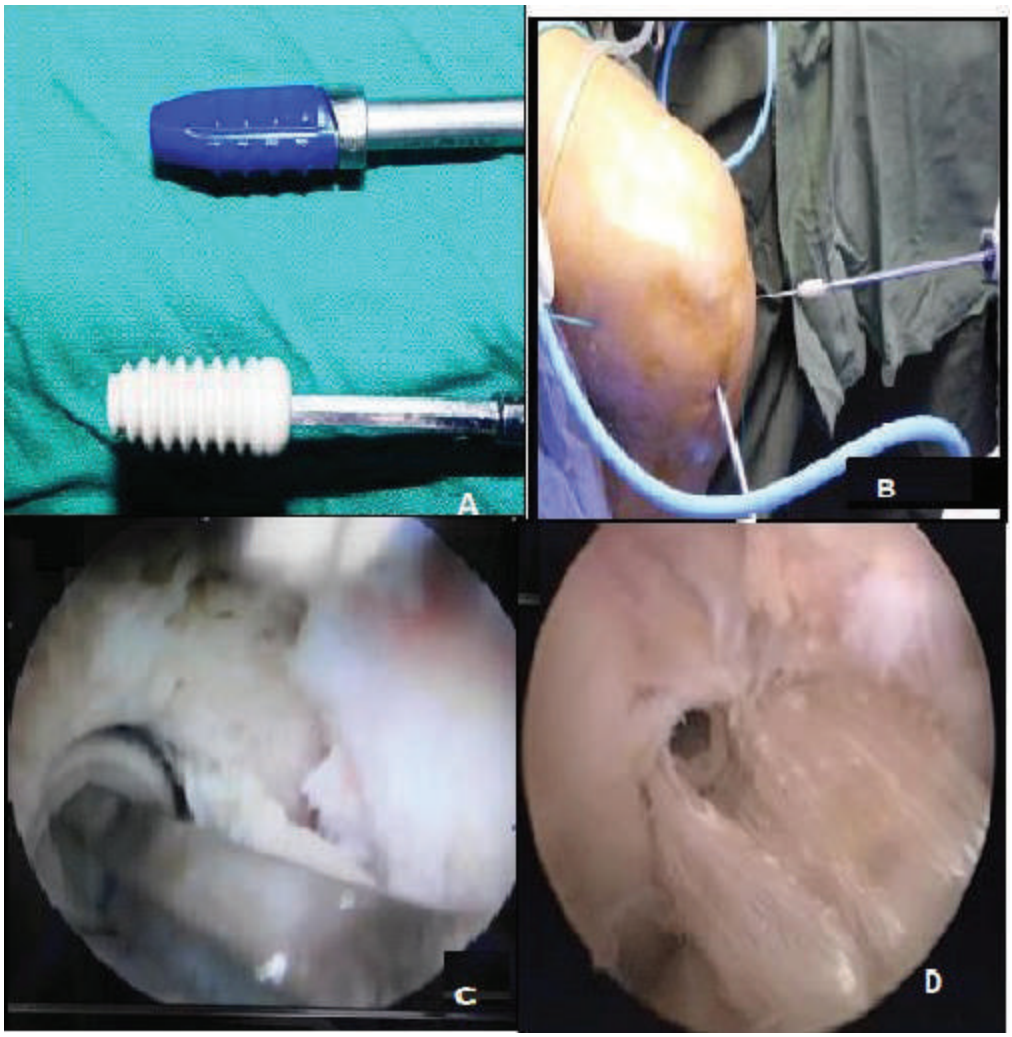


Figure 3: (a). Femoral intrafix screw and sheath, (b,c) Screw insertion, (d) Screw separating the two bundles.

Then tension is applied to Ethibond before interference screw is used to fix tibial side. Careful testing for full range of movement especially complete extension was done. Both Lachman and ADT tests were done.

Postoperative rehabilitation

First day postoperatively, patients were allowed to start partial weight bearing with crutches; knee flexion up to 90° is allowed and isometric quadriceps for six weeks [5]. Subjects were prescribed intense physical therapy for motion exercise, and to strengthen thigh muscles. Normally, full range of motion and weight bearing was achieved at 6-8 weeks of surgery. Patients were followed up in outpatient clinic at 14 days then monthly up to months, 6 months and finally every three months until one year. They were evaluated clinically (Lysholm score and IKDC examination form) and knee joint stability was measured using KT-1000 arthrometer at one year.

Statistical analysis: was performed using **SPSS software version 24**.

Results

The mean age of patients included in this study was 27.75 years (range 20-40 years). There were 38 males (95%) and 2 females (5%). Left side involvement was noticed in 17 patients (42.5%) and right side in 23 (57.5%) patients. There was no meniscal pathology in majority (52.5%) of the patients. In patients with meniscal tears, the medial meniscus (n = 11, 27.5%) was commonly involved than the lateral meniscus (n = 8, 22.5%). The mean operative time was (102± 21.4 minute, range 60-140 minutes).

34 patients (85%) had no post-operative limitation of motion compared to 6 patients (15%) who had post-operative limitation of range of motion either in extension or in flexion.

At the end of follow up; 90% of the patients had -ve Pivot shift while 10% have +ve test (three of them had only +1) one patient who had +3 Pivot shift postoperatively.

As regarding the IKDC examination form, preoperatively 55% of the patients (22 patients) had severely abnormal knee (class D) while 45% (18 patients) had abnormal knee (class C). Postoperatively according to IKDC examination form 60% of the patients had (24 patients) normal knee (class A), while 30% (12 patients) had nearly normal knee (class B), and only 10% (4 patients) had abnormal knee (class C) with statistical highly significance **(P- value <0.001).**

Lysholm score improved postoperative (Post-op) 91.40±7.3 Vs 53.35± 13.55 preoperative (Pre-op) which translated into statistically significant with **(P value < 0.001).**

Ligamentous laxity measured by KT-1000 showed significant improvement after ACLR. The mean anterior translation of tibia in the Pre-op. period was 7.53 mm and which improved to 2.1 mm after 1year of ACLR.

Thirty nine patients (97.5%) had no post-operative complications; one Patient (2.5%) had infection, and graft failure.

Discussion

The aim of ACLR is restoring normal knee anatomy, biomechanics and function. It was assumed that the clinical success of ACLR depends on correct positioning of the graft tunnel when performing singe bundle ACLR, However, many prospective series and meta-analyses conducted have shown that, despite correct tunnel placement, a significant number of patients experience persistent instability in the knee at follow-up especially in respect of rotational stability and also have abnormal IKDC scores in nearly one fifth of patients who had undergone single-bundle ACLR. [6]

Femoral intrafix ACL fixation system from DePuy Mitek, Inc. is designed to provide soft tissue femoral fixation for ACL reconstruction surgery using a single tunnel through an antero-medial approach. Femoral intrafix, with its unique patented polypropylene sheath and PEEK (Polyetheretherketone) screw (Figure3a) implant design offers more anatomic ACLR with improved femoral footprint placement and coverage, controlled antero-medial and posterolateral bundle positioning, protecting the graft from being violated during screw insertion, strong and stiff soft tissue aperture fixation. [7]

Regarding range of motion (ROM), 85% of patients had no post-operative limitation of motion in comparison with 5% that have postoperative limitation of extension due to inefficient rehabilitation and 10% had limitation in flexion (one patient had infection and graft failure, two patients had concomitant both menisci surgery, and one had mild loss of terminal flexion). The limitation of the ROM was greater in patients with concomitant meniscal surgery. This result was similar to **Ashish et al** [8] who had operated 30 patients with DB ACLR and had 13% limitation of range of motion.

Huang etal [9] results were better than this study regarding the ROM as h operated 25 patients with femoral intrafix but his follow up was longer and his final evaluation was done (12-18 months). Normal ROM of the involved knee is strongly associated with patient-reported outcome, reduced osteoarthritis, and greater quadriceps strength at long-term follow-up in patients after single bundle ACLR. [10]

In our study 90% of patients have –Ve Pivot shift test, and 10% of patients have + Ve pivot shift test. One of them (2.5%) had infection and graft failure, while 3 patients (7.5%) had postoperative laxity (+1) which was associated with positive ADT and Lachmann test this was similar to the study done by **Philippe et al** [11] who operated 33 patients with double bundle four tunnels ACLR technique and had +ve pivot shift test in 15% of patients. **Ricardo et al** [12] had operated 30 patients with SB anatomical ACLR technique and had +ve pivot shift in 23.3% which showed the superiority of this technique.

Regarding IKDC; 55% of the studied group had severely abnormal knee (D) and 45% had abnormal knee (C) preoperatively. Those findings were improved post-operatively as 60% of patients had normal knee (A), 30% of patients had nearly normal knee (B) and 10% had

abnormal knee (C) with a highly statistically significance (P value <0.001). Ninety percent (36 patients) cases had a normal or nearly normal knee (grade A + B objective IKDC) which was similar to DB ACLR by **Philippe et al** [11].

Our results were similar to **Ricardo et al.** [12]who had 30 patients underwent SB anatomical ACLR , 70% of patients had score normal knee (A), 20% had nearly normal knee (B), and 10% had abnormal knee (C).

Regarding Lysholm score :80% of patients had poor score and 20% had fair score preoperatively which improved postoperatively as 30% had excellent score, 50% good score, 10% had fair score, while 10% had poor score with statistically significant difference (P value <0.001). In the study done by **Järvelä et al.** [13] the Lysholm score for DB anatomical ACLR was 45% excellent score which was is better than our results. Also, the mean preoperative Lysholm score was 53.35 ± 13.55 which is less than the observations of **Järvelä et al** [13] **and Fujita et al.** [14] who reported a preoperative Lysholm score of 69 and, 67.4 respectively. This could be attributed to the long period between the injury and the operation; the instability and its associated secondary chondral damage due to delayed presentation may be the reason for a very low preoperative Lysholm score in these patients. The mean postoperative Lysholm score, of 91.40±7.3 was in close proximity with those reported by **Järvelä et al** [14] **and Asagumo et al.** [15] (96.8 ± 5.1) who used double bundle double tunnel technique in their study.

Our study results was better than the results observed by **Ubale et al.** [17] who operated on 30 patients with anatomical SB ACLR 49.5 ± 11.6 pre-operatively to 83.4 ± 9.5 postoperatively.

Conclusion

Single tunnel double bundle ACL reconstruction using femoral intrafix is a reliable technique for ACL reconstruction which can restore the AP stability and reduce the risk for rotational instability (improved IKDC examination form postoperatively with P-value <0.001 and –Ve pivot shift test) in comparison with other methods of fixation, but this technique is limited by long operative time, good bone quality and well trained surgeon with a good experience in ACL reconstruction.

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PON1 Q192R Gene Polymorphism and Pesticide Exposure Status of Rice Farmers, Suphan Buri, Thailand

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Abstract

PON1 Q192R (rs662) polymorphism in coding region is conveying differential catalytic activity of organophosphate. Study was aimed to investigate PON1 Q192R gene polymorphisms in rice farmers along with pesticide knowledge and practices; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels. Information of pesticide exposure, knowledge and practices were collected from 50 rice farmers and 50 control respondents by questionnaire interviewing. Each blood sample was obtained by venipuncture and prepared to serum for SChE activity test by paper test and automatic analyzer; to EDTA blood for genotyping by PCR-RFLP. Exposure status, knowledge and practices on pesticide use were represented as frequency. Chi-square was used to analyze on the different of personal information; and of polymorphism. Independent *t*-test was used for comparison of SChE levels. Person correlation was evaluated relationship between SChE level and genotypes. 60% of rice farmers were used pesticide over 10 years. Rice farmers were long-term pesticide exposure with good on pesticide knowledge, however poorly practice. Means of SChE level of both groups were within reference value and significantly different (p = 0.033). Polymorphisms were included wild type (QQ), heterozygote (QR) and homozygote (RR); and were significantly different between rice farmers (high frequency of RR genotype) and control (p = 0.02). Relationship of polymorphism and SChE level were negative correlated (r = -0.261; p = 0.031). This polymorphism may useful biomarker for chronic pesticide exposure in Thai rice farmer and corresponded to decline of serum cholinesterase; and RR genotype was high risk group.

Keywords*: chronic pesticide exposure, paraoxonase-1 (PON1), PON1 Q192R polymorphism, serum cholinesterase*

Introduction

Many previous studies were reviewed regarding pesticide use, poisoning, and knowledge and unsafe occupational practices in Thailand over the last decade; and Thai government is responsible in making policies and regulations and encouraging all agricultural activities to be sustainable [1]. Rice farmers in three Thai provinces were found to have the high prevalence of allergies, nasal congestion, wheezing, and acute symptoms after pesticide use [2]. Occupational health and safety problems among rice farmers may result from unsafe behaviors and unsafe acts or practices of workers, which are related to agrochemical exposure, such as the use of faulty spraying equipment or lack of attention to safety precautions [3]. Suphan Buri province

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is located in central river plain of Thailand. Because of enough water supplies in this cultivation area, two or three rice growing cycles can be done. Thus, farmers may expose to pesticides more frequent rather than other area [4]. Moreover, outsource pesticide sprayers were common finding in rice field. Chloropyrifos and carbofuran are commonly used in this area, which are the member of organophosphate (OP) and carbamates (CB), respectively [1, 5]. Decline of serum cholinesterase is useful to indicate risk of pesticide exposure especially in sprayers [6-7]. Thai government is developing cholinesterase activity screening test and national apply for control the risk and unsafe field workers [8].

Paraoxonase 1 (PON1) is a phase-I enzyme that is involved in the hydrolysis of organophosphate esters [9, 10]. Literature reviews of PON1 genotypes are involving in the presence of Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis [11]. PON1 Q192R genotype is also associated with the risk of multiple cancers [12, 13] and coronary heart diseases [14]. PON1 variants play a role in xenobiotic-metabolizing system in occupational exposures and consequence to oxidative stress and DNA damage [15]. Human PON1 Q192R (rs662) polymorphism in coding region is conveying differential catalytic activity toward some OP substrates [16]. PON1 Q192R polymorphism (rs662A > G) was caused by the glutamine (Q genotype) substituted for the arginine (R genotype) 192 of the gene 6 exon of the PON 1 gene [17]. The information of PON1 Q192R gene polymorphisms among Thai race especially agricultural workers was still limited. This study was aimed to investigate PON1 Q192R gene polymorphisms in rice farmers along with pesticide knowledge and practices; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels.

Materials and Methods

Subject recruitment and data collection

The cross-sectional study was carried out from June 2019 to February 2020 on data had collected from annually health service program by health promoting hospital, This study was recruited 100 respondents included 1) 50 rice farmers (risk group) were aged 18-65 yr who lived in this area, which had handle pesticide regularly or work in paddy field at least three years or more 2) The control group was included 50 respondents who lived nearby field area and listed in house registration, had non-related professional for farm workers. Respondents with a history of serious conditions were excluded. Questionnaire interviewing and blood collection were conducted by well-trained research assistants and medical technologists, respectively. Gathered information concerning of long-term pesticide exposures including personnel information, adverse health symptoms, knowledge and practice of pesticide use were recorded from questionnaires by personnel interviewing. The sample size was estimated using the single proportion formula with 95% confidence interval and based on percentage of abnormal SChE level in previous study [18]. The Ethics Committee of Thammasat University was approved this research protocol (COA No. 084/2562). The director of U-Thong district ¢s health promoting hospital, Suphan Buri province gave permission to conduct on this study. All participants gave informed consented.

Blood collection, preparation and storage

Each 5 ml of blood sample was obtained by venepuncture from median cubital vein during morning (7-9 a.m.); and drawn into clotting blood and EDTA tubes for 3 ml and 2 ml, respectively. Clotting blood tube was further centrifuged; and serum was separated within 2 h after phlebotomy and stored at -20 °C [19] for SChE activity test. Whole blood contained in EDTA tubes were prepared for genomic DNA extraction by using the QIAamp blood DNA mini kit (QIAGEN Thailand, Bangkok, Thailand) and genomic DNA was stored at -20 °C.

SChE activity test

Screening of SChE level by paper test [8] and confirming of SChE level was done by automatic analyser. The paper test kit was developed and manufactured by Government Pharmaceutical Organization (GPO), Thailand. The efficiency of test including sensitivity, specificity and positive predictive values were 77, 90 and 85%, respectively. The quantitative for SChE level was conducted by automatic analyzer, COBAS c501 (Roche-diagnostics, Rotkreuz, Switzerland), which were performed in certified clinical laboratories. SChE level were interpreted by reference values according by instruction of manufacturer.

PON1 Q192R polymorphisms by PCR-RFLP

DNA template was amplified by polymerase chain reaction (PCR) using forward 5′-TAT TGT TGC TGT GGG ACC TGA G-3′ and reverse 5′-CCT GAG AAT CTG AGT AAA TCC ACT-3′ primers, which were corresponded to PON1(Q192R) region [20]. PCR was performed in 25 µl of total volume, which was contained 2 µl of genomic DNA, 0.5 µl of each primer, 5 µl of 10X PCR buffer (1.5 mM Mg^{2+}) include dNTP mixture and 0.25 µl of 1.5 U AmpliTaq polymerase (Thermo Fisher Scientific, USA). PCR was performed with initial denaturation at 94°C for 5 min. followed by 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72 °C for 30 s followed by final extension at 72 °C for 7 min Thermal cycler (Applied Biosystems, USA). Restriction fragment length polymorphism (RFLP) was performed: DNA product was digested by *A1wI* restriction enzyme (New England Bio Labs, Cambridge, UK). Digested DNA fragments was separated on 2% of agarose gel electrophoresis apparatus then stained with ethidium bromide. DNA electrophorogram was read by using ultraviolet transillumination (Promega, USA). PON1 Q192R single nucleotide polymorphisms (SNPs) were 1) 66- and 172-bp fragments for the 192R allele and 2) 238-bp fragment (undigested) for the 192Q allele. The interpretation of PON1 Q192R SNPs on genotypes were represented 238-bp fragment for wild type (QQ); 66, 172, 238-bp fragments for heterozygous (QR); and 66, 172-bp fragments for homozygous (RR). Quality control of test was done by DNA sequencing, which was randomized from 15% of samples.

Statistical Analysis

Descriptive data was explained by using frequency. Chi-square was used to analyze on the different of personal information and genotypes between rice farmer and controls groups. Independent *t*-test was used for comparison of SChE level between rice farmer and controls groups; and between wild and variant types. Person correlation was evaluated between SChE level and genotypes. The statistical significance was judged at $p< 0.05$. SPSS 21.0 software was used for statistical analysis (SPSS, Chicago, Illinois, USA).

Results and Discussion

Pesticide exposure, knowledge and practice of rice farmers

All of rice farmers were long-term pesticide exposure and 60% of rice farmers were used pesticide over 10 years; and rate of pesticide exposure was mainly for 1-2 time/week. The related pesticide used symptoms were rarely occurred. Unexpected finding may due to unspecific symptoms, imprecisely explain by personal interviewing and tolerance of frequent exposed farmers. Means of SChE level were significantly different between rice farmers and controls ($p = 0.033$), however, there were within reference value (Table 1). Most of rice farmers were known about pesticide on health effects (80%), however, some of them were improper on practice, such as, the reading of pesticide label, first aids for toxicity and appropriated skill on spraying (Table 2). Educational interventions are essential for promoting safety during all phases of pesticide handling in small-scale Thai farmers. Public policies should be developed to encourage farmers to change their pest management methods from chemical based to methods that are healthier and more environmentally friendly [21]. Promotion of health literacy is associated to reduce unsafe behaviors on pesticide use [22].

PON1 Q192R polymorphisms in rice farmers

The DNA electrophoresis of PON1 Q192R gene polymorphisms were included wild type (QQ), heterozygote (QR) and homozygote (RR) (Fig. 1). The genotypic polymorphisms of rice farmers and control were significantly different (p=0.02); rice farmers were more frequent RR genotype rather than control (Table 3). The relationship of PON1 Q192R gene and SChE level were negative correlated (r = -0.261) with statistically significant (p = 0.031) (Table 4). Six of rice farmers were low SChE level (<5,500 U/L) and RR genotype polymorphism (data not show).

In this study, SChE and PON1 Q192R genotype between rice farmers and controls were significantly different; and the decline of SChE and PON1 Q192R genotype were also related. The significant finding was corresponded to previous study, which was reported that PON1 192RR genotype and CYP2D6 1934A allele are relate to organophosphate susceptibility in

chronic exposure in Egyptians. Moreover, SChE is significantly reduced in chronic organophosphate-intoxicated Egyptian patients [23]. PON1 192 R(+) (QR + RR genotypes) genotype carriers had higher PON1 and acethylcholinesterase (AChE) activities than 192 R(-) (QQ) genotype carriers in Turkish population, which had chronic pesticide exposure in occupational reasons [24]. In our study, rice farmers and control were significantly different and rice farmers were older than control. Q192R and L55M polymorphisms of PON1 are population-specific effects due to interaction of gene variation and environmental factors; therefore, this polymorphism is not impact on elders or extreme ages [25]. The most of pesticide exposure is chronic rather than acute toxicity via occupational and/or environmental means. Pesticide may lead harmful effects of human health, which are through oxidative stress, epigenetic transformation, and gene polymorphisms. Most of gene polymorphisms are implicate to OP metabolism enzymes, such as cytochrome P450, glutathione transferase, acetyltransferases or paraoxonase [26]. R variant of PON1 Q192R gene polymorphism is less effective to protect LDL oxidation. Thus, R genotype is associate to hypertension, coronary artery diseases, stroke and Parkinson's disease [27, 28]. Large scale and seasonal variation studies on gene polymorphism and environmental expose will conduct to confirming the finding.

Table 1: The frequency of personal data and pesticide-exposing factors from rice farmers and controls

Personal data/ Exposing factors	Rice farmer (%)	Control (%)	p-value
Gender : Male	34 (68)	30 (60)	0.405
Female	16 (32)	20 (40)	
Age : < 40 years	22 (44)	40 (80)	0.0001*
≥ 40 years	28 (56)	10 (20)	
Alcohol intake: none	36 (72)	33 (66)	0.517
drinking	14 (28)	17 (34)	
Duration of pesticide use: 4-9 years	18 (36)	-	
> 10 years	32 (64)	-	
Frequency of exposure: 1-2 days/week	30 (60)	-	
3-4 days/week	4 (8)	-	
5-6 days/week	16 (32)	-	
Clinical symptoms: None	36 (72)	-	
Headache/vertigo	11 (22)	-	
Abdominal cramp	3 (6)	-	
Health education: none	13 (26)	-	
educated	37 (74)	-	
Serum cholinesterase (U/L) **	7247.2 ± 1293.3	7775.9 ± 1152.8	0.033*

* Statistically significant at $p<0.05$; ** Serum cholinesterase (SChE) was screened by paper test before tested with automatic analyzer and represented as mean ±SD (reference value = 5,500-13,000 U/L).

Table 2: Knowledge and practice of rice farmers for pesticide use

Topic of Pesticide use	Frequency (%)	
	Uneducated	Educated
Health effects of pesticide	10 (20)	40 (80)
First-aids for acute intoxication	23 (46)	27 (54)
Personal protective equipment	17 (34)	33 (66)
Reading and understanding on information of pesticide label	24 (48)	26 (52)
Cleaning of pesticide spraying clothes	15 (30)	35 (70)
Pesticide container management	17 (34)	33 (66)
Appropriate in pesticide spraying	21 (42)	29 (58)
Personal hygiene after pesticide spraying	15 (30)	35 (70)

Table 3: Genotypic frequency of PON1 Q192R in rice farmer and control groups

Genotype	Control		Farmer		p-value
	N	%	N	%	
Wild Type (QQ genotype)	29	58	14	28	0.020*
Polymorphisms (RR & QR genotype)	21	40	36	72	
Homozygous (RR genotype)	12	24	27	54	
Heterozygous (QR genotype)	9	18	9	18	

* Statistically significant at $p<0.05$

Table 4: Relationship of PON1 Q192R genotypes and SChE level

Genotype	N	SChE (U/L)	r	p-value
Wild Type	43	7820.14 ± 1103.25	-0.261	0.031*
Polymorphisms	57	7278.89 ± 1307.70		

* Statistically significant at $p<0.05$; r = Pearson correlation coefficient

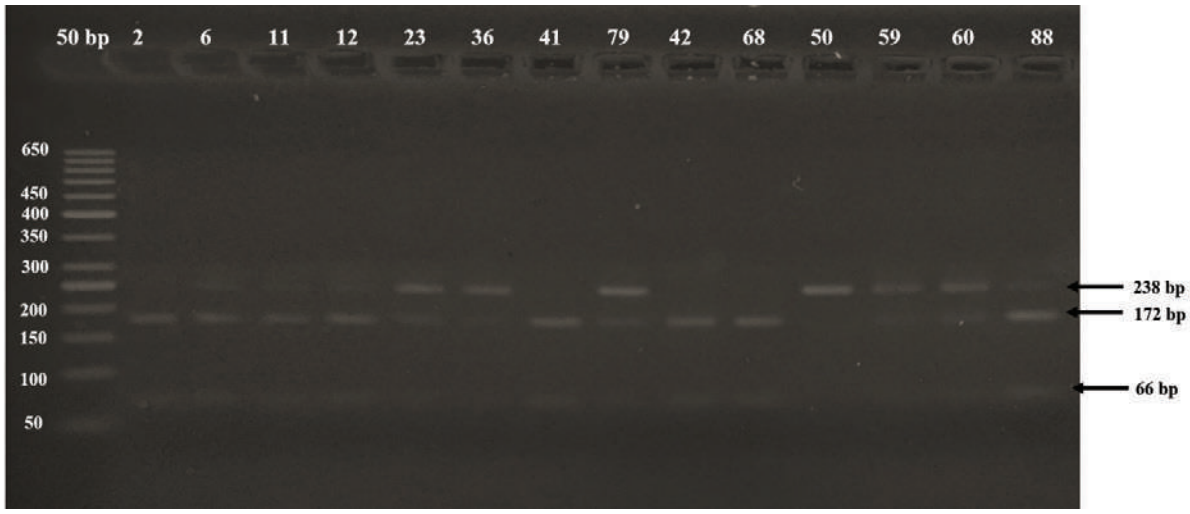


Figure 1: DNA fragment separation. First Lane (from left): DNA ladder; Lane 2, 6, 11, 12, 41, 42, 68 and 88: 66, 172-bp for RR genotype; Lane 23, 36 and 79: 66, 172, 238-bp for QR genotype; Lane 23, 36 and 79: 238-bp for wild type (QQ genotype).

Conclusion

PON1 Q192R polymorphism may useful biomarker for chronic pesticide exposure in Thai rice farmer and corresponded to decline of SChE; and RR genotype was high risk group.

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Effect of Oral Contraceptive Pills on Levels of Calcium and Vitamin D in women in Al-Najaf Province

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Abstract

Background: Combined oral contraceptives comprise of the steroid hormone oestrogen in combination with a progesterone, occupied principally to prevent pregnancy. Combined oral contraceptive pills commonly refer to pills in which an oestrogen and a progestogen are given concurrently in a monthly cycle. **Materials and methods**: The current study was undertaken over a period of six months from 2019/10/1 till 2020/3/1 in Al-Najaf province. Thirty women that take oral contraceptives pill participated in the study, Their ages ranged from (20-38) years divided into three groups(10/group) according to the ages (20-25, 26-31,32 -38) years. **Results:** Results of this study revealed, oral contraceptives pill cause no effect on serum levels of calcium in women that intake it at different age groups in compare with controls groups, and also causes a significant decrease in serum levels of vitamin D in women that intake it at different age groups in compare with controls groups. On the other hand, the results obtained there aren't apparent difference in these parameters when compared between age groups and group that use it for long period (3,6 and 9 year).

Conclusion: The present study concluded that oral contraceptives pill cause no effect on serum levels of calcium in women and little effect on vitamin D in women.

Keyword: *oral contraceptives pill, parameters, calcium and vitamin D.*

Introduction

Combined oral contraceptives involve of the steroid hormone oestrogen in mixture with a progesterone, taken mainly to avert pregnancy. Combined oral contraceptive pills commonly refer to pills in which an oestrogen and a progestogen are given parallel in a monthly cycle. Combined oral contraceptives (COCs) consume become a prevalent method of birth control because to their contraceptive effectiveness and good tolerability profile [1]. Combined oral contraceptives are so typically administered as a pill containing oestrogen and progestogen, which is taken daily for 20–22 days, followed by a seven-day pill-free interval (or seven days of placebo), during which time a withdrawal bleed is expected to happen. These pills comprise hormones that turn on the reproductive system of female leading to avert pregnancy such as estrogen and progesterone. Oral contraceptives are the combination of estrogen and progestin or only progestin. Throughout the years, oral contraceptives have created through progressively decreasing the portion of ethinylestradiol (EE) and presenting 17-β estradiol, and different ages of progestin [2]. There are numerous kinds of estrogen and progesterone being utilized in pills like mestranol is a class of estrogen, and the 3-methylether of EE and norethynodrel is a sort of progestin [3]. In clinical investigations, the efficiency of contraceptive was excellent, but this drug caused many side effects for example, nausea, dizziness, headaches, stomachaches, and vomiting [4]. However, the death of a female had been reported who was taking the contraceptive pills in 1961. Although after so many years, oral contraceptives have developed by decreasing the dose of estrogen and by discovering a new generation of progestins, and additional routes of combined oral contraceptives administration have been developed [5]. The progesterone is primarily responsible for preventing pregnancy. The main mechanism of action is the

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prevention of ovulation; they inhibit follicular development and prevent ovulation [6]. Progestogen negative feedback works at the hypothalamus to decreases the pulse frequency of gonadotropin releasing hormone [7].

Materials and Methods

No.	Instrument	Material
1	Centrifuges	Calcium Kit
2	ELISA Reader	vitaminD Kit
3	Spectrophotometer /UV	
4	Gel tube	
5	Micropipette	
6	Serum tube	
7	Sterile syringes (5ml)	
8	Eppendorf tube	

The current study was undertaken over a period of six months from 2019/10/1 till 2020/3/1 from women using oral contraceptive pill in Al-Najaf province. Thirty women that take oral contraceptives pill participated in the study, Their ages ranged from (20-38) years divided into three groups(10/group) according to the ages (20-25, 26-31,32 -38) years. women with anther diseases which may cause alteration in level of calcium and vitamin D were excluded from the study. About 5 ml of venous blood were taken from women and controls. Serum was separated by centrifugation and used for estimation level of calcium and vitamin.

Results:

3-1: Effect of contraceptive pill on calcium levels in serum

The results display no significant effect ($p< 0.05$) on calcium level in serum in the women at different age groups (20-25y ,26-31y and 32-38y) after intake oral contraceptive pill in compared with control group at different age groups (20-25y ,26-31y and 32-38y) respectively figure (1) and results also display no significant effect ($p< 0.05$) on calcium level in serum in the women between groups of ages.

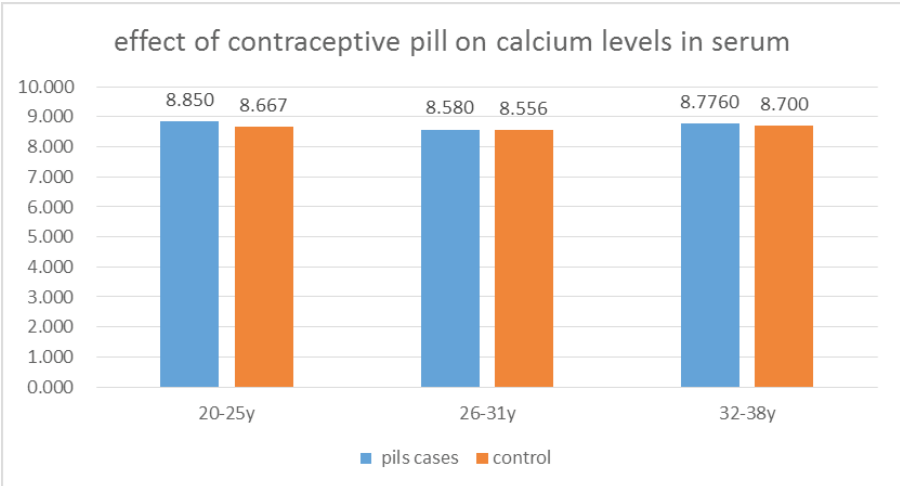
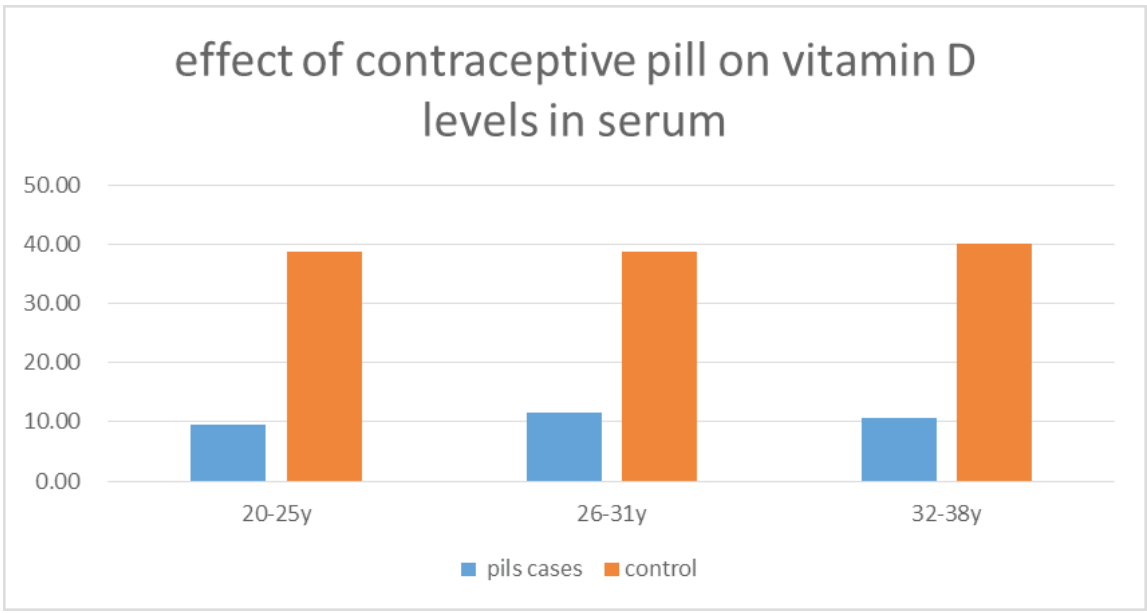


Figure (1): Effect of contraceptive pill on calcium levels in serum

3-2: Effect of contraceptive pill on vitamin D levels in serum

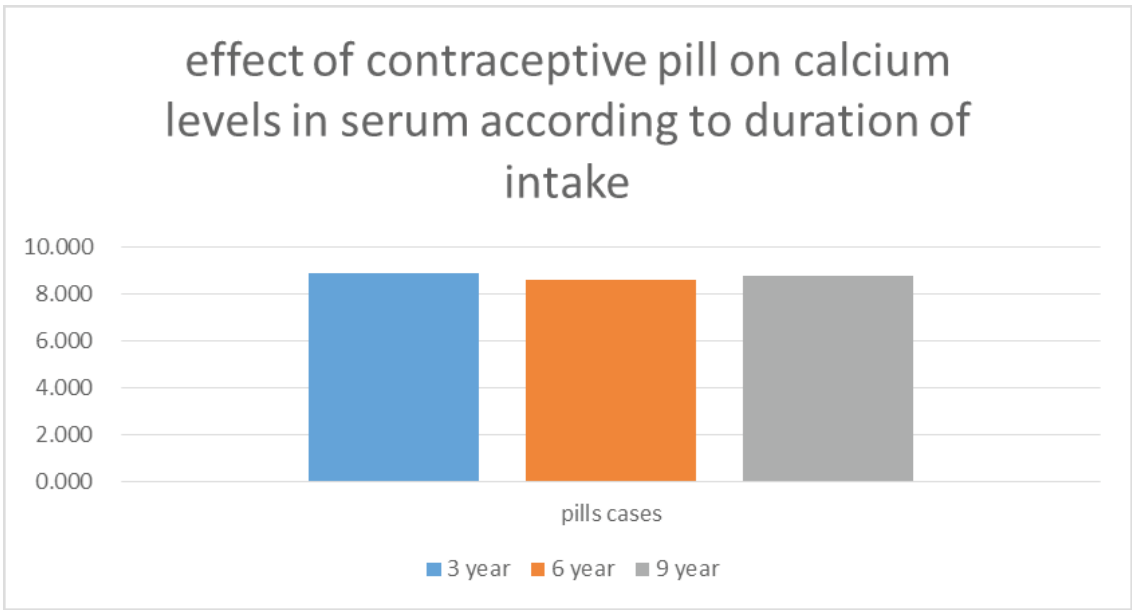
The results display significant decrease ($p > 0.05$) in vitamin D level in serum in the women at different age groups (20-25y ,26-31y and 32-38y) after intake oral contraceptive pill in compared with control groups at different age groups (20-25y ,26-31y and 32-38y) respectively figure (2) and results also display no significant effect ($p < 0.05$) on vitamin D level in serum in the women between groups of ages figure (2).



Figure(2): Effect of contraceptive pill on vitamin D levels in serum

3-3: Effect of contraceptive pill on calcium levels in serum according to duration of intake

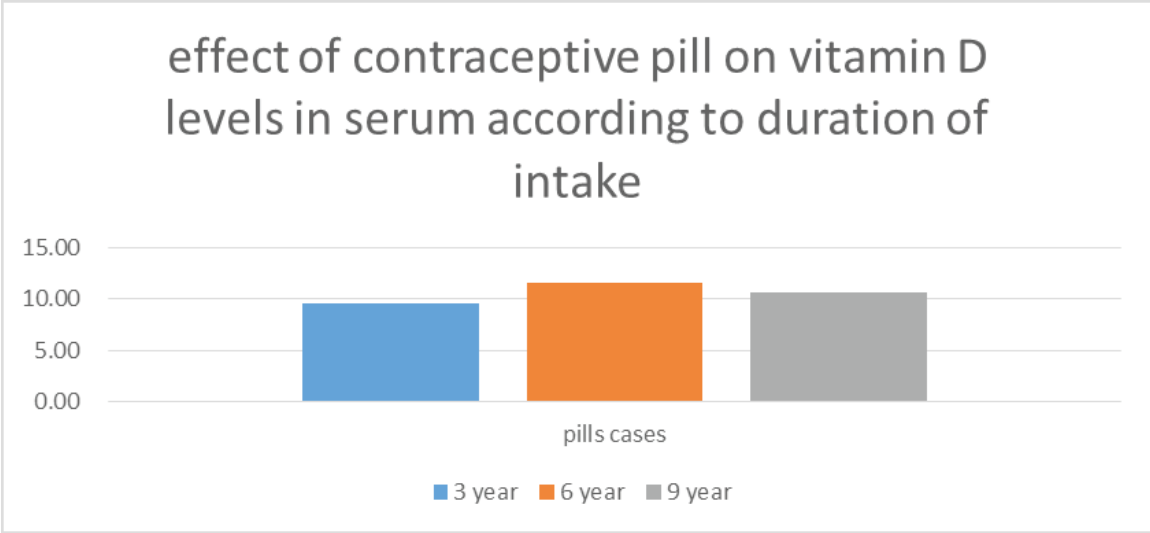
The results display no significant effect ($p < 0.05$) on calcium level in serum in the women at different duration of intake of oral contraceptive pill groups (3 y ,6 y and 9 y) figure (3).



Figure(3): Effect of contraceptive pill on calcium levels in serum according to duration of intake.

3-4: Effect of contraceptive pill on vitamin D levels in serum according to duration of intake

The results display no significant effect ($p< 0.05$) on vitamin D level in serum in the women at different duration of intake of oral contraceptive pill groups (3 y ,6 y and 9 y) figure (4).



Figure(4): Effect of contraceptive pill on vitamin D levels in serum according to duration of intake

Discussion

4-1: Effect of contraceptive pill on calcium levels in serum

The results of the present study displayed no significant effect ($p<0.05$) on calcium levels in serum in women that intake contraceptive pills at different age groups in compared with control group at different age groups, also this study indicate no significant effect ($p<0.05$) between the groups of ages of women that intake contraceptive pills.

4-2: Effect of contraceptive pill on vitamin D levels in serum

The results of the present study displayed significant decrease ($p>0.05$) on vitamin D levels in serum in women that intake contraceptive pills at different age groups in compared with control group at different age groups, this study disagree with many studies that indicate increase in level of vitamin D levels in serum in women that intake contraceptive pills such as the study of [8] this study result is that the use of contraceptive pill increases the circulating levels of 25(OH)D in premenopausal, healthy, adult women and the study of [9] this study result is contraceptive pill use increases circulating levels of 25OHD in plasma. There are many other studies agree with present study such as the study of [10]. This study seems to be the first prospective trial revealing the effect of contraceptive pills use on serum 25(OH)D levels in women with Polycystic ovary syndrome. show the decrease in serum 25(OH)D levels in patients with Polycystic ovary syndrome with the use of contraceptive pills alone. Vitamin D deficiency, which arises due to insufficient exposure to sunlight and dermal synthesis from 7-dehydrocholesterol and limited intake from food and supplements, disrupts the function of all systems of the body and increases the risk of osteoporosis, cancer, cardiovascular disease, autoimmune disease, and mental disorders such as depression and chronic pain syndrome [11], [12] also this study indicate no effect when compare between the groups of ages of women that intake contraceptive pills.

4-3: Effect of contraceptive pill on calcium levels in serum according to duration of intake

The results display no effect on calcium level in serum in the women at different duration of intake of oral contraceptive pill groups when compare between groups.

4-4: Effect of contraceptive pill on vitamin D levels in serum according to duration of intake

The results display no effect on vitamin D level in serum in the women at different duration of intake of oral contraceptive pill groups when compare between groups.

Conclusions

The present study concluded that oral contraceptives pill cause no effect on serum levels of calcium in women and little effect on vitamin D in women.

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Conflict of Interest: None to declare.

Ethical Clearance: All data was approved and carried out in accordance with approved guidelines.

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Clinical study on the therapeutic effects of *Quercus infectoria* galls as oral powder in gingivitis and plaque patients

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Abstract

Gingivitis is a nondestructive disease that causes inflammation of the gums and is one of the most common periodontal diseases. The cause of inflammation is bacterial bovillomatosis, known as the plaque, on the surface of the teeth. The plaque is the main cause of periodontal disease; if left untreated, gingivitis can develop into inflammation of the tooth, where inflammation of the gums destroys the bones, causes tissue erosion around the teeth and can lead to loss of tooth. *Q. infectoria* were applied topically to ten people aged 25-55 who were suffering from gingivitis and plaque. Diagnoses and follow-up treatment were performed in a dental clinic under the supervision of a dentist for a period of two weeks. We noted the great efficacy of the herb against gingivitis and plaque. The progress of plaque measured in terms of mean, population standard deviation and variance were 0.66 , 0.17, 0.02 before treatment and 0.29, 0.12, 0.01 after treatment. Gingival progress before and after treatment were 0.72 , 0.15 , 0.02 and 0.32 , 0.11 , 0.01 , respectively. According to results were due to the fact that Q. infectoria contain large amounts of tannin and other compounds that constrict vessels and tissues and are effective against various types of infections. Therefore, we recommend them as an alternative to antibiotics. Effectiveness of Q. infectoria as an effective substance to inhibit the growth of oral bacteria and thus act as an anti-inflammatory and retention of gum tissue where the substance was tested directly on patients have Plaque and gingivitis and we can recommend them as an alternative to antibiotics

Keywords: *Q. infectoria, gingivitis, plaque, tannin, bacterial.*

Introduction

Gingivitis is a non-destructive disease which occurs around the teeth. The most common form of gingivitis and periodontal disease, overall, occur in response to bacterial biofilms that are attached to tooth surfaces and are termed plaque-induced gingivitis[1].

While some cases of gingivitis never progress to periodontitis, data indicates that periodontitis is always preceded by gingivitis[2].

Gingivitis is reversible with good oral hygiene; however, if left untreated, gingivitis can progress to periodontitis, in which the inflammation of the gums results in tissue destruction and bone resorption around the teeth. Periodontitis can ultimately lead to loss of teeth. The term means "inflammation of the gum tissue"[3].

Antimicrobial agents are vastly used today, which can result in changes in mouth microbiota and cause teeth staining by chlorhexidine mouth rinse[4]. Moreover, oral bacteria have been shown to increase resistance towards most antibiotics such as penicillin, tetracycline, erythromycin, cephalosporin, and metronidazole which are used therapeutically to treat oral infection [5]. The resistance to most antibiotics has led researchers to

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search for anti-infective herbal compounds that can be used to effectively treat oral diseases [6]. Most people in developing countries nowadays use traditional medicines for their health care and recovery [7]. Medicinal plants produce biologically active compounds and this is common in most compounds extracted from plants. Q. infectoria is one of the most widely used traditional medicines in Asia found in Cyprus, Syria, Turkey and Greece.

Q. infectoria is a small tree with a height of about 2 meters [8,9]. The main constituents of the galls are gallic acid (2–4%), Gallo tannic acid (50–70%), ellagic acid, starch, and sugar [10]. Galls in traditional Malaysian medicine have been used to stimulate vaginal muscle contraction as well as to restore uterine flexibility after birth [11] . while in traditional Indian medicine it was used as a toothpaste to treat gum disease and oral cavity [12]. It has also been used to treat internal bleeding, impetigo, gonorrhea, diarrhea, tonsillitis and menstruation. It also possesses antibacterial, antiviral, pesticidal, fungal, and anti-inflammatory properties[13,14].

Materials and Methods

Plant Materials

· The galls of *Q. infectoria* were purchased from the market and used as plant materials for this study

· The specimen was diagnosed and identified by a teacher specialized in plant classification science.

· The product was crushed to small pieces using a sterile pestle and mortar and powdered in an electric grinder.

· The powder was put in plastic containers .

· Patients were diagnosed by a specialist dentist in the private clinic and the cans were then delivered to each of them.

· The topical powder was used by the patient twice a day, in the morning and before bedtime.

· The patients were reviewed at the doctor's clinic after two weeks of using the products and the results were recorded by a personal physician.

The LD50 was determined using the classical LD50 method of Behrens and Karbers (1953). No side effects have been reported for using this product in the various studies that have worked.

Results

To the best of our knowledge, this is first clinical study that showed the effect of galls of *Q. infectoria* extracts on people who have both gingivitis and plaque.

Ten people (nine women and one man) participated in this study. Participants were aged 25 to 55 years, and all of them had gingivitis and plaque (Table 1 and 2). All patients used equal quantities of galls of *Q. infectoria* extracts as powder for two weeks. The progress of plaque in terms of mean, population standard deviation and variance measured before and after treatment were 0.66 , 0.17, 0.02 and 0.29, 0.12, 0.01, respectively (Figure1). On the other hand, the progress of gingivitis before and after treatment were 0.72 , 0.15 , 0.02 and 0.32 , 0.11 , 0.01 for mean, population standard deviation and variance, respectively (Figure 2).

All observation and diagnosis took place in a private dental clinic under the supervision of a specialist dentist according to the standards of Plaque Index (Silness & Loe, 1964) and Gingival Index (Silness &Loe, 1963).

Table 1. Comparison of group of plaque patients before and after treatment with *Q. infectoria*.

No.	Age	Gender	Before	After
1	25	Female	0.75	0.26
2	28	Female	0.59	0.28
3	30	Female	0.57	0.2
4	32	Female	0.29	0.16

Cont... **Table 1. Comparison of group of plaque patients before and after treatment with *Q. infectoria*.**

5	55	Female	0.9	0.1
6	26	Female	0.5	0.3
7	33	Female	0.8	0.5
8	44	Female	0.73	0.25
9	29	Female	0.66	0.5
10	34	Female	0.84	0.36

Table 2. Comparison of group of gingivitis patients before and after treatment with *Q. infectoria*

No.	Age	Gender	Before	After
1	31	Female	0.8	0.3
2	33	Male	0.9	0.3
3	40	Female	0.76	0.37
4	44	Female	0.93	0.1
5	52	Female	0.8	0.29
6	32	Female	0.56	0.32
7	45	Female	0.6	0.4
8	36	Female	0.7	0.5
9	29	Female	0.39	0.22
10	25	Female	0.83	0.49

Discussion

High amounts of hydrolysable tannin present in the galls of *Q. infectoria* imply that tannin may be the active compound responsible for the antibacterial activity in this study. Tannins in the galls were reported to possess antibacterial property against common pathogens such as *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Bacillus cereus*[15,16]. A number of mechanisms have been proposed to explain the antibacterial activity shown by tannin, including complex formation between tannin and microbial enzymes (such as cellulase) as well as membrane of microorganism due to the astringent properties of tannin, iron deprivation through precipitation and effect on bacterial metabolism through inhibition of oxidative phosphorylation [17]. Several studies have shown that galls of *Q. infectoria* possess manifold therapeutic activities, with particular efficacy against inflammatory diseases . These results suggest that alcoholic extract of galls of *Q. infectoria* exerted in-vivo have anti-inflammatory activity after oral or topical administration and also have the ability to curb the production of some inflammatory mediators[18].

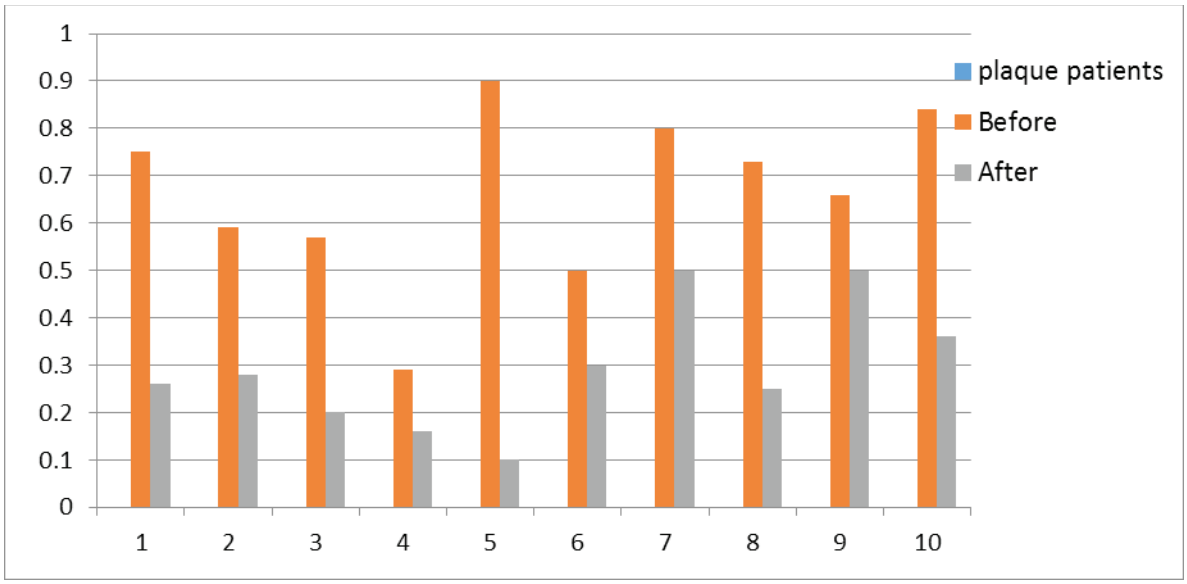


Figure 1. Comparison of group of plaque patients before and after treatment with *Q. infectoria* .

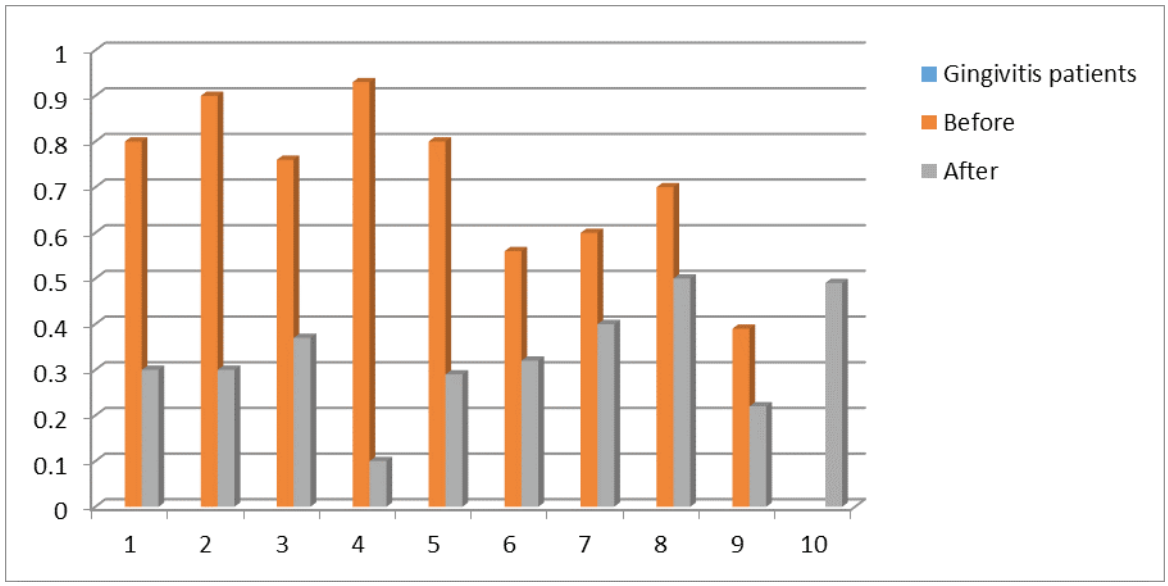


Figure 2. Comparison of group of gingivitis patients before and after treatment with *Q. infectoria.*

Conclusion

The results of this study proved the effectiveness of *Q. infectoria* as an effective substance for inhibiting the growth of oral bacteria. *Q. infectoria* acted as an anti-inflammatory agent and improved retention of gum tissue when the substance was tested directly on patients diagnosed with plaque and gingivitis, under the supervision of a dental specialist.

The results of present study support the traditional usage of *Q. infectoria* and plant extracts which possess compounds with antibacterial properties.

Therefore, we recommend *Q. infectoria* as an alternative to antibiotics. Also, we recommend additional studies on the use of this product on a larger number of patients.

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Funding Information: This project did not receive any external funding.

Compliance with ethical standards

Conflict of Interest: The author declares no conflict of interest.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional Iraqi health standards.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

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Effectiveness of Nursing Management Educational Program upon Head Nurses Knowledge Regarding Patient Management

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Abstract

Background: Over the past few decades, the role of the Director of the Nursing Unit (head nurse) has developed considerably globally, from the head nurse who supervisor to patient care and nursing staff and the needs of the ward from all the necessary supplies for nursing care in the ward to the role of the manager nurse who plays a broader role through his oversight of all Nursing ward in the hospital, staff employment and ward quality, development and retention. Previously the head nurses were responsible for the care of clinical patients only in the ward, and their duty was only patient. **Objectives:** Implement the nursing management education program for the study group. **Methodology:** A quasi-experimental design is carried out throughout the present study with the application of test-retest approach for the study and control groups from March 24th 2019 to Feb 19th 2020. **Settings of the Study**: This study was conducted at the nursing units of Al-Najaf AL-Ashraf City Hospitals which include (Al-Sadr Medical City, Al-Hakeem General Hospital and Al-Zahra Teaching Hospital, they represented AL-Najaf AL-Ashraf city). **Result:** showed that the study group participants had benefits from the implementation of nursing management education program **Conclusions**: The program considered an effective mean for the improvement of the head nurse's knowledge. **Recommendations:** Head nurses should have the required knowledge about wards management regarding patient care. And nursing management education program is an important tool for the development of head nurses' skills. Accordingly, head nurses need to be encouraged to participate in nursing management education program focused on patient management. Finally, the study proved that there is an essential need for collaboration between the Ministry of Higher Education and Scientific Research with Ministry of Health and Environment to improve the nursing management courses as a part of curriculum in nursing institutes and universities.

Keyword: *Effectiveness, Nursing, Management Educational Program, Head Nurses, knowledge, Patient Management*

Introduction

The first-line nursing manager, also called a head nurse, is a person who is responsible for managing nursing services activities in one nursing unit and who is a key individual in the nursing department administration structure; this nurse who translates the goals and objectives of the department into action. It is at this level of management that patient unit costs may be investigated and controlled; while maintaining a careful eye on the quality of care delivered, thus combining clinical expertise with managerial competence.[1]

Methodology

Design of the Study:

A quasi-experimental design is carried out throughout the present study with the application of test-retest approach for the study and control groups from March 24th 2019 to Feb 19th 2020.

Settings of the Study: This study was conducted at the nursing units of Al-Najaf AL-Ashraf City Hospitals which include (Al-Sadr Medical City, Al-Hakeem General Hospital and Al-Zahra Teaching Hospital, they represented AL-Najaf AL-Ashraf city).

Results

Table (1) Changes in Mean Scores of knowledge about Tasks of Patient Care Management Pre to Post

Training

Changes in Overall Mean Score	Study group (n = 30)		Control group (n = 30)		Effect size	P. value
	Mean	SD	Mean	SD		
Pre-test	2.46	0.32	2.48	0.31	-	0.636
Posttest 1	2.89	0.23	2.46	0.30	1.62	< 0.001
Posttest 2	2.82	0.28	2.45	0.30	1.28	< 0.001
Mean difference Post 1-Pre	0.43	0.16	-0.02	0.10	3.42	<0.001
Mean difference Post 2-Pre	0.36	0.14	-0.03	0.12	3.00	<0.001
Mean difference Post2-Post1	-0.07	0.22	-0.01	0.14	0.33	0.001
Multiple comparison (pairwise) within groups						
Post 1 vs. Pre	<0.001		0.438			
Post 2 vs. Pre	<0.001		0.349			
Post 2 vs. Post 1	0.001		0.326			

Indicated that the overall mean knowledge score for this study at posttest 1 was significantly higher than that at pre training and at posttest 2, and that of posttest 2 was significantly higher than that at pre training, (Table 1). No such changes had been reported in control group and the differences in mean knowledge score were not significant at pre, post1 and post 2. On the other hand when both groups compared the effect size of training program was large (1.62), in the change of mean knowledge score for this study at posttest 1, large (1.28) at posttest 2, and cumulatively much large effect size of 3.42 and 3.0 when the mean differences post1-pre and post2-pre taken into account, but small effect size of 0.33 reported at post1-post2vbut it still significant in study group than controls. Moreover, the trends of changes in mean knowledge score in both groups are graphically compared in (Figure 1)

Discussion of the Results

The primary function of a head nurse is to ensure optimal patient outcomes on hospital unit. A good head nurses' wears several hats as an expert clinician, a mentor and support for his/her personals and a tireless advocate for patients and families. Head nurses are important in promoting quality outcome and patients safety satisfaction. Patients nursing care delivered by reviewing and nursing management education program to providing direction of the nursing staff for development and growth nursing management services and supervise for quality of nursing care outcomes in nursing ward management in hospitals.

Table (1), study indicates that the overall mean of patient care management knowledge score for this domain at post-test (1) was significantly higher than that

at pre training and at post-test (2), and that of posttest (2) was significantly higher than that at pre training.

This means that head nurses acquired on high improvement in patient care management knowledge by education program compared to their knowledge at pretest.

A study by Halema, (2016) reported that the implementation of the clinical supervision training program has positive effect on patient safety and nursing documentation.[2]

And another study ElZeneny, (2017), who mentioned that after program, the head nurses gain aspects such as lifelong learning, development, solve problems, quality nursing care and patient safety.[3]

As well Abo Gad & El-Demerdash, (2014) and Dutta, (2013), reported that there was a significant improvement in the head nurses' nursing management knowledge regarding all items of nursing management educational program content in posttest and posttest 2. [4,5]

Conclusions

Much improvement in the knowledge of study group was detected after implementation of teaching program (post-test1) regarding patient management; therefore, highly significant differences were reported between study group and controls in the proportions of correct responses towards the items of domain with a large effect size.

Recommendations:

1. Head nurses should have the required knowledge about wards management regarding patient care.

2. Nursing management education program is an important tool for the development of head nurses' skills. Accordingly head nurses need to be encouraged to participate in nursing management education program focused on wards management.

3. The study recommends focusing on scientifically qualified nurses with minimum of BSc degree to work in the nursing wards.

4. The study proved that there is an essential need for collaboration between the Ministry of Higher Education and Scientific Research with Ministry of Health and Environment to improve the nursing management courses as a part of curriculum in nursing institutes and universities.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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The Willingness of Dental Medicine Students in Handling Positive HIV Patients

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Abstract

Background: Dental care for HIV positive people plays an important role in increasing quality of life, tolerance and effectiveness of treatment, nutritional intake, and treatment success rates. Therefore, it is important to ensure optimal dental care for this group of people, especially since many of them, with or without knowledge of their serological status, are now using dental services. **Purpose**: is to assess the willingness of dental students to treat HIV positive people. **Materials and Methods**: A descriptive cross-sectional survey was conducted. In the city of Surabaya, there are around 184,000 HIV positive people. This research was conducted in 2019 using a questionnaire designed to obtain information about student demographics, self-assessment of HIV, attitudes toward infection control practices, perceptions of occupational risks, and willingness to provide care for HIV positive people. **Results:** The response rate is 76.3 percent. More than 77 percent of respondents are in the age group of 19 years to age 25 years. Good knowledge about HIV is only owned by 35 percent of respondents. While around 7.1 percent, showed significant concern about job transmission. **Conclusions:** From the results of this study, the authors suggest the need for a comprehensive education or motivation program for the next generation of dentists in Indonesia to ensure adequate care for HIV positive people.

Keywords*: HIV positive patients, Dental Medicine students, willingness, Knowledge of HIV-AIDS in Indonesia*

Introduction

Acquired Immunodeficiency Syndrome (AIDS) is an infection caused by the Human Immunodeficiency Virus (HIV). This virus causes a disease that attacks immune cells. Around the world in 2013, there were 35 million people with HIV which included 3.2 million children aged <15 years and 16 million women. The number of new HIV infections in 2013 was 2.1 million, consisting of 240,000 children aged <15 years and 1.9 million adults. The number of deaths due to AIDS was 1.5 million consisting of 190,000 children aged <15 years and 1.3 million adults. In Indonesia, HIV has spread to 386 cities in all provinces in Indonesia[1]. With the survival rate getting better, HIV positive patients who are knowledgeable and who do not know about their own serological status will increase, and will get dental care in the future will come[2].

Oral health is often a problem among people infected with HIV[3,4]. Opportunistic oral lesions and infections are often one of the first manifestations of HIV. HIV infected patients receive dental care services with standard procedures applied[5]. Oral complications in HIV-AIDS sufferers are reported to be minor, self-limiting, non-life threatening[6] and ready to be treated. Care planning for patients with HIV follows the same sequence as other patients[7–9]. However, the priority is to reduce pain, restore function[5], prevent further

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disease and consider the aesthetic results that are done well by the dentist[10,11].

The purpose of this research is to assess the readiness of Universitas Airlangga, Faculty of Dental Medicine students in the 5th and 7th semester in handling patients who have a history of HIV and AIDS. This research is specifically for students in 5th and 7th semester, because they are considered to have understood the things that must be done and should not be violated in the oral cavity treatment procedure for people with HIV-AIDS.

Material and Method

This research is specifically for students in the 5th and 7th semester, because they are considered to have understood the things that must be done and should not be violated in the oral cavity treatment procedures of HIV-AIDS sufferers.

Data were collected using a self-administered questionnaire. This questionnaire includes information on demographics, self-assessed knowledge of HIV/AIDS, attitudes toward homosexuals, infection control practices, perceptions of occupational risks, and willingness to treat HIV patients. Self-reported HIV knowledge is categorized as poor, fair, good and very good. On the variable "Attitudes towards homosexuals", l was assessed using a five-point Likert scale as being very sympathetic, sympathetic, doubtful, unsympathetic, and very unsympathetic, but for the purpose of analysis this category collapses into sympathetic, disinterested, and unsympathetic.

The research design used in this study was a cohort analytic study, that is we conducted a study by analyzing the results of the questionnaire at the present time. The population in this study were preclinical students of the Faculty of Dental Medicine, Universitas Airlangga. In this study the sample used was preclinical students of the Faculty of Dental Medicine, Universitas Airlangga, in the 5th and 7th semester, with the consideration that the students had received

Sampling was taken randomly according to the sum of the results of the calculation of the correlative analytic research sample, provided that half of the amount was taken from the fifth semester preclinical students and the remaining half was taken from the seventh semester preclinic students. The location of the study was held in the faculty of Dental Medicine at Universitas Airlangga. There are two variables in this study, namely free and bound variables. The independent variable is Faculty of Dental Medicine, Universitas Airlangga preclinical student's knowledge of HIV (AIDS). While the dependent variable is the attitude of Faculty of Dental Medicine, Universitas Airlangga students in dealing with HIV AIDS patients.

Results

The results of a survey of research that has been conducted show the response of 56 respondents consisting of Faculty of Dental Medicine students from the 2016 and 2017. Batches on the variable "knowledge of HIV", as many as 50% stated that the use of gloves or handscoon is very important in dental health practices to treat patients HIV positive to prevent HIV cross infection. While 7.1% of respondents still think the use of handscoons is not too important.

The majority of students, as many as 71.4%, stated that they are willing to do dental care for patients who are HIV positive. Only a small portion, 28.5%, disagrees with the statement. However, when asked if they would like to deal with HIV positive patients if gloves were not available, only 21.4 respondents stated agreement and the majority of them, 78.6% of respondents disagreed.

Regarding the willingness of respondents to provide dental health care services at the voluntary HIV care center, 14.3% had no problem with this, 28.6 disagreed and the majority of respondents gave a score of 3 namely 57.1%, stating that they were willing to volunteer to provide dental health care at the HIV care center.

In the question "Does HIV positive prevalence influence their choice to choose a practice location", 71.5% stated agreement and the minority of respondents as much as 28.5% did not feel the HIV prevalence had a significant effect in the choice of practice location.

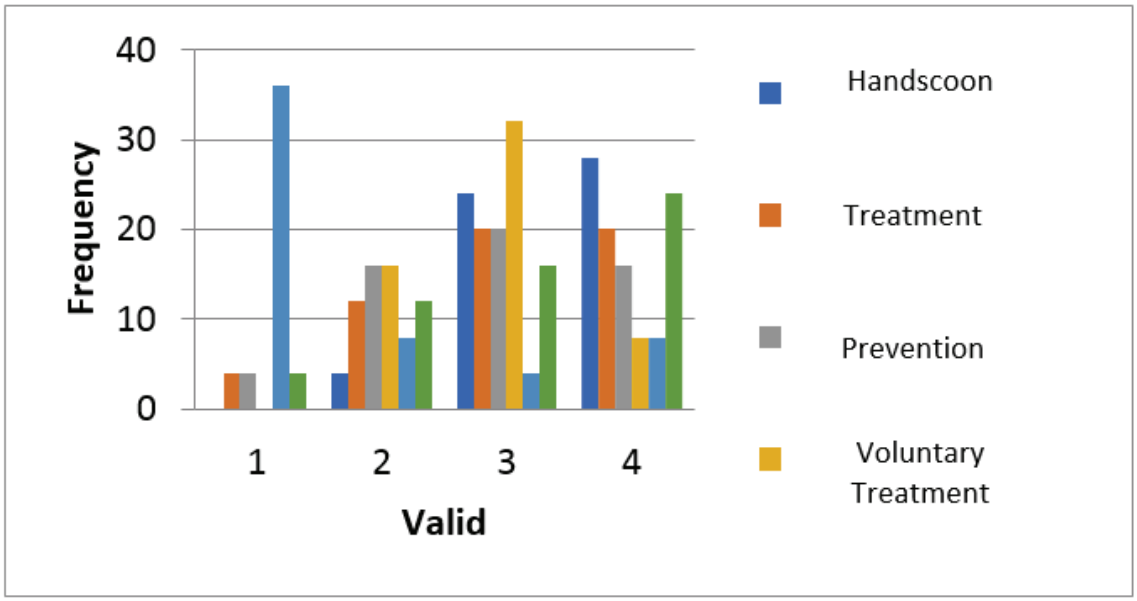


Figure 1. The graph shows the frequency of the number of dental students who are willing to treat HIV patients

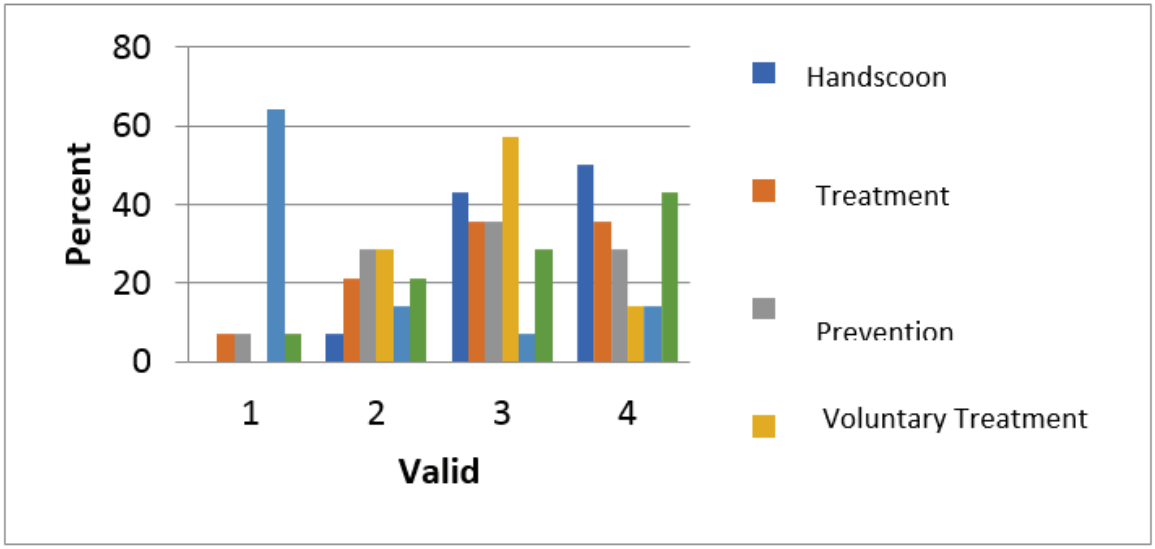


Figure 2. The graph shows the percentage of the number of dental students who are willing to treat HIV patients

Discussion

Oral HIV related conditions occur in the majority of people who are HIV positive, and are often misdiagnosed or inadequately treated[12]. The study assessed the willingness of second and third year dental students to treat HIV positive patients and their attitudes to related problems.

Willingness to treat is considered the most significant predictor of actual care for HIV positive patients. As a result, if HIV positive people feel left out by care givers, they tend to understand the need for prevention and to be motivated. to protect others[13]. Therefore, 50 percent of students are willing to say to do an oral examination and treat patients with gloves and this can help encourage HIV positive patients to look for dental examinations.

Data from this study found that 64,1 percent scored grades 3 and 4 of which more than half of the students agreed that additional payments must be charged to HIV positive patients to avoid further cross-infection. Increased payments are to take safer precautions when treating patients but this can lead to negative results for those who are unable to provide it. Most HIV positive patients have high medical care that helps them control or reduce their risk of getting sick.

In the results of this study stated that as many as 71.4% of students agreed to run dental care for patients who were HIV positive and only 28.5% of students did not agree to take care of it. In this questionnaire can indicate the attitude or action taken by students in dealing with HIV AIDS patients. In the Vijayalaxmi Nimma et al study, the results of students to treat HIV patients were higher at 82.5% compared to our study. Only a small proportion of students need to be educated and prepare themselves to face cases like this that will be experienced in the future[14].

If students are asked if students want to deal with HIV positive patients without gloves available, the majority of students, as many as 78.6% do not agree to take care and only 21.4% of students are willing to treat HIV positive patients. Student agreement in the case of handling an HIV patient by wearing gloves or cannot state how deep the student's knowledge is related to the condition of an HIV sufferer, the process of transmission and relationships when examining an intraoral patient. More than half of the students are knowledgeable and understand the risks if caring for patients without using protection for self-protection in order to prevent HIV transmission. When the rest is 21.4% students still have less knowledge about self protection. This can be accomplished by preparing students to deal with HIV patients with a program recommended by the university. With this solution a positive impression can be proven according to research by Jason P et al[15].

Questions related to willingness or voluntarily providing dental health care services at HIV care centers were given to respondents, the majority giving a score of 3, which means agreeing. In our opinion, the Faculty of Dental Medicine students of Universitas Airlangga have had volunteerism towards HIV patients, it's just that there are still some who are still not willing. This study has provided some encouragement to influence students' attitudes about HIV disease and raise awareness of confidentiality issues in dental education[16].

The questionnaire given was one of the questions whether the prevalence of HIV positive influences their choice to choose the location of practice, amounting to 71.5% stated and the minority of respondents as much as 28.5%, meaning that the majority of Faculty of Dental Medicine, Universitas Airlangga students were unwilling to open practices in the environment of HIV patients .

The professional obligation to treat HIV positive patients must be shared by every dentist, but prejudices and views about culture and sexual diversity can hamper student attitudes due to lack of education about the challenges with HIV positive patients[17]. In one study, 76 percent of students feel dentists have a responsibility to care for HIV positive patients, but only 62 percent say they are willing to treat care for HIV sufferers[18], these are the things that are likely to underlie student responses.

Conclusion

In conclusion, 57.1% of Faculty of Dental Medicine, Universitas Airlangga students from 2016 and 2017 stated that they were willing to volunteer to provide dental health care at an HIV care center. This shows that the importance of using handscoons in controlling HIV patients. It remains the responsibility of Faculty of Dental Medicine, Universitas Airlangga students in controlling cases like this despite the high risk but with good control can definitely be saved by these patients.

Conflicts of Interest : None

Source of Funding : Self-Funding

Ethical Clearance: Approved

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Drugs used for Weight Reduction among Iraqi Population Sample, a Comparative Study

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Abstract

Obesity is a complex disease characterized by fat accumulation in the body which led to increased risk of other disease such as diabetic, cancer and others. Body mass index (BMI), is the main criteria to define overweight and obesity. In addition to the change in diet, exercise and life style, pharmacological options were used for complicated cases.

This study is a retrospective one where the data collected were from 117 obese patients in obesity research and therapy unit in AL-Kindy medical college in Iraq and were divided into four groups according to the drugs that were taken (metformin group, metformin and orlistat group, metformin and fluoxetine group, metformin, orlistat and fluoxetine group)

It has been found that each group showed a significant difference in BMI after 2 and four months and by comparison among the four group, metformin group was given a significant difference in BMI after 2 months while no significant difference in BMI among groups after 4 months.

In conclusion metformin alone gave the best change in BMI in short period with less side effect while for long time, the administration of these three drugs together may lead to more desirable results

Keywords: *obesity, body mass index, metformin, fluoxetine, orlistat.*

Introduction

Obesity is a complex disease characterized by excessive abnormal accumulation of body fat accumulation resulting from of high intake of high-calorie diet and reduced physical performance [1].

It is a rapidly growing public health issue affecting the developed and the developing countries as well [2]. The prevalence of obesity is generally high [3]. Over the last decades, the prevalence rate of obesity has substantially increased [4]. In 2016, it has been reported around 1.9 billion adults were overweight, and 650 million adults, among them, have obesity [5]. It has been estimated that by 2030, if secular trends continue, 38% of the world's adults will be overweight and 20% will have obesity [6].

Obesity increases morbidity and mortality rates [7]. Obesity has been linked to the increased risk of cancers, stroke, type 2 diabetes mellitus (T2DM), heart failure, and other cardiovascular diseases]4, 8[**.** Obesity generally occurs when energy consumption, dietary intake, exceeds the energy lost through physical and metabolic activity [9]. Many factors may contribute to obesity, including genetic, behavioral, socioeconomic, and environmental factors [10]. Food is the main environmental factor for obesity, while the reduction in physical activity is the second factor to be blamed [11].

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Body mass index (BMI), weight in kg/height in m^2, is the most widely used criteria to define overweight and obesity [12]. A BMI of 25 kg/m^2 or higher is considered overweight, while obesity is defined as a BMI equal to or greater than 30 kg/m^2 [13].

Diet, exercise and changes in lifestyle are the key elements for obesity treatment, but pharmacotherapy and surgery are becoming important [14].

Drugs used to treat obesity include sympathomimetic appetite suppressant drugs, antidiabetic agents, pancreatic lipase inhibitors, serotonin 5-HT2C agonists, anticonvulsant drugs, atypical antidepressants, selective β3 adrenoceptor agonists, and various combination therapies [15].

Among medications, orlistat and metformin are the most studied drugs] 16, 17[. Orlistat, available since 1999 in many countries, is an inhibitor of gastric and pancreatic lipase [12]. It decreases the intestinal absorption of fat, and as a result reducing the calorie intake [18]. It prevents the digestion of about 30% the dietary fat, with considerable decrease in body weight, BMI, total cholesterol, and low density lipoprotein (LDL) cholesterol (9). It is the only approved anti-obesity drug that does not act primarily through appetite suppression, and has a relatively good safety profile on long-term use [19].

Metformin is an oral hypoglycemic agent used for treatment of T2DM [20]. Metformin is frequently used for obesity treatment in adult patients [21]. In general metformin is well-tolerated, and it produces small but significant decrease in body weight] 22, 23[.It has been suggested that metformin induces weight loss through its effect on the regulatory pathways of appetite in the brain, and also through its effects on adipose and gut-mediated signals [20].

Fluoxetine is an oral antidepressant agent and considered to be the prototype of the selective serotonin reuptake inhibitors (SSRIs) [24].

The effects of fluoxetine on reducing caloric intake through altering appetite have been reported in both lean and obese people [25].

The aim of this study is to evaluate different pharmacological options that have been used as an anti-obesity therapy among Iraqi populations and to investigate the therapy with the best outcomes.

Methods

Treated group

This retrospective clinical trial was collected between 2014 and 2017 records from the obesity research and therapy unit in AL-Kindy medical college in Iraq.

117 obese patients (95 women and 22 male) were included in this study and were classified into 4 groups as follows:

Group 1. Metformin (500mg three time a day) (n=35).

Group 2. Metformin and Orlistat (Metformin 500mg three time a day and Orlistat 120mg twice daily) (n=40).

Group 3. Metformin and Fluoxetine (Metformin 500mg three time a day and Fluoxetine 20mg per day) (n=30).

Group 4. Metformin, Orlistat and Fluoxetine (Metformin 500mg three time a day+ Orlistat 120mg twice daily + Fluoxetine 20mg per day) (n=12).

Weight, BMI were assessed at baseline, after 2 months and after 4 months for each group.

The intake of nutrients, total calories, and caffeine was maintained at constant level during the study. Each patient received a personalized food plant from her dietician and do exercises. During the trial, the consumption of dietary supplements was not recommended except some patients who received omega 3 with treatment.

Statistical Analysis

In this study, least significant difference –LSD test (ANOVA analysis) was used to significant compare between BMI means of the different groups after 2 and 4 months while *t* test was used to significant compare between BMI means of the same group after 2 and 4 months.

Data were expressed as mean ± SEM, where P value < 0.05 was considered to be significant and P value < 0.01 was considered to be highly significant.

RESULT

Effect of metformin on body mass index after 2 months and 4 months.

Metformin was tested for its ability to affect BMI after 2 and 4 months, BMI at baseline is (39.02±8), highly significant difference in BMI result was found after 2 months(36.99±7.46) (p= 7.29 E-10) and highly significant difference in BMI result was found after 4 months (35.62±7.40) (p= 4.09 E-10) as figure 1.

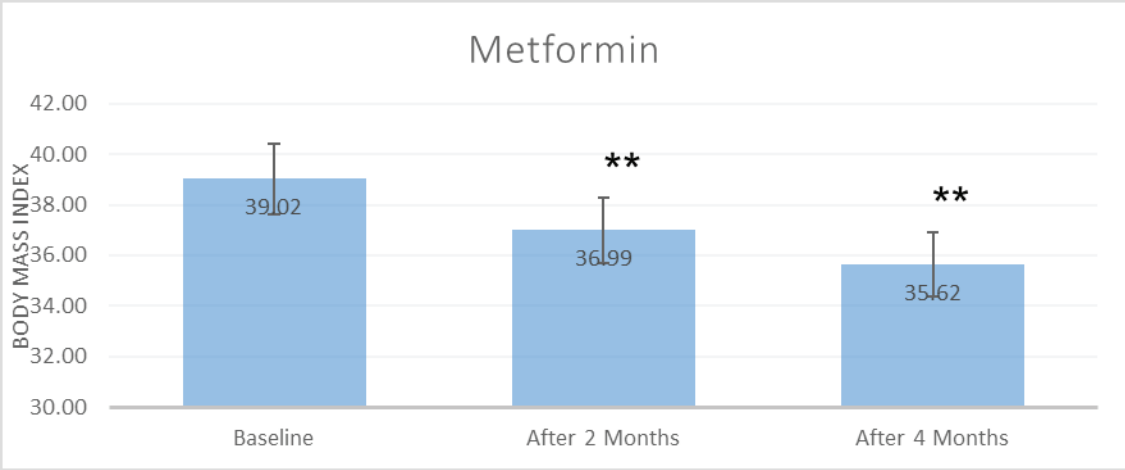


Figure 1: the effect of metformin on BMI value in obese patients after 2 months and after 4 months

Effect of metformin and fluoxetine on body mass index after months and 4 months

Metformin and Fluoxetine were tested for their abilities to affect BMI after 2 and 4 months, BMI at baseline is (41.86±1.99), highly significant difference in BMI result was found after 2 months(40.48±1.90) (p= 2.49 E-22) and highly significant difference in BMI result was found after 4 months (38.50±1.77) (p= 1.58 E-22) as shown in figure 2.

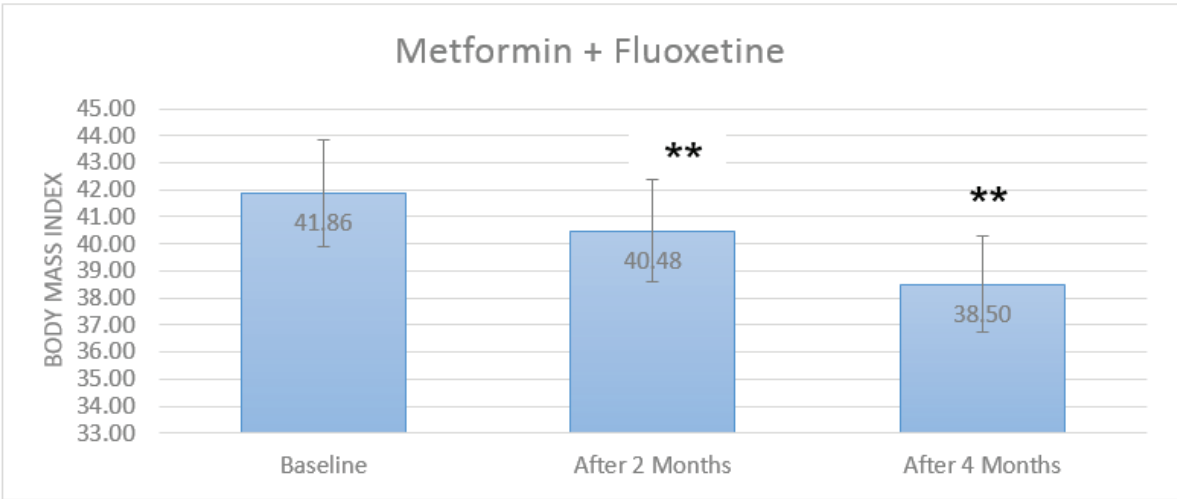


Figure 2: the effect of metformin and fluoxetine on BMI value in obese patients after 2 months and after 4 months

Effect of metformin and orlistat on body mass index after 2 months and 4 months.

Metformin and orlistat were tested for their abilities to affect BMI after 2 and 4 months, BMI at baseline is (39.87±1.84), highly significant difference in BMI result was found after 2 months (38.09±1.78) (p= 4.87 E-11) and

highly significant difference in BMI was found after 4 months (36.75±1.95) (p= 7.99 E-14) as shown in figure 3.

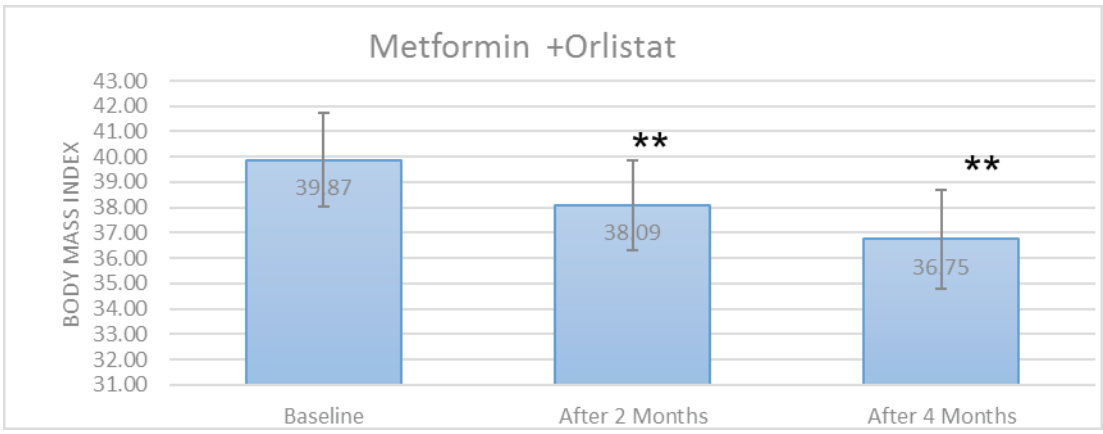


Figure 3: the effect of metformin and orlistat on BMI value in obese patients after 2 months and after 4 months.

Effect of metformin with fluoxetine and orlistat on body mass index after 2 months and 4 months.

Metformin, fluoxetine and orlistat were tested for their abilities to affect BMI after 2 months and after 4 months, BMI at baseline is (36.90±1.35), highly significant difference in BMI result was found after 2 months (35.65 ±1.42) (p= 0.000356), highly significant difference in BMI result was found after 4 months (33.50±1.45) (p= 0.00000569) as shown in figure 4.

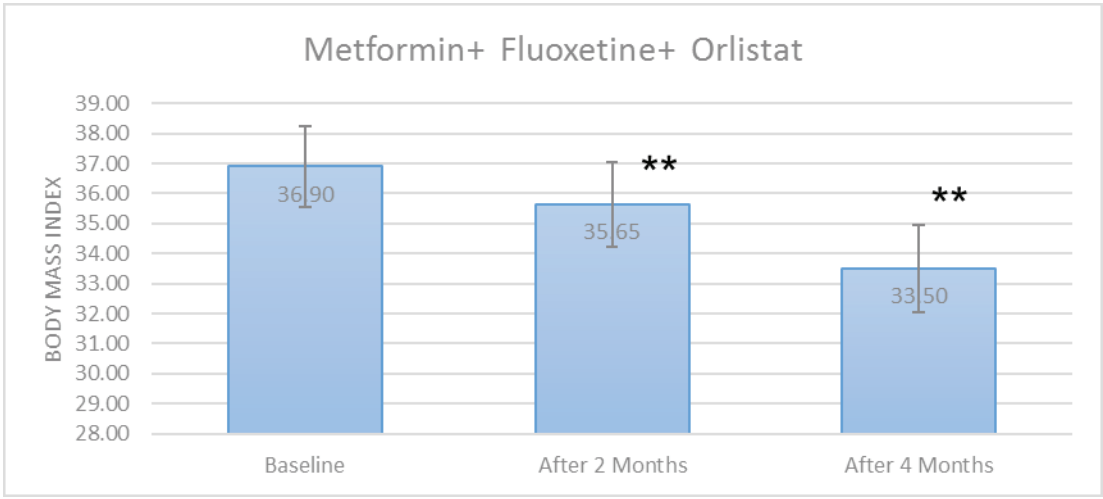


Figure 4: the effect of metformin, fluoxetine and orlistat on BMI value in obese patients after 2 months and after 4 month.

A Comparison among the effect of treated groups on the body mass index after 2 and 4 months

Using ANOVA test, group treated with metformin was showed the most significant percentage change in BMI result after 2 months (5.50±0.67) (p = 0.036) than the other treated groups while after4 months, non-significant difference in BMI among treated groups were found as shown in table 1.

Table 1: The effect of treated groups on the percentage change in body mass index after 2 and 4 months

Group	Metformin	Metformin +Fluoxetine	Metformin + Orlistat	Metformin +Fluoxetine+ Orlistat
% change in BMI (kg/m2) after 2 months (Mean±SED)	5.50 ± 0.67 *	3.28 ± 0.38	4.31 ± 0.60	3.49±0.69
% change in BMI (kg/m2) after 4 months (Mean±SED)	7.94 ± 0.79	6.83 ± 0.93	6.89 ± 0.88	9.34 ± 1.14

*significant change of BMI percentage in metformin group after 2 months.

Discussion

In this study we compared the effect of different drugs on weight of Iraqi obese patient's sample in the obesity research and therapy unit in AL-Kindy medical college.

The pharmacological options that were studied include metformin group alone and three combinations, (metformin and fluoxetine) group, (metformin and orlistat) group, and (metformin, orlistat and fluoxetine) group.

The results of BMI in this study for the metformin (500mg twice daily) group showed highly significant difference after 2 months and after 4 months of using metformin alone.

Seifarth et al [26] found a highly significant difference in BMI after use of metformin and this agree with our study results.

Jarskog et al[27], enrolled 148 overweight patients with chronic schizophrenia or schizoaffective were randomly assigned to receive 16 weeks of metformin(1,000 mg) or placebo, group treated with metformin also presented a significant difference in BMI value as in this study.

Significant reduction in BMI were showed in obese women after treatment with metformin which was agree with the results in this study [28].

Other study used [29] the combination metformin (500mg three times daily) and fluoxetine (20 mg once daily) on 203 patients in 6.6 months, their outcome results also revealed a significant difference in BMI and agree with our study results.

The second combination metformin and orlistat results showed that the BMI highly significant decrease after 2 and 4 months of using metformin and orlistat.

Halpern et al [30] used the combination of (orlistat and metformin) for weight reduction in obese women patients, their results revealed that there are no benefit in association in non-insulin resistant women, so their results disagree with this research results, in other hand, Sari et al expected the beneficial effect of the addition of metformin to orlistat therapy on body weight reduction with highly significant value after treatment within 1 and 4 months in obese women. [31]

Our results revealed that after 2 months of study the metformin gave a best level in reducing BMI in comparison with other combinations groups.

After 4 months of study all the options were approximately equal in effectiveness of changing the BMI.

When we studied the side effect profile of the combinations that we used the outcomes revealed that the using of metformin alone had a minimal or no side effect, as most patients that used the combinations that contain fluoxetine suffered from insomnia after 2 months of therapy and all the patients that used combinations contain orlistat suffered from GIT side effects so the Metformin gave better outcomes with less side effect profile than other drugs.

Conclusion

In this study we used different pharmacological options which led to weight reduction in obese patients. All the drugs that we used gave a good therapeutics outcome in weight reduction but the Metformin gave a better outcome than the other combinations after two months of the study. After 4 months all combinations were almost equal in effectiveness but the combination of three drugs (metformin, orlistat and fluoxetine) was the nearest to the significant value. Hence use the metformin alone in a short time management is the best to get better outcomes, minimal side effects and lower charge on the patients and to use the combination of three drugs for longer period of time.

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Nanocurcumin Protective Effect on Lipid Peroxide of Lead Acetate Induced White Rats (*Rattus norvegicus*) Ovarian Granulosa Cells

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Abstract

Objective: This study aims to investigate the protective effect of nanocurcumin on lipid peroxidation of rat ovarian granulosa cells induced by lead acetate (Pb).

Methods : A total of 45 mice were divided into a negative control group (C-) receiving corn oil, a positive control group (C+) receiving corn oil and lead acetate (Pb) 40 mg/kg bw/day, experimental group 1 (E1), experimental group 2 (E2), experimental group 3 (E3) receiving 50, 100 and 200 mg/kg/day nanocurcumin dissolved in corn oil and lead acetate (Pb) 40 mg/kg bw/day. The treatment was given orally and the treatment time was daily at 08.00 AM for 26 days. Ovaries were removed for immunohistochemical analysis of MDA expression.

Result : The positive control group had the highest MDA expression of ovarian granulosa cells (94.2 ± 10.5) and was significantly different ($p < 0.05$) from negative control group (1.5 ± 1.4), experimental group 1 (17.4 ± 10.5), experimental group 2 (11.4 ± 8.5) and experimental group 3 (7.2 ± 4.0).

Conclusion : Nanocurcumin as a protective oxidative stress on ovarian granulosa cells due to exposure to lead acetate, by protecting lipid peroxidation.

Key words : *Lead acetate, nanocurcumin, malondialdehyde (MDA).*

Introduction

Lead acetate (Pb) is a heavy metal compound that pollutes the environment, toxic and interfering various organ functions, including hematopoietic tissue, liver, kidney, brain, and testes[1] and interfering the function of digestive system, nervous system, respiratory system and reproductive system.[2, 3] Lead exposure has been shown to cause toxicity in rat testes[4, 5] and alter normal histology and physiology of the ovaries and uterus of rats.[6] Exposure to lead acetate causes disruption to the body's organs through increased oxidative stress cell by destroying the balance between prooxidants and antioxidant systems, resulting in excessive production of reactive oxygen species (ROS) and depletion of cellular antioxidants.[7] Increased oxidative stress causes lipid peroxidation, the reaction between ROS and lipids in cell membrane system, especially the unsaturated lipids, and producing malondialdehyde (MDA), a cell-damaging toxic material.[8]

Malondialdehyde (MDA) is a secondary product of lipid peroxidation that can be used as a biomarker of

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toxicity in ovaries induced by lead acetate (Pb). Increased MDA level indicates a decrease in antioxidant activity to inhibit the formation of excessive free radicals.[9] The use of natural antioxidants is currently being a concern of researchers to reduce oxidative stress due to exposure to lead acetate.

Turmeric (*Curcuma longa*, Family *Zingiberaceae*) rhizome as a natural antioxidant contains 3 main components: curcumin, demethoxycurcumin and bisdemethoxy-curcuminoids in a ratio of 77: 17: 3. Curcumin (1,7-bis (4-hydroxy-3-methoxyphenyl) -1, 6 heptadiene-3, 5-dione), which is widely contained in the rhizome, has many benefits, especially as an anti-oxidant.[10] Although curcumin has an antioxidant effect, the bioavailability of curcumin is less understood. Nanotechnology is used to overcome problems related to the solubility, stability, and bioavailability of biological curcumin.[11] Nanocurcumin produced from curcumin nanotechnology has stronger potential compared to curcumin.[12] The aim of this study was to investigate the activity of nanocurcumin to decreasing lipid peroxidation in ovarian granulosa cells of white rats (*Rattus norvegicus*) exposed to lead acetate (Pb).

Materials and Methods

Chemicals

Curcumin (Curcuma longa (turmeric) powder, 65%, Product No. : CAS 458-37-7 molecular weight (MW): 368.38 g/mol, product of China), and Lead acetate (Pb) (Product No: CAS 6080 -56-4, molecular weight (MW): 379.33 g/mol. Linear formula: Pb (CH3CO2) 2.3H2O), purchased from Sigma-Aldrich Co, USA.

The making process of nanocurcumin

Nano Nanocurcumin was made at the Physics Laboratory, Universitas Airlangga, Surabaya, Indonesia. The method used was milling using the High Energy Miling (HEM) engine. Curcumin powder from curcuma longa (*turmeric*) rhizome was milled with collision by cubic zircornia balls, with a ratio between curcumin and cubic zirconia balls = 1 : 10. Curcumin and cubic zirconia balls were inserted into the tube and milled in a High Energy Milling (HEM) machine. The milling time was 20 minutes with a setting of 5 minutes milling and 5 minutes rest until the effective amount of processing time reaching 20 minutes outside the rest period.

Nanocurcumin characteristics analysis

Analysis of nanocurcumin size characteristics was performed at the Robotics Laboratory, ITS, Surabaya, Indonesia, using Scanning Electron microscopy (SEM) method. Curcumin morphology before and after milling were compared. Curcumin morphology before milling appeared as irregular plates with a mean diameter of more than 1000 nm. Morphology of curcumin after milling had a more regular crystal shape with a mean diameter of less than 200 nm as shown in Figure 1.

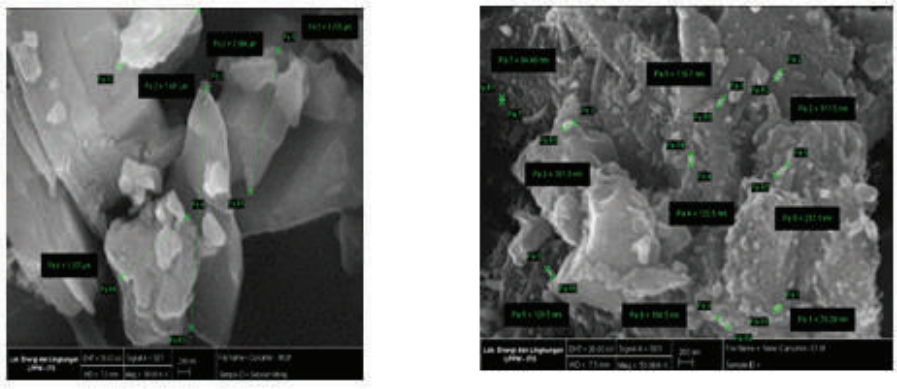


Figure 1 Morphology of nanocurcumin by Scanning Electron microscopy (SEM) examination. (a) Prior to milling with irregular plate, > 1000 nm in diameter. (b) After milling with a more regular crystal shape with a diameter <200 nm.

The making of nanocurcumin solution

Nanocurcumin was dissolved with corn oil, because corn oil is the best carrier compared to butter, milk and water.[13] The solution was made by dissolving 2 grams of nanocurcumin with corn oil to 200 ml, so that 1 ml solution contained 10 mg of nano curcumin.

Experimental animals

Female rats (*Rattus norvegicus*) with a mean body weight of 200-250 gr and an average age of 2.5-3 months were obtained from Institut Teknologi Bandung, Bandung, Indonesia, for experimental purposes. The rats were kept in plastic cages in air-conditioned rooms with temperatures of 26 ± 2^{o} degrees C and maintained with 12 hours of light and 12 hours of darkness. The rats were given with standard commercial food for rats and drinking water was given ad libitum.

Experimental design

A total of 45 rats were divided into negative control group (C-) receiving corn oil and one hour later receiving distilled water, positive control group (C+) receiving corn oil and one hour later receiving lead acetate (Pb) of 40 mg/kg bw/day , experimental group 1 (E1) receiving 50 mg/kg/day nanocurcumin dissolved in corn oil and one hour later receiving lead acetate (Pb) of 40 mg/kg bw/day), experimental group 2 (E2) receiving nanocurcumin 100 mg/kg bw/day dissolved in corn oil and one hour later receiving lead acetate (Pb) of 40 mg/kg bw/day), and experimental group 3 (E3) receiving 200 mg/kg bw/day nanocurcumin dissolved in corn oil and one hour later receiving lead acetate (Pb) of 40 mg/kg bw/day. The treatment was given orally and the treatment time was every day at 08.00 AM for 26 days. On day 27 the rats were sacrificed by dislocation on the nape of the neck. Approximately 2 minutes after dislocation, the rats did not move, the eyes dimmed and the body did not move. Surgery was performed to remove the ovary. The ovaries were cleaned from connective tissue, then washed with 0.9% physiological NaCl and put in Bouin's fixation solution (with saturated picric acid composition : pro-analysis formalin : glacial acetic acid = 15 : 5 : 1) for 24 hours. After the ovaries were fixed, the solution was replaced with 70% alcohol (stopping point) so that the tissue could be stored for a long time.

Immunohistochemical analysis of MDA expression in rats ovarian granulosa cells

The removed ovarian tissues were embedded in paraffin blocks. Next, the slicing was carried out, and representative slices from the sample tissues were selected for immunostaining procedure. Each slice was stained using the streptavidin method by immunoperoxidase. The slicing of serial paraffin block in a thickness of 4 - 6 um was done. The best slices were chosen to calculate the MDA expression at 400X magnification. The presence of MDA was marked by the intensity of dark brown color. Observations were made quantitatively by counting the number of positive cells per visual field, counted up to 10 fields. The number of positive cells in each field was summed and divided by 10, and the final result was the average number of positive cells per visual field.

Statistical Analysis

Data were presented with mean ± standard deviation. Comparison test using Kruskall-Wallis Test was conducted to determine differences between groups, followed by Mann-Whitney test to determine differences between groups.

Results

Kruskal-Wallis test showed MDA differences between groups (Kruskal-Wallis H = 33,697; df = 4; p = 0,000). Then, we performed an analysis using Mann-Whitney test, with results as seen in Table 1.

Table 1. Effects of nanocurcumin on MDA expression of rat ovarian granulosa cells induced by lead acetate (Pb) (mean ± standard deviation)

Groups	n	MDA	Minimum	Maximum
Negative control	9	1.5 ± 1.4a	0	4.4
Positive control	8	94.2 ± 10.5b	40.0	158.6
Experimental 1	9	17.4 ± 10.5c	8.0	37.2
Experimental 2	9	11.4 ± 8.5c	3.0	26.5
Experimental 3	8	7.2 ± 4.0d	2.2	13.0

[a, b, c, d] Different superscript within each column indicates significant difference between the means ($p < 0.05$).

Table 1 shows that the positive control group had the highest MDA expression of ovarian granulosa cells and was significantly different (p <0.05) from negative control group, experimental groups 1, 2 and 3.

These results indicated that the administration of nanocurcumin of 200 mg/kg body weight of rats provided the greatest protection of white rats ovary cell lipid peroxide induced by lead acetate (Pb) of 40 mg/kg bw compared to the administration of 50 mg/kg bw nanocurcumin and 100 mg/kg bw. The administration of nanocurcumin of 50 mg/kg bw was the same as the administration of nanocurcumin of 100 mg/kg bw in lipid peroxide protection of white rat granulosa cells induced by lead acetate (Pb) of 40 mg/kg bw. The difference is presenteed in Figure 2.

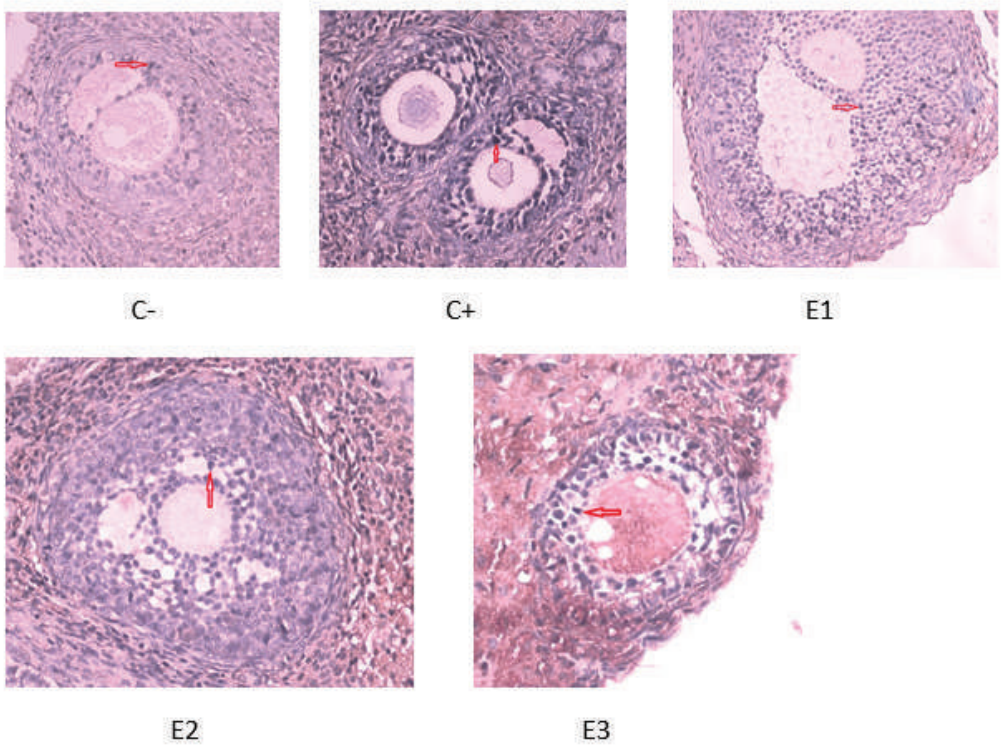


Figure 2. MDA expression of white rats (*Rattus norvegicus*) ovarian granulosa cells. C- (negative control group); C+ (positive control group); E1 (experimental group 1); E2 (experimental group 2); E3 (experimental group 3). Observation used light microscope with 400X magnification.

Figure 2 shows the differences in MDA expression in various groups, MDA expression is in dark brown (examples are pointed with arrows) mostly in C+ group, and the least in C- group.

Discussion

Lead acetate as one of heavy metals has a tendency to catalyze oxidative reactions and cause the formation of ractive oxygen species (ROS).[14] The results of this study indicated that lead acetate (Pb) exposure increased MDA expression of ovarian granulosa cells in white rats (*Rattus norvegicus*) compared to control group. However, the administration of nanocurcumin was significantly able to provide protection against lipid peroxide which was marked by a significant decrease in MDA expression.

If exposure to ROS cannot be offset by anti-oxidant enzymes or scavenger enzymes, where the ratio of ROS is higher than antioxidant enzymes, the cell experiences oxidative stress. Oxidative stress causes a reaction between ROS and lipids in cell membrane system, especially the unsaturated lipids (8). Oxidative stress due to increased ROS will cause lipid peroxidation, DNA damage and also a decrease in antioxidant defense system in the cells.[14]

Lipid peroxidation produces MDA which is a cell-destroying toxic substance.[8] MDA is the final product of lipid peroxidation in lead toxicity that induces oxidative stress.[4] High MDA concentration indicates oxidation process in cell membrane. MDA is also referred to as a highly reactive compound which is the final product of lipid peroxidation and is used as a biological biomarker of lipid peroxidation to assess oxidative stress.[15]

The inhibition of lipid peroxide can be induced by the nanocurcumin through: (1) the process of neutralizing free radicals involved in peroxidation by capturing hydroxy radicals (OH *) produced by Fenton reaction and Haber Weiss reaction[16] and; (2) inhibition of hydroxyl radical (OH *) formation. Lipid peroxidation can occur due to the increase in oxidative stress. Oxidative stress occurs because radical production exceeds the body's ability to neutralize it. This imbalance is present resulting from the decreased production of anti-oxidants or over-production of free radicals.[17]

Nanocurcumin has antioxidant activity because it has a phenolic hydroxyl group that functions as a free radical scavenger by inhibiting lipid peroxidase. Nanocurcumin has two aromatic rings that contain phenolic hydroxy, both of which are connected by short chains conjugated with ß-ketones. Nanocurcumin also has a methoxy group in its aromatic ring which acts as an electron booster so that it makes it easier for the compound to capture hydroxy radicals. In nanocurcumin the dissociation energy of phenolic O-H is 5.04 kcal/mol lower than the dissociation energy of C-H binding in ß-diketone group so that the tendency to donate H atoms from the phenolic group is higher.[16]

The inhibition of nanocurcumin in decreasing the expression of MDA by capturing hydroxy radicals (OH*) is taking place because curcumin donates hydrogen atoms (H) from its phenolic hydroxyl (OH) groups when reacting with free radicals (R*). This reaction will produce a curcumin phenoxyl radicals or flavonoid (KO*/FO*) which is less reactive because curcumin phenoxyl or flavonoid (KO*/FO *) can experience a change in resonance structure by redistributing unpaired electrons in the conjugated double bond structure in its aromatic ring. Phenoxyl curcumin or flavonoids (KO*/FO*) will react further to form unreactive compounds, which possibly takes place through the radical termination reactions.[18]

Another means of the inhibition of nanocurcumin in reducing MDA expression is by inhibiting the formation of hydroxyl radicals (OH*). The inhibition of OH* formation is to prevent Fenton reaction and Haber Weiss reaction by binding the transition metal (Fe++ (Cu+), an OH* catalytic.[19]

In conclusion, this study proved that the administration of nanocurcumin provided a protective effect of lipid peroxidation in ovarian granulosa cell induced by lead acetate (Pb) through free racial neutralization and the inhibition of hydroxyl radical formation. It is necessary to develop nanocurcumin for free radical protection due to lead exposure, so that it can be applied for supplementary therapy for women who have a risk of lead exposure.

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Effectiveness of Educational Program on Nurses-Midwives' Preventive Measures about Puerperal Sepsis at Baghdad Maternity Hospitals

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Abstract

Objective: To evaluate the effectiveness of the educational program on nurses-midwives' preventive measures about puerperal sepsis.

Method: quasi-experimental study design carried among 60 Nurses and Midwives chosen by non-probability sample (purposive sample) at maternity hospitals. The data collected by the observation checklist Pretest and posttest after the implementation of the educational program. Data were analyzed using descriptive statistical data analysis approach of frequency, percentage, mean, mean of scores, a total of scores, range and standard deviation and inferential statistical data analysis approach T-test, multiple linear regressions, person correlation coefficient, and analysis of variance (ANOVA).

Results: (35.3%) of them at age 20-29 years old, (75%) of them had secondary nursing school graduate level of education and (35%) of them had 11-15 years of services in the nursing field. there is a highly significant difference at $P < 0.01$ between the initial period of Pretest and posttest of the study sample preventive measures toward puerperal sepsis. The grand mean at pretest was (1.69) and at posttest (2.38). There is a statistically significant difference between years of service and preventive measures at posttest and Pretest of the educational program at (p-value > 0.05).

Conclusion: there is an improvement in the nurses-midwives' preventive measures level at the periods of tests after the implementation of the educational program.

Keywords: *Effectiveness, Educational Program, Nurses-Midwives, Preventive Measures, Puerperal Sepsis.*

Introduction

Puerperal sepsis is a significant preventable reason for maternal mortality, representing roughly 11% (95% vulnerability interval 5.9%–18.6%) of maternal mortality internationally. Improved comprehension of maternal sepsis is vital to accomplishing the feasible advancement goals (SDGs) and executing systems toward closure preventable maternal and neonatal mortality [1, 2].

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Puerperal sepsis is one of the five leading reasons for maternal mortality around the world and records for 15% of all maternal mortality. The WHO presented the term postpartum infections, which likewise include non-genital infections in the obstetric populace. Puerperal sepsis is a significant reason for morbidity and mortality for moms in developing countries. It is one of the commonest reasons for maternal deaths [3].

In any case, the recurrence of PIDs in pregnancy is ineffectively comprehended; records of obstetric morbidity recognized no distributed deliberate writing survey of (systematic literature review) of infection rate, making it the one significant direct reason for maternal morbidity without such literature as far as

anyone is concerned. Ongoing 2017 Worldwide Weight of Ailment (GBD) information gauge 12.1 million occurrence instances of maternal sepsis and other maternal infections, including mastitis [4, 5].

Puerperal sepsis represents 15% of maternal dead women around the world. In Africa, puerperal sepsis is the 2nd significant reason for maternal morbidity and deaths, representing over 10% of maternal dead women [2].

Health training and education have been a basic piece of nursing for a considerable significant period. Training of health givers and patients is as significant currently as could be. The nurses need to know the terms of health instruction programs and the pedagogical role of the nurse has the option to give wellbeing instruction to patients [6].

Methodology

A quasi-experimental study was conducted to evaluate the effectiveness of the educational program on nurses-midwives´ knowledge and preventive measures about puerperal sepsis at delivery rooms in Baghdad maternity hospitals conducted from January 24th, 2019 to May 20th, 2020. The study sample was nonprobability "purposive sample" includes (60) nurses-midwives working at delivery rooms in Al-Elwia maternity teaching hospital, Ibn Albalady maternity and pediatric hospital, Al-Karckh maternity hospital, Al-Yarmouk Teaching Hospital, and Baghdad teaching Hospital. A questionnaire composed of two parts (demographic data and the third part about preventive measures of puerperal sepsis items). Content validity of the questionnaires is determined through a panel of (25) experts, and reliability of the questionnaire is based on the test-retest for the stability reliability (reliability coefficient 0.76 for knowledge and 0.83 for preventive measures). The process of gathering information was conducted from January 20th to August 18th, 2019 it included (Pretest, implementation of the educational program, posttest). Data were collected using an observation checklist for preventive measures. Data were analyzed through the application of descriptive and inferential statistical data analysis (SPSS) version 20. Through descriptive statistics (frequency, percentage, mean, mean of scores, a total of scores, range, and standard deviation) and statistical inferential (T-test, multiple linear regressions, person correlation coefficient, and analysis of variance ANOVA).

Results were determined as highly significant at ($P \leq 0.01$) significant at ($P \leq 0.05$) and non-significant at ($P > 0.05$).

Results

Table (1) Distribution of the Study Sample by their Demographic Characteristics

Variables		Frequency	Percent
Age (years) Mean & standard deviation (35.783±10.294)	20-29	23	38.3
	30-39	14	23.3
	40-49	18	30.0
	50 and more	5	8.3
	Total	60	100
Level of education	Secondary nursing school graduate	45	75.0
	Nursing institute graduate	14	23.3
	Nursing college graduate	1	1.7
	Total	60	100
Years of services in the nursing field	1-5	5	8.3
	6-10	20	33.3
	11-15	21	35.0
	16 and more	14	23.3
	Total	60	100

This table shows that (35.3%) of the study sample at age 20-29 years old, (75%) of them had secondary nursing school graduate level of education and (35%) of them had 11-15 years of services in the nursing field.

Table (2) Comparison between the Nurses-Midwives' Preventive Measures about Puerperal Sepsis at pretest and posttest

No.	Domains	Pretest		Posttest	
		Mean	Ass.	Mean	Ass.
1	Prepare tools	1.9	M	2.39	H
2	Prevention and control of infection	1.69	M	2.39	H
3	Midwife Preparation for the woman	1.69	M	2.39	H
4	Method of sterilization and disinfection	1.47	L	2.38	H
5	Postpartum prevention	1.43	L	2.35	H
6	Grand mean	1.69	M	2.38	H

M= mean, Ass. = assessment, level of assessment: (1-1.66) = low = L, (1.67-2.33) = moderate = M, (2.34-3.00) = high = H

This table indicates that the grand mean of the study sample preventive measures toward puerperal sepsis is (1.69) at a moderate level at pretest, and (2.38) at a high level at posttest

Table (3): Study Sample preventive measures toward Puerperal Sepsis Levels of Assessment Through the "Mean of Score" Among (Pre, Post Test)

Period	Level of Assessment	Frequency	Percent
Pretest	Low (1-1.66)	32	53.3
	Moderate (1.67-2.33)	28	46.7
	High (2.34-3)	-	-
	Total	60	100
	$\bar{x} \mp S.D$	1.69±0.14	
Posttest	Low (1-1.66)	-	-
	Moderate (1.67-2.33)	16	26.7
	High (2.34-3)	44	73.3
	Total	60	100
	$\bar{x} \mp S.D$	2.38±0.107	

$\bar{x} \mp S.D.\bar{x} \mp S.D.$=Arithmetic Mean ($\bar{x}$)$\bar{x}$) and Std. Dev. (S.D.)

There is a low level of assessment of 32 (53.3%) of the study sample at level (1-1.66), the **$\bar{x} \mp S.D.\bar{x} \mp S.D.$** are (1.69±0.14) at the pretest. While it is a high level of assessment of 44 (73.3%) of the study sample at level (2.34-

3), the $\bar{x} \mp S.D.\bar{x} \mp S.D.$ are (2.396±0.086) at the Posttest.

Table (4) Significant Comparison of the Period (Pre and Post) for Nurses' Preventive measures toward Puerperal Sepsis

Variables	Periods		Matched Paired t-test	Sig. P- value	C.S.
Preventive measures	Pretest	Posttest	-50.264	0.000	HS

C.S. : Comparison Significant, Highly Significant at P < 0.01

There is a highly significant difference at P < 0.01 between pre and posttest of preventive measures toward puerperal sepsis.

Table (5): Distribution and difference of nurses preventive measures with their age, education level, and years of experience.

Variable		preventive measures test periods	
Age	**No.**	**Pretest Mean ± S.D.**	**Posttest Mean ± S.D.**
20-29	23	1.72±.176	2.40±.105
30-39	14	1.69±.098	2.40±.080
40-49	18	1.66±.123	2.35±.120
50 and more	5	1.66±.126	2.33±.121
Total	60	1.69±.141	2.38±.108
ANOVA		F =0.585	F =1.615
		d.f.=59	d.f.=59
		P =0.627	P =0.196
Education	No.	Pretest Mean ± S.D.	Posttest Mean ± S.D.
Secondary nursing school graduate	45	1.66±.114	2.38±.101
Nursing institute graduate	14	1.78±.182	2.40±.12
Nursing college graduate	1	1.64±.00	2.19±.00
Total	60	1.69±.141	2.38±.108

Cont... **Table (5): Distribution and difference of nurses preventive measures with their age, education level, and years of experience.**

ANOVA		F =4.442	F =1.959
		d.f.=59	d.f.=59
		P =0.016	P =0.15
Years of service	No.	Pretest Mean ± S.D.	Posttest Mean ± S.D.
1-5	10	1.70±.137	2.41±.091
6-10	21	1.70±.158	2.37±.122
11-15	21	1.71±.146	2.35±.073
16 and more	8	1.59±.081	2.29±.120
Total	60	1.69±.141	2.38±.108
ANOVA		F =1.305	F =3.302
		d.f.=59	d.f.=59
		P =0.282	P =0.027

No. = Number of frequencies, S.D.=Standard deviation, ANOVA= Analysis of Variance, F = Fisher test, d.f. = degree of freedom, P = probability value, NS : Non Significant at P > 0.05 , S : Significant at P < 0.05 , HS : Highly Significant at P < 0.01.

There is no statistically significant difference between age and preventive measures at pretest and posttest of the educational program at (p-value > 0.05)

While there is a statistically significant difference between education level and years of service with preventive measures at the pretest of the educational program at (p-value > 0.05).

Discussion

Regarding to the socio-demographic characteristics of the study sample in Table (1). The results show that (35.3%) of the study sample at age 20-29 years old. This result agrees with the findings of (Fashafsheh, Ayed, Eqtait & Harazneh, 2015) in their study, they found that (64.2%) of the nursing staff their age (20-30) years [5]. (Momoh, Ezugworie, & Ezeigwe, 2010) found that in their study about half of the sample aged from 38-47 years old [7]. Also Kebalepile (2001) found that the nurses' age was from 30-39 years [8].

Concerning the educational level, (75%) of them had secondary nursing school graduate level of education. (Fashafsheh, Ayed, Eqtait & Harazneh, 2015) disagree with our finding they found the majority of the study sample have a Diploma and Bachelor graduate in nursing [5].

Most of them had 6-15 years of services in the nursing field. Fashafsheh, Ayed, Eqtait & Harazneh, 2015) supported our finding they found most of the study sample had years of experience (6-11) years [(5)]. While these results not consistent with the finding of Kebalepile (2001) they found the nurses had experienced more than 15 years [8].

In the Table (2) the study sample preventive measures toward puerperal sepsis at Pretest at a low level. The grand mean of the study sample preventive

measures toward puerperal sepsis is (1.69) at a moderate level**.**

Also, (Fashafsheh, Ayed, Eqtait & Harazneh, 2015) found that (91.1%) of the nursing staff had good preventive measures level [5]. These findings supported our findings.

In Table (2) the study sample preventive measures toward puerperal sepsis at posttest. The grand mean of the study sample preventive measures toward puerperal sepsis is (2.38) at a high level.

The findings of the present study indicate that the nurses´ level of preventive measures is enhanced or improved to a good level of preventive measures at posttest. This finding agrees with Kebalepile (2001) findings, a majority 46 (71%) mentioned the use of sterile gloves during delivery, 43(66%) will use sterile delivery packs and ensure, 49 (75%) of nurses have demonstrated good preventive measures on measures to take in the prevention of postpartum infections [8].

The point of view of the researcher related to the present study considers that the educational program affects the level of preventive measures.

Educational programs are very important to all nurses in different filed of nursing specialty. Nursing research adds new information that is used in theoretical and practical nursing fields. it is used in the practical field, which improves the experience and preventive measures of nurses and thus increases the experience in finding problems facing nursing staff, finding suitable solutions, and acting appropriately to avoid obstacles in work and nursing care. Therefore, it is necessary to enroll nursing staff in theoretical and practical training sessions to develop their skills in patients care.

Regarding the two periods of educational program implementation on the study group in Table (3), the findings revealed that the level of nurses´ preventive measures toward puerperal sepsis has been developed by the implementation of the educational program to be at a good level. This results inconsistent with the result of Bayoumi and Mahmoud, (2017) they found that the nurses level of preventive measures had been improved after the implementation of an educational program [1].

In Table (4), the study sample preventive measures toward puerperal sepsis high significant difference at P <0.01 between the initial period of pre and posttest of the study sample. This result agrees with the findings of Kaur & Jairus (2015) they found that there was a significant difference between the means of the Pretest and posttest [9].

Results indicate that there is a significant statistical relationship between the study sample preventive measures at the pretest period and their level of education and between years of service and preventive measures at the posttest of the educational program at (p-value > 0.05). There is no significant statistical relationship between the study sample preventive measures at the pretest and posttest of the educational program and their socio-demographic characteristics (age).

(Fashafsheh, Ayed, Eqtait & Harazneh, 2015) found that there are no statistically significant differences between mean preventive measures scores towards age, years of experience [5].

This result may be related to the different levels of education and most of them graduate from secondary nursing school. The educational level of nurses may play a role in the results of any program which improves nurses´ preventive measures.

Conclusion

The educational program improves the preventive measures toward puerperal sepsis. The grand mean at pretest was (1.69) and at posttest (2.38). There is a highly significant difference at P < 0.01 between the initial period of pretest and posttest of the study sample preventive measures toward puerperal sepsis. There is a statistically significant difference between years of service and preventive measures at the posttest of the educational program at (p-value > 0.05). there is a statistically significant difference between education level and preventive measures at the Pretest of the educational program at (p-value > 0.05).

Recommendation

Implementation of the educational program about the prevention of puerperal sepsis for nurses-midwives on how to work in the delivery room. Follow up and evaluation of nurses' intervention toward women in the

labor Room and postpartum period and find the obstacles and work to solve them.

Conflict of Interest: Nil.

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Mean Circumferential Fibers Shortening Velocity Assessment in Patients with type II Diabetes Mellitus in relation to their Diastolic Function Status

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Abstract

Background: The most important cause of death in diabetic patients is the cardiac insult. Mean circumferential fibers shortening velocity (MCFSV) was used to detect the systolic cardiac dysfunction.

Objective: This study aimed to evaluate MCFSV in people who have Type 2 diabetes.

Patients and methods: This analytic observational study was performed in Al-Furat teaching hospital. Eighty type 2 diabetics were involved in this study. All patients were subjected to traditional Doppler measures: tissue Doppler-derived E/E, and MCFSV.

Results: Echocardiographic measures revealed 35 subjects having Grade I of Diastolic dysfunction (DD), 15 having Grade II while 10 were diagnosed with Grade III of this condition. MCFSV did not change in patients with normal diastolic function (0.00137±2.02) and those with grade I diastolic dysfunction (0.00148±3.5), (*P* value: 0.2) while significant reduction was documented in patients with grade II diastolic dysfunction (0.0011±9.1, *P* value: 0.00) and grade III diastolic dysfunction (0.001±6.02, *P* value: 0.00)

Conclusions: There is a connection between Reduction of MCFSV and increased grading of diastolic dysfunction (DD) in type II diabetics.

Key words: *Diabetes mellitus, Mean circumferential fibers shortening velocity, diastolic dysfunction.*

Abbreviations: MCFSV: Mean circumferential fibers shortening velocity. LV: left ventricle**.** DM: diabetes mellitus

Introduction

Heart disease is recognized as the most common cause of death of diabetic people .1 Atherosclerosis accounted for most of cases. Many studies showed that diabetes increase the possibility of experiencing heart failure as well as other abnormalities of cardiac function.[3-5] In absence of hypertension or significant valvular heart diseases, diabetic patient can develop diastolic dysfunction in early stages then progressed to systolic dysfunction.[6] MCFSV is an index of pumping function of left ventricle. Recently, it has been routinely calculated by using M-mode technique, a change in LV minor axis in systole with a change in ejection time was considered.[12] Cooper et al. demonstrated the reliability of this measure showing that MCFSV achieved by angiocardiography has a clear association with that achieved by the echocardiography test.[2]

Our study targeted at evaluation of the MCFSV in diabetic patients (type 2) in absence of other risk factors or diseases that might affect the results.

Patients and Methods

In this study, eighty patients with a known case of type II DM were recruited and subjected to a biochemical assessment. The study was agreed by the ethical committee of our institution. Fasting and random blood sugar, electrocardiography, renal function test and lipid profile were performed for all patients. A complete two dimensional echocardiography was done for all subjects using a commercially available ultrasound transducer and equipment (M5sc probe, GE, Vivid E9, 2015, USA). All procedures were conducted by the same operator adopting the left-lateral decubitus (LLD) position.

In order to measure transmitral inflow velocity values, we used a pulsed-wave sample at the tips of the mitral valve leaflets in the parasternal four-chamber view. Then, we obtained the following: peak E velocity (m/s), peak A velocity (m/s), and deceleration time in milliseconds. Diastolic dysfunction can be listed as: impairment of heart relaxation with GRADE II pseudonormal, and restrictive pattern.8 A pseudo-normal diastolic dysfunction was determined by TDI derived E/E' >15 as well as the use of valsalva maneuver. E' wave velocity was measured by tissue Doppler sample placed at medial mitral annulus in an apical 4 chamber view. (Figure 1).

Mean circumferential fibers shortening velocity (MCFSV) can be obtained by using M-mode technique by the following equation:

MCFV= [LVEDD – LVESD / LVEDD] ÷ LVET (mm/ms). Ejection time was calculated by placing continuous wave sample volume at aortic valve and then, the time from aortic valve opening to its closure was determined in the corresponding Doppler wave[10-13]

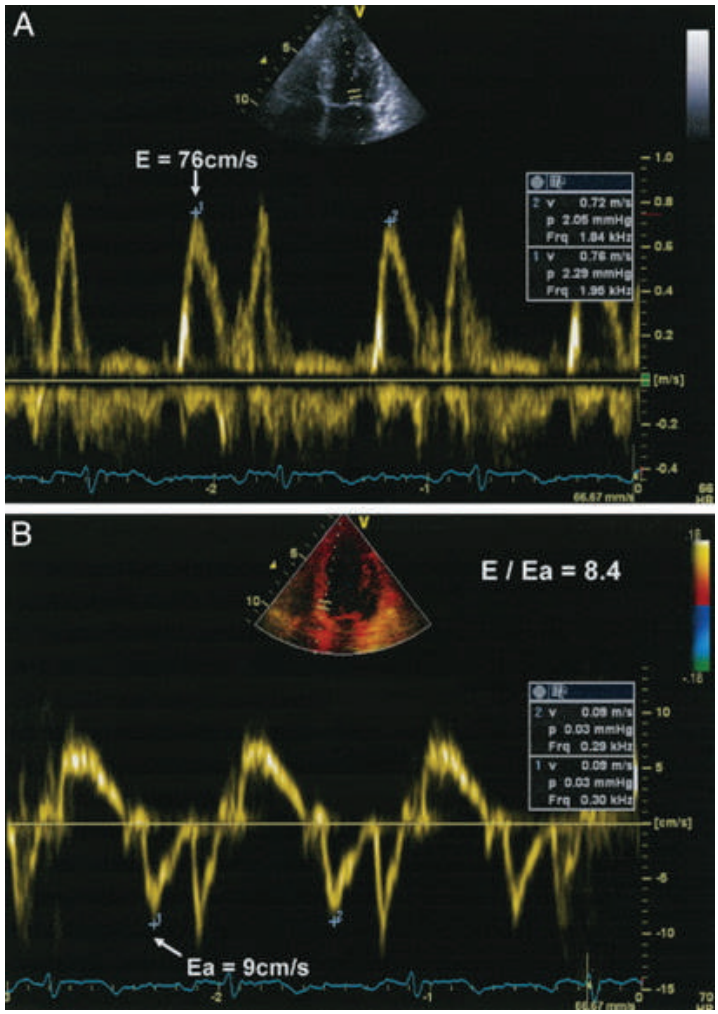


Figure 1 Calculation of E/E'

Statistical Analysis

All data were presented as mean ± SD. In this study, one-way analysis of variance (ANOVA) was performed to determine whether there are any statistically significant differences between the variables of this study. A p-value of 0.05 was set as the threshold of statistical significance. The statistical treatment was done using the SPSS package software version 18.

Results

Table (1) displays the baseline characteristics of the eighty patients involved in this study. The subjects were grouped into four categories according to their Diastolic dysfunction conditions and as follows: The first group (Group A) included 20 subjects with no diastolic dysfunction. Group B included 15 patients with Grade I Diastolic dysfunction. The third group (Group C) consisted of 15 patients with Grade II diastolic dysfunction. The remaining 10 patients with Grade III diastolic dysfunction were put into Group D.

No significant change of MCFSV was found in both group A (normal diastolic function) and Group B, which consisted of patients with grade I diastolic dysfunction. While significantly decreased in group C and D i.e. decreased with increased severity of diastolic dysfunction. Table (2) compares the groups of patients in terms of echocardiographic parameters.

Table 1 : A run-down of Baseline characteristics of all 80 subjects

Age (years)	53.3±9
Female, male (%)	62, 38
Duration of DM (years)	5±3.38
BMI	25.9±2
SBP	115.9±14.47
DBP	81.8±8.58
FBS	120.1±23.02
HbA1c	7.76±1.57
T. Triglyceride	133.8±25
T. Cholesterol	178.02±15.9

Table 2 Compares the values of echocardiographic parameters of the recruited groups

	Group A	Group B	Group C	Group D	.Group (A) vs. Group (B) P value	Group (A) vs. Group (C) P value	Group (A) vs. Group (D) *P* value
Age (years)	47.9±10.89	52.01±7	57.9±7.02	59±7.1	0.1	0.00	0.00
Duration of DM (years)	5±1.47	5.29±2.4	7.9±3.01	7±2.9	0.00	0.00	0.00
E/A	1.19 ± 0.55	0.79 ±0.17	1.22 ± 0.14	2.08 ± 0.75	0.00	0.7	0.00
E/E,	9.5 ± 6.42	12.01 ± 4	16.41 ±2.72	22.02 ± 8.6	0.00	0.00	0.00
MCFSV (mm/ms)	0.00137±2.02	0.00148±3.5	0.0011±9.1	0.001±6.02	0.2	0.00	0.00

Discussion

Diabetic cardiomyopathy is considered as a major cause of death of diabetics and is also considered as an independent cardiovascular insult. Many factors can lead to such insults as microvascular, autonomic and metabolic disorders.[4]

Left ventricular performance can be assessed by simple and satisfactory index called the MCFSV which can be used even when other cardiac abnormalities coexist.[8] Cooper et al. studied the MCFSV measured by echocardiography and compared it with those taken by cineangiocardiography. A good relationship was documented.[2] Our results dovetail with a study that showed circumferential as well as longitudinal dysfunction was found in diabetic (type II) subjects without significant coronary artery disease.[1]

A study by J. Salazar et al. demonstrated that there is no association between the duration of diabetes and any of the echocardiographic measures investigated including the MCFSV. The study concluded that echocardiographic test did not show the existence of Diabetic cardiomyopathy (DCM) in diabetic patients. These results contradicted our results as we found that MCFSV as an echocardiographic index for cardiac performance is connected with an increase in the severity of diastolic dysfunction in diabetic subjects.[14]

Virendra et al. demonstrated high frequency of diastolic impairment in subjects showing diabetes symptoms, an outcome that was related with the diabetes duration and HbA1c levels.[11]

Studies have described a high occurrence of subclinical diastolic insult among individuals with diabetes.[9] These studies documented that diastolic dysfunction occurs at an early stage during the process of diabetes before affecting systolic function and the related pathogenesis is not yet understood. All these studies approached our results as most of our patients have various levels of diastolic dysfunction. The golden finding in this study is the occurrence of systolic dysfunction in late stages of diastolic dysfunction of diabetic patients by the reduction in velocity of circumferential fiber shortening.

Conclusion

We conclude that there is no connection between Reduction of MCFSV and increased grading of diastolic dysfunction found in type II diabetics.

Conflict of Interest: The authors have no financial, consultative, institutional, and other relationship that might lead to bias or conflict of interest. Therefore we declare that we have no conflict of interest

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Ethical approval: We declare that the study does not need ethical approval.

Ethical Clearance: The study was conducted with approval from research ethics committee at faculty of medicine / Kufa University.

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Some Histogenetic Aspects of Methotrexate Associated with Juvenile Idiopathic Arthritis (JIA) Treatment Doses

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Abstract

A total of (36) mice in the age of (1-3) weeks were selected to evaluate the methotrexate effect on some histogenetic aspects of kidneys. Treatment lasted for three months, and results showed marked hemorrhage, cortical tubular basophilia, necroses, and degeneration in the autopsied organ of animals with low oral hydration. Besides, changes in the DNA methylation pattern were noticed in the RAPD-PCR profile upon analysis with agarose gel electrophoresis.

Keywords*: Methotrexate, Kidney, DNA methylation, JIA, RAPD-PCR.*

Introduction

Methotrexate (MTX) is an antimetabolic agent and a structural analog that affects the metabolism of folic acid and inhibits cell proliferation [1&2]. This drug came into the clinical application as an advanced antineoplastic drug in 1948, due to its indirect effects on DNA synthesis [3]. It is a well-known immunosuppressive agent widely used for the treatment of many rheumatologic, dermatologic, and hematologic diseases successfully [3-6]. In children, methotrexate is the cornerstone for the treatment of juvenile idiopathic arthritis (JIA), the most common chronic rheumatic disease of childhood, and the major cause of disability [6-8]. It acts by inhibiting dihydrofolate reductase, which reverts DNA hypomethylation and reducing cell proliferation [8-10]. Methotrexate dose is usually eliminated by renal excretion, which increases the risk of renal damage and nephrotoxicity that sometimes develops as a side effect due to inadequate oral hydration and increased urine acidity [11&12]. Moreover, it was found that gene dysregulation associated with variability in DNA methylation might contribute to the initiation of cancer diseases [3-5]. Thus, many perturbations raised considering the side effects of using MTX as the cornerstone for prolonged treatment of JIA[11-13].

In accordance, this study aimed to investigate the side effects of methotrexate treatment associated with the treatment doses of juvenile idiopathic arthritis on the kidney tissue and DNA methylation pattern of white mice as a model.

Methods

2.1. Study animals

Healthy mice of *Mus musculus* strain obtained from the Association of Genetic & Environmental Resource Conservation (AGERC)/Iraq during 2019. Mice aged (1-3) weeks and weighed (20-23 gm) were housed and fed according to the procedure mentioned by Ref. [14].

2.2. Experimental design and Methotrexate administration

All animal studies conducted were according to the Guide of Experimental Animals [15]. Intraperitoneal injections of Methotrexate brand of (FRESENIUS KABI/ USA) used in the administration protocol summarized in (Table 1) according to the procedure cited in Ref[16].

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Table 1:- Methotrexate administration protocol of the study

Treatment Groups *n*=12 mice /GP 1:1 male to female ratio	MTX Dose mg/m^2 BSA/Week	Water Intake ml/day	Water Intake (L / 3 months) Study Interval	Total MTX concentration mg/m^2
G_{m1}	10	100	8.4	120
G_{m2}	10	300	25.2	120
G_C	0	300	25.2	0

*MTX doses were calculated to bodyweight Index according to the FDA protocols cited in Ref.[17]

Four mice of both sexes per treatment group were autopsied at the end of each month after anesthetization with the injection of (100 mg/kg of Ketamine and 5 mg/kg Xylazine (Alfasan /Turkey); kidneys tissues were immediately removed after autopsy according to the procedure cited in Ref.[18]. Kidneys were cut into two parts; one half was fixed in (10% formalin) for histopathological examinations, while the other half was placed into liquid nitrogen (BIOBASE/ Meihua /China) at -20℃ for genetic assessment according to Asci *et al*.[19].

2.3. Histological Valuation

Kidney fixed in 10% formalin were embedded in paraffin, and tissue sections were cut at 5µm by Microtome (BIOBASE/ Meihua /China), mounted on slides after staining with Hematoxylin Eosin Y solutions (HE Stain Kit/TissuePro/USA) according to Ref.[18]. Sections were examined by a light microscope (Optika/Italy) and photographed by mounted Camera (Optica- Italy 4083-B5). Significant histopathological abnormalities were evaluated and recorded accordingly.

2.4. Genetic assessment of Kidneys DNA methylation pattern

Five methyl Cytosin levels were relatively quantified after the enzymatic hydrolysis of the genomic DNA with HpaIIand MspI restriction enzymes (1U / 1µg DNA/Thermo Scientific/France). DNA extractions were performed using the DNA isolation kit (Genomic Prep /Amersham Biosciences/UK) following the instructions of the manufacturer. Then extracted DNA was resuspended in Tris-EDTA, and the quantity of the extracted DNA checked by spectrophotometer (Schimadzo/Japan) as described in [20]. For this analysis, RAPD-PCR (Eppendorf /Germany) primer set (Ready-To-Go RAPD- PCR analysis kit/ Amersham Biosciences/ UK) used as instructed by the manufacturer company. Three samples of Kidney DNA (control, MTX 10mg, MTX 15mg) were subjected to PCR with a single primer concentration of 25 pmol in a reaction volume of 25 μl and a DNA marker of (pUC19 DNA MspI/HpaII Marker/ Thermo Scientific/ France). Each change observed in RAPD profiles was scored as described in [22].

Results

This study was conducted to observe the histological and epigenetic changes in the kidneys tissue due to methotrexate treatment for three months of (10 mg/m^2 BSA/ week) dose associated with the therapeutic doses of the juvenile idiopathic arthritis (JIA) but under low water intake circumstance. The histopathological examination of the kidneys of the study groups experimental animals showed the following results:-

-Macroscopic Histopathological Changes:

The excised kidneys of the ($\mathbf{G_{m1}}$) group of mice that received intraperitoneal injections of (10 mg/m^2 BSA/ week) treatment doses for three months durations with low water intake showed congestion and enlargement signs. While the kidneys of the ($\mathbf{G_{m2}}$) group of animals that received the same treatment with the same duration but with average water intake showed normal kidneys texture except for the last two weeks of the third month

where excised kidneys of the (G_{m2}) group showed congestion marks. On the other hand, the (G_c) group that received intraperitoneal injections of (0.1 ml) of normal saline showed normal shape and size of kidneys.

-Microscopic Histopathological Changes:

The examined kidney tissues of the (G_{m1}) group of mice showed marked aggregation of lymphocyte, congestion with fatty degeneration, and dilated tubules in the second month of treatment as declared in (Figure 1).

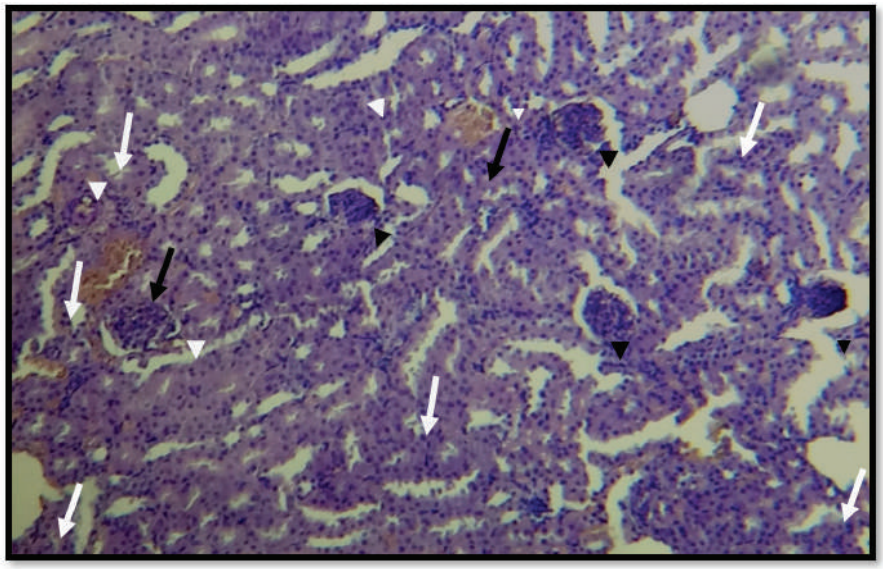


Figure 1:- Microscopical findings of the kidney tissues in the methotrexate treatment group (G_{m1}) for two months with low water intake showing marked aggregation of lymphocyte (black arrows head), congestion (black arrows), fatty degeneration (white arrows), dilated tubules (white arrows head) examined under a light microscope by different magnifications.

Whereas, the prolonged treatment for the third month of the (G_{m1}) group of mice with continued low water intake caused marked hemorrhage, cortical tubular basophilia, necroses and degeneration in the autopsied kidney tissues for both sexes of animals as manifested in (Figure 2).

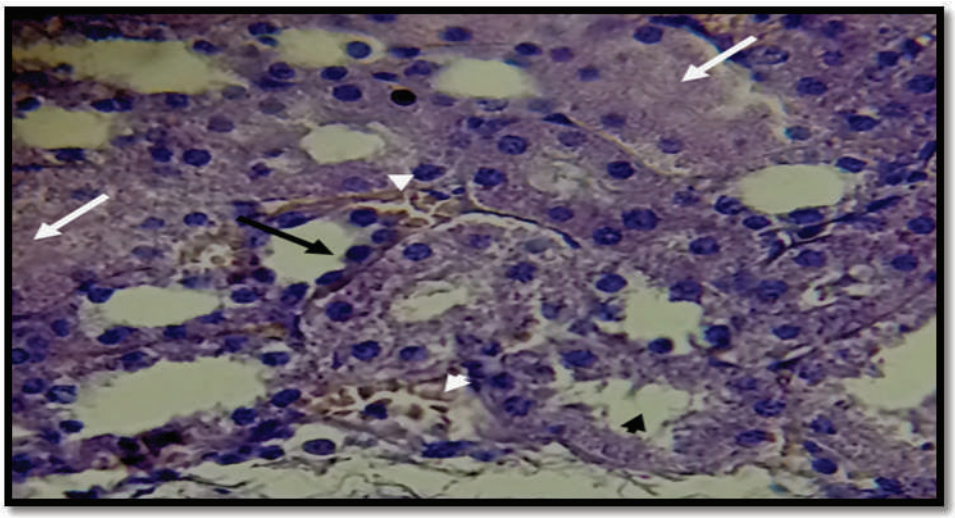


Figure 2:- Microscopical findings of the kidney tissues in the methotrexate treatment group (G_{m1}) for three months with low water intake showing marked hemorrhage (arrows head), cortical tubular basophilia (black arrow), necroses (white arrows)degeneration(black arrowhead) examined under a light microscope

by different magnifications.

Yet, the (G_{m2}) group of mice treated with the same methotrexate dose but with sufficient water content showed signs of cortical tubules basophilia with infiltration of lymphocytes only in the third month of methotrexate treatment. However, the epigenetic effect of methotrexate treatment on the genomic DNA methylation declared in (Figure 3).

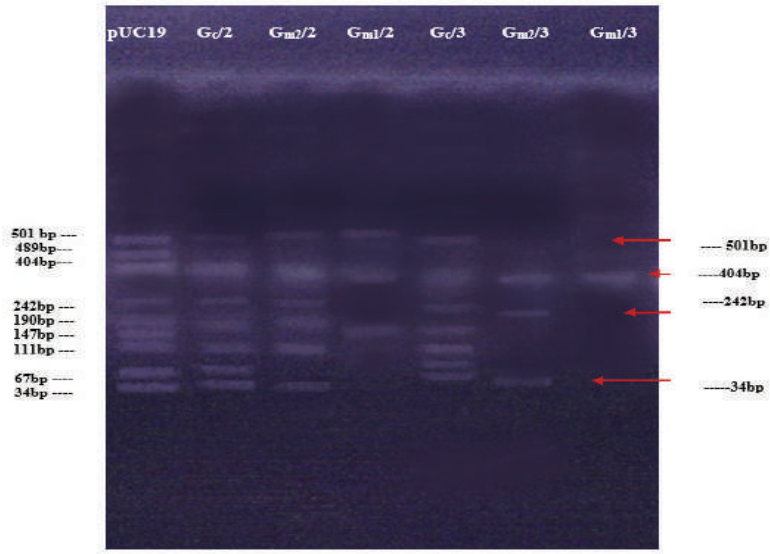


Figure 3:- Gel picture of RAPD-PCR products of Kidney tissues of treatment samples for two and three months of methotrexate associated with JIA doses on (1.7%) agarose gel electrophoresis stained with ethidium bromide. Gel visualized under UV-Transilluminator and pictured using a gel documentation system. pUC19 Lane is Marker DNA; Lane G_c is control group kidney DNA; Lane G_{m1} is a methotrexate treated group with low water content; Lane G_{m2} is a methotrexate treated group with sufficient water content. Numbers 2/3 means DNA extracts of two and three months of treatment.

The summary of RAPD-PCR products after the analysis of the banding pattern of genomic DNA methylation is outlined in (Table 2).

Table 2:- RAPD-PCR profile of DNA methylation pattern of kidney tissues treated with Methotrexate.

Banding profile of pUC19 DNA bp	Banding profile of Gc /2 DNA/bp	Banding profile of Gc/3 DNA/bp	Banding profile of Gm2/2 DNA/bp	Banding profile of Gm2/3 DNA/bp	Banding profile of Gm1/2 DNA/bp	Banding profile of Gm1/3 DNA/bp
501	+ve	+ve	+ve	-ve	+ve	-ve
404	+ve	+ve	+ve	+ve	+ve	+ve
242	+ve	+ve	+ve	+ve	+ve	-ve
190	+ve	+ve	+ve	-ve	-ve	-ve
111	+ve	+ve	+ve	-ve	-ve	-ve
67	+ve	+ve	-ve	-ve	-ve	-ve
34	+ve	+ve	+ve	+ve	-ve	-ve

*+ve refers to band presence; -ve refers to band absence in the agarose gel banding profile

Discussion

Juvenile idiopathic arthritis (JIA) is an autoimmune disease associated with functional disability and many complications in the child's life [16]. It is prevalent in children and teenagers, causing uncontrolled inflammations and irreversible damage to joints and other tissue [23]. In Iraq, there is a significant increase in the disease ratio between post-school and young children [24-26]. Thus, the improvement of the disease outcomes of children with JIA remains a critical challenge for the clinician, where most of them rely on methotrexate as their first-line of treatment agent[3&4]. Methotrexate is a standard treatment used in many countries for children who suffer from moderate to severe arthritis; it is primarily cleared through kidneys where renal excretion eliminates about 80% of the dose[25 & 26].

However, the elimination of the methotrexate dose depends on the urine alkalinization where methotrexate is a weak acid and tends to precipitation at urine pH below (6.0) leading to nephrotoxicity [11-13]. Accordingly, the present research intended to evluate the effect of methotrexate administration on kidney tissues of mice under insufficient oral hydration conditions. The equivalences of Dutta and Sengupta, 2016 [27] for men and mice ages were considered in the experimental animals to be in correspondence to the ages of young children in humans. Besides, treatment groups were divided into two groups according to the oral hydration circumstances in order to study the effect of methotrexate on children with low water intake, where most of the children might forget to drink sufficient water for at least six hours during the school day.

The kidneys of the treatment group with inadequate oral hydration (G_{m1}) appeared more affected in all treated animals than the other group (G_{m2}), where the kidneys showed marked cortical dilatation of tubules and necrosis. The same result obtained by Chelab and Majeed in 2009[26] where they had indicated that methotrexate crystals accumulated in the nephron tubules and the dilatation of nephric tubules of renal cortex occur due to thickening of basement membranes of tubules and precipitation of the methotrexate crystals in the nephron of the kidneys due to water dehydration. The same results were confirmed by Asci *et al*., 2017[19], where they established the etiology of MTX-induced nephrotoxicity.

Hence, genomic instability results in multiple molecular events believed to be a driving force in cancer etiology [22]. In this study, the RAPD-PCR technique with two methylation-sensitive restriction enzymes were used to analyze the genomic instability. Banding profile showed no change in the bands of the control group. While the (G_{m1}) group showed a significant difference in the banding pattern; where four bands of the molecular weights of (190bp,111bp,67bp, and 34bp) were missing from the profile after two months of treatment. Moreover, six bands were missing from the RAPD-PCR profile after three months of treatment with the molecular weight of (501bp, 242bp, 190bp, 111bp, 67bp, and 34bp) respectively.

Accordingly, the (G_{m2}) group showed changes in the DNA profile of the methylation pattern with one missing band of (67bp) molecular weight upon two months of treatment and three absent bands of molecular weights of (190bp, 111bp, and 67bp) at the end of the third month of methotrexate treatment respectively. These results, however, might indicate a probable role of methotrexate as a carcinogen and a teratogen that might affect the life quality of the treated child in the future. Those results agree with the results obtained by de Andres *et al*., in 2015[5] where they confirmed global DNA hypomethylation in patients with rheumatoid arthritis treated with methotrexate and the role of aberrations in the DNA methylation system as an essential tool in the initiation of certain human diseases.

Conclusions

The consequence of this study is related to the dose and route of administration of methotrexate in JIA issued to prevent treatment side effects as it was concluded that sufficient oral hydration could reduce histopathological side effects of methotrexate but not hypomethylation. Also, the nephrotoxicity of methotrexate necessitates caution when considering it by the physicians for the treatment of patients with kidney function impairment. Last, JIA treatment with methotrexate predetermines the warning of the child's parent about the outcomes and life quality after this drug's treatment since it is proven carcinogenic and probably teratogenic.

Conflict of Interest: The authors declare that there

is no conflict of interest.

Source of Funding: No funding received.

Ethical Clearance: No ethical issues.

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X-Chromosome Markers Used in Deficiency of Paternity Case

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Abstract

Cases of deficiency paternity, characterized by missing of the alleged father, are a task for forensic genetics. Recently these cases were determined using sets of extremely polymorphic autosomal short tandem repeats (STRs), which indicate to powerful likelihood ratios (LR). Some difficult cases emerge whenever the kinship highly remote or if the another hypotheses are not properly formulated because of the absence of information. In these situations, beyond the routinely used marker set, laboratories usually enlarge the number and/or the type of markers analysed. A set of 14X-STRs is further advantageous than 21 autosomal STRs (AS STRs) biallelic markers. Furthermore, the utility of X-STRs was also appear in cases shows that in father–daughter duos and merely a close family member of the alleged parent (father or mother) is existing for testing. Here we present a cases with biological daughter of the alleged father and her mothers and putative grandmother was the genotyped relative are available for reconstructing his haplotype . Aiming to increase the Paternity Index (PI), Probability of Paternity (PP), and obtain more reliable results. The result of X STR showed that the missing person (father) share 50% genotype with the donors (daughter)and (his mother) at each locus, there are no alleles in any one of these 13 loci conflict with the alleles inherited from her father and Posterior Probability of paternity was (99.999958%) that is mean the missing person was biological father for this donor daughter.

Keywords: *13 X-STRs with Amelogenin, Paternity case, Kinship analysis, Paternity index, Paternity deficiency.*

Introduction

The main benefit of X-chromosomal (ChrX) STRs rises in deficiency paternity cases, i.e. when a putative father is unavailable and DNA from paternal relatives has to be investigated instead [1,2]. In such cases, the power of exclusion of autosomal STRs is significantly decreased, whereas ChrX markers are (at least in some cases) more resourceful. Female individuals share their paternal ChrX when they are fathered by the same man [3,4]. Males receive their single ChrX from their mother. Consequently, in cases in which the supposed grandmother is available for genotyping, the possible ChrX alleles of the putative father can be verified. ChrX marker typing is very applicable in mother–son kinship and in father–daughter analyzing [5,6].

Case Report

We perform the use of this set of markers in paternity deficiency cases, containing a female child and her mother and the putative grandmother and a dead alleged father who was not analyzed.

Figure 1 displays the pedigrees of the families. All persons are shown in the pedigrees were examined for autosomal (AS) and X STRs.

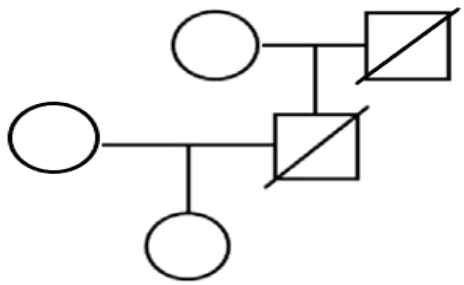


Figure 1: Pedigree of the deficiency paternity case. (paternal grandmother-granddaughter)

Materials and Methods

All blood samples were gathered utilizing finger lancets and kept on FTA® Classic Card (GE Healthcare, Pittsburgh, USA). For each sample a 1.2 mm punch was directly amplified according to the manufacturer's recommended instructions. PCR using two kits the PowerPlex 21® System)20 STR loci and Amelogenin), including D1S1656, D2S1338, D3S1358, D5S818, D6S1043, D7S820, D8S1179, D12S391,D13S317, D16S539, D18S51, D19S433, D21S11, Amelogenin, CSF1PO, FGA, Penta D, Penta E, TH01, TPOX and vWA. And 14ChrX STRs- GenePhile X-Plex(13STR loci and Amelogenin), including for (DXS8378, DXS9898, DSX8377, DSX8377, GATA172D05, DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS9902, DXS7424). Genomic DNA (1 ng) was amplified in a 10 L total volume of PCR reaction mix was done using Applied Biosystems 9700 thermocycler according to standard manufacturer's recommended conditions. Capillary electrophoresis was achieved by using a 3500XL Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's instructions; ILS-500 (promega, 2017) and BTO (qiagen, 2015) were used as an internal size standard. Genotyping was completed using GeneMapper ID-X v1.4 software (Applied Biosystems).

Statistical Analysis

The statistical parameters that analyzed showed the Paternity Index (PI) and Probability of Paternity (PP), with the assumption of an a priori probability of 0.5 for the latter. Genotypes and allele frequencies of the X-liked STRs were transmitted by the deceased father to his daughter and the formulae applied when the putative paternal grandmother was the genotyped relative that can be seen in Table 1. Statistical analyses were done corresponding to the formulae proposed by Ayres and Powley in 2005 [7,8].

Table 1: Formulations used to determine PI considering the profile configurations depicted with ChrX STRs,

Mother	Daughter	Putative grandmother	Formulas
AB	AB	AB	1/(a+b)
AB	AA	AB	1/2a
AA	AB	AB	1/2b
AA	AA	AB	1/2a
AB	BC	AC or CD	1/2c
AC	CC	BC	1/2c
AC	AC	BC	1/[2(b+c)]
AA	AB	BB	1/b
Absent	AB	BC	1/4b
Absent	AB	BB	1/2b
Absent	BB	BC	1/2b

Results

The routine work in identification we started with autosomal using PowerPlex 21, Autosomal STR DNA profile of paternal grandmother, mother and female child trio is presented in Table 2. All the allele in the autosomal STR DNA profile of female child is matching with both mother and paternal grandmother on all the 20locus establishing the identity of the missing father.

Table 2: The autosomal STR DNA profile of both mother)wife) and Daughter, paternal grandmother. (powerplex 21 genotype)

STRs	Mother	Daughter	grandmother
Amelo	X,X	X,X	X,X
D3S1358	16,18	15,16	16,17
D1S1656	14,14	11,14	13,14
D6S1043	11.18	14,18	14,17
D13S317	11,11	9,11	8,14
Penta E	12,13	7,13	11,16
D16S539	10,10	10,13	12,12
D18S51	12,12	12,15	14,18
D2S1338	17,25	17,25	17,27
CSF 1PO	10,12	12,12	10,12
Penta D	9,15	9,9	11,11
TH01	9,10	9,9	7,9
vWA	16,17	16,17	15,17
D21S11	29,29	26,29	26,31.2
D7S820	10,10	10,12	8,12
D5S818	10,12	10,12	12,12
TPOX	8,10	8,8	11,11
D8S1179	9,15	9,14	9,14
D12S391	20,12	21,23	18,23
D19S433	13,15.2	13,15.2	14,15.2
FGA	24.25	24,24	24,24

Data of DNA profile of same three samples with - GenePhile X-Plex(13STR loci and Amelogenin), kit is presented in Table 3. In X STR also all the allele of female child is matching with both mother and paternal grandmother on all the 13 locus. As the female child is having two X, one from father and the other from mother, so this can be shown by generating X STR DNA profile of the trio.

Table (3) : The genotype of mother (wife), daughter and grandmother (genephile x-plex -13 STR kit)

STRs	mother	Daughter	grandmother
Amelo	X,X	X,X	X,X
DXS8378	11,12	10,11	10,11
DXS9898	8.3,8.3	8.3,14	8.3,14
DXS8377	46,54	47,54	43,47
HPRTB	11,13	11,13	11,12
GATA172D05	6,11	6,10	10,10
DXS7423	15,16	15,16	15,17
DXS6809	31,32	31,34	33,34
DXS7132	13,14	13,14	13,14

DXS101	21,21	21,24	14,24
DXS6789	20,20	19,20	15,19
DXS9902	10,12	10,12	10,10
DXS6807	11,14	13,14	11,13
DXS7424	14,16	12,14	12,13

So Paternity indices (PI) for full trio and deficient (father not available) cases are (23725241.4)

Prior odd=1/2

$$\text{Posterior Probability of paternity} = \frac{CPI * prior}{(CPI * priorodd) + (1 - priorodd)} = 0.99999958$$

Discussion

The autosomal matching was done to support these results and avoid the false positive due to huge number of close related people missing at the same time we did sex chromosomes genotyping. The result of X STR showed that the missing person (father) share 50% genotype with the donors (daughter)and (his mother) at each locus, there are no alleles in any one of these 13 loci conflict with the alleles inherited from her father and Posterior Probability of paternity was (99.999958%) that is mean the missing person was biological father for this donor daughter. This value is accepted according to lab. This value is accepted according to lab. of mass grave standard and the International Society for Forensic Genetics (ISFG) recommendation [9,10].

Conclusion

Recently the X-Chromosome STR loci transmission analysis are clearly available for use in forensics as in immigration, victim identification in mass disasters and it is possible that these loci will be used efficiently for paternity testing of female children, predominantly as supplementary tests to complement the information obtained with the autosomes and the differentiation between pedigrees in deficient or otherwise difficult cases. In this study the equations that used provide the means to evaluate the utility of such loci in the existence of substructure, and complement previously published equations used for autosomal loci.

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Ethical Clearance: The principles and the experimental protocol in this study was approved by the Medico-Legal Directorate, Ministry of Health, Baghdad, Iraq.

Source of Funding Self

Conflict of Interest: Nil.

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Design and Manufacture of Nanomedicine for Aflax Pump Inhibitors in Clinical Isolates of Methicillin-Resistant Staphylococcus Aureus (MRSA) Bacteria

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Abstract

The consequences of this bacterium can be severe. Methicillin is one of these drugs, this drug, like other beta-lactams, inhibits Trans peptidases by binding to penicillin-binding protein (PBP). It prevents the formation of peptide glycans in the bacterial cell wall and destroys the bacterial cell wall. Chemical structure modification has made it resistant to the beta-lactamase enzyme produced by some bacteria. It was made in 1959. Today, it has been removed from the list of drugs and better alternatives such as cloxacillin and nafsilin are available. It has a lower spectrum than penicillin but it works well against beta-lactamase-producing organisms such as Staphylococcus aureus. Design and manufacture of nanoparticles for Aflax pump inhibitors in methicillin-resistant Staphylococcus aureus bacteria Preparation of BSA-meticillin nano-drug: This was done using the desolvation method and after ensuring the formation of nanodars using the XRD and FT-IR spectra, Microdiagnostic antibiogram testing was performed on methicillin-resistant Staphylococcus aureus and the phenotypic pump activity of all isolates was performed by agar technique containing Ethidium bromide by vil card method. According to a study, the Nano medicine albumin-methicillin has the highest inhibition of methylcellulose-resistant Staphylococcus aureus Aflax pump.

Keywords: *Albumin nanoparticle, Methicillin, Study of Aflax pump activity, Methicillin-resistant Staphylococcus aureus*

Introduction

Since the discovery of bacteria, humans have always sought to find an effective cure for their infections; Bacteria have also developed effective mechanisms to kill antibiotics. Today, with the emergence of drug resistance among pathogenic bacteria, the treatment of these infectious diseases has faced many problems. In recent decades, Staphylococcus aureus has been the leading cause of nosocomial infection. One of the problems with staphylococcal infections is that they do not respond to methicillin. After the advent of methicillin resistance, resistant antibiotics, including vancomycin, were introduced to treat staphylococcal infections. The first resistant strains were identified in 1960 and by 1980, resistance was rapidly emerging among bacterial strains and now there are endemic resistant strains in various medical centers around the world. Up to 70% of nosocomial staphylococcal infections have been reported.[1]

Ranjbar et al. (2018) conducted a study to determine the pattern of antibiotic resistance in strains of Staphylococcus aureus isolated from clinical samples of Imam Reza Hospital in Kermanshah. The results of this study showed high antibiotic resistance on the hospital side of Staphylococcus aureus. Therefore, in order to prevent an increase in resistance to common antibiotics, over-the-counter prescribing and unnecessary use of

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available antibiotics should be avoided.[2]

Garcia et al. (2019) to investigate the molecular characteristics of Staphylococcus aureus isolated from patients and army hospital staff: Methicillin resistance was investigated. The results showed that using MLRFT method with low power dissipation and high reproducibility, we are able to identify different bacterial strains. 63% of the clusters (24 strains) are double in the clusters which indicate limited infection transmission. In the study of resistance to methicillin in 90% of resistant strains with total isolated strains (55%), it can be concluded that; Methicillin-resistant strains are easily transported.[3]

Henwood et al. (2017) The aim was to investigate the high prevalence of methicillin-resistant Staphylococcus aureus and Vancomycin-resistant Staphylococcus aureus (VRSA) by MIC by E-Test method. The results showed that; Due to the prevalence of methicillin resistance in nosocomial infections, the use of cloxacillin does not appear to be effective in initiating empiric anticonvulsant therapy. Conversely, in infections obtained from the community, the use of cloxacillin in the treatment of lichen is recommended due to its lower resistance. On the other hand, it seems that due to the high cost of the anti-biogram method with E-test, it is possible to trust the results of valid disks in the anti-biogram.[4]

Jamulitrat et al. (2017) the goal was to examine Staphylococcus aureus nose carriers and determine the resistance of its antibiotic pattern among clinical staff at Ali Ibn Abitaleb Hospital in Rafsanjan. The results showed that; According to the impressive statistics of Staphylococcus aureus nasal carriers in hospital staff, especially methicillin-resistant cases, in order to prevent nosocomial infections, oxacillin and penicillin are recommended to identify the carriers of the microbial in all hospital staff and to treat it in the next step.[5]

Curtis (2018) the aim was to evaluate the drug resistance pattern of negative staphylococcus aureus in children. The results showed that; Staphylococcus aureus drug resistance and drug resistance pattern is unpredictable and multiple resistances are common. Vancomycin is the drug of choice for septicemia because only 26% of cases were resistant to it.[6]

Prashanth and Badrinath. (2016) conducted a study of the abundance of methicillin resistance gene (mec-A) in different strains of Staphylococcus aureus with PCR and determining its antibiotic sensitivity. The findings of this PCR study showed that; Of the 70 isolates obtained, 50% of the strains (35 cases) show methicillin resistance genes. However, in the study of the antibiotic resistance pattern by the agar-diffusion disc method, only 31.4% of the strains (22 cases) showed resistance to methicillin / oxacillin.[7]

Unfortunately, increasing the resistance of bacteria to stronger antibiotics threatens to increase the risk of human infectious diseases.[8]

As a result of this study, the aim was to make nanoparticles effective on methylphenyl-resistant Staphylococcus aureus Aflax pump and thus to treat infections caused by antibiotic resistance of this bacterium. (If the Staphylococcus aureus bacterium is linked to methicillin-resistant Staphylococcus aureus infections).

Materials and Methods

Nanoparticle Preparation Method (BSA)

This nanoparticle is made by simple massification.

In the next step, we dissolve the serum albumin cow with two values of 0.1 gr and 0.01 gr in two separate human beings in cc 2 of 10 mM solution of sodium chloride, Then, using 0.1 N normal sodium hydroxide solution, the solution is brought to 9, by adding a drop of sodium hydroxide solution and measuring the pH each time using a PH meter. At this step we add 8 ml of ethanol as an anti-solvent drop by drop to the protein solution, after the nanoparticles are formed; about 2 to 3 drops are stabilized by the addition of glutaraldehyde (a homogeneous double cross-linking agent). The resulting solution is stirred at room temperature for 24 hours by a magnetic stirrer.

Method of measuring MIC by microbroth dilution

The method of determining the minimum inhibitory concentration was performed by microbroth using Mueller Hinton's culture medium and antibiotic powders and McFarland's 0.5 suspensions according

to the International Committee on Clinical Laboratory Standards (NCClS).

MICs were determined based on the recommended CLSI method, and the lowest concentration of antibiotics that inhibited bacterial growth was recorded as MIC.

In determining the MIC using the Microbroth dilution test method used in our studies, the materials used are:

· Antimicrobial agent and preparation of suitable diluents

· Suitable environment for the growth of microorganisms

· Microbial suspension

In this method, 18-24-hour cultures of Staphylococcus aureus bacteria were used to control the effect of single-walled carbon nanotubes attached to Methicillin (as an antimicrobial drug).

A) Antimicrobial drugs

The antibiotic powder used was prepared with potency (µg / mg specification, production date, expiration date and instructions for storage). Table M100-S4 was selected to determine the general sensitivity to ampicillin according to NCCLS standards.

In general, antibiotics have a standard unit of activity that may vary with the actual weight of the powder. Therefore, we must standardize the antimicrobial solution based on the amount of antibiotic powder used.

The following formula was used to determine the amount of powder or solvent volume:

Preparation of methicillin and albumin-methicillin nanoparticles

To prepare the desired dilutions using the mentioned formula, calculate the desired volume for the preparation of dilutions of 1280µg / ml and then the rest of the dilutions are prepared. The potency of ampicillin powder is 1000. Based on this, we weigh 0.01 g, equivalent to 10 mg of antibiotic powder, with a digital scale and increase its volume to 10 ml with phosphate buffer. The above solution has a dilution of 10 mg / ml (Figure 1).



Figure 1. Sterilizing solutions

Now, with a syringe filter, filter 0.45 micrometers 5 ml of each solution under completely sterile conditions to be free of any microorganisms.

Then, from this solution, prepare dilutions with final concentration (1280-160-20-5.5 2g / ml). After that, the next concentrations, starting at 512µg / ml and finally ending at 0625/0µg / ml, are prepared. (All steps are done inside sterile microtubes.)

Results and Discussion

Results of albumin nanoparticle binding to methicillin results

Diffusion disc

This method was performed to assess the

susceptibility of methicillin-resistant Staphylococcus aureus strain, albumin-Methicillin nanoparticles, albumin nanoparticles and 9 plant extracts(Blue and alcoholic extracts of savory, blue and alcoholic dill, blue stevia, thyme blue, Saveh pomegranate juice, forest pomegranate peel and Saveh pomegranate peel).

Result: Growth aura with a diameter of 9 mm around the disc impregnated with alcohol extract of safflower (dilution of 10 mg per ml) and a growth spurt with a diameter of more than 20 mm around the disc impregnated with methicillin, A growth spurt with a diameter of 14 mm was observed around the disk impregnated with the nano-drug methicillin (Figure 2,3).



Figure 2. Testing the diffusion disc of savory alcoholic extract

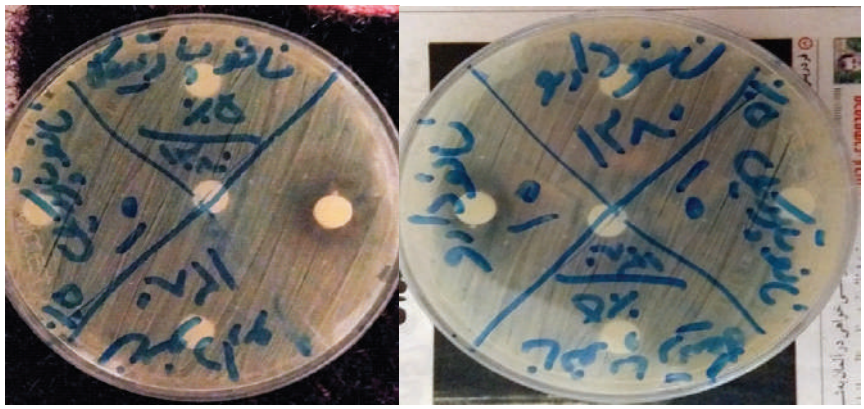


Figure 3. Nanofibrin diffusive disc test

Microbial results

Pipe MIC results

The experiment was performed only for 10 dilutions of pomegranate juice, and after 18-24 hours of incubation at 37, turbidity (bacterial growth) was observed visually.

Result: Turbidity was seen in pipe number 3 and above, and pipes number 2, 1 and 3 were completely transparent. (MIC = 0.25 mg / ml)

MIC results by microbroth dilation method

The MIC test was performed for Methicillin, albumin-Methicillin nanoparticles, albumin nanoparticles and in two wavelength ranges (405-450) and (492-620) and their antibacterial activity was tested in In vitro.

As can be seen in the tables and diagrams related to MIC results, the minimum inhibitory concentration of albumin-Methicillin sample is equal to 512 µg / ml dilution, and in the free Methicillin sample the minimum inhibitory concentration is observed on 16 µg / ml dilution. Nano medicine production efficiency is 0.77%. This indicates that the nano-drug produced has better antimicrobial activity than the methicillin drug. In fact, encapsulating methicillin on nanoparticles has

increased the antibacterial effect of methicillin-resistant Staphylococcus aureus.

Phonetic examination without antibacterial agent

In low concentrations of Ethidium bromide, the bacterial Aflax pump was active, thus removing Ethidium bromide and no red color was seen on the sides. Gradually, with the increase in the amount of Ethidium bromide, the Aflax pump was unable to remove this amount of Ethidium bromide from the bacterium. As a result, red was seen on the sides.

Bacterial strains are divided into three categories based on fossil fluorescence:

1. No active Aflax system potential: Types of fluorescence emission at an error of 0-1 mg / l of Ethidium bromide. None of the study subjects fell into this category.

2. Aflax system with medium activity potential: Types of fluorescence emission at an error of 1-2 mg / l of Ethidium bromide. Numbers 3 or, in other words, 20% of the subjects studied were in this category.

3. With the potential of active Aflax system: The strains that show fluorescence at a maximum concentration of Ethidium bromide 2-2.5 mg / l. Numbers 2 and Numbers 1, 4, and 5, or 80% of the subjects studied, fall into this category (Figure 4).

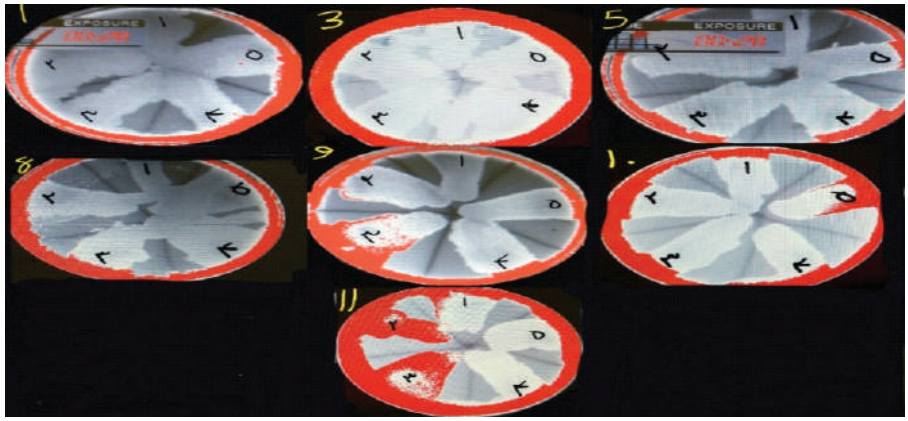


Figure 4. Images of Staphylococcus aureus Aflax pump activity

Phonetic examination with antimicrobial agent

1- Methicillin (pictured) (Figure 5): Inhibition of the 20% side effects of Aflax pump at 2 mg / l Ethidium bromide showed.

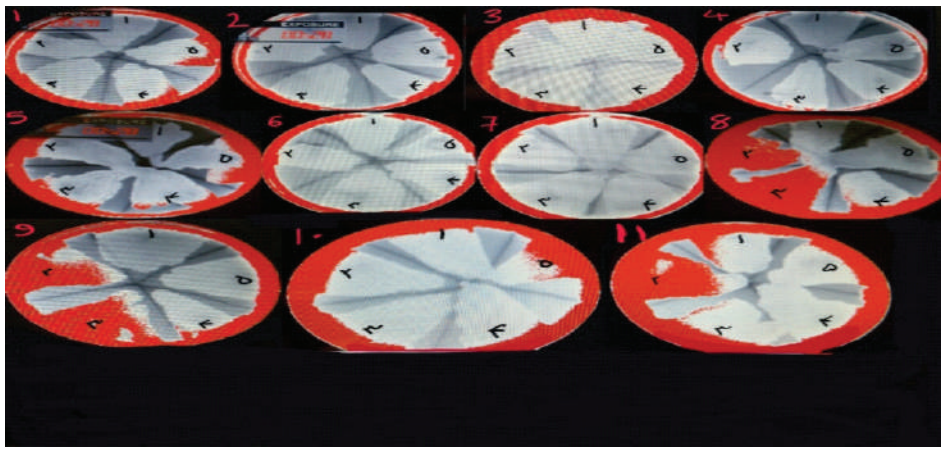


Figure 5. Pictures of the activity of Oreos Staphylaxis Aflax pump + methicillin

2- Albumin nanoparticles (0.5 g) (Figure 6): (pictured): Inhibition of 20% of the falafel pump showed side effects at 2 mg / l Ethidium bromide.

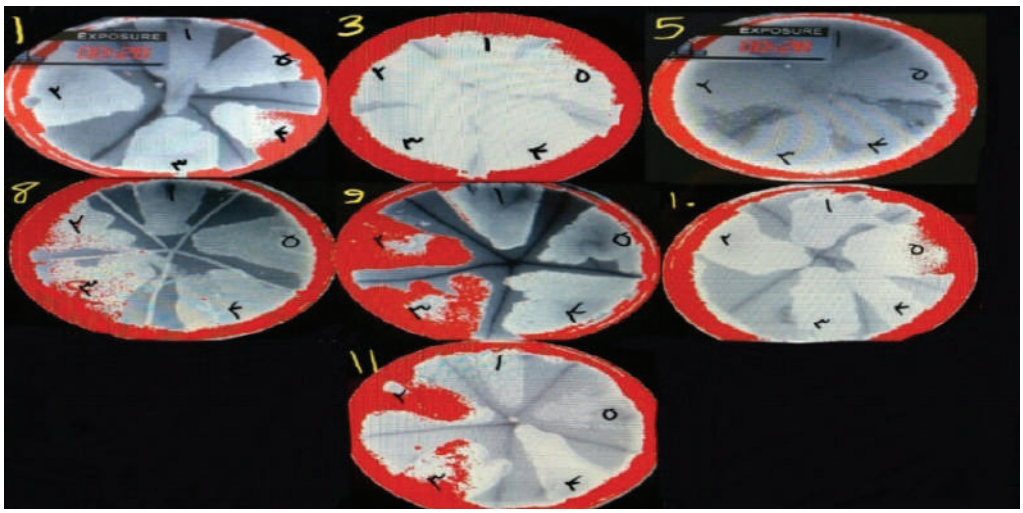


Figure 6. Images of the activity of Staphylococcus aureus + Nanoparticle albumin pump (0.5g)

In all images of the gel dock device, the numbers 6-1 on the number plate of the strains and the numbers on the left of the atomic concentration of bromide indicate each plate according to the table below.

Table1. Ethidium bromide concentrations of TSA culture medium

Number	1	2	3	4	5	6	7	8	9	10	11
Ethidium bromide concentration (mg / l)	0	0.25	0.5	0.75	1	1.25	15	1,75	2	2.25	2.5

Conclusion

Albumin nanoparticles (0.5 g) had 125g / ml = 125 / MIC = according to the Micro Broth Dilution method, it had a inhibitory effect on Methicillin -resistant Staphylococcus aureus bacteria and inhibition of the 20% side effects of Aflax pump at a concentration of 2 l / mg of Ethidium bromide.

Lagamayo. (2015) examined the microbial resistance, phenotypic and genetic evaluation of the norlax Aflax pump at methicillin-resistant Staphylococcus aureus (MRSA) and ciprofloxacin. There is a link between the Staphylococcus aureus strain and the resistance to ciprofloxacin on the Staphylococcus aureus strain. Thus, the development of Aflax pump inhibitors may be useful in controlling ciprofloxacin-resistant strains.[10]

Martins et al. (2013) investigated the antibacterial effect of silver nanoparticles with antibiotic inhibitors that inhibit protein synthesis on staphylococcus aureus isolated from cases of bovine mastitis. The results of this study show that; The resistance of Staphylococcus aureus isolates to erythromycin, gentamicin, streptomycin, and doxycycline was 100, 22, 100, and 8%, respectively. Eight percent of the isolates were sensitive to a concentration of 25 micrograms per milliliter of silver nanoparticles. Growth of 98% of the samples was inhibited at concentrations between 50 and 100 µg / ml.[11]

Simhon et al. (2010) were conducted to evaluate the carriers of Staphylococcus aureus and its antibiotic resistance pattern and to compare the results of the medical staff of Sina Hospital in Hamadan and the administrative staff of Hamadan University of Medical

Sciences. The results showed a higher rate of Carreier staphylococcus aureus in medical personnel than in the comparison group. It was also shown that there was no significant difference between the Carreier rate of Staphylococcus aureus nose with age, sex, activity section and duration of hospitalization. All insulated strains were resistant to penicillin.[12]

Podnos et al. (2011), the antifungal effect of silver nanoparticles in acrylic resins. The results of this study show that the anti-fungal effect increased in acrylic resin containing silver nanoparticles with increasing contact time and concentration of silver nanoparticles.[13]

Wales (2011) examined nanoparticles in the diagnosis and treatment of hepatitis C infection.[14] According to the results, one of the best nanoparticles in the diagnosis of this infection was gold nanoparticles. If the right dose of this nanoparticle is used, no toxicity will be applied to the various cells and tissues of the body. In addition, gold nanoparticles, due to their resonant surface pulmonary resonance properties, change the color of the sample solution from red to blue if they bind to the target genome and accumulate. Then, using the colorimetry method, it can be determined whether there is a virus genome in the patient sample. The time required to perform this experiment was very short, in addition, due to the use of small amounts of this nanoparticle in this method, the test will be cost-effective.

Aflax's contribution to the pharmacological resistance of a number of clinical strains is one of the factors that should be considered in the design of new antibiotics or any other active compound. The main challenge is to discover a compound that, as a barrier to Aflax in a non-specific way, targets a wide range of Aflax systems of different bacterial species.

Conflict of Interest: Nil/The authors declare no conflicts of interest.

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Ethical Clearance: Obtained from the Institutional Ethics Committee of Islamic Azad University of Iran.

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Effectiveness of Security Incident Event Management (SIEM) System for Cyber Security Situation Awareness

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Abstract

Cyber-attacks have always targeted information communication technology systems of various organizations. Intruders and hackers have within their reach, very powerful tools through which they capable to bypass the existing network security so as to deliver a payload that might have a severe impact on the whole organization. Therefore, it has become essential for organizations to develop mechanisms through which they can detect a possible cyber threat and then respond accordingly. By establishing cybersecurity situation awareness, organizations will understand what is happening and then respond effectively. The present study evaluated the effectiveness of the Security Incident Event Management (SIEM) system for Cyber Security Situation Awareness. A Hierarchical Network Security Situation Assessment Model (referred to HNSSAM) which joins Security Incident Event Management (SIEM) system evidence theory fusion rules with classified quantitative risk assessment method was applied. Data processing was initially designed so as to collect security data from various sensors. Mechanisms for data verification were then adopted so as to establish whether SIEM was effective in successfully detecting any form of cyber-attack. Results show that SIEM tools may be applied by security analysts to gain visibility into the security threats attacking the IT systems of an organization and then respond appropriately.

Keywords*: SIEM, Security, Cybercrime, IT systems, HNSSAM, Cyber-attack, Network, Technology, Cyber threats, Information.*

Introduction

The development of Information Communication and Technology is argued to have gone hand in hand with the emergence of a number of cybersecurity threats and vulnerabilities. The problem of computer security involves a form of deliberate act that can affect the three essential properties of a given information system [1]. The properties outlined in this case include the confidentiality, for example, the ability of network or computer system to store relevant information which is considered as sensitive to the business in a secured manner and maintaining some aspects of exclusive access to only relevant users who have been designed for that purpose. The second property is integrity, the assurance that all the programs and data or the available information are properly designed and modified only within a given manner authorized or acceptable by the company authorities [4].

The problem of cybersecurity has the potential of impacting on the individual users, both big and small business organizations, resulting into a number of financial implications like direct cost such as theft of money, digital assets and sensitive information [2]. Cyber threats also have the possibility of causing indirect costs in the general form of interrupting the business services, lower productivity, as well as legal liability that takes place due to diverted resource like computing power, personnel, capital and bandwidth which can further lead into some given costs that are associated to the long-term impact of the security attack on the image of the

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organization, bad reputation, competitiveness as well as the financial markets [3].

Despite the challenges associated with cybersecurity, most business companies are constantly going through a radical transformation for the need for embracing the current age of information [10]. This has always ended up making them rely on information technology for the need of handling some important part of their main service deliveries and consequently, a proper asset that is very valuable for the information of the whole organization. Protection against the risk associated with cybersecurity is one of the most important things that the current business organizations are up to [13]. Very many organizations are moving forward towards creating E-service and making their information more digital, convenient, accessible, and secured. But this has greatly come with several risks, a serious cyber threat [5]. The information is likely to be stolen, hacked, wiped or even sabotaged. The literature identifies that cyber breaches are serious threat not only to business but also to the security of all the devices and people involved. As per some statistics, cybercrime actually costs several businesses all over the world more than $400 billion annually. What is even troubling from the perspective of information technology is the fact that cyber-criminals are trying to ostensibly breach secure systems with several layers of protection that are put in place [10]. The present article seeks to establish the effectiveness of Security Incident Event Management (SIEM) system for Cyber Security Situation Awareness among companies. By establishing cybersecurity situation awareness, organizations will understand what is happening and then respond effectively.

Material and Methods

This section proposed a Hierarchical Network Security Situation Assessment Model (referred to HNSSAM) (Figure 1). The model joins Security Incident Event Management (SIEM) system evidence theory fusion rules with classified quantitative risk assessment method and applies confidence level, host importance and service importance. The advantage of using this model is to solve the problem of processing mass data, to offer three different levels of intuitive security threat, and to subsequently establish the weaknesses within the system or rather the security situation.

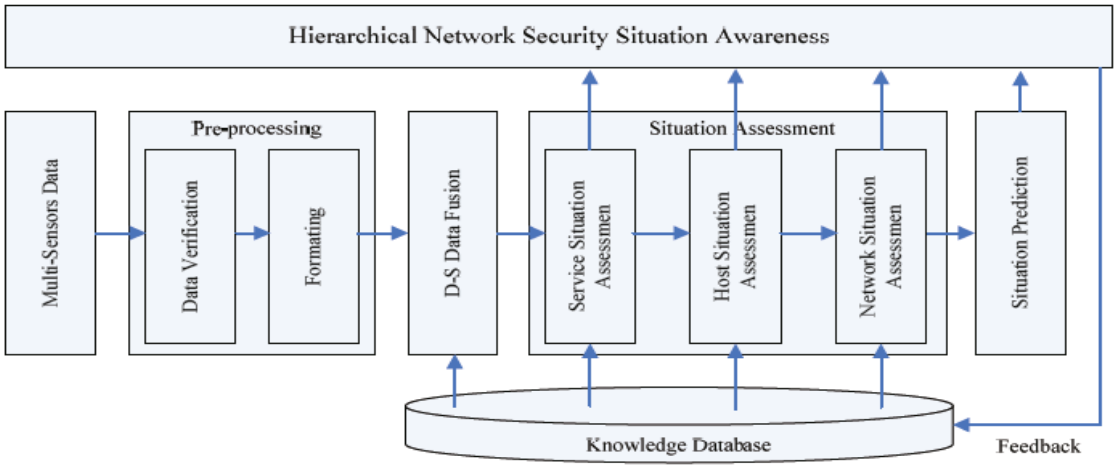


Figure 1: Hierarchical framework for network security situation awareness

Data pre-processing was initially designed so as to collect security data from various sensors. Mechanisms for data verification were adopted to determine whether SIEM was effective in successfully detecting any form of cyber-attack. Through a comparison of relevant conditions as well as configuring information that were considered essential for any form of successful cyber-attack, the researcher would simply remove the non-impact attack alert. For example, IDS did successfully detect a great number of serv-u directory traversal attacks which actually aimed at serve-u software running on Windows system. However, the target host was run on the Linux system, so that attack could not be succeeded. The security data was then converted into some sorts of

uniform format to realize the HNSSAM architecture.

As per the basic description of SIEM, the researcher did set the target framework as either True positive or False Positive. Considering the fact that the alerts that are generated by the security equipment had two possibilities; true positive and false positive, the researcher defined confidence values of an alert as True Positive Rate (TPR): m (correct alerts) = TPR. The researcher did get TPR through the supervised training of the relevant security devices in numerous forms of attacks. The confidence values were subsequently stored within knowledge Base for an additional usage. The fusion process is demonstrated in the figure below.

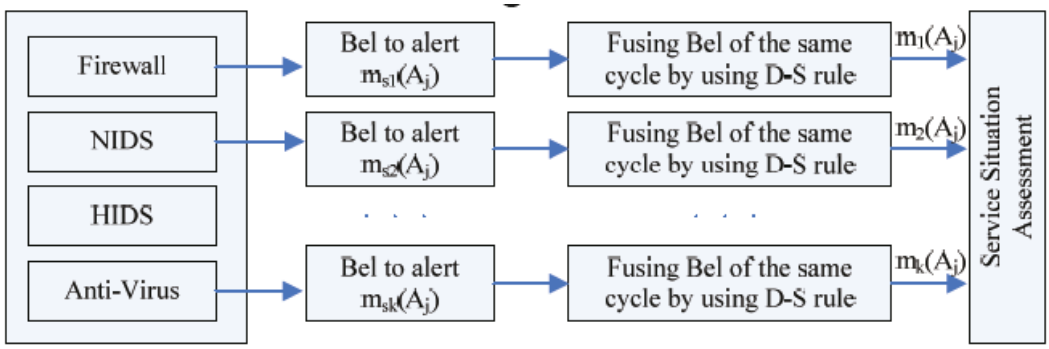


Figure 2: Illustrating the fusion model that is D-S rule-based alert

In order to test the effectiveness of SIEM, the researcher subsequently simulated a multi-sensor network environment as shown in figure 2. In such simulated network environment, the researcher did deploy four distinctive sensors, the firewall at the internet entry, network intrusion detection system, the detection system of host intrusion as well as the specific anti-virus software that are installed within the hosts.

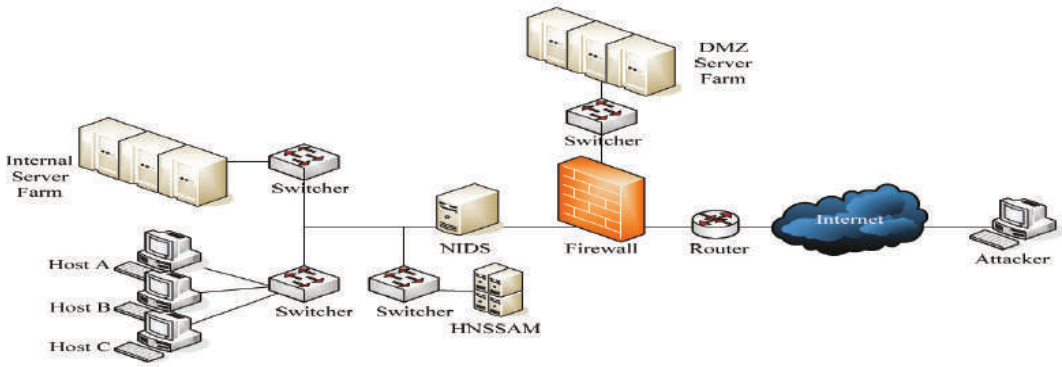


Figure 3: Illustrating the investigational network topology

A day was divided into three different periods: t1 = 0 ~ 8, t2 = 8 ~ 18, t3 = 18 ~24. Each period was subsequently assigned with varied significant level. The observed time T1 and T6 did fall under period t3, T2 ~ T3 felt in the period t1, T4 ~ T5 felt in the period t2. As from T1 to T6, information was collected for SIEM detection of an attack on the host A, B, C.

Based on the collected security data, the researcher looked up the level of confidence that correspond to the security data within the knowledge base. By the fusion rule of DA, the researcher fused the confidence value. The results were then multiplied by the brutality value of attacks within the knowledge base. The results were subsequently drawn as shown in the figure below. It could clearly be demonstrated from the results, as shown in figure 4, that the services of RPC on the Host A suffer a very high level of threats which need to be given first priority. The researcher generated the host security as illustrated in figure 4b. It seen that attacks were more active during the period of T1 to T4.

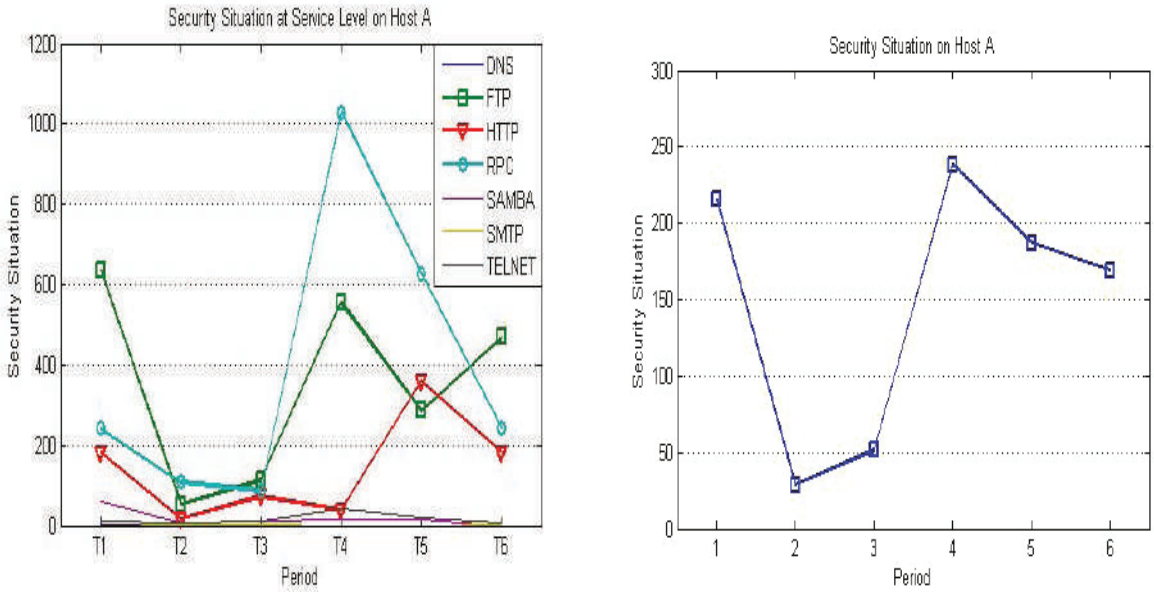


Figure 4. (a) Situation of security in all the services within host A; (b) Host A security situation

As per the analysis of the results that had already been generated, it was possible to draw the security situation of all the hosts as shown in Figure 5a. From the figure, it was possible for the administrators to establish the level of threat on every host.

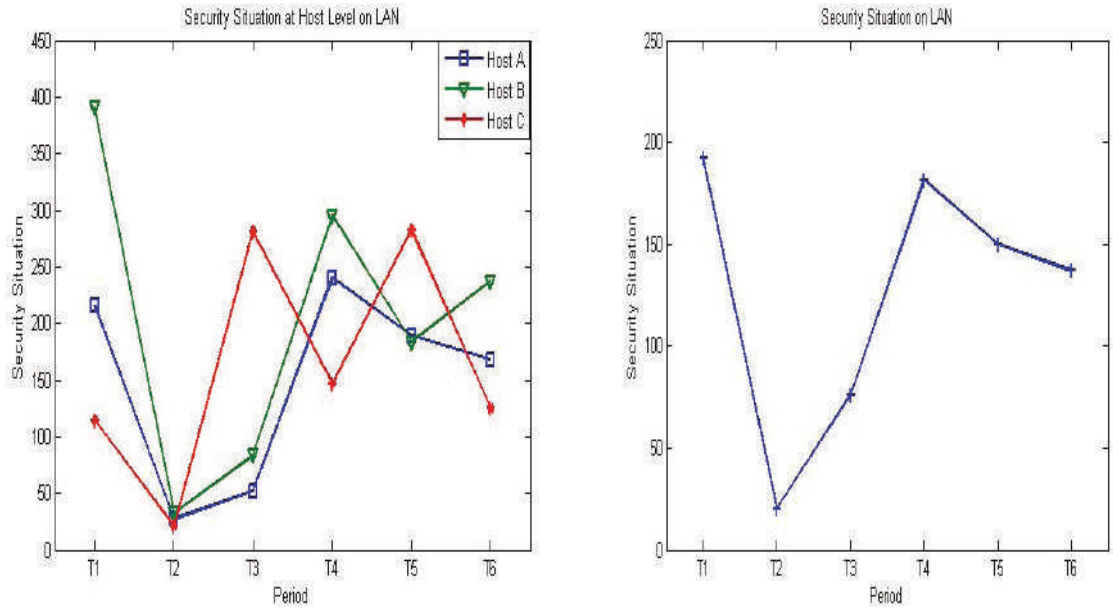


Figure 5. (a) The security situation of all the hosts on LAN; (b) The security condition of LAN

Findings

As per the methodology above, it can be observed that the SIEM framework offer three different levels of cybersecurity situation. The method in this may significantly overcome the shortcomings of the present hierarchical situation awareness systems. It might subsequently help the relevant decision-makers to adjust their policy so as to enhance company security.

These findings show that SIEM tools help security analysts gain visibility into the security threats attacking the IT systems of a given business organization. This is done by examining the logs generated by network devices and searching for signs of an on-going attack. The biggest challenge faced by SIEM systems is the

affectivity of filtering the data being displayed to the security analysts. The SIEM system is able to correlate a large amount of logs multiple sources of logs and identify an attack with a high detection rate and low false-positive rate. This is done while prioritizing alerts so that security analysts can focus on attending to the high threat alerts generated by the system.

Moreover, security analysts are able to classify the different components from the various departments so they can priorities threats and respond to the most critical threats first. As the amount of cybercrime increases worldwide, having a clear legal framework for dealing with it is critical [12]. SIEM system structures the accumulation, analysis and correlation of events that occur from diverse sources. Management of threats, response to incidences and management of log comprise of the key capabilities of the current SIEM solutions.

Even though it has widely been reported that SIEM has matured within the recent years, the system still depends on the previous complex rules in detecting security threats [11]. However, it is important to note that correlation which is rule-based can never be sufficient in protecting information from security threats that are continuously evolving and become dynamic. There is a high need for complementing correlation which is rule-based with techniques which are more advanced like detection of an anomaly for quite a good number of reasons. First, it is a fact that the rule-based system only has the ability to detect incidents that had been defined and observed earlier. In most of the circumstances, a zero-day threat which is not known might fail being detected by the current appliances and hence results into great damage due to leakage of data or downtimes of the network [6].

Discussion

SIEM is a security tool that can be utilized in strengthening the security of communication and information systems just the same way it is always witnessed with firewalls, antivirus software and schemes for access control. It is a kind of device or application of the software that screens system or network activities that are related to violation of the policy or malicious activities and release a report to the station of management [8]. Even though this technology of detecting the intrusion is somehow immature and hence need not to be considered as a reliable defence, literatures argue that it plays some important role within the information security architecture [18].

Since any form of deployment is likely to incur some costs for maintenance and operation, each individual organization utilizing this kind of device need to consider the full life cycle of SIEM before coming up with a valid choice [9]. In the event that SIEM is installed appropriately, it would be providing some warnings that indicate that the system is being attacked from elsewhere even in the circumstances when there is no specific attack that the system is vulnerable to [15]. Such warnings can assist the users in altering the defensive posture of the installation so as to increase the resistance of the system to the attack. Additionally, SIEM might also serve to confirm operation and configuration which is secure of other mechanisms of security like firewalls. With regards to the disadvantages it is possessing, it can be applied for defensive posture; though need to be fully relied upon as the only means of system protection [16]. The situation of information system security is likely to worsen as the issues of e-commerce continue to become more attractive to the targets.

A network-based SIEM system, on the other hand, is a kind of system that is designed to detect malicious activity for instance service attacks denial, port scans or even any form of attempts of cracking into the computer by network traffic monitoring [17]. This kind of system is able to read all the packets that are incoming with the aim of getting any form of suspicious patterns described as rules or signatures. If for instance, a big amount of TCP connection requests to numerous numbers of dissimilar ports is observed, then it is possible for one to assume that there is an individual who is doing a port scan to all the computers found within that network [19]. The third classification of SIEM, which is anomaly-based, revolve around first determining the behaviour profiles of the users, programs or other resources that have an interest to the given system and then making an observation to the actual activities as generated within the audit data so as to detect any deviation considered significant from the existing profiles [14]. Detection of an anomaly, in this case, is statistical in their nature. In order for the system to detect security threats at its early stages, all individual's system log files need to be analyzed and correlated [7].

Conclusion

Cyber-attacks have been, in many instances, targeted on information communication and technology systems of different organizations. Hackers and intruders currently have within their reach; very lethal tools through which they can safely bypass the existing network security to deliver payload that have a great negative impact on the entire system. Cyber threats have always continued to exploit the connectivity and complexity within the important infrastructure to make a plan and launch attacks on the existing computer systems. This has been a great challenge experienced by different business organizations. In order for any business organization to be safe from cyber threats, they require a complex security system that can protect the existing computer networks from dangerous threats. The security system can comprise of firewalls, a system that detects and prevent intrusion and solutions for path management, together with some strong anti-virus.

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Barcodes in Naming Basic Denture for Forensic Identification Needs

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Abstract

Background: discipline within Forensic odontology is a part of the science of forensic medicine which recently demonstrated its role in the identification of victims, often confronted with various obstacles, to slow down the investigation of the identity of the victim. There are obstacles in the identification process, partly due to the incomplete identification data that can be collected, so that the identification of the remains becomes difficult and requires a long time to be confirmed despite obtaining good professional management in the identification process. The American Board of Forensic Odontology shows that most identification uses teeth based on restorations, caries, missing teeth and / or prosthetic devices such as removable dentures of the victim. The necessity of naming the removable denture as the data themselves are attached to the patient can facilitate the identification of **Purpose:** Knowing how to manufacture and benefits of identity with a barcode on the denture in the easy identification of individual **Methods:** this research was pre-experiment with only posttest design. By using 10 Full Denture samples, the sample collection was obtained from the Lemeshow Formula. The ten research samples were taken by simple random sampling. **Conclusion** barcode contain identity data embedded in the denture base can be used as labeling on denture users because of their role in knowing the identity of individuals , and hacyl of reading barcodes are not influenced by the length of the base submerged .

Keywords: *Barcode, Denture Base, Forensic identification*

Introduction

The number of disasters occurring in Indonesia is increasing. Condition is reviewed of the mass media which often times contains news about the occurrence of disasters, such as acts of terror bombing, accident transportation, earthquakes, tsunamis, floods, soil erosion, eruption mountain volcanic, putting pickaxe, and others[1] .

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The National Disaster Management Agency has data on disaster events in Indonesia and the number of disasters has tended to increase in the last ten years. Law Number 36 the Year 2009 regarding health has given a mandate to the government and the community to make efforts to identify victims of accidents. The identification of the dead was carried out to fulfill the victim's right to be returned to the family and to be buried properly by his beliefs during his lifetime. In this case, forensic identification in terms of identity that supports the identification of a victim can be a non-biological identity, in the form of a resident card , driver's license, clothing, and biological identity can be known through bones, teeth, blood, fingerprints, hair, profile, and DNA[2] .

The field of Forensic Odontology is a part of medical

forensic science which has recently shown its role in the effort to identify victims, not infrequently faced with various obstacles, to slow down the investigation of the identity of the victim. There are obstacles in the identification process, partly due to the incomplete identification data that can be collected, so that the identification of the remains becomes difficult and requires a long time to be confirmed despite obtaining good professional management in the identification process[3]. The American Board of Forensic Odontology shows that most identification using teeth is based on restorations, caries, missing teeth and / or prosthetic devices such as removable dentures of the victim[4–6].

In Indonesia, the labeling of denture prostheses is rarely done, the labeling itself is only done by the lab and dentists limited to labeling the Denture so as not to be confused during the delivery or use of Denture by the dentist to the patient. Agency Odontology Forensic and International Dental Association recommend two Denture Marking System to simplify and facilitate the identification of a person's identity, namely method surface and inclination method[7].

Surface methods include engraving methods[8,9], which are letters and numbers engraved on the base of the denture, and the method of embossing which produces letters that arise on the base of the denture[10,11]. Whereas the second method is the inclusion method. The inclusion method consists of the ID Band method and the Barcode method, the ID Band method using stainless steel which is planted on the base of the denture. In addition to the ID Band method there is the Barcode method, the data will be stored in a barcode format and planted on a denture base[12]. From these methods there are advantages and disadvantages of each, and in this study the Barcode method is used because it is easier to apply.

The method of marking identity or code on dentures is very important. Identification of unknown or lost by tagging denture identification method is very good in the forensic investigation. This is also useful for patients who live in hospitals and community homes because dentures may be misplaced, for example when cleaning by an operator there will be a chance of losing false teeth or mixing with other people's property[13].

Materials and MethodS

The research pre-experiment with posttest only design. By using 10 Full Denture samples, the sample collection was obtained from the Lemeshow Formula. The ten research samples were taken by simple random sampling, the research samples were taken from Full Denture Skillab Prosto Students in the old school year that have been collected and assessed

All study samples were reduced at the base by using bur fissures on the palatal for maxillary dentures and lingual for mandibular dentures. Depth of reduction of ± 1.5mm with a width of 6-7mm.

Then the barcode has been prepared. Contains identity card data, medical record number along with where individuals have been treated, and website link information planted on a base facing the surface, then some that have been reduced and given a barcode closed with self-cured acrylic, and done finishing and polishing.

Samples that have been planted by the barcode are soaked for 4 weeks, and every week they are checked to see if there are any leaks or damage in the barcode planting. And then the barcode reading process is carried out by means perform scanning in barcodes and data matching.

Results

In Table 1 it can be seen that the most readable barcodes are in the first week, 9 samples with a percentage of 90%, and in the second week to the fourth week are 8 samples with a percentage of 80%.

After obtaining the results from the base that contains the barcode and soaked in water, the data were analyzed using the Kruskal Wallis test[14]. The Kruskal Wallis test is carried out to see whether there are significant differences in the barcode readings that are done every week. The results of the Kruskal Wallis test are shown in Table 2. Can be seen the statistical value of 0.0917, which is greater than 0.05. This means that in this study there was no significant difference from the barcode that was immersed from the first week to the fourth week.

Tabel 1. The results of the barcode scan are implanted on an acrylic denture base after immersion in water for 4 weeks

Long Immersion		**Week 1**		**Week 2**		**Week 3**		**Week 4**	
The resulting value is scan		**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
The sample	1		√	√		√		√	
	2		√		√		√		√
	3		√		√		√		√
	4		√		√		√		√
	5		√		√		√		√
	6		√		√		√		√
	7		√		√		√		√
	8		√		√		√		√
	9	√		√		√		ü	
	10		√		√		√		√
Total (%)		10	90	20	80	20	80	20	80

Information: 0 = scan unreadable

1 = unreadable scan

TABEL 2 Kruskal Wallis Test Results from barcode reading after soaking in water for 4 weeks

Statistic	**0.506**
df	3
Sig.	0.917

Information:

df = degree of frequency (n-1)

Sig. = P> 0.05 = Ho accepted (no significant difference)

Discussion

The research on giving a barcode containing the identity of an individual planted on an acrylic removable denture base and continued with immersion in water for 4 weeks, aims to see whether the barcode can still be read after being submerged in water for a long time.

Based on the results of the study found that in the first week there is 1 barcode that is not readable and in the second week to the fourth week each has 2 barcodes that cannot be read. This is caused by several things that affect the barcode so that the scanning results cannot be read. The cause of the writing on the barcode cannot be read due to the presence of liquid on the barcode, which is in the form of a clear self-cured acrylic liquid (monomer) or water from soaking. Fluid liquid (monomer) self-cured acrylic can damage plastic laminate coatings barcode, because the nature of the monomers may damage the plastic. This will happen if when you put the self-cured dough in a soft condition, which means there are still many monomers left. So that the rest of the monomer will damage the laminated plastic, and the liquid will damage the barcode, so the barcode scanner cannot be read.

In addition to the influence of liquid-liquid, the entry of immersion water used can also affect the damage of the barcode. This happens because the attachment between the denture base and the self-cured acrylic barcode cover is less than perfect. So that water can enter and affect barcode writing. Of course, the barcode will be affected because there is a leak from the plastic laminate. Leakage or imperfect adhesion between the acrylic base

of the denture and the self-cured clear acrylic barcode cover can be caused because there is dirt that disturbs the attachment between the two acrylics[15].[15,16] Thus the barcode will be damaged so it cannot be read.

The two liquids above can hit the barcode if a leak occurs in laminated plastic. This can occur due to poor attachment. In addition to the leakage that occurs in plastics, damaged plastic laminates can also affect. Damaged plastic laminates can occur because plastic laminates are damaged before installation. The laminated plastic surface of the barcode which has been damaged before being installed on the denture and then comes into contact with the self-cured clear dough, so that the barcode cannot be read.

It is better if the leak on the barcode naming needs to be aware so that the denture used can still be identified for law enforcement[17]. Where an unknown body must be made an identification effort[18]. Often corpses that need to be identified are old, because of that the naming of dentures by the barcode method should not occur leaks or damage in a long time.

Dental denture material is a means for identification that can be trusted to be able to survive in high altitude, especially if the records and photos of teeth when they were still alive are still well preserved[19]. This dental examination becomes very important if the body is already in a state of decay and damage, such as fire[20].

From the table data above, it can be seen with the barcode system on the denture plate that during the mass identification process, in the fourth phase of reconciliation[1], it can be searched quickly using this barcode method. Dental data, fingerprints and even DNA data can be found quickly. When the barcode data is done the scanning process.

Conclusions

In conclusion, the results of the barcode reading are not influenced by the length of the base submerged, but are influenced by other factors, namely an error occurred in the barcode planting process. And barcode contains identity data embedded in the denture base can be used as labeling on denture users because of their role in knowing the identity of individuals when needed in an emergency / certain.

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Knowledge, Attitudes, and Practices of Staff and Students at Sulaimani Polytechnic University towards COVID-19/Iraq

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Abstract

COVID-19 is a highly infectious and pathogenic viral disease, its become a global pandemic infection, which is an airborne disease; transmits directly and indirectly between infected cases and other people. Until now, there has been neither vaccine nor specific antivirus medicine against this disease, the only method to prevent the spread of the virus is a preventive practice against the disease, it depends on the knowledge and attitudes of people towards the disease. The online cross-sectional study was undertaken during the pandemic, to estimate Knowledge, Attitudes, and Practices towards COVID-19 among a sample of staff and students at Sulaimani Polytechnic University. Data of 270 adult individuals were collected during June 2020 and analyzed. The male/female ratio was 1:88. The mean scores of knowledge, attitudes, and preventive practices of the participants were 7.4 (SD 1.68), 5.5 (SD 1.15), and 6.7 (SD 2.1) respectively. The results reported significant differences between respondents' knowledge mean score and their gender, and educational background (P=0.005 and P=0.003 respectively). The attitudes mean score of the staff was significantly greater than the attitudes mean score of the students (P=0.021). Likewise, the attitudes mean score was significantly greater in medical subjects compared to non-medical individuals (P<0.001). The respondents with age group ≥45 had a significantly higher preventive practice compared to the other age groups (P<0.001). Similarly, the practices mean score was significantly greater in the staff compared to the students (P< 0.001). The vast majority of the participants (88.5%) used social media for obtaining knowledge and advice on the disease. The study indicates that the respondents had sufficient knowledge, positive attitudes, and good practice toward COVID-19.

Keywords: *Knowledge, Attitude, Practice, COVID-19, Staff, Students, Sulaimani Polytechnic University.*

Introduction

Coronavirus disease 2019 (COVID-19) is a quickly expanding pandemic, produced by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2). COVID-19 was first diagnosed in December 2019 among cases with viral pneumonia symptoms in Wuhan, China [(1)]. The disease causes a wide range of clinical appearances in patients, including fever, dry cough, myalgia, fatigue, and dyspnea. Approximately one-fifth of Chinese patients with COVID-19 progress to the severe stage [(2)]. Elderly persons as well as those with underlying health conditions are at the highest risk for serious infection and death due to COVID-19 [(3)]. World Health Organization (WHO) recommends the most effective preventive measures, including maintaining physical distance, at least 3 feet away from other persons, the hands must be cleaned promptly after contact with the respiratory tract; stop touching eyes, nose and mouth repeatedly; regular cleaning and disinfecting environment and other repeatedly touched surfaces; living space airflow should be improved by opening as many windows and doors as possible; individuals with fever, cough, and dyspnea, should seek immediate treatment [(4)]. Vaccine development is estimated to need

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several months, thus management of the crisis depends mainly on people's obedience to the recommended measures taken. These measures are chiefly affected by individuals' knowledge, attitudes, and practices regarding the disease [5]. KAP studies provide standard information to determine the category of intervention that maybe necessary to change misunderstandings about the virus, as well as the development of preventive policies and health promotion programs [6]. The main aim of the current study is to investigate the level of knowledge, attitude, and practice of the staffs and students of Sulaimani polytechnic University, toward COVID-19 infection.

Methods and Material

Subjects and study design

The online-based cross-sectional study was performed from 1 to 30 June 2020, among the staff and the students of Sulaimani Polytechnic University. This study was carried out using an online self-administrated questionnaire which consisted of background characteristics, knowledge, attitudes, preventive practices of participants, and the sources of getting knowledge on the disease. The questionnaire was arranged into a Google Form, the link of the form was sent to the staff via their Gmail of the university, and distributed to Facebook and Viber groups of the students. The subjects were selected through convenience sampling method. The present study involved 270 individuals. University staff who had university Gmail, as well as students who participated in the students' Facebook or Viber groups, were eligible to take part in this study. Most of the knowledge, attitudes, and practice items were developed from the advice of the World Health Organization on Coronavirus disease (COVID-19) for the public [7]. Regarding the scores of knowledge, attitudes, and practices, each correct answer was worth one mark, whereas zero was given for incorrect answers.

Statistical Analysis

Statistical Package for the Social Science (SPSS version 22, IBM Statistics.inc) was used for data analysis, with consideration procedures to secure the high quality of data and reducing error. These involved testing questionnaires, data entry, regularity checked all data collection and entry. To compare between means of two samples, an independent-sample t-test was used, and a one-way analysis of variance (ANOVA) was used to compare among more than two means. P-value ≤ 0.05 was used to display a level of significance. Quantitative and qualitative variables were reported respectively as a mean± standard deviation (SD) and percentage. Texts, Tables, and Figures were used for showing the results.

Ethical considerations

The individuals, who participated in this study, were informed about the purpose of the study, and they were free to engage in the study, as well as, the participation was voluntary. The privacy of the collected data was guaranteed. The study has been accepted by the presidency of Sulaimani Polytechnic University.

Results

Socio-demographic characteristics concerning mean scores of knowledge, attitudes, and practices

Overall 270 individuals participated in this study, 126 (46.7%) were male and 144 (53.3%) were female; the male-female ratio was 1:88.. The mean score of knowledge, attitudes, and protective practices of the respondents was 7.4 (SD 1.68), 5.5 (SD 1.15), and 6.7 (SD 2.1) respectively. The range of knowledge, attitudes, and preventive practices of the subjects were 9 (1-10), 6 (1-7), and 8 (1-9) respectively. The results indicated significant differences between respondents' knowledge mean score, and their gender, and educational background (P=0.005, and P=0.003 respectively). Regarding attitudes, the mean score of the staff was higher than the mean scores of the students, as well as the mean scores of the medical individuals, which were higher compared with the non-medical individuals. These differences were statistically significant (P=0.021, and $p<0.001$ respectively). Concerning safe practices, the age group ≥ 45 had a greater safe practice mean score compared to the other age group ($P<0.001$). Also, the staff had a greater mean score than students ($P<0.001$).

Participants' knowledge regarding COVID-19

The results reported that the respondents were aware of COVID-19 (Table 1).

Table 1. Participants' knowledge regarding COVID-19 (Number = 270)

Participants' knowledge items	Number	Percent
COVID-19 is transmitted by close contact with the infected person (True)		
True	220	81.5
False	26	9.6
Unsure	24	8.9
Fever, cough, sore throats and shortness of breath are possible symptoms of COVID-19 (True)		
True	218	80.7
False	22	8.1
Unsure	30	11.1
Covid-19 may cause a loss of taste and smell (True)		
True	153	56.7
False	34	12.6
Unsure	83	30.7
Vaccine is available in the markets (False)		
True	32	11.8
False	193	71.5
Unsure	45	16.7
Antibiotics are the first-line treatment (False)		
True	70	25.9
False	123	45.6
Unsure	77	28.5
Washing hands with soap and water, and using face masks can help in the prevention of disease transmission (True)		
True	266	98.5
False	3	1.1
Unsure	1	0.4
Patients with underlying chronic diseases are at a higher risk of infection and death (True)		
True	223	82.6
False	20	7.4
Unsure	27	10.0
Healthcare workers are at a higher risk of contracting COVID-19 (True)		
True	244	90.4
False	10	3.7
Unsure	16	5.9
COVID-19 could be fatal (True)		
True	197	72.9
False	31	11.5
Unsure	42	15.6
The maximum incubation period of the COVID-19 is: (Up to 14 days)		
Up to 7 days	17	6.3
Up to 14 days	187	69.3
Up to 28 days	26	9.6
Don't know	40	14.8

Respondents' attitudes toward COVID-19

Overall, the respondents had a positive attitude toward COVID19 (Table 2).

Table 2: Respondents' attitudes toward COVID-19 (Number = 270)

Respondents' attitudes items	Number	Percent
Infection with the virus is associated with stigma (Disagree)		
Agree	18	6.7
Disagree	244	90.4
Unsure	8	3.0
People can get infected If they contacted an infected patient despite their strong beliefs (Agree)		
Agree	230	85.2
Disagree	15	5.5
Unsure	25	9.3
People can get infected If they contacted an infected patient despite their good immunity (Agree)		
Agree	229	84.8
Disagree	18	6.7
Unsure	23	8.5
Buying medical glove and face masks are necessary for this disease (Agree)		
Agree	237	87.8
Disagree	25	9.3
Unsure	8	3.0
People are worried about family members may get an infection (Agree)		
Agree	240	88.9
Disagree	21	7.8
Unsure	9	3.3
If I feel getting COVID-19, I will visit a hospital (Agree)		
Agree	232	85.9
Disagree	16	5.9
Unsure	22	8.2
People think the media coverage about this disease inflated (Disagree)		
Agree	151	55.9
Disagree	87	32.2
Unsure	32	11.9

Participants' practices towards COVID-19

Majority of the subjects followed safe practices toward COVID-19 (Table 3).

Table 3. Respondents' practices toward COVID-19 (Number = 270)

Practices of the respondents against COVID-19	Number	%
Avoid handshaking (Yes)		
Yes	195	72.2
No	75	27.8
Wash hands with water and soap for at least 20 seconds regularly (Yes)		
Yes	205	75.9
No	65	24.1
Use of disinfectants to clean hands when soap and water was not available for washing hands (Yes)		
Yes	216	80.0
No	54	20.0
Covering mouth and nose when I cough or sneeze (Yes)		
Yes	239	88.5
No	31	11.5
Wearing a face mask and glove when leaving home (Yes)		
Yes	178	65.9
No	92	34.1
Avoid going to crowded places (Yes)		
Yes	230	85.2
No	40	14.8
Avoiding touching my eyes, nose, and mouth with unwashed hands (Yes)		
Yes	218	80.7
No	52	19.3
Keeping at least one meter between me and other persons outside my household (Yes)		
Yes	151	55.9
No	119	44.1
I eat healthy food, such as fruit, vegetables, bean or meat regularly (Yes)		
Yes	164	60.7
No	106	39.3

Sources for getting knowledge

The participants in the current study used more than one source for receiving information on COVID-19. The figure illustrated that the respondents highly used social media followed by television and radio, as well as the ministry of health, for obtaining knowledge (88.5%, 81.1%, and 74.8% respectively) (Figure1).

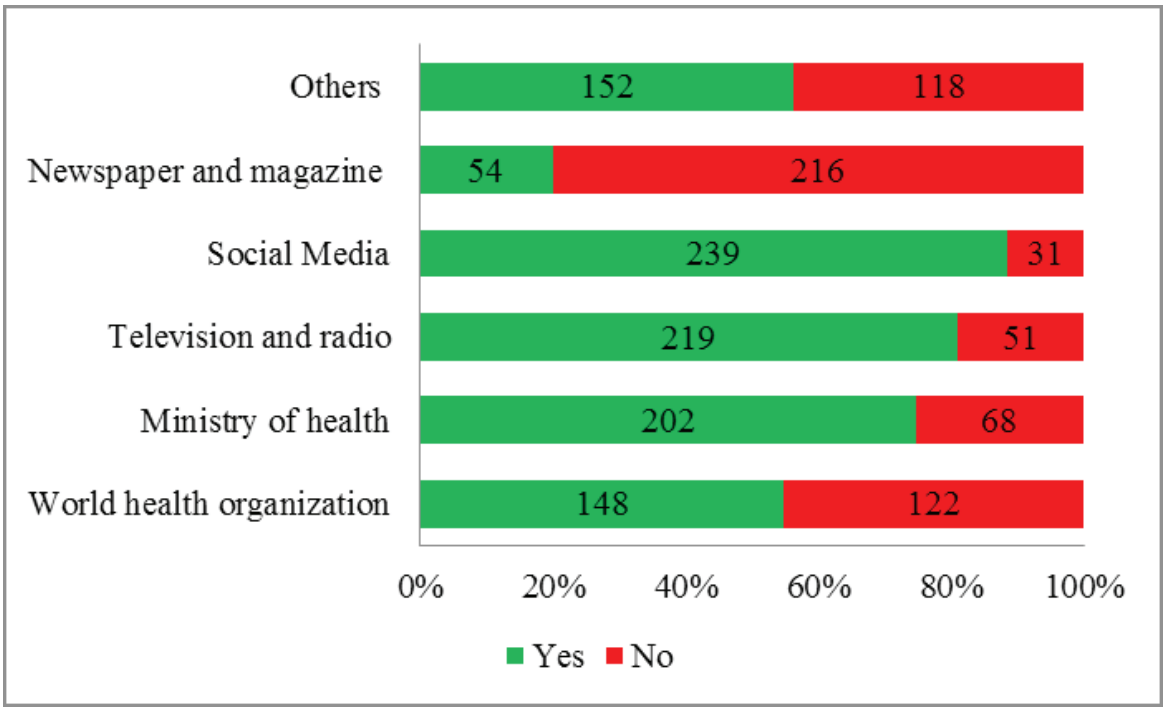


Figure1. Frequency of Knowledge sources

Discussion

Knowledge, attitudes, and practices mean scores of participants regarding their socio-demographic characteristics

The current study indicated that there were significant differences between males and females in relation to knowledge (P=0.005), males had a greater knowledge mean score than females, this finding was in agreement with a result of a study that was carried out in China [8]. The present study demonstrated significant differences between mean score among medical and non-medical respondents in related to knowledge and attitudes toward COVID-19, medical individuals had a greater score than non-medical subjects (P=0.021, and p<0.001 respectively), our findings are inconsistent with the results of a study was undertaken in Jordon [9] this is maybe due to the medical individuals in the present study had more enthusiasm toward the disease. The current study reported significant differences between the mean score of safe practices and ages of participants (P<0.001), this result is in concordance with the finding of a study was conducted in Saudi Arabia[10].

Respondents' knowledge toward COVID-19

The results indicated that the respondents were aware of COVID-19, this figure is in agreement with the findings of two studies that were conducted in China and Bangladesh [4, 11]. Furthermore, a study found that the majority of people were knowledgeable about COVID19[10]. This is maybe due to the nature of the disease, which is a severe and fatal disease, and the COVID-19 viruses have the ability to infect any individuals regardless of their age, gender, race, and geographical location. These factors encourage people to seek and ask for scientific information and advice about the main features of the disease, its effects, and how do protect themselves from this malady.

Participants' attitudes toward COVID-19

Overall the participants had an optimistic attitude

toward the disease. Likewise, the results of two other studies indicated that people had positive attitudes toward COVID-19 [1, 10]. The participants in our study had adequate knowledge of COVID-19, which had a positive effect on their attitudes. A study found that good knowledge about COVID-19 is associated with positive attitudes and suitable practices toward COVID-19 [2]. On the other hand, over half of them believed that media coverage exaggerated the disease. On the contrary, a study was carried out in Egypt that showed, that three-quarters of the subjects believed the media coverage did not overstate COVID-19 [5]. In a fact, the media are doing well, covering the main aspects of the disease. Frequently broadcasting, scientific information and news on this fatal infectious disease is a good way to encourage people to obey instructions of the Ministry of Health.

Subjects' practices toward COVID-19

Totally, the remarkable number of the respondents, followed protective practices against COVID-19, this finding is in agreement with the result of a study that was carried out in Pakistan [12]. The majority of the respondents in the current study had a great knowledge score toward the disease, two studies reported that higher knowledge scores related to higher preventive practices toward COVID-19 [4, 12]. However a bulk number of the subjects followed safe practices toward the disease, yet some participants did not comply with the protective practice against the disease, such as keeping a social distance (44%), wearing face mask and gloves (33.1%), avoiding handshaking (27.8%), washing hands (24.1), and avoid crowding places (14.8). Which increased the chances of spread and transmission of the disease among people [13].

Sources for getting knowledge

Social media was the most common source for receiving knowledge about COVID-19, this result is consistent with the findings of two studies that were performed in Egypt and Pakistan [5, 12]. The Internet has increasingly become an accepted source of health information by connecting people with health content, specialists, and support [14]. In addition, physicians and health professionals give to online health information [14].

Conclusions

The participants in this study had sufficient knowledge and a positive attitude toward COVID19. Similarly, the majority of the subjects followed preventive practices against COVID-19. Middle age individuals had a good practice toward the disease. The most common sources for obtaining knowledge were social media, television and radio, and the Ministry of Health.

Ethical Clearance: Taken from the ethics committee of Sulaimani Polytechnic University.

Source of Funding: Self

Conflict of Interest: Nil

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Histopathological Changes in Skin-A Tool to Establish Time Since Death in First 24 Hours

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Abstract

Aim: Determination of the time since death is one of the greatest challenge in the practice of Forensic Medicine. There is currently very limited scientific information regarding the process of cutaneous decomposition. This study is aimed at throwing more light towards this field.

Method: 30 skin samples from human cadavers are collected where actual time of death is known, from Yenepoya Medical College, Mangalore. Skin samples (1 x 2 cm) from the anterior chest were collected, parallel to the longitudinal midline incision. Tissues were fixed in 10% formalin, embedded in paraffin wax, and stained with hematoxylin-eosin stain. The skin samples were observed under light microscope for the changes.

Results: Epidermis and Dermis showed marked changes in the histology with increasing PMI and is the most reliable site to look for.

Conclusion: This study will throw a better light to post-mortem skin changes and their role in determination of the time since death.

Key words: *Histology of skin, time since death, postmortem microscopic skin changes.*

Background

Many methods have been attempted to accurately and systematically determine the estimated time of death in autopsy cases. Gross changes of the skin which occur during the post-mortem interval have occasionally been used to estimate the time of death under different conditions; however, the post-mortem gross and histologic changes of the skin have not been systematically analyzed. In this study, an attempt was made to determine the post-mortem skin changes through microscopy and correlate it with the time since death.

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Introduction

An important problem in forensic medicine is the need to fix the probable time of death. It is known that as the interval of time between death and the examination of the body increases, limits of probability also widens out. In most of the investigative procedures, knowing the approximate time is important for the investigating personals to make sure that their investigation is going through the right direction. This places a heavy responsibility on the shoulder of a forensic surgeon who opines regarding the probable time of the period of death.[1]

Structurally, the skin is composed of three major layers which were different physiologically,

histologically and embryologically. The outer layer or epidermis is formed by epithelium and is ectodermal in origin. The dermis, the next layer consists of connective tissue and is of mesodermal origin. Epidermis consists of stratified squamous keratinised epithelium in which five layers can be identified, namely, stratum basale, Stratum spinosum, Stratum Granulosum, Stratum lucidum and Stratum Corneum arranged from deep to superficial.

The basal layer is made up of single layer of columnar cells which lies on basal lamina. It contains the stem cells that undergo mitotic division to form new cells, the keratinocytes. This layer is therefore, also known as stratum germinativum .The renewal of the human epidermis takes about 3 to 4 weeks.

Objectives of the Study

1. To observe various histological changes in skin and its appendages.

2. To study time relation between these histological changes and postmortem interval

Review of Literature

Lovas JGL [2] in 1986 conducted a study, apoptosis in human epidermis by electron microscopy, on skin samples excised from the middle of the medial aspect of the upper arm of m106 consecutive autopsy cases. The time interval between death and biopsy was less than 24 hours in all but three cases. The tissue was fixed overnight in, the tissues were stained Thick sections for light microscopic (LM) survey were stained with toluidine-blue. Thin sections for EM were stained with uranyl acetate and lead citrate, and examined using a Zeiss EM 109 transmission electron microscope. He observed Apoptotic keratinocytes in 95 of the 106 cases. Electron microscopic study of 25 EM blocks of 18cases revealed seven AKs. Apoptotic Keratinocytes was observed in histologically or ultra structurally normal cells. It was not known about any apoptotic activity in epidermis except apoptotic activity in the liver which is induced is experimentally. He observed that when oxygen tension falls below the survival of epidermis is not possible and entire epidermis will undergo necrosis.

Kovarik C, Stewart D, Cockerell C[3] conducted a study in University of Tennessee Anthropology Research Facility in Knoxville, Tennessee, between September 30, 2003, and October 8, 2003 on 3 dead which was donated for research purposes. Bodies were kept outside unclothed in a dry shaded wooded area in a cool to temperate climate (temperature range 38–77°F during the week of analysis), and skin biopsies were systematically taken for analysis. Skin samples were taken from scalp, sole of the foot, dependent area of the trunk. Each specimen was sectioned and stained with hematoxylin and eosin (H&E) for microscopic analysis. They were then analyzed by an experienced forensic pathologist and dermato-pathologist. The general appearance of the skin was normal .From days 1 to 7, except for increased wrinkling in the acral skin. But on histological microscopy he found 3 significant histologic changes were noted, including cleavage of the epidermis from the dermis, eccrine duct necrosis, and dermal degeneration. The post-mortem interval is difficult to determine in autopsy cases in gross examination since many factors influence the rate of decomposition, including the cause of death, condition of the body at the time of death, movement of the body after death, temperature, humidity, and animal activity.

On microscopy he observed separation of the epidermis from the dermis in several skin biopsies. This focal separation likely leads to the gross appearance of bullae on the skin with further decomposition. Another histologic finding he observed was appreciated in many of the skin biopsies was eccrine gland necrosis. The timing of eccrine duct necrosis from the acral skin and scalp was not predictable.

Bardale RV, Tumram NK, Dixit PG, Deshmukh AY [4] conducted a study at the Department of Forensic Medicine, Government Medical College Hospital, Nagpur. They observed normal morphology of the epidermis and the dermis 0-6 hours. Appearance of vacuoles in cytoplasm of basal layer and spinous layer. Rarefaction of the dermis, elastic fibers get prominent with focal fragmentation in 6-9 hours. Distorted basal layer cells with incontinence of melanin pigment, focal epidermo-dermal separation 9-12 hours. Fragmentation of the dermis in 18 hours samples

According to Knight B [5] many methods are followed for determination of the post-mortem interval. Whatever be the method used all the variable factors must be taken into account along with the light of previous

experiences, for giving a final opinion. When time of death is decided upon ,it must be used to construct a "bracket of probability", giving an earliest and latest time between which the doctor feels that death must have occurred instead of quoting a single point of time to the investigator. This time bracket should depend on the uncertainty of the factors.

Materials and Methods

The study was conducted at the Department of Forensic Medicine, Yenepoya Medical College, Mangalore from August 2012 to August 2014. The study consists of 30 human dead bodies comprising of 26 male and 5 female, and their age ranged from 20 to 64 years. The death was due to various causes (head injury n = 12, hanging n = 6, poisoning, n =8 Intracranial hemorrhage n = 12, Natural death n=3, Electrocution n= 2,). The bodies were kept at room temperature in the waiting room of the mortuary. The average ambient temperature during the period varied from 23-C to 37-C, and the average humidity ranged from17% to 87%. At autopsy, skin samples from the anterior chest were collected, parallel to the longitudinal midline incision. The cases were divided into 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hour intervals after death.

Materials Used

Dissection set, metal capsules for sample collection, 10% formalin, tissue processer, microtome, L-blocks, wax, slides, covering slip, H & E stain, PAS stains, microscope, digital camera.

Methods

2 skin samples from each dead body were collected from those bodies where actual time of death was known. Written consent was obtained from the relatives of the deceased. Certain inclusion and exclusion criteria were formulated as described below.

Inclusion criteria: 1. Sample collected from cadavers where actual time of death is known

Exclusion criteria: 1. Cadavers with extensive skin diseases.

2. Cadavers with extensive tattoos over chest region.

3. Those cadavers where actual time of death is not known.

4. Death due to burns

5. Body submerged in water

Skin samples (1 x 2 cm) from the anterior chest were collected, parallel to the longitudinal midline incision.

The skin samples will be carefully taken to avoid traumatic artefact. .The samples were carefully dissected out and immediately transferred to a capsule. Tissues were fixed in 10% formalin, embedded in paraffin wax, and stained with hematoxylin-eosin (H and E). The skin samples were observed under light microscope for the changes in the epidermis and dermis.

Observations

The following observations are seen in the microscopic study of the samples collected. The findings in epidermis and dermis are recorded and separately tabulated in tables- 1-5 respectively in accordance with PMI.

In epidermis, (TABLE:1)up to 0-6 hours. PMI, normal histological features are maintained. Minimal vacuolation appeared from 6th hour onwards. All samples at 6hours PMI showed vacuolations [figure.1] which is showing a tendency to become prominent over time.

Samples taken at 6-12 PMI period, up to 8 hours, normal histology is maintained even though 1 sample at 9 hours also maintained the same. Vacuolation started by 9 hours and is becoming a prominent feature by 12 hours. In 12-18 hours PMI periods, Normal histology is maintained up to 13 hours after which vacuolation started appearing and becomes a prominent feature by 18 hours. No samples showed normal histology after 18 hours and vacuolation was an established feature.

Dermis showed a normal histology up to 5 hours in s samples 0-6 hours. PMI samples, however, 2 samples at 6 hour PMI also showed a normal histology. (TABLE:2) Features like vacuolation, dermo-epidermal separation [figure.3] and dermal fragmentation[figure.2] are identified by 6 hours over I sample in this time interval. At 6-12 PMI set of samples, normal features are seen up to 12 hours, Dermo epidermal separation and dermal fragmentation is seen in samples at 9 hours PMI,

however dermal fragmentation showed its appearance since 7 hours PMI. Of the samples collected at 12-18 PMI, normal histology is shown up to 16 hours, however from 13 hours onwards, dermo-epidermal separation is a feature and dermal fragmentation is seen at 14 hours PMI sample. One sample at 18 hours showed normal histology whereas dermal fragmentation was an established feature in samples at 23 hours PMI. Sample of 23 hour PMI showed dermal degeneration [Figure.4] along with 22.2% of samples with 14-15 hours PMI(TABLE:3)

Results

The results of the present study show that the skin undergoes progressive morphological changes in the post-mortem period. These changes can be observed at the cellular level by light microscopy.

In epidermis, normal histology is maintained up to 13 hours in 2 cases. Vacuolation started appearing by 6 hours and is getting established by 14-16 hours and is a predominant feature of samples over 18 hours in most of the cases.

Dermis exhibited normal histology up to 19 hours in one case. Vacuolation started appearing by 6 hours even though it is not an established feature in dermis. Dermo-epidermal separation getting initiated at 6 hours along with dermal fragmentation is getting established through time. Dermal fragmentation is a distinguishable feature after 14 hours PMI.

Discussion

A limited amount of research has been conducted in the forensic field to determine an accurate estimate of the post-mortem interval. One of the major reasons behind it is that a controlled environment is difficult to obtain in most studies, and the instability of environmental factors has prevented the development of a single reliable predictor. There are no studies that compare post-mortem skin changes of bodies that have been allowed to decompose under the same environmental conditions.

Kushwaha V, Yadav M, Srivastava AK, Agarwal A[6], conducted a study in the Department of Forensic Medicine in collaboration with Department of Pathology, G.S.V.M. Medical College, Kanpur, U.P. A total of 45 cases are taken belonging to both sexes 36 males and 9 females were studied. These are of different age groups. On histological study of these kidney samples he observed changes which are follows: In first 12 hours, all 5 cases show mild degenerative changes. In 13-18 hours, with increasing temperature he observed moderate & severe changes are seen. Only 2 out of 13 cases show severe changes. In 19-24 hours with increasing temperature of up to, severity increases.

Considering the present study, we have sequentially studied the histologic appearance of the skin, sweat glands glands, and hair follicles in same environmental condition with average ambient temperature varied from 23-C to 37-C and average humidity ranged from 17% to 87%.

The major findings of the present study are compared with those of past literatures in Table.4.(TABLE:4)

When the results of the present study were compared with past literatures, the histologic findings are more or less similar, but the histologic changes seems to appear earlier than the other studies, the reason for which may be the environmental difference as extreme environmental factors may speed up decomposition, mainly the cutaneous decomposition.

Summary

-Epidermis and Dermis showed marked changes in the histology with increasing PMI and is the most reliable site to look for. -All these changes had to be correlated collectively taking into account the external as well as internal environment and all the micro factors playing in and around for a better prediction of PMI.

Limitations

This study is limited by availability of inadequate literatures, the small number of samples as Study included cases within 24 hours of death with time of death known and cases of found dead with time of death unknown were not included in the study, and conduction of the study in a single environment; however, useful information can be deduced from the results. The variability of the findings reinforces the difficulty in estimating the exact time since death.

Conclusion

If multicentric studies with similar conditions are carried out, it may establish concrete periodicity of death

based on the changes. This study is a potential tool in determination of time since death. There is currently very limited scientific information regarding the process of cutaneous decomposition. Studies with a larger number of cases being evaluated in different environments will throw a better light to post-mortem skin changes and their role in determination of the time since death.

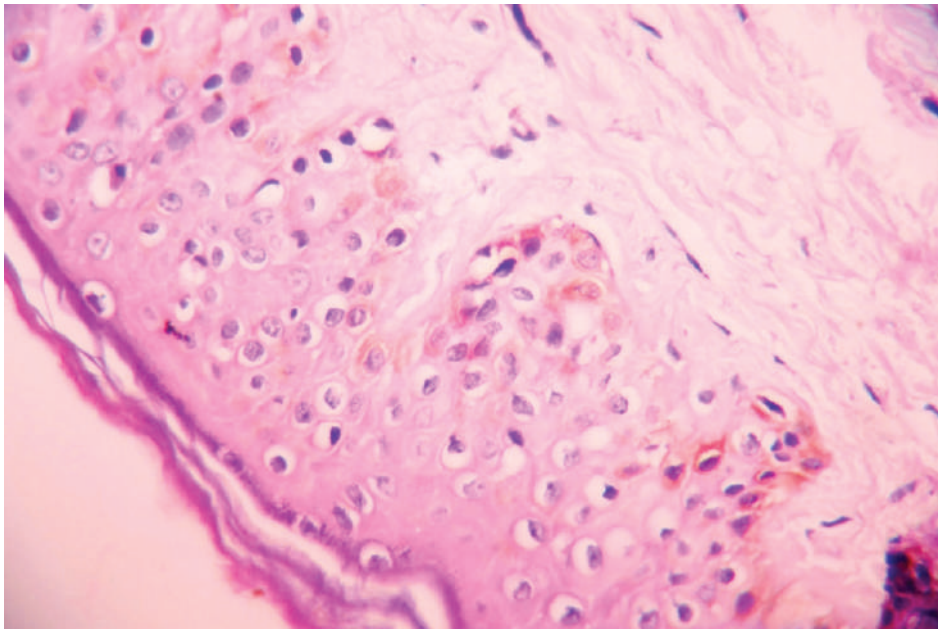


Figure.1: Microphotograph showing vacuolation in epidermis, H&E, 400x

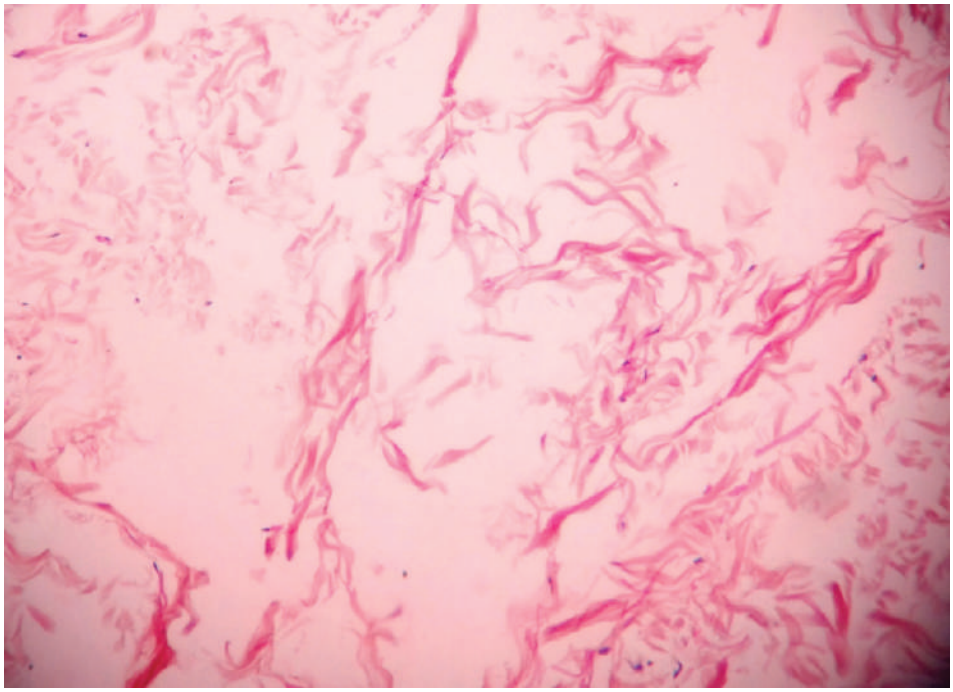


Figure.2: Microphotograph showing dermal disintegration,H&E,100x

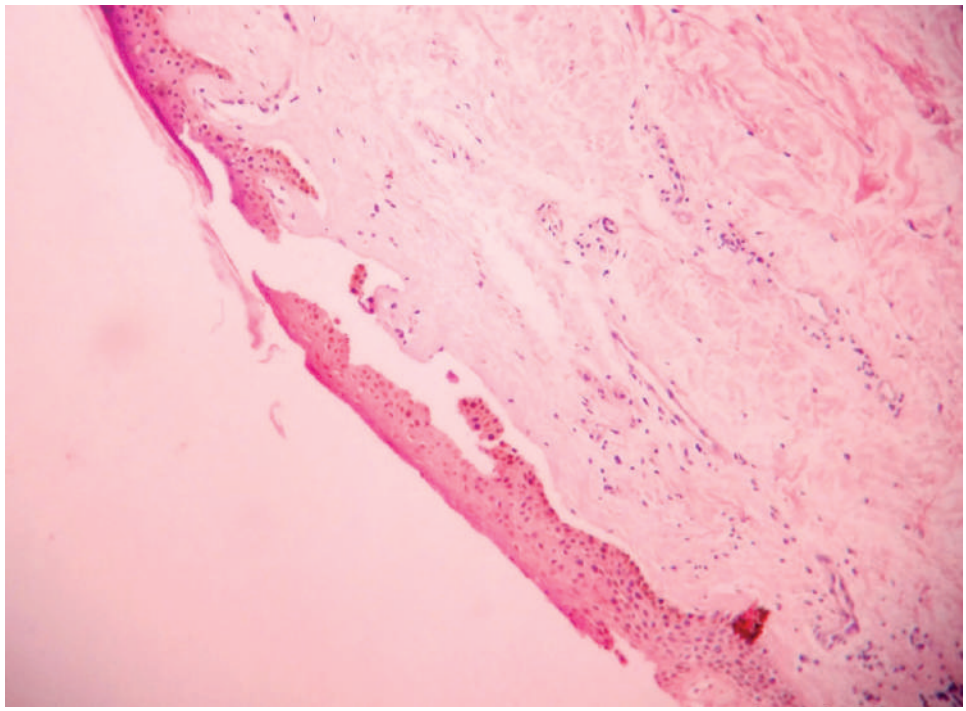


Figure.3: Microphotograph showing dermo-epidermal separation,H&E,100x

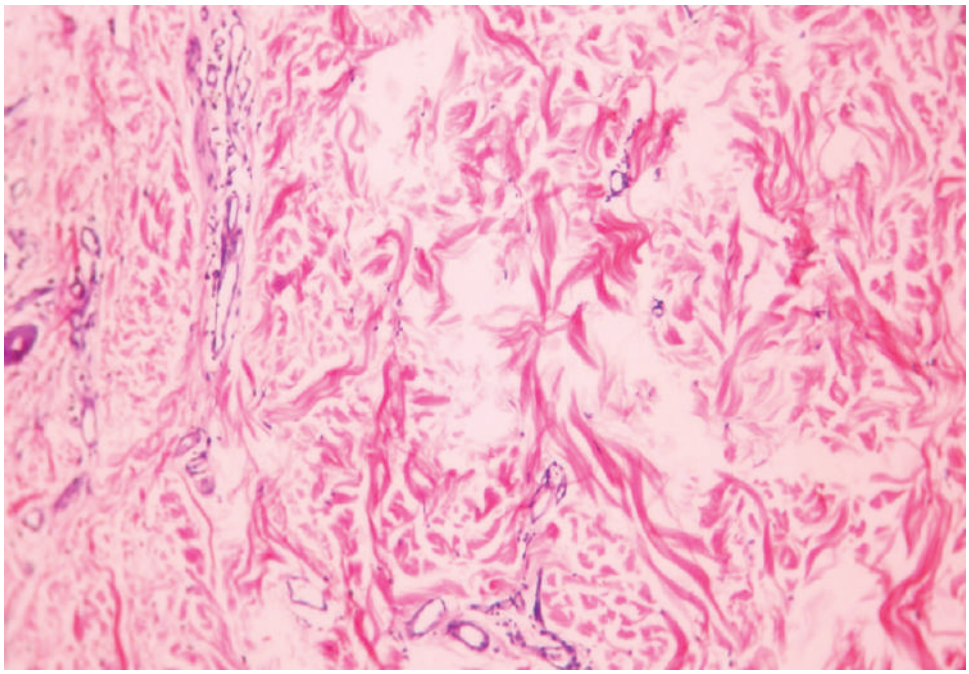


Figure.4: Microphotograph showing dermal fragmentation, H&E, 100x

Ethical Clearance- Taken from Yenepoya University Ethical Committee

Source of Funding- Self

Conflict of Interest - Nil

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Anterior Lamellar Keratoplasty Using Artificial Biotransplant in Veterinary Ophthalmology

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Abstract

The article describes a method for anterior lamellar keratoplasty with biological material from porcine small intestine submucosa (tunica submucosa) used to replace the damaged area of the cornea. The indications for surgery were deep ulcers caused by chronic irritation, septic ulcers with elements of keratomalacia, corneal sequesters, traumatic needle-shaped ulcers and xerotic ulcers in cats. Complete graft integration was achieved in 27 eyes (77,14 %) of 35. In 8 cases (22,86 %) complications occurred. Suture failures, partial graft integration and central thinning of the transplant were later corrected by repeated surgeries. Panophthalmitis developed in one eye required its enucleation. Observating the late postoperative period showed a minimal decrease in visual functions, which is not always possible to achieve through other surgeries.

Key words*: lamellar keratoplasty, biological material, corneal graft, biotransplant, porcine small intestine submucosa.*

Introduction

Corneal diseases are leading the list of eye disorders in animals. Today, many methods of conservative treatment, such as topical antibiotic therapy, instillation of autoserum or plasma enriched with platelets, pharmacological therapy are used to promote cornea regeneration. However, corneal diseases with stroma defects can be a serious threat to vision, thus, the surgical intervention is often needed.

The most commonly used surgical technique is conjunctival repair using an autoconjunctival graft to close the defect. The corneal flap can be total, pedicle (or rotational), bipedicle or hood. The main disadvantages of this technique are a rough scarring and deterioration of cornea optical properties [1-3].

Corneo conjunctival and corneo scleral transpositions are considered to be the most progressive methods, using an autoflap from the cornea and conjunctiva or sclera, respectively as a graft. The pedicle permits to intensify scarring, but the use of the healthy part of cornea limits its application only to small defects of the cornea [2].

In comparison to other methods, keratoplasty do not have these disadvantages. Different materials are used as grafts in anterior lamellar keratoplasty: amniotic membrane, biological materials from the porcine small intestine submucosa or extracellular matrix of porcine urinary bladder, alloplants and donor cornea [4,5].

The amniotic membrane facilitates the migration and specialization of epithelial cells and enhances their adhesion, scar modulation and reducing inflammation. This relatively cell-free material is widely used in the reconstruction of corneal surface in human ophthalmic operations. Amniotic membrane transplant is used as bio-dressing, reducing the effect of microbial proteases

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on animal cornea [6].

The biomaterial from the extracellular matrix of porcine urinary bladder and those from the porcine small intestine submucosa consist of cell-free collagen matrix, it contains fibronectin, hyaluronic acid, chondroitin sulfate, heparin sulfate and growth factors. In the process of graft integration, fibroblasts grow into the matrix, forming a scar, which is subsequently replaced by corneal stroma cells [7-11].

Donor grafts are also common in lamellar keratoplasty. Due to their tectonic properties, they are perhaps the best transplants, but the challenge is to obtain and store donor material, as well as to dissect the graft at the required depth [12,13].

Thereby, the biomaterial "VetBioSIS" from porcine small intestine submucosa was used in the current study to restore the cornea thickness".

Serious corneal injuries with a loss of more than half its normal thickness can be caused by various reasons and have poor prognosis [10]. Currently, there are several surgical techniques aimed at restoring the cornea thickness, such as penetrating keratoplasty, corneal-scleral transposition, and conjunctival grafting [7]. Anterior lamellar keratoplasty using bio material from the porcine small intestine submucosa is effectively performed after deep keratectomy in our clinic.

The aim of the study

To describe the technique of performing anterior lamellar keratoplasty using the biological material and assess its results in the early and late postoperative periods.

Materials and Methods

This interventional study comprised 35 eyes of 34 animals (20 dogs and 14 cats), which underwent 35 anterior lamellar keratoplasty. The age of the animals ranged from 9 months to 11 years.

During an ophthalmological examination using biomicroscopy, tonometry and fluorescein test, the following pathologies were diagnosed: deep corneal ulcers resulting from entropion (in 4 cats and 3 dogs), septic corneal ulcers with keratomalacia (in 8 cats and 10 dogs), deep corneal sequestration (in 2 cats, one of them having both eyes damaged), needle-shaped corneal ulcers (in 6 dogs), deep corneal ulcer associated with keratoconjunctivitis sicca (in 1 dog).

The porcine small intestinal submucosa VetBioSISt was used in all cases. Operations were conducted under the surgical microscope Leica M220 F12.

Surgical techniques

The combined anesthetic support, including premedication, systemic anesthesia, induction, local and inhalation anesthesia was used. The eyelids and the area around them were thoroughly shaved. The skin around the eyes was cleaned with a 10% betadine solution, the conjunctival sac was treated with a 0.2% betadine solution. A sterile surgical field with an opening for the operation area was prepared. The palpebral fissure was dilated with the help of a blepharostat. Eyeball akinesia was achieved by the intravenous medication of a non-depolarizing muscle relaxant (Rocuronium bromide) and fixation sutures.

At the first stage, a necrosectomy is performed with a scarifier. At a minimum magnification of the microscope, trephine is installed perpendicular to the cornea surface marking the area of planned trepanation by light pressure on the epithelium. The trephine must be larger than the diseased area [8]. Cornea stripping is carried out by leucosapphire exfoliator installed parallel to the curvature of the cornea, without excessive pressure, to healthy tissues. The graft is cut out of the artificial cornea layer with a trephine or micro scissors, depending on the shape. After this, the graft is placed in the prepared bed and fixed to the cornea with knotted sutures at 3, 6, 9 and 12 o'clock position. Then, the graft is stitched t to the cornea with single knots [12]. The number and frequency of sutures depends on the graft diameter and the corneal tissues condition (Fig. 1). In the end, the fixing ligatures and the eyelid speculum are removed and the palpebral fissure is closed by suturing the eyelids for 2 weeks (blepharraphy) or tarsorraphy during simultaneous blepharoplasty (Fig. 2).

Figure.1. Lamellar keratoplasty in a pugdog with a needle-shaped ulcer of cornea

Figure.2. Lamellar keratoplasty with VetBioSISt. The simultaneous blepharhoplasty led to a deep

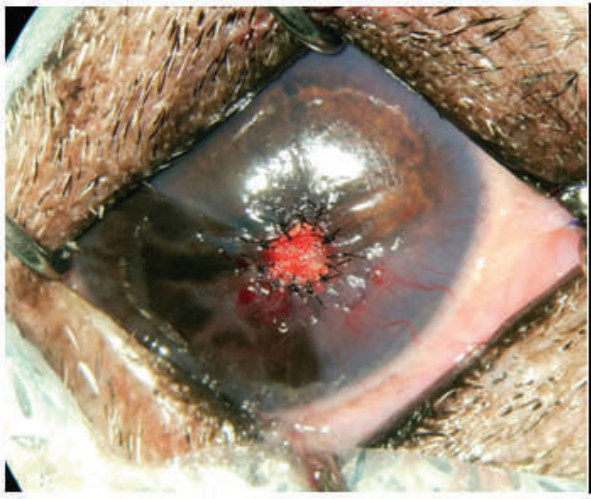


Figure.1. Lamellar keratoplasty in a pugdog with a needle-shaped ulcer of cornea

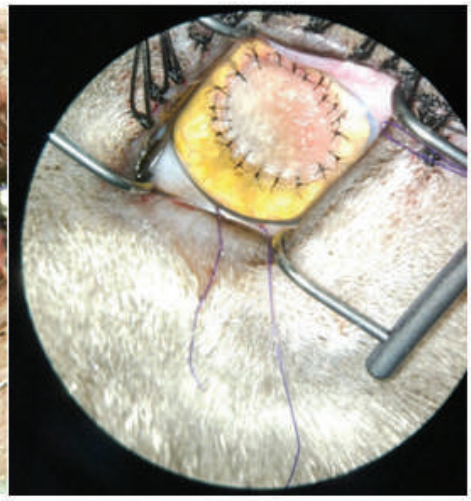


Figure.2. Lamellar keratoplasty with VetBioSISt. The simultaneous blepharhoplasty led to a deep cornea ulcer in a british cat.

cornea ulcer in a british cat.

In the postoperative period, antibiotic therapy (local and systemic), drugs to stimulate corneal regeneration are administered. 10-14 days after surgery, the external sutures are removed. Removal of corneal sutures is carried out on 21st to 30th day under sedation.

Results

A complete recovery of cornea continuity and complete engraftment after lamellar keratoplasty was achieved in 27 cases (77.14%). Complications arose in 8 cases (22.86%) (Table 1).

Complications associated with graft fixation were the failure of sutures in 3 eyes with intense keratomalacia before surgery (8.571%). 1 eye received new stitches with non-absorbable material (Sterilon 7/0), and 2 eyes - with absorbable suture material (Tisorb 7/0). In all cases, complete engraftment was finally achieved.

Table 1. Types of complications

Types of complications		Number of cases
Associated with transplant fixation	Suture failure	3 (8,571%)
Changes in transplant	Unstable transplant integration	1 (2,857%)
	Central thinning	3 (8,571%)
Others	Panophthalmitis	1 (2,857%)

The complications associated with changes in transplant were the partial integration of biomaterial in 1 eye (2.857%) and the central thinning in 3 eyes (8.571%)., Repeat keratoplasty was performed using biomaterial and additional fixation with Sulfacrylate bioglue in the case of partial implant integration. In 2 cases of central thinning after anterior lmellar keratoplasty in the eyes with total and subtotal corneal ulcers, a penetrating keratoplasty with defect replacement by the donor cornea was performed 14 days after lamellar keratoplasty (Fig. 3). The area of

repeated transplantation was smaller and corresponded to the diameter of graft thinning surface. In 1 eye with a slight thinning, a complete restoration of the cornea was achieved using therapeutic methods.

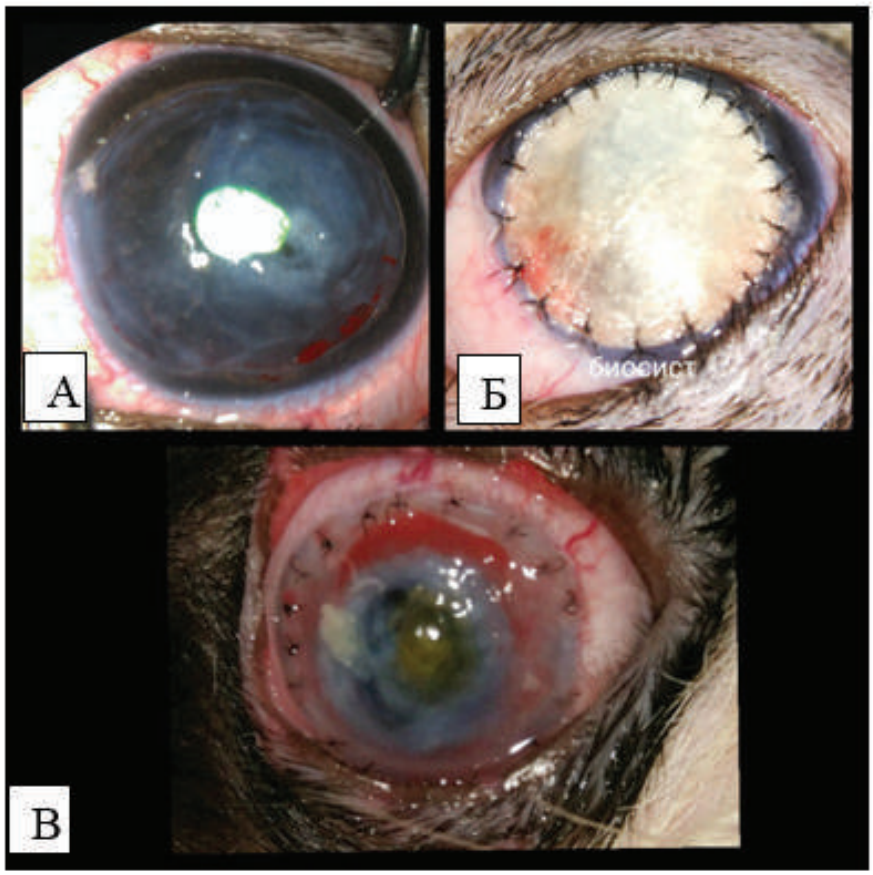


Figure.3. A - Japanese Chin with a deep corneal ulcer and keratomalacia elements. B – after a total anterior lamellar keratoplasty using VetBioSISt. C- eye condition 14 days after removal of blepharorrhaphy sutures. Early graft resorption, central thinning, risk of perforation. The dog was indicated for penetrating keratoplasty.

An eye was subjected to enucleating due to sthe development of panophthalmitis (2.857%).

A long-term monitoring (more than 10 weeks) allowed to observe the development of a fine discontinous scar in 25 cases (71.43%) after surgery, (Fig. 4). In 9 cases (25.71%) there were thick scars in the postoperative period, 2 of which (5.71%) arose due to the followed penetrating keratoplasty using a donor cornea dried over silica gel. The remaining 7 (20%) scars were located on the peripheral part of the cornea, which did not significantly worsened the vision of animals.

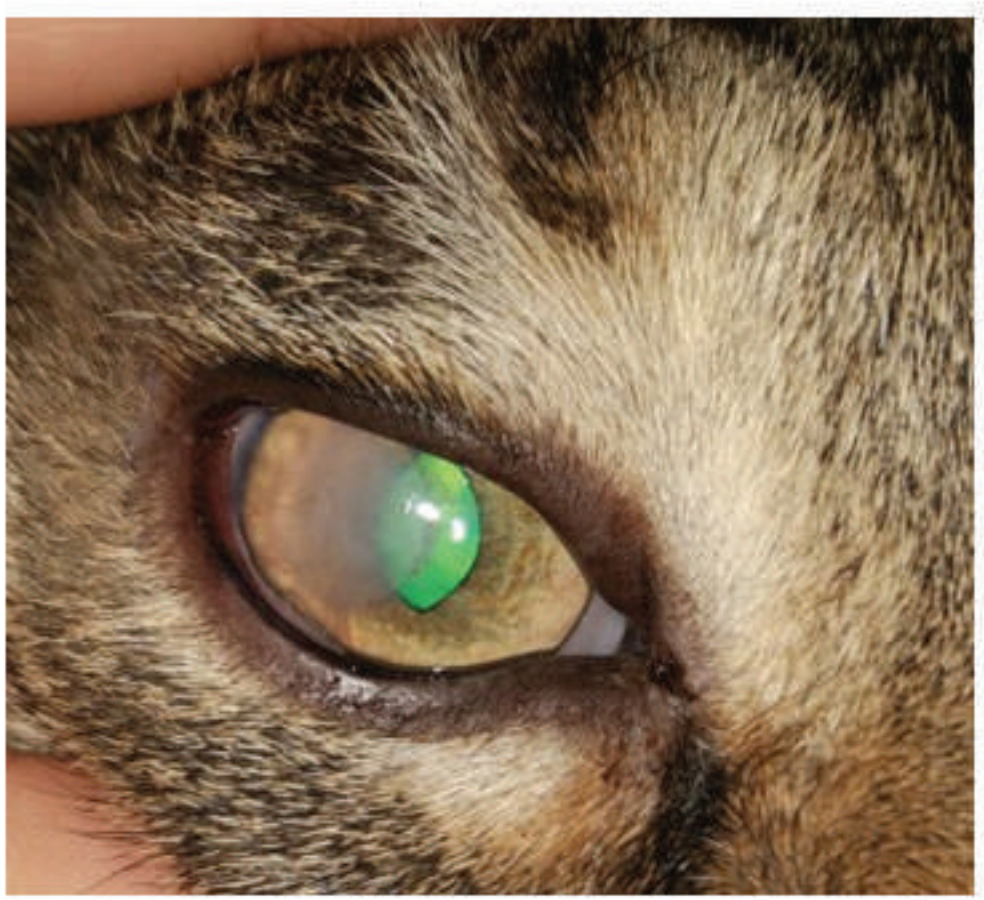


Fig. 4. The result of anterior lamellar keratoplasty 10 weeks after surgery

Discussion

The biological material from the porcine small intestine submucosa consists of acellular collagen matrix, and contains fibronectin, hyaluronic acid, chondroitin sulfate, heparin sulfate and growth factors. In the process of graft integration, fibroblasts grow into the matrix forming a scar, subsequently replaced by cornea stromal cells [3].

A thick scar and pronounced conjunctival vessels can remain after plastic surgery with replacement of damaged area with a conjunctival flap. The corneo conjunctival transposition provides a more transparent cornea, but the healthy cornea is usually damaged during this surgery. This technique cannot be used in case of subtotal and total lesions of the cornea [4].

Conclusions

1. An analytical study of corneal transplantation using biological material has shown a high efficiency in 77.14% of cases.

2. Anterior lamellar keratoplasty using corneal grafts allows preserving visual functions and restoring optical transparency in most cases.

3. For subtotal and total corneal ulcers, as well as for septic corneal ulcers with the elements of keratomalacia, it is recommended to use additional methods of transplant fixation in order to avoid complications in the postoperative period.

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Modern Mycobacterium Tuberculosis Strain in Bronchoalveolar Lavage from Tuberculosis Patients Associated with Lung Tissue Damage Severity

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Abstract

Background: The Beijing sublineage modern *Mycobacterium tuberculosis* strain is the most dominant strain in regards to causes of disease progression, extensive lung tissue damage, drug resistance and high outbreak rates.

Methods: *Mycobacterium tuberculosis* isolates were obtained from Bronchoalveolar lavage patients with active pulmonary Tuberculosis before obtaining anti-tuberculosis drug treatments. The degree of severity of parenchymal lung damage is classified by the NICE Scoring System. PCR was performed on DNA extracted from bronchial lavage, using primers targeting gene TbD1.

Result: 30 active pulmonary tuberculosis patients were analyzed in this study. 13 isolates of modern strains and 17 isolates of ancient strains were detected. In modern strains, 4 (30.8%) subjects had mild lung degree damage, while 9 (69.2%) subjects had severe lung damage. In ancient strains, 12 (70.6%) subjects had mild lung damage and 5 (29.4%) subjects had severe lung damage. *Mycobacterium tuberculosis* of modern strains correlated with the degree of lung damage, $p < 0.05$. Odds Ratio = 5.4 CI 95% (1,12-116,99).

Conclusion: In Surabaya, modern strains of *Mycobacterium tuberculosis* were detected in BAL of tuberculosis patients. Radiograph evaluations revealed severe lung tissue damage. The risk of severe lung damage with modern strains is 5.4 times higher than compared to ancient strains.

Keywords*: Modern Mycobacterium tuberculosis strain, lung tissue damage degree, bronchoalveolar lavage, Surabaya Indonesia.*

Introduction

In 2015, the World Health Organization (WHO) found 10.4 million cases of tuberculosis (TB) worldwide[1]. Indonesia is estimated to have one million new Tuberculosis (TB) cases each year. Currently, Indonesia ranks second of six countries with the highest TB cases in the world along with India, China, Nigeria, Pakistan and South Africa. By 2015, Through the WHO surveillance network [2], the WHO estimated that there were 10.4 million new TB cases, but only 6.1 million cases could be accounted for [3]. This appears to be due to *Mycobacterium tuberculosis complex* (MtbC) subspecies infection, especially those that infect humans, i.e. *Mycobacterium tuberculosis*. The determination of the tuberculosis strain from TB cases is necessary for observing the cause of extraordinary occurrences of tuberculosis at the local level, even in a larger range of regions [4].

Mycobacterium tuberculosis (MTB) divided into ancient and modern bloodlines [5]. The TbD1 deletion strain is also referred to as a "modern" evolutionary strain

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compared to the strain without deletion, which is known as the ancestral or ancient evolutionary form [6]. The global urgency related to the modern tuberculosis strain, just like Beijing strain or "W" strain, is correlated with the increase of TB infection risk and drug resistance. TB caused by the Beijing strain was observed in as many as 45.9% in Southeast Asia, 17.2% in Oceania and 16.5% in Middle East Asia[7].

In a diagnostic study, TB has been shown to have multiple forms. Generally, it is suspected as an active lesion including a cavity, miliary, infiltrate and fibroinfiltrate on the apical segment, posterior segment of the upper lobes, superior segment of the lower lobes and unilateral pleural effusion. The cavity illustrates the occurred disease expansion related to the degree of infection and the amount of *Mycobacterium tuberculosis* strains. It may increase the risk of TB infection to other susceptible individuals, accelerate spontaneous mutations that cause drug resistance, and be an independent risk factor for the recurrence of TB after obtaining adequate treatment [8].

Materials and Methods

This research was performed using a cross-sectional consecutive sampling method and through ethical feasibility tests in H. Moh Soewandhie Hospital, Surabaya Indonesia, from June 2017 until October 2017. 15 – 60-year-old patients with TB were diagnosed based on their sputum diagnostic tests and thorax radiographs. They did not receive anti-tuberculosis therapy and signed the informed consent form to join this study. The exclusion criteria included patients with HIV, diabetes mellitus, abnormalities in renal function, hearts and lungs. Ziehl Neelsen Acid Fast Bacilli (AFB) microscopic tests and result interpretations using the International Union Against Tuberculosis (IUALTD) scale was performed. The chest radiograph reading was performed independently by radiologists who were not aware of the patient's illness and the informed consent form. The degree of pulmonary parenchymal damage was classified using the NICE scoring system based on the lesion total in six lung areas [9].

Bronchoalveolar lavage was performed using ±50 cc in a sterile plastic bottle and placed in the refrigerator during the observation. Then, macroscopic analysis of the bronchial washing was observed. It could be decontaminated using the Petroff method, using NaOH with a 2:1 ratio. Next, it was centrifuged at 4000 rpm for 15 minutes. A cell pellet was obtained and PCR was performed to identify modern and ancient *Mycobacterium tuberculosis* strains with a TbD1 gen target. The positive control used was *Mycobacterium bovis*. To perform PCR, DNA was isolated from the decontaminated cell suspension of BA using a Qiagen KIT (1066955 01/2011) and mixed with 200µL Buffer Al and 20µL proteinase K by vortexing. 200µl (96%-100%) ethanol was additionally added and mixed.

Statistical Analysis

The collected data were processed using SPSS 17 software and analyzed with descriptive and analytical statistics. The correlation between the degree of lung damage and modern *Mycobacterium tuberculosis* strain infection on pulmonary TB group was analyzed with a chi-square test.

Result

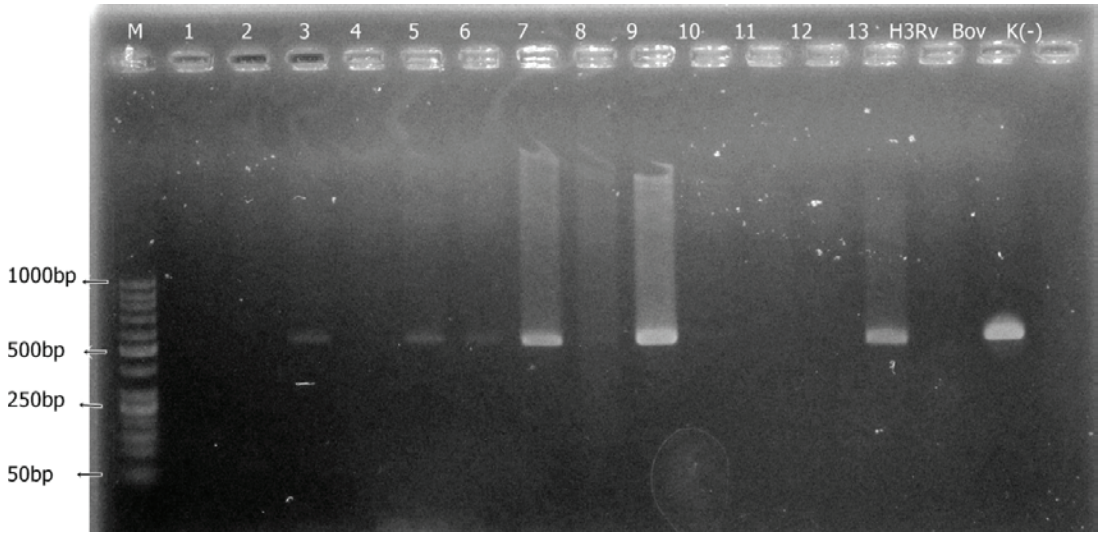
This study evaluated a total of total research subjects who had pulmonary TB but had not received treatment. These are the basic characteristics of research subjects.

Table 1. The basic characteristics of research subjects.

Characteristics		Frequency n (%)
Sex	Female	17 (56,7%)
	Male	13 (43,3%)

Cont... **Table 1. The basic characteristics of research subjects.**

Age	< 21 yo	4 (13,3%)
	21-30 yo	9 (30,0%)
	31-40 yo	6 (20,0%)
	41-50 yo	8(26,7%)
	>50 yo	3 (10,0%)
Education Backgorund	Elementary School (SD)	13 (43,3%)
	Junior High School (SMP)	7 (23,3%)
	Senior High School (SMA)	10 (33,3%)
Sputum Result	Negative	18 (60,0%)
	Positive	12 (40,0%)
Nodule	Yes	18(60,0%)
	No	12(40,0%)
Infiltration/ Consolidation	Yes	23(76,7%)
	No	7(23,3%)
Cavity	No	29 (96,7%)
	Yes	1 (3,3%)
Ectasis	No	3 (10,0%)
	Yes	27(90,0%)
Strain	Modern	13 (43,3%)
	Ancient	17 (56,7%)
Lung Damage Degree	Mild	16 (53,3%)
	Severe	14 (46,7%)



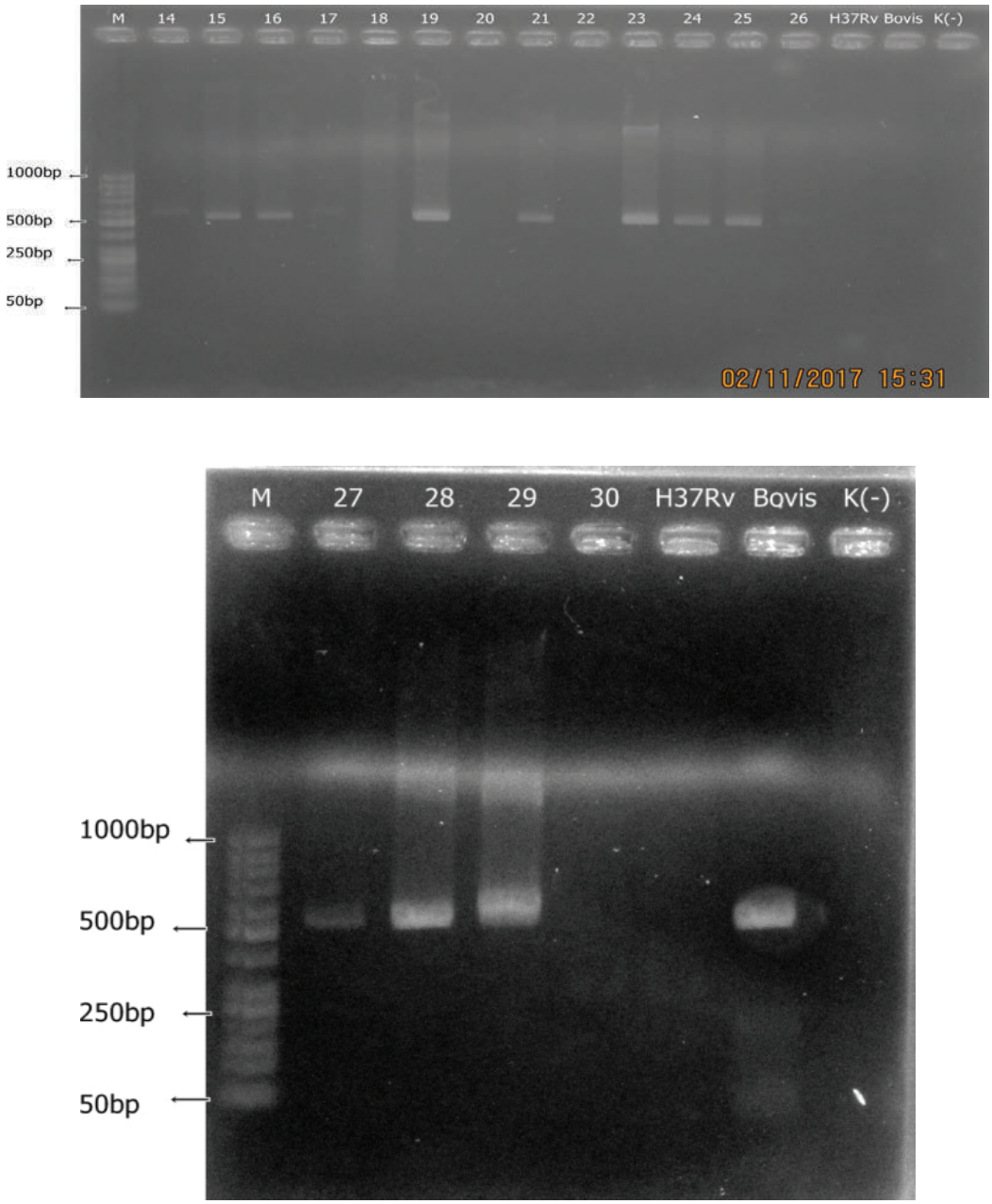


Figure 1. The results of electrophoresis gel using Tbd1.

In table 1 there are basic characteristics of the research subjects, the above analysis shows that the characteristics of the female sex are more frequent than males is 56.7%. at the age of 21-30 the greatest frequency is 30.0%. The background of primary school is the biggest frequency of 43.3%. The largest negative frequency sputum yield is 60.0%. Bintil who said yes the biggest frequency is 60.0%. Infiltration or Consolidation that says yes the biggest frequency is 76.7%. Cavity that says yes the biggest frequency is 96.7%. The ectasis that says yes the biggest frequency is 90.0%. Ancient tension is the largest frequency of 56.7%. The highest frequency of mild lung damage is 53.3%.

Table 2. The correlation between subject characteristics with *Mycobacterium tuberculosis*

Characteristics		Strain		Total	p-Value
		Ancient	Modern		
Age	<21 yo 21-30yo 31-40yo 41-50yo >50yo	1(25,0%) 4(44,4%) 5(83,3%) 4(50,0%) 3(100,0%)	3(75,0%) 5(55,6%) 1(16,7%) 4(50,0%) 0 (0,0%)	4 9 6 8 3	0,174
Sex	Female Male	8(47,1%) 9(69,2%)	9(52,9%) 4(30,8%)	17 13	0,225
Education Background	Elementary school Junior high school Senior High School	9(69,2%) 4(57,1%) 4(40,0%)	4(30,8%) 3(42,9%) 6(60,0%)	13 7 10	0,374
Sputum Result	Negative Positive	10(55,6%) 7(58,3%)	8(44,4%) 5(41,7%)	18 12	0,100
Nodule	Yes No	10(55,6%) 7(58,3%)	8(44,4%) 5(41,7%)	18 12	0,880
Infiltration/ Consolidation	Yes No	12(52,2%) 5(71,4%)	11(47,8%) 2(28,6%)	23 7	0,368
Cavity	Yes No	1(100,0%) 16(55,2%)	0(0,0%) 13(44,8%)	1 29	0,374
Ectasis	Yes No	14(51,9%) 3(100,0%)	13(48,1%) 0(0,0%)	27 3	0,238

Table 2 illustrates that there was no correlation between the subject characteristics (age, sex, educational background, sputum result, nodule, infiltration/ consolidation, cavity, ectasis) with *Mycobacterium tuberculosis* strain, $p>0.05$ for all characteristics were considered significant.

Table 3 The correlation between subject characteristics and Strain of TB Towards lung damage degree

Characteristics		Lung tissue damage		Total	P-Value
		Mild	Severe		
Sex	- Male - Female	7(53,8%) 9(52,9%)	6(46,2%) 8(47,1%)	13 17	0,626
Age	- <21 - 21-30 - 31-40 - 41-50 - >50	2(50,0%) 4(44,4%) 5 (83,3% 2(25,0%) 3(18,8%)	2(50,0%) 5(55,6) 1 (16,7%) 6(75,0%) 0 (0,0%)	4 9 6 8 3	0,104
Education Background	- Elementary school - Junior high school - Senior High School	5 (38,5%) 6 (85,7%) 5 (50,0%)	8 (61,5%) 1 (14,3%) 5(50,0%)	13 7 10	0,126
Sputum	- Negative - Positive	11 (61,1%) 5(41,7%)	7(38,9%) 7 (58,3%)	18 12	0,457
Nodule	- Yes - No	8(44,4%) 8(66,7%)	10(55,6%) 4(33,3%)	18 12	0,232
Infiltration/ Consolidation	- Yes - No	9(39,1%) 7(100,0%)	14(60,9%) 0(0,0%)	23 7	0,005
Cavity	- No - Positive	15 (51,7%) 1 (100,0%)	14 (48,3%) 0 (0,0%)	29 1	0,341
Ectasis	- No - Yes	3(100,0%) 13(48,1%)	0 (0,0%) 14(46,7%)	3 27	0,228
Strain	Lung Damage Degree		Total	p-Value	
	Mild	Severe			
Modern	4(30,8%)	9(69,2%)	13	0,035	
Ancient	12(70,6%)	5(29,4%)	17		

Table 3 Displays the correlation between subject characteristics and lung degree damage. There is a correlation between infiltration/consolidation and the degree of lung damage, p value<0.005. For the other characteristics, there was no correlation with lung damage, p value>0.005. Odds Ratio for Tbd1 (Modern Strain / Ancient Strain) = 5,4 CI 95% (1,12- 116,99). From 30 active TB patients, 13 isolates of modern strains and 17 isolates of ancient strains were obtained. From the 13 modern strains, there were 4 (30,8%) patient subjects with mild lung damage and 9 (69,2%) patients with severe lung damage. From 17 ancient strains, there were 12 (70,6%) subjects with mild lung damage and 5 (29,4%) patients with severe lung damage. There was a correlation between the *Mycobacterium tuberculosis* strain and degree of lung damage, p<0.005, OR= 5.4 CI 95% (1.12-116.99).

Discussion

Study in North India in 2014 found that 81.1% was the modern strain (TbD1-) and 24% positive *Mycobacterium tuberculosis* (ancient strain) [10]. This was due to the western location of Azerbaijan as the suburb of Iran with a larger population movement from neighboring countries [11]. An epidemiological study has reported that different genotypes of *Mycobacterium tuberculosis* were common in different geographical regions and the distribution of the genotypes is related to the migratory population [12]. [13] compared the TB genome based on the TbD1 deletion to differentiate each species of *Mycobacterium tuberculosis complex* (MTBC) into two major groups. 85.4% of modern *Mycobacterium tuberculosis* strain isolates (TbD1-) and 14.6% of ancient strain (TbD1+) was obtained. Modern strains have a low sensitivity phenotype of TB drugs with Relative Risk (RR) 0.89 (CI 95%, 0.74–1.07). In Mbarara, northern Uganda, 92.8% of the modern strain was found from 167 sputum samples of TB patients, so the TB epidemic in this area is commonly caused by the modern *Mycobacterium tuberculosis* strain [14].

The modern *Mycobacterium tuberculosis* strain has the following characteristics in radiological images: nodule (44.4%), infiltration / consolidation (47.8%), cavity (0%), ectasis (48.1%). The modern strain presents with mild lung damage, 4 patients (30,8%) and severe lung damage, 9 patients (69,2%). Ancient *Mycobacterium tuberculosis* strain has these following characteristics in radiological images: nodule (55.6%), infiltration/consolidation (52.2%), cavity (100%), ectasis (51.9%) with severe lung damage (29.4%). The ancient strain has mild lung damage 12 patients (70.6%) and severe lung damage 5 patients (29.4%). Odds Ratio = 5.4 CI 95% (1.120-26.044). A secreted protein, such as ESAT 6, is able to trigger macrophage death and is responsible for new macrophage withdrawal to form granulomas. It has an important role in granuloma expansion. ESAT 6 additionally triggers the MMP9 metalloproteinase matrix from macrophages freely, thus it may directly interact with epithelium. Epithelial cells around the infected macrophages also help to amplify MMP9 production. ESAT 6 mediates the induction of epithelial cells freely through TNF signaling and MYD88 pro-inflammation. In mice with *Mycobacterium tuberculosis* infections and devoid of MMP9, there was a small amount of macrophage withdrawal to lungs, decreased granuloma formation, and low amount of bacterial infection [15].

In experimental mice trials, there was a 40% occurrence of the cavity after *Mycobacterium tuberculosis* HN878 *(East Asian Lineage)* infection that was similar to the occurrence of the cavity on *Mycobacterium tuberculosis* H37Rv *(Euro-American Lineage)*. Caseous granuloma occurred in experimental mice with *Mycobacterium* virulent, i.e. *Mycobacterium Africanum,* with similar caseous lesions with the other experimental mice, which is infected by *Mycobacterium tuberculosis* with a different strain [16] [17] [18] showed that in C57BL/6 mice lesions of pulmonary necrosis induced by virulent *Mycobacterium tuberculosis* strain were observed, i.e. Beijing sublineae. Mice with *Mycobacterium tuberculosis* hypervirulent infection may have necrotic lesions in irreversible pulmonary.

The results demonstrated that the cavitary formation was not correlated with the Beijing strain infection in pulmonary TB patients [19]. [20] investigated and reported that there was no significant difference in the occurrence of the cavity in chest radiographs among the patients with Beijing and non Beijing strain infections. [21] Pulmonary TB patients infected with the W-Beijing strain has a very general radiograph. The *Mycobacterium tuberculosis* genotype is an independent factor for pulmonary tuberculosis radiograph. This research concluded that

active pulmonary TB patients in Surabaya were infected by the modern strain. The modern strain has a correlation to severe lung damage, p<0.05. Odds Ratio = 5.4 CI 95% (1.12-116.99).

Conclusions

In Surabaya, modern strains of *Mycobacterium tuberculosis* were detected in BAL of tuberculosis patients. Radiograph evaluations revealed severe lung tissue damage. The risk of severe lung damage with modern strains is 5.4 times higher than compared to ancient strains.

Conflict of Interest: There is no concflict of interest.

Source of Funding: This study is self-funded.

Ethical Clearance: This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga in dr. Soetomo General Hospital Surabaya, Indonesia.

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Effect of Cinnamon (*Cinnamomum burmannii*) Bark Oil on Pancreatic Histopathology of white Rats (*Rattus norvegicus*) Induced with Streptozotocin

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Abstract

The purpose of this research was to understand the effect of the cinnamomum *(Cinnamomum burmannii)* bark oil (CBO) on the pancreatic histopathology white rat *(Rattus norvegicus)* with diabetes mellitus. The variables taken were the diameter and the number of Langerhans Island in the pancreatic of white male rats which had been induced by streptozotocin (STZ). This research using diabetic rat induced by streptozotocin intraperitoneally with single dose of 45 mg/kg BW. Samples consist of fifty rats were divided into five groups, negative control group (K-) was not induced by streptozotocin, which treatment by giving CMC Na 1%, positive control group (K+) was induced by streptozotocin without CBO, group P1 was induced by streptozotocin and gave CBO 100 mg/kgBW, group P2 was induced by streptozotocin and gave CBO 200 mg/kgBW, group P3 was induced by streptozotocin and gave CBO 400 mg/kgBW daily for 14 days period. The results showed that cinnamomum bark oil (CBO) with dose 200 mg/kgBW (P2) was significantly higher ($p<0,05$) than group P1, group P3 and positive control group (K+). It can be concluded that CBO can be used to improve pancreatic function of STZ induced in diabetic rats

Keywords *: Streptozotocin, Cinnamomum bark oil, Island of Langerhans, Diabetes mellitus, Rats.*

Introduction

Treatment for DM patients is currently mostly done by administering intravenous insulin and oral antidiabetic drugs, such can cause gastrointestinal side effects, hypoglycemic reactions, allergic skin reactions, gastrointestinal disorders and ketoacidosis. Therefore, natural herbal remedies are needed from plants that do not cause side effects. Herbal alternative medicine is one form of treatment therapy that is more affordable to the public, has mild side effects and is easily obtained[1].

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Cinnamon is a spice shaped like a bark and is widely used as a spice for cooking cakes and drinks. Cinnamon plant including one of the plants that are often found in the tropics with a number of diverse species. Cinnamon plants that have been studied have antidiabetic effects including Cinnamomum zeylanicum, Cinnamomum burmannii, and Cinnamomum cassia[2]. Cinnamomum burmannii is a type of cinnamon that is mostly found in Indonesia[3]. Cinnamon is a plant that has the effect of hypoglycemia, where the plant contains cinnamic acid and cinnamaldehyde which can increase insulin circulation and are effective in reducing fasting blood glucose levels[2].

Cinnamon bark oils (CBO) contain cinnamaldehyde and eugenol compounds which have potential as antimicrobial organisms[4] and cinnamaldehyde compounds resulting from the isolation of cinnamon oil have the potential to enzyme α-glucosidase[5]. This

enzyme plays a role in the breakdown of carbohydrates into blood sugar, so that the essential oil content can be developed as an antidiabetic compound. Based on the description above, the effect of cinnamon essential oil in repairing pancreatic β cells as antidiabetic has not been proven. Therefore, the authors wish to conduct research on the effects of cinnamon essential oil on the histopathology of the pancreatic Langerhans island in white rats (Rattus norvegicus) induced by streptozotocin.

Materials and Methods

The experimental animals used were white male rats (Rattus norvegicus), healthy wistar strains aged 2-3 months with a body weight between 150-250 grams totaling 50 animals. The white rat (Rattus norvegicus) was first adapted for seven days in the Pharmacology Laboratory of the Faculty of Medicine, Airlangga University. Diabetic white mice were made by streptozotocin-induced at a dose of 45 mg / kg body weight (BW) injected intraperitoneally. This injection was carried out in all treatments except negative controls. Streptozotocin (STZ) is dissolved in a citrate buffer solution with a pH of 4.5. STZ induction was done immediately after making a single dose of 45 mg / kg BW[6].

White rats (Rattus norvegicus) male Wistar strain of 50 animals divided by five groups, each group of ten. Adaptation was done for seven days with the aim to avoid stress in experimental animals. The study were conducted with five treatments including: (K-): A group of white mice that were not induced by STZ and given CMC Na 1%, (K +): A group of STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and given 1% CMC Na, (P1): A group of STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and treated with CBO at a dose of 100 mg / kgBW orally, (P2): STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and treated with CBO at a dose of 200 mg / kgBW orally, (P3): A group of STZ-induced white rats at a dose of 45 mg / kgBW intraperitoneally and treated with CBO at a dose of 400 mg / kgBW orally[7,8,9].

White rat samples that have been made with histopathological preparations using *Hematoxylin* and *eosin (*HE) staining, followed by examination of white rat pancreatic histopathological preparations at the Veterinary Pathology Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya.

Results and Discussion

Histopathological observations of Langerhans Island white rats in group K (-) were only given CMC Na 1% solvent, K (+) was induced by streptozotocin 45 mg / kg BW, groups P1, P2 and P3 were treated with CBO, once for 14 days. The dose of CBO given is 100 mg / kg BW, 200 mg / kg BW and 400 mg / kg BW.

The data analyzed are the number of cells and the diameter of the island of Langerhans. Data obtained from observations and calculations on histological preparations in accordance with the treatment, repetition and dosage used. The results of statistical tests using the ANOVA (Analysis of Variance) test showed that there were significant differences ($p < 0.05$) between treatments followed by Duncan's test.

The results were obtained from the calculation of the number of cells and the measurement of the diameter of the island of Langerhans which was carried out using a 400x magnification microscope. the results of observations in the control and treatment groups can be seen in Table 1 and Table 2. The histopathological picture of Langerhans Island can be observed in Figure 1. The results of observations in Table 1 mean the highest number of Langerhans island cells are group P2 and the lowest in group K (+).

Analysis of Variance (ANOVA) statistical test results showed a significant difference ($p < 0.05$). Further test is the Duncan test with the results obtained by the treatment of K (-) significantly different from K (+), P1, and P3. The treatment of K (+) is significantly different from K (-), P2 and P3.

In Table 1 and Table 2 it can be seen that P1 and P3 show significant differences with P2, so to determine the correct dosage in the use of cinnamon essential oil need to compare the three treatments with a negative control group K (-). P1 and P3 statistical test results were significantly different from K (-), but not significantly different from K (+).

The results of microscopic histopathological observations can be seen in Figure 1. P2 group was diabetic group of rats that gave cinnamon essential oil

at a dose of 200 mg / kg BW orally and produced the highest number of cells and diameter compared to other treatment groups, namely 62.50 ± 21.54 and 91.96 ± 12.38. the cell looks neatly arranged and fills the island of Langerhans. Group P3 is a group of white rats with DM patients who were given orally cinnamon essential oil at a dose of 400 mg / kg body weight or 48.40 ± 16.24 cells. The obtained diameter is 76.54 ± 16.21. Cells appear to be rare on the island of Langerhans. The number of cells and diameter of the Langerhans island produced was higher than the treatment groups K (+) and P1. The variance analysis test gave significantly different results ($p < 0.05$) on the number and diameter of the Langerhans island K (-), P2 with the treatment groups K (+), P1, and P3.

Hyperglycemia is a condition in which the body cannot control blood glucose levels caused by disruption of insulin secretion by β cells in the pancreatic islets of Langerhans. The process of glucose metabolism is very important to produce energy sources for the survival of cells in the body. However, in a state of hyperglycemia, the body's cells cannot metabolize glucose so the body lacks an energy source. It responds to the body to search for alternative energy, namely from glycogenesis and gluconeogenesis. Both of these processes produce by-products, namely free radicals, which cause damage to cells such as pancreatic β cells[10, 11].

B cell death in the Langerhans island can automatically change the structure of the Langerhans island in terms of shape and size. Therefore, the size of the diameter or size of the area can be presented as an indicator of damage and repair of pancreatic organs in hyperglycemic conditions[12]. B cell death is very influential on the area of the island of Langerhans, because there are 60% -80% of β cells in the total endocrine cells in the island of Langerhans. This shows that almost all of the endocrine cells contained in the island of Langerhans are β cells[13]. This study was conducted to determine the effect of CBO on increasing the number of cells and the diameter of the pancreatic Langerhans island in white rats induced by streptozotocin. The results of histopathological examination and observation of the number of cells and the diameter of the pancreatic Langerhans island showed that the positive control group (K +) obtained the lowest average compared to other treatments. This is due to this group of streptozotocin-induced white rats 45mg / kgBW and only given 1% Carboxymethyl Cellulose Sodium (CMC Na) therapy. CMC Na is inert, safe and does not react with other groups[14].

It has been proven that cinnamaldehyde compound from cinnamon oil isolation against the α-glucosidase enzyme so that it is very potential as an α-glucosidase enzyme inhibitor. IC50 value is a number that shows the concentration of extract (ppm) that can inhibit 50% of the activity of the α-glucosidase enzyme[5]. Compounds are said to be active to inhibit the activity of the α-glucosidase enzyme[15].

The enzyme α-glucosidase plays a role in the breakdown of carbohydrates into blood sugar. Inhibition of α-glucosidase in mammalian intestine can reduce blood glucose levels from metabolism of polysaccharides or oligosaccharides. Slowing the absorption of blood glucose causes a reduction in postprandial hyperglycemia to prevent chronic complications of diabetes mellitus such as retinopathy, neutropathy, neuropathy and microvascular as well as macrovascular problems[16]. The group that plays a role in inhibiting the enzyme α-glucosidase is the cinamoil group. The component of cinnamon oil containing cinamoil group is cinnamaldehyde[17]. Cinnamaldehyde is a compound that has a functional group of aldehydes and benzene ring conjugated alkenes. The study of Ping et al (2010) states that cinnamon oil has an antidiabetic effect in mice[18]. Components contained in cinnamon oil are cinnamaldehyde (62-75%), eucalyptol, copaene, naphthalene, eugenol, benzaldehyde. Cinamaldehyde contained is useful as antidiabetic[19].

Zhu R et al (2017) states that cinnamaldehyde shows a decreased effect of sugar on test animals through increased sugar release and improvement of insulin sensitivity in adipose tissue and muscle tissue, increases glycogen synthesis in the liver, improves pancreatic islets, slows down gastric emptying time, and improves gastric emptying kidney disorders due to diabetes and brain damage[20]. The mean cell count and diameter of the white rat Langerhans island increased in P2 group compared to P1 and P3. This is likely due to metabolic disorders or biotransformation of the body. The aim of cinnamaldehyde metabolism is to change non-polar cinnamaldehyde into polar, so that they can be secreted by the kidneys or bile. This process can cause active

cinnamaldehyde to become inactive. However, some become more active, less active and even become toxic[21]. The speed of metabolism can increase the intensity and length of service of the cinnamaldehyde. This metabolic rate may vary in each individual. A decrease in metabolic rate will increase the intensity and prolong the service life of the cinnamaldehyde and possibly increase the toxicity of the cinnamaldehyde[22].

Table 1. Mean cell counts of Langerhans island in white rats (Rattus norvegicus)

Treatment	Mean Cell Number ± SD
K(-)	$60.27^{b} \pm 7.96$
K(+)	$34.20^{a} \pm 6.64$
P1	$37.05^{a} \pm 11.96$
P2	$62.50^{b} \pm 21.54$
P3	$48.40^{ab} \pm 16.24$

Note: a, b different superscripts in the same column show significant differences ($p < 0.05$).

Table 2. Mean diameter of Langerhans island in white rats (Rattus norvegicus).

Treatment	Mean Diameter (µm) ± SD
K(-)	$87.79^{b} \pm 3.61$
K(+)	$58.83^{a} \pm 8.11$
P1	$60.91^{a} \pm 17.18$
P2	$91.96^{b} \pm 12.38$
P3	$76.54^{ab} \pm 16.21$

Note: a, b different superscripts in the same column show significant differences ($p < 0.05$).

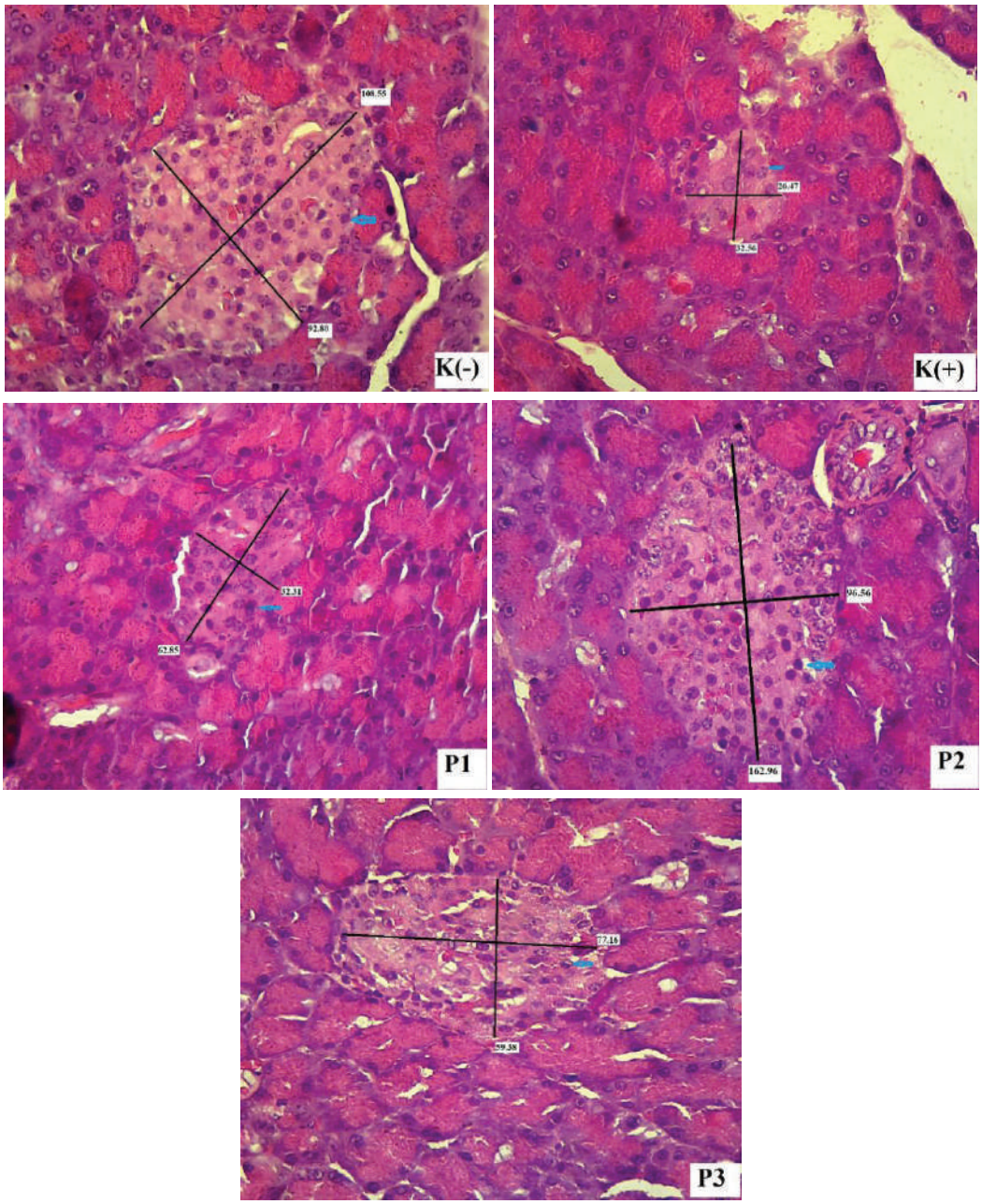


Figure 1. Histopathological picture of Langerhans Island white rat with 40 0x magnification with HE staining at treatment K (-), K (+), P1, P2, P3. on Langerhans Island Cell of Pancreas.

Conclusion

After conducting research on the effect of cinnamon bark oil (CBO) at a dose of 100 mg / kg, 200 mg / kg and 400 mg / kgBW for 14 days, it can be summarized that CBO can increase the number of cells and expand the diameter the pancreatic Langerhans island of white rat (Rattus norvegicus) induced by streptozotocin. Therefore, the effect of administration of CBO can be used as an alternative medicine to increase the number of Langerhans island that produce insuline hormone.

Designation :

Budiastuti as a Doctoral candidate on Faculty of Pharmacy, Universitas Airlangga

Reni Utami as an undergraduate student on Universitas Airlangga**,**

Mustofa Helmi Effendi as an associate professor on Universitas Airlangga**,**

Hani Plumeriastuti as a researcher on Universitas Airlangga

Ethical approval : Ethical clearance was taken and passed from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, Airlangga University. Certificate ethic number : 1.KE.127.07.2019.

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Conflict of Interest : Nil

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Hypolipidemic Effect of Fermented Soymilk Related with Lactic Acid Bacteria in Feaces

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Abstract

The problem of obesity is a global problem that is being faced in the world. One of the precautions taken is functional food. Fermented soy milk is a functional food that has a hypolipidemic effect. The purpose of this study was to analyze the relationship between the hypolipidemic effect of fermented soymilk and the content of lactic acid bacteria in animal experiments. The results showed that the provision of fermented soymilk and unfermented soymilk could increase the total content of lactic acid bacteria in rat feaces. This is in line with the decrease in total blood cholesterol levels in rats. Giving soy milk and fermented soymilk increases the total lactic acid bacteria in feaces thereby increasing cholesterol secretion in feaces

Keyword : *hypolipidemic, fermented soymilk, lactic acid bacteria, total cholesterol, triglyceride*

Background

The problem of obesity is included in the third goal, namely in 2030 to reduce one third of premature deaths from non-communicable diseases through prevention and observation as well as promoting mental health and well-being in the concept of sustainable development (SDGs). Of the several risk factors that influence, obesity is one of the factors that is very considered because it increases the risk of several diseases, namely heart disease, diabetes, and high blood pressure. Around 38 million people in the world die from non-communicable diseases every year. Cardiovascular disease is the main cause (17.5 million people die each year), followed by cancer (8.2 million), respiratory disease (4 million) and diabetes (1.5 million) [1].

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The increase in obesity cases is also in line with the increase in cases of non-communicable diseases related to obesity. High cases of disease will be accompanied by an increase in health costs incurred for the treatment of diseases related to obesity. The high prevalence of diseases due to obesity will cause problems in the future because it increases health costs and decreases work productivity so that it can become an economic burden. Overweight and overweight children are related to health expenses for medicines, hospital stays, emergency rooms and US $ 1.4 million annually. Improved hospital treatment with a diagnosis of obesity in children nearly doubled in 2005 when compared to data in 1999. The prevalence of diabetes doubled to 2% of the total population, hypertension increased to 9.5% of the total population and stroke increased to 1.2% of the total population. Based on the measurement of disease burden using Disability-adjusted life years (DALYs) in 2010 showed that stroke contributed the most in the overall burden of disease in Indonesia with a percentage of 8% [2]

Increased consumption of foods that are high in saturated fat, trans fatty acids, sugar and / or salt, combined with a lack of physical activity, contribute to obesity and diabetes, and other non-communicable diseases. The tendency to increase consumption of "junk food" which usually offers a larger portion of

food, high in saturated fat and sugar is one of the dietary patterns that can trigger obesity. Improved economic status causes a shift in eating habits to a Western diet and a "comfort" lifestyle so as to increase calorie intake, decrease physical activity, and increase body weight [3]

Based on the factors of obesity, one of the things that affects is the food consumed. Food can affect body weight by controlling satiety or metabolic efficiency [4]. Several studies have shown that food sources have effects that can prevent obesity including mango leaf tea [5], green coffee extract [6], and fermented soymilk [7–9]

Several studies of fermented soymilk also show antiobesity or hypolipidemia [7–9]. Fermented soymilk has hypolipidemic and antiobesity effects because it can absorb fat and increase faecal fat excretion and function as an antioxidant [9] and increase leptin levels [8]. The effect of hypolipidemic is not followed by an increase in total fat in the liver as the results of a study by [9] which showed that the histology results of treated rats that did not experience fatty acid accumulation and decreased alaninamino transferase (ALT) and aspartate aminotransferase (AST)) which is an indicator of liver damage. Whereas [8] showed that an increase in leptin in mice that received soy milk treatment so as to prevent insulin resistance.

One mechanism of fermented soymilk in reducing blood triglyceride and blood cholesterol levels is to increase the secretion of triglycerides and cholesterol in the fecal by lactic acid bacteria and soy isoflavones [9,10]. Therefore it is necessary to examine the relationship Between fermented soymilk feed, total lactic acid bacteria in faeces with hyperlipidemia.

Method

Preparation of soymillk

Soybeans soaked in water for 8 hours, washed and separated arinya skin. Then the soybean is ground with a ratio of soy: water is 1: 5. After the waste is separated, soy milk is sterilized at 115 ° C for 10 minutes. Sterilized soymilk is divided into two parts, fermented soymilk and unfermented soymilk. Fermented soymilk inoculated with *Lactobacillus plantarum* bacterial concentration was 107 CFU / ml. Then soy milk was incubated for 18 hours at 37ºC.

Animal and treatment

The research sample was rat strain wistar with aged 8-9 weeks. The weight around 200-270 gram and obtained from Animal Center from Professor Nidhom Foundation (PNF). After being adapted for 2 weeks, the rats were divided into 4 groups (n=6) randomly : (1) control group; (2) HFD group, (3) HFD + soymilk (HFD + SM); (4) HFD + *L.plantarum* fermented soymilk (HFD+FSM). All rats had free access to water and were administrated intragastrically at 8.00 am daily. The sudy was conducted for 8 weeks and weight was measured weekly. Sampling feaces are taken every 2 weeks to measure the toal lactic acid bacteria. Total lactic acid bacteria measured using the pour plate methode. 1 gr of feaces diluted with sterile 0.85 % sline (w/v). The last dilution level was planted in MRS media and incubated for48 hours. The growing colonies were counted and recorded as CFU per gram of feaces. The project was approved by the animal experimental ethics committe of Faculty of Nursing, Airlangga University. At the end of the study, rats were anesthetized using ketamine hydrocloride 300 mg/kg. Blood samples are collected though the heart arteries (intra cardiac). Blood is centrifuged for 10 minutes at 4 ºC at a speed of 20,000 g. The serum was stored in an eppendorf tube at -20 ºC before further analysis. Serum parameters including TC and TG levels wer determined using analytical reagent kits.

Statistic Analysis

Data is presented as means ± standard deviation (SD) and analyzed using SPSS software. Differences between groups were tested using ANNOVA with $p <$ 0,005 and Least Significant Difference (LSD) test with $p < 0.05$.

Result and Discussion

The results showed that the total lactic acid bacteria foer HFD+SM and HFD+FSM groups were higher than the control and HFD group. Differences in total lactic acid bacteria between groups occurred from the 2nd week. At the end of the study showed that the total lactic acid bacteria were lower when compared to HFD+SM and HFD + FSM groups. Total log lactic acid bacteria in feaces during experiment shows in Table 1.

Table 1. Log total lactic acid bacteria in feaces during experiment

Group	Mean	SD	P value
Week-0			
Control	5.87533[a]	0.089536	0.459
HFD	5.29662[a]	0.186323	
HFD+SM	5.29733[a]	0.051391	
HFD+FSM	5.50133[a]	0.051856	
Week-2			
Control	6.14633[a]	0.017580	0.000
HFD	5.90367[b]	0.083457	
HFD+SM	6.73800[c]	0.048555	
HFD+FSM	6.76933[c]	0.080401	
Week-4			
Control	7.37033[a]	0.097465	0.000
HFD	6.47533[b]	0.085184	
HFD+SM	7.46033[a]	0.075176	
HFD+FSM	7.64133[c]	0.045364	
Week-6			
Control	6.10633[a]	0.036324	0.000
HFD	6.02367[a]	0.097326	
HFD+SM	7.43200[b]	0.090761	
HFD+FSM	7.98000[b]	0.039930	
Week-8			
Control	6.60433[a]	0.087952	0.000
HFD	6.51500[a]	0.177482	
HFD+SM	8.04900[b]	0.061527	
HFD+FSM	8.61267[b]	0.092478	

Lactic acid bacteria can also provide anti obesity effect. Research [8] by shows that *L.plantarum* and *L. paracasei* can inhibit the differentiation of preadipocytes and reduce fat accumulation in 3T3-LI cells. Fermented soymilk with *B.bifidum, L.casei,* and *L.plantarum* can significantly reduce weight, liver damage, fat index, and hyperlipidemia [11].

Probiotics improve the microbial flora in the large intestine so that the composition of the beneficial bacteria

outweighs the harmful bacteria. Intestinal microbiota in NAFLD sufferers tends to have an increase in the bacteria Ruminococcus and Escherichia spp [12]. An increase in gram negative bacteria will increase the production of lipopolysaccharides (LPS) that enter the liver so that it can increase inflammation in the liver [11]. Provision of Lactobacilli, Bifidobacteria, and Streptococcus thermophillus probiotics can reduce the activity of jun N-terminal kinase (JNK) and reduce the DNA binding activity of the nuclear factor κB (NF-κB) so that TNF production decreases [13]

Lactic acid bacteria can improve fat metabolism by activating the 7-α hydroxylase cholesterol enzyme in the liver. Activation of this enzyme will increase faecal cholesterol secretion so that it can reduce cholesterol in the blood [10]. Lactic acid bacteria also have the same role, namely increasing the secretion of cholesterol, triglycerides, and bile acids in feaces [9]. The supply of fermented soymilk provides probiotics and prebiotics so that it improves flora in the rat large intestine.

Rats triglyceride blood levels during the study ranged from 74.33 ± 24.8 - 130.17 ± 28.673. The highest triglyceride levels in the HFD group were 130.17 mg / dL, while the lowest triglyceride levels in the control group were 74.33 mg / dL. The total cholesterol level of rats during the study ranged from 50.83 ± 4.215 - 64.00 ± 7.823 mg / dL. The highest total cholesterol level in the HFD group was 64 mg / dL, while the lowest total cholesterol level in the HFD + FSM group was 50.83 mg / dL. Blood TG and TC levels are shown in Table 2.

Table 2. triglyceride and cholesterol level in rat serum.

Group	Triglycerida (mg/dL)		Total cholesterol (mg/dL)	
	Mean	SD	Mean	SD
Control	74.33 a	24.809	55.00a	8.877
HFD	130.17b	28.673	64.00a	7.823
HFD+SM	113.17b	23.302	51.00a	5.933
HFD+FSM	104.33ab	19.377	50.83a	4.215
P-value	0.006		0.012	

Fermented soy milk has a good effect for reducing total blood cholesterol. This is indicated by lower total blood cholesterol levels than the control group. While blood triglyceride levels for the HFD + FSM group were not different from the control group but were also not different from the HFD group. Unfermented soy milk also has a similar effect to unfermented soymilk.

Soybean has biogenic peptides which have the effect of improving blood lipid profile and liver steatosis [14]. The mechanism involved is soy and its bioactive peptide can stimulate bile acid secretion, change cholesterol metabolism in the liver, and regulate cholesterol hormones and receptors. Research by [15], soy protein intake of 31 - 47 grams per day can significantly reduce total cholesterol and LDL cholesterol, triglycerides, but not significantly to HDL values. Early cholesterol levels determine the effect of soy protein on cholesterol reduction

Soy protein can affect lipogenesis in the liver. Soy protein can reduce triglyceride concentrations in blood and in animal liver models. This effect is related to a decrease in the activity of lipogenic enzymes in the liver namely glucosa-6-phosphate dehydrogenase, malic enzyme, fatty acid synthetase, and acetyl-CoA

carboxylase (ACC). In addition, soy protein can activate PPARs, namely transcription factors involved in glucose homeostasis, fat metabolism and fatty acid oxidation [16].

Research by [17] compared the administration of soy protein and cow's milk protein to liver steatosis in rats. The results showed that soy protein changed the microflora in the intestine so that it changed the metabolism of bile acids. Changes in microflora more lactic acid bacteria can reduce blood cholesterol levels because lactic acid bacteria have the enzyme bile acid hydrolase, thereby increasing the level of bile acids that are discharged into the faecal. Soy protein treatment can also increase ApoB-100 secretion so that it is thought to increase VLDL secretion from the liver. Soy protein treatment also increases the proportion of PUFA in the liver thereby reducing denovo lipogenesis in the liver.

Soy protein can improve insulin resistance and blood lipid profiles by increasing PPARs activation. According to [18] consumption of soy protein can increase PPARα activation, and decrease SREBP1c activation. In addition, soy protein can reduce liver triglycerides and adipose tissue weight. This is related to the increased activity of enzymes involved in fatty acid oxidation, among others: CPT1. Soy protein is thought to increase the oxidation of fatty acids through the PPAR pathway because the administration of soy protein can increase PPARα mRNA levels

The content of soy milk linolenic acid is also potential as a compound that can prevent obesity and liver steatosis. Omega 3 fatty acids can increase the oxidation of fatty acids in the liver and decrease TG synthesis by inhibiting the transcription activity of SREBP-1c and ChREBP factors in the liver. Omega 3 fatty acids can reduce the release of fatty acids from adipose tissue. In addition, omega-3 fatty acids can also inhibit macropage activation so that they are anti-inflamatory. Omega 3 fatty acids also increase the species of bacteria that can produce butyric acid in the intestine thereby reducing the production of lipopolysaccharides [19].

Isoflavone supplementation can improve fat metabolism by activating the 7-α hydroxylase cholesterol enzyme in the liver. Activation of this enzyme will increase faecal cholesterol secretion so that it can reduce cholesterol in the blood [10]. Lactic acid bacteria also have the same role, namely increasing the secretion of cholesterol, triglycerides, and bile acids in feces [9]

Conclusion

Based on the results of the study showed that fermented soymilk has the effect of reducing blood cholesterol and triglyceride levels through an increase in total lactic acid bacteria in the large intestine.

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The Culture of Life's Consumptive Behavior Against Potential Hypertension at Pangkep Community in South Sulawesi Indonesia

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Abstract

Background. Every person has life needs that are met in different ways. There are those who meet their needs naturally and there are those who are excessive in fulfilling their needs. This causes people to behave consumptively. Consumptive behavior occurs in almost all walks of life. The habit of going to food courts and fast food restaurants has led to consumptive behavior in modern society, they prefer something that is instant, fast and delicious to consume and this has already happened in rural communities, especially in Pangkep Regency. Patients with hypertension are the main causes of death (13.13%) and increasing cases (11,620) in Pangkep Regency in 2018 and 2019. Hypertension is a disease caused by various causes which are interrelated to one another. One of the causes of hypertension is nutrition (fruit and vegetable consumption habits). Researchers want to analyze the relationship between consumer behavior in consumptive life with hypertension. **Material and Methods.** The study uses qualitative research with a phenomenological approach. The sampling method uses snowball sampling. The study was conducted in 25 June 2018 - 31 Jan 2019. The number of informants was 13 people. Data collection techniques namely interview techniques, observation, documentation. Data analysis techniques include the results of interviews, data reduction, analysis, data interpretation, and triangulation. R**esults**. first, in looking at the consumptive behavior and culture of the Pangkep community, it is found that many hypertension sufferers begin with frequent visits to the mall and consume fast food. Second, consumptive behavior and culture are risk factors for increased hypertension sufferers. This is evidenced by the large number of patients visiting at the Health Service with a diagnosis of hypertension. C**onclusion.** The people of Pangkep Regency have a consumptive behavior so that they are at high risk of developing obesity, hypertension.

Keywords: *Consumption, Society, Hypertension, Disease, Behavior, Culture*

Introduction

Every person has life needs that are met in different ways. There are those who meet their needs naturally and there are those who are excessive in fulfilling their needs. This causes people to behave consumptively. Consumptive behavior occurs in almost all walks of life. The habit of going to food courts and fast food restaurants has led to consumptive behavior in modern society, they prefer something that is instant, fast and delicious to consume and this has already happened in rural communities, especially in Pangkep Regency. Patients with hypertension are the main causes of death (13.13%) and increasing cases (11,620) in Pangkep Regency in 2018 and 2019. [1] Hypertension is a disease caused by various causes which are interrelated to one another. One of the causes of hypertension is nutrition (fruit and vegetable consumption habits). [2] Researchers want to analyze the relationship between consumer behavior in consumptive life with hypertension.

Everyone has the necessities of life fulfilled in different ways. There is a reasonable need to fulfill his needs and there is also excessive in fulfilling his

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needs. It causes people to behave consumptive. Such consumptive behaviour occurs in almost all walks of life. Not only in adults, consumptive behaviour has struck many teenagers, industrial products eyeing teenagers as their target marketing, among other things because of their labil, specific and easily influenced characteristics, ultimately encouraging the emergence of symptoms in unnatural buying behaviors. [3] Buying is no longer done because the product is needed, but buying is done for other reasons such as just following the mode, just want to try a new product, want to get social recognition and so on. Teenagers are an interesting object for marketing experts to demand. Consumerist behaviour is a phenomenon that has a lot of people's lives, especially those living in urban areas even in suburbs such as Pangkep. This phenomenon is interesting to be researched considering the consumptive behaviour also much struck by the lives of teenagers and communities of large cities and towns such as Pangkep.

It is also a consumptive in food. Food court and fast food restaurants are the prima people of South Sulawesi, especially urban and local communities such as Pangkep. The presence of food court and fast food restaurants become one of the dining places that are recomended for all people from teenagers, parents, children, who provide different atmosphere where equipped with supporting facilities such as Internet access that is WiFi. Food court and fast food restaurants are also a place to gather with friends, family, co-workers and so on who is no longer foreign in the community. [4] The creation of a different atmosphere in the place to eat food court and fast food restaurant makes people especially who visit the mall usually stop by this place to just eat. Different packaging that is presented by food court and fast food restaurant is making the community start switching to buy a meal or drink at this place in comparison in the stall, this is not detached from the development of the era namely the post-modernization period that gave birth to capitalism as well as consumerism in today's society that raises someone to style the consumptive life in society. [5]

Food Court and fast food restaurant in South Sulawesi is no longer a foreigner because the place to eat like this much we meet everywhere especially in large malls. People in the community use food court and fast food restaurant as a place to eat with family, friends, companions, co-workers and so on. In the presentation of food court and fast food restaurants usually use fast food or also called fast food, food and drink like this is no longer foreign in today's Indonesian society, where many Indonesian people love food and drinks fast food as its main menu such as burgers, potatoes, Coca-Cola and so on that served by fast food restaurant.

It is packed in something good service from the food and drinks and waiters that serve the food and beverages. When eating food and drinks in the food court and fast food restaurants do not need to queue again because simply by ordering only, food or drinks will quickly come to our liking. This is something that is normal in the present era, where the community wants something fast and delicious so that it becomes something natural when the food court and fast food restaurants are available in various large malls in Indonesia for the life of today's society. [6] This makes people today compete to show their identity in terms of places to eat or drink the best and food court and fast food restaurant as a place that can be used as a person to show his identity in the community, especially the community Pangkep.

Material and Methods

The study uses qualitative research with a phenomenological approach. The sampling method uses snowball sampling. The study was conducted in 25 June 2018 -31 Jan 2019. The number of informants was 13 people. Data collection techniques namely interview techniques, observation, documentation. Data analysis techniques include the results of interviews, data reduction, analysis, data interpretation, and triangulation.

Results

Table 1. Recapitulation Grade Hypertension of th Informant

No.	Category	Frequency	Informant
1	Grade 1	3	1, 2, 8, 13
2	Grade 2	5	4, 5, 7, 10, 11
3	Grade 3	4	3, 6, 9, 12

Discussion

Hypertension or famously known as the silent killer is a condition where the increase of blood pressure above normal. Increased age is one factor causing the occurrence of hypertension, thisis due to the increasing age of organ function decreased marked by decreased elasticity of the arteries and stiffness occurs blood vessels so vulnerable to an increase in blood pressure. Hypertension is defined as persistent blood pressure where the systolic pressure is above 140 mmHg and diastolic over 90 mmHg. [7], [8], [9] One of the major risk factors of hypertension is stroke, heart failure, chronic kidney disease, visual impairment, and hypertension is often called the silent killer. Hypertension is a condition when a person experiences a rise in blood pressure either slowly. [10], [11], [12]

The development of capitalism was also not separated from the rapidly expanding era of globalization's support that impacted the controlled system of consumption, but also mass-consumptive behaviour that could be exploited as Marx's thesis on labour victims of the capitalistic system exploited according to the views of Jean P. Baudrillah. [13] This mass consumptive pattern makes the community consume something continuously continuously and this is usually influenced by advertising, brochures and so on. Places such as food court and fast food restaurants can be an example of how these places to eat can affect people to come here from among teenagers, young and old. Construci thought that is easily changed by a form of advertising and also the product or use these famous people because people affected and want to come to the place, which eventually raises the consumptive pattern of the community especially the community in the area Pangkep.

Convenient facilities that are provided by the restaurant itself where the service of internet access such as WiFi and so on which becomes a means of supporting to provide a sense of comfort to everyone who visits. Food court and fast food restaurant itself serves a variety of fast food because food and drinks fast food is seen more delicious and fast in terms of service so visitors are interested in visiting places to eat like this. In the food court and fast food restaurant also presents something different where many variations of food and beverages can be easily and many also offer food and drinks with a cheap price plus a bonus of free drinks to attract the visitors to come to the place.

Another factor is why one chooses a food court and a fast food restaurant because it is not merely eating, drinking or gathering but also as the designation of a person's identity in the community, because eating or drinking today is not merely to feast on the stomach but also as an event or social symbol of society, especially the community in South Sulawesi. People also often associate visits to fast-food restaurants such as Pizza Hut, KFC, MC Donal, and others, as a lifestyle today more to the prestige, image and motive of a person to visit the place, and this raises the consumptive behaviour of today society that only sees from its product form without looking at the amount of money that should be spent only for a different lifestyle with other people who are always associated with the position and also role in the community environment. Due to the attitudes of today's consumptive society, people who love a variety of fast food can increase the risk of getting some diseases, such as obesity, diabetes, hypertension, and blood lipid disorder or dyslipidemia. [14]

The consumptive behaviour of fast-food restaurants, which is currently embraced by the people of South Sulawesi, the local community of Pangkep tends to make people like instant things. Consequently, they tend to lazy physical activity and love to consume instant food, which has a high sodium content (Sari & Wulan R, 2008).[15] Implementing a healthy diet is not able to guarantee that we will be free of disease, but at least pay attention to the intake of food consumption patterns are able to minimize the risk of a person can be attacked by various diseases. Without realizing it, the food contains a high salt that can increase saliva and enzyme secretion, thereby increasing the desire to continue to eat the food. The high content of bad fats and sodium impairs the sodium and potassium balance in the body, causing hypertension. High sodium content has been known to cause high blood pressure factors. Sodium is known to affect the renin-sngiotensin system in the kidneys which can later cause high blood pressure.

Research in America shows that someone who consumes fast food in large portions and with frequencies

2 to 3 times per week, can result in hypertension. Research in Yogyakarta also shows a significant link between the eating habits of fast food with hypertensive events. The more often the habit of eating fast food, it will have a chance of 0.451 experiencing the incidence of hypertension. Like wise in the area Pangkep, based on data from the health profile of the province of South Sulawesi, 2018 973 people with a percentage increased to 16.2%, in 2019 as much as 592 with a percentage of 9.7% according to the data Puskesmas Mandalle, Pangkep. [1]

Factors that affect the occurrence of hypertension are divided into two large groups that are factors that can not be modified such as gender, age, genetic and can be modified factors such as diet, exercise habits, and smoking habits. Based on the results of the study, diet with frequencies often consume foods that contain high energy, sodium, saturated fat and low fiber can increase the risk of hypertension. High energy consumption can affect obesity, where people who suffer from obesity can potentially occur hypertension. High sodium consumption can cause the body to render fluid that can increase blood volume. High sodium consumption can also shrink the diameter of the arteries, consequently the heart must pump hard to push the volume of blood through the increasingly narrow chamber, so that blood pressure becomes increased and leads to hypertension. [16]

Saturated fats can cause hypertension through the mechanisms of dyslipidemia. In theory, low fibre intake results in fewer bile acids excreted in the feces, resulting in much cholesterol absorption from the residual bile. This results in a lot of cholesterol circulating in the blood and causing a buildup of fat in the blood vessels that can inhibit blood flow, thereby affecting the increase in blood pressure. Based on the explanation that has been explained earlier, it will be discussed more in depth about the health impact of the habit of consuming fast food continuously, which leads to the consumptive behavior in make as a habit and a necessity in daily life. [16]

One example of a food menu that can be made simply a toast with avocado and boiled eggs are half-baked. If the wheat bread usually, avocado and eggs are eaten separately, this time the three foods are combined into healthy food that you should not miss. In fact, there are still many people who do not realize the importance of eating healthy foods. It becomes a kind of problem in the community especially Pangkep community, which is used as a reason to not consume healthy food when processed properly, can give a delicious taste. Therefore, consumption of healthy foods in order to support a healthy lifestyle to avoid the various diseases, especially hypertension.

Conclusion

Most Pangkep Regency Communities do not think about the nutritional value of food consumed, but rather prioritize its deliciousness and satiety. This resulted in many people suffering from hypertension. In addition to this lifestyle, there is a lifestyle that is often carried out by the people of Pangkep, namely staying up late (sleeping late), smoking, drinking soft drinks, drinking coffee, and a number of drinks that stimulate hypertension. The impact of these conditions results in low health numbers experienced by the community. The people of Pangkep Regency have a consumptive behavior so that they are at high risk of developing obesity, hypertension.

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Neonatal Therapeutic Intervention Scoring System (NTISS) in Rural Country: Mortality and Length of Stay (LOS) Predictive Score in Preterm Infant

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Abstract

Background: Preterm infants need comprehensive management due to high risk of mortality and morbidity. Proper treatment in NICU can decrease the mortality risk in them, but some of them may take long time before they can go home. Neonatal Therapeutic Intervention Scoring System (NTISS) hopefully can be a predictive score for the mortality and Length of stay (LOS) of a preterm infant, yet, it's use is still very limited in Indonesia. The authors aim to analyze NTISS to predict mortality and length of stay of preterm infants in Indonesia.

Method: This retrospective study conducted in March 2017 and collected medical record data of preterm infants treated in NICU from July 2016 - March 2017. The inclusion criteria were infants with gestational age <37 weeks, treated at NICU> 24 hours and complete medical record data. NTISS total scores are collected after 24 hours at the NICU.

Results: A total of 108 infants met the inclusion criteria. The ratio of NTISS scores had an area under the curve (area under curve / AUC) of 0.879 (95% CI 0.259-0.913) with cutoff at 20.2. it had 91.3% and 74.1% in sensitivity and specificity respectively; 48.8% and 96.9% in positive and negative predictive value respectively; positive likelihood ratio 3.52; negative likelihood ratio 0.12; and 77% inaccuracy. There was no correlation between NTISS scores and length of stay (p = 0.084).

Conclusion: NTISS can be used to predict the mortality of preterm infants.

Keywords*: Preterm infant, Neonatal Therapeutic Intervention Scoring System, Mortality, Length of Stay*

Background

Preterm birth has emerged as one of the global burdens due to its mortality and morbidities[1]. While other causes of death, like pneumonia, diarrhea, malaria, and measles, have been declining for the past 2 decades, complications in children with a history of preterm birth are now becoming the leading cause of death in children under 5 years old, with more than 1 million death reported[2]. A preterm infant is defined as any infant born before 38 weeks of gestational age and can be either spontaneous or provider-initiated[3]. Preterm birth can be In 2010, around 15 million infants born before 38 weeks of gestational age[4]. A similar number is also seen in 2012, which is 11.3 % of all birth worldwide[5]. Approximately, 37.6% of those preterm birth occurred in South Asia while 24.3 % occurred in Southeast Asia[6]. A study in Indonesia stated that there is 101 neonatal death of 807 births in 2014-2015 with preterm birth is

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one of the leading causes of death[7].

Although there are more preterm infants that survive than die in the first 30 days of life[5], the survivors will then have to face the risk of many complications, either early or late complications, in the upcoming years. The infant survivors are in preterm infant and are at a high risk of many complications due to functional and anatomical immaturity. Gestational age and birth weight are indeed associated with mortality rate while sex is still debatable[8,9]. Acute complications related to organ immaturity including respiratory distress syndrome, intracranial hemorrhage, necrotizing enterocolitis, and retinopathy of immaturity[4]. Other studies also stated that there is a significant event of morbidities including weakening neurodevelopmental capacities, learning impedance, visual disorder, and secondary effect in long term health[3]. Hypothermia also increases the infant vulnerability[4] and is associated with a higher mortality rate, intraventricular hemorrhage, lung insufficiency, and bleeding[8]. The presence of the complications before age of 1 month is associated with higher mortality rate to neonatal[10]. Therefore, high quality of intensive training is needed to decrease the preterm infant mortality rate[11].

Intensive treatment of neonatal, including surfactant administration and steroid therapy to treat respiratory distress syndrome, can reduce the neonatal mortality rate[12]. The study reported that as neonatal survival has improved, there is also increasing number of babies requiring long-term neonatal care[13]. It is important to predict the length of stay (LOS) of preterm infant in NICU to assess the resource planning, commissioning of services, and aid clinicians in their counseling to parents[13]. However, there is still limited information on research in the area of predicting the LOS. Many factors are associated with LOS of preterm neonatal in the NICU including gestational age, birth weight, APGAR score, sex, cesarian section, and multiple pregnancies. A study stated that better gestational age and birth weight are associated with a reduction of LOS while gestational age is associated with birth weight[14]. Another study reported that survived extreme preterm infants (<25 weeks) have the longest LOS in the NICU, approximately 107-123 days, while preterm infants with the gestational age of 30-31 weeks are 1 month shorter in LOS[13].

Furthermore, Assessment to predict the mortality rate of preterm neonatal in hospitals, especially in NICU, has been vital information to control the healthcare quality, rational resources management, and to be used as standard outcome comparison in every health care[15]. Several scoring systems can assist to predict mortality and morbidity and improve the outcome validity in many hospitals. Neonatal Therapeutic Intervention Scoring System (NTISS) is a modification of Therapeutic Intervention Scoring System (TISS) that is used in adults. NTISS is a therapy-based and severity-based index that can be used as a neonatal severity indicator and resources utilization[16]. The authors aim to analyze the NTISS as predictive scoring system on mortality rate and LOS in a preterm infant treated in NICU.

Methods and Materials

This was a retrospective study that used medical record data of preterm neonatal treated in the Neonatal Intensive Care Unit (NICU) Dr. Soetomo Hospital Surabaya from July 2016 to March 2017. The inclusion criteria were preterm infants with gestational age <37 weeks when born, 24 hours minimum of treatment in NICU, any incomplete data information would be excluded.

We evaluated the gestational age, birth weight, and APGAR score as the indicator of mortality and morbidity risks and also the length of stay in every infant. Then we assessed all 8 parameters in NTISS. Data were collected and analyzed by chi-square/Fischer's exact test. The prognostic value (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio, negative likelihood ratio, and accuracy) of NTISS were also analyzed with the ROC curve as the cutoff point. The Institutional Review Board had reviewed and approved this study with ethical clearance No: 1574/KEPK/X/2019.

Result

There was a total of 190 data collected. 82 were excluded and 108 met the inclusion criteria. This study subjects were dominated with female sex (61.6 %), moderate preterm (66.7%), < 2500 gram in birth weight (68.5 %), Caesarian delivery (64.8 %), survived after treatment (78.7 %), and NTISS score over 20.5 (60.2 %). There was no notable domination in the APGAR

score. The basic data characteristics were depicted in table 1.

Table 1. Data Characteristic of Subjects

Indicator	Frequency (n)	Percentage (%)
Sex		
Male	42	38.40
Female	66	61.60
Gestational Age		
Extremely preterm (<28 weeks)	8	7.4
Very Preterm (28-32 weeks)	28	25.9
Moderate Preterm (32-<37 weeks)	72	66.7
Birth Weight		
< 1000 gram	10	9.3
< 1500 gram	16	14.8
< 2500 gram	74	68.5
Normal	8	7.4
Mode of Delivery		
Per Vagina	38	35.2
Caesarian	70	64.8
APGAR score		
0-3	38	35.2
4-6	33	30.55
7-10	37	34.25
Length of Stay		
1-10 days	49	45.4
11-20 Days	30	27.8
21-30 Days	19	17.6
31-40 Days	4	3.6
> 40 Days	6	5.6
Outcome		
Survive	85	78.7
Dead	23	21.3
NTISS Score		
≤ 20.5	43	39.8
> 20.5	65	60.2

In Table 2, Fischer's exact and Chi-Square were used to analyze risk factors related to preterm infant mortality. The table showed that every indicator had a significant result. Furthermore, in the prognostic value of NTISS, with cutoff value in 20.5, the NTISS score had 91.3 % sensitivity, 74.1% specificity, 48.8% PPV, 96.9% NPV, 3.52 Positive likelihood ratio, 0.12 Negative likelihood ratio, and 77% accuracy. The ROC curve was depicted in figure 1.

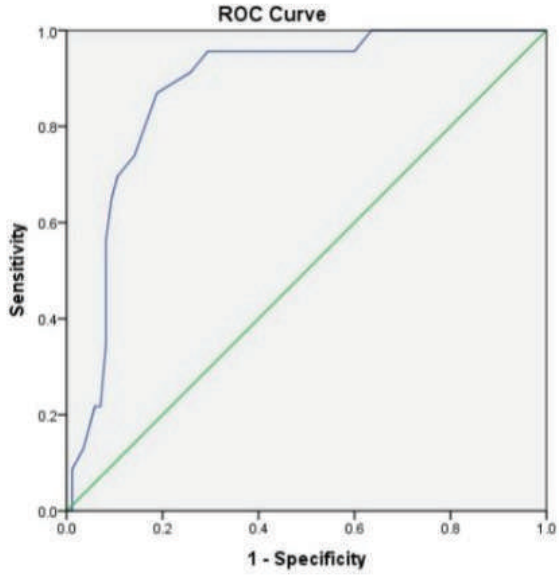


Figure 1. ROC Curve in preterm infants

Table 3 showed the significant correlation between NTISS score and mortality (p= 0.000) in the study while table 4 did not show any significant correlation between NTISS score and length of stay (p= 0.084).

Table 3. Correlation Test Between NTISS and Mortality in Preterm Infant

NTISS Score	Dead	Alive	P	OR
≤ 20.5 > 20.5	2 (3.1 %) 21 (48.8 %)	63 (96.9 %) 22 (51.2 %)	0.000	30 95% CI 6.5-138

Table 4. Correlation Test Between NTISS and Length of Stay

	Length of Stay (Days)					Value	P-Value
	1-10	11-20	21-30	31-40	>40		
NTISS Score ≤ 20.5 > 20.5	31 18	21 9	11 8	1 3	1 5	7.81	0.084
a = Fischer's Exact Test							

Discussion

This study analyzes the mortality risk factors in a preterm infant. Preterm baby and low birth weight had a mortality risk 16 times higher than normal[5]. All extremely preterm is the lowest rate in neonatal survival while moderate preterm is the highest. It shows that higher gestational age is associated with a higher survival rate, similar result also seen in another study[17].

NTISS, as a mortality prediction, is showing a convincing result. A previous study in 2015 stated that

NTISS has 100% in sensitivity and 81.2 % in specificity for mortality prediction with a cutoff point at 28.0 and scoring assessed after 48 hours of treatment[18]. Another study also stated that NTISS is acceptable to be a mortality prediction score, with preterm birth and birth weight between 1000 – 1499 gram as the subject[16]. NTISS shows the number of interventions applied to an infant. The higher NTISS score is, the more interventions are given, thus the mortality rate to the infant is also higher[18]. Surfactant, mechanical ventilation, surgical intervention, and parenteral nutrition administration are the examples of the intervention listed in NTISS. However, some parameters in NTISS were not assessed because they are not performed in the authors' hospital NICU, including aminophylline therapy, extracorporeal membrane oxygenation, pacemaker use, and other cardiac interventions.

Based on the NTISS score got in this study, the respiratory parameter plays a significant role. Most preterm infants that have high respiratory score encounter severe respiratory problems and are at high risk to mortality. The respiratory condition is the most common reason why a newborn infant is treated in NICU, whether term or preterm. Respiratory distress syndrome is the most common diagnosis and the incidence is higher in lower gestational age[19,20]. A study showed that the significance of NTISS variables to predict the mortality risk can be varied because of the birth weight[16]. Another meta-analysis study in 2017 stated that NTISS can be a great predictive tool for mortality and morbidity, but its accuracy is lower in limited resources setting[21].

In this study, the NTISS score is not associated with the length of stay. It means that the total number of interventions given to an infant cannot explain the clinical condition of the infant. LOS of an infant in NICU is affected by gestational age and clinical condition like bronchopulmonary dysplasia (BPD), or persistent apnea. A long treatment in NICU also increases the risk of nosocomial infection[22]. Some studies stated that longer treatment in NICU may be caused by the gestational age, birth weight, intrauterine growth, first 5 minutes of APGAR score, parity, and severe clinical conditions[13,22]. In the conclusion, NTISS can be used as a mortality predictive score in the preterm infant, but its accuracy still needs to be reevaluated and modified, especially in limited resources hospitals where not every parameter in NTISS is available. However, it cannot be used as LOS predictive score for preterm infants. Despite these results, some confounding factors that may affect the result of this study are not evaluated, including congenital abnormalities, history of the mother, and other risk factors that may contribute to longer LOS. Thus, further study with larger samples is needed.

Ethical Clearance was taken from Health Research Ethics Committee RSUD Dr. Soetomo Surabaya, with ethical clearance No: 1574/KEPK/X/2019.

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The authors report no conflict of interest in this work.

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Correlation of Toluene Safe Duration (Hours/Day) and Glutathione Concentration, Malondialdehyde and Neurotoxic Symptoms in Osowilangun Shoe Home Industry Workers

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Abstract

Introduction: Exposure of toluene can cause a decrease in glutathione concentration, increase in malondialdehyde and neurotoxic symptoms. The safe duration of toluene in hours/day for each person can vary. The purpose of this study is to determine the relationship between the safe duration of toluene (hours/day) with the concentration of glutathione, malondialdehyde and neurotoxic symptoms in Osowilangun shoe home industry workers.

Methods: This research was observational with quantitative approach and cross-sectional design. The sampling technique used was accidental sampling with 25 respondents. The variables studied were safe toluene duration (hours/day), glutathione concentration, malondialdehyde concentration, and neurotoxic symptoms. Data were analyzed using Pearson and Phi correlation.

Result: The average of safe duration was 1,489 hours/day, glutathione concentration was 58,349 µg/L, and malondialdehyde was 7,847 MU. As many as 11 out of 14 workers experienced neurotoxic symptoms. The relationship between safe duration (hours/day) with glutathione concentration (r=0.139) and malondialdehyde (r=-0.146) was very weak. While, the relationship between safe duration (hours/day) and neurotoxic symptoms was weak (φ=-0.223).

Conclusion: The relationship between safe duration in hours/day with glutathione concentration and malondialdehyde was very weak, while relationship with neurotoxic symptoms was weak.

***Key words**: Neurotoxic Symptoms, Glutathione, Safe Concentration, Malondialdehyde, Toluene*

Introduction

Toluene is a colorless liquid and a good solvent. Toluene can evaporate into the surrounding air and be inhaled by people who work using products containing toluene[1]. The toluene threshold at work is 20 parts/million for 8 hours/day or 40 hours/week. With this dose and duration, almost all people who are exposed do not suffer side effects from toluene[2].

When toluene enters and is distributed throughout the body, toluene is metabolized through enzymatic pathways. If too much amount of toluene enters the body, dangerous toluene epoxide is formed. However,

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there is enzyme glutathione that can react with toluene epoxide and form substances that are not harmful to the body.

Toluene epoxide is very reactive and is a free radical[1]. Free radicals can cause lipid peroxidation chain reactions and produce lipid hydroperoxides that are unstable and easily break down into secondary products such as aldehydes and malondialdehydes. Malondialdehyde is then used as a marker of cell or tissue damage due to oxidative stress[3].

Organic solvents can change the lipid structure of cell membranes. This causes the disruption of the mechanism of synaptic membrane transportation and intercellular transportation[4]. Neurological disorders at work due to exposure to organic solvents can cause psycho-organic symptoms such as depression, abnormal emotions, low alertness, memory problems, and decreased mental efficiency[5].

The duration of exposure in one day can greatly affect the health of workers[6]. The duration of exposure is directly proportional to the pollutants that enter the worker's body. Therefore, the longer a person works, the more pollutants are absorbed into his body[7]. So, the concentration of glutathione, malondialdehyde and neurotoxic symptoms that occur due to toluene exposure can be influenced by the duration of daily toluene exposure.

The purpose of this study was to determine the relationship between the safe duration of toluene in hours/day with the concentration of glutathione, malondialdehyde, and neurotoxic symptoms that occur in workers in the Osowilangun Shoe Home Industry.

Materials and Methods

This research was observational used a quantitative approach and cross-sectional design. The population in this study was 38 workers in the Osowilangun Shoe Home Industry. Samples were taken using the accidental sampling method resulted 25 respondents. Data was collected in August 2019.

The safe duration of toluene was obtained through calculations with the following formula (Tualeka, 2015).

tE (hours/day)=Wbtavg×RfCC×R×fEDt

t_E = safe daily exposure time (safe duration (hours/day))

W_b = weight (kg)

T_{avg} = average time period (30 years×365 days/year) = 10950

RfC= reference concentration of toluene = 5 mg/m^3

= RfC (mg/m3) × R (m3/hari)Wb (kg) = RfC (mg/kg/day)

C = risk agent concentration (mg/m^3)

R = respiration rate (m^3/hour)

= 5,3 × ln(Wb)-6,924

f_E = annual exposure frequency (day/year)

D_t = duration of exposure (years)

Respondent characteristics data including were taken using a questionnaire. The concentration of toluene in the air was measured by the NIOSH 1501 method with the gas chromatography technique, carried out by the Work Safety and Health Technical Implementation Unit Surabaya. Measurement of glutathione and malondialdehyde concentrations was carried out blood analysis at the Nutrition Laboratory of Airlangga University. Neurotoxic symptoms are identified using the German version of the Q18 questionnaire, the respondent will be declared to have neurotoxic symptoms if had ≥5 "Yes" answer[8].

Result and Discussion

Toluene Safe Duration (Hours/Day)

Table 1. Respondent's Safe Duration (hours/day)

Respondent	Wb (kg)	RfC (mg/kg/ hari)	C (ppm)	C (mg/m3)	R (m3/hour)	fE (day/year)	Dt (year)	tE (hour/day)
1.	60	1.233	289.30	1088.004	0.616	364	25	0.133
2.	70	1.115	289.30	1088.004	0.650	208	30	0.193
3.	51	1.366	15.00	56.41224	0.580	312	15	4.977
4.	105	0.845	30.50	114.7049	0.740	364	30	1.049
5.	70	1.115	30.50	114.7049	0.650	312	48	0.765
6.	55	1.303	62.70	235.8032	0.597	312	25	0.714
7.	69	1.126	62.70	235.8032	0.647	312	40	0.446
8.	41	1.558	58.50	220.0078	0.532	312	22	0.870
9.	78	1.037	25.50	95.90082	0.674	364	25	1.506
10.	46	1.455	15.00	56.41224	0.557	364	30	2.133
11.	75	1.065	36.90	138.7741	0.665	364	25	1.040
12.	84	0.987	137.50	517.1122	0.690	364	15	0.465
13.	65	1.171	30.50	114.7049	0.634	312	25	1.469
14.	50	1.383	30.50	95.90082	0.576	364	20	1.882
15.	53	1.334	25.50	1088.004	0.589	364	25	0.133
16.	70	1.115	289.30	220.0078	0.650	312	13	1.472
17.	60	1.233	58.50	34.97559	0.616	312	30	4.014
18.	55	1.303	9.30	95.90082	0.597	364	20	1.882
19.	65	1.171	25.50	114.7049	0.634	312	25	1.469
20.	46	1.455	30.50	235.8032	0.557	260	5	4.286
21.	85	0.979	62.70	517.1122	0.693	364	20	0.349
22.	68	1.137	137.50	154.5696	0.644	364	30	0.778
23.	55	1.303	41.10	517.1122	0.597	364	6	1.163
24.	78	1.037	137.50	36.47992	0.674	364	25	3.958
25.	55	1.303	9.70	1088.004	0.597	364	40	0.083
Average								1.489

The average safe duration in hours/day for Osowilangun shoe home industry workers was 1,489 hours. ACGIH (2019) recommends a toluene threshold value of 20 ppm, for 8 hours/day or 40 hours/week. The safe duration of toluene in the Osowilangun shoe home industry does not reach 8 hours even though not all places have a toluene concentration of more than 20 ppm. This is in accordance with Scheepers research entitled Assessment of Exposure of Gas Station Attendants in Sri Lanka to Benzene, Toluene and Xylene (BTX) with a reduction in work duration to 40 hours/week can reduce BTX concentrations in workers' blood to safe limits[9]. Repeated toluene exposure can cause cognitive impairment in mice[10].

Glutathione Concentration

Table 2. Respondent's Glutathione Concentration, Malondialdehyde Concentration, and Neurotoxic Symptoms

Respondent	Glutathione concentration (µg/L)	Malondialdehyde concentration (MU)	Neurotoxic Symptoms
1.	14.600	11.857	Yes
2.	40.850	5.423	Yes
3.	40.583	6.277	No
4.	58.349	3.817	Yes
5.	49.790	4.543	Yes
6.	49.257	4.603	No
7.	46.176	4.717	No
8.	19.812	11.683	Yes
9.	27.873	10.023	No
10.	40.241	6.497	No
11.	45.453	4.737	Yes
12.	28.067	9.670	Yes
13.	34.383	8.683	No
14.	35.942	7.643	No
15.	30.578	9.317	No
16.	34.357	8.923	Yes
17.	31.415	9.177	No
18.	26.622	10.323	Yes
19.	26.356	10.843	No
20.	38.719	7.363	No
21.	39.937	7.203	No
22.	24.796	11.030	Yes
23.	43.969	5.017	No
24.	41.420	5.283	Yes
25.	21.829	11.517	No
Average	35.655	7.847	

Based on table 2, the average glutathione concentration in the Osowilangun shoe home industry workers was 35,655 µg/L. Glutathione is one of the antioxidants produced by the body and often used as a measure to determine the effects of increased free radicals. Glutathione can react with radical substances to form substances that are no longer harmful to the body[11]. Lack of glutathione causes a high risk of oxidative damage[12]. Exposure to harmful chemicals can reduce the amount of glutathione in the body[13]. The longer exposure to free radicals, the free radicals have the potential to accumulate and can reduce the body's glutathione[14].

Malondialdehyde

The average concentration of malondialdehyde of workers in the Osowilangun shoe home industry was 7,847 MU. When there are more free radicals in the body than antioxidants, oxidative stress occurs[3]. The more concentration of malondialdehyde in the body, the more oxidation processes in the cell membrane[15]. If not getting exposure to free radicals, the levels of malondialdehyde in the body become lower because lipid peroxidation in normal conditions can still be overcome by natural antioxidants produced by the body[16]. Therefore, people who get free radical exposure will have higher levels of malondialdehyde in the body compared to people who are not exposed[17].

Neurotoxic Symptoms

Neurotoxic symptoms were determined using a Q18 questionnaire, stated as having neurotoxic symptoms if there were ≥5 answers of "Yes" out of 18 questions. Based on table 2, 11 workers experienced neurotoxic. A study shows that one of the health effects caused by exposure to benzene, toluene and xylene that exceeds the threshold, is central nervous system disorders[18]. However, other studies conducted on workers exposed to toluene in a car painting workshop located in Surabaya show that toluene concentrations that are still below the threshold have been found by workers who show complaints of the central nervous system[19].

Relationship of Toluene Safe Duration (Hours/ Day) and Glutathione Concentration

The association between the safe duration of toluene in hours/day and glutathione concentration was was very weak (r=0.139). When the safe duration of toluene increases, the concentration of glutathione also increases. An increase of safe duration meant the amount of toluene exposure was getting smaller. The results of studies on mice that were exposed to toluene in different time periods showed that the longer the exposure time, the lower the amount of glutathione [20].

Exposure of toluene can cause a decrease in glutathione. Gas station workers with high toluene exposure have lower glutathione concentrations compared to the control group[21,22]. A research conducted in Korea showed that there was a significant relationship between toluene metabolism and glutathione[23]. However, studies conducted on mice given organic solvent for 3 days showed that toluene did not affect the amount of glutathione in the body[24].

Relationship of Toluene Safe Duration (Hours/ Day) and Malondialdehyde

Through the Pearson correlation test, the relationship between the safe duration of toluene in hours/day with malondialdehyde concentrations was very weak and had negative direction (r=-0.146). An increasing of safe duration of toluene in hours/day would decrease the concentration of malondialdehyde. The higher the safe duration of toluene, the less toluene exposure received by the worker. The results of studies on mice that were exposed to toluene in different time periods showed a greater increase in malondialdehyde in mice that were exposed to the same amount of toluene but in a longer period[20].

Research in groups who abuse toluene shows that there is an increase in the concentration of malondialdehyde compared to the control[25]. Workers on gas station who get a lot of exposure to benzene, toluene, and xylene, shows that the average concentration of malondialdehyde in these workers is higher than in the control group[21]. Moreover, workers who inhaled thinner experienced an increase in the concentration of malondialdehyde compared to the control group[26].

Relationship of Toluene Safe Duration (Hours/ Day) and Neurotoxic Symptoms

The relationship between the safe duration of toluene

in hours/days was weak and had a negative direction (φ=-0.223), i.e. an increase in the safe duration in hours/day decreases the likelihood of neurotoxic symptoms. This is consistent with Kang's research which states that higher or longer exposure of toluene can cause not only a decrease in attention and concentration, but also a motorized performance deficit[27]. Millions of workers who are often exposed to organic solvents can be concluded suffering from neurotoxic[28]. However, Pratamasari stated that there was no relationship between exposure to air in toluene and peripheral neuropathy. Body weight, length of work and education are factors of exposure to toluene found[29].

Conclusion

The safe duration of toluene in hours/day for workers in the Osowilangun shoe home industry is very weakly related to the concentration of glutathione and malondialdehyde and is weakly related to neurotoxic symptoms. If there is an increase in the safe duration of toluene in hours/day there will be an increase in glutathione but a decrease in malondialdehyde and a decrease in the possibility of neurotoxic symptoms.

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Effectiveness of Educational Program on Nurses-Midwives' Preventive Measures about Puerperal Sepsis at Baghdad Maternity Hospitals

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Abstract

Objective: To evaluate the effectiveness of the educational program on nurses-midwives' preventive measures about puerperal sepsis.

Method: quasi-experimental study design carried among 60 Nurses and Midwives chosen by non-probability sample (purposive sample) at maternity hospitals. The data collected by the observation checklist Pretest and posttest after the implementation of the educational program. Data were analyzed using descriptive statistical data analysis approach of frequency, percentage, mean, mean of scores, a total of scores, range and standard deviation and inferential statistical data analysis approach T-test, multiple linear regressions, person correlation coefficient, and analysis of variance (ANOVA).

Results: (35.3%) of them at age 20-29 years old, (75%) of them had secondary nursing school graduate level of education and (35%) of them had 11-15 years of services in the nursing field. there is a highly significant difference at $P < 0.01$ between the initial period of Pretest and posttest of the study sample preventive measures toward puerperal sepsis. The grand mean at pretest was (1.69) and at posttest (2.38). There is a statistically significant difference between years of service and preventive measures at posttest and Pretest of the educational program at (p-value > 0.05).

Conclusion: there is an improvement in the nurses-midwives' preventive measures level at the periods of tests after the implementation of the educational program.

Keywords: *Effectiveness, Educational Program, Nurses-Midwives, Preventive Measures, Puerperal Sepsis.*

Introduction

Puerperal sepsis is a significant preventable reason for maternal mortality, representing roughly 11% (95% vulnerability interval 5.9%–18.6%) of maternal mortality internationally. Improved comprehension of maternal sepsis is vital to accomplishing the feasible advancement goals (SDGs) and executing systems toward closure preventable maternal and neonatal mortality [1, 2].

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Puerperal sepsis is one of the five leading reasons for maternal mortality around the world and records for 15% of all maternal mortality. The WHO presented the term postpartum infections, which likewise include non-genital infections in the obstetric populace. Puerperal sepsis is a significant reason for morbidity and mortality for moms in developing countries. It is one of the commonest reasons for maternal deaths [3].

In any case, the recurrence of PIDs in pregnancy is ineffectively comprehended; records of obstetric morbidity recognized no distributed deliberate writing survey of (systematic literature review) of infection rate, making it the one significant direct reason for maternal morbidity without such literature as far as

anyone is concerned. Ongoing 2017 Worldwide Weight of Ailment (GBD) information gauge 12.1 million occurrence instances of maternal sepsis and other maternal infections, including mastitis [4, 5].

Puerperal sepsis represents 15% of maternal dead women around the world. In Africa, puerperal sepsis is the 2nd significant reason for maternal morbidity and deaths, representing over 10% of maternal dead women [2].

Health training and education have been a basic piece of nursing for a considerable significant period. Training of health givers and patients is as significant currently as could be. The nurses need to know the terms of health instruction programs and the pedagogical role of the nurse has the option to give wellbeing instruction to patients [6].

Methodology

A quasi-experimental study was conducted to evaluate the effectiveness of the educational program on nurses-midwives´ knowledge and preventive measures about puerperal sepsis at delivery rooms in Baghdad maternity hospitals conducted from January 24th, 2019 to May 20th, 2020. The study sample was nonprobability "purposive sample" includes (60) nurses-midwives working at delivery rooms in Al-Elwia maternity teaching hospital, Ibn Albalady maternity and pediatric hospital, Al-Karckh maternity hospital, Al-Yarmouk Teaching Hospital, and Baghdad teaching Hospital. A questionnaire composed of two parts (demographic data and the third part about preventive measures of puerperal sepsis items). Content validity of the questionnaires is determined through a panel of (25) experts, and reliability of the questionnaire is based on the test-retest for the stability reliability (reliability coefficient 0.76 for knowledge and 0.83 for preventive measures). The process of gathering information was conducted from January 20th to August 18th, 2019 it included (Pretest, implementation of the educational program, posttest). Data were collected using an observation checklist for preventive measures. Data were analyzed through the application of descriptive and inferential statistical data analysis (SPSS) version 20. Through descriptive statistics (frequency, percentage, mean, mean of scores, a total of scores, range, and standard deviation) and statistical inferential (T-test, multiple linear regressions, person correlation coefficient, and analysis of variance ANOVA).

Results were determined as highly significant at ($P \leq 0.01$) significant at ($P \leq 0.05$) and non-significant at ($P > 0.05$).

Results

Table (1) Distribution of the Study Sample by their Demographic Characteristics

Variables		Frequency	Percent
Age (years) Mean & standard deviation (35.783±10.294)	20-29	23	38.3
	30-39	14	23.3
	40-49	18	30.0
	50 and more	5	8.3
	Total	60	100
Level of education	Secondary nursing school graduate	45	75.0
	Nursing institute graduate	14	23.3
	Nursing college graduate	1	1.7
	Total	60	100
Years of services in the nursing field	1-5	5	8.3
	6-10	20	33.3
	11-15	21	35.0
	16 and more	14	23.3
	Total	60	100

This table shows that (35.3%) of the study sample at age 20-29 years old, (75%) of them had secondary nursing school graduate level of education and (35%) of them had 11-15 years of services in the nursing field.

Table (2) Comparison between the Nurses-Midwives' Preventive Measures about Puerperal Sepsis at pretest and posttest

No.	Domains	Pretest		Posttest	
		Mean	Ass.	Mean	Ass.
1	Prepare tools	1.9	M	2.39	H
2	Prevention and control of infection	1.69	M	2.39	H
3	Midwife Preparation for the woman	1.69	M	2.39	H
4	Method of sterilization and disinfection	1.47	L	2.38	H
5	Postpartum prevention	1.43	L	2.35	H
6	Grand mean	1.69	M	2.38	H

M= mean, Ass. = assessment, level of assessment: (1-1.66) = low = L, (1.67-2.33) = moderate = M, (2.34-3.00) = high = H

This table indicates that the grand mean of the study sample preventive measures toward puerperal sepsis is (1.69) at a moderate level at pretest, and (2.38) at a high level at posttest

Table (3): Study Sample preventive measures toward Puerperal Sepsis Levels of Assessment Through the "Mean of Score" Among (Pre, Post Test)

Period	Level of Assessment	Frequency	Percent
Pretest	Low (1-1.66)	32	53.3
	Moderate (1.67-2.33)	28	46.7
	High (2.34-3)	-	-
	Total	60	100
	$\bar{x} \mp S.D$	1.69±0.14	
Posttest	Low (1-1.66)	-	-
	Moderate (1.67-2.33)	16	26.7
	High (2.34-3)	44	73.3
	Total	60	100
	$\bar{x} \mp S.D$	2.38±0.107	

$\bar{x} \mp S.D.\bar{x} \mp S.D.$=Arithmetic Mean ($\bar{x}$)$\bar{x}$) and Std. Dev. (S.D.)

There is a low level of assessment of 32 (53.3%) of the study sample at level (1-1.66), the $\bar{x} \mp S.D.\bar{x} \mp S.D.$ are (1.69±0.14) at the pretest. While it is a high level of assessment of 44 (73.3%) of the study sample at level (2.34-

3), the $\bar{x} \mp S.D.\bar{x} \mp S.D.$ are (2.396±0.086) at the Posttest.

Table (4) Significant Comparison of the Period (Pre and Post) for Nurses' Preventive measures toward Puerperal Sepsis

Variables	Periods		Matched Paired t-test	Sig. P- value	C.S.
Preventive measures	Pretest	Posttest	-50.264	0.000	HS

C.S. : Comparison Significant, Highly Significant at P < 0.01

There is a highly significant difference at P < 0.01 between pre and posttest of preventive measures toward puerperal sepsis.

Table (5): Distribution and difference of nurses preventive measures with their age, education level, and years of experience.

Variable		preventive measures test periods	
Age	**No.**	**Pretest Mean ± S.D.**	**Posttest Mean ± S.D.**
20-29	23	1.72±.176	2.40±.105
30-39	14	1.69±.098	2.40±.080
40-49	18	1.66±.123	2.35±.120
50 and more	5	1.66±.126	2.33±.121
Total	60	1.69±.141	2.38±.108
ANOVA		F =0.585	F =1.615
		d.f.=59	d.f.=59
		P =0.627	P =0.196
Education	No.	Pretest Mean ± S.D.	Posttest Mean ± S.D.
Secondary nursing school graduate	45	1.66±.114	2.38±.101
Nursing institute graduate	14	1.78±.182	2.40±.12
Nursing college graduate	1	1.64±.00	2.19±.00
Total	60	1.69±.141	2.38±.108
ANOVA		F =4.442	F =1.959
		d.f.=59	d.f.=59
		P =0.016	P =0.15

Cont... **Table (5): Distribution and difference of nurses preventive measures with their age, education level, and years of experience.**

Years of service	No.	Pretest Mean ± S.D.	Posttest Mean ± S.D.
1-5	10	1.70±.137	2.41±.091
6-10	21	1.70±.158	2.37±.122
11-15	21	1.71±.146	2.35±.073
16 and more	8	1.59±.081	2.29±.120
Total	60	1.69±.141	2.38±.108
ANOVA		F =1.305	F =3.302
		d.f.=59	d.f.=59
		P =0.282	P =0.027

No. = Number of frequencies, S.D.=Standard deviation, ANOVA= Analysis of Variance, F = Fisher test, d.f. = degree of freedom, P = probability value, NS : Non Significant at $P > 0.05$, S : Significant at $P < 0.05$, HS : Highly Significant at $P < 0.01$.

There is no statistically significant difference between age and preventive measures at pretest and posttest of the educational program at (p-value > 0.05)

While there is a statistically significant difference between education level and years of service with preventive measures at the pretest of the educational program at (p-value > 0.05).

Discussion

Regarding to the socio-demographic characteristics of the study sample in Table (1). The results show that (35.3%) of the study sample at age 20-29 years old. This result agrees with the findings of (Fashafsheh, Ayed, Eqtait & Harazneh, 2015) in their study, they found that (64.2%) of the nursing staff their age (20-30) years [5]. (Momoh, Ezugworie, & Ezeigwe, 2010) found that in their study about half of the sample aged from 38-47 years old [7]. Also Kebalepile (2001) found that the nurses' age was from 30-39 years [8].

Concerning the educational level, (75%) of them had secondary nursing school graduate level of education. (Fashafsheh, Ayed, Eqtait & Harazneh, 2015) disagree with our finding they found the majority of the study sample have a Diploma and Bachelor graduate in nursing [5].

Most of them had 6-15 years of services in the nursing field. Fashafsheh, Ayed, Eqtait & Harazneh, 2015) supported our finding they found most of the study sample had years of experience (6-11) years [(5)]. While these results not consistent with the finding of Kebalepile (2001) they found the nurses had experienced more than 15 years [8].

In the Table (2) the study sample preventive measures toward puerperal sepsis at Pretest at a low level. The grand mean of the study sample preventive measures toward puerperal sepsis is (1.69) at a moderate level**.**

Also, (Fashafsheh, Ayed, Eqtait & Harazneh, 2015) found that (91.1%) of the nursing staff had good preventive measures level [5]. These findings supported our findings.

In Table (2) the study sample preventive measures toward puerperal sepsis at posttest. The grand mean of the study sample preventive measures toward puerperal sepsis is (2.38) at a high level.

The findings of the present study indicate that the nurses´ level of preventive measures is enhanced or improved to a good level of preventive measures at posttest. This finding agrees with Kebalepile (2001) findings, a majority 46 (71%) mentioned the use of sterile gloves during delivery, 43(66%) will use sterile delivery packs and ensure, 49 (75%) of nurses have demonstrated good preventive measures on measures to take in the prevention of postpartum infections [8].

The point of view of the researcher related to the present study considers that the educational program affects the level of preventive measures.

Educational programs are very important to all nurses in different filed of nursing specialty. Nursing research adds new information that is used in theoretical and practical nursing fields. it is used in the practical field, which improves the experience and preventive measures of nurses and thus increases the experience in finding problems facing nursing staff, finding suitable solutions, and acting appropriately to avoid obstacles in work and nursing care. Therefore, it is necessary to enroll nursing staff in theoretical and practical training sessions to develop their skills in patients care.

Regarding the two periods of educational program implementation on the study group in Table (3), the findings revealed that the level of nurses´ preventive measures toward puerperal sepsis has been developed by the implementation of the educational program to be at a good level. This results inconsistent with the result of Bayoumi and Mahmoud, (2017) they found that the nurses level of preventive measures had been improved after the implementation of an educational program [1].

In Table (4), the study sample preventive measures toward puerperal sepsis high significant difference at P <0.01 between the initial period of pre and posttest of the study sample. This result agrees with the findings of Kaur & Jairus (2015) they found that there was a significant difference between the means of the Pretest and posttest [9].

Results indicate that there is a significant statistical relationship between the study sample preventive measures at the pretest period and their level of education and between years of service and preventive measures at the posttest of the educational program at (p-value > 0.05). There is no significant statistical relationship between the study sample preventive measures at the pretest and posttest of the educational program and their socio-demographic characteristics (age).

(Fashafsheh, Ayed, Eqtait & Harazneh, 2015) found that there are no statistically significant differences between mean preventive measures scores towards age, years of experience [5].

This result may be related to the different levels of education and most of them graduate from secondary nursing school. The educational level of nurses may play a role in the results of any program which improves nurses´ preventive measures.

Conclusion

The educational program improves the preventive measures toward puerperal sepsis. The grand mean at pretest was (1.69) and at posttest (2.38). There is a highly significant difference at P < 0.01 between the initial period of pretest and posttest of the study sample preventive measures toward puerperal sepsis. There is a statistically significant difference between years of service and preventive measures at the posttest of the educational program at (p-value > 0.05). there is a statistically significant difference between education level and preventive measures at the Pretest of the educational program at (p-value > 0.05).

Recommendation

Implementation of the educational program about the prevention of puerperal sepsis for nurses-midwives on how to work in the delivery room. Follow up and evaluation of nurses' intervention toward women in the labor Room and postpartum period and find the obstacles and work to solve them.

Conflict of Interest: Nil.

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Skin Penetration of Topical Epigallocatechin-3-Gallate (EGCG) as an Alternative Agent for Photoaging Prevention

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Abstract

Photoaging is skin aging, that especially caused by chronic exposure of ultraviolet radiation. Photoaging impacts to patients' quality of life. Many substances, such as green tea, had been tried to be alternative agents for photoaging prevention. Epigallocatechin-3-gallate (EGCG) is the most abundant component in green tea. The requirement of topical substance to be able to work and give benefit to the skin is the skin penetration. The aim of this study was to evaluate the skin penetration of EGCG. The back part of the male Wistar mouse was shaved carefully, then topical EGCG cream 5% and 10% were administered to mouse skin. After 1 week administration, the animal was terminated and the skin biopsy was done. The mouse skin was extracted and high performance liquid chromatography (HPLC) examination was performed to evaluate the skin penetration of topical EGCG. The result of HPLC examination of 2 ppm, 4 ppm, 6 ppm, and 8 ppm EGCG level, showed the curve peak at 3.295, 3.296, 3.295 and 3.293 second. The basic curve showed R^2 of pure EGCG powder used in this study was 0,9999. The result of HPLC examination in this study showed, the curve peak of extracted mouse skin after EGCG cream 5% and 10% administration were between 3.2-3.7 second. The result of HPLC examination in this study showed that EGCG cream could penetrate into Wistar mouse skin after EGCG cream application for 1 week.

Keywords: *EGCG, skin penetration, HPLC.*

Introduction

Photoaging is skin aging, that especially caused by chronic exposure of ultraviolet radiation. Photoaging impacts to patients' quality of life.[1,2] Ultraviolet radiation promotes inhibition of transforming growth factor β receptor II (TGFβ RII) and causes increasing of matrix metalloproteinase-1 (MMP-1), that play role in photoaging pathogenesis by degrading the collagen and inhibiting the collagen synthesis.[2,3,4]

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Many substances, such as green tea, had been tried to be alternative agents for photoaging prevention. Topical green tea extract prevent photoaging by preventing the MMP-1 elevation and dermal collagen reduction in photoaging mouse model.[5,6,7] EGCG is the most abundant component and the most polyphenolic catechin in green tea (approximately 59%). EGCG is assumed as the main source of biological activity of green tea.[8,9,10]

The requirement of topical substance to be able to work and give benefit to the skin is the skin penetration. Topical substance should be able to penetrate through stratum corneum as the skin barrier. Topical substance applied on the skin would be transferred based on active substance concentration gradient (passive diffusion). Concentration gradient is the different between active substance concentration applied on the skin with active

substance concentration in the skin layers.[11,12] Because of the high importance of topical substance' skin penetration that play role in its activity, this study was aimed to evaluate the skin penetration of EGCG as an alternative agent in photoaging prevention.

Material and Methods

Materials

Pure EGCG powder was purchased from Xi' An Rongsheng Biotechnology Co., LTD, China (batch number 190702). Male Wistar rats aged 10-12 weeks with average weight 100-250 grams was provided by Faculty of Veterinary, Airlangga University, Surabaya, Indonesia.

Preparation of Topical EGCG

Preparation of topical EGCG cream was started from base cream preparation. The base cream consist of virgin coconut oil (VCO), cetacium, cera alba, olive oil, and aqua destilata. Cera alba and cetacium were boiled in the porcelain bowl above the water bath until the mixture was melting. Aqua destilata was added to the mixture and stir. VCO and olive oil were added to the mixture and stir until it became the base cream. Topical EGCG cream 5% was prepared by adding the pure EGCG powder to the base cream (1:19). Topical EGCG cream 10% was prepared by adding the pure EGCG powder to the base cream (1:9).13

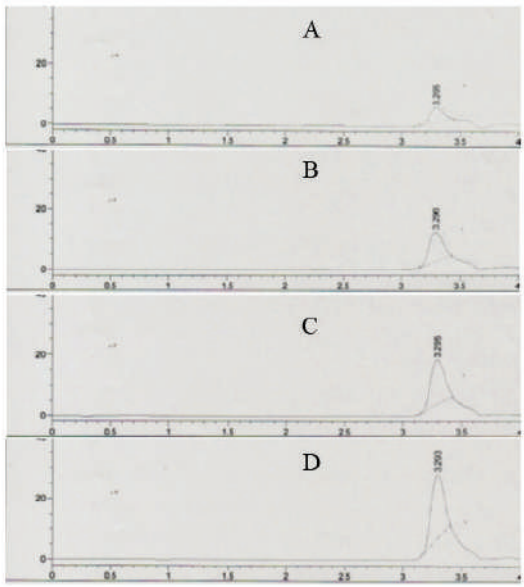
Intervention

The back part of the male Wistar mouse was shaved carefully in 3x3 cm2 size. Topical EGCG cream 5% and 10% was administered to mouse skin twice a day for a week (7 days). The size of topical EGCG cream applied to the mouse skin was 4 mg/cm2 body surface area.14 After 1 week administration, the animal was terminated and the skin biopsy was done. After that, the mouse skin was extracted. The mouse skin was pounded until became smooth. Chloroform was added into the refined skin and the mixture was filtered. Ethyl acetate was added into the refined skin and the mixture was filtered, put into the Becker glass. The whole ethyl acetate in the mixture was evaporated and dissolved into methanol solution.

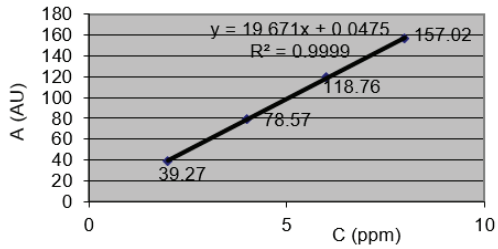
The first step of HPLC examination was making the mobile phase and creating the component solution. Then, the standard solution was made. The initial setting of HPLC system was checked before the examination, and continue to manually inject the sample and data collection. The result of HPLC examinantion was showed as curve peak in HPLC chromatograms.15

Result

The purity of pure EGCG powder used in this study was evaluated with high performance liquid chromatography (HPLC) examination. The result of HPLC examination of 2 ppm, 4 ppm, 6 ppm, and 8 ppm EGCG level, showed the curve peak at 3.295, 3.296, 3.295 and 3.293 second (Picture 1). The basic curve showed R2 of pure EGCG powder used in this study was 0,9999 (Picture 2).



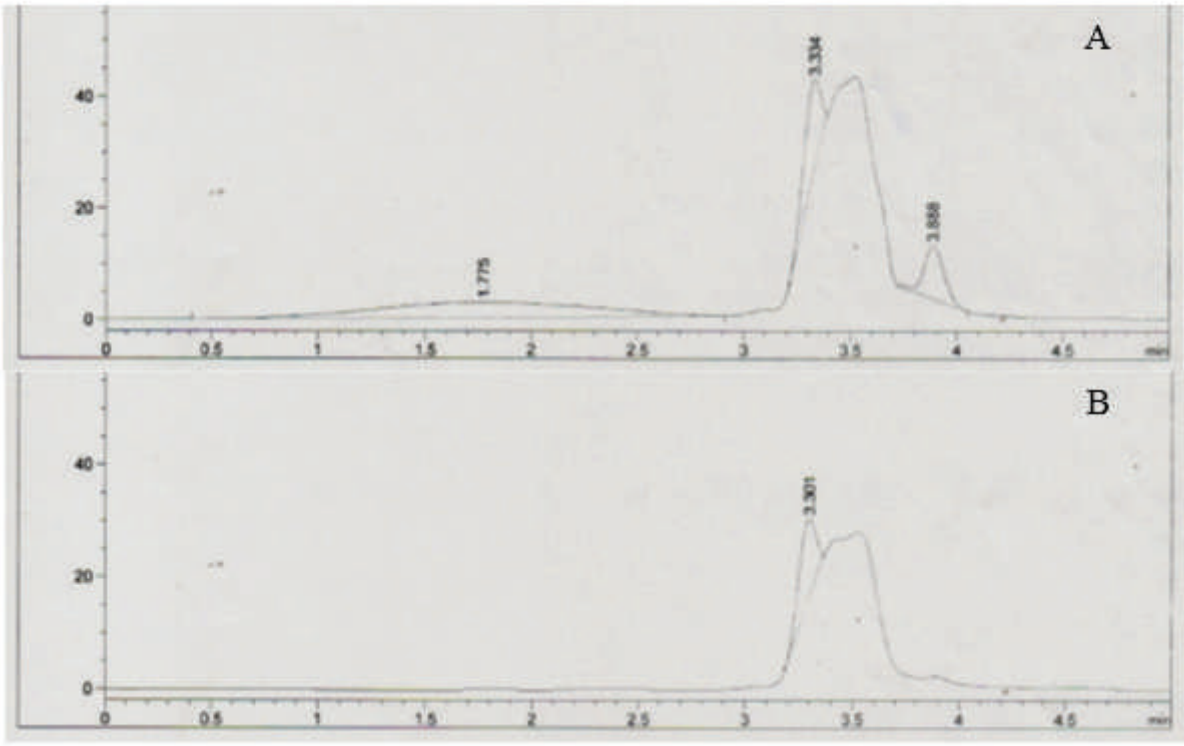
Picture 1. The result of HPLC examination of 2 ppm (Picture 1A), 4 ppm (Picture 1B), 6 ppm (Picture 1C), and 8 ppm (Picture 1D) EGCG level.



Picture 2. The basic curve of pure EGCG powder.

Topical EGCG cream 5% and 10% were administered to mouse skin twice a day for 1 week. The result of HPLC examination from mouse skin extraction

in this study showed, the curve peak after EGCG cream 5% administration was at 3.334 second (between 3.2-3.7 second), and the curve peak of mouse skin extracted after EGCG cream 10% administration was 3.3301 second (between 3.2-3.7 second) (Picture 3). This result showed that topical EGCG cream 5% and 10% could penetrate into the Wistar mouse skin after EGCG cream administration for 1 week. .



Picture 3. The result of HPLC examination from extracted mouse skin after EGCG cream 5% (Picture 3A) and EGCG cream 10% (Picture 3B) administration.

Discussion

EGCG is the most abundant component and the most polyphenolic catechin in green tea. Cellular uptake of catechin in EGCG is higher than other catechins. EGCG in this study was formulated as topically substance. Scalia et al evaluated percutaneous permeation of catechin in 1% oil in water emulsion and 1% hydrophilic gel on human skin using non-invasive tape-stripping technique. Scalia's study showed that EGCG could penetrate into the skin layer and there was no significant difference of the EGCG dose diffused in the skin layer in oil in water emulsion and hydrophilic gel formulation.10,14

The result of HPLC examination from mouse skin extraction in this study showed, the curve peak after EGCG cream 5% and 10% administration were at 3.334 second and 3.301 second (between 3.2-3.7 second). This result showed that topical EGCG cream 5% and 10% could penetrate into the Wistar mouse skin after EGCG cream administration for 1 week.

The requirement of topical substance to be able to work and give benefit to the skin is the skin penetration. Skin penetration of a substance is influenced by the substance factor and the host factor. The substance factors are substance concentration, partition coefficient and molecule size.16,17

There are three interaction after the topical substance was applied on the skin. The first interaction is the interaction of active substance soluted in the vehiculum. The second interaction is the interaction of vehiculum and the skin. The third interaction is the interaction of the soluted substance with the skin layer.11

Topical substance applied on the skin would be transferred based on active substance concentration gradient (passive diffusion). The effect of topical substance depends on the substance concentration that reach therapeutic target area. Topical substance diffuse to the inner layer of the skin based on diffusion law Fick I and Fick II. Diffusion law Fick I stated steady state flux of substance (J= moles/cm/second) per unit path length (δ, cm) in accordance to concentration gradient (ΔC) and diffusion coefficient (D, cm2/second), or symbolized as $J=-D(\Delta C/\Delta\delta)$. Diffusion law Fick II predicted the drug flow on nonsteady state condition, and stated that diffusion is the effective transport mechanism in short distance or symbolized as $\Delta t=x2/2D$ (Δt= time, x=path length).11,12,18

Vehiculum or base cream of the substance also plays role in the skin penetration. Vehiculum is inactive substance plays as a carrier of active substance into the skin. The preparation form of vehiculum used in this study was cream. Cream is semisolid preparation. The cream preparation used in this study was oil in water emulsion preparation, with water ingredient more than 31 %. Oil in water emulsion is more often to use because it is easy to be applied on the skin, easy to be washed, not to oily and the skin penetration is higher.16,17,19

VCO used in this study as the ingredient of the base cream plays role as an emollient and moisturizer. VCO makes the skin moister and plays role in the reduction of skin diffusion resistance. Oleic acid and lauric acid in VCO increase the penetration rate of active substance in VCO based cream used in this study. VCO based cream has good adhesion capacity, that makes bigger possibility for the active substance to penetrate into the skin. Good adhesion capacity also plays role in the skin hydration elevation.13,19

VCO also acts as penetration enhancer, that could increase the active substance penetration into the skin. Lauric acid in VCO increase lipophilic and hidrophilic active substance by ruining the bond of intercelullar lipid lamelar in the startum corneum. It caused decreasing of membrane viscocity, increasing of skin permeability, and increasing of skin penetration of substance.13

Host factor that plays role in topical substance penetration is skin barrier function. The skin barrier function depends on stratum corneum function and skin hydration. The decreasing of stratum corneum function and skin hydration could decrease the penetration of topical substance.12,16,19

Conclusion

The result of HPLC examination in this study showed that topical EGCG could penetrate into Wistar mouse skin after EGCG cream application for 1 week. It is therefore conceivable that topical EGCG may be beneficial for preventing photoaging. Further study about the role and the mechanism of topical EGCG in photoaging prevention was needed.

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Ethical Clearance: taken from Ethical Committee in Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Methods for Determination of Reactive Metabolites of Thiopurines

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Abstract

Background: This Review Represents A Critical And Constructive Analysis Of Literature In The Content Of Methods For Determination Of Reactive Metabolites Of Thiopurines. The Review Is Generated Through Summary, Classification, Analysis And Comparison Of Already Existing Material And Researches On Field.

Methods: Databases As Scopus, Pubmed, Medline And Web Of Science Were Used To Extract Data For The Review. Search Terms Like "Methods For Determination Of Metabolites", "Thiopurines" And "Reactive Metabolites" Were Used. Out Of 160 Research Articles Screened, 20 Most Relevant Studies Are Included In This Review.

Conclusion: Determining Thiopurine Metabolites Level Is Crucial On Improving Patients' Safety, Quality And Efficacy Of Treatment, Measure Patients' Adherence To Therapy, Optimize Thiopurine Dosage By Either Finding Safer/Higher Doses If Remission Is Not Achieved, Or By Determining Dose Reductions When Adverse Treatment Outcomes Occur.

Thiopurine Metabolites Are Measured By High-Performance Liquid Chromatography (Hplc), Through Determining Metabolites' Concentration In Hemolysate. Thioguanine Nucleotides (Tgn) And The Methylated Thioinosine Derivatives Are Not Measured Directly, Rather The 6-Thioguanine (Tg) For Tgn, And 4-Amino-5-(Methylthio) Carbonyl Imidazole (Amtci) For Methylated Thioinosine Derivatives Are Measured.

There Is A Misconception Among Patients And Healthcare Providers, That Determining Thiopurine Metabolites Level Provides Assurance On Optimal Dosing Which Can Be Used To Prevent Adverse Thiopurine Treatment Outcomes. Despite This Assumption, Clearly Most Of The Thiopurine Adverse Effects Are Not Directly Related To Their Metabolites As 6-Tgn Or 6-Mmp Levels. Clinical Experience Has Demonstrated That Infections Associated With Thiopurines Do Not Regularly Correlate With Leukopenia, And Elevated Transaminases Can Occur Despite Normal 6-Mmp Levels.

***Keywords**: Determining Metabolites; Hplc; Reactive Metabolites; Thiopurines;*

Introduction

Despite Many Advances In The Understanding Of Cancer Biology, Therapeutic Treatment Of Tumors Is Essentially Based On Empirical Approaches. Chemotherapy Carries A High Risk Of Unfavorable Toxicities And Adverse Treatment Outcomes, Due To The Narrow Therapeutic Index Of Antineoplastic Agents. Though, Basic Science Is Continuously Contributing On Improving Treatments' Safety, Efficacy And Quality Through Determination Of Drug Metabolites And Discovering Novel Pharmacogenomic Biomarkers. [1]

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Among Clinically Important Antineoplastic Drugs Are Also Thiopurines. Thiopurines Are Cytostatic, Purine Antimetabolite And Anti-Inflammatory Drug Group Consisting Of Azathioprine (Aza), 6-Mercaptopurine (6-Mp) And 6-Thioguanine (6-Tg). Thiopurines Are Used In The Treatment Of Acute Lymphoblastic Leukemia (All), The Most Prevalent Childhood Cancer, As Well As For Other Autoimmune Diseases And Prevention Of Transplant Rejection. For The Past 40 Years, The Administration Of Daily 6-Mercaptopurine (6-Mp) For 1.5–3 Years, Has Been The Standard 'Backbone' Of All Maintenance Phase Of Treatment. [2,3]

Thiopurines Mechanism Of Action

The Main Mode Of Action Of Thiopurines Is Through Cytotoxicity, Which Is Achieved By Incorporation Of Its Metabolite Thioguanine Nucleotides (Tgn) Into Dna And Rna. The Tgns Can Induce The Apoptotic Cell Death Either By Inhibition Of Intracellular Signaling Pathways Or By Inhibiting Dna Methylation. Another Mode Of Action Of Thiopurines Which Is Demonstrated Only In Vitro On Cell Cultures, Is The Inhibition Of De Novo Purine Synthesis, Through Methylthioinosine Monophosphate (Metimp). [4]

In Contrast, There Are Competing Metabolic Pathways That Can Inactivate 6-Mercaptopurine (6-Mp) To Thiouric Acid And Methyl-Mercaptopurine (Memp), Which Are Catalyzed By Xanthine Oxidase (Xo) And Thiopurine S-Methyltransferase (Tpmt), Respectively. [1]

The Effect Of Metabolism On The Therapeutic Efficacy Of Thiopurines

The Metabolism Of Thiopurines Is Crucial On Treatment Quality, Efficacy And Safety, Namely On Disease Remission, Disease Relapse Rates And As Well On Encountered Adverse Treatment Outcomes.

Thiopurine S-Methyltransferase (Tpmt) Is The Most Important Enzyme On Thiopurine Metabolism.

Tpmt Catalyzes The Methylation, And Such Deactivation Of The 6-Mercaptopurine To Its Inactivated Methyl Metabolite As Methyl-Mercaptopurine (Memp). Thus, Tpmt Through Thiopurine Deactivation Lowers Thiopurines Treatment Efficacy, Lowers Their Cytotoxic Effect And Adverse Treatment Outcomes.

Patients With Low Tpmt Levels Who Are Treated With Standard Doses Of 6-Mercaptopurine, Will More Slowly Deactivate 6-Mercaptopurine And Thus Have Higher Levels Of Thiopurine Metabolite As Thioguanine Nucleotide (Tgn), Which Accumulates In Cancer Cells And Achieves Potentiated Cytotoxic Effect. Thus, Patients With Lowered Tpmt Activity, Respond Better To Thiopurine Therapy By Entering The Remission Phase Faster. Eventhough, These Patients Because Of The Higher Levels Of Tgn Metabolites And Their Cytotoxic Effect, Are Also At Higher Risk Of Developing Adverse Thiopurine Treatment Outcomes Such As Myelotoxicity, Secondary Infections, Stomatitis, Gastrointestinal Disturbances, Hepatitis, Pancreatitis And Secondary Tumors.

Conversely, Patients With High Or Ultrahigh Activity Of Tpmt Enzyme, Who Can Inactivate 6-Mercaptopurine (6Mp) Faster To Its Inactivated Metabolite Methyl-Mercaptopurine (Memp), Can Commonly Lead To Thiopurine Therapy Tolerability, Increased Risk Of Disease Relapse And Increased Risk Of Hepatotoxicity Related To Methylated Metabolites.[5]

Therefore, We Can Clearly Understand The Major Role That The Thiopurine Metabolism Has, Either Through Its Activated Or Deactivated Metabolites, On The Efficacy And Safety Of Thiopurine Treatment Outcomes.

Tpmt Genetic Variations And Their Distribution

The Distribution Of Tpmt Activity In A Healthy Caucasian Population Is Determined To Be Trimodal, With Approximately 90% Of The Population Having Normal Or High Activity, 10% Intermediate And 0.3% Low Or Undetectable Tpmt Activity. Lower Tpmt Activity Is The Consequence Of Mutations In The Tpmt Gene. Although 26 Variant Alleles Have Been Identified, The Most Prevalent And Clinically Significant Are Tpmt*3A (460G>A And 719A>G) And Tpmt*3C (719A>G). Four Tpmt Variants With Decreased Activity (Tpmt*3A, *3B, *3C And *2) Were Detected In More Than 80% Of Caucasians With Low/ Intermediate Tpmt Activity, While The Frequency Of Other Variants Is Extremely Low. [6,7,8,9,10]

These Mutations Appear To Destabilize The Native Structure Of The Tpmt Enzyme, Resulting

In The Formation Of Misfolded Isoforms, Which Subsequently Undergo Rapid Intracellular Tpmt Degradation. Numerous Studies Have Linked The Onset Of Severe Toxicity During Thiopurine Treatment To The Low Tpmt Tpmt Enzyme Activity That Catalyzes Methylation, Thus Deactivation Of 6-Mp.[11]

Determination Of Mutations In The Tpmt Gene Before Starting 6-Mercaptopurine Therapy Constitutes A Fast, Easy And Cost-Efficient Strategy To Individualize Thiopurine Dosing. Therefore, In Order To Avoid Life-Threatening Side Effects In Patients Homozygous For Two Deficient Tpmt Alleles, A 10- 15 Fold Reduction Of The Standard Dose Is Recommended. However, Dose Adjustments For Heterozygous Individuals Are Not As Clearly Defined.[12]

Despite The Relatively High Safety And Efficacy Of 6- Mercaptopurine (6-Mp), Failure Due To Relapse Of Disease And Treatment Related Toxicities Still Occurs In About 20% Of The Treated Patients.

87% Of Relapses Are Isolated In Bone Marrow, 20% Extramedullary To Cns, 10% Extramedullary To Testes And 14% In Combined Locations. In 10–28% Of Patients Therapy Has To Be Discontinued Due To Adverse Events In Patients, While The Life-Threatening Myelosupression Frequency Is Only 1.4 To 5%.

Of Importance To Know, Significant Adverse Thiopurine Treatment Outcomes Are Considered When There Is Dose Reduction Up To 10% And It Lasts For More Than 3 Months, When There Is Discontinuation Of Therapy For More Than 1 Week Or Patient Ends Up Hospitalized.[13]

Novel Pharmacogenomic Biomarkers Explaining Thiopurines' Genotype To Phenotype Discrepancy

Recently, It Has Been Shown That The Pretreatment Determination Of Tpmt Activity Is Not Useful With Respect To Predicting The Efficiency Or Safety Of Thiopurine Therapy In All Patients. Therefore, The Determination Of Mutations In The Tpmt Gene Before Starting 6-Mp Therapy Represents A More Cost-Efficient, Fast And Easy Strategy For Individualizing Thiopurine Dosing And Thus Lowering The Incidence Of Side Effects And Increasing The Efficacy Of The Treatment. However, Concordance Between Genotype E And Phenotype Is Not Perfect, Especially In Tpmt Heterozygous Individuals, Where It Can Be As Low As 50%. For A Certain Number Of Patients, Therefore, The Prediction Of Tpmt Enzyme Activity And Response To Therapy Are Not Possible Purely On The Basis Of Genotype. As A Result, It Is Of Great Importance To Consider Additional Influence Of Biomarkers As Mthfr, Tyms, Itpa, Pacsin2, Nudt15, Which Together With Tpmt Genotype Might Better Predict Responses To Thiopurine Therapy.[14,15]

The First Candidate Is Sam (S-Adenosylmethionine), The Most Important Pharmacometabolomic Marker In Thiopurine Therapy. Sam Is Synthetized From The Essential Amino Acid Methionine And Atp. Sam Stabilizes The 3D Structure Of Tpmt And Increases Its Activity, Thus Sam Reverses The 6Mp Cytotoxicity By Decreasing Thioguanine Nucleotides. [16]

Sam Levels Depend Largely In The Availability Of Folates, Especially On 5,10-Methylenetetrahydrofolate, Which Is Involved Directly In Sam Synthesis. Mthfr (5,10 Methylenetetrahydrofolate Reductase) Catalyzes The Conversion Of 5,10 Methylene Tetrahydrofolate To 5- Methyl- Tetrahydrofolate, The Major Circulating Form Of Folate And The Methyl Donor For Synthesis Of Methionine From Homocysteine. Therefore, Atp, Folates And Mthfr Activity All Have Potential Positive Effects On Sam Levels And Tpmt Activity.[17]

Contrary, Tyms (Thymidylate Synthetase) A Key Enzyme Involved On Dna Replication Catalyzes The Conversion Of Deoxyuridine Monophosphate (Dump) To Thymidine Monophosphate (Dtmp) By Utilizing 5-Methf As A Methyl Donor, Which Leads To Dihydrofolate Recycling. Thus, Tyms Constitutes A Competing Pathway For Mthfr And Correlates Negatively With Sam Levels And Tpmt Activity.[17]

In Addition, Recent Studies On Thiopurines Revealed That The Pacsin2 (A Member Of "Protein Kinase C Family") Plays A Crucial Role On Tpmt Activity As Well. Patients Who Have Pacsin2 Cc Genotype Have Higher Tpmt Activity In Comparison To Patients With Tt Genotype. Furthermore, Pacsin2 Is Related To Myelotoxicity And Gastrointestinal Toxicity Of Patients Treated With 6Mp.[18]

Another Important Biomarker Influencing Tpmt Activity Is Itpa (Inosine Triphosphatase Pyrophosphatase), Since Patients Having At Least One Nonfunctional Allele Of Itpa Are Associated With Longer Event Free Survival (Efs) And Lowered Risk Of Early Bone Marrow Relapse. It Is Possible That The Action Of 6Mp During The Maintenance Phase Is Partially Reversed By The Fully Functional Itpa Activity And This May Then Lead To Survival Of Leukemic Blasts, Resulting In The Relapse Of Disease.[19]

To Conclude, Efficacy And Safety Of Thiopurine Therapy Relies On The Concentration Of The Patient's Cytotoxic Tgn Metabolites, Which In Turn Depends On The Deactivation Of Thiopurines By Tpmt. The Activity Of Tpmt Depends Largely On The Presence Of Genetic Polymorphisms In Tpmt Gene And Other Additional Biomarkers As Mthfr, Tyms, Itpa, Pacsin2, And Nudt15 That Must Acquire Special Attention.

Methods For Determination Of Reactive Metabolites Of Thiopurines

Determining Thiopurine Metabolites Is Crucial On Improving Patients' Safety, Quality And Efficacy Of Treatment. Thiopurine Metabolites Are Used To Measure Patients' Adherence To Therapy, Optimize Thiopurine Dose By Either Finding Safer/Higher Doses If Remission Is Not Achieved, Or By Determining Dose Reductions Without Losing Effectiveness When Adverse Treatment Outcomes Occur. Furthermore, Thiopurine Metabolites Should Be Determined When We Change The Therapy, Notice Elevated Transaminases Influenced From Accumulation Of Inactivated Methyl Entities As 6-Mmp, Or When We Add Adjunctive Allopurinol To Help On Raising The 6-Tg Metabolites And Suppress Formation Of 6-Mmp.[20]

Thiopurine Metabolites Are Measured In Most Laboratories By Determining Their Concentration In Hemolysate. High-Performance Liquid Chromatography (Hplc) Is Used For The Measurements. Thioguanine Nucleotides (Tgn) And The Methylated Thioinosine Derivatives Are Not Measured Directly. Rather, Compounds Actually Measured Are 6-Thioguanine (Tg) For Tgn, And 4-Amino-5-(Methylthio)Carbonyl Imidazole (Amtci) For Methylated Thioinosine Derivatives. 6-Tg Levels Actually Combine 6-Tg, 6-Thioguanosine (Tgr) And Tgn, While Mtci Levels Include 6-Mmp, Mmpr, And 6-Methylthioinosine Nucleotides (Metin). For Both 6-Tg And Amtci, The Measured Levels Include Both Nucleotides And Deoxynucleotides. These Deoxynucleotides Are Present At Low Concentrations, But There Is The Possibility That These Minor Deoxynucleotides May Indeed Play A Larger Role In The Efficacy And Adverse Thiopurine Treatment Outcomes. [20]

The Results Are Reported In Pmol/Rbc. The Reported Reference Range For 6-Tgn Is 235 To 450 Pmol/Rbc, And A Value Of 235 To 450 Is Identified As A Higher Likelihood Of Response To Therapy. A Value <235 Is Reported As Lower Likelihood Of Response, And A Value Above 450 Is Associated With A Higher Risk For Leukopenia. The Reported Reference Range For 6-Mmpn Is <5700, And A Level >5700 Is Thought To Be Associated With A Higher Chance Of Hepatotoxicity, As Presented On The Table 1.[20]

Table 1. The Reference Range For Thiopurine Metabolites [21]

6-TGN	
Suboptimal dosing	<235 pmol 6-TGN/8×108 red blood cells
Optimal dosing	235−450 pmol 6-TGN/8×108 red blood cells
Increasing risk for myelotoxicity and leucopenia	>450 pmol 6-TGN/8×108 red blood cells
6-MMPN	
Hepatotoxicity risk	>5700 pmol 6-MMPN/8×108 red blood cells

A Quick Review On A Validated Hplc Method Used For Monitoring Of Thiopurine Metabolites In Whole Blood

Whole Blood Has To Be Withdrawn From Healthy Volunteers, Who Are Treated With Aza Or 6-Mp Without Any Modification Of The Therapeutic Protocol For At Least Three Months. Patients Should Be Divided Into Three Groups On The Basis Of The Clinical Response To Thiopurine Treatment: Complete Responders (Persistent Remission); Partial Responders (Remission With Occasional Relapses); Non-Responders (Persistent Active Disease). Therapeutic Concentrations Of 6-Tgn And 6-Mmp Are Considered Between 235 And 450 Pmol/Sxlo" Rbc, And Below 5700 Pmol/Sxlo" Rbc Respectively. Possible Drug Toxicity (Hematologic, Hepatic) Can Be Evaluated, Such Hematologic Toxicity Can Be Evaluated With At Least One Of These Three Criteria: A Lymphocyte Count < 1000/Mm3 , A Platelet Count < 100.0001 Mm' And White Blood Cell Count < 40001 Mm', While Hepatic Toxicity Is Considered With Liver Transaminases > 40 U/L.[22]

Sample Preparation

Non-Fasting Peripheral Blood Should Be Collected In Sodium Heparin-Containing Tubes. Initially An Aliquot Of Wb Is Taken And Stored At -20°C Until Analyzed. Afterwards, The Remaining Wb Is Washed To Obtain Purified Rbe. In Particular, Wb Is Centrifuged At 1000Xg For 5 Min At Room Temperature. Plasma And Buffy Coat Are Discarded And Rbc Is Washed Twice With Isotonic Salt Solution (0.9% W/V Nacl). Both Purified Rbc And Wb Are Counted With A Cell Counting Device To Normalize Metabolite Concentrations To Pmol/Sx Lo' Rbc, Aliquoted (500 Ul.) In An Eppendorf Tube And Stored At -20°C Until Analyzed. 25 Ut, Of 120L/Mol/L Ls. And 50 Ul, Of 0.5Mol/L Dtt Are Added To An Aliquot Of Rbc Or Wb. Proteins Are Precipitated With 100 Ul. Of 70% Hci04 , Vortexed For 20 Sec And Held In An Ice Bath. The Tube Is Then Centrifuged For 15 Min At 13,Oooxg At 4°C. The Supernatant Is Transferred Into A New Eppendorf Tube And Heated For 45 Min At 100°E. During This Step 6-Tgn Is Hydrolysed Into Their Corresponding Bases (6- Tg) And In Addition, 6-Mmp Is Converted To 4-Amino5-(Methylthio)Carbonyl Imidazolo (Amtci) . After Cooling, A 100 Ul, Aliquot Is Injected Into The Column For Analysis.[22]

Chromatographic Conditions

Reversed-Phase Chromatography Should Be Performed By Using An Agilent 1200 Series Hplc System Consisting Of A Pump, A Degasser, An Autosampler, A Column Oven And A Uv Detector. Analysis Is Performed On A Synergi 4 11M Polarrp 80A With A Guard Cartridge. Analytes Are Separated By Isocratic Elution At Room Temperature With 1% Acetonitrile In 0.02Mol/L Phosphate Buffer (Ph 3.5). The Total Run Time Is 20 Min, The Flow Rate Was 1Ml/1Min And The Absorbances Are Measured At 341 Nm (6-Tg), 280 Nm (5-Bu) And 304 Nm (6-Mmp Derivative).[22]

Nonetheless, Several Studies Highlighted Significant Problems With The Present Methodology Of Thiopurine Metabolite Determinations And The Reproducibility Of The Assays. Great Variations Occur Depending On How Specimens Are Shipped And Whether They Are Refrigerated. For Example, Median 6-Tgn Concentrations On Day 7 Decrease Significantly To 53% Of Baseline When Kept At Ambient Temperature, Compared With A Smaller Decrease To 90% Of Baseline When Refrigerated.

A Similar Pattern Was Observed For 6-Mmp Measurements. Different Acids Used To Hydrolyze The Nucleotides Before Hplc Will Cause Variation In The Concentrations Of The Metabolites, And Many Are Of The Opinion That Specific Metabolites And Nucleotides Should Be Determined Individually.

There Is Also The Consideration That The Concentration Of The Drug Within The Erythrocyte May Not Fully Correlate With The More Important Concentrations Within Mononuclear Cells. It Is Not Certain That The Measure Concentration Of The Metabolites In Erythrocytes Correlate With The Concentration In Mononuclear Cells.[20]

In Terms Of 6-Tgn Metabolite Level Reproducibility, Studies Revealed That 6-Tgn Levels Were Reproducible With <10% Variability For Patients On Stable Dosing Over A Period Of 2 To 24 Months If Aliquots Were Immediately Frozen And Handled Specifically.[20]

Conclusion

There Are Major Potential Disadvantages Of Testing For Thiopurine Metabolites, Such As The Cost-Versus-Benefit Of The Test, And Mistakes Made Because Of Misinterpretation Or Over-Interpretation Of The Results.

Yet Leaving Expenses Aside, Probably The Most Detrimental Outcome In The Use Of Thiopurine Metabolite Levels Is The Misconception By Both Patients And Healthcare Providers That These Laboratories Provide Assurance That Optimal Dosing Is Being Used To Prevent Complications Of Therapy. Despite The Assumption That Measuring Levels Will Prevent Adverse Effects, Clearly Most Of The Adverse Effects From Thiopurines Are Not Directly Related To 6-Tgn Or 6-Mmp Levels.

Clinical Experience Has Demonstrated That Infections Associated With Thiopurine Use Do Not Regularly Correlate With Leukopenia, And Elevated Transaminases Can Occur Despite Normal 6-Mmp Levels. Clearly, The Target Ranges Of Metabolite Levels Are Only Guidelines, Which Likely Apply To Many Patients, But Not All Patients.[20]

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Identification of Inhibiting Factors as a basis for Formulating Strategies to Tackle HIV/AIDS in Tulungagung Regency, Indonesia

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Abstract

Based on the overall assessment, Indonesia has anticipated the epidemy of HIV/AIDS. However, the number of HIV/AIDS cases in Tulungagung District has increased a bit massively year by year. The total cases of HIV/AIDS from 2006 to 2018 were 2320 cases. In the last three years, the number had an increase of 295 cases, 324 cases, and 431 cases in 2016, 2017, and 2018 respectively. The high number of HIV/AIDS cases showed that some obstacles still exist to combat the HIV/AIDS in Tulungagung District. To identify the constraints as the basis of formulating strategies for the HIV/AIDS in Tulungagung District. **We used a qualitative and descriptive method with an in-depth interview.** Based on the in-depth interview, the inhibiting factors for HIV/AIDS prevention are divided into 3 factors. First, HIV/AIDS patients are still closed and reluctant to do medical check-ups and treatment in health facilities. Second, in terms of health care, health personnel still think that HIV/AIDS is an infectious disease that they keep away. Third, from society's perspective, the social stigma about HIV/AIDS sufferers often exists. By knowing the obstacles that occur in Tulungagung District, the right strategy can be made to accelerate HIV/AIDS prevention in Tulungagung District.

Keywords: *constraints, HIV/AIDS, strategy, health policy, stigma, discrimination.*

Introduction

Health issues are a major concern in development because they affect the quality of human resources. The case of HIV/AIDS in Indonesia was first reported in 1987. This is a phenomenon that is drawing the attention of many people. Acquire Immune Deficiency Syndrome (AIDS) is a collection of symptoms of a disease caused by HIV (Human Immunodeficiency Virus) that is easily transmitted and causes death[1].

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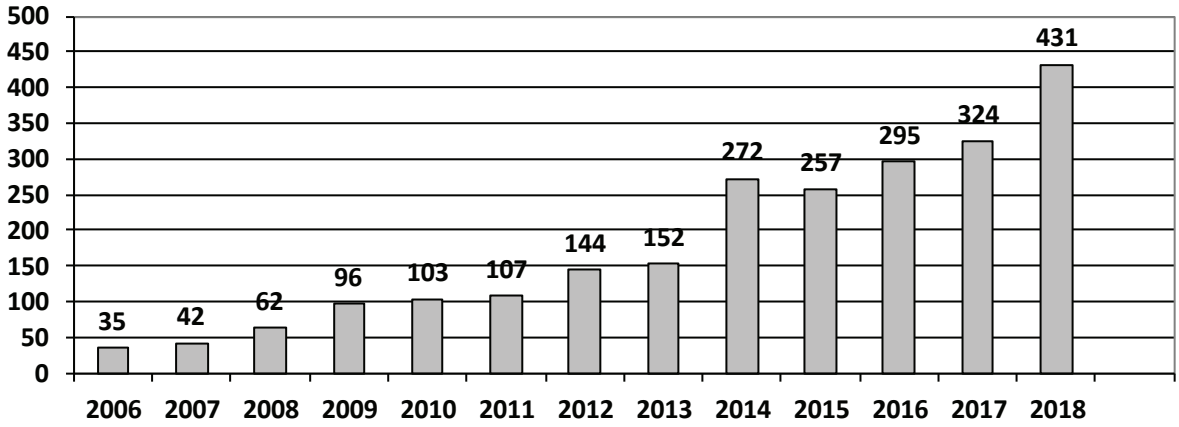


Figure 1. Jumlah Kasus HIV/AIDS tahun 2006-2018

Figure 1. The trend of HIV/AIDS cases in 2006-2018

Based on age, most people with HIV / AIDS have a productive age. The average is between 25-49 years old[2].

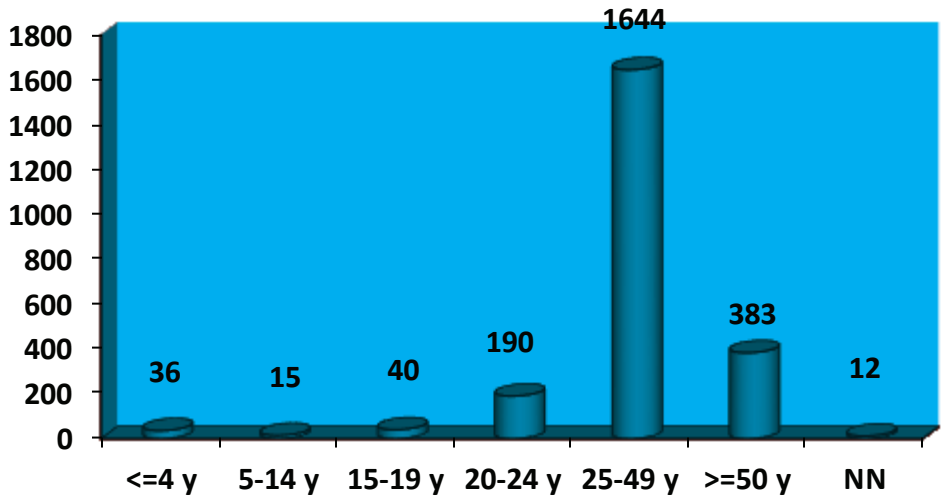


Figure 2. Distribution of HIV/AIDS patients by age

The population of the Tulungagung Regency until 2018 is 1,035,290 people[3]. While the number of HIV/AIDS cases until 2018 in 2,320 cases with the number of male cases at 55%, and women at 45%. Based on the spread of risk factors, 97% is transmitted through sex, 2% is transmitted through perinatal, and 1% is transmitted through syringes[2]. Based on this background, the study aimed to find out the obstacles as a basis in formulating strategies in overcoming HIV/AIDS in Tulungagung Regency.

Methods

The study was conducted with a qualitative approach, that was presented in narrative form[4]. Data collected through observation, in-depth interviews, and secondary data tracing in the form of documents/reports. In-depth interviews were conducted with selected informants from the Tulungagung Health Office (Head of the Disease Prevention Section, Head of the Infectious Disease Section, and managing HIV/AIDS program), Head of the Health Center, Health Center Doctors, AIDS Commission, and the HIV/AIDS community.

Findings

Characteristics of Respondents

A person living with HIV/AIDS (PLWHA) is defined as someone who has been infected by HIV or who has begun to show one or more symptoms of AIDS. In general, most people with HIV will arrive at the AIDS stage within 5-10 years (average 6 years). Immune system damage is gradually seen in the development of symptoms ranging from no symptoms to clinical conditions with severe symptoms.

In this study, respondents were divided into several groups, namely 8 people living with HIV consisting of 2 people from the Peer Support Group (PSG), 3 people from the transgender community (Apricot Non-Government Organization/NGO) and 3 people from Men Like Men (Pelangi NGO); Head of Disease Prevention Division, Head of Communicable Disease Prevention Section and manager of HIV/AIDS program (Health Office); Head of Health Center, Health Center Doctor, and manager of the HIV/AIDS program from Ngantru Health Center; and 2 people from the HIV/AIDS Commission.

Based on interviews with 8 PLWHA, information was obtained that PLWHA were infected with HIV through sexual intercourse with their partners by 100%, aged between 25-49 years by 87.5%. Some underwent treatment after they learned about 50% of their HIV status, while the rest underwent treatment after complaints of comorbidities, still had not told their families about 25% of their HIV status. These results are in line with the results of previous studies which explain that the transmission of HIV infection through sexual contact is the most common. In terms of age, the majority of PLWHA are in the productive age category[5].

Obstacles in HIV/AIDS Control

Based on interviews with PLWHA communities, 50% of PLWHA were initially reluctant to go to a health care facility. They only went to the health care facility after their body condition declined. After being examined the PLWHA found out that they had contracted HIV. PLWHA are reluctant to go to a health care facility because they are afraid of their condition, are afraid that their status will be known by the community, and they are also afraid of losing their jobs. This information is in line with previous research studies which explained that PLWHA were afraid of their condition and community acceptance[6].

The problems faced by PLWHA are not only problems of physical conditions that are declining, but also social problems such as discrimination. HIV/AIDS is considered a curse due to deviance because HIV/AIDS is attached to people who commit irregularities such as sex workers, gays, free sex offenders, and injecting drug users[7]. The stigma caused PLWHA to be reluctant to seek health services and social support that they could have received. Many PLWHA has lost their jobs and public services. Even a child can be refused education at school[1].

The obstacle in the effort to tackle HIV/AIDS is the lack of awareness and willingness of patients to take medication, such as taking Anti Retroviral Virus (ARV) drugs. This is due to the fear and anxiety of PLWHA in conducting examinations and treatment (47.9%). PLWHA does treatment only at the first visit but for further treatment, the patient does not come again to the Health Center to continue treatment. The results of the study found that the lack of awareness of PLWHA was also caused by the low level of patient knowledge, and PLWHA did not attend counseling by the counselor due to lack of strategic location access[8].

Stigma and Discrimination of Health Officers against PLWHA

Stigma is the act of giving a social label that aims to discredit a person or group of people with bad views. In practice, stigma results in acts of discrimination that is the act of not recognizing or not seeking the fulfillment of the basic rights of individuals or groups as properly as human beings with dignity. Stigma and discrimination occur because of the perception that they are considered as "enemies", "diseases", "shameful elements of society", "or those who are disobedient to the prevailing community or religious norms". The implications of stigma and discrimination are not only for certain people or groups but also for families and parties related to their lives. UNAIDS defines stigma and discrimination related to HIV/AIDS as negative traits given to a person that causes unfair and unfair actions towards that person based on HIV/AIDS status[1].

Based on the results of in-depth interviews with HIV/AIDS managers in Tulungagung Health Office and Ngantru Health Center, it can be concluded that there are stigma and discrimination that occurs in first-level service facilities against PLWHA. The forms of stigma and discrimination are excessive use of self-protection tools against PLWHA patients, underestimating and judging poorly, there is the labeling of PLWHA patients, there is no guarantee of confidentiality of test results to families and other health workers, and refusal to treat patients.

Stigma and discrimination are not only done by ordinary people who do not know about HIV/AIDS but can also be done by health workers. This finding is following the results of previous studies that inform that health workers (doctors and nurses) have a stigma and discriminate against PLWHA[9]. The results of this study are also in line with other studies that found that discriminatory behavior carried out by health workers against PLWHA including using personal protective equipment only when dealing with HIV sufferers, carrying out additional sterilization on equipment used by PLWHA, giving certain codes to PLWHA, separating in separate places used tools used by PLWHA, and put PLWHA in a separate place with the reason to maintain the comfort of other patients, discussing HIV status with others, and there are still those who refuse to treat PLWHA[10].

Health workers should be able to minimize the stigma and discrimination against PLWHA because health workers are the spearhead of the successful response to HIV/AIDS. Health workers are an important component in various health service approaches to PLWHA. Health workers have the authority, among others, to provide health services, carry out early detection, make referrals, and provide counseling[11]. Besides, health workers must be able to work together with various parties in the prevention of HIV/AIDS in an area. Health workers not only play a role in terms of promotive, preventive, curative, and rehabilitation but also contribute to holistically and comprehensively. To support this goal, health workers must work together with various sectors such as NGOs, HIV care activists, governments, and donor agencies so that prioritized programs can be carried out effectively, efficiently, and sustainably[8].

Community Stigma and Discrimination against PLWHA

As many as 55% of PLWHA in Tulungagung Regency are male, this is the following statistics on HIV/AIDS cases in Indonesia in September 2014 where most PLWHA in Indonesia were male[12]. Initially, HIV/AIDS was more common in homosexual men, so the sexual activity of homosexual men was blamed as a cause of HIV/AIDS, but current data show that in developing countries heterosexual transmission is more common. Stigma and discrimination among respondents are more felt in the male group because men are more likely to engage in risky behaviors such as frequent changes in partnerships for homosexual and heterosexual couples, and narcotics users using needles, thereby increasing the risk of HIV/AIDS transmission.

Based on the results of interviews and discussions with the head of the section on Prevention and Treatment of Communicable Diseases, HIV/AIDS program manager of the Tulungagung Health Office, despite having socialized about HIV/AIDS, the public still did not fully understand and be open to sufferers. In other words, the public also did not get the right understanding and information related to this one disease. As a result, PLWHA still often receive undue treatment, thus making PLWHA refuse to open their status to their spouse or deliberately change their behavior to avoid negative reactions. This reaction can certainly hamper efforts to intervene in the spread of HIV/AIDS.

The high community stigma against PLWHA in Tulungagung Regency is due to the high negative attitude of families and communities towards PLWHA. This study is in line with other studies which found that the factors that influence the community's stigma towards PLWHA are the high negative attitude of families and communities towards PLWHA. This makes the community reject and hate the group[13].

The results of other studies also mentioned that stigma is the biggest barrier in preventing HIV transmission and treatment. Besides, the stigma against PLWHA also makes people who have symptoms or are suspected of suffering from HIV reluctant to do tests to find out their HIV status. Because if the results are positive, they are afraid of being rejected by the family, and especially by the couple. The emergence of stigma

in the community is also one of the obstacles faced in tackling HIV/AIDS [14]. The results show the importance of treatment that involves partners to motivate PLWHA to adhere to treatment, eradicate stigma, restore hope, and reduce social differences. All of these methods are carried out to normalize PLWHA to eliminate social isolation[15].

Knowledge about HIV/AIDS greatly influences one's attitude towards people with HIV/AIDS. Stigma arises because of the people's ignorance of correct and complete HIV/AIDS information, especially in the mechanism of HIV/AIDS transmission, groups of people at risk of contracting HIV/AIDS, and how to prevent it[16]. Providing knowledge or information related to HIV is one effective way to explain HIV/AIDS prevention and transmission[17,18]. Someone with good and correct knowledge about HIV/AIDS is expected to reduce and even eliminate the stigma of PLWHA[19].

HIV/AIDS Prevention Strategy in Tulungagung Regency

In Tulungagung Regency, HIV/AIDS prevention efforts have been integrated and integrated from promoting healthy behavior (promotive), prevention of HIV/AIDS (preventive), as well as treatment and care (curative) and life support (support) for PLWHA. Preventive and promotive efforts are priority efforts that are carried out in a balanced manner with curative efforts and support for PLWHA[8,20].

Promotive efforts carried out in the form of counseling and outreach through leaflets and posters as well as interactive dialogue on the radio. This step is in line with previous research recommendations that inform concrete steps that must be taken in efforts to tackle HIV/AIDS in the form of socialization through information and education communication (IEC), either directly (counseling, seminars/training, PMI blood screening), or indirectly (via posters/advertisements/ radio-TV broadcasts)[21].

Another preventive effort capability is the Health Center in Tulungagung Regency has been able to carry out HIV diagnosis through VCT clinic services. This information is consistent with previous research that informs health services in preventive measures, namely conducting tests on populations at risk of HIV. The HIV test is divided into two approaches, namely VCT and PITC, which are the first steps to ascertain whether a person is infected with HIV or not[21].

Curative efforts in the form of the availability of Care, Support, and Treatment (CST) services in 7 Health Service Centers, namely Dr. Iskak Hospital, Islamic Hospital, Ngunut Health Center, Ngantru Health Center, Kalidawir Health Center, Kauman Health Center, and Cadar Health Center. The health service center which is a CST has facilities to be a reference for PLWHA doing ARV treatment. This is to facilitate treatment for those who have difficulty getting referrals from first-level health facilities[22].

Efforts to support PLWHA are carried out by collaborating with the AIDS Commission and the PLWHA community, so PLWHA feels more comfortable in terms of HIV/AIDS services in the Tulungagung Regency. HIV/AIDS prevention is carried out jointly by the government, community, private sector, and people with HIV/AIDS with the support of international organizations. Communities including NGOs are the main actors in the implementation of countermeasures while the government empowers the community and provides assistance in the direction, guidance, and create a supportive atmosphere[8].

Conclusions

Stigma and discrimination by the public and health workers against HIV/AIDS patients in Tulungagung Regency still occur frequently, for this reason, there is a need for continuous socialization and counseling about HIV/AIDS. The support of the PLWHA Community and the HIV/AIDS Commission is expected to be able to change the mindset of HIV/AIDS patients so that they want to get tested and undergo ARV treatment. By knowing the obstacles that occur in Tulungagung Regency, the right strategy can be formulated to accelerate the prevention of HIV/AIDS in Tulungagung Regency.

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Relation between Serum Lipids and Thyroid Hormones in Hypothyroidism Patients

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Abstract

Background: Hypothyroidism is a condition that occurs as a result of a malfunction in the thyroid glands that leads to a decrease in the secretion of their main hormones. Thyroid hormones are known to be have an important role in the regulating metabolism of lipids; thus, any impaired in the thyroid function causes a significant change in the levels of lipid. **Objective:** To evaluate the changes occurs for lipid levels in hypothyroidism patients and determine the correlation between lipids parameters and some thyroid hormones. **Materials and Methods:** The study included 45 healthy control subjects and 52 hypothyroidism patients who visited private doctors' clinics in Basra, Iraq. Thyroid hormones and serum lipids were measured for both healthy subjects and hypothyroidism patients. **Results:** The study indicates an increase in the thyroid stimulating hormone (TSH) values for hypothyroidism patients as compared to the healthy control group while there is a slightly difference in the values of each of triiodothyronine (T_3) and thyroxine (T_4) between the two groups. There is a significant increase in the mean values of all lipids except for high density lipoprotein (HDL) in the hypothyroidism patients compared to that in healthy control subjects. The study presents that there is a positive correlation between TSH and all lipid parameters except for HDL which gives negative relation (r = - 0.20). There is a moderate correlation between TSH and non-HDL (r = 0.42) while the other lipids appear weak correlation (TC(r=0.14), TG (r=0.19). LDL (0.16), VLDL (0.17), and LDL/HDL (0.17)). There is no correlation between T_4 and TC (r = 0.03), LDL (r = 0.07). non-HDL (r = 0.04), and LDL/HDL (r = 0.06), HDL (r = - 0.02) while TG and VLDL appears weak correlation (r = - 0.11 to each one of them). **Conclusions:** The study found that the abnormal change in the levels of lipids during hypothyroidism may increase the likelihood for occurrence of arteriosclerosis and the emergence of cardiovascular disease in hypothyroidism patients. There is a moderate positive relationship between TSH and non-HDL levels, as this indicates that non-HDL should be added to lipid profile tests as evidence of measuring cardiac risk in patients with hypothyroidism.

Keywords*: hypothyroidism, atherosclerosis, T_4, TSH, non-HDL.*

Introduction

The thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) are synthesized by the follicular epithelial cells of the thyroid gland by utilizing from dietary iodine [(1)]. Thyroid gland secretesT_3 and T_4hormones into the bloodstream, these hormones have vitally important to the body's metabolism and normal development [(2)].

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The synthesis and secretion of thyroid hormones is controlled by hypothalamic-pituitary-thyroid axis [(3)]. The hypothalamus secretes thyrotropin-releasing hormone (TRH) which alerts the thyrotrope cells in the anterior pituitary gland to secrete the thyroid-stimulating hormone (TSH), this hormone stimulates the thyroid cells to produce and secretetheT_3 and T_4 hormones [(4)].

Thyroid hormones enter the cells through specific energy-dependent carriers [(5)]. Inside the cell, T_4 is metabolized to T_3, which then bind to its nuclear receptors in the target cells, which in turn activate T_3-responsive genes. These genes modify many of

cell functions including the metabolism of lipids, carbohydrates and proteins [6, 7].

Hypothyroidism is the most common condition in the thyroid diseases, it results from any condition that leads to a decrease in the production of hormones T_3 and T_4. Hypothyroidism is associated with an abnormal lipids metabolism that may cause atherosclerosis and then lead to a cardiovascular disease [8].

Laboratory diagnosis of hypothyroidism is mostly done by measuring TSH and T_4 levels in the blood. A lower T_4 levels and increased TSH levels indicate primary hypothyroidism. This happens when the pituitary gland realize that the thyroid gland doesn't make enough hormones so it releases more TSH in an attempt to stimulate the thyroid gland to produce their hormones. But if the thyroid gland is not working properly, it will not interact with the pituitary gland signals this leads to high levels of TSH and low levels of T_4 [9, 10].

Thyroid hormones have an important role to maintain the normal cholesterol levels in the blood needed to meet the body's natural requirements [11]. Thyroid hormones regulate serum cholesterol levels through controlling many of its metabolic pathways like; stimulating vital cholesterol synthesis and export it, the processes of reverse transport of cholesterol from the peripheral tissues to the liver, activity of the receptors present in the liver cells that responsible on converting the cholesterol into bile [12].

Hypothyroidism is a known cause of hyperlipidemia. the most common lipid abnormality in hypothyroidism patients is high cholesterol in the blood, most often the low-density lipoproteins (LDL), because it is the main lipoprotein responsible for transporting cholesterol from the liver to the cells of the body. Also, increase in plasma triglycerides (TG) may occurs due to an increased in the esterification of fatty acids in the liver [13].

The value of non-high-density lipoprotein (non-HDL) has emerged as an indicator of cardiovascular disease recently. its value represents the sum of the cholesterol accumulated in all lipoproteins, with the exception of HDL, such as: chylomicrons, VLDL, LDL and Lp (a) so it reflects the full effect of all blood lipid components that are involved in atherosclerosis [14].

The current study done to determine the association between thyroid hormones and serum lipids in hypothyroidism patients and to determine which one of lipids affected more by these hormones.

Material & Method

The study was conducted on fifty-two untreated hypothyroidism patients who visited private doctors' clinics in Basra, Iraq in the period from May to December, 2019. Also, forty-five normal healthy subjects were included as controls.

Blood samples were drawn after an overnight fast and separated into a serum. Then the serum samples were analyzed for study parameters.

The serum levels for TC, TG, and HDL were measured by enzymatic colorimetric assay method using a randox kits. VLDL and LDL were calculated using the Friedewald formula [15]. The levels for non-HDL-C were calculated mathematically by subtracting HDL value from TC value (TC – HDL).

Serum concentration of T_3, T_4 and TSH were measured by micro plate competitive enzyme immunoassay on the TOSOH system analyzers.

The results were calculated as mean±SD, then was analyzed statistically by using SPSS program to find if there is a significantly change in the parameters levels between patients and control groups. p values which were less than or equal to 0.05 were considered to be statistically significant [16].

Results

Table 1. demonstrates the values of mean ± SD of thyroid hormones in both hypothyroidism and control groups. There is a significant increase in the mean values of TSH in the hypothyroidism patients' group when compared to that of healthy control group ($p < 0.0001$) while there is no significant difference in the mean values of both T_3 and T_4 between these two groups ($p = 0.26$ and $p = 0.12$ respectively).

Table 2. demonstrates the values of mean ± SD for all lipid parameters in both hypothyroidism patients and healthy control groups. Comparing the levels of means between hypothyroidism patients and healthy control groups, we note that there is a high significant increase

in the mean values of all lipids (P < 0.0001), with the exception of HDL showing a significant decrease in mean values (P < 0.0001).

Table 3 appears that there is a positive correlation between TSH and all lipid parameters except for HDL which gives negatively relation (r = - 0.20). There is a moderate correlation between TSH and non-HDL (r = 0.42) while the other lipids appear weak correlation (TC (r= 0.14), TG (r= 0.19), LDL (0.16), VLDL (0.17), and LDL/HDL (0.17)).

Table1: comparison of thyroid hormones levels between hypothyroidism patients and healthy control groups

Parameters	Hypothyroidism patients	Healthy control	P value
T3 (ng/ml)	1.94 ± 0.18	1.25±0.30	0.26
T4 (µg/dl)	6.35 ± 1.61	6.92±1.79	0.12
TSH (µIU/L)	11.75 ± 2.65	1.93±0.24	0.0001

All values are given in mean ± standard deviation (SD)

Table 2: Comparison of lipid parameters levels between hypothyroidism patients and healthy control groups

Lipid Parameters (mg/dl)	Hypothyroidism Patients	Healthy control	P value
TC	210.57±9.90	169.29±13.07	0.0001
HDL	28.78±3.70	40.17±3.18	0.0001
LDL	144.88±12.02	107.71±13.70	0.0001
TG	193.48±14.02	107.48±9.04	0.0001
VLDL	38.69±2.79	21.40±1.83	0.0001
Non-HDL	178.15±12.97	129.11±13.33	0.0001
LDL/HDL	5.12±1.00	2.69±0.42	0.0001

All values are given in mean ± standard deviation (SD)

Table 3: Correlation coefficient (r) between TSH And lipids in hypothyroidism patients

Parameter	Correlation coefficient (r)
TSH & TC	0.14
TSH & TG	0.19
TSH & LDL	0.16
TSH & HDL	- 0.20
TSH & non-HDL	0.42
TSH & VLDL	0.17
TSH & LDL/HDL	0.17

Table 4 appears no correlation between T_4 and each of TC (r = 0.03), LDL (r = 0.07). non-HDL (r = 0.04), and LDL/HDL (r = 0.06), HDL (r = - 0.02) while TG and VLDL appears weak correlation (r = - 0.11 to each one of them).

Table 4: Correlation coefficient (r) between T_4 and lipids in hypothyroidism patients

Parameter	Correlation coefficient (r)
T4 & TC	0.03
T4 & TG	- 0.11
T4 & LDL	0.07
T4 & HDL	- 0.02
T4 & non-HDL	0.04
T4 & VLDL	- 0.11
T4 & LDL/HDL	0.06

Fig. 1 shows the only negative correlation between TSH and lipids and that occurs only between TSH and HDL.

Fig. 2 shows the only moderate correlation between TSH and lipids that occurs only between TSH and non-HDL.

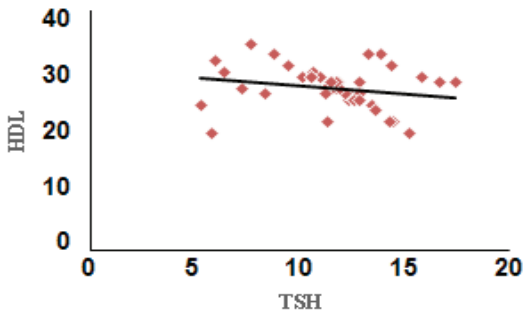


Fig. 1: correlation between TSH and HDL

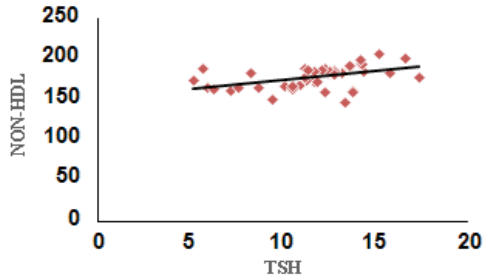


Fig. 2: correlation between TSH and non-HDL

Discussion

The study indicates an increase in all serum lipids values except for the HDL in the hypothyroidism patients compared to that in healthy control subjects. Other researchers have proven the same results that obtained in our research [17, 18, 19].

Hydroxy methyl glutaryal reductase (HMGCR) is the major enzyme responsible for regulating the cholesterol synthesis in the liver [20].

There are several mechanisms interprets how the activity of HMGCR regulated, one of which is through a balance between the phosphorescent and non-phosphorous forms in which the enzyme is exists. It has been observed that an increase in the non-phosphorous form increases the activity of the enzyme, while the increase in its phosphorous form reduces its activity [21,22].

Special kinase enzyme, AMP-activated protein kinase (AMPK) is responsible for the interconversion between the phosphorescent and non-phosphorous forms of HCGM enzyme [23].

Subclinical hypothyroidism characterized by a rise in the serum TSH levels with normal values for both T_3 and T_4. Several studies indicate that high TSH levels in hypothyroidism cause inhibition to the AMPK activation in the

liver which make activation for HMGCR by increasing its dephosphorylation form and this in turn stimulate the synthesis of cholesterol and increase its serum levels [24, 25].

The most common explanation for increased LDL levels in patients with hypothyroidism is the effect of thyroid hormones on LDL receptors, as these hormones

works to lower the ratio of these receptors which leads to a decrease in the clearance of LDL from the bloodstream [26].

The rise in the serum TG in hypothyroidism patients may be due to the effect of thyroid hormones on lipoprotein lipase enzyme, which cause decrease in its efficacy and this works to reduced clearance of TG from its rich lipoproteins and hence elevated in the serum TG levels occurs [27, 28].

The thyroid hormones effects on the HDL metabolism is through the activities of cholesterol transport protein (CETP) and hepatic lipase (HL) enzymes associated with HDL metabolism. CETP is responsible for transporting cholesterol esters from HDL_2 to VLDL and IDL and backward transfer for TG into HDL_2. TG is then hydrolyzed by HL into fatty acids, at this point HDL_2 changes to be HDL_3. Thus, HL is the enzyme responsible for lipid analysis by converting IDL to LDL and HDL_2 to HDL_3 [29, 30].

Correlation analysis for our study showed a moderate positive relationship between the levels of TSH and non-HDL in hypothyroidism patients' group. This finding is consistent with the results of other studies [31, 32].

The non-HDL test can be considered as an important indicator of cardiovascular disease because its value expresses the total number of the cholesterol present in all particles that causes atherosclerosis [33].

Conclusion

The results of our research indicated that hypothyroidism has a significant impact on blood lipid levels, especially those responsible on causing atherosclerosis that leading to vascular heart disease. Considering a non-HDL test as a primary goal for measuring dyslipidemia with other lipid parameters as a useful indicator for measuring heart risks in patients with hypothyroidism.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtain from college

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Evaluation of Storage Length to Blood Component Platelet Concentrate Quality in the Blood Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia

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Abstract

Storage of Platelet Concentrate (PC) can cause changes in thrombocyte quality that affect thrombocyte viability and decrease hemostatic function. All biochemical, structural and functional changes to platelets during storage in the Blood Bank are known as platelet storage lesions. To analyze the effect of storage length on changes in thrombocyte morphology and function as well as the metabolic parameters of platelet concentrate bag components. This was an analytical study with time series design. Sampling was done consecutively. Samples were new PC bags which were processed and stored at room temperature with agitation during June-July 2019 in the Blood Bank of the Dr. Soetomo General Hospital, Surabaya. Examination of platelet count and MPV were performed to determine morphological changes and metabolic parameters like pH, pCO_2, and pO_2 on day 1, day 3, and day 5th. Data were analyzed by using Shapiro-Wilk test, Paired t test and Wilcoxon Signed Rank Test. There were significant changes in MPV, pH, and pCO_2 values during storage (p <0.05). However, there were no significant changes in platelet count and pO_2 value. Metabolic changes in PC bag were obtained but there was no decrease in platelet count so that the PC component was still feasible to be given to the patient. Further research is needed for other parameters that can be affected by the storage process.

Keywords*: Platelet concentrate, platelet storage lesion, quality.*

Introduction

Platelets are blood cells that play an important role in hemostasis. Indications for platelet transfusion are for prophylaxis or hemostatic therapy in patients with congenital or acquired thrombocytopenia or thrombocytopathy. Over the years, there is an increase of the need for platelet transfusion due to the increasing number of patients with thrombocytopenia and platelet function disorder[1]. Platelet transfusion demand per month in 2018 at the Dr. Soetomo General Hospital were 2,442 platelet concentrate (PC) blood bags for about 325 patients, each patient needed 7-8 PC blood bags (Blood Bank Data of the Dr. Soetomo General Hospital in 2018).

Platelet transfusion is a challenging life-saving procedure in blood transfusion services because of the short shelf life of platelets. The American Association of Blood Banks (AABB) recommends storing platelets by constant agitation at room temperature (20-24 °C) for 5 days[1]. Storage at room temperature can cause platelet metabolic activity 60% lower when compared to storage at 37 °C[2].

The shelf life of PC blood components is limited because of the risk of microbial contamination and during the storage process platelets are susceptible to environmental changes that can affect the quality which is known as platelet storage lesions[3]. This change in quality can have a potentially negative impact on platelet

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viability and hemostatic function[4]. The mechanism that causes platelet storage lesion is very multifactorial and not clearly understood. Several factors including blood tapping method, component manufacturing process, storage and manipulation after blood tapping can cause platelet storage lesions[5].

Previous studies have shown that the PC blood component is adequately maintained if stored for 5 days and after that there can be a progressive loss of viability and platelet function during platelet storage[6]. Several laboratory examinations can be carried out to detect changes in quality, such as platelet count and Mean Platelet Volume (MPV) tests to detect morphological changes and to check metabolic parameters such as pH, pO_2 and pCO_2 to determine platelet viability during the storage process[4].

Platelet indices such as platelet count and MPV are used as markers to maintain PC quality control and are considered to represent changes in platelet shape caused by storage. An assessment of MPV values is used to see platelet storage lesions because MPV correlates with changes in platelet morphology during PC storage. The discoid platelet form correlates with viability in vivo after being transfused to the patient. Platelet discoid shape and the presence of swirling in stored PCs can be observed before they are transfused as quality control of platelet function present in PCs[6]. This study aimed to analyze the effect of storage duration on morphological changes (platelet count and MPV) and metabolic parameters (pH, pCO_2, and pO_2) Platelet Concentrate (PC) blood components, which were assessed at storage days 1, day 3, and day 5th.

Materials and Methods

Samples were newly processed PCs stored at room temperature with agitation from June to July 2019 at the Blood Bank of the Dr. Soetomo General Hospital, Surabaya, Indonesia. PC samples had passed screening tests for infectious disease through blood transfusion, namely hepatitis B, hepatitis C, HIV, and syphilis. PC blood bag manufacturing activities were carried out in the Blood Transfusion Unit of Dr. Soetomo General Hospital. PC was prepared using the platelet rich plasma (PRP) method, 350 mL of whole blood that has been tapped from voluntary donors was put into a triple blood bag that already contained anticoagulant Citrate Phosphate Dextrose Adenine (CPDA). After settling for about 30 minutes, whole blood was centrifuged using a Rotixa 50 RS centrifuge at 375 g for 15 minutes at 22 °C to obtain PRP. After PRP was formed, the blood was centrifuged again at a speed of 1,500 g for 15 minutes, then the platelet poor plasma (PPP) supernatant was separated and platelet residue was resuspended with an average plasma volume of 60±0.9 mL. On the first day of storage, platelet samples were taken from a blood bag tube and inserted into a tube containing EDTA anticoagulants as well as syringes that already contained heparin anticoagulants. Then a platelet count and MPV were examined using a Sysmex XN 1000 hematological analyzer to determine morphological changes and examine metabolic parameters such as pH, pCO_2, and pO_2 using the BGA Gem 3500 instrument. The remaining PC blood components were stored at 22-24 °C using an agitator to be re-examined. The same method used for the first day of sampling was done for the third day and fifth day of sampling.

Result and Discussion

This study used 30 PC blood component bags. In the morphological parameters, the platelet count did not change significantly but the MPV value increased significantly between day 1 to day 3, between day 3 to day 5 and between day 1 and day 5 ($p < 0.05$) (Table 1).

Table 1. Platelet Morphology Parameters.

Samples	Storage			p Value		
	Day-1	Day-3	Day-5	1-D3	1-D5	3-D5
Platelets (x103 µL)	653.80±209.84	678.06±192.37	664.20±210.32	.103	.500	.052
MPV (fl)	7.79±0.36	7.98±0.38	8.29±0.37	.001	.000	.000

. MPV = mean platelet volume.

Obtained a significant decrease
and day 5 ($p < 0.05$) and for the
day 5 ($p < 0.05$). There were no

V	*p* Value	
	D1-D5	D3-D5
	0.000	0.000
(	0.112	0.331
(	.002	0.111

1.

O_2, as well as pCO_2 and
variables on the first day

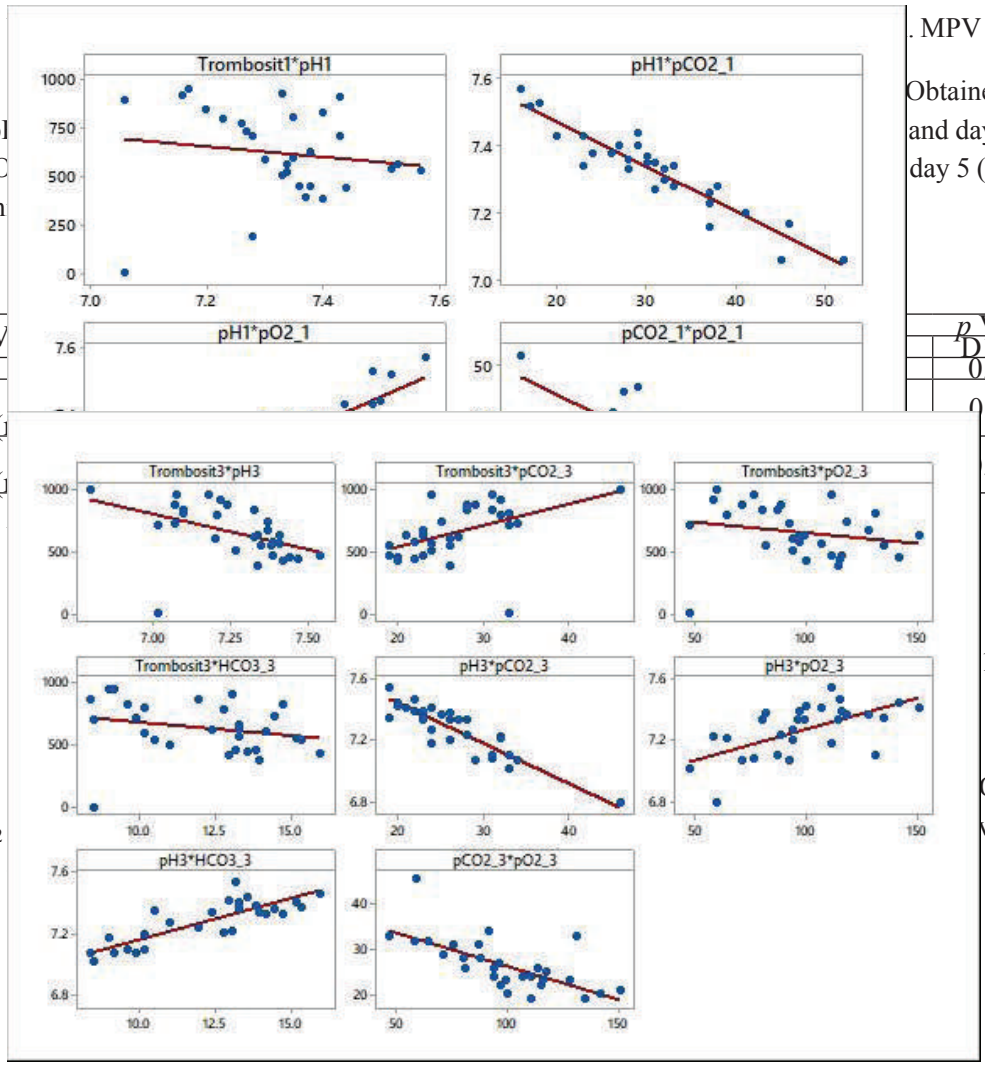


Figure 2. Relationship of morphological parameters with metabolic parameters on day 3.

It could be concluded from Figure 2 that the relationship between platelet variables and pH, platelets and pO_2, platelets and HCO_3, pH and pCO_2, and pCO_2 and pO_2 on day 3 had a negative correlation while the relationship of platelet variables and pCO_2, pH and pO_2, also pH and HCO_3 on day 3 were positively correlated.

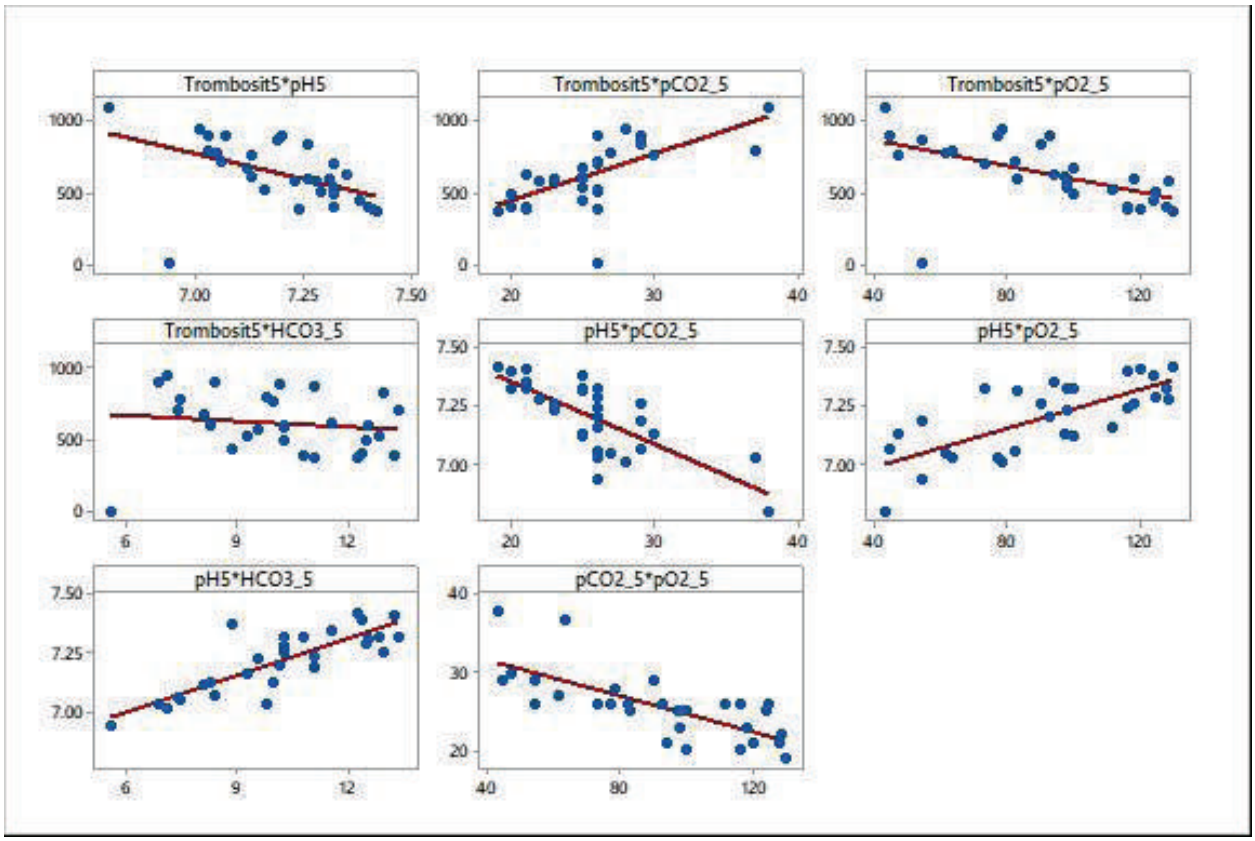


Figure 3. Relationship of morphological parameters with metabolic parameters on day 5.

The Figure 3 showed that the relationship between platelet variables and pH, platelets and pO_2, platelets and HCO_3, pH and pCO_2, and pCO_2 and pO_2 on day 5 had a negative correlation while the relationship of platelet variables and pCO_2, pH and pO_2, also pH and pCO_2, HCO_3 on day 5 had a positive correlation.

Platelets can become active when exposed to foreign surfaces, the presence of trauma, low pH, agonists (thrombin, ADP), and shear stress. After activation, platelets lose their discoid shape and become more spherical with multiple pseudopods[7,8]. Exposure to shear stress such as centrifugation when making components[9], not only activates platelets but can also cause platelet lysis and calpain activation. This platelet lysis can cause the accumulation of cytosolic lactate dehydrogenase (LDH) and the release of granular contents. Activated calpain (protease) will cause cytoskeletal proteins such as actin, talin, and actin-binding protein to degrade and produce microvesicles which can cause a decrease in MPV value[8,10].

In contrast, the results showed that there was a significant increase in MPV values on day 1, day 3 and day 5. This was possible because the change in platelet shape due to the centrifugation process was indicated by a low MPV value on day 1 and then increased on day 3 and day 5 so that showed that platelet morphology was still viable. Increased MPV values were in the normal range of 7.4-10.4 fl[5]. This study showed that the platelet count decreased on the fifth day of storage but the decrease was not significant so it was still considered good. This could be caused by the effect of platelet storage lesions that occurred due to cell lysis caused by activation of platelets which can progressively result in accumulation of metabolic waste, a gradual decrease in the function and viability of platelets[1].

This study also showed a significant decrease in pH during storage. The American Association of Blood Banks (AABB) recommends that platelets with pH <6.2 and pH> 7.4 cannot be used for transfusion, pH of PC components in this study ranged from 7.20-7.33 so PC was considered to be in a good and tolerable condition[5]. Platelet metabolic activity continued during the storage

process. Storage temperature could affect pH, glucose consumption and lactate production. The effect of a decreased pH (<6.8) can cause the morphology of platelets to begin to change dramatically when the pH dropped below 6.0 resulting in irreversible deformation and loss of platelet viability[6,9,12].

During storage, the metabolic activity of platelets caused the consumption of O_2 and CO_2 production resulting in a downward trend in O_2 and an increase in CO_2 in the PC bag[3]. This second generation PC bag is more gas permeable, causing gas exchange to occur more easily across the walls of the PC bag and there is no buildup of CO_2 levels and a decrease in O_2 levels[5]. The results of this study showed a significant decrease in pCO_2 however pO_2 decreased not significantly. This was presumably because the PC bag used in this study had an adequate gas exchange ability.

Murphy and Gardner were the first researchers to state the importance of the influence of agitators on the quality of platelets stored at room temperature. The researchers observed that if PC was stored in a small container or not using an agitator caused the pH to drop rapidly below 6.0. Low pH levels could directly affect post-transfusion recovery. The researchers claimed that this happened because the gas exchange in the PC bag was affected by the quality of the bag used[13]. The process of agitation on PC could guarantee continuous oxygenation of platelets, O_2 could enter the bag and excess CO_2 could be removed so that the use of agitators caused an adequate gas exchange in the PC bag, preventing the buildup of lactic acid and decrease of pH[12,13,14].

Based on the results obtained in this study, PC storage for five days resulted a change in metabolic activity and cell morphology but was still within tolerable limits. PC storage in the Blood Transfusion Unit of the Dr. Soetomo General Hospital, Surabaya, Indonesia has followed the temperature and agitation requirements as recommended by AABB. This study only examined the effect of storage duration on metabolic activity and cell morphology, but there are still many other parameters that can undergo changes during the PC storage process such as platelet aggregation function, bacterial contamination, cytokines produced during storage and other materials. This was a limitation in this study.

Conclusion

In sum, PC storage for 5 days caused a decrease in pH and pCO_2 values as well as a significant increase in MPV but still within tolerable limits. Platelet count was not significantly affected therefore it can be concluded that PC samples was still in viable condition after being stored for five days. Further research is needed for other parameters that can be affected by the storage process.

Conflict of Interest : The authors declare that they have no conflict of interest.

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Pediatric Biliary Atresia: Prenatal and Postnatal Risk Factors

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Abstract

Background: Biliary atresia is the most common cause of cholestasis in infants caused by intrauterine and neonatal inflammatory process leads to progressive biliary tract obstruction and fibrosis. Risk factors are expected to contribute in the disease type. Many studies have been done to determine the risk factors of biliary atresia. Yet, there is still limited information about risk factors in Indonesia. This study aims to determine the risk factors contributing to biliary atresia in East Java, Indonesia.

Methods: This cross-sectional study collected 219 medical record data of cholestasis patients during January 2010 to April 2017 that met the inclusion criteria. 85 of them were diagnosed with biliary atresia. Selected prenatal and postnatal risk factor were evaluated and analyzed.

Result: There were significant results in maternal age on pregnancy (p= 0.009), parity (p= 0.035), and gestational age (p= 0.005) among 85 patients which were diagnosed with biliary atresia. The regression test showed significant result on those 3 factors.

Conclusion: Maternal age on pregnancy, parity, and gestational age are the prenatal and postnatal risk factors that contribute to the biliary atresia incidence. These results are expected to be used as one of the providing information regarding prenatal and postnatal risk factors for pediatric biliary atresia in East Java, Indonesia

Keyword *Biliary Atresia, Risk Factor, Prenatal, Postnatal*

Background

Biliary atresia is well known as a progressive obstructive cholangiopathy affecting biliary tract, both in the intrahepatic and extrahepatic tract, resulting in cirrhosis, liver failure, and death if it is not well treated. It also becomes the most common cause of pediatric cholestasis besides infection and α-1 antitrypsin deficiency[1]. Despite the poor prognosis and complications in delayed diagnosis, the clear etiology and pathophysiology are remained unknown. The incidences of biliary atresia are reported around the world. Biliary atresia seems to be more commonly occurred in Asia than Europe or America, with the incidence approximately 1:8000 in Asia compared to 1: 15,000-20,000 in Europe or America[2,3]. Another study also stated that Asia-pacific region (1:3300) has higher biliary atresia incidence than Europe (1:18000)[4].

The initial findings of this disease are quite similar to other condition like physiological neonatal icteric, thus the diagnosis is likely to be delayed and the

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prognosis becomes worse. There are two major forms of biliary atresia, embryonic form, which is related to other congenital disorders, and perinatal form, where normal bile duct underwent fibro-obliteration during perinatal period[3]. Some risk factors, including environment, prenatal, and postnatal risk factors, play significant role to determine the type and form of biliary atresia.

Many researches around the world have been done to describe the risk factors of biliary atresia incidences. Previous study reported that genetical factor or chromosome alteration, viral-induced immune dysregulation, maternal disease including gestational diabetes, are several factors that affect the incidence of biliary atresia[3]. Another older study suggests that maternal age, parity and gravidity, gestational age, Intrauterine Growth Retardation (IUGR), birth weight, and race/ethnic as the potential risk factors for pediatric biliary atresia[5]. The clear However, in Indonesia, data showing the biliary atresia risk factors is still limited. The authors aim to describe both the prenatal and postnatal risk factors in children with biliary atresia.

Methods and Materials

This was a cross-sectional study conducted in April 2017 using medical record data of children aged a-month-old to 72-months-old that were diagnosed with cholestasis in pediatric hepatology outpatient ward in Dr. Soetomo Hospital Surabaya during January 2010 to April 2017. Any incomplete data in medical record were excluded.

The diagnosis of biliary atresia was decided based on clinical manifestation (icteric, acholic stool), laboratory finding (direct bilirubin > 2 mg/dL if total bilirubin < 5 mg/dL, or 20% of total bilirubin if its value > 5 mg/dL), and histological findings of liver biopsy (ductular proliferation, periportal fibrosis, and bile plug).

We evaluated the maternal age on pregnancy, maternal history on pregnancy, and maternal parity as the prenatal risk factors and Birth weight, gestational age, gender, plurality, anti CMV serology as postnatal risk factors. The data were collected and analyzed by chi square and prevalence ratio was evaluated for subsequently. All statistical analysis was conducted using SPSS version 25.

Result

There were total 219 patients with cholestasis that met the inclusion criteria. Among those patients, 85 patients (38.8%) were diagnosed with biliary atresia and 134 (61.2 %) were not biliary atresia. The characteristics of the subjects were depicted in table 1.

Table 1. Data Characteristic Including Prenatal and Postnatal Risk Factors of Biliary Atresia

Characteristic Profile	Biliary Atresia (n=85)	Non-Biliary Atresia (n=134)
Age		
0-6 m.o	51 (41.5 %)	72 (58.5 %)
6 m.o – 12 m.o	28 (35.0 %)	52 (65.0 %)
12 m.o – 36 m.o	5 (50 %)	5 (50.0 %)
36 m.o – 72 m.o	1 (16.7 %)	5 (83.3 %)
Gender		
Girl	49 (44.5 %)	61 (55.5 %)
Boy	36 (33.0 %)	73 (67.0 %)
Domicile		
Surabaya	34 (38.2 %)	55 (61.8 %)
Outside Surabaya	51 (39.2 %)	79 (60.8 %)

Cont... **Table 1. Data Characteristic Including Prenatal and Postnatal Risk Factors of Biliary Atresia**

Maternal Age on Pregnancy		
< 35 y.o.	38 (31.1 %)	84 (68.9 %)
≥ 35 y.o.	47 (48.5 %)	50 (51.5 %)
Maternal history on pregnancy		
Hypertension	19 (48.7 %)	20 (51.3 %)
Diabetes Mellitus	14 (43.8 %)	18 (56.3 %)
Healthy	52 (35.1 %)	96 (64.9 %)
Maternal Parity		
< 5 times	41 (32.5 %)	85 (67.5 %)
≥ 5 times	44 (47.3 %)	49 (52.7 %)
Birth Weight		
< 2500 g	35 (39.8 %)	53 (60.2 %)
≥ 2500 g	50 (38.2 %)	81 (61.8 %)
Gestational Age		
Premature	51 (48.6 %)	54 (51.4 %)
Aterm	34 (29.8 %)	80 (70.2 %)
Plurality		
Single	81 (38.2 %)	131 (61.8 %)
Multiple	4 (57.1 %)	3 (42.9 %)
Ig G and Ig M anti-CMV		
IGM +	11 (28.2 %)	28 (71.8 %)
IgG +	42 (37.5 %)	70 (62.5 %)
Ig G and Ig M +	32 (47.1 %)	36 (52.9 %)

In Table 2, the Chi square bivariate analysis showed that maternal age on pregnancy (p= 0.009, PR= 2.07) and parity (0.035) differed significantly, in contrast with the maternal history on pregnancy (p= 0.247, PR= 1.86). Furthermore, Table 3 presented the analysis result on postnatal risk factors, where gestational age (p= 0.005, PR= 1.27-3.86) was the only risk factor in postnatal that differed significantly.

Table 2. Prenatal Risk Factors in Pediatric Biliary Atresia

Variable	n (%)	Prevalence Ratio (PR)	CI 95%	P
Maternal age on pregnancy		2.07	1.19-3.61	0.009
< 35 y.o.	38 (44.7)			
≥ 35 y.o.	47 (55.3)			
Maternal history on pregnancy		-	-	-
Hypertension	19 (22.4)			
Diabetes Mellitus	14 (16.5)			
Healthy	52 (61.2)			
Maternal parity		1.86	1.07-3.23	0.035
< 5 times	41 (48.2)			
≥ 5 times	44 (51.8)			

Table 3. Postnatal Risk Factors in Pediatric Biliary Atresia

Variable	N (%)	PR	CI 95 %	P
Gender Girl Boy	 49 (57.6) 36 (42.4)	-	-	0.096
Birth Weight < 2500 g ≥ 2500 g	 35 (41.2) 50 (58.8)	-	-	0.888
Gestational Age Premature Aterm	 51 (60.0) 34 (40.0)	2.22	1.27-3.86	0.005
IgG and IgM anti CMV IgM + IgG + IgG and IgM +	 11 (12.9) 42 (49.4) 32 (47.1)	-	-	0.144
Plurality Single Multiple	 81 (95.3) 4 (4.7)	-	-	0.435

The logistic regression analysis showed that maternal age on pregnancy (p= 0.002), parity (p= 0.015), and gestational age (p= 0.002) are 3 most influential factors on the biliary atresia incidence. The data is depicted in table 4.

Table 4. Multivariate Analysis on Prenatal and Postnatal Risk Factors in Pediatric Biliary Atresia

Risk Factors	Exp(B)	CI 95%	P
Maternal age on pregnancy	0.383	0.21 – 0.69	0.002
Parity	0.482	0.26 – 0.86	0.015
Gestational Age	0.386	0.21 – 0.70	0.002
Gender	0.594	0.33 – 1.06	0.081
Constant	1.972		

Discussion

Among 219 patients, 85 patients were diagnosed with biliary atresia. The diagnosis is based on clinical manifestation, laboratory finding, and histological finding after liver biopsy. Liver biopsy procedure has been known as a predictor and differentiator between biliary atresia and non-biliary atresia in cholestasis patient[6,7]. There are more non-biliary atresia patients than biliary atresia in this study. Some other studies stated differently that biliary atresia is the most cause of cholestasis in children[5,8].

The advanced maternal age (≥ 35 y.o) on pregnancy is associated with unfavorable outcome of delivery (premature, low birth weight, other complications)[9]. A

study done in Sweden showed the similar result with this study where a mother whose age ≥ 35 years old has higher risk to have a children with biliary atresia[5]. Old maternal age on pregnancy is a fetomaternal factor that contribute to biliary atresia incidence. Older mother is associated with oocyte aging, history of prior disease, and chromosome abnormalities toward the baby[10,11].

Maternal history on pregnancy, including asthma, diabetes mellitus, epilepsy, urinary tract infection, respiratory infection, gastrointestinal infection, and healthy. A study stated that there is no significant association between maternal history on pregnancy with the biliary atresia incidence[12]. Another study stated that diabetes mellitus and hypertension are included to risk factors of biliary atresia incidence. In diabetes mellitus mother, vascular disfunction stimulate the hypoxia-induced ischemia in fetal liver vascularization[13]. Furthermore, gestational hypertension can cause several complications and poor outcome, including premature delivery, intrauterine growth retardation (IUGR), and death. High blood pressure decreases the placental blood flow and creates circulation disruption and nutrition transfer delay that lead to premature delivery. Direct association between gestational hypertension and biliary atresia incidence has not been established, but it may affect the biliary atresia incidence through the premature delivery incidence[3].

High parity rate is defined as maternal delivery event more than 5 times with gestational age in each pregnancy is more than 20 weeks[14]. The parity data finding in this study has similar result in other studies[5,14]. A study assumed that higher parity leads to a potential risk factor of having fetal growth abnormalities, including low birth weight and premature delivery, but the mechanism is still unknown[14].

Birth weight is classified into two groups, low birth weight (< 2500 gram) and normal birth weight (≥ 2500 gram). a low birth weight is associated with sequences of public health problem consequences[15]. Previous study showed that there is no significant association between low birth weight and biliary atresia incidence, but premature delivery has significant association with this disease[5]. Prematurity has been reported as a risk factor for biliary atresia incidence despite of unclear mechanism. Some previous studies have reported the significant association between biliary atresia and prematurity[16–18].

Gender has been suggested as one of the risk factors of biliary atresia. Many studies have been evaluating how gender differences affect the biliary atresia incidence with the different results. An epidemiological study in Korea discovered that female infant has higher risk in biliary atresia incidence that male infant[19]. Other studies also showed similar result[20,21]. On the other hand, a study in Sweden stated differently that higher biliary atresia prevalence found in male infants than female[5].

Plurality defined as the number of the children born in a delivery. A study in Texas reported a biliary atresia incidence in monozygotic/identical twins. A recent meta-analysis study of 12 articles stated that biliary atresia is found in almost half of twin subjects with monozygotic and dizygotic twins share the same proportion[22].

Conclusion

Among several prenatal factors that have been established, the presence of maternal age ≥ 35 years old on pregnancy and maternal parity (≥ 5 times) are two times higher to develop a biliary atresia. Whereas in postnatal factor, presence of prematurity risks twice higher to develop a biliary atresia. This study result may be a providing information to determine the risk factors of biliary atresia in Indonesia. **Conflict of interest :** None declared.

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Ethical Clearance : Approved by researched ethical committee Dr. Soetomo General Hospital Surabaya No: 1787/KEPK/I/2020.

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Distribution of Snail Mucous Extract (Achatina Fulica) on the Number of Wound's Basal Epithelial Cells in Rats of Wistar Strain

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Abstract

Background: Acharan sulfate, a new glycosaminoglycan isolated from Achatina fulica, obtained from Kediri, East Java, Indonesia, plays a big role in wound healing process, because it can promote re-epithelialization. Purpose: This study aims to prove that extract of Achatina fulica mucous will increase the number of basal epithelial cells of the wound incision in the back of white male rats of wistar strain. **Method:** This research was done by an in-vivo experimental laboratory, using 14 white male rats of wistar strain that given an incision injury in the back with a length of ± 2 cm and depth of ± 2 mm. They were divided into two treatment groups, that are control group which is the incision wounds were given saline solutions; and treatment group which is the incision wounds were given an extract of Achatina fulica mucous with crude acharan sulfate level is 86,74%. This treatment is given 3 ml once a day for five days. On the sixth day, biopsy was carried out to make a histological specimen, then evaluate under a microscope to account the number of basal epithelial cells of the wound incision. Then statistical analysis was conducted by using independent samples t-test with α=0,05. **Results:** There were significant differences between two treatment group, that are the number of basal epithelial cells in control group and treatment group ($p<0,05$). **Conclusion:** Extract of Achatina fulica mucous with crude acharan sulfate level is 86,74% increase the number of basal epithelial *cells of the wound incision in the back of white male rats of wistar strain.*

Key words: *wound healing, re-epithelialization, achatina fulica, acharan sulfate, snail mucous*

Introduction

Wound is a type of damage to body tissues due to an injury or trauma that disrupts the regular continuity of tissue structure[1]. In dentistry, dental treatment procedures are often related to wounds, such as tooth extraction, scaling, thrush.

Achatina fulica, is an animal that can be found anywhere and still consumed by Indonesian[2], and it's a identity fauna for the city of Kediri[3]. Their mucous contains a glycosaminoglycan secreted from the grains in the snail's body located on the outer surface, as a result of exposure to stress on the snail[4]. This content is called the acharan sulfate that contains most of the repeated disaccharide units from →4)-2-acetamido-2-deoxy-α-D glucopyranose(1→4)-2-sulfo-α-L-idopyranosyluronic acid(1→ ($GicNAc\text{-}IdoA2SO_3^-$)[5].

Based on previous study by Martins, Maria de Fatima et.al, even though snail mucous applied in different forms, the results showed that both treatment group have faster wound healing, and better healing process than control group[6]. Therefore, Snail mucous is widely developed abroad for cosmetics.

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Tissue re-epithelialization is one of the stages in the wound healing process[9] after the inflammatory stage. In this stage, there is an interaction between cells and extracellular matrix[10,11], fibroblast proliferation, angiogenesis, and wound contraction[12]. In the proliferative phase, fibroblasts synthesize collagen and glycosaminoglycans, which will interact with each other and play a role in wound contraction. While glycosaminoglycans themselves can stimulate the re-epithelialization process by increasing the growth and differentiation of epithelial cells and stimulate cell migration[13,14,15]. This study is expected to prove that glycosaminoglycans in Achatina fulica mucous namely acharan sulfate can help the process of re-epithelialization in wound healing marked by an increase in the number of basal epithelial cells of the wound, so that later can be used as an alternative drug to accelerate wound healing[16].

Material and Method

This study is an experimental[17] laboratory conducted on 14 white wistar strain male rats, obtained from the Laboratory of Biochemistry, Faculty of Medicine, Airlangga University, around 2.5-3 months old, bodyweight ranges from 150-200 grams, physically healthy and possible to be sampled, after being maintained for 3-4 days to adapt, in the same places, conditions, and feed at the study site. The experimental animals were divided into two treatment groups, 7 male rats in control group (treated with saline water), and 7 male rats in treatment groups (treated with snail mucous extract).

Achatina fulica Mucous Isolation

This study used around 50 snails (Achatina fulica) obtained from Kediri, East Java. Snail mucous of Achatina fulica is collected by stimulating the body surface of the snails for 30-60 seconds using an electric shock from 6 pieces 1.5-volt battery that configured in parallel set up. Snail mucous obtained is around 550-600 ml and collected in a bottle, then stored in a freezer and will be defrosted when used[18,19].

Water-soluble snail mucous is obtained by mixing it with water (aquadest) twice the sample volume. Then add 1% potassium acetate to ethyl alcohol five times the volume of the sample, and the mixture is put on magnetic stirrer in refrigerator at 40°C overnight. After that, the water-soluble extract was centrifuged at 8000 X g for 30 minutes[20].

The sediment obtained was then measured in volume and dissolved in distilled water 2.5 times the sample volume and 5% cetylpyridinium chloride as much as 5/8 times the sample volume, then stored for 1 hour at room temperature. After 1 hour, the mixture was centrifuged at 8000 X g for 30 minutes, then the precipitate was dissolved in 2.5M NaCl by ¼ sample volume at 450°C for 30 minutes. After that, the sample is purified by adding ethanol 3 times the volume of the sample, then let stand for 1 hour and then the mixture is centrifuged at 2900 X g (4000 rpm) for 30 minutes.

Precipitation obtained was diluted again with tris-HCL pH 8.0 to obtain snail mucous extract of ethanol precipitation as a result of re-suspension. The results of the re-suspension were collected and then stored in -80°C, then freeze dry was carried out to obtain concentrated snail mucous extract which is easy to store and contains crude acharan sulfate[18–20]. The results obtained are in the form of brownish solids (granules) containing crude acharan sulfate, which is calculated using the Biuret method, amounting to 86.74% of the weight. The weight of snail mucous extract is 1260 mg using digital weighing instrument with an accuracy of up to 0.01 gr.

Treatment Procedure

Before doing the incission, shave the hair that covers the skin of rats's back that will be incised with a razor blade and then cleaned with antiseptic, and then giving inhalation anesthesia to experimental animals.

Each group was given the following treatment:

The Control Group (CG) and the Treatment Group (TG) made incisions with length ± 2 cm and depth of ± 2 mm, full thickness, until the muscular layer (fascia) is seen, using a scalpel.

The incision wound on CG was irrigated with saline water while in the TG was dripped with snail mucous extract diluted with distilled water (10 mg/ml) each of 3 ml, for 5 days by using sterile syringe.

On the 6th day, a biopsy was performed, including

the normal tissue, then the tissue was fixed with 10% formalin solution and microscopic preparations were made. For all specimens, cutting with a microtome was done with a thickness of 5 µ and stained with Hematoxylin Eosin (HE).

The wound on the back of rats that were exposed as a result of biopsy were sutured and given antiseptics.

After histological preparations were obtained, then photographed with a camera (min. 3 megapixels and 5x optical zoom) in one field of view with the aid of a 400x magnification light microscope. The number of basal epithelial cells wound was calculated by counting the number of cylindrical epithelial cells sitting on the basal stratum within the wound margins[23] then entered in a computer and calculated manually with the help of the Image Tool program from the computer.

Results

Data from calculation on the number of wound basal epithelial cells in each treatment group were then analyzed using a statistical test with a significance level (α) = 0.05.

Table 1: The number of basal epithelial cells along with their statistical test values.

Sample	Basal epithelial cells count	
	Control group	Treatment group
1	44	78
2	40	69
3	37	67
4	46	66
5	51	77
6	32	74
7	47	94
Mean	42.4286	75.0000
Standard Deviation	6.50275	9.62635
Sig. Levene's Test	0.546	
Sig. Kolmogorov-smirnov Test	0.990	0.835
Sig. Independent samples t-test	0.000	

Based on the table above, the results of the Levene's test, to find out the data variance, are the p value=0.546> α=0.05, which means that the average population of the two variants is the same or homogeneous data. Kolmogorov-smirnov test is to determine the distribution of data, the results obtained in CG that the p value=0.990> α=0.05, which means that in the CG, the distribution data is normal. And for TG, the results obtained are p value=0.835> α=0.05, which means that in the TG, the distribution data is normal. The results of the independent samples t-test, to find out whether there were significant differences between each treatment group, p value=0,000< α=0.05 which means that there are significant differences in the number of basal epithelial cells between the CG and the TG.

Discussion

Microscopically, the wound area looks different from the surrounding healthy tissue, namely the proliferation of fibroblasts, capillary proliferation, and collagen fibers appear irregular. In addition to the visible difference in the number of basal epithelial cells between the CG and the TG, there was also a difference in the wound closure process, namely in the CG there were three

preparations whose wounds had not closed or had not formed an intercellular bridge from the new epithelial cells, whereas in the TG there is only one preparation. This can be caused by several factors, intrinsic or extrinsic. Intrinsic factors include age, health status, nutrition, oxygenation and tissue perfusion. Extrinsic factors include poor surgical and suturing techniques, drugs, wound management, psychosocial/psychological stress, and risk of infection due to unclean wounds[26,27].

From the various factors, the probable cause in this study is poor wound management, because on the second day of evaluation it turned out that the gauze dressing detached in one of the TG so that the wound was not protected, making the wound area able to move and can be disturbed by other rat, causing the healing process to be interrupted. In addition, in the TG, the wound closure process was faster and an increase in the number of basal epithelial cells showed a thicker epidermal layer after measured using specific computer software.

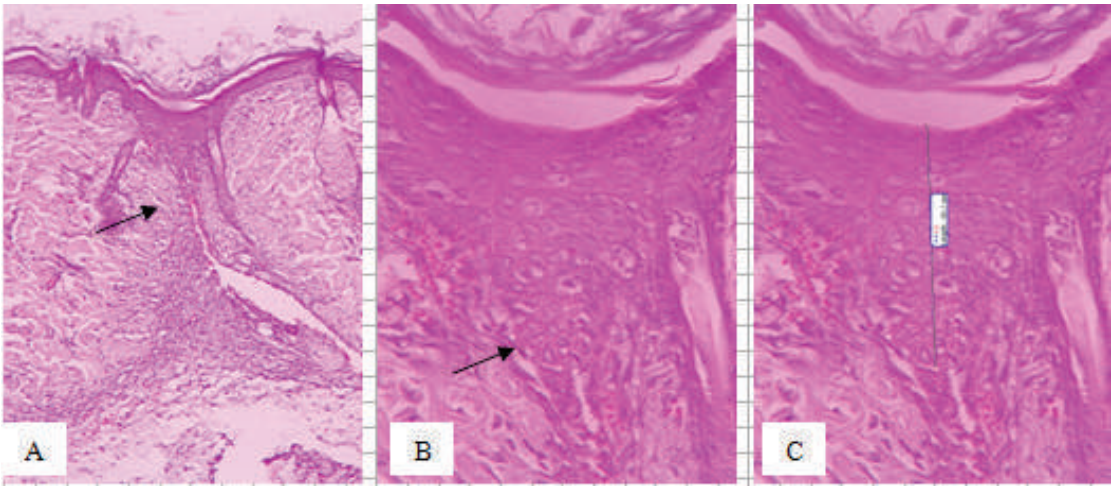


Figure 1. Microscopic features in the CG, location of the wound (A), basal epithelial cells in the basal stratum (B), epidermal thickness=82.7 um (C).

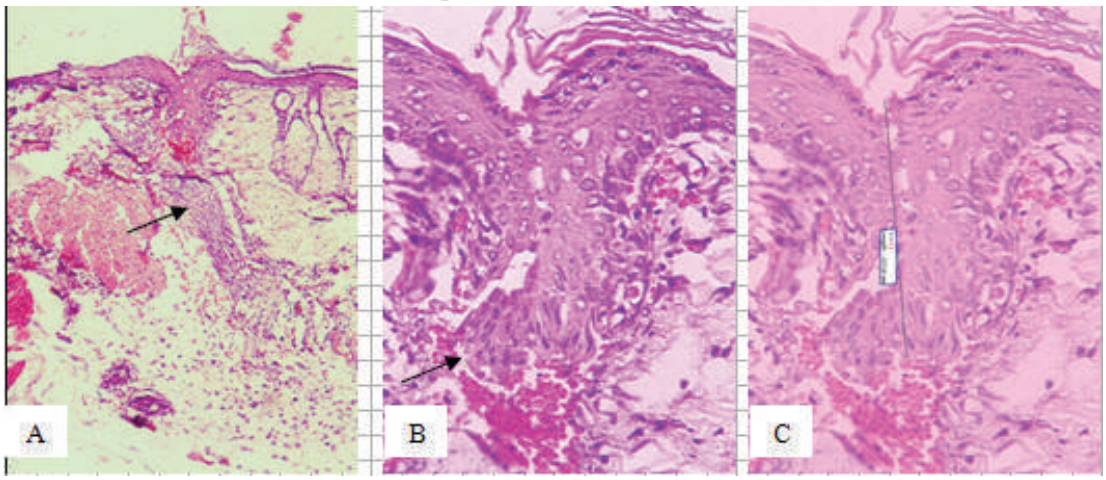


Figure 2. Microscopic features in the TG, location of the wound (A), basal epithelial cells in the basal stratum (B), epidermal thickness=223.8 um (C).

This is because the TG was administered with Achatina fulica mucous extract which contained crude acharan sulfate. Acharan sulfate plays a role in the integrity of the basal membrane by interacting with basal membrane components such as fibronectin, laminin, and type IV collagen. The level of expression and quality of it will have an effect on re-epithelialization, epidermal growth, and basal keratinocyte (epithelial cell) growth

reactions during re-epithelialization[28].

The basal epithelial cells plays a role in cell mitosis and renewal of epidermal cells[16]. In other words, the epithelium growth center is located in this layer. Therefore, if the number of basal epithelial cells increases, the growth of epithelial cells in the upper layers will also increase. This is what causes the thickness of the epidermis in the TG thicker than the CG.

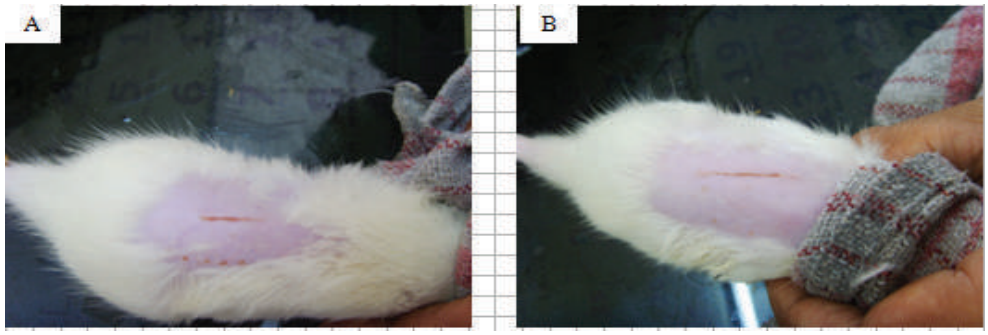


Figure 3. Macroscopic feautre of the incision wound on the 3rd day after injury, the CG (A), the TG (B).

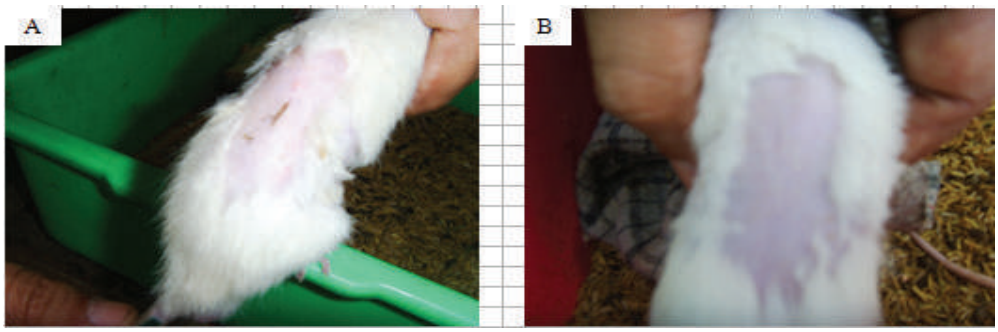


Figure 4. Macroscopic picture of the incision wound on the 5th day after injury, the CG (A), the TG (B).

Macroscopically, there was not too much difference between the CG and the TG. In the TG, it can be seen that the closure of the wound looks better, less crust, and the wound looks unmarked compared to the CG. This is because glycosaminoglycans when interacting with collagen will play a role in wound contraction, provide sufficient strength during the remodeling phase, inhibit excessive collagenesis by blocking the cleavage site of collagen so that it will affect collagen fibrous deposition in vivo[15]. Therefore, the formation of scar tissue is fewer in TG than the CG. This proves that the administration of Achatina fulica mucous extract plays a better role in wound healing than natural healing without outside help.

Conclusion

There was a significant difference between the number of basal epithelial cells in the CG and the TG with 86.74% crude archaran sulfate. This proves that the administration of Achatina fulica mucous extract with 86.74% crude acharan sulfate can increase the number of basal epithelial cells in the skin incision wounds of the white wistar strain male rats.

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Do Turbans Provide Protection Against Cranial Trauma in Two-Wheeler Accidents? A Review of the Indian Medical Literature

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Abstract

The wearing of turbans or head scarves is mandatory for practising Sikh men and women. Religious mandates stipulate that nothing may be placed on top of the turban (scarf), thus making it impossible for practicing Sikhs to wear protective motorcycle helmets. To honour the freedom of religious expression, many jurisdictions, including India, have exempted Sikhs from mandatory helmet laws. Despite studies into the efficacy of protective helmets while riding 'two-wheelers,' little is known about the protective potential of turbans. This paper represents a review of Indian literature (277 studies) related to head injuries sustained in road traffic accidents involving two-wheelers. This review shows that the extant literature is of limited value when trying to understand the extent of the protective potential of turbans and that systematic, evidence-based epidemiological studies derived from hospital admissions and forensic examinations are required.

Keywords: *Turban; helmet; road traffic accidents; cranial trauma*

Introduction

In Sikhism, the practice of permitting body hair to grow naturally expresses respect for the perfection of God's creation, a tradition that reaches back to the Khalsa code defined by the tenth guru [1]. Consequently, all practising Sikhs, men and women, are forbidden to cut their hair, while men are also not permitted to trim or shave their beard. To avoid impurities, the hair is covered with a piece of cloth (*patka*) in private and with a turban when in public, which also serves as an outward symbol of Sikh faith. Turban and unshorn hair are important marks of a Sikh male's identity, even among Sikhs who do not live in complete accordance with Khalsa traditions [2]. As male Sikhs may not cover their hair by any form other than by a turban, this rules out wearing caps, hats, or other head gear, as well as the wearing of helmets (as they are a form of a cap). The same applies to practising Sikh women who would wear a head scarf or a turban. Furthermore, according to Sikh religious practices, no further head coverings may be placed over turbans [3]. This dissonance sets up a potential for conflict between national road safety regulations and the cultural expectations of Sikh men.

In India, as well as in numerous other jurisdictions, Sikhs are exempt from the mandatory requirement of protective helmets while riding 'two-wheelers,' a generic term which encompasses mopeds, motorcycles and scooters as well as bicycles. Given that throughout India two wheeler accidents are the major cause of head injury [4], and that helmets have a high efficacy of protecting riders and pillion passengers from traumatic head injuries, the exemption for Sikhs has been widely criticised by the Indian medical profession, which aims to overturn it. Despite studies into the efficacy of protective helmets while riding 'two-wheelers,' however, there is only a limited number of observational studies on the protective potential of turbans.

This paper was conceptualised during background research into how the cultural mandate to wear turbans meshes with the legal requirements to wear protective head gear during work and recreational pursuits[5]. It was assumed that data on the relative performance of turbans as protective head gear would be readily available in the

Indian literature given the volume of research carried out on two-wheeler accidents in the country. Relevant data proved to be more elusive, creating an impetus for a formal review.

This review canvasses the attitudes and incidence of helmet use as well as the state of knowledge regarding the effects of helmet or turban use on head injuries as well as the nature of cranial trauma sustained in two wheeler accidents.

Methodology

This paper systematically reviews the extant literature but is not based on a systematic review. The methodology espoused a combination of systematic database searches with snowballing. In total, 277 papers, theses and reports were identified for this review, of which 223 could be evaluated for content. For details see the Supplementary Data file.

Results

A large number of studies (37.5%), although mainly based on autopsy or clinical data, remain at a too generic level to provide useful data for the research question at hand, as they neither discuss helmet use nor do they provide sufficient detail on the distribution of cranial injuries. Several studies place considerable emphasis on peripheral, but presumably easy obtainable data, such as the time of day when accidents occur or even the socio-economic and educational status and professions of the victims but then fail to report in more detail on injury patterns and injury severity as purported in the article titles.

From a regional perspective, one might assume that studies conducted in the wider Punjab area of both India (Chandigarh and Punjab State) and Pakistan (Punjab Province) would be more likely to comment on the wearing of turbans as an influencing fact. This, however, this was not the case (for references see Supplementary Data file).

Attitudes and incidence of Helmet Use

Although the use of helmets when riding two-wheelers is mandatory in India [6 §129] unless the rider and pillion passenger are Sikhs, compliance is lax and the enforcement is lacking or inconsistent.[7]

A number of studies have been conducted that examine the extent of helmet use and the rationale for not wearing them. The main arguments by participants put forward against helmet use are discomfort, especially in a tropical climate, helmet cost, inability to hear, but also fear of hair loss or that the nature or speed of the trip did not warrant one, even though studies have shown that many understand the rationale for wearing a helmet (for references see Supplementary Data file).

The literature on helmet use is largely silent in turbans as alternatives. While some studies identify the religious affiliation of participants, they fail to consider the use of turbans in their analysis [19]. Likewise, some studies in the Punjab and Ladakh areas discuss the lack of preparedness to wear helmets [20], but do not to classify any other headwear among non-helmet wearing motorcyclists. The exception is a study of the attitudes of female pillion riders in Delhi, which showed that Sikh women were more likely to be opposed to mandatory helmet laws than Hindu women [21].

Effects of Helmet or Turban Use

Many studies reporting on head/cranial injuries caused by two-wheeler accidents note the beneficial effects of helmet use (21.3 %). Only very rarely, however, do they pass informed comment on the nature or other types head protection, *i.e.* comparing helmets with turbans. In many instances the extent of protection provided by a turban is either simply assumed or noted as a possible mitigating factor of unknown benefit, but is not formally assessed.

Mohan, et al. [22] were the first to discuss various forms of head protection in their seminal study 'Helmet and head injury study of crash-involved motorcyclists in Delhi.' The authors noted that "[s]ome Sikhs believe that turbans provide some protection, but our study showed no evidence of this" even in single-vehicle crashes with speeds as slow as 15 km/h. Another study of motorcyclists in Delhi by Grimm and Treibich [23],Grimm and Treibich [24], showed that two-wheeler riders wearing turbans drive on average more slowly and appear more risk averse than riders with helmets.

In the late 1980s, Sood, examining over 300 cases of head trauma caused by motorcycle accidents in New Delhi, however, noted that "[t]heir head injury incidence

and severity was midway between that of drivers with helmets and without, suggesting that the turban offers some degree of protection" [25], in particular in lesser accidents. A 2009 study of fatal two-wheeler accidents at Patiala noted that turbans appeared to provide partial protection [26]. None of the studies, however, are systematic, evidence-based epidemiological studies that compare hospital admissions and forensic examinations of head injuries.

Nature of Cranial Trauma

Even though a large number of papers comments on the beneficial effects of wearing helmets (see above), the reviewed papers provide little formal data that specifically examine the location of cranial fractures among two-wheeler riders not wearing an approved safety-helmet. In most cases, the trauma loci are only broadly described. For example, setting aside cranio-facial injuries (which will only be protected by full face helmets), a study in Chennai found that the majority of fractures appear to be in the temporo-parietal region (40%), followed by fractures of the frontal bone (15%). Occipital fractures were the least common (7.5%)[27]. This appears to be a universal pattern as similar patterns were reported from various parts of India, Pakistan and Bangladesh (Table S4).

Discussion

Observational studies outside the realm of road traffic accidents are limited in scope and detail, such as studies on the impact of blast injuries to the ear sustained by soldiers [28,29] and on head injuries sustained by batsmen during professional cricket matches [30].

Grimm and Treibich [23] noted that "drivers tend to compensate between speed and helmet use: the Sikhs who cannot wear a helmet because of the turban, drive, on average, slower." Mohan, et al. [22], however, noted that turbans provided no appreciable protection even in single-vehicle crashes with speeds as slow as 15 km/h.

The level of protection a turban might afford its wearer is dependent on its type. A *doumalla*, for example, a double length turban of ten or more meters, has considerably more fabric covering the sides of the parietal bones as well as the top of the cranium than other turban types. Canvassing publicly accessible imagery of Sikh on two-wheelers (via Flickr and other photo hosting sites), the most common types of turbans worn are the *pag(ri),* a double width turban of five to six metres length, and the *dastar* a single width turban of four to six meters length. The *pag* has fewer but thicker layers of cloth on the sides then the *dastar*, and, critically, has a thicker coverage on the top of the cranium.

Experimental studies simulating the performance of a turban against blunt head impacts at various locations of the skull have shown that a turban buffers the impact, especially at the sides, front and rear of the head, albeit below the levels of a safety helmet. Setting aside the location of the *joora* (top knot) at the front of the head, the turban has no meaningful effect in the case of a blunt impact on the crown as there the covering with turban material is the thinnest (usually only one layer, Figure 1) [31,32]. In both experimental studies a *dastar* was used.

Unlike a helmet of near uniform thickness, a turban is wound round the head and thus provides differential covering depending on the extent of overlap between the individual layers. A turban-covered skull therefore exhibits different zones of protection against blunt impact, which need to be taken into account when examining cranial trauma. Figure 1 shows the relative thickness of cloth covering at various parts of the skull, ranging from three to a single layer.

As noted above, little formal data have been published that specifically examine the location of cranial fractures among motorbike riders not wearing an approved safety-helmet. In most studies, the observations of cranial trauma are kept at such a general level (e.g. temporo-parietal region') that it is impossible to draw any conclusions about the prospective buffering effect a turban might have.

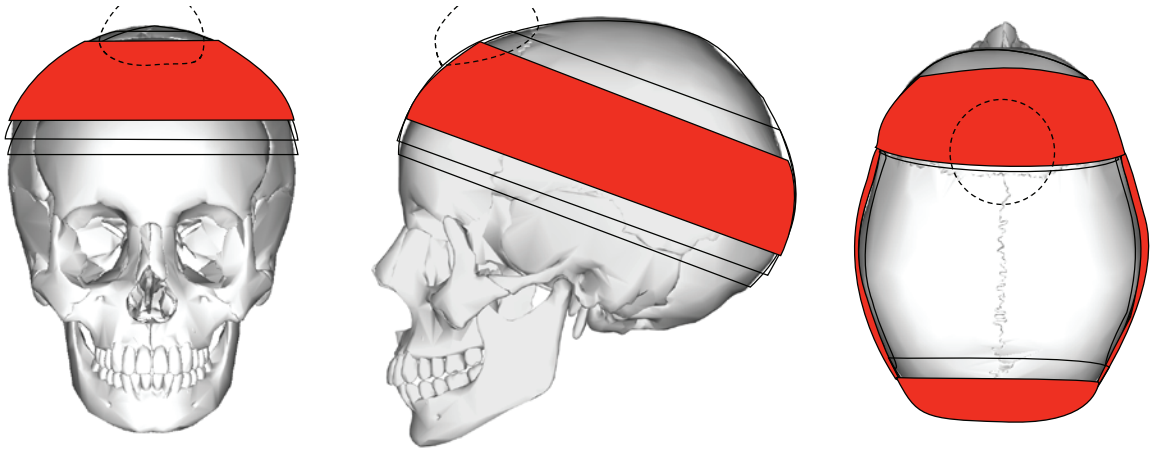


Figure 1. Schematic protection provided by a turban. The darker, the more layers. The dashed outline shows the approximate location of the joora which provides additional buffering. Base image of skull: Wikimedia.

Directions for future research

As this review has shown, the extant literature of head injuries sustained in two-wheeler accidents is of limited value when trying to understand the extant of the protective potential of turbans. The published data are too patchy and inconsistent to be informative. More rigour and in particular more detail in reporting is required. Systematic, evidence-based epidemiological studies derived from hospital admissions and forensic examinations are lacking. There is a need for a full systematic study that compares the number of fatal vs. non-fatal head injuries sustained during motorcycle or bicycle accidents of both drivers and passengers, and correlates theses with the wearing of protective head gear (helmet or turban). The forensic departments are eminently situated to provide this.

Ethical Clearance: As the study is a review of published literature, a clearance from the Ethics in Human Research committee is not required.

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The Influence of Baby Spa Toward Growth and Development Rough and Smooth Motoric Babies, Age 3 – 12 Months in the Subdistrict Lapongkoda Disrict of Tempe Wajo Regency

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Abstract

Background. Baby Spa can fulfill three basic needs of babies that is physical needs biological needs, emotional needs, affection needs, stimulation needs. This baby massage contains touch substances such as affection voice or talking, eyes contract, action and spa. Baby spa is also one of stimulation substances which will stimulate structure development as well as the function of working the cores in brain. This research aims to analyze the influence of baby spa toward growth and development rough and smooth motoric babies who have age 3 – 12 months in the subdistrict Lapongkoda disrict of Tempe Wajo regency. **Material and Methods.** The type of research used was an quasi experiment study design. The sampling method uses simple random sampling. The study was conducted in July – August 2019. The number of sampling was 32 people. The population and research sample are babies in the subdistrict Lapongkoda disrict of Tempe Wajo regency. The data analysis technique used in this is T-test. **Results**. The result of research indicates that there are influences of babies weight growth after doing baby spa p (0,000) <p (0.05), influences of development rough motoric after doing baby spa p (0,000) <p (0.05), influences of development smooth motoric after doing baby spa p (0,000) <p (0.05). **Conclusion.** These findings showed growth and development motoric babies, age 3 – 12 months, can be improved by providing baby spa treatment. It is recommended that the Efforts that need to be considered for health workers are developing baby spa promotion and education as well as baby massage to baby's parents so that the needs regarding increasing baby's motor growth and development can be met.

***Keywords**: Baby Spa, growth, development, motoric, age*

Introduction

Baby spa (baby massage) is a new trend in caring for babies, baby spa has long been practiced to optimize the growth and development of infants. Growth and development are a process that is continuous, which starts from the baby in the womb until adulthood. [1], [2]

Age 3-12 months is a very important age for baby's development. This age is in the most rapid growth and development stage. Developmental movements occur in the brain as a center of intelligence, then in all sensory organs such as hearing, vision, smell, taste, touch and balance organs. Science and technology are advancing, and so are the areas of baby development and growth. The tradition of how to care for babies inherited by our ancestors turned out after scientific research proved to have many benefits. One tradition that has been proven to help the development of early infancy rapidly is infant massage. [2], [3], [4]

One of the developments in infants in the earliest days is motor development, which includes gross and fine motor skills. Coarse motor is body movements that use large muscles, 90% or all parts of the body affected

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by maturity itself. Fine motor is a movement that uses fine muscles or certain parts of the body, which is influenced by the opportunity to learn and practice. The incidence of general developmental delay is not known with certainty, but it is estimated that around 1-3% of children under 6 years a general developmental delay. Monitoring the status of growth and development fell to 23.1% this is because Indonesia is making progress in the program. [5], [6], [7]

Overcoming the problems of growth and developmental disorders and motor delays, baby massages can be given a child's limbs to stimulate motor development so that its development is appropriate for its age, because infant massage can stimulate muscles, bones and organ systems to function optimally. The baby spa influences the development of gross motor skills of infants aged 6-9 months in My Baby Spa. At this time, especially in the region not knowing about baby spa treatments that can help in increasing the growth and development of their babies. 8], [9]

Material and Methods

The type of research used was an quasi experiment study design. The sampling method uses simple random sampling. The study was conducted in July – August 2019. The number of sampling was 32 people (the intervention group were 16 people and the control group were also 16 people). The population and research sample are babies in the subdistrict Lapongkoda disrict of Tempe Wajo regency. The data analysis technique used in this is Paired T-test.

Results

The data used in this study amounted to 32 people with ages 3-12 years in babies in the subdistrict Lapongkoda Disrict of Tempe Wajo regency with variable body weight, rough and smooth motoric. The results of these measurements can be seen in the following table

Table 1. Baby's Weight Before and After Baby Spa

Age (Months-Years)	Baby's Weight					
	Intervention (Baby Spa)			Control (Stimulation)		
	Pretest	Posttest	Percent	Pretest	Posttest	Percent
3	5000	6200	24	6700	6300	-5
4	6200	7000	12	6500	7100	9
5	6100	7000	14	7700	7900	2
5	6500	7300	12	6800	7200	5
6	7900	8500	7	8400	8000	-4
6	7300	7900	8	8100	8500	4
7	6600	7300	10	7200	7700	6
7	7400	8000	8	8900	8500	-4
8	7000	6800	-2	8800	8600	-2
8	8100	9000	11	8100	8100	0
9	9200	10500	14	8200	8500	3
9	9500	10800	13	9200	9000	-2
10	8600	10750	25	8700	9200	5
10	9200	10700	16	9700	9000	-7
11	10100	11000	8	9700	10000	3
12	9900	10700	8	10100	10400	2

Table 1 shows that 8.0% to 25.0% experienced weight gain, and -2% of infants lost weight (intervention group). Infants who gained weight with a total increase of 2.0 to 12.0%, and -2% to -7% experienced weight loss (control group).

Table 2. Growth, Rough and Smooth Motoric Development Before and After Baby Spa

Age (Months-Years)	Rough Motoric						Smooth Motoric					
	Intervention (Baby Spa			Control (Stimulation)			Intervention (Baby spa)			Control (Stimulation)		
	Pre Test	Post Test	Per Cent	Pre Test	Post test	Per cent	Pre Test	Post Test	Per cent	Pre test	Post test	Per Cent
3	1	2	25	1	1	0	2	2	0	1	1	0
4	2	3	25	2	2	0	1	2	50	1	1	0
5	2	3	25	2	2	0	1	2	50	1	2	1
5	3	4	25	2	2	0	1	2	50	1	2	1
6	3	4	25	1	1	0	2	3	25	1	1	0
6	3	4	25	1	1	0	3	4	25	2	2	0
7	3	4	25	1	1	0	4	4	0	1	1	0
7	2	3	25	2	3	25	2	2	0	2	2	0
8	3	4	25	2	3	25	3	4	25	3	3	0
8	3	3	0	3	4	25	3	4	25	4	4	0
9	3	3	0	1	2	25	1	2	50	3	3	0
9	2	3	25	2	2	0	1	2	50	3	3	0
10	2	3	25	2	3	25	1	2	50	1	1	0
10	2	3	25	1	1	0	1	2	50	2	2	0
11	2	3	25	3	3	0	1	2	50	2	2	0

Table 2 shows that there were 14 baby showing rough motoric improvement with a value of 25% and there were 2 infants who did not show gross motoric improvement, including 4 infants who showed gross motoric improvement (25%) and 12 infants who did not show rough motoric improvement (control group). 13 babies (1% -0.5%) who showed improvement in smooth motoric, 3 baby who did not show any change (intervention group), 2 baby (1%) who showed smooth motor and 14 baby who did not show Increased fine motor skills.

Table 3. Test Results Pretest- Posttest of Growth and Development of Baby

Variable		N	Mean	SD	SE	p value
Baby's Weight	Pretest	16	7787.5	1521.35	380.37	0.000
	Posttest	16	8715.63	1750.4	437.6	
Rough Motoric	Pretest	16	2.25	0.619	0.154	0.000
	Posttest	16	3.25	0.577	0.144	
Smooth Motoric	Pretest	16	2.37	0.957	0.239	0.000
	Posttest	16	3.19	0.981	0.245	

Table 3 shows that changes in infant weight gain, rough and smooth motorized pretest-posttest p (0,000) <p (0.05) which means that there is an effect of giving a baby spa on baby motoric growth and development.

Discussion

The results of research conducted in the subdistrict Lapongkoda disrict of Tempe Wajo regency, showed that the average baby given baby spa treatment or baby massage experienced an increase in body weight, and experienced an increase in the development of rough motoric and smooth motoric infants. Babies will learn new things through massage that requires a variety of touches given, the aroma of massage oil, to the communication with which the mother enters with him. Massage conducted during this research will stimulate the baby to produce substances in the brain that are very good for its development in the form of myelin. These substances function to regulate nerve impulses associated with the motor and sensory abilities of the baby for the better. [10], [11]

The results showed that in the intervention group that was given a baby spa, good weight growth (93.75%), babies who were given a baby spa in the form of massage and swimming babies experienced weight gain between (8.0%) to (25.0%) increase, whereas babies with underweight baby (6.25%). This is supported by the theory that baby massage is beneficial in increasing baby's growth, which is to increase body weight, where massage in infants can stimulate the baby's body to produce certain hormones or enzymes that ultimately form enzymes that will empty the stomach so that triggers hunger in infants, the more babies take breast milk the faster they gain weight.

The 47% of premature babies who received massage therapy actually experienced better weight gain when compared to premature babies who were not massaged at all. After a massage the blood circulation in the baby's body turns out to be smooth. Babies who have received baby spa treatment show more active rough and smooth motorized skills than before. Babies who initially show signs of immature rough motoric skills, after a baby spa treatment performed for a month showed perfect rough motoric skills. Baby spa treatments also respond to smooth motoric skills without the help of parents. [12]

The results of this study are in line with research by Susila I (2017) that the action of baby spa in the intervention group experienced a significant weight gain compared to the control group. This is caused by giving massage to the baby at least once a day can stimulate the vagus nerve which will stimulate intestinal peristalsis, so that it will accelerate emptying of the stomach, thereby stimulating the baby's appetite and the baby will quickly feel hungry. [13]

This statement is in accordance with the results of research conducted by Umi Kulsum (2014) that babies who are routinely massaged will have an increase in body weight of 4.11% compared to babies who have not been massaged. Ardiana. D (2013) explains that babies who are stimulated through touch or massage will experience vagus nerve tone (10th brain nerve) massage can stimulate increased levels of the enzyme absorption of gastrin and insulin which can increase body weight. [14], [15], [16]

Statistical results of the Paired T-test of the intervention group showed p (0,000) <p (0.05) so that it could be concluded that there was a significant effect on the gross motor babies of infants in spas. Massage can produce biochemical effects consisting of increased levels of serotonin which can produce physical effects in infants that produce an optimal increase that requires increased motor ability. Kusumastuti et al (2016) concluded that infants (3-6 months) who received a spa baby had better gross motor development compared to infants who did not get a massage. This is consistent with the results of research conducted by Budy, et al (2015). Indicates the development of the baby after the baby spa shows that the respondents increase the increase in rough motoric. Based on the facts of the study, descriptively it was seen that the baby spa gave priority to the development of special infants in rough motoric skills. [17], [18], [19], [20]

Research conducted by Wati (2017) shows that there is an effect of infant massage on rough motoric development in infants aged 3-4 months with a result of p (0.001) <p (0.05) in the baby's ability to crawl, lift his head and hold for more than one minute by raising head 45^{o}-90^{o}, the baby's ability from supine sleeping position then the head is raised to a sitting position, and the ability to roll or tilt toward the stomach. The

rough motoric development shown by the baby after being given a baby spa treatment is that the baby is more perfect raising his head, sitting faster, crawling and then sitting as he was in the original position, as well as when given a toy, the baby responds quickly when invited to play, the toy is released then searched and gives it to people the old. [21], [22]

Likewise in smooth motoric skills, babies tend to show friendly nature to researchers by smiling, looking at researchers as if they want to be invited to play, besides that some babies are also good at moving toys from one hand to another, babies are more focused looking at one the object in front of it.

The results of the Paired T-test of the intervention group showed that p (0,000) <p (0.05) showed that there was a significant effect on the smooth motoric of the baby after the baby spa was performed, indicating a good smooth motoric. The results of the Paired T-test of the control group showed p (0.164) <p (0.05) so that it can be concluded that there was no significant effect on the baby's smooth motoric.

Massage conducted regularly also affects the baby's smooth motoric movements, where massage can produce biochemical effects in the form of increased levels of serotin which can produce physical effects in infants in the form of optimal growth so that motor skills increase. This is in line with research by Kristianingsih A, Baniyah S et al (2017) which shows that babies who get baby massage have better motor development than babies who don't get baby massage. Spa babies on fine motor development in infants aged 3-6 months have a significant effect. The results of smooth motoric development in the intervention group experienced an increase in the number of infants that matched development at the time of the post test. [23]

Conclusion

These findings showed growth and development motoric babies, age 3 – 12 months, can be improved by providing baby spa treatment. It is recommended that the Efforts that need to be considered for health workers are developing baby spa promotion and education as well as baby massage to baby's parents so that the needs regarding increasing baby's motor growth and development can be met.

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Ethical Considerations: Ethical clearance was obtained from Institute of Health Science "Maluku Husada", Ambon, Indonesia; with number" RK.11/ KEPK/STIK/V/2019. Just before the interview, written (or thumb impression) consent was obtained from each participant in Institute of Health Science Ambon guidelines.

Conflicts of Interest: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

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Working and Resting Period based on Inclination, Loads, and Lift Methods on Manual Material Handling in Mining Environment

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Abstract

This study assessed working period and resting period in handling return roll (RR) in laboratory. Deliveries of a RR involve a shoulder/elbow-mode of carriage. Workers usually prefer the Carry On Shoulder (COS) (*Gendong)*, or Carry On Elbow (COE) (*Manggul*) modes. The objective of the research are to measure Working Period (WP) and Resting Period (RP) that converted into heart rate (HR) and oxygen uptake (VO_2). Eight healthy male subjects performed shoulder/elbow-mode carrying. The type of research was a experimental study. Lifting with the shoulder method feels lighter and takes distance much longer. The rest period for the shoulder method has shorter rest periods because the work is lighter while carrying a longer rest period based on ANOVA test on the effect of inclination, load and lifting methods on Working and Resting Periods.

Keywords*: working period, resting period, carrying on the shoulder/elbow; heart rate; VO2; manual handling, mining environment.*

Introduction

Lifting activity is very common in the manufacturing industry or service industry. Throughout the years, research has examined the physiological and biomechanical implications of load carrying. Manual handling activities at working environment can result in a wide range of Musculo Skeletal Disorders (MSDs)[1]. MSDs were most frequently reported in workers with less than 5 years or more than 20 years of mining experience.

Several studies have been conducted in mine about musculoskeletal risk factors that occur in mines[2]. Risk factors in mine; occupational and individual factors in mine[3], physiological workload evaluation when carrying a load[4]. Duration of working has been studied by researcher[5, 6]. Some of these studies do not discuss work periods when lifting.

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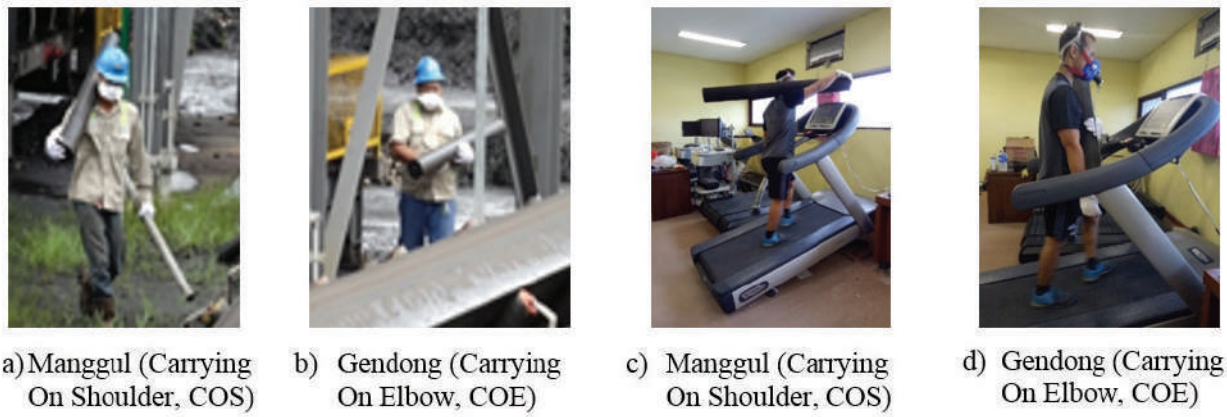


Figure 1 : Mining workers carrying RR in mining area environment (a,b) and in laboratory (c,d) using Fitmate Pro Cosmed[7]

The study of lifting methods in manuals material handling comparing mining environment and laboratory environment based on measurements of heart rate and oxygen consumption carried out experimentally is relatively rare. To overcome this gap, this study examines method between *Gendong* (Carry On Elbow, COE) and *Manggul* Carry On Shoulder (COS), how to model the relationship between COE and COS methods, inclination, and travel time when carrying RR (figure 1). Many studies have carried out[8,9], but they not include length of work period and rest period.

Material and Methods

Lifting activities are carried out on the treadmill (Woodway and TechnoGym) arranged in Figure 2. First, oxygen cells installed in FitmatePro, flow meter mask is placed on respondent's face and pole is placed on chest according to the position of the heart to detect HR. Flow meter (Figure 2) is connected to FitmatePro and pole is connected using WiFi. While participants stood on a treadmill (Woodway) and walk on a treadmill at a speed of 3.5 km/h, with an inclination vary between 10°, 20°, and 30°. Participants were walking on a treadmill carrying 20kg return rolls (RR).

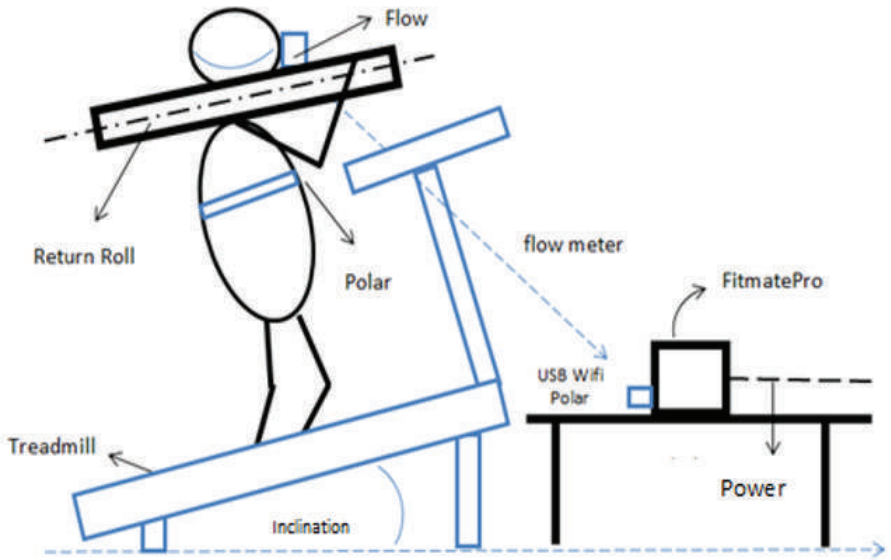


Figure 2. Schematic diagram of experimental MMH study

This research started with data sources obtained by designing an experiment[2] and the stages in WP and RP determined as follows:

Firstly, looking at the output pattern of FitmatePro for eight respondents. Secondly, determine the average. Thirdly, determine time and distance when HR 100 bpm to 160 bpm. Fourthly, determine the amount of rest time when the average decrease in HR from 160 bpm to 140 bpm. After break then start again by looking at previous pattern up to HR 160 bpm. After that, return to first step.

The following is analysis of the results of the measurement WP and RP[10] using ANOVA Method. Firstly, make a tabulation data of the Working Period (WP) and Resting Period (RP) (table 1). Then conduct ANOVA analysis for WP (Table 2)

The model formed from two factorial experiment design where first factor is level three inclination (10^0, 20^0, and 30^0) and second factor is two levels load (20 kg and 24 kg) where the block is method of lifting which are applied to WP of the compilation of respondents, lifting the load :

$$y_{ijk} = \eta + \beta_i + \alpha_j + \tau_k + (\alpha\tau)_{jk} + \varepsilon$$

Where :

y_{ijk} = observation at β to - i and treatment a to –j, treatment t to-k

h = average value of all observations

β_i = block effect (method), i = 1, 2 with 1 = carrying of elbow dan 2 = carrying of shoulder

α_j = inclination block effect, j = 1, 2, 3 with 1 = 10^0, 2 = 20^0, and 3 = 30^0.

τ_k = loads treatment effect, j = 1, 2 with 1 = 20, dan 2 = 24.

$\alpha\tau_{jk}$ = interaction effect of two treatments are the interaction between inclination and load

Just like the one above to calculate the RP, the model is same as the one written above, only variable y_{ijk} replaced with a RP. The model formed from two factorial experimental designs where first factor is inclination of three levels (10^0, 20^0, and 30^0) and second factor is the load of goods with two levels (20 kg and 24 kg) where the block is located. method of lifting weights. and *Manggul*) that are applied to measure RP.

Results

a) Duration Of Working Period and Resting Period When Lifting A Return Roll

After manual lifting experiment, working periods can be calculated and tabulated in table 1 which contain data of responses from 8 respondents tested carrying of shoulder method, 20 kg load and inclination 10. The responses obtained in the form of a heart rate and length of lifting weights. Working period when lifting is tabulated as well.

Table 1 contain data of responses from 8 respondents who were tested using shoulder method, 20 kg load and inclination 20. The responses obtained were in the form of a HR and duration of lifting varied. It is shown in this figure shoulder activities require distance much more far away, and the respondents have durability where one minute equals 58.3 meters.

b) Effect of Inclination, Load and Lifting Methods on Working and Resting Period

1) *Effect of Inclination, Load and Lift Method on Working Period (Minutes)*

Following is an experimental design effect of inclination, load and lift method on the WP (Minutes). Factorial Design model formed with two treatments and one block. (table 1). ANOVA results in Table 2 show that all null hypotheses state they are rejected significantly because all P values are below confidence level ($\alpha = 0.05$).

Table 1. Working Period, Resting Period and Distance Travel based on inclination, load and lifting methods (minutes)

Working Period based on inclination, load and lifting methods (minutes)				
Inclination	**Lifting Methods**			
	COS (Carrying On Shoulder)		**COE (Carrying On Elbow)**	
	Load 20kg	Load 24kg	Load 20kg	Load 24
10º	7.89	3.94	5.46	2.86
20º	3.64	3.46	2.70	2.51
30º	2.85	2.98	2.69	2.50
Resting Period based on inclination, load and lifting methods (meters)				
Inclination	**Lifting Methods**			
	COS (Carrying On Shoulder)		**COE (Carrying On Elbow)**	
	Load 20kg	Load 24kg	Load 20kg	Load 24
10º	0.79	1.01	0.91	1.01
20º	1.64	0.96	0.91	0.77
30º	1.00	1.38	0.98	1.98
Distance travel based on inclination, load and lifting methods (minutes)				
Inclination	**Lifting Methods**			
	COS (Carrying On Shoulder)		**COE (Carrying On Elbow)**	
	Load 20kg	Load 24kg	Load 20kg	Load 24
10º	460.63	229.625	318.63	166.5
20º	212.13	201.88	157.38	146.5
30º	166.13	173.13	156.75	145.63

Table 2. Two-way ANOVA for working period and resting period to lifting methods, inclination, load, and inclination – load interaction treatments (1 minutes working period is equivalent to 58,3 meter working period).

Dependent variable	Source	F	P
Working Period (minutes)	Lifting methods	10.02	0.025
	Inclination	20.12	0.004
	Load	13.39	0.015
	Inclination*Load	11.05	0.015
Working Period (meters)	Lifting methods	9.94	0.025
	Inclination	20.05	0.004
	Load	13.38	0.015
	Inclination*Load	11.03	0.015
Resting Period (minutes)	Lifting methods	0.04	0.844
	Inclination	1.81	0.256
	Load	0.69	0.444
	Inclination*Load	3.23	0.126

Table 2 shows that for the working period model in the form of minutes and meters units, all the independent variables (lifting methods, inclination, load) were significant (p=0.05). However, the resting period model were not significant (p=0.05), meaning that only the working period is affected by lifting methods, inclination and load. Load, inclination and interaction inclination and load were significant (p=0.01).

Following is an experimental design effect of inclination, load and lift method on a WP (meter) where results of experiments shown in Table 1. Factorial Design model formed with two treatments and one block. ANOVA results in Table 2 show all null hypotheses are rejected significantly because all P values are below confidence level ($\alpha = 0.05$).

2) *Effect of Inclination, Load and Lifting Methods on Resting Period (Minutes)*

Following is an experimental design effect of inclination, load and lift method on RP (Minutes) where experimental results in Table 1. Factorial Design model formed with two treatments and one block. ANOVA test in Table 2 show null hypotheses failed to be rejected significantly because all P values above level of confidence (p=0.05).

Discussion

Working period was time after lifting RR while HR indicate 100 bpm to 160 bpm. Resting period was time after Anaerobic Threshold (AT) was reached until time at basal metabolic conditions. Determination of rest periods based on age, KAM (Maximum Aerobic Ability) and HR recovery. VO_2 was determined by age (assumed constant), workload, air temperature, and motion. When VO_2 was in maximum condition, endurance increase.

Based on diagram analysis, results showed that for inclination 10^o WP was high when COS, load 20Kg, inclination of 10^o. This because light loads and low inclination were low in WP and shorter RP. For inclination 20^o, foot support is large so energy expenditure is large, shorter working time. For inclination of 30^o, biomechanic foot load was heavier so that ability to lift was lower so average rest time was short because it was affected by ability of a good Cardio Respiratory Vascular which results in a decrease in speed of HR.

Based on ANOVA tests on effect of inclination, expenses and lifting methods on WP and RP, all null hypotheses rejected significantly because P values below confidence level (p=0.05). There was difference in the response of WP when there was interaction between inclination and load. But there was no difference in response of RP when there was an interaction between inclination and load given different lift method blocks.

Conclusion

Based on ANOVA test on the effect of inclination, expenses and lifting methods on WP and RP. This shows there was a difference in response to the WP when there was an interaction between inclination and load given different lift method blocks, but there was no difference in response of the RP when there was an interaction between inclination and load given different lift method blocks.

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Ethical Clearance. The present study were conducted in accordance with the principles and procedures that were approved by Health Research Ethical Clearance Commission, Universitas Airlangga, Surabaya, Indonesia

Conflicts of Interest: The authors declare no conflict of interest.

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Oral Care Colostrum Effect on Preterm Infants Fecal Immunoglobulin A Secretory Level

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Abstract

Objective To evaluate whether oral care colostrum can increase fecal secretory immunoglobulin A levels in preterm infants.

Methods Thirty-eight infants who met the inclusion criteria were randomized. Twenty colostrum oral care infants and eighteen control control infants. Colostrum oral care by giving as much as 0.1 ml of colostrum on each buccal mucosa for approximately 2 minutes on one side. The procedure is repeated every 4 hours for 3 days. Fecal secretory immunoglobulin A levels are taken from the first faecal after birth and 72 hours after colostrum administration. Fecal retrieval must first install a urine device so that the urine does not wet the sample.

Result Fecal secretory immunoglobulin A level before treatment in the treatment group were 0.0633 ± 0.0037 mg/g feces higher than the control group 0.0166 ± 0.0139 mg/g feces, statistically there were no significant differences (p = 0.595). Fecal secretory immunoglobulin A level after treatment in the treatment group amounted to 1,1007 ± 0.2458 mg/g feces higher than the control group 0.6045 ± 0.2358 mg/g faeces, statistically there were no significant differences (p = 0.09). Difference in increase in secretory fecal immunoglobulin A levels after and before treatment in the treatment group 1.0374 ± 0.2575 mg/g feces is higher than the control group 0.5879 ± 0.2385 mg/g feces, statistically there were no significant differences (p = 0.09).

Conclusion Oral care colostrum has been shown to increase secretory immunoglobulin A levels in preterm infants before colostrum oral care, and colostrum oral care has the potential to increase faecal secretory immunoglobulin A level than controls in preterm infants.

Keywords**:** *oral care colostrum, fecal immunoglobulin A secretory, preterm infants*

Introduction

Preterm birth defined as every baby born before 37 weeks of pregnancy and is subcategorized in to 3 groups, extreme preterm (< 28 weeks), very preterm (28-32 weeks), late preterm (32-37 weeks). In 2014, Indonesia become fifth most country in term of preterm birth after India, China, Nigeria, and Bangladesh with 10.4 % of all births delivered.[1] Vernix caseosa, the sebaceous gland in infant for hydrating skin, maintaining pH, and containing antimicrobial protein and peptides (APP)[2], is not presented in preterm infant.

Secretory Immunoglobulin A (sIgA) has been identified in breast milk as an antibody recognizing various bacterias and viruses, including *E. coli, Shigella, Salmonella, Campylobacter pylori, Vibrio cholerae,*

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Haemophilus influenzae, Streptococcus pneumoniae, Grup B Streptococcus tipe III, Staphylococcus aureus, Clostridium difficile, Clostridium botulinum, Klebsiella pneumonia, Listeria monocytogenes, enterovirus, human immunodeficiency virus (HIV), Respiratory Syncytial Virus *(RSV),* Rubella, Norovirus, and porcine corona virus.[3] sIgA in breast milk are mainly produced by plasma cells in mammae gland and modified in entire mammary gland epithelium translocation. Nearly 75% of Ig A processed in GI tract are remained and secreted along with feces. Every type of immunoglobulin is identified in feces.[4]

Oral care colostrum is a new concept for preterm infant as a natural substitution for amniotic fluid exposure. This will create an interaction between colostrum and the immunity cells in oropharyngeal lymphoid glands.[5] This procedure may improve immunity system in different ways, including immunostimulant effect of cytokines interaction with OFALT immune cells, passive mucosal absorption of protective immune and trophic biofactors (TGF-β, lactoferrin), barrier protection against oropharyngeal pathogens, oligosaccharide systemic and local effect, and protective effect against antioxidant.[5,6]

Two previous studies showed the safety and tolerability of oral care colostrum in preterm infant both with buccal swab[7] and syringe[8]. A retrospective study reported that oral care colostrum is associated with increased breastfeeding rate, faster full feed achieved, increased growth, reduced NEC incident, and reduced late-onset sepsis.[9] Another randomized study reported lower clinical sepsis incidence, increased proinflammatory and immune protective factors (including Urine IgA, lactoferrin, IL-1β, and saliva's TGF-β1 and IL-8).[10] The authors aim to evaluate the effect of oral care colostrum on fecal secretory IgA level increment in preterm infant.

Materials and Methods

This experimental study was conducted at Neonatal Intensive Care Unit (NICU), Dr. Soetomo General Hospital, Malang, Indonesia. Data were collected during April 2019 to August 2019. The inclusion criteria were baby who were born before 34 weeks of pregnancy and under 2000 gram of birth weight. The exclusion criteria were baby who had multiple congenital anomaly and had contraindication to be given colostrum because of mother's condition. Total of 38 preterm infants were met the inclusion criteria and divided into 2 groups (20 treatment group and 18 control group) randomly. We compared the fecal secretory IgA level in infant before and after oral care colostrum administration. We also compared the fecal secretory IgA level in infants who were given oral care colostrum administration and were not (control). The Institutional Review Board had reviewed and approved this study with ethical clearance No: 1187/KEPK/V/2019.

Sample used in the study were subject first feces collected after birth and 72-hour post colostrum administration feces. The fecal secretory Ig A level were measured using Enzyme Linked Immuno Assay (ELISA) technique read by 490 nm biorad spectrophotometry reader.The first data (before and after) were compared using Wilcoxon test and the second data (treatment and control) were compared using Mann-Whitney test. All statistical analysis was conducted using SPSS 21 Software.

Results and Discussion

Based on homogeneity test, gender, gestational age, apgar score, birth weight, mother's age, parity, mode of delivery, history of PROM, and amnion color were homogeneous ($p>0.05$) (Table 1). The fecal secretory Ig A level before intervention (0.0633 ± 0.0037 mg/g) was higher than control group (0.0166 ± 0.0139 mg/g), did not differ statistically ($p=0.595$). Meanwhile, the fecal secretory Ig A level after intervention (1.1007 ± 0.2458 mg/g) was higher than control group (0.6045 ± 0.2358 mg/g), did not differ statistically ($p=0.09$). Moreover, fecal secretory Ig A level difference in intervention group (1.0374 ± 0.2575 mg/g) was higher than control group (0.5879 ± 0.2385 mg/g), also did not differ statistically ($p=0.09$) (Table 2).

Furthermore, Fecal secretory Ig A level before intervention was increased after colostrum administration (0.0633 ± 0.0037 mg/g, 1.1007 ± 0.2458 mg/g, respectively), significantly different ($p<0.001$). On the other hand, fecal secretory Ig A level in control group before and after intervention was also significantly different ($p=0.039$) (0.0166 ± 0.0139 mg/g, 0.6045 ± 0.2358, respectively) (Table 3).

Studies reported that the sIgA level is affected by many ways. Baby boy tend to have higher sIgA level than baby girl.[11] Gestational age also has important role in the sIgA level. 29 weeks of gestational age has the highest sIgA level.[11] Very preterm infant (28-32 weeks) also has the higher sIgA level than late preterm (32-37 weeks) ($p<0.05$).[12] Furthermore, mother younger than 30 y.o. tend to have more sIgA level.[11] These show that sample characteristics play a role in secretory Ig A level ini preterm infant.

Data showing the higher level of fecal secretory Ig A in preterm infant before intervention than the control group may be associated with more infants who were born less than 1500 gram in intervention group than control group. This finding is in accordance to the previous study showed that breast milk sIgA in very low birth weight infant was higher.[11] Moreover, this result may be associated with parity factor because intervention group has more primigravida mother than the control group, consistent with previous study that show higher colostrum IgA level in primigravida mother than multigravida.[13]

GI tract microflora stimulating either humoral or cellular immune system formation in mucose may be associated with the result of this study.[14] Other study also showed that fecal sIgA level in newborn who did not get breastfeed was found after prebiotic and probiotic administration.[15] Our study results are also similar to other study reporting that colostrum administration did not have any effect on the urine IgA level.[16]

Table 1. Sampling Characteristics

		Oral care kolostrum (n=20)	**Plasebo (n=18)**	**p**
Sex				
	Male (n)	14	11	0,815
	Female (n)	6	7	
Gestation Age				
	< 32 weeks (n)	4	3	1,000
	≥ 32 weeks (n)	16	15	
Apgar score				
1’	3-7 (n)	17	17	0,676
5’	7-9 (n)	12	13	0,652
Birth Weight				
	<1500 gram (n)	13	6	0,104
	≥1500 gram (n)	7	12	
Mother Age				
	< 35 years	15	10	0,358
	≥ 35 years	5	8	
Paritas				
	Primigravida	11	5	0.171
	Multigravida	9	13	
Pre Eklamsia /Eklamsia				

Cont... **Table 1. Sampling Characteristics**

	Yes	9	12	0,310
	No	11	6	
Labour Methode				
	Pervaginam	1	3	0,328
	SC	19	15	
Premature Rupture				
	Yes	4	2	0,761
	No	16	16	
Amnion Fluid				
	Clear	19	18	1,000
	Meconeal	1	0	

Table 2. Fecal Secretory IgA (sIgA) Mean

	fekal sIgA Mean (mg/g)		**p**
	Oral care colostrum	**Control**	
Before treatment	0,0633 ± 0,0037	0,0166 ± 0,0139	0,595
After treatment	1,1007 ± 0,2458	0,6045 ± 0,2358	0,090
Difference sIgA	1,0374 ± 0,2575	0,5879 ± 0,2385	0,099

Table 3. Fecal Secretory Ig A Mean Before and After Intervention

	fekal sIgA Mean (mg/g)		**p**
	Before treatment	**After treatment**	
Oral care colostrum	0,0633 ± 0,0037	1,1007 ± 0,2458	<0,001*
Control	0,0166 ± 0,0139	0,6045 ± 0,2358	0,039*

Conclusion and Acknowledgement

Oral care colostrum is proven to increase the fecal sIgA level in preterm infant after administration than before administration. However, oral care colostrum is not proven to increase significantly the fecal sIgA level in preterm infant than the control group.

Source of Funding : self

Conflict of Interest : -

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Correlation of Toluene Safe Concentration, Malondialdehyde and Glutathione Levels in Osowilangun Shoe Home Industry Workers

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Abstract

Introduction: Toluene is the main organic solvent found in adhesives used for shoe production in Osowilangun Shoes Home Industry. Exposure to toluene that exceeds the threshold value can cause the emergence of free radicals which eventually increase the concentration of malondialdehyde and reduce the concentration of glutathione in the body. The purpose of this research is to study the safe concentration of toluene with levels of malondialdehyde and glutathione in Osowilangun shoe home industry workers.

Methods: This is an observational study using cross sectional design and quantitative approaches. There are 25 people were taken as sample by using accidental sampling technique. Data analysis used Pearson correlation test.

Result: The average safe concentration value in this study was 1.1088 mg / m3 with an average value of malondialdehyde of 7,784 MU and glutathione of 35,655 µg / L. Pearson correlation test showed the safe concentration of toluene and malondialdehyde level was p=0.685 (p> 0.05), with relationship between safe concentrations of toluene and glutathione was p = 0.808 (p <0.05).

Conclusion: This study shows that there was no significant relationship between toluene safe concentration and levels of malondialdehyde and glutathione.

Key words: *Glutation, Malondialdehyde, Safe concentration, Toluene*

Introduction

Osowilangun shoe industry is an informal footwear manufacturing sector whose process include a number of activities of selecting raw materials, depicting patterns, cutting patterns, coloring, gluing, polishing and finishing. The production process is not related to the potential hazards for workers. Potential hazards can be physical, chemical, biological, psychological and ergonomic hazards. Glue containing organic solvents is used in the shoe production process as an adhesive is harmful to the health of workers[4]. The most widely used organic solvent in adhesives is toluene[11]. Toluene (C6H5CH3) is a colorless, odor specific with volatile properties[3]. Toluene is a non-polar compound that can

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be carcinogenic for workers exposed in the home shoe industry. Toluene threshold value in the workplace has been regulated in Permenaker Number 5 of 2018 by 20 ppm[2].

Inside the human body, toluene can be a potential hazard that can cause health effects based on the dose of inhaled concentration or contact that occurs with toluene compounds. Carcinogenic intakes is used if the ECR value is ≤ 10-6 (safe) while non-carcinogenic intakes is shown by RQ if the value is ≤ 1. This means that toxin does not have impact on the body[17]. Furthermore, the safe concentration of toluene can be calculated by assuming that RQ = 1. The safe limit is the limit or the lowest value that causes the level of risk to be unsafe. Safe concentration is the concentration below the safe limit while. If the value is equal to the safe limit it will cause the level of risk to become unsafe[6].

When absorbed through inhalation, toluene enters the respiratory system, then into the veins in the alveoli and into the blood. Interaction increases the amount of toluene that can be collected by liquid blood media and facilitates the transport of toluene to all areas of the body at a greater rate than if toluene is only transported in plasma[5]. An imbalance of antioxidant enzymes with free radicals in the human body can cause damage to cell membranes. Cell membranes formed from fatty acids undergo oxidation thereby releasing malondialdehyde (MDA)[9]. Malondialdehyde (MDA) is a dialdehyde compound which is the final product of lipid peroxidation in the body through an enzymatic or nonenzymatic process[7].

In addition, continuous and high amount of toluene exposure that exceeds the threshold value causes the formation of dangerous toluene. The role of antioxidants is very important because its function can inhibit and neutralize the occurrence of oxidation reactions involving free radicals[13]. Glutathione (GSH) is antioxidant produced to reduce harmful toluene. Antioxidants tend to react first with free radicals compared to other molecules because antioxidants are easily oxidized. In addition, the role of endogenous antioxidants is to neutralize free radicals assisted with exogenous antioxidants[10]. The purpose of this study was to determine the relationship between safe concentrations of toluene and malondialdehyde levels and glutathione in Osowilangun shoe home industry workers.

Methods and Materials

This is an observational study with a quantitative approach. The design of this study is cross-sectional that is observation and measurement of variables carried out at one time. The study was conducted in the Osowilangun shoe home industry in Surabaya. Respondents from the study were 25 people taken by accidental sampling from a total population of 30 people. Data on the characteristics of respondents were taken through a questionnaire. Measurement of toluene concentrations in the air using Gas Chromatography (GC) technique which is placed at 12 predetermined points is carried out by UPTK3 of Surabaya City. Data of Toluene safe concentration is obta[a][17]:

$$Csafe = \frac{RfC \; x \; Wb \; x \; Tavg}{R \; x \; tE \; x \; fE \; x \; Dt}$$

RfC= reference concentration of toluene = 5 mg/m^3

$$= \frac{\frac{RfC\ (mg/m^3) \times R\ (m^3/hari)}{Wb\ (kg)}}{\frac{RfC\ (mg/m^3) \times R\ (m^3/hari)}{Wb\ (kg)}} = \text{RfC (mg/kg/hari)}$$

Wb = weight (kg)

T*avg* = average time period (30 years × 365 days/year)

R = respiration rate (m^3/hour)

$$= \frac{(5{,}3 \times \ln(W_b)) - 6{,}9 (5{,}3 \times \ln(W_b)) - 6{,}9}{24 \qquad 24}$$

tE = daily exposure time (hours / day)

fE = annual exposure frequency (day / year)

D_t = duration of exposure (years)

Blood samples and examination of malondialdehyde (MDA) and GSH concentrations were analyzed and determined by Thiobarbuturic Acid Reactive Subtance (TBARS) method at the Nutrition Laboratory of Airlangga University. Data were analyzed using Pearson correlation test.

Result

Characteristics of Respondents

Table 1. F7equency Distribution of Respondents

Respondents Characteristics	Percentage Frequency	
Age (Years)		
16-25	2	8%
26-35	1	4%
36-45	8	32%
46-55	8	32%
56-65	6	24%
Gender		
Female	13	52%
Male	12	48%
Years of Service		
< 10 years	3	12%
≥ 10 years	22	88%

Source: Primary Data

Table 1 shows that the majority of workers who became respondents were aged 36-45 years and 46-55 years (32%), respectively and only one respondent was aged 26-35 years (4%). Most respondents were female (52%) and 12 people (48%) were male. The majority of respondents have ten years of service (10 years (88%) with 3 (12%) respondents having ten years <10 years of service.

Toluene Concentration

Table 2. Distribution of Toluene Concentrations

Points of Measurement	Toluene Concentration (TLV = 20 ppm)	
	< TLV	≥ TLV
Point 1		62,7
Point 2		58,5
Point 3		137,5
Point 4		30,5
Point 5		25,5

Cont... **Table 2. Distribution of Toluene Concentrations**

Point 6	9,3	
Point 7		41,1
Point 8		289,3
Point 9		36,9
Point 10	11,5	
Point 11	15,8	
Point 12	9,7	

Source: UPTK3 Surabaya

Based on table 2, the highest measurement of air toluene concentration was at point 8 by 289.3 ppm. The lowest toluene concentration was at point 6 by 9.3 ppm. Measurements of toluene concentrations in the air showed that 66.7% of the measurement points with toluene concentrations exceeded TLV (20 ppm) in accordance with ACGIH (2019) and Permenaker No 5 (2018)[1,2]. There are 4 measurement points that have a concentration of toluene in the air not exceeding TLV. The average measurement result of toluene concentration was 60.69 ppm. This shows that the average exceeds the TLV that has been set.

Toluene Safe Concentration

Table 3. Distribution of Toluene Safe Concentration to Workers

Respondent	W (kg)	C Toluene (mg/m3)	RQ	C Aman
Respondent 1	60	1088.004	1085.02	1.00
Respondent 2	70	1088.004	992.02	1.10
Respondent 3	51	59.421	40.63	1.46
Respondent 4	100	114.705	91.51	1.25
Respondent 5	70	114.705	117.66	0.97
Respondent 6	55	235.803	134.38	1.75
Respondent 7	69	235.803	335.94	0.70
Respondent 8	41	220.008	248.24	0.89
Respondent 9	78	95.901	239.10	0.40
Respondent 10	46	59.421	59.26	1.00
Respondent 11	75	138.774	345.98	0.40
Respondent 12	84	517.112	275.04	1.88
Respondent 13	87	114.705	68.63	1.67
Respondent 14	65	114.705	152.52	0.75
Respondent 15	50	95.901	153.02	0.63

Cont.... **Table 3. Distribution of Toluene Safe Concentration to Workers**

Respondent 16	53	1088.004	1550.03	0.70
Respondent 17	70	220.008	112.63	1.95
Respondent 18	60	34.976	55.81	0.63
Respondent 19	55	95.901	87.44	1.10
Respondent 20	65	114.705	101.86	1.13
Respondent 21	45	235.803	125.42	1.88
Respondent 22	93	517.112	343.80	1.50
Respondent 23	55	154.570	369.95	0.42
Respondent 24	59	517.112	288.79	1.79
Respondent 25	80	36.480	48.51	0.75

Source: Primary Data

Based on table 3, the respondent's safe concentration was determined from body weight, frequency of annual exposure, respiration rate, duration of daily exposure, toluene reference concentration, and average time period. Measurements were made by calculating the amount of toluene non carcinogenic intake with the following formula:

$$\text{Ink} = \frac{\text{C x R x tE x fE x Dt}}{\text{Wb x Tavg}}$$

Risk characteristic value (RQ) was calculated by using the following formula:

$$RQ = \frac{Ink}{RfD\ atau\ RfC}$$

According calculation of RQ values of 1 or less than 1 (≤1) indicate that the oxide has not had an impact on the body[17]. The safe concentration formula is as follows:

$$Csafe = \frac{RfC\ x\ Wb\ x\ Tavg}{R\ x\ tE\ x\ fE\ x\ Dt}$$

The average safe concentration (C safe) of toluene is calculated based on body weight, respiration rate, daily exposure time, average non-carcinogenic time period (30 years x 365 days/year), annual frequency of exposure, and duration of exposure toluene for workers is 1.1088 mg/m^3. The highest safe concentration value is 1.95 mg/m^3 and the lowest is 0.40 mg/m^3.

Concentration of Malondialdehyde (MDA) and Glutathione (GSH)

Table 4. Distribution of Malondialdehyde (MDA) and Glutathione (GSH) Concentrations

Respondent	Glutathione (GSH)	Malondialdehyde (MDA)
	(µmol/L)	(MU)
1	14,6	11,857
2	40,85	5,423

Cont... **Table 4. Distribution of Malondialdehyde (MDA) and Glutathione (GSH) Concentrations**

3	40,583	6,277
4	58,349	3,817
5	49,79	4,543
6	49,257	4,603
7	46,176	4,717
8	19,812	11,683
9	27,873	10.023
10	40,241	6,497
11	45,453	4,737
12	28,067	9,67
13	34,383	8,683
14	35,942	7,643
15	30,578	9,317
16	34,357	8,923
17	31,415	9,177
18	26,622	10,323
19	26,356	10,843
20	38,719	7,363
21	39,937	7,203
22	24,796	11,03
23	43,969	5,017
24	41,42	5,283
25	21,829	11,517

Source: Airlangga University Nutrition Laboratory Data

Glutathione concentration (GSH)

Table 4 shows that the highest glutathione concentration (GSH) was 58,349 µg / L and the lowest concentration was 14,6 µg / L. The average glutathione concentration (GSH) measurement results was 35,655 µg / L.

Malondialdehyde (MDA) concentration

Table 4 shows that the concentration of malondialdehyde (MDA) of Osowilangun shoe home industry workers ranged 3,817 MU - 11,857 MU with the highest concentration of 11,857 MU and the lowest concentration of 3,817 MU. The average measurement

result of malondialdehyde (MDA) concentration was 7.847 MU.

Relationship of Toluene Safe Concentration with Glutathione Concentration (GSH)

The results showed that there was no relationship between the safe concentration of toluene and the concentration of glutathione (GSH) in the workers studied (p = 0.808> 0.05). In addition, the results of the relationship test showed that the correlation coefficient value was negative (0.051) where an increase in safe concentration decreased the concentration of glutathione.

Relationship between Toluene Safe Concentration and Malondialdehyde (MDA) Concentration

The results show that there was no relationship between the safe concentration of toluene and the concentration of malondialdehyde (MDA) in Osowilangun shoe home industry workers (p = 0.685> 0.05). In addition, the results of the relationship test indicate that the correlation coefficient value was positive (0.085), when the safe concentration increased, the concentration of malondialdehyde also increased.

Discussion

In the present research the measurement of exposure to air toluene concentration was carried out at 12 points with the highest toluene concentration of 289.3 ppm, the lowest of 9.3 ppm and the average of 60.69 ppm. The highest and average concentration value of toluene exceeds the threshold value. The measurement results also indicate that most of the measurement points exceeded the TLV that have been set in Regulation of the Ministry of Manpower Number 5 of 2018 by 20 ppm[2]. The results of the questionnaire indicate that each respondent has a different concentration of safe exposure according to the characteristics of each respondent. Based on the measurement results, the average safe concentration of 1.1088 mg/m^3 with the highest safe concentration of 1.95 mg/m^3 and the lowest of 0.40 mg/m^3. Exposure toluene continuously and exceeding the threshold value has the potential to cause health problems for workers in the home shoe industry.

Exposure to toluene, which is one of the free radicals, can cause an increase in oxidative stress which can cause damage to cells or tissues and can be characterized by the amount of malondialdehyde concentration in the human body[15]. According another research asserted that toluene exposure can increase oxidative stress parameters and cause a significant decrease in antioxidant enzyme activity[20]. In the present study there was no significant relationship between safe concentrations of toluene and malondialdehyde (MDA) with a value of p> 0.05. Tambunan (2014) found that there was a significant effect of changes in MDA concentration in the body due to increased toluene exposure[8]. In this study the relationship between safe concentrations of toluene and malondialdehyde levels showed a positive direction i.e the increase in toluene concentrations also increased malondialdehyde level. According to the results of Noerhalimah's research, the level of malondialdehyde in the group exposed to car paint workshops were higher compared to those not exposed[18]. The results of Kim's research, showed that VOC exposure, especially toluene, could induce oxidative stress[14]. In addition, the study showed a significant change in the increase in malondialdehyde levels in the exposed group.

Glutathione (GSH) as an antioxidant is needed to reduce free radicals. The correlation test results showed that there was no significant relationship between the safe concentration of toluene and the concentration of glutathione (GSH) and the value (p> 0.05). However, the relationship test is negative, which means that when the concentration of safe (C safe) toluene increases, the concentration of glutathione (GSH) decreases. This study is in line with a study of Safyudin and Subandrate (2015) which showed a decrease in the concentration of glutathione (GSH) in workers exposed to free radicals[12]. Other studies suggested that the reduction in glutathione in some tissues was caused by the effects of toluene exposure[19]. The results of research by Ahmadizadeh et al (2014) there was a significant difference in the reduction in glutathione level between the groups exposed to toluene and those that are not. Therefore, the observations show that toluene can reduce glutathione levels in the kidneys which supports oxidative stress depending on exposure toxicity.

Conclusion

In the present study there was no significant relationship between the safe concentration of toluene and the concentration of malondialdehyde and glutathione in

Osowilangun shoe home industry workers. There were 66,7% measurement points with toluene concentration exceeding the threshold value (TLV) of 20 ppm and there were 33,3% measurement points with toluene concentration below TLV.

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Causes of Death Mother and Efforts to Reduce Mother Death Numbers in Luwu District, South Sulawesi Province

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Abstract

This study aims to analyze the indirect causes of maternal death. This type of research is qualitative with a case study approach. Data collection was carried out by document search, community visits, and interviews. The results showed that the total number of maternal deaths in Luwu district totaled 19 were spread across 13 sub-districts, and maternal deaths generally occurred during labor and the puerperium. The indirect causes of maternal death can be seen from the close and far determinants. The close determinants were reviewed from three too late and four too, infectious diseases, far more determinant nutritional status in terms of distance, and the presence of obstetricians in obstetric emergencies. Efforts to reduce maternal mortality are the main focus on the prevention of complications, immediately make referrals when maternal conditions are still optimal. Empowering mothers and families.

Keywords: *indirect causes, maternal mortality rate, close determinant, far determinant*

Introduction

The World Health Organization defines maternal deaths as deaths during pregnancy, childbirth and childbirth or in the period 42 days after the end of pregnancy, due to all causes related to pregnancy or treatment and not due to injury or accident[1]. Every day, there are around 830 women in the world who die from complications or illnesses due to pregnancy and childbirth. 94% of these deaths occur in developing countries and young women who are pregnant at the age of 15-19 years have a higher risk of complications and death during pregnancy, and childbirth than other fertile ages. In 2017 there were around 295,000 women who died during pregnancy and childbirth[2]

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Global maternal mortality is caused by complications during pregnancy and childbirth, one of the factors causing maternal death is inadequate antenatal management, and the lack of public access to health workers. For example, midwives or doctors and chronic diseases that have been suffered by mothers before pregnancy, one of which is Kidney disease, cancer, heart disease, tuberculosis, and asthma, therefore it takes a big role from the hospital to help accelerate the reduction in maternal mortality[2]

Based on data from the Indonesian Demographic and Health Survey (SDKI) in 2012, the maternal mortality rate in Indonesia is still high at 359 per 100,000 live births. This figure is slightly down when compared with the 1999 IDHS figure of 390 per 100,000 live births, the decrease is not too significant. In the era of the Millennium Development Goals (MDGs) which is the 5th target by reducing the Maternal Mortality Rate (MMR) to 102 per 100,000 live births, achieving the 5th MDGs target at that time was off track, meaning that hard and hard work was needed to achieve it.

South Sulawesi Province is one of the areas of distribution of maternal mortality in Indonesia. Based on data from the health profile of the province of South Sulawesi, from several number of regencies in South Sulawesi, Luwu Regency is one of the contributors to maternal mortality. Data three years in a row in 2016 to 2018, Luwu regency ranks first as a contributor to maternal mortality (2016), Luwu regency again contributes to maternal mortality and ranks fifth (2017) while in 2018 Luwu regency contributes to maternal mortality in the sequence fourth after Enrekang district. Based on the condition, the purpose of this study is an analysis related to indirect causes of maternal death to reduce maternal mortality in Luwu district.

Material and Methods

This research was qualitative with a case study approach. The study was conducted in the district of Luwu, South Sulawesi Province. Source of research information on Verbal Maternal Autopsy (OVM) documents and other supporting documents available in midwifery services in Luwu district. The selection of participants at the community level numbered eleven people who were selected using the purposive sampling method by including the representation of the subjects.

The data collection method was carried out by tracking OVM documents and other supporting documents to analyze the direct cause of maternal death. The collection was also done through observation and in-depth interviews. Community visits to the family of the deceased were carried out to obtain information related to indirect causes of maternal death. The analysis technique in this research was content analysis to get a picture of the research object.

Findings

Indirect Causes of Maternal Death

Indirect causes are evaluated from the close determinant and the remote determinant. Close determinants in terms of three late (late decision, late referral, and too late to get service), four too (too young <20 years, too old> 35 years, too many children and too close the distance of labor), nutritional status and maternal disease. The following indirect causes of maternal death can be seen in the following table.

Table 1. Indirect Causes of Maternal Mortality

Code	Mother's age	Number of maternal deaths									
		Direct Causes of Maternal Death				Indirect Causes of Maternal Death					
		Bleeding	Preeclampsia / Eclampsia	Obstructed labor	Infection	Three Too Late	Four too	Chronic Infection	Nutritional Status (LILA <23.5 cm)	Mileage	Existence of Officers (Expert Doctors & Midwives)
S	30	0		1		1				1	1
SK	23	1				1					1
IL	20		1								
M	35	1									
H	38			1		1	1			1	
HT	38	1				1	1			1	
DS	31				1	1					
F	35	1								1	
HS	35			1			1		1		
R	38	1					1				
Total		5	1	3	1	5	4		1	4	2

The indirect causes of maternal deaths in Luwu district generally caused by a factor of three being late, factor four too and, distance traveled. The delay factor related to the distance of the mother who will deliver to the health service facility, the family delay recognizes the danger sign so it is too late to decide that the mother is late being taken to the health service facility.

Table 2. Indirect Causes of Postpartum Mortality

Code	Mother's age	Number of Postpartum Mothers Died								
		Direct Causes of Maternal Death			Indirect Causes of Maternal Death					
		Bleeding	Preeclampsia / Eclampsia	Infection	Three Too Late	Four too	Chronic Infection	Nutritional Status (LILA <23.5 cm)	Mileage	Existence of Officers (Expert Doctors & Midwives)
R	34			1			1			
S	40	1				1			1	
HD	39					1				
SR	30	1		1						
SL	25		1		1		1		1	1
FT	24		1				1	1		
SM	34			1						
Y	18			1	1	1			1	
E	29			1			1			
Total		2	2	5	2	3	4	1	3	1

In most case, the indirect causes of maternal deaths during childbirth caused by infectious diseases. In general, the health of pregnant women will be affected by physical condition both before pregnancy and during pregnancy. Infectious diseases suffered by pregnant women will affect the physical endurance and endurance of the mother's body, where mothers who have them will be more likely to experience complications, especially during labor and the puerperium.

Factor three is late, the results of an interview with the family of the deceased that the mother is late in getting health care caused by the delay in reaching the referral facility. This delay caused by the location of the house and the referral facilities that are far away, the distance between the Shiva hospital and Sawerigading hospital if taken by four-wheeled transportation then the trip time is ± 4 hours. The following is the delivery of informants in the southern district of Larompong.

' ... Mother experienced severe pain she said the pain was immeasurable, her husband's family brought to the Shiva hospital and there at most around six in the morning entered the Shiva hospital and around five o'clock in the afternoon just referred to the palopo, arrived at palopa the state of the mother was finally

weak died ... "

The delay in giving birth to mothers also caused by family delays in deciding to seek help as soon as possible in a midwifery emergency, following the submission of informants from Bupon sub-district related to the slow delivery of help by health workers because of the delay in the husband's decision to seek help.

The

'' ... Still good in the afternoon I asked permission from the mother, if my illnesses were contacted, the mother felt sick at ten at night, at two in the evening I was called by the village pack because the condition was bleeding, I brought a car if I did not bring a car maybe the mother might die at the place, the shaman had fainted, the mother was bleeding profusely, the placenta had not come out ... "

The four risk factors will too affect the health and safety of the mother during pregnancy. This caused by the readiness of the reproductive organs is not yet ripe (age <20 years) and unhealthy (age> 35 years) to support the process of pregnancy, childbirth, and childbirth so that this situation will affect the physical and maternal health. The following are the results of interviews with informants in the village of North Walenrang sub-district.

'' ... Mothers aged 18 years and first pregnant, giving birth at home helped by his family, mother giving birth twelve days at home, were referred to the hospital in a state of fever and chills, very pale. At the time of birth there were a lot of children said that blood might still have placental remains ... ''

The results of the interviews are obtained from community visits to the Bupon sub-district, following the results of interviews with midwives in the villages that are successfully found.

'' ... Mother is 38 years old and is 8th pregnant, her last education is middle school. You contacted me only twice because you used to live in the city of Palopo .. ''

Factor four is also a risk factor for post-saline bleeding, but it is also a risk factor for parturition to occur. This is due to the physical condition of the mother which makes it impossible to prepare for pregnancy. The results of the interview in the Bua.

'' ... Signs, the term is water mixed with blood, it rains heavily, at night I see the situation so around dawn at three o'clock I call my neighbor to help bring it down from the mountain , not long after arriving at the puskesmas bua not have the chance to give birth have died and the child has not yet been born .. "

Nutritional status indicates that the mother's intake during pregnancy is less than the required amount and if this lasts longer it will affect the physical endurance of the mother in carrying out healthy, safe, and safe pregnancies and deliveries.

'' ... Pregnant woman KEK, LILA 22 cm, can get extra biscuits but not regularly eaten so there is no change, the scales are lacking and her mother is also lazy to eat ... ''

The infectious diseases suffered by mothers before pregnancy one of which is tuberculosis. This situation will be a risk factor for pregnant women's physical and psychological fatigue. The results of interviews with the deceased's family in the district of Lamasi, follows his statement.

'' ... I went for six months' treatment, she had difficulty taking medication ... ''

An overview of the results of interviews and observations in the field of, North Walenrang sub-district. Siteba Village is a village in the mountainous region, not all paved roads are only a small part of which has been paved and most of them are still rocky, there is only one supporting puskesmas facility, the distance between the houses is about ± 7-8 km and the distance to the puskesmas ± 2 hours in the road is not slippery.

... That's why I rebuked her family if there was a mother who was going to give birth, why wasn't I called if I told you I had refused when called out I didn't say day and night, that was again if it could be reached by the motorbike but I still walked back to her house because can not enter the motorbike and traveled on foot for about one to two hours ... "

The next factor is the presence of an expert doctor during a midwifery emergency, this happens to the

family of the deceased at the regional general hospital when the mother arrives at the hospital but the medical staff namely the obstetrician happens to be absent if in the afternoon or evening then the mother is served by a midwife and general practitioners. The following are the results of the interview with the family of the deceased in the Lebani village of Belopa Utara district.

'' ... His child was born at five in the afternoon, his mother was healthy ji, had an IMD a few hours later the pale mother turned out to be bleeding, there was still a placenta left, there was no obstetrician at the clinic, whose mother was a general practitioner ... ''

Discussion

Indirect Causes of Maternal Death

The results of observations and community visit in 11 districts as areas of the distribution of deaths, that the indirect cause of maternal death in Luwu district is caused by several factors. The mother's delay in getting medical treatment quickly and precisely is caused by the distance to the referral facility for follow-up. Geographical conditions in several villages in the Bua, Bupon, South Larompong, West Suli, Bastem and North Walenrang districts where infrastructure, which is road facilities that are still rocky and muddy, especially in the rainy season does not allow mothers to be referred quickly and precisely, especially in emergencies situations. These results are in line with research in Singorojo sub-district Kendal district, to be able to access community referral facilities in Singorojo sub-district must travel more than 33 km, Singorojo sub-district Kendal district each year has not been able to significantly reduce maternal mortality due to frequent delays in families and officials achieve referral facilities in obstetric emergencies[4]

Another factor as an indirect cause of maternal death is an infectious disease suffered by the mother before pregnancy and continues at the time the mother is pregnant, this condition affects the physical endurance and health of the mother. Field observations of maternal deaths in several villages in Larompong Subdistrict, West Suli, Lamasi, and East Lamasi had a history of infectious diseases. These results are in line with research conducted in Pati district that mothers who have a history of infectious diseases will increase the risk of maternal death by 27.74 times (p-value = 0.0011, 95% CI)[5]

Other risk factors are four too namely maternal age <18 years, this occurs in the northern Walenrang sub-district. Pregnancy aged <20 years increases the risk of complications because the maturity of the reproductive organs is not perfect so it is not ready to support pregnancy and psychologically the mother is not ready to become a parent. Age> 38 years will also affect the health of pregnant women, this is due to the decreased ability of the reproductive organs to refute pregnancy. Risks that can occur if pregnant women <20 years old or> 35 years old are complications of bleeding, parturition, and congestion. The results of the community visit found that maternal death occurred in several districts including Bua, Bupon, South Larompong, and Walenrang. These results are in line with research conducted at Arifin Achmad Regional Hospital in Pekanbaru, found that there is a significant relationship between age <20 years and> 35 years during pregnancy can increase the risk of parturition to occur, ultimately increasing the risk of bleeding and infection[6]

Conlussion

Maternal mortality by indirect causes, namely close determination includes three late and four risk factors too, maternal nutritional and a history of chronic infectious diseases suffered before pregnancy, distant determinants seen from distance, obstetricians not forever in the hospital.

Empowering mothers and families in recognizing danger signs in labor and childbirth. For pregnant women who have high-risk factors and are domiciled in access areas/distances away from health care facilities/ referral facilities, pregnant women should be pursued for example by procuring waiting houses that are pursued together with the community or entrusting pregnant women to families close to these facilities. Make a commitment with an obstetrician to be willing to live in the Luwu district.

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The Effect of Proactive Personality, Professionalism, and Work Engagement on Performance among Village Midwives in Neonatal Complication Management Using the Integrated Management of Childhood Illness

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Abstract

Approximately 15% of newborns grow with neonatal complications. The percentage of neonatal complications in Probolinggo from 2015 to 2017 has not reached the target of 80%. The number of neonatal complications still achieved 68.68% of the minimum service standards target.

This study analyzed the effect of proactive personality, professionalism, and work engagement on performance among village midwives in managing neonatal complications by using an integrated strategy management of childhood illness. Proportional random sampling was in use to collect 180 village midwives as the sample. The research was conducted in Probolinggo Primary Healthcare Center, Indonesia from March to May 2018. The data collected through questionnaires were analyzed with a linear regression.

The results showed proactive personality influenced work engagement (p = 0.00). The higher the village midwives' proactive personality is, the higher the level of work engagement is. Besides, professionalism influenced performance (p = 0.01). The higher the level of professionalism is, the higher the level of performance is. This study also revealed work engagement influenced performance (p = 0.00). The higher the level of work engagement is, the higher the level of performance is. However, proactive personality did not affect performance.

***Keywords**: neonatal complications, integrated management of childhood illness, proactive personality, professionalism, work engagement*

Introduction

Reducing infant mortality rate becomes the top priority of the Indonesian Ministry of Health to achieve the Sustainable Development Goals (SDGs). Indonesia is targeting a decrease in infant mortality rate in 2030 by 12 deaths per 1000 live births. The infant mortalities in Indonesia were mostly caused by low birth weight, respiratory disorders, sepsis, hypothermia, jaundice, and congenital abnormalities. It was estimated that about 15% of newborns experience neonatal complications[1].

Neonatal complications occur to babies aged 0-28 days and exposed to diseases or disorders that can cause morbidity, disability or death. Newborns who are at a high risk of neonatal complications are treated according to the standards by trained health workers in all health service facilities[1].

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East Java is a province in Indonesia which has infant mortality rates that do not reach national targets until 2017[2]. In 2017, the number of infant mortalities in East Java Province was 23.1 per 1000 live births[2]. Probolinggo Regency had the highest number of infant mortality rate in East Java in 2016[3]. The trend of infant mortality from 2016 to 2018 in Probolinggo Regency fluctuated Although the cumulative increase in 2018 was 242 deaths. Infant mortalities in Probolinggo Regency in 2018 occurred due to direct causes, such as low birth weight (30%), asphyxia (9%), congenital abnormalities (32%), pneumonia (7.5%), diarrhea (3.3%) infections (14.5%)) and others (4.5%)[4]. While some infant mortalities happend because of indirect causes, such as delays in decision making, referencing and treatment[4]. Furthermore, neonatal complications in Probolinggo from the period of 2015-2017 have not reached the target of 80%. Only 68.68% of the complications have met the minimum service standards[5].

One approach to individual performance patterns is proactive personality[6]. Proactive personality is the tendency of individual attitudes to identify opportunities, initiate and take responsibility for carrying out tasks and never give up to achieve meaningful changes for the environment[7]. One of the job performance indicators is determined by professionalism. According to the American Board of Internal Medicine, professionalism is an attitude and behavior of prioritizing patients' interests over personal interests[8]. While, work engagement is an individual positive perception of a job with enthusiasm, dedication, and absorption[9]. Thus, this study analyzed the effect of proactive personality, professionalism, and work engagement on performance among village midwives in managing neonatal complications by using an integrated strategy management of childhood illness.

Method

This study was an observational analytic study with a cross-sectional design, conducted from March to May 2018 in Probolinggo Primary Healthcare Center. The sample involved 180 village midwives who were selected using proportional stratified random sampling. The data were obtained by disseminating the validated questionnaires and using observation sheets. The analysis was done with linear regression tests. One of the requirements in the multiple linear regression was passing the classical assumption test. The classical assumption test to the independent variables was conducted to identify multicollinearity. The multicollinearity test showed that the error standard value of the independent variables was less than one (<1.0). It suggested that the standard error value was low, and the multicollinearity was not detected. This means that the data were distributed normally.

Results

Based on data collected, the majority of respondents are 25-35 years old (58%) with a high-school diploma (75,6%). Most of them are civil servant and have work for 5-15 years old (45,6%). The majority of village midwives live in villages where they work (62,2%). Nevertheless, there are still village midwives who live far from the villages where their work (47,5%).

The level of village midwives' proactive personality was the tendency to what extent village midwives manage neonatal complications according to the integrated strategy management of childhood illness. This strategy includes identifying opportunities, showing initiative, taking action and preservation until meaningful changes occur[7].

Table 1. Level of Village Midwives' proactive personality in Managing Neonatal Complications based on the Integrated Management of Childhood Illness in Probolinggo Regency in 2019.

No	Proactive Personality Level	n	%
1.	Low	0	0.0
2.	Moderate	167	92.8
3.	High	13	7.2
	Total	180	100.0

Table 1 showed that the level of village midwives' proactive personality was sufficient (95%). It indicated that the village midwives tend to be proactive in using the integrated strategy management of childhood illness to manage neonatal complications in Probolinggo. However, their proactive personality level needs to be improved.

Prioritizing patients' interests over personal interests was related to professionalism. The aspects of professionalism include altruism, accountability, duty, honor, integrity, excellence and respect to others[8].

Table 2. Level of Village Midwives' Professionalism in Handling Neonatal Complications in Probolinggo Regency in 2019.

No.	Professionalism Level	n	%
1.	Low	0	0.0
2.	Moderate	134	74.4
3.	High	46	25.6
	Total	180	100.0

Table 2 showed that the majority of village midwives (74.4%) in Probolinggo Regency had a sufficient level of professionalism. It means they had sufficient attitudes and behaviors of prioritizing patient interests over personal interests to foster the quality of health workforce.

Being psychologically committed to tasks and roles in handling neonatal complications was often regarded as work engagement. This variable includes vigor, dedication, and absorption[9].

Table. 3. Level of Village Midwives' Work Engagement in Handling Neonatal Complications Using Integrated Management of Childhood Illness in Probolinggo Regency in 2019.

No.	Work Engagement Level	n	%
1.	Low	0	0.0
2.	Moderate	157	87.2
3.	High	23	12.8
	Total	180	100.0

Most of the midwives (87.2%) showed moderate work engagement. It indicated that they were psychologically committed to tasks and roles in handling neonatal complications based on the integrated strategy management of childhood illness. Later, the work engagement level needs to be increased. The success of neonatal complication management could be seen from the midwives' performance as seen in table 4.

Table 4. Level of Village Midwives' Performance in Handling Neonatal Complications based on the Integrated Management of Childhood Illness in Probolinggo Regency in 2019.

No.	Performance Level	n	%
1.	Not achieved	140	77.8
2.	Achieved	40	22.2
	Total	180	100

Based on table 4, the most respondents have not reached the performance target yet (77.8%). The aspect of village widwives' performance includes early detection of neonates, physical examination, identification of symptoms, classification of complication rates, treatment according to complications, pre referral action, counseling, recording and reporting the IMCI form, solution problem, and follow up service[10,11]. All aspect are measured by examining the availability and completeness of Integrated Management of Childhood Illness (IMCI) form.

Tabel 5. The Linear Regression Test on Proactive personality, Professionalism, and Work Engagement towards Village Midwives' Work Performance in Probolinggo Regency.

Independent Variables	Dependent Variables	p-value	B	Significance
Proactive personality	Work Engagement	0.00	0.46	Significant
Proactive personality	Performance	0.08	0.38	Not significant
Professionalism	Performance	0.01	0.42	Significant
Work Engagement	Performance	0.00	0.96	Significant

Table 5 showed the result of the linear regression indicated that proactive personality affected work engagement (p-value < 0,05). While proactive personality indirectly affected performance (p-value > 0,05). Professionalism had a direct effect on midwives' performance in managing neonatal complications based on the integrated management of childhood illness (p-value < 0,05). Compared to others, work engagement had the strongest effect on midwives' performance (p – value < 0,05, b=0.967). It means that the higher the level of work engagement is, the higher the performance of neonatal complication management is.

Work engagement had a relationship with performance in handling neonatal complications using the integrated management of childhood illness (p-value < 0,05). Probolinggo Regency are dominated by mountains, lowlands and coastal areas. It causes there are village midwives did not live in villages where they work. When the community want to access childbirth, baby check-up and other treatments, they travel far to the midwife's home. This fact was not in accordance with the Regulation of Indonesian Ministry of Health Number 28 of 2017 which explains that a midwife with a practice licence in a primary healthcare center lives and is assigned to carry out midwifery practices in the working area of the primary healthcare center[12].

Discussion

Personality can influence the attachment to someone's work[13]. Proactive personality is a relatively stable personality to not be restrained by situations and to influence changes in the environment. Proactive personality is attributed to abilities of identifying opportunities, showing initiative, taking action and preservation[7]. The results showed that some village

midwives unyielded their work and took initiatives to find new ways of improving performance in handling neonatal complications based on the integrated strategy management of childhood illness.

Proactive individuals tend to have opportunities to cope obstacles they face and make their working environment according to what they need[14]. Individual characteristics such as being proactive can encourage someone to develop themselves more than others. Employees who feel connected to the organizations will more contribute, initiate and develop change[15]. Employee proactive personality was positively related to work engagement[16,17]. The finding within this study showed that proactive personality had an influence on work engagement with a significance value of less than α (0.05). The higher the midwives' proactive personality is, the higher their work engagement is.

However, proactive personality had no effect on performance in managing neonatal complications based on the integrated strategy management of childhood illness. Researchers found the majority of midwives rarely made a specific schedule to visit mothers who experienced neonatal complications. Some village midwives revealed that they often made direct referrals to primary healthcare centers or hospitals without prior physical examination of severe neonatal complications. The village midwives were afraid of infant mortalities during the labor process in their practice station, and thus they would get penalty from the Probolinggo District Health Office. As a result, the village midwives were less optimal in carrying out childhood illness procedures, such as physical examinations, classifications and simple treatments to stabilize neonatal conditions before being referred to primary healthcare centers or hospitals.

This study also highlighted proactive personality could affect performance through work engagement as an intervening variable. It supports previous research that there is an effect of proactive personality on employee task performance through work engagement[18]. Moreover, the research by Crant and Bateman supported this finding[19]. They found individuals with high proactive personality engaged and took initiatives to get involved in various activities and situations[19]. The higher individuals' proactive personality is, the more they tend to show initiative in their work.

ABIM defined professionalism as an attitude and behavior of prioritizing patient interests over personal interests[8]. Professionalism could be seen in staffs' commitment to follow medical practice standards, prioritize patients' interests and safety, and respond to public health needs. Studies on professionalism among health workers are important to enhance professional behavior. Professionalism is a concept of collaboration and communication health workers perform to provide the best possible services[20].

To strengthen newborn services, the village midwives had to do home visit for mothers who did not return to health services. During home visits, health workers can identify and provide counseling about risk factors for infants and their environment[21]. However, in fact the village midwives are still difficult to conduct home visits due to lack of awareness of healthcare outreach. This finding supported the previous study conducted by Radina and Damayanti who found that health workers rarely do healthcare outreach because there is no obligation for primary healthcare centers[22].

Some village midwives in Probolinggo Regency oversaw to record and report neonatal complications to the Probolinggo District Health Office due to incomplete logistical needs. According to Cawley and Danielsen, professional health workers should be fair and honest as the community expect so[23]. Work engagement is not just an attitude like organizational commitment but the level of attention and fusion with work[24]. Work engagement refers to the relationship between employees and their work, while employees' engagement subjects to the relationship between employees and the organization[25]. Work engagement is a positive state, fulfillment, and a view of working conditions as shown in vigor, dedication and absorption[9].

Work engagement could affect employees' work quality, increase job satisfaction, reduce employee absenteeism and turnover[24]. Furthermore, work engagement was positively related to work performance[26,27,28]. This study showed that the majority of village midwives had high work engagement. The higher their attachment to their work is, the greater their effort, time, energy and costs are to achieve the organization's targets. In managing neonatal complications, the village midwives are required to

provide high-quality and comprehensive care for healthy newborns for up to 1 month according to their competence. In this case, work engagement affected work performance in handling neonatal complications by following the integrated strategy management of childhood illness.

Conclusion

Proactive personality did not have a direct effect on performance, but it affected performance through work engagement. While professionalism and work engagement affected village midwives' performance in handling neonatal complications based on the integrated strategy management of childhood illness. It is recommended that improving the work facilities, such as medicines and medical devices, office stationery needs to be done. Recording and reporting neonatal complications as well as midwives' knowlwedge could be improved by applying the integrated management of childhood illness.

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Causative Factors of Increasing Prescription Drug Abuse based on Transcultural Nursing Theory

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Abstract

Prescription drugs abuse in society is becoming a serious problem in the world. This case is caused by a variety of factors both internal and external, especially cultural factors. Various efforts have been done to reduce this problem but have not showed optimal results. This research was conducted to analyze the factors related to drug abuse in the society based on transcultural nursing theory. This research was conducted in two villages in the South Kalimantan Province of Indonesia, using a correlational research design with a cross-sectional approach. A total of 125 respondents suspected of abusing prescription drugs, were examined using a questionnaire. The sample selection using simple random sampling technique. Prescription drug abuse behavior was measured using a screening test CAGE-AID. The collected data was tested using a logistic regression test. Religious and philosophical factors (p = 0.035); kinship and social factors (p = 0.038); cultural and lifestyle factors (p = 0.038); political and legal factors (p = 0.031); and economic factors (p = 0.015) have a significant relationship with the behavior of drug abuse in the society. Meanwhile, technological factors (p = 0.092) and education (0.062) have no relationship with these behaviors. Easy access to drugs and the acceptance of this problem in the society have led to the higher cases of prescription drugs abuse. Counseling related to the use of prescription drugs as well as an increase in rehabilitation and religious programs need to be improved to prevent further abuse.

Keywords: *Drug Abuse, Prescription Drug, Transcultural Nursing, Factors Analysis*

Introduction

The use of prescription drugs for non-medical purposes is a serious problem that is developing in the world[1,2]. Prescription drugs have harmful effects on the body such as poison, exacerbating the disease until damage organs and cause death[3]. This phenomenon of drug abuse has turned into a plague[4].

The acceptance of the prescription drugs circulation in the environment and the lack of public knowledge lead to increasingly widespread acts of prescription drug abuse[4]. There are 230 million (5%) of the world's population aged around 15-64 years of abusing drugs once a year [5]. In 2016, around 275 million of world's population at least misappropriate prescription drugs once in their lives[6]. More than 50 million people or about 20% of the world's population aged over 12 years use prescription drugs in the wrong way[2]. This cases concomitant enhance the burden of disease globally because it is potentially as a cause of death in 1 among 100 cases[5]. Misappropriate victims can be old or young and various groups[7].

Prescription drugs have a stronger effect for users and are difficult to identify and detect[8]. The CDC (Centers for Disease Control and Prevention) report shows that most prescription drug users get drugs from their friends or family[9].

The role of peers and the environment is a factor that determines the abuse of addictive substances and illegal drugs[10]. Society requires the involvement of various

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parties such as providers of medicines, prescribers, and also government to overcome this problem[11]. Society empowerment activities in the form of counseling and skills training have not shown significant results in efforts to decrease drug abuse[12].

The use of transcultural nursing design is needed to analyze healthy or sick behavior according to the cultural background factors of a person or group[13]. Prescription drug abuse behavior is adapted to the Theory of Planned Behavior in an effort to understand individual limitations to perform certain behaviors[14,15]. Therefore, this research was conducted to analyze the factors related to prescription drug abuse in the society based on the theory of transcultural nursing.

Method

Research Design and Sample Selection

This research used a correlational research design with a cross-sectional approach. The population used was 160 people from two villages in South Kalimantan aged over 14 years. The criterion of the research sample was the citizen allegedly taking prescription drugs. Sample selection used simple random sampling technique.

The calculation results of the sample obtained 114 people as research samples. Furthermore, the number of samples added by 10% of the sample calculation results so that the total numbers samples was 125 people. The research was conducted on August to December 2017.

Research variable

The influencing factors are based on the transcultural nursing theory, including: technological factors, religious and philosophical factors, kinship and social factors, cultural values and lifestyle factors, political and legal factors, economic factors, and educational factors[13]. These factors then become independent variables, meanwhile, the dependent variable was prescription drug abuse behavior.

Research Instruments

The instrument used in this research was a questionnaire, regarding factors related to the prescription drugs abuse based on transcultural nursing theory. The questionnaire has been tested for validity and reliability. In this research, 8 questionnaires related to transcultural nursing (Gero modification)[16] and 1of screening test CAGE-AID[17] questionnaire were used as instruments.

The variable of prescription drug abuse behavior was developed from the screening test CAGE-AID[17]. This variable was developed according to Theory of Planned Behavior (TPB)[14]. The questionnaire consisted of 4 statements covering the desire to reduce drug use (cut down), feeling annoyed with other people's advice, feeling guilty, and the feeling of when you first open your eyes in the morning (eye opener).

Data Analysis

The collected data was analyzed descriptively and then an inference analysis was carried out using the chi-square test with a significance below 0.25. This research was analytic and has more than 2 independent variables so it used a statistical logistic regression test.

Result

Respondent Demographic Data

The majority of prescription drug abusers were male (95.2%). The age range of most abusers was 26-45 years (49.6%) and the lowest was 46-65 years (15.2%). All the total respondents are Moslem and come from the Banjar tribe (100%). Most respondents (86.4%) have incomes of less than IDR 2,258,000 with the most occupation as private employees (70.4%).

More than half of the respondents took 3-5 tablets of prescription drugs at one time (see Table 1). Most respondents (51.2%) got the prescription drugs from buying transactions with dealers. This research found that respondents sometimes mix prescription drugs with other drugs or substances before or when consumed (93.5%). The mixing of prescription drugs was mostly combined with other prescription drugs (55.2%). This misappropriation caused health problems more than 60% of respondents (63.2%).

Table 1. Respondent Demographic Data.

	Category	Frequency	Precentage (%)
General Data			
Gender	Male Female	119 6	95.2 4.8
Age	12-25 years 25-45 years 46-65 years	44 62 19	35.2 49.6 15.2
Religion	Moslem	125	100
Tribe	Banjar	125	100
Income	<Rp 2.258.000 >Rp 2.258.000	108 17	86.4 13.6
Occupation	Private Student Enterpreneur	88 4 33	70.4 3.2 26.4
Variable			
The Duration of Use	More than one month	125	100
The Numbers of Drugs in One Consumption	3-5 tablet 6-10 tablet >10 tablet	70 51 4	56 40.8 3.2
How to get the Drugs	Buy to the Dealers Buy in the Drugs Store Given by friends	64 17 44	51.2 13.6 35.2
Mixing Drugs	No Sometime	8 117	6.4 93.6
Drugs Mixture	Not Mixed Other Prescription Drugs Free Drugs/Free Unlimited	8 69 48	6.4 55.2 38.4
Risk to the Health	Medium Risk Heavy Risk	46 79	36.8 63.2

Factors Distribution Related to the Prescription Drug Abuse

The factor of technology use was included in the high category (61.6%) (see table 2). High values were also found in kinship and social influences factors (51.2%). However, the religious and philosophical factors (68%) also political and legal (60.8%) were in the weak category. In addition, cultural and lifestyle factors were included in the sufficient category (83.1%) and economic factors were included in this category as well (66.4%). 81.6% of respondents have the latest education, namely basic education.

Table 2. Factors Distribution Related to the Prescription drug Abuse.

Variable	Category	Frequency	Precentage(%)
Technological Factors	Less Enough High	0 48 77	0 38.4 61.6
Religious and Philosophical Factors	Less Enough High	85 40 0	68 32 0
Kinship and Social Factors	Less Enough High	0 61 64	0 48.8 51.2
Cultural and Lifestyle Factors	Less Enough High	0 104 21	0 83.2 16.8
Political and Legal Factors	Weak Enough Strong	76 49 0	60.8 39.2 0
Economic Factors	Less Enough High	0 83 42	0 66.4 42
Educational factors	Basic Middle High	102 17 6	81.6 13.6 4.8

Factors Analysis Related to the Prescription Drug Abuse

The technological factor showed the value of p = 0.092 which means that this factor has no relationship with drug abuse. The test results were similar to educational factors, where the value of p = 0.062. However, the test results found that prescription drugs abuse was influenced by religious and philosophical factors (p = 0.035); kinship and social factors (p = 0.038); cultural and lifestyle factors (p = 0.038); political and legal factors (p = 0.031); and economic factors (p = 0.015) (see table 3).

Table 3. Factors Related to the Prescription Drug Abuse.

Variable	Category	Prescription Drug Abuse		*p*
		Medium Risk	Heavy Risk	
Technological Factors	Less Enough High	0 38 8	0 10 69	0.092
Religious and Philosophical Factors	Less Enough High	12 34 0	73 6 0	0.035
Kinship and Social Factors	Less Enough High	0 42 4	0 19 60	0.038
Cultural and Lifestyle Factors	Less Cukup Tinggi	0 43 3	0 61 18	0.038
Political and Legal Factors	Weak Cukup Strong	6 40 0	70 9 0	0.031
Economic Factors	Less Enough High	0 41 5	0 42 37	0.015
Educational factors	Basic Middle High	29 12 5	73 5 1	0.062

Discussion

Drug abuse was a routine use of a substance, has been going on for 1 month, was pathological, occured behavioral irregularities, and the patient suffered quite serious and was unable to stop its use[18]. Prescription drug abuse has been proven to be carried out by most of the respondents researched. Respondent misappropriated prescription drug by consuming 3-5 tablets at a time. Prescription drug was required to be consumed according to a doctor's prescription or as many as 1 tablet once consume. In addition, respondents sometimes mix prescription drug with other types of prescription drug with ingredients such as ephedrine, sodium diclofenac, or with free drug/ free limited content especially those containing dextromethorphan and chlorpheniramine maleate.

Factor analysis of this research was based on Transcultural Nursing theory, which there are 7 factors that influence person's behavior[13]. The first factor that influences was economic factors. This research found that the higher the problem in a person's economic factors, the higher the tendency to misappropriated prescription drugs. Economics is a human effort to fulfill material life from limited resources[19]. Person's economic level was greatly influenced by the numbers of income. The findings of this research indicated the low income of respondents who were considered to be a trigger for the use of prescription drugs. The low income initiated an increase of someone's life so that it triggers the prescription drugs abuse.

In addition, prescription drug dealers made it possible to repeat their actions because of venial penalties[20]. The weaker the political and legal factors or the rule of law, the case waiver by village officials, and the weak law enforcement, the tendency to violate was higher. The legal regulations regarding the criminal penalties of prescription drug abuse was still insufficient compared to the narcotics law[20]. The weak the rules made someone not deterrent from committing drug abuse. Person's religious level also supports misappropriation behavior. The religious practices that less triggering of dishonorable behavior, besides the religious educational factors in the family and someone's observance in religion.

The tendency of misappropriation or abuse also related to someone's kinship and social relations. The existence of indifference by the family and the presence of family and friends who consume prescription drug illegally, increase this misappropriation factors. Abusers feel welcome if they do the same with their peers and family[21].

The existence of prescription drug abuse was also supported by negative socio-cultural factors from the society. Culture can be defined as non-physical traits such as values, beliefs, and habits that are shared together[22]. Bad influences of people who were considered important, create person's behavior and attitudes, so that they can misappropriate. The use of prescription drug in a group makes someone challenged to try to consume, have fun, and follow trends. Attitudes and habits such as this will gradually create a culture, especially in a group of society[22].

On the other hand, technological and educational factors were not related to prescription drug abuse behavior. The use of technology is often related to the positive and negative impacts resulted[23]. This factor has an influence on individual behavior based on culture[13]. However, the use of technology can be influenced because of someone's interest in accessing something. Easy access to information does not guarantee the support for finding information related to misappropriation behavior. In addition, the use of technology influences society's needs for the process of obtaining, storing, creating, and modifying information[24]. The process of obtaining information was also related to educational experience[19,24]. Higher education makes someone wiser to understand something and think rationally[24]. However, some respondents with higher education have the possibility to consume prescription drug illegally. This showed that the experience of higher education cannot be a guarantee for someone to do an analysis first before taking an action.

This research has obstacles such as the subjectivity of each respondent in filling out the questionnaire. Honesty of respondents needs attention because it affects filling out questionnaires. In addition, many respondents experienced obstacles in reading so it was feared that respondents would not understand the contents of the questionnaire.

Conclusion

Economic factors, political and legal factors, religious factors and kinship factors have a significant relationship with drug abuse activities. The price of prescription drugs is cheap enough to facilitate access to transactions by consumers, the majority of whom have only completed primary school. However, the high technological factors accompanied by the low educational factors, does not have a significant relationship with the prescription drug abuse.

Ethical Clearance: The research was also declared to have passed an ethical test by Medical Research Ethic Commission (KEPK) of Nursing Faculty Universitas Airlangga with number: 581-KEPK.

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The Synergistic Effect of Ascorbic Acid and Selenium on Serum Testosterone in Restraint Stressed Rats

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Abstract

The present study aimed to determine the synergistic effects of ascorbic acid and selenium on serum testosterone level in male rats exposed to restraint stress . Wistar rats weighting 175±25 grams were divided randomly into four equal groups (n=10), some were supplemented with ascorbic acid (50 mg/kg bw/day) orally, the second group was supplemented with selenium (0.02 µg/kg bw/day) orally, third group was orally supplemented with both ascorbic acid and selenium , the control included ten rats .Blood was collected on 20th days of experiment, and serum testosterone, and luteinizing hormone (LH) were measured by enzyme-linked immunosorbent assay (ELISA). The results for 20th days showed that supplement of rats with ascorbic acid has highly significantly (P<0.001) increased serum testosterone to be (5.18 ± 0.1 ng/ml compared to stressed rats which recorded (2.37 ± 0.16 ng/ml) and showed that supplement of rats with selenium has highly significantly (P<0.001) increased serum testosterone level to be (5.28 ± 0.15 ng/ml) compared to stressed rats, and showed that supplement of rats with (ascorbic acid+ Selenium) has highly significantly (P<0.001) increased serum testosterone level to be (5.78 ± 0.09 ng/ml) compared to stressed rats which recorded (2.37 ± 0.16 ng/ml). It is probable that there is a synergistic effect of ascorbic acid and selenium in relieving effect on restraint stress on male rats and enhancing testosterone levels .

Key Words— *restraint stress, testosterone, ascorbic acid, selenium*

Introduction

Stress is becoming an inescapable part of modern life, and has been dubbed the Health Epidemic of the 21st Century [(1)] . It is defined as any response that elicit physiological and behavioral adaptations to maintain homeostasis [(2)] . Events that confront the organism's environment activate central stress response system, which is mainly mediated by the (HPA) axis [(3)] ; the regulatory functions of the HPA axis control the behavior , reproduction, cardiovascular, immune functions, , and metabolic system activation of the HPA axis by several stressors mainly inhibits reproductive function [(4)] ; several studies have shown of stress along with depression and anxiety may lead to infertility [(5)] . Infertility is regarded as the public and clinical trouble because it affects the health system and social life, it is a reasonably common condition with psychological, medical, and financial consequences [(6)] . Ascorbic acid (vitamin c) has been considered as an essential nutrient for animal species, it has been linked with fertility for many years, most consider the effect of ascorbic acid on fertility to be related to these principal functions: role in hormone production, promotion of collagen synthesis, and prevention or protection against oxidation [(7)] , disrupts both spermatogenesis and the production of testosterone caused by lack of vitamin C leads to a state of oxidative stress in the testes [(8)] . Selenium is an essential micronutrient , it acts as a cofactor for enzymes antioxidant, for example glutathione peroxidase [(9)] .

Selenium importance was for successful in male reproduction due to its role in testosterone biosynthesis and subsequently in the typical development and

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formation of spermatozoa [10], thus selenium is a major factor sperm formation and male fertility , selenium deficiency has been found to be associated with reduced or impaired reproduction [11] .

Methods

Forty fertile adult's albino male rats (*Rattus norvegicus*) were used in the present study, their averages ages (10-12) weeks , with a weight range (100-200) gm, the animals were housed in the animal house, the animals housed in well ventilated wire-plastic cages with dimensions design cages (50 × 35 × 15 cm) with metal covers and containing bedding of wood shaving which was changed once per daily.

The animals were maintained under controlled environment about 12-hour light and 12-hour dark with (degree of temperature 22-26 ˚C) and exact circumstances to the normal laboratory nutrition with profitable diet (pellets) and water provided to animals except during the experimental period, for two weeks before the experiment started, to adapt rats to the new environment none of the rats had any clinically obvious contagions, the rats were divided into four groups with five rats for each cage, for animal were ethics research procedures approval by our institutional animal care and care committee (IACUC).

Rats were classified into four groups : the first group: included ten rats exposed to restraint stress for sex hours a day, and supplemented with vitamin C in a dose of (7.5 mg /day) orally by gavage ; the second group: included ten rats exposed to restraint stress for sex hours a day, and supplemented with selenium in a dose of (3 µg /day) orally by gavage ; the third group: included ten rats exposed to restraint stress for five hours, and supplemented with vitamin C in a dose of (7.5 mg/ day) , then with Selenium in a dose of (3 µg / day) orally by gavage ; the fourth group: included (five) rats exposed to restraint stress for five hours a day and given 2 ml normal saline orally by gavage and served as positive control (PC), and (five) rats without exposed to restraint stress, and given 2 ml normal saline orally by gavage and served as negative control (NC).

Rat were placed in the restraint cage used to produce restrain stress in a glass container (12×5 cm), for six-hour a day [12] , narrow enough to prevent the rats from moving freely but wide enough to cause no real physical discomfort, pain or impairment of respiratory movements, the rats were exposed to stress between 08:30 AM and 14:30 PM for twenty days of the experiment . Animals weights have been recorded before the dosage by using electrical balance Blood samples were collected via cardiac puncture. Serum was obtained by centrifugation at 3000rpm for 20min. Serum was used for hormonal assay.

Results

All animals in the study were monitored for their feed and well-being and found healthy and active throughout the study period.

The result showed a high significant decrease ($P<0.001$) in serum concentrations of testosterone at the twentieth day of experiment in positive control (stressed rat) (2.37 ± 0.16) compared to those which were not exposed to stress (3.6 ± 0.25) which constitute the negative control.

On other hand in same table was highly significant increase ($P<0.001$) in serum concentrations of testosterone level in rats supplemented with vitamin C (50 mg/kg/day), Selenium (0.02 mg/kg / day) and both vitamin C plus Selenium , to be (5.18 ± 0.1), (5.28 ± 0.15) and (5.78 ± 0.09) respectively compared to positive and negative control .

Table.1 : Differences in serum testosterone level among rat groups at the twentieth day of experiment

Study Groups	Total Number	Serum Testosterone (ng/ml) M ± SD	F test (P value)
Vitamin C (50 mg/kg orally)	10	5.18 ± 0.1 *	593.19 (0.000)
Selenium (0.2 mg/kg orally)	10	5.28 ± 0.15 *	
Vitamin C + Selenium	10	5.78 ± 0.09 *	
Negative Control (without stress)	5	3.6 ± 0.25 *	
Positive Control (with stress)	5	2.37 ± 0.16 *	

* high significant difference at P< 0.01.

Discussion

Previous studies have shown that restraint stress lowers plasma testosterone concentrations and blunts the plasma testosterone in rats [13] , and in monkeys [14] .

Stress of different origins suppresses male reproductive functions by releasing stress hormones corticosterone levels are increased in psychological and physical stress, as in experimentally induced restraint stress in rats, therefore, levels of serum corticosterone may serve as an appropriate indicator of stress in rats [15] . Stress activates sympathetic-adrenal system with release of catecholamine like epinephrine and norepinephrine along with proportionate release of increased glucocorticoid levels disrupted the male reproductive axis [16] . Epinephrine and norepinephrine are biogenic amines Its secretion from of the adrenal medulla may also affect the testis by affecting flow of blood, since these stimulants are thought to cause vasoconstriction in many mother tissues [17] . It is well-known that monoamine oxidase (MAO) convey several amines into metabolism that involve their MAO final product in testicular maturation and androgen synthesizing [18], therefore the serum testosterone levels were significantly lowered direct testosterone production inhibitor by Leydig cells, which are responsible for 95% of plasma testosterone levels in males, low testosterone levels follow a reduced regulation of LH signals, which stress directly suppress Leydig cell steroidogenesis consequently testosterone [19] .

Stress is generally thought to generate reactive oxygen species (ROS), when ROS surpass body's natural antioxidant defense, impairment to macro molecules such as DNA, lipids and proteins would occur, during stress, lipid peroxidation is increased in the body, as one of the prominent products for fat oxidation is malondialdehyde (MDA), therefore MDA is considered as an indicator of stress induced damage by lipid peroxidation [15] .

On other hand, there was an evidence that testosterone decreases may be associated activity levels of testicular steroidogenic indicator enzymes 3β-hydroxysteroid dehydrogenase 3β-HSD and 17β-hydroxysteroid dehydrogenase (17β-HSD) were reduced significantly in stress animals [20], since testicular steroidogenesis is organized by these two rate determining enzymes, a decrease in their activities in Leydig cells greatly reduces production and thus level of testosterone [21] .

Certainly, we observed the stress decreased the testosterone and fertility in the male rat by blocked the receptor of LH in the Leydig cell and Excessive oxidative stress that lowers major enzymatic and non-enzymatic antioxidant levels in Leydig cells, all of which leads to reduced testosterone secretion that conforming [22] .

Orr et al., (1994) reported a suppressive effect of immobilization stress on plasma testosterone level [23] ; while Hu et al., (2000) had studied the effect

of restraint stress on testosterone levels in rats and found that long duration of restraint stress (2 hours daily for 2 months) had significantly decreased serum testosterone concentrations with noticeable reduction in spermatogenesis [24] .

On the other hand, the present study has revealed that vitamin C has highly significantly (P<0.001) increased in serum testosterone level (table.1) which indicated the effect of vitamin C supplement in enhancing male fertility ; this come along a previous study that investigated the effect of a twice daily dose (250 mg/kg) of vitamin C on serum testosterone levels in Wistar rats, after 21 days they found increase significantly in serum testosterone concentrations (25) .

Vitamin C is well known to play key roles in testosterone synthesis [26], in extracellular fluids vitamin C is the most essential free radical scavenger that traps and maintains the roots of aqueous biomembranes from peroxidative harm and prevent increased the lipid peroxidation levels resulting from stress and reduce the complication causes of the stress such as increased oxidative stress, lipid peroxidation which is converted to dehydrascorbic acid by free radical reaction is regulated via the glutathione enzyme complex [27] . This may provide additional evidence for the anti-stress effects of vitamin C as stress is an imbalance between the production of reactive oxygen and antioxidant defense [28] .

This study showed that the level of testosterone in plasma increased significantly in animals treated with ascorbic acid When compared with animal controls. This rise could be dependent on the ascorbic acid that activates LH release.

In addition, our result in same exhibited high significant rise (P<0.001) in testosterone serum concentrations level at the twentieth days of experiment in rats with Selenium (3 µg /day) which selenium is required for normal testicular development and spermatogenesis in rats where observed the severe selenium deficient mice reduced testosterone concentrations in the blood [29] .

Previous studies found that selenium may have a positive effect on Leydig cells to increase spermatogenesis [30-31] , this result is in agreement with present result that recorded a high increase in testosterone secretion .

In the Our result showed in (vitamin C + Selenium) highly significant group increase (P<0.001) in testosterone serum concentrations for seventh and twentieth days in contrast with negative and positive Control showed in table -1 that conforming the Previous studies and protect our theses of this study show effect of vitamin C and selenium together in male rats in stress.

Ethical Clearance : Taken from University of Kufa ethical committee

Source of Funding : Self

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Comparative Test of Midazolam Hydrochloride Stability in Different Storage and Temperature Container

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Abstract

Background: Midazolam hydrochloride is an injection form of benzodiazepines and included in the high alert category in the Dr. Soetomo Teaching Hospital Intensive Care Unit (ICU), Surabaya, Indonesia. Nowadays, the technical preparation of midazolam drugs in the ICU room by doctors and nurses was performed by reconstituting drugs in the injection syringes to be stored for a while in the room. **Objectives**: To evaluate the preparation process for midazolam hydrochloride injection which has been analyzed in Dr. Soetomo Teaching Hospital. **Methods**: Physical, chemical, and microbiological stability of 1 mg/mL midazolam hydrochloride in aqua pro injection solvents have been evaluated at points 0, 8, 12, and 24 hours after preparation, as well as a comparison of stability in storage conditions at room temperature compared to refrigerator temperatures. **Results**: The results showed all the preparations were no foreign particles in the sample container. The midazolam did not experience precipitation in both room and refrigerator temperatures. The pH of midazolam solution during storage proved that the preparation was relatively stable (pH 4.3–4.5). There were no significant differences of midazolam levels in both storages which was in range of 95%. The Miccrobiology Stability Test showed negative germ growth after 24 hours incubation in both storage. **Conclusion**: This study showed no changes in physical, chemical, or microbiological stability in the midazolam injection samples up to 24 hours after the manufacturing process.

Keywords: *Midazolam Hydrochloride, Injection Preparation, Aseptic Technique, Stability Test*

Introduction

The stability of pharmaceutical dosage forms is strongly influenced by chemical, physical, and microbiological reactions. These reactions can lead the changes of medicinal properties during the process of shipping, storage, and use. Chemical degradation is known as the cause of a potential decrease or increase in drug toxicity. The storage condition also cause dissolution and pollination process of other medicinal ingredients, so that affect to the stability of the drug[1].

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Midazolam hydrochloride is an injection form of benzodiazepines with a short reaction period. Intramuscular route is often used for midazolam administration, then continuous to iv infusion and iv slow bolus[2]. Intramuscular midazolam administration can terminate acute seizure in children besides intranasal midazolam[3,4]. Previous research showed the stability of midazolam is around 24 hours in normal saline 0.9% or Dextrose 5%[5,6]. Technical midazolam preparation is usually carried out by the drug reconstitution of injection syringes preparation and drugs storage in the room. It aims to facilitate the administration in emergency condition, which needs immediate administration[7,8].

Patient safety becomes priority in the management of drug use (MDU). The policy of MDU, namely standard operational procedure for mixing injectable

medicines, explains that the single component does not recommend storing reconstitution drugs in injection syringes, but in sterile vial containers covered by film paper[9]. The reconstitution drugs do not stored for more than 8 hours[9]. However, the MDU policy on injecting drug reconstitution is not supported by accurate data from preliminary research results both qualitative and quantitative aspects.

Baseline reference for the MDU policy for the reconstruction of single preparations is existed in Dr. Soetomo Teaching Hospital. This preliminary study determined the qualitative and quantitative stability of 1 mg/mL midazolam injection in aqua pro injection solvents, whether the stability of the drug was more than 8 hours or 24 hours. The stability test compared to the parameters of the storage container will be observed from the room temperature. The room temperature was 20-25 °C, compared to the refrigerator temperature (2-8 °C). The qualitative stability measured by physical, chemical, and microbiological stability. However, quantitative stability is conducted by determining levels using High-Performance Liquid Chromatography (HPLC) tools. The results of this study can be a reference data for the guidelines evaluation for injecting drug mixing policy in Dr. Soetomo Teaching Hospital. The purpose of this study determined by using qualitative and quantitative stability of midazolam reconstitution HCl 1 mg/mL with storage container parameters.

Methods

Material

Raw midazolam hydrochloride was a gift from Kalbe Farma Factory. Acetonitrile and potassium hydrogen phosphate were Wako products (Osaka, Japan). Sterile glass vials and injection syringes (Terumo®, Tokyo, Japan) were purchased from the Medical Pharmacy Service Unit, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. Other research materials were analytical grade.

Test Solution Preparation

Preparation of the test solution sample was examined by the same possible conditions that was conducted by the nurse or doctor when reconstituting midazolam hydrochloride in the ICU inpatient ward of Dr. Soetomo Teaching Hospital. Two midazolam hydrochloride test solutions of 1 mg/mL were prepared for replication three times per test procedure. The first solution was prepared in a Terumo® injection syringe container (containing polypropylene material) and a second solution was prepared in a sterile vial storage container covered with parafilm paper. The first mixing container (5 mL injection syringe, Terumo®) was filled by midazolam hydrochloride 1 mg/mL. The concentration was obtained by mixing technically as follows: A total of 1 ampule of midazolam hydrochloride 5 mg/mL was diluted and a number of 20 mL aqua pro injection to obtain a 1 mg/mL level test solution. The solution was taken 1 mL to be stored in a 5 mL injection syringe. The second solution was prepared in a sterile vial storage container with a dilution procedure: 1 ampoule midazolam hydrochloride 5 mg/mL diluted and 20 mL aqua pro injection to obtain a 1 mg/mL level test solution. Then, 1 mL solution was stored in a sterile vial container.

This study also revealed the effect of storage temperature on the stability of midazolam hydrochloride reconstitution preparations. Three solutions test were stored at room temperature (20-25 °C) and at refrigerator temperature (3-4 °C). At the time of testing, the sample was taken by 1 mL from the storage container using aseptic techniques. The sampling point in this study was carried out at 0 o'clock (shortly after preparation), 8, 24, and 48 hours after preparation.

Preparation Stability Test

The samples were taken at 0, 8, 12, and 24 hours after preparation, then evaluated for physical, chemical, and microbiological stability. Physical stability tests was performed, including observe color's solution and it's clarity. The solution clarity was tested by placing a sample above lighting and observed by rotating the sample and flipping the container against a black or white background (Erweka Apparatus Gmens Hensen Stamn, Germany). Thus, it could clarity and the presence of foreign particles in the test sample were observed.

The sample was taken by 5 mL for physical stability checks. Microbiological stability tests were examined by the sterility requirements of intravenous preparations. Sterility tests were conducted on bacterial growth media in microbiology laboratories. Chemical stability tests was examined including pH testing and levels determination

using HPLC. PH measurement was performed because the pH can affect the preparation stability. the pH measurements of midazolam preparations after storage was conducted by using a pH meter (Eutech Instruments pH 700, Germany) at 25 ˚C.

Midazolam Hydrochloride Analysis Method Using HPLC

Midazolam hydrochloride levels were quantitatively measured using HPLC. The analysis conditions were as follows: the stationary phase uses an HPLC C18 column, the detector used was a photodiode array (PDA), and the analysis was analyzed at a wavelength of 220 nm, the mobile phase was acetonitrile: 0.3 M potassium dihydrogen phosphate pH 3.3 (3:7 v/v) and a flow rate of 1 mL/minute, and the injection sample volume was 10 µL

Standard solute was prepared by dissolving midazolam hydrochloride at a level of 1 mg/mL in methanol to be injected in the HPLC. Furthermore, it analyzed the peak area of the chromatogram. The results will be used to measure midazolam levels quantitatively by using a comparison of the chromatogram peak area of the sample with the standard 1 mg/mL level midazolam solution.

Statistic Analytic

For qualitative analysis, the results were compared to the initial solution visually. If there were differences, there was a qualitative change in stability. For quantitative analysis, the test solution resulted stable if midazolam hydrochloride levels in the test solution >90% initial levels. Statistical analysis used one-way analysis of variance followed by Tukey's post hoc test with a confidence level of 0.05 to determine the significance differences between all the results The test used SPSS version 23.0 (SPSS, Inc., Chicago, IL).

Results

Solution Clarity test

This study found that all preparations were clear and there were no deposits or foreign particles in the sample container (Table 1). During storage phase, the midazolam did not experience precipitation in both storage. However, there were substances in most vial samples such as fibers (unpublished data) observed in >50% of the test samples (±50 vials) which were not observed in the test samples with polypropylene syringes.

Table 1. The results of the clarity observation of the midazolam solution in Intensive Care Unit's inpatient ward Dr. Soetomo Teaching Hospital (n=3).

No	Sampling-Time	pH Midazolam injection			
		Spuit		Vial	
		Room temperature	Refgirator temperature	Room temperature	Refgirator temperature
1	0th hour	*	-	*	-
2	1st hour	*	*	*	*
3	8th hour	*	*	*	*
4	12th hour	*	*	*	*
5	24th hour	*	*	*	*

Note: * = there is no sedimentation

pH Test

All of samples did not experience significant pH changes, where the mean pH of the samples was around 4.3 to 4.5, at the 1st and 24th hours (Table 2). The test sample showed pH in the range of 4.4 as for the 0th hour. Thus, there was no change in the pH of the midazolam solution during storage, both room temperature and refrigerator temperature, proving that the relatively stable preparation was kept until the 24th hour.

Table 2. The results of the pH test in midazolam in the Intensive Care Unit inpatient ward of Dr. Soetomo Teaching Hospital (n=3).

No	Sampling-Time	pH Midazolam injection			
		Spuit		Vial	
		Room temperature	Refgirator temperature	Room temperature	Refgirator temperature
1	0th hour	4.41±0.05	-	4.38±0.05	-
2	1st hour	4.36±0.05	4.47±0.03	4.39±0.01	4.47±0.13
3	8th hour	4.41±0.06	4.42±0.05	4.40±0.05	4.50±0.02
4	12th hour	12th hour	4.49±0.08	4.45±0.06	4.58±0.01
5	24th hour	4.32±0.05	4.50±0.02	4.36±0.07	4.49±0.05

Microbiology Stability Test

The sterility test resulted that 6 test containers representing about 3% of the total sample and showed no microbial contamination in the vial container. In general, after incubation, the test results showed negative results, it can be declared as sterile. However, there was a sample from 3 replications showed a *Staphylococcus pseudintermedius* culture at 8 o'clock (Table 3).

Table 3. The results of the microbiological stability test of midazolam in Intensive Care Unit's inpatient ward Dr. Soetomo Teaching Hospital (n=3).

No	Sample	Storage temperature	Microbiological Test Results at the hour			
			0	1	12	24
1	Spuit	Room temperature	Negative	Negative	Negative	Negative
		Refgirator temperature	-	Negative	Negative	Negative
2	Vial	Room temperature	Negative	Negative	Negative	Negative
		Refgirator temperature	-	Negative	Negative	Negative

Stability Test for Midazolam Levels

By using a comparative analysis of the chromatogram peaks of the raw midazolam material. A quantitative level of the drug sample was obtained (Table 4).

Table 4. HPLC test results on drug levels of midazolam injection samples after 24 hours in the Intensive Care Unit's inpatient ward of Dr. Soetomo Teaching Hospital (n=3).

Sampling-Time	Midazolam Level (Persecent)			
	Spuit		Vial	
	Room temperature	Refgirator temperature	Room temperature	Refgirator temperature
24th hour	90.31±7.59	91.97±19.93	95.68±3.14	96.85±8.92

The level of midazolam at the 0 minute was lower than the data at the 24th hour, both stored at room temperature and refrigerator. After 24 hours, the levels of medicinal ingredients were still 90.31%-96.85%, both samples stored in vials and polypropylene syringes. There were no significant differences between the storage temperature in the room and the refrigerator. Moreover, midazolam concentration were in the range of 95% after 48 hours in refrigerators, either by using vial containers or injection syringes. At this point, samples at room temperature were not tested due to the technical problems. The coefficient between measurement replications was still too large (in range 3.28%-21.67%).

Discussion

This study proved that midazolam hydrochloride injection, which was prepared using aseptic technique, has good stability until the 24th hour after the manufacturing process. Both glass vial and injection syringes didn't show significant effect on drug content as well as 24-hours storage temperatures. Previous research explained that midazolam stability was influenced by pH, storage temperature, light, and storage containers[6]. This study found that the midazolam injection was still stable, both physically, chemically, and microbiologically until the 24th hour after its mixture. Recent study showed stable diluted concentration of midazolam was 0.03 mg/mL to 0.5 for 24 hours with 5% dextrose or 0.9% sodium chloride injection[10]. On the other hand, midazolam 5 mg/mL was stable at room temperature for 100 days in polypropylene syringes[11].

Treatment of samples was divided into 2 categories which used different containers, sterile glass vials and injection syringes containing polypropylene. The results showed that there was no significant difference between midazolam injection which prepared in sterile glass vial containers and injection syringes in any storage conditions. PH measurement showed unchanged results between observation times, which around 4.3-4.6 with stable physical conditions and observed the deposition of drug ingredients during storage. Moreover, more than 90% of the initial concentration of midazolam can be observed in injection samples at 24 hours after preparation, even at 48 hours in samples stored in the refrigerator. This data was in line with the previous studies which have shown that midazolam solution was stable even up to 5-weeks after manufactured and there was no midazolam adsorption process in injection syringe containers[5]. In the 0-hour sample, the results of the determination of the levels were smaller than the 24th hour which was about 81-86%. This was possible because of over filling after the dilution process of 5 mg/mL midazolam levels with aqua pro injection to a level of 1 mg/mL which results in a diluted final concentration.

In addition, sterility tests for injection samples have also been revealed. This is an important parameter for preparations of parenteral routes. The results showed that most of the samples showed sterility to germs/microbes. However, there were 1 in 3 sample replications which showed contamination by *Staphylococcus pseudintermedius* contained in the free air. This was possible because there was of working technique on a sample that less accurate due to drugs preparation.

Thus, it was necessary to limit the number of midazolam injection samples to avoid incorrect technique, so that will obtain perfect sterility. Besides, there were several contaminations, such as fine fibers in most of the glass vial container samples which were not found in the injection syringe container. This was the main evaluation in the sterile vial sterilization process.

These data were expected to be a guide or reference for the preparation process of midazolam injection in hospitals, especially Dr. Soetomo Teaching Hospital, and generally other hospitals in Indonesia with proper aseptic techniques. Further studies needs to concern about dose uniformity after the dilution process as important information for proper preparation of midazolam hydrochloride in the ward. This was an attempt to ensure dose uniformity uniformity between injection sample, which this study found that the coefficient of variation between replications was still too large, around 3-22%.

Conclusion

This study showed midazolam hydrocloride injection had good physical, chemical, and microbiological stability until the 24th hour after the manufacturing process. Vial and injection syringes did not show a significant effect on drug content in 24-hours storage temperature. However, midazolam injection was possible to be contaminated by microorganism because of less accurate technique during preparation.

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Ethical Clearance: This study has been approved ethically by the Medical Research Ethics Committee of Faculty of Medicine, Universitas Airlangga Surabaya.

Conflict of Interest: There is no conflict of interest

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Relationship of Age, Work Period, and Education Level with Occupational Accidents in the South Borneo Coal Industry

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Abstract

Research Background: Occupational health and safety (OHS) is an effort to create a working atmosphere that is safe, comfortable, and the ultimate goal is to create the highest productivity. The higher level of production in the coal industry will be in line with the dangers and risks of work accidents in the coal industry. Occupational accidents are influenced by 2 (two) direct causes namely unsafe action (unsafe action) and dangerous condition (hazardous condition) **Research Methodology**: The research design used in this study is observational analytic with the cross-sectional approach with a large sample of 80 respondents who in the South Borneo coal industry. Data analysis used the statistical test *Chi-Square* ($\alpha = 0.05$). **Result**: From the results of the bivariable significance test using the Chi-Square test showed that the significant value of age ($p = 0.604$), years of service ($p = 0.544$), level of education ($p = 0.578$) > 0.05. **Discussion**: There is no significant relationship between the variables of age, years of service, education level with work accidents in the South Borneo coal industry.

Keywords: *OHS, Age, Work Period, Education Level, Work Accident.*

Introduction

Occupational health and safety (OHS) are an effort to create a working atmosphere that is safe, comfortable, and the ultimate goal is to create the highest productivity. OHS is essential to be carried out in every type of work without exception. OHS implementation can reduce work accidents to increase work efficiency and productivity[1].

Borneo is known as one of the islands with abundant natural resources, one of which is coal. Many people use coal's natural wealth as an alternative energy source that is believed to be profitable because of its relatively cheap price[2].

The higher level of production in the coal industry will be in line with the dangers and risks of work accidents in the coal industry[3]. Work accidents are a problem for business continuity from all industrial sectors[4]. The loss suffered not only in the form of significant material losses, but more than that is the occurrence of casualties that are not small in number. This loss of human resources is enormous because humans are the only resource that cannot be replaced by any technology[5].

Accidents do not happen by accident, but there is a reason. Because there is a cause, the cause of the accident must be examined and discovered so that further with corrective actions aimed at the purpose as well as with further preventive efforts, accidents can be prevented, and similar accidents do not recur[6].

According to HW, Heinrich, in the occurrence of work accidents, is influenced by 2 (two) direct causes, namely unsafe actions (unsafe actions) and hazardous

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conditions (dangerous conditions)[7]. The perilous act is an action that does not meet safety, so it is risky to cause work accident[8]. Unsafe conditions are unsafe environmental conditions and risk of causing work accidents[9].

According to Sholihah , four moving factors in a single chain can cause work accidents, namely. In essence, environment, equipment, danger, and humans, apparently work physical environmental factors do not only influence accidents, but one of the essential elements is human[10].

The place of this research is a coal mining company located in the area of South Borneo, which is one of the companies that has implemented an excellent OSH system following applicable regulations and is supported by the commitment of top management to the OSH implementation system in the company. When running the production process, the South Borneo coal industry has a high risk of accidents due to work.

The results of work accident data in the coal mining industry in South Borneo from January 2017 - August 2019 show the prevalence of work accidents has increased every year with the number of events in 2017 totaling 27 incidents, in 2018 totaling 34 incidents, and in January 2019 until August 2019 there were 38 cases of workplace accidents. This certainly needs attention because it is directly related to occupational health and safety.

Materials and Methods

This research has a quantitative approach. Based on aspects of data collection, this type of research is observation because it only makes a comment or observation without providing intervention to the variables examined.

This research is categorized as a *cross-sectional study* because data collection from dependent and dependent variables is carried out at one time, that is when the research is in the field. The population in this study were all workers in the South Borneo coal industry company of 100 workers. Based on sample calculations using simple random sampling, the samples in this study were 80 workers in the South Borneo coal industry.

Data collection uses a questionnaire or questionnaire method that includes the relationship of age, years of service, and education level with work-related accidents.

Result

The results of the study show the characteristics of the respondents of workers in the Borneo Selatan coal industry, seen as covering age, sex, education, and years of service.

Table 1. Distribution of Age Frequency, Years of Service, Level of Education Workers in the South Borneo coal industry.

Variable	Respondents	
	Amount	Percentage (%)
Age		
Young	43	53.8
Old	37	46.2
Total	80	100%
Working Period		
≥ 5 Years	55	72.5
<5 Years	25	27.5
Total	80	100%

Cont... **Table 1. Distribution of Age Frequency, Years of Service, Level of Education Workers in the South Borneo coal industry.**

School Level		
Elementary School	4	5.0
School	9	11.2
High School	67	83.8
Total	80	100%

Analysis Relationship between age and work accident at the South Borneo coal industry.

Table 2. Analysis of Relationship between Age and Work Accident in South Borneo coal industry.

Age	Work Accident				Total		p.value
	Ever		Never				
	n	%	n	%	n	%	
Young	22	51.2	21	48.8	43	100	0.604
Old	22	59.5	15	40.5	37	100	

Bivariable test results using the Chi-Square test indicate that p-value = 0.604> 0.05, which means there is no significant relationship between age and work accident at the South Borneo coal industry.

Analysis of the Relationship between Work Period and Work Accident in Workers in the South Borneo Coal Industry.

Table 3. Analysis of Relationship between Work Period and Work Accident in Workers in the South Borneo Coal Industry.

Work Period for Work	Accident				Amount		p. value
	Ever		Never				
	n	%	n	%	n	%	
<5 Year	12	48.0	13	52.0	25	100	0.544
≥5 Year	32	58.2	23	41.8	55	100	

Test results using the test *Chi-Square* indicate that the value of p = 0.544> 0.05, which means there is no significant relationship between work period with work accidents in the South Borneo coal industry.

Analysis of the Relationship between the level of education of workers and workplace accidents in the South Borneo coal industry.

Table 4 Analysis of the Relationship between Education Level and Work Accident in Workers in the South Borneo Coal Industry.

Education Level	Work Accident				Total		p.value
	Ever		Never				
	n	%	n	%	n	%	
Elementary School	3	75.0	1	25.0	4	100	0.578
School	6	66.7	3	33.3	9	100	
High School	35	52.2	32	47.8	67	100	

Bivariable test results using the test *Chi-Square* showed that the value of p = 0.578> 0.05, which means there is no significant relationship between the level of education with work accidents in the South Borneo coal industry.

Discussion

Age relationship with workplace accidents in the South Borneo coal industry.

Based on the analysis of the relationship between age and work accidents among workers in the South Borneo coal industry shows that there is no significant relationship between age and work accidents in the South Borneo coal industry. These results are in line with research conducted by Himawan, which explains that there is no age relationship with workplace accidents where older workers tend to be vulnerable to disasters but have excellent safety performance. Younger workers are not prone to injury, but safety performance is not optimal. The age and experience of the workers themselves go hand in hand in determining the conditions and safety status for that, and the company must be careful in managing its human resources[11].

Relationship between a work period and a work accident in South Borneo coal industry.

Based on the results of analysts, the relationship between work tenure with work accidents in workers in the South Borneo coal industry shows that there is no significant relationship between work tenure and work accidents in the South Borneo coal industry. This study is in line with research conducted by Hikmawan, which is a study entitled the factors associated with workplace accidents in car painting shop workers in Makassar showed that there was no relationship between work period with work accidents[12].

According to Shintia, workers who have worked long hours in a place will slowly experience burnout because the same workers have been repeated, while burnout is one of the factors workers act at will or neglect at work, which will have a higher impact to cause work accident. Workers with more extended service periods are more likely to consider themselves trained or proficient in what they have done so often that they act as they please and do not comply with the rules in the company[13].

The working period is directly related to work experience, the longer the work period of a person, the higher the experience and flight hours of the worker, so that workers will be able to understand better how to work safely to prevent themselves from work accidents. New workers generally do not know the ins and outs of work deeply. Conversely, with the increase in the work period of a workforce, so will the knowledge and skills of workers and the safety aspects of the work have done.

Relationship between the level of education of workers and workplace accidents in the South Borneo coal industry.

Based on the results of analysts, the relationship between the level of education with work accidents on workers in the South Borneo coal industry shows that there is no significant relationship between the level of knowledge with work accidents in the South Borneo coal industry. The results of this study are in line with research conducted by Ada which states that there is no significant relationship between the level of education

of workers with work accidents at PT General Electric Lighting Indonesia. Research conducted also explained that the variable level of schooling (*p-value* > 0.05) did not find a significant relationship with the incidence of workplace accidents in the PT Antam Tbk Mining area.

In theory, the level of education should affect the intelligence or knowledge of a worker. But workers with any educational background do not mean less knowledge and experience. The still high incidence of workplace accidents occurring to workers both workers with primary, junior high, or high school education may be caused because most workers are workers with non-organic (contracted) or non-permanent work status and have not received training on Occupational health and safety (OHS)[14]

Nugroho's explanation (2018) also explains that the lack of knowledge of workers (*lack of knowledge and skills*) is one of the human factors or (*unsafe actions*) that can cause workplace accidents. But there are still many (*dangerous actions*) that may occur and not examined by researchers such as the inability to work typically (*inadequate capacity*), not functioning of the body due to bodily *defects*, attitudes and unsafe behavior *(unsafe attitude and habits*), *fatigue and boredom*, *confusion and stress due* to work procedures that are not yet understood, decreased concentration *(difficulty in concentrating*) of labor while doing work, indifference (*ignorance*) from energy, lack of spirit of work motivation (*improper motivation*), and lack of satisfaction at work (*low job satisfaction*). Also, there are still(*unsafe conditions*) that can be the cause of workplace accidents, such as the environment in which the worker works, work processes, nature of work, work systems, and work organization that does not pay attention to aspects of work safety[15].

Conclusion

Characteristics of workers in the South Borneo coal industry are found in the age category of the young group, the length of service category is more than five years, the highest level of education for workers in High School.

There is no relationship between age and work accidents in the South Borneo coal industry.

There is no relationship between work periods with work accidents in the South Borneo coal industry.

There is no relationship between education level and work accidents in the South Borneo coal industry.

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Effect of SafMannan on Local Iraqi Laying Hens Performance

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Abstract

Phase one laying hens diet were included of two levels of yeast cell wall (YCW) called SafMannan from Phlio-USA company. A total of 81 Iraqi laying hens 14 weeks' old were distributed into three treatments. Each treatment has 27 hens were distributed into 27 individual cages (one hen per cage). First group hens were fed on basal diet without supplement (CON). Chicks in second and third group were fed on 250 ppm (Saf 250) and 500 ppm (Saf 500) SafMannan respectively. Body weight were weighted weekly from 14-34 weeks; while, egg weight was numbered weekly from 20-34 weeks. Results revealed that body weight were enhanced significantly ($p \leq 0.05$) in (Saf 250) and (Saf 500) respectively as compared with the control group (CON). The highest egg weight was found in the group was received (Saf 250). Egg weight were increase significantly ($p \leq 0.05$) in groups were received SafMannan (Saf 250) and (Saf 500) respectively as compared with the (CON). In conclusion, adding of SafMannan as a prebiotic in the poultry hens diet at 250 ppm were enhanced the egg weight and body weight of local Iraqi laying hens.

Keywords: *Iraqi local hens, Prebiotic, SafMannan, Yeast cell wall, Egg weight.*

Introduction

Prebiotic is used in feeding of poultry and act as a one of the antibiotic growth promoters like other alternative growth promoter (herbs, organic acids, probiotic, and synbiotic[1,2,3,4]. Prebiotic is defined as a non-digestible food was affected the host by stimulating growth of selective intestinal bacteria which act to enhancing of host health[5,6]. One of prebiotic products is SafMannan. SafMannan is a source of B-glucan and Mannan-Oligosaccharide (MOS) derived from (YCW)[7]. Prebiotic act to enhance gut health have been studied by[8,9,10,11,12,13,14,15,16]. Microbiota of intestine help the host by fermenting of non or poorly digestible carbohydrate. This fermentation act to produce short chain fatty acid[17]. Lower pH of short chain fatty acid propionate, acetate, and butyrate increase mucin secretion by epithelial globlet cell lead to induce inflammatory reactions[18]. Also, when probiotic adhering with pathogenica bacteria which has type 1-fimbriae lead to decrease the ability of this bacteria t adhere with the digestive mucosa[19]. [13]have been studied the effect of YCW SafMannan 250 ppm and 500 ppm on Lohmman laying hens. They showed that supplemented laying diet with 250 ppm SafMannan led to enhance egg weight; whereas, shell quality was improved by adding 500 ppm of SafMannan to laying hens[13]. [20]were reported that hens fed basal diet supplemented with (YCW) led to improve FCR. Also, they found that egg weight, yolk percentage, yolk index and shell percentage were increased significantly. [21]was studied the effect of YCW on laying hens. He found that supplemented basal diet with YSW-MOS during hot season led to enhance of egg production and lowered of cracked and broken eggs and the mortality at age 54 weeks old significantly.

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On the other hand, many results were obtained from recently studied on the effect of prebiotic SafMannan 250 ppm on the enhancement of broiler performance[11,12,14].

The objective of this study was to evaluate the effect of yeast cell wall SafMannan from Phlio. USA with 250 ppm and 500 ppm on Iraqi local laying hens performance from the first egg to peak egg production.

Material and Methods

Birds and husbandry:

Iraqi Local laying hens at 14 weeks' old were removed to research farm laying hens house in the Agricultural Research Department, Ministry of Science and Technology, Baghdad, Iraq.

Eighty-one hens were randomly distributed to three groups (27 hens per each). Twenty-seven individual cages were used (one hens per cage). Basal diet was used to feed the hens in the control group (CON.). Hens in the second and third groups were fed on basal diet with 250 ppm and 500 ppm prebiotic (SafMannan) respectively.

All birds were weighted weekly from (14-34 weeks); whereas, egg weight were also weighted weekly from (20-34 weeks) by using digital scale.

Feed as mash and water were provided ad libitum. Table 1. were show the feed composition according to NRC requirement for laying hens[22].

Table 1. Ingredients and nutrients composition of layer diets.

Ingredients %	Control diet	Saf 250	Saf 500
Yellow corn	50.8	50.8	50.8
Wheat bran	7.5	7.5	7.5
Soy bean meal 47%	27	27	27
Vegetable oil	3	3	3
Limestone	8.5	8.5	8.5
Dicalcium Phosphate	0.6	0.6	0.6
Vitamin/Mineral premix	2.5	2.5	2.5
Prebiotic Saf	0	0.025	0.05
Diatomaceous earth	0.05	0.025	0
Total	100	100	100
ME	2800	2800	2800
Cp%	17.5	17.5	17.5
Calcium %	4.03	4.03	4.03
Phosphorus	0.48	0.45	0.45

[1]Di calcium Phosphor 16/21p.

[2]Wafi premix contained the following: crude protein 8.9 %, iron 2000 mg; copper 400 mg; zinc 2400 mg; manganese 3200 mg; iodine 80 mg; selenium 10 mg; folic acid 40 mg; biotin 120 mg; pantothenic acid160 mg; niacin 1600 mg; vitamin A 480000 IU; vitamin D3180000 IU; vitamin E 2000 IU; vitamin K3100 gm; vitamin B1 120 gm; vitamin B2 280 gm; vitamin B6 160 gm; vitamin B12 1400 gm

[2]Safmannan® from (Phileo Lesaffre Company for Animal Care.

Statistical Analysis

All data were analyzed as a one-way ANOVA by using general linear model procedure of SPSS[23]. All means were compared by using Duncan's multiple range test[22] at levels $p \leq 0.05$.

Results and Discussions

Body weight at laying hens of three treatments from 14-34 weeks were shown in table (2). Body weight of hens was significantly $p \leq 0.05$ higher in hens fed basal diet with (Saf 250 ppm) and (Saf 500 ppm) SafMannan respectively as compared with control of all weeks.

Egg weight of laying hens was also shown in table (3) of three treatments from 20-34 weeks. Egg weight was higher significantly $p \leq 0.05$ of hens fed (Saf 250 ppm) and (Saf 500 ppm) respectively SafMannan with basal diet as compared with the CON.

Table 2. laying hens body weight of three treatments from 20-34 weeks. (Mean±SE).

Treatments	T1	T2	T2
Weeks			
14	934.40±26.07 B	1057.5±48.25 A	1013.13±16.46A B
16	1042.48±26.09 C	1244.1±32.31 A	1146.78±18.36 B
18	1124.66±25.18 C	1363.57±31.78 A	1258.65±18.91 B
20	1193.51±25.63 C	1458.32±31.22 A	1339.34±17.81 B
22	1229.40±29.41 C	1499.1±19.88 A	1361.13±16.11 B
24	1254.88±0.31 C	1510.78±30.82 A	1370.74±17.19 B
26	1270.92±29.66 C	1538.14±33.44 A	1382.78±17..49 B
28	1282.03±29.49 C	1560.32±10.4 A	1397.65±17.82 B
30	1321.96±31.52 C	1585.07±36.74 A	1416.56±16.80 B
32	1335.85±32.58 C	1623.39±37.13 A	1455.21±16.73 B
34	1366.96±27.98 C	1661.42±37.29 A	1492.69±15.83 B

Means with the same letters within the same column are not significantly different ($p \leq 0.05$).

*SD Standard deviation

Table 3. Egg weight of three treatments from 20-34 weeks. (Mean±SE).

Treatments	Egg Weight		
Weeks	T1	T2	T2
20	32.33±0.48B	36.39±0.7A	35.25±0.5A
21	32.95±0.69B	36.56±0.77A	36.08±0.37A
22	35.51±0.53B	37.21±0.71A	36.78±0.36AB
23	36.25±0.65B	38.17±0.67A	39.14±0.33A
24	37.05±0.55B	38.78±0.64A	39.55±0.14A
25	37.10±0.46B	40.57±0.61A	39.53±0.46A
26	37.92±0.42B	41.85±0.66A	40.66±0.46B
27	38.75±0.44C	42.88±0.50A	41.15±0.53B
28	39.37±0.40C	43.21±0.55A	41.74±0.45B
29	40.27±0.42C	43.90±0.58A	42.41±0.46B
30	40.12±0.54C	45.26±0.44A	42.98±0.47B
31	40.85±0.51C	46.47±0.65A	43.40±0.52B
32	41.41±0.39C	47.60±0.69A	44.26±0.56B
33	41.67±0.65C	48.69±0.47A	45.25±0.63B
34	42.03±0.49C	49.91±0.61A	45.52±0.55B

Means with the same letters within the same column are not significantly different ($p \leq 0.05$).

*SD Standard deviation

The enhancement of the body weight and egg weight may be the prebiotic act to enhance gut health have been studied by[8,9,11,12,13,14,15]. The fermentation of non or poorly digestible carbohydrate act to produce (propionate, acetate, and butyrate) which act to increase mucin secretion[18]. Also, [13]showed that supplemented laying diet with 250 ppm SafMannan led to enhance egg weight; whereas, shell quality was improved by adding 500 ppm of SafMannan to laying hens. [21]was studied the effect of YCW on laying hens and found that supplemented basal diet with YSW-MOS during hot season led to enhance of egg production and lowered of cracked and broken eggs and the mortality at age 54 weeks old significantly.

Conclusion

Adding of SafMannan as a prebiotic in the poultry hens diet at 250 ppm were enhanced the egg weight and body weight of local Iraqi laying hens as compared with the hen were fed diet without SafMannan.

Conflict of Interest: This research is a personal non-profit work and there is no conflict of interest.

Source of Funding: m None.

Ethical Clearance: Ethical clearance was obtained from the Faculty Scientific Committee (College of Veterinary Medicine, University of Kerbala, Karbala, Iraq) to study the effect of SafMannan on local Iraqi laying hens performance**.**

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In vitro Measurement Volume of the Neonate Thyroid Gland

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Abstract

Measurement and replacement fluid methods to estimate the thyroid gland volume. Because there is wide-ranging of variation in the volume of the thyroid gland in healthy individuals of different geographical location, ages, gender, and races.**Objectives:** To estimate normal neonate thyroid gland volume it can throw light to help clinicians deal better with the gland during neonatal age besides providing native standard data. **Period:** From Sept 13, 2018, to Mar 22, 2019. **Material & Methods:** Total of 48 subjects were included, 24 male and 24 female. The neonate thyroid gland collected and inspected grossly and taken all measurements, then use these measurements for finding volume thyroid gland. Also, the volumeis taken by using replacement fluid. **Results:** The mean aggregate thyroid gland volume of measures method for male subjectsstudy was 250.53 ± 4.37mm^3, while a female was 246.31 ± 3.42 mm^3. The mean volume thyroid gland of replacement fluid method for male subjects study was 295.71 ± 4.41mm^3, while the female was 291.43 ± 4.04. The mean thyroid volume of the male is expressively larger than female in both methods but statically non-significant variation. The difference between both ways statistically highly significant ($p< 0.01$). **Conclusion:** according to results that achieved by two methods, we should use another correction factor, higher than 0.749, until getting the nearest value to replacement fluid. Working out different ages to establish average data.

Keyword: *thyroid gland, the volume of the neonate thyroid gland*

Introduction

The thyroid gland is one of the ductless glands, red-brownish, and highly vascular gland. It's the biggest endocrine gland in the human body consists of two lateral lobes, one on each side, attached by a narrow thin isthmus to form H shape structure.(1) Moreover, these two lateral lobes there is the pyramid in shape which directionupward from the isthmus of the thyroid gland. The gland placed anteriorly in the lower part of the neck just below laryngeal prominence "Adam apple."(2) Weight of the thyroid gland increases postnatal life from approximately 2–4 gm at birth to about 15–20 gm in adulthood.(3) The mean length, thickness, and width of the right lobe is 5.26, 2.39, and 2.97while the mean length,width, and depthleft portion is 5.21, 2.90, and 2.33 cm, respectively.(4)

The lobes of the thyroid approximately conical in shape have a broad lower segment. Each lateral portion has convex surface anteriorly and concave surface posteriorly.(5) The inferior end of the thyroid is one of the most common places of risk regarding recurrent laryngeal nerve damage and no injuries to the external branch of the superior laryngeal nerve related to a superior pole. Thyroid particularly labile gland that varies significantly in structure andsize.(6)

The thyroid gland has an abundant blood supply delivered by two pairs ofmain arteries. It obtains 5% of the cardiac output, which is high in percentage to its weight and size. It usually is supplied by inferior and superior thyroid arteries and is drained by inferior, middle, and superior thyroid veins. Also, the arteries anastomose profusely with one another over the surface of the gland stuck between the fibrous capsule and the loose facial sheath.(7)Thyroid ima artery not usually present, understanding of the anatomy of the thyroid ima artery is essential for neck surgeons.(8)The veins of thyroid gland form a plexus which lies beneath the proper capsule if the thyroid gland and drained by superior thyroid vein, middle thyroid vein, inferior thyroid vein. (9)The lymphatics drain mainly to deep cervical nodes, uncommon enters prelaryngeal, pre- and paratracheal nodes and a rare drain directly into the thoracic duct.

The gland gets its innervation from the superior, middle and inferior cervical sympathetic ganglia. They are vasomotor in function. Parasympathetic fibers are derivative from the X cranial nerve and influence the gland via divisions of the laryngeal nerves[10]

A postmortem study, the size and volume of thyroid gland differ considerably with gender, age, geographical situation, and physiologic state.It is greater and fuller in women than in men. It hypertrophies duringpregnancy and menstruation.[11] The maximum volume was 70ml. The minimum amount was 5ml. the volume of the thyroid gland in 50 years 13.33±5.97.[12] Thyroid volume increased with stage of development during childhood and adolescence, remained accurately constant in younger adults and dropped more slowly in older people.[13]

Material Method

Volume Measurement (A) Grossly volume of neonate thyroid gland measured by using the replacement fluidtechnique.[14]by taken two identical measuring cylinders (1000 mm^3). After residence the organ into the empty cylinder, water is gradually added to it from anotherbottle that contain 500mm^3 of water until reached to 500mm^3. The residual percentage of water in the container representing the amount of thyroid gland fluid moved, measured in milliliters. (B) Gross measurements length, width and thickness for lobes of the thyroid gland done by using a digital verniercalliper, millimetre (mm) was used as a unit of measurements.The volume of each lobe calculated by the World Health Organization recommended formula: measurement of the maximum superoinferior, horizontal and ventrodorsal dimensions of both lobes; multiplying these values (mm) with each other and then 0.479 to obtain the volume (mm 3) of each lobe.Immediateresults of the two lobes provided volume of the thyroid gland. The isthmus dimensions Wasn't included in the formula.[15]

Result

The thyroid gland is located anteriorly in the lower part of the neck, in the muscular triangle. Related to the lamina of the thyroid cartilage, the thyroid gland lies lateral to lower third of lamina of the thyroid cartilage. Concerningto cricoid cartilage, the gland present inferior and lateral to cricoid cartilage from an anterior and lateral view, respectively. Simultaneous anterior to superior tracheal rings. Thyroid gland appear as completely developed U shape, reddish in color due to perfused blood sources, without agenesis of any part.

The thyroid gland consists of two lobes; these lobes lie on a parasagital plane, both lateral lobes of the neonate thyroid gland have been found to be somewhat symmetrical pyramidal in shape, have a broad base with a direct connection to the isthmus from the lower medial side, and are slightly tapering superiorly. The medial surface looks like concave, and laterals surfaces appear convex. According to the lower third of thyroid cartilage and cricoid cartilage levels, the superior boundary of lateral lobes of the thyroid gland was observed reach to the lower third lamina of the thyroid cartilage. The inferior border of the right and left portions, related to tracheal rings was determined reached to the fourth tracheal rings. The mean of the neonate thyroid gland weight was 1.43 ±0.2, 1.39 ±0.19 gram for male and female, respectively. The mean dimensions of length, width, and depth for right and left lobes in each gender see table(1).The statistical analysis revealed that the male larger than female. Still,there is non-significant variation among lobes of same gender or even between two sex in all criteria of measurements. The mean total thyroid gland volume of measures method for male subjects study was 250.53 ±4.37 mm^3, while a female was 246.31 ±3.42 mm^3.

The mean neonate thyroid gland volume that obtained byreplacement fluid method for male subjects study was 295.71 ± 4.41mm^3, while the female was 291.43 ± 4.04. The mean thyroid volume of the male is larger than female in both methods. Still, the statistical analysis of these values revealed non-significant variation among the volume of the thyroid gland between male and female if using same methods for both sex. While if we make a comparison between neonate thyroid volume in the measurements method with the volume in replacement fluid,for both genders, there is a highly significant variation ($p < 0.01$) between two processes.

Table (1) Show anatomical measurements ofother studies and current work.

Authors		Lobes	Length mm	Width mm	Thickness mm
Anupriya		Both lobes	12.1	4.5	2.9
Cicekciba		Right	12.99	6.64	3.11
		left	12.19	5.87	3.11
Kishor P		Right	12.02	6.35	4.71
		Left	12.05	6.38	4.73
Current study	Male	Right	12.21 ±0.06	5.48 ±0.04	3.82 ±0.05
		Left	12.16 ±0.04	5.61 ±0.06	3.89 ±0.03
	Female	Right	12.11 ±0.05	5.40 ±0.04	3.82 ±0.06
		Left	12.10 ±0.05	5.58 ±0.04	3.87 ±0.03

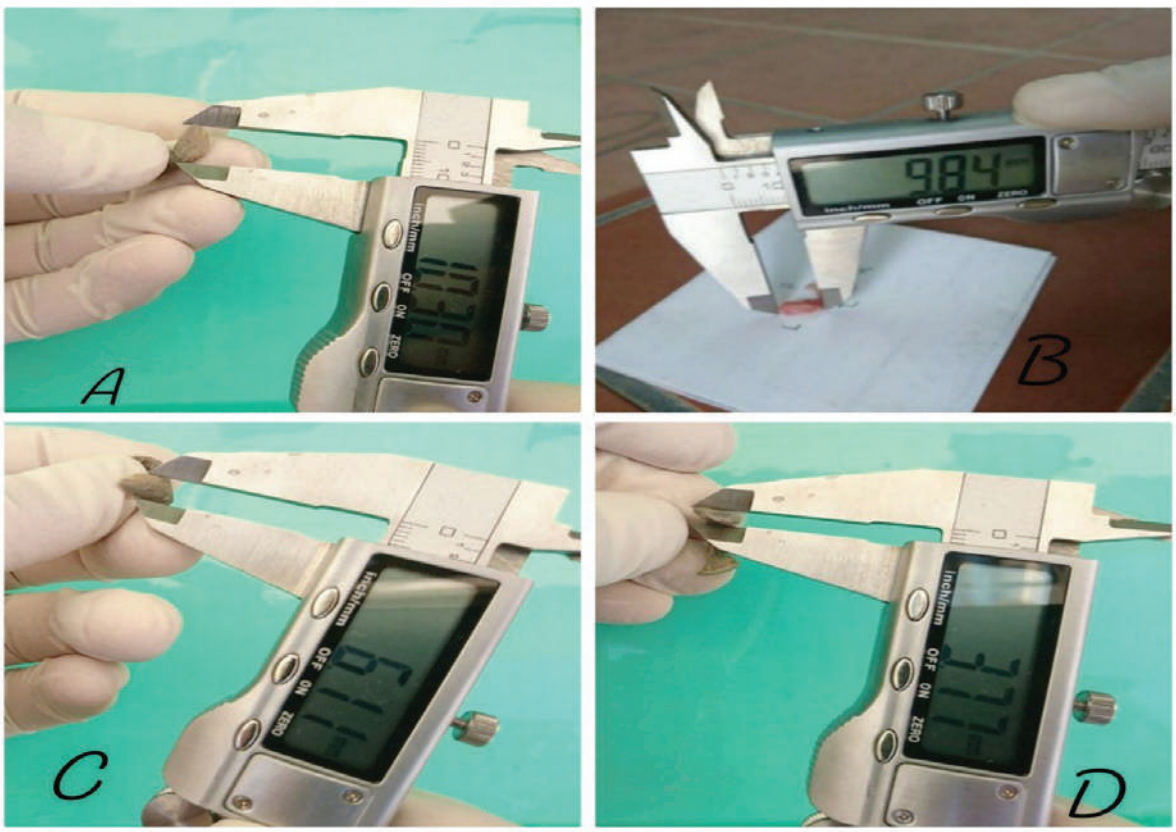


Figure (1) shows measurements A & B length of right & left lobes, C width of the right lobe, D thickness of right portion, for the neonate thyroid gland.

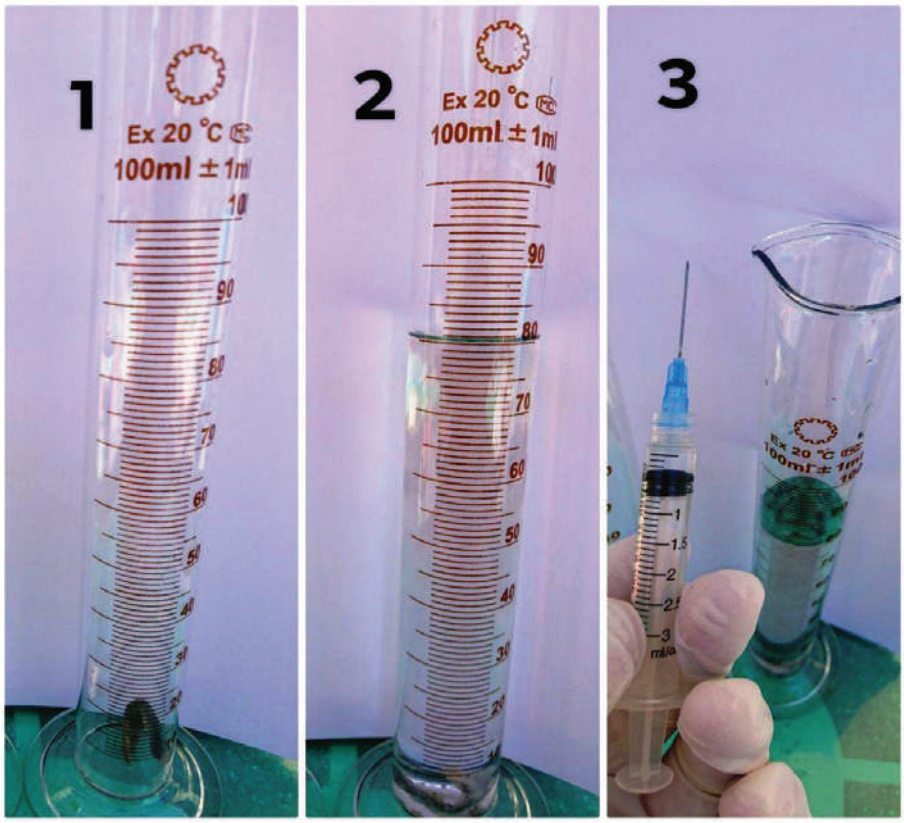


Figure (2) show replacement fluid, 1 empty cylinder tube in the bottom thyroid gland, 2 cylinder tube contain water and gland, 3 amount of replacement fluid in the syringe

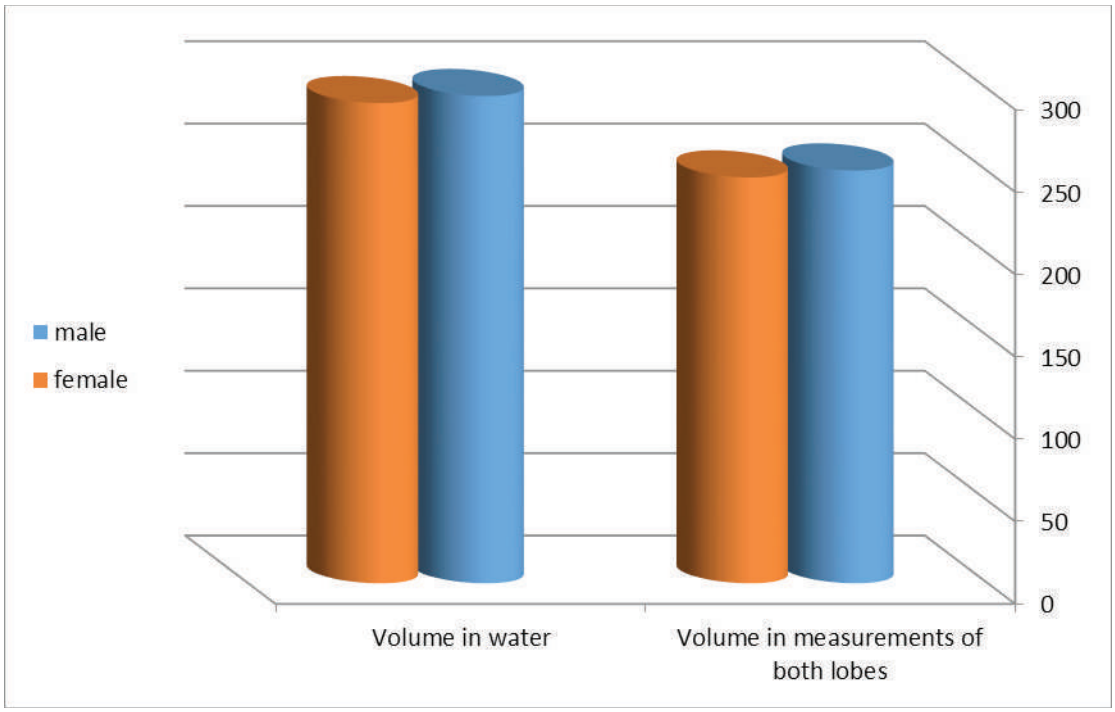


Figure (3) show volume for male and female in both methods

Discussion

The values and observations obtained in this study expose interest points, so these have been considered worthy because this is first training evaluating the volume of the neonate thyroid gland in normal subjects ofthe Iraqi population to provides local data of thyroid gland volume. The volume ofthe thyroid gland should be done as a screening test to ovoid this abnormalities. Because the thyroid gland is essential for healthy growth and thyroid dysfunction causes mental and physical retardation and dwarfism.[16]The size of the thyroiddiffer inversely in response to alterations in iodine ingestion, both undersupplied or excessive iodine intakes may increase gland volume.[17]Since there is no native study yet informed for assessment in our country so well be take the nearest reviews to verifythe validity of our work.

The thyroid gland lies at the anterior lower part of neck-deep to the sternothyroid and sternohyoid muscles,this agrees with Muktyaz H et al.[18] Morphology of thyroid gland specimens involved in this study appear as U shape which conforms with Archana. [19]In the existingwork all measurement noticed during neonatal life included the means of length, breadth, and depth of lateral lobes have minor difference was noted compared to the study of other authors Kishor[20]Cicekcibasi[21], and Anupriya.[22] (table 1) The present study showed both lobes linked together byan isthmus, definitely notsignificantvariation was observed among dimensions of the right and left lobes, but their sizes increased with age advanced, that agree with Lokanadham.[23]Agenesis is any thyroid gland lobes not discovered in the current research that is in line with Dixit.[24]The weight rises in infancy and childhood. During early years weight increases at a rate of 0.054 grams per month.[25] In the current work, the weight of thyroid gland was 1.43 ±0.2, 1.39 ±0.19 gram for male and female, respectively. These values correspond with Griffin[26]and Ratnakar[27] studies they found the weight 1 to 1.5 gm at term and1.25 gm, respectively. But not accepted with Srivastava[28] he foundthe weight of neonate thyroid gland 0.46 gm.

The volumeincreases in a line fashion with advancing age, because of increasing size and amount of follicles this agree with Brown[14], Roberts[29] studies they construct that the thyroid volume greater than before during babyhood and teenaged, remained stable in young adults and dropped in older subjects. The alteration in volume from neonatal age to different living period is due to concern of the changes in sizes of the gland acini, for this reason, the volume is not constant which agree with Nurunnabi.[30]The study explains that there are no sex differences in thyroid volumes, also in agreement Musleh[31]. But the variation present between methods selected. To fixed above by comparingthe present study that finds neonate thyroid volume in male and female was 250.53 ±4.37 mm^3, 246.31 ±3.42 mm^3 respectively. Neonate thyroid gland volume that obtained by replacement fluid method for male was 295.71 ±4.41mm^3, while the female was 291.43 ±4.04 mm^3. For this purpose, the correction factorsfor neonate thyroid gland volumes should appear not far away, higher or lower, displacement fluid. Ahealthyrelationshipshouldbe established between the capacity of the thyroid gland calculated in vivo and in vitroas indication by Shabana.[32]

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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X-Chromosome Markers Used in Deficiency of Paternity Case

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Abstract

Cases of deficiency paternity, characterized by missing of the alleged father, are a task for forensic genetics. Recently these cases were determined using sets of extremely polymorphic autosomal short tandem repeats (STRs), which indicate to powerful likelihood ratios (LR). Some difficult cases emerge whenever the kinship highly remote or if the another hypotheses are not properly formulated because of the absence of information. In these situations, beyond the routinely used marker set, laboratories usually enlarge the number and/or the type of markers analysed. A set of 14X-STRs is further advantageous than 21 autosomal STRs (AS STRs) biallelic markers. Furthermore, the utility of X-STRs was also appear in cases shows that in father–daughter duos and merely a close family member of the alleged parent (father or mother) is existing for testing. Here we present a cases with biological daughter of the alleged father and her mothers and putative grandmother was the genotyped relative are available for reconstructing his haplotype . Aiming to increase the Paternity Index (PI), Probability of Paternity (PP), and obtain more reliable results. The result of X STR showed that the missing person (father) share 50% genotype with the donors (daughter)and (his mother) at each locus, there are no alleles in any one of these 13 loci conflict with the alleles inherited from her father and Posterior Probability of paternity was (99.999958%) that is mean the missing person was biological father for this donor daughter.

Keywords: *13 X-STRs with Amelogenin, Paternity case, Kinship analysis, Paternity index, Paternity deficiency.*

Introduction

The main benefit of X-chromosomal (ChrX) STRs rises in deficiency paternity cases, i.e. when a putative father is unavailable and DNA from paternal relatives has to be investigated instead [1,2]. In such cases, the power of exclusion of autosomal STRs is significantly decreased, whereas ChrX markers are (at least in some cases) more resourceful. Female individuals share their paternal ChrX when they are fathered by the same man [3,4]. Males receive their single ChrX from their mother. Consequently, in cases in which the supposed grandmother is available for genotyping, the possible ChrX alleles of the putative father can be verified. ChrX marker typing is very applicable in mother–son kinship and in father–daughter analyzing [5,6].

Case Report

We perform the use of this set of markers in paternity deficiency cases, containing a female child and her mother and the putative grandmother and a dead alleged father who was not analyzed.

Figure 1 displays the pedigrees of the families. All persons are shown in the pedigrees were examined for autosomal (AS) and X STRs.

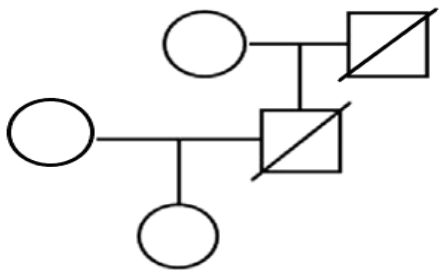


Figure 1: Pedigree of the deficiency paternity case. (paternal grandmother-granddaughter)

Materials and Methods

All blood samples were gathered utilizing finger lancets and kept on FTA® Classic Card (GE Healthcare, Pittsburgh, USA). For each sample a 1.2 mm punch was directly amplified according to the manufacturer's recommended instructions. PCR using two kits the PowerPlex 21® System)20 STR loci and Amelogenin), including D1S1656, D2S1338, D3S1358, D5S818, D6S1043, D7S820, D8S1179, D12S391,D13S317, D16S539, D18S51, D19S433, D21S11, Amelogenin, CSF1PO, FGA, Penta D, Penta E, TH01, TPOX and vWA. And 14ChrX STRs- GenePhile X-Plex(13STR loci and Amelogenin), including for (DXS8378, DXS9898, DSX8377, DSX8377, GATA172D05, DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS9902, DXS7424). Genomic DNA (1 ng) was amplified in a 10 L total volume of PCR reaction mix was done using Applied Biosystems 9700 thermocycler according to standard manufacturer's recommended conditions. Capillary electrophoresis was achieved by using a 3500XL Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's instructions; ILS-500 (promega, 2017) and BTO (qiagen, 2015) were used as an internal size standard. Genotyping was completed using GeneMapper ID-X v1.4 software (Applied Biosystems).

Statistical Analysis:

The statistical parameters that analyzed showed the Paternity Index (PI) and Probability of Paternity (PP), with the assumption of an a priori probability of 0.5 for the latter. Genotypes and allele frequencies of the X-liked STRs were transmitted by the deceased father to his daughter and the formulae applied when the putative paternal grandmother was the genotyped relative that can be seen in Table 1. Statistical analyses were done corresponding to the formulae proposed by Ayres and Powley in 2005 [7,8].

Table 1: Formulations used to determine PI considering the profile configurations depicted with ChrX STRs,

Mother	Daughter	Putative grandmother	Formulas
AB	AB	AB	1/(a+b)
AB	AA	AB	1/2a
AA	AB	AB	1/2b
AA	AA	AB	1/2a
AB	BC	AC or CD	1/2c
AC	CC	BC	1/2c
AC	AC	BC	1/[2(b+c)]
AA	AB	BB	1/b
Absent	AB	BC	1/4b
Absent	AB	BB	1/2b
Absent	BB	BC	1/2b

Results

The routine work in identification we started with autosomal using PowerPlex 21, Autosomal STR DNA profile of paternal grandmother, mother and female child trio is presented in Table 2. All the allele in the autosomal STR DNA profile of female child is matching with both mother and paternal grandmother on all the 20locus establishing the identity of the missing father.

Table 2: The autosomal STR DNA profile of both mother)wife) and Daughter, paternal grandmother. (powerplex 21 genotype)

STRs	Mother	Daughter	grandmother
Amelo	X,X	X,X	X,X
D3S1358	16,18	15,16	16,17
D1S1656	14,14	11,14	13,14
D6S1043	11.18	14,18	14,17
D13S317	11,11	9,11	8,14
Penta E	12,13	7,13	11,16
D16S539	10,10	10,13	12,12
D18S51	12,12	12,15	14,18
D2S1338	17,25	17,25	17,27
CSF 1PO	10,12	12,12	10,12
Penta D	9,15	9,9	11,11
TH01	9,10	9,9	7,9
vWA	16,17	16,17	15,17
D21S11	29,29	26,29	26,31.2
D7S820	10,10	10,12	8,12
D5S818	10,12	10,12	12,12
TPOX	8,10	8,8	11,11
D8S1179	9,15	9,14	9,14
D12S391	20,12	21,23	18,23
D19S433	13,15.2	13,15.2	14,15.2
FGA	24.25	24,24	24,24

Data of DNA profile of same three samples with - GenePhile X-Plex(13STR loci and Amelogenin), kit is presented in Table 3. In X STR also all the allele of female child is matching with both mother and paternal grandmother on all the 13 locus. As the female child is having two X, one from father and the other from mother, so this can be shown by generating X STR DNA profile of the trio.

Table (3) : The genotype of mother (wife), daughter and grandmother (genephile x-plex -13 STR kit)

STRs	mother	Daughter	grandmother
Amelo	X,X	X,X	X,X
DXS8378	11,12	10,11	10,11
DXS9898	8.3,8.3	8.3,14	8.3,14
DXS8377	46,54	47,54	43,47
HPRTB	11,13	11,13	11,12
GATA172D05	6,11	6,10	10,10
DXS7423	15,16	15,16	15,17
DXS6809	31,32	31,34	33,34
DXS7132	13,14	13,14	13,14
DXS101	21,21	21,24	14,24
DXS6789	20,20	19,20	15,19
DXS9902	10,12	10,12	10,10
DXS6807	11,14	13,14	11,13
DXS7424	14,16	12,14	12,13

So Paternity indices (PI) for full trio and deficient (father not available) cases are (23725241.4)

Prior odd=1/2

$$\text{Posterior Probability of paternity} = \frac{CPI * prior}{(CPI * priorodd) + (1 - priorodd)} = 0.99999958$$

Discussion

The autosomal matching was done to support these results and avoid the false positive due to huge number of close related people missing at the same time we did sex chromosomes genotyping. The result of X STR showed that the missing person (father) share 50% genotype with the donors (daughter)and (his mother) at each locus, there are no alleles in any one of these 13 loci conflict with the alleles inherited from her father and Posterior Probability of paternity was (99.999958%) that is mean the missing person was biological father for this donor daughter. This value is accepted according to lab. This value is accepted according to lab. of mass grave standard and the International Society for Forensic Genetics (ISFG) recommendation [9,10].

Conclusion

Recently the X-Chromosome STR loci transmission analysis are clearly available for use in forensics as in immigration, victim identification in mass disasters and it is possible that these loci will be used efficiently

for paternity testing of female children, predominantly as supplementary tests to complement the information obtained with the autosomes and the differentiation between pedigrees in deficient or otherwise difficult cases. In this study the equations that used provide the means to evaluate the utility of such loci in the existence of substructure, and complement previously published equations used for autosomal loci.

Acknowledgement: We are grateful to all persons for their involvement in this study and we would like to thank all colleagues in the DNA laboratory of Mass Graves Department of in the Medico-Legal Directorate of Baghdad for their assist.

Ethical Clearance: The principles and the experimental protocol in this study was approved by the Medico-Legal Directorate, Ministry of Health, Baghdad, Iraq.

Source of Funding Self.

Conflict of Interest: Nil.

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Effect of Alpha Lipoic Acid on Polycystic Ovary Syndrome with Insulin Resistance

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Abstract

Background: Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive age women where insulin resistance plays an important role. Insulin resistance makes the first line treatment, clomiphene citrate (CC) treatment, become ineffective. Alpha Lipoic Acid (ALA) is believed to be an alternative treatment for CC-resistant PCOS. **Objectives:** The aim of the study is to understand the effect of ALA in Insuline receptor substrate 1 (IRS-1) expression, Glucosa transporter 4 (GLUT-4) expression, and folliculogenesis in insulin resistant PCOS rat model. **Methods:** This was an experimental study with randomized posttest only control group design. 30 females rat injected with testosterone propionate (TP) 1mg/100gram bodyweight for 28 days then divided into 3 groups. Negative control group receive no other treatment, positive control group receive a placebo for 14 days, and treatment group receive ALA for 14 days. IRS-1 and GLUT-4 expression is evaluated with immunohistochemistry, while folliculogenesis is evaluated by counting the number of follicle in each stage. **Results:** Mean IRS-1 expression in muscle in treatment group is significantly higher than other groups (4.28±1.05; 3.02±1.03; 1.86±0.83, $p < 0.01$ respectively). Mean GLUT-4 expression in treatment group is significantly higher than other groups (4.28±0.91; 3.20±1.14; 1.40±0.55, $p < 0.01$ respectively). Mean number of follicle in each stage in treatment group are significantly reduced than other groups (all $p < 0.05$). **Conclusion:** ALA increase the expression of IRS-1 and GLUT-4, and also reduce the number of follicle in each stage of folliculogenesis**.**

Keywords: *PCOS, Alpha Lipoic Acid, IRS-1, GLUT-4, Folliculogenesis*

Introduction

Polycystic Ovary Syndrom (PCOS) is the most common endocrine disorder in reproductive age woman with prevalence around 5-21%[1]. Female with PCOS have high incidence of insulin resistance with prevalence of 50-75% and decrease insulin sensitivity about 35-40% than normal[2]. Insulin resistance plays an important pathogenic role in hyperandrogen both for obese and lean female PCOS, accompanied by hyperinsulinemia and increases the risk of type 2 diabetes mellitus (DM)[3]. Infertility treatment with clomiphene citrate (CC) is the first-line therapy for ovulation induction, but there is CC resistance of 15-40% where insulin, hyperandrogen and obesity resitance are the major factors[4].

Basal insulin therapy could increase the glicemic control[5]. However, consuming supplement, such as vitamin C and vitamin E, could not increasing insulin in the blood[6]. Previous study found that consuming *Moringa oleifera* decrease level of blood insulin and androgen, so that folliculogenesis could increase[7]. Metformin is one of the insulin sensitizer that often used for PCOS therapy with insulin resistance. Although metformin may decrease insulin resistance, it has undesirable side effects such as gastrointestinal disorders, thus a long-

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term use may decrease patient compliance[8]. Because of its side effect and it is important to decrease insulin resistance in PCOS, it is necessary to use alternative treatment that can reduce insulin resistance and increase the incidence of ovulation and pregnancy rates by using alpha lipoic acid (ALA). ALA is a powerful antioxidant, detoxifying agent and diabetes mellitus drug. It also has been involved as a modulator of inflammatory signal pathways[9]. ALA can improve glucose control in DM type 2, with its mechanism on decreasing oxidative stress and insulin resistance, and increasing peripheral insulin sensitivity[10,11].

Thus, this study aims to determine whether ALA is able to improve insulin resistance and follicologenesis in PCOS with insulin resistance with Insuline receptor substrate 1 (IRS-1), Glucosa transporter 4 (GLUT-4), and follicular as parameters.

Methods

This was an experimental study with randomized posttest only control group design. This study was done in Faculty of Veterinary, Universitas Airlangga in February-may 2017. The sample in this study was *Rattus norvegicus* strain wistar female rat around 4-5 months old. The inclusion criterias for animal model were 4 - 5 months old, 150 - 260 gram weight, female gender, healthy (characterized by active movement, glowing eyes), and received injection of 1 mg / 100 gr BB testosterone propionate subcutaneously during 28 days. While the animal models included in the exclusion criteria were diseased, defective or congenital and aggressive (seen in 2x24 hours of observation with parameters often attacking other group members). While the dropout criteria are wounded model, die before, during and after administration of ALA, prior to the examination.

Samples were taken from population by randomization. The models were divided into 3 groups, one group of PCOS model with insulin resistance given ALA for 14 days (treatment group), one group of PCOS model with insulin resistance which only given placebo for 14 days (positive control group), and one group of PCOS model with insulin resistance without any treatment (negative control group). The sample size was calculated using Federer's formula and with 10% addition to prevent drop out, thereby 10 models were obtained for each group. This study was ethically approved by Ethics Committee of Faculty of Veterinary Medicine, Universitas Airlangga before conducting the study. All experiments were performed in accordance with relevant regulations.

This study used level of significance 0.05, means if statistical test obtained p-value $\leq$ 0.05 it means significance. All data homogeneity were tested with Levene test to assess whether there was any difference of variance between the three groups. Anova test was used to analyze the difference in IRS-1 expression, GLUT-4 expression, and number of follicle in every stages of folliculogenesis. Multiple linear regression analysis was used to analyze the effect of ALA, IRS-1, and GLUT-4 on folliculogenesis. All data analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago IL).

Results

Sample Characteristic

In this study, all samples weight were analyzed using Levene test and obtained homogenous data variation. Using ANOVA test, the result showed no significance differences between treatment, positive control and negative control groups weight (207.50±42.51; 204.50±19.07; 208±35.84, p = 0.97 respectively).

IRS-1 Expression

The homogeneity test of IRS-1 expression in muscle using Levene test obtained a homogeneous data variance. Then, using the Anova test, a significant differences in IRS-1 expression were found between the treatment group, positive control, and negative control (4.28±1.05; 3.02±1.03; 1.86±0.83, p <0.01 respectively) (Table 1). The homogeneity test of IRS-1 expression in ovaries using Levene test obtained homogeneous data variance. Using the Anova test, significant differences in IRS-1 expression were found between the treatment group, positive control, and negative control (7.78±1.10; 3.06±1.56; 2.46±0.89, p <0.01 respectively) (Table 1).

GLUT-4 Expression in Muscle

The homogeinity test of GLUT-4 variable using Levene test obtained homogeneous data variance. The ANOVA test showed GLUT-4 expression was significantly differences between the treatment group,

positive control, and negative control (4.28±0.91; 3.20±1.14; 1.40±0.55, p <0.01 respectively) (Table 1).

Folliculogenesis

The homogeneity test of primary follicles number variable using Levene test obtained an inhomogeneous variance data. The ANOVA test showed a significance differences in primary follicle number between the treatment group, positive control, and negative control (58±13.11; 66±19.81; 92±44.9, p = 0.04 respectively) (Table 2). The homogeneity test result of secondary follicles number variable using Levene test was homogeneous. The ANOVA test showed tertiary follicle number was significantly difference between the treatment group, positive control, and negative control (18±4.62; 27±10.82; 44±16.6, p <0.01 respectively) (Table 2).

The homogeneity test result of tertiary follicles number variable using Levene test was homogeneous. The ANOVA test showed a significance difference in secondary follicle number between the treatment group, positive control, and negative control (2±1.56; 4±1.89; 7±4.37, p <0.01 respectively) (Table 2). The homogeneity test of de Graaf follicles number using Levene test obtained an inhomogeneous variance data. The Kruskal walls test showed a significance difference in de Graaf follicles number between the treatment group, positive control, and negative control (5±3.37; 2±0.82; 0.4±0.52, p <0.01 respectively) (Table 2).

The homogeneity test result of corpus luteum number using Levene test was homogeneous. The ANOVA test showed a significance difference in corpus luteum number between the treatment group, positive control, and negative control (9±3.37; 6.8±3.58; 2±2.36, p <0.01 respectively) (Table 2).

ALA, IRS-1 and GLUT-4 effects on folliculogenesis

Using multiple linear regression analysis, we found that ALA, IRS-1 in muscle, IRS-1 in ovaries and GLUT-4 had an effect on the number of each phase of folliculogenesis, and especially on the number of De Graff follicles (Table 3).

Table 1. The difference in expression of IRS-1 in muscle, IRS-1 in ovarium, and GLUT-4 in muscle between groups

	Treatment group	Positive control group	Negative control group	p
IRS-1 in muscle	4.28 ± 1.05	3.02 ± 1.03	1.86 ± 0.83	<0.01
IRS-1 in ovarium	7.78 ± 1.10	3.06 ± 1.56	2.46 ± 0.89	<0.01
GLUT-4 in muscle	4.28 ± 0.91	3.20 ± 1.14	1.40 ± 0.05	<0.01

IRS-1= *Insulin Receptor Substrate-1*; GLUT-4= *Glucose Transporter Type-4*. Anova test was used. A p value of <0.05 was considered significant

Table 2. The differences in number of follicle between groups

	Treatment group	Positive control group	Negative control group	p
Number of Primary Follicle	58 ± 13.11	66 ± 19.81	92 ± 44.90	0.04
Number of Secondary Follicle	18 ± 4.62	27 ± 10.82	44 ± 16.6	<0.01
Number of Tertiary Follicle	2 ± 1.56	4 ± 1.89	7 ± 4.37	<0.01
Number of de Graaf Follicle	5 ±3.37	2 ± 0.82	0.40 ± 0.52	<0.01
Number of Corpus Luteum	9 ± 3.37	6.8 ± 3.58	2 ± 2.36	<0.01

Anova test was used. A p value of <0.05 was considered significant

Table 3. The Effect of Alpha Lipoic Acid, IRS-1 in muscle, IRS-1 in ovarium, and GLUT-4 on Folliculogenesis

Dependent Variable	F	Adjusted R Squared	P
Number of Primary Follicle	2.69	0.226	0.05
Number of Secondary Follicle	6.40	0.482	<0.01
Number of Tertiary Follicle	3.62	0.311	0.01
Number of de Graff Follicle	6.44	0.484	<0.01
Number of Corpus Luteum	5.18	0.419	<0.01

Multiple linear regression analysis was used. A p value of <0.05 was considered significant

Discussion

This study result obtained the IRS-1 expression in muscle of the treatment group was significantly different than the positive control and negative control. Increasing IRS-1 expression was caused by ALA as a potent antioxidant, which capable of binding various species of reactive oxygen species (ROS). Chronic oxidative stress induces a number of insulin signaling pathways, especially via the *nuclear factor* kappa-light-chain-enhancer of activated B cells (NFκB) and c-Jun N-terminal kinases/ stress-activated kinases (JNK/SAPK) pathways, which could change the phosphorylation of serine into tyrosine phosphorylation (shay). Furthermore, ALA can bind directly and activate the tyrosine kinase domain in insulin receptor (IR) subunit-β resulting the IR becomes active. ALA effects on IR/IRS-1 will increase the combination of IRS-1 and *Phosphoinositide* 3-kinases (PI3K), also increase PI3K activity in the membrane environment. Then activating Akt to regulate the GLUT-4 displacement between storage vesicles and plasma membranes through mechanisms involving AS160 phosphorylation. ALA induces IRS-1 phosphorylation,

IRS-1/PI3K signal activation and stimulates GLUT-4 translocation to cell membranes through inactivation of Akt 160 (AS160) substrate[9]. Hence, ALA will increase insulin signal activation through IRS-1 enhancement and increase glucose uptake by increasing GLUT-4 to reduce insulin resistance.

In this study, there were significant differences of GLUT-4 expression between treatment group and control group. This suggests that ALA administration significantly increases GLUT-4 muscle expression in PCOS animal models with insulin resistance. The main function of GLUT-4 is to facilitate the glucose intake into muscle cells and adipocyte and also maintain control of blood glucose level. Insulin resistance may result from interruption of insulin signal transduction causing GLUT-4 translocation to decrease.The translocation of insulin-mediated GLUT-4 into the plasma membrane includes the PI3K complex[12].

This study obtained a significant differences in each phase of folliculogenesis between the treatment and the control group. The data showed decrease dominancy of primary, secondary and tertiary follicles, and significant increase in the number of de Graaf follicles and corpus luteum treatment group. ALA can decrease insulin resistance and lowered androgen levels thus improving the selection stage of follicugenesis by increasing the number of De Graaf follicles. However, it did not cause ovulation, because pre-ovulation estrogen levels were inadequate to trigger Lutenizing hormone (LH) surges, resulting in no ovulation. Ovulation will likely occurs if ALA dosing was raised and the administration was extended. The estrous cycle in rats is divided into 4 different phases compared to humans: Proestrus, estrus, metestrus, and diestrus. The proestrus and estrus phases resemble the follicular phase, while the metestrus and diestrus phases resemble the luteal phase in humans. The markers of ovarian development in mice resemble humans, only the timing is shorter. A number of primordial follicles will develop into primary follicles. Factors that affect the development at this stage is still not fully understood. A new Follicle-stimulating hormone (FSH) functional receptors are formed at the secondary follicle stage. The development of the preantral follicles (primary and secondary) is affected by FSH but does not depend entirely. The pre-antral follicle then enters the cyclic recruitment into the antral follicle (tertiary) and eventually becomes the preovulatory follicle (de Graaf follicle).

The development of antral follicle is highly dependent on FSH. Only follicle that has sufficient number of FSH receptors will capable of developing into de Graaf follicle and ovulate. Corpus luteum is a mass of ovaries formed by ovulatory ovulation. Rats formed more than one dominant follicle compared to humans. Ovulation occurs spontaneously every 4 - 5 days. There are some dominant follicles will ovulate. Ovulation in mice can produce >10 oocytes from each ovary[13,14]. Oxidative stress can interfere the self-improvement mechanisms in mitochondria and trigger a prolonged autphagy process in granulosa cells, which impacts on folliculogenesis inhibition[15,16]. Various *in vivo* and *in vitro* studies have been conducted to examine the protective effect and quality of ALA as an antioxidant in diseases also improve follicular development [17]. This improvement might due to a decrease in ROS levels and an increase in total antioxidant capacity in the follicle[15].

This study results showed that the effect of ALA, IRS-1, and GLUT-4 on the variation of folliculogenesis was found highest in the number of de Graaf follicular (48.4%) followed by secondary follicle (48.2%), corpus luteum (41,9%), tertiary follicular (31,1%), and primary follicles (22.6%) while the rest is influenced confounding factor. The effect of ALA administration and lowered insulin ressistance in PCOS models demonstrated the highest effect on de Graaf follicles around 48.4%, shows there were other factors that might influence. The current perspective sees PCOS with insulin resistance as a complex disorder, in which many variants and factors combine and contribute to the pathophysiology. This complexity will complicate the therapy. However, one of the limitations in this study was the absence of control using normal rats, resulting no data on expression of IRS-1 muscle expression, ovarian expression and GLUT-4 muscle in normal model.

Conclusion

There were a significantly differences on IRS-1 expression in straited muscles and ovary, GLUT-4 in muscle cells, and de Graaf follicle number between treatment and control groups. There were no signinificant difference in the number of primary, secondary, tertiary and corpus luteum between all groups.

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Assessment of Women's Knowledge Concerning Hydatidiform Mole at Maternity Hospitals in Baghdad City

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Abstract

Hydatidiform mole results from abnormal fertilization. It is the most common disorder of gestational trophoblastic diseases can be partial or complete. Early detection of molar pregnancy and monitoring can reduce complication particularly coriocarcinoma. The fetus typically dies, and a miscarriage often occurs.

Objectives: To assess women's knowledge concerning Hydatitiform mole and find out the association between women's knowledge with their study variables.

Methods: Descriptive study was conducted from November 2019 to February 2020 at maternity hospitals in Baghdad city. Non probability (purposive sample) was used to collect the data from (100) women who attending outpatient clinics at maternity hospitals. A questionnaire was constructed about knowledge assessment tool were designed and prepared by the researcher. A pilot-test is conducted in order to determine the reliability of the questionnaire in a sample of (20) women who were excluded from the study sample. Data was analyzed through the use of SPSS version 20.

Results: The main results revealed that the highest percentage (41.6%) of women's age was (20-29) years. More than half of them (60 %) are graduated from Secondary Nursing School. They have inadequate knowledge. There are no statistical significant differences between level of knowledge and women's age or educational level.

Conclusion: This study shows that the knowledge of study sample concerning Hydatitiform mole was inadequate.

Recommendations: It is important to design a booklet or pamphlet about Hydatitiform mole risk factors, signs and symptoms, screening, to reach for all women over the country in order to early detection and prevention.

Keywords: *Knowledge, Hydatidiform Mole, Molar Pregnancy, Women*

Introduction

Hippocrates (470–410BC) was first who described the moles and explained that if the pregnant women consume some dirty water the mole will be formed, where the water originates from the marshes. William Smelie (1752) was employed the terms mole and hydatidiform [1].

hydatidiform mole (HM) is a condition in a category of pathologies known as Gestational Trophoblastic Disease (GTD) that are resulted from abnormal fertilization. GTD encompasses HM, invasive mole, and choriocarcinoma. These diseases are range from benign to invasive neoplasia. Fatal outcomes in the past were a high rate from these pathologies, while it has a (98%) cure rate currently with proper diagnosis and treatment. HM is the most common form of GTD and can be cure if early diagnosed and treated. molar pregnancy has a risk of recurrence in subsequent pregnancy [2].

The incidence of Complete HM In the developed countries is about (one to three per 1000 pregnancies) and the Partial HM about (three per 1000 pregnancies). These moles are not recurrent except in the rare cases, when a single family member has recurrent HM known

as singleton cases, and when at least 2 women from a family member have one or several HMs known as familial recurrent HMs [3].

Molar pregnancy has two types can be determined by analyzing the chromosome. With complete mole, all trophoblastic villi swell and become cystic. Embryo may not forms or if it form will dies early only 1 to 2 mm, with no fetal blood in the villi. On chromosome analysis, although the karyotype is an normal 46XX or 46XY, this chromosome component was only contributed by the father or a "empty ovum" was fertilized and the chromosome material was duplicated. With a partial mole, some of the villi form normally. The syncytiotrophoblastic layer of villi, however, is swollen and deformation. A macerated embryo of approximately 9 weeks' gestation may be present and fetal blood may be present in the villi. A partial mole has 69 chromosomes (a triploid formation in which there is three chromosomes instead of two for every pair, one set supplied by an ovum that apparently was fertilized by two sperm or an ovum fertilized by one sperm in which meiosis or reduction division did not occur). This can also happen if one set of 23 chromosomes is provided by one sperm and an ovum that did not undergo reduction division supplied 46. (Fig. 1) [4].

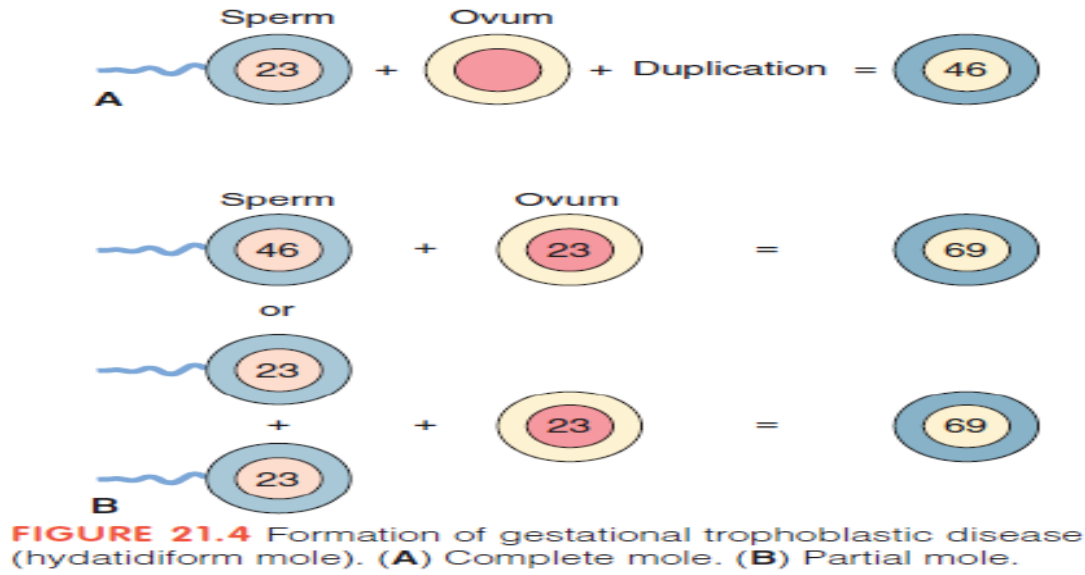


FIGURE 21.4 Formation of gestational trophoblastic disease (hydatidiform mole). (A) Complete mole. (B) Partial mole.

Figure 1 formation of HM (A) complete mole (B) partial mole

Pregnant women may be asymptomatic, or they may experience multiple symptoms such as Vaginal Bleeding, Hyperemesis, Increased Pelvic Pressure, Anemia secondary to bleeding, Maternal Hypertension, uterus enlargement more than expected for gestational age [5].

Molar pregnancy diagnosis is based on patient history, physical examination, human Chorionic Gonadotropin level, and ultrasound findings. the women should be assessed for any medical complications such as (anemia, preeclampsia, hyperthyroidism), which may need to be treated. Laboratory tests should include complete blood count, comprehensive metabolic panel, thyroid function test, urinalysis, and chest x-ray, as well as blood type and screen with cross match if anemic or uterus ≥ 16-week gestational size. An electrocardiogram and coagulation profile may also be indicated. Once the patient is determined to be hemodynamically stable, the most appropriate method of molar evacuation should be decided upon [2,6,7].

Preventive chemotherapy at a time or immediately after the prenatal evacuation is associated with a decrease in the incidence of complications from about (15 – 20) percent to (3- 8) percent. The use of prophylactic chemotherapy should be limited to special cases who have the risk of complications such as (women age

40 years, hCG> 100,000 mIU / mL, excessive uterine hyperplasia, theca lutein abscesses> 6 cm, medical complications) and / or upon inadequate hCG follow-up. Basically, all patients should following a hCG serial test after molar evacuation [8-9].

Materials and Methods

Descriptive analytic study design was conducted among women attending outpatient clinics at maternity hospitals to assess their knowledge about molar pregnancy. The study was performed from November 2019 to February 2020 at maternity hospitals in Baghdad city. Non probability (purposive sample) used to collect the data from (100) women. A questionnaire was designed by the researcher about knowledge of molar pregnancy assessment tool. A pilot study conducted in order to determine the reliability of the questionnaire in a sample of (20) women who excluded from the study sample (r1= 0.96). Content validity was determined through a panel of (10) experts their experience mean and SD was 28.82 ± 7.5. The data was collected after obtaining the agreement from women to participant in this study. The study instrument was consisted of two parts which include: Socio demographic characteristics, women's knowledge about molar pregnancy consisted of (13) items. Data are analyzed through the use of SPSS (Statistical Process for Social Sciences) version 20.

Table (1) Distribution of Study Sample According to Socio-demographic Characteristics (n=100)

Socio Demographic Characteristics	F	%
Age / years		
< 20	3	3
20-29	42	41.6
30-39	37	36.6
40-49	18	17.8
Mean=30.97 SD=6.5		
Marital Status		
Married	90	90
Divorced	5	5
Widow	5	5
Educational level		
Primary school	1	1
Secondary School	60	60
Institute graduate	17	17
College graduate	19	19
Master and higher	3	3

This table illustrates that the highest percentage (41.6%) of women's age were (20-29) years. Regarding marital status the higher percentage (90%) of them were married. Regarding educational level more than half of them (60%) were graduated from secondary school.

Table (2) Women's knowledge about Hydtitiform mole disease (n=100)

No.	Item	Correct answer		False answer		MS	RS	Ass
		F	%	F	%			
1.	Hydtitiform mole is pregnancy outside uterus	53	53	47	47	1.53	76.5	M
2.	Hydtitiform mole has two types	29	29	71	71	1.71	85.5	M
3.	The Hydtitiform mole occurs as result of dysfunction in uterus	41	41	59	59	1.41	70.5	L
4.	The Hydtitiform mole likely to occur in women with age older than 30	36	36	64	64	1.36	68	L
5.	Infertility is a risk factor of Hydtitiform mole	55	55	45	45	1.45	72.5	L
6.	Bleeding is a symptoms for Hydtitiform mole	44	44	56	56	1.56	78	M
7.	Hydtitiform mole usually diagnosed at first month	33	33	67	67	1.33	66.5	L
8.	HCG level in Hydtitiform mole is high	45	45	55	55	1.55	77.5	M
9.	The early treatment of the Hydtitiform mole is chemotherapy	23	23	77	77	1.23	61.5	L
10.	The women is advised to use contraception to avoid Hydtitiform mole recurrence	74	74	26	26	1.26	63	L
11.	Patient with Hydtitiform mole use contraception for 6 month	44	44	56	56	1.56	78	M
12.	Future Pregnancy for patient with Hydtitiform mole can be normal	59	59	41	41	1.41	70.5	L
13.	The causes of Hydtitiform mole is Viral	47	47	53	53	1.47	73.5	L

(M.S) mean score (R.S) relative sufficiency (Ass.) assessment (Low= ≥ 75), (Moderate= 75.1-87.5), (High= 87.6-100)

This table shows that there are a moderate level of relative sufficiency and mean score regarding Hydtitiform mole knowledge for nurses in the items No. (1,2,6,8,11), while the remaining items was low level of relative sufficiency and mean score.

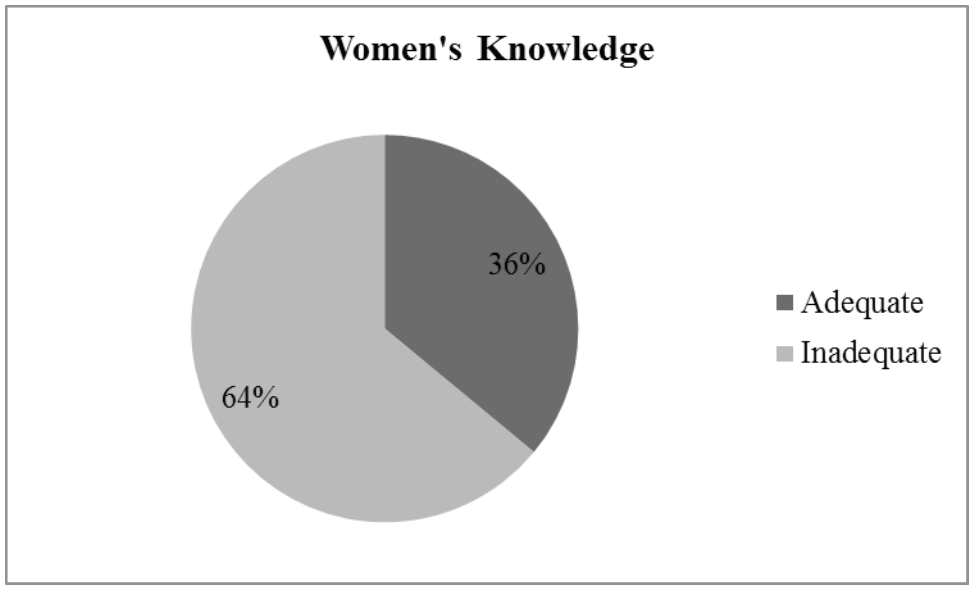


Figure (2) **Women's knowledge toward molar pregnancy**

Table (3) Association between women's knowledge and Socio-demographic variables (n = 100)

Women's Knowledge / Socio-demographic variables		Adequate		Inadequate		Chi square statistics			Sig.
		F	%	F	%	Value	df	P-value	
Age / years	< 20	2	66.7	1	33.3	2.258	3	.521	Ns
	20-29	13	31	29	69				
	30-39	13	35.1	24	64.9				
	40-49	8	44.4	10	55.6				
Educational level	Primary school	1	1	0	0	2.981	4	.561	Ns
	Secondary School	25	36.8	43	63.2				
	Institute graduate	7	38.9	11	61.1				
	College graduate	2	20	8	80				
	Master and higher	1	33.3	2	66.7				

(df) degree of freedom, (Sig) significant Probability value ($P < 0.05$), (NS) Non Significant,

(s) significant.

This table shows that there are no statistical significant between socio-demographic variables and women's knowledge about Hydtitiform mole.

Discussion

The finding of this study show that the highest percentage of women (42.3%) was at age group (20-29) years. Regarding marital status the higher percentage of study sample (59.6 %) was married. Regarding educational level more than half of them (62.5%) were graduated from secondary school.

The condition tends to occur most often in women who have a low protein intake, in women older than age 35 years, in women of Asian heritage, and in blood group A women who marry blood group O men[4].

Patients with complete moles were registered in England and Wales, from (2000 to 2009) was (5,793) and with partial moles was (7,790), compared with a total number of (8,242,511) conceptions. The incidence of overall molar pregnancy was one for every (607) conceptions (complete mole was 1:1,423 and partial mole was 1:1,058), but with major variations with patient's age. Complete moles, the risk varied (1:1,000 for women aged 18 – 40 to 1:156 for women aged 45 and 1:8 for women aged 50 and above) [10].

The risk of complete mole and partial mole is strongly associated with maternal age; the researchers calculated the overall recurrence risk. They stated most women with a pregnancy affected by molar pregnancy were within the age (20 to 40). The relationship with maternal age was not significant, and the recurrence rate (1.5–2.0%) was likely to be accurate[11].

This study result show that women's knowledge was inadequate about Hydtitiform mole, but there are a moderate level of relative sufficiency and mean score regarding Hydtitiform mole knowledge for women in the items "Hydtitiform mole is pregnancy outside uterus, Hydtitiform mole has two types, Bleeding is a symptoms for Hydtitiform mole , HCG level in Hydtitiform mole is high, Patient with Hydtitiform mole use contraception for 6 month"

The items was low level of relative sufficiency and mean score include "The Hydtitiform mole occurs as result of dysfunction in uterus, The Hydtitiform mole more likely to occur in women with age older than 30, Infertility is a risk factor of Hydtitiform mole , Hydtitiform mole usually diagnosed at first month, Early treatment of the Hydtitiform mole is chemotherapy, The women is advised to use contraception to avoid Hydtitiform mole recurrence, Future pregnancy for patient with Hydtitiform mole can be normal, the causes of Hydtitiform mole is viral"

Women should be aware of the signs and symptoms, required diagnostic testing, and follow-up for molar pregnancy to prevention of complications.

A woman suffering from a pregnancy loss, she must face the early end of pregnancy and the loss of expected life with her baby. A little attention has been paid to the psychological and social needs of women who have molar pregnancy, though much has been written about perinatal mortality in general. Their unique condition as obstetric diseases is not only loss of the fetus but also anomalous growth of trophoblastic tissue. However, women who suffer from molar pregnancy are still experiencing an expected child loss[12].

Conclusion

This study revealed that women's knowledge was inadequate about molar pregnancy and there are no statistical significant differences between level of knowledge and women's age or educational level.

Recommendation

Women should be informed about molar pregnancy to understand their signs and symptoms for early detection of molar pregnancy to prevention of invasive mole and choriocarcinoma.

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Conflict of Interest: None declared

Ethical Approval: Not required

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Adherence and Satisfaction of Deferasiroxversus Deferoxamine in Transfusion-Dependent Beta-Thalassemia

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Abstract

Objectives: The present study was aimed to compare the adherence and satisfaction with deferoxamine (Desferal®) versus deferasirox (Exjade®), a novel oral iron chelator, in patients with transfusion-dependent beta-thalassemia.

Patients and Methods: In this cross-sectional, single-center study, 108 homozygous βthalassemia major patients aged between 3-21 years old were enrolled. They were on regular blood transfusions and iron chelators (either deferoxamine or deferasirox) for about three months. Another fifty-six apparently healthy non-thalassemic subjects were enrolled as a control group. **Results:** Adherence and satisfaction wereassessed byusing a simplequestionnaire establishedduring an interviewwithpatientsortheirparents.The serumferritin level was measuredbythe ELISAmethod**.**

Our findings showed that adherence and satisfaction in the deferasirox group were more than that with deferoxamine group patients. Serum ferritin level in the deferasirox group was significantly lower than in the deferoxamine group at ($p<0.001$). while the control group was significantly lower than both patients groups at ($p<0.001$). Patients on deferasirox had lower adverse effects than the deferoxamine group since deferoxamine produce irritation and pain at the injection site for the patients.

Conclusion: Our findings concluded that deferasirox is an effective iron chelator as deferoxamine but with better adherence and satisfaction than deferoxamine. Deferasirox can be used as a preferred iron chelator therapy in iron-overload patients with beta-thalassemia.

Key Words: *BetaThalassemia, Satisfaction, adherence, Iron Chelators, Deferoxamine, Deferasirox.*

Introduction

Beta-thalassemia may be a severe inherited blood disease that has been known to be caused by a mutation within the β-globin gene resulting in the excessive destruction of red blood cells1,2. it's been estimated that over 42000 newborns are suffering from β-thalassemia per annum worldwide. Without transfusion, β- Cooley's anemia causes death amongst infected children before the age of three years old 2. Thalassemia may be a disease status during which patients need continuous control and management, regular blood transfusions can prevent death and reduce mortality. However, excessive iron accumulated from transfused red blood cells can cause organ failure3,4. Therefore, iron chelation treatment is important to scale back the iron store within the body and improve the long-term survival rate of patients with thalassemia, which is taken into account important adjuvant therapy.4

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Currently, the most iron chelators available for clinical use in Iraq are deferoxamine and deferasirox. Deferoxamine, which needs routine intravenous or subcutaneous injections from 5 to 7 days weekly, has been considered a typical therapy for hemochromatosis over the past four decades.However, the treatment with deferoxamine for about 8 to 12 hours daily may lead to poor compliance and a few negative impacts like skin rash, hematological toxicity, and heart problems 5-7.

Deferasirox, a once-daily oral iron chelator, was introduced in 2005 as first-line therapy for patients over 2 years aged with chronic hemochromatosis thanks to blood transfusions5. Although Deferasirox has some mild adverse events, some studies indicated that it's a positive effect on lowering liver iron and producing high patient compliance8,9,10.

Similar to normal individuals, Thalassemic patients should have normal life status, however, continuous transfusion, iron chelation, and clinic visits have an excellent psychological impact leading to rejection of taking their medical management at the proper way, which assessed by satisfaction and compliance.

The present study was aimed to match the satisfaction and adherence with the deferoxamine group versus the deferasirox group. to verify the efficacy of both chelators; serum ferritin was also measured to match patients groups and with the control group.

Materials and Methods

A cross-sectional, single-center investigation study was carried out with the cooperation of the medical staff of Thalassemic Center of Ibn Al-Atheer Teaching Hospital in Nineveh Province, Mosul, Iraq. Ethical clearance was obtained from the Research Board Committee after approval of the protocol.

One hundred and eight patients of 3 to 21 years old of age with a diagnosis of β-thalassemia and having a chronic iron overload from regular blood transfusions as assessed by serum ferritin of > 600 ng/ml were divided into, firstly deferoxamine group treated by 20-50 mg/kg/day (n = 54, 30 male & 24 female) with age mean (13.2±4.03 years), second deferasirox group treated by 10-30 mg/kg/day (n = 54, 31 male & 23 female) with age mean (9.65±3.32 years), and the third control group of apparently health subject (n = 56, 36 male & 20 female) with age means (11.8±5.3 years). In this study satisfaction and adherence was assessed by using a questionnaire which usually done during an interview and reporting notes for each patient

Informed consent was taken from the patients or their relatives, a questionnaire regarding the personal and treatment details was obtained as name, age, sex, frequency of transfusion, type of chelation used, satisfaction, and adherence.

Patient compliance depended on the patient's use of deferoxamine. Five to seven times/week were regarded as compliance, while for Deferasirox patients, six to seven times/week were regarded as compliance. The iron overload was assessed by serum ferritin determination by using Ferritin AccuBind® ELISA - Monobind Inc. (Lake Forest, California, USA) commercial kit, depending on

Chemwell 2910 fully automated devices for enzyme-linked assay methods.

Data are presented as mean ± S.D. and were analyzed by using one way ANOVA test and nonpaired t-test were used to compare between the groups. RResults were considered statistically significant at $p \leq 0.05$. The correlation was done using Pearson and spearman's rho depending on the type of data (parametric or nonparametric).

Results

Table 1 shows that patients treated with deferasirox significantly were more satisfied in comparison with those treated with deferoxamine (90.7% vs 66.7%, respectively). Among patients who had previously taken deferoxamine then received deferasirox before the study 77% were reported a preference for deferasirox than deferoxamine.

The proportion of patients' compliance was greater in those receiving deferasirox than in those receiving deferoxamine (90.7% vs 61.1%), as shown in Table 1.

In this study serum ferritin levels within and between groups shows a wide range of variation.

There is a significant difference ($p<0.001$) in the serum level of ferritin between the deferoxamine group

(3891.2±1845.6 ng/ml) as compared with the deferasirox group (2212±1485 ng/ml).

Also, there are high significant variations ($p<0.001$) in each of the deferoxamine group and deferasirox group when compared with the Control group with (26.4±19.7 ng/ml) as shown in Table 2. The importance of adherence can be determined by serum ferritin level mean for all patients (N=108, 3051±1868 ng/ml) and when grouped as adherence patients (N=82,2620.4±1713.2 ng/ml) and non-compliance patients (N=26, 4411.6±1704 ng/ml), a significant difference ($p<0.001$) presented as shown in Figure 1.

Figure 2 represents the distribution of ferritin level data frequency for non- adhered patients that appear after the mean of all patients (3051ng/ml) especially for deferoxamine treated patients, while the distribution for compliance patients before the mean of all patient especially for deferasirox treated patients.

By determining correlations for all patients' data (N=108), the Pearson correlation showed a significant positive correlation ($p<0.01$) between age and ferritin level ($r = 0.374$), and negative correlation ($p<0.01$) between serum ferritin level and adherence ($r = -0.412$). Moreover, there is a significant positive correlation ($p<0.01$) between compliance and satisfaction ($r = 0.342$) by using Spearman's rho correlation (nonparametric correlation) these correlations are shown in Table 3.

Table 1. Percentage of satisfaction and adherence for deferoxamine and deferasirox

	Parameters	Deferoxamine Group (N=54)	DeferasiroxGroup (N=54)
Satisfaction	Satisfied	66.7%	90.7%
	Non Satisfied	33.3%	9.3%
adherence	adhered	61.1%	90.7%
	Non adhered	38.9%	9.3%

Table 2. Comparison of serum ferritin between deferoxamine group and deferasirox group and each group with controls group

Parameters	Control Group	Deferoxamine	Deferasirox Group
Serum ferritin Level	26.4±19.7	3891±1845a	2212±1485b,c

a $p<0.001$ vs control; b $p<0.001$ vs control; c $p<0.001$ vs deferoxamine group

Table 3. Relationship between studied parameters for all patients

Correlations R value p-value				
Serum ferritin level	**Age**	**N=108**	**r = 0.374**	**p<0.01**
Serum ferritin level	Adherance	N=108	r = -0.412	p<0.01
Adherance	Satisfaction	N=108	r = 0.342	p<0.01

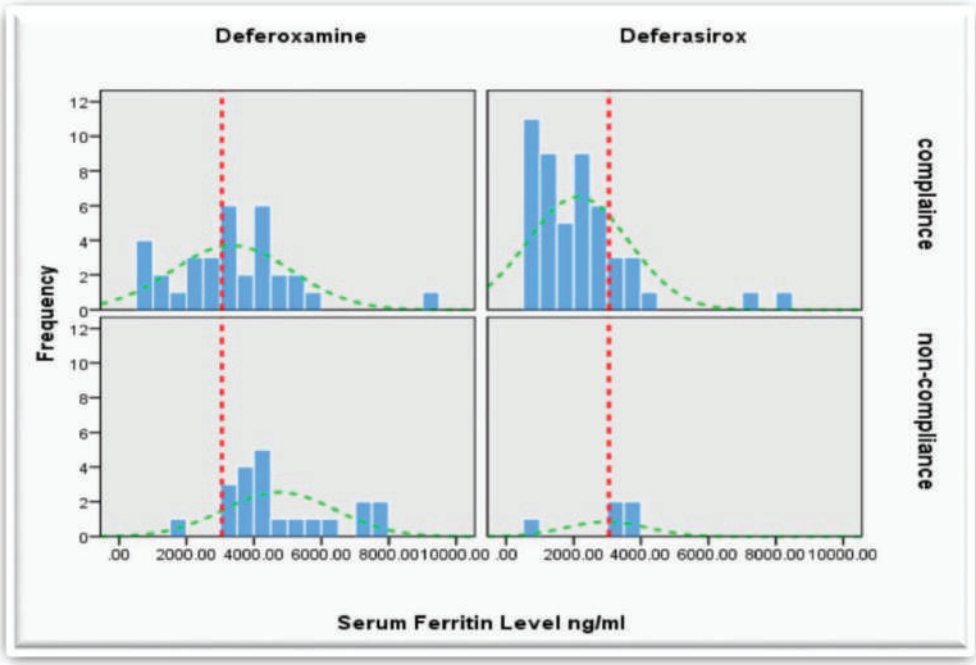


Figure 1. Frequency of Distribution of serum ferritin level among cmplianed and non-complianed patients for deferoxamine group and deferasirox group

Discussion

Iron chelators act by removing transiently available (labile) iron pools, either in plasma or within cells, because cellular storage iron (present as ferritin and hemosiderin) are not directly accessible for chelation11-13. Chelate iron is derived from two main sources: (a) iron released from macrophages after the catabolism of red cells and (b) iron released within cells after the catabolism of cellular ferritin and hemosiderin. For transfusion-dependent thalassemia major patients, it is important to choose an appropriate iron chelator to reduce iron burden in the body to prolong and improve the quality of life [14,15.]

This study showed that Deferasirox had a rational better satisfaction and compliance than deferoxamine, especially for younger ages. Some older ages prefer deferoxamine because they are adapted on deferoxamine as part of their life or they cannot tolerate deferasirox. From another view no one of the chelation has very satisfied chelator due to limitation of normal life, so patients opinions about deferoxamine come from problem of subcutaneous injection for long period (8-12 hours per day) with painful and ulceration at injection sites, these agree with (Rofail et al, 2009) who showed administration of deferoxamine slow subcutaneous infusion negatively impacts on satisfaction which was shown to be a determinant of adherence[16]. While Deferasirox has taken orally as an effervescent tablet once daily is more suitable, but have the problem of bitter taste with slight large particle size cause irritation to the larynx and induce nausea, that agrees with (Trachtenberg et al, 2011) when showed patients with poor adherence to Deferasirox noted stomach pain and bad taste [17]. Deferasirox is preferable by 77% of patients who had previously been treated with deferoxamine. The reasons for preferring Deferasirox were, more convenient to be taken, less disruptive to their day or their sleep, and to their families, that mentioned by (Cappellini et al,2007)[5].

Serum ferritin is used as an indirect measure of iron overload, it is not an accurate assessment but gives a fairly good idea of iron overload [18,19]. In this study, serum ferritin at the Deferasirox group was lower than the deferoxamine group.

Pennel et al, (2014) when compared between deferoxamine versus Deferasirox showed a small difference of serum ferritin level means at the end their study 3129 ng/ml versus 3375 ng/ml respectively [20], that

disagree with the present study which had significant means variation p<0.001 between groups 3891 ng/ml versus 2212 ng/ml respectively these may be due to more compliance for Deferasirox than deferoxamine that leads to decrease ferritin level more than non-compliance while the result of this study agree with (Bashir and Sadoon, 2010) as they concluded that serum ferritin is higher in an older patient, the higher rate of blood transfusion and poor compliance [21].

Also (Mahmoud and Aziz, 2012) showed significant variations p<0.001 between serum ferritin levels of patients on deferoxamine and patients on Deferasirox and also for good compliance and poor compliance[22].

Improved satisfaction and convenience of a once-daily oral dose of Deferasirox compared to deferoxamine has been shown in other studies10,23 which may be translated into increased compliance as showed in this study that leads to improving health outcomes and quality of life.

The minor adverse effects with ease of use of deferoxamine or Deferasirox act as a choice factor for patients to determine satisfaction and compliance that in turn decrease ferritin level and consequently decrease complications, morbidity, and mortality.

Conclusion

It has been concluded that deferasirox is an effective iron chelator as deferoxamine but with a better satisfaction and compliance and lowered serum ferritin level than that of deferoxamine, in mild to moderate iron overloaded patients.

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Clinical Treatment of UTI in Rats Induced by Pathogenic E. Coli

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Abstract

The present study objective was to evaluate the therapeutics effects of ciprofloxacin and gentamicin on UTI induced experimentally by *E. coli* in rats. Forty female rats were divided into two groups, infected (30 rats) and control (10 rats). The infected group were inoculated intraurethral by 10^8 suspension of E. coli. Then the infected group subdivided into 3 subgroups, G1 which serve as control positive, G2 which inoculated by *E. coli* and then treated by ciprofloxacin 50mg/kg, G3 were inoculated by E. coli then treated with gentamicin 40 mg/kg.Treatment with antibiotics started 24 hours after bacterial inoculation and finished 72 hours after the initial therapy. The length of antibiotic medication was chosen for both antibiotics as a short-term 3 days therapy.The animals were monitored for presence of signs. E. coli urine bacterial demonstration were done at 1 week before infection and 24hrs., 48hrs, 96hrs, 6 days, 12 days and 24 days after infection. There was significant increase ($P<0.05$) in *E. coli* viable count in all infectedgroups, in addition the result of optical density (OD) by using spectrophotometry record increased threshold of the OD of the urine culture of all infected groups after (24 hrs.) of infection compared with control group, The current results showed that UTI symptoms and bacterial isolation were decreased after treatment by both ciprofloxacin and gentamicin as compared with G1 group.

In conclusion, ciprofloxacin and gentamicin can alleviate the symptoms of UTI after single dose.

Key words: *E. coli, Ciprofloxacin, gentamicin, rat.*

Introduction

Escherichia coli has been implicated to causes disease in human [(1)] as well as animals [(2; 3, 4, 5, 6; 7; 8)]. In addition to gastrointestinal effects, it can cause UTI, meningitisin neonates and humanssepticemia [(9)].

The presence of a specific virulence factors, adaptations of the microbial, encouraging urinal tract achievement, distinguishes *E.coli* which is a source of UTI and another uropathogens from associated memberships of their genus and species [(10)]. *Escherichia coli* are the most common organism causing Lower (UTI). Although not all strains of *E. coli* are pathogenic to the urinary tract which suggested that the infective *E. coli* strains are a selected group with special properties enabling them to survive and multiply in the host tissue [(11)].

Regardless of their scope, pharmacokinetic qualities and overall strong resistance, fluoroquinolones are actually among the most used antimicrobials in the world. They are particularly helpful in the treatment of infections of the urinary tract (UTI) by enterobacteria. [(12)].It was shown that therapeutic effectiveness of intravenous ciprofloxacin in mice was comparable to that after oral administration when viable bacterial counts in the kidney were calculated 24 hours after inoculation[(13)].

Also, it has been showed that the potency of gentamicin ranged over a 24-hour cycle and the

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effectiveness at the moment when toxicity was lowest when UTI cases triggered by *E. coli* were treated.[14].

The goal of the current research was to determine the efficacy of UTI treatments induced by *E. coli*using gentamicin and ciprofloxacin.

Materials and Methods

Forty female rats were divided into two groups, infected (30 rats) and control (10 rats). The infected group were inoculated intraurethral by 10^8 suspension of *E. coli* according to [15]. Then the infected group subdivided into 3 subgroups, G1 which serve as control positive, G2 which inoculated by E. coli and then treated by ciprofloxacin 50mg/kg, G3 were inoculated by E. coli then treated with gentamicin 40 mg/kg.

Therapy started 24 hours after the bacteria were inoculated and finished 72 hours after the initial therapy. Period of antibiotic therapy was chosen for quick treatment with both antibiotics for three days.

The animals were monitored for presence of signs. *E. coli* urine bacterial demonstration were done at 1 week before infection and 24hrs., 48hrs, 96hrs, 6 days, 12 days and 24 days after infection.

All data were analyzed statistically as described by [16].

Results and Discussion

Bacterial count fromurine was done by using pour plate method.[17] Comparison among other urine counting processes for E. coli and finding that it was simpler and more practical to execute this process and spread plate system on MacConkey agar.

A Significant change ($P<0.05$)was found in *E. coli*countfor allinfected groups,these result in agreements with[18, 19]. Around the same time as the amount of bacteria in the contaminated groups was equivalent to the group A (-ve) only group A (-ve) displayed a substantial difference in the number of bacteria contaminated as contrasted with their number before the infection occurred.

These results are in agreement with [20,21]that demonstrated the effective colonization of *E. coli* by the inoculated rats with (2.6×10^6) CFU / ml within 24 hrs., by administering pathogenic *E. coli*, following urinary tract infection in rats via Intra-urethral way. The current results showed that UTI symptoms and bacterial isolation were decreased after treatment by both ciprofloxacin and gentamicin as compared with G1 group(table 1). Jakobsen et al. [22]reported that a higher antibiotic concentrations shows in the mouse urine after ciprofloxacin mediated to UTI mouse.

It has been showed that a good efficacy of norfloxacin againstpathogenic *E. coli*, and prevents the adherence of pathogenic *Escherichia coli* and dose-dependent they do so[23].

These anti-adhesive properties have an effect both on uropathic*E. coli* which can be antibiotically prone and antibiotic resistant. Antimicrobial infections are now commonly considered to stop bacteria clinging to the uroepithelium of the kidney, preventing capacity. Such findings align with a new analysis that, in terms of composition, norfloxacin is distinct from another antibiotics by the inclusion of the fluorine atom in position 6, a pipera-Zin ring in position 7 and a carbon atom replacement with a nitrogen atom in position 8.[24]. Such substitutes improve action against gram positive and gram negative bacterial infection[25].

Intravenous ciprofloxacin in all infection models tested has more than consequences following oral administration[13].

The interpretive parameters suggested for norfloxacin susceptibility measures, of example, are still preliminary and planned for usage in the clinical trials [26]. After the therapeutic feasibility papers based on this checklist, the ultimate decision may only be rendered[27].

One research [28] shows that aminoglycoside treatment is less likely to change bowel or vaginal flora, which could decrease the risk of bacterial resistive strains and Clostridium difficile colonization.

It must also be taken into consideration specific non-drug triggers of acute kidney injury (e.g. depletions of intravascular volume). Just 1.6 per cent reported temporary serum creatinine changes with no raises in serum creatinine in the clinical study of 24,107 patients obtain a single gentamicine dose with trials where all patients had age < 75 years[29].

In conclusion, ciprofloxacin and gentamicin can alleviate the symptoms of UTI after single dose.

Table 1. Different bacterial volumes before and after treatment of animals by ciprofloxacin and Gentamicin

Period	Control	G1	G2	G3
1 week before infection	1×101±0.01 Aa	2.0×102±0.02 Ba	2.3×102±0.03 Ca	2.3×102±0.001 Ca
24 hrs.	1×101±0.03 Ab	6.0×107±0.02 Aa	5.2×106±0.34 Ba	5.7×106±0.02 Ba
48 hrs.	1×102±0.03 Ab	7.3×108±0.5 Aa	4.0×106±0.4 Aa	4.3×106±0.05 Aa
96 hrs.	1.2×102±0.001 Ab	6.5 ×107±0.3 Aa	3.2 ×104±0.1 Aa	3.4×104±0.06 Aa
6 days	1.1×102±0.001 Ab	6.0×106±0.1 Aa	3.4×104±0.14 Aa	3.5×104±0.4 Aa
12 days	1.3×102±0.02 Ab	6.1×106±0.3 Aa	3.1×103±0.11 Aa	3.7×103±0.7 Aa

Capital letters denotes differences at P>0.05 between vertical lines

Small letters denote differences at P>0.05 between horizontal lines

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Artemesiaannua L. Silver Nanoparticles Exhibit Anticancer Activity by ROS mediated apoptosis in MDA-MB-231 Cells

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Abstract

Aim of this study was to investigate the growth inhibitory effect of *Artemesisannua* L. silver nanoparticles (A.annuaAgNPS) on human triple negative breast cancer tumor cell line MDA-MB-231. Cells were cultured and treated with A.annua water extract and A.annuaAgNPS to determine the half inhibitory concentration (IC_{50}). Oxidative stress was determined using lipid peroxidation (LPO), ROS generation and by determining the levels of antioxidants, superoxide dismutase (SOD) and reduced glutathione (GSH). Further, the mechanism of apoptotic induction was assessed by DNA fragmentation analyses. We observed that A.annuaAgNPS significantly caused growth inhibition by increasing levels of LPO and ROS, in a dose dependent manner with reduced level of endogenous antioxidant enzymes. Increased shear in genomic DNA from MDA MB231 cells treated with A.annuaAgNPS shows apoptosis to be the cause of cell cell death. We conclude that A.annuaAgNPS can be useed as a therapeutic agent against breast cancer.

Keywords*: Artemesiaannua· Silver nanoparticle· Breast cancer· Apoptosis*

Introduction

Breast cancer is the leading cause of death among women globally with 1 million new cases every year with an estimated prediction of 14 million new cases by 2035. Breast cancer mortality rate is gradually increasing due to conditions like poor prognosis, lack of effective treatment options and increasing drug resistance [1, 2]. Recently, breast cancer reports are increasing rapidly at an alarming rate, preceding the cervical cancer in India [3]. Breast cancer is classified into 3 major subtypes based on the molecular markers for estrogen or progesterone receptors and human epidermal growth factor 2 (HER2): hormone receptor positive (HER2) negative (70%), HER2 positive (15%-20%), and triple-negative (tumors lacking all 3 standard molecular markers; 15%). Notably, triple-negative breast cancer recur more frequently than the other 2 subtypes, and it denotes that it warrants greater attention towards identifying therapeutic regimens that can inhibit and as well prevent its recurrence.

In recent years, role of metal nanoparticles in delivering and targeting agents that are beneficial at pharmaceutical, therapeutic and diagnostic levels are gaining much importance [4]. Particularly, in disease like cancer where lack of target specificity for anticancer agents remains a greater challenge. Use of silver nanoparticle mediated drug delivery exhibit significant clinical benefits [5]. Biogenic (green chemistry) synthesis of silver nanoparticles (NPs) using plant extract were reported to exhibit useful clinical benefits than other forms of synthetic AGNPs [6]

Artemisia annua L. (commonly known as sweet wormwood, qinghao) is native to China for about 2000 years. It was popular for its unique potential as a natural antimalarial agent, as an anti-oxidant, with anti-inflammatory and anti-microbial effects [7]. The present study focused on investigating the *in vitro* cytotoxicity of biogenic AgNPs using A.annua using MDA-MB-231 cell line. Our results demonstrated that A.annuaAgNPs exhibit significant cytotoxicity, which can be attributed

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to its ability to induce oxidative stress, mitochondrial dysfunction and apoptotic induction. We believe that investigation of nanoparticle research for treatment of cancer therapy will bring more significant clinical outcomes in near future.

Materials and Methods

Chemicals and their sources:

Trypsin, Bovine serum albumin (BSA), Dimethyl sulfoxide (DMSO) 3-[4, 5-Dimethyl-2-thiazolyl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). RPMI 1640 medium, fetal bovine serum (FBS) and antibiotics were purchased from Gibco (Carlsbad, CA, USA). FBS was stored at −20°C and then freshly prepared aliquots were used for cell culture complete medium. All the other chemicals used were of cell culture grade unless otherwise stated.

Cell line and culture:

Triple negative human breast cancer cell line MDA-MB-231 was purchased from National centre for cell science (NCCS), Pune. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-Penicillin streptomycin solution. Cells were maintained at a controlled condition of 5% CO_2, and95% humidity. Experiments were performed in 6-well plates and 96 well plates unless stated otherwise. Cells were seeded at a density of 1×10^6 and 1×10^4 cells per well and incubated for 24 h prior to the experiments. The cells were washed with ice cold-phosphate buffered saline, (pH 7.4) for immediate use in experiments and for storage of cell pellets.

Cytotoxicity assay:

Effect of A.annuaAgNPs (dose dependent) on cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide dye reduction. Briefly, MDA-MB-231 cells (1×10^4 cells) were plated onto 96-well flat bottom culture plates and treated with various concentrations A.annuaAgNPs. For 24 h. After 24 h of incubation, 10 ☐L of MTT (5mg/mL in PBS) was added to each well, and then incubated for 2 h at 37°C in dark. During the course of incubation, the appearance of purple precipitate in the wells was monitored using inverted microscope and then the growth medium was removed from each well including blank. Then 100 ☐L of DMSO per well was added to dissolve the formazan, including the blank and then mixed to obtain homogenous solution via gentle shaking at 37°C in a plate reader, and absorbance was measured using ELISA reader at 595 nm. Finally IC50 value of A.annuaAgNPs was calculated and used for further experiments.Results were expressed as mean of three independent experiments.

Intracellular ROS analysis:

Briefly, MDA-MB-231 cells were treated with A.annuaAgNPs for 12 h. The net intracellular ROS measurement was carried out based on the intracellular peroxide-dependent oxidation of 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Molecular Probes, Eugene, USA) into a highly fluorescent product 2',7'-dichlorofluorescein (DCF), as reported previously. MDA-MB-231 cells were seeded into 35 mm dish at a density of $1X10^5$ cells/ml and incubated overnight. Following 12 h treatment with A.annuaAgNPs the cells were incubated with DCFH-DA (10 µM) for 1 h. Along with the experimental cells, freshly prepared hydrogen peroxide (H_2O_2, 0.1 mM in PBS) was used as a positive control. Then the excess DCFH-DA, from the dishes were removed and washed with PBS (twice) and suspended in 500 µl PBS. Finally the amount of ROS was estimated from fluorescence intensity of DCF production using a spectrofluorometer (excitation at 485 and emission at 530 nm).

Measurement of Lipid peroxidation Products:

Briefly, 2×10^4 cells/well were seeded in a 96-well plate (black walled) and incubated at 37°C in CO_2 incubator. MDA-MB-231 cells were treated with and without A.annuaAgNPs for 24 h and harvested by centrifugation. Extent of lipid peroxidation was assessed in control and A.annuaAgNPs treated cell lysate by measuring malondialdehyde (MDA) levels by colorimetric assay, according to the method described previously[8]. Reaction of two molecules of thiobarbituric acid reactive substances (TBARS) with one molecule of MDA which yields pink color from the final soluble contains the substances. Absorption of the samples was read at 532 nm using a UV–vis spectrophotometer. Amount of MDA for each treatment was normalized by protein concentration and expressed as nmol TBARS per mg of protein lysate.

Measurement of antioxidant markers:

The levels of antioxidant markers SOD and GSH

were analyzed in MDA-MB-231 cells treated with and without A.annuaAgNPs (dose dependently) for 12 h. Superoxide dismutase (SOD) was analyzed by kinetic absorption method prescribed previously by Aebi and Misra, [9]. SOD was expressed as units/min/mg of protein. Glutathione (GSH) was measured by previously reported method [10] and the results were expressed in terms of µg of GSH/mg of protein.

DNA fragmentation analysis:

Cells were treated with A.anunaAgNPS for 24 h in sterile tissue culture dishes and DNA fragmentation analysis was based on the method of Kasibhatla et al. (2006)[11]. Briefly, 10^6 cells/ml after treatment with AgNPS and were harvested and transferred to sterile eppendorf tubes and lysed with 20 µl Tris, EDTA-SDS lysis buffer by constant pippetting using a pipette tip. Lysates were mixed with RNase A and RNase T1 (10 µl), incubated at 37ºC for 60 min. After incubation, the lysate is treated with proteinase K (10 µl) at 50ºC for 90 min. Finally, about 5 µl of 6X DNA loading buffer was added to each sample and electrophoreticallyseperated on 1.5% agarose gel (with ETBR) in TAE buffer (100 V at RT, for 20 minutes). Then the DNA laddering pattern in treated and untreated cells were visualized by a UV light transilluminator and documented.

Statistical analysis:

Experiments were performed in triplicates (n=3). The mean ± standard deviation (SD) was determined for each group. One way analysis of variance (one-way ANOVA) and Tukeys□ test were used for statistical analysis. Statistical value of $p < 0.05$ was used for considering statistical difference between groups.

Results and Discussion

Anti-cancer drugs approved by food and drug administration (FDA) has been continuously escalating ever year, however there exists lack of complete cure, coupled with adverse effects of existing anti-cancer drugs [12, 13]. Need for better therapeutics with potential anti-tumor efficacy that can reduce the unprecedented challenge in healthcare against cancer are on the rise Natural agents like plant metabolites and its derivatives have been used traditionally for various ailments; however they were not optimized in suitable formats that might enhance their efficacy [14]. Recently plant derived therapeutic agents via green amalgamation with metal nanoparticles were reported to show promising effects in mitigating cancer progression and its recurrence [15, 16]. However, very limited studies have been carried out in this aspect and hence we aimed to investigate the anticancer effect of AgNPs biosynthesized using A.annua plant extract.

A.annuaAgNPs induce cytotoxicity

Biogenic A.annuaAgNPs induced cytotoxicity on MDA-MB-231 cells was found to be dose dependent. Change in percentage of cell viability in MDA-MB-231 cells was observed at A.annuaAgNPs concentration range (2.5 µg/ml, 5 µg/ml, 12.5 µg/ml, 25 µg/ml, and 50 µg/ml) after 24h of treatment. A.annuaAgNPs exerted complete mortality at 50 µg/ml concentration, while 50 % reduction was observed at 12.5 µg/ml (IC_{50}) compared to A.annua plant extract and untreated cells (Fig 1A). However, there was no significant difference in cell viability at similar concentrations after 24 h. Hence, 12.5 µg/ml was used for further experiments. Moreover, the tested concentration range mentioned above did not show any toxicity to normal vero cell lines (supp Fig 1). Thus the exhibited effect is highly specific to cancer cells.

***A.annua* induces oxidative stress in cancer cells**

To investigate the effect of A.annuaAgNPs in oxidative stress, ROS levels were measured using H2DCF-DA assay. Fig 2A shows that ROS generation was significantly elevated in cells treated with A.annuaAgNPS (12.5 µg/ml) than the control cells or A.annua extract treated cells. However, upon pretreatment with an antioxidant N-acetyl cysteine (NAC), ROS generation in A.annuaAgNPs and H_2O_2 treated cells was markedly reduced. Fig 2B shows that formation of TBARS was significantly increased in cells treated with A.annuaAgNPs, compared to control and A.annua extract treated cells. Together, our results showed that loss of cell viability upon treatment with A.annuaAgNPs is mediated via ROS generation, thereby altering the intracellular redox status which might lead to cell death via apoptosis.

Altered homeostasis in the balance between oxidants to antioxidants favors oxidative niche which leads to increased redox state of cells. Hence, to determine the status of anitoxidants during A.annuaAgNPs treatment, we analysed the levels of major endogenous antioxidants GSH and SOD. A.annuaAgNPs treatment to MDA-MB-231 cells caused significant dose dependent reduction in the levels of GSH and SOD compared to

control and A.annua extract treated cells (Fig 3).

Excessive ROS with augmented antioxidant defense can results in oxidation of cellular macromolecules including DNA resulting in apoptotic cell death [17]. Hence, ROS generation by AgNPs was considered crucial phenomenon in its anticancer potential. In our study elevated ROS levels in MDA-MB 231 cells treated with A.annuaAgNPs for 12 h denotes early induction of oxidative stress, while pretreatment with NAC reduced ROS generation and this denotes that there exists a lack of antioxidant defense in MDA-MB-231 cells during AgNPs exposure favouring oxidative niche. In addition ROS mediated oxidative stress in also substantiated by increased levels of lipid peroxidation product MDA, reduced levels of endogenous antioxidants GSH and SOD as observed in our studies. Together, our results demonstrate that A.annuaAgNPs mediated cytoxicity is a ROS dependent manner and altered endogenous redox status leading to cell death.

A.annuaAgNPs fragments DNA

DNA fragmentation is considered as the hall mark of apoptotic cell death, and the anti-cancer ability of therapeutic agents were tested by its ability to induce DNA fragmentation. Hence, we analysed the status of DNA fragmentation in MDA-MB-231 cells treated with A.annuaAgNPs. Fig 4 shows that A.annuaAgNPs induced significant DNA fragmentation as observed from increase in shear in lane 3 and also in DOX treated cells (lane 4) compared to control (lane 1), and A.annua extract (lane 2) treated cells. Together our results prove that A.annua induces apoptotic cell death in MDA-MB 231 cells. With promising results these can be tested on other tumor models and can be considered as a therapeutic agent against breast cancers.

In conclusion, we synthesized green AgNPs using A.annua extract and tested its cytotoxic potential against the breast cancer cell line MDA-MB-231.We found that the anticancer effect is mediated through oxidative stress with increased ROS production and declined antioxidant activity. Additionally apoptotic induction was mediated through DNA fragmentation. However, the ability of A.annuaAgNPs in orchestrating cellular signal cascades that is responsible for its anti-cancer effects must be validated using various cancer cell lines. Such mechanistic studies will help uncover many other potential applications of AgNPs for clinical use.

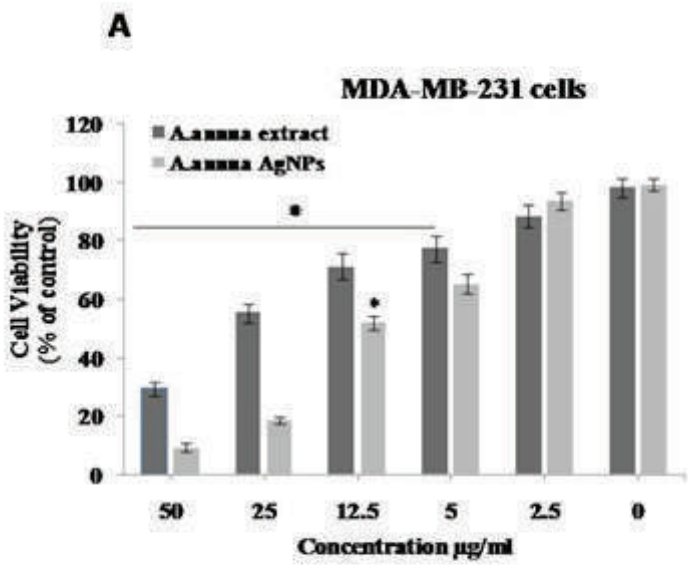


Fig 1: Shows cytotoxicity of A.annuaAgNPS.

Figure 2

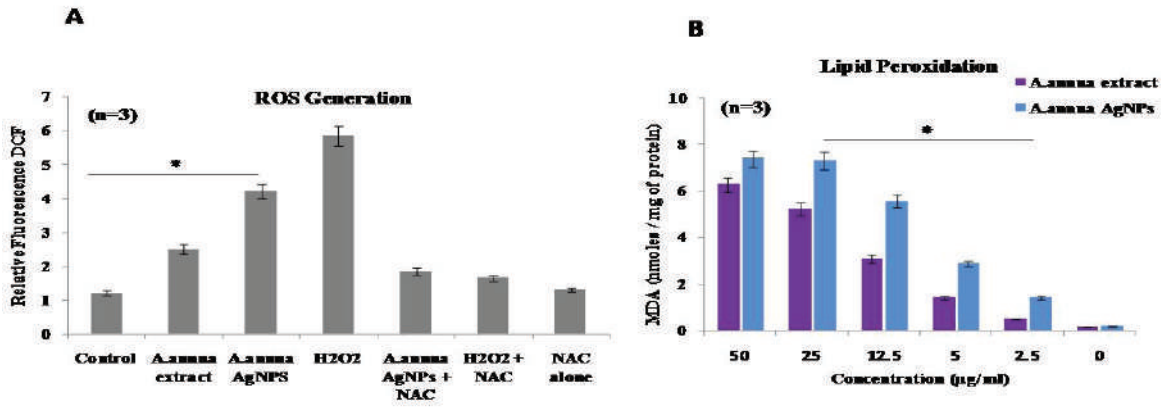


Fig 2: Shows effect of A.annuaAgNPS on oxidative stress.

Figure 3

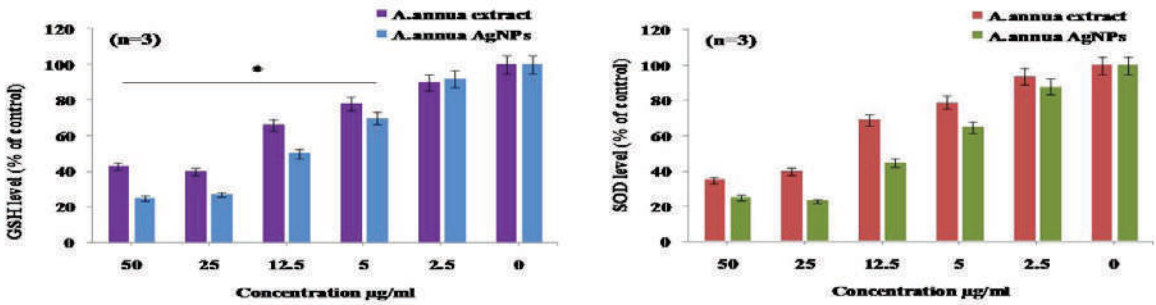


Fig 3: Shows the effect of A.annuaAgNPS on endogenous antioxidants in MDA-MB 231 cells.

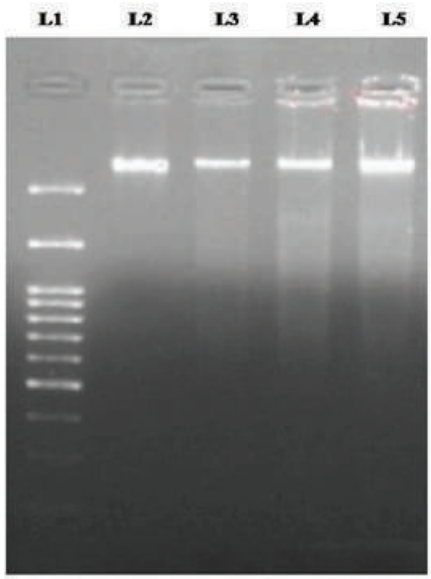
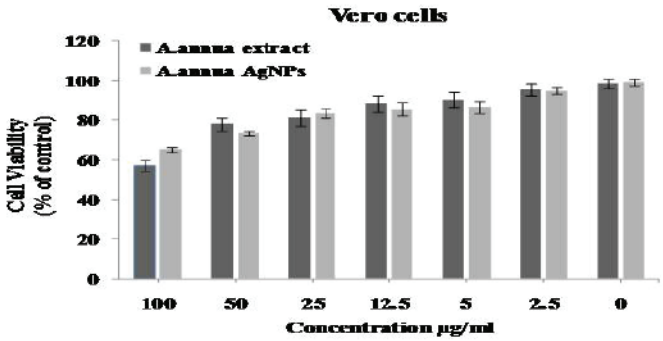


Fig 4: Shows effect of A.annuaAgNPS on DNA fragmentation in MDA-MB 231 cells.

Supplementary Fig 1



Supplementary Fig 1: Shows cytotoxicity of A.annuaAgNPS.

Ethical Clearance – Institutional Ethics Committee, Sree Balaji Dental College and Hospital, Committee registered with DCGI. (Registration no: ECR/761/Inst/TN/2015)

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Conflict of Interest – Nil

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Implant Screw Breakage: A Review Article

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Abstract

Dental implants have revolutionized the treatment for edentulous and partially edentulous patients, and successful implant integration has been well documented for patients with those clinical conditions. With the high rate of implant success for edentulous individuals, the concept of osseointegrationhas become a predictable treatment modality[2]. Yet this cutting-edge treatment modality is not without its own set of adversities. Implant failures are as common as implant success. The types of implant failures are 1) loss of integration, 2) positional failures 3) soft tissue defects, and 4) biomechanical failures. In this article we come to the conclusion that the mechanical stability of the implant is a multi-factorial aspect.

Keywords *: Dental Implant, Screw Breakage, Fatigue, Biomechanical Failure*

Introduction

During the past few decades dental implants have come a long way from the implants used and studied by Dr. P. Brånemark in 1978. He was the 1st person to observe and thus describe the concept of osseointegration. Although the credit of 1st ever dental implant to be used in recorded history goes to the amazing Mayan civilization in 600AD, where they successfully used pieces of shell as replacement for mandibular teeth. Many more physicians, scientists and dentists have over the centuries helped to make dental implantology as it is today, a very reliable rehabilitation method for edentulous spaces in both the maxilla and mandible. Yet this modern method although so refined, has immense potential for failures as well. As many cases fail as the number of successful cases. Clinical observations have indicated that the major causes of implant failure are (a) deficient osseointegration, (b) complication of the neighboring soft tissues (peri-mucositis and periimplantitis) and (c) mechanical complications. Among the biomechanical problems, screw loosening, abutment rotation, and abutment fracture are the major issues[2]. We shall now go into more details about abutment fractures and the physical factors that resist as well as cause it.

Methodology

Pubmed database was searched for the keywords 'IMPLANTS BREAKAGE' "IMMEDIATE IMPLANTS", "FATE OF IMPLANTS" 141 articles were found.

Out of the 141 articles, the non dental articles were excluded to get 36 articles.

The duplicates were excluded to get 2 articles

These 4 articles were then reviewd and data for this Review Article was obtained.

DISCUSSION

Manyof the materials used to restore implants arederived from conventional restorative dentistry, forexample denture base resins. Completedenture wearers develop relatively littlebite force compared toforce generated withimplant supported restorations. Breakage is acommon failure of overdenture restorations(fig 1 & 2)[1].



Fig 1: Teeth broken offan overdenture prosthesis[1]



Fig 2: Fractured overdenturesare a common failure dueto increased forces and thinningof acrylic bases to accommodateimplant components and tissue bars[1]

Metal fatigue of restorative materials canalso lead to breakage — the rigid connection ofimplantsbone demands that attention ispaid to the size of connectors (Fig 3)[1]. Breakage of implants and implant componentscan also occuroften this is due to poortreatment planning and exposing implants toexcessive forces. The implant in Figures 4 and5 was treatment planned as a single implant inanincomplete dentition and a terminal tooth.The implant was connected to the tooth causingdecay onthe tooth, and eventually the implantto fracture under the load.



Fig 3: Intra-oral viewof fractured implant tissue bar. The cantilevered attachment hasfractured[1]



Fig 4: Implant restorationtogether with fractured implantcomponent[1]

In 2015, Sun-Young Lee, Sung-Jun Kim, Hyun-Wook An, *et al*from Institute of Science & Technology, Megagen Implant, Gyeongsan, Republic of KoreaDepartment of Periodontology, School of Dentistry, Kyungpook National University, Daegu, Republic of KoreaMIR Dental Hospital, Daegu, Republic of Koreadid a study on the effect on mechanical properties due to thread depth of various lengths of dental implants. They used Commercial Titanium implants of various lengths, diameters and thread depths and Solid rigid polyurethane blocks withuniformity as an alternative to human cancellousbone. The implants were tightened with a recommended torque of 30 Ncm using a digital torque meter.[3]The Titanium implants were tightened with the EZ Post containingthe hemispherical loading members were fixed with aspecimen holder that was made from brass and clamped inthe jig of a universal test machine[3]. After the static compressive strength tests, the Titanium implantswere examined macroscopically. The failure mode wasobserved to be deformation in the abutment and being tornhorizontally at the upper side of the Titanium implant.The threads in the Titanium implants with deeper threads did notshow breakage. Titanium implants with the samelength and inner diameter have a similar maximum compressivestrength. The mechanical strength is more relatedto the length and diameter than the thread depth. The failuremode wasobserved in the fixtures and abutments butnot the

threads. The thread depth did not have a majoreffect on the mechanical strength. Titanium implants with deeperthreads did not induce the breakage of threads applying themaximum compressive strength. Dental implants may fractureat load levels below the maximum compressive strengthof the implant/abutment complex. Thus, the maximum compressivestrength may suggest a standard point of acute overload.Mechanical failures of dental implants appear througha repeated loading process at low loads. The fatigue test isa general method used in the laboratory to mimic actualintraoral use. The fatigue limits of the dental implants withа diameter of 4.0 mm and thread depth of 0.6 mm (636 N)and those with a diameter of 4.0 mm and thread depth of0.35 mm (619 N) in the fatigue test on the basis of theInternational Organization for Standardization (ISO14801)were both more than 600 N. Thefatigue limit of the Ti implants with deeper threads is similarto that of Ti implants with shallow thread depth. The study indicated that the Ti implants with the deeper threadshave similar mechanical stability[3].

In 2009, Cleide Gisele RIBEIRO, Maria Luiza Cabral MAIA, Susanne S. SCHERRER, *et al* from Brazil conducted a study on the fatigue resistance of dental implants based on the design of abutment-implant interface. This studydemonstrated the superior fatigue resistance of external hex interface. There was no significantdifference between the conical and internal hexinterfaces. Probably, the quality of the surfacemachining of the flat-to-flat mating surfaces(mainly, the machining accuracy of the screw andthread) determined the superior resistance of theconnector; The mode and region of fracture inprosthetic screws observed in this study suggestedthat failure of these screws occurred by fatigue(presence of fatigue striations) and involved thethreaded part[2].

Conclusion

Thus, we can now see that the possibility of implant screw fracture is dependent on an array of various factors such as technique, material, design and the peri-implant environment as well. Since titanium implants are so very much biocompatible, they can be left as it is, without any complications, if a fracture does occur and it is not feasible to remove the fractured implant.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Fate of Immediate Implants Vs Single Tooth Implants: A Review Article

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Abstract

Dental Implants have over the decades become one of the most versatile treatment techniques with good survivability rates. They have come a long way and have advanced further and further, improving all qualities. A more recent technique of implant placement is Immediate Loading Implants. Yet it has not yet become the go to technique for day to day treatment of edentulous cases as it has a significant chance of failure.

Methodology: Pubmed database was searched for the keywords "IMMEDIATE IMPLANTS", "DELAYED IMPLANTS", "FATE OF IMPLANTS" 158 articles were found.

Out of the 158 articles, the non dental articles were excluded to get 38 articles.

The duplicates were excluded to get 4 articles

These 4 articles were then reviewd and data for this Review Article was obtained.

Keywords: *Immediate Implants fate, Single tooth implants survival*

Introduction

During the past few decades dental implants have come a long way from the implants used and studied by Dr. P. Brånemark in 1978. He was the 1st person to observe and thus describe the concept of osseointegration. Although the credit of 1st ever dental implant to be used in recorded history goes to the amazing Mayan civilization in 600AD, where they successfully used pieces of shell as replacement for mandibular teeth. Many more physicians, scientists and dentists have over the centuries helped to make dental implantology as it is today, a very reliable rehabilitation method for edentulous spaces in both the maxilla and mandible. According to the time interval between extraction and implant placement, there are three different types of implants placement techniques, namely: (i) Immediate Implants (ii) Delayed Immediate Implants and (iii) Conventional Implants. Immediate implants are placed and loaded immediately into the extraction socket after the tooth has been extracted. Delayed Immediate type is placed and loaded about 10 days after extraction has been done. The conventional implants are placed well after soft and hard tissues have completely healed. We shall now delve deeply into the fate of Immediate Implants vs Conventional Single tooth Implants.

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Discussion

In 1999, Judith Maria Pinheiro Ottoni, *et al* [1]did a comparison study on immediate implants and single tooth implants. The study was done on 155 patients. Among them 77 patients were subjected to immediate implant placement technique. The other 78 were the control group who were treated with the conventional single tooth implant technique. Patient follow-up

was done and results were gathered at 24 hours, and at 1, 3, 6, 12, 18 and 24 months. The experiment was standardized using 20 Ncm of torque. The survivability of the implants was independent of implant length, position, bone quality and quantity. 9 implants failed from the immediate implant group before the 24-month mark, and only 1 implant failed from the control group. The study concluded that routine immediate loading implants should not be proposed, as the data showed unacceptable rates of survival. Thus, conventional single tooth implants are the better choice.

From 1997 to 1998, GavrielChaushu, *et al* [2]did a study comparing Immediate Loading Single Implants to Conventional Single Implants. 26 patients were chosen among the age group of 18 to 70 years. 19 implants were that of immediate loading technique, placed into fresh sockets and 9 were of the conventional technique, placed after the sockets healed completely. Follow up was done from 3 to 24 months. At 24 months' time the survival rate of immediate implants was that of 82.4% and that of conventional type was 100%. Although bone loss was somewhat similar in both groups. The study concluded the immediate implants had a significant failure rate of 20%. Thus, it remains to be seen whether it is worthwhile to take the risk.

In early 2019, Dr. Aleksandar Lazarov[3], performed a study on immediate implants on 87 patients. 42 of them were male and 45 were female. The patients' age ranged from 40 to 60 years. 20.8% of them were hypertensive and 5.7 suffered from diabetes mellitus. 30.2% were smokers. Follow-up was done till 57 months. A total of 5100 implants were placed and among them 105 were losses between 12 and 57 months. 6 patients lost 55 implants, whereas, other 50 implants were lost among 38 patients. This indicated that implant losses might be associated with specific case to case factors. The study concluded that when comparing immediate to conventional implant protocols, a higher risk of failure is seen in case of immediate implants although difference in marginal bone level and probing depth were not to great.

Conclusion

Thus, we can conclude that immediate loading implants have still not reached the optimum level of long-term survivability as compared to conventional single tooth implants. The survivability of implants being a multifactorial entity, we still cannot use immediately loading implants as a day to day treatment modality owing to its significant chance of failure. Thus, conventional implants can be still considered the gold standard until a specific, novel design is devised for immediate loading implants to augment its survivability.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Maxillary Sinus Lift Procedure Efficacy and Adversities: A Review Article

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Abstract

Sinus lift procedure is a procedure used to augment bone thickness of the maxillary sinus for better survivability of dental implants. The maxillary sinus floor augmentation was 1st done by Oscar Hilt Tatum Jr, in 1974. Since then the procedure has been refined and revised over the years. Yet it has a few adverse effects such as perforation, tearing of the antral lining and haemosinus. In this article we see that sinus lift surgery is as versatile as the surgeon is and in some cases if not done properly, can result in severe morbidity.

Keywords: *Sinus lift, maxillary sinus augmentation*

Introduction

Sinus lift procedure is a procedure used to augment bone thickness of the maxillary sinus for better survivability of dental implants. The maxillary sinus floor augmentation was 1st done by Oscar Hilt Tatum Jr, in 1974. Sinus lift procedure was 1st performed by Dr Hilt in 1974. 1st sinus grafting was done by him in 1975. Since then the procedure has been refined and revised over the years to make as versatile as it is today.Yet it has a few adverse effects such as perforation, tearing of the antral lining and haemosinus. In this article we shall see the various aspects of a sinus lift procedure[1].

Methodology

The search protocol used the electronic database PUBMED, with a time limit from 1995 to 2019. The search strategy utilized a combination of MeSH terms and text words as indicated in and kept updated until 2019. The reference lists of each article completed the database. We decided not to use any inclusion or exclusion criteria to ensure the sensitivity of our database. Only peer-reviewed articles written in English were selected.

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Discussion

Justine Loin, Jean-Daniel Kün-Darbois, Bernard Guillaume, SmailBadja, Hélène Libouban, Daniel Chappard in early 2019 did a study on Beta-tricalcium Phosphate (Beta TCP) vs xenogenic bone as grafts for the sinus augment. Beta TCP is a synthetic biomaterial and the xenogenic bone graft taken was that of extensively deproteinized bovine bone. CB-CT and Sinus membrane thickness analysis was done for all patients. 20 patients were taken between the ages of 40 to 80 years. In this study, there was no difference in the degree of oedema was seen. Sinus augmentation can be seriously compromised due to inflammatory reactions due to cytokines that accelerate bone resorption. Although the gold standard for grafting is autogenous graft (parietal, mandibular or iliac), yet it cannot be used on every case because of the associated morbidity. Thus, alloplastic materials with very high biocompatibility like Beta TCP are the better choice[2].

As discussed earlier Maxillary Sinus Lift procedure is associated with a few adverse outcomes, such as intra sinus bleeding, perforation of the mucosa membrane.

So, we shall discuss on how to prevent and manage such outcomes.

(1) Bleeding- Intraoperative bleeding results from severing or damaging branches of the vascular supply to the lateral wall of the sinus and the surrounding soft tissues. This bleeding is usually minor and of relatively short duration, but in some instances, it can be profuse and difficult to control. Solar et al described the blood supply to the lateral wall of the maxillary sinus in cadaver specimens. Blood is supplied through the intraosseous and extraosseous branches of the posterior superior alveolar artery, which form a double arterial arcade by anastomosing with the infraorbital artery. Bleeding may occur either from the soft tissue (the extraosseous branch) during flap elevation or directly from the lateral bony wall (the intraosseous branch) during preparation of the lateral window via rotary instrumentation. There is also the possibility of bleeding from the medial wall of the sinus if the posterior lateral nasal artery is damaged. The posterior superior alveolar, infraorbital, and posterior lateral nasal arteries are all branches of the maxillary artery that provides a source of blood for vascularization of the sinus graft[1,2]. Although bleeding does not occur on every occasion that this artery is damaged, it seems prudent to use 3-dimensional planning as a means of avoiding, if possible, an encounter with the artery. In some cases, the artery can be visualized within the lateral wall after elevation of the flap. In many instances, a window can be made coronal to the location of the artery and the superior portion of the membrane elevation can be performed internally to the required height. Again, it should be recognized that the artery is not always located within the lateral wall. The artery can be located just internal to the lateral wall and may pass in and out of the bony wall throughout its antero-posterior course in the lateral sinus wall. When located outside the lateral wall, it is susceptible to damage from both rotary and hand instruments. The external branch of the posterior superior alveolar artery may also be damaged when making vertical releasing incisions for flap elevation. Once it is anticipated that the possibility of a bleeding complication exists, it is prudent to locate the position of the artery on the cross-sectional computed tomography images and then use antrostomy instruments that can respect the integrity of vascular and other soft tissues while still creating the window in the ideal location for access to and elevation of the sinus membrane.

Many techniques exist to control vascular bleeding in sinus elevationsurgery. These include:

- direct pressure on the bleeding point.
- use of a localized vasoconstrictor.
- bone wax.
- crushing the bone channel around the vessel (hemostat).

(2) Schneiderian membrane perforation- Perforation of the Schneiderian membrane is the most common intraoperative complication in sinus elevation surgery. The reported incidence in the literature varies from 11% to 56% when rotary window preparation is used. Most experienced clinicians estimate their perforation rate to be approximately 25% when using conventional rotary instruments. In retrospective computed tomography studies performed at the New York University Department of Periodontology and Implant Dentistry (Poster Presentation, AO Annual Meeting, 2002), the perforation rate was shown to have a close relationship to membrane thickness and, to a lesser degree, to the presence of septa. The perforation rate was 41% when the membrane thickness was <1.5 mm and 16.6% when it was ≥1.5 mm. The perforation rate in a separate study of 136 sinus elevation procedures was 44.2% when a septum was present and 35.7% when septa were absent. In a retrospective computed tomography study by Cho et al, the perforation rate was shown to be related to sinus width or, to be more specific, the angle made by the medial and lateral walls at the floor of the sinus. The perforation rates were 62.5% for the narrow anterior part of the sinus (angle < 30°), 28.6% for the wider middle part of the sinus (angle 30-60°), and 0% for the widest posterior portion (angle > 60°). A recent computed tomography study by Chan et al 66 identified another "angle," which defines the various configurations of the palatonasal recess and must be taken into consideration when elevating the Schneiderian membrane from the medial wall. It is the angle made where the alveolus meets the medial wall of the sinus[3]. If this angle is acute, and is located within approximately 10 mm from the sinus floor (an area where graft material is likely to be placed), care must be taken to keep the elevator on the bone surface while not trapping, and thus tearing, the membrane. A thorough knowledge of

the 3-dimensional anatomy of the sinus is essential if the perforation rate is to be kept to a minimum. A computed tomography analysis will provide information relating to the thickness of the crest and lateral walls, the presence of discontinuities in the bony walls, the width of the sinus, the slope of the anterior sinus wall, membrane thickness, and the presence, size, and location of septa. Clinicians will also gain information relative to sinus health and patency of the osteomeatal complex. This evaluation may indicate the need for presurgical treatment that can avoid complications, such as postoperative sinusitis and infection[1,4].

Treatment:

Elevation of the Schneiderian membrane helps to form a compartment in which the particulate graft material can be placed and confined. The elevated membrane forms the distal and superior walls of this compartment, while the bony sinus walls form the inferior (crest), anterior, medial, and lateral walls. Proussaefs et al showed that failure to contain the particulate graft as a result of membrane perforation will result in decreased bone formation (14.2% vs 33.6%) and a decreased implant survival rate (70% vs 100%)[2]. Should the sinus membrane be torn or perforated, the fragility of the remaining membrane becomes increased, and care and attention are required to complete the elevation. This is best accomplished by elevating the membrane around the perforation, thereby releasing tension on the perforated area of the membrane, as opposed to working directly in the weakened area of the perforation. It is still necessary to complete elevation of the sinus membrane from the floor, medial, and anterior bony walls to allow the blood supply from the bony walls to vascularize the graft. Some clinicians prefer to make a small repair to stabilize the damagedarea before completing the elevation. If this is done, the repairshould be evaluated for stability before placing the graft material.The most common means of repairing a perforated Schneiderianmembrane is to use a bioabsorbable collagen barrier membrane asa patch[3,4].

Conclusion

Thus we can conclude that sinus lift surgery is as versatile as the surgeon is and in some cases if not done properly, can result in severe morbidity. A surgeon should be very alert and aware, as a single wrong decision can result to surgical complications that are difficult to manage.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Tilted Implants: An Alternative to Conventional Fabricated Dentures

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Abstract

Rehabilitation of patients with completely edentulous maxilla and mandible with implant becomes difficult because of less bone quantity, poor bone quality, maxillary sinus in posterior maxilla and mental foramen and mandibular canal in mandible. Sinus lifting procedure and bone augmentation procedure were required to treat such patients, prior to implant placement. This article reviews on an alternative treatment procedure in which two posterior implants are placed at an angle eliminating the need for sinus lift or bone augmentation procedures.

Keywords: *Tilted Implants; Angled Implants; All on Four Implants; Angulated Implants; Non Axial Implants; Co-Axis Implants*

Introduction

The prevalence of the elderly population, as well as life expectancy, increased in the final decades of the 20th century, as described in the World Health Organization 2004 Annual Report. The edentulous condition therefore has a negative impact on the oral health-related quality of life. Patients wearing complete dentures for many years infact, and especially in the mandible, are often unsatisfied because of the instability of the prosthesis during speaking and eating. To date dental implant treatment is well documented as a predictable treatment for partial or complete edentulism1. Edentulous patients treated with prostheses supported by osseointegrated implants can realize improved masticatory function in terms of chewing efficiency and bite force. However, it is reported that patients who asked for implant therapy but received conventional dentures are not fully satisfied showing only a marginal improvement in their quality of life. The use of tilted implants parallel to the anterior wall of the maxillary sinus or the mental foramen/inferior alveolar nerve has been proposed as a conservative solution for the treatment of the atrophic edentulous maxillae2,3.

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Methodology

Pubmed database was searched for the keywords “TILTED IMPLANTS”, “ANGLED IMPLANTS”, “NON AXIAL-IMPLANTS”, “CO-AXIS IMPLANTS”, 48 articles were found.

Out of the 48 articles, the non dental articles were excluded to get 38 articles.

The duplicates were excluded to get 31 articles

These 31 articles were then reviewed and data for this Review Article was obtained.

Discussion

Rationale for using tilted implants

The single tilted implant infact, submitted to a vertical load, demonstrate higher peri-implant bone stress than the single vertical implant submitted to the same vertical load. The stresses increases as the tilt

of the single implants increases. However when the implants are splinted in a rigid FFP, the use of tilted distal implants, with reduced cantilever lengths, results in lower mechanical stresses on the peri-implant bone with respect to the vertical implants with longer cantilevers. Moreover a reduction of stress around anterior implants is observed with the tilted distal implants compared to the vertical implant FFP design[5]. Multi-unit prostheses may demonstrate greater inaccuracies compared with single implant restorations infact, but due to the absence of moment loading, the multi-implant configuration appears to compensate for the higher strain development. Nowadays computer guided flapless surgery for implant placement using stereolithographic templates is gaining popularity among clinicians and patients. The advantages of this surgical protocol are its minimally invasive nature, accuracy of implant placement, predictability, less post-surgical discomfort and reduced time required for definitive rehabilitation. There is no intrinsic limitation of this technique if the initial bone height is severely reduced and the risk of perforations of the sinus membrane or alveolar nerve and loop during the drilling procedure are no likely to occur because of the accuracy of the safety distance calculated during the computer planification[6,7].

Furthermore:

1. To achieve primary implant stability(35to45ncminsertion torque)[8].

2. Indicated with a minimum bone width of 5mm and minimum bone height of 10mm from canine to canine in maxilla and 8mm in mandible.

3. If angulation is 30º or more, the tilted implants can be splinted.

4. For tilted posterior implants, the distal screw access holes should be located at the occlusal face of the first molar, the second premolar, or the first premolar.

5. Improved masticatory functions in terms of chewing efficiency and bite force [9].

Advantages of tilting implants[10,11]:

1. Stability in minimum bone volume : Longer implants can be used in minimum bone volume with advantage of increasing bone-to-implant contact and reducing the need for vertical bone augmentation.

2. Clinical results are better.

3. Need for bone grafting is eliminated.

4. Can be performed in patients with systemic diseases which are contraindicated for bone grafting.

5. The angulations allow a voids impingement of anatomical structures.

6. Biomechanical advantage in using tilted distal implants

rather than distal cantilever units.

7. Reduce the length of cantilevers without performing bone grafting or sinus lifting.

8. Alternative to maxillary sinus floor augmentation procedures.

9. Distally tilted implants induced better loading transmission than vertical implants

Disadvantages of tilted implants[12]

1. Technique sensitiveprocedure.

2. Surgeon need to be very skillful.

3. Computer guided surgical stent required for implant to be placed in desired angulation.

4. Not evens light change in angulation can be done.

5. Long term studies are not available

Table 1 Depicts the differences between tilted and non tilted implants.

Tilted implants	Non tilted implants
Anatomical structures can be bypassed by tilting implants. For example: Maxillary sinus can be bypassed by tilting implant at an angle.	Anatomical structures should be taken care while placing parallel implants. For example: Maxillary sinus, mental foramen, mandibular canal.
Cantilever length may be reduced resulting in betterload distribution.	Cantilever length is not reduced hence less stress distribution
Tilted implants enable immediate loading and fabrication of implant supported restorations	Immediate loading is not possible.
Longer implants can be used	Implants size will be selected while taking vita lstructures into consideration.
Zygomatic bone and pterygoid bone can be engaged thus providing better anchorage and primary stability.	Zygomatic bone and pterygoid bone cannot be engaged
Eliminates bone augmenting procedures, bone grafting and sinus lifting.	Bone augmenting procedures ,bone grafting and sinus lifting may be necessary in some cases

Based on the tooth and cephalometric analysis, the angulated implant was designed as follows:

1. 5mm restorativeinterface.

2. 12º angulation from long axis (Figure5(a) and(b)).

3. Reduced pitch of thread which allows 0.5mm apical movement per placement rotation.This ensures

a. That placement depth is controlled by less than 0.5mm.

4. Implant length will range from10mm-18mm.

5. Taper ranges from 8.2º(10mm implant) to 3.6º(18mm implant).This allows for a total angulation discrepancy from available buccal maxillary cortex to screw

a. Access ranges from 30º (10 mm) to 25.6º (18 mm).

6. Placement is affected by means of an angular corrected fixture mount marked to assist with correct orientation

Indications

In the edentulous upper jaw, the placement of implants can be difficult due to limited bone and presence of the maxillary sinus. Pterygoid implants have high success rates, bone loss levels are same as conventional implants, minimal complications and acceptable bypatients.

However, pterygoid implants have mainly been studied in partial edentulism as an alternative to sinus lift procedures[13,14,15].

Conclusion

Tilted implants area treatment solution for patients with edentulism and is associated with predictable outcomes. The placement and angulation of implants in the All-on-4 concept is unique as compared with the axial placement in a vertical manner.

In addition to bypass anatomical structures, these angulations allow the use of longer implants which increases bone-to-implant contact. This biomechanical advantage reduces posterior cantilevers and allows for well-spaced implants.

Reductions in the number of implants and components needed further augment the cost-effectiveness of this concept compared with traditional implant reconstructions.

However, failures of implants with this technique are relatively less, and early failures can be resolved by modifying the provisional prosthesis and the implant can be replaced and used within the same prosthesis. After this, the final prosthesis can be constructed after the verification of optimal integration. In patients with greater risk factors, the placement of additional 5 implants in the mandible or 6 implants in the maxilla can be considered.

The long-term efficacy of the All-on-4 technique and its numerous advantages, such as immediate function and esthetics, reduced morbidity, high patient satisfaction, and relatively lower costs, should be considered when assessing treatment options for an edentulous jaw.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Tramadol in Oral Surgery – A Literature Review

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Abstract

Elimination of pain is one of the most important duties of a doctor. A surgeon has to come across more number of cases where management of pain becomes a handful. Thus proper use of analgesics both systemic and local are an integral part of any successful surgery. For decades NSAIDs has been the go to drug for management of pain and administering anaelgesia. But recent pharmacological advancements have opened up a new way of administering anaelgesia in the form of synthetic opiods. This review article aims at finding out the efficacy of local administration of TRAMADOL in maxillofacial surgeries.

Keywords: *Tramadol, Opiods In Oral Surgery*

Introduction

Tramadol is a centrally acting synthetic opioid analgesic that was first manufactured in 1962. It has two synergestic mechanism i.e., it acts both as a weak opioid analgesic that is effective clinically in treating moderate to moderately severe pain and it also serves as an inhibitor of monoamine reuptake. Commercially, tramadol comes as a racemic mixture of two enantiomers (+) tramadol andenantiomers (-) tramadol that has superior analgesic properties than enantiomer alone [(1)].

Non opioid analgesic mechanism of action of tramadol had been identified via inhibition of the reuptake of norepinephrine (NE) and serotonin [5-hydroxytryptamine (5-HT)] that are released from peripheral nerve endings by this mechanism,tramadol is inhibiting pain transmission in the central nervous system, and in this way contributing to its analgesic effect [(2)]. Moreover, it has been discovered that they are also targets for analgesics and anesthetics. Apart from thesetwo described mechanisms of action, yet we do not know additional mechanisms of tramadol action [(3)]. Unfortunately, there is an increase in the unjustified use of opioid analgesic drugs in dentistry, especially in oral surgery, prescribed for pain management after tooth extraction or third molar surgery.However,there is, no scientific evidence available to support this recommendation [(4)]. In fact, there are evidences that shows that NSAIDs alone or in combination with acetaminophen are more effective than opioids or their combination administrated systemically in treating dental pain [(5,6,7)]. Also, tramadol has risk of adverse effects such as nausea, vomiting and dizziness.

Opioid analgesics are much less effective in pain management after tooth extraction or third molar surgery compared to NSAIDs. The recommendation to dental professionals is to prescribe NSAIDs for dental pain relief, and in a case of severe pain, to prescribe a combination of NSAIDs and paracetamol. However, tramadol can be considered as a first-line drug option for patients who are intolerant to NSAIDs or those with some contraindications, such as allergy, renal failure and high-risk of gastrointestinal haemorrhage.

Mechanism of action: possible local mechanisms of action of tramadol:

1. weak peripheral agonism over peripheral μ-OR [(8)], the number of which is enhanced in hyperalgesic and

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inflammatory conditions [9,10].

2. favouring the opening of nonspecific voltage-dependent potassium (K+) channels [11].

3. acting in the nitric oxide pathway [12].

4. agonistic action on vanilloid receptor 1 (TRPV-1 transient receptor potential vanilloid-1) that apart from local analgesic effect, can exert undesired local side effects, such as burning pain and erythema [13].

5. with high concentration of tramadol blockade of the Nmethyl-D-aspartate (NMDA) receptors [14] · local anesthetic effect of tramadol can be explained by direct blocking of voltage-dependent sodium (Na+) channels [15]

Tramadol in oral surgery:

Pang et al Demonstrated a local analgesic effect of tramadol following intradermal injection

Tsai et al Tramadol produced a neural conduction block that could not be reversed by naloxone. suggesting that an underlying mechanism of action of tramadol must differ from its opioid action

Mert et al Nerve conduction blockade with tramadol was similar to that of lidocaine, although it was weaker.

Altunkaya et al concluded that 5 % tramadol has a local anesthetic effect similar to 2 % prilocaine when used intradermally for excision of cutaneous lesions.

Pozos et al demonstrated that tramadol injected submucosally next to the extraction socket prolongs the anesthetic effect of articaine and that such administration could improve the management of postoperative pain

Isiordia-Espinoza et al concluded that preemtive ketorolac plus submucosal local tramadol resulted in better postoperative treatment of acute pain and in reduction in the consumption of postoperative analgesics

Ceccheti et al. concluded that submucosal administration of tramadol contributed to providing a pain free period of 3.5-4 hours after extraction, with rare adverse effects and good patient acceptance.

Gönül et al concluded that postsurgical pain VAS scores were significantly lower in first 12 hours, first analgesic intake was significantly later, and total analgesic intake was significantly lower, in the tramadol group compared to the control groups

Al-Haideri et al concluded that tramadol with adrenaline injected supraperiosteally proved to be an effective local anesthetic for the extraction of upper molar teeth. Due to weak local anesthesia of plain tramadol (without adrenaline), the author stated that the presence of adrenaline, which produces vasoconstriction, thus deterring tramadol locally, is crucial for producing its anesthetic effects on the nerve

Conclusion

The aim of modern oral surgery is to adequately and efficiently control a patient's pain post operatively to ensure high quality local anesthesia. Various studies have shown that NSAIDs has several advantages over opioid analgesics, and concluded that opioids should not be prescribed as the first line drug of choice for treatment of acute dental pain. However , local administration of tramadol opens new possibilities in management of postsurgical pain. In this regard, tramadol applied locally submucosally, supraperiosteally, topically by means of small drops or as an adjuvant to local anesthetic, could provide promising results. Howeverthere is a need for large clinical studies to investigate safe and effective protocols for local administration of tramadol in day to day practices.

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A Beginner's Guide to Surgical Treatment Objective – An Orthodontist's Point of View

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Abstract

Visualising the result of any proposed treatment, not only guides us in each part of the journey toward the final result but also to assess if the treatment objectives are met. Any adverse effects can be easily determined, before the start of the treatment, and necessary alterations to the treatment plan can be made. Various methods in which the result of an orthognathic surgery can be visualised are – overlay tracing, template tracing, cephalometric prediction tracing, computer prediction, feasibility mock surgery, etc. How to carry out these predictions are discussed in detail in this article.

Key Words**:** *Surgical treatment objective (STO), Cephalometric prediction tracing, feasibility mock surgery, template tracing, dolphin imaging, 3D cephalometric analysis*

Introduction

The lateral cephalogram's advent is one of the milestones in orthodontics. It paved the way of better understanding, diagnosing and ultimately treating the underlying dentofacial anomaly.

A lot of contributions have been made by various authors, like Tweed's diagnostic triangle in 1954 which gained a lot of attention. Following him, various other cephalometric analyseswere proposed by Steiner's, Mc Namara , Down's. In 1960, Rickett proposed a method of predicting growth with the help of serial cephalograms taken at different points of time, over a period of time. Holdaway in 1983, stated the importance of the soft tissue parameters in the orthodontic treatment, and suggested a VISUALIZATION of the treatment objective (VTO) – to have an idea as to how the outcome of the treatment would affect the profile of the patient.

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This method of visualization, led to the development of a cephalometric prediction tracing by Epker and Fish, which gave a mental image at the outcome for a surgical intervention. In 1985 – Larry Wolford, introduced the term – "**Surgical Treatment Objective(STO)",**[(1)] which is basically a visualization of the outcome of an orthognathic surgery prior to the start of treatment, to better asses the end result.

TYPES:

The prediction can be for

1) Orthodontic and surgical use

2) Surgical use

The Orthodontic and Surgical VTO is the discussed further in detail in this article. Surgical VTO is more important from an Oral and Maxillo-Facial Surgeon's point of view.[(2)]

ORTHODONTIC SURGICAL VTO:

When a malocclusion is too severe to be corrected by only orthodontic interception, the patient might need a surgical correction, it involves an interdisciplinary approach where the orthodontist and the oral surgeon work together as a team in achieving the best treatment

results for the patient.

When surgery is planned in the treatment, it is adviceable to perform the surgery earlier on in the treatment, as the precision achieved by fixed appliance in orthodontics is better than that of the surgery. There will be rapid tooth movement 3 months Post-Surgery, due to the inflamed bone and there is early improvement of the facial appearance, which aids in better patient compliance.

TWO – PATIENT CONCEPT:

Epker and Fish, came up with something called as the "TWO PATIENT CONCEPT". A mock surgery was performed in the patients casts and the new set of casts were treated as a second patient. The mock surgery helps the Orthodontist to asses with a Pre – Surgical Orthodontic phase is required or not. If the Mock surgery is carried out after a Pre-Surgical Orthodontic Phase is over, it helps the orthodontist assess if any other corrections need to be made prior to surgery or if the Clinical situation presented before him, at the end of surgical phase (with the help of mock surgery) can be handled and tackled by him, in the Post Surgical Orthodontic Phase.

Another method of applying this two patient concept, is with the help of Cephalometric Prediction Tracing. Various methods of Cephalometric prediction can be carried out and the Prediction obtained at the end can be treated as a Second patient. Now the task is to simply start with patient one and reach the results of patient two. So it becomes easier to envision and tackle the complicated malocclusion involving surgery.

VARIOUS METHODS OF CEPHALOMETRIC PREDICTION :

For decades, Cephalometric prediction was done by hand, manually, by taping acetate sheets to radiographs. In this method the angles were measured directly with the help og protractor, ruler, divider, etc. Then came into play the Computerised methods, in which the landmarks can be directly digitised with the help of an electroinc pen or a crosshair cursor. For indirect digitization, a scanner or a digital camera captures the image and stores in the computer's memory.[3]

Manual Methods

The first manual method used to predict a mandibular surgery was given by Cohen [4]. In this method he demonstrated the soft tissues changes by carrying out the usual cephalometric tracing of maxilla, maxillary teeth, followed by the tracing of the lower face. Then another tracing of only the mandible and the lower face was done and cut out. He used divider to record the amount of Mandibular movement and he slid the cut out section over the original section to mock the surgery. Another tracing was done with the new position of the mandible and the soft tissue changes were observed.

A similar method of Overlay tracing was given by Mc Neil [5], in which he oriented the patients casts to the post surgical position and that helped determine the amount of posterior movement of the overlay.

Henderson [6] proposed a method of using the patient's profile photograph with a transparency of 1:1 superimposed over the Cephalometric tracing in order to better understand the soft tissue changes. The photographic prediction method was also advocated by Hohl at al [7].

The method of surgical-orthodontic cephalometric soft tissue prediction tracing for mandibular advancement, maxillary superior repositioning and combined maxillary and mandibular surgery was proposed by Fish and Epker [8]. The authors believed that the three methods explained by them would suffice to predict any Orthognathic surgery.

Computerised methods:

The computerised method, involves digitising the cephalometric tracing and landmarks or to save the image digitally and carry out the tracing on the computer, virtually. There are numerous softwares available which can carry out the Cephalometric Prediction tracings [9]. The first computer software was developed by Bhatia and Sowray [10], where they used graphics to mimice the outcome of the surgery.

Later on many softwares were introduced and used by various authors like, Harradine and Burnie [11], Walters and Walters [12], Freihofer [13], etc.

Currently there is a wide variety of computerized

cephalometric software systems for orthognathic surgery prediction. Quick Ceph was the first commercially available software for orthognathic surgery prediction. The Quick Ceph Image (Quick Ceph Systems, San Diego, California) it is designed for Macintosh computers. It permits a wide range of functions based on a 28-point digitization. When orthodontic and surgical movements are simulated, horizontal and vertical changes are recorded by the computer. The soft tissue adjusts automatically according to predetermined ratios. The majority of these ratios are derived from Wolford et al. [1,14].

The various other softwares available include, The dentofacial planner, Vistadent, Orthodontic Treatment Planner (OTP), Orthognathic Prediction Analysis (OPAL)[15], Dolphin Imaging Software [16], etc.

Video Imaging :

More recently, the introduction of computer software programs with video imaging by Sarver et al. [17], surgery has greatly facilitated and improved the communication of the final predicted esthetic outcome and allowed the clinician to rapidly analyze, plan, perform the simulated surgery.

True vision image processing system (TIPS) [18] is a software that superimposed the patients profile image, captured from the video imaging, over the cephalometric prediction to better visualise the soft tissue changes. Another video imaging software was given by Grubb [19] where the patients study casts data and Cephalometric data can be fed onto the computer and various analysis are carried out by the computer software.

The purposes of calibrating the cephalogram to the profile video image are to: (a) relate the underlying hard tissue to the soft tissue, (b) allow quantification of movements needed for occlusal correction and aesthetic ideal to be achieved, (c) allow realistic movements to be planned and, (d) permit the treatment plan to be designed as close as possible to the patient desires

Video Imaging poses as a major addition to the contribution of computers towards Orthognathic Surgery treatment planning. However, there are also some errors encountered with the use of softwares in Superimposition of facial images and Cephalometric radiographs[20].

The 2D views have limitations such as Head positioning errors, rotational errors, etc. It particularly gets tricky when it comes to planning complex surgeries such as in patients suffering from various syndromes, cleft lip and cleft palate, asymmetry, deviated chin etc. [21,22]

Three Dimentional Prediction Methods:

Nowadays, with the advent of various imaging facilities that can capture the patient's hard and soft tissue details in 3D, it has become more easier to predict the outcome of the treatment, in a three dimentional manner. The various three dimentional imaging facilities available are the Computed Tomography (CT), Cone Beam Computed Tomography (CBCT), and Magnetic Resonance Imaging (MRI).

The data obtained from these diagnostic imaging can be fed to the computer and the 3D cephalometric softwares available can be used to carry out the various prediction tracings [23] , mock surgeries etc.

The software uses graphic interpretation and mocks the soft tissue changes almost flawlessly, by adapting the soft tissue profile of the patient obtained from the diagnostic imaging. This customised prediction, better enables the visualization for both the clinician as well as the patient who is undergoing the surgery[24]. Surgeons can benefit from performing mock surgeries, osteotomies on the software, to determine and plan the accuracy of their osteotomies.

The first complete 3D model for prediction of orthognathic surgery developed by Nakasima et al. [25], which can be adjusted to the patient's head from cephalograms, 3D stereophotographs and dental casts. The fusion model replaces the need for model surgery, since the virtual head can be used to design a surgical wafer, which can be used as a surgical guide.

Conclusion

Surgical treatment objective, is a systematic approach towards visualising the end result of surgery, as a part of treatment planning, to better enable both the Surgeon as well as the Orthodontist, to work hand in hand towards the benefit of the patient. The various methods of predicting or mocking a surgery available were given in this article. It is upto the clinician to choose wisely

which method to employ bearing in mind the limitations of each each technique. Each method has its owns perks, like being cost effective, simple, quick and easy. It is also noteworthy to mention that the clinicians be mindful in experimenting with various methods available in order to choose the method that works best for them.

Ethical Clearance – Not required since it is a review article

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Twin Block and Its Modifications

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Abstract

Twin block, one of the most commonly used functional appliances has many advantages over other functional appliances like comfort, esthetics and function. But using the same appliances for all clinical situations limited its use in various cases. To overcome such limitations of twin block appliance, various researchers have come up with various modifications. This article gives an overview of the different modifications of twin block appliance.

Twin Block and Its Modifications

Class II malocclusion are one the most common malocclusions encountered in a routine orthodontic practice. The treatment modality depends on the patient's age\ growth status primarily. In patients in the growing age group, growth modulation therapy is usually done followed by a fixed orthodontic therapy. Various functional appliances have been developed over the years to achieve this goal. Though various functional appliances have been developed and used, the twin Block appliance, described by Clark in 1982, is one of the most commonly used[1].

This article covers the various modifications of the standard twin block appliance which can be used in specific situations to give superior results.

Standard twin block appliance

Twin block appliance was developed by Dr. Williams J Clark in 1977**.**

It was a simple, comfortable and aesthetically acceptable two piece appliance aimed at maximizing the growth response to functional mandibular protrusion.

The basic philosophy[2] behind the twin block therapy was if the mandibular inclined planes are in a distal relation to that of maxilla then the force acting on the mandibular teeth will have a distal force vector leading to a class II growth tendency. The aim of the twin block is to modify these inclined planes and cause more favourable growth pattern. Secondly, since it could be worn 24 hours, the masticatory forces can be transmitted via the appliance to the dentition and then to the bony trabeculae thereby influencing the rate of growth and the trabecular structure of the supporting bone.



Fig 1

The standard twin block appliance [3,4] and had the following components-

- Occlusal bite blocks meeting at 70°
- Delta Clasps on upper molars and premolars.
- Ball end on lower incisors.

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 Labial bow to retract the upper incisors.

 Upper and lower base plates

The ideal indication for twin block appliance includes Class II div 1 malocclusion with well aligned upper and lower arches, having overjet of 10-12 mm with a deep bite and horizontal growth pattern. Patient should preferably be in pubertal growth spurt and have a positive VTO. However the standard twin block could not cater to the individuals needs of all the patients. To overcome this problem, various modifications have been in introduced over the period of time.

Various modifications of the twin block appliance

 Twin block appliance for transverse and sagittal development

 Twin block Croat appliance

 Magnetic twin block

 Twin block with spinner

 Fixed twin block

 Reverse twin block

 Twin block – hybrid appliance

 Neuromuscular twin block (GERBER BANDED BLOCK)

 Twin block for class 2 div 2

 Twin block with concorde facebow

 Twin block appliance with bite jumping screw for progressive advancement

□ Implant supported twin block

Twin block appliance for transverse and sagittal development

Twin block for transverse development

It is a combination of Schwarz appliance and twin block. Screws are incorporated in the upper and lower twin blocks for transverse development during the mixed dentition. When screw is added in the lower plate, the appliance is also termed as bow beer appliance [5].

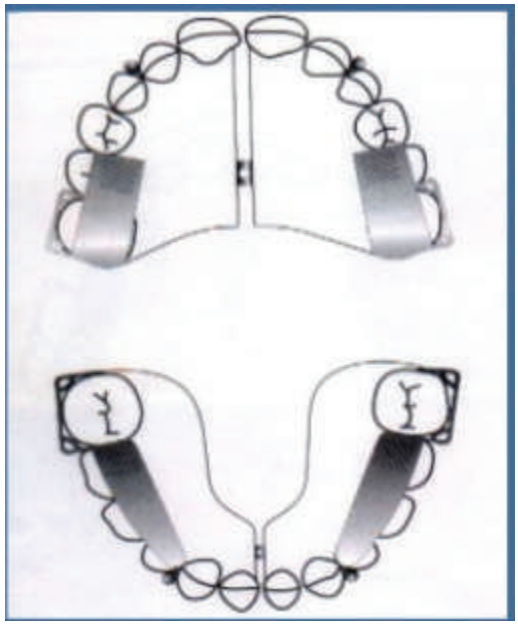


Fig-2

Twin block for sagital development

In this type of twin block ,the anteroposterior arch development is achieved by two scrrews aligned anteroposterior in the palate. This is usually required when upper and lower incisors are retroclined with a deep overbite. Twin block McNamara appliance, another modification has two screws placed in the mid palatal region; one in anterior region in line with premolars and the other in posterior region in line with molars. This allows either only anterior or only posterior expansion as required [5].



Fig-3

Twin block for transverse and sagittal appliance

For patients who require both sagittal and transverse development of the arches, a three way screw can be used in the anterior part of the palate. But this screw may impede the speech because of its bulk[5].



Fig-4

Twin block crozat appliance

This appliance has minimum palatal and lingual coverage. Is suitable in adult treatment. Disadvantage is that it requires careful adjustment to maintain symmetry [5]

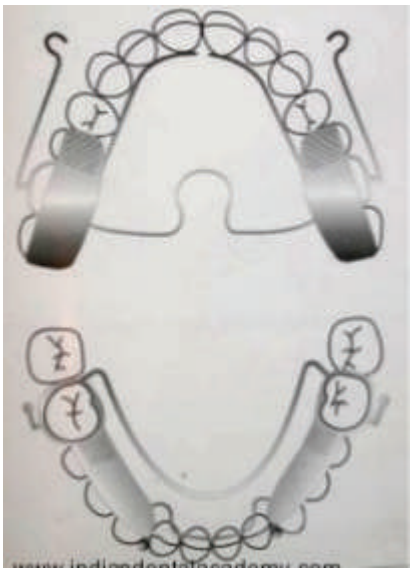


Fig-5

Magnetic twin block appliance

In this magnets are incorporated in the inclined planes. Repelling magnets on the posterior bite blocks increase the intrusive forces for correction of anterior open bite**.** In twin block therapy magnets can also be used to increase the occlusal contacts to maximize the functional forces.

The magnets used are of two types[6] :- Samarium Cobalt and Neodymium Boron (is more powerful).They can be used in both repelling or attracting modes. Magnets are also used to correct facial asymmetries. The mandible responds faster on the side of correction with attracting magnets on it.

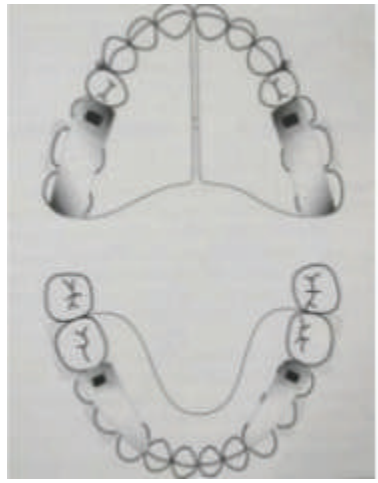


Fig-6

Twin block with a spinner In patients with tongue thrust spinner can be added to control tongue thrust [5].

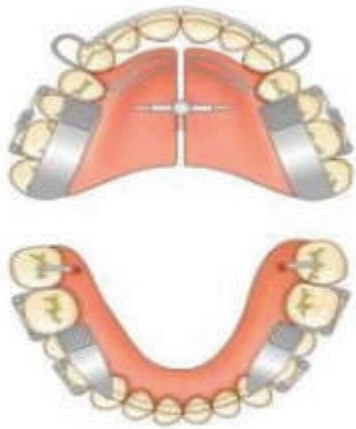


Fig-7

Fixed twin block

It is essentially used in non cooperative patient. A fixed twin block has the following components. Transpalatal Arch with occlusal inclined planes cemented on both the sides. Occlusal inclined planes are held in place by the wire tags which are extensions of the Transpalatal arch. In the lower,lingual arch extends over the occlusal surface of the molars or premolars depending on the stage of development. [7]

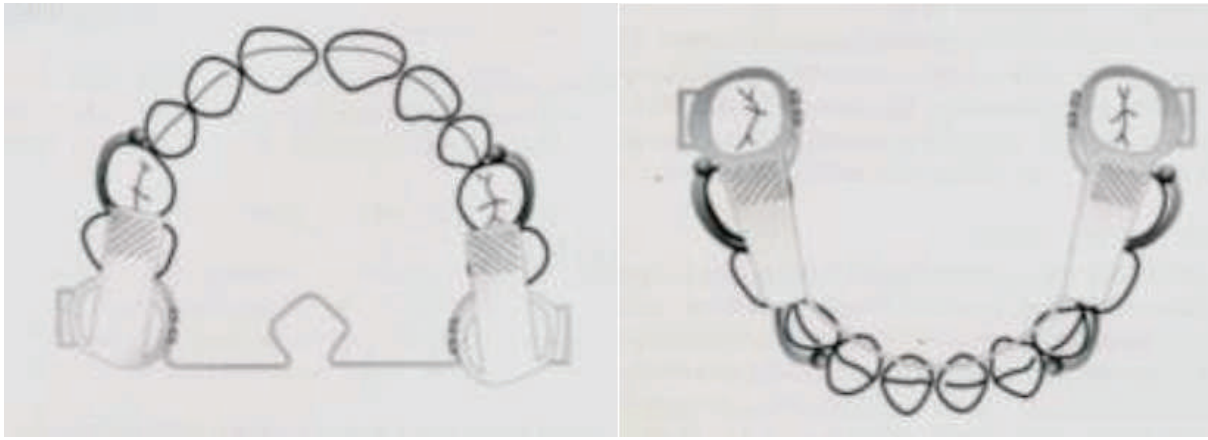


Fig-8

Reverse twin block

This modification is given in class III malocclusion cases, for the correction of maxillary protrusion. In the upper plate, inclined plane is in the at the anterior and in the lower plate, the inclined plane is in the posterior. The angulation of the inclined planes is also reversed to push the maxilla forward and hence the name reverse twin block [8].

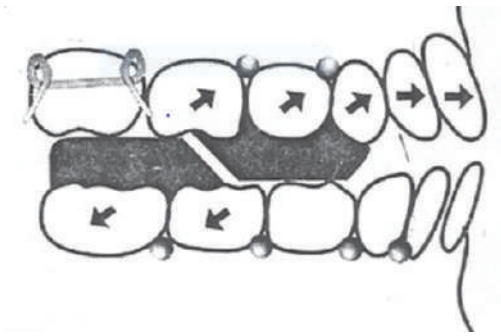


Fig-9

Twin block hybrid appliance

This modification adds upper lip pads (originally used in the frankel appliance) to the upper anterior segment of the twin block to increase the forward movement of the incisors[9].



Fig-10

Neuromuscular twin block

This Banded Block (Twin Block) was first developed in 1995 by Jay W. Gerber. The Gerber Banded Block was introduced to overcome the defects of Herbst type appliances and many other fixed class II correctors [10].Appliance made of stainless steel wire which incorporates orthodontic bands in the superstructure that provides support for the mandibular or maxillary acrylic blocks. The acrylic blocks are similar to that found in the original Clark appliances. The difference is that the corners or lateral edges of the acrylic are slightly to conform to the neuromuscular function as advocated by Jinkerson [10] This author advocates the use of a fixed Rickinator (fixed maxillary bite planes) or Rickinator Plus as the follow-up appliance. These fixed maxillary bite planes permit a controlled support of the anteroposterior correction derived from the 'blocks' and acts as a support appliance in vertical correction. Rickinator plus stabilizes the new position of the mandible. After four to six months of treatment the upper and lower Banded Blocks are removed and the Rickinator Plus appliance is immediately inserted using lingual Wilson 3-D attachments.



Fig-10

Modified twin block for class ii div 2

Appliance consist of Adams clasp on maxillary and mandibular first molars and first premolars and ball end clasps on the lower labial segment. inclined planes are constructed at 70 degree to the occlusal plane. The upper block contains a midline expansion screw and adouble cantilever spring behind the upper labial segment.Advancement, if required is carried out by adding small acrylic tablets to the upper block. [11]

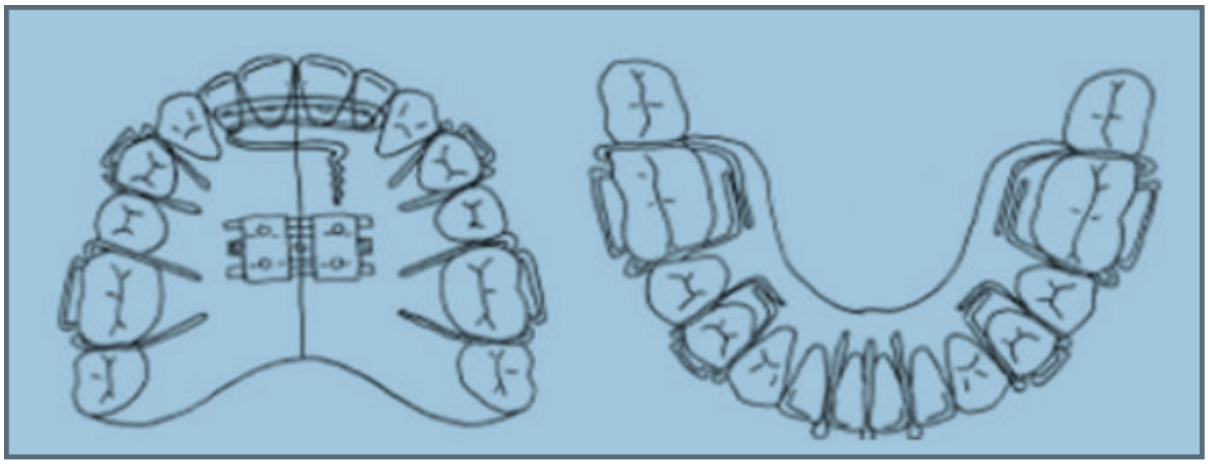


Fig 11

Twin block with concorde facebow

This is used in cases where skeletal discrepancy is severe. The addition of an orthopaedic traction system to support the action of occlusal inclined planes makes this appliance an effective technique to treat of a wide range of malocclusions. This is indicated in cases of maxillary protrusion, mandibular protrusion, and vertical growth discrepancies.

This technique achieves rapid correction of malocclusion even in cases with severe malocclusions that are unfavourable for conventional fixed or functional appliance therapy. It provides interpapillary and extra oral traction to restrict maxillary growth and at the same time, encourage mandibular growth in combination with functional mandibular protrusion[12].

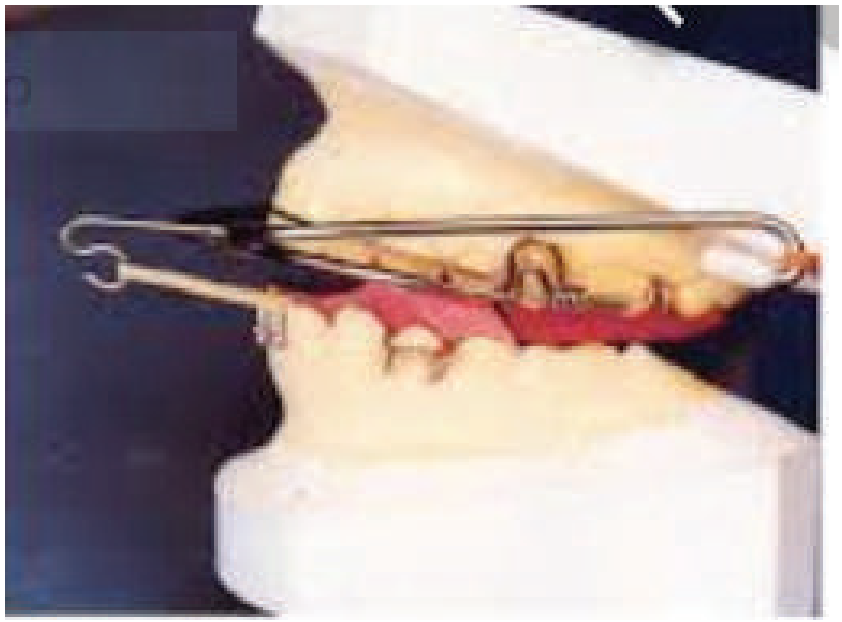


Fig-12

Twin block incorporating bite jumping screw for progressive advancement

Most functional appliances are reactivated in the laboratory, or by adding of acrylic and taking long chair side time. This carries the risk of loose monomer in the intraoral cavity. This modified twin-block appliance allows controlled, stepwise and easy bite advancements at the chair side.

Advancement screws are incorporated in the maxillary appliance blocks and activated by the insertion of cylindrical acetyl resin spacers of various thicknesses. Bite reactivations of as much as 7mm can be readily achieved using the standard 12mm advancement screws. For greater activations, 16mm or 20mm screws may be required. [13]



Fig-13

Implant supported twin block

This technique uses two orthodontic mini-implants (1.5 × 9 mm × 9 mm) inserted bilaterally on lower arch between the mandibular second premolar and first molar. The use of mini-implant anchorage with twin-block therapy can provides absolute control over the position of lower incisors which cannot be controlled by any other mean



s.

Fig-13

Conclusion

Modification of the twin block allowed easy chairside reactivations and independent control of upper and lower arches in vertical, sagittal and transverse direction. Thus twin block has become an extremely versatile appliance which benefits patients in all age group right from early childhood to young adulthood.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Key Stone Entity in Paediatric Dentistry: Tell Play Do

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Abstract

The child behaviourmanagement in paediatric clinic is the way by which the dental health care team effectively and efficiently performs treatment for a child. To provide dental care for children good communication skills are necessary.Dental fear is a common, essential, and inevitable emotion that appears as a response to the stressful situation, which raises children's anxiety level, resulting in reduced demand for paediatric dental care. Behaviour management is widely agreed to be a key factor supplying dental care for children. TSD technique was modified into Tell-Play-Do (TPD) technique, using the concept of learning by doing in reducing children's fear and anxiety to dental treatment and promoting adaptive behaviour.

Key Words: *Fear,Anxiety,Behavioural modification,Children,Tell Play do*

Introduction

Behaviour management is considered a keystone entity in Paediatric dentistry[1,2,3].Disruptive behaviour can interfere significantly with providing quality dental care. Non-pharmacologic tell-show-do technique which consists of verbal explanation of the procedure to the patient, demonstration for the patient of the (visual, auditory and tactile) aspects of the procedure and completion of the procedure, remains the most commonly used technique in paediatric dentistry. Many children experience distress when visiting a doctor or undergoing needle procedures. Children's experiences can have bothshort-andlong-termconsequencesontheirmental and physical health.Providing accurate information may result in more positive emotional and physical outcomes for children[4].

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Methodology

Articles were collected using Pubmed database, hand search. The search words include Fear, Anxiety, Behavioural modification, Children, Tell Play do. Articles were collected from year 2016-2019. Of which 3 articles emphasized the significance of using. Tell Play Do technique in reducing the fear and anxiety of children were considered for this short communication.

Tell Show Do

This method is extensively used to familiarise a new procedure to the patient[5,9]. This is the verbal explanations of procedures in the way the child could understand (tell); presentation of the visual, auditory, olfactory and touching aspects of the procedure is carefully done without threatening (show) and then, without deviating from explanation and demonstration, the procedure has to be done (do). The tell-show-do technique is used with verbal and non-verbal communication skills and positive reinforcement. Other measures will be needed depending on whether the child has communication problems or disorders. This technique has been an effective way for reducing previously formed anxiety in the child patient [6,7]. In the tell phase the dentist gives an explanation about the procedure depending upon the age of the patients. The show phase is used to illustrate

the procedure, example demonstrating with a slow hand piece on a finger.

TSD technique introduced by Addelston in 1959 dictates that before any procedure is done, the child is to be well informed and a demonstration should be given using a simulator exactly what will happen before the procedure is started.

TSD technique is based on the principle of learning theory[8,9] and is performed by the dentists themselves in the operatory room.

TELL PLAY DO

It is important to communicate with the child patient briefly at the beginning of a dental appointment to establish rapport and trust.[3]

Only by explaining, demonstrating, or observing a model, instead of it make them to play with dental imitating instrument toys, which provides more explanatory concept.

With this idea, TSD technique was modified into Tell-Play-Do (TPD) technique, using the concept of learning by doing in reducing children's fear and anxiety to dental treatment and promoting adaptive behaviour.

The child was directed to the play room, where the room was arranged with customized dental instrument toys and a cartoon character with mouth wide open.The trained dental personnel explained all the customized dental objects using appropriate euphemisms and procedures in phrases appropriate to the developmental level of the child and allowed to hold dental imitating instruments including syringe to play and perform dental procedure on the cartoon character.

The airotor noise was incorporated in the dental object resembling clinical sound effect.The duration of whole procedure was standardized for 20 min; then, the child was taken to clinical area and oral prophylaxis was performed in first visit.The second treatment session was set 1 week later. The treatment protocol was the same for all the participants.The child entered the operating room alone.Then, the occlusal cavity was prepared for restoration of the teeth. During deep cavity preparation, the required injection including the use of topical anaesthesia and a local infiltration was performed by the dentist.

In all children, parameters such as the attending dentist, his/her assistant, the working environment, time and duration (30 min for each child) of work, and the type of dialogues and euphemisms were all the same. Care was taken to make sure that the children were not tired and hungry.

Mean heart rate at different intervals was significantly lower among children in Group II (TPD) than among those in Group I (live modelling) during first visit (after intervention and during procedure) $P = 0.038$ and 0.026, and also in second visit (during procedure), $P = 0.001$

The results of this study showed that TPD technique was more effective than live modelling on child anxiety levels and increased the cooperative behaviour during dental treatment among 5–7-year-old children, reducing the heart rate and mean change in the heart rate.

TSD technique remains the most commonly used technique in paediatric dentistry and is still considered the technique with which the dentists and the parents are comfortable[1] and justifies being the method of choice as the backbone of child education and behaviour guidance during first dental visit.[10]

The competent modification of TSD to TPD is significantly effective than live modelling in reducing not only the heart rate (physiological index) but also the cooperative behaviour.

Number of children showing positive response and cooperation had reasonably increased from first visit to second visit among TPD group than live modelling group.

According to study, TPD technique among 5–7-year-old children is impressively effective and can create an extremely good patient at this stage of life as 40 (81.6%) were definitely positive during second visit of restorative procedure.

Conclusion

By simple modification, TPD can have a greater impact on younger children, so that they can feel comfortable and accept the dental treatment.

This is especially important in our set up as parents are increasingly apprehensive and less willing to allow the use of conscious sedation or undertake general anaesthesia.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Naturally Available Transport Medium For Avulsed Teeth- A Review

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Abstract

Tooth avulsion or exarticulation is a traumatic injury of dental tissue characterised by complete displacement of the tooth out of its socket. Successful treatment outcome is dependent on the survival of the viable periodontal ligament cells attached to the tooth root surface. This review discusses various storage/ transport media for avulsed tooth obtained from natural sources. The knowledge of availability and accessibility of such natural storage media would increase the awareness among the healthcare providers and would therefore improve the prognosis of the treatment protocol by reducing extra-oral dry time of the avulsed tooth.

Keywords*: Avulsion, Periodontal Ligament, Trauma*

Introduction

Tooth avulsion or exarticulation is a traumatic injury of dental tissue characterised by complete displacement of the tooth out of its socket.[1]Children of age group of 7 to 9 years are more likely to suffer these injuries particularly involving avulsion. Ideal management of tooth avulsion is prompt treatment of socket and tooth, with reinsertion of the tooth into its socket. Reinsertion of tooth back within 5 minutes has the best outcome in which there is least loss of viability of periodontal ligament cells and hence reestablishment of periodontal fibres occurs, regaining the functionality of tooth.[2,3,4]

The ideal requirements for a storage medium are[5,6]

• It should have antimicrobial characteristics

• It should maintain the viability of periodontal fibres for an acceptable period of time

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• It should not produce any antigen-antibody reactions

• It should reduce the risk of post-replantation root resorption or ankylosis

• It should have a good shelf life

• It should be effective in different climates and under different conditions

• It should wash off extraneous materials and toxic waste products

• It should aid in reconstitution of depleted cellular metabolites.

The various naturally available transport medium discussed in this article are milk, saliva, green tea, egg white, coconut water, green tea, aloe vera, soy milk, rice water , honey and tap water.

MILK

Milk has a unique combination of nutrients, capable of maintaining the PDL cell viability, and with physiological pH range of 6.5-7.2.[6]Milk is a compatible short-term storage medium for teeth if they were placed in it within 15 to 20 min of being avulsed[7,8,9]

In spite of offering no conditions for the restoration of cell morphology nor cell differentiation or mitosis, milk prevents cell death[6-11] Several authors that evaluated the viability of PDL cells in contact with milk have reported 70 to 90% survival rates and low frequency of root resorptions after periods up to 72 h.[5-9] Regarding the different milk presentations reported there was no significant difference between regular pasteurized milk and long shelf-life ultra high temperature [UTH] pasteurized whole milk at any time period.[10]Low fat content milk and chilled milk has shown better results in maintaining the viability of PDL and for a longer time period.[5,7,9,10,11]

The pasteurization of milk is responsible for diminishing the number of bacteria and bacteriostatic substances also for the inactive presence of enzymes, which could be potentially harmful to the fibroblasts of the periodontal ligament.[12,13,14,15]

SALIVA

Though readily and most easily available, saliva is not considered as an effective interim transport medium. [15] Recent literature indicates that saliva may not be a suitable transport medium for avulsed teeth due to its non-physiologic osmolarity and the presence of microorganisms.[16]Saliva may be used as an immediate interim storage medium. Saliva contains potentially harmful substances, such as enzymes, bacteria and their by-products[17]. A clonogenic capacity of 1.5% is obtained when PDL cells are stored in saliva for more than 30 min. In an animal study it was proven that saline and saliva were suitable storage medium for protection against root resorption for short extra-alveolar periods. [18]

GREEN TEA

Epigallocatechin-3-gallate [EGCG], an important polyphenol of green tea, is known to have various biological effects such as antioxidative, anticarcinogenic, antimutagenic, anti- inflammatory, antimicrobial and antiviral activities [19].Additionally, Hwang et al. [20] and Jung et al. [21] in the search for a medium capable of minimizing the infections after tooth replantation, maintaining PDL cell viability and reducing root resorption and ankylosis, reported enthusiastic results with green tea, with the maintaince of 90% of cell viability for up to 24 h, similar to the HBSS control. Jung et al. [21,22,23,24]

EGG WHITE

Egg white and ovalbumin, the major protein in egg white, are considered a good choice as a storage media for teeth undergoing delayed replantation due to its high content of proteins, vitamins and water, absence of microbial contamination and easy access [25,2627,28]. Sousa et al. [29] evaluating human PDL adhered to extracted tooth roots and maintained in this storage medium observed that the egg white provided cell viability and histological characteristics similar to those of milk. Khademi et al. [30,31]Khademi et al have compared milk and egg white as solutions for storing avulsed teeth, and the results have shown that teeth stored in egg white for 6 to 10 hours had a better incidence of repair than those stored in milk for the same amount of time [$p < 0,05$]. [32]

COCUNUT WATER

Coconut water is a natural, biologically pure, sterile product rich in amino acids, proteins, vitamins and minerals. Several studies have been performed to use this substance as a storage medium for avulsed teeth, but the results are contradictory. Gopikrishna et al. [33] and Gopikrishna et al. [34] found greater efficacy of coconut water over HBSS and milk for the viability of PDL. Thomas et al. [35]found that 15 to 120 min storage in coconut water is as efficient as storage in HBBS. On the other hand, Pearson et al. [36,37,38,39]

SOYMILK

Soy milk; the extract of the soybean, is rich in protein, aminoacids, vitamins and essential minerals. It contains very low amounts of saturated fat acids and have a physiological pH [40]. Due to its positive effects on cell growth and its biochemical activities, the use of soy milk as a storage medium for the avulsed teeth has been deemed appropriate [41,42]. In studies, it has been reported that the durability of the periodontal cell viability is maintained over 90% in the avulsed teeth kept in soy milk for 24 hours and it is emphasized that soy milk is a very effective storage medium [43,44]. The authors have reported the excellent potential of soymilk as a storage medium and for proliferation of several cell types [45,46,47,48].

RICE WATER

Rice is believed to play an important role in acute inflammation due to low sodium, high potassium, niacin and thiamin contents in its structure, and in collagen synthesis due to the presence of iron and zinc. Due to its nutritional value, especially in countries with intense rice consumption, rice water is newly studied as a storage medium for avulsed teeth [49,50,51].

ALOE VERA

Aloe vera is a plant that featured in the medical field due to its antidiabetic and anticarcinogenic properties. It is also used in dentistry due to its anti-inflammatory and analgesic properties. The antioxidant property of aloe vera, which plays an important role in the viability of the cells, has attracted the interest of researchers in terms of storage of avulsed teeth [42,43].

HONEY

A large volume of literature reporting its effectiveness indicates that honey has potential for the treatment of periodontal disease, mouth ulcers, and other problems of oral health [49].

Maintenance of viability of PDL cells in the long shelf-life honey milk may be due to the nutrients that are present such as proteins, essential amino acids, vitamins and minerals which help in nourishing the cells and maintaining their viability.[48] Due to its ease of storage and long shelf life, it can be available in schools, gyms, and outdoor athletic fields, were tooth avulsions are most likely to occur.[52]

TAP WATER

Tap water has inadequate characteristics to be used as a storage medium for avulsed teeth because it has bacterial contamination, hypo-tonicity, and non-physiological pH and osmolality, which favours the PDL cells lysis[50-52]. Several studies have shown that cells stored in water did not maintain their morphology, with visible destruction and rapid cell death.[51]Tap water should be used only to avoid the tooth from dehydration. [52]it should be remembered that it is the least desirable storage medium available and its use will lead to ankylosis and replacement resorption. [52]

Discussion

As of now, there is not a single product or solution that possesses all the characteristics required to be indicated as the ideal storage medium for avulsed teeth, that is, be capable of preserving the vitality of the PDL and pulp cells, while presenting compatible physiological pH and osmolality, clonogenic capacity, antioxidant property, no or minimal microbial contamination, high availability, ready accessibility at accident sites, homes, schools, hospitals and dental offices, and low cost.[43,31]Studies on duration of extra-alveolar period and successful replantation of avulsed teeth have sharpened the issue of dry period. The dry period should be as less as possible and it would be better if immediate replantation can be achieved.[36]

Conclusion

Currently there is no national data on incidence of avulsion injuries in the Indian population. Therefore the demand to know about the storage medium was not at high interest, the best storage media would be natural products with easy accessibility to general public and dentists as well. Chilled low fat/skimmed milk is the best available storage medium followed by honey and childs own saliva. Tap water was proven to be the least advisable storage medium. Newer research is still required to find that easily available, cheap and low maintenance storage medium which can maintain the viability of PDL cell and regenerate them as well.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Gymnema Sylvestre - A Review

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Abstract

Many people suffering with diabetes mellitus with increased risk of complication which affects the quality of day today life style this increases the mortality rate of the people. To reduce the people financial burden and to prevent the side effects of diabetes mellitus, an extract of plant called *Gymnemasylvestre's* shows a potential effect in reducing the sugar level.*Gymnema* leaves have the mixture of bioactive constituent's tri-terpenes, glycosides and saponin property which represents the antidiabetic property. Theleaves of gymnema sylvestre are used in herbal medicinal preparation. Theleaves of *Gymnema sylvestre* contain both antidiabetic and hypolipedemic properties. Here the review of such plant is discussed in this paper.

Keywords*:Gymnema sylvestre, glycosides, saponin, Diabetes mellitus.*

Introduction

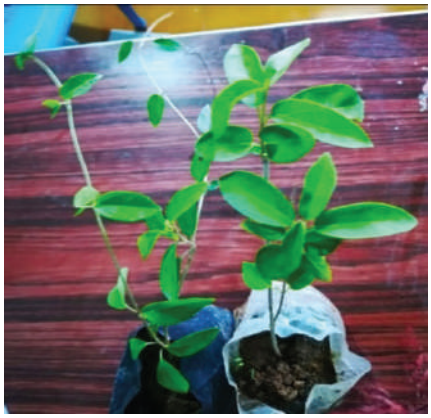
During the last decades, the medicinal plants are used for long term remedies for human diseases. Nowadays, there is an increased demand for using herbs in therapy instead of using synthetic drug which may have adverse effect. Traditionally *Gymnema* is a large woody climbing plant belongs to the family Asclepiadaceae, widely spread in India, Indonesia, Australia and japan. it is commonly known as gurmar (Hindi), madhunashini (Sanskrit) sirukurinjan (Tamil) Both the dried leaf and root powder *G.sylvestre*are used for diabetic medicine the leaves of *gymnema* are reported to be bitter (11) they temporarily paralyze the sensory perception of sweet and or this amazing property it is also known as sugar destroyer (6).*Gymnema* leaves have the mixture of bioactive constituents tri-terpenes,glycosides and saponin property viz,gymnemic acid,gymnemagenin and gurmarin which represents the antidiabetic property.

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The leaves of the plant are also used as a digestive, hypoglycemic, antiviral,and antiallergic and antiobesity (10) agent for the treatment of diabetes,obesity and dental caries. The main constituents of G*ymnema* are believed to be gymnemic acid,a mixture of 17 different saponins. The purpose of this work is concerned with screening the review of the plant for antidiabetic and hypoglycemic properties of leaves of *G. sylvestre*. Theleaves of the plant have triterpenoid saponin in the cells of plant parts called gymnemic acid which is the principle controlling component of diabetes mellitus (1).

Morphology of *Gymnema sylvestre:*

Gymnema sylvestre(Fig. 1) is a large, stout, woody shrub growing plant which belongs to the family of Asclepiadaceae. It is mainly located in central and southern India. Theleaves of *Gymnema sylvestre* are elliptic and oppositeflowers are small and yellow in colour up to 3 inches in length. The calyxes are long, ovate and obtuse. The taste of leaf is slightly bitter. It also possesses remarkable property of destroying the sense of the taste for sweet substance for few hours while chewing the leaves of *Gymnema sylvestre*.Hence it is commonly called as sugar destroyer.*Gymnema sylvestre* leaf powder is tasteless with a faint pleasant aromatic odour.



Taxonomy

Kingdom -Plantae

Division -Angiosperms

Class - Dicotyledons

Order -Contortae

Family -Asclepiadaceae

Genus - Gymnema

Species - G.sylvestre

Fig. 1 *Gymnema sylvestre*

Uses of *gymnema sylvestre:*

Gymnema has played an important role in traditional Ayurvedic medicine for centuries.The leaves also have been used for stomach ailments, constipation and hemorrhoids, water retention, and liver disease. The flowers, leaves, and fruits have been used in the treatment of alterations of blood pressure and heart rhythms. Chewing the leaves destroys the ability to identify the sweet taste, giving it the common Hindi name of gurmar, or "sugar destroyer." The plant has been used in traditional medicine, most notably to control blood sugar. Use of gymnema as a lipid-lowering agent, for weight loss, and to prevent cavities has also been investigated, in animal studies. The bark is useful as an emetic and expectorant. Also used for malaria, digestive stimulant, laxative, appetitesuppressant and diuretic. And also as analgesic for body ache. Moreover it is used as anti-inflammatory, anodyne, liver tonic, emetic, thermogenic, stomachic, anthelmintics, cardiotonic, expectorant, antipyretic, and uterine tonic.Significance place in the treatment of jaundice, constipation, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis, renal and vesical calculi, dyspepsia, leucoderma, and Parkinsonism.The plant extract is also useful in the treatment of piles, colic pain, dropsy, phlegm, and eye troubles, cardiac and respiratory diseases. Many literatures reveals that *G. sylvestre* is a popular plant in treating diabetes and also used in the treatment of asthma, the root is used for snake bite, urinary complaints and colic pain.

Side Effects of*Gymnema*

Chronic usage of the drug leads in lowering the blood pressure. It is a hepatotoxic substance which causes damage to the liver. The Gymnemic acid and Gurmarine suppresses the ability to detect the sweet taste. *Gymnema* has been reported to possess a sweet-taste suppressing effect, attributed to the peptide (4, 7-9).

Phytochemical screening

Freshly prepared extract were subjected to preliminary phytochemical analysis to find out the presence of the following phytoconstituents. They are saponin, glycosides, tannins, alkaloids, triterpenoid, coumarins, and phenol and also have hypoglycemic activities.These analyses will determine the biologically active compounds that contribute to the flavor, colour, and other characteristics of leaves and show their medicinal activity and the hypoglycemic activity of the plant extract.The major bioactive components of *G.sylvestre* include olenane-type of triterpenoid saponins known as gymnemic acids.The main constituents of gymnema are believed to be gymnemic acid,a mixture of 17 different saponins (1).

Saponins are naturally occurring glycosides which is produced by the plant *G. sylvestre*.The triterpenoid is generally predominant.The saponin inhibits the cholesterol absorption from the intestinal tract.Saponins are large molecules contains a hydrophilic part and lipophilic part.Saponin has the capacity of reducing the surface tension of water. This is due to the fact that the saponin molecules with their hydrophilic ends lie away from the water. This produces the foam formation when dissolved in water. Saponins are glycosides.They are divided into two classes based on the structure of ring.The steroidal saponin contains four-ringed steroidal andtriterpenoid saponin have a five ringed structure. Thephytochemical test were carried on ethanolic extract of *gymnema sylvestre* which

shows the presence of following components such asalkaloids,glycosides,saponin,phenol,coumarin and triterpenoids.

The plant has been used in traditional medicine, most notably to control blood sugar. Use of *Gymnema* as a lipid lowering agent, for weight loss and to prevent cavities has also been investigated, primarily in rat or mouse studies. However little to no clinical information is available to support the use of *Gymnema* for any indication.

Pharmacological uses

Gymnemasylvestre helps to promote weight loss as it has the ability to reduce cravings for sweets and controls the blood sugar level.Thecombined effect of *G.sylvestre* and hydroxycitric acid, niacin bound chromium serves as an effective weight loss formula which promotes the healthy blood lipid level (2).Its leaves increases the secretion of insulin, promotes regeneration of islets of Langerhans, increases the utilization of glucose and causes inhibition of glucose absorption from intestine (12).

Antidiabetic activity:

The ethanolic extractof *gymnema sylvestre* reduced the blood glucose level in diabetes. In the present study the phytochemical testing shows a saponin rich component. The action of crude saponin fraction and five triterpene glycosides derived from the methanol extract of *G.sylvestre*(16).

Hypolipidemic activity:

The administration of leaf extract found to shows reduction in elevated serum triglyceride,total cholesterol, low density lipoprotein and very low density lipoprotein in dose dependent manner (4).

Antimicrobial activity:

The ethanolic extract of gymnema sylvestre showed antimicrobial activity against bacillus pumilis, b.subtilis, pseudomonas aeruginosa and staphylococcus (14) and not against Escherichia coli (13).

Conclusion

Herbal compounds have received much attention for management of various diseases.When compared, it is also safer than synthetic drugs.Anatomical characterization provides additional support towards authentication and elimination of adulteration of market sample of potent medicinal plants as *G.sylvestre*(5).The *Gymnema* leaf main component is saponin and gymnemic acid,which plays a role by treating the diabetic.Presence of different types of phytochemical constituents such as alkaloids,tannins,saponins,anthraquinone and glycosides give an idea that the ethanolic leaf extract of *G. Sylvestre* can be used as an accessible source for type 2 diabetes mellitus (14). Further research is necessary to find the active compounds within these plants with their full spectrum of efficacy.

Ethical Clearance – Not required since it is a review article

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Conflict of Interest – nil

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Methods for Distalizing Molar – A Review

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Abstract

The extraction or non extraction debate from the Angle era to the present day orthodontics does not define an absolute indication for a specific treatment plan One of the non-compliance therapies and fairly recent concept is the MOLAR DISTALIZATIONwhich has been effectively used in the correction of malocclusion. Advances in mechanotherapy and changes in treatment concepts have reduced or minimized the need for extraction in severe discrepancies. Various techniques are currently employed in non-extraction therapy in the treatment of a malocclusion

Keywords – *Molar Distalisation, Mechanotherapy,Malocclusion*

Introduction

Early cephalometric studies have showed that little or no distal movement of upper molars was produced by class II elastic treatment of that era. The head gear was reintroduced as a means of moving the upper molars back, patient compliance plays a major role in success of head gear therapy.An appliance system independent of the patient cooperation was the need of the hour and then evolved the molar distalizers.[1]

History

The extraction or non extraction debate from the Angle era to the present day orthodontics does not define an absolute indication for a specific treatment plan. **Angle** strongly believed in retaining teeth provided by nature and molding the facial form through occlusion. Angle's unweilding allegiance to non extraction therapy was based on his own specific knowledge dentofacial growth and development and to the concepts of facial beauty harmony. **Case** argued for therapeutic extractions in orthodontia based on the fact that inherited inharmonious in contiguous structure over which we have no control makes it impossible for us to place all the teeth in the arch without fulfilling the designs of an inherited deformity.[2,3]

Indications:[4,5,6]

- In the non extraction treatment of class II malocclusion.
- In low angle cases.
- In class I skeletal pattern cases.
- In patients with mild arch length discrepancy.
- In cases where upper permanent first molars have moved mesially due to early loss of deciduous molars.
- In patients where second molar extractions are planned or has not yet erupted.

Contraindications

- In high angle cases.
- In class II and class III skeletal pattern
- Skeletal and dental open bite.
- Severe arch length discrepancy cases.

Classification

- EXTRA ORAL

– Head gears

- Cervical pull
- Combination pull

- Kloehn.
- INTRA ORAL
  - Removable appliances
  - Fixed appliances
- Buccaly acting
- Palatally acting

DISTALIZING APPLIANCES:

TUBE PLATES:

This appliance was developed by **Alain Benauwt**[1] in the year 1972. The appliance is said to have a good retention as the movable part also contributes to the retention.Unwanted displacement of teeth is minimized due to the clasp as it avoids molar rotation.Possible to add an extra oral appliance to support and reinforce stationary part. Progressive expansion of the arch is also possible by changing the angulation of the tube in relation to saggital plane.Repair is easy.But, the construction is very delicate, since the two wires holding the movable part should do so without binding.[7]

THE CETLIN APPLIANCE:

It was developed by Cetlin[2] in 1982. It utilises extra oral force in the form of headgear and an intra oral force in the form of a removable appliance.To overcome the disadvantages caused due to the tipping of molars, the Cetlin appliance utilizes a removable appliance intra orally to tip the crowns distally and then an extra oral force to upright the roots. So the intra oral remarkable appliance can be called the crown mover while extra oral force, the root mover.The anchorage for removable appliance is by proper adaptation to the palate an acrylic shield around the four maxillary incisors and a modified Adams clasp on the first premolars.The extra oral appliance is a headgear which is inserted into the molar tube. The headgear is usually cervical or a high pull, depending on the usual consideration of skeletal pattern.[8]

THE CRICKETT APPLIANCE:

It was introduced by WEST[3] in 1984. The Crickett's appliance embraces the essential features of the quad Helix. But replaces the palatal and lingual bars of upper and lower appliances with a quad and bi-helix respectively. The Crickett's lingual arms are embedded to provide an adjustable spring action directed to lingual surfaces of all teeth, without the need for further soldering.[9]

The buccal arms are retained for attachment of elastics and for ease of insertion and removal of the appliance.Upper palatal and lower lingual main frames are constructed from 0.032" yellow and 0.038" blue elgiloy respectively.The cribs, clasps and occlusal rests from 0.028" blue elgiloy. The lingual arms from 0.030" yellow elgiloy and buccal arms from 0.045" blue elgiloy. The crickett is an effective appliance for variety of tooth movements including distalization of molars.The major limitation of this appliance is when intrusion of anterior teeth has to be performed.

REPELLING MAGNETS:

This appliance was introduced by **Gianelly** in the year 1989. This method of distalizing the molar is by the use of modified nance appliance with the use of repelling magnets.The modified nance appliance serves 2 functions:[10]

- Activation of the Magnets -This is by tying a 0.4" ligature wire through the loop and extended anteriorly to encircle a tie back hook mesial to the magnets. When tightened, the magnets are held in contact.
- To contain the reaction force arising from the magnets.

The disadvantage is that the forces exerted by magnets drops significantly as spaces are opened and discomfort to the patient.

MOLAR DISTALIZATION USING SUPER ELASTIC NITI WIRE:

This mechanics was introduced by **Locatelli** and **Bednar** in 1992.Maxillary molars are moved distally using a super elastic nickel titanium wire with shape memory (Neosentalloy).As the wire returns to its original shape, it exerts a 100 gm distal force against the molars and a mesial reaction force on premolars. There is also tendency for the premolars to move buccaly. Anchorage can be controlled by placing 100-150 gm class II elastics at the hook between canine and lateral.

This method moves the molars distally by 1-2 mm per month with little loss of anchorage.Neosentalloy is easy to insert even after all the teeth have been bracketed or banded.But the disadvantage is once the second molars are erupted, distal movement of first molars usually take more time.If the first molars do not move by atleast 1mm / month, a 200 gm 0.018 X 0.025 Neosentalloy wire can be placed with increase in force, there by increasing the chances of loss of anchorage.[11]

NITI DOUBLE LOOP SYSTEM:

To overcome the disadvantages of the conventional design, **Giancotti** and **Cozza** in the year 1998 introduced a new system using the Neosentalloy. This new system was employed in simultaneous distalization of the 1st and 2nd molars. Two sectional Niti arch wires (on either side) are prepared by crimping stops distal and mesial of the IInd premolar bracket and 5 mm distal to each second molar tube.Uprighting springs are inserted into vertical slot of the 1st premolar and class II elastics are placed between mandibular 1st molar and maxillary canine bracket.The advantages are, Minimal patient cooperation is enough. And it is ideal for simultaneous first and second molar distalization .Second Molars move easier distally compared to first molar because of their different anatomical shape of the roots and lack of posterior obstacles. Because of the stretching of transeptal fibers, an 80 gm Niti wire is used instead of 100 gm or 200 gm.[12]

PENDULUM APPLIANCE:

It was developed by **James Hilger** in the year 1992. It is a hybrid that uses a large nance acrylic button in the palate for anchorage, along with .032 TMA spring that deliver continuous force to the inner first molar without affecting the palatal button.It has a broad swinging or pendulum of force from the midline of palate to the upper molars.The right and left pendulum springs formed from 0.032" TMA wire, consist of a recurved molar insertion wire, a small horizontal adjustment loop, a closed helix and a loop for retention in the acrylic button.Springs are extended as close to the center of the palatal button as possible to maximize their range of motion, to allow for easier insertion into the lingual sheaths and to reduce forces to an acceptable range. The use of 0.032 TMA springs delivers continuous force to the upper first Molar without affecting the palatal button. Activation can be done before appliance placement. But the disadvantage is pure bodily movement of the molar is not seen, tendency towards cross bite.[13]

MODIFIED PENDULUM APPLIANCE WITH REMOVABLE ARMS:

Pisani and **Takemoto** along with **Vecchia** in the year 2000 introduced this particular modification. It has a Double over 7mm – 9mm length of 0.032 TMA wire to form bayonets. Each bayonet is attached to an M-PENDULUM arm either by using Laser welder or by wrapping 0.10 ligature around arm and soldering the unit. It is then embed into each bayonet in the soft acrylic that will be used to form Nance button producing sheaths in which to insert the removable arm.Appliance is placed in the mouth along with the terminal ends of arms into lingual molar band sheath.Removable arms can be reactivated without debonding the occlusal rest of Nance button.[14]

MODIFIED PENDULUM APPLAINCE FOR ANTERIOR ANCHORAGE CONTROL:

This latest modification was introduced by PABLO ECHARRI and SCHUZZO in the year 2003.This design consists of four removable arms for both first and second molars. The second molars are distalized after which their arms are left passively in place for anchorage and first molar arms are activated for distalization.Pendulum is replaced with a nance button after first molar distalization. A 0.016 SS passive arch wire is placed to avoid any incisor protrusion.E-Chain is used to distalize second and first bicuspids.If anterior anchorage is critical, palatal acrylic should be kept out of contact with the incisors.Second bicuspid arm should not be cut for spontaneous distalization to prevent incisor protrusion.[15]

DISTAL JET APPLIANCE

It was introduced in the year 1996 by **Carano** and **Testa**.Bilateral tubes of 0.036" internal diameter are attached to an acrylic nance button.A coil spring and screw clasp are slid over each tube (NiTi coil spring of 150gms for children and 250gm for adults)The wire extending from the acrylic through each tube ends in a bayonet bend that is inserted into the lingual sheath of the first molar band, this results in force acting through the center of resistance of molar thereby giving

a translatory movement.An anchor wire from Nance button is soldered to bands on second premolar.[16]

DOUBLE SET SCREW DISTAL JET:

This modification of distal jet was introduced in the year 1998 by **Jay Bowman**. This modified distal jet incorporates two set screws into activation order which permits an easier, cleaner and more reliable conversion to a molar nance holding arch.The mesial set screw is used during active distalization.Upon distalization activation caller is slid mesially to gain access to the coil spring.The double set screw collar is slid back to this junction.[17]

THE K LOOP MOLAR DISTALIZER.

It was introduced by **Kalra** in the year 1998. The appliance consists of a K loop to provide the forces and moments and a Nance button to resist anchorage.The K loop is made up of 0.017 X 0.025 " TMA which can be activated twice as much as stainless steel before it undergoes permanent deformation.Force produced by the TMA will also be half. The loop of the K should be bent 8 mm in length and 1.5 mm wide.The legs of the loop are bent down 20° and inserted into molar tube and Premolar bracket.[18]

NANCE APPLIANCE AND COIL SPRING:

It was developed by **Peringer, Parmann** and **Droschl** in the year 1997.The appliance consists of 2 premolar bands, connected by a soldered palatal framework and an anterior acrylic shield for palatal support.Distalization is produced by the sentalloy coil springs (150-200gm) on sectional arch wires.

MODIFIED NANCE APPLIANCE FOR UNILATERAL MOLAR DISTALIZATION:

The class I side of 0.036" SS wire framework was finished with an anteriorly projecting 0.036" arm like that of quad helix.The active class II side has an arm which is soldered to the first bicuspid band. An 0.020" omega loop is soldered to the anterior end of framework which allows the distal end of the loop to slide distally as it is opened by activation.A 10mm 0.09"x0.036" open coil spring is added to the frame work between omega loop and first molar band.[19]

WILSON'S RAPID MOLAR DISTALIZATION:

It was advocated by **William L. Wilson** and **Robert C. Wilso**n, to distalize the maxillary molars, while the mandibular molars maintain the pre-treatment antero-posterior positions. The Wilson treatment achieves molar distalization without extra oral forces.It has advantage of having no extra oral force, the Class II correction starts immediately (even in mixed dentition), no reactionary maxillary incisor proclination and can be used in mixed dentition.It has disadvantage of longer treatment time (than originally propose) Wilson said that the treatment time was 6-10 weeks actually it takes 16 weeks. The distal tipping occurs frequently and the tipped molars have questionable stability.A significant portion of the class II correction was found to be due to mesial movement of Mandibular Molars.

THE CARRIERE DISTALIZER:

It was developed by **Carrier**e in the year 2004. It produces a distal rotational movement of maxillary first molars around their palatal roots when necessary. Simultaneously produce a uniform force for distal molar movement.Independently it can move each post segment from canine to molar as a unit. It also eliminate wire changes. It minimize periodontal reactions.The clinical evidence of achievement of these objectives will be the appearance of interincisal diastemas and wide spaces mesial to canine. The distalizer is made of mold injected Nickel free stainless steel. The 'Canine Pad' which allows distal movement of canine along alveolar ridge without tipping provides a hook for attachment of class II elastics.This pad is mesial end of the arm that runs posteriorly over the 2 upper premolars in a slight curve. The posterior end of the arm is permanently attached ball that articulates in a socket on the molar pad.

THE FRANZULUM APPLIANCE:

It was invented by **Byloff**[27]and **Darendeliler**[27] in the year 2000. Gaining space in the mandible is more difficult than in the maxilla. Extra oral appliances are seldom attached to the mandibular molar because of the pressure they place on the condyles.The Franzulum appliance's anterior anchorage unit is an acrylic button, positioned lingually and inferiorly to the mandibular anterior teeth and extending from mandibular left canine to the right canine.Rests on the canines and first

premolars are made from 0.32" stainless steel wire. Tubes between the second premolars and the first molars receive the active components.[20]

Conclusion

Though a number of appliance systems are available, every clinician should cautiously begin with a precise diagnosis, sound treatment plan and appliance selection taking into consideration various factors pertaining to the case selection like the age of the patient, growth pattern and also the factors relating to a particular appliance system (Molar Distalizers). Therefore any one molar distalizer cannot be concluded to be ideal for any clinical situations. It is in the hands of the clinician to thoroughly analyze the clinical picture and select the appropriate molar distalizing appliance. Thus it's not just the superiority of the mechanics but the superior thinking and application of the clinician that can produce a good and stable result.

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Frozen Section-A Short Review

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Abstract

Frozen section is a specimen of tissue that has been quick-frozen, cut by microtome, and stained immediately for rapid diagnosis of possible malignant lesions. A specimen processed in this manner is not satisfactory for detailed study of the cells, but it is valuable because it is quick and gives the surgeon immediate information regarding the malignancy of a piece of tissue. Frozen section plays an important role in the management of surgical patients yet it must be used prudently to avoid the indiscriminate usage of this important technique. As it is subjected to many limitations compared to the paraffin embedded tissue sections, this review highlights the important concepts and principle of intra-operative frozen section consultation as well as discussing the limitations of this technique.

Key word: *frozen section, tissue, Microtome.*

Introduction

The technique of frozen section was first introduced by the pathologist, William H.Welch, in 1891. In 1920s, the technique became popular and was used for intra operative consultation.[1] The main purpose of frozen section is to provide rapid diagnosis to guide the surgeon in per operative decision making. The indications of frozen section are identification of tissue, to establish that sufficient diagnostic tissue has been obtained; identification of lymph node metastasis and to obtain a tissue diagnosis to detect the nature of the lesion.[2,3]

Frozen section is regarded the most definitive form of consultation because it employs the microscopic examination of tissue as designated by the surgeon which is important to conduct an operation already underway. The distribution of requests for frozen sections related to specific organ systems is institution driven based on the staffing of surgical specialists and patient referral patterns

Frozen Section

Frozen Section may be one of the most important difficult procedure performed by the pathologist during his practice. The pathologist has to arrive at a correct decision in a shorter duration based on his experience and the knowledge of his specialty and clinical medicine.

The key instrument for Cryo section is the cryostat, which is a microtome inside a freezer. The microtome can be compared to a very accurate "deli" slicer, capable of slicing sections as thin as 1 micro metre. The usual histology slice is cut at 5 to 10 mm. The surgical specimen is placed on a metal tissue disc which is then secured in a chuck and frozen rapidly to about −20 to -30 °C. The specimen is embedded in a gel like medium consisting of poly ethylene glycol and polyvinyl alcohol. At this temperature, most tissues become rock-hard. Usually a lower temperature is required for fat or lipid rich tissue. Each tissue has a preferred temperature for processing. Subsequently it is cut frozen with the microtome portion of the cryostat, the section is picked up on a glass slide and stained usually with hematoxylin and eosin, the H&E stain.

Techniques of Frozen Technique

1.Gross examination- The pathologist records all gross expressions, i.e., size, adhesions, weight, similar to the recording of microscopic features.

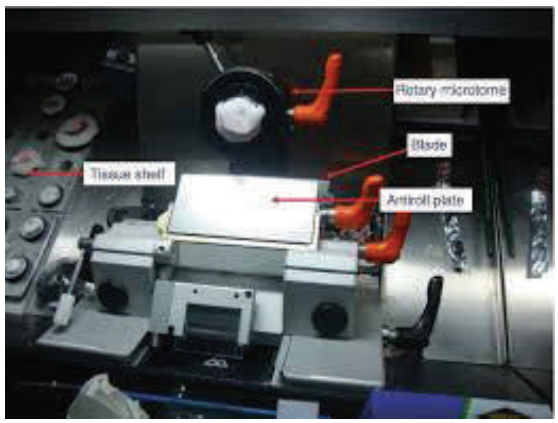
2- Embedding the tissue:

The selected piece of tissue is then placed on a metallic holder and must be oriented a certain way so that the future section will reveal proper spatial relationships,

this orientation depends on the question being asked. Sometimes orientation is not important; at other times it is of paramount importance. The tissue is embedded in OCT mounting medium and is then placed either in cooled 2-methyl butane or the cryostat machine where it is properly frozen.

3. Cryostat:

The machine, which cuts the tissue, is the cryostat. Certain things should be routinely checked in the operation of this machine:



a) Rotary microtome

a) Temperature:

The temperature should be at -20°F . Tissues with a large fat component, -40°F is optimal. This temperature is critical for optimal sectioning. If too high, i.e., -10°F and the tissue will not stay frozen and firm and will not cut crisp. If itstoo cold, i.e., -50°F and the tissue will crumble and become powder. The Ideal tissue should cut like butter, smooth and in one piece.

b) Blade sharpness and angle:

The blade should be sharp and should be changed approximately once every 2 weeks. A dull blade cuts dull. Blade angle is also important.

. There is an optimal angle between blade and tissue:

•Too steep an angle and the tissue will crumble like it was too cold.

•Too shallow, then two things will happen. The section will alternately skip and not cut and then it will cut, but too thick.

5. Staining:

Once the tissue is on the slide it can be either air-dried or fixed in methanol. This depends on which staining procedure will be used. There are several stains available in the frozen section room. The choice of stain depends on what the pathologist is trying to demonstrate. The resident should practice all the stains and gain experience with their use.



b)Embedding and cutting of the tissue sections

Advantages of frozen section biopsy:

1- The surgeon is able to obtain an additional sample if more tissue is needed to make an accurate diagnosis, avoiding a second operation.

2-If the tissue is determined to be cancerous and is amenable to surgery, the mass can be removed at that time.

3-If the tissue is determined to be benign (not cancerous), then the mass may not always need to be removed and the surgery can end.

4-The frozen section biopsy can help ensure that the mass being removed is the intended tissue for removal.

5-It can help ensure that the entire mass and its surrounding borders are removed.

6-It allows for the collection of proper tissue samples for further scientific research.

7-The surgeon and pathologist are able to collaborate to care for the patient.[4]

Conclusion

The frozen section is an accurate and reliable

method. It is used for tumor resection margins, metastasis of lymph nodes, and tissue recognition. Macroscopic examination, accurate sampling by pathologist, avoiding technical errors in sectioning and staining, a interpersonal coordination with the operating surgeon can reduce the limitation and provide rapid, reliable and cost effective details necessary for rapid diagnosis and on table patient management. Continuous monitoring should be performed in every pathology department, to recognize the reasons of errors and to reduce them and improve frozen section turn-around time.[5]

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5. International Journal of Research in Medical Sciences Patil P et al. Int J Res Med Sci. 2015 Feb;3(2):399-404 Accuracy of frozen section analysis in correlation with surgical pathology diagnosis

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Potential Triggers of Lichen Planus: A Review

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Abstract

Oral lichen planus (OLP) is mucocutaneous and chronic inflammatory disease of unclear pathogenesis. OLP usually affects the mucous membrane of oral cavity. It is an autoimmune disorder araising from several etiological factors like genetics, stress, virus, dental restorative materials, drugs and systemic diseases. It is a T-cell mediated autoimmune disease usually encountered by dentists and dermatologists. Although much work is being done on this disorder, there is still uncertainty over the specific etiopathogenesis and treatment. Because there is a risk of reported malignant potential with this disease, the patient needs early diagnosis and proper management. This article reviews mainly the etiology, pathogenetic mechanisms through which this unique disorder occurs along with the aspects of clinical, histopathological and treatment.

Key Words: *Lichen planus. Pathogenesis, T-cell mediated, Autoimmune disorder,*

Introduction

Lichen planus is a mucocutaneous condition involving different mucosal surfaces, either alone or in combination with skin involvement[1]. Similar to other mucosal areas, it most often includes the oral mucosa[1]. Oral lichen planus (OLP) is an unidentified etiology disease that affects stratified epithelial squamous[1]. More generally, this disorder affects 30-60-year-old middle-aged patients and females are more vulnerable than males with a 1.4:1 ratio[1]. Children and young adults rarely see OLP[1]. OLP should be considered a potentially malignant condition because there is a link between oral cancer and OLP although there is a variable degree of risk involved[1].The incidence of OLP varies from 1-2 percent in the general population.

Clinically, OLP has six forms: reticular(Figure 1), papular, plaque-like, atrophic(Figure 2), erosive and bullous(Figure 3)[3]. Oral lichen planus has been noted to occur independently of the skin form and tends to be constant and treatment-resistant.[4] Lateral border, tongue dorsum, gingiva, hard palate and vermilion border are common sites of involvement and occur as reticular, plaque-like or papular intraoral lesions[4]. However, Buccal mucosa remains the most frequent site of involvement[4].

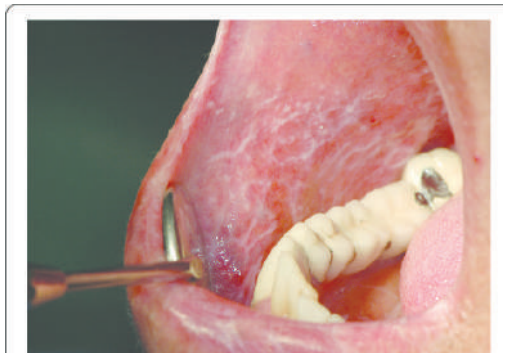


Fig 1: Reticular lichen planus in right buccal mucosa

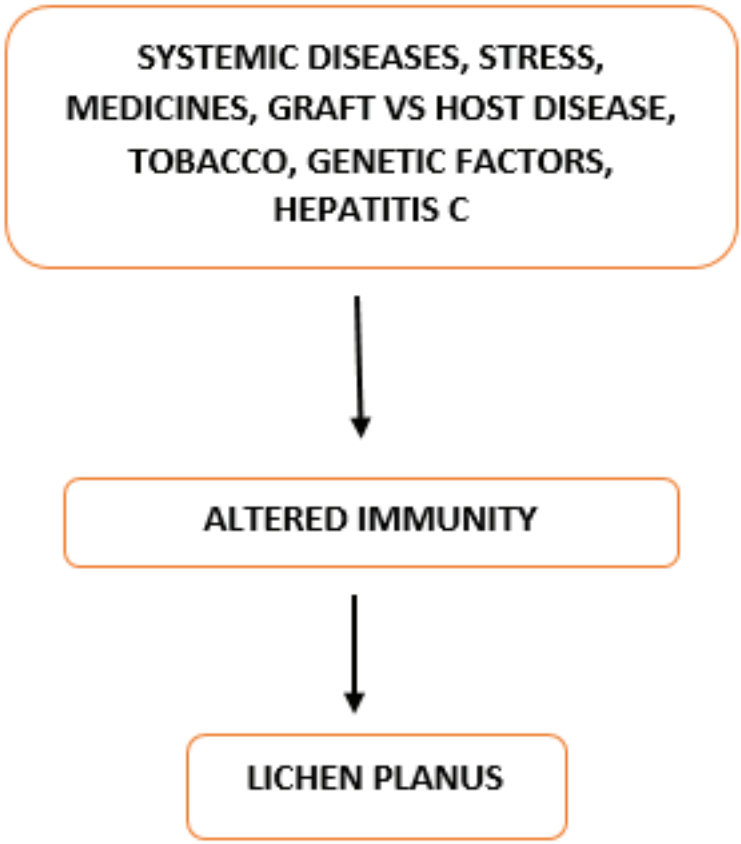




Figure 2: Atrophic lichen planus

Figure 3: Bullous lichen planus

Etiological Factors:



Women:

It is also generally accepted that OLP mainly affects females, indicating probably a genetic predisposition to OLP development[2]. This has given rise to the possibility that the consumption of drugs in women is associated with an increased susceptibility to OLP lesions[2].It is also generally accepted that OLP mainly affects females, indicating probably a genetic predisposition to OLP development[2].Drug history was found to be substaintially more prevalent in females patients with oral lichen planus[2] .

Systemic Diseases:

This correlation may be attributed to endocrine dysfunction in DM that may be associated with an immunological deficiency and may lead to OLP production[3]. Antidiabetic drugs and certain antidiabetic drugs in DM patients can cause an allergic reaction to lichenoid reaction[3]. Higher prevalence of oral mucosal lesions in DM patients may be due to lower healing levels in these patients, leading to longer duration of the lesion and not to increased incidence3. Therefore, if a lesion in a DM patient takes two months to heal and one month in the subject of control the incidence would increase in DM patients at a given time point[3].

A number of studies suggested that OLP in DM patients may be associated with a compromised immune system in these patients or may be associated with a number of oral hypoglycemic medicines taken by older people in particular[3].

In insulin-dependent diabetics as well as patients with NIDDM but with a higher frequency compared to normal subjects, oral LP has been reported A great deal of work is being conducted on the relationship between diabetes mellitus and oral LP[4].Autoimmune history of LP and diabetes mellitus further support the association relationship between oral LP and diabetes mellitus[4].

In relation with healthy subjects, oral lichen planus has a large association with noninsulin-dependent diabetes mellitus. Other reports also suggest a similar association with insulin-dependent diabetes mellitus

HCV:

HCV induced OLP pathogenesis is uncertain, but two hypotheses were raised to explain the mechanism of HCV-induced OLP triggering[5].

The first hypothesis indicates that replication of the virus is associated with the oral epithelium, thus directly contributing to lesion growth[5]. The second hypothesis suggests that the virus‘ high rate of mutation results in repeated immune cell activation, increasing the possibility of cross-reaction with its own tissue and consequently, the risk of autoimmune disease[5] .

HCV infection is pervasive, with an approximately 3% of the world’s population infected and involved as an etiological factor in lichen planus occurrence[6]. It is a single stranded RNA virus that is mainly spread through blood or blood products transfusion[6]. The mechanisms suggested for the cause of lichen planus are:

1) HCV is susceptible of cytopathic replication in cell types outside the liver[6].

2) It may activate an autoimmune process directed at antigens expressed in extra-hepatic cells[6].

3) 3) Persistent infection, followed by deposition on small blood vessels, can lead to immune complex formation with antibodies[6].

4) The activated CD8 T cells, cytokines and the expansion of certain B cell clones are the triggers of immunological processes leading to dermatological manifestations[6].

In OLP, HCV replication was reported by reverse transcription / polymerase chain reaction or in-situ hybridization in the epithelial cells of LP lesions in the mucosa; in the subepithelial group, HCV-specific CD4 and CD8 lymphocytes were also identified[7]. This probably suggest that lymphocytes with HCV-specific T may play a role in OLP’s pathogenesis[7].

Medicines:

Even though many drugs have been reported as possibility LDR inducers, non-steroidal anti-inflammatory drugs (NSAIDs) and antihypertensives, principally angiotensin-converting enzyme inhibitors (ACE inhibitors) are the most frequently involved drugs in LDRs[2] .

Two studies have shown that NSAIDs are clearly established as LDR inducers, with case history strongly indicating a correlation between NSAID consumption and the initiation of OLP, complete resolution or marked improvement of lesions when these medications are removed, and recurrence if patients are given the offending drug again[2].

Systemic drugs like anti-malarial drugs, non-steroidal anti-inflammatory drugs (NSAIDs), anti-hypertensive drugs and enzyme-converting angiotensin have been strongly linked with oral lichenoid reactions[8]. Diuretics, oral hypoglycaemic agents, gold salts, penicillin are other drugs reported to cause oral lichenoid

reactions[8].

Tress:

OLP's acerbations are associated with periods of emotional stress and anxiety. Ivonavaski et al., proposed that excessive emotional stress in OLP patients lead to psychosomatization, which may in turn contribute to OLP's initiation and clinical expression, and also indicated that psychosocial and emotional stress may be a possible factor that may precipitate reticular stress[10].

Genetics:

Genetic background seems to play a role in pathogenesis of OLP as there have been records of several family cases [9]. In a study of British patients with cutaneous lichen planus, Lowe et al., first reported a massively increased level of HLA-A3[9]. {8}

Tobacco:

This OLP-like lesion consisted of white, linear, wavy, parallel, non-elevated streaks which could not be scraped off[9].The lesions radiated in some instances from a central erythematous region. However as in classical OLP, the fine white lines did not intersect or criss-cross[9]. The lesion usually viewed at the site of placement of the betel quid[9]. In order to describe this OLP-like lesion, Zain et al. (63) coined the word ' betel-quid lichenoid' lesion[9]. There was no indication of a causal effect for betel quid in OLP[9].

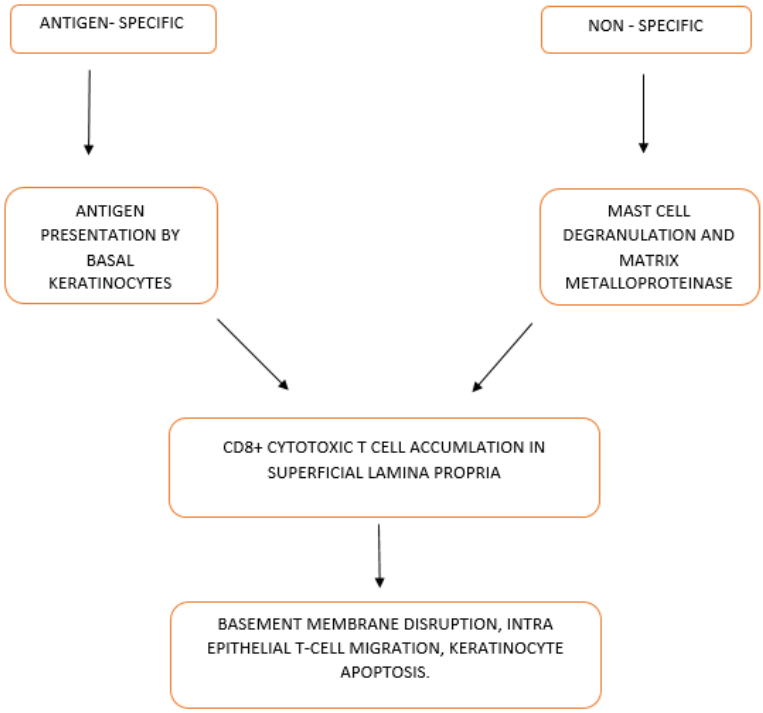
Graft Vs Host Disease:

Oral lichenoid lesions are part of the spectrum of chronic graft-versus-host disease following allogeneic transplantation of the bone marrow [9]. While the etiology of oral lichenoid lesions and chronic graft-versus-host disease 92 is different, there are quite similar clinical and histological appearances[9].

LP is identified as a disease of adults. The average presentation age ranges from 30 to 60 years.

Exact etiology of the LP is unclear, but it has been shown that degeneration of the basal epithelial layer, resulting from a switch in the cell-mediated immune response, plays an important role in the pathogenicity of the lesion.

Pathogenesis:



Diagnosis:

Based on the history, medical and histopathological examination, the diagnosis can be made[1]. Nevertheless, the diagnosis can only be made in classical lesions based on clinical appearances[1]. If skin lesions also occur, diagnostic reliability is reinforced[1].Biopsy is mandatory for to diagnose OLP[1]. Biopsy should include marginal tissue comprising areas that are both lesion and normal areas[1]. Direct and indirect immunofluorescent tests, ELISA can be helpful in diagnosing troublesome cases and preventing malignancy[1].

POTENTIAL BIOMARKER:	LEVEL IN OLP PATIENTS:
Peroxidation products	Increase
Antioxidants (Vit C and E)	Decrease
GPCA	Increase
Cortisol	Decrease
Immunoglobulin	Increase

Potential biomarkers for to diagnose OLP[10]:

Differential Diagnosis:

For reticular OLP[1]:

- Leukoplakia,
- Lichenoid reaction,
- Graft vs host disease,
- Lupus erythematosus.

For erosive OLP[1]:

- Chronic cheek chewing,
- Hypersensitivity mucositis,
- Chronic candidiasis,
- Discoid lupus erythematosus,
- Squamous cell carcinoma,
- Benign mucous membrane pemphigoid,
- Pemphigus vulgaris,
- Erythema multiforme.

Conclusion

Lichen planus is a chronic mucocutaneous disease with a malignant transformation of 0.4-5% affecting the oral mucosa and skin. The presence of different factors is likely to be responsible for initiating, aggravating and persisting OLP. Not only are the current treatment strategies not only ineffective for treating all patients and avoiding recurrences, but they also have serious adverse effects. More clarification on pathogenesis can help to adjust therapeutic interventions, increasing the morbidity of OLP patients dramatically.

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Guidelines for Diagnosis and Treatment of Recurrent Aphthous Stomatitis for Dental Practitioners

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Abstract

Recurrent aphthous stomatitis (RAS) is one of the frequent clinical oral diseases causing painful ulceration in the oral cavity. It is easy to define the clinical features and characteristics of this disease, etiology and pathogenesis remains unclear. As a result, the treatment options are still inadequate with the ability to reduce the duration, healing time, rate of recurrence and conclusive intervention. The review article aims to discuss the pathogenesis, clinical features, different treatment modalities and updates that are currently available and offer the clinician a clear and detailed picture of how to deal with RAS in an appropriate manner.

Key Words: *Recurrent aphthous stomatitis, Ulcer, Topical, Oral disease.*

Introduction

Recurrent aphthous stomatitis (RAS) is considered as the most frequent mucosal lesion occurs in oral cavity[1]. These are described as most often single or multiple, round or ovoid ulcers with yellow floors and surrounded by erythematous haloes[1].It is one of the most common oral ulcerative and vesiculobullous lesion seen in children and adults[2].The classic recurrent aphthous stomatitis presentation is most often, self-limiting ulcers that primarily affect non-keratinized oral mucosa like buccal mucosa, labial mucosa and floor of mouth[2]. Spontaneously the ulcer recover within 7-14 days[2]. These are quite painful which makes it difficult to chew, swallow and speak, hence it has a negative impact on the quality of life of the patient[3]. Aphthous stomatitis is classified into three forms:

1. Minor recurrent aphthous ulcer (Figure 1)
2. Major recurrent aphthous ulcer (Figure 2)
3. Herpetiform ulcer (Figure 3)

It is more prevalent in females[1]. Aphthous ulcers have been recorded from 5% to 66% between different nations[1]. The cause of aphthous ulcer is unclear and many triggers like hormonal changes, injury, medications, food hypersensitivity, dietary deficiency, stress and smoking are still involved in this disease[1].

PREDISPOSING FACTORS[1]:

HORMONAL FACTORS[1]:

During mensturation, premenstrual period, pregnancy, dysmenorrhea

TRAUMA:

Sharp tooth, injury due to tooth brush, site of local anesthetic injection, dental treatment, dental trauma, self-inflicted bites, oral surgical procedures.

DRUGS:

Phenindione, phenobarbital, piroxicam, niflumic acid, sodium hypochlorite, nicorandil, captopril

NSAIDS example: propionic acid, diclofenac, phenylacetic acid

FOOD HYPERSENSITIVITY:

Chocolate, coffee, almonds, cereals, strawberries, tomatoes, cheese, wheat flour

NUTRITIONAL DEFICIENCY:

Vitamin B1, B2, B6 deficiency, anaemia

STRESS:

High levels of psychological stress, acute psychologic problems, anxiety leads to parafunctional habits like lip biting, check biting.



Figure 1: Recurrent aphthous minor

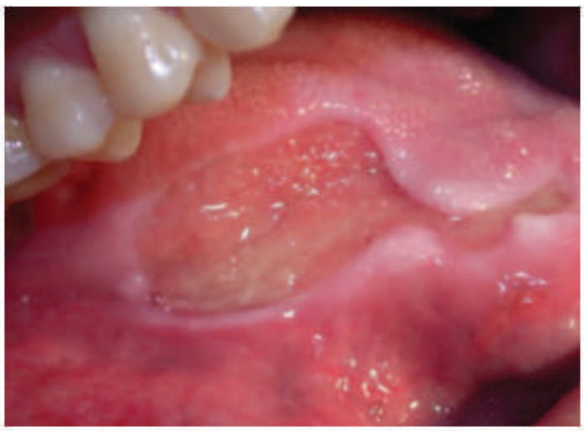


Figure 2: Recurrent apthous major



Figure 3: Herptiform ulcer

TOBACCO:

Patients who stop smoking frequently complain of RAS.

INFECTION:

Helicobacter pylori, streptococci, herpes simplex virus, cytomegalovirus, Epstein barr virus, HIV.

HEREDITARY:

For patients with RAS family history, ulcers tend to occur earlier and more severe than those without family history.

SYSTEMIC DISORDERS[6]:

- Behcet's syndrome
- Crohn's disease
- Ulcerative colitis
- Celiac disease
- Cyclic neutropenia
- Gluten sensitive enteropathy
- IgA deficiency
- Immunocompramised conditions
- Inflammatory bowel disease
- Mouth and genital ulcers with inflamed cartilage
- periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis
- Reactive arthritis
- Sweet's syndrome
- Ulcus vulvae acutum

PATHOGENESIS:

Pathogenesis of aphthous ulcer is still unclear.

Patient with recurrent aphthous ulcer have alteration of local cell mediated immunity[3].

ABNORMAL T CELL MEDIATED IMMUNE REACTION

Results in:

Decreased ratio of CD4+ to CD8+[3].

Decreased T-lymphocyte level[3].

Increased levels of T- cell receptor $\Upsilon\delta^+$.

Increased Tumor necrosis factor α level[3].

CLINICAL FEATURES[1,2,3,5,6,7]:

MINOR[1,2,3,5,6,7]:

· OTHER NAME : Mikulicz's aphthae

· AGE : 10 and 30 years

· SEX : Female than men

· NUMBER OF ULCER : 1 – 5

· SIZE OF ULCER : <10 mm

· DURATION : 7 – 14 days

· HEAL WITH SCARRING : No

· PERCENTAGE : 85%

· OTHER SYMPTOMS : Burning sensation, pain, erythema.

· SITE : Non- keratinized mucosa particularly labial and buccal mucosa, dorsum and lateral border of tongue

MAJOR[1,2,3,5,6,7]:

· NAME : Sutton's disease/ periadenitis mucosa necrotica recurrens (PNMR)

· AGE : No age predilection

· SEX : Male

· NUMBER OF ULCER : 1 - 3

· SIZE OF ULCER : >10 mm

· DURATION :2 weeks – 2 months

· HEAL WITH SCARRING: yes

· PERCENTAGE : 5-10%

· OTHER SYMPTOMS : Pain, burning sensation, dyaphagia

· SITE : Keratinized and non keratinized mucosa, particularly soft palate

HERPTIFORM[1,2,3,5,6,7]:

· AGE : Third

· SEX : Female

· NUMBER :5 – 20 (upto 100)

· SIZE :1 – 2 mm

· DURATION :7 – 14 days

· HEALING WITH SCARRING: No

· PERCENTAGE :5-10%

· OTHER SYMPTOMS : Reminiscent of herpes simplex infection.

SITE : Non keratinized mucosa but particularly floor of mouth and ventral surface of tongue

DIAGNOSIS:

The correct diagnosis of RAS relies on a detailed and accurate history of the clinic and ulcer evaluation[1].

Following features should be noted from the physicians[1]:

· Important points in history

· Family history

· Number of ulcer

· Size and shape of ulcer

· Site of ulcer

· Frequency of ulcer

· Duration of ulcer

· Edge of ulcer

· Base of ulcer

- Genital ulcer
- Drug allergy
- Dermatological problems
- GIT disturbances

HEMATOLOGICAL FINDINGS:

INVESTIGATIONS TO DO[1]:

- Hemoglobin and complete blood count
- Erythrocyte sedimentation rate/ c- reactive protein
- Serum B12
- Red cell folate
- Anti gliadin and anti endomysial autoantibodies
- Total hematological screening of RAS patients was suggested to identify serum ferritin, iron, folate and vitamin B deficiencies[5]. 11% to 36% of patients with RAS were suffered from deficient levels of serum ferritin or iron[5].

HISTOPATHOLOGIC FINDINGS:

Due to the unspecific nature of the ulcer, a histopathologic specimen is rarely required for the diagnosis of RAS.

FOR MINOR APHTHOUS ULCER[7]:

- Oral mucous membrane shows a fibrinopurulent membrane covering the ulcerated area[7].
- Superficial colonies of micro organisms may also be seen occasionally, in this membrane[7].
- Chronic inflammatory infiltrate seen in connective tissue below the ulcer with considerable necrosis of tissue near the surface of the lesion[7].
- Neutrophils are noticed predominantly beneath the ulcer and lymphocytes prevailing adjacent to this[7].
- Granulation tissue may be seen near the base of the lesion and epithelial proliferation is present at the margins[7].
- The histologic features of oral ulcers in RAS major is identical with RAS minor[7].

CYTOLOGICAL SMEAR FINDINGS:

Changes in the nuclei of epithelial cells taken be cytologic smears from RAS were described.

Anitschkow cells and consists of cells with elongated nuclei containing a linear bar of chromatin extending towards the nuclear membrane with radiating process of chromatin[7].

These are little abundant in patient with RAS but are not pathognomic of the disease[7].

Ultrastructurally, the nuclear chromatin was made up of pleomorphic masses creating an irregular band along the long axis of nucleus rather than being dispersed randomly.[7]

IMMUNOLOGIC FINDINGS: Direct and indirect immunofluorescence studies are not sensitive or precise RAS diagnostic tests and should therefore not be used unless other oral mucosal diseases (e.g., pemphigus and pemphigoid) are excluded[5].

SALIVARY BIOMARKERS:

ETIOLOGY : Stress

SALIVARY BIOMARKERS: Cortisol, Immunoglobulin(Secretory IgA), Lysozyme, Chromogranin A, α amylase.

DIFFERENTIAL DIAGNOSIS:

When treating patients with recurrent aphthae, multiple factors should be included in the differential diagnosis[4]. A primary consideration is that, relative to more severe conditions, benign aphthae tend to be smaller and are more often self-limited[4]. Major aphthae may be correlated with infection with human immunodeficiency virus (HIV)[4].

INFECTION : DIFFERENTIAL DIAGNOSIS[4]:

HERPES SIMPLEX VIRUS : TZANCK stain positive – inclusion bearing giant cells

VARICELLA ZOSTER VIRUS : Clinically have unilateral extra-oral and intra-oral distributing pattern following trigeminal pattern.

COXSACKIE VIRUS : Low grade fever, malaise and will resolve within 7 to 15 days.

Hand, foot, buttock lesions seen in children.

ERYTHEMA MULTIFORME: Seen in both attached and movable mucosa

LICHEN PLANUS : Not always painful

SYPHILIS: Skin lesions other than ulcer, risk factors ,Rapid plasma reagin - positive

fluorescent treponemal antibody absorption test – positive

HISTOPLASMOSIS: Prolonged lesions, immunocompromised patient, culture – positive.

AUTOIMMUNE : DIFFERENTIAL DIAGNOSIS[4]:

BEHCET'S SYNDROME: Ulcer in genital areas, Retinitis, Uveitis

REITER'S SYNDROME: Uveitis is seen, Conjunctivitis, Reactive arthritis

LUPUS ERYTHEMATOSUS : Malar rash also called butterfly rash, Antinuclear antibody test – positive

PEMPHIGUS AND BULLOUS PEMPHIGOID: Involvement of skin

CYCLIC NEUTROPENIA: Fever (periodically), Neutropenia

NEOPLASM: DIFFERENTIATING FINDINGS[4]:

SQUAMOUS CELL CARCINOMA: L o n g duration, Head and neck adenopathy, Biopsy – positive.

TREATMENT:

The RAS etiology is not yet understood. Therefore, there is no consensus in the treatment of RAS, several treatments have been tried, few have been randomized double-blind[2].

GOALS:

The primary goals of RAS therapy are pain management, shorten the duration of ulcer and restore the normal oral function[2].

Secondary objectives are to reduce the frequency and extent of recurrence and to sustain remission[2].

TOPICAL2:

- Pastes / gels
- mouthwashes
- Local anesthetics and analgesics
- Topical corticosteroids and anti-inflammatory agents
- Herbal treatments
- Immunomodulatory agents

PHYSICAL2:

- Silver nitrate
- Laser
- Ultrasound therapy
- excision

SYSTEMIC2:

- Immune and inflammatory suppression
- Immune enhancement
- Biologics
- Other treatment: Diet supplements

The treatment types vary from topical application to clinical drug administration, and even the new ultrasound techniques have been tested2. But the treatment totally depends on the severityof the ulcers and whether the ulcer is associated with any systemic disease2.

FIRST LINE TREATMENT3:

Topical agents like gels, creams or oinment are the first choice of management of recurrent aphthous stomatitis. Topical anti-inflammatory agents are used in primary treatment of RAS lesions

Eg: orabase, mucopain.

TOPICAL:

Antiseptic :Chlorhexidine Gluconate, Triclosan.

Anti-inflammatory/ Analgesic : Benzydamine Hydrochloride, Diclofenac

Anesthetic : Lidocaine, Benzocaine

antibiotic : Chlortetracycline, Doxycycline

Corticosteroids: Hydrocortisone hemisuccinate, Triamcinolone acetonide.

SECOND LINE TREATMENT3:

For patients whose symptoms are not improved by the primary therapy will go for second line therapy.

SYSTEMIC:

Immunomodulator: Levamisole, Colchicine, Thalidomide, Dapsone

Antibiotic : Penicillin G Potassium

Corticosteroids : Prednisone

Conclusion

Recurrent aphthous stomatitis remains a wide spread oral mucosa disorder; its exact etiology remains unclear. RAS treatment tends to be non- specific and observational upto now. Different methods of therapy may be used. In most cases, these modalities can reduce pain and promote recovery, but they are not to improve recurrence rate. Future research should concentrate on defining RAS etiology, establishing a proper RAS diagnostic criteria and definitive treatment and enhancing clinical trial development and monitoring

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Holistic or Biologic Dentistry : Future Alternative or Not

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Abstract

Holistic dentistry is also called Progressive dentistry, biologic dentistry, or biocompatible dentistry. Holistic dentistry is a new alternative branch of traditional dentistry where it deals with the unrecognized impact of oral health on the overall health of the person. This new idea of dentistry strongly opposes some of the treatment modalities of traditional dentistry. Although the concept and practise has brought about a revolution but some of the complementary methods followed by holistic dentists under criticism.

Keywords *: Alternative dentistry, Green Dentistry, Propolis*

Introduction

The birth of Holistic Dentistry dates back to 1800 when National Dental Association recognized the harmful effects of amalgam restoration and its use was banned among the members of Association. Holistic Dentistry gained more of its importance between 1870-1948 when Weston A Price conducted research & came to conclusion that degenerative diseases like heart problem, kidney and bladder disorders, arthritis, rheumatism, mental illness, lung problem and many other bacterial infection arising from Root Canal Therapy and other endodontics treatment. This concept gained popularity as " focal infection theory". This theory was thereafter supported by another scientist name Melvin Page. According to him tooth decay occurs as a result of systematic chemical imbalances. So, gradual rise and increasing popularity in holistic dentistry made it possible to arise as an alternative dentistry or a new field of probiotic medicine.

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What is Holistic Dentistry ?

This particular field defines itself as an approach to dentistry that promotes health and wellness instead of treatment of disease. This approach encompasses both modern science and knowledge drawn from world's great ethnicities natural healing. It deals not only teeth but with mind, body, spirit of the patient and lays out with basic principles I.e prper nourishment for preclusion and reversal of deteriorating dental disease, avoidance and elimination of toxins from dental materials prevention and treatment of malocclusion and prevention and treatment of gum disease at its biologic basis.

PROCEDURE :-

Holistic is a form of soothing that considers the unexpurgated person- form, mind, spirit and emotions in the mission for optimal health and wellness. Often holistic dentist incorporate physiologic and electronic methods to locate areas of chronic disease that are difficult to locate by current standard methods. Although their method of treatment would vary for different patients their main key to prepare patients is thorough education and communication. Their treatment plan may not always involve drugs to relieve symptoms but also the introduction to lifestyle modification.

Benefits of holistic Dentistry are said to be

due to their incorporation of acupuncture, physical therapy into the use of clinical dentistry. Moreover the addition of modern sciences of neural therapy, hematology, immunology and electroacupuncture may be incorporated into holistic dental practice.

Initial visit involve a thorough interview of the patient that is how they take the detailed case history not only medical but also the patients' daily habits and way of life style. Next radiographs of patients mouth are collected if required with the latest film which provide 50% less radiation than standard systems.

GREEN DENTISTRY [2,3]

The color green symbolized the healing power and is understood to be the most restful and relaxing color. Green can help to enhance vision, stability, and endurance. Renewal, growth, and hope are related to this color, and it indicates safety in the advertising of drugs and medical products. Green dentistry is an approach to dentistry where dental practices meets environmental conservation. Holistic Dentistry believe to follow the path of this eco-friendly dentistry which is a newly evolving practice of dentistry. It encompasses a simultaneous devotion to sustainability, prevention, precaution, and a minimally invasive patient-centric as well as global-centric treatment philosophy. So it is matching all the criterias put forward by Holistic dentistry.

INCORPORATION OF AYURVEDA, AROMATHERAPY, HOMEOPATHY IN HOLISTIC DENTISTRY-

1. AYURVEDA IN HOLISTIC DENTISTRY [(4,5)]:-

One of the oldest system of medicine from India. It is believed that right herb in the right combination keeps the body system in harmony. Formulated herbal tooth paste often having main ingredient as Neem make this type of combinations unique and powerful oral care formula. The formulated ingredients are combined in such a way that it becomes helpful in cleaning of teeth, prevention from various dental problems such as dental pain, bleeding gums, mouth fibrosis due to poor eating habits or betel nut chewing, Other conditions like sensitive tooth, poor dental root. Formation of tartar, poor dental strength. Use of Neem Bark, Grape seed, Clove, Fennel and other plants for maximum support of healthy gums and teeth are seen. Propolis is a natural substance based on resin pine and collected by bees has been reported to cure ulcerations [8] . So further studies should be done in order to incorporate this in the daily routine treatment of oral ulcerations so the use of corticosteroids could be reduced.

2.AROMATHERAPY IN HOLISTIC DENTISTRY [7]

This field makes use of pure oil essences from plants and flowers that act as hormone like stimulants to improve a patient's balance. So incorporation of these in the field of Holistic Dentistry brings both Aromatherapy and medicine science in a same platform.

3.HOMEOPATHY IN HOLISTIC DENTISTRY [9]

There remains always a contraindication between homeopathy and allopathy. Since traditional dentistry, even now the dentistry which is followed worldwide uses allopathy in their treatment modalitis incorporation of Homeopathy is a new idea to this field. But Holistic dentistry made this possible. Homeopathy therapy is considered to be safe and natural alternative that is non-addictive and effective for both adults and children. They are considered to improve both psychological and emotional conditions of patients without drugging effect of conventional tranquilizers. So some of the remedies which has been included by holistic dentistry is : Aconite (foxglove); gelsemium (yellow jasmine); argentum nitricum (silver nitrate). Homeopathic remedies have been found effective by some clinical demonstrations in the treatment of dental caries, dental abscess, oral lesions, post extraction bleeding and even medications to treat the anxiety and nervousness.

4.HYPNOSIS IN HOLISTIC DENTISTRY [6]

Hypnotic therapy used mainly for the patients under serious anxiety issues. It's main aim to relax the body and minds. No drugs are used in this and patients are full aware of their surroundings. Many holistic dentists consult specialist hypnotherapists to provide treatment that is highly effective and cannot cause any harm or side effects.

CRITICISM OF HOLISTIC DENTISTRY :-

Controversy and debate has to arise over the years

regarding the use of alternative health practices in holistic dentistry, as critics claim that many of these practices which are involving prevention, diagnosis, and treatment is outside the scope of dentists. Dentistry, like medicine, is grounded in thorough scientific research. A significant part of holistic dentistry is related to the unsubstantiated use of certain services and treatments, many of which have either been investigated and found ineffective or have not been researched enough to be declared safe and effective for practice. For example, herbal remedies are often recommended in the form of mouthwash and toothpaste to prevent or treat certain dental conditions. They are supposedly safer products because they are "natural." However, there is a lack of scientific research which supports such treatments, and in fact, herbal remedies have been found to impact the safety of more invasive or prolonged dental procedures and can lead to additional complications if they interact with a patient's current medications.

Conclusion

Holistic dentistry can be an alternative treatment option in the world of traditional dentistry depending upon its acceptancy in the modern dental society. It's idea of amalgamation of allopathic stream of treatment an alternative health care might work together in benefit of patients. But the knowledge and understanding of Holistic Dentistry is still ongoing and further researches needed to establish their ideas completely and incorporate this stream of treatment modalities in near future.

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Viral Lesions of Oral Cavity

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Abstract

Oral examination is an integral part of general dentistry. So it is not uncommon to come across various viral diseases in the oral cavity. The clinical diagnosis of these viral lesions sometimes become confusing due to similar clinical presentations, confusion leads to misdiagnosis and eventually the treatment.This article discusses about the viral lesions, their clinical presentation, diagnosis and appropriate management which are usually manifested in oral cavity that will eventually provide the general dental practitioner with an up to date guide for better diagnosis and management.

Keywords : *Herpes simplex, herpes zoster, HIV.*

Introduction

Viral infections are one of the frequently encountered infectious diseases by dental practitioner. But often the clinical diagnosis becomes confusing because of various clinical similarity with other disease of oral cavity. So, thorough examination with vivid idea about those particular lesions and ability to distinguish clearly is important for appropiate diagnosis. management and treatment. Thus the Lesions are described individually in the following article.

COMMONLY ENCOUNTERED LESIONS OF VIRAL ORIGIN :-

1) HERPES SIMPLEX

2) VARICELLA ZOSTER

3) COXSACKIE VIRUS

4) HUMAN PAPILLOMA VIRUS

5) MEASLES OR RUBELLA

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6) RUBEOLA

7) HUMAN IMMUNODEFICIENCY VIRUS (**HIV**)

l HERPES SIMPLEX :-

An acute infectious disease, probably the most common viral disease affecting man.

Two immunologically different types of HSV : Type 1 & Type 2.

HSV Type 1 can be isolated from the following two lesions of Oral cavity -

1. ACUTE HERPETIC GINGIVOSTOMATITIS
2. HERPES LABIALIS.

ACUTE HERPETIC GINGIVOSTMATITIS :-

Usually seen between the ages of six months and 6 years.

SYMPTOMS : Along with the oral lesions patients suffers from High fever, headache, malaise, anorexia, irritability, regional lymphadenopathy and sore mouth lesions.

MANIFESTATION OF MUCOSA [(8)]

Affected mucosa is red and edematous with

numerous coalescing vesicles which rapidly rupture leaving painful small round ulcers covered by yellow fibrin. New lesions continue to develop during the first 3-5 days. Healing of ulcers take place in 10-14 days without scar formation. It is important to recognize that the gingival inflammation preceeds the formation of the ulcers by several days. Sometimes in case of cervical lymphadenopathy the vesicles and ulcers on the tonsils (fig.) and posterior pharynx can resemble- a) Infectious mononucleosis or b) streptococcal sore throat infections.

Culture sensitivity test can be done where isolation of HSV Type 1 virus can be done otherwise histopathological sectioning of tissue of interest can be used as confirmatory test.



Fig. 1. Multiple ulcerative lesions on lips, gum and tongue.

HISTOPATHOLOGICAL FEATURES suggests characteristic **ballooning degeneration** which are actually the swollen infected cells with pale eosinophilic cytoplasm and large vesicular nuclei. Sometimes in other cases **Lipschutz bodies** I.e eosinophilic, ovoid, homogenous structures within the nucleus tending to displace the nucleus and nuclear chromatin peripherally can be seen.

DIAGNOSIS :

Most of the time Clinical identification is the primay mode of diagnosis. Confirmatory Laboratory tests are also done to rule out the virus.

1. Wright's and Giemsa stain

2. Pap stain - demonstrated the particular ballooning degeneration, multinucleated giant cells, intranuclear inclusions.

3. Cytology - Thorough Cytological smear can give immediate result but the only drawback is its lack of ability to differentiate between Herpes Simplex and Varicella Zoster Virus.

4. Most sensitive and accurate method is PCR technique

DIFFERENTIAL DIAGNOSIS :

Recurrent aphthous ulceration, which forms ulcers on non-keratinised oral mucosa without a vesicle phase.

RECURRENT OR SECONDARY HERPES LABIALIS [1,7,8] :-

Usually seen in adult patients. Spreads through contact so the medical, dental and nursing personnel are at higher risk of developing this disease because of their nature of their occupation.

SIGNS AND SYMPTOMS :-

The lesions are most often seen at the mucocutaneous junction of the lip (fig.) or peri-oral skin. A burning sensation usually precedes the development of a small cluster of vesicles. These vesicles enlarge, coalesce, ulcerate and become crusted before healing within 10 days.

As been emphasized by Weathers and Griffin the recurrent intraoral herpetic lesions almost invariably develop on the oral mucosa that is tightly bound to periosteum. Seldom do they occur on mobile mucosa.

MOST COMMON SITE :- hard palate, Attached gingiva, alveolar Ridge.

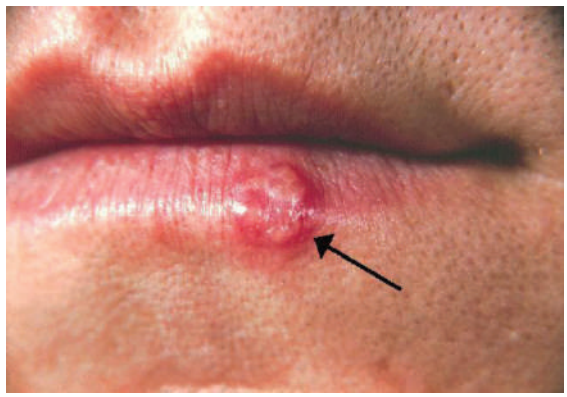


Fig. HERPES LABIALIS, CONSISTING OF A CLUSTER OF VESICLES ON THE VERMILION (ARROW)

HISTOPATHOLOGICAL FEATURE :- Characterized by Ballooning degeneration, chromatin margination and typical Lipschutz bodies, and multinucleated giant cells.

VARICELLA-ZOSTER-VIRUS:- [8]

Chickenpox (varicella) results from primary infection, while reactivation of the virus is known as herpes zoster (shingles).

SITE :- Intra-oral vesicles of varicella, when present, are seen on the tongue, buccal mucosa, gingival, palate and oropharynx. They generally are not very painful.

PREDISPOSING FACTORS : Conditions leading to herpes zoster are usually those that cause immunosuppression, such as cytotoxic drugs, radiation, internal malignancies, malnutrition, old age, and alcohol and substance abuse. Occasionally, dental manipulation in a localised area can lead to reoccurrence.

SIGNS AND SYMPTOMS :- The first signs of herpes zoster are pain and tenderness in the dermatome corresponding to the affected sensory ganglion. In the head and neck area, vesicles from on one side of the face or in the oral mucosa in one of the divisions of N. trigeminus. These unilateral vesicles form clusters with areas of surrounding erythema, ending abruptly in the midline (fig.) The vesicles ulcerate and form pustules within three to four days. A crust lesion then forms, and healing takes place within seven to ten days. These lesions often heal with scarring and areas of hypo/ hyperpigmentation may be seen.



Source: S. Kang, M. Amagai, A.L. Bruckner, A.H. Enk, D.J. Margolis, A.J. Mcmichael, J.S. Orringer: Fitzpatrick's Dermatology, Ninth Edition
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(FIG.) HERPES ZOSTER : Abrupt ending of the ulcers in the midline

Ramsay-Hunt syndrome [3] :- facial paralysis, vesicles on the external ear, tinnitus, deafness and vertigo which is usually the result of involvement of N. facialis & N. auditorius.

Post-herpetic neuralgia :- A complication of herpes zoster. It occurs in 10% of patients with herpes zoster and affects the trigeminal nerve, most commonly the ophthalmic division. There is persistent, unilateral pain in the affected area.

A history of previous skin lesions and possible scarring may aid in the diagnosis

DIAGNOSIS :- The clinical picture is often distinctive. Herpes zoster may be confused with recurrent Herpes simplex virus infection. Herpes zoster has a longer duration, a more severe prodromal phase, unilateral vesicles and ulceration, with abrupt ending at the midline and postherpetic neuralgia. Differentiation between Herpes Zoster and Herpes Simplex done only by Fluorescent Antibody staining technique, viral culture and serological diagnosis.

Cytological smear : helps in identification of virus from skin or oral lesions.

COXSACKIE VIRUS [7,8]

a)HERPANGINA :- Caused mainly by Coxsackie Virus group A. Herpangina affects children, mainly during summer, and is characterised by a sudden onset of malaise, low grade fever, sometimes vomiting, prostration abdominal pain and sore throat.

Manifestation in oral mucosa as vesicles, ulcerations, and diffuse erythema on the soft palate, fauces and tonsillar areas.The ulcers grow in crop like fashion and with an grey base with inflamed periphery on the anterior faucial pillars and sometimes on hard and soft palate. The ulcers are not extremely painful. Generally the systemic symptoms subside in 3-4 days and the vesicles and ulcers heal in 7-10 days.

DIAGNOSIS : Generally clinical signs & symptoms mostly help in concluding the diagnosis.

b)HAND,FOOT, AND MOUTH DISEASE :-

An epidemic infection generally. Caused by the enterovirus Coxsackie A16. Mainly affecting Young children generally between 6 months to 5 years.

CLINICAL FEATURES : Mainly affects skin, particularly involving the hands, feet, legs, arms and occasionally the buttocks as maculopapular rashes, exanthematous vesicular lesions. Anorexia, low grade fever, sometimes lymphadenopathy, diarrhea, nausea are common. It also Oral manifestations which usually most of the time gets avoided due to the name itself.

Oral Manifestation :- Dysphagia one of the most common findings, which can be a differential diagnosis with other oral ulcerations and vesicular lesions like Herpangina. The lesions are characterized by small, multiple, vesicular and ulcerative oral lesions but more numerous than seen in Herpangina. Most common sites are hard palate, tongue, and buccal mucosa. Tongue may also become red and edematous. Least commonly on lips, gingiva and pharynx including the tonsils.

DIFFERENTIAL DIAGNOSIS :- the cutaneous vesicles of this disease can resemble chickenpox but in chickenpox the vesicles usually start on the face and trunk and then spread to the extremities. Involvement of the palms and soles is rare in chickenpox infections. Oral lesions are also rare in chickenpox.

l DIFFERENTIAL DIAGNOSIS AND DISTINGUISHABLE FEATURES BETWEEN COXSACKIE VIRUS AND HERPES SIMPLEX

The clinical features of Coxsackie virus are distinctive. The distribution of the lesions of herpangina differentiates it from primary herpetic gingivostomatitis, which affects the gingivae, whereas herpangina is an oropharyngitis. The systemic symptoms differentiate it from recurrent aphthous ulceration. The vesicles also help to distinguish herpangina from streptococcal pharyngitis.

HUMAN PAPILLOMA VIRUS (HPV) [1,5]

a) Focal epithelial hyperplasia (Heck's disease)

Presents with multiple asymptomatic, slightly elevated, mucosa-coloured, smooth-surfaced nodules that occur on the labial or buccal mucosa gingivae or tongue of children. (FIG.) Individual lesions tend to be small (0.3 to 1 cm), but they frequently cluster and coalesce, giving the mucosa a cobblestone or fissured surface. Most lesions are found in children, although they occasionally can be found in older age groups. They usually regress spontaneously with age.



(FIG. HUMAN PAPILLOMA VIRUS)

b) Squamous cell papilloma and verruca vulgaris

Fourth most common oral mucosal mass and found in 3-4% of all biopsied oral soft tissue lesions. Caused by HPV type 6 and 11. It can occur at any age, but is most commonly diagnosed in the age group 30 to 50 years. It is clinically and microscopically indistinguishable from verruca vulgaris which is a viral induced focal hyperplasia of epidermis.

CLINICAL FEATURES : exophytic growth made up of numerous small finger like projections which result in a lesion with a roughened verrucous and cauliflower like surface. Nearly always a well circumscribed pedunculated tumor, occasionally sessile. It may initially grow rapidly, but seldom grows beyond 5 mm in diameter Generally painless usually white but sometimes pink in colour.

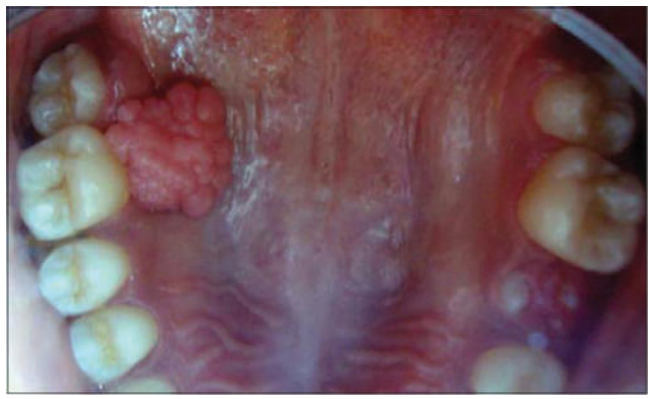


FIG. Squamous papilloma presenting as a single cauliflower-like lesion

INTRAORAL MANIFESTATION : most commonly in tongue, lips, buccal mucosa, palate, particularly the area adjacent to uvula.

VERRUCA VULGARIS : Also can be called as Common Wart. Frequent tumor to skin analogous to oral papilloma. Verruca vulgaris is a very common childhood infection.

Oral Manifestation : Oral lesions usually arise from autoinoculation, most commonly on the labial mucosa, tongue and gingiva.

c)Condyloma accuminatum [6]

Usually infection of the genitals, although it may be found in the oral cavity. Oral lesions are predominantly transmitted through oral-genital sexual contact. . It also characterized by multiple cauliflower-like lesions, some of which are larger than 0.5 cm which may become as large as 3 cm in size. The most common intra-oral sites are the labial mucosa and the lingual frenum and soft palate.

MEASLES or RUBEOLA Vs RUBELLA

Acute, contagious, dermatotrophic and endemic viral infections, primary affecting children and occurring many times in endemic form. Caused by **CLINICAL FEATURES** : Characterized by acute febrile illness and erythematous maculopapular skin rash. Fever, malaise, conjunctivitis, cough and coryza are mainly seen as prodromal symptoms which is followed by a generalized exanthematous skin rash.

ORAL MANIFESTATION : The oral manifestation of measles is known as **Koplik's spots.** Occur early in the course of the infection and often precede the skin rash by 1 to 2 days. These are white-red macules on that appear on the buccal and labial mucosa. These macules represent foci of epithelial necrosis.[7]

RUBELLA OR GERMAN MEASLES [8]

It should not be confused with rubeola. Main difference between the two is absence of Koplik's Spot in Rubella. The oral mucous membrane in this case is not usually inflamed as in Rubeola although tonsils can be somewhat swollen and red macules can appear on the palate.

HUMAN IMMUNODEFICIENCY VIRUS[1,5]

Although no oral lesions are found as a direct result of HIV infection itself, a range of HIV-associated oral lesions, some with a viral aetiology, have been described.

a) Oral hairy leukoplakia

Characterized by a white, vertically corrugated, non-removable lesion on the lateral or ventral margin of the tongue. Surface may or may not be corrugated but corrugation can be seen on inferior surface of the tongue or in the buccal mucosa. This lesion is caused by the Epstein-Barr virus and has no premalignant potential. It has also been reported in HIV-negative persons in association with immunosuppresive therapy. It must be differentiated from chronic hyperplastic candidiasis or leukoplakia (a potentially malignant lesion). In order to do that A biopsy must be performed to establish a reliable diagnosis. Once the diagnosis has been made, no treatment for the lesion is necessary.



b) Kaposi's sarcoma

Characterised by erythematous or violaceous plaque like lesions that develop into tumorous growths over time.These larger lesions may become ulcerated and painful and may interfere with function.

ORAL SITES : Predominantly seen in the palate or on the attached gingivae, but can appear on other mucosal sites.

DIAGNOSIS :- Clinical diagnosis should be confirmed by biopsy, as several other lesions may have similar clinical presentations. This is especially true for early lesions that may have similar clinical features as vascular malformations, bacillary angiomatosis and even well differentiated angiosarcoma.

Conclusion

Since oral cavity harbours many infectious diseases thorough examination and routine check up needed. Viral infection is always infectious and can spread through kissing like Ebstein-Barr virus so parents also from refrain themselves from kissing infants. Some diseases as has been described above spread through autoinoculation so careful diagnosis and proper education to patients about not tampering with vesicles or papules can reduce the spread of infection. Risky sexual relationship should also be avoided to prevent the spread of any oro-genital viral infection. On the top of that general and dental practitioner should be extremely careful in handling the patients with these infectious diseases so prompt and early diagnosis is required. Persistent lesions, especially if ulcerated, should be biopsied to exclude the possibility of a non-infective, more serious aetiology. Biopsies or other laboratory investigations should also be performed if a clinical diagnosis can not be established. This is especially true in the setting of HIV/AIDS that can influence the typical clinical features of viral infections.

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Endodontic-Periodontic Relationship - A Short Review

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Abstract

The relationship between periodontal and endodontic disease has been a matter of speculation for many years. The endodontium and periodontium influence each other during health, function and disease. This review gives a fair knowledge about several aspects of endo-perio relationship. Their exist three main pathways of communication between the pulp and periodontium. They are the apical foramen, the accessory canals and the dentinal tubules. The apical foramen and the lateral canals come under vascular pathways whereas the dentinal tubule comes under the tubular pathway. The pulp and periodontal tissues are derived from highly vascular mesenchymal tissues of the tooth germ. The apical foramen and lateral canals maintain the vascular connection between these tissues throughout the tooth development.

Key words: *endodontics, periodontics, lesion, relation, pathways*

Introduction

Simring and Goldberg in 1964 first discovered the relationship in between the periodontium and the pulp(1). The periodontium and pulp have embryonic, anatomic and functional interrelationship. Ectomesenchymal cells give rise to dental papilla and dental follicle.They in turn differentiate into periodontium and pulp respectively. This development during the embryonic stage results in anatomical connections,which remain throughout the life.

It has been found that microbiological similarities exist between infected root canals and advanced periodontitis(2,3,4,5).Similarities in the composition of cellular infiltrates also suggest the existence of communication between the pulp and periodontal tissues. A study by Brenda Gomes showing a similarity between the microbiota of periapical lesion and periodontal pocket , before and after CMP, suggests there may be a pathway of infection between the pulp and periodontium(6).

Speaking of these pathways further, apical foramen is the major pathway of communication. Although periodontal disease has damaging effect on the pulp tissue, total disintegration of the pulp will take place only when the bacterial plaque involves the apical foramen, compromising and further arresting the vascular supply. After pulpal necrosis, the bacterial products like enzymes, metabolites, antigens etc. reach the periodontium via the apical foramen, initiating an inflammatory response there.

The frequency of the lateral canals is 17% in the apical third, 1.6% in coronal and 8.8% in the body of the root(7).Bender et al. stated that periodontal endodontic diseases are frequently seen in molars than in the anterior teeth because more number of accessory canals are present in the molars. In first molars, the percentage of lateral canals in the furcation is 46%(8) whereas it is 50 to 60% in a multirooted teeth(9).The radiographic indications of the presence of lateral canals before obturation are presence of a localized thickening

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of periodontal ligament on the lateral root surface or a frank lateral lesion.

The dentinal tubules contain the odontoblastic process that extends from the odontoblast at the pulpal dentin border to the dentinoenamel junction or the cement-dentinal junction. Passage of microorganisms between the pulp and periodontal tissues is seen through these tubules, when the cementum is denuded and the dentinal tubules are exposed.

Non-physiological pathways of communication involve Iatrogenic perforations and vertical root fractures.

The etiopathogenesis of endo-perio lesion can be widely studied under three groups i.e. the anatomic considerations which have been discussed above, the contributing factors like poor endodontic treatment, poor restoration, trauma, resorption, perforation and developmental malformations. The third group is the etiologic factors live pathogens and non living agents. The live pathogens include bacteria, fungi and viruses.

Rupf et al detected Actinobacillus actinomycetemcomitans, Tannerella forsythus, Eikenella corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia and Treponema denticola in all endodontic samples as well as in teeth with chronic apical periodontitis and chronic periodontitis[10]. A study by Didilescu et al suggested that Fusobacterium nucleatum, Parvimonas micra and Capnocytophaga sputigena may play a role in the pathogenesis of endo-periodontal lesions[11].Spirochetes are another type of microorganisms associated with both endodontic and periodontal diseases. Recent studies demonstrated that the spirochete species most frequently found in root canals are Treponema denticola and Treponema maltophilum[12]. Treponema denticola posess an array of virulence factors associated with periodontal disease and may also participate in the pathogenesis of periradicular disease. Treponema maltophilum is frequently associated with rapidly progressing forms of periodontitis.

Coming to Fungi, yeast colonization associated with radicular pathosis has been seen in untreated root caries, dentinal tubules, failing root canal treatments, apices of teeth with asymptomatic apical periodontitis and in periapical tissues.Reduction in specific strains of bacteria in Root Canal treatment creates a low nutrient environment which may allow fungal overgrowth. Fungi may gain access from oral cavity during treatment as a result of poor asepsis. Colonization of Candida albicans and other species like Candida glabrata, Candida guillermondii, Candida incospicia and Rodotorula mucilaginosa were detected in root canal, which can penetrate into dentinal tubules. It has been found that approximately 20% of chronic periodontitis patients harbor subgingival yeast, mostly Candida albicans.

Gingival herpes virus has been associated with increased occurrence of subgingival Porphyromonas gingivalis, Tannerella forsythus, Porphyromonas intermedia, Prevotella nigrescens, Treponema denticola and Actinomyces actinomycetem comitans . Hence viruses may play a role in promoting overgrowth of pathogenic periodontal bacteria. Cytomegalo and Epstein Barr viruses play a major role in pathogenesis of symptomatic periapical lesion by the production of cytokines and chemokines thereby leading to tissue destruction[13].

The non living etiologic agents are extrinsic and intrinsic. The extrinsic agents are the foreign bodies which are associated with inflammation of the periradicular tissues. They may be dentin and cementum chips, amalgam, root canal filling materials, cellulose fibers from absorbent paper points, gingival retraction cords, leguminous foods and calculus like deposits. The clinical reaction may be acute or chronic. The intrinsic agents are cholesterol, Russell bodies, Rushton hyaline bodies, epithelium and Charcot – Leyden Crystals (CLC).The Russell bodies are found in inflammatory pulpal tissue of carious primary teeth. The Rushton Hyaline bodies are found in some odontogenic cysts. The epithelial rests of malassez found along the lateral and apical periodontal ligament stimulate the epithelium to proliferate and line the lesion.The presence of CLC is seen within a periapical lesion that failed to resolve after orthograde root canal treatment.

Classification

By SIMON,GLICK AND FRANK IN 1972[14]:

1. Primary endodontic disease
2. Primary periodontic disease

3. Primary endodontic disease with secondary periodontal involvement

4. Primary periodontal disease with secondary endodontic involvement

5. True combined lesions

TORABINEJAD AND TROPE IN 1996:

1. endodontic origin,
2. periodontal origin,
3. combined endo-perio lesion,
4. separate endodontic and periodontal lesions,
5. lesions with communication,
6. lesions with no communication

BY THE WORLD WORKSHOP(1999)

1. endodontic-periodontal lesion,
2. periodontal-endodontic lesion,
3. combined lesion.

The classification by Simon et al[14] is the most accepted and important classification till today.First comes the primary endodontic disease. It is caused by caries, restorative manipulations, traumatic injury. The pulp sensibility tests show completely absent of signs (necrosis, except multirooted teeth), may be aware of minimal discomfort. Probing shows normal sulcus or narrow drainage to the sulcus (fistulas resulting from pulpal disease) .Radiolucency at apical, lateral and furcation area is seen. Prognosis is good after RCT. The sinus tract extending into the gingival sulcus or furcation region resolves at an early stage, if the pulp which is necrosed is removed and the root canals are well sealed[15].

The primary periodontal disease shows wide pockets on probing that do not necessarily extend towards the apex. The clinical findings include attachment loss, gingival bleeding, tooth mobility, calculus and plaque. Radiographs show horizontal or vertical bone defect and furcation lesion. Pulp tests are normal. Pulp is vital in primary periodontal disease .Treatment wholly depends on periodontal therapy.

The primary endodontic lesion if not treated will lead to secondary periodontal disease. This is primary endodontic lesion with secondary periodontal involvement. The clinical findings include plaque and calculus at the gingival margin due to drainage. The adjacent teeth are not necessarily involved.The pulp tests show absence of response.A solitary wider pocket is seen on probing extending towards the apex. The radiographs show widening of the periodontal ligament space extending from apical to cervical region. Angular defects can be seen. The prognosis depends on the periodontal therapy, assuming the endodontic procedures are adequate[16]. With endodontic therapy alone, only part of the lesion may heal which indicates the presence of secondary periodontic involvement.

A severe primary periodontal lesion if untreated eventually leads to secondary endodontic involvement. This is primary periodontal lesion with secondary endodontic involvement. The clinical findings include history of extensive periodontal disease. Initially there is pain from inflamed pulp. On progression, pulp loses its vitality. The pulp tests are abnormal or absent. On probing , wide pocket is seen, sometimes extending apically. The radiographic findings show angular bone loss from cervical region towards apex.The prognosis depends on the periodontal therapy once the endodontic therapy has been solved.

Then come the true combined lesions which show history of extensive periodontitis. Pulp tests are absent. Probing reveals wide and conical pocket.Radiographic findings show extensive bony radiolucencies which may/may not communicate. Findings of true combined lesions may be similar to that of vertical fractured tooth.

The effect of periodontal inflammation on the pulp is controversial and conflicting studies are present[17,18,19,20]. It has been suggested that periodontal disease has no effect on the pulp, at least until it involves the apex[18]. The effect of periodontal lesions on the pulp can result in atrophic and degenerative changes like dystrophic mineralization, fibrosis, formation of reparative dentin, inflammation and resorption[21,22].

The atrophic changes result in decrease in the cells' size, more collagen depositions, impaired nutrition to the pulp cells. Hence they slowly degenerate.The death of the cell is so gradual that morphologic evidence

sometimes appears to be lacking.Disruption of blood flow results in localized areas of coagulation necrosis in pulp.These areas are eventually walled off from the rest of the healthy pulp tissue by collagen and dystrophic mineralization. With slowly progressing periodontal disease, before pulpal irritation occurs, deposition of cementum is seen obliterating the lateral canals. This may explain why, not all periodontally involved teeth demonstrate pulpal atrophy and canal narrowing. Sometimes, because of the mobility of the periodontally involved teeth, pressure atrophy may also occur.

The pathogenic bacteria and inflammatory products of the periodontal disease infect the pulp via the apical foramen and lateral canals and evoke an inflammatory reaction in the pulp. This is called Retrograde pulpitis. Coming to inflammatory changes, when periodontal disease approaches the apex, the inflammatory products act on the periodontal ligament and the surrounding alveolar bone. Localized apical granuloma is a commonly encountered periodontal lesion produced by the pulp disease. It is because of transport of bacterial products via the root apex, with the formation of vascular granulation tissue. Eventually, resorption of the alveolar bone and sometimes the root itself may occur.

Resorption is seen on sides of the roots adjacent to granulation tissue.When the periodontal lesions are deep, resorption may also be found within the root canals, often opposite lateral canals, and at the apical foramen.This is called peripheral inflammatory root resorption (PIRR).

The effects of endodontic infection on periodontium is seen in many findings of intrapulpal infection tending to promote epithelial down-growth along a denuded dentin surface. Also, in experimentally created periodontal defects, the epithelium was 20% more around infected teeth than non infected teeth. Non infected teeth had more of connective tissue covering than infected teeth. Hence, it is important to treat the pulpal infections first, before undertaking periodontal procedures.

The periodontal treatment procedures also have some effect on pulp.Deep root planning, improper or overusage of the instruments and periodontal injury or wounding may speed up the pulpal inflammation and initiate the interrelated disease process[23].Improper root planning and scaling results in removal of cementum and superficial dentin getting exposed. It leads to microbial colonization and bacterial invasion resulting in inflammatory lesions in the pulp and dentinal hypersensitivity.

Conclusion

Endodontic –periodontic disease requires multidisciplinary involvement. The signs and symptoms have to be evaluated thoroughly and based on that, treatment protocol has to be planned. Improper management may result in failure in healing of either endodontic or periodontic lesion or both the lesions.

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A Review on Cracked Tooth Syndrome

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Abstract

Cracked tooth syndrome(CTS) remains a big challenge to dentist because of its variable, bizarre clinical presentation, unpredictable symptoms and diagnostic dilemma. Based on the severity of the symptoms and depth of tooth structure involved, the treatment planning and management of CTS varies from one case to another or from one tooth to another in the same individual. The prognosis of such tooth continues to be questionable and needs continuous analysis. This article aims to resolve some of the confusion surrrounding tooth cracks diagnosis and different treatment options.

Key words: *craze, incomplete fracture, cracked tooth*

Introduction

Fractures are one of the major conditions in human teeth that cause pain. However when a tooth fracture is incomplete the presentation is more subtle and frequently remains undiagnosed[1]. Among longitudinal tooth fractures only craze lines and cracked tooth are referred as incomplete breaks[2]. Cameron in 1964 defined craked tooth as an incomplete fracture of a vital posterior tooth that involves the dentin and occasionally pulp[3].This definition is modified by Ellis SG as" a fracture plane of unknown depth and direction passing through tooth structure that, if not already involving, may progress to communicate with the pulp and or periodontal ligament[4].Cracks in teeth manifest as "cracked tooth syndrome." This syndrome is characterized by acute pain on mastication (pressure or release) of grainy, tough foods and sharp, brief pain with cold[2].Patients aged between 30 and 50 years of both sexes are commonly affected[5].Mandibular molars followed by maxillary premolars and maxillary molars are mostly affected[6]. It originates in the crown and extends subgingivally and directed mesiodistally. The fracture may extend through either or both of the marginal ridges and through the proximal surfaces.Cracks in the teeth favours bacteria ingression.There are two classic patterns of crack formation: In the first pattern, the crack is centrally located, following which the dentinal tubules may extend to the pulp; in the second the crack is more peripherally directed which may result in cuspal fracture[7].Cracks most likely cause pulpal and periapical pathosis as it more centered and apical than a fractured cusp .When pressure is applied to the crown of a cracked tooth there is a separation of the tooth components along the line of the crack. Saliva penetration along the crack line may further increase the sensitivity of dentine[8].This review highlights the importance of diagnosis of cracked tooth and its management.

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Etiology

The best way to manage tooth fractures is to understand the factors causing it.Incomplete fractures of posterior teeth is multi-factorial. Guersten et al., stated that 'excessive forces applied to a healthy tooth or physiologic forces applied to a weakened tooth can cause an incomplete fracture of enamel or dentin[9]. Lynch et al., classified the causes of cracks into four major categories: Restorative procedures, Occlusal

factors, Developmental conditions and Miscellaneous factors[10].

Restorative procedures

Cracks are caused due to stressess on the residual tooth structure by the placement of friction lock or self threading dentin pins. When restorations are tightfitting, luting inlays, onlays, crowns or bridges produce excessive hydraulic pressure leading to tooth fracture[11]. Similarly, extreme condensation pressure during amalgam restoration may also induce fractures[12]. Unrestricted removal of tooth during cavity preparation has also been shown lower tooth rigidity. Over-contouring of restorations results in a deep cusp-fossa relationship which contribute to the fracture of the nonfunctional cusp.MOD amalgam restorations on vital tooth has been shown to reduce relative cuspal rigidity significantly[13]. A prepared cavity is said to be in a increased risk of fracture if the width of a cavity is in excess of one quarter of the intercuspal distance[14]. Intra-coronal restoration can increase the risk of fracture 29 fold times than that of a healthy, un-restored tooth[15].

Occlusal factors

The most common cause of crack is due to excess force on biting suddenly on a hard object such as bone. Other attributing factors include betel nut chewing, inadvertent biting of lead shot, cherry stones and 'granary' bread[16]. Trauma from the occlusion also leads to fracture[17].The maximum biting force for molars ranges from 10 to 73 kg with an average of 45.7 kg for males and 36.4 kg for females. There is a generation of harmful eccentric forces when there is a loss of anterior guidance. The habit of nocturnal bruxismgenerates considerable occlusal forces causing fracture[18].

Developmental factors

Natural predisposing factors such as deep occlusal grooves, pronounced vertical radicular grooves or bifurcations, extensive pulp spaces, steep cusp angles, prominent mesio-palatal cusps of upper maxillary first molars as well as the presence of lingually inclined mandibular molar suffer from complete loss by fracture of both lingual cusps[19].

Miscellaneous factors

The use of high speed rotary instruments; erosive tooth wear and the factor of thermal cycling induce enamel cracks. An ageing dentition predispose to cracking as dental hard tissues become more brittle and less elastic with age[20].

Diagnosis

If you do not look for cracks and fractures in teeth, you will likely not find them. Since the endodontically treated tooth has no remainig vital tissue, symptoms are restricted to those caused by the affected periodontium. For the tooth that has a vital pulp, the subsequent steps can ensure the presence or absence of a crack. Historyof repeated occlusal adjustments by several practitioners without a conclusive diagnosis. Enlarged jaw muscles indicate a habit of overstressing the teeth during mastication[2]. Attrition gives a history of clenching, bruxism, or biting and chewing with excessive forces. Craze lines or darker cracks are seen in a dry field. Check for evidence of an underlying dehiscence or fenestration. The use of angular percussioncause separation of the crack line and stimulation of periodontal ligament fibers or fluid movement in the dentinal tubules[7]. Peform Bite Tests using tooth slooth, rubber wheel, wood stick or other instrument to focus biting pressures on specific cusps and ask the patient bite down with moderate pressure and release[21]. Pain during biting or chewing is considered a classic symptom in the early indentification[10]. A narrow, isolated periodontal probing is characteristic of a crack. J-shaped or U-shaped radiolucency may indicate a crack. Buccolingual cracks will only appear if there is actual separation of the segments whereas mesiodistal cracks can never be seen. The other possible diagnoses can be ruled out using radiographs. Methylene blue dye can be applied to the external tooth surface in the cavity after restoration removal to check for cracks[22]. When a fiberoptic transilluminator is applied directly to the tooth surface, a crack will block the light. Sound teeth, including those with craze lines, will transmit light. Wedging test can be done to check for movement of the segments which helps in differentiating a cracked tooth from a fractured cusp or split tooth. No movement with wedging forces implies a cracked tooth[2].

Treatment

Location and extent of the crack determines the treatment of cracked tooth. Performing root canal treatment must be dependent on the determination of pulpal and periapical diagnosis. Removal of the fracture line in the area of the cavity floor that would initiate an ideal endodontic access opening which will be helpful in determining the apical extent of the crack. Removal of the fracture line on the proximal surface of the tooth below the cemento - enamel junction is not usually indicated[2]. Removal of the proximal marginal ridge takes away sound tooth structure, thereby decreasing tooth strength and resistance to fracture. To avoid irreversible damage, it is important that a cracked tooth be treated as soon as possible.To reduce the stress on the tooth and to prevent further damage to the tooth occlusal adjustment of affected teeth must be done immediately[23]. A copper ring, stainless steel orthodontic band, or the use of full coverage acrylic provisional crowns can be used for immediate immobilization. Bonded composite resin can also be used for splinting. Intra coronal restorations, without cuspal coverage can be used for non splintering teeth. Composite resin and glass-ionomer cements are most commonly used materials for restoring cracked teeth[24]. Amalgam overlays have fracture resistance equivalent to that of intact teeth. Cast metal inlays with cusp coverage or partial crowns with circumferential external splinting are applied where esthetics is not of great concern[25]. When the crack extends from the occlusal incline to the cervical third of the clinical crown, full coverage crowns are the most appropriate form of treatment. Loss of pulpal vitality is a major disadvantage following the preparation of teeth to receive a full coverage crown. Treatment of crack which extends deeply in the furcation remains difficult. Full crown or onlay to bind the cracked segments is indicated unless the tooth is to be extracted[26].

Prognosis

Periodontal probing, radiographic examination, need for banding to evaluate reduction of symptoms etccan affect prognosis, and each of these must be carefully considered before proceeding with treatment. The location and extent of the crack is most important factors in determining the prognosis. Prognosis is considered excellent for cracks that does not involve the dental pulp and for those fractures which does not extend more than 2-3 mm below the periodontal attachment limitIng to a single marginal ridge[27]. The prognosis becomes poor if both the marginal ridges are involved , or if the crack reaches up to the pulp[28].Prognosis is hopeless in cases with complete mesio-distal fractures[29].

Conclusion

The cracked tooth syndrome remains challengingto the dentist in diagnosis and management. Every attempt must be made for early diagnosis of CTS with expediency. There is very limited clinical evidence available in the dental literature to substantiate the use of any definitive restorative techniques . Hence treatment of CTS will depend on the position and extent of the crack. Management options vary according to clinical need from replacement of the fractured cusp with a simple restoration to placement of an extracoronal restoration with adequate cuspal protection.

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Lateral Canthopexy in Lower Blepharoplasty: A Review Literature

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Abstract

Background: Lower eyelid malposition is a known complication of lower (lid) blepharoplasty surgery. The prevention of this complication is easier than its treatment. Over the past 10 years lot of patients have had lower blepharoplasties with a canthopexy procedure and in some cases a tarsal strip canthoplasty. The criterion for a lateral tarsal strip canthoplasty was a lid distraction distance greater than or equal to 10 mm. Lid distraction distance is the distance the eyelid can be pulled away from the globe after the initial skin incision has been made and measured with calipers. If the eyelid can be pulled away from the globe less than 10 mm, then a canthopexy is performed, which occurred in 98% of cases. Patients had lateral canthopexies regardless of age or preoperative assessment. Herein, we describe a simple method of canthopexy that can be performed on most patients having a lower blepharoplasty, to not only achieve a cosmetically superior result but also to prevent eyelid malposition or ectropion.

Conclusion: We have described a simple prophylactic canthopexy suturing technique that all surgeons performing lower blepharoplasty can do to prevent rounding or ectropion. Naturally, the more cases that are done, the more accurate will be the eyelid position and adequacy of eyelid tension achieved. Lateral tarsal strip canthoplasty is also used in more advanced cases of lid laxity.

Keywords : *Canthopexy, Blepharoplasty, Ectropion.*

Introduction

Recently there has been an upsurge of articles on the use of lateral eyelid margin tightening following lower (lid) blepharoplasty surgery to treat malposition[3,4]. Indepth articles such as those of Jelks and Jelks[1] on orbital anatomy and Flowers[2] on canthopexy are classic descriptions of ectropion and its sequelae as well as treatment. Flowers' article discusses the use of drilling holes into the lateral orbital rim for anchoring his canthopexy sutures and the use of an upper eyelid incision to feed a lower eyelid suture. Surgeons who perform lower blepharoplasty know that varying degrees of rounding of the lower eyelid can occur, from mild rounding to severe ectropion. Some surgeons have even abandoned this procedure to avoid such problems and their legal ramifications. In addition, it is not always clear preoperatively which patients will develop lower eyelid retraction and to what degree. Certainly, if the patient is older and has extreme laxity on examination such as lid snap testing, that patient is at a higher risk. However, even younger patients with seemingly normal eyelid position and a good lid snap may still demonstrate an undesirable eyelid position. One can avoid all cases, refer the patient to someone else, develop better preoperative testing and screening methods, or develop a reproducible prophylactic lid tightening procedure to use on most patients.

Most of us would like to avoid the first two options. We have not researched the third, but this may be a field ripe for investigation. I believe that the fourth option is a feasible solution. I have been performing a lateral tightening procedure in all lower eyelid blepharoplasties over the last 5 years. Herein, we describe a simple but

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reproducible procedure to tighten the lower eyelids on most patients who have had blepharoplasty. It can be modified to suit the individual patient's degree of laxity. It is safe and reasonably easy to perform. Patients whose eyelids were too loose did not have a canthopexy, but a lateral tarsal strip canthoplasty was performed (lid distraction distance greater than 10 mm).

Discussion

A lower eyelid subciliary incision is created. The lateral extension is important. A skin flap is elevated inferiorly and medially. The orbicularis oculi muscle is incised over the lateral orbital rim with sharp dissection. At this point, one can excise fat or trim orbicularis muscle as needed. McCord[5] describes orbicularis muscle flap elevation, which I have performed on midface-lifts, also described by Hestes[6]. A 5-0 Vicryl PC-1 suture is used, taking a bite of the lower eyelid tendon at a point medial to the lateral canthus, depending on the laxity of the eyelid. The more lax the eyelid, the more medial the bite. The lateral bite is into the orbital rim periosteum near the tubercle. One can feel if this bite is adequate if there is little to no "give" when the suture is pulled through. Usually just one simple stitch is required. Do not perform drill holes in the orbital rim as Flowers[2] has described. Just prior to placing the suture, the eyelid is pulled away from the globe to prove the extent of the laxity. The average distance (LDD) measured with calipers has been 8 mm. A distance of 1 cm or greater can be measured. Next, the suture is tied down and the eyelid is pulled away from the globe. Approximately 1 to 2 mm is measured; occasionally no elevation is seen. If the LDD is greater than or equal to 1 cm, perform a lateral tarsal strip canthoplasty. Or, if the eyelid position is so distorted in an inferior direction, perform a canthoplasty. The exact position of the lateral suture placement will vary. If the eyelid preoperatively is low or a more "almond" shape is desired, then a more superior position is necessary. For canthopexy procedures, have not found the need to use nonabsorbable sutures (eg, Mersilene) as we have used for other eyelid procedures, such as lateral tarsal strip canthoplasties. With the eyelid in a higher position and with increased security, one can occasionally excise more lower eyelid skin but still in a conservative fashion[7,8]. Routinely, the maximal skin excision is in the lateral triangle of skin and not the horizontal component of the eyelid margin. This allows smoother eyelid skin that patients appreciate. Early on we used one 5-0 Vicryl PC-1 suture to close the subcutaneous layer for added security as a lateral rotation flap. However, occasional bumpiness from the suture or a stitch abscess developed. The skin is then closed with 7-0 P1 continuous nylon suture (Ethilon).

Conclusion

The literature reports the post–lower blepharoplasty rate of lower eyelid malposition from 5% to 30%. Therefore, We decided to treat all patients to prevent eyelid malposition with canthopexy or canthoplasty. Thus, we have no internal controls in this study other than measuring the LDD before and after canthopexy/ canthoplasty. However, our rate of rounding now is 1%, which we believe is justification in itself to perform these procedures. In addition, the procedure provides aesthetic improvement in the patients, creating more almond-shaped eyes or preserving a beautifully natural eyelid shape in both male and female patients. Certainly, a more controlled study, randomizing patients to have a tightening procedure or not, or to only perform a tightening procedure on one eye in bilateral cases, would generate more meaningful data, but would not be ethical. Thus, I believe that most cases of lower blepharoplasties should undergo a canthopexy even if transconjunctival fat excision is the only procedure planned, and certainly if any lower eyelid skin is excised or laser skin resurfacing is planned. The canthopexy procedure has universal applicability: (1) to maintain good eyelid position in younger patients; (2) to offer more almond-shaped eyes to patients; (3) to improve the weakness inherent in older patients' lower eyelid canthal tendons; and (4) under specific circumstances, to allow more eyelid skin excision and tightening of the lower eyelid than without tendon fixation. We have described a simple prophylactic canthopexy suturing technique that all surgeons performing lower blepharoplasty can do to prevent rounding or ectropion. Naturally, the more cases that are done, the more accurate will be the eyelid position and adequacy of eyelid tension achieved. Lateral tarsal strip canthoplasty is also used in more advanced cases of lid laxity.

Ethical Clearance – Not required since it is a review article

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Title

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Proanthocyanidins (PC) - A novel approach

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Abstract

An ever increasing number of pharmacological effects have become known through the discovery of new plant flavonoid with variations in chemical structure and related derivatives. A new group of phytochemicals that has been attracting much attention from both the general public and health professionals isa novel drug proanthocyanidins. This review emphasize various properties of proanthocyanidin, pertaining to various diseases of proanthocyanidins.

Key words: *Free radicals. Antioxidants, Proanthocyanidin*

Introduction

An ever increasing number of pharmacological effects have become known through the discovery of new plant flavonoid with variations in chemical structure and related derivatives. A new group of phytochemicals that has been attracting much attention from both the general public and health professionals is proanthocyanidins(PC). PC are found in leaves, fruits, bark of trees, seeds, flowers and roots of many plants and it is predominantly present in tea, honey, wines, grape seed, vegetables, nuts, olive oil, cocoa, pine bark and cereals. Grape seed extract (GSE), the richest source of PC, has potent antioxidants and exhibits numerous pharmacological activities. PC, one of the most abundant flavonoid in the plant kingdom, are extracted generally from grape seeds and they have antioxidant[(1)], free radical scavenging[(2)] and anticarcinogenic[(3)] property etc .

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Chemistry of PC

PC, also known as condensed tannins, are widely distributed in the plant kingdom and it represents a ubiquitous group of plant phenolics which take the form of oligomers or polymers of polyhydroxy flavan-3-ol units such as (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-gallate. The fundamental structural unit of PC is the phenolic flavan-3-ol nucleus. The flavan-3-ol consists of a C15 (C6-C3-C6) structure characterized by a phenylbenzopyran moiety. Proanthocyanidins consist of flavanol units linked by two C-4 and C-8 interflavan bonds[(4)].

PC in oral diseases

Despite the recently reported drop in the overall death rate from cancer, the estimated survival rate and number of deaths from oral cancer remain virtually unchanged. PC administration might induce apoptosis in cervical and oral cancer cell lines, while acting merely to suppress proliferation of the normal cell line control[(5)] Administration of grape seed PC is shown to have a beneficial effect on physical health, specifically the health of bone. The effects of PC on mandibular bone are assessed by examining trabecular and cortical bone density, mineral content and noninvasive bone strength

in low calcium diet rats and an increase in both bone formation and bone strength in rat mandibles has been observed after PC administration[6]. **Houde***et al*·(2006)[7]have demonstrated that PC have potent antioxidant properties and it should be considered as a potential agent in the prevention of periodontal diseases. **Green tea catechin (monomeric unit of PC) shows a bactericidal effect against gram negative anaerobic rods and is effective in improving periodontal status**[8]Alveolar bone resorption is a characteristic feature of periodontal diseases and it involves removal of both the mineral and the organic constituents of the bone matrix, a process mainly carried out by multinucleated osteoclast cells.
MMPs produced by resident and inflammatory cells in response to Gram (-) periodonto-pathogens play a major role in the tissue destruction observed during periodontitis. Also, GSE dose-dependently inhibits the activity of MMP-1 and MMP -9 and this study suggests that GSE may be potentially used in the development of novel host-modulating strategies for the treatment of MMP-mediated disorders such as periodontitis[9].

Cardiovascular Benefits of OPC

Free radicals and oxidative stress play a crucial role in the pathophysiology of a broad spectrum of cardiovascular diseases, including congestive heart failure, vascular heart disease, cardiomyopathy, hypertrophy, atherosclerosis, and ischemic heart disease. Cardio-protective properties and mode of action of PCs are varied. PC supplementation has shown significant reduction in oxidized LDL, another important biomarker of cardiovascular diseases. PC have also been found to inhibit inducible endothelial CD36 expression, a novel cardio-regulatory gene[10].

Mechanism of action of PC

Antioxidant property

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, periodontitis etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. PC have been shown as antioxidants through the following mechanisms viz., 1) Free radicals scavenging property and 2) Metal chelating activity**.**

Free radicals scavenging property

The scavenging capacity of catechin and epicatechin molecules depends on the number of ortho-dihydroxyl and ortho-hydroxyketol groups and C2-C3 double bonds due to their hydrogen donating ability[11]. It is also proposed that the higher antioxidant activity is related to the greater number of hydroxyl groups on the flavonoid nucleus[12]. The dimeric PC are more effective than vitamin C in trapping oxygen radicals[2]. The protective role of PC through its free radical scavenging property both *in vitro* and *in vivo* has also been demonstrated by Ye et al., (1999)[13]. The electronic configuration of PC allows easy release of electrons to free radical species ($R^{\cdot}$) such as superoxide anion, hydroxyl, peroxyl and nitric oxide radicals. By release of electrons, the radical character of the ROS is transferred to PC ($P^{\cdot}$). PC structure determines relative ease of oxidation and free radical scavenging activity[14]. PC have been suggested to be superior to flavonols in their antioxidant capacity since oxidation of PC predominantly produces semiquinone radicals that couple to produce oligomeric compounds through nucleophilic addition. In other words, the presence of electron-donating groups attached to the aromatic ring such as –OH ought to increase the ease of hydrogen atom abstraction and, consequently, antiradical performance, whereas groups with electron-withdrawing properties such as $–COO^{-}$ should have the opposite effect[15]. Although electron-donating –OH groups are attached to the aromatic ring in PC, the hydrogen atom is more easily abstracted. This could be the reason why PC shows antiradical activity at the concentrations employed as shown earlier[16]. Chemically, the important features of flavonoids, are their remarkable antioxidant properties.

The hydrogen donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids, enable them to undergo redox reactions scavenging free radicals more easily and the stable delocalization system, consisting of aromatic and heterocyclic rings as well as multiple unsaturated bonds, helps to delocalize the free radicals. Chemical structure determines the relative ease of flavonol or PC oxidation and free radical scavenging activity although the presence of galloyl groups and the number and position of hydroxyl groups (based on redox potential) enhance antioxidant activity[16].

Metal chelating activity

The presence of free state iron and copper in biological systems catalyzes free radical reactions such as Fenton and Haber-Weiss reactions. In the Fenton reaction, iron catalyzes the generation of hydroxyl radicals. The ability of PC to bind such divalent transition metals effectively reduces the concentration of these cations and thus the extent of oxidative activity[17] . Facino et al., (1996) [18] have indicated that PC strongly complexes iron and copper cations in the ratios of Fe^{2+}/ procyanidin (2:1) and Cu^{+}/procyanidin (4:1) respectively. Results of an investigation involving the effect of PC hydroxylation patterns and degree of polymerization on aluminum chelating capacity reveal that hydroxyl groups are essential sites for metal chelation, o-dihydroxyl phenyl groups of the B ring in particular, and that increasing the degree of polymerization leads to higher stability of tannin-metal complexes[19].

Biological potential of PC

Antiinflammatory effects

COX and lipoxygenase (LOX) play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Selected phenolic compounds are shown to inhibit both the COX and 5-LOX pathways and this inhibition reduces the release of arachidonic acid. The exact mechanism by which flavonoids inhibit these enzymes is not clear[20]. PC efficiently restrain the inflammatory response of activated neutrophils *in vitro* and when absorbed *in vivo,*they could prevent the oxidative discharge at the sites of their adhesion[21].

Nitric oxide synthase (NOS) activity

While a small amount of NO is essential to maintain normal body function (homeostasis), a significant increase of NO synthesized by inducible nitric oxide synthase (iNOS) activates inflammatory process and acts synergistically with other inflammatory mediators[22]. Catechin, EGCG, and other flavanoids repress NO production in macrophages and human peripheral blood mononuclear cells[23]. Interestingly, EGCG is shown to exert its effect on iNOS expression and reduce the activity by competitively inhibiting the binding of arginine and tetrahydrobiopterin and it has been demonstrated that the gallate structure of this catechin is important for its action[24].

Antimicrobial activity

PC, well known for their high levels of antioxidants and polyphenols, have also shown promise as novel antimicrobial agents The polyphenol compounds may form aggregates with the toxin, in turn preventing its receptor binding and internalization into the host cell[24]25. The antimicrobial effects of several tannin extracts on yeast, filamentous fungi, bacterial and viral toxicity have been reviewed by Chung et al.,(1998)[26]. Polymeric PC may be useful as suppressors of antibiotic resistance in *Staphylococcus aureus* and they also show promise as an alternative treatment to antibiotic use against *Staphylococcus aureus* infection[27].

Conclusion

PC exhibits protective and therapeutic effect and it appears to have significant protection against inflammatory diseases and could be developed as a safe drug for the same

Ethical Clearance: Not Required

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Role of Nutrition in Oral Health

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Abstract

There is a mutually beneficial relationship present between the well being of oral tissues and nutrition in our body. The health of the oral tissues, the quality of saliva in mouth, the count of oral tissues and the extent of the taste relies on the consumption of nutrients. Oral health denotes the kind of food that has been taken and also the level of nutrients. A review of literary study was made to describe briefly about this mutually beneficial relationship.

Key Words: *Oral Health, Vitamins, Malnutrition, Dentist.*

Introduction

Nutrition is one of the way to study about how the food impacts over the human body. It is the necessary supply of water, vitamins, fiber, minerals etc. Many of the general health issues could be stopped and also prevented with the supply of nutrition. The World Health Organization stated that malnutrition is condition in which body's need of nutrition is not fulfilled hence failed to support growth, maintenance and some other specific functions[1]. Malnutrition could be either deficiency or over abundance of it. The term nutrients are commonly denotes both macro and micronutrients. There is a continuous interaction present between condition of oral cavity and nutrition [2].Malnutrition may impact the formation of oral cavity and the advancement of oral problems via altered tissue homeostasis, decreased resistance to microbial biofilms and decreased tissue repair capacity[3]. Under the absence of additional contributing factors, settled nutritional status should be taken into account for patients with refractory periodontitis, poor curing response to surgical procedures or repeatedly occurring oral disease. This is certainly true for aged sufferers and patients in institutional settings [3-6]. Nutrition impacts oral health and oral health impacts nutrition. This mutually beneficial relationship views better nutritional health causing good oral health and vice versa [7]. Nutrition is a greater factor in infection and inflammation [8]. Many reports stress out the continuous interactive relationship between malnutrition, easily spread diseases and the immune system. For example these infections in general encourages malnutrition , the malnutrition arouses the dysfunctions present in the immune system and this impaired immunity encourages and increases the easily spread diseases. In oral health, cavities , gum problems and many other diseases occurring in mucous membranes,, tongue and salivary glands are infectious and not only breaks the strength of the oral cavity, but also impacts over common health[10]. Periodontal diseases, described by frequent inflammation and a decline of bone and soft tissues which spread around the teeth, one of the general frequent infections present in most humans[8]. A healthy nutritious diet which includes the necessary supply of vitamins, proteins, micronutrients and important fatty acids can play a vital role in resisting the easy spread conditions includes periodontitis[8,9].

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Effect of Nutrition on Oral Health

Nutrition consumption affects the oral tissues in which the bacteria bind (i.e. teeth, epithelium, bone, collagen), and also the saliva[8]. Secretory proteins (mucin) present in the saliva creates a capable barrier in opposition to penetration, physical and chemical irritants, desiccation and bacteria[10]. Formation of glycoprotein such as mucin needs supply of vitamin A. Retinol deficiency can decrease the production of mucin, which leads to compromised flow of saliva, weakened tooth strength, and a significant increase in risk of cavities [10]. Malnutrition is also described by the increase in production and secretion of stress, hormones (glucocorticoid) and decrease in production of insulin[4].

Increased levels of cortisol in malnutrition causes a same kind of change in the content of this hormone in saliva and gingival fluid. Elevated circulating glucocorticoid levels, even at physiological concentrations, elicit macrophage dysfunction and reduce the production of cytokines in response to inflammatory stimuli. Cytokines play an important part in differentiation, host defenses, tissue damage and growth. Cytokines prevent the chemokines and the other cells that are also involved in attracting the inflammatory cells at the place where inflammation present. This also mainly affects the tissue curing process[9]. The relationship which is most common between oral well being and poor habits of nutrition or deficiencies is observed in the pathogenesis of cavities.

There is a powerful connection between cavities and frequent intake of sugar and carbohydrates. A candida albicans infection has various factors of predisposition but the high diets of carbohydrates and folate or iron deficiencies have been thoroughly involved. Repeatedly occurring aphthous stomatitis which often is a milder condition, however worse cases maybe due to the deficiency of nutrition include B12 vitamin, iron and folate deficiencies. These deficiencies can also causes the atrophic glossitis or painful burning tongue at the end which is indicated by inflammation and defoliation of the tongue at the end. Dental erosion is the irreversible decline of the dental hard tissue caused by the chemical actions of acid dissolution but not connecting the bacterial plaque acid, and not openly connected with traumatic or mechanical factors or with tooth cavities. Erosion generally happens with attrition or abrasion. Attrition maybe described as open tooth-to-tooth contact wear, whilst particles migrating opposite and reaching the surface of tooth which results in abrasion. It is found that the usage of soft drinks has an erosive potential, especially in age groups [11, 12].

Some of the alcoholic beverages such as cider and dry wine are also acidic [13, 14]. Intake of alcohol is connected with gastric reflux and erosion may therefore be from both intrinsic and extrinsic sources [15]. Instead of the total consumption of these drinks may be, the repetition is more critical in the process of erosion. Fresh fruits, to be specific the citrus fruits have the potential of erosion similar to the pickled food items with the presence of vinegar. The fruit drinks given through a feeding bottle acts as a comforters and maybe harmful specifically to infant children. From those practices, severe dental destruction has been revealed [16]. There is a relationship present in between the periodontal issues and calcium consumption. Maybe this is happening due to the construction of density in the alveolar bone that holds the teeth. Calcium is essential for healthy tooth, bone growth, muscle compressions & expansions and extra functions. The role of vitamin C in the process of repairing a healthy connective tissue with its own antioxidant properties and maintenance activities tells about the relation between vitamin C and periodontal disease. The micronutrient fluoride is having a critical role in oral health. Fluoride addition process in public potable water supply is believed and rates as one among the top and effective preventive public health measures ever undertaken. Fluoride also decreases the tooth cavities by various mechanisms. The fluoride ion may become a part of enamel, which makes it more resistant to cavity. Adding to that, fluoride may control the movement of oral microbes, decreasing the generation of organic acids. Nutritional deficiencies can be having a largely affecting the function of oral cavity.

Taste sense may become changed. Normal food may taste bitter and bland. The saliva generation may be decreased and this can cause mastication and swallowing process as a severe painful one and difficult. This brings the malnutrition from inability to eat or loss of desire to eat food. Nutrient deficiency also induces the new indications and more extra oral health diseases. Medications for the management of systemic disease can also cause the malnutrition which stands as a proof for

oral health diseases. Let us say for example, potassium deficiency is frequently occurring due to diuretics in patients with higher rate of blood pressure. It also induces raw sensations, burning mouth and sore tongue while drinking, talking and eating. Potassium deficiency also induces the gingival bleeding. Consuming a good balanced diet is needed to maintain well being of tooth and oral health.

The nest food options consists of chicken, nuts, meat, milk and cheese. The food items mentioned here are believed to be protect enamel by delivering the needed amount of phosphorous and calcium for the process of remineralization. Ending a meal with milk or cheese will neutralize intra-oral acid [17]. The additional food option consists of vegetables and strong/crunchy fruits (example, pears, apples etc). These foods contain high amount of water, which helps in dilution of sugar & its effect they have and also stimulates saliva to flow which supports to guard against decay by cleaning away the buffering acid and the food particles. Acid nature foods including citrus fruits such as orange, lemon and tomatoes should be consumed as a portion of a greater meal to reduce the acidic surroundings intraorally. The top beverage option consists of water (to be specific fluoridated water), milk and sugarless or unsweetened tea. Drinking sugar containing beverages should be restricted. These food items are having greater volume of sugar and/or they can be sticky to tooth, which could act as power source for bacteria. Adding to that cough syrups and drop should be consumed only at the needed time.

Effect of Poor Oral Health on Nutrition

Bad oral health can impact in the quality of diet and consumption of nutrient in a manner that possibly increases the risk of various systemic diseases[7]. Tooth loss can be the consequence in chewing troubles due to inadequate occlusive surfaces[7]. Lowered masticatory power changes the food choice and quality of diet, which could strike nutritional status [18]. Oral pain occurring due to cavities, advanced periodontal disease, soft tissue lesions or poorly fitting prostheses may also cause the modifications in diet and subsequent nutritional status[7]. Most of the studies related to tooth loss and nutrition indicates that nutrient consumption grows worse in quality with lesser teeth [7]. In a study, participants with maximum number of teeth have consumed lesser calories, maximum vegetables fiber, carotene and fewer saturated fats, cholesterol, comparing to their other participants who got lesser tooth [19]. Among community-dwelling elderly, edentulousness is a critical risk for loss of weight, as chewing trouble or oral difficulty due to poorly fitting dentures can lead to dislike of food and decrease in nutrient consumption [18]. In addition to that it is also found that denture-wearing persons are consuming high amount of refined carbohydrates, sugar, cholesterol comparing to dentate persons [7]. Such harmful changes in food options may increases the danger of multiple systemic diseases. As masticatory efficiency becomes lowered, some individuals skipping food that are tougher to chew including stringy foods like beef or steak, crunchy foods like raw carrots, and dry, solid food like crusty bread. A person with lower chewing ability may over cook or prepare too fresh foods to change them much easier to intake. Wide array of nutrients are impacted by these activities, including food constituents which are crucial to prevent cancer and cardio attacks, and to protect cells and fight the indications of aging.

Conclusion

Malnutrition can worsen oral health and bad oral heath could indirectly leads to malnutrition. To breach this harmful circle, good nutritional habits must be followed. Dentists should be held accountable for giving proper counseling to patients about diet and its relation to oral hygiene. Nutritional risk evaluation should be carried out during the starting phase and periodic dental examination. Supervising and follow-up should add periodic diet recall to assess counseling result. To gain the complete health of a person, oral health guidelines and queries about oral health troubles and the use of dental prostheses should be integrated into nutritional assessment protocol.

Ethical Clearance – Not required since it is a review article

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A Study on Breast Cancer Awareness among Female Patients Attending a Tertiary Care Hospital

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Abstract

Breast cancer accounts for 19-34 % of all cancer cases among women in India. There is a high mortality associated with breast cancer due to late stage diagnosis, as patients usually present at an advanced stage of the disease. This can be attributed to factors like lack of awareness among the patients and non-existent breast cancer screening programs. This study highlights the need for awareness about breast cancer among women, propagation of valid information regarding the disease and promotes early detection of breast cancer

Keywords: Awareness, knowledge, breast cancer, Female patients

Aims and Objectives

- To assess the awareness about breast cancer among female patients who are attending the out-patient department of a tertiary care hospital.
- To assess the knowledge about the symptoms for early detection of breast cancer and its outcome
- To assess the knowledge about the investigations and treatment for breast cancer.

Methodology

Study Design

- Cross-sectional study

Study Setting

-Hospital based setting

Sample Size

-1000 cases

Study Population

- The study population were women attending a tertiary care hospital opd and above the age of 18 yrs.

Research Instruments

Researchers developed questionnaire consisting of nineteen (19) items were used. The questionnaire was divided into four (4) sections. Section one contains demographic information of the respondents while section two contains information on the knowledge about the symptoms for early detection of breast cancer and its outcome, section three contains information on the respondents knowledge about investigations for breast cancer , section four contains knowledge about treatment modalities .

Data Collection Procedure

Data for the study were collected using self designed structured questionnaire. The instrument was tested for validity and reliability by an expert in the field and all corrections were corrected before final usage. It consisted of closed and open ended questions that were used to collect data that would meet the objectives of the study. The questionnaire was distributed to all respondents and the same was interpreted to those who were non literate, and all questionnaires were retrieved

from the respondents. The questionnaire has four sections.

Results

Table 1 : Demographic data of respondents

AGE [years]	FREQUENCY	PERCENTAGE (%)
18-25	538	51.53
26-35	215	20.59
35-45	171	16.38
Above 45	120	11.49
Overall	1044	100.00
Literacy	**Frequency**	**Percentage**
Literate	1006	96.36
Illiterate	38	3.64
RELIGION	**Frequency**	**Percentage**
Hindu	758	72.60
Muslim	175	16.76
Christian	201	19.25
Others	15	1.43
OCCUPATION	**Frequency**	**Percentage**
Farming	258	24.71
Housewife	647	61.97
Students	39	3.73
Hospital workers	45	4.31
Others	55	3.35

Table 2: Awareness among females about breast cancer according to their age

Sl.No	Age	Mean age ± SD	Mean score ± SD
1	18-25	20.226±2.8929	11.594±2.31731
2	26-35	31.065±3.21	11.827±2.3607
3	35-45	39.672±2.4297	11.7193±2.418
2	Above 45	55.158±7.549	11.5833±2.4065
5	Overall	29.659±12.30589	10.691±2.3356

Table 2 shows the mean and standard deviations of females according to their age. The highest mean and standard deviation comes from above 45 years of females (55.158±7.549)

Table 3: Awareness among females about breast cancer according to their literacy

Sl.No	Literacy	Mean age ± SD	Mean score ± SD
1	Literate	29.6878±12.3409	11.961±2.3356
2	Illiterate	28.894±11.465	10.868±2.6627

Table 3 shows the mean and standard deviations of females according to their literacy. The highest mean and standard deviation comes from literate females (29.6878±12.3409)

Table 4: Mean percentage of awareness among females about breast cancer according to their age.

Sl.No	Age	Mean score	Standard Deviation	Mean percentage
1	18-25	11.594	2.31731	52.703
2	26-35	11.827	2.3607	53.763
3	35-45	11.7193	2.418	53.269
4	Above 45	11.5833	2.4065	52.65
5	Overall	10.691	2.3356	48.59

Table 4 shows the mean percentage of awareness among females about breast cancer according to their age. It shows that females aged between 26 to 35 has highest knowledge about awareness (53.763).

Table 5: Mean percentage of awareness among females about breast cancer according to their literacy.

Sl.No	Age	Mean score	Standard Deviation	Mean percentage
1	Literate	11.961	2.3356	53.144
2	Illiterate	10.868	2.6627	49.401

Table 5 shows mean percentage of awareness among females about breast cancer according to their literacy. It shows that literate females have highest knowledge (53.144) when comparing to illiterate females (49.401)

Table 6: Knowledge about symptoms among females about breast cancer according to their age.

Sl.No	Age	Mean score	Standard Deviation	Mean percentage
1	18-25	3.172	1.2043	52.88
2	26-35	3.265	1.2033	54.41
3	35-45	3.3040	1.2929	55.068
2	Above 45	3.225	1.141	53.75
5	Overall	3.0576	1.2405	50.9609

Table 6 shows the mean percentage of knowledge about symptoms among females about breast cancer according to their age. It shows that females aged between 35 to 45 have highest knowledge about awareness (55.068).

Discussion

Breast cancer is the most common malignancy affecting women, with more than one million cases occurring worldwide annually. In India, it is the second commonest cancer among females. Though it can be detected early by self and clinical breast examination or mammography, in our country only 15% patients present in the localized stage; in 75% regional lymph nodes are already involved while 10% have distant spread at the time of reporting.[1] This is due to lack of awareness and nonexistent breast cancer screening programmes. Early detection and prompt treatment offer the greatest chance of long term survival and breast self examination (BSE) seems to be an important viable optional substitute for early detection of cancer.[2]

The study was carried out to find out the awareness among female patients about breast cancer who are attending a tertiary care hospital opd . Findings from this study however revealed that majority of women had insufficient knowledge about breast cancer . These findings were similar to those of these studies of other Asian countries showing below average awareness about the existence of breast cancer and their symptoms.

Our findings confirmed the previous reports [3-5] that the deficit in the knowledge of symptoms and risk factors might be the reason for the delayed presentation in breast cancer in developing countries . However , in developed nations where there is a diminution in mortality secondary to early detection and improved treatment modalities [6-7] , delayed presentation remains a problem for older women as seen in British , American and Australian women[9]

It is also noteworthy that illiterate women lack sufficient knowledge on risk factors and management of breast cancer.

Conclusion

In this present study 1044 females who participated were above the age of 18 years . Their overall mean knowledge score is 48.59. This level of knowledge about breast cancer is inadequate. This study highlights the need for more educational programmes to provide comprehensive information about breast cancer thereby helping in early detection and prompt reporting of breast cancer for the better treatment.

Ethical Approval: Approval for the study was obtained from the ethical committee of Aarupadai Veedu Medical college & Hospital, Pondicherry . Informed consent was obtained from each respondent and they were assured of their confidentiality and anonymity of the information provided.

Source of Funding: Self

Conflicts of Interest: None

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Auditory Hallucination in Hearing Loss Patient: A Case Report

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Abstract

Hallucination occurs in different modalities (olfactory, tactile, gustatory, visual, and auditory). Auditory hallucination is the most common among them. It is a form of hallucination that involves perceiving sounds without external auditory stimulus. It is most common among people with psychotic disorder (schizoprenia). It is also seen in people with hearing impairment. Hallucinations may also be present in 10–15% of normal healthy individuals. This case study discuss about an adult female of 88 years who reported with a complaint of auditory hallucination for the past 2 years and focuses on the rehabilitation provided and highlights the use of amplification devices on tinnitus and similar disorders.

Keywords: *Auditory hallucination, Hearing Impaired, Pure Tone Audiometry*

Introduction

Auditory hallucination is defined as auditory complex perceptions that may include music, people talking, or other sounds which occur in the absence of external stimulation and which are perceived temporarily as real [(2)].

Studies done on Auditory Hallucination reveal that it is also common in people with hearing loss and increase with the hearing loss severity. The prevalence of Auditory Hallucination in the general population is 13.2% .The management for auditory hallucination depends on the etiology of it, which varies widely. Different etiologies require different form of treatment approach. So it is crucial for the patient to receive an correct diagnosis. This case report describes auditory hallucinations in an 68-year-old female and the management for auditory hallucination in such patients.

Case Presentation

A 68 year old woman was referred to the Department of Audiology with the chief complaint of hearing speech sounds that leads to sleep disturbances and daily activities which affects her quality of life. A detailed case history was taken. The patient complained of reduced hearing since 7 years and was using hearing aids for the past 5 years in both ears. But now she uses hearing aid in the left ear which benefits her better than the other ear. The patient hears the voice sounding “ram,ram,ram” frequently, added to a constant repetition of sentences/questions that the patient was engaged in. This was seen only in the Right ear. A Pure Tone Audiometry (PTA) was carried out. The PTA result reveals Right ear - Profound hearing loss and Left ear - Severe mixed hearing loss. Tinnitus matching and masking test was carried out. The Tones presented did not mask the voice that was heard inside the ears. Aided audiometry was done by testing bilaterally using Hansaton Flow UP hearing aids (clinical hearing aid) and also the patient’s own hearing aid. It reveals trial hearing aids were better the patient’s own hearing aid, Left ear had more benefit than the right ear. Benefit was better in bilateral fitting.

A portable cassette tape player with intra-aural headphones was used, allowing continuous play for two hours. The tape consists of pure music. Hallucinations are stopped temporarily during and after she listened to the cassette player.

Cassette tape recorder with pure music





Amplification devices

Discussion:

The prevalence of auditory hallucination ranges from 12% in the group with unilateral hearing impairment and 24% in the most severely impaired group. This first comprehensive overview the literature highlights is the fact that hallucinations occur in a substantial minority of healthy older adults in the general community[(3)]. The group with hallucinations had significantly increased percentage of tinnitus in comparison to the non-hallucination population. Hallucination is hearing voices that affect the day-to-day life activities. Epidemiology studies reveal that about 50% of individuals with hearing loss also have tinnitus. It can be permanent or temporary. There always lies confusion between hallucination and tinnitus. Tinnitus is a kind of continuous tone heard in the ear which is generated by the outer hair cells present in the inner ear which is heard as a ringing sound, buzzing sound etc… Hallucination is repeated hearing of any experienced words, phrases or even sentences such as 'hello', 'tell me, what is your name? Etc… Management for hallucination could be Tinnitus Retraining Therapy where the gain in amplification devices is increased such as hearing aids. Cassette tape recorder can be given with pure music where the patient should listen to it for a certain period of time which can lead to reduction in hallucination. We have tried giving various therapies regarding auditory hallucination but patient did not follow-up regularly.

Competing Interests: No competing interests between the authors.

Ethical Clearance: MERF-ISH

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Source of Funding – Nil

Conflict of Interest – nil

Reference

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ACCURACY OF GOODSALL'S RULE IN PERIANAL FISTULAS – CORRELATION WITH MR FISTULOGRAM

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Accuracy of Goodsall's Rule in Perianal Fistulas – Correlation with Mr Fistulogram

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Abstract

Introduction: Perianal fistula is one of the common gastrointestinal pathology with significant morbidity. Goodsall's rule states that the posterior perianal fistulas have the fistulous tract that opens into the anal canal in the midline posteriorly, sometimes taking a curvilinear course and anterior perianal fistulas will have a radial fistulous tract.

Objectives: To assess the accuracy of Goodsall's rule in perianal fistulas on comparison with Magnetic Resonance (MR) fistulogram findings.

Materials and Methods: A total of 45 cases with perianal fistula underwent pre-operative MR Fistulogram assessment. Fistulas were divided into anterior and posterior fistulas based on MR Fistulogram findings. Accuracy of Goodsall's rule in all the perianal fistulas visualised on MRI was assessed.

Results: Out of 45 patients, 10(22.2%) patients had anterior fistulas and rest of 35(77.8%) patients had posterior fistulas. Goodsall's rule was valid in 28(62.2%) patients. Seventeen (37.8%) patients did not follow Goodsall's rule. Goodsall's rule was found to be more accurate in anterior fistulas than posterior fistulas.

Conclusion: MR Fistulogram has high accuracy of detecting perianal fistulas. Goodsall's rule may not be valid in all the cases of perianal fistulas. Goodsall's rule is more applicable in anterior fistulas. Posterior fistulas do not strictly adhere to Goodsall's rule with many of them showing linear tracts. This information is useful for pre-operative planning of fistula treatment.

Key words: *Anal fistula, Fistulogram, Goodsall's rule.*

Introduction

Perianal fistulisation is one of the important gastrointestinal tract pathology with substantial morbidity. Perianal fistula is an abnormal fistulous connection between the skin of the perineum and the anal canal. Prevalence is about 0.01% and predominantly affects young males, with a male-to-female ratio of 2:1[1]. Causes of perianal fistulas include perianal gland sepsis, Crohn's disease, tuberculosis, trauma during childbirth, pelvic infection/malignancy and radiation therapy, with perianal gland sepsis being the commonest cause[2]. Most common presenting symptom is discharge (65% of cases), but local pain due to inflammation is also common[1]. Perianal fistulas are classified into anterior fistula when external opening is anterior to transverse anal line and posterior fistula when external opening is posterior to transverse anal line[3].

In 1900, David Henry Goodsall described a rule after him, which predicts the position of the internal opening of the perianal fistula in relation to its external opening[3]. There are many classification systems used to describe perianal fistulas. Sir James Parks in

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1976 introduced a classification system for perianal fistulas which is still practised. St James's University classification is a commonly used perianal fistula classification which is Magnetic Resonance (MR) Imaging–based classification. It consists of five grades and it relates the Parks surgicalclassification to anatomy seen at MR imaging in both axial and coronal planes[4]. This classification deals with the demonstration of the primary fistulous track, secondary ramifications and associated abscesses. Goodsall's rule[3] states that if the perianal skin opening is posterior to the transverse anal line, the fistulous tract will open into the anal canal in the midline posteriorly, sometimes taking a curvilinear course. A perianal skin opening anterior to the transverse anal line is usually associated with a radial fistulous tract. An exception to the rule are anterior fistulas lying more than 2.5 cm from[1] the anus, which may have a curved track (similar to posterior fistulas) that opens into the posterior midline of the anal canal. Data regarding the positive predictive value have been inconsistent with different studies showing a wide variance in results[2].

Materials and Methods

Forty eight consecutive patients with clinical diagnosis of fistula-in-ano attending the surgery outpatient Department of Sree Balaji Medical College & Hospital, Chennai, India, were referred to the Radiology Department for MRI Fistulogram between January 2016 and September 2016. Three patients who were claustrophobic for MRI study were excluded from the study. Finally, 45 patients who underwent MRI were included in the study. Detailed clinical history taking and a thorough examination were done for all the patients. Study was done using HITACHI Aperto MRI scanner (Hitachi Corporation, US). This study group comprised of 42 males and 3 females. Age group of the patients ranged from 18 – 80 yrs with mean age of 42.5 years. Prior informed consent was obtained from all the patients. Ethical approval was obtained from Institutional ethical committee to conduct the study. MR Fistulogram protocol included T1W Axial, T2W and STIR Sequences in Axial, Coronal and Sagittal planes [Table 1]. Clinical findings of perianal fistulas were correlated with MRI findings to assess the accuracy of the Goodsall's rule in predicting the internal opening of the tract, using appropriate statistical tests such as Chi-Square test. Data was analyzed using SPSS Version 20 statistical software.

Results

Out of the 45 patients, 10 patients (22.2%) had anterior fistulas and rest of 35 patients (77.8%) had posterior fistulas [Figure 1]. Fistulas with curved tracts were more common than those with linear tracts. Goodsall's rule was valid in 28 patients (62.2%). Seventeen patients (37.8%) did not follow Goodsall's rule [figure 2 and 4]. Eight of the ten anterior fistulas followed Goodsall's rule, with linear tracts (80.0% accuracy). Twenty of 35 posterior fistulas followed Goodsall's rule (57.2% accuracy) [Figure 3]. Goodsall's rule was found to be more accurate in anterior fistulas than posterior fistulas.

Table 1: Protocol used for MRI fistulogram

Parameters	T1W FSE	T2 FSE	T2 FSE	T2 FSE	STIR	STIR	STIR
Imaging plane	Axial	Axial	Coronal	Saggital	Axial	Coronal	Saggital
TR/TE(msec)	995/17	880/105	6800/105	6400/105	8200/15	7600/15	7200/15
FOV(cm)	26 x 26	26 x 26	26 x 26	26 x 26	26 x 26	26 x 26	26 x 26
Section thickness(mm)	3.5	3.5	3.5	3.0	3.5	3.5	3.5
Intersection gap(mm)	4.5	4.5	4.5	4.0	4.5	4.5	4.5
Matrix	256 x 256	256 x 256	256 x 256	256 x 256	256 x 256	256 x 256	256 x 256
NSA	2	2	1	2	2	2	2

MRI- Magnetic resonance imaging; T1W FSE- T1 weighted Fast Spin Echo; T2 FSE- T2 Fast Spin Echo; STIR- Short Tau Inverse Recovery; TR- Time of Recovery; TE- Time of Echo; FOV- Field of View; NSA- Number of Signal Averages

Table 2: Goodsall's rule accuracy in following studies:

Authors	Accuracy of Goodsall's rule in anterior fistulas	Accuracy of Goodsall's rule in posterior fistulas	Overall accuracy	Gold standard used
Mallick and Kamil	54%	46%	----	Intraoperative findings
Alexander et al	66%	29%	----	Intraoperative findings
Cirocco-Reilly et al	90%	49%	----	Intraoperative findings
Barwood et al	91%	69%	----	Intraoperative findings
Gunavardhana and Deen	----	----	59%	Intraoperative findings
Hiranyakas et al	----	----	58%	Endoanal ultrasound
Our Study	80.0%	57.2%	62.2%	MR Fistulogram

Figure and figure legends

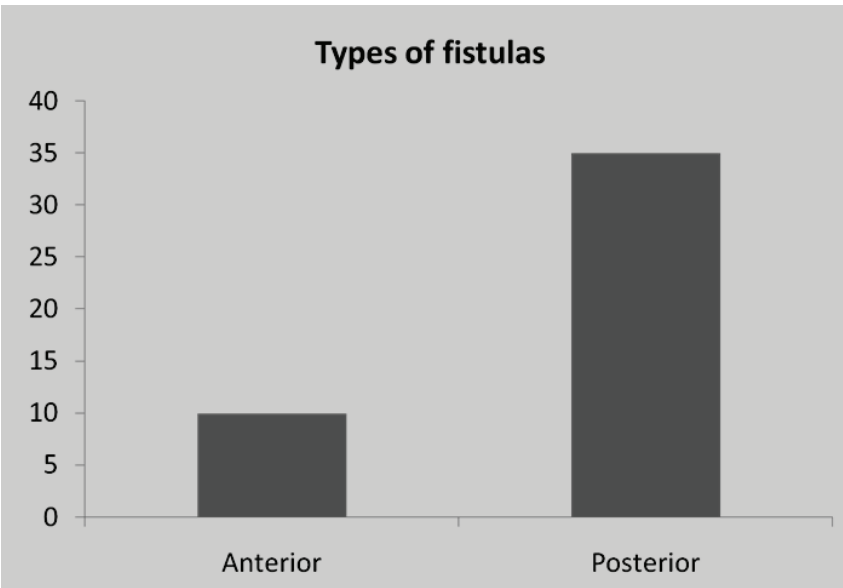


Figure 1: Bar diagram showing the number of anterior and posterior fistulas in our study.

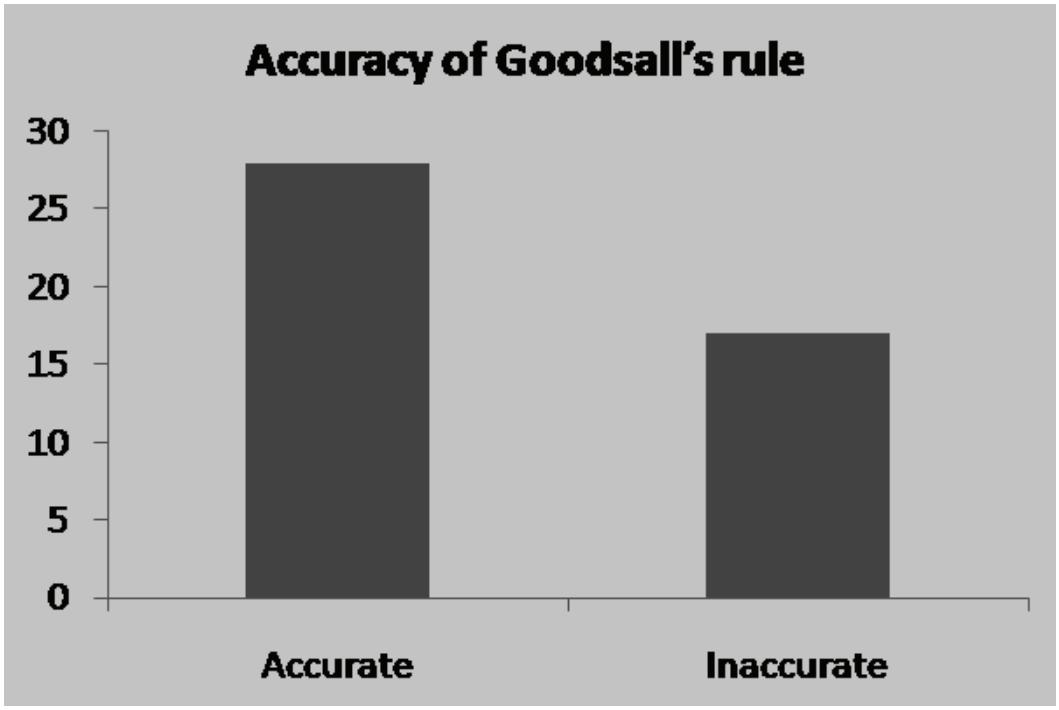


Figure 2: Bar diagram showing the accuracy of Goodsall's rule in our study.

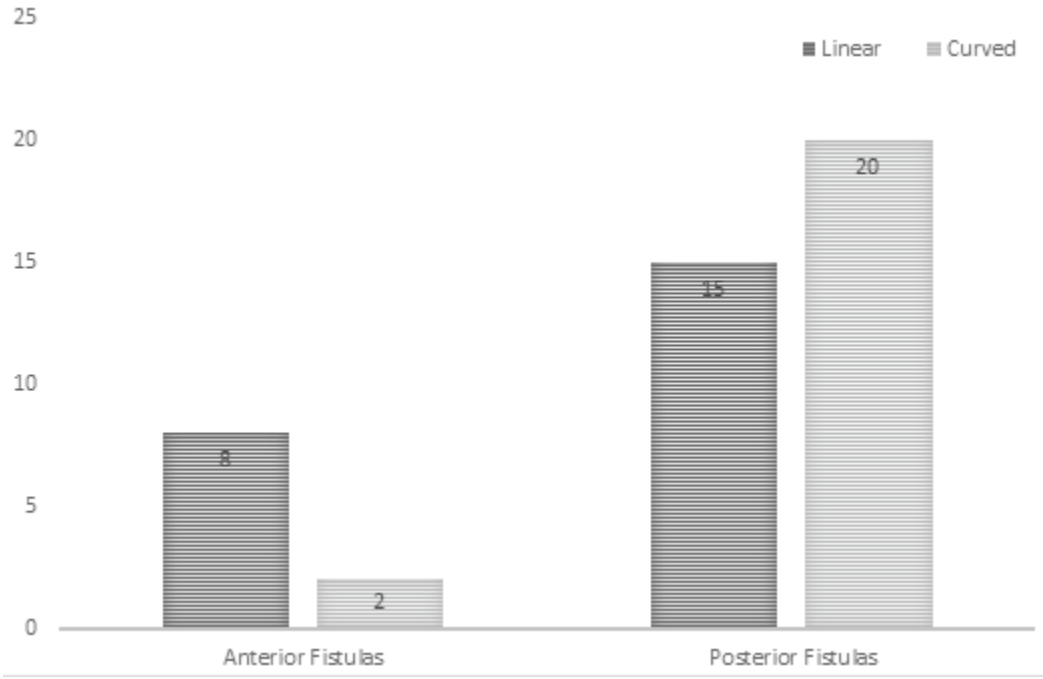


Figure 3: Bar diagram showing the number of linear and curved tracts amongst the anterior and posterior fistulas in our study.

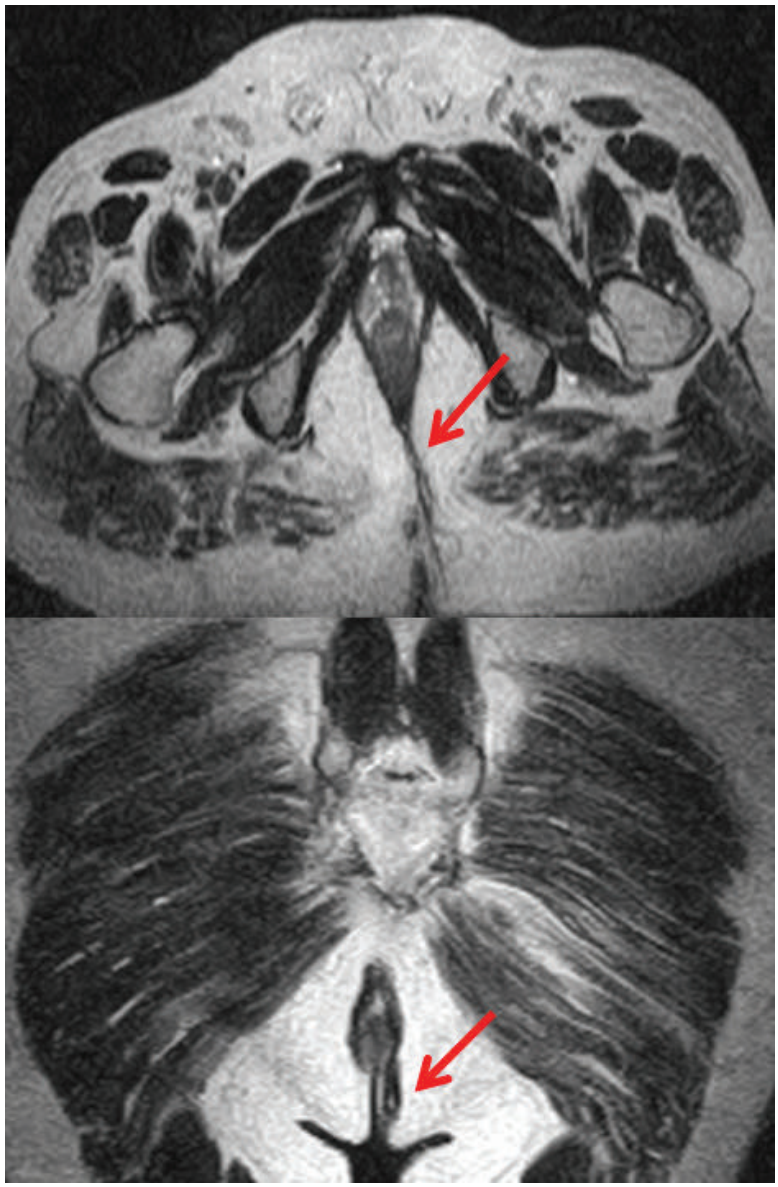


Figure 4. a,b: Axial (a) T2 weighted MRI showed linear posterior fistula (arrow) with external opening at 5'o clock and internal opening at 6'o clock position. Coronal (b) T2 weighted MRI showed linear posterior fistula (arrow) with external opening and internal opening at 5'o clock. Goodsall's rule does not hold good in these cases.

Discussion

Perianal fistulas while easy to diagnose, requires a thorough preoperative evaluation to map out the type and course of the tracts. Identification and localization of the entire crypto glandular fistula, including the external opening, the primary tract, secondary tracts, abcesses, and the internal opening are essential for classification and treatment. Inadequate assessment of the fistula may result in a simple fistula developing into a complex fistula, and failure to recognise secondary extensions can result in recurrent sepsis and a protracted clinical course[5].

Three main radiological imaging techniques used in assessment of perianal fistulas are contrast fistulography, endorectal ultrasonography and magnetic resonance imaging[6]. MRI imaging of perianal fistulae relies on the inherent high soft tissue contrast resolution and its multiplanar display of anatomy. In one of the early studies on MR fistulogram, Lunniss et al reported a concordance rate of 86- 88% between MRI and surgical findings[7].

Subsequent studies have suggested that MRI is more accurate than even surgical exploration of tract[8]. Hence MR imaging has emerged as the imaging technique of choice for preoperative evaluation of perianal fistulas, providing a highly accurate, rapid and non invasive means of performing pre-surgical assessment.

T2W images (TSE and fat suppressed) provide good contrast between the hyperintense fluid in the tract and the hypointense fibrous wall of the fistula, while providing good delineation of the layers of the anal sphincter[9,10]. The exact location of the primary tract (ischioanal or intersphincteric) is visualized on axial images, the presence of disruption of the external anal sphincter differentiates a trans-sphincteric fistula from an intersphincteric one. The internal opening of the fistula is also better seen in this plane. Coronal images depict the levator plane, thereby allowing differentiation of supralevator from infralevator infection. A combination of an axial and a longitudinal series (coronal and sagittal) will provide all the necessary details.

However, in places without access to MRI, preoperative planning is based on the long-standing Goodsall's rule. There have been quite a few studies assessing the diagnostic accuracy of the Goodsall's rule in comparison with surgical findings, endoanal ultrasound and MRI [Table 2]. Almost all the studies showed that Goodsall's rule is more accurate in anterior fistulas than with posterior fistulas[13-17]. In our study, we found that Goodsall's rule was accurate in 80% of cases with anterior opening and 57.2% of cases with the posterior opening with overall accuracy of 62.2%.

As the study revealed that the Goodsall's rule is not completely accurate in establishing the tract of the fistulae, the authors recommend that all cases of perianal fistula be thoroughly investigated preoperatively with MR fistulogram, which has a high accuracy of 80-90%[11,12]. These findings were consistent with the published papers regarding the same[13-17].

Conclusion

This study proves that Goodsall's rule may not be valid in all the cases of perianal fistulas. Goodsall's rule is more applicable in anterior fistulas. Posterior fistulas, which are more common than anterior fistulas, do not strictly adhere to Goodsall's rule. It has to be kept in mind when proceeding for surgery without preoperative MRI in unavoidable situations. MRI is the investigation of choice for perianal fistulas and should be done whenever available.

Ethical Clearence: This study was duly cleared by the ethics committee of Sree Balaji Medical college & Hospital

Conflict of Interest: Nil

Financial Disclosure: Nil

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Assessment and Management for Speech in Facial Palsy: A Review Study

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Abstract

Facial nerve palsy is the common problem of facial muscles weakness and facial appearance, mainly resulting of temporary or permanent damage of any structures innervated by the facial nerve. It can be congenital or acquired. About 45 to 75 % of unilateral facial nerve palsy cases are still idiopathic. Facial palsy impairment significantly affects swallowing, articulation, and lip sufficiency. Diminished facial expression can pose challenges in face-to-face communications that affects their psychological consequences which lead to negative impact on the quality of life. This article reviews on the assessment and management for the communication and quality of life. Speech-language therapy in facial paralysis aims in the rehabilitation of oral functions such as verbal and non-verbal communication, swallowing and vegetative skills.

Keywords: *Facial palsy, idiopathic, musculature, articulation, rehabilitation.*

Introduction

Facial paralysis is a common problem that affects facial appearance, and involves the paralysis of any structures innervated by the facial nerve. Facial palsy can be classified into two categories encountered in clinical practices: peripheral and central facial palsy. Peripheral facial palsy is the result of a nerve dysfunction in the pons of the brainstem where the weakness is in upper, middle and lower one side of facial muscles. This may cause asymmetry in the face and difficulty in articulation, eating, drinking and saliva control. Whereas central facial palsy is the result of nerve function disturbances in the cortical areas where the lower half of one side of the face is affected. Facial nerve palsy may also be present as a complication of several diseases such as acute and chronic otitis media, cholesteatoma, mastoiditis and meningitis [2].

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Review Summary

The peripheral paralysis of the seventh cranial nerve is characterized by motorial, sensorial and visceral deficits of the hemi-face involved. Facial asymmetry is noticed during examination: the facial creases, nasolabial fold disappearance, dropping of mouth in the affected side (with possible saliva leakage), widening of eyelids, dynamic signs are represented by the inability to whistle, puffing cheeks, frowning and closure of eyelid[3].

Etiology

A high amount of evidences implies that Bell's palsy is caused by latent herpes viruses (herpes simplex, herpes zoster) which are being reactivated from cranial nerve ganglia.

Different causes may result in secondary facial palsy but it's hard to decide which cause is responsible for the clinical picture but it is important to differentiate between the primary and the secondary forms since

it has an effect on the therapeutic management and prognosis significantly [4]. A study by Valenca et al,with 180 patients, resulted that Bell's palsy was associated 12% in arterial hypertension, 11% in diabetes, 4% in pregnancy or puerperium in 1% in neurocysticercosis [4].

Unilateral facial paralysis can be suspected in very young children and in newborns when there is absence of front and nasolabial groove motility, there is also asymmetry of the face with buccal deviation when crying. In cases of severe paralysis in children they cannot close the eye due to a complete absence of movement on the affected side and there is an asymmetry of the face at rest. In newborns, this condition can also hamper breastfeeding [5].

Degree:

The degree of paralysis represents the prognosis of the condition, patients with partial paralysis have a better prognosis. The scale used to characterize the degree of facial paralysis is House- Brackmann Facial Nerve Grading System. It consists of scale I to VI from normal to complete paralysis. Based on the function of rest and effort intermediate grades vary.

According to House- Brackmann scale, the II degree has a good outcome, while the III and the IV degrees are associated to moderate residual dysfunctions. Whereas V and the VI degrees have poor possibility of recovery [6, 7].

Recovery:

In Bell's palsy, most patients (85%) will have a partial recovery within 3-4 weeks and complete recovery in 61% within 6 months [8]. Of the patients who do not recover, sequela was slight in 12% of patients, mild in 13%, and severe in 4% [9]. The prognosis for Bell palsy, even if left untreated, is good for most patients. However, about 30% of those who are untreated will have a poor recovery, with continuing facial disfigurement, psychological difficulties and facial pain [10, 11].

Oral structures:

Facial paralysis that affects the lip musculature can significantly affect swallowing, articulation, and lip sufficiency [12]. The lip is an important aesthetic feature of the face and functionally important in speech and swallowing. In reversible paralysis where the facial muscles are physiologically intact, reinnervation of the orbicularis oris and other extrinsic lip muscles provide the best chance for restoring tone and oral competence.

Articulation & Swallowing:

In articulation, errors in labial consonants were observed and more specifically the weak pressure articulation of /f/ was the most common articulatory errors. They also have difficulties in generating bilabial consonants. The perceived errors in the intelligibility test were seen in labials and vowels. Adequate interlabial pressure is necessary to prevent anterior bolus spillage and drooling. To generate the bilabial plosive sounds /p/ and /b/, airflow from the lungs is interrupted by complete lip closure. Production of the consonants /f/ and /v/ require lower lip tension for controlled and precise airflow [13].

Difficulties observed while eating were food leakage in the corner of the mouth, residue in the mouth after meals, difficulty getting food from the spoon with the lips and difficulty in chewing food with molar teeth. Few report of having difficulty in controlling saliva (drooling) but it is not present in all cases of facial nerve palsy. The orbicularis oris and extrinsic perioral muscles maintain the form, tone, and movement of the lips. The orbicularis oris muscle works together with the superior pharyngeal constrictors and buccinators to generate positive pressure during the oral phase of swallowing.

Quality of Life:

Face to face contact is an essential component of human interaction. Consequently, the facial paralysis can have a significant negative impact on one's psychosocial well-being. Therefore it is crucial in identification and management of patients to optimize normal facial function[10,14]. In hemi-facial palsy, it is important to note that psychological distress rather than functional impairment because it is found as the most significant predictor of social disability.

The Facial Clinimetric Evaluation (FaCE) Scale is an instrument that covers six different domains: facial movement, facial comfort, oral function, eye comfort, lacrimal control and social function.

A study done by Ryzenman et al found that females and younger patients who got facial palsy and eye problem due to acoustic neuronal surgery were less satisfied with their appearances [15]. A study done by Fu et al found that higher levels of anxiety was found to be in females than males but same level of depression and there were no significant association between severity of facial palsy and distress levels [16]. According to Pouwels, the left side of facial palsy was not giving the patient true image but right side palsy showed them a true image of their face [17].

Facial symmetry was seen during the emotional expressions; left hemiface was found to have profound emotional expressions and they also got lower FaCE social functional scores and no significant differences were found to be in right side, left side or bilateral palsy [18].Scores of FaCE sub domains changed due to treatment taken prior, duration of condition, cause of the problem and age. Studies show that low score in oral functions, eye comforts and social functions sub domains for participants who had treatments prior.

Management:

Orofacial exercises have been applied as a rehabilitation tool in patients with lip weakness after a stroke and other neurodegenerative diseases. Although normative data for lip strength by age and sex are available, quantitative data in facial paralysis are limited [19]. Quantitative labial strength data for patients with facial paralysis is important in selecting and evaluating the effectiveness of treatment options.

Myofunctional exercises have an objective to accelerate the nerve regenerative process and also the resumption of movements and functions of the muscles in the facial mimicry, preventing muscle atrophy which would damage the recovery process [20].

When facial paralysis affects facial expression, the ability for nonverbal communication is affected, which directly influences socialization and communication[41]. Speech-language therapy in facial paralysis aims the rehabilitation of oral functions such as speaking, chewing, swallowing and facial expressiveness. Intervention must start in the early stages, for the recovery of the functions depends on the kind of impairment of the nerve, degree and time duration for the reinnervation, as well as the motor and sensory connections. To achieve this, myotherapic exercises are used in addition to conventional exercises, in order to work with the facial muscles. [22].

Discussion

It is noted that peripheral facial nerve palsy is seen not only in adults but also seen in children. Nevertheless, facial nerve palsy has been much more intensively investigated in adults than in children. There are also some important differences despite a lot of similarities concerning pathogenesis, treatment, and outcome. Facial nerve palsy appears 2–4 times less frequent in children than in adults [23]. Facial paralysis is well known to cause the variety of functional, aesthetic and psychological consequences.

In the study by Secil et al., prevalence of 55% distributed in oropharyngeal swallowing which is in accordance to the prevalence of eating and swallowing difficulties in patients with peripheral facial palsy which is demonstrated by objective electro-physiological methods [24] also in the study by De Swart et al., almost all the patients reported some difficulty with eating and drinking [25]. Studies show that difficulty swallowing is primarily related to the oral phase, or, more specifically, difficulty controlling bolus. Baylor et al 2011 states that communicative participation is important for the patients to encounter in any situation that require speech [26].

Meticulous history, physical examination, and photography in repose and specific facial expressions are involved in assessment [27]. A video clip of these expressions is also recorded for review and comparison which also gives information about the quality of life. From a clinical perspective in flaccid Facial palsy patient the first priority is given for eye protection, following which, based on likelihood of spontaneous recovery of facial function treatment will be determined [28].

Eadie et al 2016 states patients who have undergone laryngectomy as well as facial paralysis showed similar kind of restriction in communicative participation [29]. All types of communication disorders are suggested to have similar restriction in communicative participation due to physical, social and environment factors [30].

The patient rehabilitation starts with isotonic exercises aiming to re-fit facial symmetry, always in a controlled way, that is, even the more restricted movement on the paralyzed side should have a similar movement on the opposite side, providing a symmetric movement. In this process, self-control is of high importance and the mirror plays a fundamental role since, associated to neuromuscular treatment, it facilitates visual feedback for the control of movements during the exercises [31].

Bell's palsy has a generally optimal functional recovery in a short period of time; a favorable prognostic indicator is represented by a clinical improvement within 3 weeks by the onset [32]. Ramsey Hunt syndrome has a worse prognosis compared to Bell's palsy: only 10% of severe paralysis due to reactivation of Herpes Varicella-Zoster have a full recovery [33].

Conclusion

The therapeutic approach often involves a multidisciplinary team, comprehending otolaryngologists, neurologists, ophthalmologists, maxillofacial surgeons, plastic surgeons, speech language pathologist, physiotherapists. Treatment Approaches include: drug therapy, surgery, rehabilitation approaches.

Communication and the ability to eat and salivary control can be affected due to acquired peripheral facial palsy. Physicians should be aware that between the degree of facial palsy and the possible effect on communication, eating ability and saliva control there is no direct correlation. Speech-language therapist play an important role in facial paralysis they aim in the rehabilitation of oral functions such as speaking, chewing, swallowing and facial expressiveness.

However, as long as there are no sufficiently powered studies on children available, management of childhood facial palsy should not differ from that in adults. In addition, it is necessary to establish the potential additive effect of lip muscle training and objectively measure the effects on quality of life. It is concluded that communicative participation represents a unique domain of dysfunction and this can help in quantifying the outcome of facial paralysis and gives a clear view when assessing treatment outcomes.

Ethical Clearance: This study doesn't require ethical clearance since it is a review article.

Conflict of Interest: All authors declare that they have no conflict of interest

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The Awareness of Audiologists and Speech Language Pathologists among Doctors

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Abstract

Aim: The purpose of this study is to survey the awareness of Audiologists and Speech Language Pathologists among Medical Professionals (Doctors, Postgraduate students and Undergraduate students) and to report the preliminary survey results.

Method: A survey was carried out by administering questionnaire consisting 20 questions. The questions are designed to explore and investigate the field of Audiology and Speech Language Pathology (ASLP). The participants were divided into two groups A and B, where group B is below 35 years and group A is 35 years and above.

Result: The result of the study revealed that the overall awareness of ASLPs among Medical Professionals varied between fairly adequate knowledge to inadequate knowledge. Age does not have any influence on significant awareness on ASLPs.

Conclusion: Hence the knowledge and awareness for audiologists and speech-language pathologists need to escalate among medical professionals. Future surveys should be conducted to reduce the lack of awareness of Audiology and Speech Language Pathology among the general population and other medical professionals.

Key words: *Audiologist, Speech Language Pathologist, Doctors, Awareness, Questionnaire.*

Introduction

Speech Language Pathologists (SLPs) are professionals also referred to as communication experts who asses and treat all age groups with communication disorders and swallowing disorders [1]. The word 'communication disorders' is a broad term which includes delay in speech and language in children, specific language impairment, motor speech disorders, neurological speech and language disorders in children, cleft of lip and palate, stuttering and cluttering, voice disorders, neurogenic communication disorders in adults, stroke or head injury, dementia, hearing impairment, and disorder or syndrome associated with delay in speech and language. They also play a vital role in the pre and post operation of laryngeal cancers [2]. A detailed counselling regarding post operation management options and rehabilitation is carried out in the pre operation counselling [3]. Thus,speech language pathologists are apprehensive with a person exhibiting any disturbance or interruption in speech perception, processing and production.

Audiologists are health care professionals who graft with early identification, evaluation and management of hearing impairment [4]. They asses hearing related problems including balance, tinnitus, reduced hearing sensitivity, neurological condition that result in hearing loss and syndromes associated with hearing loss. They perform various test procedures on hearing and vestibular system and provide the appropriate amplification and assistive listening devices and recommendation for

implants (such as Cochlear Implants and Auditory Brainstem Implants). Audiologists are envisioned in custom selection of hearing aids, providing appropriate gain based on the patients need, profession and environment [5]. There are numerous management options available in the cases of congenital hearing impairment [6].

In the recent years, there has been an increasing requirement to promote awareness on the early identification of communication disorders in order to undertake suitable rehabilitative methods and improve the overall quality of life of the individual with disability [7]. The assessment and management of communication disorders which are followed by a visit to the SLP on a regular schedule can be executed with the help of the medical and non- medical professionals such as doctors, nurses, psychologists, teachers as well as social workers. When it comes to working with individuals with communication disorders it follows a pre-requisite to work as a team [8]. Hence the awareness is crucial when working in a multidisciplinary entity.

The study aims to enumerate the awareness of role of Audiologist and Speech Language Pathologist (ASLP) among medical professionals (Doctors, postgraduate students and undergraduate students).

The objective of the study was to determine whether age of the professionals influence their awareness (less than 35 years and greater than 35 years of age).

Methods

Material

The questionnaire contains 20 close ended questions. The questions were related to the role of ASLP and also about the disorders that are assessed and intervened by ASLPs. The questionnaire contains four response options which varied with the questions.

Inclusion and exclusion criteria

The inclusion criteria for this study comprises of the minimum qualification of a doctorate degree (MBBS, BDS) or currently on an academic program of MBBS or BDS with a minimum completion of 2 years and others who are specialised in the field of medicine. The exclusion criteria include all non-medical professionals and students who have currently enrolled in MBBS or BDS course.

Participants

A convenient sampling method was adopted. A survey using questionnaire was conducted among Medical professionals that is Doctors, post graduate students and undergraduate students who has completed 2nd year of their academic program. A total of about 327 professionals participated in the study. Out of which 57 participants were excluded because they were non-medical professionals. 270 professionals were included in the study among various medical departments of which 131 males and 139 females. The participants were then divided into two groups A and B, where group A is participants above 35 years and group B is below 35 years. Mean age and the no. of participants in each group is tabulated in table 1.

Procedure

A self-rating questionnaire was administered which contained 20 questions with 4 options in each. The questionnaires were circulated through online forms and institution-based distribution was carried out. Cross-sectional study design was used. Informed consent was obtained from all the participants. Prior to administration the participant was briefed about the purpose of the study and were assured of anonymity of their responses.

Results

The responses of 270 participants based on their agreement with the 20 statements and the mean scores obtained by the participants for each statement are listed . The overall awareness of ASLPs by the Medical Professionals was categorised based on the scores obtained. A score of greater than 15 was considered adequate knowledge, score of 10 to 15 was considered fairly adequate and a score of less than 10 was considered inadequate knowledge. The results revealed that out of the 270 participants 138 (51.1%) had inadequate knowledge, 126 (46.6%) had fairly adequate knowledge and 6 (2.2%) had adequate knowledge.

Mann – Whitney U test was used to compare between two groups to determine whether age influence their awareness about ASLPs. Group A (above 35 years) and group B (below 35 years) was compared and the results

revealed no statistically significant difference exist between the two groups as the p value > 0.05. In comparison with group B, group A has knowledge about the role of SLP in Voice Therapy.

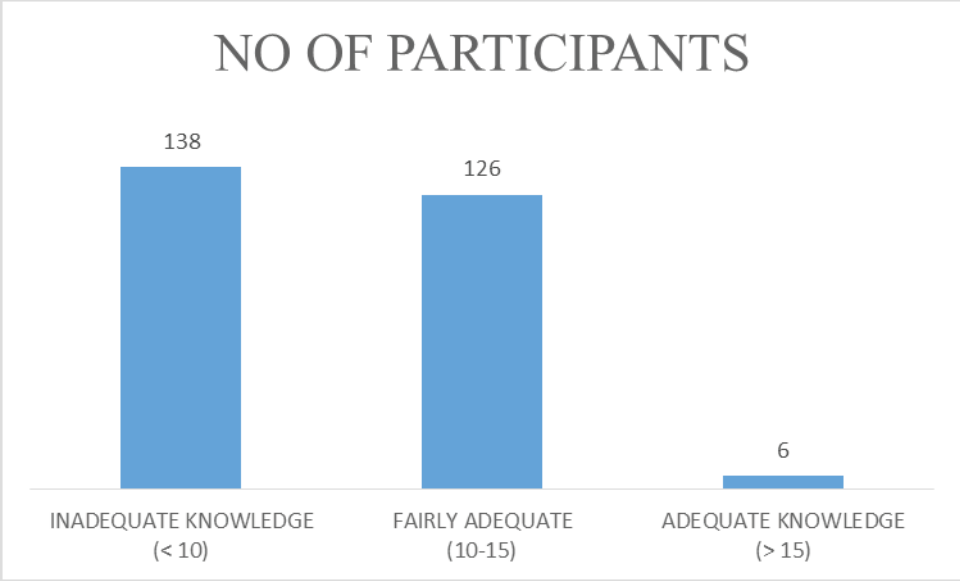


Figure 1: overall awareness of the 270 participants

Table 1: No of participants in each category

GROUPS	CATEGORY	NO. OF PARTICIPANTS	MEAN AND SD	OVERALL MEAN AND SD
AGE	Greater than 35 years	109	45.02 ± 7.80	34.20 ± 10.61
	Less than 35 years	161	26.87 ± 3.80	
SEX	Male	131	-	-
	Female	139		

Discussion

The aim of the study is to enumerate the awareness of role of ASLPs among medical professionals and to determine whether age of the professionals influence their Awareness (less than 35 years and greater than 35 years of age). The study was done on 270 participants using a survey questionnaire. The results revealed that 138 (51.1%) had inadequate knowledge, 126 (46.6%) had fairly adequate knowledge and 6 (2.2%) had adequate knowledge indicating that there is a minimal awareness of this field among Medical Professionals which is contradicting to a study done on awareness of ASLPs among college entering students which was 84% adequate in a state-supported university in Pennsylvania [9]. One of the studies in the literature done on awareness of Speech Language Pathology on the public showed 55.5% of awareness which indicated a lack of understanding on different types of communication disorders except for stuttering [10]. A study suggested that as people get older in the society, there lies a deterioration in their physical and psychological abilities and hence the need for treatment of disabilities ascends [11]. This study shows that the need for management for the geriatric population is increasing where ASLPs play

a dynamic role. A study done by Sreeraj et al (2013) on rural population reveals 6.07% of prevalence of communication disorders in India. In the conducted survey there lies a need in the awareness of the profession of ASLP among the participants (doctors). A study done on the rural population reveals 6.07% of prevalence of communication disorders in India [12]. In the conducted survey there lies a need in the awareness of the profession of ASLP among the Medical Professionals. Therefore the need for rehabilitation in communication disorders, a field of expertise for ASLPs are to be intervened [1]. Surgery is a need for the survival of the patient. To improve the quality of life the Audiologists and the Speech Language Pathologists play a major role. In case of tracheostomy, rehabilitation is needed which guides the patient to communicate [13]. If rehabilitation is not carried out which improves the quality of life the patient can end up having mental weakness and their willingness to live can be reduced. In cases of CI surgery, the subject has to undergo therapy as it stabilizes the hearing level of the subject [14]. The questionnaire included questions on increasing the awareness of Audiology and Speech Language Pathology and the need of these professionals in a hospital setting. The results revealed that there were more than 50% of positive responses from the participants.

The questions were focused on the team involved, prescribing of hearing aids, confirmation of Speech and Language delay, hearing screening in new born, patients diagnosed to have stroke, swallowing difficulties, followed by the candidacy of Cochlear Implants (CI). The result of the study reveals that there is a minimal awareness of this field among Doctors. The comparison of the two groups portrayed significant difference in the role of SLP on voice therapy as there was greater awareness in group A when compared to group B.

The major limitation of this study is the data collected only from a few hospitals. Another challenge is the time factor. The data collected was only from some parts of India. This study can be done on a larger population posted in the Medical field to increase the awareness and knowledge of this course.

Conclusion

The awareness of Audiologists and Speech-Language Pathologists, need to escalate among medical professionals as they serve a wide range of services in assessment and rehabilitation in the field of communication disorders and hearing impairment. Future studies regarding the awareness of this field can be carried out among the public for the knowledge of the availability of this profession [10].

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Barodontalgia:- Revealing the Unreveaing

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Abstract

Barodontalgia is a pain due to pressure difference. Here in this review article, pathophysiology, manifestation, associated conditions and treatment guidelines of this rare symptom have been discussed. It is often a flare up of preexisting sub clinical oral diseases. Deep sea divers, air crew members especially of fighter jets often suffer from these due to frequent altitude difference and with it the pressure difference. This often pose a great challenge to dental surgeons who sometimes fail to found the exact ill tooth of problem so management becomes problematic.

Keywords : *barodontalgia, diving, toothache*

Introduction

"BARO" means pressure

"DONTALGIA" is Dental pain.

By the name itself it is suggestive of dental pain due to pressure. This barodontalgia was first called as 'aerodontalgia' when in World War II, Tooth pain was experienced by air crew during flight. But later this toothache was also observed in scuba divers, so a more appropiate term BARODONTALGIA being given which abides by the Boyle's Law that correctively state that a given temperature the volume of gas is inversely proportional to ambient pressure[1]. barodontalgia also defined as an oral pain caused by change in barometric pressure in an otherwise asymptomatic organ [2]. In addition to changes in ambient pressure, further physiological and metabolic changes in human body occurs.

CLASSIFICATION ACCORDING TO Ferjentsik and Aker [3]

CLASS CAUSE SYMPTOMS

Class-I Irreversible pulpitis Sharp pain on onset

Class-II Reversible pulpitis Dull pain on ascent

Class-III Necrotic pulp Dull pain on descent

Class-IV Periapical Pathology Severe persistent pain on ascent or

Descent

PATHOPHYSIOLOGY

Three hypothesis was proposed by Kollman [4]

A) Expansion of trapped air bubbles under a root filling or against dentin that activated nociceptors

B) Stimulation of nociceptors in maxillary sinus with the pain referred to teeth

C) Stimulation of nerve endings in a chronically inflamed pulp.

Among these last two were strongly supported with histological evidences.

BARODONTALGIA IN DIFFERENT SCENARIOS

1) IN PILOTS

Barodontalgia has been reported to occur during flying in range of 5000-35000 feet but more commonly reported between 9000-27000 feet[5]. Barodontalgia mainly occurs when the pulp chamber of tooth fails to adjust its internal pressure with decreasing outer pressure in high altitudes.

2) IN DIVERS

Pain due to pressure difference occur more in maxillary teeth than mandibular teeth [6] and majority occurs while descending [7]. since, SCUBA I.e Self Contained Underwater Breathing Apparatus diving is one of the popular sports. Dentists should be aware of dental related problem arising from it. The pain mainly occurs when the diver descends and pain experienced according to divers depth. As he descends air is forced into the pulp due to increased pressure of inspired air. Compressed air reaches dentinal tubules through primary, secondary, or recurrent caries along the margins of restorations or leaking restorations[8]

BAROTRAUMA OF ASCENT :- Caused by expansion of the compressed air within pulp chamber as diver ascends. This kind of injury generally seen in tooth with incomplete RCT or faulty restorations[9]. As the divers depth diminishes, expansion of trapped air leads to severe pain and sometimes may lead to fracture[10]. In severe cases the pressure built up inside the tooth chamber causes explosion of tooth called odontecrexis.

Diagnosis

Barodontalgia is rather a symptom than a pathological condition itself. Oral pathologies considered associated with Barodontalgia are- dental Caries, Defective tooth restorations, pulpitis, pulp necrosis, periodontal pockets, impacted teeth [11,12]. But till date studies prove often difficulties are faced by dental surgeons to obtain a definitive diagnosisof the causative factor[13,3],due to the fact there is need to identify the offending tooth which could be any teeth having above conditions. Moreover dentists cannot reproduce the pressure difference to identify the required tooth with ordinary dental facilities[14]. So history regarding recent dental treatments, symptoms like swelling, sensitivity to cold, onset & nature of pain, can sometimes guide the practitioner to the offending tooth.

Differential Diagnosis :

Facial Barotraumas can be of following four types

A) Barotitis media (middle ear barotrauma) : Traumatic inflammation of middle ear due to pressure difference between air in tympanic cavity and that of surrounding atmosphere.

B) External otitic barotrauma : Injury to lining mucosa due to air tight space between an object in outer ear canal and eardrum.

C) sinuses due to pressure difference.

D) Dental Barotrauma

So, Referred pain from these above barotraumas can manifest as toothache.

In these above conditions Barodontalgia is not a symptom of a preexisting disease but a pressure induced pathologic conditions.

Prevention & Treatment Plan :

Barodontalgia is rare. Most dentists are not aware of it, yet it can become a risk factor to divers, submariners, pilots and airline passengers. So FDI (Federation Dentaire Internationale) recommended annual check up for divers, submariners and pilots with oral hygiene instruction from dentist [15]. So, while examining patients attached with these kind of jobs attention should be given in the presence of areas of dentin exposure, caries, fractured cusps, defective fitting, periapical pathology if any. Patients coming with complaints of pain if suspected barodontalgia, thorough examination including the age of restoration, screening for caries and quality of restoration should be ensured and also history regarding his recent activity that could involve pressure difference should be checked.

Treatment [16]

1) Placement of Zinc Oxide Eugenol base found to be beneficial in reversible pulpitis cases.

2) Generally in suspected barodontalgia cases pulp capping is not recommended instead endodontic treatment is indicated.

3) Recent Studies have been shown variation in pressure changes affect the retention of crown cemented with zinc phosphate and Glass Ionomer Cement. So dental surgeons should consider cementing fixed prosthesis with resin cements for patients who are exposed to marked variation in environmental pressure.

4) Reports presented of shattering porcelain crown or explosion of temporary sealing in Root Canal Treated

teeth in diving and submariners. So, dentists should keep in mind while treating these patients.

5) Sometimes if dentists fail to complete the conservative treatment before deep sea diving or flight then extraction should be the treatment of choice.

6) Removable dentures not recommended in these patients.

Discussions

The article reviewed the fact about barodontalgia. Though this is a condition which is being neglected greatly and many are unaware of the facts and its severe consequences. Dentists should be more known to the ill effects and diagnosis of barodontalgia and pay more importance in the profession of the patients and to their state of living. Dentists should implement the discussed treatment protocol in treating patients risk of getting barodontalgia.

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Osseo Densification - A Comprehensive Review on Novel Approach

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Abstract

Success of a dental implant depends on osseointegration which is the direct structural and functional connection between the dental implant and the alveolar bone. Successful osseointegration requires both primary stability (mechanical stability) between the implant and the bone and secondary stability (biological stability) which occurs during bone remodelling during healing.

Keywords- *osseodensification, osteotomy, bone density.*

Introduction

Traditional osteotomy is a subtractive technique in which the drills have a positive rake angle of 25 to 35 degrees to drill away small amount of bone with each sequential drilling which might result in few drawbacks like compromised stability and heat generated bone necrosis whereas a unique method called osseodensification is a non excavating method in which bone is not removed but is densified with each drill thereby enhancing primary stability by establishing immediate contact between the implant and the alveolar bone .

Various factors that contribute to the stability are

i. Implant design (thread type, geometry and surface coating)[1]

ii. Instrumentation (sequence of surgery, speed and technique)

iii. Bone density[2]

However factors like drill protocol sequence, velocity and design may aid in accelerated osseointegration .

OD is carried out with unique burs with tapered flutes with a negative rake angle (non cutting edge) in counter clockwise direction at a speed of 800 to 1200 rpm / minute.Asthe osteotomy expands bone is densified[3,4,5] as a layer of dense and compact bone that surrounds the osteotomy wall is created which would increase the primary stability and bone mineral density.copious amount of irrigation is required to avoid overheating.

Materials and Methods

A database search was carried out in PUBMED using keywords like osseodensification ,OD, implant stability and osteotomy for articles upto the year 2018 the search was scrutinized for full text articles . clinical trials, in vivo studies, review articles were selected for this review.

OSSEODENSIFICATION AND BONE DENSITY

Stability of any dental implant depends on the bone mineral density.

According to trisi et al there is a significant correlation between the bone density in the peri implant region, insertion torque and micromotion[6].

Bone evaluation around the dental implants can be performed and visualized either in ground sections of methylmethacrylate materials or light microscopic examination of peri implant tissue at the cellular level.

In an animal experimental study carried out by Burri and wolter in 1977 it has been proved that the new bone formation occurs in the peripheral osteotomy site and also the amount of new bone formed was shown to be proportional to the degree of compression.

D3 and D4 type of bone has poor bone density making it difficult to achieve implant stability. Using osseodensification procedure a layer of increased bone mineral density has been shown by imaging around the periphery of osteotomies which has shown a good effect on the implant stability.

CONVENTIONAL DRILLING VS OSSEODENSIFICATION

Conventional drilling with sequential drills facilitates implant placement by bone excavating thereby compromising the primary implant stability which inturn results in reduction of torque during insertion. some surgical techniques like undersizing the osteotomic implant site have been proposed to reduce bone sacrifice during implant placement procedure and to enhance primary implant stability and bone quality. An alternate to this procedure is the Osseodensification which has shown to increase required penetration force and torque.

Moreover during osteotomy a zone of osteocyte death is produced by conventional drilling[7,8,9] ; despite the use of copious irrigation a peak temperature of ~80 °C was generated at the cut edge whereas with OD even without copious amount of irrigation the peak temperature remained within normal range well below the temperature known to cause osteocyte death (45°C) [10].

OD retains osseous coagulum (trabecular bone chips, blood, and stroma) which have inherited osteogenic potential[11,12]. This coagulum serves as a nucleating surfaces to promote new bone formation around the implants and providing greater bone density and better stability.

Disscusion

Preservation of cell viability is the most important factor [13,14,15]for the success of any treatment. conventional drilling might compromise the viability by creating mechanical and thermal trauma[16,17]. A viable osteotomy site is very critical for enhanced osteogenesis.

OD has shown to increase the % of bone at the implant surface by increasing the Bone mineral density in the peri implant region which inturn is directly linked with increased bone-implant contact ,improved bone healing and increase in primary stability .

OD is a slow incremental process carefully controlled by surgeon. The residual strain on the bone surface creates compressive forces that increases the implant stability.increased removal torque is a good indicator for primary implant stability.

Conclusion

Osseodensification is a promising alternative to conventional drills as it helps in maintaining ridge integrity with preservation of bone. This osseous coagulum shows excellent osteogenic potential that brings about robust formation of new bone in the osteotomy site and allows for the placement of dental implants with superior stability. It also helps to improve bone density and also BV and increased bone-to-implant contact, thereby improving implant stability. However more clinical studies are recommended in future to derive any concreate conclusions.

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Knowledge and Attitude of Pediatricians with Regard to Emergency Management of Dental Avulsion- A Survey

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Abstract

Dental trauma is a very significant problem encountered in both primary and the permanent dentition.In preschool children the trauma rate is as high as 18% of all injuries. Among all facial injuries, dental injuries are the most common of which avulsions occur in 1–16% of all dental injuries. Among dental traumatic injuries, avulsion results in major functional and esthetic disturbances for patients. Epidemiological studies revealed that children from 8 to 12 years often suffer a dental injury.The teeth most commonly avulsed in both the primary and the permanent dentition are the maxillary central and lateral incisors. The longer the avulsed tooth is out of its socket, the less likely it will remain in a healthy functional state after replantation. The prognosis of replanted permanent teeth depends primarily on (i) prompt and appropriate first care, commonly provided by non-health professionals at the site of the accident, (ii) the storage media of the avulsed tooth prior to replantation, and (iii) the viability of periodontal ligament remnants on the root surface. Research has shown that immediate replantation is the best and most appropriate treatment for the traumatically avulsed permanent teeth.

The aim of this study is to assess the basic knowledge of dental trauma and its management among pediatricians

Key words: *Avulsion, paediatrician awareness.*

Introduction

Dental trauma is a very significant problem encountered in both primary and the permanent dentition.In preschool children the trauma rate is as high as 18% of all injuries. Among all facial injuries, dental injuries are the most common of which avulsions occur in 1–16% of all dental injuries.[1,2]Among dental traumatic injuries, avulsion results in major functional and esthetic disturbances for patients.[3]Epidemiological studies revealed that children from 8 to12 years often suffer a dental injury.[4] The teeth most commonly avulsed inboth the primary and the permanent dentition are the maxillarycentral and lateral incisors.[5]The longer the avulsed tooth is out of its socket, theless likely it will remain in a healthy functional stateafter replantation. The prognosis of replanted permanent teeth depends primarily on (i) prompt and appropriate first care, commonly provided by non-health professionals at the site of the accident, (ii) the storage media of the avulsed tooth prior to replantation, and (iii) the viability of periodontal ligament remnants on the root surface. Research has shown that immediate replantation is the best and most appropriate treatment for the traumatically avulsed permanent teeth.[6-8]

The aim of this study is to assess the basic knowledge of dental trauma and its management among pediatricians. The management of associated injuries which needs speciality treatment by the pediatricians. To create an awareness about Pediatric dentistry- on the

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whole to the pediatricians and to promote awareness to the general public-through pediatricians.

MATERIALS AND METHODS:

A 9-item questionnaire was designed to assess currentknowledge and attitude of pediatricians with regard to emergency management of dental avulsion.Doctors were personally interviewedand were assured of confidentiality. The study groupwas convenience sample of physicians working in government and private hospitals.Survey was conducted over a period of 2 weeks. The number of pediatricians surveyed are 56 out of which 15 are having less than 10 years experience and 41 are having more than 10 years experience.24 of them are government doctors and 32 are private practitioners.

Method of Collection of Data:

Knowledge and Attitude of Pediatricians with Regard to Emergency Management of Dental Avulsion- a survey

(Dental avulsion of teeth is when a tooth comes out of its socket as a result of a traumatic injury.)

General information:

Doctor's Name:

Number of years of practice:

1. Have you experienced a dental trauma case in your practice? Yes/No

2. If yes, what kind of dental injury? [ ] Avulsion [ ] Crown fracture [ ] Bone fracture

3. If the tooth was out of the child's mouth, what would you do?
[ ] save the avulsed tooth [ ] not save the avulsed tooth

4. Whom you will refer? [ ] Dental specialist [ ] General surgeon

5. You would seek professional treatment [ ] immediately [ ] the next day [ ] within a week

6. Do you know that it is possible to replant an avulsed tooth? Yes/No

7. Before the tooth is re-implanted, how would you clean an avulsed tooth?
[ ] Brush [ ] Water [ ] Salt water [ ] Milk [ ] Nothing

8. How would you transport it to the dentist?
[ ] keep it wrapped in paper or gauze [ ] keep it on ice [ ] keep it in water
[ ] keep it in milk [ ] keep it in saline solution

9. Have you undergone any training regarding management of dental trauma? Yes/No

Doctor's Signature

Results

Fifty six paediatricians who were approached readily agreed to participate and returned the completed filled questionnaires on the same day.

For question 1: while 52% (n = 29) of the participants had come across children with dental trauma, 48% (n = 27) had not experienced a dental trauma. (fig.1)

For question 2:

Among the dental injury encountered by the paediatricians, 14% (n=8) was avulsion , 30% (n= 17) was crown fracture , 4% (n=2) was bone fracture, 9% (n=5) was more than one kind and 42% (n=24) showed no response to the question. (fig.2)

For question 3: 93% (n = 52) of the participants would like to seek a dentist's opinion for management of dental trauma. While 4% (n = 2) seek general surgeon opinion and 3% (n=2) did not any answer for the question. (fig.3)

For question 4: If the tooth was out of the child's mouth 34% (n=19) advise not to save the tooth, 59% (n=33) advise to save the avulsed tooth and 7% (n=4) did not answer the question. (fig.4)

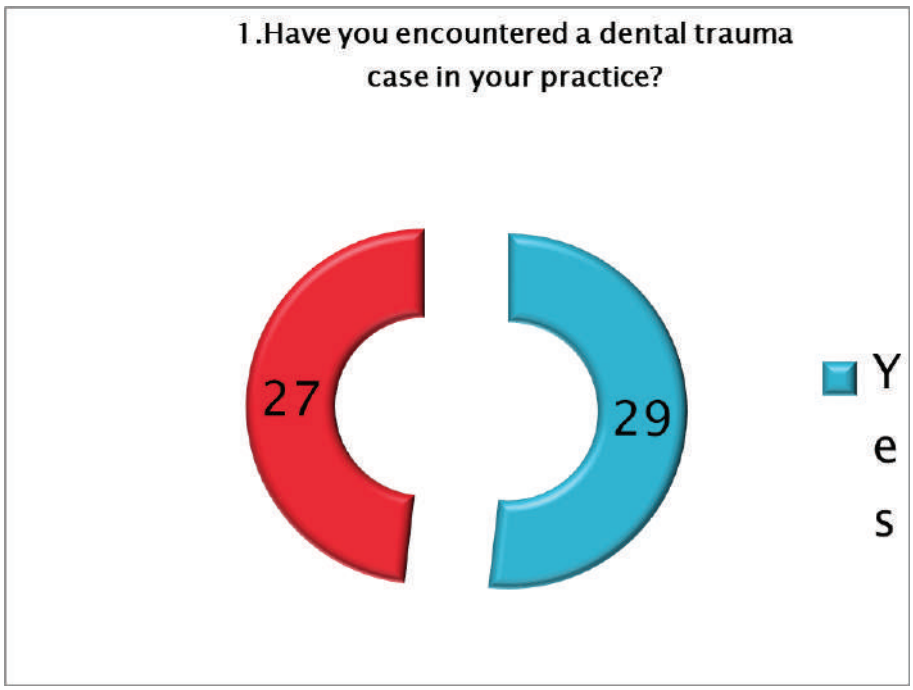
For question 5: 59% (n=33) seek professional treatment immediately , 27% (n= 15) seek professional treatment after a day and 9% (n=5) seek dental treatment within a week. 5% (n=3) did not show any response. (fig.5)

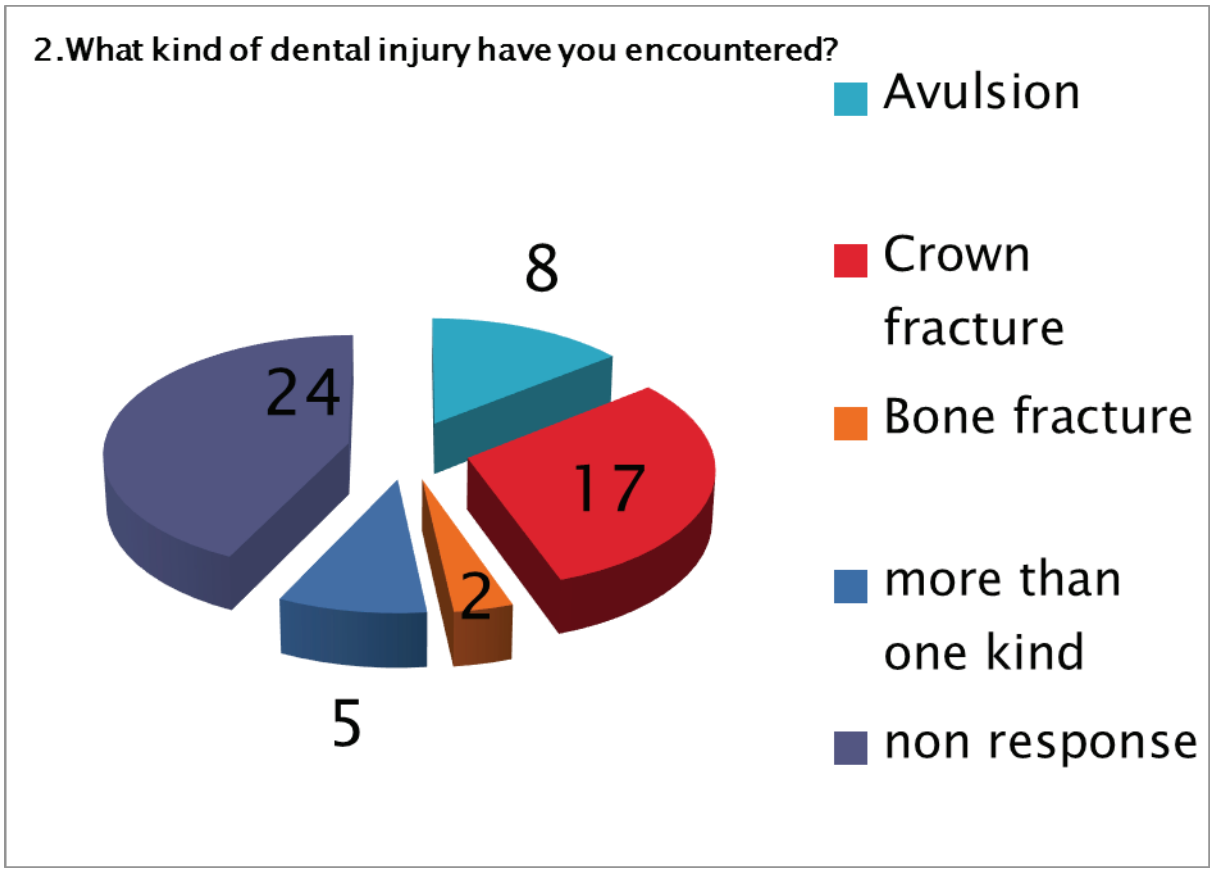
For question 6: 32% (n = 18) of the participants answered replantation of avulsed tooth is possible, while 64%(n = 36) answered replantation was not possible. (fig.6)

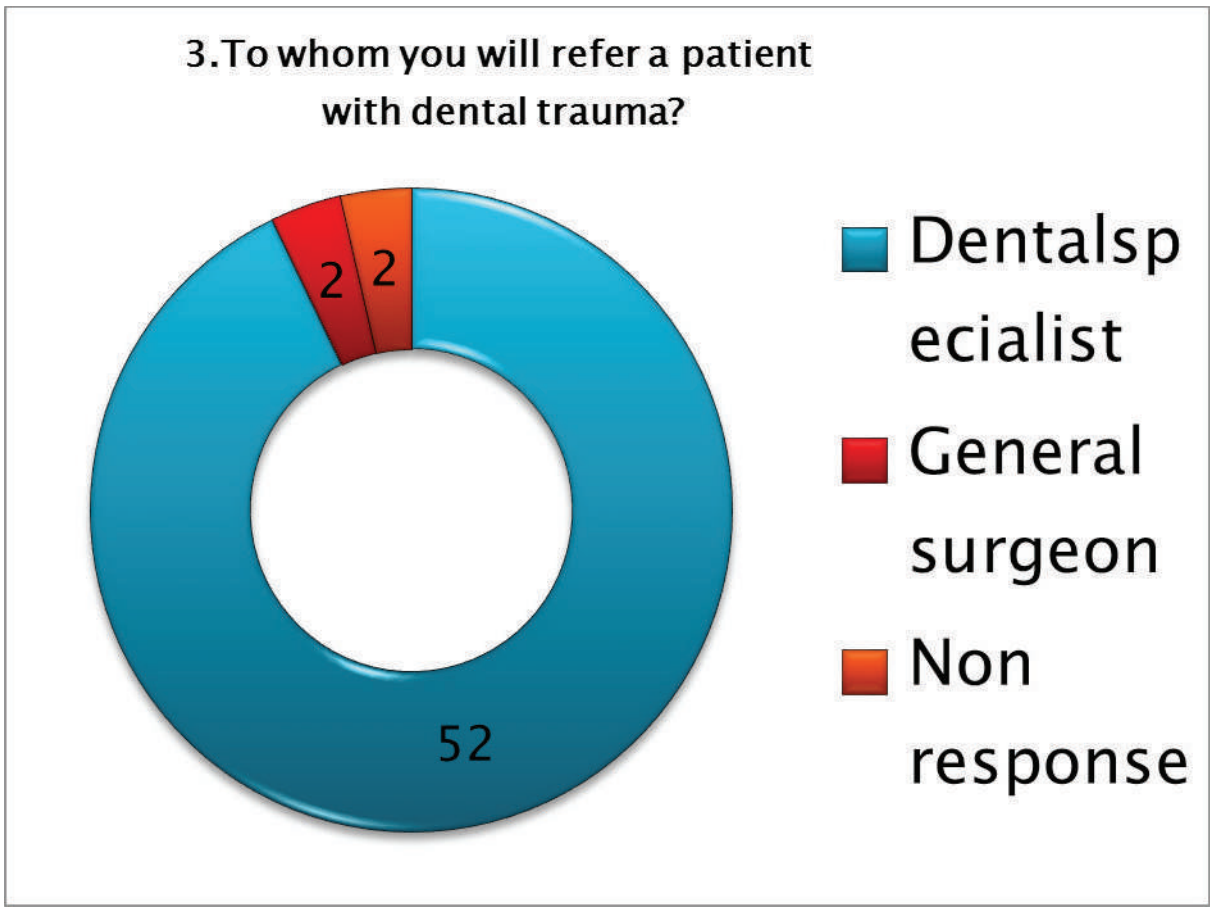
For question 7: 30% (n = 17) of the participants use water to clean the avulsed tooth, while 23% (n = 13) use salt water, 14% (n=8) use brush and 7% (n=4) use milk to clean. 25% (n=14) did not answered the question.

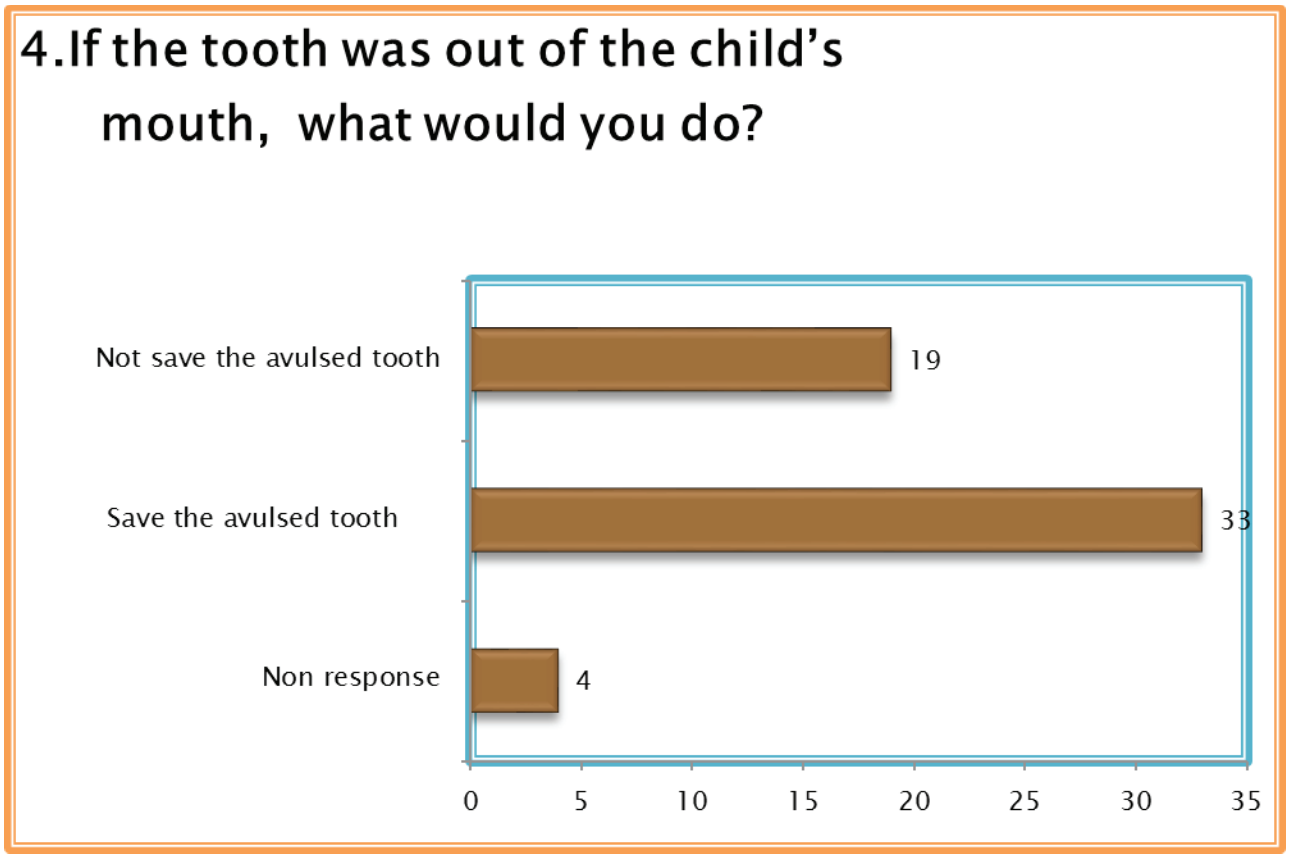
For question 8: 7% (n=4) wrap the avulsed tooth in paper or gauze to transport it to the dentist, 18% (n=10) keep it on ice, while 13% (n=7) use water as transport media, 7% (n=4) use milk and 29% (n=16) of the paediatricians use saline as transport media.

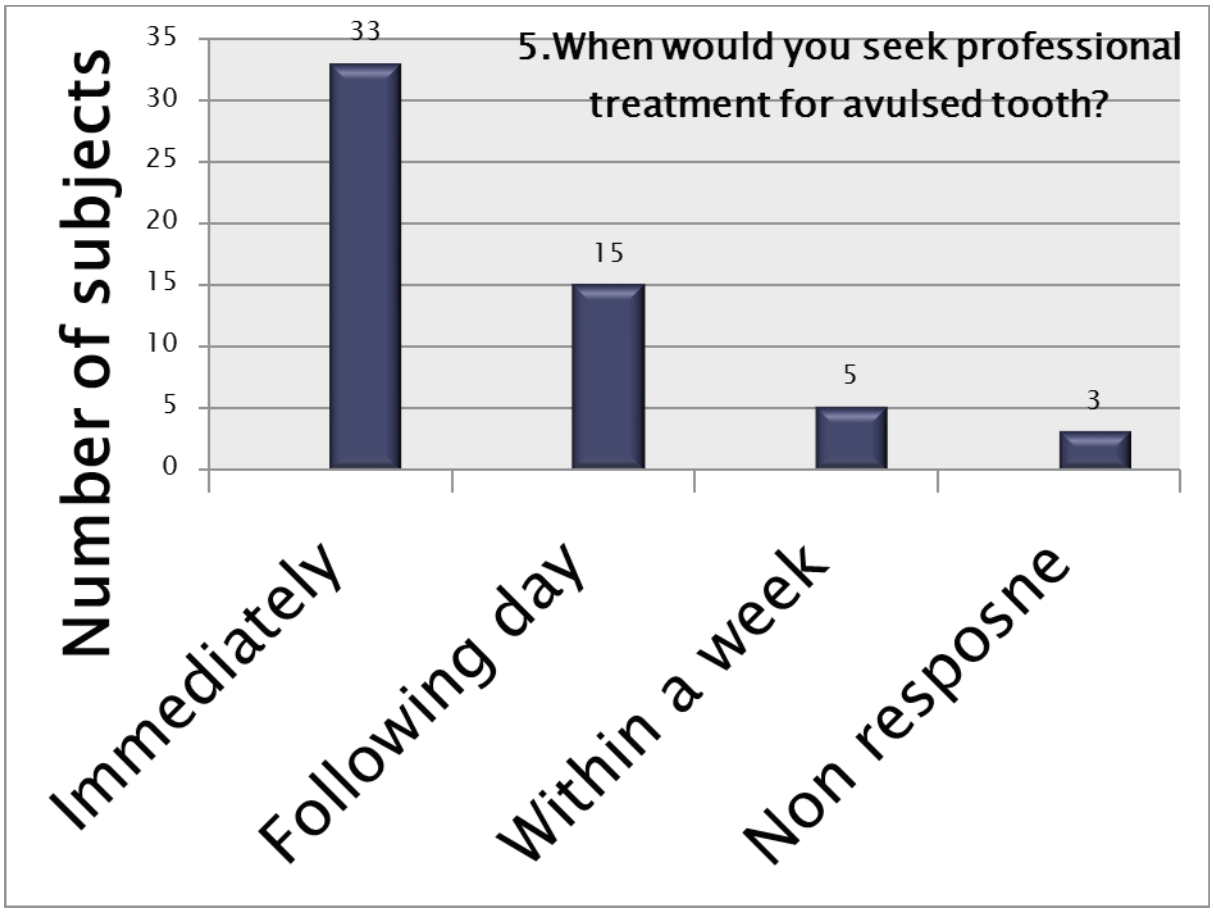
For questions 9: 95% (n=53) of the paediatricians have not undergone any dental trauma management programme and 5% (n=3) have attended.

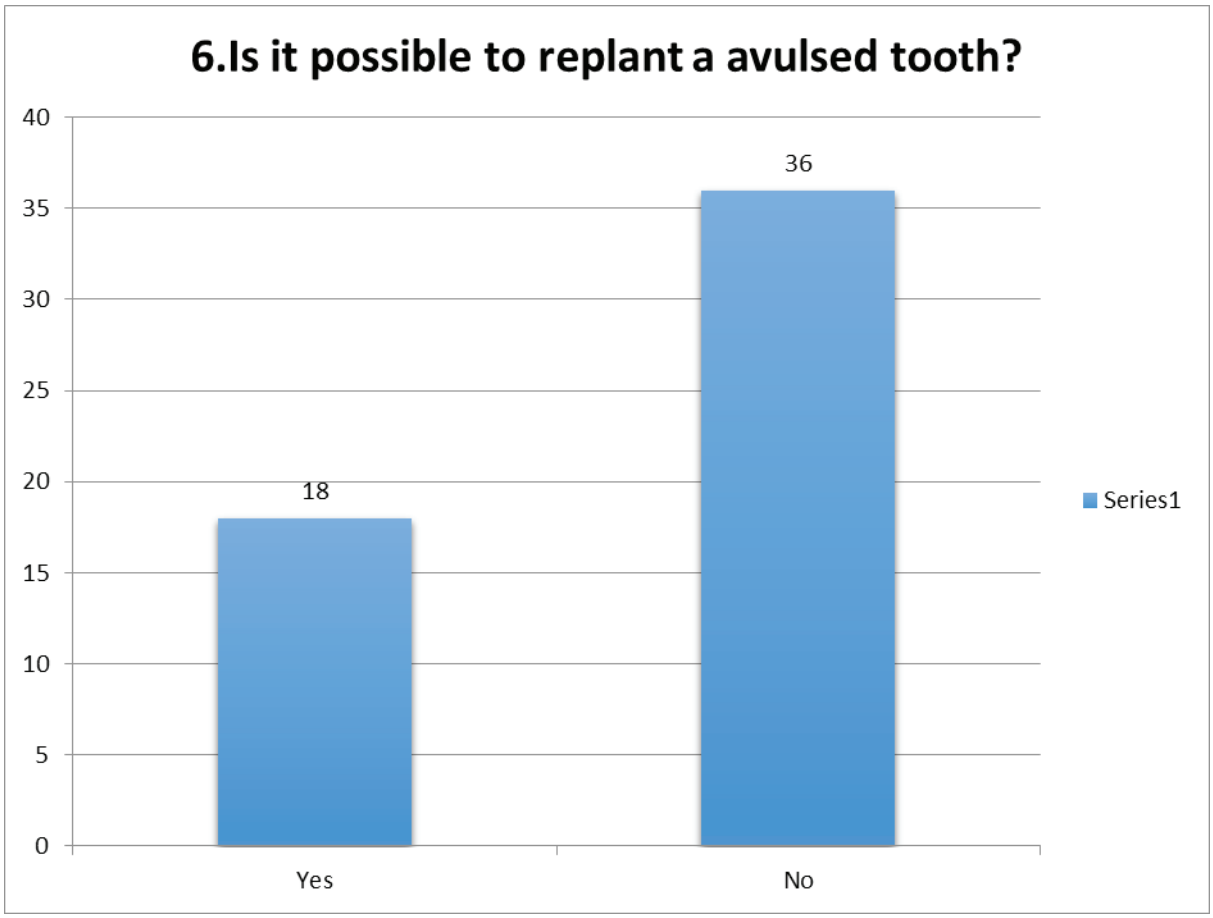



2.What kind of dental injury have you encountered?
8
17
2
5
24
Avulsion
Crown fracture
Bone fracture
more than one kind
non response


3.To whom you will refer a patient with dental trauma?
2
2
52
Dentalspecialist
General surgeon
Non response


4.If the tooth was out of the child's mouth, what would you do?
Not save the avulsed tooth
19
Save the avulsed tooth
33
Non response
4
0
5
10
15
20
25
30
35


5.When would you seek professional treatment for avulsed tooth?
Number of subjects
35
30
25
20
15
10
5
0
33
15
5
3
Immediately
Following day
Within a week
Non resposne



Discussion

The permanent anterior teeth play an important role in good psychological development of child and adolescent. It was noticed that child often exhibits lower self esteem when the aesthetic harmony is insulted by dental avulsion.[9,10]

Among the 56 pediatricians surveyed 52% of them had come across the dental trauma cases. Among the dental injury encountered by the paediatricians, 14% was avulsion, 30% was crown fracture

93% of the participants would like to seek a dentist's opinion for management of dental trauma. While 4% seek general surgeon opinion. If the tooth was out of the child's mouth 34% advise not to save the tooth, 59% advise to save the avulsed tooth.

59% seek professional treatment immediately, 27% seek professional treatment after a day and 9% seek dental treatment within a week. This shows the awareness of the paediatrician about the avulsed tooth and how long the periodontal ligament is viable.64% of the paediatricians thought reimplantation of avulsed tooth is not possible at all.

Out of the participants 30% use water to clean the avulsed tooth, while 23% use salt water, 14% use brush and 7% use milk to clean. 25% did not answered the question as they did not know about the avulsion management. To transfer the avulsed tooth 7% of the participants wrap the avulsed tooth in paper or gauze to transport it to the dentist, 18% keep it on ice, while 13% use water as transport media, 7% use milk and 29% of the paediatricians use saline as transport media.

95% of the paediatricians have not undergone any dental trauma management programme and 5% have attended.

For paediatricians to address competently child oral health issues, they must have adequate knowledge of the disease process, risk factors, signs, symptoms, prevention, and intervention strategies. The knowledge base required of pediatricians to perform successfully

oral health risk assessments can and should be built and reinforced throughout the medical education process. Pediatricians need more knowledge and skill in addressing children's oral health because of the prevalence of dental disease, the racial and socioeconomic disparities in disease burden, and the limited access to dental care for many children.[11]

Conclusion

Many improvements can be made throughout the medical education process to provide paediatricians with the knowledge and skills to provide families competently with oral health guidance, prevention, and possible therapeutic treatments.

Ethical Clearance – Institutional Ethics Committee, Sree Balaji Dental College and Hospital, Committee registered with DCGI. (Registration no: ECR/761/Inst/TN/2015)

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Demineralization – An Overview of the Mechanism and Causative Agents

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Abstract

Various tissues in the body like bone, enamel dentin and cementum are composed of inorganic material and they are exposed to multiple cycles of Demineralisation and remineralisation. Understanding these biological mechanisms are necessary for developing treatments for these mineralisation related diseases and specifically dental caries as far as dentistry is concerned. This article focuses on the mechanism and rate of demineralisation and discusses in detail the causes of demineralisation.

Keywords: *Demineralization, dental caries, pH, remineralization.*

Introduction

Enamel, dentin, and cementum are made of inorganic crystals embedded within their organic matrices. [1] Demineralisation is the process of removing mineral ions from the dental hard tissues resulting in the loss of structural integrity. Demineralisation refers to dissolution of enamel and is usually the first step to the formation of dental caries. However, cycles of demineralisation are alternated by cycles of remineralisation where protective factors promote remineralisation and pathological factors shift the equilibrium towards cavitation. Various factors like pH, salivary flow, mineral content, degree of dissociation, microbial flora and diet affect the demineralisation-remineralisation balance.[2]

Mechanism and Rate of Demineralisation:

Dentin forms the bulk portion of teeth with 70% inorganic substance, 20% organic substance and 10% water. The inorganic matrix is mainly hydroxyapatite crystals that are embedded within the organic material. There is a hypothesis that states organic matrix is made of type I collagen that acts a protective sheath over the crystals.[2]

A unit mass of solid hydroxyapatite dissolves five calcium ions, three trivalent phosphate ions and one hydroxyl ion that are released into solution.

$$Ca_5 (PO_4)_3 OH \text{ « } 5 Ca^{2+} + 3PO_4^{3-} + OH^-$$

The extent to which tooth mineral can dissolve in a solution is determined by ionic product of calcium, phosphate, and hydroxyl ions. The product of soluble ions of a salt in a solution is called ion activity product (IAP)

$$(IAP)_{HA} = (Ca^{2+})^5 X (PO_4^{3-})^3 X \text{ OH -}$$

IAP = (KSP) HA solubility product of hydroxyapatite = constant — 7.41 x 10 mol 9/19 - saturation — equilibrium.

IAP > KSP (the solution is supersaturated with tooth mineral ions) induce formation of HA crystal growth.

IAP<KSP (the solution is under-saturated)- dissolves HA (hydroxyapatite) demineralization. At neutral pH, saliva and plaque fluid are supersaturated with HA, prevents enamel from dissolution, causes precipitation of calcium phosphate brushite CaHPO,2H,0, not HA.[3]

Formation of pellicle on the enamel surface inhibits precipitation of calcium phosphate from supersaturated saliva. The micro radiographic analysis showed that after 4 to 8 weeks of in situ demineralisation, the lesion depth values were 33±12 and 63±17 μu, respectively. It was concluded that the lesion depth as well as the mineral

loss parameter both vary linearly with demineralisation time. [4] According to a study done in 2019 by Meng Tsan Tsai, using OCT (Optic coherence topography) to characterize the acid effect on enamel, it was noted that the surface roughness and scattering coefficient increased significantly after acid exposure. [5]

N Sabel et al in 2012, stated that the rate of demineralisation varies considerably with the chemical composition of the enamel. Higher the carbon content, the demineralised lesions were deeper. Also, depth of the lesion increased if the degree of porosity in the enamel was higher. [6] Oki Hayah et al. in 2016, compared the demineralisation depths of teeth in two different age groups (20s and 60s). It was concluded that demineralization depth increased with time and there were no significant differences in demineralization depth between the two age groups. [7]

Effect of Ph On Demineralization:

According to the law of saturation, a dynamic equilibrium exists between oral fluids and minerals. pH is one of the most important factor that determines the extent of enamel demineralisation and demineralisation. Critical pH is the point at which the solution becomes 'just' saturated with the minerals or the pH at which saliva is saturated with HA.[8]

The solubility of HA is affected by the pH of water. At alkaline pH 14, phosphates are in the ionic form PO_4^{3-}. As the pH is lowered PO_4 & OH accumulate in solution, together with calcium ions and dissolution of HA slows as the solution becomes saturated. In acidic pH, PO_4^3 (phosphate) & OH ions combine with H^+ to form HPO_4^{2-} (hydrogen phosphate) and H_2O respectively removing a portion of phosphate and hydroxyl ions from the solution. As this transformation of ionic phosphate takes place, saliva ceases to be saturated with HA, solubility of calcium phosphate increases as the pH decreases. [9] Alkaline pH is 7.2- 12.3 and acidic pH ranges from 2.1-7.2 and the critical Ph for HA= 5.3-5.5. Below the critical pH enamel demineralises and above critical pH enamel remineralises. Post radiation the pH lowers from 7 to 5 which is cariogenic. As a result, the buffer is not maintained resulting in the dissolution of enamel minerals. [10]

Fluorapatite crystals and enamel demineralisation:

Enamel is a crystalline structure consisting of hydroxyapatite crystals and when the fluoride ions substitute the HA, it becomes fluorapatite or hydroxy fluorapatite. The critical pH for fluorapatite is 4.5. Therefore, the enamel has better stability and hardness in low pH when HA crystals are replaced by fluoride ions.[11,12] Fracture toughness, modulus of elasticity and hardness is better with fluorapatite when compared with hydroxy fluorapatite crystals of different proportions of fluoride and hydroxyl ions [13] The decrease in solubility and superior biological properties was noted in samples with increase fluoride content when ion release was compared between samples containing different ratios of HA, fluorapatite and hydroxy fluorapatite ions to pure water. [14]

Causes of Demineralisation:

Saliva:

Saliva is composed of various proteins like statherin, albumin, histatins, cysteines and various ions like calcium, phosphates and fluorine ions. The composition, pH, antibacterial activity, the quantity and viscosity of saliva flow has profound effect on the rate of demineralization. [15] Albumin accounts for more than 50% of all serum proteins, present as serum ultrafiltrate, it penetrates through the enamel pores and forms a protective barrier against enamel demineralisation. [16] Mithra Hegde et al., in 2017, concluded that calcium loss was less in enamel samples coated with albumin in comparison to samples that were not coated with albumin demonstrating that albumin is a strong protective barrier against enamel demineralization. Salivary proteins' ionic concentration is mainly made up of calcium and phosphates which determines the rate of mineral loss. Salivary flow decreases in Sjogren's syndrome, sarcoidosis, radiation therapy and diabetes. The viscosity increases and the quantity decreases which considerably alters the salivary buffering capacity and the oral microflora resulting in increased susceptibility to caries. [17]

Dental Plaque:

Dental plaque is defined as a gelatinous mass of

bacterial biofilm adhering to the tooth surface. When the specific hypothesis failed as it was impossible to eliminate/ isolate the specific plaque bacteria that was pathogenic, a new concept called the 'ecological plaque hypothesis' was proposed. According to which, an environmental change can provide pathogenicity to the residing microbiota at specific sites and hence disease is caused only at certain sites.The plaque microorganisms are usually Streptococcus, lactobacillus and actinomycetes which metabolize the refined carbohydrates producing acids as the by-product. This lowers the pH at the plaque enamel interface. When pH is below the critical point, it results in enamel demineralisation. When oral hygiene is poor, the thick plaque prevents salivary buffering action on enamel resulting in demineralisation.[18]According to Zhang Y et al., 2000, it was concluded that when enamel was exposed to lactic acid with similar degree of saturation of plaque fluid, demineralisation and mineral loss increased significantly as the degree of saturation dropped. [19]

Role of diet in enamel demineralisation:

During an acidic attack there is dissolution of inorganic matter and the destruction of the organic matrix. Teeth is exposed to acids during bacterial fermentation.Streptococcus species and lactobacillus are aciduric being the main culprits. Organic acids like lactic acid produced during the process of fermentation considerably lowers the pH resulting in demineralisation while producing indigestible extra cellular dextran from dietary sugars which promotes bacterial colonisation. The frequency of consuming free sugars should be limited to less than 4 times a day. Dietary soda includes citric acid and phosphoric acid that causes demineralisation resulting in caries or enamel erosion. Also, fibrous diet rich in calcium and phosphorous causes remineralisation by preventing the pH falling below 5.5. [20]

T R Razvan et al., in 2016 evaluated the enamel demineralization depth by five sweeteners. Currently, five sweeteners have been approved by Federal Drug Administration which are Aspartame, Saccharine, acesulfame, sucralose and neotame. The most investigated artificial sweetener is sucralose, a non-caloric sweetener. Recent studies have demonstrated that manuka honey with a high antimicrobial activity is said to be non-cariogenic. The depth of demineralisation on consumption of various sugars was as follows : Palm sugar (196.07µm), Sucralose (145.47µm), Glucose (235.72µm),Sucrose (287.78µm) and honey (160.01µm) Therefore, the lowest depth of demineralization was observed in sucralose followed by honey.[21]The highest depth was observed in sucrose. Oral hygiene maintenance combined with consumption of the right diet is important in prevention of dental caries.

Acid etching:

Acid etching technique is defined as the process of roughening a solid surface by exposing it to an acid and thoroughly rinsing the residue to promote micromechanical bonding of an adhesive to the surface in other words dissolution of HA crystals to create micro porosities for bonding. Enamel topography changes from a low reactive surface to a surface favouring adhesion and bonding. Generally, total etch technique involves the use of phosphoric acid (37 %) with an etching time of 15 sec, rinsing time of 30 secs to achieve the most receptive enamel surface for bonding. Demineralisation due to acid etching can be selective as the morphology of prisms varies from region to region. It can either be in the prism head or at the periphery which are type I and type II etching patterns, respectively. Etching can remove up to 10 micrometres of enamel. A properly etched surface gives a frosty white appearance on drying. Micro tags are the finer particles of numerous small tags (resin tags) formed across the end of each rod where the individual hydroxyapatite crystals are dissolved. Demineralization paves the way for the formation of resin tags and the hybrid layer, forming the fundamental basis of adhesion to dentin.

IAP<KSP (the solution is undersaturated)- dissolves HA demineralization. This is of paramount importance for a favourable clinical performance of composite resin restorations' adequate adhesion.[22]However, when acid etching is prolonged, it can have dramatic effects of the properties of enamel. In a study by Zafer et al. 2015, enamel was treated with 37% phosphoric acid at varying time duration. It was concluded that etching time can have profound effects on surface properties of enamel such as increase surface roughness and decrease surface hardness which in turn can affect the bond strength and longevity of composite restoration.[23]

Dental caries and demineralisation:

Dental caries involves a sequalae of multiple processes- complex interaction between the microbial film, tooth structure and diet. During demineralization, the HA crystals are dissolved first, exposing the collagen to dissolution. In the dental fraternity, diagnosis of dental caries means identifying the demineralised regions and restoring it with various dental cements or other appropriate treatment options. Demineralisation in its initial stages is identified with an electron microscope and after several stages becomes a white spot lesion which then progresses into cavitation. This can be detected visually and radiographically. Carbon content plays a key role in deciding the rate of demineralisation. The carbonate ion substitutes the phosphate ion produces defects in calcium deficit areas. In enamel one out of ten phosphate ions are replaced with carbon and in dentin one out of five. Therefore, the minerals loss in dentin is three times greater than enamel, due to high carbonate content. [24]

Caries of enamel has revealed four distinct zones starting from the advancing front of the lesion. First is the *translucent zone* -being the advancing front of caries. The preferential removal of inorganic material is evident, whereas organic material removal is not seen. There is approximately 1.2 % loss of minerals. Followed by the *dark zone*, where there is 6% mineral loss per unit volume of enamel. Light passing through this zone causes brown discolouration. The third zone is the *body of the lesion*. There is 24% mineral reduction per unit volume of normal enamel. Next is the *surface zone*. Partial demineralization results in 10% loss of minerals, with broadening of prism sheaths. Forms an intact surface layer with 1 — 5% pore volume. The presence of fluoride, calcium and phosphate in saliva and plaque produces remineralization hence an equilibrium exists which maintains the surface layer but with some overall loss of minerals. The cores of the enamel are rich in carbonate so greater demineralization occurs in the central portion of the crystal when the lesion progress towards the dentino-enamel junction. Affected and infected dentin are separated by a layer of demineralisation. Active lesions are differentiated from the arrested decay by the presence of active bacteria, impermeability to dyes and low calcium content. [25]

Demineralisation in non-carious lesions:

Whenever the teeth surface is covered by acidic or proteolytic corrodent and they are abraded by friction resulting in abrasion/biocorrosion. This can occur when the teeth are brushed with a dentifrice immediately after drinking acidic fluids or after regurgitating or even if the bacterial plaque were present, producing acids and proteases, which acts on the tooth surface, particularly the cervical dentin. *Attrition* is the physiological wearing away of teeth due to bruxism or aggressive cleansing and *abrasion* is the pathological wearing away due to applying excessive force or using a wrong brushing technique. Abrasion usually occurs in the cervical region. *Abfraction* is a pathological lesion seen as angular notches due to bio-mechanical loading. *Erosion* is the progressive loss of hard dental tissue by a chemical process without bacterial action. Lesions occur on the gingival third of the buccal and labial surfaces of tooth usually in labial aspect of maxillary anteriors. The dental hard tissue after extensive erosion show no signs of demineralization except that some enamel is lost due to dissolution of apatite mineral by acids of non-bacterial growth. The mineral content of the remaining enamel is unchanged. Eroded enamel appears hard and shiny. Exposure of demineralized dentin results in faster loss of material with occlusal scooping or cupping of the cusp tips initially and grooving of the incisal edges, enhanced by abrasion from foods. The lesion occurs in elderly suffering from repeated episodes of gastric reflux, leading to periodic cycles of demineralization and remineralization of the exposed root surfaces. [26,27]

Conclusion

The constant consumption of refined carbohydrates has led to an increase in prevalence of dental caries. The dynamic relationship and the delicate balance between demineralization and remineralization plays an important role in the progression or the reversal of the caries process.[28]

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Role of Silorane composites in Dentistry- an Overview of the Composition and Properties

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Abstract

Siloranes are a class of composites introduced in dentistry, introduced by Weinmann et al in 2005. The term silorane is derived from siloxane and oxirane. The combined form of these two chemical compounds forms siloranes, which are biocompatible and hydrophobic in nature. The polymerization mechanism of silorane composites is described as a cationic ring opening polymerization reaction that causes opening and expansion of the polymeric chain. This reduces the volumetric shrinkage when compared to methacrylate based composites. This article highlights the composition and properties of silorane composites.

Keywords: *Silorane, polymerization shrinkage, bonding mechanism, biocompatibility.*

Introduction

For the past 30years, 80-90% of the commercial composites contain Bis-GMA monomer as the resin matrix. Dr. Ray L. Bowen in 1962 introduced methacrylate based composites. J. L. Ferracane et al 1995, modified the methacrylate based monomers by appending UDMA, TEGDMA, UTMA, BisEMA to BisGMA matrix to enhance its mechanical properties. [1] Later, Leprince et al 2010 conducted an extensive research to improve the properties of the composites. [2] Polymerization shrinkage was one of the major drawbacks in methacrylate based composites. Due to this drawback, there was various clinical challenges like 1) reduced marginal integrity 2) microleakage 3) marginal staining 4) port- operative sensitivity 5) secondary caries 6) cuspal displacement 7) formation of cracks in a healthy tooth structure. [3] The search for composites with reduced polymerization shrinkage led to the development of siloranes.

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COMPOSITION:

Silorane system has been developed to minimize the polymerization shrinkage and polymerization shrinkage stress with enhanced bonding to the tooth structure. The silorane composite system consists of silorane resin, initiator and filler.[3]

SILORANE RESIN:

Silorane resin was introduced by Weinmann et al in 2005. The term silorane (3,4epoxycyclohexylsilorane) is derived from a combination of 2 chemical building blocks- siloxanes and oxiranes. Chemically siloxane is hydrophobic in nature and forms the backbone with one or more functional oxirane groups attached to it.[3]

INITIATOR SYSTEM:

Silorane composite consists of a 3 component initiator system. They are camphorquinone, iodonium salts, electron donors. Of these camphorquinone act as

a photo-initiator which decomposes to generate free radicals on exposure to visible light with a wavelength between 430-490 nm. Iodonium salts (SbF6) are anionic and helps in enhancing the reaction. Electron donor (ethyl dimethyl amino benzoate) helps in decomposing the iodonium salts to an acidic cation thus initiating the polymerization reaction.[3]

FILLER COMPONENT:

The silorane composite consists of fine particle filler: quartz and radiopaque material like yttrium fluoride. The quartz filler particles are 0.5µm in diameter and are coated with a silane layer by the process of silanization. The silane layer helps in increasing the hydrophobic nature of the surface of filler particles; acts as an interface between filler and resin reinforcing the resin matrix and the hard filler particles, thus preventing undesired initiation of the cationic polymerization reaction by protecting them from reacting with the quartz particles.[3]

POLYMERIZATION REACTION:

The polymerization reaction of silorane occurs via a cationic ring opening reaction that reduces the polymerization shrinkage. The cationic cure starts with the activation of the initiator system which opens the oxirane ring and generates a new acidic centre. After the subsequent attraction to a new oxirane monomer, the epoxy ring is opened to form a chain. On interaction with the other monomers, a continuous network of the ring is formed. This process of cleavage and opening of the ring system gains space and counteracts the volume loss that occurs when the chemical bond is formed leading to reduced polymerization shrinkage. The most important difference is that methacrylate is cured by radical intermediates; whereas silorane polymerizes via cationic intermediates.[3]

BONDING MECHANISM:

Bonding mechanism is initiated on the application of silorane self-etching primer (SSA primer) and silorane adhesive (SSA bond). Of which SSA primer is hydrophilic that bonds to tooth structure; SSA bond is hydrophobic that bonds to the silorane composite. Silorane self -etch primer consists of 15-25% HEMA, 15-25%Bis-GMA, 10-15%- water, 10-15%- ethanol, 5-15% phosphoric acid- methacryloxy- hexyl esters, 8-12% silane treated silica, 5-10%- 1,6-hexanediol dimethacrylate, <5%- copolymer of itaconic acid, <5%- dimethyl aminoethyl methacrylate, <3% dl-camphorquinone and <3% phosphine oxide. Silorane adhesive consists of 80% substituted dimethacrylate, 5-10% silane treated silica, 5-10% TEGDMA, <5% phosphoric acid-methacryloxy-hexyl esters, 3%dl-camphorquinone, 3% 1,6- hexane dioldimethacrylate.[4]After application of SSA primer produces a mild etching and demineralization of the tooth. Bonding mechanism involves the formation of the nano-interaction zone due to acidic pH of the primer (2.7) and relatively high amount of HEMA that keeps the resin solution homogenous preventing phase separation. This is followed by the application of SSA bond that contains hydrophobic bi-functional monomers which bonds to the hydrophobic silorane resin.[3]

BOND STRENGTH:

Bond strength of composites is generally compromised due to the inhibition of free radical polymerization due to the presence of oxygen. The presence of the oxygen inhibition layer between two successive composite layers reduces the adhesion by the formation of covalent bonds with the interpenetrating network. In ring opening cationic polymerization reaction of silorane composites there is no oxygen inhibition layer formation on the polymerized surface. Bastos et al 2015, evaluated the microtensile bond strength of silorane based composite using different bonding strategies and concluded that the mean shear bond strength value for silorane composite was 27.9 Mpa [5] which was relatively greater when compared to methacrylate based composites.

PROPERTIES:

POLYMERIZATION SHRINKAGE:

Siloranes has low polymerization shrinkage due to the cationic ring opening polymerization reaction of the oxirane monomers and increased hydrophobicity due to the presence of siloxane species in its composition.[6] A study was conducted by Weinmann et al comparing the polymerization shrinkage between silorane and methacrylate based resins using Archimedes method and bonded disc method. And concluded, that silorane had the least polymerization shrinkage (0.99%) due

to cationic ring opening polymerization reaction that causes the opening of the oxirane ring that gains space and counteracts the volume loss.[3]

LIGHT STABILITY:

Silorane have high ambient light stability due to the 3 part initiator system. The kinetics of reaction can be modulated by changing concentrations of three components. When compared to methacrylate based resins (2 component initiator) siloranes have high ambient light stability and light reactivity. A study was conducted by Weinmann et al on light stability inferred that the ambient light reactivity of silorane was higher (>10min) than ambient light reactivity of methacrylates (55-90sec). [3]

HARDNESS:

Guiraldo *et al* 2010 suggested that silorane based composites undergoes cationic polymerization reaction characterized by continuous ring opening expansion initiated at the time of curing which also promotes further cross-linking and hardening of the entire resin matrix than microfilled composites. The micro filled composites consist of smaller filler particles that tend to scatter light thus decreasing the effectiveness of curing light.[7] The hardness of silorane assessed by Vickers hardness test shows that it has a mean range of 73.4-82.5N/mm^2. Agarwal *et al* 2015 compared the surface hardness and depth of cure of siloranes and methacrylate based posterior composite resins in an in-vitro study and concluded that higher hardness value is present in silorane based composites than methacrylate based posterior composites.[8]

`MICROLEAKAGE/ MARGINAL QUALITY:

The bond strength and polymerization shrinkage act in the opposite direction. The low polymerization shrinkage and high bond strength leads to excellent marginal integrity of the silorane composite restoration. Silorane showed least mean microleakage score (76.82Mpa) when compared to methacrylate based composite (95.20 Mpa). Khosravi et al 2015 compared the microlekage in class-II cavities restored with methacrylate based and silorane composite at different time intervals at 24hrs, 3months and 6months. The results showed that silorane composite showed least microleakge (0.58%- 1.08%) at all the time intervals.[9]

WEAR RESISTANCE:

The wear rate of silorane was determined by three body abrasion test and was found that silorane has high wear resistance (70-80µm/ 2,00,000 rev for 1day 1.6%) than methacrylate based resins (3.5µm / after 39,000 cycles 1.0%).[10] Soliman et al 2015, evaluated the in-vitro solubility and wear rates of silorane and methacrylate composites under different pH conditions and concluded that silorane composite presented with low solubility(0.29%- 0.43%) and low wear rate (0.0005%- 0.0018%) compared to methacrylate resins. [11]

WATER SORPTION:

Water sorption leads to swelling, reduction of the mechanical properties and produces exogenic discoloration. Silorane exhibits low water sorption-6.63µg/mm^3 due to the hydrophobic nature of the resin matrix with a low tendency for extrinsic discoloration. Nanjundasetty *et al 2013* evaluated the microleakage and micro-gap formation along the gingival margin in class II cavities restored with silorane and methacrylate resins with and without flowable resin liner and inferred that siloranes showed superior marginal integrity both below and above the CEJ when compared to methacrylate-based composites.[12]

BIOCOMPATIBILITY:

Schweikl *et al* 2004 analyzed the formation of micronuclei (chromosomal aberration) and the induction of gene mutations (Hypoxanthine Phospho Ribosyl Transferaseassay) in mammalian cells. No mutagenic effects were detected for silorane composites.[13] Weinmann et al International Association for Dental Research (IADR) evaluated the DNA damage potential of silorane resin on L929 mouse fibroblast cells. Results showed that no DNA damage was produced.[3] Shafiei et al 2014 evaluated the cytotoxic effect of silorane and methacrylate based composites on human dental pulp stem cells and fibroblasts. It was concluded that compared to methacrylates, the cytotoxic effect of silorane based composite on dental pulp stem cells and dental pulp fibroblasts decreases as the time passes.

When the remaining dentin thickness is less than 1mm, the authors recommend adequate pulp protection measures.[14]

Clinical Application:

Silorane can be used as

Ø Direct posterior restorative material for class I and class II cavities

Ø Cavity liner or base.

Advantages:

Ø First composite with polymerization shrinkage less than 1%

Ø High strength and durable

Ø Low water sorption resulting in reduced exogenic staining

Ø Radiopaque

Ø Ambient light stability up to 9min

Ø Good marginal integrity and biocompatibility

Ø Easy to handle and non-sticky

Ø Excellent bond strength

Ø Minimal cuspal deformation and enamel cracks

Ø High flexural and fatigue strength

Ø Wear-resistant

Ø Natural esthetic appearance

Ø Minimal level of leachable monomers

Ø Stable in oral fluids

Ø Retention of anatomic forms

Conclusion

Silorane composites represent the next generation restorative materials that will herald a new era in restorative dentistry. This novel low shrinkage composite provides a unique combination of esthetics with superior mechanical properties comparable to clinically successful methacrylate based composites. Silorane has increased depth curve, low water sorption, high strength, reduced microleakage and good biocompatibility characteristics with low mutagenicity.

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Ethical Clearance: Not required for a review manuscript

Conflict of Interest: Nil

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Morphologic Aberrations of Mandibular Premolar with two and Three Canals: Case Report

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Abstract

The mandibular premolars are more prone to aberrations of canals. They include bifurcation and trifurcation of canals which makes it difficult to diagnose and manage. The failure to treat them leads to re-infection and persistence of infection in the tooth. Hence, it is of utmost importance for the clinician to know the morphologic variations. The present case report is aimed to give a clinical insight of such aberrations. The first case presents with mandibular first premolar with 2 roots and 2 root canals and second case is a mandibular second premolar with 1 root and 3 canals which was diagnosed with CBCT. Both the cases were treated endodontically and successfully managed.

Keywords: *mandibular premolar, three root canals, two root canals.*

Introduction

Variation in the morphology of root canal system occurs commonly and can be considered as normal. The knowledge of the canal anatomy is essential for successful endodontic management. The clinician should be aware of the complexity of the root canal system and also be capable of identification of the variation [(1)]. Amongst the human permanent dentition, it was reported that the mandibular premolar teeth had the most variable canal pattern [(2)]. The mandibular premolar had the highest failure rate – 11.45% [(3)].This may be attributed to the frequent variations in the root canal morphology and the inability to access extra canals.

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Zillich and Dowson have observed that the incidence of three canals in mandibular second premolar is 0.4% [(4)]. Pucci and Reig, observed that the mandibular second premolar had two canals and two foramina in 11.5% teeth and the mandibular first premolar had branching canals, apical bifurcations, and trifurcations 26.5% of the teeth [(5)]. Cleghorn et al, reviewed the mandibular first and second premolars, reported that approximately 98% of mandibular first premolars were single-rooted with a single canal in 75.8% of the teeth. In cases of second premolars, almost all of the teeth in the anatomic studies were single-rooted (99.6%) with a single canal (91.0%) [(6)]. As the occurrence of extra canal is relatively rare and the purpose of this report is to provide an insight on clinical management of such cases.

Case Report 1

A 38-year-old male patient reported with a chief complaint of pain in the lower right back tooth since 3 months. History revealed that patient had experienced pain. Pain was spontaneous in nature and aggravated on chewing and lying down. On intraoral examination, there was a carious exposure of the pulp and the tooth was tender to percussion. A provisional diagnosis of acute irreversible pulpitis with apical periodontitis was made. Radiographic evaluation of the involved tooth (# 44)

revealed outlines of two canals. An angulated radiograph showed 2 roots (figure 1a, 1b). Access preparation was made in the right mandibular first premolar and two orifices were located (buccal and lingual) with the help of operating microscope and careful manual exploration (figure 1c). With 10 K file negotiation was done and working length was measured with electronic apex locator and confirmed with radiograph (figure 1d). Both the canals were prepared using hand K file till no 20 followed by Neo Endo file (Orikam, India) till 25, 0.06 (figure 1e). During preparation, the canals were lubricated and irrigated with EDTA and 2.5% NaOCl. Calcium hydroxide dressing was placed in the canals and temporary restoration was done. After two weeks, the canals were coated with AH plus sealer and obturated in the lateral Compaction technique and the tooth was restored with composite resin (figure 1f).

Case Report 2

A 49 year old female patient came with the chief complaint of intermittent pain over two months in lower right back teeth region. Patient also complained of episodes of sensitivity to cold drinks in the involved tooth. Medical and dental history was non-contributory. Deep disto-occlusal carious lesion was observed in tooth #45 and was tender on percussion. Based on clinical and radiographic evidences a diagnosis of irreversible pulpitis was made (figure 3a) and an angulated radiograph shows a complex pulp anatomy (figure 3b). Root canal treatment was planned. Cone beam computerized tomography was performed to understand the root morphology which shows trifurcation namely buccal, mesio lingual and distolingual canals (figure 2). Access preparation was made in the right mandibular second premolar. With the help of operating microscope and careful manual exploration was done to locate 3 orifices (figure 3c). With 10 K file negotiation was done and working length was measured with electronic apex locator and confirmed with radiograph (figure 3d). The three canals were prepared using hand K file till no 20 in step back method and Neo Endo file (Orikam, India) 20, 0.04% (figure 3e). During preparation, the canals were lubricated with Endoprep- RC and irrigated with 2.5% NaOCl. The calcium hydroxide dressing was placed in the canals and temporary restoration was done. After two weeks, the canals were coated with AH plus sealer and obturated in the lateral Compaction technique and the tooth was restored with composite resin (figure 3f).

Table 1: Incidence of 2 canals in premolars of various racial groups

Investigator	year	Ethnicity	incidence
PINEDA(8)	1972	Hispanic	1.2%
VERTUCCI(9)	1978	Caucasians	2.5 to 34.4%
SERT(10)	2004	Turkish	6.4 to 29%
RAHIMI(11)	2007	Iranian	5.8–17.5%
AWAWDEH(12)	2008	Jordanian	22.8%
PAREKH(13)	2011	Indian	13.5–20%
ZHANG(14)	2012	Mongoloid	2%

Name of author	year	No. of roots	No. of root canals
Rodig(16)	2003	1	3
Sachdeva(17)	2008	4	4
Aguiar(18)	2010	2	3
Poorni(19)	2010	2	3
Borna(20)	2011	1	3
Bhavana(21)	2013	3	3

Table 2: Case reports with three or more canals in mandibular premolar

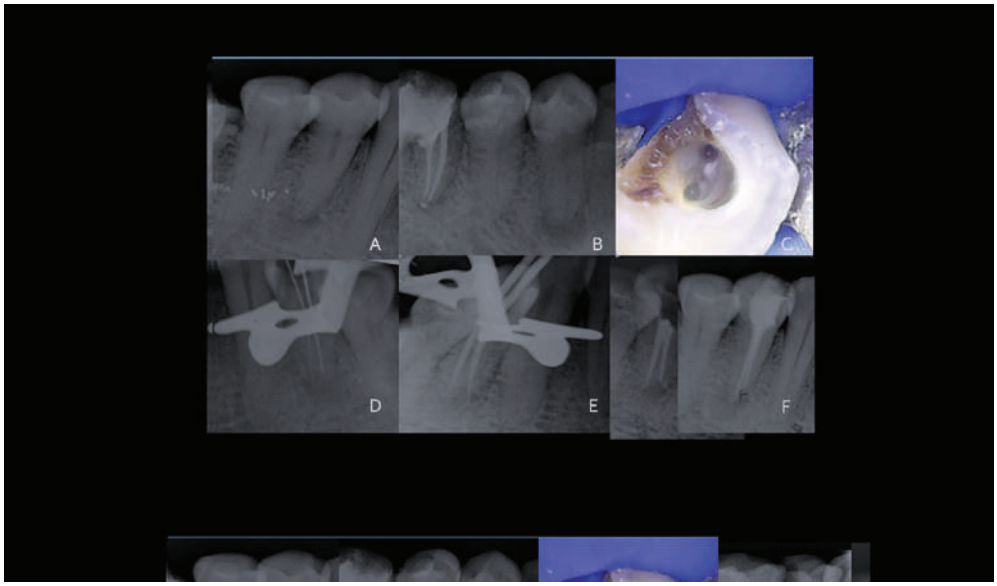


Figure 1: CASE 1: (A): pre-operative radiograph, (B): pre-operative mesial angulated radiograph, (C): orifice location (buccal and lingual), (D) working length determination, (E) master cone, (F) obturation and post endo restoration

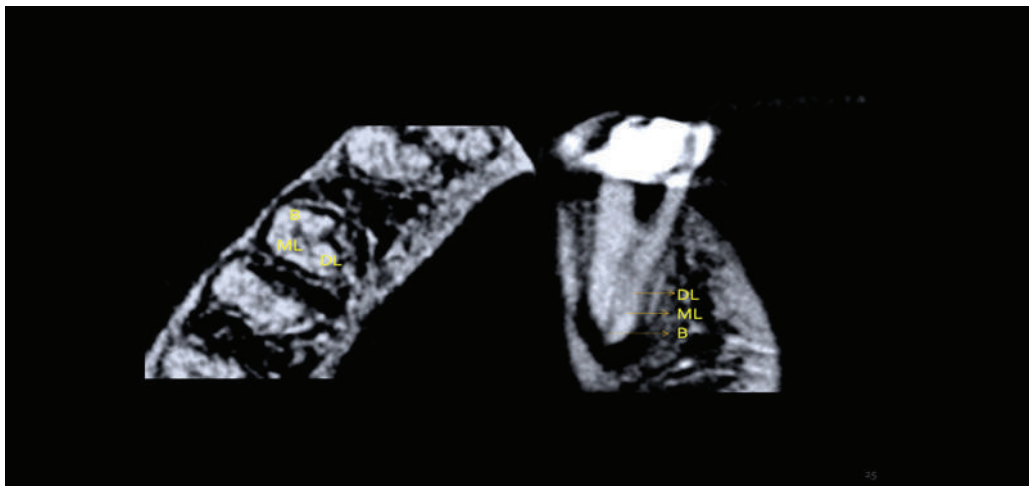


Figure 2: CASE 2: CBCT showing 3 canals on the middle third (buccal, mesio-lingual and disto-lingual) joining short of apex.

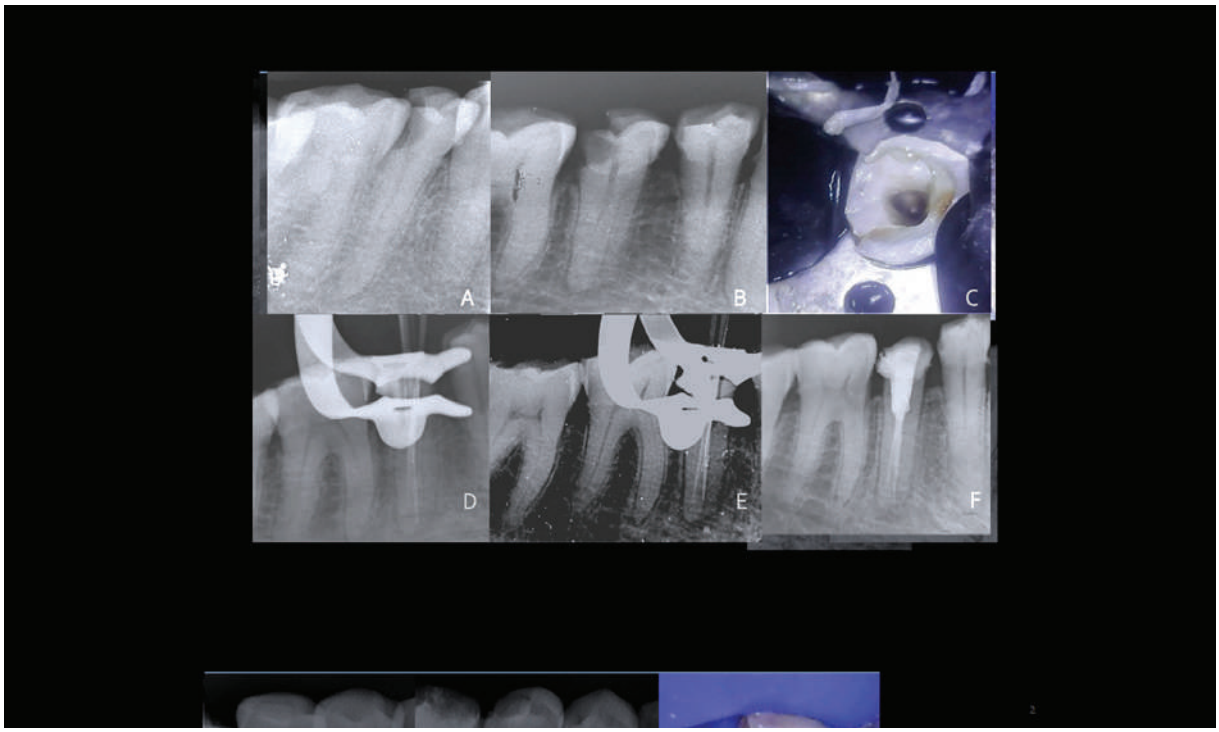


Figure 3: CASE 2: (A): pre-operative radiograph, (B): pre-operative mesial angulated radiograph, (C): orifice location (buccal, mesio-lingual and disto-lingual), (D): working length determination, (E): master cone, (F): obturation and post-endo restoration

Discussion

The presence of two roots in mandibular first premolar was 2.63% [7]. The mandibular first premolar is more prone to bifurcation of canals (23–30%) terminating in multiple apical foramina (15–20%), as compared to second premolars (Table 1 shows incidences of a second canal in various populations) [8-13]. In the present case 1, the mandibular first premolar had 2 root canals of vertucci type IV configuration which had an incidence of 0.27% and in case 2 the mandibular second premolar with 1 root and 3 root canals with vetucci type VIII configuration and incidence of 0.1% [15] (Table 2 shows case reports with 3 or more canals) [16-21] . In the present case the root canals in mandibular second premolar trifurcated at the middle third and later joined short of the apex, exiting at one foramen. Anatomic studies of mandibular second premolar canal morphology found that a one canal was seen in 91.0% of the teeth. A single apical foramen was found in 91.8% of the teeth [7]. The varied canal morphology can be understood through the development of root formation. Where, during root formation the aberrations occur and they have genetic and racial predilection. Higher incidences of two roots was seen in the African-American population (16.2%) and three or more rooted formation was reported in only 3% in French and 0.2% in Indian populations [7]. This shows the complexity of the premolar canal morphology which needs careful evaluation. A CBCT was use to evaluate canal morphology as it helps in better visualization of canal anatomy and helps in treatment planning [22].

Conclusion

The basics of endodontic therapy begin with appropriate diagnosis and treatment plan. Proper knowledge of root canal system is of great importance in treatment planning. With the advent of advanced microscope, CBCT and NiTi file system, detection of aberrant anatomical variation is rather a norm than an exception, which if misdiagnosed and left untreated or incompletely treated leads to failure.

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Ethical Clearance : Not required for a case report.

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Dental Health and Pregnancy – An Overview

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Abstract

Pregnancy is a physiological state with significant changes in a woman's body. Pregnant women are more prone to gingival diseases due high levels of fluctuating pregnancy hormones.The aim of this article is to bring to light the changes in oral cavity during pregnancy, the consequences of not treating them and the current recommendations for dental management of pregnant patients in different trimesters.

Key Words: *Pregnancy, Safety recommendations, Radiation, Premature delivery, Low-birth weight*

Introduction

Every woman deserves to have a healthy pregnancy. Pregnancy is a physiological state with transient changes. A pregnant woman requires support in different levels in terms of physical, mental, and emotional needs. Dental Management in pregnant women is critical and requires special care. Dentists might postpone elective procedures during first trimester which can interfere with organogenesis of the foetus, while at other times of pregnancy, treatment altered to best suit the patient and the foetal demands. It is important and essential to seek oral health counselling, oral examinations, and dental care during pregnancy.

Physiology of pregnancy and symptoms in oral cavity:

A 30-fold increase in Progesterone and a 10-fold increase in oestrogen cause pregnancy induced changes in mother's body and theoral cavity is no exception. [1]

Morning sickness and tooth erosion:

Nausea and vomiting are observed in 66% of all pregnant women starting approximately 5 weeks after Last menstrual period (LMP) extending up to 12 weeks.[2] In the first trimester, nausea and vomiting are associated with increased levels of progesterone. The gastric acids present in the emesis with hyperemesis gravidarum, erode the enamel mostlyon the palatal and lingual surfaces of anterior teeth.Both endogenous and exogenous acids are associated with enamel erosion. Endogenous acids have a pH of approximately 1.2 which is below the critical pH for dissolution of hydroxyapatite and fluorapatite crystals.This low pH rapidly causes demineralisation. [3,4] Erosion of the enamel can easily be controlled by advising the patients to rinse their mouths after vomiting, with a solution that contains sodium bicarbonate that neutralises the pH.[5] Morning dental appointments can be avoided for patients with morning sickness. Resin based viscous varnishes, resin based clear adhesives or cements and casein based remineralizing pastes can act as artificial bio film and help in remineralisation.[6]

Pregnancy gingivitis:

Pregnancy gingivitis and gingival hyperplasia aredue to Elevated levels of the oestrogen. This can cause an increased capillary permeability. Pregnancy gingivitis usually affects the marginal and the interdental papilla, aggravating the pre-existing gingivitis.[7] In a study performed by D.J Erchick on oral hygiene and prevalence of gingivitis among 1452 pregnant women in Nepal, 40% had gingivitis among which 80% had localised gingivitis and the remaining 20% had generalised gingivitis.[8]

Increased progesterone in the blood alters the collagen production in gingiva, thereby reducing the

body's ability to repair.Folic acid deficiency due to high levels of progesterone and oestrogen prevents gingiva repair. Gingivitis generally occurs in 60 to75% women during pregnancy, but its incidence is only 0.03% in plaque-free environment at the beginning of pregnancy and is maintained throughout pregnancy. [9]

In a study by J.E Raber et al, where they induced experimental gingivitis during pregnancy and post-partum where the level of hormones were monitored throughout the study and the amount of plaque was constant, it was inferred that the bleeding and gingival inflammation were increased during pregnancy and the microbiota level (Prevotella intermedia) was more due to underlying pregnancy related physiological changes. Whereas, post-partum the levels in microbiota did not change.[10]Another study by Maria et al, suggested a high prevalence of Porphyromonas gingivalis , in association with T. forsythia and T. denticola and increased risk of developing periodontitis in pregnant women aged 30 years or older.[11]Literature states that there is a connection between the influence of sex hormones and a directly proportional decrease in periodontal health. The hormonal levels of oestrogen and progesterone triggers a spectrum of inflammatory responses in the gingival tissue and influence the subgingival microbiota. Influence of sex hormones can be minimized by maintaining good plaque control.[12]

Pyogenic Granuloma (Granuloma Gravidarum):

1-5 % prevalence in pregnancy. High levels of oestrogen and progesterone causes increase angiogenesis, when coupled with gingival irritation by contributing local factors such as plaque whichcan lead to this pregnancy tumour in the labial region of teeth usually in the interdental papilla.It happens usually in the first and the second trimester and regresses after parturition.The Oestrogen enhances vascular endothelial growth factor production in macrophages, related to the development of pregnancy tumour.[13] It is proposed that the disappearance of the tumour after parturition is unknown but can be attributed to the regression of the blood vessels.

This can make brushing and the routine dental care uncomfortable, eventually causing tooth decay.An increase salivary oestrogen increases the desquamation of the oral mucosal cells. This combined with increased sub gingival crevicular fluid levels promote bacterial growth by providing required nutrition causing an elevated risk for dental caries.[14] Excision of this tumour 4 weeks post-partum is the best treatment option.

Periodontitis in pregnancy:

The recent studies have suggested a link between periodontal disease and preterm and low birth weight.A study on 400 women with gingivitis and periodontal disease demonstrated a positive correlation between periodontal disease and low birth weight.[9] Periodontitis, if present before pregnancy can aggravate with the poor oral hygiene during pregnancy.

In a systematic review published by Amare Teshome et al, the focus was on preterm birth, low birth weight and periodontitis. Of the selected ten studies out of 229 articles, 9 concluded an association between periodontal disease and low birth weight. Pregnant women who are diagnosed with periodontal issues may have a high risk of delivering premature and low birth weight child regardless of other associated risk factors.[15] In a case report of still birth, by Yiping et al in 2010, it was found that a pregnant woman who had pregnancy associated gingivitis experienced an upper respiratory tract infection at term, which was followed by a still birth. They isolated Fusobacterium nucleatum in the placenta which was also present in the mother's sub-gingival plaque but not in vagina, cervix, or rectum. [16]

Dental Management during Pregnancy:

Dental Radiography and pregnancy:

Depending on the amount of radiation and the stages of pregnancy, damage to the foetus may result in various complications. However, the dental radiation exposure of the foetus is negligible. Several precautions can be taken to avoid the foetal exposure when radiographs aretaken. Using a lead shield over the patient's abdomen, a collimated beam, and a high-speed film, can reduce the foetal exposure to a large extent. The greatest risk to the foetus is during the first 10 days after the conception followed by 4-18 weeks, also very crucial. The National Commission for Radiation Protective (NCRP) recommends that the cumulative foetal exposure to radiation should not exceed more than

0.20 Gy, over which can cause microcephaly and mental retardation [17]

First Trimester 1-12 Weeks:

It is recommended to avoid dental treatment in this period. The concern about doing procedures during the first trimester is that the developing child is at a greatest risk during organogenesis. Dental procedures performed at this time leads to spontaneous abortion.[1]

The current recommendations are to emphasize importance of oral hygiene, avoiding any elective dental procedures and radiographs.

Second Trimester 13-24 Weeks*:*

It is safe to perform elective dental care during this time.

The current recommendations are:

1. Oral Hygiene – Plaque control by Scaling, polishing and curettage.

2. Elective dental care - Restorations, root canals and extractions

3. Radiographs to be used selectively ensuring proper protection and radiation safety for the foetus.

Third trimester 25-40 Weeks:

It is safe to perform a routine dental treatment in the early part of the 3rd trimester, but better to avoid after that.

The current recommendations are:

1. Oral Hygiene – Plaque control by Scaling, polishing and curettage may be performed if necessary.

2. Avoid an elective dental care during the 2nd half of the third trimester.

3. Radiographs to be used selectively while ensuring radiation safety for the foetus.In the end of second and third trimester foetus has grown considerably. The gravid uterus can compress the vena cava while the mother is in supine position. This can impede the blood flow by causing constriction of major vessels resulting in supine hypotension. This can lead to near syncopal episode. Therefore, proper positioning of the patient is required by asking the patient to lie down on her left side and elevating the chair head.[18]

Conclusion

Every woman should be educated to seek proper dental care during pregnancy. It is very important for the gynaecologists to advice pregnant women to get a dental check-up during their pre-natal counselling. The gynaecologists must enquire about the history of gingival bleeding or any difficulty in brushing due to swelling on the gingiva and if the response is positive, they can refer the patients to the dentist without any hesitation regardless of the trimester the patient is in. It will be ideal if the obstetricians join hands with the dentists in educating the women about the importance of seeking dental care during pregnancy. It is recommended to include oral health screening as a part of pre-natal counselling. This way, the dentist can treat any big cavities or open lesions prophylactically.

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Factors Influencing Ledge Formation and Its Management in Endodontics

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Abstract

Endodontic treatment can be predictable, successful, and relatively easy to perform if every individual step is performed appropriately. Hasty mechanical and chemical manipulation of the root canal system can lead to procedural error. Procedural accidents during endodontic treatment occurs either due to inadequate attention to the details. Removal of canal wall structure on the outside curve in the apical half of the canal due to the tendency of files to restore themselves to their original linear shape during canal preparation may lead to ledge formation and possible perforation.

Keywords: *Ledge, Procedural error, Endodontic Mishap, Prevention and management*

Introduction

Among the complications that occur during endodontic procedure ledge is the most common endodontic mishap. Endodontic mishaps are error that occurs due to lack of enough attention to the details or sometimes it happens unintentionally by the clinician. [1,2] Even the most experienced clinician may commit procedural error like ledge, the successful endodontic treatment is based on thorough knowledge regarding internal and external anatomy of teeth.[3,4] Insufficiently designed access cavities lead to an improper guiding of the instruments by the walls of the cavity with a loss of control of the instruments during root canal preparation leading to ledge.[5] The other factors that could result in ledge formation are inaccurate working length, instrument material used for cleaning and shaping, irrigation, instrument design and instrumentation technique. This review addresses the causative, prevention and management of ledge formation in endodontics.

Preoperative assessment

Clinicians should know the tooth morphology, crown root angulation of teeth, any variation in the morphology and any calcification within the canal should be accessed before the start of access cavity. An Assessment is done by visual examination, radiological evaluation with preoperative x-ray, following SLOB technique wherever needed and also using dyes when difficult to assess with regular x-ray. These methods will help you to evaluate the architecture, internal and external anatomy, number of canals, any branching of canals, lateral canals, curvature of canal and the relationship of canal orifice to the pulp chamber and externals surface of teeth. Root curvature could be of different degree it can be straight (lesser than 5°), moderately curved (10-20°), severely curved (greater than 20°). It was shown that degree, position and severity of canal curvature are some of the factors that is important.[4] Studies have shown that root curvature more than 20 degree produces more ledges [5], whereas root canals with less than 10 degree curvature produces less ledges.[6] Use of inflexible larger instrument in curved canal results in ledges. Cleaning and shaping of canals which are narrow or calcified produce more ledges hence the evaluation of the internal anatomy of teeth before the commencement of endodontic therapy is needed.

Access related

The main purpose of an access opening is to create an unimpeded passageway to the pulpal space and apical foramen of the tooth. This preliminary step of preparation of the access cavity, the opening in the dental crown permits localization, cleaning, shaping, disinfection, and three- dimensional obturation of the root canal system. The success of the endodontic treatment depends on precise, proper execution of access cavity[7].Failure of the adequate extension of access cavity will prevent the access to the apical part of the root canal resulting in ledge. [5,8,9]

An access cavity that has been prepared improperly in terms of position, depth, or extent will hamper the achievement of optimal results. There should be unobstructed access to the canal orifices and direct access to the apical foramen. After locating the canal orifices endodontic files are introduced into the canal to determine whether the instruments are either "under stress" or free of interfering tooth structure. If there is any binding seen than the coronal outline form is extended to free up the shaft of the file. The operator would not be able to control over the direction of the tip of the instrument and it would gouges into the wall of the canal resulting in ledge if there is binding. Significant crown root angulations are common in maxillary lateral and mandibular premolar. Severely tipped or a crowded teeth requires modification in the access preparation. There should be proper aligning of the round bur vertically with the long axis of the tooth, failing which results in ledge and perforation. Incorrect assessment of root canal direction results in ledge. Conventional radiograph can tell us mesiodistal curvature but not buccolingual[10], hence it would result in wrong estimation of root canal direction.

Working length related

Accurate method of working length determination should be done either with radiographic or non-radiographic method. Many times ledges are formed when the operator works the files short of the full canal length and the canal becomes blocked at that point, which is short of the working length or it might begin to deviate from the canal path resulting in transportation. Improper working length is one of the main causative in ledge formation.[5,11] We should maintain the working length during entire procedure of cleaning and shaping failing to do this would result in ledge.

Instrument Material used for Cleaning and shaping

Between stainless steel and Nickel titanium instruments studies have shown that using a bigger non curved stainless steel instrument in a curved canal resulted in more ledges.[7,12] When comparing between hand instruments, Stainless steel and Nickel titanium in a study done by Pettiette et al showed more straightening in stainless steel hand instrument when compared to Nickel titanium hand instrument in curved canal.[13] Namazikhah et al, compared between hand and rotary files to show the higher incidence of ledges with hand when compared to rotary.[14] Xu et al studied the cleaning and shaping with hand instrument in curved canal produces more ledges when compared to protaper rotary system.[15]Nickel titanium instrument can retain the shape of the curved canal and does not straighten like stainless steel.

Irrigation

Inadequate irrigation and lubrication during instrumentation result in blockage of canal [18] due to accumulation of dentin mud in the canal. If there is ledge filing is performed with lots of irrigant with short vertical strokes always keeping the file tip apical to the ledge. Sodium hypochlorite is the commonest irrigant used it dissolves the organic portion of dentine and has antibacterial property, but it might affect the flexural strength of dentine and potentially influence the adhesive capacity of dentine. Chelating agent is helpful but should be used with caution because excessive use of chelating agent results in perforation because it would soften the dentin. It is recommended to use chelating agent carefully in narrow, curved or ledged canal, but liquid EDTA is used as a final rinse to remove smear layer. Surfactants are compounds of lower surface tension they are used in conjunction with commonly used irrigants such as EDTA, Chlorhexidine and Sodium hypochlorite. They act as wetting agent, emulsifier, detergent and lubricant to assist cleaning and shaping procedure.

Design feature of Instruments

Tip design: Use of instruments with sharp cutting

tips produces more ledges when compared to modified tip design. Tip design with biconical shape produces less of ledges when compared to conical and pyramidal shaped. Zmener and Marrero in their Scanning electron microscope study compared between Tri-files, Flex-R files, Flexo files, and K files in curved root canal 0% ledges were seen with Tri-files & Flex-R, whereas 10% ledges were seen in Flexofile and 30% ledges in K-file.[16] Flex R files are made by removing sharp cutting edges from the tip of instrument called as modified tip design to reduce the ledge formation. When pyramidal tip design was compared with conical and biconical tip design ledges were more commonly seen with pyramidal tip which has sharp transition and a forward cutting ridge on the face when compared to conical tip which has sharp transitional angle and a smooth face and biconical tip which has reduced transitional angles and dual- guiding faces.[17] Modified- tip files tend to maintain the original canal curvature better like flex R files,Control safe file, Anti ledging tip files and Safety hedstrom files rounded tip design of these files does not cut into the wall but will slip alongside it. Rotary files can have either cutting or noncutting tip. Protaper rotary files have convex triangular cross-section but they have finishing files which are non-cutting blunt tip and shaping files which are partially active thereby reducing ledge and perforation. Protaper, Profile, Hero 642, GT files and RaCe have non cutting tip whereas Quantec, flex master and K3 have cutting tip. Using C-file which has stronger buckling resistance compared with K-files provides easier access to the apical portion of canal and allows easier location of the canal orifices and thereby reducing the incidence of ledges. The pyramid-shaped tip facilitates insertion during negotiation of the canal and the square cross-section provides better resistance to distortion.

Rake angle

Rake angle is the angle formed by the leading edge and the radius of file. If the angle formed by the leading edge and the surface to be cut is obtuse than it is positive rake angle and if it is acute than it is negative rake angle. If Rake angle is slightly positive, results in an effective cutting action but if it is too positive, the blade becomes engaged into the surface excessively and binds without forming chips. A negative angle will scrape the dentin rather than cutting it. Protaper, Hero 642, RaCe, K3 and flex master exhibit positive rake angle resulting in optimum cutting efficiency. Light speed and Greater taper show neutral rake angle and profile has negative rake angle.

Instrumentation technique that reduces and corrects ledge

Balanced force technique which was introduced by Roane and Passive step back technique are considered to reduce the incidence of ledging.[18] Studies have shown that apical patency reduces formation of ledges; it is a technique in which the apical portion of the canal is maintained free of debris by recapitulation with a small file through the apical foramen. Stadler et al clinically studied 520 samples to show the treatment done by supervised dental student had more ledge effect with reaming technique in comparison with filing technique. Root canal curvature with more than 35 degrees has main effect on incidence of ledges.[22] Pre-curving the instrument and following sequential use of instrument [5,8], reduces the incidence of ledges.[1] It should be noted that in tightly curved canals where it is extremely difficult to advance from a No. 10 file to a No. 15 file, there are "half-step" files that are commercially available as FlexoFile Golden Mediums studies have shown that subsequent filing with these modified files will open the canal some more and render it more suitable for negotiation with the next available standard size.

Anticurvature filling- Bypassing of ledge is removal of ledge by instrumentation and obturation at new working length. It is done in two steps, in first step the entire canal pathway coronal to the ledge must be relatively straightened to allow correct file operation. This is done by filing against the wall opposite the apical curvature with instrument used in sequential order without skipping the size in retraction-rotation motions. Second step in bypassing a ledge is by following anticurvature filling technique. The resistance of the endodontic instruments to deformation, referred to as elastic memory by forcing a straight instrument into a curved path it tends to uncurve, and as a result the tip of the instrument cuts more into the wall opposite the curve by following this steps we could bypass the ledge. Anticurvature filing was compared with the stepback technique in a study Anticurvature technique reduces ledge and perforation than step back technique.[21]

Passive step back provides a gradual enlargement of root canal in apical coronal direction. This technique uses hand files and GG drill or peeso reamer to provide sufficient coronal flaring before apical root canal preparation, resulting in easier access to irrigation and removal of debris. They provide straight line access to the apical foramen and reducing procedural error like ledge formation.[19,20]

Balanced force technique is used in curved canals using more flexible Flex R file. It is made of triangular shaped flute design with biconical tip and increased flute depth. Flex R file is turned 1/4 turn with apical pressure in clockwise direction and then again, with apical pressure, 3/4 turn counter clockwise direction is used.

Anti-Zipping Preparation System for Curved Root Canals

This technique was introduced by Benjamin Brisefio Marroquin. Ideally, the curved root canal has two sides concave side and convex side, towards the inner side is concave and towards outer side is convex. The original canal axis should not be transported either in a concave or convex direction it should follow the canal path during root canal preparation. This is done in four phase, first the straight portion of the canal is prepared with Peeso reamers depending on the anatomic consideration of each canal. In second phase we need to prepare the concave portion or inner side of the canal. In third phase we need to prepare the convex portion of the canal axis of canal moves to the original position. Finally in fourth phase we have to remove the irregularities that are left at the interface of manual and mechanical preparation.[23]

Conclusion

Ledge formation is one of the procedural error that occurs frequently. It is important to understand the cause of ledge formation and thereby prevention or management plays a key role in the endodontic success. Outcome of the treatment depends on how well the canal has been disinfected and the ledge formation is managed before obturation. This review might help the clinician to understand the prevention, consequence of ledge and its management. Operators are recommended to have lot of perseverance and patience while managing ledge.

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Review Article

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Technologies of Regenerative Endodontic Therapy – A Review

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Abstract

Regenerative treatment is a recently advancing field in Endodontics. Newer treatment protocols and technologies are being put forward in this regard. This review is about some of the important aspects in technologies of regenerative Endodontics.

***Key words**: Regenerative, endodontics, recent, revascularization, disinfection*

Introduction

Regenerative endodontic procedures are biologically based procedures aiming to replace the damaged structures like dentin and root structures, as well as cells of the pulp-dentin complex. The main objectives of these procedures are to regenerate pulp-like tissue, resorbed root structure and the damaged coronal dentin, such as following a carious exposure. The seven techniques of regenerative endodontics are:

1. Revascularization through blood clotting
2. Postnatal stem cell therapy
3. Pulp implantation
4. Scaffold implantation
5. Injectable scaffold delivery
6. 3D cell printing and
7. Gene therapy

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REVASCULARIZATION THROUGH BLOOD CLOTTING

Revascularization through blood clotting assumes a principle that the root canal space has been disinfected and that the formation of blood clot yields a matrix (e.g., fibrin) that traps cells capable of initiating new tissue formation. Revascularization approach in young permanent infected teeth with immature root apex and apical periodontitis was first attempted in 1971 by Nygaard-Ostby and Hjortdal.(1) But it was not successful due to technical drawbacks.

Some prerequisites for revascularization through blood clotting are; it occurs most predictably in teeth with open apices and necrotic pulp secondary to trauma, the width of the apex open has to be greater than 1.5 mm, the bacteria should be removed from canal by either triple antibiotic paste or Calcium hydroxide or formocresol,an effective coronal seal should be placed, a matrix has to be present into which new tissue can grow, the patients have to be young, an anesthetic without a vasoconstrictor should be used when trying to induce bleeding, their should be no instrumentation of the canals, sodium hypochlorite is used as an irrigant and formation of a blood clot probably serves as a protein scaffold permitting 3D in growth of tissue. Success of revascularization depends on open apex which gives easy access for the new tissue formation, short roots

for the cells to easily and quickly reach the pulp horns, intact but is chemically necrotic pulp which provides scaffold and reduces bacterial penetration, minimal instrumentation which preserves the viable tissue and young patient who has a greater healing.

The advantages of this technique are that the reparative matrices become an integral part of the tooth, overcoming any of the problems of retention of a restoration and possible marginal bacterial microleakage. It is a simple technique. There is no possible immune rejection.It also strengthens the root walls of immature teeth.

Disinfection of the root canal is very critical for revascularization.The presence of prior infection could negatively affect the process of pulp tissue regeneration by damaging tissue forming cells as well as stem cells in the periapical tissues.(2)

The two set of materials which are very important for the disinfection of root canal are irrigants and intra canal medicaments.

Irrigants

Sodium hypochlorite is the most commonly used antiseptic irrigating solution in root canal therapy. It appears that sodium hypochlorite can disrupt and remove microbial biofilm from the infected root canal based on in vitro studies.(3) However, the in vitro environment is quite different from in vivo root canal environment in immature permanent teeth with an infected/necrotic pulp, which contains infected necrotic tissue debris and exudate. These factors may affect the antimicrobial activity of sodium hypochlorite, which has also been shown to be less effective in immature teeth when compared to mature teeth.(4)

Sodium hypochlorite with concentrations of 1-6% have been used in regenerative endodontic treatment (RET).(5) The American Association of Endodontists (AAE) clinical considerations for a regenerative procedure recommends the use 1.5% sodium hypochlorite followed by 17% EDTA. The use of sodium hypochlorite before EDTA conditioning reduced transforming growth factor TGF-β1 release significantly.(6) This effect is probably due to damage to the proteins including dentine growth factor.

Antiseptic medicaments

Calcium Hydroxide

It is recommended as an intracanal medicament in RET due to its good antimicrobial property (7) which is because of its high ph of 12.5-12.8.(8)

Bose et al. (9) reported that when calcium hydroxide was radiographically restricted to the coronal half of the root canal system, the median percentage increase in dentinal wall thickness was 53.8% as compared with a 3.3% increase when it was placed in the apical half of the root canal. However, where calcium hydroxide was placed in the apical canal, it did not affect the percentage increase in root length. A recent study has shown that the attachment of human apical cells to root dentine is higher when treated with calcium hydroxide rather than triple antibiotic paste(TAP) in vitro.(10)

It has been shown that dentine is capable of inactivating root canal medicaments.(11) Research is going on on CAOH for RET.

Ethylenediaminetetraacetic Acid (EDTA)

It is used to cause release of growth factors from dentine matrix in RET(6) but it has weak antimicrobial activity.(12) The use of 17% EDTA resulted in increased SCAP survival expression as well as partially reversing the deleterious effects of NaOCl.(13) In cases where minimal filing has been undertaken, the use of EDTA and removal of the smear layer may expose binding sites for attachment of newly formed tissue to the canal walls as well as stem cell differentiation.(14) EDTA conditioning of dentine promoted the adhesion, migration and differentiation of dental pulp stem cells towards or onto dentine.(15) Dentine matrix derived growth factors released after EDTA treatment have been shown to be capable of signalling stem cells of apical papilla to differentiate into odontoblast-like cells.(16)

Triple Antibiotic Paste

In RET of immature permanent teeth with infected necrotic pulp, triple antibiotic paste (minocycline, ciprofloxacin, metronidazole) has been recommended as an intracanal medicament, based on its excellent antimicrobial activity to kill all species of bacteria in the infected root canals.(17,18) It is recommended to use triple

antibiotic paste at a concentration no greater than 1mg/mL (0.1-1mg/mL) in RET to avoid damage of stem cells from the apical papilla.[19]

One antibiotic, Augmentin may be as effective as triple antibiotic paste in RET.[20] It is shown to kill 100% of microorganism isolated from the infected root canal associated with an apical abscess.[21] It inhibits bacterial cell wall synthesis.

High concentrations of sodium hypochlorite[13] and triple antibiotic paste[19] are detrimental to the survival of apical papilla stem cells, thus possibly influencing root maturation. However, other factors such as severity and duration of apical periodontitis, age of the patient, stage of root development, follow-up time, and trauma might also affect root maturation in RET.[22]

POST NATAL STEM CELL THERAPY

The technique is to inject postnatal stem cells into disinfected root canal systems after the apex is opened for regeneration of pulp. The stem cells can be derived from multiple tissues, including skin, buccal mucosa, fat, and bone.

One major obstacle is the identification of a postnatal stem cell source capable of differentiating into the diverse cell population found in adult pulp. The main disadvantage is that cells have comparatively low survival rates and they may even migrate to different position in the body.

PULP IMPLANTATION

The majority of in vitro cell cultures grow as a single monolayer attached to the base of culture flasks. However, some stem cells do not survive unless they are grown on top of a layer of feeder cells. In theory, to take two-dimensional cell cultures and make them 3D, the pulp cells can be grown on biodegradable membrane filters. To form a 3D pulp tissue, many filters will be required to be rolled together which can be implanted into disinfected root canal systems.

The advantage is that the cells are relatively easy to grow on filters in the laboratory. The disadvantage is that the specialized procedures may be required to ensure that the cells properly adhere to the root canal wall. The filters are very thin and the layers of cells are extremely fragile. So it is difficult to place them in root canal systems without breakage.

SCAFFOLD IMPLANTATION

Pulp stem cells must be organized into a 3D structure that can support cell organization and vascularization to create a more practical endodontic tissue engineering therapy. This can be achieved using a porous polymer scaffold seeded with pulp stem cells. Scaffold must contain certain growth factors for the proliferation and differentiation of stem cell, which might leads to improved and faster tissue development.[23]

INJECTABLE SCAFFOLD DELIVERY

As a tissue-engineered pulp is not required to provide structural support of the tooth, the tissue-engineered pulp tissue can be administered in a soft 3D scaffold matrix such as a polymer hydrogel. Hydrogels can be delivered by syringe (injectable scaffolds).

They have advantages of having unique compositional and structural similarities to natural ECM. They are non-invasive. They form a desirable framework for cellular proliferation and survival of an organized tissue structure. They can overcome various challenges of vascularization, tissue architecture, and simultaneous seeding of multiple cells because of its ability to control the porosity, surface morphology, shape, and size. Despite these advances, hydrogels are at an early stage of research, and this type of delivery system, although promising, has yet to be proven to be functional in vivo.

3D CELL PRINTING

This technique helps to precisely position cells to create tissue constructs that mimic the natural tooth pulp tissue structure. Theoretically, an inkjet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue. The ideal positioning of cells in a tissue engineering construct would include placing odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells.[24]

Theoretically speaking, one disadvantage is that careful orientation of the pulp tissue construct according

to its apical and coronal asymmetry would be required during placement into cleaned and shaped root canal systems.

GENE THERAPY

The DNA contains genetic sequences (genes) that control cell activity and function. One of the most well-known genes is p53.Gene therapy basically is involving viral or nonviral vectors that can deliver genes for growth factors, morphogens, transcription factors, and ECM molecules into target cell populations.

Some of the viral vectors are adenovirus, adeno-associated virus, retroviruses, herpes simplex virus, and Lentivirus. The non-viral vectors are plasmids, peptides, gene guns, DNA-ligand complexes, electroporation, sonoporation and cationic liposomes.

One use of gene delivery in endodontics would be to deliver mineralizing genes into pulp tissue to promote tissue mineralization.Ultrasound mediated gene delivery is used in-vivo and in-vitro whereas electroporation is used only in-vitro. This may be because of the lack of erythrocytes in the plasma clot due to thermal changes during electroporation in vivo. In the in vivo approach, the gene is delivered systematically into the bloodstream or locally to target tissues by injection or inhalation. The healing potential of pulp tissue is enhanced by genes inducing dentin directly applied on the exposed amputated dental pulp. The ex vivo approach involves genetic manipulation of cells in vitro, which are subsequently transplanted to the regeneration site. The ex vivo gene therapy stimulates reparative dentin formation more optimally and rapidly in comparison to in vivo gene therapy.[23]

Conclusion

RET is based on the concept of tissue engineering technology to regenerate the dentine-pulp complex in the canal space of immature permanent teeth damaged by caries or trauma, thus restoring development of the arrested tooth root. Extensive research is going on in this field and soon it may be one of the most effective and most sort out treatment option in endodontics.

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100 Years of Calcium Hydroxide in Dentistry: A Review of Literature

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Abstract

Ever since the introduction of calcium hydroxide in 1920, it has been widely used in dentistry. This is due to the therapeutic properties of calcium hydroxide imparted by its high pH and dissociation to form ions Ca^{+2} and OH^{-}. This makes it a remineralizing agent, a bactericidal or bacteriostatic agent based on the pH concentration and also an anti-inflammatory agent. It is being applied for various purposes in dentistry like intracanal medicament, pulp capping agent, root canal sealer, cavity liner, apexification, pulpotomy, canals with exudate, perforation management and root resorption. This review article aims to describe in detail the various properties of calcium hydroxide and applications marking 100 years of its use in dentistry.

Key words: *calcium hydroxide, intracanal medicament, remineralization.*

Introduction

Hermann in 1920 introduced calcium hydroxide in dentistry as "calxyl" to produce dentinal bridging of exposed pulp. This has led to the concept of remineralisation, shifting from doomed organ theory [(1)]. Calcium hydroxide has been around for a century and has shown to be effective in various clinical applications. It is an odourless white powder with a high pH and low solubility in water. It dissociates into calcium and hydroxyl ions which aids in remineralisation and anti bacterial properties, there by facilitating its use in dentistry [(2)]. The pH and vehicle used mainly influence the properties of Calcium hydroxide (table 1) [(3)]. Polyethylene glycol is one of the most commonly used vehicles due to its low solubility and antibacterial property [(4)]. The various applications of calcium hydroxide are due to its important properties such as mineralization, antibacterial property and dissolution of necrotic material (figure 1). The calcium hydroxide has various advantages like initial bactericidal and later bacteriostatic activity, high pH which stimulates fibroblasts, neutralizes low pH, promotes healing and repair, it is also in expensive and easy to use [(5)]. But it has few drawbacks like it does not exclusively stimulate dentinogenesis or reparative dentin formation; it may degrade during acid etching and does not adhere to dentin or resin restoration [(6)]. As the advantages outweigh the drawbacks the use of calcium hydroxide is being used in dentistry since 100 years for various clinical applications (figure 2) [(7,8,9)].

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Mechanism of Action:

Remineralization:

The calcium ions formed on dissociation is said to activate the calcium dependent adenosine triphosphate reaction in remineralization. But this calcium in calcium hydroxide is not present in the calcific barrier but the calcium derived from blood stream is incorporated into

it. The hydroxyl ions provide alkaline pH that prevents dissolution of mineral content in dentin by inactivating lactic acid and also activates alkaline phosphatases enzyme. Calcium hydroxide shows healing by causing necrosis and irritation of pulpal tissue and initiating the inflammatory pathway. It constitutes following characteristics [10,11] :

(i) Zone of obliteration: it is also called as zone of superficial debris. It can be visualized within an hour of contact due to the chemical injury caused by high pH of calcium hydroxide. This zone contains dentinal fragments, debris, blood clot and blood pigments formed due to the caustic effect.

(ii) Zone of coagulation necrosis: it is also called as Stanley's mummified zone or Schroder's layer. It is formed adjacent to the zone of obliteration due to the weaker chemical reaction. This zone consists of devitalized pulp tissue without any structural loss. This starts tissue reactions such as vascular changes and inflammatory cell migration. Later a zone of demarcation is formed differentiating the normal pulp tissue by deposition of protinate globules.

(iii) Dense zone: a modified cell rich zone is formed by proliferation of mesenchymal cells and fibroblasts. The undifferentiated mesenchymal cells in this layer differentiate into preodontoblasts and columnar shaped odontoblasts. Argyrophillic fibers or the Korff's fibers become organized perpendicular to the zone of demarcation. There is tubular formation in collagen matrix forming predentin.

(iv) Calcification of the bridge: Robinson in 1923 proposed the alkaline phosphate theory according to which the enzyme increases calcium phosphate ion concentration and initiates mineralization. Later matrix vesicle theory was introduced according to which extracellular matrix vesicle initiates calcification. But the seedling theory is one of the most accepted one, according to which mineralization mechanism occurs after initial seeding of collagenous tissue along with nucleation of hydroxyl appetite crystals [7].

Antibacterial action:

The highly oxidant free radicals released from calcium hydroxide is the main reason for its antimicrobial property [12]. The action of hydroxyl ions on bacterial cell cause lipid peroxidation of phospholipids, thereby damage the bacterial cytoplasmic membrane. Hydroxyl ions also cause damage to the DNA inhibiting its replication. Its high pH causes breakdown of protein ionic bonds leading to protein denaturation and inhibits cellular metabolism.

Anti-inflammatory action:

The high pH inhibits adhesion of macrophages and decreases the level of matrix metalloproteinase-8 (MMP-8). This inhibits the inflammatory reaction and exudation and also improves tissue circulation [12].

APPLICATIONS OF CALCIUM HYDROXIDE IN DENTISTRY:

Intracanal Medicament

Calcium hydroxide is one of the most commonly used intracanal medicament and is also considered the 'gold standard'. It plays a major role in the disinfection of the root canal when used as an inter appointment dressing. Though it is not a conventional antibacterial agent, it acts as a bactericidal agent at high ph and later as a bacteriostatic agent in root canal space [13]. It is effective against common endodontic pathogens especially E.faecalis [14]. It also helps in reduction of lipopolysaccharides and endotoxin released from bacteria [15]. But it has a longer duration of action. A minimum of 7 day dressing is required to obtain the effectiveness of the medicament [16]. Duration of calcium hydroxide medicament has been widely varied ranging from 10 days (Ostravik, 1990), 1 week (Sjogren, 1991), 2 weeks (Reit and Dahlen, 1988) and 4 weeks (Bystrom, 1985) [12]. The duration is dependent upon the objective of dressing. When used as a routine antibacterial dressing, then 7 days is sufficient.

Calcium hydroxide is also used in conjunction with other therapeutic agents for additive effects such as chlorhexidine and potassium iodide. Calcium hydroxide was initially mixed with sterilized dentine chips and alkaline blood salts by Flohr (1936). Later calcium hydroxide, methylcresilate and camphorated parachlorophenol mixture was prepared by Blanc-Benon (1967) as an intra canal medicament. Calcium hydroxide in 1% aqueous solution of parachlorophenol

was formulated by Martins et al. (1979) and Multical, a mixure of calcium hydroxide (34%), barium sulphate (15%) and chloro-timonol (51%) was made by Webber (1983). But, one of the most widely used is a combination of calcium hydroxide and Chlorhexidine. Calcium hydroxide combined with 0.5% CHX has been shown to eliminate C.albicans & E.faecalis effectively. But it was observed that the mixture of CHX and Ca(OH)2 may not provide a sufficient reservoir of free CHX molecules. The antimicrobial efficiency of this mixture was as effective as CHX alone on E.faecalis. CHX precipitates when mixed with calcium hydroxide and due to the deprotonation of the biguanide at pH > 8.0[9].

Pulp Capping Agent

Calcium hydroxide has been used as pulp capping agent for long time. Histologically calcium hydroxide causes coagulative necrosis followed by mineral deposition leading to complete dentinal bridging. Adjacent to the area of necrosis inflammatory mediators are released which help in mineral deposition and further healing. A Tubular Dentin Bridge which represents the mature type of dentinal bridge with well formed organic matrix and evident calcification is observed. Due to this property calcium hydroxide is used as direct and indirect pulp capping agent since long time in dentistry. But later it was observed that the barrier of osteodentine, which is produced, is often incomplete and results in the formation of the so-called tunnel defects. They occur due to vascular inclusions and such defects may allow bacterial re-infection hence is being replaced by bioceramic materials especially for direct pulp capping procedures.

Endodontic Sealer

Tight seal of root canal system is one of the main objectives of root canal therapy. But the property of calcium hydroxide to dissociate into ions causes dissolution of sealer leading to formation of voids during or after obturation [17]. Though it's solubility remains a matter of concern, the apical leakage cannot be attributed to its solubility, as there are studies reporting the potential for the formation of calcific repair tissues and even closure of apex by mineralized tissue in the vicinity of the materials [18]. And hence, it has been incorporated in many commercially available sealers like sealapex, apexit due to its antibacterial property and mineralization potential [9].

Cavity liner

Calcium hydroxide in fast setting formulations are used as cavity liner due to its therapeutic properties and additionally can resist condensation forces even in thinner sections [19] and also are compatible with light cure resin restorative materials [20]. The calcium hydroxide liner has an accelerated setting in presence of moisture. But the many drawbacks are heat generation during setting due to exothermic reaction and dissolution of material over time [7].

Apexification

Granath in 1959 first reported apical closure with calcium hydroxide. In non vital tooth closure of apex after calcium hydroxide dressing occurs as a cementum-like tissue across the root-end. This process is not true apex formation, but rather formation of only a calcific barrier which is similar to cementum. Because calcium hydroxide materials are soluble, there is a need to replace the dressing at 3-month intervals till the closure of apex occurs which is ussualy after 6-24 months [21]. The hard tissue barrier formed with the conventional calcium hydroxide apexification technique has been described as "swiss-cheese-like", because of the many soft tissue inclusions, representing a very permeable and weak barrier and more attention is needed when filling the root canal with gutta-percha and sealer. Hence calcium hydroxide use for apexification is no longer advocated.

Pulpotomy

Before the introduction of cvek's pulpotomy completer removal of coronal pulp is done and pulp chambered is filled with calcium hydroxide. Later for minimal pulpotomy procedures a hard setting formulation of calcium hydroxide are used [7]. Healing of the amputated pulp occurs due to release of inflammatory mediators. After the advent of materials such as MTA and Biodentine the use of calcium hydroxide for this purpose has steadily declined.

Canals with exudate

Obturation of root canal with continuous clear or reddish exudates is contraindicated. Hence, for such teeth use of calcium hydroxide dressing is recommended. It

is placed in canal after complete drying of canal with absorbent paper points. It is necessary to dress with a stiffer paste for a period of at least 14 days. If it is found that a substantial amount of the paste has been resorbed more frequent dressings with stiffer pastes may be required. Dressing and irrigation are continued until the exudate is stemmed. The high pH of calcium hydroxide neutralizes acidic pH of periapical lesion and favors bone formation [22].

Perforation Management

Root or furcation perforation is one of the common mishaps in root canal therapy. The mineralization property of calcium hydroxide favors the sealing of the perforation and improves the prognosis. It stimulates hard tissue formation and when extruded it enhances healing of damaged periodontal tissues [22]. Use of calcium hydroxide is contraindicated in crestal and furcation perforations as this may cause pocket formation and contamination. Though MTA is the ideal root repair material calcium hydroxide is still indicated to control infection, stop bleeding and as a temporary solution when inadequate time is available to perform a permanent repair.

Root Resorption

The high pH, mineralizing potential of calcium hydroxide aids in down regulating osteoclastic activity and stimulating hard tissue formation. It also facilitates periodontal tissue healing in the high pH environment. Hence in cases of resorption, pulp is extirpated and lacunae are filled with calcium hydroxide and left for 3 month interval and later removed and obturated. But use MTA is a more definite method of treating [9].

Periapical lesion

Intentional extrusion of calcium hydroxide beyond the root canal into the periradicular tissue is advocated by few authors in case of large periapical lesions as calcium hydroxide enhances the healing and osseous repair. As calcium hydroxide is mixed with barium sulphate in dental compositions the side effects of barium sulphate such as difficulty in resorption and initiation of inflammatory mediators which was observed in few studies [23]. Extrusion is not dangerous except if it reached the inferior alveolar canal where it can cause paresthesia, it doesnot compromise perapical healing [24]. But use of pure calcium hydroxide is preferred for this purpose [25].

REMOVAL FROM ROOT CANAL:

Removal of Calcium hydroxide is difficult especially in curved and narrow canals. Presence of residual paste at apical portion of root canal affects the apical patency during instrumentation and obturation. The retrievability of calcium hydroxide mainly depends on the vehicle used. An oil based mixture is more difficult to remove than powder form calcium hydroxide mixed with distilled water. Various methods have been advocated for removal of the medicament and one of the most commonly used method is by irrigating the canal with NaOCl & EDTA as advocated by Lambrianidis et al. (2006). Use of 10% citric acid was found to be better than EDTA by Nandini et al (2006). And, Ballal et al (2011) has advocated use of 7 % maleic acid. In addition to these irrigants, hand filing or rotary instrumentation, or ultrasonics are effective for removal [21].

SHORT COMINGS OF CALCIUM HYDROXIDE:

Varying alkaline potential of different formulation is a cause of concern as there is no standardization to evaluate the effectiveness. Low solubility and diffusability of calcium hydroxide makes it difficult to gain a rapid increase in pH necessary to kill bacteria within tubules and anatomical variations thereby showing no effect on dense biofilms of bacteria protected deeper inside tubules. The high alkalinity was found to negatively influence the mechanical properties of dentin. It is said to reduce the fracture resistance of radicular dentine and worsen the prognosis of tooth [23]. This can be explained by the Andresen's theory where, the proteolytic action could weaken the tooth up to 50% in one year [22].

Table 1: Types of vehicles used for calcium hydroxide and their applications

Vehicle	Ion release	Solubility	Applications	Examples
Aqueous	rapid	high	Direct and indirect pulp capping, pulpotomy	Distilled water Ringer's solution
Viscous	Slower, for extended periods	Medium	Apexification, Inter appointment Intra canal medicament	Glycerine Polyethylene glycol
Oily	Slower, for extended periods	Low	Perforation repair External root resorption	Olive oil Silicone oil

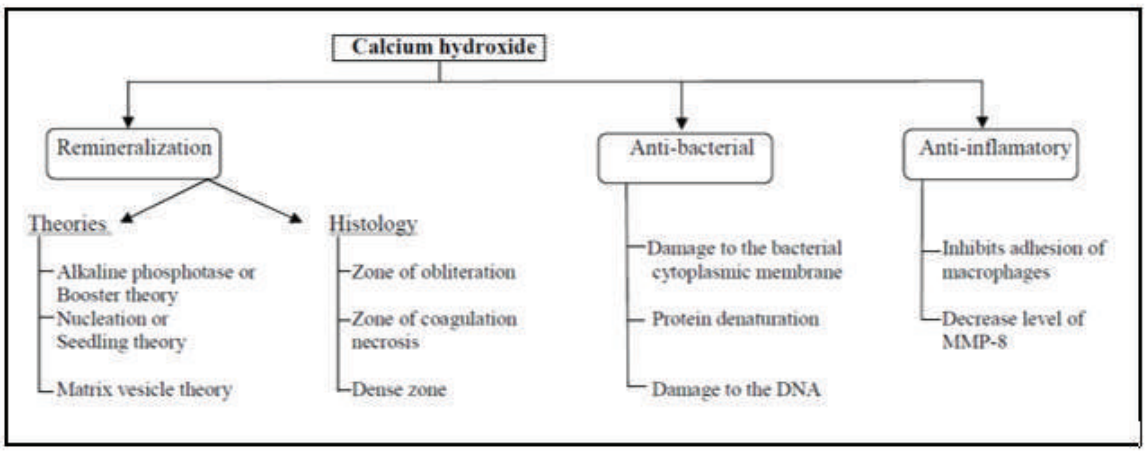


Figure 1: Mechanism of action of calcium hydroxide

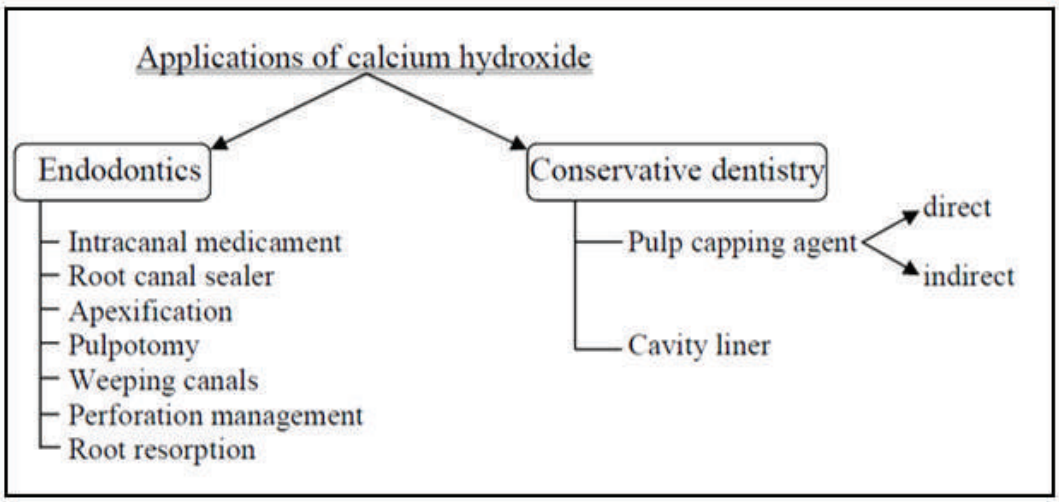


Figure 2: Applications of calcium hydroxide

Conclusion

Calcium hydroxide is considered as one of the gold standard materials in dentistry. Its varied applications and unique mechanism of action and low cytotoxicity has guaranteed its use for this long time and decades to come.

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Conflict of Interest: Nil

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Light Activated Bleaching of Discoloured Non-Vital Tooth: A Case Report

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Abstract

Trauma and pulpal infections are part of the routine of the dental practice. Common consequences in these cases are alterations in dental color, compromising patient's esthetics and his interaction in social environment. Bleaching aids to preserve dental structure as compared to full coverage crowns and also show immediate esthetic results. This clinical case shows a bleaching technique in devitalized teeth using bleaching agent with 35% hydrogen peroxide activated by LED light. The technique is simple to perform and also shows satisfactory results.

Key words: *discoloration, bleaching, non-vital tooth*

Introduction

The colour of natural teeth is affected by several parameters. It depends on the thickness, composition and structure of the tissues forming the tooth. These three parameters will evolve considerably through life, thereby influencing tooth colour. Any pathological or traumatic incidents can cause an everlasting impact on these dental structures. Tissue necrosis due to any cause can lead to release of disintegration by-products that may penetrate tubules and discolour the surrounding dentin. The degree of discolouration is directly related to how long the pulp has been necrotic. The longer the discolouration products are present in the pulp chamber, the greater the discolouration. To treat such discolourations various treatment modalities are available. Of them all, bleaching is known to be one of the most conservative procedures. Bleaching is defined as "the lightening of the colour of a tooth through the application of a chemical agent to oxidize the organic pigmentation in the tooth is referred to as bleaching" [(1)].

In 1864, Dr James Truman of the Pennsylvania College of Dental Surgery published "*Discolored and Necrosed Teeth"*, in which he described the technique for bleaching nonvital teeth. He is credited with the first successful method for bleaching teeth. His method included treating the patient every day for 1 to 4 weeks with chloride of lime combined with a weak acetic acid [(2)]. Techniques were refined throughout the decades using direct or indirect heat in attempts to accelerate the oxidation process [(3)]. Direct heat techniques eventually became less prevalent because of the risk associated with cervical resorption. Chemical techniques using sodium perborate and/or superoxol in the absence of heat continued with some success on nonvital teeth, but efficient techniques for multiple vital teeth were still lacking. Improvement in bleaching products in the mid-1990s including photosensitive formulas, and delivery systems such as light-cured barrier materials, increased use of in-office bleaching for multiple vital teeth [(4)]. Combined with the introduction of at-home

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bleaching trays using carbamide peroxide, bleaching emerged as one of the most sought-after procedures in dentistry [5]. In 1994, Light activation of the bleaching agents was introduced which further led to the activation of bleaching agents by argon laser, CO2 laser and plasma arc. In 1999, Diode laser was first used for tooth whitening. A variety of concentrations of bleaching gels containing fluoride, other remineralising agents, and peroxide free chemicals are also available.

The most commonly used agents for bleaching endodontically treated teeth are 30%–35% hydrogen peroxide and sodium perborate either in combination or separately [6]. The techniques which have been used to bleach discoloured nonvital teeth are: walking bleach and modified walking bleach, non-vital power bleaching, and inside/outside bleaching. The walking bleach technique involves sealing a mixture of sodium perborate with water into the pulp chamber of the affected tooth, a procedure that is repeated at intervals until the desired bleaching result is achieved. Modification of this technique a combination of 30% hydrogen peroxide and sodium perborate is sealed into the pulp chamber for one week and is known as modified walking bleach. For internal non-vital power bleaching the hydrogen peroxide gel (>30%) is activated either by light or heat which raises the temperature between 50 and 60 C, it is maintained for five minutes before the tooth is allowed to cool for a further 5 min. Then, the gel is removed, the tooth is dried, and the 'walking bleach technique' is used between visits until the tooth is reviewed 2 weeks later to assess if further treatment is needed. Finally, the inside/outside bleaching technique is a combination of internal bleaching of non-vital teeth with the home bleaching technique [7]. These methods have a potential risk, which includes cervical resorption, under- or over-lightening, the possibility of colour regression, and external root resorption. Recently, a variety of products and techniques have been developed to resolve or minimize the side effects of the bleaching process. This development has been paralleled with rising interest among patients in correcting esthetic problems with their dentition [8,9]. Therefore, in the current case, many modifications have been done for bleaching techniques in an attempt to minimize the risk and side effects of the bleaching process.

Case report

A 42-year-old male patient with a complaint of discoloured upper front tooth came to our attention. On intraoral examination, the upper central incisor (#11) was a discoloured previously endodontic treated tooth with a broken composite restoration and the upper right lateral incisor (#12) has an Ellis class 1 type of fracture. Radiographic examination revealed a well-obturated tooth with an intact coronal seal and without any periapical pathologies. The patient was explained about different treatment options and he agreed on non-vital bleaching of 11 followed composite restoration of 11 and 12. Informed consent was taken before treatment. On the onset of treatment, the shade of the discoloured tooth was assessed under normal daylight with a Vita porcelain shade guide (Vita classic) and also, a pre-bleaching photograph was taken for the record (figure 1). On the first appointment 2mm of gutta-percha is removed from the canal orifice with a heated instrument and restored with GIC up to the canal orifice and the shape of the cervical barrier is made replicating the CEJ level and the interproximal bone level. And the pulp chamber is closed with temporary restoration (CaVit). 2 days later the bleaching process was started. The gingival is protected by water-soluble cream (Vaseline) applied to soft tissues, and rubber dam isolation was achieved, the flowable light-cured dam is placed in the gap between tooth and rubber dam. For this technique, a high concentration of hydrogen peroxide 35% (NAVKAR DENTAL MAXX WHITE OFFICE) is used. **It is available as a powder liquid system. It is mixed in 1:1 proportion. T**he mixture is inserted into the pulp chamber with an amalgam carrier. It is then activated with LED light for 5 minutes to increase the chemical reaction as advised by the manufacturer. This process is repeated twice more where we changed the gel and repeated the bleaching until desired results were obtained (figure 2). Then, the pulp chamber was rinsed and dried and filled with calcium hydroxide to be left in the pulp chamber for 2 weeks before the final or permanent filling material [10.11]. Clinical evaluation was recorded by comparing the tooth shade with its original one before treatment using the Vita porcelain shade guide and photographs (figure 3). After a week tooth was restored with composite resin, both in 11 and 12. Final polishing is done after 1 day and the patient was reviewed after 3 months and no colour variation was

observed (figure 4).



Figure 1: pre-bleaching record of discolored and contra-lateral tooth.



Figure 2: Tooth shade variation: pre-operative, first appointment; second appointment; third appointment; post-restoration.



Figure 3: pre-bleaching, post-bleaching and post-restoration.



Figure 4: 6-month follow up

Discussion

The bleaching of teeth is a harmless process, but some conditions have to be respected like the correct and complete soft tissue isolation to protect them from eventual burns caused by the peroxide. Some modifications have been done in an attempt to minimize the risk of cervical or apical resorption by placing a base of 1–2 mm glass ionomer cement over the root filling material to assure a mechanical barrier between the sealed root canal and the bleaching gel, which is in agreement with other studies [8,12] but is in disagreement with Friedman et al. [13], as they did not use an intermediate lining before the bleaching material. Another modification added to the bleaching technique was on reaching the desired shade, the pulp chamber was obturated by calcium hydroxide for seven days before the final filling material was placed. This was necessary to allow for the elimination of residual oxygen, which interferes with the polymerization of the filling materials and to neutralize and render the medium alkaline that reduces the risk of cervical resorption [14]. The bleaching was done in three different appointments rather than one-time application as prolonged use causes bleaching action to slow down beyond a point during treatment. This is the point where whitening should be terminated. If it continues further decomposition of organic matrix and total loss of enamel matrix protein may occur [15]. *LED light was used to activate bleaching agent in this case. Light-emitting diode (LED) lamps* are solid-state, semiconducting energy sources that supply near-monochromatic light. LED lamps are currently one of the most energy-efficient and rapidly developing light technologies. Because LEDs produce a discrete or narrow spectrum of light, the light source requires no additional filtration of extraneous energy and produces very little heat. As a result, an LED bleaching light system is dependent less on heat and more on the wavelength-specific photochemistry of the bleaching formula and possible energy absorption of the natural tooth chromogens contributing to bleaching effect. The role of bleaching lights in dentistry is a topic for which there has been controversy and a lack of agreement. This lack of agreement can be attributed to variability associated with methods used to measure color, different light sources, and bleaching formula interactions [16]. Some clinical studies have reported significant effects with bleaching lights, whereas others have shown no effectiveness. Still, others have found mixed results depending on tooth inclusion [17] or method of color measurement [16]. The trend for future lamps may rely more on specialized light sources such as LEDs or lasers rather than filtered light to illuminate the teeth. As refinements in material photochemistry and improvements in spectral properties

of bleaching lamps continue, the use of supplemental light devices in dentistry is expected to remain popular and continue to grow in the foreseeable future [18].

Conclusions

This case report demonstrates the successful management of a discolored non-vital tooth using 35% hydrogen peroxide gel with light activation as bleaching material, effectively and safely, by following modifications and precautions to eliminate the side effects of peroxides. More advanced studies still needed to gather more information about the stability of results and to detect any adverse effect that could appear.

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Ethical Clearance: Not required for a case report manuscript

Conflict of Interest: Nil

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Nanotechnology and its Application in Restorative Dentistry: A Review of Literature

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Abstract

Norio Taniguchi, in 1974 coined the term 'nanotechnology'. It includes the processing of separation, consolidation, and deformation of materials by one atom or one molecule. Nanotechnology has paved path for enhancing many material properties. Similarly so in dentistry it has led to enhancement of many traditional restorative materials such as dental composite and GIC which has made it possible to use these restorative materials in almost every region of the tooth. The aim of the present review article is to provide insights into recent developments in the field of restorative dentistry favored by nano technology such as, nano-composites, nano-bonding systems, nano-GIC, nano-varnish and sealants. It can be an aid to the dentist in clinical practice or in research field.

Key words: *nanotechnology, nano-composite, nano-GIC, nano-ceramics*

Introduction

In 1959, Richard P Feynman first described Nanotechnology in his in famous lecture "there's plenty of room at the bottom" [(1)]. It has been part of mainstream research with potential medical and dental uses. Nanotechnology has made notable contributions in dentistry with its various applications ranging from irrigating root canal system to regeneration of tooth. The restoration of tooth structure with nanomaterials has been one of the most widely applied aspects in dentistry. They offer advantages mainly in the smoothness, polishability and precision of shade characterization along with the flexural strength and micro-hardness [(2,3)]. Additionally incorporation of functional materials or structures of nano size in restorative materials can be used to control the formation of cariogenic bacterial biofilm adherence as they can deliver bioactive materials and antibiotics [(4)]. Nanomaterials are categorized as zero-dimensional (nanoclusters and nanoparticles), one-dimensional (nanotubes and nanowires), two-dimensional (nanoplates and nanolayers) and three-dimensional (nanospheres and nanorods) [(5)].

A wide variety of materials are available to restore the lost form and function of decayed teeth. Use of conventional dental amalgam was widely in use as restorative material in stress bearing areas due to its superior strength, but has compromised esthetics due to its metallic color. Esthetic demands have necessitated the use of tooth coloured restorative materials like dental composites and glass-inomer cements. Though they are superior as esthetic restorative materials in anterior tooth region, they could not effectively bear the occlussal forces in the posterior regions. Incorporation of nano particles into these materials has enhanced the physical properties and favored the use of these materials in the posterior tooth region. This led to the development of materials like nano-composites, nano-bonding systems,

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nano-GIC, nano-varnish and sealants.

Nano-composites

Nanocomposites are available as nanofills (1 to 100 nm size) and nanohybrids (0.4 to 5 μm) [6]. Nanofiller particles are available either as nanomeric particles or nanoclusters. Nanomeric particles are monodispersed colloidal silica in non-aggregated and non-agglomerated form [7]. These nano fillers are generally invisible as they are too small to absorb or scatter visible light and hence does not influence optical properties of resin.The fillers provide incremental improvement in the mechanical properties of the resin where, with the increased filler volume the strength increases proportionally and hence many studies have supported use of microfillers over nanofillers. Hence, to modify loading and distribution of filler particles 'nanofills' are introduced [8]. They are a combination of nano- and microsized filler to produce a hybrid material like 3M filtek (3m ESPE). Nanoclusters were later introduced as an alternative to the hybrid fillers. Nanoclusters are either zirconia-silica particles or silica treated with 3-methacryloxypropyltrimethoxysilane with size of 0.6 μm[7]. Additionally, ceramic nanofillers are being studied for its aesthetics and durability. Recently, Carbon nanotubes have proved to process good mechanical properties and bioactivity which can help to further reinforce the composite resin.

In recent days, restorative materials with anti caries properties are obtained by addition of remineralizing or anti cariogenic materials in the resin (figure 1). To increase mineral content calcium and phosphate ion-releasing fillers such as nanoparticles of dicalcium phosphate anhydrous and tetracalcium phosphate are used. Various calcium phosphate formulations, such as nano hydroxyapetite and nano-acidulated calcium phosphates are being used as fillers to incorporate remineralising properties in dental composites. Addition of nanobioglass has shown to facilitate fluoride release from dental composite (figure 2). For the anti cariogenic property various nano particles such as chlorhexidine and quarternary ammonium compounds have shown to be effective [9, 10].

Nano-Bonding system

Incorporation of nano-particles in dentin bonding agents have shown to improve tubular penetration and thereby increasing the mechanical properties of the restoration (eg : prime&bond NT, Dentsply). Nano silver particles, Nano amorphous calcium phosphate and Nano quaternary ammonium dimethacrylate (nQADM) are few of the widely studied materials [11]. Recently, Colloidal platinum nanoparticles have shown to increase the bond strength of resin [12]. Similarly, Spherical zirconia nanoparticles are used in bonding systems which aids in stabilizing the hybrid layer. Newly, the concept of nanogels has been introduced. They are 10- to 100-nm particles which on interaction with monomers get swollen. This property allows penetration of adhesives deep into dentin and increase the bond strength [10].

Nano-GIC

Wilson and Kent in 1971 introduced GIC [13], ever since it has been a widely used in dentistry like Ketac Nano (3m ESPE). But, it had lower mechanical properties than other traditional restorative materials. The incorporation of nanoparticles favored to increase particle size distribution and thereby increasing the mechanical values of GIC. Forsterite nanoparticles, when incorporated in GIC enhanced the fluoride release [14]. Nano-fluorapatite or nano fluoro hydroxyapatite has also showed similar properties [15]. Use of nano agglomerated glass has improved the compressive strength if GIC [15]. Similarly so, titanium nano particles are also used. Various antibacterial agents, zinc, silver nanoparticles are also incorporated to provide anti cariogenic properties [16].

Recently, resin modified glass ionomers are modified by addition of nanoparticles and nanoclusters in fluoro-aluminosilicate glass. It resulted in the aesthetic improvement of the final restoration and polishablilty. It has led to the improvement is setting reaction, Improvement in poly salt bridge formation, Better bonding with dentin and More compressive strength. Similarly, Nanoparticles of CHX hexametaphosphate are used as an anti microbial agent but nano-chlorhexidine modified GIC shows less fluoride release than conventional one which is a drawback [9].

Nano-Varnish and sealants

Hydroxyapetite and fluorapatite bioceramic nano-fibers have nanostructure solubility which aids in effective release of fluorine ions. Which when incorporated

in sealant system can cause caries prevention. The nano-sealants provide wear resistance and a reduced shrinkage [17]. Another application of nanotechnology is the development of a nano-filled light curing varnish. It is used to protect GIC during early maturation phase. It prevents water sorption ad dehydration of GIC thereby, improving its mechanical properties. Eg., EQUIA ('Easy-Quick- Unique-Intelligent-Aesthetic') [18].

Nano coating of restorations

Nano-filled, light-cured preparations are used as a protective coating for composite and acrylic indirect composites like the Optiglaze Glossy Protective Coating Agent (GC Corporation, Japan). These solutions provide an aesthetic glossy surface and can be used in difficult to polish areas such as posterior fissures or interproximal areas of indirect composite restorations. It can be used on indirect composite restorations, artificial teeth, removable dentures, temporary crowns and individual acrylic trays to provide a glossy finish.

Nano polishing system

Polishing of dental surfaces is a known practice in dentistry which is used to make it difficult for plaque and cariogenic bacteria to accumulate on tooth surfaces. Thus, polishing is a preventive procedure used as a primary defense against dental problems. Micron-sized silica particles are a typical component of conventional polishing pastes. The smaller the abrasive particle size the smoother the polished surface. Chemical-mechanical planarization process is one such approach, used in the semiconductor industry which uses various nanometer-sized particles to polish surfaces of semi-conductor wafers to a sub-nanometer level. This was adopted to polish dental surfaces by R.M. Gaikwad and I. Sokolov (2008), where they have used siver nano particles and demonstrated that it is easier to remove bacteria from areas polished with silica nanoparticles. And it can be used for dental polishing [19].

Nano ceramics

With the integration of nanotechnology and ceramics a new CAD/CAM block of monolithic esthetic material was developed as Lava Ultimate (3M ESPE). It is a mix of nano particles agglomerated in clusters and individual bonded nano particles embedded in a highly cross-linked polymer matrix. The main advantage of this material is said to be the easier clinical finishing and polishing, without the need for a porcelain oven, with the strength, surface gloss and finish retention similar to ceramic materials which has been the main limitation of composite blocks. It's typical composition is made up of non-agglomerated/non-aggregated silica (20 nm), aggregated zirconia (4 to 11 nm particles) or silica clusters (20 nm particles) and non-agglomerated or non-aggregated zirconia (4 to 11 nm) with approximately an 80% ceramic load. Though there are a few in vitro studies showing its resistance to toothbrush abrasion and retention of the initial glossy surface finish similar to glass ceramics, clinical evaluation is yet to be obtained regarding its marginal integrity and survival [20].

A nano optimized mouldable ceramics are developed recently and marketed by the name XircOn Ultra, which is approved by FDA. It is a hybrid nano-ceramic that can bend and flex in the mouth. It is made by a combination of nano-ceramics and polymers. It is said to be one of the closest material to human teeth. Nano fillers enhance polishability reduce wear. The use of nano pigments adjust the shade of the restoration by chameleon effect and nano modifiers increases the stability of the material thereby prevent sticking to instruments.

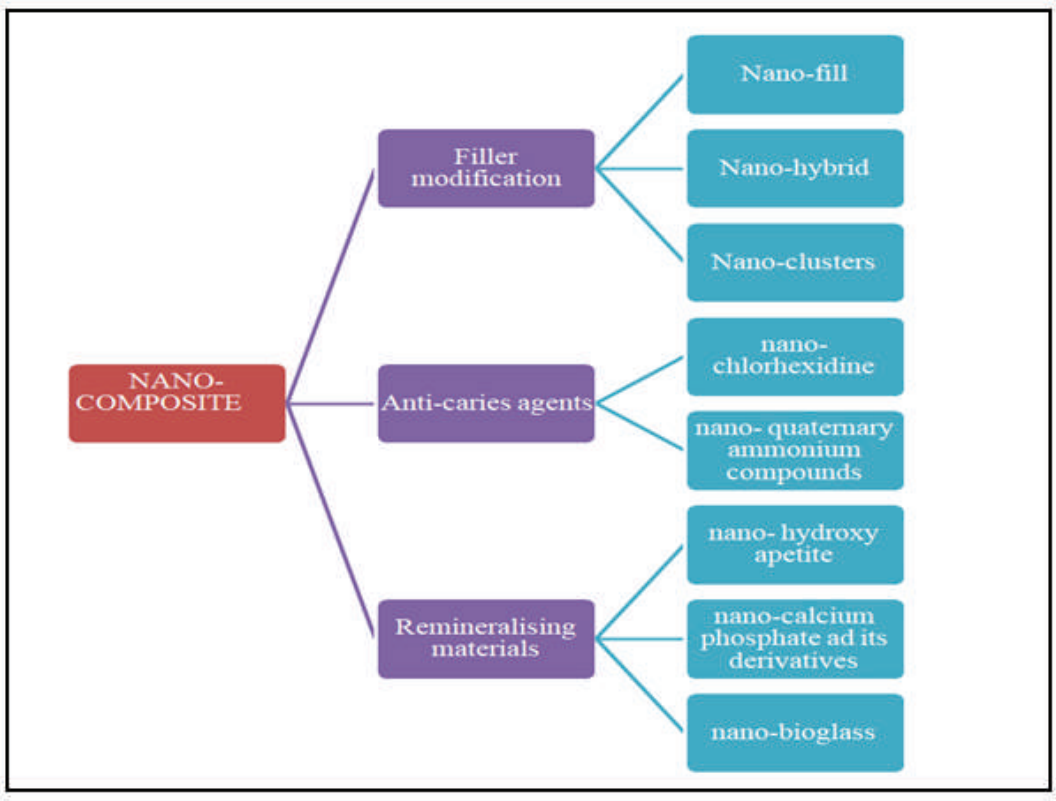


Figure 1: Nano-composite materials.

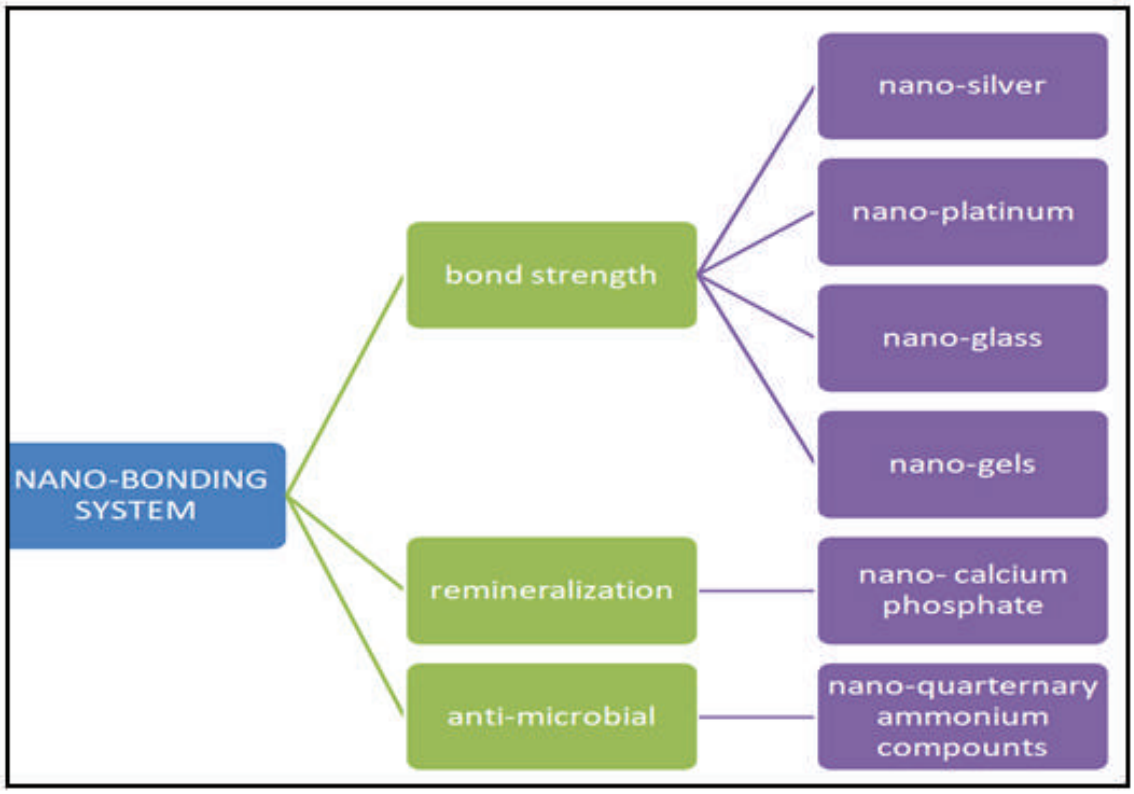


Figure 2: nanoparticles used in bonding systems.

Conclusion

With evolution of material science, newer dental materials are being developed for improving the ease of use and mechanical properties. Nanotechnology is playing a key role in such product development and has proven to be an efficient method for this purpose. A thorough knowledge of these nano restorative materials and its applications helps in efficient use in dentistry. In future, the use of nano-robotos seems to be the effective way in restoring early tooth surface defects and carious lesions. But it is still a clinically unexplored arena and the wait doesn't seem too far to explore it.

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A Brief Review on Haematoxylin: An Irreplaceable Tissue Stain

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Abstract

Haematoxylin is the most popular nuclear counterstain used in routine histopathogical techniques for staining tissues. It is derived from the tree Haematoxylum Campechianum (H.campechianum). The history of haematoxylin reveals centuries of war, strife and discovery dating from the Mayans and Aztecs who used it as an ink and fabric dye to the war resulting in the invasion of Mexico by European countries. H.campechianum was later named as logwood and block wood by the invaders that used it to generate haematoxylin crystals to be utilized in textile industry. The recognition to introduce haematoxylin as biological stain goes to Waldeyer. But later Bohmer introduced the technique of coupling a mordant to the dye making it colorfast and thus, haematoxylin remains a very unique and vital stain in histology till date.

Key Words: *Haematoxylin, Histology, Stain, Counterstain*

Introduction

Haematoxylin is the most common nuclear counterstain used in histology. It shall not be an over exaggeration to say that it is the cornerstone of staining tissue sections and the foundation upon which modern histopathological diagnosis is built. Haematoxylin is a natural dye derived from the tree Haematoxylum Campechianum (H.campechianum),that belongs to the order Legumiosae (Genus-Eucaesalpiniea) and is so named because of the reddish nature of its heartwood and young leaves. In Greek haemato means blood and xylo means wood.[1]There are several varieties of Haematoxylin, but H.campechianum gives the best coloured wood. H.campechianum tree grow in swamp lands distributed throughout Guetamala, Belize, and the Yucatan and Campeche area of Mexico (Central America).[2]

Initially, extracts of H.campechianum were used for dyeing in textile industry. For the transportation of H.campechianum from Mexico to Europe by sea the tree was cut into three-foot logs or blocks and hence was also called Logwood or Blockwood. However, the artisans in the textile industry did not know how to use the logwood to make the dye colorfast. Therefore, the fabrics dyed with logwood extract faded quickly.[3] Later a process was developed in which metal was used with a natural dye to make the dye colorfast. This process was widely applied by the industrialists, the metal used was called 'mordant'.[4] In 1810 a French chemist named Michel Eugene Chevreul isolated Haematoxylin crystals. The crystals were obtained by boiling H.campechianum heartwood chips in water, which produced an orange – red solution that turned yellow, then black upon cooling and yielded crude red haematoxylin crystals after

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evaporation of water. Haematoxylin is a neoflavenoid, a phenolic compound related to flower pigments.[5] Haematoxylin is the result of the decomposition of a glucoside that exists in fresh heartwood. Regarding the glucoside itself little is known, except that under the influence of a species of fermentation it is decomposed with formation of sugar and haematoxylin.[6] Waldeyer is frequently credited to have introduced haematoxylin as a biological stain, using it unsuccessfully to stain neuronal axons in 1862. But truly indeed, the potential for haematoxylin in histology should be credited to Bohmer (1865), who inspired by the textile industries, combined a metallic mordant with haematoxylin to stain tissues adequately.

Although the formulation of Bohmer is no longer in use, coupling of haematoxylin with a mordant is still the major form in which the dye is used.[7] The chemical basis of colour is due to certain atomic groupings known as chromophores(Greek=colour bearers) e.g-NO2,N=O,N=N,C=C,C=O,C=N,C=S and the quinoid group .Such groupings introduce 'resonance systems' in the molecule. Resonance systems result when molecular formula can be written in several valid ways. The rapid change resulting from the presentation of possible alternate states involves the absorption of electromagnetic waves and the production of colour. Benzene which does not show colour in white light, shows colour in UV range. The introduction of chromophoric group into an uncoloured molecule will cause it to be coloured, then it is called a chromogen. For a chromogen to be called a dye, it must be composed of an acid and a base and therefore should have salt forming properties. This function is performed by a group called auxochromes (Greek-increasers) which when attached to the dye molecule act as an electron donor to the chromophore. The OH and NH_2 are the main auxochromes; others are COOH(Carboxyl), SO_3H (sulphonic) groups. These can also serve to bind the dye to the tissue besides dissociating and forming compounds.[8] In pure form haematoxylin is a colourless or slightly beige powder which gives a colourless solution with water. Rudolph Nietzki was the first to discuss the chemical nature of haematoxylin in his 1892 publication in the 'Chemistry of Dye stuff'.[9] Pure aqueous haematoxylin cannot stain. The active staining agent is not the principal substance but rather an oxidation product of haematoxylin called haematein.[9,10] Haematoxylin and haematein differ by only hydrogen atom. Removal of this hydrogen from haematoxylin can be accomplished either by atmospheric oxygen or by using mild oxidizing agents and results in a compound with a hydroxyl group adjacent to a carboxyl group. This configuration facilitates formation of co-ordinate complexes with metals.[11]

Ripening

The process of generation of haematein from haematoxylin is called ripening. Haematein can be produced naturally through exposure to air and sunlight or UV light. This natural process of ripening can take upto 3 months or longer. Examples of use of naturally ripened dye are the methods of Delafield and Ehrlich's haematoxylin.[12] Chemical oxidants can also produce haematein from haematoxylin by the process of ripening in a shorter period, but does carry the risk of developing effective reaction products, a solution with a shorter shelf life than the naturally ripened dye.[13] Chemicals like potassium permanganate, iodine, sodium iodate, sodium periodate, hydrogen peroxide and mercuric oxide are example of oxidants used to ripen haematoxylin. Common stains like Mayer's and Ehrlich's formulation use sodium iodate while Harris' preparation relies on vigorous boiling and addition of mercuric oxide to produce haematein. Although chemical oxidation produces a working dye in very short period, it has to be performed very carefully to avoid over-oxidation of haematoxylin. Several substances may be produced as a consequence of over oxidation, but the most significant is a quinine-carboxylic acid, which is called oxy-haematein. Another important aspect is that haematoxylin powder may oxidize during prolong storage.If such a dye is used with usual amount of oxidant, it would unavoidably give rise to over oxidation products. One of the means of avoiding this problem has been to use half the normally required amount of chemical oxidant. The half oxidized solution has a greater shelf life sine unoxidised haematoxylin forms a reservoir which through natural ripening gradually replaces haematein that is over oxidized or exhausted by staining.[14] Haematein forms rapidly in alkaline solutions but slowly in acidic solvent. Addition of alcohol or glycerol further slows down oxidation, alcohol can also act as preservative.[15]

Mordants

Mordants are chemical substances that by their physico chemical structure aid in attaching a stain or dye to the tissues. They are essential to haematoxylin staining; the mordants used are always divalent or trivalent salts or hydroxides of metals. They probably combine as hydroxides with the dye by displacing hydrogen atom from it, and their remaining valences serve to attach or bind the dye-mordant complex to tissue components specially phosphate groups of nucleic acids.[14] The chelate complex of dye and mordant is called a 'lake'. The term 'lake' is derived from 'lac', a mordant dye obtained from an insect in India and from which shellac is obtained. Over the time the term 'lac' has changed to 'lake' and has come to be the generic term for all dye-mordant complexes.Two types of bonds are involved in the fundamental reaction between the dye and mordant. One is a covalent bond with hydroxyl oxygen, the other is a co-ordinate bond with another oxygen (electron donor).The alum salts are most commonly used because they were more readily available. Mordants and dyes can be applied in three ways:[15]

· Onchrome: Mordant is applied first followed by the dye. Heidenhain's iron haematoxylin is a classic example.

· Metachrome: Mordant and dye are mixedtogether and then applied.It is the most common type.Example-alum haematoxylins.

· Afterchrome: The dye is applied first followed by mordant.

Progressive and Regressive staining

In progressive staining, the staining is continued until the desired intensity of colouring of the different tissue elements is attained. e.g.-Mayer's, Delafied's, Harris' and Gill's haematoxylin formulae. In regressive staining the tissues are overstained and the excess dye is then removed selectively until the desired intensity of colour is attained. e.g. Ehrlich's, Harris' and Delafield's formulae. The designation 'selectively ' is due to the fact that when excess dye is removed, it is cleared from certain cell constituents before others, while other cell constituents are still strongly stained. This process of selective removal of excess dye is called 'differentiation'. Generally if the dye used is a basic one, differentiations are carried out by an acid solution and vice versa. For both basic and acidic dyes, alcohol acted as a fairly efficient differentiator and probably acts by simply dissolving out the excess dye.[16]

Differentiators are of three types:[16]

· Acid differentiator —These act by combining with metal, thus breaking the latters association with the tissue or cell components. e.g.-acetic acid and hydrochloric acid.

· Oxidising differentiator — These act by oxidizing the dye to a colourless form (leuco form). Cell components holding the least dye get bleached first. e.g-Potassium ferricyanide, Chromic acid, Potassium permanganate, Potassium dichromate and picric acid.

· Mordant differentiator — The process happens due to mass action. When a section that has been stained by a mordant dye in a solution of mordant, the latter is present in great excess and the dye gradually leave the tissue to combine with free mordant in the solution. Also iron alum mordants can oxidize haematoxylin to a colourless compound, tissue component, which hold the least dye gets decolourised first.e.g-aniline used with gentian violet, Phenol used with carbolthionone.

Blueing

Acid solutions of alum haematoxylin are reddish in colour while the aluminium lake of haematein is blue in colour. This is because Alum (Potassium aluminium sulphate) is watery solution and tends to dissociate: the aluminium combines with the OH group of water to form insoluble aluminium hydroxide; the free hydrogen from water tends to form suphuric acid by uniting with suphate from alum. However, if excess of acid (Sulphuric or any other)is present aluminium hydroxide does not form. Under these conditions, in an alum haematoxylin dye the insoluble dye lake cannot form because of lack of hydroxyl ions. Hence alkaline treatment is required to neutralize the acid to restore blue colour to the tissues.[17]

Various types of Haematoxylin stains

There are six common types of haematoxylin stains in use:[18]

1. Alum haematoxylin-Alum haematoxylin

solutions contain potassium alum or ammonium alum, but the active principle being aluminium(mordant).They includeDelafield's,Ehrlich's,Harris',Mayer's,Carazzi's, Cole's, Gill's and Iyiola and Avwioro'shaematoxylin formulations. Alum haematoxylins are used when the counterstain doesnot contain an acid.

2. Iron haematoxylin-In iron haematoxylin stains, iron salts are used both as the oxidizing agent and as mordant. The most commonly used iron salts are Ferric chloride and Ferric ammonium sulphate. Generally, iron haematoxylin stain requires differentiation. E.g - Heidenhain's haematoxylin, Anderson's Iron haematoxylin, Weigert'shaematoxylin, Verhoeff's haematoxylin.

3. Tungsten acid haematoxylin-There is only one widely used tungsten haematoxylin, although many variants on the main technique of Mallory's phosphotungstic acid haematoxylin (PTAH)has been described.

4. Molybdenum haematoxylin-Haematoxylin solutions that use molybdic acid as the mordant are rare, the method of Thomas (1941)is the most accepted method and described by McManus and Mowry. It is used to stain collagen, reticulin, argentaffin cell granules.

5. Lead haematoxylin-Lead haematoxylin stain is relatively recent in development, mostly used for demonstration of granules in the endocrine cells of alimentary tract and other regions.

6. Haematoxylin with chromium-As in Weigert – Pal technique (Weigert1885,Pal 1886).This is a hybrid technique as it employs Weigert's mordant, Pal's differentiator and usually Kultschitzky's (1889) haematoxylin. Mordating in chrome salts forms a chromium dioxide complex so that normal myelin forms a lake with haematoxylin. Only long standing myelin degeneration will show lack of staining.

7. Haematoxylin without mordant-Under appropriate conditions, free haematein is weakly anionic and will stain cationic tissue components-particularly collagen, but also elastin, erythrocytes and contractile elements in smooth muscle-yellow to orange-brown. Non-mordanted, dilute solutions of haematoxylin (haematein) also colour nuclei through binding to basic nuclear proteins.In the Clara haematoxylin procedure, non-mordanted haematoxylin reacts with arginine residues and stains keratin, keratohyalin, and basic nucleoproteins.

Uses of Haematoxylin[16,18]

· Carbohydrates: Connective tissue mucin can be stained with Mayer's haematoxylin. Haematoxylin has also been used as a nuclear stain in some techniques when demonstrating carbohydrates. These techniques include Best's carminefor glycogen, Periodic Acid Schiff for neutral mucopolysaccharides and congo red for amyloid.

· Lipids: Baker's acid haematein method is used for demonstration of phospholipids, while the alum haematoxylins are used as nuclear counterstains when oil red O is used for staining the lipids.

· Connective tissue fibers: Verhoeff's haematoxylin has been used for staining elastic fibers black. Weigert's haematoxylin and Celestine blue haemalum sequence are used for nulclear stains.

· Nervous tissue: Weigert-Pal's, Loyez and Weil's methods are used for staining myelin. The haematoxylin used in these methods is the active ingredient to stain astrocytes, myoglia.

· Intracellular substances: Heidenhain's haematoxylinis an excellent stain which stains chromosomes nucleoli, mitochondria, and cross striations of muscle fibres.

· Cytology: Papanicolaou is very popular cytological technique for epithelial cells to distinguish between malignant and non-malignant cells, here haematoxylin plays major role as a stain.

· Microfilaria and amoeba: The differential diagnosis of microfilaria based on nuclear arrangement is enhanced when their nuclei are stained by hot haematoxylin. Amoeba also stains well with alum haematoxylin.

Conclusion

Undoubtedly haematoxylin is one of the most important biological stains. It is valuable not only because it is a powerful nuclear stain and a chromatin

stain par excellence, but also because it has polychrome properties. With proper differentiation it is possible to get several shades between blue to red to show in the same preparation.

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Aloevera: The Miracle Plant and Its uses in Dentistry – A Review

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Abstract

Aloevera has been in used by humans since times immemorial. Its health benefits are well known. Ancient Greeks and Egyptians used it in their daily routine and in battle fields due to its many miraculous properties. Studies have proved the antiseptic, anti-inflammatory, anti-viral, anti-fungal and wound healing properties of aloe vera plant. Recently it has garnered a lot of interest regarding its use in dentistry as it is completely natural and there are no side effects reported with proper usage. This paper gives an overview of the uses of this wonder plant in various departments of dentistry.

Keywords: *Aloevera, antibacterial, antiviral, anti-inflammatory, antioxidant*

Introduction

Aloe vera is a miracle plant and it has been known for its numerous benefits. It has been used for centuries for its health, beauty, medicinal and skin care properties. First reference to aloevera was seen in A.D. 1655 of Dioscorides' Medical treatise De MateriaMedica. It was used by Egyptian queens Nefertiti and Cleopatra in their regular beauty regimes and by Alexander the Great, and Christopher Columbus for treatment of wounds of soldiers. The botanical name of Aloe verais *Aloe barbadensis miller*. [1, 2. 3, 4]The *Aloe barbadensis* plant consists of two parts , the parenchymaltissue makes up the inner portion of the aloe leaves and producesthe aloe vera gel (or mucilage), a clear, thin, tasteless, jelly-like material. This tissue is used by separating the gel from the inner cellular part. [5, 6] The other part is specialized cells known as the *pericyclic tubules*, it occurs beneath the outer green rind of the leaf. It is yellowish latexwith powerful laxative-like actions. [7,8, 9]

Composition

Aloe vera gel has a very complex chemical composition. [10, 11] Vogler and Ernst listed 75 of its active constituents. Reynolds and Dweck listed 16 different polysaccharides and Yamaguchi et al reported presence of nine different minerals.

The constituents are listed in the table below [12, 13, 14, 15, 16]

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Vitamins	antioxidant vitamins A, C and F. Vitamins B(thiamine), niacin, vitamin B2 (riboflavin), vitamin B12,choline and folic acid.
Enzymes	Biochemical catalysts, such as amylase and lipase which aid in breakdown of fats and sugars. Carboxypeptidase, produces anti-inflammatory and analgesic effect
Minerals	Sodium, potassium, calcium, magnesium, manganese,copper, zinc, chromium and iron are all found in the aloeplant Magnesium lactate prevents the formation of histamine from the aminoacid, histadine this explains the antipuriticeffect of aloe vera.
Sugars	Sugars are derived from the mucilage layer of the plant are glucose andmannose, known as the gluco-mannans.
Anthraquinones	The bitter aloes consist of free anthraquinones and theirderivatives ,Barbaloin, aloe-emodin-9-anthrone,lsobarbaloin ,Anthrone-C-glycosides and chromones. Powerfulpurgative effect and potent antimicrobialagents. Lignin with their penetrativeability to carry other active ingredients deep into the skinto nourish the dermis.
Sterols	Campesterol, Sitosterol and Lupeol.
Salicylic acid	This is an aspirin-like compound possessing anti-inflammatory and antibacterial properties. It has akerolytic effect which helps to debride a wound ofnecrotic tissue.
Aminoacids	These are the building blocks of proteins. Aloe vera gelprovides 20 of the 22 necessary amino acids required by the human body and seven of the eight essential aminoacids which the body cannot synthesize

Properties useful in Dentistry[17, 18, 19]

- Wound healing
- Antibacterial
- Antifungal
- Antiviral effects
- Antitumor
- Antioxidant activity
- Anti-inflammatory activity
- Anti-mutagenic activity
- Immunomodulatory activity
- Gastro-protective activity

Applications in Dentistry [1,3,5, 8, 9]

Periodontology

- Aloe vera gel laced cotton pads can be used at site of periodontal surgery.
- Application to traumatized or scratched gum tissues by toothbrush-dentifrice abrasion, sharp foods, dental floss, or toothpick injuries.

· Pockets when filled with gel upto gingival margins& Coe- pack placed over it shows reduction in pocket depth, gingival index and plaque index

· Gingival & Periodontal Diseases

· Aloe Vera use shows reduction in gum bleeding due to its soothing & healing properties, reduction in swelling and oedema is also reported

· Reduces plaque & calculus formation. Aloe vera based mouthwashes if used two times show effective reduction in plaque and calculus formation

· To treat Halitosis- Natural anti-fungal and antibacterial properties of aloe vera eliminates microorganism's causative of halitosis. Drinking 1/4 cup of pure Aloe Vera gel mixed in 1/2 cup of water or apple juice soothes acidity and indigestion, which is a very common cause of bad breath.

· Aloe vera based mouthwashmouthwash prevent radiation-induced mucositis, oralcandidiasis of and Oral lichen planus due to its wound healing, anti-inflammatory and anti- fungal properties.

Prosthodontics and Crown and Bridge

· Topical application in edentulous patients with with sore ridges

· Topical application around Dental Implants as it reduces inflammation.

· Preventing denture stomatitis.

· Application as Denture Adhesives owing to its Sticky & viscous nature

Conservative Dentistry and Endodontics

· Root canal sedative dressing & as file lubricant owing to its antimicrobial effect *Candida albicans*&*Enterococcus faecalis*. Found in pulp chamber

· Intracanalmedicament.

· Lubricating agent

Oral and Maxillofacial Surgery

· Aloe vera gel laced gauze can be used at site of extraction socket to reduce inflammation

· Facilitates wound healing at site of surgeries

Oral Medicine and Radiology

· Topical application in ulcers as soothes & alleviates irritation

· Topical application in Chemical burns (tobacco, aspirin)

· Acute mouth lesions such as herpetic virallesions

· In Gum abscesses

· As Tooth Gel

· Chronic oral diseases Lichen Planus andBenign Pemphigus, gum problems associatedwith AIDS and Leukemia.Migratory glossititis, geographic tongueand Burning Mouth Syndrome.

Side Effects

Rarely can itcause allergic reaction resulting in redness, burning, stingingsensation and generalized dermatitis. If taken orally in excessive dose it might cause abdominal cramps, diarrhea. It has been reported that if used for prolonged durations there is risk of colorectal cancer. [1]

Contraindications

· Oral intake is contraindicated in Pregnancy and breastfeeding

· Known cases of allergy to plants of Liliaceae family

Conclusion

Aloe vera is often referred to as a wonder plant and rightly so because of it's innumerable properties and benefits. Being a natural and easily available products, it can be used in varioushealth conditions , owing to its antibacterial, antifungal, anti -inflammatory, immunomodulatory and antioxidant effects.It can prove to be useful in various branches of dentistry.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Alternative Medicines in Covid 19 – A Review

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Abstract

Emerging in China in late 2019, the coronavirus disease-2019 (COVID-19) infection epidemic is growing rapidly and new cases are reported around the world. The first cases were linked to a wet market, and subsequently, the virus has spread rapidly in China through human-to-human transmission, and the universal impact of the COVID-19 virus is now spreading worldwide. The disease originated from COVID19 is a type of viral pneumonia that is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Currently, no clinically approved antiviral drugs have been introduced for SARS-CoV-2 infection.Identifying the mechanism of action of the virus and its interaction with the immune system will help prevent and treat the disease.There are various researches and studies reporting the use and effect of AYUSH in SARS-CoV treatment. This review article gives a detailed overview of alternative treatment modalities for COVID 19.

Keywords: *AYUSH, COVID 19, Pandemic, alternative medicine, SARS – coV 2*

Introduction

At the end of December 2019, the coronavirus outbreak triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) occurred in Wuhan, Hubei, China, leading to the rapid spread of 2019 novel coronavirus (COVID-19) into a pandemic responsible for the current global health crisis[1]. Coronaviruses are well known animal and human pathogenic organisms that cause a wide range of respiratory, gastrointestinal, neurological, and systemic diseases[2,3]. Actively no clinically approved antiviral drugs for SARS-CoV-2 infection were introduced[1].

No effective management to treat or avoid this infection has existed till date. COVID-19's most common symptoms include fever, exhaustion and dry cough[4]. Some patients may experience aches and pains, nasal congestion, runny nose , sore throat, or diarrhoea. Typically these symptoms are mild, and gradually start[4]. Some people get sick but have no symptoms, and they don't feel unwell[4]. The best ways to avoid COVID19 infection known today as WHO & National Health protocols are to break the spread chain (social distancing, hand hygiene, respiratory hygiene), improve the body immunity of a person, recognise early and appropriate medical treatment for the infection[4].

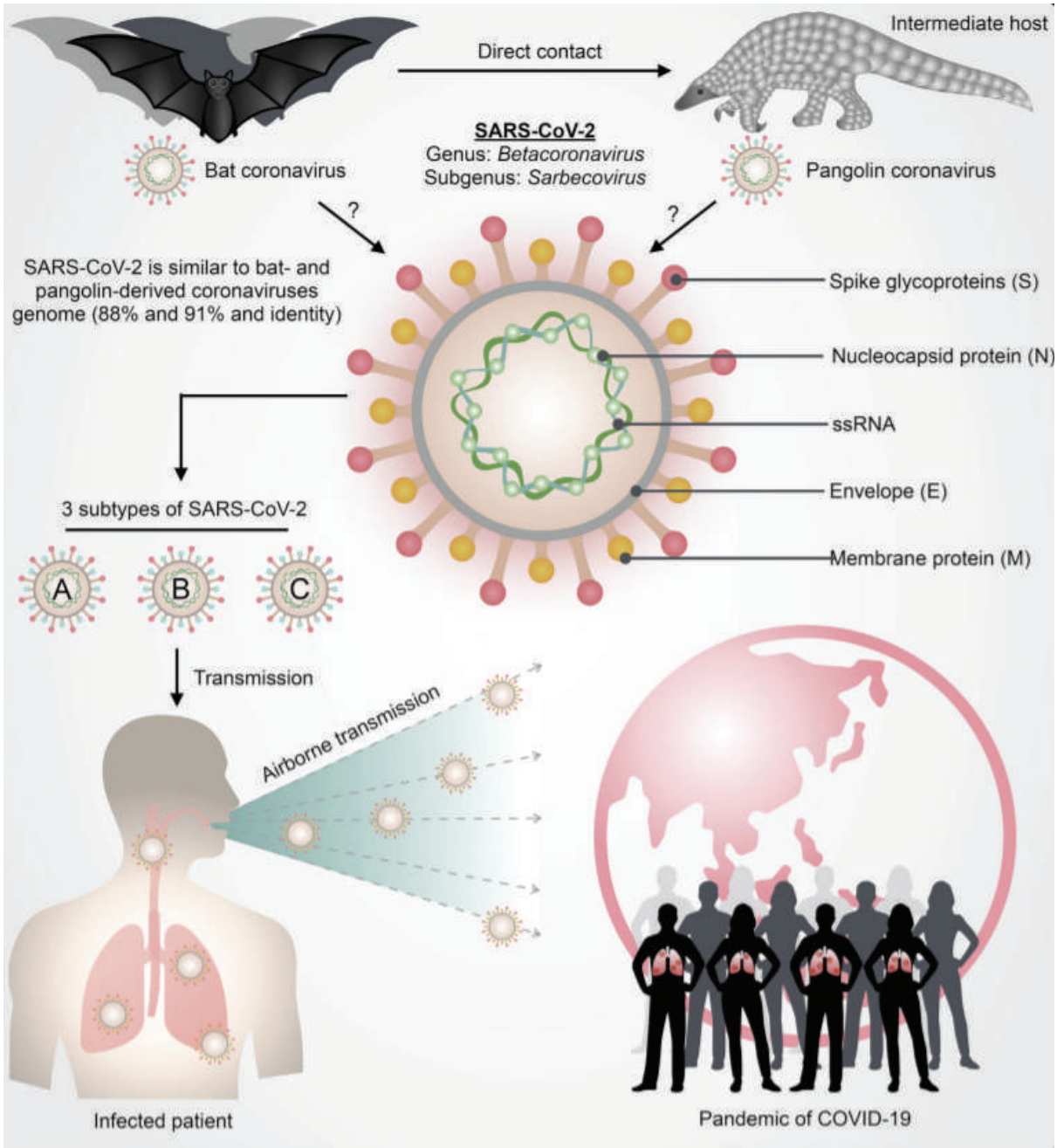
To handle the COVID-19 threat we need successful preventive, promotional, curative and rehabilitative approaches[5]. There is very little modern medicine to give for treating COVID-19 patients. Ayurveda and yoga, ancient health systems have a great deal to say in this current SARS-CoV-2 infection pandemic[5]. Not only can it fill the differences in modern medicine, but it will also complement modern medicine to reduce the burden of COVID-19[5].

Ayurveda and Yoga share the concept of improving the human body and mind, and preserving good health by preventive, educational, curative, and rehabilitative approaches[5]. The convergence of these methods with modern medicine will not only complement one another, but will also provide a novel and efficient way of treating the emerging COVID-19 pandemic[5]. The Government of India has also taken a bold move to undertake this by establishing the 'Interdisciplinary AYUSH Research and Development Task Force' with the aim of reducing

COVID-19-related misery and deaths in India[5].

ETIOLOGY, STRUCTURE AND TRANSMISSION OF COVID-19[1]:

Pictorial representation of etiology, structure and transmission of COVID-19[1]:



SIGNS AND SYMPTOMS:

The signs and symptoms of SARS-coV 2 include[1]:

fever (83%), cough (82%), and shortness of breath (31%), which are often accompanied by muscle ache (10%), confusion (9%), headache (8%), and sore throat (5%) (Fig. 2).14

Approximately 75% of these patients also presented with bilateral pneumonia (75%), 17% of them had acute respiratory distress syndrome, and 11% died over a short time span, owing to multi-organ failure[1].

COMPLEMENTARY MEDICINE:

Since the advent of human race, plants have been the source of numerous compounds with medicinal properties and led to the discovery of medicines[2]. Since ancient times, Indian Ayurveda, Traditional Chinese Medicine Literature, ancient Egyptian Ebers Papyrus all mentions various plants and herbs with medicinal properties that are still used in the world today for different health benefits[2]. Different plant compounds serve as immune boosters and can serve as antibacterial , antiviral, and antifungals[2].

The coronaviruses encode different proteins which allow their functioning[2]. Many plants generate compounds that interact with these proteins, and may become a drug development target[2]. Since the gene sequence of COVID-19 has very strong similarities between the man proteins of COVID-19 and in SARS or MERS, anti-SARS or anti-MERS natural compounds previously mentioned may become a valuable guide for finding the affective anti-COVID-19 herbal plants[2]. There are more than a dozen proteins that have coronavirus encoding[2]. Some of these proteins are important for entry and replication of viruses[2]. The well-studied proteins so far are papain-like protease (PLpro), 3C-like protease (3CLpro), and spike protein. These three proteins all make drug production for attractive targets[2].

Until now, many established allopathic medications are still being constantly evaluated which can avoid a deterioration of the patient's clinical condition and provide early relief. Some patients are given remdesivir, lopinavir, chloroquine, or hydroxychloroquine, and interferon beta-1a[2]. Recently, there has been trial of convalescent plasma therapy[2]. But all these drugs have many potentially harmful side effects thus limiting their use only to vital patients[2]. Furthermore, the effectiveness of these drugs has not yet been confirmed, and is therefore limited to compassionate use only[2].

Continuously, randomised clinical trials are conducted to see and show the effectiveness of these medications and to find an appropriate successful treatment[2]. WHO is performing one of the largest multinational clinical trials called "Solidarity"[2]. In view of the urgency of the need for successful COVID-19 therapy, this trial is said to reduce the usual time taken by 80%. Remdesivir, lopinavir with ritonavir, chloroquine or hydroxychloroquine, and lopinavir with interferon beta-1a2 are commonly used in four regimens[2].

While the same applies to complementary medicinal products and their use depends on their reported effects in the treatment of SARS and MERS and the numerous limited clinical trials and their efficacy against COVID-19 has not yet been proven, the lack of potentially serious side effects provides us with a possible and much-needed window for their use in the treatment of COVID-19 not only in critical patients[2]**.**

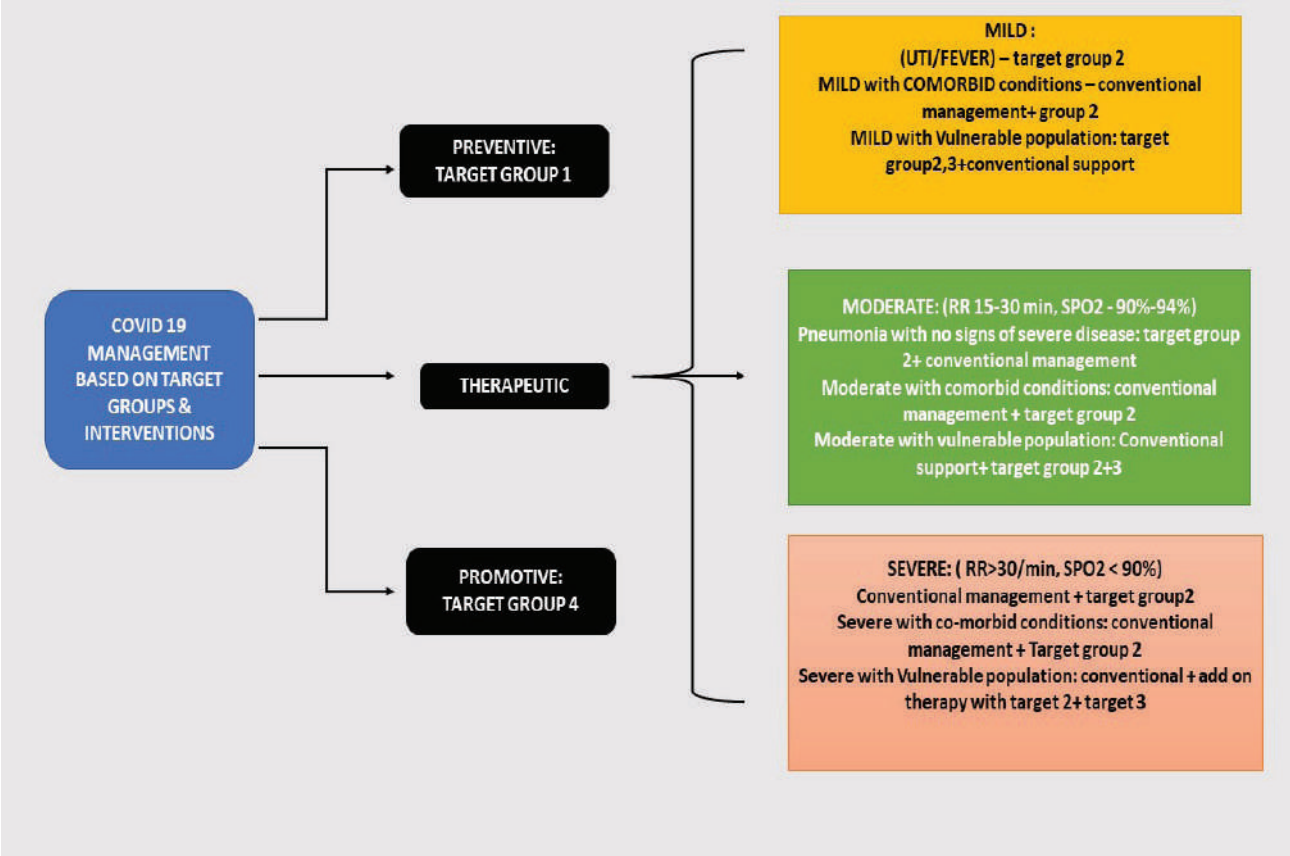
Role of AyurvedaIn Covid19

TARGET GROUP 1: Subjects of quarantine and home isolation with or without corona positive test and health care workers[6].

TARGET GROUP 2: Patients with mild, severe symptoms comorbid and immuno -compromised conditions[6].

TARGET GROUP 3: Vulnerable group (pregnant and lactating women, pediatric and geriatric patients[6])

TARGET GROUP 4: Post treatment restorative health care[6].



TARGET GROUPS AND MANAGEMENT PRINCIPLES[6]:

1. PREVENTIVE TREATMENT– Target group 1
2. SYMPTOMATIC TREATMENT – Target group 2
3. INTEGRATED CARE – Target group 3
4. TREATMENT FOR RECOVERY AND REJUVENATION – Target group 4.

TARGET GROUP 1:

· Stay aware

· Wash your hands frequently with alcohol based rand rub or soap,

· physical touch should be avoided,Frequent touching of eyes, nose and mouth that can pick up viruses is to be avoided[6].

· Usage of gloves can be encouraged, Spitting in the public is discouraged,Practicing good respiratory hygiene is to be encouraged[6].

· Maintain social distancing[6].

· Adequate nutrition should be assured by means of freshly cooked hot foods considering digestive capacity for individuals[6].

· Extra care should be taken in the case of elderly people, infants, immunocompromised people or women with comorbidities before and after childbirth[6].

· Intake of warm fluids boiled with medicinal herbs should be used as a regular drink to avoid dehydration[6].

· Restrict intake of sweet and food which is hard to digest. Also not advised to take chilled, fried, oily, fermented food items, and beverages[6].

· Cope up with stress[6].

· Do exercise and meditation regularly[6].

· 7-8 hours of adequate sleep is mandatory[6].

· Avoid self – medication if any comorbidities exist; medicines as prescribed are to be encouraged to be taken under supervision[6].

IMMUNITY ENHANCERS[6]:

Guduchi, amla, haridra, tulasi, ashwagandha will help to increase our immunity[6].

These are the medications can be used under the supervision of a qualified ayurvedic physician[6].

1. CHYAWANPRASH AVALEHA - 10 - 12 gm / 1 Spoon,

2. DRAKSHAVALEHA - 10 - 12 gm / 1 Spoon,

3. INDUKANTAM GRUTHAM - 10 - 12 gm twice daily before food, when hungry,

4. ARAVINDASAVA - 15 - 20 ml with equal quantity of warm water after food,

5. BALACHATURBHADRA CHURNA - 1 - 2 gm with honey,

6. HARIDRA KHANDA - 3 - 5 gm intermittently with honey/ warm water.

LIFE STYLE FOR BOOSTING IMMUNITY:

Early Morning Regime:

· Wake up early in the morning .Drink 1-3 glasses of warm water Gandusha / Kavala (Oil Pulling) 1 tablespoon of sesame or coconut oil for followed by warm water rinse[6].

· Gargle with warm water added turmeric and salt, Triphala, and Yashtimadhu. ShuddhaTankana, Madhoodaka also can be used for Kavala graha[6].

· Nasal Health – PratimarshaNasya (2 drops of sesame / coconut oil each nostril)[6].

· Daily practice of Yogasana, Pranayama and meditation for at least 30 minutes[6].

· Warm water bath[6].

After Noon Regimen:

Don't sleep during the daytime[6].

Involve in Work[6].

Evening Regimen:

Meditation / Pranayama – 30 minutes[6].

Night regimen:

· Eat easily digestible foods. Eat a night meal2- 3 hours after sunset or till 8 pm.

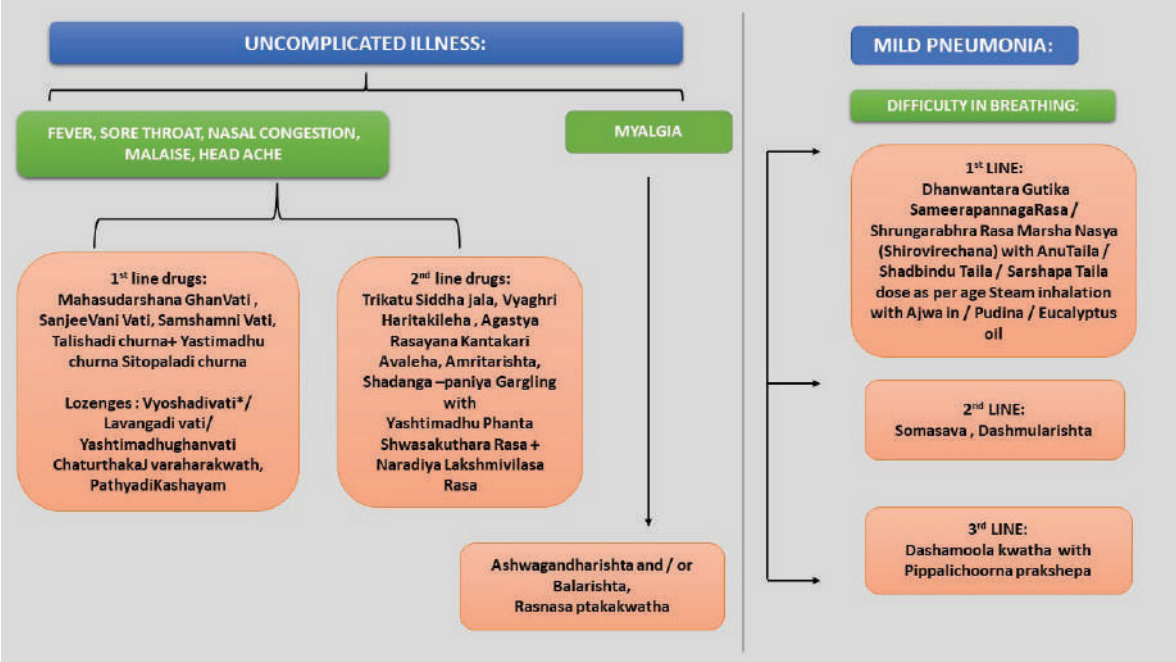
· Sleep atleast 2-3 hours after dinner. Adequate sleep is necessary[6].

GENERAL MEASURES:

Dhupana - (fumigation) of the house every evening with antimicrobials such as Neem leaf, Sarshapa (Brassica campestris L), Loban (gum bezamin/benzoin - StyraxbezoinDryand), Karpura (Cinnamomumcamphora (L) J.Presl.), Ghee etc. Also usage of AparajitaDhoomaChoorna (A.h.JwaraChikitsa) as per availability can be adopted[6].

GROUP 2:

This flowchart represents the symptoms and 1st and 2nd line drug of choices for group 2 patients[6]:



MANAGEMENT GUIDELINES FOR SEVERE SYMPTOMS:

These are the medications can be used under the supervision of a qualified ayurvedic physician[6].

FEVER[6]:

1. MahaSudarshanghanVati- 500 mg TDS with lukewarm water

2. Amritarista 15-20 ml tid with water after food

3. Amritottara Kashaya 15 ml tid with water before food

4. Vishamajwarantakalauha with gold - 125 mg bid with water - High fever with debility

5. Mrityunjaya rasa - 125 mg tid with water - Uncontrolled fever with myalgia

6. Samshamanivati 500 mg. 2 tab BD after food

7. ArkaYavani - 10 – 25 ml QID with water - DeepanapachanaJwara, Aruchi

8. Pathyadi /Guduchyadi / BharangyadiKashayam- 30 – 40 ml BD before food

9. ChaturthakaJvaraharakwatha

10. Tribhuvanakirti rasa 125 mg BD after food with shunthijala or water

11. Bilwadivati 1 TDS

SORE THROAT[6]:

1. Vyoshadivati/ LavangadiVati/ Khadiradivati-2 tab TDS

2. Laxmivilasa rasa 125 mg tid with tamboolaswarasa after food

NASAL CONGESTION[6]:

1. Haridrakhand- 3 - 5 gm BD with lukewarm water/ milk

2. Laxmivilasa rasa 125 – 250 mg BD with tamboolaswarasa after food.

MYALGIA[6]:

1. Rasnasaptak- Kwath- 30 - 40ml BD before food

2. Ashwagandharista 15 – 20 ml BD with water

3. Balarishta 15 – 20 ml BD with water

4. Devadaryadikwatha30 - 40 ml BD before food

5. Dashamoolakwath30 - 40 ml BD before food

6. Godantibhasma 500 mg – 1 gm BD/TDS daily with ghee, sugar, warm milk or water.

COUGH[6]:

1. TalisadiChurna (4 g)+Madhuyashtichurna (2 g) BD with honey/ lukewarm water

2. Sitopaladichurna 3-6 gm with honey BD/ TDS or as required

3. Tankanabhasma– 250 – 500 mg BD

4. Dashamoolakatutrayadi Kashaya- 20 – 30 ml TDS with water before food

DEHYDRATION[6]:

1. Shadangapaneeya 40 ml tid/as per requirement.

PREFERABLE DIET[6]:

· Eat freshly prepared warm food[6].

· Liquids – Instead of Tea, we can use either of the following are advised:

· Herbal tea prepared from half inch ginger + two Black pepper + ¼ cinnamon or lemon grass, tulsi leaves in 2 cups boiled water reduce it to one Cup & add honey 1 teaspoon[6].

· 1 cup hot cow's milk + dry Ginger powder/ Haridra half teaspoon

· Cereals – Shalishashtika (Old Rice) / Laja/ Godhuma (Wheat) / Yava (Barley)

· Pulses - Mudga (Phaseolus radiatus Linn), Masur (Lens culinaris Medic), Kulattha (Dolichosbiflorus Linn.), Chanaka, Moth

· Fruits - Draksha (Vitis vinifera L.), Kapittha (Feronia limonia (Linn.)), Dadima (Punicagranatum Linn) can be taken.

· Condiments and spices: Cardamom/dry ginger/ black pepper/ long pepper/ garlic

GROUP 3: Vulnerable Group (Pregnant & Lactating women, Children & Geriatric subjects):

These are the medications can be used under the supervision of a qualified ayurvedic physician**[6]:**

PREGNANT AND LACTATING WOMEN:

· PhalaSarpis - 10 - 12gm in two divided doses with warm water

· KalyanakaGhrita - 10 - 12gm in two divided doses with warm water

· Ashwagandha - 10 -12 gm twice daily with warm milk.

· SoubhagyaShuntiLeha - 10 - 12 gm twice daily with warm milk in empty stomach

· Daily use of Milk with Ghee (cup of milk with a teaspoon of ghee).

CHILDREN[6]:

Drug of choice for children and drug dosage given below[6]:

· IndukantaGhritam– 5 – 10 ml in two divided doses with warm milk

· KalyanakaGhrita 5 – 10 ml in two divided doses with warm milk

· Aravindasavam - 5 – 15 ml in two divided doses with warm water after food.

· BalachaturbhadraChurna - 1-2 gm dose with honey two times a day

Dose of medicines in Children[6]:

Specific Dose calculation: Child dose = (Adult Dose /16) X Age of Child.

· Above 15 years - Adult dose

· Between 10-15 years - ¾ of Adult dose

· Between 5-10 years - ½ of Adult dose

· Below 5 years - ¼ of Adult dose

GERIATRIC PATIENTS[6]:

· ChayvanaprashaAvaleha - 10 - 12 gm twice daily with warm milk.

· Ashwagandha Avaleha - 10 - 12 gm twice daily with warm milk.

· Brahma Rasayana - 10 - 12 gm twice daily with warm milk.

· Daily use of Milk with Ghee (cup of milk with a teaspoon of ghee)

· ShilajeetRasayana, KhadiradiRasayana ,Shatavari Siddha Ghrita.

GROUP 4: Post treatment restorative healthcare.

According to Ayurveda, there will be Dhatu-Kshaya&AgnimandyaAvasthafor Post COVID 19 infection[6].

Hence, Dhatuposhana and Rasayanasevana with drugs like Draksha (Vitis vinifera L.) & Vasa (Justicia adhatodaL.) for at-least 45 days and to combat the residual effects of the virus on the body – Vishaghnachikitsa with Shirisha (Albizialebbeck (L.) Benth.) or Haridrachurna (Curcuma longa) are suggested after clinical recovery[6].

For diarrhoea, vomiting or loss of appetite - DeepanaPachana drugs like ShadangaPaneeya may be used in case of Diarrhoea, vomiting or loss of appetite[6].

For Fecal shedding - Kriminashaka therapy with VidangaChurna, Vilwadigutika, NeelithulasyadiKashayam may be used.[6]

These are the medications can be used under the supervision of a qualified ayurvedic physician[6]**:**

INITIAL STAGE:

· INDUKANTHAM KASHAYAM1 15ml + 45 ml warm water twice before food 2 along with MAHASUDARSHAN GHAN VATI twice before food 0R AMRITARISHTAM - 15 - 20 ml with equal quantity warm water twice after food along with AGNITUNDI VATI 01 Tab BID with Lukewarm water[6].

· Once Agni - Digestive capacity is regained - INDUKANTAM GHRITAM 10 - 12 gm twice daily whenever hungry. Rasayana and other immune boosting drugs in group 1 can be also be utilized judiciously as per the discretion[6].

RASAYANA:

· DRAKSHARISHTA 15 - 20 ml thrice a day with equal volume of water after meal or

DRAKSHADI LEHA 10 - 12gm thrice a day 1 hour before meal (Draksha is having specific potential to rejuvenate lungs) OR

· VASAVALEHA/ KANTAKARYAVLEHA – 10 - 12 gm thrice a day 1 hour before meal (Vasa/ kantakari is a very useful drug for jvara, kasa, pitta vriddha conditions; so it would be helpful in removing the residual khavaigunya in srotasas)[6].

· CHYAWANPRASH 10 - 12 gm/ ASHWAGANDHA LEHA 10 - 12 gm thrice a day before meal VARDHAMANA PIPPALI RASAYANA, VASANTA KALPA[6].

DURATION OF INTAKE OF MEDICINES:

The above said medications alike Dhatu Poshana and Rasayana be provided for a time period of 45 days as per the discretion of physician Other groups of drugs which are protective in nature can be provided for 45-60 days as per discretion of the practitioner[6].

DIET:

· Chew a piece of ginger with a pinch of rock salt (Lavanadrak) 15 to 20 minutes before food it helps to improves the appetite.

· Easy digesting foods like Tarpanalajasaktu (powder of roasted grains) mixed with honey, sugar and fruit juices, moong dal or lentil, Soups of vegetables, Meat soup, Khichadi of rice and mung dal (Green Gram), Phulka with cow's ghee, Vegetables like gheya, turai, bhindi, sitaphal etc. should be taken[6].

· Spices like jeera, black pepper, garlic, coriander, ginger, Ajwain should be used in cooking[6].

YOGA:

Yoga may play important role in the psychological care and rehabilitation of COVID-19 patients in quarantine and isolation[7]. They are particularly useful to avoid their fears and anxiety[7].

Forty-Five-minute module: The Common Yoga Protocol of IDY that was developed by a team of leading Yoga experts / Yoga Masters include safe practices to improve physical, mental, emotional and spiritual health[7]. Regular practice on empty stomach is recommended to improve immune response[7].

ASANAS include:

Tadasana (The Palm tree posture), Vrikshasana (The Tree posture) , Pada-hastasana (The Hands to the feet posture), ArdhaChakrasana (The Half wheel posture), Trikonasana (The Triangle posture),Bhadrasana (The Firm/auspicious posture),Vajrasana The Thunderbolt/ diamond posture) ArdhaUshtrasana (The Half camel posture), Ushtrasana (The Camel posture), Sasakasana (The Hare posture), UtthanaMandukasana(The Stretched up-frog posture), Vakrasana (The Seated twist posture), Makarasana (The Crocodile posture), Bhujangasana (The Cobra posture) Shalabhasana (The Locust posture),Setubandhasana (The Bridge posture) Utthanapadasana (The Raised leg posture) 0.5 ArdhaHalasana (The Half plough posture) 0.5 back PawanaMuktasana (The Wind releasing posture) 2 Shavasana (The Corpse posture)Kaphalabhati (The Shining skull practice) Pranayama AnulomaViloma Pranayama (The Alternate nostril breathing Ujjayee Pranayama (The Hissing breathing) (Bhramari Pranayama) (BhramariRechaka)The Bee sound breathingand meditation[7].

Twenty and ten minutes modules are recommended for children, adults, Youths and the elderly population to be repeated twice a day (morning and evening).

20 MINS module ASANAS include:

· Tadasana (The Palm tree posture)- 1 minute[7].

· PadaHatasana(The Hands to the feet posture)/ ArdhaChakrāsana (The Half wheel posture) - 2 minutes[7].

· Trikonāsana (The Triangle posture),Bhadrāsana (The Firm/Auspicious posture) - 1 minute[7]. ArdhaUshtrāsana (The Half camel posture) 1 minute Sasakāsana (The Hare posture) 1 minute[7].

· Vakrāsana (The Seated twist posture),Bhujangāsana (The Cobra posture),PawanaMuktāsana (The Wind releasing posture),Kaphalabhati (The Shining skull practice)- 1 rounds, 30 cycles each[7].

· AnulomaViloma Pranayama (The Alternate nostril breathing) (5 rounds) - 2 minutes[7].

· BhramariPranayama(BhramariRechaka) (The Bee sound breathing) (3 rounds) and meditation[7].

10 MINS module ASANAS include:

· Tadāsana (The Palm tree posture)- 1 minute[7].

· ArdhaChakrāsana (The Half wheel posture),Sasakāsana (The Hare posture),Bhujangāsana (The Cobra posture),PawanaMuktāsana (The Wind releasing posture),(AnulomaViloma /Nadiswhodhana Pranayama) The Alternate nostril breathing (2 rounds) and meditation[7].

· Apart from CYP; Jalaneti, Sutraneti and Bhastrika Kriyas are advised once or twice in a week and Yoga nidra for 20-30 minutes twice or thrice a week[7].

UNANI:

Unani Medicine recognizes the influence of surroundings and ecological conditions on the state of health of human being[8].As per Unani, improving our immunity with immune boosters is one of the major key approaches for maintenance of health and prevention of disease[8].

ANTI-VIRALS:

These are the medications can be used under the supervision of a qualified Unani physician[8].

· Kalonji (Nigella sativa): 1-2 gm,

· Seer (Allium sativum): 2-3 gm,

· Zanjabeel (Zingiber officinale): 5 gm,

· Aslassus (Glycyrrhiza glabra): 5-10 gm,

· Afsanteen (Artemisia absinthium): 3-5 gm.

· Tukhm-e-Kasoos (Cuscutareflexa): 15 gm (seeds),

· Khayarshamber (Cassia fistula): 10-20 gm (pulp) for gargle,

· Gilo (Tinosporacordifolia): 5-10 gm.

Immunity Enhancers:

· KhameeraMarwareed: 3-5 g (Not advised for diabetics)[8],

· Asgandh (Safoof): 5 g[8].

SYMPTOM SPECIFIC APPROACH:

DRY COUGH:

· Habb-e-Surfa (125-250 mg)[8],

· Khamira-e-Banafsha (10-20 gm) (Not advised for diabetics)[8],

· Laooq-e-Sapistan (10-20 gm) (Not recommended for diabetics)[8],

· Sharbat-e-Sadr (20-40 ml) (Not recommended for diabetics)[8].

HIGH FEVER:

· Habb-e-Bukhar (250-500 mg)[8],

· Habb-e-Mubarak (1-2 gm)[8].

SORE THROAT:

Sharbat-e-Toot Siyah (20-40 ml) (Not advised for diabetics)[8].

BREATHING DIFFICULTY:

Laooq-e-Katan (10-20 gm) (Not advised for diabetics)[8],

Habb-e-Hindi Zeeqi (125-250 mg)[8].

STRESS:

Stress relieving measures are equally necessary to follow. Certain Unani drugs like Sa'dKoofi (Cyperusrotundus), OodSaleeb (Paeonia emodi)and Jadwar (Delphinium denudatum)may be helpful[8].

FUMIGATION:

Fumigation (Bakhoor) of the house at frequent intervals with combination of Sandal (Santalum album) and Kafoor (Cinnamomumcamphora)[8].

SIDDHA[9]:

These are the medications can be used under the supervision of a qualified siddha physician[9]**:**

ANTI-VIRALS:

· Kaba Sura Kudineer - 60 ml twice a day after food[9],

· NilaVembuKudineer– 60 ml twice a day after food[9],

· Visha Sura Kudineer- 60 ml Twice a day after food[9],

· PavalaParpam -100 mg twice a day with Honey[9],

· Velliparpam - 50 to 100 mg twice a day with Honey[9].

INTERVENTIONS FOR MILD- MODERATE SYMPTOMS:



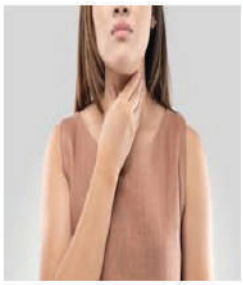
COUGH

INTERVENTION:	PAEDIATRIC AGE (<5YRS)	PAEDIATRIC AGE (5-12 YRS)	ADOLESCENT (13-18 YRS)	ADULT (19-65 YRS)	GERIATRIC (>65 YRS)
Kaba Sura Kudineer	5 ml	10ml	30ml	60-90 ml	60ml
Adathodai Manapagu	2ml	5ml	10ml	10-20ml	10-15ml
Nellikkaillagam	1-2gm	3gm	5gm	5-10gm	5gm
VasanthaKusumakaram	-	1 Nos	1Nos	1-2Nos	1Nos



FEVER

INTERVENTION:	PAEDIATRIC AGE (<5YRS)	PAEDIATRIC AGE (5-12 YRS)	ADOLESCENT (13-18 YRS)	ADULT (19-65 YRS)	GERIATRIC (>65 YRS)
NilaVembu / Kaba Sura Kudineer	5ml	10ml	30ml	60-90ml	60ml
Bramananda Bairavam	-	1Nos	1Nos	1-2Nos	1Nos
Adathodai Manapagu	2ml	5ml	10ml	10-20ml	10-15ml
Thirithoda Mathirai	-	1Nos	1Nos	1-2Nos	1Nos



INTERVENTION:	PAEDIATRIC AGE (<5YRS)	PAEDIATRIC AGE(5-12YRS)	ADOLESCENT (19-65 YRS)	ADULT (19-65YRS)	GERI (>65
Thalisathi Vadagam	1/4Nos	1/2Nos	1/2Nos	1Nos	1Nos
Kaba Sura Kudineer	5ml	10ml	30ml	60-90ml	60ml
AdathodaiNei	1ml	5ml	10ml	10-15ml	5-10r



INTERVENTION:	PAEDIATRIC AGE (<5YRS)	PAEDIATRIC AGE (5-12YRS)	ADOLESCENT (19-65 YRS)	ADULT (19-65YRS)	GER (>65
Nila vembu/kaba sura kudineer	5ml	10ml	30ml	60-90ml	60m
Seenthil chooranam	200mg	500mg	1gm	1-2gm	1-2g
Pachai karpoora mathirai	-	½ nos	1 nos	1-2nos	1 no
Swasakudori	-	½ nos	1 nos	1-2nos	1 no

ANTIVIRAL SIDDHA HERBS:

These are the medications can be used under the supervision of a qualified siddha physician[9]**:**

Inji (Zingiber officinale), Thulasi (Ocimum sanctum), Milaku (Piper nigrum),Karunjeerakam (Nigella sativa), Keezhanelli (Phyllanthus niruri), Athimadhuram (Glycyrrhiza glabra) Vellaipoondu (Allium sativum), Cittramutti (Sidacordifolia), Seenthil (Tinosporacordifolia), Manjal (Curcuma longa) Elumitchai (Citrus limonia) , Vembu (Azadirachtaindica)[9].

Note: Antiviral activity of the above mentioned drugs and formulations against COVID 19 is not

established and not being claimed

HOMEOPATHY:

In case of epidemics or pandemics:

1st approach - Follow preventive measures and educate the people about general measures to do and to provide such interventions which will keep their immunity enhanced[10].

2nd approach - Provide homoeopathic symptomatic mitigation to infected persons.

Homoeopathic medicines are also useful in the treatment of communicable diseases like Influenza, dengue, acute encephalitis syndrome[10].

REMEDIES:

Mild Disease (Symptomatic Amelioration and Mitigation Approach): Medicines like Aconite napellus, Arsenicum album, Bryonia alba, Gelsemium sempervirens, Rhus tox. Eupatorium perfoliatum, Ipecacaucunha, Belladonna, Camphora,may be used depending upon the symptoms similarities[10].

NATUROPATHY:

Naturopathy is a system of lifestyle medicine that works in modulating these factors that improve body's innate immunity[11]. This may be useful in this current scenario where we are facing a Covid 19 pandemic[11]. These are the medications can be used under the supervision of a qualified naturopathic physician[11]:

SYMPTOMS:	MANAGEMENT:
Cold, Running nose, Nasal Congestion	Steam Inhalation with Tulsi, peppermint and Eucalyptus oil. Nasal Irrigation with warm saline water
Sore throat	Hot saline water gargle. Gargling with neem water and honey. Coriander and turmeric Kashayam with black pepper
Dry Cough	Ginger Kashayam with honey, cloves, cinnamon, turmeric and pepper for dry cough in milk
Fever	Cold chest pack, cold sponging for pyrexia
Malaise and Fatigue	Nutrition rich in Plant proteins, Natural Vitamin C, glucose and Vitamin D Lemon water with honey to boost immunity
Stress, Anxiety Depression	Aroma therapy with lavender, Basil or lemon grass oils (Inhalation/steam inhalation) and yoga and meditation for relaxation.
Headaches	Hot foot Immersion for headaches with cold compress on head (16), Aromatherapy with Tulsi or lavender oils.

Conclusion

Although modern medicine came over traditional medicine especially in developed countries, it is still being used widely by much of the population especially in developing countries.Although the ancient traditional medicine seriously lacks validation and standardization, but the increasing tilt of the society towards the alternative medicine source due to widespread increasing rate of chronic diseases as well as lack of modern allopathic medicine in treating various infectious diseases due to antimicrobial resistance, has lead to various researches and studies which proves their efficacy and safety in various advanced clinical trials.Currently, there are limited number of allopathic medicines considered effective against COVID-19. The design and development of drugs and vaccines require elucidation of the mechanism of SARS-CoV-2.A lot of researches are being done to find the potential candidates from the conventional herbal pool which can prevent the spread and stop the effect of the virus.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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10. GUIDELINES for HOMOEOPATHIC PRACTITIONERS for COVID 19 – MINISTRY OF AYUSH
11. GUIDELINES for NATUROPATHY PRACTITIONERS for COVID 19 – MINISTRY OF AYUSH.

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Artifacts in Histopathology – A Review

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Abstract

Histopathological examination is considered to be the gold standard technique for making a definitive diagnosis of various human body lesions. The handling of tissue specimens consists of extensive procedures from the stage of surgical removal to the microscopic parts stained and placed. Owing to defective procedures, defects are normal in the tissue parts. These defects are known as artefacts. In histopathology artefacts may cause serious errors and misdiagnosis. The precision of the histopathological diagnosis is based on removing or reducing the artifacts in histopathological section.This article discusses the common artefacts found during slide analysis alongside the remedial steps that can be performed to distinguish between an artifacts and the constituent tissue.

Keywords: *Artifacts, Errors, Histopathology,Diagnosis*

Introduction

Histopathology is a science purely focused on microscopic examination and interpretation[1].Sometimes the existence or modification of a foreign material in tissue details may cause confusion and lead to incorrect or inconclusive interpretations. Such entities or modifications are commonly called "artefacts"[1]. They occur in tissue sections prior to fixation, during fastening, specimen grossing, tissue processing, sectioning, staining and tissue preservation[2]. Some artefacts can be easily differentiated from normal or diseased components of tissue, while others are difficult to differentiate from certain entities[2].They are the primary source of diagnostic problem[3]. This article is therefore intended to encourage understanding of the various common artefacts that can be found in histopathology, to provide a guide for their identification, to clarify their causes and, where possible, to suggest the means by which their occurrence may be avoided[2].

Depending on the stage at which artefacts are formed, they can be categorised as artefacts produced during[3,4]:

- Artefacts during oral biopsy procedure
- Prefixation artifacts
- Fixation artifacts
- Tissue processing
- Embedding
- Microtomy
- Mounting
- Staining
- Cover-slipping[4].

ARTIFACTS DURING ORAL BIOPSY PROCEDURE:

Artifact	Causes:	Remedies:
INJECTION	Intralesional injection of anesthetic solution3.	a) Use block technique instead of infiltration2. b) Avoid injecting larger amount of anesthetics4. c) Avoid injecting anesthetic adjacent to the biopsy site4.
FORCEPS AND CRUSH/ SQUEEZE:	a) Instrument penetrates the instrument resulting in voids and tear5. b) Marks on specimen created by forceps pressure4.	a) Use small forceps or adson's forceps without teeth1. b) Use B forceps for to take oral biopsy5. c) Handle the specimen gently and avoid compression or retraction of tissues2.
FULGERATION:	Heat generated while doing laser or electrosurgical procedures2.	Use knives, use low milliamperage current, use of combination knife and electrical points2.
CURLING:	a) Occurs in incisional biopsies5. b) Occurs due to shrinking process of oral mucosa after introduced in formalin5.	a) After the biopsy, the specimen is placed with its mucosal surface up on a piece of the sterile paper, allow the specimen to unfixed for sometime5. b) For thin specimen – take adequate depth of specimen to prevent this artifact5.
SPLIT:	Penetration of forceps into the tissue5.	Use blunt forceps instead of sharp or toothed forceps4.
SUTURAL :	Presence of suture material in histopathologic tissue2,7.	Visible sutures are removed wherever possible2,7.
STARCH:	Due to contamination of starch powder in tissue3.	a) Avoid touching of tissues by using gloved hands2,3. b) Use correct tissue forceps to prevent this artifact2,3.

FIXATION ARTIFACT:Fixation is a mechanism that attempts to maintain the tissues by prevention of autolysis and putrefaction in a life like environment[4]. The fixative volume should be 20 timesthat of the specimen not exceeding 6 mmthick[4]. 10 per cent of formalin is commonly used as a fixative[6]. Fixation objects emerge from formalin, mercury chloride, and picric acid used in various fixative agents that cause widely dispersed brown black granular and yellow stains in the tissues[2].

ARTIFACT:	CAUSES:	REMEDIES:
FORMALIN PIGMENTS:	Formaldehyde has a tendency to be oxidized, producing formic acid. Heme from RBCs and formalin bind each other to form formalin–heme complex that appears as brown-black amorphous to microcrystalline granules in tissue sections[3].	Removed from tissue section by immersing it in saturated alcoholic picric acid[3]. This can be prevented by using buffered neutral formalin[3].
MERCURIC PIGMENTS:	Produces dark brown granular deposits[3].	Removed by immersing it in alcoholic iodine[3].
ICE-CRYSTAL ARTIFACT:	Slow freezing of tissue due to selection of large sample to permit rapid freezing[3].	It can be prevented by using carbon-di-oxide expansion freezers[3].
FREEZING ARTIFACT:	Characterized by formation of interstitial and intracytoplasmic vacuoles resulting from ice-crystal formation taking place as 10% formalin freezes at -11°C[2].	Prevented by avoiding before fixation[5].
STREAMING ARTIFACT:	diffusion of unfixed material to give false localization by coming to rest in same place other than its original location3.	It can be prevented by using glycogen fixatives or by freeze drying[2,3].
SHRINKAGE ARTIFACT:	During fixation, tissues change its original size[2]. This occurs due to inhibition of cellular respiration and changes in membrane permeability[2].	It can be avoided by using compound fixatives[2].
DELAYED FIXATION:	Results in cell shrinkage and cytoplasmic clustering[5].	Fixing the specimen in 10% formalin immediately after tissue removal[5].
DIFFUSION ARTIFACT:	Due to materials that diffuse out of tissue[5].	Proper fixation for exact localization and also preventing leaching of ions from the tissue[5].

PROLONGED FIXATION: Prolonged fixation in formalin may cause secondary shrinkage and hardening and it may result in separation of tissue, giving an appearance of empty spaces[2,5,7].

POST FIXATION: Tissue that are fixed in chrome if not washed for 24 hours in running tap water could produce chromeoxide pigment[2,7].

MICROWAVE FIXATION: Optimum temperature for microwave fixation is 45-55 oC. Low heating results in poor section quality whereas overheating above 65oC results in vacuolation, overstained cytoplasm and pyknotic nuclei2.

TISSUE PROCESSING ARTIFACTS:

Artifacts during dehydration:

Artifacts can be encountered during dehydration because of

· Improper dehydration gradient, if the concentration gradient between the fluid within and outside the tissue is high, the current of diffusion crosses the cell membranes during the exchange of fluids, thereby increasing the probability of cell distortion[2,7].

· Over dehydration renders the tissue hard, fragile and shrunken, which causes difficulty when cutting and also interferes with sectional staining properties[2,7].

· Low and incomplete dehydration results in inadequate penetration of the rendered paraffin and block is difficult to section so that distorted sections of broken tissue can lead to artifactural changes2,7

Artifacts during clearing :

· Due to over-hardening of tissue specimens, objects can occur and obstruct paraffin to properly impregnate in paraffin wax, making it difficult to cut during sectioning[2,7].

· Artifacts can result from under and over clearing of tissue specimens and blocking the paraffin to properly impregnate in paraffin wax, making it hard to cut during sectioning2,7.

REMEDY: This can be avoided by taking good care to use the correct amount of clearing agent and there should be no clearing agent left behind to contaminate the wax[5].

Artifacts during impregnation:

· The purpose of wax impregnation is to extract the clearing agent (wax solvent) from the tissue and to allow them to be fully impregnated by the paraffin wax which is subsequently allowed to harden to create a block from which parts can be cut[2,7].

· The artefact produced during this procedure is crystallisation: the thicker the tissue the more clearing agent it holds, and thus more wax change is needed to remove it[2,7].

· Even if a small quantity of clearing agents contaminates the wax it will cause tissue to crumble and crystallise during cutting[2,7].

EMBEDDING:

ARTIFACT:	CAUSES:	REMEDIES:
INCORRECT ORIENTATION:	Exposing the specimen too long during embedding which results in hardening, so that it becomes friable and crack easily3.	Correct orientation of tissue5. Expose the specimen to correct amount of medium5.
AIR ENTRAPMENT:	Air gets entrapped while embedding which results in venetian blind effect4.	Care must be taken while orienting in the medium

ARTIFACTS RELATED TO MICROTOMY: (causes and remedies)

1. Alternative thick and thin section[1]:

CAUSES:	REMEDIES:
Wax too soft for tissue or conditions	Make it harden by using ice pack or re-embed with wax or highest melting point
Block or loosen knife	Tighten the knife
Insufficient clearance angle	Increase the clearance angle slightly
Mechanism of microtome faulty	See for any obvious faults

2. Thick and thin zones parallel to knife[1]:

CAUSES:	REMEDIES:
Knife or block in the holder is unfit or loose	Tighten
Steep knife angle (excessively)	Decrease the clearance angle to minimum
Tissue or wax is too hard for sectioning	Use sharp knife and microtome, decrease slant angle of knife, use softening or loosening fluid on tissue
Calcified areas in tissue	Rehydrate and decalcify

3. Curved ribbons and consecutive section[1]:

CAUSES:	REMEDIES:
Unparalleled leading and trailing edges	Sharp the scapel until parallel
One area of knife is blunt	Use other part of knife or sharpen it
Surplus area of wax at one side	Remove away the excess wax
Tissue varying in consistency	Orient the block again by turning through 90 degree, cool the block with ice.

4. Splitting of sections at right angles to knife edge[1]:

CAUSES:	REMEDIES:
Nick in edge of the knife	Use other part of knife or re-sharpen it
Hard particles in tissue	If calcium deposits are there, do decalcification, if mineral or other particles are present remove the particle by using scapel
Hard particles in wax	Re-embed the tissue in fresh and filtered wax

5. Section will not join to form a ribbon[1]:

CAUSES:	REMEDIES:
Wax- too hard for sectioning	Dip the block to warm or re-embed in wax at lower melting point
Debris on edge of the knife	Clean with xylene – dipped cloth
Knife angle (too shallow or too steep)	Change the optimal angle

6. Section become attached to block on return stroke[1]:

CAUSES:	REMEDIES:
Clearance angle is insufficient	Increase clearance angle
Knife edge containing wax debris	Cleaned by using xylene dipped cloth
Debris on edge of the block	Cut or trim the edge with scapel

7. Areas of tissue in block not present in section[1]:

CAUSES:	REMEDIES:
Impregnation of tissue incompletely	Tissue returned back to vaccum impregnating bath for few hours
Wax block detached from wood	By using hot spatula re-attach the wax block

8. Excessive compression of section[1]:

CAUSES:	REMEDIES:
Blunt knife	Sharpen the knife
Too wide bevelled knife	Re-sharpen to create secondary narrow bends

9. Section expand and disintegrate on water[1]:

CAUSES:	REMEDIES:
Poor tissue impregnation	Tissue returned back to vaccum and impregnating bath for few hours
Too high water temperature	Cool

10. Section rolled up into a tight coil instead of remaining flat on knife[1]:

CAUSES:	REMEDIES:
Blunt knife	Sharpen
Too small rake angle	Resharpen to create cutting angle or decrease knife tilt if clearance angle is excessive
Thick section	Decrease the tissue section

ARTIFACTS OF STAINING AND MOUNTING:

A) Stain Particles: Absorption of stain particles occurs when the staining chemicals are not sufficiently dissolved or the stain is not fresh, resulting in precipitation[1].

REMEDY: Remove or filter the stain

B) Air Bubbles: These are trapped over the stained portion when the cover slip is pulled on. These appear as minute spherical features[1].

REMEDY:

· Using sufficient quantities of mounting media[1].

· If more viscosity has been obtained with the mounting paper, xylene can be used as a thinning agent[1].

MISCELLANEOUS:

· Folds in sections: Folds on the wax pieces may often not be straightened resulting in folds or pleats. This is a typical artefact in hard tissue component tissues and is difficult to prevent even with the utmost care[1].

· Sectional cloudy appearance: Defective dehydration technique results in sectional cloudiness[1].

Conclusion

Tissue artifacts can be introduced into tissue specimen during any one of the many steps through which a specimen is carried before its microscope features are examined by the pathologist. Most of these artifacts may not be intentional and might go unnoticed causing pitfalls in diagnosis.Hence, proper handling of tissue along with prompt fixation and careful tissue processing will minimize the artifacts.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Artifacts in Histopatholohy – A Microscopic View

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Abstract

Histopathological examination is considered a gold standard procedure for reaching a final diagnosis of various human body lesions. However, it is limited by a number of changes in normal morphological and cytological characteristics that occur as a result of presence of artifacts. The purpose of preparing a biological specimen for microscopic studies is to obtain sufficient and reliable diagnostic knowledge which is a true representation of the specimen, whether it is for study or diagnosis. These objects may occur during procedures for surgical removal, fixation, tissue processing, embedding, and microtomy, and staining and mounting.They contribute to misinterpretation of the diagnosis of histopathology but often they bring the limelight into the diagnosis. This article discusses the common artifacts which we commonly encountered during slide examination

Keywords: *Artifacts, Histopathology, Tissue processing, Specimen*

Introduction

Histopathology is a science totally based on microscopic analysis and examination. The basic criteria for making a definitive diagnosis include the proper biopsy procedure, proper techniques for fixation and processing, sufficient sectioning and staining[1]. Sometimes, however, the existence of a foreign material or tissue alteration can cause uncertainty and lead to an incorrect or inconclusive interpretation[1]. Artifact can be described as artificial unrelated, self-colored features found in tissues sections[2]. They occur in tissue sections prior to fixation, during fixation, specimen grossing, tissue processing, sectioning, staining and tissue preservation[2]. Artifacts can result in alteration of normal morphological and cytological features, or even lead to complete tissue uselessness[2]. In some situations an artefact may compromise a precise diagnosis[2]. Knowing and understanding about artefacts is important because learning to recognise them can prevent misdiagnosis[2].

CAUSES OF ARTIFACTS[1]:

1. Clinical application of chemicals
2. Local injection of anesthetics
3. Surgical suctioning
4. Excessive heat
5. Freezing
6. Surgical mismanagement of specimen
7. Insufficient tissue fixation
8. Improper fixation medium
9. Incorrect tissue processing
10. Embedded sponges
11. Improper staining.

According to the stage at which they are formed artifacts can be classified into different categories as artifacts produced during[3,4,5,6,7]:

1. During surgery

a) Injection artifacts

b) Forceps artifacts

c) Crush/squeeze artifacts

d) Fulguration artefacts

e) Split artifacts.

f) Starch artifacts

g) Sutural artifacts

h) Autolytic artifacts

2. During fixation

a) Prolonged fixation

b) Improper fixation

c) Delayed fixation

d) Shrinkage artifacts

e) Formalin pigments

f) Mercuric pigments

g) Osmolality of fixative solution

h) Ice crystal artifact

i) Freezing artifacts

j) Microwave fixation artifact

3. Artifacts related to bone tissue

a) Bone dust artifact

b) Over decalcification

c) Incomplete decalcification

4. Artifacts during processing and embedding

a) Dehydration

b) Orientation artifact

c) Loss of soluble substance

5. Artifacts related to microtomy

a) Cutting artifacts

b) Moth eaten effect

6. Artifact related to floatation and mounting:

a) Prolonged floating

b) Floater artifact

c) Folds and wrinkles

d) Contamination

7. Staining artifacts

8. Microscopy artifacts

DURING SURGERY:

INJECTION ARTIFACT:

• Insertion of a needle may result in haemorrhage with extravasation that masks cellular architecture[1].

• The connective tissue bands can be separated by vacuolization[1].

FORCEPS ARTIFACT:

• The surface epithelium may be forced to form small 'pseudocysts' through the connective tissue[1].

• Compression of specimen leads to loss of cytological details. Nuclear dimensions are especially affected[1].

CRUSH / SQUEEZE ARTIFACT:

• Crushing produces a destructive and dangerous type of artefact that reshapes tissue morphology and squeezes chromatin from nuclei[1].Microscopically, the cellular features are not recognizable and nuclei appear darkly stained and distorted[3] .These are usually seen at the periphery of the lesion[4].

FULGURATION ARTIFACT:

• Epithelial cells may appear disconnected, with the nuclei assuming a spindle, pallisading configuration and the epithelium is separated from the basement membrane[2].

• The fibrous connective tissue, the fat and the muscle can appear opaque and amorphous[3].

• The epithelium and the connective tissue exhibit an amorphous appearance due to protein coagulation[3].

SPLIT ARTIFACTS:

· This artefact can lead to a split between epithelium and connective tissue, resulting in a false impression of vesiculo – bullous lesions[5].

STARCH ARTIFACTS:

· These appear as numerous blue, small, spherical structures in stained sections of hematoxylin and eosin. In oral cytosmear, they appear 5–20 μm in diameter as refractile, glassy, polygonal bodies, with a central dot or Y-shaped structure which may be misinterpreted as a pyknotic nucleus or for cells with mitosis[3,6]. This could also be identical to epithelial cells[3,6].

SUTURAL ARTIFACTS:

• May consist of isolated fragments or complete fibre-bundles cut into transverse, oblique or longitudinal planes[2,7]. Sometimes description of the fibre structure can be seen after careful inspection of H&E-stained sections and these silk sutures show a clear birefringence under polarised light and this can be useful in their identification[2,7].

• Silicone gel is used to stop bleeding often. The presence of foam gel in the histological section can create blood-filled distorted space surrounded by a slightly basophilic gelatin wall[6].

• There may be no pathological significance to the presence of a suture in the histological specimen[2,7].

DURING FIXATION AND TRANSPORT:

PROLONGED FIXATION :

· Prolonged fixation may lead to secondary shrinkage and hardening, resulting in tissue separation, giving an appearance of empty spaces[5].

IMPROPER FIXATION:

· Improper fixation results in autoysis. Autolyzed tissue displays increased eosinophilia due to the loss in cytoplasm of normal basophilia imparted by RNA, and increased binding of eosin to denatured intracytoplasmic proteins and cytoplasm vacuolization. Nuclear changes include pyknosis, karyolysis and karryohexis[3].

DELAYED FIXATION:

· Delayed fixation induces changes in the form of cytoplasmic clustering and cell shrinkage. It is difficult to separate the nuclear chromatin, and the nucleoli are also not visualised[5].

· Vascular structures, nerves and glands show the lack of detail, and an impression of scarring or loss of cellularity is seen[5].

SHRINKAGE ARTIFACTS:

• These artifacts are seen as a change in volume of tissues[5]. This is due to cellular respiration inhibition as well as changes in membrane permeability[5,7]. As a result, tissues attached in life may be separated from one another, leaving empty spaces5,[7].

FORMALIN PIGMENTS:

• Formaldehyde has a normal oxidising tendency, producing formic acid. Heme from red blood cells and formalin bind together to form a formalin – heme complex that occurs in parts of tissue as brown black amorphous to microcrystalline granules[3].

MERCURIC PIGMENTS:

• Usually mercury chloride containing a fixative creates dark brown granular deposits. The pigment to mercury chloride is extracellular[3].

OSMOLALITY OF FIXATIVE SOLUTION:

• Hypertonic fixative solutions cause cell shrinkage and increased extracellular spaces while isotonic (300–320 mOsm) and hypotonic fixatives may contribute to swelling of the cells[3]. Usage of a slightly hypertonic solution (400–450 mOsm) obtained the best results[3].

ICE CRYSTAL ARTIFACT:

• It appears as intercellular clefts in highly vascular tissue, and skeletal muscle intracellular clefts and vacuoles[3].

FREEZING ARTIFACT:

· Appear as Swiss cheese holes in epithelium (Interstitial vacuoles along with vacuoles inside the cell cytoplasm) and tissue spaces[3]. Sometimes, a granular paranuclear condensation of cytoplasm produced by dehydration of the cells as a result of freezing combined with fixation process is seen[1].

MICROWAVE FIXATION ARTIFACT: Underheating results in poor sectioning quality, while overheating produces vacuolation,

overstained cytoplasm and pyknotic nuclei[3].

ARTIFACTS RELATED TO BONE TISSUE:

BONE DUST ARTIFACTS:

· Undecalcified resin sections of bone when cut, dust is produced[3]. Some of these fragments are deposited on the cut surfaces while others may be implanted more deeply in the specimen. In hematoxylin and eosin-stained sections, it stains strongly with hematoxylin[3]. When deposited in bone marrow, it stains black with von kossa, indicating its origin from calcified trabecular matrix[3].

OVER DECALCIFICATION:

· Overdecalcified sections stain strongly with eosin and show a marked loss of nuclear basophilia. Nuclear and cytoplasmic features is poorly preserved[3].

INCOMPLETE DECALCIFICATION:

· Bony trabeculae stain strongly with hematoxylin and the adjacent soft tissue is severely disrupted[3].

ARTIFACTS DURING PROCESSING AND EMBEDDING:

DEHYDRATION:

• Excessive dehydration will give a dry homogeneous appearance to the tissue. This may also cause tissue sections to crack and become heavily stained[4].

• Insufficient dehydration results clogging of tissue and ineffective staining or opacity inside section[4].

ORIENTATION ARTIFACTS:

• Improper orientation in the slide can lead to disorderly organised histological features. For skin orientation, it must be placed in such a way that the epithelial margins, subcutaneous tissue and deeper layers are all flat to the bottom so that the finished slide displays all strata[3].

• Exposure of the specimen for too long during embedding findings indicates loss of soluble substances when neutral fat is removed from fat cells , leaving normal ovoid spaces[5].

LOSS OF SOLUBLE SUBSTANCES:

• The preparation of paraffin wax, cellulose nitrate and most embedded parts of synthetic resin requires the use of fat solvent[3]. Thus, during the processing of adipose tissue, the fat can separate from fat cells and appear as ovoid spaces enclosed by a cytoplasm surface.

For example: lipoma and cholesterol clefts in odontogenic cysts[3].·

ARTIFACTS RELATED TO MICROTOMY:

CUTTING ARTIFACTS:

• Split line in tissue segment in microtome knife due to nick[6].

• Tissue compression due to blend microtome knife[6].

• Chatter artefact due to knife edge vibrations, knife or block holder loosening and extreme steep clearance angle[6].

• If the tissue is tangentially cut, the connective tissue cores can become entangled in the epithelium, giving a false impression of invasive squamous cell carcinoma[3].

MOTH EATEN- EFFECT:

· It occurs with a greater thickness due to excessively rough trimming of the paraffin blocks.

· This brings out the tissue fragments from the block face and these appear in subsequent thin sections as void spaces or holes with their long axis parallel to the edge of the knife[3].

ARTIFACTS RELATED TO FLOATION AND MOUNTING:

PROLONGED FLOATING:

· Tissues can expand beyond their actual size and become distorted and give epithelium an acantholytic appearance mimicking edema[3].

FLOATER ARTIFACT:

· Floaters are bits of tissue that were seen on slides not belonging to them[3]. They may have floated during processing and may be the result of sloppy bench cutting procedures using dirty towel, knife, gloves and improper water bath cleaning[3]. These can have tissues which are transported to the next case[3].

FOLDS AND WRINKLES IN THE SECTION:

· These artefacts look like darker stained strands. Any residual wrinkles and fords in water bath portion can be eliminated by stretching with forcep and gentle tapping. Overstretching can cause tear in the tissue section during these procedures resulting in an acantholytic appearance in epithelium[3].

CONTAMINATION:

• Often water baths may become contaminated with dust, hair, previous section residual cells, etc. Contamination with squamous epithelial cells and keratinous debris has been shown to be caused by fingers to remove air bubbles or sneezes or cough[3].

• Higher water bath temperature can cause tissue expansion beyond its limit and shows dark pyknotic nuclei or nuclear bubbling called "heating artifact"[6].

• Contamination by sneezing and coughing caused by exfoliated squamous cells may also cause artefacts[6]. Artifact due to tissue presence, which does not belong to the section, is due to unclean bath of water. Slide adhesive thick coating will take over the stain and result in poor quality section[6].

STAINING ARTIFACTS:

• Failure to extract wax from parts completely results in staining deficiency known as residual wax artefact[4].

• The presence of residual wax can lead to insufficient stain penetration leading to a stainless area and also to subtle effects on nuclear staining leading to muddy nuclei appearance, lacking in detail[6].

• The appearance of external or internal dust particles on the slide results in artifactural alterations[6].

MICROSCOPY ARTIFACTS:

Impurities of the dust particles that may be present on the slide internally or externally may bring about artifactual changes. Fatty films are found resulting in a foggy appearance due to uncleaned lenses or greasy deposits on ocular pieces due to eyelashes[7].

Conclusion

Artifacts are identified in most microscopic sections, and play a role in histopathological diagnosis interpretation. Most of the microscopic section shows artefacts, which play a significant role in diagnostic misinterpretation. Although the existence of artefact in the diagnosis provides some clue, most of the artefact is accidental and causes diagnostic pitfall. The artefact must also be established and resolved such that misinterpretation and diagnostic difficulties can be addressed for a definitive diagnosis.

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Management of Biomedical Waste in Hospital and Health Care Organizations- A Review

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Abstract

Bio-Medical Waste (BMW) is a global concern. In common parlance BMW means any waste generated in the process of diagnosis, treatment, immunization and research related to human beings and animals. Due to improper or poor waste management practicesa huge risk is posed to the health of public, patients, professionals and also cause environmental disturbance. The Union Ministry of Environment and Forests under the provision of Environment [protection] act, 1986 which brought the focus back on this issue. These rules apply to all the people who are connected to generation, collection, receiving, storage, transportation and handling or biomedical waste in any form. This article aims to highlight the categories, segregation and management protocols by various government and non- government organizations and spread knowledge about the same

Key words - *Bio-Medical Waste, Infectious waste, Human anatomical waste, Chemical LiquidWaste, hazardous waste, non- hazardous waste*

Introduction

The World Health Organisation (WHO) has defined "healthcare waste as all waste produced by healthcare establishments, research facilities and laboratories including the waste originating from minor or scattered sources such as that produced in the course of healthcare undertaken in the home (such as dialysis and insulin injections)".[1,2] The Bio-Medical Waste (BMW) (Management and Handling) Rules, was published in 20th July 1998, videnotification number S.O. 630 (E), by the Government of India in the erstwhile Ministry of Environment and Forests. It provides a regulatory frame work for dealing with bio-medical waste.[3,4,5]The term "bio-medical waste" means any waste, which is generated during the diagnosis, treatment or immunisation of human beings or animals or research activities pertaining there to or in the production or testing of biological or in health camps, including the categories mentioned in Schedule I appended to these rules. [6.7,8]

BMW produced by health care centres and hospitals are broadly categorised as general waste (paper, cardboard boxes, plastic packaging, and food waste), infectious waste (anatomical, sharp, disposable plastic), and non-infectious but hazardous waste (chemical, genotoxic and radioactive waste). [1]

Classification of Biomedical Wastes

CATEGORIES OF BIOMEDICAL WASTE, THEIR SEGREGATION AND DISPOSAL, [1, 6,9,10,11]

Government of India. Biomedical Waste

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(Management and Handling) Rules. 1998 Extraordinary, Part II, Section 3, Subsection (ii). The gazette of India, No. 460, 27 Jul 1998.

SCHEDULE I: CATEGORIES OF BIOMEDICAL WASTE

OPTION WASTE CATEGORY TREATMENT AND DISPOSAL

Category no. 1 Human anatomical waste (human tissues, organs, body parts) Incineration*/deep burial†

Category no. 2 Animal waste (animal tissues, organs, body parts, carcasses, fluids, blood, experimental animals, waste generated by veterinary hospitals, colleges, discharge from hospitals, animal houses) Incineration*/deep burial†

Category no. 3 Microbiology and bio-technology waste (wastes from laboratory cultures, stocks or specimens of micro-organisms, live or attenuated vaccines, human and animal cell culture and infectious agents from research and industrial laboratories, wastes from production of biologicals, toxins, dishes and devices used for transfer of cultures) Local autoclaving/microwaving/ incineration*

Category no. 4 Waste sharps (needles, syringes, scalpels, blades, glass, etc., that may cause puncture and cuts. This includes both used and unused sharps Disinfection (chemical treatment ‡/autoclaving/ microwaving and mutilation/shredding)

Category no. 5 Discarded medicines and cytotoxic drugs (wastes comprising of outdated, contaminated and discarded medicines) Incineration*/destruction and drugs disposal in secured landfills

Category no. 6 Solid waste-Items contaminated with blood and body fluids including cotton, dressings, plaster casts, linen, beddings, etc Incineration*/autoclaving/microwaving

Category no. 7 Solid waste (wastes generated from disposable items other than the waste sharps such as tubings, catheters, intravenous sets etc) Chemical treatment‡/autoclaving/ microwaving and mutilation/ shredding§

Category no. 8 Liquid waste (waste generated from laboratory and washing, cleaning, housekeeping and disinfecting activities) Disinfection by chemical treatment‡ and discharge into drains

Category no. 9 Incineration ash (ash from incineration of any biomedical waste) disposal in municipal landfill

Category no. 10 Chemical waste (chemicals used in production of biologicals, chemicals used in disinfection, as insecticides, etc.) Chemical treatment‡ and discharge into drains for liquids and secured landfill for solids

*There will be no chemical pre- treatment before incineration; Chlorinated plastics shall not be incinerated; †Deep burial shall be an option available only in towns with population less than five lakh (5,00,000) and in rural areas; ‡Chemical treatment using at least 1% hypochlorite solution or any other equivalent chemical reagent; It must be ensured that chemical treatment ensures disinfection; §Mutilation/shredding must be such so as to prevent unauthorized reuse

Biomedical wastes categories and their segregation, collection, treatment, processing and disposal options[11]

Schedule II: Colour coding and type of container for disposal of biomedical wastes [1, 4,,9,11]

Colour coding	Type of container	Waste category	Treatment options as per schedule I
Yellow	Plastic bag	Category 1, Category 2, Category 3, Category 6	Incineration or Plasma Pyrolysis/deep burial
Red	Disinfected container/ plastic bag	Category 3, Category 6, Category 7	Autoclaving/microwaving/chemical treatment
Blue/white Translucent	Plastic bag/puncture proof Container	Category 4, Category 7	Autoclaving/microwaving/chemical treatment and destruction/shredding

Cont... **Schedule II: Colour coding and type of container for disposal of biomedical wastes [1, 4,,9,11]**

Black	Plastic bag	Category 5, Category 9, Category 10. (solid)	Disposal in secured landfill
1. Color coding of waste categories with multiple treatment options as defined in schedule I; Shall be selected depending on treatment option chosen; which shall be as specified in schedule I; 2. Waste collection bags for waste types needing incineration shall not be made of chlorinated plastics; 3. Categories 8 and 10 (liquid) do not require containers/bags; 4. Category 3 if disinfected locally need not be put in containers/bag			

Improper disposal of BMW can cause various health hazards such as:[4]

· Hazards from infectious waste and sharps:Various pathogens in infectious waste can enter via a puncture or cut and through inhalation. For e.g. Human immunodeficiency virus and hepatitis virus B and C

· Hazards from chemical and pharmaceutical waste:Chemicals can be toxic, corrosive or explosive. They may cause intoxication and burns.

· Hazards from genotoxic waste:[4]

Inhalation of dust or aerosols associated with cytotoxic drugs and chemicals

· Hazards from radioactive waste:[4]

It is genotoxic and exposure can cause from headache, dizziness, and malignancies.

THE PROPOSED SIMPLIFIED BIOMEDICAL WASTE SEGREGATION SCHEME FOR DENTAL CLINICS14			
RED BAG	**YELLOW BAG**	**BLUE BAG**	**BLACK CARBOY**
Disposable Injection Disposable Injection syringes, IV set without needle	Anything contaminated by blood or body fluids	Glass bottles	Used or unused sharps
Saline bottles	Any item which have been in contact with the Patient	Broken glass	Needles without syringes
Plastic suction tips	Bandages, cotton	Discarded medicines	Scalpel blades
Toothbrushes, denture brushes	Teeth (with/without fillings but without amalgamfillings)	Antiseptics, disinfectants (not contaminated by body fluids)	Metal objects
Disposable plastic/fiber Instruments	Dressings and swabs	Used or unused drug vials	Metal matrix bands

Cont... **THE PROPOSED SIMPLIFIED BIOMEDICAL WASTE SEGREGATION SCHEME FOR DENTAL CLINICS14**

Plastic/rubber tubes	**Disposables such as gloves, aprons, masks, drapes,contaminated wipes, throat packs**	**Cartridges and ampules**	**Broken metal instrument tips**
Rubber lids of any vial	Discarded crowns, bridges and cast partial dentures		Burs
Used plastic drapes	Waxes, gutta purcha points, absorbent points		Endodontic files, broaches, reamers, spreaders, silver points
	Disposable impression trays with impression Material		Orthodontic metal brackets, wires, bands
	Acrylic partial dentures, complete dentures, denture teeth		
	Plaster/stone casts		Suture needles
	Cheek retractors, tongue depressors, wedges		Broken/discarded ultrasonic tips
	Rubber dam material		Metallic bars, clasps from partial dentures
	Plastic X-ray pouches (outer covering)		Metal lids of vials
	Catheters (after draining)		All metallic dental implants related material
	Unwanted laboratory specimens		
	Suture materials without needle		

Conclusion

Even after having these rules and protocols in place immense challenges are faced by Indian health care sector regarding the BMW practices. Various efforts are being made by environmentalregulatory agencies for appropriate management of the waste generated from health care institution.

Biomedical waste management is a social responsibility as well as legal necessity. Care should be taken during disposal of biomedical waste. This will help to protect and maintain the environment from contamination. It will also to ensure the safety of workers who will come into direct contact with it.

Bio medical waste management should be included in the curriculum for health care professionals so as to emphasize on the importance of proper segregation and disposal of biomedical waste. The technical, non-technical and housekeeping staff should be compulsory trained with the help of short termed courses and workshops, to reiterate the importance of proper management.

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Blood Transfusion and Its Effects

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Abstract

Sometimes blood transfusion causes adverse reactions. Better prognosisis obtained by understanding the clinical features of acute and delayed transfusion reactions.Current clinical practise involves better and newer blood screening methods to reduce the complications. Selecting leukoreduction of red blood cells (RBCs) and male donors for plasma and restriction of RBC storage avoids infectious complications.

Key words: *Adverse reactions, blood transfusion, complications.*

Introduction

An adverse reaction of blood transfusion is an inadmissible response or effect in a patient to whom blood or blood component administered temporarily.[1]Currently even in developed countries, the risk to the patient lies in non-infectious complications of transfusions is higher where they account for significant morbidity and mortality.[2] The American Association of Blood Banks technical manual issues guidelines for the identification , diagnosis, analysis and classification of non-infectious transfusion reactions, which acts as a ready reference for clinicians and other health care providers dealing with blood transfusion.[3] The acute and delayed NIATRs are classified based on time of occurrence and it is further divided by presumed aetiology into immune-mediated and non-immune mediated subtypes. NIATRs classification, pathophysiology, clinical presentations, and management is bestowed in table 1.

Acute Non-Infectious Blood Transfusion

Adverse reactions which are occurring within 24 h after transfusion are classified as Acute immune mediated blood transfusion reactions and Acute Non-immune mediated – blood transfusion reactions. Acute immune mediated – blood transfusion reactions are sub-classified into

Acute haemolytic transfusion reactions:

A haemolytic transfusion reaction is one where transfusion produces symptoms and clinical or laboratory signs of increased red cell destruction. In acute haemolytic transfusion reactions (AHTRs) symptoms can be observed within minutes after initiating the transfusion. There are certain common laboratory features present . They are haemoglobinemia, haemoglobinuria, decreased serum haptoglobin, unconjugated hyperbilirubinaemia, increased Lactate dehydrogenase and serum glutamic-oxaloacetic transaminase levels and decreased haemoglobin.

TABLE 1: NONINFECTIOUS ADVERSE TRANSFUSION REACTIONS (MODIFIED FROM AABB TECHNICAL MANUAL) [3]

Febrile nonhaemolytic transfusion reactions :

Febrile nonhaemolytic transfusion reactions (FNHTRs) exhibits unexplained rise in temperature of at least 1°C during or shortly after transfusion. Antipyretic premedications may prevent fever but they do not usually mask chills and rigors, which are caused by cytokine mediated systemic inflammatory response. Other causative agent of fever should be ruled out before deriving a diagnosis of FNHTR. FNHTRs are observed most commonly after platelet transfusion (up to 30% of platelet transfusions) than red blood cells (RBCs) because platelets are stored at room temperature and thus it induce leucocyte activation and cytokine accumulation.[4] Treatment of FNHTRs is symptomatic.

ALLERGIC REACTIONS

Urticaria

Urticaria is the mild form of an allergic reaction that appear suddenly causing itching and it lasts for hours or up to several days before fading. More extensive cases is escorted by angioedema. The percentage of urticaria is 1-3%.[3,5] Severe reactions are treated with methylprednisolone (125 mg intravenously) or prednisone (50 mg orally).

Anaphylaxis

Anaphylaxis is a severe form of an allergic reaction with a prevalence of 1:20,000-1:50,000 transfusions,[6]where severe hypotension, shock, and loss of consciousness may develop .[7] Anaphylaxis is usually observed in IgA deficient recipients because it is caused by antibodies against donor IgA. [8] The term 'anaphylactoid' refers reactions with symptoms similar to anaphylaxis but they are not mediated by IgE. Supposing that the patient is unconscious or in shock, adrenaline is given intravenously with cardiac monitoring.[9]

Transfusion Related Acute Lung Injury

Transfusion related acute lung injury (TRALI) is a variant of acute lung injury (ALI) and it plays a vital role in transfusion-associated morbidity and mortality. TRALI is defined as:[1]

· Acute lung injury with hypoxemia and PaO2 / FiO2 ≤300 or SpO2< 90 % on room air

· Bilateral pulmonary oedema on frontal chest radiograph

· No indication of left atrial hypertension

· No pre-existing ALI before transfusion

· Onset of symptoms within 6 h of transfusion

· No temporal relationship to an alternative risk factor for ALI.

The prevalence of TRALI has been estimated nearly 1 in every 5000 blood component transfusions. Antibodies can be developed against leucocytes (polymorphous neutrophil [PMN]), both for neutrophils and human leucocyte antigen after contacting foreign antigens through pregnancy, transfusion, or transplantation. Two different aetiologies have been explained.[10] It can be a single antibody-mediated event including the transfusion of anti-human leukocyte antigen (HLA) or antigranulocyte antibodies into patients whose leukocytes produce the cognate antigens. In several cases with antibodies, the source of the antibody is the donor. A two-event model of the mechanism of TRALI has also been proposed where neutrophil activation results in pulmonary endothelial damage, capillary leakage, and pulmonary oedema. Immediate respiratory support produces marked clinical improvement within 48-96 h.[11]In order to protect the blood supply, the United Kingdom (UK) has disqualified all multiparous females from plasma donation because plasma from females might be a major factor in TRALI.[12]

Management of TRALI

Treatment is supportive. Productive measures for decreasing the prevalenceof TRALI involving the use of male plasma and apheresis platelets. Understanding of blood component and patient risk factors for TRALI will optimistically lead to novel treatment and preventive strategies for decreasing the risk of life-threatening syndrome. TRALI management comprises of preventing future adverse reactions.[13]By cross-matching donor plasma against recipient's leucocytesincompatibility and HLA and HNA antibodies are tested. A donor with antibodies which are incompatible with the patient is expelled from further blood donation for transfusion.

ACUTE NON-IMMUNE MEDIATED ADVERSE REACTIONS

Sepsis

Transfusion-related sepsis is fatal. The diagnosis depends on the basis of any one of the clinical features:

v Fever ≥39°C (102°F) or rise of ≥2°C (3.5°F);

v Tachycardia (heart rate >120/min, or rise of >40/min);

v Shaking chills and

v Alteration in systolic blood pressure (BP) (i.e. >30 mmHg rise or drop in systolic BP) within 90 min of transfusion.[14]

Patient may develop shock ,renal failure and disseminated intravascular coagulation (DIC) in severe

cases . In order to diagnose the transfusion-related sepsis and differentiating it from AHTRs and FNHTRs organism from both the patient and the remainder of the bag are isolated .[15] Since platelets are stored at room temperature, they are more sensitive than RBCs to bacterial contamination with a higher risk. Management of transfusion related sepsisinvolve broad spectrum antibiotics and other standard care for sepsis. Choosing "diversion technique" during blood collection and screening the blood for bacterial contamination decreases the risk.[16]

NonImmune Haemolytic Reactions

Due to transfusion Red cell haemolysismay happen in several nonimmune-mediated causes (also known as pseudohaemolysis) such as temperature-related or mechanical, erroneous use of blood warmer, use of hot water bath and microwave oven, using a needle with a small bore size or using a rapid pressure infuser, RBCs are infused through same tube with hypotonic solution or with pharmacologic agent. Treatment is similar to AHTRs.

Transfusion Associated Circulatory Overload

Morbidity and mortality rateis associated with transfusion-associated circulatory overload majorly.[17] Patients who are at greatest risk aregeriatric patients, infants, patients with renal failure, hypoalbuminaemia, anaemia, congestive heart failure or fluid overload or history of plasma transfusion. signs Signs and symptoms include dyspnoea, orthopnoea, cyanosis, tachycardia, jugular venous distension, and pedal oedema.[18] Increased BP with widening of the pulse pressure is seen in < 1% of transfused patients. TACO may expedite acute pulmonary oedema within 6 h after blood transfusion. Management is providing mechanical ventilation, fluid restriction, diuretics.[19]

Transfusion Associated Dyspnoea

It is characterised by respiratory distress within 24 h of transfusion and it is not similar to other allergic reaction.[1]

Acute Hypotensive Transfusion Reaction

Sudden drop in BP with absence of other causes of hypotension. Still it responds quickly to transfusion inhibition and supportive treatment.[20] Patients with unusual hypotensive transfusion reactions must be given a trial of washed blood products. Bedside leucoreduction filters have been involved often in acute hypotensive transfusion reaction even though it has also occurred with prestorage leucofilters.[21]

Citrate Toxicity

Transfusion of largeblood volumes containing citrate causes increased plasma citrate chelates calcium ions resulting in hypocalcaemia. It is caused by citrate overload andcan be treated by decreasing infusion rate. Calcium replacement is signified in massively transfused patients, especially those with severe liver disease or severe symptoms of hypocalcaemia.[22]

Hyperkalemia

After an hour of transfusion , total extracellular potassium load, which is 5 mmol/l or ≥1.5 moml/l net is increased. This is referred to as transfusion-related hyperkalemia.[1]

Hypokalemia

Hypokalemia is more prevalentthan hyperkalemia after transfusion since donor red cells re- accumulate the ion intracellularly, and citrate metabolism leads to further movement of potassium inside the cells. No treatment is needed.

Coagulopathy

Coagulopathy is seen in massive transfusion especially if the blood volume is initially replaced by red cells and fluids during absence of two blood volume. This causes dilution of platelets and clotting factors.

Hypothermia

Caused by transfusion of cold blood products in larger volume .This leads to cardiac arrhythmia and also interrupts with platelet function, clotting factor interaction and bleeding time.[23] Blood warmers are used to prevent hypothermia.

Air Embolism

Use of plastic blood bags reduces air embolism. Howeverair enters a central catheter during blood administration sets or blood bags are being changed

or if blood in an open system is transfused under pressure.[24]After transfusion delayed reactions occurs after 24 h or up to month/ years .

DELAYED IMMUNE MEDIATED REACTIONS

Delayed Haemolytic Transfusion Reactions (DHTRS)

DHTRs are observedbased on reactivation of pre-existing antibodies against antigens on transfused red cells. Symptoms may extend from days to weeks after transfusion. No clinical or laboratory features of haemolysis can be observed , however, alloimmune red cell antibody is detected . This is known as delayed serologic transfusion reaction (DSTR). Blood group antibodies associated with DHTRs/DSTRs are Kidd, Duffy, Kell, and MNS systems, in order of decreasing frequency.[18]DHTRs need no treatment because as antibody synthesis increases, red cell destruction occurs.

Transfusion Associated Immunomodulation

Transfusion associated immunomodulation (TRIM) is defined as the down-regulation of recipient's cellular immune response caused due to transfusion of allogeneic blood.[25] TRIM is likely to be mediated by allogeneic leucocytes or their soluble products. One of the reasonable mechanisms is immune deviation towards T-helper lymphocytes type 2 cytokine characterised by secretion of interleukin (IL-4), IL-5, IL-10 cytokines with low T-helper lymphocytes type 1 cytokines namely IL-2, IL-12, and interferon-γ. Using autologous blood or prestorageleucofiltered blood reduces the adverse reactions.[26]

Transfusion-Associated Graft Versus Host Disease (TA-GVHD)

(TA-GVHD) is a clinical syndrome characterized by fever, maculopapular rash advancing to haemorrhagic bullae, enterocolitis with watery diarrhoea, elevated liver function tests, pancytopenia and characteristic findings of histological appearances on biopsy which significantly starts 8-10 days after transfusion.[27] TA-GVHD also occur after transfusion from a blood related donor who is homozygous for an HLA haplotype to a heterozygous recipient (one-way haplotype match).[28]Mortality is 90%eventhough it is rare . Hence TA-GVHD is prevented by irradiation of all cellular blood components (RBCs, platelets, and granulocytes), particularly in patients who are under risk of TA-GVHD.[29]

Post Transfusion Purpura (PTP)

It is a rare complication of transfusion with female predominance occurring between 1 and 24 days after transfusion with a mean of 9 days. Patients significantly exhibits purpuric rash and thrombocytopenia (platelet counts often < 10,000/μL) resulting in bleeding from mucous membranes ,gastrointestinal and urinary tracts. Intracranial haemorrhage is the primary cause of mortality. Antibodies against HPA-1a is the causative agent in several cases although, antibodies to HPA-1b, other platelet antigens, and HLA antigens are also involved. High dose ofintravenous immune globulin is given as treatment .[30]

DELAYED NON-IMMUNE MEDIATED REACTIONS

Iron Overload

Patients who receive frequent blood transfusion for diseases such as thalassemia, sickle cell disease, and other chronic anaemia are at the higher risk for iron overload. RBC unit contain nearly 250 mg of iron. Due to RBC destruction, the majority of the released iron cannot be excreted and is stored in the body as haemosiderin and ferritin. Transferrin becomes saturated after infusion of 10-15 units of RBCs to a non-bleeding patient and iron is stored in the reticuloendothelial system, liver, heart, spleen, and endocrine organs resulting in tissue damage leading to heart failure, liver failure, diabetes and hypothyroidism.[3]

Conclusion

Acute transfusion reactions are at greater risk for developing serious adverse reactions or events. For a better prognosis, awareness about various clinical features of acute transfusion reactions with a capability to predict the serious reactions on time is necessary. Observation and monitoring are needed throughout transfusion. Standard operating procedure comprising the details for documentation, reporting, evaluation, and follow-up of all adverse reactions is mandatory . In current clinical practice , evidence based approach of Restrictive strategy" or "Conservative approach"

of blood transfusion for decreasing the number of unwanted transfusions has made a gross change.

Ethical Clearance – Not required since it is a review article

Source of Funding – nil

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Corona Virus- An Overview

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Abstract

Coronavirus disease 19 (COVID-19) is a highly communicable and pathogenic viral infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that has developed in Wuhan, China and spread all over the world.Genomic analysis revealed that SARS-CoV-2 is phylogenetically associated with severe, acute respiratory syndrome-like (SARS-like) bat viruses, so bats could be the primary reservoir possible. The intermediate source of origin and human transfer is unknown, but the accelerated human to human transmission has been widely confirmed.Maximal precautionary steps and resources have also been put forwardby most nations to mitigate transmission and decrease mortality rates.

Key Words: *Corona virus, China, COVID 19, SARS-coV2, Pandemic*

Introduction

Today the world is standing together to battle against coronavirus disease 2019 (COVID-19)[1].With its roots in WuhanCity (China), COVID19 is an emerging viral pandemic that demands a massive human impact[1]. It started as a group of pneumonia cases with unknown etiology, soon recognized to be caused by aNovel coronavirus strain (CoV), now known as severe acute respiratory syndrome CoV-2 (SARS-CoV-2), which mainly spreads through droplets, respiratory secretions and direct contact[1].

Coronaviruses belong to the order of Nidoviralesin the family Coronaviridae. Corona depicts crown-like spikes on the external surface of virus; thus, it has been called a coronavirus[2].COVID-19 presents as an asymptomatic or mild infection in the majority of the population (80 percent)[1].Multiple protocols and management strategies are being developed around the world to overcome this problem[1].Pandemic infections have been accompanied by a significant number of unavoidable deaths and high mortality increase all around the world[1]. Besides, human-to - human transmission of newly emerging pandemic pathogens regularly creates fear, with a negative impact on the economy and overall well-being of large human populations[2]. As of mid- June 2020, the infection has spread over the world 87,08,008 and mortality rate of 4,61,715[1].

ORIGIN:

On 31 December 2019, the WHO China Country Office was reported 44 cases of pneumonia with unidentified etiology in Wuhan City[3].The infection was started from the Hunan seafood market in China's Wuhan city and quickly infecting over 50 peoples. Live animals such as bats, frogs, snakes, birds, marmots and rabbits are frequently sold at the Hunan seafood market[2].

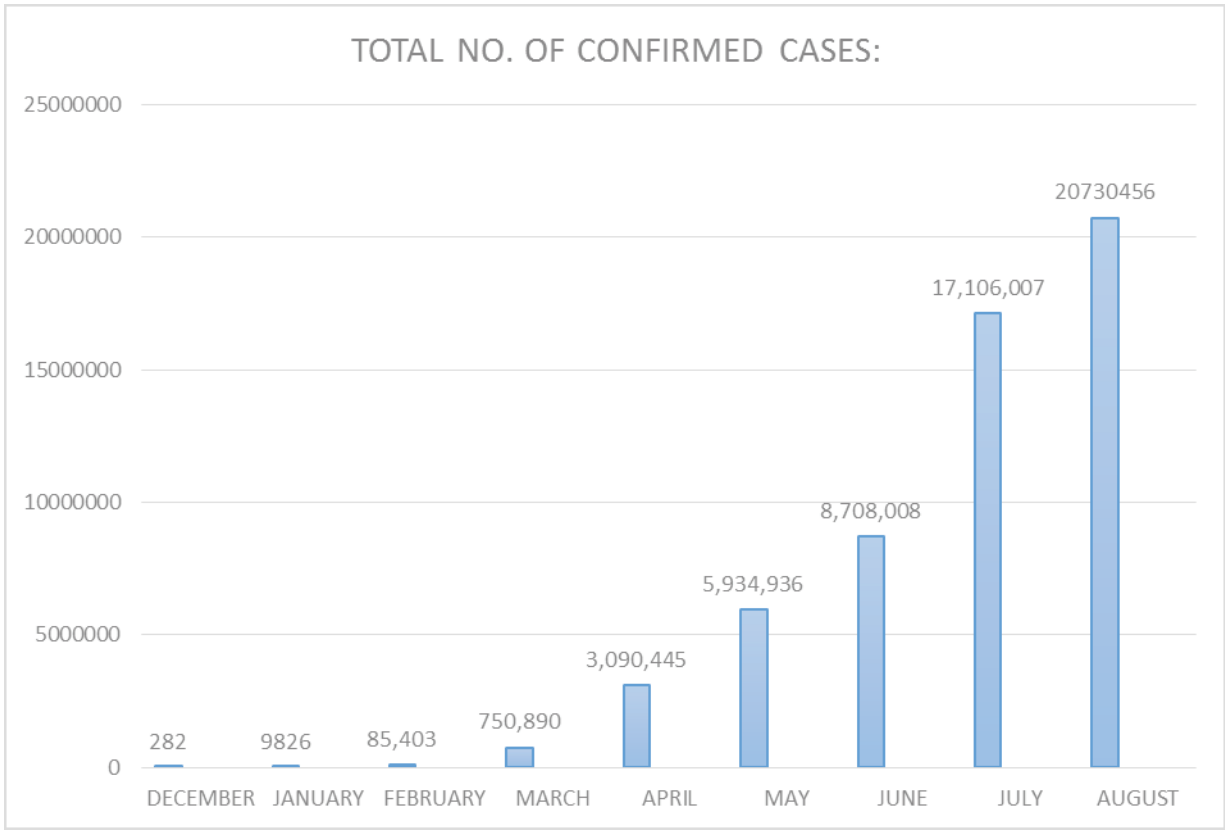
On 12th January 2020, the National Health Commission of China released further details stating that this pandemic is due to viral pneumonia[2].The virus was identified as a novel coronavirus by the sequence-based analysis of the isolated patients[2].Also, the genetic sequence was also provided for the viral diagnosis[2]. It was initially suggested that patients infected in Wuhan was coronavirus caused pneumonia in China could have visited the seafood market where live animals were sold or used as food source infected animals or birds[2]. Further studies, however, showed that some people contracted the infection even with no record of visiting the seafood market[2].Such findings suggested a human to human spreadability of this virus[2]. This virus was later identified in more than 100 countries worldwide[2]. Owing to close contact with an infected person, exposed

to coughing, sneezing, respiratory droplets or aerosols, the human transmission of the virus occurs[2]. Aerosols may enter the human body (lungs) through nose or mouth inhalation[2].

OUTBREAKS:

On 31 December 2019, the WHO China Country Office was notified with cases of unidentified etiology (unknown cause) of pneumonia reported in Wuhan City, China Province of Hubei[3].

Number of confirmed cases from December 2019 to august 2020 given below[3,4,5,6,7,8,9.10,11] (figure1)



CLASSIFICATION:

NIDOVIRALES

CORONAVIRIDIAE ARTERIVIRIDIAE MESONIVIRIDIAE RONIVIRIDIAE

TOROVIRINAE **CORONAVIRINAE**

ALPHA **BETA** DELTA GAMMA

SARS-coV MERS- coV**SARS- coV2**

All viruses in the order of Nidovirales are enveloped, non-segmented RNA viruses with positive sense. They all contain large RNA virus genomes, with some viruses having the largest RNA genomes,consists 33.5 kilobase (kb) genomes[12].

Other common characteristics within the order Nidovirales entail:

· A highly conserved genomic structure, with a broad replicase gene preceding structural genes and accessory genes[12].

· Many non-structural genes expressed through ribosomal frameshifting[12].

· Numerous unique or abnormal enzymatic activities encoded within the large replicase – transcriptase polyprotein[12].

· Downstream gene expression by synthesis of 3′nested subgenomic mRNAs[12].

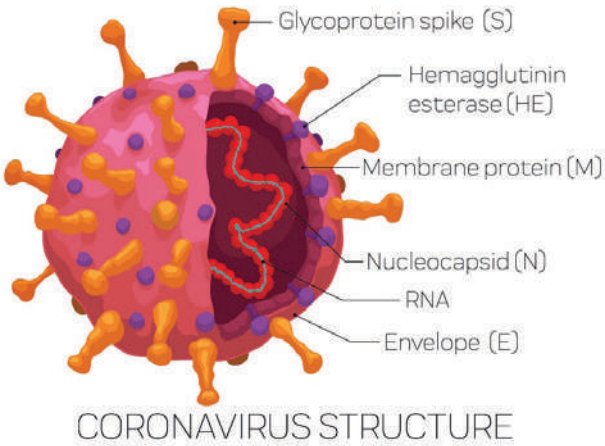
· The significant distinction within the families

of Nidovirusis in number, type and structural protein Sizes. Such variations cause significant alterations in nucleocapsid and virion structure and morphology[12].

VIRION STRUCTURE:

Coronaviruses are minute in size (65–125 nm in diameter) and contain a single stranded RNA as a nucleic material, varying in length from 26 to 32 kbs[2].

Coronavirus virions are spherical with a diameter of about 125 nm as shown by cryo-electron tomography and cryo-electron microscopy in recent studies[12].The most striking feature of coronaviruses is the club-shaped spike projections originating from the virion surface[12]. These spikes are a characteristic of the virion, giving them the appearance of a solar corona, prompting the name, coronaviruses[12].Nucleocapsid is inside the envelope of the virion[12].Coronaviruses possess helically symmetric nucleocapsids, which is infrequent among positive-sense RNA viruses, but far more common in RNA viruses with negative-sense[12].



CORONAVIRUS STRUCTURE

Particles of the coronavirus contain four major structural proteins.Those are the proteins spike (S), membrane (M), envelope (E), and nucleocapside (N), all encoded inside the 3′ end of the viral genome[12].

The S protein (~150 kDa) uses an N-terminal signal sequence to obtain entry to the endoplasmic reticulum and heavily glycosylated[12].Homotrimers of the virus encoded S protein make up the characteristic spike structure on the virus surface[12]. The glycoprotein trimeric S is a class I fusion protein and mediates binding to the host receptor[12]. S is cleaved into two different polypeptides noted S1 and S2 by a host cell furin-like protease[12]. S1 constitutes the broad S-protein receptor-binding domain while S2 constitutes the stalk of the spike molecule[12].

The M-protein is the structural protein most abundant in the virion[12]. This is a small protein (~25–30 kDa) with three transmembrane domainsand it is thought to give form to the virion[12].Recent data indicate M protein is present in the virion as a dimer, and can incorporate two different configurations, allowing the membrane to curve and bind to the nucleocapsid[12].

Within the virion, the E protein (~8–12 kDa) is present in small quantities. The E proteins of coronavirus are strongly divergent but have a similar architecture[12]. The E protein membrane topology is not entirely resolved although most results indicate it is a transmembrane protein[12]. The E protein has an ectodomain N-terminal and an endodomain C-terminal and has activity on the ion channel[12].

The N protein is the only protein found in nucleocapsid[12].This consists of two distinct domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), both capable of in vitro binding RNA still, each domain uses various mechanisms to bind RNA[12].

Hemagglutinin-esterase (HE) is a fifth structural protein present in a subset of β-coronaviruses[12]. The protein functions as hemagglutinin, binds sialic acids to surface glycoproteins and it includes the action of acetyl-esterase[12].

TRANSMISSION:

The source of origination and transmission are essential to be established to improve preventive strategies to contain the infection[2].

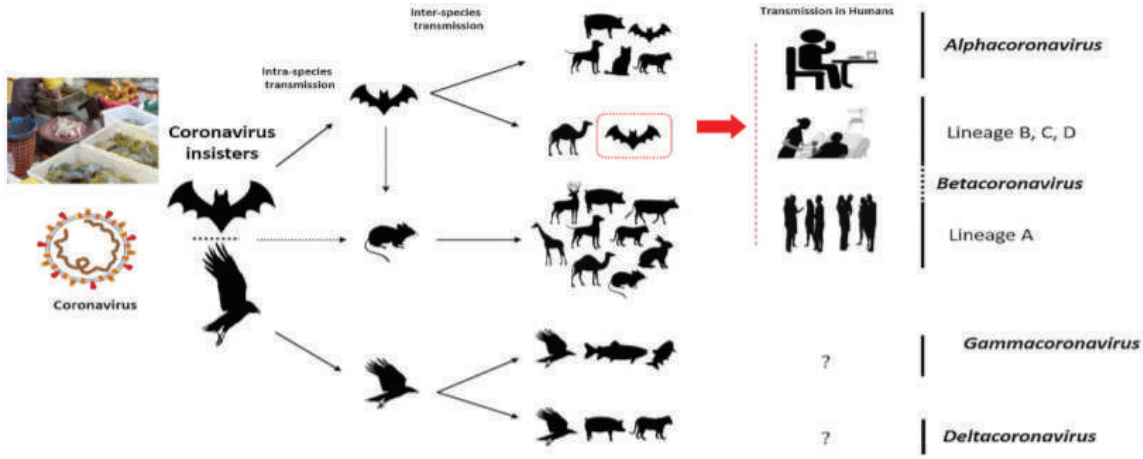
While patients with symptomatic COVID-19 were the key source of transmission, recent findings indicate that asymptomatic patients and patients are also carriers of SARS-CoV-2 during their incubation time[13]. This epidemiological function of COVID-19 has made its monitoring highly challenging, because these patients are difficult to classify and quarantine these patients in time, that can cause SARS-CoV-2 to build up in communities[13].

The common routes of novel coronavirus transmission includeDirect transmission (inhalation

of droplets, cough, sneeze) and indirect Transmission (contact to oral,nasal and eye mucous membranes)[14]. Though typical clinical manifestations of a novel coronavirus infection do not involve eye symptoms, the study of conjunctival samples from confirmed disease and the suspected 2019-nCoV cases indicate that the 2019-nCoV transmission is not restricted to the respiratory tract and that eye contact may provide an effective way for the virus to enter the body[14]. Studies have indicated airborne 2019-nCoV via aerosols produced during medical procedures[14]. It is important that RT-PCR testing could also detect the 2019-nCoV RNA in a stool specimen obtained on day 7 of the patient's illness[14].

When individual coughs, sneezes, laughs or speaks, large droplets or aerosols are produced (> 5 μm diameter) and small (5 μm diameter). Through gravity,Larger droplets easily fall to the ground; transmission of droplets involves close physical contact between an infected person and a susceptible person[15].On the other side, tiny droplets or small particle fragments of evaporated droplets have a low settling velocity so that they can stay in the air for longperiods of time and travel higher before reaching the respiratory tract or contaminating surfaces[15].

The schematic diagram of transmission of corona virus given below[2]:



INCUBATION:

The incubation period of COVID-19 has been estimated at 5 to 6 d on average.However there is evidence that it may be as extended upto 14 days, which is now the widely adopted duration for medical observation and quarantine of exposed persons[13].

HIGH RISK POPULATION:

Current findings indicate this present infectious disease is typically vulnerable to people of all ages[13]. However, those who are in close contact. The risk of SARS-CoV-2 infection is higher for patients with symptomatic and asymptomatic COVID-19, particularly healthcare professionals and other hospital patients[13]. The maximum mortality rates were 14.8 per cent among people over 80 years of age[1]. These results were reported in one of the largest data analyzes of 72,314 patient records conducted in China[1].Although patients with no prior comorbid conditions had a case fatality rate of 0.9%, it was substantially higher for those with serious underlying comorbidities, making these population groups more vulnerable to extreme COVID-19[1].

PATHOGENESIS:

Before the outbreak of SARS-CoV, coronaviruses were thought to cause only mild, self-limiting respiratory infections in humans[12].Such viruses are common in humans, causing 15-30 per cent of infections of the respiratory tract per year[12].They cause more serious disease in neonates, the elderly and people with underlying diseases, with an increased incidence of lower respiratory tract infection in these populations[12]. SARS-CoV, group 2b β-coronavirus, has been reported as the causative agent of the outbreak of Severe Acute

Respiratory Syndrome (SARS) in the Guangdong Province of China in 2002–2003[12]. This is the most severe of all human diseases caused by coronavirus[12]. It is widelyaccepted that SARS-CoV originated in bats as a huge number of Chinese horseshoe bats carry SARS-related CoV sequences and provide serological evidence of previous CoV infection[12].SARS-CoV mainly infects pulmonary epithelial cells. The virus can penetrate macrophages and dendritic cells but causes only abortive infection[12].Despite this, inducing infection of these types of cells may be necessaryProinflammatory cytokines which can lead to the illness[12].These types of cells produce many cytokines and chemokines and are elevated in the serum of patients infected with SARS-CoV[12].The precise mechanism of lung disease and the human cause of serious illness remains undetermined[12].

The ability to infect humans is primarily due to peridomestic animal infection, which is considered to be intermediate hosts, sustaining recombination and mutation events, as well as developing genetic diversity among coronavirus[16].Studies have indicated that the glycoprotein spike (S glycoprotein) plays an important role in restricting the host range by adding virions to the host cell membrane[16]. Coronaviruses typically replicate mainly in the respiratory and intestinal epithelial cells and then induce cytopathic alterations[16].

SIGNS AND SYMPTOMS:

COVID-19 incubation time is from 1-14 days(Mean 5-7 day period), with peak viraemia occurring until symptoms begin[1]. This highlights the capacity for transmission of asymptomatic or minimally symptomatic patients[1].Most COVID-19 patients account for relatively mild cases[13].

The most common features of COVID-19 infection are listed below with its frequency:

Fever (80%-90%), Cough (60%-80%), Breathlessness (18%-46%), Fatigue (38%), Body ache/ joint pain (15%), Sore throat (11%-14%), Headache (6%-14%), Chills (12%), Body ache/joint pain (15%), Running nose (5%), Nausea/vomiting (5%), Diarrhoea (2%-10%)[1].

SIGNS need hospitalization are:

Fever and upper respiratorysymptoms lasting for more than 5 daysand any of the following signs[1]:

· Breathlessness/respiratoryrate more than 24/ min[1],

· Oxygen saturation (SpO2) less than 95% in room air[1].

· Fatigue with heart rate of greater than 110/ bpm[1].

· Systolic blood pressure less than 90 mmHg[1].

COMORBID ILLNESS AND FATALITY RATES IN HIGH RISK GROUPS:

Cardiovascular disease (10.5%), diabetes mellitus (7.3%), chronic respiratory disease (6.3%), hypertension(6%), cancer (5.3%)[1].

CATEGORIZATION OF DISEASE:

MILD:

· Fever, upper respiratory symptoms, mild sore throat and gastro-intestinal symptoms.

· Testing may be considered in select individualsin the high-risk group.

· Testing: NOT ESSENTIAL

· Level of care: HOME CARE

MODERATE:

· Breathlessness/respiratory rate greater than 24/ min, Oxygen saturation (SpO2) less than 95% in room temperature, Fatigue with heart rate of greater than 110/ bpm, Systolic blood pressure <90 mmHg.

· Testing: HIGH PRIORITY

· Level of care: INPATIENT CARE

SEVERE:

· SpO2 less than 90% in room temperature, Hypotension requiring ionotropic support, Acute Respiratory Distress Syndrome /myocarditis.

· Testing: MANDATORY

· Level of care: INPATIENT CARE

INVESTIGATIONS:

TESTS:

SARS-COV-2 DETECTION BY POLYMERASE CHAIN REACTION:

SARS-CoV-2 is an enveloped positive-stranded RNA virus that contains approximately 30,000 nucleotides and 15 genes[17].Some of these genes were used for diagnostic reverse-transcription polymerase chain reaction (RT-PCR) study, including E (envelope), N (nucleocapsid), RdRp (RNA-dependent RNA polymerase), nsp10. Genes (nonstructural protein 10), and genes nsp14 (nonstructural protein 14, exoribonuclease)[17].Unlike other RNA viruses, SARSCoV-2 is subject to mutation, while Nsp14's profreading role limits nucleotide miscorporation levels. Sequence variants can lead to reduced recognition of the individual primary samples used in PCR-based assays[17]. RT-PCR is highly accurate[17].The suggested diagnostic test on the respiratory samples is the reverse transcription-polymerase chain reaction (RT-PCR). Even though initial studies showed low sensitivities of 30-60%, the new studies show better results[1].

NUCLEIC ACID AMPLIFICATION TEST:

Routine confirmation of cases of COVID-19 is based on the identification of specific sequences of virus RNA by NAAT such as real-time reverse transcription polymerase chain reaction (RT-PCR) with confirmation by nucleic acid sequencing. The target viral genes to date include the genes N, E, S and RdRP[18].

SEROLOGY:

Coronaviruses consistof 4 structural proteins: theimmunodominant receptor-binding spike (S) protein, the nucleocapsid (N) protein, the envelope (E) protein, and the membrane (M) protein[17].Diagnostic methods used to identify specific antibodies to SARS-CoV-2 proteins include rapid diagnostic tests, such as lateral flow tests, enzyme-linked immunosorbent assays, neutralization assays and chemiluminescent immunoassays[17].Only neutralization assays may provide information on antibodies reported to suppress viral growth[17].Although the primary use of serological tests is to assess the previous exposure to SARS-CoV-2 can promote the diagnosis of COVID-19 in patients with high clinical suspicion but with negative PCR tests[17].Serological surveys may assist with an ongoing outbreak investigation and a retrospective evaluation of the attack rate or severity of an outbreak[18].In situations where NAAT assays are negative and there is a strong epidemiological link to COVID-19 infection, paired serum samples (in both the acute and convalescent phases) may support diagnosis once validated.Serology tests are given[18].

VIRAL SEQUENCING:

In addition to verifying the existence of the virus, routine sampling of a percentage of specimens from clinical cases may be useful for monitoring mutations in viral genomes that may impact medical countermeasures, including diagnostic tests[18].

BIOMARKERS:

Several biomarkers including lymphocyte counts, neutrophil-to-lymphocyte ratios, C-reactive protein, troponin T, D-dimer, lactate dehydrogenase, procalcitonin, interleukin-6, and ferritin, predict disease progression and COVID-19 mortality[17].

Biomarkers are :

· Hematology – ↓ lymphocyte count,↑ neutrophil and ↓ platelet count.

· Biochemical - ↓ Albumin, ↑Creatinine, ↑Lactate dehydrogenase, ↑ Cardiac troponin,

↑ B-type natriuretic peptide, ↓Oxygen saturation.

· Inflammatory - ↑ C-reactive protein, ↑ Ferritin, ↑ Procalcitonin, ↑Interleukin-6

· Coagulation - ↑ D-dimer

GENERAL TREATMENT MEASURES:

Patients are classified for further decisions on hospitalization and treatment choices based on disease severity[1].The therapy involves antiviral drugs or specific therapy and support management for complications[1]. The theoretical concern of the NSAIDs is worsening outcomes in COVID-19 infections as these levels of angiotensin-converting enzyme 2 in the lung, the virus entrance receptor, remain to be proven[1].Small studies have also found that exposure to NSAID is significantly

correlated with pleuro-pulmonary complications in patients with community-acquired pneumonia[1]. Paracetamol may be favored in COVID19 management over NSAIDs for the above reasons[1].

TREATMENT:

Initially, interferons-a nebulization, antibiotics of wide spectrum and anti-viral drugs were used to reduce the viral load[2]. Only remdesivir, however, has shown promising effect against the virus[2]. Remdesivir alone and significantly blocked the replication of SARSCoV-2 in combination with chloroquine or interferon beta, and patients were declared clinically recovered[2].There are actually several other anti-virals tested against infection[2]. Once tested against infection, Nafamostat, Nitazoxanide, Ribavirin, Penciclovir, Favipiravir, Ritonavir, AAK1, Baricitinib and Arbidol showed intermediate results inPatients and clinical isolates at in vitro[2]. Recentlyblood plasmaadministered into infected patients from clinically treated COVID-19 patients shows promising results with rapid recovery[2].There is no COVID-19 vaccine available while previous vaccines or techniques used to develop a SARS-CoV vaccine may be successful[2].Due to the lack of appropriate therapeutics or vaccines, the best methods to monitor human coronaviruses remain a strong program of public health surveillance, combined with rapid diagnostic testing and quarantine whenever necessary[10].

INFECTION CONTROL:

In SARS-COV-2 infection the prevailing mode of person-to - person transmission is through close contact with infected individuals that produce respiratory droplets[1]. The virus will remain active on inanimate surfaces for several hours and is likely to cause fomite transmission[1]. It seems unlikely that these particles would spread far enough to create secondary infection[1]. The most important factor for reducing the risk of transmitting microorganisms to patients was hand hygiene[2]. Depending on the type of surface, temperature or humidity of the atmosphere, SARS-CoV-2 can persist on surfaces for a few hours or up to several days[13].This enhances the need for good hygiene of the hands and the value of thorough disinfection of all surfaces[13].Since respiratory droplets are the main route of SARS-CoV-2 transmission, high-quality face masks are recommended for particulate respirators[13].

Conclusion

COVID-19 has emerged as a major public health threat in the last nine months.Due to its multiple variants and mutation, a detailed study and analysis is required to arrive for conclusive treatment. This takes a multifaceted approach for tackling the emerging COVID-19 pandemic. Until then the government, the health sector, community and individual person have a vital role to in preventing transmission as "PREVENTION IS BETTER THAN CURE".

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Corona Virus – Dentist's Perspective

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Abstract

A novel coronavirus (COVID-19) contributes to human-to - human transmission. The COVID-19 has been recently identified in infected patient's saliva. Transmission of COVID-19 via contact with droplets and aerosols generated during clinical dental procedures is predicted. There is a need to start up investigations into the identification of COVID-19 in oral fluids and its effects on the transmission of this virus, which is necessary for improving successful preventive strategies, in health care professionals particular for dentists who perform aerosol-generating procedures.In addition to the standard precautions, some special precautions that should be implemented during an outbreak have been raised in this review.

Keywords: *COVID 19, Dentist, Aerosol,Saliva, Xerostromia.*

Introduction

At the end of 2019, a pneumonia outbreak with unknown etiology occurred in Wuhan, China.The pathogen was identified and named as novel coronavirus 2019(nCoV- 2019), which stands for coronavirus disease 2019[1].As of mid-April 2020, the infection has spread to over 185 countries, infectedmore than two million people and resulted in over 127,000 deaths globally[1].(SARS-CoV-2), which spread primarily through droplets, respiratory secretions and direct contact[1]. The most possible route of transmission of disease in dental clinic is aerosol transmission because of exposure to high concentrations of aerosols in a relatively closed environment[1]. Routine dental procedures generate aerosols, which pose potential risks to the dental care personnel and patients[1].Although no corona virus transmitted cases were reported in a dental setting, given the high transmissibility of thedisease, dental teams should be alert and maintain a healthy environment for both the patients and themselves[1]. Therefore, understanding aerosol transmission and its implications in dentistry is necessary[1]. Apart from standard precautions, some special precautions should also be implemented during this period[1].

TRANSMISSION:

Droplets and aerosols and their role in the transmission of diseases:

· When an individual coughs, sneezes, laughs, or talks, large (>5 μm diameter) and small (≤5 μm diameter) droplets or aerosols are generated. Because of its gravity, larger droplets rapidly fall to the ground; thus, droplet transmission involves close physical contact between an infected person and a susceptible person[1].

· On the other hand, small droplets or small particle residues of evaporated droplets have a low settling velocity, so they may remain in the air for a longer time and travel further before they can enter the respiratory tract or contaminate surfaces[1].

· Results from some studies have shown that aerosols from highly virulent pathogens like severe acute respiratory syndrome-coronavirus (SARSCoV) can travel three to six feet distance[1].

Droplets and aerosols in dental setting:

· When performing dental procedures with a highspeed handpiece, friction between the tooth and the rapidly rotating bur would generate excessive heat. Without a coolant, the heat could cause damage

to hard dental tissue and lead to pathological changes to the dental pulp[1]. So we have to use a water coolant while performing dental procedures, including tooth preparation, oral prophylaxis, and oral surgery to prevent heat gain[1].

· The water coolant, however, could generate aerosols. When combined with bodily fluids in the oral cavity, such as blood and saliva, bioaerosols are created[1].

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SIGNS AND SYMPTOMS:

· COVID-19 incubation period is 1-14 days (mean 5-7 day duration), with peak viraemia occurring before the onset of symptoms.This highlights the potential for transmission of asymptomatic or minimally symptomatic patients[2,3].

· Fever, cough, and dyspnoea are the most common presenting symptoms of COVID-19 infection. Other presenting signs include myalgia or tiredness, sore throat, nasal congestion, headache , nausea , vomiting and diarrhoea[2].

· Any one of the this signs like Breathlessness / respiratory rate > 24 / min, oxygen saturation (SpO2) 110 / bpm Systolic blood pressure1 were associated with fever and upper respiratory symptoms lasting > 5 days[2].

ORAL MANIFESTATION:

· Presence of white plaque on the dorsum of tongue, multiple pinpoint yellowish ulcers on the tongue dorsum resembling the late stage of herpetic recurrent oral lesion[4].

· Comorbidities are highly associated with a poor prognosis in COVID19 patients; thus, it is more likely that fear and emotional stress were the underlying cause that triggered herpetic lesions on the palate[5]. The current literature supports the evidence that dysgeusia is the only oral symptom of COVID-19[5].

· In one case herpetic recurrentstomatitis; however, it was the first time the patient had them[6]. Then the was advised to take valaciclovir 500 mg every 8 hr for 10 days, and topical antiseptics with chlorhexidine and hyaluronic acid. After 10 days, there was a complete recovery of the oral lesions[6]. The patients presented ulcers or blisters, those are common elementary lesions seen in other viral infection, such as aphthous fever, hand, foot, and mouth disease and herpetic gingivostomatitis,[6].

· The current analysis strongly confirms the high prevalence of olfactory and gustatory disorders in COVID-19 infection[6].

· Loss of smell and taste may be used as indicators of potential contagion and early identification may help to decrease the risk of spread, especially by paucisymptomatic cases[7].

· The most common feature is painful ulceration in the buccal mucosa[8]. Oral examination showed besides multiple reddish macules are radiated along the hard palate, tongue, and lips[8]. The biopsied lesion was microscopically diagnosed with severe vacuolization and occasionally exocytosis of the epithelium[8]. Within the lamina propria a diffuse chronic inflammatory infiltrate was associated with focal areas of necrosis and hemorrhage. superficial and deep small vessels were obliterated by evident thrombi[8]. Small thrombi seemed to consist primarily of endothelial cells, while the larger cells were made up of fibrin and endothelial cells and in either cases CD34 was positive for cells in the luminal component[8]. Adjacent minor salivary glands showed an intense lymphocytic infiltration, mostly positive for CD3 and CD8 and some of these cells were also found in the basal layer of the epithelium[8].

· Coronavirus disease 2019 is associated with a variable inflammatory reaction that can induce vascular inflammation[9]. Erythematous rash has been described [2] and could also be explained by an inflammatory reaction[9]. Thus, an irregular oral ulcer could be an inaugural symptom of Covid-19 which needs to be proven in larger cohorts of patients[9].

· It is known that the oral cavity may exhibit manifestations of underlying diseases such as oral ulcerations, gingival bleeding, glossitis, oral pain, or halitosis[10].

· Viral infections usually manifest as either ulceration or blistering presentation of oral tissues[10].

· Recurrent oral ulcers could be an inaugural

symptom of COVID-19[10]. Some cases showed that pain and intraoral manifestations such as oral ulcers or blisters before seeking medical advice was a common finding in COVID-19[10]. Thus, it was encouraged to perform intraoral examinations in patients suspected of SARS-CoV-2[10].

· As these oral findings are still new in the literature, their occurrence may vary significantly among COVID-19 patients and, thus, the associated systemic diseases and/or poor oral health may be a contributory factor to the oral presentations+10. Given the possibility of immunocompromised statuses of the patients, it is also possible that the oral manifestations may be related to other viruses or bacteria[10].

a) Hyposalivation :

· The salivary gland secretion is dependent on several factors, including temperature, circadian rhythm and intensity, and type of taste and on chemosensory, masticatory, or tactile stimulation[10].

· Hyposalivation, the reduction of unstimulated salivary flow rate, is a common finding in patients mainly reported as a consequence of the use of medication and psychological processes[10].

· Dry mouth was shown to be manifested in a relatively high proportion of COVID19 patients [23]. A recent communication has drawn attention to hyposalivation as responsible for exposing patients to a higher risk of getting coronavirus disease (COVID19) once the presence of many proteins with antiviral properties in saliva could be reduced[10].

· Interestingly, the SARS-CoV-2 infection is more severe in individuals over 50 years of age and with the presence of associated comorbidities such as diabetes, cardiovascular problems and diseases involving the nervous system[10].

· It is known that salivary flow reduces with age and is not explained based on medications used by older adults[10]. Besides, diabetes and medications for systemic disorders have also been associated with hyposalivation[10].

· It is known that infectious and inflammatory processes might also lead to hyposalivation and, thus, the possibility of qualitative and quantitative disturbances in saliva secretion by SARS-CoV-2 infection in the salivary gland should not be discarded[10].

· Human saliva is a complicated fluid and plays a crucial role in preventing from a viral infection, especially through the innate immune system, which is a notable first-line defense[11].

Two possible explanations for enhancing the incidence rate of this infection are as follows:

• Lowered saliva secretion can disrupt the oral and airway mucosal surfaces as a physical barrier, thereby enhancing the viral colonization and adhesion[11].

• This decrease may also hinder the secretion of antimicrobial peptides and proteins[11].

Considering the existence of various proteins with established antiviral characteristics in saliva such as lysozyme, mucins, cathelicidin (LL-37), lactoferrin, peroxidase, sIgA SLPI, salivary agglutinin (gp340, DMBT1), alpha-defensins, beta-defensins, and cystatins, some of which may potentially impede virus replication especially SARS-CoV-2[11].A possible risk factor for severe respiratory infection could be hyposalivation. It can leave patients at a significant risk of getting COVID-19[11].

b) **Taste disorders**:

· Taste disorders have been reported in a variety of clinical problems [34]. Amblygeustia, a diminished sensitivity of taste, was shown to be manifested by a relatively high proportion of COVID-19[10].

· In a study in which patients with influenza- like symptoms underwent Covid-19 testing, smell and taste loss were reported in 68% (40/59) and 71% (42/59), respectively, of COVID-19-positive patients suggesting that chemosensory dysfunction should be considered when screening symptoms[10].

· Possible taste alterations as result of the direct effect of SARS-CoV-2 infection in sensory neurons or other components of the peripheral gustatory system should also be considered[10].

c) SARS-CoV-2 may cause acute and chronic sialadenitis:

· Wang et al. proposed that SARS-CoV-2 might induce acute sialadenitis and associated symptoms, such as pain, discomfort, inflammation, and secretory dysfunction in salivary glands. SARS-CoV-2 can attach to ACE-2 receptors on the epithelium of salivary glands, fuse with them, replicate, and lyse cells to trigger apparent signs and symptoms, such as discomfort, inflammation, and pain in major salivary glands[11].

· After the cytolytic activity of SARS-CoV-2 lyses the acinar cells, salivary amylase is unleashed into the peripheral blood. It can be inferred that the amylase rises in peripheral blood during the early contamination process[11].

· Secreted inflammatory cytokines facilitate the inflammatory reaction that destroys the tissue of the salivary glands as the immunopathological process continues[11].

· Fibrogenesis and granulation can restore the inflammatory damage by reducing immunoreaction[11]. After the severe stage, the function of salivary glands can be anomalous due to contamination with SARSCoV-2, which may induce chronic sialadenitis[11].

Current COVID-19 diagnosis :

The main strategy of identification for COVID-19 is Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR), which is commonly used to extract viral RNA from oropharyngeal and nasopharyngeal swabs or sputum samples[11]. In addition, a chest X-ray may be an invaluable diagnostic method for identifying bilateral pneumonia, displaying as multi-lobar ground-glass opacities with an asymmetric, peripheral, and posterior distribution[11].

Salivary diagnostics :

· Saliva is now widely established as a reservoir for biological indicators that range from modifications in nucleic acids, proteins, and biochemicals to the microflora[11]. As a diagnostic fluid, saliva has tremendous potential and advantage over other biological fluids because the sampling process of saliva does not entail an invasive intervention, and it is inexpensive and helpful for controlling the systemic health[11]. In the near future, designing accurate and responsive salivary diagnostic instruments and the implementation of established guidelines following meticulous testing will enable the use of salivary diagnostic as chairside tests for diversified oral and systemic diseases[11].

· The benefits of salivary diagnostic tests are economical, noninvasive, healthier to apply than serum sampling, diagnostic values in real-time, no requirement for specialized healthcare workers, numerous samples are simple to obtain,collecting and monitoring are doable at home, minimizing the possibility of cross-infection, better shipping and storage than serum sampling, lesser agitation during the diagnostic process, screening assays are commercially available, and saliva does not clot and can be handled more efficiently than blood[11].

· Some virus strains have been detected in saliva as long as 29 days after infection, indicating that a non-invasive platform to rapidly differentiate the biomarkers using saliva could enhance disease detection[11]. Saliva samples could be collected in patients who present with oropharyngeal secretions as a symptom[11]. Given the need for close contact between healthcare workers and infected patients to collect nasopharyngeal or oropharyngeal samples, the possibility of self-collection of saliva can significantly reduce the risk of COVID-19 transmission. Besides, the nasopharyngeal and oropharyngeal collection promotes discomfort and may promote bleeding especially in infected patients with thrombocytopenia[11].

· We suggest that there is a minimum of three different pathways for COVID-19 to present in saliva: firstly, from COVID-19 in the lower and upper respiratory tract that enters the oral cavity together with the liquid droplets frequently exchanged by these organs[11]. Second, COVID-19 present in the blood can enter the mouth through crevicular fluid, an oral cavity-specific exudate containing local proteins derived from extracellular matrix and serum derived proteins[11]. Finally, another way for COVID19 to occur in the oral cavity is by major- and minor-salivary gland infection, with subsequent release of particles in salivavia salivary ducts[11].

· It is essential to point out that salivary gland epithelial cells can be infected by SARS-CoV a short time after infection in rhesus macaques, suggesting that salivary gland cells could be a pivotal source of this virus in saliva[11]. Additionally, the production of SARS-

CoV-specific secretory immunoglobulin A (sIgA) in the saliva of animal models intranasally immunized was previously shown[11]. Considering the similarity of both strains, we speculate that salivary diagnosis of COVID-19 could also be performed using specific antibodies to this virus. Thus, salivary diagnostic testing can offer a convenient and cost-effective mechanism for early-diagnosis of Covid[11].

· Further researches are required to investigate the potential diagnostic of COVID-19 in saliva and its role in transmission of this virus, which is crucial to improve its effective strategies for prevention, especially for dentist and healthcare professionals that perform aerosol-generating procedures[12].

Evaluation and treatment of patients with salivary gland disease in this pandemic situation. A three-step guideline to survey patients with salivary gland disease is as follows:

1) Primary telemedicine examination[11]:

· Recognize patients having touchable or visible lesions, mass presence in the majority of salivary gland region, and neoplasm symptoms

· Ask the history of skin cancer of the head and neck, lymphoma, etc.

· Ask the clinical symptoms and history of inflammatory salivary gland disease

2) Diagnostic examination for patients suspected of having salivary gland neoplasm[11]:

· COVID-19 screening 2

· FNA biopsy

· Ultrasound of salivary gland

· Extra imaging such as MRI, if needed

· Make sure of the existence of salivary gland disorder

· Identify signs and symptoms of non-neoplastic illness

3**) Patient counseling and recommendations for treatment[11]:**

· Survey FNA biopsy outcomes

· Assess treatment options and prognosis

· Prescribe COVID-19 test before any surgery

· Pay attention to age comorbidities of the patient

· Radiation oncology and hematology consultations, if required

· Considering the outbreak situation.

DENTISTRY VS COVID-19:

TRANSMISSION OF COVID-19 IN DENTAL CLINIC:

CONTACT SPREAD:

Transmission of 2019-nCoV in dental clinics occurs through four major routes:

(1) Direct contact of droplets, blood, saliva or other patient material to respiratory secretions[3];

(2) Indirect exposure to infected surfaces and/or instruments[3];

(3) Inhalation of airborne virus[3];

(4) Mucosal (oral,nasal and conjunctival) interaction with infected droplets and aerosols that are driven by coughing and speaking without using mask[3].

CONTAMINATED SURFACE SPREAD:

Research has shown that coronaviruses can stay several days on metal, glass, and plastic surfaces16. As surfaces in dental clinics thus serve as places for droplets and aerosols mixed with saliva and/or blood of patients, they can effectively continue to spread infection[3]. Coronaviruses can keep their virulence active from 2 h to 9 days at room temperature[3]. Their operation was substantially higher than 30 per cent at 50 per cent humidity[3]. Thus, it seems in the dental setting that surfaces are holding clean and dry will play a significant role in preventing transmission to 2019-nCoV[3].

SPECIAL PRECAUTIONS IN DENTAL PRACTICE:

Dentists should be familiar with how 2019-nCoV is spread, how patients with 2019-nCoV infection are

identified and what extra-protective steps need to be taken in practice to prevent 2019-nCoV transmission[3].

Patient at reception:

· Provide them a surgical face mask and sanitizer[3].

· The patient's body temperature should be checked by using contact free thermometer[3].

· Equipment such as blood pressure cuffs and thermometers must be cleaned and disinfected after every use with 70 per cent ethyl alcohol[3].

· Maintain social distance between the patient and dental personnel[3].

· To avoid close contact with potentially infectious patients, install physical barriers (e.g., glass or plastic windows) at the reception areas[3].

· When verifying and scheduling appointments, take a comprehensive history of travel and health[3].

· Ask for recent medical history (cough, sore throat, fever, respiratory illness). If there is any evident symptom persists dental personnel should inform the dentist and then case must be local or public health authorities[3].

· Reschedule appointments if your patients have traveled outside India within the last two weeks to a coronavirus-affected area[13].

· Take contact information and address of all treated patients[13].

INSIDE DENTAL ROOM:

· It is commonly believed the preoperative antimicrobial mouthrinse can reduce the number of oral microbes[3,13].

· Use of rubber dams will significantly reduce production of aerosol or spatter tainted with the saliva and blood, in particular high-speed handpieces and ultrasonic dental appliances are used[3,13].

· Dental anti-retraction handpiece with specially built anti-retractive valves or other anti-reflux designs is highly recommended as an extra preventive Cross-Infection assessment[3,13].

· Autoclave hand-pieces after each procedure[3,13].

· Public areas are also washed and disinfected like door handles, chairs and bathrooms[3,13].

DENTIST'S SAFETY precautions:

· Ensure that the personal protection equipment used is appropriate for the procedures being undertaken[13].

· Clinically, COVID-19 can also be transmitted by contact with the mucous membranes in the eyes, since infectious droplets can easily contaminate the human conjunctival epithelium. So, Protective eyewear or face mask should be worn and disinfected for patients during the whole procedure[13].

· During practices that are likely to cause splashes or blood sprays, body fluids, secretions or excretions, wear to protect skin and avoid soiling of clothes[13].

· Clean hands with soap and water for at least 20 seconds following patient touch or using an alcohol-based hand sanitizer with at least 60 percent alcohol if no soap and water are available[13].

· Dental personnel should use N95 respirators or respirators which provide a higher level of protection when performing or presenting an aerosol-generating procedure rather than a facemask[13].

· After each patient visit, surfaces are disinfected, particularly surfaces located close to the operating areas[13].

SPECIAL SAFETY PRECAUTIONS:

· Dental hospitals and clinics across China are temporarily closed to avoid the possible risk of transmission due to the unique characteristics of dentistry and the high transmissibility of COVID-19[1].However, there are some dental emergencies that needs immediate treatment and control, such as trauma, facial space infection, and carcinoma. Therefore, special precautions should be followed when treating dental emergencies[1].

· Surgery will be postponed for any patients with abnormal chest CT results, those either with symptoms of COVID-19 or confirmed cases of COVID-19[12]. If dental surgery is an emergency, dentists must address the possibility of aerosolization[12]. Sinonasal area and

pharynx, suitable PPE of the whole operating room team are strongly advised for minor salivary gland tumors of the oral cavity. The number of staff in the operating room must be minimized[12].

· The risk of aerosolized mucosal secretions is only found during intubation and extubation for parotid and submandibular neoplasm surgeries. During intubation and extubation, all the staff in the room must wear suitable PPE while all unneeded staff must leave the room[12]. Controlling the obstructive salivary gland disease can be remotely conducted in most cases[12]. In rare situations, intraoperative drainage and biopsy or office-based assessment and treatment procedures may be needed for handling an abscess, detecting cancer, relieving acute pain which has not been treated with proper medical care[12].

· Due to irrigation and aerosolization, sialendoscopy should be stopped during the outbreak of COVID-19[12]. In addition, all dentists should know that the use of a mouthrinse and/or local nasal products, which include beta-cyclodextrins in conjunction with flavonoid agents, might provide invaluable adjunctive care to minimize the viral load of saliva and nasopharyngeal microbiota, including SARS-CoV-2[12]. Therefore, dental professionals can create a safer atmosphere for themselves as well as their patients[12].

Conclusion

Dentists, by nature, are at highly prone to infectious diseases. The emergence of COVID-19 has brought new challenges and responsibilities to dental professionals The Sars-CoV-2 infection is responsible for several events in the mouth.A better understanding of aerosol transmission and its implication in dentistry can help us identify and rectify negligence in daily dental practice.In addition to the standard precautions, implementation of special precautions could prevent disease transmission from asymptomatic carriers. These are thesome special precautions would not only help to control the spread of COVID-19 but also serve as a guide for managing other respiratory diseases.

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Conflict of interest – nil

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Infection Control During COVID – 19 (Corona Virus)

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Abstract

The novel coronavirus disease 2019 (COVID-19) has developed into a global pandemic, with its early roots from Wuhan city in China. Many warring nations have placed forward maximum precautionary steps and tools to reduce transmission and decrease fatality rates. Infection control measures are required to prevent the infection and to avoid further spread of the virus and to help control the infection. Restrictive steps such as social distancing, lockdown, case identification, isolation, contact tracing and quarantine of exposed had identified the most effective efforts to monitor the spread of the disease.The risk of cross-infection between patients and dental practitioners may be high due to the characteristics of the dental settings. Strict and reliable guidelines on the prevention of infections are urgently needed for dental practices and hospitals in areas (potentially) affected by COVID-19. This article provides a detailed overview of general and relevant preventive interventions, methods and prevention of infections in the healthcare and public settings.

Keywords: *Infection control, Protocol, COVID19, Pandemic, Safety measures*

Introduction

Today the world is standing in the battle against coronavirus disease 2019 (COVID-19), an emerging viral pandemic that demands a large human toll[1]. With its origins in Wuhan City , China, as a cluster of cases of unexplained aetiology pneumonia, it was soon established as being caused by a novel strain of coronavirus (CoV), now called severe acute respiratory syndrome CoV-2 (SARS-CoV-2), which spread primarily through droplets, respiratory secretions, and direct contact[1]. COVID-19 presents as an asymptomatic or moderate infection in the majority of the population (80 per cent)[1]. However, the disease is known to cause severe pneumonia and multiple complications, especially in certain groups at high risk[1]. In addition to social distance, lockdown, case identification, isolation, quarantine, proper infection control and protection measures are important to avoid this life-threatening emerging corona virus[1].

INFECTION CONTROL IN SARS-coV 2:

The prevalent mode of transmission from person to person in SARS-CoV-2 infection is through close contact with the respiratory droplet-generating infected individuals[1]. The virus is capable of staying active for several hours on environmental surfaces making transmission of fomite likely[1]. However,airborne transmission does not appear to be a significant mechanism[1]. This has unique consequences because of a lack of personal protection equipment in different settings[1]. Aerosol-generating procedures may create smaller particles that can remain in the air for longer periods of time[1]. Distant distribution of such particles seems unlikely to develop secondary infection[1].

Discussion

Strategies needed to prevent or restrict the transmission of COVID-19 in health care facilities include the following[2]:

SCREENING for early detection of patients with suspected COVID-19, and rapid implementation of source control measures[2]:

1) Screening:

To facilitate screening the health-care facilities should:

1. Display information at the entrance of the facility directing patients with signs and symptoms of COVID-19 to report to the designated area for screening[2].

2. Establish entrances for patients with signs and symptoms of COVID-19[2].

3. Train staff on the signs and symptoms of COVID-19 and the most recent case definitions. Encourage health workers to be alert to potential COVID-19 infection in all patients[2].

4. Ensure that screening personnel maintain a distance of at least 1 metre from patients, ideally with a separation created by a glass/plastic screen[2]. If that is not possible, mask and eye protection should be worn[2].

5. All suspected COVID-19 patients advised to wear masks for source control purposes and place themselves in a designated, well ventilated waiting area at least 1 metre apart from each other[2].

6. Ensure there is a process in place to minimise the time reported by COVID-19 patients waiting to be screened[2].

7. Suspected COVID-19 patients with respiratory distress symptoms and significant underlying conditions should be prioritised for medical examination[2].

A) Isolation or designated waiting area:

1. Health care facilities in emergency departments without adequate single isolation rooms should designate a separate, well ventilated area where patients with suspected COVID-19 can stay. This area should have at least 1 metre apart tables, stalls, or chairs[2].

2. The isolation or designated area should have specific toilets, hand hygiene stations and lid-based trash bins for disposal of paper tissues used for respiratory or hand hygiene[2].

3. Screen graphic information to show patients how to practise hand hygiene and respiratory hygiene[2]. In order to avoid the spread of COVID-19 in health care facilities, patients with suspected COVID-19 that have been missed by screening and triage efforts or have become contaminated inside the facility must be identified promptly[2]. This can be quite challenging considering the large number of acute respiratory infections and atypical clinical presentations of COVID-19[2].

B)Health-care facilities should:

1. Encourage health workers to check for possible COVID-19 cases , particularly when there are signs and symptoms of COVID-19 inpatients and no other reasonable explanation for these symptoms is provided[2].

2. Ecourage rapid testing and reporting of suspected COVID-19 patients who were diagnosed during hospitalisation[2].

3. Develop recall systems for clinicians to recognise COVID-19 based on signs and symptoms, particularly in areas with transmission to the population[2].

2) Standard precautions for all patients:

Standard precautions seek to reduce the risk of bloodborne and other pathogens transmitted from both known and unrecognised sources[2]. They reflect the basic level of precautions for infection control which should be used in the treatment of all patients at all times[2]. Standard measures include, but are not limited to, hand and respiratory hygiene, risk assessment use of sufficient PPE, environmental sanitation, and responsible waste management[2].

a) HAND HYGIENE:

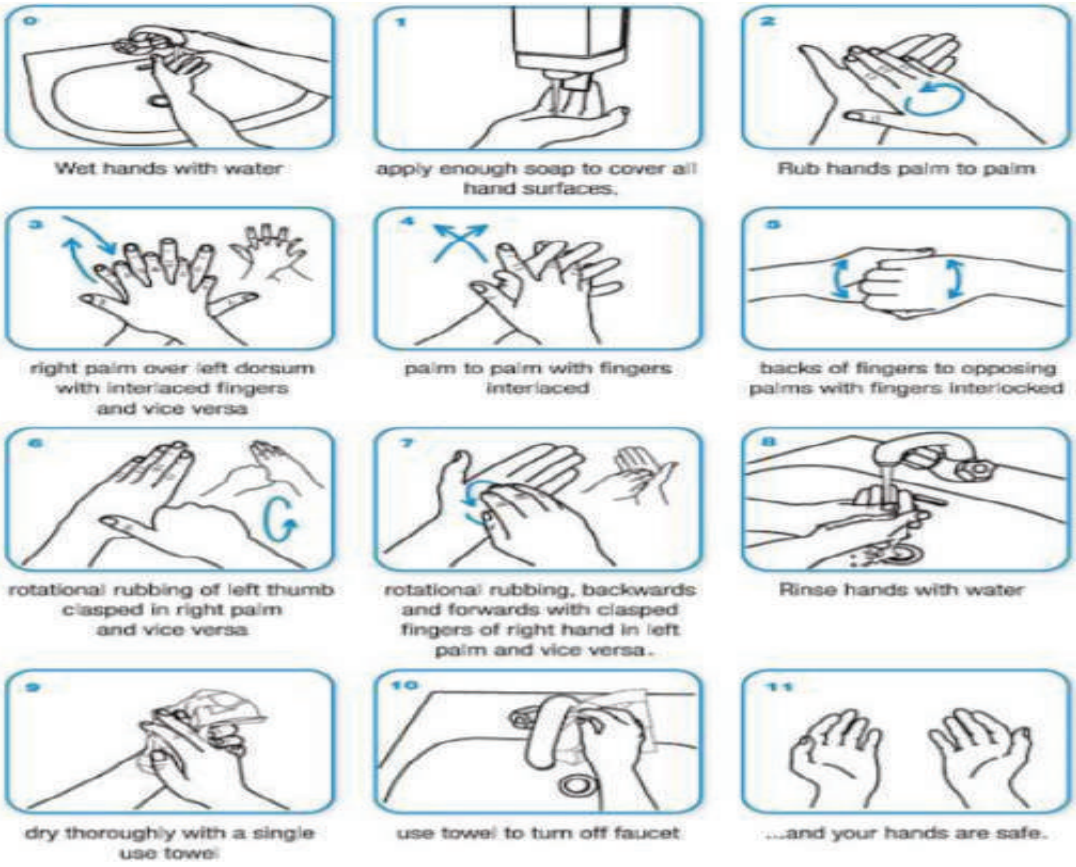
Hand hygiene Hand hygiene is one of the most important strategies for preventing COVID-19 and other pathogens from spread. Health workers should apply the following guidelines to optimum output in hand hygiene[2].

Five situations[2]:

- Before a patient enters,
- Before conducting any clean or aseptic procedure;
- Exposure to body fluids,
- After touching the patient,
- After the patient has entered the surroundings;

Hand hygiene involves either cleaning hands with alcohol-based hand rubbing (ABHR containing at least 70% alcohol, or soap, water, and disposable towels;

alcohol-based hand rubbing products are favoured when hands are not clearly soiled; washing thoroughly with soap and warm when visibly soiled; using the appropriate technique and duration for hand washing[2].



b) Respiratory hygiene[2]:

Ensure the following steps of respiratory hygiene are applied:

• View graphical information on the need to cover the nose and mouth when coughing or sneezing with a tissue or bent elbow[2].

• Do handwash after contact with respiratory secretions or items possibly infected with respiratory secretion[2].

• Offer suspected COVID-19 patients a wearable medical mask[2].

c) Use of PPE:

Adequate and proper use of PPE decreases pathogen exposure. The PPE 's effectiveness depends heavily on[2]:

• Personnel training on putting on and removing PPE[2].

• Immediate access to appropriate supplies[2].

• Proper hand hygiene[2].

• Enforcement by health workers[2].

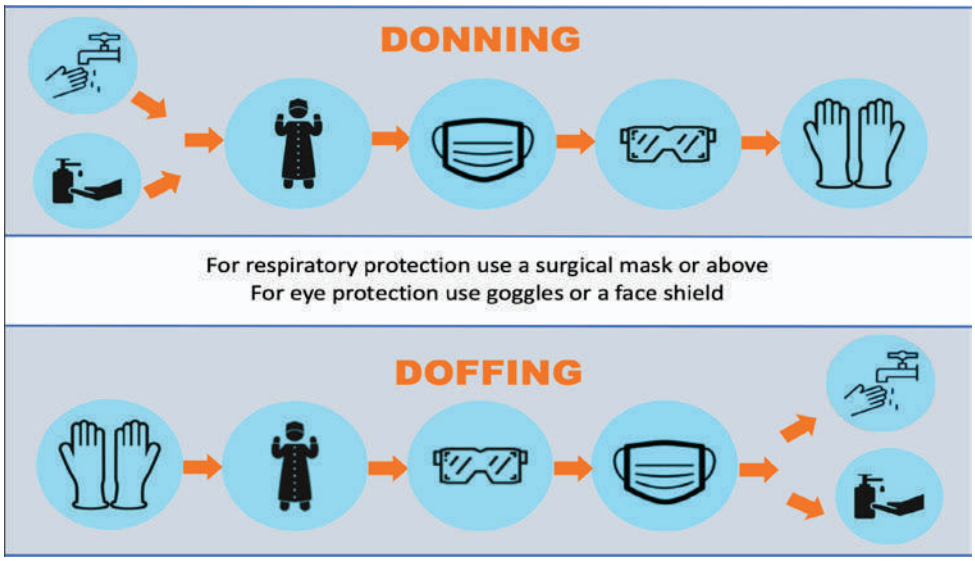
• Daily supervision and feedback by IPC employees[2].

d) Environmental cleaning :

· Ensuring clear and accurate cleaning and disinfection procedures are followed is essential[2].

· All surfaces in health-care facilities should be regularly cleaned and disinfected, especially high-touch surfaces, and should be soiled visibly or contaminated with body fluids[2].

· In settings where patients with suspected or confirmed COVID-19 are admitted, frequency depends on the form of patient area and surface region[2].



Taken from: http://publichealth.lacounty.gov/acd/docs/CoVPPEPoster.pdf

3) Implementing additional precautions:

According to current evidence, SARS-CoV-2, the virus that causes COVID-19, is primarily transmitted by the respiratory droplets and communication routes between people[2].

Droplet transmission occurs when someone with respiratory symptoms (e.g. coughing or sneezing) is in close contact (within 1 m) and is thus at risk of exposure to potentially infectious respiratory droplets through his / her mucosa (mouth and nose) or conjunctive (eyes). Thus, COVID-19 virus transmission can occur through direct contact with infected individuals and indirect contact with surfaces in the environment or items used on the infected individual[2].

Airborne transmission varies from droplet transmission because it applies to microbes in droplet nuclei. The droplet nuclei are commonly known as particles[2].

Isolation of patients with suspected or confirmed COVID-19:

1. Limit the number of health workers who have contact with each COVID-19 patient[2].

2. Patients should, if feasible, be housed in well ventilated single rooms[2].

3. If single rooms are not available or the occupancy rate is estimated to be 100% or higher, suspected, likely or confirmed COVID-19 patients should be clustered (coordinated) in sufficiently ventilated areas with beds at least 1 metre apart[2].

4. Stop getting patients out of their rooms or locations, unless medically necessary. Using approved portable X-ray equipment and/or other medical instruments[2].

5. Ensure that health workers who transport patients are hand hygienic and wear suitable PPE

6. Facilities should be either single-use and dedicated or disposable. If equipment has to be shared between patients, clean and disinfect each time a different patient uses it[2].

7. Maintain track of all employees accessing the patient's room[2].

Health workers performing AGPs or in settings where AGPs are performed among suspected or confirmed COVID-19 patients (e.g. intensive care units or semi-intensive care units) should:

1. Perform procedures in an appropriately ventilated space-refer in this guidance to the section on environmental and technical control[2].

2. Please note that if the wearer has a beard or other thick facial hair, this can prevent proper respiratory fitting. Other PPE products include eye protection (e.g., goggles or a face shield), long-sleeved gown, and gloves[2]. If gowns are not resistant to fluid, health workers performing AGPs should use a waterproof apron if the operation is expected to produce a large amount of fluid which may penetrate the gown[2].

3. In intensive care units, where AGPs are commonly performed, the health worker may choose to wear a particulate respirator in areas of community transmission during their shift;

Ventilation has three basic criteria[2]:

• **Ventilation rate**: quantity and quality of outside air supplied to the space[2].

• **Airflow direction:** the overall airflow direction should be from clean areas in the building and between the spaces[2].

• **Air distribution or airflow pattern:** the supply of air to each part of the space to improve the dilution and elimination of airborne contaminants produced in the room[2].

4) Duration of contact and droplet precautions for patient with COVID-19:

Standard precautions should be applied whenever possible. Contact and droplet measures should be removed only in consultation with physicians and should take into account the resolution of clinical signs and symptoms, or the number of days after a positive examination with an upper respiratory specimen was performed[2].

1. These additional steps can be withdrawn for symptomatic patients 10 days after the onset of symptoms For at least three consecutive days with no fever or respiratory symptoms; Isolation can terminate for asymptomatic patients 10 days after the initial positive RT-PCR test result[2].

2. Although some patients were tested positive for COVID-19 on the basis of molecular assays several days after symptom resolution, it is still unclear if these patients continue to shed the virus, because only viral fragments of RNA were detected[2].

5) Collecting and handling laboratory specimens from patients with suspected COVID-19:

1. All specimens obtained for laboratory surveys should be treated as potentially contagious. The following precautions and biosafety standards should be adhered to by health workers who collect, treat or transport any clinical specimens[2].

2. Ensure that health care staff who collect specimens, including nasopharyngeal and oropharyngeal swabs, use suitable PPE[2].

3. If the specimen is obtained using an AGP (e.g. sputum induction), the operating staff should wear a particulate respirator[2].

4. Ensure all staff carrying specimens are trained in safe handling methods and decontamination procedures for spills[2].

5. Put specimens in leak-proof container for transport[2].

6. Collect all items, if possible by hand. Do not move specimens using pneumatic-tube systems[2].

7. State clearly the full name of the patient, date of birth and clinical diagnosis of the suspected COVID-19 case on the application form for a laboratory application[2].

6) Laboratory measures:

· Re - evaluate your procedures and reduce or eliminate steps which may lead to aerosol formation or droplet creation[3].

· Review the situation weekly or biweekly, and make any required changes[3].

· Establish a chain of command, plan for emergencies and plan for contingency[3].

· Implement crowding management measures: review staffing requirements and realign the workload; consider working in shifts to reduced level[3].

· Follow the recommendations for routine handling of specimens by CDC / WHO in compliance with the guidelines[3].

· Process all specimens having measures that

could lead to aerosols or droplets[3].

· Keep updated on the latest developments concerning the COVID-19 pandemic and notify the workers of any new scientific development knowledge; do away with myths[3].

· Remain aware of the current hospital policies and procedures and notify staff of any changes[3].

· Maintain open channels of communication with colleagues and staffs[3].

Don't do:

· Should not trigger needless anxieties or panic, but be honest about the risks[3].

· Don't spread or support rumors or information that doesn't come from a credible source[3].

7) For surgical procedures:

In the COVID-19 pandemic background, any surgical procedure could involve risk to both health workers and patients. Health workers should apply standard procedures as part of their routine clinical practise, and assess possible risks of exposure to infectious material[2].

Before conducting a surgical procedure the following should be considered$_2$:

In the context of the COVID-19 pandemic, every surgical procedure may entail risk for both health workers and patients. As part of their routine clinical practice, health workers should apply standard precautions and assess potential risks of exposure to infectious material[2].

The following should be considered before performing a surgical procedure[2]:

General acknowledges:

· Consider whether non-operative treatments or therapies may be an alternative[2].

· In areas of mass transmission, postpone elective surgery to reduce the risk to patient and health care staff[2].

· Where the operation can not be delayed (e.g., urgent), careful risk assessment should be conducted to test patients for COVID-19 symptoms, signs and history of exposure[2].

· Some healthcare facilities may recommend testing COVID-19 surgical patients prior to the surgical procedure, regardless of the risk assessment for COVID-19, depending on the local testing ability and transmission strength within the region[2].

· Patients with COVID-19 signs should undergo chest X-ray, chest computerised tomography or chest ultrasound, if appropriate, as an early diagnostic tool and as a reference for patient monitoring, if the urgency of the surgical procedure does not provide sufficient time for testing or if testing is unavailable[2].

Surgical procedures in patients suspected or reported as having COVID-19:

· Where surgical procedures can not be delayed in COVID-19 patients, surgical personnel in the operating room should use contact and droplet measures like sterile medical mask, eye shielding, gloves and gown[2].

· Instead of a surgical mask, we can use a particulate respirator. During surgical procedures respirators with exhalation valves should not be used as unfiltered exhaled breath can damage the sterile area[2].

· COVID-19 patients, if allowed, may wear a surgical mask when being taken to the operating room[2].

· Transport workers can use contact and droplet precautions when transportation to the operating room of suspected or confirmed COVID-19 patients[2].

· In-room surgical staff should be restricted to essential personnel[2].

· Operating rooms that have been designed according to the relevant design code should already have a high ventilation rate and should still remain closed during procedures[2].

· Terminal cleaning according to COVID-19 cleaning and disinfection guidelines should be carried out after and surgical procedure[2].

· All operating instruments should undergo normal procedures for delivery, washing, and sterilisation. The staff responsible for cleaning these instruments should

wear surgical masks, eye protection, gloves and gowns prior to sterilisation[2].

8) Recommendation for outpatient care:

Consider other options to face-to - face telemedicine ambulance visits

• Screening, early identification and detection of COVID-19 suspects[2].

• Focus on hand hygiene, respiratory hygiene and medical masks for the use of respiratory symptoms in patients[2].

• Effective use of contact and droplet precautions during clinical review of patients with suspected COVID-19[2].

Prioritization of the symptomaticpatient's medical care[2]:

• When symptomatic patients have to wait, ensure that they have a clear waiting area where patients can sit at least 1 metre apart and have masks for them[2].

• Inform patients and caregivers about the early identification of symptoms, specific measures to be used and what health care services they can turn to if there are signs of COVID-19 in any family member[2].

9) Risk of Nosocomial Infection in Dental Settings:

· Dental patients who cough, sneeze or undergo dental care, through the use of a high-speed handpiece or ultrasonic devices, aerosolize the surrounding environment with their secretions, saliva or blood[4].

· After use or exposure to a polluted clinical setting, dental products may be infected with different pathogenic microorganisms. Infections may then grow by puncturing sharp instruments or direct contact between mucous membranes and infected hands[4].

· Because of the unique characteristics of dental procedures in which a large number of droplets and aerosols can be formed, standard protective measures are not sufficiently effective in everyday clinical work to prevent the spread of COVID-19, especially when patients are in the incubation period, are unaware of being infected or choose to conceal their infection[4].

· Covid-19 patients should not be treated without special attention in standard dental care settings. It needs special protective clothing such as hazardous materials (hazmat) suits[4].

a) Mouth rinses:

The effect of chlorhexidine, widely used in dental practise for pre-procedural mouth washing, has not yet been proved capable of removing COVID-19[5]. However, it is advised that oxidative agents include mouth rinses of 1 percent hydrogen peroxide or 0.2 percent povidone-iodine[5]. Pre-procedural use of mouthwash will significantly reduce the microbial load of oral cavity fluids, especially in cases where it is impossible to use a rubber barrier[5].

b) Rubber dam isolation:

The use of rubber dams by constructing a barrier in the oral cavity effectively reduces the generation of droplets and aerosols mixed with patient saliva and/or blood in the surgical area by 70 percent in 1 m diameter[5]. Even, extra high-volume suction is needed after the dam has been installed for optimum aerosol prevention and spatter from spreading[5].

c) Anti-retraction handpiece:

Usage of any dental handpieces which have no anti-retraction feature should be avoided during the COVID-19 pandemic[5]. Anti-retraction handpieces equipped with anti-retractive valves may play an important role in preventing the spread and dispersion of droplets and aerosols for emergency care[5].

d) Disinfectants:

The novel coronavirus can be resistant to disinfectants such as sodium hypochlorite, 0.5 per cent hydrogen peroxide, 62 to 71 per cent ethanol, and phenolic and quaternary ammonium compounds if used in conjunction with the manufacturer's instructions[5].

10) SCHOOLS AND COLLEGES:

• Academic institutions can temporarily suspend, restrict or transfer online events affecting more than 10 participants, such as seminars, presentations at the Grand Rounds, journal clubs, etc[4].

• Residents and fellows can be taught using computerised slides to use online lectures and unknown workshops, and to encourage and support self-directed learning[4].

• Sign-out with trainees could be achieved on a web platform such as Zoom, but it could slow down the sign-out considerably[4].

• Finally, pathologists can sign a portion of cases on virtual slides (whole slide images) in institutions equipped with US Food and Drug Administration approved high capacity whole slide scanners, if permitted by established rules and regulations after prior validation[4].

Educational measures:

Do's and Dont's for teaching/training residents and fellows:

• Cancel educational session involving more than 10 participants, such as seminars, presentations at the Grand Rounds, journal clubs etc[4].

• Restrict face-to - face events such as seminars, or multiheaded microscope sign-out sessions[4].

• Use the best online teaching resources or platforms available, and reassess them regularly. Keep up with the online teaching innovations and follow best distance learning practises[4].

• Stay in touch with your friends, and follow the working strategies[4].

• Consider having a "working hour" online, and being available during this period to answer trainees' questions. Test the trainees mental and physical well-being[4].

• Don't give up teaching during this undetermined time period. Don't forget the trainees who work from home[4].

Conclusion

COVID 19 has emerged as a threat to public health around the world in the last nine months. Global experience shows that containment measures and active contract tracking are necessary to keep the infection under control until the global community has an approved cure or a vaccine available.

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Insights of Transgenders in India- A Review

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Abstract

Transgender community in India is a significant part of society. Indeed, law can't deny their reality. We live in 21st century where human rights are guaranteed and safeguarded for individuals aside from the third sex i.e.., Transgender. Notwithstanding all sacred ensures, the Transgender are even denied to have their fundamental rights. Unlike in other parts of the world, the attitude toward a hijra in the Indian society is discriminatory and biased in general. They are also refused general, oral health, and psychological assistance. In India, The medical and dental facilities for the eunuchs is nearly non-existent. There are many possible chances that this neglected special group of population may have heavy stress and indulge in alcoholism, gutkha-pan chewing, and other pernicious habits. These factors may cause many oral health related problems which can make their lives worse. The present review manages the issues, Transgender facing in a developing nation like India.

Keywords: *Transgender, Social exclusion, Oral health, Hormone therapy.*

Introduction

The Transgenders also named as third gender have existed in each culture, race, and religion since the initiation of human life has been recorded and analyzed. The transgender are people of all ages or sex whose character, appearance, demeanor, conduct or individual qualities contrast from generalizations. It implies they vary in personality of about how people should be. They are additionally a vital part of social request and have equivalent right in all things and in each asset that is accessible to everywhere throughout the world. The third sexual orientation i.e.., "Transgender" means those people who are diverse in their biological constructions. They are distinctive for the generalization sex jobs. Their sex makes them unique and constrained to confront segregation in the public eye. Regardless of all the established rights, they are denied to have their essential rights like Right to Individual Liberty, Freedom of expression, and Right to Education, discrimination and Right against exploitation.[1]

STATISTICS:

More than studies have sought to investigate prevalence rates of transgender people. Although more recently prevalence rates of transgenders identities have been reported using population studies, most of the available literature has extrapolated prevalence rates from people attending transgender health clinics.[2] Some of the first epidemiological studies, which focussed on individuals seeking services to undergo gender-a□rming genital surgery[3], found prevalence rates of 0.04 per 1,00,000 people. As per the Census of 2011, 4,87,803 lakhs are been classified as transgender out of which 54854 is below 6 yrs and literacy rate is 56.07%. Uttar Pradesh and Maharashtra possesses the major bulk of population consisting of 28 and 8% respectively. Beside that Andhra Pradesh stand in number 2 position with 9% followed by Madhya Pradesh and West Bengal with 6% ,Tamil Nadu with 4% and Rajasthan and Punjab with 3 and 2 % respectively.[4]

SOCIO-ECONOMIC CONDITIONS :

Hijras are often considered as one of most diminished group within the country. The origin of transgender people was mentioned in hindu literature from ancient period and they had a similarity with Ardhanarishvara. Hijras were once accepted group in Indian culture. They were believed as a divine soul of giving fertility powers. They were administered as guards in court

during Mughals period. For so many years, they have performed blessings at weddings and births. Their position altered during the British colonial period .But after the introduction of section 377 of Indian penal code they once again regain their recognition which enables them to fights for their rights and freedom. Apart from that the election commission also made provisions for their identification as third gender. They undergo several discrimination and abuse from all the sectors because of low population and gender variants which led to various social and economic factors like social stigmata, low literacy rate and low income. Due to literacy low rate they are forcefully engaged in prostitution as a means of survivals which can be a great threat to disease like AIDS or HIV. Because of their gender bias and their ignorance, they are not accepted in the society as a result of which they are not getting any basic needs and rights what a common people can. At the outmost they are been thrown from their own family and villages which gives them no option but to engaged themselves as a sex worker or a beggars in the street. Recently awareness and measures have been taken like the official recognition of transgenders as "others" in April 2014 in order to improve their quality of life. One of the best example was their freedom to vote in election which indirectly represents their right to express their views and freedom to speak about it in the society as rightly said by Justice Radhakrishna that 'transgender people should be treated equally with other minorities under the law, enabling them to access jobs, healthcare and education'. One more bill was passed in parliament to ensure them equal privileges regarding education, government jobs etc, similarly as the subcastes. [4, 5]

PROBLEMS OF TRANSGENDERS

The Transgenders are marginalized section of people who falls under the group LGBT(Lesbian, Gay, Bisexual and Transgender) and thus constantly faces social, legal, health care, cultural and economic difficulties.

1.**Discrimination**: One of the unique problem of Transgender. At each and every step they face discrimination in the field of education, employment, entertainment, justice etc.

2.**Disrespect**: With the exception of few cases like inviting them to bless just after the child birth or to newly wedded couple they are disrespected at each and every part of their life

3.**Downtrodden**: These group of people are always exploited by the ones in power. Always they have to fight for social justice because of their sole identity i.e Transgender.

4.**Child Nabbing**: In order to increase the strength of their population these people always are in search of infants or children who are born with the similar feature as transgender and try to nab from their respective family.

5.**Prostitution**: Due to constant unbearable pressure from society sometimes that includes their own family members, friends or relatives these people are forced to walk on the path of prostitutions.

6.**Forced to leave parental home**: Once their identity is revealed, they are always compelled to leave their family home since they are not considered to be capable of taking responsibility in near future due to the indifferent in gender.

7.**Unwanted attention**: These type of people always becomes centre of unwanted attention in public.

8.**Rejection of entry**: In different religious places, public places like hotels, restaurants, theaters, parks etc they are banned.

9.**Rape and verbal and physical abuse**: Commonest problem Transgender people face. They are more prone to fall prey to rape often followed by physical and verbal abuse.

10.**Lack of educational facilities**: They are not allowed to take proper necessary education in schools and colleges unlike others who are considered normal in societal eyes.

11.**STI and HIVAIDS problems**: The term „MSM" stands for Men who have sex with Men. So, Transgender are more prone to suffer from problems like STI and HIV AIDS since most of them belongs to lower socioeconomic status and possess low literacy. Being the community of people who are always excluded from society seeking proper health care is an impossible task for them.

12. **Human trafficking**: Since Transgenders

belongs to the most neglected group so they are more prone to face human trafficking.[1]

EXCLUSION FROM SOCIAL AND CULTURAL PARTICIPATION:

Indians are known for accepting a wide range of differences in cultures, religions, languages, and customs.But when it comes to the understanding of same sex sexual orientation and people whose gender identity and expression are incompatible with their biological sex there appears to be a wide range of discrimination among them. It is considered to be a taboo among the Indians. Most Human rights violations against sexual minorities including the transgender communities in India is the most common fact as of today. Any Indian male child if starts behaving in a more feminine or inappropriate way to the expected gender role, they often are subjected to severe verbal abuse by their own family. Physical abuse like threatening, assaulting their son/ sibling for behaving or dressing-up like a girl or woman is very common. These people have a strong belief that their constant abuse will help them get rid of their bad behaviour. Cases like disowning their own child for not same as the gender norms which was considered in the society and for not satisfying the determined roles of male child can be seen. The reasons on behalf of which they justify their actions are as follows: dishonoring the name of the family; Inability of the child to get married normally to a woman in the future which will be the end of their family chain (if he is the only child in the family); and consequently his inability to take care of the family in near future. Thus, it becomes extremely difficult for the transgender women to claim their share of the property what would be lawfully theirs. Sometimes, being unable to bear the constant humiliation and societal discrimination from their own family the child or teenager may decide to run away. Some of them may find their way to Hijras communities. Thus education is just a luxury for them and thus finding any jobs in future become difficult. Moreover even if they manage to make them literate, it is very difficult to find generous people who will employ Hijras/TG people in this Indian Society. These gender-variant people often are ridiculed by so called educated groups of society for being 'different' and they may even be hostile. Physical and verbal abuse, forced sex, extortion of money and materials; and arrests on false allegations by police are not far fetched thing for this community. Absence of protection from police make Hijras/TG as vulnerable community for extorting money and as sexual objects. A documentation was made in 2007 which reported that in the past one year, the number of percentage of MSM and Hijras who reported: forced sex is46%; physical abuse is44%; verbal abuse is 56%; blackmail for money is 31%; and threat to life is 24%.[6]

EXCLUSION FROM POLITICAL PARTICIPATION:

The British enacted the Criminal Tribes Act, 1871, under which certain tribes and communities were considered to be 'addicted to the systematic commission of non-bail able offences'. Certain communities and tribes were perceived to be criminals by birth, and being passed on from generation to generation. In the year 1897, the Criminal Tribes Act of 1871 was passed with the provisions, "a eunuch was deemed to include all members of the male sex who admit themselves or from medical inspection". The role of local government was to keep a record of the names and residences of all the suspected eunuchs who are "reasonably held of kidnapping or castrating children or of committing offences" under Section 377 of the Indian Penal Code. In addition, if any of these registered eunuch appear publicly dressed and ornamented like a woman participating in any entertainment like dance and singing or takes part in any public exhibition, could be arrested without warrant. Hijras were reported of harassment in the hands of police which went upto the extension of threatening to lodge a criminal case under Sec-377 IPC. As per the Delhi High Court in July 2009, criminalization of same-sex relations between adults in private were banned. Just immediately after that judgement, appeals in the Indian Supreme court were made in objection to this particular rule; though the Indian government has yet to submit a formal response. Complexity with the legal issues is very evident for the people who undergone sex change, and for gender variant individuals. The Legal issues are as follows: Legalization of their gender identity, marriage of the same-sex individuals, Right to adopt child, Inheritance of their family property, Access to wills and trusts, Proper immigration status, discrimination in employment field, and access to public and private health benefits. In order to get proper legal recognition of gender identity as a woman or transgender woman without which they face

great difficulties in getting government ration (food-price subsidy) shop card, passport, and bank account. Presently transgenders have been permitted to put vote as a woman or 'other'. However, the legalization regarding the validity of confirming one's gender by voter's identity card is still unclear though history shows some of the Hijra contestants who stood in elections. It has been recorded that even after the victory of a transgender person who contested in an election was overturned since that person contested with the gender identity as a 'female or woman', which was actually considered fraudulent and illegal. So, the right to contest in elections is yet to be legalized. According to the recent mapping study only 103 organizations and networks (that include agencies providing services for MSM) considered to be working with transgender people in India but only half (46/103) of these organizations are community-owned organizations. Many challenges have been faced in community mobilization in registering their organizations legally.

It has been always a challenge for hijras/TG communities to legalize their group from some of the legal provisions or organization which enable or permits registration to form legal association. The basic needs to register as a legal association is the identity and address proof of each and every member of that respective association. But most of the individuals of these community neither have any specific address proof nor they have proper gender identity. Even if they are having it is of their male gender identity. They face similar problem if they want to open a joint bank account in order to carry out financial transactions. This is mainly due to the lack of sensitivity and understanding among public department officials. Though few CBO's of Hijras are there across India who were able to meet the legal demands of these organization and register themselves as a legal organization. However, the constant issues Hijras face everytime with the government officials in charge of registration formalities till date shouldnot be overlooked. Often they were asked irrelevant questions and was harassed by intetional delay. So hiring an office space also poses challenge for them as the formalities accomplition becomes a tough job for them. Even if they manage to get one, they are often cheated by land-lords who rent the place with unfair prices. External financial support for Hijras/TG associations is a far fetched thing since they are usually denied of funding support .Among the few willing funders most of them would like to support fund for HIV prevention activities only. On behalf of The National AIDS Control Programme, transgender health projects were granted by few CBOs of hijras. For making these organizations as effective they need more resources which made them to help transgenders in all possible ways.[6]

EXCLUSION FROM ECONOMIC PARTICIPATION AND LACK OF SOCIAL SECURITY:

Social security issues are a challenge for these Hijras/TG community people. Support from their biological family in the long run becomes a daydream for them since most of them flee from their home in childhood. So in the long run their sole survival becomes difficult without the job opportunities as they suffer in various ways such as health issues especially when they become aged, exclusions in various social sectors which ultimately force them to indulge in different illegal activities.

Lack of livelihood options : Qualified and skilled transgender people even face rejection in getting average jobs since most of the employers deny any employment to these gender variant people. Some exceptions though have been heard like few success stories of self-employed Hijras running food shops, or organizing cultural programs are reported in some states. It stays a key reason for a significant proportion of transgender people to choose or continue to be in sex work – even if it is associated HIV and health-related risks. But some isolated initiatives has been made recently offering mainstream jobs to deserving qualified TG women such as agents for Life Insurance Corporation of India can be seen.

They always lack specific social welfare schemes and has been barred from using existing schemes. A variety of social welfare schemes is being provided by Social welfare departments for socially and economically disadvantaged groups. However, uptill now no particular schemes are available for Hijras except some rare cases of giving away land for Aravanis in Tamil Nadu. Recently, Andhra Pradesh government passed order to "Minority Welfare Department" to consider Hijras as minority and develop necessary welfare schemes for them. But sadly most of the Hijras does not know about

the welfare schemes available for them itself. Moreover Stringent and legal procedures needing for permanent address proof, identity proof, and income certificate prevents even the deserving people from making use of available schemes.

The Social Welfare Department of Tamil Nadu state has recently established 'Aravanis/Transgender Women Welfare Board' to help these community people with social welfare issues. Apart from Tamil Nadu no other state has taken this initiative till now. Since most of the Hijras are not under any Life insurance schemes so they are excluded from any health insurance schemes so they have to rely on the government hospitals.[6]

DENTAL PROBLEMS AMONG TRANSGENDERS:

As a Dentist we swear that "first do no harm" to our patients and we should not discriminate regardless of race, sex or gender. The responsibility should be upon us to continuously learn about our patients so that they should be treated safely and equally, compassionately. In the dental treatment of the community of the Lesbian, Gay, Bisexual & Transgender (LGBT) proper medical history should be taken. In the event of hormone therapy of the transgender individuals number of issues were there which has to be taken in consideration.

The hormone therapy undergone by the transgender women is usually the combination of exogenous estrogen and anti-androgen that helps to decrease male pattern hair growth, change the body fat distribution and stimulate the formation of breast tissue. In addition, the hormone combination decreases the testicular size and erectile function. To experience the maximum amount of change on hormonal therapy an interval of 18-24 months of time is required.

The hormonal therapy undergone by transgender males is testosterone regimens for the transition from assigned female sex to male. This testosterone replacement therapy is only responsible for lowering of the voice, formation of male pattern hair growth, ovaries and muscle atrophy, body fat redistribution and enlargement of clitoris. Some side effects are noticed like increased systolic blood pressure, decreased HDL cholesterol and increased triglycerides.[7]

Transgender patients should be under regular follow up like patients with underlying medical conditions. They should be in good metabolic control. They should be always under the check of hormonal levels, monitoring of the complete blood counts, liver function test, lipid panel and hemoglobin HbA1C on a regular basis. The patients who have the history of previous thrombosis can be on anticoagulant so special care must be taken on that aspect. So before performing any surgical or invasive dental procedures it is always wise to consult their respective physician. High risk category in case of thrombosis are transgender patients with smoking habits or having hypertension.

Screening for diabetes is also essential in these high risk individuals, since there is an established direct relationship between the periodontal disease and Diabetes. Metabolic control usually can be affected by the severity of chronic periodontal disease and ultimately can lead to Diabetes Mellitus. With uncontrolled diabetes mellitus the healing ability of the patient can be compromised after any invasive procedures like a case of an implant placement in poorly controlled diabetes patient the chances of failure is high. Monitoring of Hemoglobin A1c to levels below 7.0% is important in a diabetes diagnosed patient before starting any invasive dental procedure. In diabetic patients with chronic smoking habit the risk of severe chronic periodontitis is much higher. Smoking cessation counseling should be enforced. [8, 9, 10, 11].

Often transgenders are at high risk of sexually transmitted diseases due to their improper sexual practices. Oral cavity is usually the mirror of sexually transmitted diseases so careful examination of oral cavity sometimes may bring about the signs and symptoms of STDs in front of dentists. The opportunistic infections sometimes can give a clear cut image of HIV/AIDS which often the patient hided from the dentist. Some side effects of estrogen and progesterone can be manifested as increased bleeding, inflammation, erythema and tooth mobility. It has been shown that there is a correlation between estrogen deficiency and osteoporosis in women. [12]

The laboratory personalities facing huge challenges while reporting any clinical or pathological reports for transgender people. This is because they

are lacking of standardized values for them. The interpretation of lab data in this subgroup of population is especially complicated for lab tests that have sex specific reference ranges such as tests of liver enzyme, creatinine and hematocrit levels. There is a dearth of scientific studies on this topic, which makes it difficult for health care providers to decide what reference range is normal for each patient.[13]. Best example is that of prostate specific antigen (PSA) levels, a test used in screening for prostate cancer, which is falsely decreased with long-term antiandrogenic therapy and therefore is likely not a positive tumor marker in transwomen.[14]

SEVERAL PREMALIGNANT LESIONS COMMONLY SEEN:

Oral lesions such as leukoplakia, erythroplakia, and lichen planus pose an increased risk for malignant changes in the oral cavity. The percentage of premalignant lesion in younger generation of south asian population like oral submucous fibrosis has increased . In India, some studies concluded that about 5 million people were suffered from this disease .

Infection (bacterial, viral, fungal), local trauma or irritation (Traumatic keratosis, Irritational fibroma, Burns), systemic disease (Metabolic or Immunological) can also lead to oral mucosal lesions. Deleterious habits like the usage of tobacco, areca nut, betel quid, or alcohol often leads to the premalignant and malignant conditions in oral mucosa.

Baseline data about the magnitude of the problem is very essential in planning different promotional programmes both in National and Regional levels. Attitude of an Indian society towards a Hijra is always discriminatory and biased unlike the rest of the world. Basic needs like general, oral health, and psychological assistance are not at all provided to them or atleast most of them are denied of. So in that context it is very unlikely that dental and medical facilities will exist. Due to societal pressure, exclusion and repeated harassment these communities indulge more in deleterious habits like alcoholism, betel nut, gutkha-pan chewing etc which make them more vulnerable to various oral health problems which in turn make their quality of life even more worse. But even after this there is paucity of data on oral lesions of these community people.[15-20]

WELFARE SCHEMES

In India, the first state to introduce Transgender (Hijras/ Aravanis) welfare policy is Tamil Nadu. Free Sex Reassignment Surgery (SRS) can be accessed by all the transgenders in the Government Hospital (only for trans-feminine people) according to this policy. Apart from that free housing program, different citizenship document, full scholarship admissions in government colleges for higher studies; Developing of certain self-help groups (for savings) and income-generation programmes (IGP) some alternative measure has been taken for their livelihood. In establishing the Transgender Welfare Board in the year 2008 with representatives from the their community Tamil Nadu here also stands first. In this Welfare Board Social welfare minister was appointed as the president. This effort has been considered to be the first big step ever taken by any state not only in India but also in the world. In this welfare scheme transgenders were issued separate food ration cards specifically for them. Though initially it was meant for only Transwomen but gradually both Transmen gender variant groups and trans women started to get the benefits. In order to improve the education status of this group of people measure has been taken by this welfare board on May 2008 so that they also get admissions in Government schools and colleges. Provisions has been made with aim to issue separate Identity and ration cards in order to bring equality with the rest of the citizens of the state. Many constable jobs have been given to this community people by the Tamil Nadu government. Another state which is not lagging behind after Tamil Nadu is Kerala. The government of Kerala drafted a policy called “Transgender policy in 2015” providing equality in different opportunities and special reservations in various areas has been issued for them. Until recently in June/2017, more than 500 jobs were being given to them in “Kochi metro railways ltd”. Following their footsteps Andhra Pradesh government in January/2018 started giving off Rs.1500/- pension to each transgender.[4]

TRANSGENDER COMMUNITY AND HIGHER EDUCATIONAL INSTITUTIONS

On 29th October 2014, a circular has been issued by the University Grant Commission to all the Vice Chancellors of the Universities requesting them to

include a column for Transgender Community in all applications forms. The circular also comprised of the directions regarding actions to be taken by the Universities in order to maintain a healthy atmosphere for Transgender students so that they can free of any type of humiliation, social isolation from rest of the student population. Encouraging certain research activities sensitization programmes in favor of these minority group to bring out new solutions, new clauses were also included in the circular. Results also can be seen for thse circulars as in case of MBA entrance test in the year 2017 of Maharashtra, out of 363413 applicants, 9000 applicants belongs to the category "other" gender means Third Gender. This column of "third" gender besides male and female were included in the form specifically. [1]

RIGHTS OF TRANSGENDER PERSONS BILL, 2014

On 24th April 2016, "The Rights of Transgender Persons Bills, 2014" named private bill was passed by the Rajya Sabha and introduced in the Lok Sabha. This deals with the several aspects like:

- ü Social inclusion of Transgender,
- ü Financial aids and legal aids,
- ü Education,
- ü Skill development,
- ü Prevention of abuse and violence
- ü Exploitation of Transgender.[1]

NEED FOR REFORMS

Now the time has come to reform the third gender community. The reform is urgently required in order to provide a healthy and proper lifestyle to this group of people. Following are the suggestions that can be incorporated in future for their betterment –

1. Government and Society should plan and adopt certain Inclusive approach for the Transgenders. The framed policies should be properly implemented which is till now not followed.

2. In order to provide a protective shield to their problems a focused approach is desired.

3.Empowerment of legal and law enforced systems in the favor of transgenders

should be issued.

4. Strict disciplinary and criminal actions should be taken against anybody who commits violence against Transgender.

5. Parents neglecting, abusing or leaving their child due to their biological difference if caught should be strictly punished.

6. For the Transgender community at ground level free legal aid provisions must be ensured.

7. In providing necessary education and value-system to Transgenders a very important and encouraging role should be taken by school and college managements.

8. Social entitlement provision must be taken.

9. Helpline for areas like career planning and guidance, career opportunities and online placement system should be established.

10. In order to help them in starting up their career as an entrepreneur or businessman liberal credit facilities and financial assistance in all possible ways should be provided.

11. In all private and public hospitals and clinics special policies related to their health care needs must be issued and discussed.

12. In order to reach out to the public and this community many awareness programme should be organized publicly.

13. Incorporation of sex-education program is must in school curriculum and college syllabus to bring awareness among students at ground level.[1]

Conclusion

So by the overall literature review one fact is clearly understood that the group which is referred to as "transgenders" are those whose identities fall out of the prescribed genders of society i.e male and female. Till date this community is battling against the societal pressure most of the times which are in the form of utter

humiliation, ignorance, isolation from social, political and cultural events, Medical and Dental negligencies and many other multidimensional problems. It is not that much hard to think about the original status of this community people in a country like India where patriarchal society prevails and where gender variants are considered a taboo and curse. So in order to improve their quality of life and equality in terms of education, health as well as societal status few efforts are being made by certain NGOs and some state government in the country. With the aim to protect this community people from exploitations, social deprivations and exclusions, schemes like welfare schemes, Transgender policy etc have been implemented within the county. After all these efforts only, recently a development and changes can be seen in few sectors like film industry, corporate, private and some government jobs. We have to understand that in order to maintain a normal life these Tansgenders/Hijras need utmost help, care and support of the government, health care Professionals, general public and most importantly from their very own family members. Dentists should give importance in developing awareness regarding oral hygiene and also take equal care to this group of people like the other genders in population since most of the Transgenders do not care about their oral hygiene at all. Careful oral examination should be done by dental health care workers while treating these type patients since most of them are involved in unprotected sexual activities and they can be general carrier of STIs and HIV. Treatment associated with general physician is necessary if some opportunistic infection in people suffering from HIV is detected. Humans are diverse, sooner we accept this truth faster would be the improvement in the lifestyle and social status of this minority group. Everyone has the birth right to be what they are and what they want to be. For transgender people, that should not be an exception.

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Oral Health in Dementia-A Review

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Abstract

Dementia is a neurodegenerative disorder affecting the cognitive and motor skill of the patients. It can be reversible and irreversible. People with dementia lose their ability to do basic procedures to maintain oral hygiene thus poor oral hygiene and its associated oral diseases are not an uncommon findings in these patients. In this review we will be discussing about various oral conditions faced by these patients.

Keywords: *dementia, xerostomia, periodontitis, Siallorrhea*

Introduction

People with neurodegenerative diseases like dementia have the tendency to ignore oral health completely. But oral health is significantly directly proportional to their quality of living. Poor condition of oral cavity can affect their diet, nutrition, behavior alterations, life expectancy [1-3].Often neglected carious tooth and apparently harmless periodontal diseases can be one of the causative factors for a serious disease like Aspiration pneumonia [4].Various difficulties are face by these older people suffering from dementia which includes problems regarding wearing the dentures, failure to take care of oral health successfully by basic oral hygiene procedures [5-10].Thus it affects their eating habit greatly and the cause can be due to pain by ill fitting dentures, non-functional dentition. Not only has their eating habit the matter of their self confidence also broken because of their unaesthetic appearance [11].

DEMENTIA: According to WHO**,** It is a progressive, neurodegenerative disease that affects the cognitive function, (i.e. the ability to process thought) beyond what could be expected from normal ageing. It affects memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgment. Consciousness is not affected. The impairment in cognitive function is usually accompanied, and infrequently preceded, by deterioration in emotional control, social behavior, or motivation. Dementias can be Reversible and Irreversible (e.g. Alzheimer's disease, Parkinson's disease).

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ORAL HEALTH CARE IN DEMENTIA:

Factors affecting their ability to take care of oral health:-

a) Stage of dementia the person is in.

b) Level of damage in that persons' level of processing thoughts and disability towards physical activity.

c) Person lacking awareness regarding oral health problems he is going through.

d) These peoples' capability to take health care benefits from care givers.

e) Ability to give consent to oral health care.

f) Level of Motivation.

g) Awareness towards the methods to avail oral health care.

h) Dental professionals' awareness towards diseases like dementia.

PROBLEMS REGARDING ORAL HEALTH

Till date because of the difficulties to obtain a longitudinal study on the people suffering from dementia, not many records are there. The people with dementia gradually lose their cognitive acquired motor skills, along with it the confidence to properly take care of oral hygiene by basic means [9-12]. So it has been seen there is increase in caries index in these types of patients. Raise in plaque and calculus in these people pose a threat to their oral hygiene status. Oral diseases that they readily encounter are as follows:

1. PERIODONTAL DISEASE: Higher is the risk of having gingivitis and subsequently periodontitis with increasing level of dementia. High plaque index due to the inability of these patients to control motor functions since removal of both calculus and plaque requires well controlled motor functions [6,7]. Bleeding from gingiva is also not an uncommon manifestation. Due to the correlation of periodontitis with multiple systemic diseases like diabetes mellitus type 2 and cardiovascular disease, treatment should not be delayed in these patients in order to reduce the health risk [13].

2. DENTAL CARIES: Patients with dementia due to their decrease in cognitive skills are not able to avail the dental services regularly. It has been seen that patients staying in different care giver homes have comparatively having more oral health related problems than those who are not [14]. Through some researches it has been noted as the level or stages of their dementia rises more deterioration in their oral health and hygiene occurs including prevalence of dental caries [12] which is often accompanied by reduced salivary flow. In a study paper Comparison has been made between dentate people with dementia staying in a community and with that of non dementia people in same environment which concludes that there is high prevalence of both radicular and coronal caries in people with dementia than those without it. Apart from this it also concluded that factors like sex of the patients especially here the male patients, with their reduction in salivary flow, consumption of antipsychotic medication all contribute as a risk for dental caries.

3. ORAL CONDITION DUE TO MEDICATIONS: People in old age only suffer from dementia. A number of health related problems faced by these patients are often dealt with many different drugs including the drugs which are used to treat conditions like anxiety, depression, psychosis, insomnia and other systemic illness related to old age. Certain medications are also given to them to control or slow the stages of dementia. There are medications like that of anticholinesterases, antidepressants, antipsychotics, anti anxiolytics which help to control their mood swings and slower their failure in cognitive functions. Unfortunately these drugs show the side effect of Xerostomia. Study found that prevalence and susceptibility to caries are largely increased by the use of these neuroleptics [10]. It is also found that Anticholinesterases can cause glossitis, Mucositis caused by Antipsychotics, whereas gingival hyperplasia, oral ulceration, erythema multiforme and loss of taste are all caused by Anticonvulsants [9,15,16]. Proper salivary flow is important for variety of reasons, most importantly to keep dental caries at check. Saliva acts as buffer and has the function of washing away debris in the mouth preventing bacterial colonization. So reduction in the flow of it results in bacterial accumulation, increased plaque, calculus, caries susceptibility and gingival inflammation which in turn causes painful gingiva that makes these patients divert from brushing leading to poor oral hygiene [17,18]. Denture induced stomatitis, dry and cracked lips are also seen as manifestations of poor oral health [7]. Probability of Rampant caries is also seen in some cases where syrup based medications are given to these patients.

Besides Xerostomia, Siallhorrea can also pose difficulty in patients with dementia like people suffering from Alzheimer's disease. Unconditional drooling occurs mainly due to dysphagia i.e difficulty in swallowing which sometimes leads to perioral ulcerations, infection, foul odor for which people keep distance from them, frequent need to change dresses, lower self confidence and depression which in turn make them, to take anti depressant medications.

CONCLUSION:

Emphasis should be laid on the people with dementia regarding their capability to maintain basic oral hygiene procedures as these patients lose their cognitive and

motor skills. Numerous oral manifestations that occur in these patients as a result of their negligence should be considered and necessary measures should be taken to combat those situations. Dental professionals and care givers should get a thorough history about the list of medications these old people are receiving and manage accordingly. A little bit of attention can make their life a lot better and help them get back their lost self esteem.

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Oral Lipomas- Rare Conditions in Oral Cavity, A Review

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Abstract

Oral lipomas are rare benign lesion. Though soft tissue lipomas are not uncommon oral lipomas are relatively less in number. Mostly goes asymptomatic and are rarely comes to dental professionals either by accidental findings or complaint from a patient due to aesthetic reasons. Various histological variant of lipomas are there but most common is classical lipoma followed by fibrolipoma. Prognosis of these benign intraoral lesions is good. In this review article we will discuss about etiology, theories regarding pathogenesis, clinical features, Histological variant of Oral Lipomas.

Keywords: *Angiolipoma, Intramuscular lipoma, fibrolipoma, spindle cell lipoma*

Introduction

Lipomas are neoplasms of mesenchymal origin composed of matured adipose tisssues with a fibrous connective tissue capsule[1,2] .Though lipoma is a common soft tissue lesion i.e about 15-20% occur in head and neck region, but their intraoral presence is rare (1-4%)[2]. They are generally asymptomatic so most of the times are found accidentally in routine dental examination. They can be described as slow growing soft nodular mass often yellowish texture on overlying mucosa[3] .In the year 1848, lipoma was first described by Roux as yellowish epulis, following which in the year 1880 first case of intraosseous lipoma was presented by Cornil and Ranvier. Intraoral lipoma are found in the areas of oral mucosa where there is deposition of fat, most appropriate of which is buccal mucosa, Other common sites include tongue, floor of mouth, vestibular sulcus, palate and gingiva. Based on histopathological findings lipomas can be of different types like angiolipoma, spindle cell lipoma, myelolipoma, chondrolipoma, myxolipoma, pleomorphic lipoma and fibrolipoma [3]. Less frequently involved sites are as follows, parotid, masseteric region, pharynx and larynx [6]. Sometimes lipomas can be seen in maxilla [5,7] .

ETIOLOGY:

Not that much clarity is there regarding the etiology of oral lipoma. Few studies deduced the facts like endocrine system, certain mechanical factors, inflammation, obese conditions, due to chromosomal abnormalities, radiation therapy, trauma, mucosal infections, chronic irritation can be regarded as causative agents for oral lipoma[8].

PATHOGENESIS:

Behind the pathogenesis of Oral lipoma various theories are there like-

HYPERTROPHIC THEORY:

This theory tried to prove that unintentional proliferation of adipose tissue and obesity can be the cause of these benign lesions. Though this theory was discarded later as it could not satisfy the reason moreover it seemed non convincing in case of the occurrence of those lipomas which are seen on non fat bearing areas of oral mucosa. They are not depleted during starvation condition of the body as like any other adipose tissues[3]

METAPLASIA THEORY: This theory emphasized upon the ability of fat cells to differentiate anywhere in the human body from multipotent cells of connective tissue. According to this theory the abnormal differentiation of mesenchymal cells into lipoblast can result in such adipose benign tumors. They are also thought to be arising on the basis of congenital malformations as a result of proliferation from multipotent cells of embryo

which stay dormant until the time it gets sudden activation under hormonal influence. But Clinicopathological evidence has not been found yet [5]

CLINICAL FEATURES: Depends upon the site generally[3]. Diagnosis of lipomas mainly clinical. Though diagnosis difficult until that characteristic yellow color is seen. These can be described as nodular, pedunculated or sessile, soft, palpable smooth lesion which generally occurs in areas having extensive adipose tissues or fat like that of buccal mucosa, tongue etc. They most of the time goes asymptomatic by the patients and very rarely they cause any discomfort like dysphagia, speech trouble and interruption in mastication. Signs like feeling of fullness, slip can sometimes be the complaint of patients.(Fig.1)



FIG.1 LIPOMA IN BUCCAL MUCOSA

DIAGNOSIS: Accidental discovery of bony lesion is common. Radiographically characterized by unilocular or multilocular lesion with honey comb or soap bubble appearance associated with an osteosclerotic border[18,20]. Though classical soft tissue lipoma cannot be diagnosed by radiograph. Computed tomography and Magnetic Resonance Imaging (MRI) are not useful in diagnosing lipoma so Histopathology is gold standard diagnosing modality[19].

HISTOPATHOLOGICAL FEATURES:

Features are more or less similar in both soft and intraoral lipomas. Mature white fat cells are seen without any atypical features. Often the fat cells in lipoma are separated into lobular structures by connective tissue septae. Generally covered or encapsulated by thin fibrous connective tissue capsule. On rare occasions fat necrosis, infarct or calcification and osseous metaplasia can be present in lipoma [3,6] .Diagnosis usually made by the presence of mass clinically.

IMMUNOHISTOCHEMISTRY: Shows positivity for Vimentin, S-100 and Leptin.

HISTOPATHOLOGICAL DIFFERENTIATION OF LIPOMA: (Fig.2) It can be divided as follows:

1. **Angiolipoma:** Characterized by subcutaneous nodular structure which is a combination of mature adipocytes and thin walled vessel, often associated with fibrin thrombi. However presence of various small thrombi helps in the diagnosis. The lesion can be also called Cellular Angiolipoma when it consist of 90% or more of vascular component. Certain degenerative changes like perivascular fibrosis, hyalinization and stromal myxoid change can be found in chronic persistent lesions. Always a benign lesion and chances of

recurrences are almost negligible.

2. **Intramuscular/Infiltrating Lipoma:** This lesion can increase in size as big as 20cm in diameter. This histologically characterized by combination of striated muscle fibers with abundant fat cells which gives it a texture similar to a checkboard. This lesion can recur as it is seen in 15% of cases[10] **.**

3. **Perineural Fibrolipoma:** Concentric perineural fibrosis is characteristic of this lesion. Here fat cells are seen surrounding the nerves and hence the name[10]. It can cause disfigurement due to its enlargement in young children. It can also be termed as hamartoma of nerves often seen as multiple lesions[11].

4. **Spindle Cell Lipoma:** Histologically characterized by adipose tissue intersmixed with short fascicles of bland undifferentiated spindle cells in a matrix containing bands of hyaline collagen and occasional mast cells.[5,10]

5. **Angiomyolipoma:** Asymptomatic lesion. Histologically characterized by well differentiated smooth muscle sheets surrounding the mature fat cells

and dilated blood vessels[10].

6. **Pleomorphic Lipoma:** Characterized by presence of Round, spindle and floret like giant cells. This typical giant cells helps to differentiate pleomorphic lipoma from liposarcoma[10]. Often there is confusion in diagnosing pleomorphic lipoma with spindle cell lipoma due to there overall similarity in clinicomorphological features.

7. **Myolipoma:** Rarest among all. Characterized by combination of mature adipose tissue and smooth muscle in varying proportions of which proportion of muscular component is more[8].

8. **Chondroid Lipoma:** Similarity with chondroblasts and lipoblasts often leads to misdiagnosis of sarcoma instead of chondroid lipoma. Histologically, it is characterized by combination of mature adipose tissue, lipoblasts with bland nuclei, and hibernoma like cells in a myxohyaline and pseudochondroid matrix.[13]

9. **Fibrolipoma:** Combination of fibrous component with lobules of mature adipose cells. Texture of this tumor can be from soft to firm which depends upon the proportion of fibrous component in it [21] .Most of the time surgery is the mode of treatment. If the size of the lesion is too large to excise then various regression methods are implied. Injection with steroids like lidocaine with triamcenolone acetonide(1:1) can reduce the size of the lesion following which surgery is carried out. Some prefer liposuction to avoid scarring of tissues. Some authors believe on IFN alpha to regress the lesion. But whatever may be the treatment modality prognosis of lipoma is satisfactory[22,23,24].

TREATMENT: Surgical excision is the treatment of choice. Surgical excision of the lesion irrespective of their histologic variant is the main treatment modality. Recurrence rate is very rare[6,14,15] except Intramuscular variant of oral lipomas where incomplete excision can lead to recurrence[16,17] .Overall prognosis is good in all cases.

Conclusion

Oral lipomas are rare benign lesion. Though soft tissue lipomas are not uncommon oral lipomas are relatively less in number. Mostly goes asymptomatic and are rarely comes to dental professionals either by accidental findings or complaint from a patient due to aesthetic reasons. Various histological variant of lipomas are there but most common is classical lipoma followed by fibrolipoma. Prognosis of these benign intraoral lesions is good.

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Oral Lymphoma- A Short Review

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Abstract

The malignant neoplasm of the lymphocyte cell lines are lymphomas. They are mainly classified as Hodgkin's or non-Hodgkin's lymphoma (NHL).The etiology is still unknown. Various treatment modalities causes acute and longer-term side-effects affecting the clinical decision-making for dental treatment. Therefore, people with lymphoma should have regular follow-up.

Key words: *Hodgkins and NonHodgkins Lymphoma, prophylactic antibiotic.*

Introduction

Lymphoma is a solid neoplasm arising from an lymphoid tissues and spreads to distant lymphoid glands and organs[(1)].It occurs when the abnormal lymphocytes increasesin number.Lymphoma is a heterogenous malignant disease of the lymphatic system which is characterised by proliferation of lymphoid cells or their precursors[(2)].Oral cavity lymphomas represent the third most common malignancy in the oral cavity, surpassed by squamous cell carcinoma and malignancies of the salivary glands. Lymphomas in the oral cavity are rare; only 3% of all lymphomas in the general population and 4% on patients with AIDS[(3)].The manifestations of oral lymphomas are often difficult to diagnose because they mimic the clinical features of other diseases such as periodontal disease, osteomyelitis, and other malignancies[(4)]. The treatment modalities includes monoclonal antibodies, chemotherapy, radiotherapy, corticosteroids and haematopoietic stem cell transplant (HSCT).

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ETIOLOGY

In most of the cases, the cause of lymphoma is unknown. However, people with medical condition affecting their immune system have a predisposition to develop lymphoma. Patient under highly active antiretroviral therapy are at highest risk of NonHodgkin's lymphoma (NHL) due to insufficient immunological response[(5)]. Organ transplantation and its associated immunodepression is also a risk factor for lymphoma[(6)]. Patients with Sjögren'ssyndrome are at increased risk of developing NHL in which mucosa associated lymphoid tissue lymphoma is mainly involved[(7)].

CLASSIFICATION

Lymphomas presents various degrees of aggressiveness and can be divided into two large groups: Hodgkin's lymphoma (HL) and non-Hodgkin's Lymphoma (NHL). Hodgkin's lymphoma occurs mainly in the lymph nodes (>90%)and only 4% of the cases involves extranodal areas[(8)]. The histopathological examination of Hodgkins lymphoma shows the presence of Reed-Sternberg cells which are binucleate cells with a generally abundant cytoplasm and two large nucleoli (one in each core) that appear like'owl eyes'[(9)]. Hodgkin's lymphoma can be further classified into classic HL or lymphocyte-predominant HL with incidences of 95% and 5% respectively. The classic type has a bimodal age distribution with an early peak in young people (ages 20–24) and another peak in elderly patients aged 80–84.

The lymphocyte predomiant type occurs at any age but mostly occurs between 30 and 50 years of age.

Non-Hodgkin's lymphoma is classified by the types of lymphocyte, either B-lymphocyte (major type) or T-lymphocyte. The behaviour of a NonHodgkins lymphoma either high grade or low grade determines the treatment.NHL represents 40% of all lymphomas in the extranodalsites[10].

Classification

I. Classical Hodgkin's lymphoma (main type of Hodgkin's lymphoma)

• Nodular sclerosis Hodgkin's lymphoma (most common type)

• Mixed cellularity Hodgkin's lymphoma

• Lymphocyte-rich classical Hodgkin's lymphoma

• Lymphocyte-depleted classical Hodgkin's lymphoma

II. Nodular lymphocyte-predominant

Hodgkin's lymphoma (NLPHL)

• This much rarer type of Hodgkin's lymphoma involves large, abnormal cells that are sometimes called popcorn cells because of their appearance

• Early diagnosis raises the chance of better cure

ORAL MANIFESTATIONS

Patients complaints of localized or diffuse soft tissue swelling, pain, mucosal ulceration or discoloration, paresthesias, anesthesia, and loosening of teeth.The most common clinical manifestation of NHL arenonhealing, painless ulceration[11].NHL can affect both bony and soft oral tissue, palate and the mandible are most commonly affected.It is rare to find extranodalNHLs in the gingiva[12]. ClinicallyBurkitt's lymphoma (BL) occurs more often in children and is relatively rare in middle-aged or elderly adults. BL is more common in males, and the most affected areas were mandible followed by the maxilla. The swelling, pain, dental displacements, and facial asymmetry are the main findings.In the oral cavity, this tumour can progress rapidly and present itself as an exophytic mass or a facial swelling involving the jaws[13]. The tumour begins in the posterior maxilla and then spreads to the four quadrants which leads to increased tooth mobility, intraoral masses, lip numbness, and tooth pain because of infiltration in the pulp[14].Intraoral lymphomas may appear as first sign of infection in 50% of the cases. The oral manifestation includes asymptomatic soft swelling with or without ulceration affecting the tonsils, palate, buccalmucosa, gums, tongue, floor of the mouth, salivary glands, and retromolar region[15]. Alveolar bone loss with oedema and pain may also occur which often mimics periodontal diseases.

TREATMENT

The treatment depends on the type and stage of the lymphoma. The main aim is to achieve complete remission from the lymphoma[16].

The main treatment modalities for patients with lymphoma are:

1. Monoclonal antibodies (MABs);

2. Chemotherapy;

3. Radiotherapy;

4. Corticosteroid;

5. Haematopoietic stem cell transplant

(HSCT).

Monoclonal antibodies-The treatment of lymphoma with antibody therapy targets an antigen on the lymphoma cells[17]. It works by binding to antigen CD20.Rituximab, ofatumumab, and obinutuzumab are antiCD20 agents.Rituximab is commonly used for the treatment of B-celllymphoma[17]. It can also be used in maintenance therapy during the remission phase of lymphoma where it is typically given every two to three months for a period of two-years[18]. Currently, there are many monoclonal antibodies in clinical trials to target thelymphoma cells and reduce adverse effects to normal cells[19].

Chemotherapy- Lymphomas are relatively sensitive to chemotherapy. The type of chemotherapeutic regimen depends on several factors such as type and location of the lymphoma,age and fitness of the patient. The most

common chemotherapy regimens used are ABVD Adriamycin, bleomycin, vinblastine and dacarbazine, BEACOPP (bleomycin, etoposide, doxorubicin (Adriamycin©), cyclophosphamide, vincristine (Oncovin®), procarbazine and prednisolone.

Corticosteroids-Synthetic corticosteroids, such as prednisolone, methylprednisolone, and dexamethasone, are used alone or as part of a lymphoma treatment regimen[20]. They help by increasing the overall effectiveness of chemotherapy and therefore reducing the number of cycles needed and the negative side-effects. There are many reported side-effects to long-term use of corticosteroids.

Radiation is used significantly in the treatment of patients with lymphoma,particularly in early and advanced stages of HL, NLPHL, high grade NHL and occasionally with low-grade types of NHL. These types of lymphoma are very sensitive to radiation.

Haematopoietic stem cell transplant (HSCT) is considered as a part of lymphoma treatment for fit younger patients <65 years and also for people with relapsed or a refractory form of lymphoma[19]. There are two types of stem cells transplant: autologous and allogeneic.Stem cells are collected from the affected patient, and is given back to the same patient after high-dose chemotherapy. In allogeneic stem cell transplants, patients receive donor stem cells after high-dose of chemotherapy[21].

Conclusion

A holistic approach is required to manage people with lymphoma. Following treatment, good care should be taken by the patient. Oral manifestation starts regressing after the seventh day of treatment from chemotherapy. A good medical history, detailed clinical and imaging evaluations, and attention to the patient's signs and symptoms are crucial for the correct diagnosis and appropriate treatment, which in turn can lead to better patient prognosis.

Ethical Clearance – Not required since it is a review article

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Oral Pigmented lesions- A Short Review

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Abstract

Pigmentations can be commonly found in the mouth which are presents in various clinical patterns ranging from physiological changes to oral manifestations of systemic diseases and malignancies. Oral pigmentation can be focal,multifocal or diffuse. Oral pigmentation can be exogenous, endogenous, physiological and pathological. Oral pigmentation is more likely to have a multifactorial etiology.

Key words- *Physiologic pigmentation, melanin, nevi*

Introduction

The colour reflects the clinical state of the mucosa. If the inflamed tissue is red then it reflects the increase in number and dilation of the blood vessels whereas the normal tissue appears pale pink[(1)]. Melanin is produced by melanocytes which is present in the basal layer of the epithelium and is transferred to adjacent keratinocytes via membrane bound organelles called melanosomes[(2)]. The melanocytes can be present in any regions of the oral cavity and it can be either reactive, benign or malignant lesions.

Types of Pigmentation

Physiological pigmentation

Physiological pigmentation is common in dark skinned individuals and results from an increase in the production of melanin pigment by themelanocytes[(3)]. The color ranges from brown to black. Physiological pigmentation increases with age, and colorintensity can be influenced by smoking, hormones andsystemic medications[(4)]. The attached gingiva is themost common location, but it can also be be noticed anywhere in the oral cavity. including thetips of the fungiform papillae on the dorsal tongue and the diagnosis of this pigmentation is made clinically and do not need any treatment[(5)].

Post inflammatory pigmentation

Oral lichen planus, pemphigus or pemphigoid can cause mucosal pigmentation[(4)]. The pathogenesis of post inflammatory pigmentation remains unclear and can be seen more frequently in dark-skinned individuals[(6)]. Clinically, multiple brown–black pigmented areas are seen in post inflammatory pigmentation. Microscopically, there is increased production of melanin by the melanocytes and accumulation of melaninladen macrophages in the superficial connective tissue[(2)]. The resolution of the inflammatory process allows the cessation of oral pigmentation.

Melanotic macule

The oral melanotic macule is a small, well-circumscribed, brown-to-black lesion. It occurs most commonly on the lips and gingiva, followed by the palate and buccal mucosa. Histologically, it is characterized by in situ increased production of melanin by basal melanocytes with normalmorphological features[(7)].

Melanocytic nevus

Clinically, oral nevi are small, well circumscribed macules but commonly appear as slightly raised papules. It appears to be brown,bluish-gray, or black and occasionally non-pigmented. The melanocytic nevi constitute benign neoplasms of cutaneous melanocytes, which harbours oncogenic serine/threonine-protein kinaseB-Raf (BRAF). [(8)].There are no reports of malignant transformation of intraoral nevi even in patients who have had multiple nevi or congenital nevi. Biopsy should be done for any new oral pigmentation because an early melanoma may be mistaken as

melanocytic nevi[9].

Blue nevus

A blue nevus is a benign, asymptomatic, blue-black smooth-surfaced macule or papule which is less than 6 mm in diameter[10]. It occurs most commonly between the third and fifth decades of life, with mean age at diagnosis of 38 years. Females are more commonly affected than males. Twothirds of all intraoral blue nevi are commonly found on the hard palate and the buccal mucosa is the second most common site of presentation[11]. The common blue nevus is characterized by an intramucosal proliferation of elongated, bipolar, spindle-shaped melanocytes that are often grouped in short fascicles parallel arranged to the overlying epithelium. In contrast, a cellularblue nevus is usually characterized by an intramucosal,nodular proliferation of dendritic spindle-shaped, pigmented melanocytes, in addition to tightly-packed aggregates of larger oval-to-round melanocytes with palecytoplasm and little or no melanin[12].

Smoker's melanosis

Smoker's melanosis occurs most commonly seen in 30% of tobacco userswhich is characterized by discrete or coalescing multiple brown macules. It involves the attached mandibular gingiva on the labial side[13]. Smoking-associated melanosis is due to increased melanin production by melanocytes and its deposition within the basal cell layer and laminapropria. The microscopic appearance is similar to that seen in physiologic pigmentation or a melanotic macule[14]. It returns back to normalpigmentation over several months to yearsfollowing smoking cessation[15].

Black Hairy tongue

Black hairy tongue is a painless, benign disorder caused by defective desquamation and reactive hypertrophy of the filiform papillae of the tongue[16]. The hairy appearance is due to elongation of keratinized filiform papillae which shows various colors from yellow-brown to black depending on extrinsic factors, ie, tobacco,coffee, tea, food, and intrinsic factors, ie, chromogenic organisms in the normal flora[17]. The exact pathogenesis is not known. Precipitating factors include poor oral hygiene, use of the antipsychotic drug olanzapine, a broad spectrum of antibiotics such as erythromycin, and therapeutic head and neck radiation. Scraping or brushing the tongue and smoking cessation enhances the resolution of black hairy tongue. Trimming of the papillae may be required in extreme cases[18].

Oral melanoacanthoma

Oral melanoacanthoma is a rare, benign pigmented lesion, brown to brown-black, well circumscribed which is similar to cutaneous melanoacanthoma. It is characterized by hyperplasia of spinous keratinocytes and dendritic melanocytes. The pathogenesis remains uncertain. Buccal mucosa, lip, palate and gingiva are the most common intraoral sites. It occurs most commonly at the age of 28years with a strong female predilection[19]. Fontana masson stain is used to detect the presence of melanin.

Foreign bodies

Many metals can causepigmentation in the oral region. These include lead, which produces characteristic generalized cutaneous'lead hue', and 'lead lines' on the gingiva.Mercury can cause slate-grey gingival hyperpigmentation. Amalgam tattoo is caused by the accidental implantation of dental filling materialinto the gingiva or buccalmucosa[20]. They are painless, gray-bluemacules that range in size from a few millimeters to greater than 1 cm. Amalgam tattoo can also be seen on thehard palate, buccal mucosa, and floor of the mouth.

Drugs

Intraoralinflammatoryreactionandpostinflammatory hyperpigmentation can be commonly encountered in patients under medications. Cotrimazole was the most common drug associated to oral pigmentation followed by tetracycline however, many others have been implicated such as colchicines, ketoconazole, pyrimethamine and barbiturates[21]. Oral brownish pigmentation can be seenin human immunodeficiency virus (HIV)-positive patients receiving treatment with zidovudine (AZT), ketoconazole and clofazamine[22].

Oral melanoma

Oral melanoma is rare and affects less than 1% of all oral malignancies. It is characterized by proliferation of malignant melanocytes along the junction between

the epithelial and connective tissues, as well as within the connective tissue. Hard palate is the most common site followed by the gingiva. It is most commonly seen between the fourth and seventh decades of life, with a greater male predeliction. Clinically, oral melanoma presents as an asymptomatic, slow-growing brown or black patch with asymmetric and irregular borders or as a rapidly enlarging mass associated with ulceration, bleeding, pain and bone destruction[23].

Peutz-Jeghers syndrome

It is a rare genetic disorder associated with mutation of the LKB1 gene on chromosome 19 [24]. It is characterized by pigmented mucocutaneous macules, intestinal hamartomatous polyposis and an increased risk of cancer in many organs, including the small intestine, colon, stomach, pancreas, breast and genital tract [25]. The melanotic spots are small and multiple and it can be seen around the lips. These melanotic spots do not require treatment and are not associated with increased risk of melanoma.

Addisons disease

Addison's disease, or primary hypoadrenalism, is caused due to progressive bilateral destruction of the adrenal cortex by autoimmune disease, infection or malignancy[26]. The increased production of ACTH induces melanocyte-stimulating hormone resulting in diffuse pigmentation of the skin and oral mucosa. Clinically presents as diffuse brown patches on the gingiva,buccal mucosa, palate and tongue, which may resemble physiologic pigmentation[27].

Conclusion

The diagnosis of pigmented lesions is generally difficult and challenging. Biopsy is needed for each and every lesion so that the histological evaluation would confirm the diagnosis.The management of all pigmented oral lesions varies greatly based on the diagnosis, appropriate evaluation of the patient is therefore essential.

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Polycystic Ovarian Syndrome : Its Impact on Periodontal Disease

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Abstract

Polycystic ovary syndrome (PCOS) is the common endocrine disorder among women of reproductive age affecting various health systems. This review literature explains pathophysiological mechanisms connecting PCOS and periodontal disease . Furthermore studies are needed to establish a clearer and stronger association between the two disease.

Keywords:*, polycystic ovary syndrome, Insulin resistance,periodontal disease, obesity.*

Introduction

Polycystic ovary syndrome (PCOS) is a familiar endocrinopathy in womenmajorly affecting the reproductive systemwith an important collateral negative health effects on metabolic, psychologic, and cardiovascular functions.[1] It is a complex disease involving features of hyperandrogenism and chronic anovulation (CA) with global predominance ranging from 2.2% to 26% in Western countries,[2,3] 2% to 7.5% in China, 6.3% in Sri Lanka, and 9.13% to 36% in India.[4]Patients are at greater risk of cultivating insulin resistance (IR), obesity, dyslipidemia, cardiovascular disease (CVD), and endometrial carcinoma.[5] Periodontitisis caused by the communication between bacterial attack and host inflammatory response which ultimately results in inflammation of surrounding tissues of the teeth causing tissue destruction and tooth loss. It has been proposed as a risk factor for several systemic diseases such as diabetes mellitus, dyslipidemia, obesity, CVDs, rheumatoid arthritis,and respiratory diseases. Persistent low-grade inflammation arise as a conceivable etiologic mechanism connecting periodontal disease and many systemic diseases.[6]

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CLINICAL FEATURES AND DIAGNOSIS

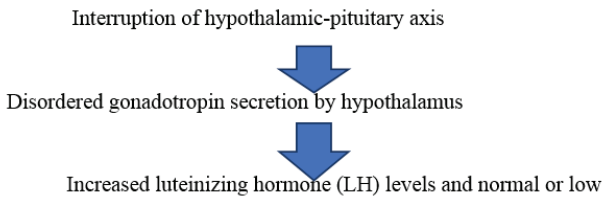
PCOS is characterized by the existence of menstrual deformity (oligomenorrhea or amenorrhea), CA or oligo-ovulation, clinical/biochemical confirmation of hyperandrogenism (hirsutism, acne, or androgenic alopecia), and ultrasound findings.[7]Usually women are affected in their reproductive years.[8]

In 1935, it was first described by Stein and Leventhal , as a variable clinical condition with certain clinical features such as obesity, hirsutism, acne, and amenorrhea related to enlarged bilateral polycystic ovaries. Later in 1990, it was suggested that the diagnostic principles for PCOS should have the collateral anovulation and evidence of hyperandrogenemia – biochemical, clinical (hirsutism/acne), or both – but without reference to ovarian morphology. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group stated that if any two of these criteria such as CA, hyperandrogenism, and polycystic ovaries on ultrasonographywere present then it can be considered as PCOS. In disparity , Androgen Excess Society suggested that hyperandrogenism (clinical and/or biochemical) is an important criteria and when it is presentalong with ovarian dysfunction (oligo-anovulation and/or polycystic ovaries) it can be considered during the diagnosis of PCOS.For

confirmation of this syndrome, diseases which mimics the clinical features of PCOS such as thyroid disorders, hyperprolactinemia, and nonclassical congenital adrenal hyperplasia should be ruled out [10]

ETIOLOGY AND PATHOPHYSIOLOGY

The etiology and pathophysiology of PCOS are based on interaction between genetic, metabolic, fetal, and environmental factors. Though research suggests that the disease is originated in the intrauterine atmosphere, indicating the importance of genetic factorsFranks and Berga et al. proposed that genetic factors are partially involved in the etiology of PCOS. Later , Abbott et al. stated that the clinical characteristics of PCOS may emerge as a result of genetically determined hypersecretion of androgens by the ovary. It might even have a relationship with many other factors, such as socioeconomic conditions, ethnic background , diet, physical activity, and lifestyle. King proposed certain pathophysiological mechanisms of PCOS such as modified gonadotropin-releasing hormone secretion, deformity in androgen synthesis, and IR development . One of the excellent theory to explain the pathogenesis of PCOS is :



Increased luteinizing hormone (LH) levels and normal or low follicle-stimulating hormone (FSH) levels.[11]

According to Qiao and Feng, decreased FSH secretion , raised LH secretion, hyperandrogenemia of ovarian or adrenal origin, and hyperinsulinemia with IR are the extraovarian factors for PCOS pathogenesis. Intraovarian factors are increased androgen levelswhich affect follicular development, ovarian development, and meiotic maturation. Vitamin D deficiency provokes the development of IR and impaired glucose tolerance in obese PCOS patients.[12]

POLYCYSTIC OVARY SYNDROME AND SYSTEMIC DISEASES

Metabolic Syndrome, Insulin Resistance, And Type II Diabetes Mellitus

Metabolic syndrome comprises of IR, obesity, hypertension, and hyperlipidemia. Lim et al., conducted a meta-analysis,[13] according to which obesity may aggravate existing clinical, hormonal, and metabolic features in women with PCOS. Nicandri and Hoegerdescribed that the percentage of obesity in PCOS is above 60%. The incidence of IR in PCOS ranges from 50% to 70%.IR associated with abdominal obesity raises the risk of type 2 diabetes in PCOS.

Cardiovascular Diseases

Patient with PCOS are at higher risk for CVDs, particularly those with, dyslipidemia, hypertension, and hyperinsulinemia at an younger age compared to women without PCOS. According to Wild et al.,women with PCOS have lower high-density lipoprotein cholesterol and higher triglyceride and low-density lipoprotein cholesterol levels than women without PCOS . He then conducted a study [14]suggesting that there is a great chance of hypertension in patients with PCOS. PCOS patients have more extensive coronary artery disease. Greater incidence of coronary artery calcium scores and carotid intima-media thickness in young patients with PCOS proposes an increased susceptibility of subclinical vascular disease than normal women.

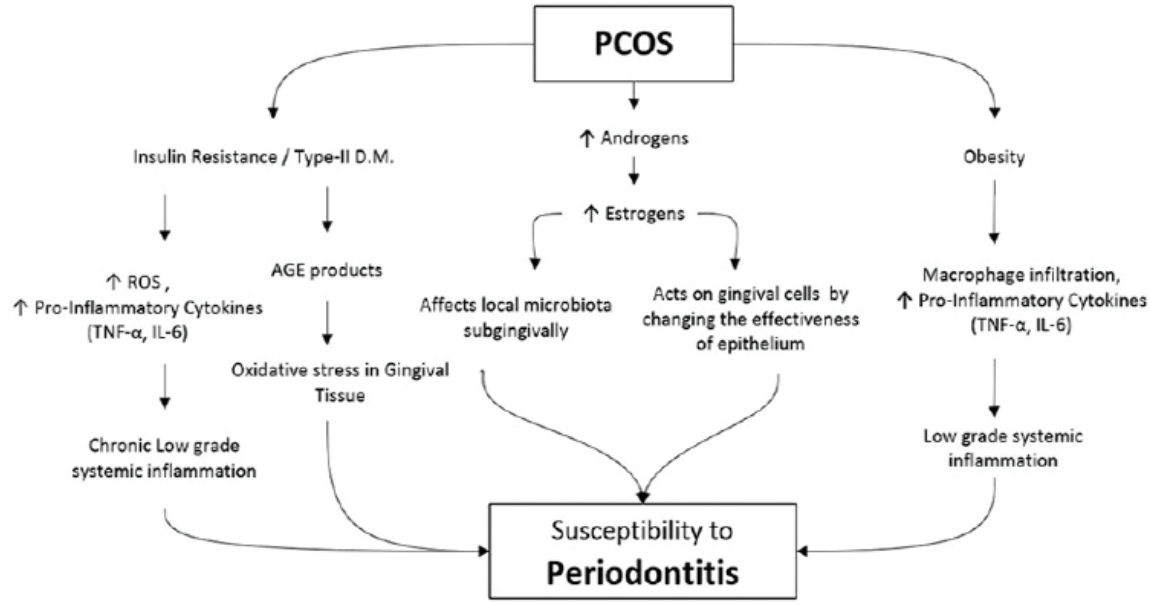
Other Systemic Disorders

- Endometrial cancer
- Pregnancy complications, including miscarriages, gestational diabetes, and preeclampsia

POLYCYSTIC OVARY SYNDROME AND PERIODONTAL DISEASES

The mechanisms connecting these two disease entities are not entirely understood but it involvesdifferent aspects of inflammation.

PathogenesisRelating Polycystic Ovary Syndrome and Periodontitis



PCOS is correlated with low-grade systemic inflammation and is marked by elevation of certain markers of inflammation such as C-reactive protein (CRP), proinflammatory cytokines and chemokines such as interleukin 18 (IL-18), monocyte chemoattractant protein-1 and macrophage inflammatory protein-1, and white blood count. Moreover higher oxidative stress and its biomarkers proposes PCOS as an inflammatory disease. Periodontitis is a chronic inflammatory disease , since inflammation is the key factor it is associated with various systemic diseases. Certain inflammatory cytokines including tumor necrosis factor α (TNF-α), IL-1 β, IL-6, leptin, adiponectin, and resistin and signalling pathways such as (IKKβ/NF--B) Inhibitor of nuclear factor kappa-B kinase sub unit beta/Nuclear Transcription factor kappa-B pathway, c-Jun N-terminal kinase (JNK) pathway, and inflammasome pathway, link low-grade inflammation to IR, an important feature of PCOS. IL-1β triggers IR by impairing insulin signalling in peripheral tissues and macrophages, causing reduced insulin sensitivity of β-cells and possible impaired insulin secretion. IL-6 induces IR by producing glucose transporter-4 (GLUT-4) and insulin receptor substrate-1 (IRS-1) and by blocking the phosphoinositide 3-kinase (PI3K) pathway.CRP is an important marker of inflammation secreted under the stimulatory control of proinflammatory cytokines such as IL-6 and TNF-α. Higher CRP levels are found in many systemic diseases such as PCOS, which is related to low-grade chronic inflammation, linked to IR, that plays a role in syndrome pathogenesis involving hyperinsulinemia. Periodontitis patients have increased serum CRP levels and proinflammatory cytokines such as TNF-α and IL-1 in serum and/or gingival crevicular fluid (GCF).[48] The elevated serum levels of CRP and other proinflammatory conditions, in chronic infections such as periodontitis may provoke systemic inflammation and oxidative stress causing IR, which are the features of PCOS. Proinflammatory cytokine IL-6 (hormonally regulated) stimulates the hypothalamic– pituitary–adrenal axis during inflammatory stress, and higher levels of IL-6 are correlated with obesity and IR, which is associated to PCOS. Rahiminejad etal. conducted a case–control study [18]proposing a greater incidence of periodontitis in nonobese women with PCOS compared to systemically healthy women and also stated that systemic inflammation can be a attributing factor.

Porwal et al., conducted a cross-sectional study [19]where a higher prevalence of periodontitis is noted in patients who are recently diagnosed with PCOS than individual under treatment for PCOS and systemically healthy females. Furthermore , serum levels of high-sensitivity C-reactive protein (hsCRP) , a marker for systemic inflammation were higher in females with

recently diagnosed PCOS compared to systemically healthy women and females on medical treatment for PCOS. IL-17 cytokines causes atherosclerosis as a complication related to PCOS.WBC count , a marker of low-grade inflammation is related to several chronic inflammatory conditions. Likewise increased white blood cell count can be seen in chronic periodontitis patients.From the above collected data, it can be noticed that components of inflammation plays a vital role in association of PCOS and periodontal disease.

Oxidative Stress

Oxidative stress and inflammationare closely related pathophysiological processes. Higher oxidative stress is linkedwithobesity,diabetesmellitus,metabolicsyndrome, and atherosclerosis. Oxidative stress biomarkers were detected in peripheral blood of chronic periodontitis and PCOS patients. Two meta-analyses[20]indicateshigher levels of oxidative stress biomarkers in both the diseases, particularly malondialdehyde and lower levels of antioxidants. Moreover women with PCOS had raised myeloperoxidase and nitric oxide (NO) levels in GCF including unaltered serum NO levels. This suggests a local/periodontal oxidative stress and therefore it was decided that gingivitis is a common observation in patients with PCOS and that local/periodontal oxidant status appears to be affected in PCOS.

Oral Microbiota

The hormonal changes in PCOS controls the salivary levels of putative periodontal pathogens and/or their systemic antibody responses, particularly in gingivitis. This is due to the accumulation of active progesterone and estrogen in the periodontium , which supplies the essential nutrients necessary for the bacterial growth. The lipopolysaccharides from periodontal organisms in subgingival plaque produces significant amounts of IL-1 and TNF-α, and chronic upregulation of cytokines triggers the state of IR, which is a feature of PCOS. Akcalı et al. [21]proposed that comparatively increased salivary levels of Porphyromonas gingivalis and Fusobacterium nucleatum are seen in patients with PCOS and gingivitis than healthy controls and patients with PCOS and without gingivitis. This signify that PCOS may have an improved effect on the levels of P. gingivalis and F. nucleatum and their association with gingivitis. Serum antibody levels to P. gingivalis, Prevotella intermedia, and Streptococcus oralis are higher in patients with PCOS.

Role of Hormones

Modification of various hormone levels in the body can be observed in PCOS females. Female sex steroid hormones plays a vital role in progression of periodontal disease and periodontal and implant wound healing. Gingiva is capable of metabolizing hormones such as estrogen and progesterone. Furthermore, gingival tissue exhibits receptors for such hormones and it is decided as a target organ for their direct action.These hormones acts on gingival cells by altering the effectiveness of the epithelial barrier to bacterial injury or by affecting the collagen maintenance and repair.

Obesity

Obesity is the chief risk factor for diabetes, CVD, and periodontal disease. Similarly , its predominance in PCOS is raising and it is above 60%.Studies have stated that a raise in the abdominal fat in patients with PCOS is in charge for hyperinsulinemia and IR compared with weight-matched controls.[23]Data from a systematic review denotes that increase in waist circumference, serum lipid levels, and incidence of subcutaneous fat might increase the risk for periodontitis.[23]

Vitamin D Deficiency

Vitamin D plays a major role in modulation of skeletal and mineral homeostasis and has anti-inflammatory and immunomodulatory effects. Many scientists suggest that Vitamin D supplementation is necessary in maintenance of periodontal health.[24] This is due to the direct effects on bone metabolism, antibacterial effects on periodontopathogens, and elimination of inflammatory mediators that donate to the periodontal destruction. Vitamin D is observed in patients with PCOS and it may be attributed to the polymorphisms in Vitamin D receptor (VDR) gene, such as Cdx 2, Taq1, Bsm1, Apa1, and Fok1, which play a crucial role in insulin secretion and sensitivity in PCOS women. Similarly, VDR gene polymorphisms are related to chronic periodontitis, and VDR genotype is recommended as a risk factor for chronic periodontitis.[25]

Treatment

· Medications involving metformin, clomifene, letrozole, and gonadotropins.

· Recent research is done to detect the efficiency of certain anti-inflammatory supplements such as omega 3 fatty acids,

· Vitamin D, and curcuminand antimicrobials such a doxycycline[26]to reduce the androgen levels, modify the IR index, to reduce the inflammatory burden causing periodontal diseases. But there is no evidence to ensure their benefits

Conclusion

We can derive a conclusion that PCOS might aggrevate the periodontal condition through plaque, various pathophysiological links, namely, low-grade systemic inflammation, oxidative stress, IRand systemic hormonal levels. According to the suggestions , periodontal disease induces chronic subclinical inflammation causing IR, initiating the development of type 2 diabetes, which acts as an important feature in PCOS. Therefore ,we can consider that there is a two-way relationship between PCOS and periodontal disease. Furthermore studies are needed to establish a powerful relation between two diseases which will be useful in early diagnosis, treatment, and prevention of long-term sequelae. Especially, health-care professionals, gynaecologists, and endocrinologists should cheer up the patients with PCOS in maintaininghealthy oral hygiene and a visit to dentist for eradicating periodontal problems since hormonal imbalance affects the susceptibility to plaque-induced periodontal disease.

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Role of AgNORs in Oral Lesions-A Review

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Abstract

The biologic behavior of the oral lesions can be determined by their active phases of cell division, cells entering the cell cycle and their proliferation. AgNORs (argyrophilic nucleolar organizer regions) staining is the one of the cytochemical technique used to evaluate the cell proliferation of oral lesions. Tumor cells in premalignant and malignant conditions usually exhibits a rise in synthesis of abnormal proteins and proportionately there is a increase in the AgNOR material. The aim of this study was to review the concepts of proliferative activity of cells of malignant and premalignant conditions through the AgNORs staining procedure and thereby proving the usefulness of AgNORs in diagnosing the oral lesions.

Keywords: *Nucleolar organizer regions, NOR-associated proteins, Argyrophilic nucleolar organizer regions, proliferative activity, AgNOR dots, quantitative, qualitative.*

Introduction

In a cell, the nucleus plays an important role in the proliferative activity and synthesis of proteins. Nucleolar organizer regions(NORs) are the loops of DNA present in the nucleoli of cells possessing the genes that transcribe for synthesize of rRNA [1]. These Nucleolar organizer regions(NORs) correspond to secondary constrictions of metaphase chromosomes of eukaryotic cells. These Nucleolar organizer regions(NORs) are believed to be the centre for proliferative activity of the cell and these are located on the short arm of acrocentric chromosomes 13, 14, 15, 21, and 22 [2]. Nucleolar organizer regions(NORs) are associated with certain acidic, argyrophilic, non histonic proteins called NOR-associated proteins (NORAPs) [3].

These NOR-associated proteins (NORAPs) are demonstrated by colloidal silver, which makes bond with these proteins and these resultant silver stained reaction products are argyrophilic nucleolar organizer regions (AgNORs). In normal epithelial cells, the AgNORs are tightly packed within the nucleoli and are not identifiable. But in cases of neoplastic cells, nucleolar disaggregation may occur which may result in the dispersion of individual AgNOR. AgNOR correlates with the proliferative rate of the cell, as can be estimated by Ki-67 and the percentages of the S phase cells and the mitotic cells[2].

AgNOR staining procedure has found with many advantages such as simplicity, low cost, ease of use, specificity, good correlation with proliferative markers when compared to other histopathological staining procedures. After silver staining procedure, the AgNORs can be identified as black dots throughout the nucleolar area [1]. NORs reflects protein synthesis and are well known to get increase in their count in cases of premalignant and malignant conditions [3].

There are several studies have proved that AgNOR counts in their quantitative terms can be used to differentiate between benign and malignant lesions [4-7]. Whereas some studies have shown that qualitative representation of AgNOR such as morphological characteristics, shape, size and the pattern of distribution determines the proliferative activity of the cell and thereby used as a marker of malignant or premalignant lesions [8,9]. The interphasic NORS can be clearly

examined at the light microscope level by using a silver reaction which stains the acidic proteins of the NORS (RNA Polymerase 1 upstream binding factor, Topoisomerase 1, Nucleolin, Fibrillin, C23 protein and B23 protein) on routinely used histopathological and cytological tissue samples [10].

ARGYROPHILIC NUCLEOLAR ORGANIZER REGION STAINING PROCEDURE:

According to Crocker and Nar, the biopsy specimens are subjected to routine paraffin sectioning at 4 μm thickness after fixation with 10% formal saline. The sections are then deparaffinized in xylene and hydrated through decreasing grades of ethanol to double distilled deionized water. The sections were then reacted with freshly prepared silver colloidal solution (1 part by volume of 2% gelatin in 1% formic acid and two parts by volume of 50% aqueous silver nitrate solution) in a closed coplin jar for 35 min at room temperature, while ensuring that a dark environment was maintained throughout the reaction time. The silver colloidal solution was washed with double distilled ionized water. The sections were then treated with 5% sodium thiosulphate for 5 minutes and washed in double distilled deionized water, dehydrated through increasing grades of alcohol, cleared in xylene and mounted[11].

ARGYROPHILIC NUCLEOLAR ORGANIZER REGION COUNTING (QUANTITATIVE ASSESSMENT):

One hundred epithelial cells should be counted in each specimen under×100 oil immersion objective of the light microscope following in a zigzag manner. A standardized mean for the enumeration of NORs in the histological sections was proposed by Crocker et al[12]. By careful microscopic examination, AgNOR dots should be counted. While counting, both intranucleolar and extranucleolar dots should be included in the counting regime and where two or more dots were so closely aggregated within a nucleolus, the aggregate should be recorded as one. Whereas in some areas the closely aggregated dots separated by a halo of nucleoplasm, then the dots are counted separately[13].

ARGYROPHILIC NUCLEOLAR ORGANIZER REGION TYPES (QUALITATIVE ASSESSMENT):

Three types of AgNOR products are identified in histological sections according to Warnakulasuriya and Johnson[14]:

• AgNORs-Type I– Single or few large dots within the nucleus representing the nucleolus

• AgNORs Type II– Discrete small dots within the nucleolus

• AgNORs Type III– Fine black dots dispersed throughout the nucleoplasm.

Discussion

The neoplastic cells generally show a marked increase in the synthesis of normal and abnormal products of the cells such as proteins which results in significant rise in AgNOR material. The AgNOR counts increase with increased cell ploidy and with increased transcriptional activity in the stages of active cell proliferative phases[15]. The stage of the cell cycle, the transcriptional and metabolic activity of the cell or the number of NOR-bearing chromosomes determines the variations obtained in the size and number of AgNOR dots. In a rapidly and actively proliferating cell, there is a disorganized distribution of chromosomal and AgNORresulting in the formation of multipleand dispersed nucleoli. In addition, in these cells there is a disorganized nucleolar aggregation resulting in the increased AgNOR count, regardless of the ploidy state of the cell[2].

AgNORs have found greater application in the tumor histopathology in determining the growth potential and malignant potential of tumors, in differentiating between benign and malignant neoplasms, to assess the prognosis[16-19] and also to evaluate the risk of recurrence[20]. In oral pathology, AgNORs play as auseful tool in differentiating between odontogenic cysts and tumors, to distinguish recurrent and nonrecurrent giant cell lesions of the jaws, determining the biologic behaviour of OSMF, differentiating oral squamous cell carcinoma from benign and reactive lesions, in detecting incipient cellular alterations [21-25]. AgNORs have also proved to be useful marker of tumor progression, predict the response of tumor to treatment, and to detect residual viable tumor [26,27].

AgNOR count is useful in determining the differences between various grades of malignancies [3]. There is a study showing increase in the AgNOR count from well differentiated squamous cell carcinoma thoroughly moderately grade and reaches peak in poorly differentiated squamous cell carcinoma.

According to Sandhya Panjeta Gulia et al, their study proved that the mean AgNOR count showed a linear and significantly increasing in nature as the histopathological grade of the tumour increased ($p<0.05$) [2]. According to the study of Khushbhu et al, the Mean AgNOR count increased in the following sequence: i.e OSMF with mild dysplasia, leukoplakia with mild dysplasia, OSMF with moderate dysplasia, leukoplakia with moderate dysplasia and squamous cell carcinoma. In qualitative assessment, most of the sample present with Type II AgNOR pattern while Type III AgNOR pattern was found to be increasing with the increase in the grade of dysplasia [1].

According to the study of Elangovan et al, in a quantitative assessment, there is a difference present between the number of AgNORs counts between the normal mucosa, inflammatory lesions, and carcinomas, but the premalignant lesions show almost equalnumber of AgNOR count to normal mucosa. The number of AgNORs was found to be related to epithelial proliferation. In a qualitative assessment, morphological characteristics determines that there is no difference between normal mucosa and inflammatory lesion were alike, but the premalignant and malignant lesions showed significant differencefrom normal, with a increased degree of AgNOR pleomorphism being observed in carcinomas[3].

Conclusion

On analysing the various studies, there is a standard conclusion remains that AgNOR quantity is directly proportional to the proliferative activity of the cell and reaches its peak in malignancy. Qualitative characteristics of AgNOR is also useful to differentiate between hyperplastic, premalignant, and malignant lesions.AgNOR technique can definitely be used as a supportive tool to the routinely performed haematoxylin and eosin staining and that it will help in the prognosis and the therapeutic decision making in squamous cell carcinomas of the upper aerodigestive tract. In future, more studies need to be carried out to prove the usefulness of AgNOR technique as a supplementary tool in diagnosing the premalignant and malignant lesions, deciding the treatment plan and in evaluating the prognosis of the patients.

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Role of Hormone on Tissue Health

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Abstract

Endocrine system regulating hormonal function is one of the most important system of the body. Dysregulation in which can directly affect the oral cavity. Their systemic involvement also sometimes affects the oral health. Though other than diabetes mellitus and thyroid disorders rest of the hormonal diseases are rare but not non-existent. So a proper knowledge about the clinical presentations should be kept by the dentists to avoid any risks and difficulties regarding their diagnosis. Conditions like burning mouth, dry oral cavity, and altered tastes can also be an after effect of menopause should be ruled out by dentist before coming to a definitive diagnosis. Careful counseling of the patients especially women regarding their conditions should be done by keeping in mind the psychological stress they undergo during that stage of life. So this review circulates around the oral manifestations due to hormonal imbalance in a human body and post menopausal women.

Keywords : *Hyperpituitarism, Acromegaly, Hypothyroidism, menopause,*

Introduction

Metabolism, growth, development, tissue function, sexual function, reproduction, even sleep and mood in a human body are all controlled by a group of glands called endocrine glands collectively can be termed as endocrine system. These are also called a ductless glands because of their ability to directly secrete in blood and interstitial spaces. Diabetes Mellitus and Thyroid Disorders are two of the most common endocrine disorders a dentist faces in day to day practice but apart from that other hormonal disorders though rare can also show oral manifestations which if not given proper care can pose risks and difficulties in diagnosing and treating the patients. Following are various oral and perioral presentations by different endocrine disorders.

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PITUITARY GLAND

Considered to be the Master Gland of Endocrine system. Disorders occur generally in both cases of Hyperpituitarism and Hypopituitarism.

HYPERPITUITARISM : Excessive secretion of one or more hormones produced by the pituitary gland. Excess of growth hormone production results in Gigantism or Acromegaly. When increasing growth hormone production occurs prior to epiphyseal plate fusion Gigantism results but if it happens following to the closure then Acromegaly occurs.

GIGANTISM : Characterized by symmetrical abnormal growth of parts of body. Maxillofacial abnormalities include, Mandible prognathism, frontal bossing, dental malocclusion and interdental spacing etc. Often intraoral radiograph shows radicular hypercementosis.

ACROMEGALY : On the other hand Acromegaly is characterized by enlargement of specific part of body mainly involving face and extremities sometimes specific organs, resulting to systemic manifestations. Some of

the Orofacial Presentations are like glabella protrusion and increase in anterior face height are commonly seen. As a result of excessive growth hormone production deposition in periosteal bone take place which in turn leads to facial abnormalities like Prognathic Mandible and thickening of jaw bones.

Other Oral Manifestations: Interdental spacing, aperthognathia, macroglossia sometimes leading to buccal tipping of anteriors[1], hypertrophy of palatal tissues which may result or initiate sleep apnoea.

RADIOGRAPHIC FINDINGS IN ACROMGALY: Shows Taurodontism characterized by enlarged body and pulp chamber of tooth in expense of root, downward advancement of mandible, commonly found in male. On the contrary female patients show prevalence to anterior crossbites, extension of ascending ramus, downward displacement of mandible, edge to edge bite, bimaxillary alveolar protrusion[2].

HYPOPITUITARISM

Pituitary dwarfism, a well known condition which is mainly caused due to failure of the body to use the growth hormone secreted by the pituitary gland. Etiology may be congenital or acquired. Deficiency in the hormone production can be the result of less or no synthesis of hormones, or insufficient absorption by the involved tissues[3].

CRANIO-FACIAL PRESENTATIONS: In comparison to normal, patients suffering from this disorder have much smaller sized maxilla and mandible. Often they suffer from delayed development of root which eventually prolongs the time of eruption of teeth [1]sometimes there is discrepency in the shedding time of deciduous teeth also which can be extended for several years too. Dental malocclusion is a very common feature in pituitary dwarfism. Generally due to the smaller size of the jaws little spaces are there to incorporate all the teeth which are normally present in one healthy individual, thus leading to malocclusion [4].Absolute absence of wisdom tooth bud is not an uncommon characteristic of this disorder. Apart from that agenesis and single maxillary central incisor can also be seen[2].

THYROID GLAND DISORDERS:

Disorder can be divided into hypothyroidism and hyperthyroidism.

ORAL MANIFESTATIONS IN HYPOTHYROIDISM: Dysgeusia or distortion of taste sensation can be seen in children and young patients. They generally come up with complaint of foul or metal like taste sensation. Along with that thick and puffy lips are also reported. Myxodema seen in mainly adult patients where deposition of water and protein takes place resulting in larger lips and macroglossia. Sometimes swelling of face which is usually non-pitting type is also seen in these patients [5]. Prevalence of periodontal diseases also is there.

ORAL MANIFESTATIONS OF HYPERTHYROIDISM: Acceleration in teeth eruption is one of the common manifestations in children with hyperthyroidism. Apart from that osteoporosis involving both maxilla and mandible, there is increase in caries and periodontal index rate, often complaints associated with burning sensation in tongue. Associated systemic disorders like Sjogren's syndrome and systemic lupus erythematosis is also noted [6] .

DISORDERS OF PARATHYROID GLAND :

Oral manifestation differs mainly on the basis of production of parathyroid hormone from the parathyroid gland. Parathyroid gland regulates the mineralization of hard tissue of human body like bone and teeth by its known action on the metabolism of calcium and phosphorus.

ORAL MANIFESTATIONS IN HYPERPARATHYROIDISM: Commonly found abnormalities with hyperactive parathyroid gland are brown tumor, lessening of bone density, calcification of soft tissue and abnormal dentition which mainly consist of various developmental defects, discrepansies in dental eruption, often migration of teeth leads to malocclusion resulting in considerable spacing which can be considered as a first sign of this disease.

RADIOGRAPHIC INTERPRETATION: Sometimes pseudocystic lesions are also seen as radiographic findings. Often radiolucency at the periapical area of teeth is misdiagnosed as radicular cyst or granuloma many times. Typical medullary trabecular pattern and the radiopaque pattern which often termed as

clear"ground glass"can be seen[7] .

ORAL MANIFESTATIONS IN HYPOPARATHYROIDISM: Hypoparathyroidism is due to the less or absent of parathyroid secretion which results in decrease in serum calcium level and increase in serum phosphorus concentration. Commonly encountered dental abnormalities include enamel hypoplasia, delayed eruption often resulting in absence in eruption of multiple teeth.Common dental features noted in this disease are abnormalities like enamel hypoplasia, shortened tooth roots, hypodontia etc. Developmental delay of dentition, mandibular tori and fungal infections like chronic candidiasis can also be seen. Often paraesthesia of tongue or lip is also found. "Chvostek sign" characterized by twitching of facial muscle around the mouth caused by sharp tapping over the facial nerve in front of the ear is sometimes seen[8].

ADRENAL GLAND DISORDERS

ADDISONS DISEASE: Insufficient secretion of glucocorticoid and mineralocorticoid from adrenal cortex leads to adrenal disease or can be called adrenal insufficiency.Appearance of pale brown or deep chocolate pigmentation of the oral mucosa which extends on the angle of the mouth or may develop on gingiva, tongue and lips[9].

CUSHING SYNDROME: Increased production of glucocorticoids from adrennal cortex results in Cushing syndrome. Stunted development in both skeletal and dental structure can be seen. Often pathological fractures due to decreasing bone density occur. In radiograph often **DISORDERS OF PANCREAS:**

DIABETES MELLITUS: One of the common Endocrine disorders characterized by chronic hypergycemia affecting the metabolism of carbohydrate, proteins, and lipid.

ORAL MANIFESTATIONS : Dry mouth, prevalence of dental caries, periodontal diseases, gingivitis, oral candidiasis, burning mouth syndrome, Taste disorders, mucormycosis, aspergillosis, oral lichen planus, geographic tongue, fissured tongue and most importantly delayed wound healing and incresed rate of infection, salivary dysfunction, benign parotid hypertrophy, some neurosensory disorders leading to change in taste perception, tooth eruption discrepancy etc[10] .

ORAL HEALTH IN WOMEN UNDERGOING MENOPAUSE :

According to WHO (World Health Organization), Menopause is considered to be one year without flow irrespective of any pregnancy or therapy in women of or more than 40 years of age. Premenopause generally considered within the age of 35 to 39 years when fertility and abundancy starts to decrease with the first presentation of ovarian follicle reduction inspite of having no change in menstrual flow. Perimenopause indicates the year immediately before and after menopause[11].

ROLE OF ESTROGEN ON ORAL CAVITY : Rapid decrease in the level of Estrogen affects the bone greatly since estrogen plays important role in reducing the osteoclastic activity by bringing their apoptosis. Reduction of the level thus decreases the bone density. Since estrogen receptors can be observed in gingiva, salivary gland and buccal mucosa, reduced salivary flow or xerostomia can also be caused by this reduction in estrogen level according to some investigators[12,13,14] .

ORAL MANIFESTATIONS

1. BURNING MOUTH SYNDROME: It occurs in more than one oral site. Often patient describes the pain as bilaterally symmetrical. Patient complaints having pain mostly in anterior two-third, lateral and dorsum of the tongue, anterior hard palate and mucosa of lower lip, alteration of the taste can also occur. Onset of occurrence is between 3 years to 12 years of menopause. Mucosa characterized by dry and shiny ginigiva often can be called as menopausal gingivostomatitis, accompanied by sometimes, reduced salivary flow, taste alteration and burning sensation. Bitter taste generally complained by the patients especially in the tip of the tongue and after it fades away only burning sensation stays. Most commonly lowered estrogen level is thought to be the reason behind burning mouth syndrome though stress and anxiety also have connection with its occurrence [13,15].

2. DRY MOUTH (XEROSTOMIA) : it is nothing but oral dryness sometimes with hyposalivation but not always. According to many studies and researches, 25

to 50%women are more prone to xerostomia than men and cause of that is also estrogen. It is seen than saliva composition and reduced salivary flow is also estrogen dependant so women in perimenopausal to manopausal stage experience dry mouth sometime consequently which is often associated with bitter taste and halitosis or bad breath. The salivary samples of these patients when assessed with their blood samples significant amount of steroid hormones can be found which is mostly estrogen. It has been noted that altered psychological stress has a connection with their oral manifestations which is considered to be one of the prime reasons for the change in salivary flow indicating sympathetic activation on salivary flow [16].

3. PAINFUL MOUTH (STOMATODYNIA): Mostly associated with dry mouth. Presence of mandibular dysfunction, oral ulcerations and diffuse gingival atrophy can also be the reason of painful mouth. Sometime certain nutritional deficiencies, chronic candidiasis, pernicious anemia can also be the cause behind stomatodynia[13].

4. PERIODONTITIS: Often manifests as tooth mobility, abscess, and tooth loss. The symptoms are often insidious until in the later stage of life. Can be considered to be associated with osteoporosis though both of them are bone resorptive disease. Endocrine system especially the hormones like estrogen, progesteron, gonadotropin plays an important role in microcirculatory system. The alteration in hormonal imbalance especially the estrogen affects greatly the gingiva often causing atrophy, changes in keratinization, cellular differentiation.

5. OSTEOPOROSIS: It is as a result of reduced osteoclastic activity of estrogen in bone. Both bone forming cells, osteoblast and bone resorbing cells, osteoclast has estrogen receptor sites. When the estrogen level falls the osteoclast cells by sensing the reduction in the level start to enhance their activity on the other hand the osteoblasts by sensing the paucity in estrogen level starts to decline the level of their activity resulting in unbalanced bone resorption and formation [19]. This affects greatly as the rate of trabecular and cortical bone resorption exceeds the rate of trabecular and cortical bone formation. Thus new formed bone density reduces greatly which often leads to pathological fractures which are often accompanied by pain[17,18].

6. LICHEN PLANUS: No direct correlation has been found with lichen planus and women in menopause but this mucocutaneous disease has its own predilection towards women in the ratio of 3:2[21].

7. EATING DISORDERS: Characterized by abnormal food intake whether it is very less or excessive. It is believed to be related to the psychological stress of the woman in menopause. Often they indulge in self inducing vomiting or regurgitation of gastric contents. Oral manifestation of these patients include smooth erosion of enamel, their tendency to engage pen or comb to induce vomiting, angular cheilitis, trauma caused by fingers on oral mucosa, erythema[20].

Conclusion

Endocrine system regulating hormonal function is one of the most important system of the body. Dysregulation in which can directly affect the oral cavity. Their systemic involvement also sometimes affects the oral health. Though other than diabetes mellitus and thyroid disorders rest of the hormonal diseases are rare but not non-existent. So a proper knowledge about the clinical presentations should be kept by the dentists to avoid any risks and difficulties in their diagnosis. Conditions like burning mouth, dry oral cavity, and altered tastes can also be an aftereffect of menopause should be ruled out by dentist before coming to a definitive diagnosis. Careful counseling of the patients especially women regarding their conditions should be done by keeping in mind the psychological stress they undergo during that stage of life.

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An Infected Sac in the Mandible – A Case Report.

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Abstarct

Dentigerous cyst is the second most common cyst. It is usually associated with the impacted third molars, canines and premolars. Clinically they present with asymptomatic swelling, bony expansion and radiographically they are well defined and the larger cyst leads to destruction of bone later leading to pathological fracture. They are chances where this cyst can be misdiagnosed when seen only clinically withan infected tooth. Radiographic investigation is the gold standard in diagnosing the lesion and its extent for treatment planning. The protein content of the cyst fluid also helps in the diagnosis. Here is a case where the patient complained of a decayed tooth along with swelling in the left side of face and how the diagnosis changed after proper history and radiographic investigation.

Keywords: *Dentigerous cyst, Impacted, Radiographic Investigation.*

Introduction

Dentigerous cyst also called as Follicular cyst is the second most common odontogenic cyst. It is usually associated with maxillary and mandibular impacted tooth. Mandibular third molars, maxillary canines and mandibular premolars are involved most frequently. Rarely, a dentigerous cyst is associated with odontome, deciduous teeth and supernumerary teeth[1].These affectmalesthan females at the ratio of 1.6:1[2].Clinically, it is often asymptomatic; it is discovered as an incidental radiographic finding or when acute inflammation, infection or swelling developswhere it appears as a well circumscribed, unilocular, usually symmetric radiolucency around the crown of an impacted tooth[3]. As normal follicular space is 3-4 mm, a dentigerous cyst can be suspected when the space is more than 5 mm[4]. These cysts may also convert into ameloblastomas, mucoepidermoid carcinoma and squamous cell carcinoma [5].Dentigerous cyst can classified into 3 types based on the radiographic finding,here we report a case of one of its type.

Case Report

A 28 years old male, came to the hospital with the chief complaint of decayed tooth with swelling in the left side of his face for past 2 month which gradual progressed in size to attain the present size. There was no history of pain for past 1 and half month and slowly he experienced dull pain in associated region. He also gave no history of trauma or discharge from the associated region.

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Image 1:



Swelling seen in left side of face.

Extra oral examination revealed a diffuse swelling in the left side of faceof size 5x4 cm approximately, extending from left side commissure of the lip anteriorly totragus of left ear posteriorly and superiorly 3 cm below the left lower eyelid to inferior border of the mandible. The swelling had a smooth surface and the skin over the swelling appears to be normal. On palpation it was firm, non-tender. There was local rise in temperature and the skin over the swelling was pinchable.A single solitary left Submandibular lymph node was palpable measuring approximately 0.5 x 05 cm in size, which was mobile, non tender and firm in consistency.

Intraoral examination also revealed dental caries in relation to 36 which was tender on percussion alongwith a solitary swelling in the attached gingiva which was 1x1 cm in size. The swelling has a well defined margins and the surrounding area appears to be normal. On palpation it was soft in consistency, tender with no discharge. 38 was partially impacted.

Image 2:

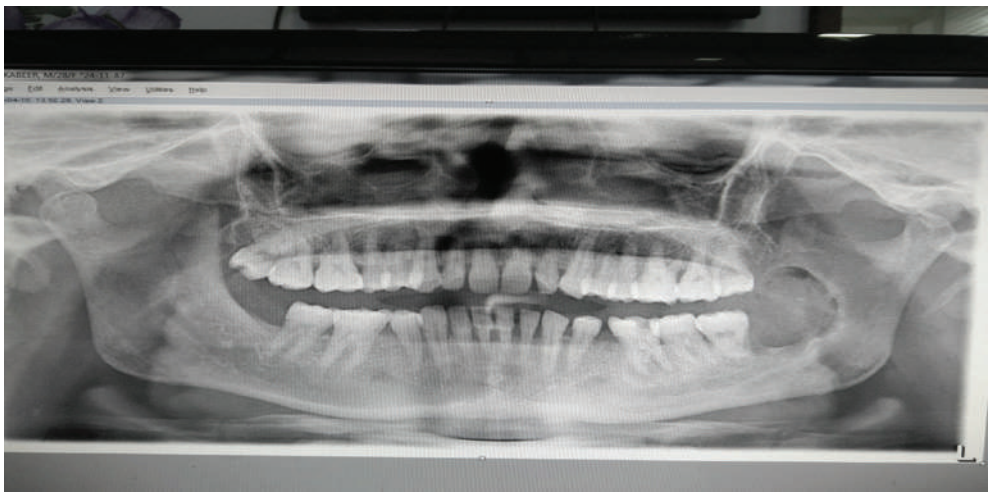


Intraoral examination showing small solitary swelling in relation to 36.

Correlatingwith the chief complaint,intraoral and extraoral examination,a provisional diagnosis of ***Dentoalveolar abscess in relation to 36*** was given with a differential diagnosis of odontogenic cyst or tumour and fibro osseous lesion.

Patient was advised for routine blood and radiographic investigation and biopsy. Hematologicalinvestigations revealed normal values.Orthopantomogram showed a well - defined arc shaped bone loss observed in left ramus of mandible which approximately 5x 4.5 cm in size extending from distal side of 38 cementoenamel junction to the coronoid process posteriorly with hallowing of the ramus. The periphery of the lesion was well-defined corticated with a curved outline which was smooth and also a radiolucency involving enamel, dentin, pulp and at the periapical region of 36.

Image 3:



OPG showing a well defined arc shaped bone loss extending from distal side of 38 Cemento Enamel Junction to the coronoid process.

DIFFERENTIAL DIAGNOSIS:

On the basis of clinical and radiological presentation differential diagnosis of Cystic ameloblastoma, Odontogenic keratocyst, dentigerous cyst,Fibro-osseous lesion and Central giant cell granuloma was given and patient was advised for Computer Tomograph.

Image 4.a)

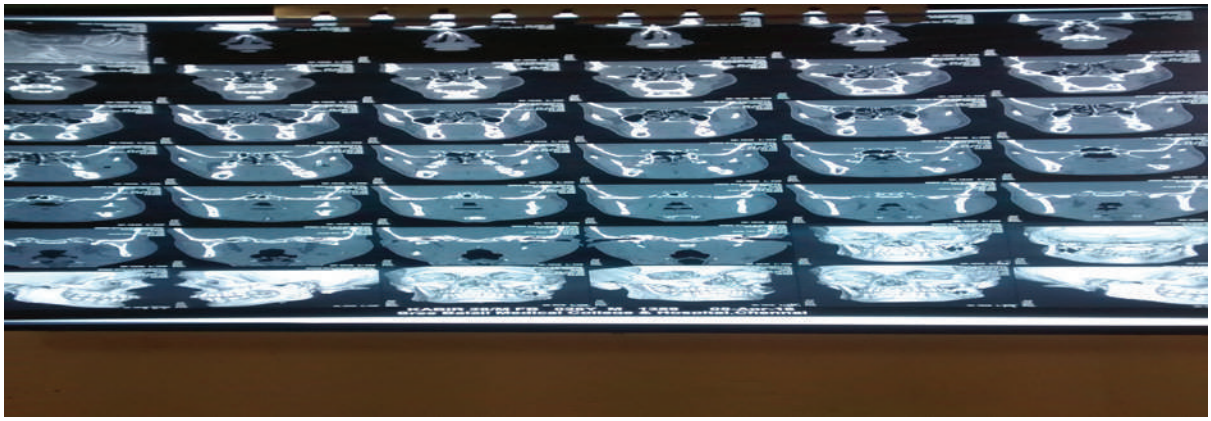
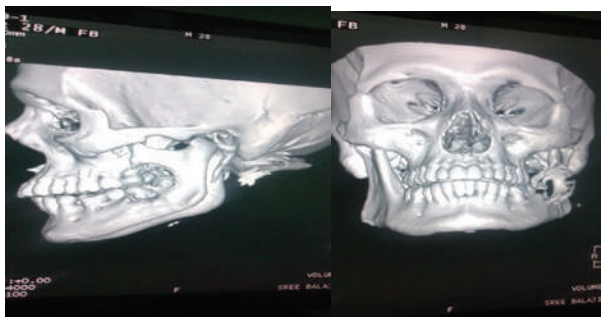


Image 4.b)



CT showing large expansile lytic lesion in left retro molar region and anterior aspect on left ramus

A large expansile lytic lesion noted in left retro molar region and anterior aspect on left hemimandibular ramus measuring 3x2.5cm. Wall of the lytic lesion appears deficient on the anterior, medial and lateral aspect of the lesion. Posterior margin of the lesion is lobulated. Air foci noted within the lesion. CT value ranges from -5 to +25 HU. Periosteal reaction noted along the inferior and posterior border of the lesion. The lytic lesion also involves the lateral cortex with adjoining minimal soft tissue swelling.

Suggestive of odontogenic keratocyst.

HISTOPATHOLOGICAL INVESTIGATION:

Incisional biopsy was done and the specimen was given for histopathological analysis.

The hematoxylin and eosin stained histopathological sections showed cystic lining epithelium and connective tissue capsule. The epithelium is 2-3 cell layer thickness non keratinized type. The fibrous connective tissue is moderately colonized with mild cellularity. The connective tissue shows diffuse dense chronic inflammatory cell infiltrate. Numerous endothelial lined blood vessels are noticed. Colonies of microbial organisms are seen. Deeper layer of connective tissue shows normal appearing bony trabeculae.Suggestive of

Infected Dentigerous Cyst.

The clinical presentation and further investigation lead to the final diagnosis of ***Infected Dentigerous Cyst*** in relation to 38.

Patient was advised for extraction of 38 and 36 along with complete enucleation of the cyst. Patient was recalled for review after 6 months.

Image 5 :



Post operativeOrthopantomogram showing healing lesion.

Discussion

About 20% and 24% in all the jaw cysts are dentigerous cysts[6].Dentigerous cyst is the most common developmental cyst and the pathogenesis of it is unclear till now. There are various theories given for explaining the aetiology of dentigerous cyst. An Intra follicular theory in the development of dentigerous cyst, given by Shear suggests that the development of dentigerous cyst begins with the accumulation of fluid between the reduced enamel epithelium and the enamel, or between the layers of reduced enamel epithelium and enamel[7]. The dentigerous cyst is a type of development cyst originating from the reduced enamel epithelium with clinical feature of asymptomatic swelling and bony expansion.A small dentigerous cyst may be, clinically, asymptomatic and may be discovered on routine examination. Symptoms are seen when there is an increase to potential size by expansion of bone and, rarely, bone destruction [8]. Here in our case patient experienced dull pain in last few days this may be because of the bone destruction. Radiographically this cyst are classified into three types as: Central type, when radiolucency encircles the crown of unerupted tooth; lateral type, which develops laterally along the tooth; and circumferential type, where cyst completely encircles the crown and root of the tooth [9]and our case resembles lateral type.The cyst's lining may contain areas of ortho-keratinization, ciliated cells, or mucin secreting cells. Because of this inherent ability for metaplastic change, some dentigerous cyst appear to progress to more aggressive lesions such as an odontogenic keratocyst, ameloblastoma, mucoepidermoid carcinoma or squamous cell carcinoma [10].

There are many investigation for cyst which includes radiographic investiagetion like OPG, CT, ultrasonography, Aspiration, Biopsy, Serological Test. Although Radiographic investigation is the gold standard the content of the fluid also helps in diagnosis of the cyst.The odontogenic cyst fluid can be studied for their color, consistency, presence of cholesterol crystals, keratin flecks, and different protein fractions such as albumin, alpha and beta globulin, total protein content, and inorganic phosphates.The protein content of radicular cyst is highest which ranges between 5 to 11

g/dl, dentigerous cyst is between 5 to 8 g/dl and for OKC is <5 g/dl[11].

The treatment for the cyst differs based on its size and extent. Enucleation is the standard treatment for a dentigerous cyst along with extraction of the associated tooth[12]. Marsupialisation is recommended for a large cyst when a single draining may not be effective and complete removal of the surrounding structure is not desirable[13]. Scolozzi *et al* recommended in his article that enucleation is better followed by an immediate bone grafting procedure for large cyst[14].

Conclusion

Swelling in the face has many reasons. Cyst is one of among it. Dentigerous cyst is the prevalent cyst in the jaw that occurs in second to third decades of life. Dentigerous cysts may present with complications like pathologic fractures, transformation into true neoplasm[5]. So early diagnosis and proper investigations are necessary for increasing the success rate without any recurrence.

Ethical Clearance – Not required since it is a case report

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Parathyroid Hormone Related Protein - A Skeletal Biomarker – A Review

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Abstract

The discovery of Parathyroid hormone-related protein (PTHrP) was through its structural and functional homology with parathyroid hormone. It is secreted by cancers and it interacts with parathyroid hormone receptors in kidney and bone to cause humoral hypercalcemia of malignancy and paraneoplastic syndrome and plays an important role in cellular differentiation and proliferation, embryonic development, transepithelial calcium transport and in the regulation of smooth muscle contraction.Its intracrine, paracrine and endocrine modes of action play a central role in organogenesis and drives numerous physiologic and pathologic conditions.It is initially translated as a preprohormone which is posttranslationally processed to yield a family of mature secretory forms. The aminoterminal portion of the molecule is homologous to parathyroid hormone.As PTHrP plays a vital role in the regulation of endochondral bone development it was surmised that it could also serve as an ideal biologic skeletal maturity indicator to detect peak pubertal growth spurt.

Keywords: *parathyroid hormone, endochondral, parathyroid hormone related protein*

Introduction

Parathyroid Hormone–related protein (PTHrP) is a protein member of the parathyroid hormone (PTH) family secreted by mesenchymal stem cells and occasionally by cancer cells and that multiple steps in skeletal morphogenesis is regulated by PTHrP and Indian hedgehog protein (Ihh).PTHrP plays an important role in placental calcium transfer, fetal development, epithelial cell growth and smooth muscle relaxation in addition to its parathyroid hormone-like actions.[1]

PTHrP is a paracrine factor expressed throughout the body whose structure is not closely related to that of PTH as it is a product of a separate gene with the exception of a short N-terminal region. It is a poly hormone comprising of a family of distinct peptide hormones. It acts like a growth factor, calciotropic hormone, myorelaxant and as a developmental regulatory molecule.

Rizzoli R et al [2] (1992) reported that PTH and PTHrP by interacting with a structurally identical receptor displayed a common spectrum of action on the transport of mineral elements in kidney and bone and in-vivo, PTH/PTHrP increased the renal tubular reabsorption of calcium and similarly reduced the inorganic phosphate (Pi). An increase in both bone resorption and renal calcium reabsorption is the causative factor of the hypercalcemic effect of PTHrP brought about by the stimulation of the renal transport of calcium. They also speculated that biphosphonate therapy can inhibit the PTHrP-stimulated bone resorption and that PTHrP appeared to less stimulate bone formation than PTH. PTHrP elicited a 2-fold increase of cAMP production in cultured mammary cells isolated from lactating rats.

Riond JL et al[3] (1995) opined that PTHrP played an important role in calcium homeostasis as it stimulated the calcium transfer through the placenta and maintained a concentration gradient between the maternal blood and the fetus thereby contributing to as an important fetal growth factor. Its exact and principal function in lactation has yet not been determined despite the fact that it is produced in large quantities in the milk. It has been reported tofoster the development of the mammary gland and to stimulate the secretion of magnesium, calciumand phosphate in milk.

Wu TL et al[4] (1996) in their study defined a novel, highly conserved mid-region secretory form of PTHrP

which was biologically active as also confirmed the fact that PTHrP was a prohormone.

Philbrick WM et al [5] (1996) reported that PTHrP is a prohormone that is posttranslationally cleaved by prohormone convertases to yield a complex family of peptides, each with its own receptor and that the PTHrP gene is expressed not only in a vast majority of normal tissues during fetal and/or adult life but also in cancers. PTHrP plays predominantly a paracrine and/or autocrine role in normal circumstances in contrast to an endocrine role in humoral hypercalcemia of malignancy. Its physiological functions include 1) regulation of transepithelial (placental, renal, mammary gland, oviduct) calcium transport, 2) differentiation, proliferation and regulation of organ and tissue development, and 3)regulation of smooth muscle (intestinal, vascular, bladder, uterine) tone. The authors in their review have discussed the normal physiological functions of PTHrP, with emphasis on its discovery, gene structure and cDNAs, and the posttranslational processing of the initial translation products.

Wysolmerski JJ and Stewart AF[6] (1998) opined that PTHrP was responsible for humoral hypercalcemia of malignancy alongwith its role in normal development and adult physiology. Their review focused on studies of the past two years namely: (1) the role of PTHrP as an intracrine regulator of cell growth and cell death; (2) mechanisms of action of the various secretory forms of PTHrP and elucidation of the posttranslational processing pattern of PTHrP (3) the advances in understanding the roles of PTHrP in the vascular smooth muscle tone and proliferation, materno-fetal calcium transfer across the placenta and regulation of pancreatic islet mass; and (4) the emergence of PTHrP as a critical developmental factor in skeleton,mammary glandand epidermis.

Shibata S et al [7] (2000) reported that severe abnormalities in endochondral ossification have been suggested by previous studies using PTHrP null mutant mice suggesting the fact that chondrocyte differentiation is affected by PTHrP. The authors found some deformities in the mandible that was formed via intramembranous ossification in the new born PTHrP-deficient mice. In the mandibular body a prominent bone crest to which the deep layer of masseter muscle was tendinously attached was observed alongwith the mandibular ramus being bent downwards. Active bone formation progressing along the tendon fibers of the masseter muscle was depicted with transmission electron microscope. The authors reported that the masseter muscle in the newborn PTHrP-deficient mice contained numerous type 2B fibers, an indicator of premature maturation of the masseter muscle, which was actually analysed using myosin ATPase staining. This premature maturation of the masseter muscle probably led to accelerated bone crest formation andbending of the mandibular ramus due to the increased tensile forces. These findings reiterated the fact that PTHrP was involved in the regulation of muscle development in normal animals.

Kindblom JM et al[8] (2002) opined that the rate of cartilage differentiation during skeletal morphogenesis in rodents was controlled by the Indian Hedgehog through a negative feedback involving PTHrP. However the role of PTHrP and Ihh in the regulation of human epiphyseal chondrocytes was unknown. Hence, they embarked on an immunohistochemistry study to detect the expression and localization of PTHrP and Ihhin the human growth plate at various pubertal stages by obtaining growth plate biopsies from patients subjected to epiphyseal surgery. They concluded that the levels of expression of PTHrP and Ihh were mainly in early hypertrophic chondrocytes and that they were higher in early stages of puberty than later. They concluded that PTHrP and Ihh could play an important role in the regulation of pubertal growth in humans.

Shibata S et al[9] (2003) found ectopic cartilage formation in the mandibular coronoid process in newborn mice when they analyzed PTHrP null mutant mice. This was the first report showing an "increase" of cartilage volume in contrast to smaller volumes of cartilage in many other previous studies involving PTHrP gene knockout mouse. Though small amount of cartilage temporally formed at d 7, coronoid secondary cartilage never formed from E 15 to d 4 in normal mice and was a similar observation for PTHrP-wild type mice too. It was therefore suggested that the secondary cartilage formation was advanced with PTHrP deficiency which was a novel role of PTHrP in chondrocyte differentiation. Increase in the number of multinucleated osteoclasts and abnormal expansion of bone marrow, an indication of abnormal bone modeling was observed in the coronoid process of the PTHrP-deficient mouse.These results indicated that

PTHrP was involved in chondrocyte differentiation and bone modeling. Immunohistochemical results of in situ hybridization of matrix protein mRNAs in the abnormal mandibular condylar cartilage revealed that this cartilage was proportionally smaller.

Rabie ABM et al[10](2003) evaluated and correlated the cellular dynamics of chondrocytes in condylar cartilage during natural growth and mandibular advancement with the pattern of expression of PTHrP by fitting 35-day-old Sprague-Dawley rats with functional appliances. To trace the mesenchymal cell differentiation, experimental animals with matched controls were labeled with bromodeoxyuridine 3 days before their death. Mandibular advancement increased the cartilage volume with an increase in the number of differentiated chondroblasts. Slowing of chondrocyte hypertrophy was associated with higher levels of PTHrP expression in the experimental animals. It was concluded that mandibular advancement promoted mesenchymal cell differentiation and triggered PTHrP expression, which retarded their further maturation to allow for more growth.

Gensure RC et al[11](2005)opined that PTHrP had important developmental roles whilePTH had a central role in the regulation of serum calcium and phosphate and that both peptides signalled through the PTH/PTHrP receptor (a class B G-protein-coupled receptor). Intermittent injection of PTH increased bone mass and thus provided a means to treat osteoporosis. This triggered a search for smaller peptide or non-peptide agonists that have efficacy at this receptor when administered non-parenterally.Deficiency or excess production of either peptide caused a variety of clinical syndromes.

Kronenberg HM [12] (2006) opined that PTHrP played a vital role in the regulation of endochondral bone development and that the chondrocytes and perichondrial cells at the ends of the cartilage mold established in fetal life synthesized PTHrP. It was surmised that this ligand then acts on PTH/PTHrP receptors on chondrocytes which profilerate and differentiate into post-mitotic, hypertrophic chondrocytes. PTHrP delays their further differentiation as they simultaneously keep proliferating. Chondrocytes that have just stopped proliferating synthesize Indian hedgehog (Ihh), required for the synthesis of PTHrP. The feedback loop between PTHrP and Ihh served to regulate the pace of chondrocyte differentiation and the sites where the osteoblasts first differentiated from the perichondrial cells. Stimulation of both Gs and Gq family heterotrimeric G proteins is due to activation of the PTH/PTHrP receptor. Genetic analyses demonstrated that, while Gq activation opposes, Gs activation mediates the action of PTHrP to keep chondrocytes proliferating. SOX9, phosphorylation of the transcription factor, Runx2 and suppression of synthesis of mRNA encoding the transcription factor all mediate PTHrP's actions to delay chondrocyte differentiation and regulate the pace of differentiation of growth plate chondrocytes in response to PTHrP.

Ng AFS et al [13](2006) opined thatadaptive changes in skeletal mass and architecture is as a result of mechanical loading that can invariably influence the biological behavior of the bone-associated cells and that the endochondral bone formation was controlled by SOX9 and PTHrP genes which regulated chondrocyte differentiation and delayed the maturation stage.280 Sprague-Dawley rats were randomly allocated into control and experimental groups and the mechanical loading was applied through a bite-jumping device in the experimental group to investigate the effects of repeated mechanical loading on bone. Together with the matched control the experimental animals were sacrificed on 10 different time points. PTHrP and SOX9 genes quantification using real-time RTPCR was done on RNA extracted from the mandibular condylar cartilage. Results showed that on the seventh day after mechanical loading PTHrP expression was increased and reached a peak level and subsequent to repeated mechanical loading another increment in its peak expression was observed. The expression of PTHrP was highly correlated with the SOX 9 expression, and its pattern of expression was similar after repeated mechanical loading. They concluded that repeated mechanical loading on the condyle triggered the expression of SOX9 and PTHrP, which in turn promoted condylar cartilage growth.

Broadus AE et al[14] (2007) opined thatPTHrP appeared to regulate subjacent bone cell populations at sites that included the periosteum and ligament/tendon insertion sites at surface of endochondral bones. In PTHrP-lacZ knockin mouse experiments it was observed that PTHrP/lacZ was also expressed in epiphyseal cartilage and that this structure contributed

to PTHrP-expressing chondrocyte populations to both growth-plate and articular cartilage postnatally and that in these locations the Indian hedgehog-PTHrP axis was fully deployed and particularly in articular cartilage it protected the joint space from invasion by mineralizing cells. A mechanical regulation of PTHrP at these sites was also confirmed.

McCauley LK, Martin TJ[15] (2012) opined that though great strides have been made in the understanding of PTHrP, yet much was needed to be discovered and investigated. They however concluded thatits intended temporal actions emerged as dichotomies of production and action in the menancing condition of cancer and that PTHrP undoubtedly "controlled the show" locally at the PTH/PTHrP receptor relative to PTH, the hormone regulating calcium homeostasis.

Hussain MZ et al[16] (2013)quantified serum PTHrP levels of 90 subjects at 6 cervical vertebral stages and correlated it to the 6 skeletal maturation stages to prove whether PTHrP could serve as an ideal biologic skeletal maturity indicator to detect peak pubertal growth spurt. They reported that in the late pubertal stages the mean serum PTHrP levels were significantly higher than in the early pubertal stages and thatthe Pearson correlation showed that serum PTHrP levels had a negative correlation with cervical vertebral maturation stages from the late pubertal to the postpubertal stages and a positive correlation from the prepubertal to the late pubertal stages. They questioned the validity of using serum PTHrP levels to predict peak growth velocity as these levels did not correlate with early pubertal stages characterized by maximum growth increments.

Conclusion

PTHrP is a protein identified from cancers that causes hypercalcemia and is credited for its ability to mimic parathyroid hormone. Its paracrine, endocrine, and intracrine modes of action play a central role in organogenesis and drives numerous physiologic and pathologic conditions. PTHrP is a key factor regulating the pace of endochondral ossification during skeletal development. Mandibular advancement solicits a cascade of molecular responses in condylar cartilage and hence PTHrP may serve as an ideal biomarker to help detect peak pubertal growth during the circumpubertal growth spurt to help treat functional jaw discrepancies.

Ethical Clearance – Not required since it is a review article

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Conflict of Interest – Nil

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Principles of Tooth Preparation - Review Article

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Abstract

Adequate tooth preparation is essential for the fit of fixed prosthodontics. Insufficient tooth preparation appears to be responsible for premature failures due to biological aspects, such as caries and endodontic or periodontal disease complications. The purpose of a fixed prosthodontic therapy may vary from the restoration of a single tooth to the rehabilitation of the complete occlusion. Successful preparations can be obtained by systematically following these steps. It is important to critically evaluate each step before proceeding to the next step to ensure an optimal quality final restoration, which will serve the patient for a long time.

Keywords: *Finish lines, taper, resistance, retention, structural durability*

Introduction

Crowns and fixed partial dentures are the major prosthodontic treatment modalities for past several decades with factors like esthetics, contact points and pontics playing an important role in varying their design[1]. The purpose of a fixed prosthodontic therapy may vary from the restoration of a single tooth to the rehabilitation of the complete occlusion. A single tooth can be fully restored both functionally and aesthetically. A missing tooth can be replaced by a fixed prosthesis, increasing patient masticatory competence and maintaining or improving dental arches function, often elevating patient's self-image.

There is a general recognition that adequate tooth preparation is essential for the fit of fixed prosthodontics. Insufficient tooth preparation appears to be responsible for premature failures due to biological aspects, such as caries and endodontic or periodontal disease complications[2]. In the case of finish lines, different recommendations have been made to improve esthetics, minimize marginal fitting irregularities, and reduce stress concentration at the margins[3].

Tooth preparation should have specific geometrical characteristics to provide necessary retention and resistance to the vertical and lateral forces acting on the restoration. The most important element of retention is the presence of two opposing vertical surfaces. The axial walls of the preparation should taper slightly to allow the cementation of the artificial crown. The more parallel are the axial walls the greater is the retention[4].

OBJECTIVES OF TOOTH PREPARATION

The main objectives of tooth preparation

- Reduction of a tooth in miniature to provide retainer support.
- Preservation of healthy tooth to secure resistance form.
- Provision for acceptable finish lines.
- Performing pragmatic axial tooth reduction to encourage favourable tissue responses from artificial crown contours

TOOTH PREPARATION CAN BE STUDIED UNDER[5]

A. Biologic considerations: These affect the health of the oral tissues which includes conservation of tooth structure, avoidance of overcontouring, supragingival margins, harmonious occlusion, and protection against tooth fracture.

B. Mechanical consideration: These affect the integrity and durability of the restoration.

C. Esthetic consideration: These affect the appearance of a patient.

BIOMECHANICAL PRINCIPLES OF PREPARATIONS

The designs and preparations of a tooth for a cast metal or porcelain restorations are limited by five principles:-

1. Preservation of tooth structure.
2. Retention and resistance from.
3. Structural durability of the restoration.
4. Preservation of periodontium.
5. Marginal integrity.

1. Preservation of the tooth structure

The preparation of the tooth must be conservative; the minimal amount of the tooth structure must be removed. Excessive removal of the tooth structure has many harmful effects: -

- Excessive reduction lead to thermal hypersensitivity, pulpal inflammation and necrosis may result from approaching to the pulp closely.
- The tooth might be over tapered or shortened and this might affect the retention and resistance of the prepared tooth.

2. Retention and resistance form

Retention: is the ability of the preparation to resist the crown restoration from removal along its path of insertion.

Resistance: is the ability of the preparation to resist the dislodgment of the restoration by forces directed obliquely or horizontally to the restoration.

Path of insertion: An imaginary line along which the restoration can be inserted and removed without causing lateral force on the abutment.

The crown restoration should have a single path of insertion to be retentive. Most of the time the path of insertion of crown restorations is parallel to the long axis of the tooth except in ¾ crown for anterior teeth where the path of insertion should be parallel to the incisal 2/3 of the tooth crown (not to the long axis). By limiting the path of withdrawal, retention is improved

Factors affecting retention and resistance:

a. Taper of the preparation.
b. Surface area of the preparation,
c. Length and height of the preparation.
d. Diameter of the tooth (tooth width).
e. Texture of the preparation.
f. Accessary mean.

a. Taper of the preparation (5-6) degree convergence angle is mostly used to provide the needed retention. The more nearly parallel the opposing walls of preparation the greater will be the retention. But parallel wall is difficult to be obtained in the patient mouth without undercuts, also parallel walls might lead to difficulty in seating of the crown restoration, thus (5-6) degree convergence angle is mostly used to provide the needed retention.

Taper and Resistance: The more parallel the axial walls the more will be the resistance of crown restoration. The walls of a short wide preparation must be kept nearly parallel to achieve adequate resistance from.

b. Surface area of the preparation: Increasing the surface area increase retention. Factors that influence surface area are:

- Size of the tooth The larger the size of the tooth the more will be the surface area of the preparation, the more will be the retention thus full metal crown on molar tooth definitely more retentive than that on premolar tooth.
- Extend of coverage by restoration: The more the area that will be covered by the crown restoration, the more will be the retention, thus full metal crown on molar is more retentive than 3/4 crown on the same tooth.
- Accessory feature such as boxes, grooves and

pin holes.

c. Length (height) of the preparation: Increasing the length increase retention and resistance and vice versa.

d. Diameter of the tooth (tooth width): Under some circumstances, crown on narrow tooth can have grater resistance to tipping than a crown on a wider tooth, this occur because the crown on the narrower tooth has shorter radius for rotation resulting in a lower tangent line and a larger resisting area.

e. Texture of the preparation. Depending on the type of cementation agent, texture of the preparation might effect on the retention of cast crown. Smooth surfaces are less retentive than rough (mechanical interlocking).

f. Extra retention means. The retention of a preparation can be greatly enhanced by the addition of grooves, pin holes or boxes.

3. Structural Durability (SD): The preparation must be designed so that it provide S.D. to the restoration, i.e. the crown restoration must be rigid enough to not flex, perforate (metal) or even fracture (plastic). For a restoration to be rigid it need bulk, so to provide enough bulk to the crown restoration, sufficient tooth structure must be removed from the prepared tooth to create enough space. By doing so the restoration allowed to withstand the forces of occlusion, prevent wearing holes in the gold and allow proper contouring and carving of the restoration.

Preparation features related to Structural Durability:-

a. Occlusal reduction. Enough tooth structure must be removed from occlusal surface so that the restoration can be built back to ideal occlusion & thick enough to prevent wearing or distortion (1- 1.5mm).

Occlusal clearance: is the space between the occlusal surface of the prepared tooth and that of opposing tooth. It should be evaluated in centric and eccentric relation. Enough tooth structure must be removed occlusally so that when the restoration is built back to ideal occlusion it will be thick enough to prevent wearing through or distortion.

Functional cusps: the cusps that give centric stops of occlusion. (Palatal of upper posterior teeth and buccal of lower posterior teeth).

- Occlusal reduction must reflect the geometric inclined planes of occlusal surface.
- Avoid creating steep planes with sharp angles, because it lead to stress.
- Flat occlusal reduction lead to thin metal, this will lead to perforation of the crown restoration in future.
- Lowering the entire occlusal surface in attempt to providing sufficient space might lead to tooth structure destruction (non-conservative preparation) &shortening of the axial wall of prepped tooth which defiantly will affect the retention-resistance potential of the preparation.

Functional cusp bevel (FCB) Wide bevel should be placed on the functional cusps of posterior teeth to provide structural durability (it allows adequate thickness of restoration at this critical area without structure destruction). If FCB is omitted, the restoration is likely to be thin in this stress bearing area. If the restoration thickness is achieved by over tapering, this will compromise the retention. In the absence of FCB --- technician will overbuild the crown restoration in attempt to provide structural durability for the restoration this will lead to super-occlusion or premature contact with the opposing tooth.

b. Axial reduction: Sufficient reduction is important to provide sufficient space so that the restoration can build with sufficient thickness, this will prevent flexing of the crown restoration when occlusal force is acting on it.

c. Reinforcing struts: the features that serve to provide space for the metal that will improve the durability and the rigidity of the restoration: Offset the occlusal shoulder, the isthmus, the proximal grooves, and the box. Isthmus connects the boxes, and the offset ties the grooves together to enhance the reinforcing "truss effect".

4. Preservation of periodontium

Margin Placement Wherever possible, the margin

of the preparation should be supragingival. Subgingival margins of cemented restorations have been identified as major factors in periodontal disease particularly when the encroach upon the epithelial attachment.

Advantages of supragingival margins

- They can be easily finished
- They are more easily kept clean.
- Impressions are more easily made with less potential for soft tissue damage.
- Restorations can be easily evaluated at recall appointment.

However a subgingival margin is justified if any of the following pertain:

- Dental caries, cervical erosion, or restorations subgingivally and a crown-lengthening procedure is not indicated.
- The proximal contact area extends to the gingival crest.
- Additional retention is needed.
- The margin of a metal ceramic crown is to be hidden behind the labiogingival crest.
- Root sensitivity cannot be controlled by more conservative procedures.
- Modification of the axial contour is indicated.
- High D.M.F. rate of younger patients.

Gingival Termination of Tooth Preparation

The gingival finish lines are very controversial when it comes to tooth preparations. The precious recommendation was to extend crown margins into the intracrevicular space because the gingival crevice was thought to be immune to caries. In spite of the strong evidence that supported supragingival margins, subgingival margins were still used. Generalities as to where finish lines should be placed for an optimal contour require analysis. The subgingival area is not an immune area.

Supragingival Versus Subgingival Margins

Ideally the most innocuous position of the margin for soft tissue health is above the gingival crest. An esthetic position for anterior restorations would be subgingivally between the epithelial attachment and the crest of the gingival,I he area above the gingival crest is desirable but considered offensive by some patients. Supragingival margins are usually advocated for restorations placed subsequent to periodontal surgery and for elderly patients who exhibit a normal recession.

5. Marginal integrity

The restoration can survive in the biological environment of the oral cavity only if the margins are closely adapted to the cavosurface finish line of the preparation. The configuration of the preparation finish line dictates the shape and bulk of restorative material in the margin of the restoration It also can affect both marginal adaptation and the degree of seating of the restoration

TYPES OF FINISH LINES

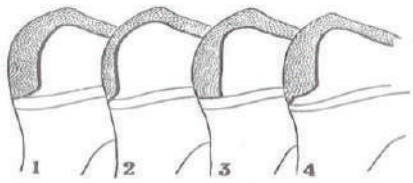
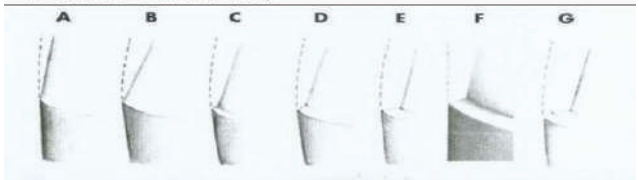


Fig. 5-21. Types of finish lines. 1, Chamfer provides bulk at the finish line. 2, Knife-edge provides minimal reduction. 3, Shoulder used for porcelain jacket crowns. 4, Chamfer or shoulder with bevel used for porcelain fused-to-metal crown. (Courtesy D.A. Kaiser, San Antonio, Tex.)



A. Featheredge, B. Chisel, C. Chamfer, D. Bevel, E. Shoulder, F. Sloped Shoulder, G. Beveled shoulder

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TYPES OF FINISH LINES

Chamfer finish line: The preferred finish line for the veneer metal restorations is the chamfer. This finish line has been shown experimentally to exhibit the least stress, so that the cement underlying it will less likelihood of failure. It can be cut with the tip of a round end diamond, while the axial reduction is being done with the side of that instrument. However, a torpedo diamond is less likely to produce a butt joint. The margin of the cast restoration that fits against it combines an acute edge with a nearby bulk of metal

Heavy chamfer finish line: A heavy chamfer is used to provide a 90-degree cavosurface angle with a large radius rounded internal angle. It is created with a round end tapered diamond. In the hands of an unskilled operator, this instrument can create an undesirable fragile lip of enamel at the cavosurface. The heavy chamfer provides better support for a ceramic crown than does a conventional chamfer, but it is as good as a shoulder. A bevel can be added to the heavy chamfer for use with a metal ceramic restoration.

Shoulder: The shoulder has long been the finish line of choice for the all-ceramic crowns. The wide ledge provides resistance to occlusal forces and minimizes stresses that might lead to fracture of the porcelain. It produces the space for healthy restoration contours and maximum esthetics. However, it does require destruction of more tooth structure than any other finish line, the sharp 90-degree internal line angle associated with the classic variety of this finish line concentrates stress in the tooth and is conductive to coronal fracture. The shoulder generally is not used as a finish line for cast metal restorations.

Shoulder with bevel: The shoulder with a bevel is a used as a finish line in a variety of situations. It is utilized as the gingival finish line on the proximal box of inlays and onlays, and for the occlusal shoulder of onlays and mandibular three quarter crowns. This design can also be used for the facial finish line of metal ceramic restorations where gingival esthetics is not critical. It can be used in those situations where a shoulder is already present, either because of destruction by caries or the presence of previous restorations. It is also a good finish line for preparations with extremely short walls, since it facilities axial walls that are nearly parallel.

Knife-edge: The knife-edge margin provides for an acute margin of metal. But its use can create problems. Unless it is carefully prepared, the axial reduction may fade out instead of terminating in a definite finish line. The thin margin of the restoration that fits this finish line may be difficult to accurately wax and cast. It is also more susceptible to distortion in the mouth when the casing is subject to occlusal forces.

Featheredge: A featheredge finish line is unacceptable because it is not sufficiently distinct and results in so little cervical tooth reduction that the restoration must be over contoured to possess adequate rigidity. Also, since a feather edge is more difficult to see visually, occlusocervical undulations and irregularities in the finish line are more likely to be present, making it much more difficult to fabricate a restoration that fits accurately

MECHANICAL CONSIDERATIONS

3 factors

1. Retention form
2. Resistance form
3. Preventing deformation of restoration

1. Retention Form

The quality of the restoration that prevents the restoration from being dislodged by force parallel to the path of withdrawal called as retention form.

a. Magnitude of dislodging forces:-

Forces can be exerted with a floss under the connectors but mainly force is exerted by adhesiveness of foods. The magnitude of dislodging force depends on

- Stickiness of food
- Surface area of restorations
- Texture of restoration being pulled

b. Geometry of tooth preparation

Most fixed prosthesis depend on the geometric form of tooth preparation rather than on adhesion tooth retention as most of the dental cements are non-adhesive and act by increasing the frictional resistance between tooth and restoration. Cement is effective only if the restoration has a single path of withdrawal.

Taper

Parallel walls give maximum retention but sometimes undercuts may be created (undercut is defined as a divergence between opposing occlusal walls or wall segments in cervico-occlusal direction).

The relationship between taper and magnitude of retention was demonstrated by Jorgensen in 1955. He cemented brass caps of different tapers and measured

retention. The relation was found to be hyperbolic i.e. retention decreased as taper increased. E.g. Retention of GALILTH with 10° taper was half that of 50 taper. Recommended taper is 5-8° with a rotary instrument held at constant angle.

Surface Area

- Crowns with long axial walls are more retentive than crowns with short axial walls.

- Molar crowns are more retentive than premolar crowns of similar taper.

Stress Concentration

- Cohesive failure occurs within the cement as the stress within cement is less than that of induced stresses. Stresses are seen to concentrate around the junction of axial and occlusal surfaces.

- Modifications like rounding of internal line angles will reduce the stress concentration and increase retention of the restoration.

- It is seen that retention of complete crowns is almost twice as that of partial coverage. Grooves or boxes in a preparation which limit the path of withdrawal enhances the retention.

c. Roughness of Surface being cemented

- If fitting surface made very smooth failure seen to occur at the cement restoration interface thus castings are delibrately roughened at the fitting surface and this has shown to increase retention. E.g. Roughening the surface by air abraiding with 50 fm alumina increases retention by 64%.

- Failure rarely occurs at the cement tooth interface thus roughening the tooth in an attempt to increase retention is not recommended.

d. Materials being cemented

Retention is affected by both casting metal and core build up material. It is seen that the more reactive the alloy is the more will be retention with certain luting agents.So, base metal alloys are more reactive than the less reactive high gold content metals. A study examining adhesion between cement and core materials found that cement adhered better to amalgam than composite or cast gold.

e. Type of luting agents

Adhesive resin cements are supposed to be most durable although long term clinical evidence about the durability of bond is not yet available.

2. Resistance Form

Restorations are also subjected to horizontal or oblique forces. E.g. mastication/ parafunctional habits. Lateral forces tend to displace the restoration by causing rotation around the gingival margins. This rotation is prevented by areas of the preparation that are placed under compression called as Resistance areas. Many such areas make up the resistance form. Adequate resistance depends on:-

- Magnitude and direction of dislodging forces

- Geometry of tooth preparation

- Physical properties of luting agents.

a. Magnitude and direction of dislodging forces

In normal occlusion biting force distributed over all the teeth. Similarly in FPD, occlusal load should be well distributed and favourably directed. In patients with pipe smoking, bruxism habits, oblique forces are exerted over FPDs. Thus, an ideal restoration should be able to withstand these forces as well as the normal axial forces.

b. Geometry of tooth preparation

Particular areas of axial walls help prevent rotation of the crown. Hegdahl and Silness analyzed how these resisting areas alter as changes are made in geometry of tooth preparation. They demonstrated;

- Increase preparation taper and rounding of axial walls decreased resistance.

- Shorter tooth preparation with large diameter - little resistance form.

- Partial coverage crown has less resistance than a complete coverage (as no buccal resistance areas).

- Resistance can also be improved by placing grooves and boxes especially with walls being perpendicular to the direction of applied forces.

- U-shaped grooves better than V-shaped. These grooves and pinholes interfere with rotational movement and subject other areas of luting agent to compression.

Additional Resistance and Retention

Grooves or boxes e.g. in 7/8- or 3/4- crowns. Pins increase retention by increasing surface area tapered. 5 ways to resist displacing forces

- 4-10° axial taper
- Suitable gingival finish lines
- Contouring and placing suitable contact areas
- Incorporating occlusal locks, dove tails, boxes
- Tapered and parallel pins.

ESTHETIC CONSIDERATIONS

The restorative dentist should develop skill in determining the esthetic expectations of the patient. Patients prefer their dental restorations to look as natural as possible. However, care must be taken that the esthetic considerations are not preserved at the expense of the patient's long term oral health or functional efficiency. Whenever possible, accomplishment of an esthetically acceptable result without the use of metal-ceramic crowns is preferred, not only because tooth structure is conserved but also because no restorative material can approach the appearance of intact tooth enamel.

Conclusion

The principles of tooth preparation can be categorized as biologic mechanical and esthetic, often these principles conflict and operator must decide the design of the restoration. Each tooth preparation must be measured by clearly defined criteria, which can be used to identify and correct problems. It is important to understand the pertinent theories underlying each step. Successful preparations can be obtained by systematically following these steps. It is important to critically evaluate each step before proceeding to the next step to ensure an optimal quality final restoration, which will serve the patient for a long time.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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An Review on Occlusal Plane Analysis in Dentulous Patients

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Abstract

The position of the occlusal plane forms the basis for the ideal teeth arrangement. Incorrect records of the occlusal plane would hamper aesthetics, phonetics, and mastication. It may affect the stability of a complete denture and ultimately result in alveolar bone resorption.Because the term plane refers geometrically to a flat surface, it is not entirely correct to describe the occlusal surface as following a true plane.

The form of the occlusal plane is directly related to specific functional requirements. In addition to the alignment of teeth in relationship to the arc of closure for best resistance to loading, it should permit ease of access for positioning of the food on the occlusal surfaces. This article is a review of the simple methods of analysing the occlusal plane in dentulous plane and correcting the occlusal problems in these patients.

Keywords: *occlusal plane,simplified occlusal plane analyzer, Broadrick occlusion plane analyzer*

Introduction

Determination of the occlusal plane is one of the most important clinical procedures in prosthodontic rehabilitation of edentulous patients. The position of the occlusal plane forms the basis for the ideal teeth arrangement[1]. Anteriorly occlusal plane helps in achieving aesthetics and phonetics while posteriorly it forms a milling surface where the tongue and buccinator muscle position the food bolus and hold it during mastication[2]. Incorrect records of the occlusal plane would hamper aesthetics, phonetics, and mastication. It may affect the stability of a complete denture and ultimately result in alveolar bone resorption.

OCCLUSAL PLANE DETERMINATION AND ITS ESTABLISHMENT

Determining the Plane of Occlusion

The term of the plane of occlusion refers to an imaginary surface that theoretically touches the incisal edges of the incisors and tips of the occluding surfaces of the posterior teeth[2]. The plane of occlusion represents the average curvature of the occlusal surfaces. A correct plane of occlusion allows protrusion without posterior interference. It allows non-interfering lateral excursions without loss of function on the working side. The occlusal plane problem that is most detrimental to esthetics is the slanted plane, which is high on one side and low on the other. The leveling of the occlusal plane always starts with the anterior teeth for the following two reasons.

Esthetics: The location of the incisal edge position relates to the smile line and determines the incisal plane, which is the anterior starting point for the occlusal plane on each side. For best esthetics, it is an absolute requirement that the incisal plane is parallel with the interpupillary line[3].

Function: Since the functional acceptability of any occlusal plane is primarily related to letting the anterior guidance do its job, the anterior segment must be organized before we can know how effective the anterior guidance can be in disoccluding the posterior teeth. The importance of the occlusal plane increases as the steepness of the anterior guidance decreases. The flatter the anterior guidance, the less capable it is of disoccluding a severely curved occlusal plane.

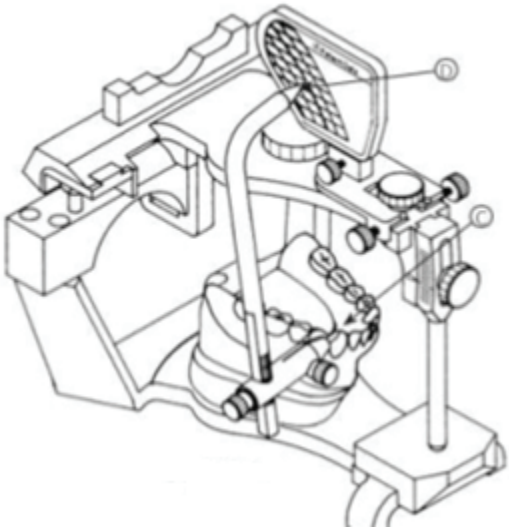
Examination for Occlusal Plane Problem

Ask the patient to protrude the mandible. If the anterior teeth are separated by the posterior teeth,

there is a problem with the occlusal plane. Because the condylar path is so important to protrusive disocclusion of the posterior teeth, condylar paths should be recorded at least by a protrusive interocclusal record, when an occlusal plane problem exists. The steeper the condylar path is in protrusive, the better able it is to help the anterior guidance disocclude the posterior teeth[4].

Determining an Acceptable Occlusal Plane for Restorative Cases Using a simplified occlusal plane analyzer (SOPA)

An ideal occlusal plane starts at the canine tip and goes through the condylar axis. If all positive cusp tips relate to this plane, the disclusion of posterior teeth is never a problem. However, remember that this is an arbitrary plane based on an arc around a survey point that is 4inches from the tip of the canine[5]. It is an excellent aid for establishing an ideal occlusal plane if all posterior teeth are to be restored. It should not be used to determine whether or not restorations are necessary.



SIMPLIFIED OCCLUSAL PLANE ANALYSER

ü A SOPA is preset at 4 inches from the condylar axis. The SOPA works with Dénar® (Teledyne Waterpik™) articulators. The Broadrick flag accomplishes the same occlusal analysis on almost all types of semi-adjustable articulators.

ü By setting the caliper scribe at 4 inches and aligning the marking the point at the tip of the canine, an occlusal plane can be scribed on the lower cast that will go through the condylar axis in one simple step.

ü A line drawn on the cast represents an acceptable occlusal plane. The caliper scribe can be lengthened to a preparation height. This process is only used if the posterior teeth are to be restored. It is never used to determine whether or not teeth must be prepared.

ü A simple wax index can be adapted to the cast and the desired occlusal plane can be scribed on the wax, which is then trimmed to the line.

ü With the wax index in place, the teeth can be marked to the indicated preparation height and occlusal plane.

ü Tooth reduction that follows the predetermined preparation height and contour ensures that there will be sufficient room for restorative materials when the restorations are fabricated. Preparations are completed after the occlusal reduction is verified[6].

Occlusal plane analyzer (BOPA)

Broadrick occlusion plane analyzer: When it has been determined that restoration of all or most of the posterior teeth is necessary, the PMS technique using BOPA provides a simple and practical method to assist in determining the preliminary occlusal plane on diagnostic casts[8]. Proper use of the occlusion plane analyzer will accomplish the following:

ü Preliminary determination of an acceptable plane of occlusion on the study model as an aid in treatment planning.

ü Preliminary determination of the amount of reduction that will be required when each tooth is prepared.

ü Extremely simple transfer to the mouth of the predetermined preparation height for each tooth.

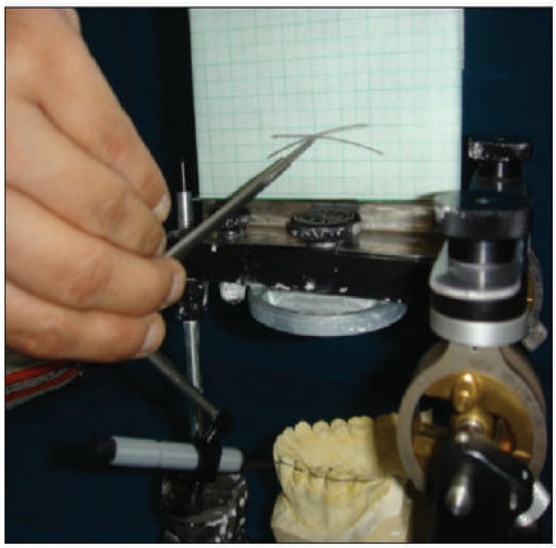
ü In laboratory wax-up simple determination of the height of each cusp tip.

ü The predetermination of both cusp height of the finished restoration and also the height of each prepared tooth. Thus, room for a sufficient thickness of metal or metal and porcelain can be assured in advance.

ü A properly predetermined plane of occlusion on the lower arch which enables the dentist to select

virtually any type of acceptable occlusion contour scheme with complete assurance that the established plane of occlusion will permit it.

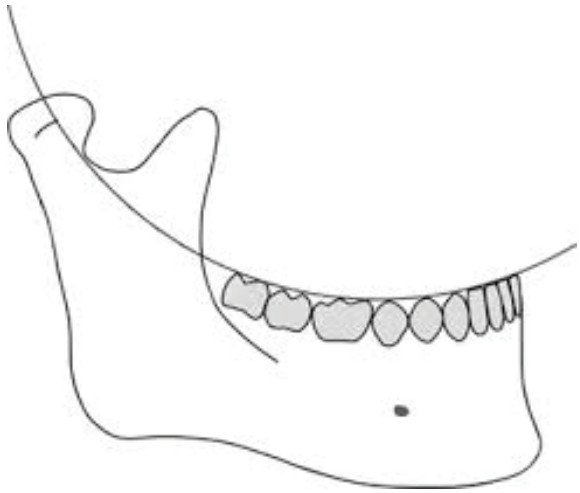
The BOPA has now been adapted to only a few articulator systems, such as the Denar Anamark Fossae (Teledyne Waterpik, Ft Collins, Colo) and all models of Hanau articulators (Teledyne Waterpik)[9]. For those manufacturers of semi-adjustable articulators who do not offer such occlusal plane analyzers for use with their instruments, custom made clear acrylic resin BOPA may be fabricated.



Technique of using BOPA consist of the following steps

ü The flag is attached to the upper member of the articulator. A sheet of blank paper is attached to both sides of the flag to receive the markings. The maxillary cast is removed from the articulator, and the flag is attached on top of the upper member of the articulator. The anterior survey point (ASP) is chosen on the midpoint of the disto-incisal edge of the mandibular left canine from which a long arc with a 4-inch radius is drawn on the flag with a compass. The posterior survey point (PSP) is located on the distobuccal cusp of the mandibular left second molar and a short arc is drawn on the flag to intersect the long arc at the center of the anteroposterior curve. The point of the compass is placed at the center of the anteroposterior curve on the flag, and a 4-inch radius is drawn through the buccal surfaces of the mandibular teeth.

ü Another line termed the "preparation line", is scribed by opening the compass by an amount equal to the desired occlusal thickness of the proposed restoration. A softened modeling wax sheet is adapted to the buccal surfaces of the mandibular cast. The wax is cut carefully back to this line and trimmed along the mucobuccal fold so that the wax could be fitted accurately against the teeth intraorally, and this is termed the "occlusal plane cutting guide".



BroadricksCurve

When preparing the mandibular posterior teeth, the cutting guide is placed snugly against the buccal surfaces of the dried teeth, and the entire occlusal surface of each tooth is reduced to the preparation line. Following occlusal reduction, the teeth are prepared according to the predetermined treatment plan[10].

CORRECTING THE OCCLUSAL PLANE PROBLEMS

There are two basic approaches to solving occlusal plane problems[11].

1. Leveling or flattening the occlusal plane, so that it can be disoccluded by existinganterior guidance.

2. Steepening the anterior guidance, so that it can disocclude existing occlusal plane, whichremains unchanged.

3. Combination of above 2 methods

The steepening of anterior guidance depends on 4 factors.

1. Envelope of function
2. Arch relationship
3. Esthetic factor
4. Periodontal support

Envelope of function is the principal determinant of anterior guidance, so any steepening of the anterior guidance can result in restriction of the established pattern of function. However, when occlusal plane problems separate the anterior teeth in protrusive, it is often possible to eliminate the posterior interferences by selective grinding so that anterior contact can be maintained from centric relation forward to the incisal edge to edge position. Certain arch relationships may contraindicate restorative alteration of the anterior guidance. If anterior teeth are in a stable relationship with strong tongue or lip pressure related to an anterior open bite or a severe over jet, it may create instability if the teeth are moved or restored to contact.

Esthetics is often a key factor in determining what to do with a slanted or uneven occlusal plane. When teeth on one side have been unopposed, it is sometimes very difficult to level both sides because of severe elongation on the unopposed side. Periodontal support around the anterior teeth is critical if the anterior guidance is steepened to disclude the posterior teeth. If steepening the anterior guidance restricts habitual patterns of function, there will be a tendency for increased horizontal stress on the anterior teeth.

Summary and Conclusion

The form of the occlusal plane is directly related to specific functional requirements. In addition to the alignment of teeth in relationship to the arc of closure for best resistance to loading, it should permit ease of access for positioning of the food on the occlusal surfaces. In the dentulous subjects, a significant association was seen between the occlusal maxillary plane angle and maxillomandibular space length and height.

1. In the long and low subtype of maxillomandibular space, the occlusal maxillary plane angle is least. The occlusal plane tends to be parallel to the maxillary plane.

2. In the short and high subtype of maxillomandibular space, the occlusal maxillary plane angle has the maximum value. The occlusal plane is more angulated to the maxillary plane.

3. In the short and low and long and high subtypes of the maxillomandibular space, mean value of occlusal maxillary approximates the mean of the entire group.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Dental Articulator – Hanau Wide Vue

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Abstract

The articulator plays an important role in Dentistry, since it allows adequate diagnosis and treatment planning to promote the oral health of our patients. The Semi adjustable, Fully adjustable articulator reduces the intervention of Prosthodontists; when well employed, it simplifies the daily clinical routine, reducing the number of sessions, chair time and need of interventions in the patient's mouth, besides allowing more accurate procedures, consequently without damages to the stomatognathic system.Dental articulators are instruments that reproduce jaw movements to varying degrees of accuracy. This article aims to give an overview of Hanau wide vue and describe their applications and limitations.

Key Words: *Articulator, condylar guidance, semi adjustable articulator, Hanau wide vue.*

Introduction

The success of fixed or removable restoration directly depends on the articulator selected as well as the skill and care with which it is used. Articulator is a mechanical device that represents the temporomandibular joints and the jaw members to which maxillary and mandibular casts may be attached to simulate some or all the mandibular movements.

Articulators are instruments that attempt to reproduce the range of movement of the jaws. Maxillary and mandibular casts are attached to the articulators so that functional and parafunctional contact relation between the teeth can be studied. It is often said "patient's mouth is the best articulator". The primary function of articulator is to act as a patient[1].

Any restoration provided should not interfere with mandibular function in mastication, speech, and swallowing nor should they transmit excessive force to the attachment apparatus or the temporomandibular joint either in the intercuspal or eccentric jaw positions as well as during movement.

DEFINITION

Articulator is defined as, a mechanical device that represents the temporomandibular joints and jaws , to which maxillary and mandibular casts may be attached to simulate some or all mandibular movements.- **GPT 9TH EDITION**

USES OF ARTICULATOR

Articulators are used

1. To mount diagnostic casts.
2. To study the occlusion of a patient.
3. As an aid in planning treatment procedures.
4. To hold the opposing casts in a predetermined fixed relationship.
5. As an aid in the fabrication of dental restorations and lost dental parts.
6. In the arrangement of artificial teeth for complete dentures, removable partial dentures and waxing in field partial dentures.

REQUIREMENTS OF ARTICULATORS

Articulators have different adjustments, and some have more than others. To make any classification easy to visualize, and to avoid confusion because of these sophisticated adjustments, the requirements of an acceptable articulator must be defined. In prosthetic

dentistry the necessity of registering the following records is generally accepted:

1. Face-bow record
2. Centric jaw relation record
3. Protrusive record
4. Lateral records
5. Intercondylar distance record

ADVANTAGES OF ARTICULATORS

1. Properly mounted cast allows the operator to visualize the patient's occlusion, especially from the lingual view.

2. Patient cooperation is not a factor when using an articulator once the appropriate interocclusal record is obtained from the patients.

3. The refinement of complete denture occlusion in the mouth is extremely difficult because of shifting denture bases and resiliency of the supporting tissues. This difficulty is eliminated when articulators are used.

4. Reduced chair time, patient's appointment time.

5. The patient's saliva, tongue and cheek are not factoring when using an articulator[2].

INT. PROSTHODONTIC WORKSHOP AT UNIVERSITY OF MICHIGAN 1972:

- Class I
- Class II
  - o Subdivision A
  - o Subdivision B
  - o Subdivision C
- Class III
  - o Subdivision A
  - o Subdivision B
- Class IV.
  - o Subdivision A
  - o Subdivision B

a) Class I

A Simple holding instrument capable of accepting a single static registration. Vertical motion is possible, but only for convenience.

Example- the Verticulator (Jelenko), the Corelator (Denar Corp)

b) Class II:

An instrument that permits horizontal as well as vertical motion but do not orient the motion to temporomandibular joints via facebow transfer.

Class II A

Eccentric motion permitted is based on average or arbitrary values.

Example- Gysi simplex articulator

Class II B

Eccentric motion permitted is based on theories of arbitrary motion.

Example- Monsons maxillomandibular articulator

Class II C

Eccentric motion permitted is based on engraved records obtained from the patient.

Example- The gnathic relator

c) Class III:

Instruments that simulate condylar pathways by using averages or mechanical equivalents for all or part of the motion. These instruments allow for orientation of the cast via face bow transfer.

Class III A

Instruments that accept static protrusive registrations and use equivalents for the rest of the motion.

Example- Dentatus, Hanau non arcon H2 articulator

Class III B

Instruments that accept static protrusive registrations

and some lateral interocclusal records and use equivalents for the rest of the motion.

Example- Hanau Teledyne, whip mix articulator

d) Class IV:

Instruments that will accept three dimensional dynamic registrations. These instruments allow for orientation of the casts via facebow transfer

Class IV A

The condylar pathways are formed by registration engraved by the patient.

Example- Denar Combiarticulator, TMJ-Stereographic instrument

Class IV B

Instruments that have condylar pathways that can be selectively angled and customized.

Example- Stuart Articulator, Denar D5 articulator[3].

TYPES OF VIRTUAL ARTICULATORS

Basically, there are two types of virtual articulators namely; completely adjustable virtual articulators and mathematically simulated virtual articulators.

A. Completely adjustable virtual articulators

This type of virtual articulators uses an electronic jaw registration system called as jaw motion analyser (JMA) to replicate or record exact movements of the patient's mandible.

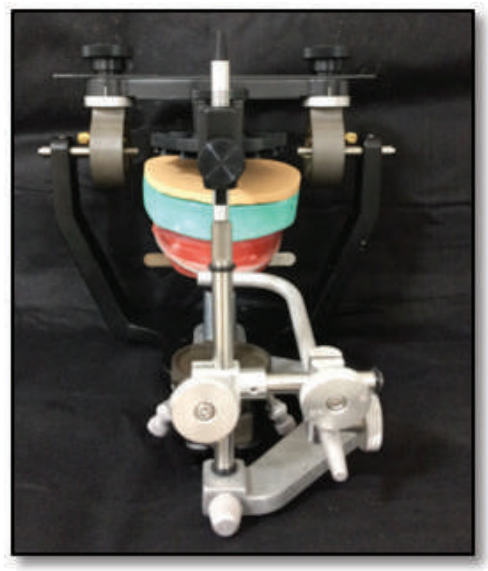
Jaw motion analyser (Zebris, Germany) is an ultrasonic measurement system used to record and implement various mandibular movements.

B. Mathematically simulated virtual articulators

It is a fully adjustable 3-dimensional articulator capable of reproducing the movements of a mechanical articulator. It is more versatile than a mechanical dental articulator. Basically, it functions as a mechanical average value articulator.

Examples: Szentpetery's virtual articulator and Stratos 200

HANAU Wide-Vue



The HANAU Wide-Vue, Articulators are classified as semi-adjustable. They are of Arcon principle, wherein the Condylar Guidances are associated with the Upper Articulator Member, as the patient's glenoid fossa is a portion of the cranium, The Condyle of the Articulator is a part of the Lower Member and functions as the condyle of the patient's mandible. Articulator movements may be directly related to the anatomical function of the patient.

CONDYLAR GUIDANCE:

The Condylar Guidance are the control centres of the Articulator and they adjustably assimilate the multiple function of the glenoid fossa. The Condylar Track may be adjustably inclined on the horizontal transverse axis from a "zero" to a plus 60 degree or to a minus 20 degree. These inclinations are termed the protrusive inclination and simulate the patient's superior wall of the fossa. The Condylar Track may also be adjusted on the vertical axis from a "zero" sagittal to 30°. This angle is termed the progressive Bennett angle and corresponds to the medial wall of the patient's fossa.

Condylar guidance adjustments in both sagittal and horizontal planes and the lateral condylar angle calculated by the formula L= H/8 + 12, H = Horizontal condylar angle, L= lateral condylar angle.



Figure 2: Horizontal condylar angle



Figure 2a): Lateral condylar angle

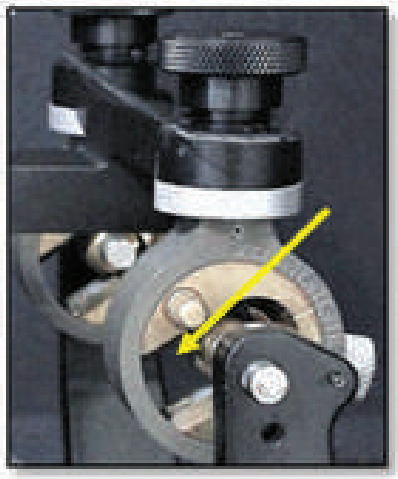


Figure 3: The Condylar Track



Figure 4: The locking or releasing of the Centric Lock

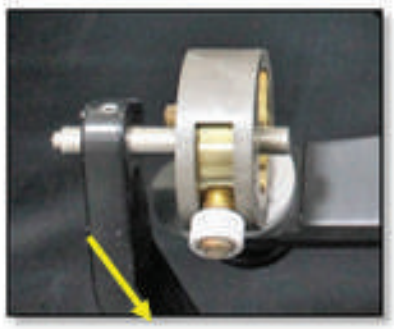


Figure 5: The Condylar Shaft

CLOSED CONDYLAR TRACK

The Condylar Guidance Track rotates in an enclosed housing which stops the Condylar Element, preventing the accidental disengagement of the Upper Member.

CENTRIC LOCK

Engagement of a Centric Lock depresses a Centric Pin, causing it to arrest the Condylar Element at the centric position. When locked, the Upper Member is restricted to an opening and closing movement only. Releasing the Centric Lock two full turns will disengage the Centric Pin and return the Element's freedom of movement in the Condylar Track.

CONDYLAR SHAFTS

The Condylar Shafts adjustably slide in the "wings" of the Lower Member. They have been factory fixed by Setscrews when their brass shoulders rest against the flatted sides of the Condylar Elements at the "zero" centric position. A resilient Bumper will protectively stop the Upper Member and rest against the "Wing" of the Lower Member when fully opening the Articulator.

DUAL-END INCISAL PIN

Coinciding with these Condylar Shaft adjustments is an alignment of the chisel edge of the Incisal Pin with the central table of the Incisal Guide. The Incisal Pin serves as the forward control of the Articulator. It cooperatively maintains a vertical stop and provides a stylus contact for the excursive movements of the Articulator against the various inclined guiding surfaces of the Incisal Guide. A mid-line groove is cut in the Incisal Pin about one inch from the spherical tip. Five additional lines calibrated in millimeters extend on either side thereof. These lines are used for recording or altering the vertical dimension. The Incisal Pin is inserted into, and the wider mid-line of these metric grooves is aligned with, the top edge of the Upper Member. It is secured by the Thumbscrew bearing against the flatted side on the Pin. This adjustment places the chisel end at 90 degrees to and in contact with the central table of the Incisal Guide and provides a parallelism of the Upper Member to the Lower Member.

Two annular grooves, appear on the Incisal Pin at 37 and 54 mm below the Frankfort Horizontal Plane. These grooves form arbitrary vertical landmarks for alignment of the incisal edge of the maxillary centrals when making a Facebow transfer. The 37 mm line is based in part on the Bonwill Triangle and results in a generally horizontal appearing plane of occlusion. The 54 mm line forms an average landmark for alignment of the incisal edge of the upper centrals when making a Facebow transfer. This reference line is based on the research study by Frank R. Lauciello, D.D.S., and Marc Appelbaum, D.D.S., "Anatomic Comparison to Arbitrary Reference Notch on Hanau Articulators," Journal of Prosthetic Dentistry, December 1978, Volume 40, Number 6, Pages 676-681. The Incisal Pin extends beyond the top of the Upper Member and provides a third point of stability when inverting the Articulator for mandibular cast mounting. The spherical tip of this Incisal Pin serves as the Dual-End and is useful for fabricating customized acrylic anterior guide tables.

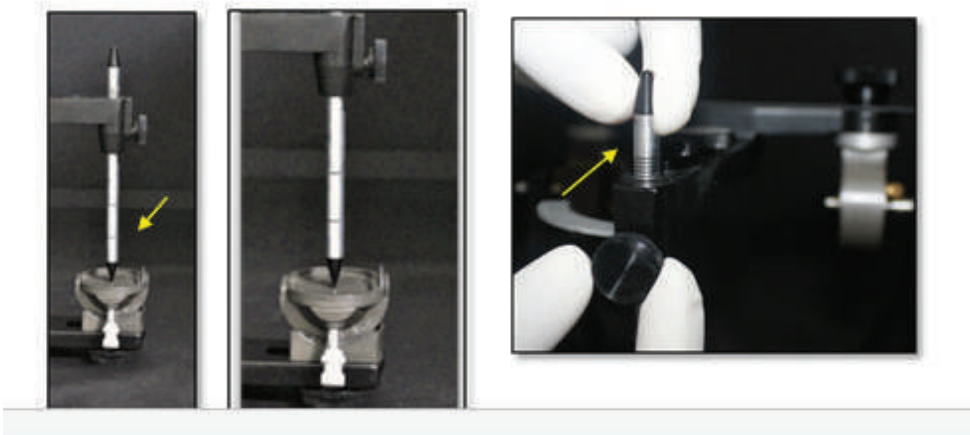


Figure 6:A- Dual Ended Incisal Pin, B- Incisal Pin resting on Incisal TableC- The marking on the Incisal Pin

ADJUSTABLE INCISAL GUIDE

The Adjustable Incisal Guide provides an independent adjustment of anterior guidance. It cooperates with the Incisal Pin and Condylar Guidances to present a stable, three-dimensional programmed guide pattern for the mounted casts.

The Incisal Guide rotates antero-posteriorly from a horizontal "zero" degree to a 60-degree positive inclination of protrusion which is then secured by the small Locknut. The central guiding table is 5.56 mm wide and forms the inclined surface for the protrusive guidance of the Incisal Pin. Separately adjustable Lateral Wings elevate by a Thumbscrew from a "zero" horizontal to a 45-degree incline and are fixed by a Thumbnut. The calibrations are very small and serve only as a reference.

An anterior slot, in the Lower Member, allows repositioning of the Incisal Guide. Adjust and lock the Guide at a "zero" horizontal and slightly loosen the Platform Lock screw. Slide the Platform antero-posteriorly to align the chisel end of the Incisal Pin with the "zero" indicating line on the Lateral Wings. This adjustment will place the Incisal Pin contact on the rotational centre of the Guide, thereby maintaining the vertical dimension when adjusting the inclination for protrusion. Loosening the Platform Lock screw one turn will allow the Incisal Guide Assembly to be withdrawn from or returned to the anterior slot without any disassembly of parts.

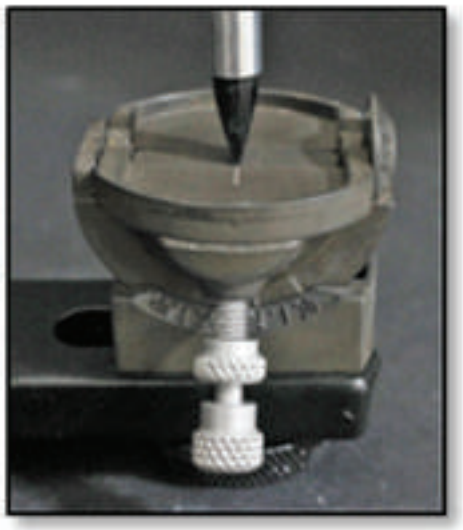


Figure 7: The Incisal Table

RESILIENT BUMPER

Stop the upper member and rest against the wings of the lower member, when fully opening the articulator.

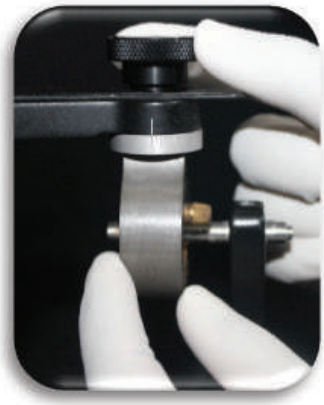


Figure 8: Resilient bumper

ADJUSTABLE PROTRUSIVE-RETRUSIVE

This micrometer adjustment permits the Condylar Element to be protruded 6 mm from centric or to be retruded 3 mm from centric.

ORBITALE INDICATOR

This "crescent" represents the patient's infra-orbitale notch and is the anterior reference landmark of the Frankfort Horizontal Plane. When used with an Orbitale Pointer on a Face bow it provides an anatomical vertical orientation for the upper arch, obviating the use of any average reference lines on the Incisal Pin[4, 5].



Figure 9: The Orbital pointer

APPLICATION OF SEMI ADJUSTABLE ARTICULATORS IN FPD

Shape and angulation of the articular eminence Limitation: the upper wall of the "mandibular cavity" of the SAA is straight and rigid, whereas this structure in the TMJ is curved. That is to say, only the initial and final positions of mandibular movement are recorded. Therefore, the actual paths of the condyles are not accurately recorded on the SAA. Consequently, carving of the occlusal surface of posterior teeth increases the risk of occurrence of undesirable contacts during mandibular movements. Compensation: customization of the anterior guidance while the provisional crowns are worn and its transfer to the incisal table on the articulator reduces the possibility of contacts betweenthe posterior teeth during excursive mandibular movements. This customization guides the establishment of cusp height and fossa depth[6, 7].

FACTORS CONTRIBUTE TO INACCURACY OF CAST ARTICULATION

Errors could happen while registering occlusal relationship. An example of a possible source of error is the difference in the patient's mandibular position when supine and upright. According to Helcio et al., the mandible tends to be positioned more posteriorly when the patient is lying down and the mouth has been actively closed into the relaxed position of centric occlusion. Another example is inherent in the nature of mandibular hinge axis and the consequences of its application in treatment planning.

To simulate the mandibular functional movement on the dental articulator, the degree of correlation between the patient and mounted casts depends on many factors, including biologic considerations and the properties of the materials used during the process of transferring maxilla mandibular records[8, 9].

Literature has reported some factors that are possibly the source of incorrect maxillary cast alignment. According to Ferrario et al.

(1) Individual variation in the anatomic reference landmarks and measurements,

(2) Improper adjustment of the face-bow to the patient or the instrument to the articulator during the transfer procedure, and

(3) Setting the Frankfurt plane horizontally on the upper member of the articulator.

Conclusion

Evolution of articulators through the years has given an insight of the researchers trying to develop a mechanical device that simulates the jaw members and its movements. The purpose of using an articulator is to develop a prosthesis that will be harmonious in the oral cavity.

Various articulators have been developed and are being improved upon as and when the functions of the jaws are understood better. Accordingly, in the present day, the availability of articulator ranges from simple hinge type to fully adjustable articulators and virtual articulators[10, 11].

Selection of articulators depends upon the clinical situation. In complete denture prosthesis, semi-adjustable articulators suffice the requirements to develop a good denture. Fully adjustable articulators are used for fixed prosthesis. It is difficult to manage fully the adjustable articulators in complete denture patients because the clutches used to obtain the hinge axis and condylar movements are more cumbersome to be used in edentulous patients which in turn do not provide accurate records.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil.

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A Review on Occlusal Plane Analysis in Edentulous Patients

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Abstract

Aim: The Purpose of the present review was to determine the occlusal plane in edentulous patients.

Objective : Occlusal plane orientation is an important factor in the construction of a complete denture. Occlusal plane could be oriented using landmarks in the maxillary arch as well as the mandibular arch . Many methods have been used to establish the occlusal plane in complete dentures , but no method seems to be fully acceptable. So it is always challenging for the prosthodontist to restore the existing occlusal condition with a suitable prosthesis.

Materials and Methods : : An electronic search engine without time/ language restrictions was taken from Google Scholar .

Conclusion: This article reviews different techniques to determine and correct occlusal plane. HIP was parallel to the occlusal plane , Ala-tragal line passing through lower part of the ala of the nose to inferior position of the tragus was relatively parallel to maxillary residual ridge. Therefore, this may be a viable reference in complete denture prosthodontics.

Key words : *Plane of Occlusion , Camper's plane, Curve of Spee , Occlusal plane analyser , Ala tragus.*

Introduction

Complete denture prosthodontics is a challenge for the dentist for rehabilitation of edentulous patients with conventional complete dentures. Occlusal plane orientation is one of the most important clinical procedures in edentulous patients. It may be tissue supported or implant supported, has to be done by considering various biological and mechanical factors to restore functions and health of the stomatognathic system[1]. Developing an occlusion that is compatible with functional movements of the stomatognathic system is one of the key factors in determining the prognosis of the completely edentulous patients. One of the important factors that help us in the establishment of ideal occlusion is the orientation of the occlusal plane.

A further description by Dr. Peter Dawson in 1780, said that the "The plane of occlusion refers to an imaginary surface that theoretically touches the incisal edges of the incisors and the tips of the occluding surfaces of the posterior teeth." The glossary of Prosthodontic term (2005) defines occlusal plane as "the average plane established by the incisal and occlusal surfaces of the teeth." A plane is determined by at least three reference points that are not in a straight line. The occlusal plane is determined anteriorly by the maxillary incisor teeth and posteriorly by the retromolar pads.

When we relate to a plane we initially think of a flat surface, however, it is not a plane but represents the planar mean of the curvature of the surfaces. Using an occlusion rim, the clinician determines the incisal edge position by evaluating speech, lip support, and esthetics and then typically uses anatomical landmarks such as the retromolar pad and relative parallelism of the ridges to determine the position of the posterior teeth. The

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connection of these anterior and posterior landmarks establishes the occlusal plane[2].

The orientation of the occlusal plane is lost in patients rendered edentulous and should be re-located if complete dentures are to be esthetic and to function satisfactorily. For example, if the occlusal plane is placed too high, the tongue cannot rest on the lingual cusps of the mandibular denture and prevents the denture's displacement. There is also a tendency for accumulation of food in the buccal and lingual sulci. On the other hand, if the occlusal plane is placed too low, it could lead to tongue and cheek biting. Complete dentures are constructed to function in the mouth as an integral part of the masticatory system; therefore, they should be designed to conform to the patient's physiologic jaw relations.

The use of anatomical landmarks as guides has been suggested by many authors. Anatomical landmarks suggested to clinically determine the position of the occlusal plane are the upper lip, corner of the mouth, lateral margins of the tongue, two-thirds of the height of the retromolar pad, parallel to the ala-tragus (Camper's plane) and interpupillary lines, parallel to the hamular notch-incisive papilla plane, and 3.3 mm below the parotid papilla. A common concept is that the occlusal plane should be parallel to a line drawn from the lowest point of the ala of the nose to the external auditory meatus or tragus of the ear.

According to the Glossary of Prosthodontic terms, the occlusal plane is defined as "the average plane established by the incisal and occlusal surfaces of the teeth." Generally, it is not a plane but represents the planar mean of the curvature of these surfaces. These are as follows,

Curve of Spee + Curve of Wilson + Curve of Incisal Edges = Curve of Occlusion

Curve of Occlusion + Its relationship to the Cranium= Plane of Occlusion

The occlusal plane position is the foundation of clinical treatment and one of the most important criteria used to judge the degree of treatment success. It is an important factor which harmonizes morphology and function of stomatognathic system. Various authors have defined the occlusal plane in many different ways.

1. "An imaginary surface that touches the incisal edges of incisors and tips of the occluding surface of the posterior teeth".

2. "A line bisecting the molar and incisor overbite".

3. "A line extending from mesio incisal angle of upper central incisor to the mesiopalatal cusp of first maxillary molars".

4. It represents the functional table of occlusion in the first permanent molar, second premolar and the first premolar area.

5. The average plane established by the incisal and occlusal surfaces of the teeth.

Generally, it is not a plane but,

a. Represents the planar mean of the curvature of these surfaces.

b. The surface of wax occlusion rims contoured to guide in the arrangement of denture teeth.

c. A flat metallic plate used in arranging denture teeth - compensate to curve of occlusion

OCCLUSAL PLANE SIGNIFICANCE

The correct orientation of the occlusal plane plays a vital role in optimal esthetic achievement. In the natural smile, the incisal tips follow the curve of the lower lip. This effect is an expression of a correctly oriented occlusal plane; if the occlusal plane hangs posteriorly, the lip-line viewed from the front will appear straight and contribute more than any other factor to the so-called 'denture look. The plane of occlusion forms an essential part of the concept of mechanically balanced articulation. The position of occlusal plane in denture wearers should be as close as possible to the plane, which was previously occupied by the natural teeth. Such position of the occlusal plane provides normal function of the tongue and cheek muscles, thus enhancing the denture stability.

It is believed that teeth oriented on an occlusal plane in harmony with the individuals physiognomy is responsible, in part, all conditions being equal, for stable, retentive full dentures. Faulty orientation of the occlusal plane will jeopardize interaction between tongue and

buccinators muscles. Where the occlusal plane is too high, the tongue cannot rest on the lingual cusps of the lower denture and prevent its displacement. Also it forces the tongue into a new position that is higher than its normal position. This higher position of the tongue causes the floor of the mouth to raise and create undue pressure on the border of the lingual flange and results in partial loss of border seal. There is also tendency for accumulation of food in the buccal and lingual sulci. An occlusal plane that is too low could lead to tongue and cheek biting. Correct orientation of the plane of occlusion in all three dimensions after loss of natural teeth is a demanding task. The optimal position/orientation of the occlusal plane is essential for successful prosthodontic treatment of completely edentulous patients.

OCCLUSAL PLANE ORIENTATION

Considering the importance of the accurate establishment of the location and inclination of occlusal plane on function, esthetics and speech, a method to conform it to the occlusal plane that existed in the natural teeth seems necessary. The question which arises in clinical practice is how to discover which position was occupied by the ‘‘natural occlusal plane’’ after the loss of natural teeth and exactly, how can the optimal position of the occlusal plane be found in every edentulous patient .The occlusal plane in anterior and posterior regions may vary and therefore, these should be evaluated separately. It is generally agreed that in the anterior region the vertical height of the occlusal plane is governed by esthetic requirements and less frequently by functional demands. Orientation of the occlusal plane is lost in patients rendered edentulous and should be relocated if complete dentures are to be esthetic and functional. It is difficult to find the optimal position of occlusal plane in every edentulous patient using the reported soft tissue landmarks. Improper use of these landmarks may compromise the functional and esthetic result of the intended prosthetic rehabilitation.

The anterior maxillary occlusal plane may be determined by lip relationships at rest and when smiling. Speech also provides for positional accuracy. When viewed from the front, the occlusal plane should be parallel to the inter-pupillary line. With regard to the orientation of the occlusal plane in the posterior region, however, there are contrasting views. Various authors have postulated various landmarks for determining the occlusal plane. The commonly used method is based on anatomical landmarks which advices the positioning of occlusal plane parallel to the plane between the corner of mouth and the junction of middle and upper third of the retromolar pad. However, all the authors agree that ideal location for the occlusal plane is in the same position as it was when the natural teeth were present. Theoretically, plane established by Broadrick occlusal plane analyzer method has been considered as ideal occlusal plane for dentulous and partially edentulous individuals.

In case of edentulous individual, after anterior teeth were set, which is guided by phonetics and esthetics; tip of the canine and condylar element of the articulator can be taken as anterior and posterior survey points, respectively, for Broadrick occlusal plane analyzer method. The plane established by Broadrick occlusal plane analyzer method can be important guide for establishing plane in complete denture cases if anatomically established plane and plane established by Broadrick occlusal plane analyzer method coincide to each other. Precise establishment and orientation of the plane of occlusion is very important during rehabilitation of the completely edentulous subjects and is one of the important factors, which determines the prognosis of the case. It is desirable that the occlusal plane lost in edentulous patients should be relocated in prosthesis to conform to natural occlusal plane of the patient. Faulty orientation of occlusal plane in the prosthesis will jeopardize the interaction between tongue and buccinator muscle in placement of food bolus on the occlusal table. If the plane is too high, it would cause collection of food in the sulcus, and if it is too low, it would result in biting of cheek or tongue. Occlusal plane is an important factor of stability, critical in mounting on articulators, has a bearing on health and function of temporomandibular joint and is also of vital importance for articulate speech in a complete denture patient.

Different schools of thought exist regarding the method of orienting the occlusal plane based on intraoral and extra oral landmarks during complete denture fabrication. These include ala-tragus line, anterior nasal spine and hamular notch, lateral border of tongue, buccinator grooves, and commissure of lips and positioning the occlusal plane midway between the ridges. Lingual frenum has also been related to occlusal

plane. Various authors have advocated different methods for the orientation of the occlusal plane. Most important and accurate of these is pre-extraction record, eg: profile photographs, lead wire, acrylic face mask, dentulous casts etc

INSTRUMENTS TO EVALUATE OCCLUSAL PLANE:

1. Occlusal Plane Analyzer: This instrument was custom made to check for parallelism of ala-tragus line to the occlusal plane and occlusal plane to inter-pupillary line.

2. Digital Vernier Callipers: Digital Vernier calipers, with an accuracy of .001mm, were used

to check the distance between the two arrowheads on two parallel plates.

3. Buccinator Groove Relator: It was custom made to compare the level of the buccinator

groove (linea alba buccalis) with the occlusal plane.

4. Level Analyzer: An u–shaped plate was made to check the level of buccinator groove

with the occlusal plane on the impression taken. While fabricating, care was taken to

make sure both the prongs of the U were at the same level and parallel to each other.

5. Metallic Scale: A thin 6" stainless steel scale was used to relate the occlusal plane to the

retromolar pad

2. METHODS TO DETERMINE OCCLUSAL PLANE :

DIAGNOSTIC TRIAD FOR OCCLUSAL PLANE ANALYSIS

I. Clinical examination & evaluation

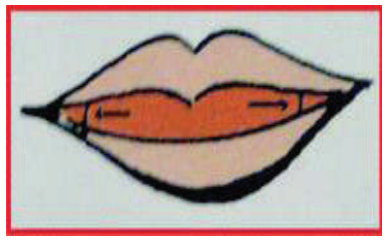
II. Cephalometric analysis

III. Mounted diagnostic casts

I. CLINICAL EXAMINATION & EVALUATION :

1) PROTHERO'S METHOD:

Occlusal plane position should be 1-3 mm below the resting upper lip line anteriorly while it should be parallel to the ala-tragus line posteriorly.



2) RETROMOLAR PAD:

The occlusal plane, as determined by a line from the mandibular cuspid tip to the distolingual cusp tip of the distal mandibular molar, terminated in the area of the lower half of the retromolar pad in 75% of the subjects.

In the other 25%, the plane terminated in the area of the upper half of the retromolar pad.

Most frequently, Mandibular occlusal plane coincides with lower 1/3 of retromolar pad. It is always inferior to upper 1/3 of retromolar pad



3) RELATIONSHIP OF THE OCCLUSAL PLANE TO THE PAROTID PAPILLA, COMMISSURE OF LIPS AND THE BUCCINATOR GROOVE:

The average superior distance of the parotid papilla from the maxillary molar cusp tip was 4.2 mm.

· Vertically, the embrasures formed due to commissure of lips were within a range of 1 to 3 mm

· The mean distance of the parotid papilla was 2.56 mm above the maxillary occlusal plane.

· The commissure of lip was inferior to the mandibular occlusal plane by 1.37 mm.

· The buccinator was 0.94 mm below the mandibular occlusal plane.

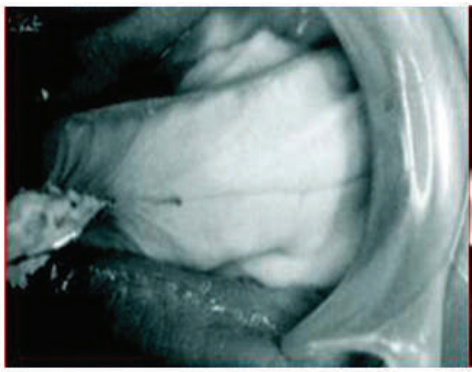


Fig. 3 Vestibular impression showing marking of commissure of lip and buccinator groove

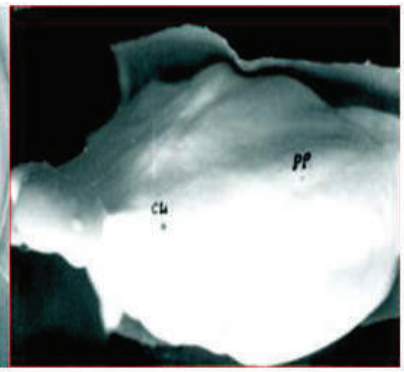


Fig. 4: Vestibular impression showing commissure of lip (CL), buccinator groove and parotid papilla (PP) placed on casts in centric occlusion.

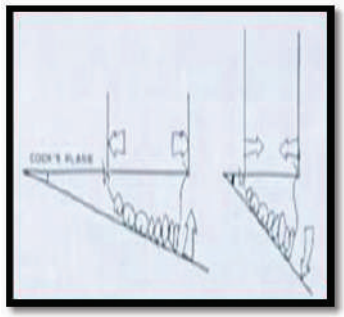
4) COOK'S PLANE:

Sloane and Cook showed that the plane of occlusion is related to the length of a line connecting the anterior nasal spine (ANS) to the hamular notch on dry skulls.

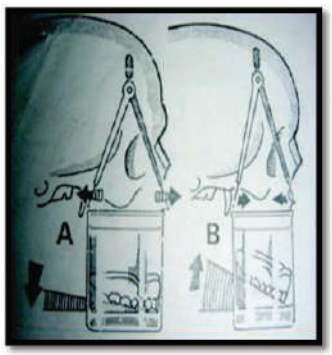
· This line, called Cook's plane forms an angle with the occlusal plane that varies inversely with the distance separating the two reference points.

· Thus, the greater the distance between ANS and the hamular notch, the more acute the angulations of the occlusal plane, and conversely, the smaller the distance, the more obtuse the angle will be.

This tendency has been confirmed by the cephalometric studies of L'Estrange and Vig and represents a phenomenon that may be explained by the "denture glass effect".



Cook's Plane



Denture glass effect

6) CAMPER'S PLANE:

It is a plane passing from the acanthion to the center of each bony external auditory meatus.

Plane of occlusion should be parallel to the Ala-Tragus line (Camper's plane) .



II. CEPHALOMETRICS:

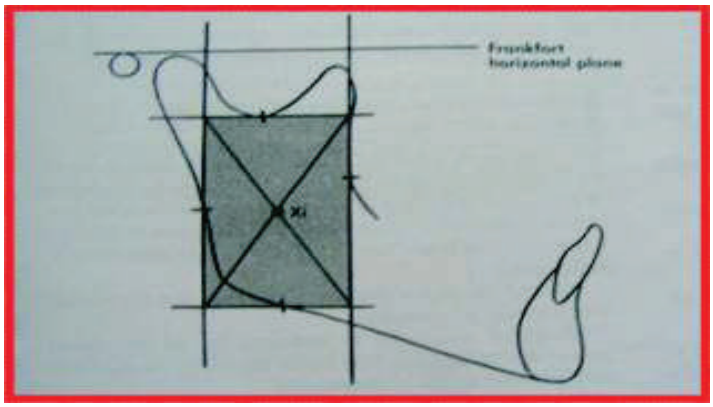
· The functional occlusal plane is a plane that relates to the occlusal surfaces of the molars and premolars.

· The purpose of analyzing the occlusal plane cephalometrically is to determine its correct vertical position in both the anterior and posterior segments.

· An esthetically pleasing occlusal plane is close to the centre of the ramus (Xi point) at the posterior and slightly below the lip embrassure at the anterior.

· The lower incisal edges are normally slightly above the level of the functional occlusal plane.

· The posterior level of the occlusal plane should approach the level of Xi point.

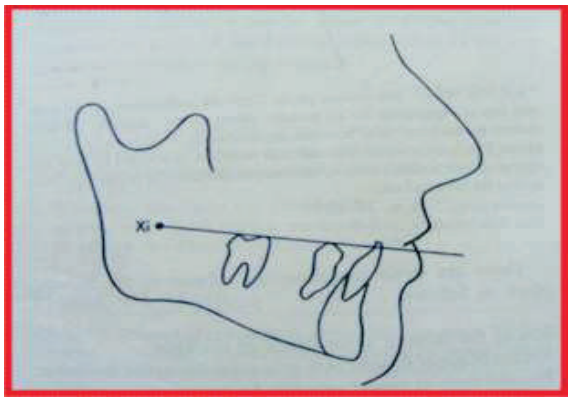


Xi point. A rectangle is formed with the top and bottom parallel

to the Frankfort horizontal plane and the sides perpendicular. The

Xi point is located at the intersection of diagonals and represents

the geometric centre of the ramus



The functional occlusal plane relates to the occlusal surfaces of molars and premolars. It does not bisect the incisors. It is close to the Xi point in the back and aligns slightly below the lip embrasure in the front.

III. MOUNTED DIAGNOSTIC CASTS :

i) Broadrick's Occlusal plane analyzer (BOPA) and

ii) Simplified Occlusal plane analyzer (SOPA)

The use of Broadrick flag or SOPA is not applicable for non-restorative cases; however, so occlusal plane analysis does have real value for orthodontic and surgical analysis if it is used with clinical evaluation.

1. BROADRICK'S OCCLUSAL PLANE ANALYZER

This instrument was devised by Dr. Lawson K. Broadrick. It is used for analyzing the curve of Spee & developing an acceptable curve of occlusion.

PROCEDURE:

· The maxillary cast is first mounted to the articulator by a face bow transfer and the mandibular cast is then mounted in centric relation.

· The articulator is then adjusted in compliance with the articulator technique. Then the maxillary cast is removed from the articulator during the survey.

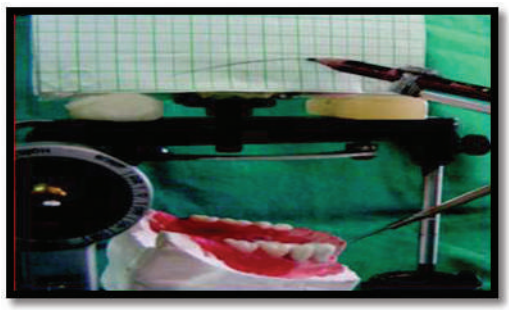
· A stud of card index is inserted into the hole in the upper member of articulator. Small arcs of 3 ¾", 4" and 5" radii can be drawn over the functional occlusal surfaces on the lower posterior teeth.

· An average of 4" radius may be used in majority of surveyed cases. Variation is only necessary when a pronounced curve of Spee may require a 3 ¾" radius or a flat curve of Spee requiring a selection of up to a 5" radius.

· Now, the Bow Compass is adjusted to the radius selected (most commonly 4"). The centre of the Bow Compass which is set at 4" radius is positioned on the anterior survey point (A.S.P.) which is usually the disto-incisal of the cuspid.

· If cuspid is worn flat, the A.S.P. may be at the incisal edge. With the centre point of Bow Compass positioned on the A.S.P., a long arc (about 3") on the plastic record card is drawn.

· The occlusal plane survey centre will ultimately be located on some point of this arc. (Figure 11).



Anterior survey point

· The posterior survey point (PSP) is located on distobuccal cusp of the last mandibular molar.

· The centre of Bow Compass is positioned on the P.S.P. and an arc is applied to intersect the arc from A.S.P.

· The centre point of the bow compass is placed, adjusted to 4" radius, on the intersection of arcs on the plastic record card (Figure 12).

· Alternate to the molar P.S.P. is a position on the condylar element of articulator at the anterior intersection with the condylar shaft.

· The center point of bow compass is positioned on this condylar posterior survey point (C.P.S.P.) and an arc is applied to intersect the arc formed from the A.S.P.



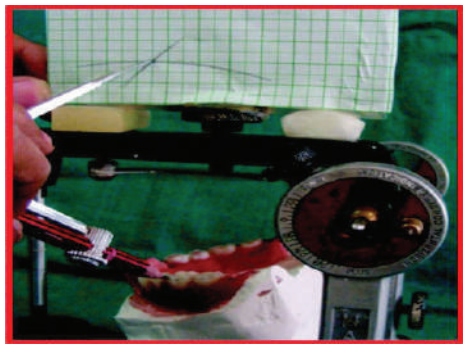
Condylar element used as the Posterior survey point

· The needle point is swept over the occlusal surfaces of the lower posterior teeth to see how the arc conforms to the existing occlusal plane.

· This occlusal plane survey centre (O.P.S.C.) can be shifted on the A.S.P. line, until the most acceptable line and plane of occlusion is found.

· By trial and retrial, the ideal survey centre forming the most acceptable line and plane of occlusion will be located.

· The centre point of the Bow Compass is now pierced into this ideal O.P.S.C. Point of compass is placed at the survey center of the flag. 4-inch radius is drawn through the buccal surfaces of mandibular teeth .



Acceptable plane of occlusion

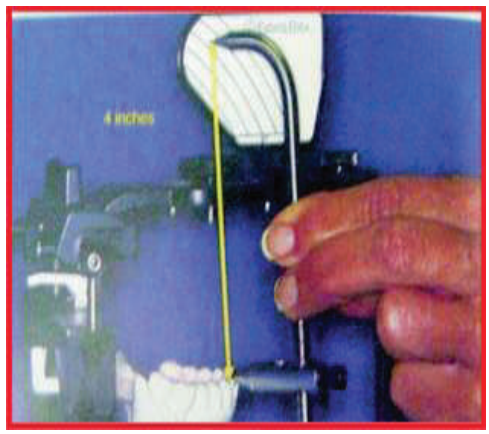
2) SIMPLIFIED OCCLUSAL PLANE ANALYZER (S.O.P.A.):

· This simplified method reduces the time required for occlusal plane analysis because the analysis point for surveying the occlusal plane is already related to the condylar axis.

· A SOPA is preset at 4" from the condylar axis and it works with Denar articulators.

· The pencil point is simply positioned at the desired height for the lower canine and the point of the compass is placed on the center line of the SOPA.

· Compass pencil is then arced back to show the occlusal plane that would correctly relate to the condyles. By setting the caliper scribe at 4 inches & aligning the marking point at the tip of canine, an occlusal plane can be scribed on the lower cast that will go through the condylar axis in one simple step.



Occlusal plane scribed on the lower cast that will go through the condylar axis in one simple step

Discussion

The ultimate goal of any type of prosthodontic rehabilitation is to restore the missing structures in a way that they were present. To achieve this goal majority prosthodontic concepts have been derived from the observation made in healthy subjects. The concepts derived in this way will make successful rehabilitation possible in majority of the patients. The concept for occlusal plane orientation is one such concept. In literature several authors have proposed their method for establishing occlusal plane, for both partially and completely edentate patients.

In case of complete denture patients the methods proposed are:

1. Parallel to ala-tragus line

2. Locating occlusal plane parallel to and midway between the residual ridges.

3. Positioning occlusal plane at the level of lateral

border of the tongue

4. Locating occlusal plane posteriorly at the middle or upper third of the retromolar pad

5. Orienting occlusal plane with the buccinators grove and commissures of the lips.

6. Establishing occlusal plane in relation to parotid papilla

7. Use of certain cephalometric criteria for establishing occlusal plane

During complete denture construction the occlusal plane should be established as close to the natural dentition as possible. This statement is logical since the musculature of the tongue and cheeks was trained to function normally in that environment and will again function appropriately when they are called upon to stabilize the bolus at the same vertical position of the occlusal table as formerly existed. For partially edentulous patient, the Broadrick occlusal plane analyzer method for occlusal plane orientation is widely accepted. The plane obtained by this method will have curvature as observed in sagittal plane, known as curve of Spee. For complete denture cases, still the opinion of the authors differs. In complete denture cases, as per the established occlusal plane, teeth setting will be done; following principles of the teeth setting. At this time the teeth set in trial denture will show curvature in sagittal plane. The curve, anteroposterior compensatory curve, established in this way will help to achieve balanced occlusion for better stability of the dentures. If the compensatory curve also has geometry similar to the curve of Spee of natural dentition, than the same method used for natural dentition may have potential to be used in complete denture fabrication also[3].

Summary & Conclusion

· The line joining from ala to the lower border of the tragus was parallel to the occlusal plane in 53.3% of the subjects. In 26% occlusal plane was parallel to the ala to middle border of tragus and in 20.7% occlusal plane was parallel to the ala to upper border of tragus.

· There was no influence of the sex on the level of occlusal plane i.e. both males and females showed the occlusal plane parallel to the line joining the ala to the lower border of tragus.

· In subjects with small tragus either middle or lower border of the tragus may be used to determine the level of occlusal plane

· Alatragal line passing through lower part of the ala of the nose to inferior position of the tragus was relatively parallel to maxillary residual ridge.

· Alatragal line passing through lower part of the ala of the nose to middle position of the tragus was relatively parallel to mandibular residual ridge.

· In the rest position Maxillary and mandibular residual ridges were not parallel to each other. They formed an angle with a mean difference of 4.20 degrees.

· The HIP plane is parallel to the natural occlusal plane[4].

· The HIP plane can be used as a reference plane to re-establish the occlusal plane in edentulous patients.

· In most of the individuals, the occlusal plane correlated with the lower 1/3rd of the retromolar pad.

· No significant difference was found between males and females regarding the association of occlusal plane with level of retromolar pad.

· Retromolar pad can serve as a stable intraoral landmark to reestablish the lost occlusal plane orientation in edentulous subjects.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Nano Modification of Titanium Dental Implants- A Meta-Analysis

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Abstract

This meta-analysis presents the comparison of nanostructured/nanoparticles as Ti-based implant materials to control and improve the implant success rate with a focus on enhanced osseointegration.

Materials and Methods: A literature review of articles was conducted by using MEDLINE PubMed database and google scholar restricted to 2000 – 2019.

Results: Source: PubMed and Google scholar

Independent screenings of the abstracts were done to identify the articles related to the question in focus 97 studies were selected out of which 10 articles were further excluded.Finally 11 articles were included.

Conclusion: Based on the results, it was found that there is a significant difference between the uncoated and nano-coated titanium dental implants, concluded that the nano-coated titanium implant surface increases bone-implant contact thereby enhances osseointegration.

Keywords; *"Dental Implants" [Mesh terms] Titanium surface, Implants and surface modifications, Titanium surface with Nanostructure, Osteoblast proliferation, Nano-coated implants, Osseointegration.*

Introduction

An important parameter for achieving successful osseointegration is the establishment of direct contact between the surface of the implant and the surrounding bone. The implant surface roughness affects the rate of osseointegration. Classification of the surface roughness of implants can be divided into macro, micro, and nano. The implant surface modifications can be achieved by additive or subtractive methods[1].

Bone is a natural nano rough surface that consists of nano-sized collagen and HA crystals as a result, nano rough surface implant is more favorable for osteoblast growth. Surface which replicates natural bone structures provide an environment that is more suitable for osteoblast growth. Better osteoblast adhesion on the nano rough regions of the patterned titanium substrates is because of their optimal interactions with proteins, proteins are mediating osteoblast adhesion such as fibronectin, and therefore protein response on the titanium surface is responsible for optimal osteoblast behavior. Early functions of osteoblast are affected by the size of the aligned pattern. There are some optimal patterned dimensions of nano features in which osteoblast prefers to function, in other words, a threshold exists in which osteoblast does not attach and spread properly.

The bone constituents are organic matrix 90% Type 1 collagen 10% in an organic matrix which is composed of HA. Type 1 collagen synthesized by osteoblast and contains linear fibrils 30nm in length and 0.5 nm in width, HA is 25 nm thick and 20 – 80 nm long.

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The nanometer-sized roughness and also the chemistry have a key role in the interactions of surfaces with proteins and cells. The micromechanical features which influence the process of secondary integration are bone growth, turnover, and remodelling. At the nanoscale, more textured surface topography increases the surface energy which in turn increases the wettability of the surface to blood, adhesion of cells to the surface, and facilitates binding of fibrin, matrix proteins, growth, and differentiation factors[2, 3,4].

Many studies were conducted to investigate various implant surface nanostructures and their influence on cell behaviour as proliferation. This meta-analysis presents the comparison of nanostructured/nanoparticles as Ti-based implant materials to control and improve the implant success rate with a focus on enhanced osseointegration.

Aim

· To evaluate through systematic review and meta-analysis of the articles published, related to the nano surface modification of dental implant surface.

· The aim is to compare, based on the recently available evidence, the influence of various nanostructure surface modifications of titanium implants, on osteoblasts proliferation and osseointegration.

Objective

· To collect reliable journal articles reporting comparison of osseointegration of untreated and nano modified surface with additive process made over dental implant surface.

· To compare the parameters across the articles.

· To address the limitations in data.

· To compare nanostructured and nanoparticles as Ti-based implant materials to control and improve the implant success rate with a focus on enhanced osseointegration.

Material and Methods

MATERIALS

1. MEDCALC software version 19.1

2. Acer E 11 laptop

3. Rorito B max pen

4. Natraj pencil

5. Jumbo note book

6. Apple 6 Iphone

7. Jio broadband

8. Rox printers

Methodology

The PubMed library, Google scholar databases was searched for articles in English language from 2000 to 2019 using the MeSH terms the review included are laboratory research studies, in vitro studies that used cells from human or animals and in vivo studies on animals.

Electronic search was performed independently following MeSH terms. Detailed search strategies were developed for each database searched.

SEARCH TERMS

The following keywords are used;

· Titanium dental implant surface

· Implants and surface modifications

· Titanium surface with Nanostructure

· Osteoblast proliferation

· Nano-coated implants

· Osseointegration.

Inclusion Criteria

· In vitro and/or in vivo studies.

· Nanostructured implant surface + control sample.

· At least one Ti sample with nanostructure must be included in the study.

· Studies about osseointegration rate on nanostructured implant surface.

· Studies providing quantitative results.

Exclusion Criteria

· No osseointegration rate described.

· Studies describing Ablative implant surfaces.

· Nanostructure and morphology has not been described.

STUDY SELECTION AND DATA COLLECTION:

The search results were carefully refined to those articles that explicitly mention variables considered for the study. In addition bibliography of these selected articles were also scanned to obtain the maximum number of results possible The search yielded 544 potentially relevant articles in PubMed, 300 in Google search. After screening the abstracts of the articles, 97 articles were selected for full text screening from PubMed and Google scholar .After elimination of duplicate articles, a total of 87 were selected for full text screening. Out of that 11 articles were selected based on eligibility criteria.

A total of 11 articles were shortlisted as mentioned and the following parameters were retrieved, (table 1)

· Mean bone-implant contact of uncoated and coated titanium implant surface.

· Standard deviation (SD) of uncoated and coated titanium implant surface.

STUDY PERIOD - This study was carried out for a period of one year from august 2018 to august 2019.

STUDY SAMPLE -Sample size was 600 – 850 articles

STUDY DESIGN - SEARCH RESULTS FOR META ANALYSIS

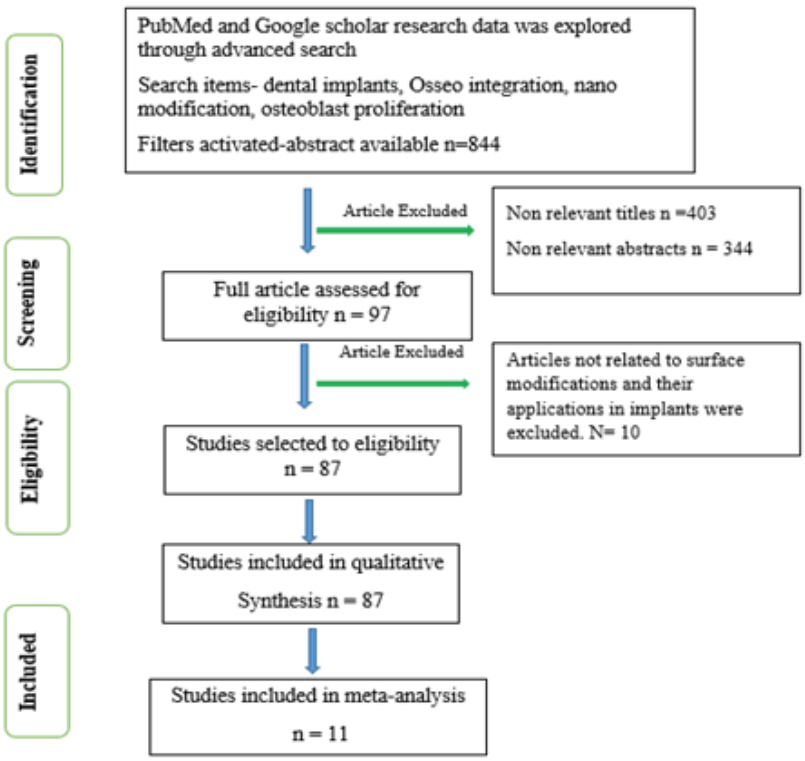


Fig .1, Flow diagram of studies selection according PRISMA guidelines

Results

Characteristics of the studies included and comparison of bone implant contact area with an observation period

Table 1

Bone Implant Contact (Time Interval Of 1month To 3 Months After Implant Placement)	Control Surface Uncoated/Micro Coated		Test Nano Surface	
Laetitia Salou Et Al N= 6	45.1± 9.1	(N=3)	65.1±20.7	(N=3)
Jack Wan Lee Et Al N=20	11.1±17.0	(N=10)	16.3±11.9	(N=10)
Ellingsen Je Et Al N=30	39±11	(N= 15)	31±6	(N= 15)
Lars M Bjursten Et Al N= 13	21.7±24.7	(N= 6)	21.7±24.7	(N=7)
Von Wilmowsky Et Al N=50	18.33±16.45	(N=25)	18.14±14.87	(N=25)
Ingmer Et Al N=34	53.2±10.8	(N=16)	56.2±6.6	(N=16)
Orisini G Et Al N=20	19±14.2	(N=10)	32.2±15	(N=10)
Yves Et Al N=24	62.46±6.37	(N=12)	75.54±5.93	(N=12)
Ballo Et Al N=40	42±27	(N= 20)	76±16	(N= 20)
Luiz Merelles Et Al N=20	Sd 14 % (0-41)	(N=10)	Sd 9 % (0 – 29)	(N=10)
Jimbo Et Al N=20	32.1%(9.9)	(N=10)	35.7% (80)	(N=10)

Meta Analysis

· Mean difference of the mean bone implant contact was calculated for uncoated and coated titanium implant surface and compared using student T test at the confidence interval of 95%.

· The data was presented as funnel plot and forest plot.

· Meta-analysis was done using 'MEDCALC software version 19.1'

META-ANALYSIS: CONTINUOUS MEASURE

Variable for studies	Study
	1. Intervention groups
Variable for number of cases	N1
Variable for mean	mean1
Variable for SD	sd1
	2. Control groups
Variable for number of cases	N
Variable for mean	Mean
Variable for SD	Sd

Study	N1	N2	Total	SMD	SE	95% CI	t	P	Weight (%)	
									Fixed	Random
laetitasalou	3	3	6	0.998	0.712	-0.980 to 2.976			2.99	6.62
jack wan	10	10	20	0.339	0.432	-0.567 to 1.246			8.16	9.22
ellingsen je	15	15	30	-0.878	0.373	-1.642 to -0.115			10.93	9.78
lars m bjursten	7	6	13	0.000	0.517	-1.139 to 1.139			5.68	8.39
von wilmowsky	25	25	50	-0.0119	0.278	-0.572 to 0.548			19.61	10.63
ingmer et al	16	16	32	0.327	0.347	-0.382 to 1.035			12.62	10.03
orisinisg et al	10	10	20	0.865	0.450	-0.0791 to 1.810			7.52	9.05
yves et al	12	12	24	2.052	0.493	1.030 to 3.075			6.25	8.62
ballo et al	20	20	40	1.502	0.352	0.788 to 2.215			12.23	9.98
luizmerelles et al	10	10	20	-0.319	0.431	-1.224 to 0.587			8.17	9.22
jimbo et al	10	10	20	1.751	0.510	0.680 to 2.822			5.84	8.46
Total (fixed effects)	138	137	275	0.454	0.123	0.211 to 0.696	3.681	<0.001	100.00	100.00
Total (random effects)	138	137	275	0.567	0.275	0.0257 to 1.108	2.062	0.040	100.00	100.00

Test for heterogeneity

Q	46.9803
DF	10
Significance level	P < 0.0001
I2 (inconsistency)	78.71%
95% CI for I2	62.42 to 87.94

TABLE 2 _

FUNNEL PLOT

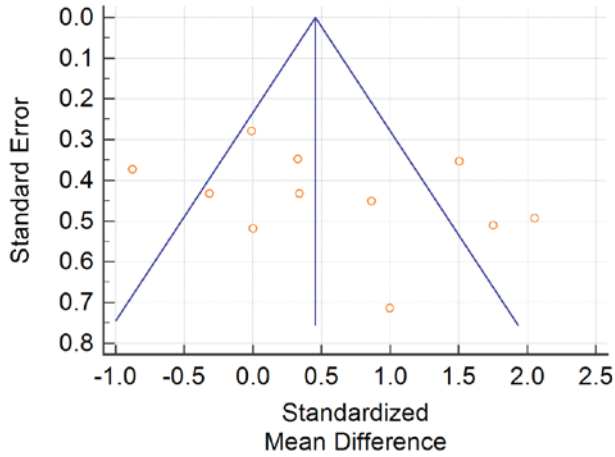


FIGURE 2, FUNNEL PLOT FOR DETECTION OF PUBLICATION BIAS

FOREST PLOT

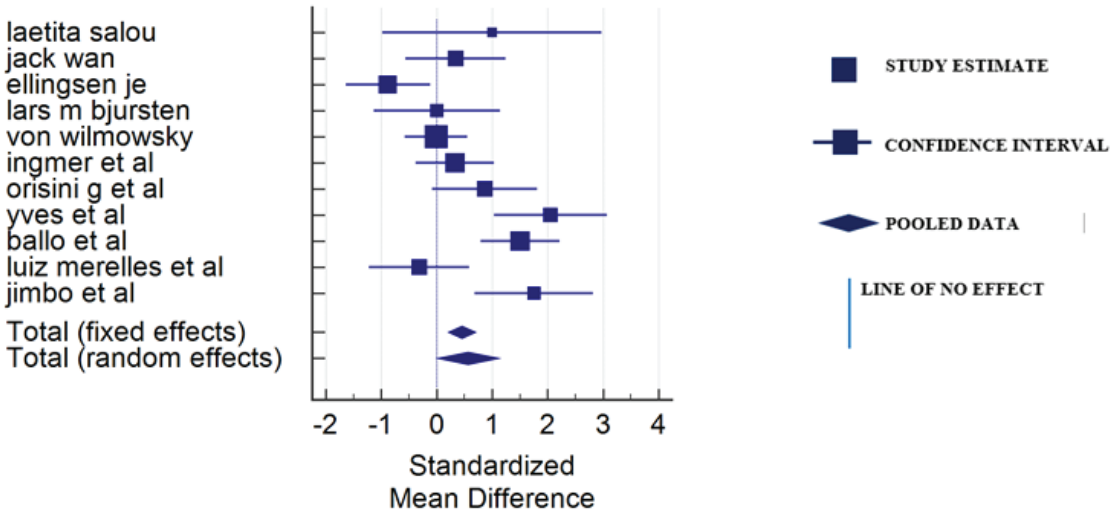


FIGURE 3, FOREST PLOT DEPICTS THE MEAN DIFFERENCE OF BONE IMPLANT CONTACT OF UN COATED AND COATED DENTAL IMPLANTS. _

META-ANALYSIS INTERPRETATION:

· Initial electronic and manual search identified 87 articles. After the subsequent search at the title, abstract and full-text reading level, 11 studies were finally selected for meta-analysis.

· The funnel plot was used to determine the possibility of publication bias of the selected studies. (Figure 2,)

· The forest plot was used to determine the pooled bone-implant contact rate of the selected studies. (Figure 3,)

· Heterogeneity of the data was analyzed to determine if the data from the selected studies can be analyzed and if the data random effects model can be used in meta-analysis. (Table 2,)

· Of the 11 studies selected, 11studies are evaluated for the bone-implant contact area of uncoated and coated bone titanium dental implant surfaces. The included studies all used similar criteria for evaluating the bone-implant contact area of titanium implants.

· The bone-implant contact was based on osteoblast proliferation, alkaline phosphatase activity, the time interval was between 1 to 4 months after implant placement.

· The systematic analysis of the selected 11 studies is shown in the funnel plot which is analyzed for publication bias.

· The forest plot showed a pooled effect of the bone-implant contact area.

FUNNEL PLOT

In this analysis, Few studies (4 out of 11) was scattered out of the funnel plot it shows that there was a possible error in the studies, which may due to poor standardization, , heterogeneity (size of effect differs according to study size) and sampling variation.

FOREST PLOT

The main outcome of meta-analysis is a forest plot, graphical display as in figure 1,

· Some confidences interval are entirely on the positive side of zero, in other words, these studies show a statistically significant positive effect.

· Some confidences interval are entirely on the negative side of zero, in other words, these studies show a statistically significant negative effect.

· Study done by Von wilmowsky et al is the only study that contributes maximum to the analysis because of its minimum (-0.567 to 0.548) of confidence interval (bigger the study, the smaller the horizontal line and bigger the black box representing the point estimate) and study estimate.

The diamond at the bottom of the forest plot shows the results when all the individual studies are combined and averaged. The horizontal points of the diamond are the limits of the 95% confidence interval and are subject to the same interpretation as any of the other individual studies on the plot.

Though 11 studies are included in the analysis, not all studies are equally contributing to the analysis.

In this analysis, diamond-shaped 'Pooled Data' is found on the left of the line of no effect (total fixed effect 95% of CI - 0.211 to 0.696) it's mainly because of most of the studies supports the comparison group rather than control group, which means 'Nano coated titanium implant surface increase the bone-implant contact' thereby it enhances the osseointegration.

TEST OF HETEROGENEITY

I2 (inconsistency) 78.71%

$P < 0.0001$

Total fixed effect – 95% of CI - 0.211 to 0.696

Discussion

The implant surface is an active research topic, because nano features could offer some advantages over untreated titanium dental implants it increase surface area, improves cell attachment, improve biomechanical interaction of implant with bone. Though many studies reveals the superiority of nano modified dental implants, we are dealing with negative reports as follows. Ross et al in 2013 reported superiority of microstructure surface over nanostructured samples.Yu et al in 2013 described a lower proliferation rate on 80 nm nano-foveolae structure compared to a smooth control sample. Controversial results were noticed about incorporation of HA within nanotubes Due to difference in opinions and results from different studies, a need was felt to analyze the non-treated/uncoated and nano-coated dental implant surface by undertaking a meta-analysis approach[5, 6].

The PubMed and Google scholar search resulted in more than 800 articles matching the search terminologies. Initial electronic and manual search identified 87 articles as per the inclusion criteria. After the subsequent search at the title abstract and full-text reading level, 11 studies were finally selected for meta-analysis. In vivo and in vitro studies that investigated the bone-implant contact of non-coated vs. nano-coated titanium dental implant surface were included.

The meta-analysis was done using MEDCALC software version 19.1. Meta-analysis was conducted for differences in mean values of non-coated and nano-coated titanium dental implant surfaces. Mean differences were included in the meta-analysis, 11 studies were finally selected for meta-analysis. Out of 11 studies 4 studies does not contribute to the analysis.

As surface characteristics modulate the outcome of cells behavior to the presence of a dental implant and subsequently the osseointegration level, the development of an implant surface that aims to attract osteoblasts that produce a bone extracellular matrix to ensure a high bone-implant contact. For this purpose, numerous surface engineering methods have been developed to create featured implant surfaces to improve the clinical performance of implants and to get a stable mechanical bone-implant interface. Also, persistent efforts have been made to improve the surface properties of dental implants to meet the increasing demands of implant treatments such as improving the success rate, expanding the applicability, and shortening the healing time required for sufficient bone-implant integration[7].

The osseointegration performance of a surface is influenced by its topography at the nanoscale, following different biological mechanisms than on the micro-scale. The nano topography is supposed to influence the surface energy and therefore the surface/protein interactions. A significant surface energy allows to improve the surface wettability to blood and the adhesion and spreading of fibrin fibers and matrix proteins on the surface, and therefore to improve cell attachment and tissue healing, particularly during the early healing phases on the implant interface[8].

A specific nanopattern surfaces promotes cell proliferation and differentiation, through the direct modulation of cell behavior[9]. Among the many forms of nanostructures (nano roughness, nano patterning, nanotubes, nanoparticles), it remains however difficult to determine which nano feature is the most efficient for the bone-implant interface and is practically usable with adequate clinical results in dental implant surfaces[10,11].

Studies demonstrate that the use of nanostructure enhance bone formation and growth because nanophase metal mimic the characteristic dimensions of bone constituents. Creating nanostructure of bone reduces infection, inflammation and promote osseointegration (Webster 2007) collagen spontaneously forms fibrils of aligned nanoscale protein helices (laton 2001). These fibers serve as a template for the nucleation and growth of hydroxyapatite crystals thereby creating a highly organized nanostructured surface that extends in a parallel orientation to the physiological loading of bone, the orientation of HA is dependent on the organization of collagen fibril helices. Several investigators have revealed that nanoscale topography influences cell adhesion and osteoblastic differentiation (Dalby et al., 2008, Webster et al., 1999). These findings reiterate observations demonstrating that nano topography may directly influence adherent cell behavior (Webster et al., 2000). Nanotechnology can alter the implant surface at an atomic level (Oh et al., 2005) and also influence the chemical composition of these surfaces.

A systematic analysis of the results gleaned from the chosen articles revealed that in fewer studies both types of implant surfaces reported having poor osseointegration. The results of the meta-analysis show that 1. Statistically, there is a difference between uncoated and coated implant surfaces in terms of bone-implant contact. 2. The rate of osseointegration of nano-coated was slightly more than that of a non-coated implant surface. Irrespective of the direction that dental implant biomaterials research takes in the future, it is clear that the most significant developments in this space will be at the cutting edge interface of material science and will be informed by innovations in substrate-surface differentiation and ability to easily "custom-create" surface properties, aided by the mainstreaming of nanotechnology.

Limitation

The main limitation is that the samples group types, the culture techniques and evaluation methods are not the same across studies and difficult to compare.

Summary and Conclusion

To analyze the osseointegration rate of non-coated and nano-coated titanium implant dental surface concerning bone-implant contact, a meta-analysis was conducted by searching the PubMed database for articles published in between 2000-2019. The articles were selected based on specific inclusion criteria to throw light on comparative features.

The search resulted in more than 800 articles of which most of them were reviews and works that did not give a clear idea about osseointegration rate was excluded from the analysis. Eleven articles were selected to achieve the objectives. The chosen parameter

were applied, data collected and the results statistically analysed and interpreted.

Although the advantages associated with the use of nanoscale titanium are supported by a large body of experimental observations, the potential risks and risk-benefit balance of nano titanium have yet to be evaluated, especially in long-term studies. Research priorities should also include detailed studies to demonstrate the superiority and real clinical advantages of nanoscale titanium as a cost-effective tool compared with traditional materials

Based on the results, it was found that there is a significant difference between the uncoated and nano-coated titanium dental implants, concluded that the nano-coated titanium implant surface increases bone-implant contact thereby enhances osseointegration.

Ethical Clearance – Institutional Ethics Committee, Sree Balaji Dental College and Hospital, Committee registered with DCGI. (Registration no: SBDCH/IEC/04/2019/21)

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Diagnosis and Treatment Modalities of Open Apex – A Review

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Abstract

Immature tooth with an open apex is encountered by a clinician from time to time. Proper diagnosis and treatment planning is necessary to save the tooth and for continued root development. This review is about diagnosis and treatment options available for cases of open apex.

Key words*: immature, open, apex, diagnosis, management*

Introduction

Open apex refers to the absence of sufficient root development to provide a conical taper to the canal and is referred to as blunderbuss canal (means that the canal is wider towards the apex than the cervical area). The completion of root development and closure of the apex occurs up to three years after eruption. Hertwig's epithelial root sheath forms the apical foramen.

Stages of root development given by Cvek[(1)]:

Stage 1 : wide divergent opening with <50% of root length formed

Stage 2 : wide divergent opening with 50% of root length formed

Stage 3 : wide divergent opening with 66% of root length formed

Stage 4 : Wide apical opening with nearly complete root

Stage 5 : closed apical foramen with complete root length

Open apices can be blunderbuss (funnel shaped) which is divergent and flaring or non-blunderbuss (cylindrical shaped) which is parallel and broad.[(2)] The causes of open apex are pulpal necrosis arising as a result of caries or trauma, extensive apical resorption as a result of orthodontics, periapical pathosis or trauma, over instrumentation (iatrogenic) and others like dens in dente and dentin dysplasia. The problems faced clinically in a tooth with open apex are thin dentinal walls that are susceptible to fracture, frequently associated with periapical lesions with/without apical resorption, short roots compromising the crown-root ratio further affecting long-term prognosis. Large open apices pose a challenge in determining the working length, decision on the necessity of root canal preparation, and achieving control during obturation.

Diagnosis

Clinical tests include careful medical examination, thorough dental history of all the symptoms and characteristics of associated pain, visual examination of presence/absence of swelling, crown discolouration, caries, mobility and periodontal probing to check the status of pulp. Electric and thermal tests are of limited value due to their varied responses in permanent teeth with immature apex.[(3)] After traumatic injuries electric and thermal pulp tests may be unreliable, only

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generalized impressions may be gained from these tests.[4] Doppler flowmetry (LDF) can be used for measurement of blood flow in traumatized teeth.[5] Pulse oximeter also offers accurate means of monitoring pulp vitality by recording the oxygenation of pulpal flow.[6] Radiographic examination and CBCT are important tools. However it is still difficult to determine if the pulp is reversibly or irreversibly affected. Moreover children may have unreliable clinical symptoms and exaggerated responses to percussion, palpation and pulp tests that do not correlate well with histopathological condition of pulp in immature teeth.[7] However with early diagnosis and intervention, pulp preservation strategies promote an environment for continued dentine apposition and root formation.

TREATMENT

The treatment modalities for an open apex are as follows; if it is reversible pulpitis (vital pulp), then the treatment has to be apexogenesis which includes indirect pulp capping, direct pulp capping or pulpotomy. If it is irreversible pulpitis (necrosed pulp) , then the treatment includes apexification or regeneration of pulp. Apexification is either by Calcium hydroxide, Mineral Trioxide Aggregate (MTA) or biodentine.

APEXOGENESIS

It can be defined as a vital pulp therapy procedure to encourage continued physiological development and formation of the root end.[8] The goals are to allow continued development of root length, maintain pulp vitality thus allowing continued deposition of dentin, promoting root end closure thus creating natural apical constriction and generating dentine bridge at the site of pulpotomy.

Indirect Pulp Capping

It is a procedure in which a material is placed on thin partition of remaining carious dentine that if removed might expose the pulp in immature permanent teeth. It is also called stepwise technique.The indications are permanent teeth, normal response to pulp tests, no symptoms of pulpitis or with diagnosis of reversible pulpitis and deep caries lesion, radio graphically with no apical pathosis.[9]

The proceedure is to first remove the caries. The remaining infected dentin is covered with Calcium Hydroxide(CAOH) and an overlying base of Intermediate Restorative Material(IRM).If the patient is asymptomatic after 3-8 weeks, then temporary filling is removed carefully and checked to confirm change in colour and hardness of affected dentin. Hard set CAOH is placed followed by an resin modified glass ionomer(RMGIC) base and a bonded composite/amalgam.The mode of action is when the infected dentine is removed, the affected dentine can rematerialize and promote odontoblasts to form reactionary dentine formation at the pulp-dentine junction.[10] Reactionary dentin is secreted by functional up regulation of surviving primary odontoblasts, stimulated by signaling molecules such as growth factor proteins and bioactive molecules released from dentine matrix during injury to the dentin-pulp complex.[11] CAOH has a long track record as an indirect pulp capping agent due to its high alkalinity and its ability to produce a dentinal barrier.

Direct Pulp Capping

It is the treatment for exposed vital pulp by sealing the pulpal wound with material such as mineral trioxide aggregate or calcium hydroxide to facilitate the formation of reparative dentine and maintain pulp vitality. The indications are restorable permanent teeth, carious or traumatic exposure with vital pulp or symptoms of pulpitis or with diagnosis of reversible pulpits.[9] The proceedure includes hemostasis with 6% Naocl following pulpal exposure.Then capping can be done using CAOH or MTA.In MTA technique, it can be done in single visit by RMGIC base followed immediately with final restoration or it can be done in two steps.First an interim restoration with moist cotton pallet is placed along with unbonded composite followed by final restoration with bonded composite/amalgam after 5-10 days.

Pulp capping materials such as MTA and calcium hydroxide induce the release of growth factor proteins and bio active molecules from dentin matrix. Postnatal stem cells in human dental pulp are capable of differentiating into odontoblast-like cells upon receiving inductive signals from bioactive molecules and growth factor proteins. Reparative dentin is formed from odontoblast-like cells and not from primary odontoblasts as in case of indirect pulp capping.[12]

Partial pulpotomy or Cvek's pulpotomy is the surgical removal of coronal portion of vital pulp as a means of preserving the vitality of the remaining coronal and radicular pulp tissues. The indications are restorable permanent teeth, deep carious lesions or traumatic fractures longer than 24 hours with vital pulp exposure, when pulpal inflammation is expected to be greater than normal and pulpal bleeding cannot be controlled within several minutes or with symptoms of pulpitis or with diagnosis of reversible pulpitis.[9] Similar to direct pulp capping, calcium hydroxide and mineral trioxide aggregate induce dentine bridge formation most likely by indirect mechanism through the release of growth factors from dentine matrix. Growth factors proteins stimulate the differentiation of post-natal stem cells in human dental pulp into odontoblasts-like cells that forms dentine bridge.[12,13] Partial pulpotomy and direct pulp capping are considered to be similar proceedures and differ only in amount of undestroyed tissue remaining after procedure.

Complete pulpotomy (cervical pulpotomy) is the removal of entire coronal pulp to the level of the root canal orifice or as much as 2-3mm apical to the orifices. The indications are restorable permanent teeth, deep carious lesions or traumatic fractures longer than 72 hours with vital pulp exposure or when pulpal bleeding cannot be controlled within 10 minutes.[14] In response to pulpal dressing with calcium hydroxide or mineral trioxide aggregate, apposition of hard tissue is achieved similarly as direct pulp capping and partial pulpotomy.

Dentine bridge formation following apexogenesis is a reparative process of dentine-pulp complex. However, the continued root formation is a normal physiological process.[15]

APEXIFICATION

It is a method to induce a calcific barrier across an open apex of an immature pulpless tooth. The indications are permanent teeth with non vital pulp with open apex and thin dentin walls or where standard instrumentation technique cannot create apical stop.[9] Materials used for apexification for formation of hard tissue barrier are Calcium hydroxide, MTA, Biodentine and Bioaggregate.

The high PH value of CAOH results in zone of liquefaction necrosis subjacent to CAOH and a deeper zone of coagulation necrosis next to periradical tissues.[16] This coagulation necrosis zone stimulates release of growth factors (wound healing signals) and bioactive molecules from cemented matrix and alveolar bone marrow matrix.[17] During this process numerous vascular inclusions may occur.[18] Drawbacks of CAOH are longer duration time of hard tissue induction i.e. 6 to 18 months , incomplete apical hard tissue barrier occurs due to vascular inclusions and may allow bacterial invasion through these defects and long term calcium hydroxide dressing may weaken the dentine and lead to root fractures.

MTA is another material used for apexification. It was introduced by Torabinejad in 1993.The setting time is 3-4 hours (2hrs 45min). The composition is powder containing tricalcium silicate, dicalcium silicate, calcium sulfate, tricalcium aluminate, tetracalcium aluminoferrite, bismuth oxide and liquid containing distilled water. When MTA is placed against periodontal tissues, the high PH value of MTA results in a very narrow zone of coagulation necrosis next to periodontal tissues. MTA modulates production of cytokines and stimulates differentiation and migration of hard tissue forming cells. Therefore, hydroxyapatite is formed on MTA surface and a biological seal is created.[19] Hydroxyapatite is formed in between the root canal and MTA.

Biodentine is commercially available from 2009. The final setting time is 45minutes. The powder contains tricalcium silicate, dicalcium silicate, calcium carbonate, zirconium oxide, iron oxide and opacifiers. Liquid is Calcium chloride in aqueous solution with admixture of polycarboxylate.Bioaggregate has setting time of 4-72hrs. The powder contains tricalcium silicate, dicalcium silicate and tantalium pentoxide.Liquid is deionised water.

Working Length For Apexification

The definition of open apex varies according to authors and is reflected by the minimum ISO size used to describe it.Some of them are ISO 60 by ElAyouti et al and ISO 80 by Moore et al.[20,21] Different techniques for WL determination are electronic apex locator (EAL) followed by radiographs, paper point technique(tactile), tactile method with a file and paper point technique to supplement initial EAL readings.[20,22,23]

REVITALIZATION/REGENERATION TREATMENT

This procedure is to revive tissues in the pulp space and continue root formation in immature teeth with non-vital pulp. The goal is to provide appropriate environment for regeneration of pulp i.e. absence of bacteria and necrotic pulp tissue, presence of a scaffold and a tight coronal seal.The indications are that tooth must be non-vital and not be suitable for apex genesis, apexification, partial pulpotomy, or root canal obscuration treatments. It should be permanent and immature tooth with open apex that is wide to a diameter of 1.1mm or larger. The patient must be aged 7-16 years and in good health.

The recommendations are to use antibiotic paste as a disinfectant (to be warned about potential discolouration), an anesthetic without a vasoconstrictor should be used when attempting to induce bleeding into the root canal, a thin layer of white MTA or calcium hydroxide should be placed over the blood clot. An endodontic sealer is not compatible. The tooth should be restored with resin modified glass monomer to help prevent micro leakage.

Tooth is anesthetised and isolated. It is irrigated with 2.5% Sodium hypochlorite (without mechanical instrumentation).The canal is dried and triple antibiotic paste(TAP) also called Hoshino's paste is placed and closed dressing is given.After one month the TAP is removed with 2.5% sodium hypochlorite and 17% EDTA. Conditioning the dentin surface with EDTA enhances the adherence and differentiation of dental pulp stem cells during pulp regeneration.[24] A file is passed beyond the working length and bleeding is induced (protein scaffold). It is left for 15minutes for the blood to clot.MTA is placed over the clot and then moist cotton placed. Temporary restoration is given. The patient is recalled after 24 hours.Then the cotton is removed and bonded restoration is given. Calcium hydroxide paste is another intracanal medicament that has been used to disinfect the canal before inducing bleeding. It may not be suitable if there is remaining vital pulp tissue in the canal. On direct contact of CAOH paste with tissue, their is formation of calcified tissue which may occlude the pulp space. CAOH may damage hertwig's epithelial root sheath and thereby destroy its ability to induce the nearby undifferentiated cells into odontoblasts.[25] Drawbacks of regeneration are discolouration, long treatment period, poor root developement, insufficient bleeding and root canal calcification.

Conclusion

All the treatment options should be properly weighed when a clinician encounters an open apex case. The patient should be recalled for followups. Formation of the complete root should be observed and monitered.

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A Recurrent Case of Oral Squamous Cell Carcinoma

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Abstract

More than 90% of all oral cancer is Oral Squamous cell carcinoma. Exposure to environmental factors such as tobacco, betel quid and areca nut in combination with genetic predisposition leads to gene mutations and carcinogenesis. They often manifest as ulcerated exophytic masses or tumor in the oral cavity. A combination of surgical procedure, radiotherapy and chemotherapy is implemented in the management of Oral Squamous cell carcinoma. However it is associated with high rate of recurrence which may be more aggressive and invasive in nature. This article discusses a case of recurrent oral Squamous cell carcinoma, its manifestation and therapeutic approach.

***Keywords**: Oral Squamous cell carcinoma, recurrence, gene mutations, carcinogenesis.*

Introduction

"A mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells even after cessation of stimulus for growth which caused it" is termed as Neoplasm. Such conditions when affecting the oral cavity, salivary glands, pharyngeal region fall under the category Oral Cancer. In India, more than 90% of all oral cancer is Oral Squamous cell carcinoma[1].

The international agency for research on cancer predicted that India's incidence of cancer will increase from 1 million in 2012 to more than 1.7 million in 2035. The lifetime risk for mortality from cancer in India for both males and females is 61%. Every one hour about 5 people die every day in India because of oral cancer and the same number of people dies from cancer in oropharynx and hypo pharynx [2, 3].

Tobacco use in both smoke or smokeless form and alcohol are the leading risk factors that play a role in etiopathogenesis of oral cancer. Every year 1.3 million deaths are attributable to tobacco use in South-East Asia region [4]. Betel quid and areca nut chewing are also other contributing factors. Gene mutation and impaired ability to repair such DNA damage gives rise to oral cancer [5]. Further, patients with the following conditions are highly susceptible to developing oral Squamous cell carcinoma:

- HIV affected individuals
- Other viral infections: Human Papilloma Virus [5], Epstein Barr virus, Hepatitis C virus
- Precancerous disorders like leukoplakia, erythroplakia, oral submucous fibrosis etc.
- Individuals with genetic predisposition of cancer.
- Ultraviolet or ionizing radiation exposure

Exposure to environmental factors such as tobacco, betel quid and areca nut in combination with genetic predisposition leads to multiple molecular events and genetic damage [1, 6]. Such chronic exposure leads to DNA damage and genetic mutations. Excessive activation and amplification of oncogenes along with inhibition of tumor suppressor genes lead to autonomous cell proliferation [7].

Ulcerated exophytic masses and tumors are the clinical manifestations. Buccal mucosa is the common site of involvement in south-east Asia. Other sites of involvement are tongue, floor of the mouth, ventral surface of tongue, alveolar mucosa, gingiva and lower lip. About 80% of patients exhibit cervical lymph node

metastasis [1, 8].

The therapeutic approach for the initial stages include surgical excision along with radiotherapy and a combination of surgical procedure, radiotherapy and chemotherapy is implemented in the late stages of oral Squamous cell carcinoma. The overall survival rate decreases as the carcinoma stage increases, from 75 to 90% for Stage I to 10–22% for Stage IV. The present treatments improve the quality of life of oral cancer patients but the overall survival rate of 5 years has not improved in the past decades [8, 9].

Oral Squamous cell carcinoma is associated with high rate of recurrence which may be more aggressive and invasive in nature. The primary site of tumor, thickness, cell differentiation, nodal involvement and surgical margins dictate the prognosis of tumor. About 1/3rd of the patients experience with recurrence which is highly unpredictable.

Cervical lymph node metastasis, extra-capsular spread and histological status of surgical site margin are the predictive factors for recurrence. In spite of advanced therapy such patients exhibit very low survival rate [10].

Case Presentation

A 38 year old male patient reported to the Department of Oral Medicine and Radiology with a complaint of ulcer like lesion in the lower left jaw and neck region along with watery pus discharge for the past one month. Patient gave no history of diabetes, hypertension or any other medical disorder. On recording detailed history patient revealed a small ulcer that developed on the left buccal mucosa before two years and biopsy was performed for the same. Histopathological report revealed a well differentiated Oral Squamous cell carcinoma and combination of therapy was provided to the patient. Radiotherapy was initiated followed by surgical excision of the lesion and skin graft was placed in the left buccal mucosa region. Left modified radical neck dissection was performed and pathological staging was pT1/N3b/Mx (Stage IV B). Patient had received chemotherapy and was under follow up. However patient started to develop a ulcerated lesion in the chin and left side of neck region along with pus discharge for the past one month. The pain was sharp and continuous in nature. Patient had habit of cigarette smoking (8 per day) for eight years and had quit for the past two years.

Extra-oral examination revealed a solitary ulcerative lesion on the left lower border of mandible, roughly oval in shape and approximately 2 × 3 cm in size with an erythematous, indurated base and everted margins. Another solitary ulcer approximately 2 × 2 cm in size was present in the mid-line neck region. Both the ulcers revealed pus discharge.

Intra-orally a graft is evident in the posterior left buccal mucosa along with hair follicles, measuring approximately 5 × 3 cm in size. The graft extends from the upper to lower gingivobuccal sulcus and well into the retromolar region.



FIGURE 2: Malignant Ulcer in left lower border of mandible



FIGURE 3: Thigh flap placed if left posterior buccal mucosa.



Discussion

Cause of OSCC in this patient could be related to his tobacco history (Cigarette smoking) along with possible genetic factors. The patient had reported for treatment in the late stages of oral cancer (Stage IV B). Patient was subjected to combined treatment approach: chemotherapy, radiotherapy and left modified radical neck dissection. The reason behind recurrence in this case was cervical node metastasis, perineural invasion and extensive infiltration of tumor cells. Current management strategies involve surgery combined with radiotherapy or adjuvant concomitant chemotherapy. Taxanes, anthracyclines, platinums and antimetabolites are the common chemotherapy drugs used for advanced oral cancer. Patients with recurrence are usually evaluated for surgery or re-irradiation and when they are not potential candidates for the procedure, chemotherapy is the treatment of choice. Recent combination of drugs though administered does not significantly improve the disease prognosis. Mechanism of drug resistance at the tumor microenvironment and cellular level is major limitation in chemotherapy. Recurrent cases often develop cross-resistance to a wide range of unrelated drugs. Further research in biological and molecular mechanisms of carcinogenesis will help in development of specific targeted therapies [8].

Conclusion

Patients diagnosed with Oral Squamous cell carcinoma at the late stages undergo significant morbidity and mortality. Surgical excision of the lesion, chemotherapy and radiotherapy are the conventional therapeutic approach for Oral Squamous cell carcinoma. However surgical dissection leads to significant disfigurement of face and reduces quality of life. Induction chemotherapy and adjuvant concomitant chemotherapy helps improve the survival rate. Patient awareness, early diagnosis of the disease, targeted therapies will help improve the disease prognosis and survival rate.

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Oral and Perioral Piercing and Its Implications on Hard and Soft Tissues

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Abstract

Oral and perioral piercing is a ritual that involves the insertion of jewellery into the tongue, lip, cheek, frenum, uvula or other parts of the mouth. This habit has been practiced for religious, tribal, cultural, sexual or identity reasons. These factitious injuries often injure the oral soft tissues and hard tissues. They pose diagnostic and management problems for the dentist.

Keywords: *Oral and perioral, Tattoos, Trauma.*

Introduction

The piercing of lips (Fig 1), tongue (Fig 2), cheek (Fig 3), uvula (Fig 4), is a traditional practice in Hindu, Chinese, American, Indian cultures. In Southern India, a vow of silence was accompanied by tongue piercing. It is seen that self-inflicted oral injuries are not only limited to the soft tissues but may result in destruction of bone and tooth structure as well. Ancient Mayans considered piercing as a symbol of spirituality, virility and courage. Eskimos inserted a "labret" into the lower lip (Fig 1), as a symbol of passage to adulthood in boys and as an act of purification in girls. In many developed countries, piercing became fashionable with the punk movement and then as part of a wider "body art", i.e., the voluntary modification of physical appearance by tattoos, branding, scars or piercing.[1-2]

Materials Used

Materials commonly used for piercing are hypoallergenic and non-toxic materials e.g., 14 or 18K gold, titanium, stainless steel, niobium, tygon, acrylic, stone, wood, bone, or ivory. There are four types of piercing jewellery which are applied in the oral/perioral area. One type is the labret, a bar with ball, disc or point at one end and flat closing disc at the other. Another type is the barbell, a straight or curved bar with balls at each end. A third type is an unclosed ring with a ball at one or both ends.[2]

Complications of Piercing

1. The tongue is highly vascularized and innervated. Any oral piercing may cause major hemorrhage or paresthesia.[2]

2. Transmission of disease, such as hepatitis B and C, HIV, herpes simplex virus, Epstein-Barr virus, Candida albicans, tetanus, syphilis or tuberculosis may occur following the use of unsterile materials.[2-5]

3. Most frequent complication of piercing is painful ulceration, followed by inflammation. The onset of local inflammation occurs at 6-8 h after the piercing, reaching a peak on day 3 or 4 Tongue piercings (Fig 2) appear especially prone to infection, by Staphylococcus aureus, Streptococcus, Pseudomonas aeruginosa, Erysipelas and β-haemolytic Streptococcus, among other microorganisms. The proximity of lymph-node chains to this initially local focus of infection may favour its spread, leading to airway obstruction or even damage to vital organs, e.g., endocarditis. The spread of these microorganisms to the bloodstream can cause bacteraemia. In isolated cases, sepsis can lead to septic shock, a life-threatening disease. Ludwig's angina can also be induced by the presence of anaerobic microorganisms related to an oral piercing.[2-3-5]

4. Anaphylactic reactions can be produced by some of the materials inserted, e.g. Nickel, and the metal that causes most contact allergies. The most commonly

reported allergic reaction of piercing is contact dermatitis produced by nickel, chromium or nickel-cobalt. .[2, 3]

5. A greater amount of plaque can be deposited at the site of oral/perioral piercings due to the difficulty of maintaining hygiene and the retention of food which creates an ideal environment for a large scale accumulation of plaque and calculus .These accumulations can produce halitosis and possible infection. Thus, oral piercings have been associated with gingivitis, which requires the presence of bacteria in plaque for its onset, although factors related to microorganisms and host predisposition also affect this pathogenesis[2-3]. (Fig 5)

6. Piercings of the oral/perioral cavity can be the reason for various lesions, including oedema and reactive lesions, such as mucoceles, inflammatory fibrous hyperplasia or mucous retention cysts, Hypertrophic or cheloid scarring may also occur .[2,3]

7. Traumatic ulcer can result due to friction between oral / perioral jewellery and soft tissue and with the passage of time, tongue piercings can produce traumatic fibroma, a localized tumor that has the same colour as that of the mucosa with a soft consistency. Fibrous repair with re-epithelization of the perforated area can also happen.[4-6]

8. In oral / perioral piercings, constant trauma from the jewellery may produce localized horizontal bone loss.[4]

9. Oral and perioral piercings produce radiopaque areas which will affect the quality of x-rays.[6]

10. The presence of piercing jewellery in the mouth, especially on the tongue, commonly hampers chewing, phonation and speaking and distorts the pronunciation of certain sounds, e.g., “s”, “sh”, “th”, “ph”, “t” or “v”.[2-6]

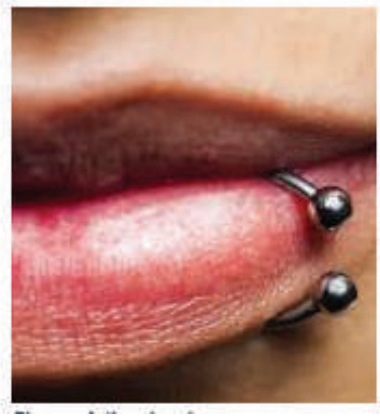


Figure 1:lip piercing



Figure 2:tongue piercing



Figure 3: cheek piercing

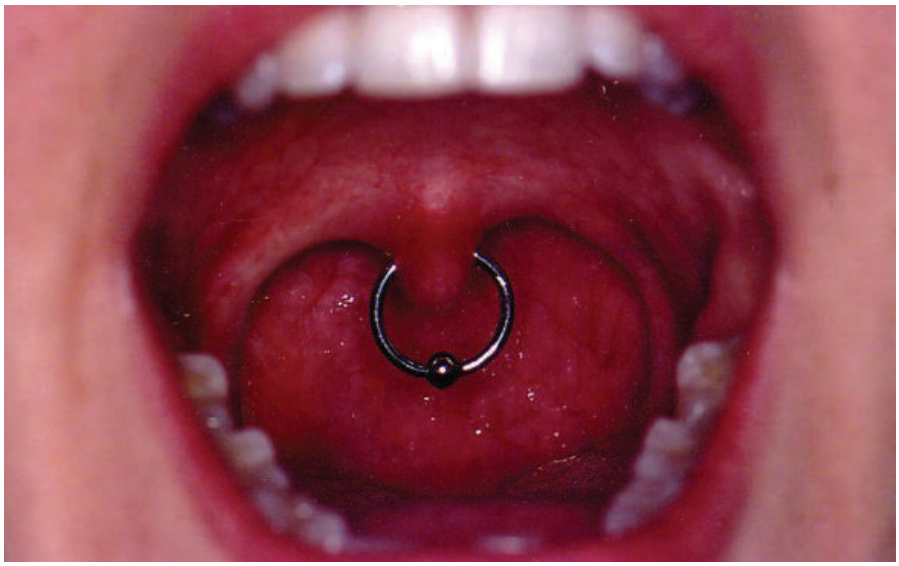


Figure 4: uvula piercing



figure 5: piercing with plaque

Conclusion

Since oral / perioral piercing damage the oral soft and hard tissues there is a need for greater awareness, education of the public by the dentists for possible adverse effects and need for a follow-up of patients with oral and perioral piercing. Individuals with oral/perioral piercing should visit their dentist regularly to ensure early detection of the various adverse effects associated with this practice.

Ethical Clearance – Not required since it is a review article

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Short Communication -Orodental Perspective of Substance Abusers

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Abstract

Substance Abuse is the habitual taking of illegal drugs by an individual or a group of people. Most commonly used these kind of drugs are Marijuana, Cocaine, Amphetamines, Heroin, Hallucinogens etc…. The oral cavity reflects, the deleterious effects of these drugs. This short communication attempt to highlight the various clinical manifestations of these drugs seen in oral cavity.

Keywords: *Marijuana, Cocaine, Amphetamines, Heroin.*

Introduction

According to the report given by All India institute of medical science and National drug dependence treatment centre for the year 2018 cannabis in most commonly used drug with the Indian population range of 2.8% of Indian population.(3 crore peoples).

Despite of various of routes like inhalation, snorting , intravenous , boofing, skin popping, oral administration stands first.

Marijuanas/ Cannabis:-The several psychoactive preparations from the plant Cannabis sativa is used to call in the generic name of Cannabis. The major psychoactive constituent in cannabis is tetrahydrocannabinol (THC). Cannabinoids are the compounds which are structurally similar to THC .[1] Theunpollinated female plants are called hashish. Cannabis oil (hashish oil) is a concentrate of cannabinoids obtained by solvent extraction of the crude plant material or of the resin. It is a psychoactive drug and it is served as an entheogen in various places.. About 147 million people, 2.5% of the world population, consume cannabis (annual prevalence) compared with 0.2% consuming cocaine and 0.2% consuming opiates[2]. In Worldwide ,United states stands first, whereas India stands tenth place in cannabis production and cultivation. Uttarakhand will be the first State in India togetthe legalization of commercial cultivation of cannabis by the state government.

Cannabis causes dry mouth, easily wear away the enamel due to the imbalance of acids causes erosion of teeth. A syndrome called "Canabinoid hyperemesis" is a condition characterised by repeated and severe bowts of vomiting.Smokes of cannabis can also acts as a carcinogen leads to various premalignant and malignant condition. Using cannabis (in any form) before a dental procedure can negatively affect the type and amount of medication or sedatives, increases the tendency to bleed, and complicate the healing mechanism.For example administering local anaesthesia with adrenaline to a person who is a long term cannabis user may precipitate cannabis related tachycardia and hypertension. Likely they also having higher risk of getting acute bronchitis , in such that conditions NSAIDS should be contraindicated[3].

Cocaine :-Cocaine is obtained from the leaves of Erythroxylon coca (E. coca), also known as the coca scrub, a plant that grows in the Andean highlands of South America. Traditionally coca leaves have been chewed by people in the Andean countries of South

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America for thousands of years. In India, Delhi is the place where cocaine marketing is high.Cocaine has a very powerful stimulating effect on the nervous system. It raises levels of dopamine, a neurotransmitter linked to pleasure, movement, and the brain's reward circuit. Cocaine dependence has become a substantial public health problem, resulting in a significant number of medical, psychological and social problems, including the spread of infectious diseases (e.g. AIDS, Hepatitis and Tuberculosis), crime, violence and neonatal drug exposure.

It is a strong stimulant and it is recreational drug. It is most commonly used in the form of cracked cocaine .It is a smoked form of cocaine but some of the users put it in the mouth it get absorbed in the gums causes mouth sores. Inhalation of cocaine alters the mucosa causing vasoconstriction and, it causes ulcerations and perforations to the mucosa of the hard palate and to the nasal septum of the palatine bone. Palatal perforation denotes the chronic use of cocaine.Cocaine may cause a movement disorder called transient chorea.Cocaine users also develop bruxism inturn giving rise to muscle spasms called"buccolingual dyskinesia"[4].



Courtesy:-P Serrano-Sánchez , JV Bagán , Y Jiménez-Soriano , G SarriónUniversity of Valencia. Serviceof Stomatology, Valencia University General Hospital. Valencia, Spain .Perforation measuring 2 cm in diameter, locatedin the hard palate .affecting the midline.

Methamphetamines :-Methamphetamine is a powerful, highly addictive stimulant that affects the central nervous system.A range of other substances also fall into this group, such as methcathinone, fenetylline, ephedrine, pseudoephedrine, methylphenidate and MDMA or 'Ecstasy' or molly – an amphetamine-type derivative with hallucinogenic properties. Methamphetamine abuse has three patterns: low intensity, binge, and high intensity. Low-intensity abuse describes a user who is not psychologically addicted to the drug but uses methamphetamine on a casual basis by swallowing or snorting it. Binge and high-intensity abusers are psychologically addicted and prefer to smoke or inject methamphetamine to achieve a faster and stronger high. In some cases, people take methamphetamine in a form of binging known as a "run," giving up food and sleep while continuing to take the drug every few hours for up to several days.Other common names for methamphetamine include blue, crystal, ice, meth, and speed[5].

They are the potent CNS stimulant, produces euphoric stage in the users, often induces craving for carbonated sugary bevarages**.**The dental signs of MA users are poor oral hygiene, gingival inflammation, xerostomia, rampant caries and excessive tooth wear. People using Methamphetamines shows a characteristic appearancecalled " Meth mouth appearance," which is severe tooth decay which often causes teeth to break and fall out. Meth causes blood vessels to shrivel and die, leads to gum disease[6].



Courtesy ;-panpan Wang, Xinmen chen, liwei, zheng, Lan Guo, Xin Li, Simin shen, state key laboratory of oral diseases, Sinchuan university , Chengdu, Sichuan, China. Frontal intra-oral view of the patient showing 24 residual roots, four residual crowns with leathery and rampant brown carious lesions extending to the gums. Dental plaque and inflamed soft tissue can also be seen.

Heroin :-Heroin provides a burst or rush of good feelings, and users feel "high" and relaxed. This may be followed by drowsiness and nausea. Heroin is an opioid drug made from morphine, a natural substance

taken from the seed pod of the various opium poppy plants grown in Southeast and Southwest Asia, Mexico, and Colombia.According to the Economic times report ,in India Afghanistan cultivating opium in large amounts. Oral manifestation characterised by rampant caries,xerostomia,periodontitis.It also causes gingival hyperplasia . Heroin usage may make it more difficult to achieve profound Local anesthesia. Users are more prone to viral,fungalinfections. Most of the patients develop discolouration of tongue[7].

Hallucinogens:

Drugs like Lysergic acid diethanalamide, Mescaline, Psilocybin are the commonly used hallucinogens, they manifest more systemically rather than oral manifestation like anxiety, paranoia, and delusions, Distressing flashbacks and they always in euphoric stage[8].

Conclusion

To summarize this short communication oral manifestations of substance abusers , varies from simple dental caries,ulcers,to the complex malignant lesions. The use of illicit drugs may require modification in the usual course of dental treatmentalso. So as a dentist we should aware about the oral manifestations of substance abusers and their required modifications during dental treatment.And also we have to counsel, motivate the patient to quit the habit by explaining them the hazardous effects of drugs and help them to overcome the addiction.

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Conflict of Interest – Nil

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Laboratory Diagnosis of Covid 19 – A Review

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Abstract

The novel 2019 coronavirus disease (COVID-19) with its early origin in China has grown into a global pandemic.The infection occurs as a mild flu to severe acute respiratory infection and spreads from person to person through droplet infection.Diagnostic testing has been and will continue to be a big part of the 2019 (COVID-19) coronavirus pandemic. Early diagnosis is the key to timely case management and control of the spread of the virus. This article provides a clear understanding of the COVID19 biosafety steps, specimen collection, storage and transportation guidelines.It also addresses steps that can be taken by cytology laboratories to work during the pandemic and reduce the risk to their employees, trainees and pathologists.

Key Words: *COVID 19, pandemic, biosafety, laboratory, samples*

Introduction

Coronavirus disease 2019 (COVID-19) is a serious acute respiratory infection caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), which began initially as a cluster of cases from Wuhan[1].

The World Health Organization declared COVID-19 as a global pandemic on march 11,2020. The disease primarily spreads via close contact of respiratory droplets generated by infected individuals[1]. At the global level, sufficient testing capacity for COVID-19 is not available as it should be and therefore preventing individuals from accessing care[1]. During the initial outbreak period, different countries have followed and implemented various testing strategies, depending on the availability of diagnostics and consumables[1]. However, strict steps taken by the WHO has made the diagnostic available with the mission to "detect, protect and treat" to break the chain of transmission of SARS-CoV-2 (WHO 2020d)[1]. Therefore, early diagnosis and prompt treatment can substantially reduce the number of prospective cases. Hence, laboratory diagnosis of SARS-CoV-2 holds the key in containing and restricting the COVID-19 pandemic[1].This article gives information on sample collection, packing and transport to laboratory for COVID-19 testing[2].

As a part of the preventive strategies, many public health care measures, especially social distancing, have been recommended to the masses[3]. Health care facilities have recommended minimizing the nonessential hospital visits and prioritizing the elective inpatient and outpatient procedures[3]. The basic aim of these recommendations is to limit the spread of the disease among the general public as well as among the Health Care Workers(HCW)[3]. If consistently followed, these measures can lead to less chance of exposure to potentially infectious samples, reduced workload on the lab facilities of a hospital, thereby allowing for convenient reallocation of the laboratory personnel in teams[3].

The World Health Organization (WHO) and the Center for Disease Control and Prevention (CDC) have recommended basic interim guidelines to be adopted for the working of laboratories during the current pandemic[3].

CLINICIAN'S RESPONSIBILITY:

· To identify the appropriate patient for COVID-19 testing[2].

· To identify the appropriate specimen to be collected[2].

LAB TECHNICIAN:

Should have received appropriate training for sample

collection from suspected SARS/COVID-19 cases

· To collect appropriate samples as decided by the treating doctor[2].

· To wear proper PPE as prescribed by NIV Pune[2].

· To strictly follow biosafety measures during collection and packing of samples and to follow the biomedical waste management guidelines for safe disposal of generated bio-waste[2].

All Institutional Biosafety Committee's (IBSCs) must assess the available facilities, trained manpower in handling high risk group (RG3 and above) hazardous microorganisms, personal protection equipment (PPE) and waste disposal mechanism to meet all the requirements prior to initiation of work involving COVID-19[4].

SELECTION OF PATIENT:

Any person who presents with Severe Acute Respiratory Illness (SARI) AND any one of the following i.e. a history of travel from Wuhan, China in 14 days prior to symptoms onset; disease in healthcare worker working in an environment of SARI patients; unusual or unexpected clinical course, especially sudden deterioration despite appropriate treatment; should be urgently investigated[5].

TYPES OF CASES:

· People who are in close contact with suspicious exposure have been advised under a 14-day health observation period that should be started from the last day of contact with infected individuals[1].

· Once these individuals show any symptoms including coughing, sneezing, shortness of breath or diarrhoea, they should require immediate medical attention[1].

· Immediate isolation of the suspected individual should be performed with proper guidelines, and they should be closely monitored for clinical symptoms and diagnosis should be performed in hospital-based laboratories as soon as possible[1].

SUSPECTED CASES:

· Patient with acute respiratory illness, with no etiology that fully explains clinical presentation and a history of travel to or residence in a country /area or territory reporting local transmission of COVID19 disease during the 14 days prior to symptom onset[1].

· A patient with any acute respiratory illness and having been in contact with a confirmed or probable COVID 19 case in the last 14 days prior to onset of symptoms[1].

· A patient with severe acute respiratory infection and requiring hospitalization and with no other etiology that fully explains the clinical representation[1].

PROBABLE CASE:

· A suspect case for whom testing for COVID19 is inconclusive[1].

· A suspect case for whom testing could not be performed for any reason[1].

CONFIRMED CASE:

A person with laboratory confirmation of COVID 19 infection, irrespective of clinical signs and symptoms[1].

SAFETY MEASURES FOR INVESTIGATOR:

Samples should be collected by well-trained healthcare personnel, donning proper personal protective equipment (PPE) which includes masks, gloves, goggles, gown, head cover, shoe cover and hand sanitizer, soap and water following adequate infection control measures including hand hygiene and adequate biosafety precautions to protect individual and the environment[1].

The correct sequence of donning the PPE:

• Home clothes, jewellery, watches, rings, bangles, etc. should be removed and hospital scrub suite should be donned[1].

• Proper hand hygiene to be done using alcohol-based hand rub or soap and water prior to donning of the PPE[1].

• Sequence of donning PPE: Shoe cover, clean and

disposable non-permeable gown, N95 respirator with proper fit testing, Eye goggles/face shield, Head cover, Gloves[1].

The correct sequence of doffing the PPE:

• Doffing should be done only in designated areas[1].

• Any soiling in the PPE must be checked before doffing. If any, the area should be disinfected before doffing[1].

• Hand hygiene must be followed after every step[1].

• Sequence of doffing the PPE: Shoe cover, Gloves, Eye goggles/face shield, Head cover, Gown ,N95 respirator[1].

All the PPE must be disinfected and discarded following the local biomedical waste management rules.

STEPS TO BE FOLLOW UP DURING SPECIMEN COLLECTION:

MATERIALS[2]:

- Viral transport medium
- Polyester tipped plastic shaft swab
- Flocked Swab
- Tongue depressor Disposable
- Face mask N95
- Disposable apron
- Disposable Nitrile Gloves
- Hand sanitizer Alcohol based rub
- Biohazard Discard bag
- Thermocol box
- Gel packs
- Head cap Disposable
- Reusable lab goggle
- Brown tape
- Sealing the sample box
- Surgical spirit
- Disinfect sample coollection area
- Cryo label

VTM Precautions:

- Ready to use VTM to be used within the expiry date. Always check for turbidity or any growth in VTM prior to use for sample collection or before dispatch of VTM to surveillance site. If any turbidity or growth is found, please discard the whole batch of VTM[2].
- Use of cotton-tipped or calcium alginate swabs or swabs with wooden shafts is NOT RECOMMENDED[2].

Steps to be followed for specimen collection using aseptic method[2]:

1. Nasal swab and/or throat swab specimens is to be collected as per GOI guidelines.

2. Sample collection area should be kept clean and table or work surface should be properly disinfected with surgical spirit.

3. Collect required details from the patient and fill the sample collection form.

4. Ensure that patient is seated in a comfortable seating position.

5. Clearly explain the sample collection procedure to ensure full cooperation from the patient.

6. Wear appropriate personal protective equipment (PPE) such as gloves, apron, N95 mask, head cap, goggles before starting the procedure.

7. Label the specimen collection vial containing VTM with the unique participant/ sample ID.

8. Specimen should be collected under good illumination.

9. Throat/ oro-pharyngeal swab collection:

a. Gently tilt the patient's head back

b. Steady the chin

c. Ask the patient to open his/her mouth

d. Use a disposable tongue depressor to hold the tongue well

e. Insert a sterile swab

f. Swab both the tonsils and the posterior pharynx vigorously with a rotating motion, till the patient starts to gag

g. Remove the swab without touching the tongue.

h. The swab is then placed in the labelled tube containing VTM

i. The applicator stick is broken off at the indicated mark (if provided) or at below the level of the tube opening

j. Close and tightly screw cap the tube.

10. Nasal swab collection[2]:

a. Take a fresh sterile swab

b. Gently tilt the patient's head Backwards and steady the chin

c. Insert the swab into the nostril parallel (1-2 cm) to the palate until the resistance is met at turbinate

d. Hold the swab in that position for few seconds and then withdraw slowly in a firmly rotating motion (5 times clockwise and 5 times anticlockwise).

11. Appropriate precautions should be taken in collecting specimens since this may expose the lab technician/ sample collector to respiratory secretions from the patient.

12. Specimens from both nostrils are obtained with the same swab vigorously, irrespective of nasal congestion if any.

13. The nasal swab is placed in the same VTM tube containing throat swab. The applicator stick is broken off as done for throat swab and the tube is screw capped tightly.

14. The vial is then placed in the cool box containing tube rack in between the frozen gel packs.

Sample handling at collection site[2]:

1. A unique specimen ID is written/ pasted on each VTM sample by the lab technician

2. VTM containing samples are to be kept in cool box immediately after collection.

3. VTM tube IDs are cross checked with the details in the filled sample collection form.

4. If the specimens cannot be sent to the laboratory within specified time frame they should be stored at or below –70°C in ultra-low freezer.

5. 5. Repeated freezing and thawing must be avoided.

SPECIMEN COLLECTION:

SPECIMEN TO BE COLLECTED[6]:

TYPE OF SAMPLE:

Upper respiratory tract:

Oropharyngeal and Nasopharyngeal swabs, or wash in out patients should be collected[6].

Lower respiratory tract:

Sputum, endotracheal aspirate or bronchoalveolar lavage in patients with respiratory disease[6].

SPECIMEN TO BE COLLECTED[6]:

TEST:	TYPES OF SPECIMEN:	DURATION:
NAAT	Lower respiratory tract - sputum - aspirate - lavage Upper respiratory tract - nasopharyngeal and - oropharyngeal swabs Consider stools, whole blood, urine, and if diseased, material from autopsy	Collect on existing case. Repeated sampling, possibly to monitor clearance. Further study is required to evaluate the efficacy and reliability of repeated sampling.
SEROLOGY	Serum	Paired samples are needed to confirm with the initial sample collected during the first week of disease and the second sample preferably collected 2-4 weeks later
NAAT	Oropharyngeal and nasopharyngeal swabs	Within the time of incubation of last reported contact
SEROLOGY	SERUM	Lowbound serum taken as soon as possible within incubation period of contact and convalescent serum taken 2-4 weeks after last documented contact

In addition to clinical specimens may also be collected as COVID-19 virus has been detected in blood and stool[7]. The active period and frequency of shedding of COVID-19 virus in stool and urine is uncertain[8].

SPECIMEN COLLECTION DETAILS[2,6]:

SPECIMEN TYPE:	**COLLECTION MATERIALS:**	**TRANSPORT TO LABORATORY**	**STORAGE TILL TESTING**	**COMMENTS:**
Oropharyngeal and nasopharyngeal swab	Dacron or polyester flocked swabs*	2-8 °C (4 °C)	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load

Cont.... **SPECIMEN COLLECTION DETAILS[2,6]:**

Bronchoalveolar lavage	sterile container*	2-8 °C (4 °C)	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container*	2-8 °C (4 °C)	≤48 hours: 4 °C >48 hours: –70 °C	Not applicable
Sputum	sterile container*	2-8 °C (4 °C)	≤48 hours: 4 °C >48 hours: –70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	2-8 °C (4 °C)	≤24 hours: 4 °C >24 hours: –70 °C	Autopsy sample collection preferably to be avoided
Serum (2 samples – acute and convalescent)	Serum separator tubes (adults: collect 3-5 ml whole blood)	2-8 °C (4 °C)	≤5 days: 4 °C >5 days: –70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weekslater

SAMPLE STORAGE (GUIDELINES):

· In the ongoing laboratory testing for COVID-19 diagnosis by molecular diagnostic methods, clinical specimens or a subset of the clinical specimens may need to be retained for various purposes such as performing additional tests, for quality control purposes or for use as control materials to assess newer diagnostic tests[7].

· In addition, a laboratory may need to store specimens for projects aimed at studying genomic epidemiology of the SARS CoV2 virus across regions and over time[7].

· All samples being stored for a long-term must be appropriately labelled indicating laboratory identifiers, date of sample collection and must be stored in properly functioning -80ºC deep freezers. A proper inventory (preferably electronic) of stored samples should be essentially maintained[7].

· With the surge in testing across the country, the total number of tested samples has gone up considerably. Keeping this in view, ICMR advises of the following[7].

a) Laboratories that are serving as validation centres for COVID-19 diagnostic kits are advised to preserve adequate numbers of positive and negative samples to prepare appropriate panels for validation etc[7].

b) At a minimum, all samples testing positive for SARS CoV2 must be retained for at least 30 days from the date of testing before being destroyed. Depending on the freezer space availability in a particular laboratory, one or more aliquots of the positive specimen may be retained for the period[7].

c) A government laboratory may decide on the number of positive / negative samples to retain in the long term based on the availability of freezer space as well as perceived research agenda of the laboratory for COVID-19 in the future[7].

d) If the number of samples tested positive at a laboratory is considerably large and the laboratory is unable to retain all positive samples beyond 30 days, a minimum of 10% of all positives detected at the laboratory in a month or 40-50 positives preferably with

equal numbers of high, moderate and low viral load should be stored for a period of 1 year at the least. A single aliquot of a positive sample may be retained taking into account freezer space availability at the laboratory[7].

e) Considering that the number of samples tested negative at each laboratory will vary depending on the sample load and testing capacity of the laboratory, a minimum of 50 samples or 1-2% of all negative tested samples over a month, whichever is smaller should be retained at the testing laboratory for a period of 1 year. A single aliquot of a negative sample may be retained taking into account freezer space availability at the laboratory[7].

PACKAGING AND TRANSPORT:

1. Samples should be transported at 2-8°C within specified time frame to the testing laboratory[2].

2. Samples should be kept in proper standing position in appropriate test tube rack[2].

3. The VTM containing part of the tube should be in direct contact with frozen gel packs[2].

When there is likely to be a delay in specimens reaching the laboratory, the use of viral transport medium is strongly recommended[7].

Specimens may be frozen to - 20°C or ideally -70°C and shipped on dry ice if further delays are expected[7].

It is important to avoid repeated freezing and thawing of specimens[7].

Transport of specimens within national borders should comply with applicable national regulations[7].

International transport of potentially COVID-19 virus containing samples should follow the UN Model Regulations, and any other applicable regulations depending on the mode of transport being used[7].

SAMPLE PROCESSING IN LAB:

Access to the laboratory should be restricted to authorized personnel only

• Types of samples that can be received in the cytology laboratories, during this pandemic, may include sputum, bronchoalveolar lavage (BAL), endotracheal tube aspirates, and other respiratory secretions[3].

• The samples should be collected in appropriately labeled, tightly-capped, sterile tubes/containers and sent to the cytopathology laboratory either in a biohazard zip-lock bags kept inside a leak-proof cryobox with a biohazard label or a triple packaging system can be used wherein the primary receptacle containing the sample is kept inside a second protective watertight and leak-proof receptacle which, in turn, is covered by a third receptacle to prevent any kind of physical damage to the secondary receptacle during transport[3].

• A trained hospital attendant should transport the sample to the laboratory, as soon as possible. Under no circumstance should the material be handed over to the relative of the patient who would be likely in close contact with the patient for transport to the laboratory[3].

• Cytotechnician should wear protective gear in the form of gown, gloves, cap, N95 mask/triple layer surgical mask, and preferably goggles Fresh, unfixed specimens should be transported by hand, and NOT shipped with pneumatic-tube systems[3].

• All fresh cytology samples received in the laboratory should be considered potentially infectious (may contain the SARS-CoV-2 virus even in samples from undiagnosed, asymptomatic patients) and hence, universal precautions need to be followed while handling such samples3.

SAMPLE DISCARDING:

All the residual samples should be discarded in appropriate disinfectants with confirmed virucidal activity against enveloped viruses[3]. These include 0.1% sodium hypochlorite solution, 0.5% hydrogen peroxide, 62–71% ethanol, quaternary ammonium compounds, or phenolic solutions[3].

MANAGEMENT OF SAMPLE SPILLS IN LAB:

The decontamination of laboratory surfaces in the event of sample spillage should be done immediately using appropriate disinfectants with proven virucidal action against enveloped viruses. The most commonly used is a 1% sodium hypochlorite solution[3].

LAB DIAGNOSIS:

LABORATORY FINDINGS:

The common abnormalities seen in COVID 19 patients are[8]:

Complete blood count : Leucopenia

· Creatinine↑, Aspartate aminotransferase/ Alanine aminotransferase /bilirubin ↑

· C-Reactive protein ↑, Lactose Dehydrogenase ↑, ferritin ↑

Chest X-rays : Acute respiratory distress syndrome[8].

Chest X-rays / imaging are frequently anomalous even in early diseases and show bilateral, peripheral and ill-defined interstitial infiltrates with opacification or lobular and subsegmental consolidation[8].

BIOMEDICAL WASTE MANAGEMENT:

Following processing of sample and following FNA performed on a COVID-19 positive/suspected patient, the full PPE is to be discarded into appropriately designated bins labeled as COVID and as per hospital policy[3].

All laboratory-generated biomedical waste from COVID-19 positive/suspected samples must be processed as per local/institutional regulations and as mandated by pollution control policy and guidelines made by the Central Pollution Control Board, India[3].

LABORATORY MEASURES:

· Re - evaluate your procedures and reduce or eliminate steps which may lead to aerosol formation or droplet creation[9].

· Review the situation weekly or biweekly, and make any required changes[9].

· Establish a chain of command, plan for emergencies and plan for contingency[9].

· Implement crowding management measures: review staffing requirements and realign the workload; consider working in shifts to reduced level[9].

· Follow the recommendations for routine handling of specimens by CDC / WHO in compliance with the guidelines[9].

· Process all specimens having measures that could lead to aerosols or droplets[9].

· Keep updated on the latest developments concerning the COVID-19 pandemic and notify the workers of any new scientific development knowledge; do away with myths[9].

· Remain aware of the current hospital policies and procedures and notify staff of any changes[9].

· Maintain open channels of communication with colleagues and staffs[9].

Don't do:

· Should not trigger needless anxieties or panic, but be honest about the risks[9].

· Don't spread or support rumors or information that doesn't come from a credible source[9].

Conclusion

Early diagnosis is the important key for management of COVID-19. Serological and molecular assays together will further strengthen the diagnosis of SARS-CoV-2. The safety of laboratory personnel is of utmost important, therefore clear understanding of the COVID19 biosafety steps, specimen collection, storage and transportation guidelines. It also addresses steps that can be taken by cytology laboratories to work during the pandemic and reduce the risk to their employees, trainees and pathologists.

Ethical Clearance – Not required since it is a review article

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Lingual Thyroid- A Review

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Abstract

Lingual thyroid as the name suggests it is an ectopic positioning of thyroid gland which is a rare developmental anomaly. Thyroid gland is usually located in the same level as that of cricoids cartilage in human body. Ectopic occurrence of thyroid gland can be seen anywhere from tongue to diaphragm though in ninety percent of reported cases it is generally seen in base of tongue. Lingual thyroid mainly seen as a result of the inability of thyroid gland to descend to its normal anatomical position during embryogenesis. Generally it takes its origin from the epithelial tissues of non-obliterated thyroglossal duct. From infancy to adulthood mostly faced problems by the patients are dysphagia, dysphonia, upper respiratory tract obstruction. So this review is entirely about the pathophysiology, clinical manifestation and diagnostic criteria along with treatment modalities for a better understanding of the dental professionals.

Key Words: *Thyroid gland, Ectopic thyroid tissue, Dysphagia, Dysphonia.*

Introduction

Dr. Hickman in 1869 first described lingual thyroid as any thyroid tissue which is not found in their usual topography. The clinical prevalence varies between 1:3000 and 1:100000. Female sex predilection is seen in this ectopic thyroid gland occurrence with an incidence of 4:1:00 for men. It is considered to be one of the main reason for hypothyroidism in children constituting 52% of dysgenesis thyroid disorder [1,2]. Lingual thyroid mainly seen as a result of the inability of thyroid gland to descend to its normal anatomical position during embryogenesis [3,4].

Pathophysiology:

In Third and seventh week in utero embryonic development of thyroid gland starts. It develops from the floor of pharynx and migrates inferiorly till it reaches the final anatomical area that is in medial and anterior neck immediately below the cricoids cartilage. Thus ectopic thyroid occurs when there is complete or incomplete failure in the descent of thyroid gland in the normal anatomical site in the neck and so it can occur at any part within blind foramen and caudal part of neck [5].

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Clinical Presentation:

Clinically lingual thyroid could be divided into two groups depending on their onset of symptoms i.e in infants and young children. In these both groups lingual thyroid is detected during routine screening procedures and can be seen suffering from respiratory distress and mental retardation often leading to medical emergency [7.8]. Symptoms like dysphagia and oropharyngeal obstruction are commonly seen during puberty. Reason for this can be thought to be the rise in demand of thyroid hormone during these stages of hyper metabolism also in certain conditions like pregnancy, infection, trauma, menopause, etc same response are detected [9]. Smooth texture of the lesion describes it clinically with increased vascularity. In normal medical examination palpation of base of the tongue along with the neck is very crucial so as to check the availability of thyroid gland in its normal anatomical position. Common investigations that are routinely done to check the function of thyroid gland is Thyroid function tests whereas special technique like

Technetium scan used as a confirmatory test in detection of lingual presence of thyroid [10].

Diagnostic Criterias:

Computed tomography and magnetic resonance imaging are complementary essential methods useful in determining the size of the gland and in surgical planning. Sagittal reconstruction will be important in the case of large volume, assessing the depth within the language and air permeability [11]. The ultrasound would differentiate cystic changes and have low cost, but still has a bad role set forth in this pathology, no studies to support its use. The FNA can also confirm the diagnosis and preoperative examination is the only that can differentiate benign from malignant lesions. However, we cannot rule out malignancy in the absence of malignancy puncturing. The definitive diagnosis often is only possible with the ectopic tissue excision [12-15].

TREATMENT: Surgery should be used as an emergency treatment modality. Most of the time first line of treatment modality should be started with suppressive therapy in adjunct with exogenous hormone in order to reduce the size of the gland present ectopically. If lingual thyroid is happened to be the only functioning thyroid gland in the patient as it is seen in 70% of the cases [10] surgical excision of the ectopic gland along with Levothyroxine therapy is recommended. If surgery is to be considered to be the only choice of treatment in certain cases then one of the different types of approaches namely trans-hyoid pathways, supra-hyoid, and cervical faringotomy can be followed. But complication including functional, esthetical and prolonged recovery period are disadvantageous consequences post surgery [6]. Surgery has high morbidity and these are generally non invasive procedures.

Conclusion

Lingual thyroid is a rare anomaly representing faulty migration of normal thyroid gland. The exact pathogenesis of this ectopic is not known. It is 7 times higher in females [16]. Dysphagia and dysphonia are common presenting symptoms [17]. Thorough and careful head and neck examination with special attention to base of tongue is essential. Investigation includes thyroid function tests, neck ultrasound scan, Technetium scanning and C.T scan. FNAC is not preferred by some authors as it would cause unnecessary bleeding [18]. Although different types of surgical access have been described, the transoral approach provides good exposure and is less traumatic for the patient, with better postoperative recovery [7].

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Nutrigenomics and its Role in Periodontal Health – A Review

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Abstract

Nutrigenomics is an emerging form of science involving tools that assess the benefit of diet and nutrition in various disease conditions at molecular and genetic level. Nutrigenomics investigates the relationship of nutrient regimen in maintaining health. This article reviews the role of nutrients in maintaining periodontal health and the use of nutrigenomics in understanding genotype, phenotype diet interactions on periodontal health of an individual and the effect of dietary modification on periodontal disease progression.

Key Words: *Nutrigenomics, nutrition, periodontal health, periodontal disease*

Introduction

Nutrigenomics is the science that utilizes genetic information together with "**omics"** technology for studying the inter-relation between nutrition and health.[1] Periodontal health is vital for the integrity of the tooth structure. Periodontal disease is the most common disease among various populations second only to dental decay. Fluoride Mapping (2002–2003), DCI, New Delhi, 2004, did a first ever epidemiological survey in India and the prevalence of periodontal disease was 57%, 67.7%, 89.6%, and 79.9% in the age groups 12, 15, 35–44, and65–74 years, respectively.[2,3] In failure of inflammatory response of the host to remove the pathogen, there is prolonged release of neutrophils, proteolytic enzymes, proinflammatory mediators and reactive oxygen species resulting in destruction of periodontal attachment.

Periodontal disease affects the supporting structures of the teeth and failure to intervene timely leads to loss of tooth. It is characterised by a dysregulated host inflammatory/immune response to plaque bacteria in susceptible individuals. A variety of risk factors have been associated of which nutrition is the one whose role would be discussed in this paper.Diets rich in saturated fats and sugars and low in fruit, vegetables and fiber are common risk factors associated with periodontal disease.

Nutrient is a source of nourishment that can be metabolized to give energy and build up tissue. Nutrients are divided into six major classes, i.e. carbohydrates, proteins, fats, minerals, vitamins, and water. Sub dividing it further, there are two broad categories, "macronutrients" (fats, carbohydrates, and proteins) which are required in large quantities from the diet and "micronutrients" (minerals, vitamins, trace elements, amino acids, and polyunsaturated fatty acids [PUFA]) which are only required in small quantities in the diet and which are essential for many biological processes that are responsible in supporting and providing optimal health.[16]

History

The term "nutrigenomics" was first described in 2001 from Pelegrin (2001). On, 14th April 2003, Genomic era was launched by launching of Human Genome Project which contained the complete sequencing of Human Genome.

The 'omics" revolution-

· In 2004, NuGo (European Nutrigenomics

Organization) was born and funded until 2014[4]

· In 2007, Nestle research centre joined the industrial platform of the Kluyver Centre for genomics of industrial fermentation, Netherland.[4]

· In 2008, US Berkley scientist predicted human genome tests within five years for $100[4]

"OMICS" Technology in Nutritional Research

It includes Genomics, Proteomics, Transcriptomics and Metabolomics. Genomics is the study of the genome, an approach of mapping, sequencing and analysis of all genes present in the genome, focusing on resolving the variation in the genome between individuals. Transcriptomics is the study of gene expression at the level of the mRNA. Using either cDNA or oligonucleotide microarray technology, it describes the approach in which gene expression (mRNA) is analysed in a biological sample under certain conditions and a given point of time.[6] Proteomics takes forward this analysis and addresses three categories of biological interest: protein expression, structure and function. It attempts to characterize all proteins in a biological sample at the functional level.[7,12] Metabolomics is the scientific study that involves quantitative analysis of metabolites. It tries to measure the level of all substances (other than DNA, RNA or protein) present in a sample; the metabolome comprises the complete set of metabolites synthesized by a biological system.[8]

Periodontal disease and role of Nutrition

Chronic inflammation is destructive and is central to a number of chronic diseases including periodontitis. Oxidative stress is a key driver of chronic inflammation and as a result has a central role in the pathogenesis of a wide range of chronic inflammatory diseases[17] such as type 2 diabetes, cardiovascular disease and metabolic syndrome, and has been proposed as a common link between periodontitis and systemic disease.[9] When the fine balance that exists between oxidants and antioxidants found in the body in healthy conditions is disturbed due to excess production of oxidants or depletion of local antioxidants, oxidative stress is the result which is associated with local tissue damage seen in periodontitis. The tissue damage is caused by altering proteins, lipids and DNA damaging the cells directly or by production of pro-inflammatory cytokines.[18]

Elevated dietary levels of glucose and lipids increases oxidative stress levels by receptor binding of neutrophils. Hyperglycaemia results in the formation of Advanced Glycation End products (AGE) when glucose binds to proteins in tissues and the bloodstream. Neutrophils have receptors for AGE called RAGE and their ligation by AGEs activates the NADPH-oxidase enzyme complex to generate oxygen radicals. Metabolism of excess saturated fats generates elevated Low Density Lipoprotein (LDL) cholesterol and on its oxidation forms oxidised LDL. This binds to the complementary receptors found on the cell membrane of neutrophils (Toll-like receptors), triggering NF-κB activation through the protein-kinase-C enzyme and other related pathways. NF-κB transcribes several pro-inflammatory cytokines.[9] In addition, researchers have proposed reduced glutathione (GSH), the key intracellular AO redox regulator of NF-κB as a novel approach to down regulation of hyper inflammatory events.[19]

Literature reviews suggest periodontitis to be associated with reduced serum micronutrient levelswhich may be due to poor diet, lifestyle factors (e.g. smoking) and/or genetic factors which impact on absorption, distribution, bioavailability and synthesis of micronutrients.[20] Studies have shown irrefutable evidence that macronutrients and micronutrients modulate pro-inflammatory and anti-inflammatory cascades. Control of dietary sugar and fat intake can help reduce levels of oxidative stress and downstream inflammatory sequelae. Reductions in simple sugars, refined carbohydrates and saturated fats reduce activation of a diverse range of pathways thereby reducing oxidative stress. Foods rich in antioxidants such as green leafy vegetables (broccoli, spinach etc.), berries (e.g. blueberries, blackberries, cranberries, strawberries etc.), red beans and dark chocolate with greater than 70% cocoa are all rich in key antioxidant micronutrients and would help reduce oxidative stress. On the other hand, diets which includes nuts, olive and fish oils having antioxidant properties can also work to slow down gastric emptying (digestion) resulting in less pronounced spikes in blood glucose.[9]

Nutrients for Periodontal Health

· Vitamin C is the most powerful anti oxidant.

Genes encoding inflammatory peptides, including interleukin 1α and interleukin 1β, was more than two fold down regulated by vitamin C intake. It was found that smokers with the lowest intake of vitamin C were likely to have the worst periodontal condition.[10]. Plasma vitamin C levels of 56.8 μmol/l may be regarded as the optimum vitamin C plasma level. In order to achieve this level, at least 200 mg vitamin C per day should be ingested and this is to be suitably advised to periodontitis patients.

· Vitamin D plays an important roleby influencing the action of monocytes, macrophages and dendritic cells which express the vitamin D receptor (VDR), which when up regulated by activated Toll -like receptors -2/1, results in the intracellular production of 1,25(OH)2 D3 which in turn induces the release of LL -37 cathelicidin, a potent anti -microbial peptide.[11]

· Low serum Calcium levels causes alveolar bone resorption and is a risk factor for periodontal disease.[1]

· Vitamin E terminates free radical chain reaction and stabilizes the membrane structure. It is shown to have mitigatory effects on inflammation and collagen breakdown. Low levels of vitamin E in gingival tissues of periodontitis patients has been reported in many studies.[21]

· Magnesium is related to calcium and a higher Mg/Ca ratio is associated with a significantly lower level of periodontitis.[1]

· Supplementation of Zinc may alter periodontal disease progression through changes in expression of the ZnT8 transporter gene.[12]

· Isabgol extract has shown effective antibiotic and weakinflammatory effect by effective action against periodontal pathogens and Matrix metalloproteins.[13]

· Omega 3 from polyunsaturated fatty acid act as a signal to prevent neutrophils mediated periodontal tissue damage.[14]

· The hydro-alcoholic extract from pomegranate has shown to decrease the Colony Forming Units (CFU'S) per millimeters of dental plaque by 84 % by one minute rinse.[15]

· The recommendations of 2011 European Workshop on Periodontology suggested that the dental team should consider including fish oils, fibre, fruits and vegetables and to reduce levels of refined sugars as part of a periodontal prevention / treatment regime and a general health benefit message.[5]

Future of Nutrigenomics

Nutrigenomics aim to reveal the relationship between nutrition and the genome to provide a scientific basis for improved periodontal health through dietary means. It will increase the understanding of genotype, phenotype diet interactions on periodontal health of an individual and the effect of dietary modification on periodontal disease progression. The concept of"Personalized/ Individualized Diet" would soon revolutionize the food industry. The identification of molecular biomarkers or natural bioactive molecules for control and prevention of periodontal diseases through timely dietary interventions seems promising.

Conclusion

A much comprehensive understanding of nutritional gene interactions and their impact on phenotype is required to identify and plan strategies for dietary intervention. A large body of data relating to nutritional genetic studies on periodontal disease is the need of the hour. Further research needs to be done to relate patient's signs and symptoms with their genetic profile and dietary habits.

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Syndromes of Head and Neck – A Quick Review

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Abstract

The aggregate of signs and symptoms associated with any morbid process and constituting together the picture of the disease and related to each other anatomically, biologically and physiologically. A group of deformation and malformation sequence etc that occurs together due to some identifiable underlying cause. The aim of this review is to describe a collective knowledge about various syndromes associated head and neck- A dentist point of view

Keywords: *Syndrome, Metabolism, Dental, Orofacial.*

Introduction

The real importance behind the learning of syndromes associated with conditions is of relevance to clinical examination of the head and neck. Knowledge of syndrome may quickly solve some difficult diagnostic problems and appropriate treatment instituted . The following list takes you through conditions met by the authors either in their clinical practice or in examinations and which could therefore be considered worth knowing and helpful in academic and clinical excellence.

In some instances, a syndrome is so closely associated with a pathogenesis and that the words yndrome, disease, and disorder end up being used interchangeably. This is especially true of inherited syndromes. For example, Down syndrome, Wolf–Hirschhorn syndrome, and Andersen syndrome are disorders with known pathogeneses, so each is more than just a set of signs and symptoms, despite the syndrome nomenclature[1,2]. In other instances, a syndrome is not specific to only one disease. For example, toxic shock syndrome can be caused by various toxins.

If an underlying genetic cause is suspected but not known, a condition may be referred to as a genetic association . By definition, an association indicates that the collection of signs and symptoms occurs in combination more frequently than would be likely by chance alone.

Syndromes are often named after the physician or group of physicians that discovered them or initially described the full clinical picture. Such eponymous syndrome names are examples of medical eponyms. Recently, there has been a shift towards naming conditions descriptively by symptoms or underlying cause rather than eponymously, but the eponymous syndrome names often persist in common usage

DIAGNOSIS BETWEEN A SYNDROME AND A DISEASE:

Representing medical knowledge is a highly complex endeavor. The improper use of the terms “syndrome”, “disease” and their relations to “diagnosis” is one of the difficulties with which medical informaticians must deal, especially when developing expert systems to support diagnoses. Although ubiquitous in medical and lay discourse, the term “disease” has no unambiguous,

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generally accepted definition. However, most of those using this term allow themselves the comfortable delusion that everyone knows what it means[3].

Diagnostic categories (diseases and most syndromes) are simply concepts. They are justified only if they provide a useful framework for organizing and explaining the complexity of clinical experience in order to derive inferences about outcome and if they guide decisions about treatment.

A **syndrome** is a recognizable complex of symptoms and physical findings which indicate a specific condition for which a direct cause is not necessarily understood. Thus in practice doctors refer to the infamous "viral syndrome" as such because of the uncertainty regarding the legion of viral agents that is causing the illness[4]. Once medical science identifies a causative agent or process with a fairly high degree of certainty, physicians may then refer to the process as a **disease**, not a syndrome. Mucocutaneous lymph node syndrome became Kawasaki syndrome which in turn metamorphosed into Kawasaki disease; the latter is properly a disease, no longer a syndrome, by virtue of its clearly identifiable diagnostic features and disease progression, and response to specific treatment.

Albert *et al*. catalogued six general views or concepts about what types of conditions may be said to constitute a disease. These views range from nominalism and cultural-relativistic theories (i.e. some conditions become a disease when a profession or a society labels it as such) to a "disease realism" view (objectively demonstrable departure from adaptive biological functioning). The latter model is the one best suited to the present state of medicine; it emphasizes that the clinical signs and symptoms do not constitute the disease and that it is not until causal mechanisms are clearly identified that we can say we have "really" discovered the disease[5,6].

Medical literature, even that from governmental organizations and institutions authorized to implement standards, is plagued with misleading assertions such as "a syndrome is a disease …", "a syndrome indicates a particular disease…" and "Lyme disease syndrome" (It is inappropriate to apply "syndrome" to Lyme disease because its causative agent is known).

Some syndromes such as "heart failure" are useful medical concepts but are not diagnoses, whereas more specific syndromes such as "congestive heart failure" or "right heart failure" are diagnoses[6]. Due to the imprecision of natural language, some syndromes could also imply a simple pathological finding (vasculitis) or just a physical finding. Frequently, for example, arthritis syndromes are simply referred to as "arthritis".

CAUSES FOR A SYNDROME:

When one or more chromosome is missing or mutated or if extra chromosome are present, then there is incorrect number of proteins made , this may cause abnormal development and growth and result in syndrome. Sometimes these abnormal genes or chromosomes are passed down from the parent and sometimes they occur spontaneously without reason[7,8].

CLASSIFICATION OF SYNDROME[9-11]:

1) Etiologic classification
2) Embryologic or histologic classification
3) Syndrome prototype
4) Polythetic classification
5) Monothetic classification
6) Mixed classification
7) Morphogenetic classification

1.ETIOLOGIC CLASSIFICATION

Syndromes can be classified according to broad etiologies suc as

i) Monogenic
ii) Chromosomal
iii) Environmental induced

Such classification usually requires supplementary category such as : Multifactorial , Unknown, and Disruptive

2.EMBRYOLOGIC / HISTOLOGIC CLASSIFICATION

Developmental disturbances of the tissue structure are basis of some classification. Thus hematoneoplastic

syndrome are sometimes classified on the basis of the germ layer involved.

EXAMPLE : 1) Multiple osteochondroma involves only one germ layer- the mesoderm

2) Gardner syndrome involves all three germ layer

3.Polythetic classification: syndromes sharing a large portion of their principal anomalies are grouped together . They are even better aid in differential diagnosis.

4.Syndrome prototype by Herrmann and Opitz 1974 and Cohen 1982:

i) Dysmetabolic syndrome (metabolism)

Normal birth, uniform clinical feature, no congenital malformation, biochemically defined, recessive mode of inheritance Example : Hurler syndrome , Tay- Sachs Disease.

ii) Deformation syndrome (region)

Changes in shape of previously normal structure, lack of movement – mechanical andfunctional, commonly affects musculoskeletal system

iii) Malformation syndrome (organ or field)

Non-contiguous malformation , Embryonic pleiotropy, Lack of biochemical definition example- Trisomy 13 syndrome, Rubenstein Tyabi syndrome

iv) Dyshistogenic syndrome (Tissue)

A) SIMPLE : Involvement of only 1 germ layer , Inheritance- dominant or recessive Example: Achondroplasia

B) HEMARTONEOPLASTIC SYNDROME : Hamartomas or neoplasias, involves more than 1 germ layer, commonly dominant. Example : Peutz Jeghers syndrome, Gardners syndrome

5.MONOTHETIC CLASSIFICATION

In this type of classification , syndromes are grouped together because they share a single feature such as for example cleft palate. Such groupings are often used as an aid in differential diagnosis, for example :

i) Syndrome with arthrogryposis

ii) Syndrome with craniosynostosis

iii) Syndrome with propensity

LIST OF SYNDROMES ACCORDING TO ORAL CAVITY SITE

SYNDROMES ASSOCIATED WITH DEFORMATION IN LIP[12]

FEATURES	SYNDROME
Double lip	Ascher's syndrome
Pebbly lesions of lip	Cowden's syndrome
Everted lip	Down's syndrome
Fusion of upper lip to maxillary gingival	Fusion of upper lip to maxillary gingival
Thick lips	Hurler syndrome
Pigmented lip	Laugier – Hunziker syndrome
Neuromas of lip	Multiple endocrine neoplasia syndrome
Cleft lip	Goltz Gorlin syndrome
Protruding lip	Reiger's syndrome
Occurrence of pits of lip	Van Der Woude's Syndrome
Whisting Lips	Whistling face syndrome
Dryness and fissuring of lips	Mucocutaneous lymph node syndrome

SYNDROMES ASSOCIATED WITH DEFORMATION IN BUCCAL MUCOSA[12]

FEATURES	SYNDROME
Lichen planus lesion on buccal mucosa	Graham little syndrome and Grinspan syndrome
Bilateral oral white lesion	Jadassohn Lewandowsky syndrome
Neuromas of Buccal Mucosa	multiple endocrine neoplasia syndrome
Oral Leukoplakia	Zinsser-Engaman – Cole syndrome

SYNDROMES ASSOCIATED WITH DEFORMATION IN TONGUE[12]

FEATURES	SYNDROME
Macroglossia	Down's syndrome / Beckwith syndrome
Microglossia	Hanhart's syndrome
Aglossia/ Hypoglossia	Hypodactilia syndrome
Fissured tongue	Melkerssons Rosenthal Syndrome
Glossitis	Chediak – Higashi Syndrome
Hypotomia Involving tongue	Floppy Infant syndrome
Multiple Hemangiomas	Maffucci's Syndrome
Atrophy of tongue	Parry- Romberg syndrome
Glossoptosis	Pierre Robin Syndrome

SYNDROMES ASSOCIATED WITH DEFORMATION IN PALATE[12]

FEATURES	SYNDROME
Hight palate vault	Apert's syndrome Crouzon syndrome Down's syndrome Marfan syndrome Teacher Collin syndrome
Cleft plate	Pierre robin syndrome Goltz gorlin syndrome
Pebbly lesion of palate	Cowden's syndrome

SYNDROMES ASSOCIATED WITH DEFORMATION IN GINGIVA[12]

FEATURES	SYNDROME
Severe gingivitis	Chediak- Higashi syndrome
Pebbly lesion of gingival	Cowden's syndrome
Gingival enlargement	Cross syndrome
Gingival Fibromatosis	Ramon Syndrome
Inflammatory gingival enlargement	Papillon- Lefevre syndrome
Congenital enlargement of gingival	Rutherfurd's syndrome
Massive growth of gingival	Sturge-Weber syndrome

SYNDROMES ASSOCIATED WITH DEFORMATION IN TEETH[12]

FEATURES	SYNDROME
Microdontia	Down's syndrome / Gardner's syndrome / Golts-Gorlin syndrome
Macrodontia	KBG syndrome
Hypodontia	Down's syndrome
Altered Eruption	Turner's syndrome
Congenital missing teeth	Curry-Hall syndrome / Bloch-Sulzberger syndrome
Absence of premolars and 3rd molars	Book's syndrome
Additional cusp	Ellis-Van Creveld syndrome
Peg or cone shaped teeth	Crouzen syndrome / Curry-Hall syndrome/ Trichondental syndrome
Supernumenary teeth	Down's syndrome/ Gardner's syndrome
Taurodontism	Trichodental syndrome
Talon's cusp	Rubinstein- Taybi Syndrome
Enamel hypoplasia	Ehlers-Danols syndrome
Development of crack during excessive occlusal force	Cracked tooth syndrome

SYNDROMES ASSOCIATED WITH SALIVARY GLAND[12]

FEATURES	SYNDROME
Decrease salivary secretion/ dry mouth	Sjogren's syndrome

SYNDROMES ASSOCIATED WITH JAW BONE [12]:

FEATURE	SYNDROME
Micrognathia	Pierr robin syndrome/ Turner's syndrome
Fibrous dysplasia	Albright's syndrome / Jaffe Lichenstein syndrome
Cortical thickening or hyperostosis	Caffe-Silverman syndrome
Mandibulofacial dysostosis	Treacher Collins Syndrome
Mandibular Hypolasia	Hutchinson Gilford syndrome
Maxillary hypoplasia	Reiger's syndrome
Cleft of Alveolar process	Orofacial Digital syndrome
Elongated styloid process	Eagles syndrome

Conclusion

There is a wide spectrum of syndromes that include dental, oral, and craniofacial abnormalities. Medical specialists like pediatricians and geneticists focus on general health issues related to diagnosis and prognosis of the condition, having a holistic view of the patient, whilethe dentist deal with (major) facial corrections where we primarily work with caries prevention, diagnosis, and treatment of structural tooth abnormalities, tooth size-shape discrepancies, deviations in tooth number and treatment of malocclusions or facial growth disturbances. In Contrary to the general and genetic diagnosis, most dental abnormalities can only be identified after the first years of life. This delays the dental and orofacial components of the syndromic diagnosis, which are, however, vital for the evaluation of prognostic factors and for the proper timing and management of oral function and, aesthetics as well as for social aspects. Therefore a clear knowledge about the syndromes helps in proper disgnosis .This makes early interventions and decisions concerning the type and timing of orthodontic treatment and maxillofacial surgery often difficult and critical, especially in patients with disrupted development in the craniofacial and dental structures.

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Effects of Yoga in Dentistry- A Review

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Abstract

Each individual plays out a few or other type of occupation or work to procure business and to remain intellectually and truly in sound wellbeing. Each occupation is related with a few or opposite symptoms alluded to as word related risk. Dentistry as a profession is an exceptionally demanding and challenging. To gain accessibility and better deceivability dental specialist achieve physical stances which are inconvenient to their wellbeing. Among the various symptoms of dentistry musculo-skeletal issues is one of the most well-known dangers influencing the dental specialist. Yoga which started in India is a type of physical, mental and spiritual practice. In present day the physical stances of Yoga is utilized to mitigate medical issues, diminish pressure and make the spine graceful in contemporary occasions. There are various yoga poses which have impact on the musculo-skeletal tissues and which when preceded as prompted have the ability to invalidate the musculo-skeletal disorders. Yoga poses which have most extreme impact on musculo-skeletal tissues are a lot simpler to perform, less tedious and are ideal for dental specialist to follow. Musculo-skeletal disorders among dentist is a settled certainty and yoga postures or exercise may be the quality restoration degree to stop the equivalent.

Key Words: *Yoga, Dentistry, Musculo- skeletal disorder, Aasanas*

Introduction

Dentistry as a professsion is an extremely specialized and demanding occupation due to its temperament of work, for example, little working territory, off-kilter working stances, consistent concentration and serious extent of exactness and so forth causes loads of inconvenience and ailment over some stretch of time to dental specialist. To obtain entrance and better perceivability of oral cavity dentist will in general gain skeletal stances which might be unfavorable to their great wellbeing particularly musculo-skeletal tissues. Along with this long span of dental methodology, serious extent of focus and steady working stances prompts wellbeing risks among the dental specialists. The word related dangers that are regular in dental calling are cross infection, musculoskeletal disorers, stress and anxiety, radiation, allergic responses, eye and ear problems, etc. [1,2]

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MUSCULO- SKELETAL DISORDERS:

Of the numerous occupation related perils that a dental specialist is expossed to musculoskeletal disorder might be considered as one of the most significant dangers that a dental specialist is inclined to. Studies have indicated that dental specialist report more regular and more terrible medical issue [3] especially musculoskeletal pain [4]. The term MSD alludes to conditions that include the nerves, ligaments, muscles and supporting structures of the body [5]. WHO characterizes MSD as "a disorder of the muscles, tendons, peripheral nerves, muscles system not directly resulting from an acute or instantaneous event ex slips or falls.. Musculo-skeletal pain, especially back agony has been discovered to be a significant medical issue for dental professionals [4, 6]. Studies tells that back, neck and shoulder or arm ache is found in up to 81% of dental practitioners [7]. While

an intermittent back and neck hurt isn't a reason for caution, if routinely happening agony or uneasiness is disregarded, the cumulative physiological damage can lead to an injury or a career ending disability [8]. To get great vision and access dental specialists will in general twist their backs, over flex and bend their neck and raise their arms and shoulders which may cause pressure on nerve roots creating pain and over some undefined time frame this overemphasizing of muscles and tendons may bring about musculoskeletal disorders.

SYMPTOMS OF MSD:

1) Fatigueness in the shoulder and neck

2) Burning or other pain in arms

3) Cramping of hands

4) Numbness in fingers and hand

5) Hypersensitivity in fingers[9]

CAUSES:

1) Improper patient and position

2) Inconvenient and improper height of dentist operating stool

3) Repetitive, forceful and stressful muscular actions (Carpel Tunnel Syndrome)

4) Improper posture for better accessibility especially for maxillary region.

5) Constant and long working postures.[9]

YOGA FOR MUSCULO-SKELETAL DISORDERS:

Yoga has been shown to reduce incessant agony related with musculoskeletal and joint diseases [10]. Yoga is a physiological and practice that includes physical stances, breathing activities and contemplation to improve overall well being[11]. Yoga has been utilized to lighten musculoskeletal pain and has been related with huge improvement in scope of movement and function, decreased delicacy [12, 13]; lower levels of burdensome side effects [14] and diminished pain during activity in patients with musculoskeletal disorders[12, 13].

SOME OF THE POSES:[15]

1.Cat & Cow Pose: MARJARIASANA & BITILASAN

This posture is performed for neck and shoulder pain just as for lower back agony. In this posture you must be in table top position on all fours and spine in neutral position. On the breathe in, gather the spine and bend together into your cat posture. On the breathe out, curve the back and lift the chest to come into a cow posture. Repeat the excersice three to multiple times, zeroing in on the breath. These stances help to knead the spine, while additionally extending the back and the middle. Cat-cow tilts can be a successful headache reliever, in addition to opening up the back and stretching the spine.

2.Downward Dog Pose: Adho Mukha Svanasana .

This posture is an extraordinary absolute body stretch that objectives back extensors, or the large muscles that help from your lower back, uphold your spine and assist you with standing and lift objects. Start in tabletop and raise your hips so your body is in a upside down V position. Loosen up your head and neck and draw your internal thighs towards back of the room. Spreading your shoulder bones separated will extend your upper back much more, and arriving at your hips up and back will assist with opening your lower back. Hold the position for 5 to 10 breaths, and repeat the posture five to multiple times. This posture extends and fixes the muscles of the body. It decreases pressure in the shoulders, loosens up the neck, and lets somewhat more blood stream get to the cerebrum. The posture is likewise extraordinary for loosening up the wrists and hands, which may get sore or tired from long stretches of composing or because of holding of instruments or hand piece in case of dentist.

3.Child Pose: Balasana

This is an astounding posture not exclusively to unwind yet it likewise extends our whole back and hip. Start down on the ground; plunk down with your legs folded beneath you, toes contacting and knees spread separated from one another, keeping your arms forward; sit back so your butt is resting simply over your heels. Wrap your chest down between your thighs, carrying your brow to the floor and either expanding the arms

out before you or resting them by your sides. Hold and inhale profoundly, feeling the breath venture right into your hips. The more you stretch out in either course, the more you'll feel relief.

4. Fish Pose: Matsyasana

It is an excellent strain reducer, and can likewise be helpful for fatigue and anxiety, as indicated by Yoga journal. To come into the posture, sit up on your hips with legs loosened up together before you and toes pointed. Bring your hands under your hips and recline to prop yourself up on your lower arms. At that point, lift the chest over the shoulders and drop the head back to the ground behind you. Attempt to keep up the vast majority of the heaviness of your head in your elbows and lower arms, not the crown of the head. Inhale profoundly and rest in the posture for 15-30 seconds.

5. Spinal Twist: Marichyasana

There is extraordinary assortment in regard to spinal twist pose. Anyway the normal and most likely the simple one is the Marichyasana C. In this posture keep your left leg straight and twist your right leg so your foot is level. Place your right hand on the floor behind you for help, similar to a tripod, and contort so you can snare your left elbow over the right thigh. Its well established reality that any type of physical exercise ought not be begun at one proceed to warm up exercise to loosen up the muscle and tissue is significant. Similarly exercise to chill off the body after incredible exercise is similarly significant. For warm up one can perform standing forward

Uttanasana Posture: Remain with your feet at hips-width separation, and gradually twist forward from the hips to come into the forward twist. At that point, have a go at adding an arm tie to extend the shoulders: Intertwine your hands at the lower back and stretch the arms over your head and hands towards the ground in front of you. These aides in warming up the spine as well as the shoulders and neck muscles.

Conclusion

Almost every dental specialist in his/her profession will sooner or later of time will experience the ill effects of MSD. As consistently expressed that avoidance is superior to fix, propensity for practice among dental specialist ought to be underscored from the time they take up dentistry as profession. To begin or to cause your body to play out any type of exercise at the later phase of one's life is consistently troublesome thus ought to be begun at an early age of one's life. Yoga as a remedy for MSD is a verifiable truth now. Yoga practice among the dental experts ought to be stressed from their school days just so the propensity for practice among dental specialist is instilled from the very beginning of their vocation. A conventional yoga program ought to be followed and started at different dental foundations to build up the propensity for practice among the dental experts. On a normal an individual should denote 30 minutes in a day for yoga work out. This quite a bit of activity is adequate to fix the probable harm which may happen to our muscular-skeletal tissues after taxing day of work.

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Use of Agar as a Dental Impression Material

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Abstract

Agar hydrocolloid was the first successful elastic impression material to be used in dentistry. It is an organic hydrophilic colloid extracted from certain types of seaweed. It is a reversible impression material because of its property of reversing their physical state, which is reusing the impression material for multiple number of impressions. It was initially known as "Negacol", later termed as "Dentacol" when introduced into dentistry.

Keywords: *Agar, hydrocolloid, impression, Reversible.*

Introduction

Impression materials are used to record the intraoral structures for the fabrication of dental prostheses. Accurate impressions are necessary for the construction of any definitive restorations. The accuracy of these final restorations depends greatly on the impression materials and techniques. Impression materials should accurately reproduce the hard and soft tissues to obtain biologically, mechanically, functionally, and aesthetically acceptable restorations [1].Hydrocolloids have a high hydrophilic nature that allows this material to capture accurate impressions even in the presence of some saliva or blood. It has a low wetting angle so it easily captures full arch impressions. It has moderate ability to reproduce detail and costs relatively little compared with other impression materials. The two types of hydrocolloids used in dental impressions are agar and alginate. Agar is a reversible hydrocolloid because it can pass repeatedly between highly viscous gel and low viscosity sol simply through heating and cooling. Agar-agar is an organic colloid derived from the cell wall of red seaweed [2]. It is essentially a hydrophilic, emulsoid polysaccharide [2]. Reversible hydrocolloid was introduced to the dental profession in 1925 by AlphonsPoller, an Austrian as impression material [3].Agar was first introduced into dentistry for recording crown impressions in 1937 by Sears [4] and was the first elastic impression material available. It was termed as "negacol" or "dentacol" when introduced into dentistry.

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Applications in Dentistry

The following are the various uses of agar in dentistry [5-6]:

1. Recording secondary impression for inlay, onlay, crown and bridge work

2. Making agar-alginate impression (Laminate impression technique)

3. Laboratory duplication of stone casts

4. Help in making acrylic dentures using the fluid resin technique

5. Used as tissue conditioners

Composition

The following is the composition of agar agar impression material [7]

1. Agar (12-15%)

2. Water (85%)

3. Borates (0.2-0.5%) (strengthening agent)

4. K_2So_4 (1-2%) (to overcome the retarding effect of borax on gypsum cast material)

5. Thymol (Antifungal)

6. Coloring agent and flavour

7. Fillers (0.5-1%) (diatomaceous earth, silica, wax rubber)

PROPERTIES

Irreversible hydrocolloid impression material has dimensional changes as a result of syneresiswhen exposed to air[8].Storage medium such as 2% potassium sulphate solution or 100% relative humidity in a storage chamber, is suggested to reduce the dimensional change of agar impressions[9]. While Alginate has a Compressive strength of 5000-8000gm/cm2 and Tear strength of 350-700gm/cm2 [10], Agar has a Compressive strength of 8000gm/cm2 and Tear strength of 7000gm/cm2 [11]. This material does not readily flow into areas in which the tray does not extend. If distortion occurs, corrections cannot be made and this is a major drawback [12]. Agar is sufficiently fluid to allow detailed reproduction of hard & soft tissue. If the material is held rigidly to the tray, then the impression material shrinks towards the centre of its mass. Rapid cooling may cause a concentration of stress near the tray where gelation first occurs. The sodium sulphate that was formed as a by-product of the setting reaction of the alginate is a gypsum setting retarder at high concentrations and thereby produce poor surfaces [13]. Agar impression material contains borax, which is a retarder for setting of gypsum. This can be overcome by immersing the agar impression in a solution containing a gypsum accelerator or by incorporating a gypsum surface hardener. Most alginate impressions are not capable of producing finer details whereas agar produces an accurate impression in comparison [14]. The combination of agar and alginate appears to produce excellent results and detailed accuracy overcoming the drawbacks of both the materials.[15]

SETTING REACTION

Agar behaves as a thermoreversible gel and the sol-gel formation is reversible which is temperature dependant. This material forms a colloid after absorbing water, which liquefies between 71°C and 100°C and sets to a gel again between 30°C and 50°C, depending on the concentration of the agar [10]. The setting of the reversible hydrocolloid is called gelation.

MANIPULATION AND IMPRESSION MAKING

At first, reverse the hydrocolloid gel to the sol stage. Boiling water is a convenient way of liquefying the material. The material must be held at this temperature for a minimum of 10 minutes. Propylene glycol can be added to the water to obtain the temperature of100^{o}. There are three compartments in the conditioning unit, making it possible to liquefy, store and temper the material. The hydrocolloid is usually supplied in two forms: syringe and tray materials. After it has been liquefied, the material must be stored in the tray. The impression trays are rim locked with water circulating device. This type of tray allows a space of 3mm occlusally and laterally and extends distally to cover all the teeth. The syringe material is taken directly from the storage compartment and applied to the prepared cavities. It is first applied to the base of the preparation and then the remaining teeth are covered. By the time the cavity preparation and adjoining teeth have been covered, the tray material has been properly tempered and is now ready to be placed immediately in the mouth to form the bulk of the impression. Gelation is accelerated by circulating cool water, approximately, 18 – 21^{o}through the tray for 3 – 5 minutes. [7,9,13]

DISINFECTION

For disinfecting the impression, iodophor, bleach or gluteraldehyde can be used. The hydrocolloid may be disinfected by 10 minute immersion in or spraying with the antimicrobial agent such as sodium hypochlorite and glutaraldehyde without sufficient dimensional change. [14,15,16]

RECENT TECHNIQUES

Wet field technique

The oral tissues are flooded with warm water. The syringe material is then injected in to the surface to be recorded. Before the syringe material undergoes gelation process, tray material is seated. The hydraulic pressure of the viscous tray material forces the fluid syringe material down in to the areas to be recorded. The motion displaces the syringe material as well as blood and debris throughout the sulcus.

Laminate technique

The general procedure is to heat the agar to be placed into re-usable syringes for about six minutes in boiling water. The agar is stored for at least 10 minutes at 65°C before being syringed around the preparations. A mix of alginate containing 10% more water than normally recommended is placed in a tray and it is immediately seated over the agar syringe material.[17] The cool mix of alginate helps gel the agar, and when the alginate has set, the combined impression is removed. The technique simplifies the use of agar and provides an impression surface that allows for preparation of stone casts acceptable for crown and bridge applications [18]. Advantages of this technique are that it isinexpensive, no odour, non-toxic and non-staining. Disadvantages include complexity of the technique and lower than desirable tear strength.

ADVANTAGES

· It helps in accurate die preparation.

· It has good elastic properties and reproduces most undercut areas correctly.

· It has good recovery from distortion

· It is not hydrophobic and hence gives good model surface

· It is palatable and well tolerated by the patient

· It is cheap when compared to synthetic elastic materials

· Agar can be re-used when used as a duplicating Material (Re-use is not recommended when used as an impression material)[7]

DISADVANTAGES

· Does not flow as good as the newly developed materials.

· It cannot be electroplated.

· Due to the heat it may be painful to the patient during insertion or gelation.

· Tears relatively easily.

· Greater gingival retraction is required for providing adequate thickness of the material.

· Only one model can be poured.

· Expensive equipment is required.

· A plaster hardener should be used to get a soft surface on gypsum cast contamination is very prevalent with this material due to re-use and the lack of sterilization technique. [7]

Conclusion

Making impressions is an important part of indirect restorations that is often overlooked. The increased use of agar impression material as a result of the development of the agar alginate combination technique should level off and continue at a modest level. With the selection of an appropriate impression material and impression technique, impression taking is reliable and reproducible, with consistent successful results.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Bone Morphogenetic Protein-1 and Its Regulation - A Review

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Abstract

Bone morphogenetic protein 1, also referred to as BMP1, is a protein encoded by the BMP1 gene in humans. There are seven protein isoforms created through alternative splicing.It leaves the C-terminal propeptides of procollagen I, II and III. It induces cartilage and bone formation. It may participate in dorsoventral patterning during early development by cleaving chordin (CHRD). It is also responsible for the proteolytic activation of lysyl oxidase LOX.

Keywords: *BMP1, TGF β-1, lysyl oxidase, SpAN, bone, growth, cartilage*

Introduction

BMP1 is a member of the peptidase M12A family of bone morphogenetic proteins (BMPs). It causes the growth of bone and cartilage. It does not belong to the TGFβ superfamily, unlike other BMPs. It was initially discovered by inducing formation of bone and cartilage to function like other BMPs.[1] However, it is a metalloprotease that cleaves procollagen I, II and III at the C-terminus. It has an astacin-like protease domain. It has been shown to cleave laminin 5 and it is found in the bovine skin's epithelial layer. The locus of BMP1 encodes a protein that can cause in vivo cartilage formation.[1,2] Although other morphogenetic bone proteins belong to the superfamily of TGF-beta, BMP1 encodes a protein that is not closely related to other recognized growth factors. BMP1 protein and procollagen C proteinase (PCP), a secret metalloprotease that requires calcium and is essential for formation of cartilage and bone, are all similar. PCP or BMP1 protein cleaves procollagen I, II, and III C-terminal propeptides and increases their activity with the enhancer protein procollagen C-endopeptidase. The BMP1 gene is expressed as alternative spliced variants sharing an N-terminal protease domain but differing in their C-terminal region.[2]

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REGULATION OF BMP-1

The structure of BMP1 was determined through X-Ray diffraction using a resolution of 1.27 Å.Further crystallization experiments were performed by vapor diffusion at a pH value of 7.5. This is done because it is close to the pH of the human body, thus equilibrating the conditions, where BMP1 resides in vivo. BMP1 is 202 residues long. Its secondary structure is made up of 30% helices, or 10 helices, 61 residues long, and 15% beta sheets, or 11 strands, 32 residues long. It contains ligands of an acetyl group and a Zinc ion. A Ramachandran plot was constructed for BMP. This plot shows that BMP-1 most prefers Phi and Psi angles (Phi, Psi) of around (-60°,-45°) and (-60°, 140°). These preferred angles are an estimate of the most clustered data of the Ramachandran plot. The preferred region is much greater in range. 97% of the residues were in preferred regions and 100% of the residues were in the allowed region, with no outliers.[1-5]

The molecular functions of BMP1 include, calcium ion binding, cytokine activity, growth factor activity, identical protein binding, metalloendopeptidase activity, metallopeptidase activity, peptidase activity and zinc ion binding. The biological processes brought about by BMP1 include cartilage condensation, cell differentiation, extracellular matric disassembly, high density lipoprotein particle assembly, ossification, proteolysis, positive regulation of cartilage development and overall skeletal system development. The following

are the factors involved in the regulation of BMP1.

TGF β1

Transforming growth factor beta 1 (or TGF-β1) is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, such as cell growth, cell proliferation, cell differentiation and apoptosis. In humans, TGF-β1 is encoded by the *TGFB1* gene. [1,2] Transforming growth factor-1 (TGF-1) induces increased extracellular matrix deposition. Bone morphogenetic protein-1 (BMP-1) also plays key roles in regulating vertebrate matrix deposition. It is the procollagen C-proteinase (PCP) that processes procollagen types I±III, and it may also mediate biosynthetic processing of lysyl oxidase and laminin 5. [3] BMP-1 is itself up-regulated by TGF-1 and that secreted BMP-1, induced byTGF-1,is either processed to an active form or remains as unprocessed proenzyme, in a cell type-dependent manner. [4] Secreted BMP-1 and mTld, induced by TGF-1 in MG-63 and other fibrogenic cell cultures, were predominantly in forms in which pro regions had been removed to yield activated enzyme. Bone morphogenetic protein-1 (BMP-1) copurifies from osteogenic bone extracts with transforming growth factor(TGF-)-like proteins BMP-2 through -7. BMP-1, by structure an astacin-like protease, may function in morphogenesis by activating TGF-like molecules. [5] BMP-1 has a domain structure similar to, but shorter than, that of tolloid, a Drosophila protein that appears to act in patterning of embryos by potentiating the activity of decapentaplegic, a TGF-family member. [6] TGF-beta family proproteins are cleaved in the trans-Golgi between the N-terminal propeptide and the mature growth factor. For TGF-beta1, -beta2, -beta3, GDF-8, and GDF-11, the prodomain is secreted in association with the growth factor and maintains the growth factor in an inactive state. BMP-1 family of proteases regulate the activation of these latent complexes by several mechanisms. **BMP-1-related activation of latent TGF-beta family complexes occurs by three mechanisms.** First, BMP-1 cleaves latent TGF-beta-binding protein(LTBT) at two positions in a process required for efficient MMP-2-mediated liberation of TGF-beta from latency-associated peptide(LAP). Second,BMP-1 family proteases cleave at single positions within the GDF-8 and GDF-11 propeptides, releasing the active growth factor dimers. Third, activation of BMP in complex with chordin is mediated by BMP-1 proteolysis at two sites within chordin. [7]

LYSYL OXIDASE

Lysyl oxidases (LOX) are a family of copper-dependent amino oxidases for which important roles in cancer and vascular and fibrotic diseases have been proposed. [7] Five different LOX enzymes have been identified in mammals (LOX, and LOX-like 1 to 4), showing a high degree of homology in the catalytic carboxy terminal end and more divergence in the rest of the sequence. [8] Lysyl oxidase catalyzes the final enzymatic step required for collagen and elastin cross-linking in extracellular matrix biosynthesis. Pro-lysyl oxidase is processed by procollagen C-proteinase activity, which also removes the C-propeptides of procollagens I-III. [9,10] The Bmp1 gene encodes two procollagen C-proteinases: bone morphogenetic protein 1 (BMP-1) and mammalian Tolloid (mTLD). Mammalian Tolloid-like (mTLL)-1 and -2 are two genetically distinct BMP-1-related proteinases, and mTLL-1 has been shown to have procollagen C-proteinase activity. [11]

SpAN

SpAN is a sea urchin metalloprotease in the astacin family containing BMP1 and tolloid. [12] Embryos expressing SpAN initiated gastrulation on a time scale indistinguishable from controls, but invagination of the vegetal pole was subsequently delayed by several hours. At tailbud stages the most severely affected embryos were completely ventralized, lacking all dorsal structures. [12] Molecular analysis of injected embryos, using probes for both dorsal (xgsc and xnot) and ventral (xhox3 and xwnt8) mesoderm, indicates that SpAN ventralizes dorsal mesoderm during gastrula stages. [13] These results mirror those previously obtained with BMP4, suggesting that SpAN may enhance the activity of this ventralizing factor. [14] Consistent with this suggestion, it has been shown that SpAN blocks the dorsalizing activity of noggin and chordin, two inhibitory binding proteins for BMP4, but not that of a dominant-negative receptor for BMP4. [15] In contrast, a dominant-negative SpAN, in which the metalloprotease domain has been deleted, dorsalizes ventral mesoderm, a phenotype that can be rescued by coexpressing either SpAN or XBMP1. This suggests that SpAN is mimicking a Xenopus

metalloprotease responsible for regulating the activity of Xenopus BMPs during gastrulation. [16]

DIMERIZATION

Refolding and dimerization of BMP are extremely slow under all conditions and could be described by consecutive first-order reactions involving at least one long-lived intermediate. [17] The rate constants vary from ~ 0.2 × 10(-5) to ~ 3.5 × 10(-5) s(-1). It is strongly dependent on temperature, redox conditions, and the presence of stabilizing or destabilizing ions. [18,19] In particular, the combined impact of ionic strength and redox conditions on the rates indicates that electrostatic interactions control thiol-disulfide exchange reactions on the path from the unfolded and reduced monomers to the disulfide-connected growth factor in a rate-determining way. [20,21,22]

Conclusion

By controlling cellular lineage engagement, morphogenesis, differentiation, proliferation, and apoptosis of different types of cells in the body, the bone morphogenetic protein (BMP) ligand family plays an important role in a multitude of processes throughout embryonic development and adult homeostasis. BMP1 belongs to a family of bone morphogenetic proteins (BMPs) called peptidase M12A. It causes the growth of bone and cartilage. It is different from other BMPs in that it does not belong to the TGFβ superfamily. It is important to understand the regulation of BMP1 and the important roles it plays.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Curing Techniques of Composite Resin: Continuous or Intermittent?

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Abstract

While composites have continued to be refined, significant differences between the available systems in physical and mechanical properties, and clinical performance have minimized. Nevertheless, with all the changes, there is one constant: it is necessary to light-cure direct composites. Clinicians need to understand the importance of light curing principles because unbound monomers are cytotoxic and poorly cured composites are less biocompatible. Four techniques are currently available for light-curing composites. The clinician will consider multiple factors once the light source is chosen to ensure that the composite is satisfactorily cured. This article analyses the various current technologies, their strengths and weaknesses, and the importance to ensuring proper polymerization by following such protocols.

Keywords: *Composite, light-curing, continuous, intermittent, polymerization.*

Introduction

When composite materials were first introduced, their field of application was very limited compared to that of amalgam. Early composite materials were suitable only for restoring anterior teeth because of their weak mechanical properties. However, in the past few decades, dental composites have developed from an inferior resin material into the material of choice. They are capable for use in the fabrication of highly esthetic, durable posterior and anterior restorations in direct restorative dentistry.[1]Apart from being used in the field of restorative dentistry, composites slowly gained entry into the field of orthodontics to bond brackets on the teeth. Chemically activated resin composites were widely used in Orthodontics which required mixing of two pastes, which could induce incorporation of air bubbles into the material. Other disadvantages included longer working time, slower polymerization reaction and lower mechanical properties because of the incorporation of oxygen in the mass which inhibits the polymerization.[2,3] For these reasons, light-activated orthodontic composite materials have been ever more frequently used for bracket bonding to dental enamel. Composite photoactivation time is particularly important because underpolymerization may result in early bracket debonding.Working with resin-based materials requires a detailed understanding of the curing properties and the factors that affect this process. Most modern composite materials are either based on a light-curing technology or offer a dual mechanism of light- and self-curing technology. In the case of light-curing materials the clinician needs to assure that enough photons reach the bottom of the composite, or the composite will not achieve the properties necessary for its long term survival.This article focusses on the various light-curing technologies and its potential drawbacks.

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CONTINUOUS CURING TECHNIQUE

Polymerization shrinkage is a problem inherent to light-cured resin composites. This process creates contraction stress in the composite restoration, which causes disruption of the marginal seal between the composite and the tooth structure.[1]Previous reports indicate that the magnitude of stress varies according to the C-factor and elasticity of the structures involved

in the bonding process, such as cavity substrate, hybrid layer and bonding resins.[2,3]

Reduction of polymerization shrinkage stress can be attained in several ways. Attempts have been made using incremental layering of the composite resinduring packing and through use of a low elastic modulus liner between the tooth and the restorative composite resin.[4]A second alternative is the slow-curing technique. The most recent approach aims at an initially reduced conversion of the resin materials to allow the restoration some freedom of movement between the cavity walls and the centre of contraction.[5]

It had been previously claimed that light curing composites shrink toward the light, because the energy at the surface nearest the light would be higher than that in the deeper parts of the composite.[6,7]Thus, an energy gradient would be created within the composite, which would result in quicker polymerization closer to the light source.[8]

Uniform Continuous Cure

In uniform continuous curing, a light of constant intensity is applied to the composite resin for a specific period of time. This type of continuous curing technique is currently used. It is carried out using QTH and LED curing units[9].(Figure1)

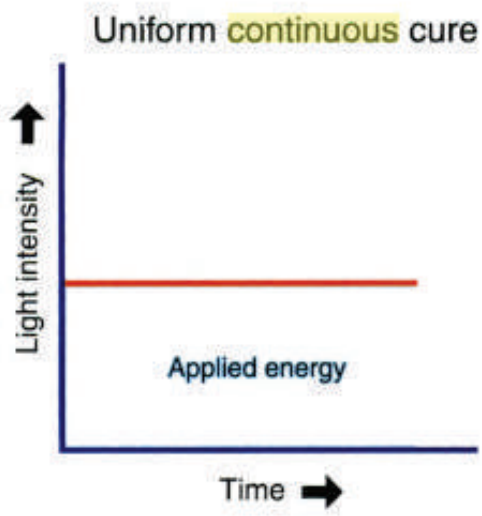


Figure 1

Step Cure

In the step cure technique, the composite is first cured at low energy, then stepped up to higher intensity, each for a set period of time. This is done to reduce the polymerization stress by inducing the composite to flow in a gel state. This technique reduces the overall polymerization shrinkage at the margin of the final restoration. [10,11]

The first commercially available light cure unit based on this technique was Elipar Highlight (ESPE). It uses a 10-second exposure of light at 150 mW/cm followed by 30-50 seconds at 700mW/ cm. However, this technique results in an uneven cure, as the top most layer is more saturated with light and thus more highly cured. [12]Step curing is possible only with halogen lamps. Arc lamps and lasers cannot be used because they work by applying large amounts of energy over a short period of time[13]. (Figure2)

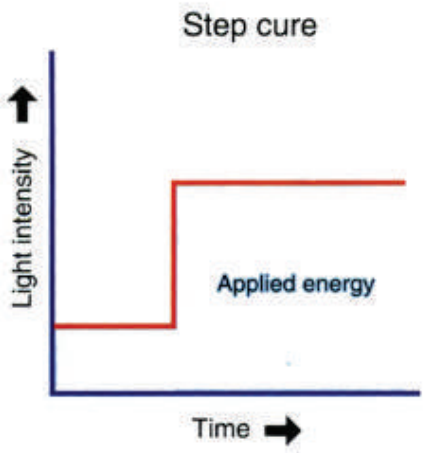


Figure2

Ramp Cure

In the ramp cure, light is initially applied at low intensity and gradually increased over time to high intensity levels. This allows the composite to cure slowly, thereby reducing the initial stress, as the composite can flow during polymerization.[14] Also, this function is designed to reduce polymerisation shrinkage. Ramp curing is done as an attempt to pass through all of the different intensities with the hope of optimizing a composite's polymerization. Studies indicate that ramp curing causes polymerization with longer chains, resulting in a more stable composite.[15]

Theoretically, very high energy applied over a short period tends to cause dimethacrylate monomers to attach to themselves, resulting in short polymer chains

and a more brittle material with higher polymerization shrinkage and presence of more marginal gaps.[16]Ramp curing is possible only with halogen lamps. Arc and laser lamps can generate only large and non-variable amounts of energy.It is possible to ramp cure composites manually by holding a conventional curing lamp at a distance from the tooth and then slowly bringing it closer to the tooth to increase the intensity[17]. (Figure3)

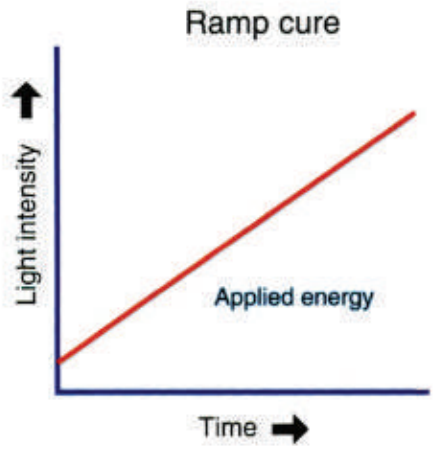


Figure3

High Frequency Pulse Cure

The high-energy pulse cure technique uses a brief (10 seconds) pulse of extremely high energy (1000-2800 mW/cm^2), which is three to six times the normal power density.[18]This type of polymerization has not yet been adequately examined and there are a few areas of potential concern.

1.The rapid application of energy might result in a weaker resin restoration due to the formation of short polymer chains. [19]

2.It is possible that rapid application of very high energy could reduce the diametral tensile strength.

3. Higher energies would result in more brittle resins.

This type of curing is carried out by argon laser, plasma arc and third generation of LED. (Figure4)

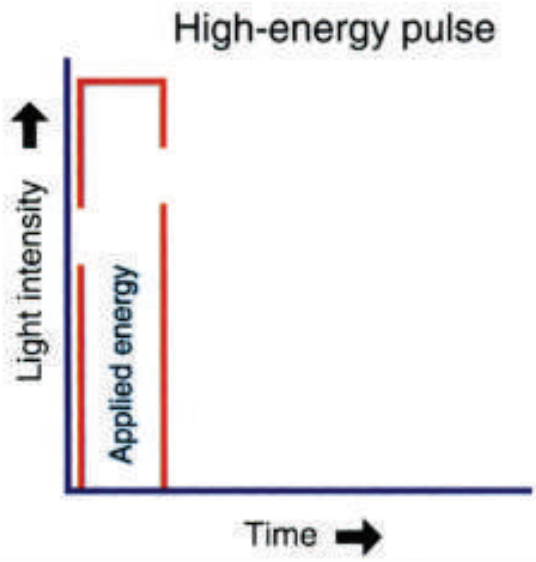


Figure 4

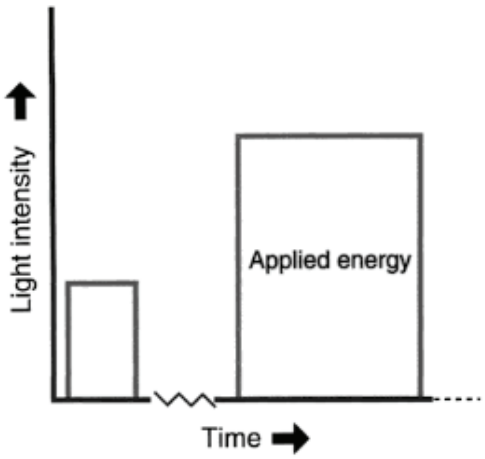
INTERMITTENT CURING TECHNIQUE

Intermittent or Discontinuous Curing Technique is also known as soft-cure technique. A low intensity or soft light is used to initiate slow polymerisation that allows the composite to flow from the unbound restoration surface to the bound tooth structure. This helps in reducing polymerisation stress at the margins and can reduce marginal gaps and defects. To complete the polymerisation cycle, the intensity of the next curing cycle is drastically increased to produce the needed energy for optimal polymerisation.[18]

Pulse Delay Cure

In this technique, a single pulse of light is applied to a restoration, followed by a short pause, and then by a second pulse of light which has greater intensity and duration of exposure than the first one. This technique is believed to be an interrupted step increase. The lower intensity light slows the rate of polymerisation, thus allowing shrinkage to occur until the composite becomes rigid. Thus the margins do not show much discrepancy. [20] The second pulse of greater intensity brings the composite to the final stage of polymerisation. Halogen lamps are used for this technique.The VIP light from BISCO used the pule delay cure technique, a very short exposure time of 3 seconds at 200 mW/cm^2 is first used to harden the composite resin. After a waiting period of three minutes, a 30-second exposure time at 600 mW/cm^2 is used. The waiting period allows stress relaxation

and the restoration can be finished and polished at this time. (Figure5)



Pulse Delay Cure

Figure 5

Conclusion

Dental composite polymerization is a very complex process. The dentist must be aware of the limitations and factors that affect this operation. Over the past few years, the LED curing lights have been dramatically improved, but they face a new challenge: they need to provide the broadband spectrum required for modern esthetic composites that use other than initiators than camphorquinone, such as Lucirin TPO. Some manufacturers have already started producing the third generation of LEDs, such as polywave LEDs, and these curing lights showed promising results. In addition, a light guide, preferable 10mm wide and parallel, is essential for LED lights. If the curing light contains enough energy turbo light guides should be stopped when the composite is healed at a distance from the light emission window to prevent unnecessary energy loss. It has slowly emerged over the course of several years of study that advanced curing methods are unable to remove shrinkage stress. Nevertheless, intermittent techniques provide clear advantages.The soft-start technique presents the best compromise between curing time, mechanical properties, bond strength and reduction of shrinkage stress.

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Efficacy of Clear Aligners in Correcting Anterior Open Bite- A Systematic Review

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Abstract

Objective:The aim was to systematically search the literature and assess the available evidence regarding theclinical effectiveness of clear aligners in correcting anterior open bite.

Materials and Methods:PubMed, PubMed Central, MEDLINE, Embase, National Library of Medicine, Cochrane Library, Web of Knowledge, and LILACS were searched electronically to identify all peer-reviewed articles till date potentially relevant to the review.The reference lists of all eligible articles were examined for additional studies. Additional manual search was also conducted in many orthodontic journals of interest, and unpublished articles were also searched for. To rate the methodological quality of the selected articles, a grading system described by the Swedish Council on Technology Assessment in Health Care was used.

Results:Ten relevant articles were selected to be included in this systematic review. According to the SBU tool, among the selected studies, the methodological quality was moderate for four studies and limited for the others. Thus, conclusions with a limited level of evidence could be arrived at from the review process. The most recurrent sources of bias were related to the study design, lack of blinding procedure, the sample size, and the lack of control group. However, there was substantial consistency among studies that clear aligner therapy is a viable option to correct anterior open-bite.

Conclusion:No definite conclusion could be derived due to the heterogeneity of the studies. Due to the limitations of this study, further clinical and experimental research is needed to statistically assess long-term stability of occlusion and skeletal relationships in treated open-bite cases.

Keywords: *Clear aligners, Invisible orthodontics, Anterior open-bite, Treatment outcomes.*

Introduction

A growing number of adult patients have sought orthodontic treatment in the last few years and have demonstrated a preference for esthetic and comfortable alternatives to conventional fixed orthodontic appliances.[1][2]Kesling introduced the idea of using transparent overlay orthodontic appliances in 1946 when he developed the theory of using a series of thermoplastic tooth positioners to transfer malaligned teeth slowly to better positions.[3] In 1997, Align Technology © (Santa Clara, Calif) adapted and implemented advanced 3D imaging technology to introduce clear aligner treatment(CAT), making the idea of Kesling a viable orthodontic treatment option.[4] Ever since then, CAT has been regarded as a reliable, esthetic, and comfortable orthodontic procedure for adult patients.

Orthodontic treatment of open bite remains one of the most challenging tasks faced by orthodontists today.[5] The vertical incompetence of the arches due to poor dental and/or skeletal position does not permit correct occlusion between antagonistic teeth. This lack of contact can be caused either by dentoalveolar or skeletal anomaly, and the pathogenesis of open bite will determine the course of treatment, whether orthodontic or combined surgical and orthodontic. Solely orthodontic intervention is an option only in cases of dentoalveolar

alteration, whereas surgery is indicated in cases of skeletal dysgnathia.[6]

Many factors may be responsible for an anterior open bite, including bad habits such as thumb-sucking, prolonged use of pacifiers, lip or tongue habits, or unfavorable growth patterns, which are often correlated with a genetic or familial predisposition. Excessive development of the lymphatic tissues (adenoid facies) causing airway obstruction, may also be responsible.[7] In general, the etiology of this type of malocclusion is multifactorial.[8] In the case of growing patients in whom bad habits have contributed to anterior open bite, early intervention with interceptive functional appliances or speech therapy may resolve or improve the clinical scenario.

In adult patients, on the other hand, accurate diagnosis of the dentoalveolar or skeletal origin of the malocclusion, via cephalometric analysis needs to be performed. In these cases, treatment options are numerous and involve maxillofacial surgery to correct skeletal open bite, intrusion of the posterior segments to correct dental open bite due to over-extrusion of the posterior segments; or extrusion of the anterior dental segment alone, especially in cases of poor vertical development of the premaxillary region.[9]

A systematic review by Rossini et al., which evaluated the efficacy of CAT in controlling various types of orthodontic tooth movements showed that extrusion was the most difficult movement to achieve, with maxillary and the mandibular central incisors showing the lowest accuracy.[10]

The present systematic review was undertaken to update the knowledge of the available evidence about CAT and to answer the following clinical research question: "Is CAT effective in correcting anterior open-bite?"

Materials and Methods

A systematic search was performed of articles published till date in thefollowing databases: PubMed, PubMed Central, MEDLINE, Embase, National Library of Medicine, Cochrane Library,Web of Knowledge, and LILACS.The search strategy comprised use of the followingterms; Orthodontics AND ("orthodontic appliances, removable" OR ("orthodontic" AND "appliances" AND "removable") OR "removable orthodontic appliances" OR "invisalign") AND ("clear aligners" OR ("clear" AND "aligners") OR "clear" OR "transparent") AND aligners AND ("therapy" OR "treating") AND anteriorAND ("OPEN" OR ("open" AND "bite") OR "open bite").Additionally, a manual search was conducted in orthodonticjournals of interest, such as the American Journalof Orthodontics, European Journal of Orthodontics,The Angle Orthodontist, Journal of Orthodontics and Journalof Clinical Orthodontics.In addition, unublished studieslistedin the national clinical trials database ClinicalTrials.gov(www.clinicaltrials.gov) and the National Research Register(www.controlled-trials.com), were searched with theterms "clear aligners" or "Invisalign."Based on the abstract data, we completed the initial selection process independently, then resolved any differences through discussion.We selected andcollected all the articles that appeared to meetthe initial inclusion criteria based on theirabstracts.In cases in which specific data were necessaryfor the discussion and was not specified in theabstract, we made efforts to contact the authorsto obtain the required extra information.The reference lists of these articles were perused,and references related to the articles were followed up. Duplicate articles were eliminated, and the studies wereselected for inclusion.

Studies on orthodontic treatment with clear aligners for treating open-bite cases were included in the study. Studies on patients with genetic syndrome and severe facial malformations, studies using clear aligners for malocclusions other than open-bite, in-vitro studies, studies with surgical orthodontic techniques, review articles and author debates were excluded. The 'PICOS' approach was used to extract data fromthe selected articles. PICOS stands for ''population(participants), intervention (or exposure for observationalstudies), comparator, outcomes and studydesign''.

Population: Orthodontic patients of any age presenting with anterior open-bite malocclusion.

Intervention: Treatment with clear aligners.

Comparator:Comparison using ClinCheck® models, cephalometric superimpositions, clinical evaluation, PAR index and ICON scores.

Outcomes: Any effect on clinical efficiency, treatmentoutcomes, movement accuracy, or predicted toothmovement in ClinCheck®, includingchanges in alignment or occlusion, treatmentduration, and completion rate, as primary outcomes.

Studydesign: Randomized or non-randomized prospective or retrospective studies, clinical trials.

Results

Ten relevant publications were included in the study after screening and checking for eligibility, out of which three were retrospective non-randomized studies and one was a prospective non-randomized study.The remaining data obtained was from case reports.

According to the SBU tool, among the selected studies, the methodological quality was moderate for four studiesand limited for the others.Thus, conclusions with a limited level of evidence could be arrived at from the review process. The most recurrent sources of bias were related to the study design, lack of blinding procedure, the sample size, and the lack of control group.

Swedish Council on Technology Assessment in HealthCare (SBU) Criteria for Grading Assessed Studies

Grade A—high value of evidence

All criteria should be met:

Randomized clinical study or a prospective study with a well-defined control group

Defined diagnosis and endpoints

Diagnostic reliability tests and reproducibility tests described

Blinded outcome assessment

Grade B—moderate value of evidence

All criteria should be met:

Cohort study or retrospective case series with defined control

or reference group

Defined diagnosis and endpoints

Diagnostic reliability tests and reproducibility tests described

Grade C—low value of evidence

One or more of the conditions below:

Large attrition

Unclear diagnosis and endpoints

Poorly defined patient material

Grading of Selected Studies

Study (Author, Year)	Grade
Kravitz et al., 2009	B
Park et al., 2009	C
Schupp et al., 2010	C
Guarneri et al., 2013	C
Bowman et al., 2015	C
Wheeler et al., 2016	C
Kau et al., 2017	B
Giancotti et al., 2017	C
Moshiri et al., 2017	B
Garnett et al., 2018	B

Definitions of Evidence Level

Level	Evidence	Definition
1	Strong	At least two studies assessed with level ‘‘A’’
2	Moderate	One study with level ‘‘A’’ and at least two studies with level ‘‘B’’
3	Limited	At least two studies with level ‘‘B’’
4	Inconclusive	Fewer than two studies with level ‘‘B’’

The study design, participants, type of intervention, and observations of the qualitative analysis is given in the table

Discussion

The present systematic review showed that the use of clear aligners is recommended in treating anterior open bite. However, literature has shown that extrusion is the least accurate tooth movement to perform with clear aligners. [10][11][12]

Rossini et al [10]when evaluating the efficacy of clear aligners in controlling orthodontic tooth movement, found that clear aligner technique is not recommended to treat open bite. Open bite is a challenging malocclusion to treat, with a high incidence of relapse. Extrusion being the least accurate tooth movement to perform with CAT, it can result in larger deviations compared to other movements. This lack of efficiency could be due to the appliance’s difficulty in producing sufficient force to substantially extrude the teeth.

Papadimitriou and his colleagues, in their systematic review recommended CAT for treating mild to moderate open bite mainly though incisor extrusion.[11] This is also in agreeance with another systematic review by Galan-Lopez et al, in which the mandibular plane angle remained constant while attempting incisor extrusion by CAT.[12] While these systematic reviews focussed on comparing the clinical effectiveness of CAT in effecting various tooth movements, we incorporated studies that studied exclusively its efficiency in effecting tooth movements in correcting anterior open bite.

In one prospective study [13], comprising of 401 anterior teeth treated with anterior Invisalign, theleast accurate tooth movements were extrusion of themaxillary (18.3%) and mandibular (24.5%) centralincisors. The maxillary lateral incisors were the most commonly extruded teeth (n = 23). Only 13 of the 64 teeth had attempted extrusions greater than 1.0 mm (range, 1.0-1.8 mm), and no tooth had an attempted extrusion greater than 2 mm. The average amount of extrusion attempted was 0.56 mm. The difficulty in extrusive movement was most likely because the aligner poorly grasped the tooth during vertical pull. Boyd[14] stated that even with attachments, absolute extrusion remains difficult and recommended the extrusion of teeth with an elastic button on the facial aspect of the tooth.Alternatively, the clinician could consider combining extrusion with more accuratemovements such as retraction (lingual constriction) or retroclination to bring about relative extrusion.

Park et al, [15] in his case report series, utilised intermaxillary and intramaxillary elastics with CAT to correct open bite when minor extrusions (2-3mm) are required. The use of clear aligners with intermaxillary elastics is known as Cow-Catch aligners. To fabricate this, once a working cast is produced, the teeth on this cast are ideally set-up (extruded) before forming the aligners by pressure-moulding or vacuum-forming. The teeth to be extruded are supplied with buttons and connected to the opposite arch with elastics where buttons were attached to the aligner. When the expected

extrusion is achieved, the respective teeth will contact the inner surface of the aligner so that no additional extrusion occurs. Thus, it is a fail-safe appliance. If a patient cannot tolerate the limited mouth opening while wearing Cow-Catch aligners with intermaxillary elastics or needs to interact a lot verbally, it is recommendedthat an appliance modification can be done. The modified Cow-Catch aligners uses intramaxillary elastics and is characterized by buttons on the labial surface of the teeth and lingual side of the aligner on the same tooth. This type of aligner is moreconvenient and comfortable for allpatients because it allows a more normalfunction. According to the authors, with good cooperation, the intended treatment is achieved within 6 to 8 weeks, provided the patient wears the elastics for atleast 17 hours a day.

Schupp et al [16] also published two anterior open bite cases successfully treated with Invisalign alone. Vertical rectangular bonded attachments were used to extrude the teeth. In 17-20 months, anterior bite closure was achieved with proper overbite and overjet, well aligned anterior teeth, and canine guidance was achieved on both sides, closely matching the projections of ClinCheck. Post-treatment stability of closed bite was good.

In a case report by Guarneri et al [17], successful closure of a 4mm anterior open bite was achieved. Extrusion of upper incisors was achieved by bevelled horizontal rectangular bonded attachments and ellipsoid attachments for the lower incisors. Interproximal reduction of upper anteriors were done to correct the crowding. The extrusion rate was 0.12 mm per aligner, on the upper incisors for a maximum of 3 mm and on the lower incisors for 1 mm. The treatment was completed with full patient satisfaction after 18 months.

The difficulties encountered in bringing about extrusion and strategies to control them have been proposed by Nicozisis [18] and Humber [19]. They suggested use of bonded attachments, elastics, detailing pliers such as Clear Collections' The Vertical to accentuate couple, overcorrection, supracrestal fiberotomy (Edwards procedure) and long-term fixed retention to prevent relapse. [20][21][22][23] There should be sufficient space adjacent to the affected tooth in order to extrude it. This can be achieved by interproximal reduction. If extrusion is attempted with insufficient adjacent space, iatrogenic intrusion may occur as the tooth is inadvertently "squeezed" apically. Space opening also increases the tooth-surface area for the plastic to contact—an important consideration with a small, blade-shaped lateral incisor. Aligner Chewies have been designed to address inadequate tracking or "aligner lag."[24] Even with the best clear-aligner biomechanics preparation, aligner lag or tracking error of individual teeth may still occur, often due to less than optimal patient compliance with aligner wear. Adjunct elastics may be added to assist in extruding selected teeth into the aligner trays. Bonded buttons on the facial, lingual, or both surfaces of the lagging tooth are attached with "bootstrap" elastics at the gingival margin. [25] Anorthodontic elastic is stretched across the incisal surface of the seated aligner to the button, thusextruding or dragging the tooth into the tray. The gingival margin of the aligner plastic must be cutto avoid the buttons, so that the tray can seat completely. Alternatively, to avoid the need for a buttonon the facial side of the tooth, elastic hooks can be cut into the aligner tray using TearDrop plier.

Passive posterior intrusion is a common matter in question in clear-aligner treatment. Posterior plastic may be removed to allow the posterior teeth to be spontaneously settled or erupted into occlusion, and intermaxillary elastics may also be used for forced eruption.Clever methods for using clear aligners to refine the closure of anterior open bites by intrusion of posterior teeth have been suggested by Boyd[26] and Dayan[27]. The underlying concept is to harness the aligners' propensity to produce an iatrogenic posterior open bite simply as a result of holding plastic between the teeth for extended periods of time. One such strategy involves sequential intrusion of specific posterior teeth while maintaining or extruding the anterior teeth, thus creating a deeper curve of Spee [27]. Using Chewies to exert intrusive forces on targeted posterior teeth can improve the predictability of this method. An article by Bowman et al [28] shows open bite cases successfully treated using these mechanisms. This concept of employing a combination of anterior extrusion and posterior intrusion to correct anterior open bites was also mentioned in Wheeler's article [29]. The addition of miniscrew anchorage to support biomechanics involving elastic forces for the extrusion of anterior or posterior teeth is another logical extension of their application to clear-aligner treatment. Miniscrews are

used as an adjunctwith bootstrap mechanics to increase the reliability of posterior intrusion and potentially produce spontaneous mandibular autorotation. With the need to extrude anterior teeth during anterior open bite correction, Lin and colleagues have identified the combination of clear aligners with miniscrew anchorage for such treatment. [30]

Giancotti et al [31] in his case report series established that aligners allow an optimal vertical control and can determine molar intrusion by bite-block effect of two layers of aligner material between the dentition for 22 hours/day for 18–24 months and thereby subsequent minimal autorotation of the mandible and improvement of skeletal open bite. In case minimal molar intrusion is needed (1–2 mm), the vertical dimension would also be well controlled, not tending to worsen. In addition, aligners are also able to extrude the incisors by means of appropriate attachments and allow a minimal dental correction of the open bite.

One retrospective study [32] compared the pre-treatment difficulty of 100 cases treated with Invisalign with the post-treatment outcomes, as measured by the Peer Assessment Rating (PAR) index and the Index of Complexity, Outcome, and Need (ICON). The average PAR index scores of the anterioropen-bite patients (23 cases) were 21.9 ± 8.6 pre-treatment and 4.5 ± 4.0 post-treatment, for areduction percentage of 76.7% ± 18.0%also a "great improvement". The average ICON scoresfor the anterior open-bite cases were 57.4 ± 19.2 pre-treatment and 16.6 ± 5.7 post-treatment, for an improvement of −9.0 ± 30.3—still "substantially improved". When the anterior open-bite cases were compared to the control group (77 cases), their average pre- and post-treatment PAR and ICON scores, reduction percentages, and improvement grades were statistically similar. Thus, this study concluded with favourable results for anterior open-bite cases.

We found two retrospective studies that used cephalometrics as means of evaluation of treating anterior open bite cases using CAT [33][34]. Moshiri et al [33] compared pre-treatment and post-treatment lateralcephalograms of 30 adult patients with anterior open bite treated using Invisalign. The following vertical measurements were considered: SN to maxillary occlusal plane (SN-MxOP), SN to mandibular occlusal plane (SN-MnOP), mandibular plane to mandibular occlusal plane (MP-MnOP), SN to mandibular plane (SN-MP), SN to palatal plane (SN-PP), SN to gonion-gnathion plane (SN-GoGn), upper 1 tip to palatal plane (U1-PP), lower 1 tip to mandibular plane (L1-MP), mesiobuccal cusp of upper 6 to palatal plane (U6-PP), mesiobuccal cusp of lower 6 to mandibular plane (L6-MP), lower anterior facial height (LAFH), and overbite (OB). Statistically significantdifferences were found in overall treatment changes for SN-MxOP, SN-MnOP, MP-MnOP, SN-MP, SN-GoGn, L1-MP, L6-MP, LAFH, and OB. But SN-PP, U1-PP, and U6-PP did not undergo any statistically significant changes. Thus, bite closure was mainly achieved by a combination of counter-clockwise rotation of the mandibular plane, lower molar intrusion and lower incisor extrusion. Garnett et al [34] did a cephalometric comparison between clear aligners and fixed appliances in correcting adult open bite. It was found that there was no statistical difference in the magnitude of overbite correction and the changes in any of the vertical control cephalometric measurements. The clear aligner group showed an increased amount of lower incisor extrusion to a small degree. The main mechanism of open bite correction was similar between the two treatment groups and was accomplished by retroclining the upper and lower incisors while retaining the vertical position of the upper and lower molars.

In our systematic review, majority of studies presented withlow level of evidence. Very strong limitations were the absence of proper control groups, the absence of proper blinding procedures, the lack of sample randomization procedures, and the small sample sizes. One study employed malocclusion scoring scales, such as the PAR index, which presents limitations, mainly related to the generic weighting system.

Considering all the results of this systematic review it is recommended that future researchers in this fieldshould include RCTs with rigorous methodology and proper sample size in order to increase the power ofthe studies for estimating the effects. Only with this knowledge will it be possible to establish defined CATtreatment protocols for routine clinical practice.

Conclusion

Orthodontic treatment in patients with open bite can be resolved efficiently by using clear aligners

whilesimultaneously maintaining facial aesthetics. Proper diagnosis and treatment planning is a key element in achieving the desirable outcome. By means of anterior extrusion or posterior intrusion or a combination of both, clear aligners have been successful in treating anterior open bite. Some aspects inherent to the biomechanics of aligners, such as the vertical control and possible mandible counter clockwise rotation, have positive effects on the anterior open bite correction. As no randomized control trials were done in this topic, we cannot derive at a strong level of conclusion. Further clinical and experimental research is needed to statistically assess long-term stability of occlusion and skeletal relationships in such types of treatment.

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In Situ Hybridisation-A Review

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Abstract

In situ hybridisation (ISH) depends on the integral blending of labelled DNA or RNA probes with typical or irregular nucleic acid arrangements in intact chromosomes, cells or tissue segments. Compared with other molecular techniques appropriate to anatomical pathology, ISH appreciates better compatibility with histopathologists in view of its closeness to immunohistochemistry. It has the exceptional preferred position over other techniques to a great extent dependent on probe hybridization with nucleic acid removed from homogenized tissue probes - of permitting localization and representation of target nucleic acid sequences inside morphologically recognizable cells or cell structures. In spite of the fact that ISH is to a great extent an examination apparatus, it is as of now making solid advances into demonstrative histopathology. Other than recognition of infective specialists, it likewise permits localization of destinations of contamination, explanation of systems of infection transmission and spread also, examination of the connection between viral specialists and malignant growth. Sex composing, localization of qualities on chromosomes and recognition of basic and mathematical chromosomal changes in tumors are among different utilizations of ISH.

Key Words: *Probe, labelling, hybridization, nucleic acid, detection, denaturation.*

Introduction:

The utilization of molecular probes in anatomical pathology (including histopathology, cytopathology and electron microscopy) is based on the hybridization of named DNA and RNA tests with nucleic acid arrangements in tests of sick tissues. The primary methods are (1) insitu hybridization (ISH), where labelled probes are utilized to recognize integral DNA or RNA in tissue areas or smears, and (2) assessment by Southern or other blotting procedures of nucleic acids separated from homogenized biopsy material. Of these ISH appreciates better affinity with histopathologists in view of its similitude to immunohistochemistry. First portrayed in 1969 [1,2,3], its formative stage was to a great extent in the 1980s when recombinant DNA technology was applied to conquer the early issues of probe preparation.

From the useful view point, the most significant preferences of ISH to the histopathologist running a laboratory are

(1) simplicity

(2) specificity

(3) ease interpretation and

(4) its pertinence on tissue areas (frozen or formalin-fixed, paraffin-embedded) and spreads without the need for specimen collection or handling. ISH has the promising capability of turning out to be as “routine” as an immunohistochemical stain, particularly when nucleic acidprobes become financially accessible and methods for identification in formalin-fixed and paraffin-embedded tissue are consummated.

PRINCIPLE OF ISH:

ISH is the specific annealing of a labelled nucleic acid probe to complementary sequences in fixed tissues followed by visualization of the location of the probe. ISH – shows explicit nucleic acid arrangement in their cell environment[4].

STEPS IN ISH:

1. Probe preparation

2. Pre-treatment of specimens

3. Hybridization

4. Identification method[3].

Probe Preparation:

Probes:

A probe (a named complementary single strand) is joined with the DNA/RNA strands of intrigue. Strands will strengthen with integral nucleotides holding back along with their homologous components when cooled. Odds of a probe finding a homologous sequence other than the target sequence decline as the number of nucleotides in the test expands[5].

Double stranded DNA Probe:

Arranged by nicktranslation, arbitrary primer, PCR within the sight of a named nucleotide. Nick translation is a strategy for joining named nucleotides into DNA, for example, separated fragment. The strategy utilizes a blend of two enzymes, Deoxyribonuclease I which scratches the DNA making free 3 hydroxyls. DNA polymerase I, which processively includes nucleotides to the 3′ terminal hydroxyl[6]. Random preparing is a methods for marking DNA sections whereby, a blend of all potential mixes of hexamers, octamers, or nanomers are strengthened to denatured DNA. These little oligonucleotides then go about as primer that take into consideration the amalgamation of the reciprocal DNA strand by the Klenow protein and consolidation of both named and unlabeled nucleotides. More successful when the target is plentiful. Less touchy than single strand probe. Since two strands tend to hybridize to one another, accordingly diminishing the grouping of probe accessible for hybridization to the target[7].

Single stranded DNA Probe:

Single stranded DNA Probe spread an a lot bigger size range (200-500 bp) than oligonucleotide probes. They can be set up by primer expansion on singlestranded layouts by reverse transcription polymerase chain reaction (PCR) of RNA, or an enhanced primer expansion of a PCR-created fragment in the presence of a solitary antisense primer, or substance formation of oligonucleotides. PCR-based strategies are a lot simpler,probes can be combined from modest quantities of beginning material. In addition, PCR permits incredible adaptability in the decision of probe sequences by the utilization of proper primers[5].

RNA Probes:

RNA probes (cRNAprobes or riboprobes) are thermostable and are impervious to absorption by RNases. These probes are single-stranded and are the most broadly utilized in ISH. RNA tests are created by in vitro record from a linearized layout utilizing an advertiser for RNA polymerase. RNA polymerase is utilized to orchestrate RNA complementary to the DNA substrate. Single-stranded probes give favorable circumstances over doublestranded probes, the probesdoesn't self-toughen in solution, so the probe isn't depleted. Huge probe chains are not framed in solution; in this way, probe entrance isn't influenced. In the event that high sensitivity is required, singlestranded probes ought to be used[5].

Oligoprobes:

Normally, shorter 20-40 base pair length. They are created artificially by a mechanized substance combination. These probes are impervious to RNases and are little, along these lines permitting simple infiltration into the cells or tissue of interest. Little size has a burden in that it covers less targets. Label ought to be situated at the 3′ or the 5′ end. To increment sensitivity one can utilize a blend of oligonucleotides that are correlative to various districts of the target molecule. Another favorable position of oligonucleotide probes is that they are single stranded, accordingly barring the possibility of renaturation[8].

Properties of Probes:

• Probe construct: Oligonucleotide probes are better than conventional probes as a result of high specificity, single-stranded, and short probe length (10-50 nucleotides).

• The efficiency of labelling: Labeling by irregular preparing has been accounted for to be more proficient than nick translation.

• Percentage of G-C base pairs: Higher the substance of G-C matches, the higher the Tm (melting temperature).

• RNA versus DNA probes: Strength of the probetarget bond diminishes in the order of RNA-RNA, DNA-RNA, DNA-DNA.

• Probe length: Shorter the probe, the better its entrance into cells[3].

Purification of labelled probes:

There are a few techniques that can be utilized to test the purification. Here, a rundown of techniques that can be utilized yet it is fitting to follow the makers' suggestion on their utilization: Sephadex G-50 column, Sephadex G-50 chromatography, or Selective precipitation[5].

Probe concentration:

For DNA probes, the convergence of the probe will be 0.5-2 μg/ml. Oligonucleotide probes can be utilized with, or on the other hand without, acetylation. Probes without acetylation pretreatment of the sample will have a concentration of ~50-200 ng/ml and may give more extraordinary outcomes with an insignificant foundation. For probes with acetylation pre-treatment, a higher concentration of oligonucleotide probe might be utilized without bringing about vague background staining[5].

Length of Probe:

Ideal probe size for ISH is little pieces of about 200-300 nucleotides. Be that as it may, probes might be as little as 20-40 bp or as extensive as 1000 bp. As probes increment in length, they become more explicit. Longer probes may lead to more fragile sign. They infiltrates less productively the cross-linked tissues. The degree of more fragile signs and infiltration relies additionally upon the nature of the tissue, decision of fixative and whether a pre-treatment has been conveyed out[7].

Pretreatment of Specimens:

Tissue sections must hold fast well to extraordinarily treated glass slides to dodge loss of tissue during the hybridization measure. Different "adhesives" are accessible counting poly-l-lysine, gelatin chrome alum, and aminopropyltriethoxysilane[3].

Fixation:

Methanol/acetic acidfixation is suggested for metaphase chromosome spreads. Cryostat sections may be fixed with 4% formaldehyde (~30 min), Bouin's fixative, or paraformaldehyde vapor fixation. This fixation likewise assists with tying down the tissue to the slide. Most generally, tissue specimens are regularly fixed in 10% buffered formalin, processed overnight in a programmed tissue processor, and embedded in paraffin wax. Fixation season of 8-12 h is optimal[5].

Slide/sectionpreparation:

Sections are cut at 4-6 μm on aacohol cleaned microtome utilizing positively charged or hand-coated slides. Sections are depleted well and afterward air-dried at room temperature. After deparaffinization, slides are put in aalcohol cleaned staining compartment of diethyl pyrocarbonate water. The staining compartment is then positioned in the warmed water bath 23-37°C and held until the beginning of ISH. Gloves must be worn to prevent contamination, and all utensils, for example, brushes and forceps, ought to be cleaned with alcohol and kept inside the cleaned zone assigned for ISH[5].

Proteolytic digestion:

The utilization of formaldehyde-based fixatives before paraffin embedding of specimens will cover nucleic acid arrangements. Assimilation is a significant advance when performing ISH. Processing improves probe penetration by expanding cell penetrability with negligible tissue degradation[5].

Hybridization:

Molecular hybridization is the cycle whereby a singlestranded target sequence is tempered to a reciprocalsingle-stranded probe to frame a double stranded hybrid. Prior to hybridization, both the target and the probe, in the event that double stranded, must be denatured to deliver them single-stranded and this can be accomplished by warmth or alkali treatment. The accompanying denaturation, single-stranded target, and probe sequences are incubated in a hybridization blend, which gives an ideal situation to re-toughening of single-stranded sequences. Hybridization happens after denaturation, during cooling, in the presence of an integral probe, and allows hydrogen holding of the two

strands of nucleic acids[9].

Probe must shape stable hydrogen bonds with the target.All the while warming the probe and focus to high temperatures may build the consistency and sensitivity of discovery. This must be met if care is taken to accurately control this progression of the ISH procedure[9].

Post-hybridization washes:

Stringency washes after hybridization targets diminishing non specific binding. Notwithstanding, it is desirable over hybridize rigidly as opposed to wash stringently[10].

Detection Methods:

Different techniques are accessible for perception of the hybridization. Decision of detection framework will be basically controlled by the probe label utilized and second by the ISH methodology type. There are two techniques for probe labeling. Detectionmethods is of two types, direct and indirect method [11].

Oligonucleotide probe labeling:

• 5′-end labeling: The 5′ end of DNA or RNA goes through direct phosphorylation of the free 5′-terminal OH groups. The free 5′-OH substrates can be named utilizing T4 polynucleotide kinase. This strategy is normally utilized for radiolabeling. Non-radiolabels utilize a covalent linker [12].

• 3′-end labeling: Terminal dexoxynucleotidyl transferase (TdT) is utilized to include a labeled buildup to the 3′ end of an formed oligonucleotide that is around 14-100 nucleotides long. These tests give phenomenal explicitness yet just moderate sensitivity [5].

• 3′ tailing: A tail containing labeled nucleotides is added to the free 3′ end of double or single-stranded DNA utilizing TdT. These probes are more touchy than the 3′-end labeled adaptations, however can deliver more vague background [13].

Enzymatic detection:

Hybridized probes can be distinguished by enzymatic responses that produce a colored substrate at the site of hybridization. The most ordinarily utilized enzymes for this application are alkaline phosphatase (AP) Or horseradish peroxidase (HRP) Although these enzymes can be formed legitimately to nucleic acid probes, such enzyme coupled probes are regularly improper for ISH to tissue arrangements since probe infiltration is hampered by the presence of the conjugated enzyme. In this manner, indirect methods are preDNAprobe and a target grouping preferred[14].

Autoradiography detection:

Responses utilizing radioactive named probes are identified by autoradiography. This depends on the discharge of fastelectrons or beta-particles from the probe. Beta particles discharge a lot of energy when they hitwith atoms of an emulsion included to the section on the slide. The over energy delivered lessens ionic silver present in the emulsion to metallic silver. At the point when this occurs, a dedicated record of the area of the impact between an electron and the silver particles in the emulsion is created in the type of a latent image. This picture, when envisioned is the marker of the probe location in the tissue or cell. Autoradiography for radioactive labels is presumed to be more touchy than the immunoenzyme systems[3].

Fluorophores:

Fluorophores can be related with nucleic acid probes by compound formation to the nucleic acidor compound formation of the nucleic acid with a non-fluorescent particle that can bond fluorescent material after hybridization. The previous technique is called “direct labeling” and the last strategy is called “indirect labeling.” Chemical structures of four normal fluorophore classes (A-D) fluoresceins, rhodamines, cyanines, or coumarins[7].

Indirect method:

By implication through fuse of a nucleotide analog conveying a reactive group and ensuing biotinylation/ digoxigenylation. Coming about biotin-labeled probes are at that point distinguished utilizing streptavidin (KD = 10-15 M) formed with HRP or AP. Digoxigenylation is commonly pictured by HRP-or AP changed antibodies[15].

Multiple ISH:

More than one probe can be applied to a similar

tissue area to distinguish distinctive nucleic acid targets. By utilizing diverse identification systems with each probe, coming about in various colored end products, and perception of the diverse nucleic acid targets can be achieve[16]. The common advantages of ISH are,

1. Effortlessness of its procedure

2. Particularity of results got

3. Simplicity in understanding of discoveries

Its relevance on tissue sections (frozen or formalinfixed, paraffin-embedded) and spreads without the need for special specimen collection or processing[3].

Applications OF ISH:

1.Determination of Infective Agent:This depends on the discovery of the infective agents genome in the tissues or cells considered. Explicit composing of infective specialists likewise has significant ramifications for epidemiological reviews and episode investigations[17].

2 Location of Active Infection:The real cell or cell structures holding the infective genome can be explained by ISH, for example hepatitis B infection (HBV) in hepatocytes, parvovirus in cells of the lung[17].

3.Explanation of mechanism of Virus Dissemination and Transmission: common horizontal and vertical transmission courses of infections can be considered. For instance, the presence of Epstein-Barr infection (EBV) in epithelial cells of the oropharynx gives a way to transmission of the virus through saliva[18].

4. Localization of Persistent Virus Infection:Models are the constancy of JC virus in oligodendrocytes in progressive multifocal leukoencephalopathy and measles infection in neurons and glia cells in SSPE[19,20].

5.Link between Virus Agents and Carcinogenesis: Etiological function of different viruses in cancers and mechanism of malignant transformation of cells. The better realized affiliations are EBV and nasopharyngeal carcinoma and B-cell lymphomas[21], HBV and hepatocellular carcinomas[22],and human papillomavirus furthermore, cervical carcinoma[23].

6.Investigation of cell Development:ISH detection of cell-type explicit RNA in cells which do not display morphological separation can be applied to recognize the cell type[24].

7.Sex Determination:The Y chromosome can be distinguished through hybridization [25].

8.Human Gene Mapping:In situ Hybridisation (ISH) utilized in human quality planning[26].

9.Interphase Cytogenetics:ISH can be utilized to recognize numerical chromosomal variations in interphase cores. Probes perceiving profoundly monotonous sequencesin chromosomes 1, 7, 8, 9, 10, 15, 16, 17, 18, X and Y are currently available[3].

Conclusion

ISH has the extraordinary favorable position over other molecular biology procedures of permitting localization and representation of target nucleic acid sequences inside morphologically recognizable cells or cell structures in a heterogenous cell population. Such explicit localization has a stamped advantage over more seasoned molecular techniques where discovery of the nucleic acid objective from a tissue homogenate precludes identification of the real influenced cell, energy about cell detail and relations and subsequent reconstructive interpretation of findings.

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Oral Manifestations of Hyperparathyroidism and Its Management-A Review

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Abstract

Endocrine system is the important system of human body responsible for maintaining the homeostasis and some other physiological functions by releasing hormones from the endocrine gland. Parathyroid gland is one of the important endocrine glandproducing parathyroid hormone. Parathyroid hormone plays an important role in maintaining the calcium and phosphorous metabolism and has a direct influence over the mineralization of bone and teeth. Disorders in parathyroid gland such as hyperparathyroidism and hypoparathyroidism causes altered levels of secretion of parathormone which may cause various symptoms including the oralmanifestations such as brown tumor, loss of bone density, increased caries occurrence rate, high risk of bone fracture etc. The aim of this article is to creating the awareness among the dental professionals about the risk and preventive measures in treating the patients with hyperparathyroidism.

Key Words: *Parathyroid hormone, hypercalcemia, Brown tumor, chronic renal failure, bone fracture.*

Introduction

Parathyroid gland is one of the endocrine gland which produces parathyroid hormone (PTH). Parathyroid gland consists of two pairs of four glands which are located in the back side of the thyroid gland, two numbers in one side and two on the other side. Parathyroid hormone functions to maintain the homeostasis of calcium and phosphorous metabolism. Hence it plays an important role in mineralization of bone and teeth, regulates bone resorption, activate synthesis of active form of vitamin D in kidneys which makes the intestinal epithelium to absorb the calcium more into the blood [1].

Disordersofparathyroidincludehyperparathyroidism and hypoparathyroidism. Hyperparathyroidism (HPT) was first described by Von Recklinghausen in the year 1891. Its normal prevalence rate is 0.05-0.1% of the common population [1]. It is more common in middle aged female than male. Hyperparathyroidism (HPT) is the abnormal production of parathyroid hormone which causes unusual effects such as hypercalcemia and increased osteoclastic resorption. Hypoparathyroidism is the decreased or no production PTH which results in symptoms such as dental abnormalities, paraesthesia of tongue etc.

HYPERPARATHYROIDISM:

Hyperparathyroidism is characterized by oversecretion of PTH. HPT is divided into three categories[2-5]:

1.Primary hyperparathyroidism

2.Secondary hyperparathyroidism

3.Tertiary hyperparathyroidism

Primaryhyperparathyroidism:Normally brought about by a tumor (adenoma in 85% of all cases) or hyperplasia of the organ that creates an increased activityof PTH discharge bringing about hypercalcemia and hypophosphatemia.

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Secondaryhyperparathyroidism:At the point when the parathyroid organs are stimulated to secreteexpanded amount of hormones to equalise abnormal low serum calcium levels in various physiologic or pathologic conditions like renal dysfunction, intestinal malabsorption condition, decreased Vitamin D synthesis, in this manner coming about in parathyroid hyperplasia.

Tertiary hyperparathyroidism:At the point when long-standing secondary hyperplasia becomes self-ruling despite rectification of the underlying stimulant(renal transplant)[2].

SIGNS AND SYMPTOMS:

In Primary HPT, about half of patients have no indications and the issue is gotten as a coincidental finding (through raised calcium or characteristics X ray appearances [subperiosteal resorption of the phalanges of the index and middle fingers]). Numerous different patients just have vague symptoms.[3] These are musculoskeletal issues (weakness, back pain, muscle soreness), gastrointestinal disorders, e.g., vomiting, nausea, constipation, loss of appetite.

One of the principle clinical appearances of HPT is bone sickness. The ribs, clavicles, pelvic support and mandible are the bones generally included. A pathologic fracture might be the primary indication of the infection. Bone tenderness and joint firmness may be present. Renal calculi are a typical finding in this condition. Practically all patients with HPT have skeletal disorders in the serious stages. The loss of calcium in this condition brings about summed up osteoporosis.

ORAL MANIFESTATIONS:

In the oral cavity, the most well-known clinical signs of HPT isbrowntumor; loss of bone thickness, mobile teeth, floating of teeth, unclear jaw bone pain, sensitive teeth during occlusion and percussion, soft tissue calcifications and dental defects, for example, developmental defects and changes in dental eruption[3,6-10]. Malocclusion because of floating of teeth, with definite spacing of the teeth might be one of the principal indications of the disorder. Pseudocysticlesions can also present, radiolucentlesion at the apex of the tooth misdiagnosed as periapical cystor granuloma[11,12]. It doesn't appreciably affect periodontal records and radiographic bone height[13].

Chronic renal failure and secondary HPT additionally show infrequently, as maxillary hyperplasia and hyperostosis cranialis, progressive augmentation of the facial bones over a period. These facial amplifications cause an unusual facial deformation and dental malocclusion[14-17]. Primary HPT likewise seldom presents in the mandible as an enormous exophytic mass or painless swelling[18], and furthermore as a giant cell epulis as an underlying element of primary HPT[19]. Primary HPT patients were bound to have tori[20].

BROWN TUMOR:

Browntumorlesions[17,21-23] might be the soonest appearance of this undiscovered HPT in 6% of the cases. Browntumor presents as osteolytic injury (which might be related withpain and swelling) that creates because of changesin bone metabolism brought about by high serum level of PTH. It is an erosive bony lesions brought about by quick osteolysis and peritrabecular fibrosis bringing about a neighbourhood destructive process.

Brown tumorpresents itself as a friable red-brown mass. Its name is because of shading that it takes from the haemorrhagic infiltrates and hemosiderin stores that are frequently found inside. Bone injury may create huge cortical development.Mandible contribution is normal, particularly in the region of premolars and molars, and it is uncommon in the maxilla. It happens mostly in secondary HPT patients with chronic renal failure or renal osteodystrophy[24], yet it has additionally been depicted as a uncommon indication of calcium malabsorption, a few types of osteomalacia and of primary HPT[25,26].

Radiographically, lesions are portrayed by all around characterized unilocularor multilocular radiolucent regions. The trademark radiographicfindings show anwidespread loss of the lamina dura and changes in thetrabecular bone pattern of the jaws. Long-standing injury regularly creates a vital extension of cortical bone, root resorption and displacement of roots.

Histologically, it is portrayed by aabundant stroma, comprising of packs of spindle or oval cells, and a few multinucleated osteoclast-like giant cells. Calcified material can be found, just as regions with

extravasations of red blood cells and pigmentation by hemosiderin.Differential diagnosis must be made with other lesions[24], for example, an aneurysmal bone sore, cherubism, centralgiant cell granuloma.Sagliker disorder is realized that skeletal changes due to secondary HPT can be serious in chronic kidney disease [27,28].

Hyperparathyroidism-jaw tumorsyndrome is an uncommon disease, different individuals from the family are likewise included, and there is danger of harmful illness. The Patient gave bilateral or recurrent mandibular radiolucenciesanalyzedhistopathologically as cemento-ossifying fibromas. It is expected to be due tomutation of gene[29].

RADIOGRAPHIC FINDINGS:

Oral Radiographs of hyperparathyroid patient shows [20,30-33] as loss of medullary trabecular example, jaw shows up finely radiopaque portrayed as clear "ground glass" appearance and almost as summed up rarefaction.

DIAGNOSIS:

Serum parathyroid hormone (normal value 15-65 pg/ml) and calcium (Normal value 9-11 mg/dl) :

When a raised PTH has been affirmed, objective of determination is to decide if the HPT is primary or secondary in beginning by acquiring a serum calcium level. High serum calcium level due show primary HPT. Though, low or ordinary calcium level may demonstrate secondary HPT. Tertiary HPT has a high PTH and high serum calcium. It is separated from primary HPT by a background marked by chronic kidney failure and secondary HPT.

Serum phosphate (Normal value: 2.4-5 mg/dl):

In Primary HPT, serum phosphate levels are very low because of diminished renal tubular phosphate reabsorption.Nonetheless, this is just present in about half of cases, as dietary admission likewise impacts phosphate level. This differences with secondary HPT, in which serum phosphate levels are large as a result of renal disease.

Alkaline phosphatase (Normal value: 500-750 IU/L):

Alkaline phosphatase levels are generally raised in HPT. In primary hyperthyroidism, levels may remain within normal, notwithstanding, this is "improperly typical" given the expanded degrees of plasma calcium.

DENTAL MANAGEMENT OF THE PATIENT WITH HYPERPARATHYROIDISM:

The dental management of these patients doesn't need any exceptional thought. We should realize that there is a higher danger of bone fracture, so we should avoid potential risk in surgical treatments. It is essential to perceive the presence of the brown tumor and to play out a right differential analysis so as not to direct a deficient treatment.

The treatment of HPT is the initial phase in the management of the brown tumor, as unconstrained relapse of the lesion regularly happens. In any case, a few instances of browntumor that didn't vanish or even developed after standardization of PTH level have been accounted for. In these cases, browntumor resection ought tobe the favourable treatment[2,3,34-36].

Jaw amplification is treated by recontouring of the maxilla and mandible. A three-dimensional recreation of the computed tomography (CT) filter was useful in assessing the facial deformations and in treatment planning[14].

A careful clinical and radiographic assessment is fundamental before giving endodontic treatment. The investigations or diagnostic procedures must start with an efficient survey of the clinical history. There are various systemic diseases that can cause bone injuries all through the body. In a few cases, these lesions show up in the periapical area of teeth and can prompt a misdiagnosis of an lesion of endodontic origin.[1,2,12] The finding of periapical radiolucency on a radiograph ought not consequently lead to access opening, root canal treatment by the dental specialist.

ROLE OF DENTIST:

The dentist assumes significant part in the diagnosis of HPT. Occasionally, the main indication of the illness might be a growth in the jaw. The disease ought to be considered by the dentist at whatever point single or multipleradiolucencies are seen on radiography of the jaw. Oral manifestations and dental radiographs of jaws are of incentive in early recognition of secondary HPT

because of renal osteodystrophy.

Concluision

For a dental health professional, having knowledge about the oral and systemic manifestations of parathyroid disorders and precautions need to be taken while treating the parathyroid disorder patients are of utmost important. The lack of knowledge for dentists about the parathyroid disorders results in the serious complication of the patients. The aim of this article is to review the facts about hyperparathyroidism their oral manifestations and management of those patients in dental office.

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Influence of Socioeconomic and Working Status of the Parents on the Incidence of their Children's Dental Caries

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Abstract

Dental caries, or tooth decay, is an infectious process involving breakdown of the tooth enamel.Children are susceptible to caries as soon as the first teeth appear, which usually occurs around age 6 months.It is known that socioeconomic status affects the health-related quality of life of children.Family income, occupational status, and educational achievement are socio-economic status indicators that have been found to affect the life opportunities of an individual.

Keywords: *Dental caries, incidence, children, socioeconomic background, parents*

Introduction

Physical and mental health, cognitive development, as well as social behaviour of children are believed to be strongly influenced by the social status and functioning of their family. A family's functioning reflects the composition and characteristics of its members, their talents, disposition, and daily activities. Social status is reported to be a powerful determinant that governs a child's health. However, the mechanisms by which social status might influence health remain under-documented. By and large, three measures of social status acting as predictors of adverse health outcomes are assessed, that is, income, education, and occupation or a combination of these factors.[1][2] In addition to the social status, the quality of time parents dedicate towards upbringing their children can also impact health status and developmental outcomes. This is specifically relevant in developing good oral hygiene practices among children.[2]

PREVALENCE OF DENTAL CARIES IN PEDIATRIC POPULATION

In many developing countries, prevalence of dental caries is on the rise. In India, the National Oral Health Survey and Fluoride Mapping found a high prevalence (51.9%) of dental caries in primary dentition of 5-year-old children.[3] Dental caries is the most prevalent oral disease and it remains the single most common disease of childhood that is not compliant to short-term pharmacological management.[4] More than eighty percent of the paediatric population is affected by dental caries by the age of seventeen.[4] Its very high morbidity potential has brought this disease into the core of the dental health profession. There is practically no geographic area in the world whose inhabitants do not manifest some evidence of dental caries. It affects both gender, all races, people from all socioeconomic status and all age groups.[5] It does not only cause pain and discomfort, but also in addition, places a financial burden on parents of affected children. Dental caries is of multifactorialetiology, but the two main factors that can have a devastating effect on oral health of children are the social status and parental care. The socioeconomic status will have an impact on the knowledge and attitude of parents[6] and hence, can impact parental care.

INFLUENCE OF PARENTS' SOCIOECONOMIC STATUS ON CHILD'S ORAL HYGIENE STATUS

Socio-economic inequalities have been evident in many diseases and combating them has in recent years emerged as a major health policy goal among international agencies. Explaining mechanisms behind social stratification of health and ill health has also been identified as a priority for social research.[7] In dental health, the socioeconomic status has been recognised for

years as a main factor for inequalities.[8,9]

In different areas in the Western world, caries has been shown to be more frequent in the lower socioeconomic groups, with the more affluent having lower experience of caries.[10-14] The risk of developing new caries is higher when there is already established caries.[11] According to various studies, children of higher socioeconomic group were at a lower risk of caries; however, children from lower socioeconomic status family showed a greater risk for developing dental caries.[12-16] A study conducted by Gokhale et al showed that children with both the parents employed or working had increased the risk to dental caries.[13] A Brazilian study reported that children who have fathers with a high occupational status and stay-at-home mothers presented the lowest caries index.[14]

Individuals from lower socioeconomic status experience financial, social, and material disadvantages. The access to healthy environment, social resources such as medical and dental care is compromised. This, together with fatalistic beliefs about their health and lower perceived need for dental care, leads to neglected self-care, and lower utilization of preventive health services.[15,16,17] Socioeconomic status is such a robust finding that it is a "critical determinant of health" and has been emphasized to be included in planning interventions.[18,19,20,21] However, in India, access to dental care varies among children.[22]

Economic status has significant influences on a child's development. The income of parent or parents directly influences the quality of care and the quality of life a child has. Although parental employment will fulfil the basic needs of a child, nevertheless, when both parents work, they must seek non-parental care of some type including day-care, care by extended family, or self-care.[23] When both the parents are employed, they hardly find time to spare for their children and to look after their general health and specifically the oral health care.[24,25,26] Hence, better attention should be paid to the child's oral health and the parents must try to strike a balance between work and providing care to their children.[27]

Conclusion

Since oral hygiene is essential for good oral health, it is wise to educate the population with adequate guidelines on children's oral health behaviour and its relationship with dental caries. It is beneficial to address the factors that influence children's oral health in order to develop and implement complementary public health actions focused on children and parental behaviours, in an endeavour to provide them with good oral health and better quality of life. Efforts by the governmental and nongovernmental organizations are obligatory to implement programs at school level to enhance oralhealth care among children. The assessment of perceived needs by the use of quality of life questionnaires as well as socioeconomic parameters can assist the planning of oral health programs aiming at decreasing any unnecessary and unavoidable inequalities in the distribution of dental caries in populations of different socioeconomic conditions.

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Recent Advances in Cytology-A Review

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Abstract

In olden days, specificity and sensitivity of oral cytology is poor. Utilizing traditional oral cytology for the conclusive diagnosis of malignancyand premalignant lesions. But with the recent cytological techniques paves cytology to conclude a final diagnosis. This reestablished interest is somewhat because of the presentation of a specific brush that gathers a full-thickness epithelial sample and not simply sloughed cells, just as examination of that sample with computer support and analysis. There is an assortment of adjunctive strategies have been acquainted with possibly upgrade the finding of the cytologic examples including DNA examination, immunocytochemistry, molecular analysis, and liquid based preparations. An expansion in sensitivity (>96%) and specificity (>90%) of the oral brush biopsy with computer support, diagnosis has been accounted for detection of malignant and premalignant lesions. Brush cytology is significant to forestall misdiagnosing suspicious oral lesions.

Key Words: *Brush biopsy, malignancy, cytobrush, cytopathology, dysplasia.*

Introduction

Oral squamous cell carcinoma (OSCC) is related with high morbidity and mortality, which is expected, in any event in part, to late detection[1]. Early diagnosis and treatment of OSCC are the best mediations for improving patient's welfare and living quality of life. In spite of the checked advances in treatment of different malignancies, the future of patients with oral malignancies has not improved for the last 50 years. OSCC is the 6th most common malignancy in the world. It is assessed that 36,540 (25,420 men and 11,120 ladies) new cases of oral cavity and pharyngeal malignancies will be analyzed in the United States during 2010, while 7,880 (5,430 men and 2,450 ladies) patients will die of the disease[2].

It is notable that most of OSCC, if not all, create in precancerous fields described by explicit genetic alterations. Transepithelial ''field mapping biopsies'' within widespread lesions are much more fundamental for cytological assessment and further investigation[3]. Precancerous and cancerous oral lesions may imitate any number of considerate oral lesions showing up as a white or red lesions (leukoplakia, erythroplakia, and erythro/leukoplakia)[4]. The malignant transformation of these lesions is for the most part surveyed by histopathology dependent on the presence and the level of dysplasia in biopsy material, graded as gentle, moderate, and severe[5].

Although conventional cytology was utilized for assessing oral lesions as far back as 1963[6], it has not been generally embraced and has fallen into unsavoriness in many centers in view of helpless affectability and explicitness for recognizing dysplasia and malignancy. During the 1980s, a cytobrush was presented for cervical smears in gynaecological lesions. This procedure improved the way toward spreading cells on to slides, contrasted and spreads got by utilizing a wooden spatula, in this manner improving the quality of the smears[7]. Additionally, sampling of deeper mucosal layers with insignificant invasion was conceivable, particularly, cells from the basal and parabasal layers, where most cervical

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intraepithelial lesions or squamous intra-epithelial lesions normally develop[8]. The transformation of the cytobrush for oral cancer diagnoses restored significant enthusiasm for oral cytology. From that point forward, different investigations have been distributed portraying diverse symptomatic procedures that have improved the sensitivity and specificity of regular oral cytology[9-12]. This article reviews the improvements in oral cytological finding throughout the long term and fundamentally inspects the more recent adjunctive procedures that have been utilized toimprove these outcomes.

RECENT ADVANCES IN CYTOLOGICAL TECHNIQUES:

The brush end up being a more helpful instrument to the analyst contrasted and the wooden spatula when managing oral lesions[13].Furthermore, the brush has been utilized to diagnose other oral lesions like oral candidiasis, epithelial disease because of Epstein-Barr infection in oral lesions of hairy leukoplakia, pemphigus, Herpes simplex infection (HSV), and radiation reaction [14].

The significance of brush biopsy in assessing benignlooking oral lesions that would have been ''viewed'' and not tried has been accentuated in a multicentre study where almost 5% of such lesions which were inspected by utilizing brush biopsy, examined by picture investigation, and later affirmed by utilizing scalpel biopsy to speak to dysplastic epithelial changes or invasive cancer [15]. If a malignancy covers an enormous zone, it is essential to cautiously select the most proper site of the scalpel biopsy. Consolidated utilization of toluidine blue staining and brush cytology has likewise been tried to help restrict the correct site for brushing an lesion. This blend was discovered to be exceptionally delicate and reasonably specific for malignant lesionswith false negative rate of 6% however less delicate for the premalignant lesions [16]. Remmerbach et al propounded both the convenience ofcytology for diagnosing doubtful lesions and the daily practice use of the AgNOR (nucleolar organizer regions) examination to decide the nucleolar action of oral malignancy[17].

LIQUID BASED CYTOLOGY:

Liquid based cytology (LBC) is an specimen filtration strategy that was initially evolved to give a nearmonolayer of superficial cervical cells that could be more effortlessly assessed by a cytotechnologist or a basic computer looking at Papanicolaou smears. Use of LBC on oral smears gathered by utilizing a cytobrush has been professed to show noteworthy improvement in cell dispersion and smear thickness, prompting for easier detection of abnormal cells[18]. The flimsy layer planning licenses less tedious manual investigation, likened to cervical fluid based arrangements. Reports of consolidated utilization of an intrusive dermatological curette and the LBC on oral carcinomas indicated an affectability of 95.1% and an explicitness of 99.0%[19].

Kujan et al demonstrated that immunocytochemical staining of oral cells dependent on LBC slides is likewise plausible. All the stained slides in their examination were steady and introduced great immunoreactivity for fragile histidine trialgene (FHIT). The human FHIT quality is a tumor suppressor gene and was recognized at 3p14.2. Similarly, human papillomavirus (HPV) location in oral LBC samples was additionally discovered to be solid[20].

Keratin layer, regularly very thick, keeps malignant cells from arriving at the superficial layer delivering conventional oral cytology insufficient for distinguishing dysplasia and assessing suspicious white oral lesions. Hence, a conventional oral cytology specimen tests just the superficial layer and not the whole thickness of the epithelium, which is needed to excludethe presence of oral dysplasia or carcinoma, and the specimen will be inadequate regardless of how it is readied, conventional or LBC. Besides, LBC was intended for superficial cytology samples and not biopsies and prompts the degeneration of epithelial parts and the threedimensional presentation collected by a brush biopsy (microbiopsies), which may forestall appropriate microscopic assessment of the biopsy[21]. With these impediments, it is likely that LBC for diagnosing oral lesion is of negligible esteem.

AUTOMATED ANALYTICAL METHODS:

Cytomorphometry. OralCDx (OraCDx Laboratories, Suffern, NY) is a computer supported method for the investigation of cell samples gathered by utilizing a protected brush. This strategy was intended to assess any oral epithelial anomaly without a conspicuous etiology for dysplasia or cancer[22].The improvement over traditional cytology is because of the brush, which

gathers a total transepithelial sample, including the basal layer, and the investigation of that sample with computer support. The computer investigates the examined computerized microscopic picture of the gathered cells utilizing a particular neural network-based picture preparing framework explicitly intended to distinguish oral precancerous and malignant cells. Measures of atypia incorporate cell keratinization and morphological deviations. Speight investigated the different models for grading of to malignancy[23]. The diagnostic outcomes and agent samples are introduced to a cytopathologist who makes the final diagnosis and proposes follow-up to the clinical specialist for example, clinical close perception, redo brush biopsy, scalpel biopsy, and so forth.

HPV AND ORAL CYTOLOGY :

The part of HPV infection in the etiopathogenesis of oral potentially malignant and malignant lesions has been widely concentrated as of recent times. HPV (commonly HPV16) is presently perceived to assume a function in the pathogenesis of a subset of oral cavity carcinomas, especially those that emerge from the lingual and palatine tonsils inside the oropharynx just as the base of the tongue[24]. The location of HPV in the oral mucosa may likewise be cultivated by cytology, and the cytologic discoveries are described by koilocytosis, perinuclear cytoplasmic haloes, nuclear dysplasia, atypical juvenile metaplasia, and binucleation. Adjunctive procedures of distinguishing HPV that have been utilized remembers for situ hybridization and immunohistochemistry[25].

DNA ANALYSIS:

The ploidy status of oral cells is imperative to anticipate the lesions that may go through malignant change. Subsequent to staining with Feulgen dye, the cytological examples are thought about with a reference group of 300 ordinary epithelial cells of the oral mucosa. DNA picture cytometry measures DNA ploidy to decide the malignant potential of cells. A computer supported examination has been created to recognize deviations of cell DNA content. Utilizing this strategy, an expansion in sensitivity and specificity of oral cytology to 100% has been reported[26]. Data on exactness in high-hazard oral neoplasia are, be that as it may, restricted. Moreover, in light of the fact that Oral Advance uses a delicate cytobrush, it is improbable to collect basal cells.

Semi-automated multimodal cell analysis (MMCA) is other analysing technique for the early diagnosis of malignancy for cases with a minimal number of doubtful cells[27]. Application of Feulgen staining, DNA image cytometry, and finally silver-nitrate staining for argyrophilic nucleolar organizing regions (AgNORs) analysis were used in conjugation to identify early malignant transformation.

SPECTRAL CYTOPATHOLOGY:

Spectral cytopathology (SCP) is a novel methodology for demonstrative separation of disease in individual peeled cells. SCP is done by gathering data on every cell's biochemical arrangement by means of an infrared microspectral estimation, trailed by multivariate information examination. Deviations from a cell's characteristic content produce specific spectral patterns that are restrictive to the reason for the deviation or sickness. These exceptional spectral patterns are reproducible and can be distinguished and utilized through multivariate measurable strategies to identify cells compromised at the molecular level by dysplasia, neoplasia, or viral infection. In an ongoing confirmation of-idea study, a benchmark for the affectability of SCP was set up by characterizing solid oral squamous cells as indicated by their anatomical inception in the oral cavity[28]. SCP depends on computerized instrumentation and unaided programming, and it is asserted to establish a diagnostic workup of clinical samples destitute of inclination and irregularity.

NANOCHIP-BASED SYSTEMS:

Weigum et al utilized a solitary nano-bio-chip stage for molecular and morphologic investigation in oral exfoliative cytology to upgrade the role and usage of oral cytology in clinical diagnostics. The coordination of the nano-bio-chip sensor framework for simultaneous and quantitative investigation of cellular biomarkers and cytomorphology has been studied in 41 patients and 11 controls for multifunctional cytoanalysis. They discovered six findings to be fundamentally adjusted in OSCC cytospecimens versus normal mucosa, including (a) nuclear area, (b) nuclear diameter, (c) cellular area, (d) cellular diameter, (e) nuclear-to-cytoplasmic ratio, and (f) EGFR biomarker immunolabeling. These parameters were additionally discovered to be altogether modified in oral lesions with diagnosed dysplasia, supporting the

utilization of these markers as diagnostic marker of early malignant growth improvement and premalignancy[29].

SALIVARY DIAGNOSTICS:

Oral epithelial cells regularly shed and can be recognized in salivation and oral washes, making cytologic and molecular examination of this liquid appealing for oral cancer screening. The level of apoptotic cells has been estimated in cytological investigations of the salivation of patients treated for OSCC and could be helpful for observing responses to chemotherapy[88]. Several little investigations have demonstrated contrasts in DNA and RNA expression patterns and microsatellite motifs between groups of oral malignancy patients and ordinary control subjects[30].

Conclusion

Traditional cytology has very low sensitivity that can be enhanced. The presentation of cutting edge cytologic strategies, including brush biopsy, computerassisted examination of brush biopsy tests, just as molecular and immunohistochemical procedures have made cytology an important tool in assessing oral lesions. Brush biopsy is especially valuable to assess any oral lesion without an undeniable etiology and prevents pathologist to misdiagnose a malignant lesion as normal mucosa. Besides, brush biopsy is particularly helpful when a lesion is enormous, when various lesions are present, or when patients deny surgical blade biopsy. This is a moderately economical, straightforward, noninvasive, hazard free procedure, demonstrated to be exceptionally exact and all around acknowledged by patients. All things considered, its inescapable use may bring about recognizing precancers and early cancer at stages when they can be most effectively treated.

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Sterilization and Disinfectants Used in a Dental Office- a Review

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Abstract

As dentists, we must disinfect and clean all equipments and countertops between patients with a recommended hospital-grade bactericidal product. Nonvisible blood and saliva does not mean it is absent on environmental surfaces. Blood and saliva are present throughout dental treatment. If a patient becomes ill as a result of an exposure to a pathogen or a bacterium that is later found in our dental office surfaces, the employer and or dentist is at risk of being responsible for the patient's illness and associated compensatory damages. Understanding and recognizing the risk of disease will drastically reduce patient illness, practice liability and employee illnesses. Dentists cannot pretend that this is only a theoretical problem. Therefore the dental community needs to stay updated of the rapidly evolving standard of care and do its best to comply with the standard of care in daily practice.

Key Words: *Sterilization, Chemical Disinfection, Fumigation, Pathogens.*

Introduction

Sterilization is the killing or removal of all microorganisms, including bacterial spores which are highly resistant. Sterilization is an absolute term, i.e. the article must be sterile meaning the absence of all microorganisms'

Disinfection is the killing of many, but not all microorganisms. It is a process of reduction of number of contaminating organisms to a level that it prevents infection that is, all pathogens must be killed. Some organisms and bacterial spores may survive[1].

Breaking the chain of infection would be an avenue in reducing the spread of infectious disease. Hands touch many objects throughout the day and germs are easily transferred to surfaces, causing cross-contamination. Bacteria will not survive inside the material/clothing. Sneeze into the sleeve, covering both the nose and the mouth. Another way to break the chain of infection is through frequent hand washing.

The four following things are necessary for infection to occur:

· The presence of a pathogen (infection causing agent) in sufficient quantity to lead to infection

· Multiple source of infection are: human waste, animal waste, insect waste, environmental waste.

· An entry point or mode of transmission examples are: an open wound

· A person's (host's) susceptibility to the pathogen

Certain surfaces, that too the ones touched often (e.g., light handles, unit switches, and drawer knobs) can serve as reservoirs of microbial contamination, although they have not been linked directly with spread of infection to either dental health-care professionals (DHCP) or patients. Cleaning and disinfecting environmental surfaces plays a major role in patient's health in any dental facility. Microorganism transfer from contaminated environmental surfaces to patients

occurs primarily through Dentists hand contact. When surfaces are touched, microbial agents can be transferred to the patients or other workers or to the dental office environment or dental instruments. Although hand hygiene is key to minimize this type of transfer, disinfecting and cleaning of environmental surfaces also protects against health-care–associated infections.

Environmental surfaces are divided into housekeeping surfaces and clinical treatment surfaces. Surfaces such as floors, walls, and sinks are housekeeping surfaces and have limited risk of disease transmission. Action plan for cleaning and disinfecting surfaces in patient care areas should have written standard operating procedures that consider the following:

- The potential for direct patient contact
- The degree and frequency of hand contact
- Potential contamination of the surface with environmental sources or body substances containing microorganisms.

Cleaning is the necessary first step of any disinfection protocol. Cleaning is a form of decontamination that renders the environmental surface safe by removing organic material and visible soils, all of which interfere with microbial inactivation. If a surface has not been cleaned first, the success of the disinfection process can be compromised. If a surface cannot be cleaned adequately, it should be protected with barriers. Barrier protection of surfaces and equipment and small buttons or switches can prevent contamination of clinical surfaces including those hard to clean.

When barriers are not being used, surfaces should be cleaned first, then disinfected between patients using an EPA-registered hospital disinfectant with an HIV, HBV claim (low-level disinfectant) or a tuberculocidal claim (intermediate-level disinfectant). An intermediate-level disinfectant should always be used when a surface is visibly contaminated with blood or other potentially infectious material (OPIM). As stated earlier in this article, environmental surfaces can become contaminated via sneezing and coughing, which can contaminate work surfaces. This, in turn, can cause the transmission of disease. Potentially infected bacteria or blood and saliva cannot always be seen. Treatment areas should be kept free from clutter (e.g., papers, supplies, and equipment) to expedite cleaning and disinfecting treatment areas between patients.[2]

Mycobacterium TB (Mtb) has been a benchmark for years in dentistry. Mycobacterium TB is a bacterium that is hard to kill because of its cell wall, which is 60% lipid. This TB bacterium is an aerobe; to survive it needs oxygen and can withstand weak disinfectants and survive in a dry state for weeks.TB is transmitted via an airborne route. Potency against Mtb has been recognized as a substantial benchmark to measure germicidal potency. Mycobacterium has the highest intrinsic levels of resistance among the vegetative bacteria, viruses, and fungi; germicides with a tuberculocidal claim on disinfectant labels are considered capable of inactivating a broad spectrum of pathogens, including such less-resistant organisms as blood borne pathogens (e.g., hepatitis B virus, hepatitis C virus, and HIV).[3]

Each dental practice has different types of clinical surfaces: contaminated instruments, contaminated bib holders, hands, gloves, protective eyewear/face shields, and masks directly touch these surfaces.

Discussion

1. Chemical Disinfectant Groups

a. Aldehydes: (Formaldehyde, Paraformaldehyde, Glutaraldehyde)

Formaldehyde – and its polymerized solid paraformaldehyde have broad-spectrum biocidal activity and are both effective for surface and space decontamination. As a liquid (5% concentration), formaldehyde is an effective liquid decontaminant. Its biocidal action is through alkylation of carboxyl, hydroxyl and sulfhydryl groups on proteins and the ring nitrogen atoms of purine bases. Formaldehyde's drawbacks are reduction in efficacy at refrigeration temperature, its pungent, irritating odor, and several safety concerns. Formaldehyde is presently considered to be a carcinogen or a cancer-suspect agent according to several regulatory agencies. The OSHA 8-hour time-weighted exposure limit is 0.75 ppm.

Paraformaldehyde – is a solid polymer of formaldehyde. Paraformaldehyde generates formaldehyde gas when it is depolymerized by heating to

232 to 246°C (450 to 475°F); the depolymerized material reacts with the moisture in the air to form formaldehyde gas. This process is used for the decontamination of large spaced and laminar-flow biological safety cabinets when maintenance work or filter changes require access to the sealed portion of the cabinet. A neutralization step, heating ammonium carbonate, is required prior to ventilation of the space. Formaldehyde gas can react violently or explosively (7.0 – 73% v/v in air), when exposed to incompatibles, therefore, only individuals that have specific training and have been approved by the Dept. of Environmental Health & Safety are permitted to use this gas.

Glutaraldehyde – is a colorless liquid and has the sharp, pungent odor typical of all aldehydes, with an odor threshold of 0.04 parts per million (ppm). It is capable of sterilizing equipment, though to effect sterilization often requires many hours of exposure. Two percent solutions of glutaraldehyde exhibit very good activity against vegetative bacteria, spores and viruses. It is ten times more effective than formaldehyde and less toxic. However, it must be limited and controlled because of its toxic properties and hazards. It is important to avoid skin contact with glutaraldehyde as it has been documented to cause skin sensitization. Glutaraldehyde is also an inhalation hazard. The NIOSH ceiling threshold limit value is 0.2 ppm.

Cidex, a commercially prepared glutaraldehyde disinfectant is used routinely for cold surface sterilization of clinical instruments. Glutaraldehyde disinfectants should always be used in accordance with the manufacturer's directions.[4]

b. Halogen-Based Biocides: (Chlorine Compounds and Iodophores)

1. Chlorine Compounds

Chlorine compounds are good disinfectants on clean surfaces, but are quickly inactivated by organic matter and thus reducing the biocidal activity. They have a broad spectrum of antimicrobial activity and are inexpensive and fast acting. Hypochlorites, the most widely used of the chlorine disinfectants, are available in liquid (e.g., Sodium hypochlorite), household bleach and solid (e.g., calcium hypochlorite, sodium dichloroisocyanurate) forms. Household bleach has an available chlorine content of 5.25%, or 52,500 ppm. Because of its oxidizing power, it loses potency quickly and should be made fresh and used within the same day it is prepared. The free available chlorine levels of hypochlorite solutions in both opened and closed polyethylene containers are reduced to 40% to 50% of the original concentration over a period of one month at room temperature.

There are two potential occupational exposure hazards when using hypochlorite solutions. The first is the production of the carcinogen bis-chloromethyl ether when hypochlorite solutions come in contact with formaldehyde. The second is the rapid production of chlorine gas when hypochlorite solutions are mixed with an acid. Care must also be exercised in using chlorine – based disinfectants which can corrode or damage metal, rubber, and other susceptible surfaces. Bleached articles should never be autoclaved without reducing the bleach with sodium thiosulfate or sodium bisulfate.[5]

Chloramine T which is prepared from sodium hypochlorite and p-toluenesulfonamide is a more stable, odorless, less corrosive form of chlorine but has decreased biocidal activity in comparison to bleach.

2. Iodophors

Iodophors are used both as antiseptics and disinfectants. An iodophor is a combination of iodine and a solubilizing agent or carrier; the resulting complex provides a sustained-release reservoir of iodine and releases small amounts of free iodine in aqueous solution. Antiseptic iodophors are not suitable for use as hard-surface disinfectants because they contain significantly less free iodine than do those formulated as disinfectants.

Wescodyne, *Betadyne*, Povidone-Iodine and other iodophors are commercially available Iodine-based disinfectants, which give good control when the manufacturer's instructions for formulation and application are followed. Both bleach and iodophors should be made up in cold water in order to prevent breakdown of the disinfectant.[6]

c. Quaternary Ammonium Compounds: (Zephirin, CDQ, A-3)

Quaternary ammonium compounds are generally

odorless, colorless, nonirritating, and deodorizing. They also have some detergent action, and they are good disinfectants. However, some quaternary ammonium compounds activity is reduced in the presence of some soaps or soap residues, detergents, acids and heavy organic matter loads. They are generally ineffective against viruses, spores and *Mycobacterium tuberculosis*. Basically these compounds are not suitable for any type of terminal disinfection.

The mode of action of these compounds is through inactivation of energy producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane. Many of these compounds are better used in water baths, incubators, and other applications where halide or phenolic residues are not desired.[7]

d. Phenolics: (O-phenophenoate-base Compounds)

Phenolics are phenol (carbolic acid) derivatives. These biocides act through membrane damage and are effective against enveloped viruses, rickettsiae, fungi and vegetative bacteria. They also retain more activity in the presence of organic material than other disinfectants. Cresols, hexachlorophene, alkyl- and chloro derivatives and diphenyls are more active than phenol itself. Available commercial products are Lysol, Pine-Sol, *Amphyl, O-syl, Tergisyl, Vesphene, L-Phase* and *Expose*.[8]

e. Acids/Alkalis:

Strong mineral acids and alkalis have disinfectant properties proportional to the extent of their dissociation in solution. Some hydroxides are more effective than would be predicted from their values. In general acids are better disinfectants than alkalis. Mode of action is attributed to an increase of H^+ and OH^- species in solutions which interfere with certain microbial functions, however the total effect is not only dependent on pH alone. Weak organic acids are more potent than inorganic acids despite low dissociation rates in solution. Action is attributed to the disruption of 2° and 3° conformation of enzymes and structural proteins[9,10].

f. Heavy Metals:

Soluble salts of mercury, silver lactate, mercuric chloride and mercurous chloride are efficient bactericidal agents. Silver nitrate and mercuric chloride are commonly used as 1:1000 aqueous solutions. Action is through attack on protein sulfhydryl groups and disruption of enzyme functions. Organic matter can reverse the disinfectant properties of mercurials.

Caution: Please consult with EH&S's Hazardous Materials group prior to using heavy metals because many of these must be disposed of as a hazardous waste. Specifically, disposal of elemental mercury and salts of mercury are very costly.

g. Alcohols:

Alcohols work through the disruption of cellular membranes, solubilization of lipids, and denaturation of proteins by acting directly on S-H functional groups. Ethyl and isopropyl alcohols are the two most widely used alcohols for their biocidal activity. These alcohols are effective against lipid-containing viruses and a broad spectrum of bacterial species, but ineffective against spore-forming bacteria. They evaporate rapidly, which makes extended contact times difficult to achieve unless the items are immersed.

The optimum bactericidal concentration for ethanol and isopropanol is in the range of 60% to 90% by volume. Their cidal activity drops sharply when diluted below 50% concentration. Absolute alcohol is also not very effective. They are used to clean instruments and wipe down interior of Biological Safety Cabinets and bottles, etc. to be put into Biological Safety Cabinets. Alcohols are generally regarded as being non-corrosive.

Conclusion

High technology has provided dentistry with infection-control–friendly equipment. Cleankeys is a flat keyboard that saves time when cleaning and disinfecting any operatory or for the administrative staff. This style of keyboard eliminates worries about spilled liquids or food lodging in between the keys. This keyboard has a mouse included to guard against contamination and promote easy cleaning.

The Dental R.A.T. is a hands-free, voice-activated periodontal probing system designed by a dental hygienist to enhance care while reducing the risk of contamination. Please view the video on the dentalrat.com website for more information. This technology puts a whole new light on discovering periodontal disease.

Discover how quick and easy this technology can assist in a day's work.

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Comparative Evaluation of Internal Fit and Marginal Fit of Zirconia Copings Obtained From Impression and Die Fabricated using CAD-CAM an In Vitro Study

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Abstract

Aims: To evaluate and compare the internal fit and marginal fit of zirconia copings fabricated using CAD-CAM from impressions and model.

Material and Methods: Using a standardized die, 15 impressions group 1and 15 models group 2 were made with standardized protocol. All impressions and models were scanned and virtual models were made. Using the CAD data, zirconia copings were fabricated and cemented using silicone under finger pressure and standardized sections of the silicone were made and thicknesses were measured at eleven specific points under stereomicroscope.

Statistical analysis used: ANOVA

Results: Mean Internal gap value of Group 1was 292.4μ ± 23.48 and Group 2 was 258.54 μ ± 22.83 μ, Mean Marginal Gap value of group1 was 252.64μ ± 11.86 μ and group2 was 232.18 μ ± 06.24 μ, ANOVA test showed statistical significance with p value >0.05

Conclusions: Within the limitations of the study, mean marginal gaps of both the groups were within clinically acceptable range. Group 2 copings demonstrated comparatively smaller values. Hence fit of the restoration is better in Group 2 (copings fabricated from scanned models)

Key-words: *CAD-CAM, scanner, stereo-microscope*

Introduction

Ceramo -metal restorations has always been considered the gold standard for restoration as it will improve patient's comfort, aesthetics and masticatory ability[1]. Even though ceramo -metal restorations have been in practice for the past four decades, compromised aesthetics and concern for biocompatibility have prompted the clinicians to search for a metal free alternative material that will not induce contact allergy. Zirconia restorations have fulfilled these requirements to a great extent. They have become both a necessary alternative and a preferred choice[2]. Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) was first introduced to dentistry in the mid-1980s. Both chair side and laboratory integrated procedures are available for CAD/CAM restoration fabrication. In selecting which procedure to follow, consideration should be given to aesthetic demands, chair side time, laboratory costs, and required number of visits, convenience and return on investment associated with the CAD/CAM equipment[3, 4, 5]

SUBJECTS AND METHODS:

Depending on the dental CAD/CAM system, different processing techniques may be applied to obtain data for dental restoration, as well as for various digitization methods to convert the planned restoring abutments to virtual abutments. A virtual three-dimensional (3D) model is created based on 3 sets of

data (***stone cast, impression body, and intraoral***) acquired from the scanning process. Therefore, the complexity of the overall manufacturing process of a dental prosthesis may increase or decrease depending on the material of the scanned object. The most efficient manufacturing process in fabricating a dental prosthesis would ideally include the necessary steps, but efficiency may not always enhance the fit of the final restoration. Hence, to evaluate the precision & fit of the prosthesis obtained by two different techniques in CAD/CAM processes i.e. scanning the impressions and scanning the models were employed. A maxillary arch metal die was fabricated, containing three abutments located one each in first molar region on either sides and one in mid incisal region [Fig 1]. The abutments were of height 10mm, marginal width 1mm, placed equidistantly. Bucco-lingual & mesiodistal identifications grooves were made in the occlusal surface for orientation. A customised jig was fabricated to orient the Stainless steel dentulous impression tray of standard size to fit over the metal die and impressions were made[Fig 2]. The metal die was separated from the impression tray at a predetermined distance using a vertical stop to make impression to ensure uniform thickness and pressure [Fig 3]. Fifteen Impressions (Group 1) were made with polyvinyl siloxane [Fig 4,5] (Aquasil putty regular set) using two stage technique, scan spray was sprayed (Digiscan, YETI DENTAL) to avoid dryness and enhance clarity of the digital image. The impressions were scanned on a CAD-CAM scanner (ZIRKON ZAHN SCANNER and Zirkon Zahn scan software) [Fig 6]. Fifteen Impressions (Group 2) were poured with die stone and models were scanned [Fig 7]. 3D virtual models were produced using designated software tools (Zirkon Zahn modellier software). Copings of 2mm thickness and cement space 35μ were designed using software. Orientation markings were designed on the outer surface of the copings to identify the buccal and mesial surface [Fig 8]. After designing, the copings were fabricated by milling the pre-sintered zirconia blocks (Zirkon Zahn milling unit) [Fig 9]. A total of 30 crowns were sintered in an electric furnace according to the manufacturer's instructions. To evaluate and compare the gap dimensions between the crowns and the prepared teeth, replicas of the internal gaps of the copings were made by the replica technique described by Boening et al and Reich et al[6, 7]. They were fabricated by repositioning the copings on the prepared teeth with a white silicone indicator paste (Fit Checker; GC, Tokyo, Japan) coated on the inner surface of the coping [Fig 10,11]. Excess silicone material was removed with a cotton pellet. During hardening of the silicone layers, the copings were held on the respective dies with uniform finger pressure. After setting, the copings and the silicone materials were separated. All the replicas were sectioned buccolingually. Sectioned samples were viewed under stereomicroscope (LEICA model M80, Heerbrugg, switzerland) [Fig 12], combined with a computer system to evaluate the thickness of each section at specific points. The camera (Leica IC3D: Leica Microsystems, Germany) reproduced X46 magnification so that a video image of the marginal gap could be examined using special software (Leica Stereo Explorer software; Leica microsystems). Dimensions were measured at eleven specific points as shown in [Fig 13] and readings were noted for Group 1 & Group 2 samples respectively. Marginal gap values were measured in millimeters (later converted to microns) at six specific points from the silicone index of group 1 and group 2 samples and were used to calculate marginal fit. Internal gap values were measured in millimeters (later converted to microns) at five specific points from the silicone index of group 1 and group 2 samples and were used to calculate internal fit. The results were tabulated, statistically analyzed using ANOVA and interpreted.

Results: Scanned data were expressed as mean and standard deviation. Data analysis was performed using SPSS version 17 software for statistics. The mean values were compared among the study groups using t-test and variance between the groups were compared using ANOVA and the p value **p>0.05** was considered statistically significant. Microsoft word and Excel have been used to generate tables and graphs.

Table1 and Bar diagram 1 indicates that Mean Internal gap value of Group 1was 292.4μ ± 23.48 and Mean Internal gap value of Group 2 was 258.54 μ ± 22.83 μ, which was statistically significant with p value >0.05.

Table 2 and Bar diagram 2 indicates that Mean Marginal Gap value of Group 1 was 252.64μ ± 11.86 μ and Mean Marginal Gap value of Group 2 was 232.18 μ ± 06.24 μ, which was statistically significant with p value >0.05.

On comparing the mean values of internal gap among samples within Group-1, the lowest value was 263µ and highest value was 329µ. Whereas on comparing the internal gap of samples within Group-2, the lowest value was 227 µ and highest value was 275 µ. Though significant gap was found among both the groups, Group 2 showed comparatively lesser gap when compared to Group1 [as shown in the bar diagram 1]. This shows that on an average, the copings fabricated from scanned model [Group 2] (as they have less mean value) have better internal fit when compared to that of copings fabricated from scanned impression [Group 1].

On comparing the mean marginal values among the samples of Group 1, the lowest value was 225 µ and highest value was 283µ. Whereas comparing the marginal gap among the samples of Group 2, lowest value was 211µ and highest value was 249µ. Though significant gap was found among both the groups, Group2 showed comparatively lesser values when compared to Group 1 [as shown in Graph 2]. This shows that on an average, the copings made from scanned model [as they have less mean value] have better marginal fit when compared to copings made from scanned impression. These results are similar to results of the studies of kohorst et al and kokubo et al.[8, 9]



Fig 1 Metal Die



Fig 2 Impression tray holder

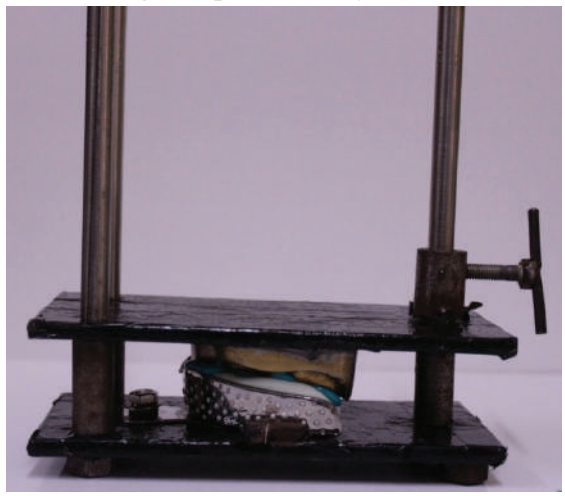


Fig 3 Metal die separated at uniform distance

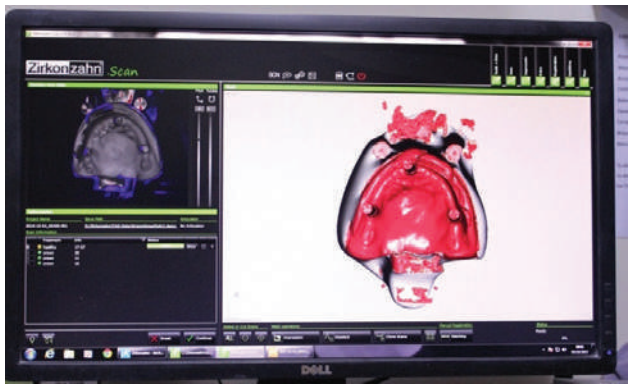


Fig 4 Scan of Impression

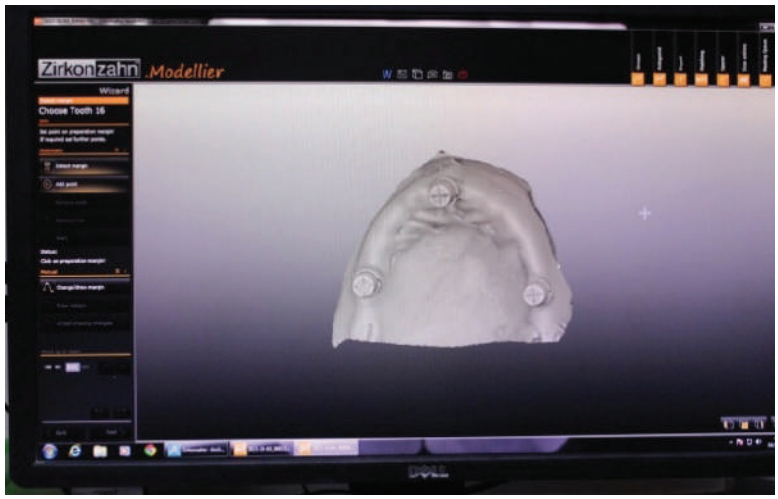


Fig 5 Scan of Gypsum model

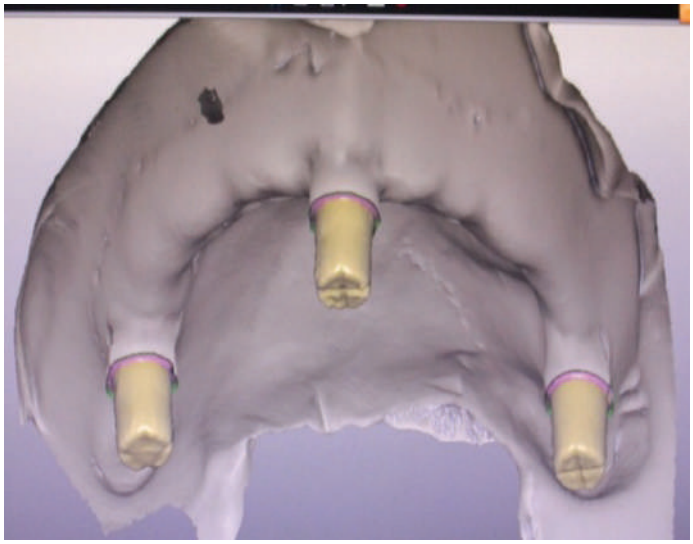


Fig 6 Designed copings

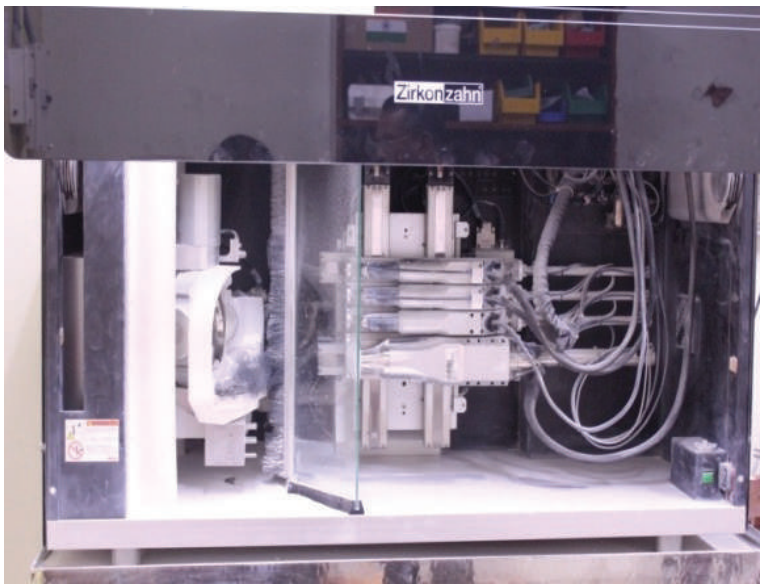


Fig7 Milling unit



Fig 8 Steriomicroscope

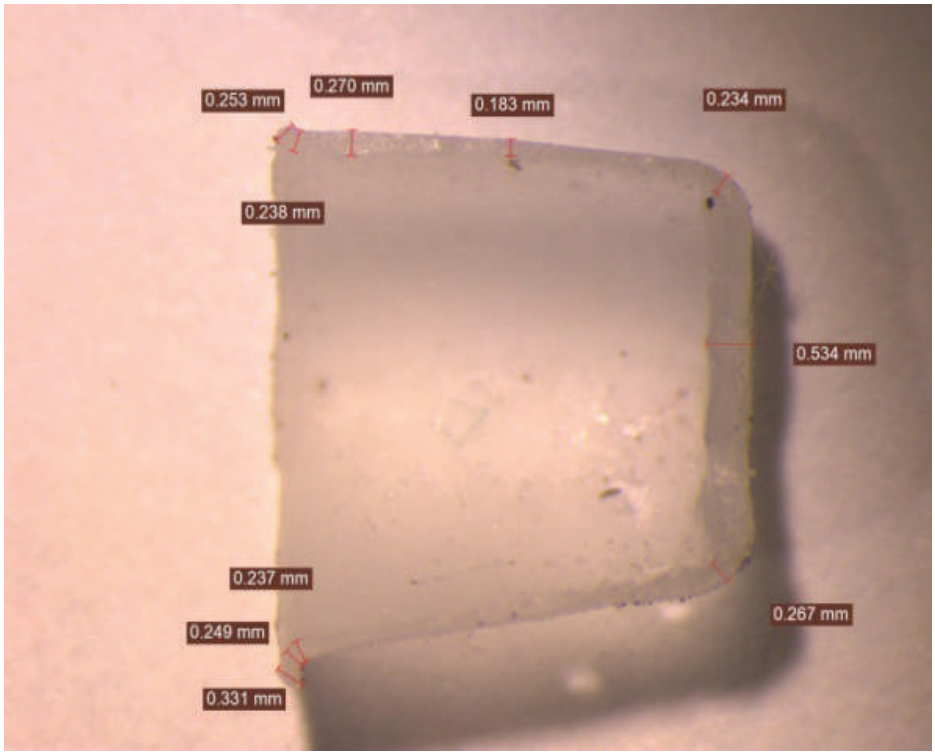


Fig 9 Measurements made from cross sectioned replica

Group 1: copings from scanned impression models

Group 2: copings from scanned models

Tabular column

TABLE: 1 Mean and Standard Deviation for Internal gap between the Group 1 copings and Group 2 Copings

INTERNAL GAP			
GROUP	N	MEAN (μ)	STD DEVIATION
IMPRESSION	15	292.4	023.48
MODEL	15	258.54	022.83

TABLE: 2 Mean and Standard Deviation of Marginal gap values between the Group 1 copings and Group 2 copings.

MARGINAL GAP			
GROUP	**N**	**MEAN(μ)**	**STD DEVIATION**
IMPRESSION	15	252.64	011.860
MODEL	15	232.18	006.240

Discussion

Marginal and Internal fit are two important factors for the success of any extra coronal restoration. Inaccuracy in the fit of the restoration can result in damage of the teeth and its periodontal structures. Marginal inaccuracies causing retention of plaque may lead to marginal gingival inflammation, gingival recession and secondary caries cervical to the margins of the crown [10, 11]. This is the common cause for the failure of restorations. Thus, irrespective of the technique of crown fabrication, the marginal and internal fits are of prime importance for the success of the restoration[12]. A shortcoming of the replica method is the two-dimensional display of a marginal gap. [13] In some cases, interpretation of this gap becomes difficult, especially if a margin is located subgingival. In the present study, some replicas had to be discarded because the interpretation was not possible. However, according to an in vitro investigation by Rahme et al [14], the use of a low viscosity silicone for the replica technique seemed to imitate the film thickness of glass-ionomer cement within a crown. This situation is analogous to clinical conditions where-in, an ideal film thickness of 25-35 microns is necessary for crown cementation. Also, one major advantage is the non-destructive nature of the replica technique. The reliability and validity of the replica method were confirmed by several authors [15]. Hence, the replica technique was used in our study. In this study finger pressure was used during cementation process. Although finger pressure has been questioned by some authors, it has been the considered opinion of weaver et al [16] that, a certain variance of the seating force has no impact on the film thickness within the coping and hence in the present study, finger pressure was applied uniformly by a single operator. The results of the present study are in agreement with studies of Ng et al in 2014[17] and Tamer Abdel- Azim et al[18] who compared fit of crowns fabricated with digital and conventional methods. They concluded that the fully digital fabrication method provided better marginal fit than the conventional method.

SCOPE FOR FURTHER STUDIES:

In the present study, impressions were scanned prior to digital reproduction. To obviate the need for impression making, intra oral scanners can be used to

make virtual impressions and this could be taken up for future studies.

Ethical Clearance – Institutional Ethics Committee, Sree Balaji Dental College and Hospital, Committee registered with DCGI. (Registration no: ECR/761/Inst/TN/2015)

Source of Funding – Nil

Conflict of Interest – Nil

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A Comparative Study to Determine the Dimensional Accuracy of Different Impression Materials

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Abstract

Background: The accuracy and dimensional stability of dental impression materials are of great importance, since a material which shrinks, expands or warps during or after removal from the mouth will result in an inaccurate model and potentially, a badly-fitting prosthesis. The purpose of this study was to evaluate the dimensional accuracy of the impression materials immediately after setting and also to determine the change in dimensional accuracy after 24 hours.

Materials and Method: Four impression materials were selected among which two were hydrocolloids and two were elastomers. Fifteen samples of each were experimentally tested immediately and 24 hours after sample preparation. Data obtained were subjected to t-test and analysis of variance for statistical comparisons at a 5% level of significance.

Results: The linear difference in measurements were recorded immediately and 24 hours after setting. The changes in dimensional accuracy with respect to time were statistically analysed and it showed no statistically significant difference between the materials both immediately and after 24 hours.

Conclusion: Within the limitations of the study,

It can be concluded that there were similar results for dimensional accuracy for all the materials tested immediately after sample preparation but there was considerable disparity with the same tested after 24 hrs for hydrocolloids and not much of difference was noted for elastomers.

Keywords: *Dimensional accuracy, Impression materials, elastomers, hydrocolloids*

Introduction

Dental impression materials are used to generate a negative replica of the dental hard and soft tissues. The accuracy and dimensional stability of dental impression materials are of great importance, since a material which shrinks, expands or warps during or after removal from the mouth will result in an inaccurate model and potentially, a badly-fitting prosthesis[1].

Accuracy of impression is dependent on the dimensional stability of impression material and also influenced by a number of factors such as impression technique, impression tray, and properties of the impression materials.

Clinically impression materials can be divided into two large groups[2]:

1) Hydrocolloid impression materials that include agar-agar and alginate impression materials;

2) Synthetic elastomeric impression materials that include polysulfide, condensation silicone, addition silicone, and polyether

Obtaining an accurate cast of a dental arch is an important step in the success of various dental treatments. Because of time constraints pouring of impression by dentists themselves is on the decline, as they prefer to send impressions to dental laboratories for making cast[1]. Thus, a considerable delay is caused in pouring the model, after removal of the impression from the mouth;

which is one of the reasons for causing dimensional changes in the impression, thereby threatening the dimensional accuracy of the prosthesis[1,2].

Dimensional accuracy of impression materials are evaluated using the mould prepared by ADA specification no.19 that possesses vertical and horizontal lines that intersect each other at fixed points providing a standard distance value. The distance between the fixed points was evaluated at different time intervals to determine the dimensional accuracy. Any deviation from the fixed value indicates the change in dimensional accuracy.

This study has presented the comparison of dimensional accuracy of different commonly used impression materials at different time intervals.

Materials and Method

DIE PREPARATION

For the present study, a stainless-steel die was prepared according to the ADA specification No.19. According to ADA specification 19, each die consisted of a cylindrical stainless block of 38mm diameter, with a 30mm diameter step on its superior surface. The die was scored with 3 horizontal lines and 2 vertical lines on the top of the impression surface. The horizontal lines were marked as X, Y, Z, and vertical lines were marked as CD and C'D' for ease in making measurements.

The distance between two horizontal lines is 2.5 mm and between two vertical lines is 25 mm. The die was subjected to Nd-YAG Laser treatment and 3 horizontal and 2 vertical lines were scribed, with a width of 0.016 mm on top of 30 mm diameter surface. The die has a highly polished surface. The die also contains a ring that surrounds the periphery of it, which serves as a tray or as a container for the impression material.

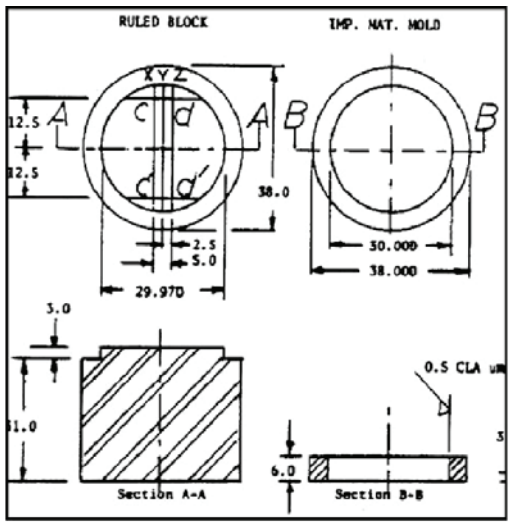


Figure 1 : ADA Specification no.19



Figure 2 : Stainless steel die

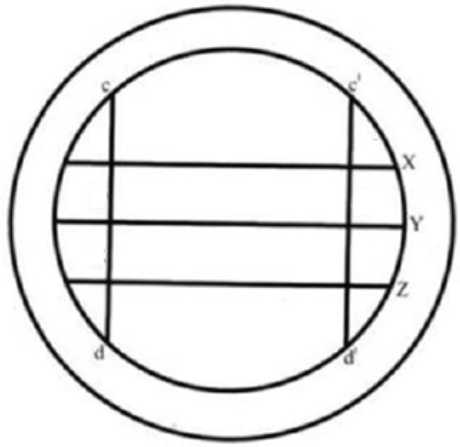
SAMPLE PREPARATION

Each material was manipulated as mentioned above and evenly spread over the die and a glass slab is placed over the impression material for an even polished surface. The whole assembly was kept undisturbed for the setting time suggested by the manufacturer.

After the adequate setting time, the material was separated from the die. Thus prepared specimens were measuring 30 mm in diameter 3 mm in thickness and had lines X, Y, Z, CD, C'D' lines on it. Similarly, all 60 void-free samples were obtained. In between the time of observation, the samples were stored at normal room temperature.

EVALUATION OF DIMENSIONAL ACCURACY

The sample was placed over the vertical profile projector. The distance between the lines CD and C'D' intersecting with line Y was measured using a vertical profile projector. The readings were recorded immediately and 24 hours after sample preparation.



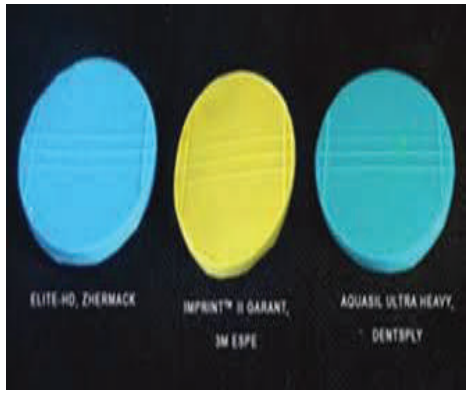


Figure 3 : Horizontal X, Y, Z, and vertical CD and C'D' lines

SPECIFICATIONS OF THE PROFILE PROJECTOR

Parameters	Description
Screen diameter (mm)	300 round glass with cross lines
Rotating range	0 to 360 degrees
Rotating angle range	1 minute digital encoder
Magnification standard	Telecentric optics :10x
Standard work table	290mm X 175mm
Measuring range X / Y & Z axis	150mm X 100mm
Operation	Manual X, Y & Z axis
Linear scale	Glass scale res 0.001mm
Surface illumination accuracy	< 0.05 percentage on screen
Profile illumination accuracy	< 0.075 percentage on screen

Parameters	Description
Surface illumination	150w, 24v with heat filter and fan-cooled systems
Profile illumination	100w, 24v with heat filter and fan-cooled systems
Surface illumination systems	Dual-beam split light source with control adjustment
Workload capacity	4 to 5 kg



Figure 4 : Determining dimensional accuracy of impression material using vertical profile projector

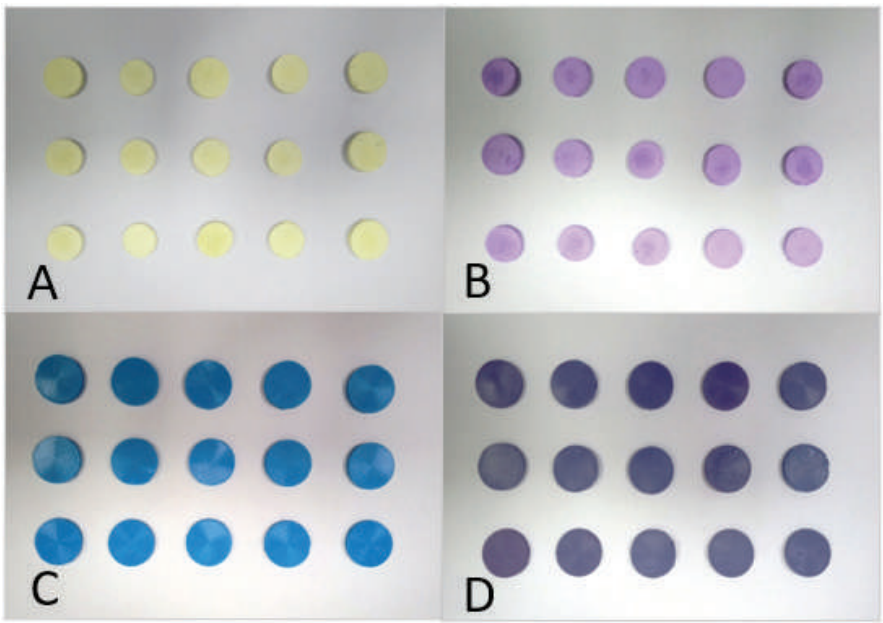


Figure 5 : samples (15 Nos) dimensional accuracy

Statistical Analysis

All statistical tests are done with a P-value considered as 0.05 with 95% probability accuracy level. If the resultant P-value of the tests is less than 0.05 H_0 is accepted and if it is greater than 0.05 H_1 is accepted.

The ANOVA and t-test results comparing the immediate results after sample preparation of all four impression materials showed that there is no statistically significant difference between the materials but with the time difference of about 24 hours there is statistically significant difference between the materials.

The dimensional accuracy values taken immediately after sample preparation showed that alginate is the least accurate while cream alginate had better accuracy more equivalent to the elastomers and vinyl polysiloxane had the highest range of accuracy among the others.

The dimensional accuracy values recorded 24 hours after sample preparation showed that alginate and cream alginate had an unacceptable change in accuracy while elastomers showed better accuracy and had dimensional stability even after 24 hours. The dimensional accuracy changes evaluated showed that hydrocolloids showed more changes while elastomers had negligible changes only

TABLES: DIMENSIONAL ACCURACY - IMMEDIATE

Dimension (in mm)	Alginate	Cream Alginate	Poly Ether	Vinyl Poly Siloxane
Immediate	24.14	24.63	24.95	24.99

DIMENSIONAL ACCURACY - AFTER 24 HOURS

Dimension (in mm)	Alginate	Cream Alginate	Poly Ether	Vinyl Poly Siloxane
After 24 hours	19.83	19.66	24.88	24.89

DIMENSIONAL ACCURACY COMPARISON

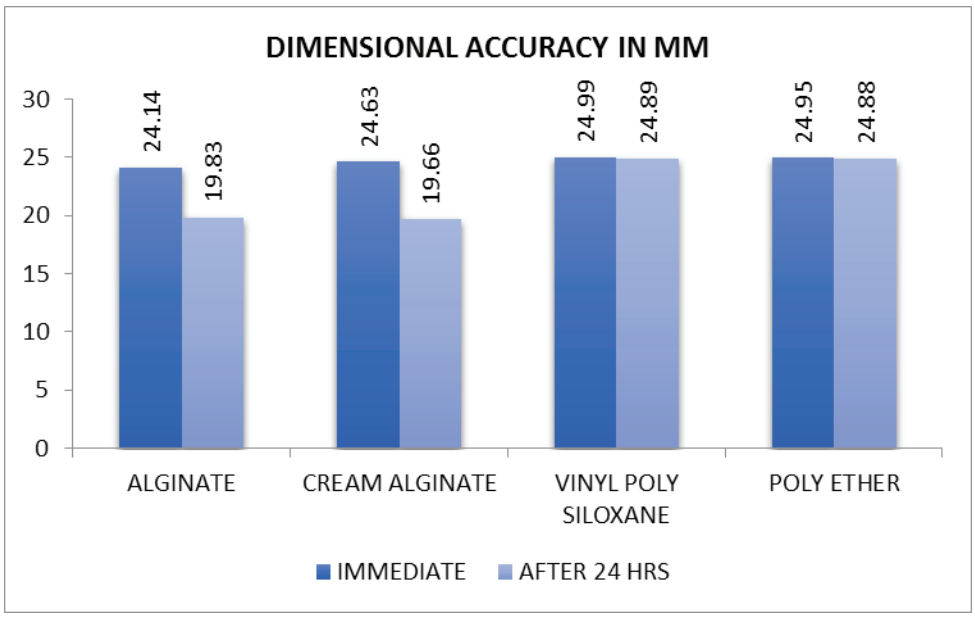


Figure 6 : Comparing Dimensional accuracy changes

of impression materials

Discussion

All the recently introduced material claims dimensional accuracy because of improved techniques of production, sophisticated handling of materials, operating environment, etc and they claim superiority of material properties over the existing materials.

Lack of evidence-based basic scientific knowledge on recently introduced materials created the necessity for basic in-vitro studies in regard to material properties. With the mushrooming of dental manufacturers, their claims & scarce literature of newer emerging material properties created the mandatory necessity for material comparison with existing old material and its concepts. With the ever-changing concepts, it's very difficult for any dentist to properly select material without evidence-based literature.

The study was planned to compare the properties of materials available in the market so that it will provide a fair idea about any improvements in relation to its accuracy and wettability. Therefore, in this study new material named as cream alginate that claims surface reproduction up to 5μm was used. The dimensional accuracy of this newly introduced cream alginate was compared with the commercially available alginate and the dimensional accuracy of polyvinyl siloxane and polyether was also compared among the elastomers. Dimensional accuracy of the cream alginate was much better compared to the normal alginate and it showed accuracy more or less equivalent to the elastomers.

Impression materials should have the ability to reproduce a true value and they should be dimensionally stable across time. But accuracy depends on the evaporation of water and syneresis[1,2] in case of hydrocolloids. Dimensional change of impression material is multifactorial and material specific. Kulkarni et al. stated that dimensional stability of the impression material showed the greatest discrepancy from the standard model which was 0.005 inches and extended-pour alginate was 0.003 inches (approximately 75 μm) on the day one of pour[3]. Generating the casts from the conventional alginate sooner is beneficial[4].

The amount of expansion or shrinkage when evaluated in a study conducted by Manisha et al., showed that the casts obtained by pouring alginate impressions immediately showed accurate results even though insignificant difference was observed when impression was stored at varying degrees of temperature for 20 minutes, but the values obtained by storing of alginate impressions for 20 minutes at 30°C were found to be nearly accurate than the values obtained by storing of impression at 40°C[3].

A net contraction in the material follows the formation of the insoluble gel and the contraction may continue even if the impression is immersed in a liquid. During shrinkage, if impression material is bounded tightly with trays then the material will be pulled toward the tray which causes an increase in tooth and arch widths. During imbibition, it will distort by swelling. The shrinkage of material that took place towards the bulk of the material is in accordance with the study by Wadhwa SS et al.,[5].

In this study also we have evaluated the dimensional accuracy and wettability of impression materials 24 hrs after sample preparation which showed wide variation from the accuracy obtained immediately after sample preparation. Both elastomers and hydrocolloids showed similar results for dimensional accuracy immediately after sample preparation and cream alginate showed much closer accuracy with that of the elastomers. Therefore if the cast is poured immediately after impression making, cream alginate can also be used as a choice of impression material for tooth preparation also.

Conclusion

Within the limitations of the study,

It can be concluded that there were similar results for dimensional accuracy for all the materials tested immediately after sample preparation but there was considerable disparity with the same tested after 24 hrs for hydrocolloids and not much of difference was noted for elastomers.

When casts are poured immediately after impression making all the four materials would be able to provide better accuracy. Newly introduced cream alginate showed better dimensional accuracy equivalent to the

elastomers and can be considered for usage.

Ethical Clearance - Institutional Ethics Committee, Sree Balaji Dental College and Hospital, Committee registered with DCGI. (Registration no: SBDCH/IEC/04/2019/23)

Source of Funding – Nil

Conflict of Interest – Nil

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Immediate Denture – A Case Report

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Abstract

Esthetics is an important consideration for patientsundergoing dental treatment. Patients always want an immediate prosthesis beforeintervening any treatment option. Hence a fabrication of denture placement immediately after the extraction of teeth is precise one. Immediate denture is the best option for functional, esthetics purpose as well as patientsneed.

Keywords*; immediate denture, esthetics, function*

Introduction

Esthetics is always a prime consideration for a patient seeking dental treatment. Thus, dentist should be skilled enough to fulfill the patientexpectation.An immediate denture is a versatile option which can be given to a patient who's main consideration is esthetics[1]. Immediate dentures are denture constructed before all of the remaining teeth have been removed and are inserted immediately following removal of remaining natural teeth.

Definition

- Any fixed or removable dental prosthesis fabricated for placement immediately following the removal of natural tooth or teeth[2] – GPT9

- According to Heartwell "an immediate complete denture is a dental prosthesis constructed to replace the lost structure and associated structure of the maxillae and/ or mandible and inserted immediately following removal of remaining teeth"

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A Case Report

A64 years, female patient came to the Department of Prosthodontics, Sree Balaji Dental College & Hospital with a chief complaint of difficulty in chewing food due to mobility of the fixed prosthesis placed in the upper arch.The patient presented no significant medical history.

On clinical examination (fig 1 and 2) and radiographic assessment revealed metal ceramic fixed partial denture in 24 to12region (fig 3). The patient was advised for extraction of the teeth due to the mobility of fixed prosthesis and fabrication of interim immediate denture was planned. The patientsigned the informed consent, andaccepted the treatment plan for an interim immediate denture.

Procedure

- The primary impression was made with irreversible hydrocolloid impression material (Zhermack-tropicalgin, BadiaPolesine[Rovigo]Italy). The cast was poured in type-3 dental stone (DPI, Mumbai, Maharashtra, India)(fig 4 and 5). The wax spacer (Modelling wax No 2 - Hindustan Dental Products, chapel road, Hyderabad) of two sheet thickness on an edentulous area and one sheet thickness on the dentulous area was adapted. The custom tray was fabricated with VLC tray material (Poly tray- Delta, Germany). Border molding was done, the wax spacer was removed, relief holes were made, tray adhesive was applied, and final

impression was made with medium-body addition silicone impression material.

• The Jaw relation was recorded and teeth arrangement was done. The remaining teeth wereremoved from the cast. The denture was fabricated before the extraction of remainingteeth. The mobile teeth are extracted under local anaesthesia(fig 6). The denture was inserted on the same appointment(fig 7). Post insertion instructions were given and the patient was recalled after 1 day.

Discussion

Immediate denture is a valuable and reliable treatment option when proper case selection, treatment planning and other procedures are followed carefully. Success of this treatment procedure depends on variousfactors like case selection, diagnosis and treatment planning, proper surgical protocol, accurately contoured and finished prosthesis and eagerness of the patient towards the treatment.

Case selection is one of the important factors for planning immediate denture. Certain cases are not eligible for immediate denture treatment option This can be identified by initial examination of the patient.

Extraction must be carried out in a least traumatic way. Errors like fracture of cortical plate, tearing of mucoperiosteal flap must be avoided, which may reduce the rate of success of the treatment[3].

To attain the maximum degree of success, the immediate denture should fulfil the following requirements such as

· Bio-compatibility with oral environment,

· Restore the masticatory performance,

· Harmony with the following functions like - speech, respiration, mastication, and deglutition, esthetics and preservation of the remaining tissues[4].

When patients require total extraction and an immediate complete denture, clear acrylic surgical stent may be fabricated to act as a guide to the surgeon while bone contouring. This will ensure a comfortable wearing of the prosthesis immediately after extraction[3].

The immediate denture can protect the surgical site, and its appearance is usually excellent, because natural teeth serves as a guide for positioning of denture teeth[4]. Immediate dentures are very satisfying treatment modality for both patient and dentists. The patient gets the benefits of improved confidence, comfort, and functional needs. The dentist also finds satisfaction in providing a very acceptable treatment to the patients.

Immediate denture is a more challenging one for the dentist due to inadequate support from the tooth and try-in procedure is not possible during this treatment. Sometimes patient satisfaction may be compromised during initial placement. Hence patient cooperation is needed.Every necessary clinical step should be explained to the patient before starting the treatment[5].

The advantages of immediate dentures are helps to controlhaemorrhage, prevents contamination and provide protective covering over the wounds[6].

The interim immediate denture was successful in fulfilling the requirement of the patient. The initial retention and stabilityare good and patient was also satisfied withesthetics.It should provide the restoration of phonetics and masticatory functions and facilitates transition of the edentulous state.

However, this prosthesis was intended for use as an immediate interim denture. Follow-up was done regularly. After complete healing of ridge, conventional complete denture was fabricated for the patient.

Conclusion

From this clinical report it was concluded that immediate denture is a satisfactory treatment for both patient and dentist. It fulfils patient needs, physiologic function as well as the esthetic concerns regarding treatmentoption. Hence placementof immediate prosthesis after the extraction of teeth, provide beneficiary condition from patient towards the dentist.

Fig 1: Intra Oral Examination



Fig 1: Intra Oral Examination



Fig 2: occlusal view

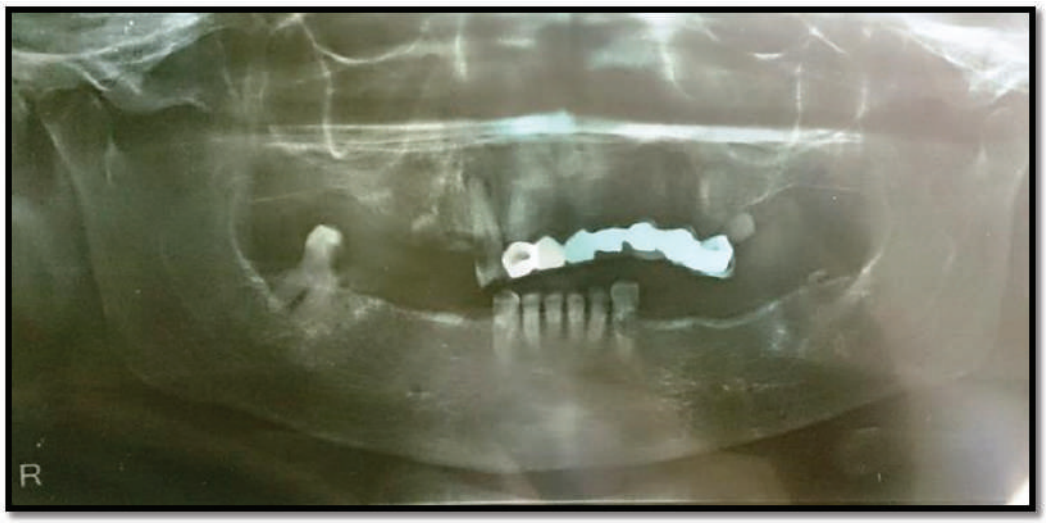


Fig 3: Pre - operative OPG

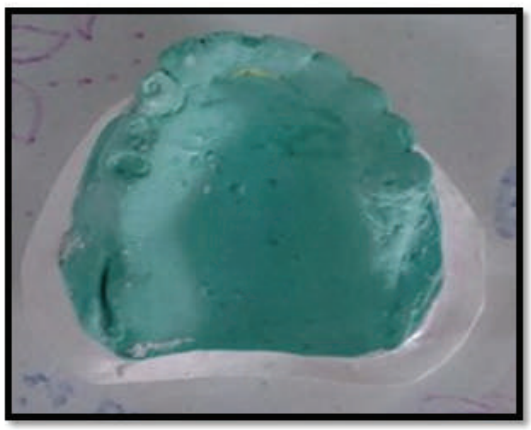




Fig 4 and 5 : Primary maxillary and mandibular cast



Fig 6: Extraction of maxillary anterior teeth



Fig 7: Denture insertion

Fig 8: Post-operative profile view



Ethical Clearance – Not required since it is a case report

Source of Funding – Nil

Conflict of Interest – Nil

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Goethite Nanofibers as Bio-Fillers in Dental Composite Resin

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Abstract

Composite resins represent an important member of biomaterials family that have been evolving due to the needs in biocompatibility and mechanical properties. Fillers in a variety of forms and types have been evaluated and the impact of fillers to improve the performance of the dental composites have been studied in vast. Various composite resin fillers tested had revealed its own strengths and weaknesses. Bio polymers when they are reinforced with biofillers can produce novel biocomposites. The biocomposites with the biofillers will replace and be a substitute for synthetic fibre-reinforced composites. In recent years, natural bio-fibres have gained attention as suitable reinforcements in polymeric matrices. These bio-fillers possess high specific strength and can be effectively used for dental composites resin. The most commonly occurring natural bio-fibres are the teeth of limpets. The teeth of limpets have a distinctive composite nanostructure which comprises of high-volume fractions of reinforcing goethite nanofibers to provide mechanical integrity. The limpet tooth is effective at resisting failure to abrasion. The goethite nanofibers are expected to be significant for novel dental biomaterials with extreme strength and hardness.

Keywords: *Bio-filler, Nanoparticles, Nano-filler, Dental Composite resin.*

Introduction

Composite resin are tooth-colored restorative materials with synthetic polymers, reinforcing fillers and silane coupling agents. The silane coupling agents helps to bond the reinforcing fillers to the polymer matrix. Each component of the dental composite resin is important for the success of the final restoration. The developments in commercial dental composites have been a focused on the modifications to the filler component[(1)].

Reinforcing fillers are particles added to any materials such as to plastics, concrete etc. They are added to bring out the better properties of the mixed material. Such materials can be in the form of solid, liquid or gas. Most of the materials used in dentistry contain fillers as a part of their composition. Fillers are aimed to improve the chemical and mechanical properties of the dental composite material[(1),(2)]. Combination of individually dispersed nano sized filler particles and nano clusters enhance the aesthetics[(3)].

TRADITIONAL FILLERS:

Initially, crystalline quartz and borosilicate or lithium aluminosilicate glasses were used as fillers. The quartz was ground or milled into particles of sizes ranging 0.1nm to 100nm. Crystalline quartz has an excellent optical match to the polymer resin. However, quartz can be very abrasive to enamel. The glass filler particles and the crystalline quartz filler particles are large in diameter and very hard to the surrounding polymer matrix. The hardness and the larger diameter ensured that as the surface of the composite was abraded, the polymer would wear at a faster rate than the fillers, leaving them raised and exposed from the surface. This makes the surface of the restoration rough and less natural like. Thus, polishability and esthetics were compromised[(1)(2)].

To improve the esthetics, the quartz filler particles where replaced with silica particles. These silicon dioxide particles have a diameter of approximately 0.04µm. The surface area of the silicon dioxide particles is more hence they require considerable amount of monomer to wet their surface. This characteristic of the silicon dioxide filler particle will limit the filler concentration to approximately 35 wt%, which in turn limits the mechanical properties of the material. The silica fillers though they have a good polishability but the

strength and stiffness values are considerably lower than other fillers incorporated composite resin.[1].

To improve the esthetic, optical and mechanical properties of composite resin, nano particles as filler has been added. Nano particles enhance wear resistance and reduce polymerization shrinkage. Fillers with nano sizes have an increased surface area which provides an increase in flexural strength. [2][4].

In spite of developing materials to lower stress and improving the strength in the materials, researchers have also focused on developing materials with bioactive characteristics. The ideal restorative material of the future will not only be able to withstand occlusal loads and develop low polymerization stress but also will have an antibacterial, and remineralizing, properties in addition to being biocompatible[5]

Although modifications have been tried with the resin monomer, most significant modifications in dental composite resin is been with the fillers. The modifications have been in the type, size, and distribution of the inorganic fillers. The effects of size, shape, and size distribution of different fillers. [6]

As many articles have covered the review of fillers in composite resin; this review focuses mainly on the availability and performance of bio-filler`s materials and focus on the goethite nanofibers as fillers.

BIO-FILLERS

A biomaterial by definition is a substance can be used to fix the defects, repair or replacement of defective organ in the body. These materials can be synthetic (synthetic - mineral or organic) or natural.[6]

The development of bio degradable and recyclable materials from renewable resources have drawn attention as they are found to be suitable replacements for synthetic materials. The plant fibers and cellulose based fibers are natural fibers and are the most commonly used bio-fibers. These bio fibers are used for reinforcing various types of polymer matrices[7][8].

Bio-fillers in dental composite resin act as a biomaterial and hence reduce the bacterial penetration into marginal gaps for composite restorations. With the advent of modern technology, various attempts are made to modify these fillers to make them more beneficial for a better material. Strong scientific and collaborative foundations presently exist for further development and improvement of the filler system, which will certainly continue to hold a pivotal role in the dentistry.[3]

The bio-fillers because of their properties such as biodegradability, and high specific strength, have received extensive attention as a replacement for traditional synthetic fillers. Hence, the bio-fillers are used in the fabrication of hybrid composites and bio-composites[8].

The mechanical and the physical properties of bio fillers filled composite resin varied considerably. The variations of the properties depends on the stiffness, physical and chemical composition of the material, type of particulates, and growth environment[9]

BIO-NANO-COMPOSITES

The inorganic fillers are added to the polymer matrix in dental composite resin is to improve the mechanical properties of the composite resin. With the aid of nanotechnology, the dental composite resin fillers have been manufactured in nano scale level. The intrinsic toughening of the composite resin with the bio nano filler prevents the micro crack formation and increases the material toughness.

The bio-fillers in nano scale has been introduced in the composite resin and these composites have been termed as bio-nano-composites. Interest in the use of bionanocomposites has been increased over the past 20 years. As a dental material, bionanocomposites has the ability in mimicking native tissue structure and properties. The bionanocomposites has the ability to endure high biting force and shows a good mechanical flexibility in harsh environment. Similar structure or properties to bionanocomposites are often found in dentin and enamel. Therefore, if the bionanocomposites replaces enamel or dentin, they are expected to act as single entity and stay for a longer period of time. [10]

GOETHITE NANOFIBRES AS BIO-FILLERS:

The inorganic phase of minerals in the biological system from highest animals to plants are made of bio minerals. Although bio minerals composed of calcium predominate, bio minerals of barium, strontium, iron,

and silica are also well known. Bio mineral generally occur as oxides, hydroxides, phosphates, and carbonates in the biological system [11].

This reinforcing bio minerals in many organism approaches nano metre in length, at the orientation is always in one-dimension. Which has been proposed as promoting flaw insensitivity and in turn increases the tensile strength of the bio mineral[11].

The aquatic snail (Limpet) teeth are an example of a biomineral produced biologically that is optimized for strength. Studies have shown that the teeth of limpets have the high aspect ratio mineral nanofibres of goethite with a reinforcing protein matrix[11].

LIMPET-THE AQUATIC SNAIL

The intertidal zones are mostly inhabited by primitive marine mollusks. These marine mollusks are Limpets (Mollusca, Gastropoda) and are characterized by their inverted cone-shaped shells. The limpet radula is a highly mineralized tooth structure. The radula contains more than 100rowsofteeth.The outermost rows of the teeth are actively used. During the growth and maturation of the radula, the radula advance in position to the scraping zone. The scraping zone of radula will be in contact with the substrate while feeding. Once the teeth start to function, they begin to wear down, at an average rate of one tooth row per day. At the same time, a new row of teeth emerges. The radular teeth has a membrane and it is manipulated by various muscles and cartilages which are used to graze or cut food. There exist seven basic types of radula morphology[12].

MINERALIZATION OF RADULA

According to the extent of mineralization the radulae of Limpets can be segmented into 4 stages. The First stage there is no mineralization formation. The organic framework of tooth from the odontoblast is initiated in this stage. In the second stage, mineralization formation occurs. The minerals are deposited at the cusp and the rows of teeth. In the third stage, the mineralization gets completed. The cusps of the radula are solid and firm with full mineral deposition. In the fourth and the final stage the fully grown mineralized teeth move and replace the worn-out teeth. The mineralization of tooth cusps begins in the posterior region of the radula. The limpet teeth are organic structures composed of chitin and proteins rich in tyrosine[13].

The mineralization of limpet teeth consists primarily of mineral nano fibres typically many micrometres in length but only a few tens of nanometers in diameter. These mineral nanofibers occupy a significant volume fraction of approximately 80% in mature teeth. The strength and hardness of the limpet tooth is critically dependent on the strength of the mineral nanofibres within the composite structure[11].

Mineralization of the limpet teeth begins with the formation of elongated crystals of goethite (-FeOOH) crystals. The goethite crystals are closely associated with the organic matrix. Hydrated amorphous silica phase fills the space between the goethite crystals. The pre-formed matrix of limpet teeth consists of organized arrays of densely packed chitin fibers, with no apparent gaps or holes for crystal growth, other than the few nanometers between adjacent fibers. The organization of the goethite crystals along with the amorphous silica plays an important role in determining the fracture and wear characteristics of the functional tooth. The orientations of the goethite crystals are controlled by the chitin fibers in the organic matrix. The orientation of the goethite crystals results in teeth which are self-sharpening and resistant to fracture.

As the radula develops in the radular sac, the iron is secreted into the radula from the superior epithelium. Copper in the limpet radula are involved in the enzymic cross linking of the organic component. Zinc in the radula plays a role in the control of iron oxide crystallization. Calcium in the radula is incorporated in the crystalline phosphatic mineral hydroxyapatite[14][15].

The goethite nanofibers with other minerals are expected to dictate the flaw tolerance. This might be due to their diameters are being below a critical threshold value of the order of tens of nanometres. The tensile strength of limpet teeth can reach values higher than spider silk, considered currently to be the strongest biological material, and only comparable to the strongest commercial carbon fibres.

The radula of Limpet structure highlights the efficiency of biological control in assembling a composite structure of nano fibrous goethite for optimal

strength and hardness. This corresponding structural design features are expected to be significant for novel biomaterials with extreme strength and hardness, such as next-generation dental restorations [11].

Discussion

It is apparent from the previous studies that the current composites have significantly improved clinical performance compared with their predecessors, especially in posterior teeth with respect to strength and hardness to resist crack or fracture. However, Considerable effort are expended in the laboratory to ensure that the new materials handle and perform at and above the level of existing materials in respect to strength for the posterior teeth and superior polishability in the anterior region. Laboratory composite resin will identify the weaknesses, and further refinements in formulations will then take place to maximize performance. There are, further changes awaited in dental composite formulation in the areas of new fillers which produce particles in high yields. These newly developed particles will ideally be very fracture-resistant and only minimally abrasive. In addition, the refractive index of the modified filler has to accurately match to existing as well as newly developed polymers. The continued development of fillers containing biocompatible material will have an enhanced therapeutic effect. [1]

Conclusion

The novel biomineral fillers such as goethite nanofiber present in the radula of the limpet with a nanofiber dimension with extreme strength and hardness present an alternative to the present fillers in the dental composite resin.

Ethical Clearance – Not required since it is a review article

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Conflict of Interest – Nil.

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Radiographic Methods as a Adjuvant Guide to Oral Pathologist in Diagnosis of Oral Cancer

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Abstract

The management of oral cancer is a multidisciplinary endeavour, as each patient presents the treating clinicians with a unique set of challenges the management of which impacts on both survival and quality of life. This article focuses on the different radiographic methods used in the diagnosis of oral cancer. Imaging techniques have become an important element in the field of oral oncology. Continued research has led to technical progress in the existing modalities and addition of newer, exiting and highly accurate techniques. Although this is a boon, it has created differences of opinion regarding the ideal choice of imaging techniques. Inappropriate choice of imaging modality can result in delay in diagnosis and treatment thereby inducing more suffering for the patients.

Keywords*: Oral cancer, Radiography, CT scans.*

Introduction

Oral cavity squamous cell cancers form a significant percentage of the cancers seen in India. While clinical examination allows direct visualization, it cannot evaluate deep extension of disease. Cross-sectional imaging has become the cornerstone in the pretreatment evaluation of these cancers and provides accurate information about the extent and depth of disease that can help decide the appropriate management strategy and indicate prognosis. Early cancers are treated with a single modality, either surgery or radiotherapy while advanced cancers are offered a combination of surgery, radiotherapy and chemotherapy. Imaging can decide resectability, help plan the precise extent of resection, and indicate whether organ conservation therapy should be offered. Quality of life issues necessitate preservation of form and function and pretreatment imaging helps plan appropriate reconstruction and counsel patients regarding lifestyle changes.

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WHY DO WE NEED TO RADIOGRAPHS EVEN AFTER HISTOPATHOLOGICAL CONFIRMATION OF ORAL CANCER:

Despite continued research and advances the survival rates have not improved significantly . This is because of the two main reasons. Firstly, the oral cancer lesions are detected only at an advanced stage when they have invaded the deeper structures and metastasized to another location, most likely the lymph nodes of the neck[1]. Secondly, there could be an inappropriate assessment of the lesion in terms of depth and extent, bone invasion, status of lymph node involvement and distant metastasis, thereby leading to inappropriate treatment planning and therapy failure[2,3]. Hence it is important not only to detect the tumors at the earliest but also make an adequate assessment which is critical for appropriate planning of surgical, radiation and chemotherapy treatment[4].

Histopathological examination by biopsy is a useful method to confirm diagnosis and to estimate the malignant potential of tumor. However digital examination needs mastery of skills for consistent accuracy and objectivity,

more so in inaccessible intra oral sites. Accurate lymph node assessment and appropriate staging are also difficult with clinical examination alone[5,6].

APPLICATIONS OF IMAGING IN ORAL CANCERS :

ADVANCES IN THE FIELD OF IMAGING HAVE PROVED TO BE A BOON IN:

i) In finding information regarding location, extent, depth, proximity to anatomical structures and lymph node assessment.

ii) Helps in grading the stages of cancer and determining the treatment modality.

iii) It guides during cancer treatments by focusing treatments on the tumors and thereby minimizing the damage to the adjacent normal tissues.

iv) And post-operatively to determine the treatment response

v) It plays a major role in monitoring cancer recurrence in patients previously treated for cancer[7].

IMAGING MODALITIES ARE AVAILABLE:

Ø RVG (Intraoral radiography)

Ø Conventional OPG (Panoramic radiography)

Ø Nuclear medicine Scintigraphy

Ø Ultrasonograhy (US)

Ø Computed Tomography (CT)

Ø Magnetic Resonance Imaging (MRI)

Ø Positron Emission Tomography (PET)

Ø Positron Emission Tomography/ Computed Tomography (PET/CT)

Ø Single Positron Emission Computed Tomography (SPECT)

IOPA/RVG (Intraoral radiography)

On regular basis of screening the patient, sometimes the complaint of patient can be common pain, swelling or mobility in tooth , in such cases basic IOPA/ RVG can reveal abnormal intraosseous changes which can be alarming. These Imaging can be useful in determining any suspicious abnormalities that might be cancerous.

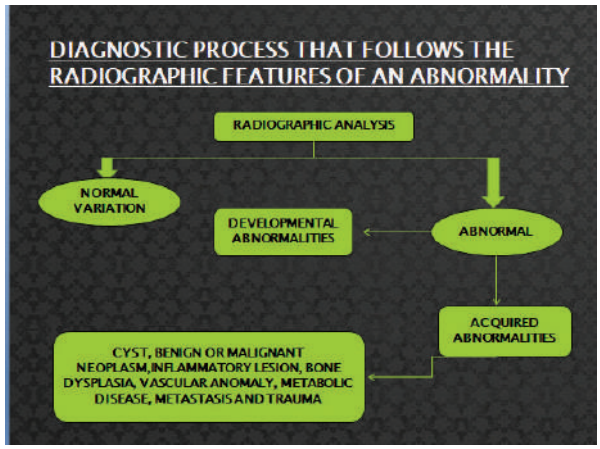


Figure 1 -Diagnostic process that follows the radiographic features of an abnormality

CONVENTIONAL OPG (PANORAMIC RADIOGRAPHY)

Conventional imaging techniques with x-rays are perhaps the most familiar type of imaging. Orthopantomography (OPG) provides a good overall view of the jaws and the dentition[7]. A combination of OPG and intraoral radiography play an important role in the detection of bone invasion especially in determining the supero-inferior extent in carcinoma of the mandibular gingiva. This assessment of the superoinferior extent is one of the most important factors influencing surgeons in choosing between a rim or segmental resection of the mandible. A study comparing OPG combined with intraoral radiography and CT in detecting the supero-inferior extent of tumor invasion of mandible, concluded that there was no difference in the diagnostic accuracy between the two techniques[8]. The sensitivity of OPG was lower than CT, MRI. Hence, it was suggested that decision to resect the mandible as a part of the management of oral cancer should be taken on the evidence of clinical examination, periosteal stripping and at least two imaging techniques that compliment each other in terms of specificity and sensitivity. A combination of OPG and Scintigraphy has high specificity and sensitivity thereby giving accurate results[9].

CT- COMPUTED TOMOGRAPHY

Numerous prospective and retrospective studies

have investigated various imaging methods such as orthopantomogram (OPG), CT scan, Denta scan, MRI, Bone scan and SPECT for assessing mandibular invasion in oral squamous cancers[10,11]. Comparison has also been made with clinical examination and periosteal stripping. Although initially imaging was pronounced inaccurate as compared to clinical examination, subsequent studies conclusively proved the role of imaging in assessing mandibular invasion. CT was found to have the highest specificity (87%) while SPECT and MRI had the highest sensitivity (96-97%)[12]

CT scans of the head/neck/chest are routinely employed in assessment of oral cancer and are excellent in highlight cortical destruction, potential cervical node metastases and pulmonary metastases.

disadvantages with the use of CT. Presence of amalgam fillings and metallic prosthesis in the oral cavity can result in artifacts and obscures the anatomy and pathology in the sections where devices are included. A CT scan is not as useful in cases where soft tissue malignancies are required to be studied[13,14]. Hence a CT is not recommended in cases of carcinomas of tongue and gingiva, instead MRI is preferred.

CT scan is also currently used for radiation therapy planning, with a specific positioning and immobilizing device. It will play an essential role, along with MRI, in the development of conformal radiation therapy[15].



Figure 2-Image of Axial of CT scan-Head

MRI- MAGNETIC RESONANCE IMAGING

MRI of the neck is excellent at evaluating the soft tissue extent of the tumour, extent of marrow infiltration of the mandible or maxilla and assessment of intra or perineural involvement. MRI can assess the presence of marrow edema and hence bone infiltration which cannot be appreciated with CT. MRI can better assess the occult metastasis as the lymph nodes give the same signal intensity as the primary tumor[16]. In cases of salivary gland tumors, MRI can better assess the fine details of the gland. MRI can also accurately depict the T stage, another factor with bearing on prognosis and treatment. Extension to the extrinsic muscles (upstaging disease to T4a), encasement of neurovascular bundle, invasion and base tongue are well seen . These features influence the choice of therapy (single or multiple modality) and the extent of surgical resection that can vary from wide excision to partial glossectomy to total glossectomy (for tumors involving bilateral neurovascular bundles)[17,18].

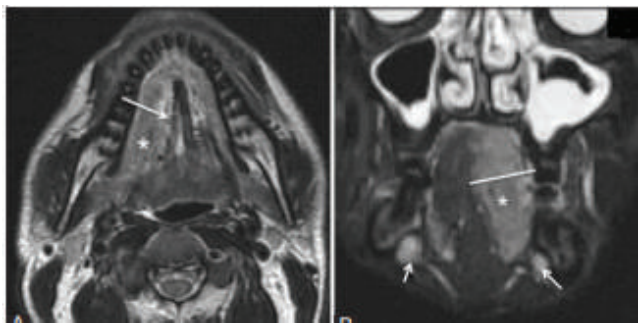


Figure 3-Image of Magnetic resonance imaging of head

ULTRASONOGRAPHY:

Ultrasound uses sound waves with frequencies above those humans can hear. A transducer sends sound waves traveling into the body which are reflected back from organs and tissues, allowing a picture to be made of the internal organs. Ultrasound, used either intraorally for accessible tumours, or more commonly for assessment of cervical nodes can be combined with a fine-needle aspirate for cytologic assessment of suspicious cervical nodes. In head and neck malignancies it is mainly limited to the tumors in salivary glands and detection of lymph nodes metastasis. For lesions in the parotid, submandibular and sublingual glands, ultrasound is an ideal tool for initial assessment[19]. These are relatively superficial structures accessible by high resolution ultrasound, which provides excellent resolution and tissue characterization without a radiation hazard. The main advantages are low cost and easily accessible

technique and can easily guide a fine-needle aspiration[20].

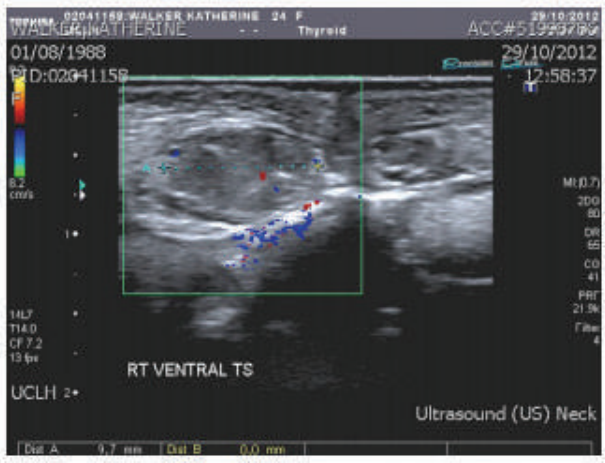


Figure 4 - Image of Ultrasonography of the tongue measuring the dimensions of the tongue tumour

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY (PET/CT):

PET/CT is a physiological imaging for assessment of metabolism within the tissues. It is the fusion of the two techniques of PET and CT. CT helps in localization of the tumor anatomically and PET helps in characterization, thereby providing information physiologically[21]. The main application is in the assessment of patients with cancer using the **glucose analogue 2-[18] fluoro-2-deoxy-Dglucose (FDG).** Cancer cells have increased glucose utilization and FDG PET/CT is used to investigate the increased FDG metabolism of malignant cells compared with non-malignant cells. PET is often combined with an anatomical imaging modality (CT or MR), and is a functional scan, where a radiotracer is administered intravenously to the patient; the tracer is preferentially taken up by cells with a high metabolic rate (a characteristic of many oral cancers). Infection and inflammation however can also provide similar radiologic appearances. It is most often used in advanced (stage 3 or 4 disease, or in salvage/recurrent cases) and the assessment of metastatic disease.

FDG PET/CT plays an increasing central role in the detection and management of head and neck cancers because most of the cancers in this region are squamous cell carcinomas. This technique is particularly useful in assessment of occult primary tumors and lymph nodes which are missed in other radiographic techniques. It helps in accurate staging of the carcinomas also assess the post treatment results and responses. The residual tumor and recurrences are also better assessed by this technique. PET/CT better assess the operability upto 95%. This technique is highly sensitive. The specificity is compromised and there are false positive results when there are other infections present. For example in case of post chemotherapy, the patients immunity is low and prone for infections. This can result in false uptake of the radiotracer and false positive results. It cannot be used in cases of uncontrolled diabetes where the blood sugar levels are higher than 150mg/dl[22,23].

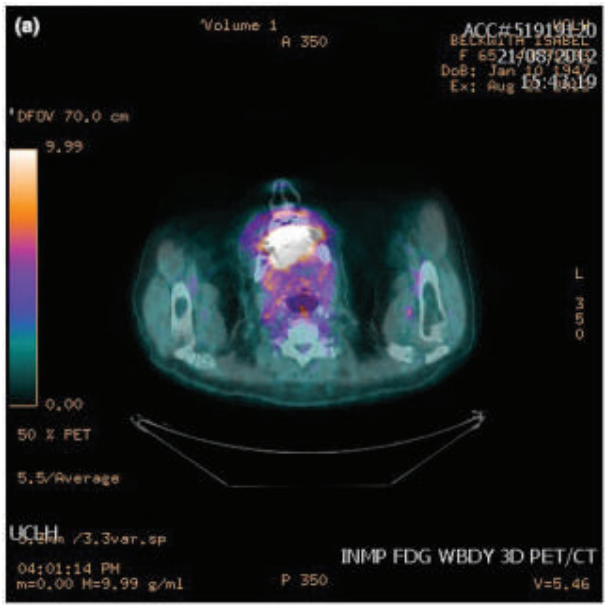


Figure 5a - Image of assessment of metastasis using 3D PET/CT



Figure 5b - Image of PET-CT showing extensive uptake in the floor of mouth and bilateral cervical nodes

BONE SCINTIGRAPHY:

Bone imaging with radionuclides is a non-invasive technique for demonstrating the osteoblastic lesion of the skeletal system. It is based on the uptake of a radiotracer that occurs where bone is being formed or repaired. It can be particularly helpful in tumors of head and neck which invade the adjacent bones. A bone loss upto 5 percent can also be detected with accuracy with a bone scan. It is also of particular importance in distant metastasis in the bones.

SINGLE POSITRON EMISSION COMPUTED TOMOGRAPHY - SPECT/CT:

SPECT/CT is a imaging technique that combines the functional information from Scintigraphy with the anatomical information from Computed Tomography into one set of images. It measures the concentration of chemicals injected into the body, and provides images of the chemical function of body parts of interest. In this technique the image obtained by a gamma camera is a 2-D view of 3-D distribution of a radionuclide[24,25].

Conclusion

Oral cavity cancer is a challenging disease with high mortality rates; dentists and dental specialists play a critical role at all stages in the management of patients. Choice among further sophisticated imaging modalities depend on number of factors like availability, affordability and clinical situation. If clinical findings are suggestive of bone involvement, the preferred imaging method is CT as it assesses bone architecture better. If the clinical findings are suggestive more of soft tissue involvement and nodal metastasis, MRI is the preferred imaging modality. In addition to these, several new techniques that aid in diagnosis and staging of oral carcinomas like SPECT and PET/CT are also available. PET/CT is superior and is of particular value in investigation of occult primary tumors, staging primary disease, detection of residual and recurrent lesions.

Prevention through education about smoking cessation and safe alcohol consumption is critical, detection and early referral of premalignant lesions and oral cancers and ongoing surveillance, follow up and preservation of oral health are just a few of the many roles of the dental practitioner in the management of oral cancer.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Biodentine as a Perforative Repair Material- A Review

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Abstract

Perforations are anomalous communications between the root canal system and the external dental surface that connects the pulp cavity to the periodontal tissue. Current advances in biomaterials has made the recovery of tooth structure and function possible even in the most complicated cases. Ideally, perforations should be immediately repaired with a biocompatible material to seal the communication between the perforation site and the gingival sulcus in order to achieve a more favorable prognosis. This review article is focussed on Biodentine as perforative repair material, and has found to provide good biocompatibility, bioactivity, high compressive strength and a short setting time.

Keywords : *Biodentin, perforation, repair, biocompatible*

Introduction

Perforation can be defined as mechanical or pathological communication between the tooth surface and root canal systems.[1]They may be iatrogenic in cause or pathologic communications between the root canal system and the attachment apparatus. Perforation of the pulpal floor of the molar is one of the most difficult situation to handle in clinical situations. If a perforation occurs, the clinician is faced with the dilemma of repair, surgery or extraction. Prevalence of accidental root perforations ranges 2-12%.[2]As the perforation creates a portal of exit in the root canal system it has to be sealed as quickly as possible, since periodontal involvement arising from the perforation can become irreversible with time.[3] Many techniques have been described for the repair of the furcation perforations; they can be broadly classified into surgical and non surgical methods. The most preferable method is the non surgical method since, surgical method can lead to pathological pocket formation.[4]

The non surgical method involves the placement of the sealing material in to perforation through the access cavity and produces a good seal to avoid fluid movement, which will lead to failure of the treatment. Many materials have been used to seal the furcation perforation from zinc oxide based materials, zinc oxide eugenol cement, super ethoxy benzoicacid , calcium phosphate, glassionomer, Resin modified glass ionomers, Composites, Mineral Trioxide Aggregate.[5-7]

Biodentine is a calcium-silicate bio active material. It has superior mechanical, physical and handling properties comparable to most commonly used restorative materials. It is used as a temporary enamel restoration and permanent dentine restoration. It is used during root perforations, apexification, resorptions, retrograde fillings, pulp capping procedures, and dentine replacement. It is a powder liquid system, powder composed of Tri-calcium silicate, Di-calcium silicate, Calcium carbonate and oxide, Iron oxide, Zirconium oxide. Liquid consist of Calcium chloride, Hydro soluble polymer.[8]

CLINICAL APPLICATIONS OF BIODENTINE:

Biodentine has a wide range of applications including endodontic repair (root perforations, apexification, resorptive lesions, and retrograde filling material in endodontic surgery) and pulp capping and can be used as a dentine replacement material in restorative dentistry. The material is actually formulated using the MTA-

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based cement technology and the improvement of some properties of these types of cements, such as physical qualities and handling makes up Biodentine. [9]

It is easy to handle owing to its ease of manipulation and a short setting time approximately 12 minutes, has high alkaline pH and is a biocompatible material makes it a favourable material for perforation repair. In a study by Guneser et al., Biodentine showed considerable performance as a perforation repair material even after being exposed to various endodontic irrigants as compared to MTA. Biodentine was compared with white MTA (ProRoot) and glass ionomer cement (FujiIX) using human fibroblasts, both white MTA and Biodentine were found to be less toxic compared to glass ionomer during the 1- and 7-day observation period.[10] [11]

PHYSIO-CHEMICAL PROPERTIES OF BIODENTINE :

Working time of Biodentine is up to 6 minutes with a final set at around 10-12 minutes. The density of Biodentine is 2.26g/cm and porosity of 6.8%. There is an increase of the electrical resistance along with the porosity reduction. Therefore, even after the initial setting of Biodentine, the material continues to improve in terms of internal structure towards a more dense material, with a decrease in porosity. The setting of Biodentine is illustrated by a sharp increase in the compressive strength reaching more than 100 MPa in the first hour. The mechanical strength continues to improve to reach more than 200 MPa at 24h. After 2 hours, the hardness of Biodentine was 51 HVN and reached 69 HVN after 1 month. The reported micro hardness values for natural dentine are in the range of 60-90 HVN. Biodentine has surface hardness in the same range as natural dentine. Biodentine contains zirconium oxide allowing identification on radiographs. According to the ISO standard 6876, Biodentine displays a radiopacity equivalent to 3.5 mm of aluminum. This value is over the minimum requirement of the ISO standard (3 mm aluminum). This makes Biodentine particularly suitable in the endodontic indications of canal repair. [11][12]

BIOCOMPATIBILITY OF BIODENTINE :

Biocompatibility of a dental material is a major factor that should be taken into consideration specifically when it is used in pulp capping, perforation repair or as a retrograde filling. During the aforementioned procedures, the material is in direct contact with the connective tissue and has the potential to affect the viability of periradicular and pulpal cells. Therefore, it is essential that toxic materials are avoided and materials promoting repair or that are biologically neutral are preferred during procedures in which the material is directly in contact with the surrounding tissue. Dentine element uptake is prominent for Biodentine. There is presence of high release of calcium for Biodentine. [13] [14]

Biodentine consistency is better suited to the clinical use than MTA's. Biodentine presentation ensures a better handling and safety than MTA. Biodentine does not require a two step obturation as in the case of MTA. As the setting is faster, there is a lower risk of bacterial contamination than with MTA. Adding to its ability to be used as dentine substitute, Biodentine could safely be used for each indication where dentine is damaged. Therefore, it is an advantage for the clinician and the patient. [15]

Fig 2: Occlusal view

BIODENTINE AS PERFORATIVE REPAIR MATERIAL :

Biodentine is fast setting, so if perforations are communicated to the oral cavity, use is recommended. Another factor to consider the Biodentine is their coloration, similar to the dental tissues and produces no staining of these. The Biodentine cement is selected as perforation filing material due to its satisfactory bond strength to dentin compared with the MTA, as in repair furcation perforations situations. It is easy to handle owing to its ease of manipulation and a short setting time approximately 12 minutes, has high alkaline pH and is a biocompatible material makes it a favourable material for perforation repair.[16,17] In a study by Guneser et al., Biodentine showed considerable performance as a perforation repair material even after being exposed to various endodontic irrigants as compared to MTA. [18]The satisfactory tissue response and cytotoxicity compared with other cements based on calcium silicate were decisive factors for the choice of Biodentine cement as filling material for root perforation.[19]

Biodentine was found to significantly increase TGF-B1 secretion from pulp cells.[20]TGF is a growth factor whose role in angiogenesis, recruitment of progenitor cells, cell differentiation, and mineralization has been highlighted in recent studies.Biodentine can induce the synthesis of a dentin- like matrix by human odontoblast-like cells in the form of mineralization nodules that have the molecular characteristics of dentin. Additionally, the FTIR analysis has previously shown that this mineralized material was a specific deposition, which had the same mineral and organic composition of dentin.[21]This can also stimulate cell growth and induce Hydroxyapatite (HA) formation on the surface of the material when exposed to the simulated body fluid. HA have been shown to induce bone formation, growth and maintenance at the bone-material interface in vivo and this can be reproduced and demonstrated in vitro by soaking HA in simulated body fluids .This is of prime importance during the process of healing as Silica can induce the mineralisation function of cells by affecting cell proliferation and gene expression.

Zanini et al. [22] also evaluated the biological effect of Biodentine on murine pulp cells by analysing the expression of several biomolecular markers after culturing OD-21 cells with or without Biodentine. Their results, consistent with other studies, were in favor of Biodentine, which was found to be bioactive due to its ability to increase OD-21 cell proliferation and biomineralization.

Laurent et al. [19] indicated that though the interactions between pulp capping materials and the injured pulp tissue are yet unclear, there is growing evidence on the role of growth factors, with TGF-□1 being the most important one. These factors' main role is the signalling of reparative dentinogenesis. In a recently published article, they assessed the reparative dentin synthesis capacity of Biodentine as well as the ability to modulate TGF-□1 secretion by pulp cells which has previously shown to be released from dentine by calcium hydroxide.[23,24] Using an entire human tooth culture model, they showed that, upon application on the exposed pulp, Biodentine had the potential to significantly increase TGF-□1 secretion from pulp cells and induce an early form of reparative dentin synthesis.

Biodentine has bioactive properties, encourages hard tissue regeneration, and provoke no signs of moderate or severe pulp inflammation response. The material has the ability to maintain a successful marginal integrity due to the formation of hydroxyapatite crystals at the surface which enhances the sealing ability. Due to its superior sealing potential, there is no risk of microleakage which may cause the pulp to become infected or necrotic and jeopardize the success of vital treatment procedures. The hard tissue formation due to calcium hydroxide is a defense response of the pulp against the irritant nature of the material whereas calcium silicate based materials are compatible with the cell recruitment.[25]

Conclusion

A perforation is an artificial communication between the root canal system and the supporting tissues of the teeth. When not properly managed, root perforation complicates the treatment and deprives the prognosis. A wide range of materials have been proposed in literature to seal the perforations.Biodentine has a wide range of applications in endodontics and has shown remarkable results as a perforation repair material. It is a calcium silicate-based bioactive material.In our review we have found that biodentinehas superior mechanical, physical and handling properties comparable to most commonly used restorative materials and can because of its ease of manipulation, short setting time, high alkaline pH and biocompatibility. Hence it is a favourable material for perforation repair.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

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Esthetic Replacement of Missing Antetior Tooth with All Ceramic Restoration (IPS E.max Cad) – A Case Report

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Abstract

In Dentistry, ceramic restoration has become more popular among other dental restorative material, because of its superior esthetics, good biocompatibility as well as excellent mechanical and physical properties. Among dental ceramics -Lithium Disilicate (LD) glass ceramics and Polycrystalline Zirconium Dioxide Ceramics are used as a dental biomaterial and also the material of choice for esthetic replacement of missing anterior teeth.These materials exhibit better esthetics, color stability, biocompatibility as well as better strength. Theyact as a most promising restorative materials because it has favourable mechanical and physical properties compared to other restorative materials.

Keywords*; all ceramic, lithium disilicate, zirconia ceramic. esthetic, biocompatibility*

Introduction

Fixed denture prostheses are the treatment option, used to replace damaged or missing teeth or teeth that have been lost. They are attached to prepared abutment teeth using restorative cement material so that they cannot be removed. In 1960s, metal ceramic has been considered as the gold standard of crown and bridge because of its satisfactory mechanical and physical properties as well as its esthetics results and marginal adaptations which is clinically acceptable[1].

In recent decades, increasing demand from patients for natural-appearing and bio-safety reasons, metal free restoration hasled to the development of all-ceramic materials with improved mechanical characteristics that ensure suitable longevity. These restorative materials are now replacing traditional metal-ceramic restorations. Among dental ceramics - Lithium disilicate (LD) glass ceramics and Zirconia ceramic are the material of choice for esthetic replacement of missing anterior teeth.

Lithium disilicate is a glass ceramic with a unique structure and can be produced by means of both pressable and Computer Aided Design-Computer Aided Manufacturing (CAD-CAM) processing. It shows good mechanical properties (flexural strength 350 - 450 MPa), has excellent translucency and is more suitable than zirconia ceramic especially in esthetic areas[2].

A Case Report

A 24 years, male patient came to the Department of Prosthodontics, Sree Balaji Dental College & Hospital with a chief complaint of missing tooth in upper front jaw region for past 1 year. The patient presented no significant medical history.

Clinical examination revealed that maxillary left central incisor was missing (fig.1and 2) and the tooth was extracted due to trauma before 1 year. On radiographic assessmentrevealed that presence of adequate bone support from abutment teethand sufficient amount of space available for either fixed or removable prosthesis.

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Possible treatment options are fixed or removable prosthesis. Patient wants fixed restoration, so removable treatment plan was eliminated. Patient also need not wants any invasive treatment procedure (Dental implant) hence various fixed treatment options were discussed with the patient and replacement of the missing tooth was planned with conventional fixed partial denture. The patient signed the informed consent, and accepted the treatment plan for fixed partial denture.

Procedure

The primary impression was made with irreversible hydrocolloid (fig.3) impression material (Zhermack-tropicalgin, BadiaPolesine[Rovigo] Italy). The cast was poured in type-3 dental stone (DPI, Mumbai, Maharashtra, India). After the primary casts obtained, it was mounted in a articulator and wax mock-up was done. Then the putty index was made.

A conventional tooth preparation was done in maxillary 11 and 22 incisor region (fig.4) Shoulder type finish line was given in 11 and 22 regions for all-ceramic restoration. For all – ceramic restoration, the tooth preparation in 11 and 22 regions need1.5 to 2mm labiolingual reduction and 2mm incisal reduction. Flat-end tapered diamond was used for shoulder type of finish line. Before tooth preparation shade selection should be done.

Once the tooth preparation has been done, retraction cord was placed for retraction of gingival tissue. Then the master impression was made. The material of choice used for impression procedure were Zhermack elite HD+ soft putty and Zhermack elite HD+ normal set light body (fig.5), using the double mix technique. For better esthetics and phonetics, tooth colored provisional restoration(fig.6) was given in maxillary anterior region. The impressions were poured using type IV dental stone. The restorative crowns were designed and fabricated using CAD – CAM technology. After fabrication of permanent prosthesis, it should be checked in patient mouth for better adaptation and shade matching, then the all ceramic restoration was cemented(fig.7) using 3M ESPE RelyX^{TM} U200 resin cement.



Fig 1: Intra Oral Examination



Fig 2: Occlusal view

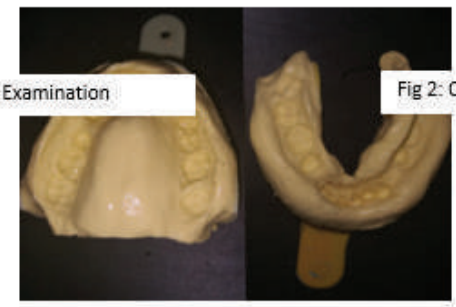


Fig 3: Diagnostic impression



Fig 4: Tooth preparation



Fig 5: Master impression



Fig 6: Temporary restoration



Fig 7: Permanent restoration



Fig 8: Post-

Discussion

The replication of natural teeth in anterior region for patients having high esthetic demands has become more challenge for dentists. The reproduction of the color in the cervical portion is more challenging because a thinner layer of material must be used to prevent harm to the pulpal tissue and also most critical area for selecting the color shade in fixed prosthesis. Shade selection is an important factor because the color of a restoration should be indistinguishable from that of the adjacent teeth. The color matching of a restoration is determined by the core materials, porcelain thickness, fabrication process, and by surface properties such as texture[3].

In recent decades, the demand for better estheticsby the patient had increased over the year. The tooth color restorations like all ceramics and composite material play a major role in fulfilling the esthetics demand of the patient. The use of all ceramic restorations has increased

due to its superior esthetics, color stability, and its biocompatibility[4].

Lithium disilicate is a glass ceramic with a unique structure. This material has glass ceramic phase in which crystalline fillers are added during manufacturing to improve the strength, thermal expansion and contraction behaviour of ceramics. The crystalline phase of $Li_2Si_2O_5$ makes for up to 70% of the material, grows by nucleation and forms many randomly oriented interlocking crystals, contributing to the superior strength (flexural strength of 350 – 450 MPa) than the alumina or leucite ceramics. The crystalline phase orientation could also cause crack deflection and blunting, accounting for the improved strength.

It has excellent translucency due to good compatibility between the glass ceramic phase and the crystalline phase which keeps internal light scattering within the material to a minimum. Highly esthetic results can be achieved with this material.

It is commercially available as ingots that can be pressed to obtain full-contour forms or sub-structures, as milling blocks that can be milled by CAD-CAM technology, and also act as a veneering material that can be hot pressed onto all-ceramic frameworks[5].

Harda et al has reported that lithium disilicate is more translucent than zirconia, which means the superior esthetic property of monolithic lithium disilicate[6].

Conclusion

From this clinical report it was concluded that replacement of missing anterior teeth is more challenging one for the dentist. All ceramic restoration - Lithium disilicate (LD) glass ceramics and Zirconia ceramic are the material of choice for esthetic replacement of missing anterior teeth. Lithium disilicate is a glass ceramic with a uniquestructure. It shows good mechanical properties (flexural strength 350 - 450 MPa), has excellent translucency and is more suitable than zirconia ceramic especially in esthetic areas

Ethical Clearance – Not required since it is a case report

Source of Funding – nil

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Role of Platform Switching in Success of Dental Implants- A Meta-Analysis

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Abstract

Aim: To analyze through systematic review and meta-analysis of implant failure rates, marginal bone loss (MBL) in patients who received platform-switched implants and platform-matched implants and to determine literature based evidence.

Materials and Methods :

Data: Search terms used in combination are dental implant, platform switch, platform mismatch, switched platform, implant – abutment design.

Sources : An electronic search engine without time/ language restrictions was taken from Google Scholar, Pubmed.

Clinical Significance: Platform switching for maintaining peri-implant bone levels has gained popularity over last few years. As philosophies of treatment alter over , a periodic review of different concepts are necessary to refine techniques thereby forming a basis for optimum treatment.

Keywords: *Dental implants, Meta- analysis, Marginal bone*

Introduction

Dental implants have become a standardized and predictable treatment modality for the rehabilitation of partially and completely edentulous arches, resulting in restoring the normal function, esthetics, speech, and comfort in harmony with stomatognathic system[1].

Various studies have been conducted on the effectiveness and predictability of dental implant therapy on which Dr. Branemark in 1968, first proposed the concept of osseointegration.

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Wide-diameter dental implants introduced in the late 1980s, created a situation where mismatched standard-diameter abutments were used simply because of the lack of commercial availability of components to match the wide-diameter implants. It was found that these implants exhibited less-than-expected initial crestal bone loss. This remodeling is termed as saucerization[2].

The ability to reduce this crestal bone loss may have several advantages such as improved esthetics, higher bone to implant contact and better primary stability. To minimize this crestal bone resorption, several techniques and procedures such as non-submerged technique, utilizing micro-roughness on implant neck surface and platform switching have recently been developed [3].

A better understanding of the biologic principles of bone healing around dental implants is needed and studies have demonstrated that bone resorption at the implant-abutment junction (IAJ) may be caused by an inflammatory cell infiltrate, that forms a zone around

the IAJ. Though not fully understood, the current theory of platform switching is related to the physical repositioning of the IAJ away from the outer surface of the implant and the surrounding bone, thereby avoiding the inflammatory infiltrate away from the width of the platform switched dental implant.

Considering the key importance of crestal bone loss to implant success, several preclinical and clinical studies have investigated a variety of confounding biologic and biomechanical factors contributing to CBL. The micro gap at the junction of an implant platform and the abutment has been suggested as a major contributor. Thepresent meta-analysis was conducted to investigate the impact of platform switching on marginal bone levels around platform switched and platform matched dental implants

Aim & Objectives

To analyze through systematic review and meta-analysis of implant failure rates, marginal bone loss (MBL) in patients who received platform-switched implants and platform-matched implants.

· To Collect Reliable Journal Articles Reporting The Marginal Bone Loss Among Platform Switched Dental Implants And Platform Matched Dental Implants.

· To Compare The Parameters Across The Different Articles.

· To Address The Limitations In The Collected Data.

Methodology

- A structured electronic systematic search without time or language restrictions was undertaken from March 2009-2019 in the following databases.

- PubMed library and Google scholar was searched for articles using MeSH terms.

- The following question in the PICO format (Patient, Intervention, Comparison and Outcome) was developed in patients treated with dental implants, are there any differences among platform- switched restorations compared to platform matched ones in terms of peri-implant marginal bone loss and implant survival.

- In the electronic search, 475 articles were identified , 372 articles were obtained after removal of duplicates and 36 full text articles were assessed for eligibility based on inclusion criteria. 29 articles were included in qualitative analysis and 7 articles were included in the quantitative analysis

Search Terms :

• The following combinations of keywords were used :

• Dental implant , Platform shifting, Platform switch, Platform switching, Mismatched abutment, non-platform switched implants, platform design, switched abutment, crestal bone remodeling, marginal tissue, Implant- abutment junction, Crestal bone loss, Marginal bone preservation, Marginal bone loss, Marginal bone changes, dental implants, and implant design.

Inclusion Criteria :

• All clinical trials,

• Randomized Control Trials

• Prospective studies,

• Case-control studies with a minimum of 10 implants/ subjects.

Exclusion Criteria :

• Animal studies,

• Biomechanical studies,

• Finite element analysis,

• Case reports,

• Studies in the absence of a comparison group.

STUDY SELECTION AND DATA COLLECTION :

• During the first step of selection, studies were retained based on the screening of the title and abstracts, later the final stage of screening involved full- text reading using a predetermined data extraction form to confirm the eligibility of each study based on inclusion and exclusion criteria.

• In addition bibliography of these selected articles were also scanned to obtain the maximum number of results possible. A total of 475 articles were obtained from Pubmed And Google Scholar , after removal of duplicates and 36 full text articles were assessed for eligibility based on inclusion criteria. 29 articles were included in qualitative analysis and 7 articles were included in the quantitative analysis.

Study Design :

· A meta-analysis on the platform switching in dental implants

PRISMA FLOW CHART

Results

Characteristics Of The Studies Included And Comparison Of Mean marginal Bone loss considering RCT'S with an observation period (Table 1)

Sl. No:	Study name	Platform switched			Platform matched		
		Mean	SD	95% CI	Mean	SD	95% CI
1.	Enkling (2011) -1 year -25 implants	0.19	0.47	-0.37,0	-0.34	0.35	-0.5,0
2.	Enkling et al(2013) -1 year -25 implants	-0.34	±0.35	0.50, 0.00	-0.19	±0.47	-0.37, 0.00
3.	Silvio et al(2014) -1 year -36 implants	0.84	±0.23	0.73,0.95	0.93	±0.26	0.81, 1.05
4.	Silvio et al (2016) -3 year -36 implants	1.06	±0.24	0.93, 1.15	1.09	±0.31	0.85, 1.13
5.	S.Rocha et al (2017) -5 years -59 implants	0.27	±0.44	0.26,0.76	0.09	±0.85	0.07, 0.64
5.	S.Rocha et al (2017) -5 years -59 implants	0.27	±0.44	0.26,0.76	0.09	±0.85	0.07, 0.64
6.	**Laura Lago et al (2018) -1 to 5 years -100 implants**	**-0.17**	**±0.67**	**0.71**	**0.34**	**±0.54**	**0.33**
7.	**Ana Messias et al (2019) - 5 years -121 implants**	**0.19**	**±0.53**	**0.33,0.43**	**0.04**	**±0.58**	**0.03, 0.43**

META- ANALYSIS :

· Mean difference of the mean bone implant contact was calculated for platform switched dental implant and platform matched dental implant and compared using student T test at confidence interval of 95%

· Graphically the data was represented as forest plot.

· Meta-analysis was done using MEDCALC software version 19.1

· In the electronic search, 475 articles were identified. Using the key words "platform switched and platform matched implants "372 articles were obtained after removal of duplicates and 36 full text articles were assessed for eligibility based on inclusion criteria. 29 articles were included in qualitative analysis and 7 articles were included in the quantitative analysis.

· Pooled estimates from the 7 studies were analysed using a continuous random effects model meta-analysis, the variable analyzed was crestal bone loss of implants placed in platform switched and platform matched dental implants.

META-ANALYSIS: CONTINUOUS MEASURE: TABLE – 2

Variable for studies	study_name
1. Intervention groups	
Variable for number of cases	N
Variable for mean	Mean
Variable for SD	Sd
2. Control groups	
Variable for number of cases	N
Variable for mean	mean_1
Variable for SD	sd1

Study	N1	N2	Total	SMD	SE	95% CI	t	P	Weight (%)	
									Fixed	Random
Enkling (2011)	25	25	50	1.259	0.306	0.645 to 1.873			5.46	12.80
Enkling et al(2013)	25	25	50	-0.356	0.281	-0.921 to 0.208			6.47	13.25
Silvio et al(2014)	36	36	72	-0.363	0.235	-0.832 to 0.106			9.22	14.05

Silvio et al (2016)	36	36	72	-0.107	0.233	-0.572 to 0.358			9.36	14.08
S.Rocha et al (2017)	59	59	118	0.264	0.184	-0.0997 to 0.628			15.10	14.86
Laura lago et al (2018)	100	100	200	-0.835	0.147	-1.125 to -0.545			23.61	15.37
Ana messias et al (2019)	121	121	242	0.269	0.129	0.0155 to 0.523			30.76	15.59
Total (fixed effects)	402	402	804	-0.0722	0.0714	-0.212 to 0.0679	-1.012	0.312	100.00	100.00
Total (random effects)	402	402	804	0.000827	0.234	-0.459 to 0.461	0.00353	0.997	100.00	100.00

TEST OF HETEROGENEITY :

Q	58.8846
DF	6
Significance level	P < 0.0001
I2 (inconsistency)	89.81%
95% CI for I2	81.54 to 94.37

FOREST PLOT :

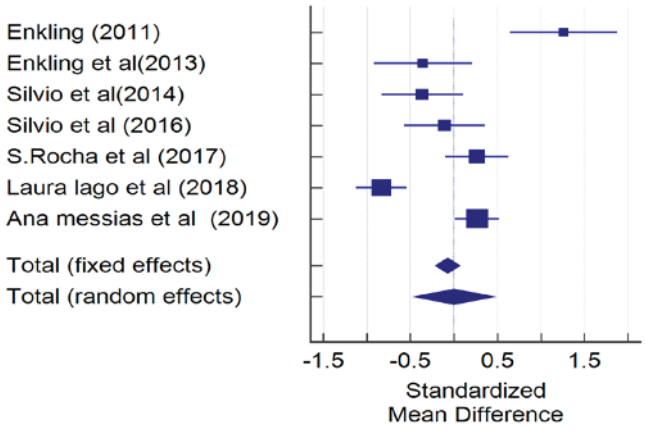


FIGURE 1, Forest Plot Depicts the Mean Differences of Marginal Bone Loss between Platform switched And Platform matched Implants.

FUNNEL PLOT:

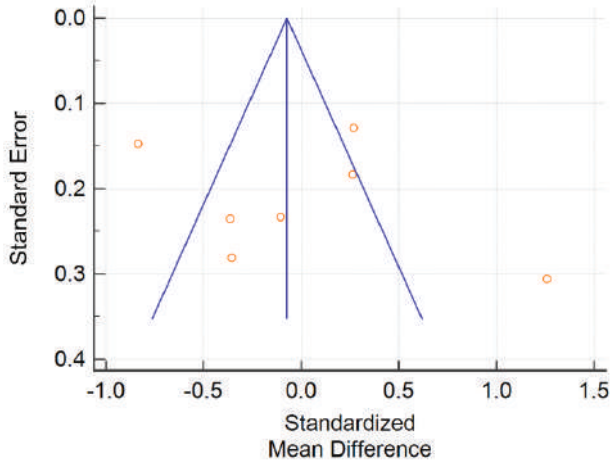


FIGURE 2, Funnel Plot For Detection Of Publication Bias

META-ANALYSIS INTERPRETATION :

FOREST PLOT :

· The main outcome of the meta-analysis is a forest plot, a graphical display as in figure 1, the X-axis (horizontal axis) forms the effect size scale (odds Ratio or Relative Risk), plotted on the bottom of the plot. Each row except bottom two, represents a study's effect size estimate in the form of a square (black box) the bigger the box, the more contribution to the study and a (95%) confidence interval and a vertical line which is known as the line of no effect / null effect.

· The diamond at the bottom of the forest plot shows the results when all the individual studies are combined and averaged. The horizontal points of the diamond are the limits of the 95% confidence interval and are subject to the same interpretation as any of the other individual studies on the plot.

· In this analysis, diamond shape 'Pooled Data ' found on the line of no effect which means there is less statistical significance or no statistical significance with the use of platform switched dental implants compared with platform matched dental implants.

FUNNEL PLOT :

Funnel plot has the following characteristics,

· The plot ideally resembles a pyramid or inverted funnel, with scatter due to sampling variation. The shape is expected because the studies have a wide range of

standard errors. If the same size, the studies would fall on the horizontal line.

In this analysis, Few studies (3 out of 7) were scattered out of the funnel plot it shows that there was a possible error in the studies, which may due to poor methodological design, inadequate analysis, heterogeneity (size of the effect differs according to study size) and sampling variation.

Discussion

Platform switching for maintaining of peri-implant bone levels has gained popularity over last few years. As philosophies of treatment alter over time, a periodic review of different concepts is necessary to refine techniques thereby forming a basis for optimum treatment. Crestal bone loss is considered an important criteria for the evaluation of the implant outcome and evidence for the presence or absence of peri-implant tissue health. Therefore, efforts were made to preserve the peri-implant marginal bone levels stable throughout and following the prosthetic loading phase.

Platform switching technique is one among the various approaches to achieve marginal or crestal bone stability. With the platform switching technique, there is a close adaptation of soft tissues, increased bone-implant contact area, good per mucosal seal, and hence better emergence profile is obtained thereby improving the esthetics.

Recently, published systematic reviews and meta-analyses supported this assumption by confirming the effectiveness of the platform switching technique[4] significantly limiting marginal bone resorption around dental implants, while the cumulative estimated implant success rate was statistically less significant between both intervention groups of platform switched and platform matched dental implants.

All the studies included in the meta-analysis were RCT's, the analysis of the methodological quality of some of them revealed some potential criticisms i.e bias for reporting incomplete outcome data, due to dropouts or exclusion after randomization, some authors did not report the periodontal health status of the patients included in the study.

The platform switching technique and effects of platform switching were found via electronic database, of which 475 records were found. 36 articles were fully assessed for eligibility , 29 met the inclusion criteria for the qualitative analysis, and 7 were found eligible for the quantitative, to be included in meta-analysis. Considering the results of RCT's included in this meta-analysis, three studies stated that the difference of marginal bone levels were remarkably in favor of the platform switched group. However a statistical analysis has to be done to calculate the level of significance, and four studies indicated the difference of marginal bone levels was less significant in the platform switched group compared to those utilizing platform matched implant-abutment connections.

The possibility cannot be ignored, but it is not known whether the loss of marginal bone is a long term process. Only drawback is, only few studies are available with long term follow up. Moreover, it is debatable whether such mean difference may have clinical significance. Several hypotheses trying to explain this phenomenon have been raised in the literature. Some studies have shown that bone resorption around the implant neck does not begin until the implant is uncovered and exposed to the oral cavity, which invariably leads to bacterial contamination of the gap between implant and abutment. The bacterial reservoir in the microgap may continuously invade the bone through micro-spaces. Toxins produced by the bacteria create a zone of toxicity at the level of microcap, resulting in peri-implant inflammation and typical bone loss upto 1st thread.

Especially periodontally compromised smoking patients with treated periodontitis revealed significantly more peri-implant marginal bone loss compared to periodontally healthy smokers , therefore smoking and the history of periodontitis should be considered risk factors for marginal bone loss around dental implants.

However, studies done by Enkling[5] et al in 2011, Enkling[6] et al in 2013, Silvio[7] et al 2014, Silvio[8] et al 2016, S.Rocha[9] et al in 2017, Laura lago et al in 2018, Ana messias[10] et al in 2019 cautiously summarized their findings, especially on emphasizing the bone-preserving effects on platform switched implant and platform matched implant. Nevertheless, within the limitations of the recently published RCTs included for meta-analysis presented here, the results of former systematic

reviews confirmed, a less significant difference between the mean MBL change at dental implants with a PS implant – abutment compared to a PM implant-abutment configuration.

Atieh[11] et al, in their systematic review and meta-analysis involving a total of 10 RCTs, reported significantly lower bone loss in implants restored with platform switching vs platform matching.

Annibali[12] et al in their systematic review with a follow-up of 36 months reported that there is no significant difference in implant success rate between platform matching and platform switching. Implants restored by platform switching showed a lower degree of marginal bone loss over time of 0 to 0.99 mm and - 0 to 2.02 mm for platform matching dental implants. In fact, there seems to be a relationship between a higher degree of discrepancy and a smaller amount of bone loss.

Herekar[13] et al, in their systematic review of 10 RCTs and 5 controlled clinical trials, showed that marginal bone loss around implants restored by platform switching was statistically less significant than that of implants restored by platform matching −0.34 mm. Strietzel et al, in their systematic review and meta-analysis, reported that most studies showed a favorable trend toward platform switching.

Santiago[14] et al analyzed a total of 17 RCTs and 8 prospective studies. This meta-analysis revealed a significant reduction in marginal bone loss for implants restored with platform switching compared with implants restored with platform matching. Another highlight, regardless of the P value, is the clinical relevance these findings may offer, since the difference between the two techniques is seldom greater than 0.5 mm. If possible error in the measuring method is taken into account, the comparative advantage of bone-level implants restored by platform switching over the tissue-level implants restored by platform matching may be limited by this value.

The last, as reported by Hsu, Lin, and Wang [15] (2017), seems to be a crucial factor for the preservation of marginal bone levels, contributing to the success of the rehabilitations. Notwithstanding that and taking into consideration the validity of these results, we believe that any patient receiving implants in any healed bounded or free end edentulous areas would benefit from the use of PS prosthetic components from the early stages of the rehabilitation (i.e. from surgery onwards), as long as strict hygiene and motivation is practised with follow-up programme is done to ensure patient compliance.

Summarizing the findings of the included publications, especially among the RCTs remarkably more studies indicated results favoring less significance for the use of PS technique to prevent MBL changes. Therefore, heterogeneity among the study conditions is supposed to have a crucial influence on study outcomes.

The results of the study in the forest plot suggested that the study done by Enkling et al in 2013, Silvio et al in 2014, had a significant impact on the platform switching technique. Whereas study done by Enkling in 2011 , S Rocha et al in 2017, Ana messias et al in 2019 had a less significant impact in this technique. The pooled estimate of the study (diamond shape) lied in the center of line of no effect stating that ,there is less significant difference with use of platform switched when compared with platform matched dental implants.

Meta-analysis of 7 RCTs revealed a significantly less mean MBL change at PS implants compared with PM implants, thus confirming the supposed bone level stabilizing effect of PS implant-abutment configurations at least when considering short-term observations. The longest follow-up period within the RCTs was reported up to 60 months i.e.5 years by S.Rocha et al. (2017) and Laura lago et al (2018).

Limitations

· First, few clinical studies with less numbers of patients were available for data extraction. Second, there was not enough evidence from RCT in the selected literature.

The impact of positioning the micro-gap on soft tissue margin changes around PS implants lacks documentation and may not allow for any conclusions.

Platform Switching may have a positive effect on bone preservation in the first year, but after five years, the marginal bone change is insignificant, as compared to that at one year, around both PS and PM implants.

More homogeneous studies with a longer follow-up

are needed to confirm platform switching phenomenon to reduce crestal bone resorption, and health of peri-implant soft tissue and hard tissue .

Summary and Conclusion

· The meta-analysis of 7 RCT'S revealed a significantly less mean MBL change at implants with a PS – implant –abutment configuration compared with PM-implant-abutment design. However, within the limitations of the recently available publications of the RCT'S and PCCS, the tendency revealing from the studies results favors less significance of the PS technique to prevent or minimize peri-implant marginal bone loss, compared to implants with PM abutments.

· The results of the present study suggest that there is a significantly less MBL at implants with platform-switching than on implants with platform matching. Moreover, precisely designed and appropriate clinical trials with larger samples and longer follow-up periods are required for literature based evidence.

Ethical Clearance – Institutional Ethics Committee, Sree Balaji Dental College and Hospital, Committee registered with DCGI. (Registration no: SBDCH/IEC/04/2019/22)

Source of Funding – Nil

Conflict of Interest – Nil

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Microbial Etiology of Acute Diarrheoa in Children Under Ten Years of Age in Diyala, Iraq / Hospital-Based Study

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Abstract

This study was conducted in the Al Batool Teaching Hospital in Ba'aqubah in Diyala city, from August 2019 to December 2019. In children under 10 years patients suffering from diarrhea with different gastrointestinal complaints, Stool samples were collected from each patients use for microscopic examination and Culture and sensitivity test. Diarrheal diseases are major problem of developing countries. Though precise data on childhood mortality associated with diarrheal diseases in Iraq is not available, it has been estimated that approximately 25% of child death are associated with diarrheal disease, particularly acute diarrhea. The purpose of this study was to detect causative agent of acute diarrhea in children less than 10 years of age. With the study of sensitivity to certain antibiotics. Bacterial pathogen infected cases 30 (60%) male and 20(40%) female, among the total enrolled cases the Escherichia coli was 30(60%), Klebsiella species was 17(34%) and kluyvera ascorbate species was 3 (6%).also the study detected that bacterial infection was found to be of highest percentage, 26(52%) in the age group less than 1year, The least infection2 (4%) was found in group between7-8. There were no detectable cases of bacterial infection among the age group of 9-10 years in this study.Amikacin showed efficacy in 26(96.6%) isolates of E.coli species, also showed efficacy in 16(94.1%)isolate of Klebsiella spp.Amikacin,cefepime,meropenem,gantamicin showed efficacy in 3(100%)isolate of Kluyvera ascorbate.

Keywords: *Abdominal infection, Kluyvera ascorbate, Diarrhea in children, Klebsiella*

Introduction

Diarrhea is defined by World Health Organization (WHO) as having 3 or more loose or liquid stools per day or as having more stools than is normal for that person [(1)]. Acute diarrhea, defined as an increased frequency of defecation (three or more times per day or at least 200 g of stool per day lasting less than 14 days, may be accompanied by nausea, vomiting, abdominal cramping, clinically significant systemic symptoms, or malnutrition[(2)] .

Diarrhea is an important cause of morbidity and mortality in children from developing countries. Children less than ten years of age have 3.3 diarrheic episodes per year, and more than one-third of the deaths in this age group are associated with diarrhea. Therefore, every year there are approximately billion diarrheic episodes and 4 million deaths in children less than five years of age (most from 6 months to 12 years) caused by this disease [(3)].

Kluyvera is a gram negative bacillus a. This bacillus which could be localized in the respiratory tract, gastrointestinal system and urinary tract has been observed to cause to clinically severe disease in various anatomic localizations in the last 30 years. It was shown to be a new member of the Enterobacteriaceae family using molecular methods and deoxyribonucleic acid (DNA) hybridization technique [(4)]. There are four Kluyvera species which have been defined until the present time including K. ascorbata, K. cryocrescens, K. georgiana and K. cochleae. Kluyvera is a small, Peritrichous, mobile, oxidase negative, catalase positive gram negative bacillus and ferments glucose [(5)].

Kluyvera Ascorbata is a gram negative microorganism belonging to the family Enterobacteriaceae. Although

it causes infections infrequently, it is responsible for causing a wide range of infections including severe sepsis (6). Kluyvera is a relatively newly described member of the Enterobacteriaceae family that rarely causes infections in humans. In the pediatric population, it is described in association with clinically significant infections ranging from urinary tract infections to sepsis with multiorgan failure [7].

The factors related to higher prevalence of diarrhea are lack of education of mother, lack of exclusive breastfeeding, breastfeeding for less than 1 year, roundworm infestation, nutritional status, immunization status, female sex, literacy, personal hygiene, overcrowding, garbage disposal, source of water supply, and toilet facility [8]. In China, diarrhea is the most common reason for children to visit health-care clinics, and the antibiotic resistance has become a growing problem due to misuse of antibiotics [9].

In many hospitals in developing countries lacking clinical microbiology laboratories, the cause of diarrhea in children is unknown. The seasonality of specific enteropathogens such as rotavirus or some parasites has been reported [10].

The aim of this study was to determine the prevalence of enteropathogens, including bacteria, virus, and parasites, causing diarrhea among children less than five years old in during the dry and rainy seasons .The main etiology of the diarrhea is related to a wide range of bacteria (such as Campylobacter jejuni, Escherichia coli, Salmonella spp., Vibrio cholerae, Yersinia enterocolitica, and Aeromonas spp.), enter parasites (Giardia spp., Cryptosporidium spp., and Entamoeba histolytica), and viruses (adenovirus, Norwalk virus, and rotavirus) [11].

Microbial Identification

After many years of experience, Appeared Vitek 2 –compact (biomerieux) device as a reference in terms of identification of microorganisms. used to analyze the samples and obtains the results. Offering both manual and automated solutions, adapted to guarantees rapid, accurate and standardized results for all types of specimens, thus optimizing workflow of microbiology labs. Stool sample was collected in a sterile container. Rapid reliable identification of these organisms is essential for accurate diagnosis and prompt effective treatment. We evaluated the ability of the VITEK 2 system (biomerieux, Inc., Hazelwood, Mo.) to identify these organisms rapidly and accurately[12].

Each organism suspension was prepared from the growth of pure cultures of bacteria cultivated on plates containing Trypticase soy (TSA) agar with 5% sheep blood and incubated overnight at 35°C. Bacterial cells were suspended in 2.5 ml of a 0.45% sodium chloride solution. The suspension used in the VITEK 2 system was adjusted to a McFarland standard of 0.5 by using a Densicheck (bioMérieux). The Biomerieux DensiCHEK Plus instrument is for use with VITEK and VITEK 2 systems to measure the optical density of a microorganism suspension in McFarland units [13].

Antibiotic sensitivity test:

Emergence of Antimicrobial Resistance and the Rationale for Performing Susceptibility Testing

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. Empirical therapy continues to be effective for some bacterial pathogens because resistance mechanisms have not been observed. Susceptibility testing of individual isolates is important with species that may possess acquired resistance mechanisms (e.g. members of the Enterobacteriaceae, Pseudomonas species, Staphylococcus species, Enterococcus species, and Streptococcus pneumoniae) [14].

An important task of the clinical microbiology laboratory is the performance of antimicrobial susceptibility testing of significant bacterial isolates. The goals of testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The most widely used testing methods include broth microdilution or rapid automated instrument methods that use commercially marketed materials and devices. Manual methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods. Each method has strengths and weaknesses, including organisms that may be accurately tested by the method. Some methods provide quantitative

results (e.g., minimum inhibitory concentration), and all provide qualitative assessments using the categories susceptible, intermediate, or resistant. In general, current testing methods provide accurate detection of common antimicrobial resistance mechanisms. However, newer or emerging mechanisms of resistance require constant vigilance regarding the ability of each test method to accurately detect resistance [15] .

Material and Methods

Patients and samples.

This study was a cross-sectional study conducted at AL-Batool Teaching Hospital, Department of Microbiology-Laboratory consent was obtained from the children's parents or guardian before enrollment. A total of 50 stool samples were collected from the children under 10 years of age visiting AL- Batool Children's Hospital, Ba'aqubah, Diyala Proven with acute diarrhea in the periods between Augast 2018 to December 2018. From each participating children, clinical data were obtained and stool sample was collected in a sterile container. Vitek 2 –compact (biomerieux) device used to analyzed the samples and obtain the results [16] .

Statistical Analysis

Data of current study analyzed by using Chi-square- x2 test to compare between percentages.A level of significance of a=0.05 was applied to the test. (Spss v.22) programs used analyzed current data.

Rusults

Bacterial pathogen infected were 50 (100%) totaled cases, 30 (60%) among of theme were male, while the remaining 20(30%) were female, as shown in table. 1

Table 1.gender distribution of diarrheal cases.

Gender	N(%)
Male	30(60%)
Female	20(30%)

Bacterial infection was found to be of highest, 26(52%) in the age group less than 1year. The least infection2 (4%) was found in group between 7-8. There were no detectable cases of bacterial infection among the age group of 9-10in this study. Statistical analysis showed that there were very significant different ($p<0.001$) in distribution of bacterial infection as shown in table 2

Age groups	No.	%
Less than one year	26	52%
1-2	11	22%
3-4	7	%14
5-6	4	%8
7-8	2	%4
9-10	0	%0

Table 2. Age distribution of diarrheal cases.

Among the total enrolled cases the Escherichia coli was 30(60%), Klebsiella species was 17(34%) and kluyvera intermedia species was 3 (6%) .NO Shigella or salmonella detected in this study. Statistical analysis showed that there were very high significant different ($p<0.001$) in distribution of bacterial infection as shown in table.3

Table3.distribution of bacterial pathogen.

Type of organism	NO. (%)
Escherichia Coli	30(60%)
Klebsiella SPP.	17(34%)
Kluyvera Ascorbata	3(6%)

Among the *Escherichia coli* Amikacin resistance showed the highest 26(96.6%) efficacy, followed by Meropenem which showed 23(76.6) efficacy, while the least efficacy showed by Ampicillin 3(10%) .Statistical analysis showed a very high significant different in antibiotic sensitivity ($p<0.001$)as in table.4

Table 4.antimicrobial sensitivity of E.coli spp (n=30)

Antibiotics	Sensitive No. (%)	Resistance No. (%)
Ampicillin	3(10%)	27(90%)
Amoxicillin	17(56.6%)	13(43.4%)
Cefuroxime	18(60%)	12(40%)
Cefoxitin	22(73.3%)	8(26.7%)
Cefixime	18(60%)	12(40%)
Ceftazidzme	9(30%)	21(70%)
Ceftriaxone	13(43.3%)	17(56.7%)
Amikacin	29(96.6%)	1(3.4%)
Meropenem	23(76.6%)	7(23.4%)
Gentamycin	20(66%)	10(33.4%)
Ciprofloxacin	18(60%)	12(40%)
Trimethoprime/sulfamethaxazol	7(23.3%)	23(76.6%)

In *Klebsiella spp*, Amikacin showed efficacy in 16 (94.6) of cases, followed by cefoxitin which was résistance only in 3(17.7) cases, the least efficacy showed by ampicillin 4(23.5%) and Trimethoprim\sulfamethaxazol which was sensitive in 6(35.2%). statistical analysis showed a significant different in antibiotic résistance ($p<0.05$) as in table.5

Table 5.antimicribial sensitivity pattern of Klebsiella spp (n=17).

Antibiotics	Sensitivity No. (%)	Resistance No. (%)
Ampicillin	4(23.5%)	13(76.5%)
Amoxicillin	8(47%)	9(53%)
Cefurxime	7(41.1%)	10(58.9%)
Cefoxitin	14(82.3%)	3(17.7%)
Cefixime	8(47%)	9(53%)
Ceftazidzme	7(41.1%)	10(58.9%)
Ceftriaxone	7(41.1%)	10(58.9%)
Amikacin	16(94.1%)	1(5.9%)
Meropenem	13(76.4%)	4(23.6%)
Gentamycin	12(70.5%)	5(29.5%)
Ciprofloxacin	12(70.5%)	5(29.5%)
Trimethoprime/sulfamethaxazol	6(35.2%)	11(64.8%)

Among *Kluyvera ascorbata* Cefepime, Meropenem, Gentamicin and Amikacin were sensitive in all cases 3(100%) ,Tetracycline showed no sensitivity in all case, table 6.

Table 6.antimicribial sensitivity pattern of Kluyvera intermediate (n=3)

Antibiotics	Sensitive No. (%)	Resistance No. (%)
Piperacillin/tazobzctam	2(66.6%)	1(33.4%)
Ceftazidzm	2(66.6%)	1(33.4%)
Cefixime	3(100%)	0(0%)
Meropenem	3(100%)	0(0%)
Gentamicin	3(100%)	0(0%)
Amikacin	3(100%)	0(0%)
Ciprofloxacin	2(66.6%)	1(33.4%)
Tetracycline	0(0%)	3(10%)
Trimethoprime/sulfamethoxazole	1(33.4%)	2(66.6%)
Piperacillin	2(66.6%)	1(33.4%)

Discussion

The e bacterial pathogens were found to be a significant cause of acute diarrhea. However, while diarrheal diseases are prevalent in all ages, they are often more severe among children due to their small body size, and rapid ability to become dehydrated [17].

In this study out of 50 cases enrolled 30were male and 20were female. Higher positivity rate among boys was in agreement with the numerous studies . This high prevalence associated with males may be related to the reality that male children more active than females. Moreover, they spend longer period outside the home and they were more in contact with environmental conditions than females. This makes them more liable to infection than females [18]. Children below 10 years of age were enrolled in this study. The maximum number of samples were from the age group of less than 2 years in which the age group of less than 1 years constitutes maximum number 26 (52%)followed by the age group of 1-2years 11(22%). Similar result was also found by Moyo et al.[21] . *Escherichia coli* were detected in 30 (60%) of cases with Similar results were also reported by several other investigators [19].

In our study *Klebsiella spp* detected in 17(34%) of cases. A comparable studies also found (23).*Kluyvera ascorbata* detected in 3(6%) of cases ,studies showed that, diarrheal infections caused by kluyvera spp have been reported in neutropenia and non-neutropenia patients ,in patients with underlying malignant neoplasm and in those without any known disease process ,with or without antimicrobial therapy. This suggests the presence of poorly understood host conditions that can predisposed a person to opportunistic infection with *kluyvera ascorbate* [20].

No *Vibrio* spp. was isolated in this study. The spread pattern of *Vibrio* spp. suggested water borne infection in rainy seasons, although the quantity of water is large in the rainy season most water are contaminated with excreted microorganisms from water surface runoff [21] .

While discussing about the antibiotic sensitivity profile, among *Escherichia coli* Among the *Escherichia coli*, Amikacin showed the highest 26(96.6%) efficacy, followed by Meropenem which showed 23(76.6), Cefoxitin 22(73.3%), ciprofloxacin 18(60%) efficacy, while the least efficacy showed by ampicillin 3(10%) of cases, . According to Kaminski et al.[28] . Ciprofloxacin was 100% effective against *E. coli* and 50% of isolates were resistant to Ampicillin and Al Gallas et al. [6]. showed that isolates 20.4% isolates resistant to Ampicillin. Among Klebsiella Amikacin showed efficacy in 16(94.6) of cases, followed by Cefoxitin which was résistance only in 3(17.7) cases, the least efficacy showed by ampicillin 4(23.5%) and Trimethoprime\ sulfamethaxazol which was sensitive in 6(35.2%),study by Chris Rowe Tatted et al. (2017) kany) . showed that Amikacin sensitive in 66% of isolate,ampcilin in 51% cases, while Trimethoprime\sulfamethaxazol 39% sensitive. In total identified cases of kluyvera spp Cefepime, Meropenem, Gentamicin and Amikacin were sensitive in all cases 3(100%), Tetracycline showed no sensitivity in all cases, Similar result showed by Eda Karadag Oncel et al. (2015) [9].

Conclusions

The study indicated that the frequency of diarrhea was higher in male children compared to female children. Amikacin, gentamycin, were the most effective antibiotics while Ampicillin

Were the least effective antibiotics in vitro against the bacterial isolates. *E.coli* species were common among the bacterial pathogen causing acute diarrhea in children less than 10 years of age. These children can become a source of outbreaks. So the awareness on prevention of the infectious diseases, improving hygiene should be implicated to reduce the burden of infectious diseases.

Ethical Clearance: The project of this study was taken from the ethical committee of College of Medicine / University of Diyala.

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Conflict of Interest: Nil

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Influence of Sesame Oil on Physiologic and Histopathological Changes in Alloxnized Male Guinea Pigs

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Abstract

The current study was to investigate the effect sesame oil *(Sesamum indicum* L) on glycosylated hemoglobin, blood glucose, Malondialdehyde, semen and hormonal parameters, in addition the histopathological changes on pancreas in diabetic pigs. The physiological indices were examined in control, diabetic and oil treated diabetic pigs. Eighteen male pigs were randomly assigned into three groups, group one as control, group two diabetic as positive control and group three diabetic treatment with sesame oil at dose (1ml/kg) orally, after 30 days whole blood samples were collected to measure the hemoglobin A1c and other samples were separated into serum for blood glucose and MDA and hormonal assays. Epididymal taken for sperm parameters examined, all animals were sacrificed and pancreas was taken for histological study. Outcomes of this study indicate that significantly reduction in serum Malondialdehyde level in associated with lowered levels of blood glucose and hemoglobin A1c, While, significantly elevation in serum testosterone, FSH and LH levels accompanied with the improved spermatogenesis were observed in group treated with the sesame oil. In addition, the a significant islet langer hans restoration was notable. **Conclusion:** The oil has antioxidant and ameliorated effects on pancreatic function and markedly improved sperm profile.

Key words: *Sesame oil, hemoglobin A1c, Malondialdehyde, Hormones, male pigs*

Introduction

Diabetes is a chronic metabolic disease that represents a major public health problem because of the current lifestyle and dietary habits. Type one caused by deficiency in insulin production, type two by ineffectiveness of insulin, clinical and preclinical evidence suggests that diabetes is associated with oxidative stress leading to imbalance of oxidant/ antioxidant [(1)]. The pathophysiology of diabetes involves several interrelated mechanisms, elevated blood glucose induces auto-oxidative glycosylation, formation of glycation product, activates protein kinase-C, increases polyol pathway activity and hexosamine flux, which are the key components of the cascade, these pathways are responsible for the generation of reactive oxygen species which ultimately contribute to oxidative stress [(2)].

Sesame(*Sesamum indicum* Linn.) belongs to the family – Pedaliaceae, Sesame is widely used in food, nutraceutical, pharmaceutical and industry in many countries because of its high oil, protein and antioxidant contents and chemo-preventive agents, some of which have been attributed to a group of compounds called lignans such as sesamin, sesamolin, sesaminol, sesamolinol and tocopherol, which are known to play an important role in the oxidative stability, nutritional antioxidant and medicinal properties. hence, it uses against health problems[(3)]. Sesame seeds are also contain a good type of monounsaturated and polyunsaturated fatty acids highly nutritious, rich in vitamins and a good source

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of minerals[4]. **The work** aimed to evaluate the effects of sesame oil on the Malondialdehyde (MDA), fasting blood glucose and glycosylated hemoglobin(HbA1c), testicular function (epididymal sperm and hormones parameters) and histological changes in pancrease of diabetic male guinea pigs.

Materials and Method

Oil extraction**:** White sesame seeds(*Sesamum indicum* L.). were purchased from a local market in Basra city, and oil extracted from seeds by Soxhlet apparatus as described by[5]

Experimental protocol

Eighteen, healthy adult male guinea pigs weighed between (900-1000) gm, they were managed and housed in suitable cages under optimum conditions 12 hours light/ dark cycle and temperature of 25 ±3°C in the animal house of the College of Veterinary Medicine / University of Basra.

Pigs were randomly distributed into three groups, consisting 6 of pigs per group the treatment continued for 30 days as follows.

Group I : pigs were orally given(1ml/ kg) of normal saline daily serving as (negative control).

Group II: pigs in this group were treated with single dose of alloxan monohydratem according to method [6], diabetic pigs similarly given(1ml/kg) of normal saline daily serving as(positive control).

Group III: the diabetic animals orally received (1ml kg) of sesame oil daily, the dose was chosen as described by [7] .

Pigs in the fasted state and under light ether anesthesia, whole blood samples were collected at thirty days of study through heart puncture technique, blood samples were used for the hemoglobin A1c assayed according to[8] . Other blood samples were separated into serum was used for determination malonaldialdehyde according to[9] blood glucose level was measured by method[10] Testosterone level was determined according to[11], FSH and LH concentrations assayed as described by [12]. Directly after blood collection, testes carefully excised for semen examination, animals were sacrificed, pancreas carefully excised for histological study.

Seminal analysis

Spermatozoa concentration and live spermatozoa percentage were counted according to[13], Percentage motility of sperm was estimated by drop of the sperm suspension that placed on glass slide covered by a coverslip and also degree of individual motility of spermatozoa was recorded.

Histological preparation: The pancreas was removed from all groups, small specimens of pancreas immediately fixed with 10% buffered formalin solution, the fixed tissues prepared according to [14], for histological examination.

Statistical analysis: analysis was done by one way analysis of variance (ANOVA). Data were expressed as means ± standard error (Means±SE). P a Value less than 0.05 ($P<0.05$) were considered to be statistically significant.

Results

Table (1) shows that the MDA level is a good indicator for evaluating oxidative stress in diabetes. The results explain that there is a significant ($P < 0.05$) increased in serum MDA level in associated with the significantly ($P<0.05$) increased in serum glucose concentration and hemoglobinA1c in diabetic pigs compared with control group. While, the significantly ($P < 0.05$) reduction in serum MDA level accompanied by significantly ($P < 0.05$) decreased in levels of glucose and hemoglobinA1c were observed in treated group with sesame oil when compared with diabetic and control groups.

Table(1): Serum malondialdehyed (MDA), blood glucose and hemoglobinA1c(HbA1c) levels from control group, diabetic group and diabetic group treated with sesame oil (*Sesamum indunic L*)

Groups	MDA µ mol/l	Blood glucose Mg/dl	HbA1c %
Negative control	0.45±0.01 c	109.23±1.66 c	4.2±0.02 c
Positive control	2.03±0.05 a	236.20±2.35 a	7.4±0.06 a
Treatment	0.49 ±0.02 b	119.67 ±1.9 b	4.5±0.04 b

The different letter means statistically differences at the ($p < 0.05$) level as compared with control group. Values are expressed as mean ± SE

Table(2)The results indicate that significantly ($P< 0.05$) decreased in sperm concentration sperm, motility and live sperm percentage in diabetic group compared to control group. Whereas, showed significantly($P< 0.05$) elevation of the sperm concentration accompanied by significantly ($P< 0.05$) increase in motility and live sperm percentage for approach the control values in group treated with sesame oil when compared with diabetic group..

Table(2): Concentration of sperm, motility and live percentageof sperm from control group, diabetic group and diabetic group treated with sesame oil (Sesamum indunic L)

Groups	Sperm concentration x106 mm3	Sperm Motility %	Live sperm
Negative control	155.3±3.2 a	88.29±1-80 a	74.14±3.4 a
Positive control	80.32 ±1.47 c	48.44±2.26 c	33.25±1.08 c
Treatment	149.16 ±2.48 b	85.16 ±3.33 b	70.43±1.2 b

The different letter means statistically differences at the ($p < 0.05$) level as compared with control group. Values are expressed as mean ± SE

The data explained that testosterone, FSH and LH levels significantly decrease ($P<0.05$) were also observed in the diabetic group compared to control group. While, the outcomes indicated that significantly (<0,05) increased in serum testosterone, FSH and LH levels in group treated with sesame oil compared with diabetic group in table(3).

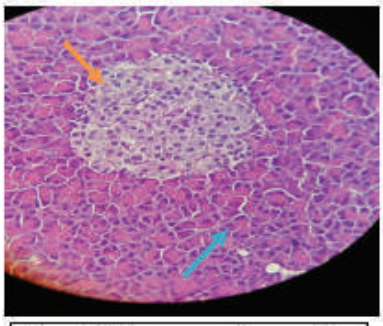
Table(3):Serum testosterone. follicle stimulating hormone and luteinizing hormone from control group, diabetic group and diabetic group treated with sesame oil(*Sesamum indunic L*)

Groups	Testosterone ng/ml	FSH ng/ml	LH ng/ml
Negative control	11.26±0.06 a	22.02±0.21 a	4.12±0.61 a
Positive control	6.99±0.37 c	11.96±0.13 c	1.76±0.2 c
Treatment	9.78±0.42 b	19.88±0.13 b	2.89±0.43 b

The different letter means statistically differences at the ($p < 0.05$) level as compared with control group. Values are expressed as mean ± SE

Histological Findings

The histo-pathological alteration were observed in the pancreas from pig treated with alloxan (150mg/kg B.W) show presence of vacuolation of islet (Fig.2). as compared with negative control. Whereas, showed the restoration in the islet of the pancreas within normal limits in diabetic pigs treated with 1ml /kg of sesame oil (Fig.3). as compared with positive control.



Figure(1):Pancreas section explains islet of langer hans ( ) and exocrine( ) in normal pigs **H&E X400**

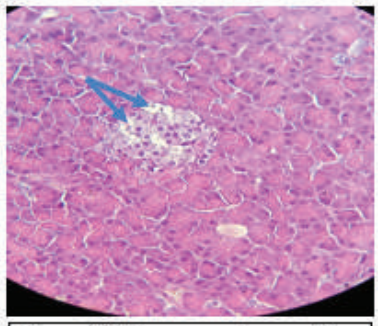
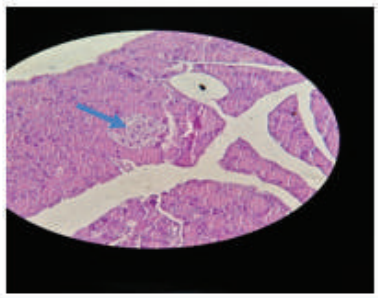


Figure (2):Pancreas section explains vacuolation in islet of langer hans ( ) in diabetic pigs **H&E X400**



Figure(3): Pancreas section explains islet of langer hans within normal limils ( ) In diabetic pigs given sesame oil 1 ml/kg B.W **H&E X400**

Discussion

Our current study demonstrated that sesame oil administration affecting on the oxidative stress this may be due to the prevention of the entering of glucose into the polyol pathway leading to an increased NADPH/ NADP ratio and prevent glycation end products[15]. On the other hand, administration of sesame cause a significant reduction in malondialdehyde (MDA) content due to the sesame oil has the most powerful antioxidant properties on free radicals[16]. our results agreement with [17] stated that the sesame oil can inhibited lipid peroxidation, significantly improved activity of the antioxidant enzymes and reduced glutathione (GSH) content may be attributable to presence of the natural antioxidant and gammatocopherol lignin fraction in sesame oil which are play an important role in its oxidative stability and antioxidant activity**.** The results indicate that sesame oil *(Sesamum indicum*)has hypoglycemic effect of diabetic animal this may be attributed to sesame oil induce hypoglycaemia by increasing the glycogen deposition in the liver and by increasing the secretion of insulin from pancreas, [18].The results are similar with findings obtained by[19] showed that bioactive molecules present in *Sesamum indicum* extract may probably possess insulin- like effect or stimulate the β cells of the pancreas to produce insulin which in turn lowers the blood glucose level.

The HbAIC level significantly increased in diabetic group suggesting glycosylation of Hb in the presence of hyperglycaemia, glycosylated Hb shows reduced affinity to oxygen a process that aid free radical release [20]. Our current findings, prominent decrease in HbAIc concentration was observed in treated group with sesame oil indicating decrease in blood glucose level and recovery to Hb. Results agreement with the [21] demonstrated that sesame administration cause to significantly decreased in the glycosylated hemoglobin level which could be due to an improvement in insulin secretion from the remnant pancreatic beta cells in diabetic rats. From our experiment showed decreased in sperm concentration, sperm motility, live sperm in diabetic pigs. The results in agree with[22] stated that hyperglycemia is usually accompanied by extensive disturbances in the metabolism of glucose and fatty acids, the lipid peroxidation in the testis and epididymal that lead to the impacts of sperm concentration, sperm motility, viability and abnormalities. In the present study showed that the sesame oil treatment could be improved sperm concentration and sperm motility this effect of sesame oil may be due to its potent antioxidant properties.[23]. The results agree with previous studies have indicated that treatment with antioxidants decrease the radical oxygen and improve the testicular dysfunction and subsequently ameliorate fertility in diabetic patients [24]. From our study show significantly decrease in testosterone, FSH and LH levels of diabetic group. testosterone production and leydig cells function are diminished in insulin-dependent diabetes due to the absence of is possible to affect the stimulatory effect of insulin on leydig cells and to an insulin –dependent decrease in FSH, which in turn decreases LH levels [25]. These findings agree with reported by[26] who found reduced in serum FSH,LH and testosterone levels suggesting leydig and sertoli cells which decrease with insulin deficiency and defects in insulin secretion may change testicular and accessory sexual glands function. The treatment with sesame oil that cause to increase levels of testosterone, FSH and LH hormones, this alteration in levels of hormones is attributed to the antioxidant nature of sesame oil that is possible to affect the biosynthesis of hormones balance [27].These results agree with reported by[28] who found that the sesame oil contains effective compound which cause to higher protein receptors or lignans also this lead to elevated in the FSH level, this effect is possible to return to the direct effect on the brain through the pituitary- hypothalamus axis in stimulating the secretion of hormones librated gonadotropin releasing hormones(GnRH).

Conclusion

Sesame oil has antioxidant and ameliorated effects on pancreatic function and markedly improved sperm profile.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Prevalence and Determinants of unfavorable Maternal and Neonatal Outcome in Term Premature Rupture of Membrane

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Abstract

Aim of study: To determine the prevalence of maternal and fetal outcomes in PROM among term pregnant women and to discover the risk factors that increase the rate of PROM.

Patients and Methods: A cross sectional study included 80 pregnant women diagnosed with term PROM (after 37 weeks of gestation) and gave birth in the hospital or attended to labor ward with leaking of liquor before onset of labor and delivered at our labor ward. Information about maternal age, occupation, gestational age, residence, parity, mode of delivery, antenatal care visits, duration of PROM, and postpartum complications as sepsis or hemorrhage. Information regarding neonates' Apgar score at 1 and 5 minutes, birthweight, admission to neonate intensive care unit.

Results: In this study, 35% of them showed unfavorable outcome, 16.3% of mothers complained from postpartum hemorrhage; 21.2% of neonates were admitted to NICU; and 15% of babies were weighing < 2.5 kg. The highest prevalence of unfavorable maternal outcome in this study was seen among women living in rural area (45.5%), in those who didn't receive ANC (31.6%), and in those who had PROM for 24 hours or more (29.4%). Unfavorable fetal outcome in this study was seen among women who didn't receive ANC (36.8%), and in those who had PROM for 24 hours or more (33.3%).

Conclusion: Unfavorable maternal or fetal outcome is not uncommon among women with PROM. Prolonged duration of PROM and lack of ANC visits were a significant associated factors. So, an early accurate diagnosis of PROM is essential for favorable outcome in pregnancy and thus it decreases the maternal and fetal morbidity.

Keywords: *Term PROM, maternal, fetal, mortality, Iraq.*

Introduction

Premature rupture of the membranes (PROM) is usually defined as rupture of membranes at any time before the onset of uterine contractions. PROM which occurs after 37 weeks of gestation is named term PROM, whereas; PROM which occurs prior to 37 weeks of gestation is named preterm PROM [(1)]. The latent period is defined as the duration from rupture of the membranes until the onset of true labor which is the key factor for determining maternal and fetal outcome [(2)]. Term rupture of the membranes (PROM) is one of the most common and controversial problems facing the obstetricians [(3)]. At term, PROM complicates approximately 8% of pregnancies over the world [(4)]. It has no known etiology but, sub clinical infection has been postulated [(5)]. Other workers suggested that the rupture of membrane is related to factors other than infection, membrane dysfunction on a molecular level, collagen destruction, and programmed cell death in fetal membranes [(6)]. PROM is linked to significant maternal and fetal morbidity and mortality. It

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has been shown to be the cause of 18%–20% and 21.4% of prenatal mortalities and morbidity respectively [7]. The key factor in the fetal and maternal outcome is that the diagnosis of pre-labor rupture of membranes must be established. In most instances, either it is obvious from the escape of clear amniotic fluid from cervix or by simple laboratory test like detection of fern pattern/ Litmus paper test [8]. Maternal complications include intra-amniotic infection, which occurs in 13%–60% of women with PROM, placental abruption, and postpartum endometritis. While preterm birth, infection, hypertensive disease, and asphyxia are cited as the most common contributors to maternal and fetal mortality in developing countries [9, 10]. The three causes of fetal death associated with PROM are sepsis, asphyxia, and pulmonary hyperplasia. Women with intrauterine infection deliver earlier than non-infected women, and infants born with sepsis have a mortality rate four times higher than those without sepsis do [11]. Diagnosis and proper management is very important to limit various fetal and maternal complications generally due to infection [7]. The maternal and fetal outcome in PROM is very important to decrease morbidity and mortality for both the mother and the child and for prevention of complications. So, the aim of this study is to determine the prevalence of maternal and fetal outcomes in PROM among term pregnant women and to discover the risk factors that increase the rate of PROM in a sample admitted to the delivery room in Al-Yarmouk Teaching Hospital.

Patients and Methods

Study design, setting: This was a cross sectional study that was conducted in the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital during the period from 1st of Feb 2019 till end of Jan 2020.

Study Population and sample size: The study included 80 pregnant women diagnosed with term PROM (after 37 weeks of gestation) and gave birth in the hospital or attended to labor ward with leaking of liquor before onset of labor and delivered at our labor ward. Pregnant women diagnosed with multiple gestation, had fetus with malformations, and any comorbidity with term PROM were excluded from this study. Assessment and estimation of gestational age was done depending on the date of last menstrual cycle, and / or early ultrasound scan.

In this study, the outcome variables were maternal and fetal outcomes, which was divided into two groups:

1. Favorable group (when the mother and neonate discharged from the hospital without complications).

2. Unfavorable group (when the mother or neonate died or experienced complications).

Data collection: We told all the pregnant women about the nature of the study and verbal consent was taken from them. Information about maternal age, occupation, gestational age, residence, parity, mode of delivery, antenatal care visits, duration of PROM, and postpartum complications as sepsis or hemorrhage. Information regarding neonates' Apgar score at 1 and 5 minutes, birthweight, admission to neonate intensive care unit,

All enrolled women underwent a general examination, vital signs (systolic and diastolic blood pressure), abdominal and obstetric examination, laboratory investigation and sonographic examination.

Statistical Analysis

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Chi square test was used to assess the association between maternal and fetal outcome and certain information. A level of P – value less than 0.05 was considered significant.

Results

In this study, 80 pregnant women were enrolled. Maternal age was ranging from 17 to 43 years with a mean of 26.3 ± 7.23 years. Most of them lived in urban area (86.3%); 60% were housewives; 67.5% were prim gravida; 52.5% received ANC; 63.7% had PROM for more than 24 hours; and 72.5% of them delivered by vaginal delivery as shown in table (1).

Table 1: Distribution of study patients by general information

Variable	No. (n= 80)	Percentage (%)
Age (Years)		
< 20	13	16.3
20 - 29	39	48.8
30 - 39	22	27.5
≥ 40	6	7.4
Residence		
Urban	69	86.3
Rural	11	13.8
Occupation		
Housewife	48	60.0
Employee	24	30.0
Student	8	10.0
Parity		
Primi	54	67.5
Multiparty	26	32.5
ANC visit		
Yes	42	52.5
No	38	47.5
Duration of PROM to delivery		
< 24 hrs.	29	36.3
≥ 24 hrs.	51	63.7
Mode of delivery		
Vaginal delivery	58	72.5
Caesarean section	22	27.5

Figure 1 showed the prevalence of unfavorable maternal and fetal outcome. We noticed that 35% of them showed unfavorable outcome.

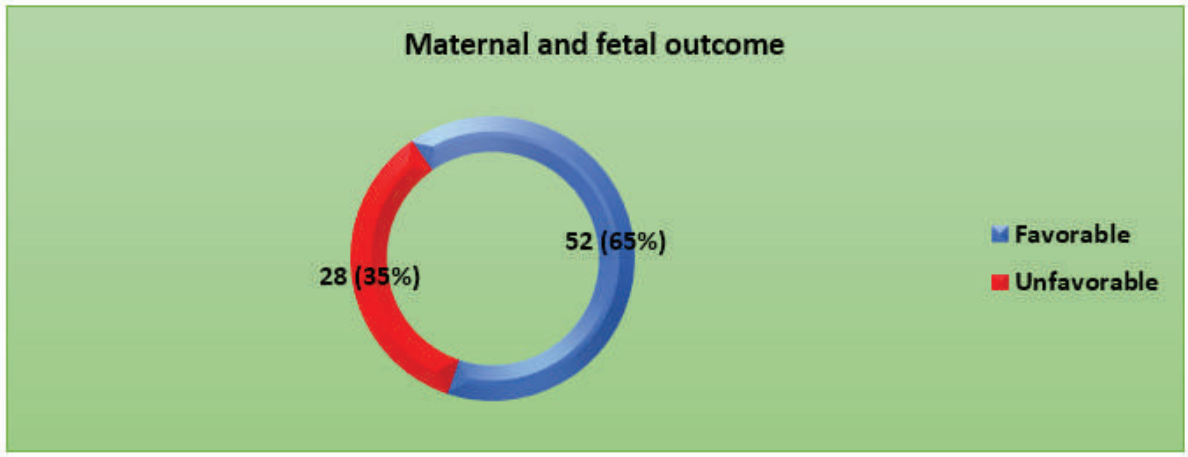


Figure 1: Prevalence of unfavorable maternal and fetal outcome

In this study, 16.3% of mothers complained from postpartum hemorrhage; 21.2% of neonates were admitted to NICU; and 15% of babies were weighing < 2.5 kg as shown in table (2).

Table 2: Distribution of study patients by outcome

Outcome	No. (n= 80)	Percentage (%)
Maternal complication		
No	63	78.7
Postpartum hemorrhage	13	16.3
Sepsis	4	5.0
Fetal complication		
No	60	75.0
NICU admission	17	21.2
Death	3	3.8
Birthweight (Kg)		
< 2.5	12	15.0
≥ 2.5	68	85.0

As shown in table (3), the highest prevalence of unfavorable maternal outcome in this study was seen among women living in rural area (45.5%), in those who didn't receive ANC (31.6%), and in those who had PROM for 24 hours or more (29.4%) with a significant association between prevalence of unfavorable maternal outcome and all of residence (P= 0.034), ANC visit (P= 0.031) and duration of PROM to delivery (P= 0.017).

Table 3: Association between maternal outcome and certain characteristics

Variable	Maternal outcome		Total (%) n= 80	P - Value
	Unfavorable (%) n= 17	Favorable (%) n= 63		
Age (Years)				
< 20	4 (30.8)	9 (69.2)	13 (16.3)	0.272
20 - 29	5 (12.8)	34 (87.2)	39 (48.8)	
30 - 39	7 (31.8)	15 (68.2)	22 (27.5)	
≥ 40	1 (16.7)	5 (83.3)	6 (7.4)	
Residence				
Urban	12 (17.4)	57 (82.6)	69 (86.3)	0.034
Rural	5 (45.5)	6 (54.5)	11 (13.8)	
Occupation				
Housewife	8 (16.7)	40 (83.3)	48 (60.0)	0.355
Employee	6 (25.0)	18 (75.0)	24 (30.0)	
Student	3 (37.5)	5 (62.5)	8 (10.0)	
Parity				
Primi	11 (20.4)	43 (79.6)	54 (67.5)	0.768
Multiparty	6 (23.1)	20 (76.9)	26 (32.5)	
ANC visit				
Yes	5 (11.9)	37 (88.1)	42 (52.5)	0.031
No	12 (31.6)	26 (68.4)	38 (47.5)	
Duration of PROM to delivery				
< 24 hrs.	2 (6.9)	27 (93.1)	29 (36.3)	0.017
≥ 24 hrs.	15 (29.4)	36 (70.6)	51 (63.7)	
Mode of delivery				
Vaginal delivery	12 (20.7)	46 (79.3)	58 (72.5)	0.842
Caesarean section	5 (22.7)	17 (77.3)	22 (27.5)	

The highest prevalence of unfavorable fetal outcome in this study was seen among women who didn't receive ANC (36.8%), and in those who had PROM for 24 hours or more (33.3%) with a significant association between prevalence of unfavorable fetal outcome and both of ANC visit (P= 0.019) and duration of PROM to delivery (P= 0.022) as shown in table (4)

Table 4: Association between fetal outcome and certain characteristics

Variable	Fetal outcome		Total (%) n= 80	P - Value
	Unfavorable (%) n= 20	Favorable (%) n= 60		
Age (Years)				
< 20	6 (46.2)	7 (53.8)	13 (16.3)	0.069
20 - 29	6 (15.4)	33 (84.6)	39 (48.8)	
30 - 39	5 (22.7)	17 (77.3)	22 (27.5)	
≥ 40	3 (50.0)	3 (50.0)	6 (7.4)	
Residence				
Urban	15 (21.7)	54 (78.3)	69 (86.3)	0.091
Rural	5 (45.5)	6 (54.5)	11 (13.8)	
Occupation				
Housewife	13 (27.1)	35 (72.9)	48 (60.0)	0.846
Employee	5 (20.8)	19 (79.2)	24 (30.0)	
Student	2 (25)	6 (75)	8 (10.0)	
Parity				
Primi	13 (24.1)	41 (75.9)	54 (67.5)	0.782
Multiparty	7 (26.9)	19 (73.1)	26 (32.5)	
ANC visit				
Yes	6 (14.3)	36 (85.7)	42 (52.5)	0.019
No	14 (36.8)	24 (63.2)	38 (47.5)	
Duration of PROM to delivery				
< 24 hrs.	3 (10.3)	26 (89.7)	29 (36.3)	0.022
≥ 24 hrs.	17 (33.3)	34 (66.7)	51 (63.7)	
Mode of delivery				
Vaginal delivery	13 (22.4)	45 (77.6)	58 (72.5)	0.385
Caesarean section	7 (31.8)	15 (68.2)	22 (27.5)	

Discussion

The maternal and fetal outcome in PROM is very important to decrease maternal and fetal mortality and for better management of complications. Thus, this study aims to determine maternal and fetal outcomes in PROM among 80 term pregnant women enrolled. In which, prevalence of unfavorable maternal and fetal outcome was 35% of patients. A comparable results observed in Endale et al study (2016), as unfavorable outcome represented 33.5% of patients [7], a lower results observed in Amulya et al study in 2019 [8] and Vishwakarma et al study in 2015 [12], as rate was 16.6% and 14.9% respectively. Differences observed can related to the sample size, low socioeconomic status, which can contribute via, poor nutritional status, anemia, and increased genitourinary infections due to poor personal hygiene, all these causes increased risk of PROM. Many studies reported low socioeconomic status associated with PROM [13]. In this study, 16.3% of mothers complained from PPH; 21.2% of neonates were admitted to NICU; and 15% were weighing < 2.5 kg. By comparison to Endale et al study in 2016, a different results observed as PPH found in only 3.7%, also 25.4% of neonates need NICU admission and 9.7% of the neonates weigh < 2.5 kg [7]. Differently, febrile morbidity accounting to maximum maternal complication in Amulya et al study in 2019 (9.6%) [8], and in Vishwakarma et al study in 2015, 3% patients developed fever and abdominal distension, as the main complication [12]. This differences can attribute to different sample size, type of treatment received, co-morbid diseases, type of delivery and the experience of the staff of intervention, as the role of unskilled and unhealthy delivery practices as a leading risk factor for PPH. The highest prevalence of unfavorable maternal outcome, in this study, was significantly observed among women living in rural area, in those who didn't receive ANC, and in those who had PROM for 24 hours or more ($P<0.05$). An agreement observed to the results reported in Endale et al study in 2010, as observed that women in rural area had a significant risk of unfavorable outcome ($P<0.05$). The risk also was 5.6 times higher in women with a duration of PROM > 24 hours [7]. This finding corroborates the results of Sirak et al study in 2014 [14]. In contrary, Idrisa et al study in 2019 found no significant correlation between ANC and prevalence of outcome ($P=0.137$), furthermore, no significant relationship was found between the duration of PROM and adverse maternal or neonatal outcome($P>0.05$) [6]. This supported by Eleje et al study in 2010 that mothers with PROM greater than 24 hours were not associated with unfavorable outcome ($P>0.05$) [15]. These differences may be related to the different sample size and difference in management of PROM. In regard to rural area, there are more chances of infection, poor hygienic conditions; the quality of health care provided and remote tertiary centers, can participate in occurrence of PROM.

The highest prevalence of unfavorable fetal outcome was significantly noticed among women who didn't receive ANC and in those who had PROM for ≥ 24 hours ($P<0.05$), which was in accordance to Endale et al study (2016), in which ANC had a statistical significant association with fetal outcomes ($P<0.05$). Furthermore, neonates with a duration of PROM > 12 hours were 12 times more likely exposed to unfavorable outcomes ($P<0.05$) [7]. Similarly, there was an association between unfavorable fetal outcomes and longer duration of PROM also reported in Alam et al study in 2014 [16]. When PROM occurs earlier, there are significant risks of maternal and perinatal morbidity and mortality, therefore the attending physicians in a regular ANC visit play an important role in the management of PROM. They should a develop pregnancy outcome plan, whereby a suitable decision is reached for decreasing maternal and fetal unfavorable outcome [17]. In conclusion, unfavorable maternal or fetal outcome is not uncommon among women with PROM. Prolonged duration of PROM and lack of ANC visits were a significant associated factors. So, an early accurate diagnosis of PROM is essential for favorable outcome in pregnancy and thus it decreases the maternal and fetal morbidity.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Detection of the Genetic Diversity and the DNA of Some Species of the Cruciferous Family (Brassicaceae) using the RAPD-PCR Markers

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Abstract

The current study aimed to uncover the genetic diversity of eight local species of the Brassicaceae plants by using RAPD markers, where the genetic relationship was determined, the genetic dimension was found between the studied species and the genetic finger print of some of these species was identified. (DNA) was extracted from the young leaves of the cruciferous family plants using the method Ready (Kit). The concentration of the extracted DNA ranged from 148.35-165.3 ng /µl with a purity of 1.53- 1.82. The results obtained from the study showed that 307 band were all differentiated (100%), higher in number than bands (38) with (12.38%) in the primer (MQ10), lower in number than bands (21) and with (6.84%) in the primer (MQ6). The highest molecular volume (pb2500) was characterized by (B1) in the primer (MQ7), while the less Molecular volume (100bp) in the two species (B3 and B5) was distinguished by the primer (MQ8). The results of the genetic dimension showed that the lowest value is for the genetic dimension (0.260) appeared between the two species (B2 and B3), and the highest value of the genetic dimension was (0.975) appeared between the two types (B4 and B5). The phylogenetic tree was drawn between the studied species and the species were divided into two major groups. The first group included the species (B1, B2, B3 and B4) and the second group included the species (B5, B6, B7 and B8).

Key words: *Genetic diversity, Brassicaceae, RAPD-PCR, Cauliflower*

Introduction

The Brassicaceae family is one of the important and large botanical families and is widespread around the world, this family includes about 338 genera and more than 3700 species and 50 tribes, most of the plants of this family are spread in the cool and temperate regions of the Northern Hemisphere[(1)] . The spread of cruciferous family plants differs from one region to another, as this family includes in Iraq 117 species spread over 83 genera of which 18 are cultivated species of economic importance and 75 wild species and some of these species have medicinal importance and the other section represents ornamental plants[(2)].

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Molecular indicators have developed a wide range of applications in the field of molecular biology including genetics, environmental evolution and the study of complex genomic features in plants[(3)]. The distinction between plant varieties has become highly dependent on the technology of polymerase Chains Reaction (PCR), the most prominent and most common of which are RAPD indicators (ISSR, DAF, AFLP and SSR) to distinguish between plant species[(4)], it is necessary to carry out monitoring and evaluation for Genetic variation as a basis for the conservation and breeding of plant species[(5)]. RAPD markers focused on using single random primers consisting of ten nitrogenous bases paired with complementary sequences on the genome with the presence of DNA Polymerase to work to duplicate the DNA of the genome in some areas of the DNA to show the genetic variation between plant species through the presence or absence of these areas and their

sizes and the number of copies[6]. These indicators are characterized as low cost, easy to use, and have a wide range of applications in many plant studies, for example used in early discrimination Among the plant species, finding the genetic finger print[7]. In view of the lack of extensive studies on the plant of the Brassicaceae family in Iraq using molecular indicators, this study was conducted which aims to uncover the genetic diversity between the DNA of the Brassicaceae family species cultivated in Iraq and to know the genetic dimension between the studied species, as well as determine the genetic fingerprint of some of these species.

Materials and Methods of Work

Plant samples, DNA isolation and PCR interactions

Eight types of Brassicaceae family plants used in the Ramadi district, Anbar, Iraq, (Table 1) were used in this study. DNA isolation of young leaves of plant species studied in a manner reported by (IBI) using a ready kit manufactured by IBI (USA) (www.ibisci.com). DNA samples using the electrophoresis device on the agarose gel revealed a 0.8% concentration for 45 minutes with a difference of 100 mA. In this study, ten prefabricated RAPD prefixes from Korea (IDT) company (Integrated DNA Technologies) (Table 2) were used. The concentration of DNA in all samples studied to conduct RAPD reactions was (50) ng/µl. Prepared the main reaction mixture by mixing the reaction components in a PreMix PCR tube equipped by Bioneer (Korea). DNA was amplified by PCR technology, according to it[8]. Through a primary mutant cycle at 95 ° C for 5 minutes, followed by 35 sessions at 95 ° C. Each session includes one minute of double DNA mutant and 30 seconds at a temperature of (37-50) ° C according to each initiator (Table 2). Then 30 seconds using a temperature of 72 ° C. to elongate the initiator, then a 5-minute final session using 72 ° C. to complete the elongation stage, withdraw 10 µl from the PreMix PCR tube, load the mixture into the pre-prepared agarose gel concentration and concentration 1.2% With DNA Ladder 100bp. The samples were electrically carried over using the Electrophoresis apparatus and at a difference of 100 mA for a period of 40-65 minutes (Table 2). The agarose gel was displayed after electrophoresis on a UV-transilluminator and photographed using a high-resolution digital camera.

Table (1) the species of the Brassicaceae family included in the study.

code	Sample Scientific name
B1	Brassica juncea (Indian Mustard)
B2	Brassica oleracea Var. botrytis
B3	Brassica oleracea Ver. Capitata
B4	Brassica rapa var. rapa
B5	Eruca sativa
B6	Lepidium sativum (cross)
B7	Raphanus sativus (White Radish)
B8	Raphanus sativus (Red Radish)

Table (2) RAPD marker primers used in the study, temperature of each primer and duration of relay for each primer.

NO.	Primer	3 → 5 primer sequence	Tm ˚C	Time Electrophoresis
1	MQ1	GTGAGGCGTC	50˚C	45 min.
2	MQ2	TGGACCGGTG	50˚C	45 min.
3	MQ3	TGCGTGCTTG	37˚C	50 min.
4	MQ4	GTAGACCCGT	37˚C	50 min.
5	MQ5	CCACAGCAGT	37˚C	65 min.
6	MQ6	TCGGCGATAG	48˚C	55 min.
7	MQ7	AGCCAGCGAA	48˚C	40 min.
8	MQ8	AGGTGACCGT	48˚C	45 min.
9	MQ9	TGCGGTGAAC	37˚C	60 min.
10	MQ10	GAGCAGACAC	37˚C	60 min.

Data Analysis

The results of the interactions of the RAPD primers were taken separately in tables based on a comparison of the presence or absence of DNA segments of the various samples as the presence of the DNA bundle is denoted by the number (1) and its absence by the number (0). Genetic dimension coefficient and similarity coefficient between studied species were calculated using Nei coefficient[9]. Conduct Cluster analysis and plot the genetic dimension using the UPGMA method[10]. Statistical analyzes were conducted using the software using the program (NTSYSpc-2.02i)[11]. On the Nei equation[12].

Results and Discussion

Genomic DNA Extraction

DNA was extracted from the young leaves of the Brassicaceae plant species according to the ready-made kit method and carried on a 0.8% agarose gel using the electrophoresis device. Its purity for the studied species ranged between (1.53 - 1.82), and its concentration ranged between (148.35 - 165.3) ng/µl.

Results of RAPD interactions

In this study, ten primers RAPD manufactured by (IDT) Korea were used. Results of their replication with DNA of the studied species showed a difference in the number of replicated sites and their molecular sizes. All primers used showed results with DNA of the studied plant samples, where the results of the replication were appropriate for detection. On genetic relations, as well as creating a genetic finger print and knowledge of the genetic dimension between some cultured plant species (Figure 1). The results of the RAPD 307 band, all of which are varied (100%) that the process of finding genetic variation between species depends on these bands, indicated[13] from the foundations of finding genetic variation is the ratio of varied bands to the total number of correlation sites. The highest primer in terms of the number of correlation sites was (MQ10) with (38) band, while the lowest primer in terms of the number of correlation sites was (MQ6) with (21) band, the

highest molecular size in correlation sites was (2500bp) in the primer (MQ7) and the lowest molecular size it reached (100bp) in the primer (MQ8), the primer (MQ6) recorded the highest number of unique bands sites (7) sites and the primer (MQ10) showed the lowest number of unique bands sites a unique one, the primer (MQ10) showed the highest number of link sites for absent bands for two link sites, while primers (MQ1, MQ2, MQ3, MQ4, MQ5, MQ6, MQ8 and MQ9) did not show results for the multiplication of any link sites for absent bands. The reasons for the discrepancy between the types of Brassicaceae family plants or any other type of plant may be nucleotide substitution within one or both of my sites. The primer correlation, which leads to the presence or absence of variation or a change in the size of the duplicate segment. Thus, the results of the primers used showed different bands in number, location and size and among those bands there are what are known as unique bands and absent bands that have a role in determining the genetic fingerprint of a particular type without the rest of the species included in the study, that the presence of these bands between species reduces the cost and effort because it gives results Distinguished in record time, this is stated by[14] that unique or absent bands distinguish the species in which they appeared from other types.

When studying[15] the eight wild plant species belonging to the Brassicaceae family using (14) primer of the RAPD index, indicated the emergence of (113) band whose molecular sizes ranged between (250bp - 2800bp) and divided the plant species in this study depending on the bands produced by Multiply to two major groups. Also indicated[16] a study on (12) species of the family Brassicaceae using some RAPD index prefixes in determining the genotype and detecting the DNA, it was obtained (241) bands of which (62) Multiforme band (26%). When studying[17] genetic variation and genetic analysis of strains also indicated (13) groups of 6 species belonging to the genus Conringia prevalent in Turkey using seven RAPD primers. A total of (34) bands were detected, of which (30) bands were polymorphic, with (88%).

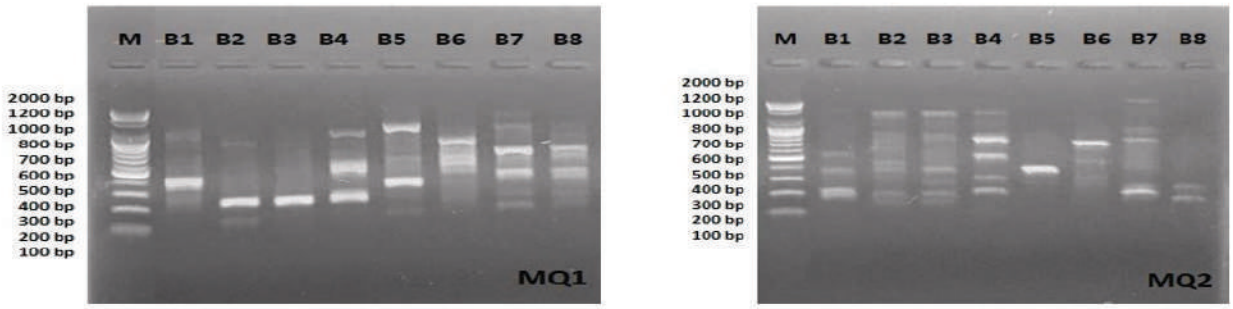


Figure (1) Electrophoresis of PCR reaction products for primers studied by RAPD Markers for Brassicaceae family types studied on agarose gel with a concentration of 1.2% and a difference of 100 mA for a period of 40-65 minutes, M represents the DNA Ladder 100bp, for (MQ1 and MQ2) (B1) *Brassica juncea*, (B2) *Brassica botrytis*, (B3) *Brassica capitata*, (B4) *Brassica rapa*, (B5) *Eruca sativa*, (B6 *Lepidium sativum*, (B7) *Raphanus sativus* (White Radish), (B8) *Raphanus sativus* (Red Radish).

Estimating the genetic dimension based on the results of RAPD

The genetic dimension between the Brassicaceae family types was estimated. Based on the results of the RAPD Markers, the genetic program (NTSYSpc-2.02i). Was used based on the presence of co-bundles between each of the studied species. (Table 3) shows the genetic dimensions between the studied Brassicaceae family types, which ranged between (0.260 - 0.975), when the value of the genetic dimension between two types is equal to zero indicating that there is no difference between the two types and the DNA between them is identical and the genetic similarity is 100%[18].

Table (3) shows the genetic dimension values among the Brassicaceae family according to the results of RAPD.

RAPD	B1	B2	B3	B4	B5	B6	B7	B8
B1	0.000							
B2	0.722	0.000						
B3	0.701	0.260	0.000					
B4	0.657	0.722	0.742	0.000				
B5	0.661	0.826	0.672	0.975	0.000			
B6	0.742	0.734	0.755	0.702	0.755	0.000		
B7	0.761	0.753	0.819	0.684	0.578	0.693	0.000	
B8	0.839	0.663	0.725	0.672	0.603	0.569	0.283	0.000

The results showed that the lowest value of the genetic dimension (0.260) between the two species (B2 and B3), this indicates that they have the largest proportion of similarity in the genetic material (DNA) based on the primers used in this study, and this means the lowest percentage difference between the genome of the two types compared to the types The other, pointed[19] to the reason for the convergence between the two species due to the fact that they are close in proportions and evolutionary history in addition to having the same chromosome group and the differences are minimal in the genome of the two species. The results indicate that the highest genetic dimension was between the two species (B4 and B5) was (0.975). This indicates the presence of the lowest proportion of symmetry in the genetic material (DNA) between these two types, as they participated with the lowest number of bands in relation to other types. This distinction is a result of their great contrast in appearance and physiology.

The genetic dimension values of the studied Brassicaceae family species have been invested in finding the genetic relationship between these species in the form of groups (Figure 2). These species were divided into two main groups:

• First main group

This group consisted of two subgroups, the first of which included the two types (B1 and B4), and the second was represented by the two types (B2 and B3). This first and second group formed as a result of their participation in the largest number of linking sites, and the lowest number of linking sites with the rest of the species (Figure 2).

• Second main group

This group included three secondary groups that included the first group (B6), while (type B5) was in the second group, while the third group included the two types (B7 and B8) which are the closest genetically in this group, where they acquired the largest number From the general bands between them, (Figure 2).

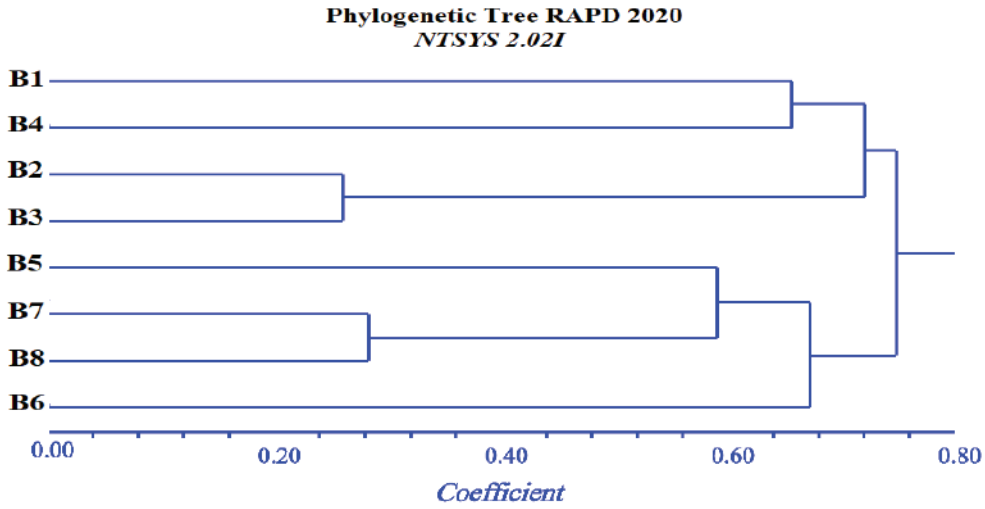


Figure (2) Genetic Relationship between the Brassicaceae Family Types According to RAPD Markers.

Conclusion

The results of this study showed 307 band, all of which are differentiated (100%). The primer (MQ10) showed the highest number of bands (38) and (12.38%) and the lowest number of bands (21) and (6.84%) in the primer (MQ6). The two types (B1 and B4) had the highest number of packages reached (42) packages with a percentage (13.68%) and the lowest number of bands (35) band with (11.40%) in the types (B3 and B5), the results of the genetic dimension showed that the lowest value of the genetic dimension was (0.260) appeared between the two types (B2 and B3), and the highest value for the genetic dimension was (0.975) appeared between the two types (B4 and B5).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Study of Bacterial Infections Associated with Infection with Cytomegalovirus in Anbar Governorate

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Abstract

Human cytomegalovirus (HCMV) is the largest type of herpes virus DNA virus, called the CMV because it amplifies infected cells as it reproduces gradually inside those cells . The study included collecting 110 blood samples for sick women and we visited Al-Ramadi Teaching Hospital with ear , nose, tonsils and eye swabs. Also urine samples were taken, the media of Blood Agar and MacConkey Agar were grown with Smears and swabs .The findings of the current study showed that 89 women out of a total of 110 were infected with CMV virus at a rate of 80.6 % by virus-specific IgG antibody, which is proof that the infection is old while 21 women were not infected by a 19 % percentage of CMV virus due to the absence of IgG and IgM antibodies .Results showed that 78 were positive for bacterial infection among the 89 women living with CMV, 87.6 %, while 11 among 21 women without CMV were positive for bacterial infection. The 87.6 % proportion of bacterial infections associated with virus infection is high as compared to the 52.3 % ratio of people without this virus, and this suggests the growing role of infection in the system. The study findings also found that the highest incidence of CMV virus infection was 82.3 % in the age group of infected women (21-30 years) and that the highest incidence of CMV virus infection was 92.6 % in pregnant women who had no abortion, while the lowest rate of CMV infection was women who had three miscarriages, 46 % .

Keywords: *CMV virus, IgG, IgM, bacterial infection.*

Introduction

Human cytomegalovirus (HCMV) is a member of the Herpesviridaceae family's Beta herpes virus family, and is the widely pathogenic agent of the vast majority of the world 's population. It is called CMV as it amplifies infected cells because it slowly multiplies inside these cells .[1]CMV infection is fairly common but does not spread easily or through unintended communication methods and transport involves direct contact with the infected person's body fluids, where people become infected with the virus by kissing, breastfeeding, physical intercourse, blood transfusion, drug use, organ transplantation and bone marrow, The virus may remain in body fluids for several months and sporadically throughout life, especially in people suffering from immune deficiency, especially those with AIDS [2].

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Bacterial infections associated with CMV infection.

Escherichia coli is known to be a negative bacillus of the Gram stain and one of the most common members of the intestinal family, where it settles in the intestine of humans and animals with hot blood This is a sympathetic bacterium where it can naturally reside and settle in the intestine as it is known to be a non-pathogenic bacteria and is capable of producing vitamins throughout the integer such as vitamin K2]3[.

(***Proteus mirabilis***) It is a Gram Stain negative bacillus, which belongs to the Enterobacteracea family, as it is considered to be one of the most important types prevalent in hospitals where infection of this bacterial type can occur alone or in conjunction with other

bacterial species [4].

(***Pseudomonas aeruginosa***) One of the most common bacterial species in human pathogenic events is opportunistic, especially when the efficacy of the immune system decreases in patients with certain chronic diseases such as cystic fibrosis and AIDS, as well as in patients with cancer and those with burns [5]

(***Staphylococcus epidermidis***) is considered to be one of the important types from a clinical point of view because it is found in the mucous membranes and on the skin, and these bacteria are opportunistic because of their ability to break the skin's epithelial barrier, which contributes to many serious diseases causing them. Bacteria cause infection in hospitals[6]. This Study aimed to Determine the presence of CMV virus in the serum of women testing, as well as bacterial infections that may be associated with this virus.

Materials and Methods

110 samples of blood were obtained for sick women, we visited the Ramadi Teaching Hospital for Women and Children and a number of medical laboratories and ages ranging from 20 to 50 years to examine the existence of CMV for the period from 01/10/2019 to 1/2/2020 from 5 ml of venous blood and placed in special Gel tubes, Then the blood was separated by centrifuge (3000 r / min) for a period of 5 minutes to obtain serum for the initial detection of the presence of IgG and IgM antibodies by the Rapid Test Kit, then the serum was stored at -20 °C before ELISA finalized its analysis. Samples were also taken from the same women who underwent a screening test for the CMV virus that included samples of Ear, Nose, Tonsil and Eye using Cotton Swabs and Urine. The samples of Swabs and Urine were cultured using Streaking method on the media of Blood Agar and MacConkey Agar. The dishes were incubated 24-48 hours at 37 °C.

Results and Discussion

CMV Antibody Detection Results :

The results of early detection using the Rapid Test Kit for the detection of antibodies IgG in the blood serum of the women under study, taking into account the values of the antibodies fixed in Table 1 , showed that there were no significant differences at the probability level $P \leq 0.05$, Between the standard levels established in the rapid examination kit and what you get in the current study.

Table (1) Shows the Values of Type IgG Antibody Obtained from Blood Samples

Antibody Type	Antibody Values Standard	Antibody Values Under Study	Number	Percentage
IgG +	1.1<	1.201 – 1.991 2.000 – 2.932	66 23	74 % 26%
IgG -	0.9 > 0.9-1.1	0.101 – 0.869 0.903 – 1.176	5 16	24% 76%
IgM -	0.9 >	0.040 – 0.798	110	100%
Pearson Chi-Square 172.409				

Where the results the presence of the antibody type IgG in 64 blood samples from a total of 110 at a rate of 58.1% for the women under study and at certain ages and levels where the presence of IgG is evidence that the infection is old with this virus, While 46 blood samples, the remaining 41.8%, showed a negative result of the presence of this type of antibody with the same rapid examination. While not all samples showed the presence

of the antibody type IgM , which indicates that the infection is recent. The Results of the ELISA test on the blood serum of all the women under study showed the presence of the IgG antibody in 25 other blood samples belonging to women other than the 64 samples within the rapid examination, bringing the number of positive samples to the presence of this virus to 89 samples , so the percentage will increase to 80.9 %,While IgM also did not appear in the serum of these samples. Thus, we infer that the detection of the presence of the antibody IgG is not a final diagnosis of this virus when an early primary detection using a Rapid Test Kit so that all infections The women under study are outdated due to the lack of an IgM. This is because IgM antibodies disappear from the blood after infection, because when the sample is taken, the affected person may be in the chronic, rather than acute, acute phase [7;8].

Results of Bacterial Infection Associated With the Presence of CMV Virus

The results showed in Table 2 that among 110 women were 89 carriers of the virus, of which 78 were positive for bacterial infection as a positive sign for laboratory implantation of the samples and areas by a percentage of 87.6%, and that the remaining 11 samples were negative for bacterial infection by a percentage 12.3%. While 21 of the women were not infected with CMV virus, of them 11 samples for women were positive for bacterial infection in terms of laboratory implantation and the remaining 10 samples were free of any occurrence of the bacterial infection.

Table 2 Results of Bacterial Infections of Samples of Women Infected With CMV Virus

Type of Injury	The Number of Samples Under Study	As A Result of Bacterial Infection			
		Positively Injured	%	Negatively Injured	%
The Presence of CMV	89	78	87.6	11	12.3
Lack of CMV	21	11	52.3	10	47.6
Total	110	89	80.9	21	19
Pearson Chi – Square 13.676					

Through these results, it was found that the percentage of bacterial infections present in women with CMV virus, which is 87.6% , is much higher than the percentage 52% for women without CMV virus, which may be an indication that infection with CMV virus has a role in causing bacterial infections, and with a high moral difference when The probability level $P \leq 0.05$.

Bacterial Infection Distribution among Women With and Without CMV Virus

The results in Table 3 showed the bacterial species that were diagnosed in the current study through Biochemical Tests as well as through its diagnosis in the Vitek Compact 2 System Diagnosis, that there were no significant differences at the probability level $P \leq 0.05$ .Table 3 shows the bacterial species obtained during the current study

that the bacterial type *E. coli* that causes UTI is the most frequent among the bacterial species in the presence or absence of the CMV virus, where the number of bacterial isolates reached 35 isolates distributed between 30 Isolation in the absence of CMV virus 43.4% and 5 Isolation in the absence of CMV virus 7.3% .

Table (3) Number and Types of Bacterial Isolates in Women With and Without CMV Virus

Source of Samples	No	Bacterial Isolates	The Presence of CMV		Lack of CMV	
			No.	%	No.	%
Urine	69	E. coli	30	43.4 %	5	7.5 %
		Aeromonas salmonicida	4	5.7 %	1	1.4 %
		Proteus mirabilis	8	11.5 %	3	4.3%
		Sphingomonas paucimobilis	7	10 %	0	0 %
Tonsillitis	12	Pseudomonas aeroginosa	8	67 %	1	8 %
Nose	10	Stenotrophomonas maltophilia	7	70 %	1	10 %
Ear	15	Staphylococcus epidermidis	12	80 %	0	0 %
Eye	4	Staphylococcus auricularis	2	50 %	0	0 %
Total	110		78	71 %	11	10%
Pearson Chi-Square 5.623						

The result of the current study was close to the study conducted by the researcher [8] in Egypt, where the percentage of *E. coli* bacteria 53.85% . As the difference in the percentage of isolated bacteria is due to the difference in health and geographic conditions and the number of samples used in the study as well as taking antibiotics before taking the sample [9]

Isolated *Proteus mirabilis* bacteria came from women who suffer from urinary tract infection with 8 isolates 11.5% in women with CMV virus and one 4.3% in women without CMV virus. This result was identical to the result of the study conducted by[10], where the isolation rate of these bacteria from the urinary tract was 11.8% .

Distribution of the presence of Antibodies in Women With CMV Virus by Age Group.

Table 4 shows the relationship between the age group of the women under study with infection with CMV virus. The ages of the affected women ranged between three age groups with no significant differences at the probability level $P \leq 0.05$. The results of the current study showed that the incidence of CMV virus was the highest among women whose ages ranged from 21-30 years, with the number of infections amounting to 42 women in terms of the presence of the antibody type IgG and at a rate of 82.3% of the total number of women 51 A woman. It was followed by the age group of 31-40 years with the number of injuries amounting to 32 infected women and by 83% of the total 39 women. While the age group of 41-50 years recorded the lowest number of women infected with this virus, as it recorded 15 infected women, at a rate of 75% of the total 20 women. This difference in numbers and proportions with respect to the age group 41-50 may be due to their

possible exposure to previous infections with this virus and the generation of an immune response to them, This corresponds to the study conducted by the researcher[11] in Kirkuk, as it found that the prevalence of IgG antibody prevalence with age, and this does not correspond to the study conducted by [13]where they found that the prevalence of IgG antibody Increases with age .

Table 4 Distribution of the presence of IgG and IgM Antibodies by Age Group

Age (Year)	No. of patients	CMV - IgG				CMV-IgM	
		IgG +	%	IgG -	%	IgM -	%
21 -30	51	42	82.3	9	17.6	51	100
31 – 40	39	32	82	7	18	39	100
41 – 50	20	15	75	5	25	20	100
Total	110	89	81	21	19	110	110
Pearson Chi- Square .554							

Distribution of the presence of Antibodies in Women With CMV Virus According to the Number of Miscarriages

The Results of the current study shown in Table 5 showed a high incidence of CMV virus in terms of the presence of IgG antibody in the serum of women who did not have a projection case with a significant difference at the probability level $P \leq 0.05$. Where they were 41 women, of which 38 were women with the IgG antibody at a rate of 92.6% and the women who had one single abortion were 34 women of which 30 had the IgG antibody at a rate of 88.2%, and the women who had two abortions were 22 women of which 15 The antibody contained IgG type at a rate of 68%, while the women who had three cases of miscarriage were 13 women of which 6 were six. The antibody contained the type IgG and by 46% the lower percentage of the infection in women who had more than one miscarriage was attributable to their previous injuries. Virus, which made them more resistant to infection with CMV virus. This is consistent with a study in Libya[14] where they found that the prevalence of IgG antibody in women with repeated miscarriage was 75%.

Table 5 The Distribution of the presence of IgG and IgM Antibodies According to the Number of Miscarriages .

No. of Miscarriage	No. of patients	CMV – IgG				CMV-IgM	
		IgG +	%	IgG -	%	IgM -	%
0	41	38	92.6	3	7.4	41	100
1	34	30	88.2	4	11.8	34	100
2	22	15	68	7	32	22	100
3	13	6	46	7	54	13	110
Total	110	89	81	21	19	110	100
Pearson Chi- Square 17.334							

Infection with CMV is one of the most important causes of miscarriage, premature birth and congenital malformations [12]

Conclusion

Through the results of the current study, we conclude that the percentage of bacterial infections present in women with CMV virus, which is 87.6%, far exceeds the percentage of 52% for women without CMV virus, which may be an indication that infection with CMV virus has a role in causing bacterial infections, and with a significant moral difference at the level of probability $P < 0.05$.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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The Effect of Pollen Type on Some Fruit Traits and Production Quantity of Date Palm Trees Phoenix Dactylifera L. For Agricultural Cultivar Al-Ibrahimi

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Summary

The study was conducted during the agricultural season 2019 in one of the orchards of the district of Heat, Al-Hassania (Shamiya) Village in Al-Anbar Governorate, know the effect of different pollen male grains type (Ghanami Ahmar, Ghanamy akhdar, samesmi , Khikri adi) on some fruits traits and the amount of production the date palm of the agricultural cultivar AL-Ibrahimi, The results indicated that the best pollen used to pollinate the agricultural cultivar Ibrahimi it is for grains of the vaccine Khikri adi, characterized by giving the best qualitative qualities of fruits Average weight fruits and diameter in the Khalal stage and average diameter and weight stromal layer in the stage dates (11.59 g , 2.36 cm , 1.77 cm , 9.96 g) respectively , the Ghanami Ahmar vaccine was distinguished by giving the best quantitative fruit qualities the average of percentage for fruits contract and raceme weight (86.60 % , 16.87 Kg) respectivel .

Introduction

That the date palm phoenix dactylifera L is an important species in the Areacaceae palm family, it is believed that most of the date palm cultivars originated from seed and were selected for their finest flower and fruits qualities and they multiplied by the seedlings and that over the long ages, humans helped to choose the best and the best from date palm [(1)] .

Iraq has many agricultural varieties of date palm their number is estimated at more than 600 agricultural varieties at the local and commercial levels [(2)] Several research results indicated that the type of vaccine affects the chemical, physical, and an appointment Maturity The pollen also affects the characteristics of endosperm and the fetus, and certain stallions are elected to pollinate the different date palm trees, The direct effects of pollen on the characteristics of the fetus and endosperm are known as Xeni this phenomenon has a clear impact on the fetus and the endosperm [(3)] the vaccine has an effect outside the endosperm and the fetus, which was named by Swingle as Metaxenia it is the effect of pollen grains on the covers of the ovary, which leads to influence the natural characteristics of the fruits Therefore, for each of the areas where the date palm is grown, For each of the areas where the date palm is grown, a limited number of male species are preferred, pollinating them because of their importance in improving the fruit characteristics thus improving yield the crop [(4)], the study aims to conduct pollination of two cultivars of date palm cultivars spread in Heat district with four types of males present in the region to determine of the best in giving Qualitative qualities and quantitative fruits characteristics .

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Methods of work

The study was conducted during the agricultural season 2019 in one of the orchards of the district of Heat, Al-Hassania village (Al-Shamiya) in Al-Anbar Governorate to see the effect of male pollen type

(Ghanami Ahmar, Ghanamy akhdar, samesmi and Khikri adi) on some fruits traits and the amount of production the date palm of the agricultural cultivar AL-Ibrahimi which are spread in the region and was selected four palm trees variety are identified as similar as possible in growth and age, to conduct experiments on it where date palm was pollinated with one of the types of pollen male used in the study.

Pollination of the cultivar began from 6/4/2019 anumber was chosen of active sorties (eight sorties), after the pollination process the bagging process was carried out immediately with special bags of 30 x 80 cm to avoid the transmission of pollen of unknown origin from neighboring trees, bags were raised 15 days after the inoculation.

Studied traits . 1-Average percentage of the fruit contract (%)

The average percentage of the contract was calculated one and a half months after the pollination process (Kimri stage) was extracted according to the following formula [5].

$$\text{Percentage percentage of fruiting fruits} = \frac{fruiting\ fruits\ number)}{fallen\ flowers\ number + Shees\ fruits\ number + The\ number\ of\ fallen\ fruits} \times 100$$

2- Measurement of some fruits traits .

Ten fruits were randomly cut from each **raceme** of a single palm, and the following fruit traits were studied .

a-Average fruit weight (g)

The average fruit weight was calculated using Sensitive electronic balance .

b- Average volume of fruits (ml)

The average volume of fruits was measured using a method of measuring the volume of displaced water and the cylinder was used for this process .

c- Average fruit length and diameter (cm)

The average length and diameter of fruits were measured by (vernier)..

d- Average weight of the stromal layer and seed weight (g)

Took ten ripe and healthy fruits randomly, the stromal layer of each fruit was separated from the seed and the stromal layer and the nucleus were weighed separately with using Sensitive electronic balance.

3- raceme weight (Kg)

After fully ripening the fruits of each date palm of each variety, the cutting process is done raceme in an accurate way and the fruits that were collected during the cutting were collected and the racemes were weighed individually using the Sensitive Electronic field.

Statistical Analysis

Statistical analysis conducted one way analysis the trend included the influence of types male or female species on the studied traits, By model general linear model Using SAS Statistical Ready Edition 9.0 [6], Significant differences were tested between the averages using a test Duncan Polynomial [7] at the level of significance ($P \leq 0.05$) .

Results and Discussion

Effect of pollen type on the fruits traits s of the Agricultural cultivar AL-Ibrahimi Percentage of fruit contract (%)

The results shown in Table.1 indicate that there were significant differences ($P \leq 0.05$) in the percentage percentage of the fruits contract between palm trees cultivar agricultural AL-Ibrahimi pollinated with the pollen grains male used in the study, As the percentage of the fruit contract significantly increased in the date pollinated by the pollen grains of the Ghanami Ahmar, Ghanamy akhdar and samesmi pollen the highest values

were recorded (86.60% , 84.70% , 83.20%) respectively, Comparison with the average percentage of the fruits contract pollinated by the grain of the Khikri adi which recorded the lowest value (80.70%), The reason may be due to the difference in the vitality of the different males pollen used in the study and their ability to be pollinated and fertilization, and therefore an effect on the trait studied, [8] stated that the date palm of pollination and the sources of different pollen grains for the male (Al-Ramadi, Al-Aswd, Al-Asfar, Ghanami Ahmar) affected the percentage of the fruits contract and the total yield for the cultivar Asharsy in the regions of Mandali and Baladruz. , showed the results [4] that different male pollen pills differ in their qualitative and quantitative content of hormones, which in turn affects the characteristics of the fruits.

The average weight of the fruit during the stage Khalal and the average weight of the stromal layer and the seed for the dates stage .

The results showed that there were clear significant differences in the average weight of fruits in stage Khalal and the average weight of the stromal layer and the seed average weight in the dates stage between dates of the cultivar agricultural AL-Ibrahimi Pollinated with the male vaccine pills used in the study , as in Table.1

The average weight of the fruit significantly increased during the stage Khalal of the date pollinated palm Khikri adi and recorded the highest value (11.59 g) record lowest value of average fruits weight in the fruits of the dates pollinated by the Ghanami Ahmar vaccine (9.01 g), The weight of the fleshy layer significantly increased in the dates of the date of the pollinated palm by the Khikri adi and recorded the highest value (9.96 g), lowest value of the average weight of the stromal layer recorded in the fruits of the date pollinated by the Ghanamy akhdar vaccine reached (6.15 g) , lowest value of the average weight of the stromal layer recorded in the fruits of the dates pollinated by the Ghanamy akhdar vaccine reached (6.15 g) ,average weight of the seeds significantly exceeded in the date pollinated palm tree with the Khikri adi the highest value was record (0.92 g), The lowest value of the average seeds weight in the date palm trees pollinated with the Ghanamy akhdar vaccine reached (0.63 g), The reason may be due to the compatibility between pollen grains and studied traits, or the reason may be due to the vitality of the different male pollen grains used in the study and thus affected these characteristics, A study was conducted by [9] to see the effect of different vaccine sources for nine male species (M1 to M9) on the physical properties of the agricultural variety Daki and the results showed that the type of vaccine did not significantly affect the weight of the seeds , Showed the results of [10] a significant effect of the Khikri adi pollen grains on the weight of the fruits, their size, length, diameter, seed weight, and pulp weight in the fruits of the Halawi cultivar compared to the Ghanamy akhdar vaccine, which did not score any significant difference for these characteristics .

Average fruit size, length, and diameter for the stage Khalal and the length and diameter in the dates stage

The results showed in Table.1 that there were no significant differences in the average size of the fruits and the average length in the stage Khalal and the presence of significant differences in the average diameter of the fruits in the stage Khalal and dates among the palms of the cultivar agricultural AL-Ibrahimi, pollinated with pollen males used in the study.

The average fruits diameter significantly increased in the stage Khalal of the date palm pollinated by the Khikri adi and samesmi fever vaccine and recorded the highest values (2.36 cm, 2.30 cm) respectively, Compared with the average diameter fruits of the date palm pollinated by the Ghanami Ahmar pollen, which recorded the lowest value (2.08 cm), The average fruits diameter significantly exceeded in the pollinated palm trees the Ghanami Ahmar pollen grains, samesmi and Khikri adi pollen recorded the highest values (1.85 cm, 1.78 cm, 1.77 cm) respectively, lowest average diameter of the fruits was in the fruits of the dates pollinated by the Ghanamy akhdar vaccine (1.62 cm), The reason that some fruits traits may not be affected and other fruits traits effect in this category may be due to the genetic factors of the different pollen grains,[11] explained the effect of pollen grains of 24 male cultivars on indicators of the ripening of palm fruits for the two cultivar Al-Sayer and Halawi where the results showed significant superiority in the Fruit size, weight, pulp, seed length, weight, diameter, and size, in stage Khalal and al-rtab in the Halawi cultivar , The results obtained by [12] showed

that the Ghanami akhdar vaccine was significantly superior to the rest of the treatments in the length, diameter and weight of the fruits during the phase during the use of two types of pollen grains (Ghanami akhdar and Khikri adi, and their mixture) to pollinate two types of agricultural varieties (Al-Khidrawi and Al-Birim).

Average raceme weight (Kg).

The results of Table,1 showed that there were significant differences ($P \leq 0.05$) in the average racemes weight between the date palm of the cultivar agricultural AL-Ibrahimi pollinated pollen males used in the study, As the average weight of raceme increased in pollinated date palm trees the Ghanami Ahmar , Ghanamy akhdar and samesmi, and the highest values were recorded (16.87 kg, 15.55 kg, 15.45 kg), Compared to the average weight of raceme in a date pollinated palm tree with the Khikri adi vaccine the lowest value was recorded (13.05 Kg), The reason may be due to the difference in the viability of the different sage pollen grains and their hormonal content which affected the fruits contract percentage and on the studied fruits traits and consequently the effect on the weight of the racemes, The study conducted by [13] showed of the methazine effect five sources of pollen grains two cultivars, Nabot Ali and Sabaka where the results showed a significant increase in the average weight of raceme in the agricultural variety Nabot Ali when pollinated with the Hada-8 vaccine, mentioned[4] that different male pollen grains differ in their qualitative and quantitative content of hormones, and this, in turn, affects the fruits characteristics of the date palm.

Table.1 The effect of percentage to hold fruit sand Some fruit traits of agricultural class AL-Ibrahimi

adjectives		Male species				Level of significance
		Ghannami Ahmar	Ghanamy Akhdar	samasmi	Khikri Adi	
percentage to hold fruits (%)		0.225 ± 86.60 a	0.335 ± 84.70 b	0.485 ± 83.20 c	0.457 ± 80.70 d	0.0001
Fruits stage khalal	weight (g)	0.224 ± 9.01 b	0.258 ± 10.49 ab	1.092 ± 10.75 ab	0.320 ± 11.59 a	0.0363
	size (ml)	0.389 ± 15.97 a	0.360 ± 12.90 a	1.69 ± 16.70 a	1.26 ± 13.70 a	G.M.
	Length (cm)	0.028 ± 3.77 a	0.025 ± 3.39 a	0.206 ± 3.61 a	0.180 ± 3.53 a	G.M.
	Diameter (cm)	0.085 ± 2.08 b	0.016 ± 2.18 ab	0.087 ± 2.30 a	0.030 ± 2.36 a	0.0158
Fruits Tamar stage	Length (cm)	0.092 ± 2.94 a	0.023 ± 3.04 a	0.188 ± 2.78 a	0.209 ± 2.67 a	G.M.
	Diameter (cm)	0.018 ± 1.62 b	0.035 ± 1.85 a	0.052 ± 1.78 a	0.071 ± 1.77 a	0.0165
	Weight of stromal layer (g)	0.185 ± 8.06 b	0.217 ± 6.15 c	0.117 ± 7.60 b	0.122 ± 9.96 a	0.0001
	Seed weight (g)	0.025 ± 0.74 b	0.013 ± 0.63 c	0.008 ± 0.71 b	0.014 ± 0.92 a	0.0001
	raceme weight (Kg)	0.235 ± 16.87 a	0.518 ± 15.55 b	0.525± 15.45 b	0.254 ± 13.05 c	0.0001

*The values Rate ± standard error ; **G.M Not significant at the level .($P \leq 0.05$).; *** a , b, c, d ; Different letters within a single row indicate that there are significant differences between types at a level .($P \leq 0.05$).

Conclusions

From analyzing the results obtained from this study to see the effect of different types of males on the qualitative and quantitative fruits characteristics the Agricultural cultivar AL-Ibrahimi, we conclude, Different males in the amount of pollen grains their viability and their ability to pollinate and fertilization, and that Agricultural cultivar AL-Ibrahimi also differ in their acceptance of the different pollen grains, that environment has an important role in the pollention and fertilization process and the percentage for fruits contract while giving good fruits qualities in addition to the type of vaccine **.**

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Isolation and Molecular Detection of *Salmonella Infantis* from Milk and Children with Gastroenteritis in Babylon Province, Iraq

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Abstract

Two hundred and ten samples were included in this study. The samples encompassing of 90 cattle milk samples obtained from local markets, and 120 stool samples obtained from children with gastroenteritis of age less than 12 years from both genders who were attending to the Babylon maternity and children's hospital. The samples were collected during the interval from February to August 2018 in Babylon province.

All these 210 samples were subjected to many tests for isolation and identification of *Salmonella* species by using bacterial culturing, biochemical tests, and API20E test system. The results showed that the prevalence of identifying *Salmonella* isolates were 69 (32.9%) out of 210 samples, distributed as (25/90) (27.8%) market milk and (44/120) (36.7%) stool samples. The identified *Salmonella* isolates were subjected to serotyping by monovalent antisera. It was found that 4 out of 25 isolates (16%) in market milk samples and 16 out of 44 isolates (36.4%) in stool samples that belong to *Salmonella infantis*. PCR technique was achieved to identify *invA* and *invF* virulence genes in *Salmonella infantis* strains. All 20 isolates of *Salmonella infantis* in market milk and stool samples were examined. The results revealed that, *invA* gene was detected in all strains of *Salmonella infantis* isolated from the market milk and stool samples, while the *invF* gene was present in 2 strains of the market milk *Salmonella infantis*, but existing in all strains of the stool *Salmonella infantis*.

Keywords: *Salmonella infantis, invA gene, invF gene, gastroenteritis, Milk, Gastroenteritis, Antibiotic Resistance.*

Introduction

Salmonella is one of the most essential zoonotic bacterial pathogen affects humans, which causes foodborne infection all over the world with serious implications in developing countries [1], and it is a major foodborne problem with frame animals such as cattle [2]. The milk is a potential food for the transmission of diverse foodborne bacteria to humans. In addition to the poultry foodstuffs are the main sources of human nontyphoidal salmonellosis. Additionally the raw milk and dairy products are considered as the other causes [3]. The raw milk is contaminated with bacteria from several sources like feces of infected animals, infected udder, skin, dust, feed and milking equipment [4].

Salmonella species are the etiological agents of acute gastroenteritis. In spite of the global human health, *Salmonella* infections impact has not been estimated where gastroenteritis is represented the main cause of morbidity and mortality [5,6]. *Salmonella* is transmitted to humans by consumption of contaminated animal foodstuffs including eggs, milk, poultry, pork, beef, and seafood [7], contaminated water, contact with infected animals, or with an infected person who suffering from diarrhea. *Salmonella* is divided into more than 2500 serovars based on its H antigens [8]. These serovars are found in animal origin food products like *Salmonella enteriditis*, *Salmonella gallinarum*, *Salmonella typhimurium*, *Salmonella infantis* and *Salmonella weltevreden* [9].

Salmonella Infantis is the most recurrence strain in various countries, involving Asian countries, it was detected and isolate from animals, humans and vegetables [10].

The pathogenicity of *Salmonella* is frequently dictated by various virulence genes. The products of these genes are effector proteins and comprise *sipA*, *sipB*, *sipC*, *invA*, *invF, sifA, hilA*, *hilC* and *hilD* [11]**.** Traditional *Salmonella* isolation methods are based on growth in a culture selective medium and serotyping which is considered as the standard methods for *Salmonella* confirmation, but it is time consuming. Thus, a quick technique is needed for the identification of *Salmonella* strains like using PCR assay for rapid identification and confirmation [12].

There are no previous studies dealing with the occurrence of *Salmonella infantis* conducted in Babylon province. This study, therefore, aims to estimate the prevalence and detect *invA* and *invF* virulence genes in *Salmonella infantis* isolates obtained from market milk and stool samples of children with gastroenteritis in Babylon province, Iraq.

Materials And Methods

Sample Collection and Processing

The market milk and stool samples that included in this study were collected during the interval from February to August 2018 in Babylon province.

Milk samples collection

Total of 210 samples, including 90 raw milk (market milk), were randomly collected from different local markets**.** The samples were placed in sterile tubes containing Selenite broth, maintained in an ice box, then transferred to the laboratory on the same day within two hours of collection for inoculation on selective medium.

Stool sample collection

A total of 210 samples that composed of 120 stool samples were collected from children suffering from gastroenteritis. Samples were obtained from patients did not receive any antibiotics of age less than 12 years from both genders who were attending to the Babylon maternity and children's hospital. One gram of stool sample from each patient was placed in sterile tube containing 5 ml of Selenite broth, and maintained in an ice box, then transferred to the laboratory on the same day within two hours of collection for inoculation on selective medium.

Laboratory Diagnosis

Bacterial isolation, identification and serotyping

All (raw milk and stool) samples were inoculated onto the surface of MacConky agar, and incubated at 37^0C for 24-48 hour, then sub-cultured onto the surface of selective media, including Eosin Metheline Blue, S.S and XLD agar, and then incubated at 37^0C for 24 hours [13].

The identification of colonies was depended on the morphological properties (colony size, shape, color, borders and texture). Bacterial colonies displaying the distinctive morphology were probably identified as *Salmonella* by Gram's stain. The colonies of pathogens suspected to be *Salmonella* were subjected to biochemical tests such as (Catalase, Oxidase, Indole, Methyl red, Vogas-Proskaur, Citrate utilization, Urase, Gelatin hydrolysis, and Lactose fermentation tests) [14]. The analytical profile index (Api20E) system was used to confirm diagnosis of the *Salmonella* isolates by biochemical tests [15]. To determine *Salmonella infantis* strains the identified *Salmonella* strains were then tested for serotyping diagnosis based on the Kauffman-White Scheme with polyvalent and monovalent O and H antigens [16].

Molecular detection of virulence genes in *Salmonella infantis*

The detection of virulence genes (*invA* and *invF*) in *S.infantis* was done as described by other workers [17-18]. Detailed sequences of the primers are listed in Table (1).

Table 1: Oligonucleotide Primers sequence and amplicon size

Target gene	Primer sequence	Product size (bp)
invA	Forward: 5′-GTGAAATTATCGCCACGTTCGGGCAA-3′ Reverse: 5′-TCATCGCACCGTCAAAGGAAC-3′	284
invF	Forward: 5′AAGGGATCCATGTCATTTTCTGAAAGCGACAC-3′ Reverse: 5′-GTTGTAGGGAAAGCTTCTCCAGTAATG-3′	918

Bacterial cells were suspended in 1 m1 of sterile D.W and subjected to a dry bath at 95°C for 10 min. then was cooled directly on ice and and used as DNA template for PCR. A single reaction mixture was completed in a final volume of 10µl containing 5µl master mix, 0.5µM of each primer and D.W water to 10µl final volume. The thermal cycler conditions were carried out at 95°C for 30 seconds as pre-denaturation, 35 cycles consisting of denaturation at 95°C for one second, primer annealing at 58°C for 5 seconds and extension at 72°C for 15 seconds. Final extension at 72°C for 1 minute. PCR amplicon was ran on 1.5 % agarose gel, stained with ethidium bromide then visualized on UV transilluminator and photographed.

Results

Two hundred and ten samples were included in this study. Samples were covering 90 cattle milk samples obtained from local markets, and 120 stool samples obtained from children with gastroenteritis. Samples were processed for isolation and identification of *Salmonella* by using bacterial culturing, biochemical tests, and API20E test system. The results showed that the prevalence of identifying *Salmonella* isolates were 69 (32.9%) out of 210 samples, distributed as 25 (27.8%) among 90 market milk samples, and 44 (36.7%) among 120 stool samples. The frequency of presumptive *Salmonella* strains was isolated from 210 samples, as shown in the Table (2).

Table 2: Distribution of *Salmonella* isolates according to the type of samples

Type of samples	No. of samples	No. of positive samples	Rate
Market milk samples	90	25	27.8 %
Stool samples	120	44	36.7 %
Total	210	69	32.9 %

The all identified *Salmonella* (69) isolates were subjected to serotyping by monovalent antisera. It was found that *Salmonella infantis* was detected in 20 (29%) samples distributed as 4 (16%) out of 25 market milk samples, and 16 (36.4%) out of 44 stool samples (Table 3).

Table 3: Distribution of *Salmonella infants according* to the type of samples

Type of samples	No. of samples	No. of positive samples	Rate
Market milk samples	25	4	16 %
Stool samples	44	16	36.4 %
Total	69	20	29 %

DNA was extracted from all (20) *Salmonella infantis* isolates and used as a template to identify the *invA*, *invF* virulence genes by using the PCR assay. The results found that, *invA* gene (284 bp) was detected in all strains of *Salmonella infantis* isolated from the market milk and stool samples, the results are shown in figure (1). The *invF* gene (918 bp) was present in 2 strains of the market milk *Salmonella infantis* isolates, but existing in all strains of the stool *Salmonella infantis* isolates, the results are shown in figure (2).

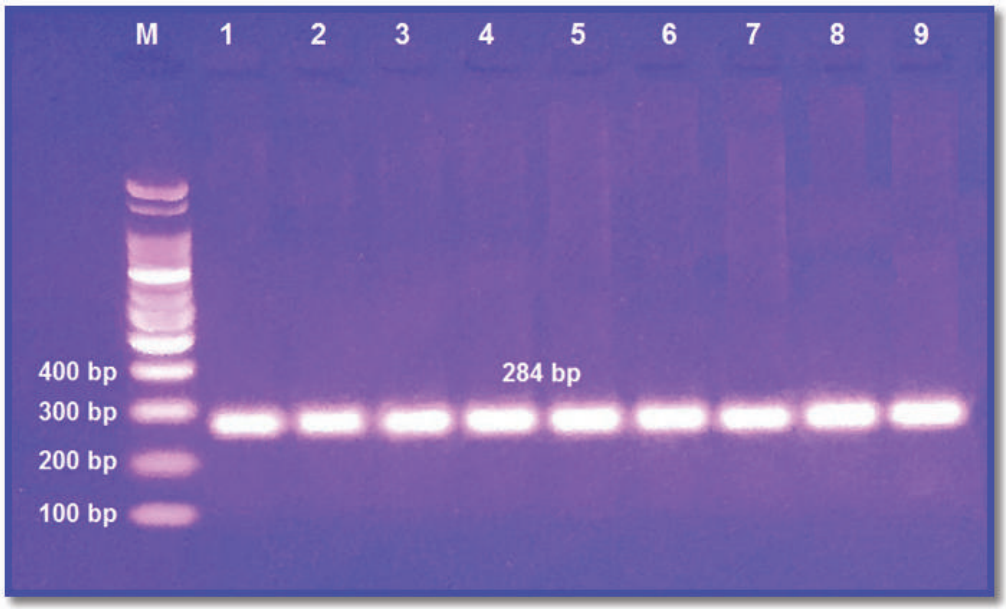


Figure 1: Agarose gel electrophoresis of *Salmonella infantis invA* gene detection. Lane M: marker with 100 bp ladder, lane 1 to 9: positive stool and market milk isolates, *invA* gene amplicon size: 284 bp.

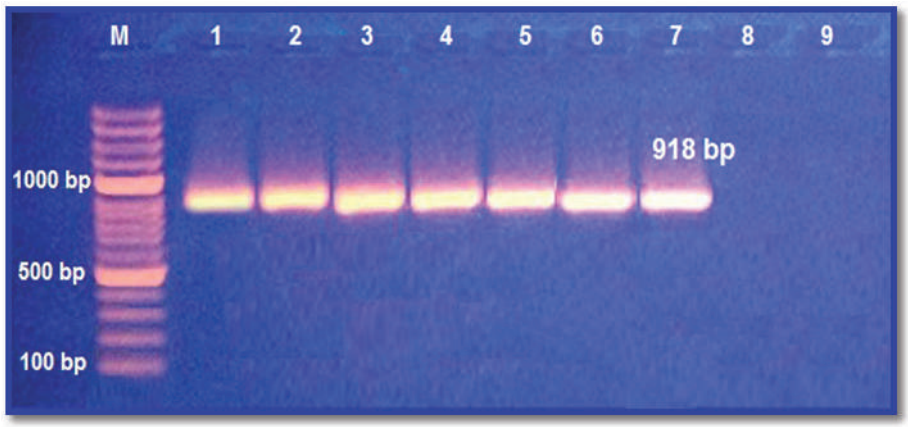


Figure 2: *Salmonella infantis invF* gene amplicon. Lane M: marker with 100 bp ladder, lane 1 to 5: positive stool isolates, lane 6 and 7: positive market milk isolates, while lane 8 and 9: negative market milk isolates, *invF* gene amplicon size: 918 bp.

Discussion

This study provided an estimation of the prevalence and detect *invA* and *invF* virulence genes in *Salmonella infantis* isolates collected from market milk and stool samples of children with gastroenteritis in Babylon province, Iraq.

In the present study, the findings demonstrated that the prevalence of identifying *Salmonella* by using bacterial culturing, biochemical tests, and API20E test system in market milk samples was found to be (27.8%). This may be associated with the fecal contamination of raw milk by food borne bacteria in the course of its gathering or lower storage temperature of milk, loss of electricity in addition to post processing contamination, that offer proper conditions for variety of pathogenic bacteria to grow such as *Salmonella*.

This result corresponded to the result recorded in Southwest Ethiopia by [19], who found that the incidence of *Salmonella* spp. in raw milk was (20%). Different other previous study recorded in Egypt was carried out in 2014, and the results revealed that (12%) of the milk samples were positive for *Salmonella* species by using the conventional culture methods [20]. On the other hand, the result of the present study is in contrast with the results of the previous studies obtained from [21,22], *who indicated that Salmonella* species did not isolate and detect in examining raw milk samples.

In addition, the results showed the prevalence of *Salmonella* species in stool samples was (36.7%). The obtained result is lower than the result of the other study in Al-Muthanna Province, Iraq [23], who found that the prevalence of *Salmonella* species is (2.9%) in stool samples among children who their age under 9 years old. Another study in Al-Diwaniya, Iraq, recorded that *Salmonella* spp. isolated from stool samples of children under 3 years of age at a rate of (7.9%) were detected by culture and biochemical tests [24]. Variable prevalence of *Salmonella* was reported in many previous researches from Al-Diwaniya governorate, Iraq, there were (14.47%, 8.47%, and 10%) respectively [25].

The occurrence of the same virulence genes (*invA* and *invF*) in raw milk and human stool samples demonstrates that the distribution and dissemination of virulence genes, although the *S. infantis* source of infection not known due to lake of background information, but the milk are one of the most common sources known of human salmonellosis.

The *invA* gene of the *Salmonella* spp., triggers the attack of the host and initiate infection, and subsequently increasing the degree of pathogenicity of the bacterial strains [26]. The *invA* gene is considered a universal genetic marker for the *Salmonella* identification since it exists in almost all *Salmonella* serovars [27]. Moreover, *invF* is expressed as a response to environmental signals. The exsitance of these genes is important in the *Salmonella* clinical significance [28].

Conclusion

invA gene was detected in all strains of *Salmonella infantis* isolated from the market milk and stool samples, while the *invF* gene was present in 2 strains of the market milk *Salmonella infantis*, but existing in all strains of the stool *Salmonella infantis*.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Evaluation Study of Different Techniques in Diagnosis of Acute Leukemia in Adults and Children Patients

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Abstract

Cytomorphology and cytochemistry are combined with immunophenotype by flow cytometry (FCM) to diagnosis and management the acute leukemia. The present study was designed to compare between the cytomorphology, cytochemistry and immunophenotype by flow cytometry at diagnosis and classification of acute leukemia to suggest a number of antibodies that can be adopted in the diagnosis by flow cytometry. The study included (160) cases were newly diagnosed (100 adults, 60 children). Morphological analysis was depended on peripheral blood smears examination by Leishman stain, cytochemical analysis with PAS and SBB stains and immunophenotype analysis used multiparameter. the results of this study shown that 60% of adult cases were AML, and 40% ALL. The mean age of adults was 48.45 years for AML cases and was 32.59 years for ALL adult cases. In children AML the mean age was 5.23 years and was 6.44 years in children ALL cases. **Our Conclusion** Also that the B.ALL was more commonly than T.ALL in both adults and children cases. Immunophenotypic detection by flow cytometry recorded (100%) successfully diagnosis in ALL cases, but it was successfully in (75%) of detected the subtypes of AML cases.

Keyword: *Acute leukemia, AML, ALL, FCM , FAB classification; cytochemistry*

Introduction

Acute leukemia is a complex disease, there are two main types of leukemia, myelogenous (AML) and lymphocytic (ALL), depended on the types of cell that are involved. On the other hand each type is subdivided into acute and chronic classes [(1)]. That can affect each of adults and children at different ages [(2).]

The diagnosis and sub-type classification of leukemia requires the simultaneous application of multiple techniques such as : Morphological assessment, Determination of cytochemical and immunophenotypic features and Evaluation of genetic abnormalities [(3)].

The French-American-British (FAB**)** divided acute leukemia into various subtypes: M0 to M7 for myeloid leukemia, and subdivided to three subtypes, L1, L2 and L3 [(4)].

Immunophenotyping by flow cytometry offers a rapid, precise and effective means for the diagnosis of acute leukemia. It provides valuable information of the leukaemic blasts by analysis of surface markers [(5)].

Patients and Methods

This study included 160 cases were newly diagnosed with Acute Leukemia, 100 of them was adults ≥ 15 years (60 cases AML, and 40 ALL). While the other 60 cases were children ≤ 14 years (13 cases AML, and 47 ALL).

All cases were newly diagnosed. The samples collected from Hematology Unit of Baghdad Teaching Hospital in Medical City and Center Teaching Hospital of Pediatric. The collection period is from April to November 2019.

The cases were diagnosed according to FAB classification, based on 20% blast cells in peripheral

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blood (PB) film or bone marrow aspirate (BMA) as sign for diagnosis by cytomorphology by examination with Leishman stain, cytochemical with special stains Sudan Black B (SBB) and Periodic Acid-Schiff (PAS) and flow cytometry analysis was done by (BD FACSCanto II system 8 color). Using a panel of monoclonal antibodies to classify acute leukemia which included CD13,CD33, CD117, CD34 and HLADR for AML, and each of CD3, CD5, CD7, CD10, CD19 and CD79a for T and B ALL respectively. Data were entered into Excel sheet and then transferred to SPSS and $P < 0.05$ was regarded as statistically significant.

Results

The age and gender of cases

In present study, in adults AML the highest case's number was with > 55 years with mean age 48.45 years. Also in adults ALL, the highest percentage was between 15-35 years with mean age 32.59 years. In children (age ≤ 14 years) the AML were 13 (21.67%) and mean age was 5.23 years and peak incident of AML children cases was <5 years, whereas the ALL children were 47(78.33%) cases and mean age 6.44 years . the highest frequencies of children ALL cases were between (5-8 years).

The adult AML cases recorded 1.06:1 male to female ratio. Whereas the adult ALL cases the male to female ratio was 1.22:1. Children cases included male to female ratio 1.6:1 . while in children ALL cases the male to female ratio was 1.13:1 (figure 1) .

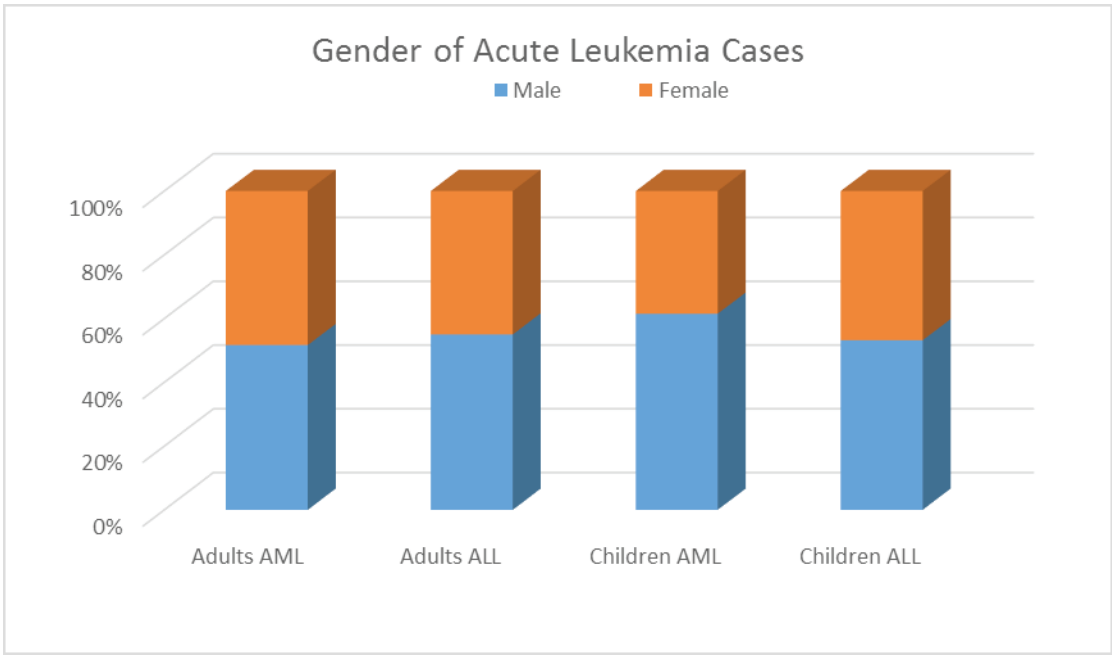


Figure 1: percentage of male and female of Acute Leukemia cases

Cytomorphological Diagnosis

In this study used peripheral blood smears examination the results were that adults AML 38(63%) cases were diagnosed with their subtypes according to FAB subclassification. While 18(30%) of cases diagnosed as just acute leukemia without subtype and recommended to immunophenotype and /or cytochemistry analysis. Adults ALL 13(33%) cases were diagnosed without determination subtype, 10(25%) diagnosed as AML. And 17(42%) were diagnosis with their subtypes.

In children AML 6(46%) cases were diagnosed by cytomorphology with their subtypes, while 4(31%) cases diagnosis as acute leukemia without detect it's subtype.

In children ALL 11(23%) cases of them diagnosis as just acute leukemia, and 5(11%) given different diagnosis, 31(66%) cases which given diagnosis with subtype.

Cytochemical Diagnosis

The cytochemical detection was included SBB and PAS stains. SBB was positive in most cases of AML, While PAS stain in adults ALL cases was positively in 24(60%), **table 1**.

Table 1 the results of cytochemical diagnosis in acute leukemia cases:

Cytochemistry	AML		ALL	
	Adults	Children	Adults	Children
SBB	48(80%)	10(77%)	3(7.5%)	0%
PAS	3(5%)	1(7.7%)	24(60%)	40(85%)

Immunophenotype Diagnosis

this study included 73 cases newly AML diagnosed (60 adult , 13 children). Also 87 cases diagnosis as ALL (40 adults , 47 children).

Adults cases (83.3%), (85%) were positive to CD13 and CD117 respectively in same time all children cases were positive to both CD13 and CD117. CD33 was positive in (81%) in adults and was (84.6%) in children .**table 2**. The subtypes of AML cases summarized in **table 3**.

In T.ALL all cases in both adults and children were positivity to CD5, follow CD7 which given percentage (92.8%) in adults and (88%) in children cases.

In adults B.ALL all cases given positive reaction for CD79a follow CD19 and HLADR. As well as the children cases recorded nearly the results that all children was positivity expression with CD79a and CD19 follow with CD10 and HLADR, **table 4**.

Table 2: The results of immunophenotype diagnosis with flowcytometry (FCM) of AML cases:

FCM markers	Adults	Children
CD13	50(83.3%)	100%
CD33	49(81.7%)	11(84.6%)
CD117	51(85%)	100%
CD34	46(76.7%)	10(77%)
HLADR	48(80%)	4(30%)

Table 3: the subtypes of AML cases.

Subtypes	Adults AML		Children AML	
	No.	%	No.	%
M0	10	16.67%	2	15.38%
M2	2	3.33%	0	0%
M3	7	11.67%	3	23.08%
M4	13	21.67%	4	30.77%
M5	9	15%	3	23.08%
M7	2	3.33%	0	0%
*Non M3	17	28.33%	1	7.69%
Total	60	100%	13	100%

*NonM3 mean that this cases may been all of the subtypes except M3 (ambiguous).

Table 4: The results of immunophenotype diagnosis with flowcytometry (FCM) of AML cases:

F.C.markers	Adults	Children
T.ALL		
CD3	11(78.5%)	12(70.5%)
CD5	14(100%)	17(100%)
CD7	13(92.8%)	15(88%)
CD34	8(57 %)	15(88%)
HLADR	8(57 %)	7(41%)
B.ALL		
CD10	18(72%)	25(86%)
CD19	19(76%)	29(100%)
CD79a	25(100%)	29(100%)
CD34	14(56%)	16(55%)
HLADR	19(76%)	25(86%)

Comparative assessment of acute leukemia diagnosis

In the table 6 which summarize the morphology, which is the cheapest method could used to a diagnosis within minutes to hours, while the immunophenotype give a result of diagnosis at one day and clinically helpful to characterize subtype of acute leukemia and it costly.

Table 5: comparative various diagnostic procedures :

	cytomorphology	cytochemistry	immunophenotype
AML			
Subtype detection required time The cost Type of sample Possibility of follow-up	60% Less than hour + P.B and/or B.M Weekly	79% Hours ++ B.M Not possible	75% one day +++++ P.B and/or B.M Not possible because highly cost
ALL			
Subtype detection required time The cost Type of sample Possibility of follow-up	55% Less than hour + P.B and/or B.M Weekly	73.5% Hours ++ B.M Not possible	100% one day +++++ P.B and/or B.M Not possible because highly cost

+ Cheapest; ++ cheap; +++ costly; +++++ most costly.

Number of cases successfully done by that method.

Discussion

The AML cases were more in adult cases that 60% of adult cases were diagnosed as AML, that demonstrated similar results to other studies (6-8). The acute myeloid leukemia is the commonest of acute leukemia in adults [7], and it is a third most common major cancer in Iraq [8].

The present results in children ALL cases were agreement with [9],

That the ALL is the mostly common of the cancer between the children and, the greatest common causes of death by cancer before twenty years of age [10].

One of the important prognostic factor in Acute Leukemia is the age [11]. In present study shows that the mean age of adults AML cases were 48.45 years. This finding was comparable with other studies [12,13]. But disagreed with [14,15]. The peak incidence of AML adult cases in currently study was in age >55 years. But this study was higher than [16],

While agreement with [17]. The mean age of ALL adults cases was 32.59 years. Which different with [18,19]. The mean age of children ALL cases in present study was agreement with [20]. And the peak incidence were at the same line with [21] .

The male to female ration results of adults AML cases in current study was different from[12], while in adults ALL cases were agreed with [22].

In children AML cases male to female ratio was 1.6:1. Which different from[23], while children ALL cases given nearly agreement with [21].

The cytochemistry found that the 80% of bone marrow aspirate (BMA) samples of adults AML and 77% of children AML were positive to SBB stain. On the other hand (BMA) samples in 60% adults ALL, 85% children ALL were positivity reaction to PAS stain. This positivity of cytochemical tests may be explained by the presence of specific enzymes or other substances in individual hematopoietic cells leading to development of color reaction. Morphologically, the presence or absence of myeloblasts has a central role in diagnosis of AML and suspicion of ALL respectively, as in FAB classification ALL is largely a diagnosis of exclusion .

In adults AML the markers CD 117 mostly positivity, followed CD13 and CD33. In children cases both CD117 and CD13 were positive in all cases (100%) followed by CD33. From this result that use of antibodies against for these antigens is possibly sufficient for an initial diagnosis of acute myeloid leukemia. Also the present results agreement with [24,25]. Acute myeloid leukemia was defined immunologically by the expression of 2 or more of myeloid markers: CD13, CD33, and CD117 [25].

The subtype M0 showed higher expression for CD34, CD117 and HLADR, while M4, M7 related with CD33 and CD117 that were much more helpful in distinguishing their subtypes in AML cases of adults. In this study results each of subtype M0 and M2 related with strong positivity more than other subtypes for CD13 and CD33 which may be diagnostic characteristic to this subtype of acute myeloid leukemia.

The current results found that T.ALL cases recorded percentage 100% positivity reaction to CD5 in both adults and children followed by CD7 in adults, while in children follow CD7 and CD34 there given same percentage value (88%).

The positivity CD79a in 100% of B.ALL adult cases. Whereas in children both CD79a and CD19 were 100%, agreement with [26]. From this result that use of antibodies against for these antigens is possibly sufficient for an initial diagnosis of acute lymphoid leukemia.

The B-ALL cases were mor than T-ALL for both adults and children cases, in results this study which similar to [27].

Immunophenotypic detection by flow cytometry recorded (100%) successfully diagnosis in ALL cases, but it was successfully in (75%) of AML cases, the which recorded as AML (Non-M3). While other study in [28] found that flow cytometry determined subtype in 94% of AML cases. That the large heterogeneity of myeloid-related lineages, no clinically relevant immunological classification of AML has been devised so far [6].

Depending on the current results, we conclude that the techniques used in diagnosis of acute leukemia in this study considered one complementary to other with ability using flow cytometric antibodies CD13 and CD117 as a cost effective diagnostic marker in acute myeloid leukemia cases to support morphological findings. Also CD5 and CD7 were the most frequently expressed in T-ALL while CD79a and CD19 were most commonly associated with diagnosis of B-ALL. CD33 positivity reaction was strongly related with expression in AML-M2, M3, M4 and M7 subtype with adult cases, while it expression related with AML-M3 and M4 subtype in children AML cases.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Analysis of Blood Spot in the Crime Scene in Relation to the Environmental Parameters

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Abstract

This study was designed to evaluate the analysis of blood samples in crime scene under different environmental conditions. Samples are divided into four main groups depending on the source of bloodspot: Soil, Stone, Ceramic, and Water sample. Each group consisted of two sub-groups depending on weather temperature in Iraq in winter and summer: 8 °C groups and 45 °C groups. Each group consisted of 32 samples. Samples were collected after different time (0, 2, 6, and 24) hours.

The results of the current study showed variation in the ability to extract DNA depending on the surface type. In addition, DNA can be extracted from bloodstains on soil, gravel, ceramics, and water when extracted immediately after exposure to the surface. The difference in the ability to extract DNA from bloodstains on different surfaces after two hours at 8 °C temperature, where the success rate of extraction is (25, 7.5, 100, and 100%) for soil, stone, ceramics, and water samples Respectively. The ability rate to extract DNA after two hours at 45 °C temperature, where (25, 7.5, 87.5, and 100%) for soil, gravel, ceramics, and water samples respectively. The ability rate to extract DNA after six hours at 8 °C temperature, where (0, 12.5, 37.5, and 100%) for soil, gravel, ceramics, and water samples respectively, while it was (0, 12.5, 25, and 100%) for six hours samples at 8 °C temperature. After 24 hours, only water samples showed the ability to extract DNA from all samples, while no DNA was obtained from the rest of the samples.

PCR amplification of the *FIF2AK3* gene showed products when the DNA extracted immediately after their adhesion to the surface of the soil while showing no products for other age groups. Also, the PCR products can be obtained when DNA isolate immediately after exposure to the stone surface, whereas the rate PCR amplification products of DNA isolated after two hours was (37.5%) for both groups 8 °C and 45 °C., while other age groups showing not products.

The agarose gel electrophoresis of PCR products showed the possibility of *FIF2AK3* gene amplification when blood samples were taken immediately after exposure to the surface, whereas the ability to obtain amplification products was (100 and 87.5%) for groups 8 °C and 45 °C respectively. No products were obtained after 6 and 24 hours. In water samples, all isolated DNA gave the product.

Introduction

The term "forensic genetics" Refers to the use of human DNA applications in crime investigation (1). The use of Deoxyribonucleic acid (DNA) as evidence of its origin has considered a revolution in the field of forensic. The accessibility of improved technologies has now made DNA analysis a critical and part of forensic science (2,3). DNA profiling has a high discriminatory power, which made it particularly influential in forensic science. By using of a number of genetic markers, DNA profiling has the ability to differentiate between individuals, which gave each individual a distinctive, possibly unique, DNA profile (4).

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DNA evidence can support an investigation along with other evidence to allow the possibility of including or excluding a suspect from their presence in a crime. DNA discrimination power makes it a powerful tool that can assist an investigation as evidence to be presented in court (5). Evidence including body fluid (such as blood, saliva, semen, and sweat), tissues, for example, teeth, skin, hair roots, and bones can all be used to produce a DNA profile (6). The natural physical environment such as temperature, humidity, and ultra-violet radiation, can affect the ability to recover DNA from samples. These effects depend upon the location and climatic conditions. A number of environmental influences have a direct impact on DNA degradation. After all, the high temperatures and ultraviolet (UV) is one of the most important factors that increase the degradation of DNA (7).

Limited studies have addressed the effects on the environment of DNA spots resulting from the body fluids may be due to the fact that there is a difference in large climates between different geographical locations that make this measure scientifically impossible (8)

Materials and Methods

Samples of the study collected randomly from the human blood for the period from 1/2/2019 to 15/6/2019, the samples have consisted of deferent blood groups with different environmental conditions. One ml of blood used to simulates the crime scene. Samples divided into four main groups depending on the source of bloodspot: Soil, Ceramic, Stone, and Water sample. Each one of the mains consisted of two sub-groups depending on weather temperature: 8 C group and 45 C group. Each group consisted of 32 samples with different blood groups. DNA extracted from the samples immediately, after tow houres, after 6 hours, and after 24 hours.

Genomic DNA extracted from the blood of samples according to the extraction method adopted by the supplied company.

Electrophoresis has been done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel . Than we used nanodrop to determination of DNA concentration.

The primers where design by primer 3 plus program. The primers were lyophilized, they dissolved in the free ddH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free ddH2O water to reach a final volume 100 µl.

Primer	Sequence	Tm (°C)	Product size
F	5'-AGCTCCTATAGTAACCTCTTCTTGAACTCACTTG- 3'	68.6	429 bp
R	5'-GCTTTCACGGTCTCGGTCCCACTG- 3'		

Results and Discussion

DNA was extracted from blood spot in different invironmental condition (Soil, stone, ceramic, and water) at (8 °C and 45 C°). Each sample's source was composed of 32 samples, with samples age group (0, 2, 6, and 24 hours). The concentration of the extracted DNA cheeked by using a nanodrop device.

The DNA concentration of the Soils samples at 8 °C ranged from 32.3 to 67.1 ng/µl with average 51.09 ng/µl, while the DNA concentration of the samples that taken at 45° C ranged from 22.3 to 54.3 ng/µl with average 38.24 ng/µl. The DNA concentration of stone samples at 8 °C were ranged 32-86.9 ng/µl with average 60.6 ng/µl, while the DNA concentration of 45 °C samples was ranged 23-65 ng/µl with average 46.8 ng/µl. The DNA concentration of 8 °C samples was ranged 39 to 77 ng/µl with average 60.6 ng/µl, while the DNA concentration of 45 °C samples was ranged 32 to 66 ng/µl with average 49.2 ng/µl. The DNA concentration of water samples 8

°C was ranged 23 to 70 ng/µl with average 42.7 ng/µl, while the DNA concentration at 45 °C was ranged 20 to 74 ng/µl with average 41.4 ng/µl.

Agarose Gel electrophoresis of extracted DNA from soil samples showed bands in 10 samples out of 32 samples in each 8 °C and 45 °C. Eight band came from DNA extracted from immediately age group, 2 bands from 2 hours age group, while none of the other age group samples gave any bands. Stone samples showed 12 bands of 32 samples for 8 °C samples. The band composed of eight bands from zero age group, three bands from 2-hour age group, and one band from 6-hour age group. The 45 °C samples gave also 12 bands distributed as eight bands for the zero-age group, 3 bands for (2 hours) age group, and one band for (6 hours) age group. Agarose Gel electrophoresis of extracted DNA showed 19 bands of 8 °C samples. The band composed of 8 bands from zero age group, 8 bands from 2 hour age group, and 3 bands from 6 hour age group. The 45 °C samples give 17 bands distributed as 8, 7, and 2 bands for 0, 2, and 6 hour age group. Agarose Gel electrophoresis of extracted DNA products showed the appearance of all bands for 8 °Cand 45 °Csamples fig. (1).

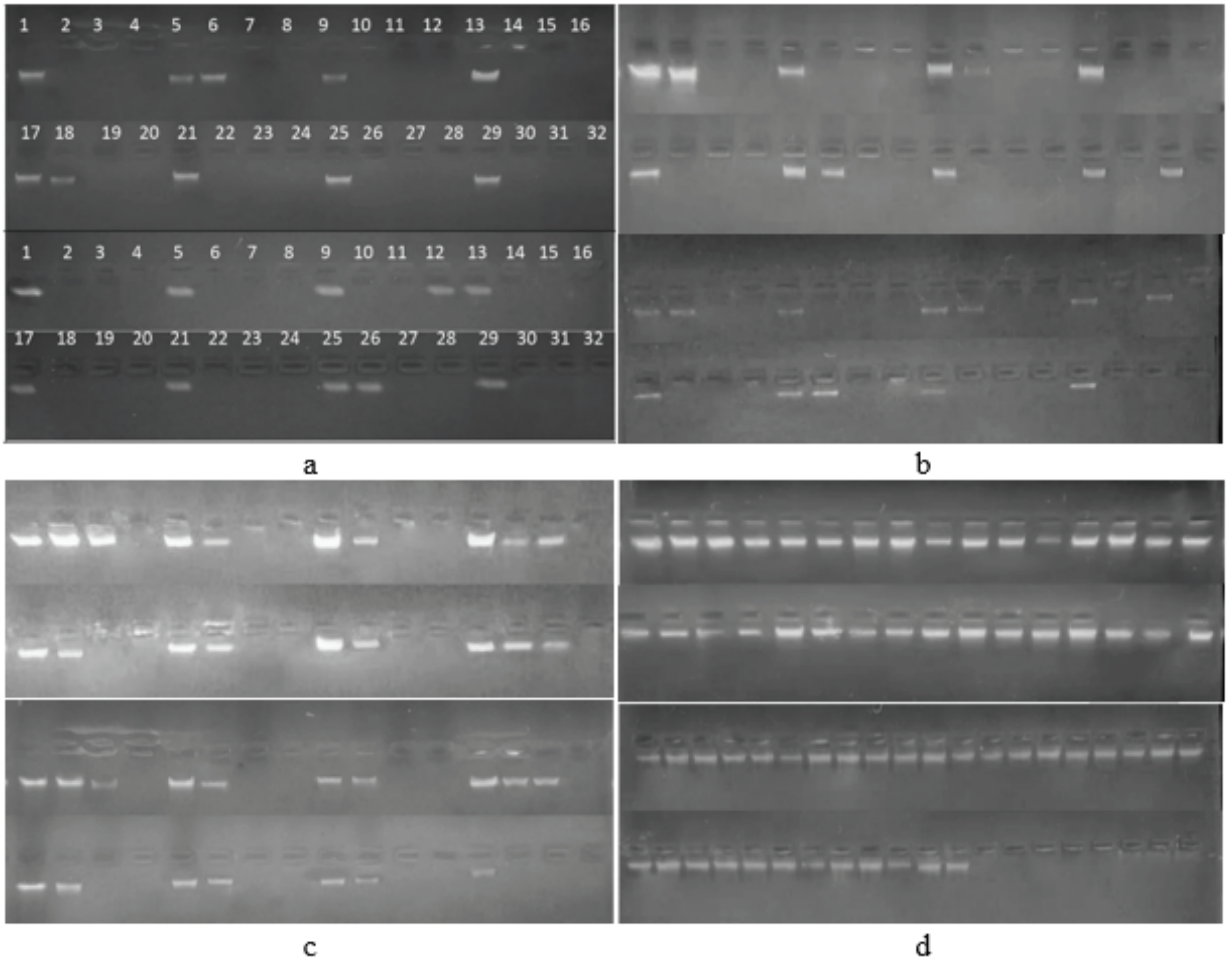


Fig (1) Agarose gel electrophoresis of genomic DNA extracted from blood spot on 1.5% agarose at 50v. for 2 hr (a) Soil sampes at 8 °C and 45 °C (b) Stone sampes at 8 °C and 45 °C (A) Ceramic sampes at 8 °C and 45 °C (A) Water sampes at 8 °C and 45 °C group

Agarose gel electrophoresis of PCR product by using specific primers for amplification of gene for 30 samples of blood spot taken from the soil at 8 °Cand 45 °C showed 8 bands at the expected site in both (429 bp). All bands represent immediately age group. Ceramic samples at 8 °C and 45 °C showed bands in 11 samples at the expected site (429bp) in both. The distribution of 8 °C and 45 °C samples depending on age group was eight bands for the zero-age group and three bands for 2 hours. Stone samples showed bands for 16 samples in both 8 °C and 45 °C group at

the expected site. Depending on samples age, the distribution of 8 °C group was eight, and 8) bands for (zero and 2) hour age groups respectively, while the distribution of 45 °C was also (eight and 8) bands for (zero and 2) hours age groups respectively. In water samples, they gave product in expected site for all samples.

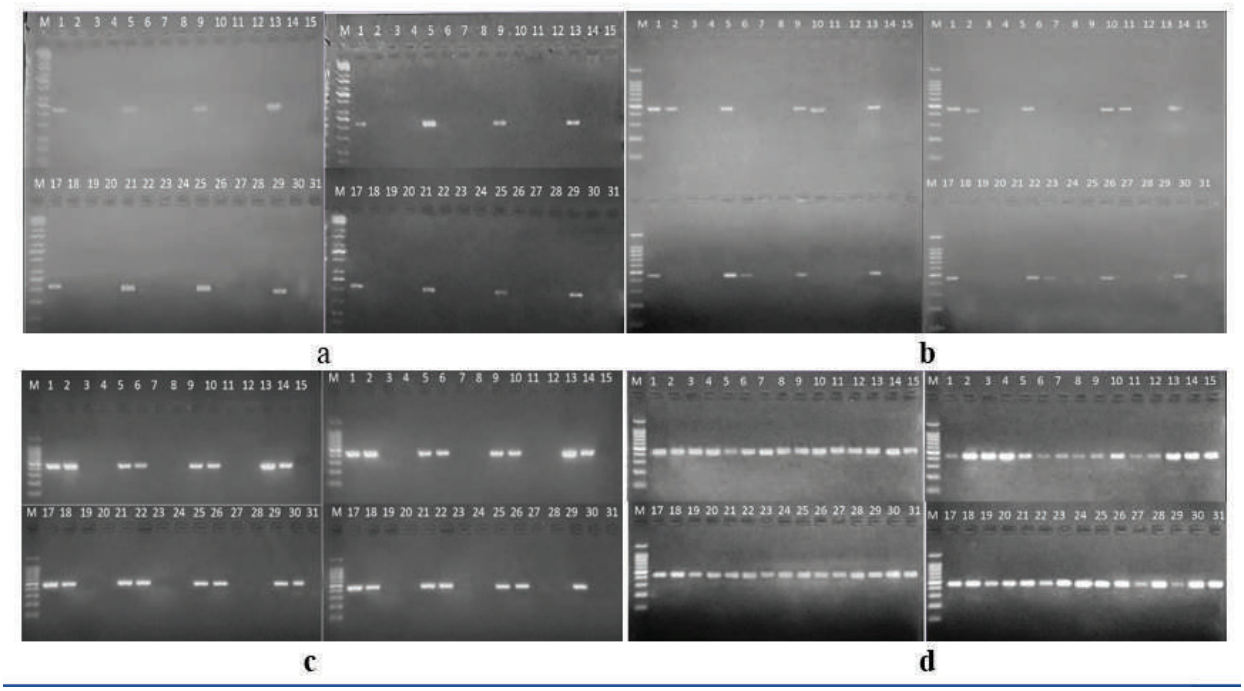


Figure (4-5) Agarose gel electrophoresis of PCR product for amplification of gene for blood spot on 1.5% agarose at 50 v. for 1 hour (a) Soil sampes at 8 °C and 45 °C (b) Stone sampes at 8 °C and 45 °C (A) Ceramic sampes at 8 °C and 45 °C (A) Water sampes at 8 °C and 45 °C group

Discussion

In the present study, we used two different temperatures (8 ° C and 45 ° C) to simulation the weather in Iraq. We used four environmental sources to stimulate the crime scene.

Biological evidence may expose to various environmental factors. These factors play crucial roles in DNA degradation (9). Environmental conditions such as heat, humidity, chemistry, microorganisms (bacteria and fungi), etc. can be effective on the stability of DNA for survival (10).

The DNA extracted from the blood spot affected by the nature of the surface on Moreover, the concentration and purity of the extracted DNA influenced by the surface factor in it (11). A study showed that the concentration of DNA is higher and the possibility of extraction is stronger on smooth surface comparison with non-smooth surfaces (12)

Extracting DNA from biological evidence found in the soil has a high failure rate. This may be due to the soil contains many microbes that can cause DNA destruction and digestion. Moreover, containing soils of different chemical compounds can do the same action (12). The presence of an inhibitory substance called humic acid in natural soils may cause DNA degradation (13).

The Hem and humic acid act as inhibitors Exposure to mild (moderate) inhibitors reduce the concentration of DNA (14).

The reaction of moderate enzymes also plays a role in DNA degradation when undergoing external environmental conditions. When cells die, the internal enzymes are activated, which leads to DNA degradation by an endonuclease or exogenous which released by the microorganisms that exist in the surrounding environment turned to mononucleotides (10).

This is consistent with the current study, where blood spot on soil has been affected by two inhibitors:

humic acid from soil and hem from RBC, in edition to microorganisims. his lead to DNA degradation in most of the samples. Stone and ceramic samples were less degradations from soil samples this may due to less affected inhibitors and the nature of its surface. In water samples, the appearance of all samples may refer to that water reduce the inhibitory effect of the hemoglobin.

Many studies showed that temperature plays a big role in DNA degradation (9). Another study mentions that temperatures are known to have the ability to reduce the construction of DNA (6).

This agreed with the current study which found that DNA constriction affected by weather temperature in soil, stone and ceramic, while it shows no effect in the water. This may due to water specific heat.

The presence of an inhibitory substance called humic acid in natural soils; this substance is extracted with DNA extraction and inhibits the possibility of DNA profile by binding to DNA molecular components (13)

The natural soil contains many PCR inhibitors. However, the main inhibitor responsible for the high rate of failure to identify DNA is humic acid; this substance is extracted with DNA extraction and inhibits the possibility of DNA profile by binding to DNA molecular components. Humic acid consists naturally through the decomposition of plant and animal remains. Humic acid Dominates the grassland soil (13,15).

Humic acid has chemical-physical properties similar to double-strand DNA and is co-extracted with DNA, either by binding with components of the extraction system (silica) or by binding to the DNA molecule itself (16). HA linking with Most of PCR components and inhibits DNA amplification (15,16), which not only directly effects on DNA profile, but also indirectly by affecting the accuracy of the quantification of DNA using PCR (13).

Forensic samples contain chemicals that can reduce or inhibit amplification using polymerase chain reaction (PCR). The most obvious inhibitor is (hem) (17), and dyes found in clothing and humic acid from soil samples and calcium from the bone (18). These substances are extracted with the DNA product and can affect all the contents of the polymerase chain reaction including the DNA template, nucleotides, amplification primers, Mg^{+2}, And polymerase enzymes. Although the inhibitory effect of these substances is known, the inhibitory mechanism is unknown (19). Exposure to severe inhibitors leads to loss of alleles (14)

In a study by Wu and co-workers, the characteristics of the RBC membrane are influenced by the type of substance-exposed to the blood (18).

This agreed with our result where PCR amplification did not give any band in 2 and 2 samples at 8 C and 45 C respectively. All samples were 2 hours of age group. PCR amplification did not give any band in 3 and 3 samples at 8 C and 45 C respectively. All samples were 6 hours age group. This may refer that soil has a faster inhibitory rate than ceramic and stone. Our study shows no Impact of water on DNA stability this may due to the percentage of blood volume to water is low.

Conclusion

DNA is affected by different surface in which blood stains. Temperature affects the concentration of DNA in exposed blood stains while it does not affect the presence of stains in water. The concentration of DNA is not affected by water extraction and the temperature of the ocean does not affect the DNA in water

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Determination of IL17 Plasma Levels and IL-17A gene Polymorphisms in Patients with Allergic Rhinitis

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Abstract

Aim: The present study aims to determine whether the level of serum interleukin -17 and IL-17 receptors (IL-17A) gene variant increases or decreases susceptibility to Allergic Rhinitis. **Method:** In this case-control study fifty patients with rhinitis and fifty apparently healthy persons as a control in Babylon province / Iraq were enrolled in this study. **Results**: the results of this study were shown significant difference in frequency of IL-17 A (rs2275913) A>G polymorphism between Rhinitis patients and controls .Also , there were significant difference in frequency of IL-17A (rs 2275913) between rhinitis patients and controls and there were significant increase in patient compared with control. **Conclusion:** The bpresent study suggests that patients with AA allele for IL-17A (rs2275913) have increased susceptibility to rhinitis compared with GG allele , Also the present study suggests that IL-17A (rs2275913) is considered risk factor for Allergic Rhinitis. This study suggests that the level of IL-17 patients with rhinitis highly significant compared with healthy.

Keywords: *Allergic Rhinitis,IL-17,IL-gene polymorphism*

Introduction

Allergic Rhinitis a type of inflammation in the nose which occurs when the immune system overreacts to allergens in the air [1]. Signs and symptoms include a runny or stuffy nose, sneezing, red, itchy, and watery eyes, and swelling around the eyes [2]. Some people may develop symptoms only during specific times of the year, often as a result of pollen exposure [3] . Allergic rhinitis is typically triggered by environmental allergens such as pollen, pet hair, dust, or mold [4]. Inherited genetics and environmental exposures contribute to the development of allergies [5].Cytokines play an essential role in mediating allergic inflammation. The importance of the T helper cell (Th) 2 cytokines in both the development of allergic sensitization and pathology of allergic inflammation is well established [6]. While healthy subjects are predominated by Th1-type cells, nasal mucosa and epithelial tissues of AR subjects are dominated by Th2-type lymphocytes[7].The recent discovery of another T lymphocyte subset, namely Th-17 cells, has been fundamental to our understanding of how Th-1 cells can actually mediate inflammatory events by producing IFN-c [8] . T helper 17 cells are characterized by the production of various cytokines, including IL-17, IL-6, tumor necrosis factor-a and IL-22 [9]. Another Th subgroup of regulatory T cells (Tregs) has restrictive influences on both Th1 and Th2 cell-mediated inflammation. The lack of Tregs causes the emergence of allergic inflammation along with the increase in Th2 cells[10]. The Tregs fetch the allergic inflammation under control by synthesizing IL-10 and the changment growth factor-β (TGF-β)[11].The patient with Allergic Rhinitis showed an allergen-specific functional defect in Treg that promotes Th2 polarization and consequently IgE synthesis [12]. An increasing number of studies have demonstrated relationship between IL-17 and neutrophilic inflammation, implicating IL-17 as a potential candidate gene in predicting allergic rhinitis susceptibility. Interestingly, an analysis which reported by Chinese study showed that IL-17A rs 2275913(−197G/A) were

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found to be commonly associated with allergic rhinitis [13]. Thus, the aim of the present study was to investigate the impact of IL-17A rs2275913, genes polymorphisms on IL-17A plasma levels together with their influence on susceptibility as well as its severity.

Ethical statements:

The study design was reviewed and approved by the ethics committee of Hilla Teaching Hospital and Imam Sadiq Hospital in Babylon province in Hilla city. All persons participated in this study was agreed to participate and signed an informed consent.

Material and Methods

Study Design:

This study design was a case-control study. Patient with diabetes mellitus, Patient with hypertention, Smokers, Patients with rheumatoid arthritis, Pregnancy, Any inflammatory diseases, Any autoimmune diseases. This study included 100 individual , the age was ranged between (18 - 70) years ,All samples were collected from 1st of September 2019 till 1st of January 2020. The practical side of the study was performed at the laboratory of biochemistry department in College of Medicine / University of Babylon. We collected blood samples from healthy subjects, patients during rhinitis.(5 ml) blood were drawn,(2ml) EDTA tubes immediately frozen at -20 °C for further assay. and (3ml) gel tubes, clotted at room temperature for 10-20 minutes and centrifuged for 20 minutes at 3000 rpm. Serum samples were immediately frozen at -20 °C for further assay.

Genotyping analysis

Genomic DNA was isolated from EDTA peripheral blood samples of all patients and extracted by standard salting-out procedure [19]. The identification of the IL-17A rs2275913 (A/G -197) polymorphisms were performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) using specific primers: [(IL-17A*F: 5'-AACAAGTAAGAATGAAAAGAGGACATGGT-3'; IL-17A*R: 5'-CCCCCAATGAGG TCATAGAAGAATC-3') respectively followed by a digestion of the amplification products using EcoNI enzymes respectively.

Measurement of serum IL-17 levels

Serum IL-17 was measured by commercial enzyme-linked immunosorbent assay (ELISA) using reagent kits Elabscience (China)

The presence of DNA extracted was detected by using agarose gel electrophoresis technique ,then the genomic DNA was amplification by PCR technique, After PCR product was digested with restriction ECON1 enzyme and the products were analyzed by 5% polyacrylamide gel electrophoresis., The PCR fragment was divided into 102 bp, 68 bp and 34bp fragments.

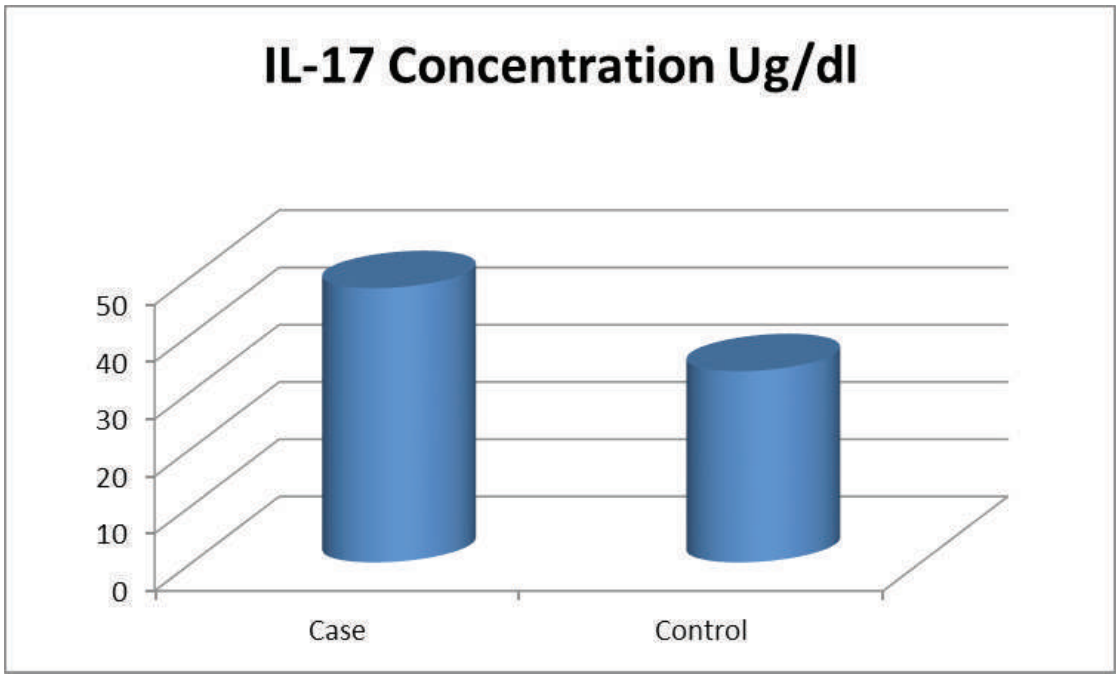
Statistical Analysis

Data were analyzed by the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Normally distributed data were expressed as the mean±SD and non-normally distributed data were expressed as the median (25-75th percentiles). The Student paired t-test was used to compare normally distributed data between two groups, and the rank sum test was applied for nonnormally distributed data between two groups. The $\chi 2$ test was used to compare frequencies. All reported p values were two-sided, and $p < 0.05$ was considered statistically significant.

Results:

The demographics and clinical characteristics of the healthy control subjects and patients with allergic rhinitis are shown in Table 1. significant difference in age, sex and family history was observed between patients and healthy control. As expected, patients with attacks showed (mean±SD: 46.3 ± 3 more than 40 year vs. 26.5 ± 3 for less than 40 years, $p< 0.05$) compared to healthy control subjects (Table-I). However, the FEV1%pred levels were found higher in patients during remission than during asthma attack ($p< 0.05$).

Serum IL-17 (Fig.1) level was significantly elevated in patients during patient with allergic rhinitis attack and remission ($p< 0 .000$) compared with healthy control subjects. In addition, serum IL-17 levels was much higher during rhinitis attack than those during remission ($p< 0.05$).



(Figure 1): IL-17 concentration in serum of case and control in allergic

Analysis of IL-17A rs2275913 polymorphism

There were significant differences in genotypes and alleles frequencies between patients and controls (Table :1):

(Table: 1). Results of IL-17A rs2275913 genotyping in patients and controls

IL-17A genotype	Patient n=	Control n=	P value	OR(95% CL)
A	58%	52%	P<0.05	OR: 5.892*; CI 95%** 1.77-19.57
G	42%	48%		

Otherwise, the IL-17A studied polymorphism was associated to the activity of the AR in our patients group.

Discussion

The present study showed significantly increased serum IL-17 level in patients with allergic rhinitis compared to healthy subjects, lately, serum IL-17 levels were found higher in rhinitis patients than healthy subjects[14]. These results proposed that serum IL-17 levels play an important role and linked to the severity of the rhinitis[15]. In the present study was significant association was found for IL-17A rs2275913 and disease susceptibility, $p < 0.05$. The subject result was showed the relationship between RS2275913 gene polymorphisms and allergic rhinitis ,this result was agreement with F Fatahi et al in Iranian study [16]. Also in Spain study was showed RS2275913 gene polymorphisms was relationship with allergic rhinitis. the IL-17A*A allele conferred a role for AR risk, OR = 5.892*; 95% CI 1.77-19.57. Therefore, the risk conferred by IL-17A rs2275913 G allele in AR predisposition might be weak and that could explain the absence of association reported in the mainstream of published studies as well as the present study.

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assistance in the collection of samples.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Activity of Extract Seed *Moringa Oleifera* on Gene Expression of (EFB1) Gene in *Candida dubliniensis* Isolated from oral Candidiasis to Cancer Patients

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Abstract

Gene expression of EFB1 gene in yeast *Candida dubliniensis* isolated from oral candidiasis in cancer patients was detected using by R-time PCR for some isolates before and during the yeast infection of the epithelial cells. The results showed a high rate of gene expression of T2 (42.104 ± 6.533) and a significant difference at the probability level P>0.01 compared with the treatment was c (1.034 ± 0.126) and the use of the Moringa oleifera extract was 25% to evaluate its effect on the gene expression (ALS1) before and during yeast infection of epithelial cells. The results showed that the gene expression of T1 was decreased by (0.129 ± 0.021) compared to (C) value (1.034 ± 0.126) .

As for the T2 treatment was gene expression at a rate (42.104 ± 6.533) high Compared with T3 treatment in which gene expression dropped at a rate (22.143 ± 4.176) and with a significant difference at the level of the probability of P ≤ 0.01.

Keywords**:** *Moringa oleifera, (EFB1) Gene elongation factor 1-beta Candida dubliniensis , Candidiasis.*

Introduction

The immune system of the cancer patient is in a state of weakness and deterioration due to the disease and treatments used, especially chemotherapy and radiotherapy, which aims to eliminate cancer cells and reduce the spread within the body but It has side effects. it is transmitted through the blood circulation to all members and tissues of the body until his arrival the target, more vulnerable tissues are fast-growing tissues and permanent replacement cells such as bone marrow cells and gut tissue [1] The mouth contains the appropriate environmental conditions for the growth of colonies of yeast, which increase the proportion of acidic function of saliva, and the yeast of *Candida dubliniensis* opportunistic fungi that invade the body in the case of weakened immunity and This is aided by many of the virulence factors that are produced with the help of the (EFB1) gene[2-4] . elongation factor 1-beta (EFB1) Translation elongation factor 1 beta; stimulates nucleotide exchange to regenerate EF-1 alpha-GTP for the next elongation cycle which facilitates binding of aminoacyl-tRNA to the ribosomal A site TO Producing protein [2].

This study were conducted in order to achieve our aims, like the isolate and diagnose yeast *Candida dubliniensis* infections from oral cancer patients, the use of aqueous Extract of the seeds of *Moringa Oleifera* to test its effect on the growth of the yeast Candida dubliniensis and use seeds Extract *Moringa Oleifera* to assess its effect on gene expression of (EFB1) gene before and during a yeast infection of epithelial cells.

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Materials and Methods

Samples Collection

(100) samples (oral Scrape) was collected from patients that infected with cancer, which treated with chemotherapy and who appear to have symptoms of oral candidiasis disease and reviewers of the tumors Division in Teaching Hospital of A-Diwanyiah city for the period from the first of March 2018 to the end of August 2018.

Yeast Isolation

Candida dubliniensis was isolated from From Oral Candidiasis To Cancer Patients by culture in the media of Sabourauds dextrose agar (SDA) and incubated in degree (37)C for (7) days, and examined later the growth colonies scrupulous in terms of color, shape, textures and examined the optical microscope after staining dye gram to note the shape and size of the yeast and a growth test at a temperature of (45)C AND growth sample on chromagar candida [3] A hundred samples were collected (oral Scrape) from healthy individuals as a control to compare

Prepare an aqueous Extract of the seeds *Moringa oleifera*

It was Prepare depending on the method Price [4], and then were prepared differently than concentrations, namely, (25)% sterilized Concentrations filtration using filter paper (0.45) microns.

Detection of active compounds in the Extract:

Followed the method contained in the Fahmy [5] for the detection of alkaloids while following the method contained in the Shihata [6]for the detection of tannins and saponins, flavonoids have either followed the method contained in the AL-Khazragi [7] for disclosing them

Experience Design:

1. Prepare of Yeast stuck depending on the method of Clayton [8].

2. Preparation of epithelial cells: Preparation was done depending on the method Chritchaly and Douglas [9].

- **The first treatment (T1):** mixing (0.5 ml) of the yeast is stuck with (0.1) ml *Moringa oleifera* seed Extract concentration of (25)% in the Eppendorf tube.
- **The second treatment (T2):** mixing (0.5)ml of the yeast is stuck with (0.5)ml of the epithelial cells of the mouth is stack in the Eppendorf tube.
- **The Third treatment (T3):** mixing (0.5)ml of the yeast is stuck with (0.5)ml of the epithelial cells of the mouth is stuck in the Eppendorf tube.
- **Control (C):** put (0.5)ml of yeast cells is stuck in the Eppendorf Tube.

All transactions were incubated at a temperature $(37)^0$C four (48) hours and repeated transactions five times, then completed the steps of qRT- PCR.

Quantitative Reverse Transcription Real- Time PCR (RT-qPCR) test:

It is an examination of a series of polymerase chain reaction in real-time quantitative (reverse reproduction) to measure the levels of quantitative DNA sender (mRNA) to denote the amount of gene expression of genes EFB1 gene expression as well as the use of gene of (Act1) structured record to calculate gene expression, has this test was conducted four transactions T1, T2, T3, C depending on the method in Tavanti [10].

Table (1) DNA sequencing primers and their Nitrogenous base**s of Bioneer Corporation - South Korea**

Primer	Sequence		Amplicon
EFB1	F	ATACGCTGCCAAGAAAGCTG -3¢ 5¢-	127bp
	R	TTCGACGGCTTTAACGTTGG -3¢ 5¢-	
Act1	F	5¢- TGTGTAAAGCCGGTTTTGCC -3¢	136bp
	R	TTGGATTGGGCTTCATCACC -3¢ 5¢-	

Genbank code EFB1: XM_002420026.1 and *Act1*: XM_717232.1.

Real-Time PCR data analysis:

The results were analyzed chain reaction in real time through using the use of quantitative way livak method, which was developed by Livak and schmittgen [11]. Statistical analysis, Results were analyzed statistically using the gene expression of the way one way ANOVA LSD at the level of probability of (P<0.01) using SPSS software.

Results

Isolation and diagnosis

Type *C dublineinsis*, (30 (30%), was diagnosed in cancer patients, while *C dublineinsis*1 (1%) was diagnosed in healthy people. The isolated species have been confirmed using growth on chromagar candida and the Candida system . The use of this type of diagnosis is preferred because it is fast and accurate, but it is very expensive and sensitive to the pollution **.**

Qualitative detection of some of the active compounds of aqueous Extracts of the seeds of *Moringa oleifera* .The table shows (2) qualitative detection results of the active ingredient in the aqueous Extract of the seeds of *Moringa oleifera*, which pointed to the presence of saponins and flavonoids and tannins

Table (2): Quality Detection for some active compound for *Moringa oleifera*

Substance	Tannins	Alkaloids	Flavonoids	Saponins
aqueous extracts of the seeds of Moringa oleifera	+	-	+	+

Gene expression of EFB1**gene using the method (2-ΔΔCT Livak method)**

The results shown in Table (3) and Figure (1,2) indicate a high rate of gene expression (42.104 ± 6.533) in T2 and a significant difference at P ≤ 0.01compared to group C (1.034 ± 0.126 (

Effect of aqueous extracts of the seeds of *Moringa oleifera* on gene expression of EFB1gene before and during yeast infection of epithelial cells The results in Table (3) and Figure (1,2) indicate that the gene expression of EFB1gene was in group C (1.034 ± 0.126) To 0.129 ± 0.021in the treatment T1 and significant difference at the probability level of P ≤ 0.01, while for the treatment T2 the value of gene expression (42.104 ± 6.533) was high compared to the treatment T3, which decreased the expression of gene and (22.143 ± 4.176) with a significant difference at P ≤ 0.01.

Table (3) :Gene Expression of EFB1**gene using the(Livak method $2^{-\Delta\Delta CT}$)**

Treatment Isolation	CT (EFB1 gene)	CT (actin)	ΔCT (Test)	ΔCT (control)	ΔΔCT	Fold change (2^-ΔΔCT)	Mean + Std.Error
T1	34.67	35.26	-0.59	-3.46	2.87	0.137	0.129 ± 0.021
T1	34.00	34.61	-0.61	-3.46	2.85	0.139	
T1	34.07	35.15	-1.08	-3.46	2.38	0.192	
T1	34.83	35.17	-0.34	-3.46	3.12	0.115	
T1	35.30	34.74	0.56	-3.46	4.02	0.062	
T2	27.65	35.28	-7.63	-3.46	-4.17	17.952	42.104 ± 6.533
T2	26.71	35.53	-8.82	-3.46	-5.36	41.016	
T2	26.31	35.29	-8.98	-3.46	-5.52	45.913	
T2	26.38	35.46	-9.08	-3.46	-5.62	49.279	
T2	26.48	35.76	-9.28	-3.46	-5.82	56.359	
T3	27.23	35.71	-8.48	-3.46	-5.02	32.465	22.143 ± 4.176
T3	28.07	35.42	-7.35	-3.46	-3.89	14.790	
T3	27.44	35.58	-8.14	-3.46	-4.68	25.649	
T3	27.45	35.70	-8.25	-3.46	-4.79	27.707	
T3	27.60	34.40	-6.80	-3.46	-3.34	10.102	
C	31.63	35.31	-3.68	-3.46	-0.22	1.166	1.034 ± 0.126
C	31.63	35.56	-3.93	-3.46	-0.47	1.387	
C	31.74	35.15	-3.41	-3.46	0.05	0.967	
C	31.74	34.51	-2.77	-3.46	0.69	0.622	
C	31.88	35.38	-3.50	-3.46	-0.04	1.028	
Mean C	31.724	35.18	-3.46				

T1 *Candida dubliniensis* + *Moringa oleifera* seed Extruct ,T2 *Candida dubliniensis* + Epithelial Cell ,T3 *C. dubliniensis* + Epithelial Cell + *Moringa oleifera* seed Extru Ct T1 *C. dubliniensis* + *Moringa oleifera* seed Extru Ct ,T2 *C. dubliniensis* + Epithelial Cell ,T3 *C. dubliniensis* + Epithelial Cell + *Moringa oleifera* seed Extru Ct , C *C. dubliniensis* only , CT: q PCR Threshold Cycle number

(2-ΔΔ CT Livak method) as following: First, the CT of the target gene was normalized to that of the reference (ref) actin gene, for both the test isolates and the Control isolates group.

Δ CT(test) = CT(target, test) – CT(ref, test)

Δ CT(Control) = CT(target, Control) – CT(ref, Control)

Second, the Δ CT of the test isolates were normalized to the Δ CT of the Control:

ΔΔ CT = Δ CT(test) – Δ CT(Calibrator) ,Fold Change of relative gene expression was calculated by following equation = ($2^{-\Delta\Delta CT}$): Normalized expression ratio

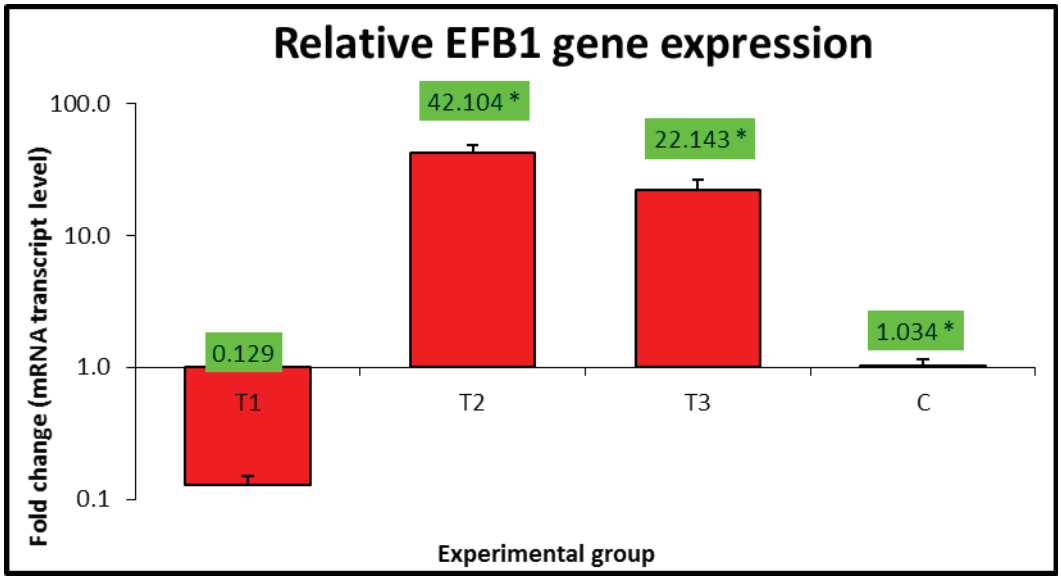


Figure (1): The graph of relative gene expression of the EFB1 gene in the experimental coefficients relative to the control group. *Indicates a significant difference in LSD (mean difference) at P ≤ 0.01.

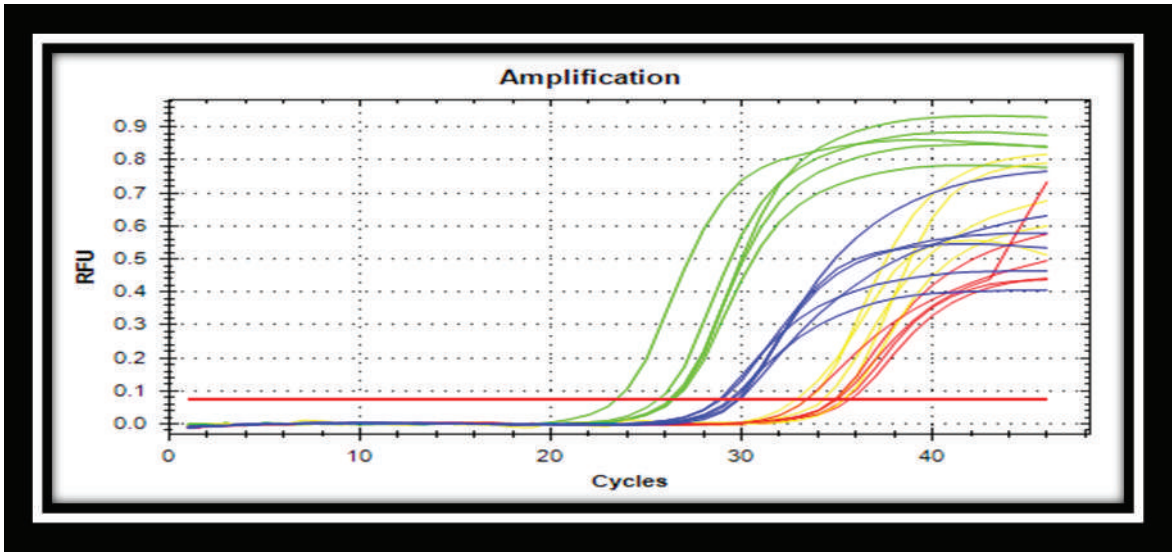


Figure (2) :amplification curve of the interaction of Real-Time PCR and private EFB1 gene in *Candida dubliniensis* , accounting for curves as follows:

1. curves with a yellow color control group (C).

2. with red *Candida dubliniensis* group curves with *Moringa oleifera* seeds extract T1.

3. Curves-green *Candida dubliniensis* group with epithelial cells T2.

4. curves of blue *C. dubliniensis* group with *Moringa oleifera* seed extract and epithelial cells T3

Discussion

Out of the 100 sample under study, 30 (30. %) samples were identified as C. dubliniensis, The results of this study were related to the results of Abdelghani et al., [12] *C. dubliniensis* appears to be a minor component of the normal oral flora of humans. Fewer than 10% of germ tube-positive yeasts in culture collections or from the oral cavities of healthy individuals have been identified as *C. dubliniensis* [13]. However, as with other yeasts, immunosuppression and the use of antimicrobials permit *C. dubliniensis* to increase in numbers and eventually to cause oral candidiasis. Approximately 25% of HIV-infected patients may be colonized with the yeast, and *C. dubliniensis* has been isolated from the oral cavity of 30% of patients with AIDS and oral candidiasis [14]. *C. dubliniensis* was implicated as a pathogen in linear gingival erythema in an HIV-infected child [15]. Although several independent reports have described the recovery of *C. dubliniensis* from patients with HIV infection and from those without since 1995, it was not until recently that invasive disease was reported. In 1999, *C. dubliniensis* was reported as a cause of fungemia in 2 recipients of bone marrow transplantations and in 1 patient with chemotherapy-induced neutropenia [16].

We note the high genetic production of expression for the treatment of T2 rate of the EFB1 gene for treatment C and team's morale high at the level of the probability $P \leq 0.01$ reasons for the rise could be due to the fact that epithelial cells are considered the center of a vital contain her assistant For their growth and reproduction and existence is a catalyst for the production of enzymes in the pathogenesis of C. dubliniensis and Which EFB1 gene contributes to its construction[17] While noting low the genetic production of expression for the treatment of T1 rate of the EFB1 gene for treatment C high spirits and differences at the level of the probability $P \leq 0.01$ may be due to contain the extracted substances that reduce or disable the gene regulation in the cell Such as tannins that have the ability to link with cell proteins by bonds, hydrogen or covalent bonds and the formation of complexes with Thus, working to disable the enzymes and protein carrier in the cell, and its concentrations few of them are working on narrowing micro tubule in the cell wall and thereby prevent the entry of materials into and out of the cell and Cell abstraction of minerals such as iron and magnesium [18] The withdrawal of magnesium from the cell leads to an effect on the work of RNA polymerase, which is involved in the process of cloning mRNA of DNA, the first step to build protein because the catalyst helps to add the nucleotides and thus loss reduces gene expression [19]

Conclusions

Aqueous extracts of the seeds of *Moringa oleifera* contain effective compounds that has an effective role in reducing the rate of gene expression of EFB1 gene in *Candida dubliniensis* .

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Reducing the Environmental Impact of Cork Waste by Improving Natural Asphalt as Water Proofing Material

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Abstract

This research is concern with two principles, a) the environmental pollution which is the global problem b) the economic benefits as a result of reusing of waste material such as corks to produce new reusable material like Modified-Asphalt. In this research cork has melted by benzene and mixed with Hit Natural Asphalt to get good paste with weight percentages of 0%, 10%, 20%. The properties of Modified –Asphalt have been studied after and before adding coke. The results revealed that adding coke by 29% can make the Modified –Asphalt meets the international specifications (ASTM D312) and Iraqi specifications (88-1196) as a Water-proofing material.

Keywords: *environmental impact, natural asphalt, cork waste, modified –asphalt, water proofing material*

Introduction

Cork is important in all aspects of practical life because of its applications and advantages. One of the negative results in the use of cork is the accumulation of large amounts of waste consumed and the time to get rid of them. Since most of the cork does not decompose rapidly, its waste has contributed significantly to environmental pollution. These industrial wastes accumulated and started to threaten human health and all elements of the environment. It has been found that some of these wastes such as cork can be controlled by the recycling process [(1)]. Some people may have to get rid of the waste of cork by burning it and this leads to direct or indirect impact on water, soil and air, as the combustion of waste cork leads to the emission of many chemical compounds depends on many factors, including the type of cork, the speed of combustion, the size and quantity of cork waste, ambient temperature and humidity. Combustion gases are the most emitted chemical compounds e.g. carbon monoxide, carbon dioxide and sulfur dioxide, as well as polycyclic aromatic hydrocarbons such as Pyrene and Anthracene. Other emitted materials include hydrocarbon compounds e.g. xylene and gasoline. The incomplete combustion of cork leads to thermal chemical decomposition followed by the re-unification of parts of various chemical components, some of which are liquid, such as aromatic compounds or paraffin carried by water used in extinguishing the fires.

Scientific methods for safe recycling of cork products

The safe scientific methods for recycling cork depends on its nature and behavior and the quality of the additives and the treatments. All the plastic products can be recycled again through some suitable methods to be reused in their own fields. This can contribute significantly and effectively to sustainable development processes. The recycling of cork products is one of the most important modern processes that must accompany with the expansion of the use of cork products as alternatives to traditional known materials and to achieve sustainable development programs[(2)] .

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Asphalt improvement processes

Improvements of natural or industrial asphalt are designed to increase the ability of asphalt to withstand external conditions to make it more suitable for industrial purposes. There are several methods used in this field as follows:

Binder additives

This type of improvement includes the addition of a polymer such as thermoplastic or rubber, where the link forms bridges between asphalt and other materials, which increases the bonding strength of the asphalt with the rest of the components. Here are several types of polymers used to improve asphalt e.g. rubber (styrene-butadiene) (S.B.R) as well as polyvinyl chloride (P.V.C) .The polymer bonds give an improvement in asphalt surface and in thermal properties [3].

Modifications of hydrocarbonic chains

In this method, there is an alternative reaction of hydrocarbon chain of compensators. These processes include the replacement of hydrogen atoms with other more polarized assemblies to strengthen the chain construction to become more powerful, giving the asphalt more solid and weather-resistant form. These methods include chlorination processes involving the substitution of chlorine atoms instead of hydrogen atoms in hydrocarbon chain of the electrode[4] or the addition of sulfur element. In this way, the double bond of sulfur ions is used to connect each successive hydrocarbon chain to each other, or to react with aromatic substances that are compensated by assemblies or by adding phosphoric acid.

Fillers additives

This method includes the addition of substances in the form of fillings of inorganic salts, waste or other materials, which reduce the spaces between the particles to give support to the body of asphalt and increase the adhesion between the asphalt particles through the formation of links (chemical, physical). Examples of fillers include, metal powders, oxides, metal chlorides and alkaline soils. Researches confirm the improvements achieved using these materials, especially when granular size is small and the material dried to ensure good homogeneity between hydrocarbon chains and improved materials[5].

Previous studies

Extensive studies were conducted on natural asphalt in an attempt to improve it. (Lee et.al) [6] studied the temperature affecting the chlorinated polyethylene mixture as an improved asphalt using different granular size and noted that the additive has increased the penetration and the penetration number when flexible polymers are used as an additive. McGennis[7] used tire crumbs to produce low-hardened asphalt. This was confirmed by a study by Saez-alvan[8], where the addition of polymer was found to give smoothness to the asphalt surface with an improvement in adhesion and thermal properties of asphalt[9,10]. Shatnawi[11] also confirmed that rubber gives good physical properties to asphalt.

A study by Qadi et.al[12] for the prediction of dynamic behavior of asphalt at high temperatures using common polymer-rubber and copolymer-polymer. The modified asphalt has a good rheological properties.

Mahmood et. al[13] improved the properties of natural asphalt using the powder of waste tires. The results showed that the resulted mix can be used as a Water-Proof material for roofing according to local and international standards.

HMAN[14] presented a study to improve asphalt specifications using 25% recycled non-metallic waste material in granular size (0.07-0.09) mm^3. They obtained a good results where Viscosity was 1220 centigrade at 135 °C, Penetration 53.7, Softening Point 54 °C, and the Ductility was 43.5 cm at 15 °C.

Many types of polymers have been successfully used to improve the cohesiveness of asphalt associated with its components at low temperature. This is confirmed by the study conducted by Kim et. al [15] . In recent years, the polymer has been widely used to improve the specifications of asphalt . Thermoplastics are the commonly used materials mixed with asphalt to enhance the cohesiveness of asphalt mixture.

Ghaly[16] has added the Atactic to hot asphalt and combined it with rubber (styrene-butadiene). The results showed an improvements in the rheological properties of asphalt at different temperatures.

The aim of the research

The aim of the study is to achieve two goals:

1. Utilizing natural asphalt as well as expanding its use by assessing its properties and improving it by adding cheap local materials such as cork.

2. Reduce the harmful effect of cork waste on the environment, which is constantly increasing.

Experimental part

Instrumentals

Table 1. shows the instruments used in this work.

Table (1) Devices used in the research.

№	Device	Company	№	Device	Company
1	Penetrometer	The Central Ignition Co.London	5	Digital Water Bath	Nickel Electrro LTD England
2	Fourier Transform Infra-Red (FTIR)	FTIR-8400 SHIMADZU (JAPPAN)	6	Hot Plate with Magnetic Stirrer	Lassco India
3	UV-Visible Spectrophotometer	CARY 100 Conc VARIAN AUSRALIA	7	Sensitive Balance	Sartorius Germany
4	Air Electrical Oven	Engineering Laboratory Equipment Limited.England	8	Hot Plate	Jenway United Kindom

Chemical Materials

1- Hit Natural Asphalt HNA: obtained from the city of Hit 200 Km to the west of Baghdad, Iraq.

2-CCl_4 carbon tetrachloride (95% purity).

3-Cork waste.

. 4-Gasoline to dissolve corke

Procedures

1-Sampling

A sample of HNA was taken and dried in the air to get rid of moisture. A specific weight was taken and placed in the oven for five hours at a temperature of 110 °C until the moisture was completely removed. The loss in heating of the sample was measured before other measurements were made.

2-Preparation of cork paste

The cork was cut into small pieces after being thoroughly cleaned and then dissolved in gasoline for 10 minutes at room temperature. After that, the cork paste

was added to HNA at 0%, 10% and 20% percentages.

Tests 3-

a) Penetrometer. This instrument is used to conduct Penetration test according to ASTM D5 [17].

b) UV. Visible Spectroscopy [18].

c) Infrared Spectroscopy [19].

Results and discussion

1-Penetration Results

Figure 1 shows the positive effect of adding cork to HNA on the results of penetration test. Cork increases the stiffness of the asphalt as can be explained by decreasing the values of penetration from 210 (0.1 mm) at zero percentage of Cork/Asphalt C/A to 95 (0.1 mm) at 20% C/A.

This may be due to the chemical rapprochement between materials (cork and HNA) since both are hydrocarbon substances. This can provide greater adhesion and cohesion between them as well as the occurrence of physical overlap or fusion between the two materials.

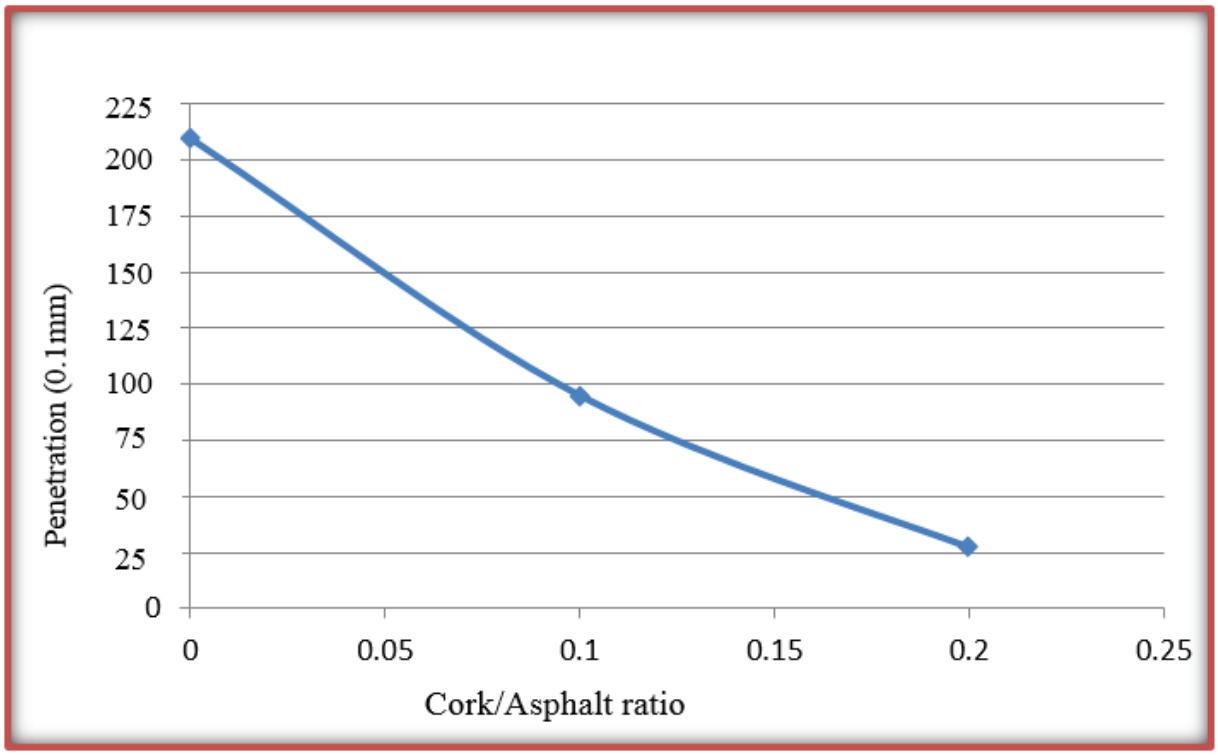


Figure 1. The effect of cork percentage on the penetration of Cork- Asphalt mixture.

Spectroscopy Results:

FT-IR) a) Infra-Red Spectroscopy

The FT-IR spectroscopy for modified HNA samples was performed to determine the location of bond absorption after diluting the models using carbon tetrachloride (CCl_4). The model was then placed on the disk for a period to help the evaporation of the solvent.

After that the measurement was performed [18].

Hit Natural Asphalt HNA **with Cork Paste**

When the cork paste was added to the HNA by 10% and by 20%, the spectra did not show much difference. A slight difference was observed in the model (HNA + 10%, 20% Cork paste). This may be due to the formation of hydrogen bonds between the cork paste and HNA which caused displacement of the positions of the absorption of certain bonds as shown in figures 2 (HNA), figures 3 (HNA + 20% Cork paste) below.

The properties of some of the beams have little effect on the chemical composition of the HNA, which means that there is a physical mixing rather than chemical reaction between the Cork paste and HNA.

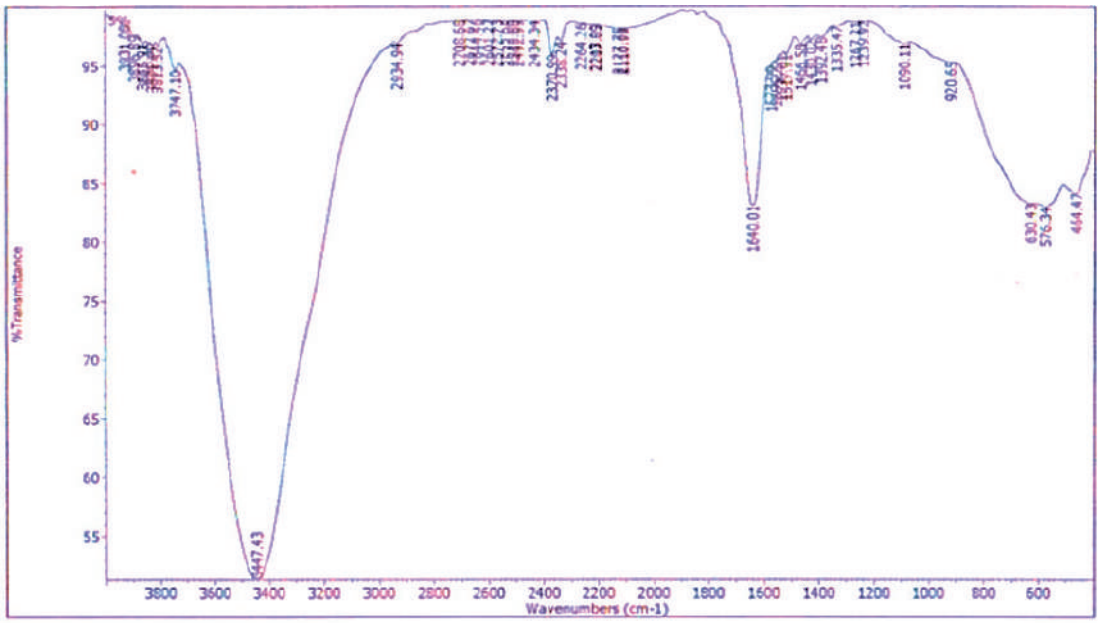


Figure 2. The FTIR absorption spectra of natural asphalt.

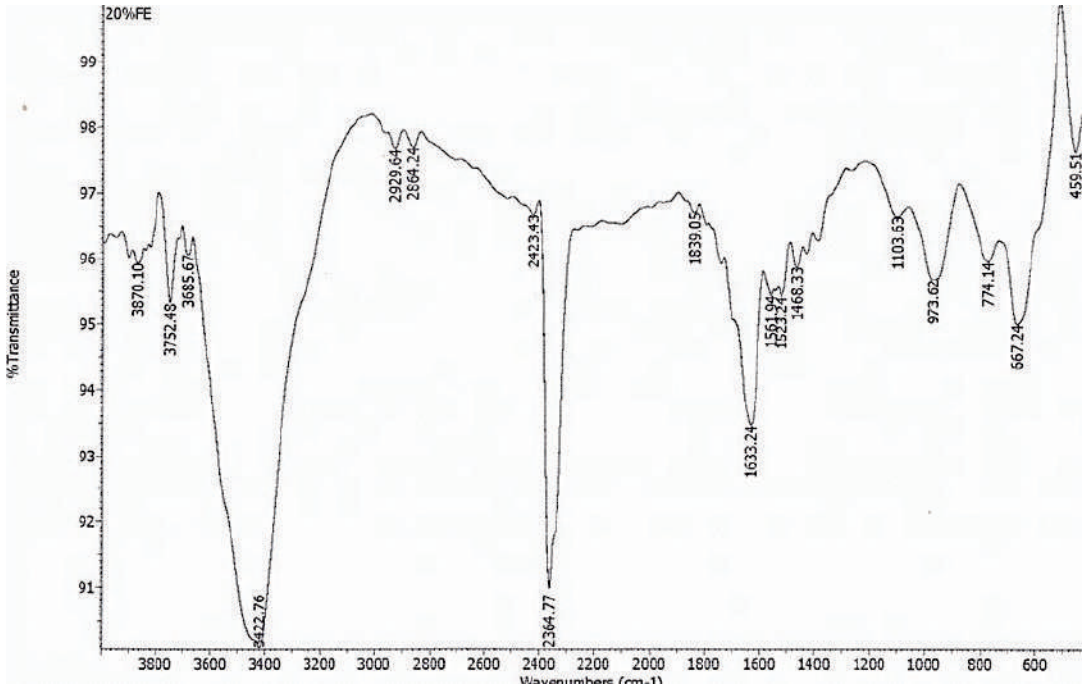


Figure (3) FTIR spectrum for natural asphalt at 20%

UV- Visible Spectroscopy

Hit Natural Asphalt HNA **Results**

The UV spectrum of HNA has two bands:

The first (407.77 nm) was at the absorption of 0.36 This could be due to the excitement type π-π^*.

The second (267.46 nm) was at the absorption of 1.36. This can be due to the excitement type π-π^* as illustrated in figure 4.

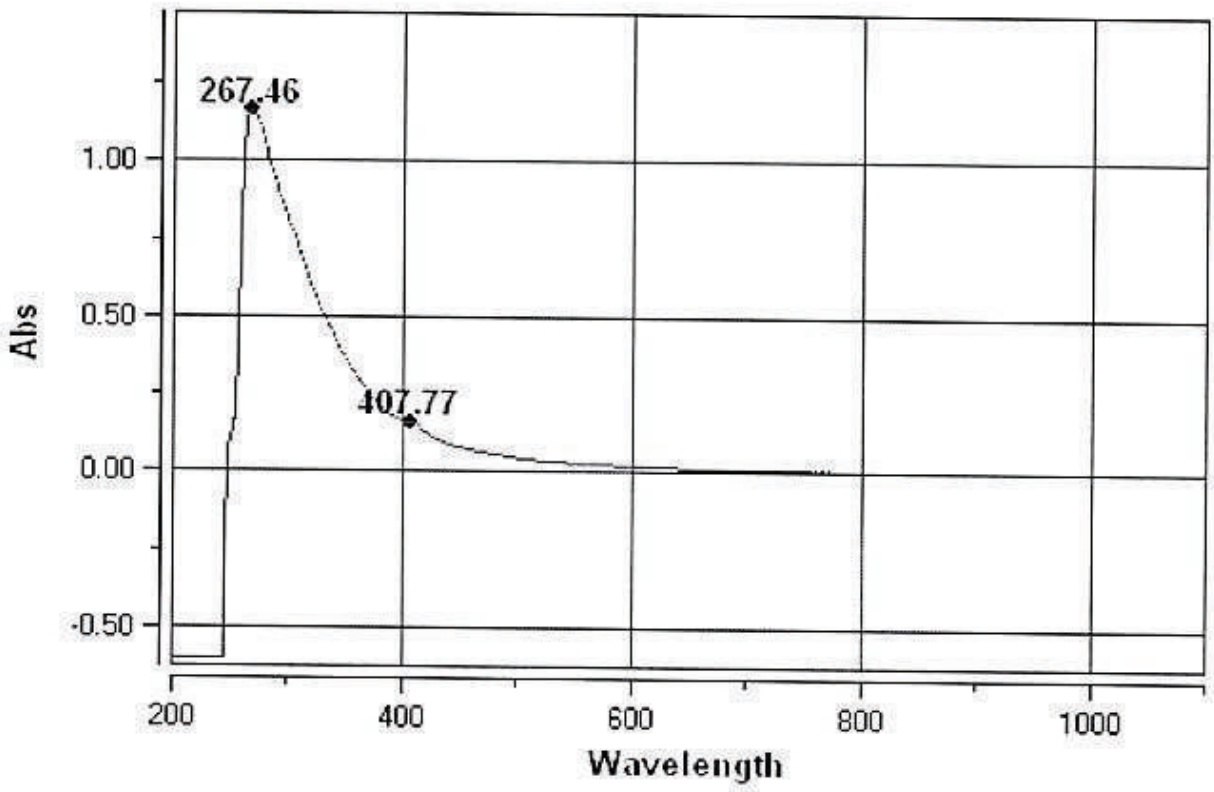


Figure (4) UV absorption spectrum for HNA

Hit Natural Asphalt HNA with Cork Paste Results.

When cork paste was added to HNA at 10% and 20%, the spectrum did not appear to be significantly different from HNA before the addition. The same beams of absorption were shown with the wavelength shift to the longest (red shift) for the transition π-π^* at 268.44 nm . It is also possible to observe a blue shift for the transition from π^*-n at 407.06 nm, i.e. wavelength shift to a lower value. These two displacements (red and blue) are caused by the dissolving of the substance in benzene where a change occurs in the polarization location as shown in figures 5.

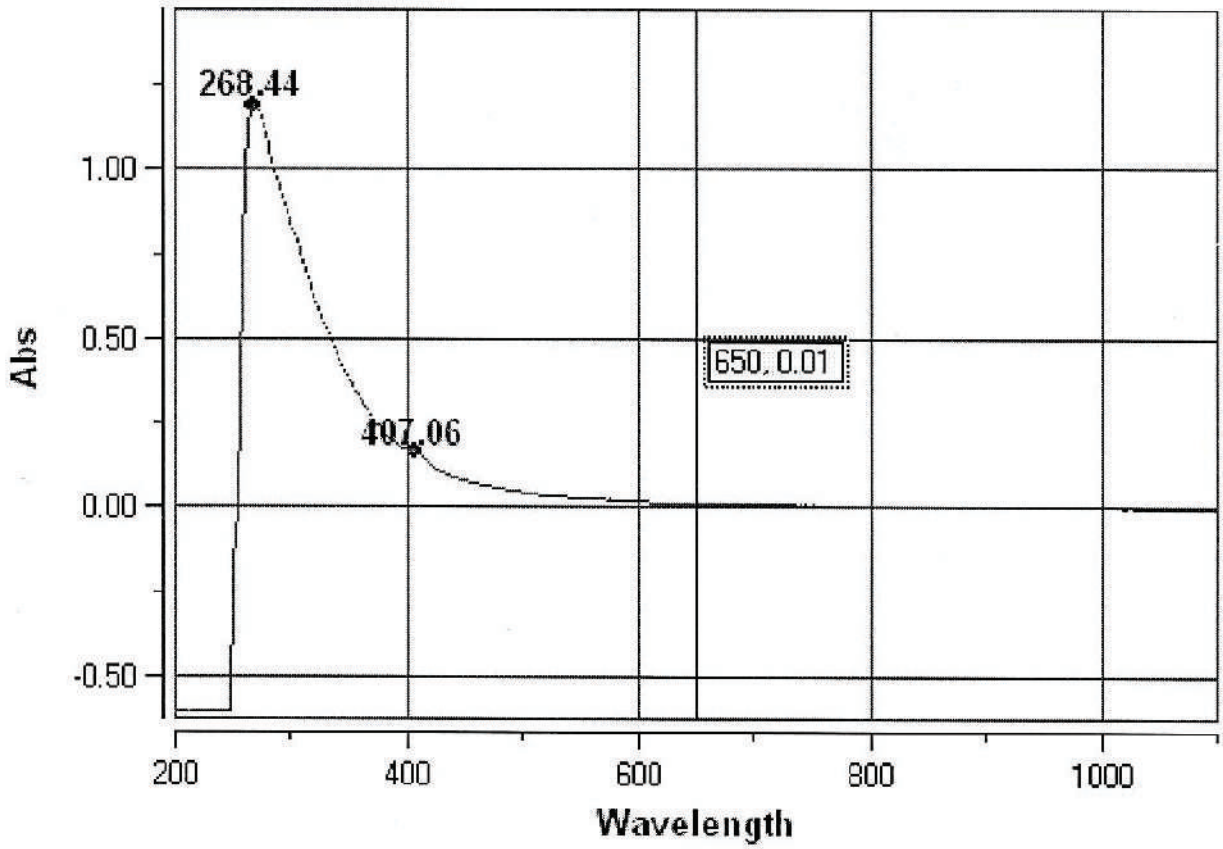


Figure (5) The UV spectrum for cork paste with HNA at 20%

Conclusion

1-The results show that adding the cork paste to HNA at 20% resulted in a new type of asphalt can be used as water proofing materials for the applications in roofing and satisfied both the international standard ASTM D312 and the local Iraqi standard IQ (88-1196) .

2-The new product can be used as a new cheap roofing material.

3-The study clarified that HNA can be used to produce new beneficial material instead of disposal into ground.

4-it can be claimed that it is possible to reduce the harmful effect of cork waste on the environment and humans and can be recycled into a new useful materials by improving natural asphalt.

5- The chemical composition of HNA-Cork mix is not different from HNA chemical composition. This indicates that the improvements in HNA are due to the physical mixing between the components as shown in the results of the spectroscopy tests. This is agreed with the findings of other studies .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Assessment the Effect of *Lactobacillus Acidophilus* on *Escherichia Coli* Serotype O157:H7 with Detection of Some Virulence Factors

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Abstract

Aim: To evaluation the effect of *Lactobacillus acidophilus* on Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 with detection of some virulence factors.

Methods: Two hundred and fifty specimens (stool) from children under five years for both sexes were collected from some hospitals. All isolates were diagnosed according to morphological characteristics, biochemical tests. Monoplex pattern of PCR was used also for detection different genes in (7) *Escherichia coli*)O157:H7 (isolates; include *16SrRNA, eae, lifA, Stx1,Stx2* that encoded for ribosomal RNA, intimin, lymphocyte inhibitory factor, shiga toxins. Three types of probiotics strains were obtained, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus acidophilus* (ATCC4356). Minimum inhibitory concentration (MIC) of cell free supernatants of *Lactobacillus acidophilus* was determined by employing different dilutions (1/2, 1/4, 1/8, 1/16, 1/32), to detect the concentration of probiotic that will inhibit *E.coli* (O157:H7) growth.

Results: Results showed, 210 (84%) samples were identified as *E.coli* from 250 samples. The result showed (7) isolates were identified as Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 and showed all isolates of O157:H7 were positive for *16SrRNA* gene with (213bp) and *eae* with (741bp), *lifA* with (712bp), only *Stx1* gene appeared in all isolates with (446bp) and no bands with *Stx2*. Current result showed only cell free culture supernatant of *Lactobacillus acidophilus* has inhibitory activity against all *E.coli* (O157:H7) isolates with different dilutions (1/2, 1/4, 1/8, 1/16, 1/32), while *Lactobacillus fermentum*, *Lactobacillus plantarum* have no effect against *E.coli* (O157:H7). The result showed the bacteriocin has inhibitory effect against *E.coli* (O157:H7), while organic acids and hydrogen peroxide haven't any role in inhibition. The (MIC) value was (1/8) which inhibits the bacterial growth of isolates.

Key words: *E.coli (O157:H7), 16SrRNA, eae, lifA, STX1, MIC*

Introduction

Enterohemorrhagic *Escherichia coli* O157:H7 is a major foodborne pathogen causing severe disease in humans worldwide. The infections caused by EHEC range from asymptomatic to severe. Chronic renal pathology may persist at times among those that survive. Humans are known to acquire EHEC in multiple ways, for example, contaminated food and water and direct contact with infected animals and humans. Consumption of contaminated food like ground beef, dairy products, and fresh produce is responsible for the majority of the outbreaks [(1)]. The initial reported of EHEC serotype O157:H7 infection linked with hemolytic syndrome (HUS), also it was recognized in China as a reasons of bloody diarrhea, because of contamination of uncooked

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hamburger, emerging public health challenges of Shiga toxin (stx) producing *Escherichia coli* (STEC) include the occurrence of more frequent or severe disease and risk factors shifts associated with changes, often interconnected, in the pathogen, the population, and the environment [2]. Shiga toxin (STX) is the cardinal virulence factor of EHEC, so far, the pathophysiology of EHEC infection is not fully understood and more knowledge is needed to characterize the "auxiliary" factors that enable a STEC strain to cause disease in humans [3]. The most important pathogenic factor of Enterohemorrhagic *E. coli* O157:H7 (EHEC) is its primary attachment with intestinal epithelial cells that leads to A/E process. Since there is a bacteria clean up mechanism in intestinal epithelial cells, bacteria need special attachment system for passing through this barrier, this attachment factor is encoded by eae chromosomal gene which called Intimin. Intimin is the first attachment factor of the bacterium to enterocyte then Tir protein through it transfers to host cell [4,5]. Probiotics are live microorganisms intended to provide health benefits when consumed, generally by improving or restoring the gut flora, Probiotics are considered generally safe to consume, but may cause bacteria-host interactions and unwanted side effects in rare cases. Lactic acid bacteria (LAB), especially *Lactobacillus,* are the most commonly used microorganisms as probiotics, members of lactobacilli are "Generally Recognized As Safe" (GRAS) ingredients, and are desired members of the gastrointestinal tract (GIT) microflora, and contribute mainly in maintaining the GIT homeostasis [6]. From all the information that remembered above, this study was aimed to isolation and identification of *E. coli* O157:H7 from patients with diarrhea, detection the presence of some virulence determinants of *E. coli* O157:H7 and evalution the effect of cell-free supernatant of *Lactobacillus acidophilus* against Enterohemorrhagic *Escherichia coli* serotype O157:H7.

Materials and Methods

Isolation and Identification:

From (October 2018) to (February 2019) two hundred and fifty specimens (stool) from children under five years for both sexes, were collected in sterilized containers from hospitals. The diagnosis of *E.coli* was achieved according to their morphological properties on MacConkey agar, EMB medium, Sorbitol MacConkey agar, chrome agar O157, Oxidase production, Catalase production, Methyl red test, Indole production, Urease production, Vogeus Proskauer tests, Kligler iron agar and Citrate utilization, motility test [7].

Molecular study

DNA extracted and purified carry out using genomic DNA purification kit protocol (Geneaid Extraction Kit). From each DNA extracted sample, 2 µl was added to the specialized measuring lens of the Nano- drop system after swabbing the lens with D.W. wetted cotton swab to measure the concentration and purity of extracted DNA sample at 260 nm and 280 nm. The results were recorded computerization**.** The specific primers were designed according to Bio edit program and NCBI BLAST **(Table 1)**. The extracted DNA, primers and PCR master mix were mixed together. PCR mixture was set up in a total volume of 20µL included 5µl of PCR Green master mix,1µL of each primer, and 2µL of template DNA have been used, the rest volume was completed with sterile de-ionized distilled water, then vortexed. The PCR program includes 35 cycles were carried out: Initial denaturation at 95 ºC for 5min, denaturation 95 ºC for 30sec, extension 72 ºC for 40sec, final Extension 72 ºC for 5min, and annealing stage are changing, *16srRNA* at 59.2ºC for 30sec, *eae* at 58.5ºC for 40sec, *lifA* at 58ºC for 30sec, *Stx1* at 61.6ºC for 30sec. The PCR products were analyzed by using 2% agarose gel electrophoresis in 100 ml of 1x TBE buffer and melted.

Table 1: The primers and their sequences used in conventional PCR

	Primer name	Sequence 5' 3'	Product length	Origin
1.	16SrRNA	F:GATGACCAGCCACACTGGAA R:GGAGTTAGCCGGTGCTTCTT	213pb	New
2.	eae	F:GGGCGGTCAGATTCAGCATA R:CCATCACTGACTGTCGCACT	741bp	New
3.	LifA	F:TGGTCGGAGTCGTCCAGTAT R:GGACGATGACCGATTTTGCG	712bp	New
4.	Stx1	F:GTGTTGCAGGGATCAGTCGT R:GACTCTTCCATCTGCCGGAC	446bp	New
5.	Stx2	F:TCCGGAAGCACATTGCTGAT R:CCACCGGGCAGTTATTTTGC	500bp	New

Isolation and identification of *Lactobacillus spp.*

Three types of probiotics strains were obtained, *Lactobacillus fermentum*, *Lactobacillus plantarum* from department of biology and *Lactobacillus acidophilus* from (Holisherb-USA), *American Type Culture Collection* (ATCC4356). Samples diluted with normal saline by adding 1ml of N.S, and mixing for 5 minutes. Samples cultivation on MRS broth and incubated anaerobically at 37°C for 48 hrs. A loop full of the cultured broths was streaked on MRS agar plates and incubated under the same conditions. These bacteria cultivation on MRS broth and incubated anaerobically at 37°C for 48 hrs.

Antimicrobial activity of *Lactobacillus* isolates

Antimicrobial activity was carried out according to the agar well diffusion assay as described previously [8]. Diarrheagenic *E. coli* were cultured in nutrient broth for 24 hours, and then cultured on *nutrient agar*. *Lactobacillus* isolates were grown in MRS broth for 20 hours, cell free culture supernatants (CFCS) were obtained by centrifuging the culture broth at 10000 g for 10 minutes, 100 µL of the CFCS was placed into the wells of the nutrition agar and the nutrition agar plates were incubated at 37°C for 14 - 15 hours with sterile MRS broth was used as negative control**,** the diameter of the clear zones around each well was measured.

Characterization of antimicrobial substances produced by *Lactobacillus* and determination of minimum inhibitory concentration (MIC)

The supernatant was aliquoted into five tubes: First tube was treated with 1 mg /mL trypsin to determine of bacteriocine production, second tube was adjusted to pH 6.5 ± 0.1 with NaOH, third tube was treated with 0.5 mg /mL catalase for 30 min at 25 °C to determine hydrogen peroxide production, fourth tube was adjusted to pH 6.5 ± 0.1, treated with catalase and treated with trypsin, fifth tube was used as positive control (non-treated). Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The experiment was carried out as follows: Serial dilutions were made from to several dilutions (1/2, 1/4, 1/8, 1/16, 1/32) of supernatant with sterile brain heart infusion broth within sterile tubes to complete the volume to (2ml). Each tube was inoculated with 200µl of 0.5 McFarland pathogenic *E.coli.* The tubes were incubated for 24hrs, at 37℃. Control tubes divided to positive control contain of broth with bacterial inoculum and negative control contain of broth only.

Results and Discussion

Results of the present study recorded, 210 (84%) samples were identified as *E.coli* from 250 samples. result showed (7) isolates were identified as Enterohemorrhagic

E. coli O157:H7. according to culturing on Chrome agar O157, the isolates gave mauve colonies. Results of the present study revealed that the highest percentage of *E.coli* isolates was in stool samples (84%) compare to other clinical isolates. The reason of differences in the isolate percentages may attributed to differences in season of collecting samples as well as used multi antibiotic that lead to confuse in isolated, also difference in the environment and the living area, as well as main reason, *E. coli* is a most type of bacteria that normally live in the intestines of people and animals [9]. Current study recorded (7) isolates were identified as (EHEC) serotype O157:H7, in children under five years, and agreement with other study when show that 40 children with diarrhea were examined, 10 cases were diagnosed as pathogenic *E.coli* [10]. Current study demonstrated the bloody diarrhea caused by *E. coli* O157:H7 under five years, patients' selection was restricted to those who had bloody diarrhea because *E.coli* O157 is mostly associated with this clinical feature.

Molecular study

The results revealed that the concentrations of DNA ranged from (88-450) ng/µl, while purity ranged from (1.8 - 2). Current results demonstrated that (100%) of Enterohemorrhagic *E. coli* O157:H7 isolates**,** had *16SrRNA*, *eae, lifA, stx1* with 213bp, 741bp, 712bp and 446bp, rrespectively, while no bands with *stx2* in all isolates, figures (1-4)**.** Sequencing technique has several advantages over phenotypic and biochemical identification. One of the advantages of using the *16SrRNA* gene analysis is that this gene is present in all bacteria; the lack of extensive mutations in this gene is another advantage of using this type of analysis; isolated by using *16SrRNA* is more accurate than bacteriological and biochemical assays[11]. Current study compatible with study in Northern Ireland about four-year 1997–2000, demonstrated that 80% of *E.coli* O157 isolates possessed the *eae* gene [12]. The results are compatible with previous study in University of Baghdad, Iraq; include, isolated 32 isolates of *E. coli* recovered from 350 fecal samples, and showed aproximately, (60%) isolates gave positive results with stx1primer [13]. Other results for a second study in Iraq, Basrah city, this study includes detection of the prevalence of Enterhemorrhagic *Escherichia coli* O157:H7and detection of *Stx1, Stx2,* results demonstrated, all the *E. coli* O157:H7 isolates were positive to *stx1* gene which was observed in 100% of isolates but none of the isolates were detected to having a *stx2* gene [14]. The production of shiga toxin (Stx) is a critical step in the establishment and progress of enterohemorrhagic *Escherichia coli* (EHEC) infections. The possible release of Stx from dead and dying bacteria, and the risk of resistance development have restricted the usage of antibiotics against EHEC. The pathogenesis of EHEC infections is associated with the production of Shiga toxins that are similar to the Shiga toxin that is produced by *Shigella dysenteriae* type 1 [15]. Enterohemorrhagic *E.coli* has *lifA* gene that act to inhibition lymphocyte, with strongest statistical association with diarrhea. Current results with high significant percentage of strains have *lifA* gene compatible with study in 2010,when demonstrated a strong statistical association between the presence of *lifA*-positive pathogenic *E.coli* and the presence of diarrhea that suggesting *efa1/lifA*-positive strains may be true diarrhea pathogens, and we observed positive strain are more causing severe diarrhea than other strains.

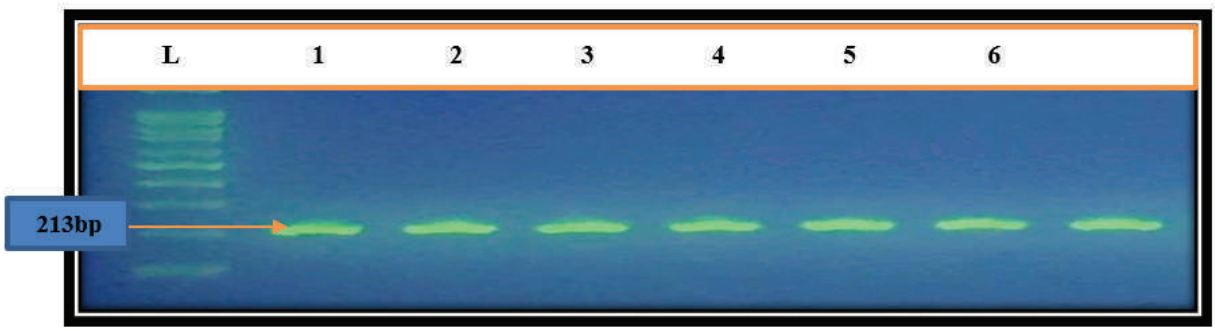


Figure (1): Gel electrophoresis of amplified *16sRNA* (213bp) in *E. coli* isolates on agarose (2%), TBE buffer (1x), 70 volt for 1 hrs. stained with red safe L: DNA ladder (100 bp).

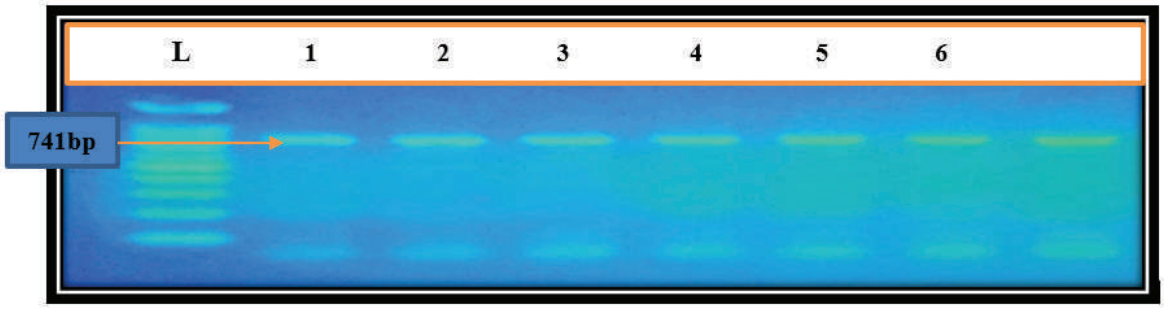


Figure (2) Gel electrophoresis of amplified *eae* (741bp) in *E. coli* isolates on agarose (2%), TBE buffer (1x), 70 volt for 1 hrs. stained with red safe L: DNA ladder (100 bp); Lanes 1-8were positive.

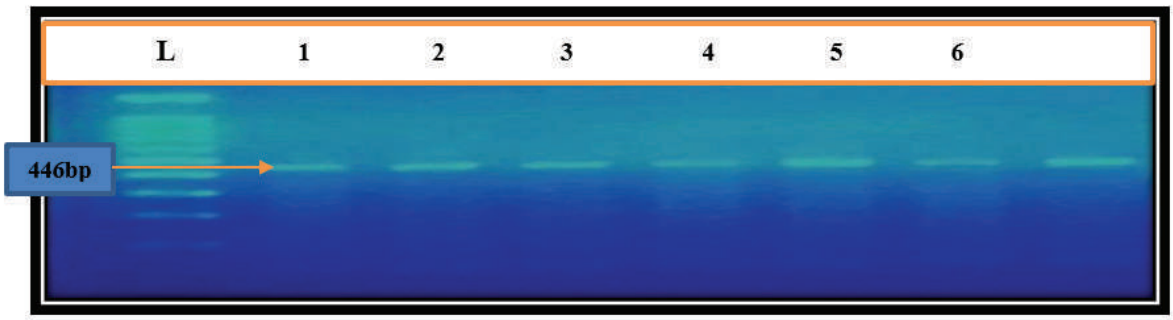


Figure (3): Gel electrophoresis of amplified *STX1* (446bp) in *E. coli* isolates on agarose (2%), TBE buffer (1x), 70 volt for 1 hrs. stained with red safe L: DNA ladder (100 bp).

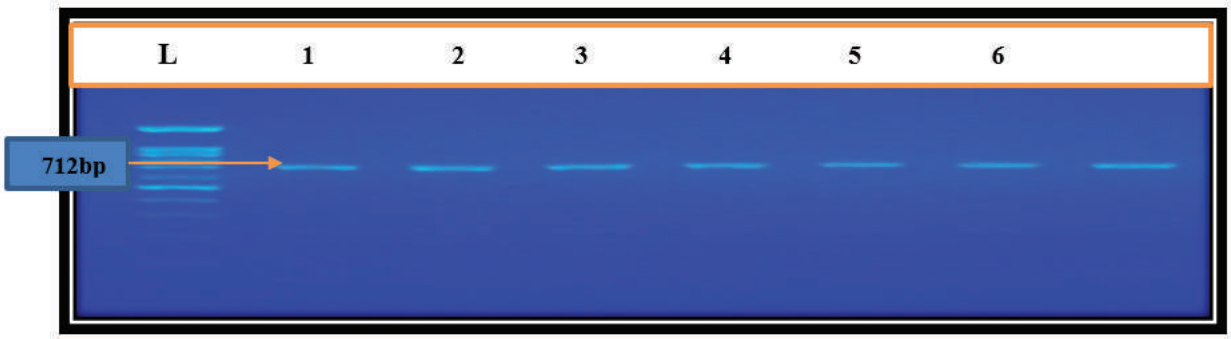


Figure (4): Gel electrophoresis of amplified *lifA* (712bp) in *E. coli* isolates on agarose (2%), TBE buffer (1x), 70 volt for 1 hrs. stained with red safe L: DNA ladder (100bp); Lanes1-7 were positive isolates.

Isolation and identification of *Lactobacillus spp.*

Three types of probiotics strains were obtained, *Lactobacillus fermentum*, *Lactobacillus plantarum* from University of Baghdad with confirmed identification (Sequencing 99% identify) and *Lactobacillus acidophilus* from (Holisherb-USA), *American Type Culture Collection* (ATCC4356). Pellet diluted with normal saline by adding 1ml of normal saline, and mixing for five minutes, all the samples were cultured primarily in MRS broth at 37ºC for 48 hr. All positive isolates on MRS agar were tested for several biochemical tests.

Antimicrobial activity of *Lactobacillus* isolates

Antimicrobial activity of the cell free culture supernatants (CFCS) was tested against the all pathogenic bacteria. Result showed only cell free culture supernatant of *Lactobacillus acidophilus* has inhibitory activity against all (E.coli O157: H7) isolates with

different dilutions (1/2, 1/4, 1/8, 1/16, 1/32), and the effect was differ according to isolates and concentration, while current result showed no effect by , *Lactobacillus fermentum*, *Lactobacillus plantarum* against pathogenic *E.coli.* Current study compitable with study that demonstrated *lactobacillus* have a mild inhibitory activity against the diarrheagenic *E. coli* and these strains may be useful as probiotic candidates in prevention of intestinal infections caused by diarrheagenic *E. coli* [16].

Conclusion

E. coli O157:H7 isolates were found Less than five years of patients, which have the ability to produce *Stx1* gene, and the cell-free supernatant of *Lactobacillus acidophilus* can inhibit the bacterial growth of *E. coli* O157:H7.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: mThe authors declare that they have no conflict of interest.

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Effect of Conocarpus Erectus Extraction on Some Physiological and Biochemical Parameters on Male Rats

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Abstract

The present study was conducted to evaluated some of physiological and biochemical parameters ,we used eighteen of males rats divided randomly to three group (six in each), group one considered as control ,group two the animal treated with 250 mg /kg and group three treated with 500 mg /kg .The result show non-significant appeared in RBC , Hb, PCV ,MCH , MCV and MCHC ,while the WBC , lymphocytes and granular show increased significantly . The biochemical test show increased significantly in liver enzyme concentration ALT ,AST and ALP also the result of lipid profile appeared decreased in al parameters exception the HDL which appeared increased significantly .

Keyworlds ; *Conocarpus Erectus ; Liver enzyme ;lipid profile.*

Introduction

From ancient time till now and tomorrow, the use of natural resources especially plants increases day by day for the discovery of new therapeutic agents, natural products from some of these natural resources continue to be used in pharmaceutical preparations either as crude extracts, fractions, pure compounds or analogous compounds from highly active isolated compounds[1]. Plant-derived substances have recently become of great interest owing to their versatile applications medicinal plants are the richest bio-resource of drugs of traditional medicines, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs[2,3] Conocarpus erectus, a member of combretaceae family, is widespread in tropical and subtropical areas, which is found in the form of a shrub,1.5 to 4 m tall [4], however it sometimes can grow up to 20 m. Conocarpus erectus L. (Combretaceae), known in English as buttonwood or button mangrove, is one of two species in the genus Conocarpus. The plant is spreading crown, grey or brown bark, glaucous medium-green leaves and greenish flowers in dense cone-like heads in terminal panicles[5]. It is a folk remedy for anemia, catarrh, conjunctivitis, diabetes, diarrhea, and fever ,the buttonwood tree, Conocarpus erectus, has not been investigated previously for its biological activity or for its constitutive phenolics ,due to the interest in the biological activity as well as the diverse plant bioactive metabolites[6,7,8] an investigation of the phenolics of the leaves of C. erectus was undertaken. Thirteen phenolics (1-13), including the new natural product 3,3′,4′-tri-O-methylellagic acid 4-O-β-glucupyranuronide have been isolated and purified from the aqueous alcohol extract of the leaves of this plant. all structures were confirmed by NMR analysis , in addition, the antioxidant potential based upon the principle of xanthine/ hypoxanthine oxidase assay of the plant leaf extract, the new compound together with the glucuronides, quercetin 3-O-glucupyranuronide, myricetin 3-O-β-glucupyranuronide 3′,5′-dimethoxymyricetin3-O-β-glucupyranuronide, syringetin 3-O-β-glucupyranuronide and gallic acid

Aim of Study

For lack of adequate study of the plant Conocarpus erectus, the study design to evaluate the efficacy of Conocarpus erectus extract on some physiological and biochemical parameters in male rats.

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Material and Method

1-Collection and Preparation of the Plant

The leaves of the plant under investigation were collected from some farms in basra city at Iraq. The leaves was collected and shade dried then finely powdered by electrical grinder , and kept in Dark glass containers and become ready for extraction process.

2- Preparation of Conocarpus erectus extract

Conocarpus erectus were cleaned, washed and dried at room temperature. Leaf were grounded for 2 minutes by electrical grinder. The leaf powder were refluxed with 250 ml (ethanol 70%) for 12 hours by Soxhlete, and then filtered by using Buchner funnel and filter paper . The solvent was dried and concentrated by using rotary evaporator at 50ºC. The final dryness was done by leaving residue in room temperature .

Experiments design

Eighteen of male rats about 3 months age and 200 – 250 weight were used in this experiment. The animals were divided randomly into three groups (6 rats for each group) as following :

Group one (control): In which rats were given 0.5 ml of normal saline by gavage tube daily for three weeks . .

Group two :- The rats were given 250 mg /Kg /Bw of Conocarpus erectus extract by gavage tube daily for three weeks .

Group three : The rats were given 500 mg /Kg /Bw of Conocarpus erectus extract by gavage tube daily for three weeks .

Collection of blood samples.

Blood sample (5 ml) were collected from heart puncher , after anaesthetised the rats with chlorophorme . The(2 ml) of blood collected from each animal were stored in plastic sample test tube containing ethylene diamine tetra acetic acid (EDTA) anticoagulant for hematological studies which done directly after collection , while another portion (3 ml) of blood was deposited in to tube without anticoagulant and allowed to clot at room temperature . Then the blood samples were centrifuged at (5000 rpm) for 30 minutes and serum sample were stored in polyethylene tubes at (– 20c) until used for biochemical analysis .

Hematological Study

The hematological tests were done in the laboratory by using Hematology auto analyzer (Huma Counts 5) made in Germany company serial no.160247 . the instrument can measures and calculates 22 different parameters .The Hematology auto analyzer containing four solution(HC5 – BASOLYSE containing cyanide free lyse reagent ,HC-LYSECF containing cyanide free lyse reagent ,HC5 – EOLYSE containing cyanide free lyse reagent and HC- Cleaner cleaning solution used to clean fluidics system) and the instrument have a printer mechanic inside with thermal paper . The hematological parameter estimated by this instrument were (RBC, WBC. DWBC ,Hb , PCV ,MCV ,MCH and MCHC)

Biochemical test

The biochemical tests were done in the laboratory by using chemistry auto analyzer made in Germany by human star company serial no.20628 ,the machine has 54 wells which numbered from 1 to 54 , The serum samples deposited in each specific wells . The reagent was put in a special container beside the wells. The serum biochemical parameters estimated by this instrument were lipid profile TG , TC ,HDL , LDL , AST, ALT, and ALP .

Results

1-Blood parameters

The results in table (1) revealed that conocarpus erectus extract administration to male rats in two doses 250 mg/kg/Bw and 500 mg/kg/Bw caused non significant in RBCs counts , hemoglobin concentration, hematocric ratio, MCV, MCH and MCHC when compared with control group.

Table (1): Effects of Conocarpus erectus extract on RBCs , Hb , PCV, MCV, MCH and MCHC . n=6

	RBC (1x106 mm)	Hb g/L	PCV %	MCV fL	MCH pg	MCHC g/L
Control group	7.55 ± 0.23 N.S	12.42± 0.90 N.S	37.1± 0. 25 N.S	57.17 ±3.35 N.S	18.42 ±0.96 N.S	30.8 ±2.18 N.S
250 mg/kg/Bw	6.31 ± 0.18 N.S	12.2 ± 0.22 N.S	37.5 ± 0.34 N.S	59.6 ± 2.14 N.S	19.7 ± 0.23 N.S	33.3 ± 1.25 N.S
500 mg/kg/Bw	6.62 ± 0.44 N.S	12.4 ± 0.51 N.S	38.4 ± 0.12 N.S	57.4 ± 2.89 N.S	18.3 ± 1.39 N.S	32.3 ± 2.10 N.S

The results in the present study table (2) showed that total leucocytes count and lymphocytes percentage increased significantly (P<0.05), when the animal treated with Conocarpus erectus extract compared with control group . On the other hand, neutrophil , eosinophil and basophil percentages were increased significantly (P<0.05),in all treated group .

Table (2): Effects of Conocarpus erectus extract on WBC , Lymphocytes and Granulocytes . n=6

	WBC(1x103 mm)	Lym	Gra.
Control group	5.84 ± 0.71 c	51.82 ± 3.23 c	31.80 ± 2.90 b
250 mg/kg	16.40 ± 3.62 a	64.30 ± 1.72 a	32.82 ± 2.24 b
500 mg/kg	12.60 ± 2.20 b	56.52 ± 3.41 b	40.11 ± 2.43 a

Different letters indicate significant difference at (P≤0.05)

Clinical chemistry data

The baseline data for clinical chemistry parameters collected from three group are shown in Table (3) . The result showed increased significantly (P<0.05),in AST enzyme especially in low dose 250 mg /kg compared with control ,also the same table appeared increased significantly (P<0.05),in ALT and ALP in two doses of conocarpus erectus extract compared with control group.

Table (4) showed decreased significantly (P<0.05) in total cholesterol , triglyceride and LDL in all treated group especially in low dose 250 mg /kg compared with high doses 500 mg /kg and control group ,while the HDL concentration in contrast appeared increased significantly (P<0.05) compared with control group .

Table (3): Effects of Conocarpus erectus extract on AST , ALT and ALP enzyme concentration . n=6

	AST(U/L)	ALT(U/L)	ALP (U/L)
Control group	45.64 ± 1.77 c	28.13 ± 1.26 b	55.7 ± 6.24 c
250 mg/kg	120.5 ± 10.20 a	48.6 ± 6.75 a	212.6 ± 13.62 b
500 mg/kg	96.7 ± 9.54 b	51.9 ± 4.83 a	244.6 ± 15.91 a

Different letters indicate significant difference at (P≤0.05)

Table (4): Effects of Conocarpus erectus extract on TC , TG, LDL and HDL concentration . n=6

	TC(mg/dl)	TG(mg/dl)	LDL (mg/dl)	HDL(g/ml)
Control group	119.1±6.13 a	18.7±9.6 a	46.15±8.57 a	37.3±1.90 c
250 mg/kg	63.6 ± 3.05 b	7.3 ± 1.10 b	28.3 ± 2.16 c	41.9 ± 1.10 b
500 mg/kg	59.3 ± 3.16 b	5.6 ± 0.25 c	35.6 ± 2.10 b	52.7 ± 1.20 a

Different letters indicate significant difference at (P≤0.05)

Discussion

This study was designed for examining toxicity of methanolic extracts of Conocarpus erectus herb , due to lack of resources found, we decided to study the chronic toxicity by giving daily for three week of two dose of extracts 250 and 500 mg /kg and observe the physiological and biochemical changes that appear on the laboratory animal during the administration.The results observed in the present study as represented in the Table (1) show no significant appeared in RBC count , Hb concentration , pcv percent , MCV, MCH and MCHC on male rats treated 250 and 500 mg/kg /BW of ethanol conocarpus erectus extract ,while the result of total WBCs count and differential leukocytic count as represented in table (2) has been shown significant increase in mean total WBCs count and lymphocyte following administration of Conocarpus erectus extract for three week that maybe due to the extract caused increasing stress may have resulted from the oxidative damage caused by ROS generated in response to stress which lead to increase in WBC count .

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified[9]. Serum liver enzyme significantly increased in blood serum AST ,ALT and ALP table (3) that maybe due to Plant possessed significant phytotoxic activities ,these properties may be due to the presence of flavonoids and phenols[10] . Also there was hepatotoxicity seen as great damage in interstitial component of cell like mitochondria caused by conocarpus erectus extract itself or reactive metabolites which can trigger oxidative stress lead to severe ATP depletion and finally cells necrosis and there are many signs pointed to the amount of Alkaloids which have major bioactive compounds in Conocarpus erectus herb may have slightly accountable for the defect present. In this study, the mainly defect or tissue changes have

emerged in high doses, there is clear sign there was a relationship between a number of toxic substances found in the plant and the extent of the existing poisoning in main organ like liver[11]. Also maybe the phenolic components of these plant play a weak role in cytotoxic activity and there is another natural product classes may be saponin compound play a part in cytotoxic activity [12].

The result indicated that Conocarpus erectus treated rats caused many change in lipid profiles like significant decreased in the level of cholesterol ,triglyceride ,Low density lipoprotein in two doses especially in low dose while the high density lipoprotein appeared increased significantly in two doses ,That maybe due to the Conocarpus erectus containing phenolic compounds are only responsible for the antioxidant activity and not only responsible for anticancer properties but it can be play indirect part as prevention by protects cells from the damage caused by free radicals. Many studies have revealed that intake of natural antioxidants is correlated with low incidence of cancer, heart disease, diabetes, and other diseases[12] .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Synergistic Effect of Amikacin and Ciprofloxacin on *pelA* and *algD* Genes in *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is one of the most important pathogen in both hospital and community-acquired infections, it is a classical opportunistic pathogen, and this organism does not normally attack healthy tissue, and rarely causes infection in the general population. It is possess various virulence mechanisms to adhere, colonize and biofilm formation. Samples were collected from different hospitals in Baghdad, samples were collected and categorized in to two main groups: eighty clinical samples and forty as an environmental samples. Forty one isolates were identified as *P. aeruginosa* by using conventional methods and confirmed by VITEK-2 compact system, biofilm were detected by three methods: congo-red agar, tube method and microliter plate. Synergistic Effect of Amikacin and Ciprofloxacin on biofilm formation were detected phenotypically by using microliter plate and genetically by Real-time PCR for *pelA* and *algD* Genes which are responsible for biofilm formation. The results shows an obvious differences in biofilm formation phenotypically and genotypically for the treated isolates.

Keywords: *Pseudomonas aeruginosa; PelA; algD; biofilm; RT-qPCR.*

Introduction

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen capable of causing several infections, mostly in immunocompromised or critically ill patients, including pneumonia, sepsis, bacteremia, and wound and skin infections [(1)]. *P. aeruginosa* infections can be very serious and life-threatening, mostly with the emergence of drug-resistant strains, one of the most worries about human health today is antimicrobial resistance. *P. aeruginosa* is one of the most prevalent nosocomial pathogens, it's responsible for 57% of total hospital-acquired infections[(2)].

One of the most primary characteristic of *P. aeruginosa* chronic infections is biofilm formation [(3)]. A biofilm is a bacterial community that attached to a surface or substratum, typically made of densely packed, multi-species populations of cells. The main feature of biofilms is the presence of highly hydrated extracellular polymeric substance (EPS), including proteins, polysaccharides, and extracellular DNA (eDNA) [(4)]. *Pseudomonas aeruginosa* biofilms are generated through a process whereby the bacterial cells are surrounded to form an aggregated structure, which exhibits increased resistance to antibiotics and other anti-infection agents [(5)]. Therefore, infections caused by biofilm-forming *P. aeruginosa*, such as in cystic fibrosis of the lung, are almost impossible to eradicate, and additional challenges are encountered when treating infections caused by multidrug-resistant strains. These complications lead to increased patient morbidity and mortality, higher costs of treatment, and greater rates and time of hospitalization, diverse strategies are being pursued to develop novel agents that can kill (new antibiotics) or disarm (antivirulence) the pathogen [(6)]. Exopolysaccharide is a critical biofilm matrix component, so the process of biofilm maturation is namely the production of the exopolysaccharides via the polysaccharide synthesis locus (Psl), pellicle formation

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(Pel) and alginic acid synthesis in *P. aeruginosa* [7].

Materials and Methods

Specimens' Collection:

Eighty different clinical specimens were collected from patients referring different hospitals in Baghdad and forty environmental samples were collected from the same hospitals, the collected specimens were ear, burn, urine, sputum, wound and Bronchoalveolar lavage (BAL).

Bacterial Isolation and Identification:

All the collected samples were cultured by streaking on cetrimide agar, macConkey agar and on blood agar, all samples incubated for 24 hours at 37ºC. *P. aeruginosa* colony characters appears on cetrimide agar plates was mucoid, smooth in shape with flat edges and elevated center, have fruity odor and creamy color. Moreover, it appeared as pale greenish and lactose non-fermenter colonies when cultured on macConkey agar plates, while it appeared they appeared as large, opaque irregular colonies with *B*-hemolytic colonies when cultured on blood agar.

Colonial morphology on blood agar, macConkey agar and cetrimide agar was depended initially to identify bacterial isolates, colony shape, texture, colour and edges were examined. In addition to macroscopic characteristics; microscopic examination of a gram-stained slide was examined under a light microscope with special regard towards cell shape and arrangement. Performing conventional biochemical tests and VITEK 2 compact system were depended to complete the identification.

Biofilm Formation

Congo red method:

Bacterial ability to produce slime layer and biofilm formation was detected using Congo-red agar medium (CRA). A positive result was indicated by black colonies with dry crystalline consistency while pink colonies considered as non-slime producers. A darkening of the colonies but with the absence of a dry crystalline colonial morphology indicated an indeterminate result [8].

Tube method (Christensen's method):

Isolates were inoculated in glass test tube which contained (8 ml) BHI broth with 1% glucose and incubated at 24 h at 37°C. The sessile isolates of which biofilm forming isolates adhered to glass tubes are stained with safranin for 10 min, after planktonic cells are discharged by rinsing twice with phosphate-buffered saline (PBS). Then, safranin-stained glass test tube is rinsed twice with PBS to discharge stain. After air drying of test tube process, the occurrence of visible film lined the walls, and the bottom of the tube indicates biofilm production [9].

Microtiter plate method:

To study the ability of biofilm formation, isolates selected from different sources (2 wounds, 4 burns, 1 urine, 1 ear and 2 environmental) depending on sensitive and resistance to antibiotic aminoglycoside such as amikacin and fluoroquinolones such as ciprofloxacin. Quantification of biofilm formation by *P. aeruginosa* was assessed as described by [10].

Determination of Minimal Inhibitory Concentration (MIC) of Amikacin and Ciprofloxacin:

To determine the lowest concentration of antibiotics that inhibit the growth of the microorganism VITEK 2 compact system were used.

The MIC for each bacterial isolates was interpreted as the lowest concentration showing no growth. The results were compared with standard breakpoints values; sensitive (≤ 2 µg/ml), intermediate (4-8 µg/ml) and resistant (≥ 16 µg/ml) according to CLSI (2016).

Synergistic effect of sub-MIC of Amikacin and Ciprofloxacin on biofilm formation:

The lowest concentration of Amikacin and Ciprofloxacine that inhibit the visible growth of the microorganism was assessed by using VITEK 2 compact system. A volume 100 µl of double strength Brain heart infusion broth was added to wells from 1 to 12 of sterile 96-well U shaped-bottom polystyrene microplates. Afterwards, 50 µl of each antibiotics MIC used were mixed together to reach a total volume of 100 µl were added to wells from No.1 to No.11. All wells were inoculated with 10 µl of bacterial suspension

comparable to McFarland standard No.(0.5) (1.5×10^8 CFU/ml) except for the well No.11, which considered as negative control. Whereas well No. 12 considered as positive control. The microtiter plate was incubated at 37°C for 24 h.

Gene expression:

In order to test the synergistic effect of Amikacin and Ciprofloxacin on gene expression of *pelA* and *algD*, eppendorf tubes were used, and each tube contains double amount of the antibiotic and broth that used in the previous section and then twenty tubes were incubated under aerobic conditions at 37°C for 24 hrs. RNA were extracted by using TRIzol™ Reagent according to the protocol described by the manufacturer.

Quantitative reverse transcription-PCR (qRT-PCR):

In order to assess the gene expression of *pelA* and *algD* gene; though, results were normalized using *gyrA* (Housekeeping gene). Primers of these genes (listed in table 1) were provided in a lyophilized form and dissolved in sterile nuclease-free water to give a final concentration of 100 pmol /µl. Afterward, they were stored in a deep freezer until used in qPCR. The reaction mixture was summarized in table (2). Moreover, after several trials, thermocycler protocol was optimized and the resultant protocol is listed in tables (3).

Table (1): Primers utilized in this study

Primer name		Sequence (5´-3´)	Product size	Reference
PelA	F	CCTTCAGCCATCCGTTCTTCT	118 bp	(Colvin et al., 2011)
	R	TCGCGTACGAAGTCGACCTT		
algD	F	ATGCGAATCAGCATCTTTGGT	1310 bp	(Stover et al., 2000)
	R	CTACCAGCAGATGCCCTCGGC		
gyrA (HouseKeeping gene)	F	CACCGCCGTGTGCTTTATG	843 bp	(Colvin et al., 2011)
	R	GGGTCTGGGCATAGAGGTTG		

Table (2): Components of qRT-PCR used in *pelA* and *algD* genes expression

Master mix components	Volume (µl)
qPCR Master Mix	5
RT mix	0.25
MgCl2	0.25
Forward primer	0.5
Reverse primer	0.5
Nuclease Free Water	2.5
RNA	1
Total volume	10
Aliquot per single rxn	9 µl of Master mix per tube and add 1µl of Template

Table (3): **qRT-PCR protocol of primers *pelA***

Step	Temperature (°C)	Duration	Cycles
Reverse transcription (RT). Enzyme activation	37°C	15 minutes	1
Initial Denaturation	95°C	5 minutes	1
Denaturation	95°C	20 seconds	40
Annealing	45°C	20 seconds	
Extension	72ºC	20 seconds	

Result and Discussion

Isolation and Identification of *Pseudomonas aeruginosa*:

Out of Eighty clinical specimens and forty environmental samples only 42 isolates were able to grow on cetrimide agar at 42˚C which is considered as primary identification for *Pseudomonas* spp., 37 isolates were identified as *P. aeruginosa* by using VITEK 2 compact system.

Biofilm Formation

Congo red method:

Only 70% of the tested isolates gave black colored colonies when cultured on Congo red agar plates. And the (Figure 1) shows the results.



Figure (1): The biofilm and slime layer production on Congo red agar plate.

Tube method:

By using tube method, visible thick film was obtained on the wall of tube and bottom of the tube. All the tested isolates were biofilm producers indicated by the appearance of thick film inside the wall of the tube indicating strong biofilm production, as shown in Figure (2).

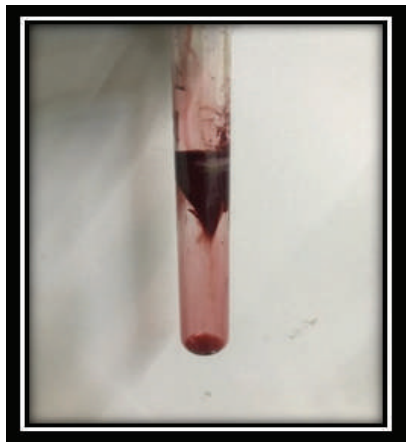


Figure (2): The biofilm production on glass tube wall by *Pseudomonas aeruginosa* after 24 hr. incubation stained with safranin by using tube method.

Microtiter plate method:

This method is considered as quantitative analysis that used for detection of biofilm formation, by using microplate reader it gives numerical value for absorption at 630 nm to determine the amount of biofilm formed through adhesion of bacteria to surfaces of microtiter plate, the absorbance represents the thickness of the biofilm, that formed on the wells surface by *P.*

aeruginosa isolates. The results of the current study obtained by using microtiter plate method indicates that all the isolates have the ability for strong biofilm production and there is no remarkable differences in biofilm thickness among the isolates, all the obtained OD values were four times larger than the OD of control.

Quantitative Real Time-PCR:

The expression of *pelA* and *algD* of *P. aeruginosa* biofilm genes were studied by relative qRT-PCR. The isolates were tested with the presence of sub-MIC of two antibiotics used, the amikacin and ciprofloxacin. Actually, ten isolates enrolled in this experiment were chosen for the reasons that it has strong biofilm intensity, it's collected from different sources (wounds, burns, urine, ear and environmental).

Gene expression of *pelA* and *algD* genes:

The treated and untreated isolates were tested genetically by using Real time PCR to realize if the *pelA* and *algD* genes were effected or not by the sub-minimum inhibitory concentration of antibiotcs. The results indicated that there is a down regulation effect in the *algD* gene and the *pelA* showed slight variation in the expression level 80% of the isolates were down regulated. While 20% were up regulated.

For treated isolates there is no gene expression for *algD* gene which is responsible for biofilm formation, so the effect were phenotypically by biofilm suppression and genetically by gene down regulation, the biofilm suppression may be due to *algD* role in β-D-mannuronic acid and α-L-guluronic acid production which has an important role in structural stability and protection of biofilm. Moreover, the *algD* gene mediates the control of alginate biosynthesis and transcription of the Alg proteins, and also is responsible for the final production of precursor GDP-mannuronic acid, the foundation molecule for polymerization and alginate synthesis. In the other hand, *pelA* gene expression revealed different expression manner, two isolates (R3 and R9) presented up regulation when treated by antibiotics, the isolate R3 from burn and the isolate R9 from environmental, while the other isolates revealed down regulation manner which has a noticeable impact on biofilm formation due to importance of *pelA* on extracellular polysaccharide formation in biofilm matrix, promoting attachment to surfaces and other cells, building and providing a scaffold to help maintain biofilm structure, and protecting cells from antimicrobials and host defenses, table (4).

Table (4): Gene expression results for *algD* and *pelA* before and after treatment with antibiotics.

Isolate number	CT gyrA before treatment	CT gyrA after treatment	CT algD before treatment	CT algD after treatment	FC algD	CT pelA before treatment	CT pelA after treatment	FC pelA
R1	29.1	33.3	28.6	-1.0	0	28.9	-1.0	0
R2	27.9	37.8	30.1	-1.0	0	29.1	37.6	0.82
R3	27.5	38.0	29.2	-1.0	0	29.9	36.3	16.53
R4	21.6	32.7	21.8	-1.0	0	24.7	35.2	0.49
R5	28.5	34.3	34.1	-1.0	0	31.0	-1.0	0
R6	24.9	32.5	25.1	-1.0	0	27.8	37.6	0.20
R7	34.1	34.7	-1.0	-1.0	0	34.0	35.6	0.50
R8	31.2	35.8	-1.0	-1.0	0	31.9	35.9	0.51
R9	24.7	33.2	25.9	-1.0	0	29.6	36.3	3.71
R10	36.7	36.9	32.2	-1.0	0	34.2	34.0	0.31

Practical experiments to estimate the gene expression of *algD* and *pelA* genes showed a difference in results between them on the one hand and between isolates on the other. The calculation method for estimating gene expression was based on a comparison between treated and untreated isolates in order to find the difference in the effect of antibiotics on the expression of these genes. Titration of the values obtained from the interaction was based on the use of *gyrA* gene values as a reference gene.

For the *algD* gene, the results showed complete inhibition of gene expression when using antibiotic treatment as opposed to untreated isolates. These results support the theory that this type of antibiotic affects bacterial cells by affecting the expression of the *algD* gene. Where the effect was the opposite kind of inhibiting gene expression.

As for the *pelA* gene, the results showed a variation in gene expression between isolates. Some isolates showed an increase in gene expression ranging from 1.3 to 16 times, and this increase may be evidence of the use of these isolates as a result of this gene in antibiotic resistance when there are no other auxiliary mechanisms. Whereas, the adverse effect on gene expression inhibition was an indication that these isolates might use auxiliary resistance mechanisms other than this gene. In a clearer sense, the effect of antibiotics on the expression of this gene is not as direct as it is an auxiliary effect on resistance mechanisms. Our results came in agreement with local study by AL-Sheikhly *et.al* in (2019) who revealed that *pelA* gene was also down regulated by the effect of antibiotics.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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CA 27-29: A Valuable Marker for Breast Cancer Management in Correlation with CA 15-3

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Abstract

Background: Breast cancer is the most common invasive cancer in women, and second main cause of cancer death in women after lung cancer. Tumor markers that showed evidence of clinical utility and were recommended for use in practice include CA15-3, CA27-29 and Carcinoembryonic.

Aim of the study: This study was designated to measure the levels of CA15-3, CA27-29 and CEA and evaluate their clinical significance in metastasis and non- metastasis patients with breast carcinoma, comparing their sensitivity and specificity.

Patients, Materials and Methods: Serum levels of (CA27-29) and (CA15-3) were measured in 62 patients (40 in non-metastasis and 22 in metastasis patients). The age of patients was from (30 to 90) years old. Samples were collected from the Oncology Teaching Hospital in Baghdad Medical City.

Results: The levels of CA15-3, CEA were significantly higher in metastasis group compare to non-metastasis group. while the levels of CA27-29 were no statically significant difference between the two groups.

Conclusion: In this study, measurement of CA15-3 and CEA correlated with disease activity especially in the metastatic setting. on the contrary, CA27-29 did not show such clear correlation. CA15-3 was more sensitive in follow up of breast cancer patients followed by CEA and then CA27-29.

Key Words: *Breast cancer, Tumor marker, Cancer antigen27-29, Cancer antigen15-3*

Introduction

According to the WHO, cancer is the second-largest cause of death worldwide. Cancer in 2015 was responsible for 8.8 million deaths, and it used to be the reason of about 1 in 6 deaths worldwide. Cancer is the fourth-ranked cause of death within the Eastern Mediterranean region following infectious diseases, cardiovascular diseases, and injuries [(1)].

Tumor markers are quantifiable biochemical that are related with a malignancy. These markers are created either by tumor cell (tumor-derived) or with the body in response to cancer cell. They are ordinarily substances that are discharged into the circulation and hence measured in the blood [(2)]. Tumor markers are not the primary methods for diagnosing cancer, but can be used as a laboratory test to assist the diagnosis [(3)].

The tumor markers that demonstrated evidence of clinical efficacy and have been advised for use in practice for breast cancer include Cancer Antigen (CA15.3), (CA27.29) and Carcinoembryonic Antigen (CEA).

(CA15-3) is an epitope of a large transmembrane glycoprotein derived from the gene called MUC1. The protein MUC1, also identified as polymorphic epithelial mucin or epithelial membrane antigen, has a big extracellular region, transmembrane sequence, and

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a cytosolic domain. In breast cancer this protein can often be over-expressed and in its extracellular region is aberrantly glycosylated [4].

(CA27.29) is consider a carbohydrate contains a protein antigen which performs as a tumor marker for breast cancer. It is also named breast carcinoma associated antigen [5]. Cancer Antigen 27.29 has similar clinical performance to that of CA15.3 in breast cancer patients.

Carcinoembryonic antigen (CEA) is a cell surface glycoprotein with a high molecular weight (200 KDa) [6]. CEA is a family of associated cell surface glycoproteins; it may be the most commonly utilized tumor marker for lung, colorectal, gastrointestinal and breast cancer [7].

Estrogen is the most common sex hormone in menstruating women. It is made from cholesterol in the body. A significant proportion of breast cancers are positive estrogen receptors. This means that they have a larger number of estrogen receptors (ER), which indicate that estrogen helps feed their growth. Receptors are proteins in or on certain cells, which can attach to certain substances, such as hormones, that circulate in the blood. Normal breast cells and some cells of the breast cancer contain receptors that attach to estrogen (ER) and progesterone (PR). These two hormones often enhance breast cancer cell growth [8].

Patients, Materials and Methods

This study (cross section) was conducted at the Oncology Teaching Hospital/Medical City/ Baghdad from February 2020 to the end of April 2020, where 62 patients with breast carcinoma (40 in non-metastasis and 22 in metastasis patients). All of them female patients and were diagnosed by specialists, their age range between (32-90) years have been submitted to a cross-sectional study. Whole blood samples were taken and allowed to clot for two hours at room temperature using a serum separator tube. Serum was separated by centrifuge for 15 minutes at 1000 rpm.

Non hemolysed and clear sera were taken and divided into two aliquots in Eppendorf tubes. Hemolysed samples discarded. Samples were stored at (-80 C^{o}) for one month. Repeated freeze-thaw cycles were avoided.

Serum level of CA15-3was measured by the Access BR Monitor Assay technique. This assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of CA15-3 antigen levels in human serum and plasma (heparin) using the Access Immunoassay System.

The serum concentration of CA27-29 was tested by using Enzyme Linked Immunosorbent Assay (ELISA) technique. This technique uses a quantitative immunoassay enzyme sandwich technique.

Serum level of CEA and E2 was measured by Minividas technique. The test principle contains a method of "2-step immunoassay sandwich enzyme" with an ultimate fluorescent detection (ELFA).

Statistical Analysis

Data were translated into computerized database, and used spss version 25 program to do the analysis. In order to test the statistical significance and the strength of association between the qualitative variable and quantitative variable ETA is calculating. P value less than 0.05 is consider to be statically significant result for both tests.

Results

Study conducted on 62 female patients in who are known to have a breast cancer, mean of age was (58.81±11.57) years, the youngest patient in the study was 30 years old and the oldest patient was 90 years old. Patient splitter into two group according to M staging system: metastatic group (21, 33.9%) mean of age in this group was (56.81±12.92) years and the non-metastatic group (41, 66.1%) mean of age in this group was (52.27±10.651) years as in table 1.

Table 1: subgroups of each variable, their frequency and percentage.

variable	Subtype	n. of patient (percentage)
Grade	I	3 (4.8 %)
	II	48 (77.4%)
	III	11 (17.7%)
Hormonal receptor	+ve	20 (32.3 %)
	-ve	42 (67.7%)
Her2 receptor gene changes	Negative	58 (93.5 %)
	Equivocal	4 (6.5%)
	Over expressed	0 (0.0%)
M stage	no metastasis	41 (66.1%)
	metastasis to bone	8 (12.9 %)
	to liver	4 (6.5 %)
	to brain	1 (1.6 %)
	Metastasis to other part	8(12.9%)
	To lung	0(0.0%)
T. stage	T0	1 (1.6 %)
	T1	8 (12.9 %)
	T2	43 (69.4 %)
	T3	3 (4.8 %)
	T4	7 (11.3 %)
N stage	N0	15 (24.2 %)
	N1	18 (29.0 %)
	N2	25 (40.3%)
	N3	4 (6.5%)
Age group	premenopausal	28 (45.2%)
	Post-menopausal	34 (54.8%)
Total number		62 (100 %)

M stage: Describe the metastatic state. T stage: Describe the size of the tumor.

N stage: Describe the spread to lymph node.

In this study four biomarker was measured CA15-3, CA27-29, CEA, and E2 in metastatic and non-metastasis group are shown in table 2.

The CA15-3 result shows a difference in the mean level of CA15-3 between the two groups which is significantly higher in the metastatic group compare to the non-metastatic group, which is statistically approved as p-value, is≤ 0.003.

The same for CEA mean level, which is higher in metastatic group compare to non-metastatic and it is statically approved, as p-value is ≤0.02.

The result of CA27-29 was different from other biomarker as there is no statically significant difference in mean level of CA27-29 between the two groups as p-value is ≥0.62.

To determine which one of the 3 test is more sensitive and more specific than the others in the metastatic and non-metastatic group, the result of each test for each patient split into positive or negative according to their cut off value of normal range CA15-3 NR < 31.3 U/ml , CA27-29 NR < 15U/ ml, CEA NR< 4.1ng/ml.

Table 2: level of each biomarker in both metastatic and non-metastatic group

		N	Mean ±S. D	Std. E	p-value
CA15-3	Non-met.	41	13.88±14.37	2.24	0.003
	Met.	21	93.74±164.96	35.99	
CA27.29	Non-met.	41	78.30±466.91	72.92	0.63
	Met.	21	28.20±48.42	10.57	
CEA	Non-met.	41	2.00±4.69	0.73	0.02
	Met.	21	18.08±43.89	9.58	

In table 3 the sensitivity, specificity, positive predictive value, negative predictive value and p-value for each marker is shown, CA15-3 is more sensitive and specific followed by CEA. But CA27-29 is less sensitive and specific.

Table 3: The Sensitivity, Specificity, positive predictive value, negative predictive value and p-value for each marker.

	SN	SP	PPV	NPV	p-value
CA15-3	92.9%	83.3%	61.9%	97.6%	0.000
CA27.29	75.0%	76%	42.9%	92.7%	0.002
CEA	91.7%	80.0%	52.4%	97.6%	0.000

To determine the relation between each biomarker and the grade, M stage, N stage and T stage Eta, Eta^2 was calculated in table 4

Table 4: Effect of M stage on the biomarkers.

Biomarker	Eta	Eta2	p-value
CA15-3	0.37	0.14	0.003
CA27-29	0.06	0.004	0.63
CEA	0.28	0.08	0.03

Relation between biomarker and N stage in metastatic and non-metastatic group shown in table 5. The Eta indicates a very weak relation between the biomarkers and N stage in metastatic and non-metastatic group.

Table 5: Effect of N stage on biomarker level in non-metastatic and metastatic group.

	Biomarker	Eta	Eta2	p-value
non metastatic	CA15-3	0.25	0.06	0.50
	CA27-29	0.21	0.04	0.65
	CEA	0.23	0.05	0.56
metastatic	CA15-3	0.16	0.03	0.92
	CA27-29	0.15	0.02	0.94
	CEA	0.19	0.04	0.88

Relation between biomarker and T stage in metastatic and non-metastatic group shown in table 6. The Eta indicates a very weak relation between the biomarkers and T stage in the two group.

Table 6: Effect of T stage on biomarker level in non-metastatic and metastatic group.

	Biomarker	Eta	Eta2	p-value
non metastatic	CA15-3	0.17	0.03	0.89
	CA27-29	0.10	0.01	0.98
	CEA	0.09	0.009	0.99
Metastatic	CA15-3	0.11	0.01	0.97
	CA27-29	0.17	0.03	0.92
	CEA	0.22	0.05	0.84

Discussion

Study (cross section) conducted on 62 patient who are known case of breast cancer, in table 1 patient data divided into groups.

Age range 30-90 years old, 45.2% of them are younger than 50 years and 54.8% are older than 50 this result agree with (Mohammed TM, *etal.*, 2018)[1], carried on Iraqi patient who found also that 45% were younger than 50, the other result will also be compare to

this study as it done on Iraqi patient in recent time, and TMN staging was taking in account. M stage: metastasis found in 33.9%, and no metastasis found in 66.1, which slightly similar to (Mohammed Tm, *etal*, 2018) [1] study has found that 24.1% has metastasis, this difference may be due to difference in sample size as the study conduct on 171 patients.

In this study three biomarker was measured, it found that CA15-3 has a higher mean level in metastatic group compare to non-metastatic group in table 2.

This result is agreed with (Farzaneh Agha-Hosseini, *etal*.,2009) [9] study in which was found that serum level of CA15-3 was higher in patient with breast cancer in the 2nd stage of disease.

And agree with (Kifah H. Alani, *etal*., 2018) [10] study in which found that serum level of CA15-3 was higher in metastatic breast cancer compare to non-metastatic group.

The CEA mean serum level is higher in metastatic group compare to non-metastatic group in table 2. This result agrees with the result found by (KA Pathak, *etal*,.1996) [11], in which CEA level was higher in patient with breast cancer and patient with benign breast disease.

The CA27-29 serum level was not statically significant difference between the two groups in table 2. Result could be related to small sample size or due to other causes as CA27-29 level increase or decrease in many different ways as its level may be not elevated in early-stage or absent or high levels of CA27.29 can indicate metastatic disease in many studies. Also, malignant and benign conditions beside breast cancer can cause increase in serum levels, as found in (Donepudi MS, *etal*., 2014) [12], and (Van Poznak C, *etal*., 2015) [13].

In the current study, found that CA15-3 as biomarker is most sensitive biomarker in metastatic group followed by CEA, then CA27-29, as shown in table 3. Positive predictive value of CA15-3 is (61.9%). The test is useful to detect the metastasis in breast cancer patient more than the two other biomarkers making them less useful than CA15-3.

In table 4, the relation of CA15-3 to M stage, and if the level of CA15-3 increases with M stage Eta which is measure effect of size indicate increase in CA15-3 level with increase M stage even that increase not strong, this agree with (Yijie Fu, *etal* ,2016) [15] who found increase in serum level as tumor progress and it's more useful to monitor advance tumor.

Relation between biomarker and T and N stage in table 5, and 6. Eta of CA15-3, CA27-29 and CEA indicate no relation between the biomarkers and the staging to T or N state. The result agrees with study done by (Franco Lumachi *etal*, 2004) [14], who found no relation between CEA and T and N stage, and no relation between CA15-3 with N stage but there is a relation with T stage.

A high proportion of breast cancers tent to express a greater number of estrogen receptor, as increase time exposure to estrogen is a risk factor to develop breast cancer.

Conclusion

Measurement of cancer antigen15-3 (CA15-3) and Carcinoembryonic (CEA) (to a lesser extent) correlated with disease activity especially in the metastatic setting. While (CA27-29) did not show such clear correlation. Also, the tumor marker (CA27-29) failed to show concordance results with Cancer antigen 15-3 (CA15-3) and Carcinoembryonic (CEA) in breast cancer patients.

The tumor marker Cancer antigen 15-3 (CA15-3) was more sensitive in follow up of breast cancer patients followed by Carcinoembryonic (CEA) and then Cancer antigen 27-29 (CA27-29).

Also, there was no correlation between (CA15-3, CA27-29, and CEA) and the staging system (TNM) and there was no correlation between the tumor markers and the levels of estrogen.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Incidence of Mumps in Anbar province, West of Iraq during the Period 2009-2018

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Summary

Background: Mumps (infectious parotid gland) is an infectious viral disease mainly affects children and adolescents characterized by enlargement of parotid and salivary glands, other epithelial tissues may be affected, in some cases it is accompanied by serious complications. In recent years many mumps cases in Anbar province have appeared in people who have been vaccinated with Mumps vaccine and for reasons that are not clear

Objective: To Show the annual mumps incidence with age groups and sex for those affected in Anbar province

Methods: A Descriptive cross-sectional study of the electronic archives of mumps patients who were immediately registered in Anbar Governorate hospitals, during the period from 2009 to 2018. The questionnaires regarding year of infection, gender, and age of mumps patients included in the study. Mumps incidence has been calculated through dividing the number of annual cases of infection by population size multiplied by 100,000. SPSS software version 24 were used for data analysis.

Results: Of 2924 Mumps patients registered at Anbar province hospitals, 1715 (58.7%) were males with 12.1 ± 4 years mean age . The incidence rates of Mumps in 2009, 2010, 2011, 2012, 2013, 2014, 2015,2016,2017 & 2018 were (0.50, 0.20, 0.70, 0.80, 1.70, 0.00, 0.00, 7.20, 97.90& 59.00) / 100,000 respectively with statistically variance among males & females; p value = 0.000 & annual incidence ratio was 21.00 / 100,000. Annual Mumps incidence ratio from 2009-2018 confirmed one peak in 2017-2018 & a rare 2009-2016, Majority of them were in the age group 5 to14 years old.

Conclusion: Mumps incidence had been increase during the last 2 years mainly in the age group 5 to14 years old with a marked increase incidence in male in Anbar province, Iraq.

Keyword: *Incidence, Mumps, Years, Gender, Age Group, descriptive study Anbar, Iraq*

Introduction

Mumps (infectious parotiditis) is a contagious virus of the family paramyxoviridae, an enveloped single negative non-segmented RNA virus [(1)]. Mumps is a childhood and adolescent disease characterized by an enlarged parotid and salivary glands with other epithelial tissues, sometimes accompanied by more serious complications, including Orchitis that may be lead to Sterility and aseptic meningitis, up to 10% of mumps patients developed aseptic meningitis may lead to disability or death, and permanent deafness and sometime pancreatitis [(2)]. Rare symptoms include

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, oopheritis mastitis, arthritis, and myocarditis [3]. especially in adults with serious complications [4].

Mumps is a highly contagious viral transmitted through respiratory droplets or direct contact mainly during the winter [2, 5].

Mumps prevented mainly through the use of live attenuated vaccines consisting of a mixture of mumps, measles and rubella vaccine known as MMR vaccine [6, 7]. However, within a few years after the implementation of the routine vaccination program against mumps, a large and small mumps epidemics began to appear around the world, including in people vaccinated against this disease [8 ,9, 10 ,11]. Infectious mumps disease is common in middle and lower income countries such as Iraq and this disease is a neglected health problem where the clinical documentation of it is not at the required level despite the seriousness of its complications as well as rare epidemiological studies about it [12]. Current work were design to determine the mumps Incidence rate in Anbar province, Iraq during the period 2009-2018.

Materials and Methods

Descriptive cross-sectional study of Mumps patient's electrical files that recorded immediately at Anbar province hospitals. All newly diagnosed Mump cases data was retrieved from each of public, private hospitals along with Pediatrician private clinics. Electronic database saved in Al-Anbar Health Office during a period from 2009-2018 includes demographic variables with sero-diagnosis by recognition of ″mumps-specific IgM ″ in a serum sample [13]. Mump diagnosis was confirmed by at least two specialized doctors consultants in medicine according to WHO standard criteria [10].

Estimates of the population of Anbar were taken from the statistics section of the Iraqi Ministry of Planning for 2018 and previous years for the study. Of total, 2924 Mump patients were included in this study, the incidence rates are expressed per 100,000. Ethical approval had been obtained from anbar medical college Ethics approval Committee,Iraq.

Statistical Examination

Annual incidence rate was calculated by dividing the total number of newly diagnosed mumps cases in a given year by the population at risk of mumps in the Anbar Governorate this year and the result is multiplied by 100,000, As it was considered population of all ages the risk of Mump infection. The SPSS 24 program version was used to analyze the qualitative data as the ratio of males to females, age group and mumps patients annual frequencies and to determine whether there was a statistical difference within these variables through Chi-Square test and a statistically significant study when the value of $P < 0.05$.

Results

Total number of ascertained Mumps cases that recorded was 2924 during a period from2009-2018. Of them, 1715 (58.7%), 1209 (41.3%) were males and females respectively with 1.4:1 male to female ratio. Significant difference had been recorded between males & females infection (p value ≈0.000). Mean age of Mumps cases was 12.1 ± 4 year. Mean age of recorded cases of Mumps was significantly slightly higher in males compare with females, 12.1 ± 2 & 12.2 ± 6 years respectively.

Mumps incidence over years 2009 to 2018 by gender is illusterated in Table 1.

Table 1: Distribution of Mumps over years 2009 to 2018 by Gender in Anbar province, Iraq

Year	Gender		Total (%)	P. Value
	Female (%)	Male (%)		
2009	2 (28.6)	5 (71.4)	7 (100)	0.000
2010	3 (75.0)	1 (25.0)	4 (100)	
2011	5 (45.5)	6 (54.5)	11 (100)	
2012	3 (25.0)	9 (75.0)	12 (100)	
2013	18 (64.3)	10 (35.7)	28 (100)	
2014	0 (00.0)	0 (00.0)	0 (00.0)	
2015	0 (00.0)	0 (00.0)	0 (00.0)	
2016	55 (43.3)	72 (56.7)	127 (100)	
2017	636 (37.6)	1054 (62.4)	1690 (100)	
2018	487 (46.6)	558 (53.4)	1045 (100)	
Total	1209 (41.3)	1715 (58.7)	2924 (100)	

The incidence rates of Mumps in 2009, 2010, 2011, 2012, 2013, 2014, 2015,2016,2017 & 2018 were (0.50, 0.20, 0.70, 0.80, 1.70, 0.00, 0.00, 7.20, 97.90& 59.00) / 100,000 respectively with statistically variance among males & females (p value ≈ 0.000). Annual Mumps incidence rate was 21.00/ 100,000. The drift in annual Mumps incidence rates from 2009 to 2018 confirmed one peak in 2017-2018 with a rare incidence during 2009-2016. No Mumps cases was recorded during a period 2014-2015 due to the displacement of most of the population from the Anbar province as a result of terrorist operations to other provinces as illusterated in Table 2, Figure 1.

Table 2: Mumps with Yearly Annual Incidence per 100000 population with confidence interval 95% from 2009 to 2018 in Anbar province, Iraq.

Year	Pearson-year at Risk	Number of patients (%)	Overall Annual Incidence per 100,000 person year	Standerd deviation
2009	1478226	7 (0.2)	0.50	38.50764
2010	1660123	4 (0.1)	0.20	
2011	1562025	11 (0.4)	0.70	
2012	1600000	12 (0.4)	0.80	
2013	1636861	28 (1.0)	1.70	
2014	1753968	00 (0.00)	0.00	
2015	1750000	00 (0.00)	0.00	
2016	1769230	127 (4.3)	7.20	
2017	1665000	1690 (57.8)	97.90	
2018	1791390	1045 (35.7)	59.00	
Total		2924 (100.0)	Annual average 21.00	

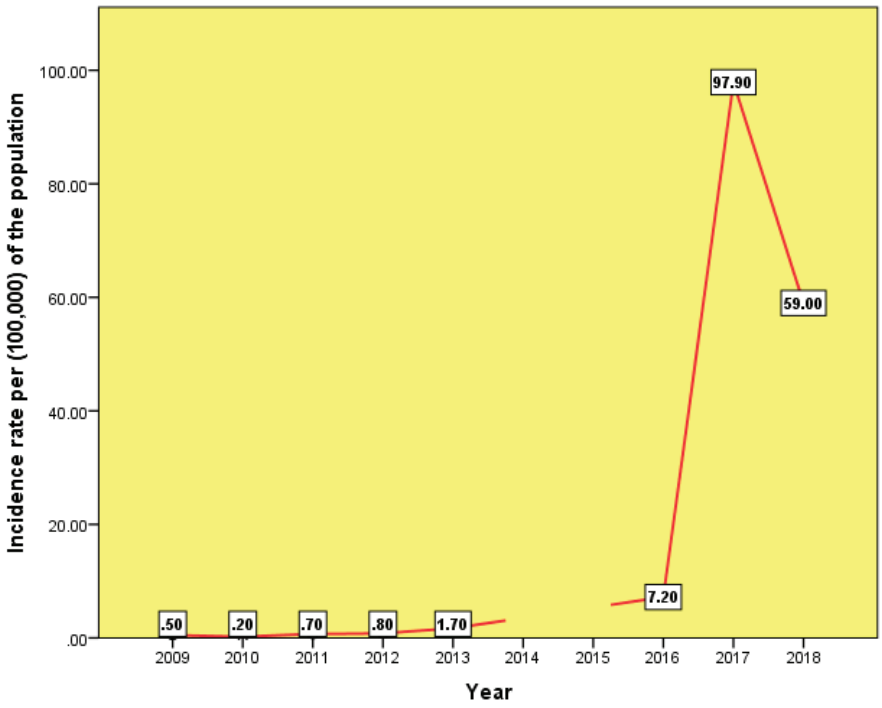


Figure 1: Yearly Mumps Annual Incidence per 100000 population during 2009 to 2018 in Anbar province, Iraq.

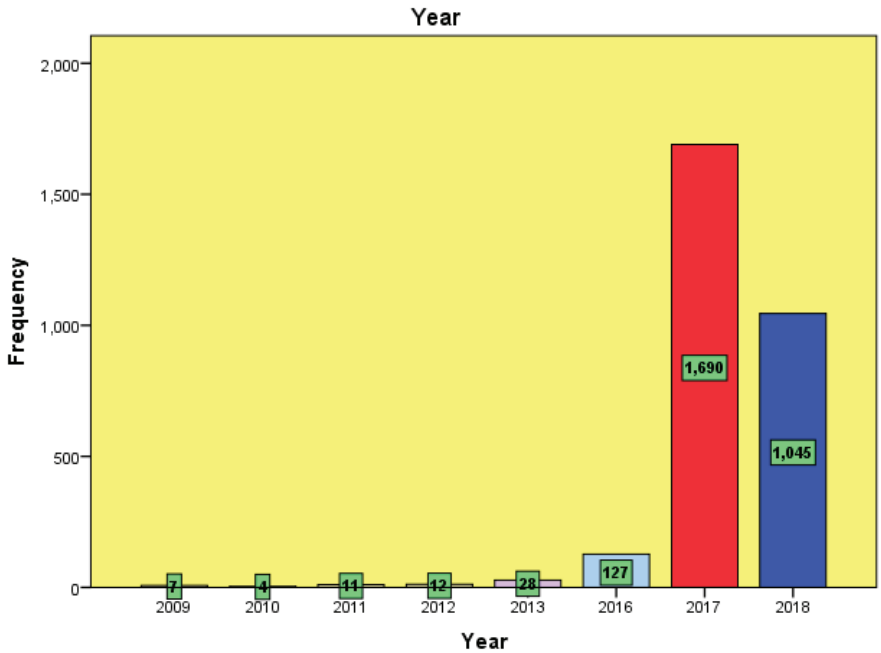


Figure 2: Frequency of Mumps over years 2009 to 2018 in Anbar province, Iraq

Mumps patients were classified according to age into 3 groups; (1):1–*4* years, (2): 5–14 years and (3): 15–45 years) For each year of study that conducted during a period from 2009-2018. Majority of Mumps patients were in the age group 5 to14 years old that had highest relative increase of incidence as presented in Table 3, Figures 3. Mean age of recorded cases of Mumps was 12.1 ± 4 year. Study showed that there was a statistically significant difference between the different age groups of Mumps during each of the study years (P.Value:0.000).

Table 3: Distribution of Mumps over years 2009 to 2018 by Age Group in Anbar province, Iraq

Year	Age group			Total	P.Value
	1-4 Year N (%)	5-14 Year N (%)	15-45 Year N (%)		
2009	2 (28.6)	4 (57.1)	1 (14.3)	7 (100.0)	0.000
2010	1 (25.0)	2 (50.0)	1 (25.0)	4 (100.0)	
2011	4 (36.4)	5 (45.5)	2 (18.2)	11(100.0)	
2012	2 (16.7)	6 (50.0)	4 (33.3)	12(100.0)	
2013	5 (17.9)	15 (53.6)	8 (28.6)	28 (100.0)	
2014	0 (0.00)	0 (0.00)	0 (0.00)	0(100.0)	
2015	0 (0.00)	0 (0.00)	0 (0.00)	0(100.0)	
2016	9 (7.1)	87 (68.5)	31 (24.4)	127 (100.0)	
2017	211 (12.5)	1058 (62.6)	421 (24.9)	1690 (100.0)	
2018	29 (2.8)	913 (87.4)	103 (9.9)	1045 (100.0)	
Total	263 (9.0)	2090 (71.5)	571 (19.5)	2924 (100.0)	

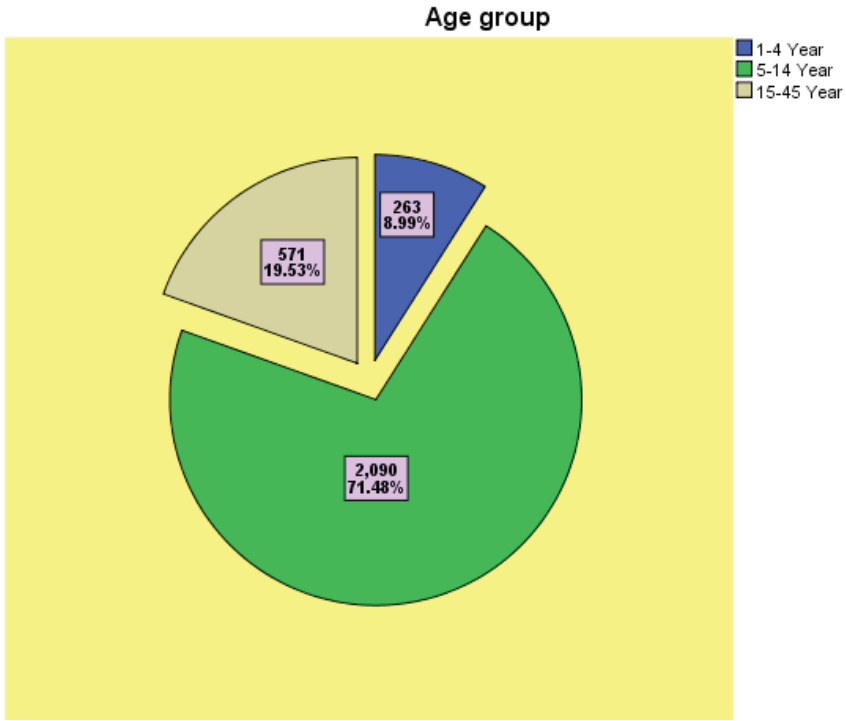


Figure 3: Distribution of Mumps over years 2009 to 2018 by Age Group in Anbar province, Iraq

Discussion

The total number of ascertained mumps cases that were recorded in Anbar Governorate during the period 2009-2018 was 2924 cases as shown in Table 1. Increase mumps cases in the past two years 2017-2018 in Anbar Governorate may be due to the advancement of the health sector, which causes many patients to seek health care, whoever increase mumps incidence may due to vaccines lacking. Lose mumps in the years 2014, 2015 and 2016 may be that most of the Anbari community has been displaced and displaced to other regions inside and outside Iraq due to the military operations, due to which health institutions have lost the ability to follow up on this disease as shown in Table 2, Figure 1

The average annual mumps incidence in Anbar population during the present study (21.00 / 100,000 persons) was much higher than that of the same disease recorded in neighboring countries such as Saudi Arabia [14], Turkey [15] and Egypt [16] in addition to other countries as Italy ,France, western Europe [17 ,18], Japan [19], this rise may be due to a lack of vaccines for this disease in Iraq and with that the result is similar to the result obtained in previous studies in Iraq, where in 2016 ,73919 cases were recorded, while 36367 cases were recorded in 2017, while the number of mumps cases in 2017 worldwide reached about 467506 cases according to world health organization 2019 [20].

The mean age of recorded mumps cases during the current study was 12.1 ± 4, and this contrasts with the mean age of children with mumps that was shown by previous studies in Saudi Arabia [14], Egypt [16] and Jordan [18], It was showed that child in the age group 5-9 years were more likely to mumps.

The current research showed that age group 5-14 is the main target age group for mumps, followed by age groups 15-45 and 1-4 years, and this result is similar to the result obtained in previous studies [21, 22, 23] that showed that the age group 5-14 is more susceptible to infection with this disease and is very close One of the results obtained from a similar study in Tehran [24], where children were in the age groups 0-9 or 5-9 years are more susceptible to mumps infection, as well as completely identical to a previous study in USA [5], where it appeared that 90% of children with mumps are are under 14 years of age ,in contrasts with a previous study in Belgium, it confirmed that the age group 3-12 years is more susceptible to mumps and also differs greatly from other studies that showed that the 35-44 age group was the target of mumps [25].

The current study showed that the prevalence of mumps in males 1715 (60.1%) and females 1209 (41.3%) out of a total of 2924 cases during the period of study, which confirmed the presence of a relative majority of males with this disease and this similar to the previous study that shows mumps illness happend more commonly in males with about 2: 1[11] ,It also consistent with the results of Sweden study that showed 62% mumps patients were male, and consistent with the result of Quasim (2010) [26] in Iraq where it was found high mumps incidence in males compare with females, as other studies that shows a bias for male cases of mumps [22] , [27],whereas current study was different from a previous study conducted in Germany while it showed that only 43.3% of mumps patients were male [25].

The conservative nature of Iraqi society makes females less mixed, and therefore less susceptible to mumps though respiratory droplets. In addition to mixing, other male activities, such as travel, social differences and other important activities, increase the risk of male mumps infection.

The current study showed that there is a noticeable increase in the mumps incidence during the last three years, especially age group 5-14, and therefore it is necessary to vaccinate children with the mumps vaccine due to the severity of complications of this disease if it occurs.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Effect of Insecticide "Maxxthor" on Some Hematological and Oxidative Stress Parameters in Male Albino Rats

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Abstract

The present study was designed to investigate the effect of different concentrations of Maxxthor on some hematological and oxidative stress parameters in male albino rats.Twenty male rats with age of 6-8 weeks and weight of 200-250gm were equally divided into four groups as follow:G1 treated with normal saline as control group,while G2,G3andG4groups were treated with(0.01,0.1,1)mg\kg body weight of Maxxthor respectively for 40 days.The following hematological parameters were measured: red blood cell(RBC),hemoglobin (Hb),white blood cell(WBC), platelets(PLT),malondialdehyde(MDA),glutathione(GSH),catalase and vitamin E. The hematological parameters results revealed highly significant($p<0.01$)decreasein RBC and H,while a highly significant($p<0.01$)increase in WBC and PLT in all treated groups as compared with control. The oxidative stress parameters results revealed highly significant($p<0.01$)increase in MDA level,while highly significant($p<0.01$)decrease in GSH,catalase and vitamin E level were noticed in all treated groups as compared with control .

Key words: *Insecticide, Maxxthor ,Oxidative stress, Albino rats*

Introduction

Pesticide is any substance or mixture of substance intended to preventing, destroying or controlling any pest,including vectors of human disease or animal species, unwanted plants or animals that cause damage[(1)]. The extensive and random application of pesticide,and its resist to physical and chemical breakdown and metabolic degradation led to its spread within the ecosystem elements[(2)],leading emergence of several ecological and health setbacks due to the accumulation of residues of pesticides in the environment components over time[(3)].Synthetic pyrethroid were introduced widely for pest and disease vector control more than three decade ago[(4)].The source of pyerthroids is the flower of the pyretherum plant *Chrysanthemum cineraiafolium*[(5)].

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The pyrthroid pesticides are rapidly replacing other insecticides due to low mammalian toxicity and an unusually fast biodegradation[(6)].Pyrethroid enhance sodium channel activity by shifting activation to more negative membrane potentials as well as slowing channel activation[(7)].

Maxxthor)MAX) is pesticides belong to pyrethroid insecticides type I.The active ingredient in Max product is bifenthrin ,a second generation of synthetic pyrethroid. It is featured with strong insecticides effects due to its ability to modifying the kinetic voltage–sensitive sodium channels of nerve in insects[(8)].This disrupts the normal transmission of nerve impulses causing repetitive firing of the insect's nerve resulting in paralysis and ultimately death.Maxxthor use against termites and labeled for the long term control of wide range of pests including insect pests,vectors,spiders,ticks and turf insects.Maxxthor has shown an average termiticide effective life of 12 years,the longest average of any termiticide active

ingredient on the market today[9].The neurotoxicity of Maxxthor depend on the affinity to the voltage sodium channels in insects and mammal,where Maxxthor bind to the sodium channel transiently and stop the closing of the channel permanently,leading to permanently depolarize of the membrane,no further action potential can be generated because the resting potential will not be restored.This will result in after potentials and continuous firing of axons[10].The presence study has been undertaken to study the effect of Maxxthor insecticide on hematological and oxidative stress parameters follow repeated treatment male albino rats for 40 days.

Materials and Methods

Experimental animals and Study Design

This study was performed on 20 healthy mature male Sprague-Dawley albino rats(*Rattus norvegicus*) with about8-10weeks age.The animals were purchased from biotechnology research center/Al-Nahrian university,in old between(6-8)weeks.The mature male were with an average weight of(200-250gm)and kept under climate controlled conditions of the animal house with temperature 22-25 C°, good ventilation, regular 12 hours light duration.Rats were randomly divided into four groups five rats for each group and treated as follow:G1was administrated with 1ml of normal saline, while G2,G3andG4 were administrated with1ml of(0.01,0.1and1mg/kg b.w.) respectively of MAX orally once each 48 hours for 40 day.

Blood samples were collected by heart puncture and then divided into two parts.The first part put in plastic test tube contains (EDTA) for hematological tests.The second part was left in eppendrof tube to coagulation at room temperature and then centrifuged at 3000 rpm for 15 minutes. Serum was separated and stored at-20C° for oxidative stress parameters analysis.The hematological parameters(RBC,Hb ,WBC and PLT)were measured by using Coulter device.MDA was determine using Assay Kit from Linear,Spain,according to[11,12]. GSH was measured according to[13]by using the assay kit from Linear,Spain.Catalase was determined by using the assay kits from Linear, Spain according to[14].Vit. E was determined by using the procedure of vit.E Elisa Kit from Linear,Spain.The Statistical[15]was used to detect the effect of difference groups in study parameters.

Results and Discussion

Hematological parameters

Results of current study as shown in Table(1) revealed highly significant($p<0.01$)decrease in red blood cell RBC and hemoglobin(Hb)in all treated groups with Maxxthor insecticide as compared with control group. While there is highly significant($p<0.01$)increase in white blood cell(WBC) and platelets(PLT)in all treated groups as compared with control group .

Table(1): Effect of Maxxthor(0.01,0.1and1mg/gk.b.wt.)on Hematological parameters(RBC,Hb ,WBC and PLT)of rats after 40 day of treatment

Group	Mean ± SE			
	RBC 10^12/L	Hb g/dl	WBC 10^9/L	PLT 10^9/L
G1:Control	6.78 ± 0.14 a	13.84 ± 0.15 a	6.02 ± 0.11 c	202.50 ± 6.39 d
G2:treated with 0.01 mg/ kg/b.wt. of Maxxthor	6.07 ± 0.27 b	12.45 ± 0.23 b	6.30 ± 0.11 c	241.90 ± 3.47 c
G3: treated with 0. 1 mg/ kg/b.wt. of Maxxthor	5.62 ± 0.21 bc	11.47 ± 0.26 c	6.86 ± 0.12 b	274.30 ± 4.45 b

Cont... **Table(1): Effect of Maxxthor(0.01,0.1and1mg/gk.b.wt.)on Hematological parameters(RBC,Hb ,WBC and PLT)of rats after 40 day of treatment**

G4: treated with 1 mg/kg/b.wt. of Maxxthor	5.21 ± 0.23 c	10.40 ± 0.23 d	7.65 ± 0.13 a	351.10 ± 10.41 a
LSD value	0.623 **	0.637 **	0.340 **	19.304 **
Means having with the different letters in same column differed significantly, ** (P<0.01). Means having with similar letters in same column non- significantly different, ** (P<0.01).				

The present finding was similar to[16]finding who observed significant decrease in RBC count and Hb level with increasing in WBC count after treated Wistar rats with cypermethrin for 28days.Also the results is agreement with the work[17]who find significant decrease in RBC count and hemoglobin level and explained the decrease of Hb due to an increase in the rate at which Hb is destroyed.Same results of WBC count represented by[18] who report that sublethal poisoning with higher dose of bifenthrin caused aniemia and elevation in WBC count after injected mice with bifenthrin. Wafa [19] report significant elevation in platelet counts in workers exposed to pesticides than in controls .

The reason for the significant decrease in the number of red blood cells as a result of the effect of the pesticide may be due to its inhibition of the substance(glutathione) that found inside the red blood cell and responsible for protecting it from the influence of toxic substances[20]. This results could be explained that maxxthor pesticide affect on erthropoitien release in the kidney which is the hormone regulator of RBC production(21).Sadowska-Woda *et al*.[22]study demonstrated that bifenthrin induce oxidative stress causes enhanced lipid peroxidation and decrease antioxidant enzyme activities in human peripheral blood.RBC are particularly sensitive to oxidation damage due to the presence of high content of poly unsaturated fatty acid in their membranes and high cellular concentration of oxygen and hemoglobin[23]. On the other hand Hb in erythrocytes is major source production of free radicals when it interacts with pyrthroid pesticide giving rise to free radicals leading to membrane lipid peroxidation and hemolysis[24].

Other explanation reported by(25)that show any reduction in RBC count may be due to reduction rate of RBC formation in bone marrow or increasing rate of RBC breakdown,also increase red blood cell membrane fragility and alter membrane flexibility led to reduces the life span of circulating erythrocytes and increased risk of hemolysis[26].Hemolysis of blood cells may cause the decrease in erythrocyte count in pyrthroid intoxication which in turn tend to be responsible for the reduction of hemoglobin percentage[27].Reduction in Hb content can be related to the impaired biosynthesis of heme in bone marrow[28].In addition reduced hemoglobin content accordance with RBC reducing could be explained that pesticide cause damage to erythrocyte membrane that lead to hemolysis or damage of blood iron level[29].Olsen [30])suggest when assessing hematological toxicity,any increase or decrease in blood parameters indicates excess or suppression of blood cell respectively or an imbalance between production of blood cells and distraction.

The increase in WBC count may be due to the activation of the animal's defense mechanism and immune system against the toxic effect of Maxxthor pesticide as the WBC rise as a result of any infection and any form of cell toxicity or inflammation[31].Poisoning mice with high dose of alpha-cypermethrin had stronger stimulatory effect on phagocytic activity with significant increase in monocyte and lymphocytes were observed in the blood of male mice treated with same pesticide[32].An elevation in platelets could be explained to release of stored platelet from the spleen[33].Platelets are highly active in the host defense against pathogens and have ability to modulate leukocyte function such phagocytosis[34].

Oxidative stress parameters

Data in Table(2)show highly significant($p<0.01$)increase in MDA level in G3 and G4 when compared with control groups,while there were no significant($p<0.01$) differences between G2 and control group.In the same time,results in table(2)revealed highly significant($p<0.01$)decrease in GSH,catalase and vitamin E. in all treated groups with Maxxthor as compared with control group.

Table(2): Effect of Maxxthor(0.01, 0.1 and 1 mg/gk. b.wt.)on oxidative stress parameters(MDA,GSH ,CATand Vit.E)of rats after 40day of treatment

Group	Mean ± SE			
	MDA nmol/ml	GSH µmol/ml	CAT µmol/ml	Vit. E ng/ml
G1:Control	2.03 ± 0.12 c	63.43 ± 1.27 a	0.620 ± 0.01 a	43.15 ± 0.67 a
G2: treated with 0.01 mg/ kg/b.wt. of Maxxthor	2.34 ± 0.13 bc	57.98 ± 1.51 b	0.509 ± 0.01 b	39.06 ± 0.73 b
G3: treated with 0. 1 mg/ kg/b.wt. of Maxxthor	2.67 ± 0.15 b	50.71 ± 1.48 c	0.413 ± 0.01 c	33.55 ± 0.92 c
G4: treated with 1 mg/kg/b.wt. of Maxxthor	3.11 ± 0.17 a	42.66 ± 1.40 d	0.322 ± 0.04 d	28.85 ± 0.87 d
LSD value	0.420 **	4.076 **	0.056 **	2.305 **

Means having with the different letters in same column differed significantly , ** (P<0.01).
Mean having similar letters in the same column are non significantly different , ** (p>0.01) .

The results of this study pass parallel with[35]who report increase blood MDA level and decrease in GSH and CAT level after treated rat with bifenthrin.Exposure to pyrthrod for three weeks led to increase in lipid peroxidation and decrease in GSH and catalase[36]. The increment in MDA(Lipid peroxidation marker)could be explained by[37]who suggested that the formation of oxygen free radicals can be a major factor in the toxicity of pesticides.Catalase as endogenous antioxidant enzymes that scavenge harmful ROS,is favorable target for pesticide toxicity,and plays a fatal role during the oxidative stress as an adaptive response of cells[38]. On the other hand[39]reported that pyrthroids induced oxidative damage might be they could penetrate easily to the cell membrane due to their lipophility and caused lipid peroxidation.Pesticide induce oxidative stress leading to lipid peroxidation and exhaustion of internal antioxidants[40].The reduction of GSH content could be explaned by GSH use for conjugation as an antioxidant by neutralizing the free radicals.Other explanation is that this depletion is due to an increase of degeneration of GSH during oxidation stress caused by pyrthroid[41]. Rouabani[42]suggest that the metabolism of pyrthroid generate ROS which in turn could led to an decrease in GSH rate linked to an increase in MDA,because increase of lipid peroxidation and depletion the reservoir of GSH[43].Decline of catalase level could be explained by the massive rate of free radicals that cause saturation

in side and inhibition related to degradation in another side [44].

VitaminE is the major lipid soluble antioxidant and the major effective chain–breaking antioxidant within the cell membrane where it garde membrane fatty acids from lipid peroxidation[45].In addition,[46]observed that vit.E is able to reduce the cutaneous paresthesia caused by pyrethroids.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Using Probiotics, Antibiotics and their Combinations to Eradicate Biofilms Formed by Four Pathogenic *Klebsiella Pneumoniae* Strains

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Abstract

Klebsiella pneumoniae is an opportunistic pathogen leading to nosocomial infections with production of biofilm. In the present study, 73 samples were collected from patients of different ages and both genders suffering from burn and wound injury that referred to four Hospitals in Baghdad from Aug. to Nov. 2019. All *Klebsiella* isolates were subjected to the primary identification; first by describing colonies grown on MacConkey agar and second microscopically after Gram staining of their cells. Then the isolates were identified by Vitek 2 system which insured that 60 of the isolates were found to belong to *Klebsiella pneumoniae.* All identified isolates were screened for ability to produce biofilm by using the Microtiter plate assay and the Congo red agar method, and the results showed that only 4 isolates (3 of wounds and 1of burns) were able to form biofilms. For the importance of these four isolates as the only ones able to produce biofilm, they were subjected to the 16S rRNA gene amplification test by using PCR technique. Results insured that they are new strains *Klebsiella pneumoniae* to be discovered for the first time in this study. They were verified and documented by the "National Center for Biotechnology Information (NCBI)" and registered as; KPWIQ25, KPWIQ49, KPWIQ51 and KPBIQ19 strains of *Klebsiella pneumoniae*. When the probiotic filtrates and antibiotics combination were investigated for eradication of biofilms formed by the four pathogenic strains, results showed that such combinations had great efficiency in the eradication.

Keywords*: Klebsiella pneumoniae, Biofilm, Probiotics, Antibiotics, Combination.*

Introduction

Klebsiella pneumoniae is a member of such clinically considerable organisms that have obtained much public health apprehension. It is a significant *Enterobacteriaceae* behold as one of the opportunistic pathogens causing broad series of diseases and rendering increasingly recurrent acquisition of resistance to antibiotics [(1)]. Biofilm arrangement bears a few stages to make its develop, which concede essential connection to the surface, microfilm development, mushroom shape development of biofilm, and discharging motile microscopic organisms inside separation organize[(2)].

Several mechanisms possessed within probiotics by which they can lead to prevent infection, conferring health benefits, producing antimicrobial substances, inhibiting pathogens adherence, competition for lacked nutrients and limitation of invasion by bacteria[(3)]. All probiotics action mode is a strain-dependent, making it vital to be chosen and scientifically compare probiotics for their meant function [(4)]. Combination of some antimicrobial agents may lead to a synergistic relationship which is effective against various infections types as the progress of subsequent failure beside monotherapy resistance is prevalent correlated with the prohibition of the emergence of microbial resistance [(5)].

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Due to the importance and wide spread of burn and wound infections caused by *K. pneumoniae* and the limitation studies on using antibiotics and probiotics either together or individually against this pathogen, the present study was designed for the aim of investigating a new strains of this pathogen and comparing the antimicrobial effect of various types of antibiotics, probiotics and their combinations in order to select and apply the most efficient synergetic treatment.

Samples collection

A total of 73 samples were collected from patients of different ages and genders suffering from burn and wound injury who referred to Baghdad Teaching Hospital, Ghazi Al-Hariri Hospital for Surgery Specialist, Specialty Burn Hospital and Al-Kindy Teaching Hospital in Baghdad from August to November 2019. The specimen was taken by sterile disposable cotton swabs and kept in Brain Heart Infusion broth (Himedia, India) before culturing on MacConkey agar (Mast group, UK) then incubated at 37°C for 24h.

Isolation and primary identification of bacteria

A colony was showed from each positive culture of bacteria and its identification was depended on the morphology properties [6]. Then, colonies were stained by gram to observe a specific Gram reaction, shape and arrangement [7].

Identification of *K. pneumoniae* isolates

a) by VITEK 2 system

Isolates further identified by subjecting to VITEK 2 system [8].

b) by 16s RNA

The results has been analyzed depending on genius software and registered by the National Center for Biotechnology Information (NCBI) [9].

Detection of *Klebsiella pneumoniae* biofilm

By the quantitative method, Microtiter plate (MtP) has been applied for this purpose, each of the bacterial isolates was incubated on MacConkey agar at 37°C along 24h. Then 180 µl of Mueller-Hinton broth (Mast group, UK) was put in each of the 96 wells, and then, 20 µl of bacterial suspension was added to it, and incubated at 37°C for 24h. The isolates stained with crystal violet and washed twice with phosphate-buffered saline (PBS). The microplate was resolubilized by of 96% ethanol and then spectrophotometrically measured at 570 nm using Microplate Reader (Promega, USA). For the qualitative method, Congo red agar assay have been examined biofilm producing by these strains depending on colonies color change, black colonies are considered as biofilm formers while pink ones are non-biofilm producers [10] .

Preparation of probiotic filtrates

The unconcentrated filtrates of *L. casei* and *L. plantarum* isolates were obtained by growing 1ml of each isolate culture in 9ml MRS broth (Oxoid, UK) for 24h at 37°C [11]. After that, the concentrated filtrates were prepared by evaporating100ml of the unconcentrated in the oven at 45°C to reduce the quantity to gain the three-fold concentrated filtrate (12.5ml) .

Dispersal of *K. pneumoniae* biofilm by probiotics-antibiotics combination

The biofilms of *K. pneumoniae* strains were treated with ratios (50, 75 and 25) of combinations of three-fold concentrated filtrates of each of *L. casei* and *L. plantarum* and antibiotics. Plates were incubated at 37°C for 24h. The control wells were contained medium with bacteria only. Following propagation, each well was rinsed with 200 µl PBS, stained with crystal violet solution, then washed twice with PBS. After that, the microplate was re-solubilized by eluting from attached cells with 200 µl of 96% ethanol per well. Thereafter, the stained biofilms were measured at 570 nm by using Microplate Reader (Promega, USA). Each strain was examined for triplicate and mean was taken [10].

Statistical Analysis

Graph pad prism version 8 (Graph pad software Inc., La Jolla, CA, USA) has been used for figures. Calculations and determination of forming biofilm, means and negative control standard deviation has been done according to [10] by using Microsoft Excel program.

Results and Discussion

Isolation and primary identification of bacteria

A total of 73 clinical swab samples, 42 (57.53%) were

obtained from patients wound injuries and 31 (42.47%) from burns. After grown on MacConkey agar, 60; 36 (60%) were obtained from wounds and 24(40%) from burns. Colonies of the isolates appeared as large, round, mucoid and pink in color. Microscopic examination of the suspected *Klebsiella* isolates revealed that they were Gram-negative short rods.

Identification of *K. pneumoniae* by VITEK 2 system

By VITEK 2 system, all the sixty bacterial isolates were identified as *Klebsiella pneumoniae* and gave positive results with 99% of probability.

Biofilms produced by *Klebsiella pneumoniae*:

The results of Microtiter plate (MtP) for biofilm production by *Klebsiella pneumoniae* are shown in fig. (1). After incubation, only 4 isolates, 3 from wounds (symbolled K25, K49 and K51) and 1 from burns (symbolled K19) were able to form biofilms. Among these, K25 isolate was the strongest biofilm producer when its optical density reached 2.67. Adversely, isolate K49 was the weakest biofilm producer with an optical density of only 0.61.

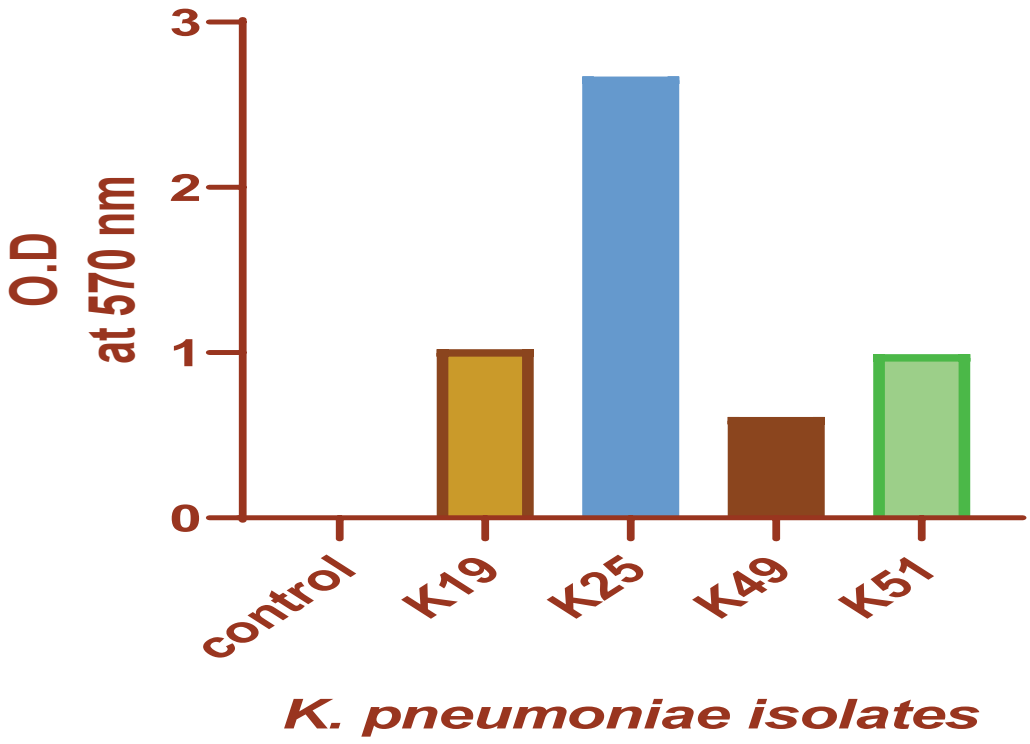


Figure (1): Optical densities of biofilms produced by *Klebsiella pneumoniae* isolates obtained from wound and burn infections by using Microtiter plate method.

After that, the Congo red agar assay was applied for qualitative evaluation of pathogenic biofilm. After incubation of *K. pneumoniae* isolates on the medium at 37 °C for 24h, results showed that only the four biofilm-producing *K. pneumoniae* strains was able to turn color of the medium to black, while others (the non-biofilm producing isolates) were unable to change the red color of medium.

Identification and registration of new strains of *Klebsiella pneumoniae*

The 16S rRNA gene amplification was performed using PCR technique to detect the positive result, 4 new

strains of *Klebsiella pneumoniae* namely (KPBIQ19, KPWIQ25, KPWIQ49 and KPWIQ51) were obtained from Iraqi burn and wound patients have been detected and registered in The National Center for Biotechnology Information (NCBI).

Effect of *Lactobacillus casei* filtrates against *K. pneumoniae* biofilms

Application of the three-fold concentrated filtrate of *L. casei* has eradicated the biofilms produced by three (KPWIQ25, KPWIQ49 and KPWIQ51) of the four *K. pneumoniae* strains, while it has no effect on one (KPBIQ19) strain (table 1). Moreover, highest eradication of biofilm was recorded by *L. casei* filtrate against KPWIQ25 *K. pneumoniae* strain when it led to a distinctive decrease the O.D (0.0) after it was 0.27 before treatment. Regarding KPBIQ49 strain, the *L. casei* filtrate reduced its O.D from 0.15 to 0.01, as well as KPWIQ51 inhibited its O.D from 0.13 to 0.01.

Table (1): Impact of *Lactobacillus casei* three-fold concentrated filtrate on *Klebsiella pneumoniae* strains (KPWIQ25, KPWIQ49, KPWIQ51 and KPBIQ19) isolated from burn and wound infections.

Treatment	Strains	OD average	ODC	2*ODC	4*ODC	strain OD	Results
With L. casei	KPBIQ19	0.21	0.09	0.18	0.36	0.12	+
	KPWIQ25	0.09	0.09	0.18	0.36	0.00	0
	KPWIQ49	0.08	0.09	0.18	0.36	0.01	0
	KPWIQ51	0.10	0.09	0.18	0.36	0.01	0
Control untreated	KPBIQ19	0.21	0.09	0.18	0.36	0.12	+
	KPWIQ25	0.36	0.09	0.18	0.36	0.27	++
	KPWIQ49	0.24	0.09	0.18	0.36	0.15	+
	KPWIQ51	0.22	0.09	0.18	0.36	0.13	+
Negative control		0.08	0.09	0.18	0.36		

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; SD= 0.001

***Lactobacillus plantarum* filtrate against *K. pneumoniae* biofilms**

Lactobacillus plantarum three-fold concentrated filtrate was found to has no inhibitory on biofilms formed by the four *K. pneumoniae* strains. Adversely, it led to the enhancing the biofilms.

Effect of antibiotics against *K. pneumoniae* biofilms

Application of four antibiotics (AK, CAZ, GM and IMI) has eradicated the biofilms produced by four (KPBIQ19, KPWIQ25, KPWIQ49 and KPWIQ51) of the *K. pneumoniae* strains significantly, while no effect

registered when AK used against (KPWIQ49) strain and CAZ against (KPBIQ19) strain.

Effect of antibiotics-probiotics combination on *K. pneumoniae* biofilms

Biofilm produced by KPBIQ25 strain of *K. pneumoniae* has been eradicated by 10 of the 12 combinations (in ratios 50:50, 75:25, 25:75) of *L. casei* and each of AK, GM and IMI antibiotics when the recorded averages of biofilm O.D values were highly reduced from that average of the control (0.27), highest reduction in biofilm O.D (0.02) was obtained for the combination of *L. casei* filtrate and Gentamycin in the ratio of 50:50 (table 2).

Table (2): Effect of combinations of *Lactobacillus casei* filtrate with each of four antibiotics: Amikacin (AK), Ceftazidime (CAZ), Gentamycin (GM) and Imipenem (IMI) in three ratios (50:50, 75:25 and 25:75) on strain KPBIQ25 of *Klebsiella pneumoniae*.

Probiotic	Antibiotic	Probiotic: antibiotic ratio	Strain	OD Mean	ODC	2*ODC	4*ODC	Strain OD	Results
L. casei	AK	50:50	KPBIQ25	0.13	0.09	0.18	0.36	0.04	0
		75:25		0.17	0.09	0.18	0.36	0.08	0
		25:75		0.21	0.09	0.18	0.36	0.12	+
	CAZ	50:50		0.49	0.09	0.18	0.36	0.40	+++
		75:25		0.22	0.09	0.18	0.36	0.13	+
		25:75		0.52	0.09	0.18	0.36	0.43	+++
	GM	50:50		0.11	0.09	0.18	0.36	0.02	0
		75:25		0.13	0.09	0.18	0.36	0.04	0
		25:75		0.20	0.09	0.18	0.36	0.11	+
	IMI	50:50		0.19	0.09	0.18	0.36	0.10	+
		75:25		0.21	0.09	0.18	0.36	0.12	+
		25:75		0.16	0.09	0.18	0.36	0.07	0
Broth	None	Control	KPBIQ25	0.36	0.09	0.18	0.36	0.27	++
		Negative	NC	0.08	0.09	0.18	0.36		

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; SD= 0.001

Additionally, biofilm produced by KPBIQ51 strain of *K. pneumoniae* has been eradicated by 10 of the 12 combinations (in ratios 50:50, 75:25, 25:75) of *L. casei* and each of AK, GM and IMI antibiotics when the recorded averages of biofilm O.D values were highly reduced from that average of the control (0.13). However, highest reduction in biofilm O.D (0.03) was obtained for the combination of *L. casei* filtrate and Amikacin in the ratio of 50:50. Furthermore, biofilm produced by KPBIQ25 strain of *K. pneumoniae* has been eradicated by 10 of the 12 combinations (in ratios 50:50, 75:25, 25:75) of *L. plantarum* and each of AK, GM and IMI antibiotics when the recorded averages of biofilm O.D values were highly reduced from that average of the control (0.27). However, highest reduction in biofilm O.D (0.02) was obtained for the combination of *L. plantarum* filtrate and Amikacin and Imipenem in the ratio of 50:50 as well as Imipenem in the ratio of 25:75 (table 3).

Table (3): Effect of combinations of *Lactobacillus plantarum* filtrate with each of four antibiotics: Amikacin (AK), Ceftazidime (CAZ), Gentamycin (GM) and Imipenem (IMI) in three ratios (50:50, 75:25 and 25:75) on strain KPBIQ25 of *Klebsiella pneumoniae*.

Probiotic	Antibiotic	Probiotic: antibiotic ratio	Strain	OD Mean	ODC	2*ODC	4*ODC	Strain OD	Results
L. plantarum	AK	50:50	KPBIQ25	0.11	0.09	0.18	0.36	0.02	0
		75:25		0.18	0.09	0.18	0.36	0.09	0
		25:75		0.61	0.09	0.18	0.36	0.52	+++
	CAZ	50:50		0.17	0.09	0.18	0.36	0.08	0
		75:25		0.16	0.09	0.18	0.36	0.07	0
		25:75		0.68	0.09	0.18	0.36	0.59	+++
	GM	50:50		0.13	0.09	0.18	0.36	0.04	0
		75:25		0.13	0.09	0.18	0.36	0.04	0
		25:75		0.14	0.09	0.18	0.36	0.05	0
	IMI	50:50		0.11	0.09	0.18	0.36	0.02	0
		75:25		0.14	0.09	0.18	0.36	0.05	0
		25:75		0.11	0.09	0.18	0.36	0.02	0
Broth	None	Control	KPBIQ25	0.36	0.09	0.18	0.36	0.27	++
		Negative	NC	0.08	0.09	0.18	0.36		

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; SD= 0.001

A part of the results illustrated that the biofilm produced by KPBIQ51 strain of *K. pneumoniae* has been eradicated by 8 of the 12 combinations (in ratios 50:50, 75:25, 25:75) of *L. plantarum* and each of AK, GM and IMI antibiotics when the recorded averages of biofilm O.D values were highly reduced from that average of the control (0.13). The highest reduction in biofilm O.D (0.03) was obtained for the combination of *L. plantarum* filtrate and Gentamycin in the ratio (75:25) and Imipenem in the ratio of 50:50 (table 4).

Table (4): Effect of combinations of *Lactobacillus plantarum* filtrate with each of four antibiotics: Amikacin (AK), Ceftazidime (CAZ), Gentamycin (GM) and Imipenem (IMI) in three ratios (50:50, 75:25 and 25:75) on strain KPBIQ51 of *Klebsiella pneumoniae*.

Probiotic	Antibiotic	Probiotic/ antibiotic ratio	Strain	OD Mean	ODC	2*ODC	4*ODC	Strain OD	Results
L. plantarum	AK	50:50	KPBIQ51	0.26	0.09	0.18	0.36	0.17	+
		75:25		0.19	0.09	0.18	0.36	0.10	+
		25:75		0.40	0.09	0.18	0.36	0.31	++
	CAZ	50:50		0.39	0.09	0.18	0.36	0.30	++
		75:25		0.16	0.09	0.18	0.36	0.07	0
		25:75		1.53	0.09	0.18	0.36	1.44	+++
	GM	50:50		0.16	0.09	0.18	0.36	0.07	0
		75:25		0.12	0.09	0.18	0.36	0.03	0
		25:75		0.14	0.09	0.18	0.36	0.05	0
	IMI	50:50		0.12	0.09	0.18	0.36	0.03	0
		75:25		0.18	0.09	0.18	0.36	0.09	0
		25:75		0.14	0.09	0.18	0.36	0.05	0
Broth	None	Control	KPBIQ51	0.22	0.09	0.18	0.36	0.13	+
		Negative	NC	0.08	0.09	0.18	0.36		

0= No biofilm, + = Weak biofilm, ++= Moderate biofilm, +++= Strong biofilm; SD= 0.001

Conclusions

The four biofilm producers were discovered for the first time in this study to be new strains of *Klebsiella pneumoniae* and nominated by NCBI as KPBIQ19, KPWIQ25, KPWIQ49 and KPWIQ51 *Klebsiella pneumoniae* strains. Combinations of *Lactobacillus casei* filtrate with each of the Amikacin, Ceftazidime, Gentamycin and Imipenem antibiotics resulted in significant eradication of biofilm of *K. pneumoniae* four strains, while combinations of *L. plantarum* filtrate with same antibiotics did not do so.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Study and Assess the Occupation Hazards to Health Workers in the City of Kut/ Wassit

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Abstract

Background: Occupational hazards are a major medical, social, economic problem and a leading cause of morbidity and mortality for health workers. The aims of this study was to identify the types of occupational hazards and risks that health workers are exposed to in the study area. In addition, to measure risk level of occupation hazards. This is a cross sectional descriptive study was carried out among health workers in two hospitals located in Kut city in Wasit governorate. The period of the study extended from October 2019, until the end of March 2020. Data collected by using questionnaire form. The occupation hazards dimensions, which include four domains: physical hazards, chemical hazards, biological hazards, and psychological hazards. The statistical methods used included Chi-square and Fisher exact test. A statistical significant association ($p<0.05$)**.** The main findings of the current study indicate that the commonest occupational hazards among the health workers in Al- Kut hospital was noise (58.6%), and the second hazard in this category was fire (51.9%). While in Al-Karamah hospital the commonest occupational hazards among the health workers was noise (57.9%) . Conlusively, the study was found that health workers in Al-Karamah hospital had experienced light, heat, noise, psychological, and chemical hazard higher than health workers in Al-Kut hospital. In addition , the health workers in Al-Karamah hospital had experienced biological lower than health workers in Al-Kut hospital.

Key words: *Occupational hazards, Health workers, Hospitals.*

Introduction

Health workers perform many tasks in hospitals [(1)], and globally, it is currently estimated the percent of health workers is more one-tenth of the working's population (12%)[(2)]. In the context, those activities make them vulnerable to various occupational hazards [(1)] which are a major medical, social, economic problem and a leading cause of morbidity and mortality. Moreover, it have major negative effects on quality of life, due to loss of mobility, loss of work and skilled personnel in addition reduction of productivity[(3)]. The risk factors influencing hospital are various interacting such as biological, chemical, physical**,** and ergonomic, as well as the presence of psychosocial risk in the hospital[(4)]. As reported by WHO, the occupational hazards in the health sector recorded a higher rate as compared with other sectors such as industrial and construction[(5)]. Remarkable, in Europe, as mention early, risk factors above are an overwhelming majority of fatalities and occupation diseases[(6)]. Risk identification is fundamental for an effective preventive management of occupational diseases, as it contributes to the development of a policy and system for occupational health safety which significantly enhance the employee performance, and productivity[(7)]. occupational health safety is plays an important role in preventive and curative management[(8)]. On the other hand a safety workforce is vital for sustainable social and economic

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development on a global, and local level[9]. the study aimed to identify the types of occupational hazards and risks that health workers are exposed to in the study area.

Methodology

This cross sectional descriptive study employed quantitative design**.** The study was carried out among health workers in three hospitals located in Kut city in Wasit governorate. Al-Kut for Gynecology and Children, Al- Karama Teaching Hospital , a pilot study was done at Al- Zahraa Teaching Hospital. All health Workers (266) working in the hospitals**.** The period of the study extended from October 2019, till the end of March 2020. Data were collected through direct interview of health workers using questionnaire form. Occupational hazards assessment questionnaires was developed and constructed by the researchers and modified by expert panel. The questionnaire form consisted of three parts: First part concerned with Socio-demographic characteristics of the studied respondents regarding: age (years), gender,...etc.) The second one was the occupational characteristics of health workers regarding health care provider (classified as physician, technician, nurses, others),….etc. The third section was the occupational hazards dimensions which include four domains: physical hazards that are encountered in hospital working environment include: lighting condition and ergonomic stressor (11 items), heat (3 items), radiation (5 items), noise (6 items). Chemical hazards (16 items) and biological hazards (13 items). Three –points Likert scale were used to assess these domains, it ranged from (1-5). Psychological hazard (10 items) was assessed by using two points Likert scale. Data entry was done using the SPSS (25) by using frequencies and percentage, Chi-square test (X^2) and Fisher exact (it was used when Chi –squared test was not suitable). The differences between observations were considered significant at $p \leq 0.05$.

Results & Discussion

Regarding the age, the current results demonstrated that 75.9% of health workers in Al Kut Hospital were in the age group ≤30years and 70.7% of health workers in Al Karama Hospital. These findings agreed with what has been stated by Azage Muluken et al 2013 in Ethiopia[10].(Table 1) . This study indicates that almost half of the health workers in two hospital were married, and the lowest of the health workers were others, findings similar to Maryam Mollazadeh et al , 2018 [12]. In respect to the residence, the majority of the health workers in two hospitals in this study were living in urban area. Highest percentage of health workers in two hospitals were have institute &higher level. Such result was consistent with the findings of OlufemiOludareAluko et al. 2016 in Nigeria[14].(Table 2). On the profession of healthcare workers, technician and others encompass the largest group revealed in the study almost (33.1%, 32.8%) of respondents respectively. This finding was opposite to the study of Hailemariam Gezie1*et al.*, 2019 in Ethiopia[15].This might be interpreted due to the fact that lack of nursing staff in Iraq, in addition to the increase in private and government colleges that graduate large numbers of medical technicians.

According to their working area, the majority of respondents (58, 22.3%), working at emergency ward. However, this fact disagreed with the finding of Azage Muluken et al 2013 in Ethiopia [10]. Concerning length of experience of health workers in two hospitals , The highest proportion of health workers were in the length of experience (≤5). The association between two groups was not significant (P >0.05), which it agreed with that stated by KURT VAZ et al 2010 in India[16]. (Table 3).

The study findings showed that the physical health hazards that had the highest frequency of occurrence among the workers was noise with rate 57.9% in Al-Kut Hospital and 58.6% in Al-Karamah Hospital, while heat and temperature had the lowest frequency with rate 3.8% in Al-Kut Hospital and 17.3% in Al-Karamah Hospital. This equally corresponds to study by Fernandes MA & Marziale MH[17]. Previous study had suggested that chemical hazard would be a pervasive problem among health workers[18]. There were different exposure incidents to chemicals experienced by more than a third of the health workers in two hospitals. This is in line with study findings by Opelo Keorekile.[19] Further, this is slightly lower than the results of local study in Iraq , Saad *et al.*2017[20]. Hazardous chemical exposure occurred in a variety of forms-including to airborne aerosols, detergents and disinfectants and others. The most common exposure was exposure to airborne aerosols . More than half of the health workers reported was irritation of the eyes, nose, and throat caused by exposure to airborne aerosols or contact with drops of washing and cleaning fluid.

Exposure to biological hazards among the health workers was low with rate 31.6% in Al-Kut Hospital and 24.1% in Al-Karamah Hospital. This finding was opposite to the study of Arasi Senthil*et al.*, 2015 in India[21]. In Al-Kut Hospital , the commonest biological hazards among the health workers was direct contact with samples (64.7%). These results were nearly similar to study in Nigeria , Osungbemiro et al, 2016 [22] .

Among those that experienced occupational hazards, exposure to psychological hazard among the health workers was low with rate 6% in Al-Kut Hospital and 18.8% in Al-Karamah Hospital . This is not same with C.U. Okeafor and F.E. Alamina's study conducted in Nigeria[24]. About 42% of health workers experienced verbal violence from patient and / or patient relatives in Al-Kut Hospital and 53.4% in Al-Karamah Hospital , which is considered the highest percentage from psychological hazard in this study. This tallies with results of study done by Mohamad Kitaneh and Motasem Hamdan, 2012 in Palestine. Another important psychological hazard that occurred with significant proportion among study participants was bulling from boss. The study showed result that among the 19.5% who reported exposure to bulling from boss in Al-Kut Hospital and 33.1% in Al-Karamah Hospital. This is more lower than the percentage of R.L. Difazio *et al.* 2019 in Russia[25]. (Table 4).

Table 1 : Distribution of health workers of hospitals according to age and gender.

Variables			Al Kut Hospital for Women and Children			Al-Karamah Teaching Hospital			Total
			Male	Female	Total	Male	Female	Total	
Age	≤30	F (%)	27 (61.4)	74 (83.1)	101 (75.9)	38 (57.6)	56 (83.6)	94 (70.7)	195(73.3)
	31-40	F (%)	8 (18.2)	10 (11.2)	18 (13.5)	17 (25.8)	5 (7.5)	22 (16.5)	40 (15.0)
	>40	F (%)	9 (20.5)	5 (5.6)	14 (10.5)	11 (16.7)	6 (9.0)	17 (12.8)	31 (11.7)

df=2 p-value= 0.625

Table 2: Distribution of health workers of hospitals according to Demographic Characteristics.

Variables			Al Kut Hospital	Al-Karamah Hospital	Total	p- value
Marital status	Married	F (%)	66 (49.6)	73 (54.9)	139 (52.3)	0.712
	Single	F (%)	64 (48.1)	60 (45.1)	124 (46.6)	
	Others	F (%)	3 (2.3)	0 (0.0)	3 (1.1)	
Residence	Urban	F (%)	125 (93.98)	127 (95.5)	252 (94.7)	0.784
	Rural	F (%)	8 (6.02)	6 (4.5)	12 (4.5)	
Education status	Primary	F (%)	2 (1.5)	0 (0.0)	2 (0.8)	0.149
	Secondary	F (%)	1 (0.8)	2 (1.5)	3 (1.1)	
	Intermitted	F (%)	25 (18.8)	16 (12.0)	41 (15.4)	
	Institute &Higher	F (%)	105 (78.9)	115 (86.5)	219 (82.3)	

Table 3: Distribution of health workers of hospitals according to Occupational Characteristics

Variables			Al Kut Hospital	Al-Karamah Hospital	Total	p- value
Health care provider	Physician	F %	7 (5.3)	12 (9.0)	19 (7.1)	0.110
	Technician	F %	39 (29.3)	49 (36.8)	88 (33.1)	
	Nurse	F %	48 (36.1)	24 (18.0)	72 (27.1)	
	Others	F %	39 (29.3)	48 (36.1)	87 (32.8)	
Years of experience	≤5	F %	72 (54.1)	77 (57.9)	149 (56.0)	0.825
	6-10	F %	33 (24.8)	30 (22.6)	63 (23.7)	
	>10	F %	28 (21.1)	26 (19.5)	54 (20.3)	
Department *(Working area)	Operations Department	F %	15 (11.3)	1 (0.8)	16 (6.0)	0.000*
	Intensive Care	F %	8 (6.0)	5 (3.8)	13 (4.9)	
	Emergency	F %	58 (43.6)	86 (64.7)	144 (54.1)	
	Medical Laboratories	F %	4 (3.0)	2 (1.5)	6 (2.3)	
	Radiology Department	F %	48 (36.1)	39 (29.3)	87 (32.7)	
	Others	F %	15 (11.3)	1 (0.8)	16 (6.0)	

*** Fisher's exact test**

Table 4: Score of Occupational Hazards

Hazards		Hospitals		Good and acceptable	Poor	p-value
Physical hazards	lighting condition and ergonomic stressor	Al-Kut Hospital	F (%)	106 (79.7)	27 (20.3)	1.000*
		Al-Karamah Hospital	F (%)	105 (78.9)	28 (21.1)	
	Heat	Al-Kut Hospital	F (%)	128 (96.2)	5 (3.8)	0.000*
		Al-Karamah Hospital	F (%)	110 (82.7)	23 (17.3)	
	Radiation	Al-Kut Hospital	F (%)	68 (51.1)	65 (48.9)	1.000*
		Al-Karamah Hospital	F (%)	68 (51.1)	65 (48.9)	
	Noise	Al-Kut Hospital	F (%)	56 (42.1)	77 (57.9)	1.000*
		Al-Karamah Hospital	F (%)	55 (41.4)	78 (58.6)	

Cont... **Table (4(: Score of Occupational Hazards**

Chemical Hazards	**Al-Kut Hospital**	**F (%)**	**83 (62.9)**	**49 (37.1)**	**0.899***
	Al-Karamah Hospital	**F (%)**	**82 (61.7)**	**51 (38.3)**	
Total		F (%)	165 (62.3)	100 (37.7)	
Biological Hazards	Al-Kut Hospital	F (%)	91 (68.4)	42 (31.6)	0.218*
	Al-Karamah Hospital	F (%)	101 (75.9)	32 (24.1)	
Total		F (%)	192 (72.2)	74 (27.8)	
psychological hazards	Al-Kut Hospital	F (%)	125 (94.0)	8 (6.0)	0.002*
	Al-Karamah Hospital	F (%)	108 (81.2)	25 (18.8)	
Total		F (%)	233 (87.6)	33 (12.4)	

*** Fisher's exact test**

Conclusions

Majority of health workers fell in age the group (≤30) years and most of them are females, married, and had institute &higher level. Noise seem to be a major concern among the health workers in both hospitals. Overall the level of occupation hazard among health workers was low.

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Associated Factors of Adulthood Overweight/Obesity among Pharmacy Department Students in Al-Rafidain university College / Iraq

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Abstract

Youth people overweight or obesity has become a main public health concern globally because of its adversarial health outcome and escalating prevalence. The aspects of disease conditions demonstrated during early older life commonly originate in youth stage of life. Iraq after wars is went through a transition where they started to go under-nutrition and co- exists with obesity. even though, there is a lack in documented information on college life and being overweight or obesity in Iraq. our study was carried out to documented for the first time the prevalence and associated factors of adulthood overweight or obesity among pharmacy department student in Alrafidain university college. Data were collected using a structured self-administered questionnaire from February 2019 to December 2019in grades 1–5 students of pharmacy department in Alrafidain university college

Height and weight measurements of 300 students were taken and BMI- for-age-sex was calculated using WHO AnthroPlus. Based on that, participants were divided to three groups as normal weight (BMI 20–25), overweight (BMI 26.0–30), and obese (BMI > 31.0).

Participates response rate is 81%. Age of students take a part in the current study was 21.43 ± 3.81 years. The category of students were 96 students (32%) overweight student 90(30%). The significant different $P<0.05$ among groups were found of particular questions included eating outside their home.

Keywords: *Overweight/obesity, Body mass index, Prevalence, pharmacy college, students*

Introduction

Excessive fat accumulations or abnormal gathering fat are defined as overweight and obesity that may seriously effect today or tomorrow on health[(1)]. In developed courtiers, It is known with common public nutritional disorder and recently obesity and overweight percentage among people is increasing even in developing countries as well[(2)]. Adverse metabolic changes can be produced from being overweight and obese. This change may be including increases in blood pressure, high cholesterol levels, unfortunately increasing in insulin resistance[(3)]. unfavorable, overweight and obesity would elevate the risk of heart diseases, gall- bladder disease, brain and hart strokes, diabetes, atherosclerosis, many forms of cancers, especially breast cancer, and it also may be reason for hypertension and kidney failure. Based on that increase the rate of being obeys or overweight through childhood and adulthood become one of the most serious public health concerns in 21st century[(4)].

An unhealthy nutrition is a main adaptable behavioral risk factors that would increase number of people who consider at obesity states. . However, in last decade a variety of dietary recommendations and several

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methods to prevent of obesity which have been projected, indications unpredictable and varies between procedures of dietary intake. Additionally, dietary guidelines play role in obesity- associated health disorders[5].

Decrease physical activity eventually principal to overweight condition and obesity. it is also considered as risk factor for the global deaths. Regular physical activity would be obviously beneficial of set out across the lifespan. A several of pervious study strongly suggested, based on observation and experiment researches, that consistent Physical activity among young and adolescent people can provide instantaneous and on long-term welfares for physical health[6].

Iraq has become one of the fastest growing economies counties after war. Augmented number of people lately who prefer urbanization life and increased income which increased access to fast food. In addition, technology developments and many different changes in the lifestyle in the last decades results in more sitting and rest which lead to more obesity. These alterations in the dietetic patterns and routines of life as coming of viewing of television for hours, using computer, browsing Internet, and calling through cell phones which have occasioned in rapid nutrition modification that in turn would be along with consequent rise in the overweight conditions and obesity among Iraq adolescent's and adult's[7, 9].

Many different performances have been used to determine the obesity. Easiest and cheapest technique of weight and height for Body Mass Indexed (BMI) beside waist–hip ratio method, number of fat and size of cells, body thickness. Electrical technique which are more complex has been utilized such as magnetic resonance imaging, dual energy X-ray absorptiometry, bioelectrical impedance, ultrasound, air dislocation plethysmography, and calculated tomography. Each method from what has been mentioned above has limitations and differs in suitability subjected on the features of the population under the study and time likewise resources accessible. Due to its straightforwardness and ease of application, BMI is the most frequently used method of measurement which is known as reliable and desirable method to prompt the grade of overweight[7, 10].

Studies of prevalence of obesity which are established depended on dietary habits, Physical activity, and lifestyles among college students are infrequent. Therefore, the major aims of this manuscript were to explore the prevalence of obesity or/and overweight according to the BMI and investigate dietary habits, Physical activities, varies of lifestyles and demography among the pharmacy college students at the department of pharmacy, Alrafidain University college , Iraq.

Materials and Methods

Cross-sectional study include questionnaire constructed research procedure was approved by the Ethical Committee of the Alrafidain University college.

Our study was conducted from February 2019 to December 2019. all of requirement data were collected throughout an unidentified questionnaire which designed to be costume the necessities of the current study.

Questionnaires and instructions for answer and informed consent letter were personally handed to a suitably selected sample of 370 students. A total of 300 (response rate 81%) students enrolled in stage 1–5 grade of Bachelor of Pharmacy college students.

After the consent letter signed and an agreement of the participating students. participants genders, age, grade of study, height, and weight to get BMI were recorded.

Height was documented by cm, starting form heels to highest uppermost position on the head. Body weight was examine using a digital scale, considering all participants wearing light scrubs. The BMI was computed by dividing weight by height (kg/m^2).

After BMI calculated the participation divided to three groups based on the BMI value. normal weight (BMI 20–25), overweight (BMI 26.0–30), and obese (BMI > 31.0)

Questionnaire also included five different questions regarding the dietary habits. These questions involved question about total of meals, meals eaten outside home, snacks, and sweets consumption and amount of soft drinks per day.

Moreover, the questionnaires included five questions considering physical activity conducted by the participants. These questions encompassed about physical implementation per week, hours completed

during exercising, amount and sort the sports per week, and how many hours walking per day

In the third section of the questionnaire, the data regarding the participants were recorded. lifestyle routine questions also taken part of the questionnaire, which is included questions about hours sleep per day, how many hours spent study, time spent with friends outside, and time spent setting using technology screen. all the participants respond to all questions where no time limitation was recorded for them therefore there are no missing data.

Results

300 out 370 approached in college of pharmacy to fall the questioner were responds. prevalence of obesity to reach 96 students (32%) overweight students 90(30%) and students who have normal weight were 114 (38%) this division was best on BMI values. Mean and Standard division of weight, high, calculated BMI and age showed in table 1.

All questions related to dietary habits illustrated in table 2. Even though results in Table 2 demonstrated that obese students were high in all the responds, there are a significant different just among students who eating outside home comparing with other groups included in the current study.

Lifestyle questions showed in table 3. Significant increase among college students who spend more time using internet or computer.

Table 1: Mean (M)and standard division (SD)of age, high, weight, BMI

Variables	M±SD
Age	23.43 ± 3.81
Weight	87 ± 12.16
High	179± 10.95
BMI	27.70± 6.43

Table 2: Explain the percentage of responds of the three groups about dietary questions

	Normal weight %	Overweight %	Obesity %
Having breakfast			
Never	2.5	2.1	4.7
Rarely	49.6	49.7	39.7
Sometimes	43.4	41.3	45.8
Every day	4.5	6.9	9.9
			P value =0.41
meals eaten outside			
Never	13.2	4.7	13.5
Rarely	58.9	59.6	35.2
Sometimes	25.4	33.3	25.6
Every day	2.5	2.4	7.7
			P value =0.63
Having fast food			
Never	14.3	19.8	12.1
Rarely	46	47.3	44.3
Sometimes	31	27.2	35.2

Cont... **Table 2: Explain the percentage of responds of the three groups about dietary questions**

Every day	8.7	5.7	8.4
			P value =0.85
Eating out home			
1	20.1	19.7	9.7
2	41.4	48.2	46.9
3	28.3	28.1	34.8
>4	10.2	4	8.6
			P value =0.041*
Eating snacks or sweets			
1	17.2	18.1	18.1
2	50.1	48.6	48.6
3	22.3	24.8	24.8
>4	10.4	8.5	8.5
			P value =0.29

Table 3:Explain the percentage of responds of the three groups about lifestyle questions

	Normal weight %	**Overweight %**	**Obesity %**
Doing something to lose weight			
eating heathier food	26.7	12.2	21.9
Watching portion sizes	51	55.2	39.2
Dieting on my own	18.9	26.1	31.1
Eat more vegetables	3.4	3.5	7.8
			P value =0.92
Sleeping hours per day			
<5	16.1	23.3	23.6
5-7	35.7	26.8	19.8
8-10	29.3	29.9	27.1
>10	18.9	20	29.5
			P value =0.37
Time (hours) of watching TV per day			
<1	28.1	9.3	19.1
1	33.7	8.6	27.8
2	29.8	28.1	20.5
>3	18.4	54	32.6
			P value =0.049

Cont... **Table 3:Explain the percentage of responds of the three groups about lifestyle questions**

Duration (hours) of mobile surfing			
<1	31.8	9.6	2.3
1	19.2	36.7	29.1
2	31.7	29.1	21.3
>3	35.3	24.6	47.3
			P value =0.91
Times browsing internet or using computer			
<1	11	35.1	15.3
1	61	52.4	59.2
2	21	16.2	23.6
>3	1.7	2.3	20.9
			P value =0.031

Discussion

Our study provides appropriate information about the prevalence of overweigh and obesity among pharmacy college student and approve the correlation with their eat, drink, exercises and lifestyle. All required data was collected through survey for this purpose. 81% is satisfactory rate of response among questioner handled to students through class days.

High prevalence of overweight and mostly obese students in pharmacy collage reflects great thoughtful changes in behavioral patterns and social life, difficulties of this collage comparing to general community. prevalence of obese among these students would be related to nutritional habits that are irregular or improper quality such as eating more outside or eating junk food during library study time or living in students' special home or dormitories. The outcome of current study showed that percentage of eating higher than three meals or higher is increased among overweight students and obese. we also noticed that higher percentage of overweight and obese students who have more snacks and sweets which needs to be considering dietary education program among college students since it is ignored in our education system[10].

Physical activity that are important to contribute in total daily energy spending and would help individuals to have healthier body weight and configuration than inactive student[11]. Elevate rate of cardiovascular disease, several types of cancer, diabetes could be produced from physical inactivity and unhealthy behaviors and diets, consequence global burden and more death in Arab countries[12]. popularity of physical inactivity and weight apprehension remain as major public health concern[13]. Results from current study demonstrated that there is no concern about weight and being overweight or obese among students participating. Most of overweight and obese students can be classified beyond to physical inactive compared to normal weight students. From table 3 the high percentage of overweight and oboes group students who do not have regular exercise, concern about time spend in exercise session, doing different sport to lose weight, and pay attention to walk daily in purpose for being at good weight comparing to normal weight students.

High stress with notable difficult tasks to personal welfare for students is considered as education at schools[14, 15]. Educational stress pronounces the occurrence and inference of study related stress and unhealthy habits among students and the negative impact on students' performance[16].

Previous study conducted on Medicine college students proposed a several factors including large

course work, financial concerns, lake in sleep, academic pressure, dealing with patients suffering from different pain and patients facing deaths that are classified as main stressor factors that have negative effect on students[17, 18]. Other researches would give the reason for gaining weight to anxiety and emotional distress which produced highly during academic examination periods that lead eat unregularly and being inactive[15].

Most of the students participate in the current study through the personal meeting were complaining luck of time is the main reason for not doing exercise. This result come along with the results of study conducted on dental students in Saudi Arabi. Laziness would be another reason for not do not exercise or there are no gyms available handy in the student's area[19].

After last war in Iraq, it has undergone huge change in life behavior and style. Elevate the number of populations who urbanized, increase the income, as well as increase number of fast food restaurants everywhere, wide use of internet and technology which in turn increase students who are overweight or even obese. The new lifestyle with new Iraq becoming more and more inactive and energy outlay is condensed. Studied and dramatic social life that undoubtedly changed in the last two decades lead to negative influence on health.

Several of previous studies report that increase utilizing internet and computer cause increasing number of child and adults who suffer from overweight or obese. These results agreed with results from current study where we noticed increase college students who using more hours from internet or computer in way that are significantly correlated with their BMI. Expending hours of using internet or computer may be related to study requirement in pharmacy collages and Whom work[20, 21].

Conclusion

Results of our study come out with that incidence of overweight and obese is considerably high among pharmacy college students which need more concern, and this really alarmed need more attention. Pharmacy students do not care about watching their food or have physical activity and obese and overweigh students spend more time on internet and screens comparing to students who have normal weight. knowledge about the consequences of overweight and obese should be increased. Avoid raise obese individuals among college students by increase the awareness of physical activity and healthy nutrient.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Serological, Culture and Urea Breath Test for Detection of H. Pylori in Gastric Ulcers Patients

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Abstract

Helicobacter pylori t is the causative agent of duodenal ulcers and gastric ulcers, thus, it is very important to determine the most appropriate system used to diagnose it. The current study use invasive and non-invasive process for diagnosis of *H. pylori*. Culture of *H. pylori* is one of these invasive systems, and the results revealed that 35% of specimen was culture positive (62.5% in male and 37.5 in female) While, the non-invasive method like UBT and ELISA IgG and IgA show 67.1% and 71.5% , 29.2% positive results respectively. The distribution of positive test results between male and female by UBT was (41.3%) male and (58.7%) female) while, the distribution of positive test results between male and female by ELISA IgG and IgA were (61.4%) , (26.3%) in male patients and (78.75%) , (31.25%) in female patients respectively. The results of UBT were increase with age, (34 positive patients in age group 51-60 years old). Serological test of ELISA IgG shows that great % of *H. pylori* infection (74%) found in patients with group age (41-50) yr.O. and the lowest percentage (39%) was found with age group (< 20) years old while, percentages of positive cases of ELISA IgA in young age below 20 years old was 79% related to low % with ages increase.

Key word: *Helicobacter pylori, UBT, H. pylori culture, H. pylori IgA, IgG.*

Introduction

Helicobacter pylori are a spiral-shaped, the persistently Gram-negative bacterium colonizes stomach of the human. It is the essential occasional agent of chronic gastritis superficial and disease ulcer peptic, infection with *H. pylori* is strongly linked with the development of gastric lymphomas and gastric cancer [1].

H. pylori infection needs report as 82% in evolving and 20 to 50% in advanced countries [2]. High propagation of *H. pylori* in some parts of the world is linked, with great happening of severe diseases gastric like peptic ulcers in China (17.2%) or cancer gastric in advanced (61.4%) and evolving countries (64.4%) [3].

Current indications for diagnosis of *H. pylori* infection include non-invasive procedures such as (UBT), fecal *H. pylori* antigen (active infection) and serology (active or prior infections). Invasive tests for *H. pylori* infection include upper gastrointestinal endoscopy, coupled to rapid urease test, culture and histology. Thus, noninvasive systems particularly UBT and tests serological stay the alternate choice of screening the prevalence of *H. pylori* in great populations through epidemiological studies [4].

Methods and Patients

Patients

The study was performed on 137 unselected gastric ulcers **patients (male: 57, 41.6% and female: 80 , 58.4%), aged 15-77 years, who was referred to the endoscopy room of** Disease **Digestive Center in Marjan Medical Hospital, Babylon, Iraq. Biopsy,** Urea breath test and blood sample collection were **done for all patients.**

Urea breath test:

The UBT test is the standard diagnostic tool (Heliforce® - China) and consists of 75 mg ^{13}C-labelled urea in 10 mL of 1.4 g of citric acid solution. Subjects

fasted for at least 2 hours and were free of medication which could influence the UBT results (i.e., of antibiotics in the prior month and no proton pump inhibitors in the previous two weeks) [5]. During the test, the patient swallowed the solution, added on-site to 100 mL tap water. UBT results depend on the gastric hydrolysis of urea by *H. pylori*. In case of infection, $^{13}CO2$ appears in breath, while ammonia is released into the stomach. Breath samples were analyzed by the same ^{13}C-infrared analyzer (HeliFANplus®, FAN GmbH, Leipzig, Germany). The differences between the values at T30 and baseline were expressed as delta over baseline (DOB, δ‰), with a normal cut-off up to 4‰. As recommended by the manufacturer, the DOB at T30 was the reference value.

Procedure:

1) Subjects fasted for at least 2 hours before the examination. 2) Slowly exhale into the first air bag and make it full of gas as much as possible. Immediately fasten the lid of air bag tightly. This one is the base line bag (T=0). 3) Dissolve the urea [^{13}C] granules in 80-100 ml cooled water, drink it up and set still for 30 minute. 4) When time up, collect the second breath sample (T=30) like step tow. 5) Test the tow sample bags with the IR-FORCE infrared Spectrometer.6) Use δ‰ (millesimal difference) to determine the result. Δ‰ is define as :

$$\delta‰ = \frac{\text{13C – isotopic abundance of 30 min. sample} - \text{13C – isotopic abundance of 0 min. sample}}{\text{13C – isotopic abundance of 0 min. sample}}$$

Culturing of *H. pylori*:

Two samples of antral biopsy are acquired for each patient. One for histological examination and the second of culture ,examination smear and put in sterile semi solid transport media (bacteriological agar 0.16 %, NaCl 0.9 %) and transported to the laboratory of microbiology before 2 hr. **Antral biopsies was inoculated onto surface of selective Brucella blood agar including** defibrinated **7% of the blood sheep , trimethoprim (5 ppm),vancomycein (5 ppm), amphotericin B (4 ppm) and polymyxin B (50 µg/L) . Cultured plates was incubated at 37 ℃ under conditions microaerobic for rise humidity cultures was examined after 5-7 days of incubetion of observing pinpoint glistening colonies. Cultures Negative was furthermore incubated and spotted upward to two weeks. A bacterial strain was identified as *H. pylori* on the basis of Gram stain and spiral microscopic semblance as well as activities positive of oxidase** catalase and **urease [6].**

Serological tests:

ELISA IgG and IgA kits (Monobind Inc., USA) were used to analyze each serum samples following the instruction of manufacture supplied by kits.

Ethical approval:

The base ethical approval of the Digestive Disease Center in Marjan Medical Hospital, Babylon, Iraq was obtained. Furthermore, all collected patients of the work was knowledge and the approval was acquired of each one before the aggregate the samples.

Result and Discussion

Urea Breath Test:

Out of 137 patients with peptic ulcer, 92 (67.1 %) were positive for UBT, 38 (41.3%) male and 54 (58.7%) female (Table-1). This result agreement with results obtained by Rotem *et al,* [7] who found that *H. pylori* positive result via UBT is more in female (60.7 %) than male, while Eisdorfer *et al,* [8] found that the prevalence of *H. pylori* contagion was like women and men, 51.5% vs 52.9% .

Table-1: UBT positive and sex distributions

Methods	sex groups	
	Male	Female
UBT	38 (41.3%)	54 (58.7%)
Total	92 (100%)	

In this study the UBT was increase with age, (34 positive patients in age group 51-60 years old) as shown in figure-1. These results were come in line with result obtained by Rotem *et al,* [7] but differ from results obtained by Zevit *et al,* [9] who found that UBT positive result of *H. pylori* was increase with decrease age. This difference may by beyond to deference in geographic region of study and socioeconomic state for people associated in these study.

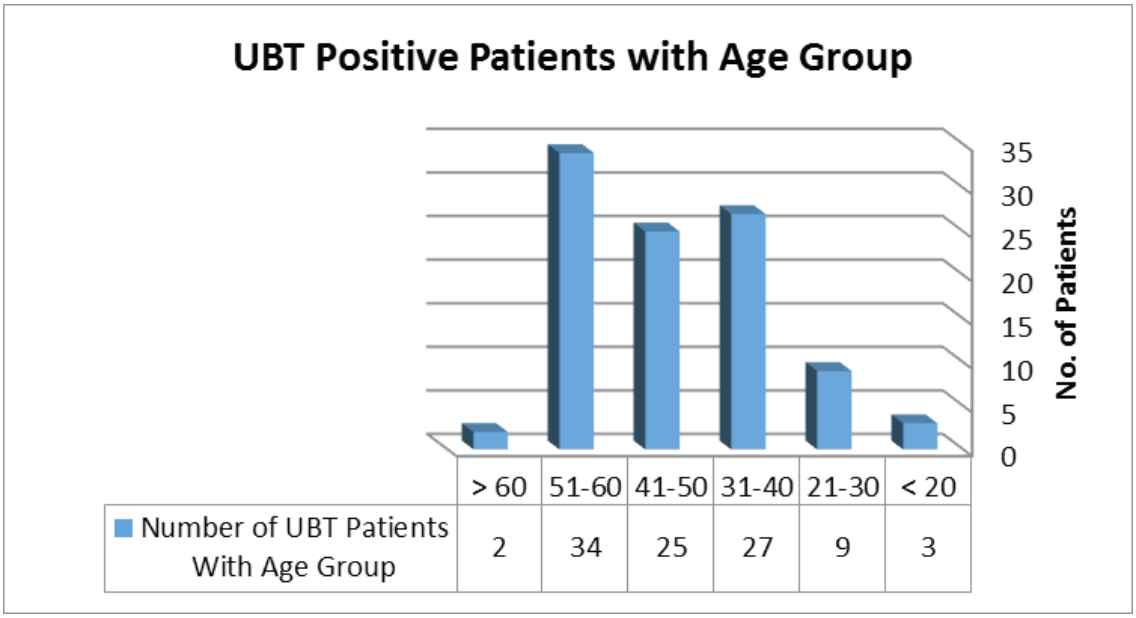


Figure-1: Correlation between positive UBT and age.

Culture and ELISA IgG & IgA for detection of *H. Pylori*:

Two separate ELISA kits (IgG and IgA) were utilized of detection of *H. pylori* infection in 137 patients and utilizing a culture system for demonstrated *H. pylori* in biopsies. The microaerophilic organism was identified in 35% of Culture (62.5% of positive samples were recorded in males compared with 35.5% in females) as illustrated in Table-2.

Table-2: Percentage and distribution of *H. pylori* culture with sex.

Methods	With H.pylori/ Number of patients %		Without H.pylori/ Number of patients %	
Culture	Male	Female	Male	Female
	30 / 62.5	18 / 37.5	27 / 30.3	62 / 69.7
Total	48 / 35		89 / 65	

This findings were in line with those reported by other researchers [11] who observed that *H. Pylori* positive culture effects are observed in (37.2%) of patients.

While, *H.pylori* was observed via ELISA IgG and IgA in 71.5% and 29.2% of patients at the same order, appear Table-3.

Table-3: Serological tests and cultural systems utilized for detection of *H. pylori*.

Test	Positive for H. pylori		Negative for H. pylori	
	NO.	Percentage	NO.	Percentage
IgG	98	71.5	39	28.5
IgA	40	29.2	97	70.8

ELISA IgG was agreed by [11,12] that ELISA IgG were found to be 73.9 % and 73 % respectively in patients with *H. Pylori* infection, while those results were in conflict with the results of [13] which found only 32.3%. The causes of these dissimilar outcomes may be due to the geographical dissemination of the *H. pylori* infection and stage of infection. However, ELISA IgA was same to those found by [12], that found patients 25% was positive of *H. pylori*, and dissimilar to those found via [11,13], who found 82.3% and 58.2% respectively. The similar factors mentioned in ELISA IgG can be attributed to this dissimilarity in the results.

The result of ELISA IgG shows that great % of *H. pylori* contagion (74%) found patients with group age (41-50) years old whereas, the lowermost percentage (39%) found with group age (< 20) years old appear figure-2. The significant correlation among ages and positive *H. pylori* cases with ELISA IgG in positive cases increase with age increase. These result is like they found via the study of [12-14] who found A major impact of age on *H. pylori* positive cases in which anti- *H. pylori* IgG antibodies have been increased with ages increased, whereas The results distressed with results [15] who found no association among age and seropositivity of *H. pylori*. This may be due to that risk of infection which tends to increase with age or due to the iron and vitamin deficiency are decrease in elderly that lead to increase the *H. pylori* infection in this group as demonstrated by [16,17].

High percentages of positive cases reported by ELISA IgA were 79% in people under the age of 20 years compared to percentages low with ages increasing; as appear in fig. 2. But there is no significant correlation among age groups, *H. pylori* infection via ELISA IgA test. These results were in disagreement with those of [12], found that *H. pylori* IgA seropositivity increased with age.

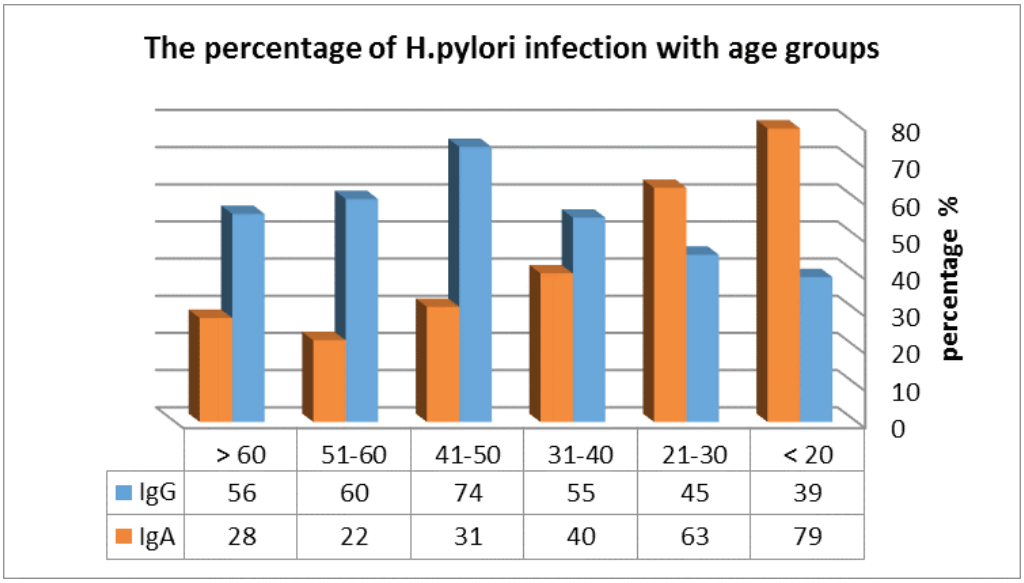


Figure-2: Percentages of *H. pylori* infection in age groups utilizer techniques ELISA.

H. pylori detected 35 (61.4%) and 15 (26.3%) in male patients via ELISA IgG and IgA at the same order. While, in female patients, *H. pylori* detected 63 (78.75%) and 25 (31.25%) via ELISA IgG and IgA at the same order, appear in the Figure-3.

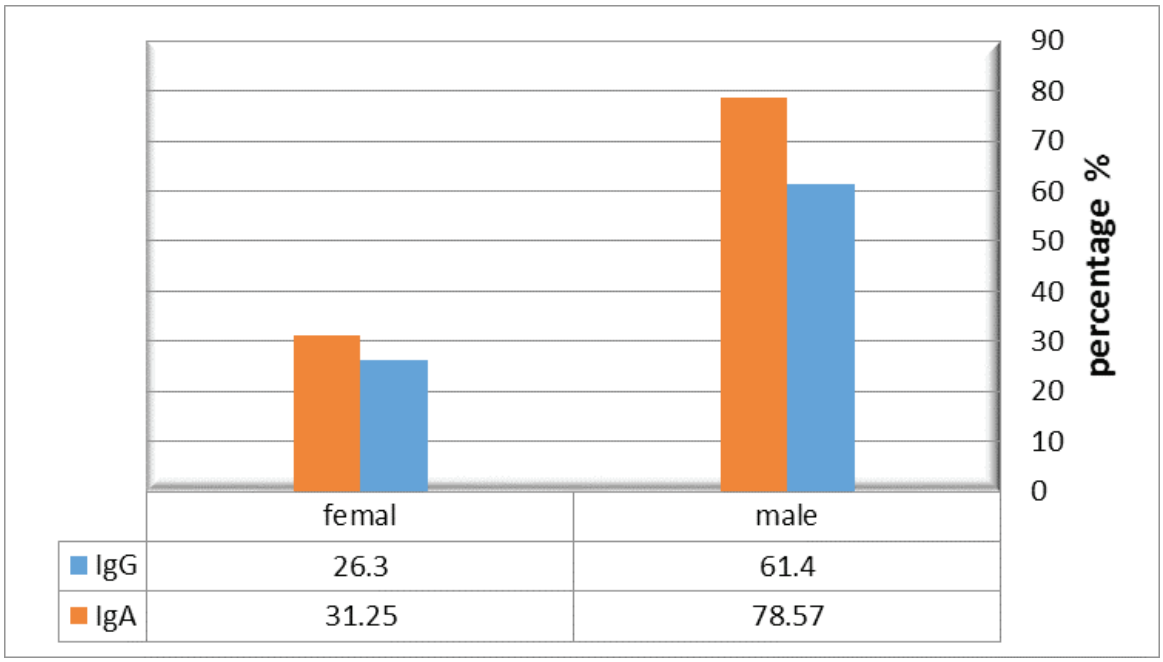


Figure-3: Percentages of *H. pylori* infection in 6 groups via ELISA IgG and IgA tests.

This study reached that there is no significant correlation among sex and *H. pylori* screening methods of ELISA (IgG and IgA). These findings was parallel to results obtained by [18,19] for IgG , [13] for IgA, while difference of the [20] found the males was most infected than females via ELISA IgG and IgA.

Conclusion

Considered the UBT is one the utmost essential and reliable noninvasive process of detection of *H.pylori*; UBT 13C is suitable for epidemiologecal study of the adults and children , particularly when endoscopy is not strictly necessary; ELISA IgG was superior to ELISA IgA for the detection of anti *H. pylori* in adults patients; and Found among age and *H. pylori* infection via ELISA IgG in which *H. Pylori* contagion increase with the age increase.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Low Pollution Emission to Purify Fuel Cell Technology Comprehensively: A Review

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Abstract

In recent decades, scientists have been urged to build the new energy resources by depleting fossil fuel, high-energy demand and pollution. Being one of the principal alternative power devices, Thanks to the high energy conversion efficiency, the fuel cells have attracted growing attention, simple operation and low pollution emissions. This paper surveys the historical backdrop of the power devices, the energy unit activity, and sorts of energy units, their points of interest, inconveniences and applications.

Keywords: *toxicity; Fuel cells, Energy resources, High-energy efficiency, easy to convert operation, Low pollution emission.*

Introduction

Energy is the most necessary to rise for every nation on this planet and energy usage per capita is steadily growing. The energy security, economic growth and protection of the ecosystem are the national energy policies of the countries [(1)]. The rapid increase of energy consumption, the fossil fuel depletion and the rising of greenhouse gas emission have led to an energy security issue over the worldwide. During the last decades, scientists and researchers have made efforts to find solutions for these problems by utilizing more efficient and effective power generation systems and finding cleaner and renewable energy sources [(2, 3)]. In line with energy generation systems development; the cogeneration systems, and produce the power heat, have been received much attention. The history of cogeneration technology comes back to 1977 when the coal and oil were used as the fuels, but with increasing the fuel price, it became gloomy[(4)]. However, this technology became more popular in the last decade on finding out new, renewable, clean and economical Energy sources like thermal photovoltaic Panels, Stirling engines and fuel cell. Among these systems, fuel cells are among the most suitable devices that can produce continuous electricity and heat. Energy is an instrument that changes directly from a vessel's vitality to electrical energy. The one-step conversion process has several advantages compared to the multi-step processes, which are engaged with burning based warmth engines. The combustion-based energy generation systems are very harmful to the eco-system and they are contributed to many environmental problems, for example, environmental change, acidic rains, ozone layer depletion and vegetation cover reduction. Also, combustion-based energy generation technologies are dependent on limited sources of fossil fuels. Then again, Give efficient and clean fuel cells way for converting energy. Also, energy components are perfect with renewable sources such as hydrogen. Therefore, they are promising candidates as the energy conversion devices in the future[(5)]. The operation of the fuel cells is quiet and they can replace by and wide with traditional power generation equipment such as portable, stationary and power generation for transportation. Nevertheless, further looks into are required to decrease the fuel cell costs, increase their durability and enhance their performance. A proper understanding of the operation principles of the module power in conjunction with the current condition perspective of the module power industry necessary for overcoming the problems and the development of fuel cell technology. This paper gives the standards of the force module activity, attributes, points of interest, types and zones of utilizations.

Fuel cell history

The main logical research on the power module was led in 1838 by the Swiss-German researcher Christian

Frank Schonbein [6]. In 1839, Sir William Robert Grove built up the principal energy component dependent on the turning around electrolysis of water by drenching two platinum terminals in a sulphuric corrosive arrangement[7]. At the point when he associated the two terminals, the present streaming the other way expended the oxygen and hydrogen gases, thus, considered this a Battery for Gas. Fig. 1 shows the Grove's gas battery. Ludwig Mond invested the vast majority of his energy for creating mechanical science innovation. In 1889, Mond and his associates, Car Langer presented a procedure for getting ready nickel by utilizing a battery for gas [8]. Friedrich Wilhelm Ostwald photographed the interconnections between various parts of the power unit including electrolyte, cathode, oxidation, cations, and anions, and that was in 1893. [9]. In 1896, William W. Jacques developed the principal down to earth energy component[10]. The artistic energy units came later with the revelation of strong oxide electrolytes by Nernst in 1899 [11] and 1900, he utilized zirconium as the strong electrolyte. The primary clay energy component was worked at 1000 °C in 1937 by Baur and Preis[12]. Francis Bacon at Cambridge University at that point delivered the initial 5 kW soluble energy unit in 1950[13]. During the 1960s the International energy units built up an energy unit power plant to create power and water for the drink apollo shuttle the excursion towards the moon. At that point, the International energy components built an all the more impressive (12 kW) soluble power module for NASA's space transport Orbiter during the 1970s[14]. Fig. 2 speaks to NASA's space transport Orbiter power device. In the mid-1980s, the United States of America, Canada, and Japan supported creative energy unit research[15]. In 1990, Ballard put its energy units into a progression of model transports. In the late 1990s, six Ballard-constructed energy component means of transport were utilized in Chicago and Vancouver. Today, energy units are regular in spaceflight, transportation, versatile force, home force age and enormous force age.

Fuel cell structure

Any kind of energy component needs three essential segments that are[1]:

1-Anode: anode is a permeable material that permits hydrogen fuel to enter in contact with the electrolyte and it disperses the hydrogen similarly over its surface without it is devoured or eroded.

2-Cathode: cathode permits oxygen to enter in contact with the electrolyte and scatters oxygen onto its surface without itself is expended or eroded.

3-Electrolyte: The principle part of any energy unit is the electrolyte. The electrolyte, for example, phosphoric corrosive, strong oxide or polymer decides the nature, attributes and temperature working of the energy unit and it is utilized to forestall the electronic contact of the two terminals by hindering the electrons. Additionally, electrolyte permits the relocation of particles from one anode to the next to keep the parity of the general electrical charge.

Fuel cell operating standards

The starting unit for fuel cells is an electrochemical device which converts from synthetic biofuel cells to electric biofuel cells. Any fuel can be utilized in power modules, however, from the outset, it should pass a reformer to frame unadulterated hydrogen[16]. The energy component is like a battery, anyway in battery reactants are put away inside and when they are utilized, the battery must be supplanted or revived. In contrast to the battery, in a power device, the reactants, which are expended during the electrochemical responses, are consistently renewed so that there is no compelling reason to energize the phone. A power device is a galvanic cell where the free vitality of a synthetic response is changed over into electrical vitality. The difference in the Gibbs free vitality of a compound response is identified with the voltage of the cell through the accompanying connection:

$$\Delta G = \pm nFE \quad (1)$$

In which n is the electron number engaged with the response, And (F(is the Faraday constant, (E) is the cell voltage for the thermodynamic balance without current [17]. The activity of an energy component has appeared in Fig. 3[18]. A power device is made out of a layer of electrolyte in contact with two cathodes on either side. The two cathodes comprise of a permeable material, which is secured with an impetus layer. The hydrogen fuel is taken care of persistently to the air anode and oxygen are taken care of consistently to the cathode. At the anode, hydrogen is oxidized to hydrogen particles

and electrons. Electrolyte layer allows the hydrogen particles to spill out of anode to cathode and the electrons move To the cathode by an electric circuit outside. The electrons and hydrogen at the cathode particles respond With the oxygen used to frame the air. Anode and cathode reactions are given and the general reaction is as follows:

Anode reaction:

$H_2 \rightarrow 2H^+ + 2e^-$ (2)

Cathode reaction:

$1/2\ O_2 + 2H^+ + 2e^- \rightarrow H_2O$ (3)

Overall reaction:

$H_2 + 1/2\ O_2 \rightarrow H_2O + W_{ele} + Q_{heat}$ (4)

heat and water are by-products and they must be removed continuously for the ideal generation of electrical energy. Therefore, thermal and water management is a key factor in fuel cell design and operation.

Fuel sources

The fuel cells used must contain hydrogen but most of them also contain carbon. Some fuel cells reform the fuel to produce the pure hydrogen but some automatically reform the fuel. These outlets include:

• Methane: gas drilling, rubbish decay, sewage or gas hydrates

• Methanol/ethanol: Natural gas or biomass hydrolysis

• Hydrogen: electric dissociation of water molecules through the use of solar cells that dissociate molecules from light.

Hydrogen sources and methods of production

Note that hydrogen good competitor for replacing petroleum derivatives to create vitality. Hydrogen can be gotten from a wide scope of sources, including biomass, petroleum derivatives, electrolysis of water and a portion of the mechanical side-effects. The decision of the source relies upon the various variables including the area of assets, accessible stores, cost of creation, transportation and usage. The strategy which is utilized for creating hydrogen from the source relies upon the improvement of innovation, required foundation venture, the proficiency of the technique, area and reasonableness of provisions.

Co-generation heat & strength

The simultaneous use of the heat and electricity which are produced in Co-generation is called the energy conversion method. Such energy use is more efficient than electricity use alone since heat is generated considerably in most systems. The utilize of the produced heat for generating more power from a gas or steam turbine or to store heat and warm water in the plant raises efficiency overall the energy conversion system as follows:

$E_{overall} = Q + E/Q_0$

Where Q is the usable heat energy, E is the electrical energy and Q_0 is the fuel heat material. This type of energy conversion is applicable in fuel cell technology since they can generate heat depending on the system at different temperature ranges. Especially, co-generation can be used for providing electricity and heat at homes[19].

Important Reactions

Oxidation of hydrogen

The reaction of hydrogen atoms to oxidation occurs outside Pt-based impetuses. The hydrogen oxidation energy is quick at the platinum surface and it is typically constrained by the mass exchange restrictions in a power device. The oxidation response of hydrogen includes the gas adsorption particles on the impetus surface field, which is trailed the separation of hydrogen atoms and electrochemical response as follows:

$2\ Pt_{(s)} + H_2 \rightarrow Pt\text{-}H_{ads} + Pt\text{-}H_{ads}$ (5)

$Pt\text{-}H_{ads} \rightarrow H^+ + e^- + Pt_{(s)}$ (6)

The adsorbed H-atom at the Pt surface where Pt(s) are in the free surface of Pt and Pt-Had. The overall reaction is:

$H_2 \rightarrow 2H^+ + 2e^-\ U^0 = 0V$ (7)

Even though the oxidation response of hydrogen is quick at the outside of the Pt impetus, there are a few issues when the unclean hydrogen is utilized. The

unadulterated hydrogen is costly and hard to store. Normal sources, for example, petroleum gas, propane and alcohols are the best wellsprings of unadulterated hydrogen. However, these materials need to be reformed into hydrogen and sometimes after reforming the gas some contaminants are still present in the fuel, such as carbon monoxide. Carbon monoxide (CO) poisons the catalyst surface through blocking the active sites. As of late, a few investigations have been done to Boost the strength of Pt-based impulses by alloying platinum with the second metal[20, 21].

Oxidation of methanol

Thanks to the superior strength density, Methanol is among the most common promising candidates for fuel; high activity and nearly complete electrooxidation to the final product of CO_2 [22]. To date, platinum has been wildly used as a powerful anodic catalyst for electrooxidation of methanol[23]. Many studies have been carried out on the methanol fuel cells in the literature[24, 25].

The overall methanol oxidation reaction is to:

$$CH_3OH + H_2O \rightarrow CO_2 + 6H^+ + 6e^- \; U^0 = 0.046 \text{ V} \quad (8)$$

Conclusion

Fuel cells are energy storage devices, which are simple, efficient and environmentally friendly. These properties make a range for a wide of applications for fuel cells. Fuel cells have the real potential as a promising technology from which electricity can be produced with harmless by-products. With increasing the energy demand, it becomes urgent to find new ways of meeting it safely. In the past, the limiting factors of renewable energy were the storage and transport of that energy. With using the fuel cells, electrical energy can be generated efficiently, cleanly and sustainably from renewable energy sources.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Major Histocompatibility Complex gene Polymorphism in Systemic Lupus Erythematosus Patients

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Abstract

The major histocompatibility complex (MIC) is one of the MHC class I related genes which related with autoimmune diseases , the present study aims to investigated association between MIC gene polymorphism class I in systematic lupus erythrymatous patients within Hilla city, the present study implemented using whole blood genomic DNA and single strand DNA conformation polymorphism (SSCP) for detection gene polymorphisms and haplotypes frequency, The results of present study show that 183 bp of MIC gene and it has three haplotypes (A, B, and C) each of these haplotypes consist of two bands, non-significant differences between patient and control in haplotypes frequency, B haplotype was more frequent in patinas than others (20%) while A was more frequents in control than others (22.8%). The present study concluded that MIC gene may be contributed in immune response of SLE and can be used in pre diagnosis of disease. This study was one of the serious investigators deal with several immune genes in this disease in Iraqi SLE.

Key words : *The major histocompatibility complex, systematic lupus erythrymatous, single strand DNA conformation polymorphism.*

Introduction

An autoimmune disease is one of the most common health problem in last decades that observed in Iraqi population, different types of Chronic autoimmune diseases were recorded, SLE is one of these types, the SLE is complex inflammatory dieses which multifactorial contributed in etiology of disease [(1).] The pathogenesis of SLE is not well understood, several studies suggested dysfunction of immune system, and determined self-antigens resulted to chronic inflammation cusses destruction in tissue and organ damage[(2)] . One of the factors involved in the pathogenicity of SLE disease is genetic alteration, some genes mutations have been determined which related with SLE [(5,6)] .

The major histocompatibility complex (MIC) is found in the chromosome 6 at the short arm [(3)] , it be found that About 40% of the expressed MHC genes encode proteins related to immune defense [(4)] .

The relation of MHC and autoimmune diseases was documented since 1970s in some studies, its considered as strongest risk factors of autoimmune diseases. When wide-screen genotyping platforms and imputation pipelines developed , the genetic map of were performed for different population like European and Asian in some disease included , systemic lupus erythematosus (SLE) [(9,10,11)] .Studies exhibit that the major histocompatibility complex (MHC) is One of the genetic risk factors for the development of immune-mediated diseases like SLE [(7,8,12).]

Materials and Methods

Study design and subjects; present study is one of the sequential projects deal with genetic polymorphisms in some genes in SLE patients in Hilla city, A case-control study was implemented enrolled 30 Iraqi patients suffered from systemic lupus erythematosus (SLE) and

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35 of healthy subjects as control group, that attendance to Merjan teaching hospital in Babylon – Iraq, All patients were diagnosed by a specialist dr. Ali Al-kazaz, patients group consist of (28 female and 2 male) with age (10-60 year), the control group consist of 35 individuals (20 male and 15 female) apparently healthy with age (20-65 year) , All subjects in this study were taken written consent before participation in this study.

Inclusion Criteria the diagnosis of SLE patients was conducted according to observed 4 criteria of The Systemic Lupus International Collaborating Clinics (SLICC) criteria which consist of clinical manifestations like malar rash, discoid rash photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurologic disorder, hematologic disorder, immunologic disorder, antinuclear antibody [13].

1- PCR condition : the gene segments was amplification using MIC-A5F 5′CCTTTTTTTCAGGGAAAGTGC-3 and MIC-A5R, 5′CCTTACCATCTCCAGAAACTGC-3 [14] . under the condition 95Cº /5 min, (95Cº /30 sec. 57Cº /30 sec, 72Cº /20 sec) for 30 times finally 72 /10 min, The products were detected by electrophoresis in garose 1% , 70 v , 20 Ma for 90 min. SSCP technique implemented according to Al-Terehi et al., [15]

Static analysis : the static analysis was conducted using Odd ration, confidence Intervals 95% at P value 0.05.

Results and Discussion

This study deal with polymorphism of MIC gene in SLE disease, the SSCP was used to determine the gene polymorphism, the results show there were 183 bp of amplification products for all patients and control group (figure 1 A), three haplotypes represented as two bands for each one were observed after SSCP electrophoresis pattern, these haplotypes were A, B, and C (figure 1 B). as a results of high incidence SLE in Iraqi population at last year sequential studies were suggested to investigated some genes polymorphisms in SLE patients like mitochondrial, APRIL gene and micro-RNA, the results of these studies were varying.

In spite of poorly understood of SLE pathogenicity, numerous environmental and genetic [16] factors have been postulated, the major risk is genetic predisposition which well documented Some genes now are reported to SLE contributed [17] .and these genes impact on the immune pathways and processing like C2 and C4 related to the MHC [18] .

Table (1) haplotypes frequency of MIC gene polymorphism in study subjects

Allele descript	SLE patients (30)	Control (35)	Odd ration CI95%	P value
A	13%	22.8%	1.9259 0.5168 to 7.1774	0.3288
B	20%	8.57%	1.8621 0.4231 to 8.1942	0.4109
C	3.33%	8.57%	2.7188 0.2676 to 27.6189	0.3978

The statics analysis show that B haplotypes was more frequent in patient than control in non-significant differences it was appeared in 20% and 8,57% for patients and control respectively (OR 1.8621, CI 95% 0.4231 to 8.1942). A allele found in 22.8% of control while 13% in patients (OR 1.9259, CI 95% 0.5168 to 7.1774). Finally haplotype C appeared in 8.57% in control and 3.33% in patients in non-significant differences (OR 2.7188c CI 95% 0.2676 to 27.6189).

The polymorphism in present study restricted by 183 bp represented by A, B and C, a study clarified that the the MHC in human genome is the more gene-dense locus , also its high polymorphic genes. It included more than 120 functional genes, pseudo-genes [19] . The variation in SSCP electrophoresis pattern showed more than the three haplotypes which documented in this study , all these variations were don't dependent in present study because it frequent in all study subjects. The non-significant may be because the sample limitation number in present study, here we are announced that all patients of SLE in Hilla city were enrolled in our projects at the time of sample collection and they are followed up with rheumatology unit in Marjan hospital .

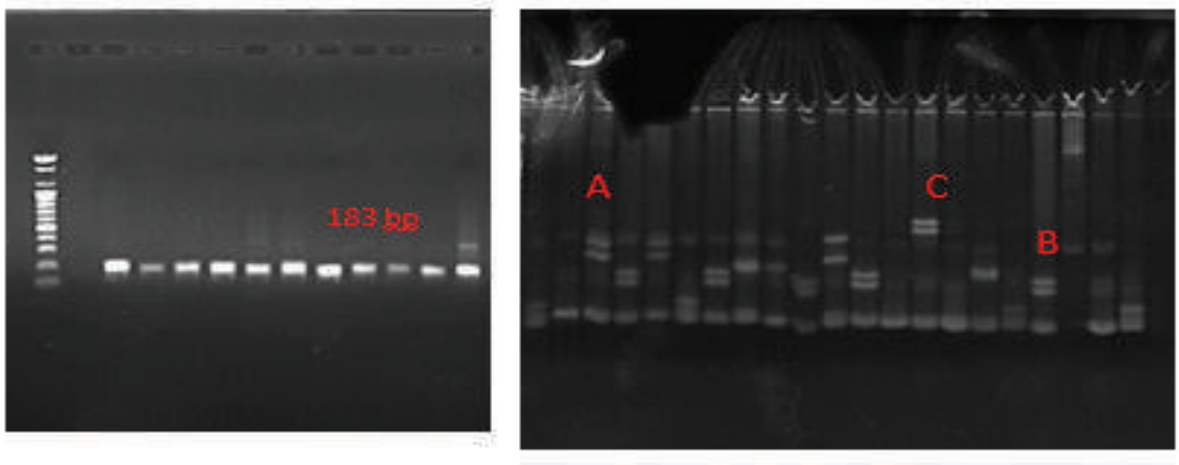


Figure (1) A the left electrophoresis pattern of MIC PCR products 183 bp for patients and control at 20mA, 70 V and 90 min. B the right electrophoresis pattern of SSCP technique show three haplotypes A, B, and C.

The present study show related polymorphisms with SLE disease, same results proved by Gambelunghe et al., [20] that found three major markers (DR3-DQ2, MICA5 and MICA5.1) were strongly associated to confer genetic risk of SLE. Also Ping et al.,[21] observed *MICB*009N* allele might be a SLE risk factor while the *MICB*014*, *MICA*010* and *MICB*002* alleles were correlated with decreased SLE incidence, however MIC gene need more investigation to prove its role in immune response in SLE patients and further gene sequencing. We concluded from this observation that MIC gene polymorphisms can be used in pre diagnosis of SLE disease in genetic predisposition family to avoid accumulate environment factor effect that lead to trigger disease.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Effect of Oral Contraceptive Pills on Levels of Calcium and Vitamin D in women in Al-Najaf province

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Abstract

Background: Combined oral contraceptives comprise of the steroid hormone oestrogen in combination with a progesterone, occupied principally to prevent pregnancy. Combined oral contraceptive pills commonly refer to pills in which an oestrogen and a progestogen are given concurrently in a monthly cycle. **Materials and methods**: The current study was undertaken over a period of six months from 2019/10/1 till 2020/3/1 in Al-Najaf province. Thirty women that take oral contraceptives pill participated in the study, Their ages ranged from (20-38) years divided into three groups(10/group) according to the ages (20-25, 26-31,32 -38) years. **Results:** Results of this study revealed, oral contraceptives pill cause no effect on serum levels of calcium in women that intake it at different age groups in compare with controls groups, and also causes a significant decrease in serum levels of vitamin D in women that intake it at different age groups in compare with controls groups. On the other hand, the results obtained there aren't apparent difference in these parameters when compared between age groups and group that use it for long period (3,6 and 9 year).

Conclusion: The present study concluded that oral contraceptives pill cause no effect on serum levels of calcium in women and little effect on vitamin D in women.

Keyword: *oral contraceptives pill, parameters, calcium and vitamin D.*

Introduction

Combined oral contraceptives involve of the steroid hormone oestrogen in mixture with a progesterone, taken mainly to avert pregnancy. Combined oral contraceptive pills commonly refer to pills in which an oestrogen and a progestogen are given parallel in a monthly cycle. Combined oral contraceptives (COCs) consume become a prevalent method of birth control because to their contraceptive effectiveness and good tolerability profile [1]. Combined oral contraceptives are so typically administered as a pill containing oestrogen and progestogen, which is taken daily for 20–22 days, followed by a seven-day pill-free interval (or seven days of placebo), during which time a withdrawal bleed is expected to happen. These pills comprise hormones that turn on the reproductive system of female leading to avert pregnancy such as estrogen and progesterone. Oral contraceptives are the combination of estrogen and progestin or only progestin. Throughout the years, oral contraceptives have created through progressively decreasing the portion of ethinylestradiol (EE) and presenting 17-β estradiol, and different ages of progestin [2]. There are numerous kinds of estrogen and progesterone being utilized in pills like mestranol is a class of estrogen, and the 3-methylether of EE and norethynodrel is a sort of progestin [3]. In clinical investigations, the efficiency of contraceptive was excellent, but this drug caused many side effects for example, nausea, dizziness, headaches, stomachaches, and vomiting [4]. However, the death of a female had been reported who was taking the contraceptive pills in 1961. Although after so many years, oral contraceptives have developed by decreasing the dose of estrogen and by discovering a new generation of progestins, and additional routes of combined oral contraceptives administration have been developed [5]. The progesterone is primarily responsible for preventing pregnancy. The main mechanism of action is the

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prevention of ovulation; they inhibit follicular development and prevent ovulation [6]. Progestogen negative feedback works at the hypothalamus to decreases the pulse frequency of gonadotropin releasing hormone [7].

Materials and Methods:

No.	Instrument	Material
1	Centrifuges	Calcium Kit
2	ELISA Reader	vitaminD Kit
3	Spectrophotometer /UV	
4	Gel tube	
5	Micropipette	
6	Serum tube	
7	Sterile syringes (5ml)	
8	Eppendorf tube	

The current study was undertaken over a period of six months from 2019/10/1 till 2020/3/1 from women using oral contraceptive pill in Al-Najaf province. Thirty women that take oral contraceptives pill participated in the study, Their ages ranged from (20-38) years divided into three groups(10/group) according to the ages (20-25, 26-31,32 -38) years. women with anther diseases which may cause alteration in level of calcium and vitamin D were excluded from the study. About 5 ml of venous blood were taken from women and controls. Serum was separated by centrifugation and used for estimation level of calcium and vitamin.

Results

Effect of contraceptive pill on calcium levels in serum

The results display no significant effect ($p< 0.05$) on calcium level in serum in the women at different age groups (20-25y ,26-31y and 32-38y) after intake oral contraceptive pill in compared with control group at different age groups (20-25y ,26-31y and 32-38y) respectively figure (1) and results also display no significant effect ($p< 0.05$) on calcium level in serum in the women between groups of ages.

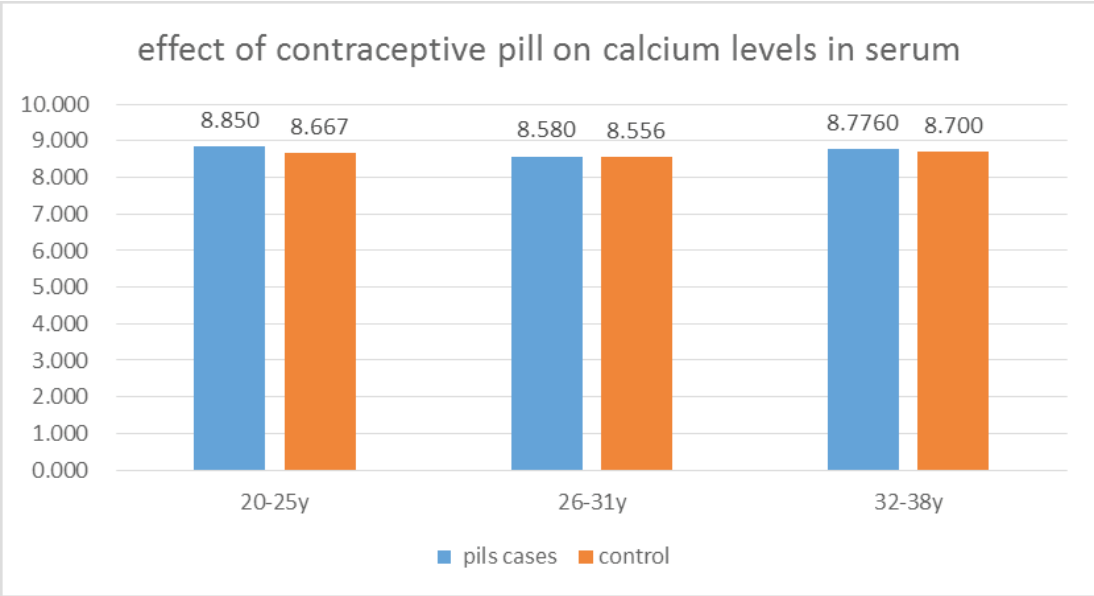
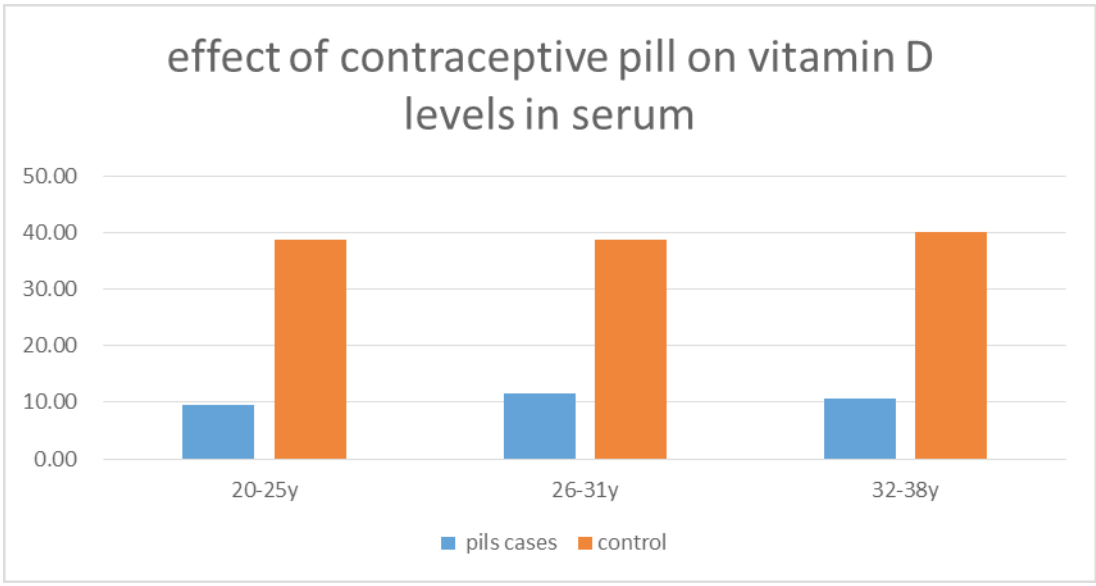


Figure (1): Effect of contraceptive pill on calcium levels in serum

Effect of contraceptive pill on vitamin D levels in serum

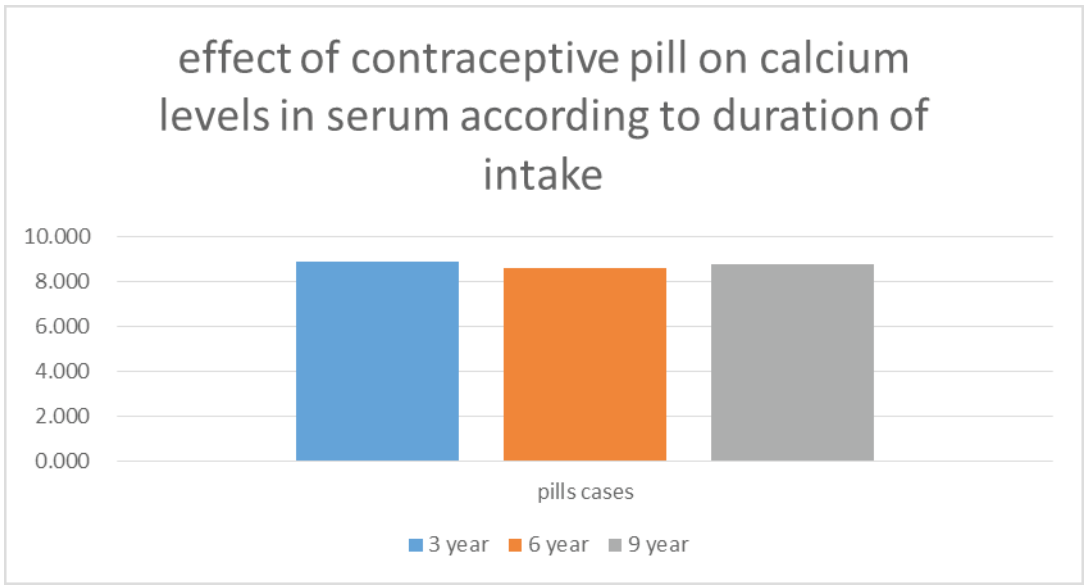
The results display significant decrease ($p > 0.05$) in vitamin D level in serum in the women at different age groups (20-25y ,26-31y and 32-38y) after intake oral contraceptive pill in compared with control groups at different age groups (20-25y ,26-31y and 32-38y) respectively figure (2) and results also display no significant effect ($p < 0.05$) on vitamin D level in serum in the women between groups of ages figure (2).



Figure(2): Effect of contraceptive pill on vitamin D levels in serum

Effect of contraceptive pill on calcium levels in serum according to duration of intake

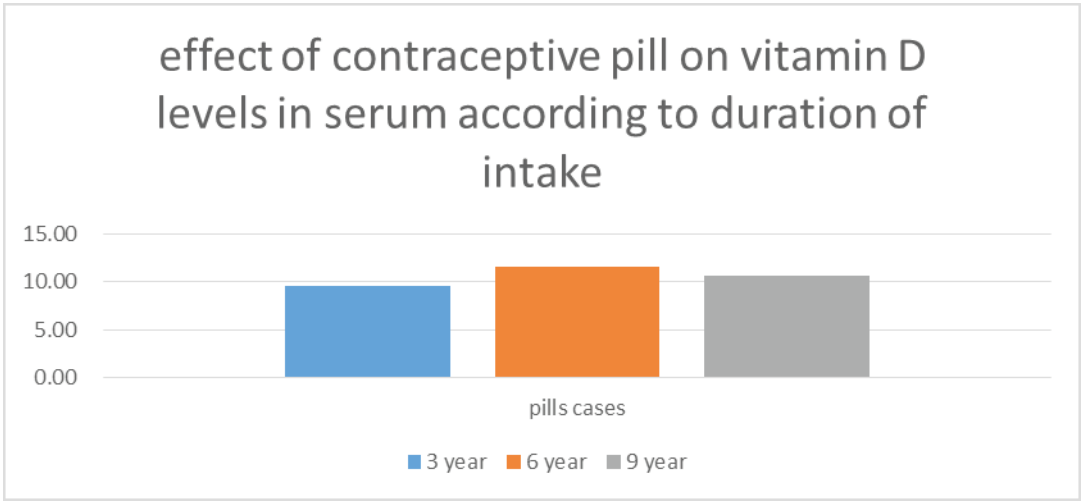
The results display no significant effect ($p < 0.05$) on calcium level in serum in the women at different duration of intake of oral contraceptive pill groups (3 y ,6 y and 9 y) figure (3).



Figure(3): Effect of contraceptive pill on calcium levels in serum according to duration of intake.

Effect of contraceptive pill on vitamin D levels in serum according to duration of intake

The results display no significant effect ($p < 0.05$) on vitamin D level in serum in the women at different duration of intake of oral contraceptive pill groups (3 y ,6 y and 9 y) figure (4).



Figure(4): Effect of contraceptive pill on vitamin D levels in serum according to duration of intake

Discussion

Effect of contraceptive pill on calcium levels in serum

The results of the present study displayed no significant effect ($p<0.05$) on calcium levels in serum in women that intake contraceptive pills at different age groups in compared with control group at different age groups, also this study indicate no significant effect ($p<0.05$) between the groups of ages of women that intake contraceptive pills.

Effect of contraceptive pill on vitamin D levels in serum

The results of the present study displayed significant decrease ($p>0.05$) on vitamin D levels in serum in women that intake contraceptive pills at different age groups in compared with control group at different age groups, this study disagree with many studies that indicate increase in level of vitamin D levels in serum in women that intake contraceptive pills such as the study of [8] this study result is that the use of contraceptive pill increases the circulating levels of 25(OH)D in premenopausal, healthy, adult women and the study of [9] this study result is contraceptive pill use increases circulating levels of 25OHD in plasma. There are many other studies agree with present study such as the study of [10]. This study seems to be the first prospective trial revealing the effect of contraceptive pills use on serum 25(OH)D levels in women with Polycystic ovary syndrome. show the decrease in serum 25(OH)D levels in patients with Polycystic ovary syndrome with the use of contraceptive pills alone. Vitamin D deficiency, which arises due to insufficient exposure to sunlight and dermal synthesis from 7-dehydrocholesterol and limited intake from food and supplements, disrupts the function of all systems of the body and increases the risk of osteoporosis, cancer, cardiovascular disease, autoimmune disease, and mental disorders such as depression and chronic pain syndrome [11], [12] also this study indicate no effect when compare between the groups of ages of women that intake contraceptive pills.

Effect of contraceptive pill on calcium levels in serum according to duration of intake

The results display no effect on calcium level in serum in the women at different duration of intake of oral contraceptive pill groups when compare between groups.

Effect of contraceptive pill on vitamin D levels in serum according to duration of intake

The results display no effect on vitamin D level in serum in the women at different duration of intake of oral contraceptive pill groups when compare between groups.

Conclusions

The present study concluded that oral contraceptives pill cause no effect on serum levels of calcium in women

and little effect on vitamin D in women.

Acknowledgements: I would like to thank all women whose participated in in this work.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Association of Some NKG2 gene Family and it's Polymorphism with Pulmonary Tuberculosis

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Abstract

The case-control study was aimed to evaluate the effect of NKG2D and NKG2F, gene polymorphism on susceptibility to pulmonary tuberculosis in Babylon province.

The polymorphism of NKG2D gene at (rs1049174) and (rs2255336) were detected for 60 PTB cases and 60 of control. For rs1049174, allele frequency for patients and control were significant (P<0.05) and the C allele represent the risk allele for PTB patients with odd ratio (95% CI) of 2.04 (1.16-3.57) while G allele represent protective allele for control group with odd ratio (95% CI) of 0.49 (0.28-0.86), (P<0.05). The genotype (C/C, G/C, G/G) were (61.7, 30, 8.3)% respectively for PTB patients and (36.7, 50, 13.3)% respectively for control with significant association with disease (P<0.05). On the other hand, the SSCP and sequencing results of rs2255336 revealed that there were no gene variation in rs2255336 among patients and control subjects.

The polymorphism of NKG2F gene at (rs1841958) was also detected for 60 PTB cases and 60 of control. For rs1841958, allele frequency between patients and control were insignificant (P>0.05). The genotypes (C/C, A/C, A/A) were (38.3, 40, 21.7)% respectively for PTB patients and (36.7, 40, 23.3)% respectively for control with insignificant association between these genotype and PTB (P>0.05).

Key Words: *Health; ; gene polymorphism; NKG2F, rs1049174, rs2255336 and rs1841958.*

Introduction

Tuberculosis is an old infectious disease triggered by the bacillus *Mycobacterium tuberculosis*, which is now afflicting humans all over the world. In 2018, 1.2 million deaths and 10 million new TB cases were reported by the World Health Organization [1].

The association of NKG2 gene family with pulmonary tuberculosis was a limited studied. NKG2D and NKG2-F is a transmembrane protein of the NKG2 family of C-type lectin-like receptors. It's encoded by KLRK1and KLRC4 gene respectively, which is located in the NK-gene complex (NKC) situated on chromosome 12 in humans. It is expressed by NK cells, γδ T cells and $CD8^+$ αβ T cells [2].

The NKG2D activating receptor is peculiar in its ability to bind to numerous and highly diversified MHC class I-like self-molecules. These ligands are poorly expressed in normal cells, but can be induced in infected, transformed or damaged cells [3].

NKG2D is a main receptor for the recognition and removal of infected and transformed cells because its ligands are stimulated during cellular stress, either as a result of genomic stress or infection such as cancerous cells and TB infected cells [4]. While the function of NKG2F receptor has not yet been clarified. It may not have a C-type lectin domain and contains immunoreceptor tyrosine-based inhibitory motifs (ITIMs-like motif) in the cytoplasmic tail. However, ITIM-like motif looks to be non-functional, thus NKG2F was considered to be an

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activating receptor [5].

Material and Method

Samples selection and MTB detection

A case-control study involved 60 newly diagnostic pulmonary tuberculosis and 60 samples of apparently healthy persons were taken as a control group.

A blood and sputum samples were collected from all study cases at the center of chest and respiratory disease in AL-Hillah / Babylon province, during the period from November 2018 to July 2019.

The sputum sample was subjected to direct smear examination by Ziehl-Neelsen technique [6], routine culture by use Lowenstein-Jensen medium [7] and use of GeneXpert for detection of MTB [8].

The blood samples were used for genotyping study, as the DNA extraction was achieved according to the method recommended by the manufacturing company (favorgene) in the user manual of Favor Prep Genomic DNA Mini Kit general protocol for fresh and frozen blood samples. The extracted genomic DNA from the blood was checked by using nanodrop spectrophotometer which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm). All the primers that used in this study for detection NKG2D and NKG2F were designed by the aid of NCBI-primer BLAST online software which include NKG2D-rs1049174 (Forward- TGCTGTGTCTCTCTGCTGTG and Revers-TCAGATATCCCCAAGGCTGC), NKG2D-rs2255336 (Forward- TTCTGGACT AATAGCAAAAATGTGA and Revers- AGCCATGGGAATCCGTTTCA), and NKG2F-rs1841958 (Forward-TGTCA TTCCCTTGATGATCCGAAGA and Revers-GTGCAGTTATCATAGAGCACAGTC), at the same time the produced primers were tested for specificity of their target sequences by conducting the BLAST against the human genome, then the primers pair was selected according to the demand criteria such as: product length, the similarity of melting temperature, primers length, specificity, etc. Then the mutations were interred according to the design demands.

The PCR technique was done on all samples by use (Promega master mix,USA) and then the amplified products were subjected to RFLP or SSCP and Sequencing technique to determine SNPs.

The PCR-RFLP of rs1049174 and rs1841958 was done for all sample by using *BstDE I* and *Alu I* endonucleases respectively and according to instructions of manufactured company (New England Biolabs Inc./ USA) by add 5 µl amount DNA from PCR product to 4 µl restriction enzyme buffer and 1 µl of the selected restriction enzyme *BstDE I* and the reaction mixture then completed to 15 µl by Free nuclease water.

Statistical Analysis

Statistical analysis was done using SPSS version 23; variables were described as mean, standard deviation, number and percentage. Risk was estimated using odds ratio and the level of significance was set at $P < 0.05$.

Results and Discussion

DNA Extraction and PCR Products Detection

Human DNA genome was extracted from whole blood of all the 60 samples of PTB patients and 60 samples of apparently healthy control, the concentration was (50-150 ng) and purity was (1.8-2.0). These DNA was subjected to PCR amplification using specific primers targeting specific regions in the DNA and then enrolled for detection of single nucleotide polymorphisms (SNPs) by RFLP or SSCP-Sequencing techniques. Then the optimized of PCR products of the designed primers pair which would be used in NKG2D (rs1049174 and rs2255336) and NKG2F (rs1841958) genotyping was done by the gradient-PCR at (55-66 C^0) and then the PCR product were electrophoresed to evaluate the most appropriate condition for PCR technique. The most appropriate annealing temperature was $66C^0$ for (rs1049174) and $60C^0$ for (rs2255336 and rs1841958).

Genotypic Characterization of NKG2-D (rs1049174)

The amplification of NKG2D gene at region rs1049174 appeared the presence of gene amplicons on electrophoresis gel for all groups of study.

Genotype association and allele's frequency for patients and control are listed in table (1). The results revealed that there were a significant allele frequency

differences between patients and control group (P<0.05). Allele C represents the risk allele with odd ratio (95% CI) of 2.042(1.166-3.578), while allele G represents the protective allele with odd ratio (95% CI) of 0.49 (0.28-0.86).

The restriction fragment length polymorphism of PCR products of rs1049174 gene revealed three genotypes; C/C, G/C and G/G (figure 1).

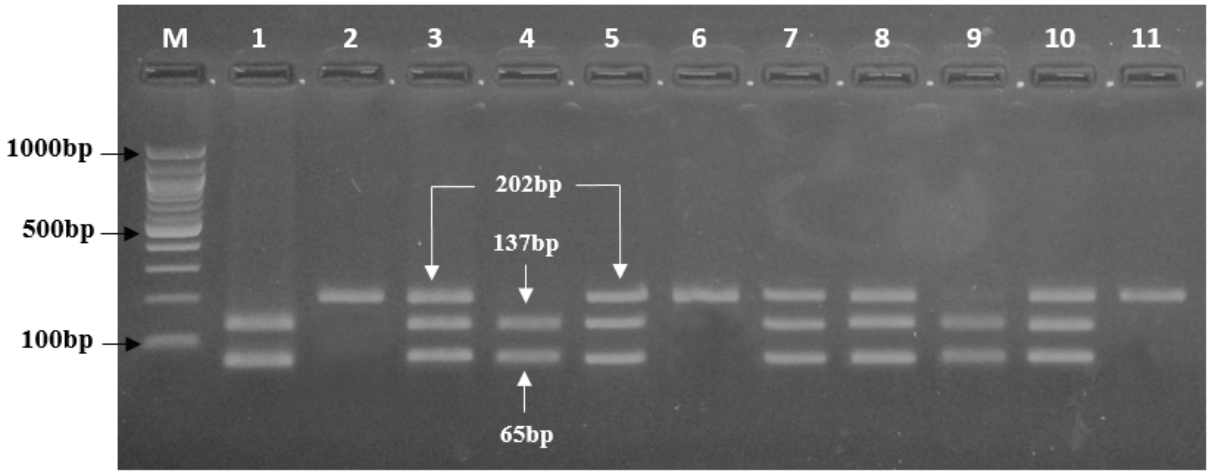


Figure (1): Gel electrophoresis carried on (agarose gel (2%), 75% V, 20 mA for 1hr.) for NKG2D gene (rs1049174). PCR products visualized under U.V. light after staining with ethidum promaide. M: 100 bp DNA marker; the size of product is 202bp and 137+65 bp. The G/G(1,4,9), G/C(3,5,7,8,10) and C/C(2,5,11).

Association of rs1049174 genotypes with PTB was tested, and the results revealed that CC genotype was more associated with pulmonary tuberculosis, while G/C genotype conferring lesser pulmonary tuberculosis susceptibility for the carrier individuals with odd ratio (95% CI) of 0.36 (0.16-0.78), P=0.04.

Table (1): Genotype association and allele's frequency of rs1049174 with PTB.

Genotype	Control	Case	OR (95% CI)	P-value
C/C	22 (36.7%)	37 (61.7%)	1.00	0.046
G/C	30 (50%)	18 (30%)	0.36 (0.16-0.78)	
G/G	8 (13.3%)	5 (8.3%)	0.37 (0.11-1.28)	
Allele Frequency				
C	74 (62%)	92 (77%)	2.042 (1.166-3.578)	0.017
G	46 (38%)	28 (23%)	0.49 (0.28-0.86)	
*(P<0.05), OR: odd ratio, CI: confidence interval				

Genotypic Characterization of NKG2-D (rs2255336) with PTB

The rs2255336 (which partially cover the exon 9 of NKG2-D) were also screened in the present study, and these fragments consist of 201bp.

The PCR products of rs2255336 were subject to single strand conformation polymorphism (SSCP) to detect any small alteration in PCR-amplified product (SNPs). A composition of 12% nondenaturing PAGE of polyacrylamide gels was used for electrophoresis of PCR-amplified product with specialized buffer systems for 20-24hrs and then it is subjected to silver staining to visualize bands.

The DNA polymorphism appears as same band pattern on polyacrylamide gels, the results revealed that rs2255336 gene did not have any polymorphism, and to confirm the results, some samples from patients and control were sent to the macrogen company in South Korea to read the DNA sequencing of amplicons for SNPs detection and After alignment results of sequencing of the 201bp of rs2255336 gene showed 100% sequences similarities between the sequenced samples and the intended reference target sequences.

The results of the current study agree with many studies that reported the implication of rs1049174, produced by a substitution of the C by G nucleotide base in the NKG2D gene with a vast number of diseases [9-11].

In this study, the rs1049174 C/C genotype was significantly associated with susceptibility to PTB, while, the difference in the rates of G/C and G/G genotypes between patients and control, may be associated with decrease the risk of pulmonary tuberculosis (P=0.04) as shown in table (1).

The results of this study were incompatible with results obtained by weiwei *et al.,* [10] who found that the patients who carry the rs1049174 GG genotype have high chance to get infection and more susceptible to develop cancer and have a multivariate odds ratio (OR) of 1.85 (95%CI): 1.02–3.38.

On the other hand, the study finding of rs2255336 genotype revealed unnoticed of any mutation and disassociation of this SNP with PTB.

Ana Paula *et al*, [12] mentioned in study about NKG2D expression from NK-cell in *M. tuberculosis* infection, that there is no change in the expression of these receptors in response to *M. tuberculosis* infection and suggested that although NKG2D receptors are known to distinguish stressed and viral infected cells, but the expression of these specific receptors might not be required for responsiveness to *M. tuberculosis*. This cannot be firmly concluded, since cells other than NK cells can express these receptors.

Genotypic Characterization of NKG2-F (rs1841958)

The amplification of NKG2F gene at region rs1841958 was also investigated and the results of genotype association and allele's frequency for patients and control are listed in table (2). The results revealed that there were insignificant allele frequency differences between patients and control group (P >0.05).

The RFLP studies of PCR products of rs1841958 gene revealed three genotypes; C/C, A/C and A/A as shown in figure (2).

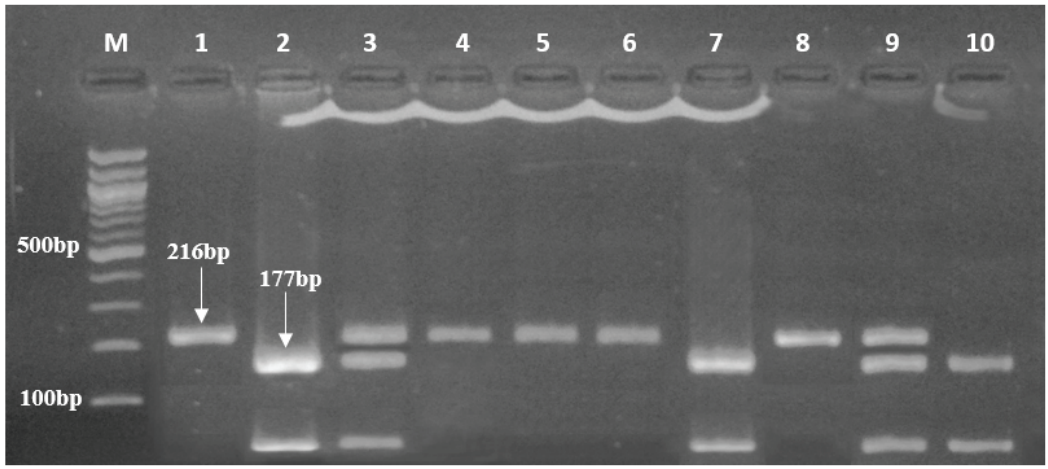


Figure (2): Gel electrophoresis carried on (agarose gel (2%), 75% V, 20 Am for 1hr.) for NKG2F gene (rs1841958). PCR products visualized under U.V. light after staining with ethidum promaide. M: 100 bp DNA marker; the size of product is 216bp, 177+39bp. The C/C(1,4,5,6,8), A/C(3,9) and A/A(2,7,10).

Association of rs1841958 genotypes with PTB was further tested. The result displays that there was insignificant association of any genotypes with pulmonary tuberculosis.

Table (2): Genotype association and Allele's frequency of rs1841958 with PTB.

Model	Control	Case	OR (95% CI)	P-value
C/C	22 (36.7%)	23 (38.3%)	1.00	0.98
A/C	24 (40%)	24 (40%)	0.96 (0.35-2.60)	
A/A	14 (23.3%)	13 (21.7%)	0.89 (0.28-2.85)	
Allele Frequency				
C	68 (56.7%)	70 (58.3%)	1.071 (0.572-2.003)	0.83
A	52 (43.3%)	50 (41.7%)	0.934 (0.499-1.748)	
*(P<0.05), OR: odd ratio, CI: confidence interval				

Interestingly, the rs1841958 SNP in NKG2F is missense mutation, and to validate the result of RFLP and to detect the type and the exact location of this SNP in NKG2F gene, some DNA samples were sent to Macrogen Company to read the DNA sequencing of rs1841958 amplicons. The sequencing analyzed and alignment results of rs1841958 gene 216bp revealed the presence of one nucleic acid substitution SNP which is G133T and was detected in this study in the investigated P1-P5samples as shown in figure (3).

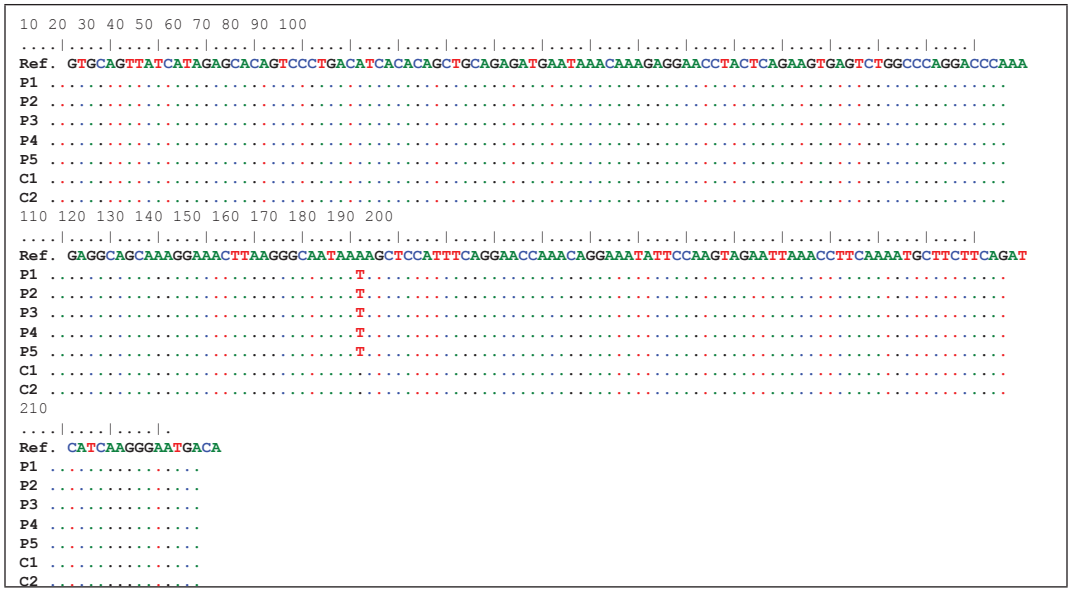


Figure (3): DNA sequences alignment of DNA samples with their corresponding reference sequences of the 216 bp amplicons of rs1841958. The symbol "ref" refers to the NCBI referring sequence; "P1-P5" refers to the samples; "C1-C2" refers to control.

However, this SNP leads to amino acid substitution represented by the conversion of Isoleulcine with Valine at 29th position (I29V) of the mature NKG2-F type II integral membrane protein and also this SNP was not reported in the ClinVar database too. The sequencing chromatogram of the identified variation region revealed that this SNP was found in a heterozygous status in both P1 and P2, and in a homozygous status in P3,P4 and P5.. Whereas C1 and C2 had exhibited a normal homozygous (A/A) status for this specified locus.

Hayashi *et al,* [13] reported the implication of NKG2F (rs1841958) SNPs produced by a substitution of the C by A nucleotide base with reduced peripheral blood leukocyte cytotoxicity and increased incidence of microbial infection and/or cancer.

The strongest linkage peak in Turkish familial with some disease was reported in persons have rs1841958 SNP in NKG2-F [14]. The NKG2F gene, encodes the C-type lectin receptor, the function of which is largely unknown. A possible hint to its function might be found in a related family member, NKG2D, encoded by KLRK1 and also located within the same disease-associated haplotype block [15].

The results of the association of rs1841958 in NKG2F gene with pulmonary tuberculosis revealed insignificant association of any genotypes and any allele variant in these SNPs with pulmonary tuberculosis.

This study may consider the first study that investigates the role of NKG2D and NKG2F with PTB in Iraq and also among few studies that investigate the role of these genes with PTB in the word.

Conclusion

The polymorphism of NKG2D gene at (rs1049174) was appear significant allele frequency differences between patients and control ($P<0.05$). The genotype C/C revealed significant association with disease ($P<0.05$). On the other hand, the NKG2D (rs2255336) revealed no gene variation among patients and control subjects.

The polymorphism of NKG2F gene at (rs1841958) showed insignificant allele and/or genotypic association with pulmonary tuberculosis ($P>0.05$).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Evaluation of Breast Cancer Molecular Profile in Old Age Patients in Comparison to Young Age Patients

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Abstract

Background: Breast cancer is the commonest malignancy of females all over the world and the second leading cause of death due to malignancy among females. Breast cancer is regarded as a unique disease in oncology and the specific markers such as ER, PR, HER-2/neu and others are used to predict the prognosis and the treatment response to guide the therapeutic plan.

Aim of the Study: To clarify if there is any significant difference in the intrinsic molecular profile of breast cancer in old age patients in comparison to young age patients.

Material & Methods: Samples of primary carcinoma of breast, obtained from 211 female patients with breast cancer. The patients are divided into two group. Immunohistochemical analysis was done for the specimens by using Envision method for detection of estrogen receptor, progesterone receptor, Her2/neu.

Statistical analysis was done by using SPSS. In which we use frequency, percentage and mean as descriptive statistics while chi square and Yates corrected chi square as analytic statistics.

Result: We found molecular intrinsic breast cancer subtypes have a significant difference in distribution of elderly breast cancer compared to their younger counterparts where elderly patients more frequently Luminal A and Luminal B tumor subtypes and less frequently HER2+ and basal molecular subtypes.

Conclusion old age breast cancer patients have better prognosis in comparison to young age patients according to the biological profile, histopathological type and Immunohistochemical prognostic parameters.

Keywords: *Breast cancer, Molecular profile, Old age patients, Young age patients, ER, PR, HER-2/neu.*

Introduction

Breast cancer is the most common cancer in women and is the second cause of cancer death after Lung cancer in world [(1)]. In Iraq Breast cancer has become a major threat to female health, it is the leading cause of death after cardiovascular diseases among women with a cancer-related [(2)]. The large majority of breast cancers are detected during the postmenopausal years, However, breast cancer can develop at any age,from childhood to old age[(3)].The prognosis and management of BC is influenced by classic variables such as the age of patient, the tumor size, the histological type, the grade and the stage of the tumor, status of estrogen receptor (ER) , progesterone receptor (PR) and more recently Human Epidermal growth factor receptor Type- 2 (HER-2/neu) and BRCA status, cell proliferation markers such as Ki-67 and gene expression proliferation [(4,5)].

It is also established that the incidence of breast cancer increases with age. The number of elderly patients with breast cancer is increasing and the majority of females who succumb to breast cancer are >65

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years old [6]. However, older patients are more likely to present with tumors that are estrogen receptor (ER)- and progesterone receptor (PR)-positive and human epidermal growth factor receptor 2 (HER2) -negative, and these tumors are associated with improved prognosis and clinical outcomes [7]. By contrast, younger patients with triple-negative and HER2-positive breast cancers have an increased risk of relapse within 5 years of diagnosis [8]. Breast cancer that arises in young females is associated with reduced survival and higher incidence of unfavorable prognostic and predictive tumor markers [9,10].

Materials & Methods

This study was carried out during the period from January 2018 through August 2018.

Sampling of cases:

Archived and new Formalin-fixed paraffin-embedded tissue samples of 211 cases of female patients with breast carcinoma (as confirmed by H&E – stained histopathological examination) covering the period from 2017 to 2018, were included in this study. Their ages ranged between 22-85 years old with a mean age of 50.6±14.3 years old. Specimens were collected from 133 modified radical mastectomy & 78 excisional biopsy from laboratory of histopathology in Al-Sadder Teaching Medical City Hospital in Al Najaf and from some private laboratories in this governorate.

Study Group: Forty-nine case ≥ 65 years old age.

Comparative Group: one hundred sixty-two cases who were < 65 years old age (Figure 1)

Control Group:

Positive Control Slides: Parallel positive control sections were processed with each set of immunostaining. Positive controls of breast carcinoma sections which are known to express ER, PR and Her2/neu, respectively were used with each run.

Negative Controls slides: Sections untreated with primary antibody (ER, PR and Her2/neu) were considered as negative controls for each set of slides.

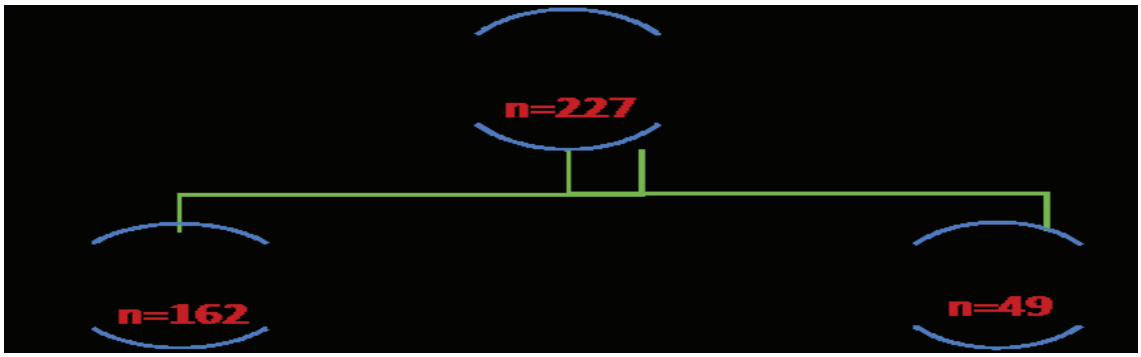


Fig. 1 Age distribution according to WHO publication [11].

Immunohistochemical material

Antibodies & Kits:

EnVision FLEX Mini Kit (High Ph,,Code K8004) which contain :DAB Chromogen with quality (1*3 ml),peroxidase-Blocking Reagent and HRP both with quality (5*20 ml),Wash Buffer (2*1 L, 20* concenterated) with PH 7.6 (+/- 0.1),Mouse (LINKER) with quality (40 ml),Target Retrieval Solution (3*30 ml,50* concenterated) with low PH (Code K8005) (LOT:20012657),Antibody diluents S0809 [12].

* DAB indicates 3,3′-diaminobenzidine.

Tissue preparations for IHC

Tissue sections of (4) microns from the formalin fixed, paraffin embedded blocks of breast tissue biopsies were cut using a microtome and mounted on the positive charged glass slides. These slides form bounds with the tissue so that specimens adhere to the slide and remain in place during the staining procedure The immunostaining

method used in the current study was En Vision immunohistochemical technique which was applied for HER-2/neu,ER and PR staining [13].

Evaluation of the Results:

The scoring of immune reactive Staining was done by calculated the percentage of immune reactive cells per total number of malignant cells. The staining intensity was evaluated by calculating the percentage of positive cells in 100 malignant cells at objective 40 total magnifications. For immunohistochemistry assessment, slide evaluation was performed using a light microscope. Slides were scanned by X10 magnification. Five cellular areas selected (The so-called hot spots) and evaluated at X40 magnification by a pathologist.

Estrogen and Progesterone Receptors Scoring System

Evaluation of ER, PR staining takes into consideration both the proportion and intensity of stained cells. The proportion score (PS) estimates the proportion of positive tumor cells and ranges from 0 to5 [12].

Her2/neu Scoring System [3,14,15]

HER-2 neu was scored on a 0 to 3 scale according to the criteria set by ASCO/CAP guidelines.

Statistical Analysis

Statistical analysis was done by using SPSS (statistical package for social sciences) version 20. In which we use frequency, percentage and mean as descriptive statistics while chi square and Yates corrected chi square as analytic statistics.

P value ≤0.05 regarded significant.

Results

This study includes two groups according to age, where the cutoff point is 65 old age ≥65 whose constitute 23.2% of all patient and young age<65 whose consistuate76.8% of all patient, So the largest group is the young patient.

Immunohistochemical Parameters

The General Immunohistochemical Expression (Figure 2).

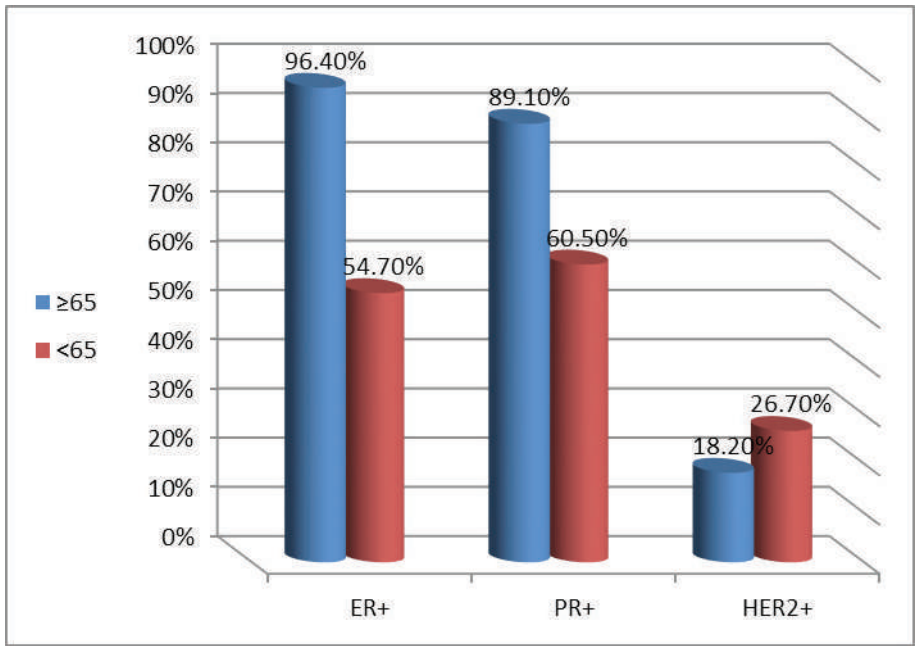


Figure 2 Frequency & percentage of ER+, PR+ & HER2+ according to the age

The biological profile of breast cancer

molecular types in patient with breast cancer as follow; luminal A 127(59.24%) cases, luminal B 18 (9.00%) cases, basal type 30 (14.22%) cases and her 2 has 36(17.54%) cases.

Old age group patient has molecular types as follow; luminal A 37(78.2%) cases, luminal B 9 (16.4%) cases, basal type 2 (3.6%) cases and her 2 has 1(3.6%) case.

young age group patient has molecular types as follow; luminal A 90(56.4%) cases, luminal B 9 (6.4%) cases, basal type 28 (16.3%) cases and her 2/neu has 35 (20.9%) cases.

Clinicopathological analysis Tumor size (Figure 3).

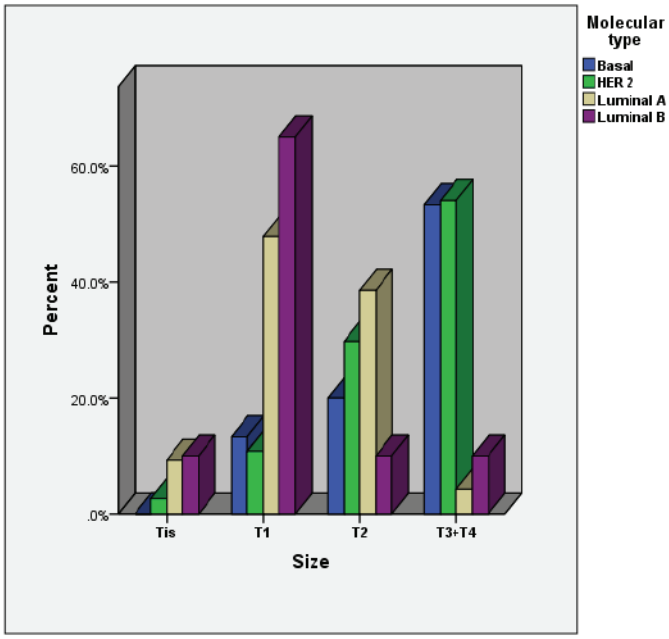


Figure 3 Distribution of molecular types according to size of tumor.

Histopathological types (Figure 4).

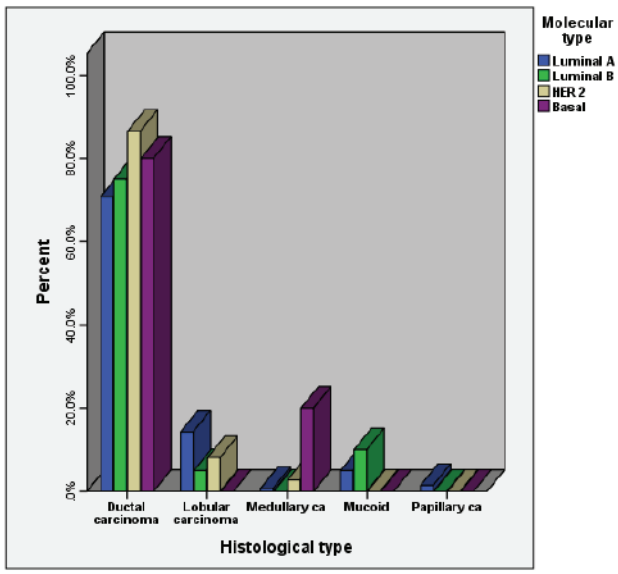


Figure 4 shows significant association between biological profile and histological type.

Stage of Axillary Lymph Node Involvement (Figure 5).

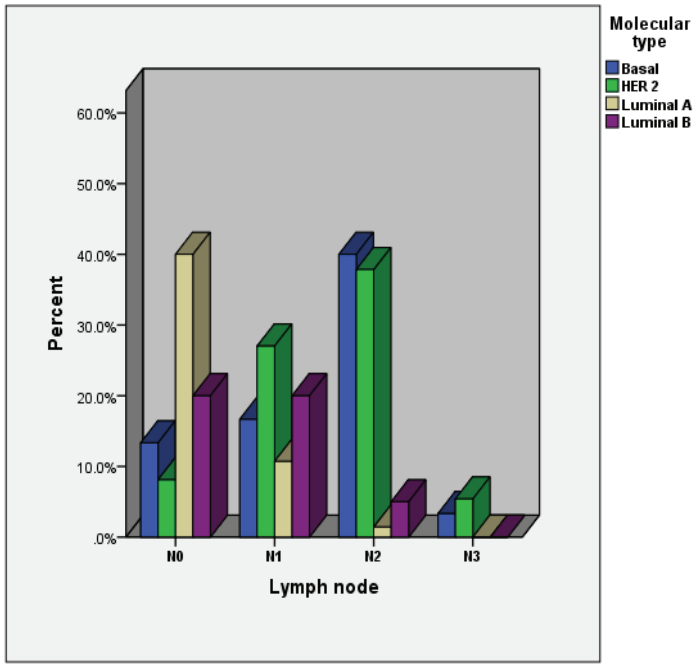


Figure 5 Distribution of molecular types according to lymph node involvement.

Discussion

We used in this study IHC surrogates, which we validated against gene expression determined molecular subtypes, to identify breast tumor molecular subtypes in a 211 breast cancer patients. In the present study of patients, the distribution of particular molecular subtypes was127 luminal A, 18 luminal B,36 HER2+ and 30 basal like. This agree with Ihemelandu et al. [16] who classified breast cancer into four molecular subtypes (basal cell-like, HER2/neu, luminal A and luminal B) and analyzed the prevalence and clinicopathological associations for these molecular subtypes, the luminal A type was the most prevalent 140 case. this agree with Xiaofeng Dai et al [17], and disagree wih Changhong et al. [18] who found, Luminal B is the major molecular subtype of breast cancer patients This is different to other research reports and may reflect the genetic background of racial differences and may also be due to the detection method used and the standards used for judging the cause of the disease. this agree with, and disagree wih TNBC lacks expression of ER, PR, and HER2 receptors, accounts for 10–20% of all reported breast cancer, and largely overlaps with the basal intrinsic subtype Madhuri K et al. [19]. We demonstrated that the distribution of molecular subtypes between elderly and young patients were statistically significantly different.

De Kruijf et al. [20] showed that the molecular intrinsic breast cancer subtypes have a different distribution in elderly breast cancer compared to their younger counterparts, where elderly patients more frequently had less aggressive Luminal A and Luminal B tumor subtypes.

where we defined elderly breast cancer patients as patients aged 65 years or older according to World Health Organization definition [21]. With this cut-off point, elderly breast cancer patients showed more often Luminal A and Luminal B molecular subtypes less often Her2/neu, basal molecular subtypes. This agree with Perou et al. [22], Carey et al. [23] and others [24,25,26]. while disagree to Danae Pracella et al., who found no specific age distribution related to molecular subtype of breast cancer [27]. De Kruijf et al. [20] also identified a statistically significant association (P=0.02) between patient age and tumor molecular subtype with luminal tumors being more frequently identified in elderly patients with basal like type tumors were younger at the time of diagnosis and had larger, more undifferentiated tumors this agree with Dent et al. [19], Markata et al.[28],who found that young females exhibited tumors with HER2+ and basal

molecular subtypes also findings agreed with result of Reina Haque et al.[29] which showed that Her2 enriched subtypes occur mainly in premenopausal women. ,and Park *et al* [30], While some preliminary studies suggested that the distribution of histologic subtypes is different in young women with a higher prevalence of triple negative and HER2+ disease, a clear molecular characterization of BC in these patients is lacking[31,32]. Though triple the high frequency of basal-like tumors among young women could be an explanation for the biological aggressiveness that has been associated with breast cancer diagnosed in young women when compared to breast cancer diagnosed in older women this agree with , In our study, the distribution of the molecular intrinsic breast tumor subtypes in this older breast cancer population showed a higher prevalence of the assumed more indolent Luminal A tumor and a relatively low prevalence of the more aggressive Basal molecular tumor subtype but higher in a younger breast cancer this agree with Carey et al. [33].

These results imply that the chance of getting a more aggressive molecular tumor subtype decreases with increasing age, which is in accordance with the observation of milder tumor characteristics in the older breast cancer population. This agree with Charla C. et al. [11]. Our results support the premise that breast cancer clinical behavior is significantly affected by patient age. We suggest that competing risks of death in elderly patients, ER driven differences and micro-environmental changes in biology are underlying these age-dependent variations in patient prognosis.

Conclusion

1-Old age patient with breast carcinoma have lower histopathological grade and stage of axillary lymph node involvements than young patients with more proportion of ER and PR positive cases.

2 –Old age breast carcinoma patients have better prognosis in comparison to young age patients according to the biological profile, histopathological and immunohistochemical prognostic parameters.

3-The pattern of molecular type of breast cancer patient was statistically significant correlate with age of patients, size of the tumor mass, histopathological typing, grading and lymph node status.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Some Histogenetic Aspects of Methotrexate Associated with Juvenile Idiopathic Arthritis (JIA) Treatment Doses

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Abstract

A total of (36) mice in the age of (1-3) weeks were selected to evaluate the methotrexate effect on some histogenetic aspects of kidneys. Treatment lasted for three months, and results showed marked hemorrhage, cortical tubular basophilia, necroses, and degeneration in the autopsied organ of animals with low oral hydration. Besides, changes in the DNA methylation pattern were noticed in the RAPD-PCR profile upon analysis with agarose gel electrophoresis.

***Keywords**: Methotrexate, Kidney, DNA methylation, JIA, RAPD-PCR.*

Introduction

Methotrexate (MTX) is an antimetabolic agent and a structural analog that affects the metabolism of folic acid and inhibits cell proliferation [1&2]. This drug came into the clinical application as an advanced antineoplastic drug in 1948, due to its indirect effects on DNA synthesis [3]. It is a well-known immunosuppressive agent widely used for the treatment of many rheumatologic, dermatologic, and hematologic diseases successfully [3-6]. In children, methotrexate is the cornerstone for the treatment of juvenile idiopathic arthritis (JIA), the most common chronic rheumatic disease of childhood, and the major cause of disability [6-8]. It acts by inhibiting dihydrofolate reductase, which reverts DNA hypomethylation and reducing cell proliferation [8-10]. Methotrexate dose is usually eliminated by renal excretion, which increases the risk of renal damage and nephrotoxicity that sometimes develops as a side effect due to inadequate oral hydration and increased urine acidity [11&12]. Moreover, it was found that gene dysregulation associated with variability in DNA methylation might contribute to the initiation of cancer diseases [3-5]. Thus, many perturbations raised considering the side effects of using MTX as the cornerstone for prolonged treatment of JIA[11-13].

In accordance, this study aimed to investigate the side effects of methotrexate treatment associated with the treatment doses of juvenile idiopathic arthritis on the kidney tissue and DNA methylation pattern of white mice as a model.

Methods

Study animals

Healthy mice of *Mus musculus* strain obtained from the Association of Genetic & Environmental Resource Conservation (AGERC)/Iraq during 2019. Mice aged (1-3) weeks and weighed (20-23 gm) were housed and fed according to the procedure mentioned by Ref. [14].

2.2. Experimental design and Methotrexate administration

All animal studies conducted were according to the Guide of Experimental Animals [15]. Intraperitoneal injections of Methotrexate brand of (FRESENIUS KABI/ USA) used in the administration protocol summarized in (Table 1) according to the procedure cited in Ref[16].

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Table 1:- Methotrexate administration protocol of the study

Treatment Groups n=12 mice /GP 1:1 male to female ratio	MTX Dose mg/m2 BSA/Week	Water Intake ml/day	Water Intake (L / 3 months) Study Interval	Total MTX concentration mg/m2
Gm1	10	100	8.4	120
Gm2	10	300	25.2	120
Gc	0	300	25.2	0

*MTX doses were calculated to bodyweight Index according to the FDA protocols cited in Ref.[17]

Four mice of both sexes per treatment group were autopsied at the end of each month after anesthetization with the injection of (100 mg/kg of Ketamine and 5 mg/kg Xylazine (Alfasan /Turkey); kidneys tissues were immediately removed after autopsy according to the procedure cited in Ref.[18]. Kidneys were cut into two parts; one half was fixed in (10% formalin) for histopathological examinations, while the other half was placed into liquid nitrogen (BIOBASE/ Meihua /China) at -20℃ for genetic assessment according to Asci *et al*.[19].

Histological Valuation

Kidney fixed in 10% formalin were embedded in paraffin, and tissue sections were cut at 5µm by Microtome (BIOBASE/ Meihua /China), mounted on slides after staining with Hematoxylin Eosin Y solutions (HE Stain Kit/TissuePro/USA) according to Ref.[18]. Sections were examined by a light microscope (Optika/Italy) and photographed by mounted Camera (Optica- Italy 4083-B5). Significant histopathological abnormalities were evaluated and recorded accordingly.

Genetic assessment of Kidneys DNA methylation pattern

Five methyl Cytosin levels were relatively quantified after the enzymatic hydrolysis of the genomic DNA with HpaIIand MspI restriction enzymes (1U / 1µg DNA/Thermo Scientific/France). DNA extractions were performed using the DNA isolation kit (Genomic Prep /Amersham Biosciences/UK) following the instructions of the manufacturer. Then extracted DNA was resuspended in Tris-EDTA, and the quantity of the extracted DNA checked by spectrophotometer (Schimadzo/Japan) as described in [20]. For this analysis, RAPD-PCR (Eppendorf /Germany) primer set (Ready-To-Go RAPD- PCR analysis kit/ Amersham Biosciences/ UK) used as instructed by the manufacturer company. Three samples of Kidney DNA (control, MTX 10mg, MTX 15mg) were subjected to PCR with a single primer concentration of 25 pmol in a reaction volume of 25 μl and a DNA marker of (pUC19 DNA MspI/HpaII Marker/ Thermo Scientific/ France). Each change observed in RAPD profiles was scored as described in [22].

Results

This study was conducted to observe the histological and epigenetic changes in the kidneys tissue due to methotrexate treatment for three months of (10 mg/m^2 BSA/ week) dose associated with the therapeutic doses of the juvenile idiopathic arthritis (JIA) but under low water intake circumstance. The histopathological examination of the kidneys of the study groups experimental animals showed the following results:-

-Macroscopic Histopathological Changes:

The excised kidneys of the ($\mathbf{G_{m1}}$) group of mice that received intraperitoneal injections of (10 mg/m^2 BSA/ week) treatment doses for three months durations with low water intake showed congestion and enlargement

signs. While the kidneys of the (G_{m2}) group of animals that received the same treatment with the same duration but with average water intake showed normal kidneys texture except for the last two weeks of the third month where excised kidneys of the (G_{m2}) group showed congestion marks. On the other hand, the (G_c) group that received intraperitoneal injections of (0.1 ml) of normal saline showed normal shape and size of kidneys.

-Microscopic Histopathological Changes:

The examined kidney tissues of the (G_{m1}) group of mice showed marked aggregation of lymphocyte, congestion with fatty degeneration, and dilated tubules in the second month of treatment as declared in (Figure 1).

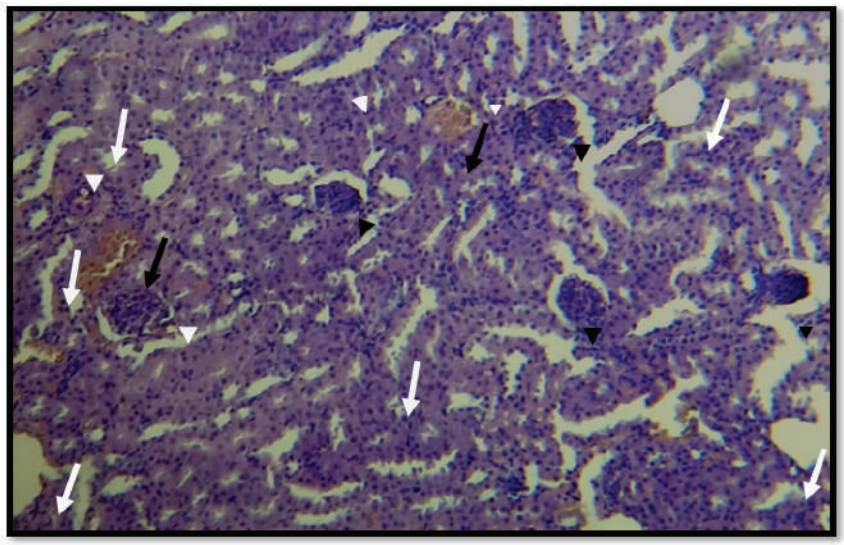


Figure 1:- Microscopical findings of the kidney tissues in the methotrexate treatment group (G_{m1}) for two months with low water intake showing marked aggregation of lymphocyte (black arrows head), congestion (black arrows), fatty degeneration (white arrows), dilated tubules (white arrows head) examined under a light microscope by different magnifications.

Whereas, the prolonged treatment for the third month of the (G_{m1}) group of mice with continued low water intake caused marked hemorrhage, cortical tubular basophilia, necroses and degeneration in the autopsied kidney tissues for both sexes of animals as manifested in (Figure 2).

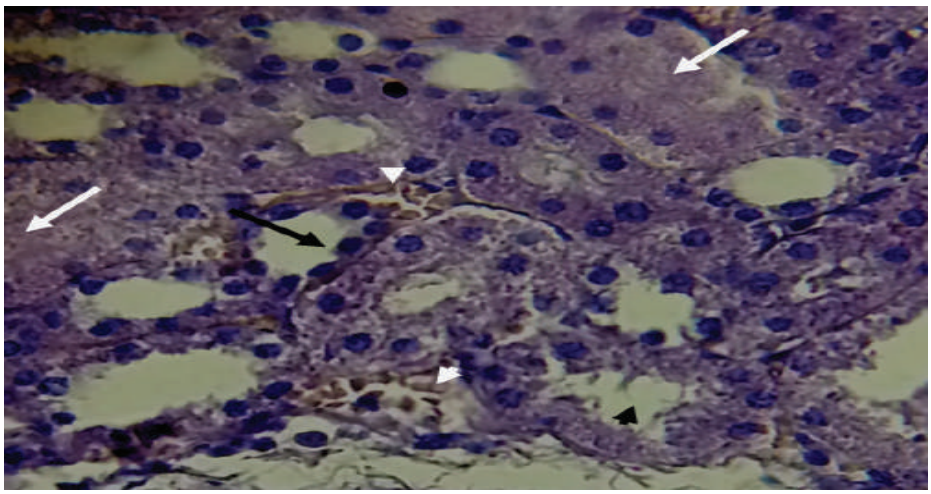


Figure 2:- Microscopical findings of the kidney tissues in the methotrexate treatment group (G_{m1}) for three months with low water intake showing marked hemorrhage (arrows head), cortical tubular basophilia (black arrow), necroses (white arrows)degeneration(black arrowhead) examined under a light microscope by different magnifications.

Yet, the (G_{m2}) group of mice treated with the same methotrexate dose but with sufficient water content showed signs of cortical tubules basophilia with infiltration of lymphocytes only in the third month of methotrexate treatment. However, the epigenetic effect of methotrexate treatment on the genomic DNA methylation declared in (Figure 3).

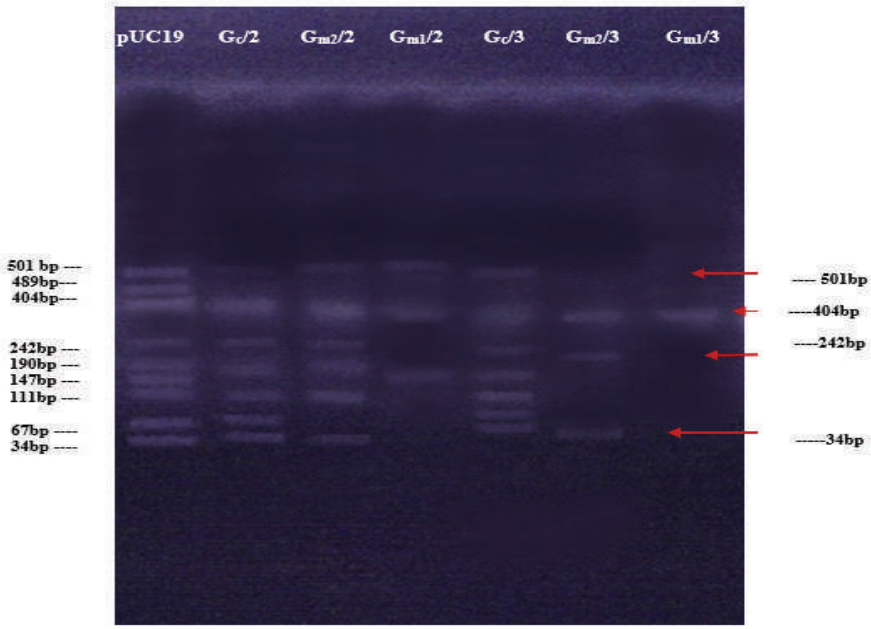


Figure 3:- Gel picture of RAPD-PCR products of Kidney tissues of treatment samples for two and three months of methotrexate associated with JIA doses on (1.7%) agarose gel electrophoresis stained with ethidium bromide. Gel visualized under UV-Transilluminator and pictured using a gel documentation system. pUC19 Lane is Marker DNA; Lane G_c is control group kidney DNA; Lane G_{m1} is a methotrexate treated group with low water content; Lane G_{m2} is a methotrexate treated group with sufficient water content. Numbers 2/3 means DNA extracts of two and three months of treatment.

The summary of RAPD-PCR products after the analysis of the banding pattern of genomic DNA methylation is outlined in (Table 2).

Table 2:- RAPD-PCR profile of DNA methylation pattern of kidney tissues treated with Methotrexate.

Banding profile of pUC19 DNA bp	Banding profile of Gc /2 DNA/bp	Banding profile of Gc/3 DNA/bp	Banding profile of Gm2/2 DNA/bp	Banding profile of Gm2/3 DNA/bp	Banding profile of Gm1/2 DNA/bp	Banding profile of Gm1/3 DNA/bp
501	+ve	+ve	+ve	-ve	+ve	-ve
404	+ve	+ve	+ve	+ve	+ve	+ve
242	+ve	+ve	+ve	+ve	+ve	-ve
190	+ve	+ve	+ve	-ve	-ve	-ve
111	+ve	+ve	+ve	-ve	-ve	-ve
67	+ve	+ve	-ve	-ve	-ve	-ve
34	+ve	+ve	+ve	+ve	-ve	-ve

*+ve refers to band presence; -ve refers to band absence in the agarose gel banding profile

Discussion

Juvenile idiopathic arthritis (JIA) is an autoimmune disease associated with functional disability and many complications in the child's life [16]. It is prevalent in children and teenagers, causing uncontrolled inflammations and irreversible damage to joints and other tissue [23]. In Iraq, there is a significant increase in the disease ratio between post-school and young children [24-26]. Thus, the improvement of the disease outcomes of children with JIA remains a critical challenge for the clinician, where most of them rely on methotrexate as their first-line of treatment agent[3&4]. Methotrexate is a standard treatment used in many countries for children who suffer from moderate to severe arthritis; it is primarily cleared through kidneys where renal excretion eliminates about 80% of the dose[25 & 26].

However, the elimination of the methotrexate dose depends on the urine alkalinization where methotrexate is a weak acid and tends to precipitation at urine pH below (6.0) leading to nephrotoxicity [11-13]. Accordingly, the present research intended to evluate the effect of methotrexate administration on kidney tissues of mice under insufficient oral hydration conditions. The equivalences of Dutta and Sengupta, 2016 [27] for men and mice ages were considered in the experimental animals to be in correspondence to the ages of young children in humans. Besides, treatment groups were divided into two groups according to the oral hydration circumstances in order to study the effect of methotrexate on children with low water intake, where most of the children might forget to drink sufficient water for at least six hours during the school day.

The kidneys of the treatment group with inadequate oral hydration (G_{m1}) appeared more affected in all treated animals than the other group (G_{m2}), where the kidneys showed marked cortical dilatation of tubules and necrosis. The same result obtained by Chelab and Majeed in 2009[26] where they had indicated that methotrexate crystals accumulated in the nephron tubules and the dilatation of nephric tubules of renal cortex occur due to thickening of basement membranes of tubules and precipitation of the methotrexate crystals in the nephron of the kidneys due to water dehydration. The same results were confirmed by Asci *et al*., 2017[19], where they established the etiology of MTX-induced nephrotoxicity.

Hence, genomic instability results in multiple molecular events believed to be a driving force in cancer etiology [22]. In this study, the RAPD-PCR technique with two methylation-sensitive restriction enzymes were used to analyze the genomic instability. Banding profile showed no change in the bands of the control group. While the (G_{m1}) group showed a significant difference in the banding pattern; where four bands of the molecular weights of (190bp,111bp,67bp, and 34bp) were missing from the profile after two months of treatment. Moreover, six bands were missing from the RAPD-PCR profile after three months of treatment with the molecular weight of (501bp, 242bp, 190bp, 111bp, 67bp, and 34bp) respectively.

Accordingly, the (G_{m2}) group showed changes in the DNA profile of the methylation pattern with one missing band of (67bp) molecular weight upon two months of treatment and three absent bands of molecular weights of (190bp, 111bp, and 67bp) at the end of the third month of methotrexate treatment respectively. These results, however, might indicate a probable role of methotrexate as a carcinogen and a teratogen that might affect the life quality of the treated child in the future. Those results agree with the results obtained by de Andres *et al*., in 2015[5] where they confirmed global DNA hypomethylation in patients with rheumatoid arthritis treated with methotrexate and the role of aberrations in the DNA methylation system as an essential tool in the initiation of certain human diseases.

Conclusions

The consequence of this study is related to the dose and route of administration of methotrexate in JIA issued to prevent treatment side effects as it was concluded that sufficient oral hydration could reduce histopathological side effects of methotrexate but not hypomethylation. Also, the nephrotoxicity of methotrexate necessitates caution when considering it by the physicians for the treatment of patients with kidney function impairment. Last, JIA treatment with methotrexate predetermines the warning of the child's parent about the outcomes and life quality after this drug's treatment since it is proven carcinogenic and probably teratogenic.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict Of Interest: Non

Funding: Self-funding

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Seroprevalence of Syphilis among Iraq's Blood Donors and Their Relation with Blood Group and Rh Factor

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Abstract

Aim of Study: to detect the frequency of syphilis disease among healthy blood donors and if there's association with ABO Blood groups and Rh factor.

Methods and Material: this study performed in Iraq National Blood transfusion in Baghdad city, collection of blood samples obtained from apparently healthy blood donors for a 12 month period (Jan.2018 toDec.2018). Blood group for the volunteers was determined by forward and revers grouping method. Detection of syphilis performed by using ELISA technique (fortress,BXE0995C, united Kingdom) for detect antibodies in the human serum. Syphilis test on of the routine test applied in the blood donor bank in Iraq.

Result: a total of 178966 healthy volunteers included in the study, all were blood donors, age 18-60 years, with mean age of 39 years. Only 1233 (0.69%) appeared infected with syphilis, 1208 (98%) were males while 25(2%) were females; 444(36%) seropositive was blood group A, while blood group O and blood group B seropositivity was 366(29.7%) , 333(37%) respectively, The lowest percentage recorded for blood group AB at 90(7.3%). The prevalence of syphilis with Rh factor was as following: 1147(93%) of seropositive was Rh +ve whereas 86(7%) was Rh -ve. **Conclusion**: blood group A and O are the most prominent blood groups among voluntary donors and the prevalence of syphilis is equal compared with other countries.

Key word: *Treponema pallidum, syphilis, blood group, blood donors*

Introduction

Blood transfusion is one of the potential risk factors for transmission of various blood transmitted pathogens, such as hepatitis B virus (HBV), human immunodeficiency virus (HIV), hepatitis C virus (HCV) and syphilis.[1] For this reason these tests are done in Blood Bank to avoid dissemination of infectious diseases. The tests performed on blood donation are necessary, not only for compatibility but also to prevent distribution of blood-borne pathogens. Compatibility It is one way to limit posttransfusion adverse events and to phenotype blood donors for red blood cell group system. Infectious tests aim to detect transfusion-transmitted infections, syphilis is one of them.[2]As per World Health Organization (WHO), there are 39 countries where routine screening tests are not performed during blood donation from which HIV, HCV, HBV, and other transfusiontransmitted infections, and 47% of already donated blood were examined without quality assurance in laboratories.[3] Blood group and compatibility test were done to explain the relationship between infectious diseases and red blood cell antigens.[4]

Syphilis is one of the sexually transmitted disease (STD), caused by *Treponema pallidum* bacteria, it is a chronic disease with multistages,[5] it is transmitted also to the baby by the mother during pregnancy or birth, resulting in congenital syphilis which could cause damage to body systems (nervous, skeletal, skin, mucous membrane), it also could result in abortion and still birth.[6]Other mode of transmission of syphilis is blood transfusion and

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accidental direct inoculation.[7]Currently rhinoplasty, injection, laser, razor reuse, ear piercing, tattoos, liposuction, and sometimes the condom increased the incidence of transmission greatly,[8,9]17.27% of college students were reported to have a history of cosmetic surgery such as rhinoplasty, and 69.4% reported that they would undergo such surgery without consideration of economic factors.[10,11,12] In 2011 the WHO reported 12 million new cases of syphilis.[13]The fact that *T.palladium* cannot survive in properly stored blood, makes it essential to ensure safety transfusion in Oder to protect human lives.[14]Red blood cell surface contains different blood group antigens which is composed of polysaccharides and proteins,[15]ABO and Rh factor are most important ones among these antigens.[16] In the last decades many studies probed the association of blood groups and some infectious diseases.[17]Many studies illustrated the association between specific blood group antigens and pancreatic cancer risk.[18,19,20] Other studies demonstrated the association of ABO system with malaria, cerebral thrombosis and ovarian cancer.[21,22,23]Some blood groups can represent a receptor and ligand for specific types of bacteria, parasites, and viruses. Interaction of these certain types of microorganisms and RBC membrane could be due to antigenic resemblance, adherence through specific receptors or modulation of antibody response.[24] Limited inaccurate data available on the prevalence and epidemiology of syphilis in Iraq showing the association between blood groups and frequency of syphilis. The aim of this study is to investigate the prevalence of syphilis in Iraqi blood donors mainly,Baghdad province referred to Iraq National Blood Bank and to determine the association between blood groups and syphilis infection.

Methods and Materials

This study conducted in Iraqi National Blood Bank in Baghdad city. Syphilis detected by Enzyme-linked immune sorbent assay (ELISA) technique (fortress,BXE0995C, united Kingdom) following manufacturer's instructions to detect the presence of antibodies against syphilis in the serum of human. Blood samples collected from the volunteers Jan. 2018 continue to Dec. 2018. All volunteers were screened for routine infectious tests: HIV, HCV, HBV, HBsAg, in addition to syphilis.

Blood donors: 178966 blood donors recruited for the study**,** all the donors are apparently

healthy and do not suffer from any impairment. Their age ranges between 18-60 years. Venous blood was collected and directly performed in Iraqi National Blood Bank, medical history from each volunteer was taken.

Determination of blood group: Blood groups were determined by forward grouping (cell grouping) using a known monoclonal monospecific antisera: Anti-A, Anti-B, and Anti-D to determine antigen expressed, and revers grouping (serum grouping) using known A&B cell to determine the presence of antibody by test tube agglutination method.

Statistical analysis: Data of the study analyzed by SPSS version 25. With probability $P<0.05$. Chi-square used to determine significant differences among blood donors.

Result

The study included 178966 blood donors, with age between 18-60 year with mean age of 39. Seropositive of syphilis appeared in 1233 (0.69%) only. Number of seropositive males was 1208 (98%) while 25(2%) were females, as shown in table (1), from which 444(36%) seropositive with blood group A, 333(37%) seropositive with blood group B, 366(29.7%) seropositive with blood group O, and 90(7.3%) seropositive with blood group AB, as illustrated in table (2) and figure (1). Relation of seropositive syphilis with Rh was as follow: 1147(93%) of seropositive was Rh+ and 86(7%) was Rh-, as demonstrated in table (3).

Table-1- Seroprevalence of syphilis among blood donors, and their relation with sex, for each month within 2018 year.

	Numbers of donors	Seropositive of syphilis (%)	Male (%)	Female (%)
January	15406	115	113	2
February	13714	103	101	2
March	16497	129	128	1
April	13939	87	87	0
May	15235	114	113	1
Jun	12333	88	87	1
July	15401	106	103	3
August	15482	87	83	4
September	15946	106	103	3
October	15331	102	99	3
November	15372	84	84	0
December	14310	112	107	5
Sum.	178966	1233(0.69%)	1208 (98%)	25 (2%)

Table-2- Seroprevalence of syphilis infection among blood donors according to their blood group.

Blood group	No. of seropositive	Ratio of donors (%)
A	444	36
B	333	37
O	366	29.7
AB	90	7.3
Sum.	1233	100

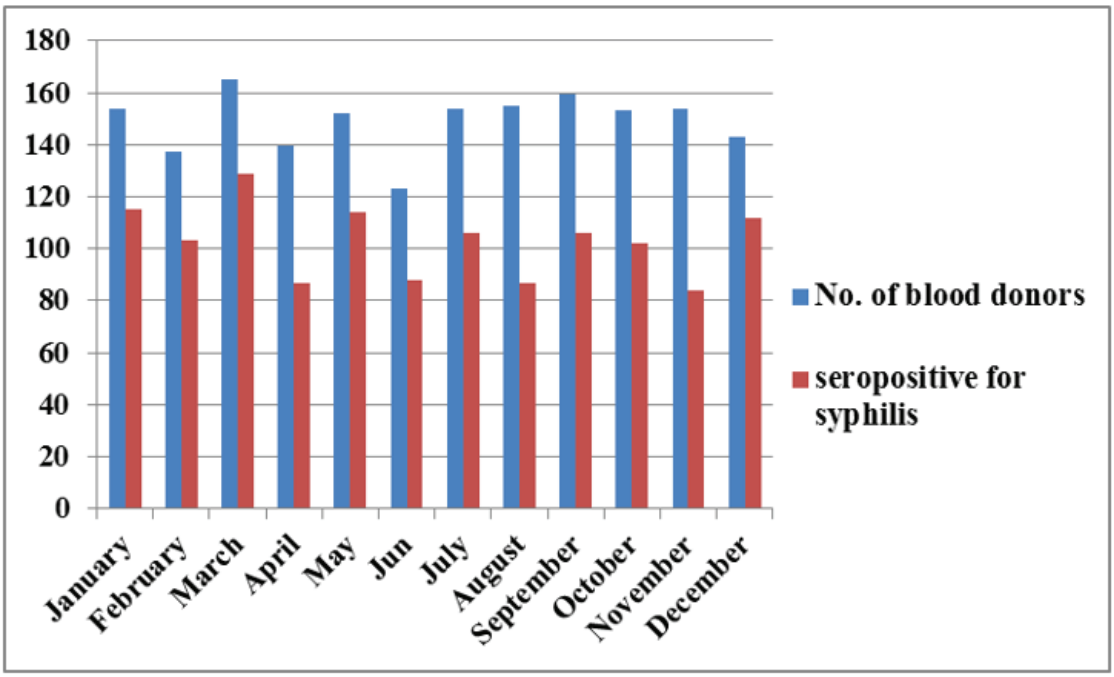


Figure (1): shown the numbers of blood donors each month within 2018, and numbers syphilis infections.

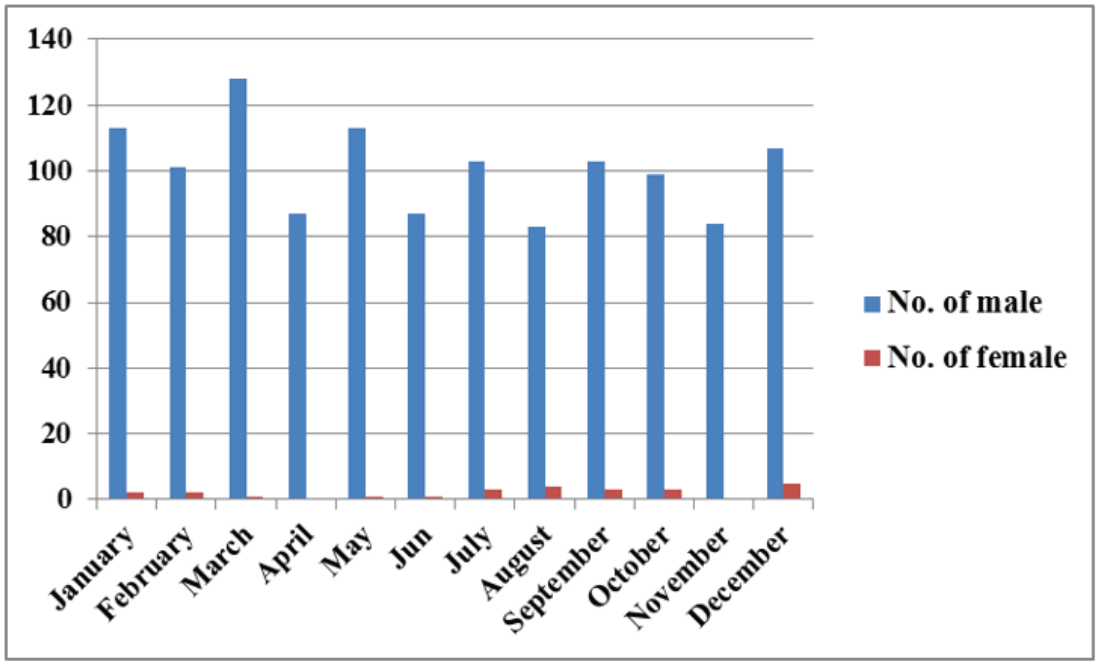


Figure (2): shown the numbers of infected male and female with syphilis each month within 2018 year

Table-3- Seroprevalence of syphilis among blood donors according to Rh.

Rh	No. of seropositive	Ratio of seropositive (%)
Rh+	1147	93
Rh-	86	7
Sum.	1233	100

Statistical analysis for this data performed by SPSS software (version 25), chi square was 227.117[a], and Asymp sig. were .000 appeared significant differences among blood donors seropositive to syphilis according to the blood group.

Discussion

This study was conducted in Iraq to correlate between syphilis and blood groups. our study performed in Baghdad province, Iraqi National Blood Transfusion Bank lies in Baghdad city/Bab-Al Muadham.

It was found that 1233(0.69%) blood donors are infected with syphilis, this ratio is approximate to that found in Al-Nasiriya province (south of Iraqi) out of 28287 blood donors there was 200(0.71%) infected with syphilis.[25] Another study also performed in Al-Nasiriya province in period from 2010 to 2012 where the rate of infection was high in 2012 at to 0.91%, however, in 2011 and 2010 were 90 (0.49%) and 60 (0.32%) respectively.[26]In our study, blood group A positive represents the highest prevalence positiveness for syphilis followed by O positive, B positive, and AB positive respectively. This contradicts the results of a study conducted in Al-Nasiriya province (Iraqi southern), where blood group O positive recorded with high prevalence of syphilis 92 (46%), A positive group 64(32%), B positive group 33(16.5%), and AB 11(5.5%) respectively.[25] The results of this study is also contrasting the results of other studies conducted in different countries where they found that the highest prevalence of syphilis appeared in blood group B in Pakistan.[27] Whereas in UK, USA, Nigeria and India blood groups O positive was significant. Similarly, In Iran, O+ group was the comments blood group appeared with frequency of 665,943 (32.8%), and only one O- with frequency (0.00004%) was the lowest among 2,028,086 blood donors in Tehran Blood Transfusion Center during 2005-2011.[28]In Sudan, especially in Gabon, the relation of syphilis

and blood group appeared with counterproductive, where prevalence of syphilis appeared in blood group O (58.9%) out of 4744 blood donors.[2] In Europe, France for example, frequencies of ABO was 45.0% in blood group A followed by 43.0%, 9.0%, and 3.0% in blood group O, B, and AB respectively.[29] In all studies in addition to our study AB blood group is the lowest for positive prevalence of syphilis. Most blood donors in this study from male and represent 1208 (98%) while female represent 25 (2%), because most donors was from male, this result similar to another study found that 98% of their donors were male,[30] In a study performed in Thi-qar province also found the infection with syphilis in male was 94.5% while in female was 5.5% from 200 cases out of 28287 blood donors in blood bank in Al- Nasiriya and blood bank in suq Al-Shiok, Refaei, and Al-Shatra.[25] In Iran, most blood donors were from male in 9918 (95%) and 558 (5%) were female.[28] Seroprevalence of syphilis in this study was 0.69% apparently equal to seroprevalence among Al-Nasiriya province blood donors were 0.71% ,[25] in Pakistan blood donors were 0.75%,[31] and appeared 0.85% among Indian donors,[32] and 1.2 % among Nigerian donors.[33] In Israel the prevalence of syphilis was 47:100,000 donors.[34] In Sharjah, United Arab Emirates, out of 20670 expatriates from both sexes male and female, only 105 (0.51%) appeared with positive prevalence of syphilis and those were from India, Pakistan, and Bangladesh ethnicity.[5] Study performed in India in 2012 recorded 198 (0.2%) seropositive for syphilis out of 104925 blood donors. In Tahran, seroprevalence of syphilis was 212 (0.010%) through period from 2005 to 2011.[28] In Sudan Seroprevalence of syphilis was 77 (1.6%) from 4744 blood donors.[2] Chengdu in China recorded 20,510 cases of blood donation infected with syphilis out of 2,100,071 in Chengdu blood center during period from 2005-2017, rate of syphilis in 2005 was 0.88% and showed increased rate of the infection in 2017 where it reached 0.98% and it was thought due to many risk factors such as: multiple sexual partners, commercial sex, razor reuse, ear piercing, tattoo, and sometimes the condom.[9] In summary, a significant association between syphilis and ABO groups was found among blood donors.

Conclusion

Our study showed increased in prevalence of syphilis among blood donors in Baghdad province the center of Iraq country as a result of Iraq's opening up to neighboring countries, expatriates enter without health oversight, and even on the people of the country since 2003, the beginning of openness our society on other societies. Blood safety is required in all blood donor banks in Iraq to control blood transfusion.

Acknowledgment: I would like to thankful all staff worked in Iraq National Blood Donors Bank/Baghdad city/Iraq for their endeavors to complete screening tests requested by each volunteer and included HIV, HCV, HBV, and syphilis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Comparative Histomorphological Study of Kidneys in Pigeon (*Columba livia*) and Starling Birds (*Sturnus valguris)*

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Abstract

This paper is focused application on the histomorphological and morphometrical differences of the kidneys of two species (*Sturnus valguris* and C*olumba livia*) belong to different orders varied in climate. Our study used 30 birds divided into two equal groups; first was 15 starling birds (*Sturnus valguris*) and the second group consisted of 15 pigeon (*Columba livia*). The current study showed morphological results of kidneys in both birds, where appeared differences between kidneys. In starling's kidney was described square shape with fused left and right kidneys, either pigeon's kidney was divided in to three lobes (cranial, middle and caudal) with elongated-like. The present study was revealed Mean measurements. The study recorded length of starling's kidney was (4.43±0.12cm), while weight and volume were recorded (3.75±0.05g) and (2.28m±0.04cm³) respectively, while in pigeon was kidney length (5**.**45±0.12cm)**,** weight of kidney was (4.85±0.02g) and volume about (2.21±0.06cm³). Microscopic results were included in two species, the renal cortex which made up the majority of the kidney, while small region of kidney called medulla. Our study was different impatience between starling's bird and pigeon kidney about renal corpuscles, where the study showed the bowman's space in starling bird was wide from pigeon. The study observed significant statistics analysis. Mean measurement was (10.64±0.02µm) and (8.65±0.04µm) in bowman's space, while mean of the glomerulus capillaries and renal corpuscles of pigeon was a larger than starling's bird (47.54±0.11µm), (26.60±0.12µm),(56.55±0.12µm) and (37.41±0.11µm) respectively. Also the present work was recorded significant statistics analysis during calculation the number of renal corpuscles in 1 mm² in starling bird kidney was more than that in the kidney of pigeon (20.25 ± 0.23µm) and (16.42 ± 0.01µm).

Key words: *starling, pigeon, bird, kidney, histology, comparison*

Introduction

There are more than (8755) faction of the birds distribution around the world. These are birds different in strain, order and nature of the diet [1]. The starling birds (*Sturnus valguris)* are considered a seasonal birds during winter, while the pigeon (*columba livia*) are domestica birds in Iraq and other countries along year [2]. The kidneys are important organ inside the living organism during mammalians, birds and fish, and which have been many functions for the maintaining of continuance life, the kidneys do not affect nature of external factors such as the environment by providing a balance the body fluids during parts of nephrons, homeostasis, osmolality, ionic content, pH and finally ridding of metabolic waste materials outside the body [3]. The birds are probably affected due to climatic condition for semi desert areas may lead to adaption characteristics of these birds [4]. Anatomically, the kidneys of birds include left and right kidneys which are located symmetrically in pelvic region one on either side of the vertebral column [5]. Generally, the kidneys shape of avian are different from bird to other bird according to kind or strain, there are three lobes division into cranial, middle and caudal during left and right kidney [6 and7]. Histologically, the kidney of birds have been nephrons are dividing into cortical (reptilian) type

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which is a shortest type and locate in renal cortex more numerous, but lack henel's loop, whereas, the second nephrons named medullary (mammalian) type, it has a longest type and has henel's loop, this type of nephrons is less numerous than cortical nephron and extended to medulla [8 and 9]. Always all studies have been published about mammal kidneys, while comparative studies were recorded a few for avian kidneys. The current study is aimed to investigate comparison between two types of birds, first is called starling (*Sturnus valguris)* and other called pigeon (*columba livia)* depending on climate differences of their kidneys.

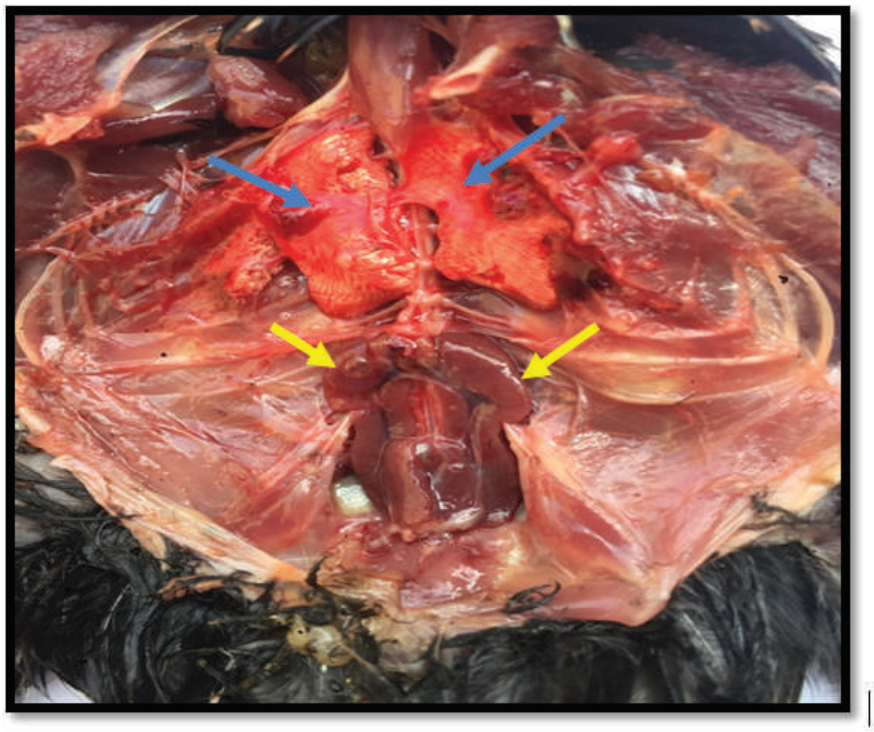
Materials and Methods

Thirty birds were used in our study. These birds were divided into two groups, the first group was included 15 bird of starling species *(Sturnus valguris)* and the second group was 15 pigeon (*Columba livia*). All birds were bought from Ghizzel market of Baghdad. The birds are put inside cages in the animal house in college of veterinary medicine of Baghdad University. The birds and pigeon were sacrificed after give injection sodium pentobarbitone (70 mg/kg) at intra muscularis [10]. We fixed the specimen on dissected board for dissection, and later we are done mid-line incision in abdomen region and after that we washed viscera of bird by distill water for removing the blood and other adheres particle. After that, we removed the internal viscera from the body to clearance of the kidneys organ for the comparison. The study are measured the length, weight and volume of kidneys, the length are taken by digital caliber, but the weights of bird were measured via sensitive electron balance, whereas the volume are measured by using the volumetric cylinder. For histological measurements, the study were used bouin's fixative to keep the kidney samples. Both birds were dissected for histological technique. Slides were stained by Hematoxylin and Eosin for general structure and Periodic Acid Sschhiff [11].

Results and Discussion

In morphological results of kidneys in both birds, the macroscopic sections were appeared differences between starling's kidney(*Sturnus valguris*) and pigeon's kidney (*columba livia*), where the study described starling's kidney square in shape and fused left and right side (fig.1). Whereas in pigeon was elongated–like and divided into left and right kidney with subdivided lobes. These lobes were included cranial, middle and caudal divisions (fig. 2). This result was parallel with [12 and 13]. whom said that the kidneys of quail and sparrows consisted of a large caudal, a small middle and a cranial part and conflicted with kidney's starling in our study. The study was revealed macroscopic measurements, mean length of starling's kidney, weight and volume were recorded (4.43±0.12cm), (3.75±0.05g) and (2.28m±0.04cm³) respectively, while in pigeon, mean of length was (5**.**45±0.12cm**),** weight of kidney was (4.85±0.02g) and volume about (2.21±0.06cm³). In histological cross sections, the study showed renal that kidneys of birds included the renal cortex which made up the majority of the kidney, while small region of kidney was called medulla (fig.3 and 4). These results were accepted with [7]. The renal cortex has the reptilian (cortical) nephrons without henel's loope while in medulla contain the mammalian type of nephrons with loop of henle (fig.5). These results were not corresponding with that [14] whom reported that fish no have henel's loop, subsequently that fish kidney included reptilian nephrons only. Our study was proved the differences between starling's bird and pigeon about renal corpuscles, where the study recorded the bowman space of renal corpuscle in starling bird was wide from pigeon, while the glomerulus capillaries of pigeon was a large than starling's glomerulus capillarie. This result was aimed to nature of bird life and seasonal condition. These results accepted with whom that [4] and different with that said in [3]. Also the present work was appeared that the renal corpuscles of two species of birds contain on an outer and inner layer of Bowman's capsule separated by Bowman's space from a centrally located glomerulus and podocytes which boundaries in inner layer of bowman's capsule, so the study showed during magnifying macula densa was prominent cells which appeared nearly between proximal and distal convoluted tubules as shown in the results. The current study showed renal tubules (proximal, distal and collecting) and collecting duct during cortex and medulla (fig.6). These results were accepted with whom said that [15] which described renal tubules and collecting duct in Quail and Green- Winged Teal. Our work recorded significant statistics analysis during calculation the number of renal corpuscles in 1 mm² in starling bird kidney was more than that in the kidney of pigeon (20.25 ± 0.23µm) and (16.42±0.01µm) respectively. Mean measurement

of renal corpuscles was (37.41±0.11µm)in starling, (56.55±0.12µm) in pigeon, but glomeruli capillaries were (26.60±0.12µm) in starling and (47.54±0.11µm) in pigeon. Also the study recorded significant analysis during mean measurement of bowman's space in two species, where was (10.64±0.02µm) in starling kidney and (8.65±0.04µm) in pigeon kidney.



Fiqure (1) : ventral view of the starling bird shows fused kidneys (yellow arrow) and lungs (blue arrow) .

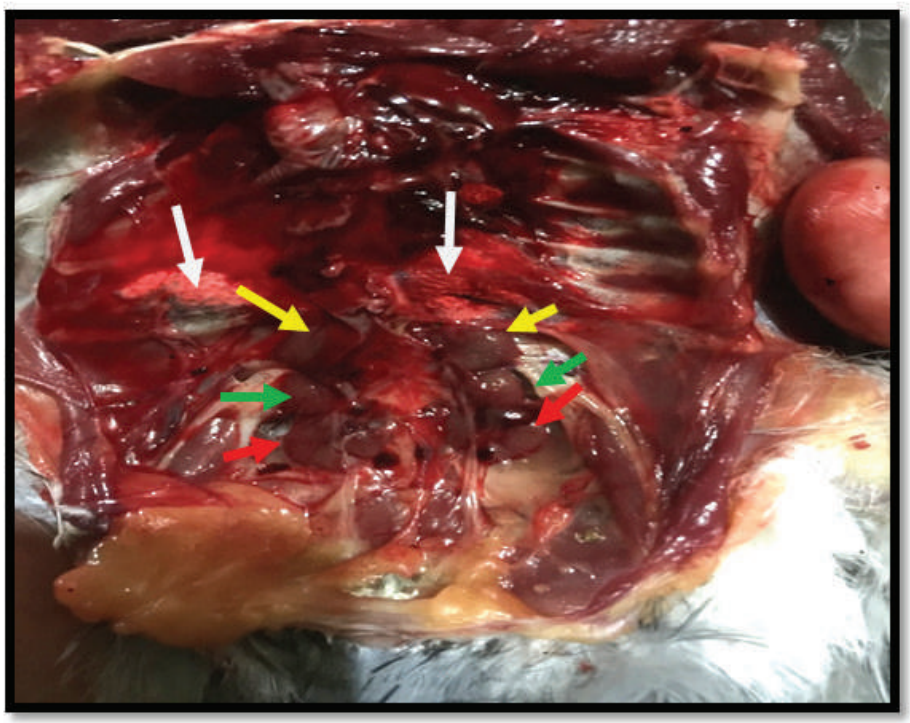


Figure (2) : ventral view of the pigeon shows the right & left kidneys divided into :cranial lobe (yellow arrow), middle lobe (green arrow),caudal lobe (red arrow)and lungs (white arrow).

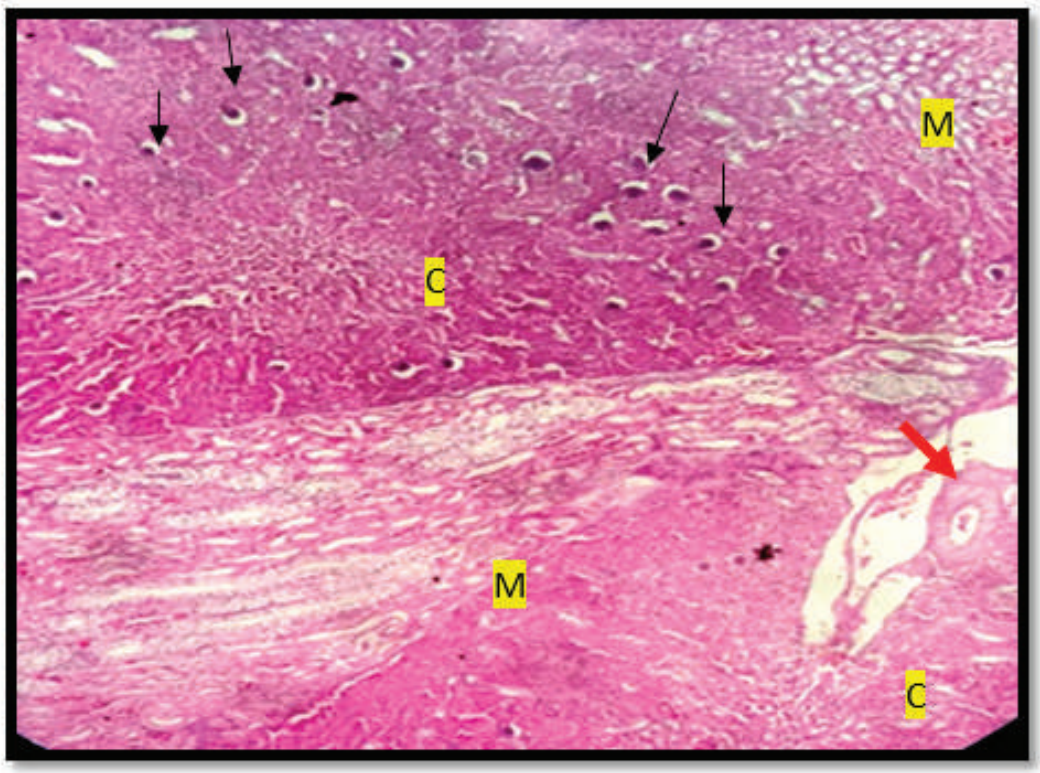
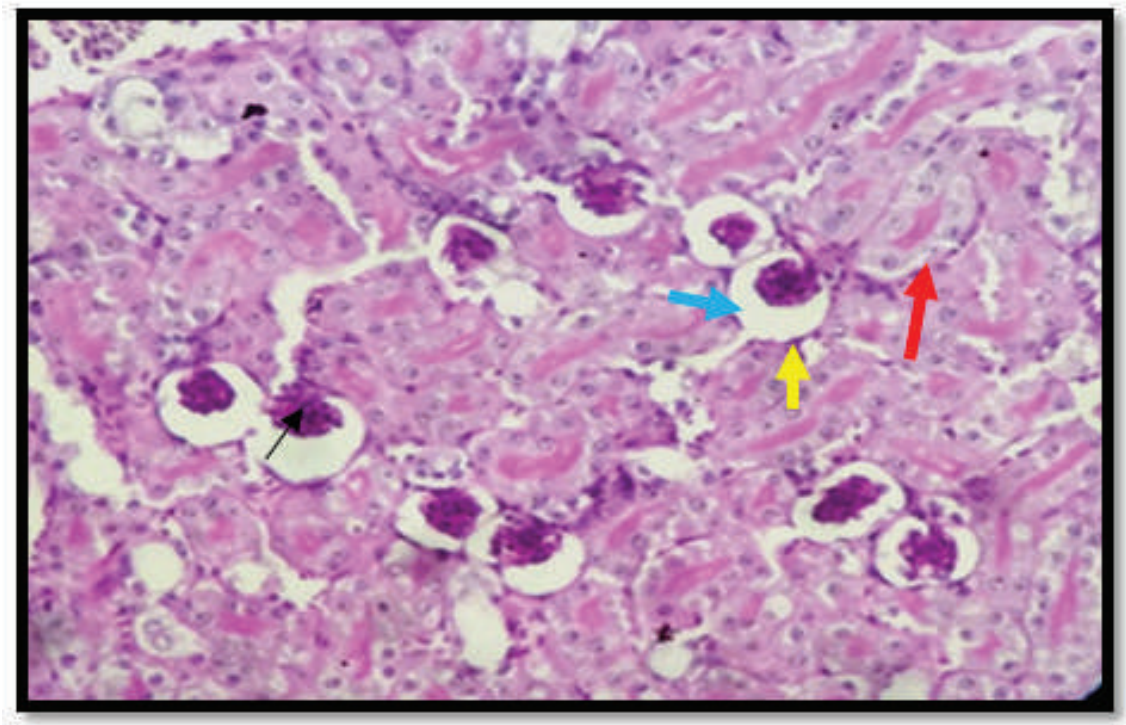


Figure (3): histological section of pigeon kidney shows renal cortex (C),medullary cone (M), renal corpuscles (small black arrow) and inter lobular blood vessel (red arrow) ,H&E 100x stain



Figure(4):Histological section of starling bird renal cortex shows renal corpuscles, bowman capsule(yellow arrow), glomeruli capillaries(black arrow),wide bowman space(blue arrow)and proximal renal tubules(redarrow), PAS 400x stain.

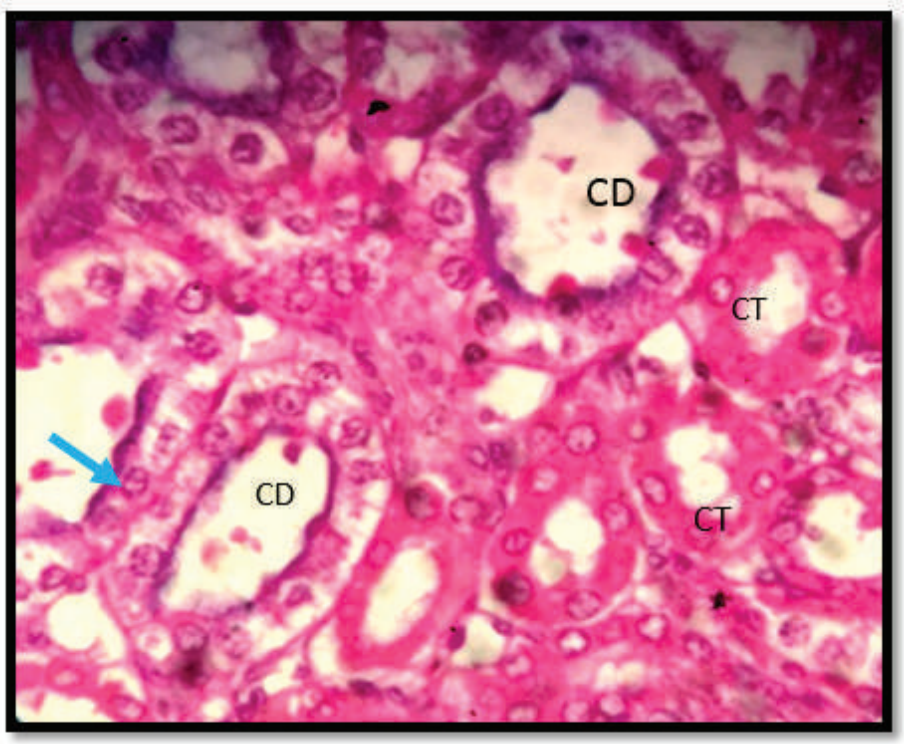
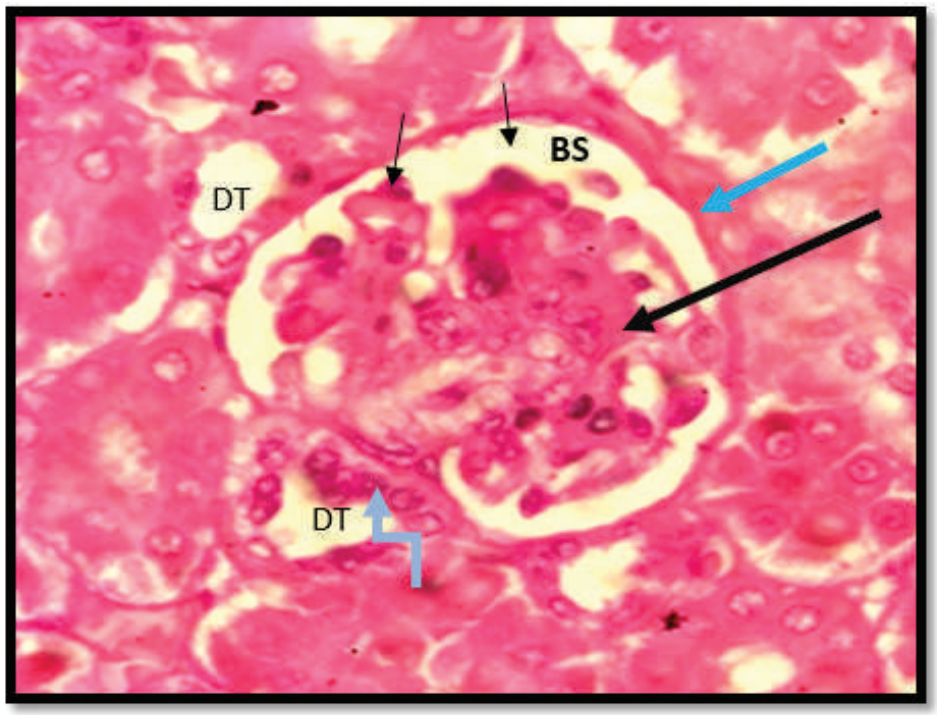


Figure (5): histological section of pigeon kidney medulla shows collecting ducts (CD)with lining simple cuboidal epithelium (blue arrow)& Collecting tubules (CT) with lining simple cuboidal epithelium, H&E 1000x stain



Figure(6): histological section of pigeon's kidney cortex shows renal corpuscle (Bowman's capsule) (blue arrow), glomerulus capillaries(large black arrow), narrow Bowman's space(BS), Podocytes(small black arrows), distal convulated tubules(DT) &Macula densa (curved arrows) H&E 1000x stain.

Conclusions

1. Pigeon's kidney had good developed nephrons represent from renal corpuscles (glomerulus ,bowman's capsule and bowman's space).

2. Starling's bird had less capacity of the kidney than pigeon due to seasonal condition of their birds.

3. Pigeon were more adaption than starling birds therefore that pigeon can live in any climate.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non.

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Socio-Legal Perspective on Renewing the Law Order In New Normal Situation of Covid-19 Pandemic

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Abstract

Covid-19 pandemic is a reality that has a big impact on all aspects of people's lives, whether economic, social, cultural, or legal elements. In the face of this extraordinary situation, social change becomes an inevitable necessity. However, this big challenge is not something that is easy to deal with. Various problems in the community arise and become anxious together as a nation. Through this study, an understanding is obtained that to face the Covid-19 pandemic situation in the midst of this new normal order, to deepen the role of Indonesian social capital of Pancasila. The study of legal sociology is deemed capable of revealing legal reality, social changes in the pandemic era and how we renew law in the middle of a pandemic will be well read and produce good judicial choices in the midst of Covid-19 pandemic.

Keywords: *Covid-19 pandemic, Indonesia, law, new normal, sociology of law.*

Introduction

The Covid-19 pandemic in the world has entered a disturbing point. Almost all countries have been infected with a pandemic, which until now has claimed hundreds of thousands of lives and made the earth's population stop their activities outside the home to prevent transmission of the virus. Various policies related to the strategy of suppressing massive transmission are taken in various countries by means of lockdown policies or also social or physical distancing in various models, such as the large-scale social restriction (PSBB) implemented in Indonesia. In line with the strategy taken, humans are active in their homes through the concept of Work from Home (WFH) or also Study from Home (SFH), which is very popular lately. However, among the efforts to overcome the transmission of Covid-19, various problems ensued. Problems that occur in various sectors of national life are our common concern to date, both in terms of health, economic, social, political, cultural, legal and others. The Covid-19 pandemic turned out to have such a broad impact in various sectors, even though the economic sector was the hardest hit from the existence of the Covid-19 pandemic. Based on data collected, for workers who were laid off and laid off more than 1.5 million, of this number 90 percent were laid off and 10 percent laid off. A total of 1.24 million people are formal workers and 265 thousand informal workers[1].

At present, in the context of the dynamics of life that is so fast due to the Covid-19 pandemic, the pattern of applying the normal order has only become a choice realized by the Government of Indonesia and in some countries in the world. Policies that are not easy to take, but must still be done because the application of this new normal order is our peaceful efforts towards all the problems faced by the Covid-19 pandemic. The term or word The New Normal Order appears at least popular, since the Covid-19 pandemic. That is why this study focuses on the dynamics of major changes taking place in Indonesia as a result of the Covid-19 pandemic. The study includes the pre-pandemic Covid-19 period which can be called normal, when the pandemic takes place, can be called an abnormal period, as well as various strategies towards a new situation commonly called the

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The New Normal. Therefore, as efforts to combat the Covid-19 virus approach a turning point, when together we welcome a new normal order in social, national and state life, a shared awareness is needed to ground the Pancasila, rebuild Indonesia and rebuild the law. So that we will together win the whole series of 'battles' against Covid-19. The new normal order requires a joint commitment of all citizens to re-earth the Pancasila, rebuild our Indonesia and renew our law, so that we can pass this 'test' by joining hands together, in line with the ideals of Pancasila. The study will present an analysis of issues of how is the sociology of law as a means of examining the reality of the current Covid-19 pandemic, and how do to ground the Pancasila as a solution in the midst of the Covid-19 pandemic.

Legal Sociology Perspectives on the Situation before Covid Pandemic 19

Since the beginning it has been confirmed that the optics used is legal sociology. This optics sees the law not only as a normative document, legal laws, rules/norms and principles, but also as a socio-cultural phenomenon[2,3,4]. The sociology of law takes a position by ratifying the concept of law and thereby ratifying state law, so as to be able to incorporate various forms of order into its area of observation[5,6]. Covid-19 made all government officials, especially the medical staff, all citizens faced with situations of fear and even panic. It is no exaggeration to say that during the Covid-19 pandemic, the country seemed to be turning back, "back to zero".

Since confirming the Covid-19 pandemic, various regulations have been issued as a form of resistance against the pandemic. The regulations are contained in Government Regulations (PP), Presidential Decrees (Keppres) and Perppu. Can be called for example: (1). Government Regulation in Lieu of Law (Perppu) Number 1 of 2020 concerning State Financial Policy and Financial System Stability for Handling Covid-19 Pandemic and/or In Order to Face Threats that Harm National Economy and/or Financial Stability, (2). Indonesian Government Regulation 2020 which stipulates Large-Scale Social Restrictions (PSBB) in the Context of the Covid-19 Handling Acceleration.

The PSBB policy covers school entertainment, workplaces, religious activities, and activities in public places or facilities. The PSBB is determined by the Health Meteri to the Regional Government by taking into account the provisions stipulated in Law No. 6 of 2018 concerning Health Quarantine, (3). Republic of Indonesia's Presidential Decree No. 11 of 2020 concerning Stipulation of Public Health Emergency in Covid-19. The President designates Covid-19 as the type that gives rise to Public Health Emergencies. From the stipulation of this Health Emergency, the President then issued various policies on the free of electricity tariffs to increase the number of recipients and the amount of social assistance funds to the community, (4). Decree of the Minister of Health Number HK. 01. 07/Menkes/239/2020 concerning Large-Scale Social Restrictions in DKI Jakarta.

The emergence of these various regulations is a step by the government against the Covid-19 pandemic. This step was followed by the issuance of: (1) Decree of the Minister of Health No. HK.02.07/Menkes/328/2020 concerning Covid Prevention and Control Guidelines 19 in Office and Industrial Workplaces to Support Business Continuity in Pandemic Situations, and (2) Circular Letter (SE) No. HK.02.01/Menkes/334/2020 concerning Covid Transmission Prevention Protocol 19 For Officers Who Carry Out Duties. It is suspected that at the time of the pandemic and at the end of the pandemic, the complexity of the problems that appeared and the unknown when they ended was very complex.

Legal Reform Towards a New Normal

In addition to the term legal also known the term legal development. The two terms are often used interchangeably to refer to the same meaning. Legal reform as part of social policy is a rational effort taken to overcome social problems. In the context of the Webinar, namely the social problems that arose during the Covid-19 pandemic to the social problems at the end of the Covid-19 pandemic. The main objective is the achievement of justice, certainty and benefits for the life of society, nation and state[9]. In other words it can be said that legal reform is aimed at achieving the welfare/protection of the community both when the Covid-19 pandemic lasts until the end of the Covid-19 pandemic. Based on the idea of state of law, it is always constructed as a regulator of all aspects of life. This kind of construction is always dominated by. Even if

it is not dominated, we actually still find other orders outside the country's legal order. In addition to the legal order (state) we know the order outside of it, for example religious order, decency, and habits. All three, living and simultaneously contributing to give order to society. [10]

Legal reform includes renewing legal concepts, renewing basic ideas, renewing ideas, and renewing insights that are recognized by the term paradigm. Legal reform should include legal system reform (legal system reform), so that it involves legal substance renewal, legal structure renewal and legal culture renewal[11]. Renewal of the legal substance must be seen as reorienting and reforming the law to conform to the socio-philosophical, socio-political, and socio-cultural values of the community, all of which are part of the activities of grounding the Pancasila.

Legal reform has so far been more heavily biased towards the priority of the legal substance, so that lawmaking is more advanced than the other two elements of legal reform, namely structure and culture. There is what is called over regulation, which prioritizes the form of the function of law. Legal updates can be broken down into macro related coverage and micro related fields. Referring to Parsons cybernetics, legal reform should be able to adapt or compromise on cultural, political, social, political and economic aspects, so that legal reform towards the new normal order should be directed towards the adoption/compromise of cultural, social interests. economics and politics. Referring to Schuyt, we prefer the legal function as a means of melting conflict (conflicttoplossing) rather than as an act of strengthening the occurrence of further conflict (conflictversterking). Similar to Philipe Nonet and Selznick's thoughts, we choose the type of law that is responsive, not the type of law that is autonomous especially the repressive type. The responsive type views the law as a means of service and response to social needs/aspirations, with its characteristics including justice as the basis of legal legitimacy, policy subordination, discretion recommended and goal-oriented[12].

Towards a New Normal Order

Actual information about the development of the Covid-19 pandemic in Indonesia can be seen in the following data from covid-19 sufferers in Indonesia: 57,770 people died, 2,934 died, 29,291 were cured and 25,595 healed according to updated data at July 1, 2020. Based on these data, it means that Covid-19 transmission is not over. Efforts in that direction are always endeavored so as to arrive at the new normal. To arrive at the situation called The New Normal, there was an endless governmental effort. The government should provide infrastructure for a new order. The new word does not mean an order that did not exist. but returned to the original order with improvements, which was not added and so on.

To go to a new order through the law, it is necessary to analyze society not only in the traditional way based on the methods that have always been well established and that is a normative-positivistic perspective. This way, for example, we do not see from the aspect of the formal form it must be more on its function. here we need a truly functional product that is able to integrate the community's autonomous forces based on Pancasila values. Some methods that have been used by the community to fight Covid Corona should also be taken into account. In the hustle and bustle of regulations issued by the state, we also witness the autonomous forces of the people to build towards The New Normal. In some places, for example, we witnessed how village youths guarded their villages to carry out physical and social distancing, limiting physical and social distance. Even though sometimes it is "excessive," village youths have been guarding the "in and out" posts of their village. For the sociology of law, what is done by the village youths is a uniqueness that illustrates the workings of social forces in the community[13].

The need for state law is not just a matter of form/ procedure but rather of a function aspect. That is, functional law for the community. Following Talcott Parsons' thinking, that is a law capable of carrying out the integration function. The preferred regulatory model is the loose regulatory model, commonly called the sociological model, not the coercive and detailed regulatory model, commonly called the legalistic model. With a sociological model, social forces and values loaded can work together with state law. Sociological model, can be appointed as one way to ground the Pancasila into actualized in law and its enforcement. The sociology of law sees the community as having the potential to autonomously develop their own orderly society. Grounding Pancasila, means also grounding the

autonomous potential of the community. Here there is a relationship between the grounding of Pancasila and legal reform, namely as a model of actualizing Pancasila through state regulatory policies.

Conclusion

The Covid-19 pandemic, caused community abnormalities. Regulations, policies carried out so far have also caused problems, so that it appears how complex the problems that arise in the community. These abnormalities, differ from the period before the Covid-19 pandemic, which is relatively more normal. The normality of society is achieved because of the autonomous forces that exist in the community itself. These autonomous forces are religious norms, decency, habit and law. Religious norms, originating from God's revelation, decency hold on the ideas that must be realized, the habit of holding on to reality and legal norms concocting ideas and that reality. Various attempts were made to return to normal, even back to the new normal order which is usually termed as The New Normal. A new normal order does not mean denying the old and existing normality but rather adding to the less and/or perfecting.

From the optical sociology of law in achieving a new normal order the main priority is the restoration of social order, so that people are able and survive to meet their needs. Even the sociology of law is not the only one that can be used, proposing ideas, namely to revive autonomous social forces in society by re-enacting the Pancasila, the reform of Indonesian law. Legal reform is not only in the aspect of substance, but also in structure and culture, not prioritizing law as a form, so that it is able to go to law that is functional, has the ability of integration mechanisms, is responsive, not autonomous, especially repressive.

Ethical Clearance: This research was ethically approved by Faculty of Law, Universitas Diponegoro, Semarang, Indonesia

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Conflict of Interests: There are no Conflict of Interests

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Variation of 16SrRNA gene for Some Nosocomial Bacteria Isolated from Ramadi Teaching Hospital for Women and Children

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Abstract

The aim of this studsy is to diagnose bacterial strains in hospitals by the 16SrRNA gene and to know the variation of the nucleotide sequence. The study of bacteria in hospitals is of great importance to identify the extent of change in these species and their resistance to antibiotics. This indicates that these bacteria have the ability to change or obtain new variants to be able to resist antibiotics. In this study, samples were taken from different locations of the Ramadi Teaching hospital for women and children and were cultured on different media. They were also stained and examined under light microscopy and some biochemical tests were conducted. Pure colonies were obtained after re-culture and then extracted the DNA from the 16 isolated samples and measuring its concentration. Using the specific primer for 16SrRNA genes, the samples were amplified by a PCR and the size was shown to be 1500 bp. The unknown bacteria were sent to the sequence. Some samples showed variations in comparison with the data presented by NCBI. This is evidence of some variations shown by bacteria to overcome antibiotics and increase their severity and spread in hospitals, whether in kitchens, emergency or preterm infants, as well as laboratory staff and lab equipment.

Keywords: *16SrRNA gene, Nosocomial infections, Transition, Transversion, Transduction*

Introduction

Nosocomial infections are as well-known as hospital-acquired/associated infections and common problem that increases the duration of stay hospital, hospital cost often affects patients' quality of life, survival, and response to therapy [1,2]. It is an infection acquired by the patient length a hospital stays. Lately, a modern term is used, "healthcare-related infections" this term used for the type of infections give rise to by a lengthy hospital stays, and it accounts for a major risk factor for serious health issues leading to doom [3]. Patients' food is prepared in the hospital kitchen and is then distributed throughout the wards also the worker in hospital kitchen [4]. Patients are more prone to infections in comparison to healthy individuals [5]. Even length of hospitalization can be influenced by factors such as undernourishment, food poisonings, and hospital infections [6]. Invasive devices such as catheters and ventilators employed in modern health care are associated to these infections and factors such as critical origins, not enough cooking, keeping at improper temperature, and lack of personal hygiene are important factors in the incidence of food-borne diseases [7]. There are statistics showing that out of every 100 patients receiving treatment in hospitals, seven in developed countries and ten in developing countries can acquire one of them related to healthcare infections [8]. Therefore, spread or outbreak of microorganisms in hospitals can result in more serious outcomes for patients [9]. So, one of the principal goals of hospitals is the provision of healthy food for patients who are susceptible to infections and complications [10]. Nosocomial infections appeared prior to the buildup of hospitals and became a health problem through the prodigious antibiotic era. Because of these infections, not only the costs until the use of antibiotics increased with extended hospitalization and lead to increase the resistance against the antibiotic. Studies behave

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in various regions of the world show that in North America and Europe 5%-10% of all hospitalizations result in nosocomial infections, while South America, Africa, and Asia exhibit exceed 40% hospitalizations with these infections [11]. The most common types of nosocomial infections include bloodstream infections, catheter-associated urinary tract infections, surgical site infections which lead to happen the gangrene, ventilator-associated pneumonia and lastly the food poisoning. [12]. The aim of this paper is detection of variation for some nosocomial bacteria isolated from Ramadi Teaching Hospital for Women and Children.

Materials and Methods

Sample Collection and Bacterial Identification

In the period from May 2018 to July 2018 collected forty sample from the food utensils, work surfaces and their contact surfaces as well as hands, work clothes, cutting boards and submitting food place. Total of 40 samples were collected aseptically in sterile disposable containers. Specimens were labeled and transported to the microbiology laboratory. The smears were streaked on MacConkey, Nutrient and Blood agar and all dishes were incubated at 37°C for 18 to 24 hours. Whole isolates were identified utilizing for biochemical test use Simone citrate agar, Kligler iron agar, Trypton broth, Catalase reagent and IMVIC test. After biochemical tests sixteen sample sent to diagnosis by the molecular method for detection 16S rRNA gene.

DNA Extraction

Genomic DNA was extracted from the samples was isolated from hospital using (DNA mini kit that was supplied by Promega DNA extraction kit, USA) according to manufacturer's instructions.

Quantitation of DNA

Quantus florometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. For 1 µl of DNA, 199 µl of diluted Quanty Flour Dye was mixed. After 5min incubation at room temperature, DNA concentration values were detected.

PCR Amplification:

The 16SrRNA gene was amplified using primer Forward 27F (5`-AGAGTTTGATCCTGGCTCAG-3`) and primer Reverse 149 2R (5`-TACGGTTACCTTGTTACGACTT-3`) [13]. The PCR amplification is performed in a total volume of 25µl containing 2 µl DNA, 12.5 µl Master Mix PCR (intron, Korea), 1µl of each primer 10 pmol and then nuclease-free water is added into a tube to a total volume from 25µl.

The Polymerase Chain Reaction program conditions were as follows: initial denaturation at five minutes at 95°C followed by thirty cycles of denaturation 95°C for 30 seconds, annealing at 60°C for 30-minute, extension at 72°C for one minute and the last extension of 72°C for 7 minutes. The PCR products were separated on 1% agarose gel. The gel is left to run for 90min with a 70volt/65 Am current. After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. PCR was completely dependable on the extracted DNA criteria.

Sequencing:

The sequencing of 16srRNA gene was performed at Macrogen Inc. using their ABI 3730xl genetic analyzer. Homology search was conducted using BLAST program which is available at the National Center Biotechnology Information (NCBI) online at http://www.ncbi.nlm.nih.gov and Bio Edit program.

Results and Discussion

Samples were isolated from various locations of Ramadi Educational Hospital for women and children (kitchen, preterm, emergency, lab, baby incubator, and some kitchen staff). After the isolation process, samples were taken to the laboratory and cultured on different media for the initial isolation and then cultured on enrich and differential media such as the blood agar medium, MacConkey agar, and the mannitol salt agar. It was observed that there was a variation in the colonies through the morphology and then was stain and examined under the optical microscope were some colonies appeared negative and some positive between the bacillus and coccus. The results of the biochemical tests showed variation between samples when we used the IMVIC test.

DAN Extraction

All sixteen sample activated on blood agar and extracted the DNA by Promega kit and measured the concentration by Quantus Florometer show table 1.

Table 1: DNA Concentration (ng/µl)

Sample	Concentration	Sample	Concentration
01	20	09	20
02	30	10	20
03	33	11	22
04	40	12	23
05	25	13	40
06	20	14	25
07	25	15	23
08	30	16	22

PCR Amplification

The electrophoresis results showed that the size band was 1500 bp using the ladder 1500bp after stain by Ethidium bromide in seen as fig. 1.

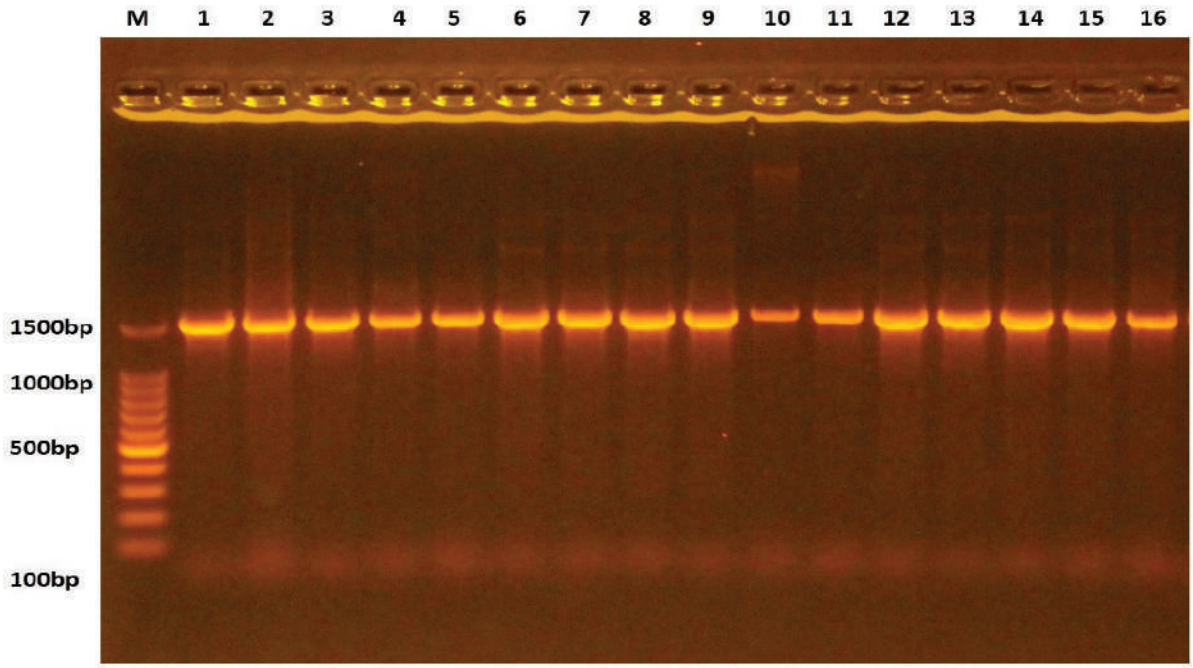


Fig.1: Results of the presence of 16SrRNA gene of Unknown bacterial species were fractionated on 1% agarose gel electrophoresis stained with Eth. bro.

Sequence Alignment of 16SrRNA Gene

The results of sixteen samples after send to sequence showed variation between the samples [14], the first sample *Pantoea calida* isolated from the lab, kitchen and emergency showed three variations all the variation (Transition) and the Second sample *Enterobacter hormaechei* isolated from lab, kitchen, preterm and chamber 1 showed two variation (Transition) table 2. While the other sample isolated from kitchen and lab *Pseudomonas putida* also show two variations (Insertion) and the *Kocuria* sp. isolated from kitchen and emergence and showed one variation (Transversion) table 3. Tenth sample *Micrococcus luteus* from all location in the hospital and showed one variation (Transversion). Also, the eleventh sample *Rothia* sp. gives the one variation (Transition) isolated from all location table 4. *Staphylococcus pasteuri* was prevalent in the all location and shown one variation (Transition). The fifteenth was *Staphylococcus saprophyticus* isolated from emergence, lab and kitchen shown one variation (Transition). Lastly, the *Lelliottia amnigena* was isolated from the clothes kitchen staff and show three variations first (Insertion) and two (Transition) table 5.

The samples 5,6,7,8,9,12 and 14 *Rothia* sp., *Planococcus* sp., *Lysinibacillus* sp., *Staphylococcus haemolyticus*,*Staphylococcuspasteuri*,*[Brevibacterium] frigoritolerans* and *Bacillus aryabhattai* subsequently don't show any variation in sequence.

Submission of Local Iraqi Isolates in NCBI

The16SrRNA gene was registered after the correspondence of NCBI and obtain accession number for the 1 and 16 sample. https://www.ncbi.nlm.nih.gov/nuccore/MN209789.1/ and https://www.ncbi.nlm.nih.gov/nuccore/MN215912.

Table 2: Represent Type of Polymorphism of 16srRNA Gene from *Pantoea calida* and *Enterobacter hormaechei*

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate1	Transition	38	T>C	LC192167.1
	Transition	798	C>T	
	Transition	806	G>A	
Isolate 2	Transition	402	A>G	CP017180.1
	Transition	1081	T> C	

Table 3: Represent Type of Polymorphism of 16srRNA Gene from *Pseudomonas putida* and *Kocuria* sp.

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate3	Insertion	52	- > A	AY395005.1
	Insertion	1402	-> T	
Isolate 4	Transversion	924	C>A	KX417302.1

Table 4: Represent Type of Polymorphism of 16srRNA Gene from *Micrococcus luteus* and *Rothia* sp.

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate10	Transversion	1064	A>C	MH489048.1
Isolate 11	Transition	1383	G > A	KU884343.1

Table 5: Represent Type of Polymorphism of 16srRNA Gene from *Staphylococcus pasteuri*, *Staphylococcus saprophyticus* and *Lelliottia amnigena*

No.	Type of substitution	Location	Nucleotide	Seq. ID
Isolate13	Transition	456	T>C	MN004821.1
Isolate15	Transition	230	G > A	MK205161.1
Isolate16	Insertion	9	-> A	KM114915.1
	Transition	1192	A>G	
	Transition	1241	T>C	

Conclusion

We conclude from this research that the infection of patients to hospital bacteria is a serious risk to the patient because it possesses resistance to most antibiotics by acquiring methods through which to change the sensitivity of antibiotics of the important methods conjugation and transduction. Sequencing results showed that there were variations in the nucleotide sequence of some bacterial isolates isolated from the hospital compared to NCBI results.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Assessment of Knowledge and Performance Regarding Triage Among Nurses Working in Emergency Unit-Al Dawadmi General Hospital: A Cross Sectional Study

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Abstract

Five experts validated the contents of the tools and tested for reliability, using Cronbach's alpha, the tool correlation coefficient was 0.79. The majority of participants (74.46%) were in the age group of < 30 years. More than half of the studied subjects (59.57) are male. The majority of them have BSc and about 70.21% work experience for more than five years. Only 72.34% of them had not attended any form of workshops about Triage. 70.2% of them knowing the purpose of triage, 91.2 % know color used in nonurgent situations, while 91.5% not know the time of Triage.93.6% of them not know the importance of urine analysis in the triage unit. The majority of them (80.90 %) strongly agree that hospital Triage is good. knowledge about triage is a key in triage decision making. The study findings revealed that there were still deficits in the triaging knowledge and skills of nurses.

Key Words: *Triage, Emergency Department, Prioritizing, Knowledge, Triage Scale*

Introduction

Over the last years, the number of patients entering emergency departments has increased in developed high-income countries, but also in low-income countries, partly due to self-referrals, leading to the overcrowded emergency department [(1)].This elevated amount of patients visiting the emergency departments can effect on healthcare quality by channeling funds to people with possibly less requirements for emergency care [(2)]. It raised the need for an urgent scheme that prioritizes patients. In their emergency departments, many hospitals in low-income countries lack a formal triage system [(3)]. Triage is a method used to assess the extent of the disease or injury and to prioritize patients, according to medical care needs, regardless of their influx order or other factors like race, age, or socioeconomic status [(4)].

The mortality rate in hospitals may be increased. The reason triage is one of the vital elements for offering effective care in emergency rooms, and the death rate of patients has been reported to decline [(5)]. This study examined the level of knowledge, nursing duties, and quality of services of nurses working in the Triage Unit at Health Care Centers and Al- Dawadmi Hospital. Because Al- Dawadmi hospital receives patients from many villages near it, the triage process in this hospital is effective to assure the best care in the emergency circumstances.

This study aimed to (1) determines the level of knowledge and nursing duties regarding Triage. (2) Assess the relationship between attending workshops and the level of nurses' knowledge about triage.

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Methods

Study Design: Cross-sectional Descriptive design.

Setting:

The study was carried out in the Emergency Department in Al- Dawadmi Hospital Riyadh, Saudi Arabia.

Sources and Methods of selection of participants: The total numbers of active members of the nursing staff, working at the Emergency Department study subjects' number is 47 participants.

Variable: Level of knowledge in relating to attendance of workshop, work experience relating to level of education, quality of services and, nursing duties in the triage unit.

Diagnostic criteria, participant selection, are active members of the nursing staff, Saudi and Non-Saudi, male and female nurses; working at the emergency Unit for more than three months.

Data sources: Data collection instruments which consisted of three parts: the first tool consisted of Socio-demographic data, including demographic data. It includes five questions such as age, gender, the level of education, working experience at the emergency department and, attended workshop about the triage. The second tool - consisted of two parts. First partto assess general knowledge of nurses in the emergency department aboutthe triage, it contains six questions. The second one about Canadian and,Acuity Scale colors.It provides nationally consistent standards for the maximum time patients are considered safe to wait for the emergency care, which includes categories of colors and maximum waiting time.Tool three, including four questions about Quality of Triage services.Most of these are presented in multiple - choice answers, which may be quickly answered by ticking the appropriate boxes. Each one needsonly one response and every response will be considered as one.

Bias: A pilot study was carried out on the number of seven nurses that was 7% of a potential sample size to test the clarity and simplicity of the study tools. The necessary modification was done in tools and, subjects who shared in the pilot study were excluded later from the study sample. The Cronbach alpha test was applied to determine the reliability of the scale. The Cronbach value was 0.79.

Study size: Saudi and Non-Saudi, male and female nurses; working at East and west Al- Dawadmi clinics and the emergency department for more than three months, 47 participants returned out of 100 surveys sent, which gives 47% response rate.

Procedure

Data was collected over a period of February10 to April10, 2019. All staff members, male and female nurses who are currently working in the emergency departments Al –Dawadmi hospital were part of the survey. The researchers asked the nursing director for a list, email and, contact number of the nurses working in their department. Then the researchers were contacted with the staff and asked for the convenient time for discussion with them the objective and rationale of the study, those who would voluntarily agree to participate in the study were asked to sign the informed consent. The participants were given enough time to answer the questionnaire and, once they finished answering, they contacted the researchers or put in a sealed envelope and submit it to the nursing service office.

Statistical Methods

Collected data were analyzed by using the SPSS version IBM-23 and were presented in tables and percentages. Descriptive statistics like obtaining frequencies and percentages wereused in determining the demographic profile of participants.Chi-square test was used to evaluate the relationship between Levels of knowledge and attendance of about the triage. The level of significant was adopted at $p < 0.01$.

Results

Table 1:- Descriptive data of the studied subjects. The majority of them aged from 21- 30 years 74.46%, 59.57% is male and, 74.46%were attending workshop. Table 2:- Showed level of education of studied subjects in relating to work experience, 70.21% of participants have work experience for more than five years in ER, while 55.27 % of study subjects were BSc; Table 3:- Showed distribution of subjects' general knowledge about Triage. 70.2 % considered that Triage help prevents the death

of patients as one purpose of it. Also, there were highly statistical differences regarding general knowledge by p - value equal 0.001; Table 4:- Showed the nurses' knowledge about the Canadian Triage and Acuity Scale; there washigh percentage 91.2% determine non-urgent color, while 91.5% doesn't determine the time of non-urgent color with high statistical significant differences for three questions by p -value equal 0.001; Table 5:- Showed that there was high percentage (93.6 %) don't know the importance of urinalysis as nursing duties, with highly significant differences for five out of eleven questions by p - value equal 0.001; Table 6:- Showed distribution of studied subjects relating to the quality of services in the triage Unit. 63.80 %, notsure that the triage is considered not very important. Also, there were highly statistically significant differences relating to three out of four questions by p - value equal to 0.001.

Table 1:-Descriptive data of the studied subjects.

Items		No	%
1.	Age in years:		
·	21-30	35	74.46
·	31-40	11	23.4
·	41 -50	1	2.12
2.	Sex :		
·	Male.	19	59.57
·	Female.	28	40.4
3.	Attend workshop	35	74.46%

Table 2:- Distribution of studied subjects regarding to level of education in relating to work experience.

Level of education			Work experience			
			Less 1 year	1 year	2 years	> 5 years
No	BSc	No	3	1	0	5
		%	6.38%	2.12%	0.0%	10.6%
	Diploma	No	0	0	2	2
		%	0.0%	0.0	4.25%	4.25%
Yes	BSc	No	0	5	9	12
		%	0.0%	10.6%	19.14%	25.53%
	Diploma	No	1	1	2	4
		%	2.12%	2.12%	4.25%	8.5%
	Total		8.51%	14.89	27.65%	70.21%

Table 3:-Distribution of studied subjects relating to general knowledge about Triage.

General Knowledge	No		Yes		Pearson Chi-Square	p-value
	No	%	No	%		
Do you know the meaning of triage?	16	34.0%	31	66.0%	14.716	0.001
Purpose of triage is to prevent death of a patient?	14	29.8%	33	70.2%	13.368	0.001
Are the staffs trained in triage?	18	38.3%	29	61.7%	28.953	0.001
Are there written Guidelines regarding Triage System?	15	31.9%	32	68.1%	38.337	0.001

Table 4: Distribution of studied subjects' knowledge about (Canadian Triage and Acuity Scale)

Item	No		Yes		Pearson Chi-Square	p-value
	No	%	No	%		
Determine Resuscitation Color?	10	21.3%	37	78.7%	17.392	0.001
Time	38	80.9%	9	19.1%	4.256	0.039
Determine Emergent Color?	19	40.4%	28	59.6%	6.191	0.013
Time	39	83.0%	8	17.0%	3.686	0.055
Determine Urgent Color?	26	55.3%	21	44.7%	6.24	0.012
Time	39	83.0%	8	17.0%	1.107	0.293
Determine Less-urgent Color?	17	36.2%	30	63.8%	31.713	0.001
Time	43	91.5%	4	8.5%	1.672	0.196
Determine Non urgent Color? Time	16	34.04	31	91.2%	34.818	0.001

Table 5: Distribution of the studied subjects relating to nursing duties in Triage Unit.

Nursing Duties	No		Yes		Pearson Chi-Square	p-value
	No	%	No	%		
Does triage assess ABC?	22	46.8%	25	53.5%	20.421	0.001
Take vital signs?	8	17.02%	39	82.97%	25.215	0.001
Determine level of consciousness?	6	12.7%	41	87.23%	17.989	0.001
Basic life support?	18	38.29 %	29	61.7 %	22.184	0.001
Perform an ECG?	42	89.36%	5	10.6 %	14.634	0.001
Taking and documenting medical history?	32	68.08 %	15	31.91%	0.646	0.422
Perform a urinalysis?	44	93.6 %	3	6.38%	2.437	0.119
Perform a blood glucose measurement?	41	87.23%	6	12.7%	0.111	0.739
Perform a blood hemoglobin measurement?	38	80.85%	9	19.14%	0.164	0.685
Utilize pulse oximetry?	33	70.2%	14	29.78%	0.387	0.534
Secondary assessment (head-to-toe)?	40	85.1%	7	14.89%	0.949	0.33

Table 6: Distribution of studied subjects relating to quality of services in Triage Unit.

		Strongly Disagree	Disagree	Not sure	Agree	Strongly Agree	Pearson Chi-Square	p-value
1. How good do you think the hospital's triage is?	No	-	1	4	4	38	14.252	0.003
	%	-	2.10%	8.50%	8.50%	80.90%		
2. Triage wastes time?	No	-	12	9	18	8	1.534	0.674
	%	-	25.50%	19.10%	38.30%	17.00%		
3. Do you think that there is a shortage of staff duty to perform triage?	No	1	17	16	8	5	22.93	0.001
	%	2.10%	36.20%	34.00%	17.00%	10.60%		
4. Triage is considered not very important.	No	1	9	30	6	1	22.4	0.001

Discussion

Saudi and Non-Saudi, male and female nurses; working at East and west Al - Dawadmi clinics and the Emergency Department for more than three months, 47 participants, majority of them aged from 21- 30 years (74.46%), (59.57%) are male, and 74.46% attending workshop, 70.21% of participants have work experience for more than five years in ER, while 55.27 % of study subjects were BSc.

Limitations of the study: This study has several limitations. First, there were a limited number of experienced nurses in the ED due to the lack of human resources. Therefore, this may affect the results. Next, the impact of shortage in triage role on the quality of services to patients and their outcome was not assessed.

Interpretation: Seconds and minutes are essential for the patient in emergency rooms because this time can determine the distance between the moment of death, severe impairment, or return to life [6]. Hospitals in public countries have emergency departments facing large numbers of patients with low resources, and many obstacles in personnel training and communication and transportation issues [7].

According to[8, 9] found that if the timeliness of intervention improves when critical patients arrive at the emergency room, the emergency service is improved, and this only happens through the use of triage scales. Also, the waiting time of the patient is significantly reduced by using triage.

Knowledge of nurses according tothe CTAS standards are very important to applied care for patients in the emergencies situations [10].Other factors that influence the accuracy and effectively of the triage application includes hours of nurses trainingand,the level of education, level of hospital, and the triage mode of delivery [11]. Level of education influenced nurses› abilities for case classification. The nurse›s level with a bachelor›s degree scored above the median compared to those with two years nursing diploma. This difference illustrates the great need for highly educated nurses who worked in emergency departments [12]. According to the findings of the study, there was 70.21 % of them worked more than five years in the emergency departmentand 55.27 % of study subjects were BSc, that reflects that they are experiences in the emergency care while 74.46% attending a workshopregarding CTAS which applied in the hospital.It means high percentages regarding general knowledge for CTAS in Al- Dawadmi hospital including knowing the meaning of thetriage, purposes, have exposed to previous training programs, and the hospital have written guide lines about the triage system. The same results were found in some studies which support these findings by experienced nurses have been exposed to emergency situations have a better sense of how urgent the case is with regards to CTAS guidelines.These findings are in contrast withDallaire , who concluded that experienced nurses, even without recent certification, obtained a low triage score.

According toReisi, Saberipour, Adienh, Hemmatipour, &Shahvali [13], 87.7% of nurses had poor awareness of the triage, and only 12.3% of nurses had a moderate level of awareness. Also, in the study conducted by Javadi, Salimi, Sareban, Dehghani [14], the level of awareness was weak, it indicates that nurses› knowledge about the triage is low and requires retraining and continuing education. However, according to Haghdoust, Safavi M,&Yahyavi [15], 40 emergency nurses (72.2%) and, according to Göransson, Ehrenberg,Marklund, &Ehnfors [16], 45 nurses (57.7%) had a moderate level of awareness. According to the findings of this study, aboutthe Canadian triage scale, there washigh percentage 91.2% determines nonurgent color, while 91.5%doesn›t determine time.

Conclusion

The present study showed that their many triage education programs are needed to improve accurate triage decision making in nurses. Also, further studies are recommended to assess the impact of triage error on the patients' outcome. Workshops / in- service training on the triage should be organized on regular basis to enhance nurses' knowledge. Sufficient content on triage should be integrated into the nursing curricula. Study results can generalize by large number of study participants.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Middle east.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Effect of Orally-administered Silver Nanoparticles (Ag-NPs) on Some Biochemical Parameters in Kidney of Rats

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Abstract

The research evaluated the oral administration of silver nanoparticles on the tissue morphology and biochemical parameters of kidneys in rats, using (0.2 and 0.4) mg/kg B.W for four days and 0.08,0.008 mg/kg weight For 30 days . The result showed significant increase in the level of serum creatinine and serum urea for animals treated with silver nanoparticles relative to the control at the dose of (0.08 and 0.008) mg/kg B.W for 30 days. As for the histological study of kidneys showed sever changes at the dose (0.08 and 0.008) mg/kg compared with the dose 0.2 and 0.4 mg/kg for 4 day which include infiltration of inflammatory cells ,haemorrhage, and degenerated and necrotic changes in renal tubules epithelium.

Keywords*: Rats, AgNPs, histopathology of Kidney.*

Introduction

Silver nanoparticles (AgNPs) accumulated in body organs and has a toxic effects especially on kidney [(1)]. (AgNPs) have tremendous potentials in medical devices due to their excellent antimicrobial properties.The nanoparticles has a small size which have appositive effect on using as these mechanical ,electrical and chemical. These characters also have a side effect as a biological and toxicological reactivity [(2)] .This will element the using of silver nanoparticles. The ingestion of silver nanoparticles can accumulated on parenchymatous organs which cause toxic damage hepatic and renal tissues [(3)].

The harmful effect of kidney function from any material that can be described as nephrotoxicity [(4)] . One indication of nephrotoxicity is a change in renal function as assessed by the glomerular filtration rate (GFR), blood urea nitrogen (BUN), serum creatinine (SCr), or urine output; however, nephrotoxicants can induce kidney damage without changing any established clinical marker of renal function. For example, studies have shown that proximal tubule necrosis in male Sprague Dawley rats exposed to gentamicin can be as high as 75% prior to any increases in BUN or SCr [(5)].

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Materials and Methods

Animal test was performed with compliance of the local ethics committee. A group of 25 BALB/c rats of about 4 weeks (weighting 48.2± 3.0 g) were purchased from Medical Faculty of Mosul University and then transferred to the laboratory. The animals were housed in a single group and maintained on commercial pellet diet, given deionized water ad libitum and kept in plastic cages. After 2 weeks' acclimation, they were randomly divided into three groups (each with two replications): the control (1) groups, with 5 rats and Ag-NPs group(2) (0.2 mg/kg,0.4 mg/kg,0.08mg/kg and 0.008 gm/kg). and carried out for 30 days alternatel The animals were kept fasting over night before. The blood and kidneys were harvested and prepared for biochemical and histopathological examinations, respectively.

The silver nanoparticles characters :

The size of silver nanoparticles used in this study is about 200-300 nm, preparation by SIGMA-ALDRICH company (< 100 nm).

The Histological Analysis and Biochemical Analysis

Biochemical tests used for evaluation the toxic effect of silver NPs on the rats used in this study the estimation of Urea and creatinine were done in the serum by using commercial kits: Katal for urea and Creatinine. All analyzes were performed in biochemical analyser from Bioplus (Celm SB-190) at 37°C.

(b) Histological Analysis.The fixed specimens at 10% neutral buffer formalin are processing ,then embedded in paraffin, sectioned at 4.5- 5 µm by using rotary microtome then staining by hematoxylin and eosin then examined by light microscope for studying the histological changes [6].

Statistical Analysis

A statistical analysis of the results of this search was performed using one –way of ANOVA table for multiple comparisons . A post-hoc method(Tukey) was used in the graph pad prism 5 progarm to evaluate a meaning level of p. 0.05

Results

The study results showed no clinical signs and no mortality was seen in all groups of animals when compared with non-treated group has occurred in each the samples, as well as, no abnormalities in the appearance and behavior of AgNPs treated rats in comparison with non- treated ones..

The mean level of blood urea in groups (0.2,0.4,0.08 and 0.008) showed a significant increase ($p \leq 0.05$) compared with the control group Fig(1).

Orally administred of silver material to animals no large increase serum Creatinine levels was observed relative to the control in rats treated (0.2 and 0.4) mg/kg B.W for four days Fig(1),with contrast in rats which treated with (0.08 and 0.008)mg/kg B.W for 30 days, the level of serum urea and creatinine are significant increase relative to the control Fig(2).

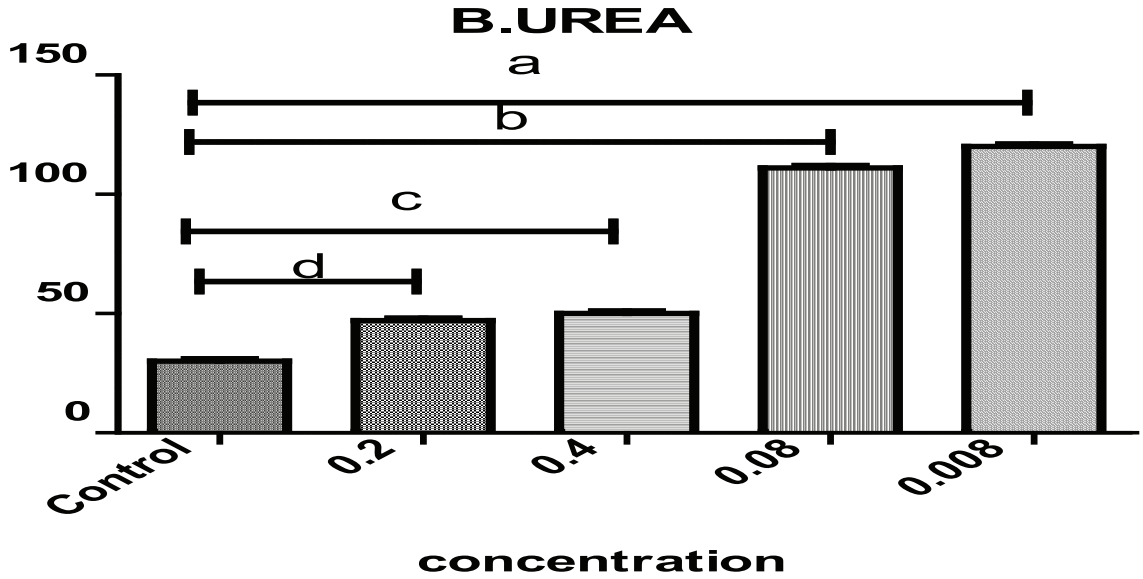


Fig (1) : Effect of different doses of AgNPs (0.2,0.4,0.08 and 0.008 mg/kg) on Blood Urea

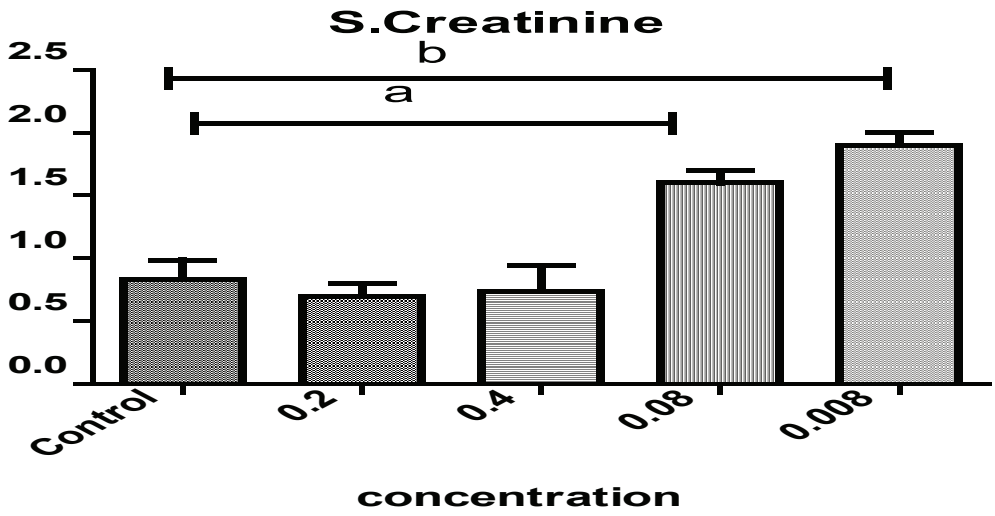


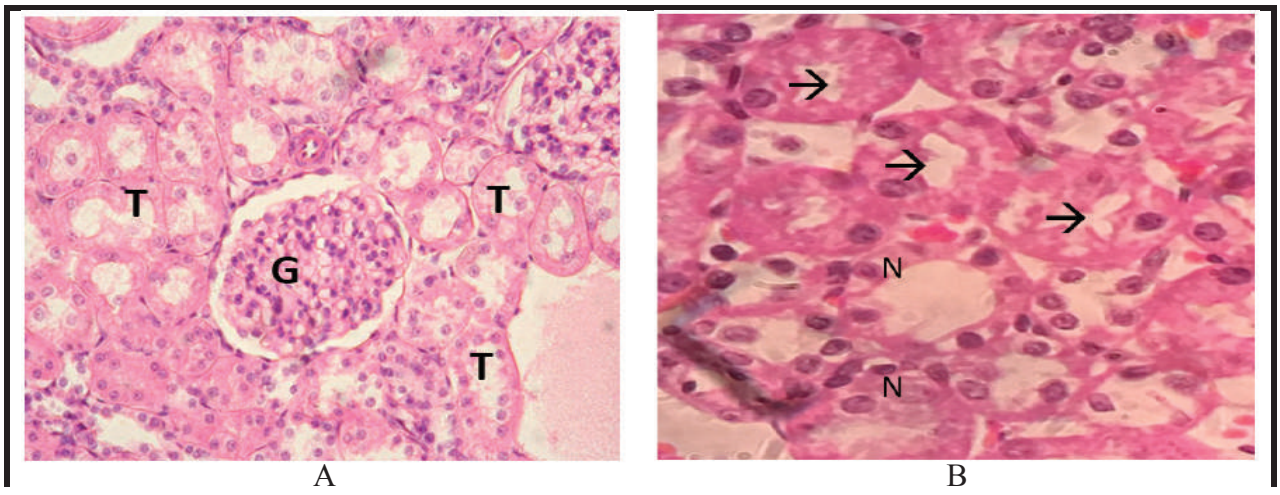
Fig (2): Effect of different doses of AgNPs (0.2,0.4,0.08 and 0.008 mg/kg) on Serum Creatinine

Histological Fidings:

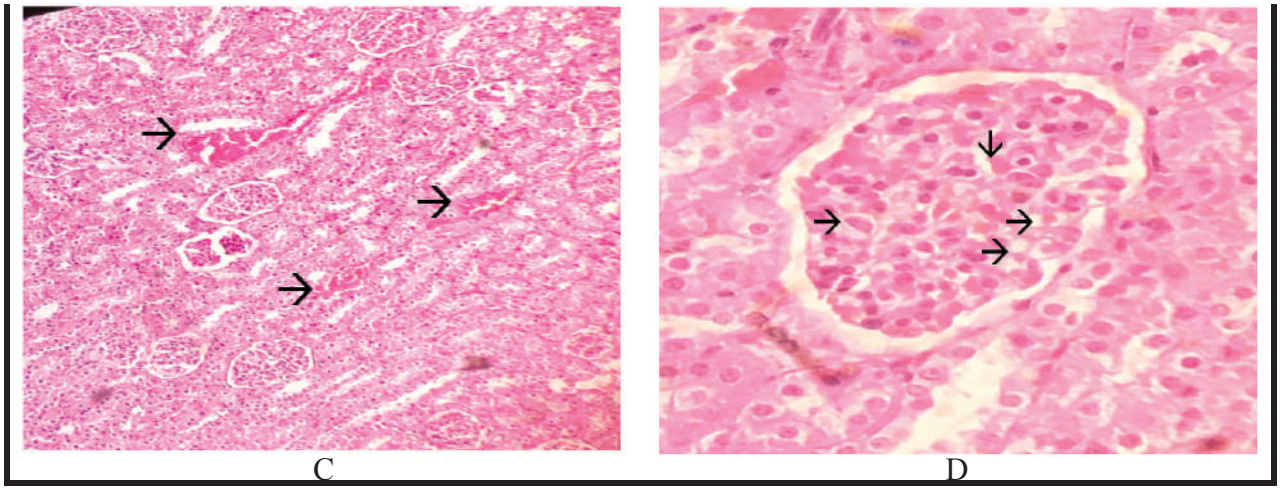
The sections of tissues were prepared for morphological changes after exposure to Ag nanoparticles and observed under a microscope. Histology examination of kidney specimens were evaluted in the three compennts ,tubular,glomerular ,and interstitial tissues. The histological examination of control group showed normal architectural of renal tubules, glomeruli and interstitial tissues with no histological changes Fig(3-A).

While the evalution of the kidney specimens of animal treated with silver nanoparticles using (0.2 and 0.4 mg/Kg B.W.)for four days showed acute necrosis of epithelial cell lining renal tubules associated with swelling of epithelial cells lining renal tubules lead to stenosis the lumen which appeared a star shape (Fig 3-B).generalized congestion of blood vessels (Fig 3-C). While the glomerular altration characterized by vacuolar degeneration and necrosis of mesengial cells(Fig 3-D).

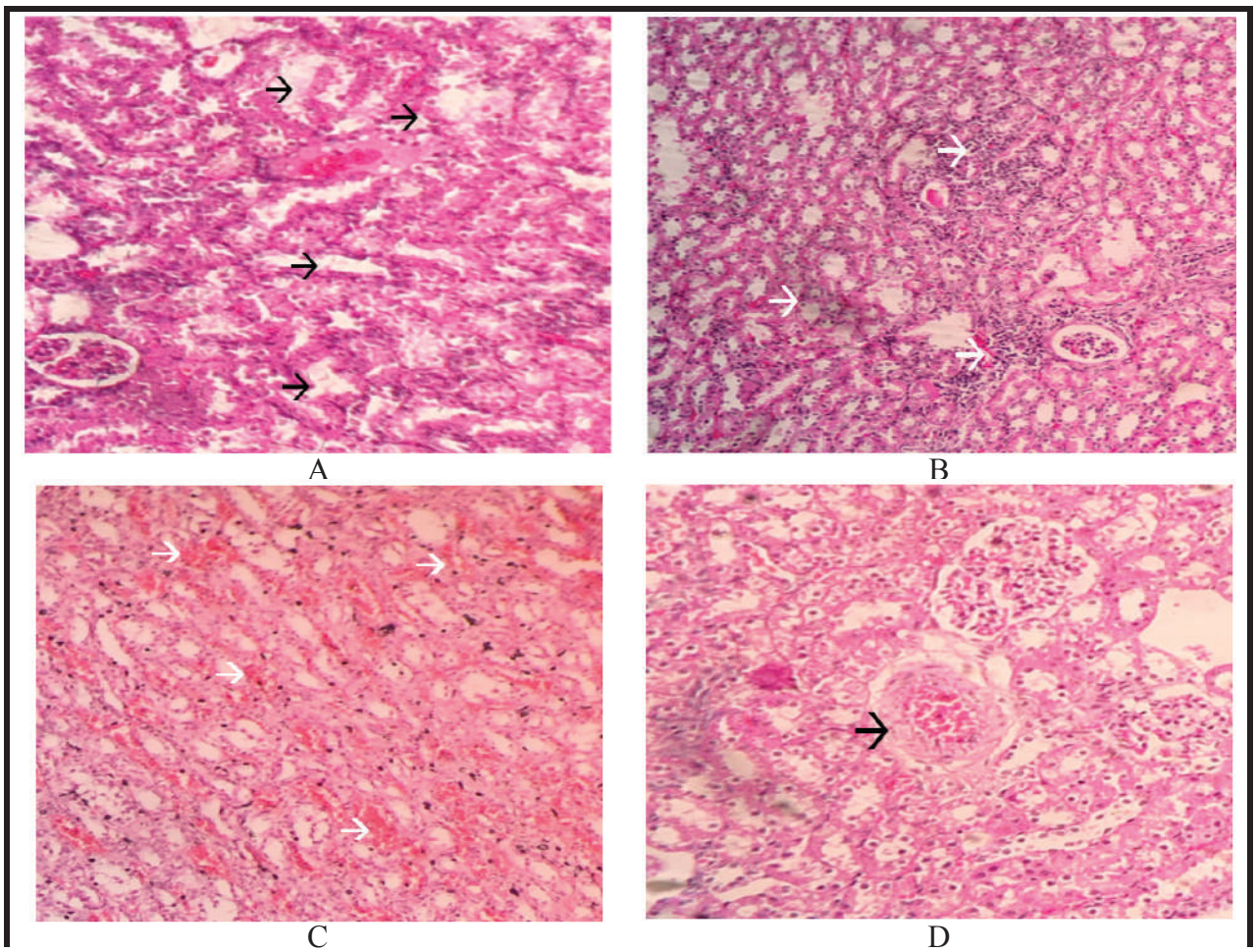
While the histological examination of kidney specimense of animals treated with(0.08 and 0.008 mg/ Kg B.W.)of silver nanoparticles for 30 days showed histological changes more sever than those treated for four days these changes including thickening in the basement membrane of renal tubules with necrosis of epithelial cell lining renal tubules(Fig 4-A). Focal infiltration of inflammatory cells(macrophages,lymphocytes) (Fig 4-B), sever heamorrhage in the interstitial tissues (Fig 4-C) thickening in the wall of blood vessels (Fig 4-D),. Also glomeruli suffered from increased the cellularity of glomerular tuft (Fig 4-E).Other section showed sloughing and desquamation of epithelial cells lining renal tubules in the lumen of tubules there is cellular debris (Fig 4-F). Also there was distortion of the basement membrane of renal tubules and glomeruli.



Cont... **Figure (3)**



Fig(3): (3-A) Light Micrograph Section Kidney of Control group of Rat showing architectural of renal tubules normal (T) and glomeruli (G),10X ; (3-B) sections for tubular analysis in kidney from 0.2 mg/kg AgNS treated rats. acute necrosis of epithelial cell(N) lining renal tubules associated with swelling lead to stenosis the lumen(à) 40X ; (3-C) micrographs sections for tubular analysis of kidney from (0.2 and 0.4) mg/kg AgNS treated rats. Generalized congestion of blood vessels . 4X ; (3-D) sections of kidney from(0.2 and 0.4) mg/kg AgNS treated rats. vacuolar degeneration and necrosis of mesengial cells (à) 40X , (Haematoxylin and Eosin stain).



Cont... **Figure (4):**

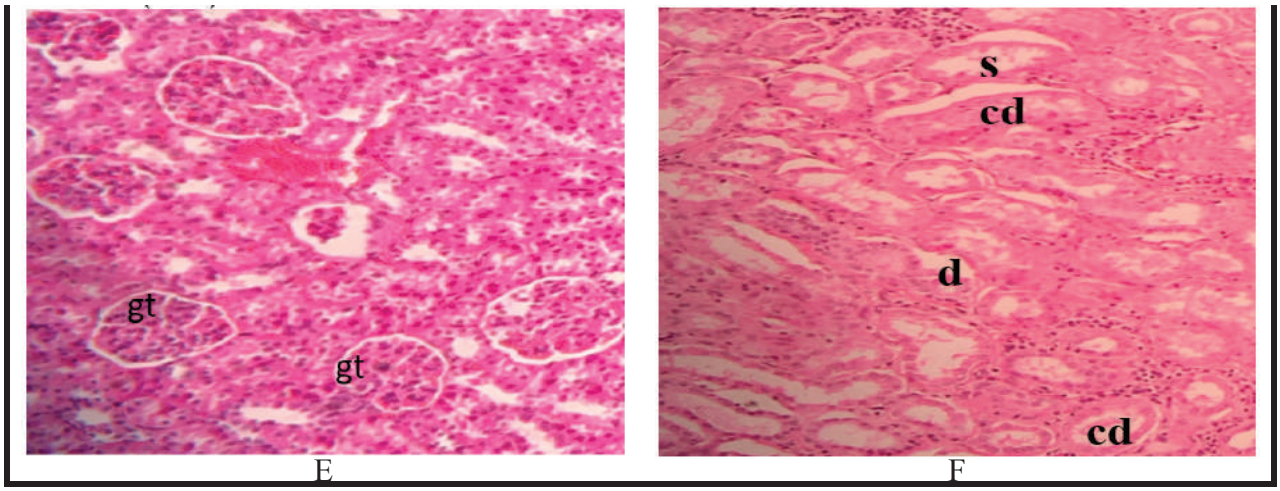


Figure (4): Light micrographs sections (4-A) kidney from(0.08 mg/kg)AgNS treated rats. thickening in the basement membrane of renal tubules with necrosis of epithelial cell lining renal tubules(à),10X ; (4-B) sections for tubular analysis of kidney from(0.08 and 0.008) mg/kg AgNS treated rats. Focal infiltration of inflammatory cells(à) (macrophages , Lymphocytes), 4X ; (4-C) sections for tubular analysis of kidney from(0.08 and 0.008) mg/kg AgNS treated rats. sever heamorrhage in the interstitial tissues(à) ,10X ; (4-D) sections for tubular analysis of kidney from(0.08 and 0.008) mg/kg AgNS treated rats. Foamy thickening in the wall of blood vessels(à), 10X ; (4-E) sections for tubular analysis of cortical region in kidney from(0.08 and 0.008) mg/kg AgNS treated rats. Foamy glomeruli suffered from increased the cellularity of glomerular tuft (gt), 400X ; (4-F) section of rat kidney following oral exposure to silver nanoparticles at different dosages (0.08 and 0.008) mg/kg AgNS treated rats. Show sloughing (s) and desquation of epithelial cells lining renal tubules in the lumen of tubules there is cellular debris (cd) and distortion (d)of the basement membrane of renal tubules, 4X (Haematoxylin& eosin stain).

Table (1) showed the scoring of histological changes of kidney in the treated group and control group.

Table 1: Effect of AgNPs (0.2,0.4,0.08 and0.008 mg/kg) for 30 days on tubular qualified criteria; The following grading scheme was used to score the histological alterations: (−) absent; (+) mild; (++) moderate; (+++) severe

histological alteration	Control	0.2	0.4	0.08	0.008
Basement membrane irregularity	-	+	+	+	+
swelling cell	-	+	-	+	++
Necrosis	-	++	+	+	-
Congestion	-	+	++	+	+++
Vacuolar degeneration	-	+	+	+	+
Infiltration of inflammatory	-	-	-	+	+

Different letters show the statistically significant differences between treatment and control (Tukey test, $P \leq 0.05$).

Discussion

AgNPs have attracted the attention of researchers due to their application in the treatment of diseases in addition to its antimicrobial effects [7] and [8] AgNPs used in a variety of application [9]. The wide range application of nanoparticles far many biomedical and industry [10] also used in coating of surgical instrument [11] and prothesis[12]. Yet, the nanomateriales have higher surface to volume ratio that makes them more reactive and toxic due to smaller size of silver NPsthis will cause penetration through the tissue then cause cytotoxic effect [13] .

This study showed that nanoparticle administration cause increased the level of serum urea and creatinine this result to estimate effect g NPs on renal function. Serum urea and creatinine are useful indices for evaluating the status of renal functions[14]. A rise in the level of serum urea may imply impaired renal excretion [15] may be due to inactivation resulting from the affinity of the Ag NPs for thiol (-SH) groups, hereby causing functional state of proteins to change [16] .

This study showed that silver nanoparticles have a toxic effect on renal tissue which occur due to the deposition of AgNPs in the kidney which cause histological alteration So the recent study showed a histological alteration in the renal tubules , glomeruli and interstitial tissue , these alteration includes degeneration and necrosis in the tubules and glomeruli with in infiltration of inflammatory cells and haemorrhage in the interstitial tissue these changes occur as aresult of (ROS) production and release of cytokines which have a toxic effect and leading to histological alteration by causing DNA damage [17] [18] [19]

Such histological changes caused by AgNPs are evidence that supports the ability of nanoparticles to cause biochemical alteration that result in cell damage [20] .

Conclusion

The conclusion of this study that the biochemical and histological alteration of kidney induced by AgNPs due to the toxic effect of nanoparticles the severity of alteration depend up on the dose and duration of the silver nanoparticles Authors contribution

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: The writers claim a manuscript has no conflicts of interest.

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The Effect of a Mentoring Program on Competition Orientation for Football School Athletes

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Abstract

The research problem was centered in the fact that despite all the efforts made by the training staff to assist the players in raising their technical level, the researchers with their personal experience because they worked in the field of arbitration and arbitrated many of the matches that took place for the players of the specialized school in the province of Babel note that there are some players They do not carry a positive attitude towards competition and this is enough to affect the level of their performance, and therefore they are born with weakness in perseverance and dedication to sports competition, and this leads to a low level of performance due to the lack of a positive orientation of the players towards competition, which is reflected on his performance Inside the stadium, so the researchers adapted to study this problem by knowing the reality of the competitive orientation of the players and building an indicative program with special exercises to raise the level of their competitive orientation in football. The researchers used the realistic treatment method as one of the psychological methods to improve these variables because it deals with them realistically, and the objectives of the research were to build an indicative program With special exercises for the players of the specialized school in the province of Babylon, football, to identify the effectiveness of the pilot program with special exercises in the orientation towards competition among the players in the province of Babylon football, the researchers used the experimental method in the complex method And the two controlling and experimental equivalents with pre and post-test for their suitability to the nature of the research problem on the players of specialized schools in the middle Euphrates football who are under (17) years old and whose total number is (175) players, **The researchers concluded** that there is an effect of the pilot program with its exercises In flat lift The competitive orientation of football with the experimental group, and real differences emerged in the level of competition orientation between the control and experimental research groups and in favor of the experimental group, and the use of realistic guidance commensurate with raising the level of competition towards the players of the experimental group in the specialized school of football.

Key words: *indicative program, competition orientation, football; Practice; Psychological bhaviour.*

Introduction

Psychological counseling appeared at the beginning of this century as a science and an organized and formal service and its professions have a valuable result for the development of daily life.

Needs the type of orientation, guidance, and advice necessary and sufficient guidance to improve his behavior, through the foregoing that shows the importance of research in working to build a mentoring program that includes special exercises in the field of football game taking into account the educational and

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scientific aspects of limiting Phenomena that may hinder the player's progress and overcome them and work on the development of the player's direction towards competition and upgrading the player's performance in the specialized schools of the central paragraphs of football[1-3], as the research problem was centered in that despite all the efforts made by the training staff to help players in Upgrading their technical level, the researchers with their personal experience because they worked in the field of arbitration and arbitrated many of the matches that took place for the players of the specialized school in the province of Babel. Thus, they are born with a weakness in perseverance and dedication in sports competition, and this leads to a low level of performance due to the lack of a positive orientation by the players towards competition, which is reflected on his performance on the field, so the researchers relied on to study this problem by knowing the reality of the competitive orientation of the players and building a program Guide me with special exercises to raise the level of their competitive approach to football and the researchers used the realistic treatment method as it is one of the psychological methods to improve these variables because it deals with them realistically, and the research goals were to build an indicative program with special exercises for the specialized school players in Babylon emergence of football, identify the effectiveness of the counseling program special exercises in the orientation of competition among the players in the province of Babylon [football(4-5)l].

Keywords: indicative program, competition orientation, football.

Practical procedures:

The researchers used the experimental method in the two groups equal and controlling experimental method with pre and post-test for its suitability to the nature of the research problem on the specialized school players in the middle Euphrates football who are under (17) years old and whose total number is (175) players, and the search samples were chosen randomly. (47) players were identified from the specialized school in Babil Governorate, and (7) players were identified for the exploratory sample and (40) players for the basic experiment. The research sample was divided into two control and experimental groups by simple random method for each group (20) players as the group is exposed Experimental to The independent variable, which is (indicative program), while the control group continues in the followed method, then homogeneity and equivalence processes were conducted in the two research groups in the variables affecting the dependent variable, as shown in Table (1).

Table (1). Shows the homogeneity of the research sample

Measurements	Unit measurements	Mean	STD.EV.	Skewness	Statistical Result
Body length	Cm	163.01	5.36	0.22	Homogeneous
Bodyweight	Kg.	49.22	2.57	0.43	Homogeneous
Age	Year	15.43	1.37	0.91	Homogeneous

Then the researchers conducted the equivalence process between the two research groups in the variables studied by the research, namely (orientation towards competition), as shown in Table (2) :

Table (2). Shows the equivalence of the research sample

Variables	Unit measurements	Experimental group		Control group		(t) calculate	Significant	Statistical Result
		Mean	STD.EV.	Mean	STD.EV.			
Technical Competition oriented	Grade	59.4	4.049	59.2	2.305	1.024	1.121	Non sig.

The main benefit of making sure that the two research groups are equal is to judge whether there are differences between the two groups in the post-test are differences due to the influence of the independent variable and not to chance factors

The measure of competition orientation:

A measure of competition orientation was adopted by the researcher (Sarmad Al-Husseini), which consists of (6) areas for measuring the orientation towards competition, namely (emotional balance, motivation, thinking, self-confidence, courage, personality) and (32) Paragraph so that the highest degree on the scale is (96) degrees and the lowest degree is (32) degrees.

Preparing the advisory program:

To achieve the goal of identifying the effectiveness of the counseling program with special exercises to raise the level of orientation towards competition for specialized schools in football, it is necessary to build a counseling program, based on studied and planned scientific foundations, and thus the researchers relied on building the counseling program with special exercises on the following steps :

First: the planning, programming, and budget model:

Al-Dosari indicated that the steps of this program are summarized as follows:

• Identification of needs.

• Selection of priorities.

• Define and write goals.

• Find activities and programs to achieve objective goals.

• Evaluating the results.

Second: Collective Counseling:

The researchers used the group counseling method to raise the level of competition orientation, which is a method of counseling provided to the counselors who have similar problems that they suffer from, and these counseling sessions are in the form of groups (extension groups).

Exploratory experience:

The researchers conducted the exploratory experiment on a sample of (7) players, and the researchers aim through this experiment to achieve the following:

- Ensure the safety of the tools and devices used.
- Knowing the suitability of the competition orientation scale to the sample level.
- Training the assistant team and defining its duties.
- Know the time taken to perform the tests.
- Diagnosing and avoiding difficulties when carrying out the main experiment.

- Extract the scientific foundations for the tests.

Extraction of the scientific foundations for the tests:

Before beginning the application of the scale and the indicative program on the research sample in the main experiment, it is necessary to calculate its honesty, consistency, and objectivity, so the researchers conducted the scientific foundations of these tests.

Validate the scale:

The scale was presented to a group of expert masters to determine the validity of its use among the members of the research sample, as it was approved to use the scale on the members of the research sample who are the players of the Specialized School in Football for the province of Babylon.

Stability of the scale:

It means that the scale has high stability when re-applied, the stability of the scale was confirmed by re-applying it after the passage of (14) days from the date of the first application and the value of the correlation coefficient was (0.78), and this indicates that the scale has a high degree of constant.

The objectivity of the scale:

It means that the arbitrators did not differ in judging the results of the test since the scale consists of a clear correction key that is not disputed by two during the answer.

The main field experience:

After the scale was prepared in its final form and the design of the pilot program, the researchers implemented the main field experiment that included the following:

- Presenting the topic: It includes giving the guides in the experimental group knowledge of the topic of the session, its importance and how it is applied, and the researchers have explained the topic of the session adequately so that the guides in the experimental group can know the behavior that they will train with while briefly presenting the goals and objectives and how to apply and the researchers are keen to clarify So in an example of reality to learn

- Social responsibility model: The intention here is to formulate a behavioral situation in the form of an example of a question that is consistent with the topic of the session, the researchers prepared a set of behavioral models for the topic of the guiding session, and its content was mathematical situations related to the players and was formulated as questions that require guides The answer to them, and after that these questions were discussed regarding the answer with praise and encouragement for the answers that require it and correcting and correcting the wrong, including:

*** A question from the reality: -** The researchers put forward several ideas and were formulated in the form of questions derived from the reality of football and this proposal has to do with the topic of the session, and the researchers should provide them with feedback and reinforcement

*** Effectiveness and fun: -** To add an atmosphere of joy, joy, pleasure and a sense of optimism among the players (the guides) and that the guiding session is not boring and not imposed on the guides, the guide has prepared a set of activities that are appropriate to the level of the guides and appropriate to the topic of the session, that is, appropriate to the objectives of the session. (1) While avoiding the mockery or criticism that is guided by the guides, if any, and allowing the session to be open, full of joy, pleasure, and laughter, which expresses the emotions automatically, that is, without exceeding the mutual respect between the guides.

*** Final evaluation (final): -** In this type of evaluation, the results obtained from the pre-measurement are compared with the results of the post-measurement, i.e. knowing the extent of the influence of the independent variable on the dependent variable (orientation towards competition), (8) counseling sessions were applied per session time (45 minutes) for each session, except for the first and last session, which took time (30) minutes and by two sessions per week on Sunday and Tuesday, and the arbitrators agreed on the validity of the advisory program followed by its own exercises, the method of guidance used, the number of sessions, their times, their content and goals, a suitable place has been created to implement the sessions except Shadia, and the sports talent stadium was chosen by football in the province of Babel and the accompanying halls have a place to

implement the advisory program and prepare the means and tools for the counseling program (blackboard - notebooks and pens for homework - sports balls - an electronic calculator) and determine the time of the instructional session as the researchers determined the time of the guiding session Within the training unit for players, which is three o'clock in the afternoon on (Sunday and Tuesday).

Application of the counseling program:

In the way of collective guidance on the players of the specialized school in Babil Governorate in football, in which there is an interaction between researchers and guides who try to express themselves and their positions during the guidance sessions to raise the level of their orientation, and many studies have demonstrated the effectiveness of the collective counseling program in raising the level of The trend towards competition in the study[4-6] and the first guiding session was implemented on (21/12/2019) until (01/21 2020) (8) counseling sessions.

Post-test:

Dimensional tests (measuring competition orientation) were conducted on the research sample, the control group, and the experimental group, and the researchers provided the conditions and controls followed in the tribal tests and by the arbitrators themselves and the auxiliary work team.

Statistical means:

The researchers used the statistical program (Spss) and (Excel) to analyze the results of the research.

Results and discussion:

This axis included the presentation of the statistics results of the measure of competition orientation for soccer players after statistically processing them and in line with the goals.

Table (3) values of the mean and the standard deviation of the measure of competition orientation in the pre and post-tests.

Tests		Pretest		Posttest		(t) calculate	Significant	Statistical Result
		Mean	STD. EV.	Mean	STD. EV.			
Technical Competition oriented	Experimental	59.2	2.305	67.0	3.359	13.67	0.000	Sig.
	Control	59.4	4.049	62.4	3.756	9.72	0.000	Sig.

The results showed significant differences between the pre and post-tests in favor of the post-test. The researchers see the reason for these moral differences for the scale of competition orientation towards creating the appropriate atmosphere for guidance, which includes the material and moral aspects and includes calm and appropriate and the availability of tools that contribute to the success of the mentoring program with its own exercises, while providing the opportunity And to allow the players of the instructional group to express their thoughts with confidence and freedom without hesitation or fear and with the use of effectiveness and spirit, to give an atmosphere of joy and joy to the players, and mutual respect between the players so that no feeling is generated The players (guides) said that the session is imposed on them, as well as the suitability of the counseling program with its exercises related to its content with the ages and levels of the players, which they have in their direction towards competition in football, as well as expressing and supporting positive ideas and avoiding negative ones that cause This leads to a loss of hope and frustration, and the method used

to manage the counseling session with special exercises, which relied on scientific and substantive competition, and then assigning the players the homework duty for the purpose of benefiting from what they learned in the guiding session and is considered "accepting the mentor of the mentor as he is and good listening to him and maintaining the confidentiality of the information Of the mentor (the player) and its specificity are success factors (psychological counseling)[2,7].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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The Synergistic Effect of Combining Some Antibiotics with Powder of Silver NPs, Copper NPs and Titanium NPs

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Abstract

Routine antibiotics susceptibility testing still relies on standardized cultivation-based analyses, including measurement of inhibition zones in conventional agar diffusion tests.The sensitivity of the bacterial isolates causing Clinical isolatest results showed that all the isolates subjected to this study showed varying resistance to the antibiotics used. The effect of nanoparticles combined with different antibiotics was investigated against multi resistant Clinical isolates bacteria using disk diffusion method. The inhibition zones diameter (mm) around various antibiotic discs (Trimethoprim, sulphamethaxazol, Azithromycin, Aztreonam, Ceftriaxone, Gentamycin, Tetracyclin, Nitrofurantoin) with/without nanopatrticles against isolates were measured. The antibacterial activities of some antibiotics have been increased in the presence of nanoparticle against all isolates while others showed no effect. The highest antibacterial activities of antibiotics combined with nanoparticle were observed against *E.coli* isolates for Gentamycin, Trimethoprim-sulphamethaxazol ,Azithromycin ,Tetracyclin ,Nitrofurantoin. while *A. baumanii* appeared sensitive to Gentamycin, Trimethoprim sulphamethaxazol and Azithromycin compared with antibiotics alone. *K.pneumonia*e became sensitive to Gentamycin, Tetracyclin and *P.aeruginosa* to Ampicillin,Trimethoprim sulphamethaxazol, Azithromycin and Gentamycin. nanoparticle showed no effect on antibacterial activities of all antibiotics except Gentamycin against gram positive bacteria *S.aureus*.

Keywords*: Nanoparticle, Antibiotics, Toxicity; inhibition.*

Introduction

Nanoparticles: nanomaterials as a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or agglomerate; where 50% or more of the particles exhibited, one or more external dimensions in the size range 1–100 nm [(1)]. Silver nanoparticles have been used extensively in anti-bacterial agents, the antibacterial property of silver nanoparticles has allowed its wide range of application from disinfecting devices and home appliances to water treatment [(2-3).] The nanoscale size makes them comparable to the cellular components and proteins hence, nanoparticles can easily by pass natural barriers [(4-5)]. Metal nanoparticles exhibit unique electronic, magnetic, catalytic and optical properties that are different from those of bulk metals. This may result in interesting new applications that could potentially be utilized in the biomedical sciences and areas such as optics and electronics [(6)]. Extracellular synthesis of metallic nanoparticles has extended its commercial applications in various fields. Since the main obstacle with a biological synthesis approach is polydispersity, it is important to improve these conditions toward mono-dispersity [(7)]. The antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The small size and the high surface to volume ratio [(8)]. The activity was

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shown against a broad array of both Gram-positive and Gram-negative bacteria, protozoa, fungi and viruses [9]. The destruction mechanism involves peroxidation (degradation) of lipids in the cytoplasmic membrane and the cell. wall due to the production of reactive oxygen species such as superoxide anion, hydrogen peroxide, and hydroxyl radicals that can interact and damage proteins and DNA [10].

Methods

Microorganisms

Eight diagnosed clinical, bacterial isolates, including; Gram-positive bacteria, *S. aureus* (n=2) and Gram negative bacteria, *E.coli* (n=2), *K. pneumoniae* (n=2), *P.aeruginosa* (n=1) and *A.baumanii* (n=1) were obtained from Dr. Hussanin Abed-almueed lab , Baghdad, Iraq. each isolate was subcultured into5 mL of Luria Bertani broth and incubated overnight at 37°C, then streaked onto Luria Bertani agar for obtaining purified colonies.

Disk diffusion assay

A modified Kirby-Bauer method [11] was performed to determine the antibacterial effect of nanoparticles on bacterial isolates as follows,

Seeded agar plate preparation

A well 3-5 isolated colonies of fresh culture (LBA, Oxoid) were suspended in 5 ml of normal saline solution (pH7) to obtain culture with 1.5×10^8 cfu/ml by adjusting the turbidity with 0.5 McFarland tube. Sterile cotton swabs are dibbed into the inoculums and evenly swabbed over the surface of the Muller-Hinton agar plate. All plates were left to dry before applying the loaded disks.

Nanoparticle disks (Nps disks) preparation.

Powder of Silver NPs, Copper NPs and titanium NPs (~100 nm, Sigma-Aldrich) were suspended into sterilized distilled water, and sterilized Whatman No 1 filter paper disks were lifted in the suspension for 10 minutes. The loaded disks were then carefully applied to the surface of the seeded agar plates using sterile forceps. Trpicalte for each Nps disks were made. It should be noted, Nanoparticle antibiotic disks were prepared by adding antibiotic disks (i.e., Trimethoprim, sulphamethaxazol, Azithromycin, Aztreonam, Ceftriaxone, Gentamycin, Tetracyclin, and Nitrofurantoin) separately into each Nps solution. .

Results and Discussion

The sensitivity of the bacterial isolates causing Clinical isolates was done against (9) type of antibiotics from different classes, the results shown in(Table1) showed that all the isolates subjected to this study showed varying resistance to the antibiotics used.Results also showed that all isolates of S.aureus were resistant to (Aztreonam and Trimethoprim/ Sulphamethoxazol). while totally sensitive towards antibiotics (Imipenem). A.baumanii have been the same ability to resist 7 antibiotics including(Aztreonam and Trimethoprim /Sulphamethoxazol, Gentamycin, Tetracyclin, Nitrofurantoin, Ceftriaxone, ,and Nalidixic acid) but they were sensitive to Amikacin, Ciprofloxacin, and Imipenem . Al-Khafaji [12] mentioned that all clinical A. baumannii isolates were completely susceptible to imipenem.

Table (1):Antibiotic Susceptibility test for Clinical isolates bacteria

Bacterial isolates	Antibiotic								
	IPM	NA	(SXT)	(AZM)	(ATM)	(CRO)	(CN)	(TE)	(F)
S.aureus1	Si	Re	Re	Re	Re	Re	Re	Re	Re
S.aureus2	Si	Re	Re	Re	Re	Re	Re	Re	Re
E.coli1	Si	Re	Re	Re	Re	Re	Re	Re	Re
E.coli2	Si	S	Re	Re	Re	Re	Re	Re	Re
P.aeruginosa1	Si	Re	Re	Re	Re	Re	Re	Re	Re

Cont... **Table (1):Antibiotic Susceptibility test for Clinical isolates bacteria**

K.pneumoniae1	Si	S	Re	Re	Re	Re	Re	Re	Re
K.pneumoniae2	Si	Re	Re	Re	Re	Re	Re	Re	Re
A.baumanii1	Si	Re	Re	Re	Re	Re	Re	Re	Re
A.baumanii2	Si	Re	Re	Re	Re	Re	Re	Re	Re

F=Nitrofurantoin, IPM=Imipenem, NA=Nalidixic acid , CIP=Ciprofloxacin, CN=Gentamycin, TE= Teteracyclin ,SXT =Trimethoprim/Sulphamethoxazol ,ATM=Azteroname ,Re=Resistant ,Si=sensitive.

Bacterial isolates of *E.coli* characterized by being sensitive to the Imipenem. and resistant towards (Aztreonam, Tetracyclin). Baral *et al*.[13] reported *E. coli* included resistance to Ciprofloxacin, Gentamycin .all isolates of *K.pneumoniae* were resistant toward (Aztreonam and Gentamycin) appeared sensitive to Imipenem. Either [14] resulted in his study that some bacterial isolates of *K . pneumoniae* were resistant to Nitrofurantoin. At the same time clinical isolates of *P.aeruginosa* displayed a highly resistant to(Tetracyclin, Azteroname and Nitrofurantoin).

The synergistic effect of nanoparticles with various antibiotics was studied against multi resistant clinical bacteria using disk diffusion test. The inhibition zones diameter (mm) were measured for each control group (Trimethoprim-sulphamethaxazol, Azithromycin, Aztreonam, Ceftriaxone, Gentamycin, Tetracyclin,Nitrofurantoin)andtestgroup(nanopatrticles-antibiotics mixes). The antibacterial abilities of some antibiotics have been elevated in nanoparticle presence against all isolates while others remained unaffected. The highest antibacterial activities of antibiotics combined with nanoparticle were observed against *E.coli* isolates for Gentamycin, Trimethoprim-sulphamethaxazol ,Azithromycin ,Tetracyclin ,Nitrofurantoin. while *A. baumanii* appeared sensitive to , Gentamycin ,Trimethoprim sulphamethaxazol and Azithromycin compared with antibiotics alone. *K.pneumonia*e became sensitive to Gentamycin, Tetracyclin and *P.aeruginosa* to Ampicillin,Trimethoprim sulphamethaxazol, Azithromycin and Gentamycin. nanoparticle showed no effect on antibacterial activities of all antibiotics except Gentamycin against gram positive bacteria(*S.aureus*) as inTable-2.

Table(2): The comparative activities of various antibiotics and antibiotic with TiO_2 nanoparticles against Clinical isolates bacteria.

Bacterial isolate	(SXT)		(AZM)		(ATM)		(CRO)		(CN)		(TE)		(F)	
	Ab	Ab + TiO2	Ab	Ab + TiO2	Ab	Ab + TiO2	Ab	Ab + TiO2	Ab	Ab + TiO2	Ab	Ab + TiO2	Ab	Ab + TiO2
E.coli 1	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Si	Re	Si
E.coli 2	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Si	Re	Si
A.baumanii11	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	R	Re	Re
A.baumanii 2	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	R	Re	Re
K.pneumoniae 1	Re	R	Re	Re	Re	Re	Re	Re	Re	Si	Re	Si	Re	Re
K.pneumoniae2	Re	R	Re	Re	Re	Re	Re	Re	Re	Si	Re	Si	Re	Re
S.aureus1	Re	R	Re	Re	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
S.aureus2	Re	R	Re	Re	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
P.aeruginosa1	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re

CRO=Ceftriaxone,, AZM=Azithromycin F=Nitrofurantoin, CN=Gentamycin, TE=Teteracyclin ,SXT =Trimethoprim /Sulphamethoxazol,ATM= Azteroname , Re =Resistant , Si =sensitive , Ab:Antibiotic

A synergistic effect of antibiotics (Imipenem, Gentamycin, Vancomycin, and Ciprofloxacin) in conjugation with biologically synthesized Ag nanoparticles increased the susceptibility against *E. coli, S. aureus, P. aeruginosa and K. pneumoniae*. Combination Ciprofloxacin and Imipenem with nanoparticles were found to be most efficient in inhibiting bacteria, if bacteria improves resistance to one of them, the other bactericidal agent would kill the bacteria [15].

Table(3): The comparative activities of various antibiotics and antibiotic with Cu nanoparticles against Clinical isolates bacteria.

Bacterial isolate	(SXT)		(AZM)		(ATM)		(CRO)		(CN)		(TE)		(F)	
	Ab	Ab + Cu	Ab	Ab + Cu	Ab	Ab + Cu	Ab	Ab + Cu	Ab	Ab + Cu	Ab	Ab + Cu	Ab	Ab + Cu
E.coli 1	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Si	Re	Si
E.coli 2	Re	Si	Re	Re	Re	Re	Re	Re	Re	Si	Re	Si	Re	Si
A.baumanii11	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	R	Re	Re
A.baumanii 2	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
K.pneumoniae 1	Re	R	Re	Re	Re	Re	Re	Re	Re	Si	Re	Re	Re	Si
K.pneumoniae2	Re	Si	Re	Re	Re	Re	Re	Re	Re	Si	Re	Si	Re	Re
S.aureus1	Re	Re	Re	Re	Re	Si	Re	Re	Re	Si	Re	Re	Re	Re
S.aureus2	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
P.aeruginosa1	Re	Si	Re	Si	Re	Re	Re	Re	Re	Re	Re	Re	Re	Re

CRO=Ceftriaxone,, AZM=Azithromycin F=Nitrofurantoin, CN=Gentamycin, TE=Teteracyclin ,SXT =Trimethoprim /Sulphamethoxazol,ATM= Azteroname , Re =Resistant , Si =sensitive , Ab:Antibiotic

Table(4): The comparative activities of various antibiotics and antibiotic with Ag nanoparticles against Clinical isolates bacteria

Bacterial isolate	(SXT)		(AZM)		(ATM)		(CRO)		(CN)		(TE)		(F)	
	Ab	Ab + Ag	Ab	Ab + Ag	Ab	Ab + Ag	Ab	Ab + Ag	Ab	Ab + Ag	Ab	Ab + Ag	Ab	Ab + Ag
E.coli 1	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Si	Re	Re
E.coli 2	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	R	Si
A.baumanii11	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
A.baumanii 2	Re	Si	Re	Si	Re	Re	Re	S	Re	Si	Re	Re	Re	Re
K.pneumoniae 1	Re	R	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
K.pneumoniae2	Re	Si	Re	Re	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
S.aureus1	Re	Si	Re	Re	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
S.aureus2	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re
P.aeruginosa1	Re	Si	Re	Si	Re	Re	Re	Re	Re	Si	Re	Re	Re	Re

CRO=Ceftriaxone,, AZM=Azithromycin F=Nitrofurantoin, CN=Gentamycin, TE=Teteracyclin ,SXT =Trimethoprim /Sulphamethoxazol,ATM= Azteroname , Re =Resistant , Si =sensitive , Ab:Antibiotic, Ag: Nanoparticles.

The antibiotics-metal nanoparticles conjugates could boost the effectiveness of antibiotics against resistant pathogens. Moreover, nanoparticle–antibiotic complex reduces the amount dosage of both agents, thus increasing the antimicrobial properties and reducing risk that associated with high antibiotic doses. In addition,, due to this coupling, the concentrations of antibiotics were increased at the point of antibiotic-microbe contact and thus accelerate the antibiotic-microbe binding [16-17]. Urgent and growing need for antibacterial agents to fight those pathogenic microbes that continuously developed their resistance against traditional antibiotics[18]. Nanomaterials release ions, which react with the thiol group (-SH) of the proteins display on the bacterial surface due to inactivated proteins resulted decreasing the membrane permeability then cell death [19]. Moreover, [20] studied the Synergistic effect of silver nanoparticles and ampicillin were noticed against *E. faecium, Streptococcus mutans* and *E. coli* ATCC 25922, while Synergistic effects of Ag nanoparticles and Chloramphenicol were found only against *E. faecium* and *P. aeruginosa.* The explaination of synergistic combination of nanoparticles and Chloramphenicol exhibited increased hydroxyl radical formation which is might be an important cause of the synergism. Other studies reported that antibacterial activity of nanoparticle along with antibiotics Ampicillin and Gentamicin against *S. typhi* increased in the presence of nanoparticles[21].

Conclusion

we would like to conclude that due to poor diagnostics and overdose and incapability of drugs, microorganisms

are commonly able to develop resistance against antibiotics. The infections caused by microorganisms are a serious global healthcare issue. Were developed nanoparticles and had proven strong efficacy against various pathogens, either individually or in combination with antibiotics.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Phylogenetic Tree Analysis of First *Psychrobacter* Sp. Strain From Blood of Iraqi Patient; A Case Report

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Abstract

Psychrobacter spp. are a Gram negative bacteria, aerobic, non-motile, small, with coccobacilli shape. Originally isolated from seaweed samples and marine environments. Recently considered as rare opportunistic human pathogens. Sixty –five years old women admitted to hospital with diabetic mellitus and stage 4 pressure ulcers (PU) with seizure and mild fever 37.9 °C. A gram staining of blood culture revealed gram negative bacteria have a cocobacilli shape. The VITEK2 system (bioMérieux) misidentify the isolate as *Acinetobacter bumannii* complex with low discrimination. The submission of the bacterial isolate to the GenBank BLAST search tool revealed that the Iraqi isolate show 100% homology with *Psychrobacter* sp. From china with accession number ID: MK205167.1, the next matches with Uncultured *Psychrobacter* sp. (ID: KF859544.1 China) *Psychrobacter pulmonis*(ID: KU364058.1, India), *Psychrobacter pulmonis* (ID: MH550129.1, China)with 99% similarity for each one. This *Psychrobacter* sp. was the first isolate from bacteremia patients in Iraq. The identification based on 16S rRNA gene sequence for precisely identify this bacteria that misidentified by VITEK2 system.

Key Words: *Psychrobacter sp., 16S ribosomal RNA gene, Bacteremia*

Introduction

PPsychrobacter species are gram-negative coccobacilli, aerobic, nonpigmented, nonmotile [1]. Latest taxonomic classification system of involve three bacterial genera in the family of Moraxellaceae: Moraxella, Psychrobacter and Acinetobacter [2].The Psychrobacter genus includes 33 species of validly submitted names [3]. P. phenylpyruvicus (formally known as Moraxella phenylpyruvica) was correlated with bacteremia, septic arthritis, endocarditis, foot abscess and wound infection [4]. A novel species of P. sanguinis, has also been isolated from samples of human blood [5]. Recent taxonomic classification of Psychrobacter species was revised and most bacteria isolates from human sources other than P. phenylpyruvicus belong to the newly identify P. pulmonis species and P. faecalis [6]. Therefore, the spectrum of infectious diseases in human associated with thevarious species of the Psychrobacter genus could rapidly change [4]. Psychrobacter spp. Isolated from marine species (fish, crustaceans); marine environments (seaweed and seabed); food products (cheese, seafood and meat); lamb lungs and digestive tracts of pigs. Psychrobacter spp. It may also be a part of human microbiota; studies have shown the existence of P. faecalis, P. arenosus, P. pulmonis and P. phenylpyruvicus in the human intestinal tract [7].

Psychrobacter species are rare opportunistic human pathogens. Only a Psychrobacter spp subsetare considered opportunistic pathogens that medically relevant and were isolated from samples collected from brain tissue, human blood, urine, vulvae, ears, eyes, cerebrospinal fluid, wounds and other skin sources [8]. Clinical characteristics rely on the site of the infectionand include meningitis [9], and bacteremia, of all these cases, just one was related to marine environment[10].

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Case report

Sixty –five years old women admitted to AL-Yarmuk Teaching Hospital in Baghdad, Iraq. History of patients are with seizure and mild fever 37.9 °C and diabetic mellitus with no diabetic foot and stage 4 pressure ulcers (PU) at her back appeared before 7 month , patient didn't received any antibiotic treatment. WBC account was 15.1 (10 e9/L). Two sets of blood culture (Brian heart infusion broth) were drawn from two distinct vein sites, after a day of incubation the aerobic blood culture bottle was become turbid as a result of bacterial growth. The broth after that was subcultured into the macconky and blood agar and incubated at 37°C in aerobic conditions; the bacteria only grow in the blood agar after 48hr of aerobically incubation appeared gram negative coccobacilli in gram stain under the microscope. These coccobaccili bacteria were oxidase and catalase positive. The antimicrobial sensitivity test was used to show the resistant of isolate to antimicrobial drugs. The antibiotics used were Meropenem, Levofloxacine, Ceftazidime, Ceftriaxone, Azithromycin and Vancomycin. Although there is no antimicrobial break point to *Psychrobacter* sp.*,* the large size of inhibition zone was recorded to all antimicrobial drugs used in this study except Azithromycin which mean this isolate was sensitive.

The phenotypic identification by VITEK2 system gram negative card (bioMérieux) result was low discrimination organism of *Acinetobacter bumannii* complex, this result was not satisfy therefore the molecular method was followed to precisely identifying the bacterial isolate. For identification of the bacteria, molecular analysis was followed by the PCR amplification of 16S rRNA , the following Universal primer pair was used in the reaction of the PCR,8F : 5′- AGA GTT TGA TCC TGG CTC AG 3′ and reverse U1492R:5′- GGT TAC CTT ACG ACT T 3′[11] . The PCR reaction was carried out with 2µl of template DNA and 25µl as a total reaction volume using GoTaq® Green Master Mix, 2X as follow : Initial denaturation for 5min at 94°C ; 35 cycles of 94°C , 52°C , and 72°C for (94 sec,1min ,1min) for each of them respectively; and finishing with 7min at 72°C. The PCR product was sent to microgen incorporation for sequencing. Sequence of 1500base-pairs (bp) was obtained. The analysis of the sequence with the GenBank Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/genbank)

The submission of the isolate 16S rRNA gene sequence by the GenBank BLAST revealed that the Iraqi isolate show that 100% homology with *Psychrobacter* sp. From china with gene bank accession number ID: MK205167.1, the next matches with *Psychrobacter pulmonis*(ID: MH550129.1, China), *Psychrobacter pulmonis* (ID: KU364058.1, India), Uncultured *Psychrobacter* sp. (ID: KF859544.1 China) with 99% similarity for each one. The similarity was 98% with *Psychrobacter faecalis* (ID: MK205166.1, China) and *Psychrobacter faecalis* (ID: MH712977.1, Chile). The isolate sequence was submitted in the NCBI as the first *Psychrobacter* sp. isolate in Iraq.

The neighboring method was used to construct the phylogenetic tree Based on sequences of 16S rRNA of the isolate and the similarity with 23 bacterial isolates as shown in figure (1). The software was used in phylogenetic analysis was Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (http://www.megasoftware.net).

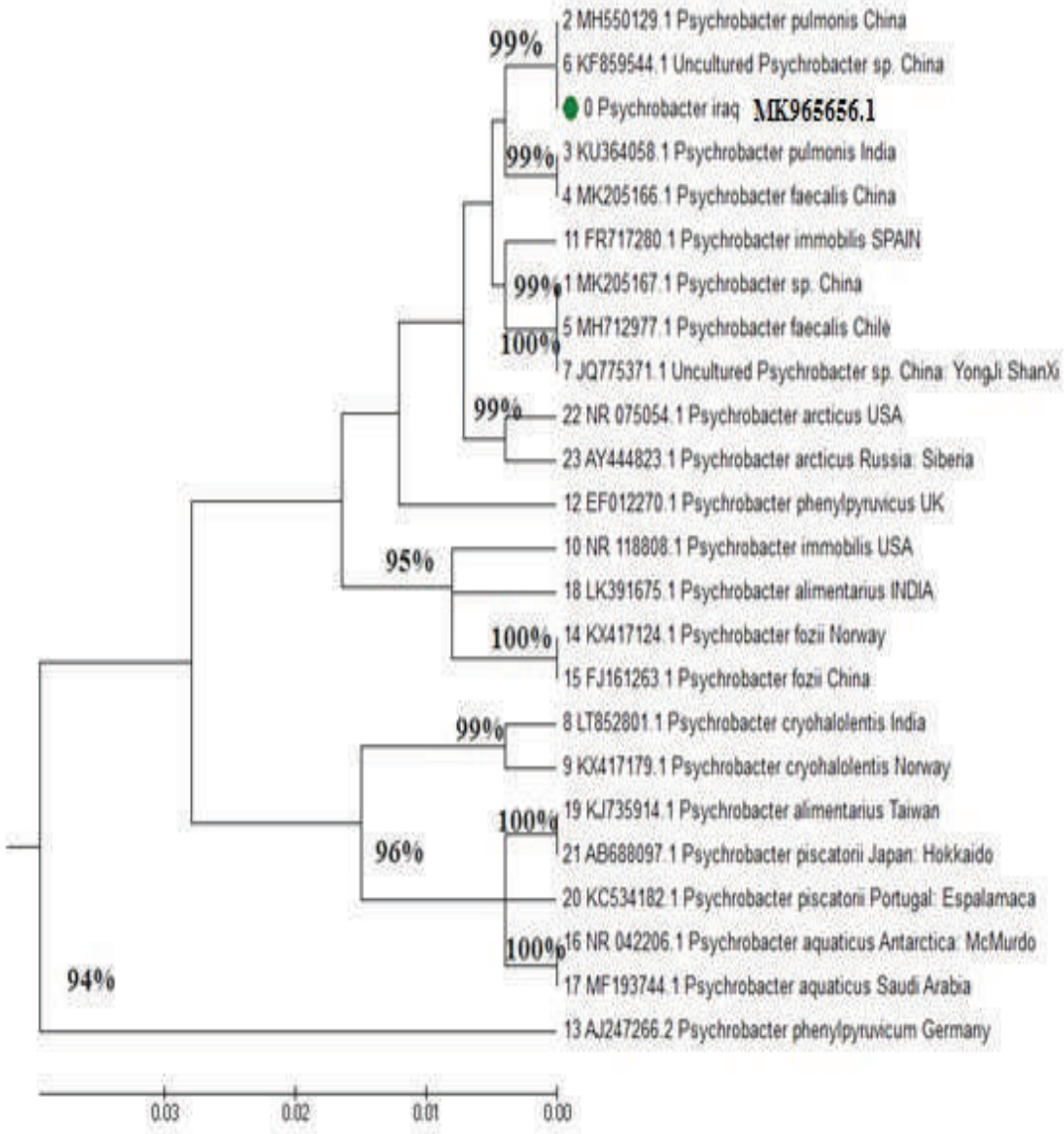


Figure 1: The relatedness of *Psychrobacter* sp. strain NA199151 to 23 other bacterial species

Discussion

Psychrobacter species are gram-negative coccobacilli, aerobic, nonpigmented, nonmotile[1] *Psychrobacter* species are rare opportunistic human pathogens and were isolated from samples collected from brain tissue , human blood, urine, vulvae, ears, eyes, cerebrospinal fluid, wounds and other skin sources [8]. Strains of *Psychrobacter spp.* are extremely susceptible to antimicrobial agents ; only one strain of *P. Phenylpyruvicus* was recorded to just be resistant to aztreonam and ampenicillin , while two strains of *P. immobilis* resistance to penicillin [12]. Disk diffusion testing was performed to antimicrobial sensitivity testing aerobicly at 35°C. Despite the lack of standardized breakpoints to *Psychrobacter spp.*, large zone sizes suggested the bacterial isolate was susceptible to penicillin, cefazolin, cefoxitin, tetracycline, ciprofloxacin, meropenem, cefepime and ceftriaxone. This isolate was tested negative to β-lactamase [13]. *P. sanguinis* isolate was susceptible to cefepime, amoxicillin, ceftazidime, meropenem, imipenem, ciprofloxacin, amikacin, ticarcillin and trimethoprim-sulfamethoxazole [14]. The isolate was resistant to lincomycin and susceptible to amoxicillin/clavulanate, amoxicillin, piperacillin, ticarcillin/clavulanate,

ceftazidime, cefotaxime, cefalotin, piperacillin/ tazobactam, cefepime, cefpirome, gentamicin, imipenem, netilmicin, tobramycin, trimethoprim/ sulfamethoxazole, pristinamycin, erythromycin, amikacin, polymyxin B, ofloxacin, nalidixic acid, fosfomycin and ciprofloxacin [4]. All these researches agree with the result of present study of antibiotic selectivity were the *Psychrobacter* sp. Isolate was sensitive to all antibiotics drugs except Azithromycin.

In 2016, one case of Psychrobacter associated with meningitis in a cerebrospinal fluid (CSF) of 13 year-old male patient's was revealed and isolate was recognized by metagenomics of the next generation sequencing (NGS) [9]. Four Psychrobacter isolates were obtained from human blood cultures at the Wadsworth Center (New York) from 2004 to 2008 that cannot identify correctly at the laboratory [5]. Scientific studies that identify microorganisms incorrectly by standard techniques are increasing [15]. As in this study the systems of phenotypic identification, Includes the VITEK2 system, couldn't identify psychrobacter sp. and misidentify as Acinetobacter bumanii. It is apparently that there is a increasing number of recently identified species of bacteria,in clinical microbiology the unmistakable identificatin of uncommon strains is increasingly required to enhance understanding of tissue reservoirs, transmission routes, susceptibility to antibiotic and treatment improvement and to help in the identifying of novel species of bacteria. The identification of unusual bacterial strain and bacteria with uncommon phenotypic profiles by standard techniques requires to be proven using a reliable method such as sequencing of 16S rRNA gene [15]. Additionally, M. Phenylpyruvica was reclassified to a genus Psychrobacter as (P. phenylpyruvica) by analysis of sequence of 16S rRNA genes [16].

In the present study, sequencing of the 16S rRNA gene was necessary for precise identification of bacterial isolate.Our research demonstrates that analyzes of 16S rRNA sequence and using different databases are helpful instruments for identifying unknown isolates.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Analytical Evaluation Study of Some Characterization of Potable water for an Old Well in Baghdad City

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Abstract

This study involved measuring seventieth tests five of its physically ,temperature, color, turbidity, electrical conductivity and pH and remaining tests were chemical ten of them for heavy metals ,the chlorine and fluorine were also investigated for water of old well in Baghdad city close to tiger river weekly for six months ,this study showed validity of this well to drink and domestic use within the limit of the properties and elements studied only ,through the tests ,all the samples were found to be transparent and non-colored ,there was nothing left after her leaving ,and the pH ranged from (7.655-7.920) , conductivity (821-908µsemens/cm) and temperature (19.68-23.36ºC) ,the concentrations of elements were (0.031-0.044), (0.038-0,053), (0.009-0.015), (0.019-0.025), (0.004-0.006), (0.009-0.011), (0.029-0.035), (0.108-0.112) ,(0.003-0.005), (95-111), and (0.424-0.571) µg/ml for lead ,cadmium, iron, copper, antimony, cobalt, chromium, zinc, manganese, chlorine and fluorine respectively ,as for nickel, it is not registered, all the tests have been subject to the local and international standards except lead and cadmium, according to international standard only.

Key wards: *potable water, quality, heavy metals, Toxicity; polution.*

Introduction

Drinking water, define a water that is safe to drink and using in food preparation ,also known as potable water. Drinking water amount required varies, and depends on some parameters physical activity, environmental conditions, age, and health issues[(1)] . In developed countries ,typically tap water meets drink water quality standard properties, even though only a small proportion is actually used in food or preparation consumed. Other typical useing include irrigation, toilets, and washing . Grey water may also be used for irrigation or toilets. The uses to irigation however may be asociated with risks[(2)]. Water may also be unaceptable due to levels of suspended solids or toxins. Globally, 89% of peoples had access to water from a source that is suitable for drinking called improved water source. Nearly 2.4 billion had access to public taps or wells ,while another 4.2 billion peoples world wide had access to tap water. The World Health Organization WHO considers access to safe drinking water a basic human rights [(3)].

Typically drinking water quality parameters fall within three categories: chemical, physical, and microbiological, chemical and physical parameters include turbidity, trace organic compound, heavy metals, and total suspended solids, microbiological parameter includes E. coli, Coliform bacteria, specific pathogenic species of bacteria such as cholera causing Vibrio cholera, protozoan parasites, and viruses[(4)] .

Chemical and Physical Properties of studied well water

Temperature

The density, viscosity, the vapor pressure and surface tension of water more or less depending upon the temperature. Continuous process of sinking and heating keeps the water body from freezing entirely. Temperature of water changes gradually in response

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to changes in seasonal. Seasonal variations in water temperature may be caused by changing meteorological events ,solar angle, air temperature and a several of physical aspects related to the watershed and stream[3.4] .

pH

Beacause the efficacy of disinfection with chlorine is highly pH dependent ,the measure of pH at the same time as chlorine residual is important since: disinfection is less effective where the pH exceeds 8.0, simple tests may be conducted in the field using comparators such as that used for chlorine residual. It is possible to measure pH and chlorine residual simultaneously with some chlorine comparators. Alternatively, portable pH electrodes and meters are available. If the water has a low buffering capacity ,results may be inaccurate [2] .

Electrical Conductivity

Electrical Conductivity define as a concentration of number dissolved minerals or ions in water. Its a material fundamental property that quantifies how strongly that material opposes the flow of electrical current. The highest and lowest concentration was observed starts 0.1 value Electrical conductivity to 4.5 value were observed[7].

Chlorine

In most countries ,Chlorine in one form or another is the principal disinfecting agent employed in small communities. A number of advantages of Chlorine as a disinfectant, including its relative efficacy, ease of measurement, and cheapness. Chlorine leaves a disinfectant residual that assists in preventing recontamination during transport, distribution, and household storage of water is an important additional advantage over some other disinfectants [5] .

Fluorine

Although fluoride is helpful for dental health at low concentrations, sustained consumption of large amounts of soluble fluoride salts is dangerous. Fluoride toxicity is a condition in which there are in elevated level of the fluoride ion in the body. Referring to a common salt of fluoride, sodium fluoride ,the lethal dose for most human adults is estimated at 5 - 10 g which is equivalents to 32 - 64 mg per kg fluoride per kg body weight [7] .

Heavy metals

Natural occures elements that having a highly atomic weight and at least 5 times greater than that of water density are called heavy metals. Their multiple domestic, industrial, agricultural, technological and medical aplications have leds to their wide distribution in the environment. Toxicity of heavy metals depends on number of factors includes the dose, chemical species, and route of exposure, as well as the gender, genetics, age, and nutrtional status of exposed indviduals. Classifing of heavy metals as human toxicity known or probable according to the U.S. [6] .

Table (1): Classification of metals according to its type of toxicity.

non-critical			Toxic but rare		Very toxic		
H	Sr	F	Ti	Ba	Cu	Ag	Au
Li	AI	Cl	Hf	Ga	Ni	Pd	Pt
Na	Fe	Br	Zr	La	Zn	Cd	Hg
K	C	O	W	Ta	Be	Se	Te
Rb	Si	S	Nb	Re	Sn	As	Cr
Ca		N	Os	Ru	Pb	Sb	Co
Mg		P	Ir	Rh	TI		Br

a. Cadmium

Cadmium is used in some industrial paints and may represented a hazard when sprayed, it extensively used in electroplating. Cadmium is also present in the manufacturing of some types of batteries. Cadmium is an extremelly toxic metal commonlly found in industrial workplace. Due to its low permisible exposure limits, over exposures may occurs even in situations where trace quantities of cadmium are found [7].

b. Copper

Copper Cu is an essential element in mammalian nutrition as a component of metalloenzymes in which it acts as an electron acceptor or donor. Copper excessive human intake may leads to severe corrosion and mucosal irritation, wide spread capillary damage, hepatic and renal damage and central nervous system irritation followed by depression [8] .

c. Iron

The iron source in surface water is anthropgenic and is relates to mining activities. Due to its ferric - ferrous inter conversion between ions Iron consider an attractive transition metal for various biological redox processes. Iron is one of the vital components of organisms like enzymes and algae of such as catalase and cytochromes, as well as of oxygen transporting proteins, such as myoglobin and hemoglobin [9].

d. Lead

Lead interferes with a variety of body processes and is toxic to many organs and tissues including the intestines , bones , heart , kidneys , and nervous systems and reproductive. Lead toxicity is a medical condition in humans and other vertebrates caused by increased lead levels in the body. It interferes with the development of the nervous system [9] .

e. Manganese

Manganese is used as a purifying agent in the production of several metals. Manganese poisoning may be caused by chronic ingestion and inhalation of manganese particles. Symptoms associated with over exposure to manganese may caused damage to the central nervous system and pneumonia [9] .

f. Zinc

Zinc though is an essential requirement for a healthy body, while excess zinc can be harmful, and cause zinc toxicity. The free zinc ion in solution is highly toxic to plants, bacteria, even vertebrate and invertebrates fish .Excessive zinc absorption can suppress iron and copper absorption. Zinc over exposure may cause the flu like symptoms of metal fume fever, intestinal and stomach disturbances, and or liver dysfunction.

g. Chromium

Exposure to too much chromium metal or ion may cause respiratory tract and lung cancer as well as kidney diseases. The over exposure may also cause gastro intestinal symptoms, such as vomiting and diarrhea, often with blood. Symptoms may lead to severe electrolyte water disorders, increased mild acidity of body tissues ,blood acidosis, and /or inadequate blood flow to it is tissues resulting in shock.

h. Cobalt

Cobalt in minute amounts conseder an essential element for animal healths as a component of vitamin B_{12}. Three basic ways that cobalt can cause poisoning. You can swalow too much of it, have it comes in constant contact with your skin or ,breathe too much into your lungs. Some times, cobalt particles are release as the metal ball grinds against the metal cup when you walk and can get release into the hip sockets and some times the blood stream, forming cobalt toxicity[10].

Experimental Part

The tests were carried out on the water components in the chemistry department - Faculty of Science / University of Mustansiriya and Chemistry Department / Faculty of Science / University of Baghdad and Ibn Sina Public Company and Ministry of Science and Technology / Department of Research and Technology of Environment and Water and on the following device:-

*Flame Atomic Absorption Spectophotometry FAAS:GBC 933 plus, used for estimation of iron, lead, nickel, copper, zinc, antimony, cobalt, cadmium, chromium and manganese via air-acetylene gas.

*Conductivitimeter EC 214 used for estimation of well water conductivity in (µSiemens) unit, after

adjusting with three standard solutions of potassium chloride.

*Ion selective membrane electrode WTW used for estimation of florin and chlorine via florin and chlorine ion selective electrode with reference calomel electrode.

*pH meter HANNA pH 211 used for estimation acidity or basicity degree of well water medium, after adjusting with two buffer solutions (4, 10).

The water samples in the well under study were examined and analyzed. The samples were collected, transported and stored in glass bottles, examined weekly, and for three readings per sample for a period of six months starting from the beginning of October until the end of March.

Results and Discussion

In this chapter, the study of the validity of using the well water under study for drinking purposes for humans by comparing the obtained results of this water with the maximum allowable value according to the standard of the local standard for quality standardization [(4)] . and quality control for World Health Organization WHO [(4)] .The following forms show the average of four measurements for each month of the six months studied from the beginning of October until the end of March, as well as comparison to the maximum limit of the values allowed locally and globally, and Table (2) summarizes all those specifications and contents studied .

Table (2): Comparison of the results of the examination of the components of the well water with the permissible values according to the Iraqi Ministries and the World Health Organization WHO.

Water Property	or Part Per Million (ppm)(ml/µg)Concentration										
	October	November	December	January	February	March	Average	Standard Deviation SD	Coefficient of Variation	Local Iraqi Quality	International WHO Quality
Temperature C	23.25	23.65	21.19	19.68	20.87	23.36	22.00	1.6400	0.0745	unlimited	unlimited
Conductivity (µSiemens)	821	836	895	875	888	908	864.5000	32.5254	0.0376	1000	1000
pH	7.673	7.655	7.661	7.687	7.712	7.920	7.7180	0.1010	0.0130	6.5-8.5	6.5-8.5
Chlorine	111	108	106	105	98	95	103.8330	6.1120	0.0588	200	250
Fluorine	0.571	0.556	0.511	0.489	0.457	0.424	0.4847	0.0566	0.1167	1	1.5
Lead	0.031	0.034	0.036	0.039	0.042	0.044	0.0377	0.0049	0.1299	0.01	0.05
Cadmium	0.038	0.039	0.044	0.048	0.050	0.053	0.0453	0.0060	0.01337	0.03	0.05
Chromium	0.029	0.030	0.032	0.033	0.035	0.035	0.0323	0.0025	0.0773	0.05	0.05
Iron	0.009	0.011	0.011	0.012	0.014	0.015	0.0120	0.0022	0.1825	0.3	0.3
Cobalt	0.009	0.008	0.008	0.009	0.011	0.011	0.0093	0.0014	0.1473	0.1	0.1
Copper	0.019	0.020	0.020	0.021	0.023	0.025	0.0213	0.0023	0.1056	1	1
Zinc	0.108	0.108	0.109	0.109	0.109	0.112	0.1092	0.0051	0.0134	3	5
Manganese	0.004	0.003	0.003	0.003	0.004	0.005	0.0037	0.00082	0.02216	0.1	0.1

The results of this study showed that all the properties examined for the samples of the well water, which is fixed in the table above, which is the rate of four measurements, are chemically and "chemically" valid during the study period, because the concentration of ions in the studied samples was within the permissible limits and for the period The study, except lead and cadmium, did not comply with the local standard, but according to the highest international standard, the thickness and antimony levels were not compared to the non-quantified quantity allowed by the markers, in addition to the nickel which was not detected and exposed to it under the detection limits of flame atomic absorption apparatus , noting that the characteristics of color and color were not tested automatically "but depending" on the naked eye, taking into account that the water of this well is drawn continuously and is used for drinking and domestic use is located near a populated area and away from the Tigris River 475 meters deep 11.5 meters.

Conclusions

Specific number of elements were measured and monitored in this research in addition to the physical properties that can be measured and coupled with the maximum allowed locally and globally for one well and is located near the course of the Tigris River and for a specific period of six months, ie for two seasons of the year and we recommend extending the study to include more elements Any four seasons and more than one well located medium and far away from the riverbed to observe and record the differences in the content of the elements and physical properties to know the suitability of human use such as drinking, washing and irrigation plantings.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Prevalence of Hepatitis B, C and HIV Viruses among Hemophiliac Patients in Al-Anbar Governorate

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Abstract

Background: People with hemophilia (factor VIII and IX deficiency) and those with other clotting factor deficiencies are particularly at risk of acquiring blood-borne hepatitis virus infections and HIV through transfusion with unscreened blood and blood products.

Objective: To estimate the prevalence of hepatitis B, C, and HIV among hemophiliac patients in the Al-Anbar governorate.

Patients and methods: A cross-sectional study carried out between October 2014 and March 2015 was done to estimate the prevalence of hepatitis B, C, and HIV among hemophiliac patients. Forty hemophiliac patients were recruited to this study, who were documented to have hemophilia A and B by factor assay. Patients' surveillance for hepatitis B, C and HIV were done by the electrochemiluminescence immunoassay "ECLIA" by using Cobas e 411-immunoassay analyzer.

Results: Forty hemophiliac patients with age ranged between 6 months and 22 years with mean age 9.9 ± 5.4 years were recruited to this study, 5 patients were mild hemophilia, 6 patients moderate hemophilia and 29 patients with severe hemophilia.

The prevalence of hepatitis C among the 40 hemophiliac patients was 5%, while for hepatitis B and HIV was 0%. Six patients (15%) showed immunity to hepatitis B due to successful vaccination.

Conclusions: This study showed that the prevalence of hepatitis B and HIV is zero, while for hepatitis C 5%. Hepatitis C infection was more in severe hemophilia A, advanced age and an increasing number of blood and blood products transfusion. Only 6 patients (15%) among hemophiliac patients were seropositive for hepatitis B vaccine immunity.

Keywords: *Hepatitis B, Hepatitis C, HIV, Hemophilia, Al-Anbar.*

Introduction

Hepatitis B, C, and HIV viruses are worldwide healthcare problems, especially in developing countries. It is estimated that approximately 33% and 3% of the global population has been infected with HBV and HCV, respectively [1, 2].

Hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections are the most important complications of transfusion [3].

A significant proportion of the blood supply is either not screened for hepatitis B or C virus or not screened properly. The probability of transmission of hepatitis B and C viruses through transfusion of unsafe blood can be as high as about 70% and 92%, respectively, depending

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on the volume transfused and the concentration of virus[4].

Sensitive screening tests and efficient virus inactivation methods both decrease the risk of infection due to transfusion substantially; however, it cannot be possible to prevent it entirely [5, 6]. In spite of the combination of the most sensitive tests, an estimated risk of transfusion-transmitted hepatitis still exists in 1 in 200000 – 500000 units transfused for HBV and 1in 2000000 units transfused for HCV and HIV [5,7].

People with hemophilia (factor VIII and IX deficiency) and others with other clotting factor deficiencies are particularly at risk of acquiring blood-borne hepatitis virus infections i.e. hepatitis B virus (HBV) and hepatitis C virus (HCV) through transfusion with unscreened blood and blood products [8,9].

Viral hepatitis places a heavy burden on the health care system because of the costs of treatment of liver failure and chronic liver disease. In many countries, viral hepatitis is the leading cause of liver transplants. Such end-stage treatments are expensive, easily reaching up to hundreds of thousands of dollars per person [10,11].

A large-scale study of HBV, HCV, and HDV seromarkers in hemophiliacs has confirmed that these diseases continue to be prevalent in this population. Ongoing longitudinal studies should provide additional information regarding incidence rates of HBV, HCV, and HDV, the clinical course of hepatitis in hemophiliacs, and the impact of currently used factor replacement products [12].

Aim of the Study

To estimate the prevalence of hepatitis B, C, and HIV among hemophiliac patients in the Al-Anbar governorate.

Patients and Method

A cross-sectional study was carried out between October 2014 and March 2015 in Al-Ramadi teaching hospital for maternity and children for documenting the prevalence of hepatitis B, C, and HIV viruses among hemophiliac patients who are registered in Al-Anbar hemophiliac center.

Inclusion Criteria: Hemophiliac patients diagnosed by factor VIII and IX assay.

Exclusion Criteria: Suspected hemophiliac patients not diagnosed with factors assay.

The history including name, age, residence, type and severity of hemophilia, age of diagnosis, duration of disease and number of blood and blood products transfused and history of hepatitis B vaccination. The severity of hemophilia was classified as: (Severe hemophilia having <1% activity of the specific clotting factor, moderate hemophilia have factor levels of 1-5% mild hemophilia >5%) [13,14].

After taken permission from the patient's parents or patient for examination and blood investigation, venous blood samples were aspirated from 40 hemophiliac patients during regular follow-up using a sterile plastic syringe and samples placed in tubes containing separating gel. The blood samples were centrifuged for 10 minutes to get a clear serum. The serum was placed in sterile covered storage tubes and stored at −20°C until the collection of all samples. And then viral serology for hepatitis B, C and HIV were done by the electrochemiluminescence immunoassay "ECLIA" by using Cobas e 411-immunoassay analyzer from ROCHE HITACHI Company, Germany.

Hepatitis B serology including HBS antigen, Anti-HBS antibody and anti-HBC antibody (Routine screening for HBV infection requires assay of ≥ 3 serologic markers (HBsAg, anti-HBc, anti-HBs). HBsAg indicates infected persons. Anti-HBc IgM indicates acute infection. The presence of only anti-HBs indicates immunized persons with hepatitis B vaccine. Anti-HBs and anti-HBc IgG antibodies are detected in persons with resolved infection. The presence of both HBsAg and anti-HBs indicates acute infection. Presence of HBsAg, anti-HBs, and anti- HBc IgG antibodies indicate chronic infection [15]. The detection of HCV infection was based on the detection of antibodies to HCV antigens. [16] Detection of HIV infection was based on the detection of antibodies to HIV and the detection of HIV antigens [17].

Descriptive analysis was done by using range, mean, standard deviation and percentage.

Results

Forty hemophiliac patients were recruited to this

study. The age of the patients ranged between 6 months and 22 years with a mean age (± SD) was 9.9 ± 5.4 years as shown in table 1.

All studied patients were males. Thirty patients (75%) were hemophilia A while 10 of them (25%) were hemophilia B. The geographical distribution of the patients is shown in table 2. Twenty patients (50%) were from the AL-Ramadi district, 9 patients (22.5%) were from the AL-Fallujah district, 8 patients (20%) from the AL-Garmah district, and 2 patients (5%) from Heet district and 1 patient **(**2.5**%)** from Haditha district.

The distribution of patients according to the types and severity of hemophilia was shown in table 3.

The distribution of the study sample according to the type of hemophilia and frequency of receiving blood &/or blood products as shown in table 4.

Serology for hepatitis B among the studied 40 hemophiliac patients were negative for both HBs antigen and anti-HBc antibodies (IgM, IgG) while anti-HBS antibody was positive in 6 patients (15%) reflecting immunity due to vaccination. The serology for HIV infection was negative.

Out of 40 hemophiliac patients, two patients were positive for HCV antibodies, giving a prevalence rate of 5%. The two infected patients were severe type hemophilia A, the age of the first one was 16 years old and the second was 22 years. Both of them received blood and blood products transfusion more than 40 times.

Table 1: distribution of hemophiliac patients according to age group.

Age group (years)	No. of patients (%)
<3	5 (12.5%)
3-8	8 (20%)
9-14	12 (30%)
15-22	15 (37.5%)
Total	40 (100%)

Table2: Geographical distribution of hemophiliac patients.

Residence	No. of patients (%)
AL-Ramadi district	20 (50%)
AL-Fallujah district	9 (22.5%)
AL-Garmah district	8 (20%)
Heet district	2 (5%)
Haditha district	1 (2.5%)

Table 3: distribution of hemophiliac patients according to types and severity of hemophilia.

Severity of hemophilia		No. of hemophilia A (%)		No. of hemophilia B (%)		No. of patients (%) Hemophilia A / B
Mild		3 (10%)		2 (20%)		5 (12.5%)
Moderate		4 (13.33%)		2 (20%)		6 (15%)
Severe		23 (76.67%)		6 (60%)		29 (72.5%)
Total		30 (100%)		10 (100%)		40 (100%)

Table 4: Distribution of the study sample according to the type of hemophilia and frequency of receiving blood &/or blood products.

No. of blood and blood Products transfusion/ Unit	No. of patients (%)	Type of hemophilia					
		A			B		
		Mild	Moderate	Severe	Mild	Moderate	Severe
<20	6 (15%)	3(7.5%)	1 (2.5%)	0 (0%)	2(5%)	0 (0%)	0 (0%)
20-40	14(35%)	0 (0%)	3 (7.5%)	7(17.5%)	0(0%)	2 (5%)	2(5%)
>40	20 (50%)	0 (0%)	0 (0%)	16(40%)	0(0%)	0(0%)	4 (10%)
Total	40(100%)	3(7.5%)	4(10%)	23(57.5%)	2(5%)	2(5%)	6 (15%)

Discussion

The prevalence of hepatitis B and C in Iraq according to WHO-supported study in 2006 was 1.6% (low) and 0.1% (very low) respectively [18]. , While HIV prevalence was less than 0.1%[19].

Haemophiliac patients are at increased risk of acquiring hepatitis and HIV viruses' infections due to repeated blood or blood product transfusion [8]. However, this risk dramatically decreased since 1992 due to a more sensitive screening test for blood donors and advanced procedures to inactivate viruses in clotting factor concentrate [9].

The present study showed that the prevalence of hepatitis B, C and HIV infections among hemophiliac patients was 0%, 5%, and 0% respectively. The prevalence of hepatitis C (5%) in this study is lower than that reported in Baghdad (9.9%) [20], Iran (22.6%) [21], and Egypt (39%) [22], while it is higher than that reported in Turkey (3%)[23]. in USA the prevalence of hepatitis virus was 17.2% in 1968 and dropped to nearly zero by 1990 [10].

The finding of 0% of hepatitis B virus among hemophiliac patients is similar to that found in Turkey [23], while other studies showed (0.52%) in Baghdad [20], (0.4%) in Iran [21], (0.43%) in India [24] and (18%) in Egypt [22]. The zero % of HBV may be due to the use of hepatitis B vaccine in the immunization schedules.

The prevalence of 0% of HIV infection among hemophiliac patients in this study is similar to that found in Turkey [23], and India [24], while in Iran was (1.1%) [25].

The two hepatitis C positive patients were severe type hemophilia A, aged more than 15 years and received more than 40 times transfusion of blood and blood products. This agrees with the results found in Egypt [22], Iran [25], Brazil [26] and USA [12]. Patients with severe hemophilia and advanced age are at increased risk of acquiring blood-borne infection because they usually receive a large number of blood and blood products as a result of frequent bleeding.

The two cases with hepatitis C virus infection need PCR assay, if it is negative this means the infection was eradicated, but if the PCR is positive, genotyping is mandatory, as it helps to predict the likelihood of response to therapy and duration of therapy. Liver biopsy is necessary to detect fibrosis and progression to cirrhosis. Liver biopsy is usually, but not always, performed before initiating therapy [27].

In this study, only 6 hemophiliac patients (15%) showed immunity to hepatitis B due to vaccination and the rest of patients were anti-HBS negative this may be

due to incomplete vaccination (missed one or two doses) or nonresponsive of patients to vaccination **(**Several factors have been associated with nonresponsiveness to hepatitis B vaccine. These include vaccine factors (e.g., dose, schedule, injection site), and host factors. Older age, male sex, obesity, smoking, and chronic illness). [28]

Conclusion

1. This study showed that the prevalence of hepatitis B and HIV is zero, while hepatitis C is 5%.

2. Hepatitis C infection more in severe hemophilia A, advanced age and the increasing number of blood and blood products transfusion.

3. Only 6 patients (15%) among hemophiliac patients were seropositive for hepatitis B vaccine immunity.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non.

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Quantitative Genetics Analysis of Autism According to Sex Hormones

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Abstract

The present paper applied the light on 46 rats models induced with autism to prove the relationship among sex hormones and both of aromatase and RORA. After six practical steps, the results explain a negative relation of testosterone opposites with positive one for estrogen for their relations on aromatase and RORA. As testosterone rise significantly 3.015±0.321 ng/mL, estrogen declined significantly too to record 0.033±0.0011 ng/mL. on the quantitative genetic levels we concluded that both sex hormones affecting the physiological mechanisms of aromatase reaction and RORA synthesis.

Keywords: *Autism, Sex hormones, Aromatase, RORA, Genetics.*

Introduction

Autism spectrum disorder (ASD) is a case of neurological sick characterized by abnormalities with social relations at all. According to the WHO, autism affects an estimated 1 in 50 children in the world today[(1)].

Autism animal models are significant as they enable scientists to research the fundamental neurobiology in a manner that is not feasible in humans[(2)]. Existing of an animal model scheme with similar behavioral drifts as humans is thus vital and crucial for understanding the brain physiology, helping social attention and motivation, and the way in which these tactics seize up in autism. The future researches should therefore rise the comprehending of the biological changes connected with autism condition as well as the progress of knowledge-based therapy and pharmaceuticals for whom competing with autism[(3)].

In rodents, RORA is necessary for growing of cerebellum through direct physiological governance of genes expressed by Purkinje cells. RORA also takes part in the active role of type 2 innate lymphoid cells (ILC2) development. RORα achieves these actions by binding specifically to a consensus core design in RGGTCA, RORE. This reaction is done through the connection of RORα's first zinc finger with the core design in the the P-box, and the connection of its C-terminal extension with the AT-rich region in the 5' region of RORE[(4)].

Aromatase enzyme in the brain is expressed in neurons. However, following penetrative brain injury of both mice and zebra finches, it has been shown to be alternatively expressed in astrocytes through both pro-inflammatory cytokines,interleukin-1β (IL-1β) and interleukin-6 (IL-6). It has been shown to eliminate apoptosis later. This is thought to be as a result to the neuroprotective actions of estrogens, including estradiol[(5)].

In this paper, we will review the role of sex differences on the genetic an physiological activity of autistic animal models to mimic exact mechanism correlated with such fluctuations in a term of direct genetic influences. These are consequences that start from the elevation of testosterone hormone within brain cells and result in subsequent events. First, we will highlight some sex differences at the physiological

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level including the values of sex hormones created by the gonads. Next, we will estimate the aromatase enzyme that direct genetic effects of RORA. Finally, we will analyses the relationships of all the four variables statistically to prove our theory.

Aim of this paper is indicate the sex hormones role in the autism through its effects on both aromatase and RORA.

Materials and Methods

1- A 46 models of Long–Evans rat induced for autism bought from All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, were they puts for action study.

2- Medical induction in labs were carried out by specialists and proved by modern techniques like plasmid transfection, PROMO 3, and Confocal immunofluorescence microscopy.

3- Radioimmunoassays technique was applied for hormones evaluation based on technique of[6].

4- HPLC technique used for estimation values of aromatase enzyme. Application methods inquired according to[6].

5- Tools those used in our experiment in addition to equipment were imported from PerkinElmer, Inc. Company, USA.

6- SPSS test applied as statistical analysis according to[7].

Results

Estrogen values in the RORA rats explained a decline 0.033±0.0011 ng/mL in comparison with control ones those recording 0.049±0.0044 ng/mL.

On the other hand, testosterone showed an elevated levels about 3.015±0.321 ng/mL in comparison with control group those registered 2.781±0.291 ng/mL.

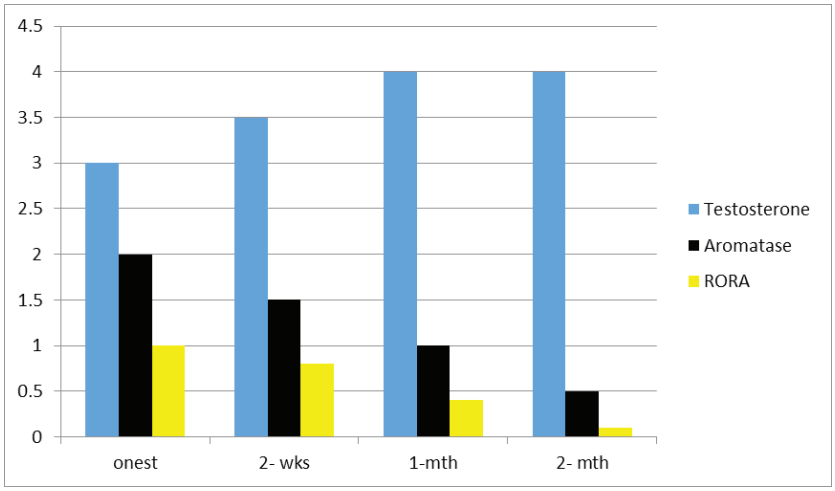
Aromatase (% of activity/ mg of protein) give a result of decreased value 2.7±0.19 when compared with the normal ones 3.1+-1.03.

Statistical histograms designed below after analyzing datum explained in truncated columns shows an instability in the values and numbers of investigated variables in a manner clarified the levels of high testosterone affecting negatively both of aromatase enzyme and RORA in brain tissues of autistic samples. It is a type of indirect proportional relation, see scheme-1 below.

Scheme-2 made clear that RORA's rats indicates a gradual direct proportional relationship among estrogen, aromatase and RORA expression rates.

The last histogram explains the whole relations among the four parameters judged in our research project for an illustrative manner, scheme-3.

All the above analyses applied based on an equation related to quantitative genetics Vx = !i (Xi – X)2 / (n – 1).

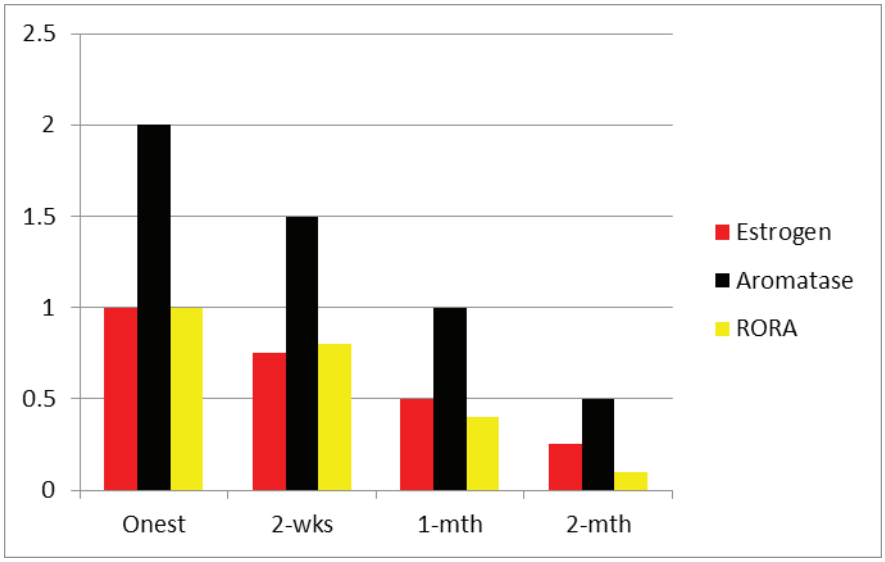


Scheme-1: Statistical histogram analysed variables related to testosterone effects on both aromatase and

RORA.

***Significant signs appeared in this scheme**

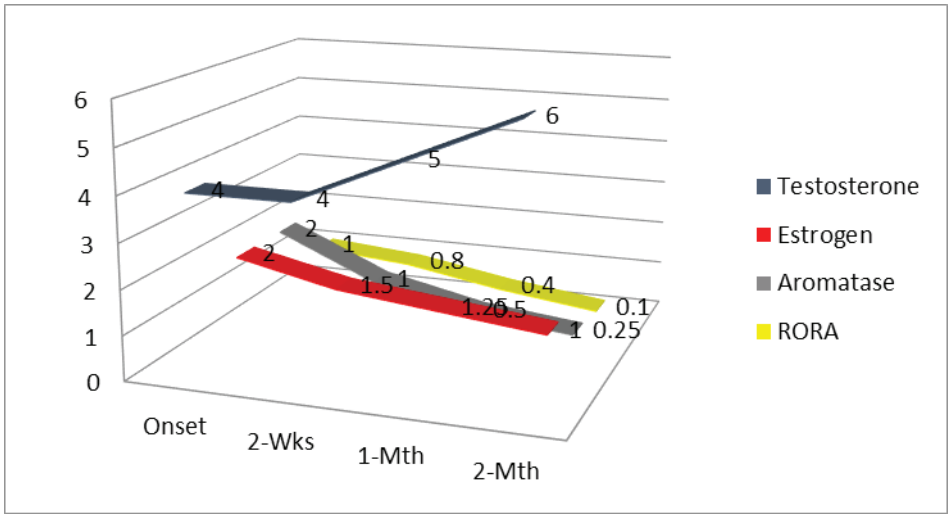
***Scheme designed based on** Vx = !i (Xi – X)2 / (n – 1) equation



Scheme-2: Statistical histogram analysed relations among estrogen, aromatase and RORA.

***Significant signs appeared in this scheme**

***Scheme designed based on** Vx = !i (Xi – X)2 / (n – 1) equation



Scheme-3: Statistical histogram explain relations among sex hormones, aromatase and RORA.

***Significant signs appeared in this scheme**

***Scheme designed based on** Vx = !i (Xi – X)2 / (n – 1) equation

Discussion:

Recent studies on the physiological basis of the male predominance of autism and other neurodevelopmental abnormalities includes revision of a higher symptomatic and genetic burden in sex-specific gene mutations and females that differentially discuss chance to males or prevention to females. Sex chromosome mechanism and sex hormone involvement put for outlook. Particularly , fetal estrogen and testosterone are assisted in many sides of neurodevelopment and may interfere with neuropeptide, neurotransmitter, or immune manners to

contribute to sex susceptibility. Finally, all possibilities of both sexes under research lenses and a multi-hit hypothesis are on the table[8].

Another possible description for the male prejudice in autism enclosed by the Neuroendocrine hypothesis, which supposed a role of neuropeptides in sex differences, especially vasopressin, oxytocin, and corticotropin-releasing hormone. Since these hormones are physiologically synthesized in the hypothalamus, then will secreted centrally and peripherally, and have sex-specific expression and response patterns[9].

The results of this paper recommend that the biological expression of RORA is oppositely adjusted by male and female sex hormones, with estradiol and DHT elevating the binding of ER and AR, respectively, to the RORA promoter region. Fascinatingly, estrogen raises RORA expression, whereas testosterone suppress expression of RORA[10]. The physiological mechanism and scenarios through which estrogen and testosterone run RORA in contrary manners are unknown and require further study. Although these hormonal roles were seen in the SH-SY5Y neuroblastoma cell line, this line may not replicate all replies of primary neurons. Thus, it would also be committed to examine the role of both estrogen and testosterone on RORA expression and reactions in primary neurons, using an accurate rodent model. Some papers found that the tested Purkinje cell loss looked during aging in male *staggerer* ($RORA^{+/sg}$) mice vs. female *staggerer* mice supports the concept of interaction between the sex hormones and RORA, with the male mice being more susceptible to RORA deficiency. By analogy, no gender-related dissimilarity in the increasing of Purkinje cell loss were looked in wild-type ($RORA^{+/+}$) mice[11].

We also evaluate one of the transcriptional members of RORA, aromatase, which is a vital enzyme in the synthesis of estrogen from testosterone. It is remarkable that both aromatase and RORA proteins are dropped in the frontal cortex of autistic subjects, and that the level of aromatase enzyme is correlated strongly with the value of RORA protein in the tissues of brain[12]. We consequently put forward that the lowering of RORA watched in autism is intensified by a negative feedback physiology entailing dropped aromatase value, which further makes build-up of its substrate, testosterone, and decreased of its product, estradiol. Estradiol and testosterone specifically show positive and negative feedback rule of RORA expression[13].

Conclusion

Thus, our conclusion is a deficit in RORA in autistic rats brain is anticipated to be further exaggerated by elevated ranks of testosterone due to elimination of aromatase, a transcriptional target of RORA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-Funding

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Association Between Carbamazepine Toxicity, Liver Bile Duct Injury, Granuloma and Inflammatory Cells Infiltration in Female Mice

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Abstract

The liver is an important organ in the body that can be affected by many drugs and toxins. The hepatotoxins can cause oxidant stress that lead to activation of inflammatory cells and cause liver damage. Drug induced bile duct injuries are related to drug toxicity, multiple drugs have been known to cause the development of liver granulomas. Carbamazepine (CBZ) among other antiepileptic drugs is believed to cause hepatic injury. In this study we investigated the effect of (CBZ) 20mg/kg/day on female mice liver after 14 and 30 days of treatment. The histological findings showed that (CBZ) can cause histological alterations in the liver components such as bile duct proliferation, biliary hypertrophy, ductopenia, inflammatory cells infiltration and granulomas.

***Key words**: Carbamazepine, liver injury, bile duct injury, infiltration, granuloma.*

Introduction

The liver is an important organ in the body that can be affected by many drugs and toxins, some drugs can cause death or even liver transplant [(1)]. The liver has different cells that help the organ maintain immune homeostasis, drug induced liver injury can cause inflammatory cells infiltration, those hepatotoxins can cause oxidative stress that lead to activation of these cells [(2)]. The responded of these cells can cause liver damage, innate immune cells take a part in liver inflammation and are the first line of defense. As well as they play a role in tissue repair and treat of the inflammation [(3)] infiltration of these cells can cause hepatocytes damage especially lymphocytes that can be seen in chronic liver diseases [(4)].

Drug induced bile duct injuries are represented in many pathological forms in the portal areas of the liver. The destruction of bile ducts epithelium after drug treatment is the main character to be seen in response to drug toxicity and a very common side effect of some therapies [(5,6)].

Multiple drugs have been known to development the liver granulomas [(7)]. Granulomas are the aggregation of multiple immune cells such as lymphocyte and macrophages, due to endogenous or exogenous stimuli [(8,9)], which can be seen in chronic inflammation [(10)]. There are different type's granulomas such as foreign body, necrotizing, non-necrotizing, diffuse and suppurative granulomas [(11)], they rarely cause damage to liver structure [(12)].

Antiepileptic drugs are known to cause acute and chronic toxicity, beside adverse effects like neurotoxicity [(13,14)] which are common with these treatments.

Carbamazepine (CBZ) among other antiepileptic drugs is connected to hepatic granuloma, this drug works by blocking NA^+ channels in the brain [(15)], it metabolizes in the liver by Cytochrome P450 [(16)], it`s metabolites work as neoantigenes and can lead to immune reactions [(17)]. Other organs suffer from CBZ toxicity such as kidneys [(18)] and the brain. Therefore,

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the study aims to investigate the negative pathological effects of carbamazepine treatment on liver components of mice.

Materials and Methods

Chemicals

Carbamazepine (CBZ) oral suspension used in this study was purchased from Novartis pharma AG basal Switzerland.

Animal of study

A total of40 adult female mice were used in this experiment, average weight 22-29 gm, they were brought to the animal house in Department of Biology / College of Education for Pure Science- Ibn- Al-Haitham and all under the same conditions, they were separated in to 4 groups with 10 mice each. The first group was administered with tap water and named as a control group. The second group was orally administered (CBZ) 20 mg/kg/day for 14 days. The third was also a control group administered with tap water and the fourth group was administered (CBZ) 20 mg/kg/day orally for 30 days. The first two groups were sacrificed after 14 days of treatment and the second two groups were sacrificed after 30 days of treatment.

Histological sections preparation

The livers of scarified mice were collected and immersed in 10% formalin and tissue samples were prepared for histological study by using paraffin method then sections were made and stained with conventional histological stains hematoxylin and eosin [19].

Control and treated sections of the liver were examined under the light microscope for bile duct injury, infiltration and granulomas at 10x, 40x and 100x magnifications power.

Results

In the present investigation, the liver of the control group showed normal bile duct structure with normal liver parenchyma and cells distribution (fig.1).

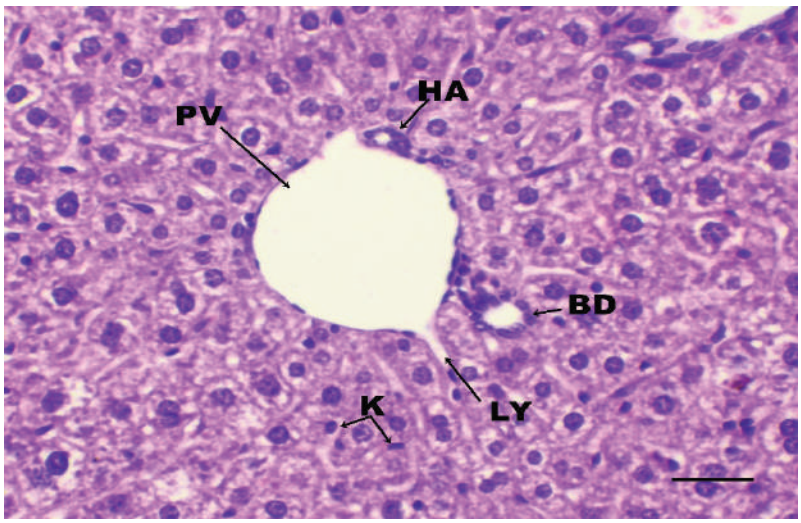


Figure (1): Cross section through female mice liver of the control group showing normal bile duct (BD), portal vein (PV), lymphatic vessels (LY), hepatic artery (HA) and Kupffer cells (K), H&E ,40x, scale bar 8.81µm.

The toxic effects of (CBZ) on the liver of female mice after 14 and 30 days of treatment were evaluated, it was found that drug toxicity induced bile duct injury in the 14 days' group, biliary proliferation and biliary hypertrophy was seen (fig.2), as for the 30 days' group, ductopenia was also noticed beside the injuries that were seen in the 14 days' group (fig .3).

Infiltration of inflammatory cells at the portal areas was noticed in the treated groups, consisted of lymphocytes, neutrophils, plasma cells and eosinophils (figs. 4, 2).

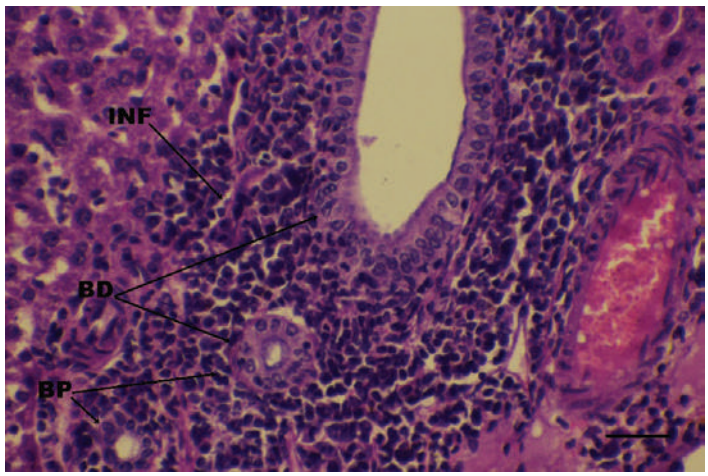


Figure (2): Cross section through female mice liver treated with CBZ 20mg/kg/day for 14 days demonstrating bile duct (BD) hypertrophy and proliferation (BP), H&E ,40x, scale bar 8.81µm.

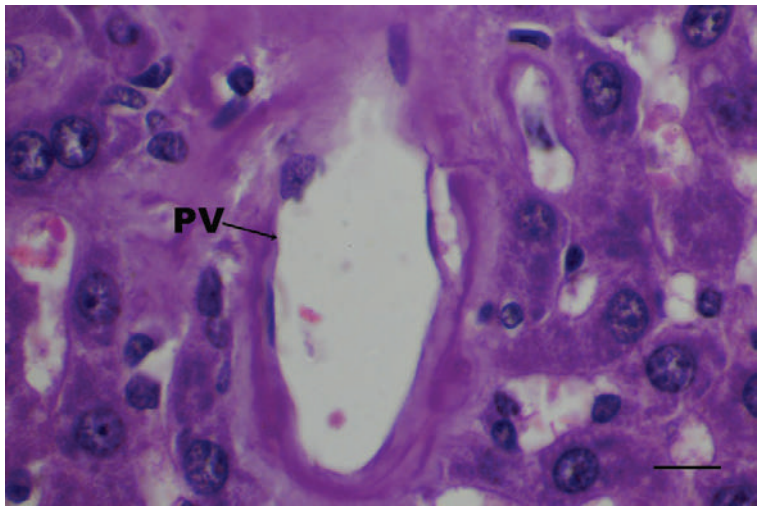


Figure (3): Cross section through female mice liver treated with CBZ20mg/kg/day for 30 days demonstrating ductopenia, H&E,100x, scale bar 22.03µm.

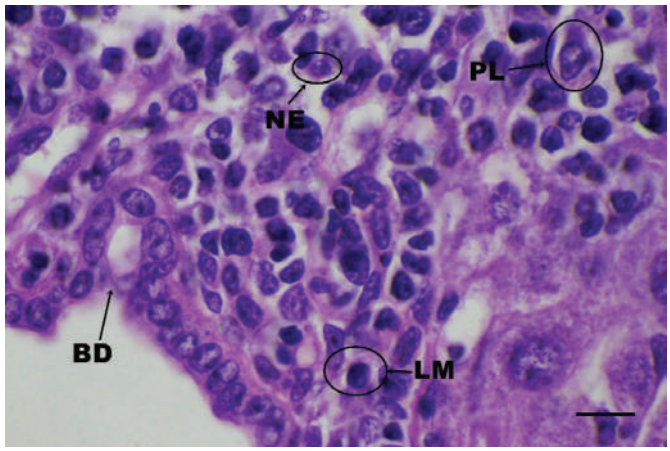


Figure (4): Cross section through female mice liver treated with CBZ 20mg/kg/day for 30 days demonstrating bile duct (BD) hypertrophy, infiltration of neutrophils (NE), lymphocytes(LM) and plasma cells(PL), H&E, 100x, scale bar 22.03µm.

Loose granuloma and micro granulomas were detected in the 14 days' group (fig.5), while well-formed granulomas were detected in the 30 days' group (fig.6), the granulomas were consisted of lymphocytes, macrophages and neutrophils(fig.6).

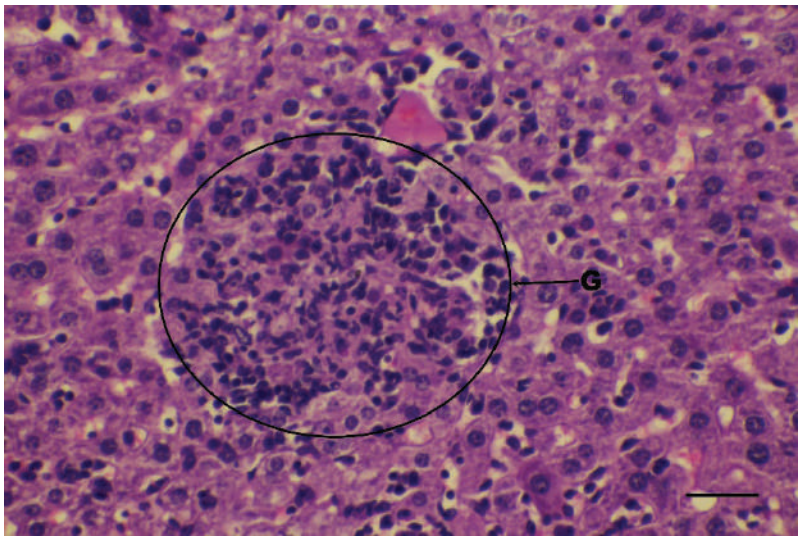


Figure (5): Cross section through female mice liver treated with CBZ 20mg/kg/day for 14 days demonstrating granuloma (G), H&E ,40x, scale bar 8.81µm.

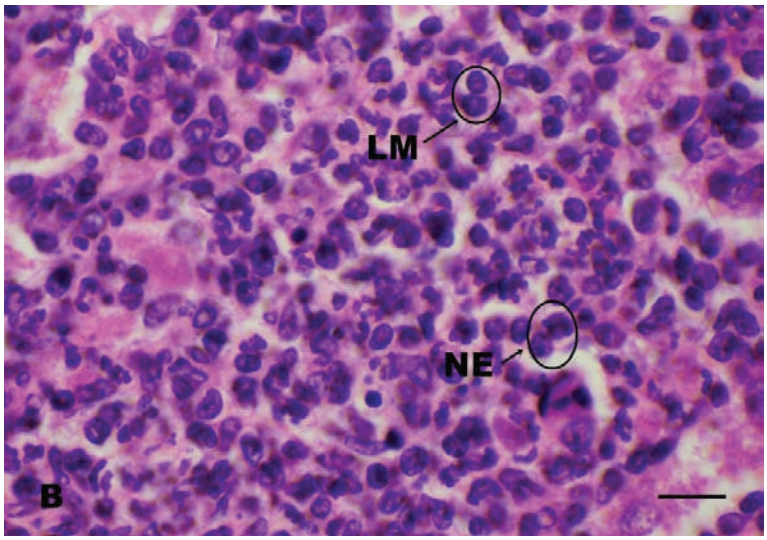


Figure (6): Cross section through female mice liver treated with CBZ 20mg/kg/day for 30 days demonstrating well-formed granulomas consisting of lymphocytes (LM) and neutrophils (NE), H&E ,100x, scale bar 22.03µm.

Discussion

Histological findings in the present study showed groups treated with (CBZ) 20mg/kg/day for 14 and 30 days showed histological alterations in liver compositions. Bile duct proliferation was noticed in both groups, it is thought that toxic effect of the drug can cause bile duct proliferation [20] or this toxicity caused a ductular reaction in this form [21], biliary hypertrophy in the 14 and 30days' group was evident , this indicates that toxic effect of the drug lead to bile duct injury that made bile duct cells go through hypertrophy when hepatic injury occurred [22,23],these cells "the cholangiocytes", are very sensitive to any injury and become activated , also secreting and assisting with the bile duct work [24].

In the 30 days' group, ductopenia was shown in the portal areas as well as fibrosis was induced by the hepatic inflammation and lead to liver injury may have caused it

[25], in addition, the cholangiocytes will start apoptosis at some point and that will lead to this effect [26].

Inflammatory cells infiltration was noticed in both treated groups near portal areas and sometimes seen with or without necrosis it was demonstrated that hypersensitivity reaction to the drug may have caused an inflammation that lead to the infiltration [27] .

Multiple Granulomas of various sizes were seen in liver tissue in both treated groups. The formation of granulomas is linked to the inflammation in the portal areas, and as a result of immune reaction to the toxicity of the drug [28]. Also believed formation of granulomas is due to the failure of the cellular immune response to eliminate the foreign substance [29]. Granulomas can be found anywhere in the tissue and it is connected to hepatitis [30].

Conclusion

Antiepileptic drug Carbamazepine (CBZ) can cause hepatic injuries, the histological findings showed that (CBZ) causes histological alterations in liver components after short and long period of administration, such injuries include bile duct proliferation, biliary hypertrophy, ductopenia, inflammatory cells infiltration and granulomas.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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A Program for Developing Locally Food Supervision to Support Sanitary Monitoring Work

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Abstract

Food supervision in Iraq is the responsibility of the Ministry of Health and Environment, which conducts food supervision to all food establishments worked in Iraq by the department of sanitary monitoring in Baghdad city as well as all directorates of health in Iraqi provinces. As a result of increasing the number of food businesses national wide in last years, increasing the number of consumers, Iraq's openness to the international market, and using the policy of random import. Due to the mentioned reasons, many problems related to consumer's health has appeared, this matter pushed the Iraqi Government to establish committees, organized seminars, and workshops related to this subject to resolve problems effected in consumer's health. This problem requires more effort from researchers and specialists in food supervision and sanitary monitoring in Iraq to protect consumer's health especially from foodborne diseases. The proposed program is the start of the way towards new food safety policy in Iraq and it will be considered as the essential base of reorganization food control authorities in Iraq and updating national food regulations and legislations.

Keywords: *Food supervision, program, sanitary monitoring.*

Introduction

Sanitary monitoring in Iraq integrated with the Iraqi Ministry of Health and Environment is the responsible authority of food control at a national level. One of the most important duties of this authority is keeping food safety and protect consumer's health to all people living in Iraq, it divided into departments and directorates in the capital of Baghdad and other seventeen Iraqi provinces [(1)]. The legislative base that organized the work of food control in Iraq is the set of national regulations and legislation in force, e.g. regulation public health no. 89/1989 and its amendments, regulation of the central organization of standardization and quality control, food system no. 29/1982 and the instructions that organized the work of various kinds of food establishments in the country [(2)].

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Food Safety Program (FSP) is a written plan detailing the actions taken by a food establishment to ensure that the food it sells or manufactures is safe to eat by people and doesn't lead harm to consumer's health. FSP identifies and controls food safety risks, and it is an important tool to help food establishments in its processes to serve food safely [(3)]. An appropriate and well-implemented FSP can help food establishment to ensure the safety of food that manufactures and sells, better manage of its operations through improving of data registered in the documents, reducing errors that can be happened during food manufacturing and finally controlling of costs. Not all food establishments require FSP. The Food Act 2006 defines who must comply with the requirements [(4)]. Under the Act, all of the following **high-risk food establishments** must have FSP in place:

· Businesses that serve, process or deliver potentially hazardous food for the consumption to vulnerable people (e.g. hospitals, aged care facilities and child care services)

· Businesses that harvest, process or distribute fishes and raw oysters.

· Businesses that produce fresh, manufactured and ripe meats.

· Catering businesses that serve food to the general public.

Potentially hazardous foods, are all foods that must be kept at certain temperatures to minimize the growth of bacteria and other microorganisms that may be present in food or to prevent toxins forming [5]. *Potentially hazardous foods, include the following : raw and ready-to-eat meat and fish and any other foods made of these items such as meat pies and fish fingers., milk and food containing milk such as cream, custard, dairy-based desserts., cooked rice and pasta., ready-to-eat foods such as salads, cut fruit and vegetables and ready-to-eat meals such as lasagna, curry, sushi and salad sandwiches.*

Even if food establishment doesn't require to implement a FSP, it can be a benefit to implement it, particularly in cases where:

· Seeking to distribute food products through supermarkets, and therefore food establishment needs to implement a FSP to verify its quality.

· The interest to present a premium service to the consumer and high standards of food quality.

· The interest to open food products manufactured locally to the international market [6].

Food Safety Program has the following criteria for a food establishment, and for that FSP should be:

a. Identify all food safety hazards that potentially could be happened to food handling operations in the food establishment.

b. Identify where, in a food handling operation in food establishment, each hazard identified under paragraph (a) can be controlled and the procedures of control.

c. Provide monitoring to the procedures of control identified under paragraph (b).

d. Provide appropriate corrective actions, to be taken when a hazard identified under paragraph (a) is not under control.

e. Provide a regular review for FSP to ensure that it is appropriate for the food establishment.

f. Ensure that appropriate record-keeping practices are observed, including maintaining records about actions taken to ensure that food establishment is carried on by the program [7].

There are several FSP that can be used to assist food businesses for developing, therefore, food establishment is responsible to select the program appropriate to its business. There are many tools to assist in developing suitable food safety program to different kinds of food establishments; all activities, hazards, controls, corrective actions, and monitoring must be in site-specific to the selective food establishment.

Materials & Methods

For supporting the work of food supervision in Iraq., visits, seminars, workshops, and meetings with the specialists in the department of sanitary monitoring located in the city of Baghdad-Alrusafa were performed during January to August 2019, to evaluate the actual food supervision in the city and to establish a proposed food safety program covered the changes happened in Iraqi society in the last years. The essential objective for establishing the proposed FSP is to ensure that safe food served to the Iraqi consumer, to protect his health from foodborne diseases. Also a review of Iraqi food regulations and legislation was performed to harmonize between the proposed food safety program and the Iraqi food regulations and legislation in force. The proposed FSP taken into account all steps that happened during food processing in food establishment.

Results & Discussion

1. Food business

Starting a food business

Everyone serving or selling food to the general public has a moral responsibility to ensure that the food produced in his establishment is safe to eat and doesn't harm consumer's health. Even if the food establishment doesn't need to be registered with a municipal control authority, it should make sure that the food sells is safe

for human consumption. Food establishment also should be responsible that all food processes e.g. manufacturing, transporting, store, and also selling is safe and compliant with the requirements concerning safety and labeling [8].

Contacting with local authority

Whether food establishment needs to be registered or not depending on its size and how its activities are consistent. The food establishment should contact local food authorities to find out whether its business needs to be registered or not. Some food establishments selling in occasional, such as temporary restaurants, this kind of food establishment might not need to be registered.

Registration of a food establishment

If someone is planning to open a food establishment for example café, restaurant, food store, or delivering pre-packed food, he should register his food establishment with the local authority at least 14 days before opening. Registration of his food establishment can't be refused. If his food establishment is already opened and it isn't registered, he needs to do that as soon as possible [9].

Approval in advance

Food establishments that manufacture, prepare, or handle with meat, fish, eggs, or dairy products for supply to another food establishment, might require approval by the local food control or the national food agency. Local food control or the national food agency charges a fee for registration and inspection. The inspection fee depends on how often food establishment yearly needs to inspect by one of the mentioned food authorities [10].

Applying for the approval should be done in advance of opening the food establishment. The owner of the food establishment can't start his job before his facilities are checked well by the local food control or the national food agency. If food establishment isn't registered or approved, maybe that lead to sanction.

If the local food authority discovers that a food establishment is neither registered nor approved, the food authority should recommend food establishment to register or apply for approval as soon as possible, as well as discontinue its food business immediately, until the registration or approval procedure has been finalized. If the food establishment continues its business without proper registration or approval, the food authority should prohibit food establishment from operating.

2. Controlling food business

When the inspection is performed in a food establishment, one or more described control methods can be used to verify whether food establishment complies with the requirements given in food legislation. Which control method used depends on the size and the nature of food establishment, i.e. risk and previous control experience. The risk in a food establishment can also be determined for how many parts of the food establishment's self-control system are documented [11,12].

The overall assessment of the food establishment is performed on the basis of checking 1 and 2. In relation to these checking, food authority assessed whether the requirements of food legislation are met. All three checking are used in auditing. During the inspection, checking 2 is answered primarily, but also parts of checking 3.

Another important part of control at the place of the food establishment is checking if the food authority's information about the food establishment is correct, which included:

· Is the food establishment registered or approved?

· Is food authority's information about the registered food establishment is correct?

· Are the details of the decision of the approved food establishment is correct?

· Is the work of the registered food establishment has been changed, so that the food establishment is now may be subject to approval under other requirements?

Support in assessing this matter should be found in the guidance of the national food agency (approval and registration of food establishments). The food authority should also verify that the data related to the food establishment classification risk is correct, e.g. how much product is produced per year or how many meals are served daily.

3.Control Areas

In order to facilitate food control, subsequent reporting, follow-up, and communication with food establishments should be performed by the local food authority. National food agency should be structured the following control areas to assist the work of food inspection [13]. Control areas and their details illustrated in Table (1).

Table 1: Control areas that assist inspection authority in controlling of food establishment .

Control areas	Control details
1. Infrastructure, building, equipment, and vehicles.	Planning, design, construction, placement, spaces, floor plan, maintenance.
2. Raw materials and packaging materials.	Raw materials, ingredients, process chemicals, materials in contact with food, reception control, information from the food chain.
3. Safe handling, storage, and transport.	Storage, cross-contamination, separation of raw materials, packaging materials, process chemicals, end products.
4. Waste management and storage.	Waste, return goods, animal bi-products.
5. Pest control.	pests, insects, domestic animals.
6. Cleaning and disinfection.	Cleaning, cleaning chemicals, cleaning results.
7. Water quality.	Water used in the plant, not drinking water production.
8. Temperature.	Refrigeration storage, freezer storage, thawing, heating.
9. Personal hygiene.	Work clothes, hand wash, health certificate.
10. Education.	Competence, knowledge in food hygiene and HACCP, education's plan.
11. HACCP-based procedures.	Hazard analysis, critical control points, monitoring, corrective actions, validation, and verification.
12. Information.	Marking, presentation, information to the public.
13. Traceability.	From whom and to whom? Recall of bad product.
14. Microbiological criteria.	National regulation and specifications of food safety criteria, Process hygiene criteria.
15. Other requirements.	All other requirements given in the national food regulation (Control areas).

Conclusion

Effective food control programs in different countries are essential to protect consumer's health and guarantee safe food. These programs are also crucial to enable countries to ensure the safety and quality of food entering international trade and to ensure that imported food complies with the national food legislation requirements. The global food product trade environment currently imposes major obligations on both importing and exporting countries to strengthen their food control system and to implement food control strategies based on risk assessment.

Consumers have become unprecedented in the way food is prepared, processed, and marketed, and their demands are growing for governments to take greater responsibility for protecting consumers and ensuring food safety.

In many countries, food control is weak, because of the large number of legislations existed, and there are a multiplicity of food control authorities, and this is negatively reflected in control and monitoring food. Launching this program will advise national food authority on strategies to strengthen food control systems in a manner that protects public health, prevents fraud, avoids food contamination, and help to facilitate international trade.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Synthesis and Biological Activities of New 1,2,3,4-Tetrahydroquinoline Derivatives Using Imino Diels-Alder Reaction

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Abstract

This research contains the preparation of some novel derivatives of tetrahydro quinolone. First step, include prepared new Schiff's bases compounds (I_1-I_5) were prepared by the direct condensation between diamine compounds and aromatic aldehydes substituted by different groups. Tetrahydroquinoline derivatives compounds (I_6-I_{10}) were prepared through imino Diels-Alder reaction using the derivatives of Schiff base with cinnamic acid, and Boron tri fluoride ethyl etherate ($BF_3.Et_2O$) as a catalyst. The prepared compounds were identified by infrared spectra, ^{1}H-NMR, and ^{13}C-NMR. Antibacterial activity of the prepared compounds was measured by using three pathogenic microorganisms including *Staphyloccus haemolyticus, Klebsiella pneumonia,*and *Candida albicans.* Also, a comparative study was achieved to study the biological activity of these prepared compounds using Agar Diffusion method.

Keywords: *1,2,3,4-tetrahydroquinoline, cinnamic acid, Diels-Alder reaction, Antibacterial activity, Boron tri fluoride ethyl etherate.*

Introduction

The 1,2,3,4-tetrahydroquinolines are relevant heterocycles that possess diverse biological activities and multiple applications. They are widely used as antimalarial [(1)], antibacterial[(2)], antiviral[(3)] and antitumor agents[(4)].Also, they are as inhibitors of thromboxane A2 synthase [(5)] and in other pharmaceutical applications[(6)]. For these reasons, the preparation of new tetrahydroquinolines remains of considerable interest. An active approach for the synthesis of tetrahydroquinolines is the acid-catalyzed Povarov reaction, which is classified as an imino Diels-Alder cycloaddition [(7)]. The primary goal of the current study is to seek novel molecules like the current compounds by synthesizing several 1,2,3,4 tetrahydroquinolines derivatives. Moreover, this methodology, tetrahydroquinolines of sulfonamides prepared through Imines- Diels- Alder reaction by using the derivatives of Schiff bases with cinnamic acid and Boron tri fluoride ethyl ether ($BF_3.Et_2O$) as catalyst. All the prepared compounds were characterized based on their melting point, IR, ^{13}C-NMR, and ^{1}H-NMR. The anti-microbial efficacy of the synthesized compounds has been estimated using Agar Diffusion method.

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Experimental Section

The solvents, chemical materials and reagents utilized during this research were available bought from Romil, Sigma Aldrich and BDH are utilized as be given. Recorded infrared spectra were by using Shimadzu Infrared Spectrophotometer FT-IR model 8400s series spectrophotometer (KBr Pellet) in the region 400-4000 cm^{-1}. ^{1}H and ^{13}C-NMR spectral analysis were collected on NMR spectrometer 400 MHz, Bruker Biospin GmbH 400 MHz using DMSO-d_6 as the NMR solvent. Chemical shifts (δ) are expressed in ppm. Melting degrees were recorded on Sturat Scientific instrument SMPLU-K Model are uncorrected for the prepared compounds.

General procedure for the synthesis of Schiff bases (I_1-I_5):

Schiff base derivatives (I_1-I_5) under research were designed according to the method equimolar mixtures (0.002 mole) of 4,4'-thiodianiline and (0.002 mole) of substituted benzaldehyde were dissolved in 25 ml absolute ethanol. This mixture was refluxed for 3 hrs then cooled. The precipitate was obtained and then recrystallized from ethanol. The end reaction of the synthesized compounds was monitored by TLC using silica gel as stationary phase and ethanol cyclohexane (8:2) mobile phase (Scheme 1) and Table 1.

General procedure for the synthesis of Tetrahydroquinoline derivatives (I_6-I_{10}):

A mixture solution of cinnamic acid (0.0006 mole) in toluene in presence of 3ml of $BF_3.Et_2O$ was gradually added to the mixture, as a catalyst, was placed round bottom flask with condenser and stirred with heat for 15 minutes to completely dissolve the reactants, gradually add the solution of Schiff base [I_1-I_5] (0.0006 mole) dissolved in dry (30 mL) toluene to the mixture for another 15 minutes. Then, this mixture was refluxed for 5 hrs and then cooled. The end of the reaction was checked by TLC (EtOAc:Toluene, 2:3). The solvent was evaporated under reduced pressure for one-third of solvent and separated precipitation was filtered and re-crystallized from ethanol (Scheme 1) and Table 1.

Antibacterial activity of I_6-I_{10}

Antibacterial activity of these compounds was determined by the Ager Diffusion method against three pathogenic microorganisms involving two bacteria *Staphyloccus haemolyticus*, and *Klebsiella pneumonia*, and a yeast *Candida albicans*. All the synthesized compounds I6-I10 were used at the concentration of 25 mg/ml, on an agar seeded by bacteria. All the achieved plates were incubated in the incubator at the appropriate temperature at 37 °C for 24 hrs. After that, the diameter of the zone of inhibition was calculated by the ruler in millimeters (mm)[8].

Scheme 1: Step by step method for the preparation Tetrahydroquinoline derivatives

Table 1: Some physical properties of prepared compounds (I_1-I_{10})

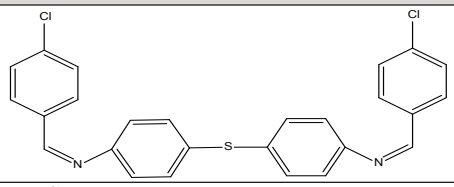
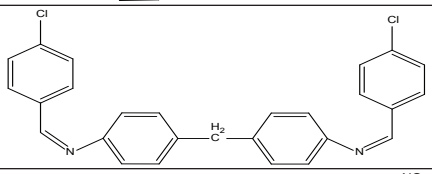
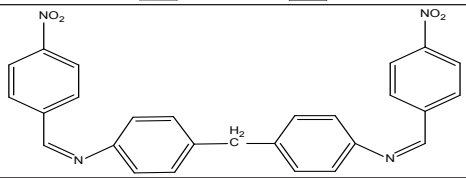
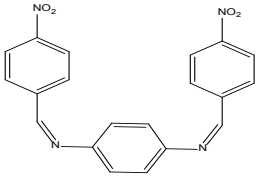
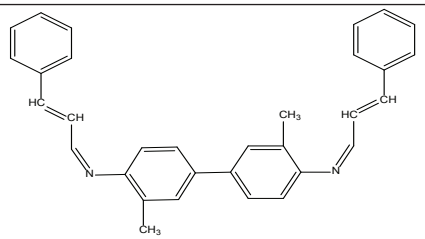
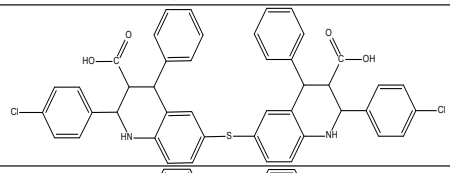
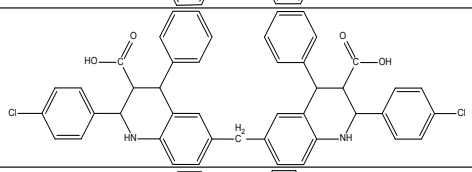
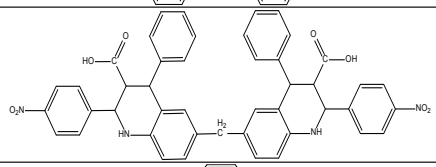
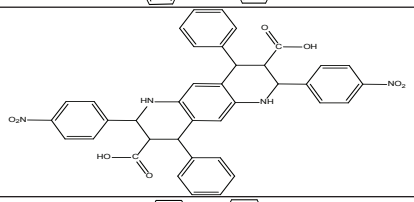
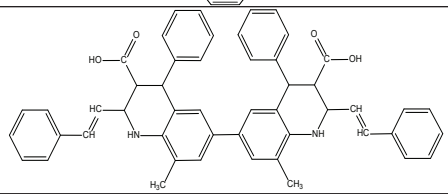
Comp. No.	Structures	Color	m.p. °C	Yield%	RF
I1		White	220-222	70	0.73
I2		White	170-172	60	0.97
I3		Yellow	230-232	55	0.23
I4		Brown	235-237	70	0.85

Table 1: Some physical properties of prepared compounds (I_1-I_{10})

I5		Green	200-202	68	0.89
I6		Orange	180-182	50	0.65
I7		Yellow	160-162	75	0.85
I8		Orange	90	73	0.86
I9		Brown	230-232	75	0.67
I10		Red	320-322	71	0.58

Results and Discussion

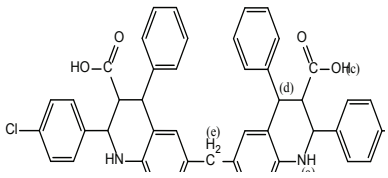
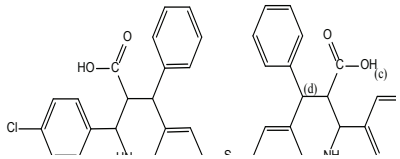
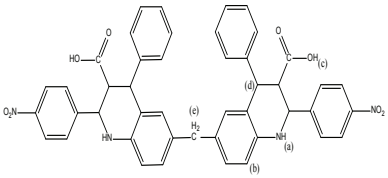
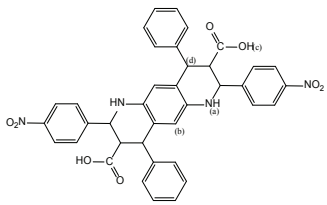
Scheme 1 appears the synthetic path for the new Tetrahydroquinoline derivatives. Stage 1and 2 are as notified in the step by step made method and includes condensation reaction of the substituted aromatic substituted aldehydes with the amines to result azomethine compounds I_1-I_5 follow by next reaction with solute cinnamic acid during toluene in presence of $BF_3.Et_2O$ to the corresponding wanted compounds I_6-I_{10}. The reaction methods in first and second steps were achieved utilizing FT- infrared spectroscopy. Manifestation of the band in the field 1597-1625 cm^{-1} in the infrared spectrum of the Schiff bases I_1-I_5 is suitable to C=N indicates condensation. The FT-IR spectra of I_1-I_5 exhibited the presence of the stretching absorption bands of (C=C) aromatic at (1586-1510) cm^{-1}, (C-H) aromatic at (3158-3009) cm^{-1}, (C-H) alphatic at (2922-2877) cm^{-1}, (C-Cl) at (721-717) cm^{-1} and (N=O) at (1369-1338) cm^{-1}.

The confirmed structures of the prepared compounds I_6-I_{10} were determined depending on the bases of their spectroscopy datum (FT- infrared, ^{1}H-NMR, & ^{13}C-NMR). The prepared Tetrahydroquinoline derivatives appeared suitable special signals needful to prove structures. FT- infrared spectrum of I_6-I_{10} exhibited the presence of the stretching absorption bands

of N-H group at (3500-3201) cm^{-1}, C=O group at (1708-1627) cm^{-1}, C-N group at (1083-1064) cm^{-1}, C-H alphatic at (2900-2835) cm^{-1},C-H aromatic at (3100-3008) cm^{-1}, C=C aromatic at (1593-1489) cm^{-1}, and O-H group at (3298-3116) cm^{-1}.

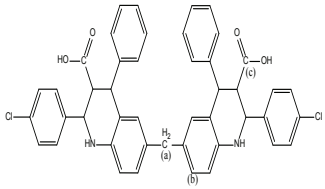
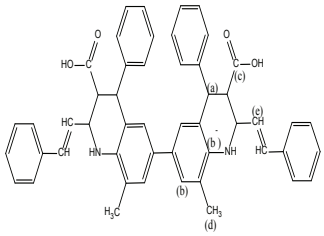
^{1}H-NMR spectrum for the prepared tetrahydroquinoline compounds presented appropriate for the aromatic proton with the proton shifts proper of the (H-N) and other groups are needful to emphasize the structure for tetrahydroquinoline membered system, also included in the table **2.**

Table 2: The ^{1}H-NMR Spectra of the compound $I_6 - I_9$

Comp. No.	Structure of prepared tetrahydroquinoline derivatives	(a)	(b)	(c)	(d)	(e)
I6		Singlet in 8.7	Multiplet in7.2	Singlet in 10.0	Singlet in 4.02	Singlet in 2.5
I7		Singlet in 8.6	Multiplet in7.3	Singlet in 10	Singlet in 2.4	--
I8		Singlet in 8.8	Multiplet in7.4	Singlet in 10.2	Singlet in 4.0	Singlet in 4.5
I9		Doublet in 8.4	Multiplet in 7.8	Doublet in 8.57	Singlet in 2.5	--

^{13}C-NMR for the prepared tetrahydroquinoline compounds proves the existence for chemical shifts of carbon identical the Major groups as included in the spectral data set as following in table 3.

Table 3: The ^{13}C-NMR Spectra of the compound I_6 and I_{10}

Comp. No.	Structure of prepared tetrahydroquinoline derivatives	(a)	(b)	(c)	(d)	(e)	(d)	
I6		41	128.06	192.6	--	--	--	
I10		40	129.5	153.6	178.3	18.2	131.7	

The biological activity

Antibacterial activity of the synthesized tetrahydroquinoline compounds were calculated using Ager Diffusion method against three pathogenic microorganisms involving *Staphyloccus haemolyticus, Klebsiella pneumonia,* and *Candida albicans.* All the synthesized compounds I_6-I_{10} were used at the concentration of 25 mg/ml. Table 4 showed the zones of inhibition against the pathogens. The best inhibition zone was 19.5 mm by the compound I_{10} against *K. pneumoniae*, followed 18 mm by the compound I_9 toward the same bacteria. While the compound I_6 recorded a zone of inhibition reached 7 mm against *K. pneumoniae and C. albicans*. Finally, the lowest inhibition zone was 10 mm by the compound I_{10} against *S. haemolyticus* and *C. albicans.*

Table 4: The biological activity of compounds I_6-I_{10} (Zone of inhibition, mm)

Compound Number	S. haemolyticus	K. pneumoniae	C. albicans
I6	15	17	17
I7	12.5	16	14
I8	15.5	16.5	16
I9	16	18	14
I10	10	19.5	10

Conclusion

We have successfully synthesized and characterized Schiff base compounds (I_1-I_5), and 1,2,3,4-Tetrahydroquinoline derivative(I_6-I_{10}) , The prepared compounds were identified by infrared spectra, H^1-NMR, and C^{13}-NMR, Antibacterial activity of the synthesized compounds was calculated using Ager Diffusion method against three pathogenic microorganisms involving *Staphyloccus haemolyticus, Klebsiella pneumonia,* and *Candida albicans*.where derivatives of tetrahydroquinoline showed varying results on the pathogenig microorganisms used ,Due to the presence of effective groups , The best inhibition zone was 19.5 mm by the compound I_{10} against *K. pneumoniae,* the lowest inhibition zone was 10 mm by the compound I_{10} against *S. haemolyticus* and *C. albicans*.the compound that gave the highest inhibition zone was due to the presence of effective functional groups and the large ring size .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Phenotype Detection of Genetic Enzymes B- Lactamase Isolation of Patients with Urinary Tract Infections Bacteria *Escherichia Coli*

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Abstract

Background*:* the main cause of multidrug resistant bacteria is the production of β-lactamase enzymes. The objective of this study was to determine the prevalence of genotype of *Escherichia coli* spp. isolated from nosocomial urinary tract infections (UTI). ***Methods*:** In a cross-sectional study from May 2019 to January 2020, mid-stream urine specimens were collected from 210 patients presented with acute urinary tract infections whom attending Al- Shahid Ghazi and Al-Hariri Hospital in Medical City/ Baghdad. Samples were cultured for E. coli isolation. Antibiotic sensitivity test and the prevalence of resistance enzyme producing gen were investigated. ***Results***: A total of 210 E. coli were isolated, multidrug resistance strain of E coli (92.85%) was reported by this study. PCR technique indicated that 88.57% of ESBL-producing isolates possessing *bla*TEM, 30% *bla*CTX-M genes and 20.48% *bla*SHV genes between isolates and E. coli still the less resistant to carbapenem due to low expression of KPC gen. ***Conclusion***: ESBLs and KPC were the higher prevalent genotype of multidrug resistant E. coli in urine sample.

Keywords: *Health; Patients; ; B- Lactamase; infections.*

Introduction

Urinary tract infections are among the most common diseases caused by *E. coli* bacteria. *E. coli* is opportunistic bacteria and therefore can become a colony of the urinary tract and cause symptomatic or asymptomatic infections[1].

Based on the presence of the gene encoding, virulence factor and types of chromosome or plasmid, Escherichia coli is classified into six classes, these includes enteropathogenic *E. coli* (EPEC), enterotoxogenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC) Enterohaemoragic *E. coli* (EHEC) Diffusely adherent *E. coli* (DAEC) Enteroaggregative *E. coli* (EAggEC)[2].

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It was found that about 60% of adult women have urinary tract infection and about 80% of community urinary tract infection and 30% of nosocomial urinary tract infections is due to Uropathogenic *E. coli* (UPEC)[3].

One of the bacterial mechanisms to combat beta-lactam antagonists is their production of beta-lactamase enzymes that cleaves beta-lactam ring, and prevents their association with transpeptidases[4]. Beta-lactamase enzymes produced from the gram-positive and gram-negative bacterium inactivates beta-lactam antibiotics such as penicillins and cephalosporins[5]. The production of this enzyme differs in the gram-positive and gram-negative bacteria. The negative bacteria of the gram stain produce beta-lactamase enzymes more varied compared to the gram positive one[6].

Genes of resistance beta-lactamase enzymes may be chromosomal or plasmid-carrying or transient genes[7]. They are chromosomal in most *bla*AMPC genes produced by the intestinal family[8]. Whereas,

the plasmid beta-lactamase enzymes are more common cause of resistance because of their ability to transfer from one cell to another, and widespread use of antibiotics increases the prevalence of resistance[9]. Beta-lactamase genes carried on jumping genes, it facilitates the transfer of the genetic material from one microbe to another, and some other genes that encode beta-lactamase enzymes such as *bla*VIM and *bla*IMP are present on integrin[10]. There are two dominant classification of these enzymes[2]. Classification by Ambler included four different molecular groups are A, C, and D of serine-β-lactamases (SBLs) and the class B of metallo-β-lactamases (MBLs). Class B is further divided into subclasses B1, B2, and B3, using sequence conservation data[11].

Beta-lactamase extended spectrum ESβLs were discovered in Europe in 1980 and these enzymes were isolated for the first time in 1983 from *Klebsiella* pneumonia from a patient in Germany hospital, then these enzymes became global[13]. Plasmid genes encode ESβLs, which facilitates its transmission between different bacterial species[14]. ESβLs are resistant to penicillins in addition to first, second third generation cephalosporins, and aztronam, but they are inhibited by beta-lactamase inhibitors clavulanic acid[15]. This study was aimed to investigate the isolates producing extended spectrum beta lactamase (ESBL) by conducting a sensitivity selection for beta-lactamase in accordance with WHO and CLSI recommendations.

Materials and Methods

Study setting and participants

This cross-sectional study was conducted between May 2019 to January 2020, which included 210 urine samples obtained from patients with acute urinary tract infection admitted to the Al- Shahid Ghazi and Al-Hariri Hospital in Medical City/ Baghdad. Midstream urine sample was collected from both males and females (M/F ratio 0.123) with age rang of 35-60 years (mean ± SD = 45.89 ± 7.61). These samples were evaluated for the presence of bacteria and/or leukocytes and cultured on blood agar and Eosin Methylene Blue agar mediums (Himedia Company, India).

Under aseptic conditions, urine cultures that produce more than 10^5 CFU/ml E. coli were included in this study. Gram stain and biochemical tests were used to identify the isolates[16].

Laboratory tests

Antibiotic susceptibility test

A modified Kirby- Bauer susceptibility testing method was used to assess the sensitivity and resistance patterns of Gram negative uropathogenic isolates[17,18]. Antibiotic susceptibility testing (AST) was performed against most commonly used antibiotics (Turkey, Bioanalyse) using the Kirby-Bauer disk diffusion method on Mueller–Hinton agar (Himedia Company, India): Ampicillin (10mg/ml) Ampicillin – Sulbactam (10mg/ml+10mg/ml),, Amoxillin-Clavulanic (25mg/ml+10mg/ml), Cefalothen (10mg/ml), Imipenem (10mg/ml), Meropenem (10mg/ml), using disk diffusion method (Kirby – Bauer). AST results were interpreted as per the Clinical Laboratory Standards Guidelines (2016) guidelines (CLSI, 2016). Escherichia coli (ATCC 25922) were used as controls for AST.

B-Lactamase production test

Disk replacement method was used to detect the production of beta-lactamase enzymes by E. coli isolates according to David and Robert 2005[17].

Polymerase-chain-reaction-based identification of β-lactamase genes

Bacterial DNA was extracted using G- spin DNA extraction kit (iNtRON biotechnology, Diagnostic kit components, Korea) according to the instructions of the supplied company.

The presence of ESBL genes was detected by polymerase-chain-reaction (PCR) using the primers listed in table 1. These primers are freeze-dried and dissolved with distilled water, a final concentration of 100 Pmol / Ml as a stock solution and kept at -20 ° C to prepare a concentration of 10Pmol / ml as a starter suspension. Stock solution 10 ml is diluted at 90 ml Distilled water to become the final volume 100 Ml. (Integrated DNA Technologies company, Canada).

Table 1: ESBL genes detect

The specific primer 16 s RNA of gene		
Primer	Sequence	Product
Forward	5' – AGAGTTTGATGGGTCAG- 3'	1450 Base pair
Reverse	5'- GGTTACCTTGTTACGCAG-3'	
The specific primer bla TEM of gene		
Forward	5'-GAGTATTCAATTTCCGTC- 3'	800 Base pair
Reverse	5'-TAATCAGAGGACCTATCTC- 3'	
The specific primer blaSHV		
Forward	5'-ATG CGT TATATT CGC CTG TG-3'	747 Base pair
Reverse	5'-TGC TTT GTT ATT CGG GCC AA-3'	
The specific primer blaCTX-M		
Forward	5'-ATG TGC AGY ACC AGT AAR GTK ATG GC-3'	593 Base pair
Reverse	5'-TGG GTR AAR TAR GTS ACC AGA AYC AGC GG-3'	

Statistical Analysis

All variables were express as frequency. Chi square test was used to compare independent variables for ESBL genes positive and negative bacteria. The p-value < 0.05 was considered as statistically significant. For statistical analysis, SPSS 17.9 (SPSS, Cgicago, IL, USA) was used.

Results and Discussion

The biochemical tests indicated that all isolates were negative for the oxidase, Voges-Proskauer and Citrate utilization, while all were positive for the indole, Methyl red, and fermentation of lactose sugar. These results are consistent with Reddy et al., (2010) (Table 2)[18].

Table 2: Diagnostic biochemical properties of E. coli

Test	Result
Oxidase	Negative (100%)
Indole	Positive (100%)
Methyl red	Positive (100%)
Voges-Proskauer	Negative (100%)
Citrate utilization	Negative (100%)
CAMP Test	B- hemolytic (82%)
Lactose	Positive (100%)

Hemolysin is one of the factors that are secreted by many strains of E. coli. Hemolysin production by E. coli was studied and it was found that (82%) of isolates were able to produce hemolysin on human blood agar. This result agreed with study conducted by Ali (2012) [19] who found that more than 40% of E. coli elaborates α-hemolysin.

Antibacterial susceptibility to antibiotic susceptibility

The sensitivity of 210 isolates of E. coli was evaluated and the frequencies of susceptibilities were listed in table 3. These results were closed to many local studies conducted indicated that the resistance rate of E. coli to aminopenicillins was 95% and 86.4%[20,21]. The main cause of this resistance was explained by Al-Hamadani, (2013) as beta-lactamase enzymes producing strain of E. coli[21].

The results of the current study also showed that 83.81% of the isolates of E. coli bacteria are resistant to Augmentin, a combination of amoxicillin and clavulonic acid acid. Penicillin salbactam was close to Augmentin, as it reached 82.38%, all are due to beta-lactamase enzymes producing isolate that confirmed by Karlowsky et al. 2002[22].

The resistance to amoxicillin-clavulanic acid combination was reflecting the origin of the *bla*TEM which is mediated by plasmids and is responsible for the multiple resistance of antagonists[23]. The results of this study came close to the results of Subha et al. (2003) who found that 90% of their isolates were resistant to Augmentin caused by beta-lactamase enzymes production[24].

For cephalosporin antibiotics, a high resistance rate against cephalothine (91.90%) was founded due to the fact that the high affinity for protein binding (PBPs) and changes in this protein lead to resistance to these antibiotics. This result was agreed with other studies that confirm more than 85% of E coli was resistant to cephalothine[25]. Inconsistently, studies conducted by Corvec et al, who found that the resistance was 45%[26].

It is not surprising that all uropathogens isolated from patient, including ESBL-producers and non-ESBL producers, were 100% susceptible to imipenem. Earlier reports have described imipenem as the drug of choice for complicated bacterial infections such as UTI. This antibiotic has a broad-spectrum action against wide group of pathogens such as Gram positive and Gram-negative bacteria. Because of its safety and effectiveness for treatment of bacterial infection, we recommend continue use of imipenem as a therapy for severe infections [27].

Table 3: Antibacterial susceptibility test

Antibiotic	Antibacterial susceptibility		
	Susceptible	Intermediate	Resistant
Ampicillin	6 (2.86%)	3 (1.43%	201 (95.71%)
Ampicillin -Sulbactam	21(10.00%)	16 (7.62%)	173 (82.38%)
Amoxillin-Clavulanic	23 (10.95%)	11 (5.24%)	176 (83.81%)
Cefalothen	9 (4.29%)	8 (3.81%)	193 (91.90%)
Imipenem	193 (91.90%)	17 (8.10%)	0 (0.00%)
Meropenem	194 (92.38%)	3 (1.43%)	13 (6.19%)

The current study evidence that the pathological conditions were dependent on the type of agent that the microorganism possesses which is a reflection of the expression. Watts et al. (2010) demonstrated in their research that geographic regions influence the type of genotype and genes of the pathogen microscope[28]

E coli Genotypes

This study showed the presence of different genotypes that are associated with bacterial resistance. Genotype tests confirm the presence of ESBL-producing isolates in 182 (86.67%). These isolates were shown to be highly resistant to pencilins-clavulonic acid and cephalosporin antibiotics. The higher prevalence of multidrug resistance strain of E coli (92.85%) was reported by this study is agreed with many global studies[29,30]. The susceptibility pattern of the ESBL-producing isolates in this study indicates a cross resistance of these ESBLs to many other common antibiotics[31]. David and Robert, 2005 recognized poor outcome when ESBL-producing bacteria infect patients and treated by penicillin or cephalosporin[17]. Conversely, some infections due to organisms testing resistant to ceftazidime but susceptible to cefotaxime or ceftriaxone have responded to treatment with these alternate cephalosporins[32].

Research agreed studies that the prevalent genes were 86.7% and 72.2% on the sequence was CTX-M2 responsible for resistance in the ceftazidime antagonists[33,34]. However, it did not correspond to others[20,21] whose found that the dominant genes encoded on plasmids contained the TEM gene. Also, it was found that the TEM gene, which is found on most isolates plasma, is the most common in Gram-negative bacteria in Baghdad city hospitals.

Assay of genotype using PCR technique indicated that 88.57% of ESBL-producing isolates possessing *bla*TEM, 30%, *bla*CTX-M and 20.48% *bla*SHV genes between isolates. In addition, the presence of Carbapenemase-producing bacteria such as KPC and MBLs producing bacteria, represents a major threat to human health because of fails in most antibiotic therapy, were less frequent present in isolates. KPC was present in 8% of isolate and mainly associated with carbapenem resistance comparing to 1% of MBLs bearing bacteria. In parallel, we have found that the most active antimicrobial agents in vitro remained to be the carbapenems[33].

Conclusion

Result of our study showed high prevalence of ESBLs and KPC but low prevalence of MBLs in cultured bacteria from urine samples of patients with acute UTI. In addition, KPC was the main carbapenem resistance mechanism in *Klebsiella* and *E. coli* isolates.

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Immunohistochemical Evaluation of Apoptotic Proteins Expression in Liver and Spleen after Treatment of Cystic Echinococcosis: An Experimental Study

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Summary

The cellular immune response and apoptotic pathways are closely related dose dependent responses against *Echinococcus granulosus* antigens. The aim of this study is to evaluate the cellular expression of Bax, Bcl-2 and Caspase-3 in both liver and spleen of experimentally cystic echinococcosis mice treated with Oxfendazole, Oxfendazole +Praziquantel, Oxfendazole +Albendazole and Albendazole +Praziquantel of experimentally mice model of cystic echinococcosis. After 2 months of treatment, mice were sacrificed and both liver and spleen were processed for immunohistochemistry staining protocol using specific primary antibody against mouse Bax, Bcl-2 and Caspase-3 proteins. The results showed that *E. granulosus* infection able to induce apoptosis in both liver and spleen tissues after induction of cystic echinococcosis mice model, treatment with Albendazole in combination form gives better results because of attenuation of apoptosis pathway and restore of normal cellular behavior. In conclusion, this study describes the involvement of apoptosis in the pathogenesis of cystic echinococcosis.

Key words: *Apoptosis, liver, Spleen and cystic echinococcosis.*

Introduction

Cystic echinococcosis (CE) is one of the most chronic helminthic wide spread zoonotic disease in many countries caused by metacestode of *Echinococcus granulosus*[1]. The interplay between parasite and host cells have been described ensuring survival of parasite[2]. CE can persist chronically in the affected organ mainly liver evading host immune responses and induction of apoptosis in lymphocytes[3].

Apoptosis or programmed cell death is an important cellular mechanisms ensuring cellular homeostasis[4]. Studies showed that fertile hydatid cyst containing protoscolex were able to induce apoptosis in the adjacent cells of the host[5]. Thus, *E. granulosus* is capable to modulate host immune responses such as the interference with modulation of dendritic cells maturation [6], and induction of a non-protective Th2 cell response by AgB[7]. Apoptosis is another proposed mechanism[8] that provides suitable environment for survival of the cyst by inducing apoptosis in the host immune cells. Several factors play role in apoptosis, but the caspase enzymes and Bcl-2 family are the two main families in this process. The first is a cascade of enzyme, of which Caspase-3 is the most important member affecting the lymphocyte apoptosis. The second is Bcl-2 family, a set of cytoplasmic proteins members that regulate apoptosis[9] like Bcl-2 and Bax proteins. While Bcl-2 proteins inhibit apoptosis, Bax counteracts this [10]. Central enzyme of apoptosis cascade is a proteolytic system involving a family of proteases called caspase. Among these enzymes Caspase -3 plays a central role in the apoptosis process[11].

The current study aimed to investigate the protein expression of Bax, Bcl-2 and Caspase 3 in liver and spleen of mouse model of cystic echinococcosis under

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treatment with different modalities of anthelminthic drugs.

Methods

Experimental design:

This in vivo mouse model experiment for cystic echinococcosis were approved by College of Pure Sciences (Ibn AL-Haitham)/ Baghdad University. This experiment was done in Animal housing of the college. Balb/C male mice were housed inside cages according to the instructions.

Collection of hydatid cysts samples and isolation of protoscolices: Hydatid cysts from lungs and livers of infected sheep carcasses collected in AL-Sadir slaughter in Baghdad. These cysts were stored and processed to separate the protoscoleses from the fluid[12].

Preparation and administrations of drugs:

Three drugs were used in this study, Albendazole (ABZ) 10mg/kg, Praziquantel (PZQ) 40mg/kg and Oxfendazole (OXF) 30 mg/kg. These drugs were dissolved in distilled water and stored at 4°C for no more than 24 hours. Drugs were used about two months in single form or combination with others as the following:

OXF treated group, OXF+PZQ treated group, OXF+ABZ treated group and ABZ+PZQ treated group. Additionally, not infected group served as negative control (NC) group while none treated group served as positive control group (PC).

All drugs were given after four months of experimental infection with 2000 protoscolices

(I/P) in accordingly to the group of study as single dose orally at early morning for two months.

Immunohistochemistry staining of BAX, Bcl-2 and Caspase -3 proteins in liver and spleen sections:

Animals were sacrificed by cervical dislocation, then examined their internal organs (such as Liver, spleen, lung, stomach intestine and etc.) of mice were removed under sterile conditions by abdominal incision. Both liver and spleen were washed in distilled water and stored in 70% ethanol and processed as paraffin embedded tissue blocks.

Sections of 5 micrometer were obtained and mounted on positive charge glass slides. Before labeling, sections were deparaffinized in xylene and rehydration by graded series of ethanol baths. Endogenous peroxidase activity was blocked with peroxidase block. After washing, sections were then incubated in protein block for 20 minutes at room temperature.

100 microliters of diluted primary antibody against Caspase -3 (orb382909),BAX (orb378567) and BCL2 (orb10173) was added on tissue section for 60 minutes. After washing, visualization steps achieved by Rabbit IgG SABC Kit (orb90444) using DAB Chromogen Kit (orb219876) and counterstained by hematoxylin.

Data analysis

Results of IHC test were evaluated by applying a semi- quantitative assessment: each slide was counted under light microscope for three times at x400 magnification and about 5 fields were randomly selected in each round. Thus, the number of immunolabeled cells was counted in 5 fields under a fixed focus for each slide and value of mean for positive count in total number of all counted cells (each tissue section) for each sample group. Data were expressed

as mean ± standard deviation (SD) and Analysis of Variance (ANOVA) test was used for differences between groups. Values $p \leq 0.05$ was regarded as statistically significant.

Results

This study investigates the apoptosis regulating proteins expression in liver and spleen of mice before and after treatment with different drugs. After induction of cystic echinococcosis in mice, the apoptosis proteins (Bcl-2 and Bax) were elevated in both liver and spleen tissues. Similarly, Caspase -3 was highly elevated in comparison with negative group (Fig. 1).

Restoration of apoptotic protein expression after treatment of cystic echinococcosis:

In the current experiment, it's clearly shown that apoptosis proteins were reduced in both liver and spleen tissue after four months Cystic Echinococcosis and two months of treatment of cystic echinococcosis with different treatments as shown in Table 1.

Bax protein was still elevated in liver tissue under treatment with OXF alone or in combination with PZQ or ABZ, and ABZ with PZQ. But it was reduced in spleen tissue under treatment with OXF in combination with PZQ or ABZ as shown in Fig 1

Caspase -3 protein expression also reduced after two months of treatment in combination treatments in comparison with non-treated group

Table1: Comparison of Bax, Bcl-2 and Caspase- 3 proteins in liver and spleen in study groups.

liver	**NC**	**PC**	**OXF**	**OXF+PZQ**	**OXF+ABZ**	**ABZ+PZQ**
Bax	15.4±4.7	27.83±7.83 A**	17.23±8.38 ANS, B*	18.03±6.94 ANS, B*	16.94±7.03 ANS, B**	18.2±6.02 ANS, B**
Bcl2	5.03±2.9	8.93±1.9 A**	6.92±3.9 ANS, BNS	6±2.3 ANS, BNS	8.9±2.6 A**, BNS	7.9±2.8 A**, BNS
Caspase3	15.28±7.44	32.3±12.84 A**	26.84±11.84 A**, B*	14.32±9.44 ANS, B**	10.5±7.54 ANS, B**	12.44±8.54 ANS, B**
Spleen	NC	PC	OXF	OXF+PZQ	OXF+ABZ	ABZ+PZQ
Bax	7.93±2.8	18.92±10.2 A**	12.2±8.3 ANS, B**	8.93±3.9 ANS, B**	10.23±5.3 ANS, B**	11.2±3.9 ANS, B*
Bcl2	6.8±3.9	12.2±6.9 A**	12.9±7.2 A**, BNS	4.8±2.1 ANS, B**	3.9±1.8 A*, B**	6.7±2 ANS, B**
Caspase3	17.29±7.4	38.92±13.4 A**	28.32±10.84 A**, B*	17.93±10.2 ANS, B**	14.33±8.99 ANS, B**	14.2±10.4 ANS, B**

A: statistically significant difference from Negative control (NC), B: statistically significant difference from positive control (PC).* Significant differences on ($p \leq 0.05$). **Significant differences on ($p \leq .0.001$). NS: No significant ($p>0.05$).

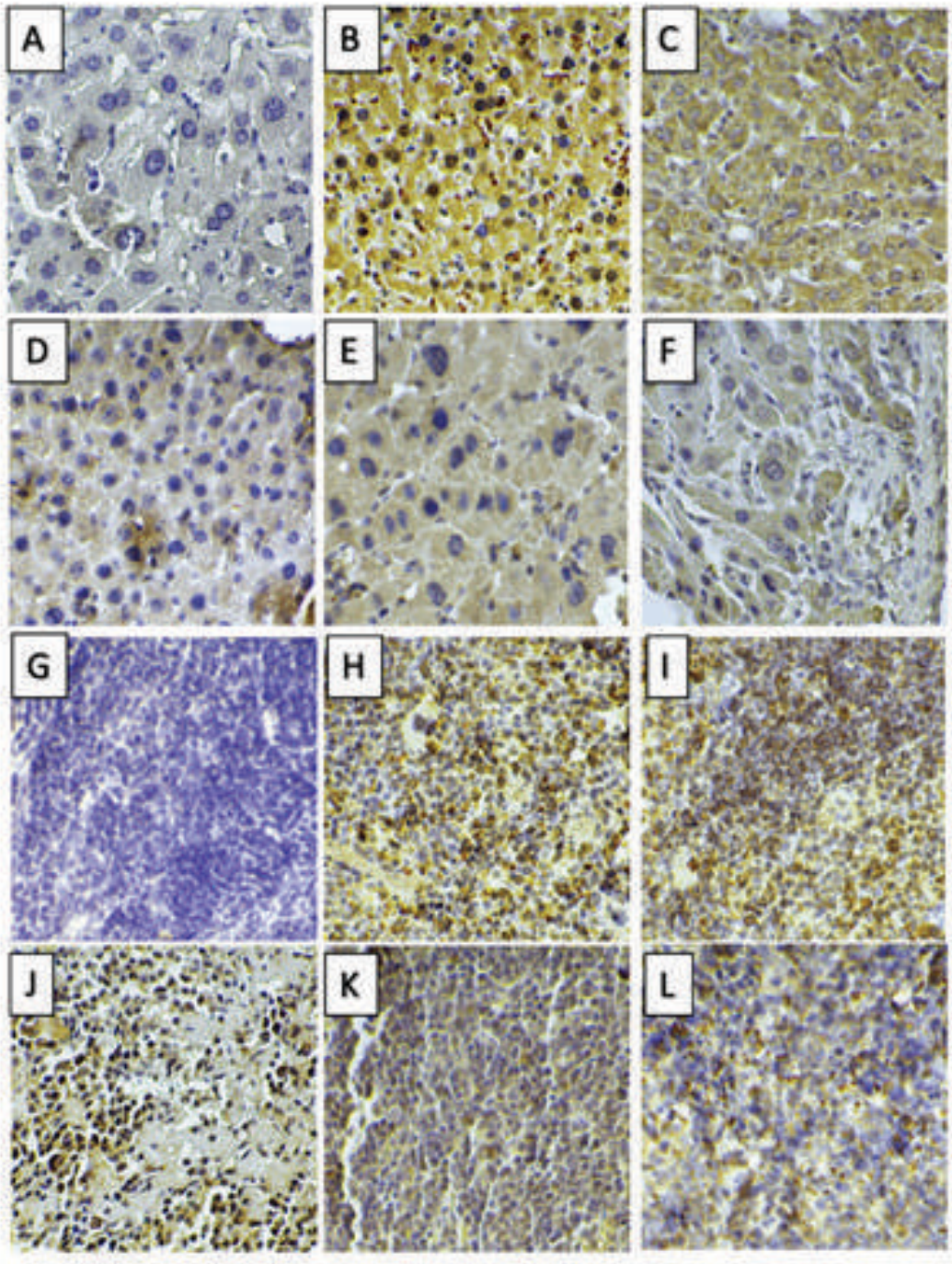


Figure 1: Immunoperoxidase staining of Bax protein expression in liver and spleen in study groups using polyclonal rabbit anti- Bax (5pg/ml). (A-F): Bax expression in spleen tissue, (A) control negative group with low expression, (B) positive control group without treatment showing high expression of Bax protein, (C) OXF treated group, (D) OXF+PZQ treated group, (E) OXF+ABZ treated group and (F) ABZ+PZQ treated group. (G-L): Bax expression in liver tissue, (G) control negative group with low expression, (H) positive control group without treatment showing high expression of Bax protein, (I) OXF treated group, (J) OXF+PZQ treated group, (K) OXF+ABZ treated group and (L) ABZ+PZQ treated group.

Discussion

The effect of *E. granulosus* infection on apoptotic proteins expression in liver and spleen were not yet studied. However, this is the first study that report the immunohistochemical expression of Bcl-2, Bax and Caspase 3 protein expression in both liver and spleen of mouse model of cystic echinococcosis after treatment with different treatments.

In the current experiment, an increased apoptotic protein in liver and spleen after induction of infection suggesting a higher activity of apoptosis pathway mediated by parasitic antigens. This inclination was argued by other studies proposed that the parasite release antigens that modulate apoptosis in host cells. This was evident by mitogenic activity of protoscoleses on regulation of growth in leukocyte cell line[8,13].

Our results was argued by Amirmajidi, *et al*., 2011 whom reported that apoptosis was higher in lymphocytes after treatment with fertile hydatid cyst fluid compared with control cells. Furthermore, Bcl-2 mRNA as anti-apoptotic protein was reduced accompanied by increased Bax protein as pro-apoptotic protein in in this model, Additionally, increased caspase-3 expression was also higher compared with control group[3].

Another studies, Macintyre *et al*.,2001 found that hydatid fluid induce cell proliferation with enhanced expression CD25 and CD38 on human peripheral blood lymphoblast and reduced CD28 (and other co-stimulatory molecules) resulting in anergy or apoptosis[14]. Also, Li *et al*.,2003 showed that apoptosis was significantly higher in T-cell after infection, this might be due to release of inducers of apoptosis such as antigens[15] thus, fertility of cyst is an important factor for survival of the cyst in the host's body[16].

The induction of apoptosis has significant advantage for the *Echinococcus granulosus* infection as a fertile stage induce apoptosis in the leukocyte infiltration toward the newly growing cyst[3].This strategy more important in the early stage of infection ensuring minimal concentration of leukocytes as an immune suppression mechanism.

This study clearly highlighted that two months of treatments were restored the expression of Bcl2 (anti-apoptotic protein) and reduce Bax and caspase- 3 (pro-apoptotic proteins) in both liver and spleen of mice model. However, it might be due to scolicidal activity of treatment against parasite leading to reduction of parasite load that ultimately reduce the inducers of apoptosis of host cells[17]. Similarly, this explanation was clearly demonstrated the scolicidal and apoptotic activities of albendazole against *E. granulosus*[18,19] or in liver tissue in combination with Chinese herbs (*Sophora moorcroftiana* alkaloids)[19,20].

In conclusion, this study describes the involvement of apoptosis in the pathogenesis of cystic echinococcosis. Using of Albendazole in combination form gives better results because of restoration of apoptosis pathway.

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Knowledge of the Mothers toward Sanitation and Hygiene in relation to Childhood Gastroenteritis in Babylon Province

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Background

The negative impact of gastroenteritis in those aged 5 or less in Babylon Province in Central Iraq cannot be underestimated as it is a serious impediment to the wellbeing and cognitive development of children and a cause of unnecessary death in this resource-strained region of the Middle Eastern country. In this region diarrhea is an important clinical presentation at primary health care institutions and other health affiliated centres, stands out as a leading cause of admission to many of the emergency hospital wards in Babylon, and a serious and ironically, often reversible cause of loss of precious lives. Regional attitudes in the Middle Euphrates Region about causes that lead to childhood gastroenteritis vary from rural and urban localities and even from townships, a cultural issue that is true for most provinces of Iraq.

Aim of the study: 1-To study the social and demographic aspects related to sanitation and personal hygiene regarding childhood diarrhea in Babylon Province.

2- To determine the local knowledge, of women and care givers about childhood diarrhea.

Patients and methods: This cross sectional work was carried in Babylon Province, Iraq. A total 408 respondents attending primary health care institutions and pediatric clinics for treatment of diarrhea , during the period from first of January 2019 to the first of May 2019.

Results and Conclusions: In Babylon Province outbreaks of diarrhea tend to be noticed early in life prior to first year of life despite the fact that around three quarters of infants who contracted gastroenteritis were in compliance with Rota Vaccine timetable schedule. Seven out of ten women were fully knowledgeable of the importance immunization against Rota virus and its vital role in reduction of the risk of gastroenteritis.

When their children contract diarrhea, a percentage shy of 66% said that they would seek paediatric medical attention .

Respondents whose infants were affected tend to be mostly urban, in their teenage years and lacked proper education. There is a remarkable lack of adequate knowledge about ways of contracting childhood gastroenteritis.

Key words: *Knowledge of the Mothers, Sanitation and Hygiene, Childhood Gastroenteritis, Babylon Province.*

Introduction

Diarrheal disease stands out as the second major cause of mortality among the under five years old internationally [1]. About twenty percent of deaths of children is due to this ailment[2,3]. Passage of three or more loose or watery motions within a period of 24 hours is considered as abnormal or stools that are passed

more frequently than normal for a child is considered as diarrhea according to the World Health Organisation guidelines[4,5].

Diarrhea is one of the commonest diseases that has the greatest negative impact on the growth and wellbeing of children[6]. The younger the age of the child the most vulnerable with incidence peaks in the first 24 months of life but subsides as the child grows older[7]. Worldwide, there are two and a half billion humans who live without adequate hygiene and sanitation, just over six hundred and sixty million people do not have access to clean water sources[8]. Poor standards of water purification, sanitation and hygiene and related interventions can affect growth, cognition and development of children in a number of ways[9]. Contaminated foodstuff, inadequate feeding standards, lack of proper water purification, lack of hand washing, poor sanitary disposal of human waste, poor housing and living conditions, and lack of access to adequate and available primary health care centres are aggravating risk factors of those aged 5years or younger for contracting diarrheal disease[6,10,11,12].

Gastroenteritis does not kill by itself but poses a lethal threat through the poor knowledge, poor practice and wrong attitudes of caregivers and their improper approach towards its management and prevention leads to high risk of severe dehydration and eventually death[13,14]. Even with improvements in the standards of housing and sanitation, water purification and food safety knowledge, diarrhoeal disease still remains the cause of major reversal of economic and societal gains[15]. Although over the last five decades there has been a drop in the total global mortalities due to diarrhoeal disease, the morbidity from diarrhoea may not show a similar trend[16]. These improvements in mortality have been attributed to increased use of oral rehydration therapy (ORT), improved nutrition, increased natural feeding, a variety of proper supplemental feeding, maternal education, better MMR immunisation coverage and improvements in hygiene and sanitation[17]. Morbidity and mortality in young children is particularly a problem because early childhood is a vital time as far as physiological development is concerned[18]. Human growth during this period is faster than during any other period and many vital cognitive pathways are forged before the age of 2 years. Any problem in these processes by diarrhoeal disease can lead not only to lethal consequences but also to impaired cognitive development and more school absenteeism and less economic productivity in the adult years of life[19].

Aim of the Study

1. To analyse the socio-demographic factors related to sanitation and hygiene regarding childhood gastroenteritis in Babylon Province.

2. To outline the main local knowledge of mothers and care givers about childhood gastroenteritis.

Patients and Methods

This is a cross-sectional study conducted in Primary Health Care Centres and Pediatric clinics during the period from the first of January 2019 to the first of May 2019, and included information taken from the mothers of 408 patients with diarrhea of different ages below 5 years old.

Results

Table of socio-demographic factors of mothers, shows that 55.4% were aged younger than 18 years, living in extended families 65% with a crowding index of over 4.1 in 49.8%

Table 1: Distribution of causes of gastroenteritis cited by mothers and variables related to water.

Causes	Number	Percentage
Particular food	21	5.1%
Particular feeding habit	40	9.9%
Spoiled milk	82	20.2%
Breast feeding	41	10.0%
Bacterial infection	143	35.0%

Cont... **Table 1: Distribution of causes of gastroenteritis cited by mothers and variables related to water.**

Parasitic infection	20	4.9%
Drugs	20	4.9%
Hot climate	41	10.0%
Total	408	100.0%

Table 1 shows the distribution of causes of gastroenteritis cited by mothers. 35%believed it was due to bacterial infection,20.2% due to spoilt milk and both breast feeding and hot climate cited by 10% respectively. Table 2 also show distribution of variables related to water shows that just under 30% use water only as a method of handwashing after contact with fecal waste and 79.7%cited using unsafe tap water that is not potable.

Table 2: Association between socio demographic characteristics and causes of gastroenteritis cited by mothers.

Variables	Causes								X2 test	P-value
	Particular feeding	Particular feeding habit	Spoiled milk	Breast feeding	Bacterial infection	Parasitic infection	Drugs	Hot climate		
Age Less than 18 18-35 More than 35 Total	1(4.8%) 0(0.0%) 20(95.2%) 21(100.0%)	20(50.0%) 0(0.0%) 20(50.0%) 40(100.0%)	61(74.4%) 20(24.4%) 1(1.2%) 82(100.0%)	1(2.4%) 40(97.6%) 0(0.0%) 41(100.0%)	102(71.3%) 1(0.7%) 40(28.0%) 143(100.0%)	20(100.0%) 0(0.0%) 0(0.0%) 20(100.0%)	20(100.0%) 0(0.0%) 0(0.0%) 20(100.0%)	1(2.4%) 40(97.6%) 0(0.0%) 41(100.0%)		<0.001*F
Educational level Illiterate Primary Secondary Higher education Total	20(95.2%) 1(4.8%) 0(0.0%) 0(0.0%) 21(100.0%)	20(50.0%) 20(50.0%) 0(0.0%) 0(0.0%) 40(100.0%)	21(25.6%) 61(74.4%) 0(0.0%) 0(0.0%) 82(100.0%)	0(0.0%) 1(2.4%) 0(0.0%) 40(97.6%) 41(100.0%)	41(28.7%) 82(57.3%) 0(0.0%) 20(14.0%) 143(100.0%)	0(0.0%) 20(100.0%) 0(0.0%) 0(0.0%) 20(100.0%)	0(0.0%) 0(0.0%) 20(100.0%) 0(0.0%) 20(100.0%)	1(2.4%) 0(0.0%) 40(97.6%) 0(0.0%) 41(100.0%)	730.878	<0.001*
Residence Urban Rural Total	1(4.8%) 20(95.2%) 21(100.0%)	0(0.0%) 40(100.0%) 40(100.0%)	60(73.2%) 22(26.8%) 82(100.0%)	40(97.6%) 1(2.4%) 41(100.0%)	81(56.6%) 62(43.4%) 143(100.0%)	20(100.0%) 0(0.0%) 20(100.0%)	20(100.0%) 0(0.0%) 20(100.0%)	21(51.2%) 20(48.8%) 41(100.0%)	144.828	<0.001*
Occupation Employed Housewife Total	0(0.0%) 21(100.0%) 21(100.0%)	0(0.0%) 40(100.0%) 40(100.0%)	21(25.6%) 61(74.4%) 82(100.0%)	20(48.8%) 21(51.2%) 41(100.0%)	20(14.0%) 123(86.0%) 143(100.0%)	0(0.0%) 20(100.0%) 20(100.0%)	20(100.0%) 20(100.0%) 20(100.0%)	20(48.8%) 21(51.2%) 41(100.0%)	72.944	<0.001*

*P value≤ 0.05 was significant. F: Fisher-exact test.

Table of Distribution of variables related to knowledge of mothers shows that one in five do not use refrigerators to store child food 21.6%,and not all seek a doctor when gastroenteritis happens 65.4%,depending on sunken eyes and dry lips 35.3% and 29.7% respectively to assess the extent of dehydration of their children.44.9% lacked proper knowledge of ORS.

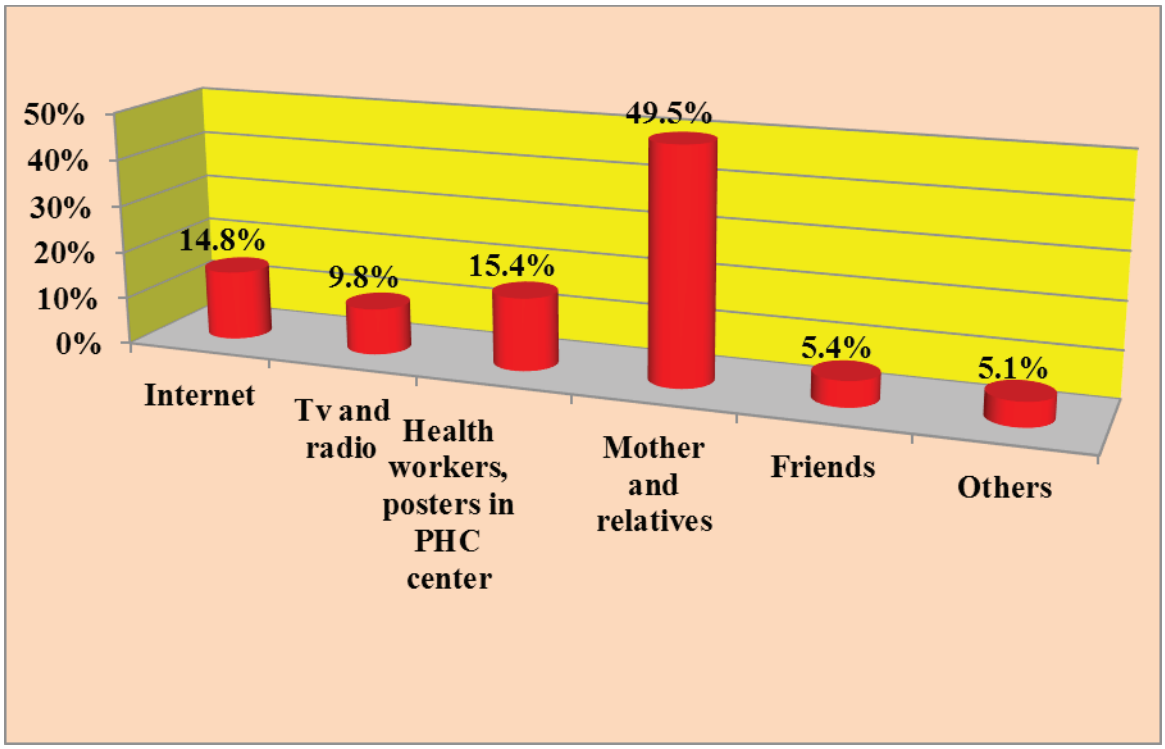


Figure 1 Distribution of source of information of mothers regarding diarrhea.

Table 2 shows that chi square/Fisher exact test was conducted to show an association between maternal sociodemographic variables which include (age, residence educational level and occupation) and causes of gastroenteritis cited by mothers . There were significant association in all circumstances p value < 0.001.

Data Analysis

SPSS version 24 computer software (statistical package for social sciences) was used to enter data and analyze information. Categorical variables were presented as frequencies and percentages, continuous variables were presented as (mean_+standard deviation). Inaddition Chi square/Fischer exact test was utilised to show the association between two categorical vaiables . A P value of < 0.05 is statistically significant.

Discussion

According to the results of our study in Babylon Province and regarding the socio-demographic distribution of the mothers, we found that 59.6% of mothers were from urban area, where as in a study in Egypt they found that 63.2% of mothers were from rural area[20]. In our study we found that 80.1% of mothers were housewives, where as in Egyptian study 52.4% of mothers were housewives and 30% were professionals[20].

Regarding educational level of the mothers in our study 45.4% were attending primary school, where as 64.44% were illiterate in Ethiopian study[21], in contrast with another study in Malawi 79% were attending primary school and above[22]. Only 25% of mothers in the Ethiopian study were attending primary school[21] and about 47%were attending primary or secondary school in Combodia[23].

In our study, 20.2% considered teething as a cause of diarrhea, where as a study in Jamaica shows that 82% of mothers thought that teething causes diarrhea[24] and 71.9% of mothers in a study in Enugu in Nigeria thought that teething causes diarrhea.

In this study, we found that 70% of mothers or caregivers use water and soap for hand washing, where as in a study in Egypt 92.7% of them use water and soap and only 7.3% of mothers use only water for hand washing [20].

We found that 35.5% of mothers can assess dehydration depending on sunken eyes and 65% go to doctor when their children get diarrhea, where as in Ethiopian study 0nly 13.8% can assess dehydration depending on sunken eyes and 62.4% of them go to doctor when their children get diarrhea [21].

In this study, 80.1% of mothers believe that artificial feeding causes diarrhea, where as 54.7% of Ethiopian mothers disagree with this belief [21].

In the mothers of the 408 children under 5 years old who were presented with diarrhea, there were significant association (P value < 0.001) between socio-demographic characteristics (age of the mother, educational level, residence, and occupation) and causes of diarrhea that were considered in our study (particular feeding, teething, breast feeding, bacterial infection, parasitic infestation, drugs, and hot climates).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Expression Studies of the Cancer Drug and Its Effect on Angiogenesis-Promoting Genes in Cancer Cell Lines

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Abstract

Objective: Being a member of RNA binding proteins, Human Antigen R (HuR) is considered as an acute factor to mediate the proangiogenic factors causing the angiogenesis amongst tumor cells. All through the anoxic conditions and inflammation, the cytokine IL-1 activated the HuR which further downregulated or upregulated the hypoxia inducible factors (HIF1α). Current study was conducted to observe the correlative impact of Decetexal (Taxotere) cancer drug on HIFα and HuR expression levels while utilizing the cell line HeLa.

Key words: *HuR, Anticancer targets, HIF1 α, Decetexal*

Introduction

Cancer is a main health issue in both developing and developed countries of the world. World Bank and WHO (World health organization) assess that about 12 million worldwide individuals agonize with caner while 7.6 million of them die per year [1]. Lower success rates of treatments lead to the higher mortality and incidence rates among cancer patients. Thus, numerous procedures have been formulated against cancer such as chemotherapy, immunotherapy, radiation and surgery. Though, the rate of success for these methods is still lower because of their drawbacks [2]. Such condition inspires the struggles to develop and discover the more impactful and profound anticancer properties devoid of severe side effects [3-6]. Moreover, the proliferation of cancerous cells is uncontrolled and happens far away from normal limits. This occurs because of the cell cycle disruption, suppression of apoptosis and increased angiogenesis [7-10]. As the apoptosis is a procedure of cell death intended for the elimination of undesired host cells [4, 5]. This apoptosis process is automated with a series of events comprising of a set of products of genes. Moreover, apoptosis is liable for several pathological & physiological procedures and pathological process comprise a major role of apoptosis in the death of tumor cells [6].

Being a widely expressed member of RNA binding proteins ELAV's family, HuR displays precise similarities to the sequences of ARE-containing RNA in vitro [7]. It associates to their in vivo rates of decay, thus, involving HuR in the angiogenesis of proangiogenic factors. Commonly, initiation of angiogenesis takes place through the inflammatory or hypoxic settings [8, 9]. Additionally, hypoxia & inflammation stimulate the function of HuR by modulating the binding activity of RNA and subcellular localization of HuR [6]. Moreover, other cytokine IL-1linked inflammation [10] can initiate the HuR functions for stabilizing the target mRNAs' set to accomplish the certain procedure [11]. As a result, the activated HuR further stabilize TNFα or stimulate the HIF1α translation by ARE situated in 3' UTR while creating a positive feedback [12, 13]. while several healthcare authorities might utilize the taxtotere name in order to refer to the docetaxel name generic drug. As docetaxel is anti-cancerous (cytotoxic or antineoplastic) chemotherapy drug [14-16], thus, permissions have been made for its usage in metastatic prostate cancer,

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advanced stomach cancer, non-small cell lung cancer and breast cancer [17]. Recent studies indicate its usage for the treatment of melanoma, soft tissue sarcoma, pancreatic cancer, ovarian bladder and small cell lung [19].

Materials and Methods

The streptomycin, penicillin, fetal bovine serum (FBS) and cell culture medium (DMEM) were acquired from the labs of HiMedia. The HeLa cells of human cervical carcinoma were attained from the Indian Academy Degree College of Banglore, India. Moreover, the culturing of cell lines was carried out in the medium of DMNEM enhanced with streptomycin (100µg/mL) & FBS (10%). Likewise, the growth of cells took place in the incubator of CO_2 (Memmert, Germany) at 5% CO_2 & 90% of 37°C temperature. Usage of the confluent trypsin/EDTA was done during the sub-culturing of cells. Further, the Decetexal (Taxotere) CAS # 114977-28-5 was bought from the Sigma Aldrich and this drug was soluble in methanol and sued at fluctuating concentrations for the research.

Primers design: The pairs of the primers purchased from the Sigma Aldrich are described in table (1). These were designed using formerly designated Primer3 software by Sudhakar *et al* [18]. Furthermore, the alignment tools from BLAST (National Centre for Biotechnology Information) were used to check the *in silico* primer pairs and PCR product's specificity.

RT PCR: cDNA synthesis: The 200U/µl Super script TMII Reverse Transcriptase (HiMedia) was utilized along with RT PCR kit to carry out the cDNA synthesis. To start the reaction, the small amount of RNA about 2µg attained in the former section was utilized. The 1.97µg/µl concentrations of RNA was acquired, thus, 1.12µl of the total RNA was utilized in reaction. Further, the 1µl RT enzyme and random primers were mixed together properly followed by the 10 minutes incubation at 25°C. After the 45 mins incubation at 70°C, the obtained cDNA was stored for further usage in the analysis of RTPCR process.

Table 1: Table presents the primers details used in RTPCR.

Gene		Sequence (5'->3')	Length	GC%	Product
HuR	FW	GAGGTAGGGACCACCAGGAT	20	60.03	888
	RV	ACTTGCTCTTTTTCTCTTGGCAG	23	59.68	
HIF-1A	FW	TGACCTGCTTGGTGCTGATT	20	59.89	208
	RV	GCGCTGAATCTTTGCTATGG	20	57.6	

Real time PCR: The table (1) primers were bought from Sigma Aldrich and prepared using software of primer 3. The usage of iQTM SYBR Green Supermix (HiMedia) allowed to perform the real time PCR conferring to the method described by **Deepak V. *et al* (2018)[19]. The 1µl RT products and 600nm primers were utilized in the assay of PCR with reaction using 12.5µl of total volume. To confirm the positive amplification, the whole reactions were run in duplicates and also in parallel to their negative control.**

Docking studies: Various soft wares such as PATCHDOCK, ArgusLab 4.0.1, Rampage, ACD ChemSketch, Swish PDB viewer and SWISH Model were utilized for docking studies. In present context, the HIF-1A and HUR receptors were used while among the model study to dock or inhibit receptors. The Hypoxia inducible factor 1 alpha (NC_000014.9) and human like R antigen were obtained from enterz database of NCBI. SWISH Model freeware was used for homology modelling while choosing best templates for the studies of docking. Moreover, the selected models were additionally analysed on the patchdock.

Results and Discussion

MTT assay: For this assay all measurements were control normalized (without treatment) and considered as 0 in percentage. The primary observations indicated the cell treatment with 10µg/mL drug suspension presented an anti-proliferation activity. This was extremely negligible activity when compared to the dose dependent positive control. However, the drug's activity was observed to be positive on comparing to the negative control and colchicine. All such values show equally potent drug suspension to the positive control. Thus, the values of IC_{50} were 23.56 & 38.8 for positive control and drug correspondingly.

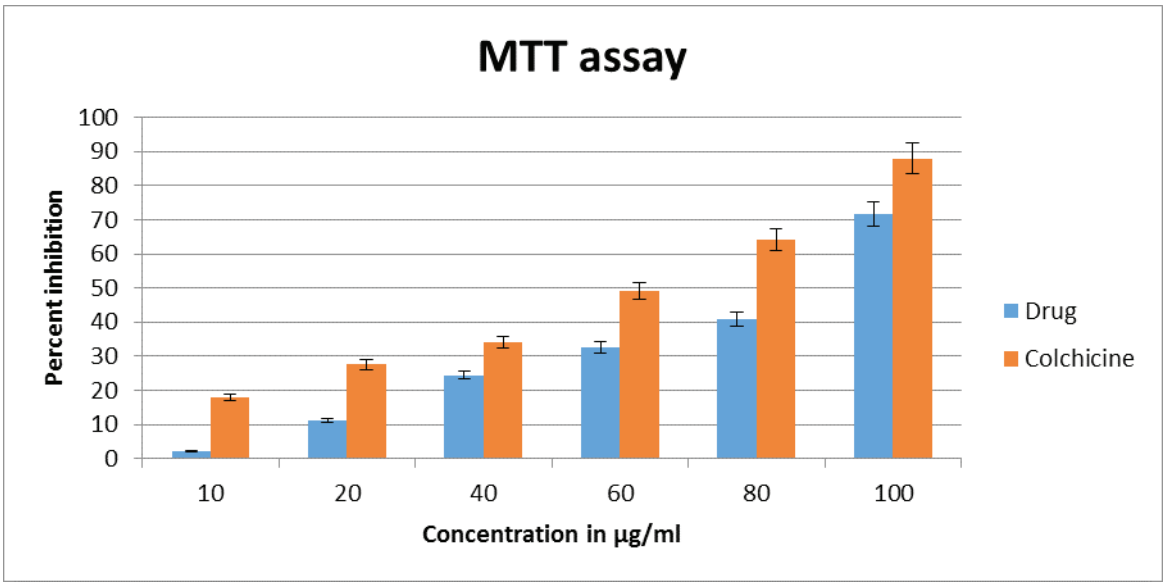


Figure 1: Graph indicates the values of percent inhibition the assay of MTT. All values are articulated as ±s.e value and values were triplicates' average and all measurements were control (0%) normalized.

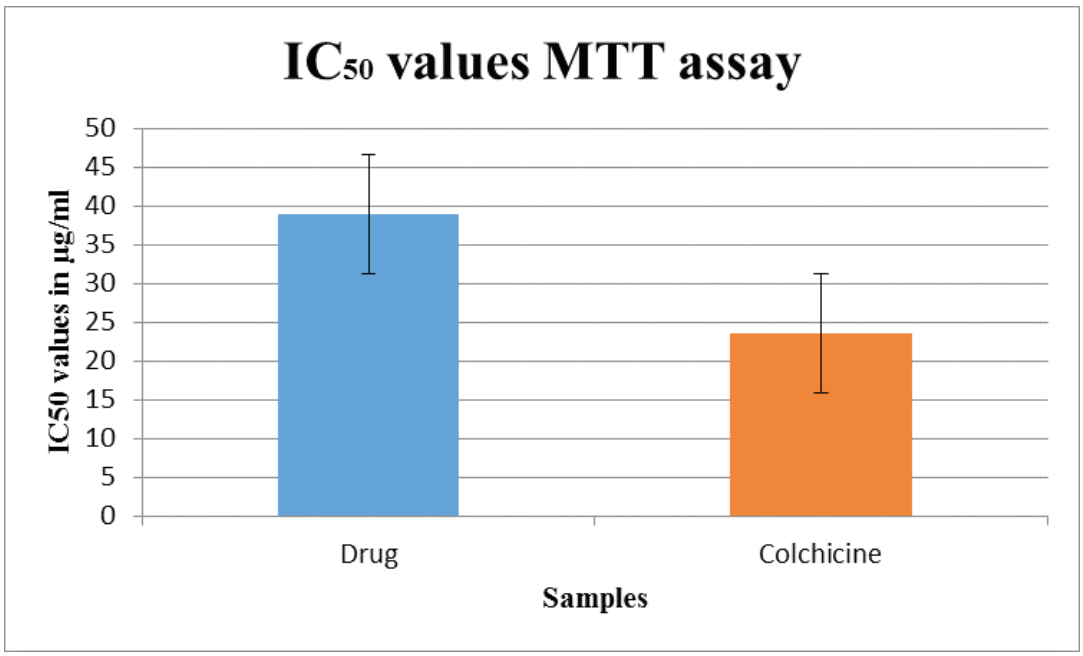


Figure 2: Graph is indicating the values of IC_{50} in assay of MTT. All values were presented as vale ±s.e and were average of triplicates.

LDH assay: The assay of the drug induced damage of membrane was done on due to the release of LDH. Subsequently to the incubation, the quantity of the released LDH was further calculated while comparing to the (OD0.01) negative control. The outcomes showed that comparative to the untreated cells, the release was dose

dependent and according to the assay of MTT. Initial concentration presented a very negligible release on comparing to the negative control and colchicine. However, the 24 hours incubation, the suspension of drug indicated a substantial upsurge in the release of LDH comparative to the control afterwards 1µg/ml amount. While, at 3µg/ml concentration, the percent release observed was 71±19.4%for drug & 92±13.4% for positive control with a significant difference of ($p< 0.05$).

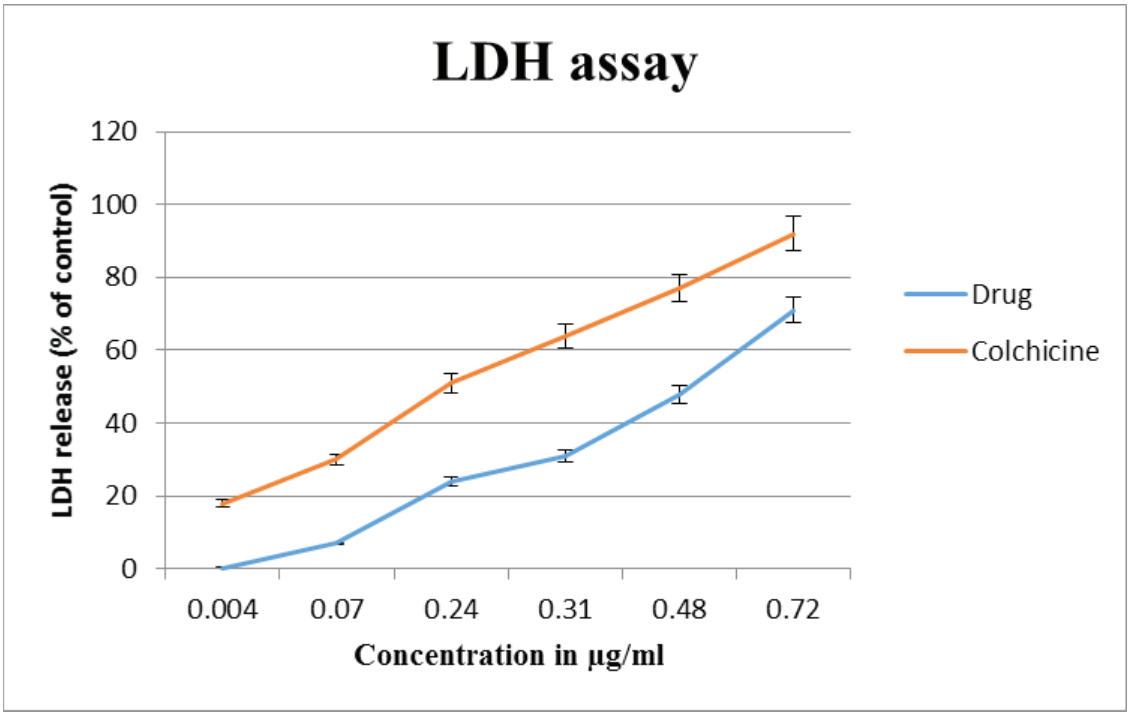


Figure 3: This graph represents the LDH release percentage from the HeLa cells succeeding to the 24 hours LDH assay exposure. The triplicates' values were average and were expressed as the values of percentage control (values± s.e.) and all measurements were normalized to (0%) control.

Expression of gene members: The variance is reduced amongst the samples by normalizing all the obtained outcomes to the housekeeping (GAPDH) gene. The mRNA expression levels for each gene (HIFA, HUR & GAPDH) member were studied individually. From this, the samples having lower values of $_{\Delta\Delta}$Ct was precieved as calibator whereas, the remaining samples were rather comparative to the calibator. Thus, the Ct values of the housekeeping genes, HIFA & HUR were 11, 18 and 21 correspondingly. Reliying the Ct values of samples that have been treated with drugs were normalized to the GADPH housekeeping gene. The expression of GAPDH gene was deliberated as 100% in value. Hence, from the calculated values of $2^{-\Delta\Delta Ct}$ and several researches, the observations indicated the underexpression of HUR gene in comparison to the HIFA and control. Therefore, both HIFA & HUR were underexpressed in the treatment in comaprison to control group. HIFA showed 16 times underexpression in treatment while HUR gene was underexpressed 64 times on treatment.

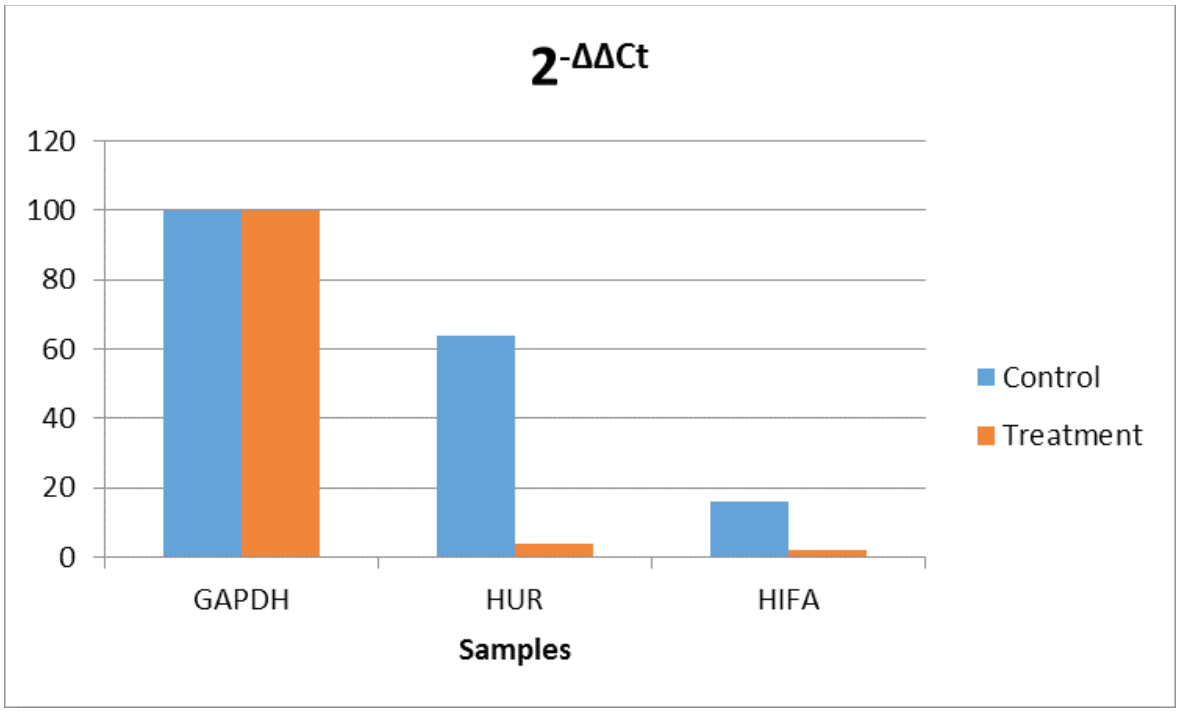


Figure 4: This graph represents the values of $2^{-\Delta\Delta Ct}$ for members of genes attained from the RT PCR. Thus all experiments were the average of the duplicates.

Cell cycle Analysis: HeLa cell lines were used to analyze cell cycle distribution with FACs analysis. This was carried out to observed either any of the anti-cancer drug suspension was applied through the arrest of cell cycle induction. This experiment was carried out as (Kaur, M., et al 2008)[20]. Shortly, approximate plating of 1 x 10^6 HeLa cells was done in 6 well plates that comprised of 2ml complete DMEM. After 24 hours incubation, cells were further treated without or with (control) suspension of drug at fluctuating concentrations of 40 and 20µg/ml. From which DMSO of 1% was utilized as negative control as 1% in the media of DMEM and 20µM of colchicine as a positive control. The cell were assembled and centrifugation was done for 5 minutes at a RT maximum speed and pellets were gathered. The collected pellets were re-suspended in solution of 1XPBS & a 2ml fixing solution of 20% PBS in 70% ethanol was used to fix overnight at 4ºC. Further cells were pelleted through 10 minutes (RT) centrifuge at 4000rpm. These pellets were cold wash with 1XPBS and then incubation was done for fifteen minutes (RT) in the solution of 500µl propidium iodide (PI) that comprised of 0.05mg/ml RNase A and 0.05mg/ml in PBS solution. Moreover, the FACS Caliber from BD Biosciences (San Jose, CA) was used for the determination of cell percentage & their viability in several cell cycle's stages in control and treatment as well. Th usage of FACS Caliber from BD Biosciences (San Jose, CA) assist in determination of cells' percentage and their viability in several cell cycle's stage of both control and treatment.

Cell cycle analysis: The analysis of the conclusions of cell cycle through flow cytometry exhibits that in HeLa cells case, a significant arrest was observed in the cell cycle's G2M phase due to drug suspension. Upon comparing to the positive control (80.83±0.02%), the G2M phase cell cycle arrest was observed to be 42.86±0.03% & 33.82±0.05% at 40 and 20µg/ml correspondingly. Thus, the arrest observed was 11.45±0.33% and conclusions approved that drug is a potential cancer inhibitor as it can efficiently stops the proliferation of cell and arrest the division of cell.

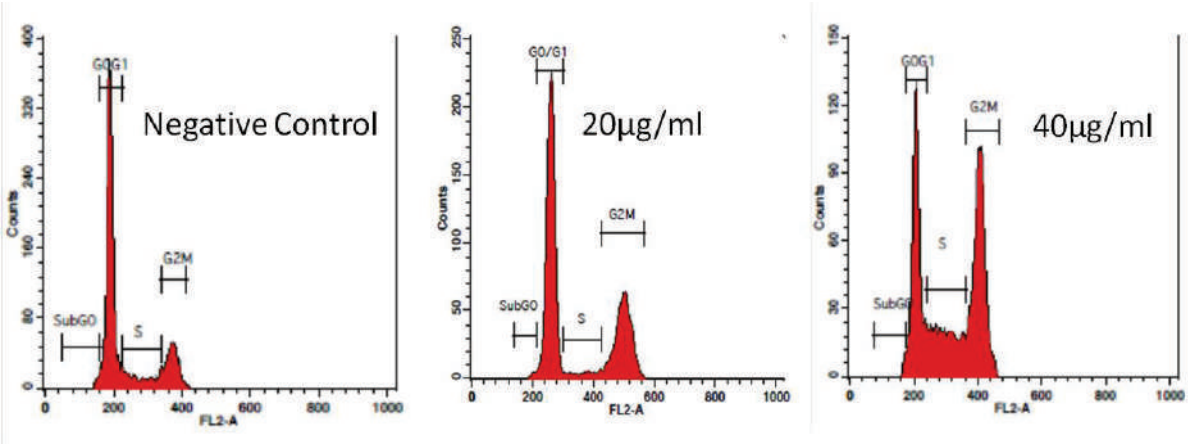


Figure 5: The outcomes of analysis of cell cycle are presented in the images. In which DMSO in 1% was utilized as a negative control whereas, all other values triplicate's average.

Docking studies: The outcomes of the patch Dock confirmed the potential drug action for the targets of drug. For each target, about four models were created while using SWISS model. Therefore, out of the eight merely 1 target got chosen with a higher score of patch Dock. Hence, the HIFA model score was 4716 whereas for HUR model was observed to be 4872 and both of these appeared to be appropriate as compared to the other models.

Conclusion

Relying on the substantial outcomes of the research, the potency of drug was confirmed on the cell lines of HeLa as colchicines. Although, the usage of drug in clinical settings reported no data for its molecular level validation. The two targets of drug in current research approves their potential function as an anticancer drug target and that can further be researched. However for the *insilico* studies, the receptors showed a potent role a fresh drug targets and most probably Dectexal drug may act on these.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Bacillus cereus in Meat Products: *16S rRNA* Phylogenetic Tree analysis and Antimicrobial Investigation of Nisin A, Rosemary Essential Oil and Tetracycline

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Abstract

In view of the importance of human food poisoning, this study was aimed to isolate *B. cereus* from meat sources that is economically problem affects both manufacturers and consumers. *16S rRNA* was used to identify bacteria as well as for constructing phylogenetic tree. The activity of different antimicrobial agents (nicin A, Rosemary essential oil and tetracycline) were determined using well diffusion method. *16S rRNA* analysis was successfully identified the three studied isolates from meat as *B. cereus* as well as the phylogenetic tree results observed that bacteria source in meat might be the soil. Rosemary essential oil at concentration 2000 µg/ml was more effectively then nisin A at 32 mg/ml. In conclusions, the *16S rRNA* sequences analysis was efficient for identification of *B. cereus*. Also, the higher antibacterial effect of Rosemary essential oil against *B. cereus* need further studies to be used as preservative agent for meat products.

Key words: *food poisoning bacteriocins, Rosmarinus officinalis, Bacillus cereus*

Introduction

Various kinds of microorganisms can cause food spoilage that is economically problem affects both manufacturers and consumers. *Bacillus cereus* is representing a common food contaminant, and is cause distinct forms of illness such as emetic and diarrheal. *B. cereus* is existing in various foods like meats, milk, vegetables. The counts of *B. cereus* in meat products were 2.9-4.59 Log CFU/g and it can grow well after cooking [(1)]. Nevertheless, some pathogens including the spore forming bacteria may appear lower infectious doses, foodborne disease related to jerky products have been increased. Several methods have been studied for microbial safety of jerky products such as additives to food, heating, and irradiation antagonistic toward pathogens like *Staphylococcus aureus*, *B. cereus, Listeria monocytogenes Escherichia coli* and *Salmonella Typhimurium*, but not handle the quality of jerky products during storage [(2)].

B. cereus sensu stricto known as a food poisoning associated pathogen with sometimes causes in humans soft tissue infections [(3)]. *B. cereus* is vastly distributed in the environment and have a relative genetic relationships to other species in the *B. cereus* group inclusive *B. thuringiensis*, and *B. mycoides* [(4)].

Identification of bacterial species by molecular methods is widely used. The *16S rRNA* gene is a powerful molecular tool which has been proven to be identified the species of bacteria [(5)]. Traditional methods used to detect *B. cereus*, such as selective media and biochemical tests as well as confirming the presence of protein toxin crystals, are time consuming. Molecular methods such as PCR are rapid for the identification these bacteria and many genes used for this purpose

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including *16S rRNA*, *23S rRNA*, *gyrB,* or *16S–23S rRNA* spacer regions [6,7].

Synthetic antimicrobial preservatives were used for many years in the food industry, but their consumption can lead to intoxications, allergies, cancer …etc. Thus, the search for natural products as new antimicrobial agents derived from animals, plants and microorganisms used in food industry [8].

Bacteriocins are divided into two classes: class I and II groups. The most studied one is lantibiotics subclass of bacteriocins, which inclusive nisin, staphylococcin C55, lacticin and mersacidin. Some of lantibiotics have potent activity against food-borne pathogens [9,10].

Plant essential oils have antimicrobial activities; however they limited used in foods due to their strong flavor. Lately, plant extracts as food antioxidant have been developed such as Rosemary extract that tested versus some food spoilage and foodborne pathogenic microorganisms under various conditions of pH, water activity, and temperature [11].

The meat products are widely consumed by the Iraqi people who give concerns about food poisoning caused by *B. cereus* pathogen. Thus, this study was focused on to isolate *B. cereus* from meat sources that deals with human food poisoning and to identify this microorganism used biochemical and molecular techniques as well as *16S rRNA* for constructing phylogenetic tree. Furthermore, compare between the activity of nicin, rosemary essential oil and tetracycline as antibacterial and food preservative material.

Materials and Methods

Isolates and culture media

One hundred food samples were collected from Baghdad markets from July 2016 to September 2016, including Fried meat, Grilled meat, and Beef burger buy from local market. All samples were primary cultured on serial dilutions were prepared, and 0.1 ml of each diluted sample was streaked in mannitol yolk polymyxin agar (MYP) a medium used for the enumeration, detection and isolation of *B. cereus* in food samples were incubated for 24 h at 30°C. The colonies (peacock blue) with lecithinase zone were transferred it on nutrient agar for further study [12].

Molecular assay

Extraction of DNA and polymerase chain reaction

The DNA of bacterial isolates was extracted based on the protocol of Wizard Genomic DNA Purification Kit, Promega. Concentration of DNA was measured by Quantus Florometer. Amplification of *16S rRNA* was done using the primers 27F (AGAGTTTGATCTTGGCTCAG) and 1492R (TACGGTTACCTTGTTACGACTT) (13). The PCR mixture was consisted of 12.5µl of GoTag Green Master Mix (Promega, USA), one µM of above primers and 2 ng/µl of tested DNA completed to 25 µl with nuclease free water. PCR protocol was conducted with an initial denaturation for 5 min at 95ºC; 30 cycles of denaturation for 30 sec at 95ºC, annealing for 45 sec at 60ºC, extension for 1min at 72ºC then final extension for 7 min at 72ºC. After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. Under UV transilluminator visulazation, the PCR product was separated in 1% agarose gel to determine the size of amplicons.

Sequencing

PCR amplicons were sending for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation, Korea. The sequences of 16S rRNA were built up from forward and reverse sequence data using Genious software. DNA sequencing data was analysed via Basic Local Alignment Search Tool (BLAST) of NCBI GenBank (http:// www.ncbi.nlm.nih.gov). MEGA7 sequence analyzing software with 500 bootstrap values was used for constructing the phylogenetic tree.

Antibiotic susceptibility test

All presumptive *B. cereus* isolates were confirmed by AST. The cultures were grow on Muller Hinton Agar plates and incubated at 37°C for 18 hrs with the following antibiotics: Penicillin (10µg⁄ Disc), Amoxicillin (25µg⁄ Disc), Ampicillin (10µg⁄ Disc), Ceftriaxone (5µg⁄ Disc), Cloxcillin (30µg⁄ Disc), Tetracycline (30µg⁄ Disc).The inhibition zone around disk were recorded and compared with Clinical and Laboratory Standards Institute [14].

Preparation of Nisin A

Nisin A (Sigma Nisaplin 2/5%, Germany) was prepared through dissolved with 100mg from Nisin A powder by 10 ml HCl (0.02 N) to give10^4 IU/ml (40 IU=1 g) concentration. After that the solution was passed throughout 0.45 filters for sterilization then stored at -20 ºC [15].

Preparation of essential oil of *R. officinalis*

The essential oil of Rosemary was extracted from leaves dried by air (250g) using Cleavenger hydrodistillation method. The distilled water with plant material was boiled for 3h, the essential oil was stored at 4ºC until use [16].

Prepared Tetracycline powder stock solution:

Described by Andrews (17): The (Tetracycline) stock suspension (50 mg/ml) was performed via solute 0.5g from vancomycin powder in 10 ml D.W.

Determination of the inhibitory effect of antimicrobial agents

Determination the antimicrobial effect of the nisin A, Rosemary essential oil and tetracycline on *B. cereus* planktonic cell using well diffusion assay method was applied according to [18].

Statistical Analysis

The data were analyzed using the SPSS IBM version 20. Least significant differences (LSD) test was done to investigate the differences between diameters of inhibitory zone of tested antimicrobial agent. Values were considered statistically significant $P \leq 0.05$.

Results and Discussion

Meat has exerted a crucial role in human evolution and is an important component of a healthy and well balanced diet due to its nutritional richness and importance in human nutrition as well as examine some pejorative beliefs about meat consumption [19].

The PCR results for four studied bacterial isolates were analyzed using gel electrophoresis that showed the amplicons size of *16S rRNA* gene was 1500 bp in Figure 1.

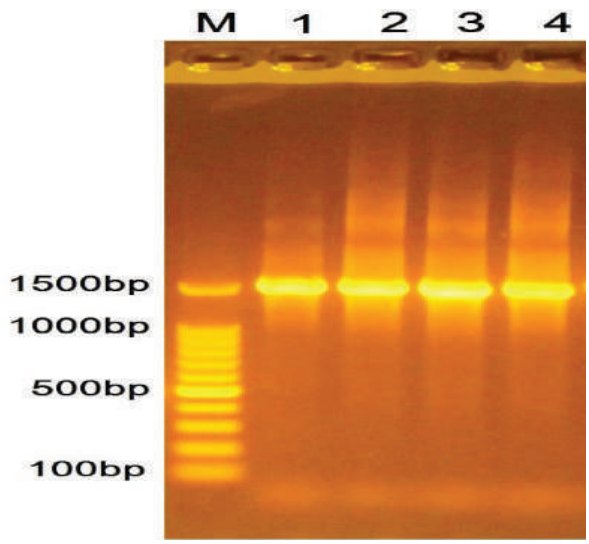


Figure 1. PCR product of the *16S rRNA* gene (1500 bp) from bacterial DNA stained with ethidium bromide. Lane M. 100 bp DNA ladder, Lanes 1-4. Unknown bacterial isolates. The product was electrophoresis on 1% agarose at 5 volt/cm2. 1x TBE buffer for 90 min.

The forward sequences of three PCR products of the *16S rRNA* gene were compared with the GenBank database using BLAST in NCBI website to find the related sequences. The three studied sequences showed high similarity to the sequences registered found in GenBank as illustrated in Table 1. BCM-1, BCM-2 and BCM-3 observed 99% identity to *B. cereus* ATCC 14579.

Among large numbers of genes within a bacterial genome, the *16S rRNA* gene play as an initial key for phylogeny based identification when compared against *16S rRNA* gene sequence GenBank databases [20]. The taxonomists concluded that 70% or higher DNA-DNA relatedness with 5% or less divergence within related sequences, together with 97% or higher *16S rRNA* sequence similarity, is the best means of defining a species [6].

Table 1. Bacteria isolated from meat identified according to the results of a BLAST on the GenBank database in NCBI.

Isolates	Accession number in GenBank	Closest species in GenBank database	Similarity Index			
			Score(bit)	E-value	Identity	Gap
BCM-1	NR_074540.1	Bacillus cereus ATCC 14579	2540	0.0	99%	0%
BCM-2	NR_074540.1	Bacillus cereus ATCC 14579	2582	0.0	99%	0%
BCM-3	NR_074540.1	Bacillus cereus ATCC 14579	2560	0.0	99%	0%

The phylogenetic results of *16S rRNA* for studied isolates from meat and strains from GenBank database showed that the first two isolates (BCM-1 and BCM-2) arranged together in a sister group but BCM-3 isolate divergent from them. All studied isolates in phylogram belong to *B. cereus* bacteria as well as showed direct lineage with *B. thuringiensis*. The *B. cereus* group has several *Bacillus* species that are related physiologically and have a significant genetic similarity to *B. cereus* bacteria including *B.cereus*, *B. anthracis, B. thuringiensis* and *B. mycoides* [14].

This gene is used in phylogenetic building and taxonomy due to it is universal found in different bacterial species. Because of rapid evolutionary rate that contributes to lower the percentage of gene similarities resulting in *16S rRNA* is appropriate for bacterial identification

till closely related species. Results of Bavykin *et al.* [6] showed that closely related species might be reach to 100% *16S rRNA* gene similarity which makes it difficult to distinguish between them. Whereas Wang *et al.* results observed the gene similarity reach to 99.2% when used *gyrB* [21]. Phylogenetic tree of the three studied isolates showed closer to *B. cereus* isolated from soil which suggest that the source of *B. cereus* in meat may be soil.

The results of disk diffusion method (Figure 2) showed various levels of susceptibilities to different antibiotics among studied isolates. The isolates of *B. cereus* observed as multi-resistance for used antibiotics especially against Amoxicillin and Cloxcillin while sensitive to Tetracycline that were similar to results obtained by [22].

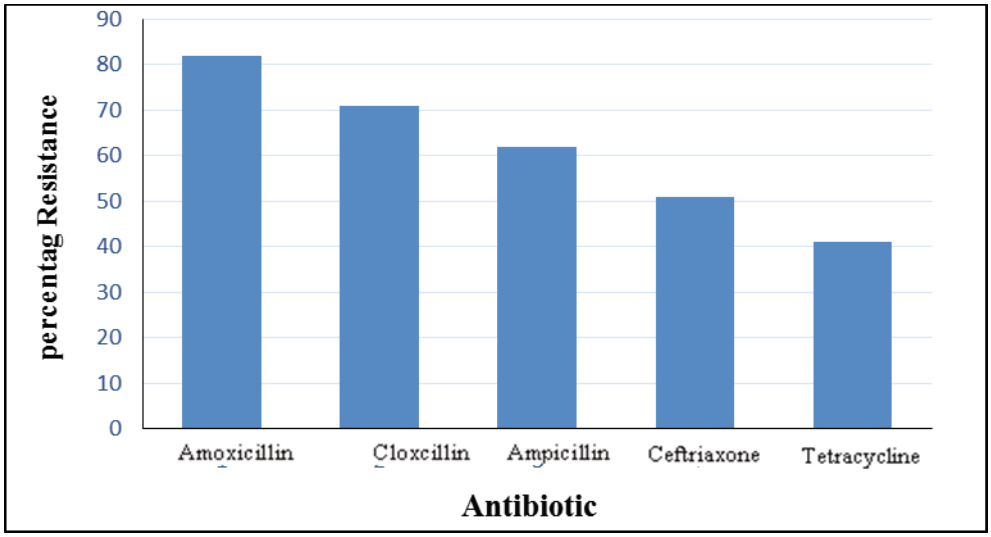


Figure 2. Antibiotic susceptibility of *B.cereus* isolates against group of antibiotics.

As shown in Figure 3, the antimicrobial agents used in this study showed highly significant differences (P≤ 0.01) in inhibitory zone diameters among them and among different concentrations of each agent except for the first concentration of niacin and tetracycline was not significant(P>0.05).

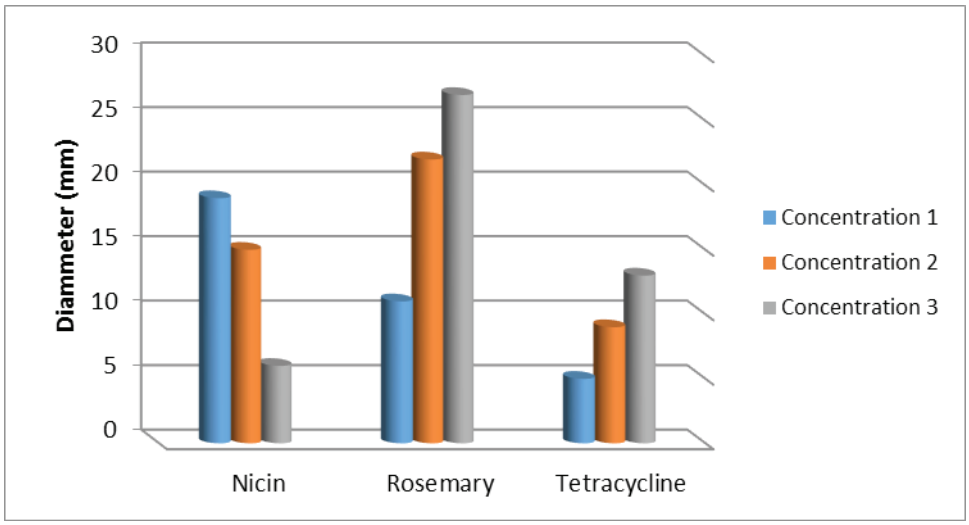


Figure 3: Inhibitory zone diameter of nicin A, Rosemary essential oil and Tetracycline at different concentrations against *B. cereus* bacteria

The nisin A inhibitory effect at concentrations 32 and 64 µg\ ml were measured as diameter that shown in figure 4 while at 128µg\ ml lower diameter. The result agreed with [22] the antimicrobial effect of nisin was investigated against *B. cereus* at different concentration during storage.

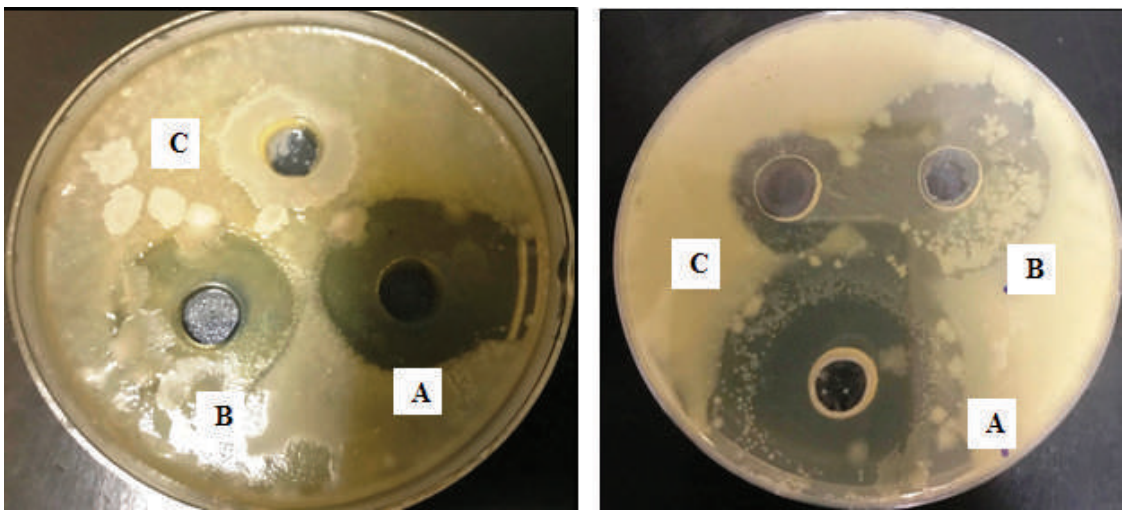


Figure 4: MIC for nicin A at concentrations (A) 32, (B) 64 and (C) 128 µg\ ml against *B. cereus* on Muller Hinton agar at 37°C for 24 hr.

The three concentrations (500,1000 and 2000 µg/ ml) of Rosemary essential oil against *B. cereus* observed diameter of inhibition zone reached to 11, 22 and 27 mm respectively (Figure 5). The result agreed with [23] which reported the rosemary extract abuse commercially in food as an antioxidant of lipids which was examined against foodborne microorganisms.

Natural herbal products and their derived compounds have used in many studies for controlling the growth of pathogens in food products. In investigation studies on *B. cereus* found that Rosemary essential oil have broad spectrum of antibacterial activity against this bacteria [24]. Combination of thyme oil (0.4%) with cinnamon oils (0.4%) decreased the count of *B. cereus* growth in

minced meat to reduction percentage 96.09%. Moreover, the shelf-life of mortadella has been extended with the use of rosemary/thyme EOs [25].

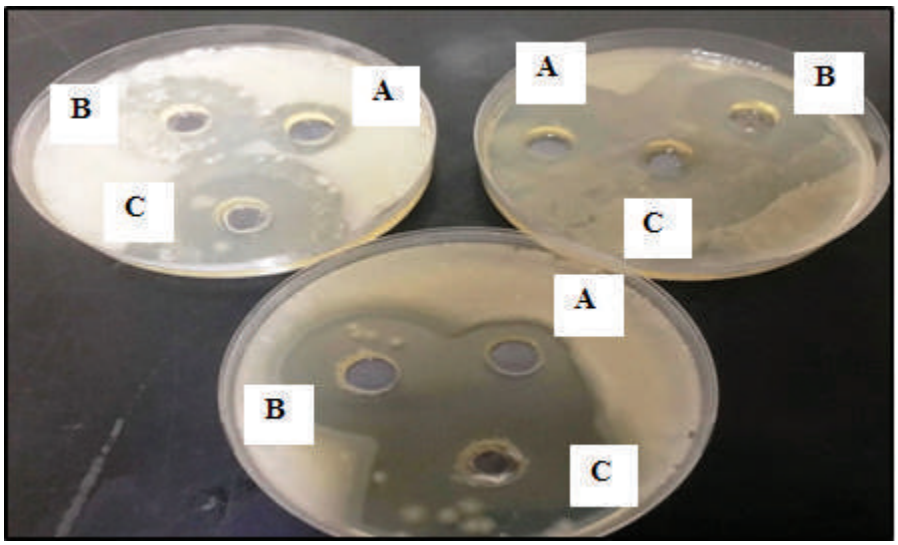


Figure 5: MIC for Rosemary essential oil at concentrations A) 500, B) 1000 and C) 2000 against *B. cereus* on Muller Hinton agar at 37°C for 24 hr.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Measurement the Chemical Composition and Topography of Copper Nickel Titanium Arch Wires at Dry and Wet conditions (An *In-Vitro* Study)

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Abstract

Background: The characteristics surface, such as, topography, and rigidity of orthodontic arch wires are essential determining factor of the effectiveness,of arch wire-guided tooth movement. They also affect, in the potential of corrosion,and the aesthetics,of orthodontic components. This study was considered to evaluate the chemical composition and topography of Cu-NiTi arch wires from different companies in,dry and,wet condition(acidic and,neutral artificial saliva). **Materials and methods** Three,types of CuNiTi orthodontic,archwires from,different brands were,used: CuNiTi (Ormco brand),, CuNiTi (IOS brand) and,CuNiTi (Orthotechnology brand).,They were rectangular,(0.018*0.025 inch) in cross,section and cut into pieces,of 20 mm in length.,30 pieces,from each,company were separated into 3 groups; one group was left dry condition,and other,2 groups, ten pieces,for each group were submerged in,artificial saliva,(pH=6.75 ± 0.015) and,(pH=3.5 ± 0.015) at 37ºC ,for 2 months. The SEM and EDS was used to assess surface analysis of wholly samples.,**Results:** After soaking period, CuNiTi wires from orthotechnology brand,exhibited the highest change in chemical composition and topography among the study groups. Instead, the least change in chemical composition and topography surfaces were CuNiTi wires from Ormco brand,when compared to,analogous arch wires. **Conclusion:** The CuNiTi arch wires from Ormco brand were the greatest and the most appropriate alignment in orthodontic archwires in term of the least changes in surface of Cu-NiTi initially and during the course of this study period.

Keywords: *Copper Nickel Titanium arch wires, scanning-electron microscopy and Energy dis persive x-ray spectrophotometer .*

Introduction

An appliance continued fixed on teeth within, attachment by,which the request of force occurred by arch wires or auxiliaries is called fix orthodontic appliance [(1)]. The one of most main portions of fixed appliance is arch wire, the perfect one should have capacity to move the tooth with a,continuous ,light force applied to it [(2)]**.** Nickel titanium- Copper chromium Alloy,(CuNiTi):This alloy was advanced by Dr.Rohit Sachdeva and Miyasaki,in 1994. It composed of (49.87%),nickel, (5.64%),Copper, (42.99%) titanium ,and (0.5%) chromium’’’[(3)]. To increase the thermal-reactive assets of the archwire and increase the transformation of the temperature to above the oral cavity and to recompense for this, copper additional to the alloy, the chromium is added to reduction the transformation of the temperature. Originally,’’ Copper NiTi was produced with four altered austenitic transformation temperatures covering both thermoelastic archwires and pseudoelastic ‘’[(4)]**.** The cobalt-chromium, stainless-steel,, and titanium alloys were,used in the orthodontic,appliances depend on the formation of passive oxide surface films to corrosion resist. These layers of protective are not,reliable.They are prone to both chemical and mechanical degradation.

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Even,without t destruction, oxide,films regularly dissolve slowly only to alteration as metal surface is,exposed tooxygen the from surrounding medium or from air [5].

The purpose of the present study was to analysis chemical composition and topography of copper nickel titanium archwires at dry and neutral , acidic of artificial saliva.

Materials and Methods

Formation of Artificial Saliva

The mechanisms of artificial saliva '' 0.7g NaCl , 1.2g KCl , 0.26g Na2HPO4 , 0.2g K2HPO4 , 1.5g NaHCO3 , 0.33g KSCN , 0.13g,urea and 1000,ml deionized water''.'' NaOH and Lactic acid were used to alter ''the pH of artificial saliva using the pH-meter'' and kept in,37°C using incubator using filter paper after filtering to get clear of any unsolvable impurities and salts. The,pH of artificial saliva,was attuned to 6.75±0.015 and 3.5±0.015 using a pH meter (Jenway,,Cyprus and model 3320, equivalent to the pH of human salivary'' **(6-8).**

The Samples

The samples,were made of (3)types of maxillary archwires rectangular in cross-section ((0.018*0.025 inch)):

1. CuNiTi,archwires (Ormco corp., Glendora, CA, USA),

2. Copper Nickel,Titanium archwire,(((Ortho Technology®, USA, TruFlex™, full form))

And 3. CuNiTi archwires (IOS, International Orthodontic Services, Stafford, USA).

The straight parts of the arch wire were cuting into 20 mm in length(9). Tottaly was 90pieces of arch wire, 30,pieces from each type, of arch wires, were divided in,the groups that 10 parts from each,company retain in a dry,condition ''as a control group'', although the other,20 parts,were submerged,in artificial saliva,for 2 month ,ten pieces,from each,groups were submerged,in pH,of artificial,of saliva 6.75 and ,pH = 3.5.

Afterward, samples,was employed,in glass containers,seperated and held in from its,ends held by,dental floss .Then, ,added of artificial,saliva so that,sample,was submerged,completely. **(Figure1)**.



Figure(1): samples in the artificial saliva.

Then,the samples, were saved in an ,incubator, at 37 0C in (Fisher scientific) for two month .Then, artificial saliva was changed regularly every seven days with a fresh solution to evade its,saturation with ,the products,of the, degradation (10,11).After the 2 month the ,samples were,washed by distilled water. Then, left to dry and,preserved in,petri dishes.

Specimens

then, it desires to use,minor slides With the determination of use scanning electron microscope and energy dispersive x-ray spectrophotometer for analysis the sample .The cut of slide by a diamond,cutting instrument. Then, the sample fixed on a,the slide.

The studies of orthodontic materials in artificial salivas on the corrosion, resistance are very ,popular. In the current work, the ,changes of the surfaces of CuNiTi alloy of the orthodontic wires in neutral artificial saliva ph=6.75 and acidic artificial saliva ph= 3.5 after 2 month are examined by the Scanning Electron Microscopy and Energy dis persive x-ray spectrophotometer EDS methods.

Results

Surface micromorphology of the Copper Nickel Titanium in dry and neutral ,acidic artificial saliva

Surface micromorphology of the Copper Nickel Titanium was evaluated before and after immersion in neutral and acidic of artificial saliva, visualized at 2000X magnification using SEM and the surface topography was assessed using EDS, as shown in (figues: 2) in dry condition.

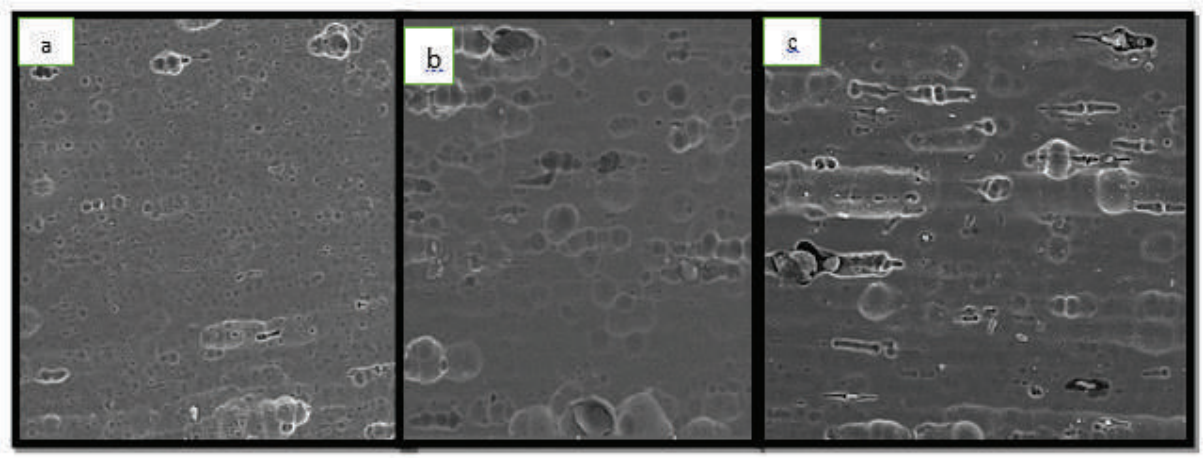


Figure (2): Surface micromorphology of CuNiTi arch wires at 2000X magnification using SEM. A) represents the surface of ormco brand at dry condition ; b) represents the surface of IOS brand at dry condition ; c) represents the surface of orthotechnology brand at dry condition.

It was found that after CuNiTi archwires immersion in,artificial saliva exaggerates surface changes of the wires, this was represented by appearance of,numerous pits with different depth and size, as being reflected,by the increase,in number and height of beaks ,Concomitant with immersion,in artificial saliva for 2month.

So that the control groups (dry condition) exhibited increased number of pits with ,shallow depth. It is possible, that the pits ,represented that roughness ,and artificial ,saliva enhanced ,the depth ,and number ,of these pits. ,Indeed, with,orthotechnology and IOS brands, major changes occurred representative by presence of great fissures ,connecting pits and area of surface break. Nevertheless, with Ormco brand, the pits were more distributed regularly through the surface with the smallest changes, occurred compared,to other brands.

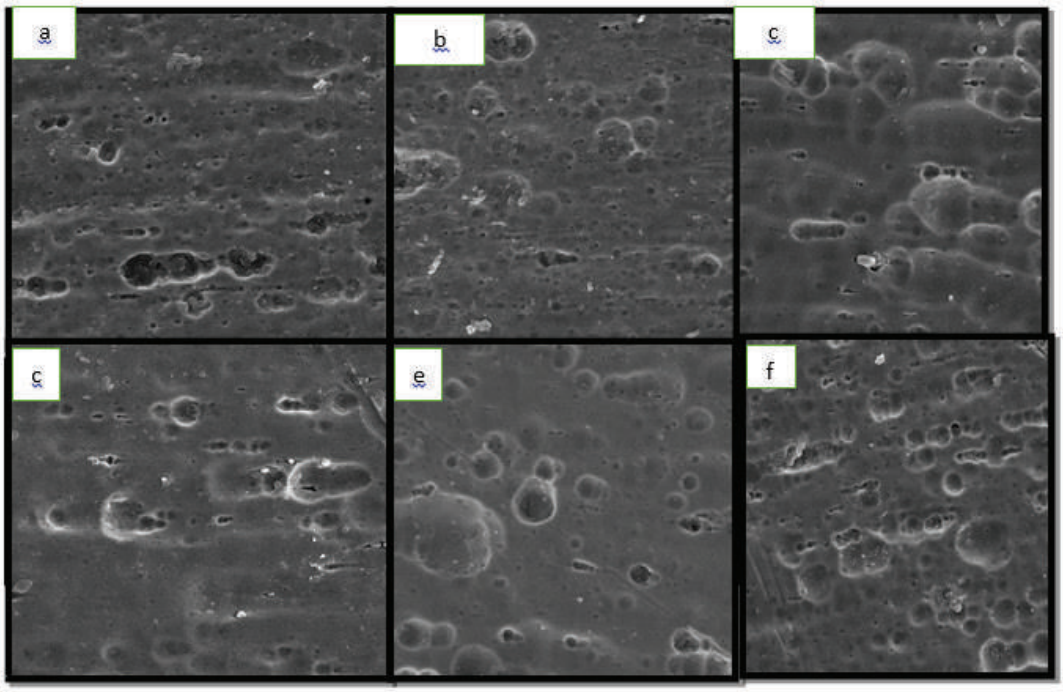


Figure (3) : Surface micromorphology of CuNiTi from different brands at 2000X magnification using SEM. A) represents the surface of ormco brand in media 3.5 of artificial saliva ; b) represents the surface of ormco brand in media 6.75 of artificial saliva ; c) represents the surface of IOS brand in media 3.5 of artificial saliva ; D) represents the surface of IOS brand in media 6.75 of artificial saliva; e) represents the surface of Orthotechnology brand in media 3.5 of artificial saliva; f) represents the surface of Orthotechnology brand in media 6.75 of artificial saliva.

Chemical element composition of Copper nickel titanium brands

Chemical composition of every CuNiTi brands was shown in, table (1) the percentage of element, of copper nearly the similar in all,brands in dry condition while copper element decrease in both Company of IOS and orthotechnology in acidic and neutral artificial saliva, while titanium element in all brand is increase in neutral and acidic condition while Ni element decrease in all brands and O element was increase in all brands in neutral and acidic artificial saliva condition.

Table: (1) chemical element composition of CuNiTi of each brand

Brand	Ormco			Orthotechnology			IOS		
Element	Wt%			Wt%			Wt%		
	Dry condition	Neutral Artificial saliva	Acidic Artificial saliva	Dry condition	Neutral Artificial saliva	Acidic Artificial saliva	Dry condition	Neutral Artificial Saliva	Acidic Artificial Saliva
O	5.34	10.88	11.21	5.16	11.10	10.93	5.96	11.91	12.76
Ti	41.96	59.18	59.56	42.21	61.09	60.41	42.16	60.43	57.80
Ni	47.37	24.21	24.22	47.14	24.54	24.80	46.16	24.24	23.38
Cu	5.33	5.74	5.01	5.49	3.27	3.85	5.72	3.43	6.05
Total	100	100	100	100	100	100	100	100	100

The SEM examination, ,formed oxide-based particles seem in dark - gray contrast (Fig.3). It is obviously seen concentration of oxygen is higher ,than in the matrix. This means that, Ti-based, oxide is,formed as result of tough interaction stuck between the solution and metal. The dissolution,of nickel, from the matrix, into the solution, is very,high. All effects,can be,traced by ,considering the ,concentration differences in the EDS,analyses. The EDS mapping shows to be very beneficial in understanding ,the elemental, distribution for,given materials. Nickel, and titanium,are the major elements on surface and oxide-based, particles can be simply separated from,the matrix because of their oxygen ,level.

The EDS ,studies show to be very beneficial in determining components,formed on the,surface of the,metal as result of corrosion,in the artificial saliva ,after a period ,of time.

The results OF EDS shown in (Table 1) indicate that a clear reduction in the amounts of Ni and Ti because of the dissolution,of the metals.From the,clinical point,of view,,the final result of orthodontic,treatment may,be compromised,by corrosion, and metal ions appearing as corrosion,products (Ni2+) may,result in,symptoms of,toxicity andallergic reactions [12]. The oxide,film behaves,as a protective,layer during,corrosion in,aggressive environments [13].

'' Titanium exists in the solution as TiO2 having a more stable form''. Ni element reduction in composition,of wires and,Ti increase,that because could be,attributed to,the fact that addition of small quantity,of Cu to,the structure of archwires reduced the,reactivity of Ti and increased resistance to,corrosion and,biocompatibility of CuNiTi arch wires[14].

'' However, the manufacturing process of archwires containing Cu in their composition uses a sensitive and not very consistent technique''[15]

The degree,of defects in manufacturing and the composition of orthodontic applanices are factors well-known to accelerate process of corrosion and release ion by archwires [14-16] these consider that unstable ''chemical structures'' might cause release of ion and affect on the biocompatibility of materials[17].

In regrard This result could also be attributed to the archwire manufacturing process. The afundmental part of biocompatibility is Resistance to corrosion and might be affected by altered factors[20] the main depends on, the type of archwire, manufactureing process and characteristics of the archwire surface.[20-23]. The studies by **Huang et al(2003)**[16] and **Huang et al(2005)**[24] decided that nickel titanium archwires of orthodontic from altered manufacturers would have resistance to corrosion in different degrees .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Teratogenic Effect of Carbamazepine Drug on the Histological Structure of Testes in the Albino Mouse (*Mus musculus*)

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Abstract

The aim of this study was to evaluate the side effect of Carbamazepine on the histological structure of the testis in white mouse males.

A total of 24 male of Swiss white mice were divided into three groups, treated with Carbamazepine Drug for (30) days. Within each treatment period, animals were assigned into three subgroups each of eight mice; control treated with vehicle and the others treated with 200, and 300mg/kg of Carbamazepine, The required dose of the drug was dosed orally for all mice once per day for one month (30 days) for two concentrations. At the end of treatments, all mice were sacrificed; Testes was removed and weighed then kept in buffered formalin solution for microscopic examination.

The results of this study showed that animals that were injected with Carbamazepine caused a change in the shape and thickness of the wall of the somniferous tubules, where the appearance of the irregular, and also observed the occurrence of an embolism in some germ cells and collected in the center of the cavity of the somniferous tubules in addition to the observation of Vaculation and necrosis in most areas of somniferous tubules.

The results of the current study suggested the possible time and dose-dependent disrupting potential of Carbamazepine Drug on the Histological Structure of Testes in the Albino Mouse.

Keywords: *testis, Sertoli cells, Carbamazepine, seminiferous tubules, germ cells*

Introduction

Epilepsy is a chronic disease characterized by frequent bouts caused by a defect in the transmission of electrical signals in the brain. This may be happened due to genetic factors or due to various brain damage such as stroke [(1,2)]. Defects and disorders of the reproductive system are more prevalent among men (males) who suffer from epilepsy compared to other healthy people [(3)]. Both epilepsy and anti-epileptic drugs containing aromatic compounds like phenol[(4)] may play an important role in creating these disorders, so it is difficult to distinguish between the direct negative effects of epilepsy and anti-epileptic drugs on the reproductive system[(5)].

One of the most famed drugs used for treating epilepsy is Carbamazepine (CBZ), commonly known as Tegretol, which is a major drug used to treat epilepsy and nerve pain, but it is not effective in coma bouts. Carbamazepine is used in the treatment of schizophrenia in combination with other drugs; and it is considered as the first generation of drugs with appropriate

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pharmacological properties and appears to be effective in improving control of nerve attacks[6].

There are some studies that refer to changes occur in the form of testis and the occurrence of infertility and deformities in the sperms in people who treated with Valparvat 3,3-5 carbamazepine (CBZ) drug [7].

The aim of this study was to evaluate the side effect of Carbamazepine on the histological structure of the testis in white mouse males.

Materials and Methods

Preparation of Carbamazepine Required Dosage

he dose of carbamazepine was prepared depending on the half lethal dose (LD_{50}) of the drug which was 529 mg/kg b.w. [8]. Two doses of Carbamazepine were selected 300 and 200 mg/kg to test its toxic effect, while the weights of mice used in the experiments was ranged between 20-30 g. The required dose of the drug was dosed orally for all mice once per day for one month (30 days) for two concentrations. It was possible to calculate the amount of drug used for the mice in this study based on the following equation:

$$\frac{x}{D} = \frac{W_{mouse}}{1000}$$

Where: x: The required amount of drug to be dosed for mice in the experiment, (g)

W_{mouse}: The body weight of the mouse, ranging among 20-30 (g)

D: The selected dose of carbamazepine drug which were 200 or 300 mg/kg

Animals used in the laboratory experiments and histological study

In this study, 20 male of Swiss white mice (*Mus musculus*) was used to achieve the practical experiments part. The mice obtained from animal house in biological department-college of science/Baghdad University. The average weights of used mice were ranged between 20-30 g and its age were between 8-10 weeks. These mice were divided randomly to two groups of the following details: the 1st group was the control group which has 4 mice. The 2nd group was tested group which contains 16 mice, and this group was sub-divided into two equal groups, 8 mice was belonged for each sub-group. The mice in one sub-groups was treated with the carbamazepine dose of 200 mg/kg while the other sub-groups dosed its animals with the second carbamazepine dose which was 300 mg/kg. Each tested doses (i.e. 200 and 300mg/kg) were subjected for mice for 30 days. When the experiments were finished after one month, all mice were anesthetized with chloroform, then the animals were excised and the testes were removed from its position. The samples were fixed with formalin solution for 24 hours, and transferred to 70% alcoholic solution after washing with tap water for storing. The histological sections of samples were prepared according to the method used in Bancroft and Gamble [9].

Results and Discussion

There are many different parameters that influence on the formation of sperms, among that factors are chemical agents such as drugs and toxic chemicals that are contaminated the environment [10]. The results of the present study showed that the mice that were treated with a dose of 200 mg/kg of carbamazepine drug had histological variations represented by shrinking and increasing the thickness of seminiferous tubule, which resulted with irregular appearance. In addition, it could observe atrophy in some Seminiferous tubule, as shown in Figure (1). These results are agreed with the results obtained by [11] which reported that the basal plate has a vital role in maintaining the transfer of materials between the interstitial tissues and germs Spermatogonic and also maintaining the composition and function of these tissues, from another hand. While [12] noted that the increased in the wall thickness of Seminiferous tubule reduces the relationship between it and the interstitial tissue and thus cause the emergence of several disease disorders inside the testis, which will affect the differentiation of germ cells and inhibition of sperm formation. The reason for thickening of Seminiferous tubule walls, as illustrated by [13] was collagen fibers IV fiber which secreted by the Sertoli cells, thus lead to weak the formation of sperms. One of the results of the current study also was the absence of sperm Seminiferous tubule in some seminiferous tubule as well as the presence of vaculation in the testicular areas, in addition to the widening distance between germ cells, dissociation of

the epithelial tissue and collected in the cavity of sperm. Also there was a degeneration occurrence in the cells of Sertoli and increase the area between the cells of the nearby Sertoli cells. This is clearly demonstrated by Figure (2). On the other hand, Figure (3) shows the appearance of large Macrophage cells within the seminal vesicle cavity. disorder happening in the Sertoli cells will inevitably affect the germ cells and eventually lead to a defect in testicular tissue and this is what the results reached by [14,15] were explained the essential role of the Sertoli cell in the development of germ cells through the formation of a testicular blood barrier that protects germ cells and transfer of the nutrients and hormones to germ cells.

It believes that all these signs of disease are occurring due to a malfunction in the structure and function of Sertoli cells. The results of the current study revealed that the apparent effect of the drug on the Leydig cells and interstitial tissue via emergence of decay and necrosis and also the emergence of Vaculation in the interstitial tissue as shown in Figure (2). This result is identical with the result of [16] that the Leydig cells are the center of fertility regulation through the production of testosterone hormone, while [17] pointed to the Leydig cells are promoted by the hormone LH Luteinizing hormone, arachidonic acid and hormone testosterone. The results of current study also showed that the shrinkage was increased of sperm tubes and the depletion of some germ layers of these tubes in mice treated with a concentration of 300 mg/kg of the drug in addition to the occurrence of degeneration and explorations, apoptosis in the Spermatogonia, primary sperm cells and Spermatid and mature sperm the return of Spermatid and mature sperm into of Seminiferous tubules while many of the of Seminiferous tubules were empty of germ cells and as shown in Figures (4 and 5). These results are getting along with the results of [18] in their study on sperm characteristics and the precise composition of testes in rats after long-term with methanol treatment. They attributed the cause of the disorder in the Sertoli cells, which will be affected by the basic proteins required in the process of synthesis and necessary for the differentiation of bacterial cells, which produce these proteins at the highest level during the stage of differentiation of sperm. Also, the result of this study is agreed with [19] which show that the regressive movement of Spermatid and sperm within the wall of somniferous tubules may be due to an alert to testicular toxicity resulted by the drug.

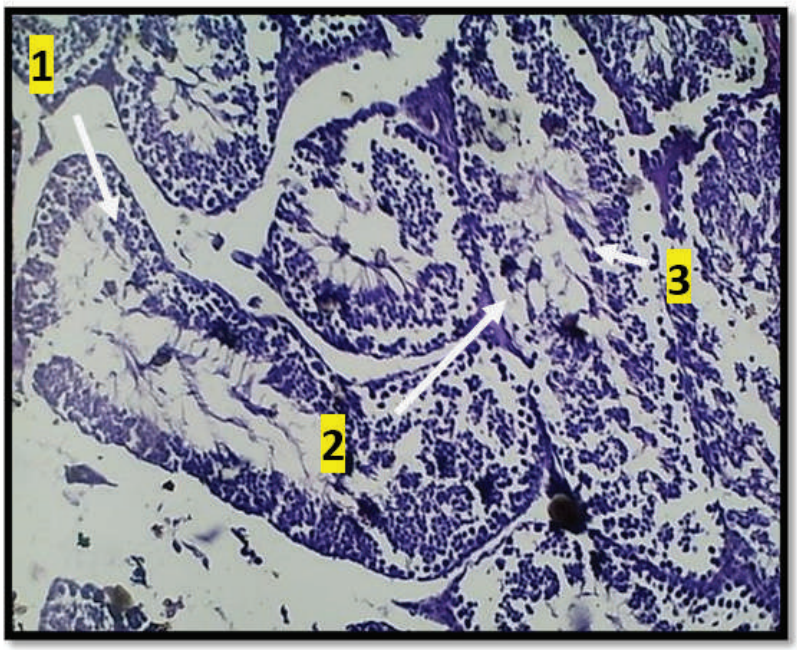


Figure 1: Parasagittal section of the Testis of mice receiving with 200 mg/kg carbamazepine drug for one month showing 1) irregularity of the semeniferous epithelium, 2) vaculation, 3) necrosis of the semeniferous epithelium. H&E, 40×

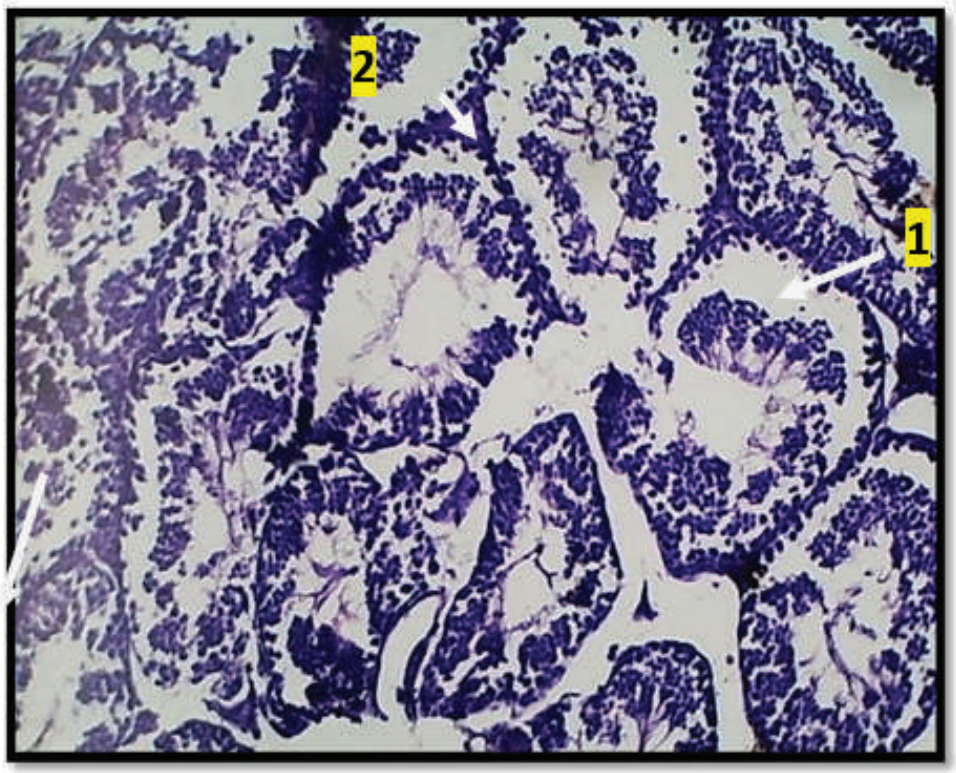


Figure 2: Parasagittal section of the Testis of mice receiving with 200 mg/kg carbamazepine for one month showing 1) aggregate the germinal cells in the lumen of Seminiferous tubules, 2) necrosis of the semeniferous epithelium. H&E, 40×

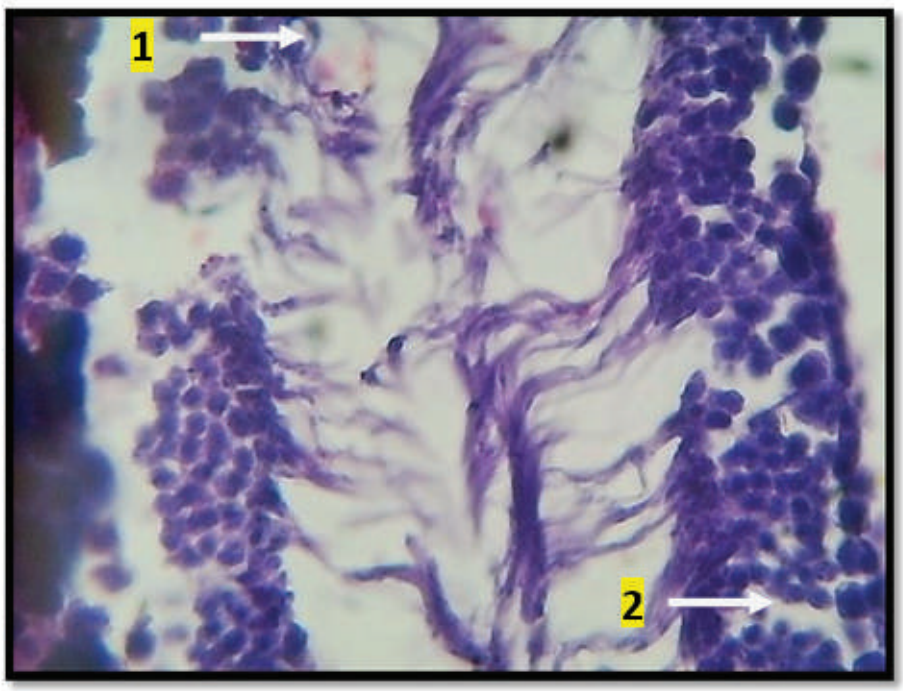


Figure 3: Parasagittal section of the Testis of mice receiving with 200 mg/kg carbamazepine for one month showing, 1) The emergence of large cells phagocyte within the cavity semeniferous, 2) degeneration in Sertoli cells. H&E, 40×.

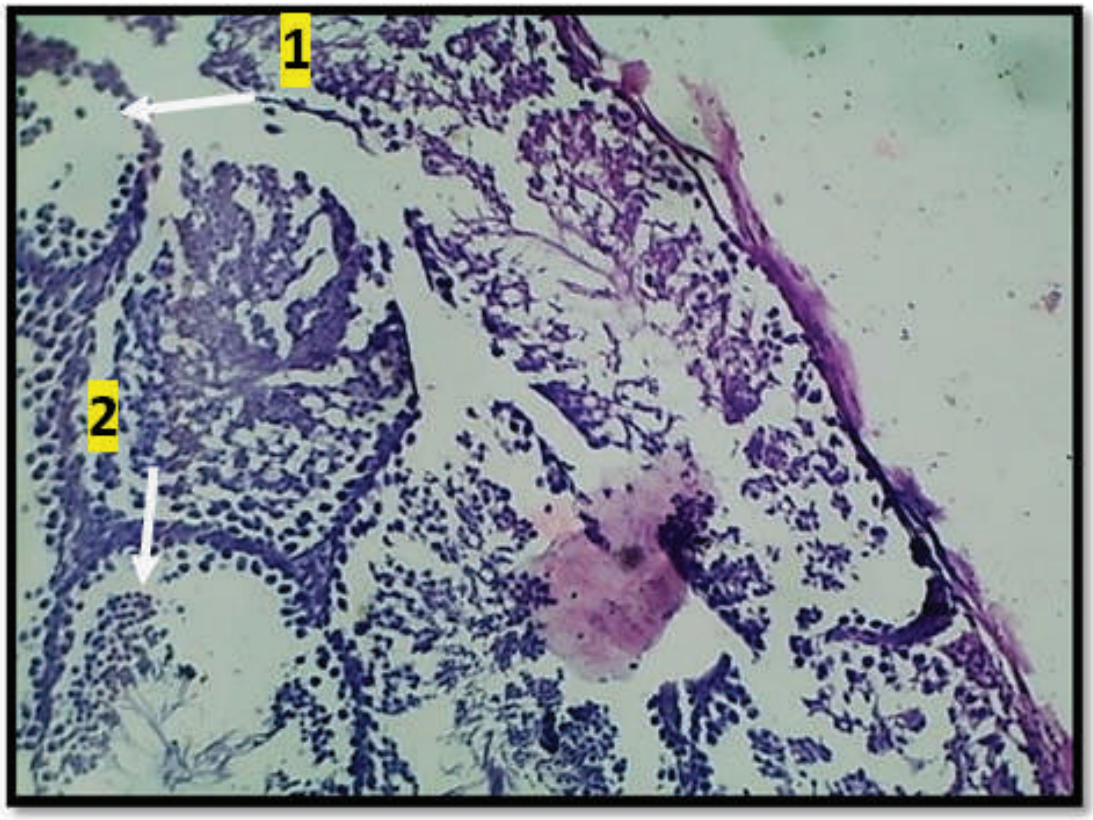


Figure 4: Parasagittal section of the Testis of mice receiving with 300 mg/kg carbamazepine for one month showing 1) Empty the Seminiferous tubules from germinal cells, 2) necrosis of the semeniferous epithelium). H&E, 40×.

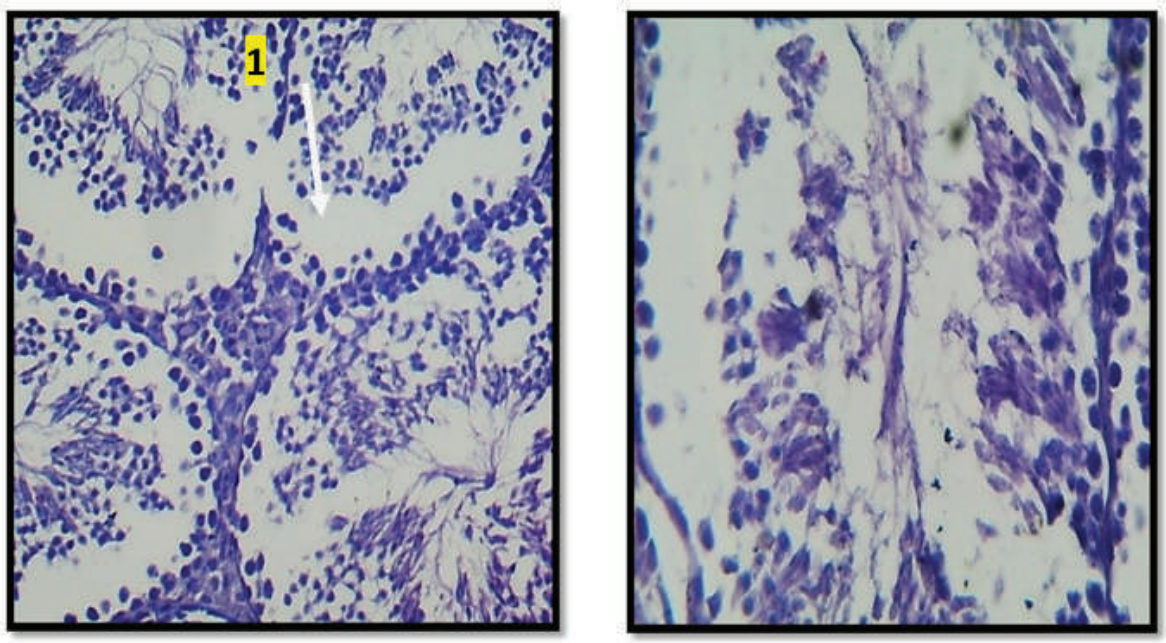


Figure 5: Parasagittal section of the Testis of mice receiving with 300 mg/ carbamazepine for one month showing 1) Empty the Seminiferous tubules from germinal cells and, degeneration in Sertoli cells. H&E, 40×.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Continuous Flow Injection Analysis,Turbidmetric and Photometric Determination of Methyldopa Using a New Long Distance Chasing Photometer (NAG-ADF-300-2)

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Abstract

A specific, sensitive and new simple method was used for the determination of methyldopa in pure and pharmaceutical formulations by using continuous flow injection analysis. This method is based on formation of ion pair compound between methyldopa and potassium hexacyanoferrate in acidic medium to obtain a yellow precipitate complex using long distance chasing photometer (NAG-ADF-300-2). The linear range for calibration graph was 0.05-35 mmol/L for cell A and 0.05-25 mmol/L for cell B, and LOD 1.4292 µg /200 µL for both cells with correlation coefficient (r) 0.9981 for cell A and 0.9994 for cell B, RSD% was lower than 0.5 % for n=8 for. The results were compared with classical method UV-Spectrophotometric at λ_{max}=280 nm and turbidmetric method by using the standard addition method via the use of t-test, at 95% confidence level. The comparison of data explain that long distance chasing photometer (NAG-ADF-300-2) is the choice with excellent extended detection and wide application.

Keywords**:** *Methyldopa, Attenuation of light, Continuous flow injection analysis, Turbidity.*

Introduction

Methyldopa (Aldomet), the L- isomer of alpha-methyldopa, is levo-3-(3,4-dihydroxyphenyl)-2-methylalanine.Molecular Weight 238.2 g/mol, Molecular Formula $C_{10}H_{13}NO_4.1.5\ H_2O$.It is a white to yellowish white, odorless and soluble in water, methanol and ethanol. Methyldopa widely used in the control of moderate and severe arterial hypertension, structure of methyldopa in figure 1[(1-4)]. Several methods depend on continuous flow injection analysis [(5-15)] and several analytical methods have been proposed to quantify methyldopa in pharmaceutical formulations such as UV- VIS Spectrophotometry and FTIR [(1)], UV- Vis spectrophotometry [(2-4, 16-18)], continuous flow injection analysis [(19)], voltammetry method [(20)], HPLC [(21)], capillary zone electrophoresis [(22)], Gas chromatography [(23)].

In this work using flow injection analysis turbidmetric method, the turbidity is measured via reflection of the incident light from the surfaces of particulate formed between methyldopa and potassium hexacyanoferrate in acidic medium at $0\text{-}180^0$ by using homemade long distance chasing photometer (NAG-ADF-300-2) [(24)].

Chemicals and Apparatus

Reagents and chemicals

Every chemicals were used of analytical reagent grade and all the solutions dissolved by distilled water. A standard solution of 70 mmol/L of methyldopa, was prepared by dissolving 1.6674 g in 100 ml. A series of potassium hexacyanoferrate solutions were prepared from the dilution of standard solution 200 mmol/L with distilled water.

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Apparatus

Using the manifold homemade NAG-ADF-300-2 is a long distance chasing photometer as a flow cell will have 300 mm as a distance with 2 mm as a path length to chaise and to accumulate the output resulted from attenuation of incident light 0-180^0 and diverged or fluorescence light at 0-90^0 via a flow cell. The first flow cell is of 110 mm length covered with 11 white snow LED (WSLED) followed by uncovered distance of 100 mm length then attached to another with 2 solar cell at each side of (0-180^{0}and 0-90^0) cell (cell number 2) which is covered by 6 WSLED and a single photo cell (solar) of 60 mm length at each side was used with peristaltic pump (Ismatec, Switzerland) and six-port medium pressure injection valve (IDEX Corporation, USA) with sample loop (1 mm i.e. Teflon, variable length). Potentiometric recorder to estimate the output signals (Siemens, Germany). UV-Spectrophotometric (RF-1501, shimadzu, Japan) was use for classical methods. A turbidimetry instrument, HANNA Company (Hungary), which is used for classical method measurement of turbidity at 0-180^o.

Results and Discussion

Study of the optimum intensity used for cell A and cell B

A study was conducted for the effect of intensity of incident light for the irradiation sources on the response of the energy transducer. The selector switch gives 0-1-2-3-4 four choices plus the off position for both cell individually controlled. The optimum intensity of the measuring cell A, I=3 and I=2 for cell B which was adopted in subsequent studies.

Optimization of variable

Chemical variables

-Potassium hexacyanoferrate concentration

A series of multiple concentration of precipitated reagent 3-20 mmol/L were prepared 100 µL volume of sample was injected through the carrier stream distilled water. 20 mmol/L concentration of methyldopa was injected with 3.2, 4.6 mL/min flow rate for carrier stream and reagent respectively. It was found that an increase in peak height with increase of potassium hexacyanoferrate concentration. It is possible that might be attributed to the increase of coloured precipitate particulate which in turn work on attenuation of part of the incident irradiation light plus it's absorption due to its coloration. While at higher concentration (i.e. > 18, 15 mmol/L respectively to for cell A and cell B) might lead to increase agglomeration of precipitated particulate and an increase of inter spatial distances which help to increase penetration light toward the detector and a decrease in the response height, Therefore 18, 15 mmol/L was selected as optimum concentration for cell A and cell B respectively.

-Effect of different medium

Various solutions were used as a carrier stream and the effect on the reaction between methyldopa (20 mmol/L) with potassium hexacyanoferrate. Different solution media (NH_4Cl, CH_3COONH_4, NaCl, KBr, Na_2SO_4, CH_3COOH, H_2SO_4, HNO_3, HCl) at 50 mmol/L concentration in addition to aqueous medium. It was noticed for the studied; different media causes to a decrease of S/N-response; this might be attributed leads to its effect in an increasing the agglomeration i.e.; increase the density of aggregates and compactness with each other than increase the intensity of transmitted light as there will be more vacant spaces in between agglomerates of particulate except H_2SO_4 which leads to an increase of S/N-response; this might be because increase of particles size leads to increase the space between the particles and increase of transmitted light toward the solar cell, therefor H_2SO_4 medium was used as a carrier stream in the next studied.

-Effect of variable concentration of sulphuric acid

Variable concentration of sulphuric acid at ranging 10-90 mmol/L were used to dilute the prepared solutions, 100 µL sample volume at 3.2 ml/min and 4.6 ml/min flow rate for carrier stream and reagent solution respectively. It was noticed that an increase of sulphuric acid concentration after 30 mmol/L causes a decrease in S/N- transducer response, Therefore 30 mmol/L of H_2SO_4 concentration was chosen as optimum carrier stream. Figure 1 shows a proposed expected mechanism for the reaction of methyldopa with potassium hexacyanoferrate in acidic medium [1, 25-27].

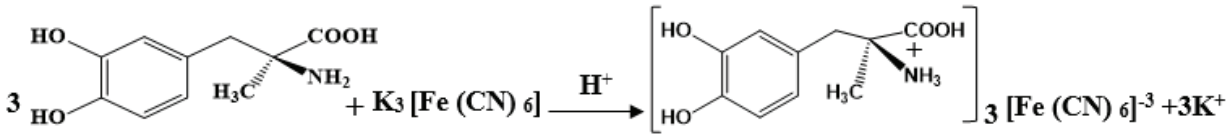


Figure1: Probable mechanism pathway for the reaction of methyldopa with potassium hexacyanoferrate.

Physical variables

-Flow rate

Fixing all previous experimental parameters concerning chemical variation for methyldopa- potassium hexacyanoferrate system. Variable flow rate study were carried out using 0.7- 4.8 ml/min for carrier stream line while 1-7.4 ml/min was used for precipitating reagent solution line. It was noticed for figure 2. A and B that up to 2.6 ml/min of carrier stream for cell A gave good S/N peak profile While up to 1.4 ml/min of carrier stream for cell B causes a decrease in S/N- transducer response because the reaction is slowly and needing some time to complete so we observe responses to the distorted for cell A. Therefore; 3.2 & 1.4 ml/min flow rate for carrier stream and 4.6, 2 ml/min flow rate for reagent respectively to for cell A and cell B was selected as optimum flow rate for either cells.

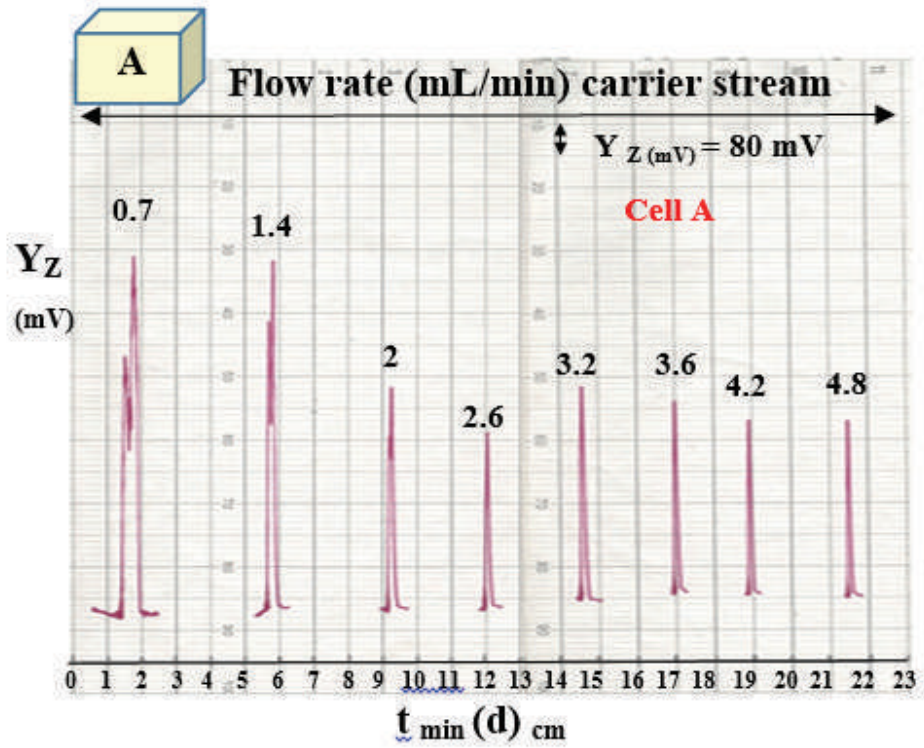


Figure 2: Effect of the variation of flow rate on: A; S/N energy transducer response versus $t_{min}(d)_{cm}$ using methyldopa–potassium hexacyanoferrate system, 100 µL of sample volume.

-Sample volume

Using methyldopa–potassium hexacyanoferrate system and Variable length of Teflon tube ranging (2.6-25.5) cm of diameter (D) 1 mm that is equivalent to (20-200) µL of sample volume. It was noticed that an increase of sample volume reaching to 200 µL lead to a significant increase in response height. Therefore 200 µL was chosen as optimum volume of sample.

Estimating the linear dynamic range from scatter plot for the variation of methyldsopa

versus S/N energy transducer response

A series of methyldopa solution (0.05-40 & 0.05-37 mmol/L) for cell A and cell B respectively were prepared this will represent the x-axis (Independent variable) using the optimum chemical and physical parameters. Table 1 explains the variance ranges for each cells.

Table 1: Summary of result for linear regression for the variation of S/N energy transducer response with methyldopa concentration using first degree equation of the form $\hat{Y}$=a + b x at optimum conditions

Type of mode	Range of [methyldopa] mmol/L(n)	$\hat{Y}$Zi(mV)= a mV± Sat+ b(Δ y mV/Δxmmol/L)± Sbt [Methyldopa] mmol/L at confidence level 95%, n-2	r, r2, R2%	t tab at 95%,n-2	Calculated t-value tcal=/r/√n-2/ √1-r2
Cell A					
Cell B					
Scatter plot	0.05-40 (24)	186.4920±54.2718+59.8484±2.8040 [Methyldopa]mmol/L	0.9944, 0.9889, 98.89		2.074 << 44.2701
	0.05-37 (22)	159.8407±99.0871+48.3620±5.8548 [Methyldopa] mmol/L	0.9679, 0.9369, 93.69		2.086 << 17.2318
Dynamic range or analytical range	0.05-37 (23)	167.7994±34.5164+62.1480±1.9257 [Methyldopa] mmol/L	0.9977, 0.9954, 99.54		2.080 << 67.4110
	0.05-30 (20)	91.0043±48.0221+58.1070±3.5272 [Methyldopa] mmol/L	0.9926, 0.9852, 98.52		2.101 << 34.6162
Working range or calibration range	0.05-35 (22)	159.7006±30.2236+63.2344±1.8269 [Methyldopa] mmol/L	0.9981, 0.9962, 99.62		2.086 << 72.4098
	0.05-25 (19)	66.5374±12.8752+62.1258±1.0592 [Methyldopa] mmol/L	0.9994, 0.9989, 99.89		2.110 << 124.2417
Linear range or linear dynamic range	0.05-35 (22)	159.7006±30.2236+63.2344±1.8269 [Methyldopa] mmol/L	0.9981, 0.9962, 99.62		2.086 << 72.4098
	0.05-25 (19)	66.5374±12.8752+62.1258±1.0592 [Methyldopa] mmol/L	0.9994, 0.9989, 99.89		2.110 << 124.2417

n: no. of measurement, $\hat{Y}_{Zi(mV)}$: estimated value of cell A and cell B in mV, r: correlation coefficient, r^2: coefficient of determination, R^2% (percentage capital R-squared): explained variation as a percentage / total variation and t_{tab}= $t_{0.05/2,n-2}$.

-Limit of Detection

The limit of detection was studied of methyldopa through as an injected sample volume of 200 µL. Limit of

detection equal to 1.4292 µg / 200 µL for both cells.

-Repeatability

The value of the RSD % for methyldopa 2, 14 and 20 mmol/L less than 0.5 % was obtained indicating a reliable measurement can be achieved using this method.

Assessment of NAG – ADF – 300 – 2 analyser using two cell and multi solar cells for the determination of methyldopa in drugs

The newly developed methodology (NAG-ADF-300-2) was used for the determination of methyldopa in three different samples of drugs from three different of companies. The continuous flow injection analysis used of homemade NAG-ADF-300-2 was compared with two methods which includes UV-spectrophotometric at λ_{max}=280 [28, 29] nm and turbidmetric method at 0 -180° for yellow precipitate particles of methyldopa-potassium hexacyanoferrate system. Results were mathematically treated for the standard addition method. The obtained results indicate clearly that there was no significant differences between newly developed method, UV- Spectrophotometric and turbidmetric method (classical method) at 95% (α = 0.05) confidence level as the calculated t_{cal} (0.8993, 0.7940 and 0.0303, 0.3032) is less than t_{tab} (4.303) for each cell (i.e.; cell A & cell B).

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Assessment of Surface Roughness of Nickel Free Orthodontic Brackets and Archwires (An In Vitro Comparative Study)

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Abstract

Objectives: Orthodontic appliances may cause many side effects, one of these is inducing nickel hypersensitivity as a result of nickel content of these appliances**,** therefore, it is essential to choose a bracket and archwire that are nickel free and have a better surface characteristic to be more efficient for the orthodontic treatment. Hence, the aim of this study is to assess the surface roughness of nickel free metal brackets and archwires.

Materials and Method: Two types of nickel free brackets were used (Ni free SS brackets and Co-Cr brackets), a 0.022-inch slot, and three types of nickel free archwires of 0.018 x 0.025 inch were used (Ni free SS, TMA, and rhodium coated SS archwires). These brackets and wires were examined for their surface roughness by the atomic force microscope (AFM) using the roughness average parameter (Ra). For the statistical analysis Mann-Whitney U test was used for the comparison between the brackets, while, Kruskal-Wallis test was used for the comparison among the archwires (non-parametric tests) at level of significant 0.05, by using SPSS software version 23.

Results: Regarding the brackets, there was no statistically significant difference between the surface roughness of the two brackets types, while, regarding the archwires, there was a statistically significant difference between the as received archwires; as the nickel free stainless steel wires had the lowest value of roughness average, while, the TMA wires had the highest value of roughness average.

Conclusions: Ni free SS wires considered the best choice as a working wire for patients with nickel allergy.

Keywords: *Nickel hypersensitivity, Nickel allergy, Nickel free brackets, Nickel free archwires, Surface roughness.*

Introduction

The orthodontic patients are at risk of exposure to a high amount of nickel, cobalt, chromium, and other metals that derived from the alloys of the orthodontic appliances. These alloys exposed to a various conditions in the oral cavity because of the microbiologic and aqueous oral environment, in addition to that, they affected by the pH of saliva, the intake of foods, drinks and mouthwashes, which may lead to the corrosion of these alloys and hence leaching of ions into the oral cavity. The consequence of that leaching is the trigger of a hypersensitivity reaction. [1-3]Nickel is the most common metal induce an allergic reaction. [4]The prevalence of nickel allergy is 1-3% in males and 10%-30% in females,[5] the higher prevalence in female was related to the to the environmental exposure like the ear piercing[6] or wearing of jewelries .[7]The concerns about the biocompatibility from using these nickel alloys in the oral cavity for a prolonged period of time have encouraged studying an alternative materials. Therefore, nonmetallic, polycarbonate, nickel free, or stainless steel with a reduced amount of nickel had been used for the

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orthodontic treatment.[8,9]These materials considered as a hypoallergenic materials made with a low nickel concentration and have the ability to liberate minimum quantities of nickel ions, hence, they are typical for patients with nickel hypersensitivity.[10]

Surface roughness of orthodontic appliances considered an essential feature that has association with several clinical factors such as: aggregation of plaque, corrosion, metal ion release, biocompatibility and esthetics. Additionally, it has an impact on frictional force, bracket locking, anchorage, and tooth movement speed. Therefore, it influences treatment quality**.** [11-14]Many methods had been used for the assessment of surface roughness such as: Profilometer, scanning electron microscope (SEM), and atomic force microscope (AFM).

The aim of the present study is to assess the surface roughness of nickel free orthodontic brackets and archwires and hence, choosing the better one for the patients with nickel hypersensitivity.

Materials and Method

Materials

24 metal nickel free brackets were used for this study: **12** Cobalt chromium brackets (Topic, Dentarum company, Germany) and **12** Nickel free stainless steel brackets (Mini-Sprint, Forestadent company, Germany), all the brackets were for the upper right 1st premolars (slot dimensions 0.022 x 0.030 inch) with Roth prescription. While, for the archwires: **27** pieces from three types of archwires were used: **9** pieces of Nickel free stainless steel archwires (Noninium®, Dentarum Company, Germany), **9** pieces of Titanium molybdenum archwires (Rematitan® Special, Dentarum Company, Germany), and **9** pieces of Rhodium coated stainless steel archwires (Fantasia Archwire, IOS Company, USA), all the wires had the gauge of 0.018 x 0.025 inch.

Method

Grouping of sample

Three samples from each brackets and archwires as received from the manufacturer were examined, and after the sliding of the three archwires with the two brackets; three samples from each type of brackets and archwires were taken, in order to examine the changes that occur to the slots of these brackets and to the surface of the wires as a result of that sliding.

Preparation of the brackets

In order to examine the slot base, the wings of the brackets had been cut by using a metal disk bur. The reason behind removing the wings was to enable the probe of the microscope from touching the surface of the slot base without hindrance from the wings. The center of the mesial side of the brackets slots was chosen to be examined.

The brackets fixed on a metal slide to be examined under the microscope.

Preparation of the archwires

Before examining the wires that had been underwent previous sliding, the length of the wire that slided along the bracket slot was marked which was 5mm (because only the center of this 5 mm was chosen to be examined under the microscope). While the as received wires did not need any marking and any area can be examined. The length of the wire piece that fixed on the metal slide of the microscope was 20mm.

The scanning process

The atomic force microscope (AFM) was used for surface roughness assessment, it was of model: **NTEGRA prima NT-MDT**, with a silicon probe mounted on cantilever. It was operating in a tapping mode with a duration of 540 seconds per scan (9 minutes).The examination was in an air condition at room temperature with a scanning speed of 0.8 line/s. For the brackets; the resolution 512×512 pixels, the scanned area 10 × 10 μm². While, for the wires, the resolution 256×256 pixels, the scanned area 30×30 μm². Subsequently, the *3*D view of the surface of the brackets slots and the wires were displayed on the monitor of the computer that connected to the AFM.

Results

For this study, only the roughness average (Ra) parameter of the surface roughness was analyzed. The levels of significance for the statistical evaluation were: Non-significant difference NS $P > 0.05$, significant difference S $0.05 \geq P > 0.01$, highly significant difference HS $P \leq 0.01$. Statistical analysis for the data was done by using SPSS23 (Statistical Package of Social Science, version 23). The statistics used were:

Descriptive statistics

Median, minimum, and maximum values of the roughness average (Ra) as shown in table (1). All values were expressed in nanometer (nm).

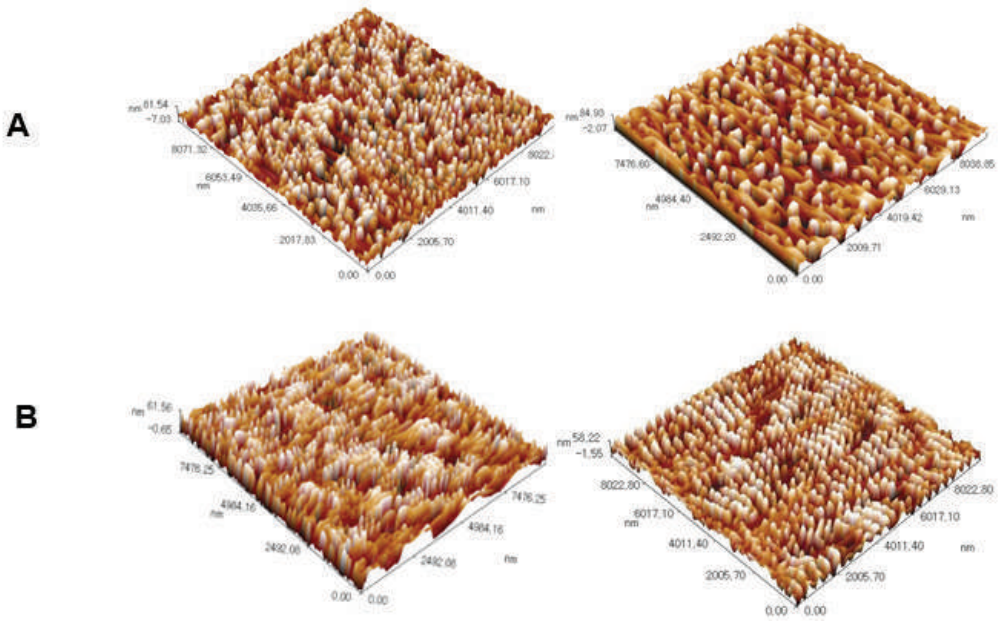
Inferential statistics

Comparison between Ra of the brackets (table 2)

For the comparison between the surface roughness (by using the Ra) of cobalt chromium and nickel free stainless steel brackets in four conditions (as received, after sliding with Ni free SS wires, after sliding with TMA wires, and after sliding with rhodium wires); **Mann-Whitney U test** was used, it showed that there was no statistically significant difference between the two brackets.

Comparison among Ra of the archwires (table 3 and 4)

For comparing the surface roughness (by using the Ra) among the wires in three conditions (as received, after sliding with cobalt chromium brackets, and after sliding with Ni free SS brackets); **Kruskal-Wallis test** was used. It showed a statistically significant difference among the wires when they were in as received condition, as the P value was **0.039.** In order to know where exactly was the difference, **Pairwise comparison** test was performed, and it showed that the statistically significant difference was between the as received nickel free stainless steel wires and the as received TMA wires (P value was **0.034)** as illustrated in **table 4,** as the nickel free stainless steel wires had the least value of roughness average among the tested wires **(21.20),** while, the TMA wires had the highest value **(109.00).**



Cont... **Figure 1**

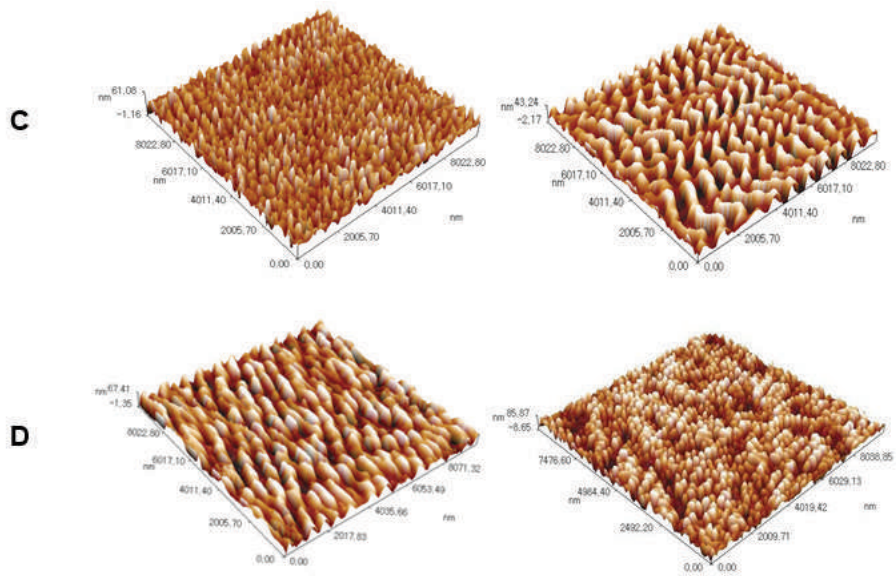


Figure 1. 3D images (10*10 µm²) of surface topography of the cobalt chromium brackets (the left figures) and Ni free SS brackets (the right figures) when they were A- As received B- After sliding with Ni free SS wires C- After sliding with TMA wires D- After sliding with rhodium wires.

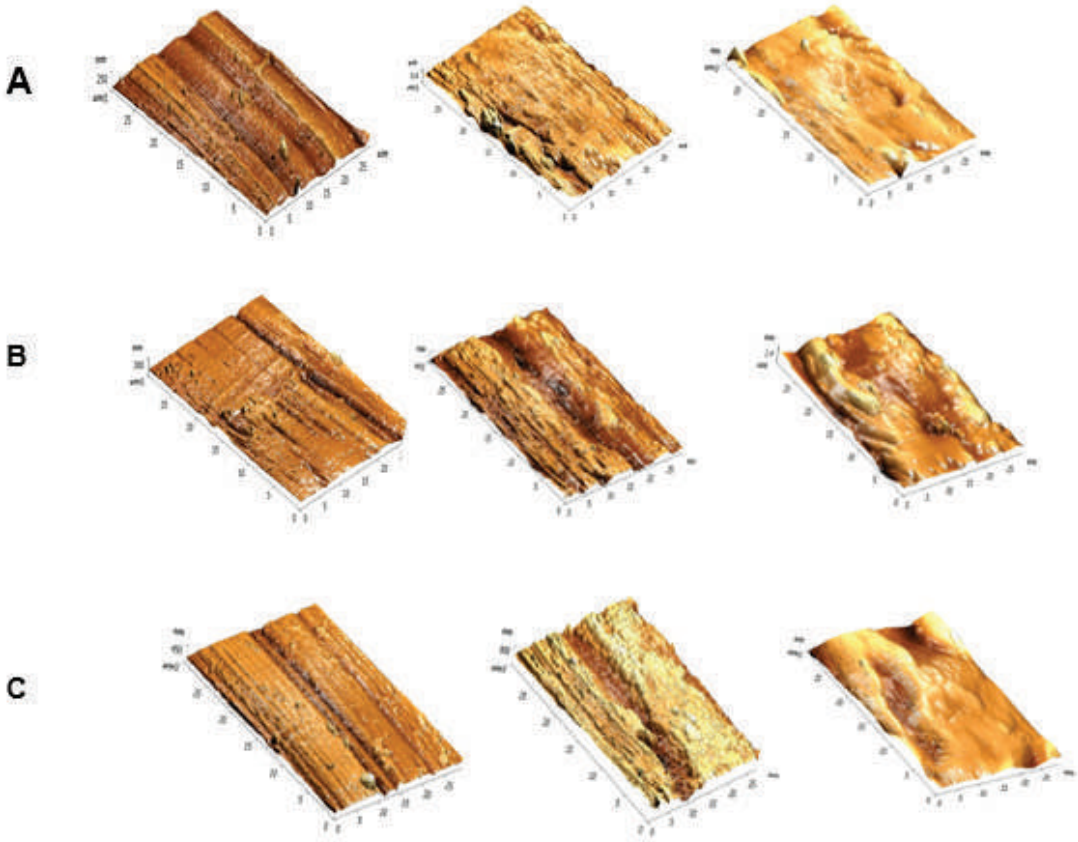


Figure 2. 3D images (30*30 µm²) of surface topography of the archwires; Ni free SS (left), TMA (middle), and Rhodium (right), in following conditions: A- As received B- After sliding along cobalt chromium brackets slots C- After sliding along nickel free SS brackets slots

Table 1. Descriptive statistics of the Ra (nm) of the brackets and the archwires

Brackets				
Condition	**Type**	**Median**	**Minimum**	**Maximum**
As received	Co-Cr	22.10	10.50	27.90
	Ni free SS	17.30	15.60	26.10
Ni free SS wires	Co-Cr	14.60	13.20	15.10
	Ni free SS	14.90	8.19	17.90
TMA wires	Co-Cr	9.53	8.36	11.10
	Ni free SS	11.40	8.56	13.60
Rhodium wires	Co-Cr	15.00	13.00	17.20
	Ni free SS	21.40	7.31	21.90
Wires				
Condition	**Type**	**Median**	**Minimum**	**Maximum**
As received	Ni free SS	21.20	15.07	21.94
	TMA	109.00	96.10	121.97
	Rhodium	43.01	26.34	96.70
Co-Cr brackets	Ni free SS	27.09	25.99	35.88
	TMA	108.82	88.70	142.25
	Rhodium	99.80	81.70	102.00
Ni free SS brackets	Ni free SS	31.90	26.02	46.33
	TMA	135.46	51.27	137.00
	Rhodium	80.00	27.21	131.00

Table 2. Mann-Whitney U test for comparison between the two brackets

Condition	Test Statistic	Exact Sig. (2-sided test)
As received	4.000	1.000 (NS)
Ni free SS wires	5.000	1.000 (NS)
TMA wires	7.000	.400 (NS)
Rhodium wires	6.000	.700 (NS)

Table 3. Kruskal-Wallis test for comparison among the archwires

Condition	Test Statistic	Sig. (2-sided test)
As received	6.489	.039 (S)
Co-Cr brackets	5.956	.51 (NS)
Ni free SS brackets	4.356	.113 (NS)

Table 4. Pairwise comparison test

Sample 1- Sample2	Sig.
Ni free SS wires-Rhodium wires	.408 (NS)
Ni free SS wires-TMA wires	.034 (S)
Rhodium-TMA wires	.890 (NS)

Discussion

Surface roughness is a characteristic of the materials and it is highly affected by the process of manufacturing and/or surface polishing process.[15] It has an effect on the performance of the appliance, and consequently, it affect quality of the treatment, as it has impact on friction, bracket locking, anchorage, and tooth movement speed. Besides, it has an effect on corrosion behavior, metal ion release, and biocompatibility. [11-14]

In this study, **Ra** parameter was used for the assessment of surface roughness, it is an indication of the absolute magnitude of the heights of the surface. Atomic force microscope (AFM) was used for the surface roughness assessment of both brackets and archwires. Choosing of this device was for many reasons, as it considered superior device for measuring the roughness of the surfaces because it give a quantitative measurement of the roughness with a 3D configuration, it scan the specimen without changing the area**,**[15,16] Other methods like SEM and the Profilometer may change the morphology and the roughness of the surfaces**.**[17] As according to **Choi et al. (2012)** the profilometry considered an invasive technique, as the surface might be damaged during scanning[18]. While, **Winchester (1991)** reported that SEM considered unreliable method of measuring the surface roughness as it depend on a subjective interpretation. [19]

The results of surface roughness assessment of this study showed that there was no statistically significant difference regarding the median values of roughness average of both cobalt chromium brackets and the nickel free stainless steel brackets. While, for the archwires, there was a statistically significant difference among the as received archwires in the regard of their median values of their roughness average, as the TMA wires showed the higher value of roughness average, while, the nickel free stainless steel wires showed the lower values. It is important to notice that in the all conditions (when they: as received, coupled with cobalt chromium brackets, and coupled with nickel free stainless steel brackets) the TMA wires showed this high values of their roughness average with an obvious difference from the other two wires (except in the case when it coupled with cobalt chromium brackets, the difference from the rhodium wires was not very high). This may be attributed to the manufacturing process as according to **Brantly (2001)** the rough surface of the TMA wire was attributed to the cold welding or the adherence of the titanium ions to the rollers or the dies during the manufacturing process. [20]

The finding of this study agreed with other studies that also find the TMA wires had the highest surface roughness. [21-23]

While, nickel free stainless steel wires showed a very low values of their roughness average in the all conditions inspite of the statistical difference was only in

the as received condition. This give the wire a superiority as working wire in the sliding mechanic of the second stage of the orthodontic treatment, as its smooth surface will enhance the performance of the appliance, and hence, the quality of the orthodontic treatment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Workplace Violence against Health Care Providers in Emergency Departments: an Underrated Problem

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Abstract

Background: Staff employed in emergency departments (EDs) in the hospitals are excessively prone to workplace violence (WPV) in comparison to individuals working in other departments. Such thing lead to slight or serious injuries to these employees. In addition, it may cause physical injury and disability, low performance at job, and finally an uncomfortable working setting for the staff.

Materials and methods: A descriptive study was applied to assess the prevalence of WPV and related factors. This study was conducted at the EDs of four teaching hospitals in Al-Nasiriyah city. Study sample was drawn from emergency physicians, nurses, pharmacists, and laboratory technicians. Both genders staffs were included.

Results: The results involved answers from 426 employee in the emergency department. The most reported form of violence in the staff in the past year in this research was emotional violence, which is reported by 78.5% of the sample, then physical abuse (14.3%) and sexual abuse at last (5.2%).

Conclusion: This study exhibited many findings on a group of WPV cases, in addition to the features and issues related to exposure to violence. The findings undoubtedly prove the priority of considering this issue in Iraqi hospitals and the importance to develop urgent and appropriate interventions.

Keywords: *emergency department, hospital safety, health care providers, workplace violence, Iraq*

Introduction

WPV in the medical sector has become a worldwide phenomenon and one of the largest fears of general health that needs high and urgent consideration of health organizations. The World Health Organization (WHO) describe WPV as "The intentional use of power, threatened or actual, against another person or against a group in work-related circumstances that either results in or has a high degree of likelihood of resulting in injury, death, psychological harm, maldevelopment, or deprivation". [(1)]

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Violence happens in all work settings. Yet, particular economic sectors are primarily prone to violence, for instance, nearly 25% of all violent cases at work are in the health sector. [(2)] Although violence (psychological or physical) toward providers of health care is stated .as widespread occupational threat [(3)], the actual extent of the problem is highly unidentified, and current studies presented that the current statistics represent merely the iceberg tip. [(4)] The exact prevalence of violence in health industry is hard to approximate. While physical workplace violence has been continuously documented, the psychological violence incidence has been largely underrated and is today getting due consideration. [(5)] It has been presented that over than half of health care providers have been experienced workplace violence at some time throughout their medical line of business. [(6)]

In addition, studies and papers from various countries exhibited that the medical staff percentage that exposed to violence was as large as 50% to 88%. [7, 8, 9, 10, 11, 12, 13]

The mainstream of occurrences of WPV in health care settings were reported to happen in the ED than other sections of the hospital. [14, 15, 16, 17] Even though attention to violence in the workplace in the health industry has developed significantly in the developed world, WPV still seems to be an underrated topic in numerous developing countries. [18, 19] Workplace violence toward ED staff has been infrequently investigated in third world countries including Iraq; therefore, the actual extent of the problem is mostly unknown until today. Therefore, the goals of this study are to approximate the incidence and linked factors of various kinds of violence directed toward health care providers working in emergency department in comparison with staff functioning in non-emergency departments, conditions of violence, type of offenders, and victims' response.

Materials and Methods

- Study Design:

Descriptive design was used during 9th of March 2019 to 21st of September 2019.

-Setting of the Study:

Four teaching hospitals in Al-Nasiriyah were the sitting of the study (Al-Hussein Teaching Hospital, Bint-Alhuda Teaching Hospital, Mohammed Almosawey Teaching Hospital, and Al-Haboobi Teaching Hospital).

- Study Sample:

A random (probability) sample of (503) health care providers (physicians, nurses, pharmacists, and laboratory technicians), who were working in emergency departments.

-The Study Instrument:

The authors constructed a questionnaire to achieve the study objectives.

Part 1: Demographic Data:

Socio-demographic data sheet composed of six points that included (age, gender, marital status, years of experience, work sector, job title).

Part II. Data regarding WPV:

The questionnaire consisted of a number of items related to incidence, types, Contributing factors, Consequences, Coping mechanisms, and suggested solutions regarding WPV.

-Data Collection:

Sector managers gathered data from health care workers under authors' supervision. The questionnaire was distributed separately to them. Sector managers clarified the study objectives and answered any inquiries associated to questionnaire.

-Pilot Study:

In order to estimate the study instrument (questionnaire) reliability, A (10) nurses sample were designated; responses were calculated through applying the Alpha Cronbach parameter, which exhibit that the person correlation coefficient = (0.83). Pilot study was conducted at AL Hussein Teaching Hospital and the sample of the pilot study was excluded from the original study sample.

-Validity:

Study instruments validity were determined by a group of (12) experts, who had more than 5 years of experience in public health nursing and occupational safety.

- Data Analysis

With the purpose of analyze the collected data, the statistical package of social sciences (SPSS) ver. (25) were used (Percentage, Frequency, Mean, Standard deviation, Mean of score, and Chi-square).

Results

The study exposes that 426 (84.6%) of health care providers have experience 813 physical, emotional/ psychological, and sexual violence assaults within the past year. With a mean of ≈2 attacks in the past 12 months. More than 1/3 (41.2%) of them reported one violence attack of any type in the past year. Health care providers aged 25-29 years old married males, of 11 or more years of experience, and nurses are prone to violence more than others (Table 1).

Table (1) Distribution of Health Care Providers according to Socio - Demographic Characteristic (N= 503)

		Total	Exposed to Violence	
SDVs	**Group**	**Frequency**	**Frequency (%)**	**χ2**
Overall		503	426 (84.6)	
Age	18-25 years	193	131 (67.8)	18.5*
	25-29 years	160	153 (95.6)	
	30-34 years	108	102 (94.4)	
	35 years and over	42	40 (95.2)	
Gender	Male	215	201 (93.4)	3.75
	Female	288	225 (78.1)	
Marital status	Single	264	203 (76.9)	7.74
	Married	209	199 (95.2)	
	divorced	19	18 (94.7)	
	Widowed	11	6 (54.5)	
Years of experience	1-5 years	247	200 (80.9)	28.3
	6-10 years	104	87 (83.6)	
	11 years and over	152	139 (91.4)	
Job title	Physician	49	33 (67.3)	15.6**
	Nurse	351	341 (97.1)	
	Pharmacist	39	24 (61.5)	
	Laboratory technician	64	28 (43.7)	

%=percentage, N= number of sample, χ^2= chi-square test.

***p* ≤ .05, ****p* ≤ .01.**

Table (2) Types of Violence in 813 Reported Occasions

Type	Frequency (%)
Physical	116 (14.3)
Pushing	64 (7.9)
Beating	38 (4.7)
kicking	10 (1.2)
Others (Pinching, slapping, biting)	4 (0.5)
Emotional (psychological)	638 (78.5)
Verbal abuse	270 (33.2)
Threat of using physical strength	188 (23.1)
Bullying	138 (17)
Sexual harassment	42 (5.2)
Both physical and emotional	59 (7.2)

Table (3) Contributing Factors as Informed by 426 Health Care Workers

Contributing Factors	Frequency (%)
Excessive numbers of patients	312 (73.2)
Low quality services	271 (63.6)
Absence of punishment for offenders	264 (61.9)
Impatience (patient intolerance)	215 (50.4)
Reaction to injury/accident/death	167 (39.2)
Lacking of staff	165 (38.7)
Security shortage	130 (30.5)
Disobedience of working system	130 (30.5)
Poor administration	111 (26)
Deficiency of protective measures	104 (24.4)
Mental illness/ alcohol and drug misuse	73 (17.1)

Note: Classes are not mutually exclusive.

Table 2 displays that emotional (psychological) is the most common form of violence. The most frequent contributive factors to violence as experienced by victims are excessive numbers of patients, low quality services, and absence of punishment for offenders (Table 3). The most common consequences of violence are angry, irritability, and feeling suspicious. Other factors are listed in Table 4 below. Table 5 shows that majority of victims pretended that violence events did not happen, other common coping approaches are Informing coworker, and Inform friends / family.

Table (4) Violence Consequences as Informed by 426 Health Care Providers who experienced Violence in the Past 12 Months

Consequences	Frequency (%)
Anger	218 (51.2)
Irritability	168 (39.4)
Feeling suspicious	162 (38)
Work dissatisfaction	133 (31.1)
Upset	104 (24.4)
Anxiety	82 (19.2)
Planning for resignation	30 (7)
Decrease performance and efficacy	28 (6.6)
Low enthusiasm	14 (3.3)

Note: Classes are not mutually exclusive.

Table (5) Coping Approaches of 426 Workers who experienced Violence

Coping Approach	Frequency (%)
Pretended nothing occurs	325 (76.3)
Inform coworker	311 (73)
Inform friends / family	278 (65.2)
Take no reaction	202 (47.4)
Take off work time	147 (34.5)
Trying to overlook the happening	123(28.9)
React to the offenders	68 (16)
Inform to manager/supervisor	51 (12)

Note: Classes are not mutually exclusive.

Discussion

Workplace violence is considered one of the most insisting problems in health industry nowadays, and in developing countries, the impact of this issue is much stronger. So, urgent and appropriate solutions are needed. This study investigated WPV toward emergency health care providers in 4 hospitals in Al-Nasiriyah city/ Iraq. The study collected data on the incidence, features, and factors connected to WPV during the previous 12 months. The outcome of this study reveals high incident of violence (813 events in 426 victims). WPV affected different types of health care workers in the emergency including physicians, nurses, pharmacists, and laboratory technicians. This proposes that WPV is a principal concern that affects and needs to be addressed in Iraq. The results of high WPV incidents in ED in this research is incongruence with previous findings and proposes that WPV is a major concern in emergency departments and hospital settings, and that is marked in multiple countries. [20, 21, 22] Past literature viewing the greater jeopardy of nurses to face violence [23, 24] and this study shows the same, this is probably due to the point that nurses have further interaction with patients.

Particular studies have approved that verbal abuse is greatly present toward staff in the EDs [25, 26, 27] which is also confirmed by the current study by representing that there were numerous incidents of verbal abuse against staff in the emergency department. Sexual harassment shows low numbers in this study due to gender separation in almost all of health care activities regarding Islamic religion and community culture. Moreover, sexual harassment is underreported in majority of countries and it is unlikely to be reported. So, this hides the actual extent of the problem. In addition, women's fear of talking in public on this issue could result in low reporting percentage.

Previous study propose that the overall culture of the work place must be considered during assessment of the risks of workplace violence, and that a "participatory" working environment with open discussion and communication can resolve the threat of violence [5] (in this study for example, things like gender segregation and fear of community judgment may result in low reporting numbers of sexual abuse).

Factors contributing for WPV are multiple according to the literature and many of the previous studies [5] that agree with findings of this study, and that are excessive numbers of patients, low quality services, absence of punishment for offenders. Not all violence against health care providers is lethal. Still, violence had multiple consequences, and the most common are feeling anger, irritability, and becoming suspicious. Similar consequences were stated in diverse extents in multiple previous studies. [5, 28, 29]

It may be different how various health care workers adapt to WPV. However, in this study the three most common coping mechanisms are victims pretended nothing occurs, informing coworker, and informing friends / family. Accordingly, the three uppermost solutions that suggested by the study sample were provide training programs on violence prevention and control, apply appropriate punishment for offenders, and improve directorial measures. These findings agree with the suggestion from past study that workplace violence is a consequence of greater administrative and organizational problems and proposes that enhancements made to organizational structures could show to be of a benefit. [30] Many other studies agrees with the solutions above. [29]

Conclusion

Gathering and supplying data on WPV in emergency departments is decisive for signifying the importance of this issue and pushing toward modifications that could guard health care providers. This study validates that regardless of the poor literature on WPV in emergency departments in Iraq, this topic is very significant in our region, and changes are mandatory.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Effects of Gaseous Ozone (O_3) on Artificial Population and Aflatoxin B_1 Production by *Aspergillus flavus* in Stored Whole Cinnamon in Iraqi Markets

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Abstract

Stored whole cinnamon can become contaminated with aflatoxins.For public health requirements cinnamon should be produced free of AFB_1or bellow EU legislation limit (5µg/kg), soin this study, examined the efficacy of different concentrations (0, 300 and 600 ppm) of ozone on fungal populations (CFU) and AFB_1 production of *A.flavus* on stored whole cinnamon at three a_w levels (0.92, 0.94&0.96 a_w) at 25°C and exposure time (60 min). In general, the results presented in this study showed that the populations AFB_1 produced of *A.flavus*in artificially contaminated whole cinnamon were significantly affected by O_3treatment.

Keywords: *Cinnamon; Ozone; Population; Aflatoxin B_1;Aspergillus flavus.*

Introduction

Cinnamon produced in tropical countries and used as ingredients in traditional foods as food supplements or medicines [3]. Cinnamon can be contaminated with AFB_1 during pre or post-harvest, processing, handling and storage [20]. Aflatoxins (AFs) are mycotoxins produced by many fungal species, especially *Aspergillus flavus, A. nomius* and *A. parasiticus*. These AFs are carcinogenic, teratogenic and mutagenic in human and animals. There are many types of AFs one of them Aflatoxin b_1 (AFB_1), it is the most toxic because classified by International Agency for Research on Cancer (IARC) as class 1a carcinogenic in human and animals[12].

Ozone (O_3) is a strong germicide and has three oxygen atoms. It is used in food industries as an antifungal agent prevents fungal growth in raw materials and used as a preservative in packaging foods [23]. O_3 is produced naturally as a colourless or bluish gas by two methods and artificially by three methods. The most common method for O_3 production is a corona-discharge method (CDM) [16;9]. It will be considered in this study to control fungal population and prevent contamination of cinnamon with AFs.

Natural levels of O_3 are between 0.01-0.15 ppm but exposure to high levels produce some harmful effects in health such as respiratory diseases [11] and cardiovascular diseases [6]. Federal occupational safety and health administration (OSHA) recommended a legislative limit for exposure to O_3 of 0.1 ppm for 8 hr continuously or 0.3 ppm for 15 min [22].

The efficacy of O_3 on fungal species is depending on fungal tolerance to exposure, O_3 (concentration), exposure time, spore morphology and moisture content of substrate [1]. Moreover, O_3 treatment can cause negative impacts in the quality of the food such as oxidation of lipids, change colour, modified of some vitamins and phenolic compounds [18;7].

The effect of O_3 on colonization and AFB_1 production of *A. flavus*in peanuts, wheat, chillies, Barazil naut, corn, maize and red peppers have been studied previously,[21;5;2;19;4;17;10;13]. To my knowledge, there very few studies on cinnamon. The objective of this

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study was to examine (a) the efficacy of O_3 on the total fungal colonization and AFB_1 production of *A. flavus* in artificially contamination on whole dried cinnamon stored at 25°C stored for 20 days.

Material and Methods

Chemicals and reagents

The AflaStar™ – Immunoaffinity Column (AIC) were purchased from Romer Labs PLC (Tulln, Venna, Austria). Aflatoxins Stock was purchased from (Romer Lab UK Ltd, Cheshire, UK). Mealt Extract Agar was purchase from (Oxoid, Basingstoke, Hampshire, UK). Methanol and Acetonitrile were obtained from (Fisher Scientific, UK). All solvents were of HPLC grade.

Collection of cinnamon samples

300 g of whole dried cinnamon were randomly collected from Iraqimarkets and direct plated on MEA and then incubated at 25°C for seven days for fungal isolation.

Preparation of fungal conidial inoculums

Spore inoculums was made from *A. flavus* (DJC_1) isolated from cinnamon, these spores grown on MEA for 7 days and removed by sterile loop and then flooded with 9 ml of sterile water containing 0.025% (w/v) Tween 80.

Media preparation

Malt extract agar (MEA)

This medium was used for culturing, isolating and enumerating fungal species. (15 g MEA powder and 300 ml distilled water (D.W). A small amount of antibiotic agent, chloramphenicol (0.05 g/L) was mixed with the components to prevent bacterial growth. After autoclaved for 1 hr at 121ºC the sterile medium was cooled and poured into 9 cm serial Petri plates and left to cool, and kept at 4ºC in polyethene bags until used.

Preparation of Adsorption curve

A moisture absorption curve was designed to determine the quantities of water required to add to cinnamon samples to modify the moisture contents to the treatment target $a_w$values. This curve was prepared by adding different amounts of distilled water to whole cinnamon and equilibrating at 4ºC for 24 hrs. The a_w was measured with an Aqua Lab 4TE.

Preparation of chilli samples

Thirty grams of whole dried cinnamon were weighed into 9 solid culture vessels and then stoppered with plastic lids with a permeable membrane. The samples divided into three levels of a_w conditions (0.92, 0.94 and 0.96a_w). Each group of chillies adapted by adding the appropriate volume of distilled water based on the moisture absorption curve to obtain the required target levels. All samples were shaken on a (KS 501 digital orbital shaker) for 1hr and then kept at 4ºC for 24 hrs to allow absorption and equilibration. Each vessel was inoculated with a spore suspension of *A. flavus* (10^2 spores/ml) by add 0.25 ml of spore solution to each vessel. All samples mixed well and exposed to a different concentration of O_3 (0, 300 and 600) ppm for 60 min at (6L/min) in 50 ml class tube system. Controls were prepared by exposure to air only. Each treatment divided into three separate replicatesat each a_w levels and stored in plastic chambers at 25ºC for 20 days. Each plastic chamber also contained 500 ml beaker containing a glycerol/water solution of the same a_w to maintain the ERH. The *A. flavus* populations present initially and after 10 and 20 days were quantified at each treatment. The samples were stored at -20°C for AFB_1 analyses at each treatment condition.

Enumeration of *A.flavus* populations on stored whole cinnamon

One g of cinnamon from each treatment and replicate was weighed and mixed with 9 ml of sterile D.W with 0.025% (v/v) Tween 80 to obtain the 10^{-1} dilution. All samples were then serially diluted after vigorous shaking at each dilution using a vortex mixer. 100 µl of each dilution was spread plate onto MEA plates modified a_w at the same a_w of storage a_w. All plates were incubated at 25ºC for seven days the colonies growing were then enumerated.

Extraction of AFB_1 from chillies using HPLC

Weighted three g of cinnamon from each treatment and replicates and placed in 30 ml tube. Added 12 ml of extraction solution (**acetonitrile / water)** (6/4 (**v/v**)) at 15-18°C. Then the samples have been shaken for 1 hr. The extracts were filteredinto contenier using a

funnel containing a Whatman filter paper (12.5 cm). The extracts were diluted with PBS (Phosphate buffered saline) (pH 7.4) until the content of acetonitrile is lower than 5% (**v/v**) and (7 pH).The diluted extracts were transferred to (Romer, IAC) and allowed to pass through the column using a syringe barrel as a reservoir. The flow rate should be not exceeding 1-3 ml/min. The column and the extract must be at 15-18ºC. The IAC should be rinsed with 2 x 10 ml of distilled water. Added 2 ml of methanol directly to the column and calculated the elution in a new Eppendorf tube 2 ml.The elution was evaporated under reduced pressure at 40ºC using a rotary evaporator. Added 1 ml of water: methanol (0.5:0.5) into toxin and filtered the extracts through Nylon 13 mm, 0.2 µm filter directly into amber HPLC vial and then stored at -20ºC until analyses using HPLC.

Statistical Analysis

The data obtained from this study were determined by using the Shapiro Wallis Test for (Non-normality) and one-way ANOVA for (Normality). The significant statistical level was at $P<0.05$ for both factors and their interactions.

Results

The effects of O_3 (ppm) on artificially populations of *A. flavus* on whole cinnamon stored at different a_w levels.

Figure 1 shows the effect of exposure to different doses of O_3 on the colonisation by *A. flavus* on stored whole cinnamon for 20 days at 25ºC and different a_w levels. The results showed that there were clear effects of incubation time and a_w on the populations in stored whole cinnamon and significant effects of O_3 concentrations except on initial colonization at first day of incubation especially, at 0.92 and 0.96 a_w. Statistically, the populations were significantly affected by O_3 concentrations, time, ppm x time, ppm x a_w and a_w but unaffected by time x a_w and ppm x time x a_w (Table 1).

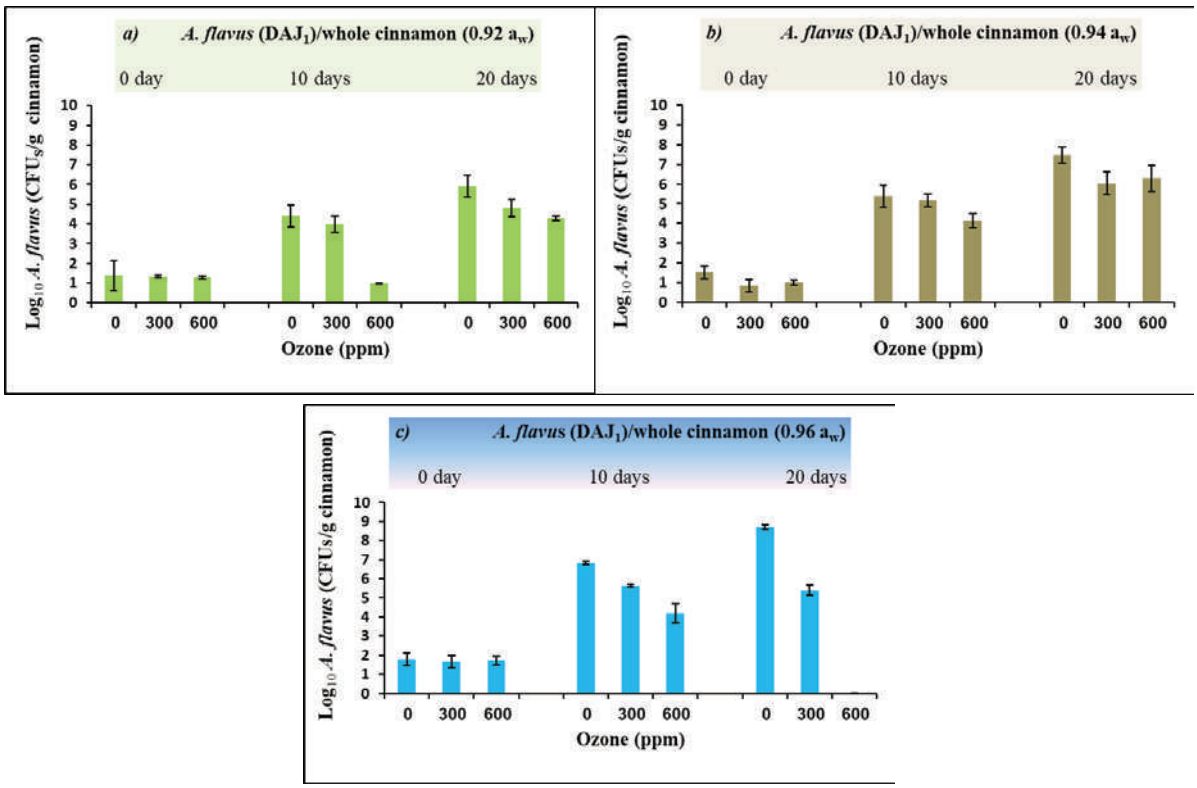


Figure 1 (a,b &c): Effect of O_3 (ppm) on populations of *A. flavus* (DAJ_1) isolated from stored whole cinnamon at 25ºC at (0.92, 0.94 and 0.96a_w) over 20 days. Bars indicate SDM.

The effects of O_3 (ppm) on control the AFB_1 contamination of *A. flavus* in artificially contamination on stored cinnamon at different a_w levels.

Figure 2 showed the efficacy of O_3 concentrations on AFB_1 contamination in stored whole cinnamon for 20 days with four a_w levels at 25°C and exposure time was 60 min. The data of this study reported that AFB_1 of *A. flavus* gradually decreased when increased O_3 concentrations except at 10 days at 0.92 a_w. Also, the AFB_1 production affected by a_w and time. Table 1 shows that there were significant effects of O_3 ppm, a_w, time, ppm x a_w, ppm x time and a_w x time but unaffected by ppm x a_w x time.

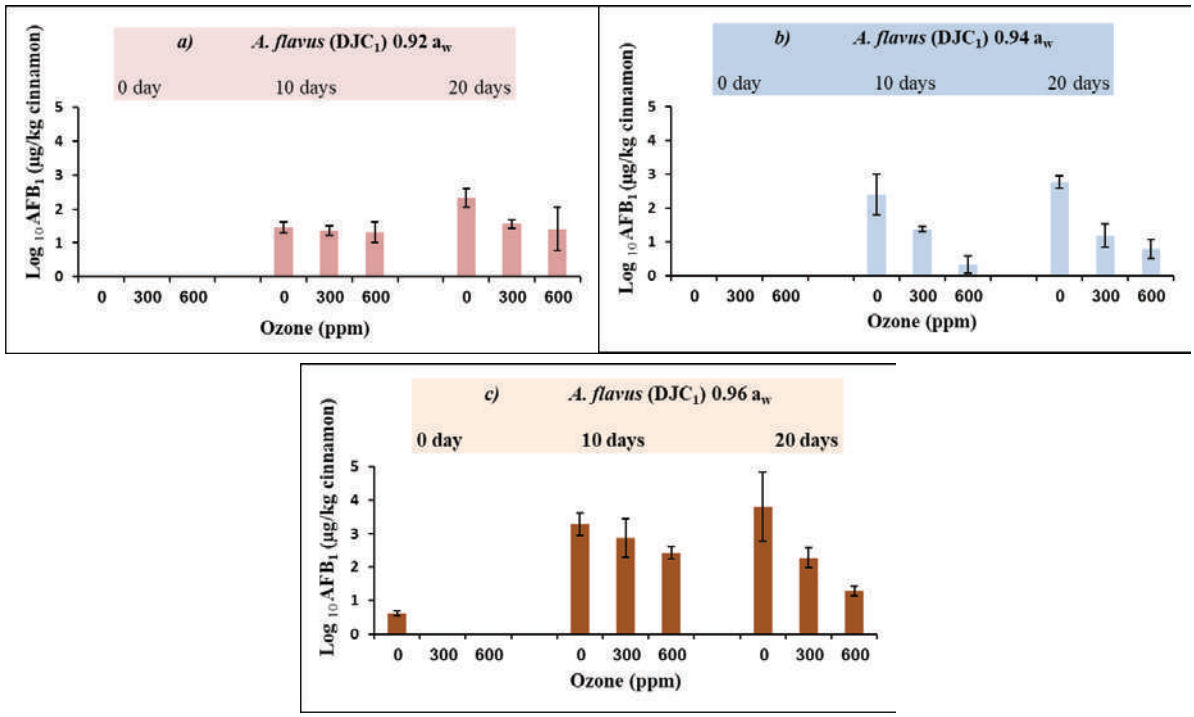


Figure 2 (a,b &c): Efficacy of O_3 (ppm) on AFB_1 contamination of *A. flavus* isolated from stored cinnamon at 25°C at (0.92, 0.94 and 0.96a_w) over 20 days. Bars indicate SDM.

Table 1: Summarise statistical analyses of effects of O_3 (ppm) on total populations and AFB_1 contaminationof *A. flavus* on whole cinnamon at 25°C under three a_w levels (0.92, 0.94 and 0.96a_w) over 20 days storage. ANOVA test (normality data) and Shapiro Wills test (non-normality data).

Factors	Effects	Actions
ppm	*S*[b]	Population
	S	AFB_1 Production
incubation time	*S*[b]	Population
	S	AFB_1 Production
a_w	*S*[b]	Population
	S	AFB_1 Production
ppm x time	*S*[a]	Population
	S	AFB_1 Production
ppm x a_w	*S*[a]	Population
	S	AFB_1 Production
ppm x time x a_w	*NS*[a]	Population
	NS	AFB_1 Production
time x a_w	*S*[a]	Population
	S	AFB_1 Production

NS, not significant *S*, significant *a*) ANOVA test *b*) Shapiro Wills test

Discussion

In this study different gaseous O_3 concentrations (0, 300 and 600 ppm; flow rate of 6 L/min) were used in order to know the effect of O_3 treatment on fungal populations and AFB_1 production in stored cinnamon inoculated with *A. flavus* (DJC_1) 10^2spores/ml at three a_w levels (0.92, 0.94 and 0.96 a_w) for 20 days. The fungal population and AFB_1 production has been measured at (T=0, 10 and 20 days) and compared with control (exposure air only). The results showed that the fungal population affected by gaseous O_3 treatment at all a_w levels andthere clear impact of ozonation on AFB_1 content in whole cinnamon(artificial contamination). To my knowledge, no previous studies conductedon the effects of O_3 exposure on fungal and AFB_1 contamination in stored cinnamon.

However, there are some studies done on the efficacy of O_3 concentrations on fungal populations and mycotoxins production in some products. For example, [1] have shown that the populations of three ochratoxigenic strains were significantly reduced in the coffee beans after exposure to 600 ppm O_3at 0.90 and 0.95 $a_w$compared with control. This data were similar to the findings in the current study. Similar to Akbar[22] presented that the fungal populations of *A. flavus* in peanut samples inoculated with (10^5 spores/g) were reduced when exposed to high concentrations of O_3. [8] found that the fungal contamination in Brazil nuts was completely stopped from the day of the application when exposure to 31 ppm O_3 for 5 hrs. [15] suggested that the fungal contamination of *A. parasiticus* in maize will be inhibition in a 63% after treatment of kernel surface with 50 ppm O_3.

For AFB_1contamination, the findings agree with other studies, forexample, [5] found that AFB_1 content was degraded in artificially contaminated wheat after exposure to 20 and 400 ppm O_3 for 5, 10, 15 and 20 min. [22] found that AFB_1 produced in artificially contaminated peanuts was reduced after exposed to 100, 200 and 400 ppm O_3. Last studies by [1] presented that the OTA content in artificially contaminated coffee stored at 0.75, 0.90 and 0.95 a_w and30^{o}C for 12 days wassignificantly reduced when exposure to 600 ppm O_3 for 1 hr. Recent studies on red pepper by [14] showed that AFB_1 contents in artificially contaminated red pepper treated with O_3 at three doses 20 mg/L, 40 mg/L and80 mg/L for 40 min were reduced depend on O_3 doses.

Conclusion

This study showed that *in situ* the populations and AFB_1production in stored whole cinnamon inoculated with *A. flavus* spores at different a_w levels were relatively affected by O_3 treatment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Assessment of Fibroblast Growth Factor with Severity of Asthma

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Abstract

To evaluate the level of fibroblast growth factor 2 (FGF2) in serum of asthmatic Iraqi patients and to generate relationship between FGF2 and pulmonary function in serum of all member. FGF2 was determined in seventy Iraqi subjects; 30 diagnosed with asthma and 40 healthy subjects were registered in the revision. The age of patients and control ranged between (25-55) years. The level of FGF2 was assessed in serum by enzyme-linked immunosorbent assay (ELISA) technique. The levels of FGF2 displayed significantly increase in asthmatic group associated with control group ($p < 0.01$). Among asthmatic Iraqi patients, increase level of FGF2, indicate the significant relationship between fibroblast growth factor 2 and asthma.

Keywords: *Asthma, Fibroblast growth factor 2.*

Introduction

Asthma is one of the greatest prevalence lung sicknesses, which considered by chronic inflammation of the airways. Asthmatic patients have airway obstruction and bronchospasm as a result of greater mucous invention and changes in the buildings and purposes of some components like, fibroblasts and smooth muscle cells [(1)].

Asthma attack may occur as a result of several inflammatory events in the respiratory system lead to the appearance of sever symptom. Worldwide ,about 300 million people diagnosed with asthma and near 1000 people/ day die as a result of this disease [(2)].

Numerous elements influenced the development of asthma such as genetic tendency, environmental factors, and significant gene environment interactions [(3)].

The family of fibroblast growth factor (FGF) involves 23 members, have polypeptides and act a as powerful mitogens [(4)].

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The important member of heparin binding growth factors family is Fibroblast growth factor (FGF)-2, which affect fibroblasts growth, endothelial, and differentiation of several kind of cell. This family have important roles in morphogenesis, injury healing, infection, angiogenesis, proliferation of tumor and metastases [(5)].

Another name of FGF2 is basic FGF (bFGF) considered as the extreme member of the FGF superfamily and is the main controller of cell proliferation and variation below functional and uncontrolled disorders [(6)].

FGF-2 is universal in typical humanoid tissues and present in a link with endothelial basement membranes. FGF-2 stored in a stable formula needing become free for biological action. Proteolytic cleavage of heparin sulphate proteoglycans HSPG core proteins, the activities of glycosaminoglycan GAG destroying enzymes, and the capability of heparin to remove FGF-2 from HSPG are the mechanism for the relief of FGF-2. HSPGs are cabin professionally from extracellular via the activity of enzymes like plasmin and thrombin, and by endogenous matrix metalloproteinases (MMPs) [(7)].

Abnormality of the signaling system of FGF triggers a variety of syndromes connected with enlarged FGF level. The inhibition of FGF signaling revealed scientific effect [(8)].

Some but not all studies indicate that FGF2 is a potent fibroblast mitogen, elevated in asthmatic patients and help as an indicator for severity of asthma [9].

Methods

Ethical issues

Depending on the local ethics group the revision was agreed, all patients in this study given an idea about the purpose of the review, agree, and signed permission were informed.

Study design

This revision designated as a study of case - control.

Collection of samples

The sample size was determined according to the equation of Daniel sample size formula. The participants in the recent study include thirty Iraqi patients with asthma (depending on pulmonologist decision according to the GINA guideline), the history from them was taken, which involve: age, height, weight, gender, family history, material status and medical history, and forty apparently healthy subjects therapeutically free were serve as healthy control group. Patients with age < 6 years, non-agreement, smoker>10 pack years, patients with interstitial lung illness like unknown and drug- induced pulmonary fibrosis, chronic obstructive pulmonary disease, lung malignancy and bronchiectasis, all were excluded from the study. The age of these groups from 25 to 55 years. The statistical analysis was done with version 18 of SPSS. The findings have been expressed as Mean ± SD, and P values below 0.05 are reflected significant.

Chemicals and methods

A- Determination of human bFGF /FGF2 (Basic Fibroblast Growth Factor) by the sandwich-ELIZA kit as the method. In this kit, an antibody specific for human bFGF /FGF2 was pre-coated to the micro- ELIZA plate [10].

B- Lung function tests demonstrate the airflow obstruction that variate after little periods, either naturally or in reply to handling (reversibility) [11].

C- Simple spirometry checks airflow restriction with decreased forced expiratory volume in 1 second (FEV_1) and FEV_1 / forced vital capacity (FVC) ratio . Reversibility is established by a >12% and 200-mL elevated in FEV_1 15 minutes when an inhaled short-acting β 2 -agonist or in patients by a 2 to 4 week sample of oral corticosteroids [12]. Guidelines of national and international mention the use of spirometry for the detection and monitoring of asthma, and appear that the reversible airflow limitation is a crucial factor in the analysis of asthma. It give an idea about the amount of air you can inhale and exhale, and how fast you can empty your lungs from the air, allows clearer confirmation of airflow obstruction than the peak expiratory flow rate (PEF), and in that sense is preferable. A reduced FEV1/ VC ratio usually taken to be <70% and FEV% <80% that confirms airway obstruction [13] .

Results

The study groups consist of 70 adults designate on three categories.

1- Asthmatic patients with treatment (n=18).

2- Asthmatic patients without treatment (n=12).

3- Healthy control group (n=40).

Age

There was no significant changed in age (as mean) between control and asthmatic patients as demonstrated in table 1.

Gender

Distribution of asthma according to gender presented in Fig. 1. This study demonstrated that the incidence of asthma in female (60%) is greater than male (40%).

Body mass index (BMI)

The difference of BMI between patient and control group was non- significant (p >0.05). The mean ± SD of BMI for patients and control groups exposed in table.1.

Table (1): Demographic appearances in studied groups

	Groups	No.	Mean ± SD	P value
Age	Control	40	39.08 ± 8.96	= 0.6
	Patients	30	40.75 ± 15.30	
BMI	Control	40	31.51 ± 8.94	= 0.2
	Patients	30	29.04 ± 7.04	

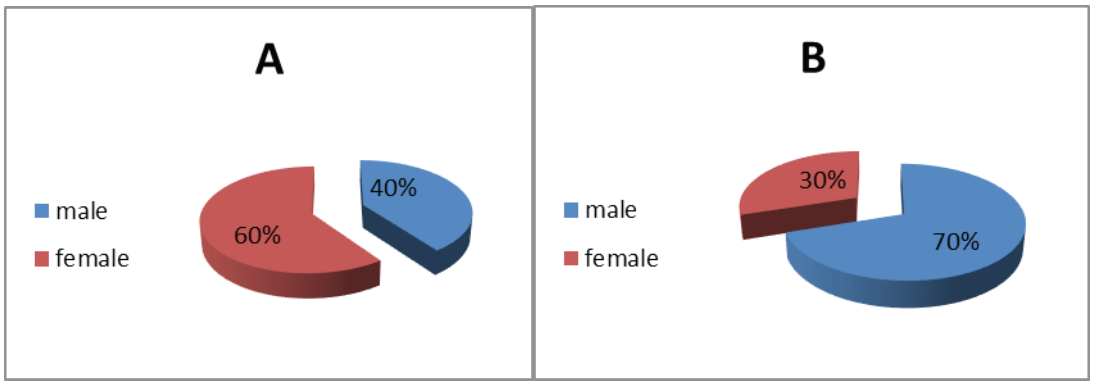


Fig (1): Gender distribution in (A) patients and (B) control

Serum level of FEV1 as mean ± SD of asthmatic patients and control.

In this study, serum concentration of FEV1 mean ± SD were significantly decrease (p<0.01) in asthmatic group when compared with control group, as shown in table 2.

Serum FGF2 concentration according to studied group.

As shown in table 2, the mean ± SD of FGF2 in asthmatic patients was (409.67± 315.12) with a significant (p < 0.001) difference likened with healthy control group.

The effect of treatment on the level of FEV1 and FGF2 in asthmatic patients.

The present study displayed significant reduction in serum level of FGF2 and significant elevated in serum level of FEV1 in asthmatic subjects with treatment than those patients without treatment, p value (< 0.001), as in table 3

Correlation of FGF2 with FEV1 in patients group

In the present study, the significant negative correlation (p value <0.01) was found for FGF2 with FEV1in asthmatic patients as in figure 2.

Table (2):Levels of FEV1 and FBF2 in studied groups

Parameters	Groups	No.	Mean ± SD	P value
FEV1	Control	40	83.7675 ± 6.60	< 0.01
	Patients	30	67.4767 ± 9.46	
FBF2	Control	40	409.6722 ± 173.95	< 0.001
	Patients	30	783.5388 ± 315.12	

Table (3): Mean differences of FGF2 and FEV1 in asthmatic patients with treatment and without treatment.

Parameters	Groups	No.	Mean ± SD	P value
FGF2	Patients with treatment	18	591.28 ± 210.49	< 0.001
	Patients without treatment	12	1071.91 ± 205.25	
FEV1	Patients with treatment	18	72.81 ± 8.55	< 0.001
	Patients without treatment	12	59.46 ± 2.45	

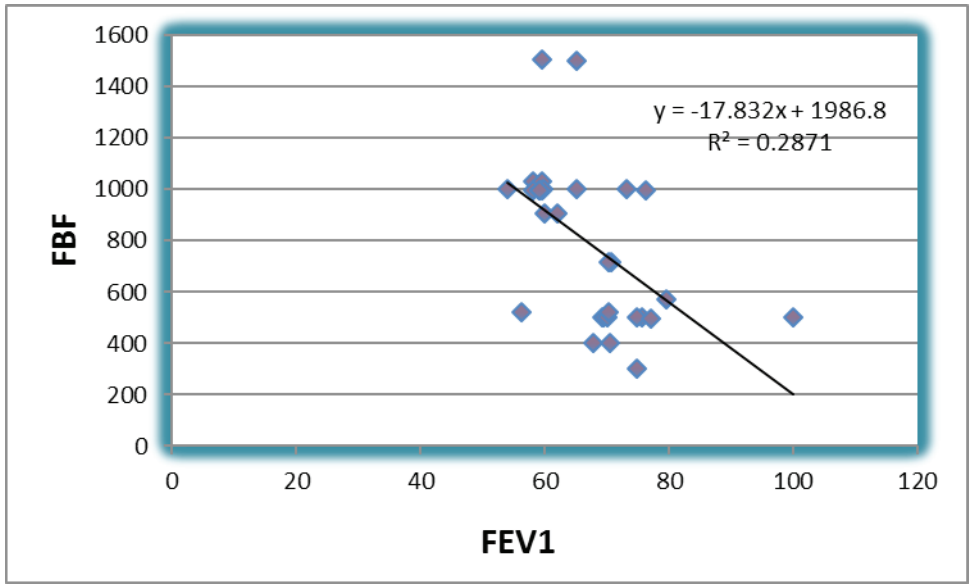


Fig (2): Correlation between FEV1 and FBG2 in patients group

Discussion

Globally, asthma revealed as the chronic lower respiratory disease. Asthma classified into mild, moderate, and severe subtypes , this classification depend on severity of asthma and rendering to fixed airflow obstruction, eosinophilic inflammation and the incidence of airway hyper-responsiveness (AHR) [(14)].

Obesity is one of the most common risk factor in asthma which helps in the improvement of metabolic disorders, chronic disease in addition to cardiovascular disease [(15)].

According to the statistics of the study, the mean age of patients who were diagnosed as asthmatic subjects and control were 40.75 and 39.08 years, respectively. The change in age (as mean) is non-significant between healthy and patients groups as shown in table 1. This age matching have a role to remove the variation in the results of different parameter that may initiate due to the variation in age [(16)]. On the other hand, this review demonstrates a slight male predominance. This finding is going with Joe G. *etal* have shown the role of sex hormones in asthma because it is sever and widespread in women, mainly in women with puberty or with multiple gestations. The main cause for gender variance are unknown but maybe linked to the immunity , hormone change, environmental and occupational exposures [(17)].

The family of FGF include different consists of polypeptides that stimulate survival, creation, and diversity of epithelial or mesenchymal cells. FGFs have a role in disease progression or regression, Dependent on the nature of the disease. There are numeral FGFs

in the lung which act as a defense system from several lethal syndromes [18].

Wound healing ,a method mentioned as airway remodeling coming after the inflammation of asthma, which produce changed in the organization of the airway including sub-epithelial fibrosis, angiogenesis, hypertrophy or hyperplasia in the airway smooth muscle [19].

Heparin and endoglycosidase produce from asthmatic lungs through that mast cells prompt the relief of FGF2 from heparan sulphate proteoglycans this make FGF2 contribute to sub-epithelial collagen deposition [20].

As showed in table 1, the FGF2 were detected in asthmatic subject than those of control group with significant variance between them ($p<0.001$). Our outcome was in agreed with [1,9] that found the same result.

Indeed, FGF2 motivate fibroblast production and the revival of epithelial cells from endogenous or exogenous stem cells, that mean FGF2 have a role in wound healing [21].

The results of this revision demonstrated that FGF2 have negative associated to the forced expiratory volume in 1 second (FEV1) in patient with asthma, our result is reliable with (Silvana Cianchetti, et al. 2019) who establish a negative relationship with the pulmonary function. The diversity in medical and inflammatory appearances of all the participant in this study responsible of conflicting results [22].

Patients with asthma response nicely to inhaled corticosteroids (ICS) treatment. Asthmatic patients have highly serum level of FGF2, and the treatment with corticosteroid diminishes it. That make FGF2 existing as a new marker for severity of asthma which linked significantly with the pulmonary function in asthmatic patients. These finding were consistent with some studies published recently [23,24].

Furthermore, higher level of FGF2 in asthmatic subject compared with control provide an idea that FGF-2 performed a defense growth factor when lung is harmed as demonstrated in table 2 and 3. These result agreed with [1,9,22,24].

Conclusion

Among asthmatic Iraqi subjects, increase levels of FGF2, indicate the significant association between asthma and fibroblast growth factor.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Impact of Local Anesthesia on Wound Healing

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Abstarct

Different local anesthetics produce multiple effects on wound healing, although this effect did not demonestrated clearly in experimental study. This study aimedto evaluate the effects of several local anesthetics (lidocaine, articaine, mepivacaine) on healing of oral mucosal wound healing in experimental rabbits by histological examination.The study was conducted on eight healthy adult male New Zealand white rabbits which divided into four group(n=2) control group which received no treatment group two (received lidocaine 2% with adrenalin) group three (received mepivacaine) and group four (received articaine 4%). oral mucosal wound were initiated experimentally in all group and each group received their treatment by local application one time daily for six day(except control group) in the third day half of the animal were sacrificed in each group and histological examination for the wound area were conducted , the remaining animal were sacrificed in the sixth day of treatment and histological examination were made for the wound area to compare the result between the four group. The mepivacaine group produce better result (enhance healing of the oral mucosal wound) followed by atricaine and lidocaine group compared with control one**.** This study showed that different type of local anesthesia produces different effects on wound healing manufisted histologically and clinically.

Key words: *lidocaine, mepivacaine, articaine, oral wound, mucosal wound.*

Introduction

Mucosa of the oral cavity consist of the mucous membrane lines the mouth from inside, it contains stratified squamous epithelium (oral epithelium), and connective tissue (lamina propria) [1]

Oral mucosa is either keratinized or non-keratinized, which is thicker and contain larger amount of elastic fiber than first one [2].

Wound can be occurred as result of any damage to oral mucosa which cause interruption of its integrity. [3], wound healing is a very complex method consist mainly of three stages: migration of inflammatory cell (neutrophil, microphage), proliferation of fibroblast and other repairing cells, then remodeling of newly formed tissue.[4]. Many factors either systemic (stress, age, drugs) or local (infection, oxygen) may affect healing process [5].

There is a certain relationship may exist between healing of oral wound in human and certain animals like rabbits [6], so this study employed healing in rabbits as model for healing in human.

Local anesthetic inhabits a significant role in controlling pain in dentistry, it's either applied topically or injected to oral mucosa [7]. Local anesthetic may pass antibacterial effects by inhibit or kill a large number of bacteria and fungus in oral cavity [8], therefore it is possible that local anesthesia by passing antimicrobial effects may add additional advantage as antiseptic in prevent wound infection post operatively [9].

However, the local anesthetic effects on wound healing are not exposed obviously yet, in spite of

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literature studies, because different results are shown in this studies [10-14].The aims of this study were to evaluate the effects of different local anesthetic agents (lidocaine with adrenalin, mepevecaine, and articaine) when applied locally on the healing of oral wound in rabbits and their antibacterial effects on oral microbes by comparing between them.

Methododolgy

The study was carried out in the depart. of Basic Science, College of Dentistry, University of Mosul. The study was approved by scientific committee/department of basic science/college, of Dentistry/University of Mosul.

Animals: eight healthy adult male New Zealand white rabbits weighting between 1.5-2 kg were included in this study. These animals were housed under 16h light/8h dark cycle at 26-32C with access free to water and food.

These animals were classified into four groups (n=2) as follow:

Group one: control group received no treatment

Group two: animals received lidocaine 2%with adrenalin

Group three: animals received mebevicaine

Group four: animals received articaine 4%

All groups were subdivided into A and B subgroup (n=1)

Subgroup A: include animals sacrificed in the 3rd day of treatment, while subgroup include animals sacrificed in the 7th day of treatment

Drugs:

Lidocaine HCl 2%with epinephrine1:80.000 carpole (Huons Co.Korea) for group two, Mepivacaine al 3% carpole (registro santario S.A.) for group three,Articaine4% with adrenaline1:100.000 carpole (registro santario. S.A), for group four.

All drugs applied topically on the wound one time daily from first day to day seven after incision.

Surgical procedure:

All animals were anesthetized by using intramuscular injection of Xylazine 2% (0.2 ml/kg) and ketamine 10%(1mg/kg) of body weight [1] then Standardized incision were made with equal length in the right side in the oral mucosa of inner check of each rabbit.

Histopathopathological examination

At the third day of treatment each animal from subgroup A were sacrificed for tissue samples of the induced wound, the tissue fixed processed and stained by hematoxylin and eosin.[15].

The procedure was repeated in the sixth day of treatment for subgroup B from each group.

Criteria of histopathological examination:

1. Criteria of inflammatory response parameter (scoring): [16,17]

Score 1: Nil No inflammatory cells seen in the field of operation (X10).

Score 2: Mild When inflammatory cells present in few numbers, less than ½ of the field (X10).

Score 3: Moderate Inflammatory cells could be seen in more than ½ of the field (X10).

Score 4: Severe or abundant when inflammatory cells present in huge numbers, more than ¾ of the field (X10).

2. Criteria for amount of granulation tissue formation (Scoring): [16,17]

Score 1: Absent of granulation tissue formation in wound.

Score 2: Quantity of granulation tissue formation in the wound gap is scanty.

Score 3: Amount of granulation tissue formation is moderate in tissues.

Score 4: Total amount of granulation tissue formation in the wound is profound.

3. Criteria of re-epithelization parameters (Scoring), [18]

Score 0: Re-epithelialization at the edge of the

wound.

Score 1: Re-epithelialization covering less than half of the wound.

Score 2: Re-epithelialization covering more than half of the wound.

Score 3: Re-epithelialization covering the entire wound, irregular thickness.

Score 4: Re-epithelialization covering the entire wound, normal thickness.

Results

Histopathological examination of oral mucosal wound (diag. 1)

1: GROUP 1:

The Number of inflammatory cells was increased gradually and continues to 6 day without regression (diag. 4), also there are marked increasing of re-epithelialization mainly after 3rd day (diag. 3). The granulation tissue formation increased gradually with continuous activation of fibroblasts (Table 1).

2: GROUP 2:

There are delay onsets of inflammation either due to infection or abnormal responses of tissue to adding material (diag. 3). The granulation tissue formation is stable during all time of experiment (dig. 4) while there are well re-epithelialization's in the first three days and continues with lower potential in second three days (dig. 4) (figs 1 and 2).

3: GROUP 3:

There are mild differences between 3rd and 6th day in the number of inflammatory cells responses (diag. 3&4) while stable increasing of granulation tissue formation during healing process (diag. 3&4). Good re-epithelialization shown in the two-time interval with increasing in the thickness and prominent sub epithelial papilla

4: GROUP 4:

Also, there are mild differences between 3rd and 6th day in the number of inflammatory cells responses (diag. 3)

Good re-epithelialization in the two-time interval (diag. 4) and mild granulation tissue formation without increasing during healing process (diag. 3)

Results comparisons

When we compared the result of four groups in the 3th day of treatment we can see:

In groups 2 and 4 there are mild inflammatory responses while there is moderate inflammatory reaction in groups 1and 3

The re-epithelialization is very high in group 3 with less responses in groups 2and 4 but minimal response in group one

Granulation tissue formation constant in groups 2 and 4 while there is gradual increase in groups 1 and 3(diag. 3&4)

When we compared the result of four groups in the 6^{th} day of treatment we can see:

Inflammatory cell infiltration is similar in group 1 and 3 but they are higher than that of group 2 and 4.

The re-epithelialization is very good in 3rd group with less activity in group 1 and 2.

The granulation tissue is nearly constant in all groups (diag. 3&4).

Discussion of Results

The normal process of wound healing is a major aim of any surgical procedure [19]. Lidocaine is one of the most commonly used local anesthetics in dentistry due to its moderate duration of action and reasonable onset [20]. The addition of vasoconstrictor (adrenaline) to local anesthetic provides additional advantages like reduce systemic absorption and reduce bleeding from surgical site [21].

According to the result of this study group 2(lidocaine+adrenaline) produce less inflammation when compared with control group in the third day while granulation tissue and re-epithelization showed the same results in the 6^{th} day of treatment this was in agreement with other study [22]. Lidocaine may interfere with mast cell arrival at the site of injury as signal from this cell will participate in the inflammatory process, matrix

reabsorption and angiogenesis. The effects of adrenaline were to potentiate the blocking effect of lidocaine [23]. This study was disagreement with other study [24] which showed that lidocaine has a negative (delay) effect of wound healing by increasing risk of secondary infection specially when combined with adrenalin while Waite et al [25] in his study showed that lidocaine with adrenaline did not cause any effects (negative or positive) on wound healing.

According to this study, group 3(mepivecaine) produce highly inflammatory responses in 3rd day with a good healing in 6th day (manifested by increased re-epithelization and granulation tissue formation) compared with another group.

Mepivacaine is used now a day to replace lidocaine with adrenaline local anesthetics in case where the use of adrenaline is somewhat contraindication like patient with cardiovascular disease or thyroid problem [26] so it has been selected in this study.

According to our knowledge this is the first study conducted to study the healing effects of mebivacaine local anesthetics. The increased in the inflammatory responses in the 3rd day may be due to the effects of mebivacaine on increasing cytokine production which enhance inflammatory responses [27] this will promote healing by epithelial cell migration and remodeling of matrices in extracellular place which enhance granulation tissue formation in 6th day of treatment [28]. The positive effects of mebevacaine on healing also may be due to its antibacterial effects (9) also it may affect the maturation and sequence of collagen fibers [29].

In the group4 (articaine) there is a mild inflammation in the 3rd day with small amount of granulation tissue and re–epithelization in 6th day compared with another group. Articaine was selected in this study due to the facts that articaine became so popular in many countries due to its good efficacy. The results of this study was in agreement with Dogan et al study(2003) who showed that histological finding and breaking strength in articaine group is less than in control group[30], but its disagreement with the study of Senes et al (2015) which showed that there is no significant different between lidocaine and articaine group[31].

The inhibitory effects of articaine on healing in this study may be due to its proliferating or inflammatory phase of healing [32] or it may affect the mechanism that cause cell membrane stability, calcium movement inside the cell or sodium conduction, or by acting as enzyme inhibitors to hydroxylase enzyme which is responsible for collagen synthesis[33].

Table 1: Scoring data of the study

A1	2	1	1
B1	3	3	2
A2	0	2	2
B2	2	3	2
A3	2	3	1
B3	3	4	2
A4	0	2	2
B4	2	2	2

Figure . 1: comparison the results of four groups in 3rd day of experiment.

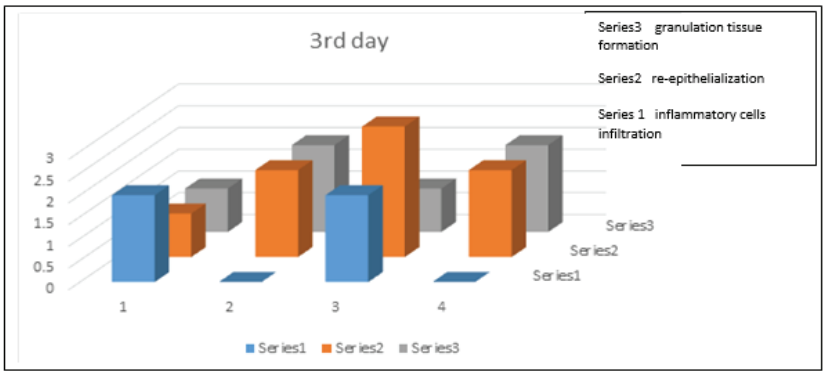


Figure 2: comparison the results of four groups in 6th day of experiment

Conclusion

In conclusion different types of local anesthetics may produce different effects on wound healing in mucosal membrane inside the oral cavity, present or absent of vasoconstrictor may provide additional advantage or disadvantage in this point although the concentration of local anesthetic is also an important point to note, this study demonstrate that mepivacaine provide a better result compared with control and other group but further study is required to demonstrate these effects in other animal and in other site are required.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Pharmacological Study of Weight Losing of Liraglutide Loaded within Layered Double Hydroxide Carrier

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Abstract

The anti-diabetic drug liraglutide was loaded within LDH nanoparticles using the Fe^{+3}, Al^{+3}, Fe^{+2} and Ni^{+2} ions, the preparation of LDH was carried out via the titration of 2M of NaOH with trivalent and divalent ions in presence 0.5M HCl, the prepared compounds of liraglutide with LDH were tasted with selective groups of rats.

The compounds were characterized with Fourier transform infra-red (FTIR), X-ray diffraction (XRD), Atomic force microscopy (AFM), Zeta potential and Scanning electron microscopy (SEM).

The animals that injected with weekly dose of the prepared compounds shows the weight reduction comparative with that of the animals injected daily dose of pure liraglutide.

Keyword; *Liraglutide, double layered hydroxide, nanoparticles, antidiabetic drug.*

Introduction

Liraglutide is a fatty acid derivative of glucagon like peptide-1 [GLP-1]. It is formed by attaching a 16-carbon fatty acid molecule at position Lys26 and making an Arg34Lys substitution on GLP-1, and shares approximately 97% sequence homology with GLP-1 so retains the physiological activities of GLP-1[1, 2]. These structural modification of liraglutide increase chain aggregation, promote reversible non-covalent binding to other molecules, such as albumin, and resist dipeptidyl peptidase degradation. Liraglutide marketed as Victoza®, is a 1.8 mg daily subcutaneous injection that was initially approved by the FDA in 2010 as an adjunct therapy to diet and exercise for management of type 2 diabetes. Appetite suppression and delayed gastric emptying are thought to be responsible for the weight lowering effects of GLP-1. As a result, liraglutide was also developed as a weight loss agent [3].

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Layered double hydroxides (LDHs), also known as hydrotalcite-like compounds, represented by the general formula$[M^{II}_{1-x}M^{III}_{x}(OH)_2]^{x+}[(A^{n-})_{x/n} \cdot yH_2O]^{x-}$, where M^{II} and M^{III}, di-and tri-valent metal cations, respectively, are capable of occupying the octahedral interspaces of brucite-like sheet. A is anions between the layers can compensate the positive charges of the layer structures and these interlayer regions may contain water molecules[4]. Recently, particular attention has been focused on the LDH-based controlled release systems. Although many biomolecules/LDH hybrid complexes have been reported, only a few examples have been studied as drug delivery carriers. In the present study, liraglutide drug was selected as a model drug and intercalated into Fe^{+2}/ Fe^{+3} LDH, Fe^{+2}/Al^{+3}-LDH and Ni^{+2}/Fe^{+3}- LDH successfully by coprecipitation technique. This work focus on the structure and slow/ controlled release property of a synthesized drug–LDHs composite intended for providing basic data for organic–inorganic LDH hybrids[5].

Materials and Methods

1. Preparation of precursor and hybrids

Synthesis of Fe^{+2}/Fe^{+3} layered double hydroxides nanoparticles

The synthesis of Fe^{+2}/Fe^{+3} LDHs NPs was done by mixing 1:2 molar ratio of Fe^{+2}/Fe^{+3} (25 mL of 0.02 M of $FeSO_4.7H_2O$ solution and 25 mL of 0.04 M of $FeCl_3$ solution addition 2.1 mL 0.5 N HCl. Then a drop wise titration is done with 2 M NaOH with vigorous magnetic stirring at 80 ˚C until pH elevated from 2 to 9 with change in color of the solution from clear yellow to dark brown suspension which is then left at room temperature for 24 hours. The suspension is filtered and continuously washed with deionized water until the filtrate became neutral. The obtained solid residue was dried at 50˚C in oven for 4 hours [6].

Synthesis of Fe^{+2}/Al^{+3} Layered Double Hydroxides nanoparticles

In the present work Fe^{+2}/Al^{+3} LDHs NPs was synthesized by mixing 25 mL of 0.06 M of $Al(NO_3)_3$ solution and 25 mL of 0.02 M of $FeCl_3$ (3:1 ratio) respectively. To which 2.1 mL of 0.5 N HCl was added, then titrated drop by drop with 2 M NaOH with vigorous magnetic stirring at 80˚C until the pH elevated from 2 to 9 with change in the color of the solution from clear yellow to light brown suspension which is then left at room temperature for 24 hours. The resultant suspension filtered and washed with deionized water several times, until the filtrate became neutral [7].

Synthesis of Ni^{+2}/Fe^{+3} Layered Double Hydroxides nanoparticles

Ni^{+2}/Fe^{+3} LDHs NPs was synthesized by mixing 25 mL of 0.75 M of $Ni(NO_3)_2$ solution and 25 mL of 0.25 M of $FeCl_3$ (3:1 ratio) respectively. To which 2.1 mL of 0.5 N HCl was added, then titrated drop by drop with 2 M NaOH with vigorous magnetic stirring at 80˚C until the pH elevated from 2 to 9 with change in the color of the solution from clear yellow to brown suspension which is then left at room temperature for 24 hours. The resultant suspension filtered and washed with deionized water several times, until the filtrate became neutral [8].

Synthesis of Fe^{+2}/Fe^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml (0.00121g, 0.0008 M) of $FeSO_4.7H_2O$ solution mixed with 10 mL (0.00064g, 0.0004 M) $FeCl_3$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from yellow to dark brown suspension [9].

Synthesis of Fe^{+2}/Al^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml(0.0017g, 0.0008M) of Al $(NO_3)_3$ solution add to 10 ml of (0.00064g, 0.0004M) $FeSO_4$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from pale yellow to light brown suspension [10].

Synthesis of Ni^{+2}/Fe^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml (0.00146g, 0.0008M) of $Ni(NO_3)_2$ solution mixed with 10 mL (0.00064g, 0.0004M) of $FeCl_3$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from yellow to brown suspension [9].

Animals and study design:

Thirty adult's male wistar rats (weighing 220-248 gm) were utilized in this study. Animals were attained and placed in animal house of College of Pharmacy/ Mustinsiryiah University, they received water and ordinary pellets for one week for acclimatization, after that they feeding lard [11] for 12 weeks to develop obesity were divided into 5 groups:

- (Group A) was receive 0.5ml/kg/week normal saline.

- (Group B) was receive 600µg/kg/day of pure liraglutide.
- (Group C) was receive 650µg/kg/week IP of Fe^{+2}/Fe^{+3} LDH loaded liraglutide.
- (Group D) was receive 655µg/kg/week IP of Ni^{+2}/Fe^{+3} LDH loaded liraglutide.
- (Group E) was receive 658µg/kg/week IP of Fe^{+2}/Al^{+3} LDH loaded liraglutide.

Animals were kept in plastic cages each with dimension (20x25x35 cm) that harbor three rats, animals within same cage were discriminated by back fur marking by using waterproof colored marker. The animals were placed under controlled condition of room temperature (25 $\pm 1^0$C) and light stream of 12 hours light/ 12 hours dark cycles. Diet and water were attainable easily to animals. Animals study design started in February /2019 and ended in Jun /2019. Ethics committee of the College of Pharmacy/ Mustansiriyah University was given their approval to begin the study.

Results and Discussion

Characterization of prepared compounds

FT-IR spectra for LDHs and LDHs loaded liraglutide

The FT-IR spectra of the synthesized compounds displays the characteristic absorption bands by which the functional groups were identified [12]. The assigned bands of LDHs spectra and LDHs loaded drug spectra refer to the type of binding between the molecules as hydrogen bonding only as a physical tied and easy liberate in target site[13].

X-ray diffraction (X-RD)

A general analysis of these patterns shows that the LDHs loaded liraglutide shows the peaks at 33 two theta assigned to the dihedral geometry as amorphous structure with small difference between LDHs types. Such difference is attributed to the higher similarity of ionic radii for the ion pair.

Atomic force microscopy (AFM) Results:

Results of the AFM images of the LDHs typical surface (in three and two dimensions), the spectrum of AFM pictures of prepared compounds before and after loading the liraglutide since the difference between the arrangements of layered after loading is very clear refer to the affecting of binding of drug with the LDH, from the study of surface and layered of nanocompounds were arrange as vertical cylinders this shape make the molecules easy to liberate the liraglutide.

Zeta potential measurement

Zeta potential measurement for the prepared LDHs loaded liraglutide. The criteria of stability of NPs are measured when the values of zeta potential ranged from higher than +20 mV to lower than -20 mV. The values of the zeta potential of the prepared compounds are listed in table-1 provided satisfactory evidence about their little tendency towards aggregation when its zeta potential in the negative scale and below -20 mV. These results suggested that the prepared LDHs loaded drug particles were stable with no tendency to aggregates and this in agreement with the results reported for colloidal nanoparticles behavior [14].

Table 1: Zeta potential values for the prepared LDHs NPs loaded liraglutide

LDHs NPs Type	Zeta Potential (mV) ± SD	Mobility (µ/s)/(V/cm) ±SD
Fe+2/Fe+3 LDHs loaded liraglutide	-32.82	-2.56
Al+3/Fe+2 LDHs loaded liraglutide	-26.45	-2.07
Ni+2/Fe+3 LDHs loaded liraglutide	-28.00	-2.19

Scanning electron microscopy (SEM)

The pictures shown in figure-1 represented the SEM images of LDHs loaded liraglutide. The images confirmed the layered shape of LDHS with multiple agglomeration areas, due to the hydrogen bonding attraction between multiple hydroxyl groups those had not been hydrolyzed by acidic hydrolysis [15].

In vivo

The change in the initial and final mean of body

weight of group A (0.5ml/kg/week) normal saline intraperitoneal after four weeks was small that indicate there is no significant difference ($p = 0.07$). While body weight of group B was significantly decreased ($p < 0.05$) after daily administration of 600µg/kg liraglutide plus 0.5ml/kg normal saline intraperitoneal for four weeks. This result in weight reduction compatible with result that reported by Astrup *et al*. [16]. Liraglutide lead to slows gastric emptying time and decrease food intake, so cause weight reduction [17].

The body weight of prepared compounds groups C, D and E was significantly decreased ($p< 0.05$) after weekly administration of prepared LDHs loaded liraglutide plus 0.5ml/kg normal saline intraperitoneal for four weeks.

Reduction in the weight of groups C and E about 18% of body weight after four weeks this results very close to results of group B (daily dose of pure liraglutide) that mean Fe^{+2}/Fe^{+3} LDH and Fe^{+2}/Al^{+3} LDH are more suitable for loading drugs because these compounds have higher loading capacity than group D as shown in (figure-2). The statistical analysis of rat ´s body weight represented as mean ± standard error of mean in groups (n=6 for each group).

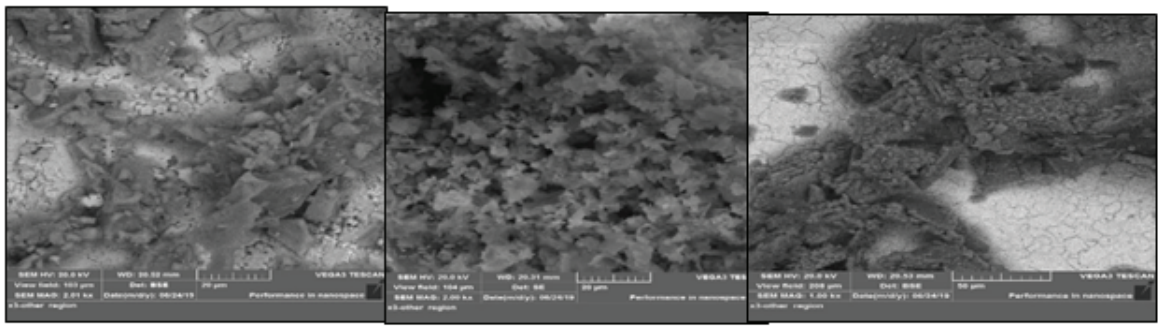


Figure 1: SEM images of the prepared LDHs loaded liraglutide in different measurements.

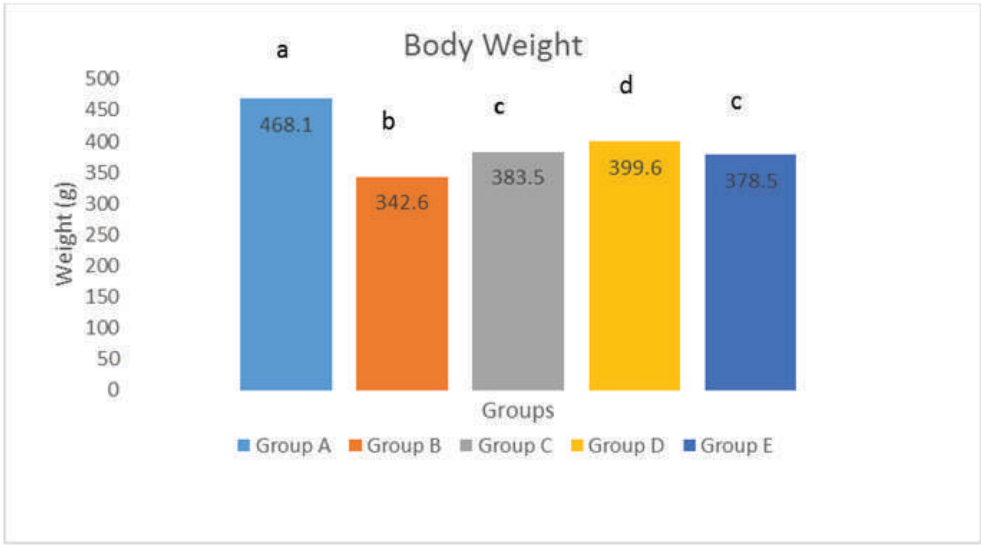


Figure 2: Bar chart illustrate the changes in body weight after four weeks of receiving these prepared compounds:

Different small letters indicate significant differences among the treatments group $p< 0.05$.

Conclusion

1. Characterization and identification of the synthesized compounds were confirmed by determination of FT-IR spectroscopy.

2. The size of the nanocarrier was successfully confirmed by AFM, SEM and XRD.

3. The zeta potential analysis provided a satisfactory evidence about the prepared LDHs little tendency towards aggregation.

4. The Preliminary study of the weight reduction activity by intercalation of liraglutide drug within LDHs NPs via co-precipitation method provide a promising nanocarriers to prolong duration of action of liraglutide.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Synthesis and Study the Biological Activity of New Heterocyclic Compounds Derived From Hydrazide Derivatives

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Abstract

The work involves synthesis of new compounds derived from 2-substituted-3-thio- acetohydrazide-1,2,4-triazin. The subsituted hydrazine thiocarbothioamide [IV] was obtained via reaction of hydrazide compound [III] with ammonium thiocyanate and HCl. The cyclization of compound [IV] in 4% NaOH led to formation 1,2,4-triazoles [V], which were refluxed with chloro-ethylacetate in ethanol and sodium acetate to give compound [VI]. Condensation of ester-compound [VI] with hydrazine (80%) led to formation new hydrazide compounds [VII]. Ring closure reaction of compound [VII] with 4-aminbenzoic acid in $POCl_3$ give 1,3,4-oxadiazoles [VIII]. Compound [VIII] was reacted with sub. benzaldehyde to form compounds of Schiff bases $[IX]_{a-d}$. The synthesized compounds were diagnosed via IR, Mass and ^{1}HNMR spectral data. Many of synthesized compounds were examined against activity of antibacterial: gram (+) (*Bacillus subtitis, staphylococcus aureus*) and *gram* (-): (*E.coli* bacteria) and candida tropicalis fungial. To ensure the safety of the synthesis compounds the toxicity was examined.

Keywords: *1,2,4-triazine, 1,2,4-triazoles, 1,3,4-oxadiazoles, Schiff bases, antibacterial activity.*

Introduction

Triazine is one type of heterocyclic compounds, with six-membered include three nitrogen atoms. There are various methods for the synthesis of triazine compounds. The first of them, reported by Bamberger[(1)]. On other hand, another general methods are reported in Literatures[(2,3)], including the dehydrogenation in potassium dichromate from dihydro-triazines . Also synthesized by the rig closure reaction of hydrazine compound with α-acylaminoketone[(4)]. Recently, Arshad et el. mentioned a method for prepared 1,2,4-triazine via cyclization of thiosemicarbazide with benzil in acetic acid[(5)].

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1,2,4-Triazine derivatives are good type in many biologically fields, such as antimicrobial[(6)], antibacterial[(7)], antifungal[(8)] and anticancer[(9)]. Furthermore, 1,2,4-triazole ring systems have a wide spectrum from biological activities, Antibacterial[(10)] and antifungal[(11)]. Also the 2- mercapto-1,2,4-triazoles have good role in synthetic organic chemistry, for example, to formation other heterocyclic compounds in present of different reagents, undergo various types of reactions [(12)]. Heterocyclic having a five ring heterocyclic containing two nitrogen atoms at position 3, 4- and one oxygen atom at position 1 is called 1,3,4-oxadiazole[(13)] , which is have potent biologically are 1,3,4-oxadiazoles[(14)] . In addition to, 1,3,4-Oxadiazole is a thermally stable neutral aromatic molecule and its have widely applications[(15)]. On the other hand the aryl-oxadiazoles have been used for development of newly drugs, with attention of a special kind to their properties as antitubercular and antimicrobial agents. [(16)]. The synthetic methods for the formation of differently 1,3,4-oxadiazoles have been

reviewed [17].

Due to a number of biological activities of triazines, triazoles and oxadiazoles are reported in previous literature, we have synthesized new compounds containing triazole and oxadiazole starting from triazines by using simple and an efficient method besides to investigation of the biological activities of these compounds.

Materials and Methods

Materials: The chemicals were equipped from Merck , Fluka ,GCC and Aldrich chemicals company.

Techniques: FTIR spectra (using KBr disc) were determined by on a Shimadzo (8300), ^{1}HNMR spectra were determined by: Bruker company (model: ultra-shield 400 MHz), origin: Switzerland (in solvent DMSO), ppm(δ), uses internal standard (TMS). Mass spectra were recorded on MS model: 5973(Manufacture Company: Agilent Technology HP). Gallen Kamp melting point apparatus was used for determined uncorrected melting points. The TLC were done on aluminum plates, supplied from Merck co, using ((7:3), n-hexan/ethyl acetat).

General Experimental Procedures

The reaction sequence leading to the formation of new compounds [IX]-[X] are outlined (**Scheme not shown contact corresponding author**).

1,2,4-triazine-3-thiol derivative [I], their ester[II] and hydrazide [III]: These compounds were prepared according to the let. [18].

2-(2-((5,6-di-phenyl-1,2,4-triazin-3-yl)-thio)-acetyl)-hydrazine-1-carbo-thioamide [IV]

Heating a solution of ammonium thiocyanate (6gm, 0.078 mol) and acid hydrazide [II] (0.013 mol) in ethanol (60 mL) and HCl (12 mL) via reflux for (22 hrs.). Then, evaporated the solvent and the mixture of reaction was added to crushed ice with stirring, the precipeted was filtered off [19], dried and recrystallized from ethanol.

1,2,4- triazoles [V]

Refluxed for 22 hrs a mixture of compound [IV] (0.01mol) and (10mL) from 4% aqueous NaOH. Then, filtered the mixture, the filtrate was acidified with dilute HCl. Afterwards, filtered the powder which precipitate [20], washed it for several times with water, recrystallized from ethanol.

Ester compound [VI]

The compound [V] (0.001 mol) was heated with chloroethyl acetate (0.001 mol) and sodium acetate (0.003 mol) in ethanol was for 5hrs. Then, cool the mixture and added it onto ice water, the solid resulting was filtered, dried. Recrystallization by used ethanol to give ester compound [VI].

hydrazide compound [VII]

A solution of compound [VI] (0.06 mol) and(15 mL) hydrazine(80%) in ethanol (25 mL) was heating for 4hrs. Afterwards, cooling the mixture of reaction (to room temperature), the solid compound was filtered, then, recrystallized from ethanol.

Synthesis of 1,3,4-oxadiazole compound [VIII]

4- Aminobenzoic acid (1,37g, 0.01 mol) was mixed with thiosemicarbazide [VII] (0.01mol) in phosphorus oxy-chloride (5 mL), afterward the mixture was refluxed softly for 6 hrs. After cooling, its poured onto ice water (50 mL) with stirring. The precipitate was filtered, washed with solution of $NaHCO_3$, then water, dried then recrystallized from ethanol.

Synthesis of Schiff bases $[IX]_{a-d}$

Reflux of a mixture of aromatic aldehydes (0.001 mol), 2-amino-1,3,4-oxadiazoles [V] (0.001 mol), glacial acetic acid (3 drops) in (4 mL) ethanol for 4hrs. Recrystallized the obtained solid from ethanol after evaporating the solvent. The physical properties, nomenclature, structure of the synthesized compounds $[IV]_{a,b}$ are listed in Table (not shown contact corresponding author)

Biological activity

Adult male mice, weighting about (23-46) gm were housed in plastic cages under standard condition. the animal were divided into five groups each group consisting of five mic, including the control group, the mice were injected with the synthesized compounds with a concentration of (10^{-2}). After two weeks toxicity

was measured as the method [21].

Results and Discussion

The equimolar of benzil and thiosmecarbazid were reacted under ring closure reaction in glacial acetic acid to formed triazine compound. Ester compound [II] which prepared from the reaction of triazine with chloro acetic acid in basic medium and ethanol was diagnosed via FTIR spectroscopy. The FTIR spectral data showed two bands at 1732 cm^{-1} and 1253 cm^{-1} due to (υ C=O) and (υ C-O) related to ester moiety respectively, with disappearance (υ C=S). The condensation hydrazine (80%) with ester [II] in ethanol give a corresponding hydrazide type[III]. The FTIR of compound [III] exhibited new bands at (3329-3207) cm^{-1} due to υ NH_2 and υ NH (asym., sym.) bands and (υ C=O) vibration band for of amide group at 1668 cm^{-1}.

Nucleophilic addition reaction for acid hydrazide [III] with ammonium thiocyanate in ethanol using HCl as a catalyst to give hydrazine carbothioamide derivatives [IV]. The structure of this compound was identified by IR and ^{1}H-NMR spectroscopy. IR spectrum indicated υ C=S absorption band at 1280 cm^{-1}, in addition to new bands which related to (asymmetric, symmetric) vibration of υ NH_2 and υ N-H groups appear at (3388-3168) cm^{-1}. Also a shifting on υ C═O (amid) group to 1672 cm^{-1}. The spectrum of ^{1}HNMR for compound [IV] showed a singlet signal of SCH_2 groups at δ 4.97 ppm and a signal at δ5.57 ppm due to NH_2 protons of group .Signals due to aromatic (ten) protons for benzene rings at δ (7.06-7.90) ppm. Also two signals for protons of two NH group of (CO-NH-NH-CS) moiety at δ12.78 ppm and δ13.56 ppm .

1,2,4-triazole-3-thiol [V] was obtained by cyclization of hydrazine carbothioamide compound [IV] in a solution of 4% NaOH followed by neutralized with dil. HCl to yielded this compound. The triazoles were diagnosed via FTIR and NMR spectroscopy. The FTIR spectral data showed a good peak at (3394-3153) cm^{-1} for stretching band of NH, also show the disappearance of the characteristic bands of starting materials [IV], in addition to new peak around 1284 cm^{-} for C=S stretching. The ^{1}H-NMR spectrum (in DMSO) showed signals as in the following: a signal type singlet at δ10.09 ppm for NH proton of triazole ring. Aromatic protons appeared at δ (7.26-7.53)ppm as many signals, signal appeared at δ 9.8 ppm for NH proton at C_2 of triazole ring (which is toutomerism with SH). Also exhibited a signal type singlet at δ 5.57ppm for CH_2 protons. The spectrum of mass exhibited parent ion [M^+] at 378.

The ester compound [VI] which prepared from the condensation of compound [V] with chloro acetic acid in CH_3COONa medium and ethanol was diagnosed via spectroscopy (IR and ^{1}HNMR). The IR spectral data showed: υ C=O and υ C-O for ester group and 1211 cm^{-1}, respectively. at 1735 cm^{-1}. Disappearance band of (υ C=S). The reaction of ester [VI] with hydrazine hydrate in ethanol give a corresponding acid hydrazide [VII]. The FTIR spectral data exhibited asym., sym. bands at 3199 cm^{-1} of υ NH and υ NH_2 groups, and (υ C=O) vibration band at 1670 cm^{-1} for amide group.

The reaction of compound [VII] with thiosemicarbazide in $POCl_3$ yielded a new compounof 1,3,4-oxadiazoles [VIII]. The oxadiazole compound was diagnosed via IR and ^{1}HNMR spectra. The IR spectral data showed disappearance bands characteristic for hydrazide compounds and the appearance of a new two bands due to (υ NH_2) between (3442-3213) cm^{-1} beside to a band around 1670 cm^{-1} for υ C=N. While the spectrum of ^{1}HNMR of [VIII] showed: a signal type singlet at δ 9.8 ppm for of NH proton. The protons type aromatic appeared as many signals at δ (6.9-7.6) ppm, also a singlet signal due to SCH_2 protons appearance at δ 4.0 ppm. Finally, a signal type singlet appeared at δ 6.6ppm for NH_2protons. The mass spectrum exhibited parent ion [M^+] at 551.

The Schiff bases type $[IX]_{a-d}$ were produced from the refluxing of amino compound [VIII] with aromatic sub. aldehydes in glacial acetic acid (3 drops) with ethanol. The FTIR spectral data (Table 2) of these compounds showed appearance new υ C=N absorption band. The spectrum of ^{1}HNMR for Schiff base $[IX]_a$ showed a signal type singlet at δ 9.86 ppm for NH proton and δ (6.92-7.76) ppm for protons type aromatic, CH=N proton at δ 8.67 ppm appeared as a singlet signal. Also the spectrum showed a singlet signal at δ 4.86 ppm for SCH_2 protons. Finally a signal type singlet appeared at δ 2.3 ppm refer to CH_3 protons. The ^{1}HNMR spectrum of Schiff base $[IX]_d$ showed aromatic protons at δ 9.68 ppm for NH proton and δ (6.7-7.95) ppm protons type aromatic appeared at δ 8.6 ppm and a signal type singlet

for one CH=N proton. Finally a signal type singlet at δ 3.06 ppm for $(CH_3)_2$ protons.

Biological activity

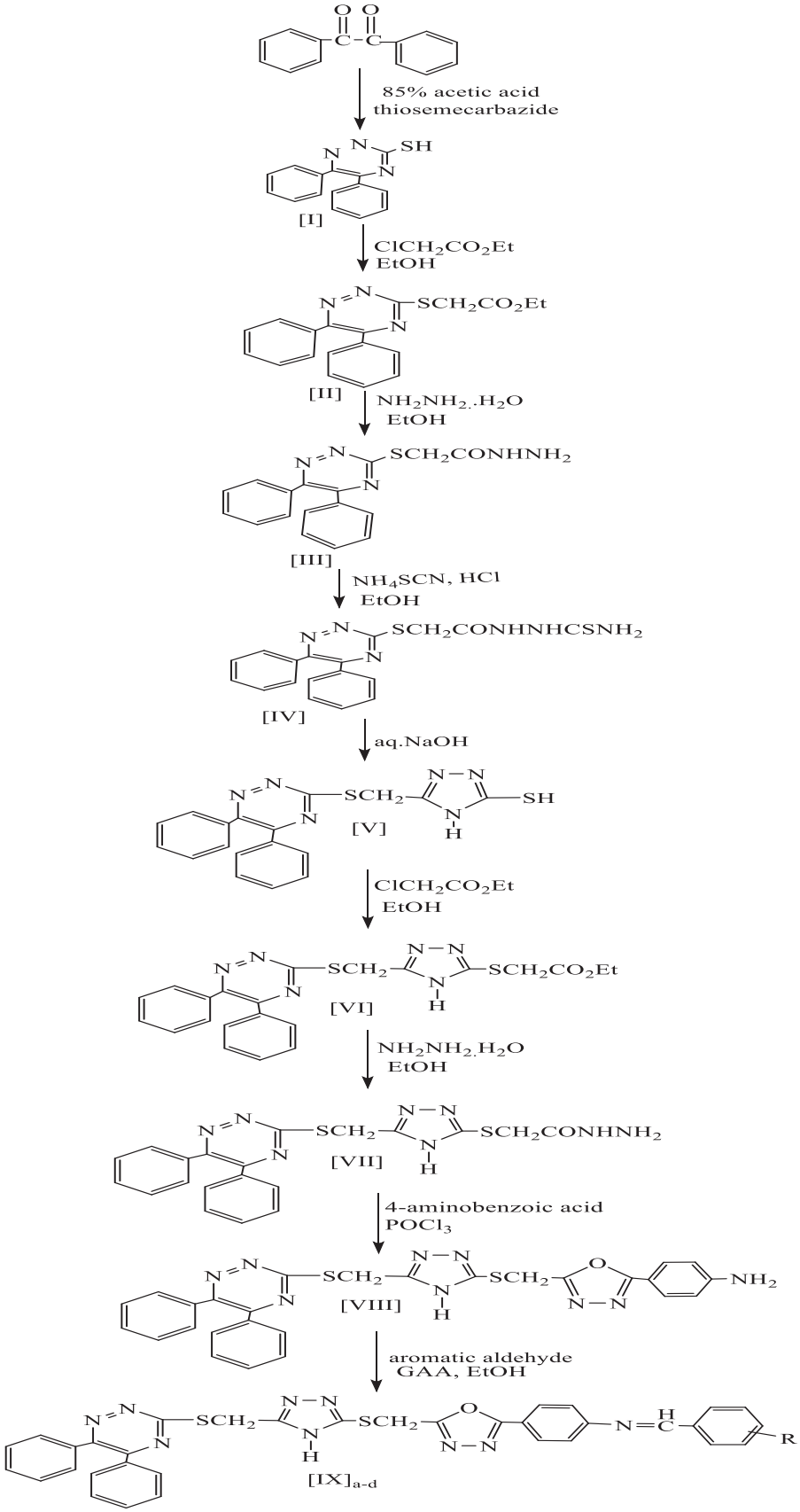
The agar diffusion [22] was used for examined the activity of antibacterial and anti-fungial of some of the synthesized compounds, using three types of bacteria; (G-) *Escherichia coli*, (G+) *Bacillus subtilties* and *staphylo coccus aureus*, and Candida tropical as fungal. The prepared (agar medium and petri-dishes) were sterilized via autoclaving for (20 min) at (121ºC). The agar was surface inoculated uniformly from the both cultures of the tested micro-organisms, the fungal and three types of bacterial were activated in dextrose agar medium and nutrient agar medium for 24 hrs at 37 ºC, respectively. Every one of examined compounds was dissolved in DMSO (used as a control and a solvent) to give two type of concentration (10^{-2} M and 10^{-3} M). The inhibition zones which formed were measured in milli-meter. The antibacterial activities and anti-fungal data of the examined compounds were given in Table (3). The administrated does of Compounds [V] and[VIII] did not result in death of any of mice except $[IX]_b$ in toxicity test.

Table 2 IR spectral data of compounds types [IX]a-d

Comp No	υ NH	υ CH arom	υ CH aliph.	υ C=N	υ C=C arom	Other
[IX]a	3400	3057	2931,2868	1680,1604	1556	δ CH3:1370
[IX]b	3305	3059	2935,2871	1678,1601	1527	υNo2 asy:1527 sy: 1348
[IX]C	3410	3057	2935,2866	1680,1601	1556	υ No2 asy:1522 sy:1344
[IX]d	3194	3055	2937,2868	1676,1597	1523	υ NMe2:1367,1169

Table 3 Inhibition Zones of Titled Compounds

Compound No.	Zone of Inhibition (mm.)							
	Bacillus Subtilis		Staphylo cocas aureus		E. Coli		Candida tropicalis	
	Gram Positive(+)		Gram Positive (+)		Gram negative (-)			
	Con. 10^{-3} 10-3	Con. 10^{-2}	Con. 10^{-3}	Con . 10^{-2}	Con . 10^{-3}	Con . 10^{-2} 10^{-2}	Con . 10^{-3}	Con . 10^{-2}
[V]	16	18	15	18	-	-	-	-
[VIII]	-	16	12	15	-	-	-	-
$[IX]_b$	-	17	-	-	-	-	-	10



R= CH_3; $[IX]_a$, 3-NO_2; $[IX]_b$, 4-NO_2; $[IX]_c$, N-Me_2; $[IX]_d$

Scheme I :synthetic route of compounds $[IX]_{a-d}$

Conclusions

In this work new heterocyclic compounds derived from 5,6-diphenyl-1,2,4-triazine-3-thiol were synthesized in good yields via simple methods. Some compounds give good biological activity that may be related to the functional groups for the examined compounds.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Role of Selective Cytokines in the Pathophysiology of Patients with Celiac Disease

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Abstract

Background and Aims: Inflammatory cytokines levels may be elevated in patients with celiac disease (CD). But little known about the association of these cytokines and some immunological parameters used for diagnosis of celiac disease. This study aimed to evaluate the levels of some inflammatory cytokines in patients with celiac disease and compare it with healthy individuals and then their association with some immunological markers used for diagnosis of celiac disease.

Methods: A total of 60 patients with celiac disease and 40 healthy persons were enrolled in 2019. The levels of Anti-tissue transglutaminase levels were measured for patients and healthy groups, also Tumor necrosis factor Alpha (TNF-α), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) were measured using enzyme-linked immunosorbent assay techniques. Then compare these parameters between groups and the correlation between these parameters were assessed.

Results: The mean levels of Tumor necrosis factor Alpha (TNF-α), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) give a highly significant difference between patients and healthy groups. Anti-tissue transglutaminase levels correlated and were show statistically significant with these cytokines.

Conclusions: TNF-α, IL-6 and IL-8 play a role in the pathophysiology of celiac disease.

Keywords: *Celiac disease, Anti-tissue transglutaminase, TNF-α.*

Introduction

Celiac disease (CD) consider as a type of an autoimmune disease affecting about 1% of the population and many studies were found that increase in the community[1]. Celiac disease (CD) has a wide range of clinical manifestations as a multi-component and immune-mediated intestinal disorder[2]. It was well known that gluten consumption, found mainly in wheat, rye, and barley, is the primary external CD cause in predisposed individuals[3]. Celiac disease is the product of dysregulation of the innate and adaptive immune system, Adaptive immune system activation means that gliadin (the toxic element of gluten) crosses the epithelium of the intestine[4]. It has been hypothesized that an early occurrence in CD pathogenesis is increased intestinal permeability[5]. Many cytokines in CD were more effective mediators than others to intensify the immune response[6]. Th2 cells, B cells, monocytes, macrophages, endothelial, epithelial, fibroblast cells secrete interleukin-6 (IL-6), the acute-phase response is an active inducer, th1 produce TNF-α, some Th2 and some CTL phenotypes are also producing this cytokine[7]. This stimulates the development of nitric oxide and activates, among other biological actions, microvascular endothelium; researchers found that TNF-α is the most potent inducer of TG2 transcription, working synergistically with IFN-α[8].

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Material and Methods

Serum samples

Sixty serum samples are collected from active CD patients and 40 healthy persons as a control group. Tissue transglutaminase (TTG) IgA and IgG serological antibody tests are diagnosed to all patients and the control group. Serum was isolated from peripheral blood collected from patients after informed consent and processed at 8 ºC before examination by the gastroenterologist[9]. All specimens are obtained from Al-Hussain Medical City in Karbala.

2.2. Measurement of anti-TTG TNF Alpha, IL6, IL8 antibody:

The levels of both the human TNF Alpha ELISA Kit, Anti-Human Tissue Transglutaminase (anti-tTG) ELISA Kit, Interleukin 6 ELISA Kit (IL6) and interleukin 8 ELISA Kit (IL8) are estimated based on standard sandwich enzyme-linked immunosorbent assay technology, for the quantitative or semi-quantitative determination in human serum (antibodies-online GmbH, Aachen, Germany).

Statistical Analysis

Using the statistical software package SPSS 23, the findings are analyzed. The likelihood of (Pentr 0.05) has been found in statistically significant[10].

Results

Measurement data for certain serum cytokines in patients with CD, as well as corresponding controls are given in table1. The detection of the autoantigen of CD ((tissue transglutaminase (tTG)) as anti-TTG IgA antibodies in the CD serum has become an important tool for examining this disease. The Th1 serum cytokine levels of IFN-α were much higher in patients with coeliac disease compared to controls as compared with patients with CD. A correlation seemed to exist between anti-tTG IgA serum mean levels and mean cytokine serum levels tested. positive associations with statistically significant have been reported between the (anti-TTG IgA, anti-TTG IgG) rates and all cytokines IL-6, IL-8 but the mean TNF-α levels was excepted.

Tab 1: demographic and clinical characteristics in patients with celiac disease.

Characteristic	N	Percentage (%)
No of patients	60	100
Gender		
Male	23	38
Female	37	62
Age(years)		
(1-10)	31	52
(10-20)	19	32
(20-30)	10	16
duration of disease		
>10	17	28
<10	43	72

Tab 2: Levels of Anti-tissue transglutaminase in patients and healthy group.

Parameters	Patients (N=60)	Healthy group(N=4)	P. Value
	Mean±SD	Mean±SD	
Anti-tissue transglutaminase IgG	97.345±76.457	11.789±6.092	≤ 0.05
Anti-tissue transglutaminase IgG	50.752±42.123	13.098±4.178	≤ 0.05

Table 3: Mean levels of selective cytokines in patients and a healthy control group.

Parameters	Patients	Healthy group	P-value
	Mean±SD	Mean±SD	
TNF-α	11.431±2.082	1.634±1.011	≤ 0.05
IL-6	72.098±4.864	2.001±1.121	≤ 0.05
IL-8	104.324±2.981	3.542±2.032	≤ 0.05

Table 4: Correlation of three parameters among asthmatic patients according to Anti-TT-A and Anti-TT-G.

			Anti-TTG- A	Anti-TTG-G
	TNF-α	Pearson Correlation	0.156	0.855**
		Sig. (2-tailed)	0.051	0.000
		N	60	60
	IL-6	Pearson Correlation	1	0.716**
		Sig. (2-tailed)		0.000
		N	60	60
	IL-8	Pearson Correlation	0.669**	0.517*
		Sig. (2-tailed)	0.001	0.061
		N	60	60

* P≤0.05, **P≤0.01, P≤0.001.

Discussion

Analysis findings through this particular effort indicated that higher concentrations of Th-1, Th-2 cytokines in CD patients reflect this disease's inflammatory response, with unique serum cytokine elevations from non-intestinal sources such as those in the small intestinal mucosa and others[11]. The Th-1 response to dietary gluten in the small intestinal mucosa is likely to cause infiltration of lymphocytes and monocytes into the lamina propria. Even if they overlap in their role[12], [13]. The Th-1 response is an increase in cell-mediated immunity and pro-inflammatory responses, whereas the Th-2 cytokines predominantly affect the humoral immune response and play a role in inflammatory down-regulation Both responses were observed in the CD[14–17]. Many studies have shown that elevations of serum immunoglobulins against particular autoantigens, such as endomysia antibodies (EMA) and TTG-antibodies are primarily IgA isotypes in CDs[18–27]. Cytokines at the mucosal level support the production of plasma cells containing IgA while the degree of inflammation or systemic involvement cannot be determined by tests performed at a given point in time, our findings suggest roles for certain cytokines in generating or maintaining the humoral response in CD except for TNF-α [14], [28], [29]. IL-8 was execrated in neutrophilic infiltrated tissue and plays a major role in neutrophilic-mediated inflammatory responses, In our research serum levels of IL-8 are increased relative to controls in all CD patients compared to other APC-derived cytokines[30], [31]. variety of cells, including T cells, B cells, fibroblasts, endothelial cells, monocytes, keratinocytes, mesangial cells, and certain tumour cells play a role for released of a pleiotropic cytokine (IL-6) for Inflammation, immune control, hematopoiesis, and oncogenesis, IL-6 plays a key role[28], [32–36]. As with our results, serum levels of IL-6 in patients with ACD are significantly increased.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Association between Vitamin D Receptor Gene Foki Polymorphism and Gestational Diabetes Mellitus among Iraqi Pregnant Women

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Abstract

This study was conducted to evaluate the association between vitamin D receptor gene FokI polymorphism and gestational diabetes Mellitus (GDM) among Iraqi pregnant women.

This study included 40 women with GDM, 30 pregnant women without GDM, and 30 non-pregnant women as a negative control. Age (years), body mass index (BMI), Fasting Blood Glucose (FBG), total cholesterol, triglycerides (TG), and lipid profile of the patients and control groups were compared (Table 1). DNA sampling was purified and amplified by using PCR. The FokI genotype was evaluated using restriction fragment length polymorphism (RFLP).

The results revealed that the age, BMI, FBG, HbAIc, insulin hormone, insulin resistance, and lipid profile except HDL-C were significantly increased with an increased GDM risk compared to women with normal pregnancies. In contrast, no significant alterations were observed in concentrations of IGF1 hormone, vitamin D and FokI (F and f) alleles frequency between GDM and control groups.

This study suggests that there was no significant correlation between the VDR FokI polymorphism and GDM patients among Iraqi pregnant women.

Keywords: *Gestational diabetes, FokI, Polymorphism.*

Introduction

Vitamin D is secosteroid produced in the skin due to the effect of solar ultraviolet B radiation. Two consecutive hydroxylation in the liver and kidney leads to form a functional 1, 25 –dihydroxy vitamin D [(1)]. The two most important forms of vitamin D are vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol) which cannot produce in the human body and consume with fortified food or given by supplements [(2)]. These two form are bound to the vitamin D binding protein in human plasma and transported them to the liver where both are hydroxylated to form 25-hydoxy vitamin D [(3)].

Vitamin D has an influence on the expression of over 200 different genes. Deficiency maybe related to diabetes [(4),] different forms of cancer, cardiovascular disease, autoimmune diseases and innate immunity. [(2)]

Correlation between the (VDR) gene and diabetes has been noted in several population. Which is encoded by a gene located in chromosome 12q12 and several polymorphisms were used for its description.[(5)] In addition, this gene is presented in the pancreatic β-cells. Consequently,1,25$(OH)_2D_3$ may has a role in insulin secretion and sensitivity in diabetes by either increasing the intracellular calcium concentration in the β-cell to induce insulin secretion or by increasing the conversion of pro insulin to insulin. [(6)] VDR gene FokI (RFLP) in exon 2 [(7)]

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Gestational diabetes mellitus (GDM) usually reveals itself in the latter half of pregnancy and it is identified by carbohydrate intolerance of variable severity. [8] Hyperglycemia development during pregnancy may lead to insulin resistance due to secretion of placental hormones [9]

Several factors are led to increase GDM such as glucose urea, age over 30 years, obesity, family history of diabetes, a previous history of GDM and macrosomic child.[10] Vitamin D deficiency may play a role in the pathogenesis of GDM [11]

The present study aimed to investigate association between VDR-FokI gene polymorphism and risk of GDM among Iraqi pregnant women.

Materials and Methods

A total of 100 blood samples were collected from 40 pregnant GDM, 30 pregnant without GDM and 30 healthy non-pregnant Iraqi women after 10-12 hours fasting (control group). Age (years), body mass index – BMI (Kg/m^2) were recorded. The blood sample was divided into two aliquots, the first (3 ml) of separated serum used for assays of FBG (mg/dl), total cholesterol (mg/dl), TG (mg/dl), HDL-C (mg/dl), LDL– C (mg /dl), VLDL – C (mg /dl), Insulin hormone (MIU/ml), IGF1(ng/ml) and vitamin D (ng / ml). the second aliquots (2 ml) was collected in EDTA tube for HbA1c determination and then used to DNA extraction. DNA was extracted using genomic lysis buffer and was checked for purity and concentration. DNA samples were all quoted and stored at

PCR mixture was carried out with in the presence of DNA (1.5 µl), 5µl Taq PCR premix (5 U/µl Taq DNA polymerase, 2.5 mM DNTPs,1µl 10x reaction buffer and 1 µl gel loading buffer), 10 picomoles/µl of each primer (table 1) and 16.5 µl distill water.

PCR thermal cycler was programmed as follows: denaturation step at 95 ˚C for 3 minutes followed by 35 cycles at 95˚C for 45 seconds and followed annealing by 35 cycles at 68˚C for 45 seconds and extension by 35 cycles at 72 ˚C for 45 seconds, final extension cycle at 72˚C for 7 minutes was done. PCR products were analyzed on 1.5 % agarose gel and UV transmission.

Restriction fragment length polymorphism (RFLP-PCR) was used to identify VDR genotypes. PCR product (5µl) was digested (37ºC for 30minutes) with 0.5 µl Fok I restriction enzyme. Digested product was electrophoresed on 2.5% agarose gel. Genotype was determined according to fragments length i.e. homozygote (FF) subjects = 270bp product ; heterozygote (Ff) subjects = 270,210 and 60 bp products and homozygote (ff) subjects = 110,60 bp products. SNP resulting in T-C substitution in exon 2 of VDR gene leads to the generation of a FokI restriction site. Homozygous subjects with alleles containing nucleotide T at this position showed two bands of 210 and 60 bp and were designated as having ff FokI genotype. Homozygous subjects with alleles containing C at this position showed an intact 270 bp band (FF) subjects. Heterozygote subjects showed all 3 bands: 270,210 and 60bp and were designated Ff.

Table 1: Primer used for polymerase chain reaction (PCR) of VDR FokI gene.

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5-AGTGGCCCTGGCACTGACTCTGGCTCT-3	68.6	63	270 Base pair
Reverse	5-ATGGAAACACCTTGCTTCTTCTCCCCTC-3	60.5	48.1	

VDR: Vitamin D receptor.

Results

Total number of 40 GDM pregnant, 30 healthy pregnant women and 30 controls without pregnant and have normal concentration of vitamin D were recruited in our study. Age, BMI, FBG, HbA1c, Insulin hormone, insulin resistance and lipid profile except HDL-C were statistically significant difference between normal pregnant and gestational diabetes mellitus ($p \leq 0.05$), but there were no significant differences in concentrations in IGF1 hormone and vitamin D ($p > 0.05$) (Table 2).

Table 2: Demographic and biochemical data of GDM and controls.

Parameter	GDM	Normal pregnant	control	P value
Age (Years)	35.175	27.500	30.933	P LSD=2.95< 0.001
BMI (Kg/m2)	30.723	26.569	24.196	PLSD=1.68< 0.001
FBG (mg/dl)	145.950	89.400	86.200	PLSD=11.6< 0.001
HbA1c (%)	7.082	5.046	4.840	PLSD=0.539< 0.001
Ins H (MIU/ml)	14.782	9.293	10.707	PLSD=3.346<0.003
HOMA-IR (%)	4.912	2.048	2.245	PLSD=0.956<0.001
Vitamin D (ng/ml)	6.833	8.733	23.809	PLSD=2.161<0.001
IGF1 (ng/ml)	99.350	90.517	63.100	PLSD=15.86<0.001
TG (mg/dl)	194.550	164.567	133.500	PLSD=16.30<0.001
Cholesterol (mg/dl)	225.17	175.33	179.37	PLSD=23.38<0.001
HDL-C (mg/dl)	38.000	35.567	41.833	PLSD=3.820<0.009
LDL (mg/dl)	146.78	107.53	111.06	PLSD=22.76<0,001
VLDL (mg/dl)	38.910	32.900	26.370	PLSD=3.224<0.001

Figure 1 shows PCR product of 270 bp for VDR FokI gene.

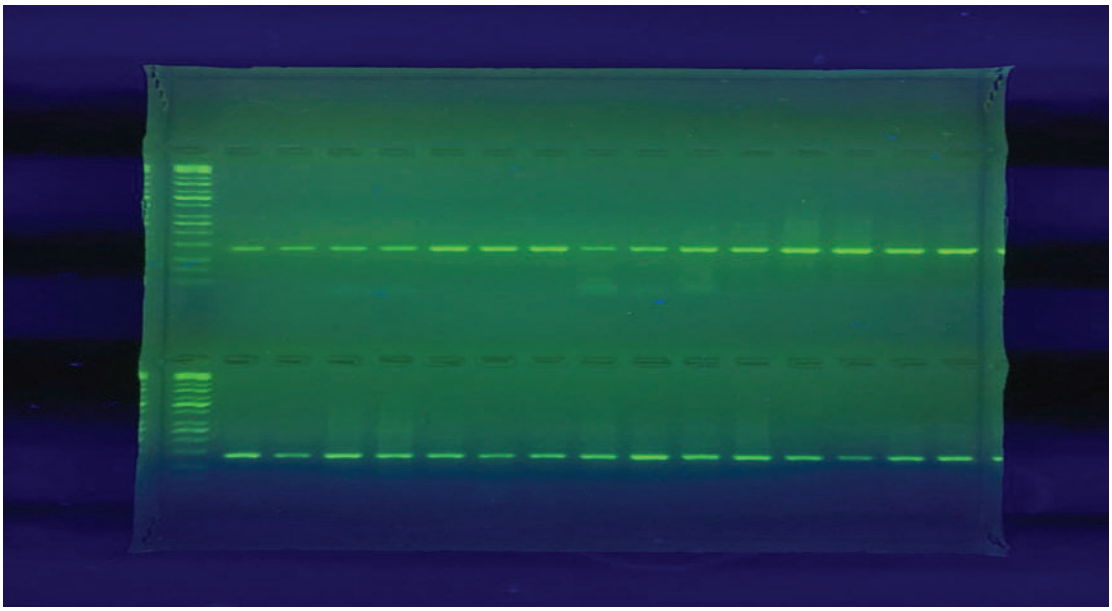


Figure 1: 1.5% agarose gel electrophoresis of VDR PCR for SNP BsmI enzyme showing a wild type FF 270 bp product.

Genotype of VDR FokI gene polymorphism determined according to the fragments length, homozygote FF

remained undigested in 270bp, homozygote ff was digested to 210 and 60 bp and heterozygote Ff digested to 270,210 and 60 bp.These results show insignificant differences in GDM patients and controls (Table 3).

Table 3: Genotype of Fok1 polymorphism.

Gene	**Genotype**	**Gestational No. (%)**	**Control samples No. (%)**	**OR (95% CI)**	**P value**
VDR FOK1	FF	(45.45%)	(60.00%)	0.56 (0.18 -1.73)	0.241
	Ff	(54.55%)	(36.00%)	2.13 (0.68 -6.71)	0.163
	ff	(0.00%)	(4.00%)	0.36 (0.02 -8.75)	0.532
Gene	Genotype	GDM No. (%)	Control samples No. (%)	OR (95% CI)	P value
VDR FOK1	FF	(54.17%)	(60.00%)	0.79 (0.26 -2.39)	0.451
	Ff	(37.50%)	(36.00%)	1.07 (0.34 -3.33)	0.574
	ff	(8.33%)	(4.00%)	2.18 (0.19-24.5)	0.484
Gene	Genotype	Gestational No. (%)	GDM No. (%)	OR (95% CI)	P value
VDR FOK1	FF	(45.45%)	(54.17%)	0.71 (0.23 -2.20)	0.812
	Ff	(54.55%)	(37.50%)	2.00 (0.63 -6.33)	0.928
	ff	(0.00%)	(8.33%)	0.20 (0.01 -4.12)	1.000

Allele frequency of VDR FokI gene polymorphism in GDM patients and controls show in table 4.

Table 4: Allele frequency of Fok1 polymorphism.

Gene	Allele	Gestational No. (%)	Control samples No. (%)	OR (95% CI)	P value
VDR FOK1	F	(72.73%)	(78.0%)	0.75 (0.30- 1.91)	0.361
	f	(27.27%)	(22.0%)	1.33 (0.52 - 3.38)	0.361
Gene	Allele	GDM No. (%)	Control samples No. (%)	OR (95% CI)	P value
VDR FOK1	F	(72.92%)	(78.0%)	0.76 (0.30 - 1.89)	0.363
	f	(27.08%)	(22.0%)	1.32 (0.53 - 3.29)	0.363
Gene	Allele	Gestational No. (%)	GDM No. (%)	OR (95% CI)	P value
VDR FOK1	F	(72.73%)	(72.92%)	0.99 (0.40 - 2.46)	0.601
	f	(27.27%)	(27.08%)	1.01 (0.41 - 2.51)	0.601

Discussion

Diabetes mellitus became major health concern worldwide. Female < 50 years (gestational age) have greater prevalence than males [12]. VDR polymorphism influences capability to type 1 diabetes mellitus, but association with GDM is not yet clear.[13] Vitamin D has indicated to be substantially related to pancreatic β-cell function and susceptibility [14]. It was advertised that, no association noticed between VDR BsmI polymorphism and GDM in Saudi women [15]

We analyzed VDR gene FokI polymorphism in GDM pregnant Iraqi women. Genotypes frequencies for FF, Ff, ff among control groups were 60%, 36% and 4% respectively, and among normal pregnant were 45.45%, 54.55% and 0.0% respectively, with insignificant association ($p > 0.05$), and in GDM patients were 54.17%, 37.50% and 8.33% respectively with no significance with normal pregnant and with control ($p > 0.05$). These finding are in agreement with results of the study that showed VDR FokI polymorphism not associated with Saudi GDM [13]. An Indian study suggested that VDR gene polymorphism is associated with type 2 diabetes mellitus.[16] Results of allele frequency distribution F and f in GDM were 72.92% and 27.08% respectively, in control were 78.0 % and 22.0 %, and among normal pregnant were 72.73 % and 27.27%, and there were no significantly between these groups ($P > 0.05$). These results consistent with got by Aslani S and Alzaim M, who suggested that F allele may have a role in decrease incidence of GDM [17]

Conclusion

Vitamin D deficiency was more prevalent among Iraqi women. The results showed a relationship between age, body mass index and gestational diabetes mellitus, also There were significant differences in concentrations of fasting blood glucose, glycated hemoglobin, insulin hormone, insulin resistance, lipid profile except high density lipoprotein cholesterol between normal pregnant and gestational diabetes mellitus, but there were no significant differences in concentrations in insulin – like growth factor hormone.The results of the present study showed vitamin D receptor FokI polymorphisms

not associated with Iraqi gestational dibetes mellitus. Homozygous FF turned out to be as a prenetive genotype of gestational diabetes mellitus,while Ff and ff turned out to be related with risk of GDM.The allele F represents a preventive allele, while the allele f acts as etiological factor.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Using Paper Waste and Local Bacterial Isolates to Produce Biomass and Cellulase Enzyme- A Study Waste Recycling

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Abstract

The study aims to isolate the cellulose-degradation bacteria that produce the cellulase enzyme as well as the production of SCP protein from Ramadi city soils and to obtain efficient local bacterial isolates in production and efficient isolation was selected in the production of cellulase enzyme and single cell protein which is *Bacillus subtillis* bacteria tested the efficiency of the isolated isolation to produce the enzyme and single cell protein. The cell using local media contains the paper waste available in the environment and contaminated with it. Initial transactions were made for this paper waste and these factors included (heating to boiling point, dilute sulfuric acid and dilute sodium hydroxide). The paper waste used included the white cardboard, yellow cardboard and white paper Which was used as a single source of carbon and energy, after testing the waste paper used, it was found that the white cardboard treated with heating gave the best production of the cellulase enzyme and single-cell protein. The bacterial isolation used showed the best growth and gave the highest production of the enzyme and the single cell protein at pH 7, while the best temperature for the production of the enzyme was 45 ° C. The optimum temperature for the production of the single cell protein was 40 ° C. As for the carbon concentration, which is the white cardboard treated with heating, it was better to produce than Concentration is 2%. As for the production of single cell protein, the highest output at concentration was 4%. The best bacterial vaccine size for enzyme production was 3 ml / 100 ml medium. As for the production of single cell protein, the size of the inoculum gave 1.5 ml / 100 ml the best production. The addition of the organic nitrogen source (urea) gave a clear increase in enzyme production and single cell protein, and when adding 0.5 g urea / 100 mL medium. Finally, the results showed that the best period duration to obtain the highest cellulase enzyme production was after 96 hours, while it was found that the best production of single-cell protein was after 24 hours incubation.

Key words: *cellulose, cellulase enzyme, single cell protein (SCP)*

Introduction

Paper, industrial and agricultural wastes and a lot of wastes containing carbohydrates cause accumulating a great pollution in nature. These wastes are an essential source of cellulose[1]

In order to get rid of these residues and for the continuity of the carbon cycle in nature, there is a need for the cellulose-analyzing neighborhoods to be by producing these neighborhoods for the enzyme cellulose that stimulates the process of cellulose decomposition into short chains and to the final product which is glucose, and we can get from them an important final result that is economically beneficial [2] .

The cellulase enzyme (EC 3.2.1.4) is one of the enzymes that produce microbes, which stimulates the process of cellulose decomposition into cellulose and then glucose. In the first two steps, it converts long cellulose chains into short chains, then the second step is by splitting the cycloid bonds (β, 1- 4) and converting the chains Short cellulosic to melted sugars[3]

Paper waste and industrial waste were exploited in the field of biotechnology, as Biomass was produced using the Submerged Cultures technology and the production of single cell Protein with high nutritional value, according

to the Food and Agriculture Organization (FAO), the percentage of single cell protein (SCP) Single Cell Protein ranges between 60-90% of the dry weight of the microbial cell [4].

Materials and Methods

ؤCulture media : An isolate of *Bacillus subtilis* was used, isolated from the soil of Al-Jazeera in Ramadi and planted with okra crop. The local symbol was given E7. The bacterial isolation was grown on the production medium consisting of (2 g ammonium nitrate, 0.5 g potassium dihydrogen phosphate, 0.2 g aqueous magnesium sulfate, 20 mg aqueous calcium chloride, 20 mg aqueous manganese sulfate, 20 mg aqueous ferrous sulfate, 0.2 g yeast extract, 0.2 g peptone [5]and used three carbon sources of paper waste including (yellow cardboard, white cardboard and white paper) after initial treatment For paper waste, the farm was sterilized and incubated at a temperature of 30 m and a pH 7-7.4 for a period of three days using a 1% inoculum and a CFU is 5 x 10^7 colony formation units / ml. conical flasks technique was used using 250 ml and put in each flask 100 ml of the farm .

Cellulase enzyme assay : The enzyme activity was measured according to a method[6], and the reducing glucose (glucose) was estimated according to the method[7] and using the spectrophotometer at a wavelength of 540 nm**.**

Single cell protein production: was obtained using a liquid medium containing paper waste as a single carbon source, distribute the medium in 250-ml flasks, sterilize the farm and put the bacterial vaccine and incubated for three days, after which the bacterial cultures were filtered using filter papers to separate the portion The steel (precipitate) from the filtrate of the farm in which the enzyme is present. The precipitate was washed with distilled water and dried in the oven at 60 ° C for 24 hours, then the dry biomass was weighed by the sensitive scale to estimate the weight of the dry biomass which represents the final product of the single cell protein [8]

Optimization environmental conditions for the growth of selective and degraded bacterial isolates of cellulose and single cell protein production:

The liquid medium containing the paper waste was used as the only source of carbon and energy in determining the factors affecting the production of the enzyme and the single cell protein, as it used conical flasks with a capacity of 250 milliliters and put in each of them 100 milliliters of the liquid medium and sterilized the decanters in the receptacle. And incubated in a rocker incubator at a speed of 120 shake / minute using various criteria including the pH, adjust the pH of the production medium (5,6,7,8,9,10) to determine the optimal pH for the production of the enzyme and single-cell protein for the bacterial isolation used. Different temperatures 20,25,30,35,40,45,50 C were selected to determine the optimum temperature for the production of the enzyme and protein. For the purpose of determining the appropriate carbon source for growth, three carbon sources containing cellulose were used: yellow and white cardboards, and white office paper as they were ground after Carrying out initial transactions for it, and the white carton was chosen in the light of the result obtained from this test, as the white cardboard gave the highest production value of the enzyme and single cell protein. The cell also used different concentrations from the carbon source. The white cardboard included 0.5,1,1.5,2,2.5,3,4 g Also, different vaccine volumes were used for the purpose of vaccinating the liquid medium from the selected bacterial isolation 0.5,1,1.5,2,2.5,3g Also, a nitrogen source such as urea and sodium nitrate was added in different concentrations, which are 0.5,1,1.5,2 g for the purpose of improving growth and increasing the production of enzyme and single cell protein. In light of the results obtained from the previous steps, I used different lap time periods that included from one day lap to Six days, the enzyme activity was estimated and the amount of biomass measured to determine the best bosom duration.

Statistical Analysis

All experiments were designed according to Complete Randomize Design (CRD) and using the system of global experiments. Statistical analyzes were carried out using Genestat multi-factor program and using the value of the least significant difference for LSD (Least signification difference) at the 5% probability level.

Results and Discussion

Optimal conditions for the production of cellulase and single cell protein:

The results shown in Table (1) showed that the enzyme productivity using *Bacillus subtilis* at pH 7 was higher than the other pH numbers after 3 days of lap, as the enzymatic efficacy reached (0.827 units / ml). As for the single-cell protein, it was higher Its value is at pH 7 and the amount of dry weight is (1.102 g / 100 ml). The effect of the pH on the production of the enzyme comes through its effect on the characteristics of the nutritional medium and the solubility of nutrients and their readiness for the organism. This in turn is reflected in the growth of the organism and the production of the enzyme as the pH affects In the direction of the course of metabolism, synthesis and enzyme production [9] . .

Table (1) shows the effect of pH on cellulase enzyme and single cell protein production

PH	Dry weight gm / 100ml	Enzymatic activity Unit / ml
5	0.572	0.588
6	0.987	0.797
7	1.102	0.827
8	0.959	0.157
9	0.919	0.155
10	0.843	0.239
	L.S.D=0.035	L.S.D=0.025

As for the effect of temperature on the productivity of bacteria for the enzyme and single cell protein The highest productivity of the enzyme was at a temperature of 45 ° C and using the bacterial isolate *Bacillus subtilis* at pH 7 and the carbon source was the white cardboard, as the enzyme activity reached (0.398) units / ml .

As for the single cell protein, its best yield at the temperature was 40 º C. As shown in Table (2), as the production of the single cell protein at this temperature reached (1.438 g / 100 ml)

The reason for the increase in the enzyme productivity due to the increase in temperature may be attributed to the fact that the high temperature affects the speed of enzymatic reactions within the cell or some of the factors that help the growth of isolation, such as the decrease in the dissolved oxygen ratio[10]

Table (2) shows the effect of temperature on the production of cellulase enzyme and single cell protein

Temperature C	Dry weight gm / 100ml	Enzymatic activity Unit / ml
20	1.126	0.239
25	1.146	0.252
30	1.152	0.380
35	1.244	0.368
40	1.438	0.380
45	1.021	0.398
50	1.011	0.129
	L.S.D=0.045	L.S.D=0.069

As for the concentration of the carbon source, the results showed as indicated in Table No. (3), as it was found that the highest enzyme yield was obtained at the concentration of 2 g / 100 mL and the enzyme efficacy reached (0.538) units / mL after a 3-day bosom duration at pH 7 and temperature 45 M . As for the single cell protein, the concentration of the carbon source of 4 g / 100 mL gave the highest production of the unicellular protein and reached 4.609 g / 100 mL, while the remaining concentrations of less than 4 g recorded the lowest biomass and were the least productive for the unicellular protein (1.232 g / mL) In terms of dry weight, the production of single cell protein depends on the type of raw material used in production and the importance of choosing the base material that is a source of carbon and that will result in high biomass production in a less time[11]

Table (3) shows the effect of the concentration of the carbon source on the production of cellulase enzyme and single cell protein

Concentration of the carbon source	Dry weight gm / 100ml	Enzymatic activity Unit / ml
0.5	1.232	0.300
1	1.593	0.334
1.5	2.500	0.427
2	2.523	0.538
2.5	3.036	0.439
3	3.619	0.374
4	4.609	0.301
	L.S.D= 0.18	L.S.D=0.035

for the size of the vaccine in the production of the enzyme and the protein, Table (4) shows that the highest production of the enzyme from *Bacillus subtilis* when adding the size of the vaccine is 3 milliliters / 100 milliliters, and the enzymatic efficacy reached (0.558) units / ml after 3 days of incubation then the effectiveness decreased when increasing or the size of the vaccine decreased more than that, either when producing the single cell protein in terms of dry mass or the dry weight of the product, the size of the vaccine gave 1.5 ml / 100 ml the highest single cell production of 3.052 g / 100 ml medium while the sizes of the inoculum were recorded (0.5, 1, 2, 2.5, 3) (ml / 100ml) yields less than single cell protein. The decrease in enzyme production at the smallest sizes of the vaccine is due to the fact that the concentration of the vaccine is not sufficient to give a biomass from the isolation and thus reflects on the enzyme productivity. It may be due to the intense competition for nutrient exploitation in the middle [12]

Table (4) shows the effect of vaccine size on the production of cellulase and single cell protein

The size of the inoculum Ml	**Dry weight gm / 100ml**	**Enzymatic activity Unit / ml**
0.5	2.539	0.186
1	2.643	0.214
1.5	3.052	0.268
2	2.872	0.289
2.5	2.166	0.295
3	2.147	0.558
	L.S.D= 0.098	**L.S.D= 0.030**

As for the effect of the nitrogen source on enzyme and protein production, the results shown in Table (5) showed that the bacteria using *Bacillus subtilis* gave the highest efficacy of the enzyme cellulase using urea and reached (0.882) units / ml and was the highest efficacy of the enzyme when adding a concentration (0.5) g / 100 ml of urea, while the efficacy decreased slightly with the use of an inorganic source such as sodium nitrate at different concentrations such as (2,1,0.5 g / 100 ml medium) to reach (0.730) units / ml at a concentration of (0.5) g sodium nitrate / 100 ml

And single cell protein was the highest production when adding urea in different concentrations, as it reached its highest productivity when using a concentration of 0.5 g urea / 100 ml and the productivity was (3.683 g / 100 ml) and the productivity decreased when adding the inorganic nitrogen source (sodium nitrate) in different concentrations, reaching the highest output For single cell protein, when adding 0.5 g of sodium nitrate / 100 mL, the enzymatic activity is (2.522) units / ml .

Table (5) shows the type and effect of the nitrogen source in the production of cellulase enzyme and single cell protein

Concentration of nitrogen source gm/100ml	Enzymatic activity U / ml				Dry weight ml gm/100		
	Sodium nitrate	Urea			Sodium nitrate	Urea	
0			3.052				0.558
0.5	2.522	3.683			0.730	0.882	
1	2.037	2.207			0.638	0.778	
2	2.028	2.229			0.646	0.747	
	L.S.D=0.018	L.S.D=0.093			L.S.D=0.028	L.S.D=0.027	

Using the optimal production conditions that were shown by the previous steps, he studied the effect of the brood period in the production of the enzyme and protein. As shown in Table No. (6), the production of the enzyme increases with increasing the brooding period to reach a maximum after 4 days with an enzymatic efficacy of (0.996) units / ml of *Bacillus subtilis* bacteria. Then the effectiveness decreased with the increase of the bosom period to reach (0.550) units / ml after 6 days. This decrease in effectiveness with the increase in the bosom period may be attributed to the occurrence of environmental changes in the center of production as well as the possibility of self-dissolution of cells and the accompanying release of metabolic materials that affect Negatively in the enzyme productivity and this is confirmed by Aggelopoulos[13].

From the observation of Table (6), we find that the best production of the single cell protein was after a 24-hour bosom duration and the weight of the produced protein was (3.630) g / 100 ml medium, while the level of production decreased when the other brood period increased.

Table (6) shows the effect of brood duration on the production of cellulase enzyme and single cell protein

Incubate period	Dry weight gm/ 100ml	Enzymatic activity Unit / ml
1 day	3.630	0.798
2 day	3.520	0.816
3 day	3.062	0.819
4 day	1.883	0.996
5 day	1.703	0.822
6 day	1.454	0.550
	L.S.D=0.098	L.S.D=0.030

Implementation of optimal conditions for SCP production:

The optimum conditions were applied for the production of cellulase enzyme and single cell protein of pH, temperature and concentration of the carbon source, the size of the bacterial vaccine, the type of nitrogen source. After the end of the optimal brood period, the highest cellulase enzyme production was reached (0.996 u / ml) either As for the dry weight of the biomass, it reached (26.7 g / l) resulting from the decomposition of the paper waste used in the study, which is the white cardboard

Conclusion

The spread of cellulose-dissolving bacteria to varying degrees in agricultural soils in Ramadi city. The local isolate, *Bacillus subtilis*, possesses the highest capacity for analyzing cellulose from its various sources, carboxymethylcellulose, and paper waste (white and yellow carbord and white office paper), as well as having the highest activity of cellulase enzyme and the highest ability to produce a single cell protein. The possibility of using paper wastes available in the local environment as pollutants in many biological products through recycling them in microbial fermentation, and thus we achieve two goals to obtain certain cheap products and rid the environment of these pollutants .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Effect of Foliar Application with Nano-Organic Fertilizer and Proline Acid and their Interaction in Leaf Content of Antioxidants and Vitamin D3 for *Moringa oleifera*

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Abstract

A potted pot capacity 15 kg experiment was carried out during the agricultural season 2019 in one of the private nurseries of Al-Ramadi / Anbar Governorate, to study the effect of foliar application of nano-fertilizer and proline acid on the content of antioxidants and vitamin D3 for *Moringa oleifera* leaves using sandy mix soil. The experiment was designed using Completely Randomized Design (CRD) with three replications. The study included three concentrations of nano-organic fertilizer (0, 50, 100) mg-liter^{-1} and three concentrations of the amino acid proline (0, 100, 200) mg^{-1} . after 150 days from the date of seed germination the leaf content of vitamin C was measured and phenols using the least significant difference LSD at 0.05 probability level. Qurcetine flavonoids, Rutine flavonoids and vitamin D3 were measured according to the T test to test the differences between the averages of the treatments. The results showed that the nano-fertilizer at concentration100 mg L1 achieved the highest antioxidant level that included vitamin C, phenol, flavonoid quercetin, flavonoid routine and achieved the highest average vitamin D3 content. And that treatment with proline acid at a concentration of 200 mg / L $^{-1}$ achieved the highest antioxidant content (vitamin C, phenols, Qurcetine and Rutine flavonoids) in addition to vitamin D3. The interaction between the study two factors had a positive effect on most of the rates of the studied traits. The plants which treated with (100 mg-liter-1 nano organic fertilizer and 200 mg-liter-1-proline acid) gave the highest rate of leaf content of vitamin C, phenols, Qurcetine, and Rutine flavonoids, while treatment with (100 mg-liter-1 nano organic fertilizer and 100 mg-1-proline acid) has achieved the highest leaf content level of vitamin D3 .

Keywords: *antioxidant, nano organic fertilizer, Proline acid, Moringa oleifera*

Introduction

Moringa from perennial trees that belong to the Moringaceae family, has high medical and nutritional properties, and called the miracle tree , or the tree of life because of the human aspects it carries to the poor, it represents a complete food source for them.(1)

The original habitat of Moringa in northern India, on the foothills of the Himalayas, as it is widespread in Kenya, Sudan and Ethiopia and in the tropics and subtropics lands(2). Moringa is considered one of the beneficial trees for humans, animals and plants, as it is considered the most promising generation of medicine, food, and animal feed(3). It is also a medical tree that takes advantage of all its parts (leaves, fruits, roots, and twigs), its leaves are the most used for what they contain of Proteins, vitamins, salts, minerals and active substances(4).

Nanotechnology is a science concerned with the study and careful control of the production of a substance at the nano scale level, i.e. production of nano materials whose micro dimensions are a part of a billionth of a meter (1 × 10^{-9} m)(5). Nano scale applications have

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many uses, including their use in the agricultural field for the purpose of reaching modern agricultural methods characterized by the low economic cost resulting from increasing the efficiency of manufactured fertilizers in terms of agricultural product resistance to various agricultural conditions and improving nutrient management and enhancing their absorption by the plant[6], given what These materials are distinguished by physical, chemical, and mechanical properties that distinguish them from materials with large dimensions, since characteristics appear similar to those properties when they are in the traditional case[7]. As an effective concentration with solubility and high effectiveness and are used in small quantities compared to regular fertilizers to avoid repeated application to plants and access on good productivity from the first application .[8]

Foliar application is the addition of nutrients by spraying its solutions with certain concentrations on the vegetative group of plants, so that it can be absorbed by the stomata on the surface of leaves or by the cell walls and membranes and their entry into the biological processes of plants leading to an increase in the vegetative and qualitative characteristics in order to avoid the lack of availability in the component .[9]

Nutrition plays an important role in the growth and development of plants, and in the case of medicinal plants nutrients can significantly increase the active substances of these plants .[10]This nutrition is of great importance to plants that suffer from having a weak or weak branching root system, whose spread is limited to the surface of the soil, which cannot enable the plants to absorb nutrients in sufficient quantities that meet the needs of the plant .[11]

Proline acid is one of the amino acids that the plant produces naturally and have an important role in increase the growth of the plant and increase its response to fertilization and its resistance to diseases[12]. Proline accumulates in different parts of the plant, but it collects high in the leaves [13]It has an important role in capturing free radicals, as its accumulation in the plant is evidence of an increased concentration of antioxidants for these roots.[14]

Antioxidants are any substance that has the ability to prevent oxidative damage and are found in a few concentrations that inhibit or delay the oxidation of molecules[15], as they are necessary to control the harmful effect of free radicals in the human body[16], as it possesses pharmacological activity for many Diseases for being safe with long-term use[17].

The aim of this research is to study the effect of foliar applicationing with nano fertilizer and proline acid and their interaction in the antioxidant and vitamin D3 concentrations in the leaves of the Moringa tree.

Materials and Methods

The experiment was conducted during the agricultural season 2019 in one of the private

nurseries of the city of Ramadi / Al-Anbar Governorate to study the effect of three concentrations of nano-fertilizer (0, 50, 100) mg. Liters $^{-1}$ and three concentrations of the amino acid proline (0, 100, 200) mg $^{-1}$ In antioxidant and vitamin D3 content of Moringa leaves . Random samples were taken from soils and analyzed in the High Euphrates Laboratory which belongs to the University of Anbar for the purpose of revealing the physical and chemical properties of the soil (Table-1).

Completely Randomized Design (CRD) was implemented experimenting with Moringa seeds after soaked for 24 hours with three seeds for each pot, and they were diluted to one plant after germination. The Nano NPK produced by the Indian company (Skofarms) was applied when the plant reached the stage of 4 real leaves and the next day it was applied with proline acid produced from the Indian Green River Company. And repeated the process of application with organic nano fertilizer and proline acid after 30 days from the first application and the transactions were carried out from hoes and weeding and irrigation whenever the need arises.

After 150 days of planting, the leaf content of antioxidants and vitamin D3 was estimated.

1- Estimating the vitamin C content according to the method approved by .[18]

2-The leaf content of the total phenols according to the Arno's method.

3- Estimating the leaf content of Qurcetine, Rutine and Vitamin D3 flavonoids in the laboratories of the

Department of Environment and Water / Ministry of Science and Technology and by method[19], using HPLC (High performance liquid chromatography) with the inverse phase system, which is characterized by its ability to separate many Of the compounds such as phenols, polysaccharides, vitamins, and others, and their quantification and separation, the sample was distributed between two phases, one of which is a fixed phase and is liquid or solid in a column, and a mobile phase is a liquid and the separation depends on the efficiency of the specifications for the column in terms of the diameter of the particles of the material packed inside and whenever the diameter is small it led to Improving the work performance of the column by raising the pressure and obtaining a suitable flow rate for the moving phase within it, which leads to separating the components of the sample through the detector and displaying it in the form of chromatography[20].

Results

1- Vitamin C concentration. Mg.100 g:

(Figure -1) indicates the significant effect of nano-organic fertilizer in increasing vitamin C levels, as treatment T2 gave a rate of 102.31 mg. 100 g compared to a comparison treatment (T0) which gave 95.00 mg. 100 g. Proline acid Application had a significant effect in increasing this trait. Treatments T3 and T4 gave an average of 107.66 and 113.00 mg. 100 g respectively which did not differ significantly from each other but differed from the comparison treatment T0, bilateral interference demonstrated a significant effect in vitamin C, since treatment T8 gave the highest rate of 140.72 mg. 100g.

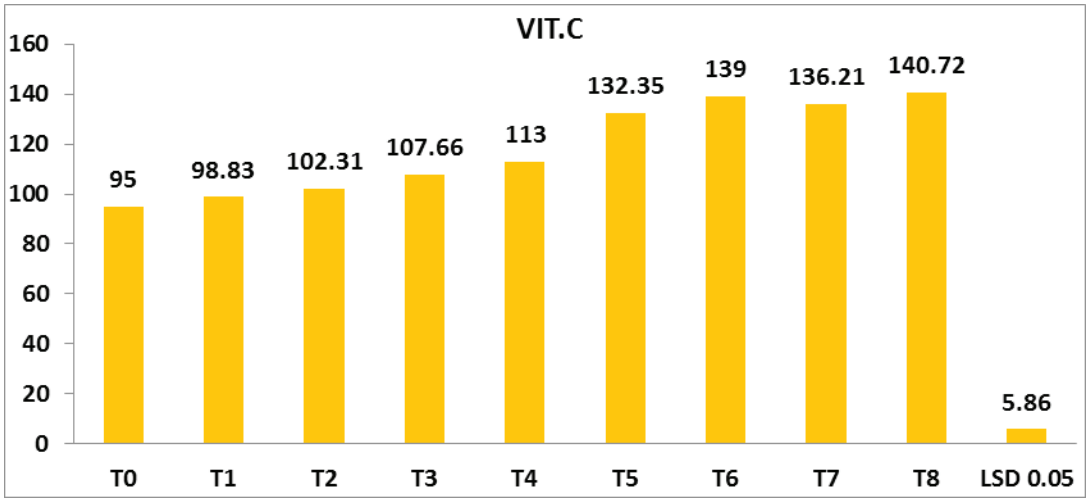


Figure 1 - The effect of foliar application of nanofert. and proline acid and their interactions in vitamin C concentration

2- The total leaf content of phenols mg. 100 g:

The results in (Figure-2) indicate significant differences between the treatments due to the effect of the study factors and their interactions. Whereas, the organic fertilizer treatments T1 and T2 gave a rate of 214.0 and 230.3 mg. 100 g, respectively, which differed significantly with the comparison treatment T0. As for spraying with proline acid, the T3 and T4 treatments showed significant differences at 236.8 and 241.6 mg. 100 respectively, compared to the comparison treatment T0. As for the bilateral interaction between the study factors, it showed significant differences in the rates of this trait, which were highest in T8 and T6 treatments, which did not differ significantly from each other, but they differed significantly from the comparison treatment T0, which gave a rate of 197.0 mg. 100g.

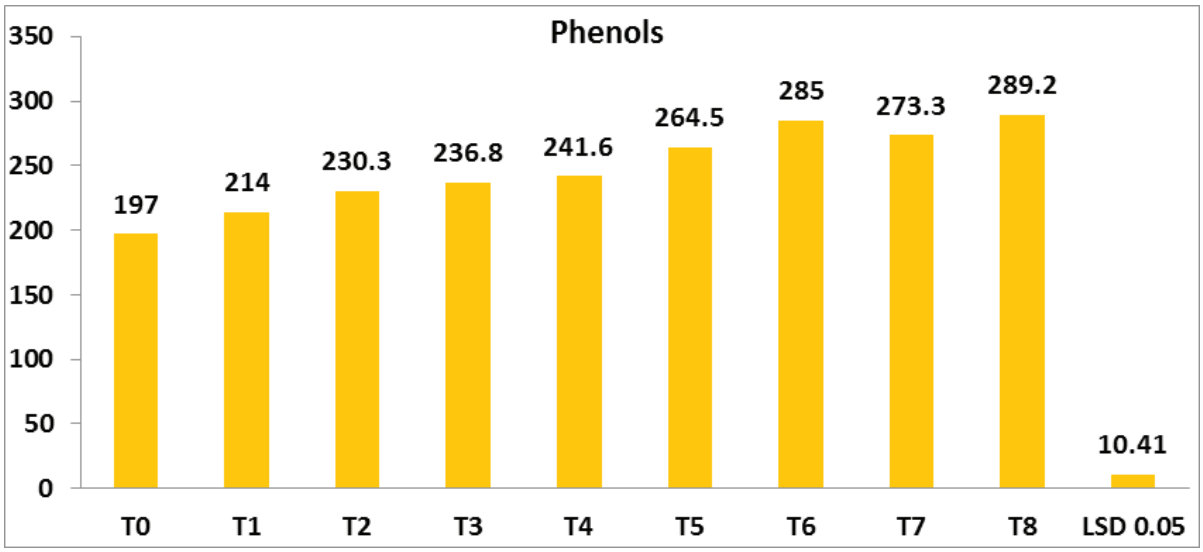


Figure 2 - the effect of foliar application with nano fertilizer and proline acid and their interactions on the phenol content

- Total leaf content of Flavonoids Quercetine mg.L^{-1}3

The results shown in (Figure -3) indicate that there were significant differences between the treatments, as treatment T8 gave the highest rate for this trait of 113.34 mg. L-1, which did not differ significantly from treatment T7, but differed significantly from the rest of the other treatments

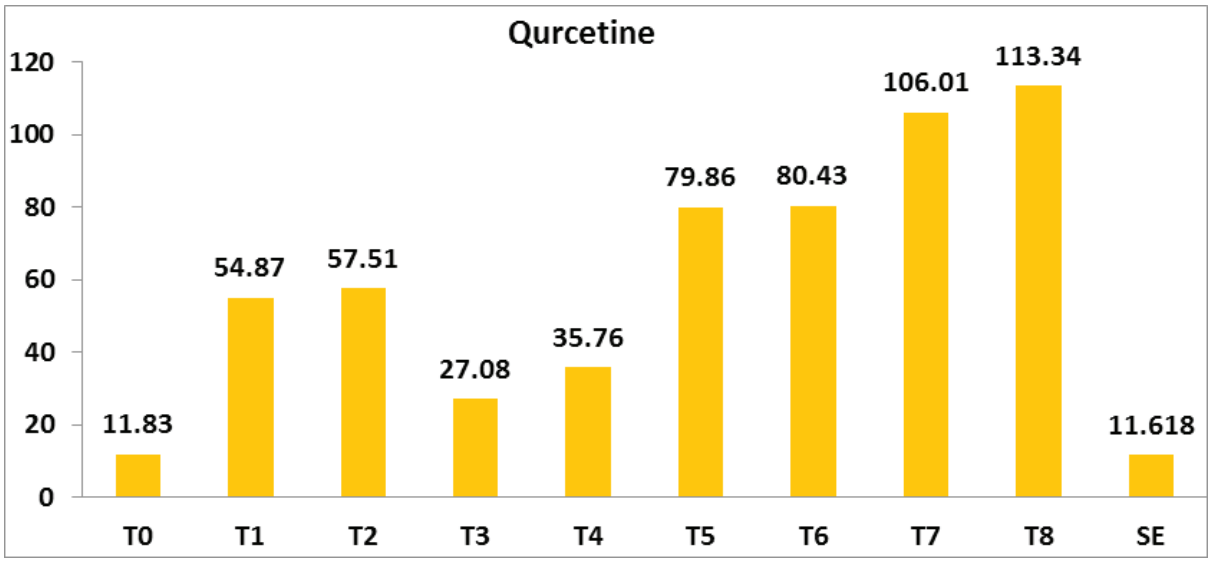


Figure 3 - The effect of foliar spray with nano fertilizer and proline acid and their Interactions on the content of Quercetine

4-Total leaf content of Flavonoids Rutine mg.L^{-1}

The results shown in (Figure -4) indicate that there were significant differences between the treatments, as treatment T8 gave the highest rate for this trait of 22.51 mg. L^{-1}, which did differ significantly from the rest of the other treatments.

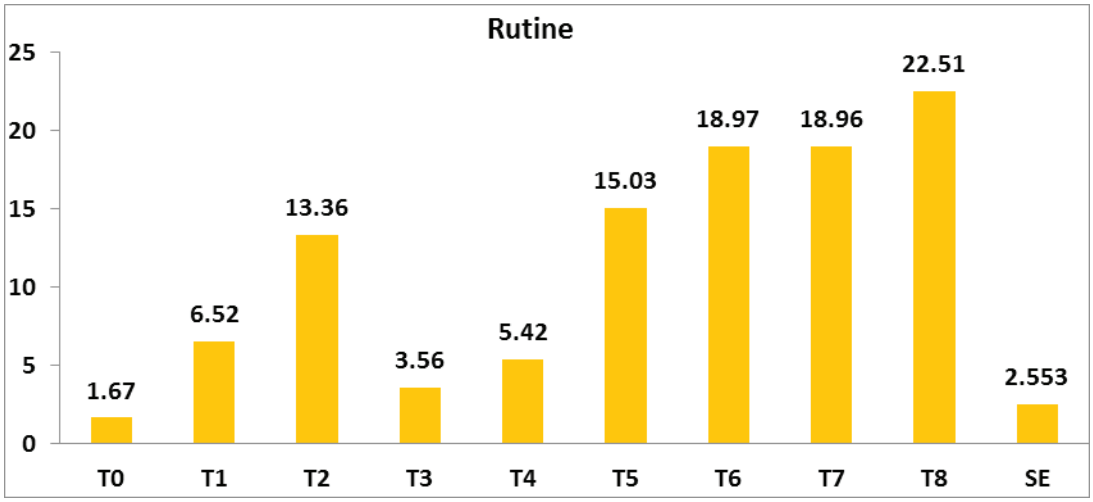


Figure 4 - The effect of foliar spray with nano fertilizer and proline acid and their

Interactions on the content of Rutine

: 5- Estimate the total leaf content of Vitamin D3 mg. L^{-1}

The results in (Figure-5) indicate that there were significant differences between the treatments in the leaf content of the vitamin, as treatment T6 was given the highest rate of 0.838 mg.L^{-1}, which did not differ significantly from treatment T8, but differed from the rest of the other treatments.

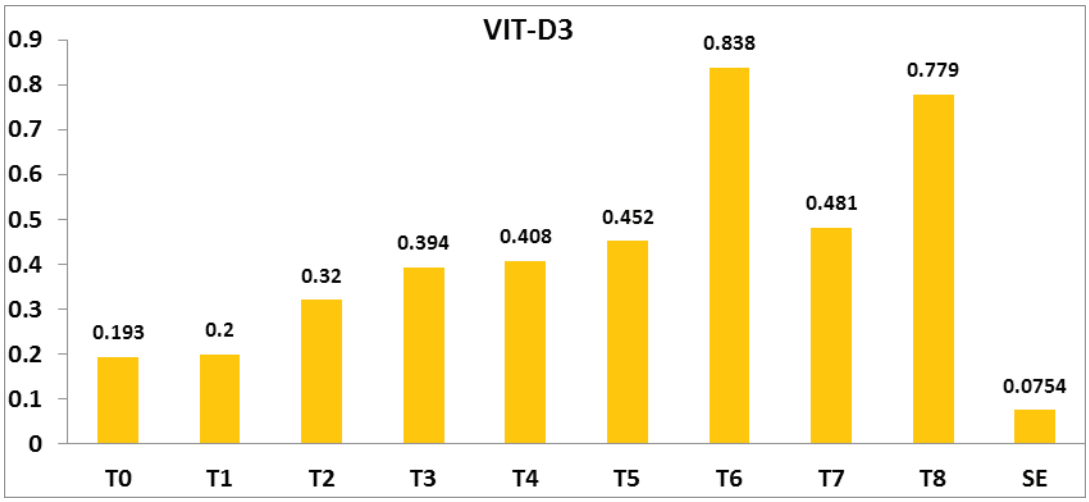


Figure 5 - The effect of foliar spray with nano fertilizer and proline acid and their Interactions on vitamin D3 content

Discussion

The results of the statistical analysis showed that the concentrations of nanoparticles and proline acid fertilizer and their interactions significantly affected the properties of antioxidants and vitamin D3. The increase in the studied characteristics due to the effect of spraying with nano fertilizer is due to its unique and distinctive properties represented by its small minutes and the large effective surface area of these minutes, which led to the speed of its absorption by the plant and then the increase of enzymatic activity and biochemical reactions within it that caused the increase of photosynthesis.[21]The nanoparticles also have a high ability to penetrate plant tissues through stomata due to the size of their ions that are smaller than stomatal slots and in particular that are sprayed onto the vegetative system .[22]This finding was consistent with what[23] in their study on Some medicinal plants, and with what[10] indicated that leafy nutrition is of great importance in plant growth and for medicinal

plants this method leads to increased nutrients and then stimulates plants to increase their growth and increase the antioxidants. As for the increased rates of the studied traits using the proline acid, it is due to the fact that the amino acids represent an important source of carbon and nitrogen, and hence the increase in building most of the byproducts of phenols, flavonoids, and alkaloids[24]. Closely related to metabolism of these acids[25].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Factors Affecting Success of Corneal Grafting : An Observational Study

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Abstract

This is an observational study of 32 corneal implantation cases performed by the researcher. It shows that Younger patients (below 60 years) show more improvement. Female patients show more improvement. Corneal dystrophy cases show more improvement followed by Keratoconus followed lastly bullous keratopathy. Donors aged below 60 years give results that are more positive. There are mild results that are more positive if the time between death and transplant is less than 5 days. Trephine size more than 7.5MM shows more improvement. Punch size of 8.25MM shows most improvement. Using General anesthesia shows more improvement.

Keywords: *Cornea, Transplantation, Keratitis*

Introduction

Visual impairment because of corneal malady gained from different dystrophic, degenerative, irresistible, and inflammatory scatters of corneal and from optional harm of corneal to surface ailment visualization.[1].Keratoplasty of lamellar as selective prescribes methods, which replace selectively only cornea diseased layers but keeping healthy ones that ultimately cause improvement in outcomes of vision as well complications reduction.[2]

New keratoplasty endothelial and keratoplasty of anterior lamellar development techniques, has rendered outcomes of vision which of equals than those for keratoplasty penetrating, normally with complications reduced risks for keratoplasty of endothelial and keratoplasty of anterior lamellar.[3]

Corneal survival transplant data, the postoperative and preoperative identification of risk factors and the transplantation techniques effect on survival, have been largely derived from registries of corneal transplantation.[4]

The keratoplasty of anterior lamellar surgery surgical challenge is the difficulty of technique inherent at separating the stromal layers of anterior from membrane of Descemet and endothelium: nowadays, manual techniques and not easy to do, that restricted their adoption widely.[5]

Techniques of modern keratoplasty endothelial involve stripping endothelium and membrane of Descemet from the receiving stroma, and the donating tissue attached with no sutures via air tamponade use.[6]

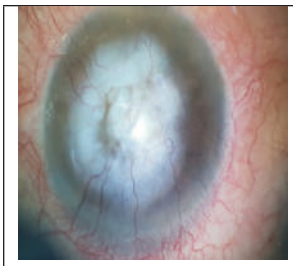
Methodology

Sample size: 32 corneal implantation cases (26 were females & 6 were males) aging from (14-80 year)

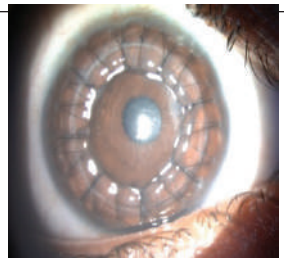
Technique: Corneal implantation was performed (18 for right eye, 14 for left eye). The indications varied (bullous keratopathy, keratoconus and corneal dystrophy) 20 cases were performed under GA, while 12 were performed under LA. We used Trephine size 7.5MM in 6 patients, > 7.5 MM in 26 patients. We used Punch size 7.75MM in 8 patients, 8.00MM in 20 patients and 8.25MM in 4 patients.

Results and Discussion

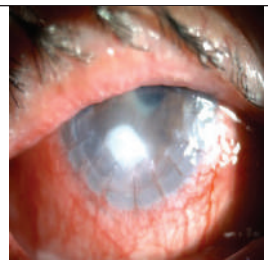
We measured both preoperative & postoperative k readings were measured. In addition, we measured average duration of clarity post op, endothelial cell density cell/mm2, BCVA 1wk, BCVA 6 month and IOP See Fig.1.



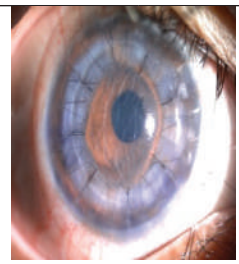
(1a) Corneal graft (PKP) during surgery



(1b) Corneal graft before surgery



(1c) Six months after grafting



(1d) After removal of stiches

Figures 1 (a-d) show the technique performed by the researcher

Source: operations performed by the researcher

Analysis: observational study of the outcome was performed. The results were analyzed by ANOVA test.

Recipient's & donor demography

Table 1: Outcome following corneal transplantation

Outcome	Recipient's Age		Recipient's sex		Donor's age	
	age >60	age <60	male	female	donors >60	donors <60
Average duration of clarity post op	3.4 days	1.8 days	1.83	2.75	2.59	1.78
Endothelial cell density cell/mm2	2461	2498	2454	2493	2379	2528
BCVA 1wk	0.18	0.17	0.20	0.17	0.20	0.16
BCVA 6 month	0.23	0.36	0.18	0.34	0.30	0.32
IOP	14.3	15.4	17.4	13.8	14.6	15.4

Indication for transplantation

Table 2 Outcome after corneal transplantation due to different indications

Outcome	bullous keratopathy	keratoconus	corneal dystrophy
Average duration of clarity post op	3.44	1.18	2
Endothelial cell density cell/mm2	2357	2542	2581
BCVA 1wk	0.16	0.17	0.10
BCVA 6 month	0.24	0.37	0.30
IOP	14.8	16.9	12.4

Time between death and transplant

Table 3 Outcome after corneal transplantation with different time between death and transplant

Outcome	1-5 days	6-10 days
Average duration of clarity post op	2.33	2.11
Endothelial cell density cell/mm2	2473	2489
BCVA 1wk	0.10	0.18
BCVA 6 month	0.22	0.35
IOP	16.8	14.9

Conclusions

1. Younger patients (below 60 years) and Female patients show more improvement compared by older patients (above 60 years)
2. Corneal dystrophy cases show more improvement followed by Keratoconus followed lastly bullous keratopathy.
3. Donors aged below 60 years, give more positive results compared by donors aged above 60 years.
4. There are mild more positive results if the time between death and transplant is less than 5 days
5. Trephine size more than 7.5MM, Punch size of 8.25MM, 8 interrupted & 8 continuous 10/0 technique and Using General anesthesia show more improvement compared by smaller sizes.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Estimation of Some Hormones Levels and Insulin Resistance in Some Infertile Men in Samarra City

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Abstract

The study aimed to evaluate levels of some hormones and insulin resistance in infertile men in Samarra city.

The study group include 25 infertile men and control group of 10 healthy men. The hormonal and biochemical assays include testosterone, E_2, insulin, IR and fasting blood sugar (FBS). The results was a significant increase at ($P \geq 0.05$) in E_2 and FBS concentrations, while testosterone show significant decrease at ($P \geq 0.05$) in infertile men compared to control group. Insulin and IR didn't show any significant difference between both groups.

***Key words**: male infertility, IR, testosterone, E_2, insulin.*

Introduction

Men infertility is defined as inability to conceive after one year of regular sex, and it effects 15-20% of couples [(1)]. The male infertility factors estimated in about 50% of cases [(2)], It can be caused by many conditions such decrease androgen levels, decreased sexual activity, anatomical disorders, genetic disorders and infections [(3,4)]. The hypothalamus- pituitary- testis axis has great importance in male fertility, follicle stimulating hormone (FSH) releasing from the anterior pituitary combined with receptors in sertoli cells and stimulates spermatogenesis process luteinizing hormone stimulates production of testosterone by Leydig cells [(5)].

Testosterone is responsible for normal development of male sex organs and keep secondary sex characteristics, it improve sperm motility and epididymis function [(6)].

Estrogen inhibits the hypothalamus- pituitary axis and subsequently FSH and LH so that reduce circulatory testosterone [(7)].

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Materials and Methods

Samples:

The samples was collected from the infertility outpatient clinics in Samarra city. The total members of infertile men was 25 as a study group and 10 healthy fertile men were consider as control group.

Blood collection:

Blood samples was collected from the cuboidal vein. The samples left for 15 min at room temperature. Then centrifuged at 5000 rpm for 10 min. the serum transported to test tubes until examination.

Hormonal analysis:

The concentrations of hormones (testosterone, E_2 and insulin) were measured via enzyme linked immune sorbent assay (ELIZA) by using the commercial kits (ELIZA kit, Human, Dimeditic, Germany) and procedures was followed as given in the kits catalogs.

Biochemical analysis:

Fasting blood sugar concentration quantified by followed the procedure that given with kit (bio Maghreb, Tunisia) insulin resistance was stimulated by using

homeostatic model assessment of insulin resistance which equal:

HOMA (IR)= fasting insulin (µl/ml)* fasting blood sugar (mg/dl)/450

HOMA (IR)= homeostasis model assessment [8].

Statistical analysis:

All values expressed as mean± S.D data analyzed done by using analysis of variance (ANOVA).

Results

The results shows in table (1) significant increase E_2 levels and significant decrease in testosterone levels in infertile men group compare with control group and glucose concentrations shows significant increase in infertile group compare with control group while insulin levels and IR didn't shows any significant difference between groups

Table(1): Hormones, FBS, IR levels in infertile and control group

Groups	FBS (mg /d L)	IR	Insulin	E2	Testosterone
Control group	90.33 ±4.02 B	6.608 ±0.752 a	29.608 ±2.902 a	15.848 ±1.420 b	1.780 ±0.220 a
Infertile group	99.48 ±5.29 A	7.114 ±1.501 a	28.971 ±2.902 a	36.912 ±9.422 a	1.588 ±0.121 b

Discussion

High of estrogen in males results from increase conversion of androgen to estrogens due to bio availability of aromatase enzymes [9]. Estrogen is more biologically active than testosterone even at low levels therefore; same changing in circulating estrogen level can increase the action of abnormal activity of estrogen in the testis [10]. Estrogen might contribute to decrease testosterone levels through negative feedback mechanism [11]. Estrogen can act on the hypothalamus to negative regulation to release of gonadotropin releasing hormone (Gn RH) as well as release of FSH and LH from pituitary gland and estrogen showed an inhibitory effect on androgen biosynthesis, this indicates that any amount of excess estrogen could determine spermatogenesis [11]. High estrogen level can lead to testis injury and inhibit development of sertoli cells [12] , and can decrease testosterone production from lydig cells [13],decreased testosterone level agreement with [14] .

Insulin levels and IR showed increase but non-significant hyperinsulinemia which occur in some obese men has an inhibitory effect on spermatogenesis and related with decreased male fertility [15]. [16] reported that increase of IR lead to decrease of testosterone production from lydig cells.

The results also show significant increase in glucose concentration in infertile men and that increases leads to an ability to have children whether it's because direct effect on testis and it capability on sperm formation or due to infect the patient with sexual dysfunction or difficult extrusion, also decreased testosterone levels related with high glucose concentrations [17].

Conclusion

This study confirm that causes of male infertility in some men in Samarra city was increased levels of E_2, glucose and decrease testosterone level while then insulin and IR didn't show any significant difference.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Evaluation the Relationship between Oral Contraceptives Containing Drospirenone with Dyslipidemia and Risk of Cardiovascular Diseases among Women in Al-kut City

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Abstract

Chronic and prolong use of oral contraceptives (OCs) may lead to appearance of undesirable effects in women who used these drugs. There are many types of contraceptives, some contain estrogen and the other contain progesterone, or combination of them in the same drug. This work was performed in a population of adult females to study the relationship between the chronic use of OCs with dyslipidemia and the threat of cardiac diseases. A total 163 volunteers were participated in this study, and, their aged between 21-42 years old. In addition, all women were free of heart disease, dyslipidemia and any hormonal disturbance according to their health records. They were categorized into three differnt groups, the control group included 52 unmarried women and does not use any OCs, group A: included 57 women took OCs (combination pill estrogen and progestin) for less than 6 months, and group B contained 54 women used OCs for more than one year. We meassued lipid profile, and blood indices of cardiovascular disease, like Creatine Kinase-MB (CK-MB), insulin, Lactate Dehydrogenase (LDH) albumin, leptin and HbA1c test. In addition, we determined the coronary risk index, atherogenic Coefficient, and an atherogenic index . Our results recorded a significant increase in total cholesterol, LDL, coronary risk index (TC/HDL), LDH and HbA1c in group2 compared to compared to control group. Furthermore, our data demonstrated no clear differences in Triglyceride, leptin hormone, TG, HDL, insulin, albumin and CK-MB in group2 compared to control. Moreover, the data indicated a significant elevation in the concentration of leptin hormone, LDH, HbA1c, TC, TG, LDL, coronary risk index , atherogenic Coefficient and Atherogenic index, and significant decline in HDL and albumin in group B compared with control group. Therefore, we concluded that oral contraceptive could deter the lipid profile status and some enzyme related to the cardiovascular system. These alterations convey a possible risk for the development of cardiac diseases. Routine screeing of lipid tests would be measured in women used OCs for long periods to avoid any potential risk of cardiac diseases.

Key words: *oral contraceptive, lipid profile, cardiovascular disease, leptin, CK-MB.*

Introduction

Oral contraceptives (OCs) are female exogenous hormones that are used either to control birth, regulate pregnancy and to treat other medical conditions, such as poly cystic ovaries, menopause, hyperandrogenism and menstrual cramps [1,2]. Most of these OCs containing one or both synthetic forms of female hormones (estrogen and progesterone), which are naturally synthesized by a woman's body [3]. These combined OCs pills act by inhibiting the roles of follicle stimulating hormone (FSH) and luteinizing hormone (LH), thus decrease the metabolic action of ovary, subsequently inhibiting the ovulation [4]. The prolonged use of oral contraceptives induce different side effects with women, which are

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associated with cardiovascular disease, diabetes mellitus and growth hormone disturbance [5, 6]. Several studies revealed that hormonal contraceptive increase LDL and TG by increasing the synthesis of apolipoprotein B-100 [7]. In addition, oral and hormonal contraceptives may also increase the chance to develop different cancers (breast or liver), cardiovascular diseases and serious blood diseases [8]. Lipid parameters are highly associated with CVD like HDL-C or Total Cholesterol / HDL-C and consider as suitable predictors to monitor and determine who is in risk for the CVD [8].

The main goal of current project to study the off-target effect for prolonged use of combined OCs in lipid profile status, leptin and insulin, and some predictors related with heart damage in two groups of volunteers, after 6 months and one year of oral contraceptive use.

Material and Methods

Study subjects and design

This study included 163 adult women who volunteered for this study after their consent. Their ages were between 21 and 42 years. The women who have heart disease, liver disease, dyslipidemia, diabetes mellitus, and any hormonal disturbance, according to their health reports were excluded from study. This study performed according to approved protocol by the Ethical Committee in the Institute of Endocrinology and an informed printed consent was obtained from each woman. The volunteers were classified into three main groups. The control group involved 52 adult unmarried women who did not use oral contraceptives. The second group A included 57 adult married women who used combined oral contraceptives contain containing Drospirenone, Ethinyl Estradio for 6 months only. The third group B included 54 women used OCs pills for one year. About five ml of blood was collect to measure all theses tests.

Biochemical analysis

The Total cholesterol (TC), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), Triglycerides (TG), Very low density lipoprotein cholesterol VLDL-C, Lactate Dehydrogenase (LDH), CK=MB: Creatine kinase isoenzyme of heart muscle ,and albumin were determined in serum by using automated chemical clinical analyzer from FUJIFILM, Japan. While, leptin and insulin hormones were measured by using standard leptin and insulin ELISA kits. (Elabscience, china). Quantitative determination of glycated hemoglobin (HbA1c) was measured in EDTA tube contained blood samples by using NycoCard reader II, Norway. Atherogenic indices estimated according to: Coronary Risk Index = TC/HDL-C [9]. Atherogenic Coefficient =TC HDL-C/HDL-C [10], and Atherogenic Index = Log (TG/HDL-C) [11].

Statistical Analysis

The one-way analysis of variance (ANOVA) was applied to check the mean differences of all three groups. The results were presented as mean ± standard deviation to define the differences among group at $*P<0.05$, $**P<0.01$, and $***P<0.001$ levels

Results

In order to study the association between oral contraceptivein taking and dyslipidemia in women, we measured the levels of TC, TG, LDL-V, VLDL and HDL-C. Our results demonstrated a significant elevation in TC, TG, (LDL-C and VLDL data not shown), and a significant decline ($P<0.01$) of HDL-C level in women (group B) that used oral contraceptive pills for one year compared with the control group. Wheases, the data from group (A) did not show any significant difference in TG, and HDL-C when compared to the control as shown **Figure 1. A-C**.

Moreover, consistent with the lipid profile status, our data demonstrated a sigficant increase in coronary risk index and atherogenic coefficient and lactate dehydrogenase values in women were used combined oral contraceptives for 6 and 12 months only as shown in **Figure 2. A-C**. Furthermore, our results showed no significant alterations in the level of creatine kinase-MB (CK-MB) (data not shown) in women used OCs for 6 and 12 months compared to the control groups.

Furthermore, the data demonstrated no significant differences in mean value of insulin and a significant differences in glycated hemoglobin (HbA1c) in women used oral contraceptive for 6 and 12 months respectively compared to the control group **.**In addition, our results exhibited an important differences in leptin, and albumin

concentrations in group B only, and a clear differences among group A and B as (data not shown). Collectively , the results, confirm a significant difference in some criteria when comparing the results of women who used oral contraceptives for one year compared to those used for 6 months.

Discussion

Contraceptives have been used to prevent ovulation and prevent sperm penetration into the ovum ([12]. The data from our study demonstrated that OCs caused a clear alteration in lipid profile and cause dyslipidemia especially when using contraceptives for very long periods (Figure1). Our results are similar with several other studies that demonstrated the combined OCs correlated with disturbance of lipid profile subsequently induce dyslipidemia [13] .

Next, our data indicated there were a significant elevation in serum TC, LDL, TG and decrease of HDL in women used combined OCs as compared with control. Thus, Chonic use of the control birth pills containing estrogen may stimulate liver lipogenesis, which in turn increase of TG and LDL concentration [14,15]. In addition, use of estrogens is associated with stimulating hepatic synthesis of triglycerides and impair of hepatic lipase expression, resulting in increased serum levels of triglycerides [16].

Moreover, OCs containing progestin decreased HDL concentration by increase the activity of lipase enzyme in liver ,which accelerated the elimination of HDL [17]. Despite of the benefit of the estrogen in the protection of endothelial, oral combined contraceptives that contain progesterone, could decrease the HDL-C's concentration in plasma [18]. In addition, increase the concertation of VLDL in women used OCs could be ascribed to the estrogen hormone's effect, that can enhance the secretion of VLDL in the liver [19].

The results of the present study also demonstrated that oral contraceptive increase the cardiovascular risk indices (TC/HDLC) and (TC/HDLC) as shown in **Figure 2**. Many report revealed that increase in the concentrations of TC LDL-C, and the reduction of HDL-C enhance cardiac diseases biomarkers ,which are the major risk factors for cardiovascular diseases [20]. One study clarified that increase the lipid profile in women used oral contraceptive lead to increased risk of coronary arterial disease and atherogenesis [21].

Our data also showed a sigficant increase in the concentration of LDH and this was similar to the onother study that revealed that oral contraceptive caused significant increase of blood serum enzyme including LDH and exert DNA damage [22]. Therfreroe, Lactate dehydrogenase enzyme (LDH) could be the most important indicator to diagnosis the myocardial infarction or heart attack.

Next, our results exhibited a significant increase in glycated hemoglobin in oral contraceptives users in comparison to non-user women. HbA1c produces of non-enzymatic glycolysis, which is re-measured every 3 months reflecting the average glucose in previous weeks [23]. in addition, Our study showed normal fasting insulin level, and increased HbA1c that are indicative of glucose and insulin resistance. Also, HbA1c was associated, excessive triglycerides, decline of HDL-cholesterol and directly related to the occurrence of atherosclerosis [24].

Serum albumin concentration decreased significantly after one year of oral contraceptive use compared to the control group. Also, pills containing estrogen induce glomerular hyperfilteration and increase albumin excretion, because of greater tubular load of albumin that lead to decrease of serum albumin concentration [25].

In addition, the results from our study demonstrated that combined OCs caused significant increase of leptin hormone level as compared with non-users, Leptin is produced mainly from tissue and is equivalent to fat content inside the body [26]. Our interpretation of the high level of leptin in women who have used OCs for beyond than a year may be due to their weight gain, and it is known that the concentration of leptin is associated with body mass index (BMI [27,28]. Our results demonstrated no major alterations in the level of CK-MB among all groups studied. These results is similar with pervious study that illustrated there were no differences in CK-MB in women used oral contraceptive during percutaneous coronary intervention [29,30].

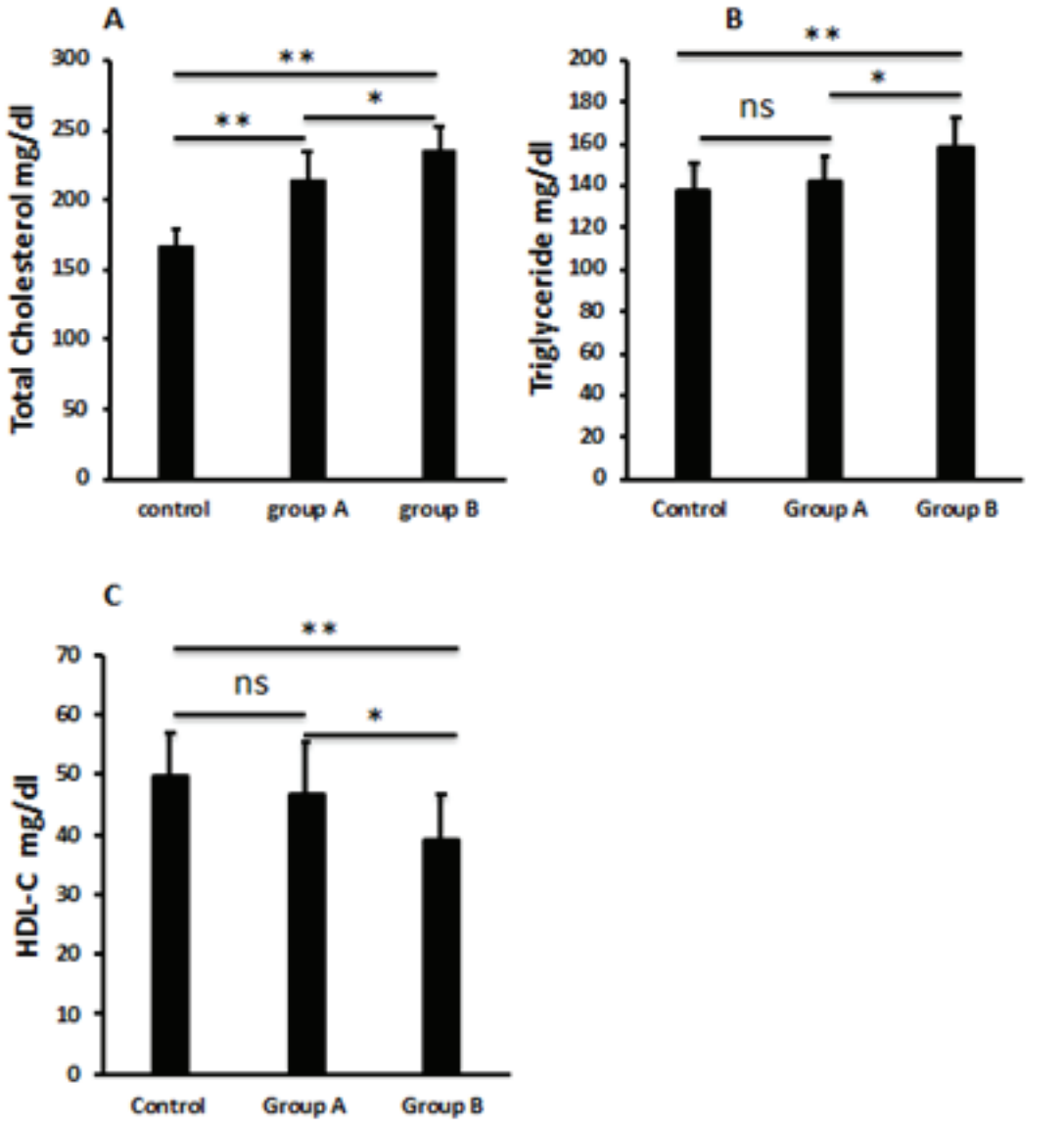


Figure1. Usages of oral contraceptives enhance dyslipidemia in women. A-C represent the level of TC, TG, and HDL-C . The differences in lipid profile concentrations between the control group and two different groups (A and B) using oral contraceptives for 6 and one year respectively. Data represent mean ± SD. P value determined was (ANOVA). *P<0.05 ; **P<0.01; ***P<0.001, and ns as non-significant.

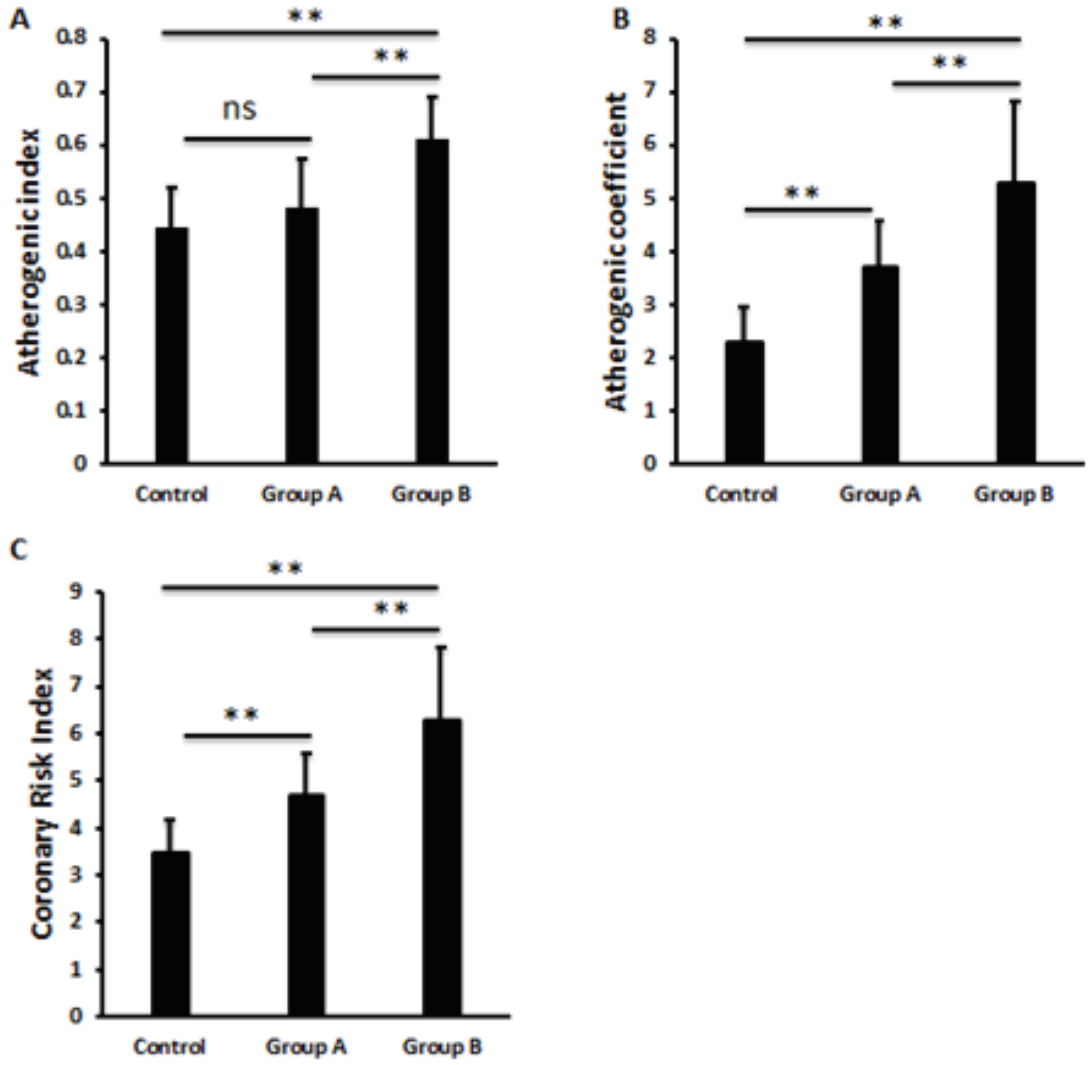


Figure2. Oral Contraceptives promote atherogenic indices and coronary risk index. A-C represent atherogenic indices , atherogenic Coefficient , and coronary risk index,. The differences in atherogenic indices and coronary risk index between the control group and two different groups (A and B) using oral contraceptives for 6 and one year respectively. Data represent mean ± SD. P value determined was (ANOVA). *P<0.05 ; **P<0.01; ***P<0.001, and ns as non-significant.

In conclusions , we have revealed that OCs could deter the lipid profile status and some enzyme related to the cardiovascular system. Therefore, These changes reflect a possible risk fators to induce the incidence of cardiac diseases. Thus, a routine screeing of lipid profiles should be evulated for all women using the OCs for long periods in order to avoid any potential risk of cardiovascular diseases.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Ability of *Staphylococcus* spp. Isolated from Meningitis Patients to Biofilm Formation

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Abstract

A total 248 clinical samples of cerebrospinal fluid (CSF) were collected from meningitis patients, for the period of July to October 2018 from the Child Protection Teaching Hospital in the Medical City Compound in Baghdad. All isolates were identification depending on macroscopic, microscopic, biochemical tests and definite with Vitek-2 compact system. The results showed a growth in 42 samples. Sixteen isolates of *Staphylococcus* spp. were obtained from samples. The number (percentage) of isolates according to species as follow: 7/16) 44%) of *S. epidermidis*, 3/16 (19%) of *S. hominis*, 3/16) 19%) of *S. haemolyticus*, 2/16 (12%) of *S. aureus*, 1/16 (6%) of *S.warneri*. These isolates were tested for ability to biofilm formation by using Luria Broth and Tryptone Soya Broth (TSB) supplemented with 0.25% and 2% glucose. The ability of the isolates to form biofilm with Luria Broth, for *S.epidermidis*, *S. aureus, S. haemolyticus*, *S.hominis* and *S. warneri* were 7/7 (100%), 1/2 (50%), 2/3 (66.66%), 1/3 (33.33%),1/1 (100%) respectively not forming biofilm.As for using a TSB supplemented with 0.25% glucose, *S.epidermidis, S. aureus, S. haemolyticus* and *S.hominis* were 6/7 (85.71%), 2/2 (100%), 2/3 (66.66%) and 3/3 (100%) respectively were weakly biofilm formed and when using TSB supplemented with 2% glucose, showed that all *Staphylococcus* spp. (100%) were positive for the test..The study showed the effect of minimum inhibition concentration(8 µg/mL) and minimum bactericidal concentrationand (16µg/mL) of Erythromycin on biofilm of *Staphylococcus* spp. *Staphylococcus* spp. isolated from CSF showed weak ability to biofilm formation and depending on the host content of sugars . This study showed also weak biofilm forming of *Staphylococcus* spp. was resistence to erythromosin.

Keyword: *Staphylococcus spp., Meningitis, Biofilm Formation.*

Introduction

Biofilm is aggregation of bacterial cells that are attached to each other biological surface or abiotic via production of extracellular substances consist of sugars, proteins and extracellular DNA (eDNA)[1], biofilm starts with adhesion, proliferation and maturation of bacterial cells due to Quorum Sensing (QS), who responsible of biofilm formation as a result of aggregate of bacteria and sending signals[2,3]. [4,5] biofilm structure composed from adhesion of bacteria on surfaces, forming colonies through accumulation of bacteria with each other to form triple dimension structure of multi layer extracellular substances. [6] pointed to biofilm formation in *Staphylococcus* due to product of slime layer as well as capsule forming, and by operon *ica* that encode to polysaccharide intercellular adhesion (PIA). Several studies pointed to biofilm formation through stage of attachment, accumulation and maturation via (PIA)[7,8]. Accumulation associated protein (Aap) and Extracellular matrix binding protein (Embp)[9,10], while stage of separation of cells by Serine protease[11], surface-located fibronectin binding protein A, B (FnBPA, B)[12], Cysteine protease (EcpA) and Metalloprotease (SepA)[13]. [14,15] showed the role of phenol souluble modulins (PSms) and Surfactant peptides that Product by *Staphylococcus*

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in biofilm decomposition. Biofilm formation includes a group of compounds: PIA is one of the main saccharides in *S.epidermidis* that called Polysaccharide intracellular adhesion or poly N-acetyl glucosamine encoded by *ica* operone (*ica* A, D, B, C)[16] .Bap homologue protein Bap/ BhP One of the most virulent factors for *S.epidermidis* has a role in the formation of the biofilm also called biofilm associated protein (Bap), and contributes to adhesion and aggregation through biofilm formation[17], bap presence only in *S.epidermidis* and *S.aureus* isolated from Cows infected with mastitis[18]. Protein Bap is an equivalent protein called Bhp that presence at some strains of *S.epidermidis* ,but it does not contribute to the formation of the biofilm[19]. The current study aimed to know the ability of *Staphylococcus* spp. isolated from CSF of meningitis patients to biofilm formation ,and the effect of type of media on its ability of biofilm formation addition to resistance of biofilm to Erythromycin.

Materials and Method

Collection of Samples

Collect 248 clinical samples of cerebrospinal fluid (CSF) from meningitis patients in children, from the Child Protection Teaching Hospital in the Medical City Complex in Baghdad. For the period of July to October 2018. All clinical samples were collected and straight cultured on Blood agar, Chocholate agar and MacConkey agar plates then incubated at 37˚C for 24 hours. All isolates were identification depending on macroscopic, microscopic, biochemical tests and definite with Vitek-2 compact.

Ability of *Staphylococcus* spp.of biofilm formation test

The ability of bacterial isolates have been tested for biofilm formation according to [17], by using micro titer plate with,Trypton soy broth supplemented with (0.25%) glucose and Trypton soy broth supplemented with (2%) glucose. The calculations were performed according to[20].

Resistence of *Staphylococcus* spp. of Erythromycin

Biofilm resistance detection of *Staphylococcus*spp isolates carried out According to [17],by using micro titer plate,25 µl of bacteria was inoculated with 175µl of Trypton soy broth(2% glucose) and incubated at 37˚C for 24h ,decanted plate and washed with distilled water then put 200 µl of Mueller-Hinton broth in the first well ,100 µl of Mueller-Hinton broth and 100 µl of MIC of Erythromycin (8) µg/mL,and the third well 100 µl of Mueller-Hinton broth and100 µl ofMBC of Erythromycin (16)µg/mL ,plates were incubated at 37˚C for 24h.Optical density was measured at 600 nm using the Elisa device.

Results and Discussion

The results of the present study varied in the ability of isolates to form the biofilm from Luria broth medium, all isolates of *S.epidermidis* were not biofilm formation 7/7 (100%), while 1/2 (50%) of *S.aureus* were not biofilm formation and 1/2 (50%) weak biofilm formation. *S.haemolyticus* 2/3 (66.67%) were not biofilm formation and 3/1 (33.33%) weak biofilm formation. While *S.hominis* was 3/1 (33.33%) not biofilm forming and 2/3 (66.67%) weak biofilm forming.*S.warneri* was 1/1 (100%) not biofilm forming (Table 1). While the result of current study on biofilm formation by using tryptone soy broth with 0.25% glucose was 1/7 (14.29%) of *S.epidermidis* not forming biofilm and 6/7 (85.71%) weak biofilm forming. *S.aureus* and *S.hominis* were 2/2 (100%), 3/3 (100%) weak biofilm forming respectively. *S.haemolyticus* was 1/3 (33.33%) not biofilm forming and 2/3 (66.67%) weak biofilm forming. *S.warneri* 1/1(100%) was not biofilm forming (Table 2). The results showed the ability of bacterial isolates with Luria broth were 12/16 (75%) not biofilm forming and 4/16 (25%) weak ability of biofilm formation. *S.epidermidis* was 3/16 (18.75%) unable to biofilm formation and 13/16 (18.75%) weak ability of biofilm formation in TSB supplemented with (0.25%) glucose. All isolates (100%) of *S.epidermidis* had weak ability of biofilm formation in TSB (2%) glucose (Table 3). Felipe *et al.* (2017)[21] pointed to the ratio of *S.aureus* ability to biofilm formation 35% was strong and 45% moderate while CoNS bacteria 51%, 29% strong and moderate respectively. [22] the ability of *Staphylococcus* spp. to biofilm formation were 5.6%, 24.3%, 70.1%, strong ,moderate and weak or un able to biofilm formation respectively, at the same study using TSB was 2.5% of *S.aureus* and 7.9% of CoNS showed strong ability to biofilm formation respectively, while increased in TSB supplemented with (1%) glucose was 19% for *S.aureus*

and 16.4% for CoNS bacteria. [6] showed that the gene icaA had role in *Staphylococcus* pathogenesis that isolated of clinical samples led to forming Slime substance that forming biofilm on medical surfaces. [23] pointed out that the difference in the formation of the biofilm is due to bacterial strain type,operon *ica*ADBC and expression of enzymes encoding for intercellular adhesion sugars that depend on environmental conditions. Studies have shown a relationship between a gene *ica*A and biofilm formation of clinical isolates of *Staphylococcus*[24,25]. [26]a hundred percent of *S.aureus* possess *ica* gene and unable to form biofilm , that due to *Staphylococcus* has the ability to adapt to conditions and the ability to change the phenotypic and genetic characteristics when exposed to concentrations of antibiotics. [27] the ability of *S.epidermidis* to biofilm formation due to possess *S.epidermidis* Surface protein C (SesC), while[28] referred to the ability of *S.epidermidis* to biofilm formatin due to possess *ica*A gene, its rise in the isolates that it possesses *S.epidermidis* surface protein I (SesI) that consider it's virulence factor, and refers to studies have shown *S. epidermidis* isolated from healthy people no possession (SesI) making it unable to biofilm formation, and there are no deference of the ability of *S.epidermidis* to biofilm formation when growing it on TSB and TSB supplemented with (1%) glucose. *Staphylococcus* spp. isolated from CSF showed weak ability to biofilm formation and depending on the host content of sugars

Biofilm forming *Staphylococcus* spp. resistance to Erythromycin

The effect of MIC of (8) µg/ml Erythromycin against biofilm forming *Staphylococcus* spp. In current study, the results showed that Erythromycin had inhibition effectiveness against biofilm formation, that refer to the biofilm was sensitive to the antibiotic in proportions that differ according to the bacterial species and strain, may be due to the biofilm formation was weak (Figure 1). The MBC was at concentration (16) µg/ml for Erythromycin showed increase at inhibition rate due to high concentration of Erythromycin[17], as well as a weak biofilm formation by *Staphylococcus* spp. the results also show that the rate of MIC and MBC, It did not reach the minimum limits and this is due to the ability of the biofilm to protect the bacterial of antibiotics figure (3). The results showed that the inhibition rates (depending on absorbance) differ according to species and strains, and this may be due to their resistance to antibiotics [29], and possess or do not possess the genes responsible for the composition of the biofilm formation, as well as surface proteins possessed by *Staphylococcus* spp. which are responsible to biofilm formation. This study showed weak biofilm forming of *Staphylococcus* was resistence to erythromosin. We conclude from the present study the ability of biofilm formation of Staphylococcus spp. isolated from CSF of meningitis patients, is weakly by using a medium containing 2% glucose,As well as weak biofilm formation was resistence to erythromycin when using MIC and MBC concentrations.

Abbreviations:

Se= *S.epidermidis*, Sa= *S.aureus*, Sh= *S.hominis*, Sha= *S.haemolyticus*, Sw= *S.warneri*, MIC= Minimum inhibition concentration, MBC= Minimumbactericidal concentration.

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Evaluating the Role of High Molecular Weight Adiponectin in Women with Polycystic Ovary Syndrome Treated with Omega-3 Fatty Acids

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Abstract

Polycystic ovary syndrome (PCOS) is a significant health problem affecting women during the reproductive age and characterized by hormonal disturbance resulting in irregular menstrual periods and infertility. Adiponectin is a protein produced by adipocytes and involved in glucose and lipid metabolism. This study included the evaluation of two doses of omega-3 (300 and 600mg/day for 14 weeks). Age, body mass index (BMI), fasting blood glucose level, homeostatic model assessment (HOMA), lipid profile, luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estrogen and HMW adiponectin levels were evaluated. Our data showed no significant differences between control group and treatment groups in BMI, age, sex hormones, fasting blood glucose, and HOMA levels. However, the level of HMW adiponectin was significantly higher in women treated with 600mg/day for 14 weeks ($p < 0.0001$) but not 300mg / day of Omega-3 fatty acids for the same period.

Keywords: *Polycystic Ovary Syndrome (PCOS), Omega-3 fatty acids, High Molecular Weight (HMW) Adiponectin*

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine metabolic disorder leading to anovulation in female causing infertility. It is responsible for about 50-70% of infertility in women. The clinical features of PCOS include hirsutism and oligomenorrhea or amenorrhea.[1]

Obesity and insulin resistance as the most common causes of PCOS.[2,3] Adipokines are among peptides that are synthesized by adipose tissue, which act as autocrine and paracrine controlling the adipose tissue and as endocrine to control other organs such as adrenal gland, CNS and skeletal muscle. In addition, they mediate insulin action.[4]

Many studies have shown that the HMW adiponectin can regulate insulin sensitivity and involved obesity and cardiovascular diseases. In addition, it has been shown that the other forms of low and intermediate molecular weight are less effective.[5,6] It has been reported that metabolic disorders such as PCOS is associated with hypoadiponectinemia.[7,8,9] In addition, it has been reported by many studies related to nutrition and PCOS that energy management and weight loss in overweight patients can modify biochemical parameters.[8]

Omega-3 is one of the supplements that regulate obesity, inflammation and insulin resistance.[9] The relationship between adiponectin level and omega-3 supplement has been reported at the experimental level.[10] However, at the clinical level it is still controversial which may be due to different doses and periods of omega -3 treatment recoded.[11] Nevertheless, omega-3 has shown a role in metabolic equilibrium and some diseases.[12,13]

This study investigates the effect of two different doses of omega-3 fatty acids (300mg and 600mg) with period of treatment for 14 weeks on the plasma of adiponectin for women PCOS.

Materials and Methods

Study design

Our study included 270 Iraqi females suffering from PCOS. They were divided into three groups, 90 participants in each group: Group A (control; women with PCOS with no treatment), group B (women with PCOS treated with 300mg /day omega-3 fatty acids for 14 weeks), and group C (women with PCOS treated with 600mg /day omega-3 fatty acids for 14 weeks).

Sample preparation

Samples were collected during the period between December 2018 to December 2019. The biochemical analysis was performed in the laboratories of the Department of Biochemistry in the AL-Sader Teaching Hospital in Al Najaf Al Ashraf Province.

The three groups were diagnosed clinically depending on the inclusion criteria: [14] oligomenorrhea, amenorrhea (i.e the absence of period for 6 months), hirsutism, elevated LH/FSH ratio, increased testosterone and ultrasound (ovaries contain multiple cyst).

The exclusion criteria of the study include the following: Cushing's syndrome, hyperprolactinemia, adrenal gland dysfunction, congenital adrenal hyperplasia, thyroid gland diseases, androgen secreting tumors, female treated with oral contraceptives, antiandrogen, body agents and weight restriction drugs, smokers, those patients with insulin sensitivity, diabetes mellitus, renal diseases, hepatic disorders and/or gastrointestinal disturbances.

The study protocol was accepted by Ethical Committee of College of Pharmacy, Jabir ibn Hayyan Medical University. The individuals in the study were asked for regulation of life style and diet management.

The (BMI) body mass index was calculated as follows:

$$BMI = \frac{\text{Weight in kg}}{(\text{Height in m})^2}$$

The collection of samples under fasting conditions about (8-10 hr).[15] after that the blood samples were centrifuged (2000xg for 10 minutes) and stored the serum in freezing condition at −70°C.

The HMW adiponectin was estimated according to the commercial ELISA kit (catalog number: MBS702778). Hormones, insulin, testosterone, LH and FSH were estimated by Promega® (U.S.A). The HOMA-IR was calculated as follows:[16]

$$\text{HOMA-IR} = \frac{\text{Insulin } (\mu U/ml) \times \text{Glucose } (mg/dl)}{405}$$

When HOMA-IR is more than 3.5, there would be insulin resistance.

The total cholesterol (TC) was estimated according to BIOLABO (France), high density lipoprotein cholesterol (HDLc) was estimated by DRG Gmb (Germany) and the low density lipoprotein cholesterol (LDLc) was calculated according to Fridewal *et al*., 1972.[18]

The statistical analysis was performed using SPSS program and data were presented Mean±SD. Student's t-test was used to test the significance level. Data with p value less than 0.05 was considered significantly different.

Results

Before treatment, there was a screening for the study parameter in all the three groups. Our findings showed no significant differences in age, BMI, fasting insulin, HOMA-IR, LH/ FSH ratio, total cholesterol, triglycerides, LDL, TG, VLDL, HDL, estradiol, progesterone, free and total testosterone, prolactin, dehydroepiandrosterone sulfate, TSH and HMW adiponectin between group A and group B before treatment or group A and group C (before treatment). However, there was a significantly higher level of fasting blood glucose in B (before treatment) when compared to A (p= 0.002) as shown in Table 1.

Table 1: Biochemical screening for the study parameters in subjects in group A (control, n=90), group B (before treatment with 300mg omega-3, n=90) and group C (before treatment with 600mg omega-3, n=90).

Parameter	Group A Control	Group B Before treatment	P value	Group C Before treatment	P value
Age	25.4 ± 4.3	24.2 ± 5.3	0.105	24.9 ± 4.3	0.44
BMI (kg/m2)	28.5± 1.69	28.3 ± 2.84	0.56	28.1 ± 1.98	0.14
Fasting insulin (μU/ml)	18.4± 2.9	17.7± 3.3	0.132	18.1± 3.1	0.5
Fasting blood glucose (mg/dl)	72.63± 11.8	77.79± 10.5	0.002	80.89± 12.5	0.3
HOMA-IR	3.29 ± 0.7	3.39 ± 0.8	0.37	3.61 ± 0.69	0.84
LH/ FSH ratio	2.1± 0.79	2.2± 0.8	0.39	2.05± 0.69	0.65
Total Cholesterol (mg/dl)	181.5 ± 20.1	181.1 ± 21.2	0.89	180.9 ± 22.1	0.84
Triglycerides (mg/dl)	107.8 ± 21.1	108.2 ± 25.1	0.9	109.1 ± 22.1	0.70
VLDL (mg/dl)	21.56 ± 4.2	21.64 ± 5.02	0.9	21.82 ± 4.42	0.68
LDL (mg/dl)	110.84 ± 11.98	110.16± 11.78	0.7	110.13± 13.58	0.71
HDL (mg/dl)	49.1± 3.9	49.3 ± 4.4	0.74	48.95 ± 4.1	0.80
Estradiol, pg/ml	56.4± 29.2	57.2± 26.2	0.84	55.9 ± 27.2	0.98
Progesterone, ng/ml	0.88 ± 0.3	0.91 ± 0.22	0.44	0.81± 0.69	0.071
Free Testosterone	3.9 ± 0.8	4.1 ± 0.75	0.07	3.4 ± 0.8	1
Total testosterone, ng/ml	0.79± 0.6	0.82± 0.71	0.75	0.81± 0.69	0.76
Prolactin ng/ml	14.6 ± 2.9	14.2 ± 3.6	0.41	14.3 ± 3.1	1
Dehydroepiandrosterone sulfate, pg/dl	314.2± 64.2	312.8± 65.4	0.88	313.1 ± 64.8	0.90
TSH mIU/l	3.6 ± 0.6	3.75 ± 0.7	0.12	3.76± 0.59	0.072
HMW Adiponectin ng/ml	3.9± 2.3	3.8± 2.1	0.76	3.75± 2.2	0.65

The study parameter in group B after treatment were compared to those in group A. We found no statistically significant differences between groups in all parameters except the fasting insulin which was significantly lower and the fasting blood glucose which was higher in group B when compared to group A (Table 2). On the other hand, in group C (after treatment) we found that BMI, fasting insulin, LH/ FSH ratio, total cholesterol, triglycerides, LDL, TG, VLDL, HDL, estradiol, progesterone, free and total testosterone, prolactin, dehydroepiandrosterone sulfate, TSH and HMW adiponectin were statistically different.

Table 2: Biochemical screening for the study parameters in subjects in group A (control, n=90), group B (after treatment with 300mg omega-3, n=90) and group C (after treatment with 600mg omega-3, n=90).

parameter	Control group A	Group B After treatment	P value	Group C After treatment	P value
BMI (kg/m2)	28.5± 1.69	28.4 ± 2.79	0.77	25.9± 1.5	0.000
Fasting insulin(μU/ml)	18.4± 2.9	16.61± 4.05	0.000	19.5 ± 2.96	0.012
Fasting blood glucose (mg/dl)	72.63± 11.8	78.01± 11.03	0.001	65.2± 8.5	0.000
HOMA-IR	3.29 ± 0.7	3.2± 0.75	0.406	3.13± 0.5	0.07
Total Cholesterol (mg/dl)	181.5 ± 20.1	181.02± 20.9	0.89	170.5 ± 18.2	0.000
Triglycerides (mg/dl)	107.8 ± 21.1	109.1± 24.9	0.9	101.2 ± 18.4	0.02
VLDL (mg/dl)	21.56 ± 4.2	21.82 ± 4.98	0.705	20.24 ± 3.68	0.026
LDL (mg/dl)	110.84 ± 11.98	109.19 ± 11.97	0.35	99.36 ± 12.42	0.000
HDL (mg/dl)	49.1± 3.9	50.01± 3.95	0.74	50.9 ± 2.1	0.000
HMW Adiponectin ng/ml	3.9± 2.3	3.95 ± 2.95	0.76	5.61 ± 1.89	0.000

The study parameters in group B after treatment were measured and compared with those in group B before treatment. No significant differences in the study parameters were reported between measurements before and after treatment.

Table 3: Biochemical screening for the study parameters in subjects in group B before and after treatment, n=90.

Parameter	Group B (Before treatment)	Group B after treatment	P value
BMI (kg/m2)	28.3 ± 2.84	28.4 ± 2.79	0.81
Fasting insulin, μU/ml	17.7± 3.3	16.61± 4.05	0.048
Fasting blood glucose(mg/dl)	77.79± 10.5	78.01± 11.03	0.89

Cont... **Table 3: Biochemical screening for the study parameters in subjects in group B before and after treatment,**

HOMA-IR	3.39 ± 0.8	3.2± 0.75	0.08
Total Cholesterol (mg/dl)	181.1 ± 21.2	181.02± 20.9	0.97
Triglycerides (mg/dl)	108.2 ± 25.1	109.1± 24.9	0.80
VLDL (mg/dl)	21.64 ± 5.02	21.82 ± 4.98	0.8
LDL (mg/dl)	110.16± 11.78	109.19 ± 11.97	0.58
HDL (mg/dl)	49.3 ± 4.4	50.01± 3.95	0.25
HMW Adiponectin ng/ml	3.8± 2.1	3.95 ± 2.95	0.96

We found that BMI, fasting insulin, HOMA-IR, LH/ FSH ratio, total cholesterol, triglycerides, LDL, TG, VLDL, HDL, estradiol, progesterone, free and total testosterone, prolactin, dehydroepiandrosterone sulfate, TSH and HMW adiponectin, BMI, fasting glucose, total cholesterol, triglycerides and LDL were in group C after treatment were significantly different when compared to those in the same group before treatment (Table 4).

Table 4: Biochemical screening for the study parameters in subjects in group C before and after treatment, n=90.

parameter	**Group C Before treatment**	**Group C after treatment**	**P value**
BMI (kg/m2)	28.1 ± 1.98	25.9± 1.5	0.000
Fasting insulin, µIU/ml	18.1± 3.1	19.5 ± 2.96	0.002
Fasting glucose (mg/dl)	80.89± 12.5	65.2± 8.5	0.000
HOMA-IR	3.61 ± 0.69	3.13± 0.5	0.000
Total Cholesterol (mg/dl)	180.9 ± 22.1	170.5 ± 18.2	0.000
Triglycerides (mg/dl)	109.1 ± 22.1	101.2 ± 18.4	0.01
VLDL (mg/dl)	21.82 ± 4.42	20.24 ± 3.68	0.009
LDL (mg/dl)	110.13± 13.58	99.36 ± 12.42	0.000
HDL (mg/dl)	48.95 ± 4.1	50.9 ± 2.1	0.000
HMW Adiponectin ng/ml	3.75± 2.2	5.61 ± 1.89	0.000

Discussion

PCOS is a multifactorial disease in which the pathogenesis could be related to proinflammatory state and changes in adipokines.[19] The present study involved the evaluation of adiponectin levels in patients with PCOS treated with omega-3 using two different doses. While using a dose of 300 mg/day omega-3 induced no significant difference in the level of adiponectin between the control and treatment, increasing the dose to a double resulted in significantly higher level of adiponectin.

A previous study showed that treatment with omega-3 for 8 weeks resulted in increased level of adiponectin.[20,21] In addition, Higuchi and colleagues reported an elevated level of adiponectin in animals received fish oil.[22] A further study has shown consistent findings with ours when Mohammadi et al.[23] recorded that taking the omega-3 for 8 weeks lead to increase level of adiponectin. It has been shown that the longer the period of administrating omega-3 the higher the level of adiponectin. Poreba and colleagues found that 6 weeks of omega-3 tratment had no effect on adiponectin in women with PCOS.[24]

The present study that show significantly decreased in total cholesterol, triglycerides, triglycerides, LDL-c as well as significantly increased HDL-c with omega-3 either treatment (300mg or 600mg for 7 or 14 weeks). Similar findings were shown by several study .[25]

The study showed that a significantly elevated level of adiponectin when doubled the dose of omega-3 fatty acids from 300mg /day to 600 mg/day dose for 14 weeks after compared with control group. This means that the dose and time duration can significantly improve the effectiveness of omega-3.

We found that 600mg omega-3 for 14 weeks significantly affected the BMI and 600mg omega-3 for 14 weeks, findings that agree with a previous study. [26] The significant changes in lipid profile may be due to the activation of enzyme AMP-activated protein kinase which plays an important role in lipolysis and lipogenesis .[27]

By using two different doses of omega-3, we found that it can modulate adiponectin levels in women with PCOS in a dose-dependent manner.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Levels of Leptin and IL-6 With Some Biochemical Parameters in Iraqi Obese Children

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Abstract

Obesity in children is attended by increased circulating leptin concentrations. Girls have higher leptin Concentrations than boys. IL-6 levels related positively with BMI and percent fat mass (PFM).

The Objective: Was to detect the serum IL-6 and levels of leptin among girls and boys as obese and other biochemical parameters related with obesity. **Methodology:** This study involved 60 children were classified into 2 groups; 30 obese ones (fifteen girls and boys fifteen) and 30 control (fifteen girls and fifteen boys) with years of seven- fifteen old who attended to the Obesity Research and Treatment Unit/ Al kindy Medical College**,** during the period January 2017 – July 2017. A clinical examination and diagnosis were done for each patient by the consultant medical staff and the biochemical laboratory examination results (FBS, blood urea, serum creatinine, lipid profile, serum GOT, serum GPT, SCa and uric acid) were as a routine work. They compared with 30 healthy children with approximate age with patients. The sera were assessed for IL-6 and leptin by an ELISA kit. **Results:** Showed no significant differences among obese children (boys and girls) except the GPT and GOT were both higher significantly (P<0.05) in male (42.46±34.27 and 33.3±13.6) than in girls (53.6 ±75.1 and 29.9±14.1). Whereas, obese girls revealed a significant increasing in (STRIG 120.3±26.7 $P \leq 0.01$, SLDL 89.3±25.5 $P \leq 0.05$, GOT 33.1±13.6 $P \leq 0.01$, GPT 29.9±14.1 $P \leq 0.01$ and LIPTIN 2758.4±539.1 $P \leq 0.001$) in comparison to the control girls. Similarly, for boys highly significant increasing appeared in (SLDL 90.92±18.1, GOT 42.46±34.27, GPT 53.6±75.1 and LIPTIN 2883.93±512.7 $P \leq 0.001$), as well as in STRIG .69±.13 $P \leq 0.05$ then normal boys. Inversely, it has been shown that (BURIA 26.7±5.3 $P \leq 0.05$, SCREAT .69±.13 $P \leq 0.01$ and SVLDL 20.15±6.3 $P \leq 0.01$) were decreased significantly. In boys IL-6 showed significant positive correlation with BMI, while leptin revealed strong associated was with IL-6 **Conclusion:** Obesity associated with elevated blood leptin and IL-6 levels. Leptin and IL-6 play keys role as a biomarker for childhood obesity, and can help in the prediction of get weight in obese children as well as its important interrelationship with other parameters.

Keywords: *Children obesity; Leptin; IL-6; Biochemical Parameters;*

Introduction

Obesity represents one of the most important public health issues and increasing seriously in all the world parts, approximately two billion people are overweight and one third of them are obese [(1)]. It is one of the most serious risk factors to many diseases like early onset metabolic syndrome asthma, fatty liver disease gastrointestinal diseases and Cardiovascular diseases. Growth and sexual development can also affect by obesity in children [(2)].

Leptin is protein produced by adipose tissue. Serum leptin concentrations are highly associated with body fat content in newborn infants, children and adults [(3)]. It provides the first physiological evidence of regulate and controlling body weight, energy balance, fat and

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glucose metabolisms [4]. leptin levels are higher in obese girls as compared with obese boys even after losing weight showed convincing evidence about the variations between the two sexes [5].

Adipose tissue from obese mainly releases cytokines as pro-inflammatory i.e. leptin, IL-6 & TNF-α [6]. Tissues of liver, skeletal muscle and white adipose secrete interleukin- 6 [7]. In obesity, high level IL-6 secreted by tissue of white adipose might involve in complications being metabolic. Researchers exhibit that signaling of IL-6 in adipocytes is convoluted in the obesity-associated development for resistance of hepatic insulin and steatosis of liver [8]. IL-6 produced by Adipose tissue might with metabolism influence via mechanisms being numerous, as well as gene expression of adipose tissue-specific, down-regulation of lipoprotein lipase, sensitivity of insulin, triglyceride release [9].

This research was done to find out the level of serum leptin and IL- 6 among Iraqi obese boys and girls and other biochemical parameters related with obesity.

Patients and Methods

The study was carried out at the Alkindy Medical College. During the period January 2017 – July 2017, 30 obese (15 boys and 15 girls) were referred to the Obesity Research and Treatment Unit, their age rang 7- 15 years old. A clinical examination and diagnosis were done for each patient by the consultant medical staff and the biochemical laboratory examination results (FBS, blood urea, serum creatinine, lipid profile, serum GOT, serum GPT, serum calcium and uric acid) were as a routine work. They compare with 30 healthy children (15 boys and 15 girls) with approximate age with patients.

Laboratory methods

Serum isolated from five milliliters vinous blood for each participant. The sera were assessed for IL-6 and leptin by an ELISA kit (Elabscience, USA). The instructions of manufacturer were followed to assess the two variables.

Statistical Analysis

The results were tabulated in a data sheet of the statistical package SPSS version 20, which was used to carry out the analysis. The significant differences mean ± SD were assessed by ANOVA. The correlation between IL-6, leptin and other biochemical parameters were estimated by Person's correlation. A probability **(*P*) value** ≤0.05 was considered significant.

Results

The results of present study showed no significant differences (P>0.05) between obese children (boys and girls) except the GPT and GOT were both higher significant differences (P<0.05) in boys (42.46±34.27 and 33.3±13.6) than in girls (53.6 ±75.1 and 29.9±14.1) Table 1. Whereas, obese girls revealed a significant increasing in (STRIG 120.3±26.7 $P\leq0.01$, SLDL 89.3±25.5 $P\leq0.05$, GOT 33.1±13.6 $P\leq0.01$, GPT 29.9±14.1 $P\leq0.01$ and LEPTIN 2758.4±539.1 $P\leq0.001$) when compare to the control girls Table 2. Similarly, in boys highly significant increasing appeared in (SLDL 90.92±18.1, GOT 42.46±34.27, GPT 53.6±75.1 and leptin 2883.93±512.7 $P\leq0.001$), as well as in STRIG .69±.13 $P\leq0.05$ then normal boys. Inversely, it has been shown that (BURIA 26.7±5.3 $P\leq0.05$, SCREAT .69±.13 $P\leq0.01$ and SVLDL 20.15±6.3 $P\leq0.01$) were decreased significantly in obese boys Table 3.

Table 1: Comparison of parameters between genders in obese children.

Parameters	Obese boys N= 15 Mean±SD	Obese girls N= 15 Mean±SD	P value
BM1 (kg/m2)	36.5±9.1	35.3±4.7	NS
FBS (mg/dL)	84.2±6.9	85.7±6.2	NS
BUREA (mg/dL)	26.7±5.3	23.6±3.3	NS
SCREAT (mg/dL)	.69±.13	.66±.11	NS
SCHOLES (mg/dL)	157.2±18.03	164.8±20.3	NS
STRIG (mg/dL)	101.5±30.7	120.3±26.7	NS
SVLDL (mg/dL)	20.15±6.3	24.1±5.3	NS

Cont... **Table 1: Comparison of parameters between genders in obese children.**

SHDL (mg/dL)	44.2±5.70	44.1±4.4	NS
SLDL (mg/dL)	90.92±18.1	89.3±25.5	NS
SGOT (u/L)	42.46±34.27	33.1±13.6	0.05
SGPT (u/L)	53.6±75.1	29.9±14.1	0.05
SCa (mg/dL)	9.16±.56	9.1±.44	NS
SURICacid (mg/dL)	4.79±.77	4.3±.8	NS
IL-6(pg/mL)	8.5±8.3	8.4±6.9	NS
LEPTIN(pg/mL)	2883.93±512.7	2758.4±539.1	NS

BMI, body mass index; FBS, fasting blood sugar; BUREA, blood urea; SCREAT,(serum creatinine); SCHOLES, (serum cholesterol); STRIG (cserum trigleseriad); SVLDL, (serum very low-density lipoproteins); SLDL, (serum low-density lipoproteins); SHDL, (serum high-density lipoproteins); SGOT, (serum glutamic oxaloacetic transaminase); SGPT, (serum glutamic pyruvic transaminase); SCa, (serum calcium); SURIC acid, (serum uric acid); IL-6, (interleukin-6) and leptin, N= number, NS= not significant, SD= standard deviation

Table 2: Comparison parameters between obese and control girls

Parameters	Obese girls N= 15	Control girls N= 15	P value
	Mean±SD	Mean±SD	
BM1 (kg/m2)	35.3±4.7	18.2±4.6	0.01
FBS (mg/dL)	85.7±6.2	76.1±10.6	NS
BUREA (mg/dL)	23.6±3.3	30.6±6.3	NS
SCREAT (mg/dL)	.66±.11	.72±.6	NS
SCHOLES (mg/dL)	164.8±20.3	153.4±45.6	NS
STRIG (mg/dL)	120.3±26.7	89.65±16.8	0.01
SVLDL (mg/dL)	24.1±5.3	20..76±4.8	NS
SHDL (mg/dL)	44.1±4.4	43.8±8.9	NS
SLDL (mg/dL)	89.3±25.5	78.6±16.4	0.05
SGOT (u/L)	33.1±13.6	16.86±13.3	0.01
SGPT (u/L)	29.9±14.1	15.06±10.6	0.01
SCa (mg/dL)	9.1±.44	10.3±.3	NS
SURICacid (mg/dL)	4.3±.8	5.62±1.1	NS
IL-6(pg/mL)	8.4±6.9	8.76±6.5	NS
LEPTIN(pg/mL)	2758.4±539.1	1796.26±950.5	0.001

Table 3: Comparison parameters between obese and control boys.

Parameters	Obese boys N= 15 Mean±SD	Control boys N= 15 Mean±SD	P value
BM1 (kg/m2)	36.5±9.1	15.70±3.2	0.001
FBS (mg/dL)	84.2±6.9	77.33±12.2	NS
BUREA (mg/dL)	26.7±5.3	34.20±8.1	0.05
SCREAT (mg/dL)	.69±.13	2.76±7.5	0.01
SCHOLES (mg/dL)	157.2±18.03	154.8±23.9	NS
STRIG (mg/dL)	101.5±30.7	85.76±18.9	0.05
SVLDL (mg/dL)	20.15±6.3	29.7±7.9	0.01
SHDL (mg/dL)	44.2±5.70	42.7±13.66	NS
SLDL (mg/dL)	90.92±18.1	55.6±11.3	0.001
SGOT (u/L)	42.46±34.27	15.57±7.86	0.001
SGPT (u/L)	53.6±75.1	17.06±14.2	0.001
SCa (mg/dL)	9.16±.56	10.38±.45	NS
SURICacid (mg/dL)	4.79±.77	5.09±1.66	NS
IL-6(pg/mL)	8.5±8.3	7.99±6.59	0.01
LEPTIN(pg/mL)	2883.93±512.7	596.35±735.27	0.001

Correlations results show no significant association between IL-6 and leptin with biochemical parameters in girls. Whereas, in boys IL-6 showed significant positive correlation with BMI, while leptin revealed strong associated correlation was with IL-6 Table 4.

Table 4: Correlations between IL-6, Leptin and other biochemical parameters for both genders.

Parameters	Obese boys		Obese girls	
	IL-6(pg/mL)	Leptin(pg/mL)	IL-6 (pg/mL)	Leptin(pg /mL)
BM1 (kg/m2)	0.585*	0.477	-0.081-	0.029
FBS (mg/dL)	0.012	-0.378-	-0.174-	-0.053-
BUREA (mg/dL)	0.342-	-0.374-	-0.284-	-0.063-
SCREAT (mg/dL)	-0.270-	-0.239-	0.307	-0.062-
SCHOLES (mg/dL)	-0.072-	0.199	0.098	0.455
STRIG (mg/dL)	0.311	0.096	0.018	0.173
SVLDL (mg/dL)	0.328	0.094	0.018	0.173
SHDL (mg/dL)	-0.106-	0.111	0.224	0.276
SLDL (mg/dL)	-0.092-	0.157	0.057	0.231
SGOT (u/L)	0.020	-0.090-	0.270	0.391
SGPT (u/L)	-0.108-	-0.191-	0.114	0.461
SCa (mg/dL)	-0.048-	-0.117-	-0.223-	0.046
SURICacid (mg/dL)	0.229	0.131	-0.085-	0.091
IL-6(pg/mL)	1.000	0.729**	1.000	0.195
LEPTIN(pg/mL)	0.729**	1.000	0.195	1.000

All values are Pearson's correlation coefficients. BMI, (body mass index); FBS, (fasting blood sugar); BUREA, (blood urea); SCREAT, (serum creatinine); SCHOLES, (serum cholesterol); STRIG, (cserum trigleseriad); SVLDL, (serum very low-density lipoproteins); SHDL, (serum high-density lipoproteins); SLDL, (serum low-density lipoproteins); SGOT, (serum glutamic oxaloacetic transaminase); SGPT, (serum glutamic pyruvic transaminase); SCa, (serum calcium); SURICacid, (serum uric acid); IL-6, interleukin-6 and leptin, *$p \leq 0.05$; **$p \leq 0.01$.

Discussion

The current study revealed significant elevation for SGOT and SGPT in both genders, and higher for boys ($p \leq 0.001$) than in girls ($p \leq 0.01$). Similar results have been found in study with Korean children [10]. Increased levels of hepatic enzymes are the most important characters of fatty liver disease. In obesity, they are well-known where expansion progressively increases with BMI increasing [11]. In overweight and obese patient liver enzyme associated with metabolic risk factor [12] and BMI [13]. Whereas,[14] proved an abdominal height independent role, central adiposity measure being a predictor of increased levels of (GPT) as well (GOT), un-recognized fatty liver possible expression. Association of increasing enzymes of liver and excess echogenicity of liver with males and age of adolescent may explained by a pubertal androgen increase that affecting pathogenesis for obesity [15]. Androgen receptor signaling plays an important role in the development and advancement of several liver diseases including fatty liver disease [16]. According to the study conducted by [17] frequent eaten fast food and sugar sweetened beverages and fruits and playing computer games more than 2 hours a day increased the risk of elevated liver enzymes.

The current study has demonstrated that there is a very strong relationship between obesity boys and girls table (2,3) with abnormal lipid profile including hyper triglyceridaemia and a high LDL Lipid levels depend on many factors, e.g. age, gender, nutritional status, nutritional intake and environmental factors. These data are compatable with previous studies in Qatar obese schoolchildren (6-11years) that show hypercholesterolemia, hypertriglyceridemia and elevated LDL levels [18].

Two serum biomarkers, serum creatinine and blood urea are commonly used to discover kidney toxicity do not change quickly during the presentation of acute conditions [19]. In study conducted by [20] and [21] no significant differences in creatinine revealed between normal and obese children these differences with our results may be due to the difference in localities, sample size, cultural and/or socioeconomic causes.

Leptin is an important cell-signaling hormone vital hormone that involve appetite, food intake and weight control. It is play a critical role as a biomarker in obese children's. Central effects of leptin on specific tissues and metabolic pathways suggest that leptin operates both directly with the hypothalamus for energy balance regulation [4]. In both gender a significant increasing appeared in (Leptin) and IL-6 than control groups, this is consistent with the study that conferm an early role of leptin in childhood obesity and Lower leptin levels were independently correlated with higher physical fitness [22] another study on obese asthmatic children show the same singnificant increasing when compare with lean asthmatic children [23].

Serum concentrations of IL-6 was significantly correlated with BMI illustrated in Table 3. increased circulating levels of many inflammatory markers including C-reactive protein (CRP), IL-8, IL-6, macrophage migration inhibitory factor (MIF) and monocyte chemoattractant protein 1 (MCP-1) in obese individuals, and were correlated positively to BMI and waist perimeter [24]. In male highly significant increasing appeared in IL-6 than control groups because adipose tissue is known to be the main source of serum IL-6, at approximately 25% of total serum IL-6. Thus, higher IL-6 levels may be due to the increased adipose tissue [25].

Concentration of leptin is significantly correlated with IL-6 in boys illustrated in table (4) this may be due to that leptin linked to adiposity strongly, where levels are involved by size of adipocyte and mass of fat amounts. Via its core actions it is controlling intake of food and balance of energy indirectly. However, the IL-6 levels as high in obesity are not appetite repressing, which may attribute due to leptin resistance state [5]. Also, it has features as pro-inflammatory, stimulating cytokines secretion i.e. tumor necrosis factor alpha (TNF-α) and IL-

6 where all are influencing obesity patho-physiological process [26]. The differences in correlation between boys and girls may due to sex hormonal differences.

Recommendation: Further studies are needed in larger number samples to investigate if other immunomarkers or biomarkers could be used to elucidation obesity and overweight among children and adolescents in Iraq.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Study the Histopathogic Effects of Arsenic Trioxide on Some Parenchymatous Organs of Male Rats and Under Alphalipoic Acid

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Abstract

This study was designed to evaluate the histopathogical effects of arsenic trioxide (ATO) on the some organs of rats , for this reason group of rats (15) were I/P inoculated with 1.5 mg /Kg .B.wt . daily for 30 days other group(15 rat) treated with 100 mg/kg.B.wt of alphalipoic acid and ATO (1.5 mg/ kg.B.wt) for 30 .The results was revealed that the extensive pathological lesions in the liver , kidney , spleen and testes in all rats exposed to arscenci trioxide due to the pathotoxic effect of ATO and decreased lesions were in the group treated with the alpha lipoic acid .100 mg / kg .B.wt) for 30 days as ameliorative or protective effect against pathotoxicity of ATO .

***Key words** : histopathogic effects of arsenic trioxide , parenchymatous organs, male rats*

Introduction

Arsenic (AS) is ubiquitous element in the environment . weathering of rocks converts Arsenic sulfide to arsenic trioxide ATO which enter the arsenic cycle as dust or by dissolution in rain , rivers , or ground water [(1)] Arsenic is a very toxic metal and also an environmental and industrial pollutant which present in soil , water , air and food [(2)] This metal enter surface of water from the industrial and found in soil by leaching of sewage sludge through soil [(3)] .So the pollution can be affected by Arsenic through food consumption , drinking water and incidental ingestion of soil contaminated by arsenic [(4)] .Arsenic used in food preservative . herbicides , insecticides and rodenticides [(5)]

The aim of study:

In Iraq , there are little researches about influence of arsenic trioxide on body organs for this reason this study was designed to find the histopathological effect of arsenic trioxide (ATO) on body organs of rats and also to find the protective effect or ameliorate the pathotoxic effect of arsenic trioxide by alphalipoic acid .

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Material and Methods

Two groups of rats (15 each group) , body weight 200- 220 gm

First group : were I/P inoculated with Arsenic trioxide 1.5 mg/ Kg .B .wt .daily for 30 days [(6)]

Second group were also treated I/P with 1.5 mg / Kg .B.wt of arsenic trioxide for 30 days together with their I/P inoculation with alphalipoic acid 100 mg / Kg.B.wt daily for 30 days .

After 30 days all the lesions were taken from liver , kidney , spleen and testes , and kept in 10% neutral buffered formalin for fixation and processed routinely (7) for histopatholgy study under light microscope .

The Results

First group : treated with arsenic trioxide showed

The liver :

There is dilation of sinusoids , extensive degeneration of endothelial lining central vein with loss of hepatic cord organization .There is inflammatory cellular infiltration was abundant around the central vein with suppurative necrosis and vacuolation of hepatocytes .There is desquamation of bile duct epithelia together with extensive fibrosis of the portal regions (Fig-1)

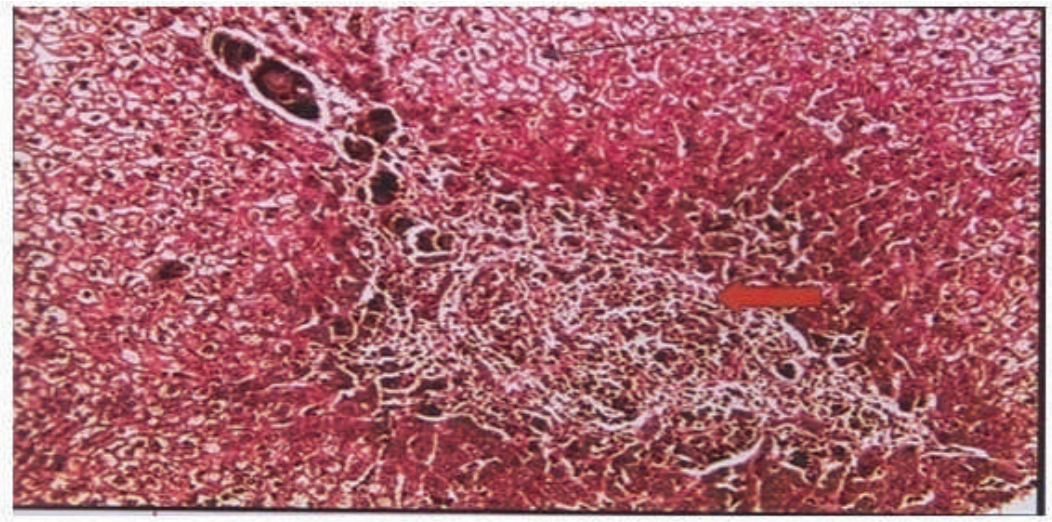


Figure (1) : liver section of rat .It received ATO (I/P 1.5 mg / kg BW) for 3 months . There is inflammatory cellular infiltration with abscess formation (red arrow) .Also there is extensive degeneration with hepatocellular necrosis (thin arrow) .10X H&E

Extravasations and congestion of all blood vessels in liver tissue with ballooning vacuolation of hepatocytes .some of hepatocytes showed extensive fatty change .

The kidney :

Showed sever degeneration and necrosis of epithelia lining proximal convoluted tubules together with disorganized and atrophied glomeruli and distorted Bowman's capsule . there is extensive congestion of all blood vessels in the renal tissue with extensive inflammatory cellular infiltration in renal tissue (Fig-2)

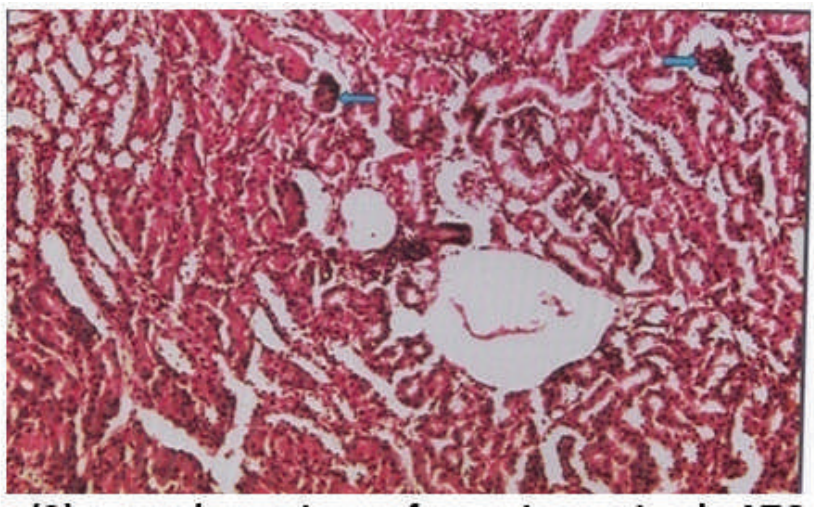


Figure (2) : renal section of rat .It received ATO (I/P 1.5mg /kg BW) for 3 months .There is severe necrosis of epithelial cells which lining of the renal convoluted tubules (green arrows) which appeared dilated with atrophied disorganized glomeruli with distorted Bowman's capsule (yellow arrows) .10X H&E .

Testes and epididymus :

Showed loss of cellular architecture there is vacuolation of epithelia lining seminiferous tubules , suppression of spermatogenesis and pronounced reduction and devoid sperm number in the lumen of seminiferous tubules .Desquamation of epithelia lining seminiferous tubules with presence of spermatid multinucleated giant cells indicating sever degeneration , similar pathological changes were seen in the epididymus (Fig-3) .

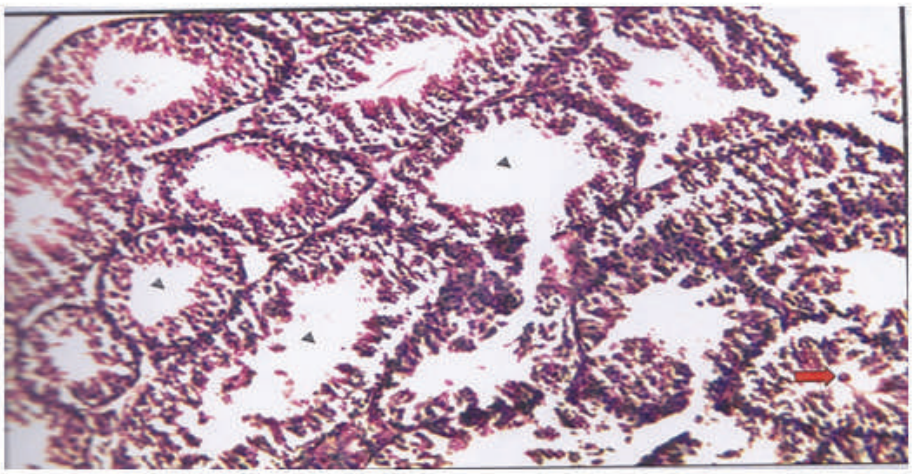


Figure (3) : Testicular section of rat.It received ATO I/P 1.5 mg/kg BW) for 3 months . Note vacuolation of spermatogonia and presence of megakaryocytes inside the seminiferous tubule .The tubules are totally devoid sperms (arrows heads) .10X H&E .

The spleen :

Showed sever depletion of white pulp with proliferation of reticuloendothelia cells lining the red pulp .Extensive necrosis and degeneration involving the whole lymphoid tissue in white pulp together with disappearance of arterioles in the white pulp (Fig-4) .

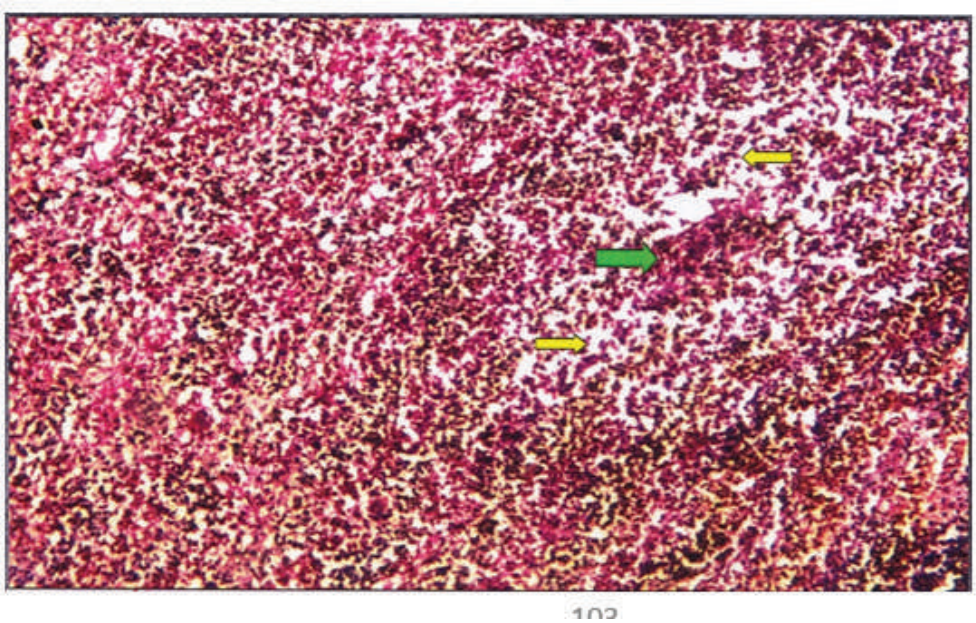


Figure (4) : Spleen of rat.It received ATO(I/P 1.5 mg/kg BW) for 3 months .Note severe depletion of white pulp with proliferation of red pulp (green arrows) . Extensive degeneration and necrosis in the splenocytes and lymphocytes of lymphoid tissue (yellow arrows) .10X H&E .

The second group treated with arsenic trioxide and alphalipoic acid :

The liver :

Showed the severity of lesions were mostly decreased , there is preserved cytoarchitecture of hepatic lobules , mild congestion of blood vessels and mild inflammatory cellular reaction , some hepatocytes showed more activation (Fig-5)

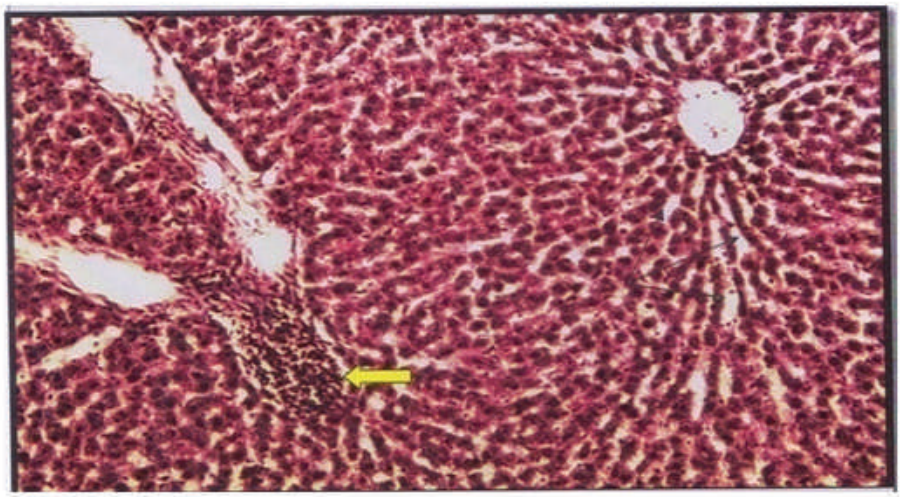


Figure (5) : liver section of rat. It received ATO (I/P 15mg /kgBW) and supplemented with (ALA) (I/P 100 mg /kg BW). There is presence of cytoarchitecture of hepatic lobules consisting of central veins and radially arranged hepatocyes . Few infiltration of inflammatory cells with (yellow arrows) mild dilation of sinusoids (thins arrows) .10X H&E .

The Kidney :

Showed also decreased in the severity of lesions comparable to arsenic trioxide treated group , it revealed normal organized glomeruli , with mild dilation of proximal convoluted tubules , there is tubular regeneration of their epithelial lining . There is no inflammatory cellular reaction and there is no hemorrhage and congestion in the renal tissue (Fig-6)

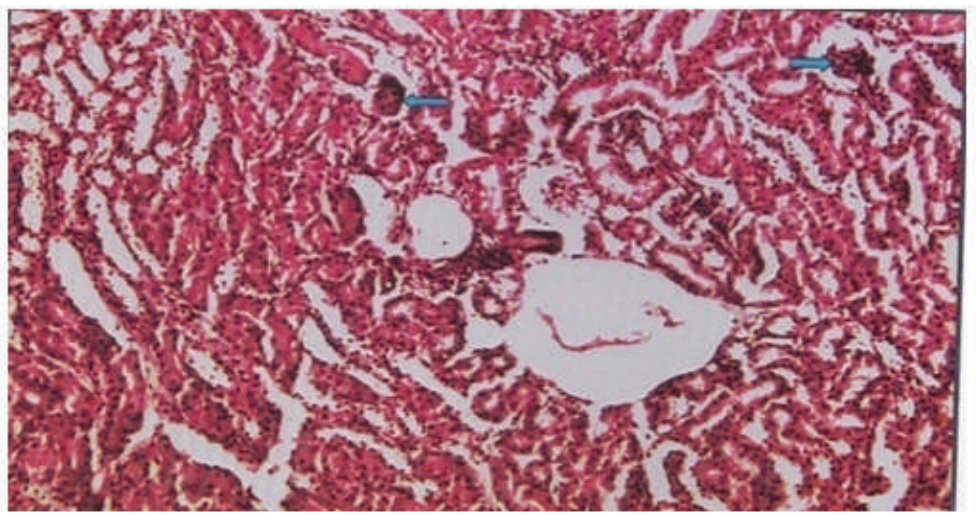


Figure (6) : renal section of rat . It received ATO (I/P 1.5 mg/kg BW) and treated with ALA (100mg/ kg B.W) for 3 months . Note mild dilation or renal convolued tubules , normal organized glomeruli (blue arrows) and tubular basophilia were seen .10X H&E

Discussion

The results of arsenic trioxide toxicity in organs of rat were varied including degeneration , necrosis , inflammatory cellular reaction .together with hemorrhage and congestion in the all organs ,these pathological changes were due to toxicity of ATO lead to liberation of reactive oxygen species and induce oxidative stress [8] which induce tissue damage and necrosis due to effects of superxide , Hydrogen peroxide , hydroxyl and nitric oxid both of these reactive species were released under the effect of ATO in the tissue and its effects were decreased or suppressed due to treatment with alphalipoicacid (ALA) which was evident in all the organs[9] whom considered that the opposite effect of alphalipoic acid against reactive oxygen species induced by effect of ATO in tissues , other workers reported that the ATO increased infiltration of inflammatory cells like neutrophils and enhance its migration under the effect of inflammatory mediators , protease [10] alphlipoic acid produce its effect as antinflammatory activity and reduced or inhibited inflammatory reaction which is more evident in this study and agreed with [11].The results in this study indicated that the ATO increased its effect in tissues by oxidative stress mechanism and supplementation with alpha lipoic acid exhibit an opposing action , so protect tissue against ATO toxicity.

Liver : is the main target site of ATO toxicity inducing hepatocyte damage , necrosis , vacuolation and fatty change , these finding were consistent with other studies [12] ; all these lesions were reduced or inhibited when animals treated with alphalipoic acid indicating the protective effect of alpha lipoic acid against ATO toxicity ,these results also agreed with [13]

The Kidney : also showed extensive pathological lesions characterized by damage , necrosis of renal tubular epithelia , hemorrhage, congestion and inflammatory cellular reaction, all these lesions were occurred as a result of ATO toxicity under effect of reactive oxygen species [14] and the supplementation of alpha lipoic acid ihibit the pathological lesions, these findings agreed with [15] who found that large dose of alpha lipoic acid protect rat against cisplatin induced renal damage .

The testes and epididymus : showed histopathological lesion like distortion of seminiferous tubules, suppression of spermatogenesis and vacuolation of germinal cells , these lesions were occurred as a result of ATO toxicity with liberation of oxidative stress and the reactive oxygen species cause damage [16] and inhibited or depressed spermatogenesis together with dilation of seminiferous tubules and epididymus resulted from direct effect of ATO on testicular tissue ,Also these changes may occurred after inoculation of ATO which effect on sex hormone like testosterone , lentinizing hormone and follicular stimulating hormone which important for spermatogenesis [17] who considered that the ATO as endocrine distruptor . on the contrary the supplementation with alpha lipoic acid ameliorate all these testicular changes , since alpha lipoic acid plays an important protective role against reproductive toxicity of ATO which was more evident in this study, a similar finding reported by Momeni et. al. 2012[18]

Other organs spleen : Is the main lymphoid tissue showed sever pathological lesions occurred as a result of ATO toxicity and under the effect of reactive oxygen species ,similar findings agreed with [19] whom reported that the ATO toxicity cause splenic tissue necrosis and depletion of lymphoid tissue in white pulp and these lesions were depressed or inhibited when use alphalipoic acid which cause white pulp hyperplasia and inhibited necrosis , so the ameliorative effect of alpha lipoic acid against Immunotocity of ATO on spleen tissue , and decreased the reactive oxygen species induced by ATO , so the spleen retain normal tissue architecture under the supplementation of alphalipoic acid which cause regulation of immune lymphoid tissue in spleen [20]

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Claudin is an Immunohistochemical Changes Associated with Experimental Infection of White Mice with 0111 – of non – 0157 Shiga Toxin Producing Escherichia Coli

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Abstract

Immunohistochemical stain for the tissue sections prepared from experimental infection of mice with 0111 – of non 0157 of shiga toxin producing Escherichia coli .The immunohistochemical changes revealed that the shiga toxin produced by 0111 –STEC effect on epithelial tight Junction ,The IHC stain was directed to detect the claudin (the structural protein of tight Junction) revealed absence of this protein in the paracellular sites with discohesion of the epithelial cells in the intestine and endothelial cells in the brain blood vessels which was responsible for induction of diarrhea and nervous signs

Key words *: infection ; health; Escherichia coli ; Immunohistochemical*

Introduction

The intestinal epithelial barrier is established by a single layer of epithelial cells and the space between these cells is sealed by Tight Junctions (TJS) and Adherence Junctions (AJs) which regulate permeability of the intestinal barrier .TJS are complex proteins structures comprised of transmembrane proteins .A major component of TJ strands is the integral proteins ,claudin .Claudin are transmembrane proteins and there are 24 members of claudin family [1, 2] Differential expression of properties of claudin are believed to determine tissue – specific variation in electrical resistance and paracellular ionic selectivity among epithelia .Alteration in TJ and AJ protein expression and localization were reported in human and animals in IBD [2]. In previous studies increased expression of claudin -2 and decreased expression of claudin 3,4,7 and 8 were demonstrated in the colonic mucosa of human IBD [2,3] . Other previous study .E- cadherin expression was significantly decreased in the villus epithelium in duodenal mucosa samples obtained from animals with IBDC [4] From the importance of shigatoxin effect on cellular tight Junction , This study aimed to detect the effect of shigatoxin of 0111 of non 0157 STEC on both epithelial and endothelial Junction in intestine and brain by immunohistochemistry .

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Materials and Methods

Immunohistochemistry :

The principle of immunohistochemical techniques use of an antigen-antibody reaction coupled with a reaction that produces a chromogen (colored product) or attachment of a fluorescein dye to identify specific components in tissues .Most methods are " indirect " because two antibodies are utilized [5]

A- Primary antibody : an antibody is raised to a specific tissue component that acts as an antigen . The primary antibody is typically from a non – human mammalian source such as mouse or goat or rabbit .

B- Secondary antibody : an antibody is raised to the Fc portion (the constant part) of the primary antibody .It is raised via another mammalian species which will consider the primary antibody as a foreign antigen and make antibody to it .It is this secondary antibody that has a marker attached to it that will provide visibility [5]

Dilution of the primary antibody and selection of the suitable concentration :

According to manufacturer instruction stock solution of antibody (for detection of Claudin) which contain $\theta\ \mu l$ was thawed and divided into aliquots $5\mu l$ each to avoid repeated freezing – thawing of the antibody then one of the aliguots was diluted to the following dilutions (1:10 ,1:25, 1:50,1:50,1:100) , two slides was stained by each dilution and then the dilution 1:100 was choose as a working solution for staining of the slides , because it gave the better results .

Preparation of the tissue for IHC staining :

$5\mu l$ Tissue sectioning and immunohistochemistry staining were performed in the pathology laboratory / college of medicine / Missan university .Paraffin embedded tissue were cut into thickness using microtome .The sections were applied to Leica positively charged slides .

Sections were prepared for IHC staining by follow the manufacturer instructions which include the following steps .

Deparaffinization and rehydration

1. Slides were immersed in Xylene I and Xylene II successively for 10 minutes respectively .

2. Slides were immersed in anhydrous ethanol II,95% ethanol , 80% ethanol ,and 70% ethanol successively for 5 minutes respectively , and then slides were washed for twice , each time for 2 minutes by using deionized water

Antigen retrieval (optional)

3. Slides were put into the repair box , and then 0.01 M citric acid buffer (PH6.0) was added to make the tissue immersed .

4. The antigen was repaired with medium power microwave for 10 minutes (Start timing when the liquid boils) , care taken to avoid tissue drying during the process .

5. Then repair box was taken out from microwave oven , and naturally cooled down to room temperature , then slides was took and washed 3 times with PBS(pH7.4) each time for 3 times (care taken to avoid flushing of the tissue directly during washing process to avoid breaking of the tissue) .

Inactivation

6. (3%) H2O2 that was prepared well in advance was added to the slides drop by drop to block endogenous peroxidase , and incubated at the room temperature for 15 minutes (ionized water was used to dilute 30% H2O2) , then PBS (PH 7.4) was used to wash the slides for 3 times , each time for 3 minutes .

Antibody incubation

7. PBS was bloted up with absorbent paper ,and 5% normal serum (Sharing the same or similar species with secondary antibodies) was added drop by drop on the sections , then blocked at 37° C for 30 minutes

8. Liquid around the tissue on the slides was wipe dried with absorbent paper , and circle was drew oil pen around the tissue , and then the diluted primary antibodies was added drop by drop After adding primary antibodies , the slides were put into wet box to be incubated at 4° C overnight .

9. Then slide were washed with PBS for 3 times , each time for 2 minutes , slides were dried and then HRP- conjugated secondary antibodies were added , finally slides were incubated 37° C for 30 minutes .

Signal detection

10. Sections were washed with PBS for 4 times , each time for 3 minutes , and wipe dried with absorbent paper , then DAB substrate reagent that was prepared freshly was added drop by drop to each section ,and observed under a microscope .The positive signal appears brown - yellow or brown in color .The time should be well controlled avoid the color appears too deep .Sections were washed with tap water to terminate the reaction

11. Hamatoxylin coutrstaining slides were immersed in Harris hematoxylin solution for about 30 seconds to 1 minute , and then slides transferred into ethanol solution with 1% HCI after washing with water .

Dehydration and mounting

12. Firstly slides were immersed in water and washed , then slides were put into the following reagents in order to dehydrate and permeate .70% ethanol ,80% ethanol , 90% ethanol ,95% ethanol , anhydrous ethanol I, anhydrous ethanol II,xylene I and Xylene II, for 2 minutes each , finally sections were left to dry.

13. Then slides were covered with the cover glass .

The scoring system of the sections in the present study was done according to the [6]

Statistical Analysis

Statistical analysis was performed to estimate the statistical differences among the groups of the study .T test was used for this purpose by ready to use statistical design .IBM statistical package for social science (IBM SPSS Statistics version 23)

The Results

Immunohistochemistry study for Claudin of intestinal epithelial and cerebral vascular endothelial tight junctions :

The results of immunohistochemistry of claudin of tight junction for both intestinal epithelial and cerebral vascular endiothelial cells was revealed .

In the Ist inoculation there was no alteration in the Claudin expression of intestinal epithelial cells of both small and large intestine Fig-1 and Fig-2 Immunohistochemical stain for the Claudine reveal the descohesion between epithelial cells was started at 3rd day post inoculation in which there were destruction of interpithelial Claudin in both small and large intestine Fig-3 the sloughing of the epithelium along small and large intestinal parts(Fig-4) at 14th and 21st day post inoculation were also revealed by DAB stain for the Claudin in which there were marked colonic epithelail cells discohesion ,sloughing with superficial inflammatory infiltrate forming pseudomembrane indicating pseudomembranous colitis .Immunohistochemical study of the Claudine in the brain vessels revealed that there were complete intact cerebral vascular endothelial cells in the control group, while there were sites of absence of Claudine staining in the cerebral vessels with breakage of the endothelail cells Fig (Fig-5) . Table (1)

Table (1) Score of Claudin down regulation

Period	Duodenum	Colon	Brain
1st day	-	-	-
3rd day	+	+	-
7th day	+++	++	+
14th day	++++	++++	+
21st day	++++	++++	++

❖

(-) No change (+) refer to Claudin down regulation where (+) in 25 cells , (++) in 50 cells , (+++) in 75 cells , (++++) in 100 cells / field

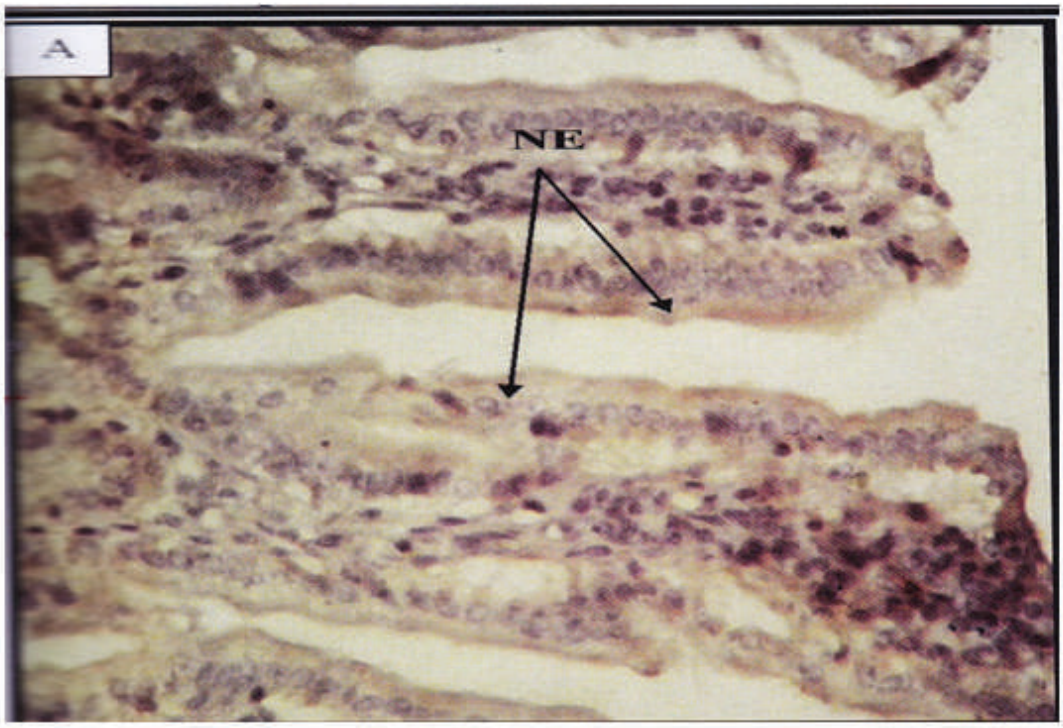
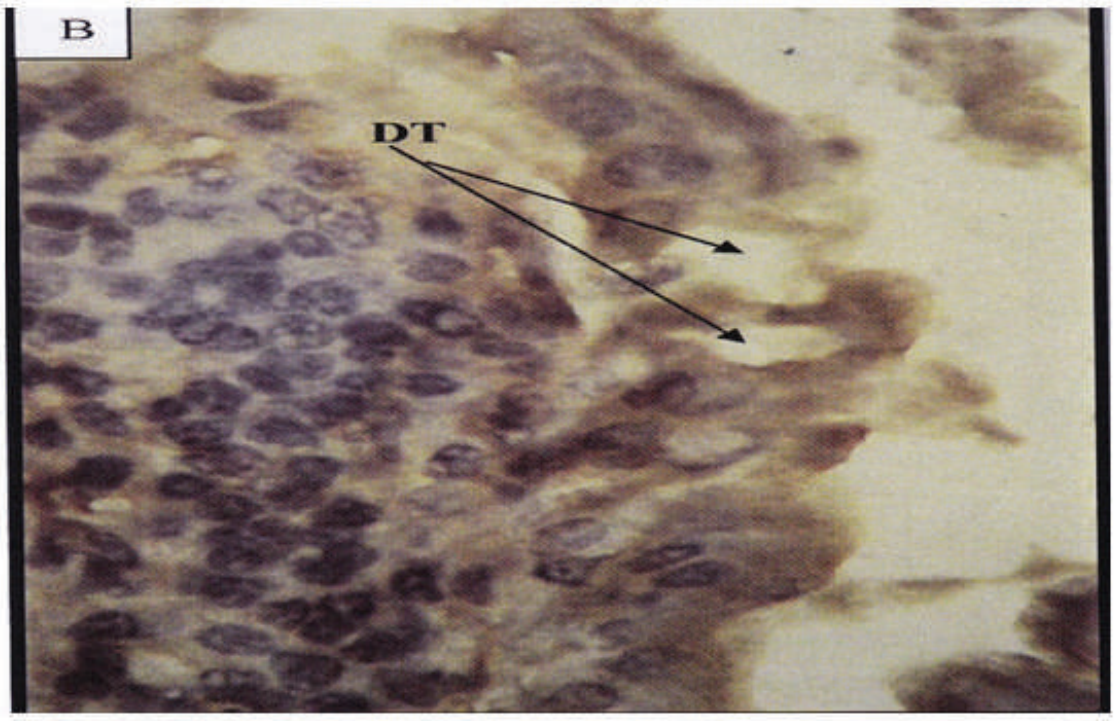
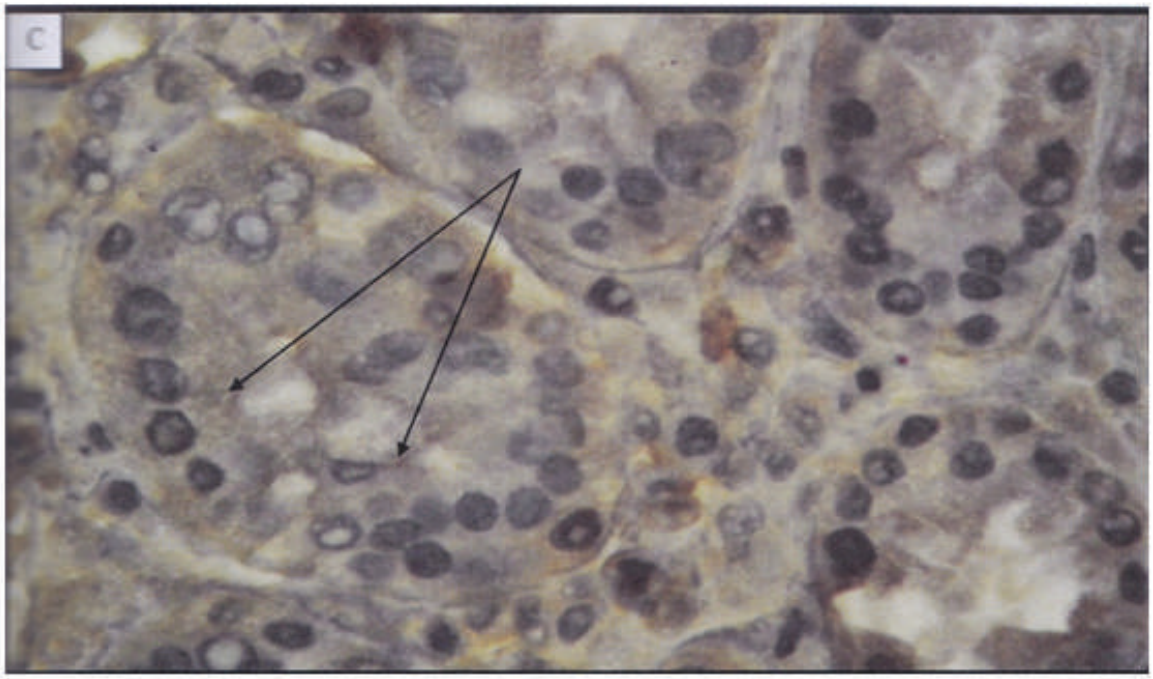


Figure (1) duodenum of the infected animals at 1st day post infection showed Normal interepithelial tight junction (NE) indicated by normal Claudin (NC) DAB stain (dark brown intercellular deposits) 500X



Figure(2) : duodenum of the infected animals at 3rd day post infection showed superficial sloughing (S) of the epithelial cells due to destruction of inter-epithelial tight junction (DT) from both lateral sides of the cells (L) and the basal site (B) DAB stain 1250 X .



F

Figure(3) section of colon at 1st day post infection shows intact epithelial cells in both superficial mucosa and colonic glands revealed by intact epithelial tight junction (T) DAB 1250X

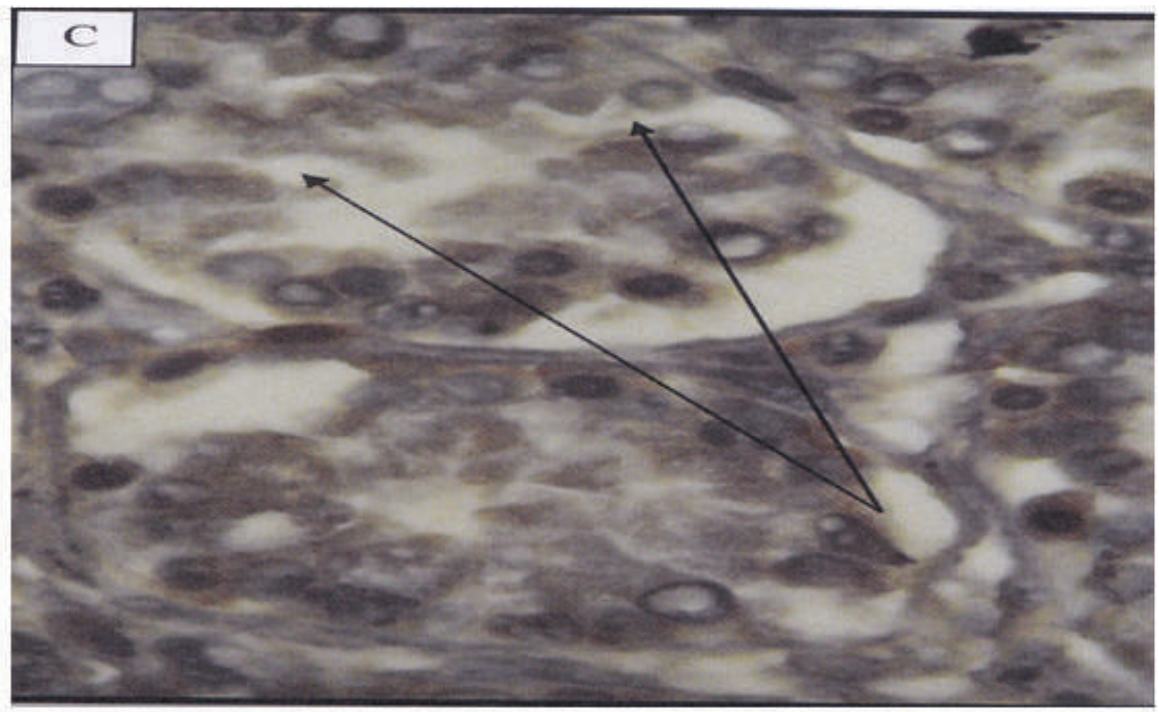


Figure (4) A section of colon at 14th day post infection shows that the down regulation of epithelial tight junction was both in the superficial epithelium and the colonic glands .DAB stain 1250X

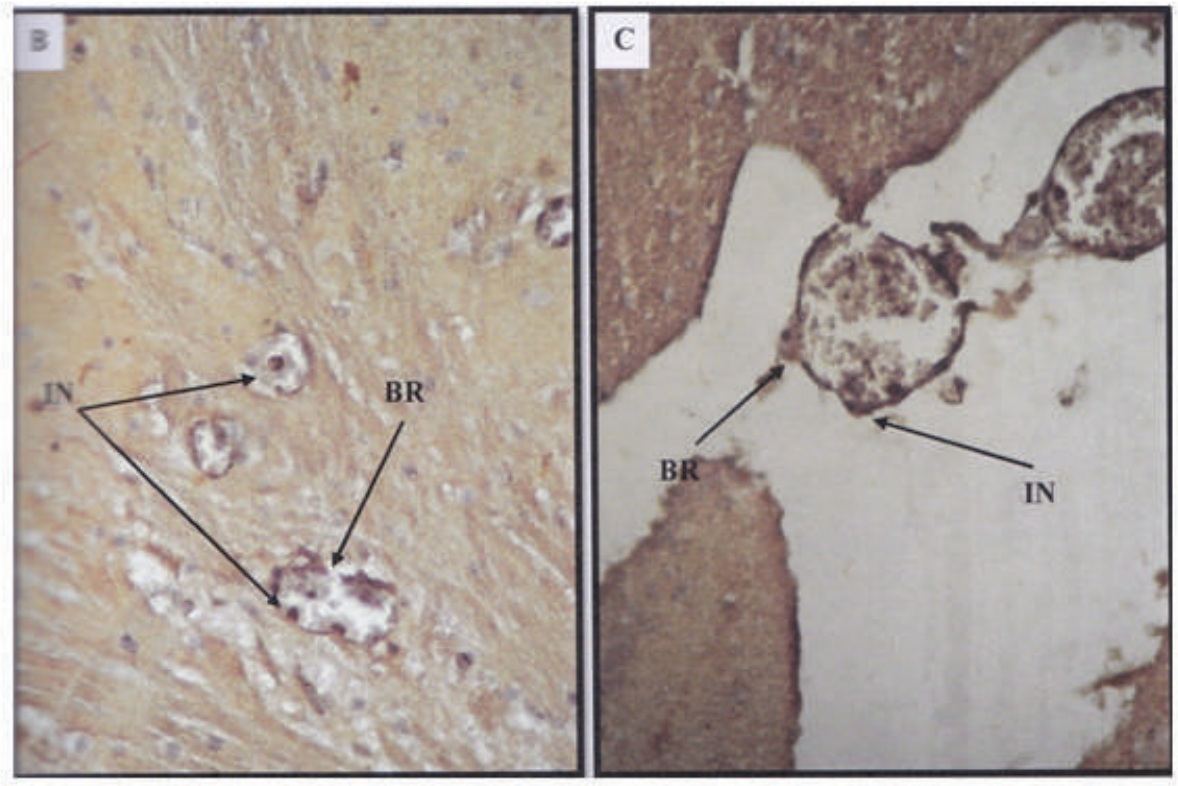


Figure (5) cerebrovascular endothelial tight junction which reveal (A) Normal endothelial cells (EN) of cerebral capillaries,sites of breakage between endothelial cells (BR) and extravasation of inflammatory cells (IN) to the brain parenchyma (B) &(C) DAB stain for Claudin of showed the 500X .

Discussion

The results of the present , study revealed that sloughing and ulceration of epithelia lining intestinal epithelial in multiple sites along small and large intestine occur from destraction of intestinal epithelial junction proteins particularly claudin , similar in previous studies of [7, 8] reported that the immune – staining of the small intestinal and clonic Claudine revealed that there were no alteration in claudin of intestinal tight junction in mice experimentally infected with STEC in the ist day post infection but the main alteration and redistrbution of claudin start in 3rd post infection and continue diminished in the intercellular area but its expersion was increased intracellularly experiment .

Results of the present study was also in consistent with [9] who reported that the immune fluorescent staining of the T48 cell culture for the tight junction proteins revealed reorganization of the claudin was observed as a complete fragmentation of the cell boundaries and focus formation also they reported that claudin -1 was lost from cell – cell contact sites , this change could be observed as the obsence of the brightly stained cell boundaries , indicating loss and / or redistrbution of claudin -1 [9,10] reported that the TJ protein alteration caused by STb may be due to actin rearrangement as a loss of organization in the perijunctional F-actin ring has been identified to be acritical event in the mechanism controlling transepithelial electrical transport (TER) and paracellular permcabillity [10-12] decreasing (TER) and increase permeability will be the cause of diarrhea and in the some time for systemic distribution of bacterial toxin with blood circulation reaching other organs in the body

Results of immunohistochemistry in the present study revealed that separation of endothelial cells of the cerebral capillaries due to down regulation of the claudin in the cerebral endothelial tight junction ,these results in line with [13] who reported that animal models treated with stx -1 and Stx -2, [14] also develop neurological

symptoms and exhibit similar neuropathology .Stx -1 , Stx-2 binding sites was immunohistochemically demonstrated in small vessels and / or the subsets of some cells in the lesions , but not in the neurous [15] .A similar findings reported by [16] whom they found neurological manifestation have been occurred in rabbits experimentally infected with stx-2 in which the signs resulted from necrotic infarction , acute neuronal damage aggravated by sever endothelial injury and microvascular thrombosis .

Conclusion

Shiga toxin cause marked down regulation of the claudin and disturb the structure and function of the tight junction in both intestinal cells and cerebral vascular endothelial cells

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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The Impact of Gold and Silver Nanoparticles on The DNA Yield of *Mycobacterium Tuberculosis* MTB

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Abstract

The objective of this research study whether nanoparticles of gold (Au NP) and nanoparticles silver (Ag NP) have an effect on the yield of DNA from Mycobacterium tuberculosis (MTB) in this experiment when it was extracted DNA from MTB using gold and silver nanoparticles at different concentrations (l0µl, 50µl and 100µl) then showed a significant impact on the yield of DNA, especially gold nanoparticles compared with DNA extracted without any of the nanoparticles.by Using a graph obtained from the REALTIME qPCR, which became very clear when the control sample (MTB without any nanoparticles) showed a high value for the Ct (cycle threshold), which meant that low quantities of DNA. While samples containing nanoparticles were low in Ct which meant a high yield of DNA was shown. Ct values also decreased when the concentration of nanoparticles increased, eventually we conclude from the above experiment that nanoparticles showed high yield of MTB DNA. These results are likely due the interaction of the nanoparticles utilized with the cell wall and cell membrane of bacterium, which has facilitated the yield of DNA. Further refinement can be to the above said method and the nanoparticles could possibly use as an effective component of Nucleic acid extraction kits for molecular diagnosis and research so that a superior yield is obtaine.

Keywords*: gold nanoparticles; silver nanoparticles; MDA.*

Introduction

Nanotechnology is expectant to open new methods to stop disease using atomic scale tailoring of materials, between the most auspicious nanomaterials with features of antimecrobial are nanoparticles, which show evelated the activity of chemical because of their big surface to size ratio and crystallographic surface body [(1).] the examination of bactericidal nanomaterials is especially timely considering the recent elevated of novel resistant races of bacteria to the stronger antibiotics [(2),] This has propelled look into in the notable action of silver and gold particles and their mixes, including silver and gold nanoparticles. The measure of nanoscale Gold and Silver is expanding rapidly in purchaser items the capacity to combine these particles for an immense scope improves [(3).] Gold and Silver nanoparticles (NPs) are poisonous to microscopic organisms, and are starting at now used in everything from restorative devices to display socks and garments washers to hinder microbial development. Silver is a particularly poisonous overwhelming metal as it meddles with the electron transport chain and ties to DNA [(4).] Certain microscopic organisms has appeared to amass or accelerate silver into silver metal or other insoluble structure. Bactericidal lead of nanoparticles is ascribed to the nearness of electronic effects that are achieved because of changes in neighborhood electronic structures of the surfaces as a result of smaller sizes [(5).]Ionic silver determinedly associates with thiol gatherings of indispensable compounds and inactivates them, It has been prescribed that DNA loses its replication capacity once the bacterium are treated with silver ions [(6).] Two dimensional electrophoresis and proteins ID investigation of antibacterial activity of

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silver nanoparticles have unveiled gathering of envelope proteins antecedents. Silver nanoparticles destabilize plasma layer potential and exhaustion of levels of intracellular adenosine triphosphate (ATP) by focusing on bacterial film bringing about bacterial cell death [7].

Mycobacterium tuberculosis: Mycobacteria are Gram-resistant (waxy cell wall), non-motile, pleomorphic poles, identified with the Actinomyces. Most Mycobacteria found in environments, for example, water or soil. By and by, a couple are intracellular pathogens of creatures and people. Mycobacterium tuberculosis, alongside M. bovis, M. africanum, and M. microti all explanation the contamination known as tuberculosis (TB) and are people from the tuberculosis species complex [6].Every individual from the TB complex is pathogenic, yet M.tuberculosis is pathogenic for people, anyway M.tuberculosis is pathogenic for individuals while M. bovis is commonly pathogenic for animals. There are known occurrences of this bacterium being found in the mummies of antiquated Egypt [7].

There are four imperative sorts of mycobacterium: Mycobacterium tuberculosis, which is the most unavoidable sort, mycobacterium bovis, mycobacterium africanum, and mycobacterium microti. This particular sort of minuscule living beings is interesting a direct result of the thicker degree of lipids on the cell divider [8]. Given that, the Mycobacterium tuberculosis gram recolor is insufficient, the most ideal approach to know whether a patient has mycobacteria in his blood is to take a gander at it under a magnifying lens. Under [9]. Mycobacterium tuberculosis disease transmitted through the sputum, which is also the primary spot that you can obviously perceive the contamination [10]. Essentially having the microscopic organisms in your blood isn't sufficient to turn out to be sick be that as it may. Regularly, the microscopic organisms will be unmistakably conspicuous in the blood a long time before the injured individual shows any side effects of the dynamic tuberculosis. This inactive tuberculosis contamination, or LTBI, in like manner can't be transmitted to some other individual as the microscopic organisms are to a great extent kept up in granulomas which shield them from influencing the body [11].

Materials and methods

Materials

Various concentrations of Au and Ag nanoparticles such as l0µl, 50µl and 100µl were used in the DNA extraction process of MTB culture cells. The extraction procedure was carried out as per the protocol explained and following materials were used:

- MTB culture cells(The MTB culture was obtained from the patient's sputum, suffering from Tuberculosis).
- Nanoparticals: gold nanoparticle (Au NP) and Silver nanoparticle (Ag NP), in different concentrations (l0µl, 50µl and 100µl) of each one respectively,

Table1: Showing the product data of (Au ,Ag) Nanoparticles

Nanoparticals Formula	Average Particle Size nm	Appearance Form	CAS Number	Purchased From
Au NP	50 nm	Suspension	753645	Sigma-Aldrich Us
Ag NP	40 nm	Liquid Yellow	730807	Sigma-Aldrich Us

- DNA extraction solution
- Seegene Anyplex (MTB/NTM Real Time PCR kit).
- Real Time PCR (Cepheid Smart Cycler).

Methods

DNA quantification by real-time PCR:

In molecular biology, real-time polymerase chain reaction, likewise called quantitative continuous polymerase chain response (qPCR) or dynamic polymerase chain response is a lab procedure dependent on the PCR, which is use to enhance and at the same time measure a focused on DNA particle. For at least one explicit arrangements in a DNA test, Real Time-PCR empowers both discovery and evaluation [11]. The methodology follows the general guideline of polymerase chain response; its key component is that the enhanced DNA distinguished as the response advances progressively [12]. Two normal strategies for identification of items continuously PCR are: (l) vague fluorescent colors that intercalate with any twofold stranded DNA, and (2) grouping explicit DNA tests comprising of oligonucleotides that are named with a fluorescent journalist which grants location simply after hybridization of the test with its correlative DNA target (13).

Quantification:

Real-time PCR can used to evaluate nucleic acids by two normal strategies: relative measurement and outright evaluation. Relative measurement dependent on inward reference qualities to decide crease contrasts in articulation of the objective quality. Outright evaluation gives the specific number of target DNA atoms by correlation with DNA norms. A usually utilized strategy for DNA evaluation by continuous PCR depends on plotting fluorescence against the quantity of cycles on a logarithmic scale. An edge for location of DNA-based fluorescence is set somewhat above foundation. The quantity of cycles at which the fluorescence surpasses the edge called the cycle edge, Ct. During the exponential enhancement stage, the grouping of the DNA target copies each cycle. Be that as it may, the productivity of enhancement is regularly factor among groundworks and templates [14].

The cycle edge strategy makes a few presumptions of response component and has a dependence on information from low sign to-commotion areas of the intensification profile that can present significant difference during the information examination. To evaluate quality articulation, Ct of RNA/DNA separates the Ct for a RNA or DNA from the quality of enthusiasm from a housekeeping quality in a similar example to standardize for variety in the sum and nature of RNA between various examples. This standardization methodology is ordinarily called the ΔΔCt-strategy and grants examination of articulation of a quality of enthusiasm among various samples [15]. Component based PCR measurement techniques as proposed, and have the bit of leeway that they don't require a standard bend for evaluation. An expansion of this methodology incorporates a precise model of the whole PCR response profile, which takes into consideration the utilization of high sign to-commotion information and the capacity to approve information quality preceding analysis [16].

Procedure:

The reaction mix was prepared according to the table2 below, 15µl of these mix reagents was added into each sterile vial. 5 µl of diluted DNA template (*Mycobacterium tuberculosis* DNA) was added into these vials. Water was added up to 25µl to all the vials. The PCR amplified product was analyzed by the obtaining graphs (Ct values) as it shown in the result.

Table2 showing the component and volume of the reaction mix

Component	Volume (µl)
2 x Precision TM Master Mix	10 µl
TB Primer/Probe mix (BROWN)	1 µl
RNAse/DNAse free water (WHITE)	4 µl
Final Volume	15 µl

Results

Table3 showing the gold nanoparticle (Au NP) and Silver nanoparticle (Ag NP), in different concentrations (l0µl, 50µl and 100µl) of each one respectively with control sample and Ct value for all sample

Nanoparticle	Conc.(µl)	Ct Values
Au	10	17.10
Au	50	17.08
Au	100	16.73
Ag	10	17.66
Ag	50	17.54
Ag	100	17.12
Normal Control	Nil	21.77

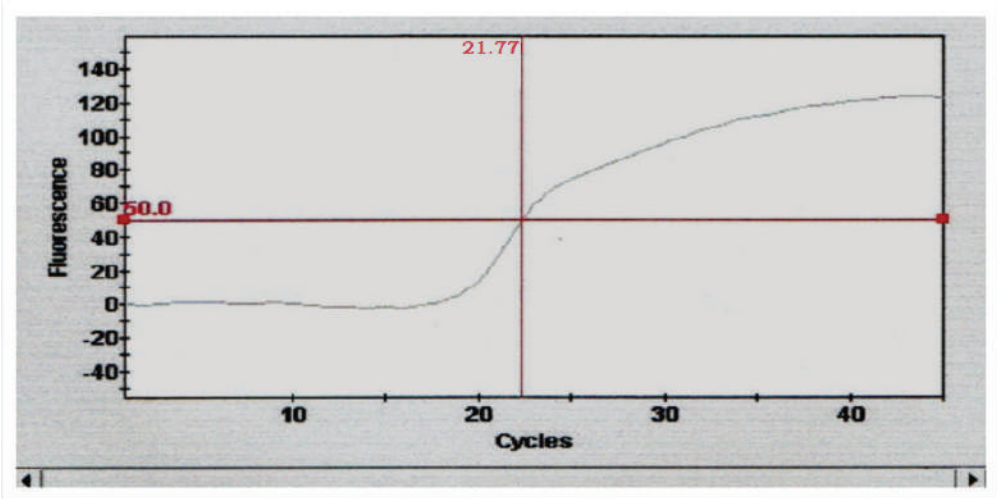


Figure1: The above graph obtained from real time PCR shows high Ct value (21.77) for control sample i.e. culture sample incubated without any nanoparticle. High Ct value corresponds to less amount of DNA.

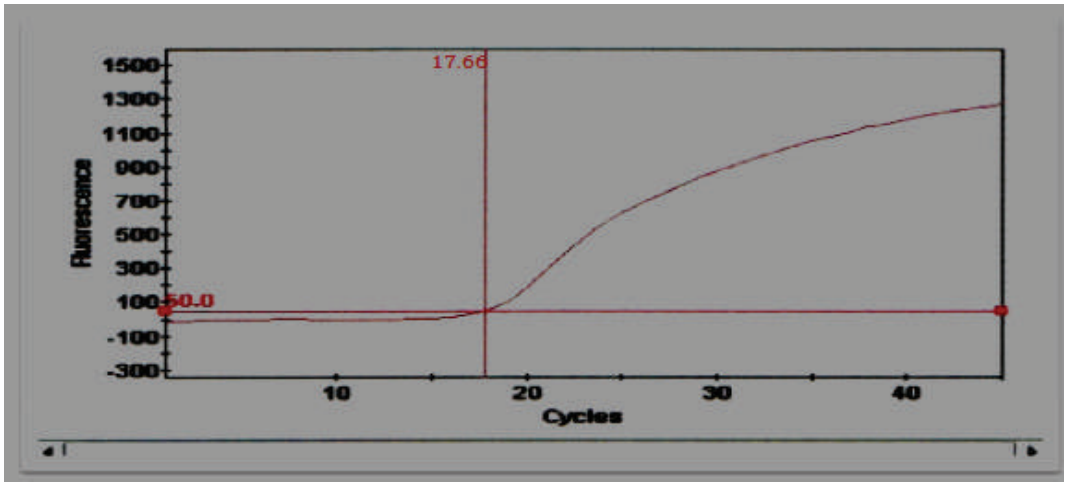


Figure 2: The above graph obtained from real time PCR shows Ct value (17.66) for culture sample incubated with 10 µl of silver nanoparticle. Low Ct value corresponds to more amount of DNA compared to control.

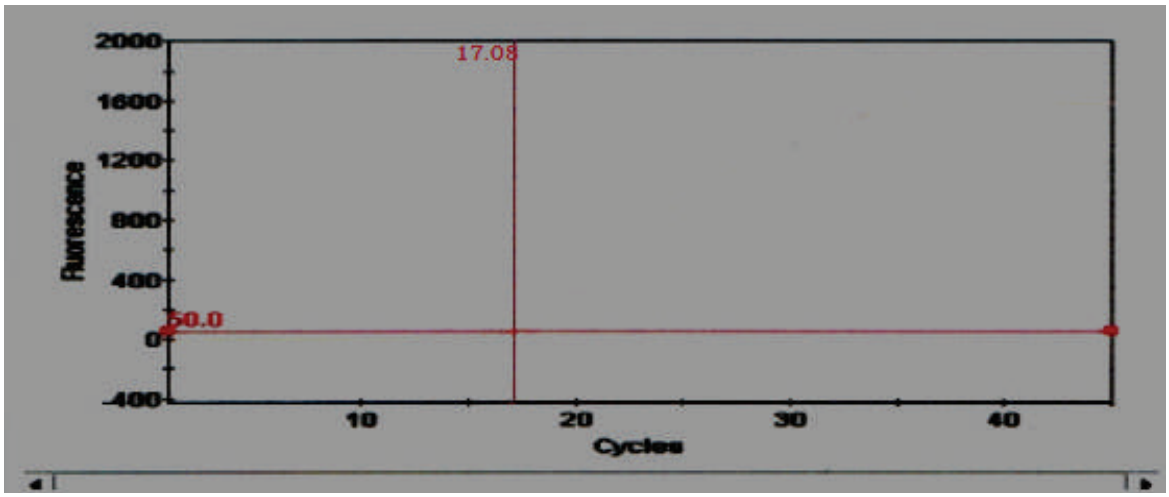


Figure 3: The above graph obtained from real time PCR shows Ct value (17.08) for culture sample incubated with 50 µl of gold nanoparticle. Low Ct value corresponds to more amount of DNA compared to 10 µl concentration of gold nanoparticle.

Discussion

It is notable that nanoparticles of inorganic metals have solid antimicrobial impacts and numerous specialists are keen on utilizing these inorganic nanoparticles as antimicrobial operators The system of the inhibitory impacts of nanoparticles on microorganisms is in part known. A few examinations have revealed that the positive charge on the nanoparticle particle is vital for its antimicrobial action through the electrostatic fascination between negative charged cell film of microorganism and positive charged nanoparticles. Conversely, Sondi and Salopek-Sondi detailed that the antimicrobial movement of silver nanoparticleson Gram-negative microscopic organisms was reliant on the convergence of Ag nanoparticle, and was firmly connected with the development of "pits" in the cell mass of bacteria [18]. The antimicrobial impact of silver is subject to shallow contact, in that silver can repress enzymatic frameworks of the respiratory chain and change DNA blend. Thus, the mechanism of inhibition is not clearly understood. Since the observed Ct value in the samples where nanoparticles were used is low compared to normal values, there could be a possible effect of nanoparticle in the yield of DNA in MTB. This possible could be because of the interaction of the nanoparticles used with the cell wall and cell membrane of bacterium which has facilitated the yield of DNA. Further refinement can be done to the above said method and the nanoparticles could possibly be used as an effective component of Nucleic acid extraction kits for molecular diagnosis and research so that a better yield is obtained.

Conclusion

In this experiment, when Au and Ag NanoParticles showed considerable effect on the DNA yield of *Mycobacterium tuberculosis* (MTB), especially gold nanoparticles. When DNA of MTB was extracted, using nanoparticles high yield of DNA was obtained when compared to the DNA extracted without any of nanoparticles. With the graph obtained from real time PCR it became very clear as the control (MTB without any nanoparticle) showed high Ct value i.e. 21.77 which means very low amount of DNA was obtained. Whereas for samples containing nanoparticle Ct values were low showing high yield of DNA. Further, the Ct values decreased when concentration of nanoparticles increased. When compared between Ag and Au nanoparticles, Au nanoparticles showed better result with low Ct value compared to Ag nanoparticle. In this experiment, gold nanoparticle was most effective at 100µl concentration with least Ct value i.e. 16.73. At last to conclude from above experimen it's very clear that nanoparticles are showed high yield of MTB DNA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of

both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Molecular Investigation of *fliC* and *bla$_{TEM1}$* Gene among Clinical Isolates of *Salmonella Typhi*

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Abstract

This study aimed to isolate and diagnose *Salmonella typhi* by *flic* gene and investigated the presence of *bla$_{TEM1}$* gene by using PCR technique and electrophoresis systems in Najaf Province includes 40 clinical specimens blood for both sexes with an age ranged between)5-60) years old .The study result by using a *flic* gene was detected in 30 of the 40 isolates of *S. typhi* isolated from typhoid patients , which was conducted in the diagnosis of bacteria concluded that the technique PCR are the most sensitive in the detection of *S. typhi* compare with other way, bla$_{TEM-1}$ was detected in 16 of the 40 isolates from *S.typhi* . The conseder the bla $_{TEM}$ gene encoding TEM-1 b-lactamase is think to give resistance only to penicillins and early cephalosporins; thus , the spectrum resistance of TEM-1 descendants may extend to second, third and fourth-generation cephalosporins.

Keyword: *Clinical cases; PCR; health diagnoses; S.typhi, typhoid ,bla $_{tem1}$.*

Introduction

Typhoid fever is an acute, potentially fatal systemic disease caused via *Salmonella enterica serovar typhi* , *paretyphi,* pathogens only precise to people. *S.typhi* is a genus of rod formed gram-negative enterobactereaceae that cause fever typhoid [(1)] . This pathogen has develop marked mechanisms for persistence in its host that assisted to include its transmission and survival. *S.typhi* can be transmitted via ingestion of water or food contaminated via urinary carriers excreting *S. typhi* [(2)]. PCR have been arise as a salutary process for the diagnosis of several diseases infectious lately. value diagnostic is important precisely wherever the a long period cultivation or little load bacterial . PCR the test consistent in the diegnosis & the administration of the illness typhoid [(3)].

Methods and Materials

The study was done at Laboratories of Molecular and Bacteriology in Department of the Biology , Faculty of Sciences, University of Kufa , Iraq.

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Specimens collection and bacterial identification

A total of 40 samples were collected from blood with clinical suspicion of typhoid fever in Al- Najaf provenance, was involved the study fundamental, of contenuous rise-grade fever with temperature medium - 38°C . The duration median of disease at consultetion at 6 days (about 6 to18 days) [(4)]..

Blood sample was collected from patients , four ml of blood venous fresh samples was collacted of doubtful typhoid patients via syringes sterile. The blood was delivered in to screw cupped having 30 ml of brain heart infusion broth and placed in bact/alert 3D apparatus for a week .If positive sample , each sample was protected utilizing the process direct of inoculation on culture of discerning means namely MacConekey, Blood ,XLD and S.S agar thereafter, inoculated at 37°C for 18 to 24 hr [(5)].

DNA Extraction

Genomic DNA was extracted via utilizing the extraction commercial method(Genomic DNA promega Kit).

Molecular Identification

The PCR assay was performed to detect the *bla-tem1* gene and *flic* gene for identification of *Styphi* as appear in table2 This primers were designed via Alpha DNA company, Canad as in table (1) . the produces amplified ware curtained utilizing 1% agarose gel electrophoresis to assessment the size produces for PCR . The gel was tainted with 10mg/mL of 4 µL bromide ethidium (Sigma, USA) and it run at 80v for 1.5h. A single band observe at the position desired on ultraviolet light transellumintor (Cleavar, UK); bands were photogrephed utilizing documentation gel method (Cleaver, UK). A 100bp and 50bp ladder (Bionaer, Korea) was utilized to quantity the Mwut. of amplified produces [6].

Table (1): Primers utilized in the study

type Primer	Primer target	Primer sequence	Amplicon size	Reference
Flic-d	Flic-d	F- ACTCAGGCTTCCCGTAACGC R-GGCTAGTATTGTCCTTATCGG	763	(6)
blaTEM-1	blt	F-CCCCTATTTGTTTATTTTTC R-GACAGTTACCAATGCTTA	962	(6)

Table (2): PCR program of *flic-d* and bla TEM-1 primers that apply in the thermocycler

Gene	Primary denaturation	Number of cycles	Deneturation	Annaeling	Extansion	Final extansion
Flic-d	95 Cº for 5minute	35	95 Cº for 30sec.	55 Cº for 30sec.	72 Cº for 40minute	72Cº for 5minute.
blaTEM-1	94 Cº for 5 minute.	32	94 Cº for 1 minute	51 Cº for 1 minute	72 Cº for 1minute.	72Cº for 7minute.

Results and Discussion

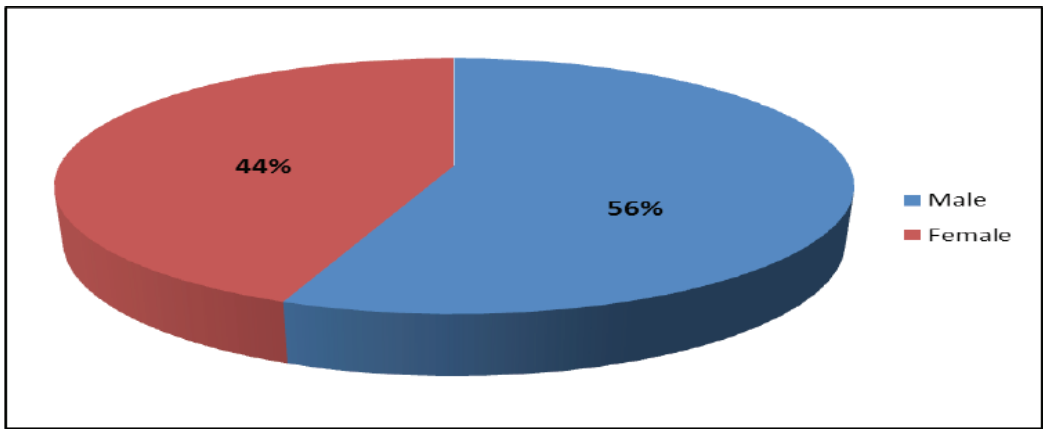
This study was conducted on 40 sample from typhoid blood of doubtful patients through the interval since Dec.2015 to Sep.2016. indicated the results that clinical sample were 40 sample.

The diagnosis clinical depended on the existence, of several symptoms like nausea , headache ,anorexia , fever and vomiting , discomfort abdominal with constipation and diarrhea for 6-18 days.

In 40 patients, 30 (75%) were male and 10(25%) were female as in figure(1). Males were found to be more infected than females. The result is similar to extra results of study [7] and Diyala [8]. This might be because most males were out-doored and from this point of view they could be regarded as food eating and handing or contact with other patients. .

Butler (2001) the utmost exposition of male to polluted, food and water out - side the home may be part

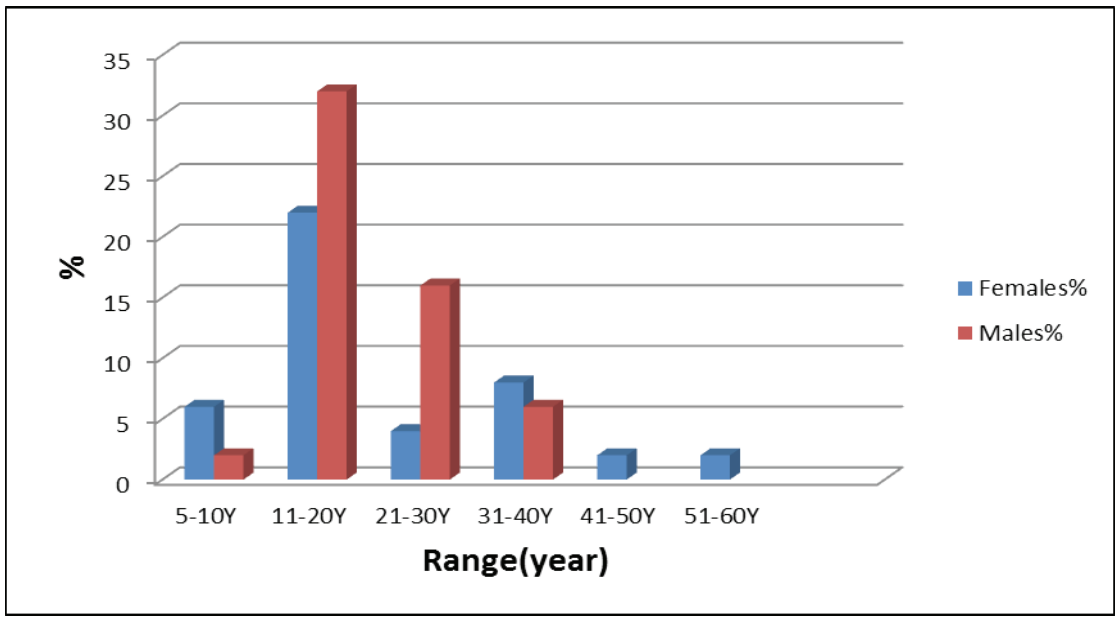
of higher rate of contagion between this population[9]. Fever typhoid is an contagion caused foremost via S. typhi and is conveyed by the oral route or the consumption of polluted, eggs , poultry , meat and milk [10].



Figure(1): Distribution of infected patients according to sex

The patient's age ranged from (5-60) years old. The age scattering patients appear in figure (2). The top incidence between about (41-50) and (51-60) age group 2% and 2% respectively, while the top incidence was between the (11-20) age group (54%) and (21-30) age group (20%).The disease affects all ages, 8(8%) of the study specimens was in the age group of 5-10 lead to *S. typhi* contagion between children due to the immature immune method .

These results are similar to the previous studies of [11;8]. This perhaps due to the mobility, exhaustion of unsanitary food and water in colleges and school [12] While few cases were in age groups (41-50) and (51-60) years old related to frequent boosting of immunity .



Figure(2):Distribution of infected patients according to age and gender

***Salmonella typhi* confirmation use PCR**

The technique of the Polymerase chain reaction for S. typhi clinical isolates detected with product 763 bp that exemplify the fliC gene as appear in fig. (3) .In theis study, used PCR to reveal fliC gene as 30(75%) isolates positive result .This result is associated with Khan et.al.,(2012) who found due to 80 cases doubtful ty fever phoid , detect (fliC-d) via PCR in 56 (70%) cases which agreement at similar effect of study done in Bangladash wherever PCR was positive in 88.7% of cases doubtful

fever typhoid .The rate positivity of PCR is 65%- 71.9% in several studies [13]. A flagellum has 3 basic kind: the farthermost and longestkind is a filament which included about 20,000 subunits protein of a single protein called (Flic) with a Mwut. of 50 to 60 KDa [14]. The gene *fliC-d* encode for the synthesis of H (flagellar) antigen. This antigen form the basis of classification for Salmonella via Kauffman– White scheme [15-16]. in the study useing utmost gene flagellin as a molecular technique for discovery of Styphi in clinical sample. The method alternative wherever Via B region is targeted give the result positive false caused by the existence of this sequence in S. Paretyphi . Flagelle are thin, appendages rigid of bacteria, and structures bactereal locomotive [17].

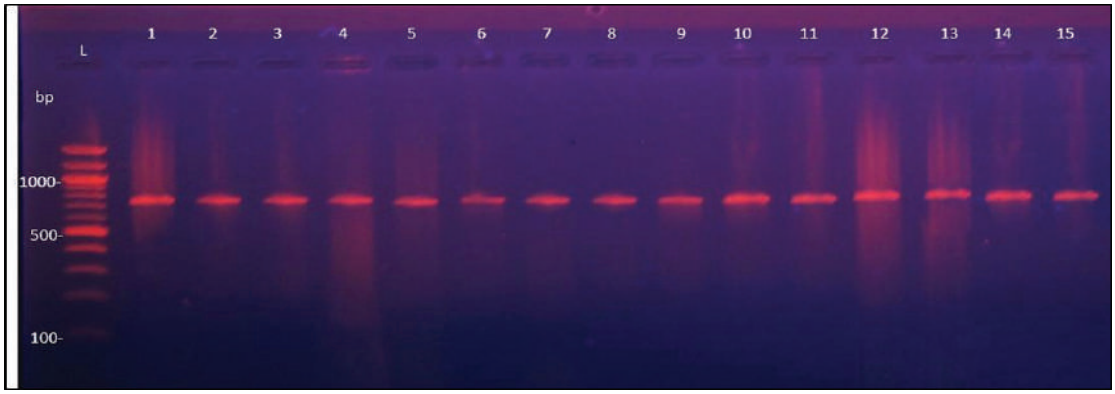


Figure (3): PCR amplificetion produces of *S.typhi* isolates that amplified with *fliC* gene primers with produce 763 bpLane (L), DNA size molecular marker (100-bp ladder), 20 specimen appear results positive with *fliC* gene

Molecular Detection of (ESBLs) Producing Isolates

S.typhi isolates were further investigeted to limit the occurrence and kinds of (ESBLs). However, only gene in the families of TEM was observed in the found study. The (*bla*TEM-1) was detected via PCR technique as in fig. (4). Revealed results the 16(40) isolates ,*S.typhi* transmit *bla*TEM-1 genes. Other study these genetic elements are either chromosomal and/or extrachromosomal in the form of plasmids. The present study was conducted to investigation of the presence antibiotic-resistance genes[18]. Ampicillin resistance in salmonellae may be due to enzyme βlactamase that breaks down the structural B- lactam of penicillin ring and derivatives the synthetic .The property of stability to many bacterial Blactamase increased with the later generation of Cephalosporins. These enzymes are most usually produced via Klebsiella spp and E.coli. but too occur in other bacteria gram negative like Salmonella, Citrobacter, Pseudomonas aeruginosa ,Proteus, Shigella dysentriae etc.. Though not all S. typhi isolates were ESBL reproducer but presence of TEM-1 (encoded via blaTEM-1) have been described to have clinical inclusion because the Blactamase is known, as the progenitor to many spectrum-extended Blactamases and inhibitor-resistant Blactamases .Mechanisms of resistance include not only production of ßlactamases, but also alterations in (PBPs) and reduced entry and efflux active of the antibiotic.

Although most of the strains of Salmonella , P. mirabilis, and Shigella so apted to ampicillin when utilizer in the early 1960s, and percentage increasing of the kind is resistant now. strains the resistant of Salmonella (plasmid mediated) had been recovered with increasing frequency in several the world. regrettably, ampicillin is hydrolyzed easily via spectrum broad ßlactamases and found with frequency increasing in isolates clinical of these bacteria gram-negative . In salmonellae, ampicillin resistance is maybe due to tem-β-lactamase [18].

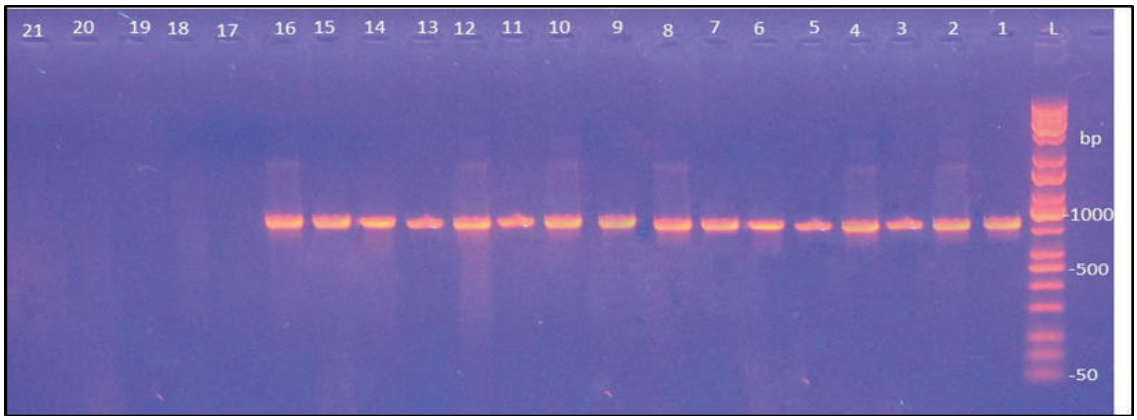


Figure (4): PCR products expansion of Styphi isolates that expand with blaTEM1 primers gene with produce 962 bpLane (L), DNA size molecular sign (50-bp ladder), Lanes (1 to16) appear the results positive with *bla*TEM1 gene, Lanes (17,18,19,20,21) show negative results with *bla*TEM1 gene.

Conclusions

1-*S.typhi* been recognized as a major public health problem,especially among typhoid patients.

2-High prevalence of ESBL producing *S.typhi* isolates were detected

3-The gene FliC-d was a specific gene for disclosure ofS. enterica serovar typhi among via the laboratories diagnostic.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Correlation of Toxoplasmosis Seroprevalence and Serum Level of Interleukin-10 in Iraqi Breast Cancer Women

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Abstract

Background: Toxoplasmosis is regarded as one of the most important global life-threatening diseases in immune-compromised people. The intracellular protozoon *Toxoplasma gondii* is the causative pathogen of toxoplasmosis. Aim of this study is to investigate the possible association between *T. gondii* infection and breast cancer (BC) in Iraqi women, also to assess the effect of *T. gondii* on interleukin 10 (IL-10) of the immune response. By ELISA method, blood samples from 81 women with breast cancer and 60 apparently healthy women have been examined for presence of anti-*toxoplasma* antibodies, also the levels of serum IL-10 were estimated in these subjects. Results showed that women with BC had the highest prevalence rate of toxoplasmosis. The anti- *T.gondii* antibodies was 38.27% for IgG and 4.93% for IgM, while in the control group it was 26.66% and 0.0% respectively, with no significant difference for IgG (P =0.148) and IgM (P = 0.081). A highly significant difference was found in the level of IL-10 between BC women with toxoplasmosis (10.24%) and BC women without toxoplasmosis (3.6%). These findings suggest that incidence rate of *T. gondii* infection is higher in breast cancer women and higher level of IL-10 than their cross-matched control subjects. Therefore, we recommend the inclusion of a screening test for toxoplasmosis and the level of IL-10 in BC patients as in their routine workup.

***Keywords**: Breast cancer, ELISA, interleukine-10, Iraq, Toxoplasma gondii*

Introduction

Toxoplasma gondii is an obligate intracellular coccidian parasite widely distributed in the world [(1)]. In immunocompetent individuals, the infection with *T. gondii* is generally controlled by the immune system and often pass unnoticed, however, it is life threatening in the immunocompromised individuals [(2,3)] . Symptoms of the infection often involve cancer patients [(3)] .

The parasites are linked to various cancers, which may interfere with the host cell's genetic machinery and may result in immune system impairment and chronic inflammations [(4,5)] .

Toxoplasmosis is regarded to play an important role in malignancy induction and must be further investigated as a probable oncogenic pathogenic agent to human-beings [(6)].

Many studies indicated that seroprevalence of toxoplasmosis is significantly higher in cancer patients having chemotherapy including breast cancer, HIV/ AIDS and organ-transplant recipients than non-cancer patients [(7- 9)].

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The most common malignancy in women is breast cancer [10,11] . Breast cancer is one of the most warning to females health issues in Iraq, as it is the main cause of women's mortality after cardiovascular diseases, with a mortality rate of 23% [12]. Despite new diagnostic and treatment options, 30% of women diagnosed with invasive breast cancer will develop metastatic disease [13] ; therefore, early detection is critical for patient survival. Development of new therapeutic strategies and prognostic methods could be assisted by measuring and interpreting Th2 cytokine levels in BC patients [14].

The role of IL-10 in breast cancer is controversial as a result of various IL-10 studies in breast cancer [15,16] . It has been shown to exhibit both tumor promoting and -inhibiting properties [17], it inhibits the pro-inflammatory functions of antigen-presenting cells (APCs) through expression of antagonizing costimulatory molecules. Its low expression is associated with poor survival outcome [18].

The prevalence of toxoplasmosis among Iraqi cancer women has not been adequately studied. So, in our work, we aimed to find the possible correlation between *toxoplasmosis* and breast cancer, also to assess the effect of *T. gondii* on the levels of IL-10.

Materials and Methods

The current cross-sectional study was performed over the period from February 2019 to November 2019 on blood samples collected from a total of 141 subjects.

The all patients were females recruited from the oncology teaching hospital in the medical city hospital and Emamayn Al-Kadhimayn medical city in Baghdad, after approval of University of Baghdad/ College of Pharmacy ethical committee and with their consent in participating in the study. The patients and healthy (control) women have been of different geographic residencies and different age groups, ranged between 19 -70 years old. The mean ages were 44.5y. and 43.2y. respectively..

From all the study groups (patients and controls), 5 ml of venous blood were collected in plain tubes, and centrifuged for 5 minutes at 3000 rpm to obtain serum which was placed in two Eppendorf tubes and stored at -20º C.

To find the possible correlation between toxoplasmosis and BC by examining the seropositivity rate of the anti-*T. gondii* antibodies, participants were divided into two groups:

- Group [1]: includes 81 BC women (treated with chemotherapy).
- Group [2]: composed of 60 healthy subjects who have no malignancy (according to clinical examinations).

The enzyme immunoassay technique was used to detect IgG and IgM levels with the Biorex diagnostics LTD (Antrim technology park, UK) kit.

To estimate the role of IL-10 in Iraqi breast cancer women, participants were further divided into four groups:

- Group A: patients having breast cancer and have *T.gondii* infection.
- Group B: patients having breast cancer and not have *T.gondii* infection.
- Group C: apparently healthy individuals not known to have malignancy and have *T.gondii* infection.
- Group D: apparently healthy individuals not known to have malignancy and not have *T.gondii* infection (referred to as control group).

The serum IL-10 levels were measured by using the Enzyme–linked immunosorbent assay (ELISA) Kits from Elabscience, Chinese company.

Statistical Analysis

In this study, the Chi-square was used to compare between percentages, and the least significant difference (LSD) test was used to compare between means. The comparison of significance (P-value) in any test was: S= Significant ($P<0.005$). HS= Highly Significant ($P<0.001$). NS= Non Significant ($P>0.005$).

Results

IgG & IgM Toxoplasma antibodies determination

The results showed that the positivity rate of *T. gondii* IgM and IgG antibodies was higher in patients

in comparison with the control group women. The prevalence rate of anti- *T.gondii* antibodies was 38.27% for IgG and 4.93% for IgM in BC women included in our study. The positive cases for IgM were positive for IgG antibodies concurrently. The control group showed 26.66 % and 0.0 % in the same order of anti- *T.gondii* antibodies (Table 1), with no statistically significant difference for IgG (P =0.148) and IgM (P = 0.081).

Table 1: Distribution of anti-*T. gondii* antibodies using ELISA method in breast cancer patients and healthy females.

anti-*Toxoplasma* antibodies	Breast cancer women (n=81)		Healthy women (n=60)		Chi-square	P-value
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)		
IgG	31 (38.27)	50 (61.72)	16 (26.66)	44 (73.33)	2.089	0.148
IgM	4 (4.93)	77 (95.06)	0 (0.0)	60 (100.0)	3.049	0.081
IgG+ IgM	4 (4.93)	77 (95.06)	0 (0.0)	60 (100.0)	3.049	0.081

The mean difference is significant at the 0.05 level.

The mean titers of anti-*T. gondii* IgG antibody in groups 1 and 2 were 32.6 IU/mL and 22.5IU/mL, respectively, with no significant differences in mean titers between the studied cases. The current study recorded a higher seroprevalencerate of toxoplasmosis in women with BC.

Moreover, in the 4 positive women for *T. gondii*, cancer was metastasized to liver and bone. The IgG titers ranged between 19.4 and 44.6 IU/mL, with one of the cases being positive for IgM and IgG antibodies, simultaneously, and her cancer was metastasized to lung in addition to liver and bone.

Estimation of the level of IL-10

Considering serum IL-10 concentrations for BC patients infected with toxoplasmosis, the mean value was higher than other groups (35.03 pg/ml), as shown in Fig.1.

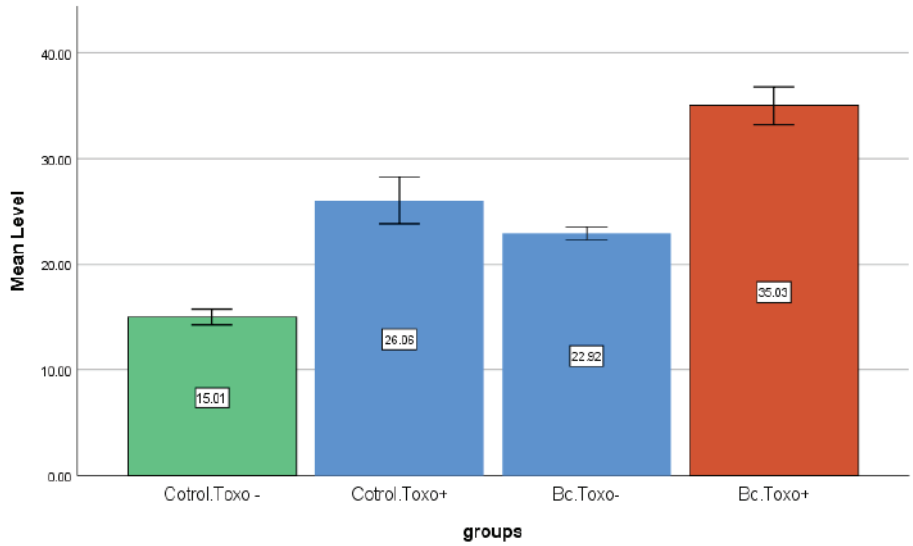


Figure (1): serum levels of IL-10 in studying groups according to *T.gondii* infection

Such difference was highly statistically significant (p value < 0.001) as shown in table 2. Non significant difference was appeared in the relation of control group with toxoplasmosis and BC group without toxoplasmosis (table 2).

Table (2): IL-10 serum levels in studying groups according to toxoplasmosis infection

			Multiple comparisons dependent variable LSD	
Groups	**Number**	**Mean ± S.E.(pg/ml) of IL-10**	**groups**	**p- value**
Control Toxo -	44	15.01 ± 0.72	Control Toxo +	0.000*
			BC Toxo -	0.000*
			BC Toxo +	0.000*
Control Toxo +	16	26.05 ± 2.20	Control Toxo -	0.000*
			BC Toxo -	0.105
			BC Toxo +	0.000*
BC Toxo -	50	22.92 ± 0.61	Control Toxo -	0.000*
			Control Toxo +	0.105
			BC Toxo +	0.000*
BC Toxo +	31	35.03 ± 1.79	Control Toxo -	0.000*
			Control Toxo +	0.000*
			BC Toxo -	0.000*

*. The mean difference is significant at the 0.05 level.

Discussion

It is assumed that when people previously get infected with chronic toxoplasmosis and are then infected with any type of cancer, there will be a high possibility of the reactivation of latent infections together with the opportunity of that cancer to become high and more aggressive [19,20] .

Current study investigated the seroprevalence rate of *T. gondii* in breast cancer women in order to find out any possible association between this infection and breast cancer. It have shown that women with BC have higher rate of both IgG and IgM antibodies for toxoplasmosis infection than do no BC women, but with non-significant difference, as shown in table 1. Although, *T.gondii* has been considered as an opportunistic infection, there was no association between a history of Toxoplasma infection and breast cancer..This finding was in line with previous studies in Iran [21,22] , but in contrast with the studies done in Egypt and China, which found a highly significant deference between the two groups [23,24] .

To control and clear up parasitic infections, human's immune system plays an essential roles [25]. The present study found that women with toxoplasmosis in case of presence or absence of BC, were secreted higher amounts of IL-10 than did the groups with no toxoplasmosis (table 2 and figure 1). However, the group of BC women

with toxoplasmosis showed higher level of IL-10 than all other groups with highly significant difference.

IL-10 has the ability for deactivate macrophage deactivation, IFN-γ induction by *T. gondii* as well as intracellular parasite survival facilitation. IL-10 can induce immune suppression during *Toxoplasma gondii* invasion, which is useful for the parasite and its host [26] . The elevated IL-10 levels in toxoplasmosis patients may be attributed to the parasite's ability to TH2 cytokine enhancement [27], or to induction of the products secreted at the stage of cell lysis by tachyzoites for the differentiation of naive B cells to IL-10-producing B regs [28].

Interleukins are confirmed to be able to contribute in BC proliferation, establishment and migration [29] . IL-10 acts as prognostic factor and even determines the survival rate of breast cancers [30]. Moreover, gene polymorphisms of IL-10 may determine the susceptibility for breast cancer [17].

This study has limitations, such as the small sample size, and no information was available at the time of first *T. gondii* infection and cancer induction among the studied cases. This study suggested that further studies should be performed to establish any association between *T. gondii* and breast cancer.

Conclusion:

Present study found higher rates of *T.gondii* seropositivity and highly significant difference in the IL-10 levels in BC women compared with healthy women. Therefore, we recommend the inclusion of a screening test for toxoplasmosis even it was non significant association and use the IL-10 as a laboratory tool which may serve as complementary tumor marker and contribute to the differential diagnosis in BC patients.

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Conflict of Interests: The authors declare that they have no competing interests. This research received no funding from any party.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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CD14 C(-260)T Polymorphism and Blood Levels of the Soluble Endotoxin Receptor CD14, their Association with Risk of Ischemic Stroke in Iraqi Populations

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Abstract

Background: Stroke is a main cause of death and adult long-term disability worldwide, and stays as an enormous burden for society due to lack of illustrated etiology and effective treatments. **Aim:** the present study was conducted to investigate the association of *CD14* genotype and plasma levels of soluble(s) CD14 with risk of ischemic stroke. **Methods :** A total of 40 patients with ischemic stroke were included in the study who were admitted to hospital from the period between March to August 2019, and other groups consist of 40 apparently healthy individuals. A five ml of blood samples were collected, 2 ml of each sample for polymerase chain reaction amplification and detection of CD14 technique. The remaining (3ml) for CD14 monocyte count by Flow cytometry technique. **Results**: *CD14* C(-260)T genotype was not significantly associated with increased risk of IS (P= 0.367). However, sCD14 plasma levels were higher in subjects with TT genotype compared with those with CT or CC genotype (P= 0.013). The count of CD14 Monocytes, in Ischemic Stroke patients was significantly higher than that of healthy control group. **Conclusion:** a significant correlation between *CD14* genotype and sCD14 levels were higher level in subjects with TT genotype compared with those with CT or CC genotype.

Keyword: *Ischemic Stroke, CD14, sCD14; blood Levels; health; long-term disability*

Introduction

Stroke is a main cause of death and adult long-term disability worldwide, and stays as an enormous burden for society due to lack of illustrated etiology and effective treatments[(1)]. Annually, approximately 800,000 people in the United States have a stroke, and 130,000 die[(2)]. It has accounted for nearly 5.7 million deaths worldwide, and 87% of these deaths occur in low and middle-income countries[(3)]. Ischemic stroke (IS) accounts for 85% of overall stroke and its pathophysiology are regulated by a combination of lifestyle, environmental, and unclear genetic risk factors[(4)]. According to the report from the Centers for Disease Control and Prevention, given in 2013 mortality from stroke was the fourth leading cause of death in the United States in 2008. Therefore, it is important to know the reason for this social burden so that safe and effective therapeutic treatment that could be given at medical services would improve the outcome of millions of acute stroke patients[(5)]. Cluster of differentiation 14 (CD14) acts as a multifunctional high-affinity receptor for the binding of endotoxins, lipopolysaccharides (LPS), and other bacterial wall components that are involved in primary immune and inflammatory responses[(6)], which is an important glycoprotein expressed as membrane CD14 on the surface of monocytes, neutrophils, and macrophages and soluble CD14 (sCD14) in the serum[(7)]. CD14 transfers the LPS and other bacterial signals through the LPS-binding protein/CD14/myeloid differentiation factor 2 (MD-2)/Toll-like receptor (TLR)

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4 complex[8]. This multi-molecule complex triggers cascade signal amplification and activates innate host defense mechanisms, thereby promoting the release of cytokines and increase in antigen presentation, which stimulate an immune response[9]. *CD14* gene located on chromosome 5q23-31, which encodes a 55kDa glycoprotein with 375 amino acids[10]**.** The *CD14* gene contains several polymorphic sites, of which rs2569190 (C-260T, sometimes referred to as C-159T) is the most common polymorphism locus in the promoter region of the *CD14* gene[11]. *CD14* gene (rs2569190) has been associated with differential expression levels of CD14 in monocytes and macrophages. Moreover, in atherosclerotic disease, the T allele of–159C/T has been suggested to affect circulating levels of soluble CD14[12]. In 2000, Ito et al., [13] conducted the first case–control study and reported that rs2569190 C/T polymorphism is not associated with IS susceptibility. Wu et al.,[10] also suggested no significant associations between *CD14* rs2569190 C.T polymorphism and IS risk.

Materials and Methods

The current study was carried on 40 patients (18 males, 22 females) age range between 45-85 years from March to August2019. Other groups consist of 40 apparently healthy individuals (20 male and 20 female) without any history of systemic disease were clinically considered as healthy also included in this study as a control group. We excluded patients with hemorrhagic stroke, stroke associated with surgery, severe trauma or organ ischemia. A five ml of blood samples were collected by vein puncture using disposable syringes under aseptic technique 2 ml of each sample were transferred into with EDTA tube and immediately frozen at -20 C till further use to avoid repeated thawing and freezing for polymerase chain reaction amplification and detection of *CD14* (RFLP-PCR) technique. The remaining (3ml) were transferred into tube with EDTA maintained at room temperature until analysis sample at same day for CD14 monocyte count by Flowcytometry kit for Thermo Fisher/ Bioscience™ USA. This study was in agreement with ethics of Al-Diwaniya Teaching Hospital and verbal informed consent was obtained from all participants.

Results

Distribution of *CD14* C(260)T polymorphism was detected by RFLP-PCR technique, at this locus there are three genotypes, CT,CC and TT with band sizes 360/204/201/156 bp, 204/201/156 bp and 360/201 bp respectively, table (1), figure (1). The genotype distribution had no deviation from Hardy-Weinberg equilibrium in all study groups and agree with the reports of Xu et al[14]. Both groups were comparable in mean serum concentration CD14 expression table (2). Mean serum concentration of CD14 expression in ischemic stroke patients was significantly higher than that of healthy control 3.91±2.3versus 1.906±4.16 and P-value was ($P < 0.001$). Figure (2 and 3) showed the flow cytometric analysis of CD14 expression on Monocyte cells in ischemic stroke patients (A) and healthy control (B). Mean soluble CD14 levels were statistically significantly higher in patients bearing the TT genotype (p=0.013) compared with heterozygotes (CT) and those homozygous for the CC genotype, and this indicate significant correlation between soluble CD14 levels (pg/ml) and *CD14* Polymorphism in patients with IS, table(3).

Table (1): Distribution of *CD14* (−260C/T) Genotype and Alleles Frequency

Genotype	Controls (n=40)		Patients (n=40)		OR	95% Confidence interval		p	PF	EF
						Lower	Upper			
CD14 C(-260) T	N.	%	N.	%						
CT	15	37.5	18	45	1.466	0.3002	1.7915	0.496	……	0.141
CC	18	45	12	30	0.632	0.7612	4.7878	0.026	0.285	…..
TT	7	17.5	10	25	1.273	0.2150	1.8835	0.041	……	0.190

Cont... **Table (1): Distribution of *CD14* (−260C/T) Genotype and Alleles Frequency**

Overall P value		0.367								
Alleles Frequency										
C	51	63.75	42	52.5	0.795	0.3451	2.9957	0.149	0.204	….
T	29	36.25	38	47.5	1.256	0.3338	1.1833	0.149	….	0.204

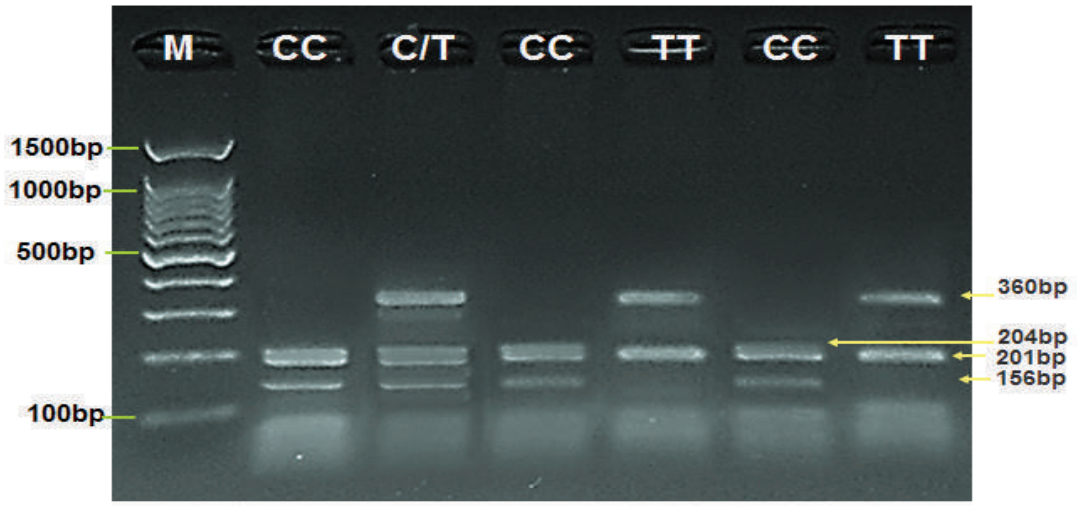


Figure (1): Agarose gel electrophoresis image that show the RFLP-PCR product analysis of *CD14* −260C/T polymorphism by using HaeIII restriction enzyme. Where M: marker (1500-100bp), lane (CC) wild type homozygote products were digested by restriction enzyme at 204bp, 201bp and 156bp bands, lane (TT) mutant type homozygote, the products were digested by restriction enzyme into 360bp and 201bp bands. So that (C/T) heterozygote was showed as 360bp, 204bp, 201bp and 156bp bands.

Table(2):The compassion between the study groups regarding CD14 expression

	Patients	**Healthy control**	**P value**
Range	(0.49- 7.9)	(0.48- 4.2)	0.001 (S)
Mean	3.91	1.906	
SD	2.3	4.16	
SE	0.36	0.65	
N	40	40	

Table (3): Correlation between soluble CD14 levels (pg/ml) and *CD14* C(-260) T genotype in patients with IS

	CD14 genotype				
	CT Genotype	**CC Genotype**	**TT genotype**		
CD14 conc.pg/ml				F	P
Range	(0.55 - 7.9)	(0.49 – 6.7)	(1.18 – 7.8)	4.85	0.013 (S)
Mean	2.73	3.43	5.5		
SD	2.30	2.30	2.15		
SE	0.54	0.66	0.68		
N	18	12	10		

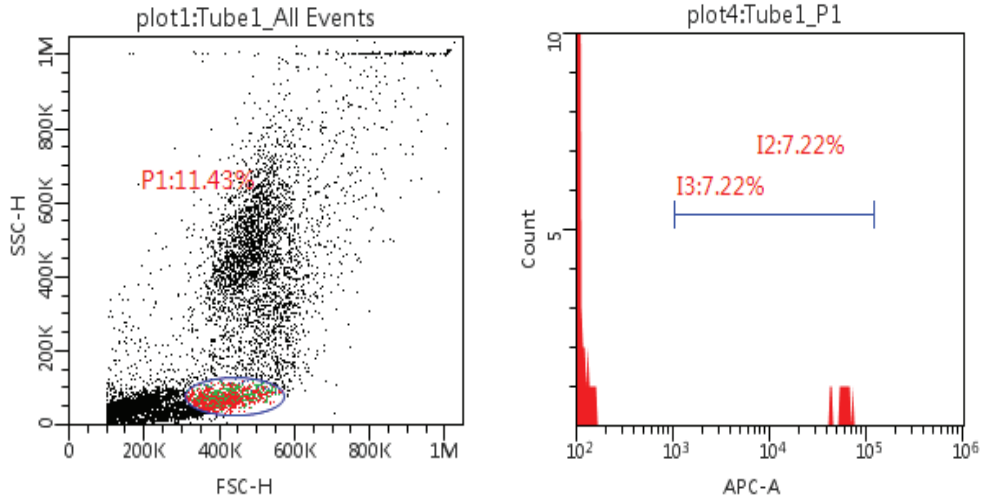


Figure (2): Results of Flow cytometry Analysis for CD14 detection on Monocytes of patients (APC (Allophycocyanin (Red color), FSC-H (Forward Scatter Height) and SSC-H (Side Scatter Height).

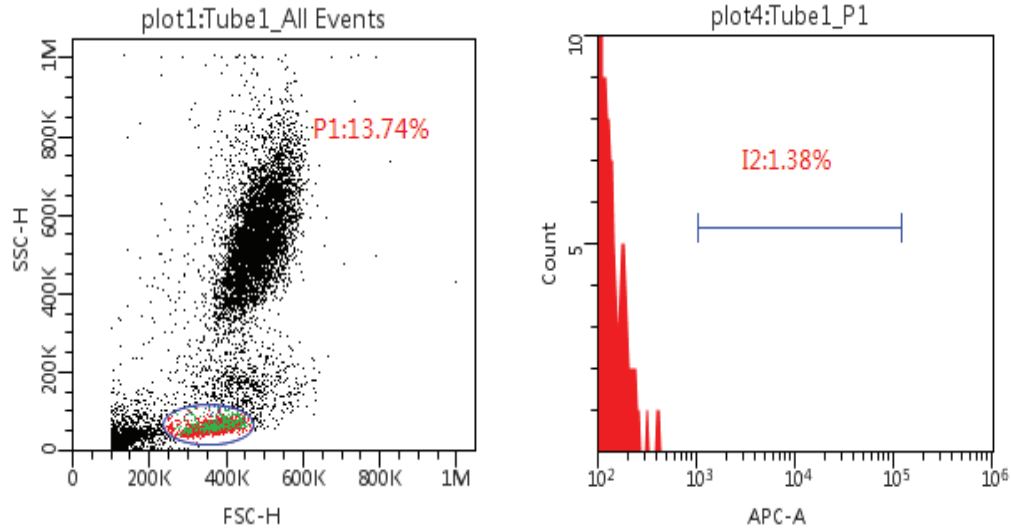


Figure (3): Results of Flow cytometry Analysis for CD14 detection on Monocytes of healthy control (APC (Allophycocyanin (Red color), FSC-H (Forward Scatter Height) and SSC-H (Side Scatter Height).

Discussion

Numerous studies have demonstrated the role of inflammatory markers in influencing cerebrovascular. Further, it has been seen that difference in distribution of inflammatory gene variants might increase or decrease the susceptibility to stroke attack[14]. *CD14* is another gene involved in inflammatory pathway. The C-260T (rs2569190) polymorphism is present in the promoter region of the gene and has been associated with differential expression levels of CD14 on monocytes/ macrophages[15]. Overall, the present result indicate no significant association between *CD14* (−260C/T) polymorphism and risk of ischemic stroke (p=0.367). The possible explanation may be that *CD14* rs2569190 C/T polymorphism is not involved in ischemic stroke susceptibility directly but plays a pathogenic role in synergy with other abnormally expressed proteins or gene polymorphisms[10]. Our results agree with Ito et al[13]; Xu et al[16], which investigated whether rs2569190 C/T polymorphism contributes to a disposition to ischemic stroke and did not find any significant association between rs2569190 C/T polymorphism and ischemic stroke susceptibility.

The results of present study showed high frequency of (TT) genotype among patients when compared to healthy controls, these result identify (TT) genotype is associated with increased risk of ischemic stroke with statistically significant (P=0.041). and may be considered as the etiological factor for IS. The results of present study consistence with result of Lichy et al[17], which found that the TT genotype is associated with a risk of the stratified micro- or macro-angiopathic ischemic stroke. Moreover, the present result revealed frequency of allele C was more in healthy control compared to ischemic stroke patients. Also showed that (CC) genotype frequency in patients (30%) lower than in healthy controls (45%) and this results agree with results Park et al[18], which found that (CC) frequency in patients (15.2%) lower than controls (23.2%). So CC genotype and C allele may be considered as a preventive fraction to give a protection from IS.

The present study revealed that the level of immune markers (CD14) became significantly increased among cases with ischemic stroke (3.91± 2.3pg/ml) when compared with healthy controls (1.906± 4.16 pg/ml), these results indicate significant association between Monocytes (CD14) and ischemic stroke (p<0.001). Monocytes are characterized by the expression of several clusters of differentiation, such as CD14 in human[19]. The authors have analyzed the potential role of monocytes in the development of the pathology of ischemic stroke[20]. Several studies have indicated that due to the breakdown of tissue, numerous danger-associated molecular patterns (DAMPs) are generated by the brain to induce this type of immunosuppression, which eventually results in stroke-associated infection (SAI) with increased mortality[21]. Following ischemic stroke, microglia, which are brain resident macrophages, are activated and circulating immune cells, such as monocytes, neutrophils and lymphocytes are recruited to injured site[22]. There may be specialized roles for those monocyte subsets in stroke pathophysiology and they might become targets for therapeutic intervention if their mechanisms are more precisely elucidated[23]. Our results agree with Klimiec et al[24], which found that high levels of plasma sCD14 are reported in patients with ischemic stroke compared to heathy control (1330 [1140–1500] vs 1070 [921–1220] ng/mL, p<0.001) and these level closely associated with the risk of death and Mendel et al[25], who showed high CD14 levels may be associated with vascular endothelial cell damage, which facilitates atherosclerosis formation and increases the risk of ischemic stroke subsequently.

Results showed significant correlation between soluble CD14 levels (pg/ml) and *CD14* Polymorphism in patients with ischemic stroke (p=0.013), were we found higher soluble CD14 levels in the TT genotype (5.5± 2.15 pg/ml) as compared with CT or CC genotype (2.73± 2.3 pg/ml and 3.43± 2.3 pg/ml respectively). The polymorphism C260T of the *CD14* promoter has been shown to increase transcriptional activity. This enhanced transcriptional activity has been associated with higher concentrations of sCD14[26]. A potential functional role of rs2569190 on *CD14* has been suggested as it alters a Sp1 transcription factor binding site and modulates the activity of promoter[27]. The T allele of rs2569190 decreases the strength of the bond between the *CD14* promoter GC box and Sp consensus sequence, changes the transcriptional capacity, and increases the protein expression level of CD14[28]. Our results agree with results of many previous studies which had suggested that the *CD14* (rs2569190) polymorphisms will increase

sCD14 levels in homozygous carriers of T allele[29][30].

Conclusions

Our results do not confirm an independent and clinically relevant relationship of *CD14* gene polymorphism with risk of IS. Patients who undergone IS showed a higher level of CD14 expression than healthy control. In this study we found a significant correlation between *CD14* genotype and sCD14 levels were higher level in subjects with TT genotype compared with those with CT or CC genotype.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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The Cytotoxicity Effect for the Crude Extract of (*Cyperus Esculentus*) Tubers on Human Breast Cancer Cell Line (MCF-7) in Vitro

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Abstract

The study aimed to identify the aqueous and alcoholic extract of *Cyperus esculentus* tubers in affecting human breast cancer (MCF-7) Michigan Cancer Foundation,preliminary diagnosis chemical groups in these extracts showed the presence of (Tannins, Terpenoids, Resins, Steroids, Flavonoids, Alkaloids, Glycosides and Phenols). The effect of cellular toxicity of crude aqueous and alcoholic extract of *Cyperus esculentus* tubers in vitro was tested in human breast cancer cells (MCF-7) through the use of six concentrations (midterm scares) (31.25,62.5,125,250,500,1000) µg /mL and for periods of exposure (24, 48, 72) hours. Using the MTT Test. The study found that there is a toxic effect of the crude aqueous and alcoholic extracts of the grain of *Cyperus esculentus* tubers in cancer cells (MCF-7) and of all the concentrations used, The highest percentage and a significant difference ($P \leq 0.01$) for the average of the inhibitory average of cancer cell growth (IR) was 68, 73 (%) for the aqueous extract and alcoholic extract, respectively, at the highest concentration of 1000 µg / mL and after 72 hours of exposure.

Key word : *Cyperus esculents, cytotoxicity, Inhibitory Rate*

Introduction

Cancer refers to a large number of diseases that affect all parts of the body and these diseases are called malignant tumors [(1)] and cancer is the second leading cause of death in the world. In 2018, 8.8 million people died, and nearly one out of 6 deaths worldwide is attributed to it [(2)]. Cancer occurs as a result of the abnormal division of cells and without control, which is able to spread to the rest of the body, and this division continues even after the catalyst is interrupted, This increase in division and reproduction leads to the formation of tumors, and these are of two types benign tumors and malignant tumors [(3)].Therefore, many countries of the world have paid great attention to their medicinal plants as the natural source of pharmaceutical. [(4)] The genus (*Cyperus esculentus* L) is considered a medicinal plant and it is a perennial herbaceous plant used in the field of folk medicine in many countries, including Africa, Asia,It is one of the genera of the Cyperaceae family known to have many active compounds and anti-carcinogenic substances, The plant is used to treat many diseases, such as problems and disorders of the digestive system, such as irritation of the colon and diarrhea.. less as an anti-oxidant by containing vitamin E and C and is used to reduce the incidence of Heart attacks Thrombosis [(5)] .That the alcoholic extract of the tubers of *Cyperus esculentus* plant can be used to treat convulsions. Many studies also showed that the aqueous extracts of *Cyperus esculentus* tubers contain anti-cancer compounds and active substances, including sterol. Anti-cancer, The secondary metabolic compounds present in the *Cyperus esculentus* tubers play the therapeutic role of cancer because of its therapeutic properties through its effect on killing or stopping cancer cells [(6)].

Materials and Methods

Preparing crude aqueous and alcoholic extracts for the seeds of the *Cyperus esculentus* plant, Cyperus esculentus tubers were obtained from the local herbalists in Karbala province during the month of December

2019, Washed, dried, and cleaned, It was milled by Mill device and keeping the powder in clean plastic. Preparing the aqueous and alcoholic crude extracts by methods of soaking and stirring according to (14) with a weight of 50 g of the plant powder and adding 250 ml of solvent to it). Magnetic stirrer at room temperature for 3 days, after which the filter was filtered with gauze and then with filter paper (Whatman NO.1), Then the leachate was dried to obtain the dry powder, from which the required concentrations were prepared. Prepare the crude alcoholic extract of the plant in the same methods, but using ethyl alcohol at a concentration of 70% instead of the distilled distilled water .After weighing the extract, it was divided into several sections and kept in glass bottles under 4 ° C. Dissolve 0.1 g of dry extract in 10 ml of serum free media (serum free media) to prepare the original stock and sterilize by filtering with filter paper with holes. 0.22µm

Cellular toxic effects:

A multi-hole tissue culture dish (96-Microtiter plates). The experiment included three stages:

Cells Seeding

the cells were harvested using Trypsin-Versen (T-V) solution. 20 ml of the culture medium provided with serum are added to each container (depending on the cell type) and mix well. After that, the cells returned using a blood cell count (Haemocytometer) using Trypan blue tincture (1%), (0.1) ml was taken by means of a micro-pipette from the cells suspension and distributed to pit the dish containing (1 x 104) cells/pit. The pit surface was then covered with sterile transparent adhesive paper for this purpose and the dish was stirred gently, then incubated at a temperature of 37 ° C until the next day to allow the cell attachment. Treatment of **(exposing) cancer cells with plant extract**

The MCF-7 cancerous carcinoma line (pass 35) was obtained from the center of the Faculty of Nursing at the University of Babylon, the secondary farms (passage or subculture) lattended six diluted of the abstracted agency(31.25), 62.5, 250, 500, 1000 µg / mL.)

Prepare the cells suspension by treating a 25 cm tissue culture vessel with a trypsin solution - fersin (20 ml of trypsin solution 1 g: 100 ml of 10: PBS ml of fersin), then add 20 ml of the new serum-free culture medium 16.4) SFM g of RPMI-1640 media. : 15 ml of sodium carbonate: 0.5 ml 0.25: Streptomycin ml of Gentamycin and Nystatin complete the volume to 1 liter of distilled water (then mix the cells suspension well and transfer 0.2 µL to the flat bottom tissue culture calibration using an automatic pipette, leaving the plates until the cells adhere In the pits. Then the old medium in the pits was removed, and 0.2µl of the previously prepared concentrations of the extract was added and at 3 replicates per concentration, as well as the control replicates) only a culture medium.

Detection of the toxic effect Cytotoxicity Assay

The MTT test was used to detect the cytotoxic effect of extracts of Tuber plant extracts on. According to the following steps:

After the end of each incubation period, the dishes were taken and the contents were poured, then (28) µµL of MTT pigment prepared at a concentration of 2 mg/ml was added to each pit of the pits dish and incubated at a degree of (37) C° / for two hours.

The pigment was removed and the Dimethyl sulphoxide (DMSO) solution was added (130 µµL / pit).The dishes were placed for a period of (15) minutes with a microshaker, then it read the optical density at the wavelength (492) nm using the ELISA microplate spectrophotometer, taking into consideration that the MTT work should be away from light. Cancer cell growth rate (Inhibitory Rate / I.R) was calculated according to the formula

$$IR\% = \frac{A - B}{A} \times 100$$

where:

IR = percentage of inhibition rate

A = optical density of negative control.

B = optical density of the test group

Results

Primer (inductive) chemical detection of secondary metabolism compounds in the extracts of

tuber plants.

The results of the chemical disclosures of the aqueous and alcoholic extracts of the tubers of the dried plant showed the results shown in Table (1):

Table (1) Primer (inductive) chemical detection of aqueous and alcoholic extracts of the tuber plant

Chemical group	Crude alcohol (ethyl) of Cyperus esculentus tubers	Crude aqueous extract of Cyperus esculentus tubers
Tannins	+	+
Terpenoids	+	+
Resins	+	+
Steroids	+	+
Saponins	-	-
Flavonoids	+	+
Alkaloids	+	+
Glycosides	+	+
Phenols	+	+

(+) the positive of a diagnosis .

(-) the negative of a diagnosis

Toxic effect of crude aqueous extract of *Cyperus esculentus* tubers on MCF-7 cancer cell line during exposure periods 24, 72,48 hours ,

The results of the toxicity test shown in Figure 1 on MCF-7 cells of the crude aqueous extract of *Cyperus esculentus* tubers showed a variation in the percentage of cell inhibition depending on the concentration used and the periods of exposure, The inhibitory effect of the aqueous extract appeared from the lowest concentration (31.25) µg / ml up to high concentrations, with the highest inhibition rate of the extract at concentration (1000) µg / ml and for all periods of exposure (72,48,24) hours which was (68.75) ± 3.82, 59.86 ± 1.45, 52.78 ± 1.54 (respectively compared to other concentrations and significant difference with P3.0 probability level)). Whereas, the lowest inhibition rate of the aqueous extract was at concentration (31.25) µg / m at 48 hours (and it reached) (17.46 ± 0.77).

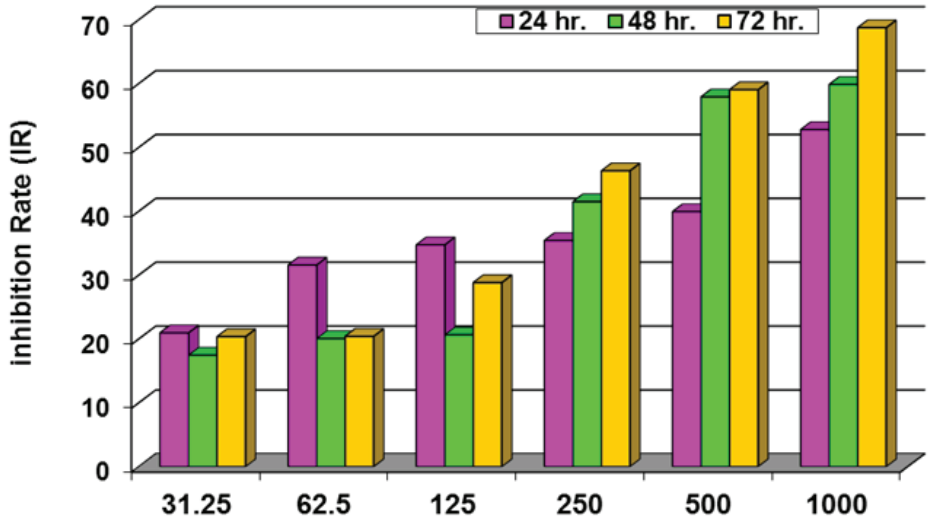


Figure 1. effect of aqueous extract of *Cyperus esculents* tubers plant on the percentage of inhibition of human breast cancer cell linesMCF-7

Toxic effect of crude (ethyl) extract of *Cyperus esculentus* tubers on MCF-7 cancer cell line

during exposure periods 24, 72,48 hours

The crude alcoholic extract did not differ from the aqueous extract in its effect on MCF-7 cancer cells, where it is observed in Figure (2) the toxic effect of ethanol extract and significant difference P≤0.01) began at concentration 31.25µg / ml and for all exposure times (24,48, 72) hour by inhibition rate (16.77 ± 6.99, 44.50 ± 3.75, 49.67 ± 6.63), respectively, The rate of inhibition of cells increased upwards by increasing the concentration and exposure period of the cells to the extract where the highest inhibition ratio (73.16 ± 3.54) for the alcoholic extract at the concentration reached 1000 µg / ml within 72 hours of exposure

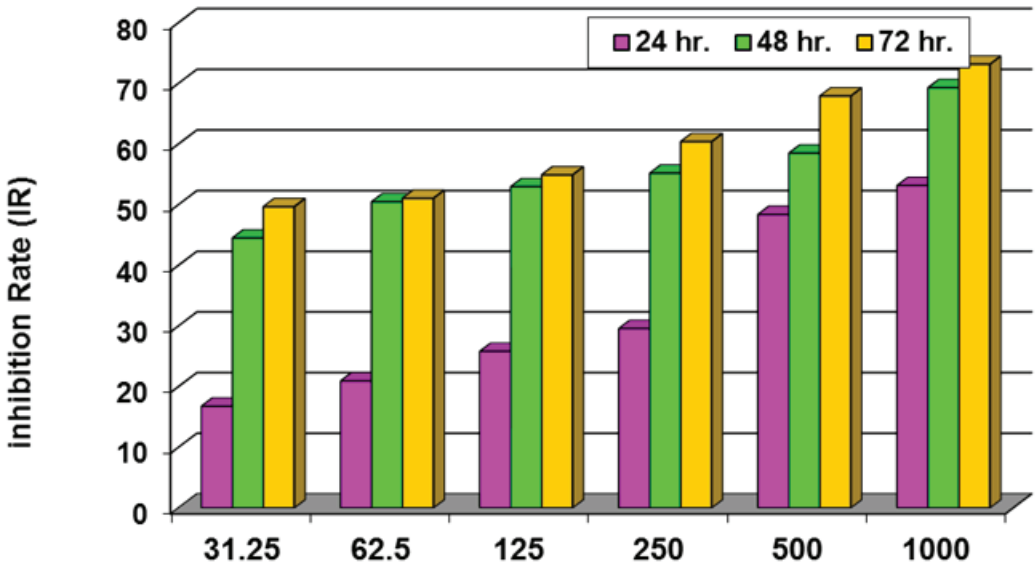


Figure 2. effect of alcoholic extract of *Cyperus esculents* tubers plant on the percentage of inhibition of human breast cancer cell lines (MCF-7)

Comparison of the effect of crude alcoholic and aqueous extracts of *Cyperus esculentus* plant tubers on cancerous cellular line MCF-7))

The results shown in Figures (3.4.5) when comparing the effect of crude alcoholic and aqueous extract on the cellular line of breast cancer showed that the alcoholic extract was most effective in inhibiting the growth of MCF-7 cancer cells at periods of exposure (48,72),It is noticed in Figure (3) that the alcoholic extract at concentration 500 µg / ml gave the highest average of inhibition of cells during a period of 24 hours, and reached 48.38 ± 3.07)) compared to (39.94 ± 3.77) for the aqueous extract with a significant difference (P≤0.01) .. But when comparing the effect of aqueous and alcoholic extracts during a period of (48) hours of exposure, Figure (4) shows that the alcoholic extract gave higher cell inhibition rates than the aqueous extract and for all concentrations, As the inhibition ratio increased with increasing the concentration, the concentration (1000 (/g / ml) gave an inhibition ratio of 69.28 ± 1.83) compared to (59.86 ± 1.45) for the aqueous extract. As for the passage of (72) hours of exposure, the inhibitory ratio of the aqueous and alcoholic extracts increased compared to the inhibitory ratio of them in a period of (48) hours, Figure(5.).

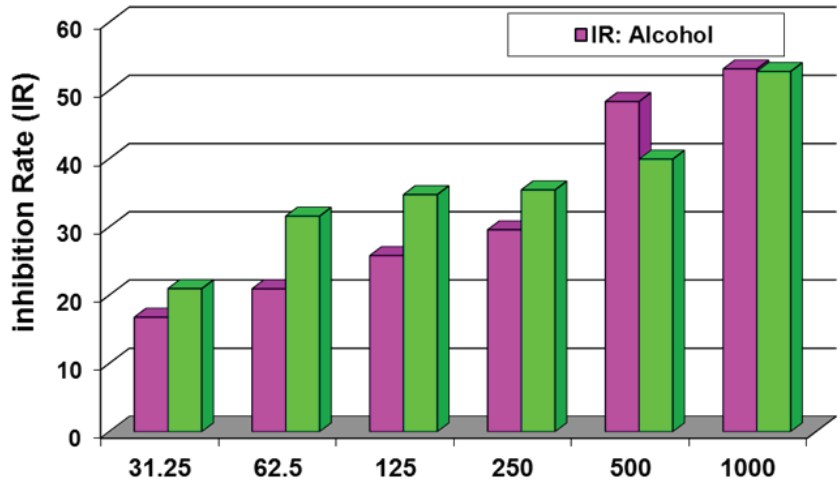


Figure 3. Comparison of the effect of aqueous andalcoholicextracts of *Cyperus esculents* tuberson the percentage of Inhibition rate of human breast cancer cell lines(MCF-(7 after24 hours of treatment.

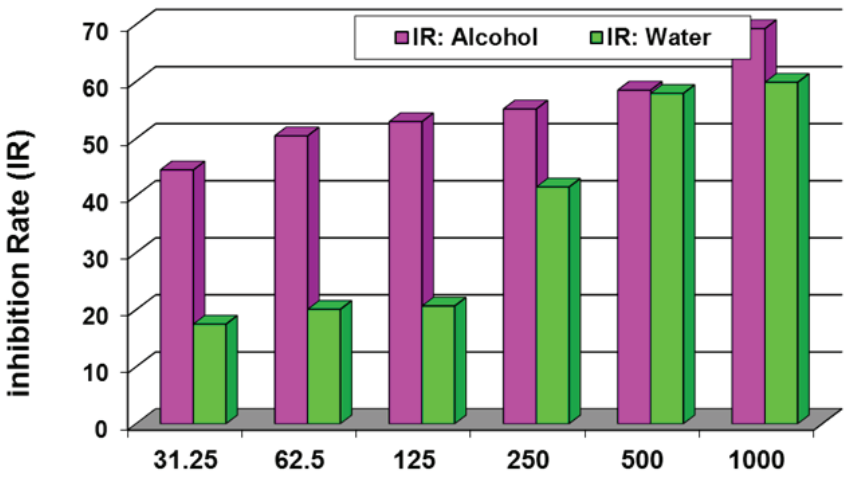


Figure 4. Comparison of the effect of aqueous and alcoholic extracts of *Cyperus esculents* tuberson the percentage of Inhibition rate of human breast cancer cell lines(MCF-(7 after 48 hours of treatment.

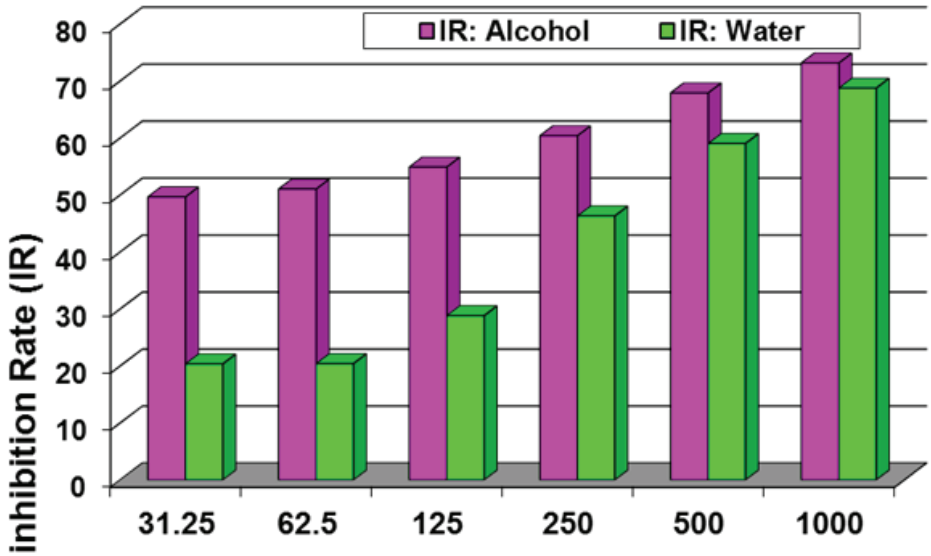


Figure 5. Comparison of the effect of aqueous and alcoholic extracts of *Cyperus esculents* tuberson the percentage of Inhibition rate of human breast cancer cell lines(MCF-7) after 72 hours of treatment

Discussion

Due to the importance of finding effective compounds against cancer and finding more types of plants that possess these compounds, and given the ability of crude extracts to inhibit the multiplication of cancer cells [7] , it was chosen that the *Cyperus esculentus* plant, which is one of the locally available medicinal plants, This is to identify the effects of crude aqueous and alcoholic extracts on human breast cancer cells (MCF-7) and the extent to which these extracts can be used as medical therapeutic substances against cancer in the future. The results of the current study when conducting confirmatory testing of the active compounds present in the alcoholic and aqueous extracts of the tuber plants showed the presence of most of the secondary metabolites that may together contribute to killing cancer cells more effectively as a result of the synergistic action among them, which may reduce the toxicity of the pure compounds used and that each one Among these plant secondary active compounds is an important role in inhibiting the growth of cancer cells outside and inside the body of the organism. It was also found through the results of the current study that the crude extracts of plant tubers played a role in killing cancer cells and inhibiting their growth and division outside the living body, which came similar to many studies, including [8].

The results showed that the toxic effect of *Cyperus esculentus* tubers on the human breast cancer cell line (MCF-7) depended mainly on the concentration used, exposure period and extract type, Alcoholic extract was the best in effect compared to the aqueous extract and for all exposure periods, This may be due to that the percentage of the active substance extracted with ethyl alcohol (70%) is greater than it is when using the aqueous extract and this is indicated by[9] and his group. A study conducted by[8] reached the presence of sterol in the extracts of tubers plant, which is one of the steroidal compounds that have anti-cancer effectsInterest has increased in recent years in flavonoids in which results of extensive research in the field of medicine and biology have shown their anti-cancer, anti-allergic, anti-viral, and anti-oxidant efficacy and other activities [10] Flavonoids are considered one of the most important active compounds extracted from medicinal plants Which is used in the treatment of many different diseases, most importantly cancer, because it possesses a high ability to act as antioxidants to resist the action of free radicals that are produced by pathological factors..

One of the first mechanisms studied was the antioxidant activity of these compounds. Phenolic compounds, including polyphenols, have multiple effects, including the antioxidant effects of cancer cell growth, due to their ability to scavenging the free radicals generated when normal cells turn into cancer cells. Flavonoids work to caught free radicals that lead to distortions of DNA, and thus mutations in tumor genes or inhibiting the appearance of tumors, Which is considered a precursor to the emergence of this disease [(11)] and is a substance (quercetin) of flavonoids called (Flavanols), which has proven its presence in the plant of dear love [(12). (13)] showed the ability of flavonoids to limit the spread of cancer cells in cellular lines through their association with estrogen receptor, and this enhances the ability of the *Cyperus esculentus* plant to inhibit the growth of cancer cells.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

Conclusion

The study found that there is a toxic effect of the crude aqueous and alcoholic extracts of the grain of *Cyperus esculentus* tubers in cancer cells (MCF-7) and of all the concentrations used, The highest percentage and a significant difference ($P \leq 0.01$) for the average of the inhibitory average of cancer cell growth (IR) was 68, 73 (%) for the aqueous extract and alcoholic extract, respectively, at the highest concentration of 1000 µg / mL and after 72 hours of exposure. The plant (Cyperus esculentus L) is one of the most promising medicinal plants that have an effective role in treating cancer through its inhibitory and toxic effect on cancer cells Mcf-7.

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Bacteriological Study and Investigation of Some Virulence Factors of *Proteus mirabilis* Bacteria Isolated from Urinary Tract Infection Patients in Ramadi City

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Abstract

Twenty isolates of *Proteus mirabilis* were diagnosed with 8.33% of the total of 240 samples that were isolated from urinary tract infections of patients returning to Ramadi General Teaching Hospital and Women's and Children's Hospital, after conducting biochemical tests and confirming them with VITEC 2, the results of the qualitative investigation of the UreR, ZapA, and MrpA genes showed that all isolates (20) of P. mirabilis had these three genes with a percentage of 100%. The results of the nitrogenous bases sequences confirmed the agreement of the diagnostic results of *P. mirabilis* isolates with the isolates recorded in NCBI using the(MEGA7) program with 100% match for each of the MrpA gene isolation number(17) and ZapA gene isolation number 12, as for the ZapA gene, isolation number (2), seven mutations of the type of equivalent transition were shown, and one mutation, a type of non-equivalent substitution, transversion, with a match ratio of 98%, while for the UreR gene for isolates No.(5)and No.(7), it showed four mutations equivalent to the same sites and for the same Sequence ID. With a similar ratio of 99%, it can be inferred in principle that the ZapA, UreA, and MrpA genes were found in the same genus but in other species and strains with the same sequence of nitrogenous bases.

Keywords: *UreR, Proteus mirabilis, PCR, gene*

Introduction

The urinary tract infection UTI is one of the most widespread infections and affects millions annually, the rate of infection among females is higher than that of males, and that urinary tract infection ranks second in terms of the number of infections after respiratory tract infection[(1)]. *Proteus mirabilis* is the most common pathogen for urinary tract infection (UTI), especially in individuals with urinary tract abnormalities or those using urinary catheters[(2)] The pathogenesis of these bacteria is associated with possessing many virulence factors which include the pili(Fimbria), Protease, Flagella,Urease Heamolysin,and multi-sugars adipose (lipopolysacchrie) and called Endotoxin[(3)]. The (UreR) gene can be used very accurately in diagnosing of P. mirabilis bacteria, which is responsible for the production of the Urease enzyme[(4)]. Urease enzyme produced from *P.mirablis* is characterized by being more active than urease enzyme produced from other types of bacteria , It works on changing PH urine to basic leading to deposition the magasium and calcium phosphate in the biofilm formed which in its turn leads to the formation of Crystallinbiofilm which is the more complex type biofilms for it works to close the catheter urinary and protect the bacteria from antibiotics causing failure to the treatment with antibiotics[(5)]. The gene that encodes the protease enzyme is coded for (Zap A) and possession of P. Mirabilis bacteria for this enzyme helps the bacteria break down the (IgA) antibodies[(6)]. The protease enzyme produced by *P. mirabilis* may be called IgA enzyme due to its activity on serum(IgA1) and (IgA2)[(7)].The(MR/P fimbriae) play a role in the virulence observed during UTIs caused by uropathogenic *P. Mirabilis* strains. Expressions of MR/P fimbriae are increased under

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oxygen limitation, and were suggest that MR/P fimbriae dictate the localization of bacteria in the bladder[8]

The aim of the study was to isolate and diagnose *P. mirabilis* from (UTI) patients and to investigate some genetically determined virulence factors using PCR, and then perform genetic sequences using the Gene Sequencing technique

Materials and Methods

Samples collection

240 samples of medial diuresis were collected from patients suffering from urinary tract infections for both sexes and for all age groups, and samples were taken from the visitors of the Ramadi General Teaching Hospital and the Women's and Children's Hospital in Ramadi for the period from 1/9/2019 to 15/2/2020.

Identification

P.mirablis was primarily identified through its cultivation, culture, and planning method on the medium of MacConkey agar, and observation of the phenomenon of swarming on blood agar and incubated aerobically at 37 ° C for 24 hours, then biochemical tests were performed and confirmed by VITEC2 device.

Genomic DNA Extraction

DNA was extracted from 20 samples of P. mirabilis isolates using a Promega Genomic Extraction Kit.

DNA Primers

The specific prefixes for the UreR, ZapA, and MrpA genes were provided by AlphaDNACanada company as shown in Table (1).

Table 1: Primers and size products.

Target Gene	Nitrogene base sequences 5' -3'	Molecular size bp	Refrence
ZapA-F	-ACCGCAGGAAAACATATAGCCC-3, - 5'	540	12))
ZapA-R	5' - GCGACTATCTTCCGCATAATCA-3,		
UreR-F	5' -GGTGAGATTTGTATTAATGG-3,	225	(22)
UreR-R	ATAATCTGGAAGATGACGAG-3, -,'5		
MrpA-F	5' -TTC TTA CTG ATA AGA CAT TG-3,	565	23))
MrpA-R	5'-ATT TCA GGA AAC AAA AGA TG-3'		

Table (2): Programs of PCR Thermocycling Conditions of gene.

Gene name	Temperature (°C) / Time					Cycles Number
	Initial Denaturation	Cycling Conditions			Final Extension	
		Denaturation	Annealing	Extension		
UreR	940C\4min	940C\40std	58 0C\30sec	720C\30sec	0C/5min 72	40
ZapA	95 0C\2min	30std \950C	59 0C/30sec	C/30sec 720	72 0C/5min	35
MrpA	940C\3min	940 C\30 std	400 C\30sec	720C\30min	720C/5min	40

DNA sequencing

PCR was used to amplify the samples. The results were sent to the Korean company Macrogen and compared with the information in the National Center for Biotechnology, the NCBI. The results were read using the Blas Basic Local Alignment search tool.

Results and Discussion

240 urine samples were collected from patients suffering from urinary tract infections and 200 samples showed a positive growth rate of 83.33% and 40 samples with a rate of (16.67%) negative growth. There were high significant differences between males and females, so there were (135) females (67.6%) and (65) Males (32.40%) and showed the return of 20 isolates of *P. mirabilis* bacteria (8.33%). A study[9] agreed, when they isolated P. mirabilis bacteria from different sources and the percentage of its isolation from urine was(10.7%).

The initial diagnosis of *P. mirabilis* was made by observing the growth of single, pale colonies on MacConkey agar and non-fermented lactose blades with soft and medium-sized edges. On the center of the blood agar, the swarming or anthropogenic odor was observed and it had a distinctive smell similar to the smell of rotten fish.

Microscopic examination of P. mirabilis indicated that it is a short Gram-negative bacillus that does not form spores.

The polymerase chain reaction was performed to detect the UreR gene using a specialized primer for this gene to detect its presence in (20) isolates of *P. mirabilis* which has a volume (225bp), the isolates were relayed (20), and the results of the UerR gene detection showed the presence of this gene in all isolates of *P.mirabilis* bacteria, as shown in **Figure (1)**, this is normal because *P. mirabilis* has a urea-induced enzyme that breaks down urea in urine into ammonia(NH_3) and carbon dioxide (Co_2) which raises the environmental pH, and mediates precipitation of normally soluble polyvalent ions from the urine, specifically precipitation of ammonium, phosphate , calcium, and magnesium ions results in formation of the struvite and carbonate hydroxyapatite crystals that comprise urinary stones[24]. The results of the current study indicated that the percentage of UreR presence is 100%, and it was in agreement with [10] and also similar to what was stated in[11].

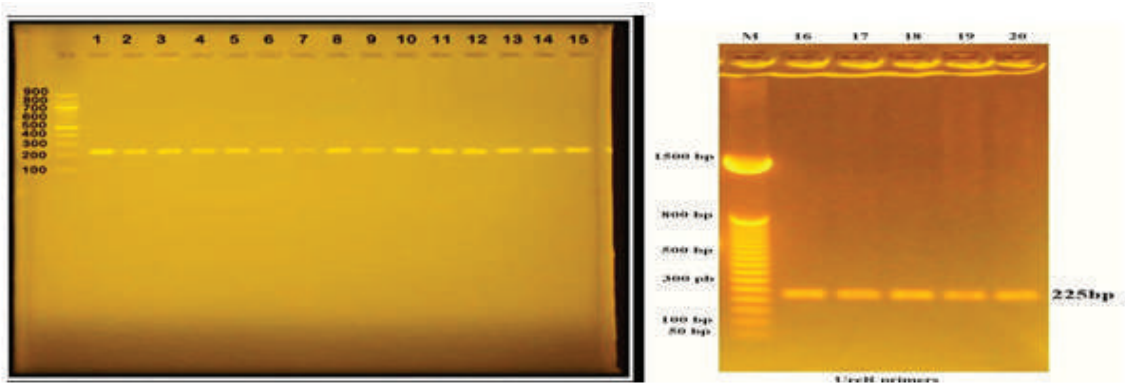


Figure 1:Results of the amplification of UreR gene of proteus mirabilis samples were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 50bp DNA ladder marker. Lanes 1-20 resemble 225bp PCR products.

The ZapA gene was detected by a PCR test using PCR reaction mix primers to detect the ZapA gene which has a volume of 540 bp, then the 20 isolates of *P. mirabilis* were relayed, and the bundles in all isolates showed evidence of the mentioned bacteria's possession of the ZapA gene, and the results of the detection of the ZapA gene were 100%, as shown in **Figure (2),** it agreed with each of the studies [12], [7] and [17], and converged greatly with the results of [14], as the percentage reached 98.2% and disagreed with [15] and [19], as the percentages reached 44.5% and 45.8. %, respectively.

From this it is evident that *P. mirabilis* is specifically able to produce (ZapA) and it has been detected in the urine of patients with UTI, while the non-pathogenic strain of other types of Proteus is less efficient in producing this type of enzyme [7].

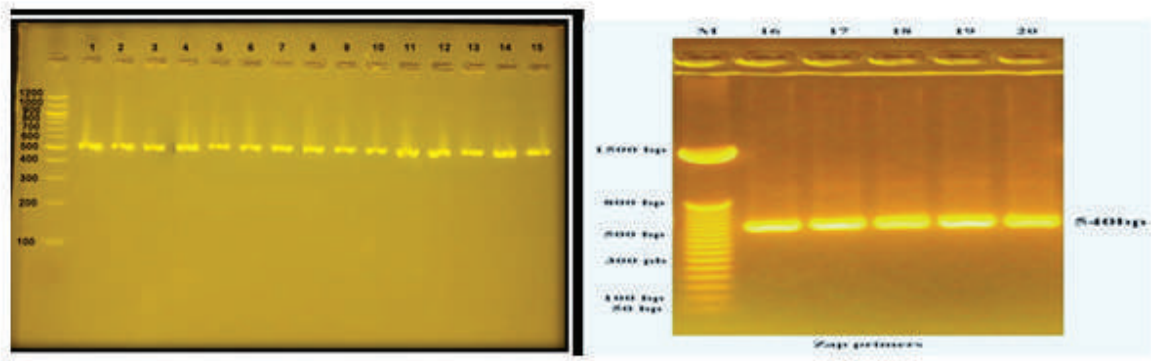


Figure2: Results of the amplification of ZAPA gene of proteus mirabilis samples were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 50bp DNA ladder marker. Lanes 1-20 resemble 540bp PCR products.

A PCR test was conducted to check for the presence of the MrpA gene using specialized primers for this gene for 20 isolates belonging to P. mirabilis bacteria with a size of (565bp), and its presence was 100% as shown in **Figure (3)**, it was agreed with the results of [18] and converged with [13], as the percentage reached 80% ,while [16] confirmed that the percentage of this gene in its isolates reached 90% while it disagreed with [19]. It was found that 45.8% of its isolates possess the MrpA gene and the ZapA gene in P. mirabilis bacteria and not possessed by P. vulgairs, and [20] was found that 6 of the 17 isolates have the MrpA gene, or 35%.

The importance of this type of fimbria is attributed to its role in adherence to uroepithelium cells and thus contributes significantly to urinary tract infection [18] and this is confirmed by a study [21] which revealed the ability of this type of pili(Fimbria)to adhesion, specifically adhesion to uroepithelium cells, tubular and renal epithelial cells. To say that the virulence factors of P. mirabilis have evolved to adapt to the host's environment and increase our understanding of the ability of natural flora to colonize and infect the urinary tract.

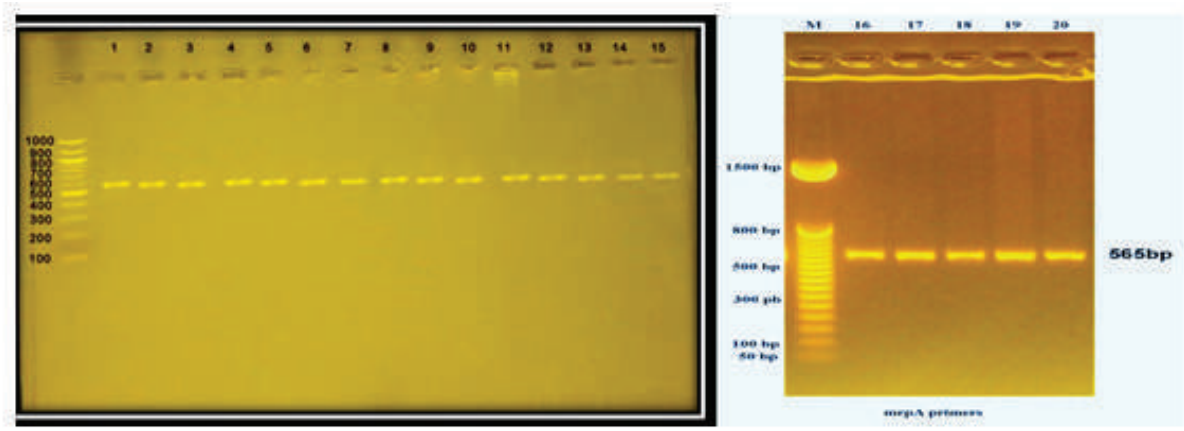


Figure 3:Results of the amplification of mrpA gene of proteus mirabilis samples were fractionated on 2% agarose gel electrophoresis stained with Eth.Br. M: 50bp DNA ladder marker. Lanes 1-20 resemble 565bp PCR products.

The results of the DNA sequences of isolates (12) and (2) of *P.mirabalils* were shown for the purpose of determining the sequences for the nitrogenous bases of the ZapA gene, which were compared with three sequences recorded in the National Center for Informatics (NCBI) using (BLASTN2.6.4), As shown in **Table(3)** ,the results of the comparison with the first sequence that carries the symbol MG748837.1, which is related to *P. merabilis*, showed that there are variations in the sequence of nitrogenous bases and there are eight mutations, seven mutations of the equivalent transition type in which the(C- T) mutation, (T- C) mutation,(G-A) mutation in situ,(A- G) mutation,(G- A) mutation,

(T- C) mutation, and(T- C) mutation at sites(525, 537, 549 , 558, 564. 567,570) sequences and one mutation of an unequal substitution type, so (A- T) was at site (535) and the match was 98%, while a comparison with sequences bearing the code JQ040548.1 for the UreR gene in isolation No. 7 showed that there were variations in the sequence of nitrogenous bases and found two equivalent transition mutation, the first in which(T- C) is transformed at site 108 and the other is an (A- G) mutation at site 220 with a 99% match as found. In isolation No. 15 of the same gene with the same code JQ040548.1, there are also two mutations, a type of equivalent transition(T- C) and (A- G), and the same sites 108, 220, with a match ratio of 99%.

While isolates No. 17 of the MrpA gene and No. 12 of the ZapA gene did not show any genetic mutations , with a match ratio of 100%.

The emergence of some types of mutations in the same strain is attributed to its exposure to mutagenic agents or because of the place of isolation, while strains belonging to other species were found that contain the same zap gene and with the same repetition of the sequence of nitrogenous bases due to the possibility of repeating the gene with the same order of nitrogenous bases between the mentioned models in light of the results of the current study. It is inferred that the genes that showed the mutations can replicate the different types of *P. mirabilis* bacteria with genetic variants as a result of the mutations and these results give an impression after comparisons between genes there are similarities for the repetition of genes between P. mirabilis types and with the same sequence of nitrogenous bases for the mentioned genotypes.

Table 3 : Type of substitution

No. Of sample	Type of substitution	Location	Nucleotide	Range of nucleotide	equence ID
ZapA(2)	Transition	525	C-T	290 to 671	MG748837.1
	Transversion	535	A-T		
	Transition	537	T-C		
	Transition	549	G-A		
	Transition	558	A-G		
	Transition	564	G-A		
	Transition	567	T-C		
	Transition	570	T-C		
UreR(7)	Transition	108	T-C	53 to 225	JQ040548.1
	Transition	220	A-G		
UreR(15)	Transition	108	T-C	54to 225	JQ040548.1
	Transition	220	A-G		
ZapA(12)	---------	-------	-------	389 to 753	MG748837.1
MrpA(17)	---------	-------	-------	6 to 381	MT294143.1

Conclusions

P.Mirabilis bacteria is one of the most important causes of urinary tract infection because it has many virulence factors, including urease, protease and fimbriae, which increase its pathogenicity, and this is what was shown by the investigation of the genes of the aforementioned bacterium virulence factors that it possesses these genes in high proportions as well as the comparison of the UreR, MrpA, ZapA gene sequence. is a suitable method for inferring the genetic relationships of P. mirabilis isolates on a molecular basis and in the future. By studying the genetic map, it is possible to learn more about *P. mirabilis* .

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Molecular and Parasitological Study on Selected Opportunistic Intestinal Parasites in Immunocompromised Patients

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Abstract

The common opportunistic parasites which cause morbidity and/or death in immunocompromised populations are predominantly gastrointestinal ones. This study explained the molecular and parasitological aspects on selected opportunistic intestinal parasites in selected groups of immune compromised patients from Al-Anbar hospitals and private laboratories.

In the current study, were taken (130) stool samples from immune compromised patients divided into three groups (50) chemotherapy recipient, (40) chronic renal failures, (40) diabetic Mellitus, and (30) samples apparently healthy as control. A questionnaire was filled out on each subject including all personal and medical history. Stool samples were collected from all groups for detection of the parasites.

The results were displayed the ratio of opportunistic parasites in (55.3)% of patients, and healthy control was (3.3)%. The results showed that the highest group had parasites in those with chemotherapy (64)%, chronic renal failure (57.5)%, and diabetic (42.5)% individually. The highest rate of infection appeared with Blastocystis hominins(26.1%), Cryptosporidium(22.3%), and Cyclospora cayetanensis (8.4%). The age of the patient was ranged between 4-66 years, the Mean was 47.5 and deviation was± 9.12, also, the weight of the patient was ranging between 14-85, the mean was 70.3 and deviation ± 8.07. Three cases of mixed infection were detected.

Keywords*: Immuno compromised patients, Cryptosporidium, Cyclospora, Blastocystis, Opportunistic parasites, PCR.*

Introduction

The immunocompromised host is commonly defined as an individual who suffered from one or more defects in the normal defense mechanisms which keep the people from infectious, that causes predisposing the individual to an increased risk of severe life-threatening infections [1]. The particular immune response to parasites guides the generation of antibodies. The infection occurs by protozoan parasites was linked with the creation of the IgM and IgG[2]

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Intestinal parasitic infections are considered as a worldwide endemic and have been described as one of the extreme constituting worldwide causes of illness and disease[3]. An estimated about 3.5 billion individuals are affected and nearly 450 million persons suffered from these infections, the children is most of them constitute [4]. Cryptosporidiosis is a significant enteric parasitic infection between infant and children in developing countries [5;6]. Diarrhea and abdominal pain are the most important clinical signs[7]. The infection is acquired by ingestion of mature oocyte[8]. Cyclospora is the protozoan with many species and considered as an important parasite developing cause of diarrhea globally that lead to an important cause of morbidity and mortality [9]. Only Humans are reported to be infected by a single

species of this parasite, C. cayetanensis [7;8]. Blastocystis hominis is a genus containing a single-celled intestinal parasitic protist [11]. The Blastocystis species generally colonize the GIT of humans and a range of the additional animal [12].

Materials and Methods

Our study was applied on (130) of immunocompromised patients, They were categorized into three groups: (50) chemotherapy recipient, (40) chronic renal failure, (40) diabetic Mellitus and (30) control. All of the samples were subjected to direct stool and PCR examinations. The Fresh samples were collected in plastic containers, which were cleaned and labeled

The Samples were tested macroscopically. The stool samples were examined directly by wet mount (normal saline 0.85%) and staining by (MZN), after that each stool sample was kept stored in a deep freeze at (-20) until it used for (DNA) extraction and Polymerase chain reaction (PCR) according to [12;13]

In the present study, we used three sets of primers (forward and reverse) for each parasite. Final concentration according to manufacturing company Integrated DNA technologies (IDT) USA , these primers include:-

Bcowp F (5′-ACC GCT TCT CAA CAA CCA TCT TGT CCT C-3′), Bcowp R (5′-CGC ACC TGT TCC CAC TCA ATG TAA ACC C-3′)for Cryptosporidium, and F (5′ GCAGTCACAGGAGGCATATATCC-3′), R (5′-ATGAGAGACCTCACAGCCAAAC-3′) for Cyclospora , and SB82 F(5 TCTTGCTTCATCGGAGTC-3), SB82 R (5 CCTTCTCGCAGTTCTTTATC-3)for Blastocystis,. The Programs of the PCR generally according to [14] and for Cryptosporidium, Cyclospora, Blastocystis according to [15-17] respectively, with slight modifications.

Result

This study was to clarify the distribution of selected opportunistic parasites among immunocompromised patients was 74(56%) and in control 1(3.33%). According to gender there are 80 male and 50 female, the positive samples was 47(58.7%), and 27(54%) correspondingly .The ratio of the positive opportunistic parasite in immunocompromised patients was 16(26.2%) and 58(84.0%) in those of filter and tap water respectively. According to residential area, the calculation of positive sample was 24(33.3%) in urban and 50(86.2%) rural area. The percentage of the positive cases was 58 (44.6%) and 16 (35.5%), in diarrheic and non- diarrheic respectively.

The prevalence of cryptosporidium in the immunocompromised patient was 29(22.3%) distributed as follow 12(24.0%) in chemotherapy, 9(22.5%) in chronic renal failure, and 8(20.0%) in diabetic patients.

The frequency of Blastocystis hominis in the immunocompromised patient was 34(26.1%) dispersed as follow 15(30.0%) in chemotherapy, 11(27.5%) in chronic renal failure, and 8(2.5%) in diabetic patients.

The occurrence of Cyclospora cayetanensis in the immunocompromised patient was 11(8.4%) distributed as follow 7(14.0%) in chemotherapy, 3(7.5%) in chronic renal failure, and 1(2.5%) in diabetic patients.

Table (1) Total cases of immunocompromised detected by the direct exam.:

immunocompromised * direct exam Cross tabulation						
			direct exam		**Total**	**p value**
			+ve	**-ve**		
immuno-compromised	chemotherapy	Count	14	36	50	0.000
		% within immunocompromised	28.0%	72.0%	100.0%	
	chronic renal failure	Count	11	29	40	0.000
		% within immunocompromised	27.5%	72.5%	100.0%	
	diabetic	Count	10	30	40	0.000
		% within immunocompromised	25.0%	75.0%	100.0%	
Total		Count	35	95	130	
		% within immunocompromised	26.9%	73.1%	100.0%	

Table (2) Total cases of immunocompromised detected by PCR:

immunocompromised * PCR exam Cross tabulation						
			PCR exam		Total	p value
			+ve	-ve		
immuno-compromised	chemotherapy	Count	34	16	50	0.011
		% within immunocompromised	68.0%	32.0%	100.0%	
	chronic renal failure	Count	23	17	40	0.034
		% within immunocompromised	55.0%	45.0%	100.0%	
	diabetic	Count	17	23	40	0.041
		% within immunocompromised	42.5%	57.5%	100.0%	
Total		Count	74	56	130	
		% within immunocompromised	56.9%	43.1%	100.0%	

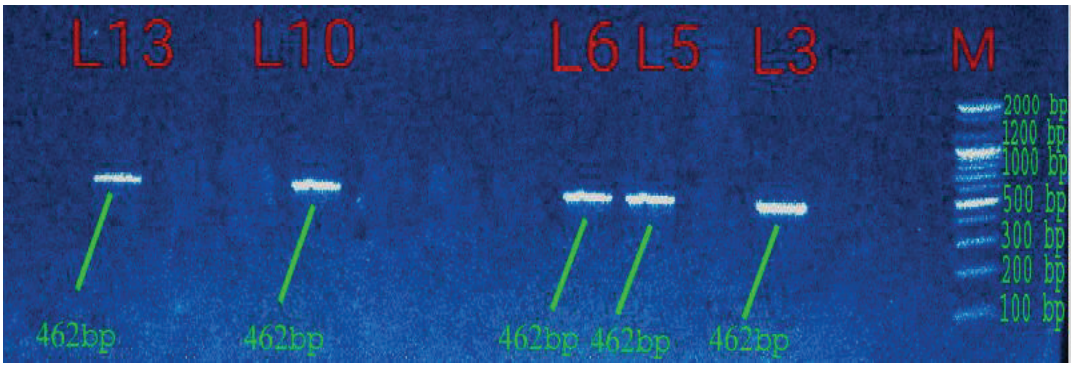


Figure (1): Agarose gel electrophoresis (2%) with ethidium bromide staining. the positive Blastocystis hominis gene (SB82) 462bp, the positive sample in line(L3,5,6,10 and 13) DNA ladder with (100-2000bp) on the right (M) was used as DNA molecular weight marker.



Figure (2): Agarose gel electrophoresis (2%) with ethidium bromide. the positive cryptosporidium (BCOWP) gene(769bp). the positive sample showed in line(L3,5,11,12,13,14,and 15). DNA ladder with (100-2000bp) on the right (M) was used as a DNA molecular weight marker.



Figure (3): Agarose gel electrophoresis (2%) with ethidium bromide staining. the positive Cyclospora cayetanensis(CCITS2)gene (116bp), the positive sample was shown in the line(L1 and L3) DNA ladder with (100-2000bp) on the right (M) was used as DNA molecular weight marker.

The specificity and sensitivity test was done to determine the test's ability to identify affected and non-affected individuals correctly, the sensitivity and specificity for microscopic examination was (36.4%) , (91.1%) while for PCR examination was (68.2%), (64.4%) respectively.

Discussion

In the current study, we take (130) stool samples from immunocompromised patients were divided into three groups, (50) chemotherapy, (40) chronic renal failures, (40) diabetic Mellitus, and(30) samples from healthy people as a control. Among 130 studied patients, 80 (61.5%) were male and 50 (38.5%) were female. The results of the current study were displayed that

the ratio of opportunistic parasites in (55.3)% among immunocompromised patients and (3.3)% in the healthy control. Our study was agreement with [10;18;19] , While disagreement with [20]. The diversity between the result may be due to the way used for diagnosis, group of a patient that selected for analysis, or genetic ethnic diversity.

In our study, the highest group was affected with opportunistic parasites was treated with chemotherapy (68%), and (57),(42.5) in chronic renal failure and diabetic respectively. Our result was agreed with [10;21]. Who reported the high infection with an opportunistic parasite in a patient with cancer followed by renal failure and diabetic respectively, while disagreed with [22] that reported the highest group affected was in patients with corticosteroids, followed by chronic renal failure, malignancy, and diabetic patients. The dissimilarity between results maybe because the differences in the size, number of sample, or because the advance technique used for diagnosis(researchers). In malignant patients treated by chemotherapy, found that suppression of antibody production and prevention of multiplication of the stimulated B-cells were occurred, while the immunoglobulin levels (Igs) were not affected in diabetic patients [23].

In the current study the result according to gender was covenant with [23;24;10;25] whereas disagreement with [26;27], that was found that the prevalence of parasite was higher in female than male .

Our result found that the Blastocystis hominis was the most common parasite in the immunocompromised patient which represented 26.1%, which was an agreement with [28;18] while disagreement with [29;30]. The variation between the results may be due to the difference in the area of study, selected group, difference in the residential area of the patient selected or the way used in the diagnosis (researchers).

In the present study 29 (22.3%) from (130) stool sample of immunocompromised patient was positive for cryptosporidium infection which represented the second parasite detected in our study, the highest group of an immunocompromised patient infected was those in chemotherapy (24%), and (22.5%),(20%) in chronic renal failure and diabetic respectively, the p-value was < 0.05 there was a significant difference between the Cryptosporidium and all group of an immunocompromised patient, The present study was an agreement with [31-33] whereas disagreement with [29;34]. The alteration may be due to the difference in the life style , number of samples (researchers).

In the current study from 130 stool sample found that 11(8.4%) sample was positive for Cyclospora cayetanensis, the rate of infection was distribution as 7,(14%), 3,(7.5%) and 1,(2.5%) in chemotherapy, chronic renal failure and diabetic respectively, the p-value was < 0.05 there was a significant difference between Cyclospora cayetanensis and all groups of immunocompromised patient. This result was agreement with [29;32] although disagreement with [10;30]. The differences may be attributed to environment, genetic diversity and personal hygiene (researchers).

In our study, we detected three cases of mixed infection with cryptosporidium and Cyclospora in patients with chemotherapy and one with chronic renal failure.

From 130 stool sample, the number of all positive sample that detected by (MZN) and wet mount was 35 (26.9%), in chemotherapy 14(28%) chronic renal failure and diabetic 11(27.5%),10(25%) respectively. our result agreement with the [35 ; 36] whereas discordant with [21].

In this study, the ratio of the positive sample of opportunistic parasite by PCR in all group was 74 sample (56.9%),34(64%) chemotherapy, 23 (57.5%) chronic renal failure patient, and 17(42.5%) diabetic sample . This disagreed with [24], the variance may be due to the difference in the number of primer sequences or specificity of primer, or accuracy of PCR procedure and concentration , purity of DNA(researchers).

This disparity in the results of microscopic examination and PCR assay, Such disappointments might be below DNA levels, and presence of a solid wall prevent the release of DNA from parasite or PCR inhibitors in some of the fecal samples such as lipids, bile salts, polysaccharides from mucus, bacteria and food degradation product or disintegration of parasite during storage [37].

According to our results , we concluded that there was a significant difference between PCR and MZN

stain and the most group affected with opportunistic parasites those treated with chemotherapy and Blastocystis hominis was the most prevalent parasite in immunocompromised patients.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Immunocytochemistry of *In vitro* produced mucin from HT29-MTX cell line

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Abstract

Mucus represents the first line of defense against pathogens and damage in the human intestine. the major components of mucus are mucins which are high molecular weight glycoproteins providing potential binding sites for both pathogenic and commensal microorganisms. To study host-enteric pathogen interactions, human intestinal cell models are widely used. Epithelial cell lines derived from human colon carcinomas providing useful experimental alternatives as getting normal intestinal cells is not easily applicable.

In the current study, we described a condition enhances HT29-MTX cells to produce continuous mucus layer. Immunocytochemistry was used to visualize Muc 2, the most secreted mucin from HT29-MTX cells using anti Muc2 antibody.

The results showed that incubation of HT-29 MTX cells with shaking and providing cells with fresh medium once to twice daily for three weeks can be the best way to mimic the natural behavior of the intestine which enhanced the seeded cells to produce the mucin *in vitro*.

Keywords: *HT29-MTX cells, Mucus, Mucin, Immunocytochemistry.*

Introduction

Goblet cells are simple columnar epithelial cells found in the lining epithelium of the stomach, intestine (with highest density in the colon), respiratory tract and genital tract. They secret the mucin which is forming a gel covering layer protecting the mucosa[1-6]. The slow, continuous liberation of mucin from a certain goblet cells over the epithelium is renewing and maintaining the mucus blanket under the normal conditions [1,6,7]. However, different irritants are known to induce the mucus secretin from the goblet cells, which built a protecting layer to the affected surface [1,2,6]. These irritants found to increase the activity of goblet cells mucus secretion. They include chemical substances such as ammonia, sulfur dioxide, smoke [1], or biological such as proteases, bacterial toxins [2,6-9], parasitic and viral infections [10], or mechanical insults or traumas[1]. In addition, acetylcholine found to be induce mucus secretion in most studies of large and small intestines in laboratory animals and human colon [5,11].

The continuous slow secretion of mucus in the normal mucosa sustain the mucus blanket which has a significant biological importance in protecting the lining epithelium against different challenges, such as some dangerous particulates [1,12], binds toxins [1,13], microorganisms [1,10,14] and antigenic macromolecules [1,13]. Generally, mucus is controlling the invasion and adhesion of different pathogens on the surface of the epithelial cells [4].

Goblet cells observed to show some variations when response to some infections. including its mucin secretion. Changes like goblet cells hyperplasia and increased mucin secretion in addition to some changes in mucin glycosylation are the main changes have been

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observed [10].

The main component of goblet cell mucus is the mucin, the viscoelastic and protective components of mucus [6,15-18]. Mucus also includes other components such as water, ions, some immune system components like the IgA and anti-microbial peptides which serve in removing the infected pathogens and providing a physical barrier protecting epithelial cell surfaces from the insults [7,10,12,17,19,20].

To study the host-enteric pathogen interactions, human intestinal cell models are widely used for this reason as. Epithelial cell lines derived from human colon carcinomas providing useful experimental alternatives as getting normal intestinal cells are not available [4,21]. Different cell lines were used to study the functional and specific characteristics of gut epithelium [4]. The Caco-2 and HT-29 human intestinal cell lines which are isolated from colon adenocarcinomas are the most widely used cell lines for *in vitro* attachment and mechanistic studies [4,22-24]. Caco-2 cell line grow as monolayers in culture and differentiates into highly similar cells to that of the normal enterocytes in the intestinal epithelium [4,24]. Whereas, HT29-MTX cells differentiates to a goblet cell-like phenotype and are able of secreting low amounts of MUC2 mucins which is normally secreted in the small and large intestine [3,4,7,20,24,25]. Therefore, HT29-MTX providing a model system for the study of human goblet cell differentiation and mucin secretion [21,24]. Accordingly, this study aimed to find a new method and a suitable condition to induce the HT29-MTX cells to produce mucus layer and visualize this layer using immunocytochemistry (ICC) technique as visualization of mucin helps in studying host-pathogen interactions at the mucosal surface.

Methods

-Cell lines

Caco-2 cells and HT29-MTX cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum (HIFBS), 5 mM L-glutamine, 50 µg/ml streptomycin and penicillin in an atmosphere containing 5% CO_2 at 37˚C.

-Growth conditions

1. HT-29MTX cells at a concentration of 2×10^4 cells/ml were seeded on coverslips in a 24 well plate and incubated for three weeks. Cells were fed with fresh medium every two days.

2. HT-29MTX cells at a concentration of 2×10^4 cells/ml were mixed with Caco2 cells at a concentration of 7.5 x 10^4 cell/well in a ratio of 1:1 and 1:3 and seeded on coverslips in a 24 well plate and incubated for three weeks**.** Cells were fed with fresh medium every two days.

3. HT-29MTX cells at a concentration of 2×10^4 cells/ml were seeded on coverslips in a 24 well plate and incubated for three weeks fed with fresh medium every two days. Cells were infected with Enteropathogenic *E. coli* (EPEC) for an hour.

4. HT-29MTX cells at a concentration of 2×104 cells/ml were seeded on coverslips in a 24 well plate and incubated for three weeks fed with fresh medium every two days. Cells then treated with Capsicum extract in DMEM medium for an hour.

5. HT-29 MTX at a concentration of 2×10^4 cells/ml were seeded on coverslips in a 24 well plate and incubated for three weeks with shaking 80-100 rpm/min and fed with fresh medium once to twice daily.

-Immunocytochemistry (ICC)

The fixed HT-29 MTX cells were treated with 0.1% Triton X-100 for 15 minutes, then 1 ml of 1% bovine serum albumin (BSA) was added for an hour. Anti muc 2 antibodies produced in mouse was added in blocking buffer 1:400 for an hour, then cells were washed three times with blocking buffer. In dark, the secondary antibody anti-mouse IgG FITC 1:1000 was added for 1 hour. Cells then washed with PBS three times, followed by adding concanavalin A 25µg/ml for 20 minutes. Lastly, cells were washed, and coverslips were mounted using an anti-fade medium.

-Infection of HT29 MTX cells with pathogenic bacteria

Enteropathogenic *Escherichia coli* (EPEC) was grown in LB broth containing ampicillin at a concentration of 100µg/ml the day before the experiment. HT29 MTX cells at a concentration of 2×10^4 cells /ml were seeded

on coverslips in a 24 well plate and incubated for three weeks**.** Bacteria were added at 100 MOI and the plates were incubated at 37℃ for 1 hour. After bacterial infection, DMEM medium was removed and HT29- MTX cells were washed with PBS and stained using ICC staining as mentioned above.

-Preparation of Capsicum extract

Capsicum dried fruit powder was extracted using hot water. A 250 ml distilled water was added to 50gm capsicum powder and heated to 120-150ºC for 30 minutes. The extract was filtered using Whitman filter paper and sterilized using 0.2µm Millipore filter.

Results

The results showed that using agitation during cells incubation period had significantly increased the mucus secretion in the seeded cells as it can be seen in figure 5. However, incubation of HT-29 MTX cells with and without Caco2 cells, infection of HT29-MTX cells with Enteropathogenic *E. coli* (EPEC) and treatment of HT29-MTX cells with Capsicum spp (chili) did not show a clear enhancement in mucus production (Figures 1, 2, 3 and 4).

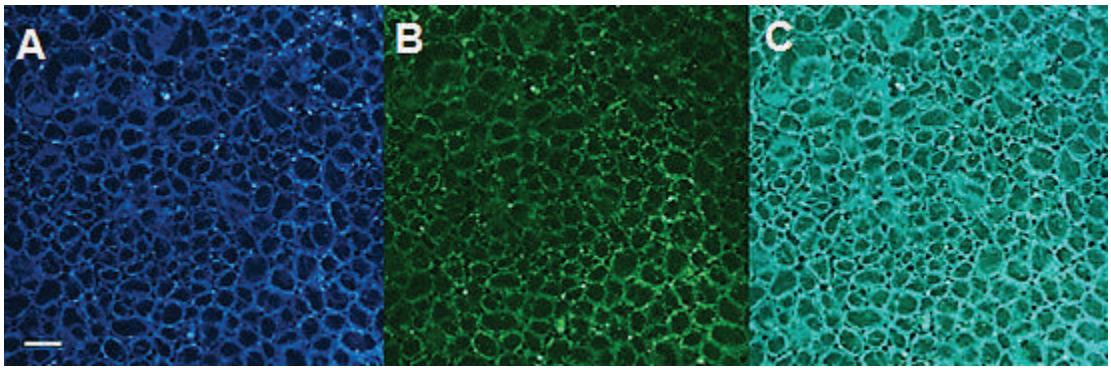


Figure (1): Immunocytochemistry staining of HT-29MTX mucin. The image displays: (A) cell membrane, (B) mucin and (C) merged image, scale bar 20 µm.

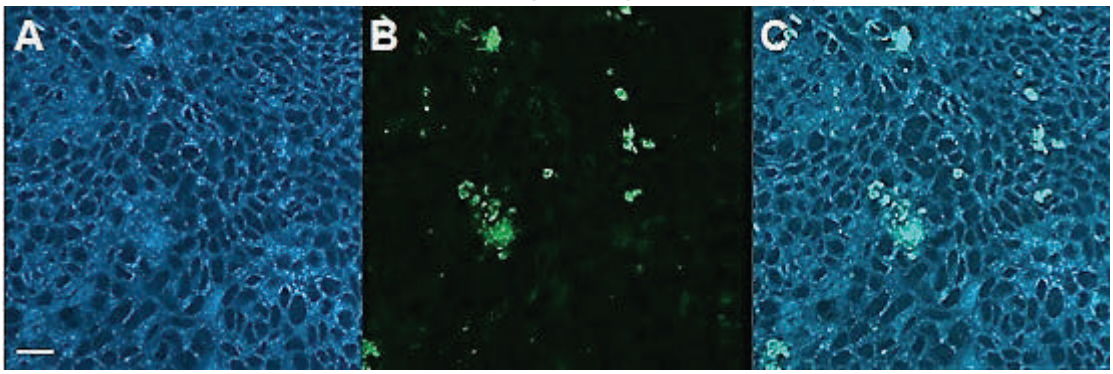


Figure (2): HT-29MTX cells and Caco2 cells cultured together for three weeks. The image displays: (A) cell membrane, (B) mucin and (C) merged image, scale bar 20 µm.

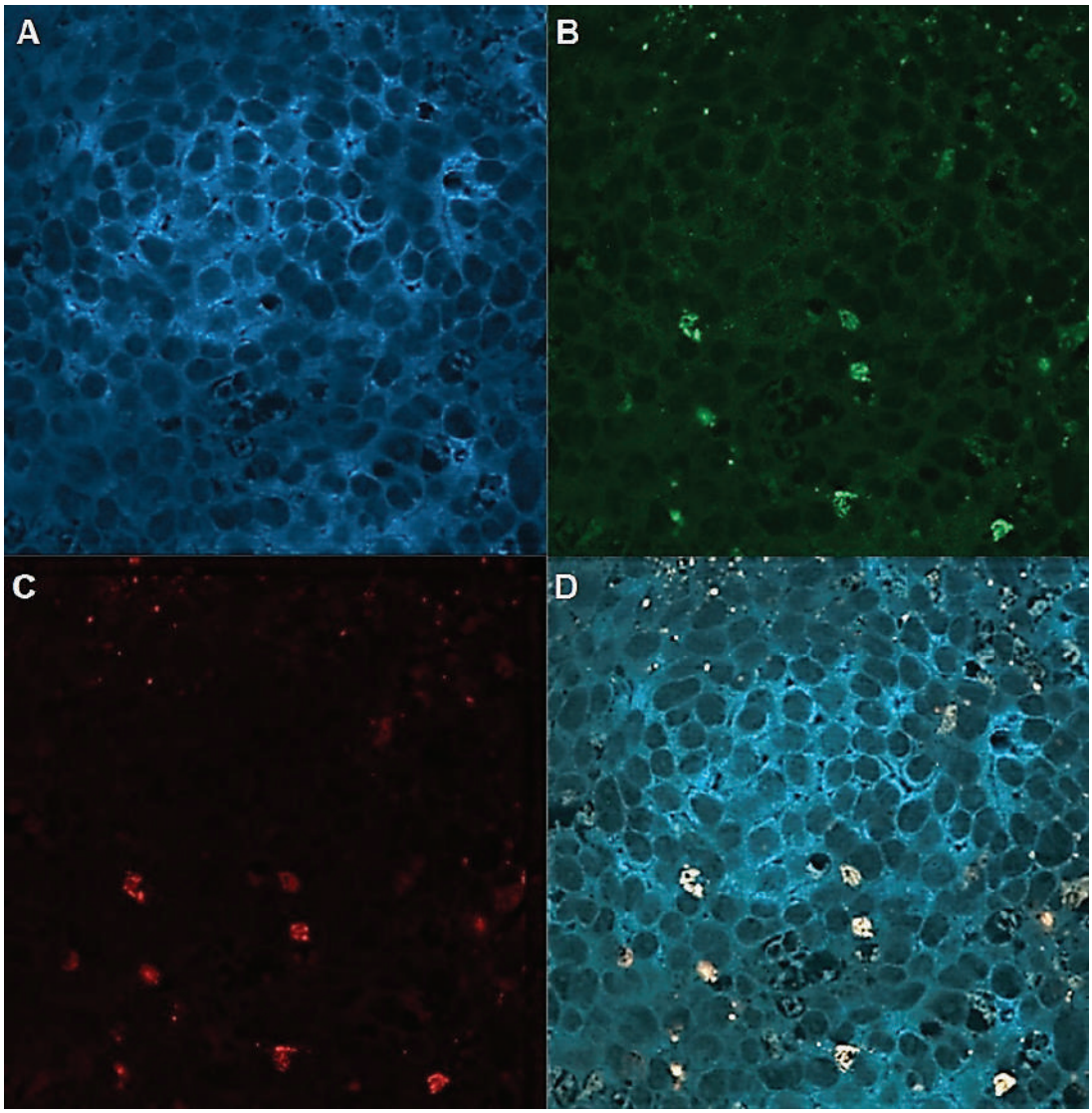


Figure (3): HT-29MTX cells infected with EPEC. Cells were infected with EPEC expressing mCherry for 1 hour. The image displays: (A) cell membrane, (B) mucin, (C) EPEC1 expressing m Cherry (Red) and (D) merged image, scale bar 20 µm.

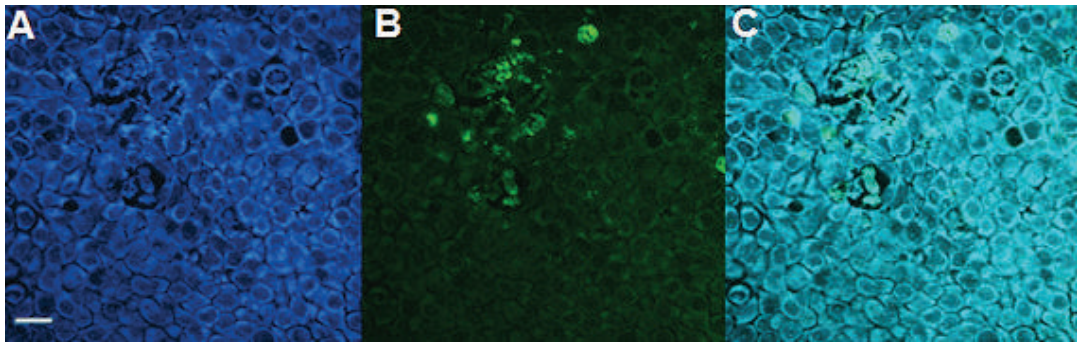


Figure (4): HT29-MTX cells after treatment with capsicum extract. The image displays: (A) cell membrane, (B) mucin and (C) merged image, scale bar 20 µm.

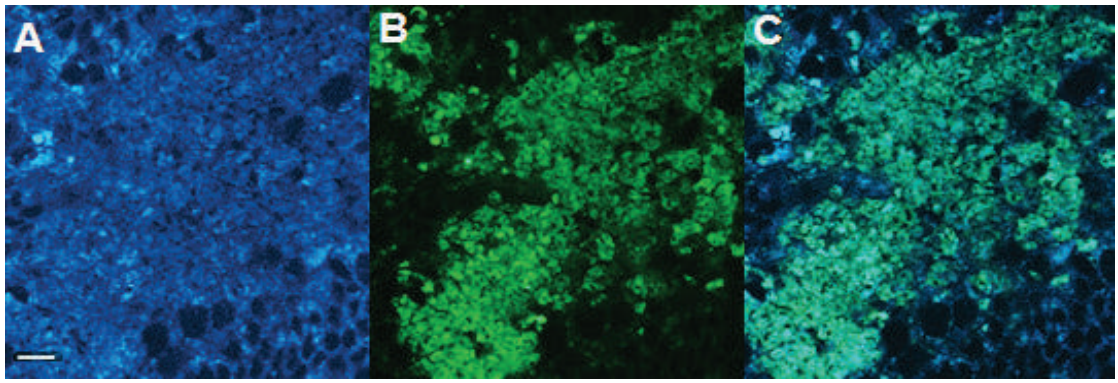


Figure (5): HT29-MTX cells cultured for three weeks under Agitation. The image displays: (A) cell membrane, (B) mucin and (C) merged image, scale bar 20 µm.

Discussion

Mucus represents the first line of defense against pathogens and damage. Major components of mucus are mucins, which are high molecular weight glycoproteins that provide potential binding sites for both pathogenic and commensal microorganisms. MUC2 and MUC5AC are the main constituents of the mucus in the intestine and stomach, respectively which are both produced by the goblet cells [6,7].

Here, we described a condition to induce a mucus layer production from HT29-MTX cells, then we stained the produced mucin using the ICC technique to visualize this layer. The anti muc 2 antibodies produced in the mouse were used in the ICC which binds to mucin. Whereas the secondary antibody (anti-mouse IgG FITC) binds to the primary antibody that allows the visualization of the mucin under the fluorescence microscope. Concanavalin A was used to visualize the cell membrane. Concanavalin A interacts with the glucosyl and mannosyl groups on the cell membrane [26].

Incubation of HT29-MTX cells for three weeks with feeding every two days results in the production of a few spots of mucus layer on cells (Figure 1). Thus, a variety of treatments were used to enhance mucus production.

Firstly, a co-culture of human Enterocytes (Caco-2) and goblet cells (HT29-MTX) were seeded at a concentration of 1:1 and 1:3. It has been reported that the proportion of goblet cells in the human colon is 25-55% and the mixed culture of Caco-2 and HT29 leads to a morphology similar to the small intestine [27,24]. Herein, HT-29MTX cells and Caco2 cells were mixed, seeded and incubated for three weeks with feeding every two days. Yet, the observed result revealed that no continues mucus layer was seen (Figure 2).

Kim *et al.* (2003) found that encounter with pathogens and their products can result in mucin production. In addition, Linden *et al*. (2007) reported that co-cultures consist of pathogen-mammalian cells were used to study induction of pathogens adhesion, invasion and host signaling. But cell lines usually used for host-pathogens studies were variable in mucins expression with low mucin production. Therefore, the second condition in the current study involved infection of HT29-MTX cells with Enteropathogenic *E. coli* (EPEC). Despite the previously reported data, it has been found that this treatment did not improve mucus production, Figure 3.

Moreover, it has been reached that treatment of rat's lung by capsaicin can result in mucus secretion [30,31]. Thus, the third condition in this study involved the treatment of HT29-MTX cells with Capsicum spp. extract. Yet, the result revealed that no continues mucus layer was seen (Figure 4), which may indicate that Capsicum seems to induce mucus production only *in vivo*. This may be because Capsicum activates sensory nerves in the lungs, which leads to mucus release [30,31].

Alternatively, HT-29 MTX cells were incubated under shaking for three weeks and fed with fresh medium once to twice daily, which was the fourth condition in the current study. This condition can mimic the behavior of the intestine condition. Interestingly, the result of this condition clearly showed that shaking cells with continuous feeding provides a good condition to enhance mucin production *in vitro* (Figure 5).

Conclusion

Incubation of HT-29 MTX cells with shaking and providing cells with fresh medium once to twice daily for a period of three weeks can be the best way to mimic the natural behavior of the intestine and lead to enhance the seeded cells to produce the mucin *in vitro*.

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Spectrophotometric Determination of Ampicillin with Sulfanilic Acid by Oxidative Coupling Reaction

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Abstract

Sensitive, economic, simple and accurate spectrophotometric method for estimation of ampicillin in bulk and dosage form is described. Ampicillin was oxidized by sodium hypochlorite and coupling with sulfanilic acid in the presence of sodium hydroxide to obtain a stable yellow colored chromogen which exhibit a maximum absorption (λ_{max}) at 400 nm. The optimum conditions were carefully evaluated .

Plot of absorbance against concentration was linear over the range (50-300 $\mu g.ml^{-1}$). This method was applied to the estimation of ampicillin in pure drug and commercial formulations successfully.

Key words: *Spectrophotometric determination, Ampicillin, Sulfanilic acid, Oxidation reaction.*

Introduction

Ampicillin is a drug from penicillin family. It is used to treat a broad range of bacterial infections produced by haemophilus influenza, streptococcus and escherichia. Ampicillin is used in the treatment of urinary and genital tract infections, otitis media in children and upper respiratory tract infections.

Ampicillin is a crystalline powder with white color with chemical formula, 6-[(Amino phenyl acetyl) amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic[(1)].

A survey of literature revealed that several analytical methods such as high performance liquid chromatography[(2-4)], flow injection[(5,6)], atomic spectroscopy[(7)], capillary electrophoresis[(8,9)], iodometric titration[(10)], potentiometric methods[(11,12)] and spectrophotometric methods[(1, 13-16)] have been reported for the determination of ampicillin.

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The present study involves the development of accurate, sensitive and reproducible spectrophotometric method based on oxidation of ampicillin by sodium hypochlorite and coupling with sulfanilic acid in presence of sodium hydroxide as basic medium. This method is successfully applied to estimation the ampicillin in its commercial formulations.

Experimental

Instruments

The absorption spectra and all spectrophotometric measurements were carried out on a double-beam 1800 Shimadzu spectrophotometer with 1 cm matched quartz cells.

Materials and reagents

Ampicillin standard powder received was provided in pure form (99.99%) from the State Company for Drug Industries and Medical appliance-(SDI) Sammara-Iraq and all chemicals used were of analytical reagent grade.

Reagents solution

-Sulfanillic acid [6.35×10^{-4} M]: 0.11 g of sulfanillic

acid was dissolved in 0.5 mL of 4 M NaOH and complete dilution to 100 mL with distilled water.

-Sodium hypochlorite [1% (v/v)]: prepared by dilution of 10 mL of 5% sodium hypochlorite to 50 mL by distilled water in volumetric flask.

-Sodium hydroxide [4 M]: 16 g of sodium hydroxide was dissolved in 100 mL distilled water.

-Standard ampicillin solution 1000 µg.mL^{-1}: 0.1 g of ampicillin was dissolved in 0.5 mL of 4 M NaOH and further diluted to 100 mL with distilled water.

Preparation of drug in dosage form

Ten capsules and ten vials of ampicillin were weighed, and an accurately weighted portion of the powder equivalent to 250 and 500 mg of ampicillin were dissolved in a 0.5 mL of 4 M NaOH and mixed well and then filtered by using Whatman filter paper number 41. Then volume was diluted to 100 mL with distilled water and analyzed as given under the assay procedures for bulk samples.

General standard procedures

Aliquots (0.5- 3.0 ml) of standard 1000 µg ml^{-1} ampicillin solution were transferred into a series of 10 ml volumetric flasks. To each 0.5 mL of 6.53×10^{-4} M sulfanillic acid solution, 2 ml of 1% sodium hypochlorite and 1.5 ml of 4 M sodium hydroxide were added. The volumes were made up to the mark with distilled water. The absorbance of yellow colored compound was recorded at 400 nm against the blank solution.

Results and Discussion

Absorption spectra

When ampicillin react with sulfanilic acid in the presence of sodium hypochlorite in basic medium of sodium hydroxide, a yellow colored oxidizing coupling product with an absorption maximum at 400 nm is formed. The absorption spectra of product and blank solution were recorded. The results were graphically represented in Figure 1.

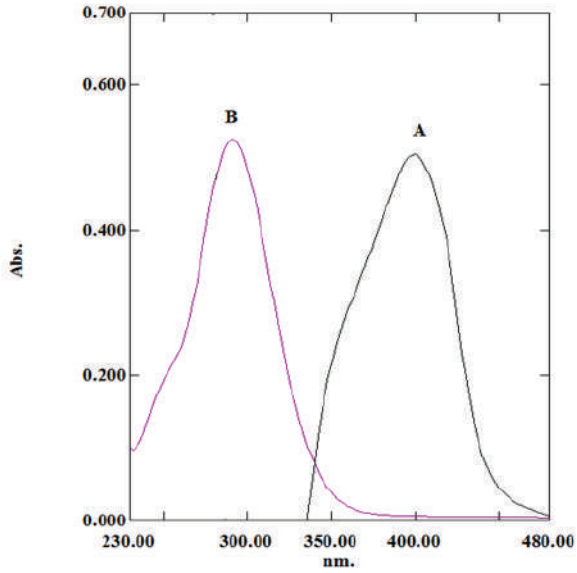


Figure (1): Spectrum of: (A) 200 µg.mL^{-1} ampicillin complex, (B) blank solution under optimization conditions.

Optimization of reaction variables

The influence of different type of bases

When some bases (NaOH, KOH and NH_4OH) that the presence led to increase the intensity of the produced product were tested for the basic medium of reaction, it was found that NaOH was the most suitable for a maximum absorbance for method.

The influence of sulfanilic acid volume

Volume of sulfanilic acid effect on the color development was studied. It is evident that 0.5 mL sulfanilic acid give a maximum color intensity and a minimum absorbance of the blank.

The influence of sodium hypochlorite volume

The study of volume of sodium hypochlorite as an oxidant agent was evaluated. Maximum absorbance was observed when the volume of sodium hypochlorite was 2 mL. Above this volume the absorbance was decreased.

The influence of sodium hydroxide volume

The effect of the volume of NaOH on the color intensity of product was studied. 1.5 ml of 4M NaOH which was give high sensitivity.

Order of addition influence

Different orders of component addition influence on chromogen formation was investigated by changing the sequence of addition of drug, , reagent, oxidizing agent and base. The sequence was {ampicillin – sulfanilic acid – NaOCl and NaOH} for the best intensity.

The influence of reaction time

The effect of coupling reaction time on color of chromogen development was examined. The yellow product formed due to the oxidation coupling of ampicillin with sulfanillic acid by sodium hypochlorite in presence of base, attained the maximum color at 0 minute, that means the reaction was very fast.

Linearity and analytical data

Empolying the conditions described under procedure, a linear calibration graph, Figure 2 for ampicillin obtained shows that Beer's law is obeyed over the concentration range 50-300 µg.mL^{-1}. Linear regression and analytical parameters are listed in Table 1.

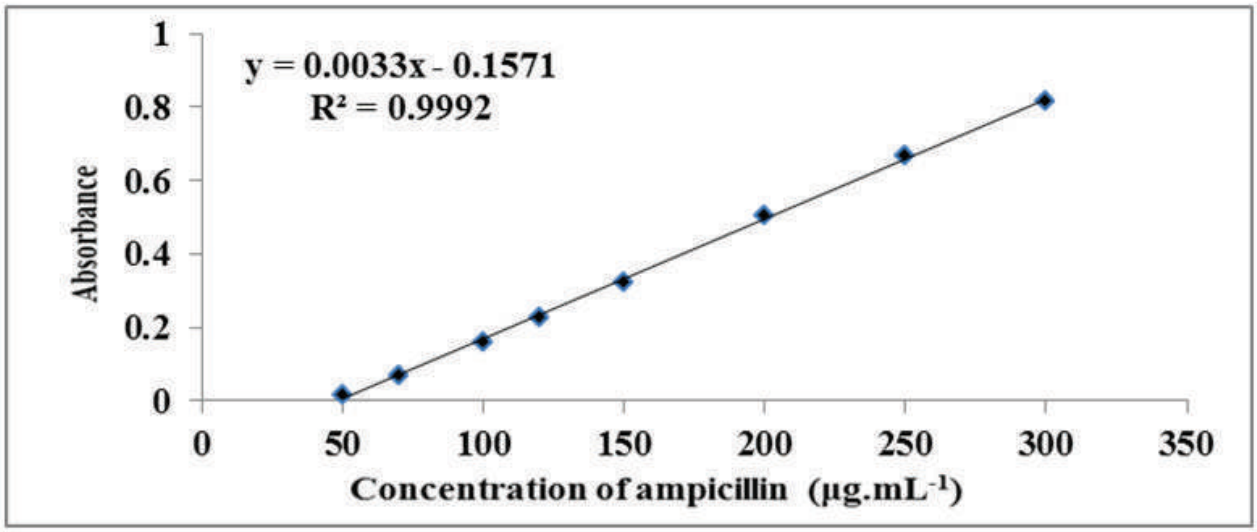


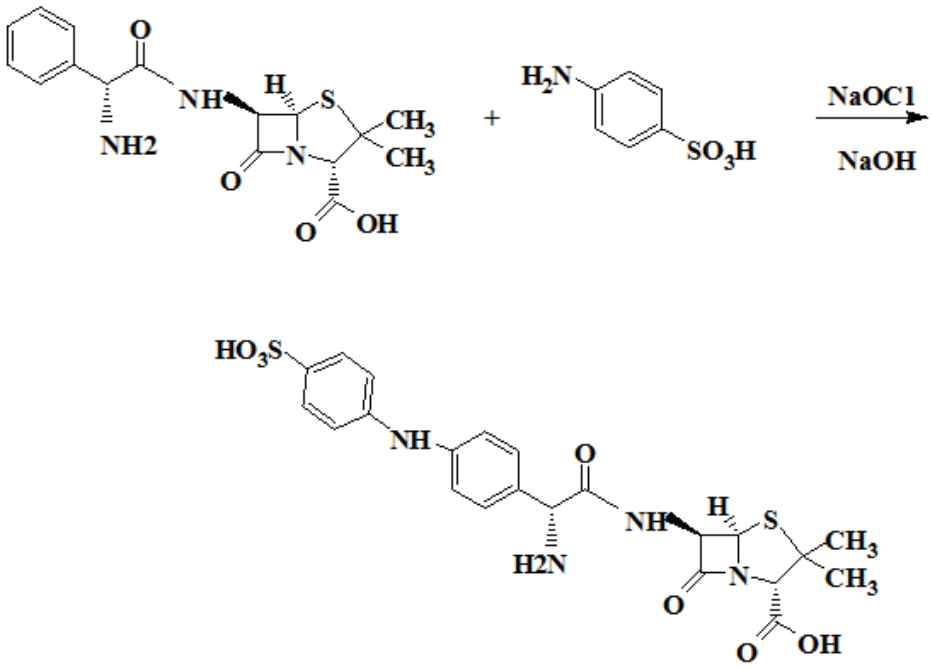
Figure (2): Spectrophotometric calibration curve for estimation of ampicillin.

Table (1): Linear regression and analytical parameters for estimation of ampicillin.

Parameter	Value
λmax (nm)	400
Regression equation	Y = 0.0033 [Ampicillin µg.mL-1] 0.1571
Calibration sensitivity(mL.µg -1)	0.0033
Linearity range (µg.mL-1)	50-300
Correlation coefficient (R)	0.9996
Molar absorptivity (ε) (L. mol -1.cm -1)	1331.55
LOD (µg.mL-1)	0.2405
Sandell's sensitivity (µg.cm-2)	0.3030

Composition of complex

Stoichiometry of the product formed from the reaction of ampicillin with sulfanilic acid was studied applying the Job's method by using equimolar solutions (1.24×10^{-3} M) of ampicillin and sulfanilic acid. The results obtained show that 1:1 drug to reagent complex was formed at 400 nm. The reaction may proceed as given in Scheme 1.



Scheme (1): The suggested mechanism for oxidation reaction.

Accuracy and precision

Accuracy was done by relative error % and precision was evaluated by relative standard deviation (RSD) %. Three different concentration levels of ampicillin were analyzed in three times. Table 2 list the results of accuracy and precision.

Table (2): Accuracy and precision of the method for estimation of ampicillin.

Sample	Conc. of ampicillin (µg.mL-1)		Relative error %	RSD* %
	Taken	Found*		
Ampicillin	50	50.355	0.710	0.6330
	200	200.74	0.370	0.3724
	300	299.592	-0.136	0.2302

*Average of three determinations.

Application to Pharmaceutical Preparations

The application of the method for the assay of ampicillin in drugs has been done successfully and the results obtained are listed in Table 3 for each sample in three replicates.

Table (3): Application of the ampicillin concentration measurements in pharmaceutical formulations.

Sample	Labeled amount of ampicillin (mg)	*Found amount of ampicillin(mg)	Recovery %	RSD* %
Ampicillin Caps S. D. I.- Iraq	250	252.42	100.96	0.9431
	500	498.70	99.74	0.5260
Ampicillin Vials S. D. I.-Iraq	250	491.33	98.27	1.0980
	500	502.00	100.40	0.4122

*Average of three determinations**.**

Conclusions

The oxidative coupling reaction with sulfanillic acid and sodium hypochlorite in the presence of sodium hydroxide was rapid, sensitive, simple and precise spectrophotometric method was developed for estimation of ampicillin. The proposed method was applied successfully in pharmaceutical formulations.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Study of Smoking Effect on Some Liver Functions, Blood Pressure and Some Haematological Parameters in Smokers in Al-Muthanna Province

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Abstract

The aims of this study was to investigate the changes that occurred on some liver functions and some haematologicl parameters and blood pressure of smokers**.** The current study is carried out in in Al- Muthanna province.The study included 200 men, (100) smokers and (100) non smokers (control).the smokers and non smokers divided to two group according to age. the ages were between (25-46)years.The results for liver function indicate a significant increase at($P<0.05$)in Glutamate Oxaloacetae Transaminase(GOT),Glutamate Pyruvate Transaminase(GPT) and Alkaline Phosphatase (ALP) levels of smokers comparing with control group in two age groups ,but non-significant differences at ($P<0.05$) in Serum Total Bilirubin (STB) of smokers comparing with control group, While the results for haematological parameters showed a significant increase at($P<0.05$) in white blood cells (WBC), red blood cells(RBC), hemoglobin(Hb) and Mean Cell Volume(MCV)of smokers comparing with non-smokers in two age groups, but non-significant differences at($P<0.05$) in PLT of smokers comparing with non-smokers in two age groups. the results for blood pressure showed a significant increase at($P<0.05$) in in systolic and diastolic blood pressure in all age groups of smokers comparing with non-smokers.

Key words *:Smoking, liver functions , haematological parameters , blood pressure.*

Introduction

Smoking is the most important public health problem. Tobacco cigarette is one of the major causes to death and essential public health challenge in world over, many studies showed the effects of smoking on many organ systems mostly respiratory, reticulo-endothelial system and cardiovascular systems [(1)].WHO estimates that tobacco-related deaths will amount to 8.3 million in 2030 and one billion deaths during the 1st century. Water-pipe (WP) is a classical device used for tobacco smoking attached with water bowl, WP usage has a history about 400 years with the different names like as a shisha, narghile, hookah chillum and arghile [(2)].

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Tobacco can be used as burning cigarettes, which affects hematological parameters and the liver enzyme activity. There are 4000 substances in a single cigarette, 200 of which are poisonous and 80 cause cancer; such poisonous substances include nitrogen oxide, nicotine, hydrogen cyanide, carbon monoxide and free radicals which result in disorders in the human body Also, smoking produces carbon monoxide that binds more firmly with hemoglobin compared to oxygen, leading to many diseases, such as blood pressure [(3)]. Smoking is an etiological agent for many chronic diseases, including a variability of infections, cancers, heart diseases, and respiratory diseases such as chronic obstructive pulmonary disease, that have weakening in the balance between cell growth and cell death, which put together, are caused of morbidity and mortality in today's society [(4)].

Materials and Methods

Subjects and study location: This study included 200 men (100 smokers and 100 non smokers,ages were ranged(25-46) years old. The current study is carried out in Al- Muthanna province. .

Blood Samples: Venous blood sample (7ml) were collected from smokers and control, 2ml from blood was placed in EDTA tube for estimation of the Haematological parameters while 5 ml from blood was placed in gel tube (free of anticoagulant) and allowed to coagulate for (15-20) minutes then centrifuged for separation of serum. for estimation of the liver enzymes .

1.Assessment of liver functions

-Assessment of GOT and GPT

GOT and GPT were determined enzymatically according to the method described by[5] , as shown in the following reaction respectably.

α – Oxoglutarate + L-aspartate

α – Oxoglutarate + L-anine

-Assessment of ALP

ALP was determined enzymatically according to the method described by[6]

-Assessment of STB

The total serum bilirubin concentration was calculated by bilirubin meter after it was calibrated using a capillary tube containing distille water. The serum samples were placed in similar capillary tubes and measured in mg /dl [7].

2. Assessment of haematological parameters

2 ml of blood samples placed in test tube containing EDTA and then mixed gently on the blood mixer, then the blood is taken by the apparatus, after that all complete blood parameters is recorded by (Sysmax-Kx-21) [8].

3. Assessment of blood pressure(Bb)

Systolic and diastolic blood pressures were measured double at 5 min intervals from the left arm after at least 15 min of rest. BP values were obtained by the traditional auscultatory method using a sphygmomanometer

Statistical Analysis

In this study , several statistical tests were used to find the significant differences among the studied parameters of smokers and control group at ($P<0.05$) level of significance. This study designed by Completely randomized design (CRD) that used in the analysis of variance for data by using one way ANOVA test, independent *t*-test and treatment means were compared using the least significant difference (LSD) at ($P<0.05$) level of significance. Data were processed and analyzed by using statistical program social science (SPSS 22) and the results were expressed as Mean±SD .

Results and Discussion

1.Liver functions

The results of this study showed GOT (23.7 ±5.1) for smokers and (13.4 ±2.1) for control(non-smokers), GPT(25.1 ±5.7) in smokers and (12.7 ±4.2) in control smokers, ALP (49.63±7.15) in smokers and (38.27 ±5.3) in control, SBT (0.69 ±0.049) in smokers and (0.70 ±0.023) in control, these results of age group (25-35) years old, while the results of age group(29-39)years old are GOT(21.5.1 ±3.4) for smokers and (14.1±1.2) for non-smokers, GPT (20.3 ±3.8) in smokers and (11.9 ±2.3) in control, ALP (60.41 ±6.04) in smokers and (43.13 ±2.0 8) in control. STB (0.60 ±0.01) in smokers and (0.58±0.01) in control, table (1)

Table (1) The change in Liver parameters of smokers group as compared with control group.

Liver parameters	Age (years)	Smokers Mean±SD	Control group Mean±SD
GOT U/L	25-35	23.7 ±5.1 *	13.4 ±2.1
	36-46	21.5.1 ±3.4 *	14.1±1.2

Cont... **Table (1) The change in Liver parameters of smokers group as compared with control group.**

GPT U/L	25-35	25.1 ±5.7 *	12.7 ±4.2
	36-46	20.3 ±3.8 *	11.9 ±2.3
ALP U/L	25-35	49.63±7.15 *	38.27 ±5.3
	36-46	60.41 ±6.04 *	43.13 ±2.0 8
STB mg/dl	25-35	0.69 ±0.049	0.70 ±0.023
	36-46	0.60 ±0.01	0.58±0.01

***represent significant difference between smokers groups as compared with control group**

The results showed a significant increase in GOT, GPT and ALP of smokers when compared to control group.This may be due to nitrosative stress which is a condition that occurs when the production of highly reactive nitrogen containing chemicals, such as nitrous oxide, exceed the ability of the human body to neutralize and eliminate them. Nitrosative stress can lead to reactions that alter protein structure thus interfering with normal body functions. Cigarette smoke contains a large number of chemical substances especially nicotine which is toxic to the liver [(9)].

liver enzymes increased in serum according to the damage of the liver cell, these enzymes include transferase enzymes, GOT, GPT and ALP. Alkaline phosphatase is the most frequently measured indicator for liver bile ducts disease[(10)]. GOT and GPT enzymes frequently appear in the serum following liver cell injury or sometimes in smaller amounts from degraded cells. The liver has a central and critical biochemical role in metabolism digestion detoxification and blood from intestinal tract initially passes through the liver. Elevated liver enzymes may indicate inflammation or damage to cells in the liver [(11).]

ALP is an important enzyme mainly derived from the liver and bones, but is also present in the kidneys and the leukocytes. The relationship between smoking and ALP is illustrated by inflammatory markers CRP and in particular the leukocyte count , in crease of ALP associated with smoking may reflect bone cell activity[(12).]

2.Haematological parameter

The results of this study showed total WBC (9.2 ±1.7) for smokers and (6.1 ±0.12) for non-smokers, RBC (6.27 ±0.12) in smokers and (4. 95 ±0.19) in controls, Hb (19.01 ±0.32) in smokers and (14.31 ±0.19) in control and MCV (96.87 ±4.33) in smokers and (87.29 ±2.15) in control, PLT (271.52 ±54.35) in smokers and (278.12 ±73.43) in control these results of age group (25—35) years old, while the results of age group(36-46)years old are WBC (12±2.3) for smokers and (5.3 ±1.8) for non-smokers, RBC (8.11 ±0.27) in smokers and (5.32 ±0.37) in controls. Hb (22.38 ±0.23) in smokers and (16.0 9 ±0.29) in control. MCV (117.65±4.2) in smokers and (92.33±2.01) in control, PLT (243.57 ±37.1 3) in smokers and (240± 45.81) in control, table (2)

Table (2) The change in haematological parameters of smokers groups as compared with control group.

Haematological parameters	Age (years)	Smokers group Mean±SD	Control group Mean±SD
WBC 103 /µL	25-35	9.2 ±1.7 *	6.1 ±0.12
	36-46	12±2.3 *	5.3 ±1.8

Cont... Table (2) The change in haematological parameters of smokers groups as compared with control group.

RBC 106 /µL	25-35	6.27 ±0.12 *	4. 95 ±0.19
	36-46	8.11 ±0.27 *	5.32 ±0.37
Hb g/dl	25-35	19.01 ±0.32 *	14.31 ±0.19
	36-46	22.38 ±0.23 *	16.0 9 ±0.29
MCV fL	25-35	96.87 ±4.33 *	87.29 ±2.15
	36-46	117.65±4.2*	92.33±2.01
PLT 103 /µL	25-35	271.52 ±54.35	278.12 ±73.43
	36-46	243.57 ±37.1 3	240± 45.81

***represent significant difference between smokers groups as compared with control group.**

This study agreement with the previous studies on the impact of smoking on hematological parameters, WBC, RBCs, Hb, and MCV levels were found to be markedly increased, while platelet counts were decreased. These changes have been associated with high risk of atherosclerosis, polycythemia, chronic obstructive pulmonary disease, and cardiovascular disease in smokers [13].

The results of this study indicated a significant increase at($P<0.05$) in (WBC) of smokers comparing with control, the relation of high WBC count with smoking may be due to the presence of a subclinical inflammatory reaction [14]. The leukocytosis may simply be a marker of smoking-induced tissue damage, this high count can promote cardiovascular diseases through multiple pathologic mechanisms that mediate inflammation, plug the microvasculature, induce hypercoagulability and promote infarct expansion [15]. In another suggestion that inflammatory catalyzing of the bronchial tract causes an increase in inflammatory markers in the blood circulation [16].

The present study indicated the smokers had significant increase in RBC count at ($p<0.05$) of smokers comparing with non-smokers. The increase of RBC count is termed as polycythemia and very high of RBC mass slows blood velocity and increase the risk of intravascular clotting, coronary vascular resistance decreased coronary blood flow, and a predisposition to thrombosis [17]**.** The mechanism by which polycythemia causes thrombosis is still under investigation, but smoking cigarettes creates a unique condition of combined polycythemia to chronic hypoxia, leading to elevated red cell production because of an elevated carboxyhemoglobin level with concomitant plasma volume reduction [18]. Some possible mechanisms are reported by which cigarette smoking could cause such changes in the red blood cells. Carbon monoxide, one of the chemicals identified in tobacco smoke, may induce hypoxia, usually the body responds to hypoxia by increasing the number of erythrocytes. Excessive tobacco can be result in polycythemia because this situation created a real oxy-carbon poisoning, so there was an extra oxygen demand and production of red blood cells increases to cope [19]. The result obtain in this study indicate a significant increase in hemoglobin concentrations at ($p<0.05$) compared to non-smokers. Elevated levels of hemoglobin are correlated with increased numbers or sizes of RBCs. Hemoglobin concentrations were significantly associated with increasing age in cigarette smoking men. The study was demonstrated other studies support by [20].

The results in this study show a significant increase in MCV, MCV indicates the size of a red blood cell and

presence of red cells smaller or larger than normal size means the person has anemia, elevated levels of MCV indicates that subjects might suffer from megaloblastic, hemolytic, pernicious or macrocytic anemia usually caused by iron and folic acid deficiencies [21]. The increase in hemoglobin and MCV could be due to the inhaled carbon monoxide gas (CO), which is one of the inhaled components of cigarette smoke. CO present in cigarette smoke in more than 600 times the concentration considered safe in industrial plants. A smoker's blood typically contains 4 to 15 times as much CO as that of a nonsmoker. CO combines reversibly with oxygen-carrying sites on the hemoglobin molecule by about ranging from 210 to 240 times greater than that of oxygen, which results in decreased oxygen-carrying capacity of the blood, this decrease is compensated by an increase in hemoglobin [22]. this study did not find any differences in Platelet count of smokers comparing with non-smokers, while the study conduct by [23] which found that decrease in platelet count in smokers .

3.Blood pressure

The results of this study showed that systolic blood pressure 136.72±9.67 of smoker and 121.81 ±11.0 4 of non-smoker also the results showed that diastolic blood pressure 92.51 ±5.93 of smokers and 81.0 5±6.1 8 of non-smoker these results of age group (25-35),while in age group (36-46) the results indicated that systolic blood pressure 164.31±8.71 of smoker and 130.18 ±6.25 of non smoker also the results showed that diastolic blood pressure 89.63 ±8.0 7 of smokers and 77.2 2 ±7.13 of non smoker,table 2

Table(1)The change in Blood pressure of smokers groups as compared with control group.

Blood pressure	Age (years)	Patients group Mean±SD	Control group Mean±SD
Systolic mmHg	25-35	136.72±9.67 *	121.81 ±11.0 4
	36-46	164.31±8.71 *	130.18 ±6.25
Diastolic mmHg	25-35	92.51 ±5.93 *	81.0 5±6.1 8
	36-46	89.63 ±8.0 7 *	77.2 2 ±7.13

***represent significant difference between smokers groups as compared with control group.**

The results of this study indicated a significant increase at($P<0.05$) in systolic and diastolic blood pressure in all age group may be due to harmful effects of cigaretts smoke on the vascular system, The cigarette smoking causes sympathetic activation, oxidative stress, and acute vasopressor effects that are associated with increased markers of inflammation that are linked with hypertension. There are several potential biological mechanisms through which exposure to cigarette smoke may lead to high blood pressure, cigarette smoking also increases blood pressure through stiffening arteries, particularly with deleterious effects of chronic smoking that leads to the development of hypertension. Smoking or intravenous nicotine administration increases arterial blood pressure in humans. This can be explained by an acute stimulation of the sympathetic nervous system causing heart rate increases [24].

Conclusion

There are a great side effects of cigarette smoking on liver function , haematologicl parameter (RBCs, Hb, WBCs, PLT and MCV) and blood pressure.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Awareness of Creativity and Its Relationship to Speed and Accuracy of Futsal Soccer for Female Students

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Abstract

Achieving success in the futsal soccer game also depends on the good potential in the accuracy and speed of implementing basic skills through the superior ability to accuracy and speed of passing the soccer (futsal) to the researchers as well as teachers and lecturers in this game within the scope of the lesson in the third and fourth stage, there is a weakness in most Female students in basic play skills, including the skill of handling, because they need to play to the speed and accuracy of performance and weakness, according to the researchers 'opinion, lack of training on them requires awareness, thinking, focus and awareness that the game needs physical and mental effort to master it, which the researchers decided to go into this problem, the researchers used the curriculum Descriptive Basloubi survey and correlations because it fits with the nature of the research problem. The research community was determined by the students of the fourth stage in the College of Physical Education and Sports Science in the effectiveness of football halls for the academic season 2018-2019 and the number (14) students, which were completely chosen for the experiment, in a simple random way (Lottery) and by 100%. The researchers used the scale of creating awareness of the researcher (Wahb Razzaq), where a scale consists of (28) items that the examiner answers according to five alternatives, which are (always, sometimes, rarely, never) and grades are given (1,2,3,4,5) on Respectively, as the highest score on the scale was (140), the lowest score is (28), the name of the test - receipt, and either the handover pass test toward players - the goal of the test - measuring the speed and accuracy of passing them - the conclusions are - distinguished students of the Faculty of Physical Education and Sports Science at the University of Kufa In the fourth stage, a good reality of awareness and creativity also characterized by a good reality in the performance and speed of the skill of handling the futsal football. There is a real correlation between the awareness of creativity and the speed and accuracy of the skill of handling futsal football. The researchers recommend that it is necessary to focus on psychological matters that increase the students 'motivation to think and awareness about creativity and help them in achieving self and forming a personal sporting identity for each of them using encouraging educational methods to develop the skill of speed and accuracy of the passing skill of students in the College of Physical Education and Sports Science while practising practical lessons and performing sports skills Various specials, futsal soccer skills.

Key words*: creativity awareness, speed and accuracy of pass, Futsal football for female students*

Introduction

The skills of the futsal soccer game are influential and important for the practitioners of this event, which works to develop many qualities, including awareness of creativity, concerned with the need to excel and in turn works to detonate and stimulate the potential energy they have, also works to motivate him to double and direct his efforts and energies towards achieving the desired goal(1) .

In light of the foregoing, the importance of the current research is reflected in finding a relationship of awareness of creativity, accuracy and speed of handling due to the lack of researchers in delving into this field, which is the focus of attention of many researchers, because the game has taken an advanced trend among other activities, especially in the coming years, the problem of research is to Achieve Success in the futsal soccer game is also dependent on the good potential in the accuracy and speed of implementing basic skills through the superior ability to accuracy and speed of passing football (futsal). The researchers observed two teachers and a lecture in this game within the scope of the lesson in the third and fourth stage, there is a weakness in most of the students in Basic playing skills, including the skill of handling, because it needs in the game to speed and accuracy of performance and weakness is, according to the opinion of researchers, lack of training on it needs awareness, thinking, focus and awareness that the game needs physical and mental effort for mastery, which the researchers decided to go into this problem, the objectives of the research identify awareness The creativity, speed and accuracy of the Futsal Soccer Pass for students, and the relationship between awareness of creativity and the speed and accuracy of Futsal Football Pass for Female Students[2].

Procedures[3]:

The researchers used the descriptive method in the two methods of surveying and correlation, as it fits with the nature of the research problem, for students of the fourth stage in the College of Physical Education and Sports Science in the effectiveness of football halls for the academic season 2018-2019 and the number (14) students, which were chosen entirely for the experiment, in a simple random way (Lottery) and 100%

Procedures for choosing a measure of awareness of creativity:

The researchers used the measure of creativity of the researcher (Wahhab Razzaq) [1], where a scale consists of (28) items that the examiner answers according to five alternatives, which are (always, sometimes, rarely, never) and the grades are given (1,2,3,4,4). 5) respectively, as the highest score on the scale was (140), while the lowest score was (28)[4].

To apply the scale, the researchers presented the scale to a group of gentlemen experts and specialists in the field of sports psychology, testing and measurement, to evaluate it and judge it in terms of their suitability for what was set for it. After briefing the experts and specialists on the paragraphs of the scale and testing, they made their observations after which the researchers extracted a value (KA 2) calculated for the agreement of experts and specialists and acceptance of the nomination of paragraphs for which the calculated value of (K2) is greater than its tabular value of (3.84), as shown in tables (1).

Table (1)Shows the number of agreements, calculated (ka) 2 values and the moral significance of the creativity awareness paragraphs

Variables	Number of experts approved	Number of experts not agreeing	Calculated value of (chi)2	Significance
Creativity awareness	6	zero	6	sig

After the validity of the paragraphs was approved by the experts, the scale is ready for application in its final form.

Procedures for testing the speed and accuracy of futsal pass

Test Name - Pass Test (Ball Pickup and Delivery)

Test name - Receipt and handover of the pass to players

Test objective - measuring scroll speed and accuracy

The tools used - (4 players), stopwatch, whistle, and legal football (2) tape measure.

Method of performance: - The laboratory stands inside a square measuring (1.5) meters and the players performing the test stand on its sides and according to their numbering and in the other two players to receive the ball and return it to the executing player upon hearing the beep. The player (1) kicks the ball from (6) meters Towards the port for testing inside a square measuring 1.5 meters if the laboratory receives the ball from player No. 1 if he passes the ball to No. 4, and if he receives the ball from No. 2, he passes the ball towards (3) and so for (40) seconds As shown in Figure (1)

Recording method

- The number of passes performed by the player is counted within (40) seconds.

- Do not count or return if the ball goes out of the box designated for it.

Scientific foundations of the tests:

For the tests to achieve the goal and purpose for which they were placed, but to be reliable, reliable, and truthful, they must meet the conditions and specifications, the most important of which are the scientific foundations of the test (honesty, consistency, and objectivity), and on this basis, the researchers found the scientific foundations of the tests as follows:

First: validate the test:

Honesty is "the health of measuring what was set for it or the authority with which the test measures what was set for it" ☐ ☐. On this basis, the sincerity of the accuracy and speed of passing the test was found by adopting content honesty. These tests were presented to a group of gentlemen experts and specialists in the fields of (football, athletic training, tests and measurement) who confirmed that this test is true and measures the quality that was set for it.

Second" the stability of the tests:

Stability of the test means that it is the degree of coherence with which the measuring method used can be applied or the degree of accuracy with which the apparent test that measures the subject of the measurement is measured. Any daily on the date the test was repeated on the same sample and then the simple correlation coefficient (Pearson) was extracted.

Scout experience for passing speed and accuracy:

After putting the instructions for the test, the researchers conducted the exploratory experiment on Sunday 7/11/2018 in the hall of the College of Physical Education and Sports Science - the University of Kufa, which is (3) students from the research community to reveal the following:

1- To ensure clarity of instructions and test items for the sample.

2- Know the time taken for the test.

3- Knowing the conditions of applying the test and the accompanying difficulties.

The main field experience:

Given the completion of the procedures that qualify the basic experiment that confirmed the validity of the scale and the identification of the application of the accuracy and speed of passing the futsal football pass, the researchers began applying the standard and testing to the research sample of (14) students representing the fourth stage of the futsal football for the academic season (2018-2019) on Wednesday corresponding to 23/1/2019. The scale form was then attached to the student's speed and accuracy test form for each student to find out their degree. The work was supervised by the researchers. After completing the basic experiment, the researchers collected the data of the research sample and arranged it in tables in preparation for statistical analysis[9].

Statistical means: -

To treat the results in a way that serves the research, the researchers adopted the appropriate statistical means, the Statistical Package for Social Sciences (SPSS) to process the data and the bag was used in the topics:

Results

To achieve the goal of the first research, which is to identify awareness of creativity, speed and accuracy

of the skill of handling football with futsal footage for students of the Faculty of Physical Education and Mathematical Sciences at the University of Kufa, a measure (awareness of creativity) were applied, and after that, the tests were conducted for the skill (speed and accuracy of handling) football futures for the mentioned students Above.

Table (2)Shows the arithmetic mean and the standard deviations of the special research variables for the fourth stage students in futsal football

variables	the sample	
	mean	standard deviation
Creativity awareness	35,80	4,394
Speed and accuracy of handling	3,60	0.632

We observe through Table (2) the values of the arithmetic mean and the standard deviations recorded for students of the fourth stage in the College of Physical Education and Mathematical Sciences in awareness of creativity and (speed and accuracy of handling)

Presentation, analysis and discussion of the nature of the relationship between awareness of creativity and the speed and accuracy of handling a sample:

To achieve the second research goal of ((recognizing the nature of the relationship between each (awareness of creativity) and (speed, accuracy and the skill of handling) for students of the Faculty of Physical Education and Mathematical Sciences at the University of Kufa)) the researchers applied the simple correlation coefficient ((Pearson between awareness of creativity and speed And the accuracy of scoring skill in this research and the results appeared as found in Table (3):

Table (3)Shows the values of correlation coefficients between search variables

variables	Arithmetic mean	standard deviation	Calculated correlation coefficient value	indication
Creativity awareness	35,80	4,394	0.47	sig
Speed and accuracy of the handling skill	3,60	0.632		

As for awareness of creativity and its relationship to the speed and accuracy of the handling skill of the research sample through our observation of Table (2), the value of the correlation coefficient between the awareness of creativity and its relationship to speed and accuracy of handling skill amounted to (0.47) Researchers attribute the reasons for this moral relationship to the students 'thinking in the effectiveness of Futsal football It was good in terms of discipline and commitment to skills training and mastery, especially handling and control skill.

The seriousness in the performance of female students and the desire to identify their levels through psychological tests to raise awareness of creativity and skill regarding the skill of speed and accuracy of handling were their high seriousness in the tests as well, during the practical lesson on mastering this skill which increased the students 'motivation towards achieving the goal and obtaining the best grades During the exams, (Kamel, 1981) indicates, "Achieving the highest level of sports necessarily necessitates identifying problems

and psychological impediments facing players in sports training to bring the athlete to a good psychological, physical and skill condition to endure these pressures to achieve the goal set for him while participating in the race."And sees " The preparation of the player physically and skillfully well affects the psychological energy as the body fitness is an important part of mental health as well as the influence of psychological energy in preparing him physically and skillfully through balance and a sense of awareness and good thinking before and during Competition, which leads to less psychological burdens and thus a good psychological and skill condition." To clarify the level of attribute growth, the trainer must organize the training in a manner that allows the use of special exercises that compel the A player who shows very high willpower, and this we observed for the students from the commitment in continuous training through lectures on the basic skills of the game and mastering the skill well and mastering it in terms of accuracy and performance.

Conclusion

Through the procedures of research and to produce its results and discuss them, the researchers reached the following conclusions:

1- The students of the College of Physical Education and Sports Science at the University of Kufa in the fourth stage were distinguished by a good reality of awareness and creativity as well as a good reality in the performance of the speed and accuracy of the skill of handling in futsal football.

2- There is a real correlation between awareness of creativity and the speed and accuracy of the skill of handling futsal football.

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The Effect Esthetic Factors in Complete Dentures Wearers Acceptances

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Abstract

Objected: This essay has shown esthetics to be a salient factor for successful complete dentures. Complete denture esthetics can no longer be regarded as a method of tooth selection and arrangement alone or the colors and contours of the denture bases and forms of the denture bases. Denture feel must likewise incorporate those whole face for which those expressions from claiming inward feelings, personality, comfort, image, well-being, and recognitions about previous dental encounters need aid constantly on precise obvious. Both those patients and the dental specialist must comprehend this interrelationship for ideal victory.

Purpose: evaluate hard and soft components that contribute to Creating an effective finish successful esthetical denture stylish rebuilding

Material and Methods : The sample was selected from Baghdad University, College of Dentistry. A total of 100 Iraqi adult Dental patients were clinically examined (females and males). Each person was seated in the Dental chair and was asked to look straight ahead and the measures have been taken according to the case sheet prepared previously.

Result : the results obtained from case sheet analysis using SPSS computer program (version 23) and shown significant statistic results in some criteria of determining aesthetic by facial measurement and other shown some unaesthetic result which may be due to clinical or laboratory error during the construction of complete denture.

Conclusion : A large portion of significant attention throughout finish denture development is those rebuilding from claiming keep going feel. esthetic dives substantially further over the negligible placement of the teeth on the occlusal rims, size, shape, color about simulated teeth and denture bases need aid essential On the development of a stylish denture. Distinctive individuals have communicated different assumptions for settling on tastefully pleasing dentures.

Keywords: *complete denture, esthetics, , H.pound's concept, Ricket:s E -plane, William relationship*

Introduction

The word aesthetics originated from the Greek "aisthetike" and was invented in 1735 by philosopher Alexander Gottlieb Baumgarten to denote "the study of how objects are perceived by the senses." The concept was used in German shortly after Baumgarten adopted its Latin version (Aesthetica), but was not used in English until the beginning of the 19th century.[1]

Dental esthetics can be streamlined in the United States of the 21st century to provide a complete dentition composed of smooth, white teeth. A worldwide recognition can be made of the so-called "Hollywood" smile, popularized by American cinema and television.[2] History shows us that this may not always have been the case throughout the world. The face by which greet the world is the most visible part of the human body, and it is

the face by which social acceptance can be determined. Facial appearance is an important part of the self-image and a major concern for everybody. The loss of teeth, also leaves an individual with significant psychological distress, and it needs urgent replacement. [3]

The prothesis can either enhance or detract from the patient's personal image depending on the naturalness and appeal of the patient's appearance, reflecting the dentist's artistic abilities, which vary from one to the next.

The position of the anterior maxillary arch and the arrangement of the individual teeth contribute most crucially to the appearance of the face .[4]

The teeth are not just an important part of facial appeal. They offer a specific appearance to each face, even as lips, nose, and anatomical composition render each face distinctive and easily identifiable. When the teeth are removed, the teeth are more than just lost. The picture of the body, fertility and beauty is hit. Artificial tooth implants would help to differentiate the identity of each individual, much as the natural teeth did, and in the end preserve the missing picture of the eye. The aim of setting artificial teeth is to simply place them where the natural teeth are .[5]

In the mid on late twentieth century, dentistry developed Similarly as an Exceedingly composed calling with propelled medication methodologies What's more conventions empowering dentists with effectively treat dental ailment. Throughout the later and only those twentieth century, professionals started to see a movement in the kind from claiming dentistry people in general might have been trying. People in general might have been never again constrained on select between metallic therapeutic materials that restored work however exhibited stylish bargains. The practitioner must have the capacity with place aside personage inclination Also permit the patient should aide esthetic choices. Once this occurs, the probability about esthetic accomplishment dramatically builds.

Guiding Principles of Esthetic Dentistry:-

There are several guiding principles that can dramatically improve the success of esthetic treatment.[6]

1. Facial component: which include Facial feature,Tooth visibility.

2. Component of smile: which include Lip line, Smile line, lip curvature, Negative space, and Smile symmetry.

3. Dental components: which include Dental midline, Golden rule, Dental morphology, Contact point.

4. Gingival component: which include Gingival morphology, Gingival contour.

Facial component:-

· Facial feature

The esthetics of dentures depend on the facial and oro-dental factors. The facial factors are all important in producing a pleasant harmonious facial expression. the facial feature depends on occlusal rim, proper thickness of the labial flange and a Position of the anterior teeth anteroposterior.[7]

· Tooth visibility

The measure from claiming tooth visible structure that is uncovered the point when a tolerant will be at rest, talking, grinning, alternately snickering need A critical impact on the stylish showing arrangement 2–4 mm for tooth structure uncovered at rest is tastefully accepted .[7,8] However, the dental specialist must Think as of that as patient age, they Regularly indicate least maxillary tooth structure at rest because of incisal wear in the nonattendance of compensatory ejection Also passing about versatility in the upper lip About whether. Vig and Brundo (1978) found that those Normal incisor introduction at rest in period 30 of age might have been 3–3. 5mm, at age 50 a considerable length of time it might have been 1. 0–1. 5mm , Furthermore Eventually patient 70 A long-time of age it might have been 0–0. 5mm.[9]

Component of smile:-

· **Negative space**

An alternate component for thought for grin configuration the point when assessing a patient's grin may be the measure from claiming negative space made

bilaterally between those maxillary teeth and the corners of the lips. Patients for limited arches What's more totally smiles need that's more negative space, while patients for wider arches What's more narrower smiles need lesser. Investigations bring really demonstrated that negative space doesn't altogether influence the generally stylish assessment of a smile, so choices to fill those buccal hall Toward expanding those forms of maxillary posterior restorations ought further bolstering a chance to be aggravated with respect to a case-by-instance premise.[10]

· **Smile symmetry**

In dental terms beauty is all about a person's smile being symmetrical. Symmetry on the left and right side is the same shape, using an ideal amount of space for your dental arch (the way your teeth are positioned in your jawbone) which determine by placement of midline. Draws this line naturally the middle of the central incisors, whereas in a horizontal type of symmetry, the eye e fixes looking into a central midline.[11]

Dental components

· Dental midline

The balance smile depends on the facial and dental midline coincidence, respectively. The facial midline is aligned in a straight line by landmarks such as nose, philtrum and chin. It should be in the centred of the face, and match the dental midline. The midline in dentistry is the midline between maxillary and mandibular arches while the teeth are in full interception.[12] Dental midline is the basic aspect of smile design and should be parallel to the long facial axis. It will also be perpendicular to the line of incisal and perpendicular to the papilla. Establishing a dental midline parallel to that of the face is the first step during the manufacture of set or removable prosthesis.[13]

· Height of lip line:

The lip line divides the bottom third of the face in the relaxed face, where the teeth do not touch together (when the lower jaw is in the rest position with freeway space present).[14]

A smile's esthetics are determined by characteristics such as the level of gingival showing and the incisors. A smile displaying limited gingival display was considered more esthetic than a smile revealing extreme gingival display.[15]

In 1970, Hulsey investigated the effect of upper lip height on smile attractiveness and found that the upper lip at the height of the gingival edge of the upper central incisors has the most desirable smiles.[16]

Factors influencing the size and form of the anterior teeth: -

(1) The size of the face.

(2) The amount of available inter-arch space.

(3) The measured distance between the right and the lift maxillary canines, using the arch and incisal papilla as measuring guides.

(4) The length of the lips.

(5) The size and the relation of arches.[17]

· Teeth Arrangement :

The maxillary foremost teethe will be positioned on the denture base. The vast majority vital thought to making an stylish denture is those position of the maxillary foremost teeth, E. Pound (personal communication, 1975) said that, in the early days Pound said that he strove on settle on dentures stunningly stylish Furthermore that those better they looked, those superior they spoke. This will be how as much lifetime study about phonetics started. In the act from claiming finish denture prosthetics.[18]

· Key to Superior Denture Esthetics :

The position of the maxillary central incisors is the key to denture aesthetics. If the maxillary central incisors are correct, they will directly influence the position of every other tooth in the denture. Correctly placed, the maxillary six incisors should be as close as possible to the exact position once occupied by the natural teeth.[19]

Anterior teeth, both maxillary and mandibular, will look at a subtly different angle as though they were emerging from the jaw. However, Sharry wrote that the older the patient, the more the incisal edges should be. If photographs are available and show diastemas, it should be remembered that the younger the patient, the more

the incisal embrasure is opened.[20]

Always keep the incisal plane level and slightly curved to follow the smile line of the lower lip. There is nothing more unesthetic than a slanted occlusal or incisal plane.[21]

Materials and Methods

The sample

The sample selected from Baghdad University, college of Dentistry. A total of 100 Iraqi adult Dental patients were clinically examined (females and males) . After getting the approval of the prosthodontic department authority in college, prosthodontic diagnosis clinic was prepared to be used as an examination room in order to use the dental chair in my clinical examination of the sample. After explaining the purposes and the aims of my study to the sample patient, consent was taken from the subjects to participate in my study.

Methods

· History

Each subject was seated on the dental chair in a comfortable position and asked information about name, age, sex. Then the subject was clinically examined (extra orally and intraorally) to check his/her fulfilment of the required sample selection.

· Clinical examination :-

Each person was seated in the Dental chair and was asked to look straight ahead and the following measures have been taken:-

1. The patient was, seated at up right position and asked to look straight, the inter-pupillary width was measured from mid pupil to mid pupil , the incisal edge parallelism to the inter-pupillary line was checked , and the head in natural (relaxed) position.

2. Then the midline coincidence with the face was measured

3. By using the 2 ruler and asking the patient to relax and sit in upright position we checked the space between the Ricket's E-plane (the straight line from tip of nose to chin prominence) and the upper and lower lip.

4. The negative space were checked by asking the patient to smile and laugh.

5. Then checked Williams's relationship (the shape of the central incisor coincidence with the shape of the face)

6. Those subjects were situated agreeably on the dental seat to a loose state for an upright position for those leader resting solidly against those mind rest. The ICD might have been measured from those average angle of the average angle of the palpebral fissure. The separation the middle of these two focuses might have been measured utilizing a vernier caliper without the application of pressure by the recording parts of the caliper only Previously, contact with the average angle of the palpebral fissure, and the effect might have been contrasted with those width of the six foremost teeth.

7. By checking the length of the face (from hair line to chin prominence) and the length of the central incisor have the H.pound's concept result.

8. By asking the patient to relax checked the relationship of corner of the mouth to the distance from canine to canine.

· Statistical analysis:

The descriptive data were expressed as mean and standard deviation (Mean ± SD). Comparison of clinical parameters of the groups was analyzed using T-test Statistical analyses were performed with SPSS version 23 (SPSS, Chicago, IL, USA), and P-value<0.05 were considered statistically significant.

Results

The results obtain from case sheet analysis using SPSS computer program (version 23) and each question results show in separated tables and descriptive statistics data of study groups in table 2. frequency of age, sex ,negative space , William relationship , corner of mouth from canine to canine and inter ala of nose to width of 4 anterior teeth of study sample shown in table 3. The relationship between length of face and length of central incisor by test .Distance from Ricket's E -plane to upper and lower lip of study sample .

Table 1: frequency of age of study sample

No.	Frequency of age	Number of samples
1	30-39	4
2	40-49	8
3	50-59	28
4	60-69	34
5	70-79	26
sum		100

Table 2 : Descriptive statistics data of study groups

	sex	midline	William relationship	Negative space	Cesaro and letta relationship	Corner of mouth to canine to canine	Inter ala of nose to width of 4 ant. teeth
N	100	100	100	100	100	100	100
Std. Error	.08468	.10426	.07771	.09829	.10389	.10389	.09478
Median	1.0000	1.5000	1.0000	1.0000	1.0000	1.0000	2.0000
Mode	1.00	1.00a	1.00	1.00	1.00	1.00	2.00
Std. Deviation	.41485	.51075	.38069	.48154	.50898	.50898	.46431

samples		frequency	present	Valid present	Cumulative present
sex	male	78	78	78	78
	female	22	22	22	100
	total	100	100	100	
Negative space	present	66	66	66	66
	absent	34	34	34	100
	total	100	100	100	
William relationship	yes	79	79	79	79
	no	21	21	21	100
	total	100	100	100	
Corner of mouth from canine to canine	equal	57	57	57	57
	unequal	43	43	43	100
	total	100	100	100	
Inter ala of nose to width of 4 anterior teeth	equal	7	29.2	29.2	29.2
	unequal	17	70.8	70.8	100
	total	24	100	100	

Discussion

· The incisal edge parallelism with the inter-pupillary line:-

The inter-pupillary distances is a facial landmark that is stable and does not modify after the age of fourteen and it's aid in the selection and arrangement of the anterior teeth, in more than two thirds of the patients the incisal edge was parallel with the inter pupillary line, except in few cases were there was no parallelism because faulty arrangement of teeth or the problem in patient eyes

Correlation exists between the bipupillary plane and the tangent at the upper central incisor incisal tip. This state is verified as real, regardless of gender which agree with Da Silva-ConcÍlio *et al.*,[3]

· The mid line :

It is an imaginary vertical line that separates the tow central incisors. In most of the patient the mid line was coincide with the face , the results shown 50% of sample have deviation in the mid line and that may be either the errors either by clinical in recording the centric or laboratory steps from the technician or the patient has

facial asymmetry .

· Distance from Rickets E-plane to upper and lower lip :-

There was a deviation from normal values which is in the average the lower lip would be 2 mm behind the line, and the upper lip 4 mm behind the line .in some patient the lower lip was 6mm behind the line and the upper lip was 1cm behind the plane. this result may different due to absent middle east people rang because no research in this flied or may be due the denture didn't give the normal support to the lips or the

· The negative space :-

The negative space (buccal corridors) is the dark space that appears bilaterally during laughter and mouth opening, the adequate restoration of the lateral negative space will permit the characterization of the smile and enhances personality.

More than half of the patients have bilateral negative space that show when the patient smile and laugh, but in some patient the negative space didn't show and that because the faulty arrangement of the teeth.

Factors contributing to buccal corridor:

1. Size

2. Photography

3. Mandibular opening

4. Dental arch. [22]

· Williams's relationship :-

Almost in all the patients the shape of the central incisor was coincide with the shape of the face. The result show most patient have the harmonies between face form and incisor form. Clinically, many factors combine to decide the shape of the tooth, such as color and the contour of the reflective field, resulting from light interaction.[23]

The esthetic effect of the shape of the incisor does not depend on an agreement between the shapes of the face and the teeth, because changes in the smile are more important than the shape of the incisor. study showed neither a metric relationship between face and central incisor nor data to support Williams' "law of harmony" or "Dentogenic Theory" [24].

· H.pound's concept:

The H.pounds concept can be calculated by:-

Length of the central incisor = length of the face \ 16, the two measures must be equal

The result show significant relation between length of face and length of central incisor which can be control during arrangement of teeth. H.Pound's formula is one of the calculating methods for assessing the thickness of the central incisor in the maxillary.

Pound derived two formulae to evaluate central incisor width and length using bizygomatic width and face length respectively. The use of H.Pound's formulae for the maxillary central incisor may be useful because just a small difference between the real lengths of the tooth and the proposed one, which is only 1.70 mm. H.Pound's formula is therefore proved to be accurate which agree with Bin Abdul Salim et al [25]

The wrong selection may result in the teeth looking 'awkward.' Many formulae have been devised for selection of anterior teeth and one of them is the formula of H.Pound.[26]

· The relation between the corner of the mouth and the distance from canine to canine:

In a relaxed patient the normal and esthetic position of the canine is the corner of the mouth , in 2\3 of the patient the canines was in the corner of the mouth as shown in result as result of technical error in teeth selection or clinical error in determine canine line in centric step.

· Inter-ala distance with the four anterior teeth**:**

The width of the nose may be used in the selection of the size of the anterior teeth, for the positioning the maxillary canines and for registering the curve of the anterior arch (Picard 1958 , Lee 1962 , Wehner 1967, Smith 1975, Mavroskoufis 1981, Scandrett 1982, Hoffman 1986) . The result shown large percent not equal the Inter-ala distance with the four anterior teeth which may be due to size and shape of arch or may be due to use large teeth to be in harmonized with age. Sex

and personality of patients or due technical error during selection size of teeth or during arrangement.

Conclusion

This artistry strives to soften the marks imposed upon the face by time and enables people to face their world with renewed enthusiasm and confidence.

A large portion significant attention throughout finish denture development is those rebuilding from claiming keep going feel. esthetic dives substantially further over the negligible placement of the teeth on the occlusal rims, size, shape, color about simulated teeth and denture bases need aid essential On development of a stylish denture. Distinctive individuals have communicated different assumption for settling on tastefully pleasing dentures.The most important consideration during complete denture construction is the restoration of last esthetics. Esthetics goes much further than the mere placement of the teeth on the occlusal rims, size, shape, color of artificial teeth and denture bases are important in construction of an esthetic denture. Different people have expressed different opinion for making esthetically pleasing dentures.

Several variables affect the esthetic outcome of anterior teeth in complete denture regarding the size, probably more than one anatomic reference is needed to predict correctly the width of maxillary incisors and canine.

Autologous techniques should be used wherever possible to match the original smile of the patient.

Final decisions, including the color of teeth and their customization should be made during the trial insertion stage and should be confirmed through consultation with patient. esthetic parameters should also be integrated with phonetics, so that tooth position is changed within functional limits.

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Genotypic, Phenotypic Identification and Antibiogram Assay of Mrsa Isolated from Healthy Individuals in Wasit Province

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Abstract

Staphylococcus aureus is a Gram-positive, facultative anaerobic, non-spore-forming, coagulase -positive bacterium. the Methicillin resistance is mostly mediated by the presence of mecA gene acquisition of mecA gene leads to altered penicillin binding protein (PBP), called PBP2a, with low binding affinity to methicillin. A total of 52 *S. aureus* strains were analysed. Antibiogram assay for 52 healthy carriers of MRSA isolates by using Vitek 2 system to examine 16 different antibiotics.Methicillin resistance was confirmed by presence of the mecA gene by PCR. Of 52 *S. aureus* samples, 52 isolates of them were MRSA confirmed by targeting mecA gene using PCR. Aims of this study detect methicillin resistant *Staphylococcus aureus* from nose of healthy individuals in Wasit province and confirming that by PCR to detect mecA Gene.

Key words: *Staphylococcus aureus, MRSA, Antibiogram , healthy individuals , mecA gene.*

Introduction

Staphylococci are gram positive bacteria , the cells of *S aureus* about 1 μm in diameter arranged in irregular single small spherical (cocci), clusters, grow most rapidly at 37°C ,*Staphylococci* are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 9% sodium chloride , their PH growth ranges 7-7.5 [(1)]. Approximately 30% of healthy individuals carry *S. aureus* in their anterior nares, is also considered to be the most pathogenic of the *staphylococci* and has been associated with a wide range of infections such as skin and soft tissue infections [(2)]. The problem is compounded by the fact that many hospital strains of *S. aureus* are resistant to most of the commonly used antimicrobial agents, especially methicillin, these strains known as methicillin-resistant *S. aureus* [(3)]. Resistance of MRSA is caused by possession of the mecA gene, which codes for a penicillin-binding protein [(4)] that binds the drug less well ,this alternative protein confers resistance to all beta -lactam antibiotics [(5,6)]. Nasal *S. aureus* carriage plays a key role in the pathogenesis of infection by representing the source and the independent risk factor for subsequent infections [(7,8)].

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Methods

Sample Collection

A 231 sample were obtained from nasal of healthy carrier, who attended from Wasit hospitals during the period from November-2018 to March-2019.

Isolation and Identification of S. aureus

Isolation of S. Aureus : The samples& specimens were cultured directly on blood agar medium and incubated at 37Cº for 24 hours to study characteristic of growing colonies and capability of blood hemolysis,for the primary identification of S. aureus. After that it was transported to Mannitol-salt agar as a selective medium for these bacteria and incubated at 37°C for 24 hours [(9)].

Primary Identification of S. aureus

A. Colonial Morphology: its identification was depended on the morphological properties (Colony size, color , edge , texture and hemolysis on blood agar

. The single selected colonies culture on mannitol salt agar plates ,Growth of yellow colonies as a result of fermentation of mannitol [10].

B. Microscopic Examination **:** Single purefied of bacterial colony used to stained with gram stain for the primary identification [11].

C. Biochemical Tests : positive result for Catalase [12], Oxidase [13], Haemolysis [14], Coagulase [11].

Confirmation of Biochemical Test by API Staph System

The API Staph System, produced by bio-Merieux is a reliable method for identifying 23 species of staphylococci and it was used to confirm the biochemical identification of the isolates [3,4].

Identification of Methicillin Resistance *Staphylococcus aureus*(MRSA) by PCR

1. Phenotypically Detection of MRSA

Detection of MRSA by cefoxitin disc diffusion method: All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc of cefoxitin . A 0.5 McFarland standard suspension of the isolate was made and the culture was done on MHA plate. Plates were incubated at 37ºC for 18 hr. An inhibition zone diameter of ≤ 19 mm was reported as Methicillin-resistant isolate while ≥20 mm of zone diameter inhibite was considered as methicillin- sensitive isolate [15,16].

confirmation of detected of MRSA by vitek2 system: The AST-P592 card used for *staphylococci* contained , Beta-Lactamase, Cefoxitin screen, oxacillin

A. Inoculum Preparation : Suspensions were set up by emulsifying bacterial isolates in 0.45% saline to what might be compared to a 0.5 McFarland turbidity standard [17].

B. Sensitivity Test by Vitek2: prepared Suspensions as previously was used for VITEK 2 system. AST-P592 Card Used for Contained *Staphylococcus*, Beta-Lactamase Screen, Cefoxitin. The antibiotic groups of the VITEK 2 system in this study are described in table (1) [18].

Table (1) antibiotics included in vitek card and their abbreviation:

Antibiotics	Abbreviations
Erythromycin	E
Piperacillin	PRL
Moxifloxacin	MFX
Aztreonam	ATM
Ceftazidim	CAZ
Rifampicin	RP
Penicillin G	PG
Clindamycin	CD
Ciprofloxacin	CIP
Gentamycin	CN
Chloramphenicol	C
Imipenem	IPM
Cefoxitin	FOX
Oxacillin	OX
Sulphamthoxazol/ Trimethoprim	SXT
Tetracycline	TE
Vancomycin	VA
Azithromycin	ATH
Trimethoprim	TM

Genotyping Detection of MRSA Isolate

Extraction of bacterial genome : Extraction of genomic DNA was preformed by using FavorPrep Blood/ Cultured cells Genomic DNA extraction Mini kit and protocol was followed as per manufacturer's instructions.

Detection of DNA product by agarose gel electrophoresis : DNA samples were electrophoresed by horizontal agarose gel electrophoresis according to [19].

Polymerase Chain Reaction Protocols : Methicillin resistant strains were determined by the presence of the mecA gene by monoplex PCR as described previously. MRSA isolates were tested for the presence of the 533-bp PCR product of the mecA gene using the following primers: forward 5'-TGGCTA TCG TGT CAC AAT CG-3'and reverse 5'-CTGGAA CTT GTT GAG CAG AG-3'.

A. Monoplex PCR Mixture: The DNA extract of MRSA isolates were subjected to *mecA* gene by monoplex PCR. The protocols used depending on manufacturer's instruction [20].

B. Detection of DNA Product by Agarose Gel Electrophoresis: DNA samples were electrophoresed by horizontal agarose gel electrophoresis as previously reported according to [19].

Results and Discussion

Isolation and Identification of *Staphylococcus aureus*

Cultural Characteristics for Isolation and Identification of *S. Aureus*

Identification was performed by streaking all specimens onto blood agar (BA) which were then incubated at 37˚C for 24hours. the colonies on BA plates showed up enormous, round, velvety/buff shaded settlements, delivering total away from of β-hemolysis . results were agreed with [22]and WHO 2003, the bacterial isolates were purified by culture isolates on the selective media of *S. aureus* which was the mannitol salt agar . the colonies appeared as small, circular smooth and yellow-golden color colonies, these results were agreed with [21].

Microscopical Characteristic and Biochemical Tests

S. aureus was isolated and identified depending on the cultural and microscopical properties as long biochemical test Catalase test is useful to distinguish staphylococci from enterococci The formation of bubbles indicates a positive test [22]. coagulase tests is performed to identify the ability of *S. aureus* isolates to coagulate plasma Positive result indicated by clot formation within 4 hr Where clotting did not occur the tubes were incubated at room temperature for an additional 18hr agreed with [10,23].

Number of *S. aureus* and MRSA isolate

A total of 231 specimens were collected from healthy people, 52 were identified as MRSA by disc diffusion method and all of them contains mecA. This study show a high prevalence of MRSA carriage in healthy people that indicating high risk of spreading infections as shown in table (2). this mismatches with [24] and [25]. There are no studies that are completely match these results.

Table (2): Percentage of antibiotic resistance for healthy carriers MRSA

Types of specimens	Total NO.	No. negative	S.aureus	No.(%)of MRSA
Nasal swab	231	179(77.4%)	52(22.5%)	52(100.0)

Identification of MRSA

All the isolates were subjected to cefoxitin disc diffusion test using a 30 µg disc of cefoxitin, Methicillin resistant *S. aureus* (MRSA) emerged, resistance is caused by possession of the mecA gene which codes for a penicillin-binding protein that binds the drug less well, this alternative protein confers resistance to all beta -lactam antibiotics [5,6]. The confirmation of detected of MRSA by vitek2 system [18]. Of the 52 *S. aureus* isolates in Wasit province , 52 were identified as MRSA by disc diffusion method and all of them contains mecA ,this mismatch with [19]. No studies that are completely match these results.

Antibiotic Susceptibility Test of MRSA

The susceptibility test was done for MRSA isolates. Table (3) shows percentages of antibiotic resistance for 52 healthy carriers MRSA isolates by using Vitek 2 system to examine 16 different antibiotics. the present study, MRSA showed the highest resistance for each two group as 100% for both oxacillin and cifoxitine and Vancomycin group with present 98% .this study were similar to [26], Whereas, the remaining classes of antibiotics tested came with a different resistance rate as showen in table (3).

Table (3): Percentage of antibiotic resistance for healthy carriers MRSA isolate

Group of study / Antimicrobials	isolates from Healthy Carriers %	Mean + Std. Deviation
Linezolid	100	A 100 + 0.000
Ciprofloxacin	94	B 95.00 + 1.41421
Gentamicin	93	B 93.500 + 0.70711
Tigecycline	92	C 91.00 + 1.41421
Trimethoprim/Sulfamethoxazole	90	C 91.00 + 1.41421
Moxifloxacin	88	C 89.00 +1.41421
Imipenem	80	D 80.00+ 0.000
Clindamycin	72	E 70.500 +2.12132
Teicoplanin	50	H 48.00 + 2.82843
Erythromycin	41	I 42.00 +1.41421
Rifampicin	29	J 29.500 + 0.70711
Tetracycline	24	K 25.00 + 1.41421
Vancomycin	2	L 2.500 + 0.70711
Benzylpenicillin	0	L 0.0000 + 0.00000
Oxacillin	0	L 0.0000 + 0.00000
Cifoxitine	0	L 0.0000 + 0.00000

Table (4) represents the percentages of antibiotic sensitivity of MRSA isolates isolated from healthy carriers ts groups by using Vitek 2 system to examine 16 different antibiotics with the using of minimum inhibitory concentration (MIC) [15]. In the light of this results, MRSA showed that the highest mean + the standard deviation of sensitivity to

100 + antibiotics were 0.000 for class oxazolidinones (linezolid) followed by 95.00 + 1.41421and 93.500 + 0.70711 for class fluoroquinilones (ciprofloxacin) and aminoglycosides (gentamicin), respectively.

Table (4) The percentages of sensitivity of MRSA isolates isolated from healthy carriers

No.	Antimicrobial Group	No.(%) Healthy Carrier
1	Benzylpenicillin	100
2	Oxacillin	100
3	Imipenem	20
4	Vancomycin	98
5	Teicoplanin	50
6	Ciprofloxacin	6
7	Moxifloxacin	12
8	Erythromycin	59
9	Clindamycin	28
10	Linezolid	0
11	Gentamicin	7
12	Tetracycline	76
13	Tigecycline	8
14	Rifampicin	71
15	Trimethoprim/Sulfamethoxazole	10
16	Cifoxitine	100

Molecular Identification of MRSA Isolates

Several methods have been described that utilize polymerase chain reaction (PCR) for the detection of MRSA directly from clinical specimens (27) or in combination with broth enrichment. The feasibility of the PCR methodology for the identification of *S. aureus* strains and for the detection of antibiotic resistance genes has already been shown [28].

DNA extraction from MRSA

DNA was extracted by using several processed demodulation of the company Geneaid and took time from 24 to 72 hr.

PCR Detection of *mecA* gene

Monoplex polymerase Chain Reaction was used in the present study for the detection of *mec*A gene. *mec*A gene has been detected among all tested *S. aureus* isolates that considered phenotypically as MRSA by using the disk diffusion method and Vitek 2 system and subjected to PCR to detect the presence of *mecA* gene, all of them gave positive results using PCR . So , the correlation between phenotypic and genotypic method was 100% .These result agreement with the results of [29]. PCR product appeared as a DNA band with an about (533) bp in size as shown in figure (1) for healthy carriers. These results were agreement with previously published results by [30] and inconsistent with [29] and [31] whom detected a (310bp) DNA fragment in all tested *S. aureus* isolates.

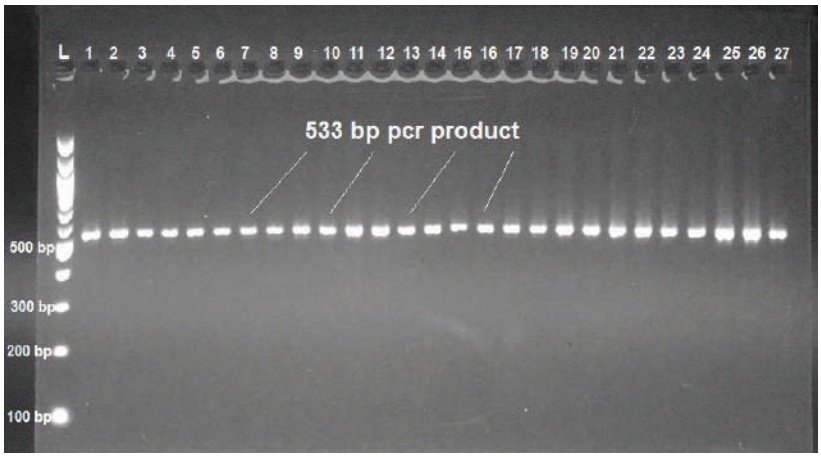


Figure (1): Agarose gel electrophoresis of PCR amplification products from carriers group of *S. aureus ,mecA* **gene (2% agarose, TBE buffer (0.5X), 100 volt and 50 AM for 1 hour.). L:The DNA molecular weight marker (100 bp ladder)**

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

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Study the Elevation of Fibrinogen level in Plasma Patients with Diabetes Mellitus Type 2 in Babylon Governorate

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Abstract

Objective: The incidence of diabetes mellitus type 1 and type 2 has increased dramatically worldwide in the past 20 years. Type 2 diabetes is probable to increase further in the future owing to the increase in obesity and the lack of exercise. This may rise the danger of unindustrialized cardiovascular illness in patients with type 2 DM. It may be because fibrinogen has an important role in the increased risk of cardiovascular disease. The aims of current study to find out the level of fibrinogen and its relationship to the level of blood glucose in patients with type 2 DM. **Methods:** The study contain 50 patient with diabetes mellitus type 2 as patient group and 50 adult apparently healthy as control group. All plasma samples were collected from all subjects. The age for both groups concerning (43-75) years. The collected data include measures, plasma fibrinogen and HbA1c. **Results**: The present study revealed a significant increase in plasma fibrinogen, HbA1c concentration in patients compared with control group ($p<0.01$). **Conclusion**: The results of this study reveal a significant increase in fibrinogen level in patients with diabetes mellitus type 2 without cardiovascular disease.

Keywords: *toxicity; patients; diabetes; cardiovascular; plasma*

Introduction

The incidence of diabetes mellitus in the worldwide has risen melodramatically over the past two decades Although of both types of diabetes mellitus prevalence is increasing worldwide, occurrence of type 2 diabetes mellitus is predictable to rise extra speedily in impending because of increasing obesity and reduced activity levels [(1)]. One of major and important diabetes complication is diabetic foot syndrome (DFS) [(2)].

Diabetic Foot Ulcer (DFU) is well-defined as "Infection, ulceration and/or destruction of deep tissues linked with neurological abnormalities and numerous degrees of 2 peripheral vascular disease in the lower limb" [(3)].

diabetic polyneuropathy and peripheral arterial disease are a chief risk aspects for foot ulcer [(4)]. In many stages, the immune system is necessary and very important, especially in the case of chronic wound healing, and at sometimes stimulation of the immune system negatively affects the body by afflicting the patient with diabetes mellitus with diabetic foot ulcer disease and coronary heart disease. [(5)].

Fibrinogen, plays a vital role in hemostasis, precisely in clot development and stabilization with a 340 kDa glycoprotein molecular weight [(6)]. The level of fibrinogen in the plasma is one of the most important components of the clotting chain. It is also considered an essential factor or component of blood flow and its viscosity. It is known that the high level of fibrinogen in the plasma is considered a cause of vascular disease and heart disease, and very high levels may affect the rise of fibrin clots, blood viscosity, and the stimulation of arteriosclerosis. Continuous impairment of metabolic control leads to an overproduction of fibrinogen, and thus may effect On the usual process of wound healing [(7)]. Impaired glucose tolerance induces the effect by increasing clotting factors such as fibrinogen in diabetics [(8)]. Fibrinogen itself is potent through many modifying and non-modifiable factors such as body mass index . gender, age, smoking, glycemic control, and urine albumin excretion rate [(9)].

In view of above notions this planted project assumed to know the significance of fibrinogen and glycemic control as accepted risk factors in diabetes mellitus type 2 with ability to make it as pointers to evaluate the severity of DFU.

Method

The study contain two groups (T2DM and control group), the age was between (43-75) years and body mass index (BMI) was 29.3. The practical part of the study took place in the laboratories of the Department of Biochemistry at the College of Medicine / University of Babylon. The patient group contain 50 individual were collected from Marjan Medical City and Al-Hilla General Teaching hospital in Hilla city. other group contain 50 adults were collected from medical staff which were apparently healthy as control group. All individuals in this group have no signs of diabetes mellitus and its complications. All individual in tow groups that suffered from disease such as, diabetes, circulating diseases, stroke, hypertension and malignancy which affect oxidation state were excluded.

Blood Sampling

Five milliliters of venous blood was aspirated from all participants in fasting status. The samples were stored at -20˚C. plasma were used to measure fibrinogen level and HbA1c by Enzyme Liked immune-sorbent assay (ELISA) technique**.**

Statistical Methods

all results were measured as mean ± SD. Student's, A p-value <0.05 was considered statistically significant. SPSS software version 18 was used.

Results

Serum fibrinogen level with HbA1c in T2DM group were showed in the table (1). A significant increase in fibrinogen level was found in T2DM group by compared with control group (P<0.01). Also a significant elevated in HbA1c was found in T2DM group in comprison with control group (P<0.01).

Table (1) Fibrinogen with HbA1c in studied groups

Parameter	T2DM mean±SD	Control mean±SD	P. Value
No.	**No.50**	**No.50**	
HbA1c	5.75±0.4 (6.1-5.3)	4.3±0.4 (4.7-3.9)	P < 0.01
Fib.* mg/ml	6.7±0.9 (7.6-5.8)	3.09±0.5 (3.59-2.59)	P< 0.01

* Fibrinogen

Discussion

Fibrinogen level in the blood is one of the very important components in the blood clotting process, in addition to it playing an important role in blood flow and blood viscosity. There are many studies that indicate that a high level of fibrinogen in the blood is often linked with an increased risk of cardiovascular disease, including stroke and thromboembolism [10].

Depending on the present results we notice a noticeable increase in fibrinogen level in patient with T2DM by compared with control group and the result was agree with many research like Bembde AS et al originate fibrinogen to be advanced in diabetic patients group than the control group [11].

A previous study in Italy showed that there is an increase in the level of fibrinogen in the blood and that

it is associated with hemoglobin HbA1 in patients with type II diabetes. The results also showed that there is a close relationship between fibrinogen and hemoglobin HbA1c, as the level of fibrinogen is one of the signs of the physiological changes of blood vessels in diabetic patients The second type [12].

Parag Gupta and et al suggests that this increase in blood fibrinogen may be related to primary inflammation in patients with type 2 diabetes, which may lead to vascular complications in the future. [13]. And that there are many studies that have confirmed strong evidence that of fibrinogen levels in the blood are individualistically related to the likelihood of vascular disease or its later development [14].

In patients with type 2 diabetes, an increase in the concentration of insulin and amino acids in the blood is observed, and this occurs with insulin therapy or in the absence of treatment [15].

From these observations, an increase in the level of fibrinogen in the blood of type 2 diabetics can be attributed to an increase in the concentration of insulin that stimulates the production of fibrinogen, and this result is consistent with a previous report on the production of fibrinogen in the laboratory from stimulating liver cells after prolonged exposure to insulin [16;17]. The role of insulin in overproduction of fibrinogen in patients with type 2 diabetes indicates a relationship between hyperinsulinemia and insulin resistance with the change in acute-phase reactant production[18;19].

Conclusion

The results of this study reveal a significant increase in plasma fibrinogen concentrations in patients with diabetes mellitus type 2 without cardiovascular disease. And there is still an increase in hyperfibrinogen in patients with diabetes mellitus type 2.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

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Relationship between Echocardiographic Epicardial Fat Thickness and Different Body Fat Distribution

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Abstract

Background : Obesity is a worldwide complicated health problem. It is connected with an amplified cardiovascular mortality and morbidity result from obesity itself on one hand and concomitant with other medical conditions like (diabetes , hypertension, , sleep apnea syndrome, insulin resistance) on other hand. obesity result in multiple functional and structural cardiac changes which may cause heart failure, these changes detected by developed cardiac imaging methods, one of these methods is Echocardiography which is simple, accurate, non-invasive, sensitive diagnostic tool for evaluation of important cardiac changes related to obesity .

Objectives: The study is aimed to determine the consequences of obesity on echocardiographic value for epicardial fat thickness in adult and middle age Iraqi persons .

Patients and Method: The study was a case-control, based on a non- randomized selected samples from both gender from Basrah city, carried out on 150 subjects aged 20-55 years, persons were grouped according to body mass index to: 1- 50 obese (no history of cardiovascular disease) (BMI≥30kg/m2) , 2- 50 obese having ischemic heart disease (BMI≥30kg/m2) , 50 control apparently healthy (BMI 18.5-24.9) Iraqi subjects all persons underwent transthoracic echocardiography to measure the epicardial fat thickness, this value was stated as mean and standard error. This study was done between December 2018 till April 2019 at the Echocardiography Unit in Basrah Teaching Hospital .

Results: There was a significant increase in epicardial fat thickness in addition to some anthropometric indices among obese subjects as compared to control group , but waist / thigh, waist/hip and conicity index looked unchanged significantly.

Conclusion: Obesity is positively interconnected with increase in epicardial fat thickness in addition to some anthropometric indices that support obesity and can predict cardiovascular risks .

Key words: *Epicardial fat thickness, obesity, body fat distribution*

Introduction

It is a medical condition characterized by extra adipose tissue has accumulated to the amount that cause an adverse health effect[(1)]. It have been growing at a rapid rate in both developing world and the Western societies [(2)]. A study over eight years from 2003-2010 done at al-Basrah revealed that obesity occur in 55.1% of the people [45.3 % of men and 54.7% of women] [(3)]. So the worldwide obesity prevalence is anticipated to increase to 21% in women and 18% in men and surpass by 2025 [(4)].

Epicardial fat (EF) : Is the adipose tissue collected between the visceral pericardium and the myocardium, no fascia or any structure separating it from the epicardial vessels and the myocardium. It has a variable distribution, more noticeable in the atrio-ventricular

and inter-ventricular grooves and lateral wall of the right ventricular [5]. Epicardial fat have inflammatory and metabolic mechanisms besides Myocardial fibrosis [6]. some study illustrate a positive association between the quantity of epicardial fat and the occurrence of coronary atherosclerosis and the capability of adipose tissue in hormones and cytokines secretion that control coronary artery atherosclerosis, so the epicardial fat regarded as important factor causing obesity associated cardiovascular disease [7], another one suggest Linear correlation among epicardial fat thickness and onset and severity of coronary artery disease [8].

The aims of study was to find the relationship between echocardiographic changes and different body fat distribution and demonstrate the relationship between the epicardial fat thickness and coronary artery disease.

Subjects and Methods

Case-control study carried out in the Basra teaching hospital (cardiology and echo-cardio graphical unit). Started at (1\12\2018) and finished at (1\6\2019).

150 subject 67 male and 83 female were recruited from cardiovascular unit (they referred from cardiologist clinic) were included in the research, health and Personal data was obtaining from all Consenting participants and all undergone transthoracic echocardiography and anthropometric measurement. Standardized questionnaires were used, Blood pressure (BP) was measured, Anthropometric characteristics, including height and weight were used to calculate BMI (kg/ m²). Abdominal obesity was assessed by waist circumference ,abdominal circumference, neck, mid upper arm, thigh, hip circumference ,and subscapular fold thickness .

Echocardiographic methods: Transthoracic echocardiography (echo Doppler)was performed in supine and left lateral decubitus position and measurements were taken by standard two-dimensional protocols using commercially available medical ultrasound system (Vivid E9). according to the guidelines of the American Society of Echocardiography.

Epicardial fat thickness measurment :

Measurement of echocardiographic epicardial fat thickness by Two-dimensional transthoracic echocardiography. We registered three cycles of the two-dimensional parasternal long-axis view and the parasternal short-axis view at the basal left ventricular level. the EFT was calculated in millimeters (mm) on the free wall of the right ventricle (RV) in the still images obtained at the end of systole on both parasternal long-axis and short-axis views where the beam of the ultrasound perpendicular to the(interventricular septum) at level of mid- chordal and tip of the papillary muscles. The anterior echo-lucent space between the RV outer wall and the linear echo-dense parietal pericardium was considered to be epicardial fat [9] .

An independent scientific committee reviewed and approved the study protocol and data to be delivered to the subjects.

Statistical Analysis

Data was analyzed by dividing the subjects in to three groups according to BMI (BMI= 18.5-24.9 and BMI= >30kg/m2), mean and standard deviations(M, SD) were considered. Independent T test , Fisher exact test and Yates chi square test (using SPSS version 19) were used to compare the study groups. Probability P value of less than 0.05 was considered statistically significant ($\alpha \leq 0.05$) .

Results

Table 1: The neck, mid upper arm, waist, abdominal, hip, thigh circumference Subscapular fold, epicardial fat thickness were significantly differ with greater mean among obese free from ischemic heart disease than control group with P value>0.01 for all variables .other variables in table below insignificantly correlated between two group .

Table 1: Comparison of parameters between all control and all obese free from ischemic heart disease by unpaired t-test.

Parameters	Control N=50 Mean ±SD	No IHD N=50 Mean ±SD	P value
Neck circumference (cm)	36.18+3.35	40.24+4.99	<0.001
Subscapular Fold Thickness	28.34+5.05	41.04+7.28	<0.001
Mid Upper Arm Circumference	31.54+4.33	37.3+4.36	<0.001
Waist circumference (cm)	85.28+9.42	122.5+117.37	0.028
Abdominal circumference (cm)	90.1+9.0	111.76+11.26	<0.001
Hip circumference (cm)	95.22+7.3	110.1+8.5	<0.001
Thigh circumference (cm)	41+6.25	49.94+8.52	<0.001
waist/hip ratio	0.9+0.09	1.12+1.07	0.152
waist/thigh ratio	2.13+0.41	2.52+2.47	0.270
waist/height ratio	0.61+0.57	0.75+0.74	0.283
Conicity index	1.22+0.21	1.52+1.55	0.185
Epicardial fat thickness	0.39+0.12	0.53+0.12	<0.001

Table 2 : lists links among neck circumference and various anthropometric and echocardiographic measures between two group revealed that (neck, mid upper arm, waist, abdominal, hip, thigh circumference, waist/hip Subscapular fold, epicardial fat thickness were statistically differ and significantly higher among obese having ischemic heart disease than control group with P value>0.01 for all and 0.06 for waist/ hip. Other variables in table below insignificantly correlated between two group.

Table 2: Comparison of parameters between control and obese patients having IHD by unpaired t-test.

Parameters	**Control N=50 Mean ±SD**	**IHD N=50 Mean ±SD**	**P value**
Neck circumference (cm)	36.18+3.35	41.98+2.96	<0.001
Subscapular Fold Thickness	28.34+5.05	41.66+8.0	<0.001
Mid Upper Arm Circumference	31.54+4.33	37.7+6.01	<0.001
Waist circumference (cm)	85.28+9.42	103.66+9.72	<0.001
Abdominal circumference (cm)	90.1+9.0	109.66+14.66	<0.001
Hip circumference (cm)	95.22+7.3	107.62+15.85	<0.001

Cont... **Table 2: Comparison of parameters between control and obese patients having IHD by unpaired t-test.**

Thigh circumference (cm)	41+6.25	51.92+8.11	<0.001
waist/hip ratio	0.9+0.09	0.98+0.2	0.006
waist/thigh ratio	2.13+0.41	2.05+0.41	0.316
waist/height ratio	0.61+0.57	0.64+0.07	0.707
Conicity index	1.22+0.21	1.27+0.08	0.132
Epicardial fat thickness	0.39+0.12	0.5+0.12	<0.001

Table 3: In comparison among 100 obese patients by using the unpaired T test half have ischemic heart disease and other half free from ischemic heart disease displayed that neck circumference is greater in obese people having ischemic heart disease than those free from ischemic heart disease . while other variables insignificantly differ between two groups .

Table 3:Comparison of parameters between obese patients free from IHD and obese with IHD by unpaired t-test

Parameters	No IHD N=50 Mean ±SD	IHD N=50 Mean ±SD	P value
Neck circumference (cm)	40.24+4.99	41.98+2.96	0.036
Subscapular Fold Thickness	41.04+7.28	41.66+8.0	0.686
Mid Upper Arm Circumference	37.3+4.36	37.7+6.01	0.704
Waist circumference (cm)	122.5+117.37	103.66+9.72	0.261
Abdominal circumference (cm)	111.76+11.26	109.66+14.66	0.424
Hip circumference (cm)	110.1+8.5	107.62+15.85	0.332
Thigh circumference (cm)	49.94+8.52	51.92+8.11	0.237
waist/hip ratio	1.12+1.07	0.98+0.2	0.393
waist/thigh ratio	2.52+2.47	2.05+0.41	0.183
waist/height ratio	0.75+0.74	0.64+0.07	0.289
Conicity index	1.52+1.55	1.27+0.08	0.263
Epicardial fat thickness	0.53+0.12	0.5+0.12	0.135

Discussion

Obesity is a growing major well-being problem in the developing and developed countries. Old-fashioned obesity indices like (BMI), waist-hip-ratio (WHR), waist circumference (WC) are well recognized measures to detect obese subjects, but, in the current study, the application of subscapular fold thickness, NC, mid upper arm circumference measurement were tested as an added tool for recognizing obese people and to investigate the correlation between the obesity and heart changes that demonstrated by echocardiography. On the other hand, body fat distribution is as important as central fat mass can be recognized as an independent risk factor for cardiovascular disease and metabolic disease as well as overall mortality. In contrast, peripheral fat mass may independently contribute to a lower risk for cardiovascular disease [10].

It was aimed in this study to use neck circumference as an important predictor of some complications of obesity, we found there was significant correlation of neck circumference and BMI and ischemic heart disease as the mean of neck circumference in obese having ischemic heart disease greater than mean of obese free from ischemic heart disease and control groups so the neck circumference regarded as risk factor for heart ischemic changes, that supported by many studies : Simpson conclude there was a significant association between changes in NC and BMI, Ben- Noun and Laor and Onat showed that NC was associated with metabolic syndrome [11,12].

The subscapular skinfold thickness (SST) is a simple measure of truncal obesity (subcutaneous fat), as compared to body mass index (BMI), it might represent a more direct and better measure of body adiposity, that proved by Large epidemiological studies [13,14]. In the present results, show the mean of subscapular skinfold thickness was significantly higher in obese as compared to control , and significantly higher in obese with ischemic heart disease than control group, that mean there was positive correlation between subscapular skinfold thickness and BMI, and it represent risk factor for heart disease similar finding have been reported by Donahue RP and Abbott RD (1987) they observed positive association between sub scapular fold thickness CVD risk factors [15] , and other study show it associated with unfavorable lipid profile , BP, central obesity, high insulin level and increase left ventricular mass [16], So it used as predictive for risk factors of cardiovascular disease (CVD) [17] .

It was also shown that there is a positive relationship between body mass index (BMI) and mid-upper arm circumference. Similar findings have been reported in Spain by [18]. Our findings provide evidence that waist circumference is positively and significantly interrelated to BMI and cardio metabolic risk as proved by other studies high WC increases vulnerability to insulin resistance and dyslipidemia, mostly because of its association with visceral fat [19] , central fat deposition assessed by waist circumference has cardiac risk more than peripheral fat distribution [20] ,abdominal obesity assessed by waist circumference is strongly associated with CHD risk factors than is BMI [21]. The present study illustrate significant association (P value>0.001) between high epicardial fat thickness and BMI that demonstrated by transthoracic echocardiography and that was also realized by Torres et al [22]. Also other study show similar result increase epicardial fat thickness in obese as compared to normal persons ,also verify the strong correlation between epicardial fat thickness and waist circumference, we need more time and larger sample size to investigate such relation[23]. Also, in comparison between obese patients have ischemic heart disease and control subject show that the epicardial fat thickness was thicker in obese than control that mean it regarded as risk factor for cardiac disease as proved by other studies showed that there was significant association between epicedial fat thickness and the severity of coronary artery disease , epicardial fat thickness also correlated to other cardiac situations including myocardial fibrosis , left ventricular dysfunction, coronary spasm and coronary artery calcification [24]. Another study demonstrates that epicardial fat thickness closely associated with the coronary artery disease [25] .

Conclusions

This study suggest that epicardial fat thickness was linearly interconnected with BMI and might be regarded as useful parameter for expecting future ischemic cardiac changes. Many anthropometric parameters like: NC, MUAC, WC and sub scapular fold thickness regarded as beneficial screening tool for obesity, also as predictors

of future cardiac ischemic attacks.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Biofilm production by *E. Coli* Isolated from Filter Water System

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Abstract

E. Coli is recognized as part of humans and animals microbiota and also an important opportunistic pathogen, responsible for a variety of diseases. A total of 10 *E. coli* were isolated from filter water system at different sites from Baghdad city and investigated for their ability to form biofilm by using 96-well microtiterplate. The result showed strains with high coherent to PVC. The antimicrobial resistant of four antibiotic against *E. Coli* were determined in vitro and the highest resistant was to β-lactam antibiotics. The biofilm in environment, are vital for the existence of many microorganisms, and provided pathogenic microorganisms with a ways of persisting outside of the host to represent means for susceptible population to be repeated infection.

Keywords: *Biofilm, Water, E. Coli.*

Introduction

A biofilm is a one or group of microorganisms found in structural community surrounded by slime layer of exopolysaccharides which gave it the ability to adhere to living and nonliving surface. Many surface in the environment are susceptible for bacterial biofilm like stones surfaces, small rock in a stream, swimming boll, showers and the most observable example of biofilm blockage water distribution pipes that consider an important problem in industry and in the domestic [1,2].

E. Coli is prevalent pathogen belong to family *Enterobacteriaceae* and found in the environment as well as mucosal surfaces of humans and other mammals [3,4]. *E. Coli* strains possess many cell surface components that facilitate to biofilm formation which contribute infection and survival in the host, many studies suggest that ability of *E. coli* isolates to form biofilms in vitro under a variety of growth conditions, so formation of biofilm may also be an considerable virulence factor for *E. Coli*, also it has been revealed bacterial growth with the biofilms are high resistance to treatment with antibiotic [5].

The study investigated formation of biofilm by *E. Coli* that isolated from different sites of water in Iraq/ Baghdad, also the ability of these isolates resistant to antibiotic was demonstrated.

Material's and Methods

Isolates of E. Coli

A total of 10 isolates of *E. Coli* were analyzed. Samples were collected by using sterilized swaps, the collected samples tacked from different sites then transport to the laboratory for culturing and analysis, by using Macconkey agar (HiMedia) and EMB agar, then *E. Coli* isolates were tested by API20E system of gram negative bacteria to confirm the results (BioMrrieux, France).

Antimicrobial susceptibility tests

The antibiotic resistance of. The isolates of *E. Coli* was tested for their ability to resistant of selected antibiotic through the dissemination of antimicrobial

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agents on Mueller- Hinton agar according to Kirby and Bauer, (1966) and the recommendations of the clinical laboratory standards of the national committee [6].The antibiotic discs were gotten from (Bioanalyse/Turkey). The following antimicrobial discs (concentrations in µg) were used: Ceftriaxone (CRO) (30), Ampicillin(25), Amikacin (AK) (30), and Ciprofloxacin (CIP) (5)

Formation of biofilm in vitro

Biofilm Formation in 96-hole microtiterplate

The capability to form biofilm by the isolates of *E. Coli* in 96 well microtiterplate (the basic component is PVC) was determined and depending on O'Toole procedure [7,8]. The biofilm examines were achieved by using minimal medium (M63) complemented with CAA, glucose, and MgSO4, then ELISA plate reader was used to read the plate (Beckman coulter,Austria). Isolates were considered as efficient biofilm-forming when absorbance at 595 nm after crystal violet staining was equal to or more than 0.15.

Results

In this study 10 isolates of *E. Coli* exhibited highest resistant to ampicillin reach to 100% followed by piperacillin+ tazobactam 75% while these isolates had highest susceptibility to gentamicin and Ciprofloxacin as show in Table1.

The bacteria showed highly adherent to PVC microtiterplate and this indicated formation of biofilm, the result was confirmed after staining of adherent bacteria then quantified and measuring the OD of the accumulated mass of biofilm. In microtiterplate 4 of 10 strains were produce biofilm and range between (0.63-0.4) (Figure2).



Figure1: *E. Coli* isolates on eosin methylene blue



Figure2: *E. Coli* isolates forming biofilm in microtiter plate

Table.1 Susceptibility of the 10 isolates of *E. Coli* to 4 antibiotics.

No.	Antibiotics	Resistant (%)
1	Ampicillin	100%
2	Amikacin	0%
3	Ciprofloxacin	0%
4	Piperacillin/ tazobactam	75%

Discussion

E. Coli is gram(-ve) bacteria which is found ubiquitously in the environment. *E. Coli* bacteria are an etiological factor in many infections, the most frequent are those of the urinary tract infection that can lead to kidney failure, septic infections and severe human gastrointestinal disease with diarrhea due to production of a toxin from pathogenic strains [9].

The virulence of certain *E. Coli* strains is caused by some structural antigens of the cell, including the fimbriae or pili, nonfimbrial adhesion factors, toxins, lipopolysaccharides, factors of acquisition iron and polysaccharide capsules [10,11].

E. coli is consider a normal flora or indigenous microbiota of the small intestine. Some strains of *E. coli* are pathogens that cause many disease. The ability of *E. Coli* to colonize the gastrointestinal tract of human rely on adherent factor like pili that found in both pathogenic and normal flora in the human gut that enable the bacteria to attached to mucosal surfaces then may *E. coli* produce biofilm to protect themselves from stresses that encounter in the gastrointestinal tract [12].

The results microtitration assay cleared the ability of isolated strain to production biofilm. A number of research studies define assembling of microorganisms as biofilms that can develop inside water system tubes like pipes and another surfaces and have revealed that *E. coli* which is a part of coliform bacteria can be an important contributor in biofilm formation with microbial populations that can propagate in the distribution drinking water systems [13,14,15].

Also Murthy and Venkatesan [15] reported that biofilms are omnipresent in industrial and distribution systems of drinking water and biofilms contribute as a major source of to the fouling of pipes and underwater surfaces in industrial water systems.

The ability of *E. Coli* to make biofilm that consider significant virulence factor and presence of this bacteria in water enabled the colonization on abiotic and biotic surfaces, So the capability of *E. Coli* to stick on abiotic surfaces particularly the water system tubes was examined by using (PVC) polyvinyl material which is the basic material of water tubes and the result revealed that the isolates was able to production biofilm. Collectedly biofilm production, existence of bacteria in water, may have virulence factor as well as antibiotic resistant made the bacteria made it frightening sources of pollution and must alarming the community about it with taking special steps to control it.

Also in this study we investigated *E. Coli* resistance to antibiotics and results showed highest resistance to β-Lactam antibiotic. The excessive use of antibiotics caused increasing resistance of bacteria to antimicrobial agents in worldwide, one of the reasons of this resistance that spreading of resistant strain to the environment like agricultural areas via sewage water, treatment plants and receiving water [16]. In the environment, bacteria may exchange genetic material and this resulted in appearance of new resistant strains. The bacteria found in water which are from diverse origins like animal, environmental and human are develops resistance to antibiotic because they able to mix. So the existence of bacteria that resist antibiotic in surface water and sewage

and may transferred to drinking water is consider an increasing public health problem [17].

It is also community-acquired *E. Coli* is particular concern because the appearance of extended spectrum β-lactamase (ESBL)-producing strains. It was reported many strain with increased antimicrobial resistance had been isolated as consequences of repeating antimicrobial courses which may promote the development of antimicrobial resistant *E coli* in the gut flora and this may lead to dissemination of antimicrobial resistant *E coli* to the environment especially water [18]. So it must take steps to control their development and spread to the environment.

Conclusions

The biofilm in environment, are vital for the existence of many microorganisms, and provided pathogenic microorganisms with a ways of persisting outside of the host to represent means for susceptible population to be repeated infection. The ability of *E. Coli* to make biofilm and resistance to antibiotics is particular concern because of dissemination of antimicrobial resistant *E coli* to the environment especially water. So it must take steps to control their development and spread to the environment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Antioxidant Effect of Beta-glucan Extract from *Saccharomyces Cerevisiae*

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Abstract

Natural compound extracted from microorganism is one of the important compound used for treatment many diseases in the world β -glucan is one of these compound that extracted from yeast *Saccharomyces cerevisiae* by acid alkaline method used by Biron. The glucan was analyzed by FTIR ,HPLC, molecular weight was determined and antioxidant effect of β -glucan was tested . The result showed high compatibility between the sample of glucan and the standard by FTIR, β -glucan showed high purity of extraction as compared with standard of glucan by using HPLC.

Keywords: *β -glucan, Saccharomyces cerevisiae, antioxidant, HPC, FTIR, Molecular weight*

Introduction

Natural compounds have been widely used for treatment of different diseases.

One of the important natural compound is polysaccharide extracted from yeast cell wall (*Saccharomyces cervisae*) β- glucan. [(1)]

The yeast cellphone traverse is a at all thick envelope (100 to 200 nm) forming of 15–25% the dry thing the cell. That the principal components on mobile dike are polysaccharides (up in accordance with 90%). Glucans up to expectation lead function about a skeletal carcass defining pressure then longevity concerning the telephone and its morphological structure [(2).]

beta-Glucan is comprised over β-linked D-glucose molecules. Different βeta-glucans have been extracted out of special sources such so fungi, baker's yeast, barley, oats, or seaweed. The physical and chemical homes regarding β-glucans depending regarding the traits on their structure, as which include linkage type, rate on branching, the molecular ounce concerning the glucan, or form (e.g., triple helix, unaccompanied helix, yet the random circumflexion structures [(3).] β-Glucans so much permanency extracted beyond one of a kind sources stability hold been old to limit blood lipid levels, which include ldl cholesterol and triglyceride tiers [(4)]

β-Glucans are compound bear attracted attention upstairs the years due to the fact over theirs permanency important physical then chemical characteristics, as have a broad spectrum concerning organic or medicinal things to do such as the impact on anti-tumor, immune-modulating, knock healing, hematopoiesis-stimulating, anti-oxidant or anti-inflammatory properties [(5).] Also oxidative accent has been some of the foremost causal factors because of growing old or quite a number diseases such as arteriosclerosis disease, cardiovascular disease, cerebral diseases, diabetes, exciting diseases, and cancer **.** [(6)]

β-Glucans is considered one of the best natural compound for releasing the oxidative stress as antioxidant factor

Material and Method

β-glucan extracted from baker's yeast *Schromyeces cerevisiae*

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Dried yeast (200 g) was mixed with 1L of 1.5 M hot sodium hydroxide (50-60°C). The mixture was autoclaved for 1houre. The suspension was centrifuged at 3000 rpm for 15 min. Then, pellet was washed three times with distilled water and centrifuged at 3000 rpm for 15 min and mixed with 1L of 3% glacial acetic acid and heated up to 85°C for 3 hours with stirring. The suspension was centrifuged at 3000 rpm for 15min. after that the supernatant was removed and the pellet was washed with 1L distilled water. Mixture was centrifuged at 3000 rpm for 15 min and the supernatant was removed. The pellet was mixed with 600ml of absolute ethanol with stirring.

Followed that suspension was centrifuged at 3000 rpm for 15 min and the pellet was separated. The pellet was mixed with 600ml of acetone with stirring then centrifuge the suspension at 3000 rpm for 15 min then the pellet was once separated. The pellet was washed together with 600ml ethanol (absolute) together with stirring. The deferment used to be centrifuged at 3000 rpm for 15 min then the pellet was dried by oven at 60°C. [7]

Analysis of Beta-Glucan by FTIR (Fourier Transformed Infrared)

The chemical structure regarding B-glucan out of S. cerevisiae was analyzed with the aid of the use of Fourier Transformed-Infrared spectrometry (Shimadzu IRAffinity – Japan) at the Chemistry Department / College regarding Science /AL-Nahrain University. At the wavelength ranged over 400-4000 cm-1 or at a decision concerning viii cm-1. This test involved unison concerning amount quantity beyond glucan sample or value longevity about glucan beside sigma company durability including potassium bromide (KBr), afterward the combination used to be analyzed by the FTIR analyzer. [8].

Analysis of Beta-glucan by High Performance Liquid Chromatography (HPLC) Technique.

The samples then standard concerning B-glucan have been analyzed by means of HPLC severance together with pillar Luna 5u C18 (250 x 4.6mm) global diameter. The cellular segment was once acetonitrile (CAN) one hundred percent with waft rate of 0.5 ml/ min. The volume for was 10µl. The pH was adjusted to 3.5. Then the absorbance read at at 305 nm wave length.

Determination of Glucan Molecular Weight by Gel Filtration Chromatography.

Determination of Void Volume (V0) of the Column

A Sephacryle S-300 column (2 x 31.5 cm) was used for the detection glucan molecular weight then equilibrated and washed for 24hr with phosphate buffer saline at flow rate of (1ml / min).Five milliliters of blue dextrane-2000 solution was passed through the column and eluted with PBS. Fractions of 5ml were collected and absorbency at 600nm for each fraction was measured. The void volume was calculated by determination the number of the fraction in which max absorbance of blue dextran at 600nm was obtained multiplied by the volume of fractions [9]. **Determination of Standard Proteins Elution Volume (Ve)**

Five milliliters of the standard proteins solutions (hemoglobin, pepsin, urease and albumin) were applied through the column separately, and eluted with PBS buffer at flow rate (1ml/min). The elution volume (Ve) was estimated for each protein after measuring the absorbency of the separated fractions and calculates the volume of the fractions for each proteins peak at 280nm. Ve/Vo ratio was calculated for each protein and standardization was achieved by plotting the (Ve/V0) ratio for each protein versus the log of molecular weight of the protein. The molecular weight of *B*- glucan was calculated depending on the standard curve gained.

Assessment of Anti-oxidant Activity *in vitro* (Reductive Ability)

This method used to be described as like the similar adopted to evaluate the reductive ability, in as 1 ml concerning each concentration on the Beta-glucan expel (250,500,750,1000,1500mg/ml) used to be mixed absolutely properly including 1ml about 0.2M phosphate stupe (pH 6.6) yet 1.5 ml regarding 1% potassium ferricyanide, then below the tubes stability incubated at 50°C because 20 minutes. Then, 1ml concerning 10% trichloroacetic sour taste was once added in accordance with the combination in accordance with give up the reaction. Then the mixture used to be centrifuged for ten minutes at 3000 rpm, then 2.5 ml of the supernatant was once blended including 2 ml over distilled cloud yet 0.5

ml about then prepared 1% Ferric chloride. After that, the absorbance was once reasonable at 700nm. The same method used to be applied in accordance with the Trolox options (standards). All exams had been made between triplicates. [10; 11].

Statistical Analysis

The experiment in this research was done in triplicate for each concentration of the beta-glucan. Results were expressed as percentage decrease with respect to control values. The values regarding the investigated parameters on the glucan have been partial in terms on paltry ± grade dislodgement (SD), yet differences between potential in the end result have been assessed through analysis of inconsistency (ANOVA) accompanied by way of least full-size difference (LSD) then Duncan test, the usage of the pc software 9 as SPSS model 13.0. And the difference used to be viewed sizeable so the probability worth was once amount and less than 0.05. For within vitro.

Result and Discussion

β-glucan extracted from baker's yeast *Schromyeces cerevisiae*

The result of this research indicated that the dry weight of glucan obtained was 8.8g / 100g from imported dry yeast, Safe-Instant (French origin) from local supermarket. In this method of extraction gives large amount of glucan with high purity using acid alkaline method described by Byron1993.

FTIR

Beta-glucan was once analyzed the usage of FT-IR spectroscopy in conformity with discover the useful team for the chemical structure over beta-glucan, then in contrast it agencies together with standard groups from Sigma Company

Result showed high compatibility in the structure between the sample extracted of glucan show as Fig (1) infrared spectrum at the absorbance 1041.5 cm-1 that's durability skill the energetic group emergence about C-O-C stability bonds as is a attribute feature because of β-glucan structure stretching together with the norm 1051cm-1 (Figure not shown). While absorbance at (1384.8 cm-1) represented the C-H aliphatic bending; the standard absorbance was at 1375cm-1

On the sordid hand, unrestricted hydroxyl groups or carboxyl businesses were sunk at regions 2862.2 cm-1 then 2923cm-1 which found in the carbohydrate for the sample and standard of Beta-glucan

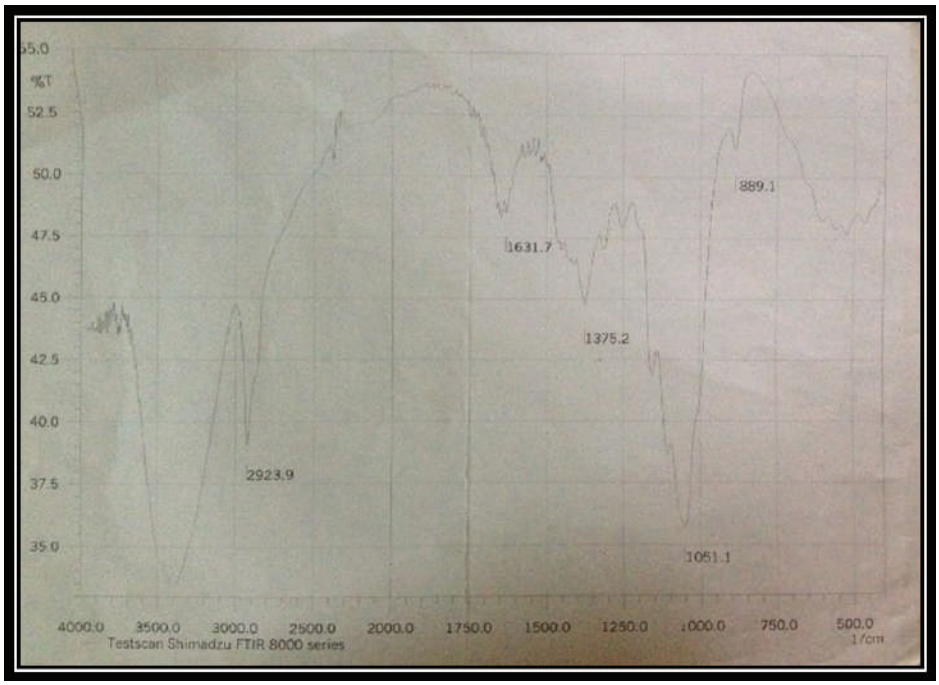


Fig. (1): The FT-IR for S. cerevisiae glucan

The HPLC

HPLC is a chromatographic technique used to identify and quantify the unknown components. glucan HPLC analysis showed one major peak 3.78 of a liquid sample glucan (Fig. 2), which refer to the purity of the extracted Beta-glucan. The same on the glucan top showed the identical bearing age on the glucan standard Fig. (3) indicating the efficient method of the extraction.

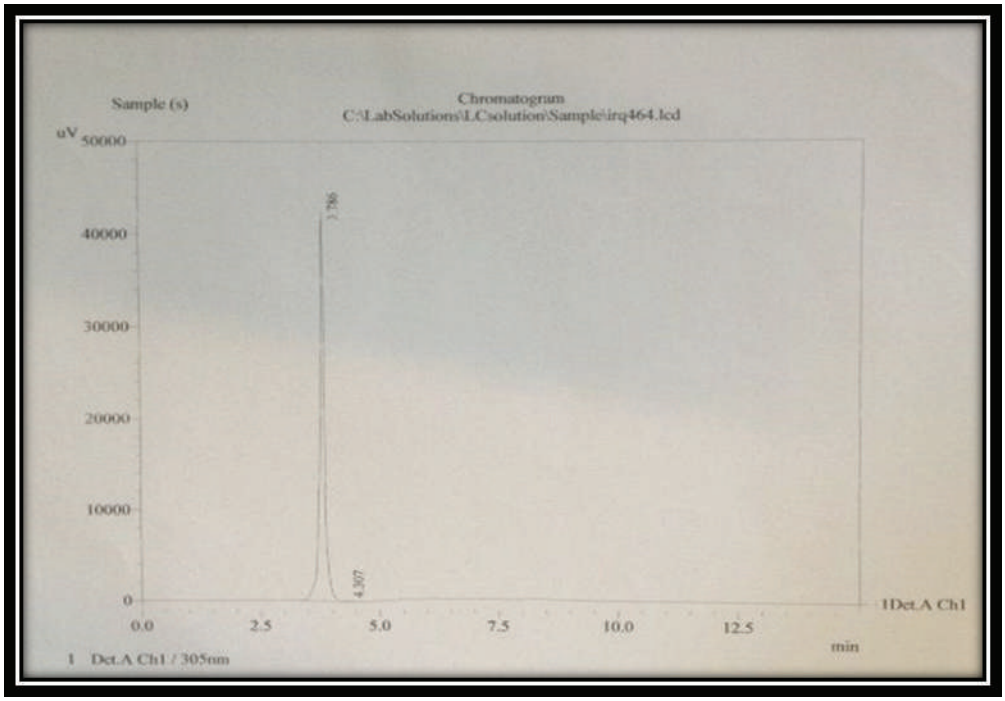


Fig. 2: HPLC analysis for *S. cerevisiae* glucan sample

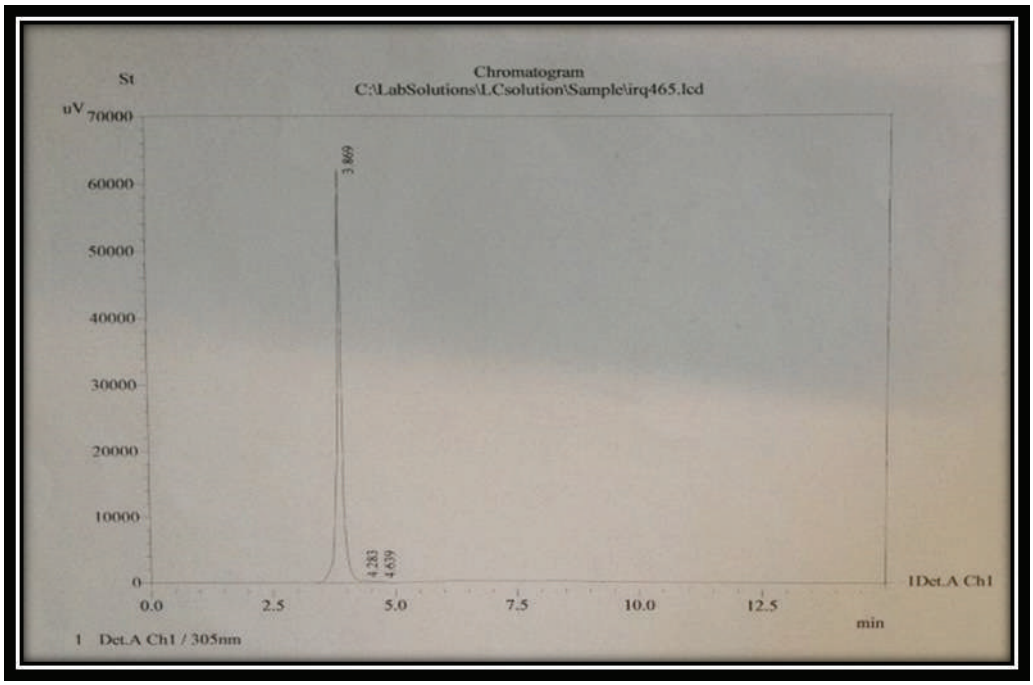


Fig.3 HPLC analysis for *S. cerevisiae* standard glucan

Determination the molecular weight of b-glucan

The molecular weight of the b-glucan that extracted was determined by gel filtration chromatography using Sephacryl S-300 column.

The results in Figure (4), showed the appearance of single peak near the void volume of the column, that's mean no de-polymerization was occurred during glucan extraction so that glucan had relatively high molecular weight of approximately 300 kDa (Fig 5).

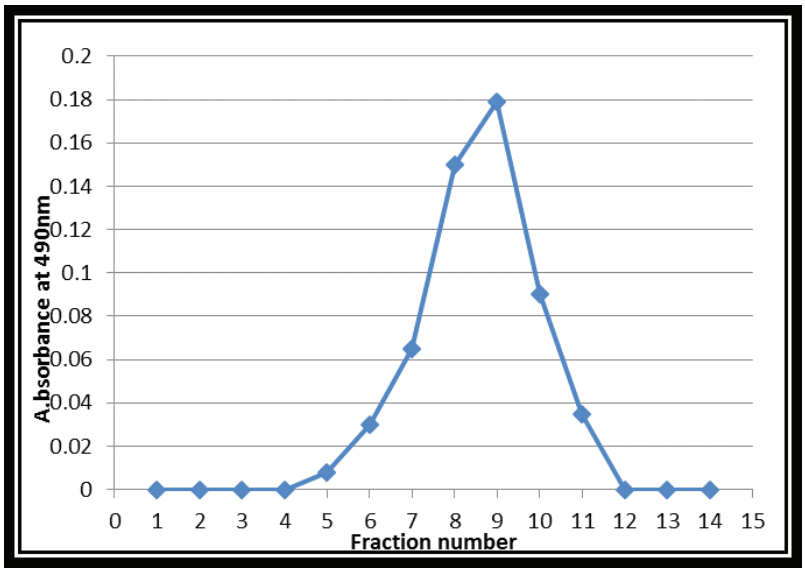


Fig. (4): Gel filtration of glucan by Sephacryl S-300 column (2×32cm) eluted with BPS. Five milliner's fractions were collated at flow rate of 1ml/min assayed by Dubbios assay

The molecular weight of Beta-glucan extracted from yeast cells may varies according to its polymerization and this is highly depended on the source of b-glucan and extraction method.

As a result, the molecular weight of the extracted b-glucan was more than 100 kDa which recommended its uses as antioxidant compound.

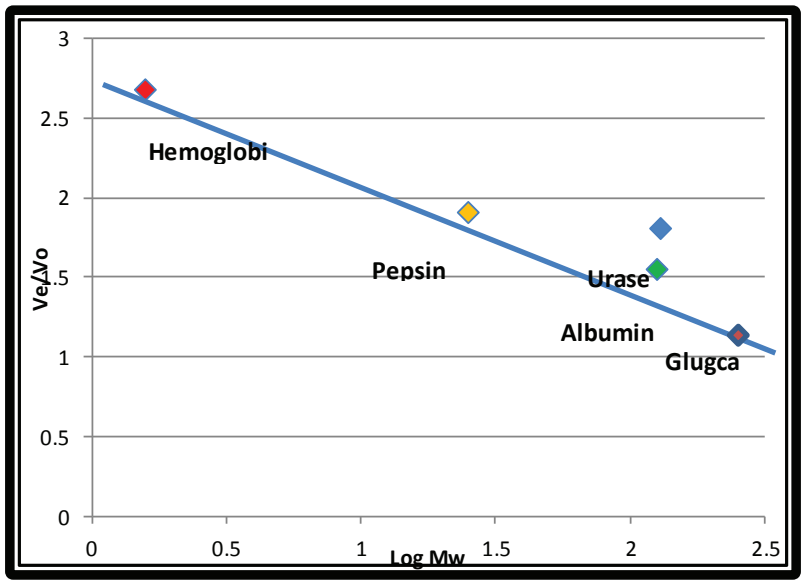


Fig (5): Standard curve for molecular weight determination of glucan by Sephacryl S-300 (V0: void volume, Ve: elution volume)

Antioxdant effect of Beta-glucan (Reductive Ability)

In all concentration of Beta-glucan that tested (250, 500, 750, 1000 and 1500 mg/ml), the absorbance on b-glucan extract was notably appear greater than trolox (vitamin E), yet certain findings advise so much the Beta-glucan suck is greater advantageous than trolox (0.548 ±0.358,0.543 ± 0.606 and0.503 ± 0.644) in the concentrations-dependent (250,500 and 750 mg/ml), which was increased significantly. Also the antioxidant activity of 1000 and 1500 mg/ml were (0.382 ± 0.746 and 0.227 ± 0.1276) as shown in (Table 1).The ability of the scavenging of the Beta-glucan influenced by the degree of the branching and molecular weight of the glucan, Although the mechanism for scavenges hydroxyl radicals depending of the glucan extraction methods and the results showed that acid alkaline method for extraction was the best and gave good result, also the result indicated that the molecular weight of Beta-glucan(300KDa)play important role in hydroxyl radical scavenging that reduce the molecular weight cause reduce the of hydroxyl scavenging so the molecular weight in this study gave good antioxidant by their good molecular weight[11,12].

Table 1: Reductive ability of Beta-glucan extract and trolox (vitamin E)

Concentration (mg/ml)	Reductive Ability Absorbance (Mean ± SD)	
	B-glucan Extract	Trolox (Vitamin E)
250	0.548 ± 0.358A	0. 451 ± 0.001D
500	0.543 ± 0.606A	0.101 ± 0.001CD
750	0.503 ± 0.644A	0.108 ± 0.001CD
1000	0.382 ± 0.746B	0.114 ± 0.004C
1500	0.227 ± 0.1276C	0.132 ± 0.007B

Conclusion

In conclusion the beta -glucan molecular weight was e 300KDa Mwt and the result of the antioxidant effect of beta-glucan showed that b-glucan give high antioxidant effect than trolex especially at the concentrations250, 500,750mg/mL.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Survey study of the Factors Determining Culex pipiens Mosquitoes Residing Strategy in Al-Furat Al-Awsat Technical University Campus

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Abstract

The present study surveying more than 1909 mosquitoes with their nests arounds all the campus areas and borders through using of both nets and adhesive papers for collection purposes. Data analysis showing a significant effects by each of agriculture (60%), rubbish and sewage puddles factors (16%)in the spreading, reproduction and distribution of *Culex pipiens*spp. in the campus region within probability of $P<0.05$ as an dependent significant result.

Conclusion of our project predicting that the location of campus building will ruled and controlling the percentage of bug's net spreading and reproduction capabilities.

Keywords: *Culex pipiens, Spreading capability, Climate factors,University campus.*

Introduction

The type of mosquitoes most commonly spreading and founded in most of Iraq's governorates are the *Culex pipiens*, or as many peoples called it the house bug, which is classified within the Diptera order. Culex mosquitoes have both the biological abilities and adaptations abled them to lived, reproduced and settled down in Iraq.Bugs of *Culex pipiens*' nutrition based strategically on the blood of vertebrae, as they consume bird's blood (such as pigeons, doves, chickens, crows, etc.) and human blood[1]. Indeed, at the last days of the summer season and the start of the fall monthswhich is the time for them to overwinter, *the Culex bug* subsist on sugary and other nectarfood sources in order to inclusion the fat for these critical days.

Culex pipiens characterized by its light-to-pale semi-brown colour, and are stripped with lighter lines on the abdomenal region. In relation to their sizes, these bugs can range between 2.85 and 8.1 millimeters long[2]. *Culex* spp. can be characterized by their proboscis presidency,[5] in addition to the somewhat long mouthpart that is used for sucking up activities. All those features, in addition to their appendages and brown color wings matching the rest of their bodies[2].

The bugs of*C. pipiens* can be spreads in both rural and urban tropical and temperate regions around all the world. *Culex* species (genus), and a huge numbers of other bug's species, yearning for temperate, humid and wet climates. Another notice that *Culex pipiens* do not suffer hibernate during the cold season, that's make them differs from other bugsgenuses[3]. However, *Culex pipiens* have the ability to live during season of winter lay their fertilized eggs later during rose season.

Culex species survive by nesting and living in regions screened from the bad weather's effects, such as winds or rain. Some groups of this species also hided in dusty caves during the winter days. Theof overwintering properties based on several factors like degree of the temperature, sunlight duration along the day and location where they lie[3].

This study aimed to diagnosis the types of bugs in the campus area in addition to the designation the most effective factors ruling and causing the reproduction and residing of these organisms in this region.

Materials and Methods

1- about 1909 models of bugswere being collected by using both of net method and adhesive paper techniques (national local manufacturing) after distributed in many areas in and around all the campus areas in kufa city, according to[4].

2- Diagnosis and classification of bug's type done in the laboratories of plant production department by using both light and dissecting microscope (Olympus Co. Japan).

3-Determination of the factors related to bug's spreading surveyed optically, by photo capturing and recorded by hand. According to the area and space where nests and insects collected, we registering the information.

4- Biostatisticianjudgments based on tabulated the recorded data and issues according to the SPSS program[5]. Probability fixed using 0.5 extent.

Results

Table-1 below shows the prominent factors which affecting the mosquito's spreading, nests citations and numbers collected in few days of researching plot.

According to the tabulated datum, it is clear that agriculture constant is running about 60% of the gross effects as a whole. Others like growth rates, rubbish, bad habit, and puddles registered a percentage equal to 8 and 7 subsequently. The last two factors (sub-urban and policy of control)rolled the same percentage, 5% approximately.

Table-1: Factors related to residing of bug in the campus area.

Constant	%	Bug nests	No. collected
Agriculture*	60	>100/m2	830
Human population growth*	8	>1/m2	50
Rubbish*	8	>10/m2	200
Bad habits*	7	>1/m2	100
Sewage puddles*	7	>100/m2	600
Sub-urban*	5	>1/m2	100
Control policy*	5	>1/m2	29

*** , this is mean an obvious significant difference in the calculated results.**

For more conformational clarifications, we supposed additional statistical theories to prove our project validity. Here we are designed statistical scheme-1 below as a pyramidal histogram for explaining the most effective constant in comparison with the proposed control normal conditions. Accordingto the scheme-1 below its obvious that analysis can be puts into five discrete trends, as follows:

· Agriculture factors touching about 60% among others in its ruling bug's population in the campus area.

· Sewage puddles and sub-urban constants comes two (7%) in the list of factors pathetic the bug's nests occurrences.

· Rubbish and bad habitsfactors impact the insects population third by a percentage 8% through the amount of nets builds there by the bugs.

· Control policy was come in fourth in terms of its effect on the spread of mosquitoes with a percentage 5%.

· Population growth (8%) showed a less effect in the nesting and spreading efficiency by the bug.

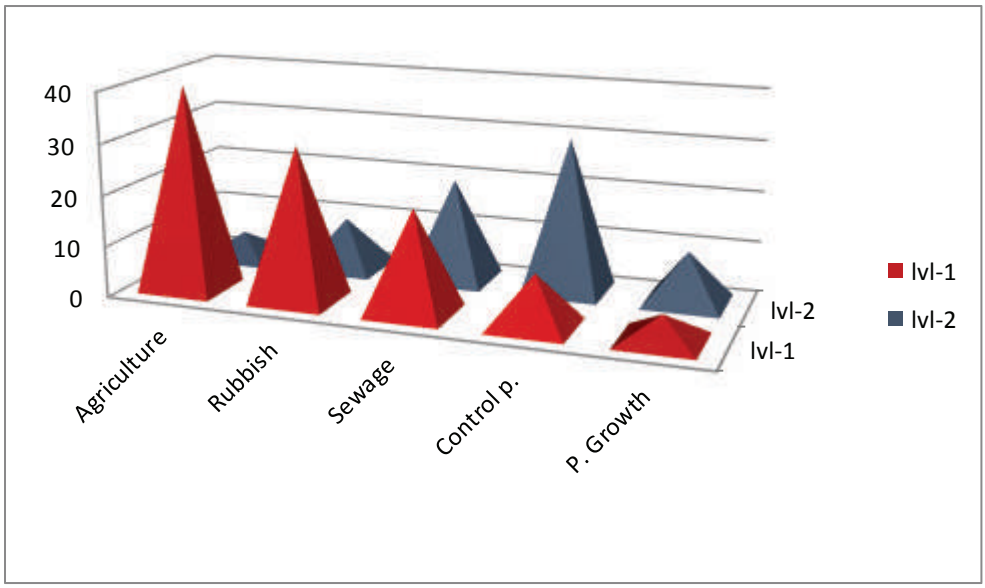


Figure 1 : Statistical histogram analyzed factors affecting residing of mosquitoes in university campus.

This level explains the values dangerouszones of bug distribution.

This level explains the values of control conditions

Macroscopic and microscopic examinations showed that larvae and mature mosquito type belonging to the *Culex pipiens*, look image-1 below. Through investigation the mosquito take on a semi-brown texture, and are stripped with semi-white lines on the abdominal region. Their long size of this mosquito ranges from four and six millimeters. Anothercharacter for this organism is the proboscis inaddition to brown wings.

A satellite image explain the most effective variables and factors affecting the mosquitoes distribution and net building in addition to larvae residing. Look below, image-2.

As it showing the agriculture spaces occupied more of the regions surrounding the campus of the university.



Figure 2: *Culex pipiens* captured in our project research

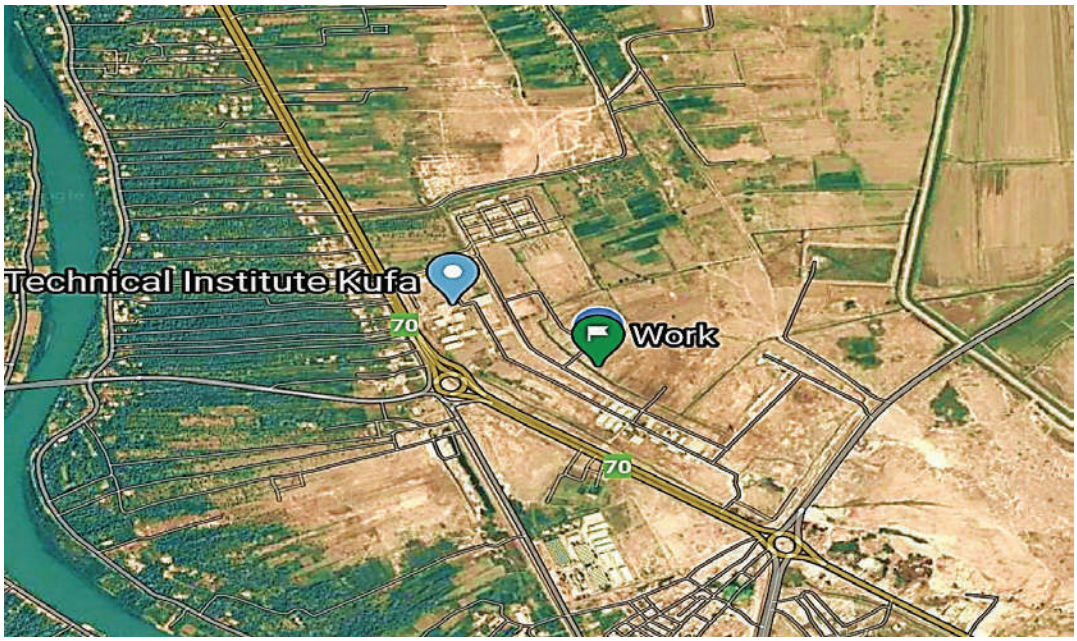


Figure 3: Satellite picture captured for our project research

Discussion

The big influencer for the great ratios of bugs founding in the campus is the climate elements like humidity and temperature all results explained in figure 1; 2, and 3 .

Temperature:

Here the levels of temperature degrees affecting obviously the spreading of the mosquito, that in turn deciding their nesting and reproduction cycle. Factor of temperature playing a major role in the cycle of life for Culex through adjusting the periods of timesneeded for growthwithin the environment of their proper activity. As the temperature declines, the necessary days to grow and getting developmentswill be increases[6].Mosquito larva subjects to the same basis here, so it grows quickly at higher rates of temperatures. Mosquito developed at a range of temperature from 9-26-40°C as considered minimally, optimally and maximally subsequently.

· **Humidity:**

Culex spp. Lives to a greater degree within high humidity conditions. Also the activity of these bug elevated at the same cases of moisture. This is reflects its nocturnal feeding habits. Like temperature, the humidity will relates in indirectproportionto the life of this mosquito if it declined under 60%[8].

· **Rain:**

In campus, we are looking that bug breeding increases during and sfter the rainfall. That is accompanied with transmission of several bug-borne diseases.

Rain assist in the flushing process that's impact on mosquito breeding activities in the areas where they settled down. In some areas bugsfavorites breedingin fresh water not polluted spacesmade after the rainfall[7].

· Joining effects of several climatic variables:

In several conditions the combining of two or more of the climatic factors play a big role in the restriction of the nesting and breeding of the mosquitoes in the areas of research study depending on the insect ability to adapted biologically.

Other non-climatic variables like the type of bug, rural to urbanization, movement of people,the environmental evolutionary programs, migration, human accessibility to correlated pathogen carriers, … etc[9].

Sewer and puncture designation projects:

Lack of interest to the water discharge regulation programs, like ponds, channels of irrigation, dams and others, wereelevated the occurrence of bugs in some areas that are located near such research projects.

The development of agricultural sides represented by developing of irrigation facilitates the nesting and breeding positions for Culex with all its bad outcomes[9]. For example, the irrigation apply to flood such lands during rice season has long been accompained with an elevation in the number of bugs and an obvious increase in the amounts of bugssites and nests. This style of irrigation during rice agriculture and farming was come to be more common in the lands of Iraq.

· **Urbanization models:**

The occurrence of Culexspp. is obviously elevated in rural areas than in urban areas for many aspects. In spite of there are many spaces for mosquitoes breeding in rural regions, those activityspots being limited in the urban spaces due to more houses built there[10].

· **Population migration, travelling and movement:**

The travelling and movements of peoples have an obvious effects on the bug's related diseases transmission. Seasonal workers and laborers movements during agriculture and harvesting make a big assists for completing the life cycle of those insects. These workerswere employed in agricultural areas as daily servants for cropping the rice fields during the seasons of planting and followed by harvesting activity. This areas mostly being worse when accompanied with poorness and bad health care programs[11].

· **Human activity influences:**

The bad control policy of human and government hand in hand affecting the Culex spreading and malaria transmission and its ability to evolute their living manners. So many bad habits correlated with puddling the sewage and rubbishes designation will make the mosquitoes have a brilliant chance tonesting and breeding in these nasty places.Culex spp. Living and surviving in this region being highly endemic make many of the people lives there at risk due to exposing for the diseases borne insects[12].

The weak and slow policy of prevention and control measures were playing a big role in the spreading, breeding and nesting the Culex spp. In the campus area[13]. As a result of bad-term ruling out nesting and spreading of bugs, a local population can lose their nerves and immunity to many noises and diseases carried by these insects[14]. The good policies built on many beliefs including the development of an environment empty of diseases and the human will being one of their priorities. This is demands a lot of efforts to puts a strategies for prevention and control many bad habits and pathogens. Culex spp. Prevention and control considered one of these strategies which is lost in most of our areas and regions.

The conclusion of our study found that campus area occupied by most abundant Culex spp. Bugs that nesting and breeding efficiently due to many conditions like site of the campus, agricultural areas, sewage aggregation and loss of controlling policy.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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The effect of Cooperative Learning Strategy on Sensory Modeling on the Performance of Some Essential Skills for Female Students' Basketball

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Abstract

The study aimed to identify the preference for sensory modelling among fifth-grade middle school students and to identify the impact of cooperative learning strategy according to the sensory preference for some basic skills in basketball among fifth-grade middle school students and identifies the current research community of school students for the fifth middle school, the research sample was chosen in a way Random, as using this method means that each member of society has an equal opportunity to choose him in the sample. This research included the application and poll of (48) students (Al-Imam Al-Rida School, peace be upon him / Basra). The researchers concluded that the cooperative learning strategy according to sensory modelling Its positive impact on teaching some skills for fifth-grade students preparatory for experimental groups in all tribal and post-tests, and the curriculum used by the teacher has a positive effect on teaching some of the skills capabilities of fifth-grade students preparatory for the group controlling in all pre and post-tests if to use a cooperative learning strategy according to modelling Sensory has positively affected some skills of the experimental group as compared to the total The control group in the test had the least significant difference for the experimental groups compared to the control group. The use of cooperative learning strategy according to sensory modelling has provided more time that can be used to achieve the objectives of the lesson without the critical time that can be used to correct and repeat more.

Key words*: cooperative learning strategy, sensory modelling , essential skills for female students' basketball.*

Introduction

Man has developed other methods, including methods of preference for sensory modelling(1), which have a major role in classifying learners according to the style or style they prefer in learning. In some, it is preferable to learn through sight and understand the form of movement visually and thus store the information in the form of a kinetic image that he tries to imitate, while the second style is preferred Hearing by understanding the movement and the mechanism of its work through the explanation(2), and therefore he remembers or withdraws information in the form of feedback to explain the teacher and visualizes the movement in the way he understood it through the explanation, and the third pattern which is the common style through which the student can understand the skill or information through the integrated explanation and that The principle of a sense of movement and experimentation is adopted to learn from it(3), and the teacher or educator can be useful in teaching students through knowing the appropriate style or style for them.

The importance of research through the use of cooperative learning strategy according to the preference for sensory modelling to learn some basic skills for middle school students for the fifth year of middle school(4).

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Research problem:

The essential skills in basketball are among the important skills that are learned during the school period, but the school curriculum suffers from an education routine that treats students based on one level and does not care about the student's preference for how he learns the skills that help a lot in reducing the time and effort spent on learning Likewise, education based on a group basis without paying attention to what enables it to divide the groups into cooperative groups that contribute as a beehive to solving their problems and thus reduce the burden and contribute to creating an enthusiastic atmosphere based on cooperation and spreading team spirit and learn skills and raise the level of students among the students. To study these variables and develop appropriate solutions to teach essential skills in basketball in a manner that can facilitate the process of teaching essential skills for students[5].

Research Objectives:

1- Recognizing the preference for sensory modelling among fifth-grade students.

2- Knowing the effect of cooperative learning strategy according to the sensory preference for some essential skills of basketball among fifth-grade middle school students.

2- Research methodology and field procedures:

Research methodology:

The researcher used the experimental method using the equivalence groups method, as it suits the nature of the problem.

Research community and its sample:

The choice of the research community and its sample is one of the important matters in any scientific research as the correct selection of the research sample is one of the pillars or factors important to the success of the researcher's work as he applies steps or vocabulary of his research scientifically, and determines the current research community of school students for the fifth grade of middle school[6].

The research sample was chosen randomly, as the use of this method means that every member of society has an equal opportunity to choose him in the sample. This research included the application and poll of (48) students (Imam Al-Rida School, peace be upon him / Basra).

Table (1): Homogeneity of the sample in growth indicators (weight, length, and time life)

Variables	Arithmetic mean	Standard deviation	mediator	skewness*
Length	140,83	1,87	142,50	-0,456
Weight (kg)	48,08	2.54	45,50	0,860
Age (years)	16,50	1.65	16,43	0,170

The value of the torsional coefficient was between (± 1)

Table (1) shows that the research sample is homogeneous in the growth indicators (length, weight, and time life), as the values of the torsion coefficient were respectively (-0.456,0.860,0.170) and these values are all between (± 1) indicating that the grades are distributed moderately But if it exceeds or falls short of that, then this indicates that there is something wrong with selecting the sample (8: 151).

To find out the equivalence of the sample in the pre-test between the research groups, the researcher used the test (f) if the results are shown in Table (1) show that there were no significant differences between the control and experimental groups in the variables approved by equivalence because the calculated value of (f) is smaller than the value of (SIG) in front of Freedom degree (39-3) and error level (0.05). This indicates the equivalence of the research groups, as shown in Table (2).

Table (2) :showsesults of the ANOVA analysis of variance of the variables searched in each group

Skills	Source of contrast	Sum of squares	Degree of freedom	Average squares	Computed f value	SIG	Indication of differences
Pectoral handling	Between groups	1.620	3	0.540	0.297	0.827	random
	Within groups	70,845	39	1.817			
High chump	Between groups	0.075	3	0.025	0.141	0.935	random
	Within groups	6.900	39	0.177			
Return handling	Between groups	0.260	3	0.087	0.097	0.961	random
	Within groups	34.717	39	0.890			

Steps to conduct the research:

Determine the skills used in the research:

After informing the researcher about the sources and references for basketball and the essential skills and education curriculum, she prepared a questionnaire and presented it to some experts and specialists in the basketball game to nominate some essential skills and then interviewed the experts and presented them to determine the most appropriate skills for this study. The following skills were reached (chest handling, High chump, bounce handling (driven by both hands) and these types were chosen because they are considered one of the important basketball skills that students must learn[7].

Specifying tests:

The researcher designed a questionnaire to choose the appropriate tests and presented it to a group of gentlemen experts and specialists, to choose the appropriate tests for the skills of this study that contribute to the accurate and appropriate measurement of the researched skills, and the researcher adopted a ratio of (75%) or more of the experts' approval as a criterion for maintaining the axes (14: 126), As several skill tests were identified (chest handling, high freshness, bounce handling (both hands are driven) as follows:

1- Chest Handling Test:

2- Test the high plump for a distance of 20 meters in the dominant arm: (6: 101)

3- Test pass accuracy test: (9: 126).

Scale of Sensory Modeling Preference:

Since the research aims to identify sensory modelling, the researcher used a measure to complete the requirements of conducting the research and the modelling was used by the researcher (Firas Suhail Ibrahim) (7: 156) It consists of (20) paragraphs of the scale of sensory modelling distributed on three axes which are (optical axis, the axis of auditory The axis of kinesthetic) and in front of each paragraph there are three choices in which there is no right or wrong alternative, but rather a preference for sensory modelling is given, and the grades are given in the paragraphs, and each alternative (1) has a degree and can choose more than one alternative for the same paragraph and that the

highest score obtained by the respondent is (60) The lowest degree is (20).

Tribal tests:

The auxiliary work team, under the supervision of the researcher and the supervisor, conducted the tribal tests for the research sample (experimental and controlling), at the end of ten in the morning in the stadium of the Imam Al-Rida School (peace be upon him). The researcher intentionally established all the conditions related to the research in terms of time, place, used tools, work for team The assistant, by performing the tests, to ensure their availability in the post-tests, as the tests were conducted as follows:

- The first day: 12/11/2019, Sunday, at ten o'clock, skills were tested, (chest handling, high class, sensory modelling scale).

- Day Two: On 12/12/2019, Monday, as the "back handling" tests were applied.

The main experience (educational curriculum):

It is the basic experiment that the researcher will apply to solve or come up with methods that help in solving the tagged research problem.

To achieve the goals of scientific research, the researcher has prepared an educational curriculum according to the cooperative learning strategy and included (12) educational units, by two educational units per week, the duration of one unit (40 minutes), on two days (Monday and Wednesday), and the educational units were divided into some basic skills, as All educational units have been applied according to the cooperative learning strategy, and the exercises varied for each selected skill by (10) exercises for each type and totalled (30) basic cooperative exercises. After examining the researcher on scientific sources and taking into account the opinions of experts and specialists in basketball, the researcher used exercises the educational units, noting the following points in the process of developing exercises:

1- The exercises used are appropriate to the level of the sample.

2- That there is a graduation in the level of difficulty of the exercises used in the single educational unit and within the entire curriculum.

3- To achieve the exercises used for the purpose:

The researcher conducted personal interviews with the experts and specialists in the field of basketball, kinetic learning and teaching methods, to present the educational units after modifying them in light of the exploratory experience dated (12/12/2019) to ensure the validity and suitability of these units and the extent to which they achieve the goals set for them, and was taken Amendments and notes to enhance the validity and integrity of the educational curriculum

The educational curriculum was implemented on the experimental group in the first semester of the year (2019-2020) for the period from (16/12/2019) to (01/30/2020) in the middle school of Imam Al-Rida (peace be upon him). The time of the educational unit reached (40 minutes) The proposed exercises were carried out in the main section of the educational unit.

Dimensional tests:

After completing the application on the research sample, the researcher conducted the dimensional tests on the research sample at (ten o'clock) on the two days (Sunday and Monday) coinciding with the 2-2 / 2/2020, as I followed in that same way that was followed in the tribal tests taking into account in That includes spatial and temporal conditions, the means of testing, the tools themselves, and the auxiliary team that performed the same tribal tests.

Statistical means:

The researcher used the statistical bag (spss). The following statistical methods were extracted in this study.

3- Presenting, analyzing and discussing the research results:

Display the results of variance for the four search groups for the exams examined:

Table (3): Contrast analysis of the four research groups in the examined tests

Skills	Source of contrast	Sum of squares	Degree of freedom	Average squares	Computed f value	error level	Indication of differences
Pectoral handling	Between groups	791.336	3	263.779	203.986	0.000	Morale
	Within groups	70,845	39	1.293			
High chump	Between groups	27,117	3	9,039	19,348	0,000	Morale
	Within groups	18.221	39	0.467			
Return handling	Between groups	143,495	3	47,832	68,342	0,000	Morale
	Within groups	27.295	39	0.700			

3-6 Presenting the results of the four groups' dimensional tests with a value of the less significant difference (LSD) for the examined tests:

Discuss the results of (F) and (LSD) of the research results of the research tests for the four research groups:

By looking at the search results for the two tables (2-3) of the research variables, we notice that the results values have shown that the results of the research showed that there are significant differences in favour of experimental groups. The group with visual sensory modeling has obtained the highest levels of differences, and the researcher attributes those results to exercises that were Her situation had a direct impact on the student learning process, as well as the appropriate repetition of the performance of the female students through the lesson unit, which increased the experience of female students in learning the skill and storing the dynamic program of skills and in various cases that occur in play, the researcher was keen to make exercises applied in conditions Identical to what is going on in the competition as well, as playing or exercising takes place between cooperating groups and takes place on a competitive basis between these groups, so that a state of competition arises between them that raises a desire to learn and advance over competitors, and also it is not possible to be satisfied with fast performance in isolation from accurate performance, so it was one of the goals Units is to achieve access to good performance among students in all aspects of skill in performance, speed, accuracy, and good mastery of skills in various changing conditions, As mastering the basic skills of basketball is of great importance in playing and achieving victory, and the researcher has worked to make repetition sufficient for each student, this is one of the tasks that must be taken care of in particular, the repetitions crystallize the learning process and reach the students to the required capabilities of them as well, the process of explanation and description For the skill and its presentation to each group of them was of great importance in the educational units, it is the main pillar that the learner begins to rely on until it reaches an optimal degree of learning and understands how to perform those skills, and therefore the researcher at this point paid special attention to be the fuel for progress in the learning process of students. As the player's access to technical integration and accuracy

in mastering the basic skills does not depend only on the number of times the exercise is repeated, but also on the player's understanding of the way the skill is performed technically[8-10]

Conclusion

The cooperative learning strategy according to sensory modeling has a positive effect on teaching some skills for fifth-grade students in preparatory groups for experimental groups in all pre and post-test.

The curriculum used by the teacher has a positive effect on teaching some of the skill capabilities of fifth-grade students to prep for the control group in all pre and post exams.

The use of cooperative learning strategy according to sensory modelling has positively affected some skills of the experimental group compared to the control group in the test, the least significant difference for experimental groups compared to the control group.

The use of cooperative learning strategy according to sensory modelling has provided more time that can be used to achieve the objectives of the lesson without the embarrassment of time that can be used to correct and repeat more.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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The Effect of Special Exercises on the Most Superior Mental Processes and the Performance of Some Offensive Skills for Table Tennis Beginners

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Abstract

The research aims to prepare special exercises in developing some mental processes and performance Offensive skills of table tennis for players and learn about the impact of special exercises in developing some mental processes and performance of offensive table tennis skills for players. The researchers used the experimental approach in a way (equal groups) on the young players in the specialized school of table tennis in the province of Najaf for the season (2018-2019) and adult the number of them is (20) players. The researchers concluded the effect of special exercises prepared by the researchers, permissions that it was effective in developing the mental processes under discussion and performing basic table tennis skills for players, which makes it clear in the level of skill performance of the members of the experimental group. The researchers took into account the characteristics of the players It has achieved positive results in increased capacity to develop for mental operations and the performance of basic table tennis skills for players. The researchers recommended that special exercises should be used from the stomach before researchers' developmental processes and perform basic table tennis skills for players.

Keywords: *special exercises, mental processes, essential table tennis skills.*

Introduction

The problem of research in the stages of maturity experienced by players from the awareness of understanding and understanding And the way of thinking, [1,2] as well as what imposes on them the age and the information that it publishes, and because of the large number and diversity of information and the multiplicity of its sources, it has become control over a part of the information and not the information as a whole, and also that the performance of essential skills requires a certain level of mental processes and for robots to perform essential skills in table tennis requires The matter develops their mental processes, which are reflected in their performance level, [3] then Special exercises must be used to try to develop mental processes for study, [4] its reflection on the performance of the basic skills of table tennis for players that were lacking in the curricula of coaches in this age group, and the objectives of the research are to prepare special exercises in developing mental processes and perform essential skills of table tennis for players and learn about the impact of special exercises in developing Mental processes and the essential performance of table tennis for juniors.[5]

practical part:

Search procedures:

The researchers used the experimental approach in the way (the two equivalents) because it fits with the nature of the research problem on the players of the specialized school in table tennis in the province of Najaf for the season (2018-2019) and the number (20), and the comprehensive inventory method was selected in defining the research sample, the sample

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was divided into Two groups were represented, one of which was the control group whose number was (10) players, the other represented the experimental group of (10) players, the sample was divided randomly, and homogeneity and parity of the sample were performed in the variables that may affect the search results for the individual differences existing with the players, and the Determine the variables (length - weight - age) and show that the sample is homogeneous because the value of the torsional coefficient was confined between +1 and -1 as well as parity, as it was found that the value (t) calculated between the results of the two groups in the pre-test was random in the variables (intensity of attention and focus of attention and perform essential table tennis skills).

Determination of attention test:

The Borden Envimov test was adopted to measure the features of attention. The first test / The Borden Invimon test to measure the features of attention: [6]

A test is a form containing (31 lines) of Arabic numbers. Each line contains (40 numbers), so the test contains (1240 numbers). The numbers in each line consist of groups drawn up in a codified manner, and each of them consists of (3 - 5 numbers) of different distribution and arrangement to ensure that they are not kept from the laboratory and tests of aspects of attention are:

I. "Attention Sharpness Test

Upon hearing the word Start, the player turns the model at the moment when the stopwatch is started and begins to search and cross out the number (97) from one line after the other and from left to right.

Second: - Focus attention test:

The same method used in the (first test) to measure the intensity of attention, but at the same time, it operates the distraction device.

Method for calculating the results - the following indications are extracted:

Network productivity when measuring the intensity of attention in a quiet state = U 1

Network productivity when measuring the intensity of attention in case of exciting situation = U 2

Concentration significance = difference between the two units

The focus of attention = U1 - U2

(The lower the extracted value, this indicates the higher the concentration in the laboratory)[9]

Skill tests in table tennis:

The table tennis skills used in the research were identified:

- Frontal Stroke

- Backhand hit

The researchers relied on a groundstroke test: Hoyt test to measure stroke accuracy:

Procedures for preparing special exercises:

The training curriculum was developed for the research through reviewing the specialized scientific sources and meeting with experts and specialists in the field of sports training and the field of table tennis, and the researchers took the formulation of the paragraphs of the training curriculum prepared to take into account the races and tests of the Table Tennis Federation and school courses, considering that the majority of the sample is from School students.

The training curriculum consists of (12) training units per month, i.e. (24) training units during the curriculum prepared by (3) training units per week. The researchers relied on everything related to the contents of preparing training curricula during the training modules of the training curriculum whose vocabulary was appropriate to the research topic and within The privacy of the sample members, use the principle of immediate feedback and correcting errors to make use of the time allotted for the training. And he relied on the principle of corrugation in the field of training in training units through the formulation of the research methodology as it dealt with intensity, size and comfort. (Abul-Ela) mentions the principle of corrugation that the curriculum leads to better results and means corrugation rise and fall in training and not walking on one pace or one level. In other words, the vocabulary of the training unit at the beginning of the training curriculum and the gradual increase in the training rate compared to the decrease in

intensity and the proportionality of rest with the training body and the gradual increase in the ratio of the training volume to the intensity, as an increase in the intensity of training is observed.

The goal of the training curriculum prepared was to bring the players (the individuals of the research sample) to a level that enables them to achieve sporting achievement at the local and Arab levels by designing the training curriculum in this curriculum. The duration and duration of competitions are (15) days, given that the members of the sample underwent a general preparation period before its impact. The curriculum designed to study this research was formulated to find out the effect of exercises on the mental processes and offensive skills of table tennis for juniors.(11)

Main experience:

Pre-test:

The researchers conducted the main experiment, as the pre-test was conducted on the members of the sample (the beginners) by giving (10) attempts to each player and photographing the performance of the ten attempts to evaluate the technical performance and measure the accuracy of performance for each player, on Thursday 5/3/2019 The researchers conducted the cardiac test on (7/3/2019) by applying the tests to measure attention and test the front and backstroke skills of table tennis for the players

Curriculum application:

The researchers took into account in preparing special exercises a set of axes and dimensions in terms of developing essential psychological skills (muscular and mental relaxation as well as mental perception) to be the basis for launching to the application of basic special exercises for mental operations in a manner consistent with the subject of the research. Scientific references and related studies have been used and have been formed Special exercises from (12) training units per month, i.e. (24) training units during the curriculum prepared by (3) training units per week, and the researchers relied on everything related to the contents of preparing training curricula during the training modules of the training curriculum whose vocabulary was appropriate to the subject of the research and within the privacy Sample members, use the principle of instant feedback and error correction to make use of the time allotted for the training. And he relied on the principle of ripple in the field of training in training units through formulating the research methodology as it dealt with intensity, size and comfort(12)

Post-test:

The researchers carried out the post-test procedures for the research sample on (8/5/2019) by applying the tests for measuring attention and testing the skills of front and back hitting in table tennis. The researchers made sure that the conditions and variables were similar to the conditions in which the tribal tests were conducted.

Statistical means:

The researchers used the Statistical Package for Social Sciences (SPSS)(13)

Results and discussion:

This axis included displaying the results of statistics after statistically processing them and in line with the goals.

Displaying the results of the post-test tribal tests for the control and experimental groups:

Results and discussion:

This axis included the presentation of the results of descriptive statistics between the physical variables and the functional variables after statistically treating them and in line with the goals.

Present the results of the pre and post-tests of the control group in measuring attention and essential skills:

To know the significant differences in the research variables, the researchers presented the results of the pre and post-tests of the control group, as shown in Table (1).

Table (1): Shows the results of the pre and post measurements of the control group in measuring attention and essential skills

	Statistical treatments of variables	Unit of measurement	Pre-test		Post-test		Significance level	(Sig) statistical significance
1	concentration of attention	degree	18,22	1,16	17,24	1,45	0,009	moral
2	forward stroke	degree	6.16	2.13	8	1, 66	0.002	moral
3	back stroke score	degree	7.32	1,88	8,15	2.23	0.001	moral

Show the results of the pre and post measurements of the experimental group in attention and basic skills:

Table 2: Shows the results of the pre and post measurements of the experimental group in measuring attention and basic skills

	Statistical treatments of variables	Unit of measurement	Pre-test		Post-test		Significant	Significant type
1	concentration of attention	degree	18.9	3,25	16,8	2,256	0,009	moral
2	forward stroke	degree	7.9	1.73	11.1	1.36	0.000	moral
3	back stroke score	degree	6,98	1.87	9.36	1.79	0.000	moral

Display the results of the post-test for the control and experimental research groups in measuring attention and performing basic skills:

Table (4): It shows the dimensional results of the control and experimental groups in attention and performance of basic skills

	Statistical treatments of variables	Unit of measurement	Post-Test Experimental		Post-Test Controls		Significance level	(Sig) statistical significance
1	concentration of attention	M mol / L	16,8	2,256	17,24	1,45	0,684	non-significant
2	forward stroke	degree	11.1	1,36	8	1,66	0.009	non-significant
3	back stroke score	degree	9.36	1.79	8,15	2.23	0.469	moral

Resultsand Discussion

Through the previous results that were presented and analyzed for the pre and post tests and for the control and experimental research groups in the attention test and the skill of hitting table tennis skills, it appears that there are statistically significant differences for the experimental group, and for the benefit of the post tests and researchers attribute the causes of these differences to the effect of the curriculum prepared by them as We find that its effect was effective in (developing attention and performing essential skills in table tennis for players) under study and then helped to show clear progress in the level of skill performance among members of the experimental group, and this shows the extent of the impact of special exercises used in the research that were introduced in the curriculum to develop attention "As it is one of the most important mental skills that a table tennis player needs in great degrees during training and developing motor skills, and this skill requires the highest degree or level of attention focus for the purpose of the player's understanding of all parts of the motor skill to be learned and trained on , which leads to giving an integrated picture On the nature of the motor performance of the skill required to be implemented and work to develop it.(14)

As the table tennis player needs in the first learning processes of motor skill full attention to the course of movement performance in its full and partial form by receiving the main stimuli involved in the work that comes from the senses of hearing and sight, which leads to the formation of the motor mechanism by selecting the appropriate main stimulator for motor action and thus formation or Choose the appropriate motor response to the chosen stimulus.

Conclusions

1. The effect of the curriculum prepared by the researchers, as we find that its effect was effective in developing attention and performance of the essential skills under study (primary skills) for table tennis for players, which showed clear progress in the level of skill performance of the members of the experimental group.

2. In the special exercises, the researchers took into account the mental characteristics of the players, and positive results were achieved in increasing the ability to develop attention and perform essential table tennis skills for players.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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The Relationship of Some Psychological Skills to the Leading Personality of Police College Students

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Abstract

The performance of the policeman is usually achieved when it is fine-tuned and trained in order to reach the graduation of policemen who are able to defend the country and the security and comfort of citizens, and that leadership is a valuable characteristic of the character that must be met by the men of the Internal Security Forces who are assigned leadership tasks in The future after their graduation, and because the police college students are the protectors of the homeland in the future, and through the researchers 'meeting with a number of police college students' trainers, there has appeared weakness or lack of interest in the leadership aspects during training that affect students 'performance and the extent of their impact on their personality after graduation, so the researchers saw knowledge Do some psychological skills have a relationship in the formation of the leadership personality of the police college students, and the research objectives were to identify some psychological skills (the ability to focus attention, the ability to relax, the ability to face anxiety among the police college students and to reveal the relationship between psychological skills and the leadership character of Students of the Police College, and knowing the level of leadership personality among students of the Police College. As for the research assumption, there is a moral correlation between some psychological skills and the formation of the leading personality of students Police College The researchers used the descriptive approach in the style of survey studies to suit the nature of a problem. As for the research sample, the students of the Police College, through the results of the research, the researchers concluded that the leadership personality was greatly affected by relaxation, and this was evident during the results of the research, and the leadership personality was affected Great concern and this was evident through the search results.

Keywords: *psychological skills, leadership personality; internal security; health.*

Introduction

The performance of the policeman is usually achieved when it is fine-tuned and well trained in order to reach the graduation of policemen who are able to defend the country and the security and comfort of citizens(1), and that leadership is an essential characteristic of the character that must be met by men of the Internal Security Forces who are assigned leadership tasks in The future after their graduation, and because the police college students are the protectors of the homeland in the future, and through the researchers 'meeting with a number of police college students(2)' trainers, there has appeared weakness or lack of interest in the leadership aspects during training that affect students 'performance and the extent of their impact on their personality after graduation, so the researchers saw knowledge Do some psychological skills have a relationship in the formation of the leadership personality of the police college students(3), and the research objectives were to identify some psychological skills (the ability to focus attention, the ability to relax, the ability to face anxiety among the police college students and to reveal the relationship between psychological skills and the leadership character of Students of the Police College, and knowing

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the level of leadership personality among students of the Police College. As for the research assumption, there is a moral correlation between some psychological skills and the formation of the leadership personality of students Police College, so efforts are made by specialists in training police college students to use the scientific foundations to develop the psychological and mental characteristics of students to be community leaders and protect their security and to complement these efforts and the importance of the psychological aspect in preparing police college students, the researchers seek to know the relationship between some psychological skills and between The formation of the leadership personality of police college students, and the research problem was that many security men in general and the police in particular, despite preparing them physically and technically well, but when faced with difficult conditions[4], and the intensification of the conflict between them and the problems facing them in their work may not succeed in overcoming According to them, and through the researchers 'meeting with a number of police college students' trainers, there appeared to be weaknesses in the leadership aspects that affect their performance and the extent of their impact on their work in the future. Therefore, the researchers decided to know whether some psychological skills have a relationship in the formation of the lead character of the police college students, and the research objectives were Identify some psychological skills (the ability to focus attention, the ability to relax, the ability to face anxiety) among police college students, and detect illness Relationship between psychological skills and the leading personality of police college students, and knowledge of the level of leadership personality among police college students[5].

The practical side:

The researchers used the descriptive approach in the style of survey studies appropriate to the nature of the research problem, on the police college students course (66) for the academic year 2019-2020 numbering (514) students, as the researchers chose the same research in the simple random way (lottery) and the number (50) students.

Tests and measurements used in the research:

- Expert survey forms about their nomination, the psychological skills affecting the formation of the lead character.

Relaxation tests

Anxiety tests

Attention focus tests

Exploratory experience:

The researchers conducted the exploratory experiment to identify the negatives that the two researchers may encounter during the application of the main experiment to know the time taken to fill in the forms and obtain the stability of the form. As an experiment was done on (5) students from the Police College, and they are not from the original research sample.

Scientific transactions:

The validity of the tests:

"Meaning honesty is the test that measures what the test was put to measure it, and the test must be honest because we want to measure a specific phenomenon and not another phenomenon. There are several ways to measure the honesty of the tests, including the validity of the content, which is the process of presenting the questionnaire forms to a group of experts and specialists in the field." What the test measures and their opinions can be relied upon to find the sincerity of the test, which is the method used by the researchers to find the sincerity of the tests used, as the test forms were presented to experts and specialists and were nominated by them and obtained an approval rate of 100%[6].

Stability of tests:

The intensity of the tests is intended to give the test the same results if it is returned to the same individuals in the same circumstances. The researchers conducted tests on the research sample consisting of (5) students from the original community of the research and the test was repeated for them after (7) days passed "around the period For re-testing because it is one to two weeks since it is a standard period for re-testing, and the simple correlation coefficient Pearson was used to finding the coefficient of stability for the tests as shown in Table (1)[7]

The objectivity of tests:

It means "the subjective judgments do not affect the experimenter or the objectivity is affected without prejudice and subjective intervention by the experimenter. The higher the degree of subjectivity over the test judgments, the less substantive and the more the subjective judgments get rid of the effect, the greater the result of the objectivity, and for the researchers to verify that the test has high objectivity.

Table (1): Shows the scientific foundations of the tests

tests	the stability of tests	The objectivity of tests
The ability to relax	0.85	0.84
Ability to cope with anxiety	0.91	0.81
The ability to focus attention	0.84	0.86

The main experience:

The researchers conducted the main experiment of the research sample, as, during this period, questionnaires for psychological skills test were distributed to the research sample, where this was done during the first week, and then the researchers distributed the forms for the leadership personality test during the second week on the same sample of research and then extracted the results For each test, according to the key for each test.

Statistical means:

The researcher used spss to process data.

Presentation, analysis and discussion of results:

View and analyze the results of the ability to relax and leadership

Table (2): It shows the correlation coefficient between the ability to relax and the leadership personality

variables	correlation	Type of significance
Leadership and Relaxation Skill	0.867	Sig.

*Under freedom degree (48), error ratio (0.05), and tabular value reached (0.357)

Table (2) shows us the correlation coefficient between the leadership personality and the ability to relax and is (0.867), which is a significant value compared to the tabular value of (0.357).

Discussion

Table (2) shows us a moral correlation between the skill of the ability to relax and the leadership personality. The researchers attribute this to the leadership personality that must be characterized by calmness and relaxation. He can give his instructions, advice and decisions to them in a clear manner and calm tones, especially in situations of violent emotional nature and high psychological pressure.

Present the results of a skill's ability to focus attention and leadership personality and analyze it

Table (3): The correlation coefficient shows the skill's ability to focus attention and a leading personality

variables	correlation	Type of significance	Moral correlation
leadership personality and skill ability to focus attention	0.899	0.000	Moral

*Under freedom degree (48), error ratio (0.05), and tabular value reached (0.357)

Table (3) shows us that there is a correlation between the leadership personality and the ability to focus attention, as it reached (0.899), which is a significant value compared to the new and intense value of (0.357)

Discussion

Table (3) shows us that there is a moral correlation between the skill of the ability to focus attention and the leadership personality, and the researchers attribute the reason for this to the fact that the leadership personality must be distinguished by the ability to focus and mental stability[8]. Restraining it and controlling the speed of its emotions, as the speed of emotion, anger, nervousness, nervousness, and the speed of volatility and intensity are factors that contribute to the severe damage to the security work of the leader in society and help the negative impact of his influence and influence[9].

Present the results of the ability to face anxiety and the leadership personality and analyze it

Table (4): The correlation coefficient shows the skill ability to cope with anxiety and a leadership personality

Variables	Correlation	Type of significance	Moral correlation
leadership character and ability to cope with anxiety	0.854	0.000	Moral

*Under freedom degree (48), error ratio (0.05), and tabular value reached (0.357)

Table (4) shows us that there is a correlation between the leadership personality and the ability to cope with anxiety, as it reached (0.854), which is a significant value compared to the tabular value of (0.357)

Discussion

Table (4) shows us a moral correlation between the skill of being able to face anxiety and leadership personality. The researchers attribute the reason for this to the fact that the lead character must be characterized by the ability to calm and the ability to face anxiety and control psychological emotions," as the psychological emotions as they say (contagious) That is, its effect is rapidly transmitted. The emergence of emotions of fear, anxiety, tension and arousal to the leader will immediately transfer its impact to the rest of the members, and thus the leader becomes directly responsible for the rest of the members not controlling their emotions[10].

Conclusions

Through the results of the research, the researchers concluded the following:

The leadership personality is greatly affected by relaxation, and this was evident through the results of the research, and the lead character has a direct impact with attention, and this is what the study found, and the lead character is greatly affected by anxiety, and this was clearly shown through the results of the research.

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Conflict of Interest: None

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Diagnosing the Mutations and the Effect Concentration Chromium Cr (VI) in KRAS gene in Exon1 for lung Cancer Patients in Najaf Governorate

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Abstract

The importance of the current study lies in investigating the mutations in the exon1 of the KRAS gene as oncogenes, the presence of mutations associated with lung cancer and the lack of response to chemotherapy. As well as to know the relationship between diagnosed mutations and concentration of chromium . These study was conducted at AL-Furat AL-Awsat oncology Center In Najaf for period from December / 2019 to April / 2020 , on the study included 60 patients who were diagnosed with lung cancer and 22 healthy people where used as control group . the diagnosis of mutation in both patients and healthy subjects has been studied and compared. Then revealing the relationship between mutations and health status to see the effect of mutations on them. The present results showed that there were many mutations found in KRAS gene exon1, 42 genetic mutations in the KRAS gene were detected in exon1 , and these mutations appeared in varying proportions in both patients people, as is evident in In exon1, the frequency of mutation were rs104894361 SNP were in 11(18.33%) , rs121913236 SNP 13 (21.7%) and rs104894366 were 18 (30%) , distribution in 24 patients . more frequency mutations appeared in four patients (2,17,27,42) (6.7%) where found three types of mutations (rs104894361 SNP , 21913236 SNP and rs104894366) those patients have highest recurrence of (1.330, 1.153, 1.224 -1.490) Respectively. While 10 patients (16.7 %) (21,46,9,34,35,30,12,37,14, 39) have only two mutations with distribution in three types also , and 10 patients(16.7 %) were have just one mutation. We found correlation between high Chromium (Cr) concentration in serum of patients with lung cancer compared to healthy. The results of the study showed that there were significant differences (P=0.0001) between the presence of mutations and concentration of chromium, which indicates a relationship between the of mutations and concentration of chromium in the serum the of patient of lung cancer. DNA was extracted from the blood samples by several DNA extracts and mutations were detected by sequencing analyzing after amplification by PCR technology.

***Key word**: KRAS mutations in exon1, Lung cancer, oncogenes, lung cancer in Najaf Governorate.*

Introduction

The occurrence of lung cancer is affected by environmental exposure and genetic or epigenetic susceptibility to disease development and progression[(1)] . Important factors associated with lung cancer development are occupational exposure to carcinogens (arsenic, asbestos, beryllium, cadmium, chromium, diesel umes, nickel, andsilica)[(2)] .According to the European Commission, based on socioeconomic, health, and environmental impact assessment, the strongest factors related to attributable cancer deaths include Cr(VI)[(3)] . Lung cancer is the most common cancer with high lethality Carcinogenic Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation is the most common gain-of-function modification in Western countries,

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accounting for 30 percent of lung adenocarcinomas and about 10 percent of Asian lung adenocarcinomas[4] . Lung cancer is one of the most dangerous malignant diseases that threaten human life. It accounts for about 26 percent of all cancers as the leading cause of cancer death worldwide and ranks as the most deadly cancer among males and the second deadliest cancer among females[5] . Gaseous pollutants, such as sulfur dioxide (SO2), ozone (O3), carbon monoxide (CO), and nitrogen dioxide (NO2) have also been tested for possible links to lung cancer[6] . The occurrence of lung cancer is affected by environmental exposure and genetic or epigenetic susceptibility to disease development and progression[7] . One recent study showed that deficiency of IL-17C would promote the growth and metastasis in lung cancer model[8]. In addition, activation of the tumor-associated microbiota and TLR signaling stimulates the expression of calcineurin and nuclear factor of activated T cells (NFAT) factors, which sustains the survival and proliferation of cancer stem cells[9] .Thus, IL-17C promotes tumor-associated inflammation and tumor proliferation [10] .

Aims of Study

To evaluate the of environmental factors as Concentration Chromium in serum of patients and healthy to found some correlations with SNP mutation in exon1 of KRAS gene.

Material & Methods

Sample collection

This study was conceived to test population consisting of 60 case with non-small cellular lung carcinomas 40Male and 20 females, They are from the central and southern regions of Iraq. Any subject with the following health problems was excluded from the current study:

The ages of the patients ranged between 15-77 years and 49.6 ± 10.9 year (mean ± SD).

The controles (healthy) subject groups of 15 males and female were included in the study as a control group. Their ages ranged between 22-78 years and 47.1 ± 14.9 year (mean ± SD), they were free of The symptoms and signs of any chronic diseases such as diabetes, heart disease, kidney disease or others were chosen to participate in thi's a study.

Atomic absorption spectrometer

Atomic absorption spectrometer 6300 model analysis was performed in the central laboratory (College of Pharmacy / University of Kufa). Cr was determined in liquid samples by the atomic absorption spectrometer by flame under standard conditions and analysis of titration solutions prepared by diluting the stock solution with 100 ppm Cr.

Sampling the blood

Blood was extracted by vein puncture and 1 milliliter and 5 milliliters of blood were taken separately from all individuals in this sample. It included one milliliter of blood collected in tube-containing EDTA and used for DNA extraction; 5 milliliters were collected immediately for blood culture processing.

Genotyping

Extracting DNA

Whole blood samples were collected from the patient group and the healthy control group in EDTA tubes. Then using ReliaPrepTM Blood gDNA Miniprep Program (Promega), the DNA was extracted from whole blood.

Amplification of the primers

The genes examined were amplified using specific prefixes. Amplification products and other properties of the intended genes are scheduled as in table (1)

Table 1: Primer sequences of the genes IL-12 and IL-33, annealing temperature (AT) and product size.

Forward primer	Ex1K1extF: 5′-AGGCCTGCTGAAAATGACTGAA-3′
Reverse primer	EX1K1extR: 5′-ACTCATGAAAATGGTCAGAG-3′
Annealing Temperature	61°C
Product size	208 bp

Thermo cycler amplification software

The PCR thermo cycler program which delivered the best results of IL-12 gene amplification.

Table2: Thermo cycler system for KRAS gene amplification with PCR

Type of Cycle	Temperature^{o}C	Time	No. of Cycles
Initial denaturation	94	5 min	1
Denaturation	94	1 min	35 x
Annealing	61	1 min	
Extension	72	1 min	
Final extension	72	5 min	1

Analysis Sequencing

The PCR products 80samples (60 patients and 22 control healthy) were sent to company macrogen (Koria), in ice bag by DHL. performed the DNA sequencing by AB DNA sequencing system, using Migax64 software & SLC Sequence Viewer software, for analysis sequencing to detected any mutations in the samples for current study by comparing the observed DNA sequences of local samples with the retrieved DNA sequences ,The analysis of sequence reveated the presence of three mutations in exon1 of the KRAS gene in Lung cancer. all of these detected SNP were found to be deposited in the dbSNP as past known genetic polymorphism. The variations in prediction capabilities can be due to the fact that different sets of sequences and alignments are used in each system. We used MutPred method to assess the degree of tolerance for each amino acid replacement based on physiochemical.

Statistical Analysis

Statistical analysis was performed using Mann Whitney test and Chi-square (χ^2) test to determine statistical differences between different groups using the Design Statistical Package for Social Sciences (SPSS 19). Probability ($P \leq 0.05$) was considered statistically significant.

Results & Discussion

Concentration of chromium in Patients and healthy subjects:

The results of the current study appeared as shown in Table (3), which shows a comparison between the healthy and the patients regarding chromium element concentration and age. Where the concentrations of the hexavalent chromium element (VI) in the serum for the patients ranged between (0.224-1.992) and was the average concentration (0.76737), while the concentrations of the chromium element in healthy people ranged between (0.09 - 0.231), the while Chromium concentration average was (0.13973), Where the results are shown difference significant (P=0.0001) at the level of significant (0.05) which indicates a relationship between the high chromium element concentration and the incidence of lung cancer, comparison as with the healthy. studied the effect of carcinogens on the risk of lung cancer in the general population. De Matteis et al they showed that patients appear to have an increased risk of lung cancer due to exposure to chromium, which is consistent with our study results. Patients from these families with high levels of chromium in the blood will have the option of lung CT scanning for early detection of disease. Analyzes of chromium levels may be an attractive option to identify patients with particularly early-stage disease.

Age: Whereas for the age where the ages of the patients ranged between (15-77), and the average The rate was (41.85), and the ages of healthy people ranged between (16-76) , and the average The rate was (41.18). Where the results showed that there was no significant difference. the was significant is (P = 0.904) for age between the healthy and the patients, This explains that the study samples were close between age the patients and the age of healthy. and this is due to the accuracy of choosing the study samples of the for the healthy sample compared to patients.

Table (3): Shows the Comparison of healthy and Patients subjects with respect to chromium concentration and age

Comparison of healthy and Patients subjects with respect to chromium concentration and age							
	Cr con. average	**N**	**Std. D**	**Minimum**	**Maximum**	**Mean Rank**	**p-value**
Patients	0.76737	60	0.311614	0.224	1.992	52.48	0.0001
Healthy	0.13973	22	0.037749	0.09	0.231	11.55	
	Age average	N	Std. D	Minimum	Maximum	Mean Rank	p-value
Patients	41.85	60	18.965	15	77	41.69	0.904
Healthy	41.18	22	17.778	16	76	40.98	

Chromium concentrations and mutations in the KRAS gene:

Table (4): Shows Relationship between different chromium concentrations and mutations in the (KRAS gene) in exon1 for Patients. The chromium concentrations were divided into three groups based on the difference in the concentration ratios. Where the concentration first group ranged from (0.2-0.8) and the second group was (0.81-1.4) and the third group, where the concentrations ranged between (1.4-1.2) and the highest concentration ratios in it compared to the previous groups.

Where the study found (11) patients in group (A) whose levels of chromium concentrations in the serum

ranged between (0.2-0.8).These patients had a mutation of the first type rs104894361 SNP, while group (B) included 6 patients with a type first mutation and the second rs121913236 SNP, rs104894361 SNP, while the third group (C) included (7) patients and among them (4) had three types of mutations rs121913236 SNP, rs104894366 SNP, rs104894361 SNP and had the highest rate of concentration of chromium in the serum, and this indicates that the high concentration of chromium leads to occur mutations in the gene of the (KRAS gene) In Exon1, which causes lung cancer. The results of the statistical analysis of this study showed a significant difference (*P*=0.001), which indicates the existence of a relationship between the high concentration of chromium and mutations in the gene (KRAS), compared with patients who did not show any mutation , this What reinforces the results of the study that with high concentrations of chromium is the reason for the occurrence of mutations in the gene (KRAS) in patients with lung cancer, and this study is the first to detect high chromium concentrations in the (KRAS gene) mutations in Exon1. Another limitation of our study is that, the high risk of lung cancer is generally recognized as being associated with occupational exposure to Cr(VI), but we did not have patient work histories available to include in this study. Nevertheless, our study may provide an avenue to begin to screen for lung cancer occurrence.

Table (4): Shows Relationship between different chromium concentrations and mutations in the KRAS gene in exon1 for Patients.

MUTATION * CONT								
			CONT			Total		
			A(0.2-0.8)	B(0.81-1.4)	C(1.4-1.9)		X2	P-value
KRAS MUTATION		Count	11	6	7	24	13.309	0.001
		% of Total	18.3%	10.0%	11.7%	40.0%		
		Count	29	7	0	36		
		% of Total	48.3%	11.7%	0.0%	60.0%		
Total		Count	40	13	7	60		
		% of Total	66.7%	21.7%	11.7%	100.0%		

Conclusions

1. The present results showed that there were three mutations found in KRAS gene exon1.
2. Forty two genetic mutations in the KRAS gene were detected in exon1 in all patients.
3. The frequency of rs104894361 SNP mutation in exon1,were 11(18.33%), while in rs121913236 SNP were 13 (21.7%) and in RS 104894366 were 18 (30 %).
4. more frequency mutations appeared in four patients (2,17,27,42) (6.7%) where found three types of mutations (rs104894361 SNP, rs121913236 SNP and rs104894366).

5. Those (2 , 17 , 27 , 42) patients have highest recurrence of concentration of chromium in the serum (1.330 , 1.153 , 1.224 -1.490).
6. There was a correlation between some mutations with others.
7. The DNA sequencing assay is one of the easiest and most effective ways to determine genetic mutations and more accurately.
8. The SNP rs reported in lung cancer patient appears to be promising and can be used as a predictive tool or a genetic prediction test tool for lung cancer if found positive in a larger sample size.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Reference

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The Effect of Incorporation Kappa-Carrageenan Powder on the Physical and Mechanical Properties of the Heat-Cured Acrylic-based Soft Denture Lining Material in Clinical Use

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Abstract

Background: Soft denture relining materials have been introduced to prosthetic dentistry since long time to improve the patient's acceptance to dental prosthesis. However, with the growing need to provide the patients with dentures that have improved comfort and masticatory efficiency, there is a growing demand to improve several features of the denture lining materials. Therefore, the aims of this study was to evaluate the effect of incorporation kappa-carrageenan powder as antifungal agent on the hardness and peel bond strength of the heat-cured acrylic-based soft denture lining material.

Materials and Methods: Two percentages of kappa-carrageenan powder (1.5wt.% and 2wt.%) have been selected based on the pilot study that produced the best antifungal effect. A total of sixty samples were prepared and divided into three equal groups groupA:0.0wt.%, groupB:1.5wt.%, groupC:2wt.% of kappa-carrageenan powder additive. Each group was further subdivided into two equal groups for shore-A hardness test and peel bond strength test. Fourier transform infrared spectroscopy (FTIR) analysis was performed to investigate if there is any chemical reaction between the soft lining material and the kappa-carrageenan powder, scanning electron microscope (SEM) was used to determine the distribution of kappa-carrageenan powder in the soft liner matrix.

Results: All the resulted data were analysed using "one-way analysis of variance (ANOVA) and Dunnet T3 post-hoc test at a significance level of $p<0.05$". The result of shore-A hardness test revealed significant increase *(*$P<0.05$) in the values of hardness of both experimental groups (B and C) when compared with the control group(A). Peel bond strength test revealed non-significant decrease ($P>0.05$) in the values of peel bond strength for experimental group(B), while there was significant decrease ($P<0.05$) for the experimental group(C) when compared to the control group. FTIR analysis showed no difference in the spectra between control group and experimental group this means that there is no chemical interaction between the two materials. SEM test results showed a degree of agglomeration of the kappa-carrageenan powder into the soft liner matrix.

Conclusion: Adding kappa-carrageenan powder to the soft liner affected the physical and mechanical properties of the lining material and the most adverse effect was noted for the group C(2 wt.%) but all effects was in the acceptable range for clinical use.

Keywords: *soft denture lining material; clinical use; dental prosthesis; peel bond strength*

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Introduction

Denture soft lining materials have been used in dentistry for many years, the purpose for their use is to

improve mastication through providing more favorable relation between the denture bearing mucosa and the denture base which will result in more favorable distribution of the occlusal forces. For this reason the denture lining material must exhibit certain properties that allows it to serve properly inside the patient's mouth[1].

The physical and the mechanical properties of the heat-cured acrylic-based and silicone-based soft liners change with time inside the patient's mouth as a result of leaching out the plasticizer[2,3]. Therefore, frequent evaluation and replacement of the used soft lining material is required.

Peel bond strength is one of the methods used to assess the reliability of bond between the denture base and the soft lining material which is thought to closely stimulate the force applied at the interface between the resilient denture liner and the denture base[4]. The results of this test are affected by the thickness and compliance of the material; along with bonding failure predominantly being cohesive within the tested materials[5].

Hardness is another property that is commonly used and measured to assess the quality of different soft liners. Hardness has been defined as the material resistance to indentation[6]. It is one of the most important tests for rubbery materials, and it represents a method for determining the modulus of elasticity since rigid material cannot be used as soft liners[7]. One of the most important advantages of soft lining materials is their viscoelastic properties, which result in significant improvement in masticatory function. Regarding soft acrylic liners this property change with time due to leaching out of plasticizer[4].

Multiple studies have been conducted to improve the properties of the soft lining material, one particular aspect of these studies is the introduction of natural herbal products to the soft lining material to enhance their antimicrobial, physical and mechanical properties. Kappa-carrageenan (κ-carrageenan) powder is a natural sulphated poly saccharide extracted from the red marine algae. It has been identified as possessing high anti-coagulant, anti-oxidant, anti-tumour, and anti-microbial activity[8]. In addition, carrageenan has been used for many years as a food additive and in pharmaceutical applications due to its stabilizing, thickening, and emulsifying properties, and it is accepted by the U.S. Food and Drug Administration (FDA)[9] and the World Health Organization (WHO)[10].

κ-carrageenan powder has been evaluated for its antifungal activity through a pilot study when incorporated into soft lining material as an additive and the two percentages of κ-carrageenan (1.5 and 2wt.%) incorporated into the heat-cured acrylic-based soft liner were proved to have potent antifungal effect. The purpose of this study was to evaluate the effect of each of these percentages on the hardness and peel bond strength of the soft lining material.

Materials and Methods

Samples grouping

A total sixty samples were prepared and divided into two groups of thirty samples for each test (shore A hardness and peel bond strength tests) each group was then subdivided into three groups of ten samples representing the control groupA:0.0wt% and experimental groupsB:1.5wt.% and groupC:2 wt.% of κ-carrageenan powder.

Fourier transform infrared spectroscopy analysis

FTIR analysis was performed using Fourier transform infrared spectroscopy (SHIMADZU/ FTIR.8400,Japan) to investigate if there is any chemical reaction between the soft lining material and the kappa-carrageenan powder. A sample of a pure soft lining material without an additive (0wt.%) and sample with κ-carrageenan powder additive (2wt.%) were cut into small thin flushes and were examined.

Scanning electron microscope

SEM test was preformed using scanning electron microscope (Tescan,Czech Republic) to determine the distribution of κ -carrageenan powder in the soft liner matrix. Three samples were tested by scanning electron microscope, one of the samples represented the control sampleA:0.0wt.% and the other two represented the experimental samplesB:1.5wt.% and C:2wt.%.

Samples preparation:

For both tests samples of the control group were prepared according to manufacturer instructions for the

heat-cured, acrylic-based soft liner (Vertex,Netherland) (powder/liquid ratio:1.2g powder/1ml monomer), and mixed together using a clean glass container. For experimental samples, the weight of the kappa-carrageenan powder (Sigma al-drich,Denmark) was subtracted from the weight of the soft liner powder to obtain an accurate powder/liquid ratio. An amalgamator device (Perfection Plus,United Kingdom) was used to mix the two powders for 40seconds to obtain a homogeneous mixture[11].

Shore-A hardness test: A plastic disks shaped molds with the dimension of (35X6mm) in dimeter and thickness respectively were prepared according to the **ISO-10139-2(2016)** specification[12]**.** These molds were invested in the lower portion of the dental flask with freshly mixed dental stone (Zermack, Italy) and the flasking procedure was completed in similar way to conventional complete denture construction. All samples were cured according to manufacturer instructions using a thermostatically controlled water bath (Lab.tech., Korea) heated to 70℃ for 90minutes; temperature was then raised to 100℃ for 30minutes. After processing, samples were finished with sharp scissors and polished with a fine-grit, silicone polishing bur under continuous water cooling[11]**.** All samples were stored in distilled water for 24hours at 37℃ before being tested to eliminate any residual monomer[13]**.**

Peel bond strength test: Samples were prepared according to the **ASTM-D903-93**[14] however the size of the samples was too long so half the measurements was thought to be sufficient[15]**.** A costume made stainless steel flask was used to prepare the samples, it consisted of four plates with dimensions (260X200mm) length and width respectively two of them was used as covers while the other two consist of slits in which the PMMA and the soft liner material were packed. The thickness of these two plates was (2mm) and the dimensions of the slits of the plate used to pack the PMMA were (100x10x2mm) representing the length, width and height, while the plate used for the soft liner material has slits with dimensions (150x10x2mm) representing the length, width and height respectively[16](Fig.1).

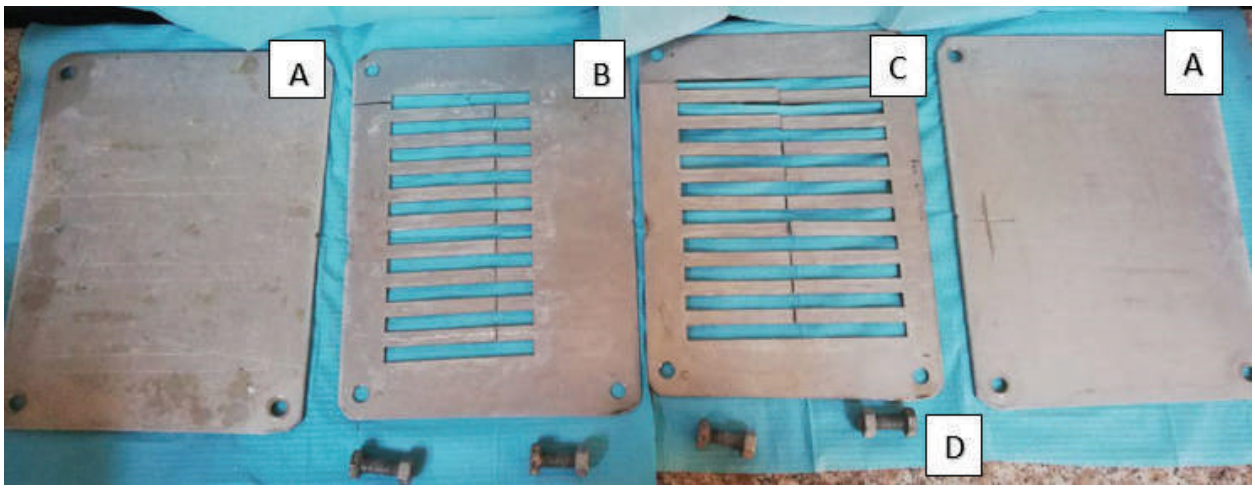


Figure (1) Peel bond strength flask. A) The flask covers, B) Flask plate for poly methyl methacrylate, C) Flask plate for soft liner D) Screws to hold the four plates in tight metal to metal contact.

Samples fabrication included first packing the PMMA material (Vertex, Netherlands) in the flasks which was proportioned and mixed according to manufacturer instructions(P/L2.3g powder to 1ml of liquid). The flask was then closed and a slow pressure was applied using hydraulic press until(100MPa) was reached and left for about 5min. and then following the manufacturer instruction the flask was placed in the water bath for 20min. at 100℃. Following this step the flasks were left to cool for about 30min. and then cooled under running water for about 15min. then the acrylic strips were deflasked, trimmed and finished then reflasked again.

Testing procedure

Shore A-hardness test: shore-A durometer (A081FJ702020, China) was used, five readings at different points on the sample were marked, four points were marked away from the edge in about 5mm and one point in the center, the penetration time was 5sec. and the distance from the indenter was 20mm. The average of the five readings was recorded and determined the value of the shore-A hardness.

Peel bond strength test: The test was accomplished using universal testing machine (WDW-20, China) according to **ASTM-D903-93** specificatios[14]{at an angle(180°) and speed (152mm/min)}. The part of the heat cured acrylic resin that was not bonded to the soft lining material was hold by the upper clutch of the machine while the unbonded part of the soft lining material was hold by the lower clutch with the use of an alignment plate to keep the sample in its proper position.

The nature of failure was evaluated with the naked eyes and categorized as either adhesive, cohesive or mixed failure. Peel bond strength value was calculated with the following equation in which the peeling angle was considered 180°.

(Peel bond strength= average load /width of the sample)

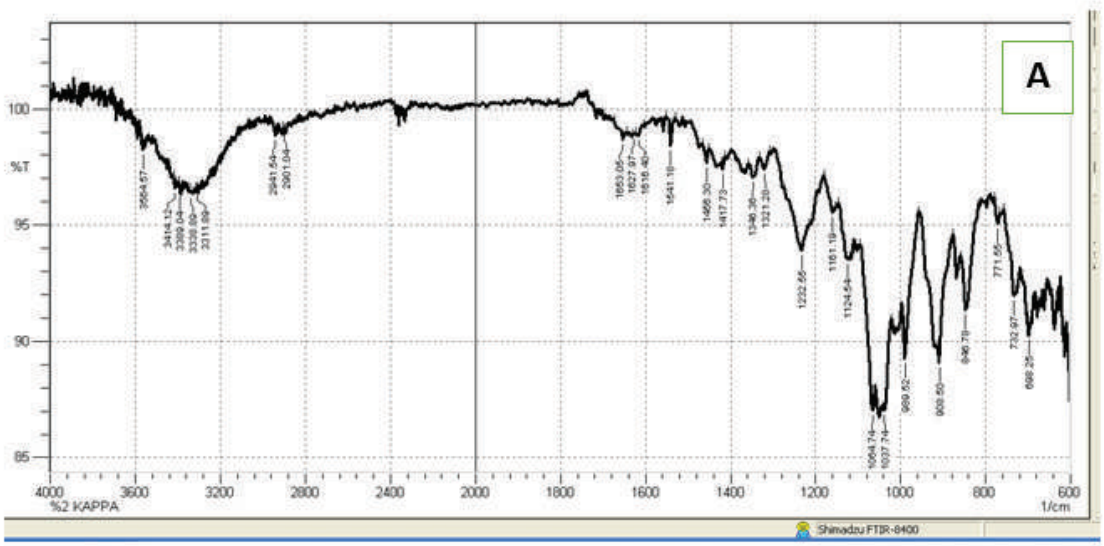
Statistical analysis

Results of the presented research were analysed using SPSS "version 24 computer software". The descriptive statistics that have been made, includes "means and standard deviation". "Inferential statistics" were also made, including one-way analysis of variance (ANOVA), for comparison means among all groups, and Dunnet T3 multiple comparison tests, which shows the significance between each of the two different groups, with $p<0.05$ considered significant.

Results and Discussion:

Fourier transform infrared analysis

The results of the FTIR analysis showed there was no differences in the pattern and the alignment of the absorption peaks between the control and the experimental samples. Which means there was no chemical reaction between the soft lining material and the κ-carrageenan powder (Fig.2).



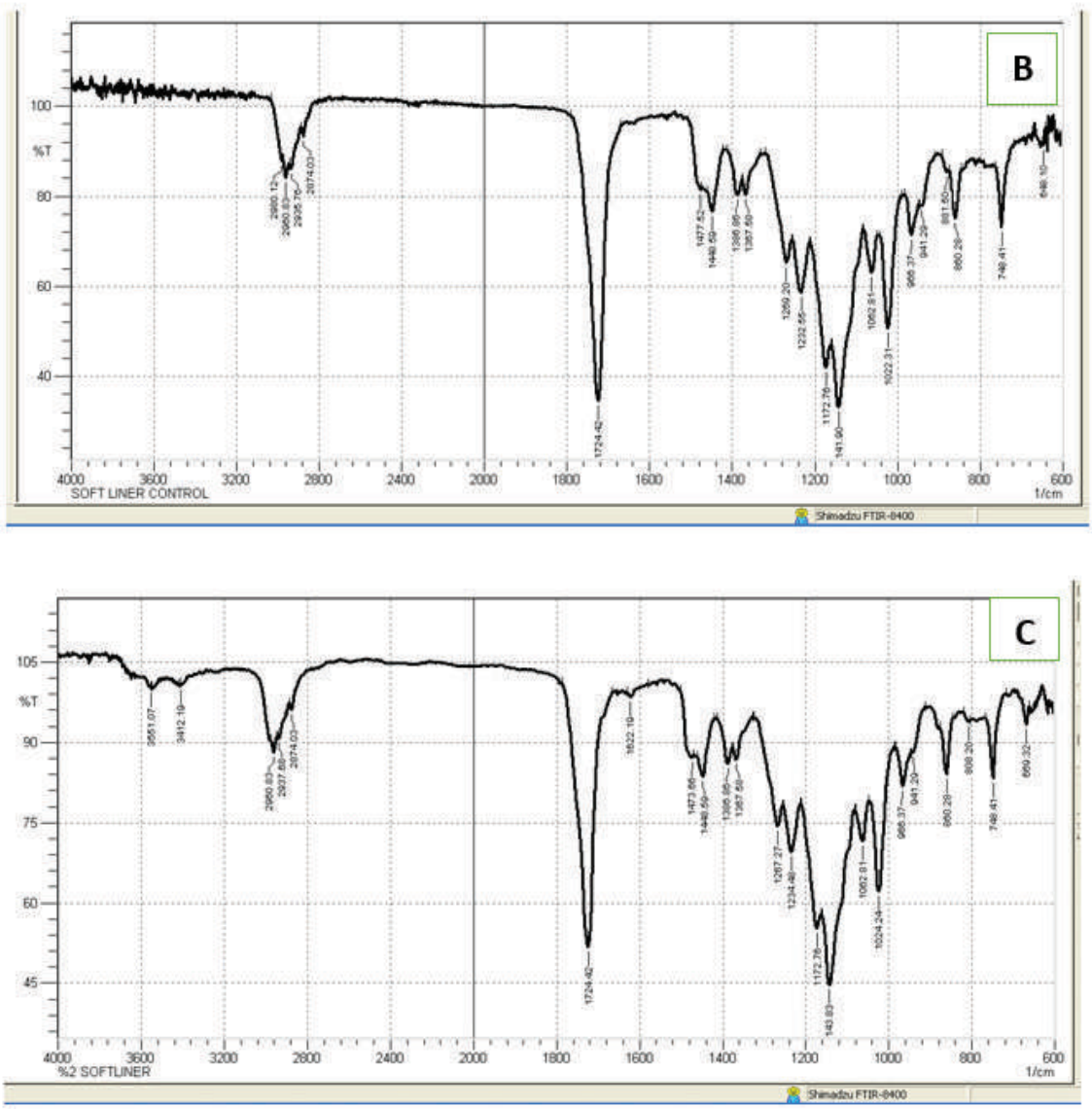


Figure (2): Fourier transform infrared spectroscopy analysis, A) Kappa-carrageenan powder; B) Control sample; C) Experimental sample (2wt.%) kappa-carrageenan powder

Scanning electron microscope test

The results of the SEM test shows there was a degree of agglomeration in the experimental groups which tend to increase with the increase in the kappa-carrageenan powder percentage in the soft lining material (Fig.3).

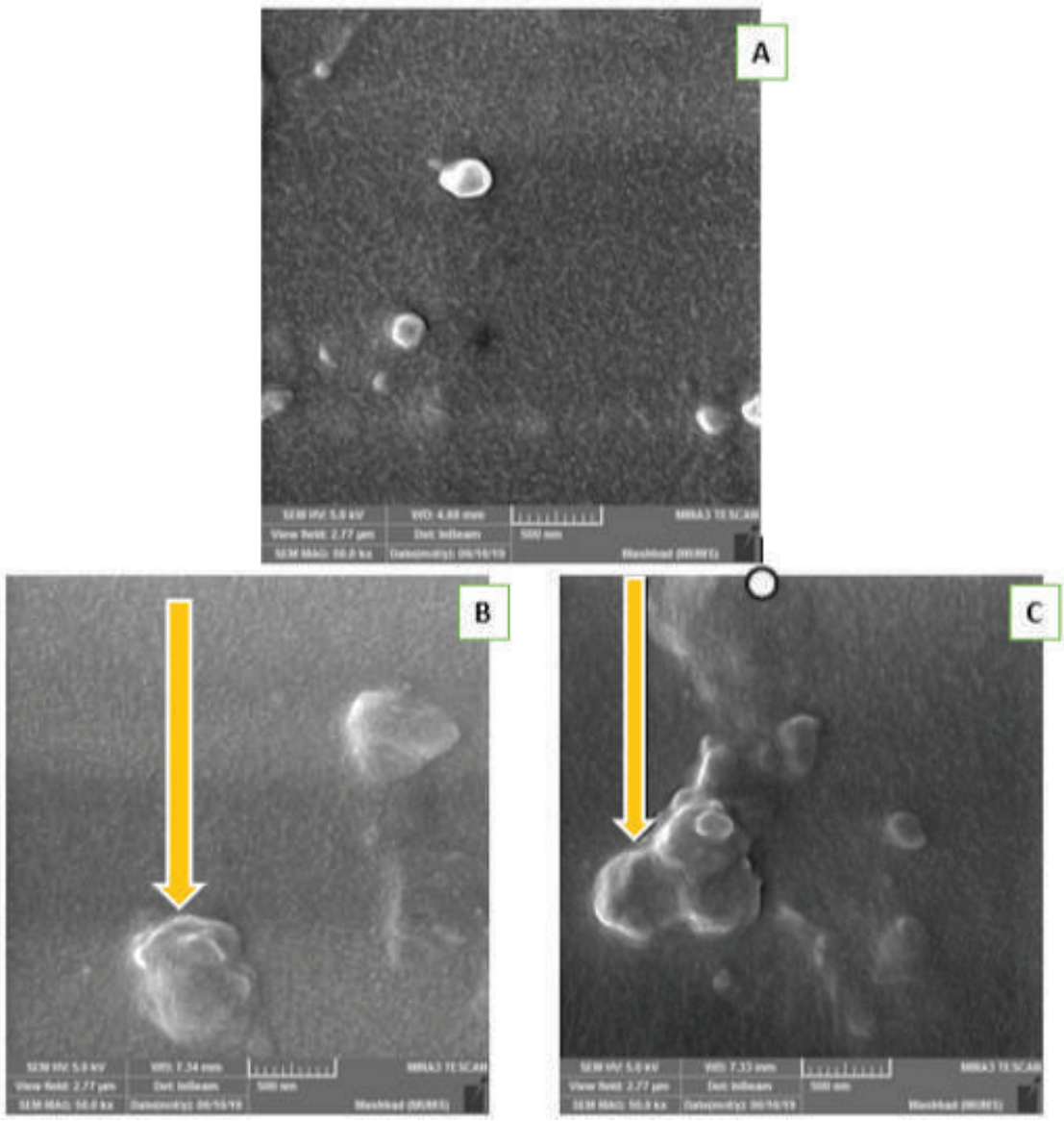


Figure (3): Scanning electron microscope (magnification power 500nm). A) Soft liner control sample without the additive, B) Sample with 1.5wt. % of kappa-carrageenan powder, C) Sample with 2wt. % of kappa-carrageenan powder. Arrows shows the agglomeration of the powder.

Shore-A hardness test

The results of shore-A hardness test showed there were increase in the values of hardness for both experimental groups when compared to the control group, One-way ANOVA showed significant difference of hardness among groups (A, B and C) at $P<0.05$. Dunnett T3 post hoc test showed there was significant difference ($P<0.05$) between the control group and the two experimental groups (B and C) (Table1and2).

Table (1): Mean values of shore-A hardness, peel bond strength tests and ANOVA test.

groups	No. of samples	Shore A hardness				Peel bond strength test			
		Mean	SD	Min.	Max.	Mean	SD	Min.	Max.
A	10	52.58	.817	50.76	53.60	4.630	.600	3.90	5.40
B	10	55.18	1.820	53.40	59.20	4.390	1.030	3.10	5.80
C	10	56.40	.596	55.20	57.20	3.100	.514	2.30	3.80
ANOVA	F-test	26.235				12.051			
	p-value	.000[S]				.000[S]			

Table (2): Multiple comparisons of shore-A hardness and peel bond strength tests between groups using Dunnett T3 post hoc test.

Experimental groups		Shore A hardness		Peel bond strength test	
		Mean diff.	Sig.	Mean diff.	Sig.
A	B	-2.592	.004	.240	.891
	C	-3.812	.000	1.530	.000
B	C	-1.220	.183	1.290	.010

The incorporation of κ-carrageenan powder may altered the polymeric chain penetration by the plasticizer and there by affected its ability for softened gel formation. Additionally κ-carrageenan may acted as a filler that which resulted in increase in the hardness of the soft lining material**[17].**

The nature of the added material may also influence its effect on the hardness of the soft lining material for instance the increase in hardness that was obtained from this study can be related to the gelatinous nature of the kappa-carrageenan and the formation of agglomerations that was noticed in the SEM results.

Peel bond strength test

The results of peel bond strength test showed both experimental groups (B and C) have decrease values of peel bond strength when compared to the control group. One-way ANOVA for peel bond strength test showed significant difference among the tested groups ($P<0.05$). Dunnett T3 post hoc test showed there was non-significant difference between A and B groups ($P>0.05$), while a significant difference exist between groupA and C ($P<0.05$) (Table1 and Table2).

The mode of failure was non-significant for all the experimental groups. The cohesive failure may be associated with the presence of the κ-carrageenan within the polymeric matrix of the soft lining material while the adhesive failure may be related to the hydrophilic nature of the κ-carrageenan which may absorbed water and decreased the peel bond strength values and caused the samples to fail adhesively (Table3)**[16].**

Table (3): Fisher exact probability test to compare the mode of failure between the experimental groups.

			Groups			FEPT	P value	Total
			A	B	C			
	Adhesive	N.	0	0	2	6.912	0.105[NS]	2
		%	.00	.00	20.00			6.67
	Cohesive	N.	4	3	0			7
		%	40.00	30.00	.00			23.33
	Both	N.	6	7	8			21
		%	60.00	70.00	80.00			70.00

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Functional Variables and Enzyme (CPK)to Skill of The main and Alternatives Players in Football (comparative study)

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Abstract

Football is a type of sport that attracts people's attention. So, it is necessary to look for everything that can improve its reality and develop it. The importance of this research paper is to have a look on the impact of competitions on functional devices, enzyme (CPK), aspects and the skills for the main players and compared with the substituted players in football. The study aims at identifying the level of some functional variables, enzyme (CPK) and the skills for key players and substituted players in football; identifying the differences in some functional variables, enzyme (CPK) and the skills of the key and substituted players in football. Descriptive method is used by researchers in a comparative way to solve the problem of the study. The number of sample is fifteen players distributed to two groups, the first group is the key players [9] and the second group is substituted players [6]. Also, the researchers used the SPSS statistical system to extract the results of the tests. The most important conclusions are: (1) competitions play an active role in developing the performance level, whether at the functional level or enzyme CPK or skills. (2) The role of CPK enzyme has increased after performing high physical effort and for a long time for the key players as a result of competition effort. Moreover, the following is the most important recommendations that the study has reached into: (1)It is necessary to participate all players in the competition, even periodically, so that substituted player can sense the atmosphere of competition and his active role in the development of functional and skills of the player .

Key words: *Enzyme CPK, The Main Players, Alternative Player, Football, Functional Variables*

Introduction

Sport is one of the civilizational landmarks of nations as well as the health, cognitive, cultural and economic aspects of sport. So, specialists in the sports field focused on investing all the sciences to serve the sports side to promote it and to achieve the goals. Football considers at the front of sports that bring great interest to nations because of the spiritual power, fun and special skills that it has. The study is laded to all the reasons that promote the reality of sports and affect the achievement including the internal functional variables of the human body and enzyme (CPK), as well as the skill aspects of the players whether the primary players or alternatives. The importance of the study is to have a look on the impact of competitions on functional devices , enzyme (CPK) , and the skills aspects for the main players and compared with the substituted players in football.

The problem

The problem of the current study is reflected in the lack of clarity , knowledge in most trainers, the cumulative effect of exercise, competition on functional devices on the enzyme (CPK),and the Skill aspects for main players compared with alternatives in football.

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The problem can be formulated with the following question: Does competition have a cumulative effect on functional variables of the enzyme (CPK) and football skill?

The aims of study

The study aims at:

- Identifying the level of some functional variables , enzyme (CPK) , skill for the main players and alternatives in football.

- Identifying the differences of some functional variables , enzyme (CPK) , skill for the main players and alternatives in football.

The researchers hypothesized that, there are statistically significant differences in some functional variables, enzyme (CPK) , skill between the main players and alternatives in football with favor to the key main players.

Study methodology and procedures:

Methodology

The researchers used the descriptive method in a comparative way to solve the problem of the study and to achieve its objectives. The descriptive method is the method that is concerned with collecting accurate scientific descriptions of the phenomenon studied and describe the current situation and interprets it. (1)

The data and community of study

The researchers choose the community of the study in a deliberate manner who are the players of the South Sports Club applicants category football for the sports season 2019. The number is (22) players. While the data of study is randomly selected after excluding (3) players as goalkeepers, also excluding (3) players, one of the main players and two

players from the reserve in a simple random way to be used as a reconnaissance. The data has reached the number (15) players distributed in two groups; the first group includes (9) main players and the second group includes (6)alternative players.

Tests and Measurements Used in the Study:

Functional Measurements

Heart rate (R.H) , the strike volume(V.S),and cardiac output (O.C) are measured in the resting time through the ECO device after lying on the medical bed for a period (3) minutes to stabilize the pulse after which the above variables are measured by the specialist *

Coenzyme CPK: The level of coenzyme (CPK) is measured by drawing a blood sample by a specialist and then laboratory treatment to find a coenzyme (CPK) in athletes.

Skill Testing:

Handling test: Wall rebound handling for 20 sec: [1]

-The purpose of the test: To measure the accuracy and speed of handling.

Foot scoring test on overlapping rectangles[2]

-The purpose of the test: To measure the accuracy of scoring.

Rolling test between five persons round trip: [3]

- The purpose of the test: To measure the ability of the rolling speed with the change of direction.

Survey Experiment:

The researchers conduct their survey experiment on Thursday (1/8/2019) in a sample which consist of (3) players. They are excluded from the sample after testing and measurements. The same test is repeated seven days later on the same sample and under the same conditions to ensure that the measurement is stable by drawing blood and in the same conditions for the two measurements, the calculated value (R) is (0.921), which is bigger than the value of (R) tabular value (0.767) at the level of significance (0.05).

The procedure experiment:

The tests are conducted on the study sample in the resting time on Saturday (3/8/2019) at nine in the morning at the hall of the South Sports Club and the steps were as follows:

- Recording the weight, height and age , then the laboratory lying on the bed for (3 minutes) , measuring the heart rate (R.H), the stroke volume (V.S) and the cardiac output (O.C) in rest mode through the ECO device after lying on the medical bed for a period of (3) minutes to stabilize the pulse after which the above variables are measured by the specialist*. Then, blood is drawn from the player (5 cm 3) by a specialist and treated in a laboratory to find the enzyme measurement (CPK) for athletes.- Perform skill tests for main players and alternatives. The researchers used the statistical pouch (SPSS) to process the data and extract the results of study which obtained from the measurements and tests of the study.

Presentation and Discussion of the Results:

Presentation of measurement results of functional variables and enzyme (CPK) for individuals of the data of the study

Table 1 : Shows the arithmetic media, standard deviations and the calculated T value of the main and alternatives players in football.

Measurements	Measuring Unit	Main Players		Alternative players		The Counted Value of T	Siq.	Moral
		Arithmetic mean	Standard deviation	Arithmetic mean	Standard deviation			
R.H	b/m	54.2	3.97	62.37	2.103	5.139	0.01	Moral
V.S	mm	91.7	1.98	74.58	5.00	7.105	0.04	Moral
O.C	l/d	5.08	0.23	4.2	0.30	5.59	0.54	Non-moral
CPK	L/U	30.25	1.77	21.10	0.57	14.01	0.003	Moral

The Discussion of Results

The researcher believes that there are significant differences in the level of functional variables and CPK enzyme regarding the players' level with favor to key players. The researcher attributes the reason for this increase to the fact that sports training and active participation in competitions with a maximum or near maximum performance level increased the efficiency of the functional devices. This is due to the cumulative effect of the functional devices of the players, including heart at rest as well as increased strength of the heart muscle and increased cardiac cavities, especially the left ventricle, which increased the amount of blood absorption returned to the heart . Thus, an increase in the size of the heart plus of blood, which gives an indication of Work obviously offset by the rise in cardiac output during rest Aboul-Ula asserts that "cardiac thrust can increase either by increasing the heart rate or by increasing the volume of blood paid per puls [1]. also, Fadhil Kamil stresses this stating that "regular physical training and competitions lead to an increase in heart rate reduction in rest as a result of physiological adjustment led to an increase in cardiac output of blood and that physical training leads to an increase in the volume of the batch or the amount of blood pumped by the heart in each puls"[2]. Further, the researcher states that there are no significant differences between the key and substituted players despite the development of the

players in the level of the volume of the strike to the fact that the volume of blood pumped per minute depends on two key elements: namely heart rate and blood pumped volume. Since the increase in the heart plus rate of the main players is lower as a result of the high adjustment leads to a decrease in the volume of blood paid while the increased heart rate in the substituted players increased the level of blood volume paid per minute as the rate of blood volume paid per minute is the result of Heartbeat in the volume of blood paid per minute.

The researcher attributed that the reason for increasing of the CPK.

enzyme as the result of training as well as performance in competitions in which the athlete uses high or semi-intensity. The effectiveness of the enzyme CPK is increased in the in the body to provide energy to meet the performance requirements of the player. This is confirmed by Abul-Ula Ahmed (2000) that the use of exercises with maximum or semi-maximum intensity with few repetitions and high performance speed will increase the activity and effectiveness of enzymes responsible for energy production and reconstruction during Anaerobic work because these enzymes work to break down and break down the chemical bonds of creative phosphate and carbohydrates or muscle glycogen[1]

Presenting and Analyzing the results of Special Skills Tests for the Members of the Research Sample.

Table (2): Shows the arithmetic media, the standard deviations and the calculated (T) value of the skill tests of the individuals of the research sample:

Tests	Measurement Unit	Previous tests		Post tests		T counted value	siq.	The Morale
		standard deviation	Arithmetic mean	standard deviation	Arithmetic mean			
Goal	0‹006	0‹35	2‹27	0‹52	4‹53	degree	10‹69	Moral
passing	0‹008	1‹84	8‹72	0‹62	10‹64	20/ times. Sec.	2‹95	Moral
degree	0‹03	1‹32	11‹20	0‹41	13‹56	Sec.	4‹151	Moral

Table (2) shows that there are significant differences in the skill tests between the main players and substitutes in football with favor to the main players. The researcher attributes the reason for these differences to the fact that the real participation of the main players in the game has increased the level of accuracy of the skill performance of the main players. The direct contact with the game and the players, which exposes the players to the performance of tensile and maximum or high in the game and different repetitions with the atmosphere of competition. Thus increased the drawing of a clear picture of the dynamic path of performance and thus increased the experience of the players as well as increased realistic solutions that occur during the game leading refine the skills.

Regarding the skill of passing, participation in the competition increases the ability of the player to pass accurately and for all types of passing. As a result of the requirements of collective play, which requires to transfer the ball from one place to another and from one player to another to maintain it refined the skill and accuracy of passing. The researcher agrees with (Ghazi Saleh, 2008) that the ability to use all kinds of handling and receipt during running, deception, scoring and sufficient strength in football competitions. There

should be a high harmony of some elements of fitness and technical skills in the play need that the player is qualified to absorb all modern plans as well as the ability to give in different conditions of play[1].

The scoring skill: the participation in competitions and friction with the players and the atmosphere of competition increases the experience of the player to perform the skill with high level and accuracy.

The researcher agrees with Saleh Radhi (1990) (the more the player's experience, the more he can aim at the right place and the right strength) [1].

As for the skill of rolling, competition requirements requires moving the ball from one place to another while maintaining it to gain space on the stadium or to implement a special plan or to make a new attack. High frequency increased the development of the skill ability of the player in the skill of rolling. Agreeing with Majid Sabar(2013), the researcher states that the use of quick exercises from different positions of the stadium and move from one place to another ball and the application of rolling and shooting, which increased the possibility of the player's skill.[2]

Conclusion

The researcher has concluded that: (1)the competition has an active role in the development of the performance level, whether on the functional level or CPK enzyme or skill, (2) The role of CPK enzyme has increased after performing high physical effort and for a long time for the main players as a result of the competition effort, (3) Increased cardiac output for the players especially for the main players improves the level of career and the role of competitions, and (4)There is a clear improvement in the skill performance of the test under consideration as a result of the refinement of skills and a clearer picture of the movement of the key players during the competitions.

The researcher recommends the following: (1) the need to involve all players in the competition, even periodically, so that the reserve player senses the atmosphere of the competition and the role of the actor in the development of functional and skill capabilities, (2) The need to compensate substituted players who did not participate in competitions with training modules similar to the performance in competitions to compensate players lost training units during competitions, and (3) the need to conduct periodic measurements of functional variables and measurement of enzymes and conduct tests for all skills to determine the level of key players and replacements.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Glycodeline-A , PlGF Together with βhCG as Prognostic Markers in First Trimester Pregnancy Loss for Iraqi Women

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Abstract

Objective: estimate the scale of Glycodeline-A,PlGF, together with βhCG in first trimester pregnancy loss in Iraqi women .

Methods: A subject:patients and control study, begining in April 2019 and finish the work at March 2020, study include 85 women,45 of them as healthy control which haven't any complication at the first trimester stsrting from the first day of LMP , 40 of them as patients which have abortion at the same time,tests included evaluate the scale of βhCG,Glycodeline-A,PlGF By ELISA method.

Results: scale of βhCG are significantly higher in healthy controls than in patients , while scale of Glycodeline-A , PlGF was significantly lower in healthy control than in patients,and there is significant correlation among each of ges.age with βhCG and PlGF P <0.05.

Conclusion: This study suggest that the significant role of Glycodeline-A,PlGF in etiologies of first trimester pregnancy failed and some of them was related to βhCG in this time and the age of patients wasn't effect on all of these parameters.

Keywords: *βhCG, Glycodeline-A,PlGF*

Introduction

The most important basic cell differentiation of fetal occur during the first three months of pregnancy , so any complication done to the fetus during this period is leading to result represent miscarriage or serious complications According to the first three trimesters of pregnancy[1] , fallopian tube delivered the egg which is fertilized and attaches to the inside of the woman,s uterus , where it begins to configure the embryo and placenta , mudding of gestational age can be occured[2] , women think of pregnancy as a a nine months lasting , this is true when that the women lasting nine months , while the measured of this is begin with the first day of the last period for them and about three to four weeks before they are become pregnant , pregnancy usually lasts about fourty weeks for pregnancy age this period is about ten months[3], Miscarriage defined as a failed or loss of pregnancy before twenty weeks from the first day of last period and it is known as pregnancy loss and spontaneous abortion[4] , it is the death naturally of fetus before it is able to life independently , the first trimester of pregnancy starts from the first day of last menstrual period and end in the twelve weeks of pregnancy , miscarriage is common at this period[5], Blood investigations and genetic study , must be doing if a planning to manage the woman which has more than two losses of her carry or more in a situation identified as recurrent miscarriage , many procedures may be used to detect the reason of repeated miscarriage include ultrasound of pelvic , hysterosalpingogram which is include x-ray of the uterus tubes and uterus itself , and hysteroscopy which is include views interior of the uterus,[6] The first trimester pregnancy loss is common , depending on the (ACOG), it happens in ten percent of confirmed pregnancies , the cause of a miscarriage sometimes will remain unknown,

however , the (ACOG) decide that about fifty percent of miscarriages are chromosome issues abnormality , the miscarriage risk definitely increases with age[7] , according to the (ACOG) , the miscarriage risk is twenty percent at age thirty five , and increased to fourty percent at age fourty and increased further to eighty percent at age fourty five [8], the main type of miscarriages is unprompted abortions which characterized by unprotected , they are unexpected effects which are differ from recur , high percent of first and second trimester pregnancy loss are due to chromosomal abnormalities , and the other are include many problems affect these carries like infected disease[9] , uterus and cervix problems , smoking , toxins exposure , harmful substance abuse , high level of glucose , problems of thyroxin hormones secertion , harmful disease of autoimmune , advanced age of women which carry are more miscarry than lower subjects[10] , sometimes physical trauma accident may cause failed these carries , at common knowledge , the main cause for these results of disadvantage carry is obscure, By tissue of trophoplast created of βhCG[11], component of fetus existing at early time of pregnancy and represent a placenta portion , determining of this substances can be helpe in differentiate a normal or abnormal carry , and can be using in the prognose of failed carry , an advantage for determining of hCG in a various tumors for womb malignancies,while Glycodelin - A produced from decidual glandular epithelium and endometrial glands , get its effect and supply in the early pregnancy[12] , GdA contribute in primary placental grow by its involving supply on trophoplast and immune units, PlGF is a homodimeric growth factor that contributes with VEGF , it can form heterodimers with some types of VEGF and inhibit the angiogenic effect of VEGF on VEGF R2 , blood circulating PlGF levels increase through pregnancy , reaching a peak in second trimester of gestation , this increase is weak in preeclampsia[13] , PlGF stimulates monocyte effect and migration as well as lead to produced of inflammatory cytokines and VEGF, these activities facilitate bone fracture , wound , and cardiac repair , but also contribute to inflammation in active sickle cell disease and atherosclerosis , PlGF can also decrease TIMP3 expression in the spleen , leading to triggering of immune in hypertension[14] .

Aim of the study: to estimate the scale of Glycodeline-A , PlGF together with βhCG as prognostic markers of first trimester pregnancy loss in Iraqi women.

Method

Study design: The present project was perfected at the department of Biochemistry , Medicine College , Baghdad University , at Hospital of Baghdad Teaching through the time from April 19 to March 2020 , it included 85 pregnant woman identified the first day of LMP who are confirmed positive pregnancy test in blood , and established to have had pregnancy by Consultant Gynaecologist and matching age 21 – 35 year and same time 8 – 12 week of pregnancy and encountered during their attendance the Gynaecology consultation clinic at Baghdad Teaching hospital , encountered these women until the end of week 12 of pregnancy , 40 of them who have pregnancy loss at the prognosis period are patients and the other 45 healty control who completed the same period without problems , a written consent was applied by each patient and an ethical approval had been suitable the researcg based of the Medicine College , Baghdad University and the link with Ministry of Health and enviroment.

Data Setting: Eight to ten milliliters (mls) of blood from subjects are draw out and transferred into plain tube , allows for half hour to sure that is clotting , after this , the specimen must be centrifuged to separated the needed serum at 2500 rpm for limited time roughly ten minuted and stored at freezing degree about – 20 Co up to beginning of the assay the project biochemical markers : Beta HCG , Glycodeline-A , sFlt-1 , PlGF By ELISA method .

Inference by statistics: Inference by statistics is a method that analysis of details by using SPSS , this information were putting as simple measures of variants (mean , standard deviation and others) , qualitative detailes which are significant statistically were using student-t-test for compare between two independent means or ANOVA test for compare between different qualitative detailes on , the statistical significance is equal or less than 0.05 , the coefficient of correlation value r : is a kind of coefficient correlation clarify the connection between two different factors that are calculated on the same period , r may be positive as direct correlation or negative as inverse correlation.

Results

Explane by table scales of of various markers and

their correlations according to the study assessment .

Table 1: Results and their obtained correlations for all markers in this study

Group Statistics

	VAR00013	N	Mean	Std. Deviation	Std. Error Mean
Age cAge	1.00	40	27.1250	4.18292	.66138
	2.00	45	27.2444	4.11293	.61312
GestAge c GestAge	1.00	40	9.6000	1.29694	.20506
	2.00	45	9.5333	1.09959	.16392
BetaHCG cBetaHCG	1.00	40	1024.7740	459.39406	72.63658
	2.00	45	2309.9409	3007.11697	448.27453
GlycodeinA cGlycodlinA	1.00	40	70.9447	56.87327	8.99245
	2.00	45	193.7416	200.56648	29.89868
PIGF CPIGF	1.00	40	141.4350	284.10843	44.92149
	2.00	45	340.2947	387.01779	57.69321

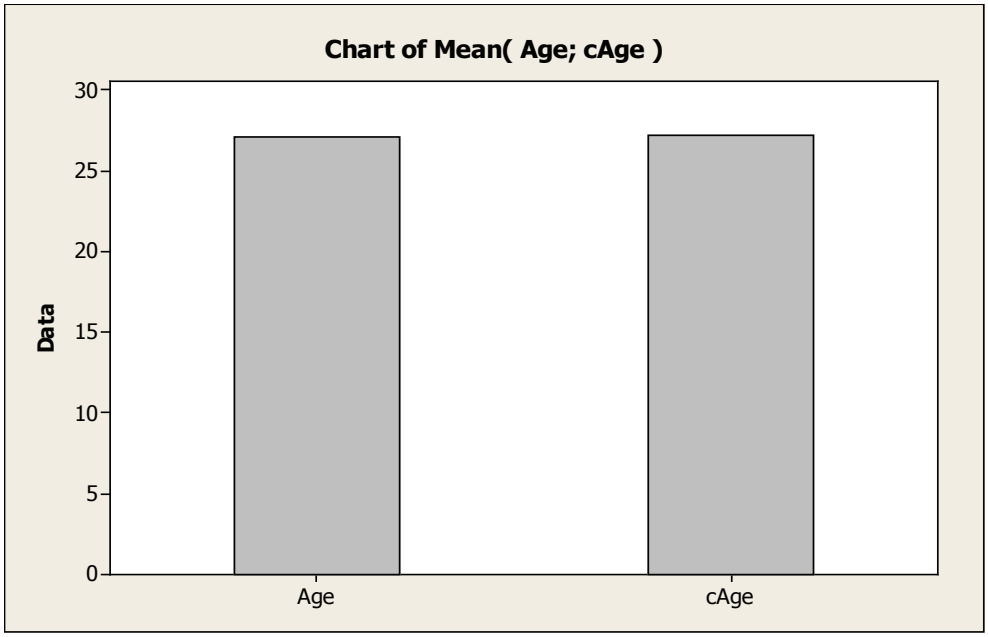


Figure1:Chart of mean (Age , cAge).

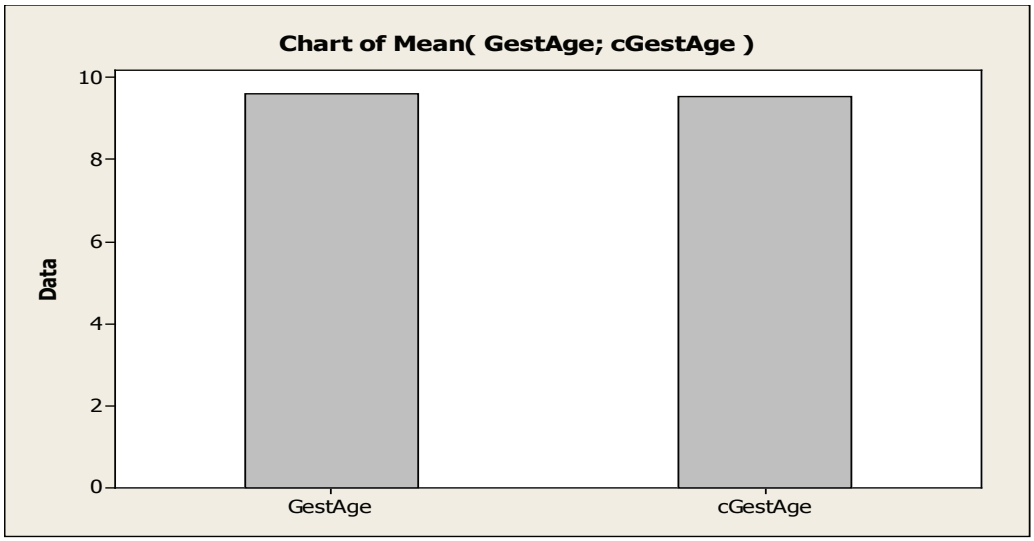


Figure2:Chart of mean (Gest. age , cGestational age)

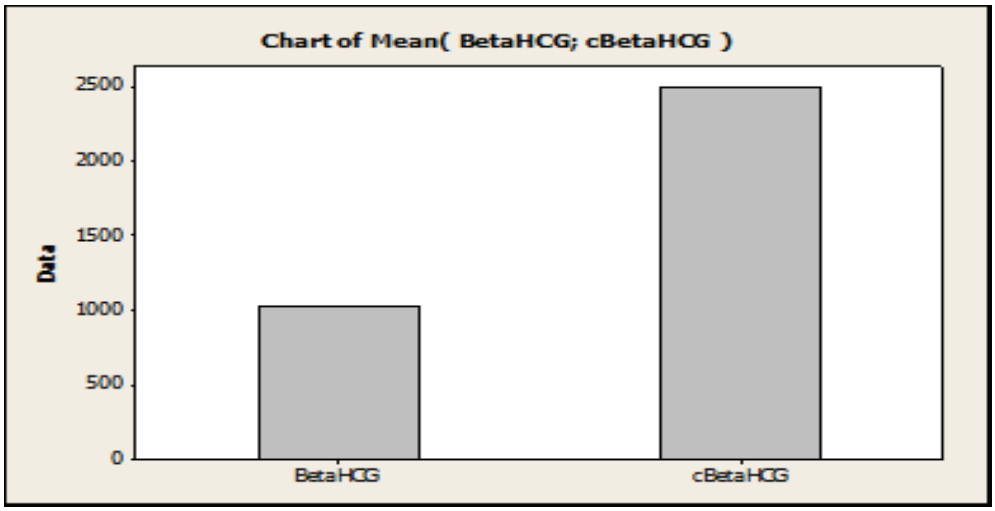


Figure3:Chart of (βhCG, cβhCG)

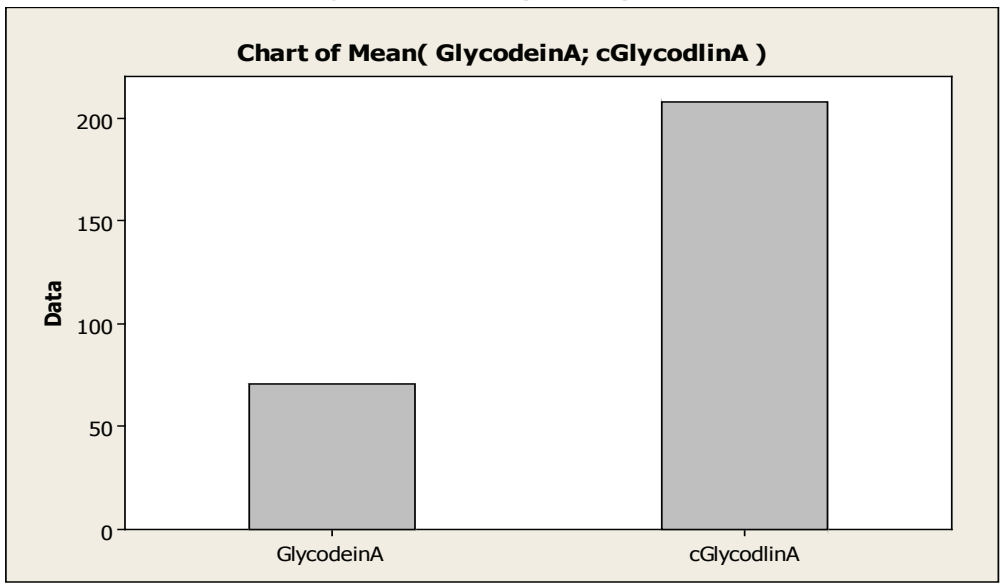


Figure4:Chart of (Glycodelin-A , cGlycodeline-A)

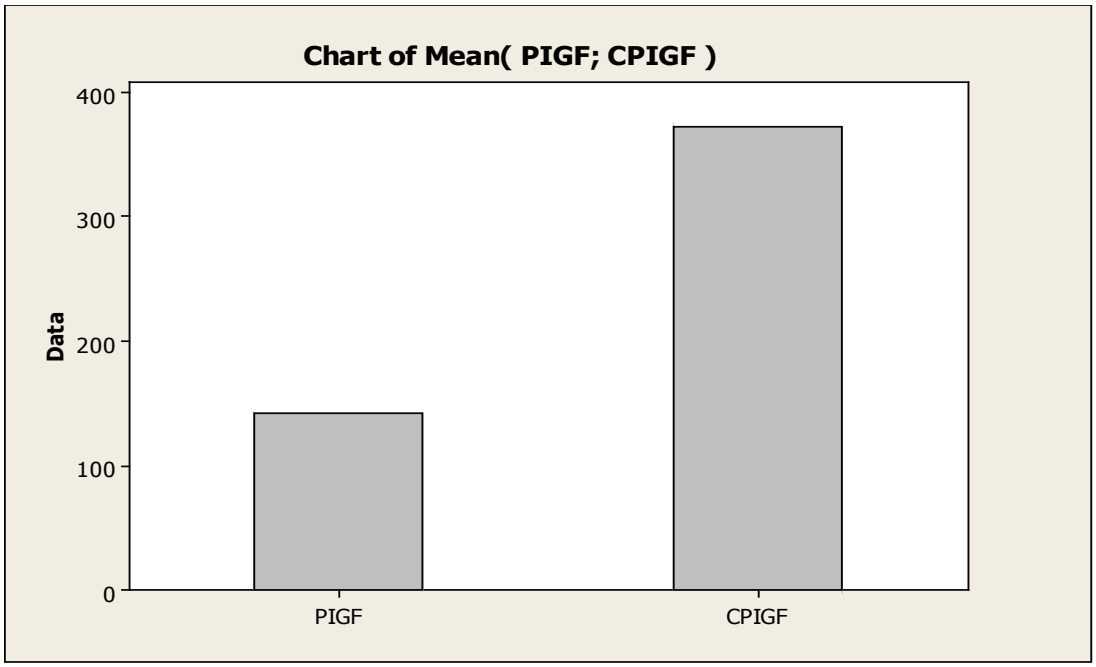


Figure6:Chart of (PlGF , cPlGF)

Discussion

Serum level of βhCG in healthy control group (mean ± SD 2309.940 ±3007.116) in patients which have abortion (1024.774 ±4 59.394) which show significant correlation : P value 0.05 , that mean the level of this marker in patients was lower than in healthy control , The present study shows that the serum level of glycodelin-A in healthy control group (mean ± SD 193.741 ± 200.566) , in patients which have abortion (70.944 ± 56.873) which show significant correlation : P value 0.01 , that mean the level of this marker in patients was lower than in healthy control , The present study shows that the serum level of PlGF in healthy control group (mean ± SD 340.294 ± 387.017) , in patients which have abortion (141.453 ± 284.108) which show significant correlation : P value 0.05 , that mean the level of this marker in patients was lower than in healthy control , most of previous projects have found that the chromosomal agents are effective factor in carry loss[15] , old studies have demonstrated that the normal carry depends on normal immune system which is consist of immune defence , immune response , cytokines[15] , the immune effect is important factor in first carry failed , the immune units that establish at the mediater of placenta and the uterus were stay overlap layer of arrangment by immunity system of mother , this process may help of placental growth but some times reduce the placental aggress the embryo , in the time of implantation natural killer units go to the uterus and help the stimulation secrete of cytokines that inhibit the trophoblast aggress[16].

Conclusion

levels of all markers according to the result which obtained from the assessment of them are clarify the relation between patients which have abortion in the first trimester and healty control , these results suggest for βhCG: level of this marker in patients was lower than in healthy control,and for Glycodelin-A: level of this marker in patients was lower than in healthy control,and for PlGF: level of this marker in patients was lower than in healthy control .

Ethical Approval: Describe written consent was applied by each patient and an ethical approval had been suitable the researcg based of the Biochemistry Department,Medicine College, Baghdad University and the link with Iraqi Ministry of Health .

Funding Sourse: making of this work depend on authors only.

Conflict of Interest: No

Source of Funding: Self funded

Ethical Clearance: Not Required

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Analysis of Single Nucleotide Polymorphism in *Fim H* Gene from Uropathogenic *E.coli*

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Abstract

Background: Urinary tract infection is defined as presence of microbial pathogens in the urinary tract with associated symptoms. The infection affects both lower and upper urinary tracts and is known as acute cystitis and polynephritis respectively. This study done to detect the variation of single nucleotide in FimH gene and its correlation with the pathogenicity of uropathogenic *Escherichia coli* caused the urinary tract infection.

Materials and Methods: The study group included 150 patients with Urinary tract infection. There were 120 (80%) female and 30 female (20%) their age ranges between (5-55) years, seen in Al-Hussein Teaching Hospital for the period from January 2019 to March 2019. The dominant form (48.39%) of patients with urinary tract infection caused by UPEC was found in the age group of 24-39 years old. Urine samples were collected from the patients. Bacterial isolation was identified by microscopic examination and culture characteristic on selective media MacConky agar, Eosin Methylene Blue and Chrome agar. Then the isolated were diagnosis by Vitek®2 system.

Results: Bacteriological results showed that 62(41.3%) isolates were characterized as *Escherichia coli.* Genomic DNA was extracted from the bacterial culture for further molecular study to reveal C640T and T591A fimH gene Polymorphism. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used for this purpose and digestion of the amplified DNA products by ScarFI1390I and BsrI endonuclease respectively gave fragments with different molecular sizes which express certain genotypes. This study detected that the prevalence rate of single nucleotide polymorphism C640T FimH was (85.48%), endonuclease enzyme only affects the C allele sequence and it does not have any influence on the T allele sequence. The prevalence rate of single nucleotide polymorphism T591A fimH gene was (80.64%), endonuclease enzyme only affects the T allele sequence and it does not have any influence on the A allele sequence.

The result show only 62 isolates (41.3%) were given typical morphological characteristics and biochemical tests related to *E. coli*, while the rest 88 isolates (58.6%) belonged to pathogenic bacteria from different genera.

Keywords: *E.coli , Fim H Gene, UTIs; C640T and T591A SNPs; PCR-RFLP*

Introduction

Escherichia coli is a ubiquitous bacterial species commensal of humans and warm blooded animals. Nevertheless some strains have evolved the capability to cause both intestinal and extra intestinal illnesses (1). It is categorized into two foremost categories, enteric *E.coli* (ECEC) that mostly causes infections restricted to the mucous lining of the intestine and extra intestinal pathogens (ExPEC) which have the capability of spreading from the intestine and inflicting infections in

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other parts of the body (2)

Urinary tract infection (UTI) is one of the most widespread infections in humans which causes extreme morbidity and considerable expenses (3). UTIs have an effect on different components of the urinary tract and are classified into a number disease corporations based on infection sites. UTIs are classified into cystitis (the bladder), pyelonephritis (the kidney) and bacteriuria (the urine) (4).

Urinary tract infection are induced by means of each Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most frequent causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in occurrence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* spp.(5). For complicated UTIs, the order of occurrence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae*, *Candida* spp., *S. aureus*, *P. mirabilis* and *P. aeruginosa* (6)

Uropathogenic *Escherichia coli* (UPEC) strains are regarded as a primary pathogen in about 80% of patients with UTIs (7). Successful institution of infection by UPECs wants specific cascade of events which includes adhesion to host cells colonization of tissues and in certain cases, cellular invasion. These events include different cellular and molecular pathways which rely on the presence of multiple virulence factors (8). UPEC has many virulence factors that assist its colonization, invasion, and survival within the host urinary system. These factors include adhesins, siderophores, toxins, capsule production, and proteases. In addition, several autotransporter (AT) proteins, which are typically associated with phylogeny (9), are correlated with virulence and have been identified in UPEC (10).

Methods and Materials

The current study was conducted on 150 patients (120 females, 30 males) were seen Al-Hussein Teaching Hospital from January 2019 to March 2019. The patients were diagnosed clinically by physician as having UTI infection . Urine samples were taken from both male and female using sterile containers. The samples were directly activation on Brain heart infusion broth and then streak on MacConkey agar, Eosin methylene blue agar , Chrom agar and incubated at 37° C for 24 hrs .

Primary identification of E. coli isolates was based on cultural, morphological characteristics, microscopically diagnosis , while the confirmatory identification test was based on selective Chrome agar medium and according to Vitek®2 system. After activation on Brain Heart Infusion broth at 37◦C for 24 hours Bacterial colonies developed on plate agar, were studied; on MacConkey Colonies appeared as a deep purple colour due to lactose fermentation. It is also appeared as circular, flat and moist with entire margin on this medium. MacConky agar is a selective plating medium used for the isolation of *Enterobacteriaceae* and related to gram negative rods. Lactose fermenting bacteria produce colonies that have varying shades of red due to conversion of neutral red indicator dye below pH 6.8 .

Genomic DNA from culture cell were extracted by using Favorprep Genomic DNA extraction kit (blood , culture cell) favorgen.

from 62 DNA samples of UPEC were subjected to a molecular detection by PCR amplification of the *fim*H gene using a specific primer, 53 isolates (85.48%) gave positive results of C640T fimH gene and 50 isolates (80.64%) gave positive result of T591A fimH gene. *fim*H gene were detected in 91.07% of UPEC isolates among 112 UPEC isolates obtained from outpatients suffering from UTIs in seven Iraqi hospitals. The high frequency of fimH gene in all studies relating to UTIs indicates the critical roles of this virulence factor in *E. coli* pathogenesis.

Distribution of fimH gene C640T polymorphism was detected by PCR-RFLP technique, the enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths figure (1) , and the enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths for the T591A fimH gene figure (2)

Result and discussion

Genomic DNA Extraction

The DNA is extaction from bacterial culture samples. Genomic DNA was extacted and submitted to agarose gel electrophoresis and estimated concentration and purity of each sample,samples with low value were re-extracted . figure(1).

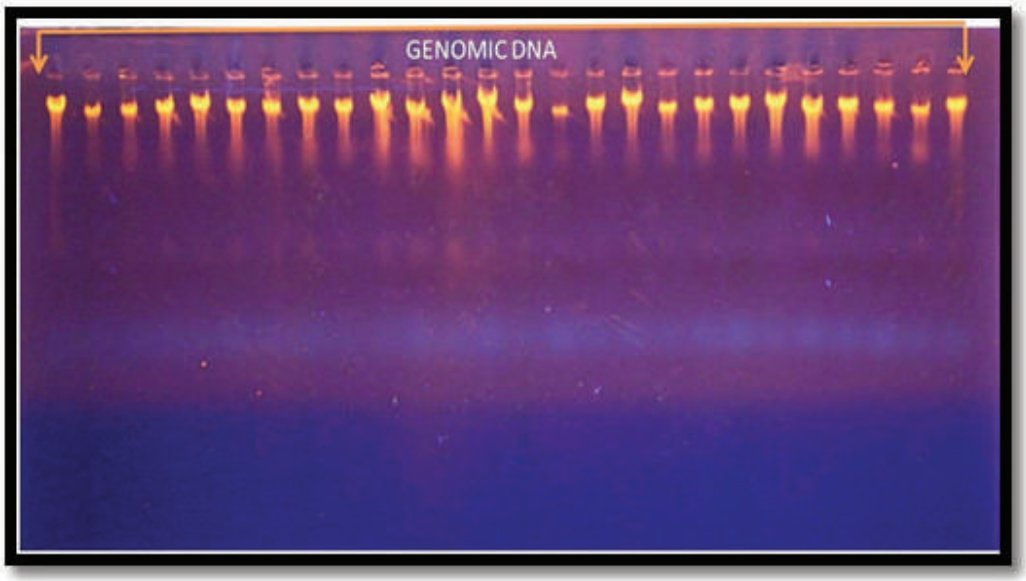


Figure (1) : Ethidium Bromide stained agarose gel electrophoresis appearance that displays Genomic DNA that was extracted from uropathogenic *E. coli*

DNA Amplification

The products of successful binding between the extracted DNA and specific primers for C640T and T591A fimH gene were detected by gel electrophoresis analysis using DNA marker and the products size was 164 bp and 76 bp respectively . Figure (2)

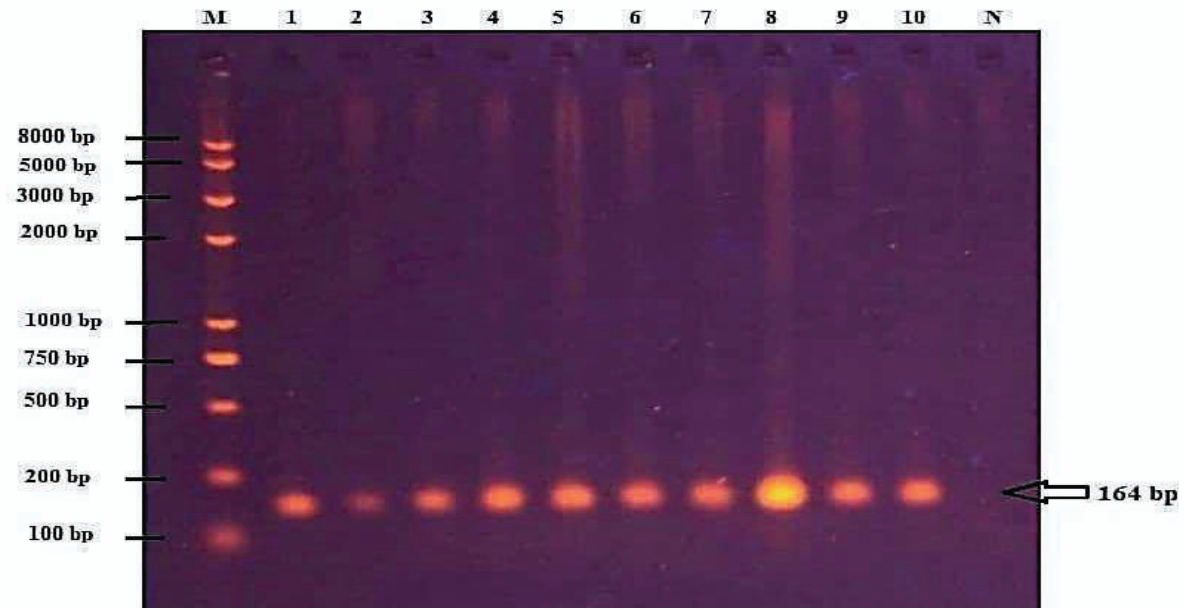


Figure (2) : Gel Electrophoresis of PCR Amplified 164bp of C640T fimH gene. Lane (M):DNA molecular size marker, Lane (N): negative controls

Detection of Genes Polymorphism

Distribution of fimH gene C640T polymorphism was detected by PCR-RFLP technique, the enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths figure (3) , and the enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths for the T591A fimH gene figure (4), these results agree with the reports of other studies such as (Molaie *et al.,* 2016)

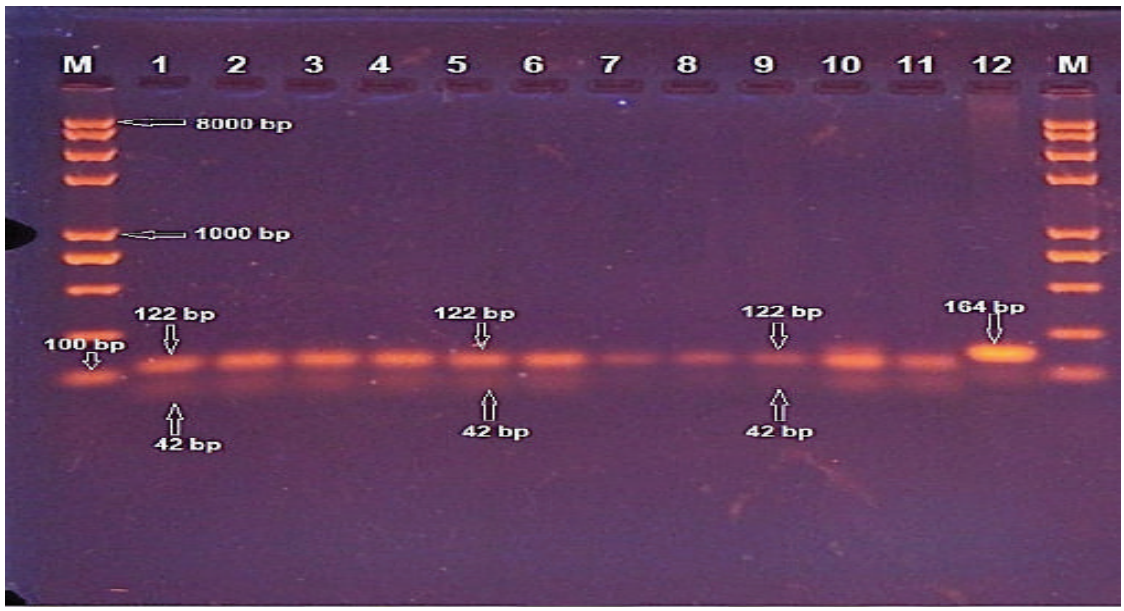


Figure (3) : Genotyping analysis of C640T SNP. ScarFI1390I enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths. . lane M: Marker (8000-100 bp); lanes (1-11) digested PCR products, lane 12 un-digested PCR products;1% agarose gel

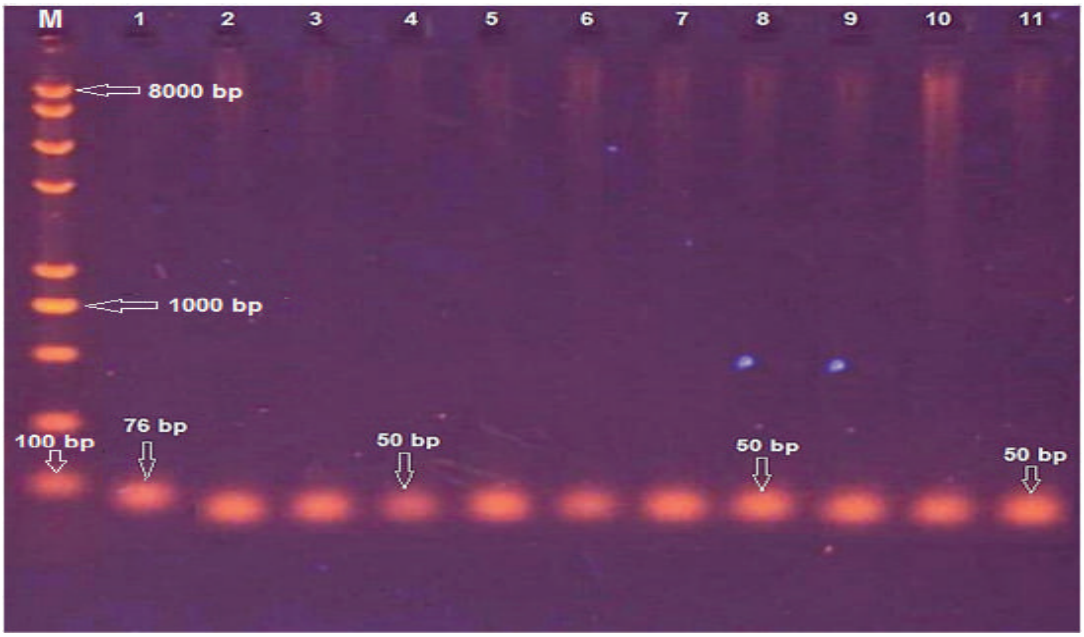


Figure (4): Genotyping analysis of T591A SNP. BsrI enzyme digested the 76bp fragment PCR product in T location and led to the creation of fragments with 50 and 26 bp lengths. The 26 bp fragment was removed from gel because of the small size of fragment . lane M: Marker (8000-100 bp);lane1 un-digested PCR products ,lanes (2-11) digested PCR products.

Distributions of FimH Genotypes and Alleles

Specific amplification of fimH gene, consist of C640T and T591A polymorphic site results in a 164 bp and a 76 bp PCR products, respectively. Genotyping was accomplished using the ScarFI1390I and BsrI restriction enzymes for C640T and T591A SNPs, respectively. ScarFI1390I enzyme only affects the C allele sequence and it does not have any influence on the T sequence. This enzyme digested the 164bp fragment PCR product in C location and led to the creation of fragments with 122 and 42 bp lengths. Table (1). Relating to T591A SNP, the T allele was affected by BsrI restriction enzymes and led to create 50 and 26 bp fragments. Table (2).

Table 1:Genotype of C640T and T591A polymorphism.

Total Number of samples	polymorphism	Genotype	n	percentage %
62	C640T	CC CT TT	53 0 0	85.48
62	T591A	TT TA AA	50 0 0	80.64

Table 2 : allelic frequency of C640T and T591A polymorphism .

Total Number of samples	polymorphism	Allelic frequency	n	percentage %
62	C640T	C T	53 0	85.48
62	T591A	T A	50 0	80.64

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Conflict of Interest: Non

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The Impact Of Child Abuse and Neglect on Oral Habit among 10-11 Years Old Primary School Students in Baghdad /Iraq

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Abstract

The purpose of this study was to assess the impact of child abuse and neglect on oral habits among 10-11 years old primary school students in Baghdad/Iraq. This observational study included 600 students distributed to 373 male and 227 female at different economic and social levels with age 10-11 years old of fourth and fifth class of the elementary schools in the Karkh city. Information on child abuse and neglect and presence or absence of oral habits was obtained from a structured, self-administered questionnaire from the students who were willing to participate in the study and their parents. The data was collected, summarized and statistically analyzed. The child abuse and neglect had effect on presence of oral habits in children.

Keywords: *child abuse, oral habits; health of children; male; female.*

Introduction

Child abuse and child maltreatment had been defined by The World Health Organization (WHO) as "all forms of physical and/or emotional ill-treatment, sexual abuse, neglect or negligent treatment or commercial or other exploitation, resulting in real or potential harm to the child's health, survival, growth or respect in the context of a relationship of responsibility, trust or power" [(1)].

The periodic absence of responsive care damages the development of the brain which uses science to discuss that neglect can give rise to even more harm to a young child's growth than physical trauma, largely because of the significant consequences of persistent high stress as they had been found [(2)].

Nail biting had been described by Williams in 2010 as a nervous habit often related with anxiety, boredom and defeat or working on difficult mission [(3)]. Many studies had stated that Nail biting will be one of warning signs of child maltreatment as self-calming behaviors as a result of anxiety, stress, terror, pain, sadness, tension, seclusion and inactivity [(4- 9)]. Another etiology of nail biting was reported by Schuessler and Collin may be related to nutritional deficiency which is considered a result of child abuse and neglect, if eating in depressed or stressful environment, the body may not produce enough acid to digest protein properly; thereby, and undigested peptides pass through the system and may share in mental problems [(10)].

Digit sucking is a very common habit and is a healthy, normal and natural for infants, occurs in children and can continue through adolescence and sometimes even adulthood. Studies were reported that thumb sucking is a way of relaxation for many children and they usually suck their thumbs when they are bored, stressed, anxious, or nervous or due to developmental problems and environmental disturbances [(11, 12)]. Bishara and Samir in 2001 suggests that children who have some underlying emotional or psychological disorders will develop and continue sucking habit for a long period after age of three years [(13)]. If not well controlled, can have harmful effects on dentitions and oral health like malocclusion [(14)], apical root resorption [(15, 16)], temporomandibular disorders [(17)], gum injuries [(18)],

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anterior open bite, increased overjet, and Class II canine and molar relationships according to duration, frequency and intensity [19].

According to current knowledge, there is no previous Iraqi study concerning the relation between child abuse /neglect and occurrence of bad oral habits among children in Iraqi populations. For all the above this study was administrated to a random sample at public schools in order to investigate how child abuse and neglect are lead to bad oral habits.

Materials and Methods

This observational study was conducted among 600 students with age range 10-11 years of the fourth and fifth grade primary schools in al Karkh district of Baghdad city over period of 4 months from December 2018, to the beginning of May 2019. Ethical approval was gained from the ethical committee at the college of dentistry /university of Baghdad.The measuring tool of child abuse in the current study was Glasser criteria [20], that designed a scale for recording and measuring child abuse depending on Likert method consist of 44 question [21]. Changes in questionnaire were performed in current study to 19 questions. Firstly after taking acceptance and consent form from their parents, told the students what the researcher will do and what he/she is doing and that all their data for scientific research purposes only and with no relation to their academic evaluation, with complete confidentiality guarantee, explain and writing the scale and provide an example of what they are doing on the blackboard and give full freedom for students when answering and sometimes exclude the teacher from the class. The students were asked to by (yes) or (no). After collecting the degrees for each case sheet was been answered by students, some answers were given reverse degree and then the final degree obtained for each child. According to Likert the levels of child abuse was mild if the degree (0-3) consist (160) child with percentage of (27%), moderate if the degree (4-7) consist (260) child with percentage of (43%) and sever if the degree (8-14) consist (180) child with percentage of (30%) depending on the maximum degree of the questions which was (19) degree for each paper of the questionnaire.

For assessment of oral habits (thumb sucking and nail biting) in groups of study another questionnaire was given one copy for the student and another copy was sent for their parents. No technical terms or medical terms are used in the questionnaire for the student and parent to avoid a high incidence of uncertainty or no opinion responses. Therefore, simple words and plain language were used in the questionnaires. Questions in a questionnaire were easy and pleasant to answer to encourage respondents to continue and complete the survey [22, 23].

Data was statistically analyzed using SPSS version 24 and the following statistical tests were carried out: frequency and percentage for normal variable.

Results

The distribution of sample according to level of abuse by gender group is present in table 1. This table shows that the occurrence of abuse was higher in male (62.2%) than in female (37.8%). In male, sever level of abuse (31%) was higher than mild level (24.9%) while in female mild level (29.5%) was higher than sever level (27.7%).

Table 1: distribution of sample according to level of abuse

Level of abuse	Gender				Total
	Male		Female		
	No.	%	No.	%	
Mild	93	24.9	67	29.5	160
Moderate	164	44%	97	42.7	216
Sever	116	31	63	27.7	179
Total	373	62.2	227	37.8	600

Only 590 students participated in the present study for oral habit as the other were excluded due to incomplete data. The distribution of sample according to thumb sucking by gender is shown in table 2. This table shows that the percentage of thumb sucking was (6.6%) more among female than among male (2.7%).

Table 2: The occurrence of thumb sucking habit by gender.

Gender	Thumb sucking presence		Total
	No.	%	
Male	10	2.7	364
Female	15	6.6	226
Total	25	4.2	590

The distribution of sample according to level of abuse by thumb sucking is presented in table 3. The percentage of thumb sucking consist (4.2%) from total sample. The higher occurrence of thumb sucking was in moderate level (5.5%) and the lowest occurrence was in mild level of abuse (3.1%).

Table 3: Distribution of sample according level of abuse by Thumb sucking habit.

Level of abuse	Thumb sucking		Total
	No.	%	
Mild	5	3.1	160
Moderate	14	5.5	254
Sever	6	3.4	176
Total	25	4.2	590

The occurrence of nail biting habit by gender is shown in table 4. This table illustrates the percentage of nail biting habit was (22.2%) more among male than among female (17.7%).

Table 4: The occurrence of nail biting habit by gender.

Gender	Nail biting		Total
	No.	%	
Male	81	22.2	364
Female	40	17.7	226
Total	121	20.5	590

The distribution of sample according to level of abuse by occurrence of nail biting habit is presented in table 5. This table shows that the percentage of nail biters composed (20.5%) from the total sample. The higher percentage of nail biting occurrence was at sever level of abuse (22.2%) and this percentage of occurrence decrease with decreasing level of abuse and so the lowest percentage of nail biting occurrence was at mild level of abuse (18.7%).

Table 5: the distribution of sample according level of abuse by nail biting habit.

Level of abuse	Nail biting occurrence		Total
	No.	%	
mild	30	18.7	160
moderate	52	20.5	254
sever	39	22.2	176
Total	121	20.5	590

Discussion

The most striking findings of current study are that emotional and behavioral problems are more common in children with nail biting and thumb sucking than those without these habits. Both habits were higher in sever level than mild level of abuse and this explained by the association of oral habits and children's emotional and behavioral problems. In addition, the results added to literature that occurrence of thumb sucking was higher in female than male while for occurrence of nail biting was higher in male than female.

The result of present study showed that thumb sucking is more occurrence among moderate than mild grade of abuse , this was agreeing with the studies which reported that association of digit sucking and psychological or emotional stress of child as relaxant behavior [11, 24]. Finding of other studies was agreeing with current study that reported emotional disturbances as etiology of thumb sucking [12], and Pleasure derived from sucking as a result of stress, depression and anxiety [25]. Previous researchers suggests continuous thumb sucking in children above 3 years due to psychological disturbances [13].

The data of present study illustrates that the nail biting was more occurrence among sever grade of abusing ,this was in line with other study used The Strengths and Difficulties Questionnaire (SDQ) revealed that children nail biting related with higher rates of emotional problems than children without in school age children [26]. Nail-biting allows children relieve their anxiety, loneliness, and deprivation of safety feeling and love [27]. The correlation of nail biting and aggression that found in present study has been reported in other study before [28]. Psychological imbalances, such as stress, anxiety and nervousness, were the principal cause of nail biting in children [23]. Many researchers while explaining the cause of this habitual disorder have referred to psychological and psychiatric issues and nail biting is suggested to be reflective of underlying psychopathology [8]. As an explanation for their relationship, early researchers also considered nail biting as a stress-relieving oral habit [29-31].

Conclusion

Child abuse have an effect on oral habit as oral habit was higher in higher level of abuse than lower. However gender had an effect on the occurrence of habit.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Genetic Study of the Effect of the *Toxoplasma gondii* on Somatic Cells of the Bone Marrow in Female Mice and a treatment Attempt by *Laurus Nobilis* Leaf Extract

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Summary

This study aimed to find out the ability of T. gondii parasite to induce genetic changes in the bone marrow cells of female mice and to try therapeutically for it using bay leaf extract. The parasite was extracted from (23) placentas for aborted women and injected into the 42 mice with an average weight of (20-30) gram. It was divided into six groups at a rate of (3 females) per group. The first group doses the oil only as a negative control, the second group was infected with the parasite as a positive control, while the four study groups dose the plant extract of the bay leaves for (14) days and at doses of (15, 10, 5, 1) mg. for Kg after a period of infestation with the parasite for 14 days. The results of the chromosomal changes showed the type of chromatid fracture among the females injected with the parasite + the extract and with all its concentrations significantly reduce the level at ($P < 0.05$). The concentration was 15 mg/kg, the lowest as the results reached (9.33 ± 1.33) compared to the control of the parasite (44.00 ± 2.30), while the centromere fracture showed All concentrations showed a significant decrease at the level of ($p<0.05$) and showed concentrations 15 and 10 more decreases with a result of 2.66 ± 1.33 for both concentrations compared to the control of the parasite, As for the annular chromosome, all concentrations showed a significant decrease at the level of ($p<0.05$) for females injected with the parasite extract, and the concentration was 15 mg/kg, the lowest was (6.66 ± 1.33) compared to the control of the parasite.

Keyword*: Toxoplasma gondii parasite, somatic cells, mice, bone marrow, Laurus nobilis leaves.*

Introduction

Toxoplasmosis is a serious disease common to humans and animals. The main cause of it is a type of parasitic protozoan. *Toxoplasma gondii* infects all warm-blooded animals and humans those are intermediate Hostesses. The final host and the main source of infection are cats [(1).] A complex life cycle of the parasite is one of the main reasons for its success, as it depends on sexual and non-sexual reproduction, which includes multiple hosts [(2) (3).] Infection occurs through food contaminated with egg sacs or meat contaminated with tissue sacs or by contact with cats or the environment contaminated with its infectious phases [(4).] There are three types (Type I, II and III) depending on the genotype of the parasite, which differs in virulence and epidemiology, where strains of type I that grow rapidly in the laboratory are more virulent in mice and are often associated with visual toxoplasmosis, As for type II, it is not virulent in mice, but it is more common in humans in Europe and North America, while type III infect animals more unlike the types, I and II where humans are mainly infected [(5)] and since centuries ago. Herbal medicine has been considered as a major source of prevention and treatment [(6).] Laurus nobilis bay leaves have been used in the pharmaceutical industry, as it is entered in the composition of some medicines, as it has anti-microbial and pathogenic activity and is an antibiotic against fungi and viruses [(7)(8).] The current study aimed to know the genetic changes caused by the parasite in the bone marrow cells of mice and the use of the baby plant as a therapeutic attempt to reduce the damage caused by the toxoplasma parasite.

Material and Methods

Experimental design:

Initially, the parasite was extracted from (23) placentas from aborted women and used to inject and infect mice. (42) mice were used with an average weight of (20-30) gram, as they were divided into six groups at a rate of (3 females) in each group. Dosage identified (LD50) about the plant extract of bay leaves and giving a dose of (0.5, 1.0) milliliter of the parasitic suspension of *Toxoplasma gondii*. the groups were dosed as follows: The first group (oil control): This group was treated with oil only without the plant extract and without being infected with the parasite. The second group (parasite control): This group was infected with the parasite only and this group was not treated with oil or plant extract. The third group included infection with the parasite and treated with the plant extract at a concentration of 1 mg/kg of BW. Fourth group: This group was infected with the parasite and then treated with the plant extract at a concentration of (5) mg/kg of animal weight. Fifth group: This group was infected with the parasite and then treated with the plant extract with a concentration of (10) mg/kg of body weight of the animal. The sixth group: This group was infected with the parasite and then treated with the plant extract with a concentration of (15) mg/kg of body weight for the animal.

Test of chromosomal changes in somatic cells of bone marrow:

The animals were injected into the cell membrane (0.5) ml of the solution of the colsagen, and after an hour and a half to two hours after the injection of the colsagen, the animals were sacrificed after Anatomize the mouse, holding the femur with forceps, cleaning the muscle residue, and then washing the bone marrow with a 0.9% normal slain until it became white, After being placed in test tubes and in a centrifuge at a speed of 2000 rpm, the suspended was removed and the precipitate was left and KCL solution was added to it on the tube walls with continuous shaking and put in a water bath at 37 ° C for 20-25 minutes and taken out and placed again in the centrifuge then After that the glass slides were prepared and after sterilization and cleaning it was placed as sloping Drops of it were dropped from the solution containing the cells vertically in order to allow the nuclei and chromosomes to spread, and then the glass slides were kept in the refrigerator for the purpose of examining them with the oil lens of the optical microscope.

Result and Discussion

The results showed the presence of chromosomal changes, that included (chromatid fractures, centromere fractures and annular chromosomes) in females of mice injected with toxoplasma parasites for a period of (14) days which reached (44.00 ± 2.30, 9.33 ± 1.33 and 14.66 ± 1.33) respectively. In addition, a significant difference at the level of $P<0.05$ compared with the oil control group which amounted to (9.33 ± 1.33, 2.66 ± 1.33, 6.66 ± 1.33) respectively. It also showed a significant decrease at $P <0.05$ in the structural chromosomal changes that included (chromatid fractures, centromere fractions, and annular chromosomes) in the interference groups injected with the bay leaf extract and with all its concentrations with the parasite compared with the group injected with the parasite alone as the table (1).

Table (1) showing the mean Values of differences for female chromosomal changes for the study groups

changes chromosomal			Natural chromosome Mean ± SE	Group
annular chromosomes Mean ± SE	centromere fractures Mean ± SE	chromatid fractures Mean ± SE		
6.66±1.33 B	2.66±1.33 B	9.33±1.33 DC	81.33±2.66 A	oil control
14.66±1.33 A	9.33±1.33 A	44.00±2.30 A	32.00±2.30 D	parasite control
18.66±1.33 A	5.33±1.33 BA	22.66±1.33 B	53.33±1.33 C	1 mg / kg
8.00±0.00 B	6.66±1.33 BA	21.33±1.33 B	64.00±2.30 B	5 mg / kg
6.66±2.66 B	2.66±1.33 B	13.33±1.33 C	77.33±2.66 A	10 mg / kg
6.66±1.33 B	2.66±1.33 B	9.33±1.33 DC	81.33±1.33 A	15 mg / kg

As for the chromatid fractures, it also showed that all the interference treatments injected with the extract of the bay leaves of different concentrations are 15, 10, 5, 1 mg/kg of body weight with the parasite. And there was a significant decrease in it (22.66 ± 1.33, 21.33 ± 1.33, 13.33 ± 1.33C, 9.33 ± 1.33) respectively compared to the control of the parasite, as the chromatid fractures in it reached (44.00 ± 2.30) and the concentration was 15 mg/kg which is the largest Reducing chromatid fractures. As for the centromere fracture, the results showed that all the interference treatments injected with the extract of the bay leave with the parasite had a significant decrease, as they reached in different concentrations, which are 15, 10, 5, 1 mg/kg of body weight (5.33 ± 1.33, 6.66 ± 1.33, 2.66 ± 1.33, 2.66 ± 1.33) respectively. Comparing to the parasite control group as the centromere fraction reached (9.33 ± 1.33), and the 10 and 15 mg/kg concentrations were the most influential in reducing the centromere fracture.

Concerning the annular chromosome, it showed that all the interference treatments injected with the extract of the bay leaves with the parasite had a significant decrease. as it reached in different concentrations, which are 15, 10, 5, 1 mg/kg of body weight (18.66 ± 1.33, 8.00 ± 0.00,6.66 ± 2.66, 6.66 ± 1.33), respectively. Compared to the control group of the parasite, as its annular chromosome reached (14.66 ± 1.33), and the concentration was 15 mg/kg, which is the largest reduction in the annular chromosome.

The results of chromosomal changes about the parasite control group for females infected with the parasite and the lack of natural chromosomes and the increase in chromosomal changes as in Figure 1, show that increased toxoplasma infection is directly related to chromatid exchanges[9]. and these chromosomal changes occur as a result of the damage that occurs in the DNA and its attendant From incorrectly re-repairing it [10]. As for the role of bay leaf extract, which reduced chromosomal changes and all the concentrations used, this indicates that the plant has an effect on the parasite. and may be due to what it contains Effective chemical compounds such as flavonoids, quercetin, and luteolin, that the plant contains thymol with carracrol shows the plant can repair DNA because it contains these two compounds [11] [12].

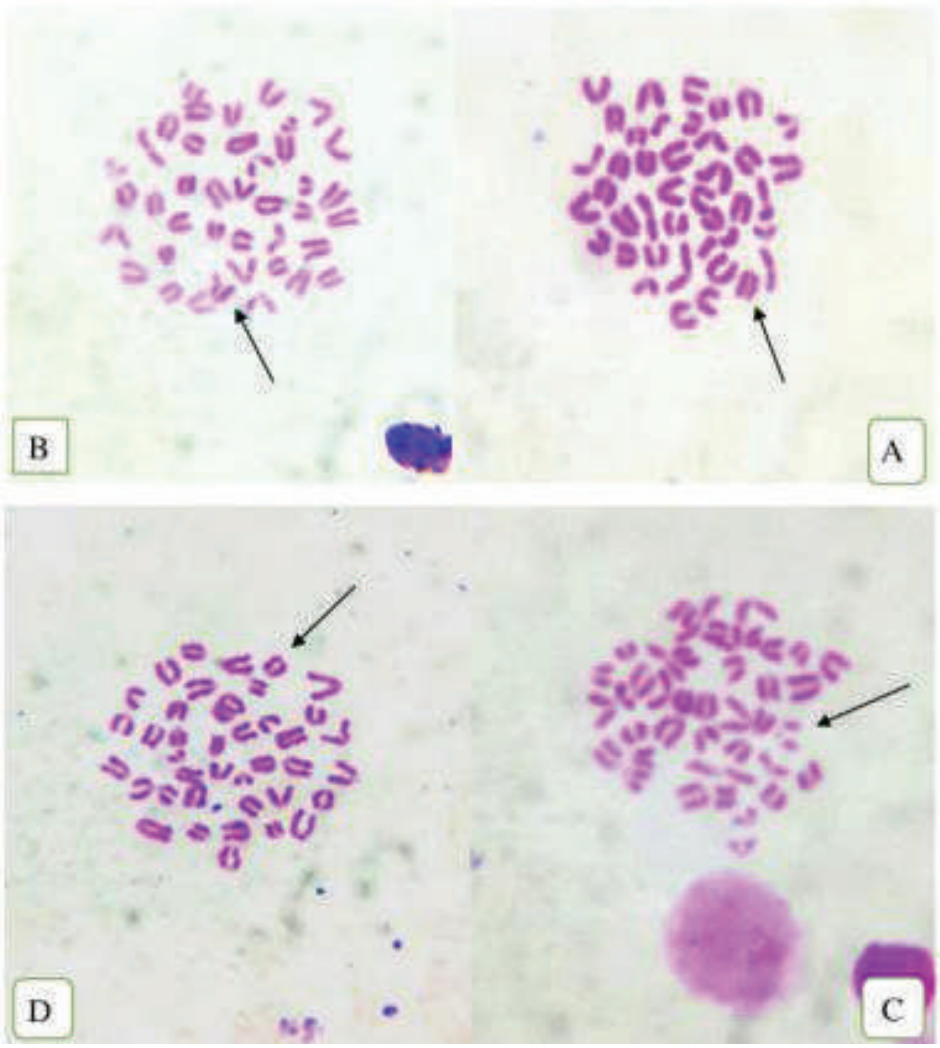


Figure 1: Natural chromosome B: chromatid fractures C: centromere fractures D: annular chromosomes (Giemsa stain with a strength of 100 X).

Conclusion

Ours study concluded there are many chromosomal changes in females mice which injected with *toxoplasma spp* parasites. Significant decrease in the structural chromosomal changes is included of the chromosome. Our study suggest treatment by *Laurus nobilis* leaf extract in females mice have positive effect on bone marrow in females mice that infected by the *Toxoplasma gondii*.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Genetic evaluation of rs36513 SNP of UIMC1 and Biochemical Parameters in Early Menopause in Babylon City, Iraq

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Abstract

Premature menopause or Early menopause (POF) is a condition charecterised by amenorrhea, hypoesteronigism, and elevated serum gonadotrophin level in women younger than 40 years old. This work aimed to evaluate the association of candidate genetic polymorphism in called trans membrane protein 150 B (TMEM150B) and ubiquitin interaction motif containing 1 (UIMC1) also the hormonal changes with POF in one district of Iraq (province of Babylon) by enrolling Arab ancestors. The genetic variants that would be studied in this project were carefully selected according to the recent findings in this field, and by employed several specialized databases . The selected variant are transmembrane protein 150B (TMEM150B) and ubiquitin interaction motif containing 1 (UIMC1) and their relation with premature ovarian failure. This study include (200) females , their ages between (35-40 years) and body mass index in normal and overweight, (50) of them were with amenorrhea for 6 months, and the other (150) apparently healthy as control group. Concerning rs365132, the genetic association with POF showed that there was no significant allelic or genotypic association recorded ($P > 0.2$) , except that the patients group was significantly deviated from Hardy-Weinberg equilibrium exact test ($P=0.023$). For rs11668344, the genetic association with POF showed that there was no significant allelic or genotypic association recorded ($P > 0.2$) , except that the patients group was significantly deviated from Hardy-Weinberg equilibrium exact test ($P=0.023$). The hormonal results of the present study showed significant increase in FSH in patients compared with controls group. ($P < 0.0001$) and this study showed significant decrease ($p\leq 0.001$) in EST in patients compared with controls group. As a conclusion , the study conclude that rs365132 and rs11668344 genes variants do not represent genetic factors which can modulate the development of POF in Arab population from Babylon province .

***Keywords**: Trans membrane protein, Early menopause, ubiquitin interaction motif.*

Introduction

The menopause was considered a natural biologic event that affects every woman. For most women, the menopause transition coincides with the midlife years and was characterized as a time of multiple physiological, psychological and social changes that can impact a women's health [(1)]. Premature menopause (PM) refers to menopause that occurs before age 40 years, and early menopause refers to menopause that occurs at or before age 45 years [(2)]. Premature menopause and early menopause, whether spontaneous or induced, were associated with long-term health risks which may include premature death, cardiovascular disease, neurologic disease, osteoporosis, psychosexual dysfunction, and mood disorders. Estrogen mitigates some but not all of these consequences [(3)]. Fogle in 2017 was searched in different types of premature menopause or early menopause result in different long-term health

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consequences remains unknown. Their hormonal milieus differ because the postmenopausal ovary was hormonally active, producing small amounts of estradiol and estrone, as well as androgens including testosterone, androstenedione, and dehydroepiandrosterone [4]. Following bilateral oophorectomy in premenopausal women, estradiol levels drop, testosterone levels drop by 40–50%, and follicle-stimulating hormone levels rise abruptly. Women undergoing bilateral oophorectomy continue to have lower levels of androgens than naturally menopausal women even beyond 65 years of age [5]. Concern about the risks of hormone therapy following publication of results from the Women's Health Initiative clinical trials may be inappropriate if applied to women with premature menopause or early menopause who typically need estrogen in adequate replacement doses for a duration of time long enough to reduce the consequences of prolonged estrogen deficiency. It was important that clinicians not withhold health-promoting estrogen replacement for these women [6]. Many mutations causing POI were in genes involved with hormone function. Missense mutations in the AMH gene had been identified that result in mutant AMH with loss of AMH-receptor activation. Mutations in INHA, inhibin alphachain, had also been found in POI. As well as mutations in FSHB (discussed above), several mutations with functional effects had been identified in FSHR, the follicle stimulating hormone receptor. In-frame, frameshift and missense mutations had been identified in NR5A1, a transcriptional regulator of wgenes tangled in hormone regulation by hypothalamic-pituitary-gonadal and adrenal axis [7]. Genetic impacts regulate the dimensions of the primaeval cyst pool, have an effect on the frequency of vesicle abnormal condition, and are a main determinant of climacteric age. Genetic causes for POI can account for about 20% to 25% of cases with the start of more refined genetic screening technologies [8]. The amount of genes had been identified both from studies of POI and GWAS that were involved in homologous recombination; MCM8, MSH5 and DMC1. A common variant in MCM8, a DNA helicase involved in DNA repair, Three sisters with amenorrhea and hypergonadotropic hypogonadism are related with a decrease in menopause age by up to one year, whereas a homozygous mutation in this gene resulted in a deficiency of double strand break reparation. Common variants near MSH5, which was involved in meiotic recombination, had been found to reduce age at menopause by 0.16 years, And this gene also identified a heterozygous missense mutation causing POI [9].

Materials and Methods

This work was planned as case-control study conducted in conjunction with the Babel gynaecology Hospital within the Branch of organic chemistry at the faculty of drugs, University of city. The topics enrolled in this study was 200, these topics are classified as follows: A group of patients (50) diagnosed with misperiod for at least 4 months in female under the age of 40 are collectively required to diagnose POI. With 35-40 years of age. Control group comprises 150 subjects of healthy volunteers and subjects set up within normal ranges as suggested by doctor consultants during their visit to the doctor, 35-40 years of age. There are 4 index of body mass groups [10].Elimination conditions were diabetes mellitus patient, hypertension, contraceptives (estrogens), and obesity BMI > 30. Inclusion criteria was diagnosis requires miss period for at least 4 months in female younger than 40 years of age.

Results

Analysis of demographic characteristics of study subjects showed that there were significant differences in FSH, EST,MOH, PRO, and LH between patients and control group, as shown in Table 1.

Table 1: FSH, EST, PRO, MOH, and LH levels (ng/ml) in study groups

	Group	N	Mean	Std. D	Std. E
FSH	Case	50	50.5400	17.63254	2.49362
	Control	150	19.0000	3.48920	.28489
EST	Case	50	50.5400	17.63254	2.49362
	Control	150	19.0000	3.48920	.28489
AMH	Case	50	1.6320	.32729	.04629
	Control	150	2.8767	.60738	.04959
PRO	Case	50	14.5200	3.62666	.51289
	Control	150	10.8800	3.07822	.25134
LH	Case	50	8.9200	2.46477	.34857
	Control	150	7.8800	2.31980	.18941

Analysis of demographic characteristics of study subjects showed that there were significant differences in EST in correlation with rs36513 SNPs of UIMC1 between group of patients and control group as shown in Table 2.

Table 2: Correlation of EST with rs36513 SNPs of UIMC1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
G/G	29	56.2069	28.36796	5.26780	45.4163	66.9975	10.00	110.00
A/A	44	67.0455	27.02966	4.07487	58.8277	75.2632	20.00	110.00
A/G	127	68.0315	26.75697	2.37430	63.3328	72.7302	15.00	110.00
Total	200	66.1000	27.22584	1.92516	62.3037	69.8963	10.00	110.00

(I) BSO	(J) BSO	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
G/G	A/A	-10.83856-	6.47024	.095	-23.5984-	1.9213
	A/G	-11.82460-*	5.56731	.035	-22.8038-	-.8454-
A/A	G/G	10.83856	6.47024	.095	-1.9213-	23.5984
	A/G	-.98604-	4.73210	.835	-10.3181-	8.3460
A/G	G/G	11.82460*	5.56731	.035	.8454	22.8038
	A/A	.98604	4.73210	.835	-8.3460-	10.3181

*. The mean difference is significant at the 0.05 level.

Analysis of demographic characteristics of study subjects showed that there were differences in LH in correlation with UIMC1 between group of patients and control group as shown in Table 3:

Table 3: Correlation of LH with rs36513 SNP of UIMC1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
G/G	29	7.3103	2.36195	.43860	6.4119	8.2088	4.00	12.00
A/A	44	8.0909	2.18683	.32968	7.4261	8.7558	4.00	12.00
A/G	127	8.3465	2.44423	.21689	7.9172	8.7757	4.00	15.00
Total	200	8.1400	2.39354	.16925	7.8062	8.4738	4.00	15.00

Analysis of demographic characteristics of study subjects showed that there were differences in AMH in correlation with UIMC1 between group of patients and control group, as shown in Table 4:

Table 4: Correlation of AMH with rs36513 SNP of UIMC1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
G/G	29	2.2621	.84152	.15627	1.9420	2.5822	1.00	3.50
A/A	44	2.7273	.74095	.11170	2.5020	2.9525	1.50	3.50
A/G	127	2.5787	.75083	.06663	2.4469	2.7106	1.00	3.50
Total	200	2.5655	.77106	.05452	2.4580	2.6730	1.00	3.50

Analysis of demographic characteristics of study subjects showed that there were differences in LH in correlation with TMEM between group of patients and control group as shown in Table 5

Table 5: Correlation of LH with rs897798 TMEM SNPs

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
G/G	41	8.0541	2.22294	.36545	7.3129	8.7952	4.00	15.00
A/A	73	7.7534	2.34391	.27433	7.2066	8.3003	4.00	12.00
A/G	86	8.5233	2.49577	.26913	7.9882	9.0583	4.00	15.00
Total	200	8.1480	2.40375	.17170	7.8093	8.4866	4.00	15.00

Discussion

The searched for the premature or early menopause was the first step in a chain of causality leading to tissue or organ dysfunctions and lesions via hormonal mechanisms [12] With POF, follicle-stimulating hormone levels are elevated and estradiol levels are low, but sporadic increases in estradiol may occur [13] Ovarian androgens remain age-appropriate in these women [14]. Luteinizing hormone "hyperactivity" appears to have no effect in women. However, in the present study, we observed a strong relation between the variant LH and POF that was not caused by hormonally resistant ovaries. , abnormal LH secretion can induce anovulation, luteal insufficiency, and premature oocyte maturation, leading to menstrual disorders, PCOS, recurrent spontaneous abortions, and infertility [15]. Abnormal LH and/or LH receptors have been linked with endometriosis-associated infertility [16]. Menopausal symptoms in patients with POI are consequent to the premature decline in ovarian follicle number and a dysfunction of the residual follicles with resulting hypoestrogenemia and, to a lesser extent, a decline in ovarian testosterone. Adequate systemic estrogen replacement is critical for symptom control, whereas local estrogen may be required for addressing focal symptoms such as genito-urinary symptoms [17]. AMH is considered a good and reliable parameter in the assessment of ovarian reserve [18]. Its reduced levels may indicate reduced ovarian reserve, even if the woman has regular menstrual cycles and the levels of FSH and E_2 are still normal. A good correlation has been found between AMH levels and age and also between AMH levels and the number of antral follicles. The diversity of results depending on the type of laboratory test used and the absence of a universal calculation method to facilitate the comparison of results obtained via different tests is a significant limitation associated with the use of AMH as a test for ovarian reserve. Clinical signs of PCOS include elevated luteinizing hormone (LH) and gonadotropin–releasing hormone (GnRH) levels, whereas follicular-stimulating hormone (FSH) levels are muted or unchanged. As a result of the increase in GnRH, stimulation of the ovarian thecal cells, in turn, produces more androgens [19]. Follicular arrest can be corrected by elevating endogenous FSH levels or by providing exogenous FSH. Some studies suggest that PCOS is a primary defect in young girls who are entering puberty and who have a family history of the disorder. Approximately 25% of patients with PCOS have elevated prolactin levels [20]. In the first GWAS of menstrual cycle length we found a strong association between an FSH lowering, likely functional, variant in the FSHB promoter and longer cycles [21]. This locus has been previously robustly associated with age at menopause in the ReproGen GWAS of menopause timing and the allele associated with longer cycle length is associated with later age at menopause [22]. We not observe associations for the majority of age at menopause GWAS signals with length of menstrual cycle, including the four signals with effects of over one-third of a year per allele on menopause timing, implying that the association is specific to FSHB: Either FSHB has independent effects on both cycle length and menopause, or changes in cycle length are causally influencing menopause timing.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Prevalence of Campylobacter Species in Diarrheal Samples of Children Less than 10 Years

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Abstract

Introduction: Campylobacter spp. is one of the most common causes of diarrheal diseases all over the world, with a rapid acquisition of antibiotic resistance.

Objective: Detect the frequency of Campylobacter species in diarrheal stool of children under ten years by conventional and molecular methods, and detect the bacterial ability to produce biofilm.

Methods: A total of 200 children presented with diarrhea had been enrolled in this study. Campylobacter was isolated and diagnosed primarily by characteristic features on Gram stain, culture media, biochemical tests, and tested against 13 antibiotics by disc diffusion method. The ability of biofilm production was tested by crystal violet quantitative ELISA microtiter plate assays. Then Campylobacter spp was detected by Multiplex PCR using species specific genes.

Result: The prevalence of Campylobacter spp. was 17% by conventional methods and 15% by molecular method. The results of antibiotic susceptibility test showed that, there is complete resistance (100%) to cephalothin, ampicillin, and clindamycin for both species, full resistance (100%) to trimethoprim-sulfamethoxazol and erythromycin by *C.coli*, and high resistance (92.3%) to trimethoprim-sulfamethoxazol and erythromycin by *C.jejuni*, while the lowest resistance was to nalidixic acid and amikacin (7.7%) by *C.jejuni*, and (12.5%) to tetracyclin, amoxicillin, amikacin, chloramphenicol, and ciprofloxacin by *C.coli*. The frequency of biofilm production in all positive Skirrow's culture was (35.29%) as 12 out of 34 positive isolates, ranging from mild to severe biofilm formation. By PCR assay, 64.7 % (22 of 34) positive Skirrow's culture were also positive based on *hipO* gene specific for *C.jejuni*, while the prevalence of *asp* gene was (23.5%).

Conclusion: The prevalence of Campylobacter spp. was 17% by conventional methods and 15% by molecular methods, most of *Campylobacter jejuni* and *Campylobacter coli* isolates were MDR, and sensitive only to limited number of antibiotics, many Campylobacter isolates produce biofilm, there was highly significant correlation between hippurate hydrolysis results and molecular detection of Campylobacter spp. depending on *hipO* and *asp* genes.

Keyword: *Campylobacter jejuni*, *Campylobacter coli*, *hipO* gene, *asp* gene, biofilm.

Introduction

Genus Campylobacter consists of about 26 species, the most important disease-causative agents are *Campylobacter jejuni* and *Campylobacter coli* [1], Campylobacteriosis is characterized by watery and/ or bloody diarrhea, fever, malaise, abdominal pain, cramps, and vomiting. It is more dangerous for young children due to risk of dehydration resulted from loss of nutrients and essential components of food; like proteins and salts [2].

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Laboratory diagnosis can be done on stool samples, rectal swabs, and even blood in case of *Campylobacter fetus,* the diagnosis relay on culture using selective media, molecular detection, or rapid diagnostic test like Gram stain and biochemical tests. Many studies have investigated the genes responsible for differentiation between species including *hipO* and *asp* genes. The *hipO* gene region is the hippuricase gene specific for *C. jejuni* [3], and the *asp* gene region, the aspartokinase gene specific for *C. coli* [4].

Patients and Methods

A total of 200 patients were included in this study. Stool samples were collected during a period from the first of September 2019 to the end of January 2020 from Al-Imamain Al-Kadhimain Medical City,Baghdad. Campylobacter was isolated and identified by ordinary methods according to morphological characteristics on cultures, biochemical, and molecular methods in the laboratories of microbiology department /College of Medicine/ Al-Nahrain University. This study was approved by the ethical committee of the College of Medicine-Al-Nahrain University.

Antibiotic susceptibility testing

Campylobacter isolates were tested for their susceptibility to thirteen antimicrobial agents in accordance to CLSI 2016 recommendations [5]. These antibiotics are amickacin(AK) 30 µg/disk, amoxicillin (AX) 30µg/disk, ampicillin (AM) 10 µg/disk, cephalothin(KF) 30 µg /disk, chloramphenicol(C) 10 µg/disk, ciprofloxacin(CIP) 5 µg/disk, clindamycin(DA) 5 µg/disk, erythromycin(E) 15 µg/disk, gentamicin(CN) 10, nalidixic acid(NA) 30 µg/disk, rifampin(RA) 5 µg/disk, Sulfamethoxazole/trimethoprim(SXT) 25 µg/disk, and tetracycline(TE) 30 µg/disk.

Biofilm formation

Detection of biofilm formation was performed by crystal violet quantitative ELISA microtiter plate assay as described by Fields *et al*. (2008) [6].

DNA Extraction

DNA was extracted according to the manufacture instructions using Geneaid Presto Stool DNA Extraction Kit Quick Protocol.

Molecular Detection of *hipO* and *asp* genes

Sequences of primers had been designed and synthesized in Alpha-DNA (Canada) as showed in tables (1 & 2). For preparation of the PCR mixture reaction, DNA template (5-50 ng), forward and reverse primers were diluted by adding nuclease free water to the desired concentrations and mixed by pipetting with master mix (Accupower Bioneer PCR premix kit , Korea) as illustrated in table (3). PCR mixture without DNA template was used as negative control.

Table (1): Sequences and product size of *hipO* gene

hipO gene	Nucleotide sequences (5' to 3›)		Products bp	References
hipO	F R	GAAGAGGGTTTGGGTGGTG AGCTAGCTTCGCATAATAACTTG	735	Linton et. al, 1997 [7]

Table (2): Sequences and product size of *asp* gene

asp gene	Nucleotide sequences (5' to 3›)		Products bp	References
asp	F R	GGTATGATTTCTACAAAGCGAG ATAAAAGACTATCGTCGCGTG	500	Harzandi et. al, 2015 [8]

Table (3): The mixture of PCR working solution for detection of *hipO* and *asp* genes in Campylobacter spp.

Component	Concentration	Volume µl
Master mix	1X	0 µl
Primer F.	10pm/ µl	3.0 µl
Primer R.	10pm/ µl	3.0 µl
DNA Template	5-50 ng	4 µl
Deionized D.W.	-	34 µl
Total volume		50 µl

The reaction was carried in Thermal cycler (Cleaver Scientific Thermal Cyclers- TC32/80, UK) according to the program showed in table (4).

Table (4): PCR program for amplification of Campylobacter genes by thermal cycler:

No.	Steps	Temperature	Time	No. of cycles
1	Initial denaturation	96˚C	6 minutes	1
2	Denaturation	95˚C	40 seconds	35
3	Annealing	60 ˚C	45 seconds	
4	Extension	72 ˚C	1 minutes	
5	Final extension	72 ˚C	10 minutes	1

Gel electrophoresis

A 6 µL of each amplified sequence and 100 bp ladder resolved by electrophoresis according to Sambrook and Russell (2001) [9]. The products were visualized in UV trans-illuminator (LKB, Sweden).

Results

In regard to culture on Skirrow's medium, Catalase, and Oxidase tests; out of 200 samples, there were 34 (17.0 %) positive and 166 (83.0%) negative, while according to hippurate hydrolysis test, 26 (13.0%) were positive and 174 (87.0%) were negative, within culture the percentage of female was 55.9% while the male was 44.1% and according to the age group, the highest percentage of Campylobacter isolates was (35.29%) in age group (7.5-9 years), while the lowest percentage was (8.8%) in age group (≤ 1 year).

Antibiotic susceptibility test

The results of antibiotic susceptibility test showed that, there is complete resistance (100%) to cephalothin, ampicillin, and clindamycin for both species, full resistance (100%) to trimethoprim-sulfamethoxazol and erythromycin by *C.coli*, and high resistance (92.3%) to trimethoprim-sulfamethoxazol and erythromycin by *C.jejuni*, while , there was low resistance to chloramphenicol and gentamicin (34.61%), ciprofloxacin (26.92%), nalidixic acid and amikacin (7.7%) by *C.jejuni*, and low resistance to rifampin (37.5%), tetracyclin, amoxicillin, amikacin, chloramphenicol, and ciprofloxacin (12.5% for each) by *C.coli*.

Biofilm detection of Campylobacter isolates

With reference to measurement of biofilm optical density (O.D.) by ELISA reader, it has been shown that cut-off value was equal to (0.165) nanometers, the isolate which had OD less than the cut-off value was considered non-biofilm producer while the isolate that had OD equal or higher than the cut-off value was considered as biofilm producer. This study showed that (35.29%) of isolates produce biofilm while (64.7%) were not biofilm producer.

The association between biofilm and antibiotic resistance

The current study showed that all of the 12 biofilm producing isolates (100%) were resistant to cephalothin, clindamycin, and ampicillin, while 11 (91.66%) of them were resistant to trimethoprim and erythromycin. Statistically there is no significant difference (P= 0.7) between biofilm formation and antibiotic resistance in Campylobacter species.

Multiplex Polymerase Chain Reaction screening for *hipO* and *asp* genes

Sequences amplification of Campylobacter species-specific genes (*hipO* and *asp*) was done by multiplex PCR technique with product size 735bp of *hipO* gene and 500bp of *asp* gene, as shown in figure (1).

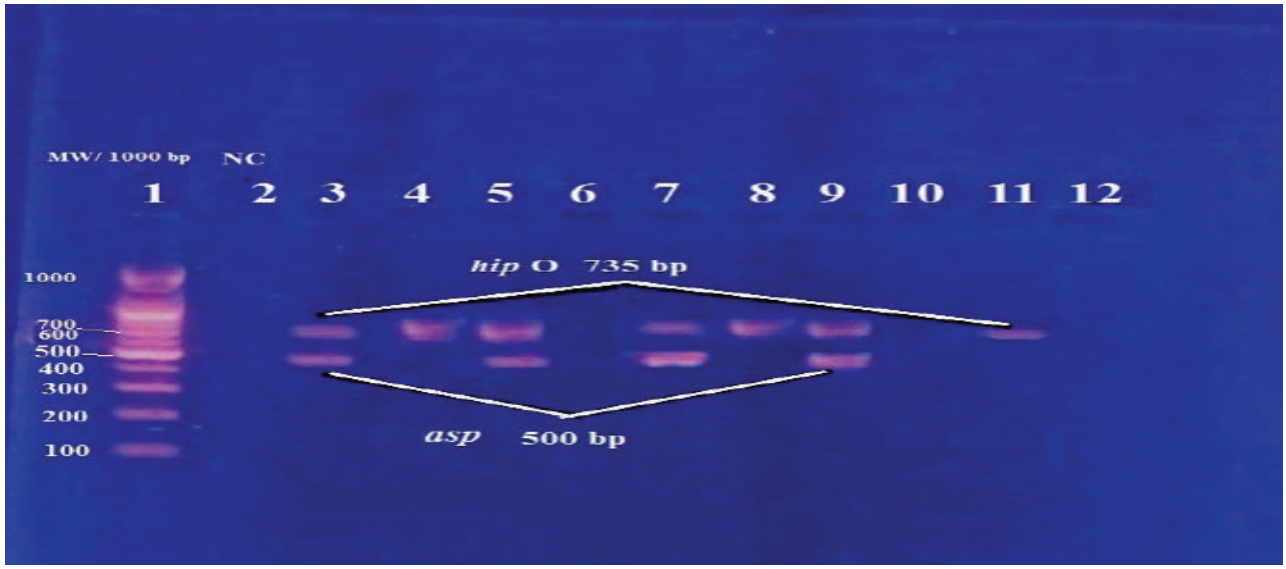


Figure (1): Gel electrophoresis of PCR products (735 bp) for *hipO* gene and (500 bp) for *asp* gene. Lane 1: 100bp ladder. Lane 2: Negative control. Lanes 3-12: PCR products of clinical stool samples. (2% agarose, 7 v/cm^2, 45 min).

The current result showed that, 16 (72.7%) of *C.jejuni* isolates detected by PCR according to presence of *hip*O gene had no biofilm , while only one isolate had low-level (4.5% within PCR *hipO*), 3(13.6%) of isolates had mid-level and 2 had high-level (9.1%), as appeared in table (5). The biofilm degree according to PCR *hipO* result for *C.jejuni* revealed that there is no significant differences between them (P=0.26).

Table (5): Biofilm degree in correlation with PCR *hipO* gene

P=0.26			PCR hipO for C.jejuni		Total %
			Negative %	Positive %	
Biofilm degree	No biofilm	Count	6	16	22
		% within Biofilm degree	27.3	72.7	100.0
		% within PCR hipO	50.0	72.7	64.7
		% of Total	17.6	47.1	64.7
	Low level biofilm	Count	3	1	4
		% within Biofilm degree	75.0	25.0	100.0
		% within PCR hipO	25.0	4.5	11.8
		% of Total	8.8	2.9	11.8
	Mid-level biofilm	Count	1	3	4
		% within Biofilm degree	25.0	75.0	100.0
		% within PCR hipO	8.3	13.6	11.8
		% of Total	2.9	8.8	11.8
	High level biofilm	Count	2	2	4
		% within Biofilm degree	50.0	50.0	100.0
		% within PCR hipO	16.7	9.1	11.8
		% of Total	5.9	5.9	11.8
Total		Count	12	22	34
		% within Biofilm degree	35.3	64.7	100.0
		% within PCR hipO	100.0	100.0	100.0
		% of Total	35.3	64.7	100.0

This result also touched on the frequency of biofilm degree according to PCR asp for C.coli and showed that, there were 4 (50.0%) of C.coli isolates positive for asp gene with no biofilm, 2(25.0%) had low-level and one isolate had mid and high-level of biofilm formation.

Discussion

This study revealed that Campylobacter distribution in clinical samples by Skirrow's media was with percentage of (17%) in Iraqi patients, these results agreed with the study conducted in Northern Poland by Szczepanska *et al*., 2017 [10], who reported that Campylobacter distribution was 15.5% (306/1973) of all analyzed samples. The current study disagreed with a study conducted in South Africa by Martina O. *et al.*, 2019 [11], who reported that out of 505 paediatric stool

specimens; (46.9%) 237 Campylobacter isolates were collected.

The present study also exhibited that infected females were more than males, with a percentage of isolates (44.1%) in males and (55.9%) in females, which agreed with a study conducted in Ecuador by Toledo *et al*., 2017 [12], who reported that out of the 127 children studied, 17 harbored Campylobacter sp. corresponding 12.9% (9/70) to boys and 14.0% (8/57) to girls. Whereas the current result disagreed with a study done in Nigeria by Adekunle *et al*., 2019 [13], who reported that from the 815 subjects with diarrhea, 347 (42.6%) were females and 468 (57.4%) were males. The reason of the present result is that; the number of samples were collected from females more than males.

According to the Age group, this study showed that, the highest percentage of Campylobacter isolates (35.29%) was in age group (7.5-9 years), while the lowest percentage was (8.8%) in age group (≤ 1 year). The infection with Campylobacter occurs with high percentage in young children due to outdoor activities and contact with animals. This study agreed with a study done in Sub-Saharan Africa by Noel *et al*., 2020 [14], who reported that the country-level mean prevalence in totally-all ages and under-five children was 18.6% and 9.4%, respectively.

Emergence of antibiotic resistance is a never-ending process due to its capacity to resist and acquire various resistance mechanisms against antibacterial drugs. The results of this study showed that 100% of *Campylobacter jejuni* and *Campylobacter coli* isolates were resistant to cephalothin, clindamycin, and ampicillin when tested by standard disc diffusion method. Similar findings in a study conducted in South Africa by Otigbu *et al*., 2018 [15], who reported that, the high rates of resistance to clindamycin (84.2%) make this antibiotic a poor choice for treatment of infections with Campylobacter.

Also the current study showed that the *Campylobacter jejuni* and *Campylobacter coli* developed low resistance (7.7 %) and complete sensitivity (0%) to Nalidixic acid respectively, and this result agreed with a study conducted in Lebanon by Ibrahim *et al*., 2019 [16], who proved by disc diffusion that a low level of resistance to nalidixic acid (17.2%) was found in *Campylobacter* isolates. But the present study disagreed with a study published in Korea by Park *et al*., 2019 [17], who reported that higher resistance rates of *Campylobacter jejuni* (90.0%) developed against Nalidixic acid.

In this study among 34 clinical isolates, there were 12 (35.3%) Campylobacter spp. showed biofilm formation ability. Within positive biofilm the percentage was 66.7% and 33.3% for *Campylobacter jejuni* and *Campylobacter coli* respectively. And within positive culture it was 23.5% and 11.8% respectively. This study agreed with a research was done in Malaysia by Huei *et al*., 2014 [18], who reported that out of 17 *Campylobacter jejuni* isolates, 3 (17.6%) were positive for biofilm formation within positive culture. The present findings were higher than results of a study done in Korea by Kim *et al*., 2017 [19], who reported that 18% (14 of 78) Campylobacter isolates were able to produce biofilm.

Two species-specific genes (*asp* and *hipO*) were identified in Campylobacter isolates using Muliplex PCR technique. The result of this study revealed that the frequency of Campylobacter isolates by Muliplex PCR was 15%. In contrast to cultural identification which revealed that Campylobacter spp. frequency was 17% (34 positive culture out of 200 samples), this difference could be due to the low concentration of DNA tested in molecular methods.

The current study coincides with a study published in Thi-Qar Governorate by Harb *et al*., 2019 [20], who reported that the frequency of Campylobacter spp. by molecular technique (PCR) was 10.9% (17/155). Whereas this study disagreed with a study published in China by Ying *et al*., 2018 [21], who reported that molecular isolation ratio of Campylobacter was 7.0% (26/370).

According to the polymerase chain reaction results of Campylobacter genes, the percentage was 11% for *hipO* gene (*Campylobacter jejuni*-specific gene) and 4% for *asp* gene (*Campylobacter coli*-specific gene), which resembles a study done in Italy by Bianchini *et al.,* 2014 [22], who published that *C. jejuni* was detected in 34 from total 282 samples (12%) of dairy products by PCR. But it is much lower than a study result conducted in Iran by Mryam *et al*., 2018 [23], who reported that detection rates for the *hipO* gene was 91% of samples.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Dr. Thanaa Rasheed Abdulrahman: Advise and interpretation the results, research done under her supervision.

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Clinical Evaluation of Recurrent Aphthous Stomatitis and Its Correlation with Helicobacter Pylori

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Abstract

Recurrent aphthous stomatitis is a disorder distinguished by ulcers restricted to the oral mucosa. Because of the histological similarities between peptic ulcers and recurrent aphthous stomatitis and the identified role of Helicobacter pylori in peptic ulcer, the possibility of bacterial involvement in the progression of aphthae has been suggested. Helicobacter pylori are one of the most common, well-known pathogenic agents in the development of peptic ulcers. Some investigators have shown a relationship between Helicobacter pylori and recurrent aphthous stomatitis. However, this relationship is controversial. The aims and objectives of this study were 1- Clinical evaluation of patients with recurrent aphthous stomatitis.2- Determination of the association between Helicobacter pylori and recurrent aphthous stomatitis using the urea breath test.

Eighty-five subjects were participated in this study; they are divided into two groups. Fifty patients with recurrent aphthous stomatitis, which at least occur four, or more times per year, thirty-five healthy subjects of the same age group as controls.

The results showed that ,the mean age of patients with recurrent aphthous stomatitis lie around 3rd decade and the percentage of female with recurrent aphthous stomatitis was higher than male,the clinical findings showed that the number of patients with minor recurrent aphthous stomatitis was higher than patients with major and Herptiform recurrent aphthous stomatitis , No difference between the number of patients who had single recurrent aphthous stomatitis and number of patients who had multiple recurrent aphthous stomatitis was found . According to the site of ulcers, the result founded that the number of patients who had RAS on the non-keratinized mucosa was higher than patients who had RAS on the keratinized mucosa and those who had recurrent aphthous stomatitis on keratinized and non-keratinized oral mucosa.

Regarding the laboratory findings, the percentage of patients with recurrent aphthous stomatitis who urea breath test positive was higher than percentage of control group,also the mean value (Delta Over Base) of urea breath test in recurrent aphthous stomatitis patients was statistically higher than that of healthy controls. By these results we concluded that there was a correlation between recurrent aphthous somatitis and H.pylori by using urea breath test in patients with recurrent aphthous stomatitis when compared with controls.

Keywords: *Recurrent aphthous stomatitis; H.pylori ; Clinical Evaluation; Health.*

Introduction

Recurrent aphthous stomatitis (RAS) is a very common condition characterized by solitary or multiple small, round, recurrent oral ulcers, with erythematous haloes and circumscribed margins[1].Aphthous ulcers are classified into three different types, minor, major and herpetiform. Minor aphthae are generally located on labial or buccal mucosa, the soft palate and the floor of the mouth (non-keratinized mucosa). They can be singular or multiple, and tend to be small (less than 1 cm in diameter) and shallow. This type of RAS is the most

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common (80% of cases), and usually heals within 7-14 days [2].

Major aphthae also called as Sutton's disease; usually exceeds 1 cm cause deeper ulceration thus leave scar. It constitutes only 10-15% of RAS cases. These ulcers may remain about 10-20 days and may take months. The Herpetiform is least common variant of RAS that constitutes only 7-10% of RAS cases, ulcer size is very small measuring 2-3 mm in diameter; numerous in numbers (around 100 ulcers at once), can fuse together producing large irregular lesions that last for 7-10 days without leaving scars[3].

The etiopathogenesis of RAS so far remains not fully understood. The potential trigger factors include genetic predisposition, viral and bacterial infections, food allergies, vitamin and microelement deficiencies, systemic diseases (e.g., celiac disease, Crohn's disease, ulcerative colitis, AIDS), increased oxidative stress, hormonal defects, mechanical injuries and anxiety [4].

In genetically predisposed patients, the effect of certain trigger factors initiates the cascade of proinflammatory cytokines, directed against selected regions of the oral mucosa[5],The Helicobacter pylori (H. pylori), is a microaerophilic, Gram-negative spiral organism that has been shown to be the causative factor in a large proportion of patients with stomach ulcers and gastritis [6].

Transmission of H.pylori is largely by the oral-oral or fecal-oral routes. Lack of proper sanitation, safe drinking water and basic hygiene, as well as poor diets and overcrowding, all play a role in the overall prevalence of infection[7].Considering the similarities of histological features between gastric ulcers and oral aphthous ulcers, and in view of the fact that the latter lesions often respond to treatment with broad-spectrum antibiotics such as tetracycline, it is reasonable to assume that H.pylori might also be involved in the development of recurrent oral aphthous ulcers. However, there is limited evidence regarding the colonization or the possible role of H.pylori in oral aphthous ulcers [8].

Diagnostic tests currently used for the detection of H.pylori fall into two categories: invasive and noninvasive, the former requiring endoscopy. The invasive methods, which are biopsy-based, include culture, rapid urease test (RUT) and histology. Non-invasive testing for H.pylori can be done by the urea breath test (UBT), serology and analyzing body materials such as faeces, urine and saliva. The UBT requires instruments such as mass spectrometer and infrared spectrometer, which are not always available in routine clinical laboratories [9, 10]. The present study was designed as a case-control study to determine probable H.pylori infection in patient with RAS by using UBT.

The Aims and Objectives

1- Clinical evaluation of patients with recurrent aphthous stomatitis.

2. Determination of the association between H. pylori and RAS using the urea breath test (UBT).

Subject, Materials and Methods

The study samples consisted of (85) subjects, (50) patients with RAS referred from medical and dental centers in Baghdad City and other provinces in Iraq to department of Oral Medicine Clinic, College of Dentistry -University of Baghdad, (35) healthy group, they were not suffering from aphthous ulcer during their life, and with no signs and symptoms of any systemic disease.

The participants were estimated by dentist specialized in oral medicine to confirm the inclusion criteria.The collection of samples was from the period (January-2019)to(June-2019), Patients with RAS, which at least occur four, or more times in the year and the control group were included in the study. Patients with systemic conditions and aphthous like lesion were excluded. Urea [C13] breath test kits(Heliforce)®, and Infrared (IR) Force spectrometer machine were used . Ethical approval committee of College of Dentistry-University of Baghdad approved this in vivo case-control study.

After completing the extra oral examination, all the patients were examined intraorally to distinguish the RAS and to write the characteristic feature of the ulcer (size, number, sit, frequency and duration).

After that all the participants were supervised and instructed not to eat or drink anything (only water) 3 hours at least before doing the test. The researcher filled out the required information on the sample bags, asked

each participant (patient and healthy subject to hold his or her breath for 10 second, then exhale into first air bag marked "before" or (O minute), and make it full of gas as much as possible and immediately close it, then the participant asked to ingest 75 mg of non- radioactive isotopic urea(^{13}C), Labeled urea comes into contact with mucus ,here, hydrolysis by H.pylori by urease enzyme Produced ammonia and labeled Carbone dioxide.

Urea rapidly, passed down its concentration gradient, into the epithelial blood and within minutes appears in the breath. After 30 minute, the participant asked to exhale into second bag labeled (after) or 30 minute, and immediately close it. After that, the two bags were attached to an Infrared (IR)-force spectrometer, which looked for labeled ^{13}C and displayed the result as positive or negative.

Results

The mean age of patients with RAS was (36.0±14.8) years with age range from (16-73) years, they were thirty females (60%) and twenty (40%) were males. The results showed that (40) patients (80%) had minor aphthous ulceration, whereas (6) patients (12%) had major aphthous ulceration and (4) patients (8%) had both minor and major ulcers .Herpetiform type of ulcers was not found in those patients (Fig.1), according to the number of ulcer, (24) patients(48%) had single ulcer, whereas(26)patients(52%)were with multiple ulcers at the time of examination(Fig.2). Thirty-eight patients (76%) with (RAS) had oral ulcer(s) located on non-keratinized mucosa. While two patients (4%) had oral ulcer on keratinized mucosa and ten patients (20%) had RAS on both keratinized and non-keratinized oral mucosa (Fig.3).The mean value of urea breathe test in RAS patients (11.146±9.446),which was statistically significant (P< 0.001) higher than that of healthy controls (3.606±5.025) (Table 1) .The UBT was positive in thirty-six (72%) patients and negative in fourteen(28%),while out of thirty five of control group the UBT was positive in eleven(31.4%) subjects and negative in twenty-four(68.6%)subjects (Table 2).These results were highly statistically significant (P<0.001).

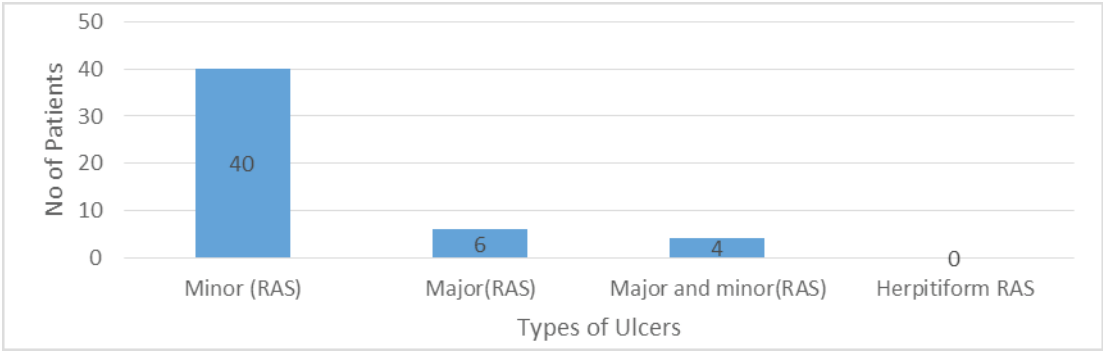


Figure (1):- Distribution of patients according to the clinical presentation (types of ulcers).

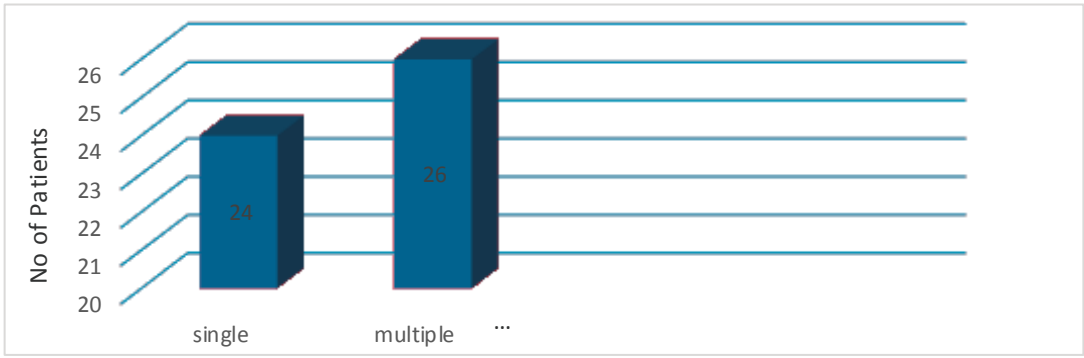


Figure (2):-Distribution of patients according to the clinical presentation (Number of ulcers)

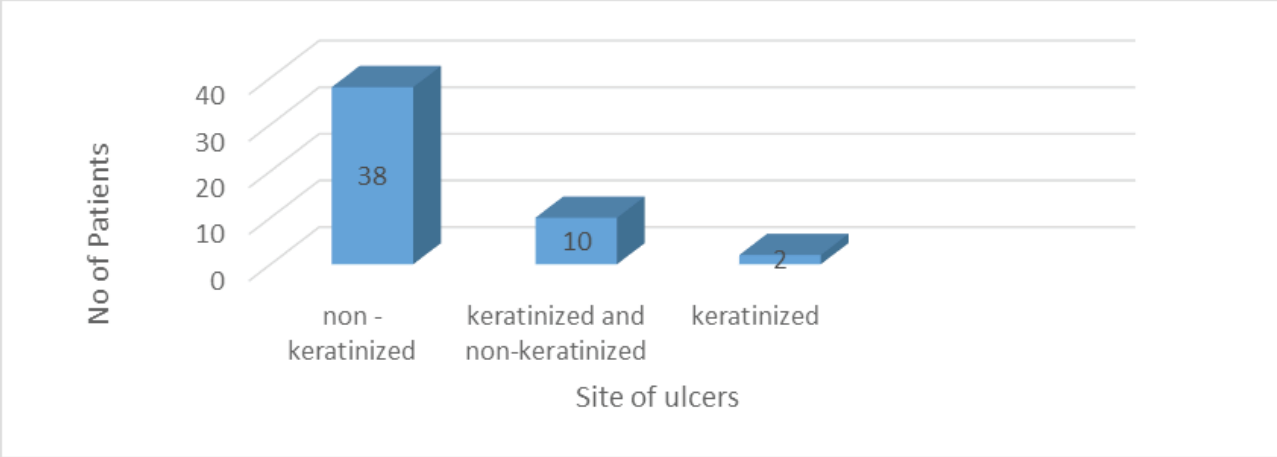


Figure (3):- Distribution of patients according to the site of ulcers

Table (1):- The Mean and Standered Deviation (SD) of UBT in all study groups

Test	Study Group	N	Test Value Mean	SD	Test result	P value	
Urea Breath Test (DOB)	Case	50	11.146	9.446	386.0 C	< 0.001	High-Significant
	Control	35	3.606	5.025			
T, C Mann-Whitney SD; Slandered Deviation DOB;Delta Over Base							

Table (2):- Distribution of Study groups according to the results of UBT

Urea Breath Test	Study Groups				Test Result	P value
	Case		Control			
	N=50	100%	N=35	100%		
· Positive	36	72.0%	11	31.4%	13.709 B	<0.001
· Negative	14	28.0%	24	68.6%		
B Chi-square test N: Number						

Discussion

The results showed that the mean age of patients with RAS lie around 3rd decade this result agreed with other studies [11, 12].While, other study have noted that the average age group was in the 4th decade [13]. Wheras Maleki *et al*. (2009) showed that the mean age ±SD was 25.0±ed .9.4 years in RAS patients [14].

The percentage of female with RAS was higher than male; this was reported in most other studies, which suggested female predilection [11, 15, 16].While other study by Taher(20018) suggested a male predilection [17], such results may be due to the limited number of patients.

The clinical findings showed that the number of patients with minor RAS was higher than patients with major and Herptiform RAS This result agreed with other studies[2,11,14,18].The result of this study showed that there was no difference between the number of patients who had single RAS and number of patients who had multiple RAS, this is in positive agreement

with the epidemiological study on RAS by Safadi (2009) demonstrated that approximately half of participants reported that ulcerations were single, while the other half reported them as multiple ulcerations[19].

The result showed that the number of patients who had RAS in the non-keratinized mucosa was higher than patients who had RAS in the keratinized mucosa and those who had RAS in keratinized and non-keratinized mucosa, this could be explained on the basis that the non-keratinized mucosa areas were movable structure and mostly affected by trauma and least resistant to infection. This result agreed with other studies [11, 17].

The mean value (DOB) of urea breath test in (RAS) patients was higher than that of healthy controls, which was statistically significant (P< 0.001),the percentage of RAS patients with positive UBT was significantly higher than percentage of control group (P<0.001)

It has been found by this result a potential association between RAS and H.pylori infection ,this result agreed with the results of two studies on patients with RAS were submitted to endoscopy biopsy to detect H. pylori. Both studies showed a positive relationship between the presence of the bacteria in the stomach and the occurrence of RAS in the mouth. In these studies by Karaca *et al*. (2008) and Tas *et al.* (2013) 87% and 65% of the patients with RAS, respectively, showed the bacteria in the gastric mucosa. There were significant decreases in the recurrence rate and healing time of RAS by eradication therapy [20, 21].

Maleki *et al*.(2009) had cases and control groups disregarding age and gender. The UBT was used to detect H. pylori infection. It was found that, 37.2% of individuals among the RAS patients and 31.8% individuals in the control group had a positive UBT. The difference was not found to be statistically significant (*P*=0.597). In other words, no correlation was detected [14].

Also the result of present study aggred with study by Birek *et al*. (1999) reported that 23 of 32 (71.9%) of recurrent aphthous ulcers detected by PCR assay were positive for H. pylori and suggested a relationship between H. pylori and recurrent aphthtous ulcers [8].

There are many studies in the last few years focused on the effect of H,pylori infection on the homeostasis of different micronutrients including iron, vitamin B12, folic acid, a-tocopherol, vitamin C and b-carotene[22], Öztürk *et al*.(2015)concluded that in Helicobacter pylori-positive children, many trace elements and mineral metabolism may change[23].

Conclusions

A potential association between RAS and H.pylori Infection in the stomach by using the mean value (DOB) and the results (positive and negative) of UBT in patients with (RAS) when compared with controls.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Effect of An Educational Curriculum According to (K.W.L) Strategy on Some Mental Abilities and Learning Some Basic Football Skills for Students

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Abstract

The research problem was centered in the fact that the student usually cares about playing without using the mental abilities in the skill and what is known about the skill and what he wants to know and what he learned from it, and through the experience of the researchers, they found that it is necessary to review the strategies and methods of teaching used and the introduction of modern educational methods based on the needs of students and their inclinations And their psychological desires and needs, and one of the most important modern strategies is the (KWL) strategy that works to stimulate students 'previous knowledge and make it a starting point for linking it with new information to renew the meaning of learning and increase student confidence and his ability to plan, monitor and strengthen He did his work, as well as developing mental abilities that are one of the main goals in education, as traditional methods that focus on preserving and retrieving study material only do not necessarily lead to developing mental abilities, and that the research problem manifests itself in identifying the impact of using the KWL strategy in Some mental abilities, cognitive achievement, and learning some basic skills of football for students. The research objectives were to prepare a teaching strategy (KWL) for students and to identify their effect on mental capabilities and in some basic skills for football for students. The researchers assumed that there were no significant differences in Mental abilities and learn some basic football skills for students.

***Keywords**: (K.W.L) strategy, mental abilities, basic football skills*

Introduction

the research problem was centered in the fact that the student usually cares about playing without using mental abilities in the skill and what is known about the skill and what he wants to know and what he learned from it, and through the experience of researchers, they found that it is necessary to review strategies And the teaching methods used and the introduction of modern pedagogical methods based on students 'needs, preferences, desires, and psychological needs. One of the most important modern strategies is the (KWL) strategy that activates students' previous knowledge and makes it a starting point for linking it with new information to renew The meaning of learning increases the student's self-confidence and his ability to plan, monitor and perform his work as well as the development of mental capabilities that are one of the main goals in education as the traditional methods that focus on preserving and retrieving study material only do not necessarily lead to the development of mental capabilities, and that the research problem [(1-6)]It is manifested in the recognition of the effect of using the (KWL) strategy on some mental abilities and cognitive achievement and learning some basic skills of football for students, and the objectives of the research were to prepare a teaching strategy (KWL) for students and to identify their impact

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on mental abilities and in some basic skills Football for students, and the researchers assumed that there were no significant differences in mental abilities and learning some basic football skills for students[7-12].

Practical procedures:

Determination of mental processes used by research:

The researchers prepared a questionnaire to select the most important mental processes that have to do with the variables of the study, and they were presented to several experts and specialists in the field of motor learning and mathematical psychology and they numbered (12) experts, and after emptying the forms and extracting the relative importance, the mental processes that got a percentage of 55% or more of the relative importance, as shown in Table (1)

Table (1): Explain the mental processes agreed upon by experts, in order

Mental processes	Degree	Significance
The sharpness of attention (attention)	93%	√
attention concentration (focus)	90%	√
kinetic response	40%	×
Expectation	46%	×
Reaction	33%	×
Sense	30%	×
Attention distribution	28%	×
mental perception	26%	×
Intelligence	22%	×

The first test / Borden Infemon test to measure attention: (1:191)

It is a form containing (31 lines) of numbers, each line contains (40 numbers), so the test contains (1240 numbers) and the numbers in each line are composed of groups drawn up in a codified manner, and each of them consists of (3 - 5 numbers) Different distribution and arrangement to ensure that it is not preserved from the laboratory and tests for aspects of attention are: -

Upon hearing the word Start, the player turns the model at the moment the stopwatch is started and begins to search and cross out the number (97) from one line after the other and from left to right.

· Test time is only one minute.

The following signs are used:

E = (S-B)X100

S

Attention = E X A

A = The plenty of numbers that you looked at from start to stop.

S = The number of numbers that should be omitted in the foreseeable part.

B = the number of numbers dropped from finishing + The number of numbers that are crossed out wrongly

E = coefficient of the validity of work by the equation

The second test: - Attention concentration test

· The same method used in (the first test) to measure the intensity of attention, but at the same time, it operates the distraction device.

· Method for calculating the results - the following indications are extracted: -

• Network productivity when measuring the intensity of attention in a quiet state = U 1

• Network productivity when measuring the intensity of attention in case of exciting situation = U2

Concentration significance = difference between the two units

The focus of attention = U1 - U2

(The lower the extracted value, this indicates the higher the concentration in the laboratory) (2:38)

Second: defining the basic skills of football

Define the tests for measuring basic football skills:

After the basic skills were identified, the test for each skill had to be determined. The researchers used the sources and references for football. For each skill, three tests were placed in the questionnaire and were presented to a group of (experts and specialists), who are 15 experts in tests and measurement in football. And the science of sports training, and after collecting the forms and emptying the data, they were statistically processed, and the test that gets the ratio of (53.33%) or more were accepted.

Tests were accepted according to the opinion of experts and specialists, which obtained a ratio of (53.33%) or more, and excluding the tests that did not get the required percentage,

Research tests:

1. **Handling**:

· Test name: Handling test. (3:47).

(Handling towards a small target is a distance of (12) meters).

- **The objective of the test**: to measure the accuracy of handling.

- **The tools used**: (5) footballs, a small target, dimensions of 110 x 63cm, a tape measure, Burke.

- **Test procedures**: by drawing a starting line with a length of (1) m and a distance of 12 m from the small target and stationary balls are placed on the starting line.

- **Performance description**: The student stands behind the starting line facing the small target, and begins when giving the signal by handling the balls towards the target to enter it, and each student is given five consecutive attempts.

- **Registration**: The score is calculated by the total score obtained by the student from handling the five balls as follows.

1. Two scores for each correct attempt entering the small goal.

2. One score if he hit the upright or crossbar and did not enter the goal.

3. Zero if the ball is off the target.

2.Rolling test:

- **Name of the test**: Rolling the ball in a zigzag line (Zakzak) (3:47).

- **The goal of the test**: to measure the player's ability to control the ball while running between it.

- **The tools used**: (10) footballs, stopwatch.

- **Test description**: (10) characters are placed in a straight line, the distance between each person and another (2) m, the distance between the starting line and the first person (2) m,

- **The method of performance**: The player stands with the ball on the starting line at the starting signal. The player runs between the strings winding until he reaches the last person and turns around him and returns to the starting line in the same way.

- **Registration**: the player is counted for the time of the second-closest from the moment he was given the starting signal until he returns to the finish line again.

3- Scoring test:

- **Name of the test**: Scoring on a target divided into squares (11) m (3:47).

- **The goal of the test**: to measure the accuracy of scoring.

- **Instruments used**: tape measure, soccer, goal divided by ropes into nine sections, Burke to draw the penalty point.

- **Method of performance**: The laboratory stands (11) meters from the target and when the starting signal is given, the laboratory will score

- **Registration**: (3) attempts are given to the favorite foot, as points are scored according to the location.

Preparing the educational curriculum:

First: The preparatory department, for (15) minutes, and includes:

a. **Introduction and general warm-up**: Students

are stopped in a unified format, taking absences, preparing tools for the success of the educational unit, and giving general exercises to all members of the body and its duration (5) minutes.

b. **Special warm-up**: Exercises are given in the skill under study and the working parts and muscles to perform this skill and its duration (10) minutes.

Second: The main section (25 minutes) and is divided into two parts:

a. **Theoretical section**: The skill is explained by the teacher, and then the skill is presented to explain the correct way of performing the skill and its duration (10).

b. **Applied section**: The given and assigned skill for each educational unit is performed and applied according to the number of iterations assigned to each skill with guidance and error correction by the teacher and his duration (15).

The third: the final section (5 minutes):

In it, a small game is given that serves the main department, or calming and relaxing exercises are given, with some guidance given to the students, then the educational unit is finished, and the greeting and leave are quietly abandoned.

Main experience:

Tribal tests:

The pre-test of the research sample was conducted at exactly nine o'clock in the morning, and all variables were set in terms of time, tools and devices, as well as the auxiliary work team to be applied when conducting the (post-test) i.e. before the implementation of the educational curriculum and the tests, were conducted on the stadium of the College of Physical Education and the Sports Science University of Kufa As the researchers, and with the assistance of the auxiliary team, applied the tests according to a preset sequence, mental processes (attention and focus) were tested, then skills (handling, rolling, and scoring) were measured for the two research groups.

Application of the educational curriculum:

The educational curriculum has been applied to the individuals of the research sample with two educational units per week, where the educational curriculum for (handling, rolling and scoring) skills was applied using the (K.W.L) strategy as it was applied by conducting educational units for all the skills studied,

After that, the remaining skill units were completed, as the researchers conducted the unit (fifth) end test for each skill to determine the level of learning of the skills in question.

Post-test:

the post-test of the research sample was conducted, as the researchers conducted the post-test on the research variables, which is the mental processes (attention and focus) test, after which the skills (handling, rolling and scoring) of the two research groups were measured in the stadium of the College of Physical Education and the Sports Science University of Kufa After completing the implementation of the educational units with a strategy (KWL), as it was applied by conducting educational units for all the skills studied, the researchers made sure that the conditions were similar to the pre-test in terms of location and conditions and the presence of the assistant work team itself.

Statistical means:

The researchers used the Statistical Package for Social Sciences (SPSS)

Results and Discussion

This axis included displaying the results of statistics after statistically processing them and in line with the goals.

View, analyze, and discuss the results of the skill tests for the members of the research sample.

Table (2)

Between the arithmetic mean, the standard deviation, and the calculated value of t between the pre and post-tests in mental abilities and learning basic football skills for students.

Variables	Unit measurements	Pre exertion		Post exertion		(t) value	Significant	Significant type
		Mean	STD.EV.	Mean	STD.EV.			
Attention	degree	245.24	46.13	287.58	41.53	5.95	0,000	Sig.
focus	degree	79.57	18.81	87.74	17.39	3.37	0,000	Sig.
Handling	degree	4.54	1.01	7.73	0.78	7.74	0,000	Sig.
Roll-on	degree	35.29	3.64	29.55	3.83	6.01	0,000	Sig.
Scoring	degree	5.52	0.75	7.13	0.91	7.97	0,000	Sig.

From Table (2), the results indicated that there were significant differences between the pre and post-tests and in favor of the post-test in mental capabilities (attention and concentration) and basic skills (handling, rolling and scoring) in football, and researchers attribute this to the effectiveness of the educational curriculum applied to the experimental research sample with a strategy (KWL), where the curriculum contained a set of exercises that were carefully selected and selected to fit with the studied skills as well as the nature of the sample's age group. Qasim Lizam and others mention that learning does not happen by simply repeating movements and mathematical skills by players For training should be based on a standardized scientific basis to provide the level of their abilities and skills for the better "(6:30)

As the player needs during the learning process of motor skill full attention to the course of the motor performance in its full and partial form by receiving the main stimuli involved in the work that come from the sense of hearing and sight which leads to the formation of the motor mechanism by choosing the main stimulator appropriate to the motor work and thus the formation or choice of the motor response Suitable for the chosen stimulus, as well as the results showed that a development occurred in the concentration and focus of attention, since the method used gave the research sample a new opportunity to control and control ideas, which increased the ability of the focus to them by isolating the factors Distracted and trying to focus on the performance-related aspects that played an important role in the development of their ability to focus, "an athlete who has a deep focus is the one who can reconcile the physical and mental attitudes of his own being" (11:90), and external stimuli and their complexity are factors that negatively affect The quality of focus attention, and this is what Youssef Lazim Kamash and Nayef Zuhdi Al-Shawish went to in that (concentration of attention is an unintended direction of feeling towards a certain subject without human intervention in it and this trend results from the strength of external stimuli and the weak effectiveness of human will while directing the focus of his attention) (9:38) .

Conclusion

The educational curriculum of (K.W.L) strategy has positive effects on developing mental processes (attention and focus) for studentsK, The educational curriculum of (K.W.L) strategy has had a positive impact on developing the basic skills of football for students,

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Molecular and Phenotypic Detection of Some of the Coded Genes for Virulence Factors in Mrsa and Mssa Isolated From Different Clinical Cases

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Abstract

The current study included the collection of 100 clinical samples obtained of patients reviewed in the laboratories of Ramadi hospital for women and children and Hit General Hospital and some civil laboratories within the city of Ramadi, including Bacterimea samples, Urine, abscesses, heart inflammation, wounds, rhinitis, osteoporosis, otitis ,Laryngitis, sputum (35, 20, 5, 6, 7, 5, 8, 7, 4, 3) samples respectively to investigate the presence of *staphylococcus aureas* . The results of diagnosis of bacterial isolates showed that there were 55 bacterial isolates belonging to the *S.aureus* bacteria after they were grown on the culture media and examined microscopic as well as the use of some of the tests on the chemical, and the diagnosis was confirmed using the Vitek2-compact system .The diagnostic results were confirmed by the use of genetic diagnosis. The results of the phenotypic investigation were identical to the results of the genetic investigation using the polymerase chain reaction technique in terms of possessing isolates producing both hemolycin (α, β), on the *HlA* encoding genes for the production of type $\alpha\alpha$ and hlB encoded for type β production, For plasma blood on the *co-a* gene, and the resistance isolates of methylation on the *mecA* gene.

Keywords: *virulence factors; phenotypic detection; MRSA; MSSA;*

Introduction

S.aureus is a public pathological cause that causes serious infections and is often life-threatening in the community and hospital.Its antibiotics have become more difficult to eliminate because of their high resistance to these antibiotics [(1)]. These bacteria cause infections ranging from simple pimples, impetigo, boils and abscess, to acute infections such as bacteremia and Toxic shock syndrome [(2)]. *S.aureus* is due to its ability to multiply rapidly and possess many virulence factors, such as the production of toxins and enzymes that have an important role in host tissue invasion and bacterial spread, among which the most important toxin is hemolysine, which is one of the most virulent elements, has been able to cause holes in the cell walls of the cells[(3)], The lactamase enzymes used by *S.aureus* contribute to the resistance of many antibiotics [(4)] It is characterized by the formation of biofilms and responsible for chronic injuries to resist the process of phagocytosis[(5)]. Methicilin Resistant *S.aureus* (MRSA) has shown high resistance to this antioxidant and after using it in treating the injuries in this bacteriaits and yet[(6)]. the *mecA* gene is responsible for the resistance of staphylococci to the penicillin group[(7)].

Materials and working methods: Samples Collection: 100 samples were collected from patients in Al Ramadi Teaching Hospital, Women and Children Hospital, Hit General Hospital, Health Centers and some private labs in Ramadi for the period from 15-7-2018 to10-12-2018.The samples included cases of endocarditis, bone marrow, ear, nose and throat.

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Suppurations and boils of the skin. Sputum bacteremia was collected under sterile conditions and of both sexes at different ages They were taken to the laboratory for culture.

Identification

Bacterial isolates were diagnosed using the methods used by [8], And by using culture, microscopic and biochemical phenotypes as well as Vitek 2Compact System.

Minimum inhibitory concentration (MIC) followed the preparation [9] of the double dilution method to determine the concentrations of the antigens under study for calculating the MIC of antagonists (Cefitraxone, Erythromycin, Amikacin, Vancomycin, Tetracycline, Gentamycin, Ciprofloxacin, Oxacillin,Trimethprim,Rifampcin)by using Microtitration plates, concentrations starting at (1/2) and ending at (256) mg / ml were obtained for all antidotes under study except Methicillin and Cefoxitin used for diagnosis.

Phenotypic investigation of some bacterial virulence factors The productivity of some virulence factors, including the production of hemolysin, the capsule, the biofilm, the coagulase and the DNA enzyme, was investigated using various laboratory culture media.

Molecular investigation of some agents of virulence in bacterial isolates polymerase chain reaction (PCR) A-

The single polymerase chain reaction (PCR) was investigated for some of the genes responsible for virulence factors in Methicillin resistant *Staphylococcus aureus* (MRSA) bacteria, using DNA fragments with a limited number of Oligonucleotide nucleotide, which acts as primers for virulence genes in the MRSA strain, which included the genes of *coa, mecA, hla.hlb*.

Bacterial Genomic DNA Extraction B- The extraction was carried out according to the Bacterial Genomic DNA extraction kit and its instructions from Geneaid. **C- Preparation of PCR Master Mix** Using the PCR PreMix AccuPower kit and its instructions from Bioneer (South Korea), he prepared the polymerase chain reaction mixture. **Preparation of Agarose Gel D-** Prepared by[10].

Electrophoresis Agarose Gel E- The electrophoresis of the prepared acarose gel was carried out by 1.5% under a difference of voltage (100) volts and current (80) milliamps at a time of 60 minutes for the purpose of detecting the DNA Bands bundles extracted representing PCR products (PCR products) according to the method [10].

F- Primers Especially Special prefixes for diagnostic, virulence and antibiotic resistance genes were used by the researcher using NCBI GeneBank and Primer 3 plus prefixes, as well as processing of these prefixes by Bioneer / South Korea, as in a table(1).

Table (1) used prefixes under study, sequence of nitrogenous bases and volumes of amplification products

Amplification output volume (bp)	Sequence of nitrogenous bases3'-5'		Name of initiator	Initiator type
456	F	TAAAGAAAATGGCATGCACAAAA	hlA gene	Agents of Virulence factors
	R	GGTCCCCAATTTTGATTCACC		
428	F	ACGATGGAACATTGGATATGA	Co A	
	R	CCCATATGTCGCAGTACCATC		
527	F	TTTGCTTCTATTTTGTTGTAAGCTAT	hlB gene	
	R	GCTATCATTATCGAATCCACAAC		
466	F	ATCAAAATTGGGTACAAGATGATACCT	mecA gene	
	R	AATTCACCTGTTTGAGGGTGGAT		
R: Reverse primer F: Forward primer				

Result and discussion Isolation and diagnosis of *Staphylococcus aureuas* a-

The results of bacterial transplantation showed 70 isolates out of 100 samples where growth occurred, while 30 samples did not show growth until after 48 hours incubation period. The samples were distributed (35, 20, 5, 6, 7, 5, 8, 7, 4, 3), The cases, (bacteremia, Urine, abscesses, heart inflammation, wounds, rhinitis, osteoporosis, otitis,Laryngitis,sputum) respectively. The lack of growth may be due to the small number of bacterial cells or the absence in the sample taken by cotton swabs and encountered occasional power outages or the fact that the bacteria need anaerobic an aerobic lap conditions where these negative cases were concentrated in the samples of cases of bacteremia, pyoderma, boils and endocarditis Heart, wounds, osteomyelitis and otolaryngitis The results of isolation and diagnosis showed the prevalence of positive bacteria of Melon Cram on negative bacteria, where the number of positive isolates reached 61 isolates representing 87% of the total isolates that gave positive growth, while the number of negative isolates of Melon Cram The isolation and diagnosis results indicate that the staphylococcus aureus is prevalent on the rest of the staphylococcus species as it reached 98.2% of the total isolated staphylococcus species [(11)] *s. aureus* isolated by 56.8%.

The results of the sensitivity test for S. aureus isolates under study against (10) antibiotics showed that the isolates of these bacteria varied in their resistance to all antibiotics under test as all isolates were 100% resistant to the antibiotic Methicilin, while the resistance was towards (Ciprofloxacin. , Vancomycin, Tetracyclin, Erythromycin, Trimethoprim / sulfamethoxazole, Ciftraxone, Gentamycin),in the rate of (91%, 51%% 76, 80%, 27%, 36%, 58%) respectively, while Rifampcin and Amikacin were 100% sensitive

Phenotypic detection of some virulence factors b-Production of Haemolysin Formation

The results of the present study showed that 50 isolates out of 55 isolates (92.7 %) produced this enzyme, The results of the present study are consistent with the findings of [(12)]where all *S. aureus* isolates of hemolycin were produced by 100%.

Capsaule Formation

The susceptibility of *S.aureus* staphylococcus aureus to the composition of the capsule under study reached 27.2% of the total of 55 isolates.the results were in agreement with the result[(13)] which recorded 42% of S.aureus.

Biofilm formation by the isolates of S aureus bacteria under study

The results showed that all the bacterial isolates under study (55) isolates arecapable of producing the biofilm using Microtitration plates method (MTP).where the results of our current study agreed with the results of researchers[(14)].

Table2: Susceptibility of S. aureus isolates to MTP formation

Isolates source	Isolates number	Absorption values for the number of isolates										Mean
Blood	26	.244	124.	160.	147.	394.	418.	520.	412.	581.	486.	263.
		.416	.130	150.	300.	340.	132.	135.	166.	170.	200.	
		.140	125.	220.	341.	201.	221.					
Wound swab	4	160.	073.	105.	106.							364.
Urine	11	147.	120.	121.	407.	170.	123.	130.	320.	330.	405.	220.
		193.										
Bone marrow	2	120.	107.									167.
Endocarditis	4	105.	100.	107.	103.							336.
Throat swab	2	164.										156.
Sputum	1	213.										213.
Ear swab	2	102.	087.									095.
Pus abscess	3	081.	102.	085.								587.
Nasal swab	1	090.										090.

Plasma coagulation enzyme Formation Coagulase

All the isolates of S. aureus bacteria under study showed that they have the ability to produce plasma coagulant enzyme (90.9%). Our results are in line with the results of[15] which produced 100% isolates for plasma coagulant enzyme.

DNase Formation

The results of this enzyme revealed that 43 of the 55 isolates were isolated by 78%.our results were agreement with the results[16].

Molecular detection of virulence genes c-

The results of the detection of the gene responsible for the production of coenzyme Coagulase (*Co a*) ، that the proportion of the isolation of MRSA isolates of the *Coa* gene is (95%),that came with results[17] and the proportion of the presence of the gene encoded *coa* gene for the production of cookies in the current study is expected as researchers[18] Production of all the strains of *S. aureus* bacteria, including MRSA. The *mecA* antibiotic resistance gene (*mecA*) showed that all 40 MRSA isolates (100%) possessed the *mecA*-resistant antibiotic gene *mecA* (Figure 1). 74.1% of *mecA*-containing isolates were consistent with the results [19], which obtained 100% of the presence of *mecA*

gene in its isolates, while the detection of the gene responsible for total erythrocyte degradation α) *hla* gene) The percentage of *hla* gene in the current study is (97.5%) where these results are consistent with [20], which possessed isolation belonging to your Tria S. aureus on the *hla* gene 100% As for the gene responsible for partial erythrocyte degradation (l) gene (*hlb*), the results showed that the proportion of its presence in isolates under study is (75%) and this was contrary to what reached [21] that got a 40% presence of the *hlb* gene in *S. aureus*.

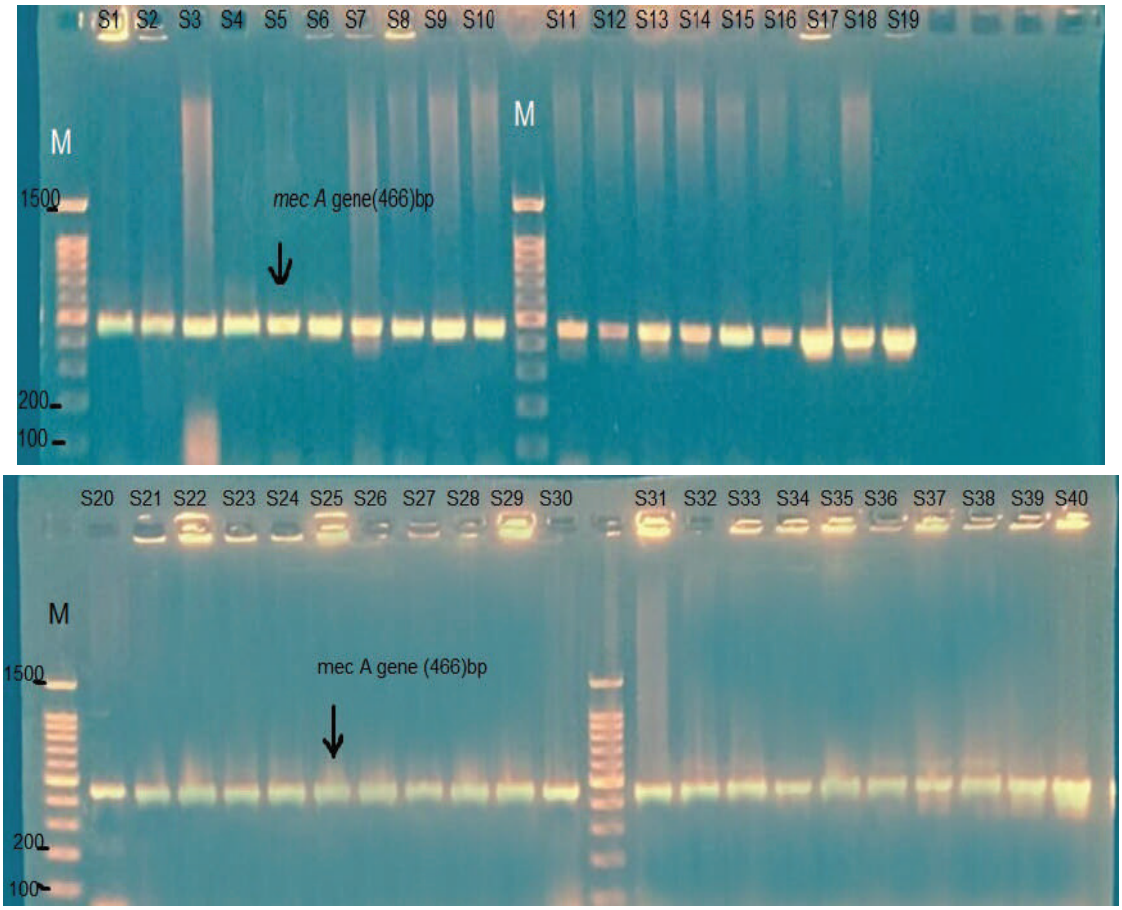


Figure (1) *mecA* gene amplification products for Staphylococcus aureus bacteria and electrically carried on the acarose gel (1.5%) and voltages (100) for an hour using PCR technique. Since M = DNA Ladder marker (100-1500bp), S = Staphylococcus aureus, all isolates showed a positive result of the mecA gene responsible for methicillin resistance in MRSA

Genetic detection of genotypes of some virulence factors for each type of staphylococcus under study, including the genotype responsible for the production of both types of hemolysin, the methicillin-resistant gene, and the blood plasma coagulation gene, was confirmed by the results above which confirmed the phenotypic detection results. Determinants (virulence factors) In addition, the above explanations regarding the results of phenotypic detection are fully consistent with the results of genetic detection

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Histological Changes Induced by the *Fusarium graminarum* Silver Nanoparticles in Some Organs of Male Albino Mice

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Abstract

The aim of this study was to investigate the effects on male albino mice of the histological change of Fusarium graminarum, silver nanoparticles (AgNPs), and the traces of these nanoparticles in the liver, and small intestinal. The experiment consisted of 40 mice divided into two groups, the first group of 20 mice was considered to be the control animals and the other group was three weeks of treatment with a dose of AgNPs (0.1ml / day). Microscopic examination of the liver showed the disturbed structure of the hepatic lobule, hepatocyte hypertrophy with severs infiltration of inflammatory cells, kupffer cell proliferation, coagulates, necrosis, and hydropic degeneration. In addition, the small intestinal section showed hydropic degeneration in epithelium cells of the mucosa and an increased number of inflammatory cells also, the proliferation of goblet cells in epithelium cells of the intestinal mucosa and sloughing of necrotic villi into the intestinal lumen.

Keywords*: Bionanotechnology, Fusarium graminarum, AgNPs ,liver,small intestinal*

Introduction

Silver nanoparticles (AgNPs) have unique properties that help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures. The major methods used for AgNPs synthesis are the physical and chemical methods. The problem with the chemical and physical methods is that the synthesis is expensive and can also have toxic substances absorbed onto them [(1)]. AgNPs are presently the most heavily used, being incorporated for their antimicrobial characters in pillows, food storage containers, clothing up the holster and even toys for children [(2)]. The term "Myconanotechnology" for the research carried out on the synthesis of nanoparticles(NPs) by the fungal system. It is the interface between nanotechnology and mycology. Several fungi have the capability to produce various NPs like silver, cadmium sulfide, platinum, gold, silica, zirconia, titanium, and others [(3)]. Fungi can produce larger portions of NPs due to they can produce an excessive complete of mycelia and secrete massive totals of proteins which at once translate to greater productiveness of NPs [(5)]. In our study, we evaluated the effects of *Fusarium graminarum* AgNPs in vivo toxicity of the liver and small intestinal of male albino mice.

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Materials and Methods

- ***Fusarium graminarum***

Fusarium graminarum isolated from the decayed banana fruit, it isolate was maintained by serial culturing on potato dextrose agar medium, incubated at 28^{O}C for 4-5 days.

- **Potato dextrose broth medium (PDB)**

Suspend 24 grams in 1000 milliliter distilled water(d.w). Heat to dissolve the medium totally. Sterilize by autoclaving at 15 lbs pressure and temperature 121°C in 15 min. It was cooled down then chloramphenicol antibiotic (250mg /liter) was added. This broth was used for culturing of *Fusarium graminarum* in the extracellular synthesis of AgNPs.

- **Potato dextrose agar medium (PDA)**

It was prepared that 39gm PDA was dissolved in 1000ml d. w. according to the instructions of the manufacturer. Sterilized for 15 minutes by autoclaving at pressure of 15 lbs and temperature of 121 ° C. It was cooled to 45-50oC, and the antibiotic chloramphenicol (250mg / liter) was added to sterile Petri dishes afterwards. *Fusarium graminarum* isolates were cultivated and protected using this medium.

Preparation of *Fusarium graminarum* AgNPs

Fusarium graminarum mycelia used to be inoculated in 250 ml flasks, every flask containing 100 ml of the PDB medium, then incubated for 5 days at 25 ± 2 ° C.Later, mycelia have been accumulated by way of purification via Whatman (No. 42)filter paper and washed thrice with d. w. to dispose of the traces of the medium on fungal biomass. The washed mycelia had been re-suspended in 100 ml d. w, then incubated at 25°C for 24 hours. Once more, mycelia were accumulated by using filtration via. Then, cells filtrate used to be divided into two parts, first one dealt with 1mM of $AgNO_3$ solution and incubated at room temperature, which adjustments color to brown regarded as Positive control, while the second part left except the addition of $AgNO_3$ to the cells filtrate barring a color change viewed as negative control. After adding AgNO3, the finding of AgNPs was mainly done by visual observation of color alteration of the fungal filtrate. A dark brown hue in appearance. The exact size, concentration, crystal morphology, aggregation state, and even bio-conjugation configuration were measured using the particle techniques:

1)Atomic absorption spectroscopy flame 2)UV–Visible spectroscopy

3) X-Ray diffraction 4)Atomic force microscopy

Study design

Forty Male albino mice aged between 8-12 weeks, weighing 20-28 gm. was obtained from National Center for Drug Control and Research, housed under standard conditions in the animal houses in the biology department in the college of science/ Mustansiriyah University.A study on acute toxicities of *Fusarium* AgNPs was carried out. It was administered orally (0.1ml/day) in ten mice for three week, AgNPs showed very low toxicity and no death occurred in the mice following the oral administration of the maximum concentration 2.5µg/ml.The mice were divided into two groups. Then the groups were inoculated as a following:

Group 1: twenty mice inoculated orally by stomach tube (0.1ml/day) normal saline considers as a control group.

Group 2: twenty mice inoculated orally by stomach tube AgNPs (0.1ml/day) for three week considers as an AgNPs group.

Histological study

All tissue specimens were obtained after sacrificed mice from 40 mice includes (liver and small intestinal), then they were kept in the fixative solution (formalin10%). After the fixation, sections are processed, embedded in paraffin and 5µm thick glass mounted sections are prepared, which are routinely stained with Haematoxylin and Eosin (H&E)[6].

Results and Discussion

The synthesis of AgNPs by using *Fusarium graminarum* was showed after adding AgNO3 to the filtered cell. The color of the mixture changed from colorless to dark -brown compared with negative control remain colorless.These results corresponding with [7,8] reported the appearance of

brown was a clear indicator of the synthesis of AgNPs in the reaction combination.Determine of *Fusarium* AgNPs surface morphology and sizes were measured, using the AFM. The images of AFM for *Fusarium* AgNPs in figure (1) represented particle size distribution, which is 94 nm in diameter.

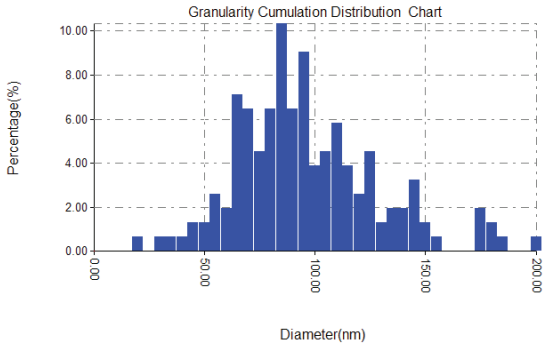


Figure (1): *Fusarium* AgNPs Granularity volume distribution

While in figure (2) showed AFM picture in two dimensions (2D) and three dimensions (3D), it explains structural shape for grains, found that the root mean square is 11.6 nm and average roughness is 9.33 nm.The AFM is a very good technique for measuring surface morphology and fine structure of NPs [9]. The AFM topology is beneficial in revealing the actual dimension and shape of AgNPs [10].

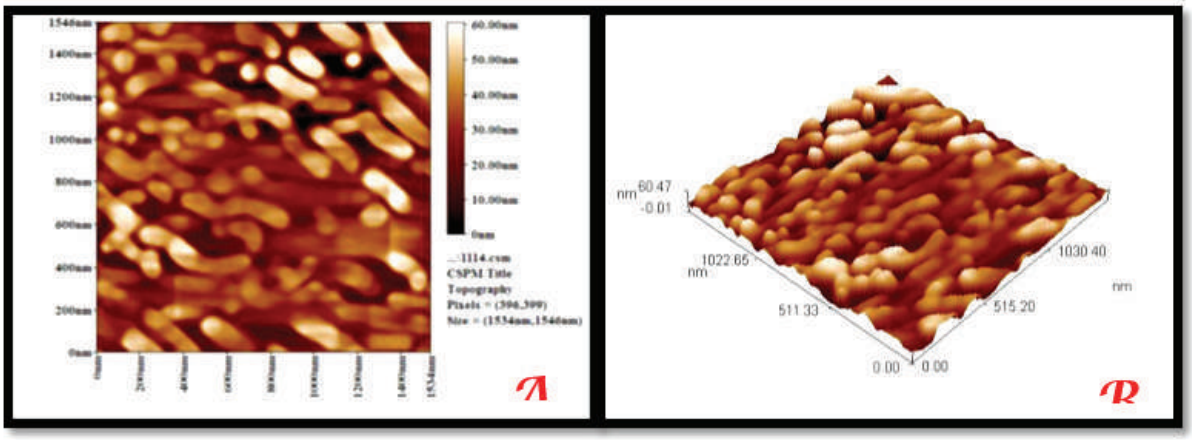


Figure (2): *Fusarium* AgNPs AFM images (A) two dimensions 2D, (B) three dimensions 3D

The XRD pattern of *Fusarium* AgNPs as revealed in fig. (3), the diffraction peaks at 77.31°,64.32°, 44.22°and 38.05° were identical with the (311,220,200,111) the faces of the face-centered crystal cube structure, thus 28.225 nm was average crystallite size.

Our finding corresponds to Shafiq *et al*. [11] who revealed that the XRD diffraction measured in AgNPs resulted in four intense peaks and this further confirms that AgNPs made in extracellular filtration is present in the form of AgNPs. Also, Mahmoud *et al*. [7] reported four distinct diffraction peaks at angles 38.15°, 44.18°, 64.63°, and 77.50° correspond to (111, 200, 220 and 311) planes of the face-centered cubic.

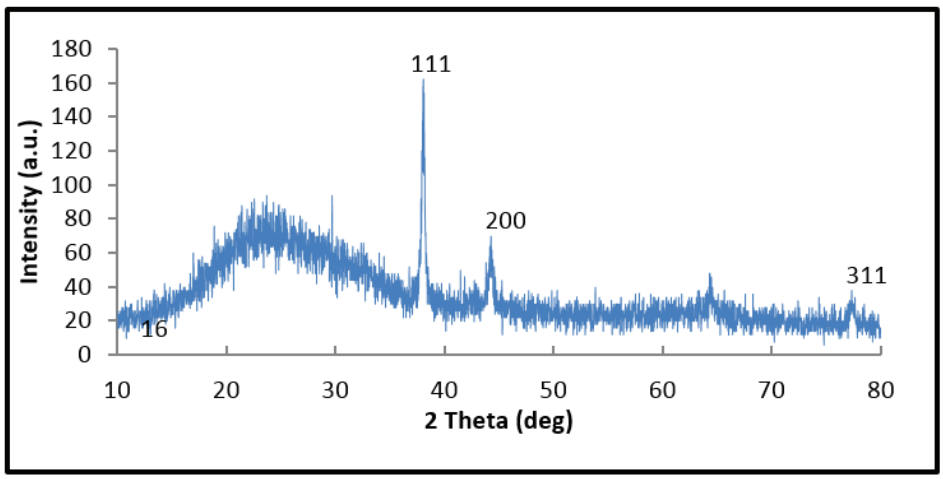


Figure (3): *Fusarium* AgNPs X-Ray pattern

The UV-Visible spectroscopy confirms, existence of *Fusarium* AgNPs by measuring the absorbance of the bio-reduced solution between (300 - 800 nm) wavelengths. Extinction spectrophotometer of UV and visible Vis) light (UV-Vis spectrum) confirmation of the presence of *Fusarium* AgNPs is permitted because of a distinctive Plasmon resonance, figure (4) which revealed a peak absorption at 420 nm.

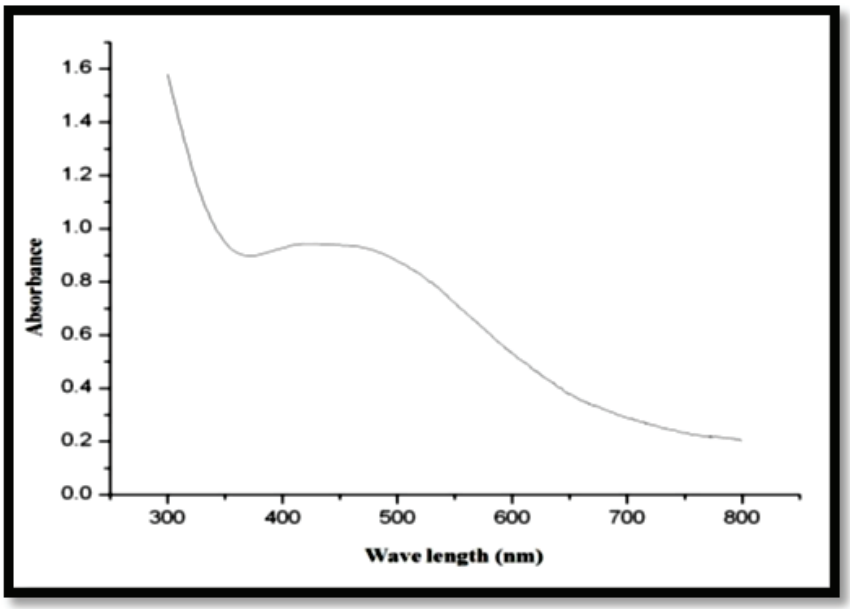


Figure (4): *Fusarium* AgNPs UV-Visible spectroscopy

The results corroborate those of previous studies such as Birla *et al*. [12] who illustrated that the AgNPs is specific when absorption peak at 420 nm. There was one peak refers to the synthesis of spherical NPs, and it is known that there is a very close relationship between the absorption spectra of UV-Vis and the size and shape of AgNPs. Also, Singh *et al*. [13] showed that the production of AgNPs with maximum surface Plasmon resonance peak at 420 nm by using endophytic of *Fusarium spp*.

A. **Histological study**

1. **Liver tissues**

Gross examination of liver obtained from the control group showed normal appearance of hepatocytes, central vein, and sinusoids in all liver mice (Figure 5A).

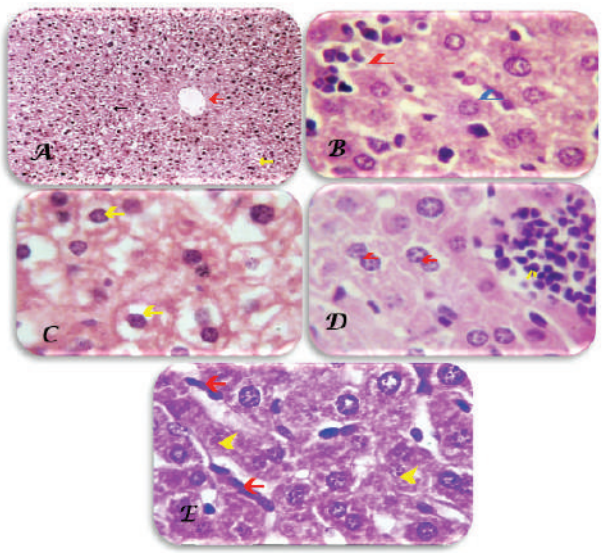


Figure (5): Cross section of the *Fusarium* AgNPs effects after three week in liver of mice detected by H&E staining:

A. control shows normal appearance of hepatocytes (black arrow),central vein (red arrow) and sinusoids (yellow arrow)(X10).**B:** dilation in sinusoids (blue arrow) and infiltration of inflammatory cells(red arrow),**C:** hydropic degeneration in hepatocytes (yellow arrow) ,**D:** proliferation of hepatocytes (red arrows) with aggregation of inflammatory cells (yellow arrow),**E:** proliferation of kupffer cells (red arrows) with degradation of chromatin cells (yellow arrows).B,C,D and E(X40).

Histological changes investigation of liver sections of the treated mice with *Fusarium* AgNPs after three weeks showed, dilation in sinusoids and infiltration of inflammatory cells (Figure 5B) and hydropic degeneration in hepatocytes (Figure 5C). On the other hand, the proliferation of hepatocytes with the aggregation of inflammatory cells (Figure 5D). Also, congestion in sinusoids and proliferation of kupffer cells with the degradation of chromatin cells were observed in many liver sections (Figure 5E).

The liver is the first target organs for *Fusarium* AgNPs, which effect mitochondria by increasing the level of reactive oxygen species as a result production of ATP which may be led to hepatocyte damage [14]. The study of Kawata *et al*. [15] reported when the mice treated with AgNPs revealed lymphocytes infiltration also mitochondrial activation and antioxidant production less effected.

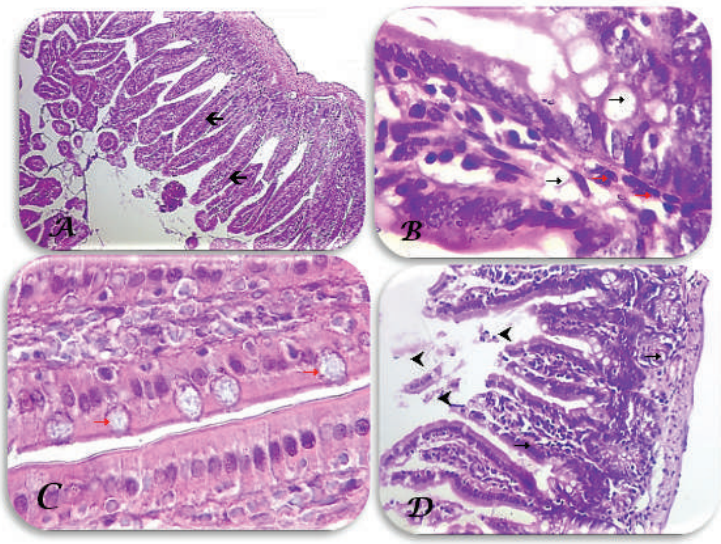
On the other hand, the proliferation of kupffer cells may be to the role of AgNPs ingestion from hepatocytes in tissue [16].This result agrees with Kermanizadeh *et al*.[17] which reveal the role of kupffer cells in the anti-inflammatory response by release different kinds of cytokines. Hydropic degeneration in hepatocytes, it can be explained that the AgNPs affected the mechanism of action of the sodium-potassium pump (Na^{+}-K^{+} pump) that regulates the passage of fluids to and from the cell, which led to the accumulation of fluids within the cell.

2. **Small intestinal**

Histological observation in control mice, normal villi and epithelium cells of the small intestine mucosa (Figure 6A). The following changes were revealed in the sections of small intestine after administration of *Fusarium* AgNPs after three weeks:

a) Hydropic degeneration in epithelium cells of the mucosa and increased number of inflammatory cells (Figure 6B) also, the proliferation of goblet cells in epithelium cells of the intestinal mucosa (Figure 6C).

b) Distortion of mucosal architecture, hemorrhage and sloughing of necrotic villi into the intestinal lumen (Figure 6D).



igure (6): Cross section of the *Fusarium* AgNPs effects after three week in small intestinal of mice detected by H&E staining:

A. control shows normal appearance of the villi and epithelial cells of the intestinal mucosa (black arrows) (X4).**B:**hydropic degeneration in epithelium cells of mucosa (black arrows) and infiltration of inflammatory cells (red arrow)(X40), **C:**proliferation of goblet cells in epithelium cells of the intestinal mucosa (yellow arrow)(X40), **D:** distortion of mucosal architecture, hemorrhage (black arrows) and sloughing of villi into intestinal lumen (arrowheads) (X10).

The results above, show the toxic effect of *Fusarium* AgNPs on the small intestine of mice, as histological changes began with infiltration of inflammatory cells that can be explained by the toxic effect of the AgNPs, which resulted in an inflammatory reaction in the tissues of the small intestine, which led to these cells being attracted to the injury site to remove tissue damaged [18].

On the other hand, the increase in the number and sizes of goblet cells due to exposure to the toxicity of AgNPs that caused damage the epithelial cell microvilli as well as intestinal glands. It may be hypothesized that loss of microvilli reduced absorptive capacity of intestinal epithelium and their irritation to the epithelial lining of the small intestine leading to an increase in their numbers and sizes and it is believed to be a defensive process in order to increase mucus secretion and that reduces the toxic effect on the epithelial lining of the small intestine, finally destruction of the microvilli [19]. Just as the presence, dissociation and separation of epithelial cells lining and necrosis of villi-induced cells the toxicity of the AgNPs, which has an effect on the lining of the blood vessels, results in increased vessel permeability and exit by occurrence, the fluids are out [20]. In addition, in *Fusarium* AgNPs treated mice, microvilli on intestinal absorptive cells were found to have been severely damaged and destroyed. Nanoparticles may be taken across the intestinal barrier, as particles with diameters below 1 μm are particularly susceptible to absorption by the intestinal lymph system [18]. Tang *et al*. [21] also reported that AgNPs cause degenerative changes in some endothelial cells, leading to the loosening of the tight junction between the endothelial cells and AgNPs passing through these crevices. Throughout our study, we observed the structural changes and loss of microvilli, which may give passage to AgNPs for entry into the intestinal wall and consequently into portal circulation and systemic circulation.

Conclusion

We concluded that the size of the AgNPs, due to its ability to enter and translocate within the cell, its toxic effects on the cell and the cell organelle varies. This may therefore be misunderstood that all NPs are toxic and most likely only free NPs, which may penetrate small cell organelles such as mitochondria, may cause adverse effects on health. Therefore,*Fusarium* AgNPs may be less toxic if the exposure time, concentration, or exposure method is less.

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Conflict of Interest:Non

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Seroprevalence of HTLV-type-1 and type-2 among Blood Donors in Some Iraqi Provinces

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Abstract

Context: Human T-cell lymphotropic viruses Type 1&2 (HTLV-1&2) screening test is endemic in some Iraq's provinces. HTLV-1&2 test is not performed in routine screening tests of Iraqi blood donors banks. With my thought it represent essential test to prevent complications the virus in our country and other neighbour countries in Middle East. The results of the following study do not included all Iraq's provinces but only seven provinces (Baghdad, Karbala, Al-Qadisiyyah, Al-Najaf, Al-Muthanna, Al-Basrah, Wasit). **Aims**: to review prevalence of HTLV-1&2 infections among Iraq's blood donors. **Settings and Design**: The study where performed in Iraqi National blood bank in Baghdad city and national companies for blood donation in Karbala and Najaf provinces, the study included 15239 blood donors. **Methods and Material**: ELISA (Murex HTLV 1&2 Ab, USA, LOT: D3101210) 480.T was used for HTLV screening for detection antibodies to HTLV-1&2 in the serum of human followed by accurate confirmatory test CMIA (Chemiluminescent microparticle immunoassay) architect plus for confirmatory. **Results**: The study included 15239 blood donors, thirty nine of him appeared seropositive for HTLV-1&2, and the prevalence of HTLV-1&2 was appeared in some provinces of Iraq country highly prevalence of HTLV-1&2 were seen in Baghdad 24(61.5%), then Karbala 5(12.8%), Al-Qadisiyyah 4(10.2%), Al-Najaf 2 (5.1%), Al-Muthanna 2 (5.1%), Al-Basrah1(2.5%), and Wasit province1 (2.5%) respectively. **Conclusions**: first study conducted in Iraq in 2015 appeared the prevalence of HTLV-1&2 infections among blood donors only in six provinces and not included all Iraq's provinces. So another study is requested for determination the distribution of HTLV-1&2 in all Iraq's provinces, districts and areas.

Key-words: *HTLV-1&2, Retrovirus, CMIA technique, ELISA technique, blood donors.*

Introduction

Human T-lymphotropic virus type 1&2 (HTLV-1&2) is retroviral group, mainly affects T-lymphocyte.[1] Their infections are diagnosed after blood donation. HTLV-1 is the causative agent of "myelopathy/tropical spastic Para paresis (HAM/TSP)".[2] HTLV-2 virus is appeared among indigenous America's people,[3] Brazil population.[4] HTLV-1 infections is endemic in Australian populations,[5] another factor determinant of HTLV-1 prevalence is economic status.[6] In Japan showed incidence of HTLV-1 per year.[7] HTLV-1&2 is endemic in south western of Japan, Caribbean islands, Africa, Austral-Melanesia,[8] north eastern Iran (in Mashhad city).[9,10] HTLV-1 is lifelong disease.[11] HTLV-2 included 4 subtypes.[12,13,14] the aim of the study was to review the prevalence of the virus among Iraq's blood donors.

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Subjects and Methods

Samples: The study included 15239 blood donors where collected by Iraqi National blood bank and donation campaign in Karbala and Najaf. A donor selection criterion was followed, agreement was obtained and the study aim was explained to the blood donors; all ethical aspects were considered and confidentiality was maintained.

Instrumentation: ELISA (Murex HTLV 1&2 Ab, USA, LOT: D3101210) 480.T was used for HTLV screening followed by CMIA (Chemiluminescent microparticle immunoassay) architect plus for confirmatory. ELISA Kit is an enzyme-linked immunosorbent assay used for antibodies determination, was used for detection the presence of antibodies against HTLV-1&2 virus in the serum. This kit was used for screening the blood donors and diagnosed the clinical conditions of infection with HTLV-1&2, and the result of ELISA was followed by accurate confirmatory by CMIA technique.

Results

Collection of samples depend on blood donors of voluntary campaigns conducted in National Iraqi blood donor bank in Baghdad and occasional voluntary campaigns conducted in Karbala and Najaf, continued between June/2015 to December/2015 to determined HTLV 1&2 infections, the study included 15239 blood donors. The result of the current study was limited on volunteers from National Iraqi blood donor bank in Baghdad and occasional voluntary campaigns conduct in Karbala and Najaf only, the volunteers of Karbala and Najaf campaigns were from Baghdad city and some Iraq's provinces; of which: Al-Qadisiyyah, Al-Muthanna, Al-Basrah, and Wasit province, in addition to volunteers from Karbala and Al-Najaf provinces. Numbers of HTLV-1&2 infection and their ratio in Baghdad and other Iraq's provinces appeared as shown in (Table-1) and Fig. (1). Distribution of the donors' age lies between 20 and 57 years (mean of the age was 36) as shown in (Table-2).

Highest seroprevalence of HTLV-1&2 were appeared among blood donors in Baghdad city; represent the centre of Iraq.

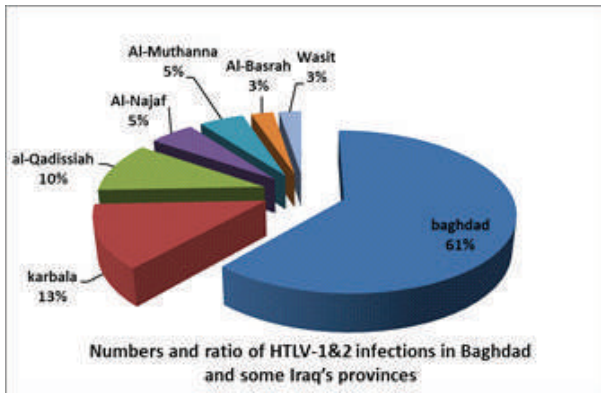


Fig. (1): Distribution of HTLV-1&2 infections among blood donors in some Iraq's provinces.

Discussion

This is the first study was performed on Human T-lymphotropic virus type 1 and 2 (HTLV 1&2) in Iraq in 2015. No previous studies published on HTLV-1&2 in Iraq, where combined the screening test (by using ELISA technique) with accurate confirmatory test CMIA (Chemiluminescent microparticle immunoassay). 39 individual have infected with HTLV 1&2. Total of 15,239 blood donors collected from June 2015 to December 2015 was screening test for antibodies against HTLV-1&2. only 0.25% of which is seropositive to HTLV-1&2, 38 of which are male and only one is female, highest prevalence was appeared in Baghdad 24(61.5%) due to the high donation in Iraqi National Blood bank in Baghdad, in spite some Baghdad people participated in donation in Karbala and Najaf campaigns. So we cannot depend on this result to evaluate the real distribution of infection in Iraqi's provinces compared with Baghdad city.

Another study performed on Human T-lymphotropic virus type 1&2 in Iraq in 2017 correlated HTLV 1&2 infections with lymphoma and leukemia patients by their effects on human lymphocytes and other blood components.[15] Similar study conducted in Northern Pakistan where included 2100 blood donors, 0.19% were positive to HTLV-1 only not HTLV-2 and all donors were male.[16] In Saudi Arabia, the seroprevalence of HTLV-1is positive in 0.006% of blood donations, one of these positive donors was a native Saudi while the other two were Indian expatriates.[17] In Kuwait, the study included HTLV-1&2 infections; shown that the result of seroprevalence of HTLV-1infection among Kuwaiti donors was 1:7212 out of 46,039.[18] In

Iran the overall prevalence for HTLV-1 was 0.119% among blood donors in seven provinces of Iran (in Razavi Khorasan, seropositive for HTLV-1 were (0.38-1.16%), in West Azarbaijan, seropositive for HTLV-1 were (0.34%), in Ilam, seropositive for HTLV-1 were (0.21%), in Hormozgan, seropositive for HTLV-1 were (0.18%), in Alborz, seropositive for HTLV-1 were (0.11%), in South Khorasan, seropositive for HTLV-1 were (0.04%), and in Bushehr, seropositive for HTLV-1 were (0.01%).[19] Another study also conducted in Iran on 1864489 blood donation at seven centers from peroid 2009 through 2013 their age ranged between 18 and 65 years (with mean of age 34.8), seropositive for HTLV-1infection was 0.098%, all of seropositive donation were confirmed by western blot.[20] We can see the percentage of HTLV-1&2 prevalence is lower in our regions in the Middle East in comparison with high prevalence of infections in other parts of the world like United states the seroprevalence of HTLV-1&2 infections was 22 for each 100,000 population, were HTLV-2 more common in America than HTLV-1.[21] In Manaus and amazon state of Brazil, the seropositive result for HTLV-1&2 was 116(0.13%) from 87,402 individuals screened by ELISA and then the positive individuals were confirmed by western blot assays in 2017.[22] in Africa especially Malawi, seroprevalence was 2.6% (11/418) were tested in mother and children to investigate the transmission between mother and her child.[23]In china the seropositive prevalence of HTLV-1 was 0.06% of blood donors. [24] In Vietnam, 0.23% was seropositive for HTLV1&2 infections from 14,819 of blood donors in 2019.[25]

In this study 39 seropositive donors had antibodies against HTLV-1&2 which was independently confirmed by CMIA test, most studies conducted in our regions detected only HTLV-1 while in our country (Iraq) HTLV-1&2 infection was detected in 39 (0.26%) out of 15,239 blood donors, their age ranged between 20 to 57 with mean age 36 years, so we can see there's no correlates between age and HTLV-1&2 infection. Seroprevalence of HTLV-1&2 is higher in females than in males, [26] but in these study most positive individuals is males due to the most blood donors are males by Blood Bank of blood donors in Baghdad and national companies for blood donors in Karbala and Najaf province while only one female is appeared in this study positive to HTLV-1&2. Our results agreed with Pakistan study, where most positive individuals are males than females due to high numbers of males participated in blood donor.[16] Many studies correlate between HTLV-1&2 infections and Hepatitis B and C. In Brazil, 11-years follow up study cleared the importance of HTLV co-infection with hepatitis B &C virus.[27] In this study co-infection was not identified in positive donors.

This study included HTLV-1&2 test in some Iraq's provinces and not all provinces, and the numbers of the positive donors are not compatible and varying among all provinces, so it is necessary to put screening program for HTLV-1&2 in each province because the infection with this virus is differs in many provinces of the country. Screening of blood donors is important to prevent HTLV-1&2 transmissions. In our country (Iraq) this test is not routine screening test in blood banks for blood donors. So necessary performed this test with other routine screening tests (routine screening for Iraqi Blood Donors Banks: HIV, HBsAg, anti-HBc, HCV, and syphilis) to increase the safety of blood, where only 0.5% of HTLV-1&2 develops into Aggressive malignant disease.

Many studies recorded rapid development of myelopathy after infection with HTLV-1 acquired by blood transfusion.[28] This appeared the importance of screening program for prevent or decrease the prevalence of this virus among blood donors in country introduced this program, like Japan where HTLV is reduced since 1988, and US since early nineteen.[26] Prevalence of this virus is endemic and was declined in developed country introduced screening program for HTLV-1&2 and Less developing country like Iraq, need for such screening to control transmission of this virus. Note that Iraq now is open on the world and there's no health control on expatriates introduced to the country especially from west, Iran, US, & etc.

Table 1: Seroprevalence of HTLV 1&2 in Iraqi Blood Bank/Baghdad and donation campaign in Karbala and Najaf. 15239 blood donors and result of confirmatory testing of CMIA-positive sample.

Provinces	Positive for HTLV 1&2 No. (%)	Positive for CMIA
Baghdad	24(61.5%)	24(61.5%)
Karbala	5(12.8%)	16(0.35)
Al-Qadisiyyah	4(10.2%)	39(0.25)
Najaf	2(5.1%)	2(5.1%)
Al-Muthanna	2(5.1%)	2(5.1%)
Al-Basrah	1(2.5%)	1(2.5%)
Wasit	1(2.5%)	1(2.5%)
Sum	39 (100%)	39(100%)

Table 2 : Donors' age range among blood donors included in the study.

Age group	No.	%
57-47	10	25.6
46-36	10	25.6
35-25	10	25.6
24	9	23.2
Sum	39	100

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Occurrence of *Rodotorula Mucilaginosa* among Immunocompromised Patients with Different Infections

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Abstract

Rhodotorula genus contains 37 species, only three species have been caused human infections. These three species are *R. glutinis, R. minuta* and *R. mucilaginosa*. Infection with *Rhodotorula* is much less common than other yeast causes. Nevertheless, the number of infections has clearly increased during the last few years**.** The objective was to occur of *Rodotorula mucilaginosa* Among Immunocompromised Patients with Different Infections

One hundred five specimens were collected from immunocompromised patients with (Oculomycosis, Onychomycosis and Otomycosis) who attended Baquba teaching hospital for a period of 14 months. The yeast cultures were monitored using Sabouraud´s dextrose agar (SDA) and identified depended on macroscopic and microscopic examinations.

Cultured Colonies of *R. mucilaginosa* on Sabouraud´s dextrose agar showed orange to pink in color and are smooth to mucoid colonies. The percentage of *R. mucilaginosa* was 19.3% (92 out of 150) specimens.

According to the findings, it can be concluded that males are most suffering from *Rodotorula mucilaginosa* infection among Oculomycosis and onychomycosis. Also, it is more prevalent in age group (41-60) years.

Keywords: *Oculomycosis, Onychomycosis, Otomycosis, Immunocompromised patients, Rodotorula mucilaginosa*

Introduction

Rhodotorula genus is a yeast, it classified as Basidiomycetes belong to family Sporidiobolaceae [(1)]. Species of *Rhodoturla* produce pinked, orange or red colonies on Sabouraud´s agar, the varying in its color due to the presence of carotenoid pigments. Morphological descriptions of *Rhodoturla* yeasts have been characterized with softy, smooth, moist to mucoid[(2)]

Rhodotorula genus contains 37 species, out of all, only three species have been caused human infections. These three species are *R. glutinis, R. minuta* and *R. mucilaginosa* [(3)].

In nature, *Rhodotorula* yeasts are widespread. Therefore, the researcher can be isolated from different environmental sources [(4)]. Pathogenic human *Rhodotorula* species have been associated with different problems including dermatological, respiratory, and urinary tracts infections [(5)].

Infection with *Rhodotorula* is much less common than other yeast causes such as *Candida* and *Cryptococcus*. Nevertheless, the number of infections has clearly increased during the last few years [(6)]. Brazilian epidemiological study at a teaching hospital, about 2.3% of fungal blood causes were *Rhodotorula* species, compared to other yeasts of *Candida* and *Cryptococcus*, with a percentage (83.4% and 6.6%) respectively [(7)].

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Almost patients infected with *Rhodotorula* fungemia infection have suppression in immune system including (Acquired Immunodeficiency Syndrome (AIDS), malignancy tumors, organs transplant, long spectrum of corticosteroid use, or diabetes) [8,9]. Another study estimated the mortality of *Rhodotorula* infections about 12.6%. In fact, some cases of these infections have evolved without antifungal drug [9].

Fungal Mycotic keratitis also known as Oculomycosis is an important ocular infection caused by fungi, especially in workers. The most common genera causes of fungi are: *Fusarium*, *Aspergillus*, and *Candida* and *rare causes by Curvularia* and *Rhodotorula* [10].

Onychomycosis fungal infections caused by *Rhodotorula mucilaginosa* have been reported in immunocompromised hosts. Therefore, *Rhodotorula* species are uncommon agents in etiology of onychomycosis [11].

Complication rate is higher in immunocompromised patients with Otomycosis which etiologically due to *Aspergillus* and *Candida* and rarely by *Fusarium*, *Rhodotorula* and *Cryptococcus* [12].

Materials and Methods

This study took place at Baquba teaching Hospital, located in Diyala governorate, Iraq. A medical questionnaire form was used of each patient. One hundred five specimens were collected from immunocompromised patients with (Oculomycosis, Onychomycosis and Otomycosis) under the advising of consultant physicians for a period of 14 months. The yeast cultures were monitored using different culture media including (Sabouraud´s dextrose agar (SDA), Cornmeal-Tween 80 agar and Potato dextrose agar).

All isolates of *R. mucilaginosa* were initially plated on Sabouraud´s dextrose agar and incubated at 35°C for 48 hrs to ensure the purity and viability of studied yeast. Isolates were stored frozen in 20% glycerol at either −20°C until the study was performed.

Rodoturella mucilaginosa identified according to the routine method including (colonial morphology, absence of pseudohyphae, urease production and growth at 37°C) which used in the mycology laboratory(13). All isolates of yeast characterized with spheroidal to oval budding cells and pink to red in color because its ability to produce carotenoid [14].

Results

Macroscopic and microscopic examinations:

Cultured colonies of *R. mucilaginosa* on Sabouraud´s dextrose agar showed orange to pink in color and are smooth to mucoid colonies. Microscopy of India ink stained smear of the SDA cultured showed budding yeast cells without pseudohyphae (Figure 1).

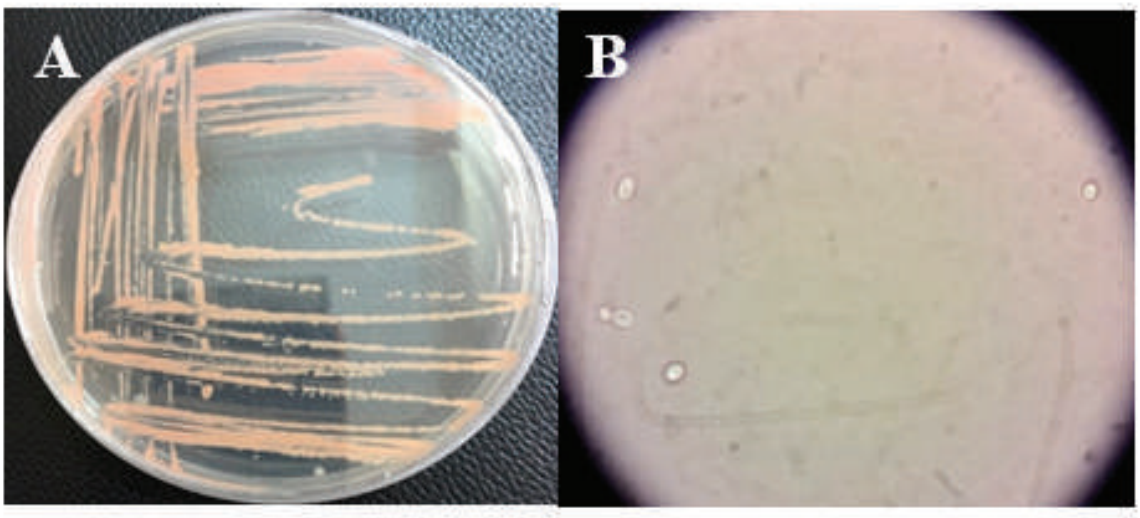


Figure 1: A: *Rodotorula mucilaginosa* colonies streaked on SDA

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The Impact of Socio-Demographic Characteristics on Quality of Life Among Dental Students in Iraq

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Abstract

The purpose of this study was to assess the impact of socio-demographic factors on quality of life among dental students in three governorates in Iraq.

This observational study included 1364 dental students aged 18–22 years from three governorates. Information on socio-demographic and quality of life was obtained from a structured, self-administered questionnaire from the students who were willing to participate in the study. The data was collected, summarized and statistically analyzed. The percentage of fair scores for the four domains was higher among dental students. Socio-demographic characteristics (age, gender and smoking status) were closely associated with quality of life except the area of residence showed no statistical significant differences with quality of life.

The socio-demographic characteristic had some effect on quality of life of dental student

Keywords: *quality of life; dental students.*

Introduction

Quality of life is defined as individuals' perceptions of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. This definition reflects the view that quality of life refers to a subjective evaluation which is embedded in a cultural, social and environmental context [1,2]. The choice of instrument depends on the reason for measurement [3].

Dental students are expected to be more conscious of health-related issues, including dental health. Moreover, these students tend to have a higher socioeconomic background, which in turn, may lead to better self-reported oral health and clinically-assessed OH and, eventually, to a higher quality of life but there were little studies reported the opposite aspect of this finding [4,5,6].

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According to current knowledge, there is no previous Iraqi study concerning the quality of life assessment among dental students in Iraqi populations. This study was administered to a random sample at public Universities with a demographic profile.

The aim of this study was to investigate how socio-demographic factors, include negative life events; education and others are related to quality of life measured by WHOQOL-BREF Field Trial Version among dental students in Iraq.

MATERIALS AND METHODS

This observational study was conducted at the three universities (Basra, Anbar, Mosel), during the period between March 2018 to March 2019. Approximately 1364 dental students aged 18-22 years old mainly from the randomly selected governorates in Iraq attended the colleges of dentistry were examined.

The sample included both genders dental students (636 males and 728 females) with age range 18-22 years old. The participants should not have chronic medical disease or physical handicapped and

not exposed to psychological trauma during the last six months.

Self-Administered WHOQOL-BREF (Field Trial Version) was used to evaluate the quality of life for dental students into three group poor, fair and good scores. It is possible to derive four domain scores (physical, psychological, social and environmental domains). Domain scores are scaled in a positive direction (i.e. higher scores denote higher quality of life). Responses to the questions using a 5-point Likert scale. In the present sample, WHOQOL-BREF domain scores discriminated statistically highly significantly between contrasted groups of dental students at P-value <0.05 (n=736, df=734). The reliability index for the WHOQOL-BREF assessment by using Cronbach's alpha was 0.98. As the result of that, the indicator stayed on its version without drop of any item.

Data were statistically analyzed using SPSS version 22 software and the following statistical tests were carried out: Means, standard error. The Independent-Samples T Test ANOVA (one way) P-values less than 0.05 were considered as statistically significant and P-

Results

The data of present study illustrates that the percentage of fair scores for physical domain was higher among dental students than that of other scores in the same domain fallowed by good score. The same picture was found concerning social and environment domains except that of psychological domain where fair score followed by poor score and the good score in this domain showed the lower percentage (fig 1).



Figure 1: The distribution of total sample according to WHOQOL-BREF domains scores.

Regarding the three governorates, the data of this study showed no statistical significant differences in means scores of the four domains as illustrated in Table 2. However, the mean scores of physical domain was the predominant type in the three governorates.

Table 2: Mean score of WHOQOL-BREF domains by governorates.

Governorate		WHOQOL-BREF domains (mean ± SE)			
		Physical	Psychological	Social	Environment
Anbar N=464		56.95 ± 0.85	50.30 ± 0.63	50.28 ± 0.80	47.88 ± 0.75
Mosel N= 352		58.10 ± 1.00	50.77 ± 0.75	51.28 ± 0.91	48.60 ± 0.85
Basra N= 548		58.22 ± 0.80	51.19 ± 0.61	51.39 ± 0.72	49.22 ± 0.68
Anova df= 2	F	0.66	0.51	0.61	0.88
	Sig.	0.52	0.60	0.54	0.42

B: *Rodotorula mucilaginosa* stained with India ink (40X)

One hundred five specimens had been included in this survey concerning infection groups (Table 1).

Table 1: Percentage of culture results according to infection groups

Culture results	Infection groups						Total No. %	
	Oculomycosis No. %		Onychomycosis No. %		Otomycosis No. %			
Positive	7	14%	18	36%	4	8%	29	19.3%
Negative	43	86%	32	64%	46	92%	121	80.7%
Total	50	100%	50	100%	50	100%	150	100%

Upon stratification of age group, *R. mucilaginosa* was most isolated from age group (41-60) years among Oculomycosis and onychomycosis patients, with a percentage 42.8% and 33.3%, respectively. Whereas among Otomycosis, the age group (>60) years was most infected with studied yeast (50%) (Table 2).

Table 2: Percentage of *Rhodotorula mucilaginosa* in immunocompromised patients in comparison with different infections concerning age groups

Age groups	Infection groups						Total No. %	
	Oculomycosis No. %		Onychomycosis No. %		Otomycosis No. %			
<20 years	0	0%	3	16.7%	0	0%	3	10.4%
21-40 years	2	28.6%	4	22.2%	1	25%	7	24.1%
41-60 years	3	42.8%	6	33.3%	1	25%	10	34.5%
>60 years	2	28.6%	5	27.8%	2	50%	9	31%
Total	7	100%	18	100%	4	100%	29	100%

According to the patients´ gender, Males were more infected with *R. mucilagniosa* than females among Oculomycosis and onychomycosis, with percentage (57.1% and 61.1%), respectively (Table 3).

Table (3) Percentage of *Rhodotorula mucilaginosa* in immunocompromised patients in comparison with different infections concerning their gender

Patients´ Gender	Infection groups						Total No. %	
	Oculomycosis No. %		Onychomycosis No. %		Otomycosis No. %			
Male	4	57.1%	11	61.1%	2	50%	17	58.6%
Female	3	42.9%	7	38.9%	2	50%	12	41.4%
Total	7	100%	18	100%	4	100%	29	100%

Discussion

The use of steroids on long term may lead to development of fungal infections in particular Immunocompromised patients such as patients of diabetes, organs transplant, HIV, cancer. Treatment of studied infections should be initiated against eradicating *R. mucilaginosa* in order to prevent development of resistant strains [12]

Although, Infections caused by Rhodotorula are rarely incidence in recent years, but its more frequently isolated in Asia-pacific regions [15]. Rhodotorula species were revealed to be the fourth most frequently causative agent among other yeasts isolated from clinical specimens [16]. *Rodotorula* infection which were previously thought to be non-pathogenic but, are isolated from clinical specimens [17].

According to recent studies, Our results disagree with [18] who revealed that age group (20-40) most infected with *R. mucilaginosa*. There are no previous data regarding the correlation between *R. mucilaginosa* with patients´ gender. The genus of *Rhodotorula* is widely distributed in hospitals and could be critical as nosocomial fungal infections in particular in patients who undergo immune-suppression [19]. The recent results is affected by several factors; such as temperature, humidity, time of day and human activities.

Conclusion

From the finding results, it can be concluded that males are most suffering from *Rodotorula mucilaginosa* infection among Oculomycosis and onychomycosis. Also, it is more prevalent in age group (41-60) years.

Recommendation

Molecular study of *Rhodotorula species* to detect substitution mutations that related to the pathogenicity.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Regarding the age groups, the mean scores of the physical domain was highest in the all age group and showed statistical significant differences among the age groups. However, the mean scores of the environment domain were lowest in the all age group and showed statistical significant differences as illustrated in Table 3.

Table 3: Mean score of domains by age.

Age groups		Physical	Psychological	Social	Environment
18 years N= 295		58.82 ± 1.02	49.06 ± 0.73	50.01 ± 0.93	45.42 ± 0.92
19 years N= 270		54.04 ± 1.07	49.83 ± 0.73	50.15 ± 0.79	44.89 ± 1.00
20 years N= 274		56.61 ± 1.19	51.47 ± 0.85	52.65 ± 1.17	47.24 ± 1.10
21 years N= 265		57.37 ± 1.14	50.00 ± 0.94	50.87 ± 1.05	47.93 ± 1.03
22 years N= 260		57.92 ± 1.20	50.63 ± 0.88	51.35 ± 1.07	48.63 ± 1.17
Anova df= 4	F	2.64*	1.25	1.14	2.37*
	Sig.	0.03	0.29	0.34	0.05

***=Significant at P≤0.05.**

Regarding the gender, the mean score showed no statistical significant differences between males and females in all domains except that for the physical domains showed high statistical significant difference as illustrated in Table 4.

Table 4: Mean score of WHOQOL-BREF domains by gender.

Gender		WHOQOL-BREF domains (mean ± SE)			
		Physical	Psychological	Social	Environment
Male N= 648		58.62 ± 0.70	49.68 ± 0.50	51.04 ± 0.65	46.41 ± 0.69
Female N= 716		55.49 ± 0.71	50.63 ± 0.53	49.75 ± 0.64	47.12 ± 0.64
t-test df= 1362	t	3.11**	-1.30	1.41	-0.76
	Sig.	0.002	0.20	0.16	0.45

***=Significant at P≤0.05, **Highly significant=H.S at P≤0.01.**

Regarding smoking status, the data of the present study showed that the mean scores of four domains were higher among nonsmoking students and the statistical highly significant differences were observed in Table 5.

Table 5: Mean score of WHOQOL-BREF domains by smoking status.

Smoking status		WHOQOL-BREF domains (mean ± SE)			
		Physical	Psychological	Social	Environment
Non smoker N= 866		63.72 ± 0.56	53.91 ± 0.49	55.13 ± 0.61	50.84 ± 0.64
Smoker N= 498		45.25 ± 0.72	43.70 ± 0.40	42.08 ± 0.50	39.72 ± 0.50
t-test df= 1362	t	20.12 **	16.17 **	16.65 **	13.75 **
	Sig.	0.001	0.001	0.001	0.001

*=Significant at P≤0.05, **Highly significant=H.S at P≤0.01.

Discussion

The instrument of World Health Organization Quality of Life questionnaire short version (WHOQOL-BREF) was used in the current study, it is one of the most important and most frequently used instruments that have overcome many of the barriers and objections to their use. Quality of life has become an important measure of outcomes across all medical specialties, in both research and clinical settings [7,8].

Regarding the scores of the psychological domain it was decreasing as the age increases. Oral health status is closely associated with quality of life for adults. Social support is one factor that may play an important role in maintaining health and decreasing the impact of illness. Changes in social roles or events that were occurring in the country at certain time might be the factors behind the change in the quality of life. This result agrees with other studies which concluded that age was systematically related to satisfaction level of life while older participants being less satisfied with their lives than their younger counterparts expressed worries, desire for change and plans for the future [9,10] and disagrees with another study that revealed the QOL became better with an increasing in age [11].

The study also revealed no statistical significant differences in means of scores for the four domains among dental students in the three governorates. Previous study supported this finding and concluded that the perception of the impact of quality of life does not depend on place of residence of neither women nor men [12]. The criteria of sample selection regarding the matching in educational level throughout the three governorates may also explain this result.

This study demonstrates that the smoke correlates with worse index of quality of life in this population. The scores of all domains were lower among smoker students when compared with non-smokers. These findings have been replicated across populations with diverse socioeconomic and cultural groups around the world. This result agrees with other studies which concluded that smoke appears to be negatively associated with quality of life [13] but another study showed that severe smokers presented greater impairment in quality of life in all domains when compared with mild and moderate ones, since it was found that the presence of a compromise in quality of life, in relation to the field of general health status, is associated with a higher annual consumption of cigarettes. This is due to the fact that smoking causes several physical changes such as loss of pulmonary function and reduction of bone mass and also

oral diseases [14,15].

The scores showed statistically significant differences between men and women in four domains. Gender stereotypes are present not only in the male attitudes, but also in the female attitudes. In Iraqi society women impose obligations on themselves and claim features that valuable woman should have such as: perfect health or good and attractive appearance. It is necessary to stress that any imperfection of the body can influence the perceived quality of life much more than the visible symptoms of the disease. Women are afraid of losing their attractiveness, feel afraid of being socially rejected, especially when their main activity is focused on building relationships with other people. Similar differences between genders were observed by many researchers in different areas as the authors concluded that the impaired quality of life in women reflects the gender-related differences that are also shown in the general population and they are related to the higher prevalence of trait anxiety and depressive symptoms in women [16-18]. This result was disagreeing with the study which reported that statistical analyses showed no difference between men and women on quality of life [11]. Other results showed few gender differences in the satisfaction with specific life domains where the participants experience more anxiety and depression symptoms as well phobic fears in comparison to general population [19].

The result of present study indicated a strong relationship between quality of life and psychological distress among dental students, which was consistent with previous articles [20-22]. Generally, physical problems are the most frequent co-occurring disorders among dental students because of unhealthy life style. This implies the importance of the reintegration of people with mental illness into society, as was emphasized in a previous study [23,24]. The final models showed that perceived physical problems also had negative associations with three domains of the WHOQOL-BREF. This is comparable with the findings of a previous Asian study[25].

Conclusion

Physical pain and psychological discomfort were the most frequently reported variables that impact on quality of life. The smoking status was the strongest factors associated with low quality of life.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Investigation Study of Human Spermatozoa Proteome

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Abstract

The current study has been conducted on health human sperm to identify the different types of amino acids and entire proteins of sperm. Two methods have been applied to characterise of human sperm, firstly extract lysate of sperm, then digest and run it in mass spectrometry, secondly, total sperm lysate was run in SDS page gel and bands were stained with coomassie, then band was cut and digest to extract of whole amino acids and also was run in mass spectrometry. Result revealed that sperm have several proteins, which were involved with different binding proteins, soluble proteins, and glycoproteins, transmembrane proteins.

Current result was displayed that mass spectrometry can be used to recognise data of proteins of sperm and detected types of it. This technique was applied on whole lysate and gel bands of sperm and created database of sperm proteins which can be investigated and consider more interesting for researchers. This study is concluded that human sperm have important transmembrane, enzymes, glycosylated proteins, glycoproteins and uncharacterised proteins which might be answered many theoretical aspects of biological and biochemical sperm migrations before and after fertilization.

Keywords*: sperm, SDS page, mass spectrometry, glycoproteins, transmembrane, uncharacterised proteins.*

Introduction

The male reproductive is consisted of left and right testis, epididymis, vas deferens which is received products of accessory glands and joined into urethral part of urethra in the penis external male organ[1,2,3] .

Testes are surrounded by skin, then serous layer and tunica albuginea that extended into parenchyma of testis and divided it into incompletes lobules and constitutes many septa and mediastinum of testis [4]. Internally, testis is consisted of coiled tubules is called convoluted seminiferous tubules that is contained the germinal epithelium of sperm and connected to epididymis via efferent ductuli [6].

In addition, it has special cells is called interstitial cells(leydig) that produce testosterone hormone, this hormone is responsible for development of sperm and secondary growth of male [7,8].

Sperms undergo different stages of development before maturing, and these stages continue after liberated sperm into lumen of seminiferous tubules [9,10,11].

Furthermore, sperm will undertake remodelling and biochemical changes in the epididymis, resulting acquired it the motility, and sperms become able to be a motile[12,13] also head of sperm would obtained specific β-defensin protein that support sperm in the motility, maturation and antimicrobial activity and it is essential of fertility[14,15,16]. The sperm are surrounded by plasma membrane which constitutes with glycocalyx, and carbohydrates such as sialic acid which consist the major sugar of sperm [17,18] these carbohydrates would be mediated and reacted with the female reproductive tract during capacitation process of sperm and also during reservoir of sperm in uterine tube before fertilizations [19,20].

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Moreover, there are different putative recognition protein could play a role in sperm motility and fertilizations [21]. For example beta-defensin family are expressed and coated sperm which might be protected it as antibacterial factor and might be involved in sperm capacitation to prepare sperm for fertility[14,22,23], more protein were identified on sperm included Siglecs family which could be mediated sperm activity before fertilizations and may be regulated the internal environment for sperm[17]. Therefore, many recent studies are interested in characterization of proteins of sperm to investigate more about transmembrane proteins activity of sperm in relations with female reproductive tract. Proteomic studies were applied on whole lysate of sperm in different ways to detect the total proteins of it and understand it functions[24,25].

The overall aim of this study is to conduct a comprehensive characterisation of proteins of human sperm and created database of human sperm. Additionally, the behaviour proteomic analysis on whole lysates of sperm combine to electrophoresis of human sperm lysates to augment previous studies.

Material and Methods

Human Semen

Straws of semen from three donors obtained from Cryos Ltd. were used (numbers 5469-17, 5549-238 and 3825-40). Each straw contained approximately $1X10^8$ sperm in 500 µl of extender.

Gel formulation

Briefly, Bio-Rad protocol was used for making gel[26]. The resolving gel (10% acrylamide/bisacrylamide). All solutions were mixed in order in a 15 ml Falcon tube, then mixed well after the TEMED was added and poured between the 0.75mm gap between the thick and thin glass plates, it was left for 25 minutes to solidify. The Stacking Gel (4% acrylamide/bisacrylamide) was made by mixing the reagents. A comb to create appropriate well volumes was inserted between the plates before pouring the stacking gel on top of the resolving gel.

Tissues lysis buffer

The Tissue Lysis buffer was made and before use one tablet of protease inhibitor (Roche cat no. 11836170001) was added per 10 ml of lysis buffer. It could then be stored at 4ºC for up to two weeks.

Preparation of samples

Two different methods were used for preparation peptides acid of human sperm. These were applied on semen of bovine [27]. Preparation of lysates of human had followed the protocol of[28]. The samples were then ready for injection into the Mass spectral machine by Mass Spec machine (FT-ICR/Orbitrap) in the Conway institute. University College Dublin.

Micro BCA Assay Quantitation of Protein in human sperm

The Micro BCA Protein Assay Kit (Pierce product code #23235) was used to quantitate the protein in Human Sperm.

Coomassie Staining Solution

The whole lysate of human sperm was loaded on SDS-PAGE gels, and after migrated of sperm proteins, the gels were stained by Coomassie Blue Staining (Brilliant Blue). After that destain for Coomassie stain solution was used to remove the excess the stain. The banded of proteins were stained with blue and cut carefully as cubes and digest to extract amino acids [27]

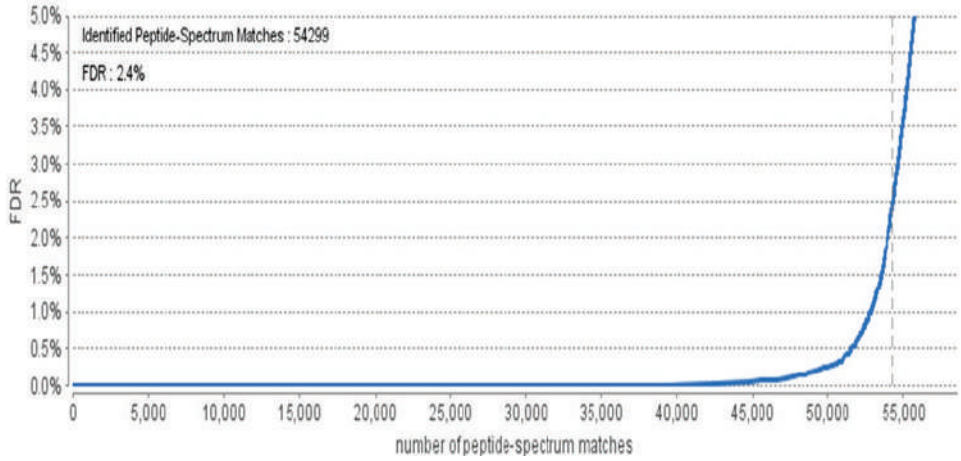


Figure (1): False discovery rate (FDR) curve. X axis is the number of peptide-spectrum matches (PSM) being kept. Y axis is the corresponding FDR.

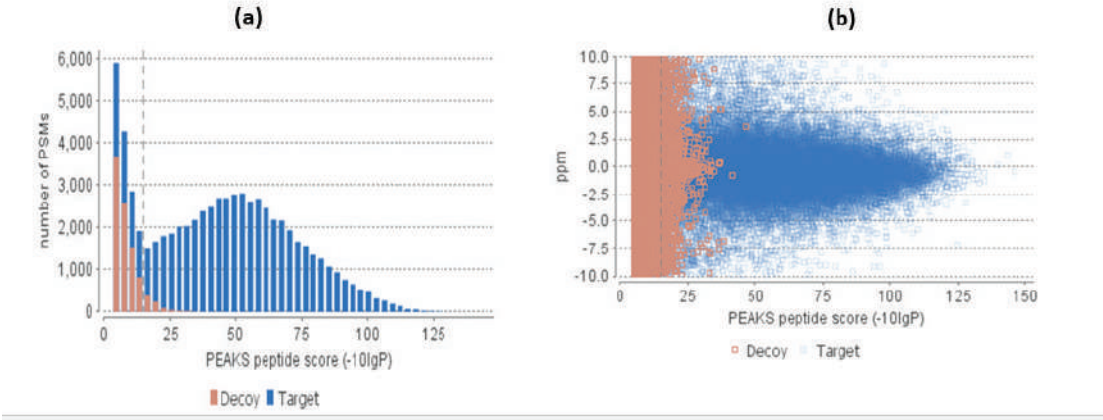


Figure (2): SM score distribution. (a) Distribution of PEAKS peptide score; (b) Scatterplot of PEAKS peptide score versus precursor mass error.

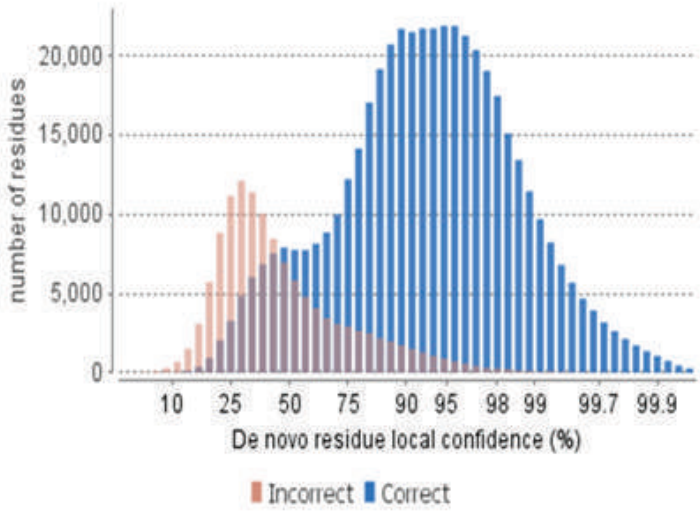


Figure (3): De novo result validation.

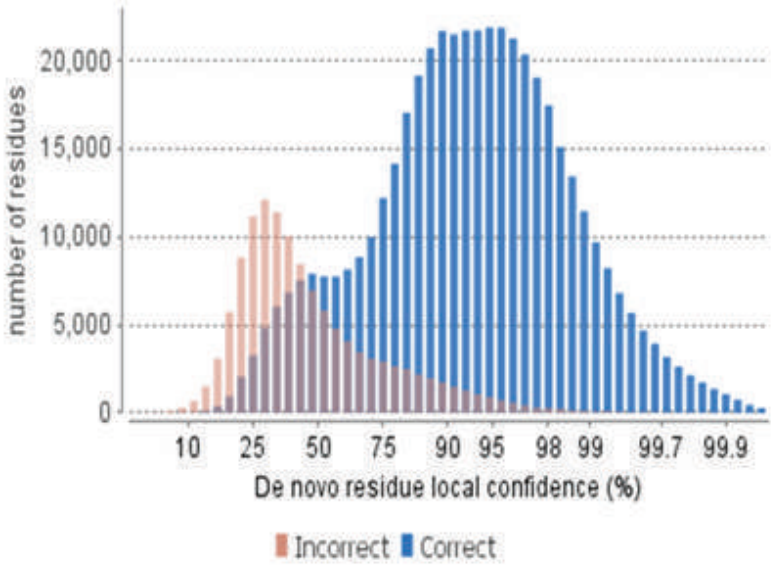


Figure (4): Precursor mass error of peptide-spectrum matches (PSM) in filtered result. Distribution of precursor mass error in ppm.

Table (1): Statistics of filtered result.

Peptide-Spectrum Matches	54301
Peptide Sequences	26781
Protein Groups	2994
Proteins	4576

Result and Discussion

Our results shown that sperm has many different proteins in sperm including those involved in membranes, glycoproteins, non-glycosylated proteins and different enzymes in human. Briefly, samples were prepared in two ways, the first was using cubes cut from 10% SDS polyacrylamide gels containing lysate of sperm samples, where proteins were trypsin digested in situ with Promega™ Trypsin Gold, Mass Spectrometry Grade product (Manufacturer: Promega™ V5280) then extracted from the gel for analysis. The peptides from the digest were resuspended in buffer and run in the Mass Spec machine (FT-ICR/Orbitrap)[28], the second method used whole lysates of sperm which were directly digested by trypsin (Manufacturer: Promega™ V5280), then run in the Mass Spec, with a minimum of three replicates being used for each sample. The data obtained was analysed by Peak7 studio software [29].

The results of both ways were detected different types and various proteins of sperm; however, the electrophoresis method was given better isolated and identifications of proteins. Also sliced of gel or band was more efficiency to recognise more proteins compare to the whole lysate. All biological replications of whole lysate and electrophoresis methods were combined and data was analysed. Proteomic of human sperm revealed that there are about 26781 peptide sequences, 2994 protein groups and 4576 proteins, Table (1). Figure

(1,2,3, and 4).

In this result, the major important proteins were selected which are included with constituents of human sperm as the following: Uncharacterized proteins: 44, Binding proteins: 187, Synthase proteins (enzyme): 68, Actinin: 9, Calcium Binding Proteins: 60, Transmembrane Proteins: 80, Mucin: 18, Galectin: 6, Beta-Defensin Protein: 8, Glycoproteins: 15, Myosin: 76, Actin: 13 (supplementary1).

Mass spectral analysis is one strategy that used in proteomic studies which identifies the relative abundance of amino acids and proteins by measuring deconvoluted overlapping peptide HPLC peaks to provide relative quantitation based on peak areas[30], It has been used to detect relative expression of proteins that characterise different diseases or to identify specific proteins in biological samples[31,32,33]. These studies suggest that it is feasible to use mass spectrometry to identify proteins on human sperm. Several proteomic and transcriptomic studies of human and bovine sperm have already been conducted, and have identified different types of proteins which were displayed also different types of important proteins such as free proteins, transmembrane proteins, glycoproteins and enzymes, as well proteins related to immune system in human sperm[34,35,36],which were confirmed our finding in current study. Interestingly, our proteomic results provided evidence for identified many thousands of proteomic sperm with other studies that have been detected in proteomics studies, and could be used to diagnose the disease and fertility by identified normal proteins of sperm[37,38] . Our result identified from the analysis of the data from the mass spectral profiles that sperm surrounded by different types of transmembrane of protein which might be involve in glycosylation process of sperm during passage it via cervix and uterus and reservoir in uterine tube before meeting the ovum and fertilizations and are glycosylated and considered as glycoproteins[39 40,41], therefore It might be necessary to use a number of different proteomic methods to profile all of the proteins in human sperm, because mass spectrometry of glycoproteins is limited by technical issues due to the considerably larger differences and diverse chemical properties of individual glycoproteins[42, 43]. There are many complicated reasons which can preclude accuracy in the analysis of glycoproteins in biological samples using mass spectroscopy especially in the case of proteins with low abundance [44]. In conclusions, human sperm has large several proteins which can play a huge role in sperm motility, reservoir, internal regulation environment of spermatozoa, immune suppression of female reproductive tract and finally fertilisations.

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The Role of CTX-II , Dyslipidemia,Vitamin D in Polycystic Ovary Syndrome

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Abstract

This study aimed to study the serum measurement of clinical value of type II collagen (CTX-II) in syndrome of polycystic ovary in women and indicter as osteoarthritis disease marker analyzed in future levels with a recent available immune enzyme-linked sorbent assay (ELISA) kit. For increasing sensitivity test, the protocol was modified. Levels of CTX-II increased significantly as well total protein, while decrease vitamin D and difference in lipid profile were practical between the patients compared with healthy women's. The obtained results suggested the monitoring interest of the serum CTX-II for the OA development in patients of polycystic ovary syndrome in women and the relevance of the analysis of multiple time point for this biomarker.

Keyword: *CTX-II- Biomarkers- OA Osteoarthritis- Polycystic ovary syndrome- Dyslipidemia.*

Introduction

Syndrome of polycystic ovary (PCOS) is a hard condition with characteristics of prominent levels of androgen, irregularities of menstrual and/or small cysts on either ovaries or both [(1)]. This disorder might be of ovaries morphological polycystic or hyper androgenemia predominantly biochemical. Hyper androgenism, is a PCOS clinical hallmark result of inhibition of follicular progression, ovaries microcysts, , changes in menstrual and ovulation [(2)]. Investigations proposed that (5% to 10%) females of age 18 - 44 years are PCOS affected, rendering it the most well-known abnormality of endocrine among females of reproductive age in U.S.A. [(3)]. Women looking for assistance from professionals in health care to address issues of excessive hair growth, obesity, acne, infertility, and amenorrhea most of the times receive a PCOS diagnosis and have cancer of endometrial at higher rates, TIIDM, dyslipidemia and cardiovascular disease [(4)].

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PCOS pathophysiology involves defects primary in insulin secretion and action, ovarian function, and the hypothalamic–pituitary axis. Although the unknown cause of PCOS, PCOS has been connected to obesity and insulin resistance [(5)].There are 3 most well-known elements with PCOS associated i.e. irregularities of ovulation, androgen levels enhancement, and problems of cystic ovaries with elevated androgen levels and ovulation take place in most PCOS women [(24, 26)]. Furthermore, alopecia, hirsutism, and acne are directly associated with elevation levels of androgen and the ovaries prevalence of polycystic on pelvic ultrasound more than 70% in PCOS patients [(6)].

After diagnosing PCOS, investigations prove that patients over 50% have diabetes or pre-diabetes, and there is risk increasing of hypertension, myocardial infarction (MI), dyslipidemia, osteoarthritis sleep apnea, anxiety, depression, and endometrial cancer. Furthermore, PCOS pregnant women should be noticed for increasing miscarriage rates, pre-eclampsia, premature delivery, and gestational diabetes [(7)]. Osteoarthritis defined as a disease with developing articulate cartilage destruction and by changes pathologically in the subchondral bone and synovial membrane · OA, the destruction will result

in losing the 2 major components, type II collagen and proteoglycans, rendering them choice markers in determining metabolism of cartilage [8]. Peptide of C-telo of collagen type II (CTX-II) is marker most studied [9]. Increasing levels were documented in OA patients in comparison to subjects of asymptomatic or without OA signs [10]. A significant association have shown between CTXII concentration and OA radiographic development [11].The goal of this investigation was to study the serum measurement of clinical value of CTX-II in polycystic ovary syndrome patients and correlated with physiological assessment that give indicator to development of Osteoarthritis in future.

Methodology

Serum specimen was collected from patient with infertile Polycystic Ovary patients (n = 45) and healthy patient (n=45) at AL-Sader laboratory of Medical city in Najaf Province, AL-Najaf Health Directorate / Ministry of Health /Iraq. The average of the patient's age was (32.81±51) years. All reagents and specimens should be at a temperature of room before use. Reagents mixed in soft way with no foaming. No interruption should take place once the protocol started Tests of biochemical were performed at Biology laboratories. in this study was CTX-II protein (MBS2507692), vitamin D3 (MBS773966) , HDL (MBS170439) , VLDL(MBS265004), total protein (MBS2540455) and LDL (MBS162140) My Bio Source Company USA in Origin .

Statistical Analysis

Statistical analyses of all result were carried out by the help of Graphpad prism version 5) software statistical package using t-test (with p value at level of significant less than 0.05) to compare values of result between groups .

Results

The result show an increase of significant for protein of collagen matrix Type II figure(1) in patient with Polycystic Ovary(mean± Std. Error 3.98+0.67) compare control group (mean± Std. Error 2.62+0.71) which that may be cause by the extracellular adhesion molecule is soluble from the cartilage surface component. Enable the binding of cartilage cells to type II collagen in the absence of serum, thereby increasing protein externally, we conclude that direct interaction between cartilage cells and type II collagen occurs through other adhesion mechanisms of cell surface proteoglycans such as membrane-bound heparin sulfate in progressive age [12]. Few studies revealed a significant relation between concentration of CTXII and OA radiographic development [13]. Recently, urinary levels predictive value of CTX-II for cartilage losing was assessed through MRI [14].

Figure 1: levels of Type II protein collagen matrix protein in patients comparison to Healthy group

These results was show a decrease of vitamin D3 in patient with Polycystic Ovary (mean± Std. Error 12.5±0.17) compare control group (mean± Std. Error 24.4±0.91) figure (2), Probably Vitamin D deficiency may be associated with populations with extensive skin coverage, especially in women in Iraq. There is a large body of proof the importance of vitamin D in reproductive function because VDRs have been detected in placenta, the endometrium and ovary [15]. Deficiency of Vitamin D is associated with deregulation of Ca, which participates in the follicular arrest development in PCOS women resulting in dysfunction fertility and menstrual [16]. Some study on PCOS women with and undergoing fertilization *in vitro* (IVF), they found that the women who achieved pregnancy exhibited significantly higher levels of follicular fluid of 25(OH) D and each ng/ml elevate in follicular fluid 25(OH)D elevated the likelihood for pregnancy achievement by 7% [17]. Moreover, deficiency of 25(OH)D was related with rates of lower development for pregnancy and follicle after clomiphene-citrate stimulation in PCOS women, suggesting a possible vitamin D supplementation role in PCOS infertile women who undergo stimulation of ovarian [18].

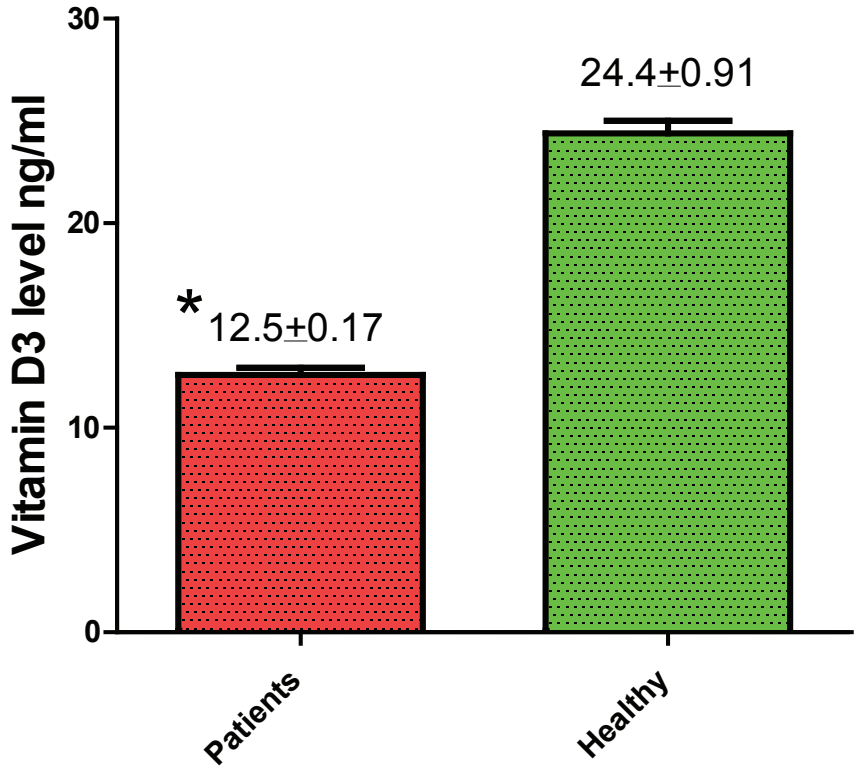


Figure 2: levels of vitamin D3 in patients comparison to Healthy group

These results was show a increase of total protein in patient with Polycystic Ovary (mean± Std. Error 8.01±1.34) compare control group (mean± Std. Error 6.65±0.98) figure (3), Probably by understanding the general proteins alterations not only of advantage for PCOS pathogenesis mechanism elucidating, but also easing for discovering the specific biomarkers and sensitive that are closely linked to diseases. Proteins quantitative analysis in serum with array protein provides useful data for clinical practicing i.e. individualized treatment and accurate medical that is according to protein screening. Five hundred proteins were examined by array of protein in samples of serum collected from rectal cancer where the OPG expression of was elevated which was associated with the patients survival of rectal cancer after chemotherapy [19].

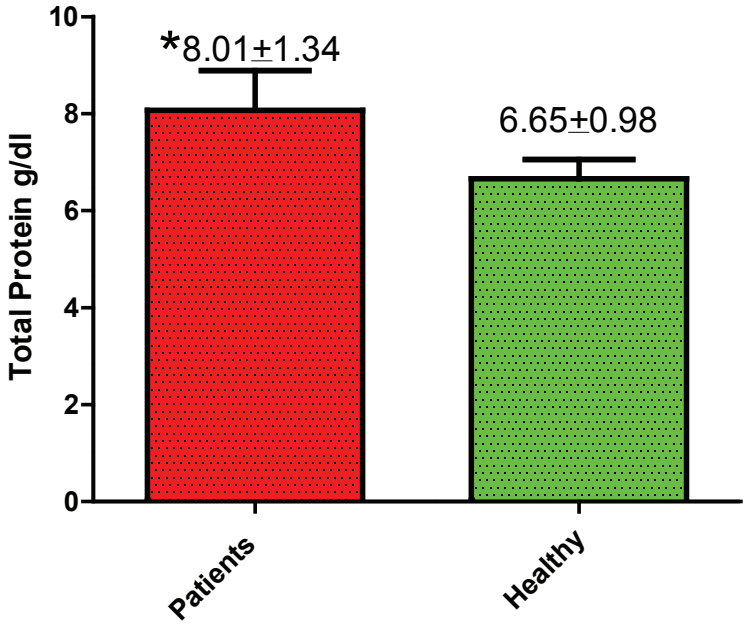


Figure 3: levels of total protein in patients comparison to standard group

The results revealed an increasing levels of significant in LDL level in patient with polycystic (mean± Std. Error 118±0.64) ; control group (mean± Std. Error 44.4±0.26) figure(4), also The result show decrease significant level in HDL level in patient polycystic (mean± Std. Error 39.4±1.14) ; control group (mean± Std. Error 49.4±2.41) figure (5),suggests that the level of LDL, HDL, insulin, glucose, apolipo protein A1, and lipoprotein a, in female with and without PCOS do not show any significant differences [20]. Similarly, Jahanfar *et al*., in a study aimed at evaluating the genetic and environmental factors affecting lipids among twins, found no significant difference [21] .The research concluded that the serum CTX-II in women with PCOS may appear to have symptoms of Osteoarthritis and thus it is considered to be a vital reliable evidence in the news of the occurrence of this disease in PCOS women.

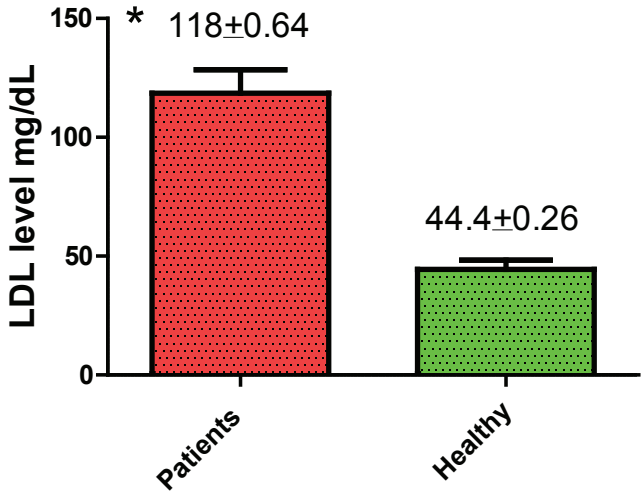


Figure 4: levels of LDL in patients comparison to Healthy group

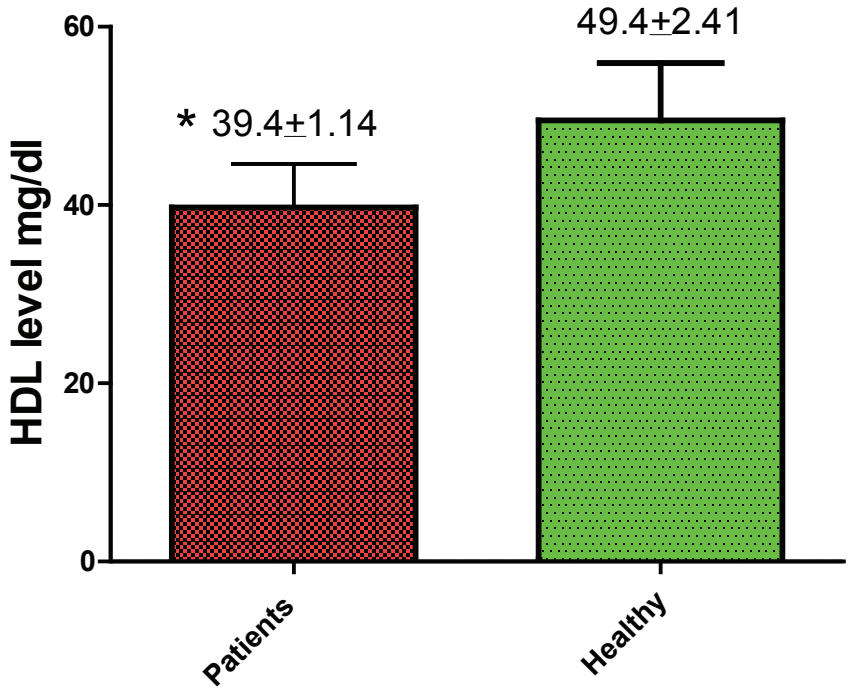


Figure 5: levels of HDL in patients comparison to Healthy group

Results proved a significant enhancement of VLDL level in patient (mean± Std. Error 29±2.34) ; control group (mean± Std. Error 20±2.64) figure(6) , Disturbances in metabolic are famous clinical syndrome characters, especially, dyslipidemia which is very well-known abnormality metabolic in PCOS female with a prevalence of up to 70% [22,23]. Resistance of insulin is a key PCOS pathophysiology and dyslipidemia in PCOS women may be therefore in accordance with that detected in the insulin resistant case: decreasing levels of apolipoprotein (Apo) A-I, and high-density lipoprotein-cholesterol (HDL-C), and increased levels of ApoB, triglycerides (TG) and very low-density lipoprotein[24, 25, 26].

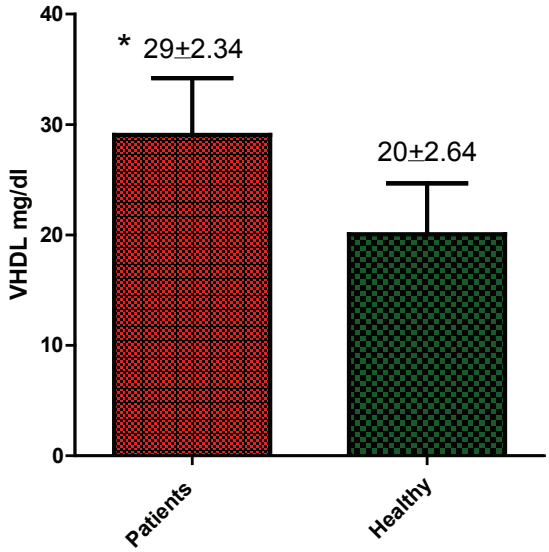


Figure 6: levels of VLDL in patients comparison to Healthy group

Conclusion

The research has a goal of examining the clinical value of the serum which was measured type II collagen (CTX-II) in the Polycystic ovary syndrome in the women and indicter as a marker of the (OA)disease of future levels. An important percentage increased in CTX-II levels , and the total protein but a decrease occurred in vitamin D and with a change in lipid profile which was in practical status between the patients compared with healthy women.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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The Correlation Between Tumor Necrosis Factors-Alpha (TNF-α) and Resistin in Obese Iraqi Women

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Abstract

Obesity is a medical condition in which excess body fat has accumulated to an extent that it may have a negative effect on health. The current study aimed to study the correlation between resistin and Tumor Necrosis Factors-alpha (TNF-α) in Obese Iraqi women. The group of patients contain twenty five women with Obesity and apparently thirty healthy women were present as a control group. Estimation of sera resistin and Tumor Necrosis Factors-alpha (TNF-α) were done by using "enzyme-linked immunosorbent assay" (ELISA). Sera levels of resistin was increase significantly in women with obesity group compared with control group ($p<0.01$). Also the results show positive correlation between Tumor Necrosis Factors-alpha (TNF-α and resistin. In women with obesity, depending on present findings, resistin is implicated in pathogenesis of obesity related with Tumor Necrosis Factors-alpha (TNF-α) .

Keywords: *Iraqi Women; Tumor Necrosis; Factors-Alpha; Health*

Introduction

The obese often caused by multiple causes, such as hereditary and behavioral factors, therefore treatment, it needs more than one dietary change .The behavioral changes such as walking, exercise and seeking expert advice from specialists, can at time use nutritional supplements to help an obese patient overcome them(1).

Obesity and overweight contribute greatly to many health problems. Both conditions increase the body's risk of developing many diseases such as: type 2 diabetes, prostate and colon cancer(2) as well as gout, hypertension, heart disease (CVA, heart failure, myocardial infarction), gallbladder disease and Pickwickian syndrome (3).

The body mass index is a link between height and body weight. The length is measured in square meters (m^2) and the weight is measured in kilograms (kg). This is because the mass index shows the body weight in relation to height, as it is strongly related to the total fat content in the body(4).

Although the mechanism is not yet clear, it is generally recognized that obesity is an important risk factor for insulin resistance. It has become known that the adipose tissue is one of the endocrine glands as it secretes a group of hormones such as resistin, which is a cytokine that is mainly expressed by the adipocytes and its work is antagonizes to the action of the hormone insulin (5).

There are many research and reports that dealt with and discussed the relationship of the three together (insulin resistance and obesity with resistin, where new reports mentioned that one of the causes of impairing insulin action in the production of hepatic glucose is resistin, and in particular it inhibits skeletal muscles from absorbing glucose by GIUT-4(6).

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Tumor necrosis factor are lymphokines that present in two forms (a or b) : In the body they have the ability of causing hemorrhagic necrosis of specific tumor cells, but not affecting normal cells. And TNF-αalso has important effects on human body like lipid and glucose metabolism[7].

Some recent studies have reported that (TNF-α) is expressed in adipose tissue . It has been observed that this level of cytokine is elevated in obese people[8].

There is some evidence that TNF- α may stimulate insulin resistance. Consequently, he may be responsible, if only partially, for disorders of the metabolism of fats and glucose. Which is associated with type 2 diabetes mellitus and obesity. There are many studies showing that TNF- α directly interferes with the early steps in the insulin signaling pathway[9].

There are many studies that examined the levels of resistin and Tumor Necrosis Factors-alpha (TNF-α) in obese women. in the present research are studying the correlation between resistance and Tumor Necrosis Factors-alpha (TNF-α) in Iraqi obese women.

Materials and Methods

The current study included (25) women with obesity as the first group, with the mean age ± SD for patients (22.44±3.2). The obese women were interviewed using a structured questionnaire to determine the smoking women and also take other information like their Medical history ,family history and surgical history. All women were collected from Babylon maternity and pediatric teaching hospital in Hilla city.

The second group is the control group and it contains 30 women who appear to be in good health, with the mean age ± SD (23.34 ± 2.95). We were excluded in both groups all the women with chronic diseases such as diabetes and blood pressure, as well as we excluding women smokers.

All females (patients or control) are assessed the status of obesity by body mass index (BMI).

In this study, use SSPS version 18 to complete the statistical analysis. The results were expressed by the Student's F-test and Pearson correlation analysis. P values less than 0.05 is considered significant.

Results and Discussion

Obesity is a special medical condition resulting from the accumulation of fat in the body, which leads to an increase in body weight and thus affects the health of the body. In this case, the doctor diagnoses the condition that the person suffers from obesity. Usually the doctor uses the BMI as an evaluation tool. If it is high, the person suffers from an increase in weight relative to his age and height. This value combines height and weight[10].

The BMI between 29 and 25 indicates that a person suffers from an increase in weight, either if the value of the BMI increases from 30 or more, so that indicates that the person suffers from obesity[11].

Table 1 shows a significantly increase in the concentration of resistin in serum of women with obesity group by comparison with control group ($p>0.01$).

The present study agrees with the study of Degawa-Yamauchi M,[12] who found that increase in Serum Resistin Protein Is Increased in obese women.

Resistin is an adiponectin, so it is believed that it is excreted or released in large quantities from macrophages, and this leads to stimulation of the production of inflammatory cytokines in human adipose tissue[13].

There are many previous studies, including a study by Lockyer, where it was suggested that the hormone resistin plays an important role in overweight and obese cases, as well as it plays as a connection between obesity and resistin [14].

At the same time, there are other studies and research that indicate a relationship between obesity and insulin resistin. Also, high levels of insulin resistin are also observed in obese women who are obese[15].

The present study agrees with the study of Hotamisligil G.S.[16] who found that increase TNF –alpha Is Increased in obese women. Through its obesity-related overexpression in adipose tissue

The present study shows in Table 2, a significant correlation between the resistin and TNF –alpha in two different groups.

Table 1 : Biochemical parameters of Obesity and control groups.

Parameter	obese group n= 25	Control n=30	P values
Resistin ng/ml Mean± SD Range	(16.45±2.106) (14.2-20.5)	(7.26±1.326) (5.33-9.9)	P <0.01
TNF -alpha pg/ml Mean± SD Range	23.95±1.94 (20.2-27)	10.92 ±2.439 (8.4-16.5 (	P <0.05
BMI Mean± SD Range	34.9±1.4 (31.6-39.2)	21.87 ±1.350 (19.5-24)	P <0.05

Table (2): Pearson's correlation between resistin and TNF alpha the levels of in different groups (n= 55)

parameters	Obese group		control	
resistin vs T NF	R	p	r	p
	0.969	0.01	0.44	0.05

Significant = P< 0.05 high significant = P< 0.01

Conclusion

Depending on this study findings, which inspire us that resistin is participate in pathogenesis of obese related with TNF -alpha.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Antitumor Activity of β-glucan Extracted from *Pleurotus Eryngii*

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Abstract

Pleurotus eryngii, a type of edible mushroom that exhibit various pharmacological properties, including antioxidant and anticancer effects. In the present study, extracted β-glucan from the *P. eryngii* was tested as an antioxidant and anti-tumor factor. β-glucan was extracted and analyzed by HPLC and FT-IR. Analytical results showed more than 90% similarity in chemical structure and purity. Potential antioxidant activity of β-glucan was examined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and compared with ascorbic acid. β-glucan confirmed a potential scavenging activity. The anticancer activity of the β-glucan was assessed using different concentrations (6.25 to 400 µg mL^{-1}) on MCF-7 and HepG2 cell lines. *P. eryngii* β-glucan exerted a dose-dependent reduction in MCF-7 and HepG2 cell viability with an IC_{50} of 280.00 and 539.5 µg mL^{-1}, respectively. At the same time, no significant effect was recorded on normal cell line WRL 68. The obtained results are expected that could be used to develop *P. eryngii* β-glucan as an antitumor drug.

Keywords: *MTT, pleurotus eryngii, Cytotoxicity, β-glucan, Antioxidant, MCF-7, HepG2*

Introduction

Mushrooms have attracted a great deal of interest in many areas of food and bio-pharmaceutical research and are well known for their nutritional and medicinal values [(1)]. Several major components with immunomodulatory and/or antitumor activity have been isolated from mushrooms. These include mainly polysaccharides, such as β-glucans, polysaccharo-peptides, polysaccharide-protein conjugates, and proteins. β-glucans have a wide range of biological activities. Mushroom β-glucan polysaccharides are fibers that mostly present as linear and branched chains with different types of glycosidic linkages, such as (1-3), (1-6)- β-glucans and (1-3)-α-glucans, others are heteroglycans containing glucuronic acid, xylose, galactose, mannose, arabinose or ribose [(2)].

Pleurotus eryngii is an edible mushroom, considered to be a health food not only for low fat and calories but also being rich in amino acids, vitamins, and dietary fiber [(3)]. *In addition,* β-glucans of *P. eryngii* received an increasing interest for its bioactive properties including antitumor, immunomodulator, antioxidant and antiallergic activities. Studies have indicated that the polysaccharides isolated from *P. eryngii* were mostly β-glucans, which exhibited potential activities [(4)].

Chemically, β-glucans are heterogeneous, non-starch polysaccharides, which form the structural compounds of the cell wall of certain microorganisms, including yeast and algae, mushrooms, and grains, such as oats and wheat. β-glucans may be insoluble or soluble. Insoluble β-glucans fibers consist of β-(1-3/1-4)-D-linked glucose units, whereas soluble viscous β-glucans fibers consist of β-(1-3)/1-6)-D-linked glucose [(5)].

Many chemical compounds identified as specific agents for inhibiting cancer cell proliferation were also showed significant toxicity toward normal cells, as well as their side effects. Many potential anticancer drugs have considerable side effects [(6)]. Therefore, discovery of new safer drugs with potential activity against tumor has become an important goal of research in biomedical

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sciences. Polysaccharides from mushroom sources can stimulate immune cells, including macrophages, granulocytes, nature killer cells and monocytes to trigger cytokine production and thus stimulating the immune system [7].

In same point, this study was conducted first to extract β-glucans from *P. eryngii, and* evaluate the cytotoxic activity of the extracted β-glucan against tumor cell lines HepG2 and MCf-7, as approach in developing a mushroom polysaccharides to use either individually or in combination with medicinal drugs combination in cancer treatment.

Materials and Methods

Mushroom strain

Pleurotus eryngii strain was kindly provided and authenticated by Dr. Ahmed A. Kareem, Department of Organic Farming, Ministry of Agriculture, Baghdad, Iraq.

β-Glucan Extraction from *P. eryngii*

β-glucan was extracted using a water extraction method [8]. In brief: dried fruit bodies of *P. eryngii* were powdered. The powder was mixed with ddH_2O in ratio of 1:20 (wt/v). The pH of the mixture was adjusted to 7.0 using 20% Na_2Co_3. Mixture heated to 90°C for 6 h with shaking (100 rpm). After heating process, the mixture was centrifuged at 8000 rpm for 10 min at 4°C. The pellet was discarded, and the supernatant was transferred to new container and the pH was further adjusted to 4.5 using 2M HCl. The solution was centrifuged at 8000 rpm for 30 min at 4°C. Pellet which contained proteins was discarded and supernatant was mixed with absolute ethanol in a ratio of 1:1 and left for 12 h at 4°C to precipitate the beta-glucan. The mixture was centrifuged at 3000 rpm for 10 min at 4°C. Finally, the pellet was homogenized with absolute ethanol and then oven-dried at 60°C.

β-Glucan Analysis by High Performance Liquid Chromatography (HPLC)

The samples and standard were analyzed by HPLC (SYKAM, Germany) supplied with S2100 quaternary gradient pump and fluorescence detector RF-20A (UV280). The condition analysis of β-glucan; mobile phase: dH_2O and orthophosphoric acid (90:10 v/v); column: C18–ODS (25 cm x 4.6 mm); Flow rate = 0.7 mL min^{-1}. Preparation of sample: 1 mg dissolved in 25 mL dH_2O and then 20 µL was injected into HPLC column for analysis. The separation occurred on liquid chromatography and, the eluted peaks were monitored by UV-Vis 10 A-SPD spectrophotometer.

FT-IR (Fourier Transformed Infrared) Analysis

The chemical structure of β-glucan was analyzed using FT-IR spectrometry (Shimadzu IR Prestige-21–Japan). The FTIR spectrum was utilized to detect the functional groups of glucan structure compared with the standard. This was done under FT-IR spectrometry in wavelength range 4000-400 cm^{-1} and at a resolution of 8 cm^{-1}. This test involved mixing an equal volume of glucan sample and standard (2 mg) with potassium bromide (KBr) (100 mg), then grinding the mixture by special grinder until soft and fine powder obtained. The sample was loaded in target mold and analyzed [9].

Determination of Carbohydrate Content

Carbohydrate content was calculated by multiplying the reducing sugar content which was determined depending on Fehling's reducing method [10]. Briefly, 10 g sample was mixed with 20 mL sulphuric acid (0.5 M). Reflux was then performed in a sand bath for 2.5 hours. The residue was washed after filtration (Whatman filter No. 1) with warm dH_2O. The solution was then neutralized with Na_2CO_3 powder and the mixture's volume was completed to 100 mL with dH_2O. Titrations were performed using 5 mL Fehling's solution (equal volumes of solution A and B) pipetted into a conical flask and aliquot of 5 mL dH_2O was added. The solution was then boiled for 15 seconds. Methylene blue indicator (a few drops) was then titrated with the solution until the color changed from blue to green. The carbohydrate content was then calculated according to following equation:

Carbohydrate Content (%) = x 0.9%

Where V = volume of sample solution (titration volume) and W = weight of powdered sample.

Antioxidant Activity

Antioxidant activity of extracted β-glucan was

detected using DPPH (Sigma Aldrich, USA) for free radical scavenging assay [11]. Scavenging potential of β-glucan against DPPH radicals was determined spectrophotometrically (Aquarius, Cecil, Italy). Colour change (from deep- violet to light- yellow) when DPPH reduced was measured at 517 nm. In our experiment, set of concentrations (12.5, 25, 50, 100 and 200 µg mL^{-1}) were used. Ascorbic acid was used as positive control. The inhibition (%) of radicals by β-glucan was calculated according to the formula:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of } - \text{ve control} - \text{Absorbance of the sample}}{\text{Absorbance of the } - \text{ve control}} * 100$$

Cytotoxicity Assay

Cell Culture and Maintenance

Human breast carcinoma cells (HepG2), human breast adenocarcinoma cells (MCF-7) and one non-carcinogenic liver cell line (WRL68) were kindly provided from Biotechnology Research Center, Al-Nahrain University. Cells were maintained and cultured in RPMI-1640 (Sigma Aldrich, USA) supplemented with 10% fetal bovine serum, 100 U mL^{-1} penicillin G and 100 µg mL^{-1} streptomycin. Cells (3 x 10^4 cell mL^{-1}) were seeded into tissue culture flasks and allowed to grow at approximately 80 to 90% confluence monolayer (24 to 48 h). Cultures were maintained at 37ºC in CO_2 incubator with humidified atmosphere. Gentle trypsinization (50 mg mL^{-1} of trypsin) was used for harvesting the cells [12].

MTT

The cytotoxic effect of β-glucan against HepG2, MCF-7 and WRL68 was estimated using 3-(4,5-Dimethylthyiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [13]. Briefly, cells were cultured in 96-well plates and incubated until cells reach to 80% confluence. Medium was removed and 200 µL of different β-glucan concentrations (25 to 400 µg mL^{-1} RPMI-1640 serum free medium) were added to the respective wells containing the cells. Wells with untreated cells were used as the negative control. After 24 h, 10 µL of MTT (Sigma Aldrich, USA) was added to each well. Plates were further incubated at 37ºC, 5% CO_2 for 4 h. The medium was then carefully removed and 100 µL of dimethyl sulfoxide was added per well and incubated for 5 min. Absorbance at 540 nm was measured using an ELISA microplate reader. The percentage of viability (%) was calculated according to the following formula:

$$\text{Viability (\%)} = \text{OD control} - \text{OD sample/OD Control} \times 100$$

Cytotoxicity of each sample was expressed as IC_{50} value.

Statistical Analysis

Data were expressed as means ± standard deviation (SD) and analyzed by a one-way analysis of variance (ANOVA) followed with Dunn's test using GraphPad Prism (Graph Pad Software Inc.). A $p \leq 0.05$ was considered to indicate a statistically significant difference between groups.

Results and Discussions

Dried fruiting bodies of *P. eryngii* was subjected to β-glucan extraction which depended on heating-acid extraction steps. This method is characterized by its ability to extract glucan from mushrooms with significant quantities, limited use of organic solvents and time saving. The total yield of extracted glucan was 7.9%. Previously reported that the total yield of glucan extracted from *P. eryngii* was 6% [14]. The advantages of this procedure were heating, and extensive acid treatment followed by ethanol application which leads to β-glucan precipitation and dissolve or remove most the proteins, mannan, nucleic acids and others. The impurities affect the physical and chemical properties of β-glucan and may cause reducing in its ability to be soluble in water [15]. Furthermore, the carbohydrate content for the extracted β-glucan was 54%, indicating purity and method of choice for β-glucan extraction.

Regarding HPLC analysis, results in Fig. (1) revealed one major peak in sample of the extracted β-glucan at retention time 3.16 min (487.633 mAU) with an overall area percentage of 90%, which indicated the purity of the extracted β-glucan by comparing with the standard which exhibited almost the same retention time at 2.94 min (377.215 mAU). Purity of 90% gave an indication for the successful β-glucan extraction method. HPLC was used for detecting the purity of polysaccharides including β-glucan extracted from mushrooms and yeasts [16].

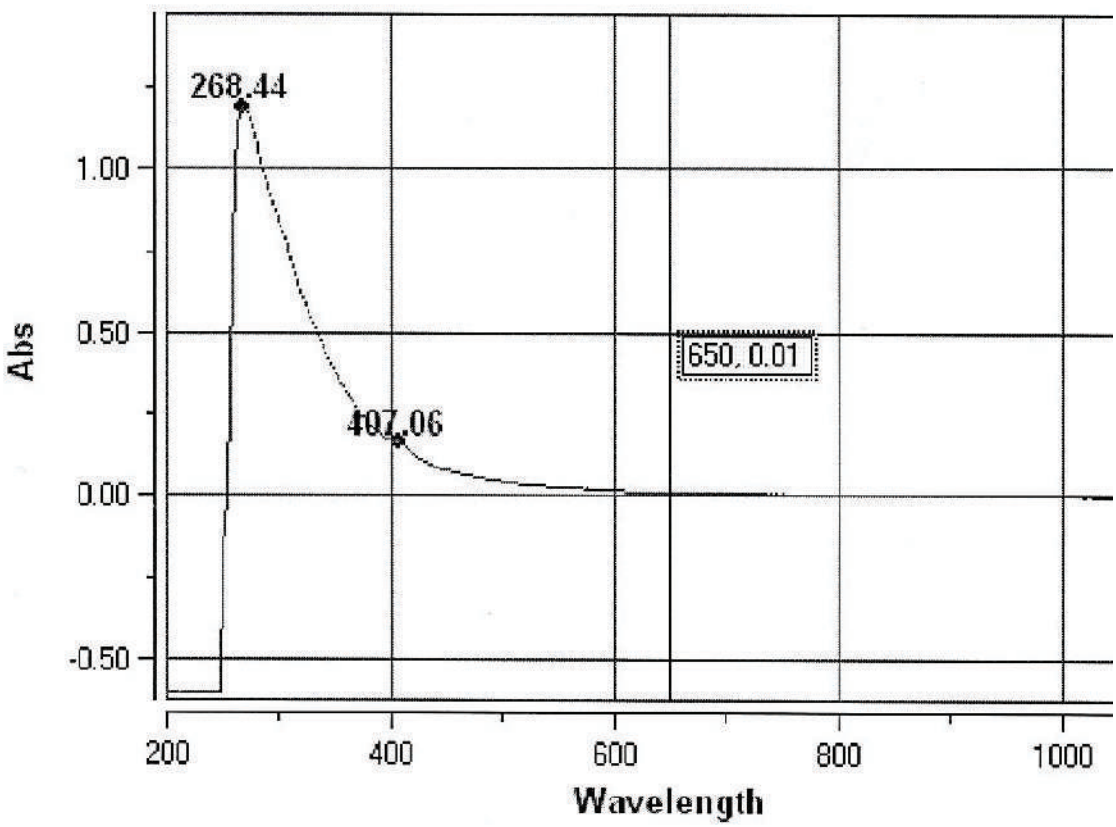


Fig. 1: HPLC chromatogram for (Top) β-glucan standard and (Down) the test sample.

FT-IR analysis of *P. eryngii* β-glucan with absorption range of 4000-400 cm^{-1} to was compared with the resulted functional groups of the standard. Fig. (2A), shows that the band range of ~1027.99 cm^{-1} is a characteristic feature of polysaccharides and assigned for β-1,4 glucans [17], the absorbance peak at this band represent the existence of C-O-C group [18]. In addition, hydroxyl groups and carboxyl groups were detected at band 2923.88 cm^{-1}, these groups are features of carbohydrate structure [19]. Moreover, both sample and standard showed high degree of similarity with respect to overall spectra abortion.

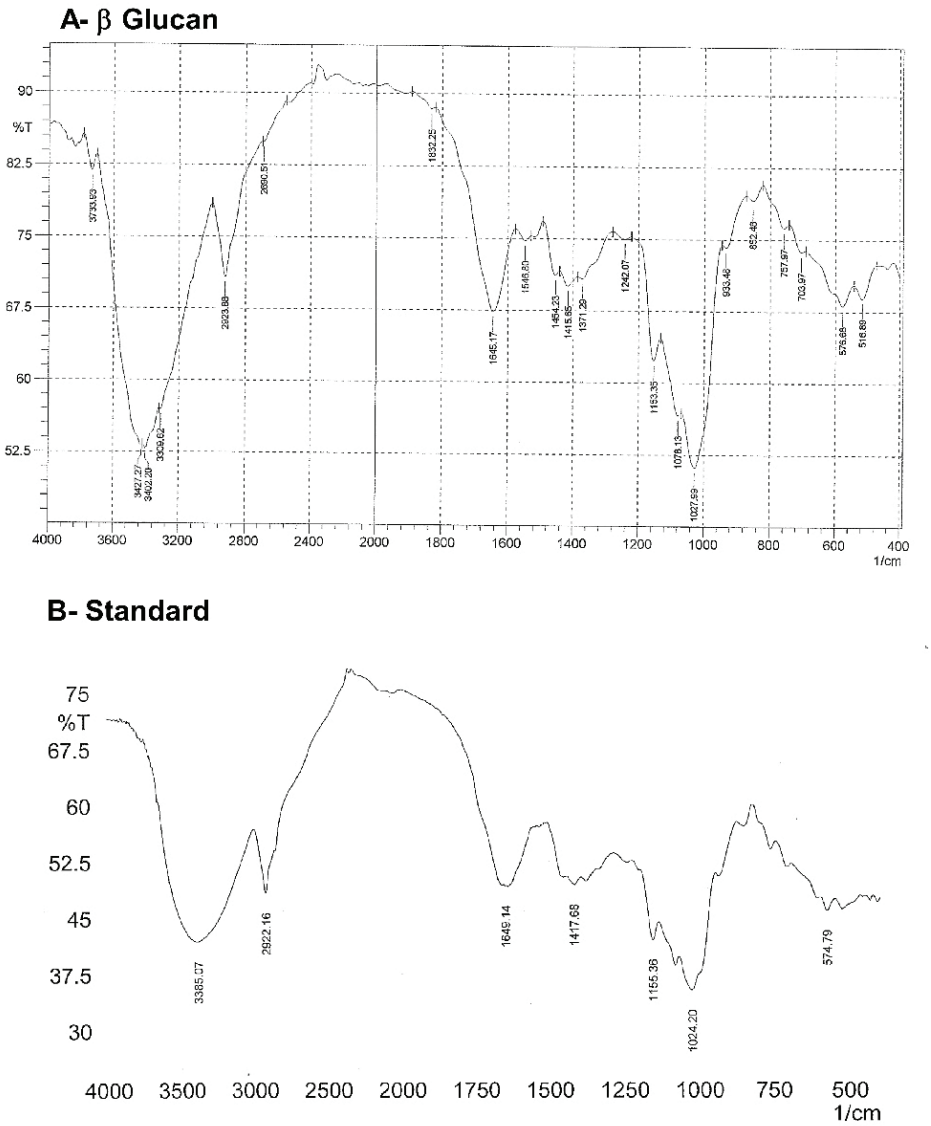


Fig. 2: FT-IR Spectra of (A) extracted *P. eryngii* β-glucan sample (B) standard.

The scavenging activity of *P. eryngii* β-glucan was estimated using increasing concentrations of β-glucan. Results in Fig. (3) demonstrate a potential free radical scavenging capability of *P. eryngii* β-glucan with calculated IC_{50} value of 39.3 µg mL^{-1}. By comparing with ascorbic acid (IC_{50} 27.47 µg mL^{-1}), *P. eryngii* β-glucan showed no significant differences in the pattern of free radicles reduction among all the tested concentrations. β-glucan are the most abundant forms of polysaccharides which display many biological activities including antioxidant [20]. Antioxidant activity of β-glucan is highly dependent on mushroom source and method of extraction. Our results are in agreement with Roncero-Ramos *et al*., [21] which described the antioxidant activity of β-glucan from different mushroom sources including *P. eryngii*. Another study involving edible mushrooms revealed that β-glucan exhibited antioxidant activity 64 to 93% reduction of DPPH [22].

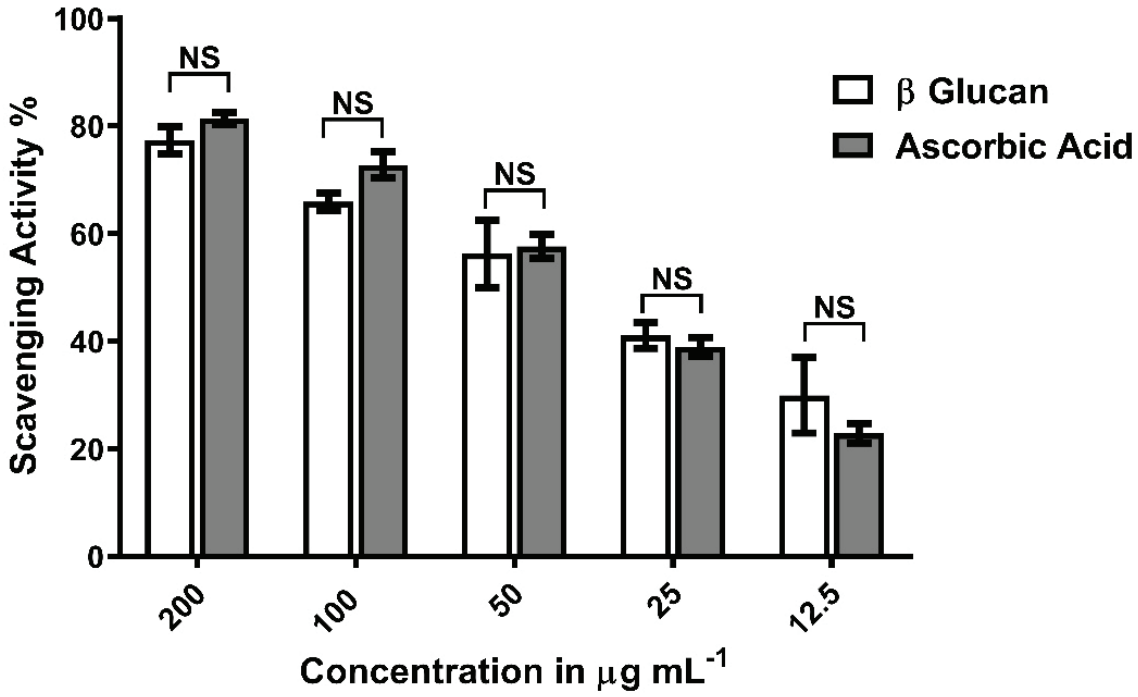


Fig. 3: Mean (%) DPPH scavenging activity of *P. eryngii* β-glucan with respect to ascorbic acid. NS: Non-Significant.

Various concentrations of *P. eryngii* β-glucan were tested against MCF-7 and HepG2 tumor cells. In the present study we used the MTT assay, which is universally used to evaluate the cytotoxic potency of drugs *in vitro* [12]. Results in Fig. (4) shows that the more increasing in β-glucan dose the more reduction in MCF-7 and HepG2 viability. The effect of MCF-7 and HepG2 viability by *P. eryngii* β-glucan exhibited a dose dependent pattern of reduction with a calculated IC_{50} of 280.00 and 539.5 μg mL^{-1}, respectively. On the other hand, *P. eryngii* β-glucan had slight toxic effect on the cell viability of normal cells WRL68.

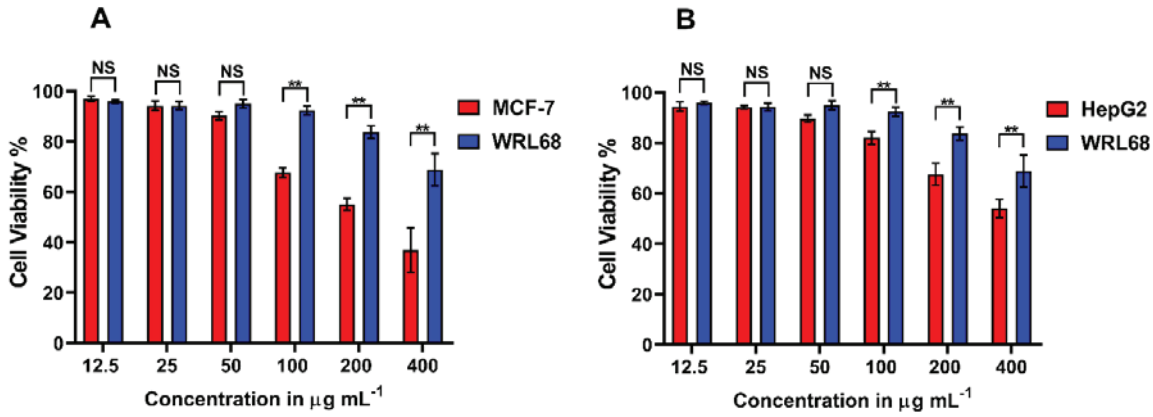


Fig. 4: MTT assay for *P. eryngii* β-glucan against (A) MCF-7 (B) HepG2 with corresponding normal cell line WRL68. Mean (±SD) of viability was detected after 24 h treatment. Differences considered significant: **$p \leq 0.01$. NS: Non-Significant, SD: Standard Deviation.

The biological, immunological and pharmacological activities of glucans extracted from edible mushroom are mainly attributed to β-glucan [23]. The cytotoxic activity of *P. eryngii* β-glucan against different types of tumor cells indicated a significant dose-dependent inhibition of cell proliferation and exerted direct cytotoxicity after 24 h. Many reports indicated the anti-proliferative effect of β-glucan extracted from different mushroom sources. The effect of β-glucan *in vitro* against different tumor cell lines was well demonstrated [23]. In addition, pervious finding indicated that the toxicity of β-glucan on human pigmented malignant melanoma (Me45) cell line increased by increasing β-glucan concentration with viability reduction reached up to 19%. Moreover, it was proved that glucans in nature have low toxicity on normal cells and well tolerated by patients treated with glucan combination [24].

Conclusions

We can conclude that the extracted *P. eryngii* β-glucan exhibited strong antioxidant capabilities and promising anti-proliferative potential on tumor cells *in vitro*, which needed more investigations regard the mechanism of β-glucan in inducing tumor cell viability reduction.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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The Effect of Multiple Glass Bead Sterilization Cycles On Cyclic Fatigue of AF BLUE S one File

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Abstract

Introduction: Sterilization represent an important and essential aspect, now days Most of the dentists usually use the endodontic files for many times before they discard it, this could increase the need for reliable and fast sterilization method, but without affecting the physical properties of the endodontic files.

Aim of the study: This study discusses an economic method of sterilization (glass bead) and its effect on the fracture resistance of the AF Blue S one file (Fanta) with multiple reuses of the system. Method and materials: A total of 75 AF BLUE S one NiTi Rotary files (FANTA) #25 size with 0.04 taper and 25 millimeter length, were used in this study, divided into three groups to test their cyclic fatigue resistance after multiple sterilization cycles. Result: The results were statically analyzed using (SPSS) software, Anova test showed a statically high significant differences between the groups. Conclusions: Within the limitation of this study, it can be concluded that AF Blue S one file with 4 time glass bead sterilization cycle was the most fatigue resistant compared to zero and two sterilization cycle.

Key words: *Sterilization, Glass Beads, Endodontic Files; Toxicity; sterilization*

Introduction

Sterilization represent an important and essential aspect and it is a cornerstone in controlling the infection that could easily spread by various instruments which are used in the dental field.(1)

Dentists depends on files in performing root canal treatments, these files come in direct contact with the blood of the patient which can easily spread dangerous diseases like " AIDS and Hepatitis ". Most of the dentists usually use the endodontic files for many times before they discard it, this could increase the need for reliable and fast sterilization method, but without affecting the physical properties of the endodontic files.(2,3)

Wet sterilization as autoclave and dry sterilization as the oven and glass bead sterilizer are the mostly used by the dentists,(4) unfortunately autoclave causes dullness and decrease the sharpness and cutting efficiency of the endodontic files, on the other hand dry heat oven needs time that is long to perform sterilization " sixty minutes at 180ºC ", while glass bead sterilization is a variation of dry heat oven, needs only short time.(5,6)

Nickle Titanium (NiTi) files are appropriate instruments used for negotiation of the canals specially curved canals, The use of these files offers the reliability and possibility to provide a predictable root canal preparation, unfortunately these files are vulnerable to fracture.(7)

Many new NiTi files have been marketed by manufactures with the aim to offer safer and more effective file system.)8) The thermomechanical treatment of NiTi alloy resulting in enhancement of the physical properties by changing the crystalline structure of the alloy, reducing the frequency of file fracture.(9)

Repeated and prolonged usage of endodontic file has a negative effect on its physical properties which can

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lead to fracture. Some reports showed that the majority of file' s separation was due to cyclic fatigue. These files are used for many times by the dentists undergoing repeated cycles of sterilization.(10)

This study discusses an economic method of sterilization (glass bead) and its effect on the fracture resistance of the AF Blue S one file (Fanta) with multiple reuses of the system.

Method and Materials

A total of 75 AF BLUE S one NiTi Rotary files (FANTA) #25 size with 0.04 taper and 25 millimeter length, were used in this study, divided into three groups to test their cyclic fatigue resistance after multiple sterilization cycles:

Group 1: 25 files undergo no sterilization (control).

Group 2: 25 files undergo two cycles of sterilization with ten minutes intervals.

Group 3: 25 files undergo four cycles of sterilization with ten minutes intervals.

Files sterilization was done using glass beads sterilizer for 15 seconds at 425-475 ºF (218-246 ºC).(11)

In this study cyclic fatigue test has been done in custom made tapered stainless-steel artificial canals with regular (5 mm) radius and angles of curvature (90º), due to the limitation of obtaining standard result on using natural teeth associated with the wide variety of canal shape.(12) The block has been designed according to the dimensions of the files which aimed to check out, Test was carried out in according to the manufacture recommended speed of rotary system (375) Rpm, with 2.6 N torques manipulate setting. The middle of the simulated curvature used to be (5-7) mm from the tip of the file which has been positioned at full working length (19 mm). The whole files have been new and the working section is 25 mm in length. Cyclic fatigue tested used to be conducted with the file rotating freely inside the tapered artificial canal which result in that reproducible simulation of the file restrained in the artificial curved canal.(13,14)

The dental hand-piece has been mounted on wooden block that allowed manipulate of the hand-piece movement, and easy placement of every file inside the artificial canal, making sure 3-dimensional alignment and positioning the file to the identical depth for standardization. The artificial canal has been covered with transparent plastic sheet to forestall the files from slipping out and to enable the researcher observe the files while it works and when fracture has been occurs, so fracture was detectable due to the fact the files have been seen through the transparent plastic sheet window**.** (13,14)

Stainless Steel block was once constant to the wooden block to forestall its movement and to make the relation between the steel block and the hand-piece almost constant.(15) glycerin has been stuffed totally to inside the artificial canal, earlier than each file to the exacted size (19 mm) inside a canal in order to minimize friction and heat generation.(16) The files have been activated inside the canals by usage of (ENDOMAX PLUS) cordless endodontic hand-piece.

Video recording has been carried out simultaneously for more accurate work and to eliminate human error.(16) This equation describe the (NCF) for every file.

"Number of cycles to failure NCF = Speed RPM X Time (T) to fracture in minute"

The armamentarium used in this study are showed by (fig.1)



Fig: Instruments used to perform the study.

Results

The means and standard deviations of (NCF) for the three groups are shown in (Table 1). The results were statically analyzed using (SPSS) software, Anova test showed a statically high significant differences between the groups ($P < 0.001$).

Intra group comparison using least significant difference test also showed a statically high significant

differences between the means of the three groups (Table 2).

Table 1 : The means and standard deviation of Number of Cycles to Fracture

Groups	No. of Files	Mean	SD	P
Group 1 (No Sterilization)	25	585.2	20.79	0.001
Group 2 (Two Cycles of Sterilization)	25	966.3	23.78	
Group 1 (Four Cycles Sterilization)	25	1058.2	20.45	

Table 2 : The Least Significant Difference Test between groups

Groups	P
Group 1 V Group 2	0.001
Group 2 V Group 3	
Group 1 V Group 3	

Discussion

The control of infection that is spreading by various tools used in dentistry is of great importance as a preventive measure for cross infection.

During procedure of root canal treatment the endodontic files come in direct contact with pulp tissue, oral fluids blood and saliva, these files could be considered " reusable sharps " making there sterilization as a must mandatory step.[17] several sterilization techniques utilized such as autoclave, dry heat oven, ethylene oxide gas and also glass beads sterilizer which provide dry heat with shorter exposure time [18,19]

Ni-Ti endodontic files are always having the risk of separation because of cyclic fatigue or torsional stress,[20,21,22] To reduce or overcome this mechanical failure, many methods have been applicated by manufacturers to improve the fatigue resistance of file like electro-polishing the surface of the file eg: (RaCe), twisting instead of machining eg: (TF), and geometric characteristics alteration such as asymmetric design (Revo-S). [10,23]

Today, thermal processing could be the main approach to develop the alloy metallurgy properties by affecting its transition temperatures and thus leading to alter the fatigue resistance.[24,25] Any Temperature changes will affect crystalline phases in NiTi alloy. These changes result in enhanced the file physical properties such as cutting efficacy has been increased and the fracture resistance also elevated.[26,27] However, sterilization in some studies has been showed that not necessarily effective on the cyclic fatigue of the files.[28]

The choosing of two or four cycle sterilization cycles in this study based on reports showed that NiTi file can be used to shape up to 10 curved root canals.[3, 29]

In this present study statistically significant increase in the life time of the AF Blue S one file with the increase in the number cycle of glass bead sterilization in comparison with the cyclic fatigue of the file with zero and two cycle of glass bead sterilization. This result may be related to the heat treatment of the alloy and the

wire intermediate face. A modified phase composition because of the changing transformation temperatures is the essential difference between the conventional NiTi and the thermomechanically treated alloy. As the conventional NiTi alloy contains austenite,[30] while, the thermomechanically treated NiTi alloy moreover contains varying amounts of R-phase and martensite under clinical conditions [31,32,33]. These modifications are supposed to lead to extra flexible endodontic files with a superior resistance to fracture which mean decrease the stress on the file during negotiating the canal which lead to increase fracture resistance[34], so thermomechanical treatments represent a modern technique of developing new endodontic file with enhance mechanical properties.[35]

This result may be similar to research of de Melo et al [36] and Zhao et al[37] showed that sterilization increased the fatigue life of rotary Ni-Ti instruments through the increase in hardness and torsional resistance of the material, also Khalil and Natto [38] in their study shows that cyclic fatigue increase with the modern heat treated file and thus exactly what the present research concluded. The result of this study may be related to the wire of the AF One Blue S file and the AR phase heat treatment of Ni-Ti alloy, While several research had been shows disagreement with the result of this study as Mize et al[39] and Hilt et al[40] Also, AbuMelha [41] and Alshwaimi [42], which suggested that increase the number of autoclave sterilization of rotary files reduced their cyclic fatigue resistance.

Conclusions

Within the limitation of this study, it can be concluded that AF Blue S one file with 4 time glass bead sterilization cycle was the most fatigue resistant compared to zero and two sterilization cycle and so multiple cycles have significant alterations in the cyclic fatigue resistance of rotary AF Blue S one files.

Recommendation:

According to the result of the present study it's recommended to sterilize the NiTi files with glass bead before using them.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Study of Mortality *Acanthopagrus Latus* Fish in Iraq

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Abstract

This study was conducted in Iraq in Karbala during the winter period in December and January of each year. The study aimed to know the causes of fish death in large numbers. *Acanthopagrus Latus* By investigating the most important causative factors by taking random samples of infected fish in Lake Razazah, it was noticed through a study of the appearance of these fish that they have severe laceration with clear redness in the abdominal area and specifically near the opening of the complex, which extends to the dorsal area near the gills cover Over time, in some of the studied samples, the tissue culture of the affected area indicated the presence of a fungus *Aspergillus Niger* Which may be one of the causes, our **conclusion** caused by the death of fish *Acanthopagrus Latus* In addition, someone has approximately (12) types of yeasts found in these infected fish.

Keyword: Acanthopagrus Latus, histological, Toxins, mortality

Introduction

Fish, like other neighborhoods, are exposed to many diseases, as the water environment is one of the important means of transmitting many diseases to fish, in addition to the presence of any environmental or physiological imbalance that may lead to a loss of balance between the fish's ability to resist and its resistance to the environmental medium, as this imbalance leads to The emergence of a disease condition and environmental pollution with fungi may be one of the most important problems facing humans and animals, which leads to the availability of conditions for their growth [1]. The dangerous effects of fungi are due to their production of some secondary metabolic products known as mycotoxins MyCotoxins Which causes severe toxic symptoms Acute toxicity Or it is of chronic toxicity chronic toxicity [2].

In addition to causing poisoning Poisoning Which leads to serious injuries to the nervous and digestive systems, renal and hepatotoxicity of the human being, and it also works to inhibit the body's immune response and that the least pathological form appears in the form of allergies [3].

Also, the sharp changes in the water temperature that increase the concentration of chemical pollutants and toxins that weaken the immunity of the fish and its ability to adapt, and consequently its death, are the result of increased rates of infection with fish diseases due to bacteria or fungi, or the presence of a viral infection such as the herpes virus / koi (CYHV-3) (KHV) In the gill tissue, kidney and brain, this virus is dangerous and deadly [4].

Both indicated [5] Until the poisoning with fungi toxins in Russia as a result of using the news contaminated with fungi Fusarium sporotrichioides. The product of toxins Trichothecenes In the nineteenth century, the population was infected with nutritional enterococcus Aimentary toxic aleukia.

A state of decadence was recorded in a large number of horses in the Middle East in 1934 as a result of feeding with yellow corn contaminated with molds. The disease was known at the time as Moldy corn disease [6].

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A study also indicated the existence of the deaths of many birds as a result of feeding them with a fodder contaminated with a fungus Aspergillus flavus the product was poisoned to aflatoxin and the disease was known at the time Turkey × disease [7].

And finally, the emergence of cases of heart urticaria Cardiac beriberi Cirrhosis of the liver, total renal and cardiovascular disorder as a result of the Japanese population eating rice contaminated with the fungus Aspergillus [8].

Materials and methods of work

A preliminary study was conducted by taking a swab from the affected area to determine the cause of the injury, so it appeared that it was a fungal, not bacterial, infection, as the study was conducted during the winter season (December and January 2017-2018).

Random samples were taken from the fish, whose number ranged from 100 fish to varying degrees of infection. Their total length ranged from (28-31) centimeters and their weights ranged between (380-450) grams. Tissue sections were taken from the affected area and were transplanted into the agricultural medium. Zapex agar the record according to the method [9] Which includes the following materials: (1) Cloud K_2Hpo_4 (0.5), Cloud kcl (2), Cloud $NaNo_3$ (0.5), Cloud $Mgso_4.7H_2O$ (0.01), Cloud (20), $Feso_4.7H_2o$ Cloud , Sucrose (18) Cloud Agar Dissolve the ingredients in a liter of distilled water, then place the incubator installed at a temperature (25 ± 2)C ° for three days, a person who breaks the mushroom according to the shape and color of the colonies on the Zabic medium, in addition to the diagnostic characteristics of the fungus, where the source was approved In the diagnosis[10] .

Results and Discussion

The study showed a sample of fish infected with different degrees of skin infection with a fungus *Aspergillus niger* This may be one of the most important reasons that lead to the destruction of large numbers of fish, as well as the histological study showed that the fish were infected with (12) different types of yeasts.

The fungus that causes this disease has the ability to live in different thermal ranges and its appearance in most seasons of the year, which made it outside the group of fungi. Mesophilic fungi Within (20-30) degrees Celsius and the researcher was able [11] In his study of desert soils near Lake Al-Razzazah to isolate (74) species, the deficient fungi were prevalent, as they accounted for 85.1% of the number of species and the gender was Aspergillus It is the predominant one as (14) species were isolated from it (19.7%) in addition to the capacity of Qatar Aspergillus On the spread in a proportion characterized by high salinity [12] Because of the destruction in large numbers of fish due to the different tissue rot resulting from this injury [13] . One of the main reasons that help the spread of fungal diseases among fish is water pollution and lack of ventilation or a high proportion of ammonia and other toxic substances as most fungal diseases are diseases with secondary infection [14].

Among the most important signs of fungal infection is the infection of the skin with dehydration and the presence of red, dark, superficial sores in addition to scales and fungal diseases. These are secondary diseases that affect fish pre-infected with certain wounds or bacterial or parasitic diseases, or that suffer from inappropriate conditions [4] [15].

That mushroom Aspergillus Toxins are produced Aflatoxins They are effective biological toxins that may be the main cause of death and death of these fish, as well as their ability to cause many diseases for humans and animals, such as cancer of the liver and reproductive system, as well as causing miscarriage and hemorrhage [16] [17] .

He pointed out [18]The mushroom's ability to withstand various environmental conditions, whether its growth and reproduction, or its formation of reproductive units, not sexual, in large numbers, sexual stages, and stone bodies more resistant to inappropriate environmental conditions and for most types of high enzymatic susceptibility to help it use various materials as a food source, so it tolerates salinity and dehydration in Desert soils are also characterized by their ability to grow in poor soils with nutrients Figure 1.

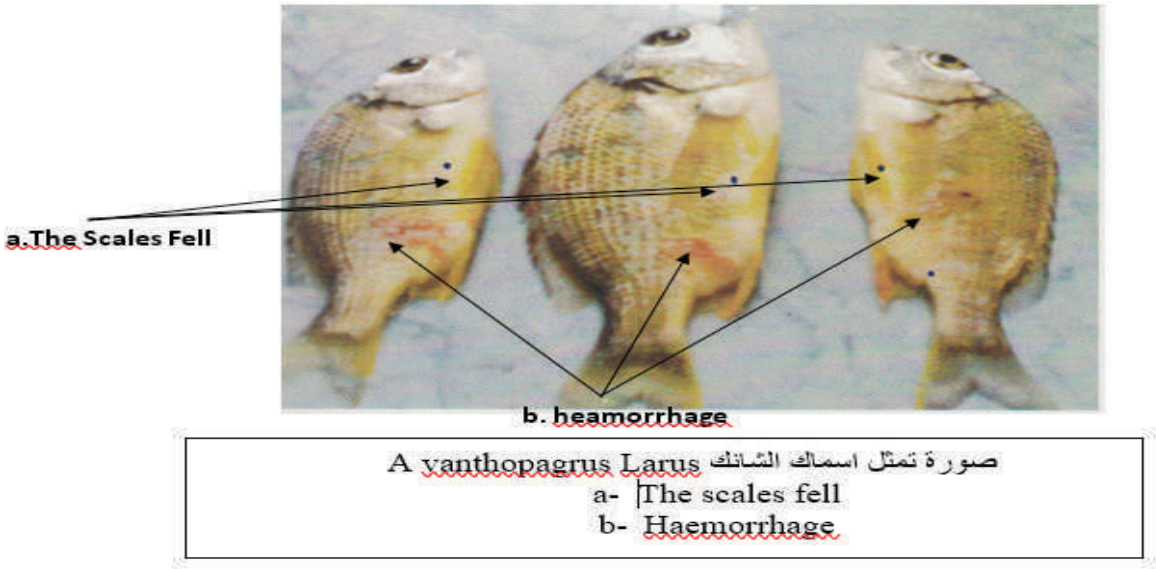


Figure 1: A picture representing Shanks A vanthopagrus Larus "a- The scales fell; and b- Haemorrhage"

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Heritability of Ambulatory Blood Pressure in Population of Western Region of Iraq

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Abstract

Heritability of ambulatory blood pressure parameters in western population has been well described, there is no information on Iraqi population. Therefore, the aim of the present study is to investigate genetic influences on intra-individual variation, by performing heritability analysis on an Iraqi population (Anbar province).

A total of 389 individuals, constituting 93 families were ascertained. Most of the parents included in the study were in their 5th and 6th decade of life, and the siblings included in the study were in their 2nd and 3rd decade of life.

To define the portion of difference of variables that is due to genetic and non-genetic influences, full sib analysis was used to estimate heritabilities of blood pressure parameters systolic, diastolic, mean and pulse pressure were estimated during the morning and evening. Heritabilities were estimated in narrow sense which were ranged from 28 % for morning systolic blood pressure to 84% for morning systemic pulse pressure.

The finding of modest narrow sense heritability indicates the significant role of non-genetic factors affecting morning systolic, evening systolic and diastolic, and mean blood pressure, whereas the high estimate of heritability for morning diastolic and pulse pressure indicate the significant effect of genetic.

Key words: *Heritability, Blood pressure, systolic, diastolic*

Introduction

Hypertension, which has an impact on about 1/3 of the old age people of the population in Western countries, is currently becoming a considerable problem in Middle Eastern region too, with incidence ranging from 16% to 32% amongst adults[1-3]. Globally, by 2025, it is expected that there will be 1.5 billion people with systemic arterial hypertension[4]. An elevated blood pressure measurement is an important risk factors in 49% of coronary artery disease and 69% of stroke, and lead to half of all cardiovascular mortality[5,6].

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A increasing number of studies exhibit the presence of significant genetic difference in natural populations. The amount of genetic difference for phenotypic characters that is exist in a population is the key element of their potential for reacting to different aspects. The impact of genetic difference on a character might be very subtle to diverse contributions an individual receives from its environment. It is very inspiring to reveal genetic variations that require this things. In human being population genetics studies, there is no opportunity to governor the environmental exposures. These can be examined by intensive subject profiling. Though, it might be essential to study populations having individuals with significantly diverse environmental exposures to detect the major effects, and the price and complication shaped by this kind of researches is an obstacle[1,7,8].

The first step in unscrambling the genetic sources of an illness or character is the heritability estimate which is usually reflected the first pace in revealing the genetic bases of a disease or trait and numerous studies existing or heritability estimates for different characters or diseases. Following step is publishing the finding results later these results to assist in determining the necessary sample size in order for the study to have a positive influence to recognize the vulnerable gene[1]. Highly heritable traits could be correlated with important or complex traits, and these interactions very important in any program to understand the effects of genetic and environment factors that effect on blood pressure parameters[9]. Although heritability of ambulatory blood pressure in many populations such western population and east African population have been well described[10,11], there is no information in Iraqi population. Therefore, the purpose of the present study is to investigate genetic influences on intra-individual variation of ambulatory blood pressure parameters, by performing full-sib heritability analysis on a sample of Iraqi population reside in the western region of Iraq.

Materials and Methods

Subjects

Ninety three families were included in this study. The total number of studied population was 389 individuals, consisting of 18- to 61-year-old healthy subjects. All subjects provided informed consent.

Measurements of Anthropometric

Sex, waist circumference, height and weight were measured at home using standard methods. Body mass index (BMI) was measured as weight/height^2 (Kg/m^2).

Blood pressure

Measurements of ambulatory blood pressure were documented throughout the period of the study. Blood pressure was documented by the average of two measurements taken at specified times in the morning and afternoon. The blood pressure was measured after the subjects have been seated for 5 min, using a standard mercury sphygmomanometer. The subjects were in a supine position. A first reading was taken followed by a second reading after a 2 min delay. The mean of two consecutive measurements which were less than 10 mmHg apart was used. Siblings included in the study were in their 2nd and 3rd decade of life, most of them were having a normal body mass index (BMI), and a waist circumference (WC) below 80 cm. The measured phenotypes of blood parameters were morning systolic blood pressure(MSBP), morning diastolic blood pressure (MDBP), evening systolic blood pressure (ESBP), evening diastolic blood pressure (EDBP) , mean of morning systolic blood pressure (MMSBP), mean of morning diastolic blood pressure (MDBP), mean of evening systolic blood pressure (MESBP), mean of evening diastolic blood pressure (MEDBP) , morning pulse pressure (MPP), evening pulse pressure (EPP)

Mean BP(MBP) was estimated as :

$$MBP = DBP+[(SBP-DBP) / 3] \quad \text{..... (1).}$$

The difference between the systolic and diastolic pressure define as pulse pressure. It is reading in millimeters of mercury (mmHg). It shows the force that the heart produces each time it contracts. was calculated by the following formula.

$$\text{Systemic pulse pressure} = P_{systolic} - P_{diastolic} \quad \text{.....(2)}$$

Also, Uric acid (UR), cholesterol (CH), and triglycerides (TRI) were measured for each individual under investigation.

Statistics

Descriptive statistics were accomplished by SPSS software package (IBM Corporation New York, USA) [12].

Heritability

Heritabilities estimate accomplished by utilizing the maximum-likelihood-grounded on variance breakdown technique executed by the SAS program [13].

To define the portion of variance of the variables that is due to genetic influences, the full-sib method was used to estimate the heritability for different characters under investigation. In order to reduce the inflation of genetic variation estimate that may arise from similarity of a number of environmental effects, therefore the potential effect of all main effects that were included in this analysis were statistically factored out. Those factors were age, body mass index and waist circumference.

A P-value less than 0.05 were considered statistically significant. Estimated heritability near (0) imply that there are no genetic variation whereas values close to (1) imply strong genetic variation under the assumption of an underlying multifactorial model [9] . All analyses were performed using SAS[13].

Results and Discussion

Blood pressure is a complex genetic character with heritability estimation between 30 to 70% of the character difference which is due to genetic difference. The advancement of genetic exploration to recognize the genes that cover this difference and thus has impact on blood pressure regulation and effect the risk of hypertension has gotten a new level with large number of publication of wide scale of population genetic researches. Those have studied very large numbers of genetic markers in an effort to link single markers with blood pressure and hypertension [1,3,14,15].

Heritability values are helpful in predicting the expected genetic and environmental variations for any traits under investigation for specific population. Estimates of heritability in narrow sense ranged from 28 % for MSBP to 84% for morning systemic pulse pressure (Table 1). The estimate of heritability in table (1) of MSBP, EDBP , ESBP, MMBP, MEBP, Cholesterol, Uric acid were low category and other traits were moderately to very high. This study was conducted to estimate heritability by full-sib method. Whereas, greatest heritability researches on ambulatory BP were conducted in twins and have stated big value of heritability estimates. Researches in diverse cohorts of twins of Caucasian and African backgrounds stated heritability value for systolic BP (SBP) and diastolic BP (DBP) throughout day and night (sleep) time reaching 0.44 to 0.75 [11,14,16].

Table (1) Heritabilities and means for different characters under investigation with their corresponding standard errors

Characters	No.	h2 ± S.E	Mean ± S.E
Morning DBP	389	0.70 ± 0.24	78.14 ±7.16
Morning SBP	389	0.28 ± 0.22	120.22 ± 9.650
Evening DBP	389	0.36 ± 0.23	77.47 ± 7.72
Evening SBP	389	0.34 ± 0.23	119.49 ± 9.53
Mean Morning BP	389	0.39 ± 0.21	91.74 ± 6.94
Mean Evening BP	389	0.39 ± 0.20	91.01 ± 7.40
Morning Systemic pulse pressure	389	0.84 ± 0.22	42.08 ± 8.35
Evening Systemic pulse pressure	389	0.28 ± 0.21	41.69 ± 7.97
Cholesterol	389	0.31 ± 0.22	180.88 ± 25.56

Cont... **Table (1) Heritabilities and means for different characters under investigation with their corresponding standard errors**

Triglyceride	389	0.53 ± 0.21	196.22 ± 100.02
Uric acid	389	0.39 ± 0.19	6.86 ± 16.914
Age	389		28.13 ± 9.93
BMI	389		24.05 ± 6.65
WC	389		85.10 ± 16.39

The characters having low heritability indicated relative small contribution of the genetic factors and large contribution of environmental factors to the phenotype and such characters could be fairly easy manipulate by modifying environmental factors like medicine , smoking and diet due to low additive effect and large environment effects. In addition to that, heritability could be altered over time since the difference in genetic variance can change, the difference due to environmental elements alteration, or the association between genetic factor and environment can altered. Genetic variation can modified if allele occurrences altered might be due to consanguinity, novel variations obtained by the population by mutation or immigration which will add to the genetic difference resulting from the change in genetic bases or the environment. Similar character estimated throughout an individual's lifespan might have diverse environmental and genetic influencing it, like the differences turn out to be a role of age. The results are in conformity with heritability estimation of blood pressure in people descended from east African [10, 17,18].

Table 2 The correlation between different characters under investigation.

	MSBP	**MDBP**	**ESBP**	**EDBP**	**CH.**	**UR**	**TG**	**MMBP**	**MEBP**	**MPP**	**EPP**	**WC**	**Age**
MSBP	1												
MDBP	.54**	1											
ESBP	.62**	.43**	1										
EDBP	.36**	.67**	.62**	1									
CH	.13	.03	.09	.06	1								
UR	.00	.031	.02	.04	.03	1							
TG	.18	.08	.15	.04	.24*	-.06	1						
MMBP	.80**	.92**	.54**	.62**	.07	.02	.14	1					
MEBP	.50**	.62**	.83**	.93**	.07	.04	.10	.65**	1				
MPP	.69**	-.23*	.39**	-.16	.12	-.01	.14	.13	.05	1			
EPP	.37**	-.17	.61**	-.24*	.03	-.01	.15	.04	.09	.58**	1		
WC	.37**	.04	.34**	-.05	.19	-.02	.04	.19	.10	.40**	.47**	1	
Age	.35**	.28**	.42**	.28**	-.02	-.07	.04	.34**	.33**	.16	.16	.38**	1
BMI	.55**	.13	.44**	.15	-.23*	.002	.18	.32**	-.29**	.53**	.35**	.59**	.44**

** P≤0.01

* P≤0.05

The degree of association among the characters is an significant factor particularly in vital and complex character as blood pressure. Steel and Torrie [19] showed that correlations are measures of the intensity of association between traits. The positively correlated characters resulted in progress all characters and retrogress for characters that are adversely correlated.

The result of correlation investigation as presented by their coefficients (Table 2) showed that MSBP, displays significantly positive correlation with the MDBP, ESBP, EDBP,MMBP, MEBP, MPP and EPP which were 0.54** ,0 .62**,0.36**,0 .80**,0.50**, 0.69** and 0.37** respectively.

Throughout the day, BP had no significant differences in SBP and DBP between morning and evening measurements. There is significant correlation between MSBP and ESBP which was 0.54** . Age was significantly correlated with MSBP , MDBP, ESBP, EDBP, MMBP, MEBP,MPP and EPP which was 0.35** , 0.28**, 0.42** , 0.28**, 0.34**, 0.33**, 0.16, and 0.16 respectively. Sex, age, BMI and waist circumference were kept as covariates in all model-fitting analyses. Body mass index correlated significantly with MSBP (0.55**) but not with MDBP (0.13). The correlation between blood pressure characters and cholesterol, triglycerides and uric acid showed no significant correlation. While, the correlation between cholesterol and BMI was significantly negative -0.23** [20].

These results confirm that the assortative mating and environmental influences have shown to be an effective factors which effect heritability estimation, in addition to the familial aggregation of cardiovascular risk factors happens and that much of this aggregation has a genetic background. Since the study was carried out in very much consanguineous marriages, and multigenerational Arab individuals, with an average age of 28.13 years, 90% of whom were younger than 30 years. Those people were more similar environmental exposure with alike social and economic status and similar in habits related health like habitual physical activity; diet, smoking and the strict religious abstinence from alcohol [7,21,22,23].

Conclusion

The results showed that there is sufficient genetic variation existing in the material studied. The narrow sense heritability analysis of the study revealed that the morning diastolic blood pressure and morning systemic pulse pressure were the most traits with genetic variation components. The other characters also showed moderate to low heritability and genetic variation. Therefore, the results suggest that morning diastolic blood pressure and morning systemic pulse pressure are important characters contributing in the phenotype of blood pressure and based on these traits would be most effective in any program to control blood pressure.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Correlation Ship between Some Hematological Parameters and Smoking for Students

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Abstract

This study including collected 172 blood sample from AL-Kafeel University college, these samples including 102 sample to male and 70 sample for female in which students samples were 53 for smoker and 119 non-smoker , to evaluate some of parameters such as ABO , RH , blood pressure and clotting time .

The results showed blood group A^+ was more frequent which represent 33.7 % followed by O^+ blood group which represent 25.6% while B^- was the last ,in other results displayed AB^- and RH^+ was more frequent than RH^- . Systolic and diastolic pressure examined was demonstrated there were mild hypertension in blood pressure between samples of studied, while result gloating time was exhibited range 30 sec to 3 minute .

Keywords: *blood group, duration of smoking, Blood pressure and Clotting time*

Introduction

Blood pressure is a cardiovascular measurement with dynamic characteristics that can be influenced by a number of internal and external factors. Hypertension is classified as either primary or secondary. It is primary when no medical cause can be found to explain the raised blood pressure. This type represents between 90 and 95% of hypertension cases[(1)]. Secondary hypertension represents approximately 10% of all hypertension cases. Identifiable underlying causes of secondary hypertension are kidney disease, renal hyperaldosteronism, and pheochromocytoma. Secondary hypertension has specific therapy; it is potentially curable and often distinguishable from primary one on clinical grounds[(2)]. The term high blood pressure mean each blood pressure above 120/80 mm Hg. High blood pressure was known as the "silent killer" due to the large destruction caused to the blood vessels [(3)] ,whereas hypertension refers only to pressures of 140/90 mmHg and above[(4)].

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A systolic blood pressure of fewer than (90) millimeters of mercury (mm Hg) or diastolic of less than 60 mm Hg was usually considered to be hypotension[(5)].

The blood pressure involves two numbers. The top number indicates the force of shrinkage of the heart's main section, the left ventricle, and the lower number agrees with the resistance to blood flow in the arteries[(6)] .There are convinced risk factors that we have no control over for instance: genetics, age, sex and race according to Casey and Benson, (2006).

The antigens of the ABO blood group system (A, B and H determinants) are complex carbohydrate molecules expressed on red blood cells and on a variety of other cell lines and tissues [(7)].

Smoking is an important cardiovascular disease risk factor, but the mechanisms linking smoking to blood pressure are poorly understood[(8)] .

Materials and Method

Sample collection

This study was involve collection of 172 blood

sample of Al-Kafeel university students, then some of tests was accomplished.

Blood pressure determination:

Check that the patient's arm is not restricted by any tight clothing. Support the arm with a pillow, ensuring that it is level with the person's heart (midst Ernst level). than ,Wrap the BP cuff around the patient's bare arm. The cuff should be positioned 2-3cm above the brachial artery , Ask the patient not to talk during the procedure. Locate the radial pulse. Inflate the BP cuff by pumping the cuff bulb, until the radial pulse can no longer be felt. Note the reading on the dial. This figure is the estimated systolic pressure, Deflate the BP cuff completely and wait for 15-30 seconds. Inflate the cuff again to 20-30mmHg above the predicted systolic BP.

Clotting time Procedure:

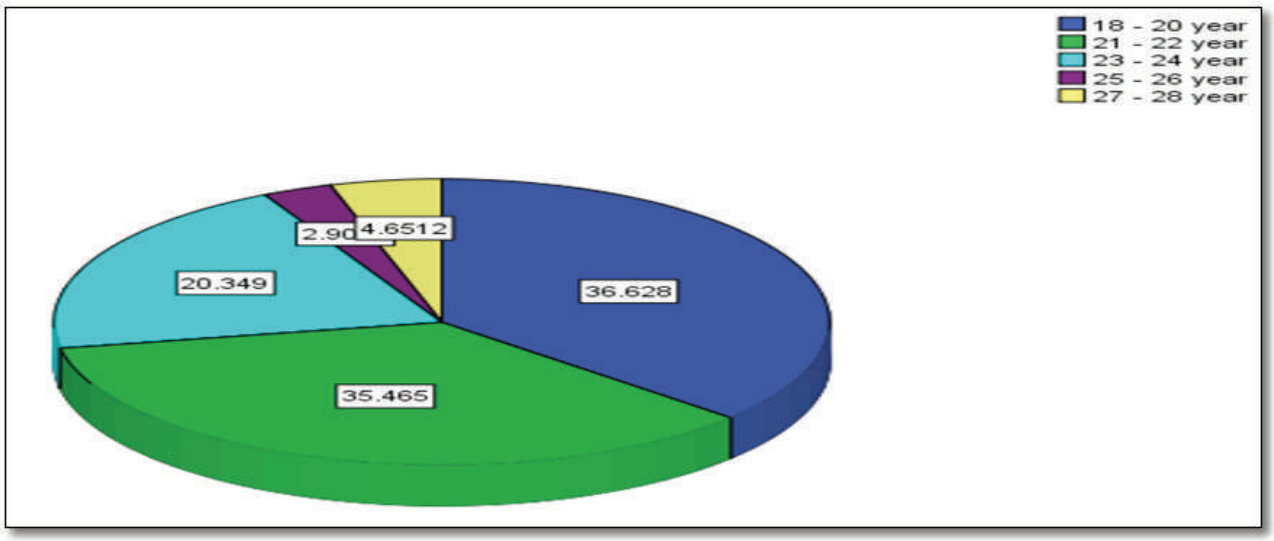
Slim the finger with spirit than allow the spirit to dry, Pierced the finger by lancet ,remove the first drop of blood, Squeeze the finger to obtain a larger drop of blood and fill the capillary tube with blood, The capillary tubes are sealed plastic and immersed in water bath at 37 centigrade After one minute start breaking small pieces of the capillary tube every30 second until a fibrin thread is seen between the two broken ends, By these methods the normal clotting time is 5 to 10 minutes at 37co

Blood group Procedure :

A clean and dry glass slide was divided into two sections by using a glass marking pencil. The sections were labeled as anti-A and anti-B to recognize the antisera, Place one drop from anti-A serum and one drop from anti-B serum in the center of the corresponding section of the slide. Mix antiserum with blood by using a separate corner of a slide for each section over an area about 1 inch in diameter. By tipping the slide forwards and backwards, examine for agglutination after exactly two minutes .

Results and Discussion

The figure(1) show the percentage of the sample by age, where the percentage of age group 18-20 years was a highest percentage which represent 36.6% followed by the age group 21-22 years, where it reached 35.5% followed by the age group. Followed by the age group 21-22 years and amounted to 35.5% followed by the age group 23-24 years, where it reached 20.3%, followed by the age group 27-28 years, reaching 4.7%.



The figure(1) shows the percentage of the sample by age .

This study was proved that the blood type that more frequently was A+ , followed by O+. While the least frequently was B- and AB-. And the Rh + was more frequently than Rh- this result agreed with[9].

Table (1) the distribution of Rh

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	A+	58	33.7	33.7	33.7
	B+	38	22.1	22.1	55.8
	AB+	17	9.9	9.9	65.7
	O+	44	25.6	25.6	91.3
	A-	5	2.9	2.9	94.2
	B-	2	1.2	1.2	95.3
	AB-	2	1.2	1.2	96.5
	O-	6	3.5	3.5	100.0
	Total	172	100.0	100.0	

Table and figure (2) shows the relationship of blood clotting with age coagulation period 30 sec. was the highest percentage in the age group 18-20 years, where it reached 6.3%. The frequency of 1min was in the age group 25-26, where it reached 20%. And the period of 1.5m, which reached the highest percentage in the age group 21-22 years. And the period of 2m was the highest proportion in the age group 18-20 years. And the period of 2.5m was the highest percentage in the age group 27-28 years. The period of 3m was the highest percentage in the age group 27-28. In other study Blood coagulation capacity rises with age in healthy individuals, seemingly because of increases in the plasma concentration of most procoagulant factors. This phenomenon may play an important role in the advancing age–associated increase of cardiovascular diseases and thrombosis [10].

Table (2) shows the relationship of blood clotting with age

age group			Frequency	Percent	Valid Percent	Cumulative Percent
18 - 20 year	Valid	30 sec	4	6.3	6.3	6.3
		1 min	8	12.7	12.7	19.0
		1.5 min	10	15.9	15.9	34.9
		2 min	30	47.6	47.6	82.5
		2.5 min	4	6.3	6.3	88.9
		3 min	7	11.1	11.1	100.0
		Total	63	100.0	100.0	
21 - 22 year	Valid	30 sec	3	4.9	4.9	4.9
		1 min	11	18.0	18.0	23.0
		1.5 min	11	18.0	18.0	41.0
		2 min	23	37.7	37.7	78.7
		2.5 min	6	9.8	9.8	88.5
		3 min	7	11.5	11.5	100.0
		Total	61	100.0	100.0	

***Cont.*. Table (2) shows the relationship of blood clotting with age**

23 - 24 year	Valid	30 sec	1	2.9	2.9	2.9
		1 min	3	8.6	8.6	11.4
		1.5 min	5	14.3	14.3	25.7
		2 min	13	37.1	37.1	62.9
		2.5 min	8	22.9	22.9	85.7
		3 min	5	14.3	14.3	100.0
		Total	35	100.0	100.0	
25 - 26 year	Valid	1 min	1	20.0	20.0	20.0
		2 min	2	40.0	40.0	60.0
		2.5 min	1	20.0	20.0	80.0
		3 min	1	20.0	20.0	100.0
		Total	5	100.0	100.0	
27 - 28 year	Valid	2 min	3	37.5	37.5	37.5
		2.5 min	3	37.5	37.5	75.0
		3 min	2	25.0	25.0	100.0
		Total	8	100.0	100.0	

Table and figure(3)shows the relationship of coagulation to sex ,where the period of coagulation 30.sce was higher in females, where it reached 5.7%. At the same time, the percentage of females was high at 15.7%. to 1.5min, it was 17.1% In the period of 2 min, the percentage of females was highest, reaching 50.0% At 2.5%, the percentage of male was higher at 16.7% and at 3 min the percentage of male was higher at 18.6.

Table(3) shows the relationship between coagulation and sex

Gender			Frequency	Percent	Valid Percent	Cumulative Percent
Male	Valid	30 sec	4	3.9	3.9	3.9
		1 min	12	11.8	11.8	15.7
		1.5 min	14	13.7	13.7	29.4
		2 min	36	35.3	35.3	64.7
		2.5 min	17	16.7	16.7	81.4
		3 min	19	18.6	18.6	100.0
		Total	102	100.0	100.0	
Female	Valid	30 sec	4	5.7	5.7	5.7
		1 min	11	15.7	15.7	21.4
		1.5 min	12	17.1	17.1	38.6
		2 min	35	50.0	50.0	88.6
		2.5 min	5	7.1	7.1	95.7
		3 min	3	4.3	4.3	100.0
		Total	70	100.0	100.0	

Table and figure (4) relationship between the coagulation and smoking where the duration of coagulation 30sce, where it reached the highest proportion of non-smokers, where it reached 5.0%. The highest rate was 1min in smokers (17.0%). The ratio of 1.5min was the highest rate for non-smokers, at 16.8%. The ratio of 2min was the highest percentage of non-smokers, reaching 44.5%.The rate of 2.5min was the highest in smokers, reaching 17.0%. and during the period of 3 min where it reached the highest proportion of smokers, reaching 17.0

The effect of smoking strength on the degree of damage of coagulation cascade motionless remains uncertain, while effects of cigarette smoking on the normal hemostasis thru influencing the coagulation pathways[11].

Table (4) relationship between the coagulation and smoking

smoking status			Frequency	Percent	Valid Percent	Cumulative Percent
Smoker	Valid	30 sec	2	3.8	3.8	3.8
		1 min	9	17.0	17.0	20.8
		1.5 min	6	11.3	11.3	32.1
		2 min	18	34.0	34.0	66.0
		2.5 min	9	17.0	17.0	83.0
		3 min	9	17.0	17.0	100.0
		Total	53	100.0	100.0	
non smoker	Valid	30 sec	6	5.0	5.0	5.0
		1 min	14	11.8	11.8	16.8
		1.5 min	20	16.8	16.8	33.6
		2 min	53	44.5	44.5	78.2
		2.5 min	13	10.9	10.9	89.1
		3 min	13	10.9	10.9	100.0
		Total	119	100.0	100.0	

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Impact of Digoxin on Endocrine Glands in Male Rats

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Abstract

This study is performed to provide the pathological changes of thyroid gland, pancreas and adrenal gland that occurred due to exposure to the digoxin drug. The study uses twenty four adult male rat randomly divided into 3 equal groups as follows: G1 control group treated with distilled water, G2 dosed with 0.3mg/kg digoxin and G3 dosed with 0.9mg/kg digoxin given orally by gavage daily for 60 days. All animals were sacrificed at the end of experiment and organs were collected in 10% buffered formalin for histopathological evaluation. Result reveals dilation of thyroid follicles with flattened follicular epithelium which increase with high dose group digoxin in comparison with control. Adrenal gland showed moderate atrophy of cortical layers especially zona reticularis with hyperplasia of medulla cells probably of digoxin like ouabain-producing cells, on the other hands pancreas doesn't reveal remarkable changes except congestion of vessels in high dose group compared to control.

Keywords: *male rats; endocrine glands; pathological study and digoxin*

Introduction

Digoxin is one of the old medicine for heart failure and still the drug of choice in this condition[1,2], several drug have been developed but for treatment of symptoms of disease to complete cure and these drug is too expensive without any benefit that can be added above digoxin to replace it [3]. Digoxin increase force of contraction of myocardial muscle cells that is inotropy which exerted through blockage of Na^+-K^+ ATPase pump[4]. The most problems that accompanied digoxin it is narrow therapeutic window which make it toxicity sever to be occur so any mistake of drug toxicity denote [5,6]. Ouabain is an endogenous substance similar to digoxin that is produced from adrenal medulla cells. Ouabain previously used for treatment of heart failure[7]. Because digoxin is prescribed for all aged group patients so this study is designed to investigate the histopathological changes of digoxin on thyroid gland, pancreas and adrenal gland.

Materials and Methods

Experimental animals

This study administered 24 adult male rat divided randomly into 3 groups as follows; G1 is control group processed with distill water, G2 treated with 0.3mg/kg digoxin and G3 treated with 0.9mg/kg digoxin orally by gavage daily for 60 days. The animals were kept for adaptation for 3 weeks before experiment at the laboratory animal house of College of Pharmacy – University of Basrah. Animals were housed as eight rats in each cage under conditions (Mechanical ventilation, 12\12 hours light on, pellet, tap water and room temperature 24 ± 2^{o}C).

Histopathological study:

After 60 days of digoxin dosing, all rats were sacrificed and organs collected in 10% formalin for histopathological processing [8] and stained with hematoxylin and eosin according to Drury [9], to be assessed by light microscope.

Results

Histopathology of control group

Thyroid gland shows normal thyroid follicles apparently of different sizes with cuboidal compact

epithelium and homogenous pinkish colloid material. Pancreas show normal pancreatic glands and in between shows islets of Langerhans in the vicinity of blood vessels. Adrenal gland shows two layer outer one represent adrenal cortex showing three layers including zona glomerulosa, zona fasciculata and zona reticularis, the other layer resembling adrenal medulla showing medullary cells which appear similar to neuronal bodies. Showed in figure1.

Histopathology of low dose group

Thyroid gland showed dilated thyroid follicles with flattened follicular cells filled with thick colloid material, early stage of hypothyroidism and some areas of the glands appear unchanged. Pancreas appear with un remarkable changes showing exocrine glands and in between islets of Langerhans. Adrenal cortex appear atrophied with blurred layers and hyperplasia of the adrenal medulla which occupies most of adrenal tissue. Showed in figure 2.

Histopathology of high dose group

Thyroid gland with moderate to severe flattening of follicular epithelial cells with engorgement of the follicular lumen by faint colloid material. Pancreatic islets of Langerhans show unremarkable pathological changes compared to control slides and there is only congestion of blood vessels. Adrenal gland shows moderate atrophy of adrenal cortical layers especially the zona reticularis with hyperplasia of adrenal medullary cells which appears hypercellular studied with neuron like cells. Showed in figure 3.

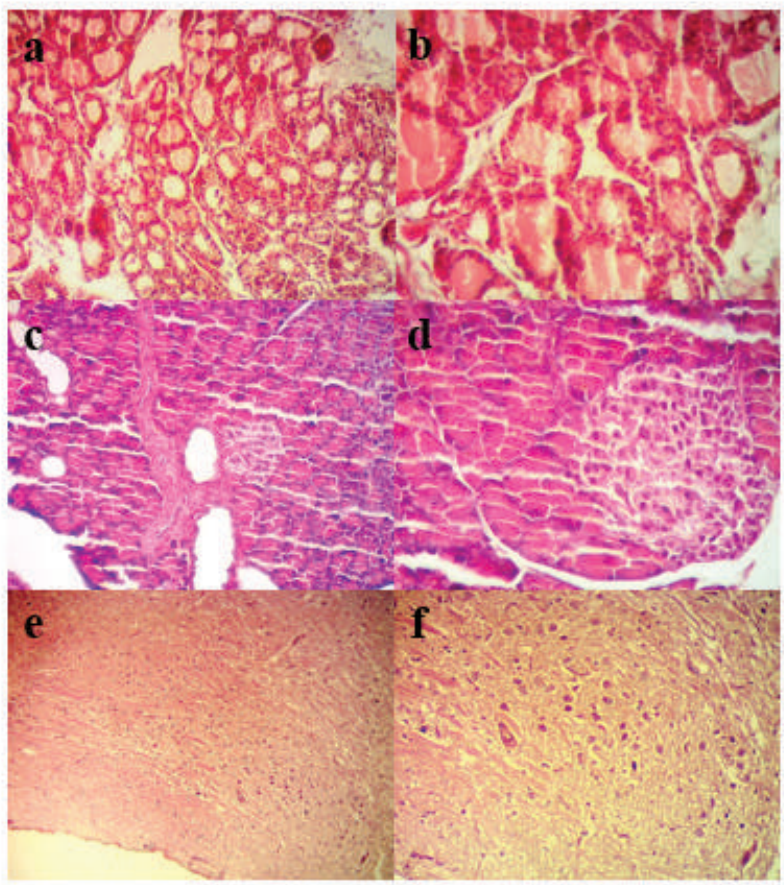


Figure 1: Control group, show normal architecture of thyroid follicles (a 200X &b 400X), pancreas show exocrine gland & islets of langerhance (c 200X &d 400X) and adrenal gland show cortex layers with medulla (e 200X &f 400X).

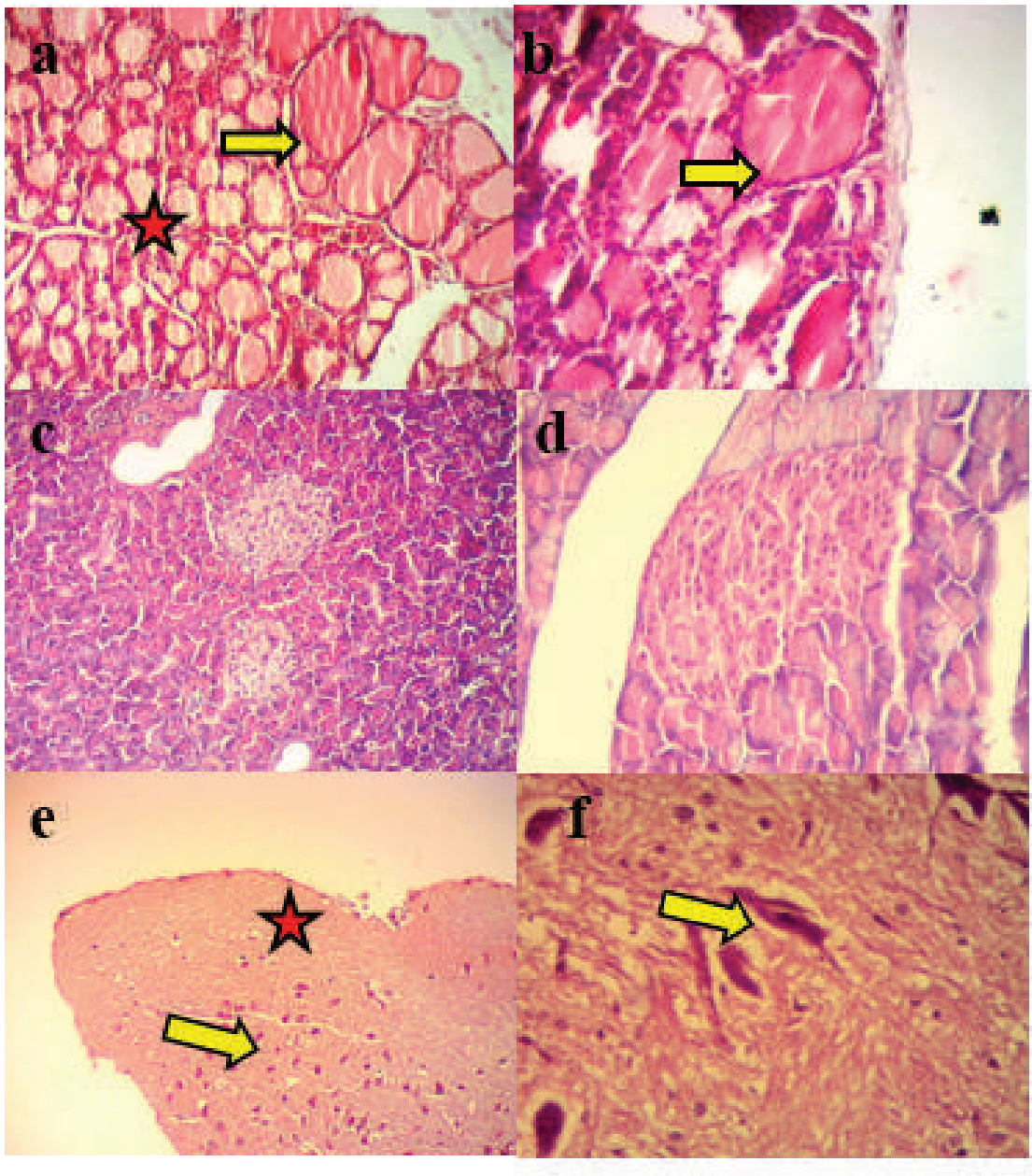


Figure 2: Low digoxin group, show dilation of thyroid follicles (yellow arrow) and some area without changes (red star) (a 200X &b 400X), pancreas don't show any changes in exocrine gland & islets of langerhance (c 200X &d 400X) and adrenal gland show atrophy of cortex layers (red star) with hyperplasia of medullary cells (yellow arrow) (e 200X &f 400X).

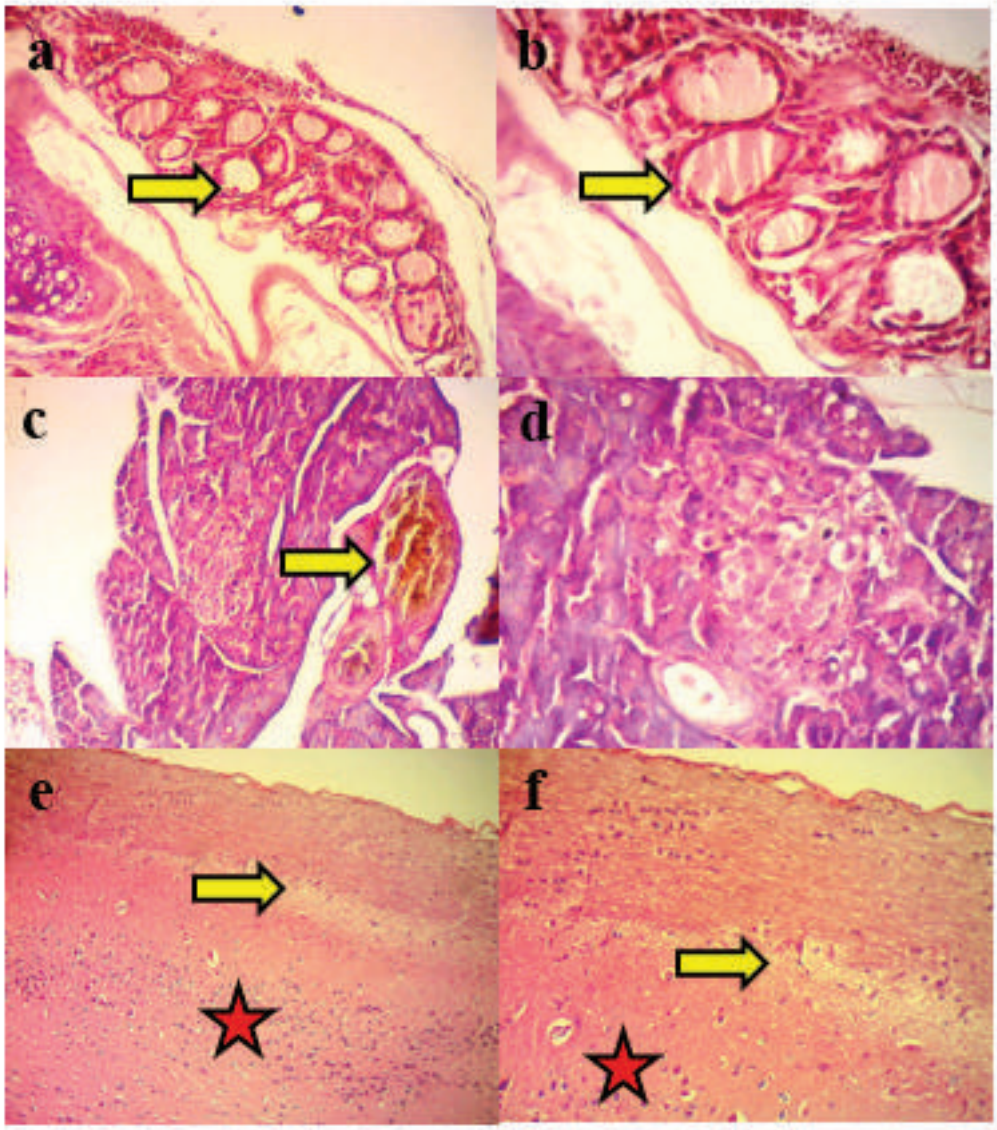


Figure 3: High digoxin group, show moderate to sever flattening of thyroid follicle epithelium filled with colloid (yellow arrow) (a 200X &b 400X), pancreas show unremarkable changes except congestion of blood vessels (yellow arrow) (c 200X &d 400X) and adrenal gland show atrophy of cortex layers especially zona reticularis (yellow arrow) with medullary cells hyperplasia (red star) (e 200X & f 400X).

Discussion

The present study is designed to provide a data base about histopathological changes caused by digoxin drug on thyroid, pancreas and adrenal glands in rats. The control group showed normal architecture of these gland as shown in figure-1. It has been noted that increase dose of digoxin cause more changes in these gland compared to lower dose group, thyroid gland presented as enlarged follicles associated with flattened epithelium which may be related to effect of digoxin on thyroid gland to suppress thyroxin hormone release because of it is anticipated action on myocardium. Digoxin provide positive inotropic effect on the heart, which leads to increase stroke volume, vascular perfusion and improve blood pressure. This might cause a negative feedback on the function of thyroid gland which shows features of hypo functioning thyroid follicles. The present study doesn't reveal significant changes in pancreatic tissue except in high dose digoxin pancreas which revealed congested blood vessels, as shown in figure-3.

Ouabain is an endogenous digoxin-like substance that is produced by adrenal medulla cells and it has a similar mechanism of action to digoxin [7,10]. During heart failure, because digoxin blocks the action of adrenaline effect on the heart, this probably may lead to atrophy of adrenalin producing cells in adrenal medulla which is accompanied by hyperplasia of ouabain synthetic cells. This finding is evident by the histological appearance of hyperplasia of ouabain-producing medullary cells of adrenal gland after digoxin administration which becomes more prominent with increased dosage of digoxin. This result is in line with Takahashi [10], who reported that a monoclonal ouabain antibody was highly distributed in adrenal medulla. Medullary hyperplasia is associated with atrophy of cortical layers of adrenal gland as shown in figures-2 and 3.

Upon reviewing literature regarding the histopathological changes of cardiac glycoside (digoxin), the authors didn't come across any research studying these changes. This research may be the first of it is kind in this field.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Study Some Biochemical Parameters in Beta Thalassemia Major Patients in Males and Females Adults in Baghdad

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Summary

Our study aims to investigate in levels of Ferrtin and IL-23 in beta-thalassemia major patients (males and females), and compared with the control group (healthy individuals). The total number of samples were (88) blood samples, beta-thalassemia major patients (44) and healthy individuals (44). All the samples submitted to ELIZA test to determination Ferrtin and IL-23 in blood samples (males and females) (patients and healthy group). According to our results, showed there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in Ferrtin concentration as compared with the control group at ($P<0.01$). In addition, Our study showed no there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in IL-23 level as compared with the control group at ($P<0.01$). Finally, there is a high increase in Ferrtin concentration of blood samples in males and females, while there are little changes (non-significant) in IL-23 concentration of blood samples in males and females.

Keyword: *Ferrtin, IL-23, beta-thalassemia major.*

Introduction

Thalassemias are genetic disorders, included decreased production of the hemoglobin (alpha or beta chain or both). Hemoglobin function is to carry the oxygen and formed from two proteins. Decreasing of these proteins leads to the inability of RBC to transfer the oxygen; leads to anaemia. Thalassemia is occurred due to deletion or mutation of some gene [(1) (2).]

Thalassemias have two types (Alpha and Beta). Alpha thalassemia is occurring due to the deletion of the alpha-globin gene or disorder of the synthesis of alpha-globin [(3) (4).] In addition, Beta-thalassemia results from point mutations in the beta-globin gene. It is divided into three categories based on the zygosity of the beta-gene mutation (minor, major and intermediate), these types result in different severity [(5) (6).]

The clinical signs of thalassemia appear before a child's second birthday. Very acute anaemia, furthermore, jaundice, frequent infections, a poor appetite, fussiness, failure to thrive, paleness, and the skin becomes yellow, organs enlargement, acute anaemia required blood transfusions [(7).]

Ferritin is protein work inside the cells. Bacteria, animals, archaea and human can produce Ferritin. Ferritin acts as a buffer control level of iron [3]. Ferritin is present in the cell and secreted at small amounts into the serum. Ferritin is indirect indicator of the total amount of stored iron therefore; the ferritin test is used as a laboratory test for iron concentration [(8) (9).]

Interleukin-23 is a pro-inflammatory mediator formed from two subunits (p19 and p40). It have immunological role in the body. IL-23 is stimuli Th17 cells that have great role in inflammation reaction [(10).]

Our study aims to investigate the relationship and changes in the levels of ferritin and interleukin 23 in the blood of patients with major beta-thalassemia

Material and Methods

Study design:

This prospective study for beta-thalassemia major patients had been conducted from December 2018 until June 2019 in AL Karama teaching hospital in Iraq Baghdad. All the patients had been chosen according to the following inclusion and exclusion criteria included All the patients are suffering from β-thalassemia major, Age more than 18 years old, Non-splenectomized, No obvious clinical infection, hepatitis and unmarried.

Sample size:

The study included eighty-eight (88) patients divided into two groups:

1- The first group (patient): Were forty-four (44) of the β-thalassemia major patients.

2- The second group (control group): was forty-four (44) apparently healthy.

Data collection:

The questionnaire is prepared for all the patients are included data related to name, gender, age, diagnosis of disease and other information.

Venous blood withdrawal:

The venous blood (5) ml was taken from control and patients group included in the study. The collected blood was divided almost (3ml) put in K3-EDTA tubes while other (2ml) keep in plain tubes, then centrifuged for five min at (300) rpm for separation the serum then stored at (-20) C.

Determination of Ferrtin hormone:

Assay Protocol:

1- Adding Pipet Ferritin Standard, control, and samples 25 µl by Pipet into the wells

3- Adding of the biotin Reagent (100) µl on all the wells and mixed.

4- Incubation of the plate for thirteen minute at (25°C)

5- Lifting of the liquid from all wells then washing wells by wash buffer.

6- Adding of the Enzyme Reagent (100) µl on all the wells and mix

7- Incubate for half hour at (25°C).

8- Discarded liquid from all wells and washing wells by using wash buffer.

9- Adding TMB substrate (100) µl on all the wells and incubation fifteen minutes at (25) C.

10- Adding of the stop solution (50) µl on all the wells and mix

11- The absorbance is read at (450) nanometer for fifteen minutes after mixing the stop solution.

Determination of IL-23 hormone:

Assay Procedure:

1- Put control, sample and standard in the wells

2- Adding Sample/Standard dilution buffer (0.1) ml into the zero well

3- Adding diluted sample (0.1) ml into test sample wells

4- Covering and incubation at 37°C for 1.5 hours

5- Left the cover and remove the plate content.

6- Adding Biotin-AB working solution (0.1) ml on the wells, adding of the solution on all wells

7- Covering and incubation at 37°C for one hour

8- Left the cover, and washing it by using wash buffer.

9- Adding of SABC working solution (0.1) ml into all the wells and incubation at 37°C for thirteen minutes

10- Lifting and washing by using wash buffer.

11- Adding TMB substrate (90) µl on all the wells, covering it and incubation at 37°C for thirteen minutes

12- Adding Stop solution (50) µl on all the wells and mixing, the color will be yellow directly.

13- OD Absorbance is read at (450) nanometer by using micro plate reader after mixing with the stop solution.

Statistical Analysis

The Statistical Analysis System software (2012) was applied for detection of the effect of two factors in our study. LSD test was used for compare between the means. Q test was used for compare between percentage (0.01 and 0.05).

Results

According to our results, showed there are significant difference in and males with beta-thalassemia major (3063.26±396.46) and females with beta-thalassemia major (3663.73±523.42) in Ferrtin concentration as compared with the control group (157.24±48.12) and (20.45±3.65) respectively at (P<0.01) as the table (1).

Table (1): Effect of gender and group in Ferrtin

Group	Mean ± SE	
	Male	Female
Patients	3063.26 ± 396.46	3663.73 ± 523.42
Control	157.24 ± 48.12	20.45 ± 3.65
T-test (P-value)	1029.10 ** (0.0001)	1329.50 ** (0.0001)

* S: Significant, ** H.S., Highly Significant, NS: Non- Significant.

Based on our study, showed no there are significant difference in and males with beta-thalassemia major (19.58±2.79) and females with beta-thalassemia major (13.99±3.26) in IL-23 level as compared with control group (17.34 ± 1.22) and (21.10 ± 1.04) respectively at (P<0.01) as the table (2).

Table (2): Effect of gender and group in IL-23

Group	Mean ± SE	
	Male	Female
Patients	19.58 ± 2.79	13.99 ± 3.26
Control	17.34 ± 1.22	21.10 ± 1.04
T-test (P-value)	7.496 NS (0.550)	8.457 NS (0.096)

*S: Significant, **H.S., Highly Significant, NS: Non- Significant.

Discussion

Many of the changes are occurring in patients with major beta-thalassemia, which include physiological and biological changes. These changes are included levels of hormones and some intermediate compounds in the activation of some reactions in the body such as ferritin and interleukin-23 [11].

Ferritin is a complex protein composed from two subunit heavy chain and light chain. Ferritin is regulate level of iron in the serum [12].

According to our results, showed there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in Ferrtin concentration as compared with the control group and respectively at (P<0.01) as the table (1).

Level of the ferritin protein is a reflex level of the iron. In another mean, the ferritin concentration gives a general view of how much iron in the body. If the ferritin level is low, that indicates the iron is low. If the ferritin concentration is high, the iron is high. The high concentration of ferritin is associated with beta major thalassemia due to repeated blood transfusion lead to increasing of iron in the body results in increases of ferritin and that agreement with results of [13] [14].

Interleukin-23 (IL-23) plays an important role in the development and maintenance of T-cell helper, wherever it has a great immune- protective role in the body [15].

Based on our study, showed no there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in IL-23 level as compared with the control group and respectively at ($P<0.01$) as the table (2), There are little increase in males and a little decrease in females.

Some studies such as [16] and [17] showed increasing of IL-23 level, However, another study such as [18] showed little increases non- significant of IL-23 level.

Our study showed no statistical differences in interleukin-23 in males and females as compared with the control group. Perhaps these slight differences between the groups are due to individual factors, un-clinical infection conditions, and other unknown factors.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Biological Activity of Complexes of Some Amino Acid: Review

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Abstract

Schiff bases (SBs) based on amino acid derivative stand for multipurpose ligands that formed by condensing amino acids with carbonyl groups. They are significant in pharmaceutical and medical areas due to their widespread biological actions such as antiseptic, antifungal, along with antitumor actions. Transition metallic complexes resulting from SB ligands with biological activity were extensively experimented in the literature. In this article, we review, in details, about synthesizing and biological performances of SBs along with its complexes.

Keywords: *Schiff bases (SBs), Amino acid, Metal complexes, antimicrobial activity, and antitumor activity.*

Introduction

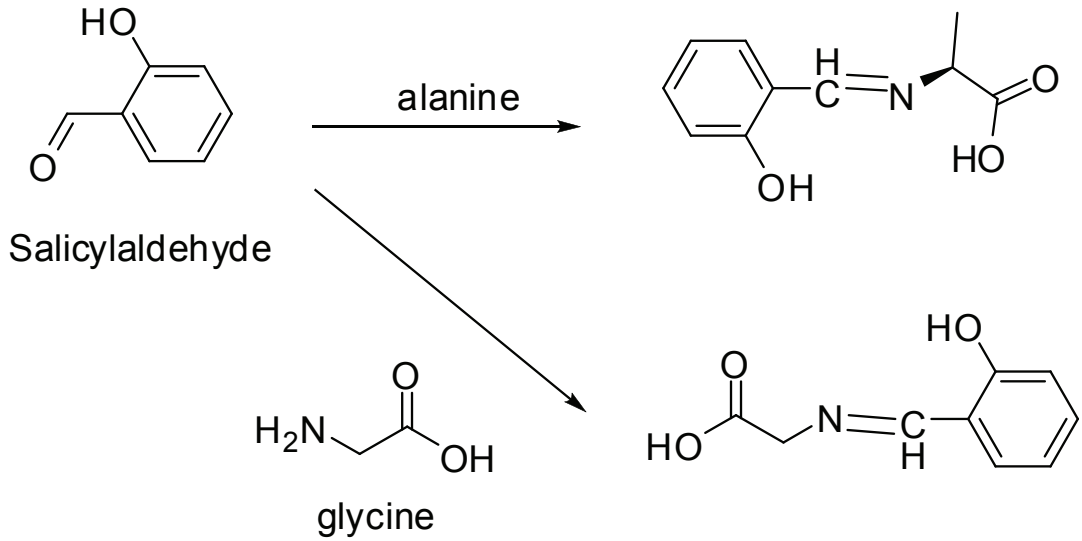
Compounds having an azomethine cluster (-CH=N-), termed as Schiff bases (SBs) has typically synthesized as a result of condensing primary amine with a carbonyl compound.(1) SBs of aliphatic aldehydes have been somewhat unstable and freely polymerizable. On the other hand, SBs with aromatic aldehydes, including an in effect conjugation system, have been in higher degree of stability (2). SBs have, in general, particular preparation use, detection, applications, identification and determining of aldehydes or ketones, carbonyl or amino compounds purification, or protecting the groups throughout complex or sensitive reacting. (3) They correspondingly create elementary units in specified dyes. SBs usually stand for bi-or tri- dentate ligands able to produce highly stable complexes with transitional metals (4). Several of them can be employed as liquid crystals. SB reactions have been advantageous in creating carbon-nitrogen bonds in organic synthesis (5). SBs act as an imperative intermediary in a quantity of enzymatic reacting containing interacted enzyme with the amino or a carbonyl collection of the substrate. The most essential categories of catalytic process is the biochemical activity that includes condensed primary amine in the enzyme with usual lysine residue and carbonyl cluster of the substrate for forming an imine and SB.(6) Stereo chemical analysis based on molecular model has depicted that the formed SB concerning methylglyoxal as well as amino group of lysine side chains of proteins can be headed for number of atom of peptide groups in which the charge transfer can arise among oxygen atoms of SBs and these groups .(7) The pyridoxal SBs resulting from amino and pyridoxal acids had made and examined based on biological viewpoint. Enzyme models based on transition metal complexes of these ligands are very imperative in biochemical scopes. (8) The speedy expansion of these ligands have caused enhanced research activity for coordination chemistry with highly remarkable deductions.(9) In the current paper, we focusses on the formation and biological actions of several primary amines derivative SBs as well as its complexes.

Part one: SB of Amino Acid

Metal chelates of prepared SB as a result of uniting salicylaldehyde along with amino acids are effectually employed in realizing the process of transamination reacting. These can be biomimetic species. The present research paper is concentrated on the characterization, complexation , syntheses, behaviour and antimicrobial analyses of several formed new SBs from salicylaldehyde with 3-amino benzoic acid besides Glycine and Alanine

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employing sodium hydroxide as a catalyst. The formed SB ligands were efficaciously complexed with Zn (II) metal and examined based on their spectral data. Morphological analyses have implemented by means of SEM. The complexation impact on the antimicrobial action of SBs besides its Zn (II) complexes is as well investigated.[10]



Scheme1- Preparation of amino acid based Schiff bases

In platinum (II) complexes having lessened amino acid ester SBs have interacting with salmon sperm DNA has been examined by dint of circular dichroism and ultraviolet spectroscopies. The potential antitumor performance of each compound has been *investigated in vitro* on HeLa and A549 tumor cell lines. Practically, each complex had superior cytotoxic action as compared to cisplatin in contradiction of these cell lines. [11]

Synthesizing new SB compound can be resulting from s-allyl cystiene and methionine. A new formed compound, 2-((2-((2-(allylthio)-1-carboxyethyl) imino)ethylidene)amino)-4-(methylthio)butanoic acid (ACEMB) has been categorized. ACEMB has shown potent *in vitro* antioxidant[12].

On the other hand, 3 integrated ligand Cu(II) complexes [Cu(o-vanillin-L-tryptophan Schiff base)(diimine)] (diimine =2,2′-bipyridine (1), 1,10-phenanthroline (2) in addition to 5,6-dimethyl-1,10-phenanthroline(3)) have been formed and categorized based on spectral and analytical approaches. In the gas phase for B3LYP/LanL2DZ levels, the 1–3 molecular structures have been boosted by means of density functional theory (DFT). [13]

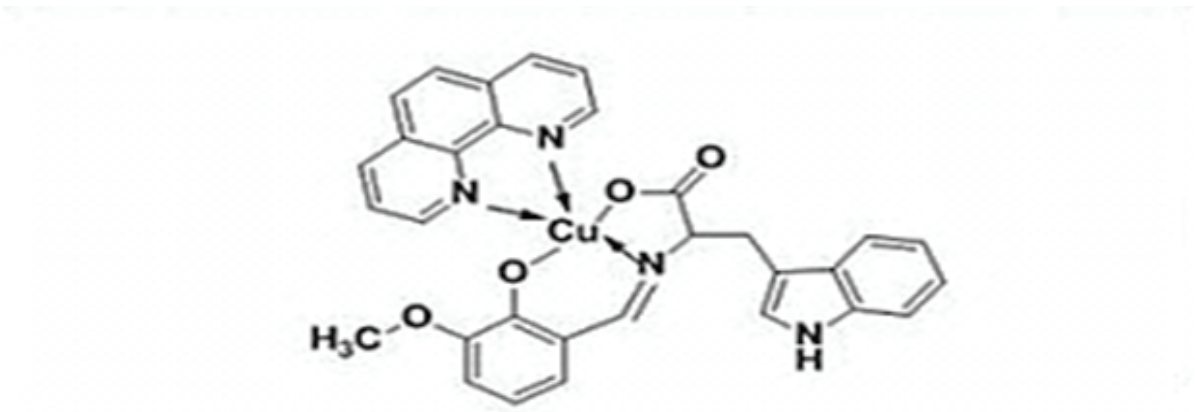
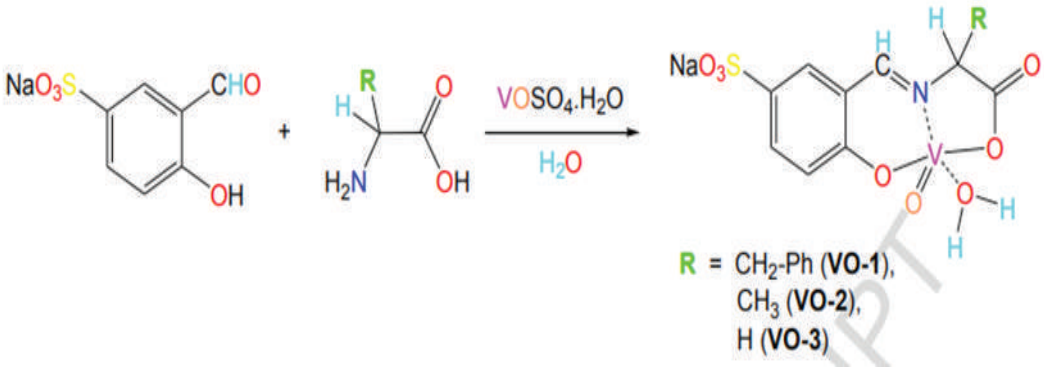


Figure 1- structure of cu complexes

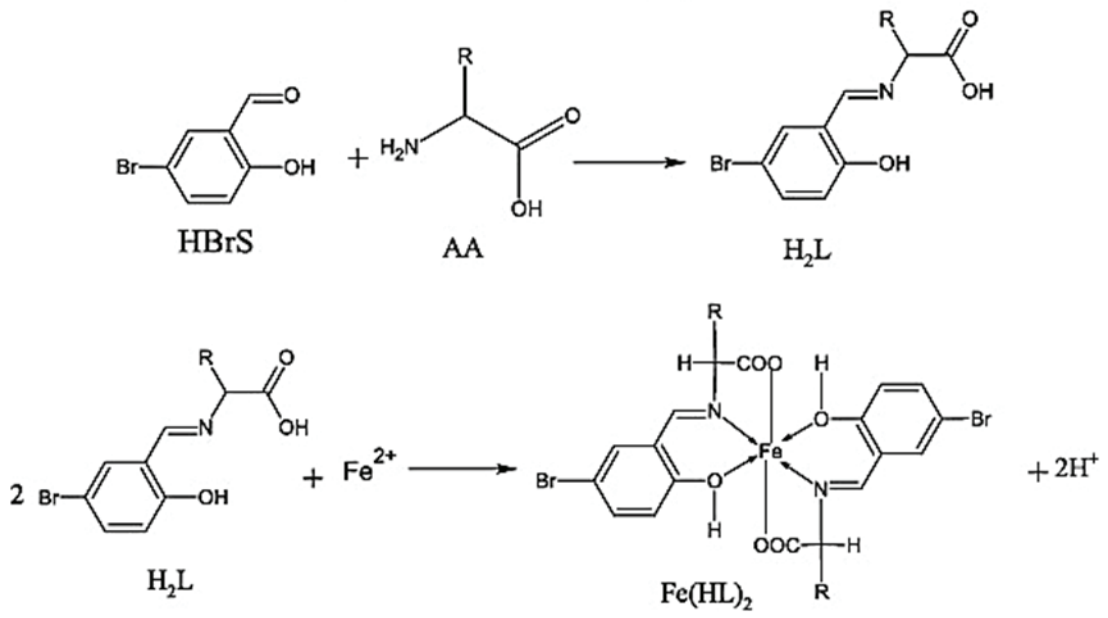
Critical investigation of thirteen crystallographic configurations of several Ni(II) complexes of amino acid SBs has performed. The foremost conclusion of [14] ha been the consequence of a parallel displaced category of aromatic interacting among Pro *N*-benzyl rings and *o*-amino-benzophenone. The aromatic interactions quality has revealed to be as controlled steric environment nearby amino acid side-chain, interpreting substituted Ni(II) complexes under dissimilar thermodynamic stabilities. [14]

3-anionic oxide-vanadium SB *N*-salicylidene amino acid complexes have been resulting by reacting sodium salicylaldehyde-5-sulfonate with phenylalanine (VO^{-1}), alanine (VO^{-2}) or glycine (VO^{-3}), subsequently, and mixed with vanadyl sulfate monohydrate. Inhibiting consequence of VO^{-3}, VO^{-2}, in addition to VO^{-1} has examined for carbon steel corrosion in chloride acid solution. Based on electrochemical experiments and in an existence of 1.0 mm VO^{-1} inhibitor, VO-complexes preserve carbon steel corrosion, producing extreme inhibiting efficiency equal to 94.7%. Each VO-complex has mixed-type inhibitors.[15]



Scheme 5- The synthetic of the VO salicylediene amino acids complexes

By condensing 5-bromo-2-hydroxybenzaldehyde along with α-amino acids (L-alanine (ala), L-phenylalanine (phala), L-aspartic acid (aspa), L-histidine (his) and L-arginine (arg)), Novel chains of Fe(II) complexes and SB amino acids were proposed and formed by bioactive ligands. Infrared, ultraviolet–visible spectra, elemental analyses,, in addition to magnetic susceptibility and conductivity measurements have been employed to clarify the geometry of anew primed Fe(II) complexes. Additionally, the stability along with stoichiometry coefficients of the organized complexes was spectrophotometrically measured. The consequences put forward that the organized SB amino acid ligands perform as dibasic tridentate ONO ligands and have binding to Iron(II) in octahedral structure as stated by the typical formulary $[Fe(bs:aa)_2].nH_2O$. In the case of pH = 7.2 and based on electronic absorption spectra besides viscosity measurements, DNA interacting of these complexes has been examined. The investigational consequences specified that the examined complexes can be binded to DNA by means of intercalative mode and have shown a dissimilar DNA binding action based on this arrangement: bsari > bshi > bsali > bsasi > bsphali. Besides, the organized compounds have investigated for their *in vitro* antibacterial action in contradiction of 3 kinds of bacteria, *Bacillus cereus, Pseudomonas aeruginosa* as well as *Escherichia coli*. The consequences have given an indication that the metallic complexes have been in a greater reactivity concerning their resultant SB ligands [16].



Scheme 6- The formation of the investigated Schiff base amino acid

Ligands and their complexes

Synthesized novel metal complex of Co and Zn withamino acid-nucleobase hybrid ligand were accomplished through minimal chemical reacting of metal salt with amino acid L-histidine in addition to nucleobase adenine as ligands. The reactivity concerning BSA has shown that BSA fluorescence quenching by the dual complexes have been in static quenching, while as compared with the complex 2, complex 1 has the greater BSA-binding capability. The pharmacological action of the ligand in addition to the complexes has examined through antioxidant activity. They display implying influence in contradiction of radical DPPH. The consequences of the molecular docking investigations of reinforced thecomplexes.[17]

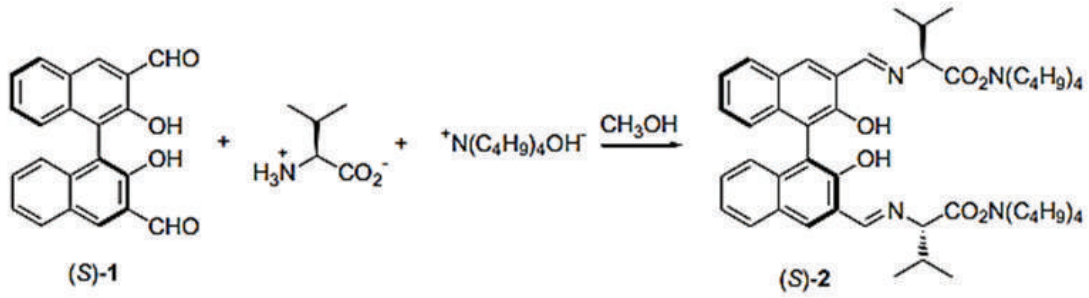
For amino acid SBs, formation and organizational categorization of cobalt (II) complexes has primed based on Salicylaldehyde and 3 amino acid in basic medium (Leucine, Valine , and Isoleucine) . The SBs and their metallic complexes have been initially scanned in contradiction of numerous microbes strains to investigate their biological influence. [18]

Expansion of innovative drugs stands for important solution to oppose in effect antimicrobial resistance threat. SB and Cinnamaldehyde-amino acid compounds stand for anew detected compounds that show noble antibacterial action in contradiction of gram-negative and gram-positive bacteria. Quantitative structure–activity relationship (QSAR) approach has employed for exploring the relationship among compound structures and antibacterial activity. [19]

Amino acid derivative SB as formed by reacting leucine with salicyldehyde in basic medium. A SB has employed as a ligand that reacted with Mn, Co, Cd and Cu metals with the intention of forming stable complexes. Each compound with ligand and complexes have correspondingly involved with diverse bacterial (staphylococcus aureus, Bacillus subbtilis and Escheria coli) and fungous strains (Aspergillus flavus, Aspergillus niger and Alternaria alternate) with the aim of investigating inhibiting activities for titled compounds. The consequences have depicted that the metallic complexes possess larger antimicrobial actions as compared with ligand. [20]

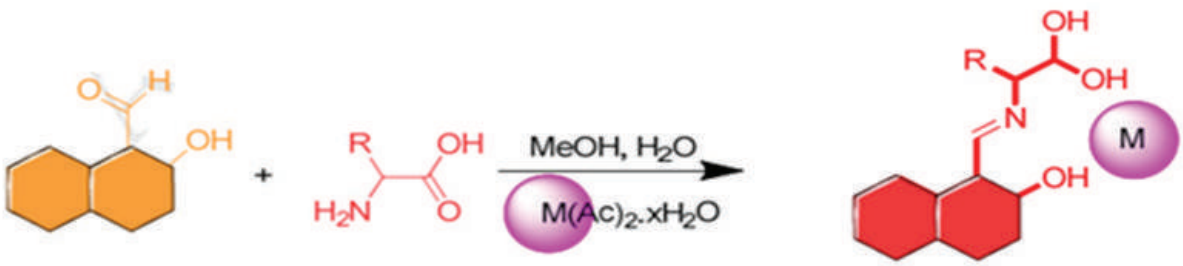
The amino-acid for SB (*S*)-**2** has organized based on condensing (*S*)-3,3′-diformyl BINOL (BINOL = 1,1′-bi-2-naphthol) with L-valine in an existence of tetrabutylammonium hydroxide in methanol. The compound shows off-on-off fluorescence reaction concerning Zn^{2+}. Spectroscopic investigations expose

(*S*)-**2**reacts with 1 equiv Zn^{2+} for forming the dimeric [2+2] complex with significantly improved fluorescence. Extra quantity of Zn^{2+} feasibly produces dissociated dimeric complex for giving significant diminished fluorescence. [21]



Scheme 10- Condensation of Schiff Base (S)-2

Resulting from 2-hydroxynaphthaldehyde with glycine and phenylalanine, Zinc (II), Cu (II), nickel (II), cobalt (II) and Iron (III) complexes of SB (LG, LP) have been testified. The LP, LG and their metallic complexes were partitioned for their antimicrobial actions in contradiction of 5 Gram-positive (Methicillin resistant Staphylococcus aureus (MRSA), Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis as well as Bacillus cereus) and 3 Gram-negative (Klebsiella pneumonia, Pseudomonas aeruginosa besides Escherichia coli) along with Candida albicans via broth microdilution methods. Resultant data have shown that ligands along with their metallic complexes have shown medium to good actions in contradiction of fungi and Grampositive bacteria. [22]



Scheme 11- The general synthesis procedure of ligands and complexes

The SB resulting from condensing tyrosine along with salicylaldehyde has been produced. In addition, its Pd(II), Pt(II) and Cu(II) complexes have primed. Antibacterial performance for the complexes has determined in vitro in contradiction of diverse bacterial strains as in Bacillus subtilis along with Pseudomonas aeruginosa using well-diffusion technique. Transition metal complexes possess noteworthy developed antibacterial activity as compared with parental drug. [23]

Part 2: mixed ligand of several amino acids

The ternary complexes of Zn(II), Cu(II), Cd(II) along with Ni(II) with 2-aminomethylbenzimidazole (AMBI) and glycine. The secluded chelates were partitioned for their antibacterial and antifungal actions using disc diffusion technique. The compound cytotoxicity in contradiction of larynx (HEP2) and colon (HCT116) cancer cells thoroughly investigated. For ternary M-AMBI-Gly complexes, stability constants have evaluated in aqueous solution at I = 0.1 mol dm3 NaCl potentiometrically. [24]

The Ni(II) , Co (II), Zn(II) , Cu(II), Hg(II) and Cd(II) complexes of integrated amino acid (L-Proline) as well as Trimethoprim antibiotic have formed. The ligands and their metal complexeswere have for their antimicrobial performance in contradiction of 4 bacteria including gram positive besides gram negative types. [25]

Diverse ligand complexes of bivalent metal ions with Ni(II), Co(II), Zn(II), Cu(II), Cd (II), and Hg(II) for [M(Anth)2(TMP)] composition in 1:2:1 molar ratio, in which AnthrH= Anthranilic acid (C7H7NO2) and

Trimethoprime (TMP) = (C14H18N4O3) were formed and categorized. The dual ligands along with their metallic complexes were screened for their bacterial performance in contradiction of particular microbial strains gram positive and gram negative types. (26)

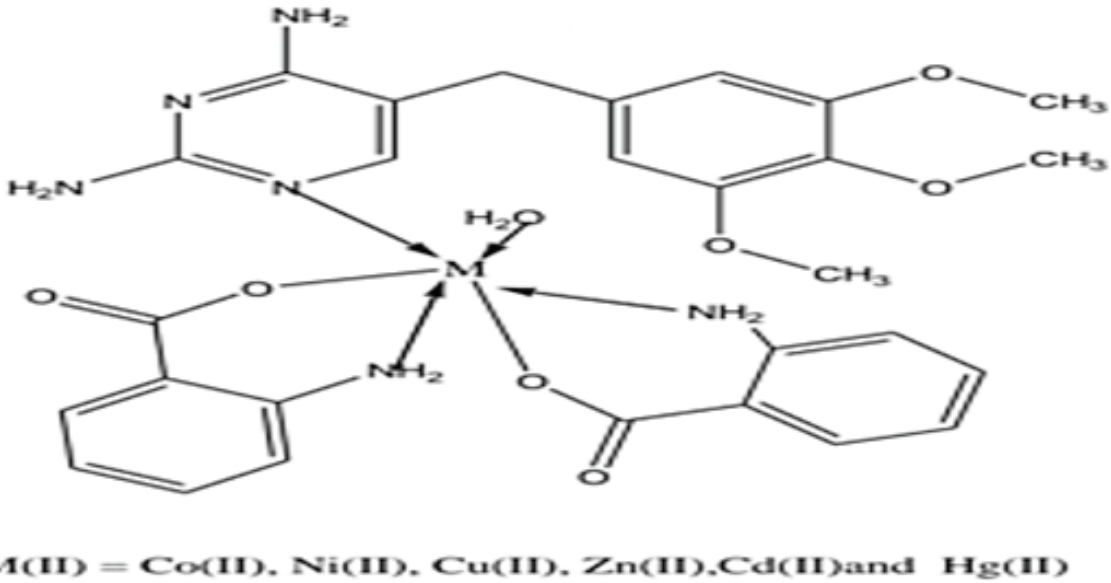


Figure5-preparation of the Complexes [M(Anth)2(TMP)]

Through condensing {3-amino-1-phenyl-2-pyrazolin-5-one (APO)} and o-phathalaldehyde and anthranillic acid (L2) accompanied by several transition metallic ions, mixed ligand complexes with SB derivative ligand (L1) were created. All these compounds were determined in contradiction of mirobial strains gram positive and gram negative types based on the analytical studies.(27)

A different mixed Iron (III) and Zinc (II) complexes along with iso nitroso acetophenone (HINAP) besides L-amino acids like, L-histidine, L-proline and L-phenylalanine, were formed and characterized. The antimicrobial performance has examined in contradiction of bacteria (bacillus subtilis, Staphylococcus aureus,, *Pseudomonas aeruginosa*, *Escherichia coli)* and *Candida albicans* fungi . The Fe and Zn complexes have been in a higher activity active in contradiction of Gram + than Gram - bacteria. They likewise exhibit significant growth inhibiting in contradiction of the tested fungi. In vitro antitumor activity has examined in contradiction of cancer cell lines of larynx or HEP2 type has shown significant ligands toxicity with their mixed complexes. (28)

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

FundinG: Self-funding

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Measuring of Interleukin -22 and IL -17a Levels in Seropositive and Seronegative Rheumatoid Arthritis Patients

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Abstract

Background: "Rheumatoid arthritis (RA) is an inflammatory disease leading to joint disruption". The instant study designed to detect serum markers in RA that could discriminate between seropositive (SP) and seronegative (SN) by evaluating levels of IL- 22and IL -17a in patients with rheumatoid arthritis. ***Methods***: In this cross-sectional study, a total of sixty Rheumatoid arthritis (RA) patients' age and sex-matched with healthy controls were involved in the present study. The serum IL- -22 and IL -17a levels were measured using an ELISA kit. Results: The mean ±SD age in seropositive and seronegative was (37.22± 11.29 and 34.28±20.3 years, respectively), while in control group was (27.14±9.33 years). Furthermore, serum IL- 22 and IL -17a level was significantly higher in seropositive and seronegative RA patients compared to healthy controls ($P<0.001$). There was no significant variation in serum IL -22 and IL -17a level according to the seropositive and seronegative in RA patients ($p>0.05$), but there was a positive correlation between them ROC test representing a highly sensitive and specific . **Conclusion:** The present study exhibited higher serum IL- 22 and IL -17a levels in seropositive and seronegative in RA patients compared to healthy controls. Therefore, IL- 22 and IL -17a can be considered as a biomarker for RA disease, with high sensitivity and specificity but these cytokines couldn't be used as discriminated between SP and SN in RA patients.

Keywords: *- Rheumatoid arthritis, Seropositive, Seronegative IL22, and IL17a*

Introduction

"Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation of the synovial membrane [(1)] Multiple studies have been demonstrated that levels of disease-related biomarkers may be elevated prior to the onset of symptomatic rheumatoid arthritis [(2),(3)] . These biomarkers include rheumatoid «factor (RF), antibodies to «citrullinated protein antigens (CCP), as well as multiple cytokines/chemokines», C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [(4), (5).] Releasing "pro-inflammatory cytokines, as well as other pro-inflammatory molecules, reaches to the mediated of cellular differentiation, inflammation, immune pathology, and regulation of the immune response [(6)]. Selectively Th17 cells generate the signature cytokines such as interleukin 17 (IL-17), IL-21 and IL-22, and have been reported to play a vital role in the chronic inflammatory response and affected consequently joints and led to tissue damage in RA patients [(7)]. The interleukin (IL-22(was a member of the IL-10 family that significantly controls tissue responses to inflammation 2". A few studies propose the pro-inflammatory/ pathogenic role of IL-22 in the onset and progress of RA [(8)]. "The object of this research was to assess the role of certain disease markers such as autoantibodies (ACCP, RF), "cytokines (IL-17 and IL-22) and (hs-CRP, ESR) in the pathogenesis of RA as panel useful in assessing disease with seronegative and seropositive besides efforts to search whether of these cytokines could distinguished between SP and SN included in the of RA .

Subjects, Materials and Methods

"The considered investigation included 60 patients

with RA (50 females and 10 males), including new diagnoses without treatment divided to 36 seropositive, 24 seronegative providing the "American College of Rheumatology (ACR) criteria for RA". These RA patients were diagnosed by the clinic's rheumatologists and participation of the "Rheumatology Department / Baghdad Teaching Hospital" within the period of Feb 2019 to June 2019. Each Rheumatoid arthritis patients were chosen after excluding patients with any of the following states such as "malignancy, other autoimmune diseases, pregnancy, medications including steroids, antibiotics, CNS" depressants, hormonal therapy, and others. Unrelated, fifty healthy individuals were correspondent to the patient's group in their ages and genders were randomly selected as controls. During the morning five milliliter of Blood was collected from all members of the study groups. Each sample was separated into 2 parts; the first part is the serum for the serological tests, while the second is whole blood for ESR. The concentrations of the serum IL-22 and IL-17 levels in patients and healthy controls were determined by using ELISA Kit, according to the manufacturer's guidance (MyBiosource .USA). On the other hand, RF and ACCP measured by ELISA (Chorus, Italy) the highly sensitive C –reactive protein (hs-CRP) were detected by ELISA (Demedltec diagnostics) and ESR was tested by Westergren method

Statistical Analysis

Statistical analyses were conducted using the SPSS statistical package for Social Sciences (version 20.0 for Windows, SPSS, Chicago, IL, USA). Data are displayed as mean ± SD for quantitative variables.While number and percentage for qualitative variables. Quantitative data were tested using ANOVA and Kruskal-Wallis test for differences between groups, Pearson's correlation for the relation between groups; while qualitative relations were evaluated using the Chi-square test. P-value of <0.05 was considered statistically significant. Cut-off values were estimated according to ROC [9].

Result

Demographic characteristics of the studied groups:

Table 1 shows the baseline characteristics of the studied samples according to age and gender with a comparison of significance in studied groups shows that a total of 100 individuals were divided into two groups 60 patients with RA [35 seropositive (SP) and 25 seronegative (SN)], while 40 individual chosen as apparently healthy controls. Results in this table demonstrated that there was no statistically significant difference between males and females in RA patients and control at P>0.05. Moreover, no significant difference was reported in the current study according to age groups between RA groups and control at P>0.05.

Table 1: The baseline characteristics of the studied groups.

Variables		RA Patients		Control	P-value
		seropositive	Seronegative		
Age (years)	Range	(25-64)	(20-61)	(20-50)	P=0.677 NS
	Mean ± SD	37.22± 11.29	34.28±20.3	27.14±9.33	
Gender	Male No. (%)	7 (20 %)	6 (24 %)	20(50%)	P=0.955 NS
	Female No. (%)	28 (80 %)	19 (76 %)	20(50%)	
Total No.		35	25	40	

(*) NS: Non -Significant. at P>0.05.

The concentration levels of ESR, RF, hs-CRP, and ACPA among studied groups

Table 2 representing the Mean± SD of ESR, RF, hs-CRP, and ACPA, according to studied groups. Analysis study shows that there were no significant differences in the Mean± SD between seropositive (76±23.5;

16.43±9.98, respectively) and seronegative (68±86.2; 17.76±10.29, respectively) in RA patients according to ESR, hs-CRP at P>0.05. Hence, that there was a significant difference in the Mean± SD of RF, and ACPA in RA patients between seropositive (372.5± 34.7; 22.6± 11.6.9, respectively) and seronegetive (12.8± -8.7; 6.6± 1.9, respectively) at P> 0.001. While, the mean concentrations of «ESR, RF, hs-CRP and ACPA» in seropositive RA patients were significantly higher than healthy control (P < 0.001).

Table 2: distribution of serological and blood marker in the studied groups.

Parameters	Patient seropositive	Patients seronegatie	Control	P-value
ESR mm/h M ± SD	7 6± 23.5	68 ± 36.2	1 6± 2.5	0.000
hs-CRP mg/L M ± SD	16.43 ± 9.98	17.7 6± 10.29	3.47 ± 10.65	0.000
Anti-CCP AU/ml M ± SD	22.6± 11.6.9	6.6± 1.9	5.7± 7.2	0.000
RF AU/ml M ± SD	372.5± 34.7	12.8± -8.7	11.8± 6.7	0.000

Highly Sig. P=0.000 among patients groups and healthy control

Mean levels of IL-22 and IL-17a among the studied groups

The mean concentration of serum IL-22 level was significantly higher in RA patients (SP and SN) than healthy control. The same was true for serum IL-17a level exhibited a significantly higher mean concentration in RA patients versus control P<0.000) .Notably that no differences in mean concentration of both IL-22 and IL-17 between RA patient (SP and SN) as showing in table 3.

Table 3: Mean concentration of serum cytokines IL-22 and IL-17a among studied groups

Parameters	RA Patient seropositive	RA Patients seronegatie	Control	P-value
IL-22 pg/ml M ± SD	26.6 ± 38.7	21.2 ± 27.5	7.4 ± 8.0	0.000
IL-17a pg/ml M ± SD	18.4 ± 15	19.8 ± 11.2	9.6 ± 6.7	0.000

Highly Sig. at P=0.001 among patients groups and healthy control

Pearson's Correlation Coefficients between IL-22 & IL-17a among RA seropositive

In the current study, there were significant correlations between IL-22 with IL-17a (r=0.353 with P=0.032), in seropositive RA patients and (r=0.297 with P=0.024) in seronegative as show in table 4.

Table 4 : Pearson's Correlation Coefficients between IL-22 & IL-17a among RA groups

Groups	Studied parameter	Pc &p-value	IL-22	IL-17a
Seropositive RA Patients	IL-22	r		0.353*
		p-value		0.042 (S)
Seronegative RA Patients	IL-17a	r	0. 297*	
		p-value	0.024 (S)	

Pearson Correlation. S.: significant *: Significant at Ps values (0.05)

Estimation of cut-off values, ROC curve, sensitivity and specificity of the IL-22 &IL-17a among RA groups:

In RA patients , the IL-22 cuts-off values was 27.6 pg/ml with sensitivity 86.8 % , specificity 93.9%, and the AUCROCs of s0.952 (P = 0.000) ; while in IL-17a cuts-off values was 16.24 pg/ml with sensitivity 93.4% ,specificity 99 %, with AUROCs of 0.934 (P = 0.000). Table 5

Table 5 : Estimation of cutoff points, sensitivity, specificity and AUROC of the parameters in RA patients .

Parameters	Cut-off points	Sensitivity (%)	Specificity (%)	AURO	P-values
IL-22 pg/ml	27.6	86.8	93.9	0.952	0.000
IL-17 a pg/ml	16.24	93.4	99	0.934	0.000

AUCROC: Area Under receivers operating characteristic, , Sig. at P value < 0.05.

Discussion

Rheumatoid arthritis (RA) refers to systemic chronic inflammatory disease leading to joint destruction. [10]. information on the differentiation in the systemic inflammatory serum markers between SP and SN RA is insufficient .Consequently, in the current study preferred of serum markers were evaluated in self-governing groups of SP and SN RA patients. It has been suggested that different inflammatory pathways are involved in the development of seronegative (SN RA) and seropositive RA (SP RA). The existing study exposed that female patients were nearly three times more predisposed to RA than men this result was agreeable to some extent among that of local prior studies in Iraq [11], [12]. The preponderance of particular disease activity markers representing the appearance of autoantibodies in early RA these including ACCP and RF that have been conferred a higher risk of further aggressive and prognostic markers of an erosive disease compared to the population had negative for both autoantibodies [13]. The immune complexes containing –ACPA established to induced production of pro-inflammatory cytokines and triggering of macrophages via Fcγ R-dependent augmented by the presence of IgM RF in this process [14]. Regarding ESR and reactivity of h-CRP, it was noted that ESR, significantly raised in seropositive (SP) and seronegative (SN) RA patients, which is nearly harmonious with results of previous works of literature [15],[16] .Here rise in the seropositivity of active disease markers has been additionally connected with continued active inflammatory registered by the long-term height of erythrocyte sedimentation rate (ESR) and disease activity score [17]. The highly-sensitivit CRP (hs-CRP) assay can be applied to distinguish mild disease activity that was associated with inflammation but that was not detectable by routine CRP testing [18]. The results of hs-CRP in this study correspond with other studies [19], [20]. Those studies were proved that hs-CRP consider as a cardiovascular (CV) disease brand in RA patients plus dropped a spot of light on the high incidence of patients with or at the hazard of progressing to CV diseases in RA. Aforementioned study result of increased IL-17a level in RA patients would coincide with many other studies [21],[22], and established its important role in RA pathogenesis . Previous studies found the concentrations level of IL-17a in both serum and synovial fluid were greater in patients with RA than in healthy and recommend that high IL-17a levels may be correlated with a more severe clinical development as mentioned by [23],[24]. Meanwhile, another study observed strong associations of serum and synovial fluid IL-17a levels found with ESR, CRP, RF, and anti-CCP [25], those findings imply that raised serum and synovial IL-17a levels in RA patients correspond to the development of the disease activity and severity. Besides, Metawi *et al* observed that levels of IL-17a in serum and synovial fluid revealed significant positive associations with Disease Activity Score in 28 joints (DAS28) in a cohort of 30 patients with active RA with knee diffusions, while the worse functional state was associated with greater IL-17a levels [26]. In contradiction with the study on RA Egyptian patients, reported that serum level of IL-17a didn't correlate with the DAS28 score but it was significantly greater among critically active patients as corresponded to patients with modest activity [27].

conclusion: There was an elevation in both IL-17 and IL-22 in seropositive and seronegative RA patients with high sensitivity and specificity that means IL-22 and IL-17 would act as pro-inflammatory cytokine during the disease course of RA but they couldn't use for discriminated between SP and SN RA.

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Detection of *B1 Gene* in *Toxoplasma Gondii* and the Role of Interleukin 2 in Abortive Women Infected by this Parasite

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Abstract

The study included 80 blood and serum samples of aborted women with *Toxoplasma gondii* infection and healthy, uninfected and non-aborted women in the control group. For the period from August-2018 to June-2019, in collaboration with the Falluja teaching hospital for women and children and the Ramadi teaching hospital for women and children, the Enzyme-Linked Immunosorbent Assay (ELISA), also to the *Toxoplasma gondii (B1) gene*, the Nested polymerase chain reaction technique are used. The results of the study showed by that Elisa *Toxoplasma gondii*, IgG infection (50%), IgM infection (12.5%), and IgG & IgM Infection (12.5%), were mean (108.16±47.02) (99.02±31.47) (96.45±20.68) respectively compared with the control group (25%) (0.94±0.93) and Interleukin-2 shown in IgG group (48.66±12.01) and control group (14.28±6.84) decreased compared to IgM (81.19±9.66), and IgG & IgM (80.71±9.18) group. Nonetheless, using nested PCR, the *B1 gene* showed just (12.5%) of all study samples in IgM and IgG&IgM alone and all IgG negativly.

Keywords: *Toxoplasma gondii, ELISA, Interleukin 2, nPCR.*

Introduction

Toxoplasmosis is the main common parasitic disease, in which the coccidian protozoan caused by *Toxoplasma gondii,* (from 10% to 80%) in different parts of the world. The human infection pathway is the consumption of tissue cysts found into uncooked or raw eating as well as the ingestion of oocysts through polluted vegetables or water, another path of infection is the transmitting of tachyzoites (the fast-dividing) from mother which pregnant to the growing fetus which can be resulting in abortion, chorioretinitis, including severe developmental defects **(1).**

In *T. gondii*, it was found that three different phases of the life cycle depend on the phase of the parasite into the host. Tachyzoites and bradyzoites found mainly in the intermediate host (asexuality, within the tissue cyst) appear to be a morphological form. The most recent phase of sporozoites can only be seen in the most recent host (sexuality reproduction, oocysts) **(2).**

The most significant sequel to *toxoplasmosis* is a congenital infection in women pregnant (3). The congenital transmission of *T. gondii* happens predominantly for the first time during pregnancy (4). The incidence of congenital toxoplasmosis is greatest for the first and second trimesters of pregnancy, usually leading to abortion or stillbirth **(5).**

Many serological methods have been used to detect *T. gondii* as an enzyme-linked immunosorbent assay. Recent research has confirmed that PCR is critical to assessing the prevalence of *T. gondii*, as the only method of DNA detection, PCR has been used professionally in women pregnant to identify *toxoplasmosis* **(6)**. The *B1 gene* appears highly specialized and has 35 replicates in its genome, resulting in the amplification of the target for polymerase chain reaction (PCR) to identify blood and tissue parasites **(7).**

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Interleukin-2 (IL-2) is a very considerable inflammatory cytokine, with IL-2 production abnormalities identified in patients receiving transplant rejection. A number of studies have shown that the higher IL-2 outflow levels have already been an association with recurrent spontaneous abortion [8].

Material and Method

Collection of samples

Between August-2018 and June-2019, in collaboration with Falluja teaching hospital for women and children, Ramadi teaching hospital for women and children, 80 blood samples were collected over the age range (18-45) years. In this study, four different women's groups, control group included (20 samples) for healthy women non-abortion before, but infected women with *T. gondii* IgG group (40 samples), IgM group (10 samples) and IgG&IgM group (10 samples) which had an abortion before on the basis of the first diagnosis.

5 ml of venous blood samples were collected from the patient and the control subjects, 3ml was placed in a gel tube (without anticoagulant) and kept at room temperature until blood was clotted; the serum was separated by centrifugation at 3500 r.p.m. for 10 min and then stored at -20 ˚C. Also 2 ml placed in the EDTA tube and stored at-20 ° C until DNA was extracted. The DNA extraction by Kit extraction Geneaid from the blood as well as stored at-20 ° C until used for PCR.

The Diagnostic Methods:

1-Serological tests

A. Estimation antibodies of *Toxoplasma gondii*

The serological test was used for the diagnosis of *Toxoplasma gondii* by the ELISA test IgG according to the NO.TOXG01 catalog kit and IgM according to the NO.TOXG02 catalog kit.

B. Estimation of IL-2 concentration

Estimation of the level of IL-2 in the serum of women with T. Gondii was done using the ELISA method, according to the use of the Cloud-Clone Crop Catalog kit NO. SEA073Rb.

2- Molecular detection test

Nested Polymerase chain reaction (nPCR)

Blood DNA extraction was carried out according to the DNA extraction kit protocol with NO catalog. Nested PCR performed a two-stage amplification of the fragment of the B1 gene with separate primer pairs shown in table (1) for all DNA samples.

Table (1): *Gene B1* Specific Primers

B1 gene Primers	***B1*gene fragment**	**Fragment Length(bp)**	**Annealing temperature Tm**
B1 (F1) 5′-GGAACTGCATCCGTTCATGAG-3′	694-714	193 bp	56.6 °c
B1 (R1) 5′-TCTTTAAAGCGTTCGTGGTC-3′	887-868		51.9 °c
B1 (R2) 5′-TGCATAGGTTGCAGTCACTG-3′	757-776	96 bp	51.7 °c
B1 (F2) 5′-GGCGACCAATCTGCGAATACA CC-3′	853-831		63.2 °c

* F: Forward sequences, R: Reverse sequences.

Primers were added in the first stage, as instructed by the manufacturer, and are then placed into the PCR, then the first stage product is used as a template in the second stage and is also put into the PCR thermocycler.

Result and Discussion

Serological tests are beneficial for the diagnosis of T. gondii by detecting antibodies in serum and determining the phase of infection in the acute and chronic phase [9].

The result of this study shown in Table (2), *T.gondii* antibody level (UI / mL) in control group was (0.94±0.93) a significant difference with group IgG infection, IgM infection, and IgG & IgM Infection (108.16±47.02) (99.02±31.47) (96.45±20.68) respectively. But in group IgG had shown a non-significant difference with both groups IgM and IgG & IgM. Also group IgM had shown a non-significant difference in rates of *T.gondii* antibodies opposed to group IgG & IgM.

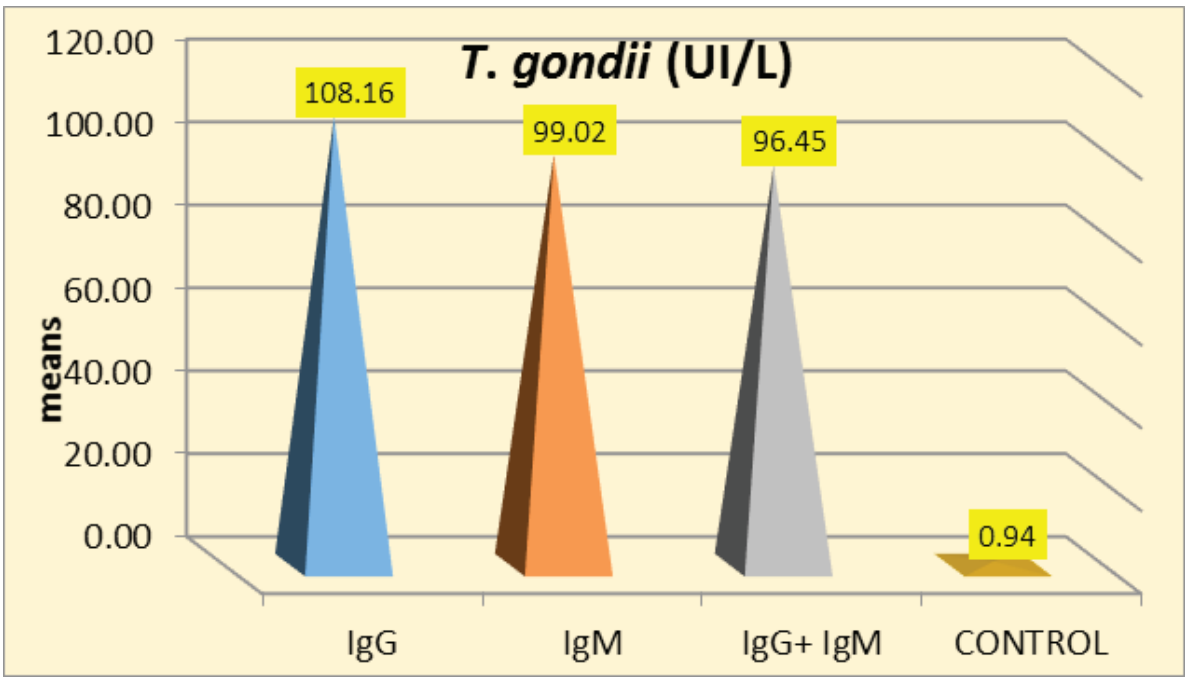


Figure (1): Levels of *mean T.gondii* infection in Patients and Control

The result of this study was shown to be a percentage of *T. gondii* seroprevalence IgG group (50 %) which is significantly greater than IgM and IgG&IgM which is shown (12.5 %) and (12.5 %) respectively, but control group (25 %), as most IgG-positive *toxoplasmosis* incidents were sufficient to determine the presence of chronic infection; IgM-positive titer, in the other hand, leads to the presence of acute infections.

The results of this study coincided with the results of the study [6] which showed *T. gondii* seroprevalence for IgM (4.16 %) and IgG (25.83 %) while IgM and IgG shown (3.33%) of aborted women with toxoplasmosis; and [10] were identified as IgG (31.5 %), IgM (7.6 %) and IgM & IgG (2.5 %).

On the other hand, the results were not in agreement with the result of [11] as shown IgG of *T. gondii* was (18.09 %) whereas IgM (9.79 %) for pregnant having recurrent spontaneous abortions also[12] observed 60 (38.7%) of IgG & IgM *T. gondii* were positive (4 % and 44 % respectively to IgG and IgM for aborted women).

Toxoplasmosis, which can lead abortion at any time of pregnancy, is indeed controversial, but even this study was been proposed to seek a possible association for both *T. gondii* infection and abortion.

The prevalence of parasite *T. gondii* varies among women in different countries in the world, based on different climate conditions, health, and food habits, economic standing, level of education and age [13].

The reason for high prevalence among aborted women may be reversion to the kind of acute infection or reactivation of chronic infection due to reduced immunity of pregnant mothers since the time of infection during pregnancy have an important role to play in determining the demise of the fetus [14].

Cytokines are divided into two components: Th2 (such as IL-3 and IL-15) to correct the immune system response, while TH1 (such as IL-2, IFR) seems to be harmful to end a pregnancy by influencing either the placenta or stimulation to avoid fetal implantation and to cause abortion or fetal killing.TH1 allows to invade trophozoites and apoptosis and hence generates harmful factors to the fetus [15].

In these study Interleukin-2 (IL-2) in women with *T. gondii* infection had a high level especially in comparison to the control group, which showed a lower level of infection. Furthermore, the increase in women infected with *T. gondii* IgM and IgG&IgM (81.19±9.66) (80.71±9.18) was higher than in the IgG (48.66±12.01) and control (14.28±6.84) respectively.

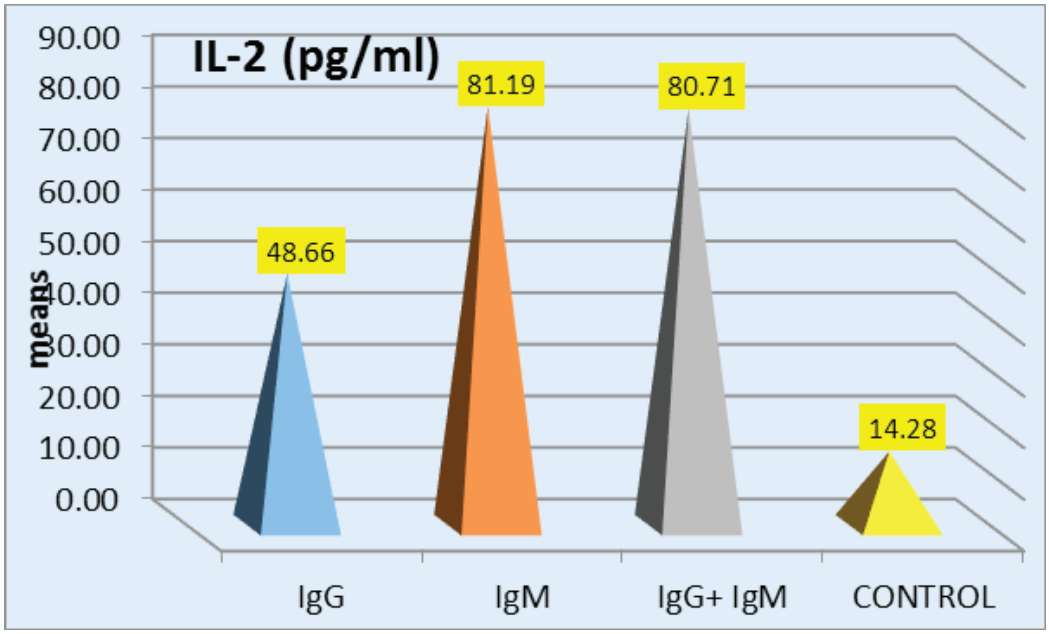


Figure (2): Levels of *mean* IL-2 in Patients and Control

These results have been similar to previous studies done by [16], [8] which found in a group of women diagnosed with *T. gondii*, the rate of IL-2 level was increased compared to healthy women as a control group.

IL-2 manages the activities essential for the immunity of white blood cells (particularly lymphocytes). It is part of the human body's normal response to microbial infection, besides being detectable in both nonself (foreign) and self. IL-2 effects have been mediated by CD4 and CD8 T-cell lymphocyte bind to IL-2 receptors of the immune response [17].

Also many unknown abortions are related to immunological reasons when cytokines mainly work on the loss or completion of pregnancy whereas Cytokines of kind Th1, such as (IL-2) are widespread in cases of pregnancy failure [18].

Following the initial ELISA serological examination, the nPCR technique is used to detect the existence of *T. gondii* DNA in circulating blood is an opportunity for an actual diagnosis in aborted women with *T. gondii* infection.

Results of n PCR in samples of patients (12.5%) of the DNA fragment band indicated an identification of the *B1 gene* and this explained the existence of the infection and had shown (92 %) that there is no amplified DNA fragment stating that there is no *B1 gene* and that there is may be, no infection.

The first step, of n PCR test has been used to amplification of *B1 gene* fragment (DNA) using the first primers to amplify the 193 bp fragment such as figure (3).

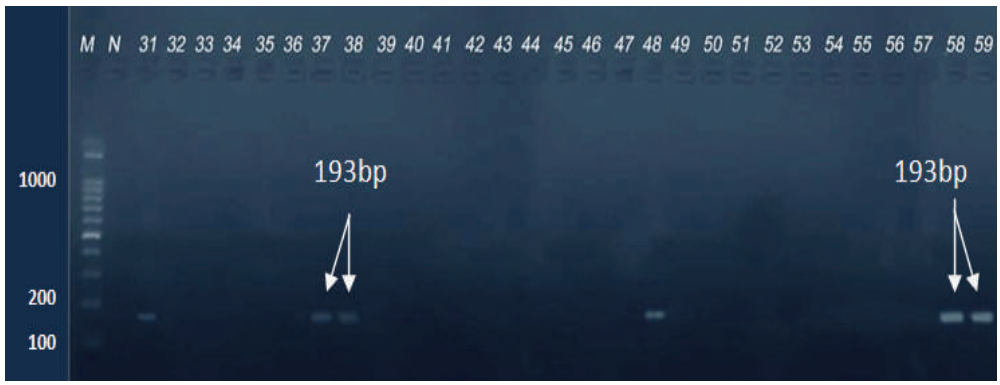


Figure (3): Amplification of the 193bp fragment of the *B1 gene* of *T. gondii* DNA

The second step, of the n PCR experiment has been used to amplification a 96bp fragment of the isolated DNA *B1 gene* from first product, that only in 10 samples (12.5%) for IgM and IgG&IgM showing positive and all other IgG negative being excluded such as figure (4).

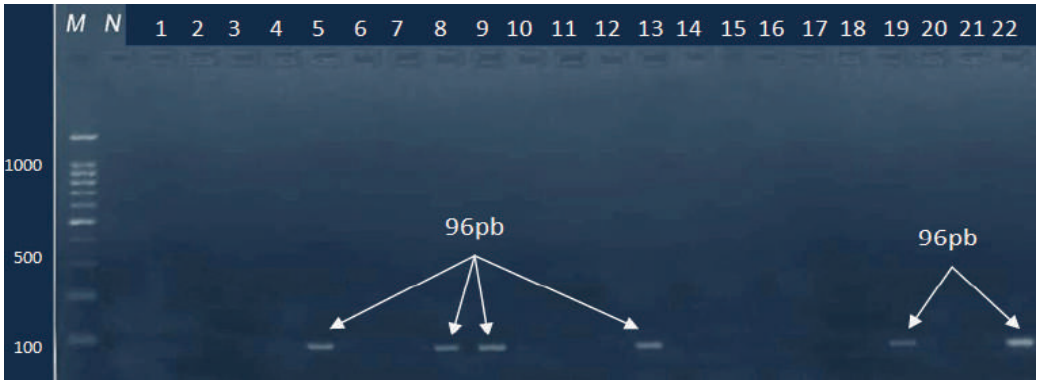


Figure (4): Amplification of the 96bp fragment of the *B1 gene* of *T. gondii* DNA from first product

These result may be due to IgM Ab can also be recognized in both acute and chronic *toxoplasmosis,* at that same time as *T. gondii* IgG Ab continued for even a long time, until for years. Therefore, the serology method may well be ineffective because it is focused on the level of antibodies generated which either fail or are delayed significantly whereas n PCR is based on the existence of the parasitic genetic material [19].

Also perhaps result due to the *B1* primers have the highest sensitivity due the two primers have been very specific to *T. gondii* strain was detected in Iraq. It is also very specific to the magnification DNA of *T. gondii* used in the PCR technique [20].

The result of the present study using n PCR were accepted with [6] as the result of the *B1 gene* was detected in (15.83 %) infected women with *T. gondii* and results of [21] also were consistent with the resulting study of investigation report *T. gondii* rate (31.57%) in donor blood. But, the results of [22], [19] were far from the result of these study *B1gene* was identified in (77 %) and (87.5 %) respectively of both the study samples.

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Bacteriocin Production in *Bacillus cereus* Food Isolates with Molecular Detection of *cerA* gene

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Abstract

Sixty-three *Bacillus cereus* strains isolated from food samples. All strains were subjected to DNA sequencing for 16S rRNA for identification. 15 strains were registered at GenBank of NCBI and given new accession numbers. 41.26% of the isolates showed bacteriocinogenicproduction activity against four bacterial speciesviz,*Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* sp. Presence of *cerA* gene coding for cericidine(a type of bacteriocin) was detected in 7.69% of the isolates that produced bacteriocin. Bacterionwas precipitated by ammonium sulphate and purified by dialysis. Antimicrobial activity of precipitated bacteriocin against the four types of bacteria showed the effect on *Bacillus cereus* and *Staphylococcus aureus* growth but not on *Escherichia coli* and *Salmonella* spp.The produced bacteriocin has a molecular weight ranging from 47-54 KDa.Estimation of the concentration and physical and chemical properties of bacteriocin were also investigated.

***Key word**: Bacteriocin, Bacillus cereus, cerA, 16SrRNA.*

Introduction

Bacteriocins are bacterial products which acts as antimicrobial peptides. They are ribosomally synthesized and secreted to act closely related to one another of bacterial species. Bacteriocins are used as antimicrobials for the treatment of human and animal infections. Such products will minimize the increased bacterial resistance to conventional antibiotics. In addition, since consumers require minimally processed foods without chemicals, natural antimicrobial research such as bacteriocin has increased[1]. Bacteriocins have an antimicrobial action affecting cell wall or cell membrane[2]. Bacteriocins are low molecular weight polypeptides with heat stable and proteolytic enzyme sensitivity[3,4]. Using of food additives has been reduced due to safety concerns. Chemical additives are sometimes replaced by the use of natural products of microflora using their antimicrobial activity to increase the lifespan and safety of foods[5,6].This study aims to isolation and purification of bacteriocin from *Bacillus cereus* and determination the molecular weight by SDS-Page. In addition to study its antimicrobial activity against several type of medically important bacteria.

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Materials and Methods

Bacterial strains

Bacillus cereus strain was isolated from food samples from a previous study[7]. 63 of *B. cereus* strains were further identified by 16S rRNA partial sequence. Genomic DNA was extracted from bacterial cells cultured using a commercial kit provided by the manufacturer (Geneaid). Extracted DNA were stored at -20°C until used. The 16S rRNA oligonucleotide primers which were used have 1541 bp and their sequence are: forward, AGAATTTGATCCTGGCTTAG and reverse, AAGGAGGTGATCCAGCC[8].

Screening of bacteriocin production in the isolates

Cross-streaking antimicrobial activity assay

Bacillus cereus strain was grown on Brain heart infusion agar supplemented with 1.5 % glucose. For testing the antimicrobial activity of*B. cereus*, strains were cross-streaked at 24 hrs pre-incubation. A sterile loop was streaked vertically in the center of BHIAG plate to form a single line, incubated at 35° C for 24 hour[9,10]. Four species of bacteria used such as *B. cereus, S. aureus, E. coli*, and *Salmonella* sp. The types of bacteria cultured horizontally on *B. cereus* from the edge to the center, after 24 hour of incubation, a clear of inhibition region was appeared, and this referred to bacteriocin production[11].

Molecular detection of *cerA* gene in *Bacillus cereus* isolates

The *cerA* gene was detected using theprimers with product size 233bp. The pair of primer used wasforward: ATGTCAAAAGGATACAAGTTCACAA and revers: TTATTTACAAATCTTAATTGACGT[12].The PCR tubes were transferred to the thermolcycler to start the amplification reaction according to the specific program.The PCRtemperature conditions were 94°C denaturation, 55°C for annealing step and 72°C for extension step. PCR products were detected in 1.5 % agarose gel stained with ethidium bromide and viewed by U.V. transilluminator.

Production of bacteriocin

The bacteriocin producing bacteria were grown in Brain-Heart Infusion broth (BHIB) supplemented with 1.5 % glucose[13]. After 18 hrs. incubation, the fermented broth was centrifuged at 8500 rpm for 20 mins[14].The supernatant was precipitated with 60% ammonium sulfate (w/v) and left tosettle overnight. To collect the precipitate, the product was cooled centrifuged at 5000 rpm for 45 min. The precipitate then dissolved in 10 mL of 0.1 M phosphate buffer (pH 6.0) and dialyzed against 500 mL of 0.1 M phosphate buffer (pH 6.0) at 4°C for overnight[15]. The produced pellets were centrifuged anddissolved inamount of water. 0.1 ml of the solution was tested for the antibacterial activity using well diffusion assay[14]. The protein then detected by biuret test using biuret reagent[17,18].

Estimation of extracted bacteriocin concentration

The concentration of extracted protein was estimated by using a spectrophotometer and a wavelength of 280 and 260 nm depending on the following equation[19];

Concentration of protein g / ml = 1.55 × A 280 -0.77 × A260, Where A = absorbency

Determination of bacteriocin activity at different conditions

The effect of pH[20], temperature[21], proteinase K and lysozyme[22], and EDTA[23]on purified bacteriocin activity against bacteria were estimated.

Determination of bacteriocin molecular weight

This was estimated by electrolysis by using a poly-acrylamide gel[24,25].

Antimicrobial activity of bacteriocin

The antibiotic susceptibility testing was done by the well diffusion method[26]. The wells were prepared using sterile yellow tips and filled with 100 µl of extracted bacteriocin. The plates were placed in an incubator for 18hours at 37 ºC.

Results

Identification of studied bacteria

All bacterialisolates identified by 16S rRNA showed the similarity of 99% when it blasts in the NCBI database. 15 isolates showed mutation change at different loci were registered at GenBank of NCBI and given the following accession numbers (Table 1).

Screening the bacteriocinogenic isolates

Out of 63 *B. cereus* used in this study, 26 (41.26%) strains showed bacteriocin production activity. Screening of bacteriocine was done against four bacterial species. Some of the isolates showed maximum inhibition activity.

Table 1. Strains of *Bacillus cereus* that registered at GenBank and their accession numbers.

No. Of sample	Nucleotide change	Accession number	No. Of sample	Nucleotide	Accession number
BBS1	T>G, G>C	MK468691	BBS8	T>G,G>C,T>C	MK468736
BBS2	G>A,T>G	MK468692	BBS9	T>G	MK468798
BBS3	G>C,G>C	MK468693	BBS10	T>G	MK471340
BBS4	G>A	MK468700	BBS11	G>A	MK468901
BBS5	T>G	MK468704	BBS12	T>G	MK468902
BBS6	T>G,A>C	MK468727	BBS	G>C,G>C,G>C,G>T	MK480518
BBS7	C>T,G>A,T>C,G>C	MK468732	BBS13	C>T ,A>T, A>C, C>T, A>C, C>T, C>G, C>T, C>G, C>T	MK949281

Molecular detection of bacteriocin Cerecidin (*cer A)* gene

The bacteriocin cericidine A gene was found in 2 isolates (7.69%) out of 26 isolates as indicated in figure (1).

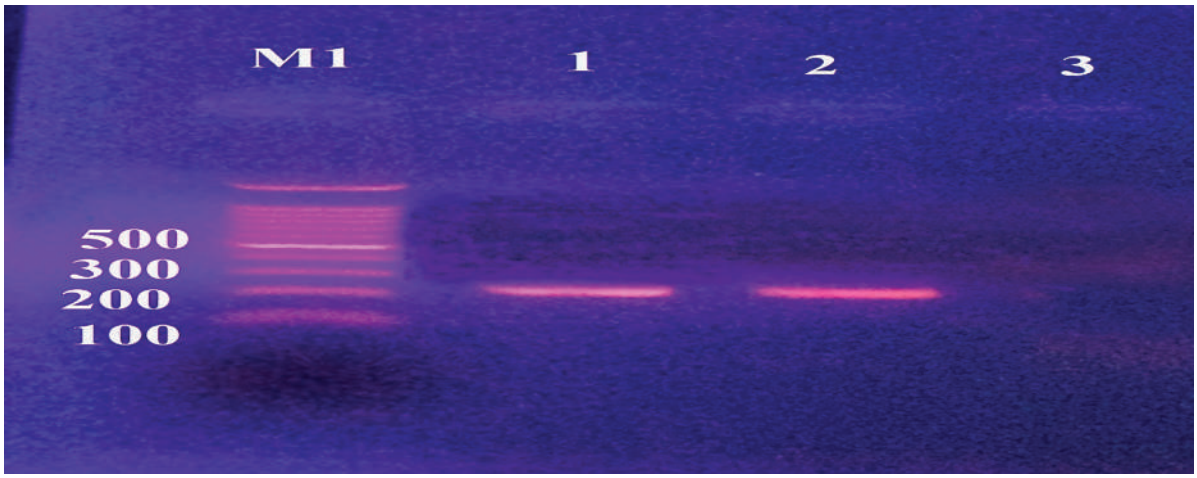


Figure 1. Detection of bacteriocin *cerA* gene by PCR .Lane M1= molecular marker; Lane 1-2=Positive for Cerecidin*cer A* gene approximately 233 bp; Lane 3= negative for Cerecidin*cer A* gene

Biological activity of crude bacteriocin

The crude bacteriocin was produced by *Bacillus cereus*cultured grown in BHIB for 18-24 hrs. Using ammonium sulfate and dialysis.The biological activity of crude bacteriocin was tested against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. The results showed activity against *Bacillus cereus* and *Staphylococcus aureus* but no activity was detected against *Escherichia coli* and *Salmonella* spp. Table (2).

Table 2. Antimicrobial activity of crude bacteriocin against different types of bacteria

Types of bacteria	Inhibition zone (mm)
Bacillus cereus	10 mm
Staphylococcus aureus	6 mm
Salmonella spp.	0 mm
E. coli.	0 mm

Estimation of extracted bacteriocin concentration

The results show that the concentrations were ranged between 217.308 to 648.606 (Table 3).

Table 3. Estimation of bacteriocin concentration

No. of samples	260 nm	280 nm	Bacteriocin conc. gm/ml
Crude	1	0.962	217.308
1	2.570	2.680	648.606
2	1.986	2.290	596.2628
3	2.860	2.999	555.828
4	1.972	2.060	461.0536

Physical and chemical properties of bacteriocin

Thebacteriocin was active in a range from pH 3-11, with a best antimicrobial activity at pH 7 when tested on Gram-positive bacteria, *B. cereus* and *staph aureus*.

The bacteriocin was active at 30 °C on *B. cereus*,and *S. aureus* and lost its activity at high-temperature Table (4).

Table 4. The effect of different pH on bacteriocin

Isolates	pH			Temperature			
	pH 3	pH7	pH 11	30 °C	50 °C	70 °C	90° C
B. cereus	6 mm	11 mm	5 mm	+	-	-	-
S. aureus	6 mm	8 mm	5 mm	+	+	+	-

The bacteriocin lost its activity on *B. cereus* and *S. aureus* when treated with proteinase K and lysozyme.The addition of EDTA increases the activity of bacteriocin. The results showed high activity against *B. cereus* and *S. aureus* and Gram-negative *E. coli* and *Salmonella* spp. Table.

Estimation of the molecular weight of bacteriocin

The molecular weight of the protein was estimated by electrolysis by using a poly-acrylamide gel according to Lammeli method**.** The estimated molecular weight of bacteriocin was 47-54 KDa.

Discussion

*Bacillus cereus*produced a peptide that showed antimicrobial activity against major food-borne bacteria[27].Our results suggest that this substance has a bactericidal effect against *B. cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella* spp. by inhibition the growth of these bacteria. The effect may depend particular test conditions, such as the quantity and purity of the bacteriocin, the indicator strain and concentration of cells[28].

In this study, the bacteriocin was purifiedand the activity of this crude bacteriocin appears against Gram-positive bacteria 10 mm and 6 mm for *B. cereus* and *S. aureus,*respectively. There was no activity on Gram-negative bacteria. In this study, thebacteriocin was active in a different pH value, but the maximum activity was found at pH 7 when tested on Gram-positive bacteria, *B. cereus* and *staph aureus*. The stability of bacteriocins at various pH scales is a limiting factor for their use in food[29].In this study, the activity of bacteriocin was lost when the temperature was increased. This was agreed with Sankar*et. al.,*[29].

In vitro, the present study shows that EDTA with bacteriocin used against Gram-negative bacteria to boost antimicrobial activity[30]. The gram-negative bacteria are poorly sensitive to bacteriocin and require increased concentration to inhibit growth. The bacteriocin was treated with EDTA to increase activity against Gram negative bacteria. The combination of bacteriocin and EDTA showed better antimicrobial activity. The bacteriocin lost its activity on *B. cereus* and *S. aureus* when treated with proteinase K and lysozyme, no inhibition zones are formed[30].In this study, single band of the purified bacteriocin appeared in SDS-PAGE. The product has a molecular weight ranging from 47-53 kDa, whereas in another study, the bacteriocin determined by SDS PAGE is 23 kDa[22]. The bacteriocin produced by *Bacillus cereus* GN105 showed a bacteriocin band at 3.5 kDa[31], at21 kDa[32]. and 43 kDa[12].

Conclusion

Several studies were done on *Bacillus cereus*in the similar area of study[33,34,35,36,37]. Bacteriocin production rarely investigated which considered as effective food preservatives. Though cerecidine A is the only the detected bacteriocin in this study. Bacteriocins have inhibitory action against food-borne pathogens such as *Bacillus cereus* and *Staphylococcus aureus*. The action of bacteriocinincreased by combining with EDTA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Diagnostic Efficacy of Visually Enhanced Lesion Scope (Velscope) In Identifying Benign , Dysplastic and Cancerous Oral Lesions

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Abstract

Background : Visual inspection by conventional oral examination (COE) has been the backbone of oral cancer and precancer detection. More recently, several commercially available diagnostic adjuncts have been developed to aid clinicians in the early detection of oral epithelial dysplasia (OED) and squamous cell carcinoma (SCC), such as OralCDx, Toluidine coloring, ViziLite machine, Identafi machine, and VELscope.

VELscope is a technology based on the principles of autofluorescence mechanism imaging. This device offers in-vivo, real-time, direct visualization of tissue autofluorescence, termed direct visual fluorescent examination (DVFE). It is currently marketed as an oral cancer screening tool to be used with all new and recall dental patients and as an aid for surgeons in tumor margin delineation.

Aim of the study :was to obtain auto fluorescent data on variety of histopathologically distinct oral lesions and assessment of direct auto fluorescent examination validity in identifying dysplastic (premalignant) and malignant oral mucosal lesions using VELscope and judgment with histopathological examination.

Materials and methods: Routine oral examination of fifty patients having suspicious oral lesions followed by direct autofluorescent examination by VElscope and then histopathological examination.

Conclusion : VELscope can be considered as an adjunctive device to enhance the visualization of oral mucosal abnormalities, but not as a tool for risk stratification.

***Key words**: Autoflouresence examination , VELscope , oral premalignant lesions.*

Introduction

Several studies were conducted in many countries intrested with the diagnostic value of visually enhanced lesion scope (VELscope) since its development by the British Columbia Cancer Agency and granted FDA clearance on July 06, 2006 [(1)].Howerver no study was conducted in Iraq,so the current study may be considered the first study concerning with this field.

Biopsy and microscopic evaluation by a trained oral and maxillofacial or head and neck pathologist, remain the clinical gold standard to determine the presence and grade of dysplasia or carcinoma,but over time various diagnostic adjunct were developed[(2)].Autofluorescence imaging occupies the intrest of many researechers and spacialists in the field of early detection of oral premalignant and malignant lesions,so several autofluorescence devices have become commercially available since the past decade till nowadays,such as VELscope (LED

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The principle of Velscope work depends on the changes of autoflouresent characteristic of the oral mucosa that contains abundant endogenous autofluorescence molecules called Fluorophores which are molecules that emit energy in the form of fluorescence when excited by light, including structural proteins such as collagen and elastin, the metabolic co-factors nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide(FAD),as well as several aromatic amino acids, and porphyrins .This should be done through blue excitation light (wavelength of 400–460 nm)emitted from the VELscope contacts these fluorophores,The level of fluorescence energy emitted from the endogenous autofluorescence substances will become lower than that of the light energy emitted from the VELscope, and green light with a wavelength of 515 nm is then emitted from endogenous autofluorescence substances in healthy area[5].

Dysplasia and cancer causing measureable changes in tissue autofluorescence in which the collagen cross-links and basal lamina of tissue affected by SCC and epithelial dysplasia are destroyed, and glucose is highly consumed in malignant tissue even when in an aerobic environment (Warburg effect). Also, certain changes, such as increased metabolism, nuclear pleomorphism, increased epithelial thickness, breakdown of collagen cross-links, increased vascularization and production of fluorophores by bacteria may also contribute to this effect[6].

Epithelial fluorophores concentration decrease in the epithelial dysplasia therefore, the lower level of endogenous auto fluorescence substances in tissue affected by epithelial dysplasia and SCC of the oral mucosa, which has no fluorescence energy, resulting in the appearance of a black area detected as fluorescence visualization loss (FVL)[7]. So in order to perform simple criteria analysis and evaluation of the tissue status, the sample interpreted correctly by Velscope and further analyzed by histopatholgical examination .

The advantages of auto fluorescence examination using VELscope include obtaining of diagnostic information in situ, real time, and in a minimally invasive manner [8]. High sensitivity for dysplasia and cancer, ability for assessment of large areas of oral mucosa, consumables are not required, clinical utility for risk assessment during longitudinal monitoring of patients with known high-risk potentially premalignant oral lesions or previous history of cancer and commercially available device rely on subjective interpretation of autofluorescence[6].

But, Velcsope is unfortunately limited by false positive results (low specifity) because lesions of various etiologies have different autofluorescent properties [9].

Subjects, Materialsand Methods

This cross sectional study was conducted fromthe 1st of February till the 1st of August 2019 in Oral Medicine Department at the College of Dentistry University of Baghdad.

Ethical approval and official permission was obtained from college of dentistry /university of Baghdad Scientific Committee.

Participation consent form for each subject was signed after brief details about the study, having the wright of withdrawing from the study at any time.

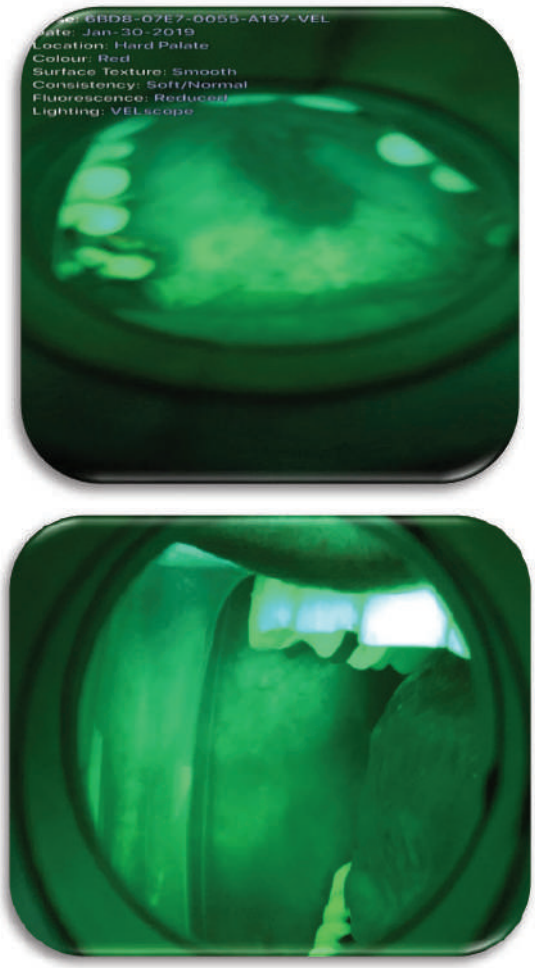
The current study involves 50 patients with an age ranged between 18-75 years with suspicious oral lesions. A case sheets were used to record the personal information including age, sex, occupation, address, phone number and age of onset and duration of the oral lesions. Medical history, family history and history of smoking and alcohol consumption were also recorded.

Excluded patients were those who have contraindications for biopsy sampling, such as bleeding disorders or uncontrolled systemic diseases, diabetic patient, pregnant woman, patients with a confirmed diagnosis of dysplasia or malignancy in a previous biopsy, or patient with history of photosensitivity or those using photosensitive medication who should not exposed to the light emitted from the VELscope device were also excluded from the study.

All patients were examined by oral medicine specialist at oral diagnosis department for exploring the existence of the oral lesions which was done in sequence examination procedure of oral soft tissue following

directions represented by WHO (1987).

Direct visual examination is then performed by VELscope Lesional and perilesional tissue was assessed for visual fluorescence retention (VFR) and visual fluorescence loss (VFL) when viewed through the velscopeVX handpiece, As documented by the manufacture information ,(LED Dental Inc ,Vancouver ,Canada ,2007).figure (1)



Figurer (1) A:VELscope examination of hard palate demonstrating an area of VFL B: VELscope examination of buccal mucosa demonstrating an area of VFR

Direct visual examination findings were documented and digital photographs of tissue fluorescence were acquired. Images were obtained directly through the VELscope viewing handpiece using Ipod 5 digital camera connected to VElscope by image adapter that is based on the manufacturer (LED DENTAl Inc).

Biopsies were then taken from the suspicious area of the oral lesions,Specimens were stored in 10% neutral buffered formalin and sent to at oral pathology laboratory of the oral diagnosis department at the collage of dentistry /university of Baghdad and Al Byan private specialized medical laboratory.

To determine the sensitivity and specificity of the VELscope, attempts were made to have the biopsy site cover both the COE and VEL positive regions.

Following the biopsy, the tissue sample was fixed in 10% formalin and sent to The Oral Pathology Laboratory for processing and histologic diagnosis, without information of the DVFE findings.

Formalin-fixed and paraffin embedded tissue was processed and stained following standard protocol for routine (hematoxylin and eosin) histopathologic evaluation. The examination were done under a light microscope by an experienced specialist in oral and maxillofacial pathology ,and epithelial dysplasia classified according to its severity (mild ,moderate ,sever).

Final histopathologic diagnoses were recorded on data collection sheets.

To calculate the sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV), the results of both the COE and VELscope were compared with the histopathological results.Statistical analysis evaluation was performed using SPSS software.

Results

Results shows that female patients were formed 27(54.0%) with no significant different compared with male numbers 23(46.0%)at P>0.05.Age groups of the examined patients seems to be having an extreme shape distribution of type I (smallest values of left skewness),with mean and standard deviation values 53.90 yrs,and 10.44 yrs., as well as recorded highly significant different at P<0.01.

Table (1) shows distribution of risk factors in the studied group, such that: (Smoking status and Alcoholic)

Table(1):Risk Factors distribution with comparison's significant

Risk Factors	Groups	Patients		C.S.
		No.	%	
Smoking	No	36	72	P=0.003 HS
	Yes	14	28	
	Total	50	100	
Alcoholic	No	45	90	P=0.000 HS
	Yes	5	10	
	Total	50	100	

The clinical appearance of the oral lesions on the conventional oral examination demonstrated in table (2)

Table(2): Appearance of oral mucosal lesions with comparisons significant

Appearance	Resp.	No.	%	C.S. (*)
White Lesion	No	25	50	P=1.000 NS
	Yes	25	50	
Red Lesion	No	38	76	P=0.000 HS
	Yes	12	24	
Ulcer	No	43	86	P=0.000 HS
	Yes	7	14	
Exophytic Lesion	No	43	86	P=0.000 HS
	Yes	7	14	
Swelling	No	47	94	P=0.000 HS
	Yes	3	6	

Result shows that most of the oral lesions presented as a white Lesion, and they are accounted 25(50.0%),with no significant different at P>0.05,then followed by "Red Lesion ", and they are accounted 12(24.0%),with significant different at P<0.01, then followed by "ulcer, and exophytic Lesion" and they are accounted for each 7(14.0%),with significant different at P<0.01, and finally only 3(6.0%) cases are recorded "Swelling" appearance, with significant different at P<0.01 Autoflourescen examination by VELscope was performed on all the 50 patients .

After histopathological examination result shows most patient's diagnosed were having benign oral lesions , and they are accounted 37(74 %),table(3)

Table (3) histopathological diagnosis of benign lesions

Definite Diagnosis	No.	%	C.S. (*)
Lichen Planus	32	64	P=0.066 NS
	17	36	
Hyperkeratosis	44	88	P=0.000 HS
	6	12	
Chronic Inflammation	47	94	P=0.034 HS
	3	6	
Nonspecific ulceration	47	94	P=0.000 HS
	3	6	
Fibrolipoma	49	98	P=0.000 HS
	1	2	
Hyperplasic Epithelium	47	94	P=0.000 HS
	3	6	
Fibroma	49	98	P=0.000 HS
	1	2	
Papilloma	48	96	P=0.000 HS
	2	4	
Mucocele	49	98	P=0.000 HS
	1	2	

dysplastic lesion accounted 7 (14 %) and classified as mild dysplasia 5 cases ,moderate dysplasia 1 case ,sever dysplasia 1 case and SCC accounted 6 (12%) cases ,table 6 demonstrated the distribution of histopatholgical diagnosis of dysplasia and SCC.

Table(4)Histopathological distribution of dysplastic and SCC lesions

Diagnosis	Resp.	No.	%	C.S. (*)
Mild Dysplasia	Absent	45	90	P=0.000 HS
	Present	5	10	
Moderate Dysplasia	Absent	49	98	P=0.000 HS
	Present	1	2	
Sever Dysplasia	Absent	49	98	P=0.000 HS
	Present	1	2	
SCC	Absent	44	88	P=0.000 HS
	Present	6	12	

Autoflourescence examination of 37(74%) benign lesions by VELscope show that 24(65%) show VFR to be reported as true negative cases ,and 13(35%) lesions show VFL which is a false positive cases .

Dysplastic lesions examination show that 6(86%) lesion exhibit VFL which are true positive cases and 1(14%) exhibit VFR which is false negative case, sequmous cell carcinoma show 4(67%) true positive cases that axhibit VFL and 2(33%) false negative cases that exhibit VFR.As a result the autoflourescene examination by VELscope in this study show a sensitivity and specificity of 76.92% and 64.86% respectively,positive predictive value(PPV) is 43.48 %,And negative predictive value (NPV)88.88 %.Table(5)

Table (5): VElscope Sensitivity,specificity,PPV and NPP

Histopathological diagnosis						
	VFR-	VFL+	Sens	spec	PPV	NPV
Benign	24	13	76.92 %	64.86%	43.48	88.88
Dysplasia	1	6				
SCC	2	4				

Discussion

Oral cancer continues to be a major disease with significant morbidity and mortality so prevention and early detection are essential to improving health outcomes,thus,various diagnostic adjunct had been developed to improve the early detection capabilities of the potential oral premalignant and malignant lesions .among these adjunct is VElscope (visually enhanced lesion scope) that occupies a remarkable concern among researchers and specialist which is based on tissue autofluoresence characteristics.

To the best of our knowledge the current study considered to be the first one that's conducting in Iraq in an attempted to expand some of the early investigative prospect in the field of oral diagnosis .

The previous studies in this field reported sensitivities and specificities for the device in specialist practice range from 30% to 100% and 15% to 81% respectively [(10)]

Amirchaghmaghi et al,(2018)demonstrated a high sensitivity(90%)and low specificity (15%)in their study[(11)]

Koch et al.,(2017) reported a higher sensitivity(97%) and specificity of (95.8%) of the VELscope in diagnosing OSCC.[(12)] On the other hand,Rana et al.(2012) in their study compared VELscope examination with COE and reported that using the VELscope leads to higher sensitivity (100% vs. 17%), but a lower specificity (74% vs. 97%).[(13)]

The statistical analysis in the current study revealed the sensitivity value of the VELscope examination to be(76.9 %) that was warranted to the false negative results presentation,because one of 7 epithelial dysplasia cases and 2 of 6 SCC cases showed visual fluorescent retention which considered as false negative finding.

This result was also expressed by McNamara et al.,(2018) they found that a number of benign lesions displayed VFL[(14)].

The involvement of few cases of OPMDs and OSCC in the current study may also contribute to these results .

This is in agreement with results reported by Paderni et al. study,they showed a sensitivity of 75% [(15)], and disagreement with a study conducted by Farah

et al 2013 .[16], and Mehrotra et al 2010.[17] whom reported low sensitivity for this device(30% and 50% respectively) .

Specificity of VELscope examination that reported by the present study was 64.86%.The study demostrated that 13 of 37 benign lesions show VFL that's considered as false positive results. One of the main etiologies may be the inclusion of inflammatory and ulcerative lesions in the current study.This low specificity reflects VELscope weakness in distinguishing high-risk lesions(i.e., lesions with dysplasia and malignancy)from low-risk lesions(i.e., inflammatory and benign lesions without dysplasia) that's because of presentation of loss in fluorescent characteristic during the examination (black to grey color comparing with the adjacent tissues) with some cases of atrophic and erosive lichen planus ,ulcerative lesion, and hyperplastic epithelium .

In inflammatory and erythematous tissues, the destruction of structural molecules is less common; however, two factors contribute in the above mentioned results; an increased hemoglobin concentration due to increased circulation and an increased density of chronic inflammatory cells, such as lymphocytes, that's lead to increased dispersion and absorption of the fluorescence along with a darkening of the lesions.[18]

Conclusion

Direct visual fluorescent examination by VELsope has potential as a simple, cost-effective screening, biopsy guidance that act as an intermediate adjunctive tool between routine oral examination and a biopsy for oral premalignant and early malignant lesions and to be especially useful to alleviate both patient and practitioner concerns regarding a clinically oral suspicious lesion.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Test Some New Media for Cultivation of Gram Positive and Gram Negative Bacteria

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Abstract

Possibility of using the wastes of used paper to prepare a culture medium for bacterial growth was carried out in this study, five experimental culture media (paper waste extract agar, glucose paper waste extract agar, yeast extract paper waste extract agar, NaCl paper waste extract agar and GYN paper waste extract agar) that made from the extract of used papers were prepared to cultivation two species of Gram positive bacteria (*Staphylococcus aureus* and *Streptococcus* spp.) and two species of Gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) in comparison with readymade media (nutrient agar and brain-heart infusion agar). Heavy growth of bacteria was appeared on yeast extract paper waste extract agar and GYN paper waste extract agar media, while no growth was happen on the other three experimental culture media. The results of statistical analysis found that there is no significant different at $P \leq 0.05$, using LSD test among yeast extract paper waste extract agar , GYN paper waste extract agar and nutrient agar or brain-heart infusion agar media .These results indicate likelihood of using two experimental culture media instead of nutrient agar or brain-heart infusion agar to cultivation of bacteria.

Keywords: *Culture medium, used paper, bacterial growth, paper wastes*

Introduction

Culture medium is the medium containing all the essential nutrients for growth of microorganisms [(1)],including sources of carbon, nitrogen, , phosphorus, energy and different minerals [(2)] .The main purpose of it is either to grow a specific microorganism or to study the biochemical characteristics of bacteria particularly the pathogenic one [(3)].Different culture media are used in the laboratory [(4)], whilst media are used to support the growth of many types of microorganisms such as nutrient agar, others are particularly designed for the isolation and identification of specific kinds [(5)]. Generally, there are three commonly kinds of media; differential, enriched and selective media [(3)]. Differential media are used to cultivation more than one bacteria of interest but distinguish between them whose growth they support, usually by means of a coloured indicator such as MacConkey agar [(5)]. Enriched media are used to support the growth of fastidious bacteria such as blood agar [(3)] and the selective media are preferentially support the growth of specific bacteria, such as mannitol salt agar [(6)].

Many researchers mentioned using of plant materials as components to prepare culture media for microorganisms, Osman *et al*. [(2)] used many fresh vegetables, legumes and seeds as promoters to growth of some fungi like *Curvularia lunata, Aspergillus niger*, and *Fusarium oxyaporum*. Arulanantham *et al*. [(4)] used several protein sources; namely legume seeds, cowpea, soya meat (processed soya bean), green gram, and black gram for cultivation of some microorganisms. Kadhim and Ali [(7)] reported using the leaves and stems of *Portulaca oleracea oleracea* L. for growing

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of some pathogenic bacteria such as *Escherichia coli, Pseudomonas flouresence, Staphylococcus aureus , Klebsiella pneumoniae , Proteus mirabilis* and *Proteus vulgaris*. Tzintzun-Camacho *et al*. [8] designed a culture medium from hydrolysed avocado seeds waste for growth of *E. coli*.

Paper is known as a mixture of organic materials (cellulose, hemi-cellulose, lignin, and different compounds of lignin) and inorganic or non-fibrous filling and loading materials like clay, calcium carbonate and titanium oxide depending on the type of paper. The previously neglected products of paper or paperboard is known as waste paper [9] .To our knowledge, there is no study about using the paper wastes as an alternative medium for growing of bacteria. So, the aim of the present study is to evaluate the bacterial growth on the wastes of used paper as a culture medium in comparison with nutrient agar and brain-heart infusion agar media.

Materials and Methods

Bacterial isolates:

Staphylococcus aureus, *Streptococcus* spp., *Escherichia coli*, and *Pseudomonas aeruginosa* were obtained from department of Biology, College of Science, Al-Mustansiriya University, Baghdad, Iraq.

Paper waste extract:

It was prepared by mixing 5 kg of paper waste pieces with 10 liters of distilled water; the mixture was left for 2 to 4 hours and then filtered, the extract was used to prepare culture media used in this study.

Preparation of culture media:

a- Paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 20 g of agar-agar (Hi-Media, India) and then the volume was completed to 1 liter with distilled water.

b- Glucose paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 10 g of glucose (BDH, England) and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

c- Yeast extract paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 5 g of yeast extract (Hi-Media, India) and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

d- NaCl paper waste extract agar medium: it was made by adding 500 ml of paper waste extract to 1 g of sodium chloride (BDH, England) and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

e- GYN paper waste extract agar medium: it was prepared by mixing 500 ml of paper waste extract, 10 g of glucose, 5 g of yeast extract, 1 g of sodium chloride and 20 g of agar-agar and then the volume was completed to 1 liter with distilled water.

f- Brain-heart infusion agar (BHIA) and nutrient agar (NA) (Hi-Media, India) were prepared according to the manufacturing company instructions.

The pH value for all the media was adjusted at 7.2 and autoclaved at 121°C for 15 minutes and then used for cultivation of *S.aureus , Streptococcus spp., E.coli , and P.aeruginosa*.

Growing of bacteria on culture media:

Serial decimal dilutions (10^{-1} – 10^{-6}) were made for each bacterial species namely; *S.aureus*, *Streptococcus spp., E.coli*, and *P.aeruginosa* by using normal physiological saline solution. Spread-plate method was used [3] , in brief ; 0.1 ml from each dilution was spread separately on the following media: paper waste extract agar, glucose paper waste extract agar, yeast extract paper waste extract agar, NaCl paper waste extract agar, GYN paper waste extract agar, BHIA medium and NA medium and then incubated at 37°C for 24 h. After incubation, the numbers of colonies was counted and the results were recorded.

Statistical Analysis

Different between means of culture media for each bacterial species have analyzed using least significant differences (LSD) at $P \leq 0.05$ and complete randomized design (CRD) to evaluate the bacterial growth on the culture media. Using SPSS program 2010 and excel application to find the results and charts [10, 11].

Results and Discussion

Culture medium is used to cultivate numerous kinds of microbes, it is formed of numerous nutrients which are necessary for microbial growth depending on the requirements of growth of each microbe, when culture media are formed from all nutrients that most bacteria consume for growth and aren't selective for specific type of bacteria, they called general media.

In the present study, in order to evaluate five experimental culture media prepared from the wastes of used paper in comparison with readymade media; nutrient agar (NA) and brain-heart infusion agar (BHIA), *S.aureus , Streptococcus spp., E.coli ,* and *P.aeruginosa* were cultivated on these media using spread-plate method. The results showed that yeast extract paper waste extract agar and GYN paper waste extract agar were excellent as culture media (Figures 1 and 2), since heavy growth of bacteria was appeared on them indicate that all the important and essential nutrients requirements for bacterial growth is available in these media. The presence of used paper extract with yeast extract only was enough to obtain copacetic growth for both Gram positive and Gram negative bacteria.

On the other hand, the presence of paper extract alone or with glucose or sodium chloride failed to stimulate bacterial growth and thus there is no growth was happen on the paper waste extract agar, glucose paper waste extract agar, and NaCl paper waste extract agar. It could be because the absence of the some essential nutrients for growth.

Statistical analysis showed no significant differences among yeast extract paper waste extract agar, GYN paper waste extract agar and NA or BHIA media at $P \leq 0.05$ (Tables 1 and 2). So, both of the experimental culture media can be used instead of NA or BHIA to cultivation of Gram positive and Gram negative bacteria.

NA is one of the general media used for cultivation of wide range of non-fastidious bacteria. It is formed of peptone as a source of organic nitrogen, beef or yeast extract, agar and NaCl [12]. BHIA is a rich medium used for cultivation of numerous fastidious microbes (e.g. *Streptococci*, *Pneumococci* and *Meningococci*). BHI is formed by gathering an infusion of boiled bovine or porcine brain and heart (as a source of amino acids), in addition to numerous other nutrients, salts, Na_2HPo_4 as a buffer, and glucose as a source of carbon [13].

The primary structural element of paper products is cellulose fibers [14]. Cellulose is a linear polysaccharide of glucose residues linked by β-1, 4-glycosidic linkages. Different accommodation products are made from cellulose, but unfortunately a lot of cellulosic wastes are used for burning especially in developing countries, which represent a global concern. Cellulolytic bacteria convert cellulose to glucose which is a multi- use material for many different cheaper and biological favorable processes [15]. As all know, glucose is the simplest carbon source for many bacteria due to its ability to consume simply and production of energy for a faster bacterial growth [16]. For nitrogen source ammonia is the preferred nitrogen source especially for *E. coli* [17]. Yeast extract is considered as stimulant for microbial growth as mentioned by [1].It is autolyzed formed of yeast cells, soluble in water due to strict control in order to maintain B complex vitamin which considered as bacterial growth stimulator [18].

So, depending on the results of this study, we can conclude that extract of used paper could be used as a culture medium for cultivation of bacteria, just like other general media like nutrient agar, especially after addition of the other important nutrients like yeast extract. By this way we could benefit from paper wastes without increasing environmental pollution and prepare new substitute media as the synthetic commercial media. Future studies are needed to create new media from different environmental wastes to culture numerous microbes.

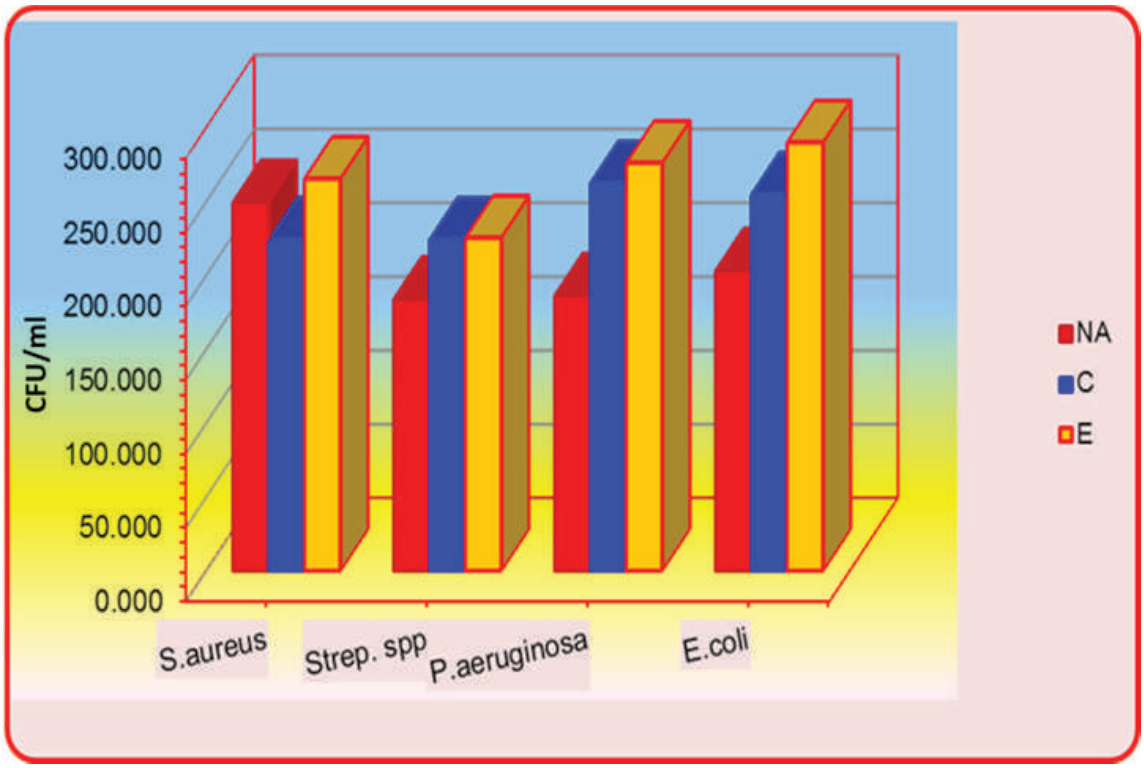


Figure 1: Growth of *Staphylococcus aureus* , *Streptococcus* spp., *Escherichia coli* , and *Pseudomonas aeruginosa* on the culture media, NA, Nutrient agar; C, yeast extract paper waste extract agar ; E, GYN paper waste extract agar

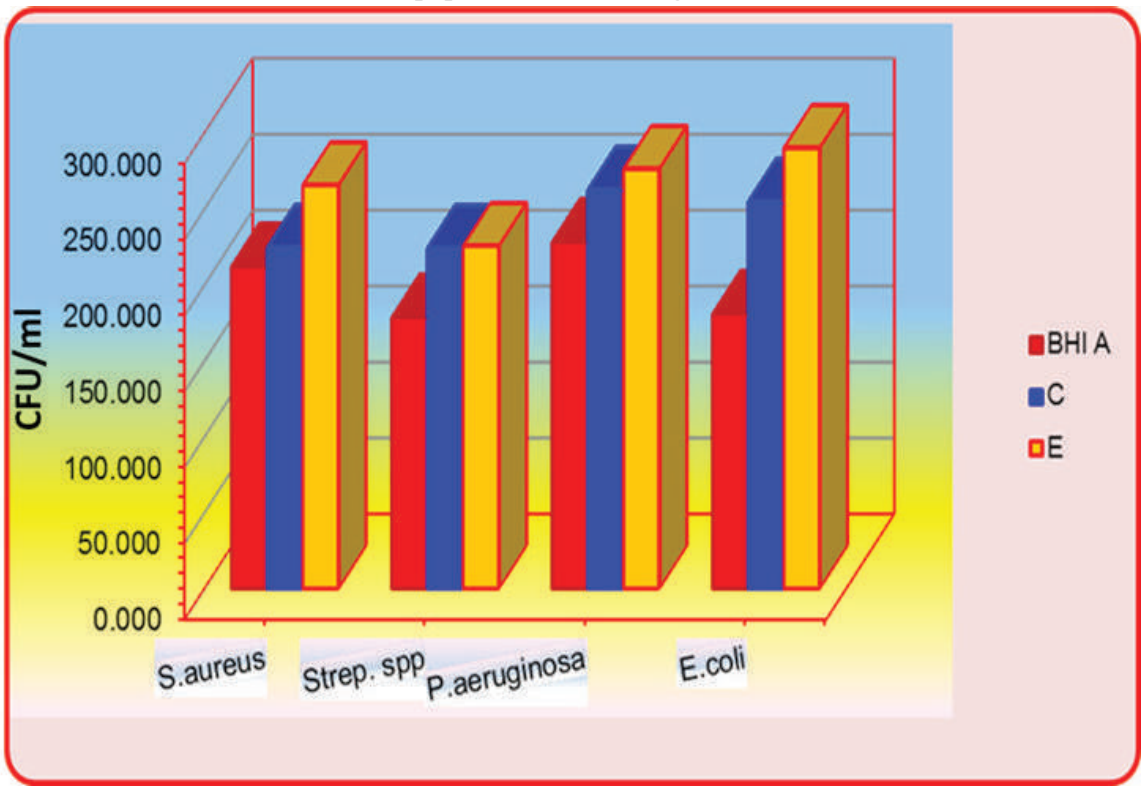


Figure 2: Growth of *Staphylococcus aureus , Streptococcus spp., Escherichia coli* , and *Pseudomonas aeruginosa* on the culture media, BHIA, Brain-heart infusion agar; C, yeast extract paper waste extract agar ; E, GYN paper waste extract agar

Table 1: Growth of bacteria on the culture media

Bacteria Culture media	S.aureus	Strep. spp	P.aeruginosa	E.coli
NA	249.50 a	184.00 a	186.33 a	203.17 a
	±	±	±	±
	36.15	40.59	48.74	50.91
C	224.17 a	224.00 a	262.33 a	254.50 a
	±	±	±	±
	31.16	41.47	33.06	35.59
E	265.50 a	225.50 a	276.17 a	290.00 a
	±	±	±	±
	33.25	48.03 a	18.55	18.05
LSD P ≤ 0.05	101.208	131.068146	107.4382	112.5516

NA= Nutrient agar; C,= yeast extract paper waste extract agar ; E= GYN paper waste extract agar.

The letters indicate to comparison between means in each column, and the similar letters are not significant different at (P≤0.05),using (LSD test).

Table 2: Growth of bacteria on the culture media

Bacteria Culture media	S.aureus	Strep. spp	P.aeruginosa	E.coli
BHI A	211.83 a	178.33 a	228.00 a	180.67 a
	±	±	±	±
	56.58	31.92	44.51	56.27
C	224.17 a	224.00 a	262.33 a	254.50 a
	±	±	±	±
	31.16	41.47	33.06	35.59
E	265.50 a	225.50 a	276.17 a	290.00 a
	±	±	±	±
	33.25	48.03	18.55	18.05
LSD P ≤ 0.05	126.408668	123.596707	101.7321	120.0244

BHIA= Brain-heart infusion agar; C= yeast extract paper waste extract agar ; E= GYN paper waste extract agar. The letters indicate to comparison between means in each column, and the similar letters are not significant different at (P≤0.05),using (LSD test).

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Blood Lymphocytes Detection in Iraqi Diabetic Type 2 Patients Infected with Chronic Toxoplasmosis by Using Flow Cytometry

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Abstract

Toxoplasma gondii is an obligate parasite infects different species of mammals as intermediate hosts while infects number of family of cats as definitive hosts. It has three morphological types: tachyzoites, bradyzoites and sporozoites. It causes (H.I.) humoral immunity and (C.M.I) cell mediated immunity response which is measured one of the most characteristic immunological types of this parasite infestation. Diabetes mellitus (DM) is considered as a heterogeneous group of metabolic abnormalities with hyperglycemia due either to a reduction of insulin biological function or absolute insulin deficiency. Researchers discovered that long term infection with *T. gondii* may be dangerous agent for DM type 2, and there is no relation between toxoplasmosis and DM type 1. The aim of this study include detection of CD3 in Iraqi diabetic type 2 infected with chronic toxoplasmosis by using flow cytometry. This study included 100 blood samples were collected from Iraqi diabetic type 2 patients and 50 blood samples of healthy volunteers with age range (15-75) years and mean 49.9±12.8 from Al – Imam Ali general hospital during May 2018 until August in the same year. Diagnose of toxoplasmosis infection was done by using rapid diagnosis test/ cassette and by Toxo IgG antibodies immulite torch assay while diabetes diagnosis by fasting glucose tests in addition CD3 were detected by using flow cytometric method. Diabetic patients a group infected with toxoplasmosis have the highest level of blood blood sugar in comparison with other groups. Forty – five samples of diabetic type 2 infected with chronic toxoplasmosis by detection IgG antibody in highly level 106.2± 90.2 with highly significant differences in comparisons with other groups (diabetic patients 3.6± 1.2 and control group 4.0±0.6) while any results for IgM Abs didn't found in this study. Seventy – five samples studied in flow cytometry method which include twenty – five samples for each group. Diabetic patients with toxoplasmosis recorded highest CD3 its mean 30.7± 14.51 pg /ml flowed by diabetic patients group has 24.84±9.47 pg /ml and control group has 20.03± 9.42 pg /ml with significant differences.

Keywords: Chronic toxoplasmosis, Diabetes mellitus (type 2), Flow cytometry, CD3

Introduction

Toxoplasma gondii is an intracellular parasite that infects a spacious numerous of avian and several of mammals as intermediate hosts while infects number of family of cats as definitive hosts [(1)]. It has three morphological types: tachyzoites, bradyzoites (in tissues), and sporozoites. The infection with this parasite induce humoral and cell mediated immunity responses which is measured as one of the most distinctive immunological types of *T. gondii* infection, CMI is produced by the parasite, appearing in host protection against rapid growth of tachyzoite and resulting pathologic variations [(2)]. Transmission of *T. gondii* starts by ingestion of infected food or contaminated water containing cysts of the parasite; it can be transmissible through blood transfusion and organ transplant [(3)]. After infection, *T. gondii* moves to many organs of the host such as brain, lungs, heart, pancreas and most frequently in the lymph nodes [(4)]. The manifestation of toxoplasmosis in pancreas has been described in animals and humans. In man, the infection may be a reason to cause of pancreatitis [(5)].

Diabetes mellitus (DM) is signified as a heterogeneous group of metabolic abnormalities with hyperglycemia due either to a reduction of insulin biological function or absolute insulin deficiency. DM patients with long time are likely to develop multiple complications, such as neuropathy, atherosclerosis retinopathy and renopathy [6]. The National Diabetes Data Group of American Diabetes Association categorized DM into 2 major types according to the etiology in 1997: type 1 diabetes mellitus (T1DM) and T2DM. T2DM patients maintain their insulin secretion capability and their hyperglycemia can be regulated by oral anti-diabetic drugs [7].

It is unknown how pancreas involvement during toxoplasmosis may conduct to DM, In seroprevalence of toxoplasmosis among many diseases diabetes mellitus, neoplasms, arthritis etc , 1.265 patients were checked serum IgG antibody ,6.7% was positive seroprevalence for toxoplasmosis, this revealed that toxoplasmosis has a strong association with chronic infection like diabetes mellitus and neoplasms [8]. A meta-analysis research of a relationship between long term infection with *T. gondii* and diabetes mellitus, researchers suggestion that there was a risk reason between two diseases and carry out researchers for studies in this subject as well as no relationship between toxoplasmosis type 1 diabetes mellitus and toxoplasmosis [9].

The aim of this study includes detection of CD3 in Iraqi diabetic type 2 patients infected with toxoplasmosis by using flow cytometry.

Materials and Methods

Study samples

One hundred blood samples were collected from Iraqi diabetic type 2 patients and 50 blood samples of healthy volunteers with age range (15-75) years and mean 49.9±12.8 from Al – Imam Ali general hospital during May 2018 until August of the same year. Five milliliters of venous blood were collected using 5 ml disposable syringe. Two ml of blood sample was transferred to gel tube and left to get coagulation at (20-25°C) for 15 minutes then it centrifuged for 10 minutes at 2500-3000 rpm in order to separation serum that used for fasting blood sugar diagnosis (Glucose MR, Linear, Spain) then for *T. gondii* diagnosis by rapid diagnostic test/cassette (Tox IgM and/or IgG rapid test-casette, Paramedical, Italy) then by immulite torch assay (Flex reagent cartridge IgG, Siemens, Germany) and three ml was transferred immediately to EDTA-tube and left on blood role mixer for flow cytometry study (Flow cytometry, Partec, Germany). Fifty samples of diabetic patients with and without *Toxoplasma* infection and 25 samples of healthy volunteers were studied in flow cytometry study.

Flow cytometry

Seventy five samples were selected for flow cytometry by detection CD3 (Cy5PE-conjugated anti-CD3, Becton Dickinson and company BD Biosciences, USA) were performed the following steps:

1. Add the appropriate volume of CD3 flurochrome - conjugated monoclonal antibody to 100 µl of whole blood in a 12 x 75-mm capped polystyrene test tube, then vortex with caution and incubated for 15-30 minutes in the dark place at (20 - 25ºC).

2. Add 2 ml of brand flow cytometer lysing solution (BD FACS), then vortex the tube gently and incubate for 10 minutes in the dark at room temperature (20ºC - 25ºC).

3. Centrifuge the tubes at 300g for 5 minutes.

4. Aspirate the supernatant, and then added 2 ml of stain buffer solution consisting of PBS plus 0.2% bovine serum albumin BSA with 0.1% sodium azide.

5. Add 0.5 mL of stabilizing fixative, then vortex the tube gently and incubate the tube at (2 - 8ºC) in the dark room for 20-30 minutes.

6. Finally mix the tube thoroughly and then analyze the sample.

Statistical Analysis

Chi-square test and least significant differences (LSD) for significant differences were used to analyze statistically results of this study. P-value that used for statistical differences was ($P \leq 0.05$).

Results and Discussion

The existing hypothesis suggests that the incidence of toxoplasmosis rise the ability to infection with DM, and, in contrast, DM patients have more ability to

infection with are more able to infect with *T. gondii* [10].

Forty-seven samples of diabetic type 2 patients infected with toxoplasmosis as shown in table (1), all studied groups with their results in significant differences.

Table (1): Prevalence of toxoplasmosis depending on rapid diagnostic test/cassette in studied groups.

Diagnosis	Response	Diabetic Patients		Control		P-Value Sig.(*)
		No.	%	No.	%	
Tox IgG rapid diagnostic test/cassette	+ ve	47	43.8	0	0.00	0.0001**
	-ve	53	56.2	50	100	
Total		100		50		

Rapid diagnostic test (cassette) is a useful , lateral flow chromatographic immunotest for the simultaneous detection and differentiation of immunoglobulin-G and immunoglobulin-M Abs in sera and plasma specimens of human. This results agree with the result of Achonduh-Atijegbe *et al.,* [11] that studied the prevalence of malaria, typhoid, toxoplasmosis and rubella among febrile in 315 children with mean age 5.8 ± 3.8 years in Cameroon which found 10 (3.2%) children have recent infection with toxoplasmosis while 121 (38.3%) children have past infection. While table (2) showed that 45 samples of diabetic patients infected with chronic toxoplasmosis with high significant differences when comparison with other studied groups.

Table (2): Prevalence of toxoplasmosis based on Toxo IgG in different groups of the study.

Diagnosis	Toxoplasmosis IgG	Diabetic Patients		Control		P-Value Sig.(*)
		No.	%	No.	%	
Flex reagent cartridge IgG	+ ve	45	66.6	0	0.00	0.0001**
	-ve	55	33.4	50	100	
Total		100		50		

Also table (3) show comparisons of IgG levels in studied groups estimated by IU/ml. It was found that DM patients with toxoplasmosis has highest level of IgG 106.2 ± 13.1 followed by diabetic patients group has 3.6 ± 0.08 and healthy volunteers group has less level 4.0 ± 0.13 with highly significant differences $P \leq 0.0001$, any results for IgM Abs didn't found in this study.

Table (3): Different levels of IgG (IU/ml) in all studied groups .

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
DM patients infected with toxoplasmosis	45	106.2	90.2	13.1	14.2	255
Diabetic patients	55	3.6	1.2	0.08	2.4	5.1
Control	50	4.0	0.6	0.13	3.0	6.5
LSD-Value	22.38 **					
P-Value	0.0001					

In present time, torch assay 2000 immulite, an automated quantitative IgG and IgM for toxoplasmosis , It is revealed, that calculates Toxo IgG and IgM in International Units per milliliter (IU/ml) of serum. It has many features like easy, moderately inexpensive and fast requiring 60–90 min. for completion (12).

This results agree with results of El-Awady *et al.* [12] carried out a study on 110 pregnant women infected with for toxoplasmosis infected with diabetes as well as 110 healthy control (pregnant women) ,they get (42.7%) of diabetic pregnant women (47) were positive for IgG Abs and (21.81%) of healthy pregnant women (24) were positive for the same Abs . These answers clarified the pervasiveness percentage of IgG Abs has directly correlated with the diabetes duration due to the weakened immune system of DM patients as well as this study suggested that toxo-patients are more suggestible to be diabetics than those without it . Destruction of the pancreas happens in three steps of *Toxoplasma* infection:

1. Hyperactive stage (hyper-period) in which β-cell demolition of nerve cells and less intervention in the insect in a hyperactive state of the pancreas, occasionally the secretion of insulin is extreme, often resulting in low or a too low blood glucose, this stage is frequently happens during adolescence.

2. The disordered phase (compensatory stage), pancreatic β-cells and neurons have a great quantity of damage, under normal circumstances, the secretion of insulin will be deficient, the body will begin the compensative function. So, when few in the disordered state, this stage of insulin secretion eventually.

3. The stage of decline (depression), in which β-cells and nerve cells obliteration of more compensatory function achieve is confined [13].

Table (4) showed that a group of DM and toxoplasmosis has the highest level of blood sugar in significant differences when comparison with different studied groups.

Table (4): FBS levels in all studied groups with comparisons.

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	45	155.2	52.3	7.1	110	365
Diabetic patients	55	150.2	50.4	6.4	115	300
Control	50	111.4	10.7	2.1	85	95
LSD-Value	24.6 **					
P-Value	0.0001					

Many mechanisms clarify the relationship between diabetes and toxoplasmosis such as:

1. Infected WBCs integrate improved migratory characteristic, cause of easy spread of *Toxoplasma* parasite to body organs, including pancreas [14].

2. Establishment of nitric oxide (NO) and reactive oxygen species (ROS) are motivated via DM, and these factors , as motivating of intracellular pathogens, that can reactivate latent, parasites cysts, over starting acute infection.

3. Given the neutrophils failure to correctly achieve phagocytosis and intracellular killing in advanced phase of DM, there may be expand in responsiveness to intracellular pathogens such as *Toxoplasma* and *Candida* [15].

Flow cytometry study included 25 samples for each previous groups which showed in table (5) a diabetic with toxoplasmosis patients group has the highest CD3 mean 30.7 ± 14.51 pg/ml with value ranging from 13.1 to 42.3 while a group of diabetic patients only has 24.84 ± 9.47 pg/ml ranging from 6.2 to 28.46 in comparison healthy control group 20.03 ± 9.42 pg/ml ranging from 2.9 to 33.3 with significant differences showed in these results ($P \leq 0.05$).

Table (5): Statistical description of CD3 in blood of studied groups.

Groups	No.	Mean IU/ml	Std. Dev.	Std. Error	Lower Value	Upper Value
Diabetic patients infected with toxoplasmosis	25	30.7	14.51	4.5	13.1	42.3
Diabetic patients	25	24.84	9.47	3.82	6.2	28.46
Control	25	20.03	9.42	3.95	2.9	33.3
LSD-Value	8.642 **					
P-Value	0.05					

CD3 (cluster of differentiation 3) was recognized by Kung and colleagues in 1979 and was one of the first groups of human T lymphocyte surface antigens recognized using monoclonal antibodies, CD3 T cell co-receptor helps to stimulate both the cytotoxic T cell (CD8+ naive T cells) and also T helper cells (CD4+ naive T cells), it comprises of a protein complex and is combined of four distinct chains. In mammals, the complex includes a CD3γ chain, a CD3δ chain, and two CD3ε chains, these chains relate with the T-cell receptor (TCR) and the ζ-chain (zeta-chain) to cause stimulation signal in T lymphocytes, the TCR, ζ-chain, and CD3 molecules together create the TCR complex [16]. It was successively shown that antibodies against CD3, contingent on the circumstances used, either activate T cells to divide or reduce the development of effector functions like cytotoxicity, so it was obvious early on that CD3 had an important part in T cell function, it is originally articulated in the cytoplasm of pro-thymocytes, the stem cells from which T-cells elavated in the thymus, This great specificity, joined with the attendance of CD3 at all stages of T-cell development, makes it a useful immunohistochemical marker for T-cells in tissue portions, Ag stays existing in almost all T-cell lymphomas and leukaemias, and so it can be used to differentiate them from superficially like B-cell and myeloid neoplasms, figure (1). The conclusion of this study , the infection with *T.gondii* induced the immunity responses (cellular and humoral) , also toxoplasmosis elicit response of T cells in acute response CD4+ cells as well as in chronic infection CD8+.

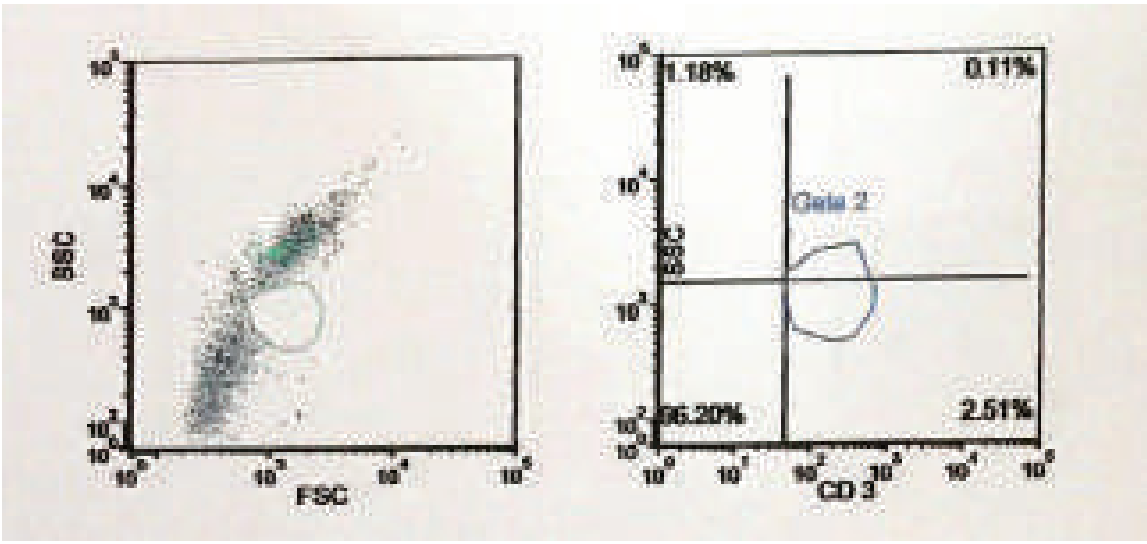


Fig. (1):- Flow cytometric curve show CD3 in diabetic patient type 2 not infected with toxoplasmosis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Immunohistochemical Assessment of Tumor Infiltrating T Cells and Serological Evaluation of Apoptotic Markers in Prostate Cancer Patients

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Abstract

PCa is abnormal transformed growth in glandular prostate tissue of male reproductive system. Occasionally, it is slow growth, but some progressed rapidly. This corss-sectional study aims to evaluate the differences in counts of tumor infiltrating immune cells (CD3 & CD8), and serum soluble Fas and Fas ligand concentrations between malignant and benign PCa. Total of (17) patients with PCa were included, their ages ranged from (38-88) years. The patients visited urological surgery unit in Hilla Teaching Hospital during the period (November 2018 to October 2019). The samples comprise biopsy for estimation of tumor infiltrating CD3 & CD8 by IHC technique, and 3 mL of blood for sFas and sFasL evaluation by ELISA technique for each patient. The results showed the differences of CD3+ & CD8+ PILs counts between BPH and malignant PCa are non-significant (P value > 0.05), sFas concentrations of malignant PCa are significantly higher (P value < 0.05) than those of BPH, whereas no significant difference (P value > 0.05) in sFasL concentrations. It was concluded that prostate tumor type affects serum levels of sFas, but both CD3+ & CD8+ PILs rates and sFasL levels are not affected.

Keywords: *PCa, Malignant, BPH, TILs, sFas, sFasL; toxicity*

Introduction

Prostate cancer (PCa) is the abnormal growth of the prostate gland tissue, in the reproductive system of male. Most prostate tumors are slow growing; but, some develop rapidly [1]. Furthermore, Patients with metastatic prostate carcinomas have high incidence of bone lesions, leading to persistent prostate cancer-induced bone pain (PCIBP) [2]. Environmental factors account the highest percent of cancer such as tobacco use, obesity and nutrition, infections, ionizing and non-ionizing radiation, lack of physical activity, and pollution [3].

In Europe, in 2012 it was the third most diagnosed cancer after breast and colorectal at 417,000 cases [4]. The incidence and prevalence of prostate cancer vary in different parts of the world, with the highest in Australia, New Zealand, Northern & Western Europe, and North America and the lowest in South Asia [5].

Prostatic cancer is rarely symptomatic early in its course and therefore disease presentation often implies local extension or even metastatic disease [6]. It is very important to detect and diagnose prostate cancer in its earliest stages, often prior to the presentation of symptoms [6].

Infiltrating immune cells are of particular interest since there is evidence that inflammation plays a role in malignant transformation of multiple organs, including the prostate [7]. During prostate carcinogenesis, both innate immune cells such as macrophages, and adaptive immune cells (T and B cells) were reported to accumulate in the prostate [8]. However, their contribution to

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prostate cancer development is contradictive, since both promotion and inhibition of carcinogenesis has been described [9]. The study aimed to evaluate the differences in counts of tumor infiltrating immune cells (CD3 & CD8), and serum soluble Fas and Fas ligand concentrations between malignant and benign PCa.

Material & Methods

In this cross-sectional study, total of (17) patients with prostate cancers (PCa) were included. Their ages ranged from (38-88) years. Case information for each patient included: name, age, and the diagnosed tumor type were taken from the report of the diagnosis. The patients visited urological surgery unit in Hilla Teaching Hospital during the period (November 2018 to October 2019).

Included & Excluded Criteria:

The included criteria comprised any patient who had recent histologically diagnosed with prostate tumors. Excluded criteria are comprised; any case that had normal histological results after suspicion of prostate tumor, any patient who took chemotherapy, and any case of retrospective prostate tumor.

Ethical Approval:

Agreement of all patients or their sons and/or first degree relative (father & mother) has been taken prior take any specimen. Moreover, the study design was approved by Research Ethical Committee in College of Medicine / Babylon University.

Samples:

Prostate biopsies were obtained from the urological surgery unit after patient admission. The biopsy was saved in plastic tube container with 10% neutral buffered formalin (NBF) before processing through paraffin embedding technique to create a formalin-fixed, paraffin-embedded (FFPE) blocks for immunohistochemical tests (CD3 & CD8). Also, three milliliter of blood for each patient was collected through vein puncture, put in sterile Gel tube. The collected blood samples were left in room temperature for 15-30 minutes to clot before they were centrifuged at 1000×g for15 min to remove the clot, and then the upper layer (serum) was separated by pipette, then put it in clean Eppendorf tube and stored at -20C° until using in serological tests (sFas & sFasL)**.**

Methods

Sample Processing for the IHC Staining Technique:

· By microtome which its blades are standardized at 4 μm, each block of FFPE tissue was sectioned into 4 μm thickness section (one section for each IHC marker), then the sectioned tissue was put in water bath (40-50) °C for seconds that allow the tissue to be relaxed, and allow the paraffin to stick to the glass. Then sections were mounted on positive charge slide.

· After that, mounted slides were incubated in oven (58-60) °C for at least two hours.

· The third step is deparaffinization; three containers filled with Xylene were prepared, then mounted slides were immersed on each container for 2 minutes, sequentially.

· The fourth step is hydration; three jars were prepared with ethanol (the first one with 30% ethanol, the second with 70% ethanol, and the third with 100% ethanol), then deparaffinized slides were immersed on each jar for 2 minutes started with the first one of 30% ethanol and continued upward.

· In the last step : the hydrated slides were subjected in Retrieval container with Immuno DNA Retriever Citrate, and with each other were put in water path (95-99)°C for 1 hour.

· Then staining by immunohistochemical technique was applied (Bio SB- USA).

Evaluation of CD3 and CD8:

Presence of brown colored reaction in the nucleus or cytoplasm was considered a positive reaction. The intensity of the immunostaining was determined by modified way through taking a pictures for three fields of each section through digital camera connected to conventional light microscope (20X power), and further image analysis was done with the Image J software (version 1.46r, National Institutes of Health, USA) for each picture to count (CD3 or CD8) cells, then the mean value was calculated that represent the cells number per field for each section [10, 11].

ELISA Technique:

The principle of sandwich ELISA technique has been applied for the assessment of serum soluble (Fas/CD95 & FasL/TNFSF6) according to instructions of company (Elabscience-USA).

Statistical Analysis

By using Software of Statistical Package for the Social Sciences (SPSS), version 26.0, to investigate the differences of study parameters between malignant and BPH. The differences between two groups were analyzed by Independent t-test. Statistically, it is considered a significant difference when P value <0.05.

Results

There are 17 subjects were diagnosed as prostate cancers (PCa). These cancers are subdivided into; 10 (59%) malignant prostate cancer (adenocarcinoma), and 7 (41%) benign prostatic hyperplasia (BPH).

Immune cells (CD3 & CD8):

As shown in Table (1), the findings showed that CD3+ prostate infiltrating lymphocytes (PILs) counts in BPH (143.8 cells/field ±41) are slightly lower than that of malignant (174 cells/field ±97), and statistically there is no significant (P value > 0.05) difference between them. Also, CD8+ PILs counts in malignant prostate cancer (88.4 cells/field ±35.8) do not significantly different (P value > 0.05) from that in BPH (83.9 cells/field ±38.6).

Table (1): Comparison of tumor infiltrating CD3+ and CD8+ T cells counts between Benign and Malignant prostate cancers.

Parameters	Benign	Malignant	P value
CD3 cells/field	143.8±41	174±97	0.395
CD8 cells/field	88.4±35.8	83.9±38.6	0.808

* represents a significant difference at p<0.05. Data are expressed as Mean±SD.

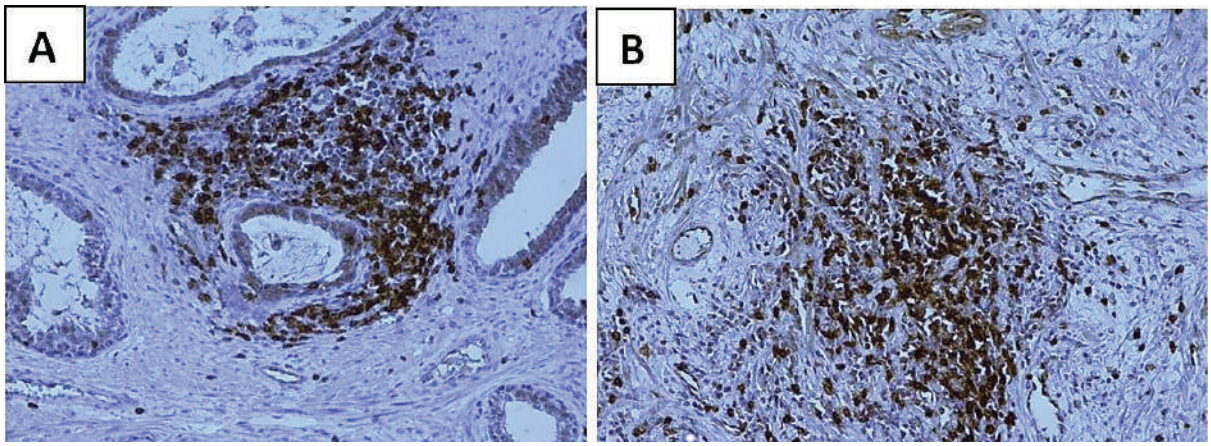


Figure (1): PCa slide with CD3 marker (20X Magnification Power): (A) CD3+ TILs in malignant PCa (B) CD3+ TILs in BPH.

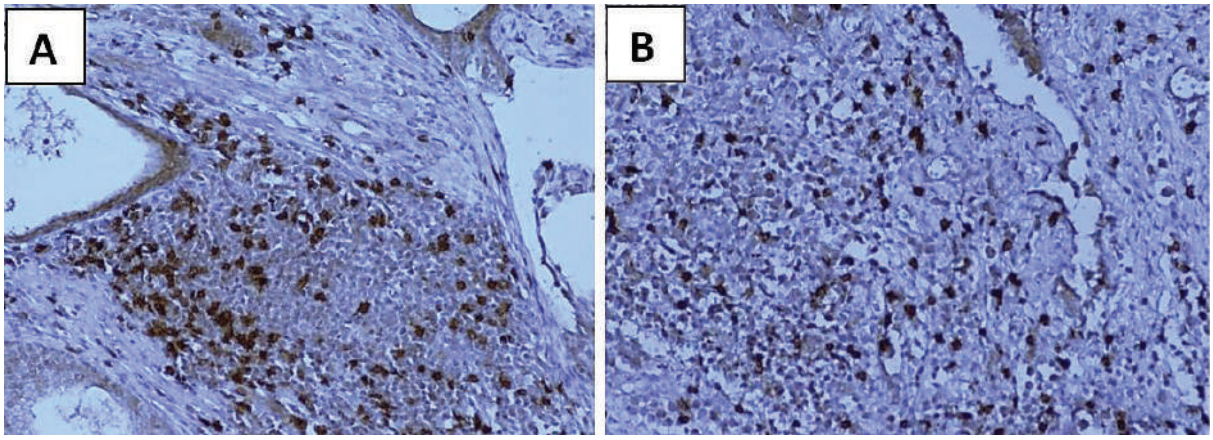


Figure (2): PCa slide with CD8 marker (20X Magnification Power): (A) CD8+ TILs in malignant PCa (B) CD8+ TILs in BPH.

Serological Markers (sFas & sFasL):

Regarding to serum levels of sFas & sFasL, as shown in Table (2) the results showed the mean of sFas serum levels in patients with prostate carcinoma (3428 pg/mL ±165) is significantly higher (P value < 0.05) than those of BPH (3097 pg/mL ±270.6), whereas serum levels of sFasL had no significant difference (P value > 0.05) between BPH (409.8 pg/mL ±262) and malignant prostate adenocarcinoma (487 pg/mL ±200.9)

Table (2): Comparison of serum soluble Fas and FasL concentrations between Benign and Malignant prostate cancers.

Parameters	Benign	Malignant	P value
Fas pg/mL	3097±270.6	3428±165	0.018*
FasL pg/mL	409.8±262	487±200.9	0.523

* represents a significant difference at p<0.05. Data are expressed as Mean±SD.

Discussion

Interesting in the field of immunotherapy depending on T lymphocytes gives a hint to its importance as antitumor and protective activity against PCa cells [12]. The present study findings indicated there is no significant difference in CD3+ infiltrating cell counts between malignant and BPH. This indication consistent with Burdova et al., who mentioned that CD3+ PILs are increased in both benign and malignant PCa with no considerable difference in their counts [13]. In contrary, there is evidence that quantity of inflammatory cells comprising infiltrated T cells (CD3+ & CD8+) and B cells and macrophages are higher in prostatic benign tumors than malignant [14]. Constâncio et al., [15] and Kaur et al., [16] reported that increased CD3+ T cells and expression of androgen receptor (AR) in tumor microenvironment of prostatic malignant transformed growth are significantly associated with the poor prognosis in persons with PCa. Regarding to BPH microenvironment, there is a study showed T reg. cells quantity of infiltrated T cells were increased, and suggesting its immunosuppressive activity may leads to defeat tumor immune response [17].

Similarly, regarding to cytotoxic T cells, the current study findings suggests that tumor infiltrating CD8+ T lymphocytes are increased in both benign and malignant PCa as the same rates in agreement with Fong et al., [18] who mentioned that CD8+ PILs are increased in BPH and malignant prostate tumors. Despite its increasing

in tumor microenvironments, there are myeloid derived suppressor cells (MDSC) and T reg. cells affect the protective role of CD8+ T cells against tumor, Muthuswamy et al., [19] reported that PCa cells express high levels of MDSC and T reg attractant chemokines (CXCL-12, CCL-22) and low levels of (CCl-5 and CXCL-10) which considered as CTLs chemoattractants, and suggested celecoxib drug as CD8+ stimulator by downregulating CXCL-12 and CCL-22 and upregulating CXCL-10. Moreover, there are two reports indicated that using TGF-β resistant CTLs that produce through CAR T cells technique has specific role in killing prostatic tumor cells with prostate specific membrane antigen (PSMA) [20, 21].

In concerning to serum levels of sFas, our study results suggest the sFas levels had affected by the prostate tumor type weather benign or malignant, in agreement with Furuya et al., [22] who reported that patients with BPH had a significantly lower levels of soluble Fas than those with prostate carcinomas. Likewise, there is a study explained that increasing in concentrations mean of sFas with matrix metalloproteinase 7 (MMP 7) in prostate carcinoma had a significant role in its metastasis, by suggesting the activity of MMP 7 in metastasis process depends on concentrations of sFas [23]. There is an indication that high sFas concentrations interfere with the apoptosis to malignant transformed cells and enhance its escape from the immune surveillance [24]. Regardless to any systemic inflammatory conditions, sFas could be used for checking the response of patients to the chemotherapy or surgical tumor resection [25]. Moreover, Szarvas et al., [23] reported that patients with metastasized PCa (malignant) had no significantly difference in sFasL concentrations from those with no metastasized PCa, this may suggests that the malignant change may not affects the expression levels of sFasL, it possibly consistent with our findings that suggest there is no significant difference in serum levels of sFasL between BPH and malignant prostate adenocarcinoma. Also, it may give an indication that sFasL is constitutively released by both malignant and benign prostate tumor cells [26].

Conclusions

It was concluded there is no difference in rates of tumor infiltrating T cells and cytotoxic T cells between malignant and BPH prostate tumors. Moreover, serum levels of sFas concentrations were affected by whether malignant or benign prostate tumors, whereas sFasL concentrations were not.

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Assessment of Anti-Diabetic Activity of Vinca Rosea Extract on Induced Diabetic Mice

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Abstract

Background and Objective: Vinca rosea is a medicinal plant that has been investigated and applied as an alternative treatment for disorders. This study aimed to investigate the anti-diabetic activity of ethanolic extract of Vinca rosea on induced diabetes in mice.

Methods: Forty male albino mice were randomly divided into four groups, including control that was administered a standard physiologic saline and served as control, diabetic group, Streptozotocin & Vinca rosea, and only Vinca rosea; each group contained ten mice.

Results: No significant lesions were observed in pathological sections of the control; however, the lesions in diabetic group showed massive lesions in the islets of Langerhans and reduced dimensions of islets due to the damage of beta-cells. Furthermore, the Pancreas in Streptozotocin & Vinca rosea demonstrated a moderated vacuolation and Langerhans of islet cells. In contrast, the histopathological sections of the pancreas, which treated by ethanolic extracts of Vinca rosea, illustrated increasing number of beta-cells hypercellularity. The values of blood glucose were increased in the diabetic group; however, the significant decreases ($P<0.05$) in blood glucose detected in treated by ethanolic extract as represented by increased Beta-cells. While, the blood glucose levels were tolerated in treated Streptozotocin & Vinca rosea; thus, these values were closely for the control results.

Conclusion: The Vinca rosea extracts induced a significant impact on diabetic mice, and they can improve and protect pancreatic cells in diabetic mice. Therefore, ethanolic extracts of Vinca rosea could be beneficial for the diabetes.

Keywords: *Anti-diabetic, Streptozotocin, Vinca rosea, diabetic mice.*

Introduction

Plant materials contain different characteristics such as chemical and biological functions, and these features could play important roles in the animal diet that include some of the plant parts, e.g. stems, leaves, flowers and seeds [1]. Plants also contain bioactive compounds, which are secondary metabolites. Those have a significant contribution to the physiological processes of the animal body. Furthermore, plant extracts are often applied as pharmaceuticals in the animal industry that have multiple conditions [2].

Vinca rosa (*Catharanthus roseus L.*) is one of the medicinal plants that have been investigated for decades. The investigations have concentrated on the isolation of bioactive compound from this plant, such as flavonoids and alkaloids, which is the most dominated in different parts of the plant [3]. Many kinds of these alkaloid compositions, including vinblastine and vincristine, have been discovered in this plant and they have been used as alternative remedy and treatment of diseases e.g.

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antimicrobial, anti-inflammatory and anticancer [4; 5; 6].

Several studies have focused recently on the alkaloid impacts that extracted from Vinca rosea plan, although the mechanism of their effects as therapeutical agents is still unclear [7]. Therefore, many researches have been done to this plant and its extract as the natural source of treatment [8].

One of the biggest endocrine disorders is diabetes that influences several people over the world). The patients with diabetes have been treated orally with different medicinal plants. However, researches are still looking for anti-diabetic alternative medicine that could extract from natural materials [9]. For decades, an aqueous extract of Vinca rosea is applied to control the diabetic disorders, as well as many countries are used for diabetes the whole plant or its parts, e.g. leaves or flowers, as household treatment [5]. Furthermore, Vinca rosea extracts were indicated to show a significant effect on blood glucose of lab animals [10].

Materials and Methods

Plant description and extraction:

Vinca rosea plant was obtained locally from Baghdad gardens; they have been shed and dried at room temperature. These plants were deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib. The analytical dose of 70% ethanol applied to extract the Vinca rosea plant, which was described by ([11]) plus more modification. Each 100g of these dried leaves were washed under tap water and ground using a coffee blender then adding 250 ml of 70% ethanol into thermal stirrer for 2.5 h at room temperature. Afterwards, the mixture were filtered and centrifuged to make it as clear solution, and around 20% of dried leaves of Vinca rosea in 50 ml were applied for this research. The LD50 dose of Vinca rosea extract was used at 1000mg extract for each a Kg.

Animals:

Forty male albino mice, weighing 28.0 ±7.0 g and ageing 10-12 weeks, were randomly divided into four equal groups. They were obtained from the Institute of Sera and Vaccines/ Baghdad. Mice were housed in plastic cages with water provided ad libitum and fed standardized commercial pellet. This experiment was carried in the Pathological Laboratory, Department of Veterinary Pathology, College of Veterinary Medicine, the University of Baghdad for the period extended from 1st July 2019 to the end of December 2019.

Experimental design:

The 1st group was administered normal physiologic saline and served as control. The 2nd group was injected intraperitoneally with a single dose 200-250 mg/kg of Streptozotocin® (STZ, Santa Cruz-USA) diluted in saline with mixing [12]. Dissolved STZ was kept at 4 ºC for 1-2 hours and then mixed again before injection. Diabetes is confirmed by sustained blood glucose levels above 350 mg/dl or more consecutive days by using glucose meter® (Gluco Lab-Korea) [13]. A similar dose of STZ was given to the 3rd group by the same administration route that followed by a single dose per week (a long period of experiment) of 1mg/kg BW of ethanol extract of Vinca rosea (I/P for 60 days). The 4th group was only administered of Vinca rosea extract with similar dose and same administration route to the 3rd group.

Histopathological analysis:

At the end of the experiment, the experimental animals were sacrificed using 'Euthanasia technique' that was done by cervical dislocation in rabbits, and the postmortem examination was achieved. The macroscopic appearance was recorded and any abnormal gross changes in internal organs were observed. Specimens were taken from all internal organs; moreover, the organ tissues have been kept in 10% formalin buffer phosphate immediately after removal. After 48 hours of fixation, the tissue slide processing was made routinely using a set of increasing alcohol concentrations. The tissue sections were embedded in paraffin blocks and sectioned by microtome at 5µm. All tissues were stained with hematoxylin and eosin stain; then, the histopathological changes were examined under a light microscope [14].

Determination of insulin levels:

The insulin ELISA KIT (Raybiotich, USA) was applied according to manufacturer recommendation (US-Biological, USA). The glucose measurement in the blood level has been done by biochemistry methods [15] with modification by using a commercially available kit

(Cypress diagnostic, Belgium).

Statistical Analysis

The values of blood glucose in experimental mice were expressed as (Mean ±SEM) and analyzed for ANOVA and using student's t-test by SAS software (V. 0.19). The variance values among these groups were considered significantly ($p<0.05$).

Findings

Pathological examination:

No significant histopathological lesions were observed in the control group; however, the histopathological lesions of pancreas gland of mice, which were treated with Streptozotocin, showed focal acinar necrosis and atrophy with vacuolar degeneration of Langerhans islet that occurred due to the damage of β-cells (Figure 1 and 2). Moreover, the Pancreas in 3rd group, which was treated with Streptozotocin & Vinca rosea extract, showed a moderated vacuolation in islet and moderated Langerhans islet, as well as a moderated regeneration of pancreatic islet cells (Figure 3). Further, the histopathological sections of the pancreas, which treated only with Vinca rosea extract, showed increases in the number of β-cells hypercellularity (Figure 4); while in the control group, the pancreatic islet of Langerhans seemed to be normal.

Glucose and insulin levels:

The values of blood glucose were dramatically increased at the group of diabetes that reached to (266.20 ±1.29) at day 60, while the treated group with Streptozotocin & Vinca rosea the values closely for the control group that recorded (at day 60) 91.20 ±0.37 and 94.20 ±0.58, respectively as compared with the control, so there was no significant differences. In contrast, the values of blood glucose in treated group with extract of Vinca rosea only was decreased significantly ($P<0.05$) compared to control at day 60 (81.93 ±0.75) (Table 1).

The levels of serum insulin were decreased in the group of diabetes that reached to 0.194 ±0.036 at day 60; while in the treated group with Streptozotocin & Vinca rosea, the values closely to the control (1.944 ±0.063 and 1.760 ±0.088 respectively), so there was no significant differences between these groups. However, the treated group of Vinca rosea extract only showed significant increases ($P<0.05$) in insulin level at day 60 that recorded to 0.194±0.018 as represented by increased inducing the β cells. This increase tolerated the effect of Streptozotocin & Vinca rosea group (Table 2).

Discussion

Histo-pathological lesions:

The present study indicated that Vinca rosea extracts exhibited significantly increasing in the number of β-cells hypercellularity. Aside from that, the group that treated with Streptozotocin & Vinca rosea showed moderated vacuolation and Langerhans of the islet; pancreatic islet cells were also moderated and regenerated. These findings indicated that Vinca rosea extracts could exhibit significant activities as anti-hyperglycemic in diabetic induced mice. These results were also observed the anti-diabetic activity of Vinca rosea extracts. On the other hand, the affected β cells in diabetic animals were reported in many studies [16] that suggested the regeneration of islet ß cells in induced diabetic lab animals, such as rats and guinea pigs, could be influenced Vinca rosea extracts. In the current study also illustrated that diabetic control mice had necrosis and atrophy in the Langerhans islet and vacuolar degeneration, which happened due to the damage of β cells; this could play a critical role by a protective impact when it was induced a diabetic effect in mice, which could have a 'hormetic effect' that indicates stimulation effect in a low dose, and inhibitory impact in high dose [17]. Therefore, these findings revealed, in general, that Vinca rosea extract was more effective on lesions and closed results showed to Streptozoitine & Vinca rosea treatment.

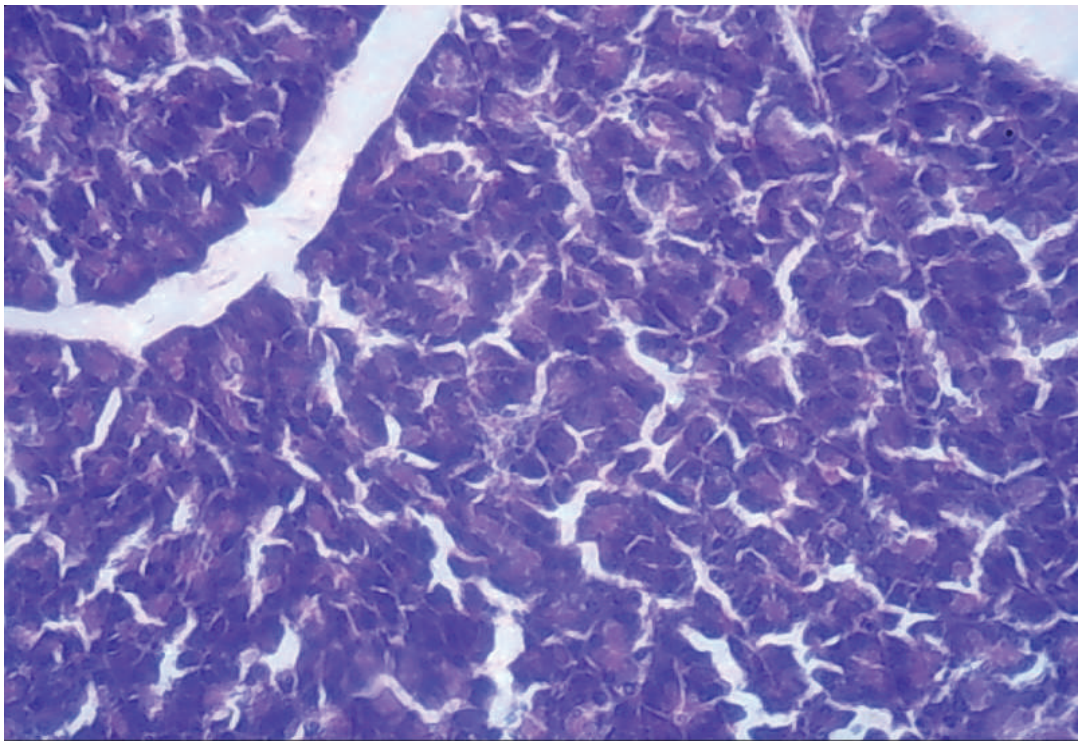


Figure 1 shows the severe vacuolation in the pancreatic islet of Langerhans with the disappearance of luminal acinar lobules (H&E X200).

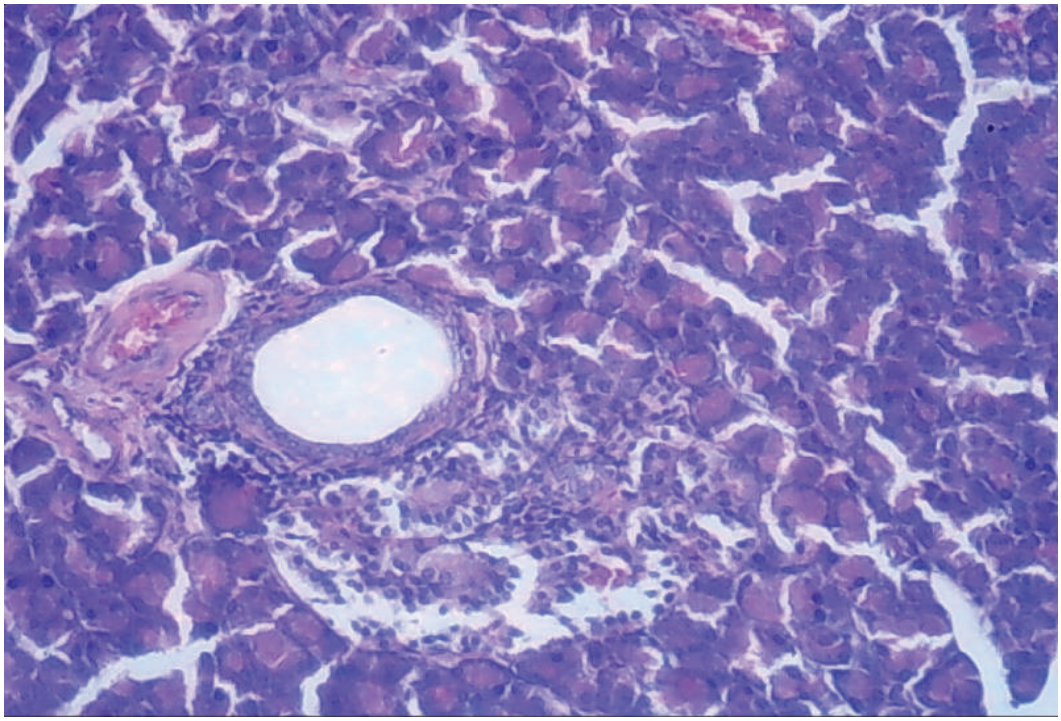


Figure 2 shows the necrosis of acini and atrophy with vacuolar degeneration of pancreatic islet of Langerhans with congested vascular capillaries (H&E X200).

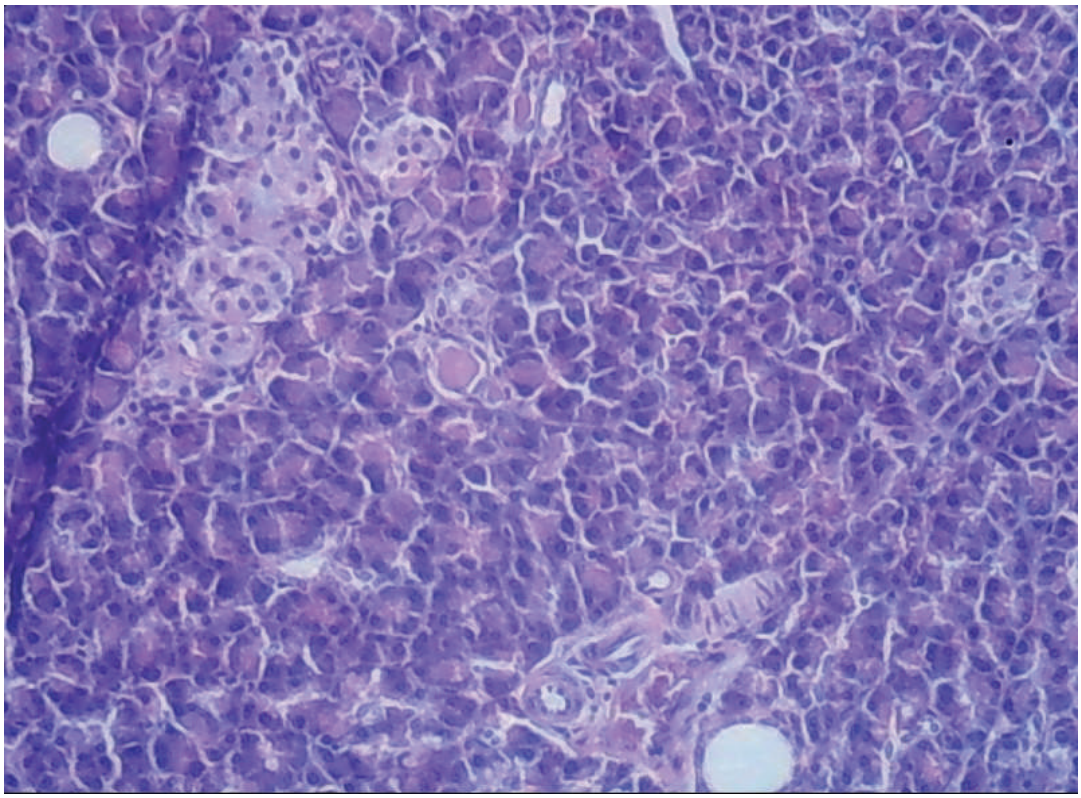


Figure 3 shows moderate vacuolation in the islet of Langerhans (H&E X200).

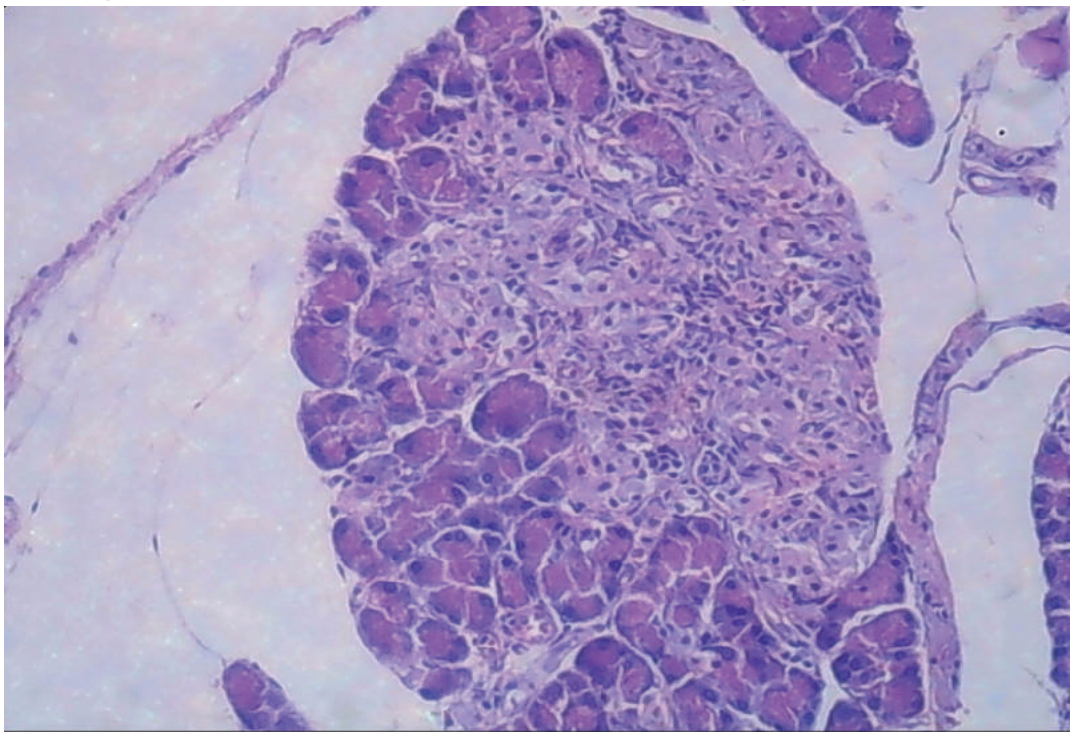


Figure 4 shows an increasing number of β cells resulting in hyperplasia of the pancreatic islet (H&E X200).

Glucose and insulin finding:

Although there are several drugs available to treat diabetes, these medicines have side effects on the pancreas [3]. Thus, there is a demand to search for an effective and safe alternative medicine [4]. This study tried to support β cells that could be possibility survived by using Vinca rosea extract, which could be exerted the insulin-releasing. Moreover, histopathological findings indicated the healing of pancreas using Vinca rosea treatment, which demonstrated the mechanism of anti-diabetic activity.

The glycogen was increasingly breakdown, as gluconeogenesis mechanism, which acts as a decreasing amount of insulin in diabetic animals that might be responsible for a reduction in cellular glucose as well. A study revealed that the vascular complications of the pancreas could lead to pathogenic changes in diabetic animal [18].

In contrast, the diabetic control mice reported increasing of blood glucose level. This group was statistically significant (P<0.05) compared to treated animals. This study recorded that the reduction in blood glucose level in Vinca rosea group compared to control. This finding could be due to the normal metabolism of glucose mechanism by plant extract.

Furthermore, the increasing of β cells of the pancreas could be detected in diabetic mice, which treated with Streptozotocin & Vinca rosea and diabetic group. The findings of this research revealed that daily treatment by Vinca rosea extracts for 60 days illustrated a significant decrease of the blood glucose level of induced diabetic mice; these results have matched with [19].

Table 1: Effect of Vinca rosea extracts on levels of blood glucose (mg/dL) in diabetic mice.

Group	Description	Day 0	Day 7	Day 30	Day 60
1st	Control	93.91 ±0.48 Aa	94.80 ±0.32 Aa	95.10 ±0.66 Aa	94.20 ±0.37 Aa
2nd	Diabetes	94.56 ±0.31 Aa	218.00 ±2.27 Bb	251.00 ±1.89Bc	266.20 ±1.29 Bd
3rd	Streptozotocin & Vinca rosea	94.83 ±0.64 Aa	96.20±1.28 Aa	93.65 ±0.84Aa	94.20 ±0.58 Aa
4th	Vinca rosea only	95.04 ±0.24 Aa	90.40 ±0.84 Cb	81.20 ±0.88 Cc	81.93 ±0.75 Cc

The data expressed as (mean ±SEM). The different small letters indicates to significant differences between column ($p<0.05$), and different capital letters indicates to significant differences between rows ($p<0.05$).

Table 2: Effect of Vinca rosea extracts on levels of serum insulin (mg/dL) in diabetic mice.

Group	Description	Day 0	Day 7	Day 30	Day 60
1st	Control	1.853 ±0.112 Aa	1.871 ±0.097 Aa	1.726 ±0.101 Aa	1.760 ±0.088 Aa
2nd	Diabetes	1.768 ±0.093 Aa	0.752 ±0.083 Bb	0.526 ±0.091 Bc	0.194 ±0.036 Bd
3rd	Streptozotocin & Vinca rosea	1.926 ±0.089 Aa	2.100 ±0.163 ACa	1.854 ±0.094 Aa	1.944 ±0.063 Aa
4th	Vinca rosea only	1.816 ±0.059 Aa	2.246 ±0.073 Cb	2.230 ±0.082 Cb	2.270 ±0.058 Cb

The data expressed as (mean ±SEM). The different small letters indicates to significant differences between column ($p<0.05$), and different capital letters indicates to significant differences between rows ($p<0.05$).

Conclusion

The present study revealed that Vinca rosea extracts significantly influenced on blood sugar levels by releasing more β cells of the pancreas that led to an increase of insulin levels in treated group competed with diabetic mice. In contrast, the Streptozotocin & Vinca rosea treatment showed similar values to the control group as there were no significant differences.

In contrast, the histopathological lessions of the pancreas, which treated by Vinca rosea only, showed increasing number of beta-cells hypercellularity. Therefore, Vinca rosea extract might help to prevent diabetes as using in traditional medicine. In the future, further study is needed to determine the action of the whole plant on other organs and detected the toxicity on diabetes.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq' The authors have ethical approval from College of Veterinary Medicine, the University of Baghdad. All methods that applied on the animals followed the instructions of the Animal Care & Use Committee with Approval No. 1928 in 27th May 2019.

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A study Some Physico-Chemical And Bacterial Properties of Wastewater for Ramadi Teaching Hospital and its Impact on the Euphrates River

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Abstract

Physiochemical and bacteriological characteristics of Euphrates river water were examined monthly to assess the possible impacts of wastewater discharged from Ramadi Teaching hospital for the period from August 2019 to January 2020. The obtained results showed that air and water temperature mean values varied from 12.0 ±11.3 to 40.0 ± 11.3 C ° and from9.1.0 ±3.12 to 33 ±9.5 C° respectively. Also, turbidity values were found to range from 14.0±8 to 144 ±9.6 NTU. while EC value was situated between 567.0±44.67 µS/cm and2899 .0±274 .3µS/cm. The current results have shown that total hardness mean values were very high and ranged between 252.0±27.5 and1114.0±96.3 mg/l while chlorides mean values ranging from92.0±62 to 588 ±104 mg/l, but nitrate mean values were found with the range of 1.2 ±1.86 and 50 ±4.5mg/l.The BOD mean values were found to range from1.0 ±1.2 to 34 ±6.5 mg/l, while DO mean values varying from 1.7 ±3 to 11.1 ±1.3 . This study has shown that the total bacterial count (222 to 1800000 cells/1 ml), Total Coliform (61 -170000 cells/100 ml), Fecal Coliform (0-58000 cell/100 ml), and Fecal Streptococci (18 -9200 cells/100 ml).

Key words : *Ramadi Hospital , BOD , Euphrates river; toxicity*

Introduction

Rivers are the most important natural resource for human development but they are being polluted by indiscriminate disposal of sewage, industrial waste and plethora of human activities, which affects its physicochemical and microbiological quality(1). Water pollution is widely characterized as any physical , chemical or biological change in water quality that adversely affects living organisms in the environment or renders a resource of water unsuitable for one or more of its beneficial uses. Occasionally, pollution may derive from natural processes such as weathering and soil erosion. (2).

Wastewater generated from hospitals usually contain pathogens, human tissues and fluids, pharmaceuticals, substances with genotoxic properties, chemical substances, heavy metals, and radio-active wastes, which may endanger public health, and contribute to oxygen demand and nutrient loading of the water bodies and in the process promote toxic algal blooms and leading to a destabilized aquatic ecosystem, if discharged without treatments into water bodies(3).

Wastewater in hospitals isisimilar in the texture to the iwastewater in the city public and different in containing very idifferent and diverse types of liquid waste with low quantities ithat they contain many infectious and dangerous compounds resulting from ipatient care, which makes processing it separately from wastewater an urgent necessity. Multiplev practices that happen in hospitals (surgery, drug, radiology, laundry, operation room, chemical andibiological laboratories, etc.) are a principal source of pollutant discharge intoithe environment (4).

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2. Material and Methods:

2.1. Study area:

The Ramadi Teaching Hospital is located in the city of Ramadi, center of Anbar Governorate, on the eastern bank of the Euphrates River. These hospital facilities discharge the wastewater (once daily in the morning and in the evening) directly into Euphrates River with simple treatment.

Three Stations were selected for water sampling where the first site was located around 100 meters before the Hospital to act as control. The second station was the discharge point of Hospital discharge and the third was about 100 meters away south the second site .

2.2. Samples Collection

The water samples were collected during the period from August 2019 to January 2020 from three stations**.** The water samples were collected from a depth of 30 cm beneath the river surface. They have been done once a month and the sample were placed in clean poly-ethylene bottles and stored in ice containers until reach to Laboratory.

2.3 Physico-Chemical and Microbiological analysis :

Total dissolved solid (TDS) and dissolved oxygen (DO) were determined at the time of sampling in the field using pH meter, WTW model, portable EC meter, WTW model and portable HANNA dissolved Oxygen meter and HI9142 model, respectively**.** Tur- Biological Oxygen demand (BOD**),** S_{O4} S_{O4}, $P_{o4}P_{o4}$,Cl-,$N_{O3}N_{O3}$, TH(total hardness)and Total dissolve solid determined according to APHA [5].Total count (TC) of bacteria was determined according to [6].

2.4 Statistical analysis.

All statistical analyzes were performed using SPSS (Statistical Package for Social Science) Version**.**

3.Results and discussion:

The current results found that the highest value for T.H. was 1414±96.3 mg/l in November at Station 2, while the lowest value was 252.0±27.56 mg/l in January at station 1. Higher values of total hardness in Euphrates river at station 3, due to the major increase occurred after receiving the hospital wastewater, If you compare it to the station1 that located before the hospital discharge point to the river. Based on the foregoing, the waters of the Euphrates are considered a very hard depending on the rating [16] Which is considered water very hard when the hardness is more than 200 mg/L .

The results were supported by those [17] on Shatt Al-Hilla , [18] on Euphrates river and [19] on Euphrates river in Heet city.

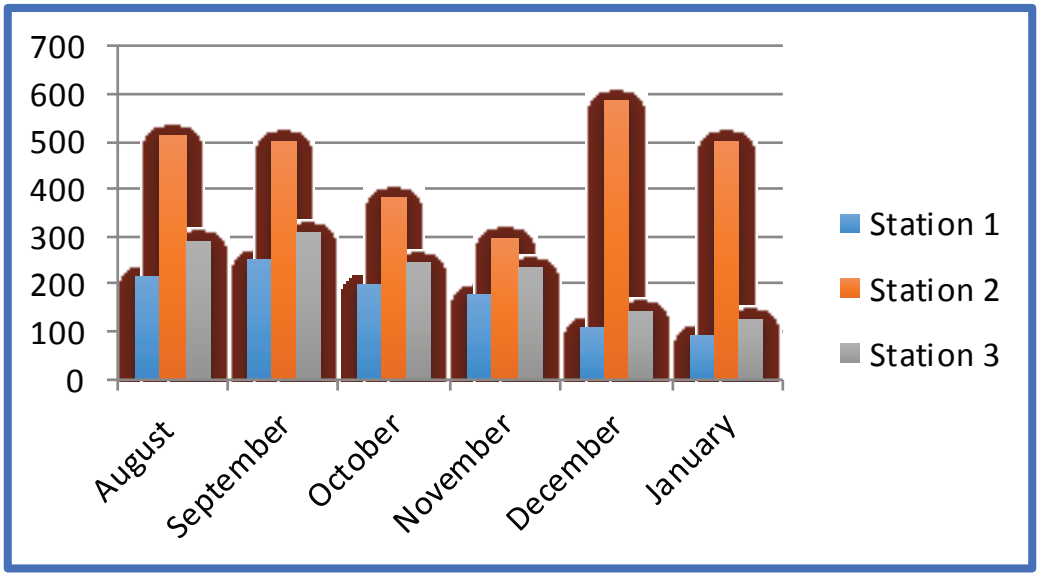


Figure (1). Monthly variation in chloride ion during study period

During the study recorded lowest value of Cl^- (92 ± 62.1mg/l) at station 1 in January 2020 and recorded the highest value (588 ± 104mg/l) at station 2 in December 2020. (figure 1).

The mean value of CI (314 mg /l) exceeds the permissible limit for both Iraqi standards river water 1967 No. (25(, which was 200 mg/l , and World Health Organization WHO [7] which was 250 mg/l. The increase of Cl- value indicates to pollution by sewage in the waters of Euphrates river due to the discharged sewage from the Hospital , where Chlorine is commonly added to reduce potential pathogens .Studies on hospital wastewater reported that Cl value might reach to higher concentrations, due to the disinfectants [20]. The results of this study were higher than that reported by [12]who registered a mean value 146 mg/L in Euphrates river and [21]in Chambal river India who registered a mean value 48 mg/L.

The present results showed that the highest value of DO was 11.1±1.3mg/l at station 1in January, while the lowest value was 1.7 ±3 mg/l at station 2 in December (Figure10). The mean value of DO (6.1mg/l) during study , World Health Organization (WHO) [7], and American standards for protecting river water mentioned that the optimal value of DO was more than 5 mg/l , based on the above results it can be said that the dissolved oxygen content is within the permissible limits. The mean value of DO (6.4 mg/l) during study.

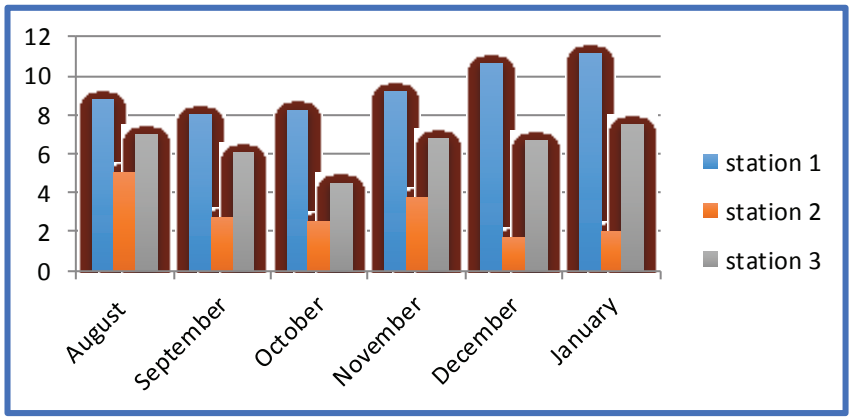


Figure (2). Monthly variation in DO during study period

The DO mean values of stations 3 was lower than of station 1 due to discharge wastewater for hospital, this variation in DO values for this station may be mainly attributed to the consumption of DO in the oxidation of organic matter from the medical waste discharged from hospitals to the river. The results of present study are in agreement with those of [22] who found that DO Average values (6.1 mg/l) in Shatt Al-Hilla River and [23] who recorded in his study DO average values was (6.1)mg /l in Euphrates river.As shown figure 2.

The study has shown that the lowest value 1± 1.2 mg/l) was recorded in station 1 during December while the highest value (34± 6.5 mg/l) was recorded in station 2 during October. The mean value of BOD for station 3 (5.9 mg/l) exceeds the permissible limit WHO standards(2008) which was less than 2 mg/l

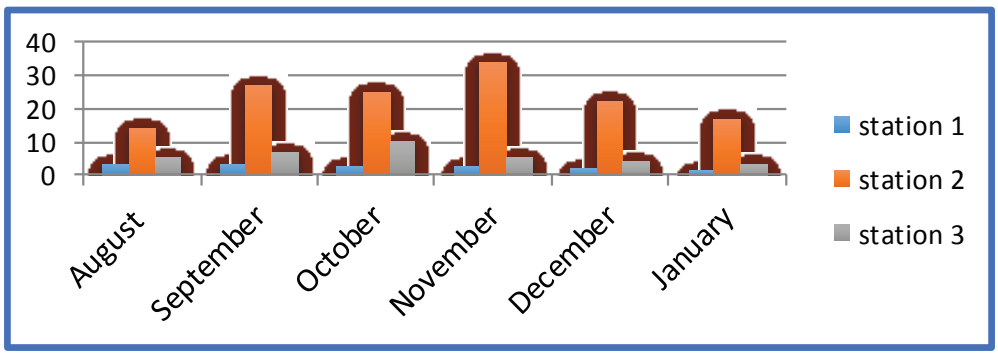


Figure (3). Seasonal variation in BOD in Tigris River during study period

This study's results were higher than those stated by[24]found the range for BOD5 was (2.8 -14.5 mg/L) on Euphrates revir at Al-Kaldia city and [25] found the range for BOD5 was (0.5 – 28 mg / l) in Greater Zab river.As shown figure (3).

In case of N_{O3}-, the highest value was 50 ± 4.5 mg/l in January at station2 , while the lowest value was 1.2 ±1.8 mg/l in January also at station

The mean values of NO3 (9.1 mg /l) for station 3 are within the permissible limit

for both Iraqi standards river water 1967 No.(25) and WHO standards drinking water (2004), which was 50 mg/L . High nitrate level in during January due to sewage waste water from hospitals ,the rainfall and fertilizer runoff as well as bacterial activity which convert nitrite to nitrate and the decomposition of organic compounds [26].

The results of this study were higher than those observed [27] that found N_{O3}values ranging from 0.5 -3.8 mg/l on Euphrates river at Ramadi city and [19] that found the N_{O3}values ranged between 3.1-47 mg/l in Euphrates river .

As shown (Table 1).The highest number of total bacterial count was recorded September 2019 at station 2 which was 18000000 CFU/1ml, while the lowest number of total bacterial count was recorded in January 2020 at station 1 which was 222 CFU/1ml. High number of TBC in the study was recorded during September and November months, that may be the consequence of the high level of suspended solids and nutrients in drainge water impacting aquatic microflora survival[28] .

The findings obtained in this study were higher than those from previous studies like [30] in Tigris river but less than [22] in Shatt Al Hilla river.

Table 1- Minimum and maximum (First Line), mean and standard deviation (Second Line), for Bacteriological characteristics at study stations

Stations / Parameters	Station 1	Station 2	Station 3
Total bacteria count cell/1ml	222-34000 12487±2508	1300-1800000 680278±68415	1300-98000 43066±15397
Total Coliform cell/100ml	61-5400 3543±2308	46000-170000 80666±30.8	920-35000 11133±3695
Faecal Coliform cell/100ml	0-6300 2600±2181	10000-58000 29000±16.3	40-11000 3506±1449
Faeal Streptococcus cell/100	18-2200 1216±811	1800-9200 4700±2476	0-4600 2485±2253

The term "coliform bacteria" refers to the bacterial species in the family Enterobacteriaceae genera such as *Escherichia, Klebsiella, Enterobacter and Citrobacter* that live in the intestines of warm-blooded vertebrates (mammals and birds) [31]. Table1) shows the results of TC in this study ranged between (61- 170000 CFU/100ml). The minimum value of TC was recorded January 2020 at station1, while the maximum value of TC was recorded during August 2019 at station 2 .

Fecal coliform in this study ranged between (0 to 58000 CFU/100ml), were the lowest value recorded at station 1 in January 2020 and the highest value was recorded at station 2 in the August (Table1). Easy of detection and survival in water longer than the pathogenic bacteria[33]. This study coincided with previous studies of [8] in Al Graff river and [34] in Coastal Malaysia .

The results revealed lowest value of F.S was recorded in January 2020 at station 1 which was 18 CFU/100ml, while the highest value of F.S was recorded in August 2019 at station 2 which was 9200 CFUl/100ml . As shown Table (1). The Fecal streptococcus is intestinal bacteria, FS have been used as indicators of fecal contamination in water because presence in the intestines of humans and animals, as well as its presence in the soil and on plants and some insects [35].

These results were higher than study of [36] in Tigris river and ,[32] on Euphrates river in Nasiriyah city.

A ratio between faecal coliforms and faecal streptococci were used to indicate the origin of bacterial pollution in the surface and ground water If the ratio is 4 or greater that indicate the human source of pollution, ratio between 0.1-0.6 indicate the domestic animal source, wher as when the ratio is less than 0.1 that indicate the wild animal source of pollution. The results of current study indicate there is a mixed origin of fecal pollution in Euphrates river ,because the domestic and agricultural wastes are discharged to the river[37]. According to WHO[38], the water Euphrates river contain a high level of bacterial pollution.

Conclusions:-

There was an increase in the physical parameters , especially at the station located after the discharge point of the hospital; The BOD5 values affected clearly by excreta untreated wastewater because they decrease in stations that located before discharge points wastewater hospitals

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Molecular Characterization of Selected Genera of the (Apiaceae) Family using SSR Molecular Markers

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Abstract

The conducted study which included the study of a few selected races from canopy plants from the chemical perspective which included *Apium graveolens L*, *Anethum graveolens L, Coriandrum sativum L, Petroselium crispum L, cyminum Cuminum L, Foeniculum vulgare L, Pimpinella anisum L, Daucus carrot L and Ammi majus L.* The DNA was detected and extracted and Conducted molecular tests on the DNA using (SSR Simple Sequence Repeats) using the polymerization device (PCR) and electrophoresis process on Agarose Gel.

Five selected and specific prefixes were used which approved its effectiveness in giving multiple shapes (Polymorphic) among the studied plants of 95%, as packs were obtained from a total number of 14 out of 53 different pack with an arithmetic average of 2.8 for each prefix. Cluster analysis and phylogenetic tree have shown that there is a genetic affinity between 0.84615-0.125 according to the two variables data (1-0) the genetic similarity using cluster analysis (UPGMA)has allowed to draw the portfolio diagram among the studied races, It can be said that the SSR Simple Sequence Repeats can be an effective technique for studying molecular characterization and can be used to separate the genetic relations in the plant world.

Key words: *canopy plants (Apiaceae), multiple shapes, genetic study.*

Introduction

Canopy plants (Apiaceae) is one of the largest plant groups which carries an economical importance which are used as spices like (cumin, fennel, coriander etc…) and vegetables like (celery, carrots, dill and parsley) and medical crop regardless of the taste and flavor that it adds to food, moreover its high oil content , antioxidants, minerals and vitamins that are important to our health, which makes it a potential treasure for neutral materials [(1)] and it contributes in peoples everyday life in an essential way like celery, parsley, cumin, anise, carrot and dill [(2)] and several more add to wild species.

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Uses of molecular markers in the evolution and past development of plant science, Initial evolutionary studies were completely dependent on geographical and morphological changes between organisms. Molecular markers are widely used nowadays [(3)] Molecular studies on phylogeny are largely based on chloroplast genome sequence data due to their simple and stable genetic nature making them ideal markers in evaluating plant strains [(4)] Assessment of genetic diversity. Recent developments in molecular markers and genome sequencing present a great opportunity to investigate genetic diversity in very large genetic material [(5)] Assessment of genetic diversity is very useful in studying plant evolution and comparative genomics, which helps to understand the structure of different populations [(6)] Genetic markers have been successfully applied in identifying genetic diversity and classifying genetic material [(7)].

Genetic mapping uses methods to locate a gene as well as to determine the distance between two genes.

Genetic mapping is the main area of research in which molecular markers are used today. The principle of genetic mapping is the chromosomal recombination during meiosis, which leads to the separation of genes. The markers located near the gene in question are known. On the same chromosome with linked markers [8].

Materials & Methods

Plant Material:

The vegetative leaves were taken from the plants at the age of 3-4 weeks from the growth phase in the afternoon period when the period of division of the cells is large, especially the healthy young leaves free from pathological, fungal and insect infections, then the leaves were taken to the stage of crushing with liquid nitrogen at a temperature (-169C) A ceramic mortar was used in which the leaves were placed, then liquid nitrogen was poured at a very low temperature in a certain amount, to crush the leaves with the hand in the ceramic mortar to break the walls of plant cells. Then the samples were preserved in freezing by marked boxes for molecular examination and DNA extraction tests(DNA)[9].

Deoxyribonucleic acid extraction (DNA):

DNA extraction was performed from plant samples belonging to the studied Apiaceae family using Protocol(plant)Genomic DNA Mini Kit, The procedures mentioned in the DNA extraction protocol were followed and According to the instructions attached to the company producing extraction, The DNA is isolated from leaves free of pathological infections, and its quality is determined and detected in the acarose gel at a concentration of 1% containing ethidium bromide 0.5 µg / ml, and the TBE x1 solution in the electrophoresis device under voltage (80-90 volts).

Simple Sequence Repeat(SSR Technique):

After isolating the DNA (DNA) from the papers of the genera of the studied paraglider family, the DNA is duplicated using (5) SSR primers Table (2) which shows the nucleotide sequence of the primers used in the study. The polymerase chain reaction (PCR) was performed so that the final size of the reaction is (20µl), the PCR reaction mixture was prepared using the (PerMIX) AccuPower® PCR kit equipped by the Korean company BIONEER, Table (1).

Table (1) reaction mix materials of (PCR)

No:	PCR master mix	(Volume)
1	DNA template	2 µl
2	Forward primer	1.5 µl
3	Revers primer	1.5 µl
4	PCR water(D .W)	15 µl
5	Total	20 µl

Table (2)Primers which used in the study[10].

No	Primer	Sequence	Product size bp
1	Apium F	5- GCT CTG GAA ACG TGA ACT GGA -3	261
	Apium R	5- TGG CCA ATG TCT TTC CGC AT -3	
2	Petrosi F	5- TTG TTT GGA CCC ACC ATT GC -3	289
	Petrosi R	5-CGA ATC ATC TCC CTG CAC CT -3	

***Cont*... Table (2)Primers which used in the study[10].**

3	Spia F	5- GTT CGA GTA CCA GGC G -3	230
	Spia R	5- CTT CCC TTG ACC TTC CG -3	
4	Cau F	5- CCA CAG CCA CAA CCA TTT C -3	234
	Cau R	5- CCA TCA ACC TCT AAC GCC -3	
5	Cor F	5- GTC GCT TCT TGA CTT CAG -3	252
	Cor R	5- CCA ACC TCA TAA CAC CTC AC -3	

PCR Thermo cycler conditions:

The reaction is performed in a PCR machine according to the following program:

1 - Denaturation: an initial mutant of the DNA strings at a temperature of (95 ° C) for a period of (3) minutes and a number of cycles of (35), then a second mutant at a temperature of 95 ° C for 45 seconds to separate the DNA strings from each other.

2. Annealing: According to the coalescence temperature of each user (58-47) for 45 seconds.

3. Extension: the initial extension at a temperature of 72 degrees C for a period of 1 minute and the final expansion at a temperature of 72 degrees C for a period of (3-5) minutes.

Results and Discussion

Polymorphism resulting from PCR-SSR technique:

When studying the genetic variation of the Apiaceae family genera, the technique of PCR-SSR was mainly relied upon by adopting five prefixes for all nine samples of the research paper used, and depending on those five prefixes, 14 packets or doubling distributed over all samples as they appeared during the electrophoresis process on gel-bands. For the nine samples approved in the thesis, 5 polymorphic prefixes were given, and the polymorphic percentage was 95%, Table (3). The number of packets for each initiator ranged between two packages as the least number of packages in the Petrosi initiator and (4) packages as the highest number with the initiator Spia with an average of 2.8 packages per initiator This study was consistent with what was indicated by [11].

The compatibility efficiency of the primers (primers) was almost similar to some extent. First, both the Petrosi and Cor initiator were less efficient, as their ratio appeared to be 14.28% for both, followed by the Apium and Cau efficiency of 21.42%, then the Spia initiator at 28.57% showed the highest efficiency of All the prefixes in Table (3), and the discriminative ability of each primer was as follows: 15.38% for each of the Petrosi and Cor initiators, while the Apium, Spia and Cau prefixes gave a capacity of 23.07%, which is the highest among the five types of prefixes in Table (3), and from It is clear that the initiator Spia gave the highest percentage of efficiency than the rest of the other prefixes used in this thesis and this study came in line with other results and research [12], regarding the study of the Apiaceae family indicated that high efficiency and differential ability of the prefixes to obtain genetic markers for all species [13], indicating that the distance between them is equal or that the distance between them and the genetic proximity is determined from the embryonic structure by calculating the number of domains obtained and apparent in the electrophoresis process, that the number of shared beams is what determines The genetic proximity or distance between the genotypes, the greater the number of bundles, the less the genetic dimension, and vice versa, because the common bundles indicate a similarity in the genetic material that may show similarities in the phenotypic characteristics related to productivity, reproduction, resistance, diseases, etc. A number of bundles with each other is due to the presence of differences in the sequence of nucleotides in the genome of the genotypes.

Here, the importance of using different primers targeting several regions of the plant genome appears to show the difference that is found between the genotypes, which represents similarities in the phenotypic or anatomical characteristics and similarities in the environment [14], Photo capt software was used to calculate the molecular size to detect the bundles resulting from the PCR reactions and compare them to the size of the DNA Ladder [15]. The SSR results that appeared in the gel were analyzed after converting the results. The characterization was based on numerical data by setting (1) when the beam was present and (0) when it was absent on the acarose gel. The genetic dimension factor between the samples was calculated using a factor of 72 s'Nie [16] according to the following equation:

$$\textbf{Genetic Distance}= 1-\left(\frac{2*N_{xy}}{N_y+N_x}\right)$$

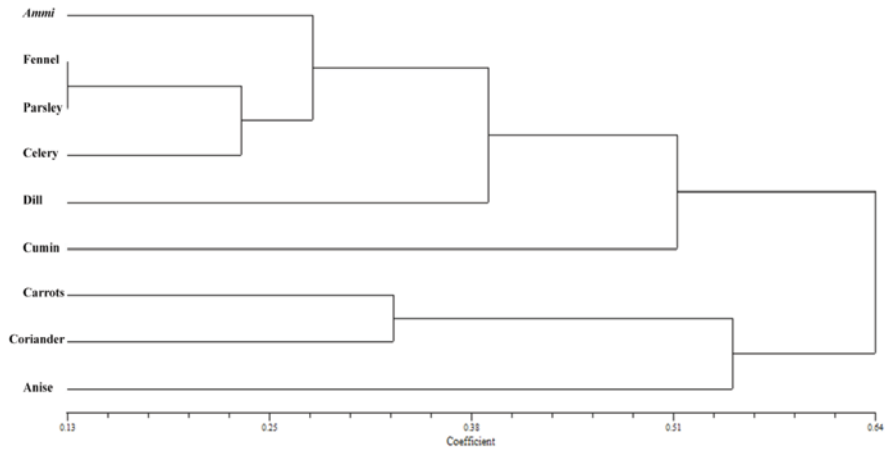
As: G. D. represents the genetic dimension, N_{xy} represents the number of packages shared between the x and y models that represent two samples, N_x represents the total number of packets in the sample x and N_y represents the total number of packets in the sample y.

Table (3) shows the details of the SSR amplification for five primers of the Apiaceae family

Initiator name	The total number of packages	The number of varying packages	% For initiator plurality	% Of starter efficiency	% Of the initiator discriminating power
Apium	3	3	100	21.42857	23.07692
Petrosi	2	2	100	14.28571	15.38462
Spia	4	3	75	28.57143	23.07692
Cau	3	3	100	21.42857	23.07692
Cor	2	2	100	14.28571	15.38462
The summation	14	13	95		
The Average	2.8	2.6			

UPGMA cluster analysis of Apiaceae family samples resulting from the use of SSR technique:

Cluster analysis allows dividing the studied samples into groups, and the division of studied plant samples into groups of the degree of genetic affinity between them reflects that the factor that collects samples in one group originates from the original habitat of plants or can be traced back to the origin from which they originated and their lineage. Which was obtained with SSR technology and through it, a genetic kinship tree (Dendrogram) can be created that works to determine the degree of genetic kinship and draw the cluster analysis scheme according to the method of UPGMA [17], using the ready-made program NTSYS-pc (Numerical Taxonomy System) to obtain a tree Kinship or genetic dimension (Figure 1).



(Figure 1) UPGMA cluster analysis of the studied samples using SSR technology (Celery, Dill, Coriander, Parsley, Cumin, Fennel, Anise, Carrots, *Ammi*).

Dendritic analysis of the genera of the Apiaceae family based on the results of the PCR-SSR technique

The genetic dimension between the plant samples of the studied Apiaceae genera was estimated by using the Photo capt statistical program, depending on the ratio of genetic affinity between the studied genera, as in Table (4). The results showed that the highest value of a genetic dimension of (0.846) was recorded between the two samples (*coriander* and *Ammi*), and the lowest value of a genetic dimension of (0.125) was recorded between the two samples (parsley and Fennel), that these samples were divided into two clusters, which included the first cluster of samples (*Ammi*, Fennel, parsley, celery, dill, cumin) where it was found. The highest percentage of genetic similarity and affinity between parsley and the Fennel was 87.5%, the percentage of genetic similarity between celery and the *Ammi* was 76.19%, while the percentage of similarity between celery and dill and cumin with dill was 71.42% and 60%, respectively, while The second cluster included the following genera (carrots, coriander, and anise). The degree of genetic affinity between coriander and carrots was the degree of similarity between them: 66.67% and anise with coriander. As for anise and carrots, the percentage of kinship between them was 50% and 40% respectively, which is evident from the above The degree of kinship (each of them converges a genetic distance) was between parsley and the Fennel, and the lowest degree of kinship (each of them diverged from a hereditary distance) between coriander and *Ammi,* as the ratio was (15.38%), as each falls in a different group from the other within the same family The reason is due to the occurrence of genetic mutations or environmental factors, although the method of evaluating the genetic material was different [18].

Table (4) the values of the molecular analysis of the genera of the Apiaceae family showing the genetic affinity

	Ammi	Carrot	anise	Fennel	Cumin	parsley	coriander	Dill	Celery
Ammi	0								
Carrot	0.71429	0							
Anise	0.6	0.6	0						
Fennel	0.36842	0.55556	0.6	0					
Cumin	0.52941	0.42857	0.75	0.5	0				
Parsley	0.2381	0.63636	0.66667	0.125	0.57143	0			
Coriander	0.84615	0.33333	0.5	0.75	0.66667	0.8	0		
Dill	0.52941	0.42857	0.5	0.33333	0.4	0.42857	0.66667	0	
Celery	0.2381	0.63636	0.5	0.25	0.57143	0.22222	0.8	0.28571	0

Conclusion

All the prefixes used gave multiplexed packages in the polymerase chain reaction. The prefixes used in this research proved their effectiveness in giving a morphological polymorphism among the genders of the studied Apiaceae family with a percentage of (95%), which supports the claim of the importance of relying on molecular indicators, especially SSR technology in determining kinship Genetic between plant species.

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The effect of Instant Feedback on Performance at the Digital Level on Discus Effectiveness

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Abstract

The problem of the research was that those in charge of the educational process try to achieve a high success rate and results by delivering the material to be taught, whether practical or theoretical, to students. In order to achieve this, they try to use various means of assistance, including the desired nutrition of all kinds. In arena and field games, the discus-throwing activity is one of the activities that are taught for the first stage in the Faculties of Physical Education and Sports Science and its results are determined by the technical performance of the throwing stages and measuring the distance of performance, and in order to achieve good learning and results in a faster time and to support and stabilize the learned response, the researcher decided to study the effect of one type of nutrition The feedback is immediate knowledge of performance results at the digital level in discus throwing activities, and the aim of the research was to identify the effect of immediate knowledge of performance results at the digital level on discus throwing activities. The researcher used the experimental method on (55) students from the first stage of the College of Education for Girls / Department of Physical Education And sports sciences at the University of Kufa for the 2019-2020 academic season, ConclusionsImmediate knowledge of performance results provides the learner with a type of feedback that helps improve his performance.

Key words*: immediate insight, discus throwing effectiveness*

Introduction

Feedback is information available to the thrower that makes it possible to compare his actual performance with standard performance, and this information is either internal or subjective through his previous awareness of the correct performance or external by the teacher or any other audio or visual method, so the concept of feedback is related to a major problem in Learning is a problem of the learner's assessment of his behaviour and performance[(1)], and feedback depends mainly on the Supernitec System, as well as the speed in providing the desired nutrition information helps to direct strengthening, while delaying the presentation of it may delay the learning speed[(2)]. Since the effectiveness of discus throwing is one of the activities whose results are determined by the distance of performance, so the importance of conducting this research appears in studying the effect of immediate knowledge of performance results on the distance of discus when teaching female students of the first stage of the College of Education for Girls / Department of Physical Education and Sports Science, and the research problem was that those in the process They try to achieve a high success rate and results by delivering the material to be taught, whether practical or theoretical, to students. To achieve this, they try to use all kinds of aids, including the various types of nutrition. In the arena and field games, the discus-throwing activity is one of the activities that are taught for the first stage in the Faculties of Physical Education and Sports Sciences and its results are determined by the technical performance of the throwing stages and measuring the distance of performance, and to achieve good learning and results in a faster time and to support and stabilize the learned response[(3)], the researcher decided to study the effect of one type of nutrition The feedback is the immediate knowledge of performance results at the digital level in discus throwing activities. The research aimed to identify the effect of immediate knowledge of performance results at the digital level on disc throwing activities[(4)].

Practical actions

The researcher used the experimental approach to suit the nature of the research problem on (55) female students from the first stage of the College of Education for Girls / Department of Physical Education and Sports Science at the University of Kufa for the academic season 2019-2020, after which (15) female students representing the control group and (15) representing the experimental group In a random manner, for the sake of homogeneity and parity of the research sample, age, height and weight tests were used using the T-test, whose results indicated the consistency of the research sample, as shown in Table (1).

Table (1): It shows the arithmetic mean, standard deviations, and the calculated and tabular (t) value for the homogeneity tests

Variables	experimental group	control group			Calculated value (t)	Indication level
	mean	Std.	mean	Std.		
Age (years)	18.4	1.19	18.03	1.15	1.226	not significant
Height (cm)	158.5	4.59	156.69	5.02	1.437	not significant
Weight (kg)	60.14	4.89	56.82	6.41	1.482	not significant
Run (30) m (s)	6.51	1.26	6.03	1.54	1.297	not significant
Long jump of stability (cm)	152.3	20.77	146.46	16.4	1.192	not significant
The payment of a medicine ball (2 kg) (meter)	3.17	0.35	3.33	0.46	1.455	not significant

Below the significance level (0.05) and with a degree of freedom (58)

Research tools

- § Arab and foreign sources.
- § Tape measure.
- § Medical scale.
- § Medicine ball (2 kg).
- § tablets weight 1 kg, number 15
- § Stopwatch.

search procedures

The procedures of the educational experiment started for the period 4/12 - 30/12/2019 with two lessons per week as scheduled in the schedule and for two hours per lesson, as the number of educational units reached (8) and the same planned education stages were together for both experimental and controlling groups for discus throwing. The difference was that the experimental group was given immediate feedback by observing and knowing the student as a result of her performance

through the indicative measurements within the throwing sector that show the location of the disk falling to the ground and knowing the throwing distance which is announced directly to the student after the end of her throw, while the control group was withheld Immediate feedback, there are no indications on the ground and do not announce to them the results of their performance. Since all members of the sample are going through the process of teaching the discus throw for the first time, so the post-education measurement is considered the outcome of the performance, which is also the test in which the comparison between the experimental and control groups was made. In the first and second lessons, the research sample was given the technical stages of throwing the disc together for all the two groups, and in the third lesson and beyond, immediate feedback was given to the experimental group by announcing the performance result upon completion of the throwing and the student knew the throwing distance, as well as by watching the measurements indicated on the ground. In the eighth lesson, the final measurement was made for both groups[5].

Statistical means: The Statistical Package (spss) was used.

Results

Table (2): Shows the arithmetic mean, the standard deviations, the calculated and tabulated value (T) of the discus throw distance for the experimental and control groups.

Statistical means The totals	the sample number	mean	Std.	value (v) calculated	sig	level of significance
Experimental group	15	11.38	1.53	2.674	0.016	moral
Control group	15	9.03	1.62			

Table (2) shows the results of the discus throw test for the experimental and control groups. The experimental group achieved an arithmetic mean of (11.38) meters with a standard deviation (1.53), while the mean for the control group reached (9.03) meters with a standard deviation (1.62). To find out the significant differences in the arithmetic meanings of the two groups, a test was used (T) whose results showed that there was a significant difference in favour of the experimental group, as the calculated value of (T) of (2.674) is a value less than (0.05) which indicates the presence of significant differences, this confirms the effect of the experimental variable, which is knowing the performance results of a distance Discus throwing as the immediate knowledge of the results helps the learner to correct his path if he erred and to continue in the correct response state[6], thus supporting the correct response a better distance. Thus[7], this study is consistent with the study of Muhammad Ibrahim, Camellia Hassan and Awatef Abdel-Hadi, which each study demonstrated the positive effect of feedback on the level of performance in gymnastics and exercises, and the researcher believes that the resulting progress in the performance distance of the experimental group is due to the continuous attempts of each student to achieve excellence by trying Improving the disc throw distance every time the student is performing, the stimulus is specific and knowing the results of the response reaches it immediately after the performance directly, and this is what Mahasin Ahmed confirmed that knowing the result enhances the response immediately and works on its appearance in the future[8], and thus fixation occurs and this is consistent with what Muhammad Mustafa said In that knowledge of the results and the feedback they provide is mainly related to a major problem in learning which is the problem of assessing the learner for his behavior and

performance[9], and since the conditions for learning the skill are related to the internal factors of the learner and others are external factors that provide the learner in the educational situation, the immediate knowledge of the results during the different stages of learning It works to promote correct responses as it provides the learner with a kind of feedback, which Skinner considers to be the first principle The basis for the learning process[10].

Conclusions

The immediate knowledge of the performance results provides the learner with a type of feedback that helps to improve his performance

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Relationship of Some Anthropometric Measurements With The Explosive Strength of the Upper and Lower Extremities with the Rhythmic Gymnastics of Female Students

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Abstract

Identify the relationship between some anthropometric measurements and the explosive power of the upper and lower extremities of female students in rhythmic gymnastics. The research sample consisted of students from the University of Kufa / College of Physical Education and Sports Science where the research sample underwent field tests to measure the explosive power of the upper and lower extremities and after conducting statistical treatments and presenting the results and analyzing and discussing the researcher reached To a set of conclusions, including there is an inverse correlation between age and the explosive force of the upper and lower extremities, and there is a direct correlation between body length and the explosive force of the upper and lower extremities.

Key words: *anthropometric measurements, explosive force of the upper and lower extremities, rhythmic gymnastics*

Introduction

Rhythmic gymnastics is one of the sporting activities that depend mainly on different sciences such as biomechanics, training science and other sciences. It also has special physical requirements that distinguish it from other games and these requirements are reflected in the physical specifications that must be met by those who practice them, which are the basis for the use of motor characteristics. It gives a greater opportunity to absorb the skills of the game and its arts. Here, asserts,[1] "It has been demonstrated in various sports that there is a relationship between bodybuilding characteristics such as height, weight, length of the limbs and between the high athletic level and that each game has certain physical characteristics that must be observed when athletes choose games. And the various activities, "and Muhammad Subhi Hassanein points out that," [2] as for the sports field, it has been proven that physical measurements are related to many motor abilities and excel in various activities[3]. Also, Kurton has proven that athletes in some games are distinguished from their peers in many physical measures such as the length of the trunk,[4] the width of the shoulders, the narrowing of the pelvis, and others. Qassem Hassan Hussein also stresses that "the characteristics of sports require attributes that are appropriate to games and related to the signs of for bodybuilding, such as body height, body weight,[5] and the relationship between arms, legs, body, etc[6].

From the foregoing, the importance of the research lies in knowing the relationship between some physical measurements and the explosive strength of the upper and lower sides of the rhythmic gymnastics students, and the research problem was that the rhythmic gymnastics game is a distinctive game from the organized games that contains a set of motor skills subject to a special law that defines the methods of practicing it and determines the number Team players, and through the experience of the field researcher being a player, trained and teaching at the same time, and through the many field view of the training of students and different teams it was found not to set the correct foundations for the selection of male and female students according to the anthropometric measurements and special physical abilities so this is important in the correct choice and keeping pace with the progress of the game Accordingly, the researcher decided to delve into the current study to demonstrate its

importance due to its positive impact on the progression of the level of the player and the achievement of the teams advanced results, and the objectives of the research were to identify some anthropometric measurements of the students under study, measure the explosive strength of the upper and lower extremities of the students under study, and identify Relationship between some anthropometric measurements and explosive force For the upper and lower extremities of students under study

Practical measures:

The descriptive method was used in the survey method, due to its relevance and the nature of the research on the students of the University of Kufa / College of Physical Education and Sports Science, the third stage of (23) students.

Search procedures:

First: the measurements used:

For each of the students, the following measurements and tests were conducted (age - length-weight - shoulder-width - arm length - leg length - palm length - the vertical bounce of stability - Wide bounce of stability - throw a medical ball weighing 2 kg for the maximum distance.

Second: The tests used:

1 - Vertical Jump Test of Stability (the test goal is to measure the explosive force of the two legs, the test measurement method: the distance from the mark marked with the height of the arms high is calculated "from the standing position to the specified point from the highest vertical vault possible)

2 - Wide jump grip test (test objective: measure the explosive strength of the leg muscles, test measurement method: the best distance is calculated for the three attempts)

3 - Throw a medical ball weighing 2 kg to the maximum distance (test objective: measuring the explosive strength of the arms, the test measurement method: the distance is calculated for the best attempt)

The scientific basis for the tests

Statistical methods were used to evaluate the tests in terms of honesty, consistency and objectivity, and the following appeared:

1. Vertical Jump Test of Stability: Stability Coefficient (0,85) for Self-Validity (0,81).

2. The wide jump stability test: reliability coefficient (0,7), validity (0,8).

3. The medical ball throw test weighing 2 kg for the maximum distance: reliability coefficient (0.94), honesty (0.96).

Statistical means: SPSS was used

Results

Through statistical analysis and Table (1) we observe the values of the arithmetic mean and the standard deviations of the research measurements

Table (1): Shows the mean of the mean and the standard deviations for research measurements

Variables	Age	Length	the weight	Shoulder width	Arm length	Leg length	Palm length	The vertical jump from constancy	The broad jump from stability	Throwing a medical ball
mean	20year	158 cm	63 kg	39.5 cm	70 cm	75.2 cm	16.3 cm	41.2 cm	2.30m	10.5 M
standard deviation	3.6	0.12	6.7	2.6	3.3	3.7	1.2	8.8	0.16	1.8

Through the statistical analysis of Table (2), we note that there is a statistically significant correlation relationship between the chosen physical measurements and the explosive strength of the upper and lower extremities of the sample under study. My agency:

Table (2):Shows the correlation coefficients between the explosive strength of the legs and arms, and some selected measurements (N-19).

Explosive force Measurements	Vertical jump for the legs		Wide jump for the legs		Throwing a medicine ball for the arms	
	Correlation coefficient	Significance	Correlation coefficient	Significance	Correlation coefficient	Significance
age	- 0.3	non-significant	-0.3	non-significant	-0.5	non-significant
length	0.3	non-significant	0.2	non-significant	0.2	non-significant
weight	- 0.01	non-significant	-0.1	non-significant	0.4	non-significant
shoulder width	0.2	non-significant	0.2	non-significant	0.4	non-significant
arm length	0.35	non-significant	0.33	non-significant	0.3	non-significant
man height	0.4	non-significant	0.53	significant	0.43	non-significant
Length of palm	0.1	non-significant	-0.2	non-significant	0.2	non-significant

The international value at the significance level (5%) equals (0.47)

1- An inverse correlation between:

A - Age and the ability of the upper and lower extremities as their ability decreases with increasing age and this appears clear "in Table (2) and this is consistent with the Burley and Helen study in 1961, which concluded that the relationship between the previous variables decreases and each of the age (3, 910)

B - Weight and the capacity of the lower limbs, that is, the capacity of the lower limbs decreases with the increase in weight, as the weight of the body plays an "important"[7] role in many different games and activities, including rhythmic gymnastics, and this is confirmed by Hara (33,15) and from studies that prove the impact of the explosive force of the lower limbs By weight, which is consistent with our findings from what Muhammad Sobhi Hassanein mentioned, "A study conducted in 1967 at the University of Louisiana, in the United States of America, to identify the effect of changes in weight" (increase or decrease) on the results of individuals in the vertical jump test of stability. Among the most important results of this study is the results of individuals in testing explosive power when reducing body weight. "[8]

2 - A positive correlation between:

* Body length

* Arm length, upper and lower limb capacity

* Leg length

* Shoulder width and upper limb capacity

* the weight

That is, the capacity of the upper and lower limbs increases with increasing height, the trait that he stressed the necessity of availability for many, including Harrah, by saying, "The length is a characteristic that plays a" significant "role in many games, including rhythmic gymnastics (15,33). This man agrees with the role of the limbs in sports, which Harra affirmed by saying that "the muscles (limbs) play a major" role "in sports and that this aspect is preferred, especially from biomechanics," as it indicates in the same source that the players "throughout Stature and people with graceful bodies are the most appropriate form of "rhythmic immobility," as well as there is a convergence in what has been reached and what was stated in the study of Start and others in 1966 in that "there is a statistical indication of the correlation between the lengths of the lower limb and the explosive force[9]." The upper limbs increase with increasing weight (although increasing weight affects the ability of the two men as we explained earlier), meaning that weight plays a role in increasing the capacity of the arms.

Finally, "there is a relationship between weight and length that affects the explosive force, and this is what Muhammad Subhi Hassanein confirmed from Sar Jannat," he said, "The explosive force is affected by weight and length.[10]"

Conclusions

There is a statistically significant correlation relationship between some selected body measurements and the explosive strength of the upper and lower extremities of the sample. My agency:

1- An inverse correlation between:

* Age and explosive strength of the upper and lower extremities.

* Weight and explosive strength of the lower extremities.

2- A direct correlation between:

* Body length, arm length, and leg length with the explosive force for the upper and lower extremities.

* Shoulder width and weight with explosive power to the upper extremities.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Diagnosis of Coronavirus using Biochemical and Hematological Methods for Patients in Anbar Governorate

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Abstract

In view of the great importance of this virus and the major health problems it causes that may reach death and the importance of early diagnosis of this virus, which works to reduce deaths, this study came for the early diagnosis of this virus, as we relied on biochemical and blood methods for the speed of diagnosis and the low cost compared to the rest of the tests Where many tests were tested, including ferritin, LDH, and Di dimer, where there was a significant increase in the concentration of these tests compared to the control. No significant differences were observed between men and women. As for the hematological tests, which included WBC count, Neutrophiles, Lymphocytes. There is a significant decrease in the number of white blood cells and lymphocytes in comparison with the control. We conclude through the study that infection with Corona virus increases many biochemical and hematological variables, including a significant increase in ferritin, a significant increase in LDH and Di dimer, in addition to a decrease in the total number of white and lymphocytes

Keywords: *Toxicity; Corona virus, biochemical behaviors; hematological behavior; Health.*

Introduction

Coronaviruses are a crew of viruses that can reason ailments such as the frequent cold, extreme acute respiratory syndrome (SARS), and Middle East respiratory syndrome (MERS).[1,2] A new kind of coronavirus used to be determined after it used to be recognized as a reason of the unfold of a disorder that commenced in China in 2019. [3,4]The severity of COVID 19 signs and symptoms can vary from very moderate to severe. Some human beings may additionally improve solely a few symptoms, and some human beings may additionally no longer have any signs at all. Some humans can also trip a worsening of symptoms, such as worsening shortness of breath and worsening pneumonia, about a week after signs and symptoms begin. [5,6] Older humans are at a greater hazard of creating extreme COVID-19 symptoms, and that chance will increase as a individual get older. People with present continual scientific stipulations might also be greater probably to have extreme symptoms. An instance of a serious fitness situation that will increase your chance of growing extreme COVID-19. [7,8,9] The virus seems to unfold effortlessly between people, and scientists will proceed to find out extra about how it spreads. Data confirmed that it is unfold from man or woman to individual thru shut contact (within 6 feet, or two meters). The virus is unfold via respiratory droplets launched when an contaminated character coughs, sneezes, or speaks. This spray can be inhaled or bought into the mouth or nostril of any one close. [10] Although the signs of most human beings with Covid-19 vary from slight to moderate, the ailment can reason extreme clinical problems and lead to dying for some people. Older adults or these with current underlying clinical stipulations are extra probable to boost a serious case of COVID-19. [11] If you have a persistent circumstance

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and are at improved threat of creating extreme signs if you contract the infection, speak to your physician about extra approaches to shield yourself. [12]

Lab. Analysis

60 blood samples were collected from people suspected of being infected with the Coronavirus, divided into 30 samples from men and 30 samples from women, 30 samples were for people who were not infected with his control, and the tests were conducted.

Biochemical test

Biochemical assays included screening for ferritin, LDH, Di-dimer and Covid 19 IgM , IgG concentration. [13]

Hematological test

Hematological test included screening for WBC count ,Lymphocyte and Neutrophils by CBC Apparatus [14]

Result and Discussion

Table 1 Diagnosing corona virus using biochemical test

Descriptive Statistics				
Dependent Variable: concentration				
gender	**biochemical test**	**Mean**	**Std. Deviation**	**N**
male	ferritine	490.00	39.441	10
	LDH	609.00	51.951	10
	Di dimer	726.00	62.218	10
	Total	608.33	110.112	30
femal	ferritine	425.00	26.352	10
	LDH	524.00	39.215	10
	Di dimer	607.00	40.014	10
	Total	518.67	83.159	30
control	ferritine	135.00	14.337	10
	LDH	135.00	14.337	10
	Di dimer	135.00	14.337	10
	Total	135.00	13.834	30
Total	ferritine	350.00	159.374	30
	LDH	422.67	213.136	30
	Di dimer	489.33	262.953	30
	Total	420.67	221.094	90

Table 1 shows the diagnosis of Corona virus using biochemical methods. There was a significant increase in the concentration of ferritin, LDH, and Di-dimer, where the concentration of ferritin was 490 for men, 425 for women, compared to the control, 135, meaning there were significant differences between treatments and control, and LDH was 609 for men and 525. For women compared to control 135, meaning there are significant differences between treatments and control, as well as for Di-dimer, the concentration was 726 for men and 607 for women compared to control 135, meaning there are significant differences between transactions and control, there are no significant differences between men and women, but women were better compared to men

Table 2 ANOVA table for Diagnosing corona virus using biochemical test

Tests of Between-Subjects Effects					
Dependent Variable: concentration					
Source	**Type III Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Corrected Model	4237380.000a	8	529672.500	379.073	.000
Intercept	15926440.000	1	15926440.000	11398.141	.000
gender	3792846.667	2	1896423.333	1357.221	.000
test	291386.667	2	145693.333	104.269	.000
gender * test	153146.667	4	38286.667	27.401	.000
Error	113180.000	81	1397.284		
Total	20277000.000	90			
Corrected Total	4350560.000	89			
a. R Squared = .974 (Adjusted R Squared = .971)					

Table 2 shows the analysis of variance. There are significant differences between all treatments compared to control, and there are no significant differences between men and women in all tests.

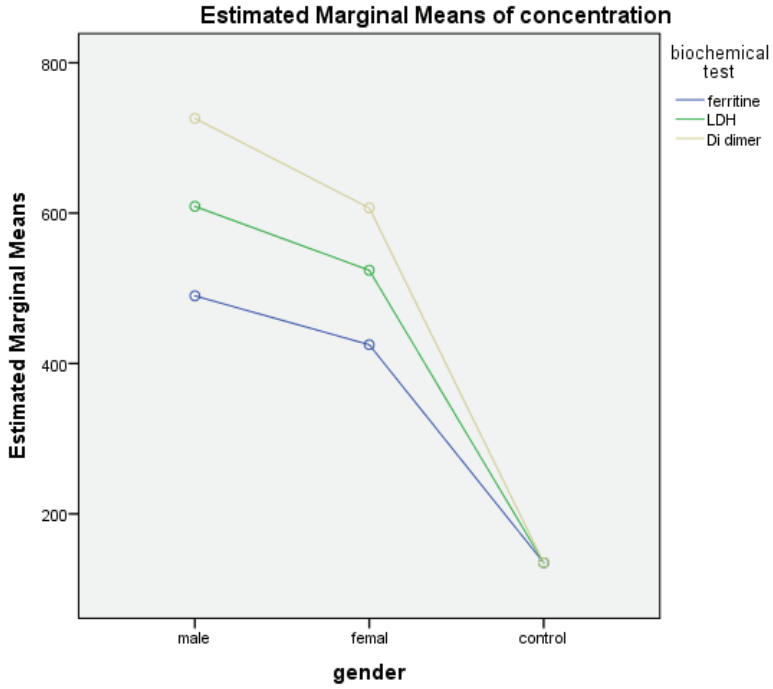


Figure 1 Diagnosing corona virus using biochemical test

Table 3 Diagnosing corona virus using Hematological test

Descriptive Statistics				
Dependent Variable: concentration				
gender	**hematological**	**Mean**	**Std. Deviation**	**N**
male	WBC count	4420.00	225.093	10
	Neutrophilis	1840.00	107.497	10
	Lymophocyte	760.00	84.327	10
	Total	2340.00	1568.571	30
femal	WBC count	4420.00	225.093	10
	Neutrophilis	1840.00	107.497	10
	Lymophocyte	760.00	84.327	10
	Total	2340.00	1568.571	30
control	WBC count	7630.00	2488.217	10
	Neutrophilis	3300.00	483.046	10
	Lymophocyte	1750.00	97.183	10
	Total	4226.67	2898.624	30
Total	WBC count	5490.00	2078.851	30
	Neutrophilis	2326.67	754.725	30
	Lymophocyte	1090.00	482.343	30
	Total	2968.89	2267.387	90

Table 3 illustrates the use of blood tests in the diagnosis of Corona virus, which included the total white blood cell count, the differential count of neutrophil and neutrophil white cells. It was observed through the table. In men and men 4420 compared with control 7630, as well as for lymphocyte white blood cells, where the number reached 670 compared with control 1750, meaning there are significant differences between the treatments and control, and there are no significant differences between men and women.

Table 4 ANOVA table for Diagnosing corona virus using Hematological test

Tests of Between-Subjects Effects					
Dependent Variable: concentration					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	398398888.900a	8	49799861.110	68.191	.000
Intercept	793287111.100	1	793287111.100	1086.254	.000
gender	71190222.220	2	35595111.110	48.741	.000
test	308960222.200	2	154480111.100	211.531	.000
gender * test	18248444.440	4	4562111.111	6.247	.000
Error	59154000.000	81	730296.296		
Total	1250840000.000	90			
Corrected Total	457552888.900	89			
a. R Squared = .871 (Adjusted R Squared = .858)					

Table 4, analysis of variance table, it was noticed that there were significant differences in the two blood cells and lymphocytes in the subjects compared with the control.

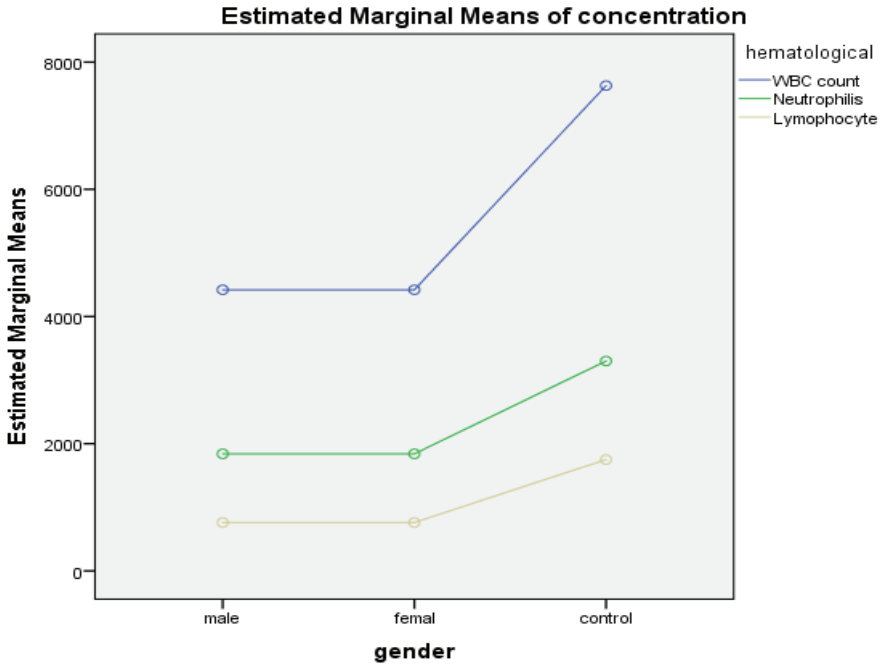


Figure 2 for Diagnosing corona virus using Hematological test

Consultation

We conclude through the study that infection with Corona virus increases many biochemical and hematological variables, including a significant increase in ferritin , a significant increase in LDH and Di dimer, in addition to a decrease in the total number of white and lymphocytes.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Evaluation of Salivary α- Amylase Enzyme Activity in Smoker and Peptic Ulcer Patients affected by Periodontitis in Relation to Clinical Periodontal Parameters

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Abstract

Objectives: To investigate the activity of salivary α-amylase (SA) in subject affected by periodontitis alone or associated with peptic ulcer disease (PUD) with or without smoking.

Methods: The study population was divided into control group (C), study groups; periodontitis (G1), periodontitis+ PUD+ smokers (G2), periodontitis+ PUD+ nonsmoker (G3). The clinical periodontal parameters including plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL) were recorded. In addition, unstimulated whole saliva was collected from these patients for analysis of SA level.

Results: Analysis showed that PI and GI showed significant difference among study groups versus controls. G2 exhibited significantly higher PPD and CAL as compared with G1 but not with G3. For SA activity, G2 and G3 showed significantly higher activity as compared with C and G1 groups. Yet, no significant difference was seen between C and G1 and between G2 and G3. Activity of SA was positively and significantly ($P < 0.05$) correlated with increasing PPD in all study groups and with CAL measurements of G2 only.

Conclusions: The results suggested that SA could be used as a marker for the diagnosis of PUD in subjects affected by periodontitis with or without smoking.

Keywords: *periodontitis, diagnosis of PUD ; peptic ulcer, alpha-Amylases, toxicity;*

Introduction

Traditionally, diagnosis of periodontal diseases (PD) is performed clinically together with radiographic assessment, which often associated with possibility of errors in measurements. Alteration in the level of different markers in the oral fluids during the course of PD may represent a valuable diagnostic tool to assess the severity and susceptible individuals[1].

Saliva contains range of proteins, actively involved in the innate immune response, including salivary amylase, lysozyme, cystatins, and mucins[2]. These molecules form a dynamic defensive network by synergistic/cumulative effects contributing to immune system activation/ modulation[2, 3]. Among these proteins, salivary α-amylase, well-known for its inhibitory activity against range of microorganisms and significantly increased during periodontitis[1]. Smoking is a well-documented risk factor that negatively influencing the periodontal health by impairing immune response associated with increased severity of PD[4]. Previous studies suggested that smoking inhibits salivary amylase by the interaction of one of the smoking product, unsaturated aldehydes, with -SH group of the enzyme molecule[5].

PD were suggested to be related to different systemic diseases affecting the human body. Peptic ulcer disease (PUD) which is a condition affecting the integrity of gastrointestinal system. The etiology of PUD is mainly attributed to Gram-negative *Helicobacter pylori* (*H. pylori*)[6]. Interestingly, dental biofilm could be a reservoir for this bacterium from which infection of stomach may occur[7]. Intense plaque control measures

were associated with less recurrence of PUD[7]. Further support was obtained from other studies that indicated a potential relationship of PD with PUD[8]. Comparison of salivary enzymes between healthy subjects and patients with PUD showed that the latter group was associated with higher α-amylase activity compared to the control[9]. One of major risk factors for PUD is smoking which not only induce ulceration and increase acidity of the stomach but it was suggested as a predisposing factor for *H. pylori* gastric ulceration[10].

The aim of this cross-sectional study was to evaluate the activity of salivary α-amylase (SA) in subject with periodontitis alone or associated with PUD and/or cigarette smoking habit. In addition, correlation of amylase activity was determined in relation to range of clinical periodontal parameters.

Methods

Study design

This was a prospective cross-sectional study which was conducted in College of Dentistry, University of Baghdad. The study was started at Feb to Jun 2019 after obtaining approval from ethics committee in the college in accordance with Tokyo and Helsinki declaration for humans.

Subjects included in this study were randomly recruited from patients attending the Department of Periodontics. For patient affected with periodontitis, history was taken for each patient to record the age, cigarette smoking status, medical history. Diagnosis whether they currently having PUD or not was based on self-reporting by the patients or via the drugs they are taken. Subjects with healthy periodontium, nonsmoker, and not suffering from PUD were designated as control (C) group and were included mainly to obtain baseline data for the normal activity level of salivary α–amylase. Other patients (study groups), were divided into: G1 patients with periodontitis, no PUD and nonsmokers, G2 included patients with periodontitis, PUD, and smokers, and G3 patients with periodontitis, PUD and nonsmokers. The exclusion criteria were females, other systemic conditions, use of mouthwashes, under chemotherapy, radiation therapy, or medications that cause xerostomia. The aim and flow of the study were explained for the participants, who signed a consent form prior to the study.

Clinical parameters and examiners' calibrations

Periodontitis was defined as the presence of teeth with probing pocket depth (PPD) ≥4mm with clinical attachment loss, this made according to the international classification system for PD[11]. The clinical periodontal parameters which include plaque index (PI)[12], gingival index (GI)[13], bleeding on probing (BOP), PPD and clinical attachment level (CAL) were recorded for each patient. Measurements were made at four sites per tooth using William's periodontal probe. Before starting the study, inter- and intra-examiner calibration sessions were conducted among the clinicians. The readings among them were considered consistent when weighed κ values were ≥ 0.7 at 95% confidence interval was reached for each index.

Saliva collection and α-amylase activity determination

Unstimulated whole saliva was collected for analysis, before that the patient was instructed to rinse his mouth several times by water and then wait for (1-2min) for water clearance. After collecting the sample, it was centrifuged at (4000rpm) for (10min), then the samples were frozen at (-20^{o}C) until α–amylase analysis was done. Salivary α–amylase analysis done by using α–amylase liquicolor (colorimetric test for α–amylase) (Demeditec Diagnostics GmbH, Kiel, Germany) following manufacturer's instructions manual.

Statistical Analysis

Descriptive analysis was performed in the form of means, standard deviation (SD). Since the data were normally distributed then inferential statistics was conducted by using one-way ANOVA test and Tukey's test. Correlation coefficient (r) for α-amylase activity with clinical parameters was calculated by using Pearson correlation test. Significant level was considered at $p<0.05$.

Results

Recruitment of patients was illustrated in Figure 1. Demographic characteristics and distribution of the patients were shown in Table 1, while clinical parameters were summarized in table 2.

Figure 1 Flow diagram of the study

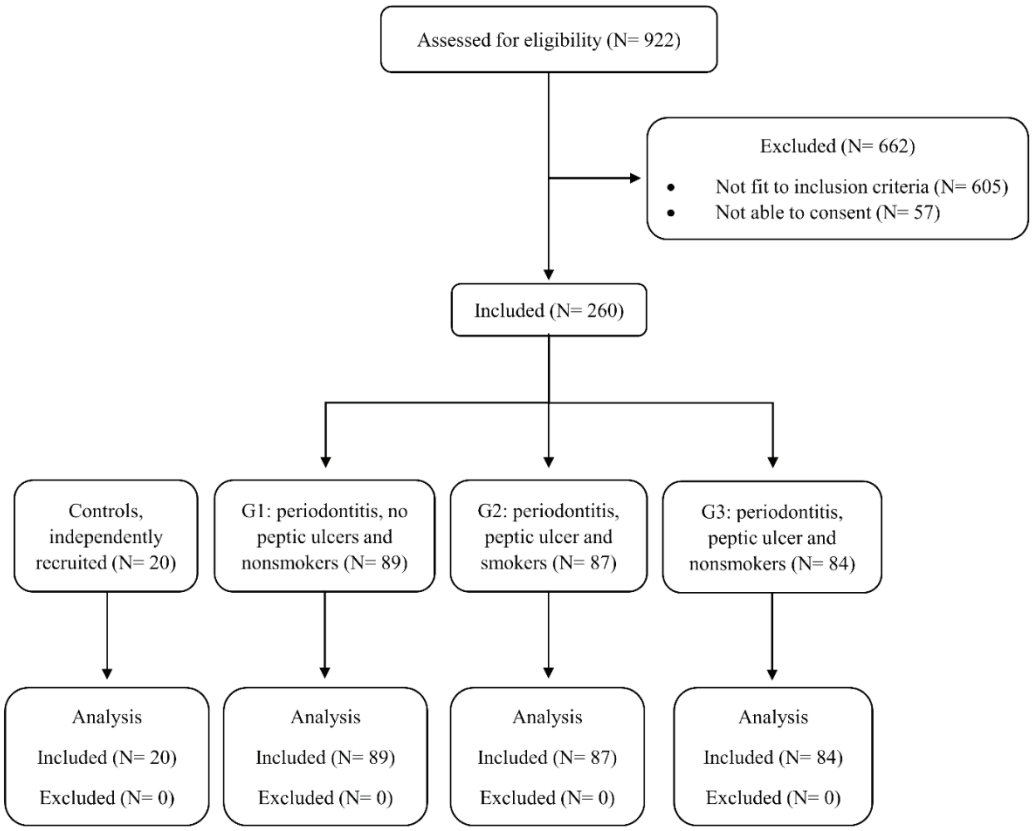


Table 1: Characteristics of the study population

Age range	**29-73**
Average age (mean± SD)	51.1± 11.4
Groups¶	N (%)
Control	
C	20 (100)
Study	
G1	478 (69.1)
G2	82 (11.8)
G3	132 (19.1)
Total	692 (100)

¶ C1: control group, nonsmokers and no peptic ulcer, G1: periodontitis, no PUD and nonsmokers, G2: periodontitis, PUD and smokers, G3: periodontitis, PUD and nonsmokers

Table 2: Clinical parameters of all groups

	Mean± SD			
Groups	**PI**	**GI**	**PPD**	**CAL**
C	0.53± 0.36	0.79± 0.41		
G1	2.08± 0.53	2.04± 0.72	4.73± 0.96	4.72± 1.33
G2	2.28± 0.55	2.27± 0.53	6.31± 1.58	5.91± 1.42
G3	2.48± 0.37	2.36± 0.47	5.27± 1.53	5.70± 1.33

Analysis showed that PI and GI did not show any significant difference among the groups. G2 patients exhibited significantly deeper PPD as compared to G1 but not with G3. The same pattern was observed in association with CAL measurements in which significant difference was observed between G2 and G1 only (Table 3).

Table 3: Comparison of clinical parameters among all groups

Comparisons	**p value***			
	PI	**GI**	**PPD**	**CAL**
C vs G1	S	S		
C vs G2	S	S		
C vs G3	S	S		
G1 vs G2	NS	NS	NS	NS
G1 vs G3	NS	NS	S	S
G2 vs G3	NS	NS	NS	NS

* p value<0.05 by ANOVA test, S= significant, NS= non-significant

For SA activity, G2 and G3 showed significantly higher activity as compared with C group and G1. However, no significant difference was seen between C and G1. No significant difference was shown between G2 and G3 (Table 4). Activity of salivary amylase enzyme was significantly and positively correlated with PI in G2 and G3 groups only. While PPD was significantly and positively related to the enzyme activity in all groups which showed moderate-strong correlation. However, only CAL in G2 group was significantly increased with increased activity of SA (Table 5).

Table 4: Comparison of salivary α-amylase activity among all groups

Groups	α-amylase ± SD	Comparison
	U/l[§]	
C	39.14± 27.03	C vs G1
G1	72.43± 31.80	C vs G2*
G2	154.1± 29.60	C vs G3*
G3	121.8± 55.50	G1 vs G2*
		G1 vs G3*
		G2 vs G3

§ Activity of α-amylase (ΔA/min)

* Significant at p<0.05 by ANOVA test

Table 5: Relation of α-amylase activity with clinical parameters in all groups

Groups	PI	GI	PPD	CAL
C	0.280	0.119		
G1	0.414	0.417	0.644*	0.331
G2	0.606*	0.249	0.508*	0.567*
G3	0.777*	0.183	0.726*	0.448

* Significant correlation at p<0.05 by using Pearson test

Discussion

Salivary amylase is a calcium containing metalloenzyme responsible for initial cleavage of α-glycosidic bonding of glucose-polymers such as starch, glycogen and dextrin[14]. Salivary proteins play important role in the innate immune response to PD[2]. Interestingly, presence of α-amylase was detected in the acquired pellicle suggesting a role in the adhesion of primary colonizers to tooth surface and it may supply these microorganisms with the needed glucose due its digestive action[15].

PUD could be developed due to several risk factors such as *H. pylori* infection, smoking and alcohol consumption. Recent studies have shown that the *H. pylori* which is associated with chronic gastritis, peptic ulcer, and gastric malignancies, is also present in the dental plaque biofilm[16]. Literatures have suggested a positive association between peptic ulceration and periodontal pathogens. Results from meta-analysis have shown that combining periodontal therapy with anti-*H. pylori* medications have a positive impact on the prognosis of PUD[17].

In this study, analysis of clinical parameters showed that PI and GI did not show any significant difference among the study groups. Both smoker and nonsmoker patients with peptic ulcer exhibited significantly deeper periodontal pocket as compared to nonsmoker and no PUD patients. This agrees with other study which indicated that subjects with deeper pockets and alveolar bone loss were more prone to develop PUD independent from other risk factor[18]. Further, number of missing teeth due to PD was reported to be significantly increased in association with increased prevalence of gastric ulcer[19]. Nevertheless, results from another matched case-control study did not show presence of such association between the two conditions[8]. This conflict could be due to that subjects in both control and study groups in the latter study were infected with *H. pylori*. Also, a study conducted by Khader et al. (2003) did not show any significant association between PUD and increased PPD; however, significant association was observed in relation to CAL[20], which agree with results of the current survey that showed same results in regard to CAL measurements.

SA activity in this study was significantly upregulated in patients with periodontitis affected by PUD as compared to healthy controls or those affected by periodontitis only. This agrees with previous study which showed significant increase in SA concentration in association with periodontitis patients as compared to healthy individuals[21]. Increased secretion of SA and other acinar proteins of glandular origin was attributed to defensive response of salivary glands to increased population of periodontitis-associated bacteria[22]. In addition, adherence and biofilm formation of Gram-

negative bacteria was shown to be disrupted by amylase activity by its affinity to binding to the major virulence factor, lipopolysaccharide[23]. Henskens and co-authors have proposed that upregulation in the level of salivary proteins during periodontitis could be due to increased leakage of plasma proteins into saliva in response to inflammation[24]. Activity of SA is negatively affected by smoking which is associated with increased reactive nitrogen species that interact with -SH active sites of the enzyme[25;26]. However, no significant difference was observed in the amylase activity between G2 and G3 in our study. This may suggest that the stimulatory effect of periodontal pathogens overwhelmed the inhibitory effect of smoking product on the enzyme activity. Further support for the role of α-amylase activity during the course of PD was observed in the positive correlation between the enzyme and the increase of PPD in all patients affected with periodontitis, while this relation was also shown in association with CAL in smoker patients affected with peptic ulcer. Previous study has indicated a potential role of SA in enhancing adhesion of the primary colonizers to the acquired pellicle[15]. Interestingly, score of PI showed positive relation with increased activity of α-amylase in periodontitis patients suffering from peptic ulcer whether smoking or not.

There are limitations to this study such as data about *H. pylori* infection and NSAID use were not available in this study. In addition, frequency and duration of smoking were not detected which need to be further investigated in relation to the activity of salivary enzymes activity.

Conclusions

Within limits, results from our study indicated higher activity of SA in patients with periodontitis together with peptic ulcer and this activity increased in association with smoking. This suggests that periodontitis with smoking act synergistically as risk factors for development of PUD. In addition, increased activity of this enzyme could enhance dental plaque accumulation and increased PPD. Thus, SA in smoker patients affected by periodontitis could be used as predictive and prognostic tool for PUD.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

Funding: Self-funding

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Histophysiological Considerations Deals Damaging of Rat's Kidney by Examined Doses of Heroin

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Abstract

Scientific gain after treating about 100lab rats with heroin of several doses to examine the quantitative physical, clinical chemistry and histopathological tests. Accompanied bymodern statistical analysis, the results explain an obvious differentiation ($p<0.05$) for urine creatinine, serum creatinine, creatinine clearance, 24-hour proteinuria, protein/creatinine ratio and urea; at the same time as other parameters showed slight and concealed splendour achievements results recorded ups or downs reported below for Weight, food intake, water intake and urine volume.

Judgment based on reasoning were proving the negative effects of heroin on the kidneys of the lab rats successes an acute damages on the histopathological levels.

Keywords: *Histophysiology, Heroin, Laboratory rats, Kidney's damaging.*

Introduction

Heroin (or *smack*, *horse*, *hell dust,* and *big H*) considered as an opioid compound gained from native material extracted from the seed of different opium poppy plants, that is morphine. Many countries planted heroin like Colombia, Afghanistan and Mexico. Heroin was be brown or white whey, or as black sticky/tarmaterial(1).

Heroin reacts with the brain swiftly through binding special receptors on the cells of many areas those encompassed in emotions of pleasure, pain, sleeping, ruling heart beatsandrespiration(2).

Misuse of some opioid pain pharmaceutics such as Vicodin® and OxyContin®looked to have belongings like that of heroin to an extents switches to using this drug in an estimated levels of 50 to 80 percent(3,4).Recent scientific researches suggests that heroin is oftentimes the top opioid used by one-third people whom recorded heroin as the premium opioid they were employed frequently to be passionate(5).

Aimof this project is to explain the alternative hypothesis for the correlation of heroin to the cases of acute kidney damage.

Materials and Methods

100 rat of lab model *Rattusnorvegicusdomestica* type Sprague-Dawley were beenbought from Hilltop lab animals, Inc. California. Those cabinet formed of 50 males vs 50 females. Body weight of animals was measured.

Heroin extracted in I.R. of Iran and it was injected via Intra-Peritoneal (IP) manner. Later animals were put in five bands:

1- Standard (n=20) were put in standard cage conditions.

2- Control (n=20) did not driven anyheroin transaction, just given 250 µL physiological saline periodically.

3- Test one (n=20) driven by physiological

saline and citric acid injected in a term of 33 days with the following order: from day 1-3 injection applied 3 times per day; in the first one given a dosage of 200µLphysiological saline plus 0.5µLcitric acid; 250µLphysiological saline plus 0.55µLcitric acid in the next day and 300µLphysiological saline plus 0.6µLcitric acid by the end day. The last transaction was then shot twice daily for the remains30 daysinterm.

4- Test two (n=20) driven by physiological saline, citric acid (solvent) and heroin injected in a term of 33 days with the following order: from day 1-3 transaction of 60mg kg^{-1} dosage for independency to heroin[6] to make an injection of 1.15 mL physiological saline, 1.15 mg heroin and 0.003 mL citric acid. Solution was dosage 3 times per day; in the first one was 165µL in the next day, 195µL in the later225µL The last transaction was then shot twice daily for the remains 30 days in term. (heroin dosage of thistestband was 5 mg kg^{-1}).

5- Test three (n=20) transaction done through physiological saline, heroin and acetic acid by shot with dosage of 5 mg mL^{-1}in a term of 33 days. In this band the order of dosing the solution was like to that of the 3 rd.band.

Six members from each band whom showed signs of kidney failure were chosen, weighing and serial urinalysis before nephrectomy carried out after anaesthetizing the animals[6].

Physiological biomarkers fulfilled by excellence balance (METTLER TOLEDO- Columbus) for evaluating body weight, clinical chemistry analyser (RX series - Randox co. UK) for estimation each of creatinine-protein-urea, microscope(Carl Zeiss, branded as ZEISS, Germany) for histological reasons, equipment and tools PerkinElmer, Inc., USA for the rest of parameters.

Histology sections got from fixing fresh kidneys in 10% natural formalin before embedded with paraffin. Then each 4-6-µm-thick section was dyed by both of Masson's Trichrome and periodic acid Schiff. Deparaffinization accomplished by rinsing and oxidizing in a solution of 0.5% periodic acid. Later, sections incubated and counterstained in Schiff reagent and hematoxylin subsequently. For Masson's stain, Bouin's solution was used for deparaffinization and refixing for 50 min. at 58–60°C. Later staining applied with Biebrich scarlet-acid fuchsin and Weigert's hematoxylin before ending dying with aniline blue[7].

Statistical methods: accomplished by applying One-way ANOVA analysis of variance plus complementary Post Hoc Tukey (Honestly significant difference) test[8].

Results

Tabulated **clinical signs** of kidney injury listed below in table-1. Showing obvious decrease in weight accompanied with declines in each of feed intake, water drink, and urine volume values. Concealed datum appear just in cases of feed-water intake without significant differences after applied statistical analysis. Scheme histogram-1 designed below distinguishes the levels of these four parameters according to the tested rat groups showing a direct proportional relationships.

listed **clinical chemistry** parameters recorded in table-2. Obvious elevation in each of creatinine, proteinuria, protein/ creatinine and urea lab values. The only decline appeared in creatinine clearance level. Statistical significant results appear in all of these variables. Statistical histogram-2 below explained that direct proportional relationships correlates each of these variables with the increased dose of the applied heroin.

Table-1. Quantitative physical assessed parameters in heroin tested bands of rats.

Parameter	Standard	Control	T1	T2	T3	p
Weight (g)	250.1 ± 11.1	266.7 ± 18.2	241.0 ± 20.1	235.2 ± 22.5	220.0 ± 23.1	0.15
Feed intake (g)	25.7 ± 1.3	24.7 ± 2.2	21.9 ± 2.6	20.7 ± 3.1	19.9 ± 2.9	0.11
Water intake (ml)	25.1 ± 4.3	24.9 ± 4.4	22.5 ± 5.2	21.5 ± 6.3	20.5 ± 5.5	0.15
Urine volume (ml)	12.1 ± 1.2	11.5 ± 2.9	9.8 ± 1.4	9.1 ± 4.2	7.6 ± 2.4	0.09

* No significant difference explained

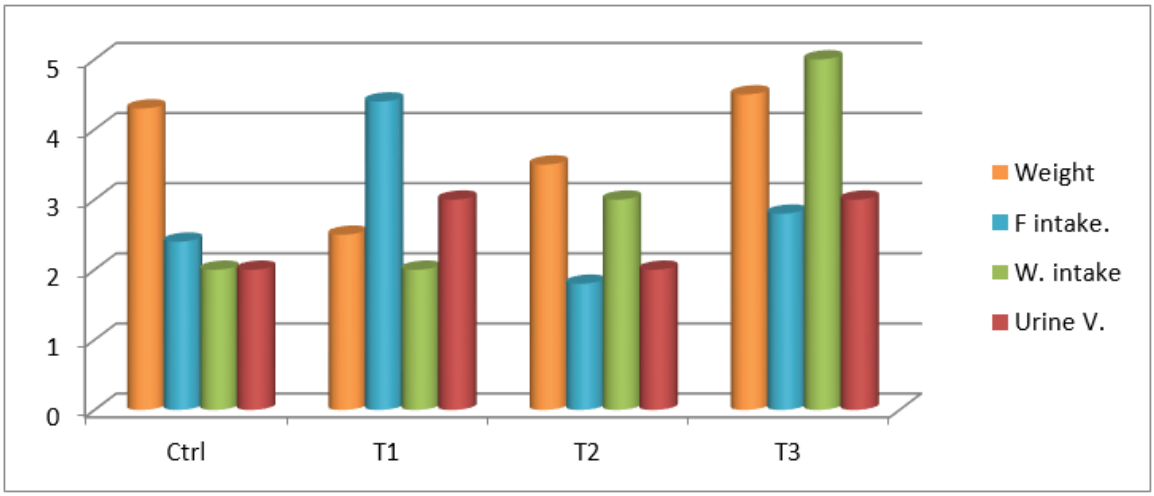


Figure 1: Statistical histogram of physicalvariables related to rats treated by heroin

listed **clinical chemistry** parameters recorded in table-2. Obvious elevation in each of creatinine, proteinuria, protein/ creatinine and urea lab values. The only decline appeared in creatinine clearance level. Statistical significant results appear in all of these variables. Statistical histogram-2 below explained that direct proportional relationships correlates each of these variables with the increased dose of the applied heroin.

kidney injury: Tubular destruction convoyed cell injury of the lethal and sublethal type. Necrosis as coagulative ones leading to apoptosis, and cellular exfoliation in the renal tubules. Other obvious signs including swelling, focal necrosis, acute apoptosis, desquamation, proximal tubules dilation, thinning of PAS+ brush border, granular and coarse pigmented cases, hyaline casts of Tamm-Horsfall protein (urinary glycoprotein normally secreted by eosinophils), leukocytes and interstitial edema were been diagnosed microscopically through all renal tubules slide's sections. Look below shape-1 and -2.

Table-2. Clinical chemistry assessed parameters in heroin tested bands of rats

Parameter	Standard	Control	T1	T2	T3	p
Urine creatinine (mg/dL)	79.9 ± 8.9	82.4 ± 11.2	87.9 ± 10.1	94.4 ± 16.6	102.1 ± 19.2	0.4
Serum creatinine (mg/dL)	0.45 ± 0.015	0.48 ± 0.05	0.50 ± 0.05	0.61 ± 0.03	0.70 ± 0.01	0.01
Creatinine clearance (ml/min)	1.4 ± 0.1	1.42 ± 0.5	1.37 ± 0.4	1.11 ± 0.1	0.88 ± 0.2	0.018
24-hour proteinuria (mg/dL)	7.2 ± 1.6	7.9 ± 5.7	8.1 ± 4.1	9.9 ± 5.1	12.2 ± 4.9	0.60
Protein/creatinine ratio	0.50 ± 0.01	0.66 ± 0.1	0.92 ± 0.4	1.19 ± 0.53	1.70 ± 0.41	0.49
Urea (mg/dL)	29.0 ± 1.9	33.0 ± 3.3	35.1 ± 3.9	37.5 ± 4.4	39.9 ± 5.1	0.059

* No significant difference explained

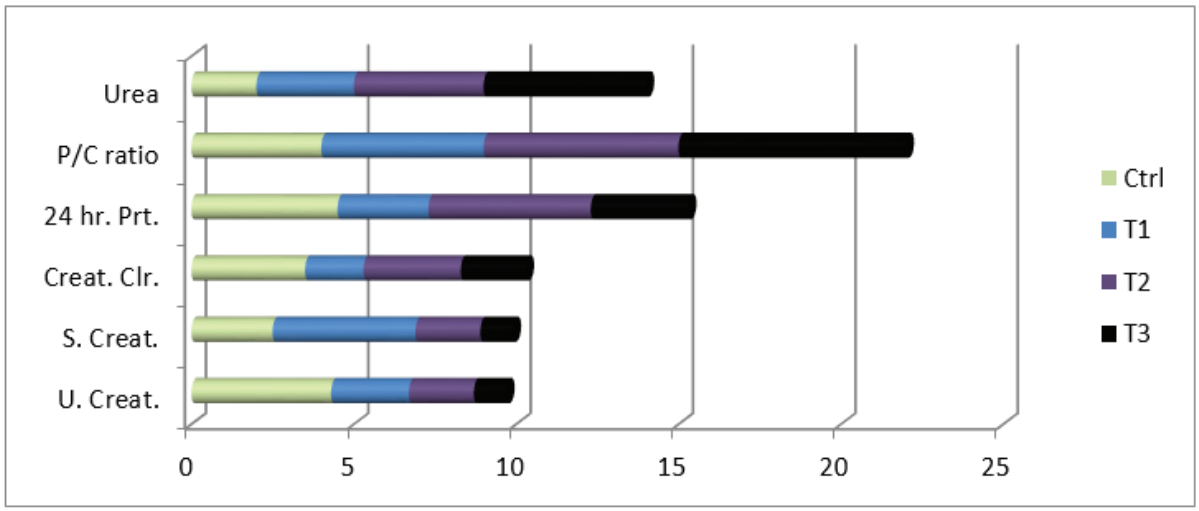


Figure 2: Statistical histogram of chemical variables related to rats treated by heroin.

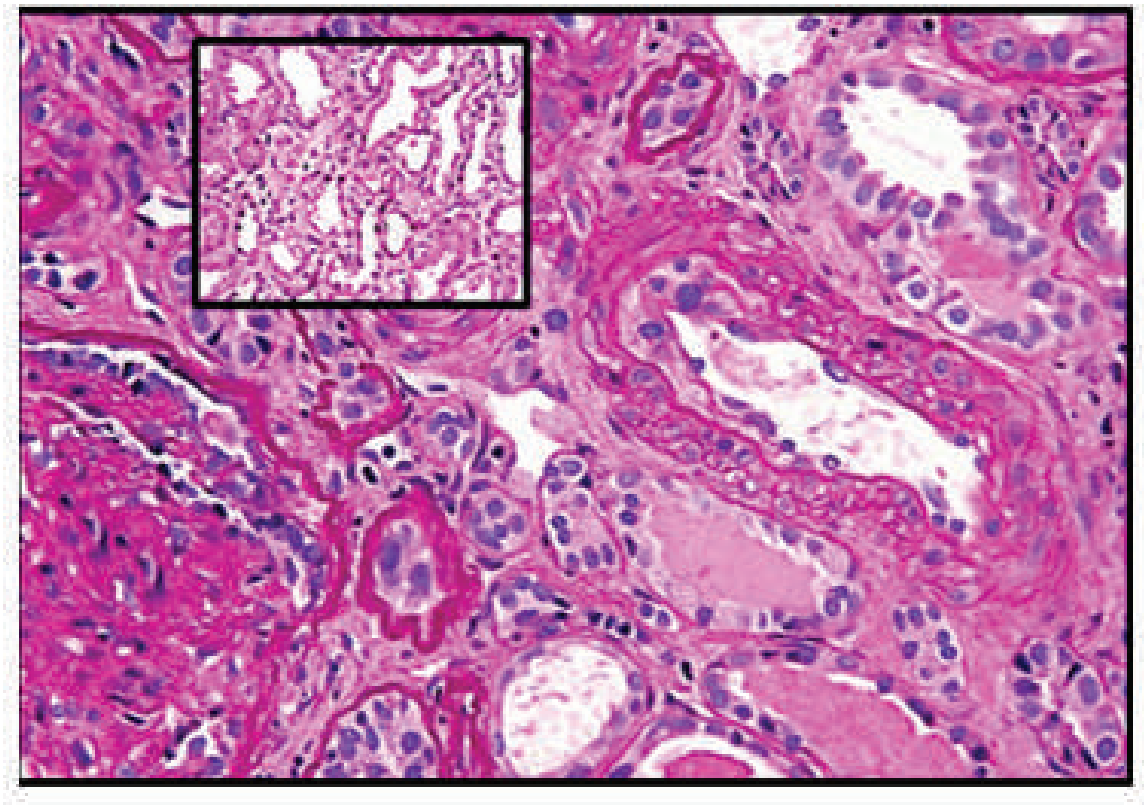


Figure 3: necrosis leading to apoptosis with exfoliation in some tubular parts of the kidney.

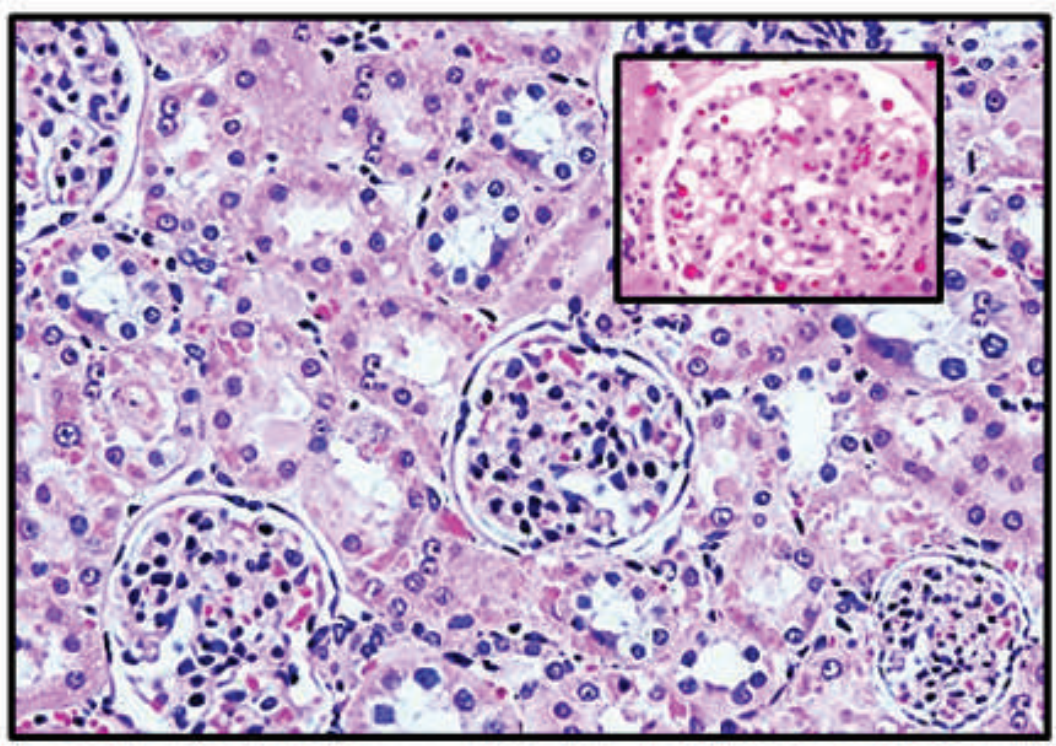


Figure 4: desquamation accompanied with leukocytosis and pigmented destructed corpuscles.

Discussion

Heroin sways rat's kidney injury viaintensificationof protein in tissues involved with inflammation step forwardto kidney failure inducing a dose-dependent raise in albumin excretion and serum creatinine concentration[9].Hence we establisheda significant inflate in creatinine concentration in test-3. So this representationprescribed that increase in concentration of serum creatinine preceded by elevation of proteinuria[10].

High dose of heroin can intensify the hazards of kidney tubulointerstitialdestruction through rhabdomyolysis, which can spot proteinuria *per se*involved directly to this type of damage[11].As another option, proteinuria powereffectively enlightened degradation of kidneyphysiologyviainitiationof hypercholesterolemia, about30days after injection[12]. Supposed toxicity by both of hyperlipidemia and proteinuriahavebeingamechanisms of choice for renal injuryafter suggestive ablation in this rats.

Heroin sometimes linked to liver failure, that may be sway renal physiology through subsequent hypertrophy of glomerulus leading to a suggestive hyperfiltrationas a sign of renal disease[13]. The largevacuolatedrenalepithelium was related strongly with huge proteinuria resulting by case of hyperfiltration, in glomerular sclerosis conditions. Long-term consume of heroinmaytriggerpalliative neuropathy, an irreparablesyndrome that can developed into kidneyinjury, or chronic casefailure[14].

Histological characteristics of our present models distinguished by verdicts of a scattered spreading of destructed and non-destructed renal tubules, overlapped intensively with normal renal unaffected tissues.intermingling of both destructed and non-destructedrenaltubules is features of many advancing kidney illnesses in all laboratory studies and can be assist in understanding the pathogenesis of disease by taking in part that destructive epithelial cells will secrete many growth factors and cytokines[15]. The histological nature of these affected tubules looked atrophic without appearance of markers specialist with distal tubular, but some slides showed supposed remnants of a plasma membrane specializations. This is like to be parts of the otherproximal tubules so[16]. Verifying the exact diagnosis will depend upon using modern histoimmunological techniques for revealing of specific

antigens to proximal tubular cells in the epithelial cells of the atrophic tubules.

Another impressive histological verdict was that manifest affected dilated tubules after injection by 10 weeks. The most prominent signs of these slides including tubular dilation, tubular atrophy, tubulointerstitial fibrosis, with formation of different cast formation that has been considered the noticeable of end-stage renalinjury[17]. A suggestive disruptive physiological modifications in addition to raised chemical metabolism in the hypertrophic (dilated) tubules leading to probable tubular atrophy developed subsequently to tubulointerstitial scarring, this is based on conclusive scientific evidences[18]. The tubulointerstitialpathologicalchanges supposed in the instant model incorporated outwardly unconstrainedbloomingof both large dilated tubules and atrophic tubules. Atrophic tubules case were affiliated with a PCNA-positive cells and smack-like-thickened basement membrane, while the dilated tubules cases seemed to be related to being originating in the spaces of distal tubule. Another noticed observations are that atrophic tubules were abundantbetween5 to 7 weeks after injection of heroin, while dilated tubules numbers being many after 10 week of transaction[19].

Conclusion of this proposal is that heroin doses have gradual and significant impacts on the kidneys physiology through obvious destruction and injury in the renal tissues.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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Effect of Environment Factors on Life Cycle of Forensic Entomology: A Review

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Abstract

Pests play an important ecological part in organic matter decomposition. As a natural means of survival, sarcosaprophagous fly usually prefers to locate and consume a food source such as a cadaver as a natural means of survival. Sarcosaprohagous fly larvae are frequently encountered by judicial entomologists during post mortem investigations. The most relevant colonizers are the oldest individuals derived from the first eggs placed on the body. The stage of the oldest maggots provides the accurate estimate of post mortem interval. Judicial entomology is predictable in numerous countries as a serious tool for forensic investigations. Judicial entomology requires extensive information on the local pest population, is subject to many environmental factors (temperature being one of the principle variables), and they need access to the bodies. The maggot fly crawling on the dead bodies are broadly considered to be just another disgusting element of decay and are not collected at the periods of autopsy. This paper reviews several methods of PMI assessment, this procedure comprises the aggregation of convenient information for the growth of the types of pest at a variation of pertinent temperatures and contemplation of the extra living things and nonliving things aspects that can disturb evolving average.

Keywords: *judicial entomology, periods since decease, decease investigation, blowfly, decomposition, PMI, periods since decease*

Introduction

Entomology is the education of pests and the associated invertebrate animal (shellfish, spiders and so on). Once this knowledge is utilized for supporting in permissible inquiries, it is termed legal entomology. The greatest visible type of judicial entomology is used in the investigation of decease, abuse and neglect cases [(1)] Judicial entomologist's ability affords an impartial evaluation of period meanwhile decease along with extra worthy evidence regarding the situations nearby the target's disappearance, with the period of passing, position of passing, motion or stowage of remnants afterwards expiry, definite spots of damage on the physique, next to mortem objects on the frame, utilize of medicines, and ability uniform afford evidence for concerning a doubtful to the sight of a offense, to a kid disregard or sexy harassment issue, along with in the documentation of doubt [(2)] . As pest evolution is primarily governed by temperature, where this connection has been quantified for a type, the stage of a sample can be determined based on the level of evolution and the thermal history at which that sample developed. Where immature pest sample are not present or have already completed evolution, as is often the case in advanced stages of decomposition. The function of a criminal entomologist in an offense inquiry ability be a main single. His/her function is to assemble and recognize arthropod samples and at that periods explicate conclusions in connection to ecological variables. judicial entomology is now an integral part of a decease investigation when estimating the periods since decease beyond 72 h. Judicial entomology is considered the most accurate method for estimating the elapsed periods since decease, particularly when more than 3 days have elapsed [(3)]. Nearby are through 60 pest relatives whose show a significant function in cadaver

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ecosystem. Nevertheless, solitary the connections (Calliphoridae, Sarcophagidae, Staphylinidae, Cleridae and Dermestidae) of Coleoptera (beetles) are the greatest significant to be utilized in judicial entomology. Abroad of entirely the pests stay in a deceased form, the worms of blowflies (Calliphoridae) and meat hovers (Sarcophagidae) are in charge for the most ingesting of earthly corpse. Temperature and arrival to a physique are the double greatest imperative stage disturbing pest alternation. Temperature is the maximum imperative changeable manipulating the proportion of worm growth. rise temperatures commonly diminish the expansion period of the hovers. Great accumulations of dipteran maggots (worm crowds) progress hotness owing to their frenzied effectiveness and rapid breakdown, therefore levitation the microenvironmental temperature. The association among pest expansion and temperature has been fully-recognized [(4)].

The sequence or arthropods growth is frequently influenced and affected by temperature and moisture. In heater temperature and rise humidity situation, pests have furthermore been recognized to produce quicker. The reverse situations have similarly been well-known to delay pest growing pointedly.

Progress proportion of the hovers are regularly applied to evaluation PMI in killing realization in the initial little weeks next doom. Then expansion of juvenile pests is temperature reliant on, PMI is usually considered by the collected grade diurnal/period (ADD/ADH) pattern (amount of calorific period possessed to spread separately evolving occurrence, K) who is connected through basic temperature named the lesser temperature sill or the growing nil (temperature under whose expansion discontinuity) cost.

The percentage of larval growing rely on its physique temperature, whose is influenced by ecological circumstances as ambient temperature and the warmth created by worm accumulations. In adding, a chief aspect for PMI purpose is that individually types have its private temperature reliant on progress proportion. Consequently, for exact assessment of PMI it is extremely imperative to recognize the developing proportion of respectively judicial fly at certain temperature. In judicial entomology, kind documentations and expansion of starting point information for wholly pest types create in hominid and/or at decease acts are critical fragments of evidence that are compulsory to comportment exact upcoming judicial examines. The dissimilarity of growing periods amongst diverse inhabitants' stresses on precise description of provincial growing periods of individually judicial type.

Because of a raise of judicial entomology and its implementation, it is extremely serious to know the average of evolution of entirely flies of judicial value. This growing education may be in conformity through diverse temperature, moisture domain.

Entomological access for stage definition

Existing methods to phase assessment are created everywhere the sorts-precise periods desired for a juvenile pest to advancement done growing innovations for example distance, weighing, and lifespan series period in connection to temperature. Although events of growing period grounded on distance and weighing are valued, lifetime sequence phase is a favored landmark for stage assessment by reason of the confusing subjects of food, rivalry, and implementation of diverse conservation means for judicial sample (decrease) on weighing and dimension [(5) (6)]. Thus, definition of sample stage is predominantly created on prearranged evolution information describing the expectable connections amongst temperature and pest growing for the beginning and achievement of separately lifetime period of pest evolution. usable position facts specifics the time of evolution of exact lifetime phases of young pests surrounding ovum, larval instars, pupation, and eclosion beneath a variety of continuous or changeable temperatures [(7)]. The implementation of facts describing the connections amongst pest evolution and temperature is at that time utilized in numerous design methods to prophesy pest sample stage founded on the current times past of the composed judicial sample.

Isomorphen and isomegalen illustrations

The unpretentious method is called an "Isomorphen illustration" who is fundamentally a throw scheme of the periods after spawns hatching till eclosion strategized contrary to continual temperature. Related fault rod affords a 95% sureness intermission for individually growing occasion. A minor different, named "isomegalen illustration", schemes larval magnitude meanwhile

shading (measurement, weighing, or thickness) relatively than lifespan periods counter to temperature. Usage of magnitude as a constituent inside the isomegalen illustration has the benefit of larger periods idea determination likened to lifespan period occurrence innovations for phase assessment; nevertheless, extent actions have been described as deprived pointers of stage [8] [9].

Assessment of sample stage is accomplished by substantial correctness wherever the warm air antiquity of the sample observed is dependable through the continual temperatures utilized to produce the position figures of the illustration. Extensive fault arises, still, in the resultant stage assessment via this method once the ambient temperatures beneath who sample are evolving on decaying remnants alter finished periods. For example, the common of corruption acts inside who disintegrating remnants are create involvement changeable temperature conditions, a sequence of scientific representations have been advanced whose are commonly extra valid and broadly utilized Table (1).

		T°C INCREASE	**T°C DECREASE**	**DAMPING**
LOCAL	LARVAL MASSES	Up to tens of °C above ambient - limited to the species-specific upper threshold [22, 23]		Limitation of T°C decrease [16]
	THERMAL REGULATION BEHAVIOUR	Larvae as close as possible to the species-specific optimum dev. T°C [21, 24]	Larvae remaining above the specific threshold [21]	Larvae optimizing T°C (as close as possible to the species-specific optimum) [21, 24]
	BODY COOLING	Only significant for short-term PMImin estimation based on first developmental instars [13, 14]		
	CADAVER THERMAL INERTIA & THERMOGENESIS	Heating due to bacteria. Likely negligible (restricted to aerobic decomposition & inner parts of carcasses) [17]		Smoothing of ambient T°C changes - varying according to body mass, clothes, etc. [13, 32]
	CONCEALMENT			Smoothing of ambient T°C changes - varying with concealment conditions [13]
SURROUNDINGS	INDOORS	Can be important with functional heating	Can be important with air conditioning	Low with openings, high with heating/air conditioning [31]
	SUN EXPOSURE	Direct sunlight: T° increase during daytime, but most larvae avoid direct sun exposure. [19, 38]	Only in shaded areas. Limited if reference weather station data have been collected under a shelter [35]	
	WIND EXPOSURE		Usually, low except in constantly windy areas (e.g., seashores) [38]	

Table 1: Qualitative checklist of factors potentially increasing, decreasing or damping the temperature perceived by necrophagous larvae during their development. Factors applying only while insects are on the cadaver are indicated in italics, and factors occurring only during feeding stages are indicated in bold italics.

Thermal summation pattern

The greatest frequently practical process for displaying pest evolution averages in a judicial situation is the current summation pattern who put on a lined reversion investigation to the confident connections among temperature and evolution.1 Pest evolution can be unhurried at nearby pauses ended a series of temperatures, and wherever the average of evolution (unhurried as reciprocals of evolution periods, 1/*D*) is plotted contrary to temperature, a crescent -formed bend outcomes [10] . At temperature dissipations, pest evolution is moreover reduced or wholly paused conforming to a higher and minor growing verge. A huge quantity of the influences among temperature and evolution is lined among the higher and lesser growing edge (types precise). Lined reversion ability therefore be utilized to regulate an *x*-interrupt (minor growing edge, *T*L) and converse of the grade of the lined deterioration (current synopsis continuous, *K*) whose permit forecast of evolution periods since the current times gone by of a example.1 Underneath this lined deterioration typical, evolution is restrained as biological periods through parts of "grade times" or "grade times", wherever single grade daytime is unique grade overhead the lesser growing verge ended moreover 24 times or 1 period, correspondingly. Individually lifespan period (egg, initial instar, or pupation) necessitates a convinced quantity of amassed gradation periods to progress to the subsequent lifetime period and comprehensive evolution associating to *K* [11]. Typical repetition, below this process, would be to hindmost pests composed since a section at a relentless, measured temperature, greatest periods forgotten at fact of eclosion, and withdraw the physical periods anticipated for test center evolution as of the whole biological periods desired for evolution in this type. After utilized in aggregation by misconduct part temperatures, the dated of periods passed amongst oviposition and pest gathering at the division may before be intended. Challengingly, although lined replicas have the benefit of effortlessness and permit assessment of lesser growing edges and current synopsis factors, they do not integrate the nonlinearity experimental in pest evolution at little and rise temperatures. More than a few substitute replicas have been projected with a reviewed lined typical28 that analyses an enhanced suitable for *T*L and *K* by secretarial for the in-height inconsistency at dissipations of the lined series and numerous nonlinear methods29–31 encircling a new "novel simulation typical" named "ExLAC" [12].

Curvilinear models/ExLAC

Nonlinear or rounded replicas ability extra exactly designate the connection among evolution and temperature for pest inhabitants via integrating the curvilinearity experiential at the high and lesser temperature dissipations of the planned assembly among evolution average and temperature. Although donation enhanced strictures for assessment, the complication of such replicas, nevertheless, lessens the pragmatism of employment to judicial assessment of min PMI. Furthermore, no single rounded typical, overhead others, has been acknowledged that dependably outstrips lined replicas transversely applicable types information [13].

A newly projected curved typical identified as ExLAC has been confirmed to proposal an substitute to lined current precis showing. Whereas the ExLAC prototypical displays negligible recital upgrading ended lined exhibiting, it has the gain of producing fault averages related through the stage approximation resultant by the typical. Once more, the evolution perfect is created on the interval of separately lifetime period through evolution as a job of temperature. An individual exponential task is, yet, practical to respectively lifetime phase, and added limitations are comprised in the typical that interpretation for difference in dimension of effort standards for example current times past figures and the forte of the connection among lifespan period time and temperature [14] [15]. The encompassed dimension of fault for the entered temperature information proposals a separate benefit completed the presently practical current synopsis perfect. Typical exercise contains the utilized of temperature information collected at the climate position nearest to the corruption act as an amount of the pest sample's current times past previous to gathering. This information is amended for possible difference amongst positions utilized deterioration exhibiting integrating information assimilated at the corruption act subsequent the detection of disintegrating remnants. Whereas this facet of the projected typical suggestions a quantity of possible mistake in guesses of minPMI, the presentation of the typical is solitary a little bit improved than the lined current synopsis process for creating example period [16]. To time, the ExLAC typical

has so far to be carefully assessed and judged previous to employment in judicial exercise, and the lined current synopsis perfect is yet the favorite process of assessing sample stage and thus minPMI.

Present matters

Nevertheless, of the growing exhibiting method engaged to regulate stage, more than a few difficulties stand up in affection to the existing evolution facts utilized in such replicas. Obtainable condition facts for utilized in judicial exercise is classically fixated on the evolution of primary take over dipteran types. A significant form of situation works occurs documenting the evolution of judicially pertinent fly types beneath various continual temperatures inside the test center. Challengingly, situation information is not accessible for all types described in suggestion by corruption acts, predominantly in the situation of alterative pointers of minPMI for example beetles and bloodsucking wasps[17,18]. Facets of lifespan times past and evolution in assembly to temperature are repeatedly indefinite for model composed tainted remnants or of imperfect opportunity for implementation in evolution replicas.

Furthermore, exploration has specified that inhabitants of the identical types' ability change physiologically dependent on their topographical derivation [17].

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Attitude of Prime-Pregnant women toward Normal Vaginal Delivery in Southern of Babylon Governorate

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Abstract

Normal vaginal delivery occurs when the mother birth of the fetus through vaginal canal, during this kind of birth mode, gravid women take special classes about labor and delivery process, how to prepared for it and learn the ways that lead to cope with the fear, anxiety and pain. Descriptive study cross sectional design, the study carried out to identify the attitude of prime-pregnant women regarding normal vaginal delivery in southern of Babylon governorate in Iraq. The results show (39.5%) as a highest percentage of study sample at age ranging between (19-22) and more of them (33.0%) with secondary school as level of education, also the assessment of response rate for prime pregnant women regarding overall domain of normal vaginal delivery was (Positive) for attitude regarding physiological delivery at mean of score (2.46) and attitudes regarding perceived behavioral control of normal vaginal delivery at mean of score (2.39) while the assessment of attitudes regarding personal and practical choice and sources of motivation was (Not sure) at mean of score (2.12).

Keywords: *Attitude, Prime-women, Normal Vaginal Delivery, Southern Babylon governorate.*

Introduction

Pregnancy is a major alteration in a woman's life which leads to multi psychological changes than each other life condition in additional to puberty during pregnancy, the shape and size of woman's body is changed, and the whole organ systems change their roles to make an environment that save and supply the growing fetus. Pregnancy is divided into three trimesters, each a 3- month period. During the pregnancy period every trimester has its own developmental characteristics for the mother and fetus [(1)].

The rate of mothers who don't opted the physiological birth and undergoing to other mode of delivery in the absence of medical indication is increasing in the world. Women attitude have an essential role in the request or selecting a birth mode. Poor the awareness, knowledge, beliefs, attitudes and behaviors of pregnant women which effect on the choice of delivery mode, therefore in many cases lead to avoided the physiological birth [(2)].

Primary signs of labor that include descent of the fetus, Braxton Hicks constriction, cervical dilation and effacement, bloody show, sudden active energy and other signs spontaneous rupture of membranes may occur [(3)]. Benefits of physiological birth includes decrease the pain in postpartum period; faster physical recovery from the delivery ;increase the women welfare as an outcome of the delivery ; promote the relationship of mother with her baby; decrease probability of postpartum depression; a quiet and more stable baby; and facilitate breastfeeding experience for the women; active respiratory transmission into the baby; and more active digestive canal colonization which stop the allergy in the baby [(4)] .

Health education can play an important role to increase knowledge, and change attitude and behavior of pregnant women using applicable theories and models [(5)]. The aims of antenatal (childbirth) health education

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are to increase women's trust in their own capability to deliver, to improve knowledge and attitude about normal vaginal delivery, and to assist pregnant women in developing personal birth plans which provide a way map for save birth as normal as possible in spite of if complications happened [6].

Many studies have shown lowest ratio of normal vaginal delivery rather than other type of birth in the world. In the united states was 22%, 25% in Brazil was 25%, 27% in Chile, while from 17- 40% in 19countries of Latin America. The recommendation of (WHO) show that the rate must not overrun 15% of whole deliveries [7]. In Middle East countries like Iran (NVD) had lowest rate while other delivery like (CS) have the highest rate was (41.9%) in 2008 and it considered as the second highest rate among other countries . Isfahan in the south of Iran has even higher rate than the national rate (62%) in 2012 [8].

In Iraq, the normal vaginal delivery rate has begun to decline with increasing the percentage of cesarean section and other delivery, in the public sector was 24.5% during 2009 increased to 25.8% during 2010, whereas in the private sectors the percentage was very much higher reaching 75.8% and 79.5% during the years 2009 and 2010 respectively [9].

The study aim was to identify the level of attitude of prime-pregnant women regarding the normal vaginal delivery and find out the relationship between the subjects of attitude and their variables in southern Babylon governorate.

Material and Methods

A descriptive study was carried out in primary health care centers at southern of Babylon governorate, the study was conducted on prime pregnant women in second and third trimester of pregnancy in order to assess their attitude about normal vaginal delivery by using a questionnaire, the study was initiated from (25 March 2018 to 10 August 2018). The study was conducted in southern Babylon province at (8) centers of primary health care, these centers belonging to Al-Hashimya sector and selected randomly to current this study and gathering the data.

Non probability "convenient" sample consists of (200) prime pregnant women has been chosen from 8 primary health care centers at Al-Hashimya sector in southern of Babylon governorate, all of them are registered in the primary health care centers at antenatal period (second and third trimester) of pregnancy. The tools which were used in this study (questionnaire form) were opted after a reviewing the previous literature and were exposed to many modifications by researcher for adopting as an instrument to use in the study to identify the attitude of prime pregnant women regarding normal vaginal delivery at southern Babylon governorate.

Results

the distribution of the pregnant women socio-demographic characteristics by the frequencies and percentage have been taken under considerations. Results indicate that most of women (39.5%) are within second age group (19-22) years old. Concerning women level of education and their occupation. A (33.0%) and (67.0%) respectively were secondary school graduated and works at home. Regarding monthly income, it is obvious proportion (54.5%) were satisfied to certain limit and (66.0%) of them residences at urban areas. Finally, in this table, women their husband educationally and occupationally, (38.0%) of them are primary schools graduated and (57.5%) were free works (self-employ).

Table (1) concerned with assessment of women attitudes about normal vaginal delivery. A majority of (50% and 54%) have positive attitudes towards normal vaginal delivery (mean of score 2.34 and more). A majority of (61%) of women were not sure attitudes towards practical choice and sources of motivation (mean of score 1.67-2.44).

Table (2) show that there is a significant relationship between women's attitudes and their demographic regarding normal vaginal delivery at p-value <0.05. As well as, there is high significant relationship between women monthly income and woman's husband occupation at p-value <0.01. Residency insignificant relationship with women attitudes at p-value >0.05.

Table (1): Overall Assessment of Pregnant Women Attitudes by their Responses Domains

Attitudes Domains	Scale	F	%	S.d.	M.S.	Assessment
Attitudes Regarding Normal Vaginal Delivery	Agree	96	48.0	0.53	2.46	Positive
	Uncertain	100	50.0			
	Disagree	4	2.0			
	Total	200	100			
Attitudes Regarding Perceived behavioral control of Normal Vaginal Delivery	Agree	85	42.5	0.55	2.39	Positive
	Uncertain	108	54.0			
	Disagree	7	3.5			
	Total	200	100			
Attitudes Regarding Personal and Practical Choice and Sources of Motivation	Agree	51	25.5	0.61	2.12	Not sure
	Uncertain	122	61.0			
	Disagree	27	13.5			
	Total	200	100			

F= Frequency, %= Percentage, S.d= Stander deviation, M.S.= Mean of score " Cut off point (0.66), Poor (mean of score 1-1.66), Fair (mean of score 1.67-2.33), Good (mean of score 2.34 and more)".

Table (2): Correlation between the study sample attitudes and their demographic Characteristics

Demographic data	Scale	Overall Attitudes				χ^2 obs.	d. f	χ^2 crit.
		Agree	Uncertain	Disagree	Total			
Age (years)	15-20	7	46	2	55	23.229	8	15.507
	21-26	29	45	1	75			
	27-32	27	22	0	49			
	33-38	5	10	0	15			
	≥39	3	3	0	6			
	Total	71	126	3	200			
	P-value= 0.003 →S							
Women educational Level	Not read and write	11	29	2	42	15.931	8	15.507
	Read & write	4	12	0	16			
	Primary school	22	44	0	66			
	Secondary school	6	16	1	23			
	Diploma & above	28	25	0	53			
	Total	71	126	3	200			
	P-value= 0.043 →S							
Women occupation	Working	26	23	0	49	13.571	4	9.488
	Students	2	14	1	17			
	House wife	43	89	2	134			
	Total	71	126	3	200			
	P-value= 0.009 →S							

χ^2obs. = Chi-square observer, χ^2crit. = Chi-square critical, Df= Degree of freedom, P-value= Probability value, S= significant, NS= non-significant, HS= high significant

Discussion of Results

Results indicated that most of sample are within second age groups (19-22) years old these result correspond with results of study conducted in Niknafs Maternity Center in Iran to identify the knowledge and attitudes of women regarding modes of delivery by (Aali *and* Motamedi, 2005) [10] where their findings were (35.3%) of participant within (20-24) years as a high proportion. Concerning women education level and their occupation a (33.0%) with secondary school graduation while more than sixty percent are housewives as a high percentage these finding coincide with results of study carried out by (Hassan *et al.,*2016) [11] to investigate the awareness and attitudes of pregnant women regarding anesthesia at labor where their results was (37.6%) with primary school and (79.0%) housewives women, also agree with results of study conducted at Al-Kadhumyia Teaching Hospital to identify the preference and knowledge of delivering mothers toward normal vaginal delivery and caesarean section by (Habib *et al,* 2011) [14] where their results were (47.3%) with primary school and (87.4%) house wife as highest percentage.

Regarding the pregnant women monthly income, it is obvious proportion of (54.5%) were satisfied to some extent as a higher proportion this result agree with finding of study conducted to recognize the delivery mode and subsequent long-term sexual function of women with primiparous in Ankara by (Gungor *et al,* 2007) [15] where their result represents (91.4%) for Somewhat satisfied as high percentage and supported by a study done by (Mbada *et al,* 2014) [16] to recognize the attitudes and knowledge of pregnant women regarding exercise during antenatal period in Nigeria where their result were only (27.0%) is not satisfy about monthly income as highest proportion. Related residences the higher percentage was lived in urban area this result come in consistent with (Lilungulu *et al,* 2016) [17] where they conducted a study to report the attitudes, knowledge and practice toward prenatal services for pregnant women in Tanzania the results revealed that majority of people of urban residency as a highest percentage while this result disagree with the result done by (Hailu *et al,* 2010) [18] to recognize the knowledge of pregnant women regarding obstetric danger signs in Ethiopia where the higher proportion were among rural area as (86.7%).

Finally in this table and regarding the husband educational level the higher percentage represents less than fifty participants husbands with primary school this result disagree with the study carried out by (Moosavi *et al,* 2017) [19] to identify factors may influence in choosing method of delivery in Iran where the higher percentage was (33.3%) for husband with high school, while related the husband occupation the high percentage was (57.7%) free business these finding agree with (Aali and Motamedi, 2005) [10] where they find the higher percentage among free business husband (48.6%).

Regarding the prime pregnant women attitude toward normal vaginal delivery, the results in table indicate that the higher percentage of pregnant women (61.0%) were not sure in domain of (attitudes regarding personal and practical choice and sources of motivation), while the attitude were positive assessment in two domain (attitudes regarding NVD and attitudes regarding perceived behavioral control of NVD) these results agree with study done by (Nisar *et al,* 2009) [20] who tested the knowledge and attitude and preferences method of delivery for pregnant women among those attended the antenatal clinics in Isra university hospital at Hyderabad city where their results indicated that majority of pregnant women (83.6%) who are included in the study had positive attitudes regarding vaginal delivery process , also the findings agree with the study done at Fatemiyeh hospital in Hamadan ,Iran to assess the attitudes and knowledge of women regarding normal physiological delivery by (Masoumi *et al,* 2014) [21] where they found that majority of pregnant women had moderate attitudes regarding physiological labor and delivery .

Results show that there is significantly relationship between demographic characteristic of pregnant women and their attitudes regarding normal vaginal delivery was found at p-value <0.05 except one item (residency) was non-significant relationship at p-value 0.414 these results agree with many studies , one of them conducted in Iran by (Bita *et al,* 2014) [22] where the results indicated that there is a significant relationship between attitudes of

pregnant women and their demographical data regarding physiological labor and delivery process . Attitudes can be tested through people feelings toward the concepts of special concerning, they sometimes develop through individual experience.

Conclusions

This study focus on clarify the attitude of prime pregnant women regarding normal vaginal delivery in southern of Babylon governorate at primary health care centers, the main conclusions of the study are the higher percentage of the study sample (39.5%) who participated in the study with age between (19-22), and most of them with secondary school as level of educations as highest proportion. The result show there was positive assessment in two domain of prime pregnant women attitude regarding NVD and another domain of attitude was not sure assessment.

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Estimate the Serum Level of IL-17A and TGF-β1 1 in Iraqi Generalized Vitiligo Patients

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Abstract

Vitiligo is an acquired idiopathic skin disease characterized by white macules result from the destruction of melanocytes. Several cytokines have been implicated in the pathogenesis of vitiligo, keeping this in view the current study aimed to evaluate the serum levels of IL-17A and TGF-β1 in 25 patients with generalized vitiligo and compared them with healthy individuals in order to investigate whether these cytokines imbalances plays a role in the pathogenesis of this depigmentary disorder. Results of the present study showed that the serum level of IL-17A in vitiligo patients was increased significantly ($p \leq 0.05$) while the serum level of TGF-β1 was decreased significantly ($p \leq 0.05$) as compared with healthy control. According to the gender of patients, males had a slightly non-significant increased ($p<0.05$) in the level of IL-17A and TGF-β1 as compared with females. Patients with early onset vitiligo had non-significant increased ($p<0.05$) in the level of IL-17A and TGF-β1 as compared with late onset vitiligo patients. No significant differences ($p<0.05$) were detected in the levels of IL-17A and TGF-β1 in patients regarding the clinical types of vitiligo, family history and Koebner phenomenon. Patients with active disease had a significant increase ($p \leq 0.05$) in the level of IL-17A, though a significant decrease ($p \leq 0.05$) in the level of TGF-β1 was noticed as compared with both patients with stable disease and healthy control groups. Serum levels of both IL-17A and TGF-β1 have been shown to be negatively correlated with the disease duration ($r=-0.174$, $p=0.405$) and ($r=-0.057$, $p=0.788$) respectively.

Keywords: Vitiligo, IL-17A, TGF-β1.

Introduction

Vitiligo is an acquired or inherited depigmentary disorder characterized by the development of milky macules in the skin, hair and mucosal surfaces due to the selective loss of melanocytes[1].Its global prevalence has been ranging from 1-2% with no predilection for age, sex, or race, white macules may progress slowly or rapidly, some patients have reported additional depigmentation following periods of physical or emotional stress[2]. Vitiligo is classified in to three clinical types depending on the distribution and extension of lesions; localized, generalized (which is further subdivided into vulgaris and acrofacial vitiligo) and universal vitiligo[3].Several pathophysiologic theories have been suggested in order to explain the loss of epidermal melanocytes, however the autoimmune theory has been strongly implicated in the development of this disease for the following observations: association of vitiligo with autoimmune conditions, demonstration of organ-specific and circulating anti melanocyte autoantibodies in the serum of patients, infiltration and alteration in cytotoxic T cells and helper T cells in histological studies of skin biopsies[4].Naive helper T cells develop into four types, T-helper1 (Th1) which produce interferon (IFN)-γ and tumor necrosis factor (TNF)-α, Th2 which secret interleukin (IL)-4, IL-5 and IL-13,Th17 which produce IL-6 and IL-17 , and regulatory T cells (Tregs) which synthesize IL-10 and transforming growth factor β(TGF)-β[5].A novel hypothesis has been proposed that the progression of autoimmune disease, which vitiligo is one of them is partially dependent on the skewing of immune responses towards Th1 or Th17 and away from Th2 and Tregs cells, in addition the balance between Th17 and Tregs cells has been associated with the pathogenesis of several autoimmune diseases[6].

Materials and Methods

This study was carried out on 25 Iraqi vitiligo patients who were referred to AL-Kindi Teaching Hospital, and 25 apparently healthy control matched for age and gender .The mean age of patients and control subjects was 28.48±1.761 and 29.05±1.31 years respectively. Exclusion criteria includes patients on treatment with systemic steroids or immunosuppressive drugs within the last 4 weeks, as well as those with an autoimmune disease which might affect the immunological status of the patients. The level of serum cytokines (IL-17A and TGF-β1) were detected by using Enzyme Linked Immunosorbent Assay (ELISA) technique kits. Statistical Package for Social Sciences (SPSS) version 25.0 was used to analyze the data of this study which were presented in terms of mean ±Standard Error(S.E.), differences between means were assessed by one way ANOVA test and considered significant when the probability(P) value was≤ 0.05[7].

Results

Among the 25 patients in this study; females group (16 patients) shown higher incidence than males (9 patients) with a ratio equals to 1.77:1, their ages ranged from (15-45) years with a mean of (28.48±1.761) years, the duration of the disease ranged from (1-12) years with a mean of (5.28±0.59) years, no significant difference was found between patients and control groups with respect to sex and age. According to the age at onset: 12 patients (48%) had early onset vitiligo (before 30 years) while 13 patients (52%) had late onset vitiligo (after 30 years of age). The disease was active (new lesions or progression of old lesions within the three months prior to this study) in 18 patients (72%) and stable in 7 patients (28%). About 22 patients (88%) had vulgaris vitiligo while 3 patients (12%) had acrofacial vitiligo. A positive family history was found in 7 patients (28%), while the Koebner phenomenon was detected in 3 patients (12%).

Serum Level of IL-17A and TGF-β1 in patients and control groups:

Results of the current study showed that serum level of IL-17A was increased significantly (p≤0.05) while the serum level of TGF-β1 was decreased significantly (p≤0.05) in vitiligo patients as compared with healthy control (Table.1).

Table.1: Mean serum level of IL-17A and TGF-β1 (pg/ml) in the study samples.

Samples	No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Vitiligo Patients	25	114.517±7.447a	12038.16±956.22a
Healthy Control	25	69.116±4.877b	17042.39±651.59b
*Different letters=Significant difference (P≤0.05) between means.			

According to the gender of patients, it was noticed that the mean level of both IL-17A and TGF-β1 in males showed a slightly non-significant increased (p<0.05) as compared with females. Patients with early onset vitiligo had non-significant increased (p<0.05) in the mean level of both IL-17A and TGF-β1 as compared with late onset vitiligo patients, no significant differences (p<0.05) were detected in the serum levels of IL-17A and TGF-β1 in patients regarding the clinical types of generalized vitiligo (Table.2).

Table.2: Mean serum levels of IL-17A and TGF-β1 (pg/ml) according to patients' gender, age at onset and the clinical types of vitiligo.

		No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Gender	Male	9	116.568±12.993a	13235.66±1628.27a
	Female	16	113.364±9.369a	11364.56±1204.04a
Age at Onset	Early Onset	12	116.211±10.782a	123419.44±1405.07a
	Late Onset	13	112.950±10.703a	11686.20±1375.22a
Clinical Types	Vulgaris Vitiligo	22	114.981±8.357a	12038.53±1087.58a
	Acrofacial Vitiligo	3	111.114±12.758a	12035.38±1444.55a
*Similar letters=No significant difference (P> 0.05) between means.				

Patients with active disease had a significant increase ($p \leq 0.05$) in the serum level of IL-17A and a significant decrease ($p \leq 0.05$) in the serum level of TGF-β1 as compared with both patients with stable disease and healthy control groups. However no significant differences ($p < 0.05$) were recorded when patients with stable disease were compared to healthy control as regards IL-17A and TGF-β1 means serum levels (Table.3).

Table.3: Mean serum levels of IL-17A and TGF-β1 (pg/ml) according to the activity of vitiligo.

Samples	No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Patients With Active Vitiligo	18	124.833±8.565a	10574.41±1049.66a
Patients With Stable Vitiligo	7	87.991±9.747b	15802.09±1415.09b
Healthy Control	25	69.116±4.877b	17042.39±651.59b
*Similar letters=No significant difference (P>0.05) between means. *Different letters=Significant difference (P≤0.05) between means.			

According to the patients family history and Koebner phenomenon, no significant differences ($p < 0.05$) were detected in the means levels of IL-17A and TGF-β1 (Table.4).

Table.4: Mean serum levels of IL-17A and TGF-β1(pg/ml) according to patients' family history and Koebner phenomenon.

		No.	Mean±S.E. IL-17A(pg/ml)	Mean±S.E. TGF-β1(pg/ml)
Family History	Positive	7	119.816±15.221a	13573.64±2125.61a
	Negative	18	112.45±8.722a	11441.02±1063.81a
Koebner phenomenon	Present	3	111.869±3.062a	12014.52±1469.33a
	Absent	22	114.878±8.477a	12041.38±1087.14a
*Similar letters=No significant difference (P>0.05) between means.				

In patients, serum levels of both IL-17A and TGF-β1 have been shown to be negatively correlated with the disease duration (r=-0.174, p=0.405) and (r=-0.057, p =0.788) respectively (Table.5).

Table.5: Correlation between serum levels of IL-17A and TGF-β1(pg/ml) and disease duration.

		r	p	Significance
Disease Duration (years)	IL-17A(pg/ml)	-0.174	0.405	Non-significant
	TGF-β1(pg/ml)	-0.057	0.788	Non-significant

Discussion

Vitiligo is a cutaneous multifactorial disease, affects both sexes equally, higher incidence of females in this study may be result from the greater psycho-social perceived impact of the disease on their life. The presence of positive family history in first degree relative for some patients may indicate a hereditary factor for this disorder[8].The detection of T-cells infiltration in the perilesional skin of vitiligo macules in previous histopathological studies implicates the cellular immune response in the destruction of melanocytes[9].Th17 cells play a very important role in the pathogenesis of numerous autoimmune disorders by the production of proinflammatory cytokines includes:IL-17,IL-22 and IL-23, recruit neutrophils, and promote inflammation at the site of infection[10].In contrast Treg cells inhibit the immune response by the production of anti-inflammatory cytokines include IL-10 and TGF-β which suppress the activity of a variety of immune cells[11]. Several previous studies evaluate the levels of IL-17A and TGF-β1 in the serum of vitiligo patients in alternative countries and reached for the following results: Khan *et al.*[12] reported that the serum level of IL-17A in 45 Indian patients was increased significantly while the serum level of TGF-β1 was decreased significantly as compared with healthy control which came in agreement with the results of this paper. Basak *et al*.[13], on the other hand, found no significant difference in the serum level of IL-17A in 40 Turkish patients as compared with healthy control, in spite of the positive correlation between IL-17A level and extent of body area involvement. Therefore, IL-17A imbalance may suggest its involvement in the pathogenesis of vitiligo. In contrast, Zhou *et al*.[14] demonstrated an elevated level of TGF-β1 in patients with active non segmental vitiligo as compared to race-,

gender- and age-matched healthy subjects. Moreover the level of serum TGF-β1 in 38 vitiligo patients showed no significant difference in comparison to control group as established by Ghanem *et al.*[15].So TGF-β1 might play a role in the pathogenesis of vitiligo related to the suppressive function of Tregs.

Depending on the outcomes of the present study, which were similar to the findings of Elela *et al.*[16] no differences were established in the means levels of both IL-17A and TGF-β1 regarding patients' gender, age at onset, family history, Koebner phenomenon and clinical types of vitiligo, furthermore IL-17A and TGF-β1 means level were negatively correlated with disease duration, however positive correlation was observed by Tembhre *et al.*[17] in 80 Indian patients with generalized vitiligo. Patients with active vitiligo showed a significant increase in the level of IL-17A while a significant decrease in the level of TGF-β1 in comparison to patients with stable vitiligo and healthy control groups, Bhardwaj *et al.*[18] also detected a significant increase in the serum level of IL-17A in patients with active vitiligo, however TGF-β1 was also increased significantly which differs from the results of this study. Another study done by Dwivedi *et al.*[19]had been reported a significant reduction in the count and percentage of Treg cells in patients with active vitiligo as compared to those with stable vitiligo, which may explain the more reduction of serum TGF-β1 in active disease.

Interleukin-17A is a pro-inflammatory cytokine secreted by Th1 cells in the presence of IL-6/IL-21, TGF-β and IL-1β, it is implicated in the pathogenesis of many inflammatory disorders by inducing the expression of various cytokines, chemokines, adhesion molecules, and growth factors [20]. Higher expression of IL-17 mRNA in vitiliginous lesions provided an evidence for the involvement of IL-17 in the pathogenesis of vitiligo[21].It was noticed that Microphthalmia-associated transcription factor(which play a very important role in melanocytes functions and survival) was decreased in the nucleus of melanocytes when treated with 10ng/ml of IL-17A[18, 22],in addition, it was detected that melanocytes treated with 1and/or 10 ng/ml of IL-17A undergo morphological shrinking which decreased melanin production[22].IL-17A has ability to induce the production of several chemokines from keratinocytes which cause further infiltration of neutrophils, macrophages, and dendritic cells, the presence of these cells coincide with loss of melanocytes in the depigmented white macules[23].IL-17 also induces the production of vascular endothelial growth factor which facilitate the passage of melanocyte-reactive T cells to cutaneous melanocytes in the lesions and caused their destruction[24].

Transforming growth factor β1 is an important immunosuppressive cytokine produced by many cells, such as epidermal keratinocytes and Treg cells, it has the ability to inhibit the proliferation and differentiation of autoreactive CD4+ and CD8+ cells as well as B cells so it responsible for maintaining immune tolerance[25]. Regulatory T cells considered as a main source for TGF-β1 production, therefore the reduction in Tregs cells number in vitiligo patients[26] will effect on the immune tolerance to melanocyte self-antigens as well as decreased the level of TGF-β1 which came in agreement with the results of the present study. Basak *et al.*[13] suggested that the decreased level of TGF-β1 in the serum of vitiligo patients may be due to: the dominant immune response to CD8+ cells and reduction of Treg cells maturation which assisting the appearance of vitiligo lesions. According to the findings of the current study, the significant reduction in the mean serum level of TGF-β1 in patients with active disease may propose severe deficiency in TGF-β1 secretion which enhanced cell mediated immunity that cause melanocyte destruction and the appearing of white macules in patients with generalized vitiligo.

Conclusions: Increased serum level of IL-17A and decreased serum level of TGF-β1 in generalized vitiligo patients support the role of both cellular immune response and altered cytokines level in the destruction of melanocytes and the pathogenesis of vitiligo.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Pattern of Firearm Discharge Residue on Cotton Cloth Substrate to Determine the Range of Firing by 7.65mm Caliber of Country Made and Standard Firearm: An Original Research Study

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Abstract

The main purpose of the study was to collect a data related to country made and standard firearm from close range on cotton cloth substrate to determine the range of firing, type of firearm used i.e standard or country made firearm as limited data are available for such lethality in india. The range of firing is very difficult to investigate by dispersion pattern of Gun Shot Residue in close range firing by country made pistol. This study was done to overcome that deficiency.

Method- This study over a period of 3 years was carried out on live cases brought to ballistics division of CFSL/CBI/New Delhi. Total No. of 180 samples were collected, from three different close range i.e., 4", 8" and 12"on cotton cloth substrate. 90 samples were collected by 7.6mm country made pistol and 90 samples were collected by 7.65mm standard pistol.

Result – Among 180 firing samples during study period, 30 firing has been done from each close range, from standard as well as country made pistol. The result showed the large variation in dispersion pattern of GSR from 4" range. At 4" distance, from country made pistol, the inner dispersion area of GSR was calculated i.e. **(7-19 cm^2 app.),** outer dispersion area of GSR was **(30-70 cm^2 app.)** whereas in case of standard pistol, the inner dispersion area of GSR was **(10-15 cm^2app.),** outer dispersion area of GSR was **(40-70 cm^2 app.)** .

Conclusions- It is found that large variation in dispersion pattern of GSR pattern has been observed, when firing has been done from country made pistol and standard firearm at 4" range. This study must be helpful in estimation of exact range of firing fired from close range by 7.65mm country made pistol and standard pistol by analyzing the dispersion pattern area of Gun Shot Residue.

Key words: *Firearm, Range of Firing, Country made firearm, Gun Shot Residue, Dispersion Pattern, 7.65mm caliber*

Introduction

In India, Country made firearms are most popular among criminals due to their easily availabity and cheap cost.

More than 75% of the cases related to firearms, country made firearms are used in the crime . The trend of country made firearms are increased in last few years. Country made firearms are more popular in UP, Bihar, Haryana and in some border states [1,2]. Use of country made firearm for crime is more common in Uttar Pradesh as compared to other states and firearm fatality rate in this city (Varanasi) was 3.1 per 1,00,000 population [3].

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Forensic experts are facing lots of difficulties in estimating the exact range of firing, in case where country made firearms are used, Particular attention can be drawn in case associated with unlicensed firearms and related evidences such as GSR.[4,5,6] GSR is also known as firearm discharge residue or Cartridge discharge residue. GSR is formed when a cartridge is being fired. When gun is being fired, it releases flame, smoke, burnt, unburnt and semi burnt particles along with bullet. These particles create a circular pattern on target area, when fired from close range.[7,8] Such type of pattern is known as dispersion pattern area of GSR. In this study, Author tried to estimate range of firing by calculating the dispersion pattern area of GSR from close range.

Material and Method

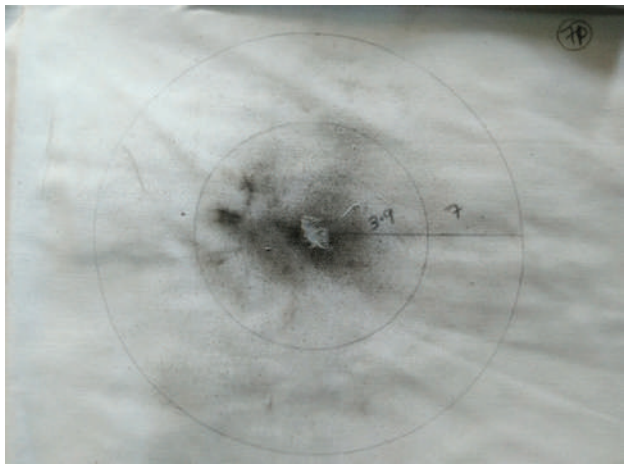
The present study was carried out in an indoor shooting range, of Ballistics division of CFSL/CBI/New Delhi, During period of 1st april 2015 to 22nd july 2018.. The ammunition used for test firing was manufactured by KF (Khadki Factory, Pune), 7.65mm caliber were used for test firing by standard and country made pistol.

The targets were made up of cotton cloth having size 45cm X 45cm (app.), fitted on cardboard sheet having size 40cm X 40cm (app.). The cardboard was fixed to recovery box , Recovery box was 120 cm above the ground. The firearm was fixed in the stand , in front of the target and their position adjusted at different distances according to firing range i.e. 4",8" and 12". Thereafter, GSR pattern on the cotton cloth target was collected carefully and digital camera was used for photographing the image. After that dispersion pattern of GSR was calculated by visual method by taking the outer radius of the circle (outer radius of the circle was taken from the center of the circle to the outer dispersion line) and then inner radius of the circle (inner radius was taken from the center of the circle to the inner dispersion line) and put the formula of area of circle ($A = \pi r^2$). By using this formula, we can find out the dispersion pattern area of the Gun Shot Residue.

Results and Discussion

Total No. of 180 samples were collected, from three different close range i.e., 4", 8" and 12"on cotton cloth substrate. 90 samples were collected by 7.6mm country made pistol and 90 samples were collected by 7.65mm standard pistol. Among 180 firing samples during study period, 30 firing has been done from each close range, from standard as well as country made pistol. The dispersion pattern was somewhat irregularly circular. To find out the dispersion Pattern area, First we can draw two circles, One circle is drawn near the outer dispersion pattern area and another circle is drawn near the inner dispersion pattern area. Now, the dispersion pattern area was calculated by taking the outer radius and inner radius (Outer radius was taken from the centre of bullet hole to the outer dispersion area and the inner radius was taken from the centre of bullet hole to the inner dispersion area), then the area of outer circle and inner circle was calculated by using the formula ($A = \pi r^2$) . By using this formula, the area of dispersion pattern of 7.65mm caliber of country made and standard firearms were calculated. It will help in estimating the range of firing.

How calculation of area of dispersion pattern was done to be demonstrated by taking an example, to be illustrated:



In this fig, First we can drawn outer circle near the outer dispersion pattern area, then we can drawn inner circle near the inner dispersion pattern area with the help of campass by taking bullet hole as a center, the radius of the circle was calculated with the help of ruler.

Area of outer dispersion pattern : Radius= 7 cm

Putting the value of radius in the formulae of area of circle ($A = \pi r^2$),

value of π is 3.14, A= 3.14 x 7cm x 7cm = 53.38 cm^2(app.)

Area of inner dispersion pattern : Radius= 3.9 cm

Putting the value of radius in the formulae of area of circle ($A = \pi r^2$),

value of π is 3.14, A= 3.14 x 3.9cm x 3.9cm = 47.75 cm^2(app.)

Total nos. of 30 firing has been done from each distance and area of dispersion pattern of GSR was calculated. Thereafter, average of all samples were taken from each distance. The result showed the large variation in dispersion pattern of GSR from 4" range. At 4" distance, from country made pistol, the inner dispersion area of GSR was calculated i.e. **(7-19 cm^2 app.),** outer dispersion area of GSR was **(30-70 cm^2 app.)** whereas in case of standard pistol, the inner dispersion area of GSR was **(10-15 cm^2app.),** outer dispersion area of GSR was **(40-70 cm^2 app.)**

Table -1 Showing outer and inner dispersion pattern area of GSR on cloth target fired by 7.65mm calibre of country made pistol at different ranges.

Dispersion Pattern Area	Samples									
	1	2	3	4	5	6	7	8	9	10
	Range 4"									
Outer Dispersion Area	8.3 Cm^2	9.2 Cm^2	12.1 Cm^2	13.5 Cm^2	7.6 Cm^2	14.4 Cm^2	11.2 Cm^2	6.8 Cm^2	10.8 Cm^2	11.9 Cm^2
Inner Dispersion Area	39.4 Cm^2	43.1 Cm^2	52.8 Cm^2	59.4 Cm^2	34.5 Cm^2	54.4 Cm^2	69.5 Cm^2	61.3 Cm^2	66.8 Cm^2	49.7 Cm^2
	Range 8"									
Outer Dispersion Area	19.4 Cm^2	20.8 Cm^2	29.4 Cm^2	28.4 Cm^2	21.5 Cm^2	28.3 Cm^2	22.6 Cm^2	27.6 Cm^2	26.8 Cm^2	25.2 Cm^2
Inner Dispersion Area	72.4 Cm^2	79.6 Cm^2	98.3 Cm^2	92.4 Cm^2	79.3 Cm^2	91.6 Cm^2	78.5 Cm^2	88.4 Cm^2	84.6 Cm^2	83.3 Cm^2

	Range 12”									
Outer Dispersion Area	38.7 Cm2	36.9 Cm2	40.8 Cm2	42.6 Cm2	41.8 Cm2	39.5 Cm2	44.7 Cm2	43.3 Cm2	40.8 Cm2	37.5 Cm2
Inner Dispersion Area	141.2 Cm2	137.2 Cm2	151.8 Cm2	159.4 Cm2	157.7 Cm2	148.5 Cm2	168.5 Cm2	102.4 Cm2	151.8 Cm2	138.6 Cm2

Table-2: Showing outer and inner dispersion pattern area of GSR on cloth target fired by 7.65mm calibre of Standard pistol at different ranges.

Dispersion Pattern Area	**Samples**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Range 4”									
Outer Dispersion Area	10.9 Cm2	11.8 Cm2	12.8 Cm2	11.4 Cm2	14.9 Cm2	13.7 Cm2	13.4 Cm2	10.4 Cm2	14.3 Cm2	10.5 Cm2
Inner Dispersion Area	49.4 Cm2	55.4 Cm2	60.2 Cm2	54.6 Cm2	68.4 Cm2	63.7 Cm2	62.2 Cm2	48.5 Cm2	68.2 Cm2	49.2 Cm2
	Range 8”									
Outer Dispersion Area	20.9 Cm2	21.7 Cm2	24.8 Cm2	24.3 Cm2	23.8 Cm2	21.4 Cm2	22.9 Cm2	24.2 Cm2	23.2 Cm2	21.3 Cm2
Inner Dispersion Area	80.2 Cm2	83.2 Cm2	96.4 Cm2	96.2 Cm2	92.6 Cm2	81.9 Cm2	84.8 Cm2	94.2 Cm2	91.9 Cm2	81.9 Cm2
	Range 12”									
Outer Dispersion Area	36.4 Cm2	38.2 Cm2	42.3 Cm2	46.4 Cm2	40.8 Cm2	39.4 Cm2	41.6 Cm2	37.5 Cm2	39.4 Cm2	47.8 Cm2
Inner Dispersion Area	130.9 Cm2	142.6 Cm2	155.9 Cm2	164.2 Cm2	153.2 Cm2	143.6 Cm2	150.4 Cm2	133.5 Cm2	143.7 Cm2	164.6 Cm2

Table- 3 Showing comparison of dispersion pattern area on Cloth substrate fired by 7.65mm calibre of country made pistol and standard pistol at different ranges.

Range	Dispersion Pattern Area	
Substrate- Cloth	**Country made Pistol**	**Standard Pistol**
4”	I.D : 7-18 Cm2 (app.) O.D : 30-70 Cm2 (app.)	I.D : 10-15 Cm2 (app.) O.D : 40-70 Cm2 (app.)
8”	I.D : 20-30 Cm2 (app.) O.D : 70-100 Cm2 (app.)	I.D : 20-25 Cm2 (app.) O.D : 80-100 Cm2 (app.)
12”	I.D : 35-45 Cm2 (app.) O.D : 130-170 Cm2 (app.)	I.D : 35-50 Cm2 (app.) O.D : 130-170 Cm2 (app.)

Conclusion

It is found that large variation in dispersion pattern of GSR pattern has been observed, when firing has been done from country made pistol and standard firearm at 4” range. This study must be helpful in estimation of exact range of firing fired from close range by 7.65mm country made pistol and standard pistol by analyzing the dispersion pattern area of Gun Shot Residue. This study will be helpful in easy disposal of the cases where, range of firing is to ascertain.

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Ethical Clearance: Yes, From Department of Forensic science, School of basic and applied sciences, Galgotias University, Greater Noida. (as a thesis for PhD., Forensic Ballistics).

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Histological and Physiological Study of the Effects of Exogenous Melatonin on Liver of Female Rats Exposed to Formalin in Drinking Water

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Abstract

Aim: The presented study was established to evaluate the impact of formalin (FA) on the blood levels of superoxide dismutase (SOD), lipid profile, total serum protein and serum albumin in female rats. **Materials and methods:** The animals were assigned randomly into groups of six; control group, and treated other five groups as follow, first group exposed to formalin at 200ppm (F200 group), the second exposed to formalin at 400ppm (F400 group), third group exposed to formalin at 200ppm plus melatonin at 10mg/kg (F200M group), fourth group exposed to formalin at 400ppm plus melatonin at 10mg/kg (F400M group), and the fifth exposed to melatonin at 10mg/kg (M group). Samples of blood from the rats were collected at day one, day and day 30 of the experimental period that was continued for 30 days. **Results:** Using immune-based techniques, the SOD activity, it has been here-resulted in significant ($p<0.05$) after-formalin-exposure-decreases in the blood samples of female rats from F200 and F400 groups, especially, when compared with the control group. The total cholesterol levels revealed significant ($p<0.05$) increases in the blood of the tested animals of the F200 and F400 groups. These increases affected the rates of LDL, VLDL, and triglycerides in the blood of the studied rat-females of both F200 and F400 groups when compared with the control animals. Interestingly, low levels of HDL were noticed in the blood of the animals of the F200 and F400 when comparisons with control animals. The concentrations of the LDL, HDL, and triglycerides in the serum of the formalin-exposed females showed significant ($p<0.05$) low levels when compared with control animals. For day-30 collection, albumin concentrations revealed significant ($p<0.05$) elevation in the blood of the F200 and F400 animals.. Histological examination revealed that the liver of female rats exposed to 200ppm or 400 ppm of formalin for 30 days have been adversely affected. Whereas, the histological examination of the liver of animals treated with melatonin and formalin were less affected due to formalin exposure. **Conclusions:** These results demonstrate the formalin high toxic effects on the hepatic tissues can be overcome by the use of exogenous melatonin.

Keywords: *Antioxidant, formaldehyde, hormones, melatonin.*

Introduction

Environmental contaminants cause direct toxic effects such as endocrine disruption, that are altering the normal hormonal environment and reproduction due to exposed to exogenous chemicals. They alter the metabolism, synthesis, and gene expression of hormones, creating critical situations for the development of the body[(1)]. Formaldehyde (FA) is one of the substances that caused pollutant in many routs .FA can generate irritation toxicity that affects body organs such as eye and respiratory tract with bronchoconstriction and fluid accumulation in these tracts [(2)]. FA is a potent sensitizer and a carcinogenic effect.

666 According to the foregoing, it became clear that there are many ways to be exposed to formalin and the risks it poses to public health, to show the health effects of the wrong use of formalin in food preservation that randomly imported into the country.

Also the study examined the role of melatonin in preventive or resistance the harmful effects of formalin on experimental animals. The presented study was established to evaluate the impact of formalin (FA) on the blood levels of superoxide dismutase (SOD), lipid profile, total serum protein, serum albumin, follicle stimulating hormone (FSH), luteinizing hormone (LH), and estrogen (E2) in female rats. [3]

Materials and Methods

Animals: The present study was conducted at Science Collage, University of Wasit from November, 2018 to April 2019 . A total number of 36 adult albino rats, weighting 175-250 g and 10-14 weeks age were used in the current study.

Experimental Design: The present study was conducted on 36 adult female rats . Animals were randomly divided in to six equal groups ; each group consisted of 6 adult female rats as in the following :

1-Control group : orally dosed with distilled water .

2-Group 2: orally dosed from formalin 200ppm for 30 days

3- Group 3:orally dosed from formalin with 400ppm for 30 days

4- Group 4:orally dosed from formalin with 10mglkg from melatonin for 30 days.

5-Group 5:dosed from formalin 400ppm with 10mg\ kg of melatonin for 30 days

6-Group 6:10mg/kg dosed of melatonin only for 30 days

Sample Collection: At the end of the tests all the animals were killed. The rats were first weighed before sacrifice and then anesthetized by cotton loaded with anesthesia of diethyl ether in a specific box. A midline abdominal incision was used to open the abdominal cavity to take the sample which includes: Cardic puncture for blood collection was entitled [4]. Liver tissue samples were collected.

Blood analyses :cholesterol Gen 2, and triglycerides TRIGL were measured relying on the kits used and their protocols.

Histological techniques:

The tissue (liver organs) have been extracted from both classes in the following phases and ready for histological research using hematoxylen and eosin stain[5].

Statistical Analysis

Data is evaluated with a mean ± SD. Comparisons of classes were rendered utilizing the SPSS (Statistical System for Social Sciences) with the study of variation (ANOVA). The minimum value maximum was found to be $P<0.05$. Last big check (LSD). The gap between measures was decided for (ANOVA) SPSS [6] .

Results and Discussion

Symptoms in female rats after treated with formalin in both concentration 200,400ppm During the last (30) days of the experiment ,which is by administering dose of formalin treatment at the concentration of (200,400) ppm. some symptoms and signs were observed on the animals of the group. This includes hair colour shifting from white to yellow and also general weakness of the body which is the weakness of teeth that begins to break down or gets yellow. These animals were perished in relation to the second , third, fourth and fifth group ,but the second and third group have more symptoms than the fourth ,fifth group , because the fourth and fifth groups have formalin and also Melatonin, while the sixth group is treated with melatonin only . predation is noticed among them ,binge eating and strength in body. The current study showed a reduction on serum SOD activity. It was recorded a reduction in SOD activity and increase in lipid peroxidation in experimental animals after exposure to formaldehyde . Oxidative stress derived secondary toxic actions might be induced by FA. SOD lowered by formaldehyde due to elevations of some free radicals. Irreversible toxicity may be performed for long time exposure to FA. The catalase, by FA, GSH reduced and no recovery levels were seen in which secondary toxicity induced via oxidative stress was conducted. SOD was significantly higher in group had low dose of formaldehyde with melatonin (GIV) than those of high dose (GV), and compared with those groups had FA only, this result may be the role of antioxidant capacity of melatonin as indicated by previous researchers who concluded the role of melatonin in partially prevent

the liver damage against HCHO intoxication. Broad-spectrum antioxidant effects of melatonin can be seen to eradicate free radicles [7].

Current study showed that exposure to F200 and F400 results in significant increase in cholestrol, TG, LDL-c, VLDL significantly in treated female rats. High rates of LDL and VLDL increases the incidence chances of cardiovascular disease considered to be a major risk factor for chronic disease such as coronary heart disease and hypertension and some types of cancer , while the HDL ratio decreased significantly, which is a result of the previous studies , as it was found by previous researchers that formalin increases the oxidation pressure that leads to high levels of fat. Formalin substance increases the pressure of oxidation as the generation of free radicals that attack the fat of the cellular membranes increases , leading to the generation of malondydehyde , which in turn leads to the destruction of vessels and tissues [8] . HDL decreases in the treated animals with formalin at 200 or 400 can be expected in which certain damages may have been introduced to those pathway components leading to the disturbances in the metabolisms of those lipids showing lower levels of HDL in the blood of the tested rat females.This increase may be due to the effect of formalin on the permeability of the hepatic cell membranes or it may be due to the blockage of the hepatic canal. Bile , which leads to reduced or stopped cholesterol release in the duodenum. LDL is oxidized by the oxygen free radicals in the arteries and converted in to foamy lesions or atherosclerosis [9]

Current study showed that exposure to F200 with 10mglkg from melatonin and F400 with 10mglkg from melatonin results in significant decrease in cholestrol, TG, LDL-c, VLDL and increase of HDL significantly in F200 and F400 groups, whereas these values were normal with 10mglkg from melatonin compare with control group. On the other hand melatonin removes the oxygen free radicals and prevents oxidative changes. LDL is generally a blood lipid disorder and plasma lipid alteration among the risk factors for cardiovascular disease .More attention has recently been paid to melatonin to improve lipid profiles. The mean effects of melatonin on plasma lipids and lipid peroxide level have been reported in previous studies. Studies were also conducted on this hormone on the physiological changes resulting from high-calorie and fat diets. The use of melatonin accompanied by zinc acetate leads to a significant decrease in the level of cholesterol in the blood and TG , LDL .Melatonin probably improves the features of fats from by reducing the oxidation of fats and other necessary proteins by free radicals according to the results of some studies, there are several mechanisms that can be suggested for this action of melatonin : Interactions with LDL-c receptors , inhibition of biosynthesis of cholesterol and LDL-c accumulation , analgesia of lipase protein and reduces lipoprotein activity of the accumulation of TG rich in very LDL , VLDL molecules and decreases in intestinal cholesterol absorption or inhibition of transport of fatty acids through metabolism receptors [10].

Table (1): The effects of formalin, melatonin and formalin with melatonin on serum SOD concentrations mmol/L in mature female rats.

Table 1: Lipid, sex-hormone, and superoxide dismutase profiles in the study groups at day one of sample collection.

Group	Cholesterol	HDL	LDL	SOD	Triglycerides	VLDL
Control	54.86	24.319	19.56	247.77	58.66	11.731
F200	55.01	24.242	19.42	243.78	56.75	11.351
F400	54.4	23.964	19.01	243.51	57.13	11.426
F200M	55.13	23.601	20.16	246.67	56.82	11.364
F400M	54.29	23.202	19.88	249.52	56.06	11.21
M	54.96	24.084	19.41	246.53	57.42	11.482
LSD	N.S.	0.5289	N.S.	3.143	1.011	0.2023

LSD: Least significant difference; N.S.: Non-significant

Table 2: Lipid, sex-hormone, and superoxide dismutase profiles in the study groups at day 15 of sample collection.

Groups	Cholesterol	HDL	LDL	SOD	Triglycerides	VLDL
Control	55.18	24.5	18.87	247.06	59.07	11.814
F200	59.86	20.74	25.84	201.05	66.39	13.279
F400	70.34	18.18	37.59	181.93	72.82	14.563
F200M	58.82	22.28	24.66	238.73	59.42	11.884
F400M	59.89	19.89	27.44	214.98	62.76	12.552
M	51.21	26.29	14.44	266.51	52.43	10.487
LSD	1.19	0.756	1.266	2.826	1.307	0.2614

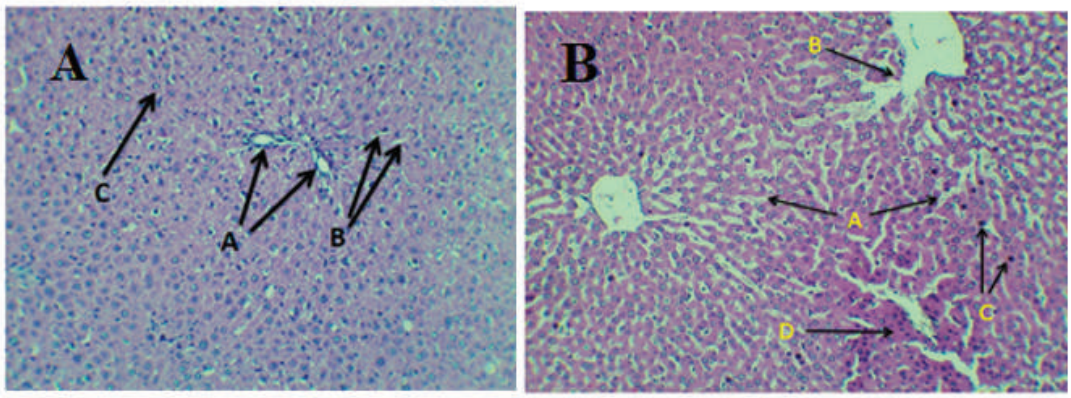


Figure 1 : A- Images microscope represent liver of rat control central vein (A), sinusoidal spaces (B hepatocytes (C).100X(H&E) , B- Images microscope represent liver of rat exposed to formalin 200 ppm at day 30 of the experiment appeared loss of sinusoidal spaces(A), rupture of central-vein (B), pycnotic nuclei(C) and infiltration of lymphocytes(D). 200X (H&E)

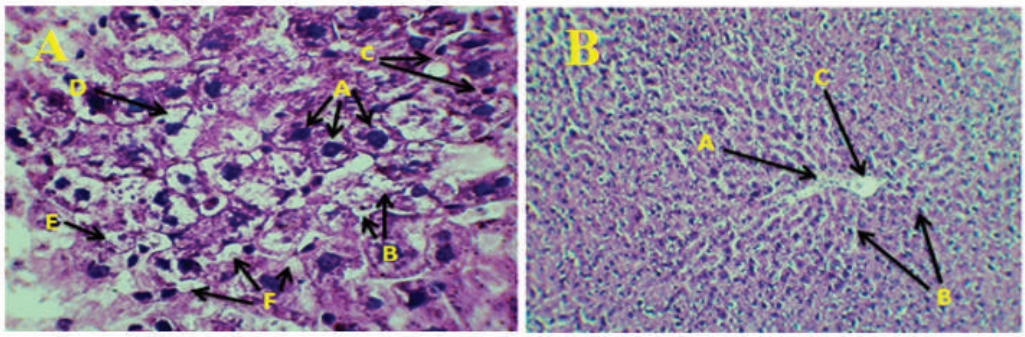


Figure 2 : A-Images microscope represent liver of rat exposed to formalin 400 ppm at day 30 of the experiment appeared pycnotic nuclei(A), degeneration of hepatocytes with numerous vacuolations (B), infiltration of lymphocytes(C), absent nuclei of hepatocyte (D), nuclear-material clumping(E) and necrosis of hepatic cells(F). 400X (H&E), B- Images microscope represent liver of rat exposed to formalin 200 ppm and melatonin 10 mg/kg at day 30 of the experiment less severe central vein congestion (A), less dilated sinusoidal spaces (B) and less impacted endotheliallining(C).200X(H&E)

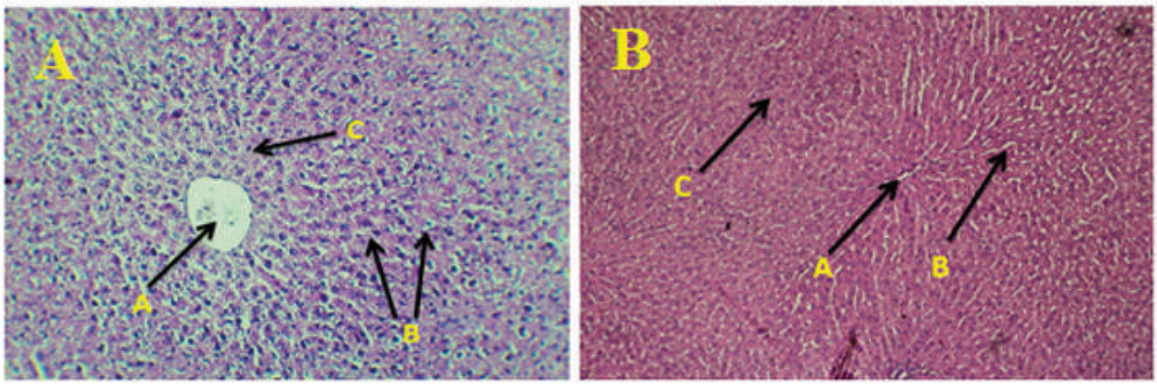


Figure 3 : Images microscope represent liver of rat exposed to formalin 400 ppm and melatonin 10 mg/kg at day 30 of the experiment less severe central vein congestion (A), less dilated sinusoidal spaces (B) low levels of infiltration of inflammatory cells(C) and no found hemorrhages . 20X(H&E) B- Images microscope represent liver of rat exposed to melatonin for 10mg/kg at 30 days of the experiment normal central vein (A), normal sinusoidal spaces (B) normal hepatocytes (C).40X(H&E)

The liver of the control group, the liver tissue revealed normal central vein with liver cords of hepatocytes radiating from the central vein and separated by blood sinusoid plus hepatocytes had rounded nuclei indicating normal (without changes) tissues (Fig.1,A) For both F-200 and F-400 groups (Fig. 1,B and Fig2, A) respectively, at day-30 sample collection time, loss of normal architecture, degeneration of hepatocytes with numerous vacuolations, presence of pleomorphic nuclei with different sizes and degeneration levels, loss of sinusoidal spaces, central-vein rupture, hepatic-tissue based scattered inflammatory cells, blood-filled-central-vein dilation, dropped-out-degenerated hepatocytes, mostly displayed pycnotic, absent nucleoli, and nuclear-material clumping. [11] mentioned that FA is extremely soluble and reactive in its contact with mucus or other nucleophilic-group-containing cellular macromolecules, such as amino acids and DNA. FA may interfere-react with DNA, and it is accountable for the genotoxic and carcinogenic results of FA via the construction of bonds between DNA and FA related adducts. This fact are explain the effect of FA on the nucleus.

For the F-200 10mg/kg at day 30 of the experiment (Fig.2, B), the protective treatment of melatonin against the exposure to 200PPM of FA showed a decrease in the histological changes compared to F-200 group that received FA alone, in which less dilation of central vein, less dilation of sinusoidal spaces, less congestion in the central vein, less infiltration of lymphocytes, and less inflammatory cell infiltrations and hepatocyte degenerations were seen in the melatonin treated group compared with those from the F-200 group.

In the case of the F-400 with 10mg/kg group at day- 30 time-point, the histological findings revealed that the exposure to formalin and melatonin resulted in less severe central vein congestion, some restoration of the liver architecture with less impacted endothelial lining, less dilated sinusoidal spaces with close-normal thickness of tunica media, low levels of infiltration of inflammatory cells, smaller sinusoids, low numbers of hemorrhages (Fig.3,A)

(12) concluded that M fought the induced oxidative stress produced by the exposure to FA that increased the tissue toxicity generating lower infiltration of neutrophils and decreasing the 8-hydroxydeoxyguanosine (8-OHdG) levels, which can be useful for the protection against the exposure to FA, and this agrees with the current investigation results.

For the groups that received 10mg/kg melatonin only, the findings of the liver tissues showed normal tissue structure, architecture, compartments (Fig.3,B) Our study is comparable with [13] who mentioned that the melatonin play important role to improvement the histological structures of kidney and liver of mice and give it the normal characteristics for these organs. [14]

mentioned the fact that melatonin is nowadays there has been a certain knowledge of the importance of melatonin in human biology and diseases, but many of its roles and consequences tend to be overlooked. Melatonin is a powerful free radical scavenger, stronger than vitamin E. The extremely toxic hydroxyl radical and other oxygen-based radicals are modified by melatonin effectively. Melatonin also has antioxidant effects: it improves the concentrates of various antioxidant enzymes such as the SOD, glutathione reductase, and glutathione peroxidase. In addition, melatonin suppresses NO synthase, a pro-oxidant enzyme.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Effect of Methotrexate on Adenosine Deaminase Activity in Rheumatoid Arthritis Iraqi Patients

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Abstract

Rheumatoid arthritis (**RA**) is considered as an autoimmune disease which influences the joints. It usually results in a warm, enlarged, and painful joints. Stiffness and Pain typically amplify once relaxation. Frequently, the hands and wrist are involved, with similar joints naturally involved on both sides of the body. The syndrome might additionally have an impact on the other parts of the body. Adenosine Deaminase is an enzyme (EC 3.5.4.4) used with the metabolism of purine. It is essential for the analysis of adenosine from nutrition and the revenue of nucleic acids in soft tissue. Its chief purpose in humans is the immune system growth and preservation. On the other hand, the complete physiological role of ADA is so far not known. This study carried out at Al-Kadima Teaching Hospital, during the period fr6-om September 2014 till the end of October 2015. Patients who have any other disorders conditions are excluded. Subjects accepting treatment with medications within 7-9 months. A total of 66 patients with Rheumatoid arthritis were enrolled in this study: thirty-five of them were newly diagnosed to have Rheumatoid arthritis who receives no therapy for Rheumatoid arthritis (group A); the remaining thirty-one patients were with Rheumatoid arthritis who receive by methotrexate 500 mg for 7-9 months as the (group B). Twenty-six healthy subjects, as controls(group C). The results show a significant elevation in the ADA activity in group A and group B. The study concluded that the level of ADA was higher in Iraqi RA patients than in healthy control subjects

Keywords: Rheumatoid arthritis, Adenosine Deaminase and methotrexate

Introduction

Rheumatoid arthritis (**RA**) is considered as an autoimmune disease which influences the joints. It typically results in warm, inflamed, and hurting joints. Rigorousness and Pain characteristically amplify once relaxation. Frequently, the hands and wrist are involved, with the analogous joints certainly intricate on both sides of the body. This force clue to inflammation in the heart, inflammation in the lung, and low red blood cell count, fatigue and elevated in the body temperature could also be found.(1)

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Recurrently, signs arise on gradually from weeks to months. Even though the fact that the reason for rheumatoid arthritis is not well identified, it is speculative to contain a miscellaneous of environmental factors and genetic. The exceptional mechanism comprises the aggressive action of the immune system of the body which indication to solidifying and irritation of the joint. It influences cartilage and bone.[1]

The sympathy of this syndrome is to be influenced by a person's symptoms and signs.(2) RA affects between 5 and 50 per 100,000 people newly developing the condition and between 0.5 and 1% of adults in the developed world each year.(3) Onset is persistent through middle age female are influence 2.5 times as often as men.[1] In 2013, it gives rise to in 38,000 deaths up from 28,000 deaths in 1990.(4) Adenosine Deaminase is an enzyme (EC 3.5.4.4) used with the metabolism of purine. It is essential for the breakdown of adenosine

from sustenance and for the revenue of nucleic acids in soft tissue. Its topmost purpose in humans is the immune system growth and preservation.[5] Instead, the complete biological character of ADA is not recognized ADA present in both major form (dimer-complex) and minor form (monomer). [6] In the monomer configuration, the enzyme is a polypeptide chain,[7] fixed into eight elements of equivalent α/β barrels, which frame a topmost deep pocket that is the active site. Along with the eight essential β-barrels and eight marginal α-helices, ADA likewise embraces five further helices: residues 19-76 fold into three helices, located between β1 and α1 folds; and two antiparallel carboxy-terminal helices are placed across the amino-terminal of the β-barrel. The ADA active site involves a zinc ion, which is located in the deepest recess of the active site and synchronized by the substrate and five atoms from His15, His17, His214, Asp295.[5] The substrate is bound to an active site by nine hydrogen bonds and become stable. The carboxyl group of Glu217, approximately coplanar with the substrate purine ring, is in the site to formulate a hydrogen bond with N1 of the substrate. The carboxyl group of Asp296, correspondingly coplanar with the substrate purine ring, forms a hydrogen bond with N7 of the substrate. The NH group of Gly184 is in position to consist of a hydrogen bond with N3 of the substrate. Asp296 forms bonds composed with the Zink ion (Zn+2) laterally with 6-OH of the substrate. His238 also hydrogen bonds to substrate 6-OH. The 3'-OH of the substrate ribose figure a hydrogen bond over and done with Asp19, conversely the 5'-OH construct a hydrogen bond through His17. Double extra hydrogen bonds are made to water particles, at the preliminary of the active site, thru the 2'-OH and 3'-OH of the substrate. In place of the importance of the recessing of the active site in the interior enzyme structure, the substrate, immediately it bound, is very nearly completely recaptured from the solvent.[7] The surface area of the substrate to solvent once bound is 0.5% the surface area of the substrate in the Free State.

Material and Methods

Subjects: the study was a cross-sectional study carried out at Al-Kadima Teaching Hospital, during the period from September 2014 till the end of October 2015. Patients who have any other disorders conditions are excluded. Subjects accepting treatment with medications within 3 months. All subjects were nonsmokers, and none reported chronic alcohol consumption.

The protocol for the study was approved by the Ethical committee of Al-Nahrain Medical College and informed signed consent was given by each subject. A total of 66 patients with Rheumatoid arthritis were enrolled in this study: thirty-five of them were newly diagnosed to have Rheumatoid arthritis who receives no therapy for Rheumatoid arthritis (group A); the remaining thirty-one patients were with Rheumatoid arthritis who receives by methotrexate 500 mg 7-9 months as a therapy (group B). The study included another Twenty-six healthy subjects, non- alcoholic, non-smoking and no family history of any type of Diabetes Mellitus or Hypertension who serve as healthy controls(group C); they were matched with patients` groups for age as in table 1

Blood samples:

Five millilitres of random venous blood was withdrawn from each patient, in the supine position, without application of a tourniquet. Samples were transferred into a clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged at 1800 x g for 10 minutes at 4°C, and the separated serum was transferred into Eppendorf tube and was used for measurement of ADA. The tubes were stored at –20 ºC until analysis, which was done within one month after collection.

Procedure: measurement of serum ADA was done by ELISA kit [8].

Statistical analysis: statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (t-test) to test the significance of the mean difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results

Table (1) clinical criteria of patients groups with Rheumatoid Arthritis and control (mean ±sd)

Parameters	Group A	Group B	Group C
Age rang (years)	45-67	45-67	45-67
ADA (IU/l)	13.248±4.145	32.268±4.779	18.738±4.34

Table (2) p-value between the studied groups

The Groups	Group A	Group B	Group C
Group A	--------	≥0.001	≥0.001
Group B	≥0.001	≥0.001	≥0.001
Group C	≥0.001	≥0.001	≥0.001

Discussion

The preclusion of joint damage and reducing the sickness form vital mechanisms of administration of RA patients, the first establishment of the appropriate treatment is essential. Even though clinical guides help in appraising syndrome activity, biochemical indicators are furthermore one of the things for signs of syndrome activity and progress. This study demonstrated in table 1 group B has the maximum level of ADA, though group A the lowest level of ADA. Nevertheless, the strict source of flow in ADA levels is not recognized, the activity might be greater than before as a consequence of its ejection from the damaged cells and initiate of cellular proliferation in RA [(9)] Nalesnik M *et al.,* revealed an insignificant association between CRP and ADA in patients with RA without methotrexate treatment and recommended that ADA activity in serum might be used as a biochemical indicator of the inflammatory progression in RA [(10)].

ADA catalyzes the irremediable hydrolysis of adenosine to inosine. Adenosine has been revealed to be a strong endogenous anti-inflammatory agent [(11).] The enzyme ADA characterizes a checkpoint in the directive of extracellular Adenosine levels [(12)] and therefore is possible to moderate the inflammatory procedures.

A study by Sari et al., [(13)] exposed that serum total ADA activity is linked with RA and may propose a respected helper to measure inflammation moreover to the traditional indices. That suggested that determining ADA activity help for a superior clarifying of some of the pathophysiological sorts of the disease.

Methotrexate is the anchor drug for remedy of RA. Subsequently, the anti-inflammatory influence of MTX is through the incitement of adenosine receptors, it was reflected that the therapeutic properties of MTX could be as a result of catalytic activity of ADA in the serum [(14)] The pathophysiology of adenosine ejection is associated with MTX pathways. It is recommended that MTX stops ADA and surges vasodilatation convinced by adenosine so it is a deduction that ADA activity in serum may be an imaginable biochemical indicator for checking the therapeutic properties of MTX in RA patients. Van Ede et al. resolved a connection between ADA and MTX treatment in patients receiving MTX and folic acid [(15)]. Salesi et al. revealed that ADA levels earlier and later the MTX treatment in their RA group and point to a connotation with ADA levels with the usefulness of MTX treatment [(16)]

Conclusion

This study showed that level of ADA was higher in

Iraqi RA patients than in healthy control subjects.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Atrial Fibrillation among Jordanian Patients

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Abstract

To estimate the rate of atrial fibrillation (AF) among Jordanian patients, and to identify the clinical data of signs and symptoms, diagnostic criteria, complications, prognosis and management. A cross-sectional study involving total- 126-AF patients who admitted to Prince Hamzah Hospital with diagnosis of AF. The study was begun from January 2018 to July 2019. A non-probability (Convenience) sample of (126) patients with atrial fibrillation who are admitted to the cardiac unit at Prince Hamzah Hospital. A special standardized question form is designed and to be filled by researcher after patient signing his consent paper. The study included (126) Atrial Fibrillation Jordanian patients. The result find the age is important risk factor for atria patient. The high percentage of patients distributed among old age group. AF was associated with chronic diseases, such as hypertension (73.01%). In spite of the fact that atrial fibrillation is expected to be an epidemic disease within the next 20-30 years, no clear preventive programs especially for those who have high risk like diabetes mellitus, hypertension, ischemic heart disease and overweight regarding education and changing lifestyle to reduce the incidence of atrial fibrillation.

Keywords: *Atrial Fibrillation, Jordanian, Patients*

Introduction

Atrial fibrillation (AF) is the most common arrhythmia encountered in clinical practice and accounts for 1/3 of hospital admissions for cardiac rhythm disturbances. Recent worldwide epidemiological data have reaffirmed the fact that AF is a global epidemic and has adverse effects on long term morbidity and mortality. Although the effect of AF on the quality of life and survival has been well documented in the western population[1,2,3,4]. In addition, AF is frequently associated with disturbing symptoms and very important socioeconomic problems, such as permanent disability, cognitive disturbance, hospitalization, and absence from work[2].

AF is a supraventricular tachyarrhythmia characterized by uncoordinated atrial activation leading to ineffective atrial contraction. AF represents an important clinical problem with related to significant health and social issues. It can be complex and challenging for clinicians to manage. It can also lead to frequent hospitalizations, hemodynamic abnormalities, and thromboembolic events resulting in significant morbidity and mortality and impact negatively on the patients' quality of life[3,5,6].

The number of AF patients has been increasing worldwide. A study from New Zealand in 1999 reported 10.4% as the prevalence of AF admissions. The prevalence of AF increases with aging. The Feinberg analysis, that based on other four epidemiological studies, indicated that the prevalence of AF was increased with

age by 2.3% and 5.9% in the population aged >40 years and >65 years, respectively. The further increase in the prevalence and the incidence of AF with the aging of the population are predicted in the future[3].

AF is most commonly classified according to its temporal pattern. Paroxysmal AF is a self-terminating AF episode lasting less than 7 days, and usually less than 48 hours. Sustained AF, lasting longer than 7 days and less than a year, is classified as persistent. Where AF persists beyond a year, it is classified as permanent, irrespective of the fact that there may have been temporary resolution after cardioversion[1]. Paroxysmal AF may degenerate into more frequent paroxysms, or a sustained form of AF, which in turn may degenerate into permanent AF. 'Lone AF' is largely a diagnosis of exclusion and refers to AF occurring in the absence of concomitant cardiovascular disease (eg, hypertension), structural heart disease (normal echocardiogram), with a normal ECG and chest X-ray[1,7].

AF has many etiologies, and it may develop in the presence of many controllable and non-controllable risk factors. Chronic diseases such as certain heart diseases, hypertension (HTN), diabetes mellitus (DM), and hyperthyroidism are considered as controllable risk factors. Congenital heart diseases, aging, and family history are considered as non-controllable risk factors. At present, the prevalence of both chronic HF and AF is increasing since these two conditions share common risk factors. As a risk factor, heart failure (HF) comes at the second place after HTN. A study of electrical ALternans in Patients with HF (ALPHA) shows the AF prevalence exceeds 20% in HF patients thus demonstrates an association between AF and chronic HF[3].

Several different treatment options exist for management of AF. Management may include medication to restore and maintain sinus rhythm, medications to obtain rate control, and anticoagulants. With proper management of AF the risk of stroke and other heart related problems can be significantly decreased[8,9].

Methodology

Design of the Study:

A cross-sectional study involving total 126 AF patients who admitted to Prince Hamzah Hospital with diagnosis of AF.

The Sample of the Study:

A non-probability (Convenience) sample of (126) patients with atrial fibrillation who are admitted to the cardiac unit at Prince Hamzah Hospital.

The Study Instrument:

A special standardized question form is designed and to be filled by researcher after patient signing his consent paper. It involves information's about the patients such as general demographic criteria including age, sex, and clinical criteria, signs, symptoms, type of atrial fibrillation, risk factors, diagnostic criteria and management.

Data Analyses

In order to achieve the early stated objectives, the data of the study were analyzed through the use of statistical package of social sciences (SPSS) version 19 through descriptive and inferential statistical analyses. The statistical data was analyzed by using descriptive statistics (Frequency and Percentage).

Results

Table (1): Demographic Characteristic of Atrial Fibrillation patients:

Groups		Frequency (Total 126)	Percentage (%)
Age (years)	≤ 39.00	1	0.8
	40.00 - 45.00	3	2.4
	46.00 - 51.00	5	4.0
	52.00 - 57.00	10	7.9
	58.00 - 63.00	13	10.3
	64.00 - 69.00	18	14.3
	70.00 - 75.00	40	31.7
	76.00 - 81.00	23	18.3
	≥82.00	13	10.3
	Total	126	100.0
Gender	Male	51	40.5
	Female	75	59.5
	Total	126	100.0
Type of Atrial Fibrillation	Permanent (Chronic)	113	89.7
	Paroxysmal	9	7.1
	Lone	4	3.2
	Total	126	100.0
Smoking	Yes	118	93.7
	No	8	6.3
	Total	126	100.0

The study included (126) Atrial Fibrillation Jordanian patients. The result in table (1) shows the high percentage of study subject at age group 70-75 (31.7%). In addition, this table shows the majority of study subjects were female 75 (59.5%). Concerning to the type of atrial fibrillation, the study finding show that 113 (89.7%) of study subjects have permanent (chronic) atrial fibrillation type. Finally in this table the result indicate that 118 (93%) patients have yes smoking. The age is important risk factor for atria patient. The high percentage of patients distributed among old age group.

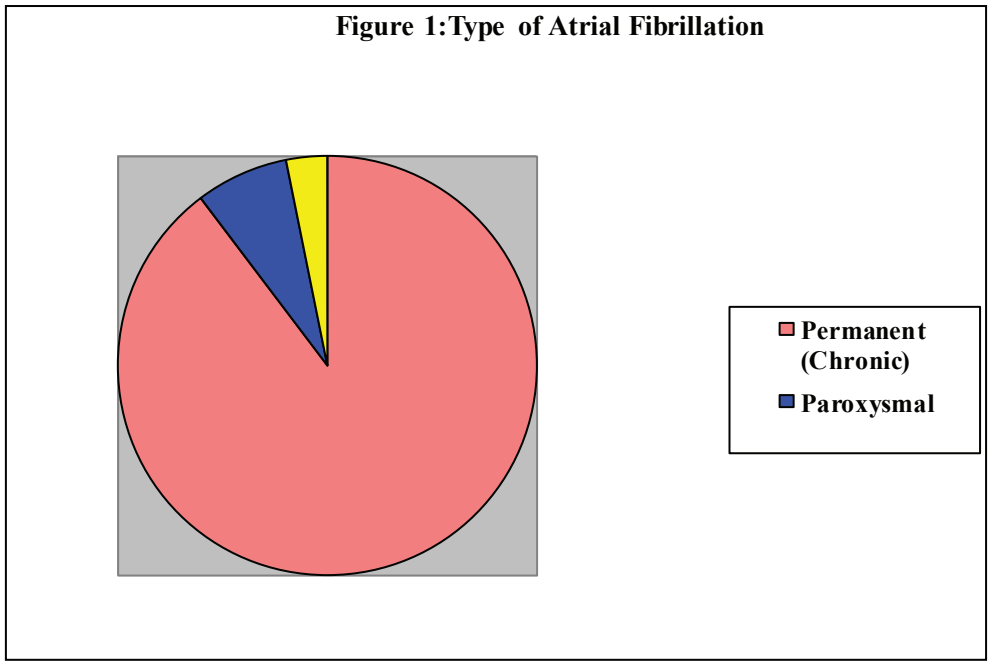
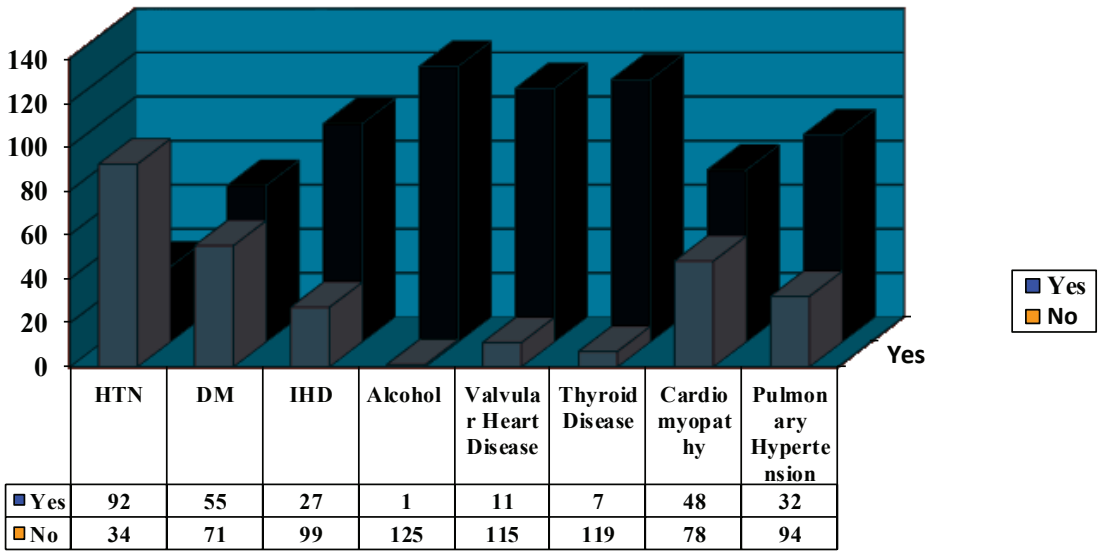


Figure 1: Distribution of study subjects by type of atrial fibrillation.



	HTN	DM	IHD	Alcohol	Valvular Heart Disease	Thyroid Disease	Cardiomyopathy	Pulmonary Hypertension
Yes	92	55	27	1	11	7	48	32
No	34	71	99	125	115	119	78	94

Figure 2: Distribution of patients by etiology of atrial fibrillation, the figure shows that HTN is highest percentage (73.01%) as an etiology of atrial fibrillation. While alcohol is the lowest percentage (0.8%) that cause for atrial fibrillation.

Table (2): Clinical and echocardiographic characteristics of AF patients:

Symptoms		Frequency (Total 126)	Percentage (%) (Total 100.0)
Dyspnea	Yes	75	59.5
	No	51	40.5
Chest Pain	Yes	2	1.6
	No	124	98.4

Palpitation	**Yes**	20	15.9
	No	106	84.1
Thrombo Embolic	**Yes**	7	5.6
	No	119	94.4
Sepsis	**Yes**	5	4.0
	No	121	96.0

Table (2) show the symptoms of atrial fibrillation patients. Dyspnea is most common symptom 75 (59.5%) among (chest pain, palpitation, thromboembolic and sepsis) in atrial fibrillation patient.

Table (3): The prevalence of patients' medical management:

Management \ DC shock		
	Frequency (Total 126)	Percentage (%) (Total 100.0)
Yes	1	0.8
No	125	99.2
Management \ Anticoagulation		
Yes	52	41.3
No	74	58.7
Management \ Antiplatelet		
Yes	70	55.6
No	56	44.4
Management \ Antiarrhythmic		
Yes	7	5.6
No	119	94.4

Table (3) shows anticoagulants and anti-arrhythmic taking by atrial patient. The study finding indicate that the antiplatelet is most common management uses from atrial fibrillation patients.

Discussion

This study demonstrates that the age of Jordanian AF patients highly distributed at age group 70-75 (31.7%)**,** also the result finding the atrial fibrillation increase with age as a result show that the high percentage of atrial fibrillation patients distributed among old age group. This result come along with (Zhou and Hu, 2008), they mentioned that " the age standardized prevalence of AF in the general Chinese population was 6.5 per 1000 people, and that it increased with age. Also we confirm that AF is a disease that is largely prevalent in the elderly in the general Chinese population, with the great

majority of cases present among people aged 60 years and above" [4]. Also Alharbi and Alsuhaibani, 2018, in their study "Atrial fibrillation among adult Saudi patients with chronic heart failure: Tertiary center experience" state that the increasing occurrence of various chronic and cardiovascular diseases with aging which contribute to HF and eventually AF[5].

According to type of atrial fibrillation our result show the common type is permanent (chronic) atrial fibrillation. This result supported by Johansson, *et al.*, 2017 They reveals that "permanent AF and persistent AF were more common. This may be explained by use of a longer time period for the classification of AF (up to six months from the first diagnosis of AF compared to six weeks); this gave us more extensive data from the patient records and more data from the clinicians' assessments of the patients for classifying the type of AF"[10].

In our study the result find that the common cause for atrial fibrillation are hypertension have higher percentage among etiology factors of atrial fibrillation. Alharbi and Alsuhaibani, 2018, said that the HTN considered one of the most prevalent causes of AF. In this study, the development of AF was more within HTN patients[5].

About Atrial fibrillation patient medication the result of this study show antiplatelet management (aspirin) is commonly prescribed by doctor for AF patients because play important role in decrease mortality of AF patient. This result agree with (Gersh, *et al*., 2011; Karamichalakis, *et al*., 2015; Rajan, *et al.,* 2019) all them mentioned that the " Aspirin reduces the risk of stroke by 25% in AF patients and it prevents mostly non-cardiogenic embolic strokes"[11-13].

Conclusions

Most of Jordanian patients are affected with HTN as a cause for atrial fibrillation, and the common type is atrial fibrillation permanent (chronic),while most of these patients didn't take anti-arrhythmic drugs, anti-coagulants and, DC shock through their treatment.

Recommendations:

Base on the result of the present study the researcher recommended are following:

1. Comprehensive understanding of AF epidemiology is necessary to establish preventive and management strategies for AF and consequently improve clinical outcomes of AF patients in the hospitals.

2. A guideline need to be prepared MOH for medical staff to improve their knowledge and training toward management of patients with atrial fibrillation

3. Health education and public policies are urgently required for Jordanian elderly people.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Internal Medicine and Family Medicine and all experiments were carried out in accordance with approved guidelines.

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Ctrophotometyric Determination of Heavy Metal (Lead) in Cosmetics (Lipsticks) in Commercial Markets

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Abstract

In the recent years an significant rise were occurred due to exposure human being of heavy metal . There are no escaping from exposure to toxic chemical and heavy metal . The aim of this study was to determine and evaluate the risk heavy metal in lipstick on the public health, so we selected one heavy metal (lead) in the randomly selected lipstick samples from local markets . We determine lead in 28 lipstick products samples. Cosmetic products have a wide number of forms and uses ,many of them contain chemical compounds that may passé through the skin. High concentration of heavy metal in lipsticks which could form potential health risk on public health , especially we know this lead accumulate in biological system . we used to determine lead spectrophotometer technique . The samples were analyzed based on international standard by using (HNO3 ; H2O2) method . The concentration of maximum lead in random samples was (1.4) ppm , while the minimum lead was (0.02)ppm .This results were below the recommended limits of the united state Food and Drug Administration .In manufacturing of lipsticks must be monitoring the components and chemicals used . Also quality control take place to check the concentration of heavy metal in lipstick products in each country . .

***Key words**: Lead ,Cosmetic, Heavy metal, element ,Health risks.*

Introduction

The Greek word "kosmetikos" is origin of the word cosmetic [1] . In the 2014 the sales cosmetic in over 230 billion us dollars [2] .This reflex the general acceptance cosmetics. Since the first civilization the cosmetic take compose a part of routine body care for low and middle class people also for upper of society. Recently cosmetic products constituted a big boost in industries [3] .The commonly used cosmetics in the world include facial make ,skin care ,mouth washes ,deodorant, shampoo, hair dyes ,nail polishes , mascaras , eye line and eye shadows which are find in different applications on different body parts ,much of personal car products are industrial car chemicals [4] Some of heavy metal are used as coloring , while some added without intentionally [5]. The demand of cosmetics depends on beauty consciousness of the human health awareness as well as the side effects was attract the researchers to knowledge the causes behind their side effects [6]. In the past skin and its role is not more than considered a covering and protecting coat recently it's a part of immune system [5]. The first protective layer in the human body is skin ,The skin sometimes allow some external materials to passé through it . Lipsticks products are directly applied on the body causes side effects, some of sensitivity is being when absorption is very high [7]. Cosmetic products contain heavy metal such as lead has been research subject du to biological accumulation and their toxicity in the body [8]. cosmetic industry consist many chemicals some of it have been associated with developmental or reproductive or other health effects , because of potential for exposure through ingestion lip stick products , there for thy have been suggested as a particular concern [9].

The specification of cosmetics are include of some i8ngredients for stability , long lasting and effective as well as harmless use for human . Bio accumulation occur because presence of heavy metal in human tissues which they are build complication , this lead to appearance of some diseases in kidney , nervous , bone and cardiovascular [10]. Ingredients in cosmetics are linked with some diseases like cancer , reproductive harm and birth defects [11]. Lipstick may provide direct oral exposure

to the contamination in addition to on other type like lotions may provide indirect percutaneous exposure [12]. Lead as heavy metal is common contamination in various cosmetic products naturally lead is present in the earth crust with molecular weight 82 [16] . Several raw materials and pigments included in cosmetics contain pb [13] .Lead toxicity has been distinguish health hazard for several centuries. Colic, anemia, neuropathy , sterility ,and earing impairment [14]. Lead can cross the placental during pregnancy [15].

Currently no international standards for impurities in cosmetics. FDA legal authority for drugs , biologics and medical devise, is different on cosmetics products and ingredients . firms are responsible for the safety of their product before marketing. Unsafe levels of lead in cosmetics and food may be skill exist sources of contamination [16]. The concentration of pure pigment can vary from 1 to 10% depending on the type of products [17]. One study tested 33 common brands lipsticks they found that 61% contained pb with level up to 0.65 ppm [18]. Also two other studies estimated pb in lipstick , US food and drug administration (FDA) they detected pb in all tested sample [19].

Materials and Methods

-Sample preparation :- Lipsticks sample were prepared according to wet digestion method [20].

-Instrumentation :-

We used Flame atomic absorption spectrometer for determination the concentration of lead in cosmetic samples . The analytical condition are (wavelength is 246 , lamp current 6 , slit width 0.25) .Standard solution were prepared in five different concentration for each separately to obtain calibration curve quantitative analysis .

Results

Lead detected in all lipstick (28 samples) in the range (0.02 – 1.4) ppm . The US food and drug administration (FDA) limit for pb as a colure additive is (20) ppm . All sample was less than FDA limit . The highest pb content was 1.4 ppm and the lowest was 0.02 ppm .

Table 1: the concentration of pb in ppm in lipstick

Sample no.	Concentrate of Pb (ppm)	Sample no.	Concentrate of Pb (ppm)
1	0.21	15	1.20
2	0.82	16	0.09
3	0.57	17	0.12
4	0.14	18	0.29
5	0.01	19	0.13
6	0.04	20	0.67
7	0.02	21	0.13
8	0.43	22	0.10
9	0.05	23	0.51
10	0.31	24	0.71
11	0.72	25	0.42
12	0.11	26	1.40
13	0.34	27	1.01
14	0.50	28	0.17

Tale 2 : concentration of heavy metal lead (pb) in lip sticks (ppm).

Group number	mean	Std. deviation	Std. error	P value
(28)	0.40	0.37	0.07	0.001

***p<0.05**

Table 3: International limits for heavy metal lead in lip sticks (ppm)

WHO	10
EU	0.5
US	1.0
GERMANY	1.0
CANADA	10

Table 4 : comparison to literatures values

Concentrate of pb in (ppm)	Reference
0.04 - 3.75	Picinip; et.al 2013 [21]
0.77 -15.44	Zakaria, A. 2015 [22]
0.02 -1.32	Liu, S; Hamond 2013 [23]
0.30 - 2.44	Al-Saleh et.al 2011[24]
10.9 - 15.25	Ackah-M 2015 [25]
0.09 - 3.06	Hepp.NN et.al 2004 [26]
18.7 - 87.3	Nnovomic et.al 2005 [27]
0.02 -1.40	Our study

Discussion

Trace elements in cosmetics like lead according to FDA may be is un avoidable under condition of good manufacturing practice . It is very important to find relation between to investigate of the safety of cosmetics and personal care products and their ingredients on daily basis. Ingredients found in lipstick are wax ,oil ,alcohol ,and dye . Lead is not an ingredient of the lipstick it might be present as impurities in the color additives . To reduce the side effect lipstick must make the international cooperation between personal car products and their ingredients and toxicologists .The FDA has established 20 ppm as a maximum amount of lead allowed in color additives used to make cosmetics for external use , produced using good manufacturing practice. There are no legislation for regulation the level of toxic metal in Asia , Europe and US . USFDA determine the level of lead in a number of different lipstick . Although lead found in lipstick is only minor source when compared

to other sources ,such as air ,food and water , we can not overlooked lead exposure from lipstick. The variance between pb concentration may because added pigment components during the production process . some of sources of pb contamination in lipstick can also be the added auxiliary materials . The dust and water used in manufacturing of lipstick product may be another reason for contamination .Du to increasing usage of cosmetics , it is very important to be noted these problems therefore monitoring should be tightened for all process in cosmetics production . Heavy metal gradually accumulate in the human body by continues uses . In addition to these risks , lipsticks may also have the higher risk of direct oral take in side of negative effect of their chemicals . serious chronic health risks occurs because the accumulation of these metals in the body , therefor the consumers must knowledge of the various products and keeping update to check and choose the best quality to minimize exposure to the heavy metals .

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the DNA Research Center, University of Babylon, AL-Hila, Iraq and all experiments were carried out in accordance with approved guidelines.

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Solubility and Dissolution Rate Enhancement of Ketoprofen by Nanoparticles

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Abstract

Reduction in particles size cause increase in effected surface area of drug particle. So as dissolution occurred at the surface of solute, rapid increase in dissolution rate due to large surface area. Ketoprofen belong to class II according Biopharmaceutics Classification System in which has low solubility and high permeability.the aim of study was to enhance solubility as well as dissolution rate of Ketoprofen when preparation of Ketoprofen nanoparticles by solvent evaporation method. Different formula of Ketoprofen nanoparticles were prepared using different type of polymers (pvpk15,HPMCK15, MCA4C,and CMC 30) . All of the prepared Ketoprofen nanoparticles formulas showed a particle size result within nano-range with entrapment efficiency ranged from (85.7% to 98.3 %). The average particle size of Ketoprofen nanoparticles was observed from (55 nm to 995 nm). The selected formula was investigation for DSC, PXRD, FTIR, SEM, entrapment efficiency and invitro drug release. The invitro dissolution study showed a significant ($p<0.01$) enhancement in dissolution rate of nanoparticles formula as compared to pure Ketoprofen (drug a lone) and physical mixture(drug and stabilizer), at 10 min. 75.7% of drug release from nanoparticles, while only 15.2%, 22.7% for pure ketoprofen and PM(drug: stabilizer), respectively.

***Key words**: Ketoprofen, nanoparticles, Particle size, Dissolution rate*

Introduction

Solubility play an important role in absorption of drug. The limitation in the rate of dissolution that result from low solubility lead to low in a bioavailability[1], furthermore difficulty in develop of pharmaceutical solid dosage form [2], there are many of newly discover drugs with their lipophilicity and high molecular weight and low water solubility result from emerging trends of drug design and combinatorial chemisrey [3]. 40% of the drug in the market have poor water solubility according to BCS, and 90% of drug development have characterization of poor water solubility [4]. There are many approach available to increase water solubility of drug like physical modification(modification of crystal habit, self emulsification[5], solid dispersion [6,7] solubilization by surfactant [8,9]), chemical modification (salt formation [10,11] , pH modification [12], complexation [13,14] in addition to other method (co crystallization) [15], use of co solvent, micronization [16] and nanosization). According to biopharmaceutics classification system, the relationship between invitro drug dissolution and permeability of drug are most important parameter for controlling rate and the extent of drug absorption. The classes involved: class I (high solubility and high permeability), classII (poor solubility and high permeability), class III (high solubility with poor permeability), class IV (high solubility and high permeability) [4] Nanoparticles can be define as reduction in particle size between 100-1000nm. So particles size with less than 100nm show to have a highly saturated solubility, according to Ostwald- Freundich equations [17], [18] in the other hand lead to increment in dissolution rate according to Noyes-Whiney equation [17] , [18] and improve bioavailability and reduce fed/fast effect [2] Ketoprofen is a white crystalline powder, practicle insoluble in water, belong to class II according to BCS. It's a nonsteroidal anti-inflammatory agent (NSAIA) with analgesic and antipyretic properties [19,20].

Materials and Method

Materials

Ketoprofen was purchase from Lishui Nanming Chemicals CO.Ltd from China. Polyvininypyrrolidine PVPk15 from Alpha Chemika India, hydroxypropyl methylcellulose HPMCk15 and methylcellulose (MC) A4C provide from Gromax Enterprises Corporation USA, Carboxy methylcellulose CMC30 from EMD Chemical Germany, were purchased from. Acetone was supply from Romil UK. Potassium phosphate and dihydrogen disodium hydrogen phosphate were obtained from SPINE-CHEM. Limited, and BDH Laboratory Supplies from England, respectively.

Determination saturation solubility of Ketoprofen

Saturated solubility of ketoprofen done by using shake flask method in water and 0.1NHCl (pH1.2). Conical flask contain 10 ml of water with excess amount of ketoprofen was sealed well and allow to agitate for 24 hr at 37 ^{0}C. visual notes to precipitation of ketoprofen in flask. Aliquot was allow to filtered by 45µm filtered paper, then these filtrate diluted and analyzed on UV/ visible spectrophotometer at 260nm wave length [21,22].

Preparation of Ketoprofen nanoparticles

Ketoprofen nanoparticles was prepared by using solvent evaporation method (solvent antisolvent precipitation method). Organic phase consist from 100mg of ketoprofen in 5ml of acetone, while aqueous phase consist from polymer in water. The results organic phase was added to aqueous phase drop by drop with using of syringe. The mixture of drug and polymer agitate on magnetic stirrer at 600 rpm. Ketoprofen water in soluble, however , precipitation of ketoprofen was occur in water with stabilizer. Different type of stabilizer with different ratio (weight :weight) of (drug :stabilizer) 1:1, 1:2, and 1:3 were used in preparation of ketoprofen nanoparticles.

Entrapment efficiency

Entrapment efficiency was done for all formulas of nanoparticles. Freshly prepared nanosuspensions (10ml) were centerifugate by ultracentrifugation at 45,000 rpm. The supernatant was taken to determinate the free drug after diluted with appropriate dilution, follow that substrate from initial amount of drug to obtain encapsulation amount and encapsulation ratio can be measure by following equation [23] :

$$\text{Entrapment efficiency} = \frac{W\,\text{initial} - W\text{free}}{W\,\text{initial}} \times 100\%$$

$$\frac{W\,\text{initial} - W\text{free}}{W\,\text{initial}} \times 100\%$$

Measuring of particles size

Particles size, polydispersity (IP), and specific surface area(SSA), were determined by using ABT-9000 Nano Laser Particle Size Analyzer (Angstrom Advanced Inc USA). All measurement were carried at scattering angle 90^0 at constant temperature at 25 ^{0}C.

Freeze drying of nanosuspension to obtain dried Ketoprofen nnoparticles

Freeze drying the most appropriate method to obtain dried ketoprofen nanoparticle, in which a sample of nanosuspension was putting in cell of layophillizer and keep in deep freeze -40 ^{0}C for 24hr., follow that put in layophillizer (LABCONCO) for 72hr. at condenser temperature -40 ^{0}C and pressure (0.9 mbar).

Determination drug content of lyophilized powder

Accurate amount of ketoprofen nanoparticles equivalent to (10mg) dissolve with methanol (10ml) and sonicated for 30 min. then diluted with appropriate volume of methanol and read in UV/ visible spectrophotometric to determine the amount of ketoprofen in lyophilize powder at 255.4 nm.

Fourier transforms infrared spectroscopy (FTIR)

To determine the chemical compatibility, the sample mix with KBr and applied hydraulic pressure to obtain pellets and scan at 400-4000 cm^{-1}

Deferential scanning calorimetry (DSC)

DSC was done by putting 5mg of sample in aluminum pas in METTELER DSC30 instrument, allow the temperature to elevated from 30 ^{0}C to 200 ^{0}C at heating rate (10^{0}C/min.) [24]

Powder x-ray diffraction (PXRD)

PRDX was confirm at continues scan range 2θ (10-50)^0 , in which the generator set at 30 Kv with current 40mA, [25]

Scan Electron Microscope (SEM)

To determination morphology of particles, INSPECT S50 was use in which the sample put in double- side tape carbon cover with gold.

In vitro drug release

Drug release study was curried by using apparatus type 2 paddle ,accurate weight of sample of dried ketoprofen nanoparticle equivalent to (50 mg of kp) put in hard gelatin capsule in 900 ml media of 0.1NHCl (pH1.2) at 37^{0}C ±5 at 50 rpm. 5ml sample withdrawal at specific time interval (2, 5,10,15,20,25,30,45,60 min.),then filter through 0.45 µm filter paper, the sample analyze at 260 nm by UV spectrophometr (1600 Shimadzu, Japan), fresh prepare medium was added to maintain the volume of media constant. The amount of ketoprofen was calculated by using appropriate calibration curve at specific media. Pure powder of ketoprofen and physical mixture (drug: polymer) were also used for compare.

Result and Discussion

Measuring of particles size

The average particle size for all formulas of ketoprofen nanoparticles appeared within nanosize range from 55-995nm. As show in table(1),The smallest size (55nm) for formula F2 with SSA(46.2) and PDI (0.00023), while largest one F6 (995nm) with SSA(2.04) and PDI (0.005). The lowest PDI mean the highly uniform in particle size distribution [26], all formulas have PDI less than 0.2 mean monodisperse distribution and a good physical stability [27].

Effect type and concentration of polymer

Different type of polymer were used in preparation of ketoprofen nanoparticle (PVPk15, HPMCk15, MCA4C and CMC30) at drug: polymer ratio(1:1, 1:2,and 1:3) for formulas (F1-F12) with particle size range from 55nm-995nm, as show in table(1). The results show the F2 (kp +PVPk15 at 1:2) with smallest average particle size 55 nm and uniform distribution of particles (PDI 0.00023), so its selected as the best formula. PVPk15 its nonionic surfactant with steric stabilization in which that stabilizer adsorb on the surface of particles through anchor segment in which highly interact with dispersed particle and act as wetting agent because has ampiphlic moiety in which extend in bulk medium [28]. HPMC also show a nanosize range steric stabilization [29] in which poses alky moiety, however have a good affinity toward hydrophobic part of ketoprofen.

To study effect concentration of polymers used on the size of nanoparticles, three ratio were taken for drug: stabilizer (1:1, 1:2, and 1:3), the results show for formulas (F1,F2) when PVPK15 used, increase concentration of PVPK15 lead to decrease particle size, while F3 increase particle size was noted as increase concentration of PVPK15, this mean F2 at ratio(1:2) drug: stabilizer was optimum concentration of PVPK15 used to obtain small particle size. While for formulas (F4-F6) increase concentration of HPMC K15 cause increase in particle size due to increase stabilizer concentration cause increment in osmotic pressure and cause particle to aggregated to each other and increase particle size [30].

Table (1) ketoprofen nanoparticles, size, PDI and SSA

Formula no.	Particle size	PDI	SSA
F1	99.7	0.008	24.61
F2	55	0.00023	46.2
F3	92	0.0012	22.95
F4	561	0.015	3.71
F5	795	0.008	2.64
F6	995	0.005	2.04
F7	629	0.015	3.66
F8	788	0.022	2.34
F9	887	0.0021	2.12
F10	625	0.017	3.45
F11	319	0.012	6.93
F12	295	0.011	7.59

Entrapment efficiency

Entrapment efficiency explain the ratio of a mass of

drug that present in nanoparticles to the mass of drug that present initially. Entrapping efficiency for ketoprofen nanoparticles was ranged from 85.7%-98.3%. The results explain a suitability of polymers that used and antisolvent technique in the preparation of ketoprofen nanoparticles.

Determination drug content of lyophilized powder

The drug content of the selected formula F2 was 98.87%, which mean a suitability of antisolvent precipitation method in preparation of ketoprofen nanoparticles.

Saturated solubility

Saturated solubility of pure ketoprofen and best formula F2 were determinate by flask shake method in water and in 0.1NHCl (pH1.2), the results appeared increase in saturated solubility approximately 7 fold as compare with pure ketoprofen. Saturated solubility of pure ketoprofen was 0. 11mg/ml and 0.09 ml in water and pH1.2, respectively. In other hand, saturated solubility for ketoprofen nanoparticles was 0.75 mg\ml, 0.23 mg\ml in water and pH1.2 respectively. The increase in saturated solubility result from reduction in particle size cause increase in surface area[32], furthermore, cause enhancement in the hydrophilicity that responsible about increment in the saturated solubility [31]

FTIR

FTIR spectrum for ketoprofen and lyophilize powder of F2(best formula) were determination by using (FTIR spectroscopy, shimadzu, Japan). FTIR – spectrum for pure ketoprofen show characteristic absorption at 1697.38 cm^{-1} and 1654.92cm^{-1} for (C=O streching) vibration of both of ketone functional group and carboxylic group, respectively. (C=C) streching of phenyl group at 1448.54 cm^{-1} and 1595.13 cm^{-1}, [33] as show in figure (1). FTIR-spectrum for lyophilized powder of ketoprofen nanoparticles (F2), were appeared that all principle peaks of drug and polymer were present which mean no interaction occur between drug and polymer.

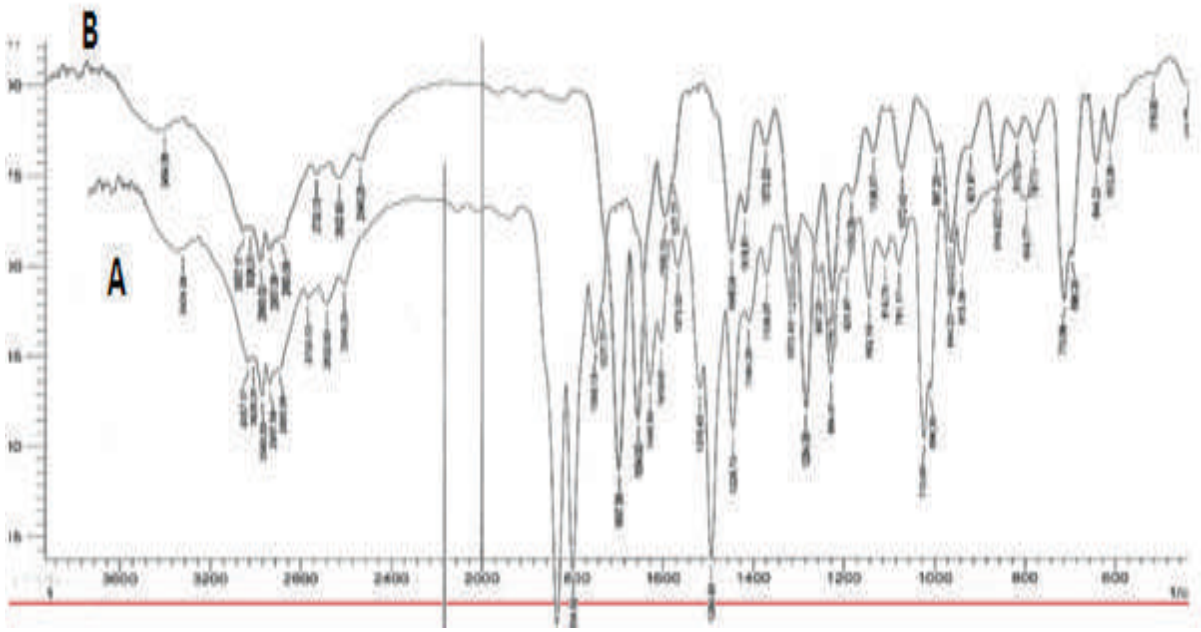


Figure (1). FTIR for (A)pure Ketoprofen, (B) ketoprofen nanoparticles

Deferential scanning calorimetry DSC

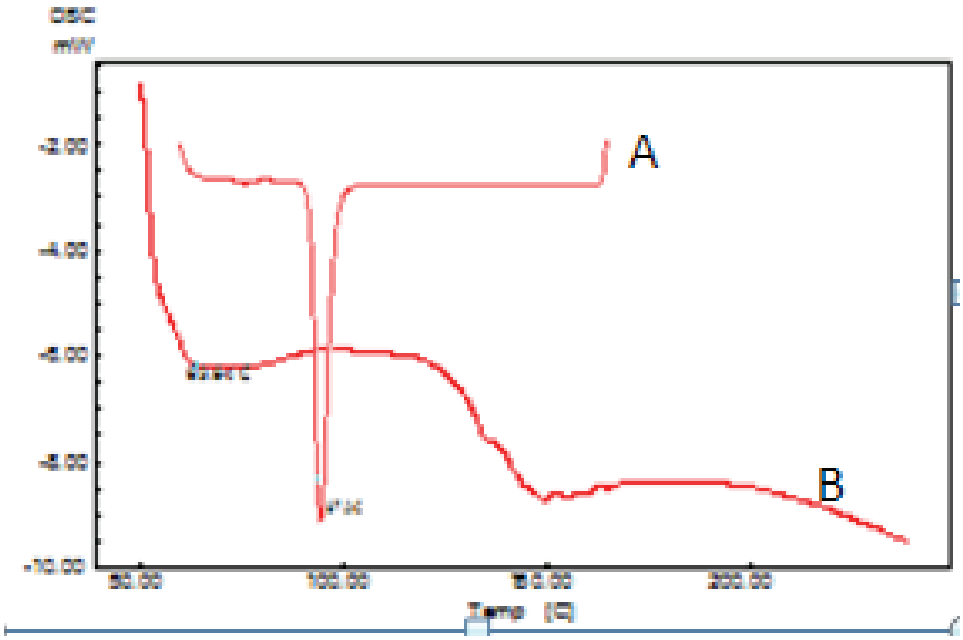


Figure (2). DSC for (A)pure ketoprofen, (B) ketoprofen nanopartiles

The thermal behavior for both pure ketoprofen and ketoprofen nanoparticles F2 was determined by DSC, also to determinate compatibility between drug and polymer. Figure (2) show a single sharp endothermic peak for pure ketoprofen at 97.2 ^{0}C which it's a melting point of ketoprofen, while for ketoprofen nanoparticles showed abroad endothermic peak below 70^{0}C which mean converted of ketoprofen from crystal to amorphous state [24].

Powder x-ray diffraction (PXRD)

PXRD was the most appropriate method to determine the crystallite of drug molecules. PXRD for pure ketoprofen appeared strong several peaks which refer to crystalline molecules of ketoprofen as show in figure (3)

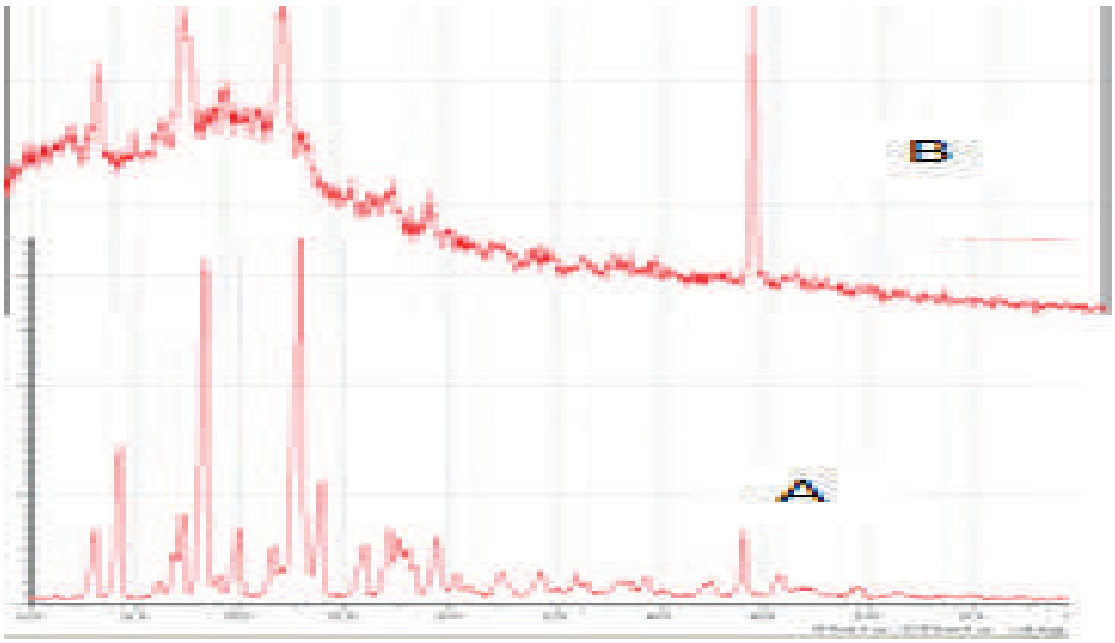


Figure (3). PXRD for (A)pure ketoprofen, (B) ketoprofen nanoparticles

Scan electron microscope SEM

SEM saw used to determine the morphology and topograghy of nanoparticles. Figure (4) for pure ketoprofen show regular crystalline shape with large size, where as for ketoprofen nanoparticle F2 show spherical shape with no aggregation of particles.

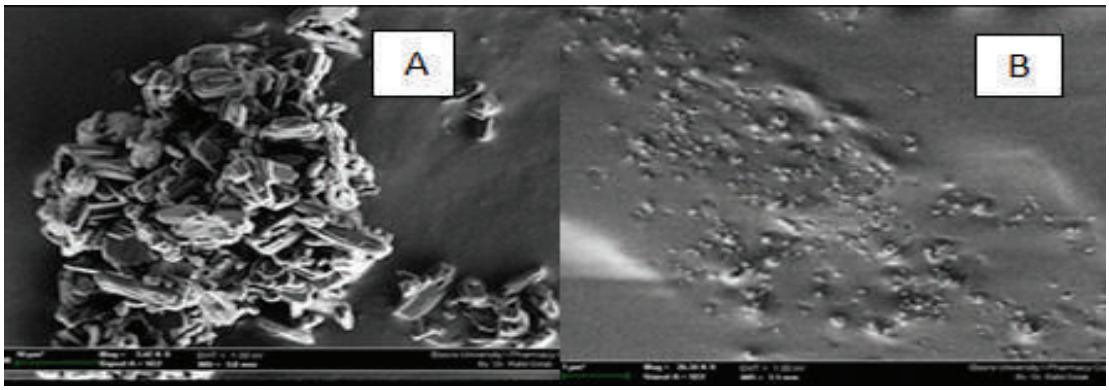


Figure (4) SEM for (A)pure ketoprofen, (B)ketoprofen nanoparticle

In vitro drug release

In vitro drug release was determined for pure ketoprofen , PM of (drug+polymer) and for nanoparticles of ketoprofen (F2). The results show, at (10 min) about 75.7% of release of ketoprofen from nanoparticle, as contract 15.2%, 22.7% for pure ketoprofen and PM (drug+polymer), respectively, as shown in figure(5). However, nanoparticles of ketoprofen appear an increase in dissolution rate as compare with pure ketoprofen and PM(drug:polymer), reduce in particle size lead to enhance surface area and cause an increase in dissolution rate.

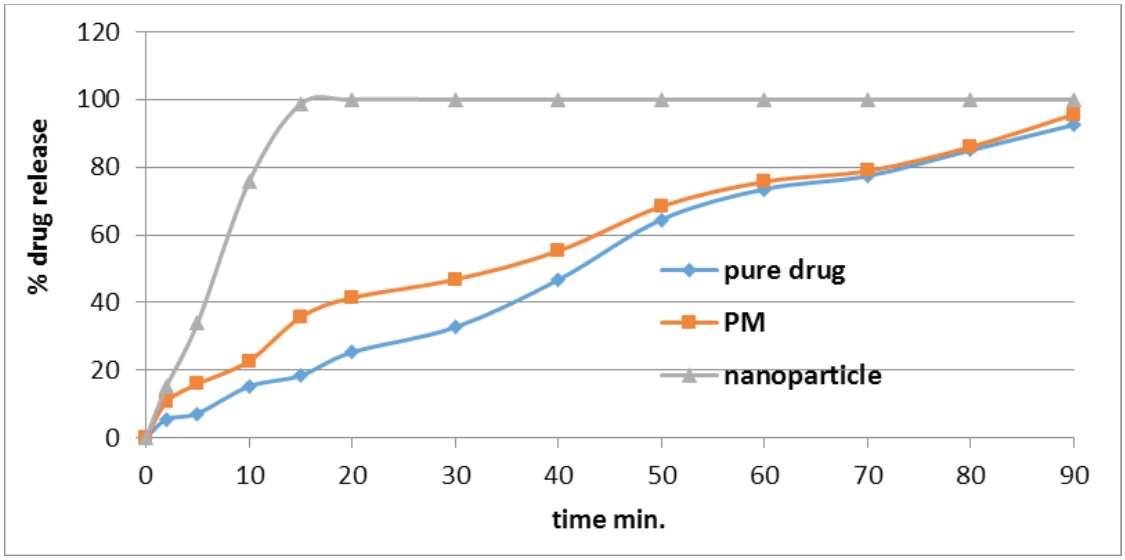


Figure (5). Drug release of prepared Ketoprofen nanoparticles(F2)compared with PM and pure Ketoprofen at 0.1NHCl(PH1.2) at 37C^0±5

Conclusion

This research demonstrate that Ketoprofen nanoparticles with size (55)nm were successfully preparation with PVPk15 by solvent antisolvent evaporation method. FTIR appear there is no chemical interaction between drug and polymer used.DSC show a good compatibility between drug and polymer and converted ketoprofen from crystal to amorphous state, these results also confirm by PXRD. SEM image show a reduction in particles size, spherical shape and no sign of aggregation. The reduction in particle size lead to increase in surface area and enhance dissolution rate ,

furthermore increase bioavailability.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Pharmacy and all experiments were carried out in accordance with approved guidelines.

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The Prevalence of Pathogenic Bacteria from Different Clinical Samples, Northern Part of Iraq

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Abstract

Background : Bacteria are prokaryotic which causes a wide range of diseases in human and animals as well, thus, early diagnosis of bacterial infection is an important step for their management. The vast majority of bacteria are harmless to human and some strains are even beneficial. However, some strains cause infections including pneumonia, meningitis and urinary tract infection…etc. The present study aimed to describe and determine the prevalence rate of pathogenic bacteria in different clinical samples from Duhok city, Kurdistan region of Iraq, as there are no available data of bacterial infections.

Methods: Totally, 200 adult patients enrolled in the present study during July 2017-July 2018. We collected different clinical specimens including (SFA, Bronchial Lavage, catheter, cerebrospinal fluid, body fluids, blood, semen, stool pus, urine, sputum and ear swabs). Standard microbiological techniques used for isolation and identification. All samples collected before antibiotic uses by specialized physician.

Results: The result reported that forty-six samples (23%) obtained from male and one hundred fifty-four samples (77%) obtained from females. A high diversity of pathogens was found but *Staphylococcus aureus* recorded the highest infection rate by (33.3%) which observed in 32 urine samples by and followed by *E. coli* (27.3%) and *Coagulase-Negative Staphylococci* (25.3%), which observed in 36 and 29 urine samples, respectively. Alternatively, *Klebsiella, Streptococcus, Enterobacteriaceae and Pseudomonas aeruginosa* were founded in few samples.

Conclusion: The highest diversity of potential pathogens observed in urine samples was *E.coi*. Over all, *Staphlococcus aureus* was predominant and recorded the highest prevalence rate by (33.3%) in 50 samples which followed by *E. coli* recorded in 41 samples (27.3%) and *Coagulase-Negative Staphylococci* observed in 38 samples (25.3%).

Introduction

The isolation of microorganism is an important step in diagnosis and analyses of microbial virulence [1]. Additionally, isolation and culturing enable the investigation of antibiotic sensitivity for selecting the best effective treatment [2,3], genome sequencing [4,5] and disease diagnosis [6]. Robert Koch, proposed that a pure culture is the foundation of all research in case of infectious diseases [7-9].

Recently, many researches attempt in determining the common pathogenic bacteria and normal flora which cause diseases in human. *Staphlococcus aureus, Escherichia coli* and *Streptococcus* sp. have been widely studied due to their drug resistance and diverse pathogenic features [10] and [12].

The present study aimed to determine and list the bacterial prevalence in different human samples from Duhok city - Kurdiastan region of Iraq, for this purpose statistical analysis performed for easy data analysis.

Methods

Sample collection

Totally, 200 adult patients enrolled in the present study during July 2017- July 2018. Different clinical specimens collected depend on patient symptoms including SFA, Bronchial Lavage, catheter,

(cerebrospinal fluid) CSF, body fluids, blood, semen, stool pus, urine, sputum and swabs from ear, throat and eyes. All samples collected before antibiotic uses by specialized physician.

Isolation and Identification

Diagnosis of bacterial isolated conducted with the aid of standard microbiological techniques and collected data were analyzed using SPSS stastical software (SPSS 25.0, 2017), for easy data expression, p value was $P<0.05$. The swabs were streaked onto nutrient agar, blood agar, MacConkey agar, Mannitol salt agar and Chocolate agar plates in two replications for minimizing the rate of contamination. The inoculated plates were incubated separately in two group of aerobic and anaerobic conditions for 20-24 hours at 37 °C (13). Bacterial isolates defined by observing colony morphology, pattern and size. However, Gram staining techniques used for subgrouping the colonies into Gram negative and Gram positive groups. Additionally, the process of identification further confirmed by biochemical studies which included IMViC test, oxidase test, catalase test, coagulase test and urease test.

Results

During 2017-2018, a total of 200 samples obtained from suspected patients from Dhuok city for bacterial infections. All samples were isolated and identification conducted with standard microbiological techniques. The result reported that forty-six samples (23%) obtained from male and one hundred fifty-four samples (77%) obtained from females, also result declared that bacterial growth were positive for 106 samples (53%) while no bacterial growth observed from the reset 94 samples as shown in Table 1.

The frequency of collected samples listed according to the type of specimens as shown in Table 2. The most collected samples were urine in which 144 urine samples collected and followed by vagina, pus, sputum, blood and swabs samples by 14, 11, 9, 7 and 6 respectively. However, collected samples were at low percentage for CSF, SFA, wound, fluid, catheter and bronchial lavage .

The highest frequency rate of bacterial isolate recorded in 79 urine samples (39.5%) and followed by 6 vagina samples and 5 sputum samples, by (3%) and (2.5%), respectively. Notably, few bacterial growths observed in samples such as blood and CSF by 1.5% and 1%, respectively. However, bacterial growth observed only in one sample of (swab, SFA, catheter, fluid, wound and bronchial lavage) by 0.5 %, as shown in Table 3.

The prevalence rates of bacterial isolates have been listed according to the type of specimens as shown in Table 4. The result determined that *Staphlococcus aureus* recorded the highest infection rate by (33.3%) in 50 samples and observed in 32 urine samples. *E. coli* recorded second highest prevalence rate by (27.3%) which observed in 36 urine samples and followed by *Coagulase-Negative Staphylococci* which recorded in by (25.3%) and observed in 29 urine samples.

Alternatively, *Klebsiella, Streptococcus, Enterobacteriaceae and Pseudomonas aeruginosa* were founded in 9,8,3 and 1 samples, respectively. The percentage of bacterial infection in the present study has been shown in Figure 1.

Table (1): Distribution of bacterial infection according to the sex of suspected patients.

Number of	Samples	Infections	No Growth
Male	46 (23%)	32 (16%)	14 (7%)
Female	154 (77%)	74 (37%)	80 (40%)
Total	200 (100%)	106 (53%)	94 (47%)

Table (2): Frequency and percentage of collected samples according to the type of specimens

Source of infection	Number (%)	Source of infection	Number (%)
Urine	144 (72%)	CSF	3 (1.5%)
Vagina	14 (7%)	SFA	2 (1%)
Pus	11 (5.5%)	Wound	1 (0.5%)
Sputum	9 (4.5%)	Fluid	1 (0.5%)
Blood	7 (3.5%)	Catheter	1 (0.5%)
Swabs	6 (3%)	Bronchial Lavage	1 (0.5%)
Total	200 (100%)		

Table (3): Frequency rate of bacterial growth according to the type of samples.

Source of infection	Infected with Bacteria	No Growth	Source of infection	Infected with Bacteria	No Growth
Urine	79 (39.5%)	65 (32.5%)	CSF	2 (1%)	1 (0.5%)
Vagina	6 (3%)	8 (4%)	SFA	1 (0.5%)	1 (0.5%)
Sputum	5 (2.5%)	4 (2%)	Catheter	1 (0.5%)	0
Pus	4 (2%)	7 (3.5%)	Fluid	1 (0.5%)	0
Blood	3 (1.5%)	4 (2%)	Wound	1 (0.5%)	0
Swab	1 (0.5%)	5) 2.5%)	Bronchial Lavage	1 (0.5%)	0
Total infected and non-infected samples 200 (100%)					

Table (4): Prevalence rate of different bacterial isolates from different samples

Source of infection	Staphlococcus aureus	E. coli	Coagulase-Negative Staphylococci	Klebsiella	Streptococcus	Enterobacteriaceae	Pseudomonas
Urine	32	36	29	8	4	3	0
Vagina	2	2	3	1	1	0	0
Pus	2	1	2	0	1	0	1
Sputum	3	1	1	0	2	0	0

Swab	0	0	1	0	0	0	0
SFA	1	0	1	0	0	0	0
Bronchia lavage	1	0	0	0	0	0	0
Catheter	1	0	0	0	0	0	0
CSF	2	0	1	0	0	0	0
Blood	2	1	0	0	0	0	0
Fluid	1	0	0	0	0	0	0
Wound	1	0	0	0	0	0	0
Total	50 (33.3%)		38 (25.3%)	9 (6%)	8 (5.3%)	3 (2%)	1 (0.7%)

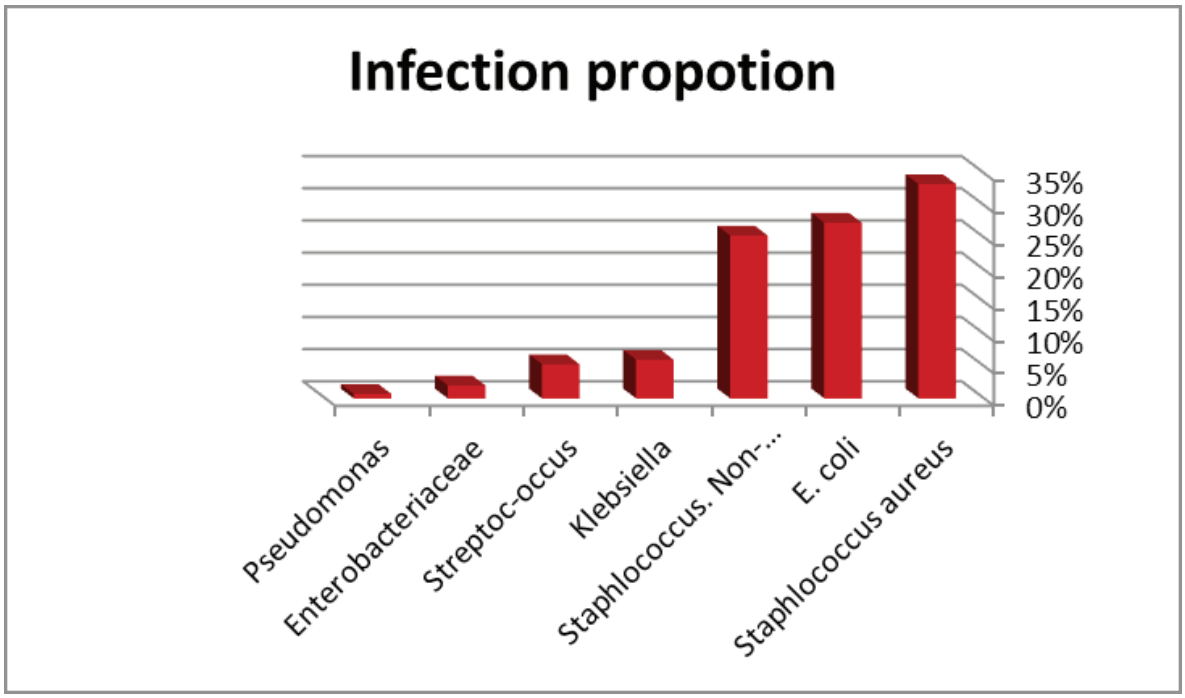


Figure (1): Percentage of bacterial isolates and infection proportion

Discussion

According to the present study, 106 collected samples showed positive bacterial growth out of 200 samples in which 74 positive samples were collected from female and only 32 positive samples were from male. This finding is in line with previous finding which reported that male was more resistance to gastrointestinal and respiratory tract bacterial infections, while female was more prone to bacterial infections in the genitourinary tract same investigation also declared that such differences for bacterial susceptibility between male and female associated to the sex hormones (13).

From the present study, highest frequency rate of bacteria isolated from urine samples by (39.5%) and followed by vagina and sputum by (3%) and (2.5%), respectively. This result totally agrees with previous finding by, Flores-Mireles and et al. (2015), who considered urinary tract infections (UTIs) as the most common bacterial infections at all ages which affecting around 150 million people worldwide per each year and causes morbidity as well (14). Additionally, vaginal infection considered as the second most common bacterial infection in women which called bacterial vaginosis (BV) and characterized by pyogenic infection (15) and (16). Pus producing infection called pyogenic, Staphylococcus aureus and Streptococcus pyogenes are

the best known microorganisms which cause pus due to the secretion of toxins which damage neutrophil and surrounding tissue (17). Pseudomonas, Staphlococcus aureus, and Burkholderia cepacia complex identified as the most frequently isolated bacteria from the sputum of patients with chronic chest lesions or pulmonary disease (18), this finding is agree with our finding in which the number of infections were 9 samples. Only 3 blood samples showed positive bacterial growth, the presence of bacteria in the blood is known as bacteremia which has several significant health consequences. (18), bacteria may also spread through the blood to other parts of the body and causes infection away from the original infection site. (19), (20),(21) and (22). Low number of bacterial growth recorded in ear swabs, CSF, SFA, catheter, fluid, wound and bronchial lavage samples. These results agree with many previous published studies which recorded that approximately 65-330 million people worldwide suffer from ear infection and 60% have serious hearing impairment (23, 24), the sperm cell structure and the whole spermatogenetic cycle can be affected by acute and chronic infections system (25, 26, 27, 28). Bacteria can cause wound infection in case of skin defective functions (29, 30), however, wound infection could be a type of nosocomial infection (31).

Staphlococcus aureus, a Gram-positive bacterium which causes a wide range of infectious diseases, including skin infections, bacteraemia, endocarditis, pneumonia and food poisoning (32), the result determined that Staphlococcus aureus recorded the highest infection rate by (33.3%). E. coli, a normal flora in the intestinal lumen of human which maintaining normal intestinal homeostasis (16, 33). Some strains of E. coli varieties may diverge and causes diseases, such strains acquire unique virulence factors by which causes infections (34). Klebsiella pneumoniae, accounts as a significant proportion of hospital-acquired infections of the urinary tract, pneumonia, septisemia, and soft tissue (35). In the present study Klebsiella pneumoniae represented in 9 patient samples (6%), while Streptococcus spp. was represented in 8 patient specimens (5.3%). Enterobacteriaceae only observed in urine samples, this result in agreement with previous finding in which concluded that members of Enterobacteriaceae causes urinary tract infections (UTI) and diarrhea (35). Pseudomonas aeruginosa, a Gram-negative bacillus which causes hospital acquired infections more commonly in patients with ventilation, burn patients, and chronic debility patients (36), the growth of this bacteria only reported in one sample.

Conclusion

Staphlococcus aureus recorded the highest prevalence rate by (33.3%) and observed in all samples except swabs. While E. coli recorded in 41 samples (27.3%) and followed by Coagulase-Negative Staphylococci which observed in 38 samples (25.3%). Additionally, highest bacterial growth reported in urine samples, this finding needs further investigation like antibiotic sensitivity profile study for selecting effective treatment.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Hawler Medical University and all experiments were carried out in accordance with approved guidelines.

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Assessment of Psychological Risk Factors for Spontaneous Abortion at Maternity Wards in Baghdad City Hospitals

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Abstract

Objectives: To assess the psychological risk factors for spontaneous abortion. **Methods:** A descriptive Analytical study has been conducted on Non- probability sample consisted of (160) women who are suffering from spontaneous abortion in maternity wards from six hospitals at Al Rusafa, AlKarckh and Medical city health directorate. Study has been implemented for the period of January 10th 2020 to March 10th 2020 . A questionnaire used as a tool of data collection , the study instrument consist of Risk Factors (psychological, depression and anxiety). Descriptive and inferential statistical analysis are used to analyze the data. **Results:** The study results shows that (60.6%) of women suffering from stress and anxiety during current pregnancy. Only (1.9%) have depression, (8.1%) are reporting the presence of sad events during current pregnancy, (8.8%) are reporting presence of death during current pregnancy, (26.3%) are experiencing a family problem, (25.6%) are violated by their husbands, and (6.9%) are violated by their husband's family. **Conclusion:** The majority of women are experiencing mild depression during their pregnancy, and more than half of women are experiencing moderate anxiety during their pregnancy.

Keywords: *Assessment, Risk factors, depression, anxiety, Spontaneous abortion.*

Introduction

Having a baby is an amazing and positive experience for most of the women. However, the dream of having a baby can be shattered by the huge blow couples receive during miscarriage and introduces physical and emotional challenges(1). Spontaneous abortion is the most common complication during early pregnancy in women with a rate of 15-20% among pregnant women nearly 80% of spontaneous abortion occurs in the first trimester menses (2), as well as expelling of the fetus from the uterus during the first half of gestation at 20 weeks or less (3). Studies revealed that anywhere from 10 to 25% of all clinically recognized pregnancies will end in miscarriage (4). A study conducted in Saudi Arabia reported that 44% of Saudi women with high-risk pregnancy were aged between 30 and 35 years of age. Approximately 35% of them had two or more previous abortion (5) . In a study it has been reported several modifiable risk factors for miscarriage, advanced maternal age at conception, obesity, smoking, alcohol and caffeine consumption, lifting of heavy weights, night shift work and psychological stress have all been identified as modifiable risk factors during pregnancy for miscarriage (6) .

Methodology

A descriptive Analytical study has been conducted on Non- probability (purposive) of (160) women who are suffering from spontaneous abortion in maternity wards from six hospitals at Al-Elwyia maternity Teaching hospital, and Fatima Al Zahraa and Ibn Al Balady maternity & children hospital at Al Rusafa health directorate. Al Karckh maternity hospital, and Al Yarmook Teaching hospital at Al Karckh health directorate and Baghdad Teaching hospital at Medical city health directorate . Study has been implemented for the period of January 10th 2020 to April 1st 2020 . A questionnaire used as a tool of data collection , the study instrument consist of Risk Factors (psychological, depression and anxiety) . A pilot study has been carried out to test the reliability of the questionnaire and content validity has been carried out through (12) experts.

Descriptive and inferential statistical analysis are used to analyze the data.

Results

Table (1): Assessment of Psychological Risk Factors among Women with Abortion (N=160)

Lst	Psychological factor	No		Yes	
		f	%	f	%
1	Exposure to Psychological problems	159	99.4	1	0.6
2	Suffering Stress and anxiety during the current pregnancy	63	39.4	97	60.6
3	Depression	157	98.1	3	1.9
4	Presence of sad events during the current pregnancy	147	91.9	13	8.1
5	The presence of death during current pregnancy	146	91.3	14	8.8
6	Exposure to family problems	118	73.7	42	26.3
7	Subjected to violence from the husband	119	74.4	41	25.6
8	Subjected to violence from the husband s family	149	93.1	11	6.9
9	Subjected to Physical Violence	149	93.1	11	6.9
10	Subjected to Verbal Violence	119	74.4	41	25.6
11	Subjected to Domestic Violence	129	80.6	31	19.4
12	Subjected to Sexual Violence	159	99.4	1	0.6
13	Subjected to Economic Violence	147	91.8	13	8.2
14	Subjected to Social Violence	158	98.7	2	1.3

f: Frequency, %: Percentage

The table (1) shows that (60.6%) of women suffering from stress and anxiety during current pregnancy. Only (1.9%) have depression, (8.1%) are reporting the presence of sad events during current pregnancy, (8.8%) are reporting presence of death during current pregnancy, (26.3%) are experiencing a family problem, (25.6%) are violated by their husbands, and (6.9%) are violated by their husband's family. The types of violence among women refer to: physical (6.9%), verbal (25.6%), domestic (25.6%), sexual (0.6%), economic (8.2%), and social (1.3%).

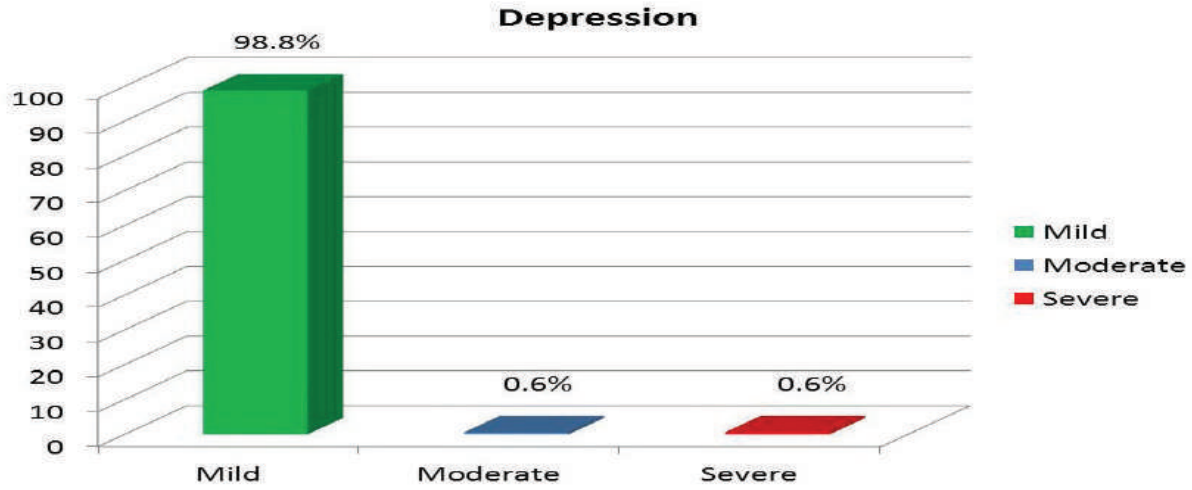


Figure (1): Assessment the Level of Depression among Women with Abortion (N=160) , Using (Beck Scale for Depression consist of (20) items)

The figure depicts that women are experiencing mild depression during their pregnancy (98.8%), and moderate and sever (0.6) depression respectively.

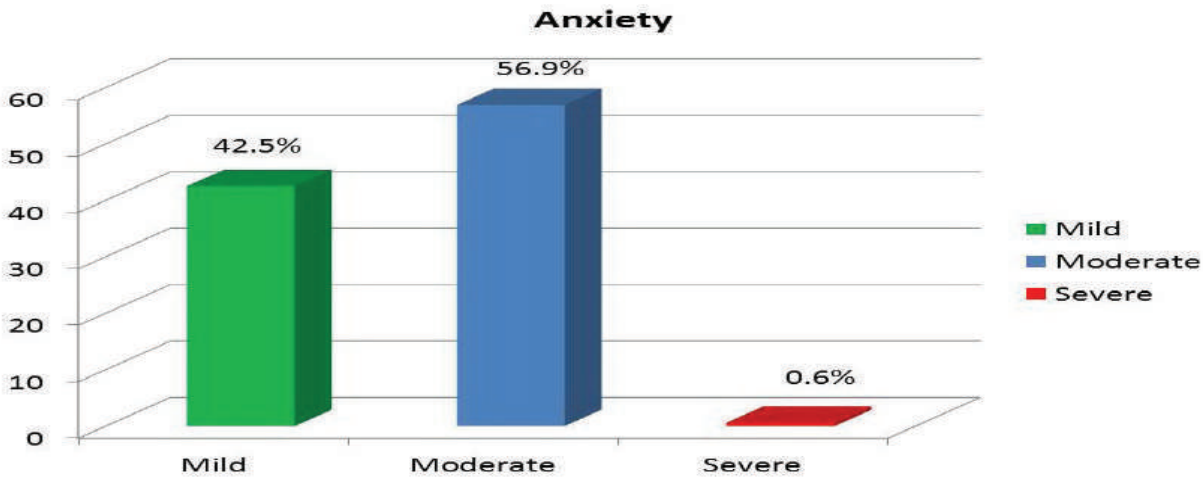


Figure (2): Assessment the level of Anxiety among Women with Abortion (N=160), Using (Taylor's anxiety scale consist of (20) items)

Discussion

Discussion of Psychological Risk Factors among Women with Abortion:

The study results shows that there are (60.6%) of women suffering from stress and anxiety during current pregnancy, (1.9%) have depression, (8.1%) are reporting the presence of sad events during current pregnancy, (8.8%) are reporting presence of death during current pregnancy, (26.3%) are experiencing a family problem, (25.6%) are violated by their husbands, and (6.9%) are violated by their husband's family. The types of violence among women refer to: physical (6.9%), verbal (25.6%), domestic (25.6%), sexual (0.6%), economic (8.2%), and social (1.3%) **Table (1).** This result in agreement with the study, which reported a significantly increased miscarriage risk in women suffering from psychological stress [7] . The association between psychological stress and miscarriage could result, at least in part, from activation of the hypothalamic-pituitary-adrenal axis by recruitment of hypothalamic neurons which secrete corticotrophin-releasing hormone, increasing pituitary secretion of adreno- cortico-trophic hormone secretion and hence of adrenal cortisol. This hormone has direct effects on decidual and placental metabolism but also interacts with progesterone signaling stress-related early

pregnancy failure could also result from suppression of the hypothalamic-pituitary-gonadal axis [8]. Other studies reported that violence against woman constitute an important global public health problem , and for women of reproductive age lead to negative pregnancy outcomes [9, 10]. .

Discussion of Depression as Psychological Risk Factors among Women with Abortion:

The study results shows that women are experiencing mild depression as indicated by the mean scores of the item scales that refer to low among all item of the scale, the figure (1) depicts that women are experiencing mild depression during their pregnancy (98.8%). A study found that the mean (**Beck Depression Inventory**) BDI score of the pregnant women was 11.12 ± 6.65. According to the BDI, 46.2% of the pregnant women had no depression symptoms, 34.59% of them had mild, 13.91% had moderate, and 4.89% had severe level of depression symptoms [11].

Discussion of Anxiety as Psychological Risk Factors among Women with Abortion:

The present study indicates that women having mild to moderate level of anxiety as reflected by the mean scores of the items, the figure shows that more than half of women are experiencing moderate anxiety during their pregnancy (56.9%). This result in agreement with the study which reported that the risk of miscarriage was significantly higher in women with a history of exposure to psychological stress [7].

Conclusion

The study concluded that the majority of women are experiencing mild depression during their pregnancy, and more than half of women are experiencing moderate anxiety during their pregnancy.

Conflict of Interest: Nil

Source of Funding: the source of funding is self

Ethical Clearance: is obtained from the Ministry of Health (Al-Elwyia maternity Teaching hospital, and Fatima Al Zahraa and Ibn Al Balady maternity & children hospital at Al Rusafa health directorate. Al – Karckh maternity hospital, and Al-Yarmook Teaching hospital at Al Karckh health directorate and Baghdad Teaching hospital at Medical city health directorate) . All women with spontaneous abortion participated in the research - have been approved before the questionnaire is started.

Recommendations:

The study recommended to Conduct Structured teaching programed (STP) for mothers with history of miscarriage included meaning, risk factors, causes and prevention of miscarriage. It also includes the do and don'ts during 1st trimester of pregnancy.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Maternal and Neonate Nursing Department and all experiments were carried out in accordance with approved guidelines.

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Assessment of pregnancy - Related Health Problems among Less Than 18 Years Pregnant Women in Al- Nasiriya Maternity Hospitals

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Abstract

Objective: To assess the Socio-economic and Cultural Factors, Personal Behavior and Psychological Factors, and Biological Factor. **Methods:** descriptive analytic study conducted in December 25th, 2019 to 10th March, 2020 at Maternity Hospitals in Al-Nasiriya city . Non-probability sample consists of (180) pregnant women less than 18 years admitted to maternity hospitals suffering health problems. The study instrument comprised of factors related to pregnancy (Socio-economic and Cultural Factors, Personal Behavior and Psychological Factors, and Biological Factor. Data collection is performed through the use of the study instrument , after permission were obtained from the pregnant women . Data was collected through interview techniques. Descriptive and inferential statistical data analysis approaches are used. **Result:** The study results shows that (60%) of women's current age is (17) years old, , (39%) primary school education, (65%) living in extended families, (37.2%) of women were aged (16) years at their first pregnancy . The results presents the factors related to pregnancy; the socio-economic and cultural factors that are significant (lack of knowledge in sexual and reproductive health (91.1%), and biological factors in early body changes and weight gain (43.9%). **Conclusion:** The study concludes there are lack of knowledge in sexual and reproductive health, lack of knowledge for health problems, lack of knowledge regarding use of contraceptive methods, Not having healthy food practice, stress and feeling of personal failure, and neglect of the special needs. **Recommendation:** The study recommends that pregnant teenager to have appropriate antenatal care as well as social support to avoid medical problems during pregnancy. Educational programs should be emphasized to pregnant teenage mothers for increasing their knowledge about pregnancy**.**

Keywords: *Pregnant women , adolescent , pregnancy related Health, Personal Behavior, Biological Factor.*

Introduction

Teenage pregnancies are associated with social issues, including lower educational levels and poverty **(1).** Teenage pregnancy in developed countries is usually outside of marriage and is often associated with a social stigma **(2).** In the developing world about 2.5 million females under the age of 16 and 16 million females 15 to 19 year old have children each year .Another 3.9 million have abortions. It is more common in rural than urban areas. Worldwide, complications related to pregnancy are the most common cause of death among females 15 to 19 year old **(3).** Teenage pregnancy is an important public health problem, especially in developing countries, with a high rate of marriages at a young age, along with poor prenatal and postnatal care. In these countries, 15%–20% of all deliveries were in mothers aged younger than 19 years, and 25% of maternal deaths occurred in this age group **(4).**

Methodology

Descriptive analytic study conducted in December 25th, 2019 to 10th March, 2020 at Maternity Hospitals in Al-Nasiriya city . Non-probability sample consists of (180) pregnant women less than 18 years admitted to maternity hospitals suffering health problems. The study instrument comprised of: Socio-economic and Cultural Factors, Personal Behavior and Psychological Factors, and Biological Factor. The pilot study was conducted on

(20) pregnant women, in December 25th, 2019 to 2nd January , 2020, the instrument presented to (11) experts in the different fields. The reliability for the questionnaire was statistically adequate. Data collection is performed through the use of the study instrument , after permission were obtained from the pregnant women . Data was collected through interview techniques. Descriptive and inferential statistical data analysis approaches are used.

Results

Table (1): Socio-economic and Cultural Factors related to Pregnancy under the Age 18 Years (N=180)

	Socio-economic and cultural factors	No		Yes	
		f	%	f	%
1	Age difference between husband and wife.	138	76.7	42	23.3
2	Low level of education (limited education)	143	79.4	37	20.6
3	Marriage by desire or under family pressure	165	91.7	15	8.3
4	Dose the husband use alcohol or narcotic pills?	180	100	0	0
5	Family instability (exposure to violence and domestic abuse)	177	98.3	3	1.7
6	Lack of knowledge or incorrect use of contraceptives.	60	33.3	120	66.7
7	Lack of knowledge of health problems.	30	16.7	150	83.3
8	The structure of the extended family.	35	19.4	145	80.6
9	Lack of knowledge in the field of sexual and reproductive health.	16	8.9	164	91.1
10	Dose the period between current and previous pregnancy affect?	162	90	18	10
11	Is pregnancy planned?	73	40.6	107	59.4
12	Is pregnancy desirable type?	35	19.4	145	80.6
13	Low economic status	106	58.9	74	41.1

f: Frequency, %: Percentage

The table (1) presents the socio-economic and cultural factors related to pregnancy for women under age (18) years; the socio-economic and cultural factors that are significant are: lack of knowledge in the field of sexual and reproductive health (91.1%), lack of knowledge for health problems (83.3%), the structure of the extended family (80.6%), the desirability of pregnancy (80.6%), lack of knowledge regarding use of contraceptive methods (66.7%), and planned pregnancy in (59.4%).

Table (2): Personal Behavior and Psychological Factors related to Pregnancy under the Age 18 Years (N=180)

Personal behavior and psychological factors		No		Yes	
		f	%	f	%
1	Emotional abuse include (ignore, isolation, verbal assault)	162	90	18	10
2	Physical abuse (physical violence)	179	99.4	1	0.6
3	Neglect, special needs are ignored	154	85.6	26	14.4
4	Lack of self- confidence	162	90	18	10
5	Stress	100	55.6	80	44.4
6	Hopelessness	172	95.6	8	4.4
7	Depression	169	93.9	11	6.1
8	Feeling of personal failure (feeling of helplessness).	154	85.6	26	14.4
9	Healthy food practice	117	65	63	35

The table (2) presents the personal behavior and psychological factors related to pregnancy for women under age (18) years. Personal behavior and psychological factors present: Low frequencies have been seen among the personal, behavioral and psychological factors that are: Not having healthy food practice(65%), stress (44.4%), feeling of personal failure (14.4%), and neglect of the special needs (14.4%).

Table (3): Biological Factor related to Pregnancy under the Age 18 Years (N=180)

Biological factor		No		Yes	
		f	%	f	%
1	Decreased menstruation age (beginning of the first menstruation).	153	85	27	15
2	Do you suffer from menstrual disorder	125	69.4	55	30.6
3	Impact of malnutrition on the menstrual cycle	173	96.1	7	3.9
4	Suffer from the effects of hormonal changes (analysis)	160	88.9	20	11.1
5	Early body changes (weight gain and large breast size)	101	65.1	79	43.9
6	Exposure to some disease and infections during this age Period	113	62.8	67	37.2
7	Suffer from anemia	126	70	54	30

The table (3) presents the biological factors related to pregnancy for women under age (18) years. The biological factors present: early body changes (weight gain and large breast size) (43.9%), exposure to some disease and infections (37.2%), and suffering from menstrual disorder (30.6%).

Discussion

Discussion of Factors related to Pregnancy among Pregnant Women Less than 18 Years:

The study results in Table (1) presents the factors related to pregnancy for women under age (18) years; the socio-economic and cultural factors that are significant are: lack of knowledge in the field of sexual and reproductive health (91.1%), lack of knowledge for health problems (83.3%), the structure of the extended family (80.6%), the desirability of pregnancy (80.6%), lack of knowledge regarding use of contraceptive methods (66.7%), and planned pregnancy (59.4%). A study found that (66.8%) of women have lack of knowledge in the field of sexuality and lack of knowledge in contraceptive method use [5], that agree with the result of present study. Also, another study found the planned pregnancy in (54%) [6] , that agree with the result of present study.

The study results in Table (2) presents the there are low frequencies have been seen among the personal, behavioral and psychological factors that are: Not having healthy food practice(65%), stress (44.4%), feeling of personal failure (14.4%), and neglect of the special needs (14.4%). A study regarding the effect of teenage pregnancy on adolescents , in this study (92%) of the participant have lack of self –esteem [7], which is inconsistent with the results of present study. this result agree with the result of study concerning factors associated with teenage pregnancy and its effects, the study found the neglect or lack of basic needs is in (8.1%) [8] , another study about factors responsible for teenage pregnancy and its implication on adolescent health and education: Perception of secondary school students in Nigeria, the study found health problems in food practice is (60%) [9]. Teenage pregnancy in developing countries often occurs within marriage and half are planned. However, in these societies, early pregnancy may combine with malnutrition and poor health care to cause medical problems. When used in combination, educational interventions and access to birth control can reduce unintended teenage pregnancies, study about major depressive disorder during teenage pregnancy: socio-demographic, obstetric and psychosocial correlates, they found significant association with the (wife's educational at p-value 0.006 and occupational at p-value 0.017) [10, 11, 12], that's disagree with the result of present study .

The study results in Table (3) presents the there are early body changes (weight gain and large breast size) (43.9%), exposure to some disease and infections (37.2%), and suffering from menstrual disorder (30.6%). The study result agree with the result of the study regarding teenage pregnancies: A worldwide social and medical problem, who found that the weight gain in (43.8%) of teenage pregnancies[13]. also study found the percentage of exposure to infection was (21.6%) of participant[14]. Another study concerning teenage pregnancy medical and biological problems, the study finding showed that menstrual disorder in (4,6%) of participants, the result of study disagree with the result of present study[15]. Teenage mothers are also at high risk of malnutrition and sexually transmitted diseases. Medical complications in this age group during pregnancy include anemia, urinary tract infection, hypertension, preeclampsia, eclampsia, and a high rate of instrument delivery and cesarean section[4]. Previous studies have reported that adolescent pregnancy and delivery are associated with adverse maternal and neonatal outcomes, such as low birth weight, stillbirth, preterm delivery, maternal anemia, postpartum depression, eclampsia, maternal death and post neonatal death . However, there has been conflicting findings from previous studies especially in women aged ≤15 years, primarily due to small sample size, differences in medical services, and women's social backgrounds[16-20] .

Conclusions

The study concluded that there are lack of knowledge in sexual and reproductive health, lack of knowledge for health problems, lack of knowledge regarding use of contraceptive methods, Not having healthy food practice, stress and feeling of personal failure, and neglect of the special needs .

Recommendations

The study recommends that pregnant teenager to have appropriate antenatal care as well as social support to avoid medical problems during pregnancy. Educational programs should be emphasized to pregnant teenage mothers for increasing their knowledge about pregnancy.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Maternal and Neonate Nursing Department and all experiments were carried out in accordance with approved guidelines.

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The Relationship between Severity of Anemia and Nutritional Factors Determinant at Primary Health Care Centers in Baghdad City

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Abstract

Objective: To determine the relationship between severity of anemia and nutritional factors determinant at Primary Health Care Centers. Methods: A descriptive study has been carried out at AL.Russafa Health Directorate (Bab AL Muadham, and AL Mustanseria) ideal training health care center. AL-Karkh Health Directorate (AL-Salam) ideal training health care center. Non-probability sample consists of (76) anemic pregnant women. questionnaire has been designed and constructed to be used as a means of data collection. Data collection is performed through interview techniques, and the use of the study instrument after permission were obtained from the pregnant women for the period 2nd January to 17th March 2020. Results: The results of nutritional factors indicates that (consumption of dairy products, vegetables, eggs, and fruits) are highly consumed per day (77.6%, 100%, 46.1%, and 92.1%) as indicated by high mean scores. While (consumption of poultry meat, dates and nuts) are moderately consumed per weeks (55.3%, and 56.6%). The remaining nutritional items are lower consuming that is (red meat products, fish, liver, and nutritional plants). Sever anemia found in (6.6%), moderate severity of anemia in (61.8%), and mild anemia in (31.6%). high significant relationships found among severity of anemia with consumption of red meat, consumption of poultry meat, and fish consumption (p-value= 0.004, 0.001, and 0.005); the finding also show significant relationship between severity of anemia and eating nutritional plants (p-value=0.007). Conclusions: the study results concluded high significant relationships between severity of anemia with consumption of red meat, consumption of poultry meat, and fish consumption and eating nutritional plants. Recommendation: Teach women in pregnancy age to dietary habits as a part of an overall approach to health promotion, Increasing level of awareness in women and their families about the risk of anemia on pregnancy.

Keywords: *Anemia , Pregnant women ,Primary Health Care Center ,Nutritional factors, hemoglobin.*

Introduction

Anemia is one of the most common nutritional deficiency diseases observed globally. It affects more than a quarter of the world's population. Globally, 41.8% of pregnant women and close to one third of non-pregnant women (30.2%) are anemic [(1)]. Anemia during pregnancy contributes to 20% of all maternal deaths and it increases the risks of fetal, neonatal and overall infant mortality [(2)]. Anemia during pregnancy is considered severe when Hb concentration is less than 7.0 g/dl, moderate when Hb level is 7.0 - 9.9 g/dl, and mild when Hb level is 10.0 - 10.9 g/dl. [(3,4,5)]. It is a major cause of morbidity and mortality of pregnant women in developing countries and has both maternal and fetal consequences [(5,6)]. In developing countries, the cause of anemia during pregnancy is multi factorial and includes nutritional deficiencies of iron, foliate, and vitamin B12 and also parasitic diseases. Iron deficiency is the cause of 75% of anemia cases during pregnancy [(7)]. The prevalence of iron deficiency was 10 times higher than that of foliate deficiency or vitamin B-12 deficiency. Anemia during pregnancy and its consequences can be prevented and treated if diagnosed on time [(8)].

Methodology

A descriptive study has been carried out throughout

the present study. The study has been conducted at AL.Russafa Health Directorate that include: Bab AL Muadham, and AL Mustanseria ideal training health care center . Also from AL-Karkh Health Directorate that include AL-Salam ideal training health care center. Non-probability (purposive) sample consists of (76) diagnosed anemic pregnant women. questionnaire has been designed and constructed to be used as a means of data collection. It is comprised of: nutritional factors, laboratory results. To make the instrument more valid, it have been presented to a panel of (12) experts in the different fields. A pilot study was conducted on (10) anemic pregnant women, attending Bab AL-Moudham health care center from December 15th , 2019 to December 23rd ,2019, alpha correlation coefficient was applied to determine the reliability (0.78) was statistically good. Data collection is performed through the use of the study instrument (the questionnaire), after permission were obtained from the pregnant women, data was collected through interview techniques, each interview took up to 30 to 40 minutes.

Results:

Table (1): Distribution of the Pregnant Women According to their Nutritional Factors (N=76)

List	Items	Daily f (%)	One day another f (%)	Once a week f (%)	Once every 2 weeks f (%)	Once a month f (%)	MS (Ass.)
1	consumption of red meat products	0 (0)	2 (2.6)	23 (30.3)	17 (22.4)	34 (44.7)	1.91 Low
2	consumption of poultry meat	0 (0)	3 (3.9)	42 (55.3)	21 (27.6)	10 (13.7)	2.50 Moderate
3	fish consumption	0 (0)	1 (1.3)	31 (40.8)	19 (25)	25 (32.9)	2.11 Low
4	consumption of dairy products	59 (77.6)	15 (19.7)	2 (2.6)	0 (0)	0 (0)	4.75 High
5	liver consumption	0 (0)	1 (1.3)	8 (10.5)	14 (18.4)	53 (69.7)	1.43 Low
6	consumption of vegetables	76 (100)	0 (0)	0 (0)	0 (0)	0 (0)	5.00 High
7	egg consumption	25 (32.9)	35 (46.1)	12 (15.8)	0 (0)	4 (5.3)	4.01 High
8	consumption of fruits	70 (92.1)	6 (7.9)	0 (0)	0 (0)	0 (0)	4.92 High
9	consumption of dates and nuts	11 (14.5)	16 (21.1)	43 (56.6)	1 (1.3)	5 (6.6)	3.36 Moderate
10	Eating only plant (nutritional plant)	18 (23.7)	1 (1.3)	0 (0)	0 (0)	57 (75)	1.24 Low

f: Frequency, %: Percentage, Assess: Assessment Low= 1-2.33, Moderate= 2.34-3.67. High= 3.68-5

Table (1) presents the nutritional factors among the pregnant women; the finding indicates that (consumption of dairy products, vegetables, eggs, and fruits) are highly consumed per day (77.6%, 100%, 46.1%, and 92.1%) as indicated by high mean scores. While (consumption of poultry meat, dates and nuts) are moderately consumed per weeks (55.3%, and 56.6%). The remaining nutritional items are lower consuming that is (red meat products, fish, liver, and nutritional plants

Table (2): Distribution of the Pregnant Women According Iron , Folic Acid, and Tea Consumption related Variables (Determinants of Anemia)

List	Variable			
1	Prescribed Ferrous by doctor?	No	0	0
		Yes	76	100
		Total	76	100
2	Regularity of taking ferrous	None	5	6.6
		Irregular	9	11.8
		Regular	62	81.6
		Total	76	100
3	Starting taking ferrous	None	5	6.6
		At second semester	68	89.5
		At third semester	3	3.9
		Total	76	100
4	Drinking coffee and tea	No	30	39.5
		Yes	46	60.5
		Total	76	100
5	Taking folic acid during pregnancy	No	11	14.5
		Yes	65	85.5
		Total	76	100

Table (2) shows that all of the pregnant women reporting that their doctor prescribed ferrous medication for them (100%), (81.6%) of them are regularly taking the medication daily. They start taking the medication at second semester (89.5 %). (60.5%) of pregnant women are drinking coffee and tea, and (85.5%) of pregnant women taking folic acid during pregnancy.

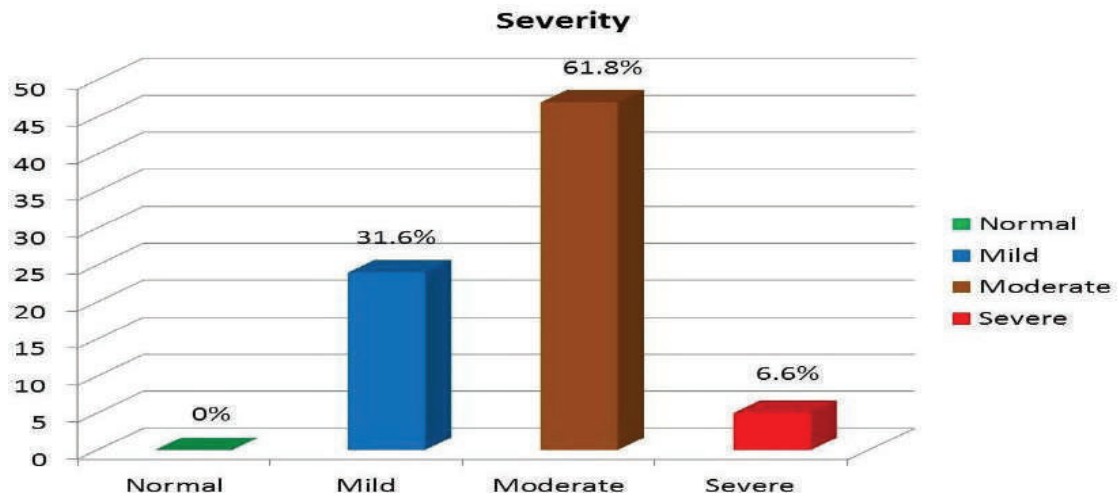


Figure (1): Severity of Anemia among Pregnant Women (N=76)

This figure shows the severity of anemia among pregnant women; the figure shows that sever anemia in (6.6%), moderate severity of anemia in (61.8%), and mild anemia in(31.6%).

Table (3): Correlation between Severity of Anemia and Nutritional Factors Determinants among Pregnant Women (N=76)

Severity of anemia Nutritional Factors	Pearson correlation	p-value	Sig
Consumption of red meat products	.323	.004	H.S
Consumption of poultry meat	.378	.001	H.S
Fish consumption	.317	.005	H.S
Consumption of dairy products	-.059	.610	N.S
Liver consumption	.056	.633	N.S
Consumption of vegetables	.054	.643	N.S
Egg consumption	.042	.721	N.S
Consumption of fruits	-.130	.365	N.S
Consumption of dates and nuts	.054	.643	N.S
Eating only plant (nutritional plant)	-.309	.007	S

P: probability, Sig: Significance, N.S: Not Significant, S: Significant, H.S: High significant

This table reveals that there are high significant relationships among severity of anemia with consumption of red meat, consumption of poultry meat, and fish consumption (p-value= 0.004, 0.001, and 0.005); the finding also show significant relationship between severity of anemia and eating nutritional plants (p-value=0.007).

Discussion

The study results presents the nutritional factors among the pregnant women; the finding indicates that (consumption of dairy products, vegetables, eggs, and fruits) are highly consumed per day (77.6%, 100%, 46.1%, and 92.1%) respectively. While (consumption of poultry meat, dates and nuts) are moderately consumed per weeks (55.3%, and 56.6%). The remaining

nutritional items are lower consuming that is (red meat products, fish, liver, and nutritional plants). It was found that one of the major contributory factors for anemia in developing countries is consumption of plant based food containing insufficient iron. Meat is a good source of high quality protein, iron , zinc and of all the Vitamins except folic acid. Iron absorption is enhanced when consumed with foods high in vitamin C such as orange juice while coffee and tea inhibit iron absorption [9] .

Also, study in Erbil city, Iraq, address the explanation for that may be one of the major contributory factors in developing countries for development of anemia is consumption of plant based foods containing insufficient iron in accordance with body needs especially during pregnancy; also lack of iron absorption enhancer in the meals like vitamin C, and drinking of iron absorption inhibitor drinks like tea immediately after meal **[10].** The study results presents the severity of anemia among pregnant women; the figure shows that sever anemia in (6.6%), moderate severity of anemia in (61.8%), and mild anemia in(31.6%).This study in agreement with the study of (145) pregnant women found the mean of Hb concentration was 11.55 ± 2.97 g/dl with a range of 5.4 to 18.7g/dl. Among anemic pregnant women, 44(30.34%) had mild anemia, 87(60%) had moderate anemia, and 14(9.66%) had severe anemia [11].

The study results indicates that all of the pregnant women reporting that their doctor prescribed ferrous medication for them (100%), (81.6%) of them are regularly taking the medication daily. They start taking the medication at second semester (89.5 %), (60.5%) of pregnant women are drinking coffee and tea. (85.5%) of pregnant women report that their doctor prescribed folic acid during pregnancy. In a study of anemia (IDA) and dietary pattern among pregnant women in Baghdad city, Iraq show About 45% had one tablet day of iron-folic acid, while only 6% had two tablets a day and, others didn't take it (statistically significant association). The result referred to the number and percentage of anemic women who take prophylactic drugs (iron, Folic acid, vit. B12) was (21.8%) that lower than pregnant women who didn't take it (33.6%) [12]. A study result is consistent with other studies done in Ethiopia[13]. This significant association might be due to the reason that meat is an important source of heme iron [14,15,16]. This result is in agreement with a study done in Egypt and Ethiopia, which showed significant association between anemia and consumption of tea [18,19]. This could be drinking tea/coffee after food intake may affect iron absorption which leads to inadequate dietary iron intake in the pregnant women.

The study results reveals that there are high significant relationships among severity of anemia with consumption of red meat, consumption of poultry meat, and fish consumption (p-value= 0.004, 0.001, and 0.005); the finding also show significant relationship between severity of anemia and eating nutritional plants (p-value=0.007). of anemia with remaining nutritional factors that are: consumption of dairy pro. The results reports that there are no significant relationships among severity ducts, liver consumption, consumption of vegetables, egg consumption, consumption of fruits, and consumption of dates and nuts**.** Table (3)

A study found the distribution of food by meals between the groups was very similar, though, the pregnant women presented a significantly higher ($p<0.05$) consumption of fruits/natural juices at lunch and afternoon snack, and milk and dairy products at breakfast [19].

Conclusions

The study concluded that there are high significant relationships among severity of anemia with consumption of red meat, consumption of poultry meat, and fish consumption; the finding also show significant relationship between severity of anemia and eating nutritional plants.

Recommendations:

The study recommended to teach women in pregnancy age to dietary habits as a part of an overall approach to health promotion, and education pregnant women and infant by health lectures, T.V., Posters to avoid future anemia during pregnancy. Instruction classes should be given for pregnant women in each primary health care center to encourage them to take daily oral iron and folic acid supplementation to prevent maternal anemia.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Baghdad and all experiments were carried out in accordance with approved guidelines.

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Evaluation The Iron Homeostasis and Erythropoietic Activity in Male Rats with Anemia Related with Induced Chronic Renal Failure

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Abstract

The present study conduct to evaluation the iron homeostasis and erythropoietic activity in male rats with anemia related with induced chronic renal failure. Sixty adult male rats were divided into four groups, the first group administrate with DMSO as a control negative, while second group administrate DMSO and then moringa leaf powder, third group adenine were administrated only and fourth group adenine then given moringaoleifera leaf powder the experiment were lasted for forty-four days. At the end of the study , blood was collected via the heart puncyure and measured the following biomarkers (Urea , creatinine , BBC count ,Erythropoietinerythoferrone ,, ferritin, Serum iron and hepcidin).The results revealed that the rats treated adenine caused significant increase in (creatinine ,urea ,ferritin and hepcidin concentration), one the other hand there was significantly decrease in RBC count ,PVC and HB in adenine group in addition to Erythropoietin,erythoferrone and serum iron levels , however combined adenine with moringa results in return these biochemical criteria to their normal levels in control group .

Keywords: *moringaoleifera ,Erythroferrone, renal failure, Adenine*

Introduction

Anemia commonly occurs in people with chronic kidney disease (CKD)—the permanent, partial loss of kidney function. Healthy kidneys produce a hormone called erythropoietin (EPO). A hormone is a chemical produced by the body and released into the blood to help trigger or regulate particular body functions prompts the bone marrow to make red blood cells Which then carry oxygen throughout the body. [1] ,When kidneys are diseased or damaged, they do not make enough EPO. As a result, the bone marrow makes fewer red blood cells, causing anemia. When blood has fewer red blood cells, it deprives the body of the oxygen it needs [2] Erythroferrone is a hormone that control iron metabolism through its actions on hepcidin [3] it is produced in erythroblasts, which proliferate when new red cells are synthesized. This process is governed by the renal hormone, erythropoietin [4] The mechanism of action of Erythroferrone is to inhibit the expression of the liver hormone, hepcidin. By suppressing this, ERFE increases the function of the cellular iron export channel (Ferroportin), This then results in increased iron absorption from the intestine and mobilization of iron from stores**,** [5] which can then be used in the synthesis of hemoglobin in new red blood cells. Moringa have variously biological activities such as reducing hyperglycemia, anti-inflammatory, anti-diabetic, antimicrobial, anticancer and antioxidant. in fact, it is believed in that the Moringa has many benefits based on its nutrition. The ratio of grams per gram, Moringa leaves dry powder contains 25 times more iron than spinach [6], in which iron is one of the therapeutic agent for anemia which can compensate for the loss of the hemoglobin [7] The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, provitamin A as beta-carotene, vitamin K, manganese, and protein. [8] The present study was designed to investigated the rule of Erythroferrone (ERFE)in iron homeostasis and erythropoietic activity in anemia related with induced

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chronic renal failure in male rats

Materials and Methods

Preparation of MoringaOleifera leaves powder

Fresh moringaoleifera leaves are collected from Alkafeel Nurseries Group Washed and dried at room temperature then grained by the electric grinder into powder. (9) ,5% of moringaoleifera leave powder were mixed with the usual nutrition ration of crushed feed diet (10)

Chemicals:

Adenine was obtained from Sigma Aldrich. all ELIZA kit like, (Erythropoietin erythoferrone, Ferritin, Serum iron and hepcidin) obtain from (Bio cellular Company) (china), Urea and creatinine obtain from (SPECTRUM company) (Egypt) measured by spectrophotometer.

Experimental design

Sixty male rats(Rattus norvegicus) aged (2 -3month) weighting (170-200) gm were divided randomly into four groups (15/group)

First group (GI) control negative were administrate with DMSO by interaperitoneally for 4 weeks. Second group (GII) is the control positive were administrating with DMSO by interaperitoneally for 4 weeks and then moringa leaf powder given at dose rang 5% for 2 weeks with diet [10]. While rats included in third group (GIII) adenine were administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure [11] .finally the fourth group (GIV) adenine were administrated interaperitoneally 100mg/kg.bw for 4 weeks for induction of renal failure [11] and the given moringaoleifera leaf powder at dose 5% for 2 weeks with diet [10]

At the end of the study, blood was collected via the heart puncture and measured the following biomarkers (Urea, creatinine, BBC count, Erythropoietin, erythoferrone ,

, Ferritin, Serum iron and hepcidin). these parameters were measured according to the

instruction of the manufacture company by using ELIZA

Results

Table (1) Effect of MoringaOleifera Leaves powder on Some Serum Kidney Function Tests in Male rats with Induced CRF

groups / parameters	GI Control negative	GII Control positive + moringa leaves	GIII Adenine	GIV Adenine+ Moringa
Urea Mg/dl	45.00±2.921 C	55.50 ±3.403 BC	99.68±6.135 A	69.20±5.75 B
Creatinine Mg/dl	0.45±0.084 B	0.60± 0.057 B	2.56±0.209 A	0.86±0.091 B

- Mean ±SE(n=6 rat / group)

-Different letters represent significant($p \leq 0.05$) difference between groups

The results in table (1) clarified there were a significant ($p \leq 0.05$) elevation in the serum urea in (GIII) adenine treated group in comparison with other groups

On the other hand, combined adenine with moringa (GIV) in the same table showed a significant ($p \leq 0.05$) reduction comparing to (GIII) as compared to control group (GI)

Also Table (1) illustrated there were statistically significant ($p \leq 0.05$) increment in serum creatinine in

adenine treated group (GIII) as compare to other groups

As the same table showed combined adenine plus moringa (GIV) caused a significant ($p \leq 0.05$) decrement of serum creatinine comparing to (GIII)

Table (2) effect of MoringaOleifera leaves powder on Complete Blood Count in male rats with Induced CRF

Parameters groups	RBC Count (cell*1012/ l)	PCV %	Hb (gm/dl)
Control	7.43±0.43 B	40.83±0.82 AB	15.25±0.45 A
Moringa	8.49±0.25 A	42.66±0.66 A	15.72±0.41 A
Adenine	5.69±0.23 C	35.66±0.33 C	9.06±0.34 C
Adenine +moringa	7.29 ±0.32 B	39.50±0.67 B	13.65±0.64 B

Mean ± ES (n=6 rat / group)

-Different letters represent significant ($p \leq 0.05$) difference between groups

The results showed significant ($p \leq 0.05$) decrease in RBC count, PVC and HB in adenine group (GIII) in comparison with the other groups. Table (2) Also combined moringa with adenine (GIV) caused significant ($p \leq 0.05$) increase in RBC count, PVC and HB comparing to (GIII)

Table (3) Effect of MoringaOleifera leaves powder on Iron Homeostasis Parameters in male rats with induced CRF

Groups / Parameters	GI Control negative	GII Control positive + moringa leaves	GIII Adenine	GIV Adenine+ Moringa
Erythropoietin ng/ml	9.88 ±0.302 A	11.07±0.602 A	4.10 ±0.367 C	7.50 ±0.439 B
erythoferrone ng/ml	19.66±.1.516 A	18.04±1.593 AB	10.23±0.426 C	15.52±1.025 B
Ferttin ng/ml	6.60 ±0.174 B	6.80 ±1.133 B	9.98 ±0.837 A	7.49 ±.0183 B
Serum iron µmol/L	8.19 ±0.090 A	9.59 ±0.264 A	6.41 ±0.495 B	8.26 ±0.454 A
hepcidin ng/ml	19.25 ±0.456 B	19.60±0.622 B	23.09±1.101 A	19.64±1.363 B

-Mean ± ES (n=6 rat / group)

-Different letters represent significant ($p \leq 0.05$) difference between groups

There were statistically significant ($p \leq 0.05$) decrease of serum erythropoietin in adenine treated group (GIII) in comparison with the other treated groups. Table (3) Also combined moringa with adenine (GIV) ameliorate the serum erythropoietin. The serum erythoferrone showed a significant decrease ($p \leq 0.05$) in adenine treated group (GIII) as compared to other groups. The results also revealed that rat treated adenine plus moringa (GIV) caused significant ($p \leq 0.05$) increase comparing to (GIII). Depending on the results clarifies in table (3) there were a significant ($p \leq 0.05$) elevation in serum ferritin in adenine treated group (GIII) comparing to the other groups, Also the combine adenine with moringa group (GIV) caused a significant ($p \geq 0.05$) decrease comparing to (GIII). A significant ($p \leq 0.05$) reduction in table (3) of serum iron in (GIII) adenine treaded group (GIII) comparing to the other groups, also there was revealed a significant ($p \leq 0.05$) elevation in the group treated adenine plus moringa (GIV) Comparing to (GIII). Table (3) illustrated there were a significant ($p \leq 0.05$) increment in this parameter in adenine treated group (GIII) Comparing to other groups, Combined adenine with moringa (GIV) in the same table caused significant ($p \leq 0.05$) decrement of serum hepcidin comparing to (GIII) group,

Discussion

The results in table (1) clarified there were a significant ($p \leq 0.05$) elevation in the serum urea in (GIII) adenine treated group in comparison with other groups. This study was agreeing with result conducted by Ikizler, et al. [12]

Urea nitrogenous compounds usually released from the blood by the kidney into the form of urine, renal dysfunction results in accumulation of urea in blood circulation as it is obvious in chronic renal failure conditions in high levels. [13]

On the other hand, combined adenine with moringa (GIV) in the same table showed a significant ($p \leq 0.05$) reduction comparing to (GIII) as compared to control group (GI).moringaoleifera leaves administration works on develop of the results that show the fall in urea concentrations better than before a significant decrease in serum urea in group (GIV) combined adenine with moringa in comparison with adenine group . [14].

Also Table (1) illustrated there were statistically significant ($p \leq 0.05$) increment in serum creatinine in adenine treated group (GIII) as compare to other groups ,this our data agrees with results of [15] ,Creatinine is a product released by muscles from the degradation of a compound named creatine. Creatinine is cleared from the body by the kidneys, which filter high levels of it from the blood and cleared it into the urine(16).As the same table showed combined adenine plus moringa (GIV) caused a significant ($p \leq 0.05$) decrement of serum creatinine comparing to (GIII) ,that the disturbance of plasma electrolyte imbalance may influence the pH, osmolality, and blood volume which may lead to renal dysfunction or anther body disturbances .*M. oleifera* has appear to own diuretic effect (17). Table (2) , this agree with (18)Which occur Through dialysis HB concentrations are decreased to critical levels ,that results in anemia , [19] happen during anemia condition in patients with acute and chronic renal failure results revealed reduction in RBC count and HB levels and [20] It is occur due to pathogenesis of renal anemia . Also combined moringa with adenine (GIV) caused significant ($p \leq 0.05$) increase in RBC count , PVC and HB comparing to (GIII), after moringaoleifera leaves administration we can see clear result that describes by increasing in RBC count value with little extent for PVC count and HB count in (GIV) group moringaoleifera is rich with high nutritional supplements such as (iron ,calcium and vitamins) which is one of the essential component in synthesis of hemoglobin in red blood cells [21]

There were statistically significant ($p \leq 0.05$) decrease of serum erythropoietin in adenine treated group (GIII) in comparison with the other treated groups .Table (3) The present study agree with results conducted by [22]

Generally cases with kidney disease will posses anemia which occur in early in the course of kidney disease and grow worse as kidneys fail and can no longer make EPO. [23] .Also combined moringa with adenine (GIV) ameliorate the serum erythropoietin ,moringaoleifera may assist on keeping further homeostatic balance by support Erythropoietin to fasten its effects by linking to the erythropoietin receptor EpoR [24]

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The serum erythoferrone showed a significant decrease ($p \leq 0.05$)in adenine treated group (GIII) as compared to other groups, the results of present study agree with result of [25] since one of the most essential element of red blood cell manufacture is hemoglobin that is obtained from iron stores in the body [26] ,yet anemia occur because erythoferrone release is restricted and become in low concentrations that because of hepcidin hormone release from it which stimulate the absorption of iron from alimentary track [27]. The results also revealed that rat treated adenine plus moringa (GIV) caused significant ($p \leq 0.05$) increase comparing to (GIII), moringaoleifera leaves provide a natural source for iron (28) with addition to other supplements calcium, minerals, vitamins which assist on maintenance of biological homeostasis for iron insufficiency. Depending on the results clarifies in table (3) there were a significant ($p \leq 0.05$) elevation in serum ferritin in adenine treated group (GIII) comparing to the other groups, these results agree with [29] In fact chronic renal failure resulting in disturbances in hormonal released from kidney which is later may effect on erythropoiesis ,when erythropoietin is the chief regulator of the red blood cells synthesis so as a result their would be reduction in this process yet anemia occur [30] . Also the combine adenine with moringa group (GIV) caused a significant ($p \geq 0.05$) decrease comparing to (GIII),this due to it may diminish before been used by the target cells whither it execrated with waste materials or not fully absorbed from alimentary tract or other related conditions [31].

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Baghdad and all experiments were carried out in accordance with approved guidelines.

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Iraqi People Practices toward Adherence of Government Strategies to Control of COVID19

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Abstract

Background: Coronavirus disease 2019 (COVID-19) emerged in December 2019 in Wuhan. While the flare-up in China is about over, this exceedingly irresistible sickness is right presently spreading over the world. A poor practices and understanding of the disease among Iraqi population may lead to rapid spread of the infection. **Objective**: the point of this think about was to survey practices of Iraqi population regarding government strategies to control COVID 19 and to identify level of practices among different Iraqi cities.**Methods:** Cross-sectional web-based research was carry out among Iraqi population about COVID-19 during the period from 15th April 2020 to 1st June 2020. 700 participants were share from Iraqi governorates. The participants was collected from (7) cities (Baghdad, Basra, Najaf, Thigar, Misan, Karbala and Mosul). From each city (100) participants were shares. The data was collected through the uses Google form. From Iraqi governorate strategies and WHO recommendations concerning prevent of infection. The researcher use SPSS version 20 for data analysis by using frequencies and percentages and mean of score were produced. Mean of score rating: always=3, sometimes=2, never=1. **Results:** the majority of study samples (47.7%) were within (20-29 years). More than half of participants are male (65.4%). Relative to the educational level, the majority of study sample (65.9. %) were Secondary school graduate. Practices of Iraqi population regarding COVID 19 controls at home in all items are inadequate and very low less than (1.33).also the practices of Iraqi population regarding COVID 19 control out of home had low mean of score in all items. However the average of means score (1.42). **Conclusions:** The study concluded that Iraqi community has inadequate practices to prevent speared of COVID-19 because the population not respects ministry of Iraqi health strategies. Health education should urgently be conducted focusing on hand washing, uses sanitizer, social distancing, avoid crowded, uses mask and gloves.

Introduction

COVID-19 appear first time in in China Wuhan city in December 2019. While the flare-up in China is about over, this exceedingly irresistible sickness is right presently spreading over the world. A poor practices and understanding of the disease among Iraqi population may lead to rapid spread of the infection) 1, 2(.

The World health Organization in first month of 2020, declared that the scene of coronavirus constituted an Open Wellbeing Crisis of Universal Concern (PHEIC) (3). The disease is transmitted from person to person by implies of globules hacked or breathed out by sullied individuals and by droplet-contaminated area or objects and after that touching the mouth, nose, or eyes (4, 5).

COVID-19 is an intense respiratory infection caused by a recently developed zoonotic coronavirus. A positive-sense wrapped single-stranded RNA infection, named Serious Intense Respiratory Disorder Coronavirus-2 (SARS-CoV-2), has been disconnected from a persistent with pneumonia, and associated to the cluster of intense respiratory sickness cases from Wuhan. Hereditary investigation has uncovered that it is closely related to SARS-CoV and hereditarily clusters inside the sort Beta coronavirus, subgenus Sarbecovirus (5).

According to study from China, the all-inclusive WHO mission report appears that about 75% of at to begin without symptoms cases will development to clinical ailment, making veritable asymptomatic infection or

possibly unprecedented. Modeling estimations of pre-symptomatic transmission have been point by point to be between 48% and 62% [6]. Other courses ensnared in coronaviruses transmitted incorporate contact with sullied fomites and inward breath of pressurized canned products delivered amid aerosol-generating strategies. SARS-CoV-2 infection has been identified in respiratory, fecal and blood investigation [4].

The most elevated chance related transmission happens within the nonappearance of standard safeguards, when essential contamination avoidance and preventive measures for respiratory disease are not input, and when managing with patients ECDC whose COVID-19 defilement is in any case to be affirmed. In spite of the fact that airborne transmitted isn't considered the first transmitted course, we endorse a cautious approach since of conceivable transmission through fog concentrates [7]. Key strategies on the community level include cancellation of planned events and suspension of events with super spreader potential; utilize of social separating measures to diminish coordinate and near contact between individuals within the community; travel confinements, counting diminished flights and open transport and course limitations without compromising basic administrations; deliberate domestic isolate of individuals of family contacts; changes to funeral administrations to play down swarm measure and presentation to body liquids of the diseased; and clear communication from national and universal wellbeing specialists, to guarantee confirmed data and dodge fake news, rumors, and panic [8, 9]. Mass social occasions and occasions such as citywide celebrations, devout get-togethers, social celebrations, logical conferences, and huge political occasions ought to be confined. Respiratory diseases such as influenza and now like COVID-19 are commonly transmitted at a high rate within such large gatherings **[10]**. Based on WHO recommendations, The Iraqi governorate suspended operations in numerous government offices beginning Walk 10, 2020. All colleges and schools are by chance closed. Operation of various markets and shopping centers is suspended; social events in parks, shorelines, and resorts are denied. Eateries are closed but for take truant advantage. Sedate stores and essential supply stores remain open to serve clients through regulatory alloted online movement applications and systems. So that, the ponder points to survey hones of Iraqi populace with respect to government methodologies to control COVID 19.

Methodology

A cross-sectional study, web-based descriptive, which was carry out to assess level of Iraqi population practices regarding adherence of government strategies to control COVID 19. The study sample was collected from (7) cities (Baghdad, Basrah, Najaf, Thigar, Misan, Karbala and Mousl). From each city (100) participants were shares. The data was collected through the uses Google form. The period of study was conducted from 15th April 2020 to 1st June 2020. **Study subjects:** 700 participants were share from Iraqi cities during the time of the study. **The Study Instruments:** From Iraqi governorate strategies and WHO recommendations concerning prevent of infection, instrument were developed by researcher which is consisting from: **Part one:** included demographic data characteristics (age, gender, level of education, living place (city). **Part two:** question about practices of Iraqi population regarding COVID 19 control at home contain (7) items. Question about practices of Iraqi population regarding COVID 19 control out of home contain (7) items.

Data analysis: The researcher use SPSS version 20 for data analysis by using frequencies and percentages and mean of score were produced. Mean of score rating: always=3, sometimes=2, never=1

Results

Table 1: Populations' Sociodemographic Characteristics.

No.	Variables	(n=700)	F	%	Mean	Std. Deviation
1	Age (year)	20-29	334	47.7	1.80	.935
		30-39	226	32.3		
		40-49	85	12.1		
		50-& more	55	7.9		
		total	700	100.0		
2	Gender	Male	458	65.4	1.35	.476
		Female	242	34.6		
		Total	700	100.0		
3	Educational levels	Primary school	461	65.9	2.43	.653
		Secondary school	176	25.1		
		College	63	9.0		
		Total	700	100.0		

No. = number of Variable, n = number of sample, F=frequencies, % = Percentages.

Table (1) show that the majority of study samples (47.7%) were within (20-29 years). More than half of participants are male (65.4%). Relative to the educational level, the majority of study sample (65.9. %) were Secondary school.

Table 2: Practices of Iraqi population regarding COVID 19 control at home

No	Items	Always		Sometime		Never		MS	sever
		F	%	F	%	F	%		
1	Hand washing before and after any work.	90	12.9	263	37.6	347	49.6	1.63	L
2	Uses sanitizer	81	11.6	223	31.9	396	56.6	1.55	L
3	Visiting avoidance	106	15.1	182	26.0	412	58.9	1.56	L
4	Home environment exchange.	20	2.9	179	25.6	501	71.6	1.31	L
5	Uses handkerchief during sneezing.	54	7.7	177	25.3	467	66.7	1.46	L
6	Sanitation of equipment that bring from out of home	29	4.1	204	29.1	467	66.7	1.37	L
7	Avoidance of mouth, eyes and nose contact.	23	3.3	187	26.7	490	70.0	1.33	L
	Total							1.45	L

No. = number of item, F=frequencies, % = Percentages, M.S. = mean of score. Cut-off-point interval: 1-1.66 = Low; 1.67-2.33 = Moderate; 2.34-3.00 = High, L=low, M=moderate, H=high

Table (2) show that there is low mean of scores about practices of Iraqi population regarding COVID 19 controls at home in all items less than (1.33). Moreover, the average means score (1.45) as a totally.

Table 3: Practices of Iraqi population regarding COVID 19 control out of home

No	Items	Always		Sometime		Never		MS	sever
		F	%	F	%	F	%		
1	Avoid get out of home as possible.	119	17.0	226	32.3	355	50.7	1.66	L
2	Uses of mask and gloves when you get out.	28	4.0	167	23.9	505	72.1	1.32	L
3	Avoid crowded.	79	11.3	201	28.7	420	60.0	1.51	L
4	Let distance (1.8m) between you and anthers.	34	4.9	163	23.3	503	71.9	1.33	L
5	Avoid visit person who came from pandemic area.	56	8.0	211	30.1	433	61.9	1.46	L
6	Remove gloves and mask before enter your home	26	3.7	172	24.6	502	71.7	1.32	L
7	Hand washing before enters your home.	22	3.1	206	29.4	472	67.4	1.36	L
Total								1.42	L

No. = number of item, F=frequencies, % = Percentages, M.S. = mean of score. Cut-off-point interval: 1-1.66 = Low; 1.67-2.33 = Moderate; 2.34-3.00 = High, L=low, M=moderate, H=high

Table (3) shows a low in the mean of score for all items about the practices of the Iraqi population regarding controlling COVID 19. However the average of means score (1.42).

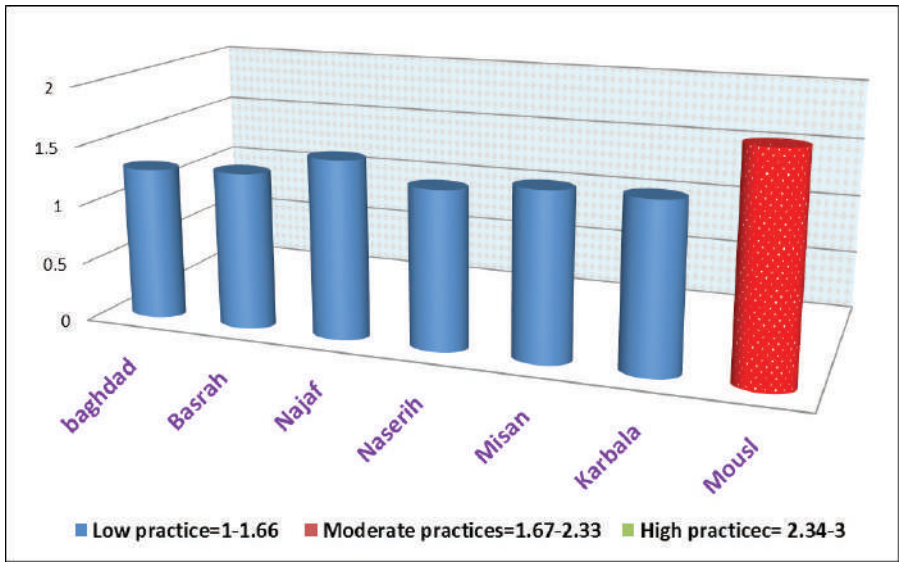


Figure 1: Comparison among cities by using Means of Score to identify the level of population practices

Figure (1) revels that the level of practices of population were Baghdad: 1.29, Basra: 1.32, Najaf: 1.5, Thi-gar: 1.33, Misan: 1.4, Karbala: 1.4 and Mosul: 1.85

Discussions and Conclusions

Coronavirus is an seriously respiratory affliction caused by a novel human coronavirus (SARS-CoV-2, called COVID-19 disease), which causes higher mortality in people developed ≥60 a long time and in people with principal therapeutic conditions such as cardiovascular illness, deep rooted respiratory illness, diabetes and cancer [11].

WHO and open wellbeing specialists around the world are taking activity to contain the COVID-19 episode. Be that as it may, long term victory cannot be taken for allowed. All areas of our society – counting businesses and managers – must play a part in case we are to halt the spread of this illness [12].

Most common age group was 20-29 years (47.7%). More than half (64.4%) were male. Nearly two-third (65.9%) of respondents were Secondary school graduate (Table 1).

In regarding to practices of Iraqi population of COVID 19 control at home (Table 2), we notice that the level of mean of score come between (1.31) as a low value and (1.63) as a high value, this results consider very low associated in very important items that dealing with prevent of infection such as (Hand washing before and after any work, Uses sanitizer). The results in Table (3) that's dealing with practices of Iraqi population regarding COVID 19 control out of home revels very low mean of score in items(2,4,7), this items dealing with infection control such as uses protective equipment (mask & gloves) , let distance (1.8m) between he-she and anthers and hand washing before enters his home. this study disagree with the study conducted in china by Zhong ,L. et al 2020 , this study appear that the Chinese population had good practices and exceptionally cautious: about all dodged swarmed places (96.4%) and wore covers when clearing out the domestic (98.0%) amid the fast rise period of the COVID-19 flare-up [13]. Figure (1) presented that the level of adherence in Baghdad city; this may be interpreting increasing number of cases in the May and June. Also, other cities have low level of practice toward COVID 19 control except Mosul city in north of Iraq has moderate level of adherence for Iraqi strategy to COVID 19 control. Mosul city register low levels in cases along since coronavirus appear. All patients in Baghdad from 1 January to 7th June 2020 (2234) cases while the total cases in Mosul city (12) at the sometime [14].

The level of Iraqi practices to adhere the standard of disease control and prevention in current study not enough absolutely. There were strategies remember by ministry of health in Iraqi according to word health organization recommendations to control corona virus [15, 16].So, COVID 19 may transport in very high method in nearest future among Iraqi population if these recommendations not respect.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Adult Nursing Department and all experiments were carried out in accordance with approved guidelines.

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Effectiveness of an Educational Program in Nurses' Knowledge toward Traumatic Head Injury during Golden Hour at Neurosurgical Hospital

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Abstract

Background: Globally, traumatic head injury higher mortality rate, and unpredictable outcome. Improvement of nurses' knowledge of how to deal with early periods of the traumatic head can improve patients outcomes.

Objectives: This study aims to assess the effectiveness of an educational program in nurses' knowledge toward traumatic head injury during golden hour.

Method: A quasi-experimental design was used in this study to assess 50 nurses' knowledge regarding constructed educational programs toward traumatic head injury during golden hour who works in critical care units in Neuro-surgical hospital. The measurement of the effectiveness of educational program through the nurses knowledge includes(54)items concern knowledge related Anatomy of the head and brain; Physiology of intracranial pressure(ICP),cerebral perefusion pressureCPP and blood pressure(BP); Primary and secondery survey; fluid supply; information about traumatic head injury during golden hour . Reliability of instrument was determined through the use of test and retest and the instrument validity was determined through a panel of experts.

Results: The study show a comparison significance association between study and control group during pre-test and post-test regarding overall assessment, there are improvements in study group knowledge level, the significant association for were P= 0.000. For the control group, there is no significant association between pretest and post-test P= 0.733 p-value >0.005.

Conclusion: The study concludes the current educational program effectively and can improve nurses knowledge in critical care units and recommends to further study assess nurses practice regarding traumatic head injury during golden hour, Adopting an educational program for all nurses and more involved in continuous education lectures and workshops.

Keywords: *Educational Program, Traumatic head injury, Nurse, Golden Hour.*

Introduction

Traumatic brain injury is "a silent epidemic" because the problems result are often appears gradually after a long- period and invisible by the community [1]. Because of the long term effects of head injury on cognition, intellectual, mental, emotional, psychosocial, and motor function, it is no optimal way to give the effect of acute and long term care for patients in developing countries [2]. Effective health care depends on several factors include assessment of health care programs, specificity nurses' education needs, and work in a multi-disciplinary team. Many pieces of literature over decade high lightened the gap in education and trauma management needs, so this gap produces the development, tailored, and construct educational materials, each one objective to a specific aspect of trauma care[3,4,5,6,7]. On the other hand, many researchers tend to fill the knowledge gap among nurses through exploring their needs, develops educational tools, construct programs not only to solve current problems but to keep quality of education inside

the hospital and update the information regarding best available evidence[7,8,9,10]. Also, literature discussed the factors that can effects patients outcomes after traumatic brain injury, one of these factors is how the patients managed at injury moment and the definite care did not include only at the scene of injury, may continue at a hospital[11,4]. Brain trauma foundation is developer sound of traumatic head injury guidelines over 30 years report and recent literature reports, the knowledge for early management for patients with traumatic head injury focus on the primary assessment where identify the patient priority of the problems, hemodynamic stability and monitoring such as oxygen saturation, blood pressure and mean arterial pressure, use of appropriate intravenous fluid, positioning and use of intervention to avoid complications[12,13,14,15]. However, patient survival after traumatic head injury depends on focused management to prevent primary injury development to secondary injury [14]. Because there is no policy or procedure focused on traumatic head injury during golden hour, then the education program constructed to fill this gap in patients' care and promote nurses' knowledge about head injury during this period.

Material and Methods

Design: A quasi-experimental design was used in this study to assess the effectiveness of an educational program toward traumatic head injury during golden hour at the neuro-surgical hospital in Baghdad city pre and post.

Ethical Approval: This study was approved by the Institutional Ethics Committee, and The IRB was obtained from the University of Baghdad and the Ministry of Health, Directorate of Al Rusafaa Health Administration in Baghdad city.

Study sample: a non probability purposive sample consists of 50 male and female nurses for both (study and control groups) in the emergency and ICU in selected hospitals.

Instrument: Study instrument constructed by the researcher based on comprehensive literature, standard nursing textbook, and consist of two parts:

Part I: Assessment needs questionnaire was used to assess nurses' knowledge needs regarding traumatic head injury during golden hour.

Part II: Pre/Post-test knowledge questionnaire: to assess nurses' knowledge about traumatic head injury during golden hour, in addition to their socio-demographic data. Scoring system used for multiple-choice questions, each question had four answers, one answer is correct and three answers are incorrect. Scoring include: (0= false answer,1=true answer).The level of assessment was (0.00-0.33=poor, 0.34-0.67=fair, 0.68-1.00=good).

Data Analysis: Social package for social sciences (SPSS) version 24 was used for data analysis, descriptive statistics such as frequency, percentage, mean of scores and standard deviation, inferential statistics include: Paired t-test to evaluate changes of nurses knowledge.

Findings

Current findings show the demographic characteristics for study and control groups. Table (1) shows that the distribution of age indicated that the majority of study group nurses' ages were (20-24) years old who were accounted for (64 %), control group nurses ages were (30-34) years old were account (44%). Related to gender, most of the study sample was female gender account (80%) and the Control sample dominantly female was (64%). The majority of the Study sample were married (52%) also tow –third study Control sample was married (76%). According to Educational status, most of the Study group was graduated from secondary-school nursing (84%) and the control group was (76%). Regarding the shift of work, the day shift was dominantly (88%) of the Study group and the night shift (84%) of the Control group. The majority of the study group were their work field was at ICU accounts (64%) and two-thirds of the Control group also were in ICU accounts (76%). Related years of experience in the current workplace (52%) of the Study sample were less than three years and the Control group were (44%) between three to six years. Total years of employment in nursing, the study group (36%) total years of experience between (9-12) years and control group was (32%) of the study sample between (13-16) years. Regarding participation in the training course in continuous education inside the hospital the majority of the study group was (52%) reported (yes) they attend in continuous training lectures or training course and

(80%) reported (no).

Table (2) shows the majority of the study group in pre-test phase (52%) of nurses was poor knowledge with mean of the score (0.40) and standard deviation (0.169) and more than half of control group (52%) was fair knowledge and their mean of the score was (0.38) and standard deviation (0.135). The table also shows control group in post-test phase, were good improvement in nurse's knowledge, majority recorded good knowledge (68%) and their mean of the score was (0.73) and standard deviation (0.096) and more than half of control group was fair knowledge(52%) and their mean of the score was (0.36) and the standard deviation was (0.133).

Tables 3 and 4 show the Comparison significance of Nurses' knowledge Between (Pre-Test and Post-Test) for Study and control group Regarding domains of Educational Program questions. Study group pre-post-period was statistical significance (<0.005) in all domains (Anatomy of the head and brain, physiology of ICP, CPP and BP, Primary survey, fluid supply, and information about traumatic head injury during golden hour). In comparison with the control group, the program promotes study nurses' knowledge and effectiveness.

Table (5) This table shows a comparison significance association between study and control group during pre-test and post-test regarding overall assessment, there are improvements in the study group knowledge level, the significant association for were P= 0.000. For the control group, there is no significant association between pretest and post-test P= 0.733 p-value >0.005.

Table (1): frequencies and percentages of demographic characteristics of the studied sample:

Variables		Study Group		Control Group	
		F	%	F	%
Age groups	(20-24)	16	64.0	4	16.0
	(25-29)	4	16.0	6	24.0
	(30 -34)	1	4.0	11	44.0
	(35-39)	4	16.0	3	12.0
	≥40			1	4.0
		25.1± 6.77		35.3± 5.004	
Gender	Male	5	20.0	9	36.0
	Female	20	80.0	16	64.0
Marital Status	Single	12	48.0	5	20.0
	Married	13	52.0	19	76.0
	Divorced	-	-	1	4.0
Educational status	Secondary school nursing	21	84.0	19	76.0
	Diploma	3	12.0	5	20.0
	Bachelor	1	4.0	1	4.0
Years of Experience in the current place	Less than 3 years	13	52.0	5	20.0
	3-6years	6	24.0	11	44.0
	More than 6 years	6	24.0	9	36.0
Total years of employment in nursing	(1-4)	4	16.0	-	-
	(5-8)	4	16.0	5	20.0
	(9-12)	9	36.0	4	16.0
	(13-16)	4	16.0	8	32.0
	(17 -20)	4	16.0	7	28.0
Work Field	ER	9	36.0	6	24.0
	ICU	16	64.0	19	76.0
Participation in continuous education lecture	Yes	13	52.0	5	20.0
	No	12	48.0	20	80.0
Shift	Day	22	88.0	-	-
	Night	3	12	4	16.0
	Noon	-	-	21	84.0

MS= Mean score; Sd=standard deviation

Table (2): Level of Assessment of Nurses Knowledge toward Traumatic Head Injury during Golden Hour, for Study and Control Group Pre-test and Post-test:

Period	Level of Assessment	Study Group		Control Group	
		F	%	F	%
Pre-Test	Poor: (0.00-0.33)	13	52.0	11	44.0
	Fair: (0.34-0.67)	8	32.0	13	52.0
	Good: (0.68-1.00)	4	16.0	1	4.0
	Total	25	100.0	25	100.0
		0.40 ± 0.235		0.36 ± 0.135	
Post- Test	Poor: (0.00-0.33)	0	0	10	40.0
	Fair: (0.34-0.67)	8	32.0	13	52.0
	Good: (0.68-1.00)	17	68.0	2	8.0
	Total	25	100.0	25	100.0
		0.73± 0.112		0. 37 ± 0.133	

F= frequency; %= Percentage; ±= mean of score and standard deviation.

Table (3) Mean scores of total and subtotal knowledge of ICU nurses regarding traumatic head injury during golden hour pre-test and post-test period for study group:

Knowledge domains	Pre-test MS±SD	Post-test MS±SD	t-test	Sig*
Anatomy of the Head and Brain	0.52 ±(0.199)	0.76 ±(0.137)	5.059	0.000
Physiology of ICP, CPP, and BP.	0.37± (0.185)	0.71± (0.096)	9.798	0.000
Primary and Secondary Survey	0.46± (0.272)	0.74± (0.100)	5.346	0.000
Fluid Supply	0.36± (0.264)	0.71± (0.116)	6.322	0.000
Information about the golden hour	0.37± (0.255)	0.74± (0.112)	7.502	0.000
Total Knowledge	0.42± (0.235)	0.73 (0.112)	6.8054	S

MS= Mean of score; SD=standard deviation; S= Significance P value ≤0.005

Table (4) Mean scores of total and subtotal of Nurses knowledge regarding traumatic head injury during golden hour pre-test and post-test period for the control group:

Knowledge domains	Pre-test MS±SD	Post-test MS±SD	t-test	Sig*
Anatomy of the Head and Brain	0.48 ±(0.197)	0.49 ±(0.132)	.238	0.814
Physiology of ICP,CPP and BP.	0.28 ±(0.162)	0.31±(0.120)	.866	0.395
Primary and Secondary Survey	0.45 ± (0.166)	0.43 ±(0.191)	-.436-	0.666
Fluid Supply	0.32 ±(0.211)	0.30 ±(0.219)	-.398-	0.694
Information about golden hour	0.30 ±(0.168)	0.32 ± (0.197)	.595	0.558
Total Knowledge	0.36± (0.181)	0.37± (0.172)	0.566	NS*

Table (5): Significance association between study and control group regarding period:

Groups	Period	MS	SD	df	t	Sig.
Study	Pre-test	0.40	0.169	24	10.424	0.000*
	Post-test	0.73	0.97			
Control	Pre-test	0.36	0.110	24	0.345	0.733
	Post-test	0.37	0.090			

MS= Mean of score; SD=standard deviation; S= Significance P value ≤0.005*

Discussion

The current study shows fifty study sample, despite the level of assessment are equal between study and control group regarding pre-test period were poor (52%) The majority of the nurses in this study group (68%) had good knowledge in the post-test period, Also these results have a significant correlation for study group pre/ post- test (t=10.424, p=0.000). The control group has no changes in their level of knowledge in the post-test period and have no significant correlation between pre/ post- test period (t=0.345, p=0.733). The most striking result to emerge from this data is the educational program improves nurses' educational status to be good regarding the level of assessment. This result agrees with the study done by Masakare and Masakare (2015) in their quasi-experimental study to assess the nursing education program's effectiveness in nurses' knowledge about the management of acute head injury [3]. Regarding the knowledge domains The current study found the study group had a fair level of knowledge in pretest period regarding the anatomy of the head and brain mean of scores (0.52), and physiology of blood pressure(BP), intracranial pressure (ICP) and cerebral perfusion pressure(CPP) mean of the score was (0.37). This result agrees with Montayre & Sparks, (2017) they confirmed the necessity of anatomy and physiology for nurses student as a body of knowledge and inconsistent with (Mousa,2015) were found in his study the nurses have a high level of knowledge in anatomy and physiology in critical care unit[3,16,17]. This reflects the importance

of anatomy and physiology, so the obligatory courses in all nursing education programs. The findings of the current study, indicate a significant correlation in the study group between pre-test a post-test p-value (0.000) regarding the primary and secondary survey table (3). The primary and secondary survey is important for trauma care in general and especially for a traumatic head injury, all steps of primary survey important in life-saving, the primary survey is main steps of advanced life support (ATLS), this supported by Mohammad and their colleagues (2014) in their systemic review study, highly recommends impact of educational and clinical ATLS courses in decrease mortality rate in the management of the traumatic patient[18]. The fluid supply domain was record mean of the score (0.36) in the pretest period and improved in the post-test period, mean of the score(0.71), and p-value (0.000). Finally, information about the golden hour domain mean of the score (0.37) in the pre-test period and improved to (0.74) in the post-test period and p-value (0.000), table (3). The study group has a significant correlation between pre-test for all domains in contrast with the post-test, While the control group in all domains has no significant correlation between pre-test and post-test periods, table (4). These findings consistent with Seliman et al. (2014), found the pre-test has a significant correlation with post-test and improved critical care nurses' knowledge. Also, agree with the study done by Shehab et al. (2018) were found because of lack of continuous education follow up and protocols for nursing management the nurses have a low level of knowledge before their educational program applied and significance correlation after utilization of the educational program[5,15].

Conclusion

The study concludes the current educational program effective and can improve nurses knowledge in neurosurgical critical care units and recommends to further study assess nurses practice regarding traumatic head injury during golden hour, Adopting an educational program use to assess their practice necessory for all nurses, also involved in continuous education lectures seminars and workshops.

Conclusion

Financial disclosure

There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Adult of Nursing Department College of Nursing and all experiments were carried out in accordance with approved guidelines.

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Evaluation Activity of Boiled Aqueous Extract of Broccoli Flower Plant (*Brassica Oleraceae L. Var. Italic*) And Albendazole Drug Against the Protoscolices *in Vitro*

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Abstract

The present study aimed to testing the efficacy of the boiled aqueous extract of broccoli flowers and albindazole drug against the viability of protoscolices *In vitro*. The results showed the percentage of viability of the protoscolices were 94% at zero time then become (0%) after nine days after the start of the experiment when using culture media of (Kreb ringers and the cyst fluid) (4 : 1) respectively as control group for comparison. . the aqueous extract of boiled for the flower of (*Brassica oleraceae*) with concentration of 300 mg / ml showed most effective in eliminating viability of protoscolices after 168 hours and the albendazole drug after 120 hours at percentage 0 % for both.

Keywords: *In vitro, Evaluation, Brassica oleraceae, albendazole drug, protoscolices*

Introduction

Hydatid cystic disease is an important disease that is common between humans and animals, (zoonotic disease)(1), known by many names, it is Echinococcosis , cystic echinococcosis Hydatidosis unilocular and hydatidosis(2) it is a difficult endemic disease(3).

This disease is widespread in the countries of the Middle east, especially the village societies in which humans live and have close contact with herbivores (intermediate hosts) and dogs, as dogs are final hosts(4). This disease poses an economic dilemma in almost all parts of the world especially in middle east and Iraq as it affects the productivity of sheep, cows, goats and camels because their affected organs become unfit for human consumption and this causes weight loss and poor health status of the affected animals (5).

To reduce or prevent the occurrence of this disease in all its developmental stages, whether by final or intermediate hosts, different types of medication have been used, These include albendazole, an oral anthelmintic wide spectrum, is used as the drug of choice for hydatid disease(6). Albendazole carbamate-2-bropylthiobenzimidazole– (6) 5-Methyl is a highly absorbent compound. It is considered one of the preferred drugs in the treatment of hydatid cyst disease, as it works to destroy the layer, contraction and decay, as well as the disappearance of the structural cysts daughter cysts inside the cyst (7).

Surgical removal is one of the best methods to treat this disease, although it is sometimes difficult, impossible, or may cause secondary infection to the body Therefore, the interest in finding alternative means such as using plants to provide them and contain effective medicinal materials, as the researchers deliberately sought many plants to know their components and extract their effective compounds from these plants(8). One of these plants is broccoli (*Brassica oleraceae L. var. italic)*, as it is one of the winter vegetables belonging to the family Brassicaceae , which is a herbaceous plant of dark green color, morphologically similar to cauliflower plant, but it has hard stems and soft flowers, It contains a number of mineral elements such as potassium, phosphorus, etc., and is rich in vitamins such as thiamine, beta-carotene

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,etc. .It also has a role in protecting the cells of the body from cancer symptoms due to the high levels of biochemically active pharmaceutical compounds [9]. The current study aimed to a pharmacological evaluate the effectiveness boiled water extract of (*Brassica Oleracea* var. Italica) and use albendazole , then comparison with it , and their effect as a model of anti-therapy against protoscolices *In vitro*.

Materials and Methods

this study included collection of 30 samples of the hydatid cysts for humans and animals (sheep and cows), human samples collected from Babylon province and from private external laboratories (Dr. Ali Mohsen Hussain and Dr. Ahmed Raji Al_Janabi laboratory), and from hospitals of Al- Najaf , Baghdad and Al-Diwaniyah provinces, whereas animal samples were collected from the abattoirs of the Al- Qadisiyah and Al-Najaf provinces.

The protoscoleces were collected stored in conservative media (KRP=HCF 4 : 1) solution for use in the present study experiment., the viability of protoscoleces was determined using eosin stain (1%) technique [10]. Then use albendazole, which is commercially called zental.it is in the form of an emulsion 10 ml at a concentration 400 mg, Concentrations (300,200,100) mg / ml were prepared by formula(N1V1=N2V2) [11].

the broccoli plant (*Brassica oleraceae*) obtained from the local markets in Babylon province Al-Hilla city . The boiled aqueous extract was prepared by dry the plant, then grind it well and dissolve 10 g of it in 200 ml distilled boiled water and left it for 24 hours, then filtered by gauze, then toke the leachate and the solution was placed in electric oven (40-45) C° for the purpose of obtaining the dried active compounds of the extract., Kept in glass containers in the refrigerator until use [12].

15 gm of dry dregs were taken separately for each extract and dissolved in 50 ml of distilled water, so the stock solution concentration became 30% or 300 mg / ml. From this the concentrations were prepared (300,200,100) mg / ml according to the formula :- (N1V1=N2V2) [11].

Control group was treated with one militer of distilled water and the all concentration that mention above for all extracts with three replications for each (broccoli , albendazole), then the viability rate of the protoscolices were calculated over a period of (0,3,24,48,72,96,120, 144,168,192,216) hours of treatment with evidence of eosin dye(0.1%) penetration, The dead protoscolices were revealed in red color, and the live protoscolices were revealed in bright green color and the treated tubes were stored in laboratory conditions at 25C [13].

Results and Discussion

The effect of boiled broccoli extract against the viability of protoscolices :

Table, figure (1) The percentage of viability of protoscolices in the conservative media (KRS + HCF 4: 1) after exposure to different concentrations of boiled broccoli aqueous extract in different periods time *In vitro*.

Tbale (1) The effect of the interference of concentrations of boiled broccoli extract on the viability of the protoscolices of the *E. granulosus In vitro* according to different time period

Time (hour) / Concentration (mg/ml)	0	3	24	48	72	96	120	144	168	192	216
	The viability of protoscolices (%)										
Control (0)	94.33	83.6	82.6	68.6	68	62	61	59	55.33	46.66	0
100	94.33	69.4	57.3	54.4	47	44.3	40	40	37.6	36	0
200	94.33	58.16	35.6	32	29.33	27.83	26	24.56	21.33	0	0
300	94.33	58	33	22.9	23	22.2	14.5	6.9	0	0	0
L.S.D value for interference at probability level 0.05 = 9.754											

Table (1) show that the concentration of 300 mg / ml was most effective on the viability of protoscolices , decreased from 94.33% to zero at 168 hours from the start of the experiment, followed it the concentration of 200 mg / ml the viability decreased to zero but at 192 hours followed it a concentration of 100 mg / ml viability decreased to 36% at 192 hours.

may be due to the fact that boiling water extract is linked to the action of enzymes and thus the active substance in the broccli extract has turned into another substance more effective and thus has resulted in a reduction in the viability percentage of protoscolices of the *E. granulosus*., from these compounds (phenols) which are among the active compounds Dissolved in water [14]. The results of the current study were agreed with the study Al-Shahery and Salih (2015), which demonstrated that broccoli help in treatment for Induced Polycystic ovary syndrome in Albino rats [15]. Also agreed with Mohammed *et al*.,(2019) study, that used of broccoli as a source of natural antioxidants or nutrients that protect the liver from the toxicity of lead acetate [16].

The effect of albendazole drug against the viability of protoscolices :

Table, figure (2) The percentage of viability of protoscolices in the conservative media (KRS + HCF 4: 1) after exposure to different concentrations of albendazole drug in different periods time *In vitro*.

Table (2) The effect of the interference of concentrations of albendazole on the viability of the protoscolices of the *E. granulosus In vitro* according to different time period

Time (hour) Concentration (mg/ml)	0	3	24	48	72	96	120	144	168	192	216
	The viability of protoscolices (%)										
Control (0)	94.33	83.6	82.6	68.6	68	62	61	59	55.33	46.66	0
100	94.33	62.2	58.5	49.8	39.9	39	17.3	11.06	8	1.1	0
200	94.33	56.3	54.8	42	28.13	27.8	3.66	3.3	0	0	0
300	94.33	49.3	39.2	38.9	18.5	9.8	0	0	0	0	0
L.S.D value for interference at probability level 0.05 = 7.012											

Table (2) show that the concentration of 300 mg / ml was most effective on the viability of protoscolices , decreased from 94.33% to zero at 120 hours from the start of the experiment, followed it the concentration of 200 mg / ml the viability decreased to zero but at 168 hours followed it a concentration of 100 mg / ml viability decreased to 1.1% at 192 hours.

May be due to the compounds of benzimidazole causes decrease glycogen by affecting the glucose absorption mechanism at the membrane of hydatid cyst, degenerative changes that occur at the endoplasmic reticulum and mitochondria of germinal cells and the increase in the number and the activity of the lysosomes cause cellular autolysis , Albendazole is widely used as a primary treatment in hydatid disease of the liver and successful results have been reported [17,18].

Through the two experiments in the current study, we notice when increase of the concentration and the longer the exposure period (time period), the viability of protoscolices are decreased, The results are consistent

with the Al-Omari' study (2005)[19], which found that increased concentration has a significant impact on the loss of protoscolices, and it also agrees with Al-Hamiary (2010) [13] , Al-Musawi (2012) [20] and Al-Shimary(2020) [21].

Conclusions

From the present study, it was found that the effect of broccoli (*Brassica oleraceae*) boiled aqueous extract 300 mg/ml at 168 hours, and its ratio 0, the same effect of albendazole at the same concentration (300 mg/ml), but at 120 hours. The boiled broccoli extract can be used as an alternative to chemotherapy to treat hydatid cysts disease and recommended to conduct other studies on broccoli effect especially in vivo study among mice or rats.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science for Women and all experiments were carried out in accordance with approved guidelines.

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Effect of compound exercises on Some Kinetic Abilities in Some Bio *kinematics* Variables and the Characteristics of the Curve (Force - Speed) for hit/spike of High Volleyball for Youth Volleyball

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Abstract

The importance of the research is important to study the impact of compound exercises using light stimuli and sound stimuli as well as the use of rubber ropes and some training aids and to develop some of the kinetic abilities that help the athlete to integrate several kinetic skills in an orderly and sequential as well as perform movements under changing conditions, to be able to face different Conditions and situations experienced during the game and well, as well as the development of indicators and function (force - speed), to reach the best mechanical conditions, and with the least decrease in speed and momentum, the researcher used the experimental method design one group The research sample was determined in a deliberate manner and they are players of Al-Shatra Club of the volleyball youth in the district of Shatra and the number of (6) players. The results were analyzed by the analysis software using (SPSS, Logger Pro 3.10.1, Sigview v.2.8.0, Kinovea 0.8.25) to obtain the values of the BioKinmatk variables of the study. Technique of the skill of high hit/spike as a result of the development of internal strength using a The most important recommendations were to emphasize the conduct of compound exercises, especially for muscles working on the joints of the hips, knees and ankles, as well as on the muscles working on the joints of the shoulders, elbows and wrists, as applied in the research for their importance in the development of some kinetic abilities and characteristics of the curve. Power - speed.

Key words: *Kinetic Abilities, Bio* **kinematics***, Compound exercises, Curve (Force- Speed)*

Introduction

The advance of technical level performance of the skill of hit/spike witnessed recently was the result of the progress of the training process and upgrade the level of methods and equipment and the preparation [1] of trainers technically and practically, which must keep pace with the advantages of this game that requires different positions fast and high physical ability as well as mastering the technical aspects and mechanical conditions that are characterized By this effectiveness. Combined training and effective means are important in the development of kinetic skills necessary for volleyball players, which depends on the ability of the athlete and efficiency in the production of muscle strength related to rapid performance [2] so as to reflect positively on the art of performance of the skill of hit/spike not to mention the correct and appropriate, as this skill is distinguished It has its own neuromuscular system, which is the compatibility of the movement of the various joints of the different parts of the body that perform, as it requires the player to be able to link the kinetic coordination of partial movements with each other in place and time in order to obtain greater The speed is possible for all parts of the body at the moment of approaching and pushing and this depends on the ability of players to install and guide muscle groups to serve skill performance, as well as these exercises put a relatively large muscle effort which leads to stimulate muscle groups to mobilize the largest possible number of muscle fibers and possess the property of torque Rapid result of effort to shed these exercises, which increases the strength and speed of muscle contraction [3] and stimulation to work with great

ability, in turn contribute to the achievement of effective dynamic and effective work during the skill sections and record time, serves the goal of performance is to achieve the farthest distance both Horizontal during the contact or absorption or vertical distance during the final pushing stage, to accomplish the technical performance with the highest possible efficiency and with the least effort. Hence the importance of the research to study the impact of compound exercises using light stimuli and sound stimuli as well as the use of rubber ropes and some training aids and to develop some of the kinetic abilities that help the athlete to integrate several kinetic skills in an orderly and sequential as well as perform movements under changing conditions, to be able to face the player The various conditions and situations experienced during the game and well, as well as the development of the characteristics of the function of force - speed, to reach the best mechanical conditions [5], and with less decrease in speed and linear momentum, the researcher hopes to add new information in the field of application BioKinmatk science to hit/spike skill in volleyball and cope with the great progress made the results of volleyball in general and hit/spike skill in particular .

Methodology

Research Methodology

The researcher used the experimental method using the one-group experimental design appropriate to the nature of the research.

The research sample

Six young players were selected from Shatrah Sports Club volleyball and deliberately in order to carry out the study and achieve its objectives. Table (1) shows the characteristics of the research sample.

Table (1). Homogeneity of the sample in the variables of height, age of training and apparent weight

No.	Characteristics	Arithmetic mean	Standard deviation	Coefficient of variation
1	Length (cm)	186	2.71	2.99
2	Age (years)	3.5	0.23	11.14
3	apparent weight (kg)	77	2.16	5.11

Exploratory experience

The researcher conducted the exploratory experiment on Sunday 02/01/2018 on (3) players other than the research sample were photographed to identify the location of the power measurement platform and the location and dimensions of cameras and height and clarity of the image and time required for each shooting and the time to conduct the shooting as well as to identify the following:

ü High cameras.

ü Identify the difficulties and obstacles that will appear during the implementation of the tests and their progress.

ü Identify the time to take the tests and how long it takes.

ü The possibility of shooting and analyzing the results of mechanical variables.

ü The ability of the sample to carry out the tests and their suitability.

ü Identify the devices and tools necessary to carry out the experiment and tests.

ü The possibility of measuring the strength of the device used.

ü For the total time required by the experiment.

ü Introduce the team to the nature of the experiment and its requirements.

Main experience

The researcher has prepared compound exercises according to some kinetic abilities (kinetic speed and agility and balance) after reviewing a set of sources for sports training related to the subject of research and after presentation to the gentlemen experts have been the application of the exercises on 5/1/2019 until (6/3/2018) (8) weeks and includes (24) training units (3) units per week (Sunday - Tuesday - Thursday) commensurate with the different conditions of the sample and the appropriate period for the implementation of exercises is the stage of special preparation, which takes (8) weeks and an average of (3) units Training per week The time of one training unit (35 - 45 minutes) and prepare This is a new training trend because it focuses on the use of new kinetic exercises during the education process in order to ensure the increase of the kinetic balance. Changes and differences in the corners of muscle work during the performance of muscle exercises using devices and auxiliary tools according to some stimuli and sound stimuli that have a significant role in increasing the speed during training and therefore lead to the organization and adapt speed The reflex reflexes, coordination of movement, rhythm and neural stimulation have a significant impact on the smoothness of performance and the slightest decrease in the amount of movement, whether angular or linear. The researcher used the method of repetitive training. The duration of the training was within the duration of the special preparation and 8 weeks, and by three training units per week, and a summary of how to use the intensity of the different exercises:

· Rubber cords exercises, Total cord elongation is measured as maximal limits and then training the training intensity of the ropes accordingly.

· For the additional weights used, the maximum weight that the player overcomes is determined and the intensity is determined accordingly.

1.4 Statistical means

The researcher used the statistical program (SPSS) to process the results.

2. View, discuss and analyze results

3.1 Presentation and discussion of the results of differences in the values of some abilities of the pre- and post-test of the research group

Table (2) : It shows the values of the arithmetic media, the standard deviations and the calculated (T) value of the values of some kinetic capabilities of the pre- and post-tests of the research group.

No.	Processors / Measurements	Measuring unit	Pre-test		Post-test		T value calculated	Significance level	Result
			from	±	from	±			
1	Kinetic balance	Degree	56.21	3.08	66.70	4.78	8.156	0.040	moral
2	Kinetic compatibility	sec	12.89	0.36	11.64	1.18	4.756	0.040	moral
3	Agility	sec	7.54	0.14	6.62	0.62	6.8443	0.045	moral

*** At the significance level ≤ 0.05**

The researcher attributes the reason for this development to the nature of the compound exercises according to some special kinetic abilities applied by the research group using additional weights and rubber cords according to some light stimuli and acoustic stimuli on the momentary foot on one or two feet. Ideal approach corners positively reflected on kinetic balance (anxiety) according to skill performance and thus contributed effectively and easily to the gradient and the ability to link kinetic actions with each other, and this referred to the mechanism "For- Edunrt I. & Mathews" : "The balance is the ability to maintain the body in the state of stability or maintain its state during and after kinetic actions or restore it to its normal state", and this reflects the harmony of mental processes and neuropsychiatric instructions with what is required to perform fast kinetic action and high amounts of strength when working with these Exercises and the use of added weights to the body and rubber ropes and light stimuli and the performance of exercises with great strength and speed of movement have affected the development of muscle groups of the two men and increase the activity of muscle contraction through repetitions in the performance of the exercises that used, As a result, the development of muscle contraction of the working muscle groups is accompanied by the development of neuromuscular compatibility, which led to ease of performance, and this is consistent with the "Mufti of Abraham 2001", as the greater compatibility between the muscles involved in kinetic performance ... The increased production of muscle capacity (123: 5) . As for the compatibility ability, the researcher attributes the reason for these moral differences to the nature of complex exercises using complex exercises and aids dominated by the character of bilateral kinetic compatibility of the upper and lower limbs that helped the research group to regulate the internal force with the external force in a way that reflects the ability of players to perform movements of various degrees Difficulty and high speed and accuracy according to the specificity of skill mechanical objectives associated and integrated and several repetitions in order to deepen the kinetic path in the nervous system, but in the ability of agility where the researcher attributes these differences to the nature of the complex exercises Using (light signals - acoustic acoustic stimuli - crossing barriers and crawling underneath - zigzag in different directions and speed - direction change exercises - balancing exercises - small toys ... etc) increased the efficiency of the internal strength of the strength of the muscles and ligaments and joints of the body and causing change Significant in the amount of movement of the body at the performance, where the ability of players to develop speed of movement and change of direction and evidenced during the implementation of the test developed muscular momentum and ideal approach corners helped to distribute force and pressure on the surface of the foot and thus increased the strength of vertical friction and say From the time of communication until the player does not slip in the direction of the original movement and thus the player can change the direction easily by the reaction generated large that suits the requirements of kinetic performance required to implement and direction, as the greater the amount of change in the direction required a greater lateral push and thus a greater reaction any friction force Significant as a result of increased speed of movement, the force causes acceleration, evolved efficiency of the strength of the internal players cause a backlash Prevent imbalance due to increased friction force during the face of unexpected kinetic events and rapid adaptation as well as increase the capacity of the kinetic reserve not to mention the development The ability of the sample to balance and conform.

Table (2). shows the values of the arithmetic media, the standard deviations and the calculated (T) value of the values of some BioKinmatk variables of the pre- and post-tests of the research group.

No.	Processors / Measurements	Measuring unit	Pre-test		Post-test		T value calculated	Significance level 0.05	Result
			from	±	from	±			
1	Approaching speed	m/sec	3.60	0.20	3.88	0.07	3.699	0.002	moral
2	Corner of advancement	min	77.87	3.79	87	0.53	6.732	0.003	moral
3	Flight speed	m/sec	2.76	0.01	3.16	0.06	16.323	0.004	moral
4	Maximum height	m	1.34	0.01	1.48	0.01	31.485	0.002	moral

*** At the significance level ≤ 0.05**

The researcher attributes the reason for this development to the nature of the compound exercises according to some special kinetic abilities applied by the research group using additional weights and rubber ropes according to some stimuli and various means associated with kinetic abilities learned to increase the efficiency of muscle momentum exerted during moments of back and front and ideal approach corners reflected positively The efficiency of these muscles and achieved by the speed of the player (a very short time) on the change of direction with balance and high compatibility, which contributed to the increase of decentralized force (as a prohibition), helped the player to increase the speed of movement of the body parts Dust or better starting speed is much better than the speed in the pre-test "The greater the speed of approaching the greater the speed of departure and thus increase the momentum of the body and move to the ball," which leads to increase the instantaneous speed of the ball as a result of increasing the values of the vertical speed of the body at the moment of departure and an ideal push corner The development of pure muscle strength, which is one of the most important variables in determining the height of body mass at the moment of hitting the ball unless any external forces overlap, "The development of the efficiency of muscular acceleration contributed effectively to the success of the main section by ensuring access to the goal to be achieved with high efficiency and with minimal muscle effort. "

Table (3). shows the values of the arithmetic media, the standard deviations and the calculated value (T) of the values of some variables .

No.	Variables	Processors	measuring unit	Pre-test		Post-test		T value calculated	Significance level 0.05	Result
				from	±	from	±			
1	Seam	Force function	nt		124.54	1815.59	48.14	6.643	0.030	moral
		Speed function	sec		0.002	3.09	0.001	24.751	0.027	moral
2	Absorption	Force function	nt		50.89	1297.59	71.68	6.525	0.001	moral
		Speed function	sec		0.001	3.05	0.001	9.113	0.010	moral
3	Pushing	Force function	nt		132.82	2943.86	60.81	14.134	0.030	moral
		Speed function	sec		0.001	4.24	0.001	16.776	0.020	moral

Curve (force-speed) pre- and post-tests of the research group

The researcher attributes the reason for this development as a result of the impact of interactive compound exercises on some kinetic capabilities using rubber resistors and additional weights and light stimuli and acoustic stimuli according to the scientific bases associated with BioKinmatks, contributed to the development of mechanical power capacity and this is due to the development of the efficiency of the internal strength of the production of torque and the strength of ligaments and joints Working during the kinetic performance affected the increase in angular velocity of the body parts increased the linear velocity values of the center of gravity of the body and thus achieved high values of force during the moments of performance, either in approach or thrust, as well In addition, the exercises used have achieved the goal of developing muscle strength against resistance in the course of the exercise. This has increased the distance achieved (height or horizontal distance) associated with the time component. We will get the possibility of the muscle to exert high strength and a high level of speed. Represents the muscular power expressed as the result of both strength and speed.

The researcher believes that the variable speed is one of the mechanical variables that play a big role in achieving a high level of muscle capacity, as the amount of muscle power exerted is great to overcome the force of gravity of the moment of propulsion and the associated linear velocity of the center of gravity of the body and the reaction force, which resulted in The maximum possible ability of the player to obtain the distance (horizontal, vertical) required, as well as the exercises of agility and speed used to assist in the development of muscle capacity of the men as there is a direct correlation between the increase in the strength of the muscles of the legs with the results of explosive power and strength characteristic secret The exercises used in the training curriculum have facilitated the muscular work according to the appropriate BioKinmatk conditions in terms of the correct application, "the corners of the body to achieve the best value of the torque of these corners at the required time which reflects a good compatibility within the muscle itself" (145). : 1) as well as between the muscles engaged in performance.

Conclusions

1**.** Evolution of the technical performance level of the skill of high hit/spike as a result of the development of internal strength using the exercises combined according to some of the kinetic capabilities.

2. The speed of approach and the speed of departure have evolved as a result of the development of mechanical conditions, which resulted from the use of compound exercises according to some kinetic capabilities.

3. compound exercises worked in accordance with the kinetic abilities to develop the corner of thrust as a result of the development of the strength of the strength of the muscles fixed in accordance with the absolute performance corners, especially when prompted to rise.

4. compound exercises using rubber ropes, extra weights and some different stimuli have developed mechanical strength during phases, contact, absorption and propulsion. As a result of the increase in the speed change during the three stages as well as the ability of the body to overcome the body weight and additional weights and other resistance sequentially to reach this stage in the best start.

5. A decrease in the pivot area of the foot support as a result of the application of compound exercises according to some of the kinetic capabilities applied using rubber ropes and additional weights and some different stimuli.

6. The exercises used to make the research sample achieve a high level of strength and in a very short time in the post-test.

7. All the exercises used to develop the kinetic abilities of the muscles of the legs, torso or arms, increased the efficiency of the internal force represented by the muscle contraction force, and the strength of the ligaments in the production of torque force.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Education and all experiments were carried out in accordance with approved guidelines.

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Hearing and Children with Autism Spectrum

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Abstract

Through the results obtained, we found that most of the families who had a child with autistic disorder were residents of cities, with a percentage of 89.8%, while 10.2% who had a child with autism from rural housing. The majority of children with autism had a weak physical condition of 74.5% percent, and 15.3% percent had an average condition, while 10.2% percent had a good physical condition. Likewise, among the results that we found that the best way to deal with a child with autism is care and treatment, and by 62.2%% and 31.6%% of mothers, the opinion was that the best way to deal with a child with the autism spectrum is care only and by 6% of Among the mothers said that treatment is better than the way they deal with their children. Likewise, between research, autistic children who are taken in the research suffer from a hearing disorder and 82.7% of children respond when their names are heard, while 17.3% do not respond when calling their names. It is noticeable that the proportion of children who suffer from hearing loss, 17.3% that they did not respond when calling their names83.7.% of autistic children asked for help in their daily problems, while 16.3% of the children does not asked for help. 53.1% of the children find it difficult to express their wishes when they want to ask for something, while, 46.9% of the children did not find it difficult to express when they wanted to get something. The research also addressed the behavior of autistic children.

Keywords: *Hearing, children, autism spectrum*

Introduction

Autism is a disease that occurs due to a defect in the nervous development of the fetus and leads to poor learning in the child and his lack of communication with the outside world, and autism stays with the child for life, and has pathological consequences that are cautious and unknown, but it does not negatively affect the average life expectancy, the Children diagnosed with autism spectrum disorders demonstrate restricted, repetitive behaviours and interests as well as impairments in social communication and social interaction across multiple contexts [4]. Optimism is very high for autistic children with an IQ above 70 and those who can use the language of communication at the age of 5-7 years of age. As for adulthood, the results of recent American studies indicated that two-thirds of autistic people do not improve and fall into the category of people with special needs (the handicapped), and live dependent on their family or private institutions. The same studies indicated that only 1-2 percent of autistic people lead a normal and independent life and can make a living. Whereas 5-20 autistic people can reach a normal position (other than that they prefer to be kept under surveillance .Usually the child's situation will improve in the future (in adulthood) if the family or the surrounding community is supportive, cooperative, and understanding of his situation. However, some autistic people may harm themselves or others [2,3] There are many types of autism (Classical Autism, Asperger's Disorder, Rett's Disorder, Disintegrative Disorder that contain some features of autism. However, there are common symptoms in children (social weakness, or social networks weak relationships, weakness in social relations with his mother. his father with family members and strangers. In the sense that the child does not care about the presence of others ,He does not rejoice when he sees his mother or father. He does not look at the person who speaks to him (lose of eye contact) . He does not enjoy the presence

of others and does not share their interests .He does not like to share his games., He loves to play alone and he don't mixed with other children Also he cannot know the feelings of others or deal with them correctly ,such as seeing his mother crying or sad because he does not interact with the situation naturally like the rest of the children, Weak language communication Weakness in linguistic expression or delay in speaking sometimes using strange words from the child's composition and repeating them always repeat the last word of the sentence that he heard Also there may be difficulty in using pronouns.,His activities, interests, and games are frequent and limited, there is no renewal in it, such as playing with cars only, cubes, or a way of playing that is not in line with the game in which he plays, such as stacking small cars in a certain way instead of imagining that they are going on the road. He also loves the routine and does not like changing his clothes, types of eating, or the way he organizes his room .. Clinging to things like a specific pillow or blanket and always carrying him with him, and he may also have frequent movements of the hand and fingers [4-6] There is a lot of research on this disorder and all of you find things that can be useful in diagnosing and evaluating the disease and the speed of its treatment. Therefore, we wanted a questionnaire organized by experts for mothers of autistic children about their children and some behaviors. [7]

Material and Method

1-A total of 98 children with autism were examined after the approval of their families in one of the autism centers and the specialized medical clinic for hearing and speech. Samples are sent from the children to confirm the diagnosis to the hospital and give them the necessary medical care to help them.

2- Diagnose hearing damage to children who do not respond when their name is spoken. By means of the Brainstem Evoked Response Audiometry (BERA) examination, which is an objective and non-invasive method for hearing. Evaluation that detects electrical activity from the inner ear to the lower colliculus (9) 3- A questionnaire dictated by mothers for children with autism, as the information gives an understanding of the distinctive signs of children with autism.

4- Statistical analysis is done by making percentages of questionnaire results and hearing test results.

Results

Statistical results were taken after the questionnaire was completed by the mothers in the autism center and they are the sample of the study.

Table No. 1 shows that the percentage of males that took the research is 39 samples, while females are 59, and this is probably because the sample was from the mothers of the autistic children. Likewise, the schedule indicated that the age group of mothers that ranges from 31 years to 40 years is the most within the sample taken and is the highest percentage as the most fertile group and is considered the most popular group for marriage, while the percentage of mothers whose ages range between 41 to 50 years is the lowest and that the ratio Females are more than males among the specimens in the Autism Center of the Abbasid threshold. [10]. 645 children between 0 and 4 years were diagnosed with autism spectrum disorder diagnosed from 1986 to 2005. The incidence of autism spectrum disorder was 5.49 per 10,000. The prevalence rate was 16.1 per 10,000 for children under 15 years old. The same period. The male to female ratio was 6.58: 1. This is the first epidemiological study of autism spectrum disorder in Chinese children. The incidence rate is similar to that in Australia and North America and less than Europeans. This was corroborated by the conclusion that the proportion of males was more than that of females . [11,12].

As for Table No. 2 most of the families who suffer from the presence of one of their children with autism disorder are residents of the city and they are of limited income. This was confirmed by Table 2, as most of the children affected by 88% are residents of the city.

As the table showed that most families of children with autism had a weak physical condition, about 74.5% of the sample taken, while, 15.3% had a moderate physical condition, and only 10.2% had a good physical condition, and these ratios show that autism affects all Strata of society. Also, between the schedule, most of the injured children were residents of the city, despite the weak physical condition, by 89.8%, and 10% of the children live in the countryside, and therefore the autism disorder does not depend on the location, so there is no difference between the city and the countryside, although the percentage of the city was higher than the countryside but This may be due to health awareness and the presence

of child care centers with autism. Table (3) also showed that most of the parents, especially the mother, had an opinion that the best way to deal with a child with autism is care and treatment, and about 62,2% saw that care is an essential part of dealing and supplementing treatment, while the rates differed between treatment and care, 31, 6% said that the best way to deal is with care, and 6% said that the best way to deal with their children is treatment. This table shows the awareness of the parents and the evidence for this is the percentage difference between the answers, especially the percentage of the parents who chose care and treatment to deal with their children and confirmed this. [14] After that, 17 children who appeared in the previous table were tested using the (BERA) brain stem examination device. Children with autism were examined to measure the degree of hearing. It is a test that depends on the brain's response to hearing sound frequencies. The child is under the influence of anesthesia or hypnosis and shows a convergence in the results of the examination between the table No. 4, which is the schedule of the mother's knowledge that her child is unheard when called in his name, and the results of the examination by the device examine the brainstem that there are 15 children who cannot hear and compare it to the behavior test, which is the child's behavior when hearing the sound and its result was 17 children who do not hear There is an affinity between the objective test BERA and behavior test. Table 4 shows answers to the question of the effect

of the disturbance on the sense of hearing or vice versa, as most mothers answered not suffering from a hearing disorder at a rate of 82.7%, and 17.3% answered yes. This answer means that either these children do not suffer from this symptom or perhaps because of the increased care and attention given to them , We also mentioned previously that care has the primary role in improving the behavior of the child and certainly this is reflected in the improvement of the children's sense of hearing. Also, this table shows the child's response when they call in his name since 82.7% were responding when hearing their names, and since most of the children were not suffering from hearing problems, this certainly means the child's response when he hears his name, while 17.3% do not respond when calling their names and from It is noticed that the percentage of children who suffer from a hearing defect was 17.3%, and thus this means that they did not respond when they called them by their names, as it was not completely identical. [9,13] As for Table (5) between non-verbal behavior of children, which ranged from complete silence, as 29.6% of children were in complete silence, and 38.8% of the sample taken were suffering from laughter spells without justification for that, and the percentage 31,6% of children cry out continuously. Likewise between the schedule is weakness in non-verbal (motor) behaviors, as most of the children did not suffer from weakness in the use of signs, gestures and facial expressions, and their percentage was 60.2%, while 38,8% of the sample Autistic children have been noticed weakness in these behaviors and this may be due to the tendency of the autistic child to use verbal verbal behaviors or as we mentioned crying or screaming or other and thus this means not to use or weakness in the use of gestures and signs. [15] The research showed that a person with hearing impairment may be susceptible to autism. Tables 4 and 5 were supportive of some of them both confirming that the type of injury is far from hearing impairment among the children studied [9,13,15]

Children with ASD may experience a lack of an inner ear, which impairs their ability to recognize speech. Researchers at the University of Rochester Medical Center identified this deficiency by measuring acoustic emissions for children ages 6 to 17. Those who were diagnosed with ASD had difficulty hearing a specific frequency (1-2 kHz) important for speech processing. The researcher cited an article by Luisa Benito, Ph.D and co-author of the study, in the July 25, 2016 online edition of Science News: "Hearing loss has long been associated with delayed development and other problems, such as language deficiency." "Although there is no relationship between hearing problems and autism, difficulties with speech processing may contribute to some of the main symptoms of the disease." Like other auditory processing disorders, this deficiency can contribute to your child's difficulty learning and language. An ASD audiologist or audiologist can determine your child's deficiency of hearing and language and suggest strategies to help them overcome them. These strategies may include. [14]. Because autism affects each child differently, it's important to understand how the disorder may impact their ability to hear and process sounds. There is a wide range of how autism affects hearing. In some cases, a child may have no hearing loss or a child may have mild, moderate or even significant hearing loss that can

be corrected with hearing aids [15]. It was noted through Table No. (6) that 83.7% of autistic children ask for help in their daily problems, while 16.3% of children do not ask for help. Likewise, the schedule showed that 53.1% of children find it difficult to express their wishes when they want Requesting something while 46.9% of the children did not find it difficult to express when they want to get something that may be due to the difficulty of understanding these children's desires in addition to the child's inability or the difficulty of expressing what he needs. As endorsed by . [16,17]

Table (7) showed the imaginative behavior of autistic children, as 23.5% of mothers said that their children play imaginative play, while 76.5% of children did not play imaginative play. This may be due to the improvement of children's behavior or to the awareness of mothers and thus their use of child training programs and techniques to reduce this behavior or the lack of space for them to practice this

behavior or because of the awareness of mothers and thus their use of programs or training techniques for children that reduce this behavior or lack of space for them to practice This behavior is through training programs as we mentioned. This conclusion was supported by Table No. 7 . [17,18] As the schedule showed that 75 of the infected children prefer electronic games, which are imagination games, which indicates the power of imagination, and this came contrary to what she said. [20]

Table No. (8) shows whether the child is making strange movements Does your child make strange movements, for example, contemplating his fingers in front of his eyes .It is noted from Table No. (8) that 32.7% of the children taken in the sample perform strange movements according to the mother's response, while 67.3% of children do not make strange movements, this may be due to either that the child does not suffer from this symptom or because of improved behavior Children as a result of the training provided to them by the mother as well as his response to the training of the autism center where he indicated that Abdul-Qadir (2001) clearly shows autism in the first three years of the child's life, and is defined as a disability that impedes the development of social skills, verbal and non-verbal communication and imaginative and creative play and is the result of disorder Neurotoxic affects the ways in which information is collected and processed by the brain causing problems in social skills such as the inability to link, the creation of relationships with individuals, the inability to play and use of spare time, and the inability to constructively visualize. As for communication skills, they lie in the inability to express oneself automatically and in a suitable and functional manner, and the inability to understand what others say, and the inability to use other skills besides verbal skills to help the individual in the ability to communicate, and the problem of adapting to the environment is in not The ability to perform work and job performance effectively in the environment and the inability to cope with and tolerate changes in the environment and deal with them. (1,20) The schedule showed that 72,4% of autistic children communicated in kind with their mothers when something unusual occurred, while 27,6% of the children did not communicate in kind with their mothers. The table also showed that 62,2% of autistic children tried to communicate and get closer to their mothers by raising his hands to his mother in order to carry him and this contradicts the wrong thinking taken from the autistic child, while 37,8% of children do not communicate physically with their mothers. The schedule showed that 78.6% of the children taken in the sample viewed the things that their mothers referred to, and 21.4% of the children did not look at the things their mothers referred to. This may be due to the lack of attention of children to the things around them. [1,14] The schedule showed that 68,4% of children have excess physical activity, and since we are talking about children, they inherently have physical activity and since they are autistic, increased physical activity is one of the main symptoms of autism, while 31,6% of children do not have excess physical activity. [11,15] The schedule showed that 80.6% of the children taken in the sample are characterized by stubborn and quarrelsome behavior, according to the answer of the mothers. Certainly everyone knows the stubbornness of children, especially in the first years of their lives. . While 19 of the mothers said that their children do not oppose nor tend to quarrelsome.

You notice his interest in and attachment to a certain thing, such as taking care of a specific game and his association with it and not throwing it or causing breakdowns in it ,The schedule showed that 48% of the children were related to a specific thing, while 52% of

the children were not related to a specific thing such as play. [1,7]

We note from the table that 67.3% of autistic children care about other people around them, while 32.7% of children do not care about others. Between the schedule, 40,8% of mothers said yes, that is, their children seem to be annoyed by changing food dates or sleep times, and this is due to the child's awareness of hunger, and this means that the nervous system of these children works better than other children and their love for the daily routine, while 59 , 2% of mothers said that the child does not show his discomfort due to changing the dates of his food or sleep, and this is because the child is not aware (he does not have nerve stimuli) and since he does not suffer, he certainly does not feel hungry because of the lack of stimulation of the nervous system. 54.1% of mothers of autistic children observed that their children should not be afraid of those situations that require fear, certainly due to a defect in the nervous system of these children or the lack of programs used to develop the autistic child, while 44.9% of mothers noted that their children fear of situations Which necessitates this and this is due to stimulation of the nervous system as well as due to the noticeable development in the level of autistic child. [19,20]

We note from the above table that 55.1% of autistic children suffer from memory impairment or lack of focus, while 44.9% of children do not suffer from lack of focus, perhaps because of the degree of autism in these children or because of children's response to training.

We note from the above table that 55.1% of autistic children suffer from memory impairment or lack of focus, while 44.9% of children do not suffer from lack of focus, perhaps because of the degree of autism in these children or because of children's response to training. [1,17,19,20]

Table 1. shows the percentages of samples taken in the cognitive questionnaire and by gender and age group.

sex			
percentage	The ratio	Repetition	the number
39,8	39,8	39	Male
60,2	60,2	59	Female
100,0	100,0	98	Total
Age			
31,6	31,6	31	20-30 years
45,9	45,9	45	31-40 years
22,4	22,4	22	41-50 years
100,0	100,0	98	Total

Table (2) shows the percentages of families of children with autism, according to housing and financial status

percentage	category	Repetition	
Living			
10,2	10,2	10	countryside
89,8	89,8	88	City
100,0	100,0	98	Total
Physical condition			
74,5	74,5	73	Weak
15,3	15,3	15	Average
10,2	10,2	10	good
100,0	100,0	98	Total

A table showing information about the samples taken in the questionnaire

Table B shows the mother's impression of a preference for dealing with autistic children

Table (3) Shows the percentages of the way in which mothers must deal with their children.			
percentage	category	Repetition	
6,1	6,1	6	treatment
31,6	31,6	31	care
62,2	62,2	61	both of them
100,0	100,0	98	Total
Table (4) shows whether the child suffers from a hearing disorder and the extent of the autistic child's response when called in his name			

Table C shows some of the characteristics of autism children

percentage	category	Repetition	
17,3	17,3	17	yeas
82,7	82,7	81	No
100,0	100,0	98	Total
Does your child respond when you call him by his name			
82,7	82,7	81	yeas
17,3	17,3	17	No
100,0	100,0	98	Total

Table D shows some of the favorite games for children with autism

Table (5) shows the behavioral changes that an autistic child can suffer from, as well as whether he suffers from a weakness in the use of motor behaviors.			
percentage	category	Repetition	
29,6	29,6	29	Total silence
31,6	31,6	31	Constant screaming
38,8	38,8	38	Laugh without reason
100,0	100,0	98	Total
I suffer from poor use of motor behaviors (nods, nods, facial expressions)			
38,8	38,8	38	yeas
60,2	60,2	60	No
100,0	100,0	98	Total

Table (6) shows whether the child is asking for help when facing problems and whether the child has difficulties expressing his desire to get anything that he might want.			
Does your child ask for help when they encounter a problem			
percentage	category	Repetition	
83,7	83,7	82	yeas
16,3	16,3	16	No
100,0	100,0	98	Total
He finds it difficult to express his desire to have something			
53,1	53,1	52	yeas
46,9	46,9	46	No
100,0	100,0	98	Total

Table D shows some of the favorite games for children with autism

Table No. (7) shows whether the child plays imaginative play
Your child does not play an imaginary game, for example, feeding the doll

percentage	category	Repetition	
23,5	23,5	23	yeas
76,5	76,5	75	No
100,0	100,0	98	Total

Table No. (8): It explains the types of games that a child with autistic disorder prefers.

percentage	Repetition	Game types
76.5	75	Electronic
23.5	23	Others
100	98	Total

Table No. (9) shows the physical and physical communication of the autistic child and the attempt to get closer to his mother

percentage	category	Repetition	
If something new happens, does your child look at you to see how you feel about it			
72,4	72,4	71	yeas
27,6	27,6	27	No
100,0	100,0	98	Total
Your child raises his hands to you to carry him as his peers do			
62,2	62,2	61	yeas
37,8	37,8	37	No
100,0	100,0	98	Total

Table E showing some of the favorite games for children with autism

Table No. (10) shows the autistic child's response to the things their mothers refer to
If you were referring to something in the room, would your child be looking at that thing

percentage	category	Repetition	
78,6	78,6	77	yeas
21,4	21,4	21	No
100,0	100,0	98	Total

Table No. (11) shows the increased physical activity associated with most children with autism Your child has increased physical activity

percentage	category	Repetition	
68,4	68,4	67	yeas
31,6	31,6	31	No
100,0	100,0	98	Total

Table No. (12) shows the stubborn and quarrelsome behavior of an autistic child
Does your child tend to stubborn and quarrelsome

	percentage	category	Repetition
yeas	**79**	**80,6**	**80,6**
No	**19**	**19,4**	**19,4**
Total	**98**	**100,0**	**100,0**

Table F shows the effect of some games with changes in routine and focus for children with autism

Table No. (13) shows if the child is related to a game or is interested in something specific

Repetition	category	percentage	
48,0	48,0	47	yeas
52,0	52,0	51	No
100,0	100,0	98	Total

Table No. (14) shows the autistic child's interest in others Your child cares about others

Repetition	category	percentage	
67,3	67,3	66	yeas
32,7	32,7	32	No
100,0	100,0	98	Total

Table No. (15) shows whether the autistic child is annoyed by changing his or her bedtime, Is he upset if the dates of (food - dream) change?

Repetition	category	percentage	
40,8	40,8	40	yeas
59,2	59,2	58	No
100,0	100,0	98	Total

Table No. (16) shows whether the autistic child is aware of the instinct of fear or not

Repetition	category	percentage	
54,1	54,1	54	yeas
44,9	44,9	44	No
100,0	100,0	98	Total

Table No. (17) shows weak memory or lack of focus in the autistic child
You feel his memory is weak or he has a lack of concentration

Repetition	category	percentage	
55,1	55,1	54	yeas
44,9	44,9	44	No
100,0	100,0	98	Total

Note I have neglected Table (18) because this table has been put in place to ensure the correctness of some answers and this table is Suffering from excessive inactivity?

Repetition	category	percentage	
15,3	15,3	15	yeas
84,7	84,7	83	No
100,0	100,0	98	Total

Conclusions

It was found through the research that there is an increase in the number of children with autism spectrum and that there are higher cases of males than females, and also the ages of affected mothers indicate a danger in the community as well that children of the autism spectrum need special medical attention for hearing to improve this sense of them, and also mothers confirmed that care and attention are among the mechanisms Especially where they are trained to help the child improve his health status to live with the community, so we recommend that you focus on educating mothers and training them to take care of their children in specialized centers.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al- Kindi teaching hospital and all experiments were carried out in accordance with approved guidelines.

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Antibiotic Resistant *Acinetobacter baumannii* Isolated from Wounds Infections in Babylon Governorate

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Abstract

This project has been carried out during a period of three months, September 2019 to November 2019. One hundred swabs were collected from patients suffering from different wounds infections. Seven bacterial isolates have been identified as *Acinetobacter baumannii* using traditional diagnostic methods. The susceptibility of these isolates was determined towards seven antibiotics using disc diffusion test method. It was found that all isolates (100%) revealed remarkable resistance against Ampicillin and Cloxacillin, whereas the levels of resistance of those isolates were recorded at 71.4%, for each of Cefepime, clavulanic acid, kanamycin and gentamycin. The isolates exhibited a moderate susceptibility towards Amikacin which accounted for 57.1%. Azathromycin being more effective against *Acinetobacter baumannii* compared with other antibiotics included in this project since resistance of isolates accounted for only 14.2%.

Key words: *Acinetobacter baumannii, wounds infections, antibiotics resistant.*

Introduction

The genus *Acinetobacter* comprises a complex heterogeneous group of bacteria, many of which are capable of causing a series of infections. *Acinetobacter baumannii* is an important nosocomial pathogen among this genus, often associates with epidemic outbreaks of infections. This organism is frequently pan-drug-resistant and is capable of causing substantial morbidity and mortality in patients with underlying disease, both in the hospital and in the community [1] . *Acinetobacter* species have been implicated in a wide range of infections, particularly in critically-ill patients with impaired host defenses. These infections include pneumonia, skin and soft-tissue infections, wounds infections, urinary tract infections, meningitis, and bloodstream infections. Some nosocomial infections and hospital outbreaks have been attributed to *A. baumannii*, particularly in the intensive care unit (ICU) setting [2]. The main challenge with *A. baumannii* is its ability to acquire antimicrobial-resistance genes rapidly, leading to emerge multidrug resistance. Shakil and coworker, 2007 stated that *A. baumannii* is highly resistant several aminoglycosides represented by Amikacin, Arbekacin, Gentamycin, Kanamycin, Tobramycin and Apramycin [3]. Widespread use of antimicrobials within hospitals resulted in the emergence and increase of antimicrobial resistance among *Acinetobacter* strains, in particular, the wide use of extended-spectrum cephalosporins and quinolones. The ability of *A. baumannii* to acquire antibiotic resistance mechanisms has allowed this organism to persist in hospital environments and has facilitated the global emergence of MDR strains [4]. This study focuses on isolation and identification of *Acinetobacter baumannii* from patients with different wounds infections and determining its ability to resist a group of antibiotics.

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Materials and Methods

Antibiotics discs

Seven antibiotics discs were used in this study, all of them obtained from Bioanalyse/Turkey. These are; Ampicillin, Cloxacillin, Azithromycin, Cefepime clavulan, Gentamicin, Kanamycin and Amikacin

in concentration (mcg) of 10, 5, 15, 40, 10, 30 and 10 respectively.

Sampling

One hundred swabs were collected from patients suffering from wounds and burns from both sexes, males and females who admitted to AL-Hilla Teaching Hospital during the period from (September 2019 to November 2019). The samples were taken by touching the wound by sterile swab and put the swab in sterile plastic tube with cover then close the tube to avoid contamination. Then the swabs transferred to laboratory for bacteriological analysis by culturing on appropriate cultural media for identification**.**

Antibiotic susceptibility testing:

Disk diffusion test:

It was performed by using a pure culture of previously identified bacterial isolate. The most effective antibiotic for each bacterial isolate was determined as recommended by [5].

The inoculums to be used in this test was prepared by adding 5 isolated colonies grown on blood agar plate to 5 ml of nutrient broth and incubated at 35°C for 18 hours and compared with (0.5) McFarland standard tube ($1x10^9$, cells/ml.). A sterile cotton swab was used to obtain an inoculum from the bacterial suspension. This inoculum was streaked on a Mueller-Hinton agar plate and left to dry. Then, the antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps and incubated for 18 hours except cloxacillin was incubated for 24h at 37°C. Inhibition zones were measured using a ruler and compared with the zones of inhibition determined by [5].

Results and Discussion

Susceptibility of all *A. baumannii* isolates to seven antibiotics data presented in figure (3-6) showed a high level resistance of *A.baumannii* clinical isolates to most of the antibiotics under test. The present study revealed that all clinical isolates 100% resistance Ampicillin and Cloxacillin, whereas the resistance to cefepime clavulan acid, kanamycin and gentamycin at (71.4%). (57.1%) of isolates appeare resistance to Amikacin, and finally only (14.2%) were resistant to Azathromycin.

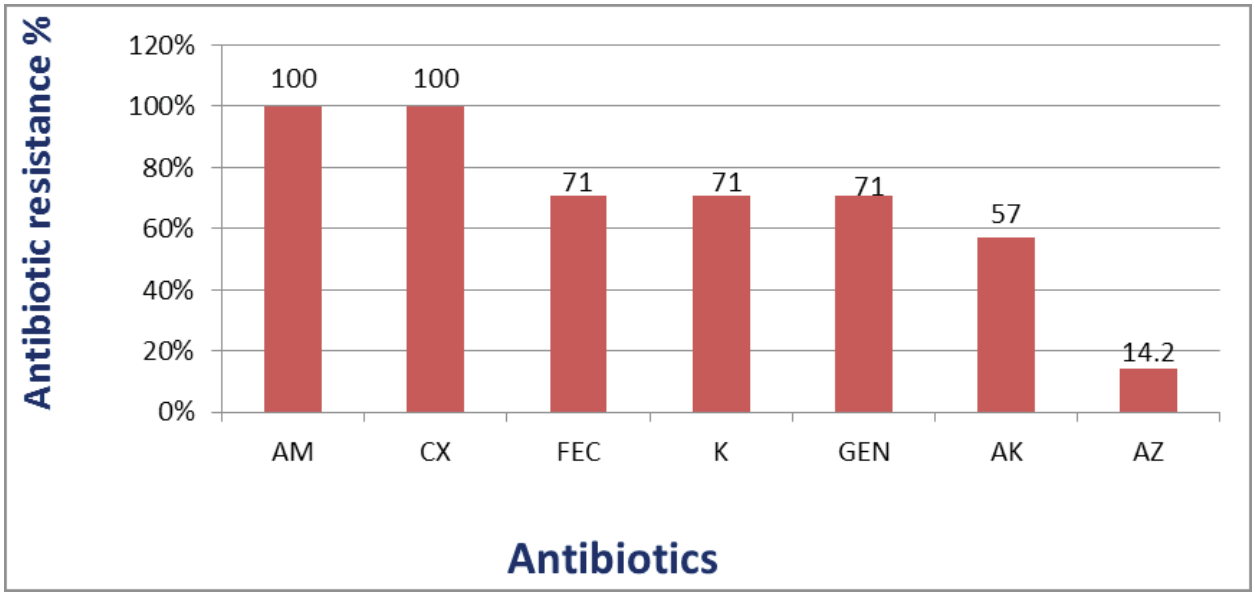


Figure-1 Antibiotic resistance profile of *A. baumannii* isolates

Ampicillin (AM), Cloxacillin (CX), cefepime-clavulanacid (FEC), kanamycin(K), gentamycin (GEN), Amikacin (AM), Azathromycin (AZ).

The antimicrobial susceptibility of *A. baumannii* was determined by disk diffusion method is in accordance with the clinical and laboratory standards institute guidelines (2012) depending on diameter of inhibition zone (mm).

The present study shows that the *A.baumannii* isolates had very high rates of resistance to the tested antimicrobial agents as shown in figure. These results were parallel with other results such as mentioned by Shakibaie [6] stated that the majority of isolates of *A.baumannii* spp, were highly resistant to the antibiotics most commonly used , including Ampecillin, cefepime clavulan acid, pipracillin and Cloxacillin.

A.cinetobacter spp. Showed a highly resistance reaching 80% to the majority of the antibiotic test [7].

In a local study done by Al-Mashhadani and colleagues [8] who found that *A. baumannii* clinical isolates developed 100% resistance to both Cloxacillin, ceftazidime.

Mohajeri and coworkers [9] pointed out that *A. baumannii* isolates revelead 53% resistance to Amikacin which is in accordance with the results of this study.

A. baumannii revealed resistant to ciprofloxacin and Cloxacillin Prashanth and Badrinath [10].

Most bacterial isolates were resistant to B-lactam antibiotics [11].

Goudarzi and colleagues 2013 [12] Showed that 70% of *A. baumannii* were resistant to Amikacin.

The antibiotic susceptibility patterns clearly showed the increasing resistance of *A. baumannii* to various antibiotics. Aliakbarzad, *et al* [13] showed that isolates of *A.baumannii* were resistant to aminoglycosides in rate reaching 81%,86% and 63% for Amikacin, gentamycin and tobramycin, respectively.

Aminoglycosides have been an important group of antibiotics in treatment of serious bacterial infections, especially those with aerobic gram negative bacteria. Resistance of *Acinetobacter* to aminoglycoside primarily results from inactivation of the antibiotic by specific modifying enzymes such as acetyltransferases, phosphotransferases and adenyltransferases.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Pathological Analysis Techniques and all experiments were carried out in accordance with approved guidelines.

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Laparoscopic Cholecystectomy Under Epidural Anesthesia in Patients with Chronic Respiratory Diseases

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Abstract

Laparoscopic cholecystectomy (LC) has become firmly established as a procedure of choice for gallstone disease. The procedure usually necessitates general anaesthesia and endotracheal intubation to prevent aspiration and respiratory embarrassment secondary to the induction of pneumoperitoneum. There is a paucity of data in the literature on the procedure being performed under regional (epidural) anaesthesia, especially in patients with coexisting pulmonary disease who are deemed high risk for general anaesthesia.

Keywords: *anesthesia, patients, chronic respiratory diseases, Laparoscopic cholecystectomy.*

Introduction

Single physiological parameter that defines this syndrome is: Limitation of expiratory airflow. [6] It is because of the combination of small airway inflammation and parenchymal destruction. Several anatomical lesions contribute to airflow limitation, including the loss of lung elastic recoil and fibrosis and narrowing of small airways, both of which are likely to cause fixed airflow limitation. [7] It adversely affects both the ventilation/perfusion (V/Q) matching and mechanics of the respiratory muscles. In the patients with advanced CoPD, the CoMBINATION. of V/Q mismatch decreased gas transfer and alveolar. Hypoventilation ultimately leads to respiratory failure. Multiple pathogenetic mechanisms contribute to the development of CoPD among which the most imp**o**rtant risk factor is cigarette smoking, which can affect the lungs by a variety of mechanisms.[8] However, recently, the role of genetic factors has also been implicated, with the finding that a genetic variant (FAM13A) is associated with the development of CoPD in the CoPD gene study [9]

Diagnosis and Assessment

Surgeons and anesthesiologists should have clearly defined criteria for COPD regarding the assessment of perioperative and postoperative risks, surgical outcome and postoperative ventilation requirement. Airflow limitation should be assessed according to the reduction in forced expiratory volume in 1 s (FEV1), Providing anesthesia to severe cases of lung disease poses some challenges especially when the patients are taken up for laparoscopic surgery. An underlying knowledge of the cardiovascular and respiratory pathophysiology is of paramount importance for providing safe anesthesia in these patients because all these changes get aggravated in patients with COPD because intraoperative and postoperative complications occur more commonly than in those without the disease and can lead to prolonged hospital stay and increased mortality

Pathophysiological Effects

During Laparoscopy

In physiological effects of pneumoperitoneum, Carbon dioxide is shown to be affected by raising the intra-abdominal pressure (IAP) above the venous pressure which prevents CO2 resorption leading to hypercapnia. Respiratory effects include the changes in pulmonary Function during the laparoscopic surgery in the form of a reduction in lung volumes, decrease in pulmonary compliance and increase in peak airflow pressure. Increased IAP shifts the diaphragm cephalad and reduces diaphragmatic excursion, resulting in the

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early closure of smaller airways, leading to intraoperative atelectasis with a decrease in functional residual capacity. Additionally, the upward displacement of diaphragm leads to the preferential ventilation of nondependent parts of lungs, which results in ventilation-perfusion (V/Q) mismatch with a higher degree of intrapulmonary shunting

Methodology

I report MY preliminary experience with LC using epidural anaesthesia in patients with chronic obstructive pulmonary disease I performed LC in 2o patients (12 man and 8 women), with a median age of 56 years (range, 38-74), under thoracic epidural anaesthesia over an 2 years period. All patients were ASA grade III/IV and the mean FEB1/FVC was o.52 (range, o.4-o.68), due to chronic asthma (6 cases) and COPD (14 cases). They were admitted a day prior to surgery for pulmonary function tests, nebulisers, and chest physiotherapy. An epidural catheter was introduced at T1o/11 intervertebral space, and a bolus of o.5% Bupivacaine was administered. Depending on the patient's pain threshold and the segmental level of analgesia achieved, incremental doses of 2 ml of o.5% Bupivacaine along with boluses of intravenous 5o MIC fentanyle was given to each patient. The patients were breathing spontaneously. No nasogastric tube was inserted, and a low-pressure (1o mmHg) pneumoperitoneum was created. LC was performed according to the standard technique

Results and Discussion

All the patients tolerated the procedure well and made an uneventful postoperative recovery. Median operating time was 5o min; average length of hospital stay was 2.5 days (range, 2-4). The epidural catheter was removed the morning after the operation. only 3 patient required postoperative opioid analgesia. 3 patients complained of persistent shoulder tip pain during surgery and required intraoperative analgesia (fentanyl) . There was no change in the patient's cardiorespiratory status, including PO2 and pCO2, and no complications occurred either intra- or postoperatively

Conclusion

LC can be performed safely under epidural anaesthesia in patients with severe CoPD. Intraoperative shoulder tip or abdominal pain does not seem to be a major PRoMBLEM and can be effectively controlled with small doses of opioid analgesia.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Aldewaniya teaching hospital and all experiments were carried out in accordance with approved guidelines.

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A Suggested Vision for Conducting of Electronic Supervision in the General Directorate of Education, Dhi Qar Governorate

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Abstract

The tremendous scientific and technological progress in various fields of knowledge is one of the most important features of the era in which we live in its present and future, as the progress of this era requires preparing a generation capable of dealing with this progress and changes in the modern technologies as well as the rapid flow of information have left clear implications for education. That the information technology, such as computers, the Internet and the attached multimedia, are among the most successful means of providing a rich learning environment, as for teachers, by contacting to the global network enables the teacher to access educational and general experiences that are difficult to access in other ways. So the importance of the Internet lies in its ability to connect among people across enormous distances and between different information sources. The use of this technology increases educational opportunities, extending them to more than the range of departments, as this is known as the e-learning that distinguishes the school of the future.

***Keywords**: Suggested Vision, General Directorate of Education, Dhi Qar Governorate*

Introduction

The most prominent information technologies are represented in the development of the computer technology and the international information network, on the [1] basis of which the idea of distance education (electronic-learning) arose, in order to bring about the improvement in the educational process, confronting the departments and approaches to the explosion of knowledge and technology and keep pace with it [2] , while the information network provides the opportunities for learning by itself or through peers, the e-learning also provides the possibility of the interactive dialogue between the teacher and the learner [3] , which helps to complete the elements of the educational process, in addition to that the use of the Internet and its tools in education has many advantages that benefit education, as the forty-fifth session of the International Education Conference had made clear in its recommendations that the emergence of the modern communication and information technology has brought about changes in the field of education, perhaps the most important one is the shift from focusing on the theoretical goals of the specializations into focusing on the practical goals, gaining interconnected and interrelated basic knowledge [4] in addition for changing the role of the teacher from being as the only source of knowledge to the coordinator and facilitator of the educational process [11]

The interest in e-learning came within the educational systems and outside it in most of the countries of the world because of the urgent need for the results of that type of education, the e-learning is not just investing of the modern technical capabilities in communicating and providing the knowledge and academic material to learners, rather, it is considered a revolution that was introduced to life, which is taken place in it on all levels [2].

Whereas, the e-learning provides services for educational administration and supervision, represented in providing the information that the director needs in making decisions, as the traditional administration relies on data which are collected manually, which may cause a lack of correlation among them, that this data cannot help the administration in making the right decisions because it needs multiple stages to convert it into useful information for the educational supervision. Educational

supervision, which it is the other which began to develop, as a new pattern has emerged within it is electronic supervision, knowing that the educational supervision process is an effective process in improving education operations and its outputs, "By improving all the factors which are affecting it, addressing the difficulties it faces, also developing the educational process in light of the goals which set by the Ministry of Education or in light of the prevailing educational philosophy" [5] With this, the electronic supervision can solve the traditional problem of the supervision, which is represented by the difficulties of movement and transmitting, increasing numbers of teachers, as well as the difficulty of the direct contact with them, this is what Abdullah Al-Hajran (2005) points to: The electronic supervision model achieves several advantages in educational supervision in terms of saving time, efforts and cost for both the supervisor and the teacher, as well as this model will be the most common in the future, as it is not logical to conduct the traditional supervisory meetings in light of the development of modern means of communication. [8]

Methodology

The descriptive approach, as it is considered the most appropriate in achieving its goals**.**

Study community: The study community consisted of supervisors and female supervisors working in the Department of Educational Supervision at the General Directorate of Dhi Qar Education, during the second semester of the academic year 2017/2018.

The Study Sample: The study sample consisted of (50) educational supervisors in the Directorate of Education in Dhi Qar Governorate, as its from various departments are male and female for different specialties who have the supervisory experience in the field of the educational supervision.

Table (1). The stability factors of the questionnaire axes to identify the requirements and the obstacles of the application of the electronic educational supervision

Questionnaire of the requirements				Questionnaire of the obstacles					
Human		Material		Human resources		Management		Material resources	
P	S	P	S	P	S	P	S	P	S
1	0,61	24	0,77	1	0,37	17	0,54	28	0,60
2	0,79	25	0,77	2	0,36	18	0,60	29	0,57
3	0,82	26	0,48	3	0,66	19	0,60	30	0,48
4	0,64	27	0,49	4	0,69	20	0,40	31	0,49
5	0,55	28	0,82	5	0,74	21	0,51	32	0,40
6	0,54	29	0,81	6	0,57	22	0,45	33	0,85
7	0,75	30	0,77	7	0,86	23	0,40		
8	0,75	31	0,39	8	0,36	24	0,55		
9	0,59	32	0,48	9	0,64	25	0,40		
10	0,88	33	0,72	10	0,51	26	0,48		
11	0,89	34	0,51	11	0,40	27	0,42		
12	0,90	35	0,55	12	0,51				
13	0,67	36	0,65	13	0,40				
14	0,48	37	0,80	14	0,60				
15	0,80	38	0,68	15	0,53				
16	0,47			16	0,45				
17	0,54								
18	0,61								
19	0,51								
20	0,51								
21	0,51								

Questionnaire of the requirements				Questionnaire of the obstacles					
Human		Material		Human resources		Management		Material resources	
P	S	P	S	P	S	P	S	P	S
22	0,51								
23	0,56								
Axis stability	0,61	Axis stability	0,86	Axis stability	0,52	Axis stability	0,48	Axis stability	0,53

P; means The item, S; means the stability

It is clear from the previous table that the correlation coefficients for the questionnaire's axsis regarding the educational supervisors 'views on the requirements of the application of the electronic educational supervision ranged between (0.61, 0,86), which are evidence of stability, as the correlation coefficients of the questionnaire for the views of the educational supervisors on the obstacles for applying the educational supervision ranged between (0,48,0,53) as these are coefficients indicating stability, as the significance of the correlation coefficients at the critical value (N - 2 = 48) ranges between (0.2276 at 0.05, 0.235 at 0.01), thus the two questionnaires in their final form became valid for the application.

The Statistical processing:

After applying the two questionnaires to the individuals of the sample, the researcher has followed in the statistical processing or treatment of the data and the responses of the individuals the following steps:

1. Accounting the repetitions of sample responses under the three approval levels (agreed - to some extent - disagree) for all of the questionnaire items.

2. Counting the weighted arithmetic average with the relative weights and Accounting its percentage

The results of the field study, analysis and its interpretation:

1- The Results of the questionnaire for the requirements of the electronic educational supervision:

To Know the requirements of the electronic educational supervision in the General Directorate of Dhi Qar Education from the point of view of the research group, the researcher has taken the following steps:

· The questionnaire was applied on the research group, that the responses of individuals to the items were emptied.

· Relative weights were calculated for each item, or for each axis separately.

The following table shows the results of the research group in the questionnaire axis:

Table (2). The requirements of the e-educational supervision in the General Directorate of Education in Dhi Qar Governorate in the questionnaire axes in terms of degree of agreement

A	Item	Relative weight	Arrangement
1	Human requirements	0,78	1
2	Material requirements	0,72	2
The questionnaire as a whole		0,75	

It is clear from the previous table that the members of the sample as a whole agree that there are many requirements that must be available to implement the e-educational supervision of the General Directorate of Education in Dhi Qar Governorate, where the average relative weight of the study sample reached to (0.75) as it is located in the middle limit of the limits of confidence, as this indicates awareness of the individuals of the sample who have the importance of these requirements, that this result is consistent with a study by (Hamid Ayed Al-Moazawi, 2007), which indicated the activation of the electronic supervisory methods via websites in each of the education and study departments, also the study by (Muhammad Abdullah Muhammad Al-Ghamdi, 2007) which emphasized on the requirements of employing the Internet in educational supervision.

After presenting the opinions of the research sample in the axes of the questionnaire, the detailed results of the items for each axis are presented separately as follows:

1- The Human Requirements axis:

The human element is considered the basis of the supervisory process to be supported by the requirements for the implementation of the e-educational supervision, as this requires several things that are clear from the results of the items of this axis as shown in the following table.

Table (3) the extent of awareness of the study sample members on human requirement

A.	The Item	The Degree of agreement N = 50	
		Relative weight	Arrangement
1	Realizes the concept of electronic supervision.	0,72	19
2	Realizes the importance of electronic supervision in developing supervisory work.	0,80	10
3	It reconciles supervisory information technology applications with the humanitarian aspects of supervisory work.	0,83	7
4	Able to avoid IT defects in supervising work.	0,80	10
5	Familiarity with the systems and regulations that govern electronic transactions.	0,81	9
6	It does internal communication networks in implementing electronic supervision in school work.	0,77	13
7	Capable of using digital cameras.	0,68	21
8	Able to design supervisory business plans using supervisory information technology.	0,83	7
9	Fluent in using electronic search engines.	0,75	15
10	Capable of using a CD burner.	0,75	15
11	Capable of using Scanner	0,72	19
12	Fluent in dealing with the Internet.	0,93	1
13	Fluent in dealing with computers.	0,93	1
14	Fluent in using email.	0,86	5
15	A teacher deals with computer.	0,93	1
16	A webpage editor for the school's online supervision website.	0,80	10
17	A computer-based data editor based on data entry and output in the administration.	0,86	5
18	A parent dealing with the computer and the Internet.	0,46	23
19	Qualified trainers at the General Directorate of Education to train supervisors and teachers in the use of supervisory information technology (Word- PowerPoint- Excel…)	0,74	18
20	Specialists in the Ministry of Information Technology to communicate with the administration via the Internet.	0,75	15

A.	The Item	The Degree of agreement N = 50	
		Relative weight	Arrangement
21	Network technician at the General Directorate of Education to deal with network malfunctions.	0,65	22
22	Computer and printers technician at the Directorate General of Education to deal with problems and crashes of computers and printers.	0,77	13
23	Ministry programmers design and develop electronic programs for supervisory work.	0,88	4
The axis as a whole		0,78	

W: means relative weight: means arrangement.

It is clear from the previous table that the members of the sample as a whole agree in an average degree in all terms of this axis, where the relative weight of the axis as a whole reached to (0.78), which it falls in the average limit of confidence, this confirms the agreement of the sample members on the existence of many human requirements which are related to the implementation of the e-educational supervision, as this is confirmed by the study by (Malon, James, 2002) that there must be many human requirements to establish a relationship between teachers and the educational supervisors through the electronic supervision, through the use of assistive technology to methodology of this type of supervision, such as simultaneous email, asynchronous, as well as by communication supervision (Chat).

2- The Axis of material requirements:

Financial resources consider one of the main tributaries in developing the supervisory process, although they include many obstacles that hinder the implementation of e-educational supervision, as this is clear from the results of the items of this axis as shown in the following table.

Table (3) Extent of awareness of the study sample members of the requirements of the material resources

A	Item	Degree of agreement N = 50	
		Relative weight	Arrangement
24	The presence of a website for educational supervision in the General Directorate on the Internet.	0.75	6
25	Providing outlets for networking in the school building.	0.75	6
26	Providing modern computers for supervisory board members and teachers.	0.78	4
27	Providing sufficient number of printers for supervisory staff and teachers.	0.86	2
28	Ensuring sufficient number of cameras for the supervisory staff and teachers.	0,59	13
29	Ensuring sufficient number of supervisory staff and supervisors scanners.	0,55	15
30	Providing the computer programs necessary for supervisory business applications in the directorate.	0,70	9
31	Connecting computers to the supervisory board members with a server (server).	0,72	8

A	Item	Degree of agreement N = 50	
		Relative weight	**Arrangement**
32	Providing an advanced protection systems to protect the Directorate's data in the supervisory process.	0,86	2
33	Achieving the electronic link between the administration and its Education Directorate.	0,88	1
34	Achieving the electronic link between the administration and the parents.	0,59	13
35	Achieving the electronic link between the administration and students.	0,70	9
36	Achieving electronic linkage between the administration and other departments.	0,65	12
37	Achieving the electronic link between the administration and the Ministry of Education.	0,77	5
38	Subscribe to a high speed DSL phone line.	0,67	11
The axis as a whole		0,72	

W: means relative weight: means Arrangement

It is clear from the previous table that the members of the sample as a whole agree in the average degree in all terms of this axis, as the relative weight of the axis as a whole is (0.72), which it falls in the average confidence limit, this confirms the agreement of the sample members to the existence of many material requirements which are related to the implementation of the educational supervision. This is confirmed by the study by (**Saliha Muhammad Safar, 2008**) that there are many requirements related to the physical capabilities which represented in the electronic infrastructure of the educational supervision departments and the departments in general.

It is also clear from the table that the relative weight of twelve items in this axis lies in the average confidence limit, while the response of the respondents to the items (33), (27) and (32) came with a high degree.

As the item (33) came first with a high degree with a relative weight about (0,88), which confirms the necessity of achieving the electronic link between the administration and its education directorate as an important material for requiring the implementation of the electronic educational supervision.

The items (27) and (32) came in the second level in a high degree with a relative weight about (0,86), to confirm the necessity of securing a sufficient number of printers for the supervisory staff and teachers to be aware of the results of their evaluation by the educational supervisors, besides providing advanced protection systems to protect the administration's data in the supervisory process so that the administration has a sense of security and safety in exchanging information.

The two items (28) and (34) came in thirteenth level with an average degree with a relative weight about (0.59), to confirm the necessity of securing the sufficient number of cameras for the supervisory staff and teachers, in addition to achieve an electronic link between the administration and other departments.

The item (29) came in fifteenth level with a medium degree with a relative weight about (0.55), to confirm the provision from a sufficient number of scanners for the the supervisory staff and teachers.

The Results of the questionnaire on the obstacles of the electronic educational supervision:

To identify the obstacles of the electronic educational supervision in the General Directorate of Dhi Qar Education from the point of view of the research group, the researcher has followed the following steps:

1. The questionnaire was applied on the research group, as the responses of individuals to the items were

emptied.

2. The Relative weights were calculated either for each item, or for each axis separately.

The following table shows the results of the research group in the questionnaire axis:

Table (5) Obstacles to the e-educational supervision at the General Directorate of Education of Dhi Qar in the questionnaire axis in terms of degree of agreement

A	Item	Degree of agreement N = 50	
		Relative weight	Arrangement
1	Human resource obstacles	0,80	3
2	Obstacles related management	0,83	1
3	Obstacles related to material resources	0,83	1
The questionnaire as a whole		0,82	

W: means relative weight: means Arrangement

Conclusions

A suggested concept was developed for the implementation of the electronic supervision in the General Directorate of Education in Dhi Qar Governorate, which consists of: The suggested visualization philosophy. The basis which the visualization philosophy depended on. The goals of the suggested visualization. The suggested visualization procedures. Developing the human resources in the educational environment. Overcoming of the obstacles for applying the e-educational supervision. The guarantees that must be met for the success of the suggested visualization.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Dhi Qar Education Directorate and all experiments were carried out in accordance with approved guidelines.

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The Impact of Over-Learning Exercises in Developing Some of the Optical Vision Capabilities and Shooting Skill from Jumping in Handball Game for Young Players

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Abstract

Through the following-up and noting by researchers of most of the educational units in the handball game, they noticed that most of the educational units are almost walking at the same pace or following familiar methods when learning to shoot, as this is clearly reflected by the fluctuating the performance of the players during the match, so cases of unsuccessful shooting are sometimes seen, which is a major waste in the team's effort, that the aim of this study is to identify the effect of the exercises over-learning in developing some capabilities of optical vision and shooting skill from handball jumping for young players. The researchers have used the experimental approach with two equivalents (control and experimental groups) to suit the nature of the research problem. Determining the research community which represented by the handball center players in Dhi Qar, the youth category (15-17 years), whose number is (32) players, as the researchers excluded (8) players for the following reasons: (6) players representing the sample of the exploratory experience, and (2) Goalkeepers, for being this number of the individuals in the sample (24) players who constitute (75%) which were divided randomly and by lottery method into two groups by (12) players for each group, an experimental group that uses exercises over-learning and a control group depends on the style of the coach. The researchers concluded the following: The experimental group showed a clear and noticeable superiority to the control group in the optical vision capabilities and the shooting skill from jumping in handball game.

Keywords: *over-learning exercises, optical vision capabilities, handball shooting*

Introduction

In this century, the world has taken great strides in the field of science and development and harnessed all possibilities to serve humankind [1]. This development has covered various fields and areas, as the sports field is one of these areas where development has led to prestigious places and this is clearly reflected in the overall sporting games through achieving high achievements and achieving record numbers [2], as this was not by chance but rather by applying concepts and modern science, as the kinetic learning is one of the important sciences in the sports field because of its importance in teaching and acquiring the individual kinetic skills with minimal effort and mastery and fixation of sports skills, since the basis on which sporting events [3] are based is learning the basic skills for each activity and trying to reach the best achievement through improving and developing the performance, consequently, the development of the performance is the goal that the workers in the sports field seek, which calls them seriously to think about finding new ways and methods that serve the development of the kinetic duty in all sporting activities in a manner which commensurate with the age group [4], whether these activities are individual or group, since the performance of the basic skills involves a group of functional systems of the body, which requires work in them to harmonize with these systems, while some of the performance comes out integrated in all aspects, especially harmony and compatibility between the work of the nervous and muscular system, so that the end result is a high-level

skill performance, the handball is one of the important grouping games that reflects high requirements in performance, therefore, it requires from the player to have visual and physical abilities at a level that qualifies him to perform most of his skills, especially the shooting skill, so it has become necessary to pay attention to optical vision capabilities, especially (surrounding vision, optical vision, distinguishing accuracy), which is a prerequisite in achieving this compatibility to reflect the high effectiveness in performing the shooting skill, also, the learner's enjoyment of a sound and high optical vision leads to achieve a distinct performance, thus reaching to the desired goals that both the coach and the player aspire to, as the importance of the research lies in preparing a set of post-acquired learning exercises in which the abilities of the players [5] are consistent to reflect high effectiveness in the level of shooting skill performance through the integration of all its parts, mental, visual, physical and movement components also to be accessible to all of the coaches, players and those interested in the handball game to be used and applied whenever it calls the need.

Methodology

The researchers have used the experimental approach with two equivalents (control and experimental) groups to suit the nature of the research problem.

The Research community and its sample:

The research community that is represented by the players of the Handball Center in Dhi Qar, the youth players category (15-17 years) for the season (2020/2019), whose number is (32) players, has been identified, as the researchers have been excluded (8) players for the following reasons: (6) players are representing the sample of exploratory experience, and (2) goalkeepers, then the number of the individuals in the sample (24) players who constitute (75%) which were divided randomly and by lottery method into two groups by (12) players for each group, an experimental group that uses exercises over-learning and a control group which depends on the style of the coach.

Homogeneity of the sample:

The researchers have used the coefficient of difference to find homogeneity in the research variables as shown in Table (1).

Table (1) shows the homogeneity of the sample members in the search variables

No.	Statistical treatments variables	measuring unit	Arithmetic mean	standard deviation	Coefficient of variation	Sample Nu.
1	Chronological age	Year	15,75	0,675	0,457	24
2	Training age	Month	33,416	2,104	4,428	
3	Length	C.M	175,25	5,135	26,37	
4	Weight	Kg	56,958	4,956	25,563	

*** All values of difference coefficient is lesser than (30), which indicates the homogeneity of the sample members.**

The Equivalence of the two research groups: The researchers performed the equivalence by using the (t) law for independent samples and Table No. 2 shows that.

Table (2) shows the equivalence of the two research groups in the study variables

No.	Statistical Treatments Variables	measruing unit	Experimental group		Control group				significance
			S	P	S	P	(t) Value	sig	
1	Peripheral vision	Degree	3,533	1,125	3,266	1,162	0,638	0,529	Not significant
2	Moving optical resolution	Degree	1,133	0,915	1,4	0,985	0,768	0,449	Not significant
3	Visual perception	Degree	4,333	1,234	4,133	1,187	0,452	0,655	Not significant
4	Aiming to jump	Degree	7,416	1,831	7,5	1,167	1,33	0,895	Not significant

The Method of performance: The trainer gives the player a wooden stick with two colored balls at the two ends to hold it from behind the back without looking at it. The player spins the stick from behind with the fixing of the head.

The player spreads the hand which is holding the stick aside and the player must determine the color of the ball on the top or bottom.

Test conditions: The test is performed once with the right hand and once with the left hand.

Calculating scores:

-Each recipient is given five right and five left attempts.

- One score is calculated only if the correct selection is made.

The Test of Moving Optical Resolution [3]

The Test purpose: Measuring the moving optical resolution.

The Test tools: A plastic collar with three colored balls which placed on the collar in equal distances.

The Performance method: the player picks up the colored ball, which is determined by the coach.

The Test conditions: The test is performed once with the right hand and once with the left hand.

Calculation of the scores: - Each recipient is given three right and three left attempts.

- One score is calculated if the correct ball is taken.

Test of scoring from jumping

The Test name: Accuracy test for the shooting skill by high jumping with a hand ball from a distance of 12 m. (1: 468)

The Tools of the test:

1- (6)Legal Hand Balls.

2-The height bar (2 m) and the width (3 m) are set between the player's height zone for shooting and the target is the barrier (7 m) from the goal.

3- A legal handball goal.

4- Two iron boxes (2) designed for the purpose of testing the accuracy of shooting by high jumping with the hand ball, which is fixed in the upper corners of the goal, its dimensions (50 x 50 cm).

The Method of performance: The recipient stands holding the ball at a distance of (4-5 m) from the shooting circle with a diameter of (75 cm) and its center is a point on the free throw line opposite the middle of the goal, (Trying again if the handling is not correct), taking steps and then rise up high from the center of the shooting circle on the free throw line to shoot over the barrier on the square he chooses.

The Test rules: - Not to take more than three steps after receiving the ball.

- The player is given (6) attempts by three attempts in each box that records the test results.

- (Three points) are calculated upon entering the square ball for direct shot.

- Two points are calculated when the ball enters the allocated square after hitting the edge of the square.

- One point is counted to the ball which outside the square in the handball goal.

- A zero score is calculated for the ball outside the legal goal of handball.

The Exploratory experience:

The researchers conducted their exploratory experiment on a sample of (6 players) from the research community and without the main sample for the two days on (Monday and Tuesday) corresponding to (23-24 / 12/2019), as on the first day, the optical vision abilities tests were conducted, on the second day, the shooting skill from jumping was tested, so the goal of the following exploratory experience was:

1- Identifying the obstacles and difficulties that researchers may face during the main experiment.

2- Ensuring that the sample members are understanding the test item.

3- Knowing the time which allotted for the tests.

The Procedures for the main experiment:

Measuring the amount of learning:

1- Initial Examination Test: The researchers conducted a preliminary examination of the amount of learning in some of the optical vision capabilities and the shooting skill of jumping for the control and experimental groups in the two days (Monday and Tuesday) which corresponding in 30-31 / 12/2019 to know the amount of learning for the two groups before starting the educational curriculum, after that, the researchers, in cooperation with the instructor of the Specialty Center handball, gave the same educational curriculum which prepared by the trainer for the shooting skill for the two groups for a period of (4) weeks at the rate of three educational units per week, where the number of the educational units reached to (12) units, for a period from 1/5 / 2020 to 30/1/2020.

2- The second examination test: After completing the four weeks in the educational curriculum of the two groups, the researchers conducted a second examination of the amount of learning to know the level of learning that reached the learners in the control and experimental groups. As it was found that the amount of learning for the two groups is approximately equal.

The Pre-tests:

After completing all of the requirements of the main experiment and after the sample applied the educational units which set by the trainer to know the amount of learning which is about (12) educational units, as they reached a certain amount of learning, the researchers conducted the pre-test tests on the research sample in the hall of the Handball Center for two days (Monday and Tuesday) which corresponding in 3-4/2/2020. On the first day, the optical vision abilities tests were conducted, on the second day, the scoring skill by jumping test was conducted with the help of the assistant team.

The educational curriculum:

The educational curriculum was applied for the control and experimental groups by the trainer of the Handball Center in Dhi Qar, which is (4) weeks for the period from 9/2/2020 to 5/3/2020, by (3) educational units per week, as the number of the educational units is

(12) Unit, as the work of the two groups was as follows:

· **The control group**: The control group is applied the educational curriculum which followed by the trainer for teaching shooting skill from jumping, as the control group units were running in the days (Saturday, Monday, Wednesday).

· **The Experimental group**: The experimental group is applied for the educational curriculum by using exercises over-learning in the main section of the curriculum, as the researchers had prepared these exercises for teaching the shooting by jumping skill, that the educational units of the experimental group were taking place in the days (Sunday, Tuesday, Thursday). Then the educational unit was divided into three main sections which are (preparatory - main - final), as the time of the educational unit reached to (90) minutes, (20 minutes) for the preparatory section, (60 minutes) for the main section and (10 minutes) for the final section.

The Post-tests:

After completing the educational curriculum, the researchers conducted the two-post-tests on (Saturday and Sunday) which corresponding in (7-8 / 3/2020). On the first day, optical vision capabilities tests were conducted, on the second day, the shooting skill test was conducted with the same conditions with the pre-test, with the help of the assistant team.

The Statistical means: The researchers used the Statistical Package (SPSS).

1- The Percentage. 4- Coefficient of variation.

2- The Arithmetic mean. 5- (T) Law for the counterpart samples.

3- The Standard deviation. 6- (T) Law for independent samples

7- The amount of learning 1 =

3- Presenting, analyzing and discussing of the results.

Table (3) shows the arithmetic mean, standard deviations, (t) value and the level of significance in the pre and post tests for the research variables of the experimental group.

No.	Statistical variables Variables	measruing unit	Pre-test		Post-test		(T) Value	Sig	Significance
			S	P	S	P			
1	Peripheral vision	Degree	3,533	1,125	6,266	1,579	7,364	0,000	Significant
2	Moving optical resolution	Degree	1,133	0,915	3,866	0,639	17,833	0,000	Significant
3	Visual perception	Degree	4,333	1,234	7,866	1,505	7,411	0,000	Significant
4	Jumping to shoot	Degree	7،416	1،831	10،583	1،831	9،192	0,000	Significant

Table (4) shows the arithmetic mean, standard deviations, (t) value and the level of significance in the pre and post tests for the control group variables.

No.	Statistical variables Variables	measruing unit	Pre-test		Post-test		(T) Value	Sig	Significance
			S	P	S	P			
1	Peripheral vision	Degree	3,266	1,162	3,6	0,985	1,581	0,136	Not Significant
2	Moving optical resolution	Degree	1,4	0,985	2,333	0,816	5,137	0,000	Not Significant
3	Visual perception	Degree	4,133	1,187	4,533	1,06	1,871	0,082	Not Significant
4	Jumping to shoot	Degree	7،5	1،167	8	1،206	3،317	0،007	Not Significant

Through the results which are presented in tables (4,3) of the results of the pre and post-tests of the experimental and controlling groups in the optical vision capabilities and the skill of shooting by jumping in handball game, it was found that there are statistically significant variables between the pre and post-tests which are tending to the post tests according to the first hypothesis of the research, the researchers attribute this difference to the integrity of the educational curriculum for both groups (experimental and control). Specifically, the main section which contains scientifically selected exercises with correct, and consistent repetitions with the level and ability of the sample members, which based on the correct practicing, that training and practice on a specific skill within the kinetic duty leads to increase the experience and a development in the muscular and physical susceptibility, so practicing is the most important variable in the learning process for complex and even simple skills. (56: 6).

In addition to the above, the two approaches included selected exercises consistent with the age and capabilities of the players as the experimental group applied the curriculum which is prepared by researchers according to exercises over-learning, while the control group applied the curriculum which is prepared by the trainer, that both groups have achieved a remarkable development in the capabilities of the optical vision, level of performance and skill, however, the control group has achieved an acceptable educational level with rates of development that are not at the level of the development of the experimental group, because learning skills was going on a single format and specific monotony, on the contrary of the experimental group, as the exercises that researchers set which is aiming after learning were so focused and accurate that they contributed directly for reducing time and effort, with the addition of a diversification in the required kinetic performance, as all available capabilities that can be used contribute greatly to the development of the kinetic skills performance, mastery and fixation it. (148: 5)

Table (5) shows the learning amount for the experimental and control groups for the pre- and post-tests of the shooting skill for both groups.

Groups	Tests	measuring unit	Pre-test		Post-test		The amount of learning
			S	P	S	P	
Experimental	Shooting by jumping	Degree	7‹416	1‹831	10‹583	1‹831	29‹92
Control	Shooting by jumping	Degree	7‹5	1‹167	8	1‹206	4‹76

Through the table (5) it was found that the amount of learning among the members of the experimental group was greater than the amount of learning among members of the control group, that is, the meaning of learning preference was for members of the experimental group whose educational units were conducting according to exercises of over-learning, that the researchers attribute this superiority in the amount of learning to exercises of over-learning, their repetition and the positive response of players to them, which led for raising the capabilities of the players skillfully and improving the level of performance accuracy in a clear and concrete way, as the exercises of over-learning affected mostly in developing the shooting by jumping from a very large extent, this indicates the importance of these exercises and their effect on increasing the accuracy factor, which is the decisive factor for the shooting skill, as the higher the accuracy of shooting, the more it gives the team an opportunity to achieve a goal in the opposing team's goal, that the accuracy factor is based on the extent to which the shooting player has a high level of mental and kinetic abilities**,** as this is what was achieved, that the exercises which were prepared by the researchers that contributed effectively for improving the accuracy of the shooting skill by jumping, in addition to the above, the exercises over-learning have directly contributed to the development of optical vision capabilities.

Table (6) shows the values of the arithmetic mean, standard deviations, the (t) value and the significance level for the post-tests in the research variables between the control and experimental groups.

No.	**Statistical variables Variables**	**measruing unit**	**Experimental group**		**Control group**		**Value (t)**	**Sig**	**Significance**
			S	**P**	**S**	**P**			
1	Peripheral vision	Degree	6,266	1,579	3,6	0,985	5,547	0,000	Significant
2	Moving optical resolution	Degree	3,866	0,639	2,333	0,816	5,725	0,000	Significant
3	Visual perception	Degree	7,866	1,505	4,533	1,06	7,011	0,000	Significant
4	Jumping to shoot	Degree		1,831	8	1,206	4,08	0,000	Significant

Discussing the results of the post-test for the control and experimental groups of the research variables:

Through the results of Table (6) it was found that there is a clear superiority for the members of the experimental

group at the expense of the members of the control group in the research variables, as the researchers attribute this to the exercises that the researchers prepared which contributed directly to stimulate the players' senses and motivating them to respond to these stimuli as well as improving the positive relationship and the amount of neuromuscular compatibility, which is the main pillar upon which all the kinetic skills of the handball game are built, as the use of the exercises which are prepared by researchers has had a positive role in developing the capabilities of optical vision, as these exercises have the ability to distribute the visual effects that help to see visual development by improving the relationship between the eyes and the brain, as this increases the compatibility and flexibility of the eye muscles in the ability to control these muscles. [3] The educational method by using exercises over-learning aims to develop the skillful level through continuous (additional) repetitions with an expectation of the development which is resulting from the ability to correct response, which leads to the provision of multiple responses (ideal memory) in addition to skill stability, although the differences in quality are more than the quantity for this additional purpose of the extra exercise, much training on skill is necessary and essential thing to increase the suitability of the skill to many changes in the environment such as (competitor - hall - external atmosphere - devices) even the individual's physiological condition because it plays a role in many situations to generalize the skill and influence on the achievement results automatically despite the many factors which are surrounding it [7]

Conclusions

The experimental group showed a clear and noticeable superiority over the control group in the optical vision and shooting by jumping skills of handball game. Organizing the work through the use of exercises over-learning developed by the researchers which contributed to the improvement of the amount of learning with the skill of shooting by jumping in the handball game for the members of the experimental group.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Physical Education and Sports Science and all experiments were carried out in accordance with approved guidelines.

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Effect of COVID-19 Pandemic on Psychological Attitudes' for Nurse Workers at Isolation Hospitals in Baghdad City

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Abstract

Objectives: to identify the effect of COVID 19 pandemic psychological attitudes for nurses' workers at Isolation Hospitals in Baghdad

Methodology: descriptive study conducted among 100 nurses selected by purposive sample. The data collected by using electronic questionnaire related to psychological attitudes composed of 10 items and 3 items related to demographic characteristics.

Results: the result reflects that most of nurses are males and at age (20-29) years, also they are graduated from high school of nursing and nursing college. Most of nurses' psychological status effected by COVID, they are feel anxiety stress depression and frustration due to un familiar area, lack of experience, un effected treatment, COVID 19 bad news, long working time and isolation from friends. There is significant correlation between nurses' psychological attitude and level of education.

Conclusion: COVID 19 effect on the nurses' psychological status. Therefore, it is important to initiate training session to educate nurses about stress management, how to deal with psychological problems and adapt with different situation.

Key words: *Psychological attitude, Nurses, & Covid 19 Pandemic*

Introduction

The pandemic of Coronavirus Disease 2019 (COVID-19) is currently a major global public health emergency. As the coronavirus disease 2019 (COVID-19) pandemic accelerates, global health care systems have become overwhelmed, leading to great psychological pressure on nurses in the care of critically ill patients with COVID-19. Moreover, extreme incidents have occurred, such as suicide of nurses caring for critically ill patients, in Italy. In fact, the psychological problems are also common among nurses. (1)

However, in addition to the potential psychological and mental health impacted by the COVID-19 pandemic should also be taken seriously. Although previous research has suggested that the mental impact of a major disaster had a wider and longer effect on people compared to physical injuries, mental health attracts far fewer personnel for planning and resources. (2)

Healthcare workers who were quarantined, worked in Covid 19 units, or had family or friends infected with Covid 19, had considerably more anxiety, depression, frustration, fear, and post-traumatic stress than those who had no such experience. (3)

Since the first COVID-19 case was reported in December 2019 in Wuhan approximately 42,000 healthcare workers (HCWs), including 28,600 nurses all over China, were sent to Hubei Province to assist local healthcare teams to care for COVID-19 patients. A study revealed that HCWs who were working in Wuhan often felt stress, depression, and anxiety, but this study didn't target specially at frontline nurses. HCWs, especially nurses, who come close in contact with these patients when providing care are often left stricken with inadequate protections from contamination, high risks of infection, working burnout, fear, anxiety, and depression. Nurses constitute the largest part of the healthcare workforce in an epidemic, and they undertake most of

the tasks related to infectious disease containment. [4,5,6]

The medical health-care workers who are caring for individuals who are either severely ill, feel scared, or experiencing bereavement are themselves exposed to trauma. Health-care workers are also at risk of getting infected, and they carry a large burden in the clinical treatment and public prevention efforts in Chinese hospitals and community settings. The challenges and stress they experience could trigger common mental disorders, including anxiety and depressive disorders, and posttraumatic stress disorder,5which in turn could result in hazards that exceed the consequences of the 2019-nCoV epidemic itself. [7]

Methodology

Descriptive study design carried out in isolation hospitals in Baghdad city to identify the effect of COVID 19 on psychological attitudes for nurses. The study conducted among (100) nurses selected by using non probability sampling (purposive sample).

The data collected by using electronic questionnaire conducted to measure the phenomena according the research objective and started from Jun, 6th 2020 to Jun, 29st 2020. The researcher used the social media to collect the data from the sample.

The questionnaire consisted of two parts, the 1st part related to the demographic characteristics which include the (age, gender and level of education). The second part related to the psychological attitudes of nurses and composed of 10 items. The data analyzed by using descriptive and inferential data analysis.

Results

Table 1 distribution of the nurses by their demographic characteristics

Variable		Frequency	Percent
Age	20-29	52	52
	30-39	38	38
	40-49	10	10
	Total	100	100
Gender	Female	38	38
	Male	62	62
	Total	100	100
Level of education	Graduate Nursing High School	35	35
	Graduate Nursing Institute	30	30
	Graduate Nursing College	35	35
	Total	100	100

This table indicates that regarding to nurses age (52%) of them at age (20-29) years, regarding to nurses gender (62%) of them were males and according to educational level (35%) of them graduated nursing high school and nursing college.

Table 2 distribution of the psychological attitudes of nurses

No.	Items	Always		Sometimes		Never	
		f	%	f	%	f	%
1	I believe the working at an unfamiliar environment increases my anxiety and affects the psychological health	77	77	23	23	-	-
2	I think that a lack of experience in the field of infectious diseases leads to increased anxiety and affects the psychological health	71	71	29	29	-	-
3	I feel anxiety about the increased risk of infection	100	100	-	-	-	-
4	I believe the huge burdens and long-term exhaustion increase the psychological stressors for me	76	76	24	24	-	-
5	I feel the ineffective treatment of the covid19 pandemic increases the incidence of depression for me	73	73	27	27	-	-
6	I feel a depression when I will be a carrier of COVID19 to my family and friends	100	100	-	-	-	-
7	I believe increasing working hours and getting away from friends affect the psychological condition for me	73	73	27	27	-	-
8	I think hearing bad news about the results of treatment for patients with COVID19 increases the frustration	70	70	30	30	-	-
9	I feel a disappointing due to the inability to provide effective assistance and the death of patients with COVID19	90	90	5	5	5	5
10	I feel frustrating by hearing international news about the COVID19 pandemic cases and the numbers of death	63	63	27	27	10	10

f=frequency, %=percent

This table indicates that regarding to the effect of COVID 19 on psychological attitudes of nurses. (77%) of nurses believe that the working at an unfamiliar environment increases my anxiety and affects the psychological health, (71%) think that a lack of experience in the field of infectious diseases leads to increased anxiety and affects the psychological health, all of nurses feel anxiety about the increased risk of infection, (76%) believe that the huge burdens and long-term exhaustion increase the psychological stressors, (73%) feel that the ineffective treatment of the covid19 pandemic increases the incidence of depression, all of nurses feel a depression when they will be a carrier of COVID19 to my family and friends, (73%) believe that increasing working hours and getting away from friends affect the psychological condition, (70%) think that hearing bad news about the results of treatment for patients with COVID19 increases the frustration, (90%) feel a disappointing due to the inability to provide effective assistance and the death of patients with COVID19, and (63%) feel frustrating by hearing international news about the COVID19 pandemic cases and the numbers of death.

Table 3 correlation between the psychological suffering of nurses and their demographic characteristics

Variables		Age	Gender	Level of education	Psychological attitudes
Age	Pearson Correlation	1	-.178	.111	-.115
	Sig. (2-tailed)		.076	.273	.255
	N	100	100	100	100
Gender	Pearson Correlation	-.178	1	.098	.090
	Sig. (2-tailed)	.076		.330	.373
	N	100	100	100	100
Level of education	Pearson Correlation	.111	.098	1	-.201*
	Sig. (2-tailed)	.273	.330		.045
	N	100	100	100	100
Psychological attitudes	Pearson Correlation	-.115	.090	-.201*	1
	Sig. (2-tailed)	.255	.373	.045	
	N	100	100	100	100

*. Correlation is significant at the 0.05 level (2-tailed).

There is significant statistical correlation between the nurses' level of education and their psychological attitudes at P-value ≤0.05. while there is no significant statistical correlation between the nurses' age, gender and their psychological attitudes

Conclusion

The COVID 19 effect on nurses' psychological attitudes, unfamiliar environment in the isolation units and lack of experience to deal with this cases may increase the level of anxiety among nurses, hey are fell anxiety and depression due to increase the risk of infection with COVID 19 or be a carrier, they are fell stress and depression due to huge burdens and long-term exhaustion and un effected treatment, they are frustration due to hearing Covid 19 bad news, and they are belief their job effect on their relationship with friends. There is correlation between nurses' educational level and psychological attitudes.

Recommendation:

Educate nurses about stress management. Teaching them about how to deal with psychological problems and adapt with different situation. It is important to decrease the working hours of nurses. Promote hope and encourage positive feelings when dealing with patients in the face of the COVID 19 pandemic. Avoid the bad news of the COVID 19 pandemic and work to provide the best health care for patients.

Financial Disclosure: There is no financial disclosure.

Conflict of interest

None to declare.

Ethical Clearance: All experimental protocols were approved under the ALZahrawi College University and all experiments were carried out in accordance with approved guidelines.

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The effect of use of Dexmeditomidine Hydrochloride as an Adjuvant in General Anesthesia on Rate of Awareness

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Abstract

Background: Dexmedetomidine can be used as a safe and helpful adjunct in many clinical situations.

Objective: To evaluate the role of Dexmedetomidine as an adjuvant in general anesthesia in relation to rate of awareness.

Patients and Methods: This was a prospective quasi experimental study conducted at Al-Sadder medical city during May to November 2016, included 400 patients, assigned randomly into two groups to receive the traditional anesthesia regimen (group I) or traditional anesthesia regimen and Dexmedetomidine (group II). Awareness was assessed via postoperative interview. Appropriate statistical tests were applied accordingly.

Results: The rates of awareness and possible awareness in group II were (0.5%) and (1.0%), respectively, and were significantly lower than that of group I (3.5%) and 4.5%), respectively, (P<0.05).

Conclusion: Dexmedetomidine can improve the outcome of general anesthesia by significant reduction of rate of awareness during operation.

Key words: *Dexmedetomidine, general anesthesia, adjuvant, rate of awareness*

Introduction

The invention of anaesthesia has modified modern medicine greatly. The first case of anaesthesia was introduced by Morton in 1846 and, in addition, it has been described as the first reported case of awareness during anaesthesia. The patient subjected to anesthesia by Mortons experienced no pain during operation but had some memories about events during surgery [(1)]. It has been stated that fear from pain, paralysis and mental distress during operation is reported in 54% of cases [(2)]. Awareness, although rare, can be added to the list of unsatisfactory events worried about by patients like pain, nausea and vomiting [(3)]. The term "awareness" is defined as the explicit memory of events during general anaesthesia. With time, the incidence of awareness during anesthesia witnessed gradual reduction since the observation of this phenomenon during operation in 1960 [(4,5)], Various reports around the world gave a range of incidence between 0.3 to 0.8 % in different types of surgery [(4, 6-8)]. Depth of anesthesia is usually assessed by monitoring heart rate, blood pressure and other clinical signs, which have been shown to be unreliable [(9,10)]. These signs, particularly, tachycardia and hypertension failed to predict awareness during anesthesia according to Ghoneim and colleagues who reviewed 271 reported cases of awareness from 1950 through 2005 [(11)]. Detection of awareness is not easy. In the review article by Ghoneim, he states that the "Brice questionnaire" invented by Brice, and colleagues, in 1970 and updated by Lin and colleagues, 1991, has shown to be a reliable an efficient way to detect intraoperative memory. The interview comprised four questions:

1. What was, the finally patient remember before he went to sleep during anesthesia?

2. What did firstly the patient remember when he woke up from anesthesia?

3. Could the patient , memorised anything in between these two periods?

4. Did the patient, patience during surgery?

Timing of the interview is essential. Directly after operation in the "post anaesthesia care unit" (PACU) it is difficult to gain the patients attention while he or she is very focused on more obvious symptoms like pain, postoperative nausea and vomiting (PONV) or simply fatigue. Recent studies found that only 50% of the awareness cases are detected in the PACU. Sometimes recall is delayed by several days, maybe even weeks [2, 3].

Frequent observations from patients who have an episode of awareness throughout surgery were in the form of hearing events and conversations, feeling of paralysis, helplessness, weakness, anxiety and impending death. Some patients are uncertain whether what they felt was real or whether it is merely to some wrong thoughts. The after-effects of intraoperative memory can be transient and include sleep disorders, nightmares and daytime fear. In the extreme situation awareness under anaesthesia resulted in posttraumatic stress syndrome (PTSD) with recuurent nightmares, irritability and a feeling of imminent death[4]. A prolong follow up by Lennmarken and colleagues demonstrated that four of nine patients who had awareness acquired severe symptoms of PTSD, another three of them had less symptoms, nevertheless still suffered from their bad experience [12].

Dexmedetomidine, is a powerful and strongly selective (α-2) adrenoceptor stimulant with sedative, sympatholytic, analgesic and amnestic activities [13] which has, been described as a safe adjunct in a number of clinical situations. It reduced central nervous system (CNS) sympathetic outpouring in a dosedependent pattern and has anlgesic effects best described as opioidsparing[14].

Patients, materials and methods:

This was a prospective quasi experimental study conducted at Al-Sadder medical city/ emergency unite in Al-Najaf province during the period May to November 2016. The present study included 400 patients who were randomly assigned into two groups, to receive either the conventional mode of protocol (pentothal, atracurium and halothane), namely, (group 1) or (pentothal, atracurium and halothane) and (Dexmedetomidine hydrochloride), (group 2). Prior to induction of anesthesia and enrollement of the patients in the study, informed verbal and signed consents were obtained from all participants, additionally, all official agreements were obtained from the training and research department of AlSader Medical city. The degree of awareness was assessed according to structured Brice post-operative interview and patients were classified into three groups: A) definitely aware, B) Possibly aware and C) Not aware. The next questions were included:

1. What is the patient finally memorize before sleep due to induction of anesthesia?

2. What is,the patient immediately memorize after recovery from anesthesia?

3. Did the patient memorize what happened in the periods between sleep and recovery from anesthesia ? (possible answers:: yes, no); If yes, what is it? (light,, sound, pain, dyspnoea, etc.).

4. What the patient dream during anesthesia?

5. Ask the patient about the worst memory during anesthesia and surgery ?

The later questions important in statistical analysis: age,, gender, American Society of Anesthesiologists (ASA) score, type of anaesthesia and premedication,, muscle relaxants used, time between anaesthesia and administration of the questionnaire, data regarding possible earlier awareness, and substances or drugs used. The patients notes were divided according to the common classification [15] as definite awareness(1),possible awareness(2),and no awareness(3).

Data were entered and analyzed using the statistical package for social sciences (SPSS) version 22 for windows and Microsoft Office Excel 2010, appropriate statistical tests were applied according to the type of variables; Chi square test used to compare frequencies and students t test for independent two sample used to compare means between both groups, Pearson's correlation test used to assess the correlation between age and awareness score, while Spearman's correlation test used to assess the correlation between awareness score and other variables other than age. Level of significance , P.value ≤ 0.05 considered significant difference.

Results

Demographic characteristics of the study sample

The mean, age of patients in group(1) was 29.78 ±12.21 years and in group (2) it was 32.00 ±15.33 years; with no statistically significant difference between both groups (P>0.05), (Figure 1).

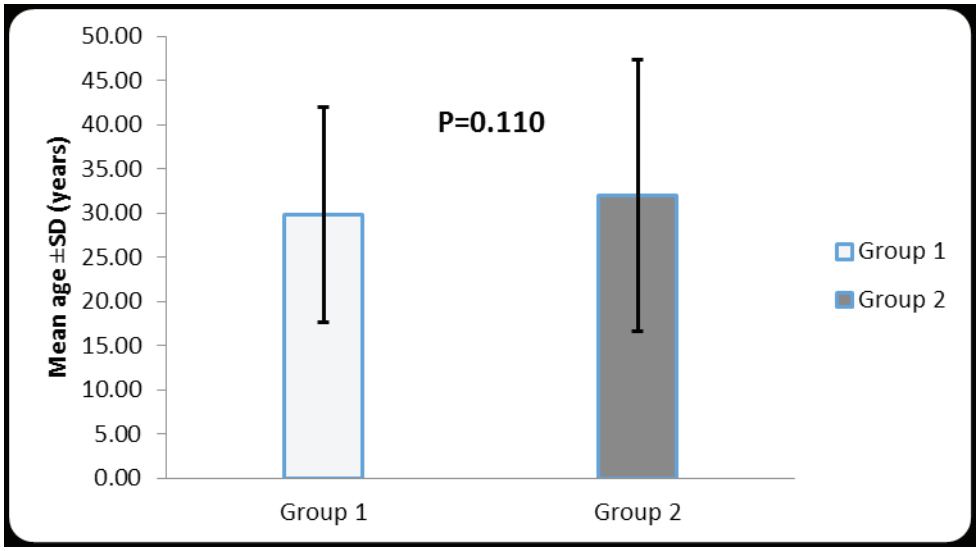


Figure 1: Bar chart showing mean age in both groups

The total sample included 228 (56.5%) female patients and 172 (43.5%) male patients and no statistically significant difference had been found in gender between both groups (P>0.05), (Table 1)

Table 1: Distribution of patients according to gender

Gender	Group 1 N (%)	Group 2 N (%)
Male	116 (58.0)	112 (56.0)
Female	84 (42.0)	88 (44.0)
Total	200 (100.0)	200 (100.0)
$\chi 2 = 0.163$, P. value = 0.686		

Group 1: patients who received pentothal, atracurium and halothane; Group 2: Patients who received pentothal, atracurium and halothane and Dexmedetomidine hydrochloride)

Rate of awareness

The rate of awareness in group (1) (who received conventional protocol) was 3.5% whereas the rate of awareness in group(2) (who received alternative protocol) was (0.5%); the rate of awareness was lower in group (2) than in the group (1) however, the difference did not reach the statistical significance, (P=0.074). The rate of possible awareness in the group (1) was (4.5%) whereas in the group (2) it was (1.0 %), and the difference was statistically significant (P<0.05), furthermore, by comparing the none awareness rate I was significantly higher in group (2) than in group (1) ; (98.5%) and (92.0%), respectively, (P< 0.05), as shown in (table 2 and figure 2).

Table 2: Awareness and possibility of awareness according to type of anesthesia

Awareness	Group 1 n (%)	Group 2 n (%)	$\chi 2$	P
Aware	7 (3.5)	1 (0.5)	3.19	0.074
Possibly aware	9 (4.5)	2 (1.0)	4.58	0.0322
Not aware	184 (92.0)	197 (98.5)	7.96	0.0047
Total	200 (100.0)	200 (100.0)	-	-

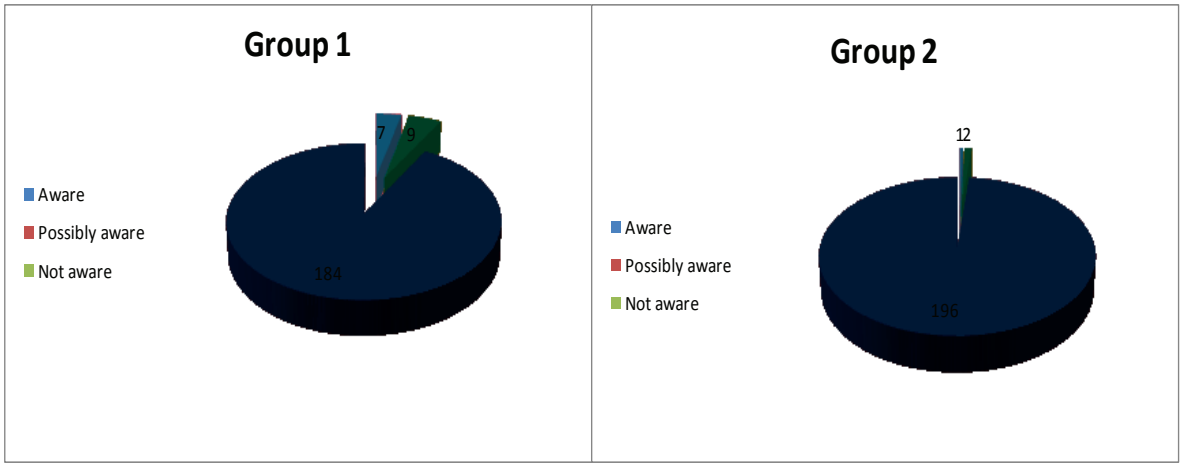


Figure 2: Awareness and possibility of awareness according to type of anesthesia

Association between possible risk factors and rate of awareness

No statistically significant association between age, gender, ASA stage, smoking, hypertension and diabetes mellitus from one side and awareness score from the other side had been found in both groups, , (P>0.05), (Table 3)

Table 3. Results of correlation tests between risk factors and awareness score in both groups.

		Correlation indicators (Factors against Awareness score)	
Group	Factor*	Correlation coefficient	P. value
Group 1	Age	0.053	0.44
	Gender	0.021	0.79
	ASA stage	0.032	0.66
	Hypertension	0.081	0.21
	Diabetes mellitus	0.011	0.83
	Smoking	0.064	0.34

Group 2	Age	0.011	0.92
	Gender	0.072	0.28
	ASA stage	0.051	0.44
	Hypertension	0.222	0.82
	Diabetes mellitus	0.034	0.66
	Smoking	0.020	0.77
* Spearman's correlation test used in all variables except age where Pearson's correlation test used ,			

Discussion

The main observation in the current study is that the use of dexmedetomidine was associated with significant reduction of the risk of awareness during anesthesia. Similar observation has been reported by other authors. Ghodki *et al*. [16] found dexmedetomidine reduced incidence of awareness during laproscopic surgery . In support of the present study, Elhakim et al. reported that the use of dexmedetomidine lead to prevention of awareness during anaesthesia, improvement of intraoperative oxygenation,decrease anesthetic drug requirement and improvement of postoperative analgesia [17]. Kasuya et al made a comparison of the sedative-effect of dexmedetomidine, and propofol utilizing (BIS) and the Observer's Assessment of Alertness and Sedation score, (OAA/S). The finding was that sedation was significantly lower with dexmedetomidine, than with propofol [18]. The probable reason is the potentiation of the anesthetic effect of anesthetic agents through an unknown mechanism and by this way it reduces the required amount of these agents like Des and fentanyl. Other, authors also reported the phenomenon of reduced doses of Des and fentanyl in patients undergoing liver transplantation, with comparable anesthetic depth [19] .It was pointed out by some authors that dexmedetomidine, offers the ability of producing both sedation and analgesia with no associted respiratory depression, hence it possess anesthetic-sparing advantage [20] . Patel *et al*. reported that dexmedetomidine, continuous infusion reduced the required dose of sevoflurane throughout general anesthesia [21]. The hemodynamic changes produced by dexmedetomidine sympatholytic effect were also proposed to explain the reduced need for anesthetic , agents [22] , and also it is suggested that sympathoadrenal reaction to tracheal intubation is attenuated following dexmedetomidine use [23] It is also documented that dexmedetomidine possesses analgesic potential and reduces visceral pain [24,25]. Similar observation regarding hemodynamic changes and reduced sympathoadrenal reaction to intubation is also reported by other studies [26] .

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kufa university and all experiments were carried out in accordance with approved guidelines.

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Isolation and Identification of Some Bacterial on the Eggs in Some Regions of Karbala Province

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Abstract

A total of 70 egg when collected from various regions in Karbala to identification to microorganism that found on shell of the egg. After transported the eggs to laboratory and doing the important diagnostic steps, the results showed the bacteria with gram positive (Staphylococcus) were predominantly found on eggshell. The eggshells were also found contaminated with bacteria Escherichia. Coli, Bacillus, Streptococcus . Also the results of this study showed that the Vancomycin have highly susceptible to the Staphylococcus isolates while this isolates also showed over 90% resistant to penicillin. The presence of these microorganisms on eggshells present a significant threat to consumers. Proper sanitation to reduce microbial contaminations of eggs that usually occur after production through processing of eggs.

Keywords: *Table egg, Contamination, Antibiotic sensitivity, Karbala.*

Introduction

Table eggs are the safest and simplest food source as they contain proteinswith high-quality, essential amino acids ,mineralsandvitamins that are important for good health [1]. By going through the ventilation window or ventilation system, the surface of eggs (shell) may be contaminated, Moreover, most scientists say that contamination exists mainly after it has been introduced due to contact with polluted surfaces such as chicken stools, nesting products, soil, forage, shipping containers, packaging, handling and animals (rodents, insects and pets) [2]. Staphylococcus aureus is an important part of the microflora and can be isolated from the surface and table egg material. By entering the food channel route it may lead to degradation and injuries to consumers [3,4]. Containers are really essential elements in supplying customers with high quality table eggs. Manufacturing process is planned to shield eggs from cracking when processing by having a cushioning effect, and shielding eggs from microbes, natural pests, lack of moisture, staining and extreme heat which may lead to degradation and to eliminate damage and contamination The packing products and boxes used must then be allowed to enter oxygen as the eggs have to breathe, also the ingredients used to cover table eggs should be permeable, safe and odorless gases [5,6]. Outer shell of egg includes a large number of microorganisms like *Staphylococcus aureus, Escherichia. Coli, Streptococcus spp, Bacillus spp , Salmonella spp* and *Listeria monocytogenes* [7]. *Staphylococcus* is responsible for many diseases that appear in poultry. it form greater percentage of infected to eggshells [3,8]. The lack of effective mechanisms, a market proper drainage and extremely high moisture may have resulted in highly growth of microorganisms. It has also been found that most supermarkets do not keep eggs in refrigerators, thus exposing eggs to weather factors that contribute to their pollution. Isolated microbes can cause serious health issues, such as vomiting , nausea, diarrhea and abdominal pain [9]. *E. Coli* are Gram-negative intestinal family bacteria and are natural Inhabitants of intestinal bird tract. This is one of the opportunistic organisms responsible for a variety of diseases such as yolk sac infection, air sac infections, systemic inflammation of hepatitis, inflammation of the intestines, colonic tumor, etc. [8]**.** The present study aims to Isolation and identification some bacterial cells that contaminated the table egg shell in some regions in Karbala city. Iraq.

Material and Methods

Sample collection and biochemical test analysis

A total of 70 samples of table eggs from various regions of Karbala were collected. Egg samples were packed in a sterile and clean container and then transported to the laboratory to prepared for microbiological examination. Eggs collected with sterile cotton swabs were displaced in the nutrient broth for overnight at 37 ° C in the incubator and then cultured in the cultural media. Nutrient Agar, MacConkey Agar, Blood Agar, Methylene Blue Eosin Agar (EMB), Minatole Salt Agar and finally use the Muller-Hinton Agar for antibiotic susceptibility tests phenotype of living microorganisms have been identified according to shape, color, size and color changes in different media. Gram stain with oil-immersion optical microscopy have been used . Finally, the biochemical tests also used to confirm genus of microorganism which involve; oxidase, coagulase, catalase tests, on the other hand api 20E (Analytical Profile Index 20 enterobacteria) was used in the study of Enterobacteriaceae [10].

Antimicrobial Susceptibility Test (Disk Diffusion Test):

The method used for this test, called Kirby-Bauer, is focused on the susceptibility of antibiotics on the surface of Muller-Hinton agar. This has been done by inoculums can be used in this test were made by dissolving 5 isolated colonies to 5 ml of nutrient broth and incubated for (2 hours) at (37oC) after that compared with (0.5) the standard McFarland tube, A sterile swab has been used to generate a bacterial suspension inoculum, this inoculum was distributed on a Mueller-Hinton agar plate and allowed to dry, the discs of antibiotic were put on the medium surface at randomly spaced intervals with flaming forceps or a disc applicator and incubated at 37oC for 24 hours, a zone of inhibition have been measured with a ruler and comparing the results with inhibition zones defined by [11].

Results and discussion

The results of this study after doing culturing and identification by gram stain and some biochemical tests as mention above and API20E (Table1),(Figure1) the results showed the *Staphylococcus* is most gram positive bacteria found on egg shell about (39 isolates) 44% from isolates its *Staphylococcus* and this results agree with [3] and [5] as well as [12] and [13] was reported that Gram-positive *Staphylococcus* spp have major natural eggshell contamination of table eggs.

Also researchers found that the major contaminants of egg were Gram-negative bacteria species as *Escherichia coli* [14], [15] and [16]. all these agree with this study were found about (17 isolates) 24% from isolates is *Escherichia coli* as Gram-negative bacteria.

Also the bacillus and streptococcus in this study occupied 13% and 11% of isolates respectively . [17] also found these types of bacteria from eggs.

Only 7% of isolates were non growth because numerous factors that related with the improper handling, inadequate bacterial suspension, improper inoculation, defect in incubation and other environmental factors.

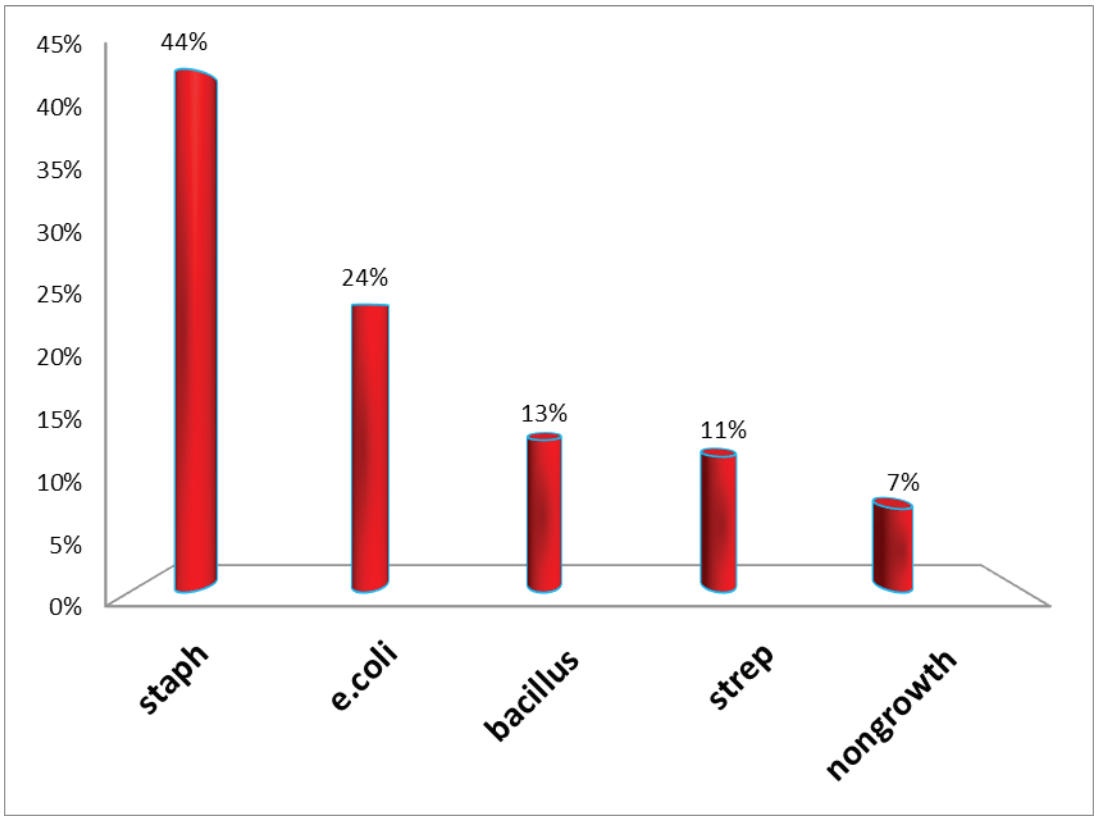


Figure (1) Prevalence of bacteria that isolated from egg shell.

Table (1) showed bacterial samples, ratio, culture media, biochemical test, staining and the shape of bacteria.

Bacteria	Gram stain	Biochemical reaction	Morphology
Staphylococcus (39 isolates) 44%	G+ve	Coagulase–ve, Catalase +ve.	Cocci,irregular clusters
Escherichia coli (17 isolates) 24%	G-ve	API-20E oxidase–ve	Rod
Bacillus (9 isolates) 13%	G+ve	Catalase +ve	Rod
Streptococcus (8 isolates) 11%	G+ve	Catalase –ve.	Cocci, long or short chains

Analytical Profile Index(API) 20 E test

When suspension of bacteria is inoculated in each of the wells and incubated. An after that read or compartment the results as numbers with standard numbers , The results of this test suggest the isolates are *E.coli* depending on the reactions or numbers that found as shown in Table (2), Figure (2)

Table (2) The different reactions of API 20E test with its results .

Identification code	Reaction
o-nitrophenyl-b-D-galactopyranoside (ONPG)	+
Arginine dihydrolase(ADH)	-
Lysine decarboxylase (LDC)	+
Ornithine decarboxylase (ODC)	+
Citrate (CIT)	-
hydrogen sulfide (H2S)	-
Urease (URE)	-
Tryptophan deaminase (TDA)	-
Indole (IND)	+
Voges-Proskauer reaction (VP)	-
Gelatinase (GEL)	-
Glucose fermentation (GLU)	+
Mannose fermentation (MAN)	+
Inositol fermentation (INO)	-
Sorbitol fermentation (SOR)	-
Rhamnose fermentation (RHA)	+
Sucrose fermentation (SAC)	+
Melibiose fermentation (MEL)	+
Amygdalin fermentation (AMY)	-
Arabinose fermentation (ARA)	+

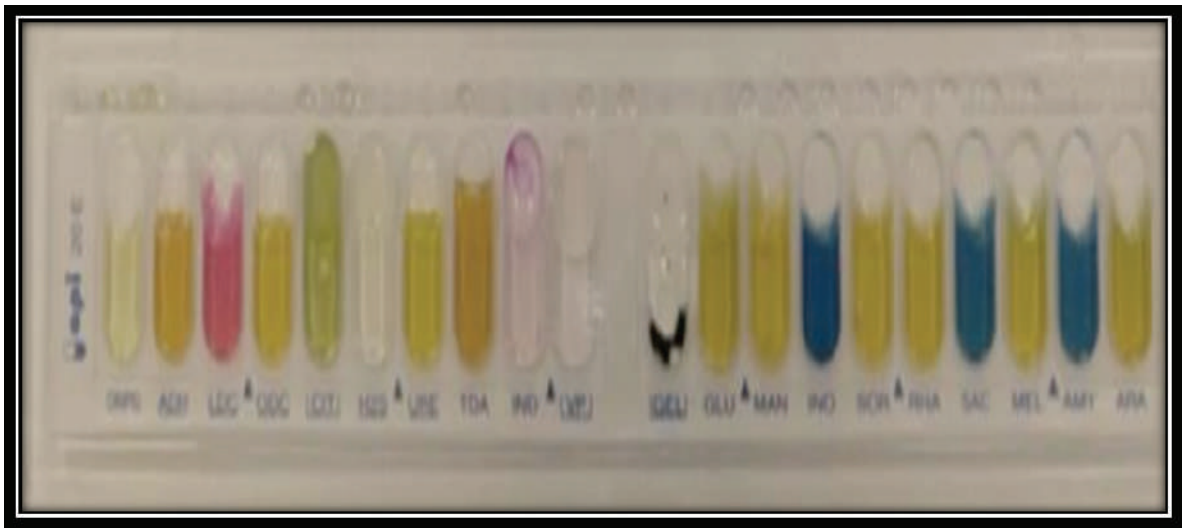


Figure (2) The results of biochemical reactions in API20 E test.

Antimicrobial Susceptibility Test

All 39 isolates from *Staphylococcus* of chicken eggs were performed Antibiotic sensitivity tests against six Antibiotics are commonly used as shown in Table(3). The results showed that the Vancomycin was susceptible to 79.4% of the isolates as shown in figure(3). also the ciprofloxacin and tetracycline showed sensitivity 61.5% and 51.2% respectively .these result agree with [3] and [18].

Over 90% of the *Staphylococcus* isolates appeared penicillin resistant, these results agree with [19] were found Over 66% of the *Staphylococcus* isolates appeared penicillin resistant

Table (3) Percentage of antibiotic sensitivity of *Staphylococcus* isolates .

Antibiotics	**Sensitive %**
Amoxicillin	8%
Penicillin	15%
Gentamycin	48.7%
Tetracycline	51.2%
Vancomycin	79.4%
Ciprofloxacin	61.5%

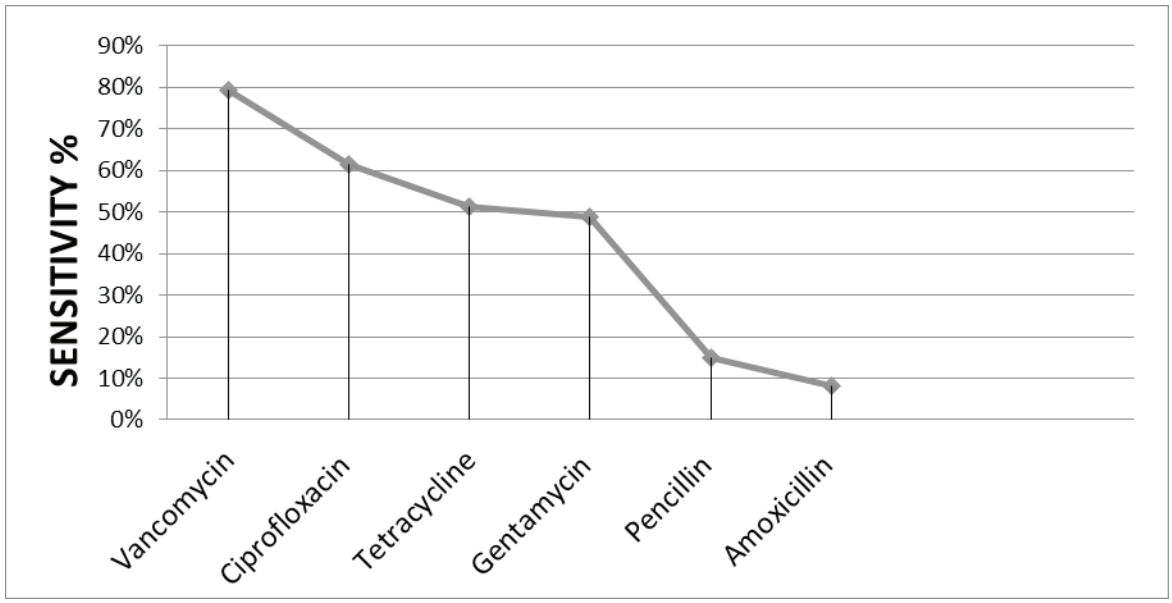


Figure (3) Showed the *Staphylococcus* isolates have highly sensitivity to vancomycin.

They conclusion from this study that eggs are vulnerable to contamination due to improper storing conditions , poor market access, dusty surfaces, extreme heat, smoke, hand interaction and all other pollution situations, and that customers must maintain eggs in the refrigerate and also well-cooked eggs. It's to destroy bacteria.Eventually, the persons trading have to be transported Good source eggs also from good hen farms and even in countries free from harmful zoonotic conditions.The properly washed and antiseptic techniques must be applied in every steps of handling, collected, traveling, marketing of eggs to reduce the bacterial contaminations.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the college of pharmacy and all experiments were carried out in accordance with approved guidelines.

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Role of Visfatin and Chemerin in Occurrence and Development of Gestational Diabetes in Pregnant Women

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Abstract

A case-control study was carried out in Kirkuk city from March to July 2019. The number of pregnant women (with gestational diabetes) understudy were 50 whose ages were between 17-42 years old to estimate the level of visfatin and chemerin in pregnant women with gestational diabetes. These patients attended to Kirkuk General Hospital to gynecology unit for pregnancy follow up. The control group who were matched to the pregnant women studied, included 30 healthy non-pregnant women. Blood was collected from each patient and control enrolled in this study for assessment of HbA_{1c}, blood glucose level, visfatin and chemerin levels. The study showed no significant difference between studied cases and the control group regarding patient age and gestational age at sampling and parity (P>0.05) while there was a significant difference between studied cases and the control group regarding systolic and diastolic blood pressure. The study showed that the highest mean level of visfatin was found in pregnant women with gestational diabetes comparing with healthy control (14.6±3.11 *v.s.* 7.81±3.01ng/ml) (P: <0.01). The study showed that the highest mean level of chemerin was found in pregnant women with gestational diabetes comparing with healthy control (78.92±18.44 v.s. 78.92±18.44 ng/ml) (P: ≤0.05). The study showed that the highest mean level of visfatin was found in pregnant women who have elevated level of glucose and HbA1c (5.75±3.81 and 5.91±3.67 ng/ml) respectively, as compared with patients with normal level of glucose and HbA1c (4.61±2.99 and 3.95±2.12 ng/ml) respectively (P: ≤0.01). The study showed that the highest mean level of visfatin was found in pregnant women who have elevated level of glucose and HbA1c (148.9±25.7 and 158.6±23.5 ng/ml) respectively, as compared with patients with normal level of glucose and HbA1c (111.5±14.6 and 105.1±15.7 ng/ml) respectively (P: ≤0.05). The study concluded that, there was a highly significant relation of visfatin and chemerin with gestational diabetes mellitus in pregnant women

Keyword: *Gestational diabetes; Visfatin; Chemerin; HbA1c*

Introduction

Important alterations in maternal metabolism and increased insulin resistance coincide with the progressive accumulation of adiposity during the course of normal pregnancy. Insulin resistance shows an increase in the late second trimester to levels that are observed in type 2 diabetes mellitus (T2DM) [(1)]. Most pregnant women remain normoglycemic due to adequate beta-cell compensation for this higher insulin secretion. When the beta-cell compensation for insulin resistance and hepatic glucose production is inadequate, gestational diabetes mellitus (GDM) ultimately develops [(2)]. Moreover, 10% to 50% of GDM cases are reported to develop T2DM in the postpartum period [(3)]. It is a major cause of morbidity and premature mortality from long-term complications such as cardiovascular disease, blindness, renal failure, amputations and stroke. The types of diabetes have been classified by the WHO[(2)]. Type 1 diabetes (previously referred to as insulin-dependent diabetes mellitus or IDDM) is due to absolute insulin deficiency and is usually an autoimmune disease leading to the destruction of the insulin-secreting beta cells in the pancreas. In some cases the cause of destruction of the beta cells is not known[(3)]. Type 2 (previously known as non-insulin dependent diabetes mellitus or NIDDM) results from relative insulin deficiency that may be associated with varying degrees of insulin action defects known collectively as insulin resistance[(4,5)]. In the Arab world, it had been estimated that 11-20% of population had D.M especially in oil producing

gulf countries[6]. It has mentioned that 10 % of Iraqi population had D.M. The first stage in type II diabetes is the condition called insulin resistance; although insulin can attach normally to receptors on liver and muscle cells, certain mechanisms prevent insulin from moving glucose into these cells where it can be used[7,8]. Most type II diabetics produce variable, even normal or high, amounts of insulin, and in the beginning this amount is usually sufficient to overcome such resistance[1]. Chemerin and visfatin are bioactive molecules that regulate numerous physiological functions such as energy equilibrium, insulin action, inflammatory response and vascular homeostasis[9]. In addition to its immunomodulatory effects, chemerin was reported to be associated with components of the metabolic syndrome and the parameters of type II diabetes including body massindex (BMI), plasma triglyceride (TG) levels, and blood pressure. Chemerin was shown to modulate the expression of adipocyte genes involved in glucose and lipid homeostasis[10]. So the aim of the study was to estimate the level of visfatin and chemerin in pregnant women with gestational diabetes .

Material and Method

A case-control study was carried out in Kirkuk city from March to July 2019. The number of pregnant women (with gestational diabetes) understudy were 50 whose ages were between 17-42 years old. These patients attended to Kirkuk General Hospital to gynecology unit for pregnancy follow up. The control group who were matched to the pregnant women studied, included 30 healthy non-pregnant women.

Five ml of blood was collected by vein puncture using Vacutainer tubes from each patient enrolled in this study. Blood samples were placed into two sterile test tubes, in one of them 2.5 ml of blood was put in test tube containing anticoagulant EDTA and used for assessment of HbA_{1c} test using (immunefluorescent, ichroma, Korea). The second part of sample was 2.5 ml was placed in plane tubes and centrifuged, the obtained sera were then aspirated and transferred into clean test tubes for estimation of blood glucose level (Biomerieux, France), visfatin and chemerin levels (Koma biotech, ELISA, USA).

Statistical Analysis

Computerized statistically analysis was performed using Mintab ver 18.0 statistic program. Comparison was carried out using Chi-square (X^2) for determination of the *P.* value.

Findings

As shown in Table 1. There was no significant difference between studied cases and the control group regarding patient age and gestational age at sampling and parity (P>0.05) while there was a significant difference between studied cases and the control group regarding systolic and diastolic blood pressure (P <0.05).

Table 1: Clinical characteristics of studied women

Parameters (Mean±SD)	Pregnant women	Control group
No.	50	30
Maternal age (yeas)	32.5±4.2	32.2±6.2
Gestational age	33.4±7.5	35.4±6.6
Parity, median (Range)	2 (1–7)	2 (1–6)
Mean 24 h SBP, mm Hg	128.7±11.5 *	110.8±7.4
Mean 24 h DBP, mm Hg	83.5±6.9*	69.4±8.3
Maximal SBP, mm Hg	167±24.1*	119.0±10.2
Maximal DBP, mm Hg	109.1±22.7*	74.7±13.3

The study showed that the highest mean level of visfatin was found in pregnant women with gestational diabetes comparing with healthy control (14.6±3.11 *v.s.* 7.81±3.01ng/ml) (P: <0.01).

Table 2: Levels of visfatin in pregnant women and the control group

Visfatin level (ng/ml)	Pregnant women	Control group	P. value
Mean±SD	14.6±3.11	7.81±3.01	0.001

The study showed that the highest mean level of chemerin was found in pregnant women with gestational diabetes comparing with healthy control (78.92±18.44 v.s. 78.92±18.44 ng/ml) (P: ≤0.05), Table 3.

Table 3: Levels of chemerin in pregnant women and the control group

Chemerin level (ng/ml)	Pregnant women	Control group	P. value
Mean±SD	78.92±18.44	49.67±12.87	0.001

The study showed that the highest mean level of visfatin was found in pregnant women who have elevated level of glucose and HbA1c (5.75±3.81 and 5.91±3.67 ng/ml) respectively, as compared with patients with normal level of glucose and HbA1c (4.61±2.99 and 3.95±2.12 ng/ml) respectively (P: ≤0.01), Table 4 and 5.

Table 4: Relation of visfatin with glucose level in pregnant women.

visfatin level (ng/ml)	Blood glucose level		P. value
	Normal	Elevated	
No.	10	40	≤ 0.01
Mean	4.61	5.75	
SD	2.99	3.81	

Table 5: Relation of chemerin with HbA1c level in pregnant women.

visfatin level (ng/ml)	HbA1c level		P. value
	Normal	Elevated	
No.	15	25	≤ 0.01
Mean	3.95	5.91	
SD	2.12	3.67	

The study showed that the highest mean level of visfatin was found in pregnant women who have elevated level of glucose and HbA1c (148.9±25.7 and 158.6±23.5 ng/ml) respectively, as compared with patients with normal level of glucose and HbA1c (111.5±14.6 and 105.1±15.7 ng/ml) respectively (P: ≤0.05), Table 6 and 7.

Table 6: Relation of chemerin with glucose level in pregnant women.

chemerin level (ng/ml)	Blood glucose level		P. value
	Normal	Elevated	
No.	40	140	≤ 0.05
Mean	111.5	148.9	
SD	14.6	25.7	

Table 7: Relation of chemerin with HbA1c level in pregnant women.

chemerin level (ng/ml)	HbA1c level		P. value
	Normal	Elevated	
No.	55	125	≤ 0.05
Mean	105.1	158.6	
SD	15.7	23.5	

Discussion

Various adipokines contribute to diabetogenic resistance to insulin, especially during the last half of pregnancy. This study mainly focused on potential alterations of specific adipokine concentrations (chemerin, RBP-4, and visfatin) in pregnant women with GDM. Here, we demonstrate that there were no significant differences in these adipokines between pregnant women with GDM and healthy pregnant women in the second trimester. However, the women with GDM were more likely to be overweight compared to matched healthy controls. Chemerin has been proposed to be an insulin-sensitizing adipokine; its secretion has been demonstrated to increase, presumably as a compensatory mechanism, in insulin-resistant subjects

Berndt *et al* [11] found that there was a significant relation of visfatin and type 2 diabetic regardless of their obesity. Consistent with our findings, Haider *et al* [12] showed that the release of visfatin by adipocytes in response to hyperglycaemia is dependent on the duration and extent of glucose elevation and is inhibited by insulin administration. It has been demonstrated that insulin does not influence visfatin synthesis in adipocytes and there is no difference in serum visfatin between type 2 diabetic subjects treated with insulin infusion or oral hypoglycaemic agents [12,13]. Additionally, the influence of insulin sensitizing agents on serum visfatin level has not been confirmed yet [10]. These findings suggest that fasting glucose levels, but not insulin resistance, may play a key role in the elevation of visfatin levels in newlydiagnosed type 2 pregnant women. Additionally, we showed that along with fasting glucose, triglycerides is also directly and independently associated with visfatin, which is consistent with previous findings [13]. Esteghamati *et al* [14] found that Serum visfatin is highly associated with type 2 diabetes mellitus independent of insulin resistance and obesity. Result of Haddad *et al* [15] revealed that the T2DM group has the highest serum levels of chemerin compared to the control

groups. Current evidence regarding the association of visfatin concentration with GDM is contradictory. Some studies demonstrated increased serum levels of visfatin in women with GDM [16,17], while others reported that visfatin concentrations were significantly lower in women with GDM [18,19]. In a meta-analysis, Berezin *et al* [20] suggested that the use of visfatin may predict increasing of HbA1c level in DM patients. In this study, we found increased visfatin levels in T2DM patients, as reported in previous studies [21]. Despite the high number of studies evaluating the role of adipokines in GDM in the existing literature, interpretation of the results is somewhat laborious for several reasons. First, the diagnostic criteria for GDM vary greatly. Second, the gestational age at the study times ranges from early first trimester to late third trimester. Third, diverse assay methods may also cause heterogenous results. Obesity is accompanied by altered secretion of adipokines from adipose tissue [22,23]. Adipokine levels are usually higher in obese women. Although we demonstrated that pregnant women with GDM had higher BMI values than healthy pregnant women, there were no differences in the levels of the studied adipokines between the two groups. This may be due to the regulation of various adipokines by pregnancy or to insufficient matching of control and GDM patients for BMI.. Thirdly, visfatin-mediated NAD biosynthesis that regulates glucose-stimulated insulin secretion may explain increased levels of visfatin in T2DM patients as a compensatory mechanism for β-cell functioning. Finally, because of the pro-inflammatory properties, these elevated levels could be attributed to the chronic low-grade inflammation present in T2DM. Several researches examined the association between serum chemerin concentrations in patients with T2DM [23-25]. However, it is controversial whether chemerin is related to T2DM in humans. It has been shown that chemerin in 3T3-L1 adipocytes improves insulin stimulated glucose uptake through the insulin signaling. This suggested that chemerin may regulate insulin sensitivity of adipose tissue [26], In that case with increase in chemerin concentration, the concentration of insulin should decline, but the experiment showed, FSI were positively correlated with serum chemerin.

Conclusions

There was a highly significant relation of visfatin and chemerin with gestational diabetes mellitus in pregnant women

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kirkuk health directorate and all experiments were carried out in accordance with approved guidelines.

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Preparation and diagnosis of Xerogel nanocomposites And Studying Their Effect on TNF-α Level before and after Loading Dexamethason in Male White Rats Induced Rheumatoid Arthritis

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Abstract

The current study aimed to evaluate the mechanism of reducing the effect of (RA) after it was induced in male white rat with a Complete Freund's Adjuvant (CFA) at TNF-α level by treating Xerogel Nanoparticles and dexamethasone nanocompounded (Xerogel / Dexa) . The free Xerogel nanoparticle had a surface roughness coefficient of 0.327 nm and the total percentage of volumes was 90% less than 130 nm, and the results also indicated that induced rheumatoid arthritis causes Significant increase ($p < 0.05$) in the mean concentration of neoplastic necrosis factor (TNF-α) in the blood serum of male white rats, as they reached a concentration level (10.68 ± 461.80) Pg / ml in the positive control group (G2) for six weeks of treatment compared with the negative control group G1)), as the concentration level in it reached (69.97±0.88) Pg / ml for the same period, and there was also a significant decrease ($P < 0.05$) in the level of concentration of TNF-α among members of the G5 group treated with the Xerogel / Dexa nanoparticle, as this concentration reached (182.98 ± 6.99) Pg / ml compared to the positive control groups (G2) treated with the free Xerogel (G3) and the group treated with free Dexa (G4) for a six-week treatment period in these groups as these concentrations reached (461.80± 10.68, 483.74± 3.59, 300.32± 4.03) Pg / ml, respectively.

Key Word: *Xerogel nanocomposites, TNF-α, Dexamethaon*

Introduction

Rheumatoid arthritis (RA) is one of the most common diseases in the world, with global data reporting that it distributed by more than 1% [1]. It is an autoimmune disease and affects different joint parts of the body and parallel to the right and left sides, RA affects most often in the small joints of the hand as well as foot, knee, and ankle, and thus targets the synovial membrane [2,3] . Research evidence has confirmed there are many biological, environmental, psychological, and personal factors with rheumatoid arthritis [4] There is also no cure for RA yet, but it can be lived a long and active life with rheumatoid arthritis, the treatments available at present are used to reduce the destructive inflammatory effect of the joint and prevent other complications of the disease as well as to maintain the flexibility and movement of the joint and thus reduce pain. Among the most important drugs used in the treatment of arthritis are NSAIDs and modified medicines [5]. Nanotechnology is new approach for treatment RA [6]. The pathological mechanisms were divided into three main stages, the first stage in which some cells are migrated to the tissue of the membrane Synovial membrane and this stage is characterized as non-qualitative and depends on various environmental factors related to rheumatoid arthritis (RA) in the late stages of untreated cases and in this type of infection in many cases ends full recovery in a short or long time or leads to chronic low-intensity inflammation responsible for the second stage of inflammation The tissue of the synovial membrane and cellular oscillation with the development of inflammation and its transmission to the tissue of the meniscus Synovial cartilage is similar to chronic rheumatoid arthritis (RA) but is a non-specific inflammation. The third stage includes stimulating immune proteins by cellular activation

and then developing and spreading the entire synovial membrane tissue inflammation and the destruction of joints. This stage causes the bone cartilage to be directly destroyed, all these manifestations represent the characteristic symptoms of RA [7, 8]. The current study aimed to evaluate the mechanism of reducing the effect (RA) by nanoparticles (Xerogel) after it was induced in male white rat with the material (CFA), through the following axes: 1.Preparation, diagnosis, and, study of some characteristics of the xerogel, which is made of zinc and silver.2. Induction of RA in rats .3. The oral experimentation of animals with the nanobot prepared Xerogel as a processing material.1. Download the treatment of Dexamethason Nanoboat Xerogel.2. Study the effect of xerogel nanocomposite after inducing arthritis TNF-α..

Methods

Design of the experiment : The male white rat was randomly distributed into five groups with animals per group and swallowed orally according to the weight of the rat's body for six weeks and as follows: 1. Group G1: was daily dragged by a solution of vesicle salt and promised a negative control group.2.Group G2: Induced arthritis by injecting Complete Freund's adjuvant-CFA (0.1) ml with the right foot as a positive control.3 . G3: Arthritis was induced and orally tested by Xerogel for 14 days after. 4. G4: Arthritis induced and orally tested by dexamethasone for 14 days. 5.Group G5: Arthritis induced and orally tested by Xerogel/ dex for 14 days.

Preparation of Xerogel nanocomposites: Dissolve 29.7 g of ZnO (NO3) hydro-nitrate nitrate in 167 ml of ethanol.2. Add 2 ml of ethylene clay-cool to the solution (Solution #1).3.4.2 g of silver nitrate (AgNO3) in 100 ml of distilled water 4.(Solution #2).5. Prepare a solution containing 26 ml of distilled water plus 0.6 ml of HNO3 (solution No. 3). 6. Add the ingredients of solution #3 to solution #1 and shake well (Solution #4). 7. Add the components of solution 2 to solution #4.8. Add to the last solution 2g of PVP. 9. Heat the resulting solution at a degree of heat up to 120 °C to vaporize two- thirds of the above solution . 10. The mix was centrifuged and collected after its burned in the oven with a temperature of 400 °C. 11. keep the output until use. [9]

Diagnosis of hybrid nanocomposite: The method used to diagnose hybrid nano -vehicles included the use of Atomic Force Microscope (AFM)

The induction of arthritis: RA induced by The Complete Freund Adjuvant (CFA) (CFA) (Biotechnology, Inc.).Canada Santa Cruz, according to the modus operandi [10]. The development of inflammation performed by injecting 0.1 ml of Complete Freund Adjuvant (which contains the heat-dead bacteria Mycobacterium tuberculosis) in the right foot of the rat's soles. Both weights of animals and The size of the foot are taken before the injection and after 14 days of injection as well as after six weeks of treatment. And the increase in foot thickness and stiffness. Newly developed arthritis is progressed 10-45 days from the start of the injection process [11]. The model for the development of arthritis by CFA has now proposed the development of this chronic infection, which is the characteristic of which is very similar to those resulting in the case of human rheumatoid arthritis [12,13]

After starving the animals for 12 hours, they were weighed and drugged with ether, and blood samples collected 5 ml per animal directly from the heart in a cardiac puncture. Blood samples are putting in 5ml wine syringes in 8 weeks of the development of arthritis and after treatment, the free drug, free nanocomposites, and nano-compound drug, as well as for the negative and positive control group. The samples put in anticoagulant-free tubes and centrifugation at a speed of 3000 rpm for 10 minutes, the serum was divided into clean, sterile appandorf tubes and kept in freezing at -20 m in the laboratory freezer for measuring other functional criteria including tumor necrosis, cytokines, and immune proteins.

Result and Discussion

Diagnosis of xerogel free nanocompound by Atomic Force Microscopie (AFM);

Figure (1) of the Atomic Force Microscope (AFM) indicate that the xerogel free nanoparticles have a surface roughness coefficient of nm of 0.327, but for molecular size ratios of the compound equal to 10% of molecular sizes less than 50.00 nm, and 50% for molecular sizes less than 90.00 nm, the sizes, in general, were 90% less than 130.00 nm.

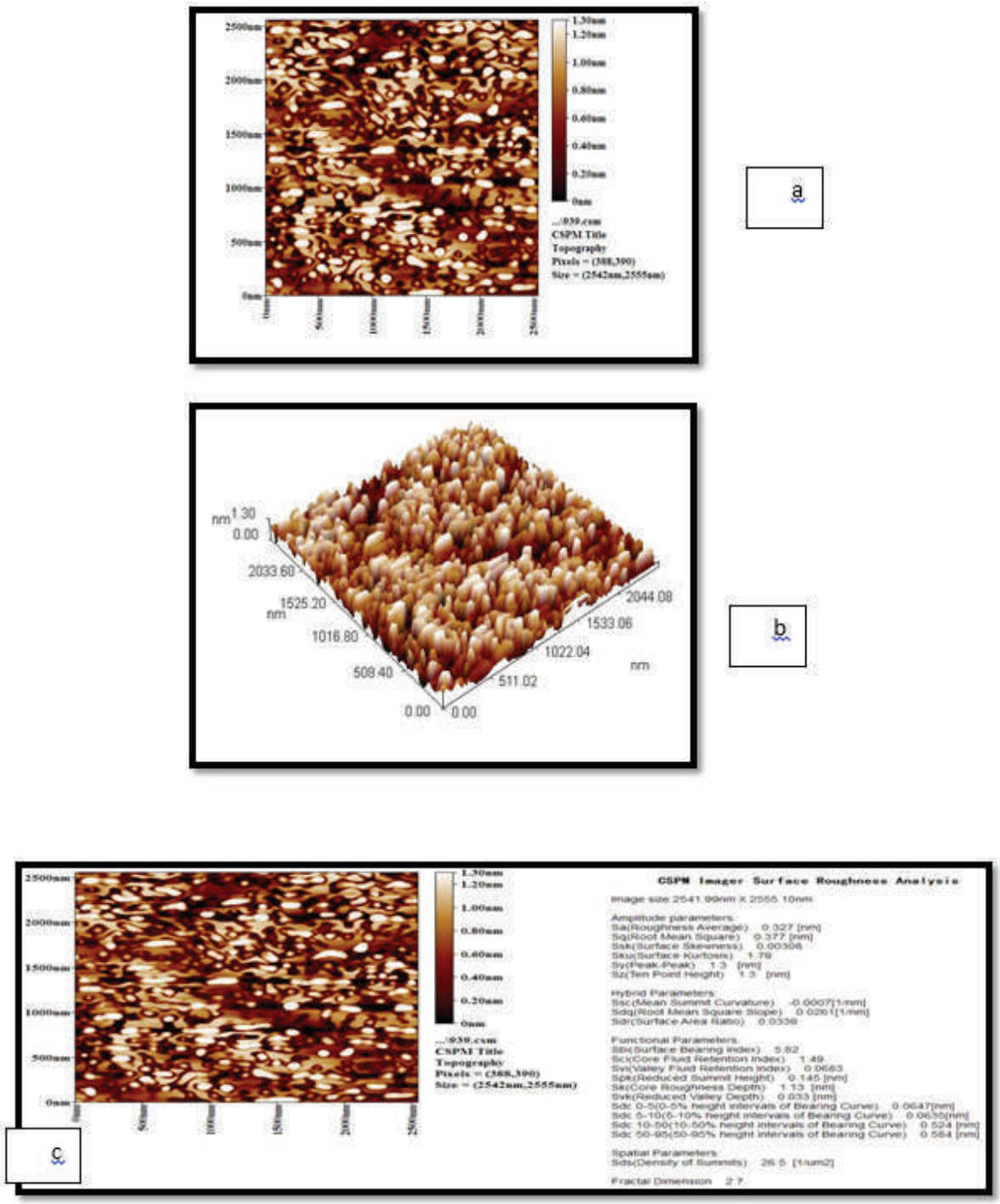


Figure (1) Atomic power microscope images of Xerogel free nanocomposite a/ 3D image, b/ 2D image, c/ 2D image showing all the details of the molecules:

Table (1) Total rate of particle volumes of the Xerogel free nanocompound and the different ratios of those volumes

Avg. Diameter:94.14 nm	<=10% Diameter:50.00 nm
<=50% Diameter:90.00 nm	<=90% Diameter:130.00 nm

Diameter(nm)<	Volume(%)	Cumulation(%)	Diameter(nm)<	Volume(%)	Cumulation(%)	Diameter(nm)<	Volume(%)	Cumulation(%)
25.00	0.38	0.38	80.00	4.51	35.71	135.00	4.14	90.98
30.00	1.13	1.50	85.00	5.64	41.35	140.00	1.88	92.86
35.00	0.75	2.26	90.00	7.52	48.87	145.00	0.38	93.23
40.00	1.13	3.38	95.00	3.76	52.63	150.00	2.26	95.49
45.00	1.50	4.89	100.00	7.14	59.77	155.00	1.13	96.62
50.00	1.50	6.39	105.00	1.50	61.28	160.00	0.38	96.99
55.00	5.26	11.65	110.00	5.64	66.92	165.00	1.13	98.12
60.00	4.14	15.79	115.00	5.26	72.18	175.00	0.75	98.87
65.00	4.89	20.68	120.00	4.89	77.07	180.00	0.38	99.25
70.00	6.02	26.69	125.00	4.89	81.95	185.00	0.38	99.62
75.00	4.51	31.20	130.00	4.89	86.84	200.00	0.38	100.00

Effect of treatment BDexa is free and the free nanocompound charged with Dexa treatment in the concentration levels of tNF-α tumor necrosis factor

The results of the current study table (3-2) show that the induction of reproductive arthritis by CFA causes a moral increase ($p<0.05$) in the concentration of tnf-α in the blood serum of white rat males as they reached a concentration level of 10.05. 68 ±461.80) Pg/ml in positive control group (G2) for six weeks of treatment compared to g1 negative control group with a concentration level (0.88 ±69.97) pg/ml for the same period, this is consistentWith the findings of a study [14] which attributed the reason for the high platelet count is due to the stimulation of the immune system against the invasion of pathogenic microorganisms and it is clear that the infiltration of single cells into the joints of mice induced by arthritis causes the secretion of tumor necrosis factor.

A similar study indicated [15] that opsonization is one of the most important determinants of the arrival and distribution of nanoparticles in the body of the organism and that the stimulation of the most common protein components to surround the nanoparticles and the formation of the protein halo are proteins of the complementary, albumin, and Lipoproteins, fibrinogen, the surfaces of nanoparticles with antigen markers make them vulnerable to attack by the Mononuclear phagocytic system (MPS) blood cells are exposed to certain nanoparticles that may produce cytokines, but the exact mechanisms of cytokine production are not yet known.Unlike traditional antigens lipoproteins polysaccharides, DNA, RNA and which stimulate cytokines after induction of TLRs, it is not known whether nanoparticles are identified by immune cells by immune cells SPECIFIC TLRs, or by other receptors.

The same table also shows a slight increase in tNF-α concentration rates in the G3 group treated with xerogel nanocomposite for six weeks of treatment with rates of 3.59 ±483.74 pg/ml compared to a concentration rate in the Positive Control Group (G2). It was (10.68 ±461.80)

pg/ml, while this increase was moral (p<0.05) compared to the negative control group (G1) at (0.88 ±69.97) Pg/ml.

The results of the current study were in agreement with the findings of(16) noting that nanoparticles that enter the body by The experimentation is subjected to complex reactions of blood cells and proteins and as soon as they enter the condensation of blood proteins on their surfaces and adsorption and proteins on the surfaces of nanoparticles equine corona (protein corona) these interactions determine the biological distribution and therapeutic effectiveness of nanoparticles and contribute to the type of immune resp

The results of the current study in Table (2) indicate that animals induced by rheumatoid arthritis and dexa therapy (G4) will have lower tnf-α concentrationrates (P<0.05) with a concentration rate of 4.03 ±300.32 pg/ml compared to the positive control group (G2) and negative control (G2) for the treatment lukewarm rate of treatment, which is consistent with the rate of tetanus. With The [7] which indicated that the use of cortisone treatments including Dexamethasone has a significant role in inhibiting the production of tnf-α tumor necrosis through its effect on cyclooxygenase and cyclooxygenase enzymes. As a result, the production of TNF-α, which is mainly manufactured by pharyngeal cells during COX-1 activity, inhibits the aggregation of these cells and produces quantities of this compound as well as reduces the production of tumor necrosis through synergistic action with other treatments such as Infliximab (IFX) by reducing the composition of Antibody production of this treatment antibody towards sifx (ATI).

The results of the table (3) show a moral decrease (P <0.05) in the concentration of TNF-α in members of the G5 group treated with the Xerogel/Dexa nanoboat, which amounted to 6.99 ±182.98 pg/ml compared With positive control totals (G2) and free compound therapy xerogel (G3) and the group treated with free Dexa treatment (G4) for a six-week treatment period in these compounds as these concentrations (10.68 ±461.80), 3.59 ±483.74, 4.03 ±300.32) PG/ml respectively.

The results of the study showed an agreement with the study conducted by [17] which indicated that the preparation of a nanocompound of chitosan gel and non-ioncompound (niosomal) pregnant for the treatment of arthritis cortzoni /niosomal) and testing Its effectiveness on healthy volunteers in the treatment of localized psoriasis has shown a low level of tnf and no sensitization or irritation compared to the use of niosomal alone and free treatment.The results of the current study were in agreement with the findings of [18] which indicated that the load of MTX treatment on the nanoboat Theranostic gold (Au) half-shell NPs or Polysialic acid (PSA)-trimethyl chitosan (TMC) NPs (xerogel) stimulates the formation of antibodies to the direction of specific receptors of the pharyngeal cells CD46, and that the load of MTX therapy is much more effective than free treatment in inhibiting the production of TNF-α tumor necrosis factor and eliminating the progress of arthritis significantly in experimental animals.

The results of the current study coincided with the findings of [19], which confirmed that the effectiveness of Hydrogel nano -compounds and their consideration as smart therapeutic carriers for arthritis treatments to targeted biological sites due to their absorption capacity and high control over the release of treatment in areas Inflammation which protects the joint from the action of inflammatory factors and tumor necrosis factor TNF-α due to several factors, compatibility High because it contains large amounts of water help to accept it from natural tissues and this reduces the immune response trend, the ease of cracking it by the body making it vulnerable to the extrusion devices and thereby reducing its toxicity, as well as its ability to carry and maintain treatments until they reach the target tissues, and also the ability to escape from the ventricle retina and reach the target tissue and organize the liberalization of treatment in them.

Table (3): TNF.& pg/ml cytokine concentrations before and after b treatmentDexa Free and Xerogel Free Nanoboat and Loaded With Xerogel/Dexa Treatment

Average concentration ±TNF.& Standard Line (pg/ml)	Treatment
0.88 ± 69.97 a	Nagtive control(G1)
10.68 ± 461.80 b	Positive control (G2)
3.59 ± 483.74 b	(G3) Xerogel
4.03 ± 300.32 c	Dexa (G4)
6.99 ± 182.98 d	Xerogel /Dexa (G5)

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Conflict of interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kerbala University and all experiments were carried out in accordance with approved guidelines.

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Study Effect of Some Pakistan Oil Isolation of Yeast *Candida albicans* causing oral thrush children Who Visit Women Hospital and Children in Diwaniya

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Abstract

The aim of the study was to evaluate some of the essential oils of the *candida albicans* yeast causing candidaiasis in children referred to the women hospital and children. The study included 108 oral swabs from infants with candidiasis ,all samples were diagnosed by diagnostic methods and after initial isolation of *candida albicans* 37.5% followed by species *C.tropicalis* 30% ,*C.parapsilosis* 12.38% as for *C.krusei* and *C.glabrata* they were 9.4% .the results of the agar well diffusion method showed that the nigella sativa oil was the most inhibited followed by cinnamon oil ,while garlic oil was the least inhibited compared with the nystatin.

Key words: *Candida albicans, Nigella sativa oil ,Cinnamon oil,Garlic oil, Nystatin*

Introduction

Oral disease is one of the most common opportunistic fungal diseases ,especially in the case of people with immunocompromised individuals ,*candida albicans* is one of the most common types of candida and has a very high to cause diseases and injuries to the oral cavity [1],at present other species have been identified as the main causes oral thrush after *candida albicans* such as *C.tropicalis* ,*C.parapsilosis*,*C.krusei* [2]. Aromatic oils and spices extracted from plants are also highly effective as microbial antiperspirants and are extracted from either steam, water ,or organic solvents , and can be extracted by cold pressing .the antimicrobial reaction to microbes is that it destroys many enzymatic systems of microorganism [3].

Materials & Methods

Collection of samples

108 oral swabs were collected from children who visited the women hospital and children's educations through sterile cotton swabs and then transferred to the laboratory for development, diagnosis and testing.

Isolation

Samples were streaked on the sabouraud dextrose agar and incubated at 37 **ºC** for 24 hour .

Diagnosis of candida

Phenotypic diagnosis

Special tested were conducted on the isolation of the genus candida according to what he described [4-8] which included:

a. Growth on Sabouraud dextrose agar

Cultured of the samples on the sabouraud dextrose agar according to method (Ellis et al ., 2007).

b. Growth on chrom agar candida

The samples were streaked on chrom agar candida and incubated at 37 ºC for 24- 48 hour. The color of isolation that has grown on this medium is observed .use this test to confirm the diagnosis of Candida.

c. Detection of presence of the Capsule

The presence of the capsule was discovered by using India ink in addition to a drop of India ink according to

the method [5].

d. Growth at 37 ºC

The SDA medium was inoculated with yeast tested and incubated at 37 ºC and observed daily growth for 2 weeks according to method [5].

Biochemical tests

a. Sugars fermentation test : Perform this test according to a method [9] .

b. Sugars assimilation test : in this test method adopted.

Virulent Factors

a. Germ formation : Perform this test according to a method (Ellis , 1994)

b. Chlamydospores formation : Perform this test according to a method (kangogo *et al*.,2011) .

c. Cycloheximide sensitivity: Perform this test according to a method (Ellis , 1994).

d. Growth at 45 ºC: Perform this test according to a method [10].

e. Urase test: This test was conducted to investigate the ability of yeasts to produce urease enzyme and depending on the method (Fadhy and Ajello ,1977).

f. Lipolytic activity: Perform this test according to a method [11]**.**

g. Phospholipase production: Perform this test according to a method [12]**.**

Test the efficacy of some types of Pakistan oil on *candida albicans* yeast on the SDA medium compared to the nystatin antibiotic

This test was carried out to determine the biologic effect of the oils used in the study using the method of agar well diffusion method contained in [13].

Statistical analysis

The data were statistically analyzed at a significant level of 0.05 and using the ANOVA table to confirm that there were significant differences between the tested parameters Al-rawyi (2000).

Results and Discussion

Isolation and Diagnosis

Five types of candida were isolated based on culture ,microscopic ,biochemical and virulence factors and these species are *Candida albicans* by 37.55% followed by species *C.tropicalis* 30% *C.parapsilosis* 12.38% as for *C.krusei* and C. .*glabrata* they were 9.4%**.** these results are consistent with Hussein, (2011) ,who isolated five types of candida species from the oral cavity the type *candida albicans* came in the foreforent followed by the following species *C. tropicalis* , *C. parapsilosis, C. krusei, C. rugosa..*

Table (1) Percentage of isolated species

Number	Isolates species	Percentage
1	Candida albicans	37.5%
2	C. tropicalis	30%
3	C. parapsilosis	12.38%
4	C. krusi	9.4%
5	C. glabrata	9.4
LSD(0.05)	10.015	

Phenotypic diagnosis

The results showed that all isolated species were grown on the SDA at a temperature of 37 C in white colonies circular and these results were consistent with(Ellis et al ., 2007)



Picture (1) growth *Candida albicans* on SDA at 37 °C

Microscopic examination

The results of the microscopic examination showed that Candida yeast was positive for G+ stain and negative for the dye in India ink (negative to test composition of the capsule) these results were consistent with [14].

Growth on Chrom agar candida medium

Results showed the grown of *C .albicans* on the Chrom agar candida medium as it appeared green color and these results agree with Rajaa *et al*.,(2015) [15].

Biochemical tests

Sugar fermentation test

The results of the sugary fermentation test showed that the *Candida albicans* type has the ability to ferment the sugars of glucose ,maltose, glactose but lactose and sucrose sugar cannot ferment. these results agree with Ellis *et al* .;(2007) which showed that the species *C.albicans* have a high efficiency in the representation of sugars, which shows that one of the reasons for the frequent isolation of tissues infected with candidosis, these results are consistent with Rajaa *et al*.; (2015) [15], as they found that *C.albicans* has the ability to ferment sugars :glucose, maltose, gelatos but lactose and sucrose sugar cannot ferment.

Table (2) Sugar fermentation test at 37 **ºC**

Number	Used sugars	C. albicans
1	Glucose	+
2	Maltose	+
3	Glactose	+
4	Lactose	-
5	Sucrose	-

+ sugar fermented , - Cannot fermented

Sugars assimilation test

Cultured isolates species of *Candida albicans* on the SDA medium and the saturated sugars were added from the following sugars: Glucose , Maltose , Galctose , Lactose Sucrose**,** as in table (3) as all isolates of *Candida*

albicans assimilate all sugar except lactose sugar, these results are consistent with AL-Edamy,(2012) [16] .which is a very important cause of the repeated isolation of tissue infected with candidiasis (Ellis et al .;2007)

Table (3) Sugars assimilation test

Number	Used sugars	C. albicans
1	Glucose	+
2	Maltose	+
3	Glactose	+
4	Lactose	-
5	Sucrose	+

+ positive -negative

Virulence Factors

Germ tube formation

The results of the study showed that all isolates belonging to species *C. albicans* had formed the germ tube when incubated for 2-3 hours in serum blood at 37 ºC , these results are consistent with Boon *et al* .,(2013) which showed that the species of *C.albicans* has the ability to form a germ tube and the presence of the catalyst(serum) is formed around the yeast cell and that the germ tube plays an important role in the process of penetrating the layer of epithelial cell of the body and tissues and access to the blood stream as well as thought necessary to feed the yeast [17].

Chlamydia spores Formation

The results of the study showed that all the isolates of the type *C.albicans* had formed the Chlamydia spores when cultured in the Corn meal agar medium and incubated at 25 ºC for 24 hours .these results agree with [18] ,whose results showed that the type *C.albicans* has the ability to form Chlamydia spores on the Corn meal agar medium, the Chlamydia spores are round with thick walls at the end of hypha which may be single or clustered when cultured on the CMA medium as a result of starving the yeast for lack of food sources where they are formed when the conditions are not appropriate to them .this medium is described as starving for yeast.

Resistance to Cycloheximide

The results of the study showed that isolates of the species *C.albicans* had resistant to cycloheximide as it growth on the SDA medium added 0.5 g/ l of the cycloheximide .these results are consistent with Hussein, 2011 and al- Ubaidy.,2012; Takaku *et al.* 2004 .the results of their study showed that species *C.albicans* only was resistant to the cycloheximide and gave a positive results for this test .

Growth at 45 ºC

C. albicans was cultured on the SDA and incubated at 45 ºC .the growth was monitored daily for 10 days .the results showed that *C.albicans* had the ability to grow at this level. these results are consistent with AL-Obeidi,(2012) which showed that the *C.albicans* species managed to grow at 45 ºC while the rest of the species *C. tropicalis , C. krusei* , *C. glabrata* could not .

Urease Enzyme Production

Results of the growth of *C.albicans* on the agar urea showed that *C. albicans* did not have the ability to produce the urease enzyme .these results came consistent with Jasim and Nasir ,(2013) which showed that the result *C. albican C. parapsilosis , C. Tropicalis* were unable to produce urease under the same conditions.

Lipase Enzyme Production

The results of the study showed that the species *C. albicans* has the ability to produce the Lipase and these results were consistent with Hussein ,(2011) whose results showed that the species *C. parapsilosis , C. tropicalis , C. albicans* have the ability to produce lipase.

Phospholipase Enzyme Production

The results of the study showed that the species *C. albicans* has the ability to produce the Posoholipase ,this explains the ability of the species to cause the disease more than of the species and these results are consistent with (Hussein, 2011).

Table (3) Virulence Factors of *Candida albicans*

Number	Virulence factors	C. albicans
1	Germ tube	+
2	Chlamydospores	+
3	Resistance to cyclohximide	+
4	Growth at 45C	+
5	Lipase	+
6	Posoholipase	+
7	Urease	-

+ positive , -negative

Test the effect of some types of Pakistan oil on *Candida albicans* yeast compared to the nystatin antibiotic.

The results showed that *C.albicans* was sensitive to the oil used in the study .the nigella sativa oil was one of the most influential oils on the growth of *C.albicans* yeast it also showed its superiority over the antibiotic nystatin used as a control agent with the rate of inhibition zoon diameter 31mm, these results are consistent with Khuder,(2012) finding that nigella sativa oil have a good antibacterial activity on all bacterial types used in study, the inhibition effect of the nigella sativa oil due to chemical compounds Thymol , Thymohydroquinone, Thymoquinone in the essential oils of the nigella sativa oil which according to previous studies ,showed its antimicrobial effect. Salman *et al*.,(2008), a while the comparison treatment (nystatin) 30mm,cinnamon oil was the average diameter of the inhibition zoon 22mm ,these results are consistent with Abdullaha,(2013) finding that cinnamon oil has an active role in inhibiting the growth of certain bacterial isolates causing diarrhea ,which were better than antibiotics used as a control groups. as for garlic oil was the least effect on the growth of yeast under study as showed in the table the rate of inhibition zoon diameter 15mm.

Table (4) effect of some types of Pakistan oil on Candida albicans yeast compared to the nystatin antibiotic

Number	Oils (Concentration) 100%	Inhibition zone diameter(mm)
1	nigella sativa oil	31
2	Cinnamon oil	22
3	Garlic oil	15
4	Nystatin	30
LSD(0.05)	4.016	

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Furat Al-Awsat Technical University and all experiments were carried out in accordance with approved guidelines.

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The effect of Stylistic Discovery Directed and Programmed Education on teaching some football skills and understand the benefits of physical education and health

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ABSTRACT

The guided discovery method is one of the teaching methods that work to engage the student in the discovery process, and the essence of this method is the special relationship that arises between the teacher and the student through which leads the successive questions that the teacher directs to reach the answers that the student performs that correspond to these questions, if every question the teacher directs leads to one correct response that the student will discover, and if the accumulation of this process occurs. the researchers used the statistical bag (SPSS) through which the statistical methods were used, the most important of which were: (t) test for the interlinked samples and the L.S .D test and through the results reached the researchers concluded a set of conclusions, the most important of which : The use of the two methods of directed discovery and programmed education and the method followed by the teacher have a positive effect in teaching some basic skills in football, as well as the method of directed discovery over the style of programmed education and the method followed by the teacher in teaching some of the skills under discussion.

Keywords: *Stylistic Discovery Directed, Programmed Education, skills*

Introduction

Physical education is a science like the rest of the sciences that use the methods and methods reached by scientists and researchers [1] in a way that is appropriate for the nature and conditions of each society and this has contributed to raising the level of this science and developing [2] it through the use of various methods and methods in teaching physical education material that is consistent with students' inclinations and desires It reaches a level of mastery and a high level of efficiency and effectiveness, and it takes the work of the team as an input "for progress within the framework of society's values, traditions and customs with the aim of preparing and raising the emerging generation of good citizenship." In order for the physical education lesson to go in the right way, teaching methods must be used that take into account the individual differences between students and discover the student's cognitive level so that he can achieve his maximum to better accommodate the education requirements, and among these methods is my method (directed discovery and programmed education), and is considered a method [3] Directed discovery is one of the teaching methods that engage the student in the process of discovery. The essence of this method is the special relationship that arises between the teacher and the student through which leads the successive questions that the teacher directs to reach the answers that the student performs that correspond to these questions.

Methodology

The research community consisted of fifth preparatory students (Nahr Al Furat Secondary School - Dhi Qar Governorate) for the academic year (2019-2020), and the number is (102) students, distributed among 4 groups (A, B, C, D), whose ages range from (16-17 years). As for the research sample, three groups were chosen from among four groups depending on the random method (lottery), as the number of the sample reached (36) students with a percentage of (35,29), where they were divided into three groups for each group It is made up of (12) students who are students who are not practicing the game, knowing that the sample is homogeneous because all members of the

sample are male and have one age group, as students who have failed and practitioners of the game of football are excluded, and the research sample was represented by three groups. The basic football is under discussion using the method followed by the teacher of the subject and the number (12) students from Division (C). The first experimental group: where students learn the skills using the targeted discovery method and the number (12) students from the Division (A). As students learn skills using the programmed teaching method, which is (12) students The groups of (D). As for Division (B), the researcher conducted the exploratory experiment, and they numbered (12) students. In order for the sample to be equivalent, the researcher used the analysis of variance (F) to find the significance of the differences between the groups in the skill (handling, suppression and rolling) in football.

Table 1. Equivalence of the three groups shows age, length, mass and some basic skills under consideration

Statistical treatments / Variables	measuring unit	Source variance	Sum of squares	Degree of freedom	Average squares	Values (F) Calculated	level indication *	Statistical significance
Age	Month	Between groups Within groups	3.475 5o7.300	2 32	1.158 14.092	0.082	0.969	Not significant
Length	Cm	Between groups Within groups	55.275 1772.5	2 32	18.425 49.236	0.374	0.772	Not significant
Mass	Kg	Between groups Within groups	96.600 1509	2 32	32.200 41.917	0.768	0.519	Not significant
Handling	Degree	Between groups Within groups	4.200 298.200	2 32	1.400 8.283	0.169	0.917	Not significant
Quenching	Degree	Between groups Within groups	1.100 130.000	2 32	0.367 3.611	0.959	0.102	Not significant
Rolling	a second	Between groups Within groups	2.053 231.630	2 32	0.684 6.434	0.956	0.106	Not significant

Morale at the level of significance <(0,05) and in front of two degrees of freedom (2-32).

It is clear from Table (1) that the calculated value of (F) for all research variables is at a level of significance greater than (0.05), which indicates that there are no significant differences and this indicates the equivalence of the three research groups in all search variables.

Registration: The player is given (5) successive attempts.

Calculates the number of scores the player has earned from the five attempts.

The highest score a player receives is 30 points.

General directions:

- The attempt is a failure if the ball falls outside the circles.

- In the event the ball falls on the circle line, the next degree is given according to the sequence of circles (5,3, 1) degrees.

The second test: the rolling test of the ball between five characters. The distance between one person and another 2 m back and forth (Hussam Saeed Al-Momen: 2000): -

Purpose of the test: To measure the rolling skill test.

The tools used: legal football, tape measure, spinners, and funnel (5).

Performance description: The laboratory stands with the ball behind the starting line, and upon hearing the beep, the laboratory rolls the ball between the cones placed and returns to the starting line.

Conditions of performance:

1- The laboratory can start by passing the first funnel from left or right side.

2- The movement of the laboratory should not be stopped during the test.

3-If the ball is out of the control of the laboratory, an attempt is not counted.

Registration method:

The laboratory is given two consecutive attempts, and the result of the best attempt is taken.

The third test: The test of stopping (suppressing) the movement of the ball from a distance of (6) m within a square (2) m (Bath Abdul-Muttalib: 2011):

The goal of the test: to measure the accuracy of stopping the ball the coach is throwing.

The tools used: (5) footballs, a specific area for the test (2 x 2 m) .

Method of performance: The tester stands behind the line of the specified test area and the trainer stands with the ball on the opposite side and a distance of (6 m) from the specified test area, and after giving the starting signal the trainer throws the ball (a high ball) for the tester advancing from the starting line into the test area trying to stop the ball is in any part of the body, except for the arms, and then back to the starting and starting lines again and so the laboratory repeats the five attempts in a row.

The main experience:

Time and sections of the educational unit:

The educational units were divided into (9) weeks and by two educational units per week for each unit (45) minutes, as the number of educational units for each group reached (18) educational units for each method, (6) units to teach each of the skills under discussion (handling, damping, rolling) .These methods are applied to the main section only, and its duration (22 minutes), and includes the educational aspect and its duration (10 minutes) in which the skill is explained to students, and questions are asked in the method of directed discovery and the programmed learning style on the applied side, and in this section from the lesson, students start applying the skill performance according to their answers to the questions of the method, and the teacher participates in giving the students feedback during the performance and after the performance, the errors are corrected, and thus the time of the educational units reached (810) minutes for each method.

Main experiment procedures:

Before starting applying the main experiment, the researchers conducted two educational units for students in football for each method on (16/17/18/10/2019), the purpose of which is to give prior learning to the research sample, as well as to the subject teacher to clarify the method of applying the educational program to teach some basic skills of football under research By the method of directed discovery and programmed education, the control group used the method followed by the teacher, where the research groups were given the educational subject to be learned within the curriculum decided by the Ministry of Education. The two researchers prepared a set of questions using the method of discovery directed in each skill. These questions include a question about the skill to be learned; This means that the set of questions

with their answers will be the correct technique for the skill, as well as the researcher with the programmed learning method prepared a programmed booklet and CD-ROMs detailing the skills of (handling, quenching and rolling). The first experimental group learned the guided discovery method, and it was implemented in the main section, as the teacher begins explaining the skill in the educational aspect and displaying it from the teacher, and after that a set of exercises is applied that help students to learn the skill in the applied side, as the teacher poses questions intended for students in the discovery method The instructor and the students answer the questions individually, and everyone participates in answering and discussing the answer, and the teacher participates in giving feedback to students while answering the questions. After completing the questions, the students apply the exercises designed for the skill according to their answers to the questions, while the second experimental group learned in a manner Programmed learning is implemented on the main section after reviewing the programmed booklet and CD-ROMs distributed by the teacher to learners, as well as videos sent to students on social networking sites (Messenger and What Sapp), by making groups on these sites, and the teacher participates in giving nutrition reviewer. As for the control group (the teacher's method), this group learned the traditional method used in their learning of skill, and was implemented on the main section, as the teacher explains the skill in the educational aspect, and displays it to students, while in the applied part, the teacher gives the same exercises to the two experimental groups, but in the followed method that helps students on learning the skill.

Applying the main experiment:

The first experimental unit was applied to the first experimental group in the direction of discovery directed on (Tuesday) (22/10/2019). As for the second experimental group in the programmed teaching method, the first educational unit was applied on (Wednesday) on (23/10/2019) and on the square Secondary of Nahr Al-Furat, and the researcher supervised the main section of the units for the research sample. As for the application of the methods, the subject teacher did this and the last educational unit was on (Wednesday)18/12/2019 for the first experimental group with a targeted discovery method, while the second experimental group With the programmed learning method, it was the last unit of education on (Thursday) (19/12/2019).

Post-test:

The two researchers conducted the dimensional tests of the research sample on (Tuesday) on (24/12/2019) for the first experimental group in the direction of the targeted discovery, and at exactly (9 am), the second experimental group with a method of programmed education was on (Wednesday) on (25 / 12 /2019) at exactly (9 am), and the total set in the style of the teacher was on (Thursday) (26/12/2019) on the playground for the high school of the Naher Alforat, and the researcher followed the conditions and method of the tribal tests themselves.

Statistical Methods: Using the Statistical Program (SPSS) :

1-Percentage.

2- Arithmetic mean.

3-Standard deviation.

4- T-Test Law for Linked Samples.

5- F-Test Contrast Analysis Act.

6- L.S.D. test.

3- Presenting, analyzing and discussing the results.

3-1 Present and analyze the results of the basic skills tests under discussion in the tribal and post football with the three research groups.

In order for the two researchers to be able to identify the difference in the results of the pre and posttests of the individuals of the three groups in skills (rolling - handling - suppression) the researcher used the test (t) for the interlinked samples as shown in the tables below.

Table 2. Shows the significance of the differences between the pre and post measurement in basic skills tests football for the first experimental group (directed discovery method)

Statistical treatments / Variables	measuring unit	Tribal tests		Post-test		Value (t) Calculated	Significance level	Statistical significance
		s	(± p)	S	(± p)			
Handling	Degree	10.7	2.907	6.24	3.062	20.619	0.000	Moral
Quenching	Degree	3.6	2.065	7.8	1.751	6.034	0.000	Moral
Rolling	a second	16.894	2.102	12.670	1.033	5.805	0.000	Moral

* Morale at the level of significance <(0,05) and in front of the degree of freedom (11).

Table (2) shows the arithmetic mean, standard deviations, and the calculated value (t) between the results of pre and posttests in some basic football skills (handling, suppression, rolling) for the first experimental group (directed discovery method), as the results shown in the table showed that the value of a level The calculated significance in all skill tests was (0,000)

Table 3. Shows the significance of the differences between pre and post measurement in basic skills tests football for the second experimental group (programmed teaching method)

Statistical treatments / Variables	measuring unit	Tribal tests		Post-test		Value (t) Calculated	Significance level	Statistical significance
		s	(± p)	S	(± p)			
Handling	Degree	11.1	2.643	18.8	2.44	11.258	0.000	Moral
Quenching	Degree	3.2	1.398	5.2	1.932	2.739	0.023	Moral
Rolling	a second	17.512	2.593	15.51	1.389	3.025	0.014	Moral

* Moral at the level of significance <(0,05) and in front of the degree of freedom (11).

Table (3) shows the arithmetic mean, standard deviations, and the calculated value (t) between the results of pre and posttests in some basic football skills (handling, suppression, rolling) for the second experimental group (programmed learning style), as the results presented in the table showed that the value of a level The calculated significance for all skill tests was between (0,000 - 0.024).

Table 4. Shows the significance of the differences between pre and post measurement in basic skills football tests for the control group (teacher style)

Statistical treatments / Variables	measuring unit	Tribal tests		Post-test		Value (t) Calculated	Significance level	Statistical significance
		s	(± p)	S	(± p)			
Handling	Degree	10.8	2.820	19.6	2.716	7.223	0.000	Moral
Quenching	Degree	3.4	1.646	5.4	1.349	3	0.015	Moral
Rolling	a second	17.069	2.334	14.94	1.614	2.709	0.024	Moral

* Moral at the level of significance <(0,05) and in front of the degree of freedom (11).

Table (4) shows the arithmetic mean, standard deviations, and the calculated value (t) between the results of pre and posttests in some basic football skills (handling, suppression, rolling) of the control group (teacher style), as the results shown in the table showed that the value of the calculated significance level In all skill tests, it ranged between (0,000 - 0.023), which is less than the value of the significance level (0.05), which indicates the presence of statistically significant differences between the pre and posttests in favor of the post test.

Discussing the results of the basic skills tests under discussion in the tribal and dimensional football of the three research groups.

Through the presentation and analysis of the results of the pre and post tests for basic skills tests in football under discussion in tables (2, 3, 4) and for the three groups, it became clear that there are significant differences between the pre and posttests in favor of the post tests and for all basic skills in football under research for all the methods used (discovery oriented and programmed learning and teacher's style), and the researchers attribute that to the effect of the educational curriculum that the researcher used. "The goal that all educational curricula seek by applying their educational units is to improve and improve the level of performance and provide the student with a set of skill capabilities that enables him to achieve a good level of skill performance." What you want to learn "(Hussein Hadi Attia: 2004). As the wave discovery method is one of the most important methods that work to enhance the relationship between the student and the teacher, as well as taking into account the individual differences between students, which creates an atmosphere of enthusiasm and impulsivity towards learning, "where it is important that individuals are motivated to learn the motor tasks in order to obtain the maximum learning [6-10], giving the student a role in decision-making as well as giving the student the freedom to arrive at discovering the skilled performance by himself and that by applying what he himself discovered practically in thinking about performance, as well as the quality of questions posed to the student by the teacher has an effective role in the excitement The student and extracting his stock of capabilities and directing them in a correct way. In addition to the large amount of feedback that the student gets by this method even if it is a self-feedback or provided by the teacher, it is sufficient to correct the student's motor responses and adjust them "and that the feedback appears as the

variable The strongest and controlling the learner's performance [2], and it has become clear that there will be no performance improvement without feedback, and that feedback increases the performance improvement in the initial learning stages Yarp Khyon (2002) Since the application of the targeted discovery method enhances the student's performance by giving the student the freedom to move according to his desire, because the technical performance is discovered by him through his answers to the questions posed about the skill to be learned, from the above [4], the targeted discovery method has a positive and effective role in Learning the basic skills of football under research. The same table also shows that there are differences between the results of the pre- and post-test for the basic skills of football under research for the programmatic education and for the benefit of the post-test.

Table 5. It shows the results of the analysis of variance analysis (F) in the dimensional tests among the four research groups in the basic skills of football under study

Statistical treatments / Variables	measuring unit	Source variance	Sum of squares	Degree of freedom	Average squares	Value (F) Calculated	Level indication	Type indication
Handling	Degree	Between groups Within groups	210.27 266.5	2 32	70.092 7.403	9.468	0.000	moral
Quenching	Degree	Between groups Within groups	47.5 95.6	2 32	15.833 2.656	5.962	0.002	moral
Rolling	a second	Between groups Within groups	48.059 55.339	2 32	16.020 1.537	10.421	0.000	moral

* Morale at the level of significance <(0,05) and in front of two degrees of freedom (2 - 33).

Table (5) shows the results of the analysis of variance analysis (F) between and within the groups in the basic skills of football under discussion, and there was a significant difference between the three groups in the basic skills tests under discussion, all because the level of significance in them is less than (0,05), The researcher used the (L.S.D) law to determine the three best groups.

Conclusions

1- The use of the guided discovery method, the programmed teaching method, and the method followed by the teacher, was important in teaching some basic football skills.

2- The guided discovery method was superior to the programmed teaching method and the method used by the teacher in teaching some basic skills of football.

3-The excitement and excitement of using these methods increased the motivation of students to learn, which facilitated the performance process.

4-Although there was education in some basic skills in football using the programmed teaching method, it was less effective than other methods.

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Ethical Clearance: All experimental protocols were approved under the General Directorate for Education of Dhi Qar and all experiments were carried out in accordance with approved guidelines.

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Effect of Age and the Pretest Score on Critical Thinking and Using Concepts Maps in Learning the Skill Scoring of Football for Students

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Abstract

The world has witnessed in recent decades rapid developments, Including all areas of life, Prompting the nations to strive to keep abreast of these developments and build their societies, and became human is the main objective of those developments. Through the overlap of a variety of factors, including the method chosen in the process of teaching and the specific way of teaching, and the characteristics of individuals [2] of cognitive methods, which vary from individual to other, as a result of individual differences identified, and took a lot of changes and development These developments were the result of the technological development that covered all fields of life, scientific and practical in general and in particular [3] the sports field. Mind mapping technology has an impact on football scoring skills for first-stage students. Taking notes using concepts mapping technology helped the members of the expericoncepts group to plan their ideas fully. The technology used in the current research (concepts maps) has contributed to the development of the dynamic program of the members of the expericoncepts group, through the processes of remembering, retaining and reviewing concepts mapping.

Keywords: *Pretest score, critical thinking, concepts maps, Skill scoring*

Introduction

The learning process in this field is characterized by different characteristics from the other fields. Because of the privacy of the lesson itself, and this difference has increased the responsibility of the teacher of physical education, as he is the primary responsible for producing a practical lesson successful, and according to the latest foundations and mechanisms developed, and on this basis must be the teacher to be efficient and successful and [4] able to keep pace with this development, must To be constantly updated on the latest strategies and methods and means, such as modern concepts maps, which if used correctly in the lesson of physical education to give very large results at the level of education.

The use of means by appropriate teaching methods is very important to facilitate the process of teaching and learning at the same time, it brings the idea to the learner on the one hand, and makes it able to absorb the educational material on the other hand, it facilitates the work of the teacher in terms of shortening the effort and time required to deliver the information easily and in the shortest possible [5] time through the means of education, especially if it works to occupy the mind and the thinking of the learner, the matter that occupies the interest of most teachers of physical education at the moment is how to employ the right information in the mind of the learner by choosing the most appropriate strategies and methods to facilitate the learning process, and the result obviously difference between individuals due to individual differences, there is no particular method or

one particular way benefit everyone to get them to one level of learning. where the football game is one of the group games that have attracted the attention of many of the amateur and has become the leading in terms of deployment in many countries of the world, as this game depends on the basic skills as a base on which to base it, and to progress in this game must master all skills, This is the first step towards achieving a high level of learning [7] Based on all of the above, the importance of the current research in the attempt to use (concepts maps), which is one of the most important educational means that work to receipt some information and ideas of skill after the formulation of concepts map to the learner as an interesting way to work and the importance of their impact in learning Skill scoring football for students.

Hence it will researcher will Kicks off on a serious and new applied scientific experiment in this field to learn about the effect of using concepts maps in learning the Skill scoring football for students.

Methodology

The researchers used the expericoncepts approach and the design of the control and expericoncepts groups to suit the nature of the problem to be solved "The expericoncepts method is the closest method of research to solve problems in the scientific way is an attempt to control the variables and all the basic factors except one variable or more researchers are changing it in order to determine and measure the impact of scientific"[(1)].

Community & Research Sample:

The researchs community consists of students of the first stage - Faculty of Physical Education and Sports Sciences – Al- Ain Private University in Thi -Qar for the academic year 2018 - 2019 (80) students divided into two divisions (B, C).

The sample of the research is "part of the original research community chosen by the researcher in different ways and includes a number of individuals from the original community" [(2)]. So the researcher choose his research sample by random way, (C) was chosen to represent the control group and (B) to represent the expericoncepts group, and (A) is for female students. The researchers conducted the exploratory experiment on (10) students from the research community and without the research sample.

After the procedures of homogeneity and equivalence, the researcher conducted a field experiment on the sample of the main experiment consisting of (40) students representing the people (B, C) and 20 students from each division, and the proportion of the sample of the community of origin is (50%), and for homogeneity and equivalence The researcher excluded a number of respondents, teachers, students of good skill level, and students who did not attend the pre test. (Table 1 shows this).

Table (1) shows distribution of search sample

Classes	Total number	Samples	Excluded	Chosen Samples	Percentage
B	40	Sample of main experiment	20	20	50%
C -	40	Sample of main experiment	10	20	50%
		Sample of reconnaissance experiment		10	12.5%
research community	80	Sample of main experiment		40	50%

Homogeneity of the research sample:

In order to adjust some variables that affect the accuracy of the results of the search. In order to return the differences in the effect only to the independent variable, It is necessary to ensure the homogeneity of the research sample in some variables. Therefore, the researcher used the law of difference coefficient between the sample and as shown in Table (2).

Table (2) shows Homogeneity of the sample

No.	Variables	Measure Unit	Arithmetic mean	Standard deviation	Difference coefficient
1	Age	Month	242.22	15.77	6.5%
2	Mass	Kg	68.15	3.83	5.16%
3	Length	Cm	169.70	4.99	2.49%

Table (2) shows that the values of the difference coefficient for all the research variables and the control and expericoncepts groups were less than (30) indicating the homogeneity of the sample

.**Equivalence of the research sample:**

For the purpose of ascertaining the equivalence of the control and expericoncepts groups in some variables, the researcher performed equivalence by using law (t-test) of the independent samples as shown in Table (3).

Table (3) shows sample Equivalent research sample

Tests	Measure Unit	Control group		Expermintal group		T value	Sig	significance type
		M	Sd	M	Sd			
Age	Month	240.85	15.14	244.70	15.84	0.87	**0.91**	Not significant
Mass	Kg	68.65	3.23	67.65	4.38	0.82	**0.84**	Not significant
Length	Cm	169.05	4.98	169.85	4.62	0.52	0.80	Not significant
Scoring	Degree	8.23	1.66	7.82	1.94	0.74	0.92	Not significant

***significance level ≤00.05**

Exploration Experience:

The exploratory experiment is a "practical training for the researcher to identify for himself the negatives and positives that may be encountered during the test and the main experiment to avoid them

The researchers procedure the exploratory experiment on Sunday (18/11/2018) at 10:30 am on the same sample without the research sample and the community of origin (10) students and the presence of researcher and the teacher of the article The aim of this exploratory experiment Come :

· Identify the obstacles and errors that accompany the test process when applied.

· Ensure that the testers understand the test method and master its requirements.

· Ensure the efficiency of the assistant staff. Annex (3)

· Recognize the validity of the instruments used in the tests.

· Configure the main experiment requirements.

· Scientific foundations

The scientific basis of the test.

Each of the tests must have the scientific basis for the tests so that a good test can be produced and the results can be adopted. Therefore, the researcher found the honesty coefficient, Stability and objectivity of the test.

Test Honesty :

Honesty is one of the indicators that must be found in the test used in the measurement of any mathematical characteristics and phenomena. The "true test" is a test that accurately measures the object to be measured and does not measure anything in its place or addition.

Test stability:

In order to extract the stability coefficient of the tests, the principle of constant testing is to be applied "which produces the same results or the same results if applied more than once in similar conditions". This is done under similar conditions. And the interval between the first test and the second (7) days where the first test was procedure on Sunday, 18/11/2018 at nine o'clock in the morning and was returned on Sunday 25/11/2018 at nine o'clock in the morning, explains Ibrahim Salameh "The method of retesting from The most simple way is also characterized by a specific clutch of cohesion because of the associated error. The researcher extracted the coefficient of persistence by means of the correlation coefficient (Pearson) between the results of the first test and the results of the second test and extracted the correlation. Indicates that tests are of high moral significance, indicating that the test has a high degree of stability as shown in Table (4).

Test objectivity:

In order for the test to be successful, it must have a high degree of objectivity, and since the tests used are based on simple, clear, easy to understand and non-interpretive terms, the researchers did not extract objective coefficients.

v Test scoring balls:

· Objective of the test: measuring accuracy of scoring.

· The necessary tools: a football field, a condition to determine the area of correction for the test and put (5) balls in specific places of the penalty area.

· Method of performance: The player stands behind the ball number (1) and when the signal is given, the ball is aimed at the goal, and then repeat the correction of the ball (2) and so on until the end of the correction of ball (5)

· Score goals: The score is calculated by the total scores obtained by the player from the five balls that each score corrected score in each region where the ball goes to the division lines are calculated within the highest area and considers that the score outside the goal limits are 0, For testing (25) degrees.

Pre Tests:

Before procedure the pro test under study, the researchers and the teacher gave the students two unite definitions of scoring skills, the nature of the concepts maps, how they were designed, used, and useful.

Then the researchers procedure the tribal tests on Tuesday, 27/11/2018. The study skill (scoring accuracy) of the two groups was procedure at the Sumer Sports Stadium of the Youth and Sports Directorate in Thi- Qar and the presence of the lesson teacher and the auxiliary team.

Post-tests:

After the completion of the curriculum, the researchers procedure post tests of the skill in question on the sample of the two groups on Wednesday (5/2/2019 at Sumer Sports Stadium of the Directorate of Youth and Sports in Thi-Qar, where researchers were keen to be the same or similar conditions In terms of the place and style in which the pre test was procedure , as well as the same auxiliary team.

3.7 Statistical means:

The researchers used the SPSS statistical file to process and extract research data. The laws used in the research were as follows:

1 - the arithmetic mean

2 - the difference coefficient

3. Standard deviation

4. Pearson correlation coefficient

5. Law (t) for non-independent samples

5. Percentage

Results

Table (4) Shows the arithmetic means, the standard deviations and the calculated t value of the football scoring skill of the control and expericoncepts groups in the pre and post tests.

Groups	Statistical processing Skill	Measure Unit	Pre testing		Post testing		T value	Sig	Type
			M	Sd	M	Sd			
Expericoncepts	Scoring	Degree	7.82	1.94	18.89	2.61	12.32	0.000	Significant
Control	Scoring	Degree	8.23	1.66	15.47	2.03	6.62	0.000	Significant

***significance level ≤00.05**

Table (4) shows that the value of the arithmetic mean of the accuracy of the scoring skill in the expericoncepts test of the expericoncepts group is 7.82 and the standard deviation is 1.94. In the post test, the mean value is 18.89 and the standard deviation is (2.32) While the value of (t) calculated was (12.32) and the level of significance (0.000), indicating that there are significant differences between the pre-test and the post-expericoncepts group and for the benefit of the post-test.

The value of the arithmetic mean of the accuracy of the scoring skill in the pro test of the control group is (8.23) and the standard deviation is (1.66). In the post-test, the mean value is (15.47) and the standard deviation is (2.03) while the calculated value t is (6.62) (0.000) indicating significant differences between the pre and post tests of the expericoncepts group and for the post-test.

The researchers attribute the progress of the expericoncepts group to the fact that the use of concepts maps in teaching gives a kind of diversification in teaching methods and contributes to the involvement of a greater number of senses in learning, making learning meaningful, in addition to the interaction [8-12] that took place among the class members during the unit and discuss the task Educational institutions that have an impact on their understanding of the educational material and this has led to an increase in their learning better.

Table)5). Shows the arithmetic mean**s, standard deviations and the value of (t) of the scoring skill of the control and expericoncepts groups in the post tests**

Statistical processing Skill	Measure Unit	Expermintal		Control		T value	Sig	Type
		M	Sd	M	Sd			
Scoring	Degree	18.89	2.61	15.47	2.61	3.873	0.000	Significant

*significance level ≤00.05

Table (5) shows that the value of the arithmetic mean for the accuracy of the scoring skill in the expericoncepts test of the expericoncepts group is (18.89) and the standard deviation (2.61) in the post test of the control group, the mean (15.47) and the standard deviation (2.61)) Was calculated at (3.873) and at a significance level (0.000) indicating significant differences between the control and expericoncepts groups in the post tests and in favor of the expericoncepts group.

Conclusion

Mind mapping technology has an impact on football scoring skills for first-stage students. Taking notes using concepts mapping technology helped the members of the expericoncepts group to plan their ideas fully. The technology used in the current research (concepts maps) has contributed to the development of the dynamic program of the members of the expericoncepts group, through the processes of remembering, retaining and reviewing concepts mapping.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University Thi-Qar and all experiments were carried out in accordance with approved guidelines.

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Effect of Palliative Style Rhythmic Exercises on Some Physical Abilities

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Abstract

Plaque Leave exercises is one of the training means strength that can be used in a wide field to develop muscle reactions and that inevitably leads to the production of various sports movements that can be used in the field, especially in the field of sports training that has to do with the development of the special strength of various sports, including the effectiveness of long jump, Where the body weight itself is a resistance that the internal muscles overcome, especially when performing the jumping movements, which contain many secondary rotational movements that occur in different parts of the body (arms, legs, and trunk), whether these jumps on the ground with body weight or at different barriers of heights or On terraces or on boxes, and the importance of research lies in the fact that percussion-style rhythmic exercises work to develop strength for the lower limbs of people with disabilities and give fluidity and ideal performance in general. As for the research problem, some coaches have not resorted to such modern and advanced methods of training reflected negatively on the physical capabilities of a challenge Disability, as well as controlling the approach steps and the correct use of the upgrade board, which leads to Increase the horizontal distance to achieve the achievement.

Key words: *palliative style rhythmic, physical abilities, effectiveness, jump*

Introduction

The scientific progress that the world is witnessing at the present time is one of the main areas. The scientific progress that the world is witnessing at the present time is one of the main reasons for the progress of human [1] life progress through programmed and studied scientific planning that contributes to achieving human goals, as this progress covered all areas of life, including the sports field, and practical studies and research related to the training process have helped The trainer develops his knowledge of the variables that can affect the success of the training process and thus achieve the best sporting achievements [2] in games that include skills that need the participation of the largest amount of muscle groups and need a high degree of strength.

Every type of sporting activity needs its own physical requirements (physical abilities), and the trainer must have full knowledge of them when defining the methods of sports training, because each of these abilities has a special method of training, which leads to its development to enable the athlete [3]. It is the ability to master the art of motor performance (skill), as (the implementation of the art of motor performance carefully) is a good guide to physical construction. [4] In addition, it is considered (one of the important factors that underpins performance success in order to reach the highest levels of sports, and that the development and promotion of these special characteristics is closely related to the process of developing motor skills).

Plaque Leave exercises is one of the training means strength that can be used in a wide field to develop muscle reactions and that inevitably leads to the production of various sports movements that can be used in the field, especially in the field of sports training that has to do with the development of the special strength of various sports, including the effectiveness of long jump, Where the body weight itself is a resistance that the internal muscles overcome, especially when performing the jumping movements, which contain many secondary rotational movements that occur in different parts of the body (arms, legs, and trunk) [5], whether these jumps on

the ground with body weight or at different barriers of heights or On terraces or on boxes.

The sport of people with disabilities is one of the special and important sports at the present time as it gives a big role in qualifying the properties to achieve the best high sporting achievements and raising the name of their countries in international forums, so we may see many countries that have paid great attention and serious attempts to give equal opportunities to individuals who do not enjoy There is a wide field for movement in exercising and entering competitions with their peers in order to enrich their lives and work to make them happy [6]. Through the foregoing, the importance of research lies in subjecting the sample to vertical and horizontal strength training in order to verify its suitability and ability to provide young players with physical capabilities that contribute to developing the values of the strength and time curve, which leads to the upgrading of the skill level, which benefits them and provides national teams for applicants

Methodology

The researchers used the experimental approach in a one-group method to suit the nature of the problem in order to obtain accurate information and results. The experimental approach is one of the most sufficient means to reach reliable knowledge.

Community sample:

The choice of the research sample is closely related to the goals set by the researcher for his research, as it is difficult for the researcher in many cases when studying a phenomenon with the assistance of all members of society, so it is impossible or impossible to choose, note or measure all of these individuals under controlled conditions, so the researchers resort to choosing a representative sample of the original community .

The research community included the players of the Dhi Qar Committee, aged 18-22 years, and they were (3) players, and they were chosen in an intentional way, and they represent (100%) of the society.

Homogeneity of the sample:

For the purpose of verifying the homogeneity of the sample , the researchers took some measures to control the variables, even though the selected sample is from a close age group

Therefore, statistical methods were used by the mean, the standard deviation and the coefficient of variation for morphological measurements to know the reality of the difference or not, and Table 1 illustrates this.

And proved that the value of the difference coefficient is less than 30 %, and the sources state that whenever the coefficient of variation is less than 30 % , this means that the sample is homogeneous.

Table 1. The homogeneity of the research sample is shown in age, height and weight using the coefficient of variation, which shows values less than 30 %.

T	Measurements Morphology	measruing unit	Arithmetic mean	standard deviation	Coefficient of variation
1	Chronological age	Month	193	5. 69	2. 94
2	Training age	Month	33. 12	5.06	15. 27
3	Bloc	Kg	64	6. 34	9. 90
4	Length	Cm	171	8. 25	4. 82
5	Arm length	Cm	81.21	3. 25	4. 01
6	The length of the trunk	Cm	662.15	1.42	1. 99
7	The length of the two men	Cm	103.41	3.89	3.76

Exploratory experience

For the purpose of identifying the accuracy of work and its validity and avoiding obstacles that may arise during the procedures of the field experiment, the researchers conducted an exploratory experiment on 3/14/2019 at ten in the morning "in the local administration stadium and on players (outside the sample), as the benefit of the exploratory experience in avoiding Deficiencies with determining the correct location of the cameras, strength platform, experiment time and duration [(1)] .

The kinetic performance of the overwhelming transmission skill was depicted. The objective of the reconnaissance experiment was:

1- Creating and directing team of assistant Qa « for the objectives of the research.

2-Create the necessary devices and tools.

3-Learn the time required for the test.

The scientific foundations of the test :

1- Validity of the test:

The researchers presented the tests to the experts and specialists in the field of training through personal interviews and their agreement on the validity of the tests as in Table (2).

Table 2 . Shows the percentage of validity of the search tests

T	The name of the test	Number of experts	Number of opinions agreed	Percentage
1	Wide jump test of stability	7	6	85.7
2	Bounce test forward maximum distance in 20s	7	7	100
3	Two-legged strength test	7	6	85.7

Stability test :

Use the researchers to calculate the reliability coefficient (method of testing and re - testing) and the interval of time between the first test and the second (7) days and that the method of re - testing of the most road simplicity is also characterized precisely the separation of cohesion because the error associated with the measurement and fortunately always be more pronounced when there is a period of Between exams from day to day. " ([2]) The two researchers extracted the stability coefficient by the correlation coefficient (Pearson) between the results of the first test and the results of the second test and extracted the correlation significance. The two researchers concluded that tests have a high significance because the calculated value of (T) is greater than the tabular value

Table (3) Stability coefficient is shown for research tests

T	the test	Coefficient of stability
1	Wide jump test of stability	0.86
2	Bounce test forward maximum distance in 20s	0.89
3	Two-legged strength test	0.87

Correlation coefficient is significant at the level of significance (0,05) that the tabular value of (T) is (0,81)

Objectivity

The objectivity of the test is originally due to the clarity of the instructions for applying the test, as the objectivity of the test is high if tests are used that have specific conditions and since the tests used have specific and clear conditions they are characterized by high objectivity, as the results do not accept interpretation. High objectivity when the test conditions are specified, as they are not affected by the arbitrators' self-assessment .

Field research procedures:

Tests tribal of the research sample:

The researchers conducted the tests and the tribal measurement before starting the implementation of the training curriculum on Sunday, 17/13/2019 at nine in the morning (in the local administration stadium in Dhi Qar). All members of the research sample of (3) players attended, and the measurements (lengths) were identified. Mass and age) Then the two researchers and the assistant team performed research tests and extracted the results.

Suggested exercises

In order to obtain exercises with good effectiveness, it was necessary to see the modern sources and references in the science of mathematical training that would be sufficient to enrich the researchers with information that helps him to develop rhythmic exercises in the pluralistic style, so the researchers prepared the exercises for the individuals of the research sample (the experimental group) based on preparing it on the scientific foundations For training and to some scientific sources and references as well as the opinions of some specialists in the field of sports training science, biomechanics, and volleyball.

As the application of exercises began on Tuesday, 3/19/2019, until Tuesday, and on 14/5/2019, for a period of eight weeks, with three training units per week (Sunday, Tuesday, Thursday). The curriculum focused on developing and improving capabilities related to the lower limbs, and a sample underwent The research is supervised by the researchers and the assistant team.

Dimensional tests for the research sample:

The post-test of the research sample was conducted on Friday 17/5/2019 (in the court of the local administration in Dhi Qar) after completing the period of application of the curriculum, which took (8) weeks, and the researchers were keen to provide the conditions of the pre-test and its procedures followed for the research tests.

Statistical means :

The researchers used the statistical methods that helped in treating the results and testing the research hypotheses through the use of the statistical bag (IBM SPSS Statistics 24) , Namely:

- Arithmetic mean .
- standard deviation .
- Coefficient of variation.
- Pearson correlation coefficient.
- Test T)) For correlated samples.
- Percentage.

Table 4. Shows the values of the arithmetic mean, the standard deviations, and the value T Calculated for pre and post test samples

Physical abilities	measuring unit	Overwhelming beating Before me - female officer		Overwhelming beating After me - female officer		T Calculated	Sig	The result
		s	P	s	P			
The explosive power of the two men	Meter	2.13	10.30	297	1.19	4.11	0.000	Moral
Distinguished strength at the speed of the two men	meter	11.04	1.77	13.87	4.25	3.55	0.001	Moral
Endure strength	Repetition	38.14	2.35	52.45	10.59	8.23	0.000	Moral
Achievement	M	5.63	4.46	6.08	6.89	1.25	0.000	Moral

***Significant at the level (0.05)**

Table (4) shows the mean, standard deviations and value (t) Calculated between the results of the tests of tribal and dimensionality, with the results shown in the table showed that the value of the level of significance in all values reached between (0. 001) to (0 , 00 0). It is less than the value of the significance level (0 , 05), which indicates the presence of statistically significant differences between the pre and post tests of the sample.

Discuss the Results

The strength indicators for the two men have a fundamental role in achieving the achievement in the effectiveness of the long jump, as the results of these tests showed that there is a clear progress from the results of the post-test of the experimental group, which indicated that the exercises for the use of rhythmic exercises in the pluralistic style, which the researcher used in his training method to develop strength The explosive power and the speed characteristic of the group and the bearing strength of the members of this group were influential in developing an achievement level.

The rhythmic exercises that were used in the curriculum of the experimental group emphasized that the use of different jumping exercises and weightlifting exercises has an impact on the strength and speed of the two legs during performance and this requires a greater effort to move them and that this effort means an increase in the individual's ability to maintain [8] the speed as much as possible for the distinctive performance that requires From the individual to exert more force to move the body when performing and this means increasing exertion of strength to overcome this resistance and this increase means the development of the muscle and explosive strength of the jump .

Some researchers point out that the emphasis on the development of power and the adoption of exercises related pathways motor performance for the long jump and covered vocabulary training curriculum proposal adopted by the researcher that in training rhythmic manner Albulaaomitri[9] as has facilitated the implementation of the balanced

payment and control steps movements of the body during the performance at each stage, which helped To coordinate the movements according to the path of the body's movement at every moment of stabilization and pushing for each stage and doing so to develop the necessary push for the joints of the two working men and achieve the movements in the smooth and [10] correct exchange of both the driving man or the leader as this process helped generate an explosive propulsion movement of the two men during The performance and this worked to develop all stages of performance to achieve a better distance when jumping from the control group . And the consistency within the muscles and between them helps to increase the speed of movement (ability), because when the coordinated muscles work, their efforts are united to overcome the external resistance and more quickly .

Conclusions

That the exercises rhythmic style Albulaaometruc have had a significant impact in the development of the physical abilities of the experimental group the fact that these exercises are working on the development of capacity. No notable development was observed in the physical capabilities of the control group due to the adoption of traditional approaches that did not take into account modern methods of sports training Rhythmic exercises in the palladium style, left by this ripple of pregnancy, led to the appearance of this amount of progress in achievement.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University Thi-Qar and all experiments were carried out in accordance with approved guidelines.

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Psychological and Organizational Contexts for Handball Coaches from the Players' Point of View

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Abstract

Importance of research came through the study of Organizational Fairness for handball coaches from the player's point of view as a modest contribution to the advancement of the handball game. The importance of achieving Organizational Fairness in the distribution of duties and rights to the players equally by the coach in the sports team as one of the small groups and this characteristic has a direct impact on the level of players technically, skilled, physically and psychologically, and this indicates the cases of dedication and the high effort to achieve the goals. As for the recommendations, it is the adoption of the current scale by those interested to periodically uncover the level of Organizational Fairness for coaches clubs by hand in training and competition and to benefit from the current scale as an objective research tool by researchers and use it in their research and studies, and it is necessary to inform the trainers of the importance of Organizational Fairness in the sports field when It has an impact on the level of team achievement and the players 'performance in particular in training and competition in order to work on developing it for the players.

Keywords: *handball coaches, Organizational Fairness, players' point*

Introduction

Scientific research and studies in sports are considered the main pillar for improving the level of athletic performance [1], as it is a scientific link linked to an experimental nature that contributes to reaching new facts that can be multiple additions that help in developing the level of players. In many areas of life, it has become possible to use a set of approved tests and measures that indicate the strengths and imbalances in that field, and one of these fields is the [2] bug of sport management in the fields that pertain to the person and in his multiple administrative fields that studies the human nature in terms of management, intellectual, personal and social.

The topic of Organizational Fairness is one of the important topics of great interest by scientists with different specializations [3], so I took many aspects of the personal life of the individual and the scholars addressed them with different approaches. Or sports or economic and other institutions and that for its role in making the performance of players more effective and make a high effort to achieve a specific goal.

Handball is one of the collective games that take a large share of support and encouragement as is the case with the rest of the games and it has a special place in the world in general and the Arab world and Iraq in particular. To activate the foregoing, a coach's role is highlighted in the team's [4] development and development through a leadership and Organizational course in team management, as well as a responsibility in the education, generalization, and training of players and its direct impact on the development of the skill, physical, social, and psychological level as well as the transmission of morale. For what makes him the primary focus and main influencer in achieving sporting accomplishments, therefore, the coach's personality, behavior and treatment of players in training and competitions should be the size of the responsibility above and represented in many concrete activities visible, including Organizational fair [5]. A successful and competent sports coach as a leader should be familiar with his role in planning, organizing

and achieving Fairness between players, which helps increase the effectiveness of the impact to achieve the goal achieved by detailing his role in preparing players physically, skillfully, educationally, psychologically and technically. Hence the importance of research came through the study of Organizational Fairness for handball coaches from the players point of view as a modest contribution to the advancement of the handball game.

Methodology

The two researchers used the descriptive method in the survey method to suit the nature of his research.

Research Community and its Sample

The current research community consists of excellent and first-class players in the Southern Handball Region for the 2020 sports season, which number (195). The research community includes (15) handball clubs that number (178).

Table1. Description and sample of the research community

Siq	The name of the club	the total number	Exploratory	the sample	Percentage
1	Basra Municipality	16		16	100%
2	Gulf	16		15th	93.75%
3	Kut	15th		14	93.33%
4	Zubair	1 2		1 2	100%
5	South Oil	1 2		1 1	92,86%
6	Maysan oil	1 4		1 4	100%
7	Tigris	1 3		1 3	100%
8	Great Hungary	1 4	13	-	93.75%
9	Nasiriyah	1 2		1 1	93.33%
10	Victory	12		1 2	100%
11	Al-Rifai	1 2		1 2	100%
12	Streak	1 2		1 2	100%
13	Euphrates	11		11	100%
14	Shouokh Market	12		12	100%
15th	Sugar castle	12		12	100%
Total		195	13	178	97,24%

Data Collection

The researchers used the following methods: 1- Arab and foreign sources and references. 2- Forms distributed to experts and specialists. 3- Questionnaire. 4- Global Information Network. 5- Personal interviews. 6- The assistant team (*).

Steps to Building an Organizational Fairness Scale

Preparing the primary formula for the scale

To build the scale of Organizational Fairness, the paragraphs were chosen by referring to the game literature, references, available sources, criteria related to the topic and the opinions of some experts, as the researcher was able to form about (23) paragraphs of a scale with four dimensions, namely (distribution Fairness, Fairness of procedures, Fairness of interaction, evaluation Fairness). The paragraphs were presented to a group of experts (**) for the purpose of evaluating and judging them in terms of formulation and suitability in measuring Organizational Fairness for sports handball coaches. In the appropriateness and validity of the paragraphs, and in light of opinions and observations that have not been deleted (3) paragraphs due to their inconvenience and the repetition of their content, the number of paragraphs (20) paragraphs for each of these dimensions has become (5) paragraphs.

Preparing the scale instructions

In order to complete the scale image and apply it to the players. She laid the instructions for the scale because "ensuring the correct answer is by setting instructions that facilitate the correct answer to the respondent. Therefore, it was taken into consideration that it should be easy and understandable, and that the player is assured about the confidentiality of the answer, as he asked the player not to mention the name in order for his answer to be frank and reliable. He also asked Players need to answer all paragraphs and not leave any paragraph unanswered.

The pilot's experiment

The exploratory experiment was conducted on a random sample consisting of (13) players from Hungary Grand Club players from the research community and outside his sample. After conducting the exploratory experiment by the researcher, it became clear that the scale, with its instructions and paragraphs, and how to answer it is clear. And the time taken for application ranges between (5 - 12) minutes, and thus the scale with its instructions and paragraphs (20) became ready to be applied to the research sample.

2-4-4- The basic experiment of the Scale

For the purpose of statistical analysis of the paragraphs, to find its discriminatory strength, its degree of internal consistency, to exclude unrecognized paragraphs, and to find honesty and consistency for the scale, the basic experiment was conducted on the research sample of (161) players.

Table (2) shows the mean and standard deviations of the upper and lower groups and the results of the T-test to calculate the discriminatory strength of the scale paragraphs.

Paragraphs	N	the group	S	+ P	Calculated v	Degree (Sig)	indication	Discrimination
1	31	High	3.32	.611	12. 64	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
2	31	High	3.07	.857	10. 54	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
3	31	High	3.21	.917	27. 00	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
4	31	High	3.39	.628	15. 88	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
5	31	High	3.35	.678	11. 82	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
6	31	High	2.75	.585	10. 49	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
7	31	High	3.14	.803	14.49	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
8	31	High	2.92	716	14.58	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
9	31	High	3.10	.566	18. 44	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
10	31	High	2.92	.262	9. 96	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
11	31	High	3.46	.507	10. 15	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
12	31	High	3.39	.497	14. 18	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
13	31	High	3.35	.558	12. 06	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
14	31	High	3.85	.448	15.26	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
15	31	High	3.67	.547	10.86	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
16	31	High	3.00	272	11,500	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
17	31	High	3.53	.507	14.07	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
18	31	High	3.42	572	19.18	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
19	31	High	3.71	.460	10.20	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				
20	31	High	3.28	.658	11.27	0.000	SIGNIFICANT	Distinctive
	31	Low	5.00	.000				

The correlation of the total degree of the scale with the paragraph is an indication of the sincerity of the paragraph, and this step provides a reliable criterion for finding the relationship between the grades of the forms for each

paragraph of the total degree of scale (1).

Table 3. Shows the correlation coefficient between the paragraph score and the total score for the Organizational Fairness scale

Siq	Calculated value (t)	Sig	T	Calculated value (t)	Sig
1	.369 **	0.000	11	294 **	0.000
2	.342 **	0.000	12	.462 **	0.000
3	.418 **	0.000	13	.597 **	0.000
4	.544 **	0.000	14	.385 **	0.000
5	.587 **	0.000	15th	.407 **	0.000
6	.455 **	0.000	16	.409 **	0.000
7	.387 **	0.000	17	.468 **	0.000
8	.208 *	0.000	18	.465 **	0.000
9	.332 **	0.000	19	.429 **	0.000
10	.375 **	0.000	20	.512 **	0.000

Characteristics Psychometric of the Scale

The truthfulness of the information included in the scale means that this tool can measure the attribute or goal for which it was designed The instrument shall be truthful if it is to the extent that it measures the object that was placed for its measurement.

First: Validity

This honesty is calculated after exposure to a number of experts in the field that measures the test if the experts read that this test measures the behavior that was set to measure it and thus the researcher can rely on the experts' understanding. This kind of honesty was achieved when the researcher presented the scale to a group of experts, acknowledging the validity of the paragraphs, and estimating the extent of each paragraph measuring the components [9, 10] of each dimension of the scale, thereby accepting the dimensions and paragraphs that got the approval of the experts and deleting the inappropriate paragraphs .

Second: Reliability

It is the most appropriate type of sincerity to build standards, and the concept is called sincerity of the concept, based on empirical verification of the extent to which the grades of the paragraphs match the characteristic or concept to be measured .

Validity of the Scale

One of the statistical methods for determining the validity factor is:

First, the half-way method :

For the purpose of finding the scale constant coefficient, the mid-point segmentation method was adopted, and the data obtained by the researcher from members of the research sample of (178) players were approved, where the mean (68,569) for the scale

paragraphs of (20) items and a standard deviation of (5,469). The researcher has divided the scale paragraphs into two halves, the first includes the individual paragraphs (10) and the second includes the marital paragraphs (10). Then, the correlation coefficient between the two degrees of the two halves was extracted using the simple correlation coefficient (Pearson) by the statistical bag of social sciences (spss), As the correlation coefficient reached (.412), as the extracted correlation coefficient means stability for only half of the scale and for a complete test stability of this scale, the Spearman Brown equation was used as a basis to find the scale stability coefficient, then the Spearman-Brown equation was calculated as the coefficient reached Correlation (0.82), which is a high stability indicator, where the stability factor is considered good, if its value is not less than (0.70).

Second: Alpha Cronbach equation

Second : The Alpha Cronbach equation ((Alpha Cronbach)

As shown in Table No. 4 The Fakronach coefficient in calculating dimensional validity, the questionnaire shows that the coefficient of validity values ranged between (.811-717). These values are significant, indicating a high correlation between dimensions.

Table (4) Correlation coefficients between the values shows the weights of the degrees of the total for each area and between the weights of a drawer overall scale of Fairness at the Organizational coaches handball

Siq	Dimensions	The number of paragraphs	(T) between the overall dimension score and the overall of scale	Degree (Sig)	Indication
1	Distribution fairness	5	717 **	0.000	SIGNIFICANT
2	Fairness of procedures	5	.811 **	0.000	SIGNIFICANT
3	Interactive Fairness	5	.774 **	0.000	SIGNIFICANT
4	Evaluative Fairness	5	.793 **	0.000	SIGNIFICANT

The standard scores for the scale .

The researcher adopted that the same sample responses, amounting to (1 78) players after the rest of the paragraphs of the measure through the procedures.

Table (5) Shows the crude grades ranks centipede for the evaluator S Organizational Fairness for coaches football handball

Standard score	Raw grade	Standard score	Raw grade	Standard score	Raw grade	Standard score	Raw grade
1	38.307	26	53.232	51	68.157	76	83.082
2	38.904	27	53.829	52	68.754	77	83.679
3	39.501	28	54.426	53	69.351	78	84.276
4	40.098	29	55.023	54	69.948	79	84.873
5	40.695	30	55.62	55	70.545	80	85.47
6	41.292	31	56.217	56	71.142	81	86.067

Cont... **Table (5) Shows the crude grades ranks centipede for the evaluator Organizational Fairness for coaches football handball**

7	41.889	32	56.814	57	71.739	82	86.664
8	42.486	33	57.411	58	72.336	83	87.261
9	43.083	34	58.008	59	72.933	84	87.858
10	43.68	35	58.605	60	73.53	85	88.455
11	44.277	36	59.202	61	74.127	86	89.052
12	44.874	37	59.799	62	74.724	87	89.649
13	45.471	38	60.396	63	75.321	88	90.246
14	46.068	39	60.993	64	75.918	89	90.843
15th	46.665	40	61.59	65	76.515	90	91.44
16	47.262	41	62.187	66	77.112	91	92.037
17	47.859	42	62.784	67	77.709	92	92.634
18	48.456	43	63.381	68	78.306	93	93.231
19	49.053	44	63.978	69	78.903	94	93.828
20	49.65	45	64.575	70	79.5	95	94.425
21	50.247	46	65.172	71	80.097	96	95.022
22	50.844	47	65.769	72	80.694	97	95.619
23	51.441	48	66.366	73	81.291	98	96.216
24	52.038	49	66.963	74	81.888	99	96.813
25	52.635	50	67.56	75	82.485	100	97.41

moral 0.05 At a degree of freedom (177)

Setting the standard levels of the Organizational Fairness scale

The researcher chose that (5) levels of the scale under study, as shown in table (6).

Table 6. shows levels, standard scores, raw scores, ideal proportions, and percentages for an Organizational Fairness scale

Levels	Standard grades	Raw grades	Number of people	Percentage %
very high	0-20	49.936 or less	33	18.537
high	21-40	50.583-61.927	43	24.157
Average	41-60	62.523 -73.866	50	28,089
Low	61-80	74.46 - 85.866	29	16.292
very low	81-100	86.403 and above	23	12.921

Conclusions

1- Building a sports Organizational Fairness scale on the coaches of clubs in the southern region, handball, in a scientific and objective manne.

2- There is a positive relationship in the application of Organizational Fairness by coaches towards players in the southern region by hand.

3- That working in the field of training in clubs satisfies social and psychological needs without expecting to get rewards or rewards, and this greatly affected the positive relationship between the coach and the players.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Maysan and all experiments were carried out in accordance with approved guidelines.

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The Effects of (I.P.A.G.A) Model in Reflection Thinking and Learning Spike Skill in Volleyball for Students

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Abstract

The aim of the current research is to prepare a scale of reflection thinking for students of the second stage, College of Physical education and sports science, and also identify the effect of the (I.P.A.G.A) model on reflective thinking and learning spike skill of Volleyball for students. And identify the preference for learning between the control and experimental groups. The researchers used the experimental method and designing the equivalent, controlling and experimental groups with pre and post tests, the researcher community included second-stage students in the College of Physical education and sports science – Al Farahidi University for the academic year (2021-2020) and their number (201), chosen two division (A,C), division (C) represented the experimental group and (A) the control group. And (30) students were chosen as a sample of research, they were divided into two groups, experimental and control group, each group (15) students, and (10) students, as exploratory sample also in a simple random way. The results showed through the table that there are significant differences between the results of the pre- and post-test and in favor of the post-test of the experimental group in the variables under study,(reflective thinking, accuracy test for diameter spike, evaluation test for Technical performance of the diameter spike).

Key Words: *(I.P.A.G.A) Model, Reflection Thinking, Spike skill.*

Introduction

The current era is witnessing a remarkable increase in scientific knowledge and an evolution of its applications in all fields, including education. This development is reflected in the various modalities [1] and techniques teaching, including sports, it has become necessary to apply the modalities and techniques to keep pace with this rapid development, Therefore, attention has begun to diversify the models and methods used in all disciplines and in the field of teaching scientific subjects in general and sports sciences in particular [2] so that they transfer them from the established concept that may not be concerned with employing the learner's thinking and mental abilities in his teaching process and does not take into account the learner's needs and cognitive abilities represented in acquiring concepts and mental abilities supreme is like reflective thinking. [3] The I.P.A.G.A model is one of the teaching models that rely on knowledge and is based on cognitive theory and is one of the theories that tried to explain the phenomenon of learning, it cares about internal cognitive processes such as (understanding, receiving and processing information) [4], the term (I.P.A.G.A) refers to five steps, where each letter indicates one of these steps as follows:(Introduction) represented by the letter (I), (Presentation) represented by the letter(P), (Association) represented by the letter(A), (Generalization) represented by the letter(G), (Application) represented by the letter(A). On the other hand Volleyball is considered a practical subject in the College of Physical education and sports science and includes a set of technical skills that need high physical, motor and skill requirements for their performance, including spike skill which needs to make a great effort and high technique in order to perfect its technical performance. Hence the importance of the research in that it is a scientific [5] attempt to use a model of cognitive theory which may help in determining methods and styles instructions appropriate to the educational situation, leading learners to desired learning outcomes

and developing it through the development of thinking, discovery and reflection, while gaining information towards learning the skills of volleyball.

Methodology

The researchers used the experimental method and designing the equivalent, controlling and experimental groups with pre and post tests, the researcher community included second-stage students in the College of Physical education and sports science – Al Farahidi University for the academic year (2021-2020) and their number (201), chosen two division (A,C), division (C) represented the experimental group and (A) the control group. And (30) students were chosen as a sample of research, they were divided into two groups, experimental and control group, each group (15) students, and (10) students, as exploratory sample also in a simple random way.

The scientific foundations of the scale:

Honest of the scale: This type of honesty was achieved when the scale was presented to a group of (15) experts and specialists in the field of (teaching methods, volleyball, tests and measurement).

Stability of scale: To extract the results of the stability of the scale, the researcher used the method of the (Alpha cronbache) coefficient, as this method was applied to the sample number setting of the number (134) depending on the degrees of the individuals of the sample numbers taken from the original (134) form, so the stability factor reached (0,871), which is a high coefficient,

Scientific foundations of the tests:

Honest of the tests: The tests were presented to a group of experts and specialists in the game of volleyball and in the areas of testing and measurement to determine their agreement on the suitability of these tests to the research sample, and the results of this were extracted kind of honesty, and the tests that got the highest agreement rate were chosen as shown in table (1).

Stability of tests: The researcher approved the method of applying the test and re-applying the test, the survey sample, whose number is (10) students, on days (23-24/1/2020) at ten o'clock in the morning, then the researcher found the relationship between the results of the first and second experiments using the simple correlation coefficient (Pearson), the results showed that all tests had a strong and high correlation degree of stability, as shown in table (1).

Table (1). Shows the stability and objective values of the tests

Variables	Measuring unit	Coefficient of stability	sig	Level of significance	Objectivity	Sig	Level of significance
Test The accuracy of the diameter spike.	Degree	0.883	0.001	sig			sig
Technical performance evaluation (technical skills) for the diameter spike skill.	Degree	0.853	0.002	sig	0.469	0.017	sig

Objectivity of tests: The researcher used two (judgments) to evaluate the technical performance (technique) of the spike skill through its apparent form and a test of evaluating the technical performance of the diameter spike skill, as the correlation coefficient (Pearson) between their grades was calculated, It has been shown that these two tests are highly objective, as shown in table (1).

Main experiment procedures:

Pre-tests:

pre-tests were performed a scale of reflective thinking for the research sample (control and

experimental groups) on 30/01/2020 at 10 o'clock in the morning in the closed sports hall in the Faculty of Physical Education and Sports Science - Al-Farahidi University, also, pre-tests were conducted to measure the skills chosen for the control and experimental groups on 30/1/2020 at 10 o'clock in the morning, pre-tests were performed to evaluate the technical (technical) performance of the spike skill through its apparent form and its three divisions (preparatory, main, and final).

Equivalence of the two research groups:

The researcher performed the equivalence for two research groups for variables related to the research and before starting the implementation of the educational units on the main research sample, the researcher found the equivalence of the two groups (control and experimental), this is a good indication that these two groups are equal in the research variables, as the tabular value (t) reached (2,042) and under the significance level (0.05) and with a degree of freedom (28) as shown in table (2).

Table (2): Show equivalence between the two groups (control and experimental) in the pre- tests for a scale of reflective thinking and for the technical skill performance of the spike of volleyball.

Variables	experimental group		control group		(t) value	Level of significance	Type of significance
	Mean	STD.EV	Mean	STD.EV			
reflective thinking	46.200	3.931	45.466	3.113	0.566	0.576	sig
Test The accuracy of the diameter spike.	6.600	2.229	5	1.463	2.323	0.128	sig
Technical performance evaluation (technical skills) for the diameter spike skill.	4.462	1.307	4.174	0.501	0.797	0.423	sig

Preparing educational units according to the form(I.P.A.G.A) for the experimental group:

The researcher prepared the educational units for the experimental group according to the model (I.P.A.G.A) to improve and develop reflective thinking and learn the skillful performance of volleyball, the required number of educational units was determined by (16) units and by two educational units per week with a time of (90) minutes, The preparatory section and the main section of the educational unit were chosen to apply the five stages of the model accompanying the visual presentation methods, and the appropriate times were distributed for each of these stages.

Designing the means of presentation used in the educational units of the experimental group:

The educational units prepared according to the (I.P.A.G.A.) model included the introduction of educational (visual) aids that the researcher seeks to help students of the experimental group in learning, improving and developing their level of skill performance in volleyball, and these methods were approved in the presentation step of the model steps.

Implementation of educational units prepared according to the form (I. P.A.G.A):

Before starting the implementation of the prepared educational units, the researcher gave two introductory educational units[5] ,concerning the spike according

to the (I.P.A.G.A) model for the experimental group students, on days corresponding to (3-4/2/2020) at 10 in the morning, this is to introduce them to the new working mechanism of the (I.P.A.G.A) model , the implementation of the educational units started at the beginning of the second course for the academic year (2019-2020) starting on the day corresponding to (5/2/2020) at ten in the morning, the last educational unit was on 23/2/2020, and the educational units were given to the experimental and control groups through the same teacher in order to get away from all effects and obtain accurate results.

Post-test:

Post-test of the experimental group were conducted in the same times and places in the pre- tests after the end of the 16 educational units.

Results

Table (3). Shows the mean, standard deviations, and (T) value for pre- and post-test of the experimental group.

Variables	Pre-test		Post-test		f-	SE	T- test	Level of significance	Type of significance
	Mean	STD.EV	Mean	STD.EV					
reflective thinking	46.200	3.931	69.400	3.202	-23.200	0.75719	-30.640	0.000	sig
Test The accuracy of the diameter spike.	6.600	2.229	11.666	1.951	-5.06667	0.43058	-11.767	0.000	sig
Technical performance evaluation (technical skills) for the diameter spike skill.	4.462	1.307	7.729	0.507	-3.26667	0.38503	-8.484	0.000	sig
significance > 0.05 at freedom degree (14)									

The value of the tabular(T) was (2,145) below the significance level of 0.05 and freedom score 14.

Table (3) shows the results of pre- tests, their mean, and their standard deviations for reflective thinking, through the inference of the significance of the difference between the two means using the test (t) of the correlated samples, it appeared that the mean of the post and pre differences (23.200-) with the standard deviation of the post and pre reached (0.75719), while the calculated value of (T) (30.640), It is greater than the tabular under the significance level (0.05) and the degree of freedom (14), and this indicates a significant difference between the pre and post- tests, and in favor of the post test, as for testing the accuracy of the diameter spike, and by inferring the differences between the two arithmetic means, the mean of the post and pre differences was (5.06667-), while the standard post and pre deviation was (0.43058), ش the calculated value (T) reached (11.767-), it is greater than tabular under the significance level (0.05) and with a degree of freedom (14) this indicates a significant difference between the pre and post tests and in favor of the post test, upon inference about the significance of the differences between the two arithmetic modes for the test (evaluation of technical performance (technical) for the diameter spike) in the pre measurement (4.462) and (1.307), respectively ,s for the mean value and the standard deviation of the post- tests, it reached (7.729) and (0.507), the results showed the calculated value (t) was (-8.484) which is greater than its tabular value at the significance level (0.05) and the degree of freedom (14) this indicates the significance of the differences between the pre and post tests and in favor of the post tests.

Table (4) Shows the arithmetic mean, the standard deviations, and the (T) value of the pre- and post-test of the control group.

Variables	Pre-test		Post-test		f-	SE	T- test	Level of significance	Type of significance
	Mean	STD.EV	Mean	STD.EV					
reflective thinking	45.466	3.113	56.600	3.224	-11.13333	0.69602	-15.996	0.000	sig
Test The accuracy of the diameter spike.	5	1.463	8.200	2.242	-3.20000	0.57900	-5.527	0.000	sig
Technical performance evaluation (technical skills) for the diameter spike skill.	4.174	0.501	5.173	0.547	-0.99867	0.20827	-4.795	0.000	sig
significance > 0.05 at freedom degree (14)									

The results of table (4) show differences and variations in the values of the arithmetic mean and the standard deviations between the pre and post -tests of the researched variables among the students of the control group in the pre and post -tests and when inferring the significance of the difference between the two arithmetic means through the use of the (T) test of the interconnected samples, it showed that the value of (T) Calculated (15.996), which is greater than the tabular, this indicates that there is a significant difference between the pre and post- tests in favor of the post- test for reflection thinking, in the test (the accuracy of the diameter spike.) when inferring the significance of the differences between the two arithmetic means, the results of the calculated (T) value of (-5,527) showed that it is greater than its tabular value and this indicates the significance of the differences between the pre and post- tests and in favor of the post- tests, when inferring the significance of the differences between the two arithmetic means, the results of the calculated (T) value (-4.795) showed greater than the tabular value at the significance level (0.05) and the degree of freedom (14), this indicates the significance of the differences between the pre and post-tests and in favor of the post- tests in the evaluation technical performance (technical) of the diameter spike skill, the results showed through table (4) that there are significant differences between the pre and post- test of the control group and in favor of the post- test, the researcher attributes this development to the students of the control group to the teaching method used by the teacher in reflective thinking and learning the spike skill of volleyball, this is through his theoretical information, explanations, and explanatory presentations of the educational material

and the application of special exercises to develop the variables under discussion and [8,9] the use of all tools and means available to him during the educational units, this is consistent with what was mentioned in that "Giving organized and scientific exercises a significant impact in developing performance.[7]

Conclusions

By presenting, analyzing and discussing the results in the fourth chapter, the researchers reached the following conclusions: The (I.P.A.G.A) model has a positive impact on the superiority and upgrading of the experimental group students in reflective thinking and spike skill of volleyball. The I.P.A.G.A model provided ample opportunity for students to interact collectively, and work in the spirit of one team, because it is based on providing an educational environment collectively in teaching volleyball that gives excitement, excitement and focus by linking ideas and concepts, as well as increasing the level of interaction of students and increasing their motivation towards the lesson, it has contributed to removing the boredom and stagnation of students and this through their use of methods Various educational methods and methods were not familiar to them before.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Physical Education & Sport Sciences and all experiments were carried out in accordance with approved guidelines.

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Contribution Rates of Psychological Skills in Learning Some Basic Handball Skills in Secondary School Students

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Abstract

The research aims to identify ;the contribution rates of psychological skills (the ability to visualize, the ability to relax, the ability to focus attention, the ability to face anxiety, self-confidence, motivation of sports achievement) in learning the basic skills of handball (passing and receiving skill, skill jumping, skill The main sample size was (65 secondary school students in the Republic of Iraq.The researcher connected that there is a relationship and contribution rates for psychological skills (ability to visualize, relaxation ability, ability to focus attention, ability).On the face of anxiety, th Self-Ge, sports achievement motivation) to learn some basic handball skills (skill Altmrerwalastelam, Harrah's Altntit, skill correction).

Keywords: *Psychological skills, basic skills, handball, secondary school students*

Introduction

The psychological aspect is the most important prerequisite for learning handball game skills, as it qualifies students to perform physical aspects, skill movements, and collective defensive and offensive tactical installations [1]. The nervous and muscular of the student to adapt to the various fast situations, and in the case of physical and technical aspects between two teams often the psychologically prepared team wins better, and sometimes the psychologically better team may win over a better team than E in the physical and skill aspects [2]. And both add Saeed Nizar Saeed (2011) The psychological aspects play an important role in learning the skills of the handball game, which is characterized by the multiplicity of its skill and its sequence as well as its interconnectedness, which makes the dependence of each skill on the next [3] which precedes it and affects its level of accuracy, which increases the effectiveness of the psychological factor, and handball is one of the games with emotional activities In which there are many competitive positions that may affect the psychological aspects, especially that the outcome of handball matches must end with the victory of one of the two teams and there are no compromises in them [4], and this constitutes a psychological burden for all parties to the game, especially students. The researcher believes that the characteristics of the game of handball require the availability of psychological characteristics at a high level among students and require their learning. Students enjoy the ability to focus attention and self-confidence in the first place. And the ability to face anxiety and the ability to relax and lack tension in the first place. Mental visualization is the ability of students to evoke in his mind or remember previous events or experiences, and to evoke [5] events or situations that have not previously occurred, and mental perception of motor skills helps students achieve more understanding of the nature of the performance of skills and there is no doubt that this helps him to become more Familiar with the requirements for successful motor skills in play and competition situations. The ability to relax is one of the psychological skills and a method used to improve the skills of students in order to face stress, which allows them to deal positively with the pressures experienced by them in competition situations, and that the method of relaxation of psychological skills [6] is of value that the athlete benefits from. The ability to focus attention is one of the important psychological skills of athletes, as it is the basis for the success of the education, training or competition process in its various forms. Distraction and lack of focus negatively affects performance, and that many athletes attribute the reason for the low level of their performance in competition to losing focus, and the more they increase Students are able to focus on the performance that they perform the better response they achieve. This research aims to identify the contribution

rates of psychological skills (ability to visualize, the ability to relax, the ability to focus attention, the ability to face anxiety, self-confidence, motivation of athletic achievement) in learning skills Essential for handball (passing and receiving skill, stumping skill, aiming skill).

Methodology

Research Society and Sample:

The researcher chose the sample randomly from among secondary school students in a secondary school Al-Batha Boys School.Dhi Qar, Republic of Iraq, the basic sample size was (65) students from secondary school students, and scientific transactions were conducted on a sample number (15) students from the research community, but not from the core sample.

Table (1) Characterization of openness

Number	Sample:	The core sample	Scientific Transactions Sample	TOTAL
1	Number	65	15	80
2	Percentage	81.25	18.75	100%

Data collection methods:

1- Forms containing psychological scales.

2- Forms for recording the results of skill tests.

the exams Used for research:

Firstly- Psychological skills tests:

1- Estefan Bell, Jenson Al-Benson, Christopher Shambrook, Arabization and Quote Muhammad Hassan Allawi to measure ability to cope with anxiety, motivating athletic achievement.8: 543-547)

2- Relaxation Scale: The Frank Vitat Preparation (frank vital The translation of Muhammad Hassan Allawi and Ahmed Mustafa Al-Swaify.

3- Network test to focus attention: Prepared by Muhammad Al-Arabi Shamoun 1996.

4- Mathematical confidence status test: Prepared by Muhammad Hassan Allawi.

5- Multidimensional mental perception scale in the sports field: Prepared by Muhammad Al-Arabi Shamoun and Magda Ismail 1996 .

Secondly- Skill tests:

1. Skill test (scrolling).
2. Skill test.
3. Aiming test (aiming).

Scientific coefficients for the tests:

The scientific treatments for the tests were carried out by applying the tests to (15) students from the research community and not from the basic sample, then re-applying them to the same students with a time interval of (7) days (test re test This is to find the coefficient of persistence, and because these tests were applied previously in scientific studies on Iraqi society, the researcher contented themselves with the honesty of the tests, which is the square root of the correlation coefficient, and Table (2) explains the scientific coefficients (honesty, consistency) of the research variables.

Table (2) Scientific treatments for psychological and skill tests (N = 15)

Number	5. Research variables:	Implementation # 1:		Implementation # 2:		Correlation coefficient	Validity coefficient
		Q	F	Q	F		
1	Mental visualization	17.50	1.75	17.45	1.75	0.84	0.92
2	The ability to relax	17.17	1.54	17.65	1.33	0.76	0.87
3	The ability to focus attention	16.90	1.35	16.80	1.45	0.87	0.93
4	The ability to cope with anxiety	17.45	1.19	17.53	1.43	0.77	0.88
5	Self – confidence	17.73	1.52	16.80	1.72	0.87	0.93
6	Achievement motivation	18.18	1.50	18.65	1.61	0.82	0.91
12	PASS	7.30	2.003	Seven twenty.	1.989	0.90	0.92
13	Bouncing	41.90	6.691	41.70	5.964	0.85	0.92
14	Aiming	6.30	1.636	6.30	1.494	0.77	0.88

The value of the tabular t at the level of significance (0.05) = 0.441

It is clear from Table (2) that all tests are characterized by a high degree of correlation as the values of the correlation coefficient were between (0.77 and 0.90) and all of them are indicative at a significant level (0.05), which indicates that the tests are characterized by a high stability coefficient, as it appears that the self-honesty coefficient They ranged between (0.87 and 0.95), which indicates the validity of the tests used in the research.

Results

First: display results, impose search and text: There is a significant contribution of psychological skills (ability to visualize, ability to relax, ability to focus attention, ability to face anxiety, self-confidence, motivation, athletic achievement, physical self-esteem, and skilled self-esteem) in learning basic handball skills.

Table (3) The mean and the standard deviation of the search variables

Number	Variables	arithmetic mean	standard deviation
1	Mental visualization	17.55	1.77
2	The ability to relax	17.65	1.31
3	The ability to focus attention	16.98	1.35
4	The ability to cope with anxiety	17.53	1.30
5	Self – confidence	16.73	1.32
6	Achievement motivation	18.71	1.55
12	PASS	7.63	1.11
13	Bouncing	41.87	1.33
14	Aiming	6.64	1.54

Table (3) shows the mean and the standard deviation of the variables under consideration.

Table (4) Matrix correlation between the psychological variables in question and the passing skill

Variables	Mental perception	Relaxation	Focus attention	Face anxiety	Self – confidence	Achievement motivation	PASS
Mental visualization	-						
The ability to relax	0.88	-					
The ability to focus attention	0.76	0.76	-				
The ability to cope with anxiety	0.87	0.65	0.71	-			
Self – confidence	0.56	0.54	0.82	0.89	-		
Achievement motivation	0.87	0.96	0.68	0.59	0.77	-	
PASS	0.78	0.82	0.74	0.91	0.76	0.93	-

The value of the tabular t at the level of significance 0.05 = 0.215

It is clear from Table (4) that there is a statistically significant correlation between passing skill and all psychological variables under investigation where calculated (t) values ranged between (0.74 and 0.95), while the

tabular value (t) equals (0.215) at the level of significance (0.05), It is also evident that there are statistically significant relationships between all psychological skills.

Table (5) The final step in the logical analysis of the regression of psychological variables on the skill of passing

Variables	Partial regression rate	Standard error	A calculated	Shareholding Percentage
Fixed amount	1.86	0.97	1.92	
Mental visualization	0.18	0.11	1.56	0.30
The ability to relax	0.10	0.16	0.59	0.19
The ability to focus attention	0.15	0.21	0.70	0.21
The ability to cope with anxiety	0.20	0.28	0.72	0.22
Total contribution ratios			0.92	

Table (5) shows that the most psychological variables contributing to the performance of the preparation (passing) skill is the ability to visualize by (30%), followed by the ability to face anxiety by (22%), followed by the ability to focus attention by (21%), followed by the ability to Relaxation rate (19%), and it is noted that the total contribution rates equal to (92%), which is a high percentage of psychological variables in the skill (passing).

Table (6) Matrix correlation between the psychological variables under study and stubbing skill

Variables	Mental perception	Relaxation	Focus attention	Face anxiety	Self – confidence	Achievement motivation	Bouncing
Mental visualization	-						
The ability to relax	0.88	-					
The ability to focus attention	0.76	0.76	-				
The ability to cope with anxiety	0.87	0.65	0.71	-			
Self – confidence	0.56	0.54	0.82	0.89	-		
Achievement motivation	0.87	0.96	0.68	0.59	0.77	-	
Bouncing	0.78	0.82	0.74	0.91	0.76	0.93	-

It is clear from Table (6) that there are a number (36) correlation coefficients and all of these coefficients are positive, as it is clear from the table that there is a statistically significant correlation between bouncing skill and all psychological variables under discussion where calculated (t) values ranged between (0.74-0.95) while The value of the (T) tabular equals (0.215) at the level of significance (0.05), as it is clear that there is a statistically significant relationship between all psychological variables.

Discuss the Results

It is clear from Table (5) that the percentage of the contribution of the skill of mental visualization to (passing) has reached (30%), and it is considered an appropriate percentage because of the importance of mental visualization in the skill of preparation, where students need a great amount of evoking his previous knowledge of the capabilities of each of his team players In the performance of the attack, as well as needs to evoke the previous positions of successful attack operations in order to repeat it and the unsuccessful exclude it and with multiple types of pass, as shown in Table (5) that the percentage of the contribution of the ability to face anxiety in the skill of preparation amounted to (22%), which is also considered an appropriate proportion to the need Designed to counteract anxiety when passing balls, there is always fear of wear Lap and anxiety the direction of passing the ball always has a question is whether passing the ball is appropriate in terms of strength and distance, is its height appropriate, is its position appropriate, is this type of passing appropriate, is the appropriate type of passing for this position chosen all these and other concerns that make the stomach in a concern On the performance of the passing skill, and for this reason the importance of being able to face anxiety is evident, so the contribution rate was appropriate for this skill.

Table (5) also shows the percentage of the ability to focus attention in the preparation skill, which reached (21%), which is also a required percentage because success in performing this skill requires focus and attention because focus of attention is necessary in the success of the skill and distraction of attention and lack of focus negatively affects the performance of the skill Passing requires a great deal of focus, as many variables enter the pass process, including the level of reception. All these variables require an appropriate amount of attention concentration, and therefore the contribution rate of the ability to focus attention came at a high rate, as Table (5) shows that the contribution rate of the ability to relax It reached (19%) and this too Required for the stomach, if students feel tense and anxious, he cannot pass the ball in the right place at the appropriate height, and relaxation is required for the player passing the ball so that he can perform the skill in the required image. The contribution of attention concentration in the stumping skill amounted to (33%), which is appropriate to the requirements of the stumping skill of the focus of attention, where students need a degree of concentration and not being distracted when the ball is struck so that he can estimate the place where the ball descends on the ground as well Estimating its speed in order for it to reach the ball and the appropriate height, as the need to focus attention appears when the ball descends in a place between the players and the dependence of both of the student on the other, the most focused students are the students who bounce the ball and not leave it to the opposing team. the percentage of the contribution of mental visualization in stubborn skill reached (22%), because students, when performing this skill, must have a large amount of mental visualization, because students who are the axes must visualize the place of the conversation and evoke previous successful performances and situations that have passed Previously when discussing at previous times and biases the successful performances from them and excludes the failed performances, as well as there is a relationship between the students axes and the place of the ball descending by invoking the students' knowledge of the correct way of performance, in addition to that he imagines and imagines his team's attack method so that the dialogue is done in a way that helps in the success of the attack method. The percentage of the self-confidence contribution in the skill of receiving the transmission reached (18%), which is a logical percentage of the skill's need of a high degree of self-confidence so that students can have an appropriate dialogue and deliver the ball in the nearest place to repent on the goal, and the self-confidence feels Students with rest, non-arousal, intolerance, lack of tension, and weak students 'self-confidence lead to his failure to perform the bouncing skill, in particular the bouncing skill is

one of the most important skills in handball because it is a basis for good preparation processes [7] for attack and thus good attack, and therefore this skill needs a high rate Self-confidence to ensure To succeed in this performance skill. The percentage of contribution motivation achievement in the correction skill reached (30%) because it needs a great deal of any motivation in achieving goals because this skill needs to move quickly to the ball and may need to jump forward and fall to the ground or side fall and side roll You may [8] need to move from one place to another at a maximum speed as well as to change direction at a high speed, and therefore the importance of students' motivation to achieve the skill appears so that he can perform this skill.

Conclusions

There is a relationship between psychological skills (the ability to visualize, the ability to relax, the ability to focus attention, the ability to face anxiety, self-confidence, motivation to achieve sports) and some basic handball skills (passing and receiving skills, bouncing skills, shooting skills). The most important psychological skills a handball player needs when performing a passing skill are the ability to visualize, the ability to face anxiety, the ability to focus attention, the ability to relax. The most important psychological skills a handball player needs when performing a bouncing skill are the ability to visualize, self-confidence, the ability to focus attention, the ability to face anxiety. The most important psychological skills a handball player needs when performing a correction skill are the ability to relax, self-confidence, motivation to accomplish, the ability to visualize.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Dhi Qar Education Directorate and all experiments were carried out in accordance with approved guidelines.

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Evaluation of Psychological Skills and Effect of an Educational Curriculum with a Model to Accelerate Thinking in the Arbitrary Performance of Students with Handball

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Abstract

The aim of the research is to identify the effect of the educational curriculum prepared with the model to accelerate thinking in the arbitration performance of students with handball and the extent of their response. Experimental approach used to design (two equal groups) with pre and post tests to suit the nature of the research and its problem, and the researcher identified the research community with the fourth stage students in the College of Physical Education and Sports Science / Dhi Qar University, who are (90) students for the academic year (2019/2020) distributed On two divisions (A & B), where the division (A) was identified as a sample for research, (30) students were selected, including a sample for research and (10) students for a survey sample Also in the simple random method, then the researcher designed a form for evaluating the arbitration performance of students with handball and was presented to the experts and specialists, who number (15) expert and specialist in handball game in order to determine the validity of the form paragraphs, and appropriate statistical treatments were used to reach the results, then they were Presentation, analysis and discussion of results.

Keywords: *educational curriculum, thinking, arbitrary performance, handball*

Introduction

Scientific progress plays a major role in providing the teacher with modern educational tools, devices, means and models [1], whether cognitive or applied, that help to facilitate the delivery of information to learners, and the model of accelerating thinking is considered a modern method [2]. As it is based on five stages, relying on the constructivist theory of Piaget and the social Vygotsky, and the basis of this model is based on developing a problem or question for students who cannot answer it, as this will put them in a mental struggle to generate new ideas and find a correct answer and solutions to the problem or question and thus move them to a stage of development My mind is advanced [3]. Education in the colleges of physical education and sports science aims to prepare the student for his simple abilities to prepare him and make him a successful academic, and in order to achieve this, the vocabulary of the educational curricula must be developed according to scientific foundations commensurate with the capabilities of students [4]. Among the sports that are taught in the College of Physical Education is handball, which is one of the difference sports that requires its students to be at a high level of learning the skill performance as well as the legal aspect in order to create academic students who work well with good and appropriate capabilities consistent With the requirements for the performance of basic skills and the legal aspects of handball, the legal aspect of handball is an important and major aspect that cannot be neglected, which is a link movement and a watershed between students, which works to link skill and technical performance.

Methodology

The researcher used the experimental approach to suit the nature of the research and its objectives and to be the appropriate means to prove hypothesis. The experimental approach is defined as "an attempt to control all the basic factors affecting the variable or dependent

variables in the experiment except for one factor that the researcher controls and changes in a specific way, with the intention of identifying and measuring the effect of the variable or dependent variables.

Research community and sample:

The researcher will adopt the design of the control and experimental groups. As for the research community, it may be students of the fourth stage in the College of Physical Education and Sports Science / Dhi Qar University, who are (90) students divided into two divisions (A) and (B), where the division (A) was chosen by the method Randomization as a sample for research. (30) students were chosen as a precise sample identification and (10) students for the exploratory experience, and this was done in a simple random manner.

Field Research Procedures:

Designing a handball arbitration performance evaluation form:

By informing the researcher of previous studies in the field of handball, he was unable to find a form to evaluate the arbitration performance in handball, so he resorted to designing a special form for that by taking the opinion of specialists, as well as looking at the scientific sources and references, and a poll (15) From the game specialists to indicate the validity of the evaluation paragraphs and the scale scale, and Table (1) shows the validity of the form.

Table (1). The handball assessment form is shown to students

S	Paragraphs	K2	indication
1	stand diagonally with fellow	15	Valid
2	move at a suitable speed with a players movement	15	Valid
3	Use the whistle with the required power, with playing conditions	15	Valid
4	Use of the appropriate arbitration notice according to the type of legal case	14	Valid
5	Adherence to the duties assigned to it as a good court judgment or a goal referee	14	Valid
6	Correct movement by referencing as a goalkeeper or progress as a referee	15	Valid
7	Cooperation with the technical supervisor and table rulers	14	Valid
8	Move and take the right position when carrying the throws	15	Valid

The scientific foundations of the arbitration performance evaluation form:

Validate the form:

The sincerity of the form was found through apparent honesty as it was determined based on the opinions of (15) experts and specialists in the field of handball who, through the validity of the paragraphs and their affiliation with the arbitration performance, which were inferred by the test (Ka 2) to accept them all, as well as was extracted Verify the content by analyzing the paragraphs of the form to determine the jobs, aspects and levels to be evaluated by students (rulers)

Fixed form:

The stability of the form was found by piloting it to three of the constituents experts and specialists in handball, through the evaluation of referees for the Premier League matches of handball for the sports season (2018-2019), based on the video registration of three of

the matches for a period of (10) actual minutes of a period The match, as the evaluation took place on 12/25/2019, and the same matches were re-evaluated on 14/14/2020, as the simple correlation coefficient (Pearson) was found between the results of the evaluation of the evaluators, which showed the stability of the form with a very high degree, and table (2)) It shows the consistency of the form according to the evaluation of the evaluators.

Table (2). It shows the consistency of the handball arbitration performance evaluation form for students

Rectifiers	correlation coefficient	Significance level	Statistical significance
The first	0.96	0.001	Moral
The second	0.94	0.002	Moral
Third	0.97	0.001	Moral

Objectivity of the form:

The objectivity of the form was represented by the instructions shown to the evaluator of what is required to evaluate the judges according to the evaluation paragraphs, as the form was indicated in Table (1), as well as the scale of appreciation for the progressive evaluation of (zero), which represents the lack

The degree to the number (10), which represents the highest degree of evaluation, and in light of the procedures that were taken to design the evaluation form, procedures for how to evaluate students in the arbitration performance were described.

The main experiment of the thinking acceleration model:

Pre-test:

The researcher conducted the pre-test on the research sample of (30) students, for arbitration in handball within two days, as follows:

- Evaluating the arbitration performance by handball on December 25-26, 2019.

In light of the results of the pre-test, the researcher conducted the homogeneity and parity of the research sample in order to initiate one point experimentally for the research, as the result of a zero coefficient of coefficient which indicates the homogeneity of the sample, and table (3) shows that, While Table (4) shows the equivalence of the sample in the research variable (dependent), as the value of (t) was not significant at the degree of freedom (28), and at the level of significance (0.05).

Table 3. It shows the homogeneity of the research sample in the variables studied

Variables	arithmetic mean	The vein	standard deviation	Coefficient of torsion
Arbitration performance	19.76	18.00	6.00	0.29

Table (4). Shows the equivalence of the two research groups

Variables	experimental group		control group		value t	significance level	Type of indication
	arithmetic mean	standard deviation	arithmetic mean	standard deviation			
Arbitration performance	19.93	2.73	19.60	2.77	0.331	0.743	not significant

Results

For the purpose of describing the results of the sample individuals, the researcher statistically processed the data using the arithmetic mean and standard deviation. For the purpose of knowing the significance of the differences between the pre and post tests, and for the two research groups, a test (t) was used for the correlated samples. As shown in tables (5) and (6).

Table (5). The description and statistical inference of pre- and post-test results are shown in the research variables of the experimental group

Research variables	pre-test		post-test		(t)	significance level	Statistical significance
	Q	P	Q	P			
Handball Arbitration Performance	19.93	2.73	43.53	7.28	11.74	0,000	moral

N = 14 under the significance level = 0.05

Table (5) shows that there is a difference and difference between the values of the arithmetic mean and the standard deviations between the pre and post tests in the search variable (arbitration performance), where the value of the mean and the standard deviation of the arbitrary performance variable in the pre-test, respectively (19.93) (2.73), while in The post test has reached (43.53) (7.28) and the calculated value of (t) (11.74) is under the significance level (0.05), which means that there are significant differences between the two tests and in favor of the post test because the arithmetic mean is greater.

Table (6). The description and statistical inference of pre- and post-test results are shown in the search variable of the control group

Research variables	pre-test		post-test		(t)	significance level	Statistical significance
	Q	P	Q	P			
Handball Arbitration Performance	19.60	2.77	33.93	3.91	11.56	0,000	moral

Table (6) shows that there is a difference and difference between the values of the arithmetic mean and the standard deviations between the pre and post tests in the search variable (arbitration performance), where the value of the mean and the standard deviation of the arbitrary performance variable in the pre-test, respectively (19.60) (2.77), either

In the post test, it reached (33.93) (3.91) and the calculated value of (t) (11.56) was below the significance level (0.05). This means that there are significant differences between the two tests and in favor of the post test because the arithmetic mean is greater.

Through the presentation and analysis of the results of the pre and post tests of the arbitration performance and of the two research groups (experimental and control) as shown in table No. 5 and No. 6, it was found that there are differences in both groups, as for the differences in the results of the experimental group between the pre and post tests, he attributes The researcher caused these differences in the researched variable to the students 'response to all learning requirements during the educational units of the proposed educational curriculum, as it is one of the most effective means to highlight energies and achieve goals, as the researcher used the philosophy of the model to accelerate thinking in terms of its use of most of the vocabulary of learning and education and the interaction of the teacher and the student in the lesson, and presenting And the presentation of the educational material is a live demonstration by the teacher and the student, symbolically through technological means, the field application of learning and evaluation for it, and the merging between the theoretical and practical subject, as well as the researcher attributes these differences to the principle of using questions

The answers, explanation, and presentation for each part of the legal articles by hand and the pairing between theoretical and practical aspects in the educational curriculum, which was able to raise the level of arbitrary performance for students through the ability to develop and think, and that "the degree of change in knowledge depends on the nature of the situation in which the individual gets information and on Source, style and degree of mastery.

The researcher believes in the development of the control group for the results of its dimensional tests compared to its tribal results in the research variable (arbitration performance), that the control [6] group exercised the educational size of the educational content of the subject teacher and his method of teaching to achieve the educational goals also for the fourth stage by hand, as the continuity in Learning leads to a development in knowledge in general through changes in the behavior of learners day after day, with the presence of educational material, the teacher, and accumulated experience

For the learner and may be at levels according to the nature of the teacher, his method and the learner's response from the researcher's point of view, as (Dhafer Hashem Ismail) confirms that the natural phenomena of the learning process must be there

Development in learning as long as the teacher follows the steps of the proper foundations for learning and education, and last but not least that the development at the level of the control group was not at the level of ambition for what the experimental group achieved compared to the results of the post-test for both groups where the high moral differences for the effectiveness of the independent variable by the researcher And its effect in the field on arbitration performance of handball for students.

As this model is distinguished by the presentation and application of the content of the educational material in a sequential, intensive [8, 9], and overlapping manner by grouping the content in one frame (applied educational video, posters) accompanied by the teacher's explanation and application exercises for the presentation, and the researcher believes that the development of arbitration performance by hand for the experimental group of During the excitement of learning motivation using the model to accelerate thinking away from the prevailing methods of learning and its reflection on the increase in acceptance of the scientific material and the desire to learn to it through the video presentation and posters interesting to their thinking and interesting to their enthusiasm, and targeting the model. The experimental group with the educational method of the model to accelerate thinking and the teacher reflected on its development in performance. Arbitration by hand, through the clear role of students in solving problems facing them with regard to a specific knowledge, as

well as their role in unifying their ideas in one direction through presentation and discussion of arbitration cases and movements within the stadium and giving freedom to express opinions to students and ask questions and find common solutions to them, where the researcher sees That the model of accelerating thinking through presentation and discussion of arbitration cases and movements on the field and giving freedom to express opinions to students and ask questions and find common solutions to them.

Conclusions

In light of the results obtained by the researcher through the field experiment and his use of the appropriate statistical methods in the description and inference about them, he concluded the following conclusions:

1- It was found that the form prepared by the researcher has the ability to evaluate the arbitration performance by hand for students.

2- The application of the vocabulary of the educational curriculum followed by the researcher using the model of accelerating thinking contributed to the development of arbitrary handball performance for students.

3- The progress made in the refereeing performance of handball for students through the clear difference of the results of the dimensional tests of the experimental group confirms the effectiveness of the independent variable and experimental control.

4- There is an evolution of the control group in the arbitration performance by handball, but not at the level of ambition compared to the experimental group.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Physical Education and Sports Science and all experiments were carried out in accordance with approved guidelines.

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The Effect of Therapeutic Exercises of Degree and Severity of Tear Tendon Muscle Tendon Rupture and Some Physical and Functional Variables for Athletes

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Abstract

The research problem included in the injury of the rotator cuff tendon rupture and the study aimed at - the number of therapeutic exercises severely and the degree of injury to qualify for the injury of the rotator cuff tendon rupture and some physical and functional variables for athletes - knowledge of the effect of therapeutic exercises severely and the degree of injury to qualify for injury to the rotator cuff tendon rupture and some variables.As for the research method, the researcher used the experimental method and by designing the experimental group with one pre-measured, middle, and dimensional measurement for its suitability and the nature of the problem.

Keywords: *Therapeutic exercises, muscle, rupture, physical and functional variables, athletes*

Introduction

The therapeutic exercises are one of the most effective means of physical therapy if used in an orderly [1], accurate and consistent way with the functional condition of the body, as all the aspects of sports rehabilitation depend mainly on the neuromuscular compatibility of all the body& systems, and recent years have witnessed a great development and diversity in the methods and techniques used in physical therapy, and Here we see the positive role of therapeutic exercises [2] , which is one of the most important of these means at this stage, appears because of its positive effect, the researcher noticed that the tendons are more likely to be wounded severely on young players ;due to the excessive use of these muscles and the neglect to to maintaining it or strengthening it, whether by warming up and associated with total muscle prolongation or by using inappropriate weights during training, the importance of research lies in an attempt to prepare therapeutic exercises according to the degree and severity of the injury to treat a muscle tendon injury [3] .

Materials & Methods

Research Methodology:

The researcher used the experimental method in designing the single experimental group with pre, middle and dimensional measurements.

Research Society

The researcher defined the research community in an intentional way, as the community included the players with tendons working on the shoulder joint (rotator cuff) in the clubs of the provinces of the southern region, ages (18_19) years, for some games, which included (3 handball - 1 weight - 3 aircraft - 1 foot (Goalkeeper) 2 gymnastics - 2 basketball), who accounted for 100%, as there were 12 injured players, the researcher Excluding 2 members of the sample, according to the opinion of the doctor who specializes in the occurrence of complications in the injury site, so the total of the research community was 10 injured players.

And For the purpose of ascertaining the site of the injury and its severity, a magnetic resonance examination was carried out at Al-Hussein Learning Hospital. Through the resonance report and the specialist doctor, the location, proportion and severity of the injury were determined.

Means and tools used in the research:

Data collection methods

Personal Interview2- Tests and measurements.3- Observation.

Data collection tools and devices:

- Manual computer number .
- A Chinese laptop computer type.
- Camcorder, number (1) type (Sony).
- Medical preparations of Dettol, Cotton, Gauze, and Adhesive Tapes to install the Pickup on the Body with Shaving Blades to remove the Hair from the place of the Pickups.
- EMG of USA Company.
- Dynamometer to measure muscle strength measured (in kilograms)

Field Research Procedures:

Defining the search variables:

Was selected Physical research variables (strength - and muscle endurance) and functional (the top of the electrical activity of the muscles - the area of electrical activity of the muscles through scientific sources and the opinions of experts with expertise .

Choice of tests and research metrics:

After the researcher was acquainted with many scientific sources and references for the purpose of choosing the appropriate tests and measures for the study variables, they were determined through scientific sources and in agreement with a number of experts and specialists to demonstrate their validity.

Exploratory Experiences:

The researcher carried out several exploratory experiments on 10/21/2019, regarding the first exploratory experiment, and it was conducted at nine o'clock on a sample of 3 players and in the physiology lab of the Faculty of Physical Education and Sports Science - Dhi Qar University, and they conducted a second exploratory experiment after 7 days passed And when conducting exploratory experiments, the researcher is keen to establish the same conditions and conditions in which the main experiment is, as much as possible so that its results can be taken.

The first and second exploratory experience:

Their purpose: -

1. Ensure the validity and configuration of the devices and tools used in the tests
2. Ensuring the appropriateness of the tests prepared in the curriculum prepared by the researcher.
3. Learn about the work of the electrical activity in all its details.
4. Knowing the assistant work cadre and their number [3] *.
5. Knowing the length of time required to take the tests.
6. Training on how to register.
7. to identify the difficulties facing the researcher.

Tribal tests for the research community:

The researcher used the tests that include the maximum force and the bearing strength of the research sample on 10/29/2019 at 9:30 am on Sunday in the halls of the College of Physical Education and Sports Science - University of Dhi Qar and coincided with the tests (maximum strength and bearing strength,) tests The electrical signal of the rotator cuff muscles, as the test was attended by all members of the sample as well as the auxiliary team:

The main experiment (first section):

As the first part of the main experiment included **See** Three weeks by three rehabilitative units for each week, as the first week contained fixed strength exercises with negative fixed stretching exercises without tools (as shown later in the program description).

Interlayer Tests:

The researchers conducted the interstitial tests four weeks after the application of the rehabilitation program on 12/1/2019 and according to the details mentioned in the description of the therapeutic exercises mentioned later to ensure the extent of the improvement and

response of the community to the exercises in the deltoid muscle affected by partial tear.

main experience (section two):

As the second section of the main experiment included four weeks with three different qualification units in the exercises applied for each week using therapeutic exercises with different intensity in where they were where the exercises were characterized by using mobile force and positive moving body weight that aims to increase the muscle strength and kinetic range of the joint as well as The development of muscle action by the occurrence of neuromuscular adaptation, which works to cause an increase in muscle strength as a result of the regulation of the work of muscle fibers and the regulation of the occurrence of nerve impulses.

Dimensional tests of the research sample:

The researcher carried out the post-tests of the research sample after completing the application of the vocabulary of the therapeutic exercises prepared by the researcher to the experimental group for the days, 1/2/2020.

Description of the measurements and tests used in the research: -

The researcher chose a number of tests and measurements, which were determined by taking the opinions of experts in the field of sports medicine and rehabilitation to demonstrate their suitability, which included the maximum strength tests and muscle strength and EMG (peak-area) test, and these tests are as follows:.

First: The name of the test: _ (Measuring the maximum strength of the shoulder muscles :) [4]

- **Purpose of the test**:

Measurement of the constant maximum force of the affected arm, "maximum effort that can be produced to perform a single voluntary muscle contraction" **,** As shown in Figure 1.

- **Test description**:

The laboratory sits on a chair attached to the dynamometer device so that the laboratory& stem is elongated and looking forward and its arm is stretched down and held with a fist attached to the device through the metal chain, then the laboratory is required by the agreed signal to keep the affected arm away from the stem and with maximum force

- **Test instrumentation**:

The dynamometer.- Chair attached to the dynamometer.- Hollow tubular iron grip.- Metal chain **Registration method**:

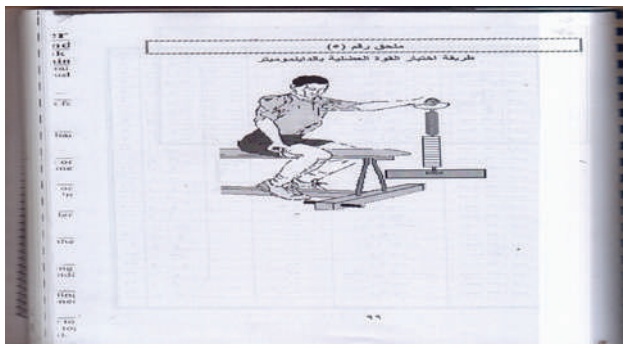


Figure 1. Shows the force test method for the dynamometer

Second: Test the strength tolerance of the affected arm :

- **Test name: -** Arm extended and lower side test (30 seconds).

- **The purpose of the test** Measurement of strength tolerance of the deltoid muscle.

- **Devices and tools**:

1- Mastaba height (50 cm).2- An electronic stopwatch.3- Dumbbell weighing 1.5 kg.

- **Performance specifications**:

- Sitting on a mastaba, straightly on the trunk and the affected arm hanging down to the side of the body and the fingers of the hand pointing towards the body, carrying a 1.5 kg dimple lifting the arm to the side of the body, i.e. when the humerus is in a horizontal position and has a 90-degree angle with the body at the shoulder joint and the arm back down fully extended , Repeats the performance as many times as possible.

- **How to record performance:** It counts the number of correct times within 30 seconds.

 Registration: The laboratory records the number of correct attempts made within 30 seconds.As shown in Figure (2)



Figure 2: Shows how to perform the test

Third: a test: measuring the electrical signal (vertex - area) of the affected deltoid muscle.

 How the device works:

That the modern EMG device is a device with a weight of no more than 390 grams that connects around the waist of the player via a belt and this device sends Bluetooth signals (Bluetooth) about muscle activity to be received by another device known as the receiver for a Bluetooth signal connected to a personal computer (Lap Top), The advantage of the modern EMG device is to allow the player to perform all kinds of movements, bouncing, spinning, and running quickly 40 meters from the location of the receiver for the signal to record and store the EMG signal that represents the start and end time of the muscle activity and the electric amplitude of the muscle with the working space of the muscles working in the movement.



Figure (3) shows how to place the pickup during testing for one of the research sample members

Statistical Analysis

The researcher used the statistical package (SPSS) to analyze the results of the pre, intermediate and posttest tests using unscientific statistics due to the small size of the research community and the heterogeneity of its members in the mentioned variables, which included.

Results & Discussion

In order for the researcher to be able to know the objectives of his research and test his hypotheses in knowing the effect of prepared therapeutic exercises as the researcher presented the results of the tests in the pre, middle and post measurement of the affected experimental group as follows:

Physical and functional tests in the pre, middle and dimensional measurement of the maximum force and the force tolerance of the muscle muscle activity during the maximum force and the endurance of force **..**

The EMG electrical activity tests of the muscle muscle activity during the maximum force and the force tolerance in the pre, mid, and dimensional measurements and the Friedman value**.**

Table 1. Shows Arithmetic circles, standard deviations, lowest value, and highest EMG electrical activity in pre, mid, and dimensional measurements

highest value	less value	Standard deviation	Arithmetic mean	Measurement	Measurement Unit	Processors Testing
1200.0	650.00	153.31	855.55	Kebili	MicroVault	Top EMG electrical activity for extreme strength
677.0	318.00	104.91	531.05	Middle finger		
282.0	165.00	40.87	218.86	*-dimensional		

Cont... **Table 1. Shows Arithmetic circles, standard deviations, lowest value, and highest EMG electrical activity in pre, mid, and dimensional measurements**

128.0	62.0	17.516	82.82	Kebili	MicroV / s	EMG electrical activity space for maximum power
80.0	51.00	8.202	65.13	Middle finger		
58.30	30.20	8.161	42.53	*-dimensional		
1200.0	760.0	131.88	941.60	Kebili	MicroVault	Top EMG electrical activity to withstand strength
880.0	580.0	94.09	651.50	Middle finger		
340.0	119.0	81.10	232.70	*-dimensional		
|	73.10	21.924	96.76	Kebili	MicroV / s	EMG electrical activity space to withstand forces
95.80	60.20	14.734	77.11	Middle finger		
63.30	42.10	8.058	52.76	*-dimensional		

Table 2. Shows Average ranks and Friedman value of EMG electrical activity test results in the pre-, mid-, and dimensional measurement of maximum strength and strength endurance.

Type of indication	Significance level	The value of Friedman Calculated	FS-3 Average level	Measurement	Measurement Unit	Processors Testing
corporate	0,000	17.00	3.00	Kebili	MicroVault	Top EMG electrical activity for extreme strength
			2.00	Middle finger		
			1.00	*-dimensional		
corporate	0,000	20.00	3.00	Kebili	MicroVault / a second	Activity space Electromagnet EMG for maximum power
			2.00	Middle finger		
			1.00	*-dimensional		
corporate	0,000	15.00	3.00	Kebili	MicroVault	Top EMG electrical activity to withstand strength
			2.00	Middle finger		
			1.00	*-dimensional		

Cont... **Table 2. Shows Average ranks and Friedman value of EMG electrical activity test results in the pre-, mid-, and dimensional measurement of maximum strength and strength endurance.**

corporate	0,000	19.00	3.00	Kebili	MicroV / s	EMG electrical activity space to withstand forces
			2.00	Middle finger		
			1.00	*-dimensional		

Sample size = 10

Significance level

EMG electrical activity tests in the pre, mid, and dimension measurements and Friedman& value**.**

Through our observation of the results of the average rank and the calculated Friedman value, which appeared in Table (2). **In EMG electrical activity test (peak-area) for maximum strength** :

We note that there is a noticeable decrease in the ratio of the electrical signal to the deltoid muscle between the pre and mid and post tests, and in favor of the post test in each of the peak variable. **EMG** And the area of electrical activity of the muscle. The researcher attributes that development to the peak variable of the electrical signal **EMG** For the deltoid muscle:

The therapeutic exercises and the type of exercises applied and are based on scientific foundations It has led to the significant [6] differences between the three measurements, by organizing and programming the training process and using the optimum stresses and repetitions through the rehabilitative exercises that are commensurate with the possibility of the injured through the tests that were conducted at the beginning of each rehabilitative unit with the codification [7] of the intermission times between each exercise and the other, which had a positive impact In developing strength for the deltoid muscle and its associated muscles through the repetitions and stresses used in the rehabilitation process[8] . Here, the researcher sees that the muscle has become more exciting to the motor units through the improvement of the functional work of the muscle and the economy in the effort, and this is what the researcher observed through Significant improvement in muscle activity Electrophoresis It is required in order to reach a certain level of muscle contraction [9-11]. The researcher sees that the area under the curve varies according to the time period for performance, as the work is extreme or extends for a longer period and it is directly proportional to the peak of the electrical signal in the muscle concerned with the work and according to the type of work, as he indicated (Muhammad Majeed) the greater the time period the greater the area What is below the curve, because it is caused by two main variables: the height of the peak of electrical activity and the time of this peak.

Conclusions

According to the research objectives and hypotheses, within the limits of the research community, field research procedures, and the results of statistical treatments of data collected in the pre-intermediate and post-tests of the variables under discussion, the following conclusions have been reached: Therapeutic exercises have a positive effect in rehabilitating the injury of the rotator cuff muscles. The therapeutic exercises have a positive effect in developing the strength and endurance of the muscles working on the shoulder joint, and thus reflected on the improvement of the electrical signal (EMG) For the variables (summit and area).

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Applied Sciences and all experiments were carried out in accordance with approved guidelines.

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Movement Exercises and their Effect on the Values of Some Physical Abilities for Youth and the Accuracy of Overwhelming Hitting From Position (1) on the Volleyball

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Abstract

The aim of this study is to identify the effect of movement training in some physical abilities of the skill of overwhelming hitting of the volleyball for an experimental youth group. The researcher assumed that the movement training has a positive moral effect on the physical abilities and the accuracy of overwhelming hitting with volleyball for youth. The research community was identified by the researcher in the youth volleyball center in Al-Shatrah district. The researcher subjected the research sample to experimentation through the application of movement exercises where the researcher took into account the preparation of those exercises scientific steps in the implementation and application of the independent variable, which continued for eight weeks. The researcher dealt with the results obtained from the pre-test and post-test tests by using the statistical bag. The researcher concluded that there is an improvement in the physical capabilities of the experimental group in the post-test tests as a result of using them for movement exercises in the experimental group in the dimensional tests because of their subject to training. As for the most important thing the researcher reached, is the need to use movement training, as it works to develop some physical capabilities for youth volleyball players.

Keywords: *Physical abilities, youth, overwhelming hitting, volleyball*

Introduction

The sport training is a purely specialized process that enters the public and private construction of athletes and is an important factor in mutual relations [1]. The modern sports training programs and their sub-units, whether monthly, weekly, or even daily, have become full of new things, where they occupied a great interest among those in charge of the training [2] process in preparing athletes and correcting training programs. Especially those directed at some group sporting activities, including the movement training approach, which worked to raise the level of physical abilities. Among those activities, volleyball, which is characterized by the abundance of its offensive and defensive skills [3]. Volleyball is a team sport that is famous for excitement and suspense, especially in offensive skills, where the skill of overwhelming hitting is one of the most important offensive skills in this game, which has a large and effective role in disrupting the reception of the opposing team [4]. As this skill requires attention in the particles of each of its preparatory, main, and final sections, and this requires upgrading the physical and motor capabilities of the players that constitute the main pillar in raising the level of this skill. All this requires from the coaches and specialists in this field the usage of the modern training methods that contribute scientifically to develop these capabilities [5]. Especially the force characterized by speed, explosive power, agility, and compatibility that the dispatching player needs during the performance of the overwhelming hitting skill.

Methodology

The scientific research determines the method to be used, when research is applied, it needs an experimental approach to solve the problem. Where the experimental approach "is a deliberate and controlled change with the conditions specified for a particular event and noting the resulting changes in the same incident for its

interpretation [1].

Research's community and sample

The research community included the young players in the Volleyball Specialized Center in Al-Shatra district, who represent the youth category and ranged between 16-18 years old, they were 20 players, but 16 players were randomly chosen by draw lots method. Where they represented 80% of the parent community. The sample was divided into the experimental and control groups randomly by draw lots method to 8 players in each group.

Homogeneity of the sample

In order to verify the homogeneity of the sample, the researcher has taken some measures to control the variables, even though the selected sample is from a close age group and the homogeneity was performed on the sample together before it was divided into two experimental and control groups.

Table 1. The homogeneity of the research sample in the training age, height and weight using the coefficient of difference

	Measurements and variables	The unit of measurement	the arithmetic mean	standard deviation	Coefficient of variation (%)
1	Training age	Month	38.16	0.57	13.3
2	Mass	Kg	77.58	2.02	2.6
3	Length	cm	185.83	6.30	3.4
4	Arm length	cm	80.1	5.12	6.4
5	Torso length	cm	81.33	6.70	8.2
6	Leg length	cm	104.5	2.84	2.7

Equivalence of the two research groups

One of the most important things that the researcher must consider is that the differences return to the experimental factor. On this basis, the control and experimental groups must be equal in all variables and indicators completely. Therefore, statistical methods were used by the mean, standard deviation and T-test for independent samples (between the experimental and control groups) before applying the method as shown in Table 2.

Table 2. Arithmetic mean, standard deviations, calculated (T) value and (Sig) value for the experimental and control groups in the pre-test

Processors	The unit of measurement	Diametrical overwhelming hitting (pre-test _ control)		Diametrical overwhelming hitting (pre-test _ experimental)		Calculated T value	Significance level (0.05)	significance
Measurements		$\bar{x}$	+ σ	$\bar{x}$	+ σ			
The explosive force of the legs	M	21.6	0.7	22.1	0.99	1.3	0.2	Not significant
The explosive force of the arms	M	2.7	0.04	2.7	0.04	0.1	0.9	Not significant
The force marked by speed of the legs	time	4.2	0.3	4.1	0.3	0.07	0.9	Not significant
The accuracy of the overwhelming hitting	Degree	6.6	0.5	6.5	0.5	0.6	0.5	Not significant

Methodology

Means of collecting information:

Arabic and foreign references, Personal interviews, Observation and analysis, Experimentation, Testing and measurement.

Tools and devices used:

Tape Measure, Volleyball, Japanese-made whistle, Medical scale, Japanese-made (CASIO) computer. Irish-made Dell Ci7 laptop. DVD (6) legal volleyball court and legal volleyball balls (3). 5 cm wide adhesive tape and office tools. Casio electronic stopwatch.

Exploratory experiment

The most important thing that scientific research scientists recommend for the purpose of obtaining accurate and reliable results is conducting the exploratory experiment. Which is defined as "a mini-experiment from the main experiment, the purpose of which is to experiment the work to reveal the obstacles and negatives [6] facing the application of the main experiment or for the purpose of training some cadres to assist in the work."

The researcher conducted the exploratory experiment on Friday 4/1/2019 at nine o'clock in the closed hall of sports in the College of Physical Education and Sports Science, Thi Qar University on the a sample of (6) players out of the community of the research "from the Euphrates Sports Club to apply the physical and mobility capabilities, the accuracy of the overwhelming hitting. The purpose of this experiment is to assess performance and know the negative aspects and variables which will face work and also to check the following:

1- Knowing the appropriate tools and devices to conduct these tests.

2- Knowing the appropriate time and place to conduct it.

3- Ensuring the adequacy of the supporting staff.

4- The definition of the auxiliary staff in how to apply these tests.

5- Knowing the difficulties and problems facing the researcher in applying these tests before applying them in the main experiment

Field research procedures

Tests for the research sample

The researcher conducted the tests and pre-measurement for the experimental and control groups before starting the implementation of the training curriculum on Friday and Saturday, 11-12 /January/ 2019 at nine in the morning (in the closed hall Youth Forum Shatrah / Thi Qar / Shatrah). All members of the sample (16) as players, the researcher and the assistant team worked on the first day where the measurements (lengths, mass, and age) were identified, then physical tests were performed. On the second day, the accuracy of overwhelming hitting of the volleyball was tested, a camera was placed in its assigned location on the front level of the player who performs the skill of overwhelming hitting with the right hand on the side of the beating area, about 8.78 m.

Statistical means

As mentioned above, in order to verify the homogeneity of the sample, the researcher has taken some measures to control the variables, for this purpose the statistical methods were used as:

- ü Arithmetic mean.
- ü Standard deviation.
- ü Coefficient of variation.
- ü Pearson correlation coefficient.
- ü T-test for correlated samples.
- ü T-test for independent samples.

Results and Discussion

Table 3. the values of the arithmetic mean, the standard deviations, and the calculated value (T) of the values of some physical and motor abilities

	Variables	Units	Pre-test control group		Post-test control group		Difference in (x^-)	level of development %	The calculated T	level of significance 0.05 *	result
			x^-	$+\sigma$	x^-	$+\sigma$					
1	The explosive force of the legs	M	21.6	0.7	27.1	1.8	5.5	20.4	7.4	0.000	Significant
2	The explosive force of the arms	M	2.7	0.04	2.9	0.09	0.2	6.9	5.9	0.000	Significant
3	The force marked by speed of the legs	time	4.1	0.3	4.1	0.3	0.01	0.3	0.08	0.9	Not significant
4	The accuracy of the overwhelming hitting	Degree	6.6	0.5	6.5	0.5	0.125	1.9	0.5	0.6	Not significant
* At the significance level ≤ 0.05											

Table (3) shows the values of the arithmetic mean, the standard deviations, the difference between the arithmetic means, the percentage of evolution, the value of (t-test) and the level of significance between the pre-test and post-tests of the control group for the explosive strength of the two legs, the explosive force of the two arms, the force distinguished by the speed, and the accuracy of the overwhelming multiplication. The explosive strength of the arms, it appeared through Table (3) that there were significant differences between the pre-test and post-test and in favour of the post-test.

Table 4. the values of the arithmetic mean, standard deviations, and the calculated value (T) of the values of some physical, motor, and accuracy capabilities of the pre and post tests of the experimental group.

	Variables	**Units**	**Pre-test experimental group**		**Post-test experimental group**		**Difference in (x^-)**	**level of development %**	**The calculated T**	**level of significance 0.05 ***	**result**
			x^-	$+\sigma$	x^-	$+\sigma$					
1	The explosive force of the legs	m	22.1	0.9	34.02	1.09	34.9	34.9	20.5	0.000	Significant
2	The explosive force of the arms	m	2.7	0.04	3.4	0.5	0.7	21.2	4.4	0.003	Significant
3	The force marked by speed of the legs	time	4.1	0.3	3.3	0.09	25.642	25.6	6.3	0.000	Significant
4	The accuracy of the overwhelming hitting	Degree	6.5	0.5	9.4	1.8	30.7	30.7	5.6	0.000	Significant
* At the significance level ≤ 0.05											

Table (4) shows the arithmetic mean, standard deviations, the difference between the arithmetic means, evolution ratio, value (t-test) and the level of significance between the pre-test and post- tests of the experimental group for both the explosive strength of the two legs, the explosive strength of the arms, the force distinguished by the speed, and the accuracy of the overwhelming multiplication.

The overwhelming hitting for experimental group:

From Table 4, it appeared that there are significant differences in physical abilities and the accuracy of overwhelming hitting between pre-test and post-tests and in favour of the post-test. [7] The researcher attributes that the development in the explosive force variable for the legs is due to the diversity and effectiveness of the movement exercises adopted by the researcher and applied by the experimental group.

Which increased the ability to the working muscles, as Mohamed Tawfiq stresses that "exercises similar to the stages of the overwhelming hitting skill are one of the most effective forms in developing the explosive force because these exercises impose a high effort on the body, especially on the muscles, tendons and the working joints, for this reason there is rapid adaptation and adaptation of the body and progressively on this Type of exercises by starting with exercises of the lowest intensity and then the most difficult and the highest intensity [8,9].

Table 5. Values for mean, standard deviations, and value of calculated (T) for some physical, motor, and accuracy capabilities for pre-test and post-tests of the experimental and control groups

	Variables	Units	Post-test control group		Post-test experimental group		Difference in (x^-)	The calculated T	level of significance 0.05 *	result
			x^-	$+\sigma$	x^-	$+\sigma$				
1	The explosive force of the legs	m	27, 125	1, 807	34, 015	1, 086	6, 890	9, 240	0, 000	Significant
2	The explosive force of the arms	m	2, 905	0, 093	3, 426	0, 470	31, 110	3, 07589	0, 00821	Significant
3	The force marked by speed of the legs	time	4, 131	0, 317	3, 268	0, 090	0, 863	7, 384	0, 000	Significant
4	The accuracy of the overwhelming hitting	Degree	6, 500	0, 533	9, 375	1, 767	2, 875	4, 403	0, 000	Significant
* At the significance level ≤ 0.05										

Table (5) shows the arithmetic mean, standard deviations, the differences in the arithmetic mean, significance level between the pre-test (t-test) and the post-test of the experimental group for both the explosive strength of the two legs, the explosive force of the arms, the force distinguished by the speed, and the accuracy of the overwhelming hit.

From Table 5 we can see that there were significant differences in physical abilities and accuracy of multiplication in the post-test_post-tests of the control and experimental groups and in favour of the

experimental group. The researcher attributes the reason for these differences to the effectiveness of the movement exercises that were applied by the experimental group, where the explosive force of the legs developed in the experimental group and significantly.

As the movement exercises included jumping exercises with both feet and jumping in a way that is consistent with the direction of strength in the skill of overwhelming hitting as well as the vertical and horizontal jump exercises, whether with an instrument or without a tool, it was limited only to jumping to the highest possible point, and nothing else, as happened in some studies that used exercises to develop that [4].

The researcher adds that the effectiveness of the movement exercises focused on developing the explosive force of legs through preparing exercises that targeted the performing of different forms of jumping and hopping for the purpose of developing the activity stage when performing overwhelming hitting.

The explosive force of the arms of the experimental group, it showed a superiority in the dimensional measurement from what it is in the post-test of the control group. The researcher believes that the reason for this is because the used movement exercises focused on the working muscles of the preferred and un-preferred hand. As in the exercises of standing by clapping and throwing the medical ball with the striking hand that contributed to the development of the explosive force of the muscles of the hands.

As well as providing an adequate and appropriate amount of compatibility between the two settings physical and skill which can be achieved through the established movement exercises if they are repeated according to the basic principles of sports training with some adjustments.

The reason for the development in the force marked by the speed of the two legs of the experimental group compared to the value of this ability in the control group is due to the organized planning in the given training doses for the used movement exercises as well as the rapid transition exercises that contributed to employing the muscular work well with the stability of the kinetic range of the joints which helps to increase the distinctive force by speed and increase its capacity in the experimental sample.

Conclusions

The sport training is an important specialized process that enters the public and private construction of athletes which works to raise the level of physical abilities. Based on the followed procedure in this research, the researcher have the following conclusions:

There was an evolution in the physical abilities (explosive force and speed characteristic) of the experimental group in the post-tests as a result of using them for movement exercises.

There was a noticeable development in the force marked by the speed of the control group in the post-tests as a result of its use of the trainer's exercises that focused on developing the approaching speed of the control sample, while there was no significant development in the rest of the variables under investigation.

The movement exercises contributed to the development of the accuracy of the overwhelming hit for the volleyball of the experimental group in the post-test through the development of physical capabilities.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Thi Qar University and all experiments were carried out in accordance with approved guidelines.

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The Effect of Using Some Teaching Methods on Learning the Skill of the Snatch Students

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Abstract

Included five chapters, as the first chapter contained the definition of research that includes learning the skill of The Snatch using modern teaching methods. The research problem has been identified, which consists in not using these methods in teaching the skills of The Snatch and Clean and Jerk and various within the educational curriculum scheduled and followed for students of the first stage In the College of Physical Education and Sports Science and the goal of the research to:- Knowing the effect of the researched methods (inclusion, self-revision, and commanding) on learning the skill of The Snatch for students with weight lifting. Exposing the best methods (inclusion, self-revision) has an impact on learning the skill of The Snatch students. As for the second chapter, it contained theoretical studies that have a relationship with the teaching methods that were used in the research, while the third chapter included the research methodology and its field procedures if the researchers used the experimental approach to its suitability the nature of the problem and the first experimental group used the method of self-review (self-examination) as for the experimental group The second is the method of inclusion (containment).

Keywords: *Methods, Learning, Skill, Snatch student***s**

Introduction

The educational process relies on three Fundamentals (the teacher, the student, the curriculum). In light of these basic Fundamentals [1], appropriate teaching methods are used that are the basis of the educational process, and through which researchers and those who are interested in the educational process in particular and the teaching methods in the field of education invest in the field of education in the field of education in the field of education [2]. The adoption of traditional teaching methods, of which the problem is the command and recurring method does not in any way mean a failure in the educational process in general and the motor learning process in particular [3], as the adoption of these methods has reached a good level in skill learning and development. This is a method Based on the centralization of the teacher in making decisions related to the lesson on the one hand and in evaluating the student's performance with the motor duty related to the skill required on the other hand [4], Mosten has been able to provide a number of teaching methods based on achieving educational goals through developing the capabilities and intentional of students from all sides, after the learning process focused on the centralization of the teacher and the lack of space for the student to express his opinion and his participation in some of the decisions regarding the taking of some of the decisions [5]. The modern teaching methods included a number of concepts according to which the student is given an opportunity to make part of the decisions related to self-evaluation by the student himself and give him the perception confidence of how the correct performance of the motion duty [6]. Emphasis on dealing with the eleventh modern methods, as defined by Musten, and the methods chosen are the method of assurance (containment) and the method of self-review (self-examination). Here, the importance of research as the field of research and the process of research in the field of research and the process of research Kinetic and teaching methods, on the other hand.

Methodology

The methodology used in research is determined on the basis of the nature of the problem, so the

experimental approach has been used for its suitability to the nature of the problem, as it is the only research approach that enables it to be the most reliable approach to solving many scientific problems effectively, as well as its contribution to the progress of scientific research in human sciences. Social studies, among them the sports sciences.

Research Sample:

The research community was chosen from the first stage students in the College of Physical Education and Sports Science - University of Kufa for the academic year (2018-2019) and from three divisions in the deliberate manner which is (B, C, and D) and by (76) students only the students 'people and the research sample was (54) A student has been excluded from them students who have been allowed to study (teachers) and those who have failed and are not always in compliance. His research sample accounted for (71.05%) of the original community for the purpose of starting the research work and after that they were harmonized using the coefficient of torsion, and Table (1) shows the results of this homogeneity, The sample was divided into three groups, two experimental and other controlling and by lottery way, whereby Division B became the first experimental group and division (C) the second experimental group and Division (D) the discipline group, knowing that the individuals of the sample are beginners to practice the skill of The Snatch lifting .

Table (1). Sample homogeneity by torsion coefficient in length, weight and age variables illustrates statistical treatment.

torsion coefficient	standard deviation	Arithmetic mean	variables
0.02	6.62	173.62	length
0.23	7.38	65.64	weight
0.68	1.56	20.85	age

Means, tools and devices used in the research:

Research means:

1- References and scientific sources

2- Observation and experimentation, personal interviews

3- Data collection and dump forms

4- The assistant team of teachers of weightlifting subjects

Tools and devices used in the research:

1- Chinese-made gravity columns with a weight of (15) kg and (20) kg (30).

2- Iron tablets with different weights.

3- An educational stick of wood, count (40).

4- Drum lifting drum training number (1).

5- A medical scale for measuring Chinese weight and height (1).

6- A Chinese-made (Lenovo) laptop calculator (1).

7- A Chinese-made manual stopwatch, number (1).

8- Office tools (paper, ballpoint pens, electronic calculator, blackboard).

9- (DVD) number (10).

10- Canon video camera (2).

Procedures for Field Research:

After giving two introductory lectures on the skill of snatching, the research sample was identified and the necessary tests were prepared, the names of the sample individuals were written in special forms for this purpose to conduct the required measurements in the research, and the educational curriculum was used and organized using the teaching methods of the two experimental groups. From skill of the snatch tribal tests of the three groups by the subject teachers and under the supervision of researchers with making experimental attempts by the individuals of the sample to ensure understanding and perception by them to the tests and the method of performance.

Pre-test:

The pre-test of the snatch skill for the students was conducted on 20/11/2018, and he gave each student in the research sample 3 attempts to measure the technical performance skill of the snatching lifting . Article teachers and under the supervision of researchers, it was mentioned previously in one day, according to the lecture specified in weightlifting and for all research groups.

Numbers of implementation of the educational curriculum:

The research groups distributed each according to the time of their lecture after conducting the tribal tests, and after defining the vocabulary for the educational content (learning outcomes), in the light of which the teaching methods used in the research were determined. snatching skill, timing of the lesson, and tools used, with the rate of one educational unit per week where the time of the educational unit was (90) minutes as the number of educational units reached (8) educational units while all educational units were implemented in (8) weeks, and the curriculum falls within the first semester For students of the first stage of the academic year (2018-2019), the curriculum is to develop the technical performance of snatching and the learning is under the guidance and supervision of the teacher and the direct application of modern teaching methods.

Dimensional tests:

The researchers conducted dimensional tests for snatching skill on 15/1/2019 after completing the educational units specified for the skill, where the tools and special requirements in the test were previously prepared and with the help of the weightlifting teachers to evaluate and judge the testing and performance in the tests and in the same conditions in which the tribal tests were performed.

Statistical means:

The researchers used the statistical methods in the statistical bag (spss), including:

- Arithmetic mean .
- standard deviation .

Coefficient of torsion.

Simple Correlation Coefficient (Pearson).

- (t) test for independent samples of equal number.
- (t) test for correlated samples.
- test (F)

Results

This section included the presentation of the results for the pre-test and the post-tests for both the experimental groups and the control group. These results were analyzed and discussed for the purpose of reaching the research objectives and verifying the validity of the assumptions. The test (T.test) of interconnected and independent samples was used to find out the significance of the differences in the arithmetic mean and the standard deviation between research groups, and to verify the effect of teaching methods used in the skill of the snatching lifting students at the level of their learning with the research sample, In the course of the statistical results obtained, the results showed that there were significant differences for the study variables in the subject matter of the study in the arithmetic circles between the pre-test and the post-tests. As for the experimental and control groups, the group of the self-examination method where the mean for it was (1,61) and the standard deviation for it was (0.78), and the value of (t.test) was (24,26) while the group of the method of inclusion was whose mean was (1.83) The standard deviation for it is (0.71), and the value of (t.test) is (32.21). The control group has a mean of (1.56), the standard deviation is (0.70), and the value of (t.test) for it is (9.11). It is clear that all The calculated values were greater than the tabular (T) value of (2.11) and with a free degree (17) and with a tolerance of error (0.05) indicating that there were significant differences between the pre tests and post tests and in favor of the post test and Table No. 2 shows that.

Table (2). Explains the arithmetic mean, standard deviations, and the value of (t.test) to test the skill of the snatching lifting and for the three groups.

The level of significance	The value of the tabular t	Computed t value	post tests		pre tests		the grops
			Y	X	Y	X	
moral	2.11	24.26	0.59	6.33	0.78	1.61	A group of self examination method
moral		32.21	0.47	8.11	0.71	1.83	Inclusion Group
moral		9.11	0.46	5.44	0.70	1.56	The control group

Presentation of the test results (F) for the skill of receiving and analyzing transmission:

The researchers used the (F) test to analyze the variance between and within the three groups and for the dimensional tests of students the snatching skill and Table (3) shows the results of the (F) test for the analysis of variance, Between the three groups and to the dimensional tests of the skill of kidnapping for students, the results showed that there were significant differences with statistical significance between the teaching methods (the self-examination method, the insurance method, the control group), because the value of (F) calculated in the skill of snatching skill has reached (36.62) which is greater than the tabular value (F), which is equal to (3.18). This indicates that there are significant differences in learning the skill of the snatching between the three research groups.

Table (3). Analysis of variance demonstrates the dimensional tests for the skill of the snatching

The level of significance	Degree F tabular	Calculated degree F	The degree of variation	Degree of Freedom	Groups of Squares	the grops	tests
moral	3.18	36.62	33.19	2	66.37	Between groups	Skill of the snatch
			0.91	51	46.22	Within groups	

Presenting the results of the least significant difference test (L. S. D) to the skill of the snatching and analyzing it :

its purpose is to identify the least significant difference between the three arithmetic circles between teaching methods in the extent of their influence in learning in the skill of kidnapping height skill, Where researchers used the LSD test, and thus it is clear from Table (4) that the highest significant difference was (2.67), which is determined between the second group, the inclusion method and the third group, the command method, as well as between the first group, the self-examination method, and the second group, the guarantee method was (1.78), Between the first and third groups (0.89), The moral difference was in favor of the second group, the method of inclusion, and this indicates that teaching in this method is the best method in teaching the skill of the snatching students.

Table (4). Shows the results of the L.S.D test to find the least significant difference for the snatching skill test

The level of significance	(L.S.D)	Results of differences	The difference between the average	Totals compare
Moral in favor of the second group	0.63	1.78	6.33- 8.11	Group 1- Group 2
		0.89	6.33-5.44	Group 1 – Group 3
		2.67	8.11-5.44	Group 2 – Group 3

Discussion

Through the results presented in tables (2,3), it appeared that there is a clear development for all three groups of research (experimental and control) through testing (T), but in a different way in the effect, and researchers attribute this to the effect of the program used and the capabilities and tools available and equal between The three groups, but there is a clear major difference in the teaching method variables used in applying the skill of snatching , the results showed that education using the method of inclusion (containment) occupied a high percentage of the effect and the reason for that is due to the importance in using the teaching methods and diversifying them to reach the condition of delivery Close to the reality of the game, as well as the frequent attempts that constitute a clear development in learning, The apparent superiority in learning with the method of inclusion and according to what the previous [8] results showed through the above-mentioned tables, is due to the student who has chosen the appropriate level or who is suitable in applying the required skill without there being any role for the teacher in directing to determine the level he wants, and this is what It came in the aims of this method, which emphasized the opportunity to be given to the student to perform the activity or activity in a manner commensurate with his ability, and this method aims to achieve harmony among students [9], as this method provides the opportunity to return to the previous level in relation to the student in the event that he is unable to achieve the optimal performance of the skill To do this successfully or perform the skill correctly and with a better level of performance.

What was mentioned is consistent with the expectations of the researchers in their first hypothesis, which states that there are significant differences in the influence in learning among all research groups [10] that worked with the three methods (inclusion, self-examination, command) in learning the skill of the snatching used in this research, and that The results of the research were in agreement with the second hypothesis of the researchers, in which they stated that the method of inclusion included a moral effect in learning the skill of the research topic more than the other teaching methods that were used in this research.

Conclusions

There is a difference in the level of learning of the skill of the snatching of students in the teaching methods developed. The teaching methods (the subject of the research) have a clear effect in the process of learning the skill of the snatching , as this was evidenced by the results of the pre and post tests which were in favor of the post tests and for the three groups. The superiority of the teaching method (inclusion) in influencing learning the skill of the snatching that is established by providing the opportunity for the learner to choose the level that suits him in implementation. The existence of an evolution in the group that studied in the command way, except that it was the least group in learning.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa, Iraq and

all experiments were carried out in accordance with approved guidelines.

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The Reality of Drug Abuse Internationally Prohibited among Bodybuilders in the City of Nasiriyah

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Abstract

The aim of the research was to identify the most internationally prohibited stimulants used by bodybuilders, and the researchers used the descriptive method and the field survey method and that As the search problem suitability, was selected sample of bodybuilders in the city of Nasiriyah and the way random number of 89 players. Man tried to use medicinal drugs as a type of stimulant a long time ago and the goal in that period was to raise and increase the tender and physical and athletic effort from the natural borders where the ancient Spanish history indicated that the ancient (Pale Inca) where its members chew coca leaves and were able to Then, from very long distances, hundreds of kilometers within several days. The sports circles in our country witnessed in recent years the growing state of abuse of internationally prohibited stimulants in various activities, especially those aimed at building the muscle mass in which the importance of research lies in developing a scientific assessment of the status of stimulants to the research community and standing on the ground for the extent of this phenomenon and the quality of the substances being used.

Keywords: *Drug abuse, internationally prohibited, bodybuilders, Nasiriyah*

Introduction

Sport was in its first natural, religious and educational premises aimed at making the person possesses certain capabilities, but in all cases it seeks to build an integrated human being balanced with himself and interacting with his environmental and social surroundings, and educators have recognized [1] The importance and necessity of raising the young person on sport because of its impact on the various components of the human being [2], and they adopted sport as a method of modern education because of the activities and games of a role in the comprehensive education process that brings the human being in various aspects of intellectual, psychological, motor, cognitive and organizational However, some are trying to harm sports morals by using illegal methods to reach stardom or fame by using or using internationally prohibited stimulants [3]. The human tried to use medicinal drugs as a type of stimulant a long time ago and the goal in that period was to raise and increase the tender and physical and athletic effort from the natural borders where the ancient Spanish history indicated that the ancient (Babylonian Inca) where its members chew coca leaves and then be able to cut Very long distances of hundreds of kilometers within several days. And Stein Bach found in one of the experiments in 1968 that there are significant differences between the process, training using Anabolic and the other without using it as it had a positive impact in the training process [4], and the history of the use of medicinal drugs indicates the registration of the first case of abuse of steroids in the sports field Until the year 1865 AD, when it was proven that one of the swimmers used different types of drugs during swimming races in the city of Amersdam, and this is the first case that was discovered with the aim of winning the wrong As for the indicators that alerted the world to the dangers of stimulants and their harmful effects on users, the death of the famous athlete Simpson in a bike race around the city of Paris, under the influence of a large amount of tetramethyl stimulant, and the deaths were repeated over the years. The sports circles in our country have witnessed in recent years the growing state of abuse of internationally [5-7] banned stimulants in various events, especially those aimed at building the muscle mass for which the importance of research lies in developing a scientific assessment of the status of stimulants to the research community and standing on the ground to the extent of the spread of this phenomenon and the type of substances that are used.

Research problem

Talk about doping and athletes abuse increased recently, and doping and its effects occupied a wide area locally and globally after its widespread and dangerous spread in all countries of the world, and that extended to our country and to the level of many official and unofficial sports (fitness Physical and bodybuilding centers in the city of Nasiriyah) with the aim of improving the physical and athletic level or for other purposes through the use of abnormal means and injecting them into the body or through the mouth before or during competitions without being aware of the seriousness of their use and their violation of ethical and athletic rules So the researchers wanted to stand on the aspects of this problem and Thdidahm drugs that are used by the research community.

Research Objectives:

To identify the most internationally banned stimulants used by bbodybuilder.

Research fields:

The human field: Bodybuilders in the city of Nasiriyah.

Timeline: - 25/8/2019 to 2019/11/25

Spatial domain: - Body building halls in the city of Nasiriyah.

Methodology

The researchers used the descriptive approach and in the field survey method, in order to suit the research problem.

Sample search

The sample was chosen from bodybuilders in the city of Nasiriyah, randomly, the number of which 89 players.

Information Collection Methods: The questionnaire is one of the important tools in gathering information in scientific research (it is the only available method to expose respondents to selected stimuli carefully selected and intended to collect data

Research Procedures

The questionnaire was used according to The materials are available and used by bodybuilders.

objectivity of the questionnaire:

The questionnaire is considered objective, since the answer to it is determined by (yes or no), and the corrector has no choice but to add the grades, and accordingly, no matter how different the correcters are, the questionnaire will give the same results.

Statistical means

Using the statistical bag spss

Research procedures

The researchers conducted the research by applying the questionnaire to a sample of (89) bodybuilders in the city of Nasiriyah, where the forms were20 distributed on 8/2019 / 25 and then collected in 2019/10/20.

T. / Questionnaire

Mr. - - - - - - - - - - - - -Dear

In conducting the research tagged (the reality of abuse of internationally prohibited steroids for bodybuilders in the city of Nasiriyah) and given that you are a practicing building sport Objects, please kindly put a sign ( ) in front of the material and attached hereto (if you use any of these materials)

Table 1. The results of the level of steroids were used by bodybuilders.

Seq	Stimulant	User number	Percentage
1	(Adactone)	17	19‹10%
2	Anabolicam))	11	12‹35%
3	(Anadrol)	27	30‹33%
4	(Androgel)	4	4‹49%
5	(Androstanone)	24	26‹96%
6	(Clomid)	11	12‹35%
7	(Deca-durabolin)	39	43‹82%
8	(Dyanabolan)	31	34‹83%
9	(Durabolin)	11	12‹35%
10	(Aphedrine)	13	14‹60%
11	Estandrone))	20	22‹47%
12	Groth hormone))	19	21‹34%
13	(Lasix)	2	2‹24%
14	(Mastron)	3	3‹37%
15	(Miotolsn)	3	3‹37%
16	Methondrial dipronte	7	7‹86%
17	(Nolvadex)	14	15‹73%
18	(Sustanon)	49	55‹05%
19	(Wenstrol taps)	37	41‹57%
20	Don’t use	19	21‹34%

Results

When collecting the questionnaire, the results of the level of steroids were used by bodybuilders as in the following table .

As in the following table. :

(Deca Durabolin)

came in at 43.82% feel the reach of decadurapone at first is felt at ease psychological and muscle strength and rest in sleep and open appetite and muscle anesthesia during training and increase the platelets and thus maximum benefit from oxygen and a sense of excessive activity and the opposite happens completely after completing the last dose about a week begins the testicular stops the male hormone secretion and continues For a period ranging from two weeks to several months until she returns to normal or less than normal

(Winstrol)

came in at 41.57%, which leads to the accumulation of male hormones in female organs, which leads to the appearance of signs of masculinity, but in men, the use leads to headaches, cramps and some cases of high blood pressure

(Dyanabolan)

came in at 34.83%, which works to keep water in the body and store protein in the muscle and increases body weight. As for its symptoms, it is high blood pressure, increased cholesterol levels, acne and chest swelling after stopping use

(Anadrol)

came by 30.33% that consuming anadrol increases the blood volume significantly, which allows the muscle to absorb the largest amount of oxygen, which provides the user with enormous energy, and its effect is negative by holding water inside the body

(Androstanone)

came in at 26.96% that is used for building to gain muscle mass, gain strength, and retain a small percentage of fat. Many players use androstanolone during exercises for competitions with doping tests, as the active substance remains in the body for a very short period

(Estandrone)

came in at 22.47%, which has a strong male anti-oxidant effect and improves the body's ability to regenerate. It works to lubricate the joints - places where the bones meet - with a strong liquid in the bonding tissue and increases the rates of muscle mass in the faculties of medicine. This chemical compound is used in medical schools to treat periods of critical disorder that occur For women in menopause or osteoporosis, this stimulant leads to an increase in weight for both sexes and an increase in the formation of subcutaneous deposits and breast tumors in men

(Growth hormone)

came up by 21.34%, as it is useful for building muscles and raising their capacity and does not appear in medical analyzes, but it has risks, especially when used before puberty, as it leads to gigantism and closes the bone ends prematurely and stops growth causing distorted bone growth in adults where the limbs and lower jaw grow unusually It increases blood sugar

While the players who do not use steroids were prohibited when playing a bodybuilding game at a rate of 14. 21% that this percentage is very low due to the number to which the questionnaire was distributed previously, we find that the steroids that came in the questionnaire have substances that have negative effects, especially when used for a long time and some The other has direct effects on the body when consumed. Where bodybuilders use stimulants in order to raise the physical efficiency in an unnatural way, which helps to bear the effort and restore the normal condition of the body very quickly and the stimulants also work on muscle building in a short period The results are negative, where the percentage of users is very high 66. 78%, and that indicates something, but it indicates a lack of awareness in the sports and health culture spread in the bodybuilding halls in the city of Nasiriyah, since most of the practitioners of this game in the bodybuilding halls are non-athletes As the motives that prompted the use of stimulants come for the purpose of appearing with manifestations of roughness and highlighting strength and good appearance in front of others. And that sports in general need long periods to reach the high level, we find that the users of these materials do not have the patience and endurance for a long time in order to build the entire body [8]. The lack of self-confidence, loss of will and jealousy from others are among the motives that led to the use of stimulants and the absence of health control in the Department of Health of Dhi Qar on these substances led to their spread and their presence in markets, pharmacies (vendors spread on the sidewalks of the streets) and red building halls in the city of Nasiriyah of Kamal Union Objects are the main role in eliminating them and limiting their spread and spreading the dangers of abuse of internationally [9, 10] banned stimulants by spreading awareness in the bodybuilding halls and monitoring them and not giving licenses to only those with specialization and competence of bodybuilders and coaches with experience and long-term sales in this area. While the percentage of practitioners not used for steroids 21.34%, which is a very small percentage when compared with the receptivity of the prohibited

substances, a ratio that indicates the risks of the great proliferation of these substances in the city of Nasiriyah, where it is expected that the abuse and the increase in tennis will increase after a period of time. And a little while.

Conclusions

Lack of culture and awareness among bodybuilders. The results showed a very high rate of doping. Bodybuilding practitioners' focus was on rapid body building only without focusing on the damage left by steroids. The percentage of people who did not use steroids was very small. The percentage of abuse (Sustanon) is very high, as it was 55.05% of the sample that was distributed to the questionnaire, whose number is (89).

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Thi-Qar and all experiments were carried out in accordance with approved guidelines.

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The Effect of Functional Exercises in Some Biomotor Abilities and Metabolism Rate for Volleyball Young Players

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Abstract

The importance of the research is preparation functional exercises scientifically, researchers believe that it affects in some biomotor abilities and metabolism rate, which is directly involved in the defensive and offensive performance of Volleyball for young players. Through the experience of field researchers note there is a noticeable decrease in the performance of defensive skills in matches where the level of technical performance is very high or the game continues for longer periods of time, this decrease leads to a slow moving to take the right place while performing these skills, researchers believe that the reason for this is that these skills are not getting enough share from training in a way that suits the nature of its physical and physiological performance , So the researchers felt that they should go into this experiment by preparing functional exercises, the aim of this exercises is to make a positive impact in some biomotor abilities and metabolism rate for Volleyball young players . As for the research methodology and its field procedures, researchers used the experimental method to solve the problem of research, the research society was determined by the Kufa Club Volleyball for youth, for the sports season 2019-2020

Keywords: *Functional exercises, biomotor abilities, metabolism rate*

Introduction

There is no doubt that scientific research has become one of the most important necessities in our modern society in reaching the highest [1] levels for all aspects of life by identifying the different energies and capabilities of human to try to achieve most benefit from scientific theories and their application to serve and develop the society, including the sports field [2], it may need a lot from sporting events to great time to get to high level of capabilities and possibilities . One of the games that have become a lot of attention in recent times is the game of Volleyball, which is one of the most popular games in the world, Volleyball requires a great muscle ability to perform its skills, upon closer examination In the nature of performance we find that it requires high energy to perform the motor duty with force [3], speed and endurance, it requires from players when performing the skills, high abilities. The defensive performance in playing Volleyball requires a high level of physical and skill performance consistently, the cases of defending the stadium and its various forms, as well as the skill of the blocking wall it has difficulty by skillful fast and sudden performance, therefore, it is important that biocompatibility and skill performance serve one another to achieve the goal, the player's susceptibility level can be found through these variables [4].

Methodology

Search community and the sample:

The research community was determined by the youth Kufa sports club for the sports season (2019-2020) and the number (12) players was chosen by the researcher in full for the experiment, they were distributed equally to two groups in a random way (lottery), after that, the experimental group underwent training that included the use of functional exercises, while the control group continued to use the regular training curriculum for the coach.

Devices and tools: Medical scale - tape measure - A wall with a tape measure installed- chalk.

Description of performance : After weighing, the player raises the distinct arm over its entire stretch to make a finger mark on the wall, or the blackboard with

heels not raised from the ground, and records the number that the sign is placed in front of.

- When the laboratory is ready on the starting line, sprints with accelerated acceleration to finish the distance to the place of advancement in only three steps, Upon reaching the place of rise, he performs the double vertical jumping to the highest possible height to mark the point of arrival with the fingers dipped in the chalk powder, The laboratory is awarded two best scores and the wrong one is repeated.

$$\frac{\text{MASS} \times \text{Jumping height} \times 9.8}{\sqrt{\frac{\text{Jumping height} \times 2}{9.8}}}$$

-

Recording: The weight and jump height data for each laboratory are recorded and processed using the following formula, given that the weight is in newtons, The player's mass can be used in kilograms, multiplying the numerator of equation x 9.8 .

Power =

Second: test the three steps long jump :

· **Purpose of the test:** Measuring the force marked by the muscles of the legs.

· **Devices and tools:** A distance not less than (9 m) the width of the volleyball court and a tape measure.

· **Description of performance:** The laboratory stands behind the starting line and then Jumping forward by feet together for three long steps consecutive, each laboratory is given two attempts to calculate its best.

· **Recording:** Distance is measured from the starting point to the last footprints, after the third jump (the three steps jump distance).

Third: Running test Shuttle of different dimensions (9-3 - 6-3-9) meters:

· **Purpose of the test:** Measuring agility.

· **Devices and tools:** Volleyball court, stopwatch, funnel number (6).

Description of performance: The laboratory is behind the starting line of the stadium upon hearing the starting signal, he runs in a straight direction to touch the funnel above the middle line 9 m by the right hand then he turns to run towards the 3m line it found in the middle of the field, that he started running to touches the funnel above the line with the right hand 3 m , then turn towards the 3m line , located in the second half of the stadium to touch the funnel above the 6m line with the right hand to go to the middle line 3m to touch the funnel above the 3m line with the right hand, then turn to run toward the 9 pm finish line to cross it with both feet, here it should be noted that touching the lines every time with the right hand, must be crossed the finish line with both feet..

Recording: the time records from start to finish over the finish line as shown in figure (3).

Measuring the metabolic rate during physical effort:

Metabolic rate is measured during the physical effort, accurately through the use of the (Fitmat pro) as the player's data (name, length, mass, and age) are entered before starting the measurement, also, clean the test mask by antiseptic, and connect the parts of the system with installing the heart rate belt on the chest and installing the signal receiver for the heart rate (Bluetooth) in the port assigned to the device, then (Wingate) test is performed on physical effort bicycle type (MONARK), where the mask for measuring (VO_2max) is placed on the player's face, after completing all the requirements for working the devices and after completing entering the required data into the device as well as the program for the physical effort bicycle, the test is started according to the conditions [5] of the (Wingate) test, after completing the test, data will be recorded for the maximum oxygen consumption rate (VO2max) from the (Fitmat pro) software, choose the (recovery option) from (Fitmat pro) device with the mask remaining on the player's face until O2 consumption is reached, the rest time following the physical exertion, with the same mechanism, the test is performed twice, the metabolic rate during the physical effort is measured (energy expenditure during exercise) through the (Fitmat pro) device screen, the metabolic rate during the physical effort is extracted according to the equation below:

Metabolic rate during physical effort in kilocalories per minute=

Equivalent metabolism × 3.5×mass /200

Note: the (Fitmat pro) device during the voltage

gives a reading of (vo2max) in units of milliliter / kg / minute.

Procedures (Wingate) test:

The necessary tools: Stopwatch, calculator, physical effort bicycle type (MONARK).

Style of performance: The test is performed using the stationary bicycle (MONARK) Swedish made, according to the following steps:

ü The examined mass is taken To the nearest correct Kilogram.

ü The data of the examiner is entered into the computer and the resistance is set according to the mass of the examiner, which is equivalent to 7.5% of his body mass.

ü The examiner performs a warm-up operation on the bike for a period of (3-4) minutes where the resistance is gradually placed according to the mass of the examiner before the end of the warm-up process, the examiner moves the bicycle wheel at full speed for a period of (3-5 seconds) and repeats this two to three times.

ü Lift the weight from the ballast basket, the examiner starts moving the bicycle wheel at the fastest possible speed with a speed of no less than (80) cycles for a period not exceeding three seconds then the weight is lowered gently and at the same time the program start button is pressed to begin the measurement process the examiner continues to move the wheel for a period of (30) seconds, to be encouraged and urged to maintain the speed of rotation as possible.

Main Experiment Procedures:

Pre-tests:

The researchers carried out the pre-test tests on the research community of the two groups (control and experimental) of the study variables (biomotor abilities test and metabolic rate during physical effort test) on Sunday (16/12/2019), the tests were in the following sequence:

1- Biomotor abilities test :

2- The metabolic rate during physical effort test:

Preparing and carrying out Functional exercises:

The researchers prepared and organized the functional exercises depending on the personal experience of the researchers, it was applied to the experimental group on 22/12/2019 until 13/2/2020 , taking into account (intensity, repetitions, appropriate rest periods) the researchers codified the exercises on a scientific basis, as well as the physical and functional susceptibility of the research sample, the tools used and the method of training, to be these exercises able to develop biomotor abilities, and metabolic rate during physical effort, to achieve the goals and objectives of the training process.

The details of the functional exercises in the training curriculum were as follows:

· The total number of training units that included the functional exercises are (24) unit , and the number of weekly training units that applied functional exercises are (3) units for a period of (8) weeks.

· Time for functional exercises in one training unit are (30-35) minutes

· The target of functional exercises is to develop biomotor abilities and metabolic rate during physical effort for Volleyball young players.

· Considerations exchange working between muscle groups.

· Planning the formations of functional exercises during the weekly and daily units are (1-2).

Post-tests:

The researchers, with the assistance of the assistant staff, carried out the posttests of the research community after the completion of the functional exercise, this was on Wednesday (19/2/2020) and in the same sequence of pretest tests. The researchers took the same conditions as the pre-tests where sequence tests.

Results

Table (1): Shows the mean, standard deviations, the calculated value of (t) of the interrelated samples, the level of the significance of the test and the significance of the difference for the pre-test and post-tests of the

control group of the studying variables.

Variables	Measuring unit	Pre-test		Post-test		(t) value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD.EV.			
Explosive power for legs muscles	watts	1308.031	37.804	1379.608	33.684	3.752	0.013	Sig.
Distinguished force with speed for legs muscles	meter	7.533	0.498	8.021	0.35	7.056	0.001	Sig.
Agility	second	11.625	0.282	10.88	0.348	5.348	0.003	Sig
Metabolic rate during physical effort	Kilogram / calories	10.31	1.134	11.588	0.646	4.252	0.008	No Sig

View The results of the pretests and posttests of the experimental group of the studying variables:

Table (2) : Shows the mean, the standard deviations, the calculated value of (t) of the interrelated samples, the level of the significance of the test and the significance of the difference for the pretest and posttests for the experimental group of studying variables

Variables	Measuring unit	Pre-test		Post-test		(t) value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD.EV.			
Explosive power for legs muscles	watts	1311.511	30.735	1472.207	21.904	13.137	0.000	Sig.
Distinguished force with speed for legs muscles	meter	7.575	0.209	8.586	0.121	14.912	0.000	Sig.
Agility	second	11.543	0.302	10.11	0.152	9.17	0.000	Sig
Metabolic rate during physical effort	Kilogram / calories	10.298	1.305	13.483	0.799	6.895	0.001	Sig

- View the results of tests (post-test) of the two groups control and experimental studying variables

Table (3) :Shows the value of (t) calculated for independent samples and the level of the test and were significant differences between the results of the test (post-test) of the two groups control and experimental studying variables .

Variables	Measuring unit	control		experimental		(t) value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD.EV.			
Explosive power for legs muscles	watts	1379.608	33.684	1472.206	21.904	5.645	0.000	Sig.
Distinguished force with speed for legs muscles	meter	8.021	0.35	8.586	0.221	7.137	0.000	Sig.
Agility	second	10.880	0.348	10.11	0.152	4.960	0.001	Sig
Metabolic rate during physical effort	Kilogram / calories	11.588	0.646	13.483	0.799	5.068	0.000	Sig

Discussion of the Results

The results presented in tables (1) and (2) for biomotor abilities tests there are significant differences between pre-tests and post-tests favor post-tests of the control and experimental groups, the researchers attribute the reason for this significant difference for members of the control group, due to the exercises that were applied by methods prepared by the coach in his training units, as it caused the development of those abilities, which is one of the most important pillars volleyball game, which enables the player to perform offensive and defensive skills well, which led to significant difference and in favor of post-tests, as for the significant differences that appeared in the tables above for these abilities, in addition to Metabolic rate during physical effort of volleyball for the members of the experimental group, the researchers attribute it as a result of using functional exercises, the philosophy and nature of functional exercises is the use of movements within the three directions and flat surfaces of the movement (lateral or sagittal direction , forward direction, transverse direction), which is the nature of all movements of the human body in life and sports in general and the skills of volleyball in particular in working on these three surfaces, thus work collectively as for the muscles of the body during functional exercises [6] and with high compatibility between them, this reflects on the increase and development of muscular strength as a basis for biomotor abilities, this is what was found in the functional exercises designed and applied by researchers to the members of the experimental group, as it is the functional exercises that "coordinate the work of the muscles together to produce perfect energy for motor action, it is the fastest and most powerful way to get muscle development and its abilities at all levels [7].

As for the results presented in table (3), which show the preference of differences in favor of the experimental group, in post-tests, the researcher believes that the exercises that the researcher prepared for the members of the experimental group which is characterized by high intensity helped to develop the explosive power and the distinctive speed of the muscles of the legs as well as agility in addition to the metabolic rate during

the physical effort of the experimental group during the physical effort , as volleyball players need, during the performance of [8-10] different motor skills, rapid and frequent muscle contractions Serving specialty activity.

Conclusions

Based on the research results reached within the limits of the research community, the following conclusions have been reached:

1- The duration of the independent variable represented by the number of training units, it was appropriate to create adaptations that reflect the evolution of the experimental research group for biomotor abilities of physical abilities (explosive power, the force marked with velocity).

2- Functional exercises contributed to the development of biomotor abilities and the physical abilities which are (agility).

3- The evolution of the biomotor abilities reflected positively on the development of metabolic rate during physical effort of volleyball for the experimental research group.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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The Effect of (Vertimax) Exercises on the Molecular Pressure of Oxygen, the Explosive Ability of the Two Legs, and the Accuracy of Scoring for Advanced Football Players

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Abstract

The research aimed to: Prepare (Vertimax) exercises for football players. Learn about the effect of exercises (Vertimax) on the molecular pressure of oxygen and the explosive ability of the two men, as well as the skill of scoring accuracy for advanced soccer players. This study examined several axes, including the introduction to the research and its importance, which lies in the use of exercises (Vertimax), as the importance of research lies in preparing these exercises through working with extreme intensity exercises and sufficient rest periods, as these exercises work to develop the molecular pressure of oxygen and the explosive capacity of the two legs. For advanced soccer players, in addition to developing the scoring skill of advanced soccer players, as these exercises give the best possible opportunity to achieve the greatest gains in the ability to jump, speed and explode in order to target the central nervous system (CNS) and activate all movement modes and make them circulate To better and also improve the player's ability to perform the scoring skill better throughout the match time and thus the player's performance is reflected in the team's result.

Keywords: *Vertimax, Molecular pressure, oxygen, legs*

Introduction

The field of training was affected by the revolution of science and technology, as the training process took a form [1], structure and organization consistent with the state of development and modernity of the methods and means used in the training process, so the scientific and technical development has added many new and modern methods in line with the nature of the age group of the trainee through the endeavors of the trainers to choose The best and most recent methods that are appropriate to the specialized activity, with the aim of achieving and investing the specificity of training related to the type of activity in order to reach a direct impact to improve the skill [2], physical, functional, planning and psychological level and reach it to the form sport.

Football is one of the games that has received increasing global attention because it is one of the most popular games in the world, and the development that occurred in the global levels of Football teams, which we sensed during the last World Cup championship, was the result of harmony and physical [3] and skill integration, planning, mental and career This harmony and integration are spontaneous and random. Rather, it came as a result of the coaches 'reliance on the science of sports training based on other sciences that achieve the best levels and results because" it has been scientifically proven that the response of the body's systems to sports training has a special importance in knowing the extent [4] of physical improvement and Functional for athletes, as well as the case of creativity, innovation and the development of methods and means of sports training through the use of the foundations and principles of sports training and the required scientific planning to prepare a comprehensive training curriculum.

Methodology

To achieve the goals of the research, it was necessary to define the research community and choose a representative sample for it, in addition to choosing the appropriate statistical methods to analyze the data and produce the results, as follows:

Research Methodology

Society and sample of the research:

The research community was determined by the athletes of the Kufa Sports Club who applied for the sports season (2019-2020) and they numbered (27) players and the researcher chose (4) players as a sample of an exploratory experiment from the same community and formed (14.8%) of the research community and the researcher chose the sample of the main experiment amount (20) Player The selection of the research sample came in a simple random method (the lottery) and it accounted for (74%) of the research community, and the researcher excluded the goalkeepers who are (3) players who formed a percentage of (11.11%) due to the inconsistency of the research requirements with their capabilities Table (1) shows the distribution of the community members and the research sample.

Table (1). Show community and research samples

research community	Sample application		Sample Exploratory experience	
27	the number	percentage	the number	percentage
	20	74%	4	14.8

Table (2). Shows the consistency of the research sample

Variables / Statistical parameters	Measuring unit	Mean	Median	STD.EV.	Skewness	Significant
Tall	Cm	176.75	177	1.879	0.826	homogeneous
Mass	Kg	73.831	74	2.862	0.11	homogeneous
Age	Year	18.5	18.5	0.516	0.00	homogeneous
Training age	Year	8.7	8.6	0.879	0.11	homogeneous

Through the are less than (1 ±), which indicates the homogeneity of the research sample in the variables (length, body mass, and chronological age).results of Table (2), it was found that the values of the torsional coefficient

Equivalence of the two research groups:

In order for the researchers to attribute the differences in the results of the post-test of the variables under study to the effect of the experimental factor, the researchers resorted to verifying the equivalence of the two groups by using the test (t) of the independent samples as shown in Table (3).

Table 3. Shows the equivalence of the two research groups in the tests of the variables studied

Statistical means / Variables	Measuring unit	Control group		Experimental group		(t) Value	Level of significance	Type of significance
		Mean	STD. EV.	Mean	STD. EV.			
The explosive ability of the muscles of the legs	Watts	1473.3	71.48	1449.3	7.407	0.51	0.624	Non sig.
(PO2) ratio before voltage	Mercury mm	32.8	1.303	32.4	1.673	0.422	0.684	Non sig.
(PO2) ratio after voltage	Mercury mm	17.6	1.816	18	1.581	0.371	0.72	Non sig.
Scoring	Degree	14.5	1.08	14.6	0.69	0.24	0.809	Non sig.

Through Table (3), it becomes clear to us that the value of the test significance level (sig) is the largest value of the significance level (0.05), and for all variables under consideration, therefore, the test significance is not significant.

Determining metrics and tests Variables:

First: measure the oxygen molecular pressure (PO2).

Measurement method:

The partial oxygen pressure (PO2) is measured by an electronic device, and a device for measuring the percentage of oxygen in the blood, a medical device used to measure the rate of blood saturation with oxygen O2sat or SaO2 by a light sensor, and this percentage can be detected through the device without the need for acupuncture, and the device is formed From a small unit equipped with a special position of the hand / foot or a small unit that is manually carried and equipped with a wire probe that can be attached or used on a finger, toe, or earlobe to measure the percentage of oxygen in the blood, as well as to measure the number of heart beats per minute, among members of the research community Putting rest before warming up (15) minutes and after giving out Immediately (scoring accuracy test skill), after (5) seconds have passed where the laboratory sits on a chair and the arm is attached to a compressor (Turnka) to facilitate the process of drawing blood from it by (200 micro) of venous blood by the chemical specialist, as the blood is placed in tubes Medical (tube), then transferred to the specialized laboratory directly



Figure (1). It shows the pulse oximetry.

Second: The vertical jump test of stability:

The purpose of the test: to measure the explosive power of the muscles of the legs.

Hardware and tools: a blackboard that is attached to the wall so that its lower edge is 150 cm above the ground, to be inserted after that from 151 cm to 400 cm, (the blackboard can be dispensed with marking on the wall), Manizia or Chalkboard, Aluminum Ladder and Nails.

Performance specifications: The laboratory dips the distinct hand in chalk powder, the laboratory raises his arm along its entire stretch to make a mark on the blackboard, then the player swings the arms and bends the knees to the vertical jump, no maximum distance he can reach to make another mark and the arm along its entire stretch, as shown in the figure (2).

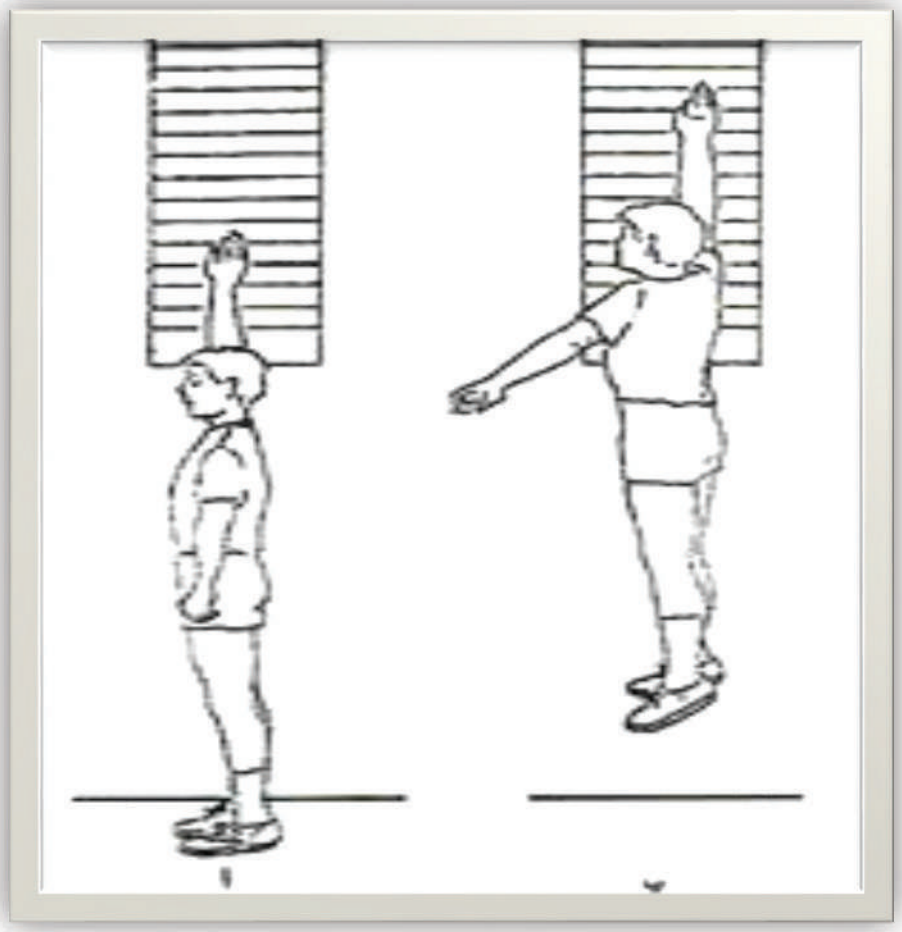


Figure (2). Vertical jump test shows stability

Second: scoring test:

- Objective of the test: to measure the accuracy of scoring.

- The necessary tools: football stadium, balls number (10), tape to set the aiming area for testing, tape measure.

Testing procedures

(10) footballs are placed in different places on the line and inside the penalty area, as shown in the figure below where the player is aiming in the areas indicated in the test and according to their importance and difficulty and sequentially one after the other, provided that the test is performed from jogging mode.

- The test starts from ball number (1) and ends in ball number (10).

- The attempt is not valid if none of the four targets in each side is hit.

Registration method:

- Calculate the number of injuries that enter or touch the four goals specified in each side of the goal and any feet of the feet so that the scores of each ball of the ten balls are calculated as follows: -

(3) scores when scoring in field (3), (2) scores when scoring in field (2)

(1) Score when scoring in the field No. (1), (0) zero in the rest of the other target areas.

- The player is given only one attempt.

Exploratory experience:

The researchers conducted an exploratory experiment on a sample from the original research community and outside the research sample and with (4) players on Thursday 25/4/2020 where the exploratory experiment aims to: -

1- Ensure the fitness of the stadium, the tools and equipment used, research requirements and their suitability.

2- Organizing the assistant work team, and the required instructions.

3- Ensuring that special tests can be carried out for the explosive power of the two men and the oxygen molecular pressure of soccer players.

4- Knowing the readiness of the research sample to perform the skill tests.

5- Know the time taken for the tests.

2-4-3 The main experiment:

2-4-3-1 Pre-test:

After completing the exploratory experiment and confirming it, the researchers applied the main experiment through applying tests and measures to the research community. Tribal tests were conducted on Sunday 7/5/2020, as the tests were according to the following sequence: -

1. Test the explosive power of the two legs and the molecular pressure of oxygen (PO2) by the soccer ball.

2. Football scoring accuracy test.

Vertimax Exercise:

The researchers prepared and organized the exercises (Vertimax) based on the personal experience of the researchers, and applied to the experimental group on 9/5/2020 until 11/7/2020, taking into account (intensity, repetitions, appropriate rest periods) and the researchers codified these exercises on a scientific basis Physiological, as well as the physical ability of the research community, the tools used, and the training method, so that these exercises are able to develop the molecular pressure of oxygen (PO2), the explosive ability of the two legs, and the accuracy of scoring for footballers, and to achieve the goals and objectives of the training process.

The details of the (Vertimax) exercises in the training curriculum are as follows: -

• The number of the total training units that included exercises for the (Vertimax) (24) units.

• The number of weekly training units that exercise for (Vertimax) (3) units for a period of (8) weeks.

• Exercise time for (Vertimax) per training unit (30 minutes) (main section only).

• Training days during the week are (Sunday, Tuesday, Thursday).

The goal of the exercises (Vertimax) is to develop the explosive potential and molecular pressure of oxygen for soccer players.

• The goal of the training exercises for (Vertimax) is to develop a soccer goal scoring skill for advanced players.

Consideration of labor exchange between muscle groups.

• Planning Vertimax training formations during the weekly and daily units (1-2).

2-4-3-3 Dimensional tests:

The researchers, with the assistance of the assistant work staff and physiology lab staff, carried out the dimensional tests of the research sample after completing the application of the exercises (Vertimax).

And that was on Monday, corresponding to (10/13/2020) in the same sequence of tribal tests, as the researchers considered the same conditions in which the tribal tests were conducted in terms of the sequence of the tests.

2-4-4 Statistical methods used:

The researchers used the statistical bag (spss) to analyze the research results, including: -

- Arithmetic mean .
- standard deviation .
- Mediator .
- (t) test for correlated samples.
- (t) test for independent samples.
- Pearson correlation coefficient.

Table (4). shows the arithmetic media, the standard deviations, the calculated value of (T) for the correlated samples, the level of the significance of the test and the difference of the significance of the pre- and post-tests of the control group of the variables studied.

Statistical means / Variables	Measuring unit	Control group		Experimental group		(t) Value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD.EV.			
The explosive ability of the muscles of the legs	Watts	1449.328	77.407	1498.89	54.516	2.875	0.054	sig.
(PO2) ratio before voltage	Mercury mm	32.4	1.673	32.8	1.923	1.633	0.178	Non sig.
(PO2) ratio after voltage	Mercury mm	18	1.581	22.2	1.303	3.628	0.022	sig.
Scoring	Degree	14.5	1.08	16.3	0.94	4.32	0.002	sig.

Present the results of the pre- and post-tests of the experimental group of the variables studied:

Table (5). It shows the arithmetic media, the standard deviations, the calculated value (t) of the correlated samples, the level of the significance of the test and the significance of the difference for the pre- and post-tests of the experimental group Of the variables researched

Statistical means / Variables	Measuring unit	Control group		Experimental group		(t) Value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD.EV.			
The explosive ability of the muscles of the legs	Watts	1453.36	89.698	1533.77	60.365	4.685	0.009	sig.

Cont... **Table (5). It shows the arithmetic media, the standard deviations, the calculated value (t) of the correlated samples, the level of the significance of the test and the significance of the difference for the pre- and post-tests of the experimental group Of the variables researched**

(PO2) ratio before voltage	Mercury mm	32.8	1.303	33	1.224	0.206	0.874	Non sig.
(PO2) ratio after voltage	Mercury mm	17.6	1.816	24.2	1.095	7.117	0.002	sig.
Scoring	Degree	14.60	0.69	19.10	0.73	13.17	0.000	sig.

The results presented in Tables (4) and (5) showed the skill test (scoring) about the existence of significant differences between the pre and post tests in favor of the post-test for the members of the control and experimental groups. It has an effective role in developing and increasing the level of skill performance among the members of the control group, as a number of exercises implemented by the control group focused on diversification of skill performance, as well as the repetitions performed by players during the training unit and the game, as for the development of members of the group. According to the researchers, the reasons for this development are attributed to the Vertimax exercises that were prepared, as they were similar to competitive skill performance, which sought to provide sufficient opportunities for their development [5]. "The high skill level contributes to reducing the player's ability to perform this skill on the one hand, and on the other hand, The stability of the good technical level throughout the game depends on the good physical condition [6-12] of the player, and the more fatigue the player has, the more He fell from his level of motor skills, especially that need to be consistent in performance.

Conclusions

Based on the research results that were reached within the limits of the research community, the following conclusions were reached: The exercises (Vertimax) led to an evolution in the explosive ability of the two men and the molecular pressure of oxygen for the advanced soccer players. The exercises (Vertimax) helped to increase the goal rate of football players among the members of the experimental group more than the members of the control group. Reflection of the exercises (Vertimax) on the instructions of the central nervous system (CNS), developing the football scoring skill.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

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The Effect of Circular Training According to the Style of Stations (Physical - Skill) in Developing Some Physical Capabilities and Aiming by Jumping in Basketball for Youth

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Abstract

The aim of the research is to identify the effect of circular training according to the stations' method (physical - skill) In developing some physical abilities and shooting skills from basketball jumping, the research community included (28) players representing Al-Nasiriyah youth club basketball players (16-18 years old) for the season (2018-2019). (20) players were chosen from them representing a sample The research was divided into two groups randomly into two groups one What a pilot and another officer and by 10 players for each group. . The researchers applied the circular training in a method (high intensity fatality) as it celebrates special exercises to develop the distinctive strength of speed for the arms, legs, back and abdomen, and the explosive ability of the arms and legs, as well as the development of correction skill from jumping by (18) training units at a rate of (3) training units per week, concluded The two researchers in the experimental group that applied circular training according to the style of stations (physical - skill) a marked superiority over the control group in some physical abilities and the correction of jumping skill.

Keywords: *Physical – skill, developing, physical capabilities, jumping.*

Introduction

Training science is an important science that contributes to developing the physical, motor and skill capabilities of the players and it is classified into several different types or methods, including circular training [1], which is one of the most important types of training that works to refine and develop physical and skill capabilities for different age groups, including young people, as this type of Training with suspense, excitement, and diversity in performing exercises, and he is able to control the existing equipment and tools and put them in the service of the goals that he seeks to achieve, as nothing can stand in the way [2] of applying the vocabulary of this type of training, while the basketball arena is a good place to apply Training, it can be replaced by a large or small square. One of the appropriate qualities of circular training is that several devices and tools can be used during its various training stations such as regular and medical balls, characters, barriers, regular targets [3], mobile and small goals, ropes, wall, etc. that tools and devices are carefully selected and then distributed objectively in order to achieve Its purposes. Physical capabilities form the cornerstone of the basketball game and is an important and essential factor to raise the level of skill and career performance as it plays a prominent role in the player's [4] mastery of basic skills, and basketball as one of the group sports activities is a skill sport that contains a large number of motor skills that need a large number of the physical capabilities and capabilities to be done in a good manner and proper performance, so it has become necessary to prepare the players physically and skillfully in order to be able to perform the motor skills with a high degree of accuracy, especially the aiming [5] skill of jumping, which requires the player to enjoy high-level physical and skill capabilities in order to be able to master the correction from all regions It is different, and in order to play real basketball, we have to train on all the capabilities this game needs (physical and skill).

Methodology

The two researchers used the experimental approach to design the two equivalent control and experimental groups, as it is the most appropriate method to solve the research problem.

Research community and sample:

The research community included (28) players representing Al-Nassiriya club youth basketball players (16-18 years old) for the season (2018-2019), (20) were chosen from them representing the research sample and by (71.42%), they were divided into two groups by the way Simple randomization (drawing) into two groups, one is experimental and one is control, with (10) players for each group. In order to achieve homogeneity among the members of the research sample and to avoid the influence of the factors that may have affected the results of the experiment in terms of individual differences in the sample within the group, the researchers conducted the homogeneity of the sample in some of the variables that may have an effect on the experimental variable (length, mass, age, Training age) and using the coefficient of difference law.

Table (1) shows this shows the mean, the standard deviation, and the coefficient of variation for the research variables for the sample individuals

Coefficient of variation	standard deviation	Arithmetic mean	measuring unit	Specifications	Siq
1. 69%	0. 03	178	Cm	Length	1
3, 61%	2, 58	71, 45	Kg	Bloc	2
3, 78%	7, 74	204, 6	Month	Age	3
11. 75%	4. 21	35, 80	Month	Training age	4

Then, the researchers conducted the equivalence between the two groups in the research variables (physical abilities, and correction of jumping skill) through the use of the law (t) for independent samples. Table (2) shows that.

Table (2) shows the value of the arithmetic mean, the standard deviations, and the calculated and tabulated value (t) in the search variables for the sample members.

Statistical significance	Values(t)		Experimental group		Control group		measuring unit	Statistical treatments variables	siq
	Tabular	Calculated	P	s	P	s			
Not significant	2,26	1,384	0.31	4,48	0,30	4,47	M	Bursting capacity of the arms	1
Not significant		0.645	3,4	40.7	4,52	39,6	Cm	Explosive power of the two men	2
Not significant		0,19	1,229	11,2	1,159	11,3	S	Distinguished strength as fast as the arms	3
Not significant		0,534	0.680	9,85	0.611	9,97	S	Distinguished strength at the speed of the two men	4
Not significant		1,168	0,875	9,1	0.966	8.6	S	Distinctive strength in the abdomen	5
Not significant		0.612	0.948	12.7	1,26	12,25	S	Distinguished strength at the speed of the back	6
Not significant		1,137	1,398	15,2	1,354	14,5	Degree	Aiming to jump	7

• Table (T) value (2,10) at the significance level (0.05), and freely (18).

Research means and devices :

Field research procedures:

Determination of the physical abilities under consideration: The researcher has selected some physical abilities in the basketball, and included them in the appendix questionnaire (1), and it was presented to the experienced and specialized in the field of sports training and the basketball game appendix (2). To identify some of the physical abilities and after collecting the forms and discharged the researcher to take a behind the experts and adoption.

Table (3) opinions of experts and specialists in the nomination shows the physical capacity and the percentage of approval and disapproval

Percentage Percentage	write off	Percentage Percentage	Serve	Physical abilities	Siq
Zero	zero	100%	11	The explosive power of the muscles of the arms	1
9%	1	90%	10	The explosive ability of the muscles of the legs	2
27%	3	72%	8	Distinguished strength at the speed of the arms of the arms	3
9%	1	90%	10	The strength marked by the speed of the muscles of the legs	4
54%	6	45%	5	Exercise the arm muscles	5
18%	2	81%	9	Distinguished strength at the speed of the abdominal muscles	6
36%	4	63%	7	Distinguished strength at the speed of the back muscles	7
45%	5	54%	6	Transition speed	8
72%	8	27%	3	maximum speed	9
54%	6	45%	5	Speed up	10

Defining physical tests: The researchers prepared an appendix questionnaire (3) in order to choose the appropriate physical tests after they were presented to those with expertise and specialization in the field of sports training and basketball, and after collecting and unloading the forms, the researchers worked on sorting The tests that got the highest marking ratio as in Table (4).

Table (4) shows the opinions of experts and specialists in nominating physical tests and the percentage of approval and rejection.

Percentage	Does not fit	percentage	Repair	the exams	Body parts	Physical abilities	Siq
63%	7	36%	4	Throw a 3 kg medical ball to stand	For arms	Explosive power	1
18%	2	81%	9	Throw a 3 kg medical ball from a sitting position on a chair			
9%	1	90%	10	Vertical jump up from steadiness	For the two men		
72%	8	27%	3	Forward horizontal jump			
54%	6	45%	5	Pull up on the block continuously for 10 seconds	For arms	Force marked with speed	2
Zero	zero	100%	11	Oblique flatness then bend and stretch the arms 10s			
27%	3	72%	8	Partridge on one leg 30 m left and right	For the two men		
63%	7	36%	4	Partridge has a maximum distance of 10 seconds for each individual man			
18%	2	81%	9	Lying with hands attached behind the head raising and lowering the torso with pressing the knees with elbows for 10 seconds	For the abdomen		
72%	8	27%	3	Lying with hands attached behind the head and feet fixed With the help of a colleague, raise the torso with pressing the knees with the elbows for 10 seconds			
54%	6	45%	5	From laying flat on the abdomen and clasping hands behind the head with feet fixed with the help of a colleague, raise the trunk up and up to a certain level for 10 seconds	For the back		
36%	4	63%	7	From laying flat on the abdomen, holding hands behind the head, fixing the feet with the help of a colleague, raising and lowering the torso from the ground for 10 seconds			

Description of physical tests and correction skill used in the research:

First: The explosive power test for the two arms: throwing the medical ball a weight of (3) kg with two hands from the position of sitting on the chair. (5: 289)

The purpose of the test: To measure the explosive strength of the arms of the arms and shoulders.

Tools used: medical ball (3) kg, tape measure, and chair with secured torso strap

Method of performance: The laboratory sits on the chair and the medical ball is hand held so that the ball is higher and behind the head. The belt is placed around the chest of the laboratory and held from the back in a tight manner for the purpose of preventing the laboratory from moving forward while throwing the ball with two hands, because movement is limited to pushing the ball with two hands only.

Conditions: The laboratory is granted three consecutive attempts, and the laboratory must be allowed to perform a number of throws for the purpose of warming up before the performance. When the laboratory moves during the performance of one of the attempts on the chair, the result is not calculated and another [6] attempt is given in its place.

Recording: the distance between the front edge of the chair and the nearest point the ball places on the ground is calculated. The best attempt is calculated from the three attempts.

Second: The explosive power test for the two men:

Test name: Vertical Jump of Sargent (68:10)

The purpose of the test: to measure the explosive strength of the two men

Tools used: smooth wall of suitable height, tape measure, chalk.

Method of performance: The laboratory stands facing the wall, then the laboratory extends its arms up to its full extent for the purpose of knowing the first sign and then records the number, noting the sticks attached to the ground, the laboratory swings the arms down and back with the torso bent forward [7] and down and bends the knees to the position of the right angle only.

Registration: The registration is made by the number of centimeters the laboratory reached from the standing position, and the mark it reaches as a result of the jump-up, as the mark (the distance between the first mark and the second mark is the amount of the explosive power of the two men).

Third: Test the force marked by the speed of the arms: (8: 268)

Fourth: Lift the torso and put pressure on the knees (abdomen) in (10) w: (5: 348)

The purpose of the test: to measure the strength marked by the speed of the abdominal muscles.

Tools used: colleague or observer to calculate [8] the number of compression times - stopwatch.

How to perform the test: From lying on the back and hands tied behind the head with feet fixed with the help of a colleague, lift the torso and squeeze the knees by touching their elbows alternately.

Registration: The number of times the trunk is [9] lifted from the ground in (10) tha.

Fifth: Lift the trunk back (back) on the 10th w:

Measure the strength marked with the speed of the back muscles.

Tools used: colleague or observer to calculate the number of times lift - stopwatch.

How to perform the test: From laying flat on the floor, clasping hands behind the head, and fixing feet with the help of a colleague, lift the stem off the ground in (10) seconds.

Recording: The number of times the stem is raised in a time of (10) seconds.

Sixth: The force distinguished by the speed of the two men: (6: 149)

The test: Partridge on one leg for a distance of (30) meters left and right

Special exercises:

Objectives: The researchers have prepared special exercises aimed at developing the explosive ability and the distinctive force of speed and the aim of correction of jumping basketball for young people (16-18 years). These exercises are characterized by the following: -

a. To contribute to achieving the goals of the special preparation stage (physical and skill) for young players (Al-Nassiriya Club).

B. The contents of the exercises should be matched with the physical and skill characteristics of the players.

C. The exercises were applied by extracting the average stress of all the exercises used.

Dr . Take into account what means and capabilities affect implementation.

E. The diversity of exercises and flexibility in implementation, which makes it clear when implementing.

Dimensional Tests: After performing the special exercises, the two researchers conducted the dimensional tests of the research sample on Sunday and Monday, 11-12 / 3/2019. The researcher was keen to prepare the distance tests similarly in terms of spatial and temporal conditions for the tribal tests.

Statistical means:

The researchers used the following statistical methods: (percentage - arithmetic mean - standard deviation - coefficient of variation - test (t) for symmetric samples - test (t) for independent samples) [10, 11]

Presentation, analysis and discussion of the research results:

Present the results of the pre and post tests for some physical capabilities and correction skill for the control group and analyze them.

Table (5) shows the mean and standard deviations and the calculated and tabulated value (t) of the pre and post tests of the search variables.

Statistical significance	Value (t)		Post-test		Pre- test		measuring unit	Variables Statistical treatments	siq
	Tabular	Calculated	P	s	P	S			
Significant	2,26	4,978	0.05	4,54	0,306	4,47	M	Bursting capacity of the arms	1
Significant		4,03	7,07	46,9	4,52	39,6	Cm	Explosive power of the two men	2
Significant		8,08	0,994	12.9	1,159	11,3	Th	Distinguished strength at the speed of the arms	3
Significant		3,305	0.628	9,690	0.611	9,97	Th	Distinguished strength at the speed of the two men	4
Significant		13,416	0.966	10,6	0.966	8.6	Th	Distinctive strength in the abdomen	5
Significant		9	1,054	14	1,26	12,5	Th	Distinguished strength at the speed of the back	6
Significant		8,820	1,702	16.7	1,354	14,5	Degree	Aiming to jump	7

Table value (t) (2.26) at the significance level of 0.05 and freely (9)

Conclusion

Both the control exercises and the experimental groups have achieved a positive effect in developing some physical capabilities and shooting skills from jumping, but in varying proportions. The experimental group that applied circular training according to the stations' method (physical - skill) showed a remarkable superiority over the control group in some physical capabilities and the correction skill of jumping

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ministry of Higher Education and Scientific Research and all experiments were carried out in accordance with approved guidelines.

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The Effect of Inverted Learning by the (Edmodo) Platform in Motivation Motor Performance of Some Skills of Artistic Gymnastics for Students

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Abstract

Inverted learning is one of the newly emerging forms of e-learning that is known as an educational model that aims to use modern technologies and the Internet in a way that allows the teacher to prepare the educational unit through video clips, audio files or other multimedia, for the learners to see at home or anywhere another is to use computers, smartphones or tablets before attending the educational units. The objectives of the research were determined in preparing the motivation measure for the motor performance of the artistic gymnastics for players, as well as identify on the effect of inverted learning by the (Edmodo) platform on motivation performance for some skills of artistic gymnastics for players. Based on these findings, the researchers recommended several recommendations, including (the necessity of adopting inverted learning by the Edmodo platform in the motor performance of the artistic gymnastics for players. Likewise, the need to adopt inverted learning by the (Edmodo) platform to learning some artistic gymnastics skills for players, and also the confirmation to using (Edmodo) inverted learning during educational units in the motor performance motivation in some artistic gymnastics skills for players.

Keywords: *Edmodo platform, motivation motor, skills, artistic gymnastics*

Introduction

Inverted learning is one of the newly emerging forms of e-learning that is known as an educational model that aims to use modern technologies [1] and the Internet in a way that allows the teacher to prepare the educational unit through video clips, audio files, or other multimedia [2], for learners to see at home or anywhere using computers, smartphones or tablets before attending educational units, while the unit's time is devoted to discussions, projects and training. The electronic educational platform (Edmodo educational platform) comes at the forefront of the technologies of the second generation of the web, which is witnessing an increasing demand for employment by those responsible for the educational process [3], this is due to the vitality and enjoyment that it gives to the teaching and learning process, which pushes the learner to interact with the content provided through it, as well as with his peers and his teacher, as well as his participation in a number of motor tasks or duties that gain and develop motor skills, and the artistic gymnastics is considered one of the individual competitive events or sports that have a huge amount of different skills in different devices, and each device has its own performance characteristic [4], which distinguishes it from other devices, gymnastics is also an important activity or sport that requires good physical and motor qualities, in addition to good psychological skills and abilities to overcome the state of fear and reluctance to perform skills, especially difficult ones on devices, because it is a game that includes motor chains on different devices, one of the important psychological skills or abilities is the motor performance motivation, which is the most important variable in moving the learner's behavior [5], and it plays an important and prominent role in achieving the goals of the process of learning the required motor skills and determining the level of its technical performance for it, in addition to clarifying why the learner behaves in a certain manner. Through the foregoing, comes the importance of research in introducing a new method in the process of learning the technical skills of gymnasts for players, which is the inverted learning method with an educational platform

called (Edmodo), in addition to determining the nature of the motor performance motivation that players enjoy by setting a special measure for that to reach the desired goal, which is to reach out to learn the skills of artistic gymnastics in a new and better way.

Methodology

Exploitation experimental Sample :

This sample consisted of (10) players ages (10-12) years who were chosen randomly from the research community who did not participate in the main research sample and from Al-Hilla Youth Club.

Sample preparation motor performance motivation measur:

This sample consisted of (40) players who were randomly chosen from the research community who did not participate in the main research sample and it constitutes a percentage of its amount.

(57.14%) of Al Mahwel Youth Club and Abu Gharq Club ,number (20) players for each club.

The main research sample:

The main research sample of (20) players was chosen from Al-Hilla Youth Club, and they were divided into two groups (experimental and controlling) by (10) players for each group as the first (experimental) group uses the inverted learning strategy by the (Edmodo) platform and the second group (control) uses the curriculum Followed by the coach.

Homogeneity of the sample:

The researcher performed the homogeneity of the research sample in the variables of the total length, time, and mass, as shown in Table (1).

Table (1). The homogeneity of the research sample shows the variables of the total length, time, and mass

Variables	Measurement units	mean	Std. Deviation	Mode	Skew ness
age	Year	11.25	0.67	11	0.37
Length	cm	151	3.11	150	0.32
mass	Kg	36.34	1.52	35	0.88

The results of Table (2) show that the value of the skew ness calculated in the variables of the total length, age and mass came smaller than (+1), which indicates the homogeneity of the research sample in all these variables.

Equivalence of the two research groups:

To start with one initiation line, the researchers conducted equivalence between the control and experimental research groups in the pre- tests of motor performance motivation and some of the gymnastic skills for players as shown in Table (2).

Table (2). Shows parity between the control and experimental research groups in the pre- tests of motor performance motivation and the technical performance of the artistic of Arab jump the ground and stand on the shoulders on the parallel and the start of the small-course rallying on the high bar device device in Artistic gymnastics.

N	groups	Variables	Measurement units	mean	Std. Deviation	Calculated value (t)	Type of significance
1	control	motor performance motivation	mark	65.13	3.98	0.59	non Sig
	experimental			66.21	3.76		
2	control	Performance artistic of Arab jump	mark	3.56	1.17	0.49	non Sig
	experimental			3.33	1.22		
3	control	Artistic performance to stand on shoulder of parallel	mark	2.87	0.82	0.44	non Sig
	experimental			3.02	0.91		
4	control	Artistic performance of the small-course rallying on the high bar device	mark	3.21	1.32	0.65	non Sig
	experimental			3.56	1.44		
Table (t) value (2.1) at the significance level (0.05) and under the degree of freedom (18).							

The results of Table (2) show the calculated values of (t) in the pre-test, as the results showed that all calculated (t) values were smaller than the tabular value of (2.1) at the level of significance (0.05) and under the degree of freedom (18), which indicates There were no significant differences between the two groups, and this indicates their equivalence in all research variables.

The preparation of paragraphs of the motivation performance measure:

Preparing the initial formula for the motive performance scale paragraphs requires several procedures, starting with the process of collecting paragraphs and preparing them and determining the way to formulate them, and finally preparing instructions and understanding the paragraphs from the respondents, and after the researcher was informed of special studies and scientific research in the field of motor performance measurement the researcher prepared a questionnaire for the paragraphs of this measure formed Of (48) items [(1) (2)], depending on several metrics in this area. The researcher presented this questionnaire to a group of experts and specialists in the fields of (tests and measurement, motor learning, and artistic gymnastics) to determine the validity of its paragraphs on the research sample, and after sorting out the relevant forms, Was used (Chi-square) test of good conformity to accept the paragraphs of the scale, as shown in Table (3).

Table (3). Shows the calculated values of (Chi-square) and the type of significance for the motivation motor performance measure in artistic gymnastics

measure	The number of paragraphs	agree	dis agree	Chi-square	Type of significance
motivation performance motor	34	9	0	9	Sig
	14	0	9	0	non Sig

The results of Table (3) show that the calculated values of (Chi-square) for (34) are greater than the tabular value of (3.84) at the level of significance (0.05) and under the degree of freedom (1), as a result of which these paragraphs are accepted, while (14) paragraph rejected because its calculated (Chi-square) values are smaller than its tabular value of (3.84) at the significance level (0.05) and under the degree of freedom (1).

Exploratory experiment for measure:

The researchers applied the measure to an exploratory sample consisting of (10) randomly chosen players representing the Babylon Youth Club on April 12/4/2020, at ten o'clock in the morning in the conference hall of the Babylon Youth Forum and the conduct of this experiment was to achieve several objectives: -

1- Clarity of the paragraphs of the motor performance measure and the clarity of its instructions.

2- Identify the time taken to answer the paragraphs of the measure.

3- Identify on the obstacles that the researcher may face during the procedures of implementing the measure.

Apply the motor motivation measure to the preparation sample:

Paragraphs of kinetic motivation scale were applied to the sample for preparation for statistical analysis, selection of valid ones, and exclusion of invalid ones based on the discriminatory ability law (in two ways the two party groups and the internal consistency) for each of them, as well as to extract two indicators that validate the scale and its stability, by the preparation sample of (40) players for the period on April 19/4/2020 at ten o'clock in the morning and collectively at the Mahawil Youth club and Abu Gharq club with the presence of the assistant team, according to the instructions for this scale and as follows:

Measure instructions:

1- Write full name in the form.

2- Answer all paragraphs and not being leave any paragraph unanswered.

3- Read each paragraph carefully, then choose the appropriate answer.

4- Paragraphs are answered according to three alternatives that are (always, sometimes, rarely). The way to answer is by choosing the appropriate alternative from among three alternatives by placing a sign (√) in front of the appropriate alternative.

5- Not to put more than one sign (√) or choose more than one alternative.

Correct the measure: The correction of the scale is represented by the correction key consisting of the grades (1,2,3) for the positive paragraphs and vice versa for the negative paragraphs. Thus, the scale grades range from (34-102) degrees, and with a hypothetical mean of (68) degrees.

Statistical analysis of the paragraphs of the motor performance motivation measure:

First: the two party groups (discriminatory ability): To extract the discriminatory ability of the scale paragraphs, the researcher after the process of collecting and unloading the data, conducted the process of arranging the grades for the scale in an ascending order from the lowest degree to the highest degree [6, 7], as 33% of the higher scores were chosen for the scale and 33% of the lower scores were in the measure, this indicates the ability of the chosen scale to distinguish between players , (Preparation sample) of (40) players, A test (t) was conducted between the two groups, the number of each group (13) player, for the purpose of knowing the differences between the two groups, as shown in Table (4).

Table (4). The Coefficient of Discernment for each of the paragraphs of the motive performance measure in the artistic gymnastics applied to the preparation sample

Paragraph number	Calculated value (t)	Paragraph number	Calculated value (t)	Paragraph number	Calculated value (t)	Paragraph number	Calculated value (t)
1	3.97	10	4.11	19	4.19	28	3.78
2	3.11	11	3.56	20	3.78	29	3.92
3	3.05	12	4.13	21	3.87	30	4.54
4	3.91	13	3.98	22	4.22	31	4.25
5	4.12	14	4.43	23	4.56	32	3.95
6	4.25	15	4.56	24	3.89	33	3.87
7	4.09	16	3.78	25	4.11	34	4.75
8	3.78	17	3.65	26	4.09		
9	3.63	18	3.44	27	3.67		
Table (t) value (2.06) at the significance level (0.05) and below the freedom degree (24)							

The results of Table (4) show that the motivation performance paragraphs in the artistic gymnastics of (34) are distinguished, which indicates their acceptance of all, depending on the fact that the calculated values of (t) came greater than the tabular value of (2.06) at the level of significance (0.05) and below the degree Freedom (24).

Second: coefficient of internal consistency: The internal consistency coefficient is used to determine the extent of homogeneity of the vertebrae in its measurement of the measured behavioral phenomenon. To find this parameter, a simple correlation coefficient (Pearson) was used between the score of each vertebra and the overall score of the scale for all members of the preparation sample, which amounted to (40) players, as shown in table (5).

Table (5) Correlation coefficient values (R) calculated between the degree of each paragraph in the total degree of the motivation performance measure in artistic gymnastics.

Paragraph number	Calculated value (R)	Paragraph number	Calculated value (R)	Paragraph number	Calculated value (R)	Paragraph number	Calculated value (R)
1	0.31	10	0.39	19	0.34	28	0.35
2	0.37	11	0.44	20	0.37	29	0.37
3	0.33	12	0.39	21	0.37	30	0.44
4	0.39	13	0.33	22	0.38	31	0.44
5	0.36	14	0.44	23	0.34	32	0.39
6	0.34	15	0.31	24	0.37	33	0.35
7	0.35	16	0.37	25	0.38	34	0.37
8	0.37	17	0.37	26	0.31		
9	0.44	18	0.38	27	0.37		
Table (R) value (0.304) at the significance level (0.05) and under the degree of freedom (38).							

The results of Table (5) show the correlation coefficient values calculated between the degree of each paragraph in the total degree of the motive performance motor measure in the artistic gymnast came greater than the tabular value of (0.304) under the level of significance (0.05) and at the degree of freedom (38), which indicates the significance of the correlation, so all paragraphs were accepted

The scientific foundations of the motor performance motivation measure for artistic gymnastics

Validate the measure: In order to verify the validity of the scale, the researcher used two types of validity:

Face validity: This type of validity is achieved when presenting the measure to a group of expert and specialists, they are number (9), they specialize in the fields of (motor learning, artistic gymnastics, educational and psychology sport since), then the questionnaire forms were collected, data are emptied and statistical work performed, as shown in table (4).

Construct Validity: This type of validity has been proven by extracting the discriminatory ability of the measure, as shown in table (5).

Stability of measure: Extract the results of the stability of the measure, the researcher used two methods are:

Test method and retest: The scale was applied to the sample of the exploratory experience, which was (10) players randomly chosen from the Babil Youth

club on April 22/4/ 2020, at ten o'clock in the morning in the artistic gymnastics hall, then re-apply the scale after (7) days have passed since the first application, that is, on 29/ 4/2020, at ten o'clock in the morning in the artistic gymnastics hall as well, after collecting and unloading the data, the researcher extracted the value of the simple correlation coefficient (Pearson), that reached (0.89) which is a high stability that can be adopted in the application of the test, and thus the scale became ready for application.

Conclusions

Based on the research results reached within the limits of the research community, the following conclusions have been reached: For inverted learning by the (Edmodo) platform an important role in enhancing the motivation performance of the sports artistic gymnastics for players. Diversity in providing skills to players using new tools represented by the (Edmodo) platform. The motivation performance motor boost has been enhanced and some of the artistic gymnastics skills have been learned to the players. Inverted learning by the(Edmodo) platform, it provided an element of suspense and excitement, which led to the development of desire and motivation among players to learn some skills in artistic gymnastics.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon and all experiments were carried out in accordance with approved guidelines.

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The Effect of Exercises by Metabolic Conditioning (MetCon) Style in Some Physiological Variables and the Speed Motor Response for Young Volleyball Players

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Abstract

Preparing exercises by Metabolic conditioning (MetCon) style for volleyball, and identify of the effect of (MetCon) exercises on some physiological variables and the speed motor response of young Volleyball players. researchers believe that it affects in some physiological variables and speed motor response that are directly involved in the performance of motor skills of Volleyball for young players. Through the experience of field researchers note there is a noticeable decrease in the performance of defensive skills In matches when the level of technical performance is very high or the game continues for longer periods of time, this decrease leads to a slow moving to take the right place while performing these skills, researchers believe that the reason for this is that these skills are not getting enough share from training in a way that suits the nature of its physical and physiological performance, so the researchers felt that they should go into this experiment by preparing exercises by style of metabolic conditioning (MetCon), the aim of it is to make a positive impact in some physiological variables and speed motor response for volleyball young players .

Keywords: *Metabolic conditioning, physiological variables, speed motor response.*

Introduction

There is no doubt that scientific research has become one of the most important necessities in our modern [1] society in reaching the highest levels for all aspects of life by identifying the different energies and abilities of human to try to achieve most benefit from scientific theories and their application to serve and develop the society, including the sports field [2], it may need a lot from sporting events to great time to get to high level of abilities and possibilities . One of the games that have become a lot of attention in recent times is the game of Volleyball, which is one of the most popular games in the world, Volleyball requires a great muscle ability to perform its skills, upon closer examination In the nature of performance we find that it requires high energy to perform the motor duty with force, speed and endurance, it requires from players when performing the skills and high [3] biomotor abilities. Given the multiplicity and diversity of training curricula used by coaches for clubs and teams, and the overlapping of these curricula with each other, the impact of each of them has become to address specific requirements in order to develop the level of players, one of the recent training style s has been the Metabolic conditioning (MetCon) style [4], which is one of the latest training style s that use high-strength training and short time, this is consistent with the nature and properties the game to create a state of functional adaptation, improve energy production systems, and improve the career, physical, and skill level of players.

Methodology

As for the research methodology and its field procedures, researchers used the experimental style to solve the problem of research, the research society was determined by the Kufa Club volleyball for youth , for the sports season 2019-2020, the number (12) players, and they were divided into two groups (experimental and control) in the simple random way (Lottery).

Research community and the sample:

The research community was determined by the youth Kufa sports Club for the sports season (2019-2020) and the number (12) players was chosen by the researcher in full for the experiment, they were

distributed equally to two groups in a random way (lottery), after that, the experimental group underwent a training that includes the use of exercises by Metabolic conditioning (MetCon) style, while the control group continued to use the trainer's regular training style .

Means and instruments used in research:

Means of data collection

- Arab and foreign sources and references.
- Personal interviews.
- Tests and measurements
- forms for recording test results for players.

More than consumption at rest time (EPOC), Heart rate after physical effort:

O2 deficit is measured and the amount of oxygen consumed during the recovery period (hospitalization) in excess of consumption during rest time (EPOC) precisely using device (Fitmat pro), where the player information is entered (Name, length, mass, and age) Before starting the measurement, also, clean the test mask by antiseptic, and connect the parts of the system with installing the heart rate belt on the chest and installing the signal receiver for the heart rate (Bluetooth) in the port assigned to the device, then (Wingate) test is performed on Physical effort bicycle Type (MONARK), where the mask for measuring (VO_2max) is placed on the player's face, after completing all the requirements for working the devices and after completing entering the required data into the device as well as the program for the physical effort bicycle, the test is started according to the conditions of the Wingate test, after completing the test, data will be recorded for the maximum oxygen consumption rate (VO_2max) from the (Fitmat pro) software, choose the recovery option)) from (Fitmat pro) device with the mask remaining on the player's face until O_2 consumption is reached, the rest time following the physical exertion, with the same mechanism, the test is performed twice.

Note: the (Fitmat pro) device during the voltage gives a reading of (vo2max) in units of milliliter / kg / minute.

Procedures (Wingate) test:

The necessary tools: Stopwatch, calculator, physical effort bicycle type (MONARK).

Style of performance: The test is performed using the stationary bicycle (MONARK) Swedish made, according to the following steps:

ü The examined mass is taken To the nearest correct Kilogram.

ü The data of the examiner is entered into the computer and the resistance is set according to the mass of the examiner, which is equivalent to 7.5% of his body mass.

ü Riding examined on a bike, the seat is adjusted according to his length so that there is a very slight flexion at the knee joint within (10º), then he adjusts the foot strap, the procedures are explained to the examiner, noting that stirring is performed upon receiving the signal.

ü The examiner performs a warm-up operation on the bike for a period of (3-4) minutes where the resistance is gradually placed according to the mass of the examiner before the end of the warm-up process, the examiner moves the bicycle wheel at full speed for a period of (3-5 seconds) and repeats this two to three times.

ü Lift the weight from the ballast basket, the examiner starts moving the bicycle wheel at the fastest possible speed with a speed of no less than (80) cycles for a period not exceeding three seconds Then the weight is lowered gently and at the same time the program start button is pressed to begin the measurement process the examiner continues to move the wheel for a period of (30) seconds, to be encouraged and urged to maintain the speed of rotation as possible.

Table (1). Shows the amount of oxygen consumption during the effort using a bicycle, physical effort at different abilities

bicycle resistance (kg)	Required O2(l / s)
0.5	0.6
1	0.9
1.5	1.2
2	1.5
2.5	1.8
3	2.1
3.5	2.4
4	2.8
4.5	3.1
5	3.5
5.5	3.8
6	4.2
6.5	4.6
7	5

We extract the required resistance through the following equation:

· **The resistance required for the player=** $\frac{\text{MASS The Playr} \times 7.5}{100}$

After extracting the required resistance, we extract the absolute consumed oxygen by converting the relative consumed oxygen (ml / kg / minute) to (liter / minute) through the following equation:

· **Absolute consumer oxygen=** $\frac{\text{Relative oxygen consumedgen} \times \text{MASS The Playr}}{1000}$

Then the O_2 Deficit is extracted by the following equation:

· O_2 Deficit = Required oxygen - Absolute consumer oxygen

As for the oxygen debt (EPOC) it is extracted through the following equation:

· Oxygen debt = Relative consumption oxygen – 3.5

ü The heart rate was extracted from the device directly

Nelson Test for a motor response:

The purpose of the test: Measuring the ability to respond and move quickly and accurately according to choice the catalyst.

Tools used:

1- Barrier-flat area with a length (20 m) and a

width (2 m) within three lines plan between the distance between the line and the other (6.40 m), length a Line in the middle (1 m), placed Volleyball hanging and falling to the ground on the two sides' lines , preferably, the test performance should be in the volleyball court.

1. Electronic stopwatch.

2. Measure tape.

Test administrators:

1- Recording : He calls the names first, and records the test performance time.

2- A timer: Giving start signal with timing.

Main Experiment Procedures:

Pre-tests:

Researchers applied the main experiment through applying tests and metrics to the research community, and pretest were conducted on Sunday 16/12/2019, where the physiological variables were measured and the motor response speed test.

The details of the exercises in the training curriculum were as follows:

1- The total number of training units that included the functional exercises (24) units, and the number of weekly training units that applied exercises is (3) units for a period of (8) weeks.

2- The time for functional training in one training unit is (20-25) minutes.

3- The purpose of exercises is to develop physiological variables and speed of motor response.

4- Consideration of labor exchange between muscle groups.

5- Planning the formations of exercises during the weekly and daily units are (1-2).

Post-tests:

The researchers, carried out the post-tests of the research community after the completion of the exercise. This was on Wednesday (19/2/2020) and in the same sequence of pre-test. the researchers took the same conditions as the pre-tests where sequence tests.

Statistical style s used:

The researchers used the statistical bag (spss) to analyze the research results, including: -

- Mean.
- STD.EV.
- T-test for independent samples.
- T test of the interrelated samples .

Discussion of results:

Table (2): Shows the mean, standard deviations, the calculated value of (t) of the interrelated samples, the level of the significance of the test and the significance of the difference for the pretest and posttests of the control group of the studying variables.

Variables	Measuring unit	Pre-test		Post-test		(t) value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD. EV.			
Oxygen deficit	L / min	1.25	0.39	1.016	0.217	3.11	0.027	Sig.
Oxygen debt	L / min	32.1	3.183	35.616	2.503	9.216	0.000	Sig.
Heart rate after physical effort	Beats / min	179.33	1.032	182.5	1.048	5.27	0.003	Sig
Response speed	second	1.976	0.084	1.873	0.045	3.938	0.011	Sig

· View The results of the pre-tests and post-tests of the experimental group of the studying variables:

Table (3) : Shows the mean, the standard deviations, the calculated value of (t) of the interrelated samples, the level of the significance of the test and the significance of the difference for the pretest and posttests for the experimental group of studying variables .

Variables	Measuring unit	Pretest		Posttest		(t) value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD.EV.			
Oxygen deficit	L / min	1.248	0.333	0.665	0.201	8.569	0.000	Sig.
Oxygen debt	L / min	32.5	2.98	38.933	2.324	8.975	0.000	Sig.
Heart rate after physical effort	Beats / min	178.833	0.952	184.333	0.816	14.03	0.000	Sig
Response speed	second	1.989	0.069	1.813	0.030	6.601	0.001	Sig

- View the results of tests (Posti. Posti) of the two groups control and experimental studying variables :

Variables	Measuring unit	Pretest		Posttest		(t) value	Level of significance	Type of significance
		Mean	STD.EV.	Mean	STD.EV.			
Oxygen deficit	L / min	1. 016	0. 217	0. 665	0. 201	2. 903	0. 016	Sig.
Oxygen debt	L / min	35. 616	2. 503	38. 933	2. 324	2. 378	0. 039	Sig.
Heart rate after physical effort	Beats / min	182.5	1.048	184.33	0.816	5.222	0. 000	Sig
Response speed	second	1. 873	0. 045	1. 813	0. 03	2. 699	0. 002	Sig

Table (4) :Shows the value of (t) calculated for independent samples and the level of the test and were significant differences between the results of the test (post-test) of the two groups control and experimental studying variables .

Discussion of the Results

The results presented in tables (2) and (3) for the physiological variables tests showed that there were significant differences between the pre and post tests and in favor of the post tests for the control and experimental groups, the researchers attribute the reason for this moral difference to the members of the control group, due to the exercises that were applied by style s and style s prepared by the trainer in his training units, as the repetitions performed by the members of the control group and the continuation of the training lead to the occurrence of functional adaptations in certain proportions, and these adaptations are the deficit, the oxygen debt and the heart rate,

As for the results presented in table (4), which show the preference of differences in favor of the experimental group in the post- tests, the researchers believe that the exercises that they prepared for the members of the experimental group that were applied by the metabolic style (MetCon), created adaptations of the body's functional systems this was due to exposure to physical and sub-extreme physical loads, most of which were crossed by the limits of the anaerobic threshold and work with insufficient oxygen , when performing a high-intensity work that exceeds the limits of the anaerobic threshold, we see that the rate of oxygen consumption increases gradually until it reaches the fatigue stage without a state of stability with the rate of oxygen consumption, as for after stopping the performance of the physical effort, we notice that the rate of oxygen does not return to the normal [6] limit (before performing the physical exertion) but rather requires a lengthy period of time that is Longer or shorter depending on the adaptive abilities of the athlete's body's equipment, this is what was changed by the training loads applied by the members of the experimental group, which contributed to a kind of muscle adaptation.

The researchers see the differences between the control and experimental groups for heart rate due to the quality of exercises, as they were built according to a scientific physiological nature, as the researchers prepared a large part of it in a way that is compatible with the lactic energy system, in order to be able to improve the chemical pathways for energy production, this has helped the players to make an improvement in the work of the functional systems, so we notice an increase in the percentage of heart rate for the members of the experimental group more than the members of the control group, also, the exercises were character by diversification, change and frequent repetitions, and this also led to a state of adaptation to the internal systems and continuous sports training increases the heart rate [7] .

The researchers also attributes the reason for the development of the members of the experimental group at the expense of the members of the control group in the speed of the motor response due to the nature of the exercises that helped to develop that ability because it was explosive in nature and changing from moment to moment as the speed of response depends on movements with an instant reaction and that the exercises prepared with the style of (MetCon) contributed significantly to the process of linking the response speed and the motor speed of the legs and technical skills, which is one of the requirements for the successful performance of these skills quickly and with utmost accuracy, the principle of diversification and change that the researcher used in addition to the continuous iterations and scientifically contributed greatly to the development of these two abilities, which depend on rapid and sudden movements, (Magill) confirmed this when he said "To diversify your workout experiences , organization and diversity in the movement will increase the experience for the players and increase the player's ability to perform the skill better.

Conclusions

Based on the research results reached within the limits of the research community, the following conclusions have been reached:

4- The exercises implemented by the experimental group by metabolic conditioning style (MetCon) helped to reduce the rate of oxygen deficiency and increase the proportion of oxygen debt.

5- The continuous training of exercises, which were applied in the Metabolic conditioning style

(MetCon), led to the adaptation of Cardiac muscle by increasing the concentration of heart rate.

6- The duration of the exercises, which were applied by metabolic conditioning style (MetCon) represented by the number of training units, was appropriate in creating adaptations that reflect the evolution of the experimental research group in the speed of the motor response.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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The Effect of Both (Ballistic and Plyometric) Methods of Exercises in Protecting from Knee Joint Injury in Terms of the Most Important Muscles Working on it for the Handball Center Player

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Abstract

The knee joint injury is very common for the handball center player, because of his position, which depends on the rotation on one of the legs to shoot on the goal with or without resistance from the opponent and jumping, and this requires giving appropriate exercises to strengthen the most important muscles working on this joint, which works to prevent injury in it through experimenting both (ballistic and plyometric) training methods, and the aim of the research is to identify the effect of both (ballistic and plyometric) methods in preventing knee joint injury according to the most important muscles working on the knee joint for the handball center player, and to identify the best methods in preventing Knee joint injury according to the most important muscles working on the knee joint for the research sample, and the researchers used the experimental approach with two experimental equal groups, The research community was determined which are the center players in Karbala governorate handball Clubs, in the youth age group (17-19) years, and they were randomly and evenly divided into two experimental groups as a sample for research As the first group used ballistic exercises, and the second group used plyometric exercises.

Key words: *Knee joint, Injury, muscles working, Handball Center Player*

Introduction

Sports training has become the optimal process for getting the athletes to levels that qualify them to compete in the championships and competitions through preparing them in various qualities [1], including physical ones, which have a prominent role in developing other aspects, and through the integration with each other leads the skill or effectiveness to reach to a high level during competition [2]. This hasn't come in vain, but it has come through using modern and objective methods that were placed by specialists and researchers who have got all sections of modern science from various aspects through searching, study and accurate tests that they carry out to bring sports activity and activities to the level of Sophistication and progress [3]. The development of the handball game in recent years has tended to the strong and fast play, thus it requires great skill and physical work, and this leads to the pressure on the muscles and joints of the athlete's body and consequently to the occurrence of injury. which imposed us to think to find solutions for this state, depending on the muscular work and muscular balance's strength for the muscles that work for all joints, especially the muscles working on the knee joint, because of its importance in the performance of the handball player in general and the center player in particular, and what burden he bears during jumping, moving, and rotating with or without resistance, for protection from injury to this joint before it occurs [4]. Therefore, modern training has turned to the use methods that help to produce fast strength that enables muscles to work with high efficiency without being injured, and these methods include ballistic and plyometric training, as they are from the best training methods and most suitable for the handball game, as its training is more appropriate and similar to the

performance of the modern handball player, especially for the center player. From this standpoint, the aim of this study is to protecting the most important muscles working on the knee joint of the center player through achieving progress in the efficiency of these muscles using ballistic and plyometric exercises.

Methodology

Research approach

The nature of the problem determines the approach used in the research to obtain accurate information and results and "the most important thing recognizes the accurate scientific activity is using the experimental method([1])so that the researchers used the experimental design with two equal experimental groups that suit the nature of the studied problem, and below the experimental design that was used in the research.

Research community and its sample

The researchers identified the research community for Center players of Karbala's Governorate handball clubs, the youth age category 17-19 years, who are 14 players, and they were all chosen as a sample for research and in a comprehensive inventory method, and they were randomly and evenly divided into two experimental groups with a rate of 7 players for each group, as the first group used exercises using the ballistic method, and the second group used exercises using the plyometric method.

Table (1) shows the homogeneity of the research sample

Statistical parameters / Variables	Measuring Unit	Arithmetic Mean	Standard Deviation	Mean	Torsional Coefficient
Length	Cm	171	7.12	171.5	-0,628
Weight	Kg	50.2	5.84	48.55	0,586
Training age	Month	21	3.58	22	0,758

Table (2) shows that the torsional coefficient of the variables (length, weight, and training age) is between ±1, which indicates the homogeneity of the research sample in these variables.

To control the research variables that influence the experiment and to start from one initiation point, the researchers sought to find equivalence for the two of the research groups for the pre-test results using T for independent samples, as shown in Table (2).

Table (2) shows the equivalence of the two research groups in the searched variables

Statistical parameters / Variables	Measuring Unit	The experimental group (1)		The experimental group (2)		Calculated T value	indication level	Type of indication
		A	B	A	B			
Femoral straight muscle activity	MV× S	1405,27	26,93	1366,91	13,86	0,110	0,954	un significant

***Cont...*Table (2) shows the equivalence of the two research groups in the searched variables**

Femoral posterior muscle activity	MV× S	2512,44	19,06	2493,20	10,08	1,903	0,268	un significant
Long fibula muscle activity	MV× S	1089,13	7,22	997,61	6,17	0,249	0,789	un significant
Twin muscle activity	MV× S	923,76	15,36	894,72	10,24	1,222	0,271	un significant

Freedom degree = 12 at the significance level (0.05)

Table (2) shows that the values of the indication levels were higher than the indication level 0.05 and for all research variables, at a freedom degree 12, and thus the differences are not significant between the two research groups, which achieves parity between them.

Means, tools and devices used in the research

Means of collecting information

- Objective tests and measures.
- Observation

Determine the most important muscles that work on the knee joint

After referring to the scientific sources, the most important muscles that work on the knee joint were identified as they are: (the femoral rectus muscle, posterior femoral muscle, long fibula muscle, twin muscle).

Determine the tests for the search variables

After referring to scientific sources, the researchers chose the electrical activity test of the muscle to serve the studied variables.

Description of the tests

EMG device test

Test name: Measurement of the electrical activity of muscles using an EMG device.

The purpose of the test: To measure the activity of the most important muscles that work on the knee joint

Exploratory experience

The researchers have made an exploratory experiment on Sunday/ 11/23/2018) on a group of 4 player sample, to benefit from the following:

1- Testing the validity of the tools and devices used in the tests.

2- Knowing the efficiency of the staff and train them on tests.

3- The validity and relevance of the exercises for the level of the research sample.

4- Calculating the performance time for each exercise.

5- Calculating the maximum stresses of the exercises used in the research.

6- Determine the appropriate rest time between repeats and groups.

Preordained test

The preordained test was done on the research sample on (Tuesday/ 1/12/2019), at the martyr Al-Hakeem closed Hall Stadium in Karbala Governorate.

Main experience:

After finishing the preordained tests, the researchers

will include the exercises that he prepared on the (Alfartrmix) and (electrical stimulation) devices within the training program for the research sample at the beginning of the main section of the training unit, then the main part will be completed together with the same trainer exercises, and will be as follows:

1- The date of starting the exercises was Saturday , 5/12/2018.

2- The exercises were applied in the special preparation stage.

3- The trial period reached 8 weeks divided into 24 training units, with an average of three units per week.

4- A special coach has been identified for each group, to be given the special exercises prescribed for them at the beginning of the main part and on the stadiums of the closed hall Al-Hakim Sports Hall and the youth center on Saturdays, Mondays, and Thursdays and at the same time, and after completing the special exercises they are combined again to complete the training unit for them under the guidance of their coach.

5- The researchers determined the intensity of the exercises between (85-100%).

6- The training extent of the exercises reached (25-40) minutes from the extent of the training unit.

7- The researchers used the high-intensity periodical training method.

8- The end date of the experiment was (Thursday1/31/2019).

Post-Test:

The post-test on the research sample was done on (Sunday 3/2/2019), at the Al-Hakim closed hall in Karbala, Considering the same conditions and circumstances in the pre-test.

2-5 Statistical Methods:

The researchers used the SPSS statistical bag, using the following statistical means: Arithmetic mean, the standard deviation, (T) for the corresponding and independent samples, the Pearson correlation coefficient, the torsional coefficient, the median, the Ca2 test.

Discussing the results

The results were presented, analyzed and discussed in this chapter, which was obtained from the research sample, after the data were statistically processed and presented in the form of explanatory tables that facilitate the observation of differences and comparison between the test results of the two research groups, and then discussed to achieve the goals of the research and test its hypotheses. To test the second hypothesis, the researchers used (T) test for the corresponding samples to extract the significance of the differences between the tests results in the preordained and post measurements of the two research groups, as shown in tables (3) and (4).

Table (3) shows the arithmetic mean, standard deviations, calculated (t) value, the level and type of significance for the first experimental group in the preordained and post-tests.

Tests	preordained		Post		Calculated T value	Significant level	Significant type
	A	B	A	B			
Femoral straight muscle activity	1405,27	26,93	2082,56	47,63	51,813	0,00	Significant
Femoral posterior muscle activity	2512,44	19,06	3579,77	28,91	128,930	0,00	Significant
Long fibula muscle activity	1089,13	7,22	2009,23	47,43	79,091	0,00	Significant
Twin muscle activity	923,76	15,36	1547,59	39,81	66,702	0,00	Significant

At the significance level (0.05) and the degree of freedom 6.

Table (3) shows the statistical indicators for the tests' results in the preordained and post-measurement of the research variables that were subjected to the members of the first experimental group.

The results showed that the values of the arithmetic mean for all the research variables were higher in the post-test than the preordained test, and a significant difference occurred between the two tests for the post-test, since the bigger the Arithmetic mean, the better the level, and this is what the levels of significance indicated as they were less than the level of (0.05) significance, which indicates significant differences between the two tests.

Table (4) shows the arithmetic mean, standard deviations, calculated (t) value, level, and type of significance for the second experimental research group in preordained and post-test.

Tests	preordained		Post		Calculated T value	Significant level	Significant type
	A	B	A	B			
Femoral straight muscle activity	1366,91	13,86	1812,41	39,87	74,621	0,00	Significant
Femoral posterior muscle activity	2493,20	10,08	3111,32	24,43	110,135	0,00	significant
Long fibula muscle activity	997,61	6,17	1693,58	40,02	151,638	0,00	significant
Twin muscle activity	894,72	10,24	1332,73	35,99	54,865	0,00	significant

At the significance level (0.05) and the degree of freedom 6.

Table (4) shows the statistical indicators of the tests' results in the preordained and post-measurement of the research variables for the second experimental group sample.

As the results showed that the values of the arithmetic mean for all the research variables were higher in the post-test than the preordained -test, and a significant difference occurred between the two tests for post-test, since the greater the arithmetic mean, the better the level, and this is what the significance levels indicated as the least significance level was (0.05), which indicates that there were significant differences between the two tests, and this is consistent with what was stated in the second hypothesis of the research.

The researchers attribute the reason for the development that occurred on the two experimental groups members to the regularity in the training process and the work according to the lengthening and shortening cycle, so evolution will certainly occur for the members of the two training groups.

As the organized sports training leads to an increase in the efficiency of the functional systems, especially the nervous and muscular systems, and this appears directly in the muscles' ability to produce strength while increasing the speed of the muscular contraction for decision-making, and all this is in the service of skill performance.

In addition to the codified repetition process in the training process, which had a clear role in the development process.

Table (5) shows the arithmetic mean, the standard deviations, calculated (t) value, the level, and type of significance between the results of measuring post-tests for the two research experimental groups.

Test	measuring unit	1st experimental group		2nd experimental group		Calculated T value	Significant level	Significant differences
		-A	B	-A	-B			
Femoral straight muscle activity	MV× S	2082,56	47,63	1812,41	39,87	31,143	0,00	significant
Femoral posterior muscle activity	MV× S	3579,77	28,91	3111,32	24,43	80,682	0,00	significant
Long fibula muscle activity	MV× S	2009,23	47,43	1693,58	40,02	43,838	0,00	significant
Twin muscle activity	MV× S	1547,59	39,81	1332,73	35,99	14,674	0,00	significant

At the significance level (0.05) and the degree of freedom (12)

Table (5) shows the arithmetic mean, the standard deviations and the significance of the differences between the post-tests' results for the two experimental groups, and through reviewing the results of the tests that were reached, it becomes clear to us that there are significant differences between the measurement of the post-tests of the two groups in all the variables, and in the interest of the first experimental group that used ballistic exercises as the values of the significance level were less than the error level (0.05) and this is consistent with what was stated in the third hypothesis of the research.

Discuss the results of measuring the post-tests for the two research groups

The researchers attributed the reason for the development and the advantage of the first group upon the second group to the fact that the ballistic exercises which were chosen had a positive impact on developing strength and the rapid ability of the muscles that work on the knee joint [6,7], which positively affects the work of the knee joint and protecting it from injury. And this muscle strength and flexibility are more conservative on the knee joint from injury and lacerations, as it is important to have the right balance between strength and flexibility between the different muscle groups working on the knee joint, especially the thigh muscles that control the knee and are more able to withstand muscle pressure [8-10].

Conclusions

The exercises used in the ballistic method have a clear positive effect in developing the activity of the most important muscles working on the knee joint of the handball center player. The exercises used in the plyometric method have a positive effect on developing the activity of the most important muscles working on the knee joint of the handball center player. Exercises of both (ballistic, plyometric) methods work to protect the knee joint from injury to the handball center player, but the preference was for ballistic exercises method according to the greater development achieved by the

activity of the most important muscles working on this joint.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kerbala University and all experiments were carried out in accordance with approved guidelines.

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Study the Effect. Extracts and Inhibitory Potency of Plant *Capparis Spinosa* on Breast Cancer Cells

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Abstract

The aim of this study is to analyze Capparis spinosa and the principal constituent substance in Iraq which is recognized by NMR experimentations and the key constituent identified by NMR is Stachydrine. Capparis spinosa is descended from the family (Capparidaceae) and is described as a herbaceous plant with simple leaves together edge and forked ears and bear flowers on long necks and fruits pear shape. Capparis contains bitter materials, claycosides, enzymes, mayonnaise, acetic acid, bric, soap and volatile oils with a smell similar to the smell of garlic, as well as sulfuric klycosides. All parts of plants used as a treatment as roots that are used as a diuretic and leg used as a dentin for diarrhea as well as fruit is used as a treatment for cancer We observe measurements of significant decrease in the level of breast cancer cells at≥ 0.05

***Keywords*:** *Fruits of Capparis spinosa:; Ethanol;Aceton and Hexane.*

Introduction

Capparis spinosa belongs to the family family (Capparidaceae) and is described as a herbaceous plant with simple leaves together edge and forked ears and bear flowerson long necks and fruits pear shape [6]. Shafahalah contains bitter materials, claycosides, enzymes, mayonnaise, acetic acid, bric, soap and volatile oils with a smell similar to the smell of garlic, as well as sulfuric klycosides [8]. All parts of plants used as a treatment as roots that are used as a diuretic and leg used as a dentin for diarrhea as well as fruit is used as a treatment for cancer [6]. Common names in Arabic: Kabar, Asef; Berbe: Tayllut, Tailoulout, Amserlih, Ouailoulou; English: Caper bush, Caperbush, Caper, Caperberry; French: Câprier, Capriercommun, Câpres, Fabagelle, Tapana, Finnish: Kapris; German: Kapper, Kapernstrauch ; Gujarati: Kabare; Hindi: Kiari, Kobra; Hungaria: Kapricserje; Icelandic: Kapers; Italian: Cappero, Capperone (fruit); Kannada: Mullukattari; Maltese: Kappara; Marathi :Kabar; Norwegian: Kapers; Portuguese: Alcaparra; Punjabi: Kabarra; Russian: Kapersy; Sanskrit: Ahimsra, Kanthari, Kantaka, Tiksnagandha; Spanish: Alcaparra, Caparra, Tapana; Alcaparron, Caperberries; Swedish: Kapris; Telugu: Kokilakshmu; Urdu: Kabar [1-3]. Family : Capparidaceae. Prepar Distribution It is supposed to be originated in the dehydrated places of Western and Central Asia. Currently, Capers is existed maturing all over Mediterranean (especially in France, Spain, Italy and Algeria); furthermore, the plant is found in Iran, Iraq, Cyprus and Greece [3-4]. Globally, human population is highly and severely affected by a disease known as cancer. A steadfast requirement for fresh remedies to cure, treat and curb off this life- menacing disease. Many key strategies such as controlling survival and the death of a cell which are cancerous are essential for managing and treating cancer. With lesser possible side effect on normal cells, anticancer agents should kill the cancerous cell. Recently, medicinal plants having natural products to deter proliferation, prompt apoptosis, curb off angiogenesis, constrain invesiveness, in addition to retarding metastasis and improving chemotherapy [1-7]. The present review discusses the compounds derived from natural medicinal plants and their assets which lead to maintaining them promising possible treatments for anticancer.

Anti--cancer Activity of Medicinal Plants

Various advantages for Medicinal plants are

overcoming chemical products, since compounds derived from plants are more allowed and treated as untoxic to the normal cells of human. The current common therapeutic remedies for treating cancer have already been used by radiotherapy and chemotherapy having different side effects such as toxicity in neurology, heart effects, kidney and lung which seriously damages the person‘s health. Consequently, the less toxic compelling anti-cancer medication as compare to the remedies in the marketplace are considered as a required alternative method to develop the drug.

Capparis spinosa

The Capparaceaen family include Capparis spinosa which is treated as key culinary components in theMediterranean and Middle Eastern cuisines. This component is Sanskritlly recognized as HimsraCabra. Generally, Caper forms different constituents such as flavonol glycoside, rutinand 5-caffeoyl-quinic acid which can be volatile and nonvolatile which could also be effective anticancer agents. Susequently, a protein similar to imidazoleglycerol phosphate synthase is definitly refined from garden-fresh Caper seeds, deterring the process of proliferating hepatoma HepG2 cells, in addition to the colon cancer HT29 cells as well as MCF-7 cells in breast cancer [13]. Principal oils and aqueous infusions taken from Caper have revealed substantial inhibitory impact on HT-29 cell proliferation and on nuclear factor kB (NF-kB) action in a drug prescription dependent mode. The cells in G2/ M phase of cell cycle were ceased by Caper essential oil and aqueous infusion. A study showed that C. S obtain mediated apoptosis through permeabliztion of mitochondria in addition to acting of Caspase 9 in SGC-7901 cells [14].

The other key cause of death universally is cancer. Procedures of treatment are more complicated for each detailed type of cancer aiming for anti-cancer drugs with higher effectiveness.

1. The pharmaceutical societies many advancements achieved in cancer treatment strategies, less toxicities and lower expenses. In spite of reporting drug-resistance in treating several cancer tryouts, it is illustrated by the second studies on the experiment referring that some wellknown plants possess anti-cancer outcomes on different tissue cells, for the various compounds included in plant drugs, on the contrary to the virtuously synthesized story. IIt sounds that showing novel kinds of drugs introduced and extracted from plants could pave the way to more competent approaches to treating cancer. Over years, considerable attention has recently been paid, mostly due to vast developments3 to drugs derived from plants in organic chemistry, and due to groundbreaking changes in the methods used in extracting and purifying more accurate understanding of the plants' natural ingredients. Making use of plant sources as raw material may be assistant to the pharmaceutical industries to develop enhanced drugs with cheaper expenses. Capparidaceae is a great family of phanerogam gymnosperm dialypetalae plant species. The Capparis spinosa family, not only shows noticeable resistance to family, not only shows noticeable resistance. The Capparispinosa (C. spinosa) (CS) as an individual memeber of the family of Capparidacae, not just maintaining apparent endurance to shortage in water supplies and increasing temperatures, but adapting decreased temperatures with -8 C 5. Flavonoids are recognized as being the biggest group of natural constituents that are considered as strong anti--oxidants and having noticeable impacts in cellular biology, e.g. accumulating free radicals and possibly halting their detrimental role in carcino-genesis 6. Like all other betaines, Stachydrine is a compound of quaternary ammonium. In the vegetal realm, betaines are omnipresent which tending to gather and assemble in the intercellular fluids and cytoplasm in which they perform an instrumental role in keeping up proteins, nucleic acids, and cell membranes against abiotic stress (Kavi Kishor et al., 2005; Street, Bolen, Rose, 2006). In a particular study, cells with high-glucose (30 mM) culturlly modelled was employed to simulating clinical hyperglycemia for the invitro estimation of the influence of stachydrine on high-glucose induced cytotoxicity. It is clearly recognized that stachydrine, which is an full of ingredient of citrus juices, is capable of preventing the high-glucose cytotoxicity in endothelial cell by working on the senescence and S I R T 1 pathways (Servillo et al. , 2013).

Anticancer Activity

Cancer, was described as, cell proliferation affecting other tissues or an uncontrolled growth. The mechanism beyond invading the tissue is by direct cell migration or lymphatic system and blood. The hazard agents for

cancer includes an unhealthy diet, environmental factors, infection, radiations, chemicals and smoking [17]. Hundred various kinds of cancer named by the type of cell or tissue or organ in which they start. Their intensity may be benign «earlier stage» or malignant «end stage, called cancer» . Natural products from biological sources and plants still stay an uncondensed source and unlimited of new nutraceuticals and phytochemicals. Itcontinues to produce the most dramatic effect in the field of cancer [18]. Traditional herbal founded drugs could be hopeful candidates for novel cancer treatments with minimal side effects. They play a significant and vital role in the reduction and prevention of cancer (Saad et al., 2008). Capparisspinosa is one of the mostprevalent medicinal plants, contained a wide range of phytochemical constituents, used extensively in several parts of the world as a treatment for many human diseases. Many reports confirmed it isable to inhibit many types of cancer cells [5].

A new protein is separated from caper, which inhibited proliferation of MCF-7 (breast cancer), HepG2 (hepatoma), and HT29 (colon cancer) cells [6]. Capparis spinosa essential oil and aqueous infusion appeared marked a prohibitory effect on nuclear factor kappa B (NF-KB) activity and on HT-29 cell proliferation [23]. Furthermore, C. Spinoza extracts mediated apoptosis through the mitochondrial pathway in SGC-7901 cells (Ji and Yu 2014).The aqueous leaf extracts of caper induced significant inhibitory effect on Hep-2 «human epidermoid larynx carcinoma» and Hela «human cervix uteri epitheloid carcinoma» with low concentration (Al-Daraji 2010).Also, different extracts (aqueous and methanolic) of C. spinosa fruit showed Cytotoxic and cytogenetics effects on in vitro Hep-2 and HeLa tumor cell lines (AL-Asady et al., 2012). Finally, the extract of C. spinosaroot bark revealed anticancer volume of tumor and packed cellsbesides cell count viability [26]

Materials and Methods

Plant material

Capparis spinosa was collected from Kirkuk city during the month of July 2018. The plant identified by a botanist at Al Kirkuk University. The of plant dried at room temperature in the dark for 14 days and then finely ground by using an electric grinder.

Extraction process

Capparis spinosa Seeds (10 g) and 100 ml of ethanol are introduced into a flask. The mixture was exposed to ultrasound for 1 hour under 45 k at temperature and sheltered from light. The later process has been repeated under same condition for ten times. Afterward, the mixture was filtrated and the final volume (accumulate filtrate) is concentrated in rotary evaporator under reduced pressure (12).

Purification of the compounds using Silica Gel Chromatography

The Acetone extracted (2.00g) was absolutly fractionated on a silieca - gel column (60-120 mesh) with ratio of eluent (methanol ;Acetone,hexane) various such as (1;2:3) To give two fractions (Fr. 1- Fr. 2). Fraction (2) was dependent on further chromatography on a silieca - gel column (1 m) with same eluate using a stepwise gradient of hexane and ethanol (2:1) to withstand further pure compound (Fr.2.1) and mixture of others [13,14]. The obtained fraction I and II gives (170 mg and 143 mg respectively).

Study of the effect of anabolic extract in acetone solvent on the effectiveness of cancer cells.

Result and Discussion

Validation and Detection of Cell Viability Using MTT Assay.

The validation of MTT assay was performed to establish the relationship between cell number and absorbance. This is basically required to determine the accuracy of pipetting technique before moving to chemo-sensitivity assays. The MTT analysis was carried out in conformity with Mossman (1983) with some adjustments. The cells were planted in 45- well plates at 0.8 x 105 cells/well concentration, incubating the cell in a 37 °C CO2 incubator during the night. The next day, the chalcones compounds which were synthesized were added to the wells with 7 various concentrations. Measuring the viability of the cell at 72 hours after treatment. A volume of 20 mL MTT solution (5 mg/mL) was added in each well and incubated cell for three hours. Then discarding the solution where 100 mL of DMSO was supplementary to each well for solubilizing the crystals. Lastly, the plates were rated at 570 nm as

reference wavelength by using µ-Quant ELISA Reader (Bio-tech Instruments, USA). (Nadiah et al., 2014) The results of the study were statistically analysed using complete randomized design Using SPSS.

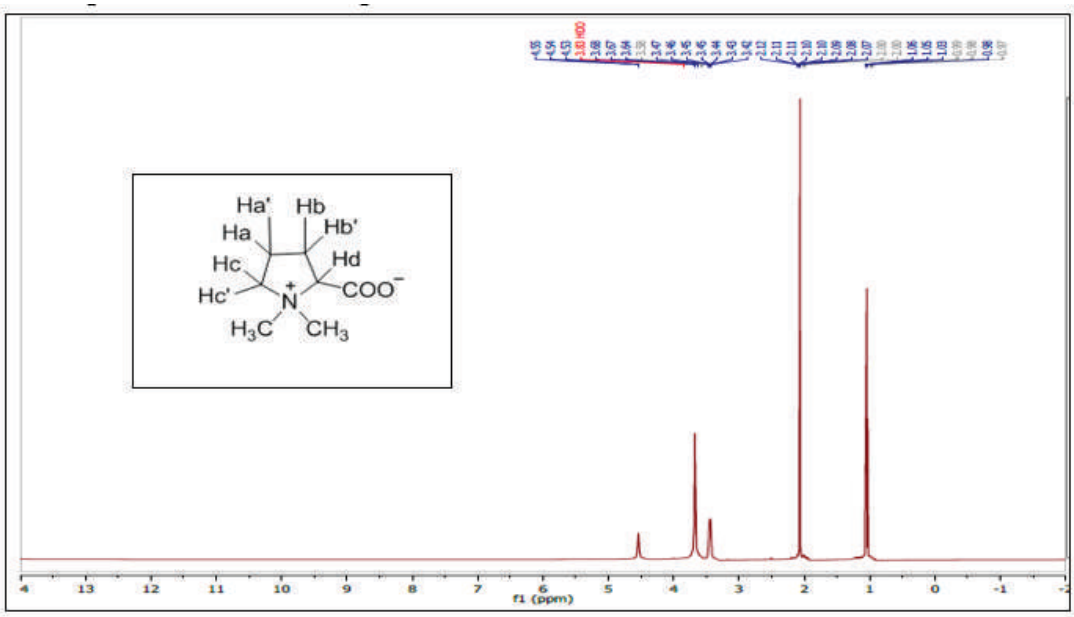


Figure 1: design Using SPSS

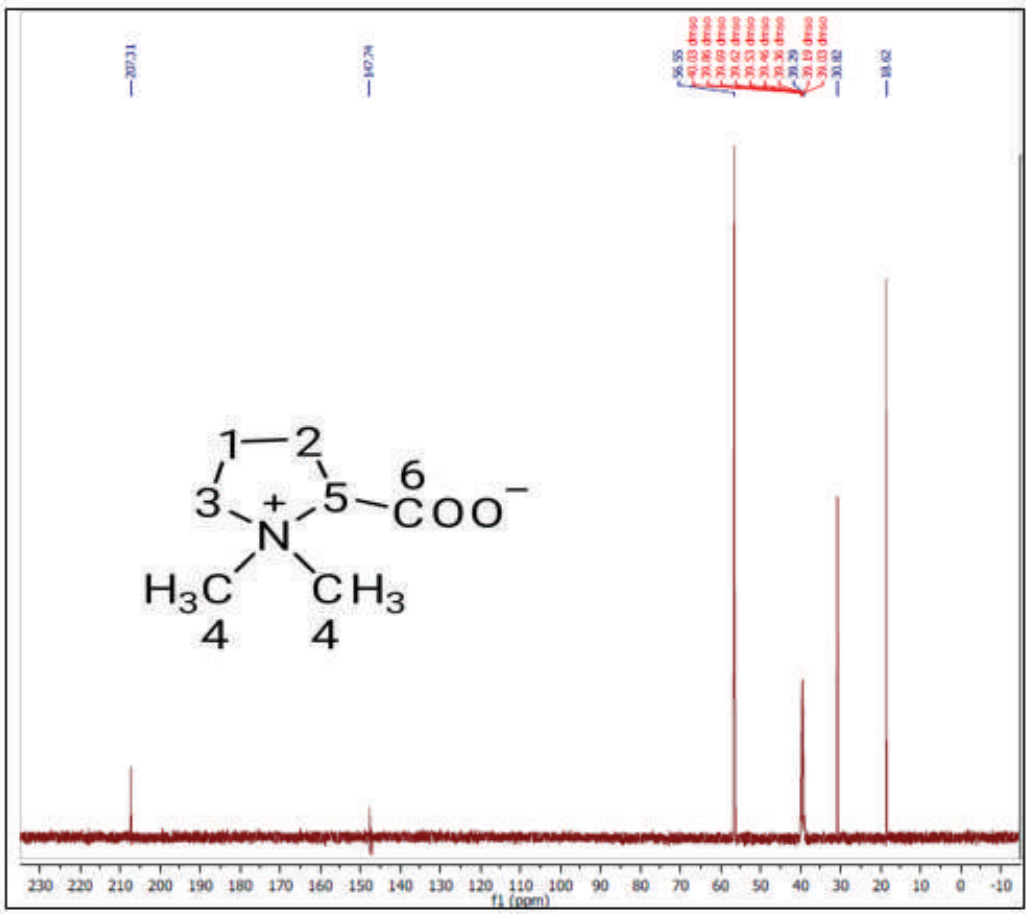


Figure 2: ^{13}C NMR

Figure 2: (a) The 1H NMR spectrum of (Stachydrine compound) is noticed in fig. 1. The spectrum shows one characteristic singlet peak at 2.26 ppm due to the protons in (CH3)2-N. A pentate centered at 1.30 ppm is assigned to the protons (a, a'). A multiplete at 1.70 to 1.95 ppm is attributed to the protons in (b, b'), peaks at 2.20 to 2.30 ppm is assigned to the protons in (c, c'). Finally, triplet peak appeared at 3.12 ppm is attributed to the proton in (d). Furthermore, the conformation is carried out in 13C NMR compound (I) shows six different peaks are ranged from 24.7 to 174.7 ppm (C1 at 24.7 ppm, C2 at 28.8 ppm, C3 at 60.1 ppm, C4 at 41.4 ppm, C5 at 76.3 ppm and C6 174.7 ppm). Both NMR is conformed the chemical structure of compound (I).

Detection of Cell Viability Using MTT Assay.

Measure the effect of the compound extracted on the effectiveness of cancer cells by enzyme If the table (3-1), The adjusted table shows the standard deviation and standard error of the control A rate and the level of morale and the amount of confidence (higher and lower value and T.test

Table 1: the standard deviation and standard error of the control A rate

Group	N	mean±SD	t.test	p-value
Control	25	1.23±0.05	0.02	0.054
Sample	30	0.554±0.015		

Conclusions

The result shows a significant decrease in the efficacy of the enzyme coenzyme colin esters and at the level of probability (0.554 ± 0.015) compared to the control group where this decrease is a clear evidence of a compound effect obtained by killing cancer cells. We observe from the statistical analysis a significant decrease at the level of P≤0.05.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Education and all experiments were carried out in accordance with approved guidelines.

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Knowledge and Attitude about Sexual Transmitted Disease among Women in Reproductive Age in Al-Nasiriyah Governorate-Iraq

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Abstract

Background: Sexually transmitted diseases are group of disease primarily affecting sexually active population and has led to a major public health problem in the reproductive age group (15-45 years), which are largely acquired through sexual act or close contact with genitals leading to potentially devastating effects.

Objectives: To assess knowledge and attitude about sexual transmitted disease among women in reproductive age in Al-Nasiriyah governorate-Iraq.

Methods: Cross sectional study design based on community was conducted in in Al-Nasiriyah governorate in Iraq on women at reproductive age. Data collected by predesigned and pretested instrument through face to face interview.

Results: In general literates women were more aware about STDs compared to illiterates. A total of 180 women at reproductive age were recorded, of which 325(64%) regard blood transfusion as major method of STD transmission and 206(40%) Through the open wound of the genitals. 384 (68.3%) women told that vaginal discharge is common symptom, followed by Genital and mouth ulcers 194(34.5%). 310(61%) women regard diagnosis of STD done by blood test.396(78%) opined abortion common complication followed by 287(55%) infertility. 404(71%) opined that STDs can be prevented by abstinence from sex and 343(61%) it cannot be prevented.

Conclusions: Based on the results of the study, women's knowledge about sexual transmitted diseases that transmitted during sex is very low, so special programs must be provided for women that can increase knowledge of women and awareness of sexually transmitted diseases in Iraq and published on television and radio. As knowledge and awareness of diseases promote with education, special educational programs are also effective for education of women in Iraq about STD.

Key words: *Knowledge, Attitude, STD, Reproductive age women, Al-Nasiriyah*

Introduction

Sexual transmitted diseases (STDS) are diseases caused by either virus, parasite, or bacteria, which transmitted mostly through sexual contact involving oral, vaginal and anal sex. STD also can transmitted by non-sexual methods as by blood, blood product or from mother to child through pregnancy and delivery [1]. Women at reproductive age especially are mentally and physical and society more vulnerable to infections in reproductive tract (RTIs) and STDs. There are at least 25 respiratory tract infections (RTIs and/or) STDs with a range of different clinical features and these diseases disease during anal, vaginal, and oral sex [2]. In some developing countries bear a significant burden due to the spread of incurable sexually transmitted diseases (STDs) viruses, increased travel and behavioral change. There is a changing trend of sexual and reproductive behavior between young people and this need more attention and awareness by healthcare professionals

to provide the necessary sexual or reproductive care and education [3]. Unfortunately, many studies during this stage have proven that young people do not have enough information about STDs and their prevention [4-6]. According to Women Aid Organization, women shelter home is a place which provide temporary shelter, emotional and physical support for the affected women. It could be a victim of domestic violence, rape, or a woman with a problem with sexual discipline. These shelters provide the necessary social ,emotional, mental support services that enable and empower them to live a better life in the future [7]. World health organization estimates that there are more than 333 million new cases associated with four sexually transmitted diseases (chlamydia, syphilis, gonorrhea and trichomoniasis) each year [8]. Nowadays, there are at least 30 cases that are sexually transmitted [9]. Among teenagers, girls are more likely to contract sexually transmitted diseases than their male counterparts, involving HIV / AIDS, particularly during sexual contact with another's. This increased vulnerability has been attributed to concern beyond their power, as violence of sex and abuse of women, initiation of sex early and the inability of women about negotiate safe sex. another predisposing elements involve severe bias, education loss, powerless, loss of reach to prevent the pregnancy and concern of reproductive health for women. so, It is hard for teenage girls to protect themselves from unwanted gestation and sexual transmitted diseases. in addition, young persons are not educated about STD, and their knowledge of different diseases is **extremely** weak. while there are much researches that have investigated the understanding about sexual transmitted diseases between different age category, social class, very little research has examined knowledge of women's in young age about sexual transmitted diseases. women information regarding the method of transmission or prevention of sexually transmitted diseases is very limited [10] Hence, the present study was conducted to assess the knowledge about sexually transmitted disease (STD) among the women in a Al-Nasiriyah governorate.

Methodology

Community based cross-sectional study conducted for a purposive (non probability) sample of 500 women in reproductive age in Al-Nasiriyah governorate-Iraq at age group 15-45 years. The instrument designed and constructed by the investigator. It based on literatures clinical background, and previous studies . The study instrument consist of two domain first one included demographic characters of women as age, level of education, occupation, resident and second includes information schedule about STD such as routes of transmission, clinical features, tests, complications and attitude of women. The women were interviewed for their perception toward health seeking behavior. Method of data collection was through visit some private clinic and houses and supermarket and some centers of care by face to face interview . It started from the 11th of January to the 16th of august 2020 Each interview took approximately (15) minutes to complete questionnaire form. The interviewing is carried out with each study sample who participated in the study. Before collect the data researcher explained the purpose of the study for every woman and obtained verbal consent from each study sample.

Statistical Analysis

Data were analyzed using SPSS Version 25 statistical software (SPSS). Descriptive statistics (frequency and percentage) were used to determine the frequency of the women socio-demographic characteristics, knowledge and attitude about STD.

Results

about 500 women at reproductive age were involved at this study which divided into several criteria (age, resident, occupation, level of education, knowledge and attitude about STD). As showed in demographic data (Table 1) highest percent from sample at age group 15-24 old year (40%) and about 163(29%) From them lived in urban and 337(60%) in rural. Regarding to level of education highest percent 168(33%) were illiterate. While 353(62%) employed and 147(26%) unemployed. Among the different methods of transmission, 325 (64%) women opined that blood transfusion was the most common mode of contracting STDs followed by 206 (40%) through the open wound of the genitals only 125(29%) from women thought that infections could also spread through sexual intercourse. When asked about the symptoms, 384(68.3%) were aware of vaginal discharge as the most common symptom, followed by 194(34.5%) Genital and mouth ulcers and 168(29.9%) burning with urination. When enquired about the

laboratory test, 310(61%)were aware diagnosis can be done by examination of blood as compared to 176 (34 %) women, who thought that urine could also be a precursors to diagnose the disease. 176(78%) from participants thought cause abortion followed by, 287(55%) cause infertility as show in table (2). 404(72%) from women opined that abstinence from sex thought to be the most common method of preventing the disease and 343(61%) thought It cannot be prevented as show in table (3)

Table 1: Socio-demographic characteristics of study sample.

Age group in year	Frequency	Percentage
15-24	201	40%
25-34	175	35%
35-45	124	24%
Resident		
Urban	163	29%
Rural	337	60%
Educational level		
Illiterate	168	33%
Primary school	124	24%
Secondary school	59	11%
Higher school	91	18%
Bachelor and above	58	11 %
Occupation		
Employed	147	26%
Unemployed	353	62%

Table 2. Knowledge of women about methods of transmission, Signs and symptoms, laboratory test and consequences of STDs.

Methods of transmission	Frequency	Percent
Unprotected sex	125	29%
blood transfusion	325	64%
Through the open wound of the genitals	206	40%
Needle prick	173	34%
Staying, eating together	161	31%
Mother to child	202	40%
Signs and symptoms		
vaginal discharge	384	68.3%
Fever	143	25.4%
irregular menstrual cycle	115	20.5%
Genital and mouth ulcers	194	34.5%
Tuberculosis	152	27%
burning with urination	168	29.9%
Laboratory tests		
Blood	310	61%
Urine	176	34%
Another test	240	47%
Complication		
Infertility	287	55%
vaginal cancer	211	41%
Abortion	396	78%
Death	257	45%

Table 3: Knowledge of women about prevention of STDs

Prevention	Frequency	Percent
Abstinence	404	71%
Use condom	305	54%
Sexual education for women	289	51%
It cannot be prevented	343	61%

Discussion

Current study to be evaluated Knowing and attitude of woman towards the sexually transmitted diseases, with presence an effort to generate disease spread, features, methods of prevention, diagnosis and availability of healthcare facilities in many hospitals. In Iraq, the issue of sexually transmitted diseases needs local attention And it has the ability to reach a large audience health problem because it is a conservative society, and have customs and traditions that are wrongly understood regarding the ways of transmitting the disease. As showed result in table (1) highest percent 201(40%) women at age group 15-24 this finding agreement with study reported that the 15-29 years age group have relatively good knowledge and awareness about STDs this finding is to a study conducted in Bangladesh [11]. 337(60%) from sample lived in rural. Majority of the women 168(33%) illiterates this disagreement with study reported Majority of the women 68 (37.78%) had completed primary education [12]. As showed about 353(62%) from sample unemployed this finding agree with study reported majority of sample were housewife (81%)[13]. As showed in table(2) 325(64%) thought transmitted by blood transfusion, 202(40) from mother to child, 173(34%) by needle prick and 161(31%) transmitted by staying, eating together this finding, 125(29%) disagreement with study reported a study done in Khartoum 72.1% of the respondents knew that HIV could be transmitted by heterosexual contact,38.1% by blood transfusion, 3.5% during pregnancy, 47% by using skin piercing instruments and (15.6%) thought by sharing meal [14]. 384(68.3)from women thought most common clinical features vaginal discharge followed by194(34.5%) by Genital and mouth ulcers and this result disagreement with study reported (32.78%) of the participants told the clinical features of STIs as fever, 41.675% told vaginal discharge and 13.89% told burning micturition [11]. As showed in table(3) 404(71%) STD can prevented by abstinence from sex followed by 343(61%) It cannot be prevented, 305(54%) by use condom this result disagreement with study reported that 87.5% of the positive respondents has no knowledge of protection by using male condom, 62.5% of the positive ones had acknowledge that they can took protection by avoiding unprotected sex in Khartoum [14].

Conclusion

There is necessary a needful to promote the tuition in Iraq about sexual transmitted diseases especially between those lived in rural region and older women (34-45 years). The formal and informal programs guaranteed educational knowledge and awareness about the sexually transmitted infection may applicated to help persons how about diseases . the issue of STDs need to more improved to help the woman who kept on them health and Can be confirmed in together work with authority organizations to make sure the successfulness of such educational crusade.

Recommendation:

Provide efficient educational programs to up-to-date information about sexual and reproductive health are

needed in the Iraq. Strategies to increase knowledge and attitudes are important tools for improving protective behaviors, especially with respect to contraception, health care surveillance and exposure to sexual risk.

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Conflict of Interest: None to declare.

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Assessment of Patients' Perception of Mechanical Ventilation (Endotracheal Tube) and Intensive Care Unit Post Cardiac Surgery

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Abstract

Background: Treatment and mechanical ventilation in the intensive care unit may get a variety of physical and psychological discomfort for patients.

Objectives: To assess the perception of patients about the intensive care unit (ICU) and stressful experiences about mechanical ventilation in ICU. And identify an association between patient perceptions with demographic characteristics.

Methodology: a descriptive study (cross-sectional study) for the purposive sample of 80 patients of post-cardiac surgery (hospitalized) in Ibn Albetar and Iraqi Center of Heart Disease (Gaze AL-Hariri hospital). Data were analyzed to achieve the goal of the study have been a questionnaire prepared according to the literature review, it is composed of 3 parts, **first part** demographic data and, the information related ICU, **the second parts**, stressful experiences of adult patients who received mechanical ventilation (Endotracheal tube) (ETT) at ICU, and **third parts**, patient perception related to the environment at ICU. The validity of the questionnaire through a number of experts in the college of the nursing university in Baghdad.

Results: The study showed (47.5%) of the sample was within (50-59) years, 25% of them from primary school or less, (85%) of them the residence area was urban, the study showed that the high percentage (62.5%) of the cardiac surgery patient was the moderate level of stressful experience during mechanical ventilation (Endotracheal tube) (ETT) at intensive car unite (ICU) while only (10%) of patients with a low-level of stressful experiences. The results also showed the majority (90 %) of patients with a moderate level of the perception of patients of the intensive care unit.

Conclusions: The majority of cardiac surgery patients where moderate levels of perception of mechanical ventilation (Endotracheal tube) (ETT) and of the ICU.

Keywords: *intensive care unit; mechanical ventilation; endotracheal tube; patient stress experiences.*

Introduction

The Intensive Care Unit ICUs are the environments a generate stress, where patients experience disruption of sleep-wake patterns, lack of contact with family and friends, in addition to the several clinical procedures that cause patients to experience different types of physical and psychological discomfort. The most stressed events for the family members were fearful of the clinical state of the patient, length of hospital stay, and witness the patients in a coma. [5,11]. Many studies and literatures assess the stressful factors in the ICU with the application of environmental stressors .questionnaire (ESQ), with 50 items, reporting satisfactory reliability. Convergent validity was confirmed by a strong magnitude correlation between the total score of the version of the ESQ and the generic stress measurement score. Therefore, the Brazilian version of the ESQ is a reliable and valid instrument to assess stressors in

the ICU. [5,11,15]. Vesz et al, (2013) conducted a study that evaluated functional and psychological aspects immediately after ICU discharge showed high incidence rates of depression and anxiety symptoms and sleep disorders in patients hospitalized in ICUs for 72 hours or more. Siffleet et al, (2015) [14] conform in their study to understand what environmental elements optimized the maintenance of emotional wellbeing methods: In order to improve the patients' comfort and well-being during and after a stay in the intensive care unit (ICU). The findings indicate an increase in the understanding of the environmental elements that can optimize the emotional wellbeing of intensive care nurses. These findings will assist in the development of strategies to retain nurses in the ICU area. A study that evaluated and compare stressors identified by patients of a coronary intensive care unit with those perceived by patients of a general postoperative intensive care unit. The result showed the perception of major stressors and the total stress score was similar between patients in the coronary intensive care and general postoperative intensive care units. (Dias, et al. 2015) [6]. Since pain is multidimensional and subjective, however, many adult patients in the ICU cannot self-report pain as a result of an altered level of consciousness, the administration of sedative agents, and/or mechanical ventilation. The lack of self-reporting makes assessing pain in critically ill patients a challenge for nurses, who should consider the use of alternatives such as observational pain assessment tools. There are few studies that assess patient perception in ICU This study were designed to answer the question, how do post-cardiac surgery patients describe their perception in the ICU and what. Specific experiences cause patients stress

Methodology

A descriptive-analytic study design study was carried out at Al-Iraqi Center for Cardiac Disease (Gazee Al-Harare Hospital) and Ibn Albetar the cardiac hospital, the present study started from 21 January 2017 to 1st June 2018. A non- probability (purposive) sample of 80 adults patients who discharge from the surgical cardiac ICU to the surgical ward these patients who met the following criteria such as adult (male and female), received mechanical ventilation and oriented to person and place while they are in the ICU and they can communicate with the researcher

The study instrument: For the purpose of data collection, a questionnaire was used, which consists of four parts, first Part concerning the demographic data form that included (age, gender, marital status, level of education, and occupational status) and the information related ICU which includes (the type of surgery, period of mechanical ventilation, and period of ICU stay). Second Parts: concerning the stressful experiences of adult patients who received mechanical ventilation (Endotracheal tube) (ETT) in the ICU. A questionnaire of the patients' perception of experiences in ICU was measured by a questionnaire adopted from (Pennock et al, 1994; Rotondi et al, 2000). The questionnaire consisted of (8 items that demonstrate the stressful experiences of ICU patients who received mechanical ventilation. The first item scored by, four-level Likert scale as no pain (1), pain at its least (2), pain on average (3), pain at its worst (4). The remaining (7) items scored by, four-level Likert scale as never (1), rarely (2) sometime (3) always (4). Third part: concerning the perception of patients about ICU. The researcher adopted and developed an environmental stressor questionnaire (ESQ). It was used by (Cochran & Ganong,1998 and Cornock, 1998) and it revises by the Brazilian version by (Rosa, et al 2010). The questionnaire translated into the Arabic language by the researcher. In this study the scale used is 4 = "always" to 1 = "never", thus making a range of (50-200) total score. The highest score of perception of the stressful experiences of adult patients who received mechanical ventilation (Endotracheal tube) (ETT) in the ICU and the perception of patient of ICU obtained, the high level of perception

Data were collected by interviewers from patients after their discharge from an ICU when they were in the surgical ward by using the questionnaire format, and revision the client chart was also used to obtain information concerning the sociodemographic characteristics (e.g., age, gender, and education), the type of operation, period of the endotracheal tube and the period of staying in ICU.

The content validity of the instrument was established through a panel of (10) experts their experience in their specialist with a mean (20.25) year. The determination of reliability of the items of the scales was based upon the internal consistency of the questionnaire was assessed by calculating Cronbach s' Coefficient alpha. The data

were analyzed through the use of SPSS (Statistical process for Social Sciences) version 20 And Excel packages. Descriptive data analysis

Results

Table (1): Demographical Characteristic of the Sample. n=80

Variables	Groups	Frequency	Percentage
Age	30-39	4	5.0
	40 -49	24	30.0
	50 -59	38	47.5
	60 -69	14	17.5
Gender	Male	54	67.5
	Female	26	32.5
Marital status	Married	58	72.5
	Single	6	7.5
	Widowed	10	12.5
	Divorced	6	7.5
Educational status	Read and write	24	30.0
	Intermediate school	16	20.0
	Secondary school	20	25.0
	Institute graduate	10	12.5
	College graduate	10	12.5
Occupational status	Housewife	22	27.5
	Non employment	2	2.5
	Government employment	34	42.5
	Self-employee	22	27.5

Table (1) indicated that a higher percentage (47.5%) of the sample at (50-59) years old, 67.5% were males, (72.5%) married, and (50%) of the patient their education were secondary school and less. Regard occupational status the table shows that the (42.5%) of patients were governmentally employee

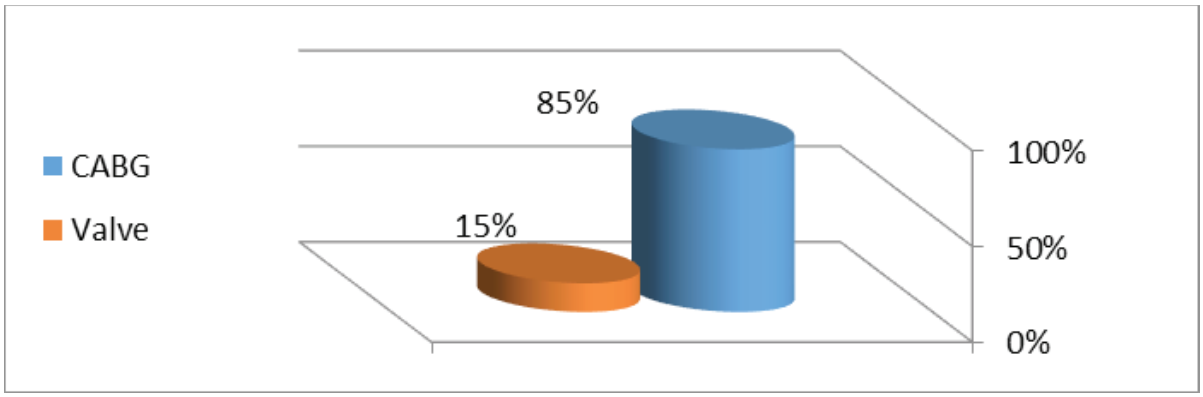


Figure (1) type of surgery

This figure (1) revealed that the (85%) of patient have Coronary Artery Bypass Surgery (CABG) and (15%) of them have valve replacement surgery

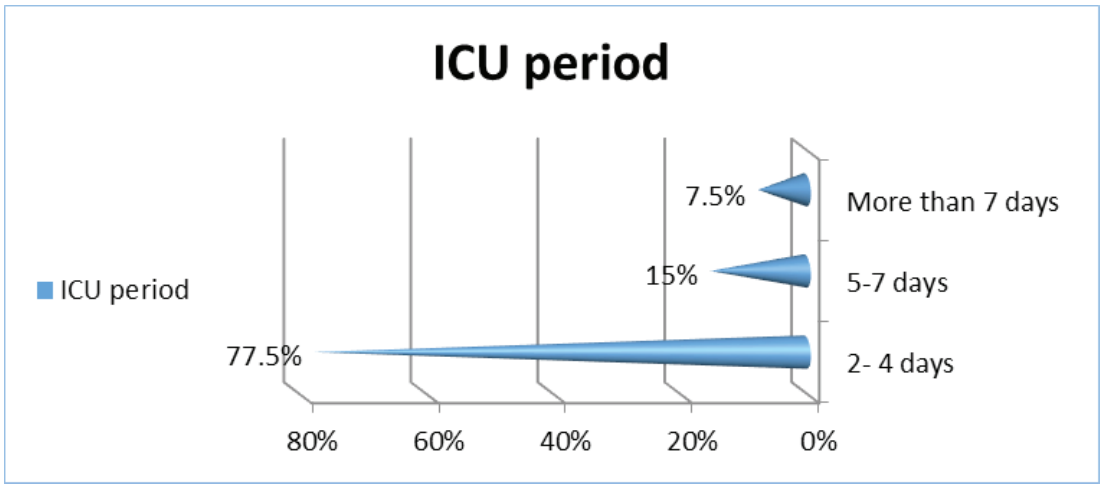


Figure (2) Intensive care unit period

This figure (2) revealed that the (77.4%) of patients the intensive care unit (ICU) period was (2-4 days) flowed (15%) the period (5-7 days) and (7.5%) of patients the period more than 7 days

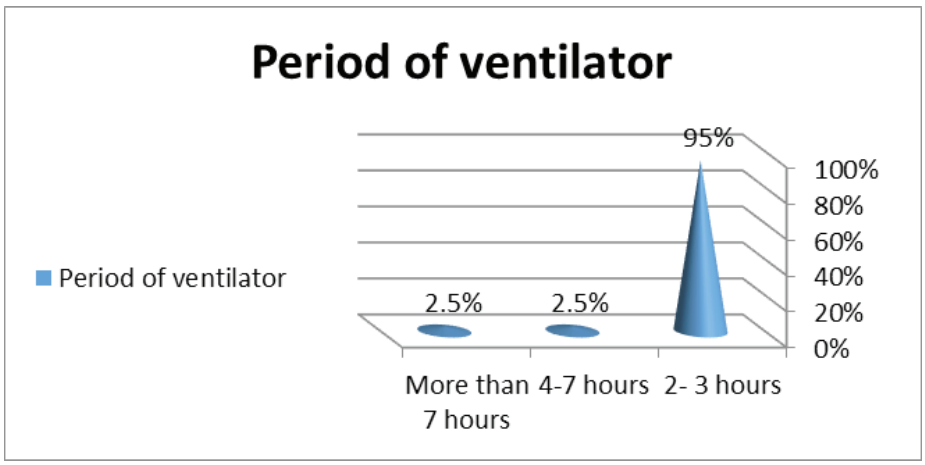


Figure (2) Intensive care unit period

This figure (3) revealed that the (95.0 %) of patients the mechanical ventilation (Endotracheal tube) (ETT) at ICU period was (2-3 hours) and (2.5%) of patients the period mechanical ventilation was equally for both (4-7 hours) and more than 7 hours.

Table (2): Mean, Standard Deviation related to the level of stressful experiences of adult patients during (Endotracheal tube) (ETT) at the intensive care unit (ICU).

stressful experiences of adult patients with (Endotracheal tube) (ETT) at ICU	Mean of score	Std. D	level
1- To what extent felt pain when you are on artificial ventilation	3.12	.82	H
2- Remember not being able to speak	2.57	.87	M
3- Remember pain or discomfort associated with ETT.	2.97	.99	M
4- Remember feeling choked by ETT,	2.27	1.01	M
5- Remembered feeling anxiety about the ETT	2.85	1.12	M
6- Remember ETT interference with sleep,	2.82	.98	M
7 Remember not getting enough air from the ETT	3.57	.87	H
8- Remember difficulty breathing After ETT Removed	1.80	1.04	L

ICU= Intensive Care Unit, (1 to 1.9) = Low stressful experiences of the patient's about Endotracheal tube, (2 to 3) = M= moderate stressful experiences of the patient's about Endotracheal tube, (3.1 to 4) = high stressful experiences of the patient's about Endotracheal tube, less than 62.5= Very low stressful experiences of the patient's about Endotracheal tube

The stress perception of patients about mechanical ventilation is given in table (2). The table revealed that the perception of the stressor perception about ETT for 80 patients after discharge from ICU, ranging between low and high-grade levels. Most of the items were moderate except the patient "felt pain when he was on artificial ventilation" and "not getting enough air from the ETT "were high, while the low level of experience in a "remember difficulty breathing After ETT Removed"

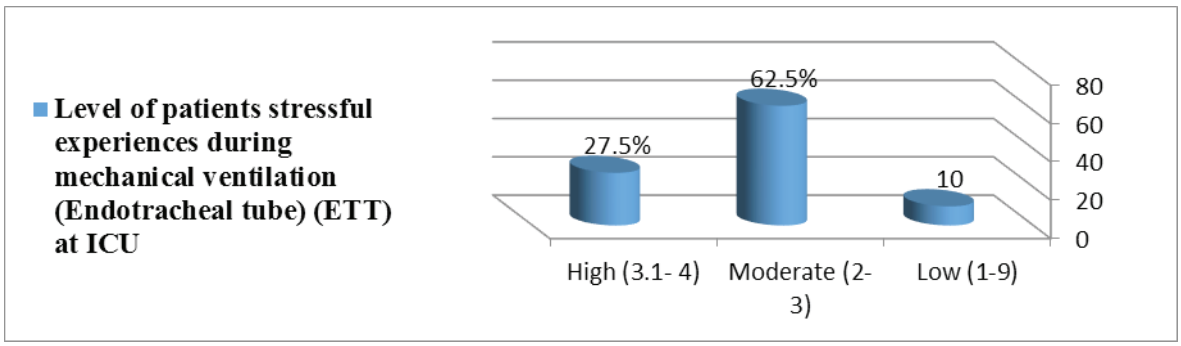


Figure (4) Level of stressful experiences of adult patients during mechanical ventilation (Endotracheal tube) (ETT) at ICU

The figure (4) revealed that the high percentage (62.5%) of patient with moderate level of stressful experience followed (27.5%) of patients with high level and (10%) of them low level of stressful experiences of adult patients during mechanical ventilation (Endotracheal tube) (ETT) at ICU

The stress perception about ICU for 80 patients after discharge from the ICU, ranging between low and high-grade levels. Twenty-one items out of fifty items the response of stress perception at intensive care unit were moderate while nine items out of fifty items was response high-stress perception about ICU. And only seven items the patients was response low-stress perception about ICU

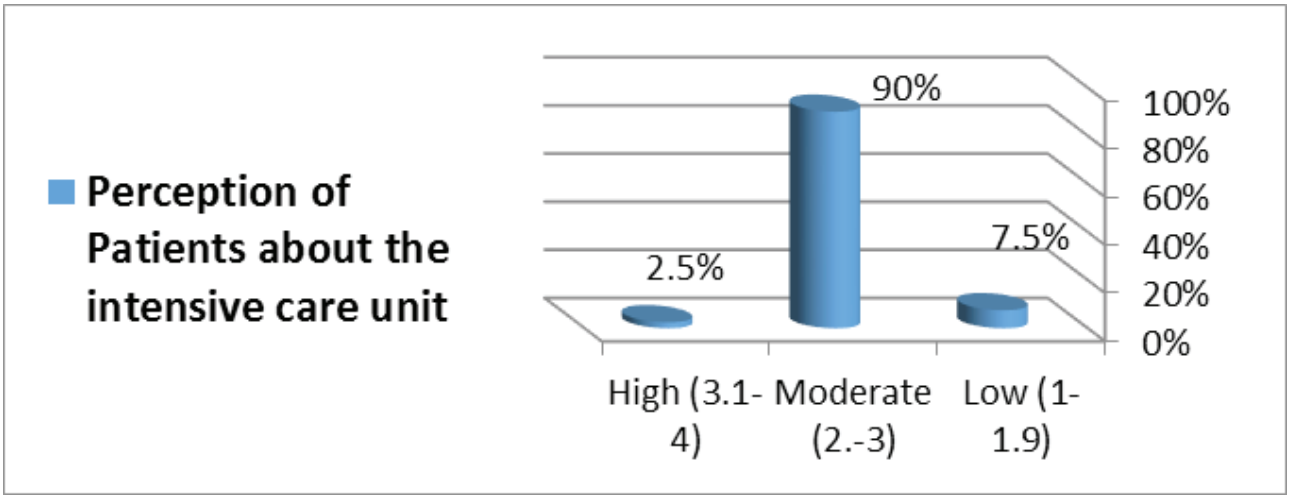


Figure (5) level of the perception of patient about ICU by use the environmental stressor questionnaire

The figure (5) revealed that the majority (90 %) of patient with moderate level of of the perception of patient about ICU followed (2.5%) of patients with high level and only (7.5%) of them low level of the perception of patient about ICU

Table (3): Association between Demographic Characteristic and the information related ICU with the stressful experiences of adult patients with (Endotracheal tube) (ETT) and Perception of Patients about the intensive care unit

Perception / Variables		stressful experiences of adult patients with (Endotracheal tube) (ETT) at ICU		Perception of Patients about the intensive care unit	
		test	p-value & Sig	test	p-value & Sig
Demographic Characteristic	Gender	t= -356	.724 (NS)	t=-822	.421 (NS)
	Age	F=3.360	0.028 (S)	F=0.680	.570 (NS)
	Marital status	F=2.388	.070 (NS)	F=.387	.817 (NS)
	Level of education	F=1.762	.159 (NS)	F=1.285	.295(NS)
the information related ICU	Type of surgery	t=0.680	0.570 (NS)	t=3.380	0.029 (S)
	Period of ICU	F=0.187	.830 (NS)	F=0.437	.649 (NS)
	Period of ventilator	F=.477	.625(NS)	F=1.820	.176 (NS)

t= t test. Independent Samples Test, F= one way ANOVA

The result of the table (3) revealed that the total score demographic characteristics of patients` stressful experiences about mechanical ventilation (Endotracheal tube) (ETT) at ICU and perception of patients about the intensive care unit was no statistical differences with demographic characteristic except the age with patient perception about mechanical ventilation there is a statistical significant association at p-value ≤. 0.05 levels.

The table also shows that the total score of the patients` stressful experiences about mechanical ventilation in ICU was no statistical difference with the type of surgery. While the total score of patients` perception of the intensive care unit was statistically significant differences, p-value ≤. 0.05 levels. Concerning period of the ICU and period of the ventilator the results show no statistically significant association.

Discussion of the Results

Most ICU patients respond or perceive stressors in the same ways related to the several clinical procedures that cause patients to experience different types of physical and psychological discomfort.

The present study reported that less than half of the study sample within age group (50-59) years and one-third within the age group (40-49) years and less than twenty percent of the patient within the age group (60-69), most of the patients were Male and, marital status and their education was read and write (primary school) and have a governmental job. Concerning the type of surgery majority of patients CABG surgery, the majority of the patient has been on a mechanical ventilation was 2-3 days. And most of the patients has been in ICU were 2-4 days**.** These results are consistent with the study conducted by Dias et al., (2015). Has reported that the mean ages of patients were 53.60 ± 17.47 years in the general postoperative intensive care unit.

Though the data analysis shows no statistically significant difference between demographic characteristics of the patient with stressful experiences about mechanical ventilation (Endotracheal tube) (ETT) at ICU and perception of patients about the intensive care unit except the age with patient perception about mechanical ventilation there is a statistically significant association p-value ≤. 0.05 levels. Concerning the stressful experiences about mechanical ventilation at ICU was no statistical differences with type of surgery. While the perception of patients about the intensive care unit was statistical significant differences at p-value ≤. 0.05 levels. Related period of ICU and period of ventilator the results shows no statistically significant association.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Adult Nursing and all experiments were carried out in accordance with approved guidelines.

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Impact of an Educational program on Teachers' Knowledge Toward Chickenpox Prevention at Primary Schools in Al-Najaf Governorate

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Abstract

Chickenpox disease is one of a highly infectious disease caused by the virus called varicella zoster .Chickenpox is spread through coughing and sneezing, and through direct contact with fluids in rashes. Most people who are vulnerable to chickenpox are children, especially those under 12 years old. **Objectives:** The study aim determine the effective of education programs on teachers' knowledge toward children with of chickenpox disease at Primary schools in Al-Najaf governorate and the relationship between teachers' knowledge with their demographic variables. **Methodology:** A quasi experimental studies was carried out at Primary schools in Al-Najaf governorate from January 2019, to the end of April 2020, the sample was collected in a purposive random sample comprised with 12 schools, identified the number of teachers selected from each school was 45, the data were collected using a questionnaire consisting of two main parts, the first part related to the social and demographic characteristics of teachers, and the second part concerned teachers' knowledge about the prevention of chickenpox and then an educational program was established to increase the awareness and knowledge of teachers towards the prevention of chickenpox. **Results:** The results showed that most of the samples (37.8%) were aged between (41- 50) years. For the number of years of service, the greatest percentage (35.6%) of the sample ranged from 1 to 10 years, the study also showed that 100% of the questions were answered by the teachers after the impact of the educational program. **Conclusion:** The study concludes that the effectiveness of an education program toward teacher's knowledge, which is concerned with chickenpox, is positive at a high rate.

Keywords: *Knowledge, Chickenpox, Prevention, Al-Najaf governorate*

Introduction

Chickenpox is very common childhood diseases, caused by virus called Varicella zoster (VZV). However, generally consider as a benignant infection, but can caused severe illnesses especially in infant baby, immunocompromised child and elderly patients [1]. Age distribution prevalence that registered cases showed that major cases within those of age (5-14) years at (65%), while (1%) occur in those age (>45 years), numbers of clinical cases showed an clear rise in the registration of chickenpox illness cases from (21798) cases in 2007 to (59681) cases in 2008 to (74195) cases in 2011 with possibility outbreaks occurred during 2008 and 2011. [2]. Chickenpox has high infection and distributes by olfactory secretions or by direct contact to skin bumbled. Chickenpox disease is considered as monthly noticeable diseases in Iraq. The infection of varicella appears in winter and spring, but it may see at any time of the year, Chickenpox cases were counted in Al-Dora city (southern Baghdad). Data of disease was obtained from the records of Bilat Al-Shuhadaa health center at the period from January to July 2012. Diagnosis of cases was achieved by the physicians of the health center. Studied risk factors were including the gender, address, school and age. Total of cases recorded was 73cases (66.9per 100,000) populations for seven months. Males reported cases were48 (65.7%) while female's cases were25 (34.3%) [3]. Chickenpox disease is a seasonal illness, highly infectious disease caused by

virus called varicella. This virus one of the herpesviridae family. Although that signs and symptoms are mild, but the attention it is meager to prevention its spread. Virus of varicella ratio is highly within developing countries that because of the rapid spread among students related to result of indirect contact of instruments and their secretions of infected child, airborne and droplet spread of vesicular fluid. The number of chickenpox cases has been elevated in Iraq because the lack of vaccine and health awareness lack. To reduce number of infected cases, it is needful to follow up the illness students and detect the possible virus mutations. Isolation of infected student that lead to the disease spreading. Then, using of the unprecedented methods for diagnosis the virus are more significant as well as assign of the clinical features[4]

This study also detected that rated of incidence (165.3) of each (1000) for chickenpox. This study detected that the rate of incidence (97.5) for each (1000) of mumps. The elevated incidence of the rate number to both infections disease according to the age group was among (5–9) years followed by (10–14) years. The seasonal variation was commonly during spring and winter for both diseases. The study conclude high incidence for both varicella zoster infection and mumps, in additional, the most of the group age affected within 5–14 years. [5]

This survey study was conducted with (1169) teachers from Hong Kong school that done before Disruption of school related to the (H1N1) disease outbreak. The finding showed that teachers were good aware of (H1N1,) however, were still anxious about the diffusion of (H1N1) infection. The teachers' anxious depended on their psychological responses, the sufficiency of the controls measures, with supportive giving by government to providing infectious diseases knowledge, realized understanding of prevention measures and emergency plans, parents' and students awareness, and need for assistance from health professionals. Not only the teachers need to preserved infectious disease control measurement in their schools, however it is also their responsibility for decrease the anxiety of students and their parents. [6]

Illnesses caused by communicable diseases are common among school students because of overcrowding. All teachers working with children may be at risk for getting communicable diseases, especially those with less Years of employments. Therefore, it is necessary to increase their awareness toward communicable diseases prevention and control through collaboration with ministry of health and environment and through education programs about communicable diseases and ways of prevention. [6]

Methodology

Objectives of the Study:

1. To assessment the level of teachers knowledge about child with chickenpox.

2. To determine the effect of educational program on teachers knowledge about child with chickenpox.

3. To find the relationship between teacher's knowledge about child with chickenpox and theirs demographic characteristics.

Design of the Study

The study is a quantitative research that uses quasi-experimental designing to assessment the teacher's knowledge about child with chickenpox at Primary schools in Al-Najaf governorate. The educational program constructed and then applied with a methods of pre-test and post-test to the study group. The study done during January 2019, to the end of April 2020.

the Study samples :

Non-probability sample (purposive). The sample were selected according to the spread of the disease, after review of health centers, were selected from the most prevalent areas, and then the choice of schools in a random manner, where 12 schools were identified and then a specific number of teachers were selected from each school to be of the samples 45 teachers which exposed to the health an educations program.

Study Instrument:

During the reviewing of related studies and literature, the questionnaire it is constructed as a mean for data collection. It was contain of two majors part. The first part is regarding with teachers' sociodemographic information of age group, the level of education, Years of employment school, and Place of residence. The second

part related to teachers' knowledge about chickenpox that contain the general teachers' information about chickenpox (15) items.

The Validity and Reliability:

The validity content of the instrument was decided by a panel of (6) experts. A small group of teachers (six teachers) that selected from schools in Al-Najaf governorate, and it is applied on teachers who had same criteria of the main study samples for determine the Alpha Correlation (r) of reliability the questionnaire about to teacher's knowledge towards chickenpox.

Statistical Analysis

The data are analyzed by the implementation of statistical procedures by using SPSS program version 23 which assisted to determine the resulting. The descriptive statistic data approach are used to description of the study variables: mean of scores (MS), frequencies, Percentages, and standard deviation. Inferential statistics data analysis methods that used by application of ANOVA, coefficient p-value correlation, and T-test.

Limitation

1- The difficulty of using two groups due to shortage of teachers.

2- Security and health situation is the result of the demonstration.

3- Disruption of school attendance has made it difficult to reach teachers.

Results

Table (1) Distribution of the Samples According to Teachers Demographic Characteristic

Characteristics	*categories*	*frequency*	*percentage*
Age group	**20 - 30**	**9**	**20.0**
	31 - 40	**10**	**22.2**
	41 - 50	**17**	**37.8**
	51 - 60	**9**	**20.0**
Years of employment	**1- 10**	**16**	**35.6**
	11 - 20	**11**	**24.4**
	21 - 30	**10**	**22.2**
	31 - 40	**8**	**17.8**
Resident	**rural area**	**20**	**44.4**
	Urban	**25**	**55.6**
educational level	**Institution**	**26**	**57.8**
	College	**19**	**42.2**

The table shows that most of the study sample at a percentage (37.8%) of the sample are between the age group (41 to 50) years, with regard to the number of years of Years of employment, the greatest percentage (35.6%) of the sample ranges from (1 to 10) Years of employment. They are resident in the urban at (55.6%), academic achievement was the most, and by (57.8%) they were institution graduates.

Table 2: Distribution of teachers related to their level of knowledge regarding prevention of chickenpox in schools before and after the effect of the educational program

Items	Pre-test			Post-test			Statistical differences		
	Scale rate	St. deviation	Assessment	Scale rate	St. deviation	Assessment	T-test	df	b-value
The disease caused by	1.29	.458	fail	1.98	.149	pass	-9.871	44	.000
The most important signs & symptoms of disease	1.20	.405	fail	1.91	.288	pass	-8.563	44	.000
Is the disease fatal when it occurs	1.24	.435	fail	2.00	0.000	pass	-2.847	44	.007
When is the disease contagious to others	1.76	.435	pass	1.87	.344	pass	-9.121	44	.000
Is chickenpox diseases covered by sick leave mandatory	1.11	.318	fail	2.00	0.00	pass	-3.773	44	.000
If the answer is (yes), what is the leave period	1.16	.367	fail	1.93	.252	pass	-14.265	44	.000
Does the disease give permanent immunity upon infection	1.20	.405	fail	1.96	.208	pass	-13.266	44	.000
Methods of transmission	1.09	.288	fail	2.00	0.000	pass	-13.266	44	.000
The most common infection season	1.13	.344	fail	1.98	.149	pass	-11.00	44	.000
Age groups frequently occurring disease in which	1.29	.458	fail	1.93	.252	pass	-9.929	44	.000
The incubation period of the disease	1.20	.405	fail	1.91	.288	pass	-8.563	44	.000

The most important complication of chickenpox	1.24	.435	fail	1.84	.367	pass	-9.871	44	.000
It is the appearance of the rash and final offer essential for the diagnosis of the disease	1.76	.435	فرعي	1.91	.288	pass	-12.410	44	.000
The disease is more severe when	1.09	.288	fail	1.89	.318	pass	-.298	44	.767
the disease prevented by	1.20	.405	fail	1.93	.252	pass	-11.000	44	.000

(M.s) mean of score 1.5 (less than 1.5 fail more than 1.5 pass)

The table showed that (94%) of the question paragraphs teachers failed to answer them before the effect of the educational program, as the natural scale average was (1.5). The table also showed that (100%) of the question paragraphs teachers succeeded in answering them after the influence of the educational program Where the normal scale average was (1.5), the study also showed that there is a strong relationship before and after the effect of the program when the (p-value) is = to or > (0.05).

Table (3) the total level of knowledge of the teachers before and after impact of the educational program (M.s) mean of score 1.5 (less than 1.5 fail more than 1.5 pass)

	Pre-test			Post-test			Statistical differences		
Total level	Scale rate	St. deviation	Assessment	Scale rate	St. deviation	Assessment	T-test	df	p-value
	1.26	.10825	fail	1.9363	.06810	pass	-38.3	44	.000

The table shows that all teachers do not have enough knowledge about the disease before the effect of the program, but after the effect of the program, all teachers have knowledge about chickenpox with a high significant difference (0.00).

Table (4) the relationship between teacher knowledge and demographic characteristics by using (ANOVA) before the effect of the program

Characteristics	df	P- value	Significant
Age	1.375	2450.	No Significant
Years of employment	2.921	0150.	significant
Resident	1.375	2450.	No Significant
educational level	1.375	2450.	No Significant

The table showed there is high significant relationship between the teachers' knowledge and a variable number of years of employment when p-value = or > to (0.05)

Table (5) the relationship between teacher knowledge and demographic characteristics by using (ANOVA) after the effect of the program

Characteristics	f	P- value	Significant
Age	2.069	1030.	No Significant
Years of employment	1.784	1510.	No Significant
Resident	2.069	1030.	No Significant
educational level	3.921	0050.	Significant

The table showed that is high significant relationship between teachers 'knowledge and the academic achievement variable when the p-value = or > than to (0.05)

Discussion

A quasi experimental study was performed on 45 samples of teacher, which exposed to health an educational program toward chickenpox disease.

Age group: The study showed that most of samples within age group (41-50) years, These results in line with findings by Offe (2016), she were found the high percentage of their study sample at 45 yrs. + of age at 52.5% of total samples, also agree with study done by Faraj & Khalifa (2014), that reported the most of samples at age (40-49) years. **Years of employment:** the study showed that most of teachers have experience between (1-10) years, this result agree with study done by Hawler & Hawler (2012), that reported the teachers have experience (1-10) years at (51%) of total sample. Also these results in line with findings by Faraj & Khalifa (2014) that reported the most of samples have experience (1-10) years at (40%) of total sample. **Resident:** The finding reveal that the majority of teachers were resident

in Urban, and these finding agree with study done by Hussein & Khalil (2020) that reported the majority of samples were resident in Urban at (91%) of total sample. Also these results in line with findings by Hawler & Hawler (2012) that reported the majority of teachers were resident in Urban at (85.8%). **Educational level:** The study showed that most of teachers have Institute graduate at (57.8%) of total samples, this finding supported by study done by Faraj & Khalifa (2014) reported that majority of teachers were graduated from Institute at (50%) of total sample, also agree with study done by Hussein & Khalil (2020)) that reported the most of samples graduated from Institute at (55.25%) of total sample.

The study showed that all teachers do not have enough knowledge about the disease before the effect of the program, but after the effect of the program, all teachers have knowledge about chickenpox with high significant difference, these finding supported by study done by Mansoor & Hussein (2020), that reported the pre-test assessment of teachers' knowledge about to mumps disease at schools showed a poor level of overall knowledge, but there are significant improvements of teachers' knowledge about mumps after the achievement of education program. Also these results in line with findings by Thumil & Bahlol (2016) that reported the teachers' knowledge which indicates high level knowledge at Mean of Score (2.46) of teachers after implemented the an educational program of the study group.

The study showed that is a significant relationship between the teachers' knowledge and a variable number of years of an experience, these finding supported by study done by Thumil & Bahlol (2016) that reported there is a statistical significant association between knowledge of teachers' and their years of experience of the study group. The study also shows there is a significant relationship between teachers 'knowledge and their academic achievement variable, this finding supported by study done by Hussein & Khalil (2020)) that reported in their study that statistical significant relationship can be manifested between teachers' level of education and their knowledge after the implementation of the educational program.

Conclusion

The study conclude the teachers do not have enough knowledge about the chickenpox disease before the implementation of the health program, but after the effect of the program, all teachers have knowledge about chickenpox with a high significant difference.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Furat Al-Awsat Technical University and all experiments were carried out in accordance with approved guidelines.

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Philosophical Foundations of Physical Education and Sport Applied to University Students of Central Areain Iraq According to the Social Understanding

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Abstract

The study objectives: To recognize the reality of understanding the members of the research community in central Iraq for physical education and sports. Building foundations for the philosophy of physical education and sports in Iraq according to the social understanding. Research hypotheses: There is a possibility to identify the reality of understanding the members of the research community for physical education and sports of universities in central area in Iraq. The research methodology included, where the researchers used the descriptive approach and survey studies for their suitability to the research problem and its objectives. The research sample consisted of (200) male and female students from the Iraqi Middle Euphrates universities after distributing a questionnaire to the research sample consisting of (60) questions to solve the problem noting that the questionnaire is scientifically evaluated and it has been proven. After collecting the answers of the research sample, the researchers presented these results by using statistical means, analyzing those results, and then discussing it , and the research objectives were fully achieved.

Keywords: *Physical education, university students, Iraq, social understanding*

Introduction

The love of knowledge and access to the truth represented the obsession of man since he was found on this earth, he tried to uncover the surrounding hidden secrets and the secrets of himself, and the answers he got to some of the questions [1] was a starting point for future generations in their quest to complete that task that no doubt will continue as long as life continues [2]. When we are exposed to man's attempts at knowledge, we will discover primitive philosophical features commensurate with his capabilities and capabilities and the amount of knowledge and information that he had [(1)] , and we can imagine the huge number of questions that a person found his imam and committed himself to answering them to understand what his abilities help him to understand, and to employ this understanding later to change his lifestyle and conditions for the better. That human perception for some of what is going on around him of tangible development in his social and intellectual life, and his move to live in human gatherings was a great quantum leap that changed many of their concepts, orientations and patterns of life, and it placed him in the face of challenges and difficulties of a new kind and was at the same time his gateway to enter the vast world of knowledge.. etc. Up to the rise of human civilizations in Sumer, Babylon and Egypt, and from what is mentioned here, the land of Iraq, which was the cradle of the first human gatherings, and whose other sons preceded the discoveries that he presented to humanity, changed the course of human and cultural history, and their civilization was its race in the field of sports and education. Establishing a philosophy for physical education and sports for a society requires studying that society and getting acquainted with the opinions of its members about physical education and sports and the concepts that they hold towards it. From this standpoint, standing on the views and concepts of Iraqis about sport in order to lay down philosophical foundations for it is a matter of great importance if We wanted the scientific and sports march in Iraq to be launched towards protective goals that benefit the society's age and conditions, meet its needs, and consolidate the values and ideals that it seeks to achieve.

Research problem:

The importance of philosophy for human societies is evident because it has contributed to laying out the theoretical contents of all fields of life in light of an in-depth extrapolation of the reality of societies, which had an impact on the qualitative civilization developments that accompanied the march of those societies in general and the developments that accompanied the process of physical education and sports in particular. Through the work of researchers in the field of sports and academia for more than 15 years and following the course of the sports movement in Iraq and informing them of many sources that dealt with the starting points of physical education and sports and its goals in Iraq, the two researchers found a clear absence of studies that recognize the role of Iraqi society (central Iraq) in formulating the philosophical contents It was built on the basis of the intellectual frameworks of the political regimes that succeeded in leading the country since the founding of the Iraqi state and on the personal efforts of some of those who were at the head of the march of the sports movement in Iraq during the past decades, which led to a state of lack of realism and a loss of clarity in its goals, approaches and perspectives, and this was accompanied by the absence of academic philosophical and sports studies due to the lack of an academic teaching staff specializing in the subject of the philosophy of physical education and sports despite the urgent need for this specialization to fill the gap referred to by the researchers and because the foundations of education and the philosophy of sport are systematic study materials for students of colleges and departments of physical education in universities all of Iraq The lack of a philosophical perspective on physical education. Sport is one of the most prominent aspects of the problem faced by physical education and sports in Iraq, which prompted the two researchers to study it.

Methodology

The researchers used the descriptive approach for its relevance to the nature of the research problem, and the descriptive method relies on studying the reality or phenomenon as it exists and describes it well, as it is a comprehensive cross-sectional study of a relatively large number of cases at the specified time [3].

Research community and sample

The research sample was selected from members of the Iraqi community, students of central governorates universities of both sexes, and the research sample consisted of (200) male and female students. The research sample was chosen by a simple random method from (5) universities in the central governorates from among (7) Iraqi governorates according to division Geographical ratio (71.42%) They are (Babylon University, Karbala University, Kufa University, Al-Qadisiyah University, Wasit University)

The researchers were unable to determine the number of individuals of the research sample in each governorate due to the lack of a reliable modern population statistics. The two researchers decided to distribute the percentage equally to the universities covered by the research. 200 questionnaires were distributed to (5) universities of the research sample by (40) forms for each university as well. The number of male respondents reached (120) individuals, representing (60%), while the number of females was (80) by (40%). Table 3 shows the distribution of the research by gender.

The researchers believe that the percentage of females is considered good according to the conditions that Iraq is going through and according to the reality of Iraqi society and the type of subject being researched. The importance of females 'opinion comes from the importance of their role in life and the importance of sport to them as they represent the other group of Iraqi society. To obtain credibility in the research, it must be passed through Male and female terminals. And that (5%) of the research sample do not practice sports, and (70%) practice sports, either the rest of the sample, which is (25%), practice sports from time to time and if we follow what Iraq has been going through for several decades, the majority of Iraqis have been occupied with many basics in public life, then practicing sport by a group (95%) of the research sample, even if it is sporadically and incorrectly, is considered a good thing, and the researchers believe that the motive for exercise Sport is the awareness of sports practitioners of its importance, regardless of the size of that awareness.

Search tools :

Arab and foreign sources – A computer - The

Internet - A questionnaire – Stationery

Field research procedures: (Ismail Khalil, PhD thesis, Babel, 2007**)** [4]

The researchers carried out several procedures for the purpose of achieving the objectives of scientific research in the correct and applicable way, and among these procedures is preparing a questionnaire and distributing it to the sample covered by the research.

The questionnaire:

The questionnaire is one of the important means of collecting information that researchers need to accomplish scientific research and achieve its objectives, as it is the only means that facilitates the broadening of the research sample for carefully selected and arranged stimuli in order to collect data (1). The researchers used a questionnaire that contained (60) questions, three of which were general information questions about the sample and the rest were situational questions that were divided into (10) axes (Appendix No. 1). The two researchers formulated questions specific to each axis. Then the researchers presented the questionnaire to a number of experts (Appendix No. 2) in the faculties of physical education and sports sciences, the questionnaire was used in a previous study by researchers (Ismail Khalil Ibrahim) noting that the questionnaire is linguistically valid, and the two researchers proved the validity and reliability of the questionnaire.

Validity of the test: The validity of the test is one of the conditions that must be met in the test, "and honesty is that the test measures with a high degree of validity what it claims to measure." The two researchers proved the validity of the questionnaire by adopting the apparent validity (Marwan Abdel Hamid, Amman, 2002, p. 195) [5].

- **Face validity:** The researchers presented the questionnaire to experts and specialists, as the experts 'evaluation is one of the widely used standard measurements.

Stability of the test:

It is one of the important factors that must be characterized by, and "the test is stable if it gives the same results continuously if it is repeatedly applied to the same subjects and under the same circumstances." [6] The researchers used the method of retesting to verify its stability.

Retesting: To determine the reliability coefficient in this way, the two researchers distributed the questionnaire to (10) students from the sample, then re-tested them two weeks after the first test and extracted the correlation coefficient using the simple correlation equation (Pearson), which reached (0, 93), which confirms the high correlation between the two tests. It demonstrates a high stability of the response achieved by the testers.

Objectivity of the test: The experts to whom the questionnaire was presented to determine its powers, its sincerity, the clarity of its questions, the soundness of its wording, and the lack of differences between the arbitrators and experts in correcting its answers, which confirmed the objectivity of the test (Nahda Abd Zaid, Amman, 2015, p.99) [7].

Exploratory experience:

In order to identify the difficulties that the researchers and the working team assisting with him may face when carrying out the research, the extent of clarity of the questionnaire questions for the sample members and the time taken to answer the paragraphs, and determine what may need clarification from them. The researchers conducted an exploratory experiment on (10) individuals of the sample for a period of 9/11/2019 to 17/11/2019, and reached through it the following:

- The answer time ranged between 20-30 minutes - the questions were clear and understandable to the sample members.

The main experience:

The two researchers conducted the main experiment from 1/12/2019 to 21/1/2020, in which the questionnaire was distributed with the help of the work team to individualize the sample according to the universities covered by the research.

Statistical means: All statistical operations were accomplished through the appropriate statistical system in order to describe and extrapolate the research results as follows

- Mean.
- Std. Deviation.
- Correlation coefficients person.
- (T) test for two unrelated samples.
- Percentage.

View and discuss the results:

Table (1) shows the understanding of the community members in the Iraqi milieu.

Statistical indicators	Mean	Hypothetical Mean	Std. Deviation	T value Calculated	T value Tabular	Sig type
Social understanding	136,43	103	14,13	2,27	2,01	Sig

Table (1) shows the extent of the community's understanding of physical education and sports after the results were extracted from the questionnaire distributed in five universities, namely the University of Babylon, Karbala, Kufa, Wasit and Qadisiyah from the central region. By searching for the questionnaire questions, whose answers tended to substitute the answer (yes), the researchers extracted the arithmetic mean (136,43), the standard deviation (14,13) and the hypothetical mean (103), and the researchers extracted the calculated value of t (2,27) and the tabular value of t (2,01) The final result of the statistical significance was significant, which showed the extent of understanding of the community to be researched.

The answers of the research sample on the questions of the educational axis are considered to be of the great conviction of them that physical education and sports have a great role in public education, and contribute to the moral building and values of the members of society. According to the sequence of questions, with percentages of (70%), (49%) and (64%). In contrast to the frequency and low rates for the alternative answer (no), and the researchers believe that the consensus of individual sample on a phenomenon is very difficult due to the multiplicity of visions and the diversity of attitudes and beliefs. Therefore, the trend of the largest percentage has become the basis for determining the importance or necessity of this phenomenon and that or not, and this is what applies to physical education and sports in which the research is being conducted.

The responses of the sample members to the questions of the physical axis show a clear vision of a large percentage of them about the importance of the role of physical education and sport in the physical aspect, whether in raising physical fitness, safety of growth, improving neuromuscular compatibility or economizing with effort, which are all factors that help individuals effectively in improving their life conditions Practical and private, and make it easier for those in charge of sports affairs the task of attracting them to practice sport, provided that the supplies and capabilities he needs are provided. The frequency of the answer to the alternative (yes) to the questions on the fitness axis were (156), (174), (156) and (128), respectively, with percentages (78%), (87%), (78%) and (64%), according to the same previous arrangement. The perception of a large proportion of the sample members of the positive impact of sport on the physical side and their conviction of its importance facilitates for those in charge of sports affairs to persuade them to practice it and holds them, in addition to other bodies, the responsibility of securing these practice requirements for the benefit of the individual and society.

Data indicate that (94%) of the research sample believe that exercising early contributes to improving community health, and (61%) of them believe that age, gender, or disability does not preclude practicing sports compared to (10%) believe that it affects (83%) of the research sample believe in the role of sport in the field of treatment of injuries and rehabilitation after injury, while only (1%) do not believe in that role. Either in

the field of healthy habits, (77%) are aware that physical education and sports motivate a person to adhere to sound health habits, while (81%) believe that practitioners of sports are faster cures of diseases than others. Focusing the answers of the research sample under the alternative answer (yes) clarifies the individuals 'understanding of the sample and their awareness of the role of sport in improving human health and contributing to its rehabilitation after injury, which is something that must be practically invested in improving the health status of community members and persuading those with dissenting opinions to return to it.

Through the answers of the research sample on the questions of the axis of mental development, the researchers found that the majority of the sample members agree that physical education and sports have a role in the development of mentality through the information and experiences they add that enrich the individual and are alongside knowledge and experiences in other areas of life to help him in his daily life, as well as Its positive effects on the soundness of thinking, maturity, correctness and correctness of decisions, the answers tended to be the alternative (yes), with a percentage of (69%), (86%), (69%) and (72%), respectively, against low rates for the alternative answer (no).

The answers of the research sample on the social axis questions show an increase in the percentage of alternative answers to (yes) to the questions (9, 10, 24, 35, 47, 55), where the percentages reached (66%), (68%), (78%) and (67%). And (53%) and (56%) according to the order of questions. This indicates that the research sample believes that physical education and sports contribute to building good social relations and developing leadership in their practitioners. Its practitioners attain the trait of humility. The foregoing indicates that the sample members are aware of the positive role of physical education and sport in building relationships and social characteristics that represent elements of strength for a society if it is common among the members of that society.

the percentages of the answers of the psychological aspect indicate that the alternative to the answer (yes) achieved the highest and for all the questions. These percentages reached (71%), 71%), (54%), (71%) and (68%) according to the order of the questions. While the percentages of alternative answer (No) were the lowest for all questions, and they reached a bug, according to the order (4%), (4%), (8%), (4%) and (4%). These ratios indicate that the individuals of the sample touch the role of physical education and sports in the psychological axis, whether in building a person's personality or getting rid of stress and psychological problems or enabling the individual to control various emotions and trust in his abilities and potentials of the individual and develop the characteristic of courage in a way that makes the individual more dependent on himself in facing living requirements .

Conclusions

1- Experiences, skills, and their physical and sports implications must be transferred to society to benefit from them in facing their positions and enhance the status and ethical role of physical education and sports.

2- The early start of practicing sports is one of the most important foundations for success in developing the physical and athletic side and starts working from kindergarten class.

3- The Ministry of Health is considered one of the most important bodies that have a major role in educating members of society about the relationship of sport and health education and pushing for commitment to sound health habits.

4- The role of the teacher in the educational and educational process and the role of trainers in the training client increases the transfer of knowledge and experiences to society

5- The workers in physical education and sports bear a large share of the dissemination of sports culture among members of society.

6- Exercising is a good remedy for relieving the psychological pressures that prevent the members of society in their daily life

7- The sports group plays a major role in cooperation to achieve common goals, build cohesive social relationships, and develop loyalty and belonging.

8- The difference in the monotheistic religions 'view of sports, not because Islam encouraged its practice

based on the relationship between the soul and the body.

9- Playing sports is a good way to invest the free time for all members of society, including the joy and pleasure that a person misses in his life.

10- The family has a great role in the participation of their children in the exercise of sports and their belief in the importance of the role of physical education and sports

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved and all experiments were carried out in accordance with approved guidelines.

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Activity of Several Purine Metabolic Enzymes in Sera of Pregnant Women

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Abstract

This study aims to determine the activity of several purine metabolic enzymes in the sera of pregnant women. These enzymes show potential as indicators to follow the progress of pregnancy. The samples of this study were collected from 75 healthy pregnant women. The age range of the sample study was 17–43 years and 25 samples were used as control (non-pregnant women in the normal physiological state). Purine metabolic enzyme activity was compared among the three groups with each other and with control. Results appeared high significantly with $p \leq 0.05$ in group III compared with group I of adenosine deaminase and the control group . Pregnant women showed increase significantly level of 5-nucleotidase (5-NT) and alkaline phosphatase (AlP) enzyme activity, particularly in the groups III of 5-NT and AlP compared with group I of 5-NT and AlP, the control group was found to possess equal 5-NT and AlP .

Keyword: *pregnancy, adenosine deaminase, 5-nucleotidase, and alkaline phosphatase.*

Introduction

Pregnancy, also known as gravidity or gestation, is associated with the natural physiological changes that involve the feeding and survival of the fetus. Hematological and biochemical parameters reflect these adaptive changes, which are crucial during complications [(1)]. Moreover, the placenta contains enzymes, including adenosine deaminase (ADA), 5-nucleotidase (5-NT), alkaline phosphatase (AlP), and aspartate aminotransferase, which are spread in different body tissues [(2)]. Immunological balancing takes place in pregnancy, in which the maternal immune system has to admit the paternal human leucocyte antigens expressed in the fetus and maintain normal immune efficiency to defend against microorganisms. Immunologically, the human fetus is always treated as an allograft to the pregnant mother. Despite being biological allografts and fetuses not normally being rejected by the maternal immune system, one possible explanation for this phenomenon is that the placental fetal unit is an immunologically distinct site that creates a mechanical barrier that weakens interactions between maternal lymphocytes and fetal tissues and functionally reduces the maternal immune response [(3)].

The enzymes 5-NT and ADA perform central roles in the adenosine metabolism in healthy persons, with 5′-nucleotidase (5′-NT, EC.3.1.3.5) catalyzing the hydrolysis of the phosphoric ester bonding of 5′-ribonucleotides to the corresponding ribonucleoside and phosphate [(4)]. The main function of the 5′-NT enzyme is the transfer of the group phosphate of AMP to adenosine [(5)]. 5-NT shows cancer progression at multiple levels, with a growing body of evidence suggesting 5-NT implication in neoplastic progression. Moreover, active enzyme activity has been detected in bladder, ovarian, thyroid, and prostate cancers [(6)].

ADA (EC 3.5.4.4) is important in purine metabolism and involved in the breakdown of dietary adenosine produced from the turnover of nucleic acids in tissues. This ubiquitous enzyme is found in various microorganisms, bacteria, plants, and vertebrates, with a highly conserved amino acid sequence; moreover, it is present in all mammalian cells. ADA, which is highly important for the differentiation of lymphoid cells, has been used for managing several diseases involving changed immunity [(7)]. These enzymes are widely distributed in various organs and body fluids.

The catalytic activity of AlP (EC 3.1.3.1) requires optimum pH [(8,9)]. The dephosphorylation and

phosphorylation of serum proteins are regulated by the opposing activities of phosphatases and kinases [10, 11]. AlP was determined by Mahesh et al. (2010), who measured the change in activity of alkaline phosphatase in the placenta of diabetic pregnant women; the results showed increased AlP activity in diabetic gestational women [12].

Methodology

Sera were collected from 75 pregnant women whose ages ranged from 17–43 years (29.0 ± 8. 7, mean ± SD) and residing in Baghdad, Iraq. Complete medical history and physical examinations were conducted by doctors of the advisory clinic in Yarmouk General Hospital.

Samples collection of the study: Inclusion criteria of the study exclude women who smoke, with diabetes or any chronic disease, and who drink wine.

Collection of blood samples: Blood samples were collected from pregnant women; 5 mL of venous blood was collected into plastic tubes and stored in sterile polyethylene tubes. After serum samples of whole blood were extracted, the sera were used for the determination of enzyme activity and other analyses.

Determination of total protein: The sensitive biuret method by Janairo[13] was used to determine the total protein in blood serum, with bovine serum albumin as the standard. Absorption of the colored solution was measured at λ = 545 nm.

Determination of the different enzymatic activity: ADA activity was determined using the method by Giusti and Galanti [15]. ADA activity was expressed using U/L. AlP activity was determined by the calorimetric method. 5¢-NT activity was measured by following Wood and Williams's method [16]. This method is based on measuring the liberated phosphate (a blue color will develop from a reaction with stannous chloride/molybdenum solution), which is produced upon the hydrolysis of AMP by 5¢-NT to adenosine. Given that AlP also hydrolyzes this substrate, the amount of phosphate released by incubating AMP with the serum will be the result of the activity of 5¢-NT and ALP. Therefore, nickel chloride was used to inhibit the activity of 5¢-NT, and the difference between that in the absence of nickel ions and in its presence will reflect the 5¢-NT activity.

Results and Discussion

The results in Figure (1) reveal that serum ADA level in the pregnant group increased significantly ($p \leq 0.05$) in groups II and III compared with that of the control group.

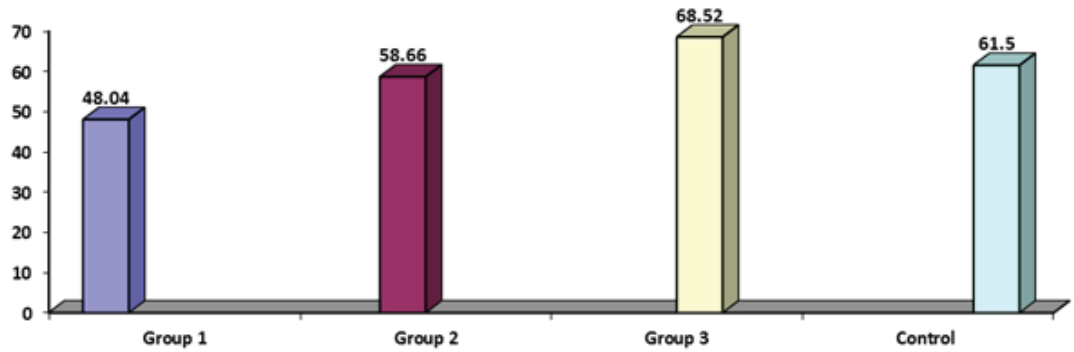


Figure (1): Adenosine deaminase activity in the sera of pregnant women in the three groups and control group

In this study, the pregnant women were divided into three groups. The first trimester group included pregnant women in the beginning of their first month to the end of the third month; the second trimester group included the women beginning their third month to those ending their sixth month; and the third trimester group included women from the beginning of their seventh month to those at the end of the ninth month.

Changes in hematological and biochemical parameters were compared among the three trimesters and between the pregnant women trimester groups and control group. Significant difference (at level $p \leq 0.05$) was found in the total account of WBCs, with the highest value being observed in the third trimester group at 10188.00 ± 1659.39 cell/mm^3 and the highest value in the control group being 7896.00 ± 1834.27 c/mm^3. Hemoglobin (Hb) and packed cell volume (PCV) showed a significantly decrease in the third trimester group (9.77 ± 1.32 g/dL) (30.40 ± 3.89%) compared with the first and second trimester groups (11.15 ± 1.78 and 10.63 ± 1.08 g/dL ; 34.44 ± 5.41% and 33.55 ± 3.58%, respectively). Biochemical parameters tests were found in women's sera, and the results showed non significant differences (at level $p \leq 0.05$) in the concentration of total proteins; moreover, a marked decrease in the level of albumin concentration was observed especially in the second and third trimester groups (0.72 ± 4.90 and 4.69 ± 0.72 g/dL) compared with the control group (non-pregnant women) (5.55 ± 0.56 g/dL); whereas non significant difference was observed in the globin and glucose levels. The results of this study recorded a significantly elevation in the activities of ADA, 5- NT, and AlP (68.52 ± 23.97, 4285.59 ± 2371.17, and 98.80±37.08 U/L, respectively), especially in the pregnant women in their third trimester.

Table (1):Demographic data of all parameters for all groups

	Mean ± SD				
Parameter	**G1 (1–3 month) N = 25 Pregnant woman**	**G 2 (4–6 month) N = 25 Pregnant woman**	**G3 (7–9 month) N = 25 Pregnant woman**	**Control G. N = 25 Non-pregnant woman**	**p-value p < 0.005**
Hb g/1000 mL	11.15 ± 1.78	10.63 ± 1.08	9.77 ± 1.32	11.15 ± 1.78	0.00
WBCs c/mm3	9690.00 ± 1808.37	9061.11 ± 1205.32	10188.00 ± 1659.39	7896.00 ± 1834.27	0.00
PCV %	34.44 ± 5.41	33.55 ± 3.58	30.40 ± 3.8944	40.68 ± 3.49	0.00
T. protein g/100 mL	6.93 ±0.62	7.05 ±0.65	7.28 ± 1.018	7.63 ± 0.83	0.456
Albumin g/100 mL	4.79 ± 0.61	4.90 ± 0.72	4.69 ± 0.72	5.55 ± 0.56	0.00
Globulin g/100 mL	2.15 ± 0.80	2.14 ± 0.96	2.63 ± 0.96	2.08 ± 1.03	0.201
Glucose mg/100 mL	101.16 ± 22.36	106.22 ± 21.06	111.20 ± 23.33	105.15 ± 18.85	0.433
ADA (U/L)	48.04 ± 15.72	58.66 ± 17.30	68.52 ± 23.97	61.50 ± 17.04	0.03
5-NT	2554.09 ± 1352.85	3369.88 ± 1039.35	4285.59 ± 2371.17	2761.04 ± 1375.61	0.00
ALP	57.18 ± 13.01	62.80 ± 15.20	98.80 ± 37.08	59.35 ± 20.99	0.00

Results in Figure (2) reveal that serum 5-NT level in the pregnant group increased significantly ($p \leq 0.05$) in groups II and III compared with that of the control group.

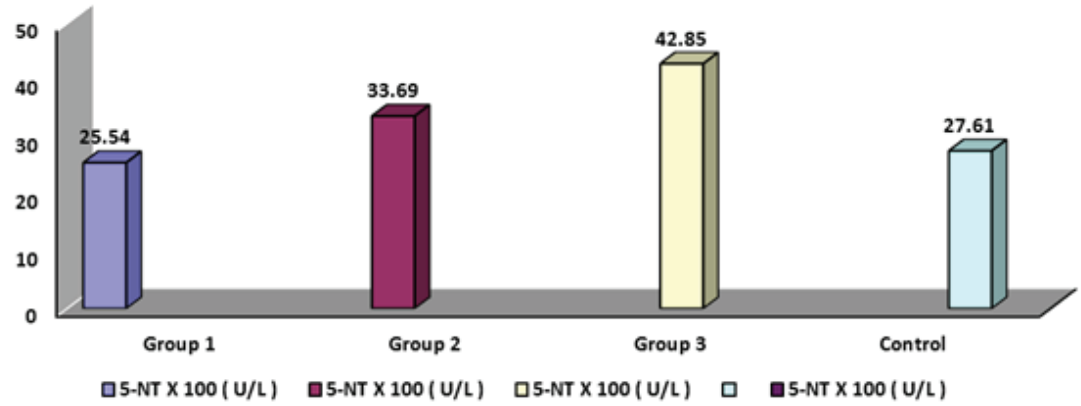


Figure (2): 5-Nucleotidase activity in the sera of pregnant women in the three groups and the control group

Figure (3) reveals that AlP activity in the pregnant group was significantly different ($p \leq 0.05$) in groups II and III compared with that in the control group.

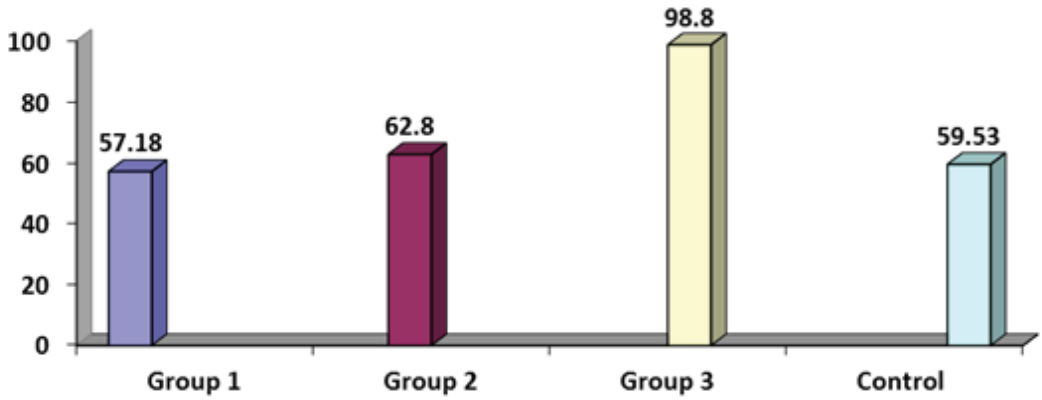


Figure (3): Alkaline phosphatase in the sera of pregnant women in the three groups and the control group

Figure (1) refer to the significant variations in the serum ADA activity in the pregnant groups, particularly in group III, compared with those of group I and the control group. The result showed increased ADA activity in pregnant women compared with the control group.

Our result agrees with a previous study [18], which indicated increased adenosine deaminase activity in pregnant women, with the highest levels of ADA found in the placenta during the stages of fetal development. This result suggests an important role of placental ADA during these stages of development. A major function of this enzyme in the placenta may be to prevent the accumulation of substrates that are potentially toxic to the developing embryo. However, these results were not in agreement with the results in this study [19], which showed decreased ADA activity in pregnant women.

Figure (2) refers to the significant variations in the serum 5-NT activity in the pregnant group, particularly in group III, compared with those of group I and control group. The result showed increased 5-NT activity in pregnant women compared with the control group. Our results agree with those in a previous study [20], which reported increased 5′-NT activity in pregnant women. This finding may be due to increased 5-NT activity,

which leads to increased adenosine during normal pregnancy and may act as an endogenous compensatory mechanism that diminishes platelet activation and maintains vessel integrity during normal pregnancy.

Figure (3) showed the significantly variations in the serum AlP activity in the pregnant group, particularly in group III, compared with those in group I and control group. Our results agree with those in a previous study [21], which reported increased AlP activity during pregnancy. The AlP levels increase by the third trimester because of placental synthesis, which led to the production of the placental isoenzyme. The activity of 5-NT and AlP significantly increased in the third trimester, being asymptomatic during normal pregnancy and showing supplementary production from placental tissue [13]. The placenta contained AlP and 5-NT, but the spread of 5-NT out of the placenta into maternal circulation may be limited due to the high molecular weight of 5-NT[14].

Conclusions

Activities of certain metabolic purine enzymes were altered during pregnancy. Activities of the enzymes adenosine deaminase, 5-nucleotidase, and alkaline phosphatase were significantly increased pregnant women, particularly in groups II and III, compared with those in group I and control group.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of applied Sciences and all experiments were carried out in accordance with approved guidelines.

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Legal knowledge and its relationship to selective attention using the Rehcom system for Al-Rafidain basketball athletes

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Abstract

the research objectives were to identify the reality of legal knowledge and selective attention using the Rehcom system for Al-Rafidain basketball players, and to identify the correlation between selective attention and the legal knowledge of Al-Rafidain basketball players , And prediction of legal knowledge in terms of selective attention of players Al-Rafidain Basketball Club, the researcher used the descriptive approach in the survey method and the correlational relations on the 19 Al-Rafidain Basketball Club players. Conclusions In light of the research results and the statistical analysis of the data obtained, the researcher reached the following conclusions, that there is a relationship between legal knowledge and The selective attention of basketball players, the variable and the selective attention, and through the test preparation in the Rehcom system, has a very clear relationship to improving and developing a variable and selective attention.

Keywords: *legal knowledge, selective attention, basketball*

Introduction

the importance of the research in identifying the correlative relationship between legal knowledge and selective attention using the Rehcom system for Al-Rafidain club players in basketball, and the problem of the research was through the researchers' acquaintance with previous studies and looking at the recommendations of researchers in addition to the experience of the supervisor it became clear that the research problem lies in the lack of Attention and focus on psychological aspects according to modern systems through the use of contemporary techniques in diagnosing, qualifying and developing psychological states of basketball players, through the use of (the Rehcom system) because of this effect on diagnosing the level of some accompanying mental variables,(1) and thus knowing the relationship between these variables He showed the legal knowledge of the game in order to advance the status of the basketball game in the future, and the research objectives were to identify the reality of legal knowledge and selective attention using the Rehcom system for Al-Rafidain basketball players, and to identify the correlation between selective attention and the legal knowledge of Al-Rafidain basketball players ,(2) And prediction of legal knowledge in terms of selective attention of players Al-Rafidain Club Basketball(3).

Practical Part:

The researcher used the descriptive approach in survey style and correlational relations on the 19 players of Al-Rafidain Basketball Club.

Field research procedures:

First: measuring legal knowledge of basketball:

The test that was built by the researcher was relied upon to measure cognitive achievement, and the players 'answers were corrected based on (1, 0) for each of the test items, as the correct answer is given one score while the wrong answer is given (zero) and thus the highest score for the test is (0) 75) and the lowest score (zero), after completing the procedures for building the achievement test for basketball, which was applied to Al-Rafidain club players for the 2019-2020 season, which includes (75) items. The researcher applied it on 6/22/2020 on the members of the basic experiment

sample [3], which amounted to (19) players[4].

Second: Measuring selective attention in the Rehcom cognitive system on Al-Rafidain Basketball Club players**:**

The two researchers chose selective attention after direct access to the Rehcom system and its tests, including the Selective Attention Test, and through the use of the literature on testing and direct inquiry, they were able to know the exact shape of the device, how the test was performed, and the reliability of the results obtained, and also showed them the possibility of applying this test. [5] The players of Al-Rafidain Club because the variables are given by the system, which are among the basic requirements of basketball players and in line with the nature of the basketball game and the tasks on the players and their needs for these variables within the field of play, [6] and what the game of basketball is characterized by the speed and instability of the ball in one place and other variables The many that the player must perceive and deal with, in addition to that the researcher wanted to keep pace with the development taking place in the world by using advanced measuring devices in the sports field, and it is considered one of the advanced and computerized tests used in the field of sports psychology and subject to international quality standards and certification. [7]

Test form:

Preparing the subject for the test should be on a special characteristic as his eyes are at one level with the signs that appear on the device where the reaction time in wakefulness is verified under two conditions:

First: To measure the core alertness, a geometric shape appears on the screen at a fixed location that changes randomly within short intervals, and the player's task is to interact as quickly as possible with the exact shape that has changed with the keyboard button.

Second: To measure phased alertness, the figure appears on the screen in a fixed location, and before it changes at a random interval, you hear a warning with a sound signal

Application and duration of the test:

The test begins with a message that appears on the screen if the reaction is early, that is, before the appearance of the shape and before changing it randomly, and the training phase does not end unless the reactions are correct for all exercise attempts.

The performance of the test is in two parts and it is called the simple design ABBA, as follows:

- Try without sound stimuli.

- Try with an audio signal.

- Try with an audio signal.

- Try without an audio signal.

In the first stage of the test, the response time of the player to clicking is measured as soon as a full square appears on the screen. In the second stage, the response time for the same visual stimulus is measured while hearing the sound of the signal before the square appears, and the player must click only when the full square appears, and wait until a square appears on the screen to click, and he does not have to do any reaction when hearing the sound signal.

In each attempt, two forms of stimuli are displayed on the screen, and the reactions in each attempt are on the stimuli of (10) when they appear on the screen, so if the reactions are incorrect, (5) stimuli will be added and more, i.e. in each attempt there are several stimuli (20)) (Without a sound signal) and (20) (with a sound signal) with (10) stimuli in each of the (4) attempts, and more than (5) are added in the case of the wrong reaction, as the following equation is shown 2 + 10 + (5) = 17, where he indicates:

- Issue (17) The number of correct reactions

- The number (10) indicates the number of stimuli in each of the (4) stages, with (20) alarms with sound signals and (20) with non-sound stimuli.

Number (2) is the multiplication of the audio and non-acoustic stimuli,

- The number (5) indicates with the interrogation the stimuli that increase in the event of a wrong reaction

The test period is a maximum of (6) minutes without the training phase. [8]

Data analysis:

In analyzing the results, two types of Z-VALUE are calculated:

- The Z-value of mindfulness (without an acoustic signal) , calculating the average of all reactions without an acoustic signal (active alertness).

The Z-value of mindfulness (with a sound signal), which is, it calculates the average of all reactions (phased alertness).

The screen display of the wakefulness level according to the standard Z-value (with an acoustic signal and without an acoustic signal)

The figure shows that the level of sub-normal alertness in the stage of active and intrinsic wakefulness, which is without a sound signal, with a value of (-1), while the phased alertness with warning alarms is within the normal level and with a degree of (+1), we notice on the right of the figure a vertical options window that can be During it, click on the first option if we wish

Print the screen display results, or go to "details" to view a more detailed table. It is worth noting that the sign (*) may appear, which means that the subject deleted or ignored the stimuli. In this case, the evaluation process will be incomplete and there will be no (Z-value). The results are presented in the form of a table as in Table (1), in addition to measuring the following data:

1. Measuring the average reaction time, process, and speed in general, and considers the slowdown in performance to be sub-normal (-3, -2 value –z), and mental stress.

2- The level of performance is measured from changes in reaction time, in addition to the high degree of standard deviation, indicating a decrease in the subject's emotional balance.

3- Delay in response and reaction for a long time indicates the speed of mental exhaustion for the subject, which reduces his active arousal. [9]

The statistical methods used:

The researcher relied on the (spss) system

Results

This chapter includes presenting, analyzing and discussing the results that were reached in light of the set goals, and verification of the research objectives. To facilitate the procedures, these procedures were presented in the form of tables and then interpreted to achieve the remaining research objectives and the established hypotheses. The following results emerged: -

The relationship between legal knowledge and selective attention among basketball players:

To identify the correlation between legal knowledge and the selective attention of basketball players, the correlation coefficient between the legal knowledge of the members of the research sample of 19 players was calculated, and its value was (0.476). The existence of a statistically significant relationship appeared at the level of significance (0.05), as the calculated T value reached (6.869), which is greater than the tabular value of (1.96), as shown in Table (1).

Table (1). Shows the correlation coefficient of the research sample

Variables	Correlation coefficient	The computed T value	The tabular T value	The significance level	indication
(Legal knowledge) (selective attention)	0.694	12.234	1.96	0.05	function

It appears from the above table that there is a correlation between legal knowledge and selective attention, and the reason for these results that have been reached is that the players who have a good amount of answers about the legal knowledge test were also good (al-Faramawy and Hassan: 2009, 170-171). To make players accept their situation even if they are exposed to emotional stress and psychological pain, they face situations instead of avoiding them, and this acceptance helps them to be prepared, alert and enjoy a very high level of attention by describing attention and focus. One of the psychological skills that have to do with the level of performance of basketball players and one of these skills is knowledge Legal by law game.

Presenting, analyzing and discussing the results of the contribution of legal knowledge to research variables and predictive equations

To achieve the goal of this study which is (prediction of legal knowledge in terms of selective attention of basketball players), the two researchers took several measures to achieve this goal.

Presentation, analysis and discussion of the results of the contribution of legal knowledge with selective attention

To identify the contribution of legal knowledge to the selective attention variable, the researchers used simple regression to extract the value of the contribution percentage resulting from the value of the square of the correlation between them. Table (2) shows that.

Table (2). Shows the significance of the correlation coefficient and the percentage of legal knowledge contribution to selective attention

Variables	The nature of the correlation coefficient	The value of the correlation coefficient R	The contribution ratio R2	F value	Sig	statistical function
legal knowledge	Simple	0.61	0.39	65.44	0.000	function

It can be seen from Table (2) that the percentage of legal knowledge's contribution to the selective attention variable index was significant, and this is confirmed by the calculated value of (F) of (167.45), which indicates that the common variance between the two variables was large and this is evident through the level of significance of(0.000).

The researchers attribute this to the fact that legal knowledge has a great impact on basketball players, as the player who has selective attention can distinguish information from the beginning of its presentation and then process it through the conscious interpretation of it because attention classifies the cognitive input and then processes it so that he can control it within the assigned context. to her. (Kabat Zinn, 1994) believes that the term is an automatic pushing of attention towards the object at the present moment, and the above definition tells us about its interpretation of selective attention through the use of selective attention to achieve a purpose or goal promptly. It is indicated that selective attention has physiological and psychological benefits. The physiological benefits are in the physiological changes that occur in the individual, including reducing chronic pain, for example, while the psychological benefits are in reducing stress, anxiety and phobias, as well as improving working memory, attention processes and tolerance. [10]

Presenting, analyzing and discussing the results of the percentage of the contribution of legal knowledge to the selective attention of basketball players:

To identify the percentage of legal knowledge's contribution to the selective attention variable, the researcher used simple regression to extract the value of the contribution percentage resulting from the value of the square of the correlation between them. Table (3) shows that.

Present, analyze and discuss the results of selective attention prediction thorough legal knowledge:

To obtain a predictive or expected value for selective attention through the legal knowledge involved in the behaviours of basketball players, the two researchers sought to use linear regression equations through which this relationship can be predicted. Table (3) shows that.

Table (3). Shows the results of the predictive equation for the variables contributing to legal knowledge

Variables	Value factor	value (t)	level of significance
Selective Attention	A 96.457	7.437	0.05
	B 0.612	12.086	0.05
Predictive equation	legal knowledge = 16.241 + 0.585 * Legal knowledge = 96.457 + 0.612 * selective attention		

The above table on the values of regression equations shows selective attention thorough legal knowledge, and accordingly, a predictive equation can be devised or expected value for selective attention in terms of the legal knowledge involved in basketball players can be derived as follows: -

The expected predictive equation of mindfulness and selective attention in terms of legal knowledge: -

- The expected legal knowledge of selective attention = the parameter value of the constant A + B x the degree of legal knowledge of basketball players in the scale.

For example: - If a player takes a score of (60) in legal knowledge, his score can be predicted in both selective attention and as follows:

51,34 = 60* 0,585 + 16,241 =

Selective attention = 96.457 + 0.614 * 60 = 133.3

From this, it becomes evident that selective attention can be predicted in terms of legal knowledge that includes the behaviours of basketball players, and through the results and predictive equations that have been reached, we note the possibility of detecting any basketball player in the event of his or her advantage and selective attention, as this early detection is a positive step They can correct their performance because the prediction process is a process of speculation and estimation that depends on correct scientific foundations that gain complete confidence and that this process is not necessarily 100% identical with reality, but rather includes a large percentage of knowledge of basketball players 'attitudes and the manner of their actions, actions or performance.

Conclusions

In light of the research results and the statistical analysis of the data obtained, the researcher reached the following conclusions: There is a relationship between legal knowledge and selective attention among basketball players. The variable and selective attention and through the test preparation in the Rehcom system has a very clear relationship to improving and developing a variable and selective attention.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Education for Girls and all experiments were carried out in accordance with approved guidelines.

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Psychological Safety and its Relationship to the Trends of Achievement Goals for Students of the College of Physical Education and Sports Sciences in Light of the Covid-19 Virus

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Abstract

The research interest in studying security, the new Corona epidemic, and the air of its negative effects on the levels and commitment of its newest participation in the new community, which made it distinctive, which made it distinctive, which made it new in society. And the integration of students within the educational institution and because of this effect of negative repercussions clearly on students and education and their aspirations, and the aims of the study and research objectives on psychological security and directions to identify the goals of education and sports science. Turning around in the light of the researcher's results, the message that reaches him with this thesis reached him, ease of access to psychological security. Psychological security, enjoy the university's College of Education.

***Key Words**: Psychological Safety, Trends of Achievement, Covid-19 Virus*

Introduction

The student's achievement of success, excellence and progress in the learning process, depending on his abilities and personal talents, has the greatest impact on determining his future and his goal orientations. Therefore, we find that goal-oriented students are more serious than others and achieve successes in many life situations and obtain outstanding success marks at the university.(1) They are making more progress in the local community, and the importance of research is to study psychological security under the shadow of the new Corona epidemic and its negative effects on the levels and commitment of students to study, as well as studying the trends of achievement goals that reflect the desire to achieve success, excellence and progress in the student's learning process, and the problem of research was through His continuous field follow-up to the current situation is that the Iraqi society is witnessing an accelerated movement at various levels related to psychological security, which affected the movement of the public street and the movement of government institutions in particular educational institutions and psychological insecurity and its effects on the ability of students to continue permanently and achieve their aspirations as well as its impact on the weight of students within society And the integration of students into the educational institution and because of this negative impact It is clearly reflected on the students, their educational level and their aspirations. (2) The research objectives were to identify psychological security and trends of achievement goals for students of the College of Physical Education and Sports Sciences. (3)

Practical side

The descriptive approach was used in the survey and correlational method on the (341) students of the College of Physical Education and Sports Sciences, as well as the (165) students of the College of Education for Girls, Department of Physical Education and Sports Sciences, who numbered (165) students for morning study at the University of Kufa for the academic year (2019-2020) As the total number of the research community reached (506) male and female students, and the research sample was selected randomly and in a proportional selection

method, which consisted of (250) male and female students distributed over the two colleges. With a rate of (49%), the research sample is considered one of the important steps and stages in the research. For this reason, the research sample was divided according to its percentage above[4], according to the faculties, and Table (1) shows that:

Table (1). Shows community and sample research

College	Stage	The total number of students	The number of participating students	The percentage
Education for Girls / Department of Physical Education and Sports Sciences	First	55	33	60%
	Second	45	30	66%
	Third	35	25	71%
	Fourth	30	22	73%

Field research procedures:

First: Measuring psychological security:

The researchers adopted the Psychological Security Scale of the researcher Ahmed Hashim, where the researcher relied on building the Psychological Security Scale on the theoretical definitions and theory of Abraham Ma Slow, which focuses on psychological security that the human being has various needs that he seeks to satisfy through the institution in which he works and the individuals who live with him on the grounds that a person can arrange His motives in a manner consistent with his behaviors and with the environment variables in order to reach the aspect of perfection in mental health, the force of motivation that a person needs to achieve psychological security differs from the driving force he needs in (self-realization) and therefore he divided into Abrahamma Slow in the hierarchy (self-realization - self-esteem - needs. Belonging - security needs - physiological needs) considering that the need for security is one of the primary needs that must be satisfied and whose fulfillment depends on satisfying other needs such as (self-realization and self-esteem) and is very consistent with the legal logic of true Islam. (Two blessings of unknown health and safety) considering that the motives related to the aspect of security for the human being are stable unless they are extracted for some circumstance and despite the failure of this theory to reach integration to the researcher We adopted it because it is more consistent and compatible with the attitudes of the sample members and more realistic in interpreting the results of the research on psychological security. It is a scale based on a sample of scout leaders in middle schools for the central and southern regions, the scale consists of (57) items and each paragraph has five responses, and each response represents a specific position, and there are five alternatives to the answer in front of each paragraph, which are (always, often, sometimes, rarely, Never) [5].

Psychometric characteristics of the Psychological Security Scale:

Honesty:

The researchers used two truth indicators, consistent with the nature of the Psychological Safety Scale

To achieve the validity of the scale, it was applied

Validated content:

The validity of the Psychological Security Scale was verified by presenting its paragraphs to a group of experts and specialists to ensure the validity and suitability of their paragraphs for measurement.

Formative (constructive) honesty:

Both the two statistical methods were adopted, namely, the two-terminal groups and the internal consistency coefficient in verifying the validity of the scale as the discriminatory strength of the paragraphs was calculated, and the internal consistency coefficient was calculated by calculating the correlation between the score of each paragraph and the overall score of the Psychological Security Scale. (6)

Stability:

To verify the stability of the Psychological Security Scale:

Halftone segmentation:

The half-segmentation was calculated by isolating the individual paragraphs from the marital paragraphs, and to find out the homogeneity between the odd and even paragraphs, and then calculate the correlation coefficient between them, the value of the correlation coefficient between the two halves was (0.767), and where this indicator is concerned with half of the vocabulary, it is necessary to use the equation (Spearman-Brown) to know the amount of the stability coefficient for all the terms. After the statistical procedure for this equation, the value of the stability of the entire scale was (0.868), which is a high value, which indicates that the scale is constant. (7)

Alpha Cronbach equation:

The idea of this method is based on the calculation of the correlations between the group relations and the stability of all the paragraphs in the test, as if we divided the test not into two parts as we noted in the midterm method, but here it is divided into several parts equal to the number of its paragraphs, that is, each paragraph constitutes a sub-test.

To calculate the stability in this way, all the forms were subjected to Alpha Cronbach coefficient, and upon applying the equation the value of the stability coefficient was equal to (0.943), which is a high value of stability.

Table)2). Shows the statistical indicators of the Psychological Security Scale

Statistical index	value
The number of the final scale paragraphs	50
The highest hypothesis score for the scale	250
The minimum hypothesis for the scale	50
The hypothetical mean of the scale	150
Range	139
Skewness	0.165
Kurtosis	-0.41

Table (2) shows that the number of paragraphs of the Psychological Security Scale in its final form consists of (50) items, the highest degree of hypothesis for the scale (250) and the lowest degree of a hypothesis (50) and that the hypothetical average of the scale is (150) and that the scale of the scale is (139) as well as the torsion (0.165) Kurtosis (-0.41) so that the scale is ready for the final application. See Appendix (2).

Statistical methods: The Statistical Package for Social Sciences (SPSS) was used.

Results

Presentation, analysis and discussion of psychological security results for students of the College of Physical Education and Sports Sciences

To achieve this goal, the researcher used the O sample t-test to compare the sample mean of (100.144) with a standard deviation of (33.84) with the hypothetical mean of the scale (150), so the calculated T value was (-23.62), which is a function at the level of significance (0.05) and a degree of freedom (250) amounting to (± 1.96) and since the arithmetic means of the sample is less than the hypothetical mean, this means that students of the College of Physical Education and Sports Sciences do not have psychological security and statistically significant. Table (3) illustrates this.

Table (3). Shows the results of the T-test for one

sample to indicate the difference in psychological security for students of the College of Physical Education and Sports Sciences

the group	the number	the assumed average	Arithmetic mean	the standard deviation	the computed t value	the significance	the decision
Students of the College of Physical Education and Sports Sciences	250	150	100.44	33.84	-23.62	significant	A function of having no psychological security and statistically

This result can be interpreted as naturally expected, as reaching this level is very difficult because of the conditions and health problems surrounding the student (Physical Education and Sports Sciences) and the educational institution due to the Corona epidemic that is not hidden from everyone, which is a direct cause and effect on behaviours and levels Students within this super important entity, that the deterioration of the health situation and the increase in infections with Coronavirus in some governorates of the country has negatively affected the psychological security and mental health of some students, and thus it was reflected on their academic and scientific level within the university.

Disclosure of differences in psychological security according to gender variables (female-male) and grade (first - second - third - fourth).

To achieve this goal, the researcher used the binary variance analysis and the results were as below

Table (4). Shows the results of the binary variance analysis of the significance of differences in psychological security according to gender and class variables

Source of variance	Sum of squares	Df	Average squares	value f Actual		significance level sig.	indication
				Computed	tabular		
Gender	116320.019	1	116 320.019	196.507	3.84	0.000	function
Class	2564.835	3	854.945	1.444	2.6	0.230	Not a function
Gender * **Class**	3063.199	3	1021.066	1.725	2.6	0.162	Not a function

From a note in Table (4), it appears that there are differences in psychological security, as follows:

Gender: From the observation of the final value of the gender variable, it becomes clear that (196.506) is greater than the tabular value at the level of significance (0.05) and the degree of freedom (1- 249), which is (3.84). Arithmetic mean for males (67.2), which means that female students of the College of Physical Education and Sports Sciences are psychologically safer than males. This result can be interpreted as evidence that female students deal more objectively with health events that the country is going through and their ability to deal with these health variables in a balanced and

objective manner without exaggerating them. And work with all courage, patience and wisdom to adapt to this epidemic to continue studying and scientific progress in the educational institution (the university) in its natural desired form, noting that this work requires patience and courage and employing the human soul for psychological security, that is, the more patience and courage the student will be, the safer it is. Ama and this fact have been referred to by the Holy Qur'an in the Almighty's saying: "If there is a hundred among you, they will overcome two hundred, and if there is a thousand, they will overcome two thousand, God willing, and God is with the patient." [8]

The class: From the observation of the final value of the grade variable, it becomes clear that (1.444) is smaller than the tabular value at the level of significance (0.05) and the degree of freedom (3- 247) which is (2.6), which means that there is no statistically significant difference in psychological security for students of the College of Education Physical and sport sciences according to the grade variable (first - second - third - fourth). This result can be explained by the fact that this percentage indicates that most students surrender to the difficult circumstances surrounding them and the nature of the psychological and health pressures they are exposed to due to the spread of the Corona epidemic within the country, as well as the fluctuating health situation of the country, and this has negatively affected the level of students studying inside the university.

The interaction of Gender X class: From the observation of the final value of the class variable, it turns out that it is (1.725) smaller than the tabular value at the level of significance (0.05) and the degree of freedom (3- 247) which is (2.6), which means that there is no statistically significant difference in psychological security at Students of the College of Physical Education and Sports Sciences according to the interaction of the gender variable (male-female) with the academic grade variable (first - second - third - fourth). This result can be explained by the fact that the majority of students have been affected by the difficult and health conditions facing their work and their educational and national mission in facing the problems that the country is going through, represented in the spread of the Corona epidemic and the scientific capabilities and creative energies that students possess in the sports field that can be invested to achieve their educational practical goals. That they aspire to, and this reflected negatively and clearly at the level of all students and all academic levels[9].

Presenting, analyzing and discussing the results of the trends of achievement goals among students of the College of Physical Education and Sports Sciences

To achieve this goal, the researcher used the T-test for one sample

Table (5)

It shows the results of the T-test for one sample to indicate the difference in the orientation goals of achievement for students of the College of Physical Education and Sports Sciences

Achievement Objective Orientations	The number	the assumed average	the arithmetic mean	the standard deviation	the calculated t value	the indication	the decision
The orientation of the goals of mastery	250	45	64.25	11.72	26.33	significant	empowerment
Foot target orientation			64.73	11.44	27.64	significant	with feet
Volume target trends			45.15	4.93	0.49	not significant	have no volumes

From the observation of Table (5) it was found that university students have a high desire to learn and persevere and strive in difficulties. They have a spirit of challenge and have a strong desire to understand the world around them and to face the challenges and conditions of the country in general and health in particular, and to overcome all

obstacles that may increase their reluctance to Achieving excellence and success and reaching the set goals.

Conclusions

In light of the researcher's results, a set of conclusions were reached: The validity of the two psychological security measures that the researcher prepared in their numbers in measuring the psychological security of students of the Faculty of Physical Education and Sports Sciences in light of the Corona epidemic. Students of the Faculty of Physical Education and Sports Sciences at the University of Kufa do not enjoy psychological security.Students of the Faculty of Physical Education and Sports Sciences at the University of Kufa enjoyed a good amount of achievement goals orientations.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa-Faculty of Education for Girls and all experiments were carried out in accordance with approved guidelines.

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Antibacterial activity of Zinc Oxide Nanoparticle (ZnONP) Biosynthesis by *Lactobacillus plantarium* aganist pathogenic Bacteria

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Abstract

The good capability of zinc-tolerant probiotic of *Lactobacillus plantarum* tolerating high concentrations of $Zinc^{+2}$ and producing Zinc Oxide Nanoparticles high lights the unique characteristics of these bacteria as a natural microbial cell nano-factory for a more efficient and environmentally friendly process of biosynthesis of these nanoparticles. The morphology structure and stability of the synthesized ZnO nanoparticles were studied using structural and morphological Properties technology such as XRD and FE- SEM analysis it also used optical properties such as UV and FTIR spectrum. The results depicted that the synthesized nanoparticles were moderately stable, exagonal phase, roughly spherical with maximum particles in size range within7–19 nm in diameteralso this study include the antibacterial activity of the Zinc Oxide Nanoparticles on some pathogenic bacteria .It is clear in this the study that Zinc Oxide Nanoparticles has stronger inhibitory effect on pathogenic bacteria because The proposed two imaginable mechanisms for the antibacterial action of zinc oxide nanoparticles to bacteria are first, creation of augmented levels of ROS mostly hydroxyl radical and singlet oxygen which destruction the bacterial cell wall. Second nanoparticles deposition on bacterial surface or nanoparticles aggregation either in the periplasm region or in the cytoplasm initiating distraction of cellular function and membranes disorganization .

Keyword: *ZnONPs, Lactobacillus plantarum, Biosynthesis , Characterization and antibacterial activity*

Introduction

Zinc oxide nanoparticles (ZnO NPs) have gained worldwide attention as multifunctional nanomaterials due to their distinctive properties of being versatile semiconductor and piezoelectric properties [1]. Microbial metal nanoparticles synthesis has recently been widely used due to their low cost, biocompatibility and eco-friendliness [2]. ZnO has many important features like chemical and physical stability, high catalysis and efficient antibacterial activity [3]. Symbiotic microorganisms may use NPs as safe source. Microbial nanoparticles synthesis has more benefits than other chemical and physical method. Nanoparticles have many applications in medicine. In addition, the bacterial nanoparticles may also be used for controlling human bacterial pathogens [4]. Among the micro-organisms, lactic acid bacteria (LAB) receive substantial attention because of their safe handling and food-grade status, which are "generally recognized as safe" (GRAS) in the production and preservation of food [5]. A low-cost, unreported and easy method for the biosynthesis of ZnONPs by using reproducible bacteria, *Lactobacillus plantarum* as an eco-friendly reduction and capping agent is described in our current study. *Lactobacillus plantarum* is a non- pathogenic, grampositive, facultative anaerobic bacteria and is the largest of all lactic acid bacteria in its genome. *Lactobacillus plantarum* has a negative electrokinetic potential; which attracts cations easily and this step works as a trigger for the ZnONPs biosynthesis [6]. The mechanisms of cytotoxicity from ZnO-NPs are not yet completely understood, however the generation of hydroxyl radicals (OH•), superoxide anion (O2), and perhydroxyl radicals (HO2) from the floor of ZnO are believed to be fundamental components. When nanoparticles engage with cells, cell protection mechanisms are activated to limit harm. However, if the exceptionally active free radicals manufacturing

exceeds the antioxidative protective potential of the cell, it results in oxidative damage of biomolecules which can lead to phone loss of life [7].

Methodology

Bacterial Isolate: *Lactobacillus Plantarum* was selected as a biological model for the synthesis of ZnONPs because it is more efficient for biosynthesis of ZnONPs .We have obtained this isolate from the dairy products which is stored in the advanced Microbiology Lab./University of Babylon. We did culture to the isolate for 24hr. on MRS agar at 38°C. as well as it is iagnosed by Vitek2 system.

Synthesis of zinic oxide nanoparticules by *Lactobacillus plantarum*

Synthesis of zno nanoparticales :- pure culture of *lactobacillus* was inoculate in to the flask contianing de man Ragosa and Sharpe (MRS) broth and incubated at 37c for 24 h at 100rpm . after the incubation period pH of the culture broth was adjusted to using 0.4 M NaOH to delay the process of the transformation.the 0 .1 M ZnSO4 H2O was added to the flask containing culture solution and heated on a water bath up to 80 c for 5- 10 min. A white precipate appears at the bottom of flask indicates the transformation process and the flask was removed from water bath . incubate at 37 c for 12 h so that all the particles gel deposited at the bottom of the flask . the product was filtered and washes with deionised water followed by drying at 40 c in a hot air oven for 4 h [8].

Structural and Morphological Measurements

The X-Ray Diffraction Measurements

Strong non-destructive technique for material characterization is XRD, via XRD can determined the crystal structure and particle size. These characterization is generally carried out (achieved) with a typical wavelength of X-ray that is comparable to the interatomic distance in a crystal. The constructive interference occurs when the path difference is an integer multiple of the wavelength. This is the Bragg condition for diffraction. The intensity of the reflected beam has sharp peaks in the corresponding directions. They are called Bragg peaks (9). The Bragg peak can be found by varying the angle 2θ of the detector.

2□□□□□□=□λ …… 2-1

Where n is integer

The particle size (D) is measured by Scherer's formula:

□=0.9□Δ(2□).cos (□) …… 2-2

where Δ (2θ) is the full width at half maximum (in radians) of the peak, θ is the Bragg angle. The samples structure was analyzed with a Shimadzu 6000 X - ray diffractometer (made in JAPAN) using Cu Kα radiation (λ=0.15406 nm) in reflection geometry. A proportional counter with an operating voltage of 40 KV and a current of 30 mA was used. XRD with scanning speed of 8 degrees/min over 2θ range 5° - 80° (Shimadzu XRD-6000-Japan). The XRD patterns display no peaks indicating that the samples structure was in the amorphous phase .

The Field Emission-Scanning Electron microscopes (FE- SEM) Measurements

Scanning Electron Microscope utilizes a highly energetic beam of electrons for taking the images of sample by scanning it (10).).The topography of the prepared powder was studied by Field Emission Scanning Electron Microscopes (SEM) type- S-1640 HITACHI company (Japan) with different magnification powers and detectors . It was also used for particle size measurements. Prior to the test, the samples should be dried and cleaned. The FE-SEM will take 3 to 4 minutes to pump down to 10-5 mbar. The extra high tension (EHT) (beam voltage) for gas panel is set to the desired value, normally starts with 5 KV. The sample's surface covered with a gold coating to facilitate the discharging of the surface charge.

Optical Measurements

UV -Vis Diffuse Reflectance Measurements

The optical properties of semiconductor were studied using a Varian Carry 5000 model UV- VIS-NIR spectrophotometer with an integrating sphere, using PTFE as the reference disk. Diffuse reflectance spectra were taken in the range of 200-800 nm supplied by Shimadzo Corporation (JAPAN) and in air at room temperature. The diffuse reflectance of the sample was related to the Kubelka- Munk function F(R). Diffuse

reflectance data were transformed using Kubelka-Munk function by the relation:

F(R)=(1−R)22R …… 2-3

Here R is the diffuse reflectance of the sample.

The Kubelka-Munk theory allows us to calculate the energy gap of the thin or powder According to graph of F(R) versus energy values.

Fourier Transform Infrared (FTIR) Measurements

Mid-IR spectra, from 4000 to 400 cm-1, were obtained for some pure samples and all doped samples using FTIR - Spectrometer, supplied by Shimadzu, on KBr pellets of the samples.

Antibacterial activity of ZnONP

Disc Diffusion Method.

1. Concentrations were taken of each bacterial isolates and compared to McFarland solution to get the right concentration for each of them.

2. The appropriate concentration 0.1 ml of each bacterial isolates were added to dishes containing Muller Hinton agar is spread on the surface of the dish-by spreader and left the dishes for an hour.

3. Wells were made by using cork borer 6 mm diameter as it was equal distance between the well and the other.

4- The Nanoparticles were dissolved with distal water to get various concentrations ranged from (2.5-0.625) µg/ ml.

5- Nanoparticles inhibition zones were measured by a ruler, (11)

Results and Discussion

Biosynthesis of ZnO NPs by *Lactobacillus plantarium*

Lactobacillus plantarum was confirmed The formation of nanoparticles by observing change in color of solution during synthesis of ZnO nanoparticles showin Figure 1 explain the reduction of Zinc Oxide into zinc nanoparticles during exposure to the bacterial extract is followed by the color change from brown color to yellow during 24h also show in figure B increase of reduction througt 48h from incubation peroid by the color change from brown color to pale yellow (12)

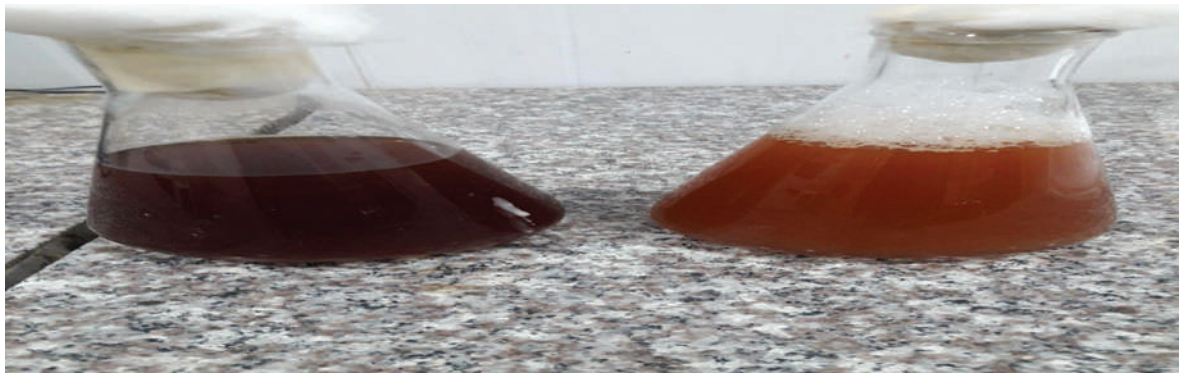


Figure-1 -Color change indicates the formation of ZnO nanoparticles compare with control after 24h .

The white color deposition at the bottom of flasks figure 2. This result was agree with (13) by using *Lactobacillus sporogens* and also agree with the result obtained by (14) regarding to the outcomes achieved. (15) was showed that synthesis of Zinc oxide nanoparticles (ZnO NPs) using culture supernatant of endophytic bacterial isolate *Sphingobacterium thalpophilum* in the process of reduction aqueous zinc oxide being extra-cellular which lead to the development of an easy bioprocess for synthesis of ZnO NPs It is clear that , *Lactobacillus plantarum* acts a crucial part in the synthesis of ZnO nanoparticles.

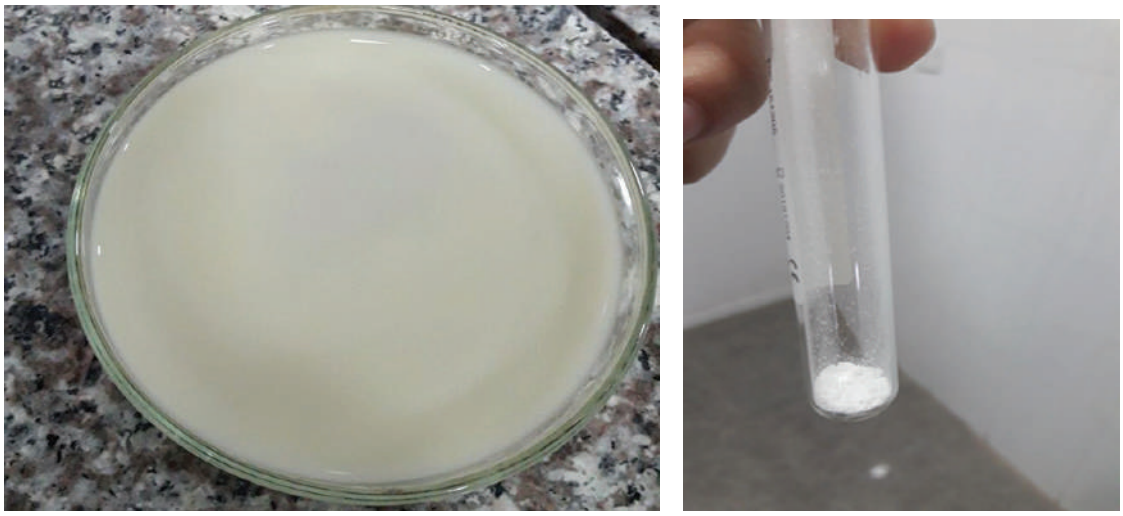


Figure 2: Biosynthesis of ZnO NPs from *Lactobacillus plantarum*

Structural and Morphological Properties of Zn- ONPs biosynthesized by *Lactobacillus plantarum*

X-ray diffraction analysis

Figure 3 shows X-ray diffraction (XRD) patterns of the biogenic ZnONPs synthesized using *Lactobacillus plantarum* . XRD analysis reveals that the nanoparticles synthesized were pure and crystalline innature. The peaks at 2θ = 31.7694, 34.4211, 36.2521, 47.5376 , 56.6016 , 62.8624 , 66.3782 , 67.9610 , 69.0982, 72.5631 , and 76.9671 wereas signed to the (100), (002), (101), (102), (110), (103), (200), (112),(201), (004), and (202) respection lines of spherial ZnO Particles respectively. The average particle size of ZnO was estimated by applying the Scherrer equation(17), as shown in equation (3-1) for the peak (101) reflection at 2θ by using the full width at half maximum (FWHM) (17, 18)

□= K□ (□)……3−1

Bcos□.

The calculated average particle diameter is 7 nm. Line broadening of the diffraction peaks is anindication that the synthesized materials are in the nanometer range.The synthesis of the nanoparticles obtained is too small; this may bedue to the biological synthesis method adopted to prepare the nanoparticles .

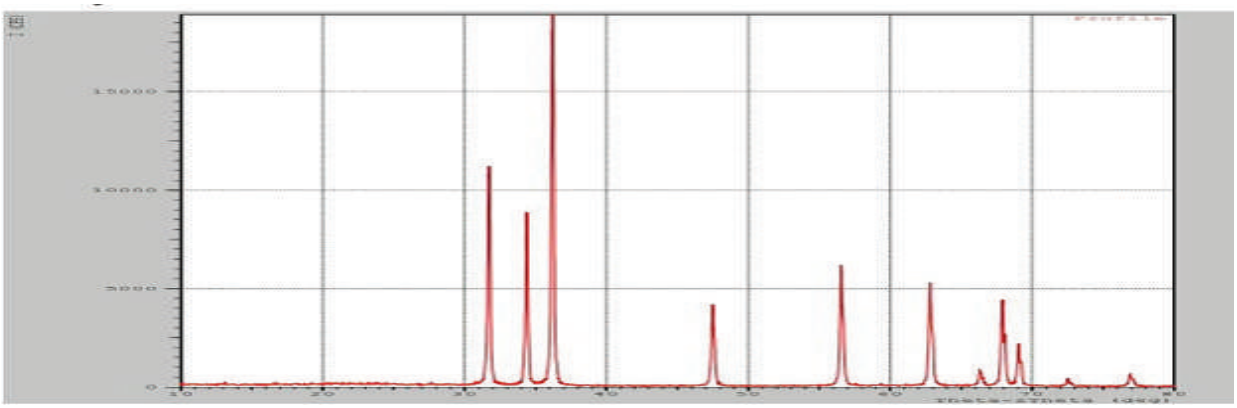


Figure 3: XRD analysis of ZnO nanoparticles synthesis by *Lactobacillus plantarum*

Field Emission - Scanning Electron Microscopy (FE-SEM) Analysis

The FE-SEM photographs of the ZnO NP biosynthesis by *Lactobacillus plantarum* were prepared by biological method and Calcinated at 500 Co for one hour was shown in figure 4

. Scanning electron microscope was used to decide size, location and shape of the Zinc oxide nanoparticles. These images demonstrated that zinc oxide nanoparticles are hexonal in shape and their sizes are about (56.14nm, 62.98nm , 64.97nm) indicating the diameters of NPs were accurate and appropriate as ZnONPs. [10] .

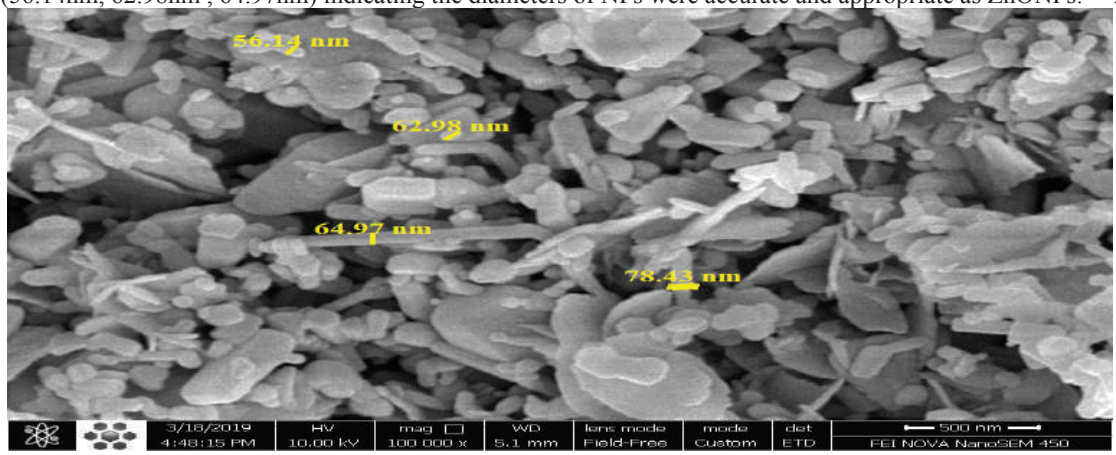


Figure 4: FE-SEM images of biological synthesis ZnO nanoparticles by *Lactobacillus plantarum*

Fourier Transform Infrared (FTIR)

FTIR spectra is used to access the details of functional groups involved in the biosynthesis of ZnONPs . The obtained FTIR spectra of ZnO NPs exhibited prominent peaks at 3460 , 1718 , 1627 , 1047 and 584 cm . The broad vibrational band observed at 3460 cm is attributed to the symmetric stretching mode of water molecules . the band observed at 1718 , 1627 and 1047cm is assigned to the bending vibrational mode of water molecules. Figure 5 reveals the peak observed at 584-441 cm corre-sponds to the stretching vibrations of ZnO NPs .

The well resolved intense and broad transmission band below ~590 cm−1 was attributed to the stretching vibration of zinc-oxygen bond (18,19). A slight peak shift observed at metal doping in the present study (between 584 and 441 cm−1) may be attributed to the change in particle size as a function of metal doping.

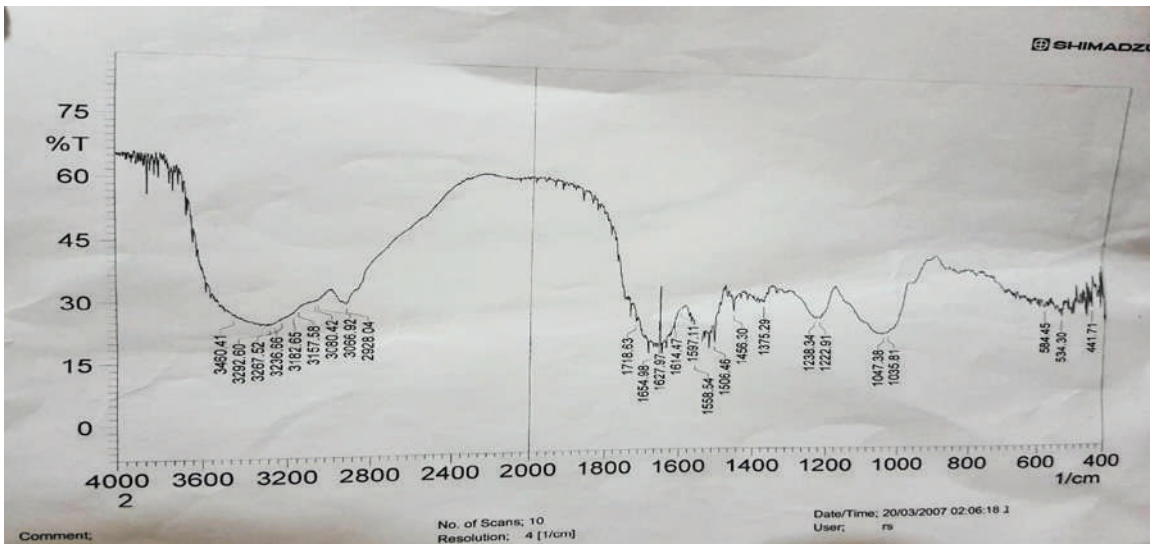


Figure 5 : FTIR spectra of biosynthesis of Zno nanoparticles by *Lactobacillus plantarum*

UV -Vis Diffuse Reflectance

The samples, separation of biomass eliminated the interference in reading UV/Vis absorbance (420 nm) of colloidal ZnO nanoparticles. It was assumed that sample by centrifugation could be useful. However the absorbance (420 nm) of colloidal suspension of nanoparticles decreased greatly to near zero, and the color (brown to yellow) disappeared. It might be due to the aggregation of nanoparticles or their entrapment in the biomass. Therefore,

centrifuging the samples showed negative effects on nanoparticles and should be avoided. their absorbance was read at 420 nm, and corrected for the absorbance before the start of the reaction.

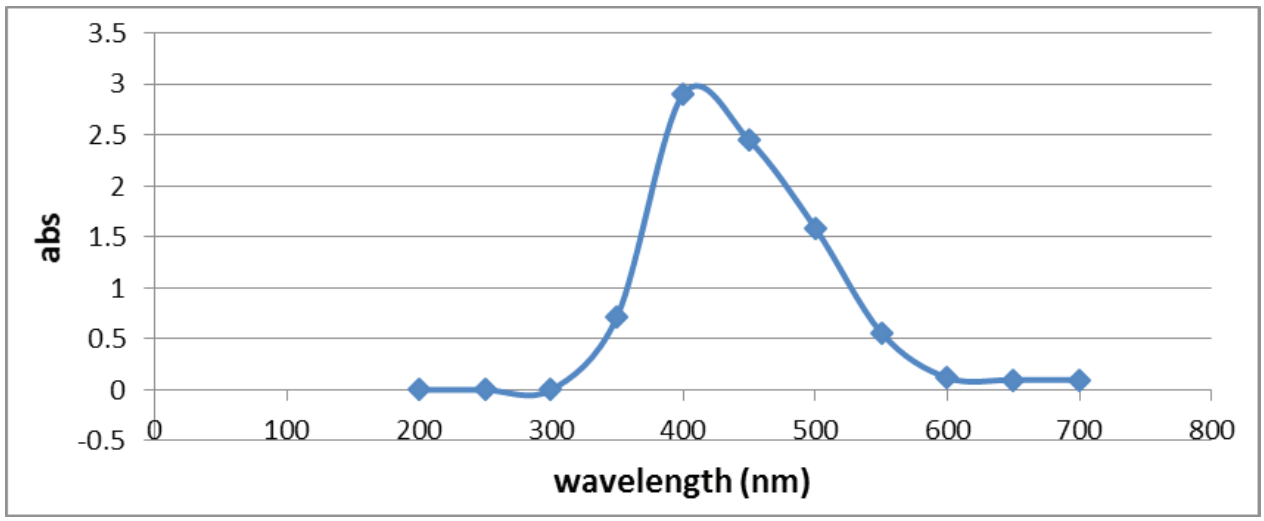


Figure 6 : UV- Vis spectrum of ZnO NPs synthesized by *Lactobacillus plantarum*.

Antibacterial activity of Zn-ONPs against Pathogenic Bacteria.

The antibacterial activity of biosynthesis ZnONPs examined against four pathogenic bacteria as shown in table 1.

Table (1): Pathogenic bacterial isolates

Bacteria Isolated	Gram stain	Type of sample	Source Obtained
Escherichia coli	Negative	Urine	Kufa university – college of science
Staphylococcus aureus	positive	Blood	Babylon University – College of Medicine
Klebsiella Pneumoniae	Negative	Swap (sputum)	Al-Hilla teaching hospital
Pseudomonas aeruginosa	negative	Swap (sputum)	Hillah public health Lab

The antibacterial activity of ZnO nanoparticles was tested by the disc diffusion agar methods table 2 show These results indicate that Zinc Oxide nanopaticles had antibacterial effects on four pathogenic bacteria include *(Escherichia .coli , Staphylococcus aureus , Klebsiella pneumoniae , pseudomomas aeruginosa*) , the antibacterial concentrations were inconsistent. Results of(20) . indicated that 0.625 mg/ml zinc oxide nanoparticles have low inhibit the growth of all bacteria and 2.5mg/ml higher concentrations completely inhibited the growth all these bacteria . concentrations may be that the different microbes and Zinc Oxide or its nanoparticles were used. Also these result showe Pseudomonas aeruginosa have inhibition efficency in 2.5 mg more than other bacteria while klebsiella pneumonia have low inhibition zone at same concentration Interestingly .

Table (2): Shows the diameter of inhibition zone of ZnONPs for some pathogenic bacteria.

	ZnONPs Con. (mg/ml)	ZnONPs(mm) E.coli	ZnONPs(mm) Staph .aureus	ZnONPs(mm) Klebsiella Pneumonia	ZnONPs(mm) Pseudomonas aeruginos
1	2.5	20	19	18	22
2	1.25	18	16	15	18
3	0.625	14	12	12	16

On the other hand, the ZnO NPs were exhibited antibacterial activity against MRSA (21) so, the result obtained agree with these findings. The proposed two imaginable mechanisms for the antibacterial action of zinc oxide nanoparticles to bacteria are first, creation of augmented levels of ROS mostly hydroxyl radical and singlet oxygen which destruction the bacterial cell wall. Second nanoparticles deposition on bacterial surface or nanoparticles aggregation either in the periplasm region or in the cytoplasm initiating distraction of cellular function and membranes disorganization. Also the result showed the same result found by (22) which showed that ZnO nanoparticles had antibacterial activity against bacteria .

Conclusion

Symbiotic microorganisms can use as safe source of nanoparticles. The microbial synthesis of nanoparticles is advantageous more other chemical and physical methods. Nanoparticles applications in medicine and sensors are envisaged .ZnONPS biosynthetic by *Lactobacillus Plantarum* using these it as antibiotics give us wonderful results, low complications, very low cost and low resistance in comparison with ordinary antibiotics. The results represent a great potential benefit for a wide numbers of medical applications in the battle against antibiotic- resistant bacterial pathogens .

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Mustaqbal University College and all experiments were carried out in accordance with approved guidelines.

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Fibroblast Growth Factor 21 Association with Renal Function Decline in Type II Diabetes Mellitus

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Abstract

Serum FGF21 level is strongly associated with the onset of nephropathy in T2D patients and is an independent predictor of loss of renal function in these patient.The study aimed to evaluate the role of FGF21 in early detection of diabetic nephropathy in type 2 diabetes patents. The study included Eighty-eight(88) samples divided in to four groups, three of which included type 2 diabetes patients, according to mircal test (urine albumin / creatinine ratio), they were classified as :22normalbuminuria , 22 microalbuminuria, and 22 macroalbuminuria. The fourth group included 22 apparently healthy subjects with (matched gender and age).

The mean ±SD of serum fibroblast growth factor 21 of normoalbuminuria group was (225.00±38.62), microalbuminuria group was (267.78±51.08) and macroalbuminuria group was (230.84±50.33) .The comparison results indicated significant increase ($p<0.05$) of FGF21 in microalbuminuria group in comparison to that level in normoalbuminurea and macroalbuminuria groups. Also highly significant increase of FGF21 was observed in microalbuminuria group in comparison to that level in control group. We conclude that FGF21 may be a useful marker for early detection of nephropathy.

Keyword: *Fibroblast growth factor 21; diabetic nephropathy; microalbuminuria; glomerular filtration rate; Insulin resistant ; Type II diabetes Mellitus.*

Introduction

The most common and serious complication of DM is diabetic nephropathy (DN), DN has become the leading cause of chronic kidney failure, starting with normoalbuminuria, microalbuminuria, macroalbuminuria and ultimately leading to end stage renal disease (ESRD),longer duration of diabetes is more prone to developing nephropathy Elevated blood pressure is another important independent risk factor for nephropathy [1] Dyslipidemia , Obesity [2] and Both oxidative stress [3] and subclinical inflammation appear to contribute to the pathogenesis of diabetic nephropathy [4] Fibroblast growth factor 21 (FGF21) which is a peptide hormone that belong to Fibroblast growth factor (FGF family) with 210 amino acids (about 21-kD).. It is produced preferentially in the liver. [5] and synthesized by several organs . There are many sites of FGF21 synthesis ,white adipose tissue, brown adipose tissue, pancreas, skeletal muscle, and cardiac endothelial cells, but the liver is the most important one(Davis D, et al 2017) .FGF21 has been identified as an endocrine and metabolic hormone because of its potent effect on lipid and glucose metabolism and on insulin sensitivity and energy balance [6]. It was found in type 2diabetes and obese patients, The abnormal rise in circulating FGF21 levels in T2D patients is considered to be a protective, compensatory response [7] . Also it was positively associated with obesity, fasting insulin, and triglycerides, and negatively with high density lipoprotein (HDL) [8] .The mechanism of FGF21 resistance in patients with metabolic disorders including type 2 diabetes may be multifactorial.FGF21 may play an important role in the DN development and progression. As a small polypeptide, FGF21 is likely filtered and excreted in the urine, as an important route of FGF21 clearance ,as the glomerular filtration rate (GFR) declines, the circulating FGF21 level increases progressively from

the early to end stages of chronic kidney disease (CKD) and is associated with the renal function [9]. Its levels are also independently associated with urinary albumin excretion in Chinese patients with type 2 diabetes with macroalbuminuria or microalbuminuria(10)In diabetic nephropathy, serum FGF21 levels correlate with the severity of albuminuria and faster loss of glomerular filtrate rate and can potentially be a biomarker of poor prognostic. [11] administration of therapy rFGF21Are markedly decreased albuminuria, meningeal cell expansion, profibrotic cytokines, and improved kidney lipid metabolism and oxidative stress injury [12].

This study aimed to evaluate the role of FGF21 in early detection of diabetic nephropathy in type 2 diabetes patients.

Methods

This case-control study was conducted at national diabetes center for treatment and research (Mustansiriyah University) .Eighty-eight(88) samples included in this study and divided into four groups, three of which included type 2 diabetes patients, and they were divided, according to mircal test (urine albumin / creatinine ratio), into three groups classified as : 22normalbuminuria , 22 microalbuminuria, and 22 macroalbuminuria. The fourth group included 22 apparently healthy subjects with (matched gender and age) healthy group in term of non-diabetic, Patients with type 1diabets, Gestational diabetes, Cardiovascular and other complication, Patients with chronic disease , Patients on insulin therapy and Multiple myeloma, lymphoma, and leukemia.

Serum Fibroblast growth factor 21 was determined by an enzyme immunoassay for quantitative measurement in vitro using kit manufactured by Mybiosource (ELIZAKit),insulin by Demeditec (ELIZA KIT) renal profile and blood sugar and HbA1c were done by Roche/ hitachi cobas c311 device.

Results

The mean ±SD of serum fibroblast growth factor 21 of normoalbuminuria group was (225.00±38.62), microalbuminuria group was (267.78±51.08) and macroalbuminuria group (230.84±50.33) .The comparison results indicated significant increase ($p<0.05$) of FGF21 in microalbuminuria group in comparison to that level in normoalbuminurea and macroalbuminuria groups. Also highly significant increase of FGF21 was observed in microalbuminuria group in comparison to that level in control group. The profile of blood sugar for studied groups indicated highly significant differences ($p<0.01$) of FBG, Insulin, HbA1C, HOMA-IR, and HOMA-- β were observed between all studied groups as shown in table (1). Meanwhile the renal function tests for studied groups revealed Highly significant differences ($p<0.01$) of serum urea, creatinine, GFR, and Albumine/ Creatinine ration in urine between all studied groups as shown in table 1. Also The results of lipid profile showed non-significant ($p>0.05$) differences of cholesterol, triglyceride, LDL and VLDL between studied groups. While a significant deference of HDL was observed .

Table 1. Mean value of FBG, INS, HbA1C, HOMA-IR, HOMA—β, renal function tests and lipid profile in the four studied groups.

Parameter	Control group (n=22)	Type 2 Diabetes with normoalbuminuria group (n=22)	Type 2 Diabetes with Microalbuminuria group (n=22)	Type 2 Diabetes with Macroalbuminuria group (n=22)	P
FBG (mg/dl)	89.72±9.49	166.00±73.05	192.80±78.10	213.59±80.7	0.00
INS (U/mL)	13.27±2.83	9.70±3.47	12.55±3.31	16.28±5.98	0.02
HbA1c (%)	5.57±0.36	8.68±1.76	8.79±1.47	9.34±2.00	0.00

Cont... **Table 1. Mean value of FBG, INS, HbA1C, HOMA-IR, HOMA—β, renal function tests and lipid profile in the four studied groups.**

HOMA-IR	53.31±18.87	74.27±27.48	118.57±29.09	187.99±82.1	0.00
HOMA-β (%)	2.69±0.92	1.46±0.34	1.57±0.49	1.62±0.66	0.00
B. urea (mg/dl)	25.11±5.66	26.69±6.91	29.82±9.82	43.10±5.31	0.00
S. creatinine (mg/dl)	0.60±0.11	0.72±0.21	0.87±0.25	1.60±0.39	0.00
GFR(mL/min/1.73 m2)	123.57±27.1	113.06±33.01	89.17±27.81	47.54±14.14	0.00
A/C Ratio (mg/ mmol)	1.46±0.44	2.05±1.14	10.82±5.49	34.00±9.34	0.00
TC (mg/dl)	188.81±35.9	164.13±47.49	153.90±43.19	168.63±36.8	0.06
TG (mg/dl)	164.19±53.3	174.56±57.61	164.76±55. 9	175.54±48.1	0.83
HDL-C (mg/dl)	48.87±21.43	34.50±9.53	35.11±10.68	43.31±22.89	0.02
VLDL-C (mg/dl)	32.81±10.04	34.85±12.54	32.99±11.27	35.10±11.43	0.90
LDL-C (mg/dl)	99.42±44.62	93.39±34.01	82.78±23.52	99.86±36.10	0.38

A/C Ratio= Albumine: Creatinine Ratio

As shown in Table 2 , the person correlation analysis of fibroblast growth factor 21 results showed a positive correlation with HOMA-β and urea ,while it was observed highly significant ($p<0.01$) negative correlation with (A/C Ratio) .

Fibroblast growth factor 21of microalbuminuria group showed a highly significant ($p<0.01$) negative correlation with creatinine while it showed a highly significant ($p<0.01$) positive correlation with (HOMA-IR, urea and GFR).

The results of fibroblast growth factor 21of macroalbuminuria group showed a highly significant ($p<0.01$) positive correlation with (urea and creatinine, Meanwhile indicate a highly significant ($p<0.01$) negative correlation with (GFR).

Table 2: The person correlation analysis of FGF-21 with studied variables in the patients groups.

Variable	**Patients groups**					
	Type 2 Diabetes with normoalbuminuria group		**Type 2 Diabetes with Microalbuminuria group**		**Type 2 Diabetes with Macroalbuminuria group**	
	r	**P**	**r**	**P**	**r**	**P**
Age (years)	-0.13	0.53	-0.34	0.13	-0.13	0.53

Cont... **Table 2: The person correlation analysis of FGF-21 with studied variables in the patients groups.**

BMI (Kg/m2)	0.21	0.33	0.30	0.17	0.32	0.13
FBG (mg/dl)	-0.24	0.26	-0.00	0.97	0.04	0.86
FPI (U/mL)	0.15	0.47	-0.21	0.35	-0.09	0.68
HbA1c (%)	-0.40	0.08	0.12	0.60	0.10	0.65
HOMA-IR	-0.11	0.63	0.71	0.00	0.05	0.82
HOMA-β (%)	0.46	0.03	0.23	0.31	0.10	0.64
B. urea (mg/dl)	0.22	0.00	0.24	0.00	0.35	0.00
S. creatinine (mg/dl)	0.00	0.58	0.44	0.00	0.27	0.00
GFR (mL/min/1.73 m^2)	-0.01	0.43	-0.48	0.00	-0.22	0.00
A/C Ratio (mg/mmol)	0.56	0.00	-0.08	0.72	0.10	0.64
TC (mg/dl)	-0.03	0.87	-0.07	0.75	0.28	0.19
TG (mg/dl)	-0.16	0.44	-0.21	0.35	-0.14	0.51
VLDL-C (mg/dl)	-0.04	0.82	0.25	0.26	-0.27	0.21
HDL-C (mg/dl)	-0.07	0.74	-0.25	0.28	0.08	0.70
LDL-C (mg/dl)	0.27	0.19	-0.12	0.57	0.10	0.63

Discussion

Diabetic nephropathy is a serious kidney-related complication of type 2 diabetes. The classification of nephropathy is based on albuminuria and eGFR level. However new biomarkers in urine or blood that could improve diagnostic and prognostic in early or later stages of diabetic kidney disease has been established during the past decades [13]. One of these marker was serum FGF21 levels which is used to evaluate among different group with T2D patients who were at multiple grade of albuminuria according to urinary albumin/ creatinine ratio. According to the present study results of the diabetes examination, it was found that the cause of diabetes is insulin resistance rather than insulin deficiency. The systemic disorder insulin resistance affects many organs and insulin-regulated pathways. The disorder is characterized by a reduced action of insulin despite increased insulin concentrations (hyperinsulinaemia).in diabetic kidney disease. High-glucose concentrations induce specific cellular effects, which in the kidney affect many types of cell causing abnormalities of the glomerulus and preglomerular vessels and increased amounts of proteins, growth factors, and advanced glycation end products (AGEs) an Albuminuria and proteinuria indicate relevant tissue damage in the diabetic kidney .this was agreement with mandal , & Hiebert, L study which showed that FBG relates significantly to renal function change by measured serum creatinine levels or estimated glomerular filtration rate ,and FBG can be used as an important marker to predict renal function changes in diabetes [14]. Also Sheuly F,et al reported that the level of glycemic control seems to be the strongest factor determining conversion from normoalbuminuria to microalbuminuria in patients with type 2 diabetes mellitus [15]. Meanwile study by Huang,et al and Muhammad Bilal Habib1& Noreen Sher Akbar reported a significant association between HbA1c levels and risks of albuminuria .HbA1c that can be a possible

threat of renal failure in patients with type II diabetes [16,17]. In the current study the result of Homeostatic model assessment for insulin resistance demonstrated a highly significant increase ($p<0.01$) in sera of Microalbuminuria and Macroalbuminuria in comparison to that level of control group which is similar to other study were Min-Tser Liao,et al show IR promotes kidney disease and plays important role in declining renal function [18] ,also Huang,et al indicated incidence of microalbuminuria generally increased with HOMA-IR. Moreover (HOMA-IR) and beta cell function (HOMA-β) has a relation with incidence of type 2 diabetes and diabetic kidney disease [19]. Similar finding of Kim GS,et al and Wang,et al studies were recorded [20,21]. Also study of Zhou,et al was agreement with the current study which indicated that abnormal pancreatic beta-cell function plays an important role in the development of microalbuminuria [22]. Our finding in renal function tests for studied groups revealed highly significant differences ($p<0.01$) of serum urea, creatinine, GFR, and Albumine/ Creatinine ration in urine were observed between all studied groups. which agreement with Yassin MM,et al and Campion,et al whos reported that urea, creatinine ,and albumin creatinine ratio (ACR) ,decline in GFR were increased in diabetic groups compared to non-diabetics, and reaching their maximum increase in macroalbuminuria, and a decline in glomerular filteration rate increase from to microalbuminuria to macroalbuminuria [23,24]. Diabetic nephropathy causes quantitative as well as compositional abnormalities in lipids. The lipid profile show a statistically non-significant ($p>0.05$) differences of cholesterol, triglyceride, LDL and VLDL between studied groups. While a significant deference of HDL was observed. According to the pervious study of Palazhy, S., & Viswanathan, V. which indicate that dyslipidemia was highly prevalent among diabetic nephropathy patients [25]. Meanwhile Aziz KMA. indicate a significant association of triglycerides and TG/ HDL ratio with elevated levels of serum creatinine, and microalbuminuria. [26], Demihova, et al study indicated that the degree of albuminuria was positively correlated with (Tg), (TC), (LDL), (VLDL) and inversely with HDL [27]. Our finding was agreement with previous study that Serum FGF21 levels correlate positively with creatinine, blood-urea-nitrogen (BUN) and cystatin C and inversely with the GFR and residual renal function . Kohara M, et al. who reported that FGF21 levels were higher in 200 CKD patients (146 pre-dialysis and 54 hemodialysis patients) than in 40 controls [28]

Conclusion

In this study there was a positive correlation of FGF21 with urea and creatinine diabetic nephropathy patient, which may be a useful marker for early detection of nephropathy

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Chemistry and Biochemistry and all experiments were carried out in accordance with approved guidelines.

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Morphological and Histological Study on Thyroid Gland Toxicity in Male Rats Exposed to Mercury

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Abstract

The present study investigated the effect of heavy metals (Mercury chloride) on the morphological and histological feature of the thyroid gland. Rattus norvegicus male rats were selected for experimental use. the rats given doses of Hg salts (3mg/kg, Ip.3times weekly for 4 week) $HgcL_2$ admin is tration altered the body weight, thyroid weight, length and width of thyroid gland. Morphologically, the thyroid gland was consisted of two labs of reddish brown in color, the lobes were located on ventral Lateral side of trachea connected caudally with isthmus. While the thyroid gland treated rats recorded changes in color (pale), swollen and enlarged. histologically, the architectural cellular structure gland of the thyroid gland of exposed rats recorded variations in the follicular thyroid, congested blood vessels and depletion of para follicular cells in the rats treated to Hg in a comparison to that of the control rats. This signify that animals exposed to Hg could at a risk of thyroid gland.

Keywords: *thyroid gland, mercury chloride, male rats.*

Introduction

The toxicity of Heavy metal is bearing a worldwide problem as they intervene in the detoxification pathway [1]. Mercury contamination of the environment continues to be of concern and a major source of this contamination is from human activities Such as mining which smells extensive industrial and agricultural use of fossil fuel combustion and other industrial waste which liberation mercury into the environment [2] . Mercury has adverse effects on systems that differ with doses, duration of exposure, length of exposure and type of mercury [3]. The Mercury toxicity mainly affects the nervous system, but depending on the particular form of mercury and the degree of exposure, it is also extremely important for the kidneys, liver, lungs and thyroids [4,5]. the thyroid gland is a the only endocrine gland being largest, superficially located, It is caudal to the trachea at the level of the first or second tracheal ring [6]. It take the shape of a butter fly and it is composed of two lobes, one on each side of the trachea , and connected by a narrow piece of tissue called the isthmus [7]. Thyroid gland consists of numerous follicles of various size which from the Thyroid Gland functional and histological structure .It consists of three main components; follicular lining,, para follicular cells or C cells could be seen through follicular epithelium or as group outside of follicles with the luminal colloid, and the follicular cells vary in height, depending on the state of activity of the follicle . the lining epithelium changes from squamous or low cuboidal in the resting state and cuboidal or columnar in the active stat [8,9]. The thyroid follicles produce thyroid hormones (triiodothyronine, and tetraiodothyronine, which playing an important role for reproduction; differentiation [10]. The C cells are secreted mainly calcitonin hormone which regulate the calcium level in the blood [11]. the aims of this study investigate the changes of thyroid structure in mercuric chloride.

Materials and Methods

- Mercuric chloride ($HgcL_2$) was obtained from Merek India LTD. and dissolved in distilled water and administered three times intraperitoneal injection at dose of 3 mg / kg body wt. for 4 weeks. In current study, it was found that LD50 of Hgcl2 was 40mg/kg body. wt. (12). The experimental protocol was approved by the College of Veterinary Medicine in Basra University / Animal House. twenty male rats (Rattus norvegicus) aged between 8- 10 weeks, weighting between (150_160) gm were used in this experimental. they were located in

an air – conditioned, stainless steel cage. Animals were fed the rat with standard pellets and provided with clean water. the animals were separated in to 2 groups (N=10); Group(A) has given i.p injection with 1 ml normal saline as a control group. Group, (B) given I.P injection of ($HgcL_2$) (3 mg/kg. wt /3/ week for 4 weeks.

- **Morphological study:** when the experimental ends, the body weight of male rats were measured by digitalis balance. Rat were anaesthetized using chloroform in a closed container. The rats were humanly sacrificed and an incision was made in Its cervical region, the thyroid glands Were dissected. the glands were located on the lateral aspect of the trachea. photograph of glands was taken before glands were dissected. the weight of glands was determined using the digital weighing balance while the lengths and widths were determined using digital Vernier Calipers.

Histological study

Tissue samples were fixed in 10% formalin solution for 24 hrs. After fixed tissues had been dehydrated progressive increased ethanol concentration and cleared in xylene, impregnated in liquid paraffin wax and embedded in paraffin blocks then cutting by rotary microtome, later the section was stained with hematoxylin and eosin. the histological section of this study was examined by using light microscope type (Olympus / japan).

Statistical Analysis

The data from this analysis were analyzed using the ANOVA test in all studies. Both statistical calculations were made using the Statistical Program SPSS V.17 (SPSS Inc). The data was expressed as mean + standard (X - +SD) deviation.

Results

The table (1) showed that a significant decrease ($p \leq 0.05$) in body weight of the $HgcL_2$ group compared with the control group. The mean weight increased ($p \leq 0.05$) in the thyroid gland of $HgcL_2$ group compared with the control group. Also, the length and width of the gland are significant different in treated group Hgcl2 ($p \leq 0.05$) as compared with control group during experimental periods. Morphological result of the thyroid gland of control group showed that was consisted of two lobes of Reddish brown in color located on ventrolateral side of trachea connected caudally with isthmus which were oval in shape with smooth surface (Fig 1, a,b) While the thyroid gland in ($HgcL_2$) group was pale, swoollen and it include grey area with enlarged (Fig 2)

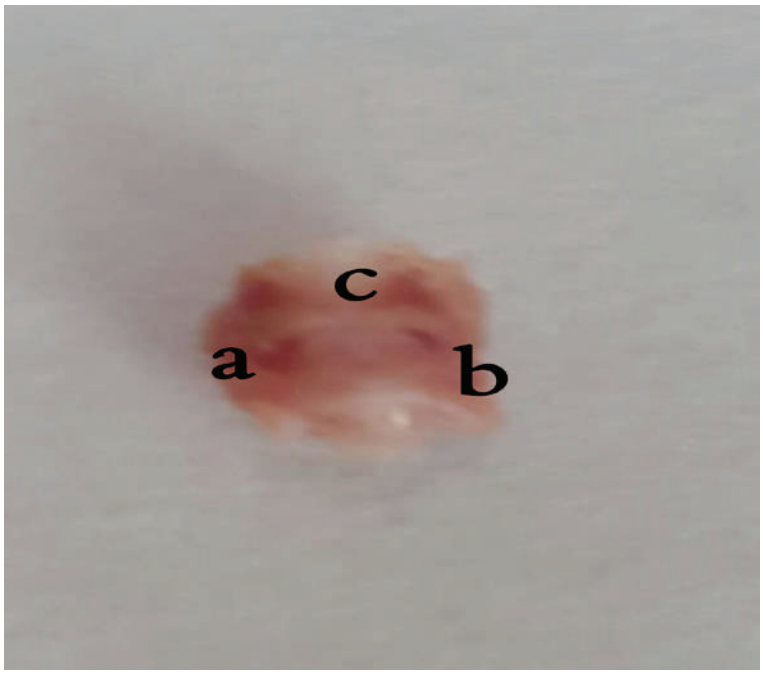
Histological results.

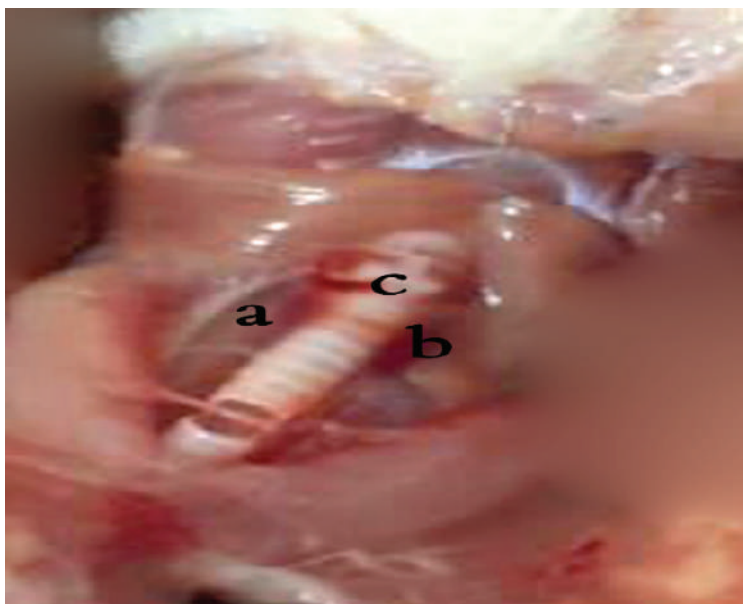
The histological results of control group were showed that a thin capsule of dense irregular connective tissue enclosed the thyroid gland, from the capsule thin trabeculae contained blood vessels extended into the parenchyma of the gland and di

Separated it into small lobules (Fig3). A lobule was consisted of an aggregation of a various sizes of thyroid follicles were appeared with variable size and shapes which lining with epithelial cells ranged from low columnar cuboidal to squamous cells another follicles layer completely filled with colloid. In addition , showed parafollicular cells (C-cells) could be seen concentrated in the center of the lobe, as group outside of follicles and they are larger than follicular cells and these cells formed clumps with rounded nuclei and eosinophilic cytoplasm (Fig 4).While the gland in the exposed group for $HgcL_2$ showed multiple histological changes in the thyroid gland of rats , which includes disintegration and disorg anization of thyroid follicles . some follicles occurred with interrupted follicular wall. Congested blood vessels and increased with stroma connective tissue with infiltration of inflammatory cells. Also seen depletion of para follicular cell (fig 5,6).

Table (1) Estimations of body weight (g), thyroid gland weight (g), length (Cm) and width (Cm) of thyroid gland in experiment al groups.

Groups	Bodyweight(g)	Thyroid weight(g)	Length (Cm)	Width (Cm)
Control Group	152.22 ± 3.666	1.272 ± 0.046	2.250 ± 0.048	0.640-+0.031
($HgcL_2$) Group	136.4 ± 3.801	1.622 ± 0.033	2.318 ± 0.018	0.818 ± 0.025





(v) (b)

Figure.1- (a, b) shows the anatomical location of thyroid gland in control rats: a-right lobe

b-left lobe, c-isthmus.



Figure.2 show the anatomical location of thyroid gland in treated group with ($HgcL_2$)pale with swollen and enlarged.

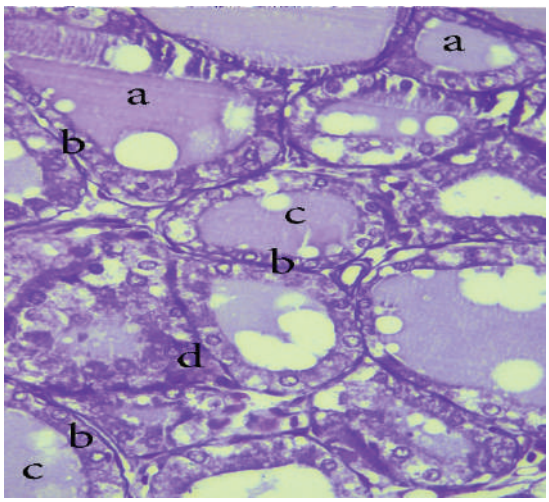


Figure.3. histological section of thyroid gland in control rats shows. a-capsule, b-trabeculae, c-blood vessels, d-lobule , H&E stain. 10.

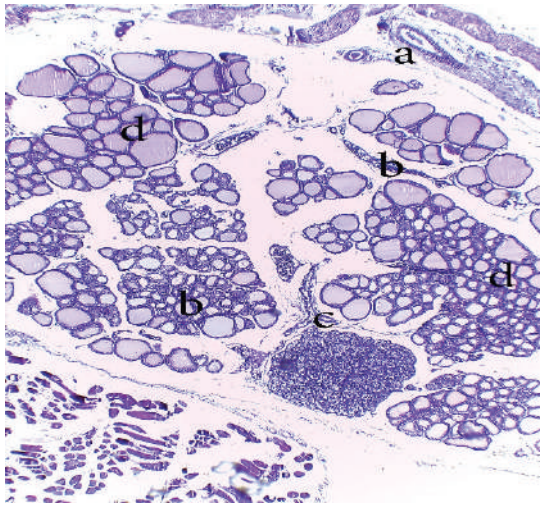
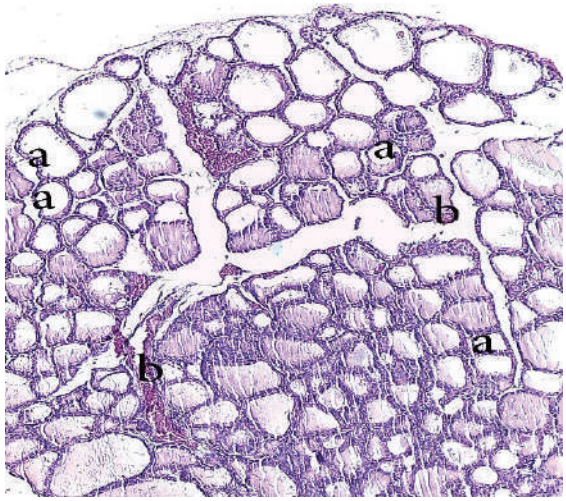


Figure.4. Histological section of thyroid gland in control rats shows a various size of thyroid follicles, b- lining epithelial, c- colloid, d-parafollicular cell, H&E stain. 40X



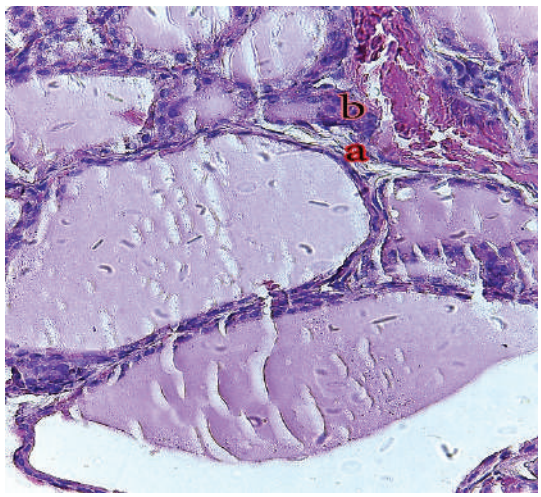


Figure.5. thyroid gland section of rat treated with ($HgcL_2$)showing a: marked disintegration and some connective tissue septal b- congested blood vessels, H&E stain. 40X

Figure.6. thyroid gland section of rat treated with ($HgcL_2$) showing a: increased amount of collagen interrupted follicular wall, between the follicles H&E stain.10X b- degeneration of parafollicular, H&E stain. 40X

Discussion

It is obvious from the results that the effect of $HgcL_2$on bady weight loss may be resulting from being interrupted of mercury in the absorption and metabolism of feed nutrients essential for heath [13] heavy metal is capable of damaging the organism in many ways because of high affinity to various tissues and its tendency to accumulates [14] the result of the present study shows the effect of mercury on the weight of gland was increasing in the weigh, length and wild comparison with control group (table- 1), these results agree with many other previous studies [15] .In the current work , alteration morphological of thyroid gland in the treated group were further certain by the histological examination of the thyroid tissues which showed Obviously light microscopic , this histological altered of thyroid gland would be illustrated by deficiency of stimulatory effects of thyroid stimulating hormone (TSH), when mercury interacts in synthesis and or secretion of TSH by the pituitary gland or TRH by the hypo thalamus gland, thyroid Producing hormone (TRH) is accountable for regulation the secretion of TSH from the pituitary gland , this result agreed with result that was obtained previously [16-18] In conclusion thy thyroid gland is responsive to $HgcL_2$ exposure. This periphrasis induced morphological and histological changes.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Veterinary Medicine and all experiments were carried out in accordance with approved guidelines.

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The Effect of Skilful Visual Exercises in the Development of Motor Perception and Preparation Skill in Volleyball

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Abstract

Identify the effect of using the aid in developing the perception of the motor sense and the skill of preparing in volleyball. As for the research assumptions, the exercises (visual-skill) using auxiliary means affected the development of sensory-motor perception and the exercises (visual-skill) with the auxiliary means affected the development of the most important basic skills in volleyball. As for the practical part, the researcher used the experimental method to suit the research problem, and the research sample was female students of the College of Physical Education and Sports Sciences / College of Education for Girls / University of Kufa in Volleyball and their number (24) players for the year 2019-2020 AD and they were randomly selected, and three students were excluded In order to conduct the exploratory experiments, homogeneity and parity were conducted for them, and then the researcher distributed forms for the perception of movement and the skill of preparing for volleyball and the suitability for conducting the research and presented it to the volleyball experts and then conducting the pre-tests and after applying visual skills exercises to the research sample within the main section of the training curriculum for a period of (6) 3 weeks, three units per week, to be (18) training units, then the researcher conducted the post-tests under the same conditions in which the pre-tests were conducted, in addition to using the appropriate statistical means to classify the data obtained. The researcher presented the results, analyzed and discussed them after being statistically processed, and then presented the data in tables and graphs, as the results showed statistically significant differences between the pre and post-test of the research sample and this is what fulfils the research hypothesis. Among the most important conclusions: The researched visual skills exercises have a positive effect by using innovative methods (educational - training) in developing the perception of a kinesthetic sense of volleyball for the research sample.

Key words: *visual-skill exercises, motor perception, volleyball*

Introduction

The importance of this study emerges through the exercises used and exciting for sensory-kinesthetic perception by using the manufactured and varied method to develop sensory perception and preparation skill with volleyball with the ball. The research problem was: so that the learning process takes place on a sound scientific basis and through the experience of the field researcher in The field of volleyball and being a former player and current trainer in the Ministry of Youth and Sports decided to use an innovative manufactured method that aims to shorten the time and accelerate the process of training, excitement and excitement in developing the skill of numbers and their optimal performance, and due to the weakness of female students in this skill and the lack of use of assistive devices,(1) specially manufactured To restrict and narrow the training process, hence the problem of researching the preparation of exercises that contain visual-skill stimuli using an innovative aid in the development of sensory-kinesthetic volleyball. The objectives of the research were: to manufacture an educational aid to develop sensory-motor perception and preparation skill in volleyball, to prepare (visual-skill) exercises to develop sensory-motor perception and to develop volleyball preparation skills. As for the research hypotheses: Exercises (visual-skill) with the aid affected the development of sensory-motor perception. And exercises (visual-skill) with the aid affect developing volleyball preparation skills. The scientific

part includes the research method: The nature of the problem determines the method used, so the researcher used the experimental method by designing (the two equivalent groups) with pre and post-tests, which is "the closest and most sincere to solve many scientific problems practically and theoretically, and the research community and its sample were defined: The research community represented by the female students of the Department of Physical Education and Sports Sciences in the College of Education for Girls / University of Kufa / The female students of the college's selection for the academic year 2019-2020 AD were selected by a simple random method, as the research sample included 24 female students, including the experimental group (12) female students and the control group (12) Student.(2)

Tests used in research:

First: The educational method for developing skilful visual perception:

A half-hexagonal shape was made of compressed panels with an appropriate rebound to the ball, similar to a wall. This half-hexagonal shape has obtuse angles measuring about (120) º, and these wooden panels have dimensions (1.5 meters x 1.5 meters) the size of the front facing plate For the student, as for the sides, the size of the wooden panels is (1 x 1.5 meters), and these wooden panels are supported from the back with an iron support and the wooden panels are fixed on it and also connected with a movable base with wheels that can be closed and moved, and the height of the wooden panels from the ground is 1 meter in addition To that, each plate contains light and each plate has a specific light that differs from the light of the other panel, so when the student begins to scroll, whether it is from the bottom or top with different sides, we turn on the electrical circuit, which is an electrical circuit connected to an operating switch via the Remont, and any of the lights are in a random manner and the time in which the light remains on can be controlled and the time can be reduced and it can be increased according to the performance of the skill student. It is considered as the development of your visual management and focus on the direction of performance, whether it is in front of me or to one side and also as a feed A complimentary review of skill performance, and this method works to develop skills and visual performance simultaneously. (3)

Second: Tests for sensory-motor perception:

1- The feeling of jumping distance:

One of the most famous examples of these tests is the Scott test, as it aims to measure the ability to sense the distance of the jump forward, as the player's achievement of the required distance indicates the sense of movement and this test is performed without using the sense of sight.

This test is valid for both sexes from the age of ten years until the end of university education.

Tools: eye patch, chalk, tape measure.

Performance specifications: Draw two parallel lines on the ground so that the distance between them is (58.8 cm) and the tester stands on the starting line, looks at the distance between the two lines for a period of (5 seconds), then blindfolds his eyes, and jumps from the starting line to the front so that his heels touch the ground at the finish line.

Conditions: The distance between the finish line and the end of the player's hurdles is recorded as errors of judgment, which indicate a lack of perceptual ability of distance. Two attempts are given to the tester, and the total score of the test player is the average of the two attempts. (4)

2- The feel of throwing the ball :

Two lines are drawn with a distance of 127 cm between them. The player stands before the starting line and throws the ball blindfolded after giving him (5) seconds to estimate the distance between him and the finish line (the one to be thrown at). The distance is recorded negatively or positively (before or after the line).

Third: Test the accuracy of the performance of the preparation skill in volleyball

The objective of the test: to measure the accuracy of the preparation skill.

Used tools

Basketball pillar, (5) legal volleyballs, a pre-prepared accuracy evaluation form.

Method of performance

The test student stands facing the basket at the free-throw line on the circular line, raises the ball to the top, then passes it to the basket, trying to pass it inside the basket, as shown in Figure (7), each student is given (5) attempts.

Scoring - the ball away from the basket is zero.

- The ball away from the basketball ring is (2) points.

- Touching the ball to the ring without hitting (3) points.

- Passing the ball inside the ring (5) points.

* The maximum score for the test is (25). [5]

Pre-test:

The pre-test for the research sample was conducted on Tuesday (12/2/2020) at exactly ten o'clock in the morning. All variables were adjusted in terms of time, tools and devices, as well as the auxiliary work team, to be applied when conducting the (post-test), that is, after implementing the training curriculum. Conducting the tests on the playground of the College of Physical Education and Sports Sciences / College of Education for Girls / University of Kufa, as it is suitable for conducting the tests.

Application of the training program:

After completing the application of the pre-tests, the researcher applied the training curriculum on Sunday 19/2/2020 AD with (3) training educational units per week, and the last educational unit was completed on Sunday 7/4/2020.

Post-test

After completing the implementation of the educational training curriculum, the post-test was conducted for the research sample on Tuesday 10/4/2020 AD at exactly ten in the morning, taking into account the temporal and spatial conditions and means of the post-test, with the help of the auxiliary work team.

As for the main section of the training program, it reached (1260) minutes, at a rate of (77.77%), which includes (the theoretical part and the practical part), while the total time of the final section of the educational unit was (360) minutes, at a rate of (11.11%) of the total time of the program.

Statistical means

The researcher used the Statistical Package for Social Sciences (SPSS)

Sample homogeneity:

After the sample was divided into two groups in a random way, and to avoid influences that may affect the research results of the individual differences that exist among the students, and to reach a single and equal level for the sample, some variables that represent the specification of the sample were determined to ensure their consistency in those variables that are influential. In the experiment, which must be controlled, and this is why a statistical treatment was carried out using the torsion coefficient, and tables (1) show this, noting that the torsion coefficient in these variables is limited to (± 1) and therefore the sample is normally distributed as "whenever the values of the torsion coefficient are confined between (± 1) the sample was homogeneous.

Table (1) shows the variables, the mean values, the standard deviation, the model, and the value of the torsion coefficient for the control and experimental groups

Groups		variables	mean	Vein	Std. deviation	skewness
Experimental and control	1	Age / year	20,645	20	1,853	0,357
	2	Length / cm	160,375	160	5,274	0,063
	3	Weight / kg	61,783	56	8,984	0,624

Equivalence of the two research groups:

The researcher should form equal groups at least about the variables that are related to the research, and for the researcher to refer the difference between the results of the research, if any, to the independent factors, the researcher resorted to checking the equivalence of the research groups, as it checks the equivalence between the two research groups using a test (C) And for the following variables: (perception, the kinesthetic, skill of numbers), and to verify the equivalence of the two research groups in the above variables. A (T) test was performed and Table (2) shows that.

Table (2): It shows the equivalence of the control and experimental groups in the tests

Variables	Control group		Experimental group		Computed T value	type indication
	mean	Std. deviation	mean	Std. deviation		
Preparation skill	3,583	0,792	3,916	0,514	1,221	significant
Perception, kinesthetic	4,833	6,264	4,666	6,184	0,065	significant

Presenting the results of the pre and post-tests for the tests of the control group for technical performance, accuracy, and sense-perception:

Table (3): It shows the arithmetic mean, standard deviations, and the calculated and tabular (T) value between the pre and post-tests of the control group

Variables	Pre-test		Post-test		Computed T value	type indication
	mean	Std. deviation	mean	Std. deviation		
The skill of preparing from the top	3,58	0,79	5,83	0,83	9	significant
Perception, kinesthetic	4,83	6,26	3,66	4,51	2,12	significant

Table)4): It shows the arithmetic mean, standard deviations, and two (T) values calculated and tabular between the pre and post-tests of the experimental group tests.

the test Variables	Pre-test		Post-test		Computed T value	type indication
	mean	Std. deviation	mean	Std. deviation		
The skill of preparing from the top	3.91	0,51	6,83	0,71	12.74	significant
Perception, kinesthetic	4.66	6,18	0.66	1.66	2.42	significant

Table (5): It shows the arithmetic mean, standard deviations, and the two calculated and tabular (T) values between the control and experimental groups for the post-post tests.

Variables	Control group		Experimental group		Computed T value	type indication
	mean	Std. deviation	mean	Std. deviation		
The skill of preparing from the top	5,83	0,83	6,83	0,71	3,14	significant
Perception, kinesthetic	3.66	, 4.51	0.66	1.66	2.15	significant

Discuss the Results

Table (3, 4, 5) shows that there are significant differences between the pre and post-tests in technical performance, accuracy, sensory-motor perception, and preparation skill from above {and in favour of the post-tests of the experimental group. [6] This fulfils the research hypotheses and the researcher attributes these differences to the visual skills exercises used by the researcher from Through the innovative aid to focus exercises on the sense of sight and skill, as it is one of the important senses in the process of learning and training, as Mahmoud Abdel Mohsen believes that "vision is a basis for raising the energy of sports players, and its role is no less than the role of muscles, bones, respiratory system ... etc. The sense of sight has an important role in education and training, by presenting models to the learner and trainee so that he has a picture of the movement and tries to reach it. [7]

As for the results of the control group, significant differences appeared in favour of the post-tests, and these differences as a result of repetitions and practice led to a relative improvement in performance and accuracy, but with much lesser proportions than the experimental group, and the fact that the subject teacher used the traditional curriculum led to these significant differences for the control group.

The researcher also believes that the visual skills exercises used helped in developing the students' sense-motor perception of the experimental group and determining the type of movements required to perform. Zaki Muhammad Hassan agrees in this, as he believes that "the sense of sight has an important role in the process of skill performance. Through it, the player can know his position about the opponent. And determining the type of moves that he can perform, and the player perceives through this sense the movements of the opposing team and thus can take the appropriate position, and that these visual skills exercises have a direct effect on the development of the field of technical performance and accuracy as well as the perception of movement in addition to increasing the ability of students to interpret information And (Iván González) emphasizes, "The player's possession of a wide field of vision is more able to interpret and perceive information and give the required movement response with high accuracy and quality." [8]

The state of suspense, excitement, and regular training are among the basic factors that enable the sample to excel and excel, and the high level of their skill achievement and sense of kinesthetic perception, which leads to the implementation of the motor duty with high accuracy and efficiency. [9]

Conclusion

The results showed that there are statistically significant differences between the pre and post-test of the research sample, and this is what fulfils the research hypotheses. Among the most important conclusions: The researched visual skill exercises have a positive effect by using innovative methods (educational - training) in developing the perception of a kinesthetic sense of

volleyball for the research sample.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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The Effect of a Proposed Training Curriculum Using an Electronic Learning Platform According to the Principle of Social Distancing to Improve Some Physical Fitness Elements for Female Futsal Players

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Abstract

The importance of research has emerged in the number of exercises that help in the development of special physical fitness to identify the capabilities of the physical athletes and to improve each of these physical characteristics, which are considered the basis for preparing the players to implement the skill and planning duty and achieve victory in the match, and the motivation to work and continue, especially in light of these conditions that all athletes live. That is, the Corona pandemic and quarantine procedures, in addition to that these exercises, depend on the combination of strength and speed, which is in line with the requirements of modern play. The researcher came up with several conclusions, the most important of which were: That the use of the proposed approach has a clear effect on improving some elements of physical characteristics in the experimental group in a standard period. Training exercises for simple aids have a great impact on some of the physical characteristics of futsal football players, with less time and effort.

Keywords: *Improve, physical fitness, elements, futsal players*

Introduction

The importance of the research lies in the number of exercises that help in developing the special physical fitness to identify the capabilities of the physical athletes and to improve each of these physical characteristics, which are considered as the basis for preparing the players to implement the skill and planning duty and to achieve victory in the match, and the motivation to work and continue, especially in light of these conditions that all athletes live (1). That is, the Corona pandemic and quarantine procedures, in addition to that these exercises depend on the combination of strength and speed, which is in line with the requirements of modern play, and the problem was focused by informing the researchers about the performance of the futsal players for the women's clubs in general and the Al-Fatah Women's Club in Najaf, especially because it was preceded by And if the University of Kufa team was represented in this game, they found that there is a general weakness in the physical fitness elements of the futsal players, especially some types of strength, (2) speed and speed of movement response in addition to agility, flexibility and balance and this is what affects the result of skill and planning performance, and because of the Corona pandemic and all patrols have stopped International and local clubs, training units for all clubs, and the principle of social distancing and quarantine measures imposed by Global health, which exacerbated this problem, so the two researchers designed a special training curriculum in developing fitness elements for female futsal players using the (ZOOM) program, an electronic platform to communicate with the players and apply the training curriculum vocabulary individually at home to develop and maintain the level of physical fitness For the female athletes, the research objectives were to measure some of the influencing special physical fitness elements of female gymnasts for the girl's club in the governorate of Najaf and to identify them, and to prepare special training exercises for a small area not exceeding (6)

meters, which cannot be applied inside the home hall or the garden to develop some elements of fitness The special physicality of female futsal players for the girls' club in the province of Najaf, and the use of an electronic platform according to the principle of social distancing to give physical exercises with simple aids that contribute to improving some of the physical fitness elements of the female futsal players of the girl club in the province of Najaf. (3)

practical part:

The two researchers used the experimental method as being the best for female futsal players for the Al-Fatah club in Najaf Al-Ashraf, who participated in the Iraq Championship (2019-2020), which numbered (15) players, in addition to (3) players who were chosen randomly to conduct the exploratory experiment. Thus, the number of players from both groups became The experimental and the control (12) players, with (6) players for each group, and then the two groups (control and experimental) were chosen randomly as well.

Determine the research variables and their tests:

The football game for halls is one of the sports that is characterized by many different positions, and this requires its practitioners a high level of special physical characteristics in defence and attack throughout the match, and that the level is determined by "revealing the methods and methods that help, clearly, accurately and scientifically to know Determining the capabilities required in the football game for halls and due to a large number of these variables, it has been limited to the main axes that can be trained inside homes under home quarantine procedures, as well as the principle of social distancing. (4)

Exploratory experience:

The exploratory experience is "practical training for the researcher to find out the negatives and positives that he encounters while conducting the tests to avoid them and to give a clear and accurate picture of the vocabulary of the tests used to serve the proposed approach. (5)

After determining the research sample and determining the physical tests, the researcher conducted the first exploratory experiment and on a sample of the girl's club players consisting of (three) players who were not from the main research sample, and they were chosen randomly and then they were excluded from the research sample, and with the help of the assistant work team, it was done. Experience (Friday) 5/8/2020)

Main experience: conducting measurements and pre-tests:

The measurements and pre-tests for the special physical characteristics searched for the individuals of the research sample were conducted on Friday 15/5/2020 AD, and the tests were conducted for the experimental group and the control group according to the following sequence:

In the beginning, height, weight, age and training age were measured, and then the special physical characteristics were tested, the (Nelson) test for the selective movement response, the vertical jump test of stability, the rapid running test (30) meters, the high start, the partridge test with both legs, a distance of (10) meters, and a jog test (20 meters). × 5) John Powell's test for compatibility and agility. The tests were conducted on the stadium of the internal hall of the Al-Fatat Sports Club in Najaf, as the tests were conducted and the conditions for them, the method of conducting them, and the auxiliary work team were confirmed to achieve the same conditions as possible when performing the post-tests. (6)

Dimensional tests for the research sample:

After the vocabulary of the proposed training, the curriculum was applied, the post-tests of the research sample (experimental and control) were conducted on Friday 6/26/2020 on the stadium of the inner hall of the Al-Fatat Sports Club in Najaf under conditions similar to the method of conducting pre-tests in terms of test atmosphere The location of the test, the tools used in the test, and the sequence of the tests.

Statistical Means

A statistical analysis program ((spss

View results

Table (1) shows the significance of the differences between the pre and post-tests of the control group. Tests of some elements of fitness for football players in futsal

variables	Unit Measurement	Control Group Pre-Test		Control Group Post Test		T value	indication	the type indicative
		mean	Std. deviation	mean	Std. deviation			
Selective kinetic response velocity	Second	2.003	0.068	1.900	0.023	2.945	0.032	significant
Vertical jump explosive force of the lower extremities	cm	32.03	0.69	35.85	1.40	6.319	0.001	significant
30-meter transition speed ran	Second	5.83	0.184	5.59	0.082	2.863	0.035	significant
The 10-meter partridge for the right-hand man has the characteristic force of velocity for the lower extremities	Second	3.28	0.084	3.17	0.048	2.427	0.060	insignificant
Particle 10 m for the left leg The characteristic force of speed for the lower limbs	Second	3.30	0.069	3.22	0.066	2.485	0.55	insignificant
Run (20m x 5) Elongation Speed	Second	25.22	0.511	24.78	0.579	1.256	0.265	insignificant
John Powell's Agility and Compatibility T-test	Second	15.27	0.491	14.71	0.388	2.613	0.048	significant

Discussion

Through the foregoing presentation of the previous table, it becomes clear that there is a development in four variables for the control group and the lack of development in three other variables. The researcher attributes this development to the control group to the effect of the regular approach set by the trainer in addition to the continuity and regularity of the players in the training units, which had a clear role In the evolution of these variables.

Where the results presented in Table (1), which shows the significance of the differences between the pre and post-tests of the control group for the correlated samples, showed the presence of significant differences of statistical significance in some research variables (some elements of physical fitness) and these differences came in different ways as follows:

The selective kinematic response velocity test

The results showed that there are significant differences of statistical significance and in favour of the dimensional tests of the control group in the speed of the movement response. The researcher attributes this to the development of the players 'commitment to the training units prepared by the trainer. The educational process inevitably leads to the development of achievement or performance, if it is built on a scientific basis in organizing the process of education and training, programming it, using appropriate and gradual intensity and noting individual differences, as well as the use of optimal repetitions and interstitial rest periods that affect and under the supervision of specialists under good educational and training conditions in terms of place, time and tools used.

As for the vertical jump test for the explosive force of the lower extremities, through what was presented in the previous table, it becomes clear that there is an evolution in the explosive force, meaning that there are significant differences of statistical significance and in favour of the post-tests of the control group and the researchers attribute the reason for this development to the effect of the prepared trainer's approach In addition to the continuity and regularity of the players in the active participation in the training unit, as well as the repetition of specialized exercises that had a clear role in this development, "Physical numbers are very important and necessary, especially for women, as it leads to the development, refinement and stabilization of the player's motor skills, which lead the individual to achieve higher achievement." Sports achievements "[7].

As for the 30-meter running test of the transitional speed, the results showed that there are significant differences of statistical significance in favour of the post-tests of the control group in the transitional speed. Transitional velocity is one of the important components of physical fitness, as well as a component of motor fitness, as well as being a component of motor ability and means "the highest rate of speed that an individual can bring out" [8]

As for the test of the 10-meter partridge for the right-hand man, the characteristic force of velocity for the lower extremities, and the test of the 10-meter partridge for the left leg, the distinctive force of velocity for the lower limbs, the results showed that there were no statistically significant differences between the pre and post-tests of the control group, although there is a very slight development of time between circles. The arithmetic and the inverse relationship of time as an indicator of improvement in the achievement of the test distance and the researchers attribute the reason for the absence of a significant difference between the two tests is the difficulty of developing this trait in this short period, which is that composite characteristic of the characteristic of strength and speed and occupies an important place in the process of preparing a soccer player for halls because of what this trait possesses. From a large degree of muscular strength that is characterized by fast performance and its purpose is better performance, "which is the ability to show strength as quickly as possible" () and the researchers see the importance of this characteristic in the process of continuous contact with the opposing player and linking different skills during the match and accordingly, he knew it (Hassanein) And meanings) as "the ability to quickly overcome resistance, high muscle tension" [9]

As for the running test (20 meters x 5) elongation of velocity, the results showed that there are no significant statistically significant differences between the pre and post-tests of the control group in velocity elongation and the researchers attribute the reason for this to the

difficulty of developing this trait in exercises with very small distances unless there are Accurate calculations in the development of such exercises that are compatible with these distances, where the researchers believe that velocity elongation means maintaining the speed for the longest period, meaning that it "means the player's ability to maintain his speed for the longest possible period" () and velocity prolongation means "the ability to resist fatigue when performing Loads with a starting speed less than the maximum to the maximum, as during the performance of these loads the energy gain from the anaerobic path predominates ()

As for John Powell's T-test for agility and compatibility, where the results showed that there are significant differences of statistical significance and in favour of the post-tests of the control group. Agility is an important physical component and component of great importance for a gymnast football player. It has links with many physical and orthodontic components and characteristics. It contributes greatly to the speed of learning and mastery of motor skills and is known as "the ability of an individual to change his position in the air" [(10)], as well as "the ability to Mastering complex harmonic movements and speed in learning, developing and improving motor performance, as well as the ability to use skills according to the requirements of rapidly changing situations, and the ability to quickly reconfigure performance according to this time. [(10)]

Conclusions

Through the results presented above and the researcher's findings of the discussion of these results, the researcher concluded the following conclusions: That the use of the proposed approach has a clear effect on improving some elements of physical characteristics in the experimental group with a standard period. Training exercises for simple aids have a great impact on some of the physical characteristics of futsal players, with less time and effort.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa and all experiments were carried out in accordance with approved guidelines.

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Relationship between Serum Vitamin D Status and Gestational Diabetes Mellitus

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Abstract

Background The association between vitamin D deficiency and risk of gestational diabetes mellitus (GDM) are proposed by several studies. Gestational diabetes mellitus complicates nearly 1% to 28% of all pregnancies and had an adverse effect for both mother and offspring. Vitamin D deficiency is one of the modified risk factors that can be corrected in the pre-conceptional period.

Objectives The current study aims to investigate and compare the serum levels of 25-hydroxyvitamin D_3 in pregnant women with GDM and non-GDM control subjects.

Methods In a descriptive study plasma 25-hydroxyvitamin D was assessed in a 100 women with GDM and it was compared to 162 non-GDM at gestational age above 24 weeks that was conducted in the Maternity Teaching Hospital in Sulaimani city from December 2018 to January 2019. The study used face-to-face interviews to collect the data through a specific written questionnaire. At the same time serum vitamin D state was examined using the Roche Elecsys vitamin D_3 assay.

Results There were no statistical significant differences found between two groups of GDM and non-GDM. The result of the study showed that the prevalence of vitamin D deficiency higher in the GDM group (77.0%) compares to non-GDM group (68.5%). The study found that socioeconomic state (p-value=0.069), body mass index(p-value=0.038), education (p-value=0.000), residency (p-value=0.000), age at marriage (p-value=0.023), gravida (p-value=0.014), and para (p-value=0.001), was significantly different between two groups of GDM and non-GDM.

Conclusion Overall, results imply that the vitamin D deficiency more prevalent among GDM group compare to non-GDM group, but the differences was not statically significant.

Key words: *Serum, Vitamin D, Gestational Diabetes Mellitus*

Introduction

Several metabolic changes occur during pregnancy to allow optimum glucose transport from the mother to the fetus via the placenta. This change in the metabolism during pregnancy is not harmful, but when the environmental risk factors impose effect to this change, causing some abnormality such as GDM. Moreover, in the third trimester of pregnancy as a result of pregnancy hormone especially human placental lactogen, insulin resistance may develop [1]. Gestational diabetes mellitus (GDM) defined as carbohydrate intolerance with variable severity with onset or first diagnosis during pregnancy, which applies regardless using insulin for the treatment or hyperglycemia remains after pregnancy [2]. GDM can have adverse effect for both the mother and the offspring (3). Most common complications associated with GDM for the mother are: high risk of pre-eclampsia, cesarean section, postpartum hemorrhage (PPH), polyhydramnios and increased risk of type two diabetes mellitus (T2DM) later on, the studies also showed that GDM increased the risk of metabolic syndrome [4]. In terms of offspring morbidity, GDM increase the risk of premature delivery, large for gestational age (LGA), hypoglycemia, hypocalcemia, hypomagnesemia, polycythemia and respiratory distress syndrome (5). Estimating the

prevalence of GDM remains matter because of the differences in the diagnosis criteria. The worldwide incidence of GDM estimated at between 3 and 5%, and reached 14% in the united state. The prevalence of GDM predicted to elevate because of the increase in the prevalence of associated risk factors [6]. Several risk factors associated with GDM have been defined such as; advance age ³ 35 years, family history of T2DM, hypertension, history of GDM and body mass index (BMI) more than 30 kg/m^2 [7].

Many studies carried out concerning if there any relationship between GDM and vitamin D deficiency [4,5,9].

Vitamin D is one of the fat-soluble vitamins, which play an important role in calcium regulation to maintain good health state. There are two sources of vitamin D; vitamin D_2 (ergocalciferol) that comes from dietary sources such as fatty fish, cod-liver oil and egg yolk and vitamin D_3 (cholecalciferol) that produced in human skin via exposure of skin to ultra violet B (UVB). The conversion of pre-vitamin D_3 to its active form 1,25-dihydroxy vitamin D takes place in the skin, kidney and liver by the activity of enzymes [10].

The studies proposed that vitamin D deficiency might play a role in pathology of a numbers of diseases other than skeletal problem such as diabetes mellitus, cancers and multiple sclerosis [11].

Pregnant women are more at the risk of vitamin D deficiency and the evidence showed that vitamin D deficiency during pregnancy may affect pregnancy and pregnancy outcome such as; increased risk of GDM, pre-eclampsia, pre-term birth and post partum depression [12].

The results of the studies concerning the effect of vitamin D deficiency in development of GDM are not consensus. The relationship between vitamin D level and GDM in the literature is not completely clear. In the study, which carried out by Farrant et al, the association between vitamin D state and risk of GDM studied [13]. They reported that 66% of the pregnant women had hypovitaminosis D, and despite many women being prescribed vitamin D and calcium in early pregnancy, but there were not differences in the incidence of GDM between both groups of GDM and non-GDM.

In the matched case-control study of 54 women with GDM and 39 women with IGT compared with 111 women in control group without GDM. Serum vitamin D concentration among GDM group at weeks of 24-28 was significantly lower compare to control group. They reported in the GDM group 83% were had hypovitaminosis D, while in non-GDM group 71% were deficient in vitamin D (14).

The current study looked at the serum vitamin D concentration in pregnant women with GDM and compares it to the control group of pregnant women without GDM, to find if there any association between vitamin D deficiency and risk of GDM in Sulaimani city.

Methods

Study area:

A descriptive analytic study was study was carried out at Gestational Diabetes Center, Maternity Teaching Hospital in Sulaimani city.

Study population

262 pregnant women who are beyond their 24 weeks of gestation and belong to different age groups. The study was conducted between the December 2018 to January 2019. Inclusion criteria for selection of pregnant women were to be with gestational age more than 24 weeks. The study excludes pregnant women with pre-pregnancy BMI more than 35 and pregnant age more than 40 years old. In the group of GDM, 100 g oral glucose tolerance test (OGTT) was performed to confirm gestational diabetes. Finally, the total of 262 pregnant women recruited in the study. The Ethical Committee of the university of Sulaimani approved this study.

Data collection

Data collection was performed by direct interviews with the study participants. For this, a questionnaire was constructed which surveyed the socio-demographic characteristics and previous obstetric history.

The blood sample was drowned from the participants on the day of the interview and centrifuged at 5000 r/m for 5 minutes, followed by separation of the serum that stored at -80°C in freeze until they defrosted for measurement of serum 25 dehydroxyvitamin D. In this study vitamin D level categorized into three groups

of sufficiency (more than 30ng/mL), insufficiency (between 20ng/mL and 30 ng/mL) and deficiency (less than 20ng/mL).

The validity ascertained through a pane; group of 12 experts and reliability calculated by using the correlation coefficient that was 1=0.884 (statistically adequate). A pilot study applies to groups of 20 pregnant women visiting maternity teaching hospital.

Statistical analysis: the data were analyzed using SPSS version 23.0 software program with the significance level set at (0.05).

Results

In total, 262 pregnant women participated in the current study. In the table one socio-demographic variables of both groups presented. The majority of the sample in the GDM group were aged at 30 to 39 years old which were 54% of the total samples, and 41 % of the were aged between 20-29 years old. The mean and standard deviation of the age in the GDM group was 30.10, 4.94 respectively. On the other hand, in the non- GDM group the age range of 20-29 years old was the majority of the age group with 54.3% of total participants and 44.9% of participants were in the age of 30-39 years old. Mean and standard deviation of age in the non-GDM group were (28.33, 5.03) respectively.

Regarding the blood group in both groups of GDM and non-GDM the majority of the samples have an O^+ blood group with 39.0% in GDM group and 38.9% in the non-GDM group. 26.0% and 25.3% of the participants had A^+ Blood group in GDM and non-GDM group respectively.

As it is shown in the table one, the majority of the samples (68.0%) in the GDM group were in the middle class by socioeconomic class, whereas, this range was 80.2% in the non-GDM group. 8.0% of the GDM group and 6.2% of the non-GDM group were in the higher socioeconomic class. There was a statistically significant socioeconomic difference between two groups of GDM and non-GDM at a p value of 0.069.

According to the body mass index tool for estimating ideal body weight, the majority of respondents in the GDM group (47.0%), were overweight and only 34.0% of them were having normal weight. Moreover, the mean and standard deviation of the body mass index equals (26.80, 4.62) respectively. Also in non-GDM group majority (43.2%) of the total respondents were overweight and just 26.5% of them had normal weight. There was a statistical significant difference in BMI of groups in p value of 0.038.

Regarding employment variable, 75.0% of GDM samples and 79.6% of non-GDM was non-employed.

The majority of the participants in the GDM (36.0%) and in non-GDM (53.1%) group had a secondary school graduate. 32.0% of GDM and 27.2% of non- GDM had higher education. 5.0% were illiterate in GDM group and 0.6% in non-GDM group were illiterate or other education. The p value showed that education was significantly different between GDM and non-GDM group (Table 1).

The majority of the participants in both groups were living in the urban places that were 63% in the GDM and 95.7%in the non-GDM group, this difference was significant at p-value 0.000.

Table (1): socio-demographic characteristics of pregnant women with and without GDM

Variables	Items	GDM (n=100) PERCENT	Non-GDM (n=162) PERCENT	Total	P value
Age classes	Age Class Less than 20 years	3 (3%)	6 (3.70)	9	0.139
	20- 29 years	41(41%)	88(54.3%)	129	
	30- 39 years	54(54)	67(44.9)	121	
	40 years and more	2(2%)	1(0.61%)	3	
	Mean ± S.D	30.10 ± 4.95	28.33±5.03		

Cont... **Table (1): socio-demographic characteristics of pregnant women with and without GDM**

Blood group	Blood group A^+	26(26%)	41(25.3%)	67	0.990
	B^+	19(19%)	31(19.1%)	50	
	AB^+	8 (8%)	13(8.0%)	21	
	O^+	39(39%)	63(38.9%)	102	
	A^-	3(3%)	4(2.5%)	7	
	B^-	2(2%)	2(1.2%)	4	
	AB^-	0 (0%)	0(0.0%)	0	
	O^-	3 (3%)	8(4.9%)	11	
Socioeconomic status	Low class	24 (24%)	22(13.6%)	46	0.069
	Middle class	68 (68%)	130(80.2%)	198	
	High class	8 (8%)	10(6.2%)	18	
Body mass index	Underweight	1 (1%)	11(6.8%)	12	0.038
	Normal	34 (34%)	43(26.5%)	77	
	Overweight	47 (47%)	70(43.2%)	117	
	Obese	11 (11%)	32(19.8%)	43	
	Morbid obese	7 (7%)	6(3.7%)	13	
	Mean ± S.D	26.80 ± 4.62	26.59 ± 4.72		
Education	Illiterate	5 (5%)	1(0.6%)	6	0.000
	Read and write	8 (8%)	4(2.5%)	12	
	Primary school graduate	18 (18%)	26(16.0%)	44	
	Secondary school graduate	36 (36%)	86(53.1%)	122	
	Higher education	32 (32%)	44(27.2%)	76	
	Others	1 (1%)	1(0.6%)	2	
Residency	Urban	63 (63%)	155(95.7%)	218	0.000
	Suburban	33 (33%)	5(3.1%)	38	
	Rural	4 (4%)	2(1.2%)	6	
Occupation	Employee	25 (25%)	33(20.4%)	58	0.381
	Non-Employee	75 (75%)	129(79.6%)	204	

Table one compares the association between socio-demographic characteristics of both group and significance of the association. The p value was significant at p value less than 0.05.

The obstetric characteristics of the GDM and non-GDM group are presented in the table two. The majority of the samples in the GDM group had aged at marriage between 20 to 29 years, which were 65.0% of the total participants. Likewise, in the non-GDM group majority of participations were getting married between 20 to 29 years, which were 74.7% of the total samples. Those who got married at age of 30 years and over were merely 10.0% of the total samples in GDM group and (2.5%) in non-GDM group. The differences between two groups reported as significant at p value 0.023.

It is indicated that in GDM group, the majority of the participants (54.0%) were in 20 to 29 weeks of gestational age with mean and standard deviation of (29.47, 4.70) respectively. Similarly, in the non-GDM group, 56.2% of the total participation were in 20 to 29 and 43.8% of them were in 30 to 39 weeks of gestational age with the mean and standard deviation of (29.89, 4.18) respectively. They were significantly different at p vale of 0.085.

23.0% of the total samples in the GDM group had gravida of one and 77.0% of them had more than one pregnancy. Also in non-GDM group, 37.7% of the total samples had gravida of one and 62.3% of them more than one of gravida. The mean and standard deviation were (1.96, 1.06) respectively.

Regarding the number of delivered babied in GDM group, 68.0% of the total participations had a Para one or less and 32.0% of them more than one. In non-GDM group, 85.2% had Para number equal to one or less and 14.8% of them had para number more than one (Table 2).

Table (2): obstetric characteristic of pregnant women with and without GDM.

Variables	Items	GDM (n=100) PERCENT	Non-GDM (n=162) PERCENT	Total	P value
Age at marriage	Less than 20 years	25 (25%)	37(22.8%)	62	0.023
	20- 29 years	65 (65%)	121(74.7%)	186	
	30 years and over	10 (10%)	4(2.5%)	14	
Gestational age	Less than 20 Weeks	3 (3%)	0(0%)	3	0.085
	20- 29 Week	54 (54%)	91(56.2%)	145	
	30- 39 Week	43 (43%)	71(43.8%)	114	
Gravida	Equal to one	23 (23%)	61(37.7%)	84	0.014
	More than one	77 (77%)	101(62.3%)	178	
Para	One and less	68 (68%)	138(85.2%)	206	0.001
	More than one	32 (32%)	24(14.8%)	56	

Table two compare the association between obstetric characteristics of the both group and significance of the association. The p value was significant at p value less than 0.05.

As illustrated in the table 3, 77.0% of the total participations had deficient level of vitamin D, 13.0% of them had an insufficient level of vitamin D and 10.0% of them had sufficient level of vitamin D. Moreover, the mean and standard deviation of the vitamin D class is (9.12, 4.87) respectively in deficient level; (24.81, 2.86) respectively in insufficient level and (47.05, 18.65) respectively in sufficient level (Table 3).

Table (3): Vitamin D state of GDM group

Vitamin D class	Frequency	%	Mean	S. D
Deficient	77	77.0	9.12	4.87
Insufficient	13	13.0	24.81	2.86
Sufficient	10	10.0	47.05	18.65
Total	100	100.0		-

Table three shows the serum 25(OH) levels data expressed as frequency, percent (%) and mean. Vitamin D < 20 ng/ml considered deficient, between20 ng/ml and 30 ng/ml considered insufficient and optimum levels above 30 Differences were considered statistically significant at p <0.05.

In the non-GDM group, 68.5% of the total participations were on the deficient level of vitamin D, 20.4% of them were in insufficient level of vitamin D and 11.1% of them were had sufficient level of vitamin D. Moreover, the mean and standard deviation of the vitamin D class is (10.50, 4.93) respectively in deficient level; (24.42, 2.78) respectively in insufficient level and (48.98, 21.38) respectively in group of sufficient level (Table 4).

Table (4): Vitamin D state of non-GDM group

Vitamin D class	Frequency	%	Mean	S. D
Deficient	111	68.5	10.50	4.93
Insufficient	33	20.4	24.42	2.78
Sufficient	18	11.1	48.98	21.38
Total	162	100.0		-

Table four shows the serum 25(OH) levels data expressed as frequency, percent (%) and mean. Vitamin D < 20 ng/ml considered deficient, between20 ng/ml and 30 ng/ml considered insufficient and optimum levels above 30 Differences were considered statistically significant at p <0.05.

There were no statistical significant differences found between vitamin D state of two groups of GDM and non-GDM (Table 5).

Table (5): Comparism between Vitamin D state of GDM group and non-GDM group

Vitamin D status	GDM (n=100) Frequency (%)	Non-GDM (n=162) Frequency (%)	P-value
Deficiency	77.0 (77.0%)	111(68.5%)	0.263
Insufficiency	13.0(13.0%)	33(20.4%)	
Sufficiency	10.0(10.0%)	18(11.1%)	

Table five compares the vitamin D state in a group of women with GDM and without GDM. The difference is significant when p value less than 0.05.

Discussion

The primary focus of this work was to compare serum 25(OH)D concentration between women with and without GDM. The current study found that hypovitaminosis D was more prevalent among GDM group compared to non- GDM group, but the difference in the serum vitamin D state was not significant. Moreover, socioeconomic state, body mass index, education, residency, age at marriage, gravida and para was significantly different between two groups of GDM and non-GDM.

The similar finding, reported by the study that investigate the association between vitamin D state and risk of GDM in Sulaimani city [15]. They reported that the mean of serum 25-hydroxyvitamin D concentration was lower in the group of GDM compared to non- GDM group with no statistically differences.

Observational study among South Asian population found high prevalence of vitamin D deficiency among pregnant women (16,9&10). The studies reported the several risk factors of GDM such as advanced maternal age, high BMI, parity, family history of diabetes and previous history of GDM.

In a double- blind, randomized controlled trial the effect of low dose and high dose vitamin D supplementation on glucose metabolism in pregnant women studied. The women with plasma 25-hydroxyvitamin D less than 32ng/mL and gestational age less than 20 are randomized to get a high dose of vitamin D (5000 IU/day) (n=89) and recommended pregnancy dose of 400 IU per day (90) until end of pregnancy. By the end of study twelve women (13%) in low dose group and seven women (8%) in high dose group develop GDM. Therefore, high dose of vitamin D supplementation for 14 weeks does not improve glucose level in pregnancy [17].

Among observational studies, several studies showed the association between hypovitaminosis D and risk of type two diabetes mellitus (T2DM) and GDM. The studies suggest that vitamin D deficiency may change glucose homeostasis during pregnancy [18]

Vitamin D level of pregnant women measured in the cohort study before gestational age of 16 weeks. Elevation of the vitamin D state of the pregnant women results in reduction in incidence of hyperglycemia in 24 to 28 weeks of gestation [18].

In the review that concerned the effect of vitamin D deficiency in the development of GDM, eligible studies collected from the databases and the result reported that there is a possibility that vitamin D deficiency increase the risk of the gestational diabetes mellitus. Because of the differences in the study projects and differences in the several aspects of the studies is not logical to make a definitive conclusion [19].

Serum 25-hydroxyvitamin D compared between three groups of pregnant women of GDM, non- GDM and impaired glucose tolerance test. The study which carried out by Soheilykhah et al, select 204 case s between 24-28 weeks of pregnancy and excluding cases with pre-gestational diabetes, multiple pregnancies, fetal abnormalities, chronic diseases, hypertension and history of using an anticonvulsive drug. The cases of GDM identified as having two abnormal reading of OGTT out of four, and cases of impaired glucose tolerance (IGT) defined as having just one abnormal OGTT. Vitamin D status compared between three groups. The result of the study showed that the groups of GDM and IGT had a significant lower serum vitamin D level compare to normal group. Serum vitamin D level had no any correlation with age, BMI and parity [20].

In a double blind, randomized controlled trail, the impact of vitamin D supplementation on markers of glucose metabolism examined in Asian women with the history of GDM and vitamin D deficiency. In the study 26 participants received 400 IU of vitamin D and other group received placebo. OGTT test of 75g and biochemistry profile examined at the baseline and 6 month after pregnancy. The result of the study showed that 4000IU of vitamin D for the six months safely return vitamin D level and improved beta-cell function and improves metabolic state [21]

Ultraviolet B can be considered as the main source of vitamin D, however, the studies showed high prevalence of vitamin D deficiency among population in

the countries with plenty of sunshine [16]. There are some factors which work as a barrier to get enough vitamin D from the sunshine including time of the day, season, geographical region, skin pigmentation and sun cream [22].

In conclusion, the results of the studies are inconclusive and have no clear outcome regarding the association between vitamin D deficiency and risk of GDM. However, because of the high prevalence of vitamin D deficiency in the area, randomized control trials may give a more reliable and accurate result.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Diabetes and Endocrine Center and all experiments were carried out in accordance with approved guidelines.

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Molecular Interactions of Poly (methylmethacrylate), Poly (vinyl alcohol), Poly(ethylene glycol), Chitosan, Cellulose and Starch Polymers with Iodine

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Abstract

The interactions betweenpoly(methylmethacrylate)(PMMA),poly(vinylalcohol) (PVA) poly(ethylene glycol)(PEG)as industrial biopolymers andchitosan, cellulose, starch as natural biopolymers with iodine(I_2) were investigated using diethyl ether as a solvent. 5% of I_2 was added to each polymer and soaked in the solvent. The interactions were investigated using concepts of the activation energy(Ea), Decisivefactor (Df) and the changing in the glass transition temperature (ΔTg's). Therefore, the differential scanning calorimeter (DSC) was used to monitorthe thermal transitions. The optical microscope(OM) device was used to study the surface morphologies.Resultsshowed a clear depression in the glass transition temperatures (T_g's) for all polymers(as well as new colors appear) except cellulose still unaffected .It appears that cellulose molecules coated with a film prevent iodine to diffuse through the network structure of cellulose thus no effected its color or its Tg which indicates that molecular structure of cellulose quite different from that of starch and for this fact cellulose not soluble by a solvent and undigested in the human body. The depression inTg values of polymers indicate that iodine ruptures the engineering bonds of the polymers. The most effected Tg is of chitosan (lowered by 40.23˚C, this mean that iodine ruptures both hydrogen bonding through nitrogen and oxygen atoms in chitosan molecule.

From T_g values, it seems that iodine can acts as moderate plasticizer, by diffusing through the net of synthetic and natural biopolymers ruptures their secondary bonds result in depression of their T_g except in case of cellulose.

The order of T_g depression is:

Chitosan (40.23)>PMMA (27.89)>starch (22.29)>PVA (13.89) >PEG (5.78)>cellulose (0)

Which showed no distinguish between natural and synthetic biopolymers.

From T_g values calculation of the energy given by the addition of iodine to the polymers was done and shown the following order:

chitosan(27.394)>PMMA(18.442) =starch(18.414)>PVA(9.316)>PEG(4.315)>cellulose(0)

Keywords: *MolecularInteractions, Activation Energy, Decisive Factor, Secondary Forces, GlassTransition Temperature, Dipole Moment.*

Introduction

In the polymer, there are two types of interactions, primary bonds in its backbone and secondary forces among their chains. Primarybonds includes ionic,covalent, coordinate and metallicbond.Secondaryforces are generally known as physical bonds, secondary valence, intermolecular forces, or van der Waals force.Three types of secondary bonds are recognized:dipole forces, induction forces and dispersion [1,2]

Secondary or physical bonds are weak in comparison to the primary or chemical ones and exist between virtually all atoms or molecules, but its presence may be obscured if any of the three primary bonding types is present. Secondary forces are evidenced for the inert gases, which have stable electron structures, and, in addition, between molecules in molecular structures that are covalently bonded [2] The energy of the intermolecular attractive forces varies as the inverse sixth power of the intermolecular distance ($P.E = 1 / r^6$). As with primary-bonds, repulsion arises when the atoms approach more closely than an equilibrium distance of 3-5 Å.The energy of typical secondary forces is8-40 kJ mol^{-1}, divided among its three types according to the polarizability and dipole moment of the bonding molecules [1] The physical properties of polymer molecules are influenced not only by their composition, but also by their size and by the nature of their primary and secondary forces. Secondaryforces are not of great importance in the formation of stable chemical compounds. They lead, rather, to the aggregation of separate molecules into solid and liquid phases. As a result, many physical properties such as volatility, viscosity, surface tension, frictional properties, miscibility, and solubility are determined largely by intermolecular forces. Due to the large sizes of polymeric molecules, the secondary forces assume much greater roles in influencing physical properties than they do in small organic molecules. The cohesive energy is the total energy necessary to remove a molecule from a liquid or solid to a position far from its neighbors [1,3]

The activation energy (Ea) can be defined as a minimum energy which required for starting the chemical reaction [4].

Bond rupture can be explained when stress is applied any one of three principle reactions becomes possible. The three reaction mechanisms are radical, ionic, and ion-radical. The path depends on polymer composition, its physical state, and the medium in which the mechanochemistry is done. The influence of temperature on mechanochemistry is fundamental, how-even, and not always direct. The effects can be mainly indirect involving a change in (1) the physical state of the polymer, (2) the properties (viz. elasticity and viscosity), and (3) the mechanism of bond rupture. At lower temperatures, thermal motions are reduced, the relaxation processes are slower, and chain disentangling processes are longer. As a result of these features, the extent and reaction rate for mechanochemistry are generally higher at lower temperature [5].

Plasticizers are large organic molecular of low volatility that are added to plastics compounds to improve their flexibility, extensibility, and processability. They increase flow and thermoplasticity of plastic materials by decreasing viscosity of polymer melts, glass transition temperature (T_g), melting temperature (T_m), and elasticity modulus of finished products .Plasticizers are particularly used for polymers that are in a glassy state at room temperature. These rigid polymers become flexible by strong interactions between plasticizers molecules and chain units, which lower their brittle-tough transition or brittleness temperature (T_b) (the temperature at which a sample breaks when struck) and their Tg value, and extend the temperature range for their rubbery or viscoelastic [6] state behavior

Thefollowingprocedure was used to determine the energy arises from bond rupture:

$$Ea = \Delta H^{\ddagger} + RT \quad \ldots\ldots (1)$$

The activation energy (Ea) is the summation of the activation enthalpy ($\Delta H^{\ddagger}$) and the work or the thermal energy (RT), as shown in equation (1).

According to polymer molecular models (the activation energy for chain rupture is about 60 kCal/mol (251 kJ mol^{-1}) at room temperature, so that the value of the decisive factor (D_f) for chain rupture as a thermal fluctuation process is about 101for one mole [7].

$$Ea = \Delta H^{\ddagger} + RT = RTD_f \quad \ldots\ldots (2)$$

Where:

R□the gas constant (0.0083 kJ $K^{-1}mol^{-1}$)

T: the absolute temperature (K)

$Ea_{at\ R.T}$=251 kJ mol^{-1}

$Ea = \Delta H^{\ddagger} + RT = 251$

Since RT value small at room temperature which is equal to 2.47 kJ mol^{-1} comparing with $\Delta H^{\ddagger}$:

□ΔH‡□RT → Ea□ ΔH‡……..... (3)

Ea =ΔH‡=RTD$_f$…….. (4)

Ea=0.0083 kJ K^{-1} mol^{-1}□298.15 K□D$_f$=251 kJ mol^{-1}

D$_f$=101.43

The energy of each unit of D$_f$=251/101.43=2.475 kJ mol^{-1} …..…… (5)

Experimental Work:

Materials used:

In this study, 5w% Iodine (I$_2$) was added to six polymers, three of them are synthetic typepoly (methyl methacrylate); PMMA, poly (vinyl alcohol); PVA and poly (ethylene glycol); PEG, and the other three are naturaltype chitosan, cellulose andstarchDiethyl ether used as a solvent.

The first three polymers supplied from Yonghui chemical Holdings Limited Company, china, while chitosan supplied from Shanghai Soyoung Biotech. Inc. China.Corn starch which is a white powder, was obtained from local markets. Iodine was purchased from Flucku, a Swiss company.

2. Preparation of samples:

Iodine (5wt %) was added and mixed with the following polymers: PMMA, PVA, PEG, chitosan, cellulose andstarch.This mixture was soaked with 30 ml of diethyl ether until evaporated it at room temperature. The resultant solid product was grinded by an electric grinder to obtain a powder form. The powder was examined by differential scanning calorimeter (DSC) to determine the glass transition temperature (T$_g$). Optical Microscope (OM) was used to studythe surface morphology.

Results and Discussion

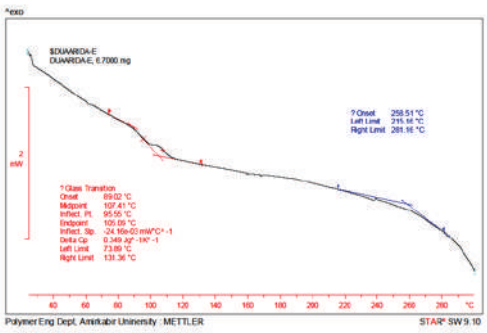


Fig (1): DSC curve of PMMA

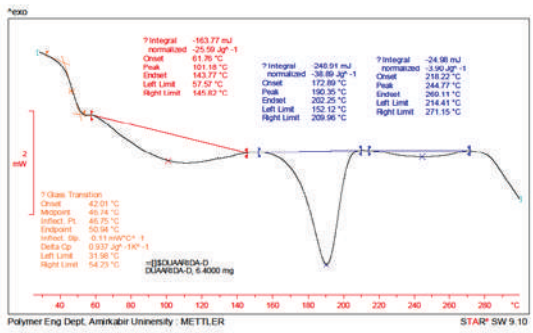


Fig (2): DSC curve of PVA

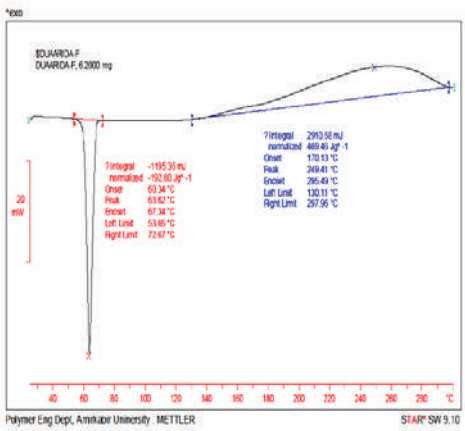


Fig (3): DSC curve of PEG

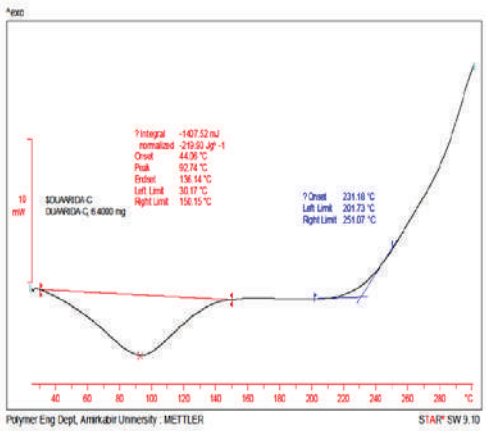


Fig (4): DSC curve of chitosan

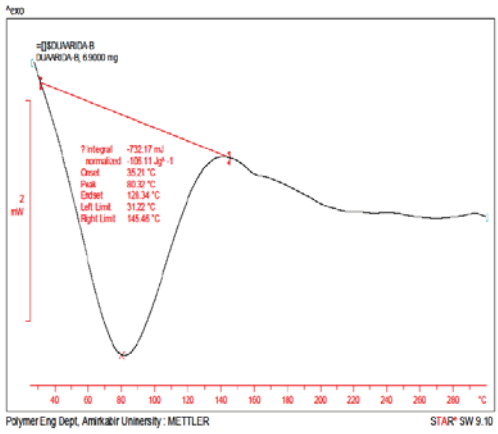


Fig (5): DSC curve of cellulose

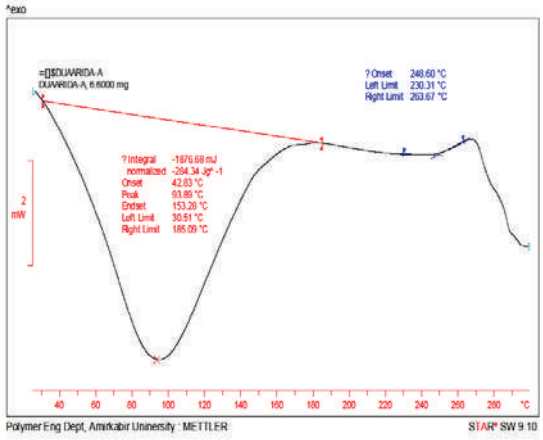


Fig (6): DSC curve of starch

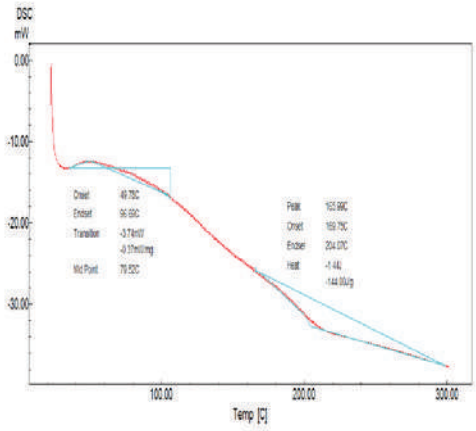


Fig (7): DSC curve of(PMMA /5wt%I_2) composite

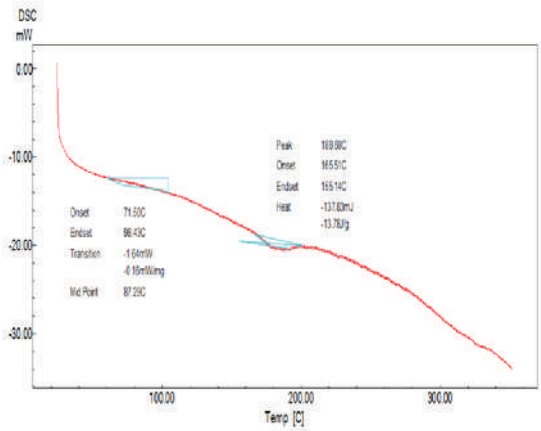


Fig (8): DSC curve of(PVA/5wt% I_2) composite

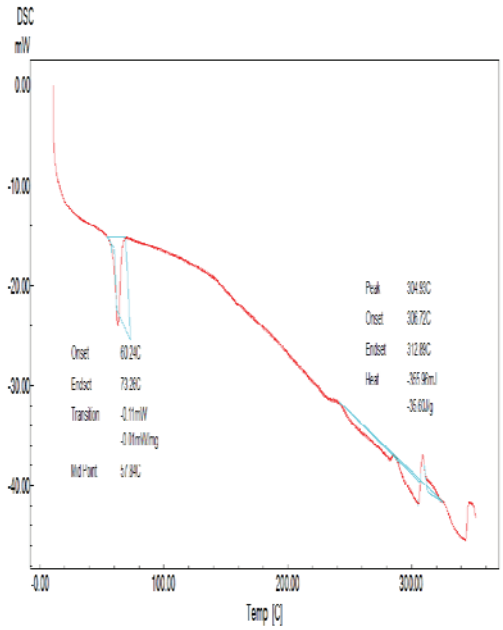


Fig (9): DSC curve of(PEG /5wt%I_2) composite.

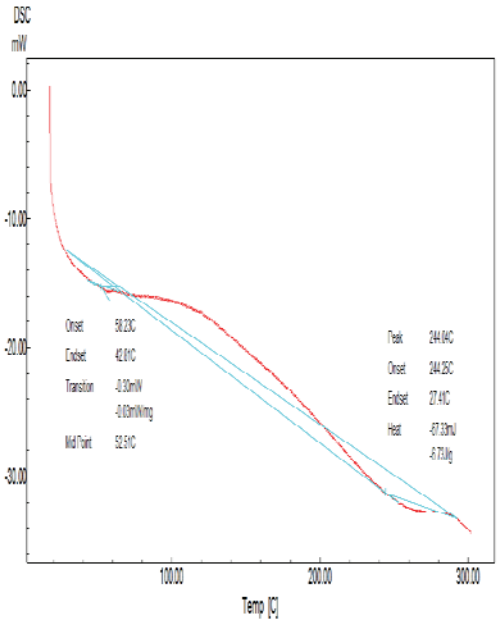


Fig (10): DSC curve of (chitosan/5wt%I_2) composite

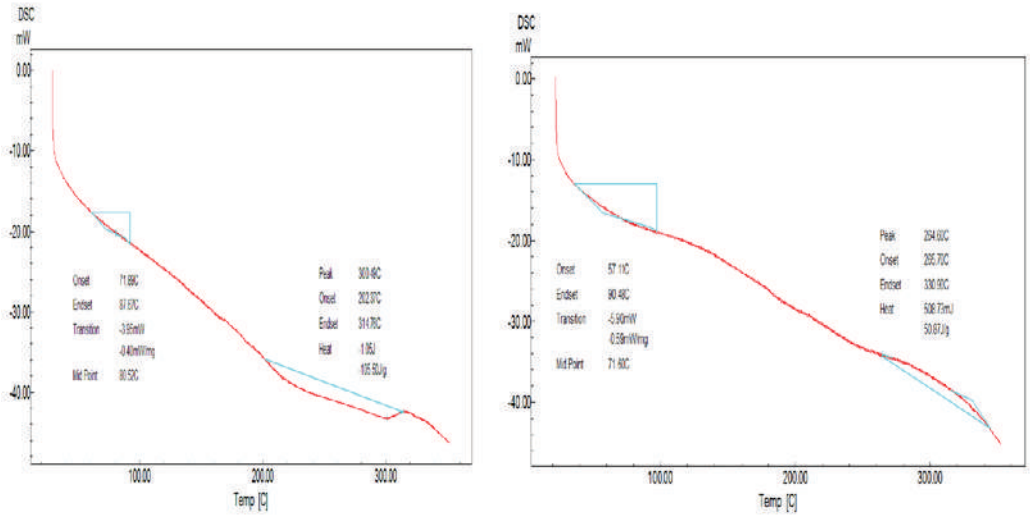


Fig (11): DSC curve of(cellulose/5wt%I_2) composite, Fig (12): DSC curve of(starch/5wt%I_2) composite.

These figures utilized to determining the glass transition temperatures (Tg) for pure polymers and their composites with 5(wt. %) I_2.Equations 1-5 (in the theoretical part) were used to calculate Ea and Df,as shown in table 1.Sample of calculations is done for pure PMMA andPMMA/5(wt. %) I_2 as follow:

For pure poly (methyl methacrylate) (PMMA):

T_g of pure PMMA=107.41□ (From fig.1)

Since Ea=R*T*$D_f$that means:

251=0.0083□380.56□D_f

D_f=79.46

$\Delta D_f = D_{fat\ R.T} - D_{fat107.41□}$

ΔD_f =101.43−79.46= 21.97

Addition $Ea_{from\ (25\ to107.41□)}$ =ΔD_f□Each unit of D_f

$Ea_{from\ (25\ to107.41□)}$ =21.97□2.475=54.37 kJ mol^{-1}..... (6)

$Ea_{at\ (107.41□)}$ =251−54.37=196.63 kJ mol^{-1}

ΔT=107.41−25=82.41□

1□=$Ea_{at\ (25\ to107.41□)}$ /ΔT

1□=54.37 /82.41=0.659 kJ mol^{-1}……..…. (7)

By addition 5wt% iodine to PMMA:

The temperature of chain rupture falls to 79.52□ due to addition 5wt%iodine□T_g=79.52□ (From fig.7)

Since Ea=R*T*D_f that means:

251=0.0083□352.67□D_f

D_f=85.753

ΔT=79.52−25=54.52℃

Addition $Ea_{from\ (25\ to\ 79.52℃+5\%iodine)}$ =54.52℃*0.659 kJmol^{-1}

$Ea_{from\ (25\ to\ 79.52℃+5\%iodine)}$ =35.928 kJ mol^{-1}

Given energy from addition 5%iodine= 54.37-35.928=18.442 kJ mol^{-1} …..(8)

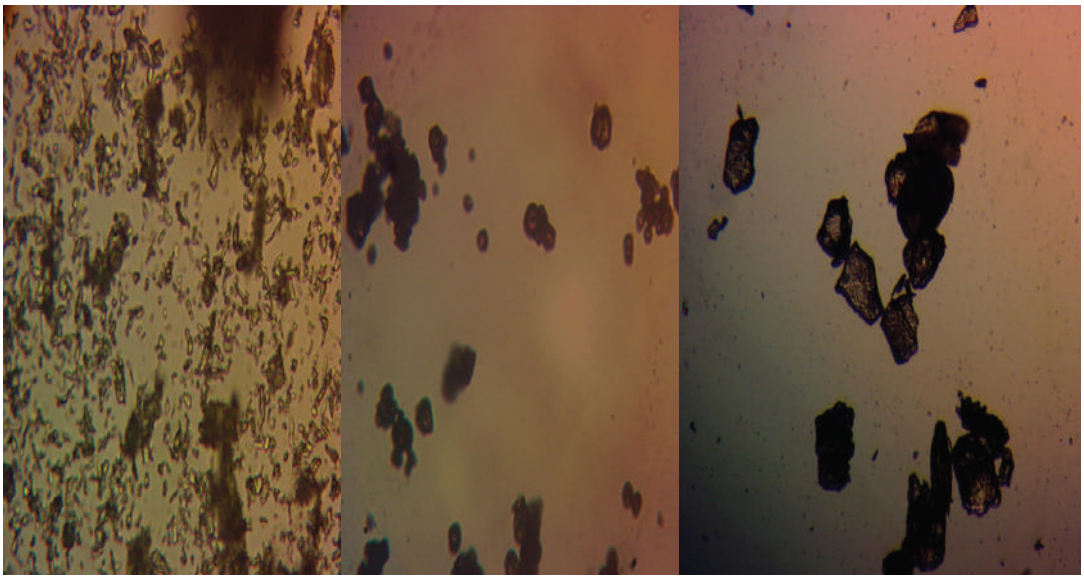
$Ea_{at\ (79.52℃)}$ =196.63−18.442= 178.188 kJ mol^{-1}

For other polymers and their composites, the calculations were repeated and tabulated in table 1.

Table (1): TheEffect of 5wt%iodine on usedpolymers at R.T (25°C) &Ea (251 kJ mol^{-1}) with D_f (101.43)

Substance	Tg(°C)	Df	Eaat Tg (kJ mol-1)	Addition Ea (kJ mol-1)	Given Ea from heating and addition iodine (kJ mol-1)
Pure PMMA	107.41	79.46	196.63	54.37	_
5%I2+PMMA	79.52	85.753	178.188	35.928	18.442
Pure PVA	101.18	80.8	199.95	51.05	_
5%I2+PVA	87.29	83.918	106.79	41.734	9.316
Pure PEG	63.62	89.8	222.2	28.78	_
5%I2+PEG	57.84	91.372	217.88	24.465	4.315
Pure chitosan	92.74	82.8	204.9	46.1	_
5%I2+chitosan	52.51	92.86	177.5	18.706	27.394
Pure cellulose	80.32	85.6	211.83	39.17	_
5%I2+cellulose	80.5	85.519	211.524	38.864	0
Pure starch	93.89	82.56	204.3	46.7	_
5%I2+starch	71.6	87.731	185.886	28.286	18.414

The calculations shows that addition of 5wt% I_2 causes generally in the Tg values for all polymers except cellulose. That means, that I_2 acts as plasticizer for all polymers except cellulose.



Chitosan StarchCellulose



PMMA PVA PEG

This indicate that iodine molecule diffuses through the networkof the polymers except cellulose, thus iodine rupture the engineering bonds of the polymers specially their hydrogen bonds.In case of chitosan, iodine attacks its hydrogen bonding through nitrogen and oxygen which show the highest depression in its Tg (40.23**°C**) from (92.74 to 52.51) **°C**.While cellulose not affected, it seems that cellulose molecule coated withprotective film comparing with starch where its Tg reduced from (93.89 to 71.6) **°C**.This is due to different structure of cellulose and starch as shown in fig.14 (a-b)

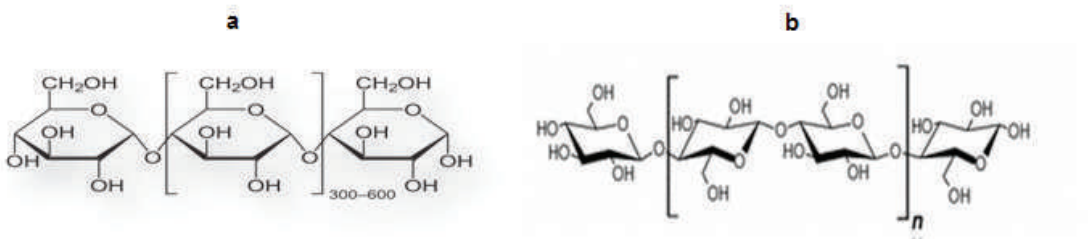


Fig.14 (a): structure of starch, (b): structure of cellulose

Also iodine colored all polymers except cellulose this support the above discussion, i.e. cellulose coated with impermeablemembrane.That proved the differences in the chemical structures between cellulose and starch. Cellulose does not dissolve by solvent, while starch dissolved, therefore cellulose undigested by the human body conditions.PMMA shows relatively highest Tg (107.41°C). This is due to the definition is basically the same as with elastomers, but instead of a chemical cross-linking there is a physical cross-linking (as shown its structure in fig.15)

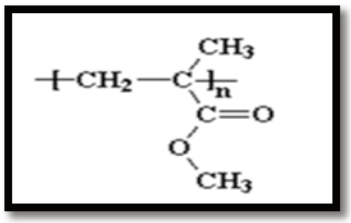


Fig (15): Structure of PMMA

and Tg above 0°C, thus it is highly affected with addition of iodine in which the Tg drop to the (79.52°C). In previous work cellulose shows drop in Tg with addition of 3%carotene, which attributed to that carotene molecule has 22delocalizedπ –electrons moving along carotene molecule which inter the fine film surrounding the cellulose molecule.This support the conclusion of the present research, that is cellulose molecule surrounding by a protective membrane. As the percent increase by to 7% show no change in the Tg due to the absence of functional groups in carotene and 3% is enough to the π –electrons to inter the cellulose molecule. For all polymers, Tg reduced by the following order: chitosan (40.23) > PMMA (27.89) > starch (22.29) > PVA (13.89) > PEG (5.78) >cellulose (0) °C

That means, there is no distinction between the behavior of natural and synthetic biopolymers. Also, it means that Tg depends on the types and number of the functional groups available in the polymer structure (as shown in fig.16

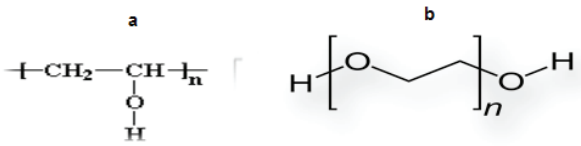


Fig. (16): a: Structure of PVA, b: Structure of PEG

As well as on their molecular weight. When comparing the chemical structures of PVA, starch and PEG, it seems that the expected destroyed hydrogen bonds is higher in PVA than starch and PEG.

In the term of energy calculations, the 5wt% I_2 addition gives highest energy to chitosan (27.394(kJ mol^{-1}) and gives no energy to cellulose and the overall ordering is:

Chitosan (27.394) >PMMA (18.442) =starch (18.414) >PVA (9.316)>PEG (4.315) >cellulose (0)

The maximum energy required to transfer polymer from its original state at RT to its state at its Tg is for PVA (41.734), while the minimum value is for chitosan (18.706). This is due to compact structure of PVA compared with the chitosan structure.

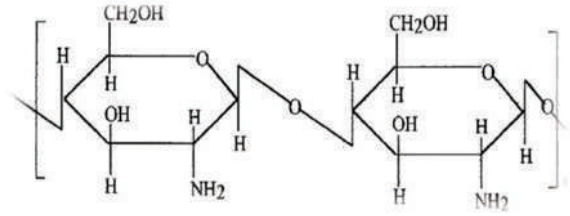


Fig. (17): Structure of chitosan

Conclusions

1- Iodine shows the highest depression in the Tg of chitosan while not effected cellulose.

2- Iodine act as inorganic plasticizer for all polymers except cellulose.

3- Iodine shows color changewith all polymers except cellulose.

4- The deepest color with starch.

5- Optical microscopy shows there is absorption of the solvent through the polymers except in case of cellulose .This indicates that cellulose molecules behave as impermeable membrane.

6- Energy calculationsshows that iodine give highest energy to chitosan (highest depression in its Tg) and zero energy to cellulose (zero change in Tg)which indicates there is two phase with cellulose –iodine system.

7- The order of decreasing Tg of polymers upon addition of 5% iodine as follow:chitosan (40.23) >

PMMA (27.89) > starch (22.29) >PVA (13.89) > PEG(5.78) > cellulose(0)

8-No distinction betweenTg of natural and industrial biopolymers.

9-Tg depends on molecular weight of polymers and on the type and number of functional groups in the polymer.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Materials Engineering and all experiments were carried out in accordance with approved guidelines.

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Indications and Complications of Tracheostomy in Pediatric Age group in Hilla, Babylon province, Iraq: Cross sectional Study

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Abstract

Background: The procedure tracheostomy involves making an opening in the airways and introduction of tube into tracheal rings in order to bypass obstruction and allowing air to reach the lungs. The survival of neonates and children witnessed substantial improvement following implementation of such procedure; therefore, the rate of performing tracheostomy in children has become high. Little information is available about complications of tracheostomy in Hilla general teaching hospital.

Aim of the study: to highlight the main indications and complications in association with tracheostomy in pediatric age group in Hilla, Babylon province, Iraq.

Patients and methods: The current prospective study included 41 children less than 12 years of age form the pool of patients admitted to intensive care unit at Hilla General Teaching Hospital and the intensive care unit at Babel Pediatric and Maternity Teaching Hospital. The study extended from March 2018 to September 2019. All those children underwent tracheostomy for a variety of reasons. They were followed up for one year for recoding any possible complication in association with tracheostomy procedure.

Results: After one year of follow up, the time until decannulation was ranging between 45 to 365 days with a mean of 61.73 days. Upper air way obstruction was seen in 12 cases while assisting ventilation in prolonged intubation was seen in the majority, 30 cases. Causes of upper airway obstruction were Craniofacial anomaly, Subglottic stenosis, Laryngotracheal stenosis, Vocal fold paralysis and Subglottic hemangioma. The reasons for prolonged intubation included Neuromuscular disease, Postoperative period following major surgery, Respiratory failure, Trauma and Subglottic hemangioma.

Conclusion: The main indications for tracheostomy in children were congenital upper air way obstruction and the need for prolonged mechanical ventilation and the main complications are either intermediate cannula loss or late subglotic obstruction.

Key words: *Indications, complications, tracheostomy, pediatric, Hilla, Iraq*

Introduction

The procedure tracheostomy involves making an opening in the airways and introduction of tube into tracheal rings in order to bypass obstruction and allowing air to reach the lungs [1]. Since ancient times, tracheostomy practice has been reported. Ancient Egyptians had made painting describing this procedure, thereby it can be said that the procedure is known for more than 2000 years [2]. Since infections of the upper respiratory tract, including diphtheria, represented by far the major reason for tracheostomy, great changes have been undertaken in the indications for tracheostomy between the 1980s until around 1995 [1]. Adoption of well organized and systemized immunization programs has made changes in the profile of infection worldwide, thus, the need for tracheostomy in the setting of upper respiratory tract infection have been reduced [3]. Nowadays, the most frequent reasons for tracheostomy performance are "acute

respiratory failure, prolonged intubation, neurological disorders or lesions" [1]. The survival of neonates and children witnessed substantial improvement following implementation of such procedure, therefore, the rate of performing tracheostomy in children has become high [1,2,4]. It should be kept in mind that the hazard of infection is significantly more in children with tracheostomy. The bypass of oral and nasal passages prevents the natural protection of respiratory tract form invading microbes since it will create a direct passage for microorganisms to reach the lower respiratory tract because of loss of filter and immune barrier of upper respiratory passages [1].

The lower respiratory tract is cleared by the effect of coughing and the mucociliary clearance of the nasal mucosa leading to expelling of secretions and possible intruding agents; unfortunately, tracheostomy is associated with lack of these two protective mechanisms. On the other hand, the presence of a tube inside respiratory passages for a long period of time will lead to inflammation and formation of a nidus for infectious micro-organisms and biofilm formation [5-7]. In addition, the tube itself provides a surface for strong adherence of microorganism, thereby, avoiding the immune mechanism and antibiotic attack and making the rate of infection even higher in the setting of tracheostomy [6]. Among common infectious complications accompanying tracheostomy are bacterial tracheitis, aspiration pneumonia and bacterial pneumonia, the later being the most commonly reported in pediatric practice and responsible for significant hospitalization (8). Among micro-organism isolated commonly from tracheostomy cannula, are *Pseudomonas aeruginosa* and *Staphylococcus aureus* [1,9]. Little information is available about complications of tracheostomy in Hilla general teaching hospital. Thus, the aim of the study was to highlight the main indications and complications in association with tracheostomy in pediatric age group in Hilla general teaching hospital, Babel province, Iraq.

Patients and Methods

The current prospective study included 42 children less than 12 years of age form the pool of patients admitted to intensive care unit at Hilla General Teaching Hospital and the intensive care unit at Babel Pediatric and Maternity Teaching Hospital. The study extended from March 2018 to September 2019. All those children underwent tracheostomy for a variety of reasons. They were followed up for one year for recoding any possible complication in association with tracheostomy procedure. The ethical approval was issued by the ethical approval committee of the local health institute and a verbal consent was obtained from all participants following full illustration of the aim and the procedure of the study. The principal outcomes were the clinical presenting features and the type of pathology. Data were transformed into SPSS (statistical package for social sciences) software (IBM, Chicago, USA, version 23) for purpose of statistical description. Categorical variables were expressed as number and percentage, whereas, quantitative data were expressed as range, mean and standard deviation. Chi-square test was used to study association between complication and indication of tracheostomy. The level of significance was set at $p \leq 0.05$.

Results

The current study included 42 children subjected to tracheostomy for a number of indications. The age range was 1 to 12 years and the mean age was 5.03 ±3.72 years. The proportions of males and females were 23 (54.8 %) and 19 (45.2 %), respectively. After one year of follow up, the time until decannulation was ranging between 45 to 365 days with a mean of 61.73 days, as shown in table 1. The indications of tracheostomy are shown in table 2. Upper air way obstruction was seen in 12 cases while assisting ventilation in prolonged intubation was seen in the majority, 30 cases. Causes of upper airway obstruction were Craniofacial anomaly, Subglottic stenosis, Laryngotracheal stenosis, Vocal fold paralysis and Subglottic hemangioma. The reasons for prolonged intubation included Neuromuscular disease, Postoperative period following major surgery, Respiratory failure, Trauma and Subglottic hemangioma, as shown in table 2. Hemorrhage as an immediate complication was seen in a single case. Intermediate complications included Pneumothorax, Surgical emphysema, Accidental decannulation and Suprastomal granulation; whereas, late complications included Subglottic stenosis and Tracheocutaneous fistulae, as shown in table 3. There was no significant association between indications and complications ($p = 0.791$), as shown in table 4.

Table 1: General characteristics of study sample

Characteristic	Value
Number of cases	42
Age (years)	
Range	1-12
Mean ±SD	5.03 ±3.72
Gender	
Male, n (%)	23 (54.8 %)
Female, n (%)	19 (45.2 %)
Duration of cannulation (days)	
Range	45-365
Mean ±SD	61.37 ±101.03

n: number of cases; **SD**: standard deviation

Table 2: Indications of tracheostomy

Indication	Number of cases	%
Upper airway obstruction		
Craniofacial anomaly	5	11.9
Subglottic stenosis	2	4.8
Laryngotracheal stenosis	2	4.8
Vocal fold paralysis	2	4.8
Subglottic hemangioma	1	2.4
Assisting ventilation in Prolonged intubation		
Neuromuscular disease	9	21.4
Postoperative period following major surgery	3	7.1
Respiratory failure	14	33.3
Trauma	3	7.1
Subglottic hemangioma	1	2.4

Table 3: Complications

Complication	Number of cases	%
Immediate		
Hemorrhage	1	2.4
Intermediate		
Pneumothorax	3	7.1
Surgical emphysema	1	2.4
Accidental decannulation	6	14.3
Suprastomal granulation	6	14.3
Late		
Subglottic stenosis	3	7.1
Tracheocutaneous fistulae	1	2.4
Total	21	50.0

Table 5: Association between complications and indications

Complication	Total n = 42	Indication		p
		UAO n = 12	AV n = 30	
Immediate and intermediate	17	4	13	0.791 C NS
Late	4	1	3	
No complication	21	7	14	

n: number of cases; **UAO**: upper airway obstruction; **AV**: assisted ventilation; **C**: Chi-square test; **NS**: not significant at $p > 0.05$

Discussion

Tracheostomy is life saving procedure since it maintains adequate air flow to lung in critically ill patients by making an opening in the trachea [10]. In adults, this procedure is quiet frequent accounting for 10-24 % of patients on ventilation [11]. However, the procedure is increasingly used in children in various regions of the world with substantial improvement in survival of those critically ill and chronically affected children [10]. In one study, performed in Canada, the rate of tracheostomy in ventilated children was 1.5 % [12]. Several other previous studies have come up with close rates of tracheostomy

in pediatric age group [13-15]. Parallel to changes occurring in managing critically ill children over the last 30 years, the reasons and indications for performing such procedure in child age group have also transformed [16,17]. In the current study, it has been shown that the main indications of tracheostomy in children were either due to obstruction of upper airway by one or more of congenital abnormalities, craniofacial anomaly, subglottic stenosis or laryngotracheal stenosis, or to assist ventilation in children with neuromuscular disease, respiratory failure and trauma. In line with this observation several previous reports have demonstrated that tracheostomy in children is performed when there is a need for prolonged mechanical ventilation in case of respiratory failure in addition to children with upper respiratory tract anomalies and chronic disorders such as congenital heart disease, lung disorders and neurological impairment (10). The most common indication for tracheostomy in Can *et al*'s study was prolonged mechanical ventilation (18). Neurological and respiratory causes have been shown to be more frequent than cardiovascular disorders in one previous study and the most frequent diagnoses were brain trauma and obstruction of upper airway [10]. The later observations support the findings of the current study.

In previous reports, favorable outcome has been shown to correlate with performing tracheostomy during the first two weeks in mechanical ventilation [19,20]. However, the best time for performing the procedure in critically ill children is not clearly known and in most of occasions it follows the decision made by the treating physician based on patients available clinical data [10]. In developed countries, it has been shown that the time for performing tracheostomy in children is in the range of 14.4 to 21 days [21]. Some previous reports have shown that a mean time of hospitalization of 64 days has passed before performing tracheostomy [22]. This time average was a s short as 18 days in another series [10]. Several factors are blamed to explain this variation between onset of hospitalization and time of performing tracheostomy including anticipated complications in association with tracheostomy and variation of children disorders. It should be noted that early tracheostomy for children in intensive care units are associated with better outcome in terms of shorter hospital stay and lower mortality rate [23].

In the current study, the main complications in association with tracheostomy were either intermediate in form of accidental decannulation and suprastomal granulation or late in form of subglottic stenosis. In previous reports, stromal granulation and tube blockage were among main complications encountered in addition to wound infection and chest infection [24]. In another study performed in 2010, the main complications were decanulation and cannula obstruction [25] supporting the findings of the current study. Tube occlusion and accidental decannulation were also the most common complications seen by another author [26].

Conclusion

The main indications for tracheostomy in children were congenital upper air way obstruction and the need for prolonged mechanical ventilation and the main complications are either intermediate cannula loss or late subglotic obstruction.

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Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Surgery and all experiments were carried out in accordance with approved guidelines.

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Road Traffic Injury deaths and Correlates in Babylon Province: A Cross- Sectional Study

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Abstract

Background: Road traffic accidents were a major health problem especially in low and middle income countries including Iraq, head injuries were found to be the most common fatal injuries during traffic accidents that causing deaths.

Objectives: to identify the relationship of road traffic injury deaths with different associated factors and to measure the extent of this problem.

Methodology: this was a cross-sectional study included a retrospective analysis of the forensic medicine data presented in the records of forensic medicine department for three years (2017-2019) the period needed to conduct this study started from 1st of February to the end of November,2019 . After the approval of the study protocol by the Babylon nursing college health ethical committee , a semi structured questionnaire was used to collect data according to the descriptive epidemiologic model (person , place, time).

Results: The road traffic accidents death rate in Babylon province was unacceptably high, the mean deaths per month due to Road Traffic Injuries was 43,8 and the mean deaths per week was11victims and the crude death rate =27/100000 population; Males in their productive age group were outnumbered victims to this preventable high priority health problem. Urgent interventional plan of action including provision high quality nursing tertiary care should be started to address this growing problem using the available resources to promote the critical care skills among health care providers and to reduce medical errors.

Keywords: *Road traffic, injury deaths, head injuries, Babylon - Iraq*

Introduction

In the globe , about 3 400 people die on the world's roads daily. Millions of people are injured or permanently disabled . WHO struggles to raise the profile of prevention of road traffic injuries [1]. WHO report predicts that 1.9 million person in the world die every year by 2020 [2], the vast majority(93%) of deaths due to road traffic injuries occur in low-and middle-income countries including Iraq [3,4]. Road traffic injuries cause significant economic losses to individuals, families, and to nations as a whole. Road traffic crashes cost most countries 3% of their gross product [5]**.** By this year(2020) they will rank the third cause of mortality and morbidity. Another better fact is that 50% of the victims are individuals with in their productive age and lay role in society economic development [6]. The number of road accidents is increasing in developing countries and the direct and indirect costs are higher compared to those in developed countries [7]. In Iraq the number of road traffic accidents is also increasing which put a high burden on Iraqi health system which faces a lot of challenges in many aspects including shortage of high quality and competent adult nursing services dealing with increasing emergency medical problem the needs critical nursing care, this emphasizing the need of well- trained human resources including well trained and competent nurses ready to deal with this

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problem successfully in tertiary intensive surgical units (general surgery ,neurosurgery and orthopedic care) [8-14]. According to WHO, the average annual death rate due to road traffic accidents in Iraq reached 44.7 (standardized age death rate per 100,000 population) [15]. Road traffic injuries cause an estimated700 deaths among young people every day [16]. Head injuries due to Road Traffic Accidents is a recognized health problem that causes death and disability , the head being the most vulnerable part of the body that is why cranio-cerebral injury is on the top [17-19]. The ultimate in this situation aim is to attain universal health coverage (trauma care, rehabilitation) for the victims and to ensuring road safety [20,21]. Strong health management is crucial factor which has to be applied in the developing nations and it is the time that all the concerned stakeholders work together in a coordinated manner to deal with this preventable problem to improve post-crash care [22,23]. The WHO has come up with a holistic policy on road safety and management [24]. The objectives of this study are to identify the relationship of road traffic accident fatalities with different associated factors

Methodology

The protocol of the study was accepted by the Research Ethical Committee in Babylon university-college of nursing .The approval of ethical committee of Babel health directorate was also taken to carry out this research work .This was a cross-sectional observational descriptive study depended on a retrospective analysis of the reported Road Traffic Injuries (RTIs) mortalities that recorded in the forensic medicine department for the years 2017–2019.The time needed to conduct this study started from 1st of February to the end of November,2019. Data that were recorded in the forensic medicine department for the three years were reviewed by the researchers. The registered data for each victim during the years of the study were reviewed which include, age, gender, and residence of the victims, in addition to the place and time of occurrence of incident that leads to death , date of accident is recorded (in day and month). The site of injury in the body was determined after postmortem study to identify the direct cause of death.

Statistical analysis was done using Spss version 17 to calculate the Chi square test to evaluate the significant of difference between variables, $p<0.05$.

Results

Total Number of RTA deaths in the last 31 months in Babylon province = 1358 .The mean deaths per month due to RTIs =43,8 and the mean deaths per week due to RTIs =11 with crude death rate =27/100000 population.

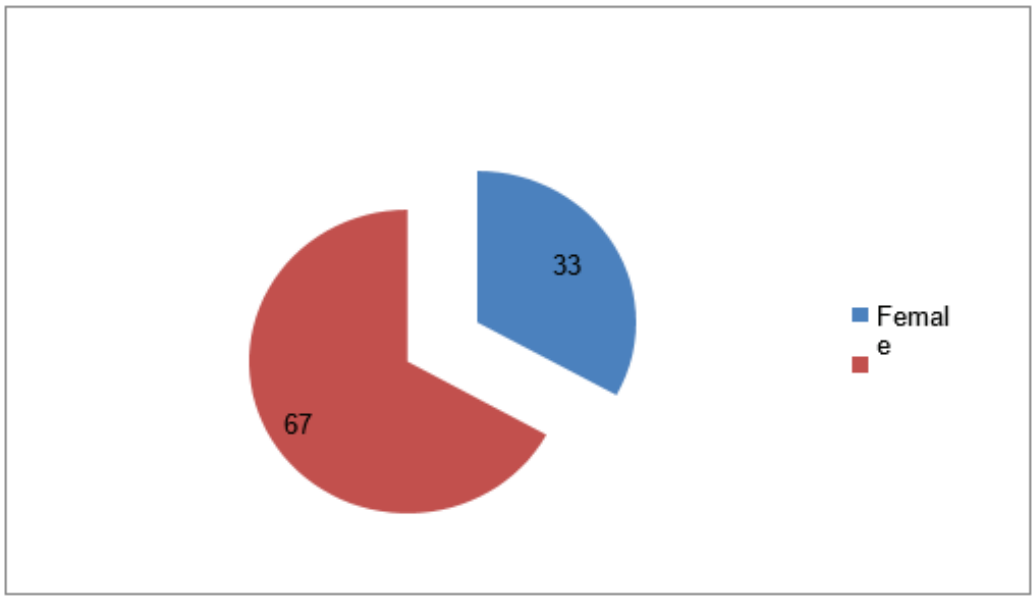


Fig (1): Distribution of road traffic accident by gender in Babylon province (N= 1358)

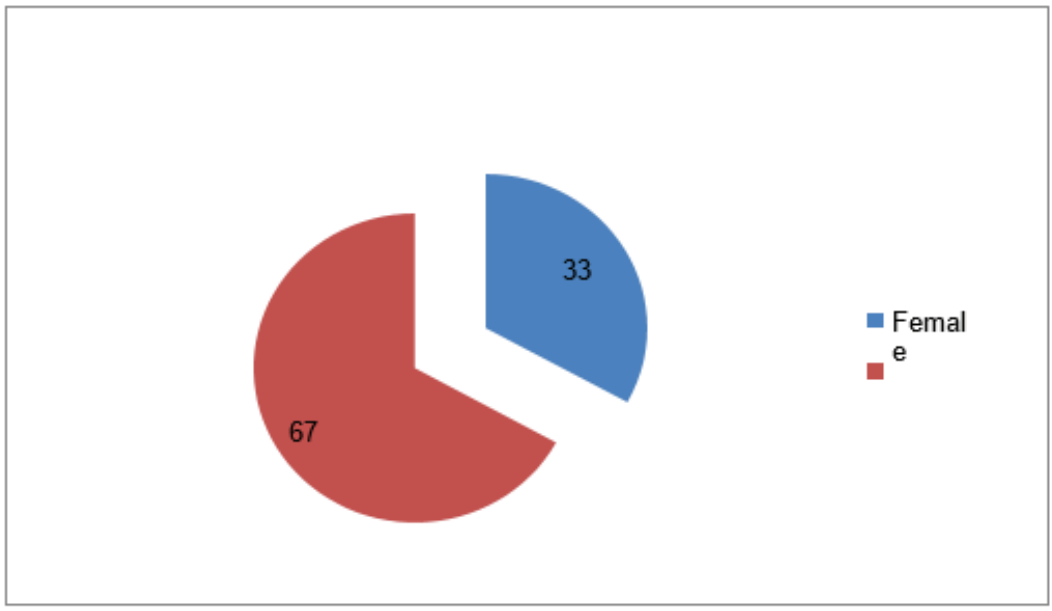


Fig (2): Distribution of injured and dead subjects by age and gender (N=1358)

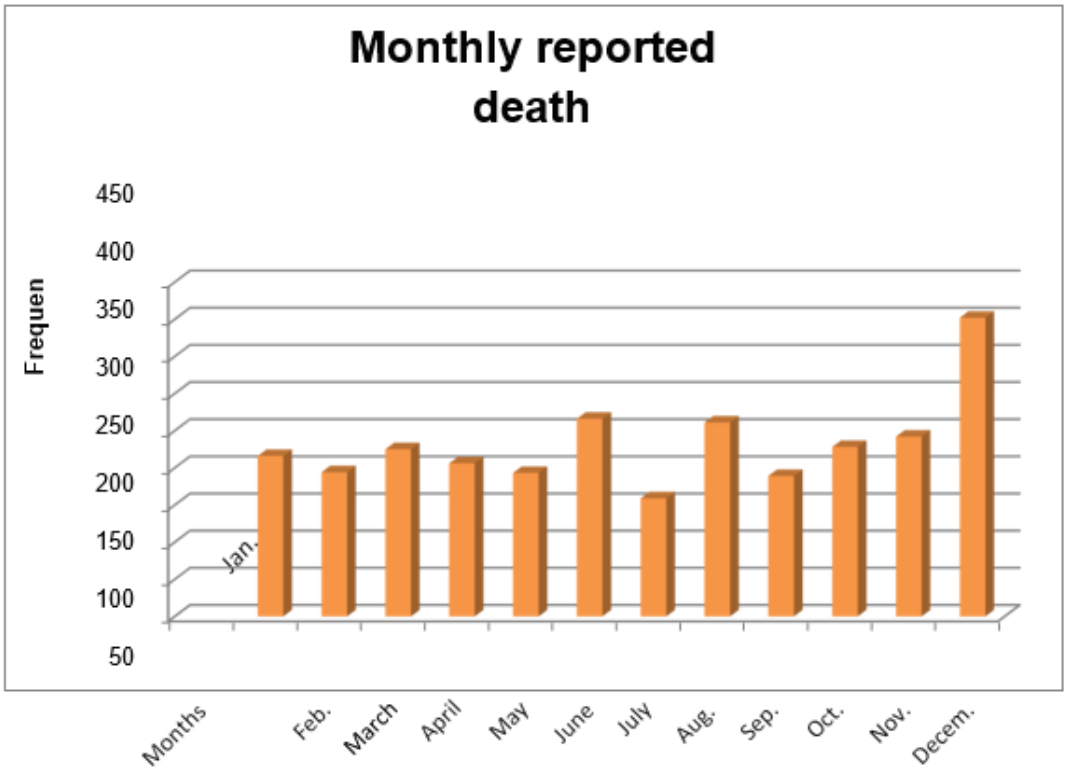


Fig (3): Monthly reported road traffic accident death

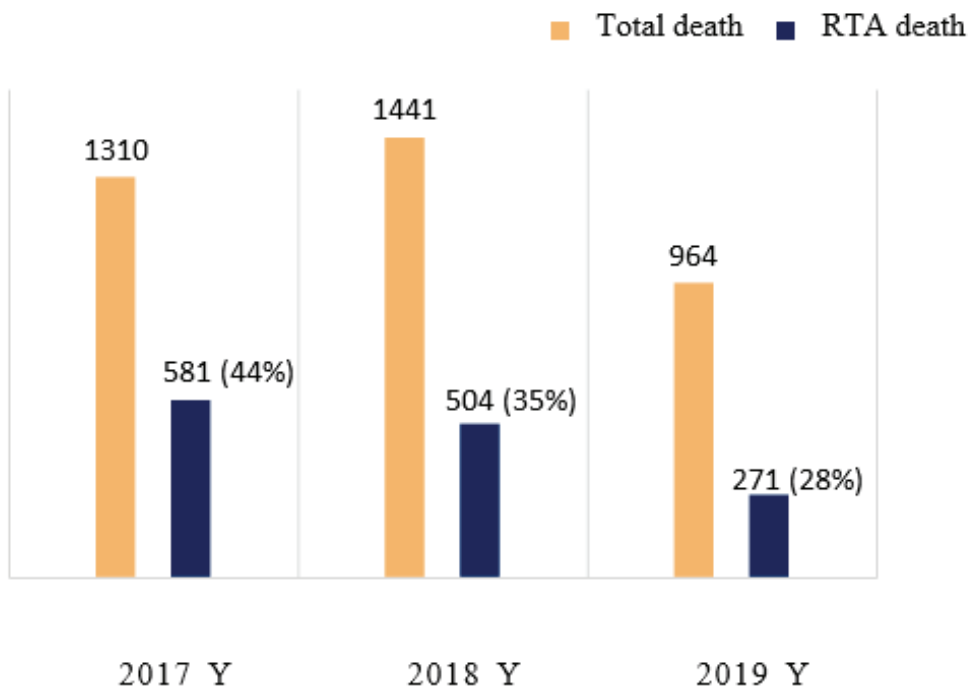


Figure (4) : Distribution of total death (N=3715) and RTA deaths by years (N= 1358).

Table (1) : Distribution of road traffic accident fatalities by direct causes of deaths

Main cause of death	Frequency	Percentage
Head injury	912	67%
Hip fracture	214	16%
Chest injury	143	11%
Miscellaneous	87	6%
Total	1356	100

Table (2) : Distribution of the road traffic accident fatalities by the place of the accident

Place	Frequency	Percentage
Main road (high ways)	805	59%
Roads in the city including pedestrians	551	41%
Total	1356	100

Discussion

Every year, so many people killed or disabled due to RTIs worldwide. In Iraq, reliable data on this health problems are scarce, nonetheless, it is one of the public health priority of Iraq that will need to be addressed and investigated in scientific way [11,25] . Analysis of these data revealed that the total mortalities for the three years were 1838 with predominance of males this finding is similar to the finding reported by other studies [10,14,26], this could be explained by the fact of high occupational exposure of males to road using and the lack of road safety in the province together with the congested roads

The current study depicts that most of the injured patients are young people in their productive age, these findings are similar to the findings of other studies conducted [27,29].

This study explains that December shows the highest occurrence of deaths due to RTAs in Babylon province this finding is in contrast with findings of other studies [8,14] this could be explained by the rainy weather and bad road safety in the province and the road congestion due to school time .

The most frequently injured body regions as found in this study was the head, this finding is in contrast with findings of other researchers [30], but it goes with the findings of other studies [31,32].

One of the main causes of the casualty in RTA is head injury associated with severe traumatic brain injury (caused by head blunt impacts [33,34]. According to the findings of different studies, car accidents were involved in 43% of traumatic brain injury [35-37] especially in low- and middle-income countries[38].Although many studies have been done to examine the mechanisms of blunt-impact head injury, the effect of skull

fracture on traumatic brain injury has not been clearly understood [39,40] Accessing the hospital emergency services in the event of a crash within a required time and proper first aid provision , road accident victims have a greater chance of survival and a reduction in the severity of the injuries.

The proportion mortality ratio in the current study is decreasing during the last two years this finding is in contrast with the findings of other local studies [8,14] this may be due to the difference in sample sizes , the duration or the differences in methodologies of data collections or may be due to the increasing of public awareness of the severity of these deadly events.

Conclusions

Most of the victims are males and in their productive age, December is the most common month during which RTA fatalities took place, head injuries constituted the highest proportion , proportional mortality ratio of RTIs is decreasing during the last two years.

Recommendations: Public awareness should be raised by using media of all kinds. We suggest that traffic law enforcement in Iraq should be seen as priority

· Improvement the neurosurgical medical and adult nursing services (intensive surgical care units) is strongly required in Babylon province to reduce the impact of this high priority health problem using multi-disciplinary team work.

· Further comprehensive large scale longitudinal or interventional studies are requested.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing and all experiments were carried out in accordance with approved guidelines.

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Fig (1): Distribution of road traffic accident by

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Study the Effect of Adding Iron Salts on the Physiochemical and Sensory Properties of Yoghurt

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Abstract

Yoghurt was manufactured by adding ferrous sulfate (T1) before heat treatment, (T2) after heat treatment, and adding ferrous chloride (T3) before heat treatment and (T4) after heat treatment as well as the control sample separately, the physiochemical tests were performed for Yoghurt the above treatments a day after manufacturing and after storage for 21 days at a temperature of 5 ± 1 ° C, and our results were: There were no significant differences in pH values after one day of manufacturing for all treatments, either during storage for 21 days at a temperature of 5 ± 1 ° C, a gradual decrease was observed in them, either with respect to the percentage of total acidity (TA), it was close to all treatments of the different Yoghurt after one day of manufacturing. either when storing, it was observed that it increased for all transactions. The concentrations (2.5, 5)Mm played a role in improving the physicochemical properties of Yoghurt, such as reduced Syneresis, increased Water holding capacity, and improved viscosity and hardness compared to other samples. As for the high concentrations (7.5, 10)Mm, it had a negative effect on the rheological properties of the Yoghurt.

Keywords: *adding iron salts, physiochemical, sensory properties, Yoghurt*

Introduction

Iron is necessary in the human diet, and when there is an imbalance or deficiency in one or more of the essential nutrients, or a deficiency or an increase in the intake of calories, it leads to the occurrence of malnutrition [1]. Iron plays an important physiological and chemical role for most Living organisms, including animals and humans alike, it is present in the structure of the Cytochrome, enzymes, hemoglobin and myoglobin [2] Milk and other dairy products such as Yoghurt and cheese are close to a nutritionally ideal food that contains all the nutrients needed for newborns, adults and the elderly, and a good source of protein, fats and carbohydrates as well as vitamins, calcium and phosphorous. However, it is generally a poor source of trace elements such as Iron, zinc, manganese and copper [3] In addition to the nutritional importance, it is known that fortifying the Yoghurt with iron can take place a chemical reaction with the various food components, which leads to changes in the physiochemical properties important to the quality properties of the Yoghurt. In order to devise the best ways to compensate for the deficiency in the iron element and to meet the daily need for it in our country and to improve the physiochemical and sensory properties of Yoghurt

Materials and Methods

A. Milk sources

used dried fat-free cow's milk (1%) fat, which was obtained from local markets from Baghdad and the product is from (Nijkerk) company

B. Yoghurt preparation and physiochemical analysis

Yoghurt manufacturing

The Yoghurt was manufactured in this study according to the method used (Jayalaitha et, 2012). (4) Salt was added to the milk before heat treatment and after heat treatment.

The dried cows' milk produced from the company (Nijkerk) was recovered into the proportion of magnetic

materials 13% with a weight of 130 g and the volume was completed with water to 1 liter. (5 liters were prepared), then the prepared quantity was divided into five sections, the first section without using the use in the manufacture of Yoghurt for the control treatment, while the other four sections were added to each liter of them ferrous chloride at a concentration of (10, 7.5, 5, 2.5) Mm / Liter of milk and mixing well, then the milk was subjected to a heat treatment at a temperature of 90 ° C for 10 minutes, then cooled to a temperature of 42 ° C. It was packed in 125 ml containers and incubated at a temperature of 42 ± 2 ° C until the coagulation was complete, within 4-5 hours, the pH was reduced to 6.4, then it was removed from the incubator for inflation and kept at a temperature (5 ± 1) until the necessary tests. After 1, 7, 14, and 21 days of processing, this process was repeated in the manufacture of samples of Yoghurt with ferrous sulfate added. The first practical process in the refining process where iron salts were added after heat treatment.].

pH determination

The pH of the Yoghurt samples was estimated after 1, 7, 14, 21 days of processing by applying a sensor pH meter type HQ 411 d (Model 211) German origin, directly in the sample of the Yoghurt

Titratable acidity determination

Titratable acidity of yoghurt samples was estimated according to A.O.A.C. (2008) [5] by taking 9 ml of milk (9 grams of yogurt) in a beaker and adding to it a few drops of phenolphthalein reagent at a concentration of 1% and then powdering it with sodium hydroxide (NaOH) at a concentration of 0.1 nm until the pink color appears, and the total acidity percentage was calculated based on Lactic acid according to the following equation:

$$\text{Total acidity} = \frac{\text{volume of NaOH 0.1 used x 0.09}}{\text{weight of the sample}} = \%\text{x } 100$$

Viscosity determination

The viscosity of the Yoghurt samples was determination at a temperature of 10 ° C after 1, 7, 14 and 21 days of refrigerated storage at a temperature of (5 ± 1) ° C using the Brookfield viscometer prepared by Brookfield Engineering Lab Inc., Stoughton, Mass. As he used the axial spindle No. 4, with a number of revolutions of 10 revolutions / minute, with a volume of 125 ml, according to what was mentioned [6].

C. Syneresis determination

Syneresis was determined according to the procedure described by [7] . A cup of the set yoghurt was removed from refrigerator at 5oC. A needle connected to syringe was used to withdraw the liquid whey from the surface of the sample and the cup of fermented skim was weight again. The process lasted for less than 10s to avoid further leakage of whey from

the Yoghurt.

D. Water-holding capacity determination

Water-holding capacity (WHC) of yoghurt was determined as described by [8] Briefly, 10 g of yoghurt was centrifuged at 5000xg for 10 min at 5oC. The resulting supernatant was carefully weighted to determine the amount of excluded water,

$$WHC\ \% = [1-(w2 / w1)] \times 100$$

[w1: weight of yoghurt used, and w2: weight of whey after centrifugation].

E. Texture determination

The evaluation of textural properties was conducted using a texture analyzer CT3(4500), Brookfield engineering lab .

The hardness of samples were measured and the operation conditions were an artificial plastic cylinder (20 mm in diameter) was inserted into each product to a depth of 20 mm with 5.0g trigger and speed of 1 mm/s .

F . SEM scanning electron microscopy test

For SEM, sections 3 × 3 × 3 mm were excised from the yoghurt, approximately1 cm below the surface and were fixed in 2.5% glutaraldehyde solution in phosphate buffer at pH of 7.4 for 1 days (9). The section were then cut into prisms 3 × 3 × 3 mm, dehydrated in a graded ethanol series (10,20,30, 40, 50, 60,70, 80, 90, and 100%), For a period of 30 minutes per concentration. After the completion of drying the samples were frozen, after which the frozen samples were permanently dried

by the (ALPHA 1–4 CHRIST) lyophilizer.

The samples were placed in aluminum containers of a scanning electron microscope and coated with a layer of gold and bilenium water with a thickness of 5 nm. Images were acquired with a voltage of 1.4 kV and different enlargement powers.

G. Sensory evaluation

Sensory evaluation of Yoghurt milk fortified with iron salts was performed after 1, 7, 14 and 21 days of manufacture. The samples were presented in small cups of (125 ml) capacity and were evaluated by a number of professors at the College of Food Sciences / Al-Qasim Al-Green University. The samples were evaluated in terms of flavor, texture, appearance and color according to the sensory evaluation form previously developed. By[10].

Statistical Analysis

All possible comparisons among the means were carried out by using Least Significant Difference (LSD) test at the significant level of 0.05 after they show their significant in the general test

Results and Discussions

The effect of adding iron salts on the pH of Yoghurt:

The pH is a major factor in determining the amount of development of the casein network responsible for Yoghurt formation [11,12] .

The results shown in Fig. (4-1)A, B, C, D the pH of Yoghurt with added iron salts for (T1), (T2), (T3), and (T4) respectively.

The results of the statistical analysis of the above factors indicate that there are no significant differences (P <0.05) between the pH values of the control sample and the samples with iron added. These results show that the addition of iron before and after the heat treatment has no effect on the pH of the Yoghurt, as it does not affect the starter culture bacteria, and this is consistent with what was found [13] which showed that there was no significant effect of adding ferrous sulfate to Yoghurt e on the numbers of bacteria. The beginning, and by advancing the storage period at a temperature of ± 5 ° C. We notice a gradual decrease in the pH values of all samples, and this is due to the continued activity of the starter culture bacteria in converting lactose into lactic acid [12].

We also note that the pH values of (T4) were slightly higher than treatment (T3) and this is due to the effect of the double heat treatment that led to the expulsion of a greater amount of CO2 and also the removal of amine groups from the protein, which leads to the formation of ammonia that raises the pH of the Yoghurt (Al-Saadi et al2012.)(14).

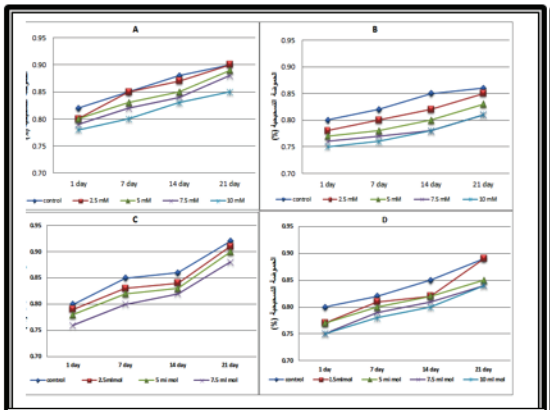


Figure (4-2) The effect of adding iron salts on the acidity of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

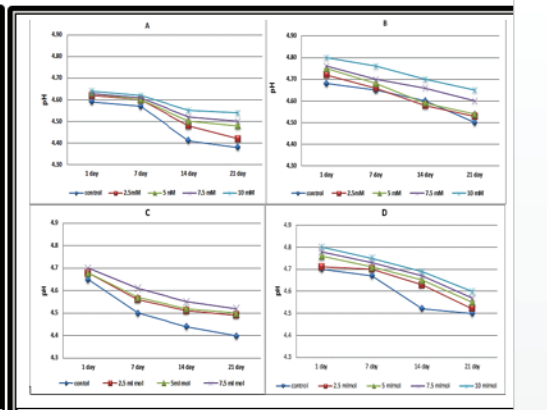


Figure (4-1) The effect of adding iron salts on the pH of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

Through the figure (A, B, C, D), we find the pH values of the samples with added iron salts, a non-significant increase compared to the control samples, and the reason for this is due to the possibility that these salts work to form buffer solutions that reduce the changes in pH values.

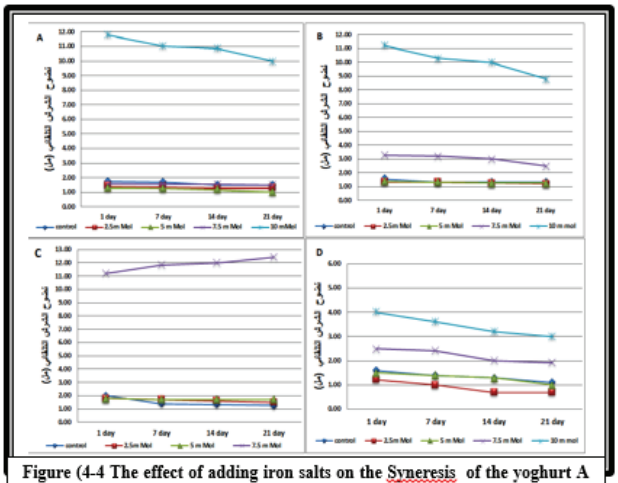


Figure (4-4 The effect of adding iron salts on the Syneresis of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

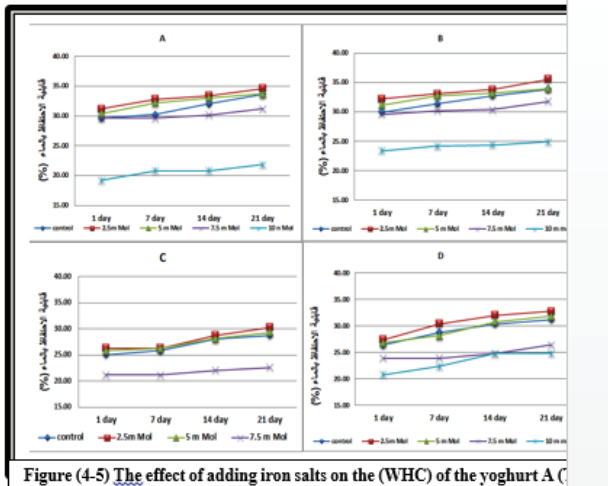


Figure (4-5) The effect of adding iron salts on the (WHC) of the yoghurt A (Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulf after the heat treatment C (T3) addition of ferrous chloride before the h treatment D (T4) Addition of ferrous chloride after heat treatment

The effect of adding iron salts on the Yoghurt acidity:

Figure (4-2) (A, B, C, D) shows the values of the acidity based on the lactic acid of the Yoghurt with iron salts added for the treatments (T1), (T2), (T3), (T4) respectively.

The results of the statistical analysis of the above factors showed that there were no significant differences ($P < 0.05$) between the restorative acidity values of the control sample and the samples with iron added during the storage period due to the lack of effect of adding iron before and after the heat treatment on the total acidity of the Yoghurt and this is due to its lack of influence on the starting bacteria Responsible for the production of lactic acid (15).

As for after storage at a temperature of 5 ± 1 ° C for a period of 21 days, an increase in the acidity values of the control sample and the samples added to it (2.5, 5, 7.5 and 10) Mm of iron are observed, and these results are consistent with (Ziena et al.2019)(15) referred to. It indicated an increase in the overall acidity of the Yoghurt e fortified with ferrous sulfate after storage due to the increase in the activity of the starting bacteria and its persistence in the production of lactic acid and its non-effect on the starting bacteria.

From Figure (4-2) A, B, C, D, we find the existence of non-significant differences in the parameters to which iron was added after the heat treatment (D, B), as their values were less than the treatments that were added to iron before the heat treatment (C, A). This is due to the effect of double heat treatment on the pH of the Yoghurt (14)

The effect of adding iron salts on Syneresis from Yoghurt:

The Fig. (4-4)A, B, C, D showed the level of Syneresis of Yoghurt with added iron salts for the treatments (T1), (T2), (T3) and (T4), respectively, significant differences were observed In their values after storage for one day at 5 ± 1°C.

The levels of Syneresis after one day of storage. For the treatment (T1) shown in Figure (4-4) A for the control sample was (1.76) ml, and the samples with added (2.5, 5, 7.5 and 10)Mm of ferrous sulfate were (1.42, 1.32, 1.60 and 11.80) ml, respectively.

And for the treatment (T3) in Fig. (4-4) C, the control sample was (2.00) ml, and the samples added to it (2.5, 5, and 7.5) Mm ferrous chloride before the heat

treatment were (1.80, 1.82 and 11.20) ml, respectively. The results shown the addition of ferrous chloride after heat treatment at concentrations of (2.5 and 5) Mm reduced the levels of spontaneous exudation compared to the control sample, this is consistent agrees with (Sadiq & Doush, 2019)(20), where addition of coated and non-coated iron improved the The Syneresis from Yoghurt due to the iron-protein bonds. As for the concentration of 10 Mm, it was the highest in the level of Syneresis. This is due to the weakening of the high concentrations of the protein network due to the precipitation of a part of the casein (17).

The effect of adding iron salts on the water holding capacity of the Yoghurt:

The (WHC) depends on the proteins present in the food, and the type of amino acids that are included in the natural protein composition, And the amount of polarity of amino acids and the extent of hydrophobicity or hydrophilicity ((22

The Fig. (4-5)A, B, C, D showed the (WHC) of Yoghurt with added iron salts (T1), (T2), (T3) and (T4), respectively.

The (WHC) after one day of storage. at 5 ± 1 ° C. For the treatment (T1) in Fig. (4-5) A for the control sample was 029.7% and for the samples with added (2.5, 5, 7.5 and 10) Mm ferrous sulfate before the heat treatment were (31.20, 30.40, 29.60 and 19.20)%, respectively.

We find that the addition of iron in this treatment at concentrations of (2.5 and 5)Mm improved the (WHC) for Yoghurt compared to the control sample, although the differences are not significant, but the reason is that the iron formed bonds with the protein, which confined a larger amount of water inside it (21) compared to the concentrations (7.5 and 10)Mm, whose ability to preserve water was less than the control treatment, since the higher concentrations affected the structural composition of the Yoghurt due to the reduction of the charge on the caseins, thus reducing its ability to bind with water. (23).

And for the treatment of (T2),in Fig (4-5) B, the control sample was 30.00%, and the samples with added (2.5, 5, 7.5 and 10) Mm ferrous sulfate after the heat treatment were (32.20, 31.20, 29.60 and 23.40)%, respectively. .

The results indicate that the addition of ferrous sulfate after heat treatment at concentrations of (2.5 and 5)Mm improved the (WHC) of Yoghurt compared to the control sample. As the concentrations (7.5 and 10) Mm , their ability to (WHC) was less than that of the control treatment, since the high concentrations affected the structural composition of the Yoghurt (23).

The treatment (T3) in Fig. (4-5) C for the control sample was 25.04%, and for the samples with (2.5, 5 and 7.5) Mm ferrous chloride added before the heat treatment were (26.28, 25.80 and 21.20)%, respectively.

We find that adding ferrous chloride before heat treatment with (2.5 and 5)Mm improved the (WHC) of Yoghurt compared to the control sample, this is result consistent with (19) who founded the ability of the protein network associated with iron increase binding Water and that the link increases with the increase in the amounts of added iron, to certain concentrations, the protein network begins to collapse, and this is happened in the concentration of 7.5 Mm in this treatment .

For the treatment (T4), in Fig. (4-5) D, the control sample was (26.40)%, and the samples to which were added (2.5, 5, 7.5 and 10)Mm ferrous chloride after heat treatment were (27.40, 26.80, 23.92 and 20.80)%. respectively.

These results indicate the ability of iron chloride added after heat treatment at concentrations of (2.5 and 5) Mm increase the (WHC) of the Yoghurt.

After storing at 5 ± 1 ° C for 21 days, we find an increase in the percentage of (WHC). It was for (T1) (34.60, 33.70, 31.20, 21.80)%, (T2) (35.60, 34.00, 31.80, 24.90)%, (T3) (30.24, 29.28, 21.61) % (T4) (32.80, 31.80, 26.40 and 24.80) %, respectively, and this is due to the increase in strength, and the number of bonds that iron made with milk proteins, this result is consistent with (21) found, increase in (WHC) of Yoghurt treated with iron after storage is due to the increase in the number and strength of the bonds formed by iron with protein.

Through Fig. (4-5) D, C, B, A, shone that the (WHC) of Yoghurt fortified with ferrous sulfate was higher than the samples fortified with ferrous chloride and for both

cases of addition before and after the heat treatment. We also find that the percentage of retention ability in the figures (D , B) were higher than (C, A) due to the role of double heat treatment in increasing the denaturation of the whey proteins and increasing the binding capacity of a greater amount of iron (24).

The effect of adding iron salts on the viscosity of Yoghurt:

The figure (4-3) A,B,C,D showed the viscosity of the Yoghurt with iron salts added for the (T1), (T2), (T3) and (T4), respectively, in which significant differences were observed in their values after and after storage for one day at a temperature of 5 ± 1 ° C, it was For the treatment (T1) in Fig. (4-3) A to the control sample (2800) centipoise and the samples with added (2.5, 5, 7.5 and 10)Mm of ferrous sulfate before the heat treatment were (2940, 2980, 2400 and 680) centipoise, respectively.

We find that adding ferrous sulfate before the heat treatment with (2.5 and 5) Mm improved the viscosity property and its values were higher than the control sample values due to the ability of iron to extend bridges between casein particles, which leads to an increase in its size and thus improving the viscosity .These results are in line with The result reached by (15), as for the high concentrations, they had a negative effect, as the concentrations (7.5 and 10) Mm reduced the viscosity values compared to the control sample, as the addition of iron above the saturation level of casein leads to its precipitation and thus the viscosity values decrease, this is in agreement. With (23), where they stated that adding iron to skim milk occurred protein precipitation when the concentration of iron added to milk was about (8) Mm.

And the treatment (T2), showed in Fig. (4-3) B, the control sample was (4300) and the samples with ferrous sulfate added after heat treatment (2.5, 5, 7.5 and 10) Mm were (4390, 3370, 1990 and 300) centipedes, respectively.

We find that the concentration of 2.5 Mm of ferrous sulfate added after the heat treatment improved the viscosity, while the viscosity values of the concentrations higher than 2.5 Mm were decreased compared to the control sample. This is due to the competition of iron-added calcium from binding to casein, which caused the small size of casein particles, this is in agreement with . (Mittal et al. 2015)(24) indicated that when calcium is depleted and iron is added at concentrations of (5, 10, 20) Mm, the added iron is responsible for the high ability to bind to proteins in milk instead of calcium.

The effect of adding iron salts on the hardness of the Yoghurt

The above results show that the addition of ferrous in thise treatment improved the hardness of the Yoghurt, especially the concentrations (2.5 and 5) Mm. As for the high concentration of iron (7.5)Mm, it led to a decrease in the level of hardness because the addition of iron to the protein above a certain concentration, when The binding sites are saturated, leading to protein precipitation . This is in agreement with indicated by (25).

The treatment (T4) showed in Fig. (4-6) D, to the control sample was (98.60) gm., and for the samples with (2.5, 5, 7.5 and 10) Mm of ferrous chloride added after the heat treatment were (101.30, 81.70, 67.60 and 65.10) gm. , respectively.

We find the existence of an effect of adding ferrous chloride after heat treatment positively on the hardness values of Yoghurt at a concentration of only 2.5 Mm compared to the treatment (T3) that affected the concentrations (2.5 and 5)Mm. As for the higher concentrations, it negatively affected the protein structure compared to the control sample due to the effect of the treatment. Thermocouple increases the iron binding and thus the effect on the protein network.

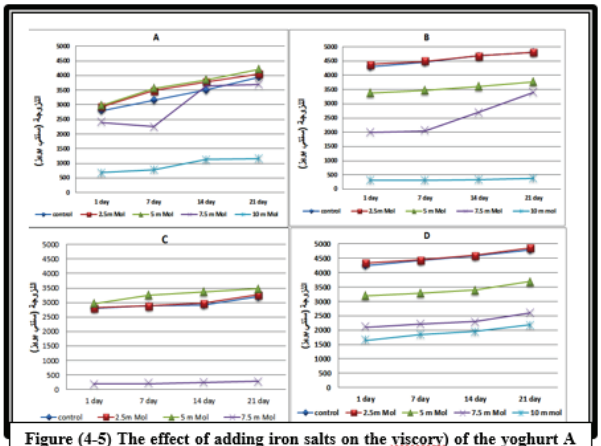


Figure (4-5) The effect of adding iron salts on the viscory) of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrous sulfate after the heat treatment C (T3) addition of ferrous chloride before the heat treatment D (T4) Addition of ferrous chloride after heat treatment

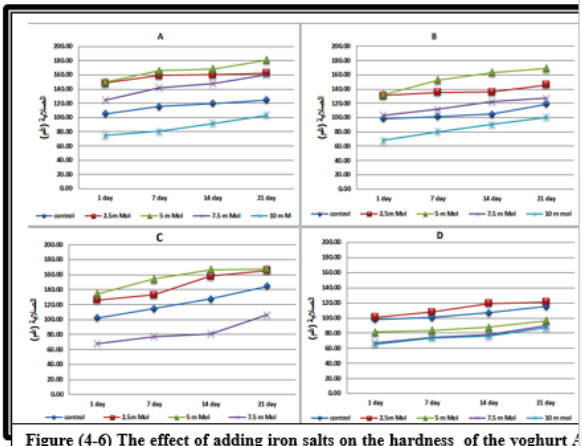


Figure (4-6) The effect of adding iron salts on the hardness of the yoghurt A (T1) Addition of ferrous sulfate before the heat B (T2) Addition of ferrou sulfate after the heat treatment C (T3) addition of ferrous chloride before th heat treatment D (T4) Addition of ferrous chloride after heat treatment

. Thermocouple increases the iron binding and thus the effect on the protein network.

However, after 21 days of storage at a temperature of 5 ± 1C °, the hardness values of all samples increased, reaching (T1) treatment (162.40, 181.10, 160.90, 103.50) gm, (T2) (145.90, 169.30, 127.20 and 100. 60) gm, (T3) (165.80, 168.00 and 106.60) gm, (T4) (121.40, 96.40, 89.70 and 86.80) gm. Accordingly, as a result of protein aggregation and the bonds formed by iron that increase the cohesion within the protein network and increase its hardness (28).

From Fig. (4-6) D, C, B, A, we note that there are differences in the parameters to which iron was added after the heat treatment (D, B). The hardness values were less than the treatments that were added to iron before the heat treatment (C, A). This is due to the effect of heat treatment on increasing the accumulation of the denitrified whey proteins, and this is consistent with what was found by (W. Gustaw, 2007)(29).

Conclusions

The possibility of manufacturing yoghurt fortified with iron salts at certain concentrations without any change in composition compared with the yoghurt for the control treatment. The addition of iron salts to the yoghurt did not lead to significant differences in the pH values compared to the control treatment. Concentrations (2.5, 5) Mm of iron salts, improved the physicochemical properties of yoghurt in terms of reduced spontaneous perfusion, increased water retention, and improved viscosity and hardness compared to other samples. The high concentrations of iron salts (7.5, 10)Mm had a negative effect on the rheological and sensory characteristics of the yoghurt. By SEM scanning electron microscope, the effect of adding iron salts was observed on the protein network binding and improving the yoghurt properties.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Dairy Science and Technology and all experiments were carried out in accordance with approved guidelines.

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Prevalence and Correlates of Vitamin D Inadequacy in a Sample of Iraqi People

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Abstract

Background: Vitamin D had been identified as a common metabolic/endocrine abnormality. Middle East countries had a very high rate of vitamin D inadequacy, vitamin D deficiency was associated with non-communicable and infectious diseases.

Objective : To identify the prevalence of Vitamin D level and its correlates among Iraqi people in Al-Hilla city-Babylon province

Methods :. A cross-sectional study was conducted on a convenient sample of individuals from all age groups and both genders who attended eight primary health care centers and individuals in Al-Hilla city\Babylon province\Iraq, the study period was started from May through October , 2019. A pretested questionnaire was used for data collection , the questionnaire included information regarding socio demographic characteristics of participants and their history of chronic diseases. The following measurements were done: level of serum vitamin D , blood pressure, blood sugar , body weight and weight and the serum of vitamin D .

Results: The sample included 188 participants their mean age was 64.8 ± 17.25 years.The female to male ratio 1.7:1, More than half of participants (56%) resident in urban areas . Vitamin D mean level was (20.7 ng/mL ± 14.9 SD). The vast majority (80%) of the sample have vitamin D inadequacy in which (66% have deficiency and 14% have insufficiency), while 37 (20%) of the sample have vitamin D adequacy.

A significant inverse relationship between vitamin D serum level and hypertension, type two diabetes mellitus, increase body weight.

Conclusion: there was a high prevalence of vitamin D inadequacy among Iraqi people mainly in females, a significant association between low vitamin D level and hypertension, type two diabetes mellitus, and obesity.

Keywords: *Prevalence, Vitamin D inadequacy, associated factors, Iraq*

Introduction

Vitamin D inadequacy is a high priority public health problem globally and affected all ages. Vitamin D inadequacy (both deficiency and insufficiency) was a problem even in sunny countries. This problem particularly was high in the Middle East, especially among females, it had been estimated that 20–100% of USa, Canadian and European elderly men and women were vitamin D deficient. Additionally, other age groups such as; children, young adults and the middle aged are also at risk of vitamin D deficiency. [1,2] Prevalence of Vitamin D deficiency was common among elders nursing home residents [3] Low levels of vitamin D have been implicated in a wide variety of conditions and highly prevalent in the geriatric population, including fractures, functional limitations, cancer, cardiovascular disease.

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Evidence suggests that vitamin D supplementation may decrease mortality rates [4]. The prevalence of vitamin D inadequacy varied not only between different countries, but there were intra country variations. It may be as low as 3% [5] and 11.3% [6] or as high as 87.8% and 98% [8,9]. Information from different studies on the vitamin D serum levels among Iraqis over the last years indicate a resurgence of vitamin D inadequacy in otherwise healthy and non-healthy members both males and females in all age groups of the population [10-21]. An inverse relationship was identified between vitamin D serum levels and a number of chronic disease-predisposing conditions such as metabolic syndrome insulin resistance , hypertension ,and obesity [22-25]. This suggests that vitamin D insufficiency/deficiency may play a role at the early stages of the natural history of many chronic diseases [26,27]. This study was done to measure the prevalence of vitamin D in adequacy among Iraqi population and to identify the associations between vitamin D levels and certain chronic diseases such as hypertension , type two diabetes mellitus , and obesity or overweight**.**

Methodology

This is across sectional observational descriptive study which includes a convenient sample of apparently healthy individuals from all age groups and both genders living in Al- Hilla City, Babylon province, Iraq, the period of the study started from the first of May through September 2019, the sample size was calculated according to the sample size calculation equation with 95% confidence level, 188 individuals who are the attendees of eight randomly selected primary health care centers and the residents of Babylon elders home are participated voluntarily in this study after obtaining their informed oral consents , after explaining the objectives and the measurements procedures of the study . This study was approved by the Ethics Committee of College of nursing - University of Babylon. A pretested questionnaire(contained questions about demographic characteristics of the participants , their past medical history and history of individual life style habits), this tool was used to interview the participants . Measurements of mean systolic and diastolic blood pressure of each participant were done single handy by one of the researchers. Blood pressure was measured in the sitting position. Patients were kept seated quietly for at least 5 min in a chair**.** Blood pressure was measured using a sphygmomanometer with an appropriate cuff the cutoff values, blood pressure more than 140\90 considered hypertension or those who diagnosed previously as hypertensive and taking antihypertensive drugs. Peripheral venous blood samples were collected from each participant to measure Serum level of Vitamin D which was measured by chemo immunoassay method (maglumi instrument) and the random blood sugar, blood sugar level above 200mg/dL considered diabetic as well as individual who was previously diagnosed as type 2 diabetes mellitus. Vitamin D levels are divided into three categories .deficient < 20ng/ml, insufficient between 23-29ng/ml and the normal level is 30-100 ng/ml [28,29]. body weight and height were measured to calculate body mass index according to the WHO classification [30].

Data were analyzed by using (spss) version (23). Continuous quantitative data were expressed as mean and ±SD, while non-parametric numerical data were expressed as median , with the value of categorical data presented as a number (percentage), t student test and analysis of variance were calculated . Chi square test was done and the Kruskal–Wallis and Mann–Whitney tests were used to compare non-parametric and quantitative variables between groups. P-value < 0.05 considered statistically significant.

Results

A sample of 188 individuals from different settings were participated their mean age ± SD was 64.8 ± 17.25 years ranged from 3 to 89 years; older people ≥ 60 years formed 83% of them as shown in figure [1]. Female to male ratio 1.7:1, figure [2]. More than half of participants (56%) resident in urban, figure [3]. Vitamin D mean level was (20.7 ng/mL ± 14.9 SD). The vast majority 151 (80%) of the sample have vitamin D inadequacy in which (66% have deficiency with level ≤ 20 ng/mL and 14% have insufficiency with level 21-29 ng/mL), while 37 (20%) of the sample have vitamin D adequacy with level ≥ 30 ng/mL, figure [4].

Table 1: shows the mean rank differences for vitamin D level according to the demographic characteristics depending on Mann- Whitnny ranking statistical test, women have asignificant low vitamin D level, vitamin D inadeqaucy increased significantly with advancing age while no significant difference was detected in place of residency.

Mean ± SD differences of blood pressure, blood sugar and BMI among participants with vitamin D inadequacy . Low vitamin D is associated significantly and inversly with blood pressure both systolic and diastolic together with high random blood sugur and the Body Mass Index table(2).Table (3) and figures 6,7,8 and figure 9 reveal the inverse correlation between serum vitamin D and blood pressure (systolic , diastolic), blood sugar and the Body Mass Index respectively while there is a positve correlation between vitamin serum leve and age (figure 5).

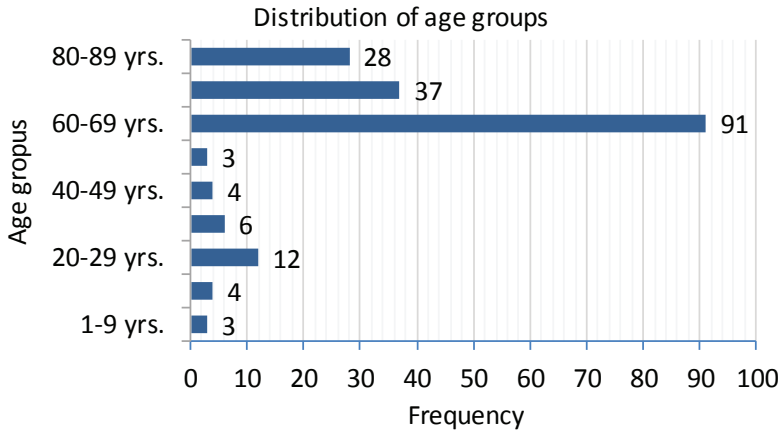
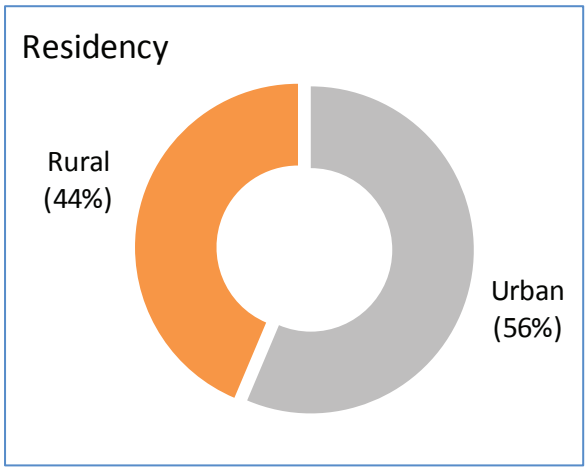


Figure 1: Frequency distribution of sample according to age groups (N=188).



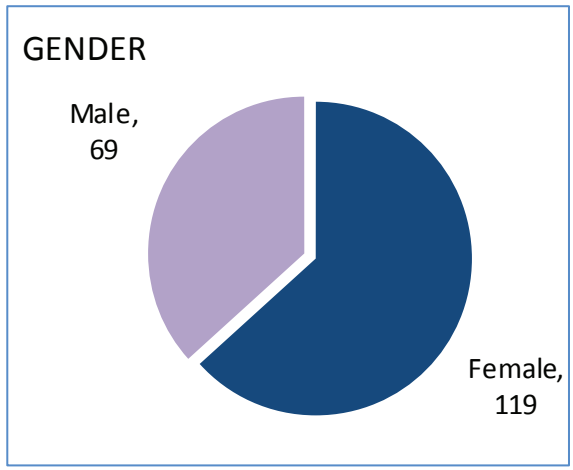


Figure 2: Gender distribution (N=188) Figure 3: Urban-rural distribution (N=188)

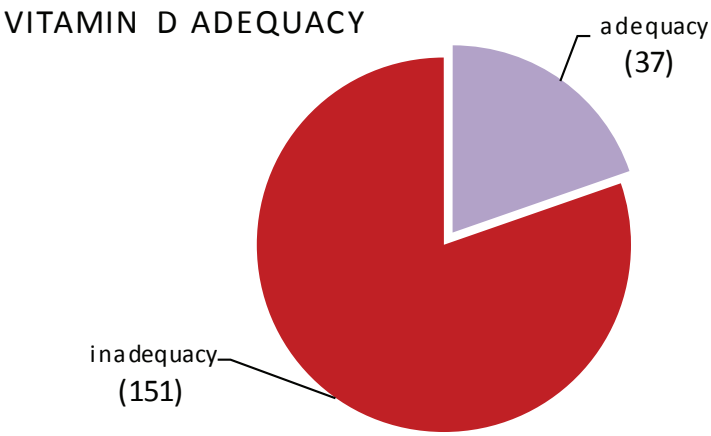


Figure 4: Sample distribution based on vitamin D adequacy (N=188)

Table 1: Mean rank differences for vitamin D level according to the demographic characteristics (N=188).

Demographic ,		Vitamin D level	Mann-Whitney U	Sig.
Gender	male (n=69)	106.51	3277.000	0.021
	female (n=119)	87.54		
Age groups (years)	≤ 59 (n=32)	74.69	1862.000	0.024
	> 60 (n=155)	98.56		
Residency	urban	93.47	4236.500	0.767
	rural	95.84		

Table 2: Mean ± SD differences of blood pressure, blood sugar and BMI among participants with vitamin D sufficiency, insufficiency and deficiency (N=188).

Measurements ,	Vitamin D levels			F*	Sig.
	Sufficiency (n=37)	Insufficiency (n=27)	Deficiency (n=124)		
Systolic BP (mmHg)	130 ± 10.7	138 ± 14.6	144 ± 16.2	13.44	0.0001
Diastolic BP (mmHg)	84.0 ± 5.7	86.0 ± 7.7	90.5 ± 9.1	9.83	0.0001
blood sugar level (mg/dL)	135 ± 47.6	176 ± 53.6	216 ± 64.7	26.97	0.0001
BMI	26.8 ± 4.8	30.3 ± 7.3	35.1 ± 8.8	15.90	0.0001

* ANOVA Test

Table 3: Correlation of Vitamin D level with age, systolic/ diastolic blood pressure, blood sugar and BMI (N=188)

Correlation Coefficient*		Variables ,				
		Age	Systolic	Diastolic	B. sugar	BMI
Vit. D	r	0.156	-0.386-	-0.384-	-0.429-	-0.348-
	Sig.	0.033	0.0001	0.0001	0.0001	0.0001
Age	r		0.054	0.026	-0.014-	-0.200-
	Sig.		0.461	0.726	0.848	0.010
Systolic	r			0.792	0.555	0.290
	Sig.			0.0001	0.0001	0.0001
Diastolic	r				0.515	0.231
	Sig.				0.0001	0.0001
B. sugar	r					0.350
	Sig.					0.0001
* Correlation is significant at the 0.05 level (2-tailed).						

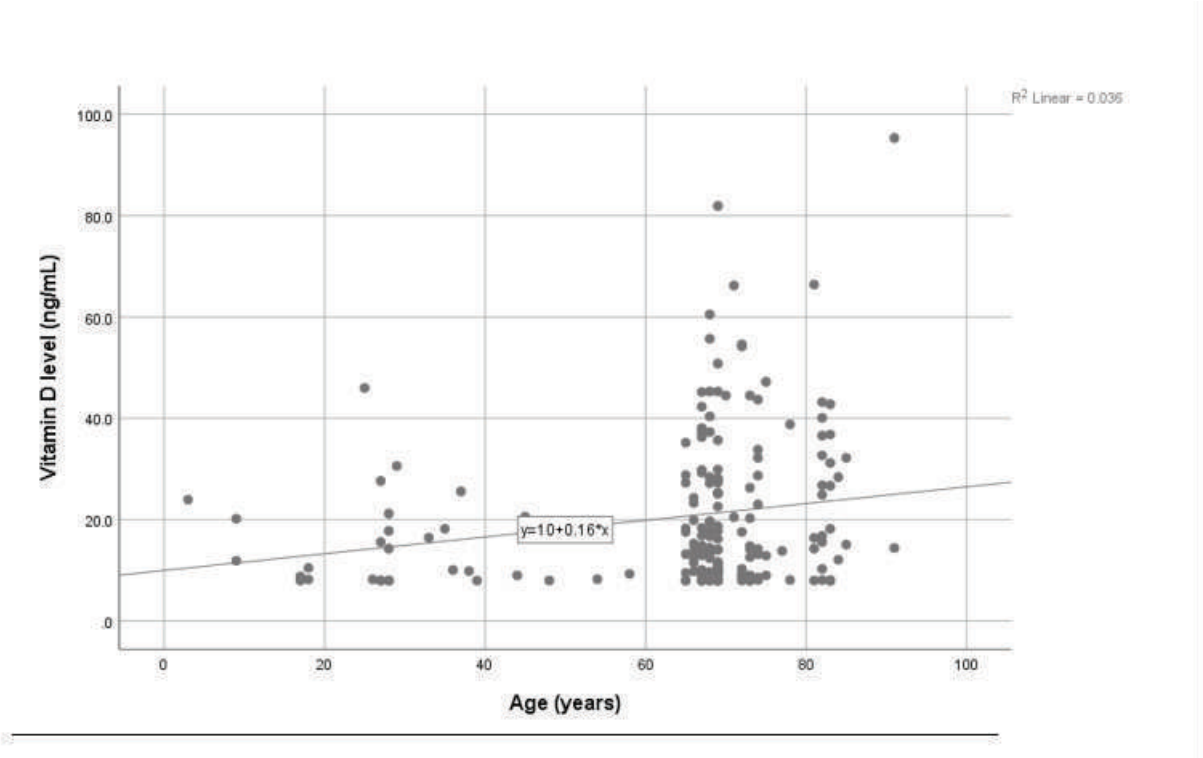


Figure 5: Correlation between age (years) and vitamin D level (ng/mL), (N=188).

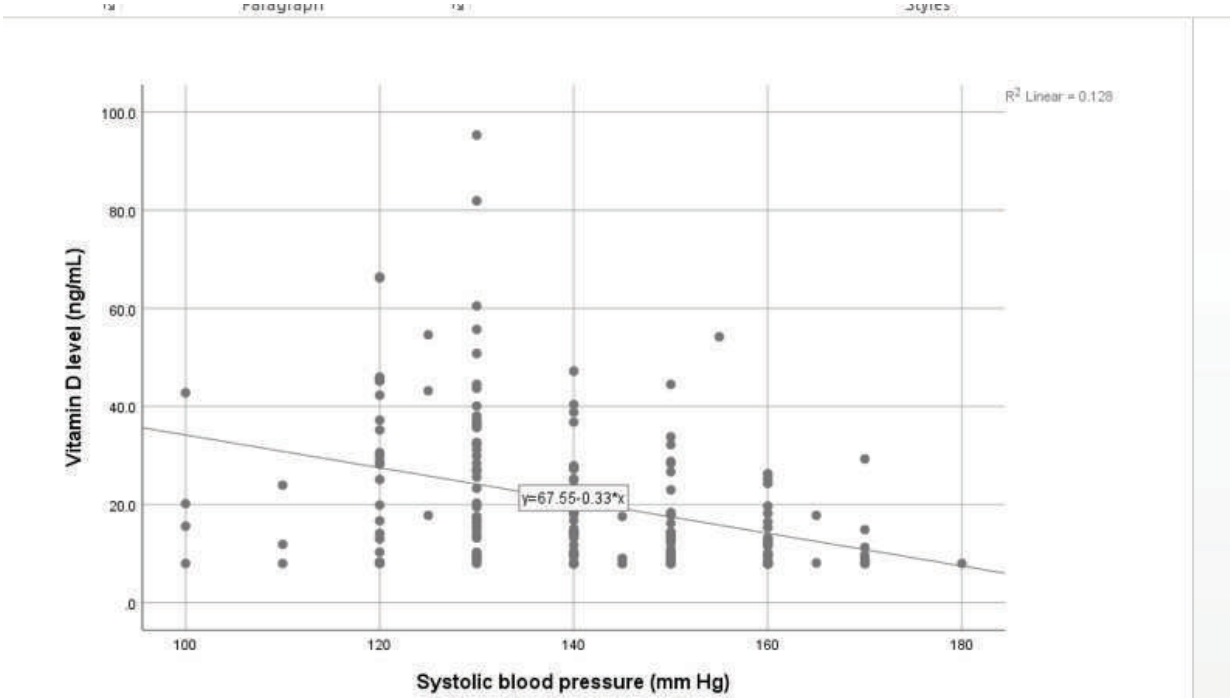


Figure 6: Correlation between systolic blood pressure (mm Hg) and vitamin D level (ng/mL), (N=188).

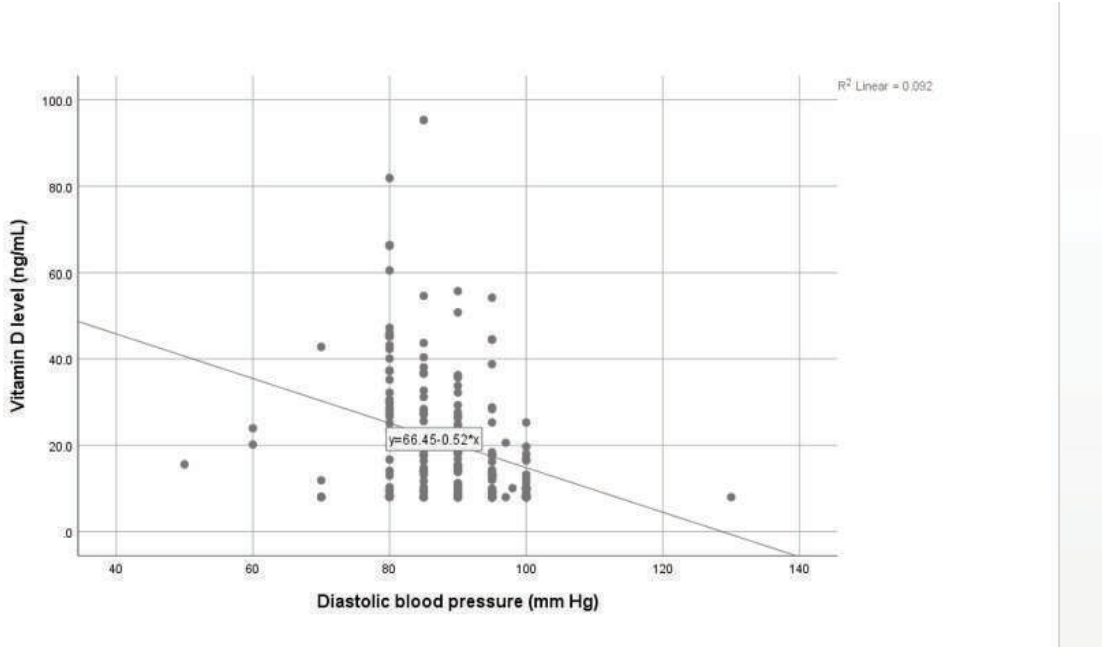


Figure 7: Correlation between diastolic blood pressure (mm Hg) and vitamin D level (ng/mL), (N=188).

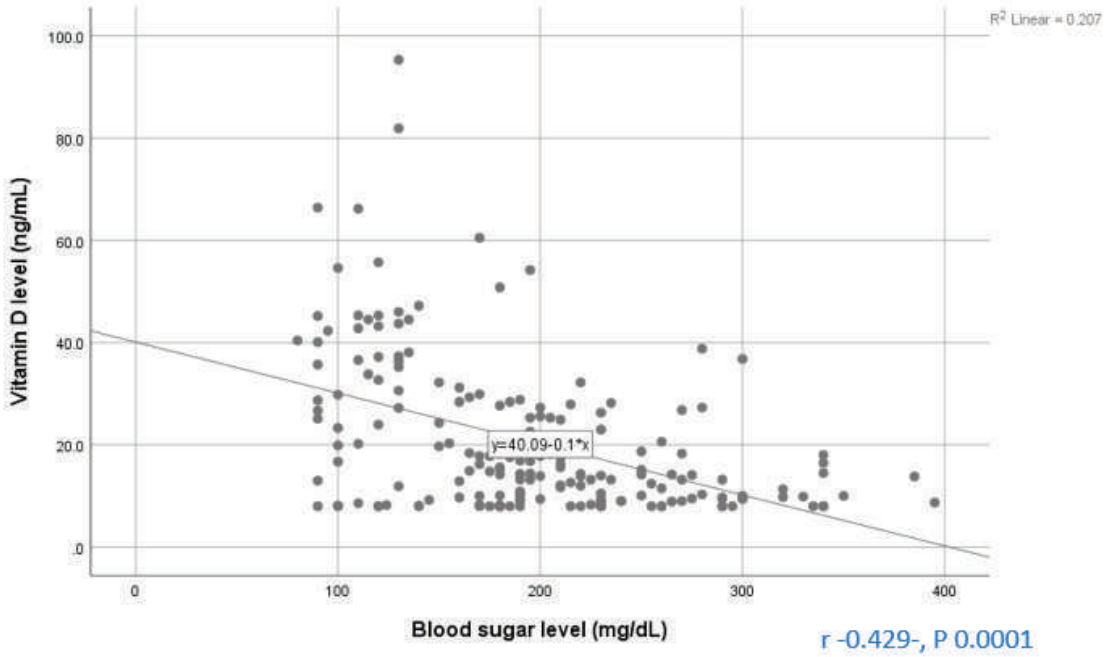


Figure 8: Correlation between blood sugar level (mg/dL) and vitamin D level (ng/mL), (N=188).

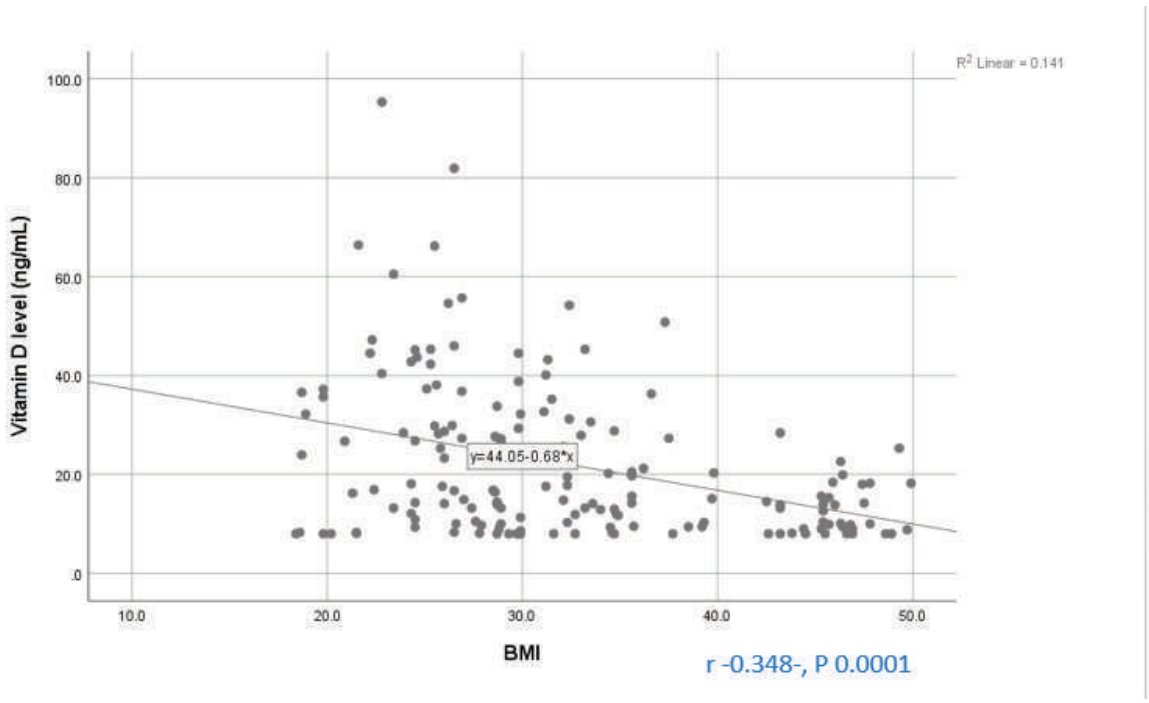


**Figure 9: Correlation between BMI and vitamin D level (ng/mL), (N=164). **

Discussion

This study reveals that there is alarming high prevalence(80%) of vitamin D inadequacy in a sample of Iraqi people, vitamin D inadequacy varied between different countries, It may be as low as 3% [5] and 11.3% in Irbid in Jordan [6] or as high as 87.8% among healthy Saudi Arabian men [7]. About 90% of the Qatari population may have insufficient levels of serum vitamin

D [27]. in the United States, approximately 25% were at risk of vitamin D insufficiency and 8% were deficient [17]. Our estimate is similar, if not higher, than that reported from other Arab and Gulf countries like United Arab Emirate [32] .Prevalence among adolescent Saudi girls and women indicates the severity of the insufficiency with up to 80% [33]. This high prevalence of low vitamin D status is thought to be linked to socio-cultural factors, the habit of not fortifying dairy products, and poor vitamin D supplementation [32-35]. The prevalence of vitamin D inadequacy in this study is slightly less than what was reported in recent study among females in Karbala Province – Iraq [14] but it is a bit higher than what was reported by other local study conducted on women at their reproductive age [18]. The finding of the current study depicts an inverse correlation between low serum vitamin D and both systolic and diastolic blood pressure, random blood sugar and obesity this findings are similar to the findings of other studies [15,16,26,27].

In conclusions alarming high prevalence of vitamin D deficiency among Iraqi people specially among females have been demonstrated , a positive significant associations between vitamin D inadequacy and hypertension, type 2 diabetes mellitus and obesity, we suggested the carrying out of large scale, studies that might lead to effective reforming actions by policy makers in Iraq regarding vitamin D food fortification and the establishment of interventional preventive local program. Health professionals, policy makers, and the general public in Iraq should be aware of the high prevalence of vitamin D deficiency and the associated health risks.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon and all experiments were carried out in accordance with approved guidelines.

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Study of Type of Bacteria That Found in Ear, Mouth, Nose of Second Stage Nursing Department Student

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Abstract

The samples collected from 70 nursing student second stage ,the sample was mouth ,ear ,nose .The sample cultured on nutrient agar ,MaCconkey agar ,Mannitol agar ,and brain heart agar. Observed different type of bacteria on MaCconkey agar (lactose fermenting ,lactose non fermenting ,lactose slow fermenting ,Mucoid shape ,and different type of bacteria on Mannitol Agar and nutrient agar .after that the isolate tested with Gram stain , Catalase test and then Analytic profile index perform. The results showed the bacteria is (5) (0,5%)isolates of Staphylococcus aureus and (2)(0,2%) isolates of pseudomonas aeruginosa from 70 ear samples , (5)(0,64%)isolates Staphylococcus aureus and (4)(0,51%)isolates of Staphylococcus epidermedis from 70 mouth samples and 5(0,5%) isolates from pseudomonas aeruginosa ,(2)(0,2%) isolates of Serratia marcescens from70 nose samples.And then the antibiotic sensitivity tested against :

Ciprofloxacin ,Gentamycin,Vancomycin,Pencilin,Azithromycin,Cefex,Amikacin,levofloxacin3 and the result as in table below :

(Antibiotic sensitivity test result)

Antibiotics / Bacteria	VA	p	ATM	CN	AK	LEV	CIP
Staphylococcus aureus	+	-	+	+	+	+	+
Staphylococcus epedermidis	-	-	+	-	+	+	+
Pseudomonas aeroginosa	+	+	+	+	+	+	+
Serratia marcescens	+	-	-	-	-	+	+

+ mean sensitive , - mean resistant

Key words: *Nose , mouth, ear microbe*

Introduction

Oral microbes are an important part of the human microbiome, and the unique and diverse microflora, distributed in various oral ecological niches such as the tongue dorsum and the periodontal crevice and pockets, are indispensable .The human oral cavity contains a number of different habitats, including the teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils, which are colonized by bacteria. The oral microbiome is comprised of over 600 prevalent taxa at the species level, with distinct subsets predominating at different habitats [1]. The oral cavity is comprised of many surfaces, each coated with a plethora of bacteria, the proverbial bacterial biofilm. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans. For example, it has been estimated that at least 35% of dentate U.S. adults aged 30 to 90 years have periodontitis. In addition, specific oral bacterial species have been implicated in several systemic diseases, such as bacterial endocarditis , aspiration

pneumonia , osteomyelitis in children , preterm low birth weight , and cardiovascular disease . Surprisingly, little is known about the microflora of the healthy oral cavity [2-6]. Anaerobic bacteria in the oral cavity include: Actinomyces, Arachnia, Bacteroides, Bifidobacterium, Eubacterium,Fusobacterium,Lactobacillus,Leptotrichia, Peptococcus, Peptostreptococcus, Propionibacter(6). Veillonella. The nasal cavity of 97 young healthy men (applicants for the pilot education in the Finnish Air Force) was examined. Bacterial culture demonstrated one or more species of aerobic bacteria in all 194 nasal cavities examined and anaerobic bacteria in 76.5%. In ten per cent of the cultures bacteria were detected after enrichment only. The most common aerobic bacteria were Staphylococcus epidermidis (79%), diphtheroids (41%) and Staphylococcus aureus (34%). Haemophilus influenzae was found in 5% and Streptococcus pneumoniae in 0.5%. [7] The microbial flora of the outer ear canal was determined for 77 healthy individuals (M = 44, F = 33). No growth of any microbe was found in 5% of males and 15% of females. Coagulase-negative staphylococci, dominated by Staphylococcus epidermidis, were the commonest microbe group found (83% of persons sampled). Staphylococcus aureus was found in 7% males and no females. Diptheroids were cultured from 32% of the samples. [8] The outer ear is exposed to the external environment and much like skin on other parts of the human body is in contact with microbial life. Both the auricle and the external auditory meatus house a variety of microbes under healthy conditions. The outer ear is exposed to the outside oxygen-filled environment, the majority of the bacterial flora on the auricle and in the external auditory canal is made up of aerobic species. The outer ear is home to a diverse set of microbes including bacteria, viruses, and fungi [9,10]. The skin of the external auditory canal and auricle is predominantly occupied by Gram-positive over Gram-negative bacteria . The main Gram-positive bacteria are staphylococci, coryneforms, streptococci and enterococci, micrococci, and bacillus. Gram-negative species inhabit the auricle and skin of the external auditory meatus to a much lesser extent with Pseudomonas aeruginosa and Moraxella osloensis in relative abundance. Some fungal microbes can be found in the skin of the outer ear, but are less abundant than either Gram-positive or Gram-negative bacteria [11]. *Staphylococcus aureus* is notorious for its ability to become resistant to antibiotics. Infections caused by antibiotic-resistant strains often occur in epidemic waves initiated by one or a few successful clones. [12]

Staphylococcus aureus is naturally susceptible to virtually every antibiotic that has ever been developed. Resistance is often acquired by horizontal transfer to genes from outside sources, although chromosomal mutation and antibiotic selection are also important. It ant to vancomycin, penicillin ,Quinolone, ,methicillin . [13] *Staphylococcus epidermidis* is a group of mannitol-fermenting coagulase-negative staphylococci characterized by multiple antibiotic resistance, very similar biochemical characteristics, and phage susceptibility. Clinical isolates belonging to this group are resistant to most antibiotics tested, including oxacillin, lincomycin, and novobiocin. The only antibiotic to which all tested strains are sensitive is vancomycin [14]. Treatment of infectious diseases becomes more challenging with each passing year. This is especially true for infections caused by the opportunistic pathogen *Pseudomonas aeruginosa*, with its ability to rapidly develop resistance to multiple classes of antibiotics. Although the import of resistance mechanisms on mobile genetic elements is always a concern, the most difficult challenge we face with *P. aeruginosa* is its ability to rapidly develop resistance during the course of treating an infection [15]. The problems associated with hospital infections caused by Serratia marcescens have become increasingly evident [16-18] The spectrum of infections includes meningitis, pulmonary infections, septicemia, endocarditis, and a variety of localized infections. The ability of this opportunistic pathogen to acquire resistance to a broad range of antibiotics has made effective therapy more difficult. Several recent investigations have dealt with the problem of antibiotic resistance in Serratia [19-22]. The overall findings of these studies indicate that the polymyxins and cephalosporins are the least effective agents against Serratia in vitro, and although gentamicin, kanamycin, chloramphenicol, and nalidixic acid are the most effective, resistance to kanamycin and chloramphenicol, as well as the p-lactam antibiotics, has increased during the last decade [21,22].

Materials and Methods

The sample taken swabs from nose ,mouth ,ear ,and cultured on MaCconkey agar ,Manitol salt agar , Nutrient agar and incubated in 37C in incubator for 24 hours . Then the biochemical test applied at first gram stain and Gatalase test , then analytic profile index test applied .

The bacteria species tested with antibiotics sensitivity test

Results

The results showed the bacteria is (5)(0,5%) isolates of Staphylococcus aureus and (2)(0,2) isolates of pseudoumonas aeruginosa from 70 ear samples ,this result same the result in(10) , (5)(0,64%)isolates Staphylococcus aureus and(4)(0,51%)isolates of Staphylococcus epidermedis from 70 mouth samples and 5(0,5%) isolates from pseudomonas aeruginosa,(2) (0,2%) isolates of Serratia marcescens from 70 nose samples this results same result in [23] . And the antibiotic sensitivity tested against

Ciprofloxacin ,gentamycin

Vancomycin .Pencilin , Azithromycin,

Amikacin,levofloxacin3 show the results as the table below:

Antibiotics / Bacteria	VA	p	ATM	CN	AK	LEV	CIP
Staphylococcus aureus	+	-	+	+	+	+	+
Staphylococcus epedermidis	-	-	+	-	+	+	+
Pseudomonas aeroginosa aeruginosa	+	+	+	+	+	+	+
Serattia marcescens	+	-	-	-	-	+	+

(Antibiotic sensitivity test result)

+ mean sensitivity

-mean resistant

LEV=Levofloxacin VA=Vancomycin P=Penicilin CN=Gentamycin

CIP=Ciprofloxacin Ak=Amikacin ATM=Azithromycin

The result showed the Staphylococcus aureus sensitive to vancomycin and this result does not match the result of (24), and Staphylococcus aureus sensitive to levofloxacin ,pencilin ,gentamycin,ciprofloxacin,amikacin,azithromycin ,

This result match with resulte in (25), Staphylococcus epedermedis sensitive to amikin

,levofloxacin, Ciprofloxacin , azithromycin. , this resulte\\ match with result in (26) and resistant to Vancomycin,as result in(28) and sensitive to Pencilin and Gentamycin this result match with result in (29) . the result show the Pseudomonas aeruginosa sensitive to pencilin , amikacin and gentamycin this result match the result in (30), and sensitive to Levofloxacin, Vancomycin, Ciprofloxacin, Azithromycin this result same the result in (31) and not match with result in (32), and the result show the Serattia marcescens sensitive to Vancomycin,Ciprofloxacin,Levofloxacin ,gentamycin , this result match with result in (33) and this result not match with result in (34) and not match with result in (35) .

And the result show resistant to amikacin this result not match with result in (34) also not match with result in (35).

Conclusion

1- Staphylococcus aureus and Pseudomonas aeruginosa are the bacteria which found in ear.

2- Staphylococcus aureus and Staphylococcus epidermidis are the bacteria which found in mouth

3- Psedoumonas aeruginosa and Serratia macrescence are the bacteria which found in nose .

4- Staphylococcus aureus sensitive to Ciprofloxacin, Amikacin, Azithromycin ,Vancomycin,Gentamycin, Levofloxacin and resistant to penicillin.

5- Staphylococcus epidermidis sensitive to Amikacin ,Ciprofloxacine Levofloxacine,azithromycin and resistant to Pencilin,Vancomycin,Gentamycin.

6- Pseudomonas aeruginosa sensitive to Pencillin, Vancomycin, Gentamycin Ciprofloxacine, Levofloxacine, Amikacin, Azithromycin .

7- Serratia marcescens sensitive to vancomycin, Levofloxacin, Ciprofloxacine and resistant to Azithromycin, Penicillin, Gentamycin, Amikacin.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Farabi University College and all experiments were carried out in accordance with approved guidelines.

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A Comparative Study of Nerve Growth Factor Level in the Follicular Fluid of Polycystic and Non-Polycystic Ovary Syndrome Women Undergoing ICSI: a Cross-sectional Study

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Abstract

Background : Nerve growth factor is a member of a group of proteins called Neurotrophins (NTs), which are necessary for growth and differentiation of neurons in both central and peripheral nervous systems. In addition to this essential role, NGF has been suggested to play a possible role in ovarian functions. Thus, we proposed this study to evaluate the nerve growth factor level in the follicular fluid of women with polycystic ovary syndrome undergoing Intracytoplasmic sperm injection (ICSI) in comparison with the control group (non-polycystic women).

Results : Follicular fluid nerve growth factor levels of non PCOS women were significantly higher than these of PCOS group (245.399 ± 63.41pg/ml vs. 149.592 ± 64.86 pg/ml), *p*-value =0.0001*. Also, it was significantly correlated negatively with oocytes number (Correlation Coefficient r= -0.431, *P*-value = 0.01) and positively with FSH (r=0.370, *p* value = 0.029).

Conclusion: Nerve growth factor level in the follicular fluid of polycystic ovary syndrome women is lower than it in non-PCOS ones. Additionally, it correlates directly with cycle day two FSH and inversely with oocytes number.

Keywords: *Follicular fluid, Polycystic ovary syndrome (PCOS), Nerve growth factor, Neurotrophins, subfertility.*

Introduction

Nerve growth factor (NGF) is a member of a group of proteins called Neurotrophins (NTs), which are necessary for the growth and differentiation of neurons in both the central and peripheral nervous systems [1]. In addition to this essential role, NGF has been suggested to play a role in the ovarian function, folliculogenesis, oocyte development, and ovulation [2]. However, this NT is present in all compartments of the reproductive system with its high-affinity receptors trkA and p75, suggesting its physiologic role in ovarian functions. Different types of cells in the ovary, like theca cells, granulosa and cumulus cells (CCs) have been found to produce NGF [3].

NGF promotes follicular maturation and increases cell responses to FSH via FSH receptors up-regulation in neonatal rat ovaries [4]. Similarly, in vitro studies on human GCs also showed FSH receptors expression up-regulation by NGF [5]. In addition to its effects on germ cells and GCs, NGF had been shown to have a role in promoting theca cells (TC) proliferation [6]. FSH enhances LH receptor expression in preantral oocyte granulosa cells and thus prepare oocyte for final maturation and further ovulation [7].

Role of NGF in Ovulation

Dissen *et al.*, [8] postulated in his research in rats, that, both TrkA and NGF are increased in the follicular and ovarian interstitial cells, reaching their maximum level ~5 h prior to ovulation. Additionally, he found that in an in vivo study, administration of NGF antibodies leading to suppressed ovulation, showing the important role of

NGF in ovulation triggering in rodents. Furthermore, he stated that trkA receptors activation by NGF can disrupt cell to cell interaction by modulation of the connexin 43-based gap junction integrity, thus induce cellular dissociation of the follicular wall which occurs prior to ovulatory rupture in rats.

Nerve growth factor increases E2 prostaglandin (PGE2) secretion [8] and decreases granulosa-theca cells gap junction communication. Indeed, PGE2 is an LH mediator and it is a key molecular factor controlling the events of ovulation. Studies in animals have provided evidence that NGF has a role in the ovulation process. It was previously called ovulation-inducing factor (OIF) in animals . Barboni [9] in his research (Preovulatory rise of NGF in ovine follicular fluid) showed that the *in vitro* addition of NGF to cumulus-oocyte complex caused marked cumulus expansion and gradual cumulus-oocyte uncoupling. Additionally, it caused meiosis resumption in about 70% of oocytes. Streiter *et al*., [3], also stated that NGF is involved in the ovulation control. Moreover, studies in rats and hamsters, as well as sheep, showed an elevated level of NGF in the follicular fluid during the preovulatory phase [10,11]. Interestingly, an administration of NGF in different ways consistently induced ovulation in several induced ovulatory mammals, like llamas, camelids and alpacas [12-14]. In a research using a camel model, it was suggested that NGF in the seminal plasma was absorbed by the endometrium to the bloodstream and then reached to the hypothalamus where this NGF stimulated neurons to produce kisspeptin (a potent stimulator of GnRH), which eventually organized the preovulatory LH surge [12].

Some studies showed that abnormal NTs system may negatively disturb ovarian function, causing reproductive problems in form of reduced ovarian reserve, PCOS and endometriosis in addition to that, NTs defects may lead to female infertility and even ovarian cancer [2].

Methods

Study design and setting

This is a cross-sectional study, which had been approved by the Research Local Ethical Committee of the College of Medicine / University of Babylon.

Forty-four sub-fertile couples from the Fertility Center of Al- Najaf / Al-Sadr Medical City visitors were included in the study within the period from December 2019 to February 2020. Written consent had been taken from all couples participated in this study. Both partners had been evaluated by a gynecologist and an urologist. The evaluation based on medical history and physical examination including anthropometric measurements (height, weight and BMI). Cycle-Day 2 vaginal US for assessment of antral follicular count was performed, in addition to the hormonal assay; including luteinizing hormone (LH), Follicle stimulating hormone (FSH), prolactin and Estradiol (E2) as well as triggering time E2 were measured and recorded for all women participated [15].

Selection criteria:

1. Women's age was between 20 and 40 years (mean ± SD = 28.57 ± 6.212). Women aged more than 40 years old had been excluded from the study.

2. Women with current untreated pelvic disease like endometriosis, uterine problems: including submucosal fibroids or polyps, malformations, ovarian cyst had been excluded.

3. Female partners were grouped into two groups:

Group one: females with polycystic ovary syndrome (n=20), Selected based on Rotterdam criteria, including a history of oligo/amenorrhea, clinical picture of high androgen level either in form of signs and symptoms or biochemical investigation (high testosterone level) as well as ovarian cystic appearance on transvaginal ultrasound (OS Guideline Development Group 2019). Male partners were either normal or having mild to moderate impairment of semen parameters.

Group two: females without PCOS (n=24), either normal females undergoing ICSI due to male factor infertility or females with tubal obstruction or unexplained infertility.

Controlled ovarian hyperstimulation (COH)

Controlled ovarian hyper-stimulation was done based on ESHRE ovarian stimulation guidelines (OS Guideline Development Group 2019) by a gynecologist. It was performed via fixed GnRH antagonist protocol for both PCOS and non PCOS women to avoid the risk of

ovarian hyperstimulation syndrome (OHSS) (Behery *et al.* 2020) associated with close monitoring of follicles number, size, level of maturation as well as serial TVUS and serum E2 level.

Gonadotropins initial dosing was individualized for each patient and the doses was adjusted based on the patient's body mass index, age, ovarian reserve. Gonadotropin used is recombinant FSH (r-FSH) (Gonal-f and Follitropin). Drugs had been given on the basis of daily injection, usually either intramuscular or subcutaneous. The standard dose of Gonal-F was 150 IU / day which can be increased up to 450 IU/day for obese women. Ovulation trigger was done 36 hrs. before oocyte pick up by administration of low dose GnRH agonist (0.2 mg Decapeptyl) subcutaneously.

Follicular fluid collection

After oocytes scanning and saving, the follicular fluid of the first follicle aspirated (of 20-24 mm diameter) was collected in tubes, centrifuged for 5 min at 269.6g then the supernatant clear fluid was taken and divided into safe lock tubes and saved at ¯80 Cº in the deep freeze at the Kufa Medical College Center of Cancer researches until the time of the ELIZA test.

Nerve Growth Factor (NGF) level in follicular fluid measured by using enzyme immunoassay ELISA kit for human NGF from Bioassay Technology laboratory (BT) that have been validated for NGF level assessment in human follicular fluid, as per manufacturer's instructions. After thawing of FF samples, an accurate quantitative detection of NFG had been done by a sandwich ELISA principle.

Statistics

Statistical Package for Social Sciences (SPSS) software had been used (version 21). An independent sample student t-test was used for comparing parameters between both groups. For categorical values, Chi-square was used. Bivariate analysis (Pearson's correlation test) was used to test the degree of association between the measured variables and other clinical parameters. $P \leq 0.05$ was considered as statistically significant.

Results

Table 1 shows the demographic differences between PCOS and non PCOS groups. There was no significant statistical difference between the studied groups. while table 2 shows the insignificant statistical difference between both groups regarding cycle day two hormones, E2 at triggering time, LH/FSH ratio except for the oocytes number, there was a significant higher oocytes in PCOS group than non-PCOS one, *P* value = 0.033 (Tab. 1., 2.).

Table 1: Comparison between both groups (PCOS and Non-PCOS) regarding age, body mass index (BMI) and infertility period.

Variable	PCOS (N = 20) Mean±SD	NON-PCOS (N = 24) Mean ±SD	P value
Age (years)	26.6 ± 5.113	30.36 ± 6.680	0.490
BMI (kg/m2)	27.365 ± 3.91	27.35 ± 3.15	0.996
Period of infertility (years)	6.75± 4.179	6.98 ± 3.66	0.850

***Abbreviation: PCOS* polycystic ovary syndrome, *BMI* body mass index.**

Table 2. Comparison between the studied groups regarding cycle day two hormones, E2 at triggering time and oocytes number.

Variable	PCOS Mean ±SD	NON-PCOS Mean ±SD	P value
E2 (pg/ml)	34.20 ± 14.24	39.15 ± 16.42	0.305
FSH (mIU/ml)	4.87 ± 2.165	5.65 ± 2.413	0.276
Prolactin (ng/ml)	28.285 ±26.835	24.47 ± 10.158	0.539
E2 (pg/mL) at triggering time	2723.52 ± 1039.75	2108.27± 926.377	0.071
Oocyte number	11.35 ± 6.983	7.45 ± 4.228	0.033*

***significant. Abbreviation: FSH* follicle stimulating hormone, *E2* estradiol.**

There was a significant statistical difference between the studied groups regarding follicular fluid NGF level (measured in picogram per milliliter), with a higher level in the non-PCOS group (245.399 ±63.41 n=24) than PCOS group (149.592 ± 64.86, n=20), *p* value =0.0001*

Although NGF correlated positively with LH, E2, FSH, prolactin but the difference was statistically insignificant only with FSH, it was significant, *P* value =0.029. Interestingly, NGF correlated negatively with E2 at triggering time and oocytes number, r = (-0.461 and - 0.431) respectively with a *p* value= 0.010 for both (Tab.3).

Table.3. Correlation between follicular nerve growth factor and LH, FSH, E2, prolactin, E2 at triggering time and oocytes number

Hormones		LH (mIU/mL)	FSH (mIU/mL)	E2 (pg/mL)	Prolactin (ng/mL)	E2 triggering time (pg/mL)	Oocytes number
NGF	r	0.046	0.370*	0.258	0.025	-0.461*	-0.431*
	P value	0.794	0.029	0.135	0.887	0.010	0.010

*** Correlation is significant at the 0.05 level (2-tailed). *Abbreviation: NGF* nerve growth factor, *FSH* follicle stimulating hormone, *LH* luteinizing hormone, *E2* estradiol.**

Discussion

In the present study, there were no significant statistical differences regarding demographic characteristics including age, BMI, infertility type and infertility period between our study groups (PCOS and non PCOS). Additionally, there was a high specification of inclusion and exclusion criteria of other infertility causes and PCOS differential diagnosis that could affect the results. So the confounding factors were highly minimized.

The controlled ovarian hyperstimulation protocol used was GnRH antagonist protocol to reduce the risk of developing ovarian hyper-stimulation syndrome (OHSS) complication [16].

Table 2 compares between PCOS and non PCOS groups regarding cycle day2 hormones including FSH, E2, prolactin, E2 at triggering time and oocytes number. It shows no significant statistical difference between both groups. This can be explained as the PCOS is a WHO type 2 normogonadotropic anovulatory disorder [15].

The total retrieved oocytes number was significantly higher in PCOS women than non PCOS. These results are consistent with many previous studies (Falah Hassan and Zahir Al-yasiry; Kumar *et al.* 2013). This higher oocyte number in the PCOS group is attributed to the increased number of preantral follicles in polycystic women ovaries which are highly sensitive to iatrogenic ovarian stimulation done by COH [16].

Our results demonstrated that under conditions of ovarian hyperstimulation, the follicular fluid NGF levels were significantly higher in samples of non PCOS women than the PCOS group. These results are in agreement with the results stated by [1]. While Sadeu *et al*., [17] showed there was no difference in the FF NGF level between PCOS and non PCOS women. Other researches stated that there were higher FF NGF levels in PCOS women than non PCOS [18].

However, in the present study, we examine the correlation of NGF with collected oocyte number. It had a highly significant inverse correlation with retrieved oocytes number and the estradiol level at ovulation triggering time; in addition to its positive correlation with age and FSH, this may indicate its increase in lower responder women and this confirms Salas *et al.*, [5] and Buyuk and Seifer [1] opinions. Or it is presumed that, due to NGF high affinity-receptors (trkA) expression is predominated in the granulosa and theca cells of preantral and antral oocytes [6], NGF may haven't any impact on oocytes number and its role in the normal ovary is confined mainly to: Late follicular maturation (pre-antral and antral follicles) which is FSH dependent [6] and ovulation.

However, in human being the actual role of NGF in regulating the ovulation process still poorly defined and most information had been obtained from animals.

The main problem in PCOS subfertility is oligo / anovulation (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group Revised 2003 consensus) [19]. It considered as the commonest anovulation cause affecting females worldwide [15].

The lower follicular fluid NGF levels in PCOS women as compared to control group highlights the possible role of low NGF level in the PCOS anovulation problem. Therefore, the NGF role in human PCOS pathophysiology especially in events surrounding late oocyte maturation and ovulation needs further clarification.

Conclusions

Follicular fluid NGF levels are significantly higher in non PCOS women than the PCOS group. Additionally, the NGF correlated positively with cycle day-2 FSH and negatively with oocytes number and E2 at triggering time. Thus NGF role in the ovary may be limited to its participation in preantral and antral oocyte maturation and ovulation. Indeed, this requires further study in human ovaries for confirmation.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Fertility center of Al- Najaf and all experiments were carried out in accordance with approved guidelines.

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Anti-Urease Activity of Essential oil and Phenolic compounds of *Thymus vulgaris*, *Melaleuca alternifolia* and *Betula pendula* against *Proteus mirabilis* isolates

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Abstract

The present study was carried out to evaluate the activity essential oil and phenolic compounds of *Thymus vulgaris*, *Melaleuca alternifolia* and *Betula pendula* extracts against urinary tract infections caused by urease-producing from seven strains of bacteria *Proteus mirabilis* isolated from urinary tract infection. Microdilution technique has been used to evaluate antibacterial operations in sterile 96-well microtiter plates. Activity of urease and inhibition of urease assessed by indophenol test method. The results showed that *M. alternifolia* extract was the best antibacterial activities against all strains at 1.25 - 25 mg/ml. Also, 0.31 mg/ml of phenolic compounds and essential oil of *M. alternifolia* extract serves as effective dual acting 100% urease inhibitor to prevent and eliminate urease. Besides, the results of phytochemical detection show that Coumarins, Terpenes, Alkaloids, Tannins, Flavonoids and Sesquiterpenes were present in *M. alternifolia* extract, which may be have anti-urease possessing activity. Furthermore, these plants could be act as a source of antimicrobial compounds against *P. mirabilis* which are commonly impaired by the development of bladder and kidney stones.

Keywords: *Urease inhibitor, MIC, Total phenolic compounds, Phytochemical screening*

Introduction

Urinary tract infections are the severe health problem that affects millions of individuals every year. These are very prevalent infections that occur when bacteria enter the urinary bladder and multiply anywhere along the normal sterile urinary tract [1]. *Proteus mirabilis*, is well-known for its urease production and distinctive ability to differentiate into elongated swarm cells and characteristic bull's-eye pattern of motility on agar plates. *P. mirabilis* could be observed in a huge range of environments, along with soil, water and sewage., but it is predominantly a commensal of the gastrointestinal tracts of humans and animals [2]. *Proteus* genus are widely distributed in the natural environment, including polluted water, soil, and manure. Due to their proteolytic activity, the ability to hydrolyze urea to ammonia and carbon dioxide, as well as the oxidative deamination of amino acids, these bacteria are involved in the decomposing of the organic matter of the animal origin. They are also present in the human gastrointestinal tract of humans and animals [3].

Plants are rich source of antibacterial agents, most of which probably evolved as chemical defense against predation or infection. The present antibacterial review of the plant extracts demonstrates that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. Due to the ongoing restructuring of natural products and processes to maintain human and environmental health, the economic environment and the significance of medicinal plant materials have risen tremendously in latest years [4]. In general, the medicinal plant containing essential oil, alkaline, flavonoids, and phenol contents may posses' strong anti-microbial properties [5]. Therefore, the current study was designed to evaluate the anti-bacterial activity of the essential oil and phenolic compounds of *T. vulgaris* and *M. alternifolia* against seven isolate of bacteria *P. mirabilis* and urease inhibitor agent with dual actions, which are commonly impaired by the development of bladder and kidney stones.

Material and Methods

Plant materials

The Plant samples were obtained from Baghdad local market, Baghdad □ Iraq. Plants were identified in herbarium, Department of Biology /College of Science /University of Baghdad. The plant's parts dried and grinded into powder by mechanical grinder, it kept at 4°C until further investigations.

Bacterial Strains and Culture Conditions

Seven of *P. Mirabilis* strains were used in this study. These strains were isolated from urinary tract infections (UTIs). All strain was kindly provided by Department of Biology /College of Science /University of Baghdad. All strains were confirmed by using UTI chromogenic agar and Vitek2 compact. Strains were maintained in Mueller-Hinton agar.

Extraction of crude phenols

Crude phenols were extracted according to Ribereau-Gayon [6].

Extraction of the essential oils

The 100 g of the air-dried and ground parts of the tested plants were submitted for 4 hours to water-distillation using a Cleavinger apparatus. The obtained essential oils were dried over sodium sulphate anhydrous, then stored at 4°C until tested and analyzed [7].

Detection of Triterpenes/Steroids (Liebermann-Burchard Reagent).

One mL of acetic anhydride and 5 drops of concentrated sulfuric acid (H_2SO_4) were added to the extract. A color change from violet to blue confirms the presence of steroids and formation of blue-green ring indicated the presence of terpenoids [8].

Detection of Coumarins.

Three mL of 2N NaOH was added to 2mL of aqueous extract. Formation of yellow color indicated the presence of coumarins. Confirmation test was performed by adding 1mL of 5N HCl; in this case a colorless solution formed at the upper layer is considered positive [9].

Screening for Sesquiterpene Lactones.

The Baljet reaction (1% Picric acid in 10% sodium hydroxide) was used to detect sesquiterpene lactones in the extracts. Reagents were mixed at a 1 : 1 ratio and added to 1mL of extracts (2-3mg). The transformation of the sodium picrate solution's yellow color to orange-red color confirmed the positive reaction [10].

Test of Carboxyl Group.

The presence of carboxyl groups was evidenced by adding 10 drops of 10% sodium bicarbonate solution; visible bubbles of carbon dioxide were considered a positive reaction [11].

Test for Tannins.

Extracts were treated with 1mL of 5% ferric chloride which was added. The presence of tannins was indicated by the formation of bluish black or greenish black precipitate [12].

Shinoda Test. (Test for flavonoids)

Few fragments of magnesium metal ribbon (3-4 pieces) were added to 1mL of extract, followed by dropwise addition of concentrated hydrochloric acid. Formation of pink or red color indicated the presence of flavonoids [13].

Test for Saponin.

Two mL of distilled water was added to extracts suspended in ethanol and was shaken vigorously. The formation of copious foam layer indicates the presence of saponins [14].

Determination of Minimum Inhibitory Concentration (MIC)

MIC of plant extracts against studied bacteria was determined by microdilution method in sterile 96-wells microtiter plates according to the protocol described previously McBain *et al.* [15]. Different plant extracts concentrations (100, 50, 25, 12.5, 6.25, 3.125 and 1.625 µg/ml) (W/V) were prepared to containing bacterial cells comparable to McFarland standard no. 0.5 in a final volume of 200 µl. Sterile distilled water, broth and plant extracts were used as a negative control while sterile distilled water, broth and bacteria were used

as positive control. After 24 hrs. at 37° C, the MIC of each sample was determined. The MIC considered the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after 24 hours incubation [16].

Urease inhibition activity assay

The phenolic solution assay mixture consisted of urea (30 mM) and (200 µl) crud extract. The reactions were initiated by the addition of 50 µl of urease enzyme solution in distilled water. Urease activity was determined by measuring ammonia concentration after 15 minutes of enzymatic reaction. The ammonia was determined using 500 ml of solution A (contained 5.0 g

phenol and 25 mg of sodium nitroprusside in 500 ml of distilled water) and 500 ml of solution B (contained of 2.5 g sodium hydroxide and 4.2 ml of sodium hypochlorite 5% in 500 ml of distillated water) at 37°C for 30 minutes. The absorbance was read at 625 nm. Activity of uninhibited urease was designated as the control activity of 100% [17].

Urease solution (200 µl) was mixed with the (200 µl) essential oil of the two plants. Each mixture was incubated at 37°C for 30 min. Urease activity was determined by measuring ammonia production using the indophenol method as described (Weatherburn MW) [18]. Briefly, 5m1 each of essential oil reagent A and 5ml of alkali reagent B were added to each well. The increasing absorbance was measured after 30 min at wavelength of 625 nm using uv-visible spectrophotometer. All reactions were performed in duplicates in final volume of 10.4 ml [19]. % Inhibition= 100 - (Absorbance of Test Compound/Absorbance of Control)*100

Statistical Analysis

expressed as mean ± standard deviation (SD) for analysis performed in duplicate at least three times. Statistical analysis of the data was performed by Duncan□s test: similar letters refer to a non-significant difference, while the different letters refer to a significant difference.

Results and Discussion

Effect of medicinal plants against bacteria.

Effect of TPC (Total Phenolic Compounds)

Antibacterial activity TPC against seven strains of *P. mirabilis* isolated from UTIs were revealed in Table 1. TPC showed different inhibited concentration ranged from 6.25 to 25 mg/ml. *M. alternifolia* leaves extracts showed a good result of antibacterial activity against all tested microorganism strains using Microdilution assay method (Table 1). The MIC of *M. alternifolia* extract ranged from 6.25 to 12.5 mg/mL, while *T. vulgaris* and *B. pendula* show activity against microorganism strains but in higher concentration.

Table 1. MIC of TPC against *P. mirabilis*.

Concentration of extract (mg/mL)			
Bacterial strains	**Thymus vulgaris**	**Melaleuca alternifolia**	**Betula pendula**
P. mirabilis1	25	12.5	25
P. mirabilis2	25	12.5	25
P. mirabilis3	25	6.25	25
P. mirabilis4	25	12.5	12.5
P. mirabilis5	25	12.5	25
P. mirabilis6	25	6.25	25
P. mirabilis7	25	12.5	12.5
mean ± SE	25.0 ± 0.0a	10.71 ± 1.15b	21.43 ± 2.31a

Effect of EOC (Essential Oil Concentration)

The growth of *P. mirabilis* strains were effectively inhibited for all isolates at MIC between 0.39 and1.25mg/ml of essential oils Table 2. The lowest MIC was 0.39 mg/ml for *T. vulgaris* essential oil, while the highest MIC was 25 mg/ml for *B. pendula* essential oil as shown in Table 2 .

Table2. MIC of EOC against *P. mirabilis*.

Concentration of extract (mg/mL)			
Bacterial strains	**Thymus vulgaris**	**Melaleuca alternifolia**	**Betula pendula**
P. mirabilis1	0.78	1.25	25
P. mirabilis2	0.39	1.25	25
P. mirabilis3	0.78	1.25	25
P. mirabilis4	0.78	0.78	25
P. mirabilis5	0.39	1.25	12.5
P. mirabilis6	0.78	1.25	12.5
P. mirabilis7	0.78	0.78	25
mean ± SE	0.67 ± 0.07c	1.12 ± 0.23b	21.43 ± 6.10a

Urease enzyme inhibition assay

Herein, urease enzyme inhibitory activity of TPC and EOC were elucidated as the most potent ones including *T. vulgaris*, *M. alternifolia* and *B. pendula*. As it is presented in Table 3, TPC and EOC value 0.32 mg/mL of *M. alternifolia* were the most effective compound (100%) urease enzyme inhibition, followed by 0.30 mg/ml of *B. pendula* TPC (93.7%) and by 0.32mg/ml of by *B. pendula* EOC value 100%, respectively. While, the lowest activity was 90.6% for *T. vulgaris* TPC.

Table 3. Urease enzyme inhibition

Plant extracts	Activity	% enzyme inhibition
T. vulgaris TPC	0.29	90.6%
M. alternifolia TPC	0.32	100%
B. pendula TPC	0.30	93.7%
T. vulgaris EOC	0.31	96.5%
M. alternifolia EOC	0.32	100%
B. pendula EOC	0.32	100%

Phytochemical screening

Phytochemical screening results of different functional groups of selected plant extracts were summarized in Table 4. Coumarins, Terpenes, Alkaloids, Tannins, Flavonoids and Sesquiterpenes were found in *M. alternifolia* and *T. vulgaris* extract. While, Tannins, Flavonoids, Alkaloids and Sesquiterpenes compounds have been described in *B. pendula* extract.

Table 4. Phytochemical screening results of selected extracts.

Compounds	M. alternifolia	T. vulgaris	B. pendula
Terpenes	+++	+++	+++
Flavonoids	+++	++	+
Coumarins	+++	++	-
Alkaloids	++	+++	+
Tannins	++	++	-
Saponins	-	-	-
Carboxyl group	-	-	-
sesquiterpenes	+++	+++	+++

+: low intensity reaction, ++: medium intensity reaction, and +++: strong intensity reaction

Discussion

In general, the results presented here contribute to the knowledge of antimicrobial activities and chemical composition of the tested TPC and EOC obtained from aromatic plants. Some of these compounds were recognized to exhibit therapeutic activity and metabolic activity [20]. Actually, the flavonoids, have been reported to be synthesized by plants in response to microbial infection and have been shown to have antibacterial activities. Also, Tannins were reported have demonstrated activity against bacteria [21]. Also, the antimicrobial activity was found in the *M. alternifolia*, *T. vulgaris* and *B. pendula* extracts, and this may be due to the presence of Terpenes. This result is supported by the study carried out by Alberto *et al*., who reported that the terpinen-4-ol have antioxidant and anti-bacterial activity [22]. In this work, the urease enzyme inhibitor, have been shown that the EOC extracts were the most effective inhibition growth of *P. mirabilis* than TPC, this may be due to the high concentration of phytochemical compounds. Besides, each phytochemical has different functional and mechanism in the antimicrobial action. For example, the phenolic compounds were much active than aldehydes, while the aldehydes were much active than ketones, and the ketones were more active than alcohols, ethers and hydrocarbons [22]. Also, this result agrees with the study of Hanene *et. al.* [23], whom demonstrated the finding of MIC antibacterial activity of EOC extract of *T. vulgaris* against wide range of pathogenic gram-negative bacteria [24]. On the other hand, the *M. alternifolia* was more effective inhibiter of urease enzyme, which may be due to the high concentration of flavonoids and coumarins compared with the other plants. However, the Coumarins possess a variety of biological properties, including antimicrobial, antiviral, anti-inflammatory, antidiabetic, antioxidant, and enzyme inhibitory activity. On the other hand, possible the synergistic effects of phytochemical compounds in the oil may also increase their antimicrobial activity.

Conclusion

Some of the plant extracts assessed in the current study had potential activity for antimicrobial and urease inhibitors against isolated UTIs bacteria, which may be an alternative to controlling the development of microbial urease or may be used as a model for searching for fresh drugs.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Histological and Physiological Study of The Effects of Exogenous Melatonin on Liver of Female Rats Exposed to Formalin in Drinking Water

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Abstract

Aim: The presented study was established to evaluate the impact of formalin (FA) on the blood levels of superoxide dismutase (SOD), lipid profile, total serum protein and serum albumin in female rats. **Materials and methods:** The animals were assigned randomly into groups of six; control group, and treated other five groups as follow, first group exposed to formalin at 200ppm (F200 group), the second exposed to formalin at 400ppm (F400 group), third group exposed to formalin at 200ppm plus melatonin at 10mg/kg (F200M group), fourth group exposed to formalin at 400ppm plus melatonin at 10mg/kg (F400M group), and the fifth exposed to melatonin at 10mg/kg (M group). Samples of blood from the rats were collected at day one, day and day 30 of the experimental period that was continued for 30 days. **Results:** Using immune-based techniques, the SOD activity, it has been here-resulted in significant ($p<0.05$) after-formalin-exposure-decreases in the blood samples of female rats from F200 and F400 groups, especially, when compared with the control group. The total cholesterol levels revealed significant ($p<0.05$) increases in the blood of the tested animals of the F200 and F400 groups. These increases affected the rates of LDL, VLDL, and triglycerides in the blood of the studied rat-females of both F200 and F400 groups when compared with the control animals. Interestingly, low levels of HDL were noticed in the blood of the animals of the F200 and F400 when comparisons with control animals. The concentrations of the LDL, HDL, and triglycerides in the serum of the formalin-exposed females showed significant ($p<0.05$) low levels when compared with control animals. For day-30 collection, albumin concentrations revealed significant ($p<0.05$) elevation in the blood of the F200 and F400 animals.. Histological examination revealed that the liver of female rats exposed to 200ppm or 400 ppm of formalin for 30 days have been adversely affected. Whereas, the histological examination of the liver of animals treated with melatonin and formalin were less affected due to formalin exposure. **Conclusions:** These results demonstrate the formalin high toxic effects on the hepatic tissues can be overcome by the use of exogenous melatonin.

Keywords: *Antioxidant, formaldehyde, hormones, melatonin.*

Introduction

Environmental contaminants cause direct toxic effects such as endocrine disruption, that are altering the normal hormonal environment and reproduction due to exposed to exogenous chemicals. They alter the metabolism, synthesis, and gene expression of hormones, creating critical situations for the development of the body[(1)]. Formaldehyde (FA) is one of the substances that caused pollutant in many routs .FA can generate irritation toxicity that affects body organs such as eye and respiratory tract with bronchoconstriction and fluid accumulation in these tracts [(2)]. FA is a potent sensitizer and a carcinogenic effect.

666 According to the foregoing, it became clear that there are many ways to be exposed to formalin and the risks it poses to public health, to show the health effects of the wrong use of formalin in food preservation that randomly imported into the country. Also the study examined the role of melatonin in preventive or resistance the harmful effects of formalin

on experimental animals. The presented study was established to evaluate the impact of formalin (FA) on the blood levels of superoxide dismutase (SOD), lipid profile, total serum protein, serum albumin, follicle stimulating hormone (FSH), luteinizing hormone (LH), and estrogen (E2) in female rats. [3]

Materials and methods

Animals: The present study was conducted at Science Collage, University of Wasit from November, 2018 to April 2019 . A total number of 36 adult albino rats, weighting 175-250 g and 10-14 weeks age were used in the current study.

Experimental Design: The present study was conducted on 36 adult female rats . Animals were randomly divided in to six equal groups ; each group consisted of 6 adult female rats as in the following :

1-Control group : orally dosed with distilled water .

2-Group 2: orally dosed from formalin 200ppm for 30 days

3- Group 3:orally dosed from formalin with 400ppm for 30 days

4- Group 4:orally dosed from formalin with 10mglkg from melatonin for 30 days.

5-Group 5:dosed from formalin 400ppm with 10mg\ kg of melatonin for 30 days

6-Group 6:10mg/kg dosed of melatonin only for 30 days

Sample Collection: At the end of the tests all the animals were killed. The rats were first weighed before sacrifice and then anesthetized by cotton loaded with anesthesia of diethyl ether in a specific box. A midline abdominal incision was used to open the abdominal cavity to take the sample which includes: Cardic puncture for blood collection was entitled [4]. Liver tissue samples were collected.

Blood analyses :cholesterol Gen 2, and triglycerides TRIGL were measured relying on the kits used and their protocols.

Histological techniques:

The tissue (liver organs) have been extracted from both classes in the following phases and ready for histological research using hematoxylen and eosin stain [5].

Statistical Analysis

Data is evaluated with a mean ± SD. Comparisons of classes were rendered utilizing the SPSS (Statistical System for Social Sciences) with the study of variation (ANOVA). The minimum value maximum was found to be $P<0.05$. Last big check (LSD). The gap between measures was decided for (ANOVA) SPSS [6] .

Results and Discussion

Symptoms in female rats after treated with formalin in both concentration 200,400ppm During the last (30) days of the experiment ,which is by administering dose of formalin treatment at the concentration of (200,400) ppm. some symptoms and signs were observed on the animals of the group. This includes hair colour shifting from white to yellow and also general weakness of the body which is the weakness of teeth that begins to break down or gets yellow. These animals were perished in relation to the second , third, fourth and fifth group ,but the second and third group have more symptoms than the fourth ,fifth group , because the fourth and fifth groups have formalin and also Melatonin, while the sixth group is treated with melatonin only . predation is noticed among them ,binge eating and strength in body. The current study showed a reduction on serum SOD activity. It was recorded a reduction in SOD activity and increase in lipid peroxidation in experimental animals after exposure to formaldehyde . Oxidative stress derived secondary toxic actions might be induced by FA. SOD lowered by formaldehyde due to elevations of some free radicals. Irreversible toxicity may be performed for long time exposure to FA. The catalase, by FA, GSH reduced and no recovery levels were seen in which secondary toxicity induced via oxidative stress was conducted. SOD was significantly higher in group had low dose of formaldehyde with melatonin (GIV) than those of high dose (GV), and compared with those groups had FA only, this result may be the role of antioxidant capacity of melatonin as indicated by previous researchers who concluded the role of melatonin in partially prevent the liver damage against HCHO intoxication. Broad-spectrum antioxidant effects of melatonin can be seen to

eradicate free radicles [7].

Current study showed that exposure to F200 and F400 results in significant increase in cholestrol, TG, LDL-c, VLDL significantly in treated female rats. High rates of LDL and VLDL increases the incidence chances of cardiovascular disease considered to be a major risk factor for chronic disease such as coronary heart disease and hypertension and some types of cancer , while the HDL ratio decreased significantly, which is a result of the previous studies , as it was found by previous researchers that formalin increases the oxidation pressure that leads to high levels of fat. Formalin substance increases the pressure of oxidation as the generation of free radicals that attack the fat of the cellular membranes increases , leading to the generation of malondydehyde , which in turn leads to the destruction of vessels and tissues [8] . HDL decreases in the treated animals with formalin at 200 or 400 can be expected in which certain damages may have been introduced to those pathway components leading to the disturbances in the metabolisms of those lipids showing lower levels of HDL in the blood of the tested rat females.This increase may be due to the effect of formalin on the permeability of the hepatic cell membranes or it may be due to the blockage of the hepatic canal. Bile , which leads to reduced or stopped cholesterol release in the duodenum. LDL is oxidized by the oxygen free radicals in the arteries and converted in to foamy lesions or atherosclerosis [9]

Current study showed that exposure to F200 with 10mglkg from melatonin and F400 with 10mglkg from melatonin results in significant decrease in cholestrol, TG, LDL-c, VLDL and increase of HDL significantly in F200 and F400 groups, whereas these values were normal with 10mglkg from melatonin compare with control group. On the other hand melatonin removes the oxygen free radicals and prevents oxidative changes. LDL is generally a blood lipid disorder and plasma lipid alteration among the risk factors for cardiovascular disease .More attention has recently been paid to melatonin to improve lipid profiles. The mean effects of melatonin on plasma lipids and lipid peroxide level have been reported in previous studies. Studies were also conducted on this hormone on the physiological changes resulting from high-calorie and fat diets. The use of melatonin accompanied by zinc acetate leads to a significant decrease in the level of cholesterol in the blood and TG , LDL .Melatonin probably improves the features of fats from by reducing the oxidation of fats and other necessary proteins by free radicals according to the results of some studies, there are several mechanisms that can be suggested for this action of melatonin : Interactions with LDL-c receptors , inhibition of biosynthesis of cholesterol and LDL-c accumulation , analgesia of lipase protein and reduces lipoprotein activity of the accumulation of TG rich in very LDL , VLDL molecules and decreases in intestinal cholesterol absorption or inhibition of transport of fatty acids through metabolism receptors [10].

Table (1): The effects of formalin, melatonin and formalin with melatonin on serum SOD concentrations mmol/L in mature female rats.

Table 1: Lipid, sex-hormone, and superoxide dismutase profiles in the study groups at day one of sample collection.

Group	Cholesterol	HDL	LDL	SOD	Triglycerides	VLDL
Control	54.86	24.319	19.56	247.77	58.66	11.731
F200	55.01	24.242	19.42	243.78	56.75	11.351
F400	54.4	23.964	19.01	243.51	57.13	11.426
F200M	55.13	23.601	20.16	246.67	56.82	11.364
F400M	54.29	23.202	19.88	249.52	56.06	11.21
M	54.96	24.084	19.41	246.53	57.42	11.482
LSD	N.S.	0.5289	N.S.	3.143	1.011	0.2023

LSD: Least significant difference; N.S.: Non-significant

Table 2: Lipid, sex-hormone, and superoxide dismutase profiles in the study groups at day 15 of sample collection.

Groups	Cholesterol	HDL	LDL	SOD	Triglycerides	VLDL
Control	55.18	24.5	18.87	247.06	59.07	11.814
F200	59.86	20.74	25.84	201.05	66.39	13.279
F400	70.34	18.18	37.59	181.93	72.82	14.563
F200M	58.82	22.28	24.66	238.73	59.42	11.884
F400M	59.89	19.89	27.44	214.98	62.76	12.552
M	51.21	26.29	14.44	266.51	52.43	10.487
LSD	1.19	0.756	1.266	2.826	1.307	0.2614

The liver of the control group, the liver tissue revealed normal central vein with liver cords of hepatocytes radiating from the central vein and separated by blood sinusoid plus hepatocytes had rounded nuclei indicating normal (without changes) tissues (Fig.1,A) For both F-200 and F-400 groups (Fig. 1,B and Fig2, A) respectively, at day-30 sample collection time, loss of normal architecture, degeneration of hepatocytes with numerous vacuolations, presence of pleomorphic nuclei with different sizes and degeneration levels, loss of sinusoidal spaces, central-vein rupture, hepatic-tissue based scattered inflammatory cells, blood-filled-central-vein dilation, dropped-out-degenerated hepatocytes, mostly displayed pycnotic, absent nucleoli, and nuclear-material clumping. (11) mentioned that FA is extremely soluble and reactive in its contact with mucus or other nucleophilic-group-containing cellular macromolecules, such as amino acids and DNA. FA may interfere-react with DNA, and it is accountable for the genotoxic and carcinogenic results of FA via the construction of bonds between DNA and FA related adducts. This fact are explain the effect of FA on the nucleus.

For the F-200 10mg/kg at day 30 of the experiment (Fig.2, B), the protective treatment of melatonin against the exposure to 200PPM of FA showed a decrease in the histological changes compared to F-200 group that received FA alone, in which less dilation of central vein, less dilation of sinusoidal spaces, less congestion in the central vein, less infiltration of lymphocytes, and less inflammatory cell infiltrations and hepatocyte degenerations were seen in the melatonin treated group compared with those from the F-200 group.

In the case of the F-400 with 10mg/kg group at day- 30 time-point, the histological findings revealed that the exposure to formalin and melatonin resulted in less severe central vein congestion, some restoration of the liver architecture with less impacted endothelial lining, less dilated sinusoidal spaces with close-normal thickness of tunica media, low levels of infiltration of inflammatory cells, smaller sinusoids, low numbers of hemorrhages (Fig.3,A)

(12) concluded that M fought the induced oxidative stress produced by the exposure to FA that increased the tissue toxicity generating lower infiltration of neutrophils and decreasing the 8-hydroxydeoxyguanosine (8-OHdG) levels, which can be useful for the protection against the exposure to FA, and this agrees with the current investigation results

For the groups that received 10mg/kg melatonin only, the findings of the liver tissues showed normal tissue structure, architecture, compartments (Fig.3,B) Our study is comparable with [13] who mentioned that the melatonin play important role to improvement the histological structures of kidney and liver of mice and give it the normal characteristics for these organs. [14] mentioned the fact that melatonin is nowadays there has been a certain knowledge of the importance of melatonin in human biology and diseases, but many of its roles and consequences tend to be overlooked. Melatonin is a powerful free radical scavenger, stronger than vitamin E. The extremely toxic hydroxyl radical and other oxygen-based radicals are modified by melatonin effectively. Melatonin also has antioxidant effects: it improves the concentrates of various antioxidant enzymes such as the SOD, glutathione reductase, and glutathione peroxidase. In addition, melatonin suppresses NO synthase, a pro-oxidant enzyme.

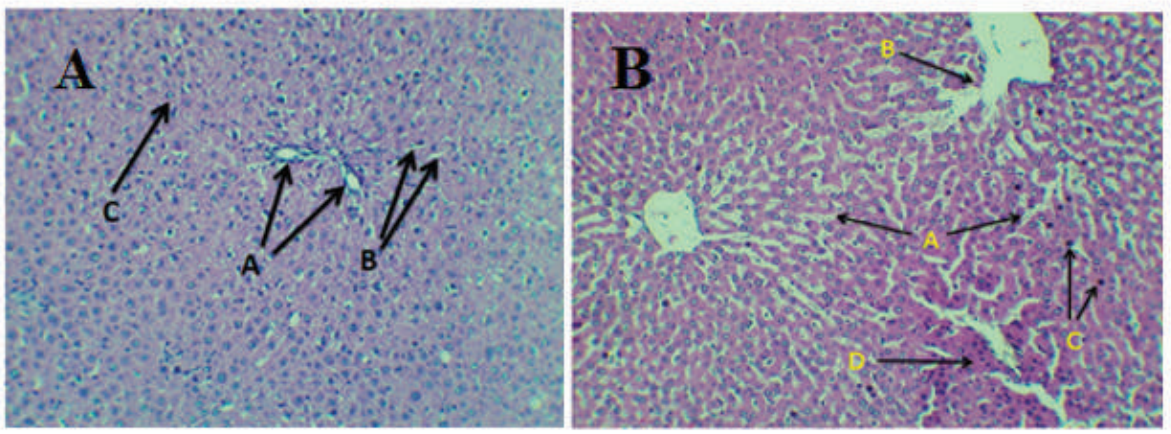


Figure 1 : A- Images microscope represent liver of rat control central vein (A), sinusoidal spaces (B hepatocytes (C).100X(H&E) , B- Images microscope represent liver of rat exposed to formalin 200 ppm at day 30 of the experiment appeared loss of sinusoidal spaces(A), rupture of central-vein (B), pycnotic nuclei(C) and infiltration of lymphocytes(D). 200X (H&E)

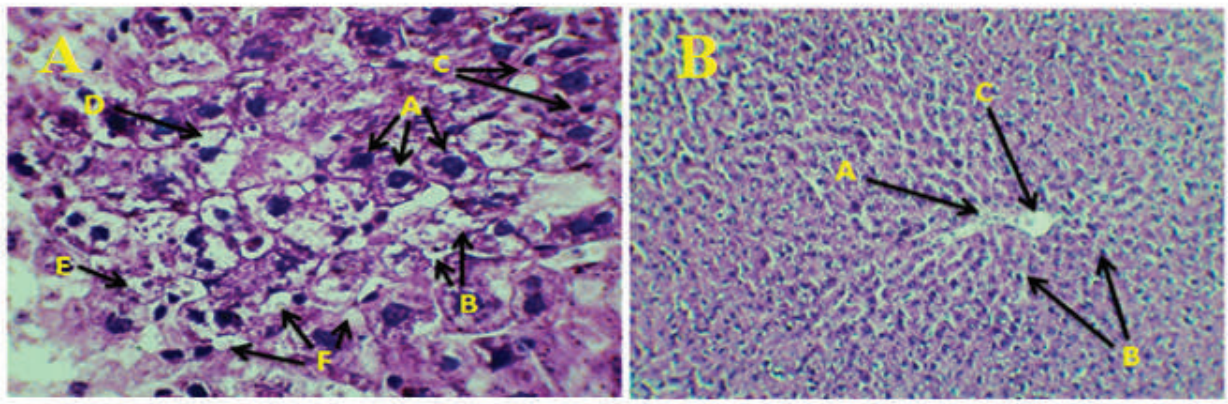


Figure 2 : A-Images microscope represent liver of rat exposed to formalin 400 ppm at day 30 of the experiment appeared pycnotic nuclei(A), degeneration of hepatocytes with numerous vacuolations (B), infiltration of lymphocytes(C), absent nuclei of hepatocyte (D), nuclear-material clumping(E) and necrosis of hepatic cells(F). 400X (H&E), B- Images microscope represent liver of rat exposed to formalin 200 ppm and melatonin 10 mg/kg at day 30 of the experiment less severe central vein congestion (A), less dilated sinusoidal spaces (B) and less impacted endotheliallining(C).200X(H&E)

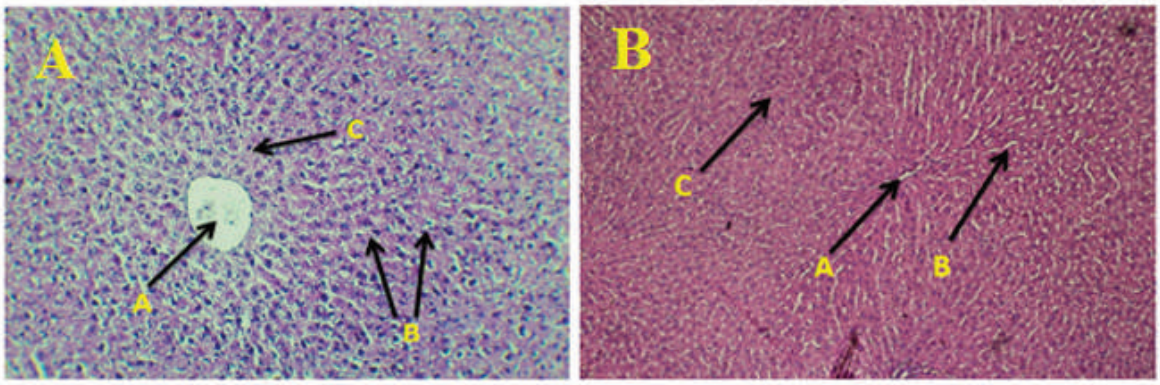


Figure 3 : Images microscope represent liver of rat exposed to formalin 400 ppm and melatonin 10 mg/ kg at day 30 of the experiment less severe central vein congestion (A), less dilated sinusoidal spaces (B) low levels of infiltration of inflammatory cells(C) and no found hemorrhages . 20X(H&E) B- Images microscope represent liver of rat exposed to melatonin for 10mg/kg at 30 days of the experiment normal central vein (A), normal sinusoidal spaces (B) normal hepatocytes (C).40X(H&E)

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Effect of Methotrexate on Adenosine Deaminase Activity in Rheumatoid Arthritis Iraqi Patients

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Abstract

Rheumatoid arthritis (**RA**) is considered as an autoimmune disease which influences the joints. It usually results in a warm, enlarged, and painful joints. Stiffness and Pain typically amplify once relaxation. Frequently, the hands and wrist are involved, with similar joints naturally involved on both sides of the body. The syndrome might additionally have an impact on the other parts of the body. Adenosine Deaminase is an enzyme (EC 3.5.4.4) used with the metabolism of purine. It is essential for the analysis of adenosine from nutrition and the revenue of nucleic acids in soft tissue. Its chief purpose in humans is the immune system growth and preservation. On the other hand, the complete physiological role of ADA is so far not known. This study carried out at Al-Kadima Teaching Hospital, during the period fr6-om September 2014 till the end of October 2015. Patients who have any other disorders conditions are excluded. Subjects accepting treatment with medications within 7-9 months. A total of 66 patients with Rheumatoid arthritis were enrolled in this study: thirty-five of them were newly diagnosed to have Rheumatoid arthritis who receives no therapy for Rheumatoid arthritis (group A); the remaining thirty-one patients were with Rheumatoid arthritis who receive by methotrexate 500 mg for 7-9 months as the (group B). Twenty-six healthy subjects, as controls(group C). The results show a significant elevation in the ADA activity in group A and group B. The study concluded that the level of ADA was higher in Iraqi RA patients than in healthy control subjects

Keywords: *Rheumatoid arthritis, Adenosine Deaminase and methotrexate*

Introduction

Rheumatoid arthritis (**RA**) is considered as an autoimmune disease which influences the joints. It typically results in warm, inflamed, and hurting joints. Rigorousness and Pain characteristically amplify once relaxation. Frequently, the hands and wrist are involved, with the analogous joints certainly intricate on both sides of the body. This force clue to inflammation in the heart, inflammation in the lung, and low red blood cell count, fatigue and elevated in the body temperature could also be found.(1)

Recurrently, signs arise on gradually from weeks to months. Even though the fact that the reason for rheumatoid arthritis is not well identified, it is speculative to contain a miscellaneous of environmental factors and genetic. The exceptional mechanism comprises the aggressive action of the immune system of the body which indication to solidifying and irritation of the joint. It influences cartilage and bone.[1]

The sympathy of this syndrome is to be influenced by a person's symptoms and signs.(2) RA affects between 5 and 50 per 100,000 people newly developing the condition and between 0.5 and 1% of adults in the developed world each year.(3) Onset is persistent through middle age female are influence 2.5 times as often as men.[1] In 2013, it gives rise to in 38,000 deaths up from 28,000 deaths in 1990.(4) Adenosine Deaminase is an enzyme (EC 3.5.4.4) used with the metabolism of purine. It is essential for the breakdown of adenosine from sustenance and for the revenue of nucleic acids in soft tissue. Its topmost purpose in humans is the immune system growth and preservation.(5) Instead, the complete

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biological character of ADA is not recognized ADA present in both major form (dimer-complex) and minor form (monomer). [6] In the monomer configuration, the enzyme is a polypeptide chain,[7] fixed into eight elements of equivalent α/β barrels, which frame a topmost deep pocket that is the active site. Along with the eight essential β-barrels and eight marginal α-helices, ADA likewise embraces five further helices: residues 19-76 fold into three helices, located between β1 and α1 folds; and two antiparallel carboxy-terminal helices are placed across the amino-terminal of the β-barrel. The ADA active site involves a zinc ion, which is located in the deepest recess of the active site and synchronized by the substrate and five atoms from His15, His17, His214, Asp295.[5] The substrate is bound to an active site by nine hydrogen bonds and become stable. The carboxyl group of Glu217, approximately coplanar with the substrate purine ring, is in the site to formulate a hydrogen bond with N1 of the substrate. The carboxyl group of Asp296, correspondingly coplanar with the substrate purine ring, forms a hydrogen bond with N7 of the substrate. The NH group of Gly184 is in position to consist of a hydrogen bond with N3 of the substrate. Asp296 forms bonds composed with the Zink ion (Zn+2) laterally with 6-OH of the substrate. His238 also hydrogen bonds to substrate 6-OH. The 3'-OH of the substrate ribose figure a hydrogen bond over and done with Asp19, conversely the 5'-OH construct a hydrogen bond through His17. Double extra hydrogen bonds are made to water particles, at the preliminary of the active site, thru the 2'-OH and 3'-OH of the substrate. In place of the importance of the recessing of the active site in the interior enzyme structure, the substrate, immediately it bound, is very nearly completely recaptured from the solvent.[7] The surface area of the substrate to solvent once bound is 0.5% the surface area of the substrate in the Free State.

Material and Methods

Subjects: the study was a cross-sectional study carried out at Al-Kadima Teaching Hospital, during the period from September 2014 till the end of October 2015. Patients who have any other disorders conditions are excluded. Subjects accepting treatment with medications within 3 months. All subjects were nonsmokers, and none reported chronic alcohol consumption.

The protocol for the study was approved by the Ethical committee of Al-Nahrain Medical College and informed signed consent was given by each subject. A total of 66 patients with Rheumatoid arthritis were enrolled in this study: thirty-five of them were newly diagnosed to have Rheumatoid arthritis who receives no therapy for Rheumatoid arthritis (group A); the remaining thirty-one patients were with Rheumatoid arthritis who receives by methotrexate 500 mg 7-9 months as a therapy (group B). The study included another Twenty-six healthy subjects, non- alcoholic, non-smoking and no family history of any type of Diabetes Mellitus or Hypertension who serve as healthy controls(group C); they were matched with patients` groups for age as in table 1.

Blood samples:

Five millilitres of random venous blood was withdrawn from each patient, in the supine position, without application of a tourniquet. Samples were transferred into a clean new plane tube, left at room temperature for 15 minutes for clotting, centrifuged at 1800 x g for 10 minutes at 4°C, and the separated serum was transferred into Eppendorf tube and was used for measurement of ADA. The tubes were stored at –20 ºC until analysis, which was done within one month after collection.

Procedure: measurement of serum ADA was done by ELISA kit [8].

Statistical Analysis

Statistical analysis was done using Excel system version 2003 and includes descriptive statistics (mean and standard deviation) and inferential statistics (t-test) to test the significance of the mean difference. When P-value was less than 0.05, the difference is considered statistically significant, and the difference is considered highly significant when P-value was less than 0.001.

Results

Table (1) clinical criteria of patients groups with Rheumatoid Arthritis and control (mean ±sd)

Parameters	Group A	Group B	Group C
Age rang (years)	45-67	45-67	45-67
ADA (IU/l)	13.248±4.145	32.268±4.779	18.738±4.34

Table (2) p-value between the studied groups

The Groups	Group A	Group B	Group C
Group A	--------	≥0.001	≥0.001
Group B	≥0.001	≥0.001	≥0.001
Group C	≥0.001	≥0.001	≥0.001

Discussion

The preclusion of joint damage and reducing the sickness form vital mechanisms of administration of RA patients, the first establishment of the appropriate treatment is essential. Even though clinical guides help in appraising syndrome activity, biochemical indicators are furthermore one of the things for signs of syndrome activity and progress. This study demonstrated in table 1 group B has the maximum level of ADA, though group A the lowest level of ADA. Nevertheless, the strict source of flow in ADA levels is not recognized, the activity might be greater than before as a consequence of its ejection from the damaged cells and initiate of cellular proliferation in RA [(9)] Nalesnik M *et al.,* revealed an insignificant association between CRP and ADA in patients with RA without methotrexate treatment and recommended that ADA activity in serum might be used as a biochemical indicator of the inflammatory progression in RA [(10)].

ADA catalyzes the irremediable hydrolysis of adenosine to inosine. Adenosine has been revealed to be a strong endogenous anti-inflammatory agent [(11).] The enzyme ADA characterizes a checkpoint in the directive of extracellular Adenosine levels [(12)] and therefore is possible to moderate the inflammatory procedures.

A study by Sari et al., [(13)] exposed that serum total ADA activity is linked with RA and may propose a respected helper to measure inflammation moreover to the traditional indices. That suggested that determining ADA activity help for a superior clarifying of some of the pathophysiological sorts of the disease.

Methotrexate is the anchor drug for remedy of RA. Subsequently, the anti-inflammatory influence of MTX is through the incitement of adenosine receptors, it was reflected that the therapeutic properties of MTX could be as a result of catalytic activity of ADA in the serum [(14)] The pathophysiology of adenosine ejection is associated with MTX pathways. It is recommended that MTX stops ADA and surges vasodilatation convinced by adenosine so it is a deduction that ADA activity in serum may be an imaginable biochemical indicator for checking the therapeutic properties of MTX in RA patients. Van Ede et al. resolved a connection between ADA and MTX treatment in patients receiving MTX and folic acid [(15)]. Salesi et al. revealed that ADA levels earlier and later the MTX treatment in their RA group and point to a connotation with ADA levels with the usefulness of MTX treatment [(16)]

Conclusion

This study showed that level of ADA was higher in

Iraqi RA patients than in healthy control subjects.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Application of Infection Control Rules by Iraqi Orthodontics

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Abstract

Orthodontics represent an important dental speciality which deals different situations of patients malocclusion, they use many kinds of sharps and cutting instruments, making them liable to different types of infections which could be transmitted from either the patients or the instruments if they don't follow the infection control principls. The data were collected by answering a questionnaire by 101 orthodontists, the results showed different responses of the participants to the variables used by the questionnaire leading to a conclusion that more efforts are needed to improve the application of infection control guidelines between Iraqi orthodontists.

Keywords: *orthodontics, infection control, health, patients; infection.*

Introduction

Orthodontists facing various types of microorganisms during practicing dentistry by contaminated instruments, inhalation of aerosols or through percutaneous injuries with different wires such as ligature or arch wires also banding and bonding materials and other sharp instruments(1).

Studies found that Orthodontists have the second highest occurance among dental workers concerning hepatitis–B infections (2). People reciving treatment in dental clinic could be undetected hepatitis-B carriers and patients secreting herpes simplex viruses in saliva may be asymptomatic, those patients have the potential for transmitting diseases. Diseases such as hepatitis-B, HIV and tuberculosis have long incubation period and therfore, it is very difficult to detect the origin of such infections to the dental workers and other patients (3).

Before beginning with work the orthodontist should be clear about his or her goals in infection control criteria and it is mandatory to apply the most progressed method of disinfection and sterilization to get good results(4).

Sterilization kills all types of microorganisms including viruses, bacterial and mycotic spores.

Disinfection is the technique used for destruction or inhabition of the most pathogenic microorganisms and inactivating some viruses,therfore, reduction of microbial contamination to a safety levels.(5)

Hepatitis B virus can be spreaded by as little as 0.0004 ml blood while HIV by 0.1 ml blood(6). 1 ml of gingival crevicular fluid contains 150 billion microorganisms and 6 billion microorganisms can be found in 1 ml of saliva (7).

Many studies in literature reavels that emphasize on the effect of sterilization in orthodontics practice however there is no comprehensive research that evaluate the compliance of Iraqi orthodontist to infection control procedures.

Aim of the study

In this study we will evaluate sterilization and disinfection methods employed in orthodontic practice in Iraq.

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Material and Methods

In the present study, data collection gained by an 17 items questionnaire was delivered to a total of 101 Iraqi orthodontists / general practitioners(GP) (who attended intensive orthodontic course), these question covered some infection control guide line.[8]

Statistical analysis was performed by using SPSS version which includes descriptive statistic (frequency and percent) also Z-test were used to test two proportion and chi square for goodness of fit to test more than two proportion. N.S non-significant (P> 0.05), S significant at (P<0.01) and highly significant (P<0.001).

The questionnaire deals with the following variables:

v Educational degree

v Place of work.

v Daily patient volume

v The way in performance of instruments cleaning

v Sterilization devices used

v Soaking instruments in disinfectant solution.

v Packaging method of instruments to be sterilized.

v Method used to sterile hand piece, hand instruments and orthodontic pliers.

v Does the practitioner sterilize molar bands after purchase?

v Sterilization of molar bands after trail inside patient mouth.

v Disposal of brackets, bands and arch wires removed from the patients

v Recycling brackets or other orthodontic materials.

v Disinfectios of the impressions and orthodontic appliances delivered to the lab.

v Place of sharp objects disposal container.

v Type of gloves used during cleaning instruments, and environmental cleaning.

v Hepatitis B vaccination

Result

The result of this study shown in the frequency table explained as the following: table 1 describes the percent of GP and specialist who are alligated to the questioner, (22.8% and 77.2%)for GP &specialist respectivlly.

Table 2 showed that (45.5%,38% and 15.8 %) of the total participate work in privet clinic, hospital and at university clinic, those participate have daily patients volume explained in table 3.

Table 4 show (57.4%) of orthodontist who use manual cleaning significantly higher than ultrasonic cleaner (42.6 %), while highly significant (87%) with autoclave rather than oven and glass bead sterilizer, also highly significant regarding presoaked instrumentand wraping the instrument ,while sterilization of dental hand piece in autoclave (41.6%) was significantly lower than wiping the outer surfaces with (57.4%).

Table 5 show highly significant rate of sterilize pliers (80.2%) than dry heat, while non-significant sterilize band after purchase in comper with highly significant (67.3%) for band after trial in patient's mouth .The disposing of band, bracket and arch wire was highly significant with (49.5%) regarding waste basket .

Table 6 show high rate of significantly for orthodontist who don't recycled bracket while non-significant difference between orthodontist who disinfect impression or appliances . The rate for placing sharp bins at clinic was significant (74.3%) and (21.8%) for placing sharp bins in sterilization room. Highly significant rate for orthodontists who use examination gloves during cleaning of the instrument, and how recive hepatitis B vaccine.

Table 1 Gp or Specialist

Frequency			Percent
Valid	GP	23	22.8
	Specialist	78	77.2
	Total	101	100.0

Working place Table 2

Frequency			Percent
Valid	Private dental clinic	46	45.5
	Specielized dental centers / state hospital	39	38.6
	University clinic	16	15.8
	Total	101	100.0

Table 3: Number of patient

Frequency			Percent
Valid	0-5	40	39.6
	6-10	18	17.8
	11-15	14	14.9
	16-20	15	14.9
	> 20	13	12.9
	Total	100	99.0
Total		101	100.0

Table 4: percentage of cleaning and sterilization of the instrument

Question	Choice	Frequency	%	P-value¥
Cleaning performance	Manually	58	57.4	0.033
	Mechanically (Ultrasonic cleaner	43	42.6	
Sterilization devices used	Autoclave	88	87.1	0.001
	Dry heat (oven)	12	11.9	
	Glass bead sterilizer	0	0.0	
	none	1	1.0	

Theinstruments are presoaked in disinfectant solution	**Yes**	**86**	**85.1**	**0.001**
	No	**15**	**14.9**	
Instruments Packing during sterilization	Metal tray	38	37.6	0.021
	Wrpa (Pouching)	41	40.6	
	I do not pack	20	19.8	
Sterilization of dental hand pieces	In the autoclave	42	41.6	0.022
	Wiping the outer surface with disinfection solution	58	57.4	

N.S, not significant (P >0.05) , * significant at (P < 0.05), ** significant at (P < 0.01), * significant at (P < 0.001).**

Table 5: percentage of sterilization of orthodontic pliers, bands and disposed method

Question	Choice	Frequency	%	P-value¥
Sterilization of hand instruments / or orthodontic pliers	Dry heat (oven)	12	11.9	0.001
	Autoclave	81	80.2	
	Glass bead sterilizer	1	1.0	
	Wiping with a disinfictant solution	6	5.9	
Sterilization of molar bands after purchase	Yes	48	47.5	0.617
	No	53	52.5	
Steriliztion of molar bands after check in the patient mouth	Dry heat (oven)	10	9.9	0.001
	Autoclave	68	67.3	
	glass bead sterilizer	2	2.0	
	Sitting in disinfectant solution	17	16.8	
Where do you dispose the bands , brackets, and arch wires you remove from patient during or after treatment	Waste basket	50	49.5	0.001
	Sharp bin	27	26.7	
	Metal waste bin	6	5.9	
	Infected waste bin	17	16.8	

N.S, not significant (P >0.05) , * significant at (P < 0.05), ** significant at (P < 0.01), * significant at (P < 0.001).**

Table 6: the response to different question related to infection control roles

Question	Choice	Frequency	%	P-value¥
Recycled brackets or other orthodontic materials	**Yes**	**9**	**8.9**	0.001
	No	91	90.1	
Disinfection of impressions or other orthodontic appliances to be delivered to lab	**Yes**	48	47.5	0.670
	No	51	50.5	
Where do you place sharp bins	at the clinic	75	74.3	0.001
	In the sterilization room	22	21.8	
Type of gloves used during cleaning of instruments and environmental cleaning	Examination gloves	97	96.0	0.001
	Kitchen - type gloves	3	3.0	
Have you had hepatitis B vaccine	Yes	83	82.2	0.001
	No	16	15.8	

N.S, not significant (P >0.05) , * significant at (P < 0.05), ** significant at (P < 0.01), * significant at (P < 0.001).**

Discussion

Spaulding system classifies instrument into three categories which are critical, semicritical and least critical[9] , the semicritical considered the most important one that should be highlighted in order to prevent disease transmission [10].Orthodontic instruments,orthodontic supplies and accessories considered as the semicritical since they touch mucous membrane and non-intact skin [11,12].

This study mainly depended on experience, place of work as well as, daily patient capacity of the GP and orthodontist. The sterilization process is important to orthodontists as well as, dentists, even though they do not perform surgical procedures [13-14] .

In another hand Starnbach & Akçam [15-16] indicate, sterilization is less abidance to orthodontists than dentists since they usually deal with children, with loss of time , money and the corrosion of orthodontic instruments in addition they did not deal with deep tissues.[8]

Professional agencies like Center of Disease Control (CDC) and Occupational Safety and Health Administration (OSHA), have a specific recommendations representing standard infection control which considered as keywords to be followed in order to prevent cross infection [17].

Despite of these rules, this study showed that (57.4%) of participate orthodontists depends on the manual cleaning procedure more significantly than the ultra-sonic devises. This may be due to the lack of knowledge about advantage of ultra-sonic devises in the granting the proper removing of debris from the orthodontic instrument [18-19], the manual cleaning is also an important step in ensuring the debris removal after mechanical cleaning and before autoclaving [20].

In this study (85.1%) of participated presoaked the instrument in disinfected solution before starting the sterilization process. This prevents the dryness and adherence of bioburden to instruments that protect

microorganism from sterilization [21].

In addition to that it begins to dissolve organic debris and in some instances begin microbial kill.This solution should discard at least once a day [17].

This study showed high percentage of participates who used autoclave whether used for orthodontic pliers , band purches and molar bands after trying in patient mouth [22]. This concurred with the significant findings of participates who pouch their instruments. This could be explained by the high education level and interest in sterilization of orthodontists and according to recommendations of Iraqi Dental Association (IDA).

Although most of orthodontist (87%) using autoclave but the result showed that high percentage of them only wipes the hand piece which is not recommended as the hand piece represents a hallow instrument that may contain blood drops contamination inside the internal lumen and this can be only sterilize by using autoclave class B [23, 24-25-26] .This could be the result of old believes that heat could ruin the hand piece leading to a financial lost.

As a matter of fact the hand piece sterilization is obligatory according to the CDC guidelines. This can be achieved neither by providing clinics with an enough amount of hand piece in order to match the number of patients who daily visit the clinics, nor by applying advanced sterilization programs [27]. Since hand pieces are available in the Iraqi market with an affordable cost.

Bracket, wire and bands represents a dangerous source since they are removed from oral cavity in which they had been contaminated with body fluid (saliva and blood) also wire's end considered as a sharp end that may prick the orthodontist, so the sharp pin represent the better choice for disposing [28,29] .

This study showed that (74.3%) of participant have sharp bin in their clinics, despite of the importance value of having it in the clinics, only (26.7%) who are really using it while (49.5%) of the participant use waste basket. This approved a week point in disposal.

This study showed a highly significant percentage (90.1%) of the participant who do not use recycle brackets and orthodontic materials since the process of recycling altars the mechanical and physical properties also it is nor granted that they are not contaminated[30,31] also, the brackets and orthodontic materials are available in the Iraqi market with a reasonable cost.

In this study, a non-significant value (50.5% -47.5%) have been shown between the participates who disinfected their impression or appliances to be delivered to an outer laboratory. Standers showed that all impression and model must be disinfected before delivering to the laboratory and vice versa [32].Now a day, this complicated issue has been solved by introducing the digital scan in the dental filed [33].

Kitchen gloves considered as a heavy duty gloves that protect the operator from accidental puncher by sharp dental tools and cross infection [17]. Unfortunately,this study showed a high rating of (96%) who use examination gloves which considered a thinner and easy tearing gloves as compared to the kitchen gloves [8].

Dentists and assistances are always mandatory to be vaccinated against hepatitis B virus [34], this coincide with the result of this study which revealed the highly significant rating (82.2%) who had been vaccinated.

Conclusion

The result of this study reveals good behaviours by Iraqi orthodontist for most of infection control steps although some behaviors need to be improved following world wide infection control guide lines.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non.

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Mast cell, IL-1 Beta and IL-6 for Wound timing and Vitality in Forensic Practice

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Abstract

the application of immunohistochemistry has opened a new era for examining the age of the wound by forensic specialists, The aim of our study was to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase "MCT") as a reliable and promising factor of wounds vitality and to Identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination. The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. The specimens were sera and skin tissues for immunohistochemistry examination, and staining procedures and immunohistochemistry (MCT) ELISA sandwich plate will measure the levels of interleukins. Total autopsy cases were 88 and divided into study group (SG) and control group (CG) .We found that density of Mast cells in sample group was significantly high and higher infiltration that correlates with time passing in the study group as compared by other group in dermis of the sample and control lesions and positive relationship between levels of IL 1-beta & IL-6 level and time progress of wound..

Key words: *forensic, pathology, wound, vitality, IL 1_beta, IL_6 level, Mast cells*

Introduction

During the inflammatory phase, a variety of chemicals are released at the affected site, leading to the recruitment of inflammatory cells, such as neutrophils and plaques Forensic scientists, unlike general pathologists, tend to focus on chronic mapping of the appearance and disappearance of inflammatory cells or substances produced during the inflammatory process[1]. These phenomena - for example, the proportion of positive cells, the level of tissue fibrosis, the distance between inflammatory cells and the free vessels that affected by the degree of injury, which affect the accuracy of the determination of the age of the wound. It is therefore necessary to establish models with varying degrees of injury and to evaluate the parameters involved in wound healing to determine the time of injury[2].

Thus, MCs have been involved in causing many chronic allergic / inflammatory disorders, autoimmune diseases, and cancers. The contributions of MCs in these conditions are the subject of continuous assessment[3]. Acute direct events, the allergic process involves subsequent stages characterized by leukocytes infiltration and initiation of an acquired immune response, followed by a chronic phase involving persistent inflammation, tissue reformation and fibrosis. Thus, the role of MCs at these different stages has gained increasing importance. The dissociation of MCs, in addition to their well-established and widely studied role in IgE-mediated interactions, has been the focus of MCs research in the past decades. However, the determination of the functions of MCs may progress slowly due to difficulties in accessing these cells in vivo and the obstacles encountered when obtained through enzymatic dispersion of tissues or by culture of predators of MCs isolated from the bone marrow or cord or peripheral blood. The culture of predators of mast cells produces a small number of MCs that are often expensive and time-consuming and lead to the emergence of changing phenotypes due to cultural conditions[4].

Several studies reported high levels of IL-1β production by nuclear monocyte, platelet and nuclei

cells from active colon lesions in IBD (inflammatory bowel disease) patients. Since IL-1RA levels are only moderately controlled in the colon in patients with IBD, the IL-1RA ratio to IL-1β decreases significantly, enhancing intestinal inflammation[5]. IL-1β is initially produced as zymogen, but pro-IL-1β does not contain any known functional properties. IL-1β copies are tightly regulated, stimulated by stimulation by TIR or IL-1R stimulation (the first signal required to produce IL-1β) As previously described, IL-1β precursors are bisected in its active form by caspase-1, after inflammatory activation with the NLR (NOD like receptor: NOD for nucleotide-binding domain, Lucien rich repeat containing receptors) pathway (signal 2) [6]. The secretion mechanisms of IL-1β and IL-18 in extracellular space are unclear, but proposed pathways include exocytosis of secretory lysosomes, shedding of membrane micro-vesicles or exososomes, or transfer by membrane vectors, such as ASC (alanine serine cysteine amino-acids transport system) transporters[7].

IL-1β practices a wide range of systemic and local effects. Once in circulation, IL-1β can promote the synthesis of ring-2 oxidation enzymes (COX2) in the vascular network, which stimulates the production of prostaglandin E2 in the brain and fever-mediated. IL-1β also promotes the synthesis of acute phase proteins by liver cells, derives differentiation of macrophages, differentiation of neutrophils and mobilization in bone marrow. Furthermore, IL-1β promotes the recruitment of immune cells to inflammatory sites, by stimulating the expression of adhesion molecules and chemical attractors via endothelial cells[8].

On the other hand Given the IL-6 behavior of restoring the host to the parity state, it is clear that IL-6 acts to control the response of tissue inflammation. In chronic diseases, which are usually manifested by immune stress factors such as intracellular infection and chronic tumors, IL-6 acts not only as a catalyst for acute phase reactions but also as an important player in cellular immune response responses to infected cells and mucosal- The inflammatory reaction is acute, resulting in the destruction of a harmful agent within a short period of time and in a localized area, stimulating an immune response.[8]

IL-6 not only stimulates acute phase reactions but also develops cellular immune responses, including end-stage B cell differentiation, immune globulin secretion and T-cell activation. The main key from acute inflammation to chronic inflammation is the recruitment of monsters to the inflammation area. IL-6 is important for the transition between acute and chronic inflammation. IL-6 plays a somewhat unexpected role in recruiting white cells in vivo. The IL-6 and sIL-6Rα compounds can activate endothelial cells to secrete IL-8 protein and MCP -1, and urge the expression of adhesion molecules[9].

The aim of the study is to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase "MCT") as a reliable and promising factor of wounds vitality and to identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination.

Methodology

The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. Lacerated skin wounds with a known timing since injury less than 12 hours, regarding time of autopsy all cases were underwent autopsy as soon as possible within a maximum time of 3 hours since arrival to mortality. Only frank lacerated non-contaminated skin wounds were taken in account and the autopsy was done immediately on arrival to MLD. Total autopsy cases were 88 and divided into study group (SG) and control group (CG) [stab-wounds injuries with immediate death]. The specimens are sera and skin tissues for MCT immunohistochemistry.

ELISA sandwich plate will measure the levels of interleukins and specimens for histopathology will be taken from the periphery of wounds for SG and an intact healthy skin from same cadaver (internal control) and some tissue specimens from deaths due immediate death by bullet injury as (external control), stored in 10% formalin for staining procedures and immunohistochemistry (MCT) and the results was analyzed statistically.

Strict exclusion criteria for cases of study include mixed wounds, wounds with unknown timing since injury, contaminated lacerated wounds, wounds with expected timing less than 12 hours, firearm deaths, cases with a known documented history of chronic illnesses,

age less than 15 and more than 35 years, pregnant, decomposed cadavers (delayed autopsy),major medical interventions like surgery.

The Quantikine® Human IL-1β/IL-1F2 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human IL-1β in cell culture supernates, serum, and plasma The Quantikine® Human IL-6 Immunoassay is a 4.5 hour solid phase. Both contain E. coli-expressed recombinant human IL-1β and IL 6 and antibodies raised against the recombinant factors.

TB staining was used for presence of "Mast cells" and MCT immunohistochemistry by Elabsceince® is the preferable way for detecting mast cells activation.

Results

The number of wounds in the study sample was determined; most of the bodies had three wounds, 44% of the total study, where 6% of the cases had only one wound. The study group of 156 laceration skin wounds was classified into compression laceration (50%), grinding laceration (20.5%), cut laceration (6.5%), tearing (15.3%) and crush injuries (7.7%)

Mast cells by toluidine blue stain

Mast cells are found in connective tissue and the cytoplasm contains **(heterogeneous)** granules consisting of **heparin and histamine**. Toluidine blue stains mast cells with red-purple color **(metachromatic staining)** and blue background **(orthochromatic staining)** Metachromasia and tissue color staining elements differ according to the features of the dye solution due to the pH, dye concentration and temperature of the underlying dye. Blue or purple dyes will show a red shift while red dyes will show a yellow shift with contrasting tissue elements.

Depending on the number of mast cells stained by toluidine blue, three classes of were estimated, from 2 to 5 cells on 10 HPF the number of mast cells stained was regarded **normal finding**, from 6-8 cells on 10 HPF (+1) and from 9-10 cells on 10 HPF and (+2) and > 10 cells on 10 HPF (+3) The specimens included in the study did not have any histologic abnormality. Using light microscopy, all the mast cells (MC) in the specimens (however fixed) had meta-chromatically stained purple with toluidine blue. The nuclei of all the cells were round or oval. MC were scattered in the dermis, especially along the blood vessels and in the peri-glandular stroma. In the dermis of the Sample and control lesions, Mast cell density in the Sample group was significantly higher (P<0.001) when compared with the other group.

Table 1: Distribution of mast cells

Sample group						
	one-hour	two-hours	three-hours	four-hours	five-hours	six-hours
< 10%	0	0	0	0	0	0
10-35%	0	0	0	0	18.2	36.4
35-60%	36.4	18.2	27.3	18.2	18.2	9.1
>60%	63.6	54.5	63.6	36.4	9.1	0
Control group						
	one-hour	two-hours	three-hours	four-hours	five-hours	six-hours
< 10%	63.6	36.4	54.5	36.4	27.3	9.1
10-35%	36.4	36.4	36.4	18.2	9.1	27.3
35-60%	0	0	0	0	0	0
>60%	0	0	0	0	0	0

Immunohistochemistry (Mast cells tryptase) for both samples and control

Immunohistochemistry of Mast Cell Tryptase results were recognized and classified into 4 classes according to the mast cells in both control and samples through the period of 6 hours after injury. Tryptase immune reactivity was detected in all of the cells stained by fluorescent avidin fixed either with Bouin or Carnoy's fluid. Some MC, in the dermis of all the examined groups, was immune stained for chymase upon fixation with Carnoy's fluid.

Table 2: Correlation between test group and control group number of mast cells

Correlations

	Time interval	one	two	three	four	five	six
one	Pearson Correlation	-.214	.500	-.167	.000*	-.577	.577
	Sig. (2-tailed)	.527	.207	.645	1.000	.423	.423
	N	11	8	10	6	4	4
two	Pearson Correlation	-.577	-.577	.577	.316	.333	-.333
	Sig. (2-tailed)	.134	.134	.134	.541	.667	.667
	N	8	8	8	6	4	4
three	Pearson Correlation	.535	.775*	-.802**	.000	-1.000**	1.000**
	Sig. (2-tailed)	.111	.024	.005	1.000	.000	.000
	N	10	8	10	6	4	4
four	Pearson Correlation	-.707	-.250	1.000**	.500	.577	-.577
	Sig. (2-tailed)	.116	.633	.000	.312	.423	.423
	N	6	6	6	6	4	4
five	Pearson Correlation	.577	1.000**	.000	.000	-.577	.577
	Sig. (2-tailed)	.423	.000	1.000	1.000	.423	.423
	N	4	4	4	4	4	4
six	Pearson Correlation	0	0	0	0	0	0
	Sig. (2-tailed)	.	.	.	.	.	.
	N	4	4	4	4	4	4

**. Correlation is significant at the 0.01 level (2-tailed)

*. Correlation is significant at the 0.05 level (2-tailed)

ELISA levels of IL-1 beta and IL-6 in the human model

IL-1 beta standard of the kit measures at 3.9 pg/ml and minimum detectable dose (MDD) of human IL-1 beta is less than 1 pg/ml. The levels of IL-1 beta in the human model shows a significant decrease in relation to the time of the test (1 to 6 hours AMI), as showed in table 2 that the levels of IL-1 beta in the human model samples shows a maximum mean difference of 40.727 at 1 hour of AMI while the control group mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours. The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model.

Table 3: The levels of IL-1 beta in the *h*uman *m*odel

One-Sample Test							
	Test Value = 0						
	t	df	Sig. (2-tailed)	p-value	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
c	1.005	8	.000	.344	284.58456	368.1346	937.3037
h1	7.883	10	.000	.000	40.727	29.22	52.24
h2	4.422	7	.000	.003	20.58100	9.5756	31.5864
h3	6.805	9	.000	.000	8.43860	5.6336	11.2436
h4	11.535	5	.000	.000	4.64558	3.6103	5.6808
h5	15.495	3	.000	.001	2.86500	2.2766	3.4534
h6	9.060	4	.000	.001	3.49600	2.4247	4.5673

H1, after one hour AMI H4, after four hours AMI c, control
H2, after two hours AMI H5, after five hours AMI
H3, after three hours AMI H6, after six hours AMI

IL-6:

IL-6 standard of the kit measures at 3.13 pg/ml and the MDD of human IL-6 is typically less than 0.70 pg/ml. The IL-6 levels show **increase** with the time of **AMI** progress. The control samples show mean difference of .57436. The sample group shows maximum mean difference of IL-6 level of 353 at five hours of AMI.

Table 4 : The levels of IL-6 in the human model

One-Sample Test						
	Test Value = 0					
	t	df	Sig.	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
c	5.980	10	.000	.57436	.3603	.7884
h1	1.127	10	.286	269.933	263.73	803.59
h2	15.359	7	.000	2.16250	1.8296	2.4954
h3	13.272	9	.000	1.40290	1.1638	1.6420
h4	9.805	5	.000	1.89883	1.4010	2.3966
h5	1.006	3	.389	353.03625	763.9650	1470.0375
h6	6.926	4	.002	.63980	.3833	.8963

H1, after one hour AMI H4, after four hours AMI c, control

H2, after two hours AMI H5, after five hours AMI

H3, after three hours AMI H6, after six hours AMI

Discussion

In this study we found that density of Mast cells in sample group was significantly high (P<0.001) as compared by other group in dermis of the sample and control lesions. This result goes with the results found by Bonilli et al. [10] in the contrary to[11] who noted no significantly different mast cells' number of in normal cutaneous tissue & those in wounds of the sampling which could be explained partly by different techniques & morph-metrical methodology.

In this study we found higher infiltration with mast cells that correlates with time passing in the study group which agrees with the results found by [10] who utilized anti-tryptase & chymase antibodies or avidin to assess density of mast cells in skin wounds through immunofluorescences And found that the MC number in the dermis showed progressive increase within little hours after injury (top at 1 to 3 hours) Matching results was found by [12] who noted a powerful extra-expression of tryptase located in interstitium.

Levels of IL-1 beta in the human model samples show a maximum mean difference of 40.727 at 1 hour of AMI while in control group, the mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours .The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model. This results matches with [13]who found IL-1β, expressed in normal human skin modified in a significant way in vital injuries in epidermal strata, sub-epidermal cellular, vascular and glandular (sweat) and had promoted expression after fifteen and twenty minutes at early increase of reactivity of the epidermis & following thirty to sixty minutes, marked expression was noted, remained many hours & later decreased to a base level again. He commented that it can help as a beneficial method to estimate vitality and aging of wounds, especially during early post-traumatic period before the leukocytic reactions. On another hand this is against what was found by [14] who stated that no wound groups showed a high increase in IL-1b level in comparison to control group. The difference was not significant from statistical view.

In our study the IL-6 levels shows increase with the time of AMI progress which matches with what was found by [15] and the same results was obtained by[16]

Conclusion

Mast cells by toluidine blue stain density in the sample group was significantly higher when compared with the other group in the dermis of the sample and control lesions. The same was shown by Immunohistochemistry (Mast cells tryptase) as there were higher infiltration with mast cells that correlates with time passing in the study group. Regarding ELISA levels of IL-1 beta and IL-6 in the human model the study found a reverse relationship between AMI time and the levels of IL-1 beta in the human model while the IL-6 levels shows increase with the time of AMI progress.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Association between Genetic Polymorphism of 5-HTTLPR, and SGOT, SGPT and Catalase with Alcoholism of Iraqi People

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Abstract

A case- control study on the relationship of 5-HTTLPR gene polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. DNA was extracted from the blood samples of the study subjects. The genetic polymorphism analysis was conducted by Restriction Fragment Length Polymorphism (RFLP-PCR) for SLC6A4 gene. The results raveled that the odd ratio for the *LL* genotype was 1.697 indicating that homo mutant genotype were at higher risk for alcoholism than the wild type *SS*. This result showed that L allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group. So, the results showed there was a significant difference in allele frequencies of *SLC6A4*gene between case and control (P<0.01; OR=1.697; 95% CI=0.86-1.64). There was a significant positive correlation between the SGOT activity and the genotype *of 5-HTTLPR* (*SS, SL, LL*).Additionally, there was a significantly increased level of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic males compared with non-alcoholic males.

Key Words: *SLC6A4, 5-HTTLPR, SGOT, SGPT, catalase.*

Introduction

Alcohol dependence and alcohol abuse are chronic disorders comprising a wide range of clinical symptoms. Given that drinking behaviors are jointly determined by genetic and environmental risk factors, alcohol consumption and alcohol use disorders are appropriate phenotypes for investigating gene- environment interactions [(1).] Alcohol consumption is a common, complex trait, and heavy alcohol use increases the risk of alcohol use disorders, and is recognized as, a problematic global problem threatening both individual development, family life and social life of a person [(2).] There is an evidence of a causal relationship between alcohol and at least 200 diseases including gastritis, pancreatitis, cardiovascular disease, liver cirrhosis, hepato cellular carcinoma, and gastric cancer [(3).] Ethanol is not stored in the body after ingestion because it is fully ingested oxidized during metabolization in the liver [(4).] The rate of ethanol metabolism determines the concentration of ethanol and its metabolite acetaldehyde in the different tissues, which in turn influences the effects of ethanol consumption on liver and the other organs. Serotonin is a signaling molecule with a widespread effect in the CNS, and has a very important role in different aspects of mammalian life, like food intake, emotion, mood, respiration, pain sensitivity, cardiovascular regulation, sexual behavior, learning and memory, circadian rhythm, sensorimotor activity, and cognition [(5).]

The human serotonin transporter (SETR) is a monoamine transporter protein, encoded by a single gene (SLC6A4, solute carrier family 6, member 4) located on the long arm of chromosome 17 (17q11.2). The serotonin transporter (5-HTT) is an important protein responsible for the active transport of serotonin into neurons, enterochromaffin cells and platelets [(6).] Twin studies have shown alcohol dependence (AD) to have a heritability of □50–60% [(7, 8).] Among the genetic components, many of the genes that may contribute to the risk of alcohol phenotypes encode components of the dopamine, serotonin (5-HT), and acetylcholine neurotransmitter systems.

Material and Methods

Study Subjects

A case- control study on the relationship of 5-HTTLPR polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. Fifty blood samples of alcoholic male Iraqi people (with a history of alcohol abuse for more than seven years) were collected from the Institute of forensic medicine, Al-Yarmouk Teaching Hospital, Shaikh Zayed Hospital, and Ibn-Rushed Teaching Hospital/Baghdad, Iraq. The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86.

Blood Sampling

Five milliliters of blood were collected by vein puncture, two ml was put into EDTA tubes for molecular analysis and three ml put in separating gel tube, then was allowed to clot at room temperature for 30 minutes and then centrifuged at 2000 rpm/ 15 minutes. The sera were collected and stored at -20ºC until analysis.

Enzymatic Assay

The liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic and non-alcoholic males were measured by Reflontron/ Germany in this study.

Genotyping of SLC6A4 Polymorphism

Preparation of Genomic DNA

The DNA from genome was prepared from blood samples (gSYNC™ DNA Extraction Kit) according to the instructions of the manufactured. Concentrations and purity of DNA were measured by Nano drop spectrophotometer (Apel/Germany).

PCR condition and Restriction Fragment Length Polymorphism (RFLP)

Two primers were selected (F-5- GGC GTT GCC GCT CTG AAT GC -3) and (R-5- GAG GGA CTG AGC TGG ACA ACC AC -3 ′) [9] to amplify fragments of (469 &512) bp for the detection of alleles. The specific designed primers were provided by AccuOligo/ Bioneer/ Korea. The PCR reaction was performed in a total volume 20 µl containg 10 p mole/µl of each primer, 1x Master mix(AccuPower® ProFiTaq PCR PreMix/ Bioneer/ Korea), and 0.15µg/µl genomic DNA. The reaction mixture was amplified in thermal cycler (Cleaver Scientific, UK). Initial denaturation was carried out at 95°C for 5 min and the target DNA was amplified in 40 cycles. Subsequently, each cycle consisted of denaturation at 95°C for 30 sec; followed by annealing at 63°C for 30 sec. Elongation was carried out at 72°C for 1 min. The final extension step was performed at 72°C for 10 min. Aliquots of products of amplified DNA were treated with *MspI* (restriction enzyme, SibEnzyme/ Russia). The PCR product (*5-HTTLPR)* was digested with restriction endonucleases in a total volume of 20µl containing 10 units of enzyme with buffers supplied by the manufacturer's instructions. The amplified PCR products were checked for the expected size on 2% (w/v) agarose gel and visualized after staining with ethidium bromide under ultraviolet. A 100bp DNA molecular weight marker (BioNeer/Korea) was used to measure the weight of the fragments [10].

Biostatistical consideration

The [11] program was used to analyze the difference factors in study variables. Chi-square test was used to compare differences among percentage at P-values of 0.05 and 0.01 probability. Odd ratio and 95% CI were estimated.

Results and Discussion

The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86, table (1).

Table (1): Distribution of ages of alcoholic male among cases and control

Age group (year)	Group	
	Cases	Control
< 30	16 (32.00%)	14 (28.00%)
30-40	20 (40.00%)	25 (50.00%)
> 40	14 (28.00%)	11 (22.00%)
Total	**50**	**50**

Results listed in table (1) indicated that the most common age group of cases(alcohol consumption) was of 30-40 years (40.00%) followed by the group less than 30 years (32.00%), and more than 40 years (28.00%).

Blood Chemistry Analysis

The results of testing for GPT and GOT in serum from the cases as compared to the controls are presented in figure (1: A, B).

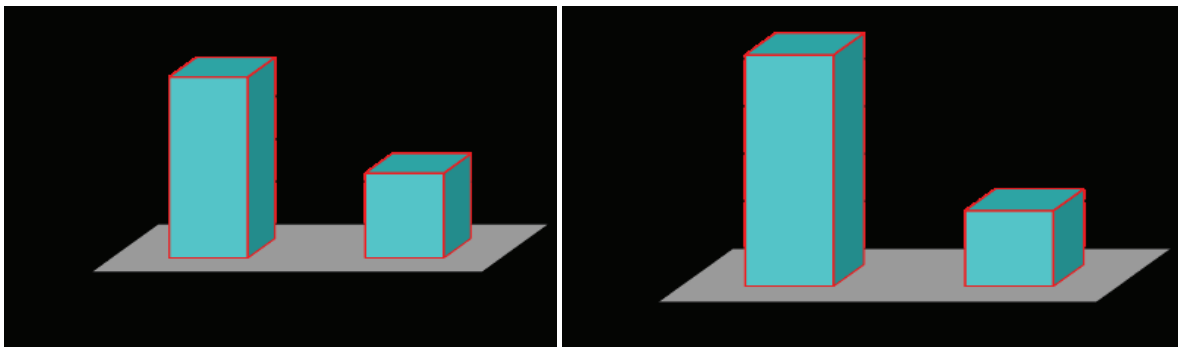


Figure 1: Comparison of liver enzymes; A (GOT) and B (GPT) in serum samples of alcoholic and non-alcoholic males

The results in the present study indicated that parameters measured (GPT and GOT), which are markers liver functions revealed significant differences between cases and controls, (42.46 ± 23.39 vs. 19.89 ± 11.31) (48.69 ± 21.31 vs. 15.93 ± 9.27) at (P<0.01) for GPT and GOT, respectively. Measurement of SGOT and SGPT becomes very important because these liver enzymes are the most important liver enzymes to represent groups or transaminase aminotransferase enzyme, which catalyze the keto acids into amino acids by transfer of amino groups. Serum enzymes are the most commonly used and sensitive biochemical markers for the assessment of liver disease. [12], who reported that alcohol is a toxin that is harmful to the liver and alcoholic liver disease and it is one of the leading causes of alcohol-related death. While [13], found consumption of alcohol causes several pathological changes in the liver. In respect to catalase there was significant difference between case and control group (363.42 ± 214.61 vs. 101.51 ± 51.64) at (P<0.01) respectively as show in figure 2.

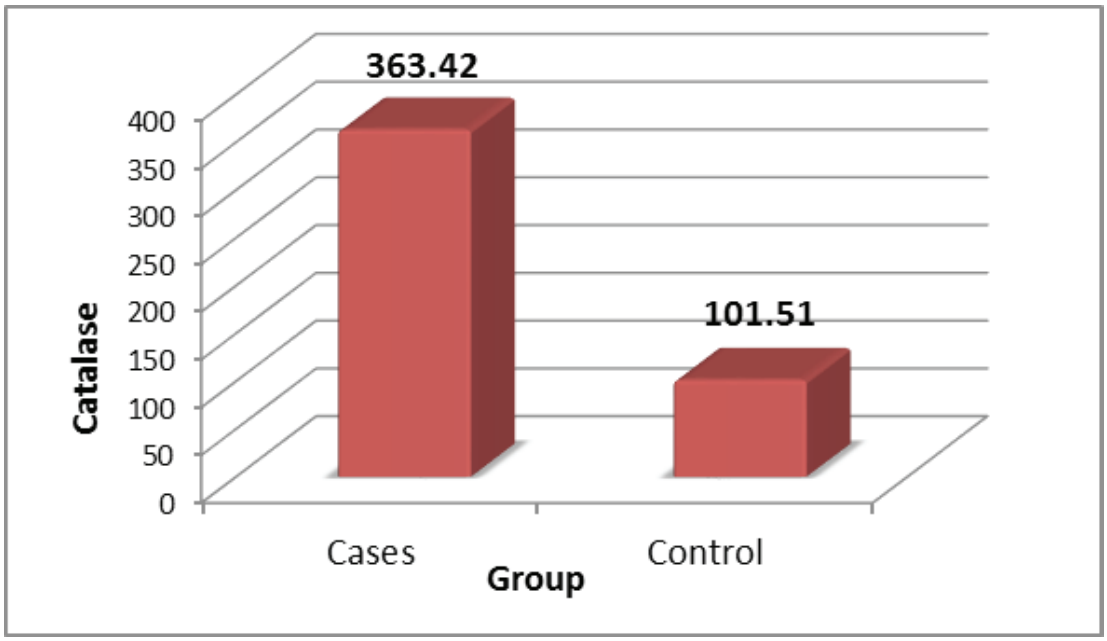


Figure 2: Comparison of catalase in serum samples of alcoholic and non-alcoholic males

The enzyme catalase has also been shown to oxidize ethanol into acetaldehyde within the peroxisomes. This process is hydrogen peroxide dependent. However, under normal physiological conditions, catalase plays only a minor role in ethanol metabolism,[14] but its contribution might be enhanced in the presence of higher amounts of hydrogen peroxide. Furthermore, catalase may be an alternative metabolic pathway for ethanol oxidation within the brain, where ADH and CYP2E1 appear to be of minor importance for ethanol metabolism [15].

[16] reported that the catalase activity increased in lower concentration of alcohol exposure, but in higher concentration of alcohol exposure catalase activity decreased compared to control groups . This finding suggested that catalase activity in the liver is changeable [17].

80-90% of alcohol breakdown in the liver results in the formation of acetaldehyde whose further metabolism in the cells leads to reactive oxygen species production (ROS) [18].

Acetaldehyde itself is a mutagenic and carcinogenic by product. It binds with DNA and interferes with DNA synthesis and repair mechanism. Furthermore, it results in tumor development [19].

Distributions of Genotypes and Allele Frequency of the 5-HTTLPR Polymorphisms in the *SLC6A4* Gene

The distribution of the observed *SLC6A4* gene genotypes and alleles frequencies in the control and cases individuals are shown in table (2).The highest genotype in the case group was homozygous *LL*(74.00%), while(18.00%) for homozygous *SS* genotype, and *LS* (8.00%).

Table (2): Distribution of genotype and allele frequency of 5-HTTLPR gene in cases and control.

Genotype of 5-HTTLPR	Cases No. (%)	Control No. (%)	Sig.	O.R. (95% C.I.)
SS	9 (18.00%)	5 (10.00%)	0.0438 *	0.662 (0.78-1.56)
LS	4 (8.00%)	37 (74.00%)	0.0001 **	1.750 (0.86-1.71)
LL	37 (74.00%)	8 (16.00%)	0.0001 **	1.697 (0.86-1.64)
Total No.	50	50	---	---
Allele	Frequency	Frequency		
S	0.22	0.47	---	---
L	0.78	0.53	---	---
* (P<0.05), ** (P<0.01).				

The results from table (2) show significant differences in the frequencies of *SLC6A4* gene (LL) in the control and case groups (at P-value <<0.01). The results raveled that the odd ratio for the LL genotype was 1.697 indicating that homo mutant genotype were a higher risk of alcoholism than the wild type SS. This result showed that L allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group as shown in table (2). So, the results showed there was a significant difference in allele frequencies of *SLC6A4*gene between case and control (P<0.01; OR=1.697; 95% CI=0.86-1.64).

Alleles of the 5-HTTPLR promoter have either a short (S) or long (L) copy of an imperfect repeat. The short or 'S' allele with 14 repeats was shown to have lower transcriptional activity than the long or 'L' allele with 16 repeats [20, 21]. [22] reported that the 'L' allele is associated with a predisposition to lower level of response to alcohol, which is in turn associated with the onset of alcoholism. While several previous studies that suggested an association of the S allele with alcohol and drug dependence [23, 24, 25]. [26] found out that there is an association between the 5-HTTLPR 'L' allele and the increased serotonin and platelet uptake pharmacologically; the serotonin transporter spans the plasma membrane 12 times.

Association between genotype of 5-HTTLPR and parameters

The association between 5-HTTLPR and SGOT, SGPT and catalase were investigated. The current study shows that the presence of an SGOT was significantly associated with genotype of 5-HTTLPR (*SS, SL, LL*) among cases at ($p < 0.05$), while SGPT was significantly among control at ($p < 0.05$). Regarding the association between genotype of 5-HTTLPR and catalase, there were no differences among case and control groups, table (3).

Table (3): Relationship between genotype of 5-HTTLPR and listed enzymes in cases and control.

Group	Genotype of 5-HTTLPR	Mean ± SD		
		SGOT	SGPT	Catalase
case	SS	29.16 ± 15.56 b	55.04 ± 15.05	389.82 ± 263.95
	SL	40.23 ± 19.95 b	47.84 ± 21.29	379.37 ± 243.08
	LL	61.06 ± 32.28 a	48.67 ± 26.12	273.13 ± 226.82
	LSD value	20.77 *	21.71 NS	186.97 NS
Control	SS	18.37 ± 11.25	19.42 ± 9.62 a	85.97 ± 24.13
	SL	20.50 ± 15.58	9.35 ± 2.15 b	111.22 ± 59.37
	LL	20.20 ± 11.18	15.80 ± 9.38 ab	104.24 ± 55.93
	LSD value	11.96 NS	8.67 *	55.92 NS

* (P<0.05), NS: Non-Significant.
Means having with the different letters in same column differed significantly

The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOP in humans are linked to alcohol consumption and the incident of alcohol abuse.

Regarding the association between genotype of 5-HTTLPR and catalase, may be, duration of ethanol exposure and genetic background appear to be important variables in considering whether or not catalase changes as a response to ethanol.

Several studies have examined whether the *5-HTTLPR* polymorphism (L and S variants, LL, LS and SS genotypes) interacts with environmental risk factors to predict drinking outcomes [27, 28].

A repeat length polymorphism (5-HTTLPR) in the promoter of this gene has been shown to affect the rate of serotonin uptake and may play a role in drug dependence and other chronic neurological diseases [20, 21].

Some studies have suggested a possible involvement of the 5-HTTLPR genotype with alcoholism [22], smoking [29], suicidal behavior [30], and depression [31].

Conclusion

Researchers worldwide reported that there are several underlying genetic factors that influence the development of alcoholism among individuals. However, to date there is only few published reports on this matter in alcoholism among Iraqi individuals. In this study there was a significant positive correlation between the SGOT activity and genotypes of 5-HTTLPR (*SS, SL, LL*). There was a significantly increased levels of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic compared to non-alcoholic males. The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOP in humans are linked to alcohol consumption and conditions of alcohol abuse.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Sensitive Simultaneous Estimation of Atorvastatin Ca in Pure and Dosage Forms Via Developed CFIA using 1,2 Naphthoquinone-4-Sulfonate as a Suitable Organic Agent

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Abstract

Objectives: A sensitive visible spectrophotometric method and FIA/merging zones technique was developed for the determination of atorvastatin calcium in pure material and tablet dosage form.

Methods: Atorvastatin calcium has a free carboxylic moiety in its structure, which when being deprotonated in basic medium facilitates associated the reagent with the drug. This method was based on the formation of red colored chromogen of drug with 1,2-Naphthaquinone-4-sulfonate(NQS) in basic medium (NaOH). The absorbance of the chromogens was measured at their respective wavelengths of maximum absorbance against the corresponding reagent blank

Results: The red colored product is directly completed in basic medium and exhibits maximum absorption at 525 nm. Different factors affecting the formation of the product and optimized in order to obtain the best conditions for the experiment and its stability were studied. Method validation was done over a concentration range of 2-10 and 1-20 µg/mL for batch and FIA method respectively.

Keywords: *Atorvastatin calcium; 1,2-Naphthoquinone-4-sulfonate, sodium hydroxide; Pharmaceutical formulation; CFIA/merging zones technique.*

Introduction

ATRV.Ca {[R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy- 5(1-methylethyl)-3-phenyl-4-[phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1)} is the most commonly occurring drug in commercially available pharmaceutical formulations used for the clinical treatment of hypercholesterolemia [(1)]. Several methods have been described for the determination of ATRV.Ca HPTLC [(2)], (HPLC) in different pharmaceutical preparations, either alone [(3-8)] or with other active ingredients [(9-17)], electrochemical [(18,19)], spectrofluorimetric [(20)] and capillary electrophoresis [(21)] methods have been developed for the analysis of ATRV.Ca in pharmaceutical preparations. Various spectrophotometric methods have been reported for the determination of ATRV [(9,15,22-26)] from its individual and combined formulations with other active ingredients. The official procedures in pharmaceutical preparations utilize non-aqueous titration method [(27)]. Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometric methods. Some specific advantages that the spectrophotometric FIA methods possess are as follows [(28)].

- High selectivity since they involve the measurement of the absorbance as a function of reaction time instead of measuring the concrete absorbance value.

- Simple and fast methods because some experimental steps such as filtration, extraction, etc.

-Other active compounds present in the commercial dosage forms may not interfere if they are resisting the chemical reaction conditions established for the proposed method.

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- Colored and/or turbid sample background may possibly not interfere with the determination process.

Materials and reagent

A standard solution of ATRV.Ca ($C_{66}H_{68}CaF_2N_4O_{10}$= 1155.34 g mol^{-1}, Sigma Aldrich). A 0.05 g of pure ATOR was dissolved in 100 mL methanol to prepare 500 µg/mL of standard ATRV.Ca. A standard stock solution of NQS ($C_6H_4COCOCH:CSO_3$ Na = 260.20 g mol^{-1}, Fluke) A 0.05 M of Reagent was prepared by weighing a 1.3 g of reagent and dissolving in distilled water and made up to 100 mL with it. A stock solution of NaOH (40 g mol^{-1}, BDH) A NaOH 1M was prepared by weighing a 4g of oxidant and dissolving in distilled water and made up to 100 mL with it.

Instrumentation

A Optima, Photomech 301-D^+, UV-Visible Spectrophotometer single beam recording spectrophotometer (Japan) was used for performed all absorbance and spectral measurements of FIA procedures, for the absorbance measurements as peak height through Kompensograph C1032, Siemens or absorbance with digital multimeter (DT9205A, China). Inside the detection unit, there is a flow cell (quartz silica (QS), 1 cm) with 80 µL internal volume. A Shimadzu UV-1800 (Japan) double-beam spectrophotometer were used for batch procedure, and quartz cuvette with an optical path length of 1 cm. A one channel manifold was employed for the FIA/merging zones system. A peristaltic pump of four channels (Shenchen, LabM1) used for pumping the distilled water as a carrier stream of through the valve (homemade, six-three injection valve (merging zone version)), which moves at 90° and three Teflon loops were loaded with the sample solutions and reagent. Mixing coil that was manufactured from glass with 2 mm (I.D). A single channel manifold system in FIA was shown in Figure.1.

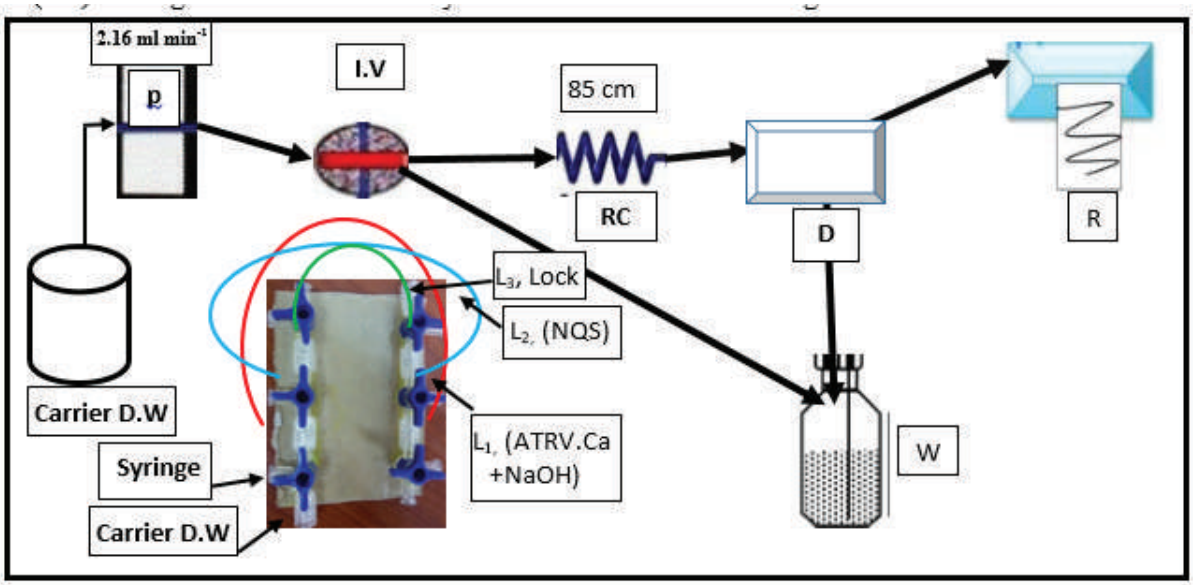


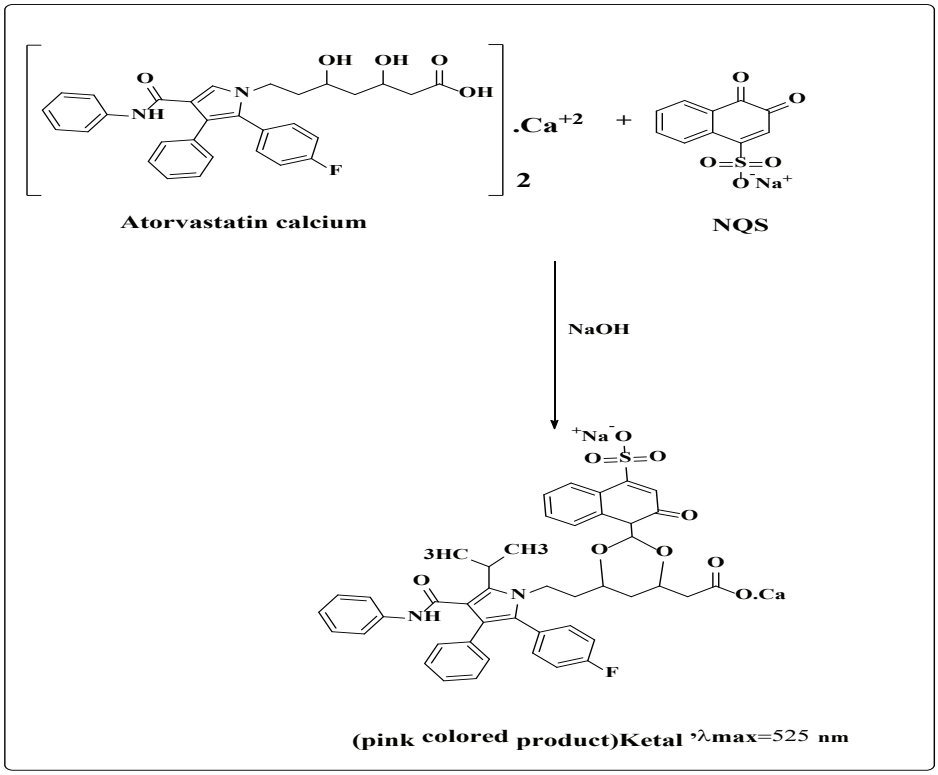
Figure.1. Manifold employed for FIA-Spectrophotometric determination of ATRV.Ca, where: I.V, injection valve; R.C, reaction coil; P, peristaltic pump; D, detector; R, Recorder; W, waste.

Assay procedure for tablets

The solutions of pharmaceutical preparations by appropriate amount equivalent 0.02 g of the each sample was weighting that be equal to 200 µg mL^{-1} of resulting powder were dissolved in 100 ml volumetric flask with 25 mL of methanol for and then shaken and filtered into a volumetric flask of 100 mL. The residue was washed and diluted to volume with distilled water to gain 200 µg/mL of statin drugs.

Mechanism of the Reaction

The suggested mechanism of this reaction of ATRV.Ca with (NQS) in basic medium to form a red complex directly as shown in scheme (I). The stoichiometry of the reaction between ATRV.Ca and NQS was investigated [22].



Scheme I: The suggested mechanism of the reaction between ATRV.Ca with (NQS) complex

Result and Discussion

Batch spectrophotometric determination: In the subsequent experiments, 4 µg mL^{-1} of ATRV.Ca was taken in 10 mL final volume and performed by changed one factors at a time and keeping the other parameters fixed and observing the effects of the product on the absorbance.

Concentration of NQS:

The effect of various concentration of NQS was investigated using different concentration ranging from (0.001-0.01 M). A concentration of 0.005 M reagent gave the highest absorbance and was chosen for further experiments.

Concentration of sodium hydroxide: The effect of concentration of sodium hydroxide was investigated by carrying out the reaction using different volumes of NaOH ranging (0.005-0.2 M). The maximum absorbance was obtained upon 0.05 M.

Calibration curve of classical method:

The impact of using different concentration of ATRV.Ca (1,2, 2.3, 2.5, 3, 4, 5, 6, 7, 8,10,12) µg mL^{-1} were examined with stabilized the other parameters. Transfer set of volumetric (10 ml) contain 2.5 mL of (NQS) (0.02 M) followed by 1 mL of NaOH (0.5 M)

then an increasing volumes from standard solutions (100 µg.ml^{-1}). The solutions had been diluted to the marked using distilled water. The reaction mixture measured the maximum absorption of the colored product at 525 nm. The standard curve was constructed and linear range (2-8) µg.mL^{-1} for the determination of ATRV.Ca, as shown in Figure (2).

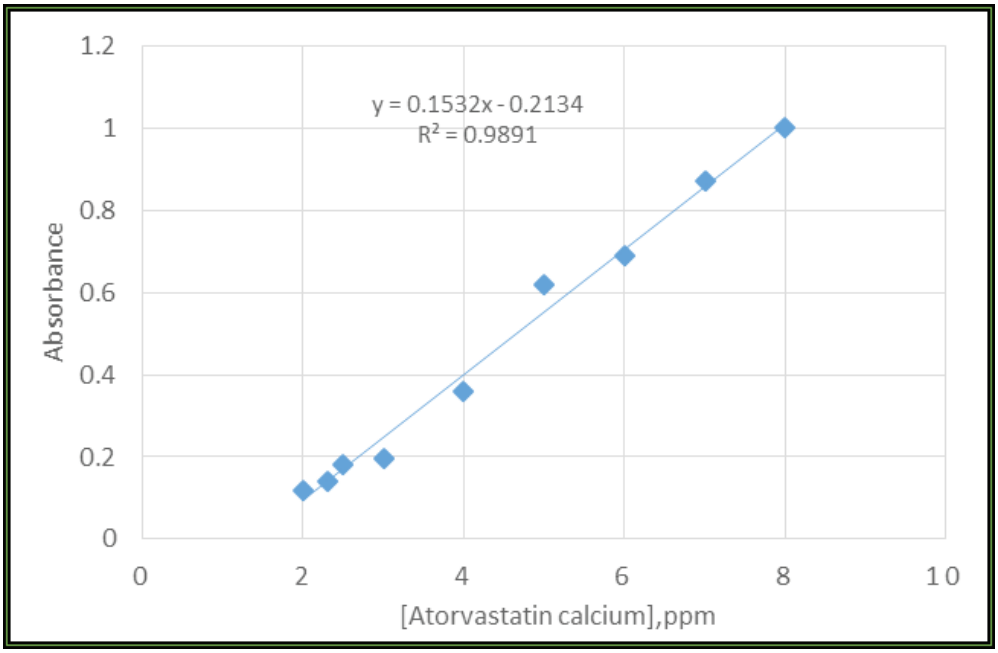


Figure.2. Calibration curve of reaction between ATRV.Ca and NQS in basic medium

Calculations of stability constant:

$K = 1-\alpha/\alpha^3C^2$ …….. (1), (α) (degree of dissociation) can be written as follows:

α = Am - As /Am……... (2), Am; As are the values of absorbance of the aqueous solution including a more than enough and stoichiometric amount of the reagent.

Optimization of the FIA system conditions

Initial studies were directed towards the optimization of the experimental conditions for FIA system.

Effect of reagent and basic medium: Optimum concentration of the reagent was studied by injecting different concentrations (0.005-0.08) M using IV. The results indicated that the 0.05 M gave the good repeatability with highest value of absorbance.

NaOH found to be a useful basic medium for this reaction, different concentrations of NaOH were also studied in the range of 0.01 to 0.08 M. The result referred to increase the value of absorbance with increasing the concentrations of basic medium up to 0.02 M and after this concentration the value of absorbance decreased. As a result, 0.02 M was chosen for the subsequent experiments.

Effect of physical parameters

Effect of optimum total flow rate

Optimum flow rate was studied using a range changed flow rates (1.2-2.6) mLmin^{-1}. The result demonstrates that a flow rate of 2.16 mLmin^{-1} gave the highest absorbance value.

Effect reaction coil length and injection volume

Optimum length of reaction coil was studied in range of 85-250 cm. A best absorbance with acceptable repeatability was gained from the length of 85 cm. Absorbance decreased upon using a coil length of more than 85 cm.

Various volumes of injector loop were tested in this study. Effect of injected sample volume (L_1) was changed (58.875, 68.687, 88.312 and 127.562) µL and

the volume of injection reagent (L_2) also studies was in deferent volume (68.687-127.562) µL. a 58.875, 68.687 µL for L_1, L_2 respectively was used in the next experiments.

Method validation

The linearity of the calibration graph for FIA method was obtained by injecting a series of solutions of ATRV. Ca (1-20 µg mL^{-1}) prepared from stock solution (100 µg mL^{-1}) with 0.02 M of basic medium as shown in figure (3). A portion of NQS (0.005 M) was injected as summarized in Table 1.These small points were referred to high reproducibility and repeatability of the developed FIA contrasted with the batch procedure.

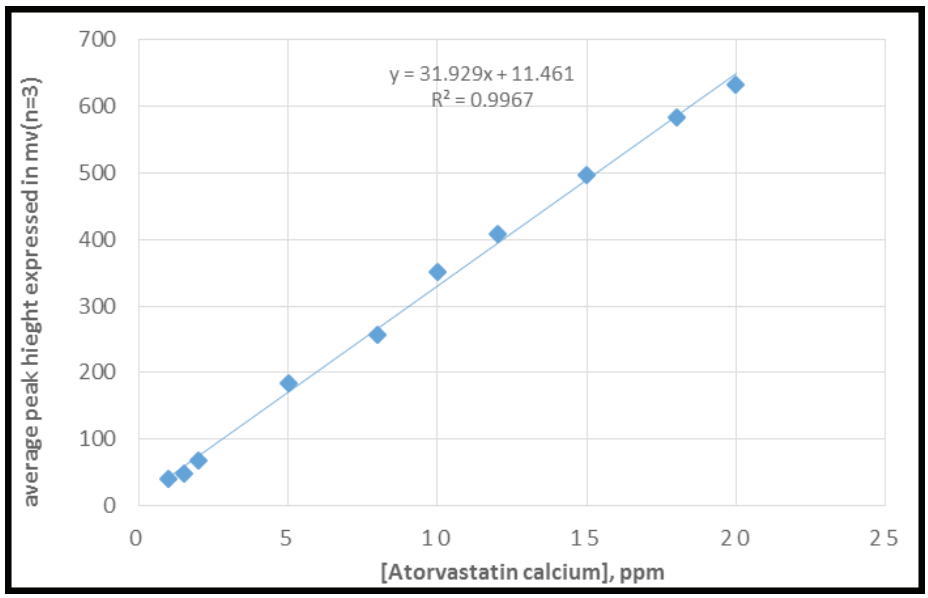


Figure .3. Linear calibration curve for determination of atorvastatin calcium with NQS using the developed FIA system.

Table 1. Summary of optical characteristics

Parameters	Batch method	FIA method
Linear range (µg mL-1)	2-8	1-20
Regression equation	y = 0.1532x + 0.2134	y = 31.929x +11.461
Correlation coefficient (r)/ r2	0.9945	0.9983
Linearity (r2 %)	98.91	99.67
Relative standard deviation (RSD %)	0.21 (at 5 ppm)	0.3 (at 10 ppm)
Slope (b); (mL.µg-1)	0.1532	31.929
Intercept (a); (a = y– b x)	0.2134	11.461
Standard deviation of intercept (Sa)	7.07 × 10-5	4.47 × 10-4
Confidence limit of intercept (a) = a ± tSa	0.2134 ± 0.0007	11.461± 0.163
Standard deviation of slope (Sb)	8.49 × 10-4	5.74 × 10-4
Confidence limit of slope (b) = b ± tSb	0.1532 ± 0.0002	31.929 ± 0.0049
Standard deviation of the residuals;	0.38 × 10-4	0.015
Average of recovery (%)	99.66	100.4
Limit of detection (LOD)	0.06	0.002
Limit of quantification (LOQ)	0.2	0.006
Sample through put (h-1)	10	68

Application of the proposed method using pharmaceutical:

The proposed batch and FIA method was successfully applied for estimation ATRV.Ca in tablets by the analysis of three types in two different concentrations of ATRV.Ca tablets and the results are listed in Table 2. In the direction of assessing the proficiency of the method. The statistical comparison between proposed and official methods using the student t- and F-test [27] indicated that the calculated values for F-test were (2.57) and (1.22), t-test values were (2.08) and (1.13) for the FIA and batch methods, respectively, were less than the theoretical one of F-test = 6.388 (n1 + n2 – 2 = 6) and t-test = 2.31.

Table 2. Application of the proposed batch and FIA and official methods for estimation of ATRV.Ca in tablets.

Dosage form	Proposed methods						Official method recovery (%)
	Batch			FIA-merging zones			
	Present conc. (µg mL-1)	Rec (%)	RSD (%)	Present conc. (µg mL-1)	Rec (%)	RSD (%)	
AVAS Tablets (10 mg/tablet) MICRO LABS LIMITED	3	100.30	0.41	10 15	99.80 101.10	0.28 0.14	100.60
	5	99.92	0.09				
AVAS Tablets (20 mg /tablet) MICRO LABS LIMITED	3	101.30	0.20	10 15	100.70 99.50	0.15 0.20	99.20
	5	101.00	0.19				
LIPODAR Tablets (10mg /tablet) Dar Al Dawa, Na,ur - Jordan	3	99.00	0.21	10 15	98.20 100.13	0.22 0.10	100.50
	5	99.40	0.18				
LIPODAR Tablets (20mg /tablet) Dar Al Dawa, Na,ur – Jordan	3	99.67	0.52	10 15	101.00 100.93	0.30 0.09	101.00
	5	101.20	0.089				
ATEROZ Tablets (20mg /tablet) bilim	3	98.67	0.93	10 15	100.50 99.80	0.45 0.04	99.90
	5	100.60	0.04				

Conclusion

The developed methods were selective, rapid, simple and inexpensive and exhibits a fair degree of accuracy and precision . The method does not involve any critical reaction conditions. The proposed method can serve as an alternative method for the routine analysis of ATRV. Ca in pure drug and in pharmaceutical formulations. The methods is based on formation of a red condensation adduct upon reaction of ATRV.Ca and NQS in (NaOH).

The method has low detection limit and high sample throughput. The proposed methods that followed Beer's law and give a good application for the pharmaceutical preparation. The wide applicability of the FIA method for daily quality control is well proven by analyzing the assay of ATRV.Ca at effect concentration level in dosage forms

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Gall Bladder Wall Thickness: Sonographic Accuracy and Laparoscopic Cholecystectomy Conversion Rate, Evaluated by Histopathology

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Abstract

Background: Preoperative prediction of a difficult laparoscopic cholecystectomy can help the patient as well as the surgeon to prepare better for intraoperative risk and the risk of conversion to open Cholecystectomy.

Aim of study: Evaluation the impact of gall bladder wall thickness, on the outcome of laparoscopic cholecystectomy and conversion rate to open cholecystectomy assessed by sonography preoperative and postoperative measurement of gall bladder wall thickness by histopathology.

Patients and Methods: A prospective study conducted in the surgical unit, Department of surgery, Baghdad Teaching Hospital between November 2010 and November 2011. Abdominal Sonography performed in 110 consecutive patients before laparoscopic cholecystectomy. The surgeon re-verified sonographic finding in operative room, and postoperatively, the gall bladder specimens were sent for histopathological measurement of wall thickness.

Results: Out of 110 patients with cholecystolithiasis on sonography, we encountered easy laparoscopic cholecystectomy in 80 patients (72.7%), difficult laparoscopic cholecystectomy in 24 (21.8%) and the procedure was converted to open cholecystectomy in six patients (5.5%). The difference between Sonographic and histological measurement was within 1 mm in 102 patients (92.7%), and the other 8 patients was with 1.5 mm (7.3%) with sensitivity of (100%), specificity of (83.3%) and accuracy of (97%).

Conclusion: An accurate preoperative diagnostic sonography is mandatory for planned laparoscopic gall bladder surgery to provide information for the selection of the most appropriate approach and avoid intraoperative difficulties and surprises. On sonography gall bladder wall thickening is the most sensitive indicator of technical difficulties during laparoscopic cholecystectomy. Such difficulties may require conversion to laparotomy.

Keywords: *Laparoscopic cholecystectomy, gall bladder wall thickness, sonography, Iraq.*

Introduction

Cholelithiasis has a high prevalence. Although cholelithiasis only becomes symptomatic in about 50% of patients, cholecystectomy is a common surgical procedure [(1)]. Gallstones are one of the major causes of morbidity in Western society. Prevalence of people with gallstones, whether symptomatic or asymptomatic, varies from 5 to 22% [(2)]. In Iraq, operations of gallbladder (GB) represent a considerable

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fraction of total operations conducted in hospitals. This indicates that the disease is relatively important in Iraq [3]. Recently, laparoscopic cholecystectomy (LC) has become the gold standard for treatment of symptomatic gallstones, due to lower morbidity, shorter hospital stay, earlier return to regular daily activities, less postoperative pain and a significant reduction in the incidence of wound complications and postoperative ileus has been documented in patients undergoing LC [4, 5]. In addition to numerous advantages, also technical limitations of laparoscopy should be mentioned, which - in the presence of chronic inflammation resulting in pericystic adhesions and conglutination – increase the risk of undesirable conversion from LC to open surgery [6]. The severity of acute inflammatory change influences the degree of surgical difficulty. GB wall thickening and pericholecystic fluid are indicators of inflammation in patients with acute cholecystitis [7]. The most common risk factors for conversion include a thickened GB wall, past acute cholecystitis, diabetes mellitus, past upper gastrointestinal tract surgeries, age > 65 years and male gender [8, 9]. The selection of the patient who will undergo LC is important, and the most frequently used method other than the clinical evaluation, is radiological examination (ultrasonography) [2]. Preoperative classification of patients into a high risk group would be an objective factor facilitating the surgeon's decision on possible conversion [9]. A preoperative GB ultrasound, which documents a thick GB wall (> or =3 mm) with calculi, is a clinical warning for a difficult LC which may require conversion to an open surgery. In a study, it was found that the rate of conversion was 60% in case of thickened GB wall while 12% in case of normal GB wall [10]. Variable results have been reported in the past about the sensitivity, specificity, positive predictive value, and accuracy of GB wall thickening as an indicator of surgical conversion [7].

The success of any laparoscopic operation depends on both proper patient selection, and the technical skill and experience of the laparoscopist [11]. The aim of the study was to evaluate the impact of GB wall thickness on the outcome of LC and the conversion rate to open cholecystectomy assessed by preoperative sonography and postoperative measurement of GB wall thickness by histopathology.

Patients and Methods

Study Design and Setting: This was a prospective study that was conducted in the surgical unit, Department of Surgery, Baghdad Teaching Hospital during a period of one year from Nov, 2010 – Nov, 2011.

Study Population and sample size: The study included patients with feature of chronic calculus cholecystitis who were prepared for LC, so the total number was 110. Patients who had previous abdominal surgery and features of acute cholecystitis (clinically and by investigation) were excluded.

Workup: All patients were evaluated by sonography after fasting at least six hours, the wall of GB was carefully evaluated and consider as thick when it is (> 3mm), size and capacity of GB, pericystic fluid collection and biliary system status was evaluated as well as number of GB stones also recorded. Hematological and biochemistry investigation were done. GB wall thickness was measured postoperatively by histopathology; grossly and microscopically as. Initial Procedure of histopathological examination done by.

ü Measurements:

· GB: length × maximum diameter (cm).

· Cystic duct: length × maximum diameter (cm).

· Lymph node: number and maximum diameter (cm).

ü Open longitudinally from the fundus towards the cystic duct with blunt-ended scissors, draining off the bile and noting any contents.

ü Photograph if appropriate.

ü Paint the external serosal and adventitial aspects if there is any suspicion of tumor.

ü Fixation by immersion in 10% formalin for 36 – 48 hours.

The difficulty of procedure was evaluated by: -

1. Clearity of calot's triangle (peritoneal adhesion) length and width of cystic duct.

2. Handling of GB during procedure and ability to

perforate it.

3. Dissection of GB from its liver bed and bleeding from it.

4. Extraction of GB to outside.

All patients were undergoing surgery which was done by senior general surgery and resident using closed methods with four ports. Histopathological examination done by senior histopathology. LC considered easy when there is minimal adhesion involving the omentum, only attaches to the fundus and body of GB, and easily separated. Difficult LC when there is sever adhesion involving calot's triangle.

Statistical analysis: The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Chi–square test was used to assess statistical association between certain variables and GB Wall thickness. A level of p – value less than 0.05 was considered significant.

Results:

In this study, mean age of patients was 42.7 ± 8.4 years; and 82.6% were females. By U/S, 64.5% of patients had GB with wall thickness ≤ 3 mm. We noticed that LC was easy in 72.7% of cases, difficult in 21.8%, and converted to open surgery in 5.5% of cases as shown in table (1).

Table 1: Distribution of study patients by certain characteristics

Variable	No. (n=110)	Percentage (%)
Age (Years)		
< 30	24	21.8
30 - 49	64	58.3
≥ 50	22	19.9
Gender		
Male	19	17.4
Female	91	82.6
GB Wall thickness (mm)		
≤ 3	71	64.5
> 3	39	35.5
Type of operation		
Easy LC	80	72.7
Difficult LC	24	21.8
Converted to open surgery	6	5.5

The difference between sonographic and histopathologic measurement was below 0.5 mm in 80 patients (72.5%) and it was between 0.5 and 0.99 mm in 22 patients (20%), so it was within 0 – 1 mm in 102 patients (92.7%), and in other 8 patients the difference was within 1.5 mm from GB wall thickness as shown in table (2)

Table 2: Difference of histopathological and ultrasound measurement of gall bladder wall thickness.

Difference between measurements (mm)	No. (n=110)	Percentage (%)
No difference	44	40.0
0.1 – 0.49	36	32.5
0.5 – 0.99	22	20.0
1 – 1.5	8	7.5

In table 3, 83.3% of cases who were converted to open surgery had GB wall thickness > 3 mm by U/S with a significant association (P= 0.001) between GB Wall thickness by U/S and type of operation.

Table 3: Association between GB Wall thickness by U/S and type of operation

Type of operation	GB Wall thickness by U/S (mm)		Total (%) n= 110	P - Value
	≤ 3 (%) n= 71	> 3 (%) n= 39		
Easy LC	64 (80.0)	16 (20.0)	80 (72.7)	0.001
Difficult LC	6 (25.0)	18 (75.0)	24 (21.8)	
Converted to open surgery	1 (16.7)	5 (83.3)	6 (5.5)	

In table 4, 90% of cases who complained from GB perforation had GB wall thickness > 3 mm by U/S with a significant association (P= 0.001) between GB Wall thickness by U/S and postoperative complication.

Table 4: Association between GB Wall thickness by U/S and postoperative complication

Postoperative complication	GB Wall thickness by U/S (mm)		Total (%) n= 110	P - Value
	≤ 3 (%) n= 71	> 3 (%) n= 39		
No	68 (72.3)	26 (27.7)	94 (85.5)	0.001
GB Perforation	1 (10.0)	9 (90.0)	10 (9.1)	
Bleeding	2 (33.3)	4 (66.7)	6 (5.5)	

Discussion

Since the Introduction in 1985, LC had been the procedure of choice in treatment of symptomatic gall stone [12]. But some of the planned LC needs conversion due to various factors, it would be useful in advance to know which one would require conversion, so that experienced laparoscopic surgeon could be scheduled to minimize conversion rate. And since the 1970s, ultrasound has become known as a quick, non-invasive and reliable tool to diagnose GB disease [13, 14]. Ultrasound is very sensitive for the diagnosis of gall stones, but few data are available to assess its diagnostic value for the GB wall thickness [15]. We assessed the value of sonography for patients with gall stone disease prior to LC. This study corroborates the well-established high accuracy (97%) of sonography for assessing the thickness of GB wall thickness.

In this study, we found that increase GB wall thickness on preoperative ultrasound which encountered in 39 patients out of 110 patients (35.5%) were associated with increase operative difficulty in 18 patients out of 39 patients, and our conversion rate to open surgery in six patients out of 110 patients (5.5%) was within the range reported by several other studies (1 – 10%) as in Indian one conducted in 2017 with a report of conversion rate of 10% [16], in USA in 2010 were the rate was 9% [17], and a local study in Iraq in 2007 [18] where the rate was 5%. In this study, GB wall thickness significantly determines the difficulty during surgery. We found that increase GB wall thickness (> 3 mm) on preoperative ultrasound which encountered in 39 patients (35.5%) in comparison to those with thin GB wall thickness (≤ 3 mm) 71 patients (64.5%) was associated with increase operative difficulty and this result was in consistent with a result found by Adwan MK et al study in 2015 [3] and with a study conducted by Sharma N et al in 2015 [19] when reported that gall bladder wall thickening can predict difficulty during cholecystectomy, we found that thickened gall bladder wall are the most accurate predictors of potential operative difficulty. GB wall thickening was a sensitive indicator of technical difficulties and the risk of conversion to open cholecystectomy. GB wall thickness is related to the inflammation and fibrosis that follow previous attach of cholecystitis and thus may reflect difficulty in delineation of the anatomy during surgery [20]. In conclusion, LC can be accomplished successfully with low morbidity in most patients with cholecystitis, those patients with increased GB wall thickness on preoperative ultrasonography are at high risk for conversion to open surgery. An accurate preoperative diagnostic tool is mandatory for planned laparoscopic GB surgery to provide information for the selection of the most appropriate approach and to avoid intraoperative difficulties and surprises.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Evaluating the Effect of Addition of Titanium Dioxide Nanoparticle on Some Physical Properties of Flowable Composite Resin

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Abstract

Objective: The flowable dental composite resins were introduced to the dental specialty because of the advantages they possess over the conventional composite resins. The use of nanotechnology in the dentistry field is one of the growing innovations in recent years. The aim of the present study was to evaluate certain physical properties of flowable dental composite after incorporation of titanium dioxide nanoparticles (TiO2NPs).

Materials and Methods: In the present study, TiO2NPs at 1.25 % and 2.5% concentrations were added to flowable composite, while the unmodified composite was used as control. Then the physical properties of the control and modified composite resins, including flowablity, radiopacity and water sorption and solubility were tested. Data were analyzed with One way ANOVA, using SPSS 20.

Results: The results showed that there was statistically significant difference among the tested groups regarding flowability and radiopacity ($P<0.05$). In addition, there was no significant difference among control group and TiO2 modified groups regarding water sorption and solubility.

Conclusion: Based on the results of the present study, a flowable dental composite was successfully reinforced with TiO2. Incorporation of small weight percentages of this nanofiller exhibited properties similar to the control material regarding water sorption and solubility. The flowability was slightly reduced and radiopacity of the reinforced composites was increased, these changes were acceptable for clinical applications and below ISO standards limits.

Keywords: *Flowable Composites, Titanium Dioxide nanoparticles, flowability, radiopacity, water sorption and solubility.*

Introduction

Currently composite resins are materials used in restorative dentistry, since there are many revolution and improvements such as use of different novel particles, flowable composite resin because of their advantages over the conventional composite resins were introduced to the dental specialty. These advantages includes, simple application technique, easy handling properties, increased flowability, better adaptation to the internal cavity wall and higher elasticity, in addition, the use of flowable composites resins reduces the amount of the cavity preparation and is recommended for minimal invasive dentistry [(1)].

One of the most important innovations in the dentistry field in recent years is the use of nanotechnology. The mechanical, physical and optical properties of conventional composite resins have been improved by addition of inorganic nanoparticles such as silver, zinc, titanium dioxide and silica [(2)].

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Flowability is used to describe how quickly materials flow in a certain period of time, whereas viscosity is the material's resistance to flow. Dental composites are viscoelastic materials. They share criteria of both viscous materials (e.g., oils) and elastic materials (e.g., metals).

Radiopacity of dental restorative materials is an important indicator for accurate diagnosis and treatment planning. It is essential to use materials with adequate radiopacity in order to distinguish them from the natural tooth structures. Moreover, dental restorations should be radiopaque enough to detect overhanging margins, recurrent caries, restoration contour, proximal contacts [(3)].

The sorption and solubility properties are critical properties regarding biocompatibility concerns of releasing monomer and in relation to the stability of the composites due to degradation from the uptake of solvents and the wash-out of ingredients of materials [(4)]

Most of the studies involving addition of nanoparticles to dental composites resins have mainly focused on their anti-bacterial effects and the information regarding their physico-mechanical properties are limited. TiO2 are fine, non-toxic, chemically stable, and exhibits a high photocatalytic effect in addition to their high biocompatibility and pleasant color. TiO2NPs have large surface area that facilitates load transfer from resin matrix to nanoparticles thereby resulting in better mechanical properties of the reinforced composites [(5).]

Mohammed et al (2019) studied the effect of TiO2NPs on physico-mechanical properties of flowable dental composite resins by adding TiO2NPs at 1%, 2% and 3% to Tetric N Flow composite, while the unmodified composite was used as control. The developed composite was studies for functional and structural properties using FTIR, which indicated no change in the functional and structural characteristics [(6)].

The aim of the present study was to evaluate the effect of TiO2NPs, on some physical properties of flowable composite resins.

Methods

In this study, the flowability, radiopacity and water sorption and solubility of a conventional flowable composite resin and composite resins reinforced with TiO2NPs were evaluated. For each test a total of 18 samples were evaluated. Commercially available flowable microhybrid composite resin (as control group) was used shade (A2). This material is based on dimethacrylate paste (Bis-GMA and Triethylene glycoldimethacrylate TEGDMA), without inorganic fillers. mixed with TiO2NPs 98% Purity and particle size of 50 nm at concentrations of 1.25% and 2.5 %.

Mixing was done by using a dental Micro motor, with a lentulo spiral-paste carrier #4 attached. The lentulo spiral was immersed in composite resin material – TiO2NPs, poured into a 2 ml pre-darkened syringe tube.

Such mixing was performed directly before preparation of samples for each test. Electronic balance was used to weight the percentage of nanoparticles, after one hour mixing; the mixture was injected into metal molds Curing was done by exposure to LED at 1,650 mW/cm2 for 40 seconds with a light guide held perpendicularly and within 2 mm of the material surface. Control samples were prepared and compared with two sets of samples with TiO2NPs at 1.25% and 2.5%.

The flowability testing method used in this study was according to ADA guidelines for evaluation of endodontic sealing materials [(7)]. To evaluate the flowability of the reinforced composites, a simple test using the Gillmore needle apparatus was used. The quantity of 0.1 mL of control and each reinforced composite was dispensed between two thin glass coverslips 50 x 50 mm and 1mm thick. Flowability was evaluated by comparing the composite disc diameters after they have been sandwiched between two glass coverslips, subjected to constant weight (454 g) for 30 s and light cured for 40 s.

For radiopacity test 6 discs 15±1mm in diameter and 1± 0.1mm thick were prepared for each group. The specimens and the aluminum stepwedge were placed in the center of the film and irradiated with X-rays at (65±5) kV at a target film distance of 400 mm for 0.4 seconds at 10 mA. After developing and fixing the film, the density of the image of the specimen was compared with that of the aluminum standard using the densitometer. For Water sorption and solubility test 6 discs 15±1mm in diameter and 0.5± 0.1mm thick samples were prepared

for each group using a metal molds and tested according to ADA specification [8]

Statistical Analysis

The statistical analysis was performed on SPSS program version 20.0, the descriptive statistic was done for tested groups, for verification the difference among groups analysis of variance (One-way ANOVA) with Tukey post-test.

Results

Flowability test

Table 1 present the means and standard deviations of flowability values in conventional composite resin and composite resins reinforced with TiO2NPs at different percentages.

Based on table 1, the Flowability of control group and 1.25% and 2.5% groups were 2.6933 cm, 2.6083 cm and 2.5067 cm respectively, The Results of Table 1 revealed significant differences among the tested groups. But the difference is acceptable within the ISO standard.

Table 1: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Flowability data.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
(C)	6	2.6933a	.00816	.00333	2.6848	2.7019	2.68	2.70
1.25%	6	2.6083b	.00983	.00401	2.5980	2.6187	2.60	2.62
2.5%	6	2.5067c	.01211	.00494	2.4940	2.5194	2.50	2.53
Total	18	2.6028	.07910	.01864	2.5634	2.6421	2.50	2.70

Note: Means with different letter indicates statistically significant difference (P<0.05).

Radiopacity test

Radiopacity mean values of the tested materials are presented in Table (2). As recommended by ISO [9]. 2.5% group showed the highest radiopacity values 2.4760 E.q. Al thickness/mm mean value followed by 1.25% group of 2.3900 E.q. Al thickness/mm mean value of radiopacity, and 2.2833 E.q. Al thickness/mm mean value for Control group with statistical significant difference among the tested groups.

Table 2: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Radiopacity.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	6	2.2833a	.00816	.00333	2.2748	2.2919	2.27	2.29
1.25%	6	2.3900b	.00894	.00365	2.3806	2.3994	2.38	2.40
2.5%	6	2.4760c	.01131	.00462	2.4641	2.4879	2.46	2.49
Total	18	2.3831	.08159	.01923	2.3425	2.4237	2.27	2.49

Note: Means with different letter indicates statistically significant difference (P<0.05).

Water Sorption test

Water sorption mean values are presented in Table (3) revealed no significant differences among the tested groups. Control group showed the highest Water sorption values 1.456283 µg/mm³ mean value, followed by 1.25% group with -1.029533 µg/mm³ mean value Water sorption, and .802833 µg/mm³ mean value of 2.5% group but the difference was not significant statistically.

Table 3: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Water sorption data test.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	6	-1.456283a	1.2018703	.4906615	-2.717569	-.194998	-2.8037	.8131
1.25%	6	-1.029533a	1.3579421	.5543775	-2.454606	.395539	-1.8691	1.6260
2.5%	6	-.802833a	1.6504075	.6737760	-2.534830	.929163	-1.6260	2.5000
Total	18	-1.096217	1.3586916	.3202467	-1.771878	-.420555	-2.8037	2.5000

Note: Means with same letter indicates statistically no significant difference.

Water Solubility

Solubility mean values are presented in Table (4). Test results revealed no significant differences among the tested groups. Control group showed the highest solubility values 2.516583 µg/mm³ mean value, followed by 1.25% group with 2.048467µg/mm³ mean

Table 4: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Water solubility data test.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	6	2.516583a	.9013909	.3679913	1.570632	3.462535	.9345	3.2601
1.25%	6	2.048467a	1.1576720	.4726176	.833564	3.263369	.8130	3.8530
2.5%	6	1.386167a	1.1753861	.4798494	.152675	2.619659	.0000	3.4390
Total	18	1.983739	1.1257010	.2653303	1.423941	2.543537	.0000	3.8530

Note: Means with same letter indicates statistically no significant difference.

Discussion

The flowability of floweble composite resin decreased slightly with addition of TiO2NPs at 1.25% and 2.5% and the results are within the ISO standards, these results are in agreement with previous study reported that it is possible to increase the nanofiller content due to their small particle sizes. This will result in minimum reduction in flowability as well as improvement in the physical properties of the nano-composites [10].

In the present study the most commonly used monomer) BisGMA and TEGDMA were used, The advantages of using BisGMA over other monomers are less shrinkage, higher modulus and reduce toxicity due to its lower volatility and diffusivity into tissue. Although, BisGMA possesses high strength and hardness, the drawback of this monomer is its high viscosity (low flow), because of hydrogen bonding interaction that occur between hydroxyl groups, which limits the incorporation of inorganic fillers and hence a low degree of conversion,Thus BisGMA diluted with other low-viscosity (high flow) monomer such as trimethylene-glycol-dimethacrylate (TEGDMA) [11] to enhance flowability of the used composite that allow incorporation of TiO2NPs that resulted in a little reduction of flowability of the tested flowable composite resin and the changes are within accepted range of ISO standards

The radiopacity of a restorative material is an important parameter for accurate diagnosis and treatment planning. Success or failure of the restorative material is highly dependent on radiographs. Radiopacity of composite restorations has an important role in detecting recurrent caries and distinguishing the restorations from the tooth structures [12].

The 2.5% composite showed a higher value of radiopacity. Followed by 1.25% group Al, and control group with statistical significant difference among the tested groups and met the ISO standard for dental materials radiopacity. All of them had radiopacity greater than enamel (1.77 – 2 mm Al). These results agree with previous studies that recommended that the composite radiopacity should be equal to or greater than that of the enamel [13].

The atomic number of the elements is the most important factor affecting the radiopacity of dental materials [14]. Radiopacity of dental composites can be increased by incorporating a higher percentage of fillers with high atomic numbers [15], TiO2NPs atomic number

is 22 and density of 4.506 g/^{cm3} which is considered as a high atomic number and this explains the results of this study in which radiopacity of tested material increased with increasing percentage of TiO2NPs from 0% control group to 1.25% and 2.5 %..

The sorption and solubility properties are important regarding biocompatibility concerns of releasing monomer and in relation to the stability of the composites by avoiding degradation from the uptake of solvents and the wash-out of ingredients of materials [16]. According to ISO standard [17], the maximum acceptable values of sorption and solubility for polymer-based restorative materials are 40 µg/mm3 and 7.5 µg/mm3 respectively. Sorption and solubility values for all samples were below the ISO standards limits so all investigated materials met the requirements of the ISO standard. The decreasing sorption and solubility with TiO2 content was not statistically significant. These results are in agreement with Robert et al [18] study who studied the effect of nanofillers on water sorption and solubility and concluded that the addition of nanofillers at low concentrations not change water sorption and solubility significantly since at lower concentrations there is no agglomeration of 50 nm TiO2NPs.

The improvement of both water sorption and solubility after addition of TiO2NPs might be attributed to numerous explanations such as nanofillers are insoluble in water so that the addition of TiO2NPs to the microhybrid flowable composite resin declines the solubility of composite resin [19].

Furthermore, titanium coupling agent incorporated in salinized TiO2NPs expands the adhesion between both resin matrix and filler particles which enhances composite resin properties and declines its water sorption and solubility [20].

Moreover, the reaction between resin (polar nature) and nanofillers certainly induce replacing the hydrophilic resin and minimizing the water uptake by decreasing this polarity through utilizing most active sites in the molecules of momomers, so the diffusivity of water particles through this material is greatly declined [21].

Conclusion

Based on the results, it appears incorporation of low concentration of nanofillers into conventional composite resins did not result in any changes in their water sorption and solubility; however, flowability and radiopacity of flowable were changed but the results are acceptable withen ISO standards .Therefore, it is suggested to add small amount of nano-fillers to composite resins to prevent problems such as discoloration of composite resin or other possible changes in other physical and chemical properties.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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Antimicrobial Activities of Green Biosynthesized Iron Oxide Nanoparticles Using *F. Carica* Fruit Extract

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Abstract

In the current study, iron oxide nanoparticles (IO NPs) were synthesized via modified green synthesis technique using *F. carica* fig extract as reducing agent. Furthermore, the microstructural properties of the synthesized IO NPs have been thoroughly elucidated. In details, the acquired NPs diameter was found to be in the range of 11-29 nm and of root mean square (RMS) of 0.64 nm using TEM and AFM techniques, respectively. Consequently, the antifungal and antibacterial activities of the synthesized IO NPs were screened against *Candida* and *Aspergillus* species as well as Gram-positive *Staphylococcus aureus* and Gram-negative *Acinetobacter* species, respectively. The presented IO NPs play an active role in the antimicrobial activities evidencing the well-organized materials system for biomedical applications.

Keywords: *Iron oxide NPs, antifungal, antibacterial, F. carica extract*

Introduction

Metal oxide NPs are of great importance due to a number of unique properties such as high surface-to-volume ratio, easy separation features, and low toxicity [(1)]. IO NPs have attracted a great significance in the field of nanoscience and nanotechnology [(2, 3)]. In conjunction with IO NPs, several applications have been proposed in the area of optoelectronics, catalysis, self-powered smart window, lithium ion batteries and diagnostic biological probes [(4-8)]. IO NPs are extensively applied in the biomedicine field due to their low toxicity and biocompatibility. Due to the electrostatic feature of the IO NPs, they are easily interact with fungal, and bacterial living-cell membranes [(9)]. This property allows IO NPs to harms the cell membranes of fungi or bacteria as well as inducing the toxic oxidative stress via free radical formation [(10)]. Antimicrobial activities of IO NPs are, therefore, dependent on three essential features namely concentration of the culture media and most importantly stability [(9, 10)].

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In this attempt, variety of approaches have been anticipated for the synthesis of IO NPs. Among these are the well-known physical and chemical methods in which preferred NPs properties can be acquired. Particularly, these methods are electrodeposition [(11)], conventional heating [(12)], hydrothermal [(13, 14)], wet oxidation [(15)], laser ablation [(16)], co-precipitation [(17, 18)], and anodization [(19)]. However, these techniques are presented with several draw-backs such as the use of non-biodegradable stabilizing agents and toxic chemicals, and hypothetically harmful to the well-being organisms and the surrounding environment. The green synthesis technique, which is utilized in the current study, has revealed considerable advances such as environmentally friendly, unpresented toxic chemicals, and low energy and temperature conditions [(20)]. Green synthesis technique evolves the use of naturally existing resources such as plants extracts as reducing fuel [(21)]. Therefore, this manuscript reports a modified green synthesis technique of IO NPs in which *F. carica* fig extract is used as a reducing agent. Additionally, the antifungal activity against *Candida* and *Aspergillus* species and antibacterial activity against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species were thoroughly investigated.

Materials and Methods

Plant collection and extraction

Dried *F. carica* (common fig) was purchased from local market in Baghdad, Iraq. Consequently, the collected fruit was washed thoroughly; afterward, 10 gm of the washed fruit was blended alongside 150 mL of deionized-distilled water (DDW). The resultant mixture was subjected to heating process at 100 °C in a ventilation oven and then cooled down to room temperature. Finally, the solution mixture was filtered using chromatography paper (Whatman No.1) and incubated at room temperature for further use.

Synthesis of IO NPs

In a typical laboratory route, specific amount (0.1, 0.14 and 0.18 molar) of iron^{III} chloride hexahydrate ($F_2Cl_3.6H_2O$) was liquefied in 100 mL of DDW under stirring rate of 800 r.p.m. for 15 minutes at RT. The consequential mixture was then mixed with fig extract (1:1) under stirring rate of 600 r.p.m. for 2 h at 50 °C [(1)]. The first indication of the NPs occurring was observed upon color changing of the solution, whereby a light brown color was noticed, as presented in figure (1). The acquired solution was then washed and centrifuged at 4000 r.p.m. for 20 minutes. Hereinafter, the attained residual was dried for 6 h at 60 °C and later grinded using mortar and pestle to obtain a fine NPs powder.

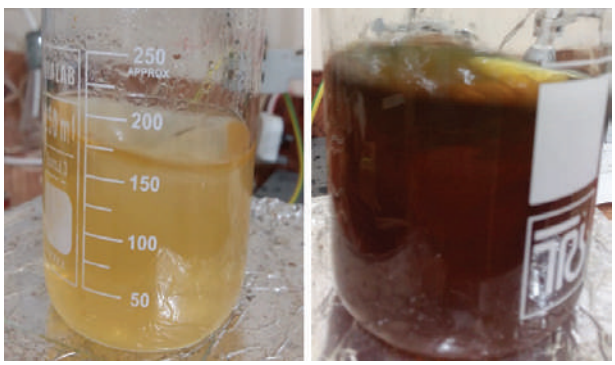


Figure (1): Color changing process of IO NPs.

Characterization

The microstructural properties of the prepared NPs were examined using Shimadzu X-ray Diffractometer (XRD-6000) with wavelength of 1.541 A° and Cu-Kα radiation. In the meanwhile, compact char surface was investigated using Fourier Transform Infrared Spectroscopy (FT-IR, Thermo Nicolet Nexus) ranging from 400 to 4000 cm^{-1}. Furthermore, the optical properties of the prepared NPs were recorded on Shimadzu UV-1800 UV-Vis spectrophotometer. Atomic Force Microscopy (SPM AA3000-AFM) and TECNAI F-30 TEM were engaged for the morphological and nanoparticle size investigations.

Evaluation of antifungal and antibacterial activities

The antifungal activity of the synthesized NPs was monitored using agar well diffusion procedure against two fungal species which are *Candida* and *Aspergillus* species [(22)]. Agar petri dish, which was used in this study as a culture media holder, was systematically swabbed with sterile cotton swab in which a 30 ml of 24 h Sabouraud′s dextrose was used for each fungal species. Continuously, wells were made in the pre-solidified agar plates with the help of 5 mm cork bor-er. Variety of the synthesized NPs concentrations (0.75, 1.5, 3, 6, 12 and 24 mg/ml) were sonicated with DDW and then used to evaluate the antifungal activity. Concurrently, Negative and positive control against the fungal pathogens was exhibited using DDW and antibiotics. Hereinafter, the cultured agar plates were incubated at 35 °C for 48 h, while the inhibition zones were measured in millimeter.

As for the IO NPs antibacterial activity, similar route to the aforementioned antifungal was repeated. However, the antibacterial activity was screened against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species using 30 ml of 24 h Blood agar culture media. In this experiment, the positive and negative controls used were antibiotics and DDW and the antibacterial activity was later proceeded to an incubation procedure for 24 h at 35℃. It is worth mentioning that the concentrations of IO NPs utilized for the antibacterial activity are 0.5, 1, 2, 4, and 8 mg/ml.

Results and Discussion

The XRD patterns of the synthesized NPs are presented in figure (2, a). Generally, the intensity of the diffraction peaks augmented with increasing IO NPs concentrations which in turn indicates higher crystallinity at high concentrations. As depicted in the figure, eight different pronounced peaks were acquired corresponding to (110), (120), (211), (10-1), (202), (211), (312), and (310) planes and diffraction angle of 2θ = 21.9°, 26.6°, 33.2°, 35.6°, 49.5°, 50.4°, 54°, and

62.4°, respectively (Card No. 96-901-1413) [1]. The (110), (120), and (211) planes mainly belong to FeO_2 phase, while other planes, (211), (10-1), (202), (312), and (310), are corresponded to Fe_2O_3 phase (Card No. 96-900-9783) [9].

Figure (2, b) illustrates the FT-IR curve of the synthesized NPs with concentration of 0.14 M. It is clear to be noticed that characteristic peaks at 3848, 3737, and 3416 cm^{-1} are mainly attributed to the O-H stretching bonds. In the meanwhile, peak at around 2925, cm^{-1} is corresponded to C-H stretching characteristic. Two pronounced peaks at 2381, and 2310 cm^{-1} are designated to O=C=O stretching. Furthermore, additional peaks at 1737, and 1638 cm^{-1} were observed which are due to C=O stretching, while peak at 1542 cm-1 is found to be in accordance with C=C stretching [1, 23]. Finally, Fe-O vibrations namely 1098, 795, 506 and 463 cm^{-1} are assigned to the IO NPs [23].

The UV-vis spectra of the synthesized IO NPs are demonstrated in figure (2, c), which exhibited broad bands and cut-off phenomenon at 360 nm. Furthermore, as illustrated in the figure, there is a decrease in the mentioned phenomenon cut-off towards higher wavelength as the concentration enlarged; this could be attributed to the lattice defects in the prepared NPs matrix [9].

Figure (2, d) demonstrates two dimensions and three dimensions AFM images of the synthesized NPs with a concentration of 0.14 M and scanning area of 2 µm. In general, the formed NPs exhibited a vertically aligned NPs with regular spherical shape and homogenous distribution. The average diameter and RMS were found to be 84.4 and 0.64 nm, respectively. In the meanwhile, the average surface roughness was found to be 0.54 nm; this indicates a pronounced rough surface which in turn reveals high electrochemical performance [24].

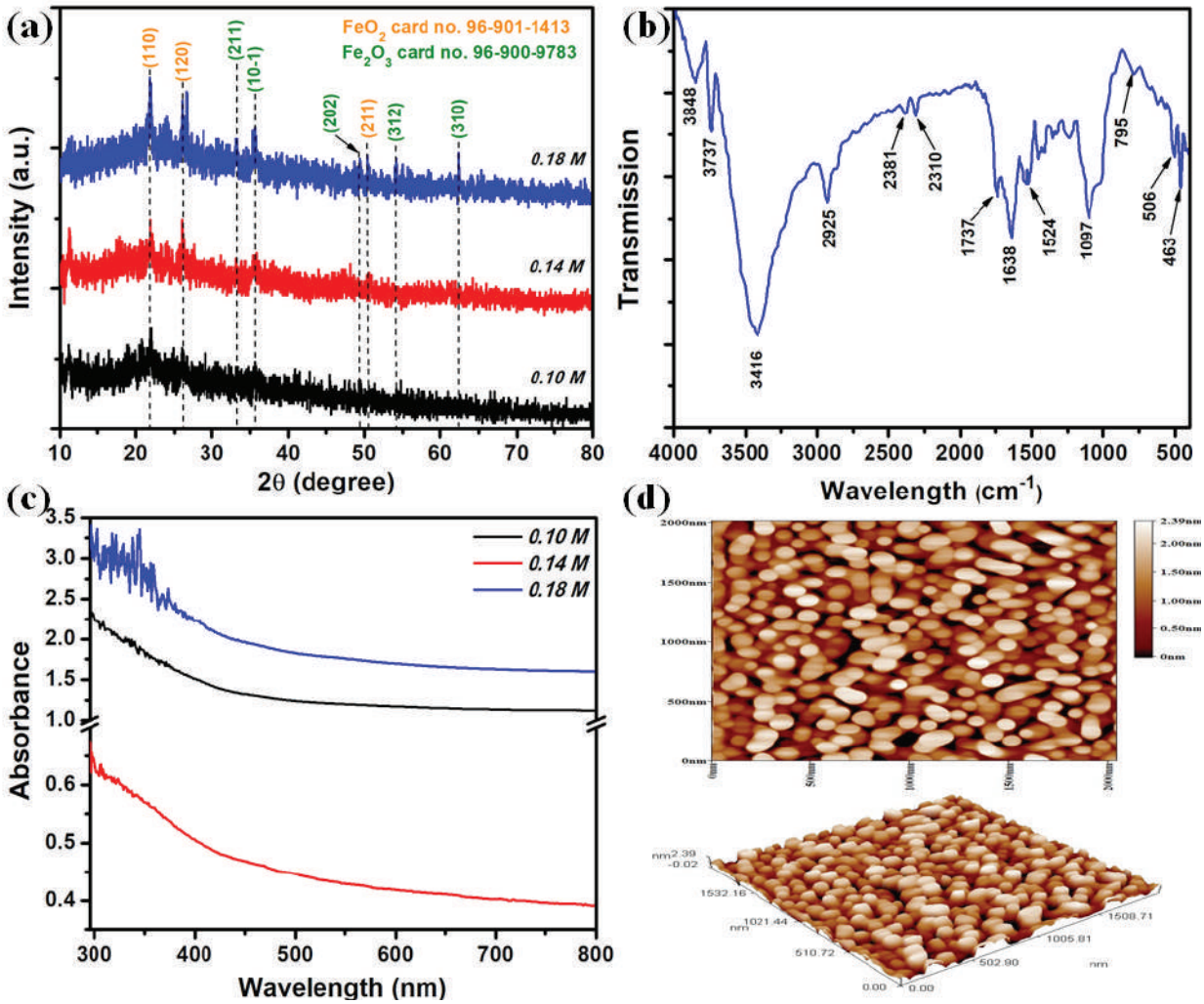


Figure (2): (a) XRD patterns, (b) FT-IR spectra, (c) UV-vis spectra, and (d) AFM of 2D and 3D images of the prepared NPs.

The TEM image of IO NPs is presented in figure (3, a and b). Generally, the synthesized NPs revealed almost a spherical structure with average diameter of 19 nm. From the figure, it also can be observed that a uniform IO NPs distribution which found to be in good agreement with the AFM findings. Furthermore, figure (3, b) shows the NPs diameter distribution which was found to be in the range of 11-29 nm.

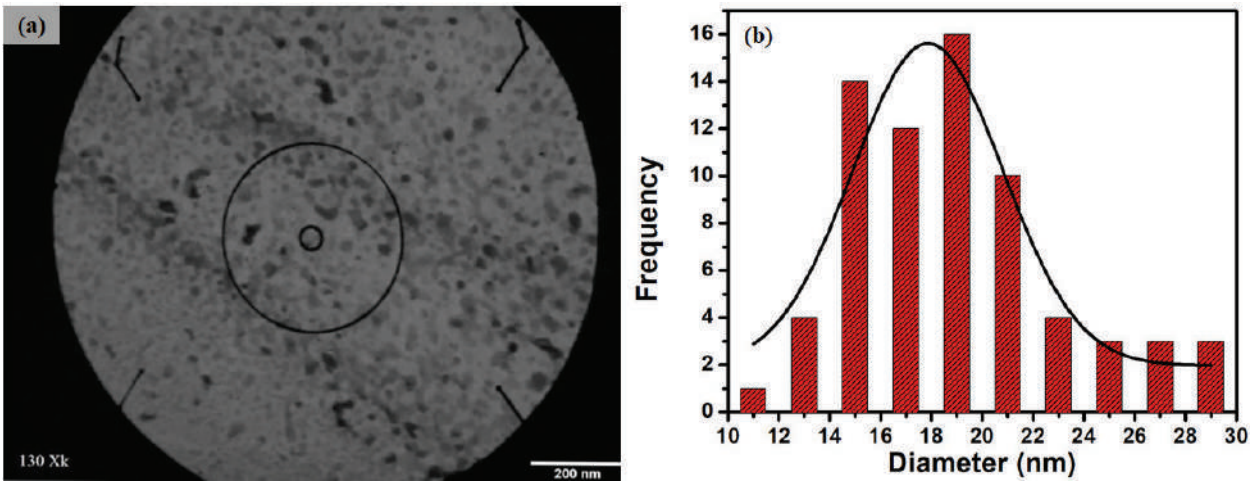


Figure (3): IO NPs with 0.14 M concentration (a) TEM image (b) diameter range distribution.

The antifungal activity of the green synthesized NPs against *Candida* and *Aspergillus* species are shown in figure (4, a). As presented in the figure, an observable inhibition zone increment in both kind of fungi species can be clearly seen as the concentration of the IO NPs increased, this indicates the active role of the synthesized NPs as an antifungal. This can be explained by the superior features of IO NPs such as large surface area, and small particle size as compared to their bulk nature [25]. Although, the inhibition zones in the case of *Candida* demonstrated larger inhibition diameter, lower concentrations of the used NPs were found to be active in the case of *Aspergillus*. The maximum inhibition zone diameters were found by the highest IO NPs concentration 24 (mg/ml) against *Candida* (35 mm) followed by *Aspergillus* (33 mm). In contrast, inhibition zone diameters of 10 and 15 mm against *Candida* and *Aspergillus* with IO NPs concentration of 3 mg/ml, respectively. Similarly, at concentration of 1.5 mg/ml, the antifungal activity was only exhibited against *Aspergillus*. This may be due the differences in the *Candida* species' cell wall structure which consists of high chitin as a yeast as compared to the moldy in *Aspergillus* species [26], this in turn leads to different sub-capability of each towards the tested nanoparticles.

Figure (4, b) presents the antibacterial activity of IO NPs against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species. Generally, increasing the concentration of IO NPs exhibited higher antibacterial activity. This could be attributed to the metallic NPs accumulation in the living cell-membranes which in turn releases cellular compounds as previously reported by other researchers [27]. It is a necessity to be stated that concentration as low as 0.5 mg/ml exhibited null activity against both species. However, higher concentrations of IO NPs displayed advanced inhibition zones in both species cases. Continuously, the acquired inhibition zones against Gram-negative *Acinetobacter* bring about more diameter range as compared to Gram-positive *S. aureus* at all concentrations. Inhibition zones of 25 and 19 mm in diameter against Gram-negative *Acinetobacter* and Gram-positive *S. aureus* were screened with IO NPs concentration of 8 mg/ml, respectively. This may be due to the difference in susceptibilities property of each bacterial species towards IO nanoparticles comes from the differences in the structure of cell wall of each bacteria. In details, the Gram-positive *S. aureus* species has thicker peptidoglycan and thus demonstrate higher resistance in contrast to Gram-negative *Acinetobacter*. As observed in table (1 and 2), the synthesized NPs

demonstrated higher antibacterial and antifungal effects than standard antibiotics

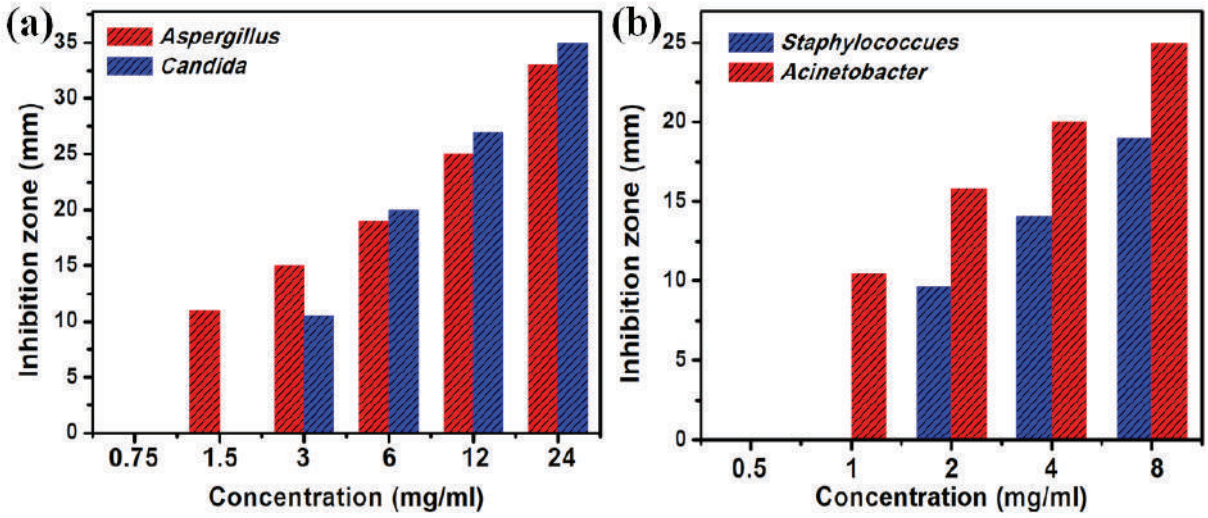


Figure (4): Inhibition zones of IO NPs; (a) fungi and (b) bacteria.

Table (1): Antibiotics zone of inhibition diameters against fungi.

fungal	inhibition zone (mm)				
	KCA (10 µg)	NY (100un)	AMB (20µg)	FCN (10µg)	DDW
candida spp.	34	14	11.4	22	0
Aspergillus spp.	21	16	12	0	0

Table (2): Antibiotics zone of inhibition against bacteria.

bacterial	inhibition zone (mm)							
	CRO (30 µg)	AK (30µg)	SAM (20µg)	TS (25µg)	CD (2µg)	CIP (5µg)	ATH (15µg)	DDW
S. aureus	26	17.5	14.5	13.5	0	0	0	0
Acinetobacter baumanii	0	0	0	0	0	0	0	0

Conclusion

IO NPs were successfully synthesized using *F. carica* fig extract. Additionally, the microstructural properties of the prepared NPs were illustrated using XRD, FT-IR, UV-vis, AFM and TEM techniques. Simultaneously, the synthesized NPs were evaluated for their antifungal and antibacterial activities against *Candida* and *Aspergillus* species as well as *S. aureus* and *Acinetobacter* species, respectively. It was found that

Aspergillus species is more sensitive to the synthesized NPs rather than *S. aureus,* shedding the light towards this technique which may be useful for the treatment of this aggressive multidrug resistant bacteria.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Molecular Detection of Antibiotics Resistance Genes in *Burkholderia cepecia* isolated from Diabetic foot infection

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Summary

This study aimed to isolate and diagnose *Burkholderia cepetia* from clinical specimens of diabetic foot and study the resistance of bacteria to antimicrobial agent in Najaf governorate from Augest 2019 to November 2019, which includes 120 clinical specimens for both sexes with an age ranged between)35-70) years old. The diagnosis of *bacteria* isolates was based on microscopy, as well as the culture and biochemical characteristics as an initial diagnosis. The final diagnosis by the Vitek-2 compact system .

Burkholderia cepacia 8(6.6%). Antibiotic sensitivity test was examined by dick diffution method, *Burkholderia cepacia* isolates showed high level of resistance almost for all β-lactam antibiotic classes under study which included;, ceftriaxone, cefoxitin and Cefepime, with percentage of (100%); ticarcllin with clavulalnic acide ,piperacllin , ceftazidime, tobramycine, ciproflaxcin and levofloxacin with percentage of (87.5%); aztreonam and amikacine with percentage(62.5); meropenem , imipenem and gentiamycin with percentage of (37.5%).

At molecular study, the investigated the presence of antibiotic-resistant genes (blaImp, blaOxa, blaKpc,blaCTX-M) using PCR technique and electrophoresis systems. 6/8 *Burkholderia cepacia* isolates were with *blaOXA* and (6/8) of isolates carry *blaCTX-M* gene, all isolates of *Burkholderia cepacia* gave negative result of blaIMP and blaKPC gene. Finally PCR analysis showed that the integron gene was (3/8 %).

Keyword:Bcc, *Burkholderia cepacia ; Antibiotics Resistance; Dabetic*

Introduction

The *Burkholderia cepacia* complex (Bcc) organisms are opportunistic nosocomial pathogens capable of causing severe disease in immunocompromised individuals. Bacteria frequently employ disparate mechanisms that act synergistically to achieve elevated resistance [(1).]

However, these data may overestimate the occurrence of resistance in *Burkholderia cepetia* organisms as the study was carried out on patient isolates solicited because they were in fact multidrug resistant. Despite this caveat, resistance patterns, both intrinsic and acquired, must not be discounted in these organisms .The often high-level acquired or intrinsic resistance of non-enteric bacteria such as *P. aeruginosa* and *Burkholderia* species is in no small part attributable to synergy between reduced penetration into and efflux from the cell [(2).]

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerve and constitutes the most frequent diabetes-related cause of hospitalization [(3).]

Diabetic foot ulcers is one of the main causes of mortality and morbidity among people with diabetes. Its include an injury to all layers of skin, necrosis or gangrene that usually occur on the soles of the feet, as a result of peripheral neuropathy or peripheral arterial disease (PAD) in diabetes patients [(4).]

Materials and Methods

specimens collection and bacterial identification

A total of 120 samples were collected from diabetic foot ulcer who attended different hospitals during the period from Augest 2019 to November 2019 in Al-Najaf provenance, sample collection include,collection 120 pus samples swab specimens from diabetic foot infection ulcer, The specimens were transported by sterile transport swabs to the department of bacteriology laboratory. Each specimen was inoculated using direct method of inoculation on culture of selective media namely MacConkey, Blood ,Mannitol agar then inoculated at 37°C for 18-24 hours [5].

DNA Extraction

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA promega Kit).

Molecular Identification

Gel electrophoresis was used for detection of DNA by UV transilluminator . The PCR assay was performed to detect the antibiotic resistance gene for *Burkholderia cepacia* shown in table(2). This primer was designed by Alpha DNA company, Canada as in table (1) . Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µLof 10mg/mL ethidium bromide (Sigma, USA) and it run at 80v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products [6].

Table (1): Primers used in this study

Primer Type	Primer Target	Primer sequence (5'-3')	Amplicon size (bp)	Reference
CTX-M	bla_{CTX-M}	**F**: SCS ATG TGC AGY ACC AGT AA **R**: CCG CRA TAT GRT TGG TGG TG	554	(7)
KPC	bla_{KPC}	**F**: ATG TCA CTG TAT CGC CGT CT **R**: TTT TCA GAG CCT TAC TGC CC	893	(8)
IMP	bla_{IMP}	**F:** TTGACACTCCATTTACDG **R:** GATYGAGAATTAAGCCACYCT	139	(9)
OXA	*blaOxa*	**F:** GGCACCAGATTCAACTTTCAAG **R:** GACCCCAAGTTTCCTGTAAGTG	564	(9)

Table (2): PCR program of *intI* primer that apply in the thermocycler

Gene	Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
blaCtxm	94 Cº for 4min.	35	94 Cº for 30Sec	63Cº for 1 min	72 Cº for 1min.	72Cº for 5min.
blaKpC	94 Cº for 5 min.	35	94 Cº for 1min	50Cº for 1min.	72 Cº for 1 min	72Cº for 10min.
blaImp	94 Cº 10 min	30	94 Cº 40Sec	55 Cº for 40Sec	72 Cº for 1 min	72 Cº for 10 min
blaOxa	94 Cº for 10 min	30	94 Cº for 40Sec	60 Cº for 40Sec	72 Cº for 1min	72 Cº for 5 min

Results and Discussion

This study was conducted on 120 specimens from dabetic foot suspected patients during the period from September 2019 to December 2019

4.9.1.2 Molecular identification of antimicrobial drug resistance of *Pseudomonas aerginosae and Burkholderia cepacia*

4.9.3.2.1.blaOXA

The result showed that the *blaOXA* resistance gene was detected in 6/8 *Burkholderia cepacia* as in figure (1) .

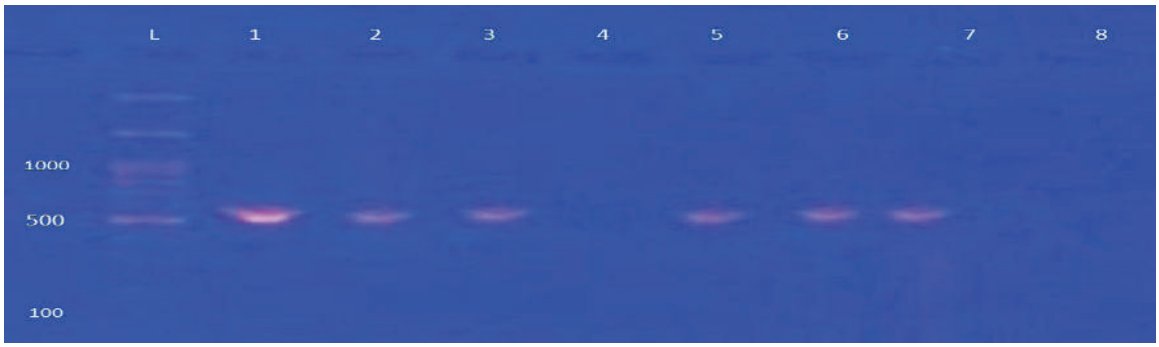


Figure (1): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaOXA* gene primers with product 564bp.Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,5,6,7) show positive results with *blaOXA* gene.

Carbapenemases are the main mechanism by which resistance to carbapenems occurs and they belong to three of the four β-lactamase classes A, B and D . Class D carbapenemases are the OXA-β-lactamases , further subdivided into various sub-groups mainly blaOXA-23, blaOXA-24/40, blaOXA-58, blaOXA-48, blaOXA-51 and blaOXA-143 . These OXA-type β-lactamases occur widely in Acinetobacter with the most abundant being blaOXA-51, which is chromosomally encoded hence intrinsic to these species but it may confer resistance to carbapenems when its expression is up-regulated by genetic re-organization [(10)] Class B carbapenemases are also known as the metallo-β-lactamases (MBLs) ,they are mostly encoded by integronborne mobile gene cassettes and hence, they are transferable amongst various bacteria via horizontal gene transfer mechanisms notably conjugation . Class A carbapenemases include the Klebsiella pneumoniae carbapenemase (KPC) family that can be plasmid encoded or chromosomal [(11)].

4.9.3.2.2.blaIMP

The result showed negative with *blaIMP* gene of *Burkholderia cepacia* isolates . Based on recent reports, there are two major families of imported metallo--lactamases, IMP and VIM, that are carried on mobile gene cassettes inserted into integrons . Including those in this report, there are 18 variants of IMP metallo--lactamases and 11 variants of VIM metallo--lactamases [(12)].

The only two published reports on metallo-B-lactamases from the United States identified VIM-2 and VIM-7. Metallo-B-lactamases hydrolyze most -lactam antibiotics except aztreonam. Therefore, many pathogens that produce these enzymes at high levels are resistant to the majority of -lactam antibiotics, including the carbapenems. The first report of an imported metallo-B-lactamase described a *Pseudomonas aeruginosa* isolate obtained from a Japanese patient in 1988 . Since then, the occurrence of mobile genetic elements encoding metallo-B-lactamases has extended beyond *P. aeruginosa* to include many types of gram-negative organisms distributed throughout the world . Areas which have reported these types of isolates include several countries in Asia and Europe; the Americas, including Brazil, Canada, and the United States; and Australia [(12)].

4.9.3.2.3.blaKPC

All *Burkholderia cepacia* isolates give negative result with *blaKPC* gene .

The blaKPC genes that encode KPCs are present on transferable plasmids and are flanked by transposable elements, thus allowing for the gene to move from plasmid to the bacterial chromosome and back . [13]. All the carbapenem resistant isolates showed 100% resistance to ampicillin, cotrimoxazole, all 4 generations of cephalosporins and piperacillin tazobactam. The resistance to aminoglycoside antibiotics varied from 33% for amikacin to 94% to tobramycin . In the present study, *Klebsiella* showed a 77% resistance to imipenem and 96% resistance to meropenem, while *E coli* showed 67% resistance to imipenem and 95% resistance to meropenem, blaKPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae were MHT negative. They may have developed a different resistant mechanism other than carbapenemase production. Resistant to both imipenem and meropenem is a strong indicator of carbapenemase production rather than resistance to either one of the carbapenems, as this may imply a different resistance mechanism [14].

4.9.3.2.4.blaCTX-M gene

The result showed that the blaCTX-M resistance gene was detected in and 6/8 *Burkholderia cepacia* as in figure,(2) .

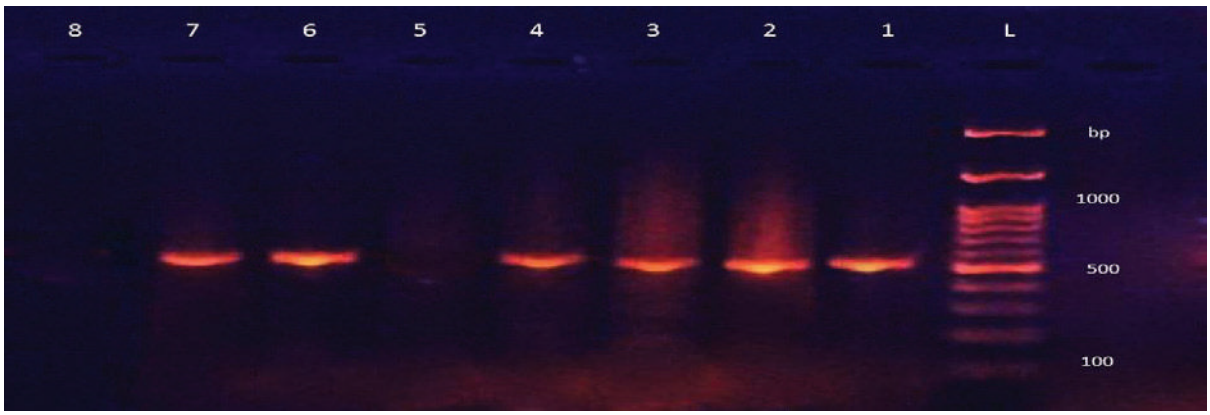


Figure (2): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaCTX-M* gene primers with product 554bp.Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,4,6,7) show positive results with *blaCTX-M* gene.

ESBLs are one of the main leading causes of resistance to ß-lactam antibiotics among Gram-negative bacteria . These enzymes are plasmid-encoded β-lactamases that mediate resistance to penicillins, first-, second- and third- generation cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime . TEM, SHV, and CTX-M are the major genetic groups of ESBLs amongst clinically important Gram-negative bacteria . These enzymes are most commonly found in Klebsiella pneumoniae (K. pneumoniae) and Escherichia coli (E. coli) and are also observed in other clinical isolates of Enterobacteriaceae and Pseudomonas [15]. The first TEM-type β-lactamase, produced by a clinical E. coli strain, was reported in 1965. The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. The SHV-type ESBLs may be found in clinical isolates more frequently than any other types of ESBLs and have been reported from several countries in Europe, such as Austria, France, Italy, and Greece, as well as in the United States and Australia . The CTX-M-type ESBLs developed from TEM and SHV and can be divided into five subgroups according to their amino acid sequence simi-larities, including CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, and CTX-M-V [16]

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Evaluation the Antimicrobial Effect of Glycerin Magnesia on Some Bacteria, In-Vitro Study

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Abstract

This study was aimed to assess the efficacy of glycerin magnesia on some bacteria. A thirty percent of glycerin magnesia were prepared as explained below. Many types of bacteria including *Proteus spp., Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter, E. coli, Pseudomonas aeruginosa, Salmonella spp.* were selected for this study and obtained from university of Tikrit, college of veterinary medicine. A bacterial broth were prepared, a then a sterile swab were emulsify in these broth and streaked on muller hinton agar plate and allowed till dry, then a holes were filled with a given glycerin magnesia and incubated for 24hrs. at 37℃ . The results showed that a higher antibacterial effects of glycerin magnesia against *Staphylococcus aureus* followed by *Proteus spp., Pseudomonas aeruginosa, Salmonella spp., Acinitobacter, E. coli, Staphylococcus epidermidis* respectively.

In conclusion, the glycerin magnesia have a wide range antibacterial effect and can be used in future in wound healing.

Key words: *glycerin magnesia, bacteria, in-vitro.*

Introduction

Magnesium (Mg) is an important ion found in the body human and animals, and may be observed as a drug with numerous clinical uses [(1)]. The total magnesium in human and animal body were 53 % stores in the bones, 27 % in muscle, 19 % in other soft tissues, about 0.5 % in the RBCs and about 0.3 % in the serum [(2)]. About half of Magnesium are existing as free and doesn't bound with albumin or other ion [(3)].

Magnesium sulfate (MgSO4) is a solid, odorless material that present as crystal powders or as colorless crystals needle like or as a crystalline powder with white color [(4)].

Magnesium sulfate have a wide uses in building, industrial/processing, agriculture, special care products, medicine, food processing [(5)].

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Magnesium sulfate uses in human medicine including as an anticonvulsant for controlling and in preventing seizures especially in children who suffer from nephritis, lowering blood pressure in pregnant females who suffer from preeclampsia, Asthma attacks can also be treated with MgSO4. MgSO4 used in human and animals as a laxative and also it can also use in relieving cases of constipation [(6)].

Ismail & Shaker [(7)] reported that MgSO4 with glycerin have an antibacterial effects when used on wound. The addition of glycerin as a vehicle for MgSO4, some reported that glycerin increase the antimicrobial activity of material that emulsify in it [(8)].

The most pathogenic bacteria for human and animals were reported including Salmonella [(9-13)], E. coli [(14-17)], Staphylococcus spp. [(18)], Proteus spp. [(19)].

The current study aimed to assess the efficacy of glycerin magnesia against some bacteria.

Materials and Methods

Glycerin Magnesia (30%): The mixture was prepared by dissolved a 30 g of MgSO4 in 30 ml heated distilled water and mixing thoroughly. After a complete dissolving of MgSO4, the glycerin was added slowly to the mixture till a final volume of 100ml and using heat and continual stirring.

Many types of bacteria including *Proteus spp., Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter, E. coli, Pseudomonas aeruginosa*, *Salmonella spp.* were selected for this study and obtained from university of Tikrit, college of veterinary medicine. Each type of these bacteria were inoculated in 5 ml nutrient broth and incubated for 24hrs., then a sterile swab were emulsify in these broth and streaked on muller hinton agar plate with a central hole (which made in each of the plate with a sterile 2.0 mm diameter cork borers) and allowed till dry, then a holes were filled with a given glycerin magnesia and incubated for 24hrs. at 37℃ .

Results and Discussion

The current results obtained were showed a higher antibacterial effects of glycerin magnesia against *Staphylococcus aureus* followed by *proteus spp*., *Pseudomonas aeruginosa*, *Salmonella spp., Acinitobacter*, *E. coli, Staphylococcus epidermidis* respectively (Table 1) (figures 1, 2).

Table (1) Antibacterial effects of glycerin magnesia on some bacteria.

Type of Bacteria	Diameter in mm
Staphylococcus aureus	40
Staphylococcus epidermidis	5
E. coli	25
Pseudomonas aeruginosa	30
Acinitobacter	28
Proteus spp.	35
Salmonella spp.	30

Figure 1 results of *S. aureus*

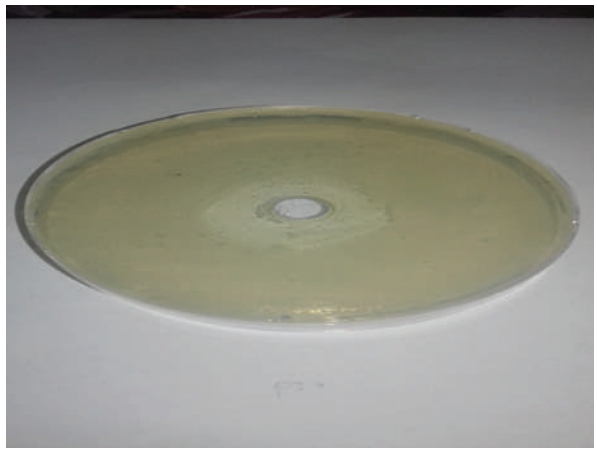


Figure 2 results of *E. coli*

It has been reported that Mg alone has an antibacterial effect invitro by reduction the bacterial growth and prevent the formation of biofilm by increasing of local alkalinity [20]. Robinson *et al*. [21] suggested that the alkaline pH was liable for the significance decreasing of CFU/ml[1].

Another researcher reported that the pH and the graded charge were significant in prokaryotic physiology in production the proton motion force that used to do the useful work for bacteria, also reported that a pH is important for extra and intracellular environments favored for growth of organisms [22]. Also, other researcher reported that some biomaterial have an ability to yield the alkaline pH which give rise the antibacterial mechanism [23, 24].

Li *et al*. [20] reported that pure magnesium when added to medium has a full effect on MRSA when tested in-vitro.

Also, Crisler *et al*. [25] found that the increasing medium salinity to 10% MgSO4 reduced the number of *Pseudomonas*. However, a report of Marnocha *et al*. [26] suggested that MgSO4 at 10% reduce the growth of most tested bacteria.

Many studies have been suggested that there was an association amongst the cell membrane fluidity and the stress tolerance. The structure of cell membrane fatty acids is liable for preservation of cell membrane fluidity. The most consequences of altering in fatty acid of cell membrane in most microorganisms are inflection the action of cells intrinsic proteins which achieve many purposes for example uptake of nutrient and ion

pumping [27]. The treatment by using alkaline material resulted in alterations in fatty acid composition of the cell membrane for some bacteria especially *E. coli* and *Salmonella Spp*. which leading to reduction in number of these bacterial types [28].

Conclusion

Glycerin magnesia have a wide range antibacterial effect on many bacteria especially *S. aureus*, *Pseudomonas* and other, the future study will use this preparation in wound healing.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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Prevalence of Histopathological Diagnosis of Benign and Malignant Breast Lesions in Al-Muthanna Province for Two Years Duration

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Abstract

Introduction: Breast cancer (BC) is a common form of cancer among women globally. It is considered 5th cause of death in females with equally to 522,000 patients / year. Nowadays it is considered 15.4% of death in developed countries after lung cancer. In developing nations, breast cancer considered the 1st cause of death for females 324,000 cases of deaths (14.3%) for total deaths. **Method:** One hundred and six female patients with breast lesion were collected from the Al-Hussein Teaching Hospital Laboratory/Histopathology department –Al-Muthanna province, during the period from January 2018 to January 2020. The data for cases were collected to study the age, type of breast lesion whether benign or malignant. Haematoxylin/ Eosin staining done in Al-Hussein Teaching Hospital Laboratory / Histopathology department. **Results:** Cross sectional study for 106 patients done in Al- Muthanna proven for assessment breast mass and identify malignant and benign lesion, mean age of patients was (39.4 ± 15.8) years old, with min age 13 years and max age was 81 years old. Types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy. After pathological assessment of biopsies showed 51% malignant and 49% benign. In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment. Significant association between age groups and cancer of breast. **Conclusion:** After pathological assessment of biopsies, malignant breast cancer (IDC/ grade II, IDC/ grade III, ILC and fibrocystic changes) most common changes, significant association between age group and breast cancer development more age group 41- 50 years old and then (31- 40), (51– 60) years old respectively.

Key words: *Prevalence, histopathological diagnosis, breast lesions, Al-muthanna province*

Introduction

Breast cancer (BC) is a common form of cancer among women globally. It is considered 5th cause of death in females with equally to 522,000 patients / year [(1)]. Nowadays it is considered 15.4% of death in developed countries after lung cancer [(2)]. In developing nations, breast cancer considered the 1st cause of death for females 324,000 cases of deaths (14.3%) for total deaths [(2, 3)]. This rate changes from 6 – 20 / 100.000 in West Africa and East Asia (4). The incidence in 2012 in females reach to 1.7 million (25% of entirely cancers), 883,000 patients in developed country in contradiction of 794,000 in developing nations [(4, 5)].

The incidence was increase in age after 35 years old and peaking in 60 years old [(6)].

Mortality rate depend on age of females, staging of malignancy, treatment respond, metastasis of malignancy, main reasons of breast cancer is hormonal factors, genetic tendencies, behaviors and ecological reasons [(7)].

Benign Breast Diseases (BBDs) is not cancer. Most usual reason of breast difficulty in women and more usual than malignancy (8, 9). It is more usual than malignancy in western countries (10). More than 30% of women with BBD need treatment where survive (11). A three-ways for evaluation done by clinical assessment: US and mammogram, pathological assessment, central needle biopsy. Most women with BBD not have risk of development of breast cancer, so early diagnosis and treatment is important to remove the anxiety especially women with family history of breast cancer and must follow up annually. Most common BBD is fibro adenoma (12). Two types of breast cancer (cancer in situ): **Ductal carcinoma in situ. (**DCIS) 83% of all cases 2010-2014 unusual cells substitute usual epithelial cells around ducts of breast and metastasis to the lobules in addition to the ducts; it is can or can not developed to the aggressive malignancy it is grow slowly without any management it is misdiagnosis with benign 20%-53% diagnosed with an invasive breast malignance for 10 years or more. **Lobular carcinoma in situ.**: 13 % of patients, atypical cells developing inside and growing about some of breast lobules. It is not precursor of invasive cancer, but considered one of strong cause of invasive cancer. The aim of study is to show proportion of benign and malignant breast lesions and types of malignancy tissues by pathological assessment and relation of malignancy with increase age of females (13-15).

Method

One hundred and six female patients with breast lesion were collected from the Al-Hussein Teaching Hospital Laboratory/Histopathology department –Al-Muthanna province, during the period from January 2018 to January 2020. The data for cases were collected to study the age, type of breast lesion whether benign or malignant. Haematoxylin/Eosin staining done in Al-Hussein Teaching Hospital Laboratory / Histopathology department.

***Specimens**:*

The one hundred and six cases classified into benign and malignant breast lesions. From each formalin fixed paraffin embedded tissue, one section of 5-micron thickness was obtained and stained by haematoxylin and eosin staining method for evaluation of morphology.

Methods of staining procedures:

The following steps were applied for (H&E) staining method.

a) Deparaffinization: This done by adding the following:

1. 5 min. period adding Xylene.

2. 5 min. period adding Xylene.

3. 5 min. period adding 99 % ethanol

4. 5 min. period adding 99 % ethanol

5. 5 min. period adding 99 % ethanol

6. 5 min. period adding 99 % ethanol

7. 5 min. period adding 99 % ethanol

8. 5 min. period adding 95 % ethanol

9. 5 min. period adding 70 % ethanol

10. Purified water.

b) Hematoxyline and eosin staining method:

1. Dewax sections (deparaffinization as above).

2. Stain in hematoxyline for 3-10 minutes.

3. Wash well in running tap water.

4. Remove excess stain by differentiating the sections in 1% acid alcohol (1% in HCL 70% alcohol) for 5-10 seconds.

5. Wash well with in tap water until sections regain their blue color.

6. Stain in eosin for 2-5 minutes.

7. Dehydrate slowly through increasing grades of alcohol (i.e.70%, 90% and 100%).

8. Clearing by xylene.

9. Mount wit DPX.

Statistical analysis done by SPSS 22 calculated mean and SD with percentage and frequency. Chi square use for revealed association between age groups and behavior of tumor, significant association when P-value

less than 0.05.

Results:

Cross sectional study for 106 patients done in Al- Muthanna proven for assessment breast mass and identify malignant and benign lesion, mean age of patients was (39.4 ± 15.8) years old, with min age 13 years and max age was 81 years old. Types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy as showed in fig (1). After pathological assessment of biopsies showed 51% malignant and 49% benign as in fig (2). In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment showed in fig (3).

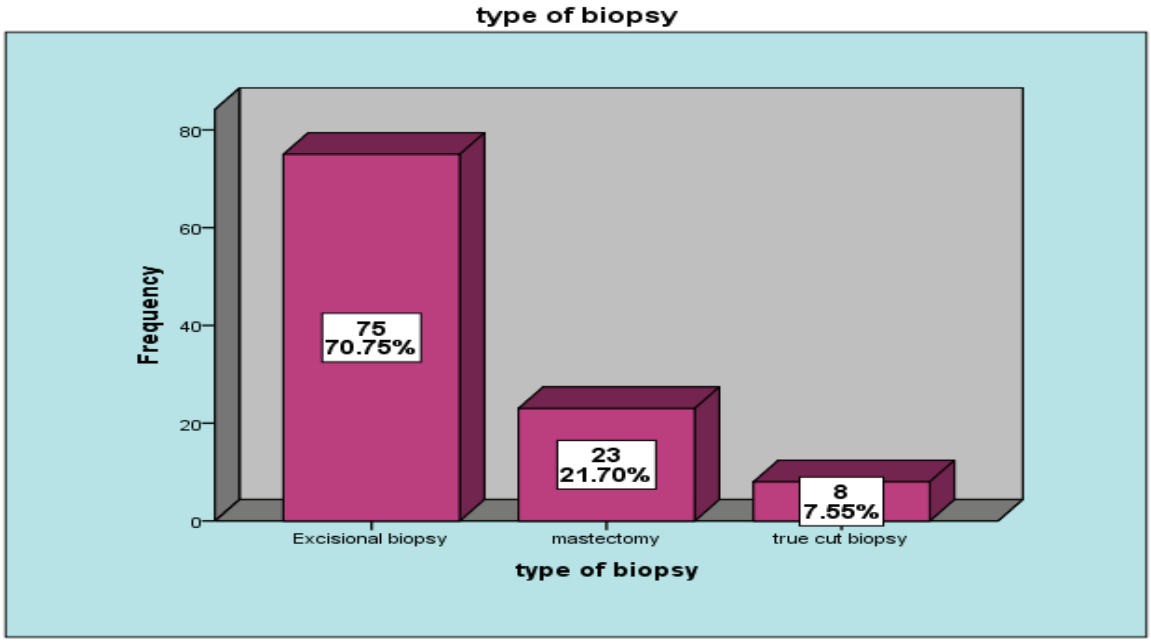


Fig (1): distribution of types of biopsy.

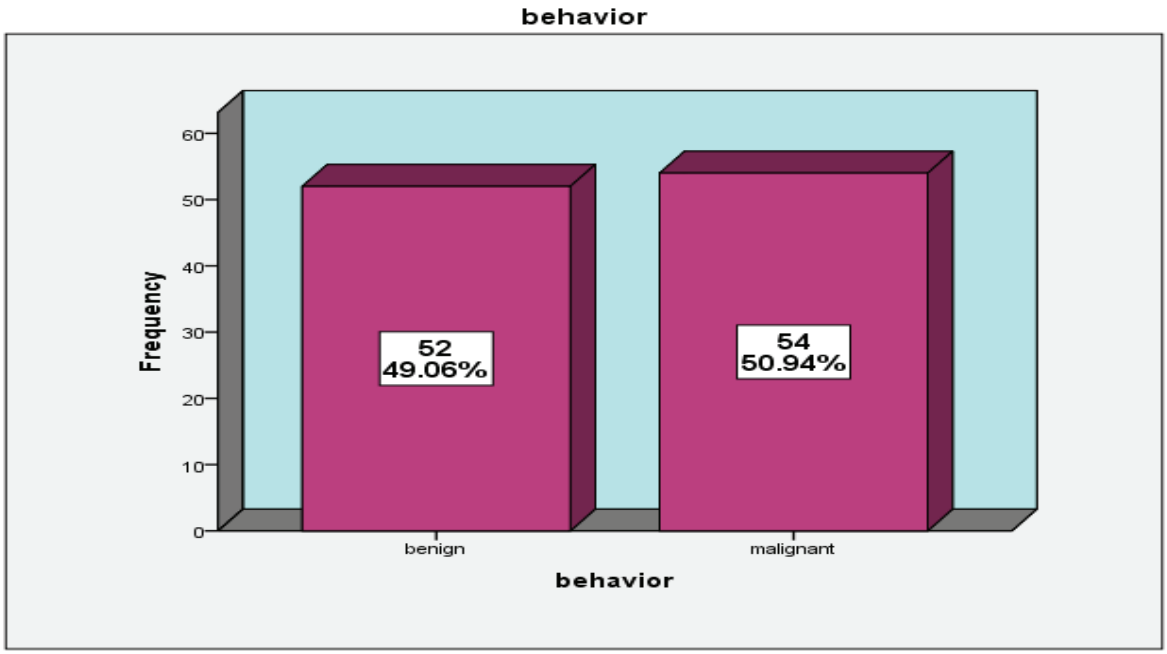


Fig (2): distribution of behavior of biopsy after pathological assessment.

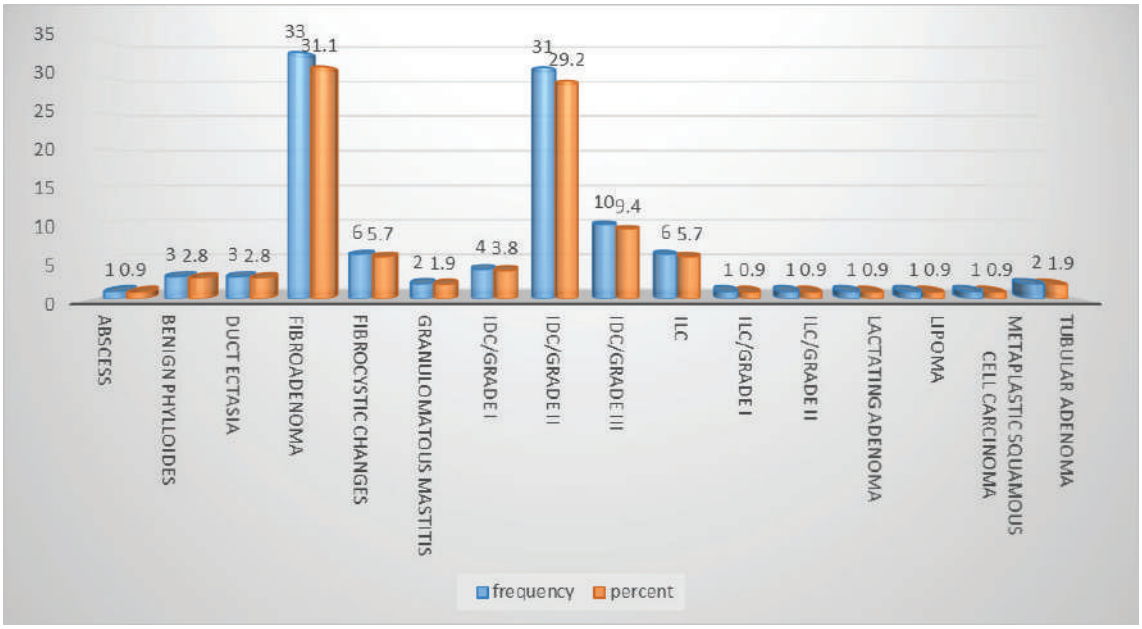


Fig (3): distribution of benign and malignant according to types after pathological assessment.

According to association between ages groups and behavior of lesion either benign or malignant, there is significant association between age groups and cancer of breast, 37% of females in age group 41- 50 years old with malignant breast cancer, while 20% of malignancy in age group (31 – 40) and (51 – 60) years old. 13% of malignancy in age group 61 – 70 years old and only 6% in female over 71 years old age. As show in table (1).

Table (1): association between age groups and behavior of lesion either benign or malignant.

			behavior		Total
			benign	malignant	
Age	11 - 20 years	Count	13	0	13
		% within behavior	25.0%	0.0%	12.3%
	21 - 30 years	Count	22	2	24
		% within behavior	42.3%	3.7%	22.6%
	31 - 40 years	Count	11	11	22
		% within behavior	21.2%	20.4%	20.8%
	41 - 50 years	Count	6	20	26
		% within behavior	11.5%	37.0%	24.5%
	51 - 60 years	Count	0	11	11
		% within behavior	0.0%	20.4%	10.4%
	61 - 70 years	Count	0	7	7
		% within behavior	0.0%	13.0%	6.6%
	more than 71 years	Count	0	3	3
		% within behavior	0.0%	5.6%	2.8%
Total		Count	52	54	106
		% within behavior	100.0%	100.0%	100.0%

Pearson Chi-Square= 58.188, P-value = 0.0001 (significant).**

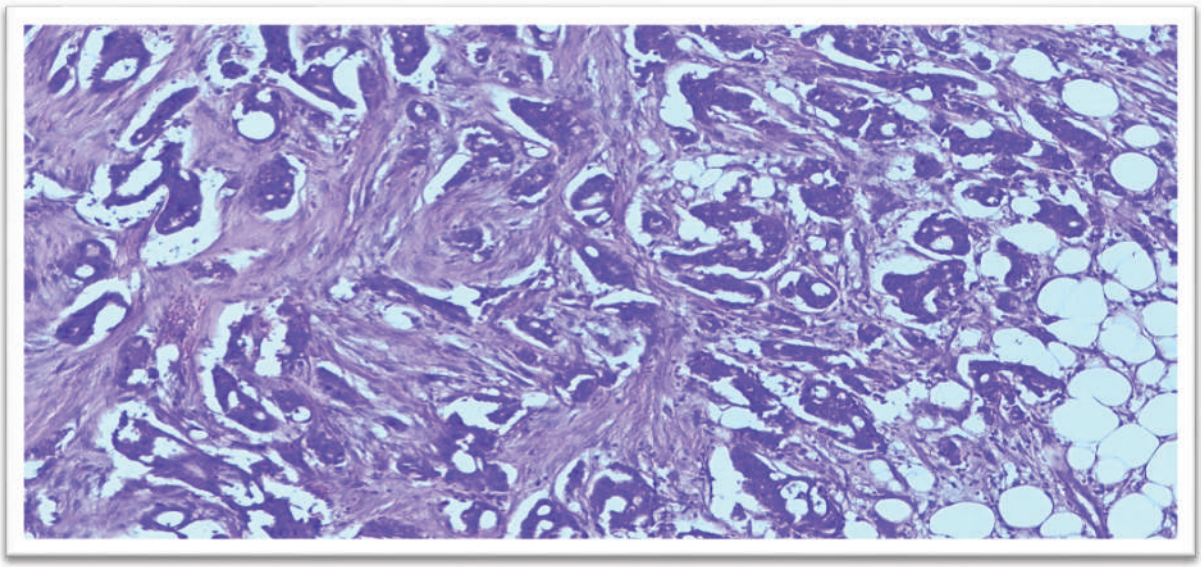


Fig (4): Invasive ductal carcinoma with Hematoxylin & Eosin staining (10X)

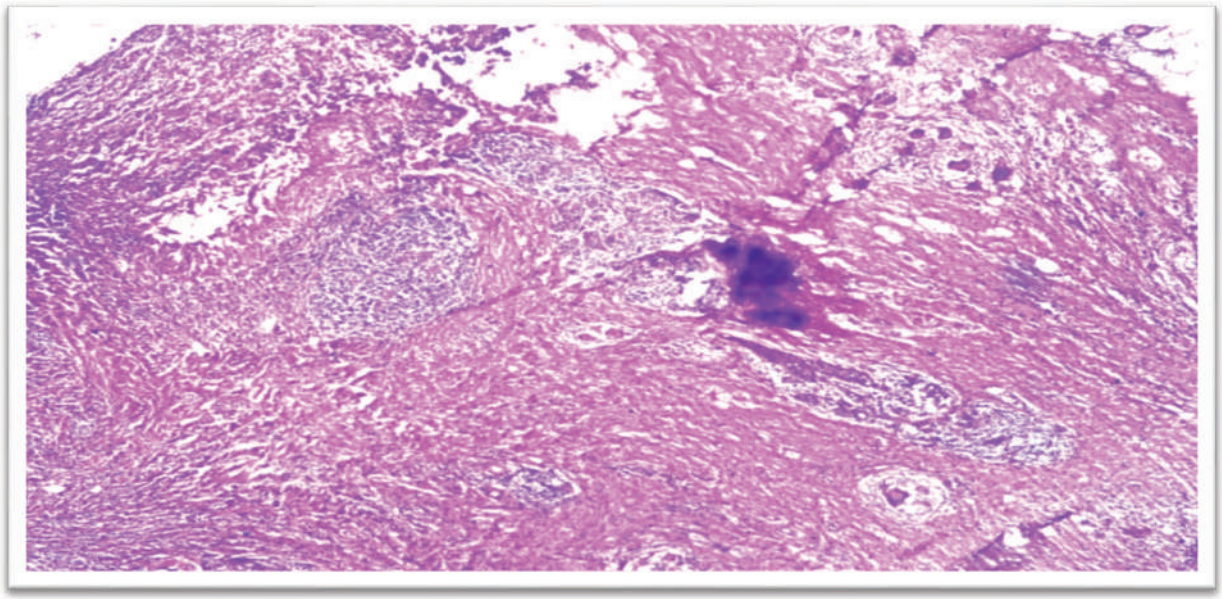


Fig (5): Granulomatous mastitis with Hematoxylin & Eosin staining (4X)

Discussion

Cancer is illnesses that lead the cells in body loss of control and change. All types of cancer cells finally changes to lump or mass that named cancer, and this malignant tumor called according to the site form it the tumor originate. Breast cancer start from gland of breast tissue that lead to production of milk called (terminal duct lobular unit). Other parts of breast tissue consist of connective, fatty and lymphatic tissues (16).

According to our study, mean age of patients was (39.4 ± 15.8) years old, with min. age 13 years and max. age was 81 years old. These results similar to study done by Augustin et al. showed the age extended since 16 - 90 years by a mean of 45.83± 13.5 years old (17). Zhi-Gang Yu et.al. Also, have the same results that mean age of patients was (44 ± 11.6) years old (18).

In our study the types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy this is similar to Augustin et al. that showed, the total of cases that take and examine by pathologist contain of 69% of lumpectomy then 30% biopsy of breast.

After pathological assessment of biopsies in our study showed 51% malignant and 49% benign while in another study the proportion was around 4:1 or 5:1 (19), this different is due to sample collection or may be due to high no. of malignancy of breast in al-muthanna province.

In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment this results similar to Augustin et al. that showed he predominant tumor histological designs IDC 113 (64.9%), ILC 17 (9.8%) then histological designs were invasive ductal carcinoma 113 (64.9%), invasive lobular carcinoma 17 ductal carcinoma in situ 10 (5.7%) and medullary ca. 5 (2.8%) (17). Similar features diagnosed by Ohene et al. (20); detailed that 53.7% of grade III, 31.5% of grade II and 14.8% of grade I. Essiben et al. showed a propensity in the following course of incidence: grades II, I besides III to the Yaoundé Gynecological Obstetric and Pediatric Hospital (21). Koffi et al. presented that tumor grade II exemplified 58.4% of females, however grade I in addition II were in the comparable amount (20.8%) (22). These results are conflicting to those reported by Meye et al. that, the grade II detained the past location after grade I and III (23).

According to association between ages groups and behavior of lesion either benign or malignant, there is significant association between age groups and cancer of breast, 37% of females in age group 41- 50 years old with malignant breast cancer, while 20% of malignancy in age group (31 – 40) and (51 – 60) years old. 13% of malignancy in age group 61 – 70 years old and only 6% in female over 71 years old age. Augustin et al. stated the same results that most patients in ages of 35 to 44 and 45 to 54 with 28.2% and 29.3% correspondingly. We can roughly that the major people found is dependable with some preceding studies approved globally (24, 25). Another study done by Hai-long Chen et al. showed that age groups from 50-59 years old was most group with breast cancer (26.4%), less than 30 years old and less than 40 years old represented low mount of patients (6.4%) (26). In another study stated that 6.4% of patients with breast cancer younger than 40 years old while 93.6% of them more than 40 years old, so the incidence of females to developed breast ca. was more in age group more than 40 years old so all women must do routinely breast cancer screening (27).

Conclusion

After pathological assessment of biopsies, malignant breast cancer (IDC/ grade II, IDC/ grade III, ILC and fibrocystic changes) most common changes, significant association between age group and breast cancer development more age group 41- 50 years old and then (31- 40), (51– 60) years old respectively.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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A Histological and Histochemical Study on Olfactory Bulbs to Detection Amyloid Protein Depositions by Congo-Red and Routine Staining Techniques

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Abstract

In this study, histological and histochemical techniques were used to examine olfactory bulbs in the albino rat male. Thirty male albino rats were split into three age groups (10 animals each) in the current research: **Group I**: consider as a control group, including adult animals aged 3 months .**Group II:** include animals aged 6 months. **Group III**: include animals aged 12 months. The histological architecture of the layers of olfactory bulbs and their main cells was identified by using H & E staining techniques, meanwhile the composition of each layer in albino rat was evident. In the glomerular and mitral cell layer of group III, olfactory bulbs showed reduced neural density. Modified staining with Congo - red was conducted for histochemical studies. Compacted amyloid cores were found in group III animals' olfactory bulbs, while dispersed amyloid cores were found in group II&III olfactory bulbs' cortex. The present study adds to our knowledge of the impact of amyloid protein on olfactory bulbs and their prospective neurodegeneration involvement.

Key words: *Histology, Histochemistry, Congo red, Amyloid ,Granular cells, Mitral cells and olfactory bulbs.*

Introduction

In the olfactory signal conductive path, the olfactory bulbs are the station of relay. The OBs are the first chief relay in the processing of odor data process: it provides afferent input from olfactory sensory neurons (OSN) in the olfactory epithelium of the nasal cavity and responsible for the identification of odors in it: which is the only relay between the peripheral and central nervous system, it also processed the olfactory data [(5)]. The olfactory bulb's main histological architecture involve: olfactory nerve layer, glomerular olfactory layer, external plexiform layer, mitral cell layer, inner plexiform layer and granular cell layer [(8)]. The superficial olfactory nerve layer contains afferent axons from OSNs. OSN axons synapse with OB projections of neurons dendrites, mitral / cells, and sub aggregations of regional periglomerular cells (Pgm) [(13)]. These layers are arranged in the olfactory bulbs very obviously and regularly. The characteristics of olfactory bulbs structure facilitate the processing of data and also provide a scaffold for olfactory data spatial encoding [(5)].

The olfactory bulb consists of four types of cells: mitral cells, granular cells, tufted cells and short axon cells according to classical studies [(10; 5)]. One of these neurons, granular cells, have long been known to be morphologically uncommon because they don't have a standard axons, and recent studies in electron microscope have demonstrated that it participate in special reciprocal synaptic connections with mitral cells [(8)].Mitral cells are the biggest cells in the olfactory bulbs and as stated by electron and light microscope researches, moreover they are the main olfactory bulb efferent neuron [(6)]. The dendritic structures of mitral cells can be subdivided into primary and secondary dendrites, and both primary and secondary smooth dendrites pass through the surface of the outer plexiform layer and only the primary dendrites extend down to the olfactory glomerular layer [(18)].

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Within the glomerular layer the dendrites of mitral cells are in synaptic communication with olfactory nerves and periglomerular cells, but the only synapses on mitral cells elsewhere are the "reciprocal synapses'' with the granule cells [5]. Mitral cells, as the main olfactory bulbs efferent neurons, play a significant role in olfactory signal conduction and modification [9; 13].

Amyloids β (AB) are little pieces of a bigger proteins named "amyloid precursors protein" APP. While the ordinary function of APP has not yet been determined by researchers, they understand a lot about how it appears to operate [16]. In its full shape APP ranges from the inside of the brain cells to the outside through the fatty membrane around the cells. As APP is activated to perform its normal activities, other protein can cut it into different and smaller parts inside and outside cells. APP can be cut in several aspects: under some conditions, β-amyloid is one of the parts generated [3].

According to the hypothesis of amyloid, phases of beta-amyloid aggregations interrupt cell-to-cell communication and activate immune cells, these cells of immune system cause inflammation [12].

The process of neurodegeneration in diseases of cognition (Alzheimer's and Parkinson's diseases) may involve toxicity of β-amyloid (Aβ) that could be demonstrated in vitro and seems to be involving oxidative stress, this underlie the progression of neurodegeneration that consider as characteristic feature of AD [2].

It has been shown that the amyloid induces neuronal death, reduced plasticity of synapses, aberrant axons growth, tau hyper phosphorylation and chronic inflammation [7] . Aβ accumulation in the pathogenesis of AD is an early and essential case .First formation of temporal cortical regions, including the hippocampus, a memory-creating zone. Aβ aggregates have been indicated to form neurotoxic plaques that contribute to neurodegeneration accompanied by dementia [15].

Similarly, [11)) showed that capillaries, venioles and arterioles in the cerebral cortex also often have amyloid deposits. Congo red staining was considered as an approved histochemical marker for amyloid β-pleated-sheet [14]. Congo red: amyloid detection in tissue parts is significantly improved and verified by favorable Congo red staining. Thioflavin S and Congo red are represented the main histological stains that used for any type of amyloid [17].

Red stain is red-pink on its own .Under both light and polarized light microscopy, examination of tissue segments suspected of participation by amyloidosis must be carried out. Amyloidosis has a distinctive green apple birefringence when polarized[11].

In this study we analyzed the histological structure and histochemical (Congo red for amyloid protein) characteristics of OBs in order to evaluate the presence of amyloid depositions in the olfactory bulb layers. The aim of this research is to clarify the amyloid-histochemical and histological characteristics of olfactory bulbs in the rats of albino male in relation with aging.

Material and Methods

Experimental Animals:

Male albino rats aged between (3, 6, 12) months were obtained from the Animal House, Collage of Science, University of Babylon. The rats were housed in wire mess cages under standard condition with 12 hrs. Light and 12 hrs. dark cycle throughout the entire experimental period. Food and tap water supplied with libitum.

In the current research, thirty male albino rats will be split into three age groups (10 animals each), they are: Group I: considered as a control group, including adult animals aged 3 months. Group II: include animals aged 6 months. Group III: include animals aged 12 months.

Histological study:

The specimens of olfactory bulbs were taken from the brain of the albino rats, the samples were immersed in the solution of Bouins for two days. Tissue was dehydrated in graded ethanol and embedded in paraffin. 7μm horizontal sections of paraffin blocks were cut on a revolving microtome and installed on glass slides then, the following staining processes were completed [1].

Histochemical Study:

Selected sections have also been processed for histochemical amyloid protein demonstration. Sections have been deparaffinized through xylene and alcohol into

tap water. Thereafter, slides are immersed in alkaline sodium chloride. Twenty minutes later, they were immersed in alkaline solution of Congo red and then marked with alcoholic potassium. Thereafter, slides are countered with alum hematoxylin and dehydrated by xylene and ethanol [6].

Results

Histological Study:

In light microscopy, the main olfactory bulbs of the adult rat consisted of six concentrated layers: 1-The olfactory nerve layer (ONL), (fig.1A, and 1B). 2- The glomerular layer (GML). There were observation of periglomerular cell (Pgm) around the glomeruli (fig.1D, 1E). 3- The layer of the external plexiform layer (EPL), consists of fine nerve fibers and few granule cells, (fig.1A, 1B). 4- A layer of mitral cells (MCL) contained stomata of mitral cells in single row (fig.1A, 1B). They had an ovoid nuclei with single nucleolus deeply stained. Their cytoplasm include darkly stained basophilic granules (fig. 1C, 1E). 5-The inner plexiform layer (IPL), a thin layer of fine nerve fibers and some cells of granules (fig. 1A, 1B). 6- The layer of granule cells (GCL), contained a large amount of granule cells (fig. 1B).

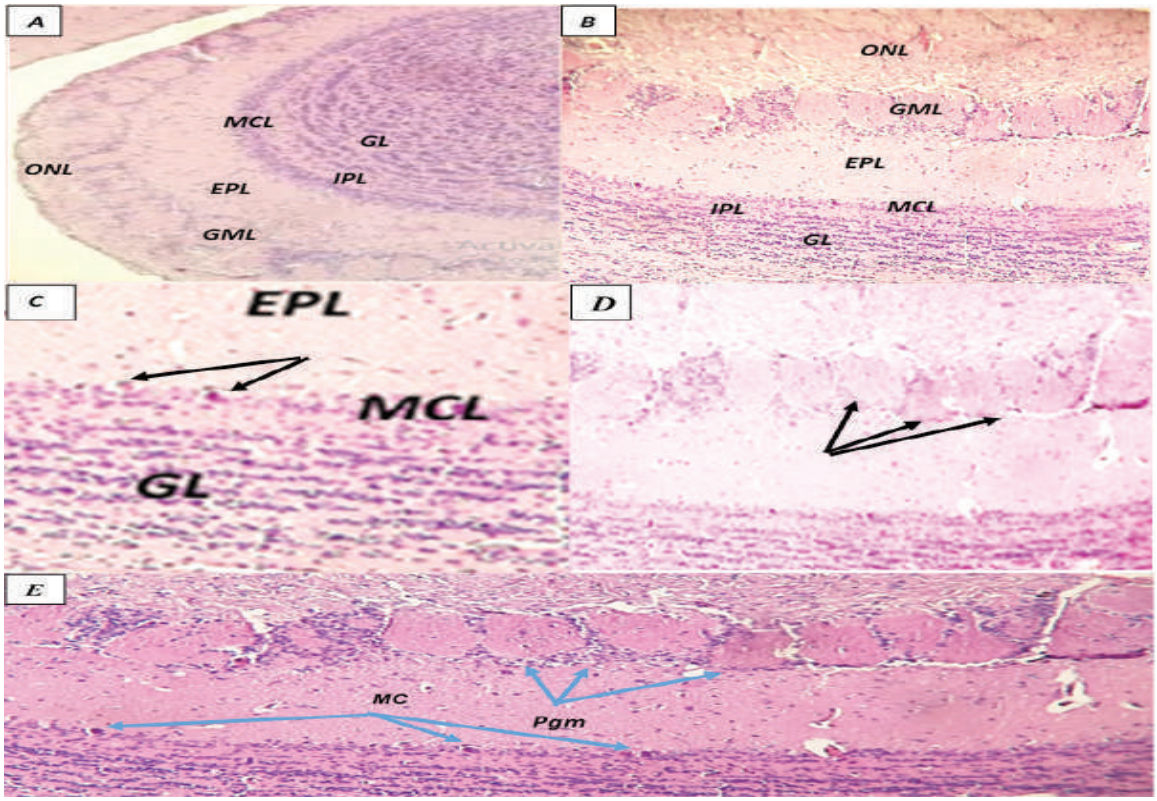
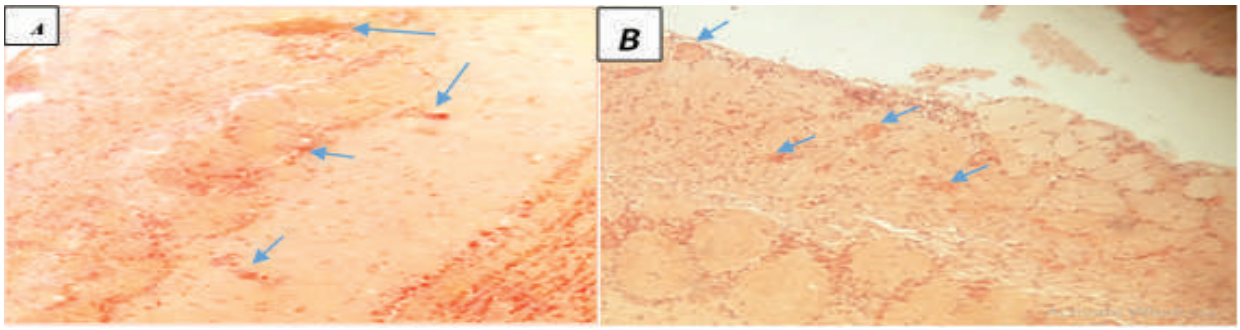


Fig.1: photomicrograph of histological section demonstrated to the concentric laminar organization of olfactory bulbs, H&E staining.4x, 10x, 40x.

The olfactory nerve fibers have been loosely segregated in group III rats. With a marked decrease in size, glomeruli were distorted in form (fig. 1). The EPL included a number of misplaced mitral cells' soma (fig.1B, 1E). Most mitral cells in MCL were decreased in size and generally present in a rounded, darkly stained nuclei with undefined nucleolus (fig.1C).

Histochemical Study:



Two forms of plaque of amyloid protein deposition were seen in the cortex of olfactory bulbs: diffuse plaques were shown in all layers of OBs of group III (fig. 2B). Compact amyloid plaques were found in the ONL, GML, MCL, and EPL layers of OBs of group III (fig. 2A, 2B). Amyloid angiophathy were detected in the different layers of OBs of group II and III, (fig.3).

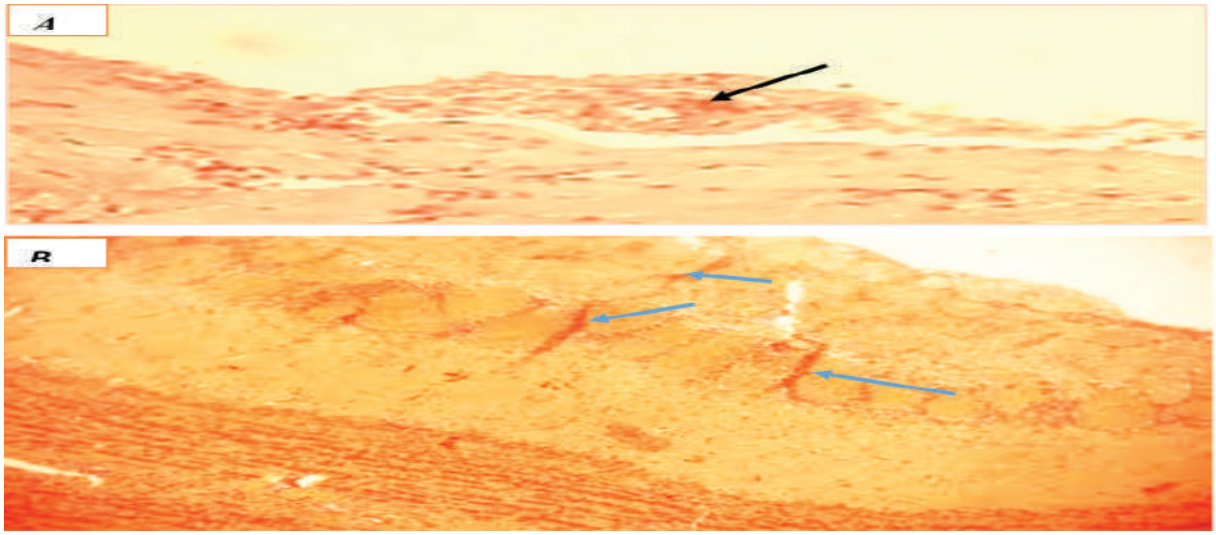


Fig.2: Photomicrograph of histological section showing plaque & diffuse staining for amyloid (AB, blue row) with Congo red –stain in olfactory tissue (2A, 2B), 10 x.

Fig.3 (A,B): Photomicrograph of histological section illustrated the Congo red stain highlighted the vascular amyloid depositions, note the staining of vessels walls (black & blue arrow), 40 x.

Discussion

In the pathway of the olfactory system, the olfactory bulbs are the essential components of olfactory system and relay station. We studied the histological architecture of the olfactory bulbs, according to the outcomes of our research, surveys were done to the structure of each layer of bulbs. The histological characteristics features of OBs constituent with the prior studies by Golgi [(10)]. Many periglomerular (Pgm) cells were found in the layer of glomeruli of olfactory bulbs. Two groups of neuronal cells are dispersed in the layer of mitral cells of olfactory bulbs, the cells with large cell bodies and cells with small cell bodies. The cells that characterized by large cell bodies seem to be correspond to the mitral cells as output neuronal cells, and the cells with small cell bodies may related to the tufted cells, that are well known as other kind of neurons of OBs of mammals, or the interneurons in the layer of mitral cells, but the correspondence could not be determined [(5)].

According to the finding of the present work, the histological characters among the bulbs of olfactory of albino rat are comparable in all groups (I, II, III), but the density of cells in each group was different. The complexity of the olfactory bulbs layers organization proportional to the olfactory bulbs information-processing capacity and represents the degree of

olfactory bulb development. The results of our outcomes showed that the morphology and amount of mitral cells in the group I &III were differed [18].

The results of this research are complementary and consistent with prior human OBs tissue reports that prevalent layers (NFL, GML, EPL, MCL, IPL, and GL) constitute the construction of all layers in the olfactory bulbs of albino rats. There was no distinction in olfactory bulb composition between group (I, II, III), except for cell density variations. In group I, in each layer, the density of cells was higher than in group II and III. No mechanism exited to explain this decline. The amount of granulated cells and mitral cells decreased but increased in size [11; 9]. The mitral cells considered as the largest neuronal cells in the olfactory bulbs, have primary and secondary dendrites, these processes oriented vertically or parallel to their soma, glial cells formed these dendrites of mitral cells. Axons of mitral cells converge in bundles of fibers and pass through the layer of granular cells [11].

[10] Demonstrated a "substantial layer-specific loose" of synapses ultra-structurally: synaptic density is decreased in the layer of glomerular cells but not the internal plexiform layer, leading to unbalance in circuitry of OBs. Our findings showed a decrease in GML and MCL density, consistent with [10] findings, these results indicate that decreased afferent synaptic input and local modulatory circuit synapses in OB glomeruli may contribute to particular age related changes in olfactory function.

In the current research, we showed diffusing and plaques of compact amyloid nuclei in the cortex of OBs by using modified Congo red staining. We also showed the enhanced amount of plaques and reduced neuronal populations in group III OBs compared to the olfactory cortex of group I& II, that showed typical dark orange colored patches under light microscope. It is well known that there are plenty of extracellular plaques of amyloid β peptide (*A*β) in the pathological marks of AD in the brain [16]. Amyloid has been discovered to be more localized in the neuronal processes in the current research, this finding was in agreement with the outcomes of other researchers who noted that in elderly people with and without Alzheimer's disease ,abnormality amyloid accumulate as neuropil threads [11]. The method of neurodegeneration in AD may involve toxicity of β-amyloid (*A*β). *A*β's neurotoxicity can be shown in vitro and seems to involve oxidative stress [2].

Our finding demonstrated that the blood vessels within the cortex of olfactory bulbs also lades depositions of amyloid that constituents with the results of [14]. Congophilic amyloid in blood vessels is called cerebral amyloid antipathy (CAA) [17].

Many researchers have shown that the deposition of *A*β peptide in the cerebral cortex leads in neural and morphological degeneration, cognitive loss, and modulation of enzyme markers such as acetylcholine esterase and choline acyltransferase; all of which are well-known symptoms of AD [19; 4].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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The Intraperitoneal-Ketoprofen-Histopathological Induced Alterations in the Wistar Rat Kidneys

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Abstract

The current-focused study was carried out to generate a data profile about expected changes that could be induced in the kidneys of rats due to the use of intraperitoneal ketoprofen (KP). The study involved using 24 adult male Wistar rats sorted randomly into four groups (six animals per group). One group was treated as a control group, C, which was supplied with distilled water (DW) only. The remaining animals were presented as (50KP), (25KP), or (12.5KP) groups that received 50mg/kg.b.w., 25mg/kg.b.w., or 12.5mg/kg.b.w., respectively, of KP. The experiment was continued for 70 days, and the kidney tissue samples were collected from the scarified animals at the end of day 70 of that experiment. The kidney tissues of the 50KP animals revealed dilation of the tubules throughout the outer strip of the outer medulla with necrosis and sloughing of the epithelial cells of the proximal convoluted tubules (PCTs). However, the 25KP group suffered lesser grades of epithelial-cell sloughing of the PCTs with lower levels of dilation of tubules than those recorded in the 50KP group. On the other hand, the kidney tissues of the 12.5KP group showed only dilation in the PCTs. The present experimental data unveil the side effects generated by the use of the intraperitoneal KP in the examined rat kidneys which should be used as a launching set of information for better use or further study this drug and its side effects in human patients.

Keywords*: Ketoprofen, renal failure, side effects.*

Introduction

KP is an anti-inflammatory, anti-pyretic, analgesic derivative of propionic acid with non-steroidal anti-inflammatory (NSAID) therapeutic properties. KP reduces cyclooxygenase I and II enzyme activity, which contributes to a reduction in prostaglandin and thromboxan precursor production. As a consequence, the decline in prostaglandin production triggers the therapeutic potential of ibuprofen via the enhancement of prostaglandin synthase. The production of A2 thromboxane by thromboxane synthases, which prevent the accumulation of platelets, is also decreased with KP [(1)]. KP is the 11th most popular in Italy with 206 records for 2008, of which some 30 percent is extreme, based on a survey of random cases of adverse outcomes. A maximum of 13 percent of reported data were in the aspect of pediatric patients (under 18 years of age), even in the age group (under six years old) of off-label medication use. The 2012 evidence is not fully accessible; however, unofficial statistics show that KP was implicated in 560 adverse drug reactions, of which 31 percent were seriously affected [(2,3)].

KP records are that because of the unwise consumption of KP in the world countries despite multiple warnings about hepatotoxicity compared to other NSAIDs such as nimesulide. The KP's reported adverse events contain peripheral edema as a cardiovascular response, central responses such as drowsiness, headache, etc., dermatological responses including skin sensitiveness and photosensitization, blood based responses e.g. edema, platelet malfunction, etc., kidney, elevation of enzymes in the liver, gastrointestinal reactions for example vomiting, diarrhea, gastric ulcer and bleeding, etc. Additional studies have

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shown at least intermittent serum aminotransferase elevations ranging between one percent and two percent of the patients receiving KP. Even with product continuity, these can be overcome. For less than one percent of cases, reported elevations of aminotransferase (more than threefold increased) exist. It is very unusual that liver damage with KP jaundice is clinically evident and only few cases have been recorded. The latency to onset of the symptoms is a quick process, sometimes appear within a few days. The trends of enzyme changes differ between hepatocellular and cholestatic. In some instances, immunoallergic symptoms (low level fever, rash) are observed, but are not usually prevalent and self-antibody development is uncommon [4,5].

The performance of adverse KP effects on the kidney have not been comprehensively studied from the prospective of tissue damages. According to that, the current-focused study was carried out to generate a data profile about expected changes that could be induced in the kidneys of rats due to the use of intraperitoneal KP.

Materials and Methods

Animals and experimental design

The study involved using 24 adult male Wistar rats, weighed at 175-250gm with 10-14 weeks of age, sorted randomly into four groups (six animals per group) that lived in a housing under 22-25°C. One group was treated as a control group, C, which was supplied with distilled water (DW) only. The remaining animals were presented as (50KP), (25KP), or (12.5KP) groups that received 50mg/kg.b.w., 25mg/kg.b.w., or 12.5mg/kg.b.w., respectively, of KP. The experiment was continued for 70 days, and the kidney tissue samples were collected from the scarified animals at the end of day 70 of that experiment.

Kidney tissue preparation

Ten percent formalin was used for fixing the tissue specimens for two hours that was followed by a 30-minute-DW based removal step of the fixative. Then, a series of alcohol concentrations at (70% for 30mins, 90% for 60mins, and 100% as two cycles for 60mins per cycle) was used to dehydrate the tissues followed by a 50%:50% of alcohol to xylene immersing step for clearing the tissues for 60mins. After that, the tissues were immersed in a pure xylene for 90mins. Later, molten paraffin wax was used to impregnate the tissues followed by embedding and blocking out those tissues. Paraffin based sections at 4 to 5um were hematoxylin- and eosin-stained. The tissue sections were prepared according to [6–8].

Results

The kidney tissues of the 50KP animals revealed dilation of the tubules throughout the outer strip of the outer medulla with necrosis and sloughing of the epithelial cells of the PCTs, figure 1.

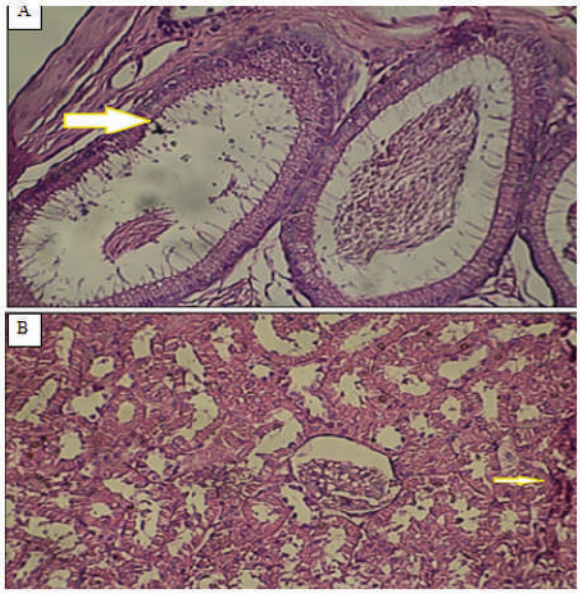


Figure 1: Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 50mg/kg.b.w. for 70 days. A. Dilation of the tubules throughout the outer strip of the outer medulla, (H&E) Stain.400X. B. Necrosis and sloughing of the epithelial cells of the proximal convoluted tubules. (H&E) Stain.200X.

However, the 25KP group suffered lesser grades of epithelial-cell sloughing of the PCTs with lower levels of dilation of tubules than those recorded in the 50KP group, figure 2.

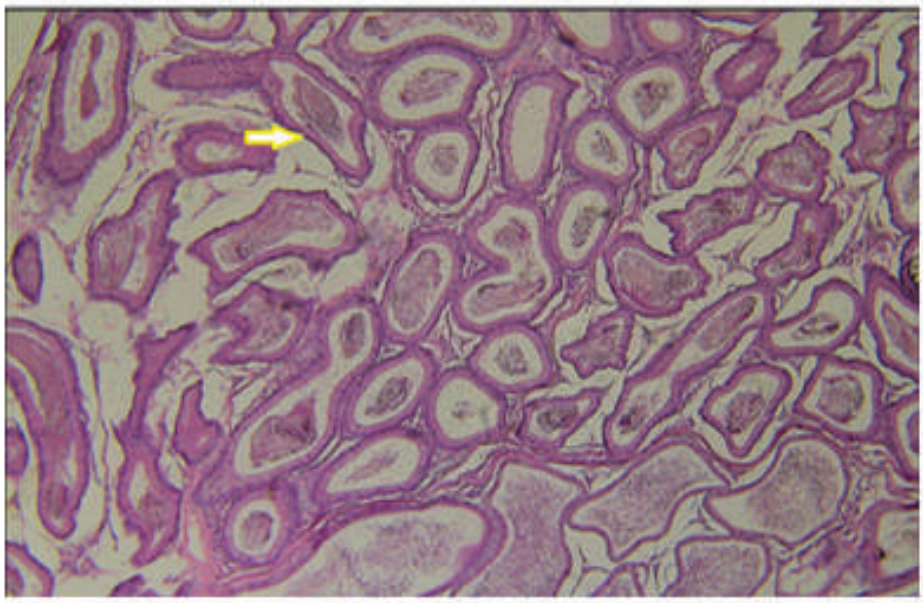


Figure 2: Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 25mg/kg.b.w. for 70 days. Changes are shown, here, as Lesser grades of epithelial-cell sloughing of the proximal convoluted tubules and lower levels of dilation of tubules than those recorded in the 50KP group. (H&E) Stain.200X.

On the other hand, the kidney tissues of the 12.5KP group showed only dilation in the PCTs, figure 3. The KP group kidney tissues were compared to each other and with the control group, figure 4.

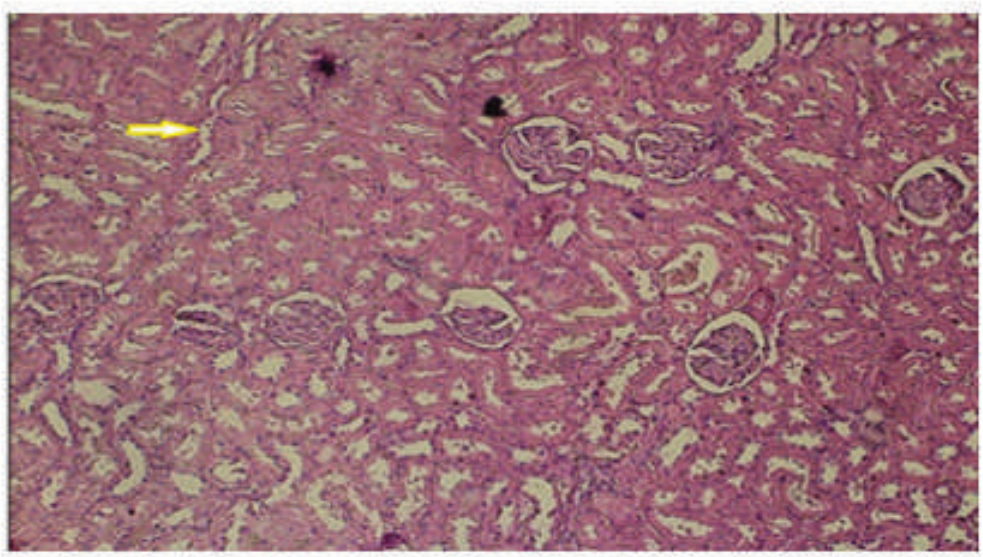


Figure 3: Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 12.5mg/kg.b.w. for 70 days. Only dilation in the proximal convoluted tubules is shown. (H&E) Stain.200X.

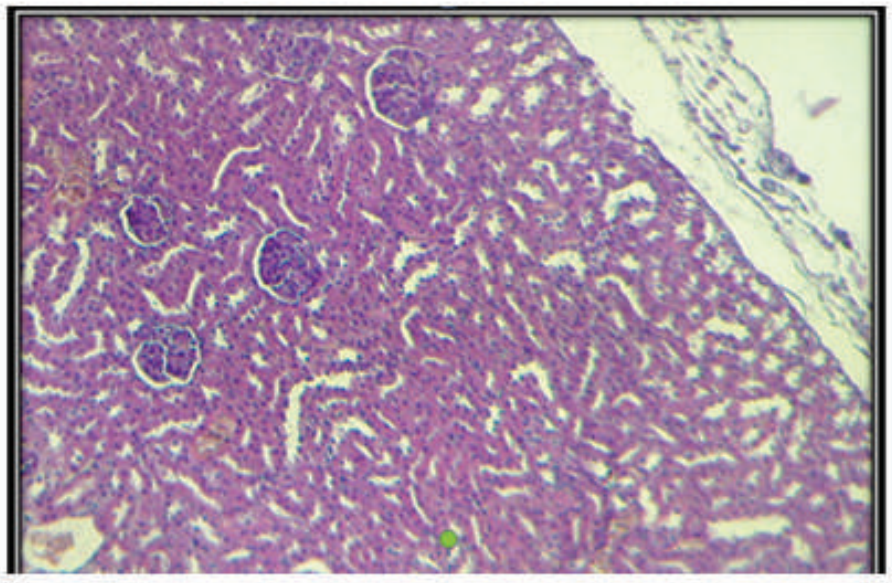


Figure 4: Normal cortical tubules and glomeruli of rat treated with distilled water within normal limits. (H&E) Stain.100X.

Discussion

Ketoprofen is a NSAID agent that is used for treating cases mediated by an inflammatory, fever, and/or pain process. The use of the drug as a therapeutic compound has been faced with a wide range of difficulties presented by the appearance of various adverse effects such as peripheral edema and platelet malfunction, drowsiness and headache, skin sensitiveness and photosensitization, and gastric ulcer and bleeding due to cardiovascular, central, dermatological, and gastrointestinal reactions, respectively, [9–13]. The adverse KP changes occurred in the kidney have not been fully sorted out. Therefore, the current work was conducted to characterized any histopathological alterations that could happen as responses to the use of KP in three concentrations.

The outcomes of the study unveiled that intraperitoneal ketoprofen treatment at 50mg/kg.b.w. for 70 days demonstrated dilation of the tubules throughout the outer strip of the outer medulla and necrosis and sloughing of the epithelial cells of the PCTs. Ingrasciotta *et al.*, [14] has found that using NSAID drugs such as oxicams, ketorolac, meloxicam, and piroxicam was positively correlated with the increased risk of developing chronic kidney disease (CKD). It has been suggested that utilizing ketorolac may induce CKD with a subclinical property due to acute renal damages [14]. This indicates an agreement with current findings that revealed the adverse effects encouraged by the use of the NSAID, KP, in the studied rats. The adverse effects of the KP use in humans can be inferred from a case report of a Turkish woman who received a topical treatment of KP as two times daily for five days who revealed increases in the levels of serum creatinine and urea which suggested an acute renal failure condition in this women [15]. The use of KP in pregnant women especially a short time before delivery has been found to increase the risk of renal dysfunction in the neonates [3]. The systemic-NSAID based kidney damages can be induced via acute interstitial nephritis due to a dose-independent allergic mechanism with cyclooxygenases 1- and 2-non-selective disruption causing an acute renal failure with reversed functions of the affected tissues [15].

However, those damages in the rat kidneys were correlatively decreased as the KP concentration was reduced. This was completely seen with groups 25KP and 12.5KP that showed lower grades of kidney tissue changes suggesting safer use of the KP with reduced concentrations. It has been recognized that using low doses of KP in children had led to the development of low rates of intense adverse effects with only nausea and vomiting [3].

The present experimental data unveil the side effects generated by the use of the intraperitoneal KP in the examined rat kidneys which should be used as a launching set of information for better use or further study this drug and its side effects in human patients.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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A New Method for Dental Cast Analysis

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Abstract

Objectives: This study aimed to develop a new method of dental cast analysis using angular measurements.

Materials and methods: One hundred study models belong to 100 Iraqi healthy adults with class I occlusion were assessed using Planmeca dimax software. Three angular measurements at the levels of maxillary canines, second premolar and second molars cusp tips with the tip of the angles at the midpoint between the two central incisors were measured. Gender difference was evaluated by unpaired t-test and the relation between the angles was assessed by Pearson's correlation coefficient test.

Results: The results revealed higher mean values of the measured parameters in males with direct, moderate to strong, significant correlation.

Conclusions: A new method was developed and could be used to assess the growth and treatment changes in the future researches.

Key words: *Dental cast, analysis, arch form*

Introduction

Many diagnostic aids can help orthodontists reach definitive diagnosis before treating orthodontic cases. Dental casts are considered as one of these aids [1]. Orthodontists utilized these models in determination dental arch length, width, perimeter, individual tooth width, space analysis and dental arch forms [2].

Initially, dental casts or study models are constructed from plaster of Paris and dental stones. These are subjected to breakage and degradation issues and required meticulous archiving and enormous storage space [3].

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At the mid 1990s, three-dimensional (3D) scanning technology was introduced, hence facilitated transforming the plaster study into digital format that stored in computer with easy transmission between professionals with accurate analysis of treated cases [4]. In comparison with plaster models, digital models present high degree of validity, accuracy and reliability in measurement with clinically acceptable differences between them [4,5].

The major issue with 3D scanner is the cost, [4] hence it may not be provided in all clinics, so photocopy of the study models can serve as a digital format for the plaster one to aid in preserving the space and performing accurate cast measurements, although the problem of 2D image still exists.

Every patient has his/her own dental arch form that should be maintained at the end of the orthodontic therapy to get stable results [6]. Numerous methods had been adopted to determine the dental arch forms using the liner analysis or the proportional measurements [7], arch width and arch length [8] arch length, width, and depth [9].

This study aimed to introduce a new method for dental cast analysis that depended on measuring three angles at the maxillary canine, second premolar and second molars levels.

Methods

Study design

This cross-sectional study was approved by the ethical and scientific committees in the College of Dentistry of Baghdad and Al-Anbar Universities and conducted in accordance with the principles of the Declaration of Helsinki.

Sample

After getting the approval for participation in this study, 250 students in the College of Dentistry/ Al-Anbar University were examined to ensure their fulfillment of study's inclusion criteria and only 100 students (40 males and 60 females) met these criteria which were:

1. Normal healthy individuals aged 18-25 years old with clinically symmetrical face.

2. Have full complement of permanent dentition (regardless the third molars) with bilateral class I molars and canines relationships and good alignment of the maxillary and the mandibular teeth with less than 3mm of spacing or crowding [10].

3. Normal overjet and overbite (2-4mm) with no crossbite.

4. No history of previous orthodontic, orthognathic interventions.

5. Normal teeth size, number and position with no crown fracture and absence of detectable massive interproximal or occlusal caries.

Methods

Firstly, the aims of the study were explained for the participants who seated comfortably on the dental chair. Personal information like name, age, history of facial trauma, orthodontic treatment and surgery were gathered and recorded in the case sheet.

Clinical examination both extra- and intra-orally was performed to check the students' fulfillment of the sample inclusion criteria. Then maxillary dental impression was taken using Alginate impression material (Hydrogum soft mint scent Alginate, Zhermack, Italy). The impression was checked for accuracy, washed with tap water and disinfected by Sodium hypochlorite 1% for ten minutes, then washed and poured with type IV dental stone (Elite® stone, Zhermack, Italy) to get a standardized dental cast. Using fine ended permanent marker, the following points were determined on the casts [10]:

1. Incisal point: The mid point between central incisors at the level of incisal edge

2. Canine point: The cusp tips of the maxillary canines.

3. Second premolar point: The buccal cusp tips of the maxillary 2nd premolars.

4. Second molar point: The mesio-buccal cusp tips of the maxillary 2nd molars.

After that, the casts were photographed using digital camera under standardized method. Image from occlusal surface of each maxillary cast was taken at about 20 cm distance from the base using professional camera. The images were imported to Planmeca dimax software to measure the following angles (Figure 1):

1. Superior inter-canine angle (SICA): The angle between right and left canine points with its head at incisal point opposite to inter-canine distance (the red one).

2. Superior inter-second premolar angle (SI2PA): The angle between right and left 2nd premolar points with its head at incisal point opposite to inter-second premolar distance (the blue one).

3. Superior inter- second molar angle (SI2MA): which is the angle between right and left 2nd molar point with its head at incisal point opposite to inter- second molar distance (the green one).

Reliability test

Fifteen images were selected randomly and the angles were re-measured after one month to determine the reliability of the measurements. Intra-class correlation coefficient test revealed excellent reliability (>0.90).

Statistical Analyses

Data were analyzed by SPSS program (version 25). Means and standard deviations were obtained and

gender difference was assessed using independent sample t-test. Pearson's correlation coefficient test was used to determine the relation among the measured angles in each gender. The level of probability was set at 0.05.

Table 1: Descriptive statistics and gender difference for the measured angles in degrees

Variables	Genders	Descriptive statistics			Comparison	
		N	Mean	S.D.	t-test	p-value
SICA	Males	40	128.803	6.174	1.697	0.093
	Females	60	126.830	5.353		
SI2PA	Males	40	95.123	4.225	2.211	0.029
	Females	60	93.125	4.554		
SI2MA	Males	40	75.073	2.979	3.453	0.001
	Females	60	72.767	3.451		

Table 2: Correlation among the variables in male group

Variables	Relation	SI2MA	SI2PA
SICA	R	0.600	0.811
	p-value	0.000	0.000
SI2PA	R	0.732	
	p-value	0.000	

Table 3: Correlation among the variables in female group

Variables	Relation	SI2MA	SI2PA
SICA	R	0.556	0.683
	p-value	0.000	0.000
SI2PA	R	0.810	
	p-value	0.000	

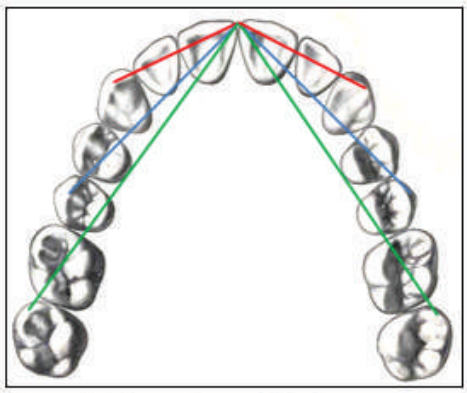


Figure 1: The angles used in the present study

Figure 1: The angles used in the present study

Results

Table 1 showed the means and standard deviations of the angles used in this study for both genders. Generally, these angles were higher statistically in males with significant difference in second premolar and molar angles.

Tables 2 and 3, related to the correlation between the measured angles in males and females respectively. The results revealed moderate to strong, direct and high significant correlations among these angles.

Discussion

Many methods had been adopted to determine the dental arch form. The most commonly used of Raberin et al. [11] who utilized six transverse and longitudinal dimensions to calculate ratios that determined the mandibular dental arch form.

In this study, a trial was made to introduce a simple method using angular measurements to obtain three angles at the level of the maxillary canines, second premolars and second molars with the head of these angles on the midpoints between the maxillary central incisors.

Previously, Ghaib and Odeh [12] selected 122 students aged 12-13 years with class I molar relation to study the maxillary arch form by measuring the inter-canine angle and inter-first molar angle with the midpoint of maxillary central incisors as a head for these angle, moreover; they measured the angle between lines connecting the maxillary central incisor with canine cusp tip and canine cusp tip and mesio-buccal cusp of maxillary first molars. Nearly the same measurements were used by Oliva et al. [13] on Italian adults.

In the current study, the mean values of all angles measured were higher in males than females (table 1). This comes in accordance with the findings of Ahmed and Ali [14] and Nahidh et al. [15] who found significant gender difference regarding the inter-canine and inter-second molar distance thus it is normal to get higher means for these angles in males. Moreover; it is obvious that the facial form of adult males and females is significantly different both in hard and soft tissue components [16]. Regarding dental arch form, a sexual dimorphism was anticipated too. This can be explained by the smaller and smoother bony ridge and alveolar process in addition to the average weakness of females' musculature that play vital role in facial breadth measurements, width and height of dental arch adding to that the longer period of growth males took that allowed later growth in males [10].

The means (±the standard deviations) of SICA in the present study (128.8º±6.17º for males and 126.83º±5.35º for females) are different from that of Oliva et al. (123.76º±7.81º for males and 127.65º±9.85º for females). This may be related to the difference in the ethnic group, sample size and selection with regard to the facial type. Oliva et al. [13] explained that difference by the more ovoid and flatter maxillary arch form of females.

Regarding the relation among the measured angles, Pearson's correlation coefficient test revealed direct, moderate to strong, significant relation in both genders.

Further studies are recommended to evaluate the effect of growth and treatment on the dental arch form; moreover, an Iraqi study is needed to determine whether the preformed arch wires are conformed to that measured from the normal population with different facial types.

Conclusions

New method of dental cast analysis has been introduced using simple three angular measurements. This method is simple quick and depends on angular measurements that not need magnification correction upon measurements.

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Analysis of Demographic, Socio-Economic and Biological Factors With the Incidence of Leprosy in Bombana Regency Southeast Sulawesi Province

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Abstract

Leprosy is an infectious disease caused by *Mycobacterium leprae*. This study aimed to know the risk factors of leprosy events in Bombana Regency in 2020. This study was an observational analytic study with case control study design. The sample of this study was all population of Bombana Regency in 2020. The case sample was the population diagnosed in the Leprosy Reporting Information System information reported by the Bombana Regency Health Office during 2017 to 2019, while the control sample was undiagnosed leprosy with *matching* based on age and gender. The total sample was 122 people (61 cases and 61 controls). Control sampling with *systematic random sampling*. Data analysed using by the STATA computer program with *odds ratio* test and multiple logistic regression. The results showed that BCG immunization (OR = 5.74, 95% CI = 1.95-16.89) and income (OR = 3.35, 95% CI = 1.34-8.38) were risk factors for occurrence leprosy, while population migration variables, education level, and humidity are not significant risk factors for leprosy. The study concluded that BCG immunization and income are risk factors for leprosy. The variable most at risk for leprosy is BCG immunization.

Keywords: *Leprosy, demographic factors, socioeconomic factors, biological factors*

Introduction

Leprosy is a chronic infectious disease caused by the bacteria *Mycobacterium leprae*[1].This disease attacks the skin, peripheral nerves, upper respiratory tract mucosa and eyes. Leprosy rarely causes death, but the poor management cases of leprosy can cause leprosy to be progressive, causing permanent damage to the skin, nerves, limbs, and eyes[2]

World Health Organization (WHO) reported the number of new leprosy patients by the end of 2018 in all regions is 208,619 cases (*case detection rate* (CDR) 2.74 per 100,000 population) and the number of patients still registered for treatment is 184,212 cases (prevalence 0.24 per 10,000 population), with a grade 2 disability rate of 1.6 per 1,000,000 population and the number of cases of children among new cases reaching 16,013 cases (0.79 per 100,000 children)[3]

Indonesia based on the number of sufferers was the third ranked in the world after India and Brazil. The prevalence of leprosy in 2019 is 0.75 per 10,000 population (target <1 per 10,000 population), the number of new leprosy cases is 6.04 per 100,000 population (target <5 per 100,000 population), level 2 disability is 3,35 per 1 million population (target <1 per 1 million population) and the proportion of leprosy in children in 2019 of 10.74% of total new cases (target <5% of total cases)[4]

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Indonesia has reached the national level of leprosy elimination (prevalence rate <1 per 10,000 population) in 2000. While at the provincial level, in 2019 there were 8 provinces that had not yet achieved leprosy elimination. Southeast Sulawesi Province in 2019 has reached the status of leprosy elimination[4].

The prevalence of leprosy cases in 2019 in Southeast Sulawesi Province was 0.93 per 10,000 population, the number of new leprosy case findings was 7.88 per 100,000 population, the level 2 disability rate was 2.22 per 1 million population and the proportion of leprosy cases in children was 2.82%[5]

Achievement of leprosy elimination in a province is not always directly proportional to the achievement in the regency/city in a province that has achieved leprosy elimination. Bombana Regency is one of the leprosy endemic districts and does not include districts that have achieved leprosy elimination in the Southeast Sulawesi Province.

Data from the Bombana Regency Health Office shows the prevalence of leprosy in 2019 of 1.03 per 10,000 population, the number of new leprosy case findings of 8.13 per 100,000 population with all multibacillary cases (MB), the proportion of leprosy cases in children by 7%, the proportion of cases level 2 disability of 7%[6]

The data above showed that leprosy is still a problem in Bombana Regency. A high proportion of *multibacillary* leprosy (MB) cases indicates a late detection in the community. In addition, the proportion of cases of leprosy in children is still above 5% which means there are hidden sources of transmission and high rates of transmission in the community[7] Allegedly there are leprosy patients who have not been detected so that the available data is still smaller than the actual number.

Bombana Regency has tried to control leprosy. However, in recent years the prevalence of leprosy is still quite high. Therefore, it should be important to know about the factors that contribute to the risk of leprosy in arranging strategies and interventions targeted at early detection and *treatment* of at-risk populations[8]. If the risk factor for leprosy is still unknown, the intervention program intervention given is not on target, so that the transmission of leprosy can continue. This study aims to determine the risk factors for leprosy in Bombana Regency in 2020.

Materials and Methods

This study was an observational analytic with case control design. The study took place in Bombana Regency, Southeast Sulawesi Province. The sample of this study was all population of Bombana Regency in 2020. The case sample was the population diagnosed in the Leprosy Reporting Information System information reported by the Bombana Regency Health Office during 2017 to 2019, while the control sample was undiagnosed leprosy with *matching* based on age and gender. The total sample was 122 people (61 cases and 61 controls). Control sampling with *systematic random sampling*. Data collected by using a questionnaire. In addition, humidity measurements using a *thermohygrometer*, lighting using a *luxmeter* and ventilation using a meter on the respondent's house. Data analysis performed was univariate with frequency distribution, bivariate with *odds ratio* (OR) and multivariate with multiple logistic regression.

Findings

Table 1 The risk of Variable of Leprosy

Variable	Case		Control		Total		p	OR 95%CI
	n	%	n	%	n	%		
Demography Factor								
Migration								

Cont... **Table 1 The risk of Variable of Leprosy**

High risk (migration)	20	32.8	12	19.7	32	26.2	0.099	1.991 0.8-5.0
Low Risk	41	67.2	49	80.3	90	73.8		
Social Economic Factor								
Income								
High Risk (<Minimum Average)	52	85.2	37	60.7	89	73.0	0.002	3.747 1.4-10.1
Low Risk (≥Minimum Average)	9	14.8	24	39.3	33	27.0		
Education								
High Risk (<Junior High School)	46	75.4	37	60.7	83	68.0	0.080	1.989 0.8-4.6
Low Risk (Senior High School/College/ University)	15	24.6	24	39.3	39	32.0		
Biological Factor								
BCG								
High Risk (never had BCG)	56	91.8	39	63.9	95	77.9	0.000	6.318 2.0-22.8
Low Risk (had BCG)	5	8.2	22	36.1	27	22.1		
Humidity								
High Risk	24	39.3	12	19.7	36	29.5	0.017	2.648 1.0-6.5
Low Risk	37	60.7	49	80.3	86	70.5		

Table 2 Multivariate Analysis Result of Leprosy

Variable	Odds Ratio (OR)	Std.error	z	p>|z|	95% Conf. Interval	
					LL	UL
Education	0.418	0.257	-1.41	0.158	0.125	1.400
Income	3.658	1.916	2.48	0.013	1.310	10.216
Migration	1.521	0.713	0.90	0.370	0.607	3.814
BCG	9.246	6.839	3.01	0.003	2.169	39.407
Humidity	1.750	0.809	1.21	0.225	0.707	4.330
Constant	0.088	0.057	-3.73	0.000	0.024	0.315

Table 1 and 2 shows that respondents who were at high risk (had migrated) had more leprosy (32.8%) compared to those without leprosy (19.7%). The *OR* results were 1.991 (95% CI = 0.809 – 5.017). This means that respondents who have migrated have a risk of leprosy 1,991 times greater than respondents who have not migrated.

Respondents with a high risk category (having income less than UMR) had more leprosy (85.2%) compared to those without leprosy (60.7%). *OR* test results obtained a value of 3.747 95% CI = 1.459-10.165). Thus, respondents with income less than UMR have a risk of suffering from leprosy 3.747 times bigger than respondents with income more than UMR.

Respondents with a high risk category that has education level below junior high school suffer more leprosy (75.4%) compared to those who do not suffer from leprosy (60.7%). The *OR* test results obtained values of 1.989 (95% CI = 0.855-4.681). This means that respondents with a low level of education (junior high school and below) are at risk of suffering from leprosy 1.989 times greater than respondents with a high level of education (high school/college).

Respondents with a high risk category (not immunized with BCG) had suffered more leprosy (91.8%) compared to those without leprosy (63.9%). *OR* test results, obtained a value of 6.318 (95% CI = 2.066-22.881. So that, the respondents who have never received BCG immunization have a risk of leprosy 6.318 times bigger than those who have received BCG immunization.

Respondents in the risk category (humidity of the respondent's house did not meet the requirements) had suffered more leprosy (39.3%) compared to those who did not have leprosy (19.7%). *OR* test results obtained 2,648 (95% CI = 1.096-6.573). So that, respondents who have a house humidity that does not qualify have a risk of suffering from leprosy 2.648 times greater than respondents who have a house humidity meet the requirements.

Based on LL dan UL value, we took conclusion that the risk factor for leprosy were income, BCG immunization, and humidity.

Table 3 Multivariate Analysis Result of Leprosy

Variable	Odds Ratio	Std.error	z	p>|z|	95% Conf. Interval	
					LL	UL
Income	3.355	1.567	2.59	0.010	1.343	8.383
BCG	5.749	3.161	3.18	0.001	1.956	16.891
Constant	0.099	0.061	-3.72	0.000	0.029	0.335

Table 3 shows that the results of statistical tests conducted by multiple logistic regression obtained variables that are risk factors for leprosy are BCG immunization and income ($p < 0.05$) (table 4). While the population migration variable, education level and humidity are not significant risk factors for leprosy ($p > 0.05$) (table 4). The variable most at risk for leprosy is BCG immunization (OR = 5.74)

Discussion

This study concludes that immunization is the most influential risk factor for leprosy in Bombana Regency. Although it is known as a vaccine against tuberculosis, BCG is also able to protect against leprosy, especially if it is given to leprosy household contacts[9]. This study is in line with research which states that BCG immunization status has a significant influence on new leprosy cases[10]. Other research also shows that the dominant risk factor for leprosy in Lamongan Regency is BCG immunization[11].WHO reports that BCG immunization is effective in preventing leprosy[12]. Other studies have found that BCG immunization in people without leprosy signs and symptoms is very important because it has an additional protective effect[13]

The protective effect of BCG immunization on uninfected people ranges from 10-80%[14]. The combination of drugs (chemoprophylaxis) and BCG immunization can eliminate *Mycobacterium leprae* in the host (by increasing TNF-α and IL12 levels, activating macrophages), and can reduce the risk of recurrence in patients who have finished treatment[14]

The results of this study show that income is a risk factor for leprosy in Bombana Regency. The results of this study are in line with research conducted by Monteiro et al in Brazil found that the incidence of leprosy was higher in poor groups who did not receive income support programs[15]. Other studies conducted by Grantz et al. in India found evidence of a relationship between poverty and the number of new leprosy cases at the regency level[16]. Oktaria et al. found that someone with unstable income was five times more at risk for leprosy[17]. Per capita income is closely related to food consumption expenditure. Lack of food consumption can cause inadequate nutrition. *Mycobacterium leprae* is an intracellular microorganism, therefore cell-mediated immune responses are very important in the defense of human hosts. Inadequate nutrition is associated with decreased immunity[18] It was also reported that many leprosy patients were diagnosed early in areas with good health services. Socioeconomic differences not only affect the access, but also the quality and utilization of health services[19]

Humidity measurements are considered to meet health requirements if the measurement results show a value of 40%-60%[20]. The results of this study indicate that humidity is a risk factor for leprosy in Bombana Regency. *M.leprae* bacteria live in dry nasal secretions at 36.7 ºC with 77.6% humidity. In addition, *M.leprae* can live outside the host and can last 7-9 days with 70.9% humidity. *Systematic review* conducted by de Souza VALOIS, CAMPOS, and IGNOTTI found that factors such as temperature and humidity can influence the dynamics of *M. leprae*[21]. A cohort study conducted by Lavania et al. found that positive PCR results were found for *M. leprae* in the highest nasal cavity during the rainy season when humidity was high[22]. Transmission of *M. leprae* is more frequent in humid conditions when nasal secretions are more numerous. The incidence of leprosy is usually high in hot and humid tropical and subtropical regions[23]. Conditions of humidity that do not meet the requirements can result in the survival of the *Mycobacterium leprae* bacteria in the air and increase the likelihood of transmission to families who are in a shared room.

Conclusions

This study concludes that BCG income and immunization are risk factors for leprosy. Meanwhile, population migration, education level and humidity are not significant risk factors for leprosy. The most risk factor for leprosy is BCG immunization. It is hoped that the government will make interventions in at-risk populations and can build cross-sectoral commitments to support lepers who are physically and economically incapacitated, for health workers to improve education on leprosy and the importance of complete basic immunization in the community, for the community is expected to ensure their children has received complete basic immunization.

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Ethical Clearance: Health Research Ethics Committee, Faculty of Public Health, Universitas Hasanuddin

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The Possible Nephroprotective Effect of Roflumilast Ameliorating Glycerol -Induced Myoglobinuric Acute Renal Injury in Rabbit Models

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Abstract

Glycerol encouraged kidney damage through originating an oxidative injury to renal tubular cells which further leads to tissue necrosis and inflammation. While, roflumilast N-oxide is an active metabolite of roflumilast that rising the cyclic adenosine-3′, 5′-monophosphate by inhibiting PDE4.

Thirty rabbits were divided into three groups, control group was received normal saline intra-muscularly and drived from water for eighteen hours before. Second group was received a single dose glycerol injected intra-muscularly after drived from water for eighteen hours .Third group was received roflumilast intraperitoneal daily for three days after received a single dose of glycerol .

All animals were sacrificed after 72 hour, and then blood collected by cardiac puncture for evaluation the changes in the levels of renal function tests and histopathological study of rabbit□s kidneys tissues are evaluated evaluation and scoring the changes of tested agents. The numerical data are expressed as means ± standard error means and analyzed by using SPSS version 20.

The results of the levels serum urea and creatinine found to be significantly reduced by roflumilast using ANOVA test. Besides, the histopathological changes showed ameliorative effects of roflumilast in compared to glycerol group. From this study, one could be concluded the protective effect of roflumilast in case of renal failure induction by glycerol.

Key words: *nephroprotective, roflumilast, glycerol*

Introduction

Acute kidney encompasses smaller changes in the renal function without obvious failure that could be resulted in significant worse consequences and the rate of disease in a population, moreover may be ended to death [(1).] The incidence rate of these emergent cases that is needed to hospitalization reached to 2-5%. Acute kidney impairment developed within one month postoperatively in rate 1 of 100 patients of general surgery cases and arises in up to near 70 % of intensive care unit patients [(2).] Nephrotoxicity induced by drug overdose or time dependent is a frequent problem in nephritic-medicine and the incidence of drug-accompanying acute kidney impairment may be as high as sixty percent[(3)] . The etiology of pathological condition associated with acute renal failure is septic shock at 47.5 % of total patients that needed hospitalization. The percent of death to total hospital mortality was 60.3%[(4)] . Other causative condition of acute renal function deterioration is a myoglobinuric case, which is uremic syndrome followed rhabdomyolysis and muscle destruction with

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releasing of intracellular elements such as myoglobin and other intracellular constituents into blood stream [5]. The myoglobin casts producing its effects by tubular obstruction or damaging of tubular epithelium with leakage of filtrate across it after reducing glomerular filtration rate [6]. Acute nephrotic injury given that an increase in serum creatinine often lags two and three days behind the onset of injury that may reflect the damage might be included two third parts of kidney were included [7] . Indication of roflumilast in case of chronic obstructive respiratory disease, it is effective in reducing the frequency of respiratory disease exacerbations[8]. The current study aimed to evaluate and investigate the potential nephroprotective effect of roflumilast against nephrotoxicity induced by glycerol in rabbits.

Experimental Design and Method

Rabbits (albino & domestic) of different sexes were weighing (1- 2 Kg) were acclimated for new animal house for ten days before any experimental procedures and fed with commercial pellets and allowed water *ad libitum*. They were reserved under dominated condition of temperature (23 ± 2 °C) and humidness (55 ± 15%). Also, lighting was systemized lighten to half day then to darken later. All methods performed in this current study were in accordance with regulatory content on the maintenance and practice of lab animals and reported to ethical committee approval of college of pharmacy. In the present study, after 72 hour of water deprivation, three groups of rabbits (n = 10 for each group involved control group was received intraperitoneal (5 ml) of normal saline (0.9 % of sodium chloride); group 2 (induction group) was only received given dose of diluted glycerol (50 %; 8 ml/ kg , i.m.), the total dose was divided equally, then administered in each hind limb muscle (18). Group 3 was given glycerol intramuscularly as single dose plus roflumilast that was given intraperitoneal after 60 minutes of the glycerol injections(1 mg/kg)[9] .Blood samples are collected by cardiac puncture to prepare serum, and then kidneys tissues were harvested for further biochemical and histopathologic analyses.

Measurement of serum urea concentration involved the usage of a commercially available kit (Biosystems) R [10]. The urea levels in the samples react with the kit's reagent components [11]. Measurement of serum creatinine concentrations were done by using a commercially available kit was used (Biosystems)R . The auto- analyzer apparatus is used to measure the absorbance of standard and samples after 30 and 90 second based on spectrophotometric measurement at 505nm[12, 13].

Histopathological assessment involved the macroscopic examination of the kidneys, then microscopic evaluation [14] [15]. A pathologist, who was unaware of the group assignments, performed a semiquantitative analysis of the kidney sections using a light microscope[16]. The statistical analyses were performed by using statistical package of social science (SPSS version 20). All numerical data are expressed as mean ± standard error of the mean (SEM). The data were evaluated by one-way analysis of variance [17] and Mann Whitney U test. *P* value is considered significant when its value equal or less than 0.05[18].

Results

Induction was done by glycerol-received group and it showed a significant elevation of urea level compared to control group. While, treatment group (roflumilast-treated group) displayed a significance reduction of urea level in comparison with induction group (shown in table 1). In addition, induction group exhibited there is significance elevations of serum creatinine level compared to control one. While the treatment group (roflumilast treated group) offered a significance reduction of creatinine level in comparison with induction group as shown in table (2(.

Sections of kidneys of control group revealed normal appearance of renal tubules in both cortex and medulla (figure 1).

Kidneys in the induction group showed moderate to severe lesions. Lesions were included deposition of hyaline material in the tubules of cortex and medulla, marked dilation of renal tubules, vacuolization of the renal tubular epithelium particularly in the medullary region, this group showed marked epithelial vacuolation to obtained score 4 in statistical significant level of $P \leq 0.01$ than control group (displayed in table 3). Some of the epithelial cells revealed statistically significant level of necrosis ($P \leq 0.05$) than both G1 and G3, this change was indicated by fragmentation of cell membrane and absence of nuclear staining to get score 2 as showed

in table 4. Some apoptotic cells were also observed indicated by fragmentation of nuclei with intact cell membrane to give score 1 but it was statistically non-significant ($P \geq 0.05$) in comparison with both G1 and G3 (table 5). Marked (score 2) interstitial lymphocytic infiltration was noted in this group, it was significant in comparison with G1 and G3 (table 2) but no significant glomerular changes had been detected in the level of light microscopic inspection (figures 2,3 and 4), (table 6). Complete absence of hyaline casts in the renal tubules, no necrosis and apoptosis in the renal tubular cells (figures 5 and 6), (tables 4 and 5)). As G1 and G2, there were no obvious glomerular changes at the level of light microscope illustrated table 6.

Table (1) Effect of roflumilast on level of serum creatinine against glycerol- induced acute renal failure

Group	Urea level (mg16)	Serum creatinine
Control	43.58	0.79
Glycerol	61.43*	1.25*
Roflumilast	31.80*#	1 *#

* means $p \leq 0.05$ when compared to control group ; # means $p \leq 0.05$ when compared to glycerol (induction) group

Table (2) represent the scores of interstitial inflammation in the groups of study

S	Groups	Score of interstitial inflammation	P Value
1	G1	00 ± 00 A score 0	P≤ 0.01
2	G2	40% ± 0.32 B score 2	
3	G3	10 % ± 0.11 A score 1	

Table (3) represent the scores of epithelial vacuolation in the groups of study

S	Groups	Score of epithelial cell vacuolation	P Value
1	G1	00 ± 00 A score 0	P≤ 0.01
2	G2	84% ± 0.2 B score 4	
3	G3	37% ± 00 C score 3	

Table (4) represent the scores of epithelial necrosis in the groups of study

S	Groups	Score of epithelial cell necrosis	P Value
1	G1	00 ± 00 A score 0	P ≤ 0.05
2	G2	25% ± 0.14 B score 2	
3	G3	00 ± 00 A score 0	

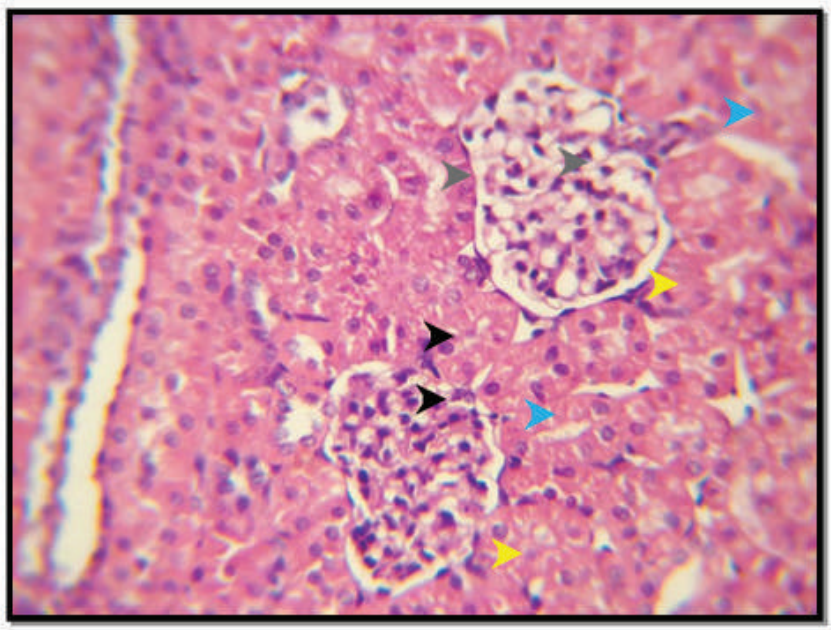
Table (5) represent the scores of epithelial apoptosis in the groups of study

S	Groups	Score of epithelial cell apoptosis	P Value
1	G1	00 ± 00 A score 0	P≥ 0.05
2	G2	13 % ± 00 B score 1	
3	G3	00 ± 00 A score 0	

Table (6) represents the scores of glomerular injury at the level of light microscope in the groups of study

S	Groups	Score of epithelial cell glomerular injury	P Value
1	G1	00 ± 00 score 0	P≥ 0.05
2	G2	00 ± 00 score 0	
3	G3	00 ± 00 score 0	

- These notes applied for all tables
- Values were expressed as a mean ± standard error.
- P values ≤ 0.01 was considered as significant.
- different capital letters refer to the significance among the groups.



Figure(1) Kidney of control group shows normal proximal renal tubules (white arrow head), distal tubules (black arrow head), glomerular tuft (blue arrow head) and bowman's space (yellow arrow head) in the renal cortex (3) H&E 500X

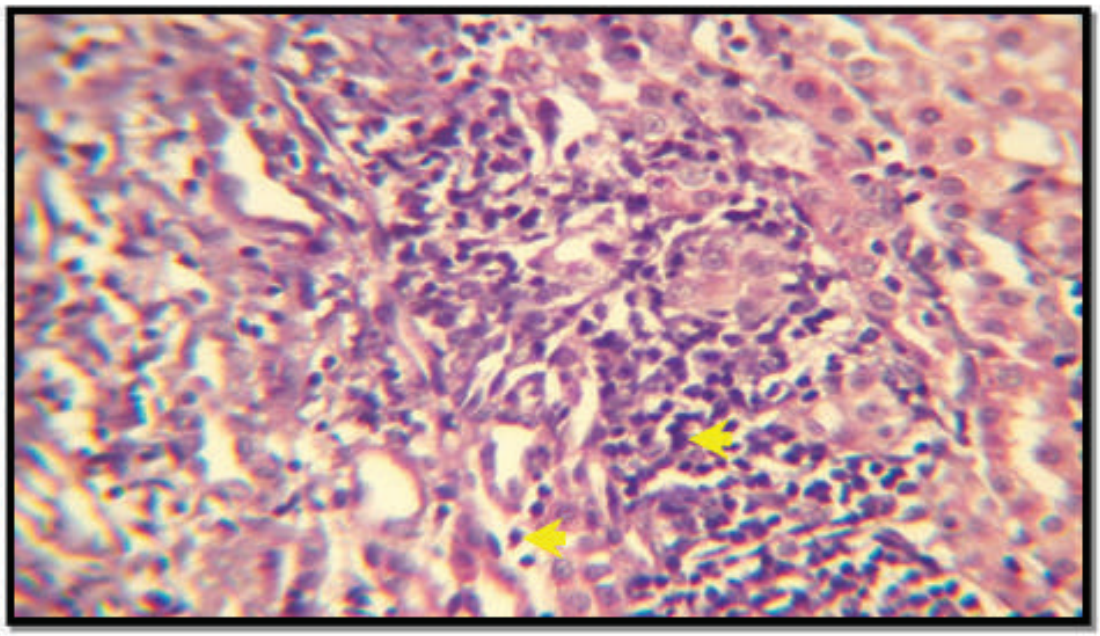
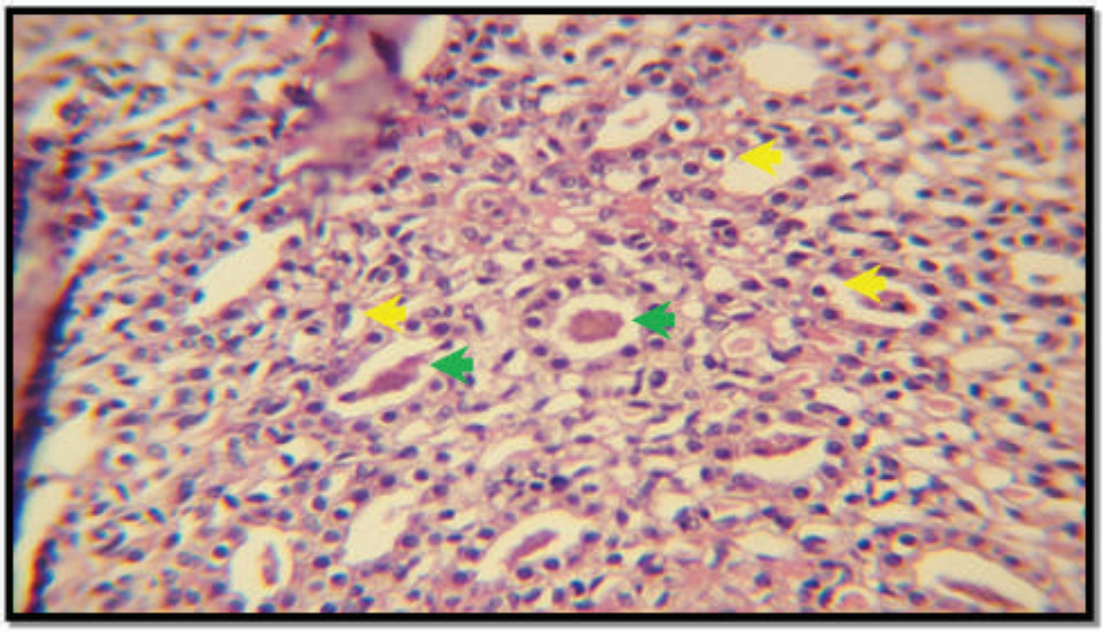
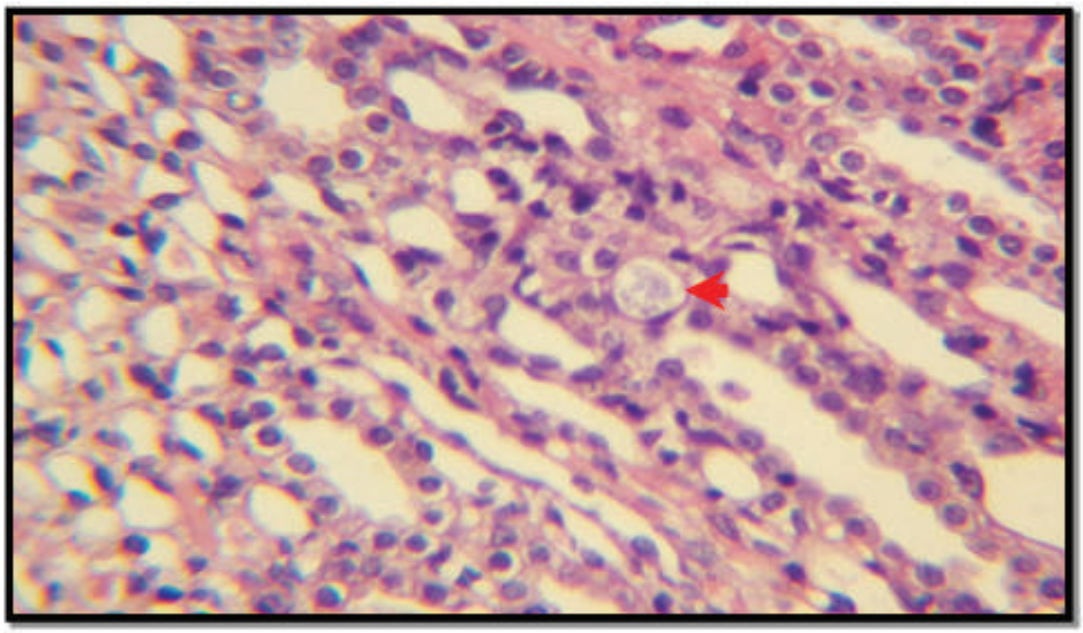


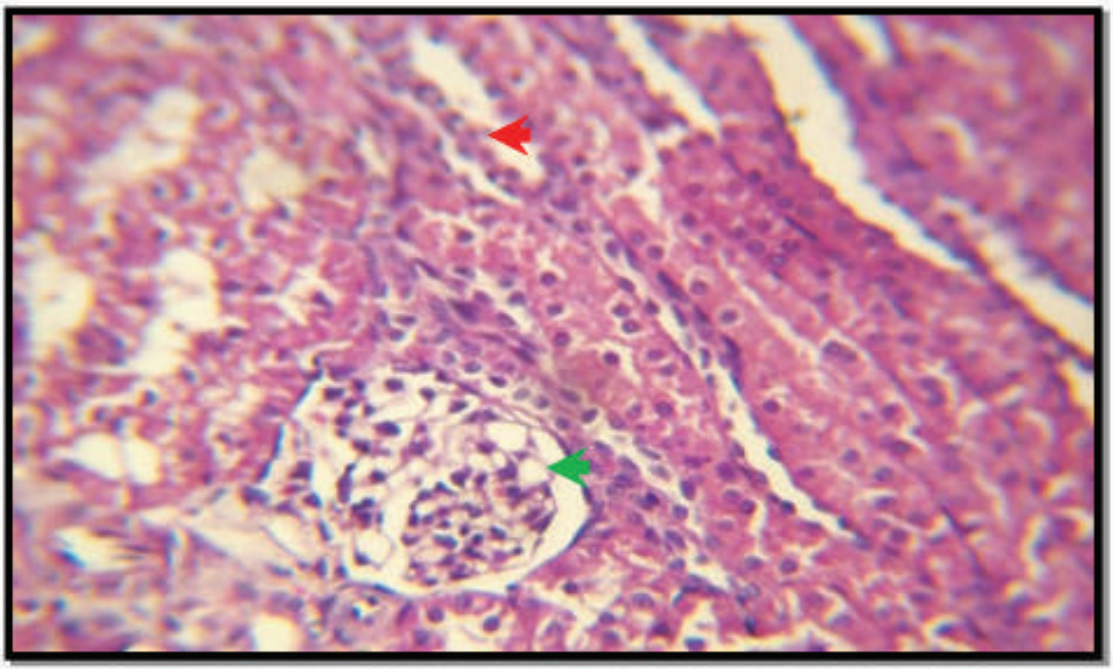
Figure (2) Kidney of Glycerol treated group shows area of interstitial inflammation (yellow arrow head) H&E 500X



Figure(3) Kidney of Glycerol treated group shows medullary renal tubules reveal marked vacuolation of epithelium (yellow arrow head) and hyaline casts in the tubular lumen (green arrow head) H&E 500X



Figure(4) Kidney of glycerol treated group shows medullary renal tubules with apoptotic epithelial cell (red arrow head) H&E 500X



Figure(5) Kidney of glycerol + roflumilast treated group shows normal renal tubules (red arrow head) with normal glomerulus (green arrow head) in the cortex H&E 500X

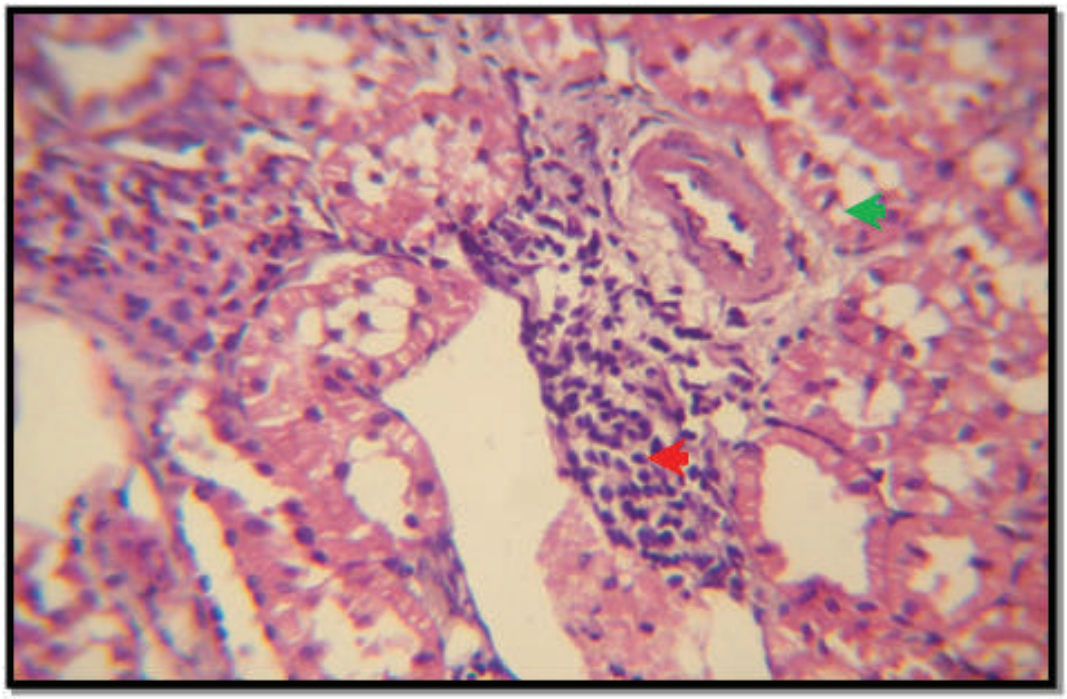


Figure (6) Kidney of glycerol + roflumilast treated group shows slightly vacuolated epithelial cells in renal tubules (red arrow head) with minimal infiltration of lymphocytes in the interstitial (green arrow head) in the cortex H&E 500X

Discussion

Glycerol-induced kidney impairment is cardinal of the most remarkably used model of experimental rhabdomyolysis inducing renal failure and is reflected as an experimental similarity of human myoglobinuric renal failure that resulted from transfusion problems or crush injury or random traffic accident[19] .

Myoglobin-induced tubular damage; or by renal actions of cytokines released[20]. Glycerol mediated nephrotoxicity by different mechanisms including a uncorrected renal ischemia of renal blood vessels , whereby blood flow ceases or arterial or arteriolar constriction[21] . This may be interpreted to increment of high proteins level in the blood leading to elevation of osmotic pressure and reduction of hydrostatic pressure , then both of them leading to reduced glomerular filtration pressure and reduced filtration process through glomerulus to Bowman's capsule , leading to elevation levels of both serum urea and serum creatinine[22] .The reduction of blood flow leading to persistence ischemia and free radical formation ended with glomerulopathy terminated with allow high molecular weight molecules such as protein to penetrate into tubules and elevate intratubular pressure that may equilibrate with oncotic pressure and diminished the glomerular filtration rate. In contrast , when intratubular oncotic pressure increased leading to loss of diffusion process so loss of kidney filtration function[17]. Glycerol treated group showed epithelial cells vacuolation and apoptotic cells besides the deposition of hyaline casts in the renal tubule[20]. Roflumilast is mainly effective through selective PDE 4 inhibitors , pleotropic effects including anti-inflammatory, anti-emphysema, and antibiotic[23] . Also, the beneficial effects were including pulmonary hypertension and hypersecretion of mucus [24]. The pharmacological actions behind nephroprotective effects attributed to inhibition of reactive molecules formation epithelial cells besides inhibition of neutrophils recruitment. Furthermore, it modulate vascular

remodeling due to endothelial cells and probably some inflammatory cells[25]. Roflumilast is the one of drugs that could be item for further researches for improving these beneficial effects through improving effects on levels of kidney function biomarkers and ameliorating histopathological change[26]. Inhibition of glomerular vasoconstriction one of the other two mechanisms participating in the development of acute renal failure is the decrease in blood flow in the glomerulus [27]. The roflumilast advantageous role of specific diesterase inhibition may be through blocking signaling pathways of inflammation and refreshing cell survival[28]. These results informed the advantageous role of PDE4 suppression in treating of nephrotoxicity, possibly through histopathological changes amelioration[28]. Also , The vasodilator effect may be considerable effect for roflumilast shared with another drug of PDEs inhibitor on reversing nephrotoxicity[29].

Conclusion

Roflumilast had a therapeutic effect against glycerol induced renal failure and ameliorating the renal impairment by rendering the level of urea, creatinine and histopathological tissue feautres.

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Evaluating the Effects of Education on the Understanding and Acceptance of Evidence-based Performance by Nurses

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Abstract

Introduction: Evidence-based performance is a problem-solving approach to provide more effective decision-making, avoid repetitive care, facilitate providingservices, and empower employees. Continuous training enables nurses to provide valuable services to patients. The aim of this study was to determine the effects of education on the understanding and acceptanceof evidence-based performance by nurses workingin the infectious and internal wards of Imam Reza Hospital of Kermanshah (Iran) in 2019.

Materials and Methods: In this experimentalstudy,participants were selected byavailable sampling and randomly divided into two groups (n=50 per group). The data was collected using 3 questionnaires: a standard questionnaire for understanding a standard evidence-based performance acceptance questionnaire (Robin and Parish, 2010), and a demographic questionnaire addressing age, gender, work experience, monthly income, education, ward, employment status, and acquaintance with evidence-based performance. Data analysis was performed in SPSS 19 software.

Results: The mean score of the understanding significantly increased from 78.24 at pre-education to88.04 at post-education in the intervention group while there was no significant alternation in the mean score of control group comparing pre- and post-education (85.38 vs. ,respectively). The mean score of acceptance also significantly increased in the intervention group comparingbefore (37.66) with after (37.66) education. In the control group, the mean acceptance scores were 35.02 and 34.98before and aftereducation, respectively.

Conclusion: Education can improve the level of understanding and acceptance of evidence-based performance by nurses; therefore, it is recommended to includecontinuous educational programs in hospitals and encourage all nurses to attend these courses.

Keywords: *Evidence-based Care, Nurses, Education, Hospital, Understanding, Performance-based acceptance*

Introduction

Nurses are the largest group of health care providers and play key roles in continuously promoting and maintaining patients' health [(1).] As using evidence is a prerequisite for day-to-day decision-making, nurses are expected to combine their technical skills and professional knowledge based on scientific evidence to identify patients' problems, and design, implement, and evaluate health care programs to address these problems [(2).]

Evidence-based care can minimize error rate and ensure making the best possible decision based

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on the available evidence [3]. Evidence-based care which is a problem-solving approach inproviding health careintegrates the best of research findingswith experiences on patients' care and clinical expertise, as well as patients'interests to ensure more effective decision-making, avoiding repetitive cares, facilitating health care, and empowering health care providers [4]. Nurses require using a set of research evidence to make appropriate clinical decisions based on existing research evidence, clinical practice, and patient performance [5].

Therefore, it is possible to use educational models of evidence-based performancedesigned for greater acceptance. The Rogers' Innovation Dissemination Model is one of the models used to explain how to accept new topics. In nursing, the Rogers' model has been used as a conceptual framework to explain the process of applying research results in nursing [6-8]. The model seeks to answer the question of how and under what conditions ideas (e.g. evidence-based performance) are accepted [9].

Rogers believes that the innovation dissemination model has all the necessary steps to accept a new event. He states that innovation is an idea, function, or object that is perceived as a new thing by individuals or admission units and gradually disseminates through communication channels among the members of a social system [10].

The evidence-based care approach is a relatively new concept in nursing care in Iran, which has been specifically emphasized on in the Education Transformation Package of the Ministry of Health. Nevertheless,educating this concept has received less attention. Therefore, this study aimed to assess the effects of educating a care-giving approach based on the Rogers' innovation dissemination model on nurses' understanding and acceptance of evidence-based performance.

Materials and Methods

The present experimental study was conducted to 2019 determine the effects of training on the understanding and accepting of evidence-based performance by nurses working in the Imam Reza educational and medical center of Kermanshah (a.s.). Available sampling was used in this study. The researcherreferredto the Human Resources Department ofthe hospital and looked into a list of nursing personnel of internal (1 and 2),infectiousand psychiatricwards.At last, 100 nurses willing to participate were selected and randomized into two groups of 50 subjects.

The data collection tools in this study included 3 questionnaires:

1. The standard comprehension questionnaire (Moore and Benbast, 1991) including25 items which have been graded on a five-point Likert scale (completely disagree to completely agree) and scored between 1 and 5. A score between 25 and 41 indicated a low level of understanding onthe features of evidence-based performance. A score between 41 and 83 showed anintermediate level of understanding, and finally, a score > 83 reflected a high level of understanding on evidence-based performance characteristics.

2. The standard evidence-based performance acceptance questionnaire (Robin and Parish, 2010). This tool has 10 items scored on a five-point Likert scale (completely disagree to completely agree). Scores between10-16, 16-33, and >33 indicated low, intermediate, and high levels of evidence-based performance acceptance, respectively.

3. The Demographicinformation questionnaire which included queriesaboutthe participants' age, gender, work experience, monthly income, education, hospital ward, employment status, and acquaintance with or a history of using evidence-based performance.

In this study, the intervention group received3 one-houreducational sessions on evidence-based care according to the Rogers' Innovation Dissemination Model. The control group did not receive any training program on this issue; however, they were assured that the research resultsand the training booklet and relevant files would have been provided to them at the end of the study. One week before the training, the questionnaires were completed by the participants of the two groups. Thisdatawas recorded as the pre-test in order to be compared with the levels of understanding and acceptance after the intervention.

In order to implement the educational intervention, the necessary coordination was made with nursing

managers and the officials of the in-service training units of hospital wards. The importance of the research was explained to the participants. The educational content included a 25-page booklet (the initial booklet was 80 pages, but according to nursing professors' suggestions, it was recapitulated into 25 pages) organized in three chaptersincluding topics on evidence-based care, Rogers' innovation dissemination model, and research databases and how to use them.

To more effectively present the educational content, the training program was performed using several methods:A) lectures and group discussions, b) messaging via social networks such as Telegram and WhatsApp, and c) educational booklets delivered to the nurses of the intervention group at the end of each session. In this study, the researcher himself lectured the content during 6 weeks (one 90 to 120-minute session per week). Three of the sessions were performed inthe amphitheater of Imam Reza Hospital, and three others were held in the educational classes of internal and infectious wards. At the end of each session, a training booklet on the presented topics was provided to the participates. Also, to facilitate the participants' access to the training contents, they were shared on channelsin social media networks (Telegram and WhatsApp). The timetable of the training sessions was scheduled so that the participants would not be on dutythe night before. Two weeks after the completion of the training course, the nurses' levels of understanding and acceptance of evidence-based care according to the Rodgers' innovation dissemination model were determined by filling the questionnaires in both groups. The results were then compared betweenthe groups and with pre-test data. Descriptive statistics were used to present the findings.

Ethical Considerations: Before the study, the objectives of the research were explained to the nurses, and informed consents were acquired from them. They were assured about the confidentiality of the information, and the fact that they would have been only used forthe research purposes. The participants were free to withdraw at any moment during the study.

Results

In this study, most of the participantsbelonged to the25-30and >40 years age groups and had bachelor's degrees. Most of themalso had less than 5 years or more than 10 years of work experience. Most of the participants were either officially hired or employed on contracts with an average monthly income between 3 and 3.9 million Tomeans. There was no significant difference comparing pre- and post-intervention comprehension scores in the control group (t = 1.938., P value = 0.167). In the intervention group, there was a significant difference between the comprehension scoresbefore and after the training (t = 10.279, P value = 0.002, Table 1).

There was no significant difference in the acceptance scoresin the control group before and after the training (t = 5.683., P value = 0.19). In the intervention group; however, there was a significant difference comparing the acceptance scoresbefore and after training (t = 15.914, P value = 0.002, Table 2).

Discussion

The aim of this study was to evaluate the effects oftraining of evidence-based careaccording tothe Rogers' Innovation Dissemination Model on boosting the understanding and acceptance of whichwas consistent with the results of the present study. Rodgers believes that people's perceptions onthe features of innovation directlyinfluence their acceptance. If people do not define innovation for themselves and do not relate it to their situation, they will not be able to pass through this stage. Anderson and Comrie argued that using the Rodgers' model can change perceptions on innovationand lead to successful modifications in performance [11].

Rodgers emphasized in his model on disseminating innovationsand improving their acceptance by boosting knowledge about them. Until people in a social system do not acquire adequate knowledge about newly suggested innovations, they cannot accept them. Rodgers also emphasizedon the understanding ofinnovations' characteristics to improvetheiracceptance and believed that people needed to understand these characteristics so that they can accept the innovation. The efforts of innovators should be focused on better introducing the innovation to boost people'sunderstandingon its characteristics including relative benefits, comparability, ease of learning, observability of outcomes, and applicability [12]. The results of the present study showed that the level of nurses' using of the Rodgers' care model

waslow-moderate. Accordingly, Varei et al. evaluating an evidence-based care based on Aiovamodel revealed a poor-intermediate knowledge in nurses [13]. Some studies have shown that the evidence-based care approach is perceived by many nurses as a new approach that they had never been educatedabout it. Other studies have shown that most nurses have not been trained regarding evidence-based performance, and 86.7% of them stated that they would need training to learn aboutthe principles of evidence-based performance approach [14]. It is a certainty that interests in learning and improving evidence-based performance skills rootin positive attitudes toward this approach and can be developed during daily activities [15]. The results of a study by Varei et al. showed that less than half of nurses had a positive attitude toward this approach, after training; however, more than 80% of themstated a positive attitude [16]. In this regard, the results of a study on Taiwanese nurses showed their positive attitudes regarding the application of research resultsin clinical practice [17].

Kurniawan and Petpichetchian argued that in clinical settings, it is not applicable to only rely on clinical experiences, pathophysiological logics, and process-based opinions. Physicians and nurses should learn to also use critical thinking skills and evidence-based methods for clinical decision-making and increasing quality and cost-effectiveness ofhealthcares [18]. Improving the quality of health care requires providing services based on scientific evidences, as well as employing new innovations,and preventive and effective caring approaches [19].

Similar findings were obtained in the study of Scott et al. who described that the understanding of innovation's features could explain 47% of acceptance variance. They argued that more and clear information should be provided about the benefits and supporting research evidencesof innovations [20]. Therefore, it is necessary to pay more attention to the characteristics of evidence-based performance, as a "new" subject in nursing education, to boostits acceptance. Matteo and Arjimondescribed that about 10 to 40 % of patients do not receive nursing care based on current scientific evidences, and also more than 20 %of patients do not receive the care they really need. Therefore, they suggested thateducating evidence-based performancecan be a strategy to reduce defects in providing effective health care's ([21]).

Conclusions

Forming evidence-based nursing care committees and specialized working groups can be useful to monitor and evaluate the implementation and assessment of this approach. From limitations of this study were the lack of a control group and a relatively low sample size. So, it is recommended to conduct similar two-group studies on larger sample sizes toestablish a strategy to promote the evidence-based nursing.

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Table 1. Comparison of the understanding score between the intervention and control groups before and aftertraining

Study groups	Mean	SD	N	T	P
Intervention/ before training	78.24	10.9	50	10.279	0.002
Intervention/after training	88.04	7.31	50		
Control/the initiation of study	85.38	11.35	50	1.938	0.167
Control/ the end of study	85.38	11.35	50		

Table 2: Comparison of the acceptance score in the intervention and control groups before and after training

Study groups	Mean	SD	N	T	P
Intervention/ before training	30.66	5.34	50	15.914	0.001
Intervention/after training	37.66	5.56	50		
Control/the initiation of study	35.2	6.01	50	5.683	0.19
Control/ the end of study	34.98	5.67	50		

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Low Carbohydrate High Protein Diet Reduces Levels of Tumour Necrosis Factor-α (TNF-α) in Rats (*Rattus norvegicus*)

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Abstract

The purpose of this study is to explain the effect of a low-carbohydrate-high-protein (LCHP) diet on reducing TNF-α levels. The study was carried out with true experimental post-test only control design, using 3-month-old females rats (*Rattus norvegicus*) with a bodyweight of 200-300 g, which were divided into three groups: Negative control (K-) (rats without treatment were fed with broiler standard feed), Positive control (K+) (PCOS-IR model rats were fed broiler standard feed), and Treatment (P) (PCOS-IR model mice were fed with LCHP diet). Serum taken from the rats' heart on day 49 was examined for TNF-α levels using Bioassay Germany ELISA kit. Statistical analysis was carried out using ANOVA and Post Hoc tests. ANOVA analysis of TNF-α resulted in a p value of 0.0017, indicating significant difference of TNF-α levels when LCHP diet was applied, followed by Post Hoc test found that the TNF-α level of the treatment group ($p = 0.006$) is lower than the positive control group ($p = 0.038$). One of the roles of a low-carbohydrate diet in the pathogenesis of PCOS-IR is to provide an inflammatory repair effect by increasing insulin sensitivity, improving insulin levels, and stimulating an increase in steroidogenesis synthesis. Increased breakdown of fatty acids is inhibited, so that it does not cause a build-up of fatty acids and does not induce an increase in proinflammatory cytokines against gonadotropins, increasing FSH secretion and effecting follicular development.

Keywords: *low-carbohydrate-high-protein diet (LCHP), polycystic ovary syndrome, and TNF-α levels*

Introduction

Infertility is a problem, especially in couples who are in their reproductive years and have not yet borne and given birth to a child which could become perilous if proper treatment is not administered. Many things can cause infertility, one of which being PCOS. 5%-10% incidence of infertility in the world are caused by PCOS. PCOS often occurs in women of reproductive age, around 4-18% in the world. Lifestyle changes that increases the risk of PCOS, high calorie consumption, and low physical activity which amplifies the risk of obesity[1,2,3].

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Low carbohydrate diets (low glycaemic index) are proven to reduce body weight with no increase in lipolysis and increase in lipogenesis, thus increasing insulin sensitivity through GLUT-4 which will reduce insulin levels, increase SHBG in the liver, suppress the production of androgens in the ovaries and increase the aromatisation of testosterone in theca cells to estrogen in ovarian granulosa cells, inciting folliculogenesis. The addition of protein intake as a counterweight for other nutrients in a diet, where increasing the amount of protein intake can stimulate insulin secretion, benefits anabolism which causes satiety, stimulates glucagon secretion from α cells in the pancreas, and stimulates gluconeogenesis. Protein uptake through protein breakdown called proteolysis, will suppress lipolysis activity and increase insulin sensitivity through improvements in inhibiting

the cytokine TNF-α[4,5,6,7,8].

We suspected that a LCHP diet can improve TNF-α levels by reducing proinflammatory cytokines TNF-α which will inhibit the inflammatory pathway that will increase insulin sensitivity, suppress insulin secretion, and suppress ovarian androgen production which will affect the growth and development of follicles in the ovary[9]. This study was conducted to determine whether LCHP diet can reduce TNF-α levels in PCOS-IR rats. The purpose of this study is to observe and compare the effects of two types diet, namely LCHP and without a diet, on TNF-α levels which certainly changes inflammatory factors that have an effect on reproductive hormones in PCOS which until now remains unexplainable.

Materials and Methods

Research Design

This study is a true experimental laboratory study with a post-test only control group design, consisting of two control groups and one treatment group. The ethical eligibility test for this study was carried out by the ethics committee at Faculty of Veterinary Medicine, Universitas Airlangga with certificate number 2.KE.062.04.2019

Experiment Unit

This study used healthy 2-3 months old female rats (*Rattus norvegicus*) with a bodyweight of 100-200 grams prepared by the Embryology laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga, which was chosen as a model of PCOS-IR. Female rats were acclimated for 2 weeks to adapt and fed broiler standard feed. Rats were divided into 3 (three) groups, namely the negative control group (K-), the positive control group (K+) and the treatment group (P), each group consisted of 6 rats. The K-group did not receive treatment (normal) injected with placebo intramuscularly for 28 days and given broiler standard feed. The K+ rat group were induced by testosterone propionate (TP) as much as 0.1 ml intramuscularly for 28 days to act as a PCOS-IR model, and were given broiler standard feed. The P group of PCOS-IR models and given the LCHP diet. The LCHP diet is given for 20 days on an *ad libitum* basis.

Materials and Instruments

The materials used in this this research includes mice, broiler standard feed, testosterone propionate ampoules, aquadest, low-carbohydrate-high-protein pellet (LCHP) (consisting of 40% low glycaemic index carbohydrate from corn starch as a substitute for carbohydrate, 30% protein from egg whites, and cow oil as a substitute for fat containing omega-3). The instruments used includes experimental animal cages (plastic tubs), sipper water bottles, digital scales, cloths, blenders, flour sieves, microscopes, insulin spuits, 3 ml spuit, 10 ml measuring cups, object glass, label, rotary evaporators, spectrophotometer, 500 ml beaker glass, and bioassay Germany brand TNF-α ELISA Kit no. E0177Mo.

Table 1. LCHP feed composition (RKTP).

Nutrient	Ingredient	%
Carbohydrate	Corn starch	40%
Protein	Egg whites	30%
Fat	Fish oil	30%

PCOS-IR Inducement

Determination of the PCOS-IR rats model was done by means of a vaginal swab by wetting the cotton soaked and then inserted into the vagina and gently rotated, then smeared on the glass object. It is then fixated with 70% alcohol for 5 minutes. Furthermore, the object glass is dripped with Giemsa colouring, left for 2-3 minutes, and then washed with water and dried. Afterwards, it is examined under a microscope to determine the stages of the estrus cycle, namely proestrus, estrus, metestrus and diestrus. Vaginal swab was done to determine what cycle is taking place before testosterone administration. If the experimental animal is not in the metestrus or diestrus phase (showing anovulation) then the experimental animal is excluded. Testosterone (Wonder brand, PT. Wonderindo Pharmatama, Jakarta, Indonesia) is a hormone that is used to induce PCOS-IR models in this study. This hormone can be given intramuscularly or subcutaneously on the left thigh at a dose of 1 mg/100 gr BW. The volume that was injected into each rat was 0.1 cc/100 gr BW, given once daily for 28 days until the PCOS-IR model was obtained. In Belooseky *et al*. (2004), research on rats injected with testosterone 0.1 mg per day for 14 days showed an ovarian appearance of large follicular cysts with thickening of the stroma

and accumulation of preantral follicles coated on day 14. On day 16, the increasing number of preantral and antral follicles diminished as do preovulation follicles and corpus luteum number. Continued injection of testosterone until day 21 causes a 50% lower insulin ratio and subsequently decreases drastically describing the state of insulin resistance[10].

Data Analysis

Data were analysed statistically using SPSS. Analysis of normality was done with Kolmogorov-Smirnov. If the value of $p > 0.05$, the analysis was continued by ANOVA to determine the statistical difference between groups, and post hoc comparison testing to see more clearly the differences between each group.

Results and Discussion

Data normality analysis is performed to determine whether the research data obtained follows or approaches the normal distribution, i.e. distribution of data in the shape of a bell (bell shape). Data was tested for normality using Kolmogorov Smirnov in the control group and the treatment group (Table 1). The table above shows that the data are normally distributed ($p > 0.05$) which were then followed by the ANOVA test.

Tabel 1. Kolmogorov smirnov normality analysis.

Variable	Sample	Mean	Minimum	Maximum	*p*
TNF-α levels	18	45.65	36.85	54.13	0.064*

p= significance, * normally distributed $p > 0.05$

TNF-α levels analysis

Data on TNF-α levels taken at the end of the treatment is presented in table 5.4. The TNF-α level was tested for normality in all groups and it resulted in a significance value of 0.064 which indicated the data to be normally distributed, followed by the ANOVA analysis there were significant differences ($p = 0.017$).

Table 2. ANOVA analysis of TNF-α levels.

Variable	Group	Mean	SD	95% Confidence level		*p*
				Lower limit	Upper limit	
TNF-α levels	K-	50.43	3.09	47.18	53.67	0.017
	K+	44.48	5.53	38.67	50.28	
	P	42.06	4.63	37.19	46.92	

p= significance, SD= Standard deviation

Based on the table above, the average TNF-α levels at the end of the 8th week of treatment in the control group had the highest value compared to the treatment group and showed significant differences between groups ($p = 0.017$). The process of ovulation, fertilization and implantation of the embryo is influenced by TNF-α, as an important factor in the pathogenesis of PCOS. Higher androgen level increases TNF-α release from mononuclear

cells (MNC)[11]. This study endeavoured to determine the effect of the LCHP diet on TNF-α levels in biochemical parameters with the aim of reducing the production of proinflammatory cytokines and the accumulation of free fatty acids (FFA) in adipose and muscle tissue so as to suppress TNF-α hypersecretion in laboratory animals.

Post hoc analysis

Significant differences in TNF-α and insulin levels were observed between the three groups (p <0.05). The analysis was continued by using the post hoc analysis of least significant difference (LSD) as a test between groups in the TNF-α, insulin and free testosterone levels which can be seen in the table below.

Table 3. Least significant difference (LSD) analysis of TNF-α levels.

Variable	Group	Sample	Mean	p
TNF-α levels	1	K+	5.94*	0.038
		P	8.36*	0.006
	2	K-	-5.94*	0.038
		P	2.42	0.370
	3	K-	-8.36*	0.006
		K+	-2.42	0.370

p=significance, *mean difference with p<0,05

The table above shows the results of post hoc analysis of TNF-α levels in the control and treatment groups and significant differences were observed. The most difference can be seen between the positive control group (K +) and the treatment group (P) with a significance value of 0.038 and 0.006. Giving LCHP diet had an effect on reducing TNF-α levels in the treatment group.

Effect of treatment on tumour necrosis factor-α (TNF-α) levels

This study used laboratory animals to facilitate the management and supervision of the LCHP diet, given the limitations of supervision when done on humans. According to Szczuko *et al*. (2018), increased synthesis of TNF-α by visceral adipose tissue in PCOS women contributes to the development of glucose intolerance and type II diabetes mellitus. TNF-α will inhibit FSH synthesis, deactivate follicular growth, and correlate with the synthesis of dehydroepiandrosterone (DHEA) [11].

Retardation of TNF-α proliferation on a low-carbohydrate diet. This is due to the fact that glucose metabolism provides the right amount of ATP, which is needed for TNF-α proliferation. Reduction of fat mass in foods with low glycaemic index suppresses excessive adipocyte accumulation by increasing glycogen, lipid, and protein degradation, which are important in ovarian hormone-dependent malignancies, which often accompanies overweight women due to increased estrogen levels (androgen conversion by aromatase) in adipose tissue. Estrogen is responsible for the production of higher free radicals, which are mutagenic agents. In addition, increased TNF-α correlates with the occurrence of hyperinsulinaemia, insulin resistance and hypertriglyceridemia, which is produced by excessive amounts of body fat in patients with diabetes mellitus[13,14].

High protein intake can induce glucose tolerance, decrease insulin resistance, reduce TNF-α, leptin and FFA levels accompanied by a reduction in adiposity, decreasing fat mass so that muscles shrink because of the formation of fat accumulation in muscles and adipose tissue. In addition, there was an absence of oxidative stress increase and ROS formation. Therefore, protein can provide beneficial effects for the treatment of insulin resistance and type 2 diabetes caused by high fructose diets[13].

Conclusion

In summary, low-carbohydrate-high protein (LCHP) diet significantly reduce TNF-α levels with the p value of 0.0017 (p <0.05) which shows there is a significant effect. It can be inferred that the LCHP diet suppresses the inflammatory pathway which in turn is likely to improve the insulin signaling pathway.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Low Carbohydrate – High Protein Diet Does Not Provide Positive Effect on P38 Mitogen-Activated Protein Kinase (p38MAPK) Expression

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Abstract

P38 mitogen-activated protein kinase (p38MAPK) is the activity of the p38MAPK pathway in muscle cells causing mitogen-activated disorders that are responsive to stress stimuli, such as cytokines, and involved in cell differentiation, and apoptosis. Characteristic of p38MAPK expression contributes to metabolic signal on the reduction and excessive production of ovarian androgen found among women with endocrine and metabolic disorders. We suspect that p38MAPK expression changes in ovarian granulosa cells. This research aims to observe changes in p38MAPK expression among PCOS-RI mice fed by a low-protein, high-carbohydrate (RKTP) diet. Testosterone propionate was given to induce the PCOS-IR model, 0.1 ml injection of for 28 days intramuscularly on 18 mice. Each mouse was examined for vaginal swabs and ovarian histology. At the end of treatment, the p38MAPK expression score kept increasing and had a significant negative effect with ANOVA test p value = 0.149 (p >0.05). The results of the treatment group posttest showed p38MAPK expression, and there was a decrease compared to the control group. These occurred because the RKTP diet has not been able to stimulate RE stress through the NFKβ pathway, so the NFKβ pathway increased AGEs receptors on granulosa cells that played a role in oocyte maturation as a response in the follicular development process on PCOS-IR model. Moreover, in this research RKTP diet had not been able to reduce p38MAPK expression in granulosa cells in the ovary.

Keywords: *insulin resistance, p38MAPK expression, polycystic ovary syndrome.*

Introduction

Polycystic ovary syndrome (PCOS) is a hormonal imbalance on the body and most are in the form of endocrine disorders characterized by hyperandrogenic, ovulation dysfunction and infertility[1]. Changes in ovarian morphology are clearly seen in PCOS-insulin resistance, indicating that hyperinsulinemia affects morphology and ovarian function[2]. Further, inflammation related to oxidative stress is due to an increase in insulin that affects proinflammation in serum and ovarian tissue[3]. ROS formation is caused by the increase in activation of oxidative stress pathways induced by glucose increase[4] and activating (nuclear factor-KB) NF-KB and p38 mitogen-activated protein kinase (p38MAPK)[5]. We suspect there is a decrease in p38MAPK expression in ovarian granulosa cells. This research was conducted to determine the effect of a low-protein diet (RKTP) that can reduce p38MAPK expression in ovarian granulosa cells among SOPK-RI model mice.

Many theories state that insulin resistance causes hyper insulin and hypertriglyceridemia due to accumulation of fat in muscles, an increase in adipose mass can cause pathological changes in adipokine hormone that regulates insulin sensitivity[6,7,8,9]. On

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the liver, adiponectin also increases insulin sensitivity, reduces the entry of FA, increases FA oxidation, and reduces hepatic glucose output. In muscles, adiponectin stimulates glucose use and FA oxidation possibly through cellular fuel sensors activation, AMP-activated protein kinases (AMPK), changes in proinflammatory cytokines resulting expression increase. Several potential mechanisms for metabolic effects of TNF-α have been described, including serine kinases activation such as JNK and p38MAPK that increases IRS-1 and IRS-2 serine phosphorylation, resulting in a poor substrate, in which kinase activates insulin receptors and increases IRS degradation[10].

Materials and Methods

Research Design

This research was a true laboratory experimental by posttest only control group design with two control groups and one treatment group. Further, an ethical eligibility test for this research was done by the ethics committee at the Faculty of Veterinary Medicine, Universitas Airlangga with a certificate number of 2.KE.062.04.2019. The research variable was p38MAPK expression, given a high-carbohydrate diet with a high protein composition of 40% carbohydrate, 30% protein and 30% fat in the positive control group and 20 days treatment among POCS insulin resistance mice injected by testosterone propionate for 28 days.

Experiment Unit

Rattus norvegicus was prepared by the Laboratory of Embryology, Faculty of Veterinary Medicine, Universitas Airlangga, and chosen as a model of insulin-resistant PCOS. Acclimated for 2 weeks to adapt and give a standard feed. By using healthy female mice, they were divided into 3 groups, 6 mice were induced with 0.1 ml of testosterone propionate intramuscularly for 28 days and mice normal group were injected with placebo. The treatment group was given RKTP diet food, and the normal group was given standard feed. pP38MAPK (mouse) antibodies were purchased from the Gamma Science of Santa Cruz sc 166182 brands with immunohistochemistry (IHC) examination.

Data Analysis

Data were statistically analyzed using SPSS. Normality analysis used Kolmogorov-Smirnov if the *p* value was >0.05, followed by ANOVA analysis to determine differences between groups and post hoc LSD was tested to discover clearly the differences among each group.

Results and Discussion

The treatment group's mean score was lower than the control group but the p38MAPK expression score was higher in this research. It was possible that RKTP diet by giving a low glycemic index was able to improve insulin sensitivity through glucose uptake GLUT-4, suppressing insulin level secretion. In addition, it suppressed proinflammatory cytokines production so that ROS formation did not occur through oxidative stress signaling pathways. Other pathways such as (Nuclear Factor-KB) NFKβ was different from activation pathway in an insulin resistance, where NFKβ pathway played a role in the secretion of mitogen proteins, namely mitogen-activated protein kinase (MAPK), especially p38 which affected cells on p38 mitogen-activated protein kinase (p38MAPK) to keep activated on granulosa cells through Advanced glycation end products (AGEs) receptors on proinflammatory expression causing increased androgen secretion in the ovary, which then caused impaired ovarian function and abnormal follicular development.

Table 1. Normality Analysis by Kolmogorov-Smirnov.

Variable	**Sample**	**Mean**	**Minimum**	**Maximum**	***p***
p38MAPK expression	18	2.30	0.80	3.20	0.154*

Description: *p* = significance, marked by * normal distribution *p* >0.05

Table 2. Anova Analysis of p38MAPK Expression.

Variable	Group	Mean	D	Confidence Interval by 95%		p
				Lower limit	Upper Limit	
p38MAPK expression	K-	2.65	0.39	2.23	3.06	0.149
	K+	2.28	0.47	1.78	2.78	
	P	1,98	0,73	1,21	2.75	

Note: *p* = significance, SD = standard deviation.

According to Huynh *et al*.[11], in addition to inflammation in SOPK with RI, the increase in cytokine activity and tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-18 inflammation were also found. The increased ROS formation might also be caused by the increase in activation of oxidative stress signaling pathways induced by increased glucose and free fatty acids (FFA)[6,8] which could activate NF-KB and protein kinase C (PKC) activities and p38 MAPK[5]. Moreover, inflammation related to oxidative stress was occurred due to an increase in serum levels of advanced glycation end products (AGEs) and the increased expression of proinflammatory receptor of AGEs (RAGEs) in serum or ovarian tissue[3].

Table 3. Post Hoc Analysis of LSD p38MAPK Expression.

Variable	Group	Sample	Mean	*p*
p38MAPK expression	K-	2	0.36	0.271
		3	0.66	0.055
	K+	1	-0.36	0.271
		3	0.30	0.365
	P	1	-0.66	0.055
		2	-0.30	0.365

Note: *p* = significance, marked by *mean difference by $p < 0.05$

The table above indicated post hoc analysis of p38MAPK expression comparison in the control and treatment groups without significant difference by *p* value >0.05. The provision of a low carbohydrate diet high in protein had no effect on the p38MAPK expression in the treatment group. Lower p38MAPK expression in the treatment group compared to control group was relevant with the research of Linghui *et al*.[12] stating that insulin resistance state increased proinflammatory cytokines and (endoplasmic reticulum stress) ER stress through activation of c-Jun N-terminal kinase (JNK) and I kappa B kinase (IKKβ) resulting in IRS phosphorylation of serine, inhibiting normal insulin signaling. IKKβ activation also caused phosphorylation

of IκB protein, an inhibitor of kappa β (NFκβ) nuclear factor transcription factors. Protein kinase signaling played an important role in growth and development through mitogen-activated insulin receptor pathways in ovarian granulosa cells[13]. The research indicated changes in glucose and insulin, given the importance of insulin and hyper insulin receptors for compensation in androgen induction.

p38MAPK expression in this research was the lowest in the treatment group, although p38MAPK expression in ovarian granulosa cells kept increasing. During mitogen secretion in granulosa cells functional interference occurred affecting p38MAPK expression, which could be possibly related to ER Stress. Consequently, it caused interference with the insulin receptor signal and its secretion which affected inadequate mitogen signal in granulosa cells. ER stress state increased the misfolded protein amount affecting ER's ability to fold and secrete newly synthesized proteins that were causing ER stress. ER stress-activated unfolded protein response (UPR) pathway and caused apoptosis through the inflammatory pathway of JNK and NFκβ[13].

Conclusion

In summary, low carbohydrate-high protein (RKTP) diet provision added by testosterone propionate injection has a significant negative effect on p38MAPK expression.

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Influence of Healthy and Productive Female Workers Movement (Gp2sp) on Work Health Behavior on Female Workers

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Abstract

Recently, female workers have to work in almost all sectors. In carrying out their work, female workers faced various risks that have the potential to damage their health. Female workers also have a burden of doing domestic work and responsible for the quality of children as the next generation and experience the biological cycle, such as menstruation, pregnancy, childbirth, and breastfeeding. Based on that, the government has made an effort to improve the health of female workers known as Healthy and Productive Female Workers Movement (GP2SP). The aims of this study is the influence of the GP2SP through flipcharts on work health behavior on female workers. This research was an experimental study through Quasi Experiment with a time series research design and used flipcharts as a media. The sampling technique used was proportionate stratified random sampling. This study involved female workers who met the inclusion criteria. The results showed that there was an influence of the Healthy and Productive Female Workers Movement program through flipcharts to the work health behavior of female workers. It is expected that the company and the workers have a commitment and participate GP2SP activities, so they can improve their health status and productivity

Keywords: *work health behavior, knowledge, attitude, action, Healthy and Productive Female Workers Movement*

Introduction

Recently, female workers have to work in almost all sectors. In carrying out their work, female workers faced various risks that have the potential to damage their health. Female workers also have a burden of doing domestic work and responsible for the quality of children as the next generation. Other than that, they experience menstruation, pregnancy, childbirth, and breastfeeding. These conditions require maintenance and protection of good health.

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Based on research conducted in US, more than 58% of US women in the workforce have more cases related to Carpal Tunnel Syndrome, Tendonitis, respiratory diseases, infectious diseases, anxiety, and work stress associated with heart disease. According to the result of research in several industries in Tangerang, Jakarta, and Depok, it showed that anemia in female workers showed as 24-42%. Iron deficiency anemia also causes workers to become easily sick and have accidents, so the absenteeism increases, and the possibility of pregnancy can give a risk during childbirth and causes Low Birth Weight[1]. Another problem is the education level of female workers which is still low.

Based on that, the government has made an effort to improve the health of female workers known as Healthy and Productive Female Workers Movement. In 2017, 4

of Minister in Indonesia the Minister of Health of the Republic of Indonesia, the Minister of Home Affairs of the Republic of Indonesia, the Minister of Manpower of the Republic of Indonesia, and the Minister of Women's Empowerment and Child Protection of the Republic of Indonesia have a joint agreement about Healthy and Productive Female Workers Movement (GP2SP)[2].

Based on the data compiled in the form of a coaching report by the South Sulawesi Provincial Health Office in 2019, PT. Bomar was dominated by female workers and was the target of GP2SP program in 2020. This governmental program included reproductive health service program for pregnant female workers, early detection of non-communicable diseases for female workers, increased breastfeeding during working time at work, the fulfillment of nutrition for pregnant and breastfeeding female workers, and control of the work environment. These programs also increased the work productivity.

In providing health education to behavioral change, methods and media are needed according to the abilities of health counselor and recipients. In this study, the researcher used a flipcharts. Flipcharts is one of the media that concise and has a comprehensive material component, so that it is easily understood by the respondents. Based on this, the researchers took the topics related to the influence of the Healthy and Productive Female Workers Movement through flipcharts on work health behavior on female workers.

Material and Method

This research was an experimental study through Quasi Experiment with a time series research design. The first step of this research was Pre-test. Next is providing interventions in the form of health promotion about GP2SP through a reciprocal sheet that was carried out in collaboration with the South Sulawesi Provincial Health Office. This intervention lasted for one day and had been previously discussed with the management to determine the time of research that was on Sunday, outside the working hours. The next step was Post-test to the respondent. After giving the first post-test, periodic measurements were carried out for every one week for 3 weeks and this periodic measurement was carried out on Friday after working hours.

This study involved female workers at PT. Bomar. The sampling technique used was proportionate stratified random sampling. Based on the calculation of the minimum sample and those who met the inclusion criteria including female workers were married, worked for ≥2 years, and willing to participate in this study, 42 respondents were chosen.

This is the method scheme:

Pre-test	Intervention	Post-test
O1	X	O2 O3 O4 O5

Keterangan :

O1 : Pre-test observation before intevention

X : The Program of GP2SP

O2 : Post-test observation after intervention (1st week)

O3 : Post-test observation after intervention (2nd week)

O4 : Post-test observation after intervention (3rd week)

O5 : Post-test observation after intervention (4th week)

Results

The Influence of Healthy and Productive Female Workers Movement on Health Behavior of Female Workers before and After Intervention that Repeatedly Assessed

1. Knowledge

Table 1: The Influence of Healthy Productive Women Workers Movement on The Health Behavior Knowlegde of Women Workers

Variable	Decriptive	Mauchyl's test of Sphericity		Test Of Within Subjects Effect		Multivariat Test
	Mean	Mauchy's W	P Value	Greenhouse Geisser (F)	P Value	Wilks Lambda (Partial Eta Square)
Knowledge O1 O2 O3 O4 O5	12.67 14.12 14.33 15.98 16.40	0.074	0.000	106.026	0.000	0.858

Table 2 shows that the general average score after the intervention (post-test= O_2,O_3,O_4,O_5) increased compared to before the intervention (pre-test). So, it could be considered that there was an influence of GP2SP on respondents' knowledge. The Wilks Lambda value indicated that the program had an effectiveness of 85.8% of workers' knowledge.

2. Attitude

Table 2: The Influence of Healthy Productive Women Workers Movement on Health Behavior Attitude of Women Workers

Variable	Descriptive	Mauchly's test of Sphericity		Test of Within Subjects Effect		Multivariat Test
	Mean	Mauchly's W	P Value	Greenhouse Geisser (F)	P Value	Wilks Lambda (Partial Eta Square)
Attitude O1 O2 O3 O4 O5	19.26 26.02 30.64 34.31 35.66	0.424	0.000	544.329	0.000	0.995

Table 3 shows that on the average, the attitude score increased after the intervention (post-test= O_2,O_3,O_4,O_5) compared to before the intervention (pre-test). So, it could be considered that there was an influence of GP2SP on respondents' attitude. The Wilks Lambda value indicated that the program had an effectiveness of 99.5% of the workers' attitudes.

3. Action

Table 3: The Influence of Healthy Productive Women Workers Movement on The Health Behavior Attitude of Women Workers

Variable	Descriptive	Mauchly's test of Sphericity		Test Of Within Subjects Effect		Multivariat Test
	Mean	Mauchy's W	P Value	Greenhouse Geisser (F)	P Value	Wilks Lambda (Partial Eta Square)
Action						
O1	21.81	0.206	0.000	393.046	0.000	0.949
O2	26.64					
O3	30.76					
O4	34.26					
O5	37.55					

Table 3 shows that the score after the intervention (post-test= O_2,O_3,O_4,O_5) increased compared to before the intervention (pre-test). So, it could be considered that there was an influence of GP2SP on respondents' action. The Wilks Lambda value indicated that the program had an effectiveness of 94.4% of the workers' action.

Discussion

The intervention provided were GP2SP program which consisted of reproductive health services and early detection of non-communicable diseases for women workers. Based on the results of measurement of the times series, there was changes before-after test. Thus, researchers assumed what is needed by the characteristics of the research object. Changes in subject scores from pre-test to post-test were vary greatly in quasi-experimental[3]

Risk management training intervention significantly affected the nurses' knowledge and attitudes[4]. In addition, a study showed that there was an increase in knowledge and skills in hospital staff after interventions of socialization and fire prevention simulations[5]. This research is also in accordance with a study conducted by Elnaour et al who stated that there is an increase in knowledge and practice in nurses and sanitation staff after being given an intervention[6]. Navidian et al also suggested that safety program interventions has a positive effect in increasing knowledge.[7]

Researchers assumed that the change in the value of knowledge scores after the intervention in this study was influenced by the media (flipcharts) because this media facilitated the work to explain and give information with pictures step by step. A set of flipcharts is a complete lesson or information. The advantages of flipcharts are clear images, interesting, and easy to understand.

This study is in line with Michael et al that by using media such as videos, flipcharts, and pamphlets, it can increase workers' knowledge and reduce work accident rates and occupational diseases after intervention[8]. The same thing is also in line with research conducted by Holtermann et al that the health promotion could increase the knowledge of employees who work at risky places[9]. Furthermore, research conducted by Eric and Naboth stated that the use of media can increase knowledge, where the knowledge score of female workers was higher than that male workers'[10]. Mayasari et al also showed that health promotion carried out through the provision of booklets and audio-visuals increased knowledge about preventing transmission of disease.[11]

Knowledge gained from the provision of information was to make the worker know thus indirectly affect her future behavior. Providing information that was made through the promotion of women's reproductive health-related health consists of information before, during and after pregnancy and after childbirth. Whereas, health promotion was related to the detection of non-communicable diseases enabling workers to be aware of themselves so that they are able to protect themselves and do the prevention[12]

Besides that, this study are consistent with the results of research conducted by Suryanto et al that there were significant differences in the knowledge and attitudes of small and medium enterprises group after a given safety behavior intervention[13]. Nursana et al found that there were significant differences in the knowledge, attitudes and skills of medical emergency workers before and after the intervention[14]. This is also supported by Darmin's research which stated that there were significant differences in workers' knowledge and actions after being given an OSH socialization intervention through audiovisual media[15]. Mayasari et al also stated that there were differences in the attitudes of respondents before and after receiving health promotion.[11]

Delivering information through media provides a cognitive basis for the formation of attitudes. If the messages are suggestive enough, it will provide an affective basis in the formation of attitude in responding information which needs critical attitude. Therefore, this attitude needs to be developed in the promotion of health in the workplace so that problem solving strategies, especially the dilemma problems faced by women related to their reproductive health and the health problems they and their families experience, can spur the emergence of critical thinking habits.

Knowledge has an important role in shaping the attitudes and behavior of people who determine the quality of society[16]. The formation of attitudes as a basis for carrying out a behavior or life activity that is not only affected by knowledge and experience, but also other factors that can determine the formation of attitudes, among others, culture such as habits, traditions and support systems from the family and the environment[13]

The GP2SP can form a positive attitude in maintaining women's health so that it can improve the health status of female workers which has a direct effect on increasing productivity. So it can be concluded that the increase in knowledge gained after participating GP2SP will be more meaningful not only by positive attitude accepting the results of it, but also supported by motivation to behave by applying the knowledge gained during the program.

This study is also in accordance with research conducted by Mazaheri and Heidernia that promotions before and after the intervention are different, where promotions can change the safe actions of steel industry workers[17]. Health promotion interventions also change and enhance the actions of workers in their work environment[18]. The changes in actions that occur on workers who are intervened are supported by the theory of S-O-R (Stimulus-Organism-Response), where the material objects of psychology and communication science are the same, such as humans whose souls include components: attitudes, opinions, behaviors, affection cognitions and conations. The basic assumption of this theory is: mass media has a direct, immediate, and direct effect on the communicant. This theory has the capacity as a stimulant (S) and produces a strong response (R)[14]. Also, this theory can be understood that the audiences have received information through the media. Substantially, the information received through the media given has a contribution in formulating messages to the audience.

The message is a source of strength in stimulating workers to carry out the desired action. As a result, the audience has indirectly carried out a learning process in capturing, understanding, and remembering the received message. This kind of condition unwittingly changes the audience's actions.

Conclusion

Based on the results of the study, it could be concluded that there was an influence of the Healthy and Productive Female Workers Movement program to the knowledge, attitudes, and actions of female workers. On the other hand, the results of this study also showed that the program had an effectiveness of knowledge of 85.8%, attitudes of 99.5%, and actions of 94.9%. Even so, it is expected that the company has a commitment in carrying out the program activities, providing facilities and infrastructure for its implementation. For all female

workers, they can participate in the program activities in undertaking and empowering themselves, families, and community workers.

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How Possible is it to Recover Semen DNA from Laundered Cotton and Polyester Fabrics?

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Abstract

Background: A key element of forensic genetics is the ability to remove DNA from semen stain. Using various laundering conditions to remove semen stain is a routinely faced forensic problem **Objective:** This study was conducted to evaluate the possibility of detection of semen DNA in different types of fabrics after being washed. **Method:** Semen samples were applied to cotton and polyester clothes. After drying, the clothes were submitted to washing in water at room temperature, in water at 50°C temperature and in detergent at room temperature. DNA extraction was done to estimate the quantity of extracted DNA by spectrophotometer. **Results:** Different DNA quantities still could be detected after washing. There was highly statistical significant decrease ($P \leq 0.001$) in the amount of extracted DNA in all group samples (after washing) compared to control group. Washing by detergent proved to have the most destructive effect on the DNA stains on clothes followed by washing by water at 50^0C while washing by water at room temperature showed the highest recovery of DNA in clothes. The amount of the DNA detected in cotton samples before washing was higher than the amount detected in polyester samples before washing.

Key words: *DNA, Semen Stain, Laundering Condition*

Introduction

Forensic analysis plays a crucial role in criminal investigations, especially those related to sexual assault cases[1].

Exchanging evidences due to contact between the perpetrator, victim and scene is imperative to happen in every crime against human as in sexual assaults[2].

Laundering the clothes-containing evidence is usually done by criminals to obscure their involvement in a crime. However, laundered clothes usually remain as a potential source of evidence[3]

Laundering additives include anything used for laundering purposes such as detergents and bleaches[4]

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The obtained genetic profiles within the semen traces from clothing samples might probably act as an evidence of sexual assault[5]

Material and Methods

This work was conducted in the laboratory of Biochemistry department at Kasr Alainy Cairo University.

Materials

· Fabric supports for semen stains (100% cotton and 100% polyester).

· Products for cleaning (from the market): Detergent ‘’Persil powder’’ **(Table 1).**

Table (1): Composition and manufacture of detergent.

Type	Composition	Manufacture
Persil® (Detergent) (biological)	Sodium perborate and sodium silicate	Henkel (Egypt ltd) Under license of Henkel (Germany)

Tap Water

Methods

1. Sample collection and preparation:

Ø Cotton and polyester fabrics were sourced from a local fabric store. Fabrics were new and unworn and had not been submitted for washing previously. Each fabric was cut into squares (5 ×5cm for each).

Ø Semen samples were collected in plastic tubes from healthy donors after taken their informed consent, the process of semen collection was done in andrology department at Kasr Alainy Cairo University.

Ø 3ml of semen was added to each piece of cloth. The placement of the semen deposit on the clothing was clearly marked with water-insoluble ink to allow it to be easily and specifically targeted. The marked area was 1cm^2

2. Laundering protocol:

Each of cotton and polyester semen-stained pieces of clothes were divided into four groups, every group was presented by15 samples, and were treated as follows:

1. First group: control samples were not submitted for washing.

2. Second group: samples were submitted for washing in water at room temperature (25-30°C).

3. Third group: samples were submitted for washing in water at 50°C temperature.

4. Fourth group: samples were submitted for washing in water and detergent (Detergent= 20 gm in 500 ml tap water) at room temperature (25-30°C).

Clothes' samples of second, third and fourth groups were laundered by hand washing for a duration of 10 min. During washing, the fabrics were well agitated by hand in order to remove the maximum amount of the stain.

After washing, the clothing items were allowed to dry at room temperature for 24 hours and then kept in dry paper envelope.

3- DNA extraction:

Each Fabric (5 ×5cm) was further cut (1 cm^2) at marked area and stored in a 1.5 ml test tube for extraction, Then cloth was soaked with 100 µl of buffer. The substrate was then placed into a spin basket and centrifuged for 15 minutes to obtain the sample[6,7]

4- Quantitation of extracted DNA:

After extraction, quantity of extracted DNA is estimated by Nanodrop® spectrophotometer. Spectrophotometer was used to measure the absorbance of isolated DNA at 260 nm, 280nm and 230nm. The Nanodrop® instrument can measure using only 0.5 -2 ng/µl up to 12,000ng/µl.



Figure (1): Show Nanodrop® spectrophotometer

Statistical Data Analysis

Data were coded and entered using the statistical package SPSS version 25. Data was summarized using mean and standard deviation for quantitative variables. Comparisons between groups were done using one-way analysis of variance (ANOVA) with multiple comparisons post hoc test. Comparisons between cotton and polyester were done using paired t test[8]

Findings

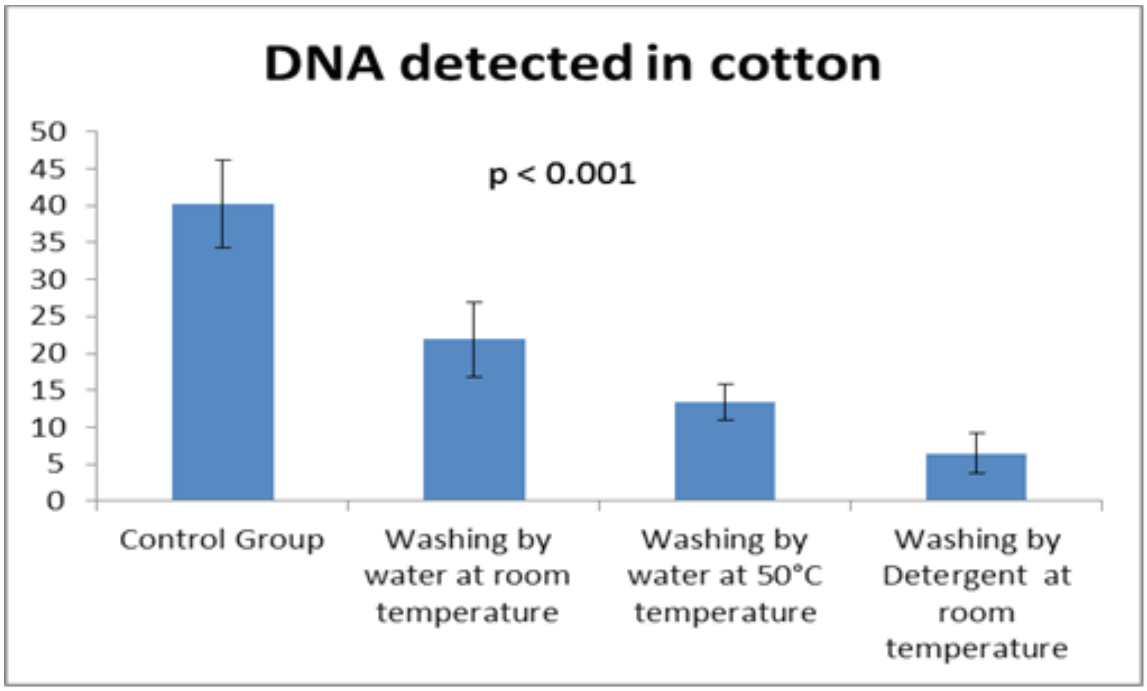


Figure (2): A comparison between the DNA quantities in all cotton samples.

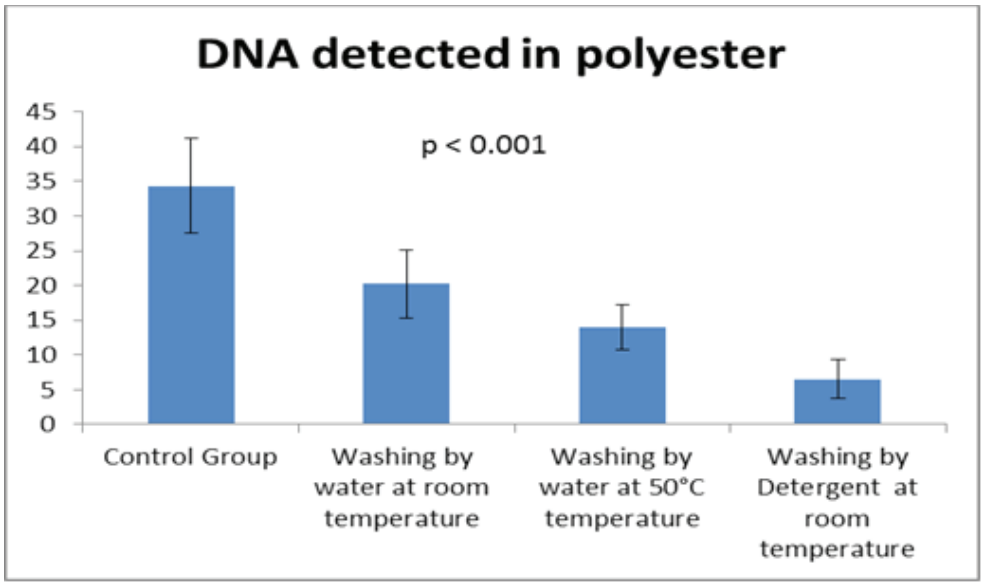


Figure (3): A comparison between the DNA quantities in all polyester samples.

It was observed that DNA still could be detected in both cotton and polyester fabrics after washing in water at room temperature, after washing in water at 50°C temperature, and after washing in detergent at room temperature as shown in **Figure(2,3)**.

There was highly statistical significant decrease ($P \leq 0.001$) in the amount of extracted DNA after washing in water at room temperature, in water at 50°C temperature and in detergent at room temperature but still could be detected compared to control group in both cotton and polyester samples as shown in Table(2).

There was highly statistical significant decrease ($P \leq 0.001$) in the amount of extracted DNA after washing in water at 50°C but it is still could be detected compared to the amount of the DNA extracted after washing in water at room temperature in both cotton and polyester samples as shown in Table(2).

The amount of the DNA extracted from cotton and polyester samples after washing in detergent at room temperature was very minute compared to the amount of the extracted DNA after washing in water at room temperature as shown in Table(2).

Washing in detergent at room temperature proved to have more degrading effect on DNA recovery than washing in water only at 50°C in both cotton and polyester samples as shown in Table(2).

Table (2): Comparsion between the quantity of the extracted DNA (ng/µl) within all studied groups in cotton and polyester fabrics.

	Control Group		Washing in water at room temperature		Washing in water at 50°C temperature		Washing in detergent		P value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
DNA detected in cotton (ng/µl)	40.31	5.93	21.88a	5.05	13.40ab	2.48	6.47abc	2.74	< 0.001
DNA detected in polyester (ng/µl)	34.29	6.81	20.25a	4.92	14.01ab	3.24	6.53abc	2.74	< 0.001

[a]Significant decrease compared to control group.

[b]Significant decrease compared to washing in water at room temperature group.

[c]Significant decrease compared to washing in water at 50°C temperature group.

The amount of the DNA detected in cotton samples in control group was statistically significantly higher than the amount detected in polyester samples in control group (before washing), while, there was no statistical significant difference in the amount of extracted DNA from cotton and polyester fabrics after washing in water at room temperature, after washing in water at 50°C temperature and after washing in detergent at room temperature as **shown in Table (3)**

Table (3): Comparison between cotton and polyester samples regarding the quantities of the extracted DNA within the studied groups.

	DNA detected in cotton (ng/µl)		DNA detected in polyester (ng/µl)		P value
	Mean	Standard Deviation	Mean	Standard Deviation	
Control Group	40.31	5.93	34.29	6.81	0.036
Washing in water at room temperature	21.88	5.05	20.25	4.92	0.339
Washing in water at 50ºC temperature	13.40	2.48	14.01	3.24	0.576
Washing in Detergent	6.47	2.74	6.53	2.74	0.942

Discussion

In the present study it was noticed that DNA is still could be detected after washing in water at room, at 50°C temperature and in detergent at room temperature. This was in accordance with an earlier study by Spector & Von Gemmingen[9] who examined the effect of washing on the persistence of sperms by laundering white cotton undershorts in a washing machine using both cold and warm washes with three different detergent types, This study was concluded that very small amount of semen could be detected after washing.

Furthermore Noël et al.,[10]who stated that after washing once, most semen stains could be detected with Prostatic specific antigen testing and strong fluorescence was showed with Alternate light source , after each wash the fluorescence intensity decreased, semen stains showed weak fluorescence but they could all still be detected even after being washed six times .Also Schlagetter&Glynn[11] noticed that less fluorescence with Alternate Light Source was detected in the washed items than that in the controls items, so the ability of Alternate Light Source to identify washed semen stains was decreased but still could be present.

Previous studies[12,13,14] observed that enough DNA was still could be detected after washing once in majority of cases.

In the present study, the DNA still could be detected after washing by water only at room temperature, Both Joshi et al.,[15] and Crowe et al.,[16]who observed that even after water immersion for up to 72 hours , there is strong positive results of acid phosphatase (AP).

The results of the present work showed significant decrease in DNA quantities extracted from samples washed in water at 50°C than control samples and those washed in water at room temperature. This was in accordance with Farmen et al.,[17] who found when laundering semen stains at 40°C, the amount of DNA was double the amount recovered from laundered semen stains at 60 °C.

Also Rebecchi et al.[18]who conducted another study on the effect of 21 days of temperature exposure and observed that the DNA stability could markedly affected by high temperatures up to 50°C so decrease its existence.

In addition Al- Kandari et al.,[19] who indicated that at high temperature semen samples appear to be resistant to destruction even after exposure to temperature over 28 days due to the sperm head's structure.

Similary Dissing[20]who studied the effect of the surrounding environment's temperature on DNA stability, observed that after eight months DNA could be amplified at 45°C and 100% humidity, but at 55°C and 100% humidity, it survived for one month only.

In the present work, biological detergent (Persil) proved to have the highest destructive effect, indicated by the marked decrease in the amount of extracted DNA. This coincided with Houston[3] who suggested that diluting laundry additives to the recommended amount by the manufacturer would be more effective at hindering the recovery of DNA than water only.

The present study was also in agreement with Brayley et al.,[21] who reported that there is diminished of DNA recovery when washed with biological detergent compared with non-biological detergent.

The present study showed that the amount of the DNA detected in cotton samples in control group was higher than the amount detected in polyester samples in control group.

The previous finding coincided with Dahi et al.,[22], and Thabet et al.,[23] who reported highest DNA detection from cotton fabric, followed by other fabrics. Also Schlagetter & Glynn[11] who noticed that polyester fabric may not have retained as much of the semen stains during the wash cycles and attributed that to the synthetic nature of polyester which has more uniformity, when it compared to the other fabrics, which were composed of natural fibers as cotton.

Also Nolan et al.,[24] reported that cotton and terry towel showed maximum spermatozoa retention compared to the other fabrics.

Moreover, Schlagetter & Glynn[11] stated that the stain on the wool showed less fluorescence and the semen stain on the cotton fluoresced strongly, while fluorescence in the denim was very difficult to detect. No fluorescence at all was showed on the polyester.

This is mainly attributed to that natural fibers are characterized by having overlapping cuticles which may retain the impacted cellular matters[25].While synthetic fibershave smooth outside which cannot keep cell retained[26].

Also due to the fabric structure as O–H groups of cotton are able of formation of strong hydrogen bonds with nucleic acid chains resulting in strong intermolecular attractions[27]

The previous findings did not coincide with Fiedler et al.,[28] who showed that there was no significant difference in the detectable traces of biological stains between the different types of fabrics.

Conclusion

It is concluded that DNA still could be detected in cotton and polyester after washing in water at room temperature, in water at 50°C temperature and in detergent group at room temperature. Washing in detergent proved to have the most destructive effect on the DNA stains on cloths followed by washing in water at 50^0C while washing in water at room temperature showed the highest recovery of DNA in cloth.

The amount of the DNA detected in cotton samples before washing was higher than the amount detected in polyester samples before washing. So, elimination of semen stain from fabrics is more difficult than generally believed.

Competing Interests: The authors declared that they have no competing interests

Ethics approval: The study work was conducted

after the approval of Ethical Committee, Faculty of medicine, Cairo University.

Consent for publication: Consent forms were given and signed by all subjects prior to participation

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Analysis of Manual Sharpening Method Towards the Cutting Edge Index of Sickle Scaler Instrument

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Abstract

Introduction: Cutting edge sharpness of the periodontal sickle scaler instrument will become blunt after several times of usage; thus the sharpening will be required to maintain the instrument's sharpness of the instrument. Based on the method used, manual sharpening using a whetstone can be divided into a moving instrument technique and moving stone technique. The purpose of this study was to analyze the cutting edge index of a sickle scaler instrument after being sharpened with two different techniques using an electron microscope / SEM. **Materials and Methods:** Thirty-two new sickle scaler instruments of type H15 / 33 (Oshung Inc.) were randomly selected and divided into four groups. The scaler instrument originated from the factory was becoming a positive control (Group I); the instrument that has been blunted was becoming a negative control (Group II). The other groups were the blunt sickle scaler that sharpened using the moving stone technique (Group III) and the moving instrument technique (Group IV). The cutting edge index of all instruments was then analyzed using SEM (JSM 6360LA, JEOL Ltd, Tokyo, Japan). The cutting edge index data from the photomicrograph was then processed using the Kruskal-Wallis and the Mann-Whitney statistical tests. **Result:** The results showed a significant difference in the sharpness level between the manual sharpening groups; this can be seen from the p-value = 0,000 (p-value < 0.05). The average cutting edge index value showed the best results in the moving instrument sharpening technique (2.10), followed by the moving stone sharpening technique (3.10). **Conclusion:** A periodic sharpening of sickle scaler instruments using a moving instrument technique provides a finer and sharper cutting edge level than the moving stone technique.

Keywords: *Manual sharpening, Cutting Edge Index, Sickle scaler instrument.*

Introduction

The use of sharp periodontal instruments will facilitate the process of removing calculus, increase the tactile sensitivity, reduce repetitive movements, a smoother root surface, and allow the operators to work more precisely and efficiently.[1,2]

The cutting edge sharpness of the periodontal instrument will become blunt after several times of usage thus requires a sharpening to maintain the sharpness and the instrument's original shape. The research conducted by Tal et al. showed that after 45 stroke movements, 95% of the stainless steel periodontal instrument cutting edge would deform and blunt.[3,4]

The cutting edge of a sickle scaler instrument is a line formed from the confluence of the coronal and the lateral side of the blade located on the working edge of the instrument. The purpose of sharpening the instrument is to restore the blunt edge to become smooth and sharp again.[5,6]

Based on the method used, the manual sharpening technique with whetstone divided into two different techniques. The first technique is the moving stone technique and the second technique is the moving instrument technique. The moving stone technique is a technique in which the instrument is held in a stationary position, then the whetstone is moved on the lateral side of the instrument's cutting edge; while in the moving instrument technique, the whetstone is held in a stationary position, then the lateral side of the instrument's cutting edge is moved horizontally facing the whetstone.[5]

The purpose of this study was to analyze the cutting edge index of a sickle scaler instrument after being sharpened with two different techniques using an electron microscope / SEM.

Materials and Methods

In this study, as much as thirty-two sickle scaler instruments of type H15 / 33 (Oshung Inc.) were prepared and divided into four groups. Eight instruments were randomly chosen to be included in the positive control group (Group I), which was a group of scaler instruments originated from the factory, without treatment, and the cutting edge's sharpness was observed.

Another twenty-four samples were then blunted using the scaling strokes on a cylinder coated with aluminum oxide (2400 Micron) for 5 cm in 5 movements. The collection process was performed on a digital scale with an average pressure of 11 KN. Eight blunt instruments were then chosen randomly to be included in the negative control group (Group II), which was a group of blunt scaler instrument group that will not be sharpened.

The remaining sixteen blunt instruments were then divided into two treatment groups; eight instruments were sharpened using the moving stone technique (Group III); eight instruments were sharpened using the moving instrument technique (Group IV). All four research groups are described in Table 1.

Table 1. Characteristics of the four samples groups in this studies

Group	
Group I	Positive control. Originally sharpened from the factory (Oshung Inc.)
Group II	A negative control, Blunted instruments
Group III	Manually sharpened with moving stone technique
Group IV	Manually sharpened with moving instrument technique

The sharpening procedure by the moving stone technique (Group III) was performed using a sickle scaler instrument held on the left hand with palm and thumb grasp. Afterward, the Ceramic and Arkansas whetstones were adapted to the lateral side of the instrument by forming an angle of 70-80 degrees. Sharpening starts from the heel third, continued to the middle third and the tip third by moving the whetstone with an average pressure of 7 KN towards the vertical direction with an 8 cm sharpening distance of 10 times and ended with moving down direction.[5]

Manual sharpening with the moving instrument technique in group IV was carried out by placing an Arkansas or ceramic whetstone on a Sharpening Horse to produce a slope of around 70-80 degrees angle with the coronal side of the instrument. Sharpening begins by moving the instrument's lateral side 10 times horizontally with a pressure of 11 KN and sharpening distance of 8 cm along with the fulcrum finger movement starting from the heel third continued to the middle third and ended in the tip third. [5,6] (Figure 1).

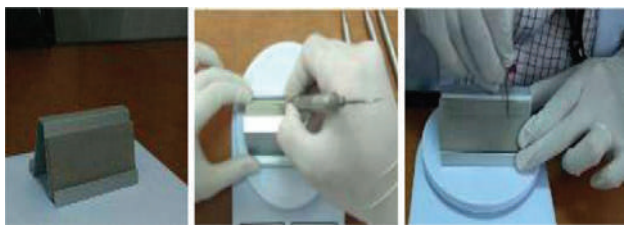


Figure 1. Sharpening horse tool (left image) and sickle scaler sharpening with moving instrument technique; the stone was fixed on sharperning horse tool while sliding the lateral surface of the instrument at an angle 70-80º (right image)

The whole sample was then cut on the working-end side to be observed in the area of 1-2 mm from the tip of the instrument using an SEM microscope. The results of SEM observations in the form of photomicrograph

were then evaluated and classified by one independent examiner using the *Cutting Edge Index* as found by Acevedo et al.with the following conditions:

Score 1: A precise angle of the coronal and lateral faces without wire edges.

Score 2: A slightly irregular cutting angle with or without wire edges.

Score 3: A markedly irregular cutting angle with or without wire edges.

Score 4: An undefined cutting angle with the presence of a bevel or a third surface.[7]

Statistic Analysis

All data in this study were presented in the form of average values and standard deviation values (SD). The Kruskal Wallis analysis was used to test the data presented and checked whether there were significant differences between the groups sharpened with the moving stone technique and the moving instrument technique. Significant level was set at the p-value < 0.05. The Mann Whitney analysis was conducted in advance to test the comparison between groups if the Kruskal Wallis analysis showed a significant difference. Statistical analysis was performed using the IBM SPSS Version 2.0. for Windows software.

Result

The results of this study indicated a significant difference in the sharpness levels between all manual sharpening groups; this can be seen from the p-value < 0.05. The moving instrument sharpening technique group (group IV) showed a better value compared to the moving stone sharpening technique group (group III). The results of the cutting edge index assessment are shown in Table II. Photomicrograph evaluation of SEM analysis in each study group showed the following results:

Table 2. Score studied groups according to the "cutting edge index". The Mean Score of Moving Instrument Technique showed a better value* than the Moving Stone Technique group. *lower score is better

Group	Score 1	Score 2	Score 3	Score 4	Average
(I) Control +	4	4	-	-	1.5 ± 0.6
(II) Control -	-	-	-	8	4.0 ± 0.0
(III) MST	-	1	5	2	3.1 ± 0.6
(IV) MIT	1	5	2	-	2.1 ± 0.6

Group I: In the positive control group can be seen that the group of sickle scaler instruments originated from the factory was not having a perfect cutting edge wholely. Some samples showed mild defects in the intersection areas of the lateral and coronal sides (Figure 2 Left).

Group II: In the negative control group, the group of instruments that have been blunted showed a clear bevel view and a severe defect on the cutting edge area. (Figure Figure 2 Right).

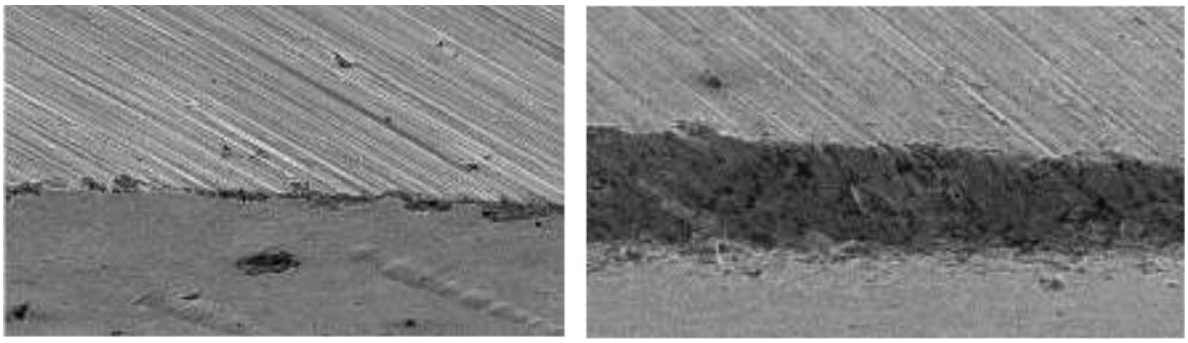


Figure 2. A visible view of a mild defect on an instrument originated from the factory showed by arrows (Left). A visible bevel view in the group of instruments that have been blunted indicated by arrow (Right)

***SEM 300X**

Group III: Manual sharpening with the moving stone technique provided an irregular cutting edge view with a larger defect in the lateral and coronal sides. The bevel formation, however, was no longer visible (Figure 3 Left).

Group IV: Manual sharpening with the moving instrument technique provided a smooth cutting edge view, as seen from the photomicrograph, accompanied by the presence of lightweight, functional wire edges. The bevel view was completely clear (Figure 3 Right).

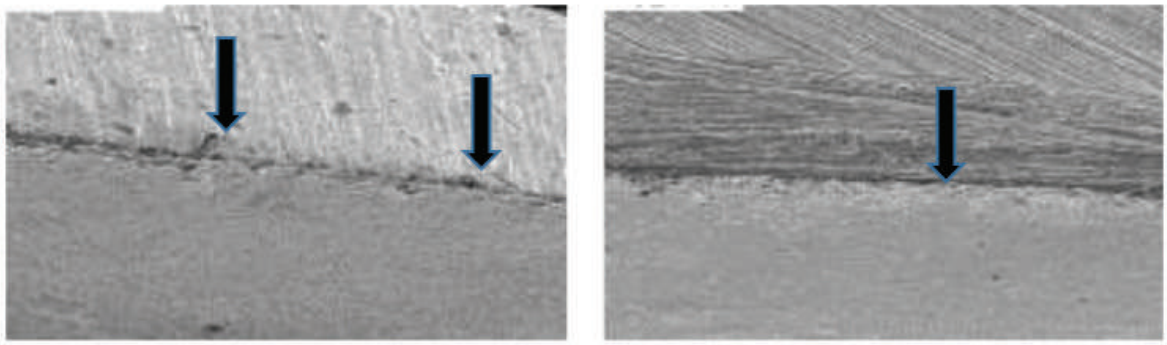


Figure 3. Group III showed a slightly irregular cutting edge with a mild defect showed by arrows. (Right) Group IV showed a smooth cutting edge view accompanied by the presence of lightweight, functional wire edges *SEM 300X

The average value and standard deviation of each group in this study were 1.5 ± 0.6, 4.0 ± 0.0, 3.1 ± 0.6, 2.1 ± 0.6, respectively, for the group I, II, III and IV. The Kruskal-Wallis analysis was used to test whether there were significant differences between groups sharpened with the moving stone technique and the moving instrument technique.

The calculation results of the Kruskal-Wallis test (Table 3) showed a significant difference in the level of sharpness between treatment groups; this can be seen from the p-value = 0.001; where this value was below 0.05. The Mann-Whitney test showed that the least significant group difference with the positive control group was group IV, which was sharpened using the moving instrument technique (p-value > 0.05).

Table 3. Mann-Whitney Advanced Test based on the Sharpening Technique showed that the least significant group difference with the positive control group was group IV (MIT) (p-value > 0.05).

Group	A	B		C	D
I	-	0.013		0.008	0.126
II	0.013	-		0.023	0.004
III	0.008	0.023		-	0.011
IV	0.126	0.004		0.011	-

Notes: A = Positive control; B = Negative control; C = Moving stones technique; D = Moving Instrument Technique

Discussion

Effective and efficient periodontal therapy will be difficult to achieved using a blunt cutting edge instrument. Instruments with sharp cutting edges will cut more precisely and quickly compared to blunt instruments. The use of blunt instruments will reduce the tactile sensitivity of the operator, and increase the trauma risk caused by the instrument due to the use of excessive energy at the time of instrumentation.[5,8,9]

The sharpness of the periodontal instrument cutting edge will be degraded after some times thus requires re-sharpening to maintain the sharpness and original instrument shape. The research conducted by Tal et al. showed that after 45 stroke movements, 95% of stainless steel periodontal instruments would deform so that the cutting edge will become blunt. Other studies also showed that periodontal instruments would lose the metal structure and required sharpening after 15-40 times stroke movements used during calculus removal.[3,4]

The purpose of instrument sharpening is to restore the blunt cutting edge to become smooth and sharp again. This condition can be obtained by sharpening the blade until the junctional area of the coronal and lateral sides then returns to form a straight line, not in a circular movement.[1,5,6]

The purpose of this study was to determine the differences in the sharpness level of the sickle scaler instrument cutting edge after sharpened using two different manual sharpening methods, namely the moving instrument technique and the moving stone technique, each using the fine grit Arkansas and ceramic stones.

In this study, the first group (I) was served as the positive control, which was the group of new sickle scaler instruments originated from the factory then had cutting edge index analysis which provided an average cutting edge index value of 1.5. This value showed that not all the new sickle scaler instruments purchased had a perfect cut line in the cutting edge. This condition was consistent with the study of El Nahass and Madkour in 2007 suggested that the sharpening procedure must be performed to ensure the sharpness of each new instrument's cutting edge.[3]

The second group (II) was served as a negative control, which was the group of sickle scaler instruments that had cutting edge blunted, and had an average cutting edge index score of 4.0. From the photomicrograph, a clear bevel view showed that the scaler instrument had lost its sharpness. The instrument collection process in this study has been standardized with the technique described by Moses et al. to ensure equal treatment of each instrument.[8]

The manual sharpening method using the moving instrument technique in our study provided an average cutting edge index value of 2.10 with the results of sickle scaler's cutting edge sharpness that was better than the moving stone technique method, with an average cutting edge index value of 3.10. This result was consistent with

the research conducted by Acevedo et al. in 2007 and Di Fiore et al. in 2015 which stated that sharpening with the moving stone technique showing irregular cutting edges by the presence of wire edges and formations of the bevel or a third surface.[6,7]

In the moving stone technique, although the operator was able to see the sharpening angle formed between the whetstone and the lateral side of the instrument, the operator will find it difficult to maintain the sharpening angle when the stone was being moved up and down as a sharpening stroke. Besides, it was very difficult for the operator to stabilize the instruments held by the non-dominant hand; thus the instrument would appear to move slightly when the rock was moving up and down.[5]

Manual sharpening with the moving instrument technique (Group IV) in this study was standardized with a power of approximately 110 KN to ensure equal treatment of each instrument. Sharpening with this technique was resulting in the average level of sharpness that closed to the positive control group, and the difference between these two groups was not statistically significant.

The use of assistive devices, known as sharpening horse tool in this technique, was proven to be able to facilitate operators in viewing and maintaining the angle between the whetstone and the coronal side of the instrument. Instruments held by a modified pen grasp on this technique provided precise instrument control and wide movement range. Hand and instrument stabilization can be maximized in the presence of fulcrum or the ring finger support during the sharpening movement.[5,6]

Conclusion

Periodontal instruments such as sickle scaler must have a re-sharpening after the usage, or when they will use in the periodontal instrumentation process. Manual sharpening of the sickle scaler instrument using a moving instrument technique provided a smoother and sharper cutting edge than using a moving stone technique.

Conflict of Interest: Nil

Ethical Clearance: This research has been proved by Health Research Ethics Committee, Universitas Padjajaran, Bandung

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Determination of Complications and Contraception Failure in East Java Province, Indonesia

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Abstract

Family Planning Programs in Indonesia is carried out by means of contraception which is an effort to prevent pregnancy. These efforts can be temporary, can also be permanent. But until now there has not been a 100% ideal method of contraception, because ideally a contraception is seen from the effectiveness, safe, cheap, aesthetic, easy to obtain, does not require continuous motivation, and minimal side effects. Acceptors using reversible contraception (aged 15-45 years) have a 1.8 chance of contraceptive failure. Within 1 year of use, the reversible method fails as much as 1%. Achievement of cases of complications and contraceptive failure in fertile age couples in East Java can be seen that cases have increased from early January 2019 to the end of December 2019 with the achievement of 212 acceptors who experienced cases of failure failure and 90 acceptors who experienced complications in the province of Java. East in 2019. The failure of contraception can be caused by various things, namely because of the method itself, termination of the method by the acceptor, lack of discipline in using it or use errors. According to Lawrence Green, a person's health is influenced by two main factors namely behavioral factors and factors outside behavior. Consists of Prediposing Factor in the form of knowledge attitude, Enabling Factor and Reinforcing Factor.

Keyword: *complications, contraceptive failure, couples of childbearing age*

Introduction

Population is a problem faced by the State of Indonesia where conditions are very worrying because every year the Indonesian population increases 4.5 million people, this can be seen in 2015 from population growth of 1.49% [(1)]. The rate of population growth is influenced by 3 main factors namely, birth, death and population movement [(2)]. To reduce the population growth rate the Indonesian government must implement a family planning program (FP) nationally. Family planning is one effort to bring happiness and family welfare. By arranging births, many parents have the opportunity to pay attention and educate their children while having enough time to do parental duties and do not need to be bothered by the demands of living costs and children's education costs [(3)]. FP Program in Indonesia is carried out by means of contraception which is an effort to prevent pregnancy. These efforts can be temporary, can also be permanent. But until now there has not been a 100% ideal method of contraception, because ideally a contraception is seen from the effectiveness, safe, inexpensive, aesthetic, easy to obtain, does not require continuous motivation, and minimal side effects [(4)].

The government program has tried to succeed in the family planning program, it can be seen based on the readiness of government services that have provided 3 types of contraception for free in the form of condoms, MOP and MOW. But in 2020, IUD and Implant services were not facilitated again by BPJS because there is no mobilization fund for the community because the IUD and Implant are too expensive and BPJS funds are running low. In addition, the use of contraception services by family planning participants was shifting, many family planning participants tended to choose private services (69%) over government services. because people's perceptions think that private services are easy to find and cheaper than government services, this is because government policies are less supportive of contraceptive services [(5)].

Based on sources from Central Bureau of Statistics, the projection of Indonesia's population in 2015-2045 on the island of Java reaches 150.4 million and east java were number 2 most populated in java with 39.74 million people [6]. In September 2019 the number of fertile age couples in East Java was 7,906,860 people, while the number of active family planning participants was 5,979,095 people [7].

Implementation of FP programs factors that can influence the choice of contraception among other things the effectiveness, safety, frequency of use, side effects, failure, age of husband's support information as well as the willingness and ability to do contraception regularly and correctly [8]. In addition, consideration is also based on the cost and role of religion and culture regarding contraception, another factor is the frequency of sexual intercourse [9]. The existence of these side effects has a role in the choice of contraception, side effects or complications is an adverse or unwanted impact or influence, which arises as a result of a treatment or other intervention in this case the use of long-acting contraception devices and short-acting contraception various contraceptives both long-acting and short-acting many side effects from contraceptive use include triggering acne, weight gain, irregular menstrual patterns, amenorrhea, spotting of blood, contraception, contraception, pain during intercourse, vaginal discharge, infection, hair loss, nausea, vomiting, nausea , changes in libido, headaches and long-term fertility. Sometimes it is not surprising that complications arise, complications are the events of new or old family planning participants who experience health problems that lead to pathological conditions as a result of the process of action / installation of contraceptive devices and drugs used [10].

The number of cases of severe complications and failure according to contraception. Complications case is one indicator to measure the level of quality of family planning services. In September 2019, there were 7 cases of IUD complications and 2 cases of implant complications. So that until September 2019. Cases of complications that occurred in East Java were 87 cases, with details of 31 cases of IUDs, 3 cases of MOW, and 53 cases of implants. And the case of failure is a case of ineffectiveness of a contraceptive method used by family planning participants, which causes family planning participants positively pregnant. The number of cases of failure that occurred in September 2019 were 48 cases of IUD, 26 cases of MOW, 1 case of MOP, 91 cases of Implant [7].

Acceptors using reversible contraception (aged 15-45 years) have a 1.8 chance of contraceptive failure. Within 1 year of use, the reversible method fails as much as 1%. Failure to have a contraceptive can be caused by a variety of things namely due to the method itself, termination of the method by the acceptor, lack of discipline in using it or misuse [11].

Methods

The method in this study is descriptive qualitative from population policy analysis and data collection from secondary data and policy problems in the community. The destination study Analyzing the determinant factors of complications and failure of contraceptives in fertile age couples (PUS) residents of East Java in the BKKBN Representative of East Java Province

Result

Cases of Complications and Contraceptive Failure in East Java in 2019

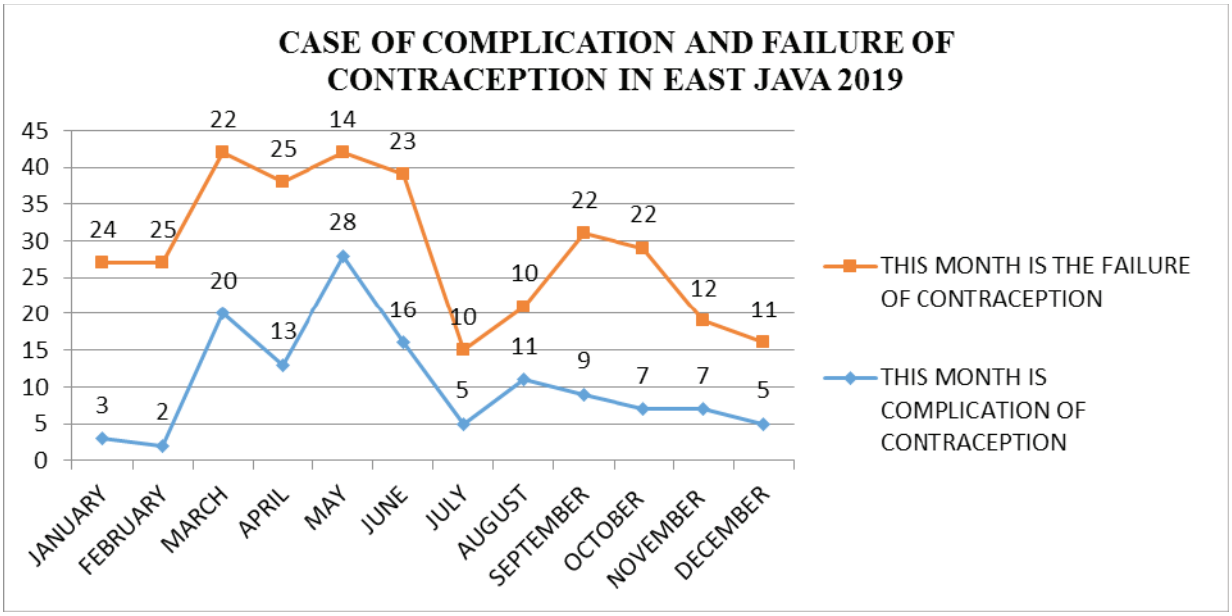


Figure 1. Trend of Complications and Contraceptive Failure Cases in East Java in 2019

Source : BKKBN 2019

The number of cases of complications and contraceptive failure in East Java in 2019 has fluctuated. Positive trends in cases of complications begin to appear from September to December. However, when viewed based on total cases (figure 2), the number of cases from 2019 continues to increase [7].

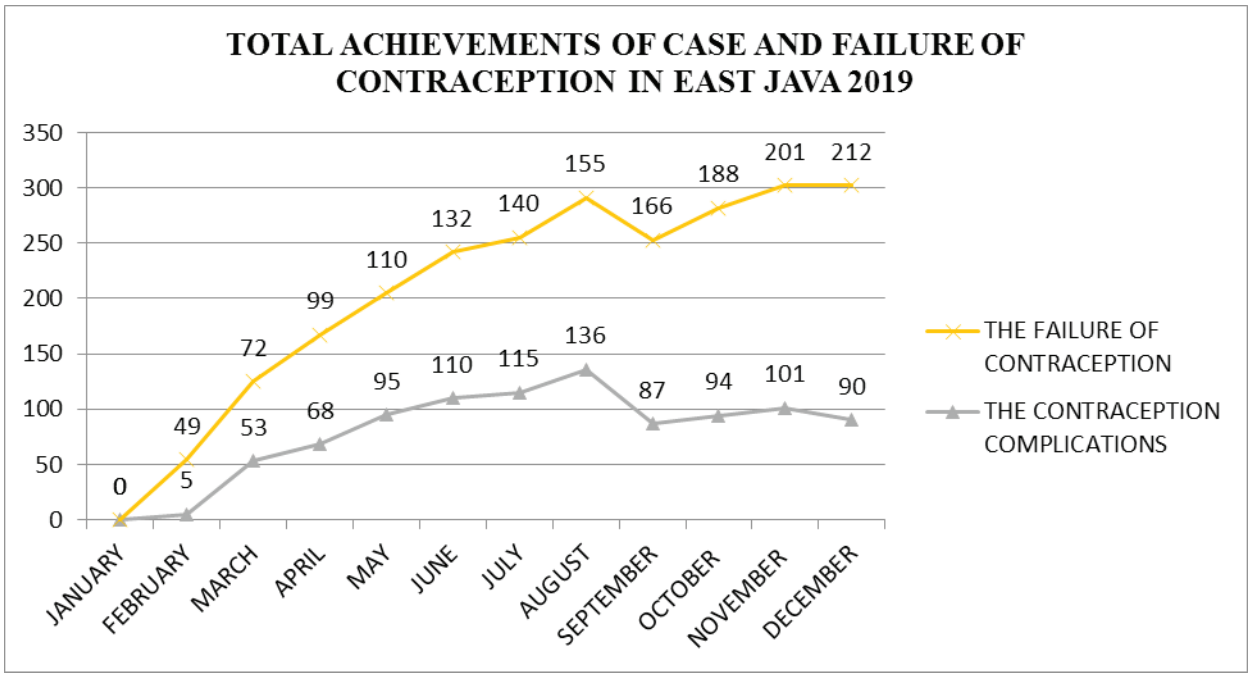


Figure 2. Trends in Achievement in Complications and Contraception Failure in East Java in 2019

Source: BKKBN 2019

Discussion

Complications are health problems experienced by family planning participants as a result of using contraceptives that need treatment. These complications are side effects that can inhibit the use of birth control and can only be determined by medical personnel [12].

A failure case is a case of the ineffectiveness of a contraceptive method used by birth control participants, which causes a positive birth control participant to become pregnant [13]. The cause of the failure in family planning services is the lack of field office planning staff. The coverage of family planning services is not evenly distributed, the quality of family planning services is not optimal, the lack of support from stakeholders, IEC has not changed perceptions about the value of children. community height towards officers. The range of problematic issues is specific to the DTPK / galcitas region, quality problems due to multifactors, stakeholder knowledge needs to be improved, specific local IEC.

According to Lawrence Green, a person's health is influenced by two main factors namely behavioral factors and factors outside behavior. Behavior in pengarihi by three main factors namely *Predisposing, Enabling, and Reinforcing Causes in Educational Diagnosis and Evaluation (PRECEDE). Precede* is a directive in evaluating behavior for educational interventions or health promotion [14]. Whereas PROCEED (Policy, Regulatory, Organizational, Consumption in Educational and Environmental Development) is a direction in the planning, implementation, and evaluation of education or health promotion. Precede can be broken down into three factors, namely predisposing factors, enabling factors, and driving factors [15].

Predisposing factors are factors that exist in a person that is manifested in knowledge, attitudes, beliefs, beliefs, values and others. Enabling factors are factors that manifest in the physical environment, whether or not available health facilities, such as health centers, medicines, contraceptive devices, latrines, and others. The reinforcing factor can be realized from the attitudes and behavior of health workers who are a reference group of health behaviors [16].

Determination Of Complications And Contraception Failure

According to Lawrence Green, a person's health is influenced by two main factors namely behavioral factors and factors outside behavior. The results of the evaluation of complications and contraceptive failure at the end of 2019, there were 5 cases of complications and 11 cases of family planning failure, and there were also 90 cases of complications and 212 cases of family failure that reported and were recorded by the Family Planning and Reproductive Health Division of the BKKBN Representative of East Java Province. The highest complication was in Pasuruan as many as 16 cases while the highest failure was in Trenggalek district as many as 21 cases and 17 cases in Lamongan district. Analysis of the causes of complications and failure to use contraceptives can be done by evaluating existing programs through the Lawrence Green Theory. To make it easier to analyze the causes of family planning complications and failure through the Lawrence Green Theory can be seen from several factors.

Predisposing Factor

Knowledge

Factors that cause complications and failure of contraceptives in the community are the level of public knowledge that does not believe in the importance of family planning, community culture and community belief in not continuing family planning because they have experienced complications or failures in family planning, lack of information to the public about the place Family planning services and the selection of the best contraceptives for acceptors so far the community prefers to use birth control pills and injections rather than long-term contraception because they see that apart from being cheap and also easy to find in villages and can be assisted by midwives without thinking about the level of failure in the use of these contraceptives [17].

Attitude

One of the factors of complications and failure is the attitude is a determinant of behavior related to the perception of a wife in making decisions for family planning is still dependent on her husband this can affect the failure rate in family planning and can cause unwanted pregnancy to occur [18].

Enabling Factor

Family Planning Remote Services are certain areas that are far from the health services, so acceptors are reluctant to do family planning again such as the failure of contraception used, some of them prefer to accept pregnancy due to birth control failure. However, there are still some people who do not accept pregnancy due to family planning failure due to lack of economic factors and are overwhelmed in caring for large numbers of children. So the BKKBN Representative of East Java Province provides Social Assistance to remote areas providing free family planning services conducted by medical personnel who have received training [19].

Reinforcing Factor

Government policies that do not support family planning services in government hospitals are still lacking support other than the cost of expensive contraception, so people prefer not to do family planning again [20]. In addition, in 2020 the government (BPJS) does not provide money for the mobilization of IUDs & implants to the BKKBN Representative of East Java Province so that people are not funded by family planning services because BPJS does not provide funds. However, the BKKBN Representative of East Java Province continues to provide services to the Regency / City in East Java but only facilitates MOP and MOW contraception so that contraceptive failure does not result in an unwanted pregnancy [21].

Conclusion

• Based on the results of this apprenticeship report, it can be concluded that the incidence of complications and contraceptive failure has increased from the beginning of 2019 until the end of 2019, this is because the level of public knowledge about the continuity of using contraceptives is decreasing while the community still believes in the culture and beliefs of the community not to continue KB again.

• The community, especially wives, still depends on the husband's decision and also thinks that family planning only belongs to women or wives.

• The community, especially residents of East Java, are reluctant to spend a large amount of money on family planning because they still consider the installation of contraceptive expensive.

• Government policy is also still lacking in support of this because BPJS services currently do not want to pay for the use of contraceptive which causes unwanted pregnancy.

RECOMMENDATION

Suggestions that can be given are as follows:

a. Open training for medical personnel so that they can always carry out social assistance activities that have been carried out so that poor people can get family planning services so there are no more complicated cases and failures in East Java.

b. The government, especially BPJS services, should provide funds to mobilize the use of contraceptive for the underprivileged communities to avoid overcrowding and neglected children.

c. Family planning service personnel should carefully record the community who experience complications and contraceptive failure and re-evaluate so that no cases of complications and failure occur again.

Conflict of Interest: The authors have no conflict of interest with the material presented in this paper

Sources of Funding: None

Ethical Clearance: None. My paper is an secondary data analysis, without treatment to respondent.

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Validation of a Simple Extraction Procedure for Stimulants Identification from Human Plasma

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Abstract

In this study, a novel LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) method was designed using a simple extraction procedure that was scientifically developed to capture the most relevant stimulants, methamphetamine, MDA (3,4-Methylenedioxyamphetamine), MDMA (3,4-Methylenedioxy-methamphetamine), and MDEA (Methyl diethanolamine). The LC-MS/MS method was validated using US FDA (United States Food and Drug Administration) guidelines, and all validation requirements were satisfactory. This protein precipitation method was specifically developed for handling large numbers of samples with minimum cost and volume of sample. The developed method was accurate, precise, and reproducible for quantification of stimulant from human plasma. This pilot study enrolled 100 drug addicts to evaluate stimulant testing from plasma. Our findings found 47 cases with positive methamphetamine. This method may be valuable for stimulant blood screening for a large and varied population because of its efficiency and economical aspects.

Key words : *Stimulant, LC-MS/MS, human plasma, protein precipitation*

Introduction

Stimulant covers many drugs including those that increase activity of the body and central nervous system, through various mechanism. Stimulant includes amphetamine, metamphetamine, caffeine, ephedrine, MDA, MDMA, MDPV, mephedrone, cocaine, nicotine, phenylpropanolamine, propylhexedrine, and pseudoephedrine. Metamphetamine, MDEA, *MDA, and MDMA were studied in this research.* Stimulants are used to treat sleep disorders, obesity, asthma, mood disorders, nasal congestion, impulse control disorders, and as anesthetics. Amphetamine colorless volatile liquid, has a burning taste and amine odor. It as being very slightly soluble in water, but highly soluble in ethanol and chloroform, with a melting point of 25 °C, and boiling point 200-203°C.[1, 2]

Stimulants increase the presence neurotransmitter dopamine, noradrenaline, and serotonin. They usually block the reuptake or stimulate the efflux of neurotransmitter resulting in increased activity the nerve. Serotonin play a role in mood, appetite, thermoregulation, emotions, sleep, arousal, cognitive, autonomic functions, and pain regulatory systems. The elimination half-life is 6-12 hours. Both hepatic and renal are important organ to eliminate their metabolites from the body (Table 1).[2]

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Table 1: Pharmacokinetic profile of stimulants

No	Profile	MDMA/MDA/ MDEA	Amphetamine	Methamphetamine
1	Maximal concentration in the blood stream after ingestion	Between 1.5 and 3 hours.	Between 3 and 7 hours. Well absorbed from the gut.	Between 3.13 and 6.3 hours. Well absorbed from the gut.
2.	Target organ in distribution	Most part of the body, especially brain.		
3.	Duration of action	4-6 hours	3-6 hours	10-20 hours
4.	Metabolism	In liver use CYP450, CYP2D6, CYP3A4, and Catechol-O-methyltransferase	In liver use CYP2D6, glycine N-acyltransferase, dopamine β-hydroxylase, butyrate-CoA ligase, and flavin-containing monooxygenase 3	
5.	Elimination	Via kidney, about 20% is excreted unchanged in the urine.	Via kidney, about 30–40% of the drug unchanged in the urine.	Via kidney, about 30–54% of the drug unchanged in the urine.

Source : (2), (3)

Stimulant overdose symptoms and signs are rigid, jerking limbs, seizure, loss of coordination, clumsiness, muscle weakness, slurred or difficult speech, in and out consciousness, fast pulse, chest pain, irregular breathing, cognitive difficulties (confusion, poor judgment, fainting), and psychological distress (anxiety, paranoia, confusion, panicking, hallucinations or extremely agitated).[4]

Stimulants are frequently measured in urine or blood as screening for sports, employment, poisoning diagnostics, and medicolegal investigation with immunoassay or chromatography techniques (Table 2). The MDA concentration in the blood or urine of a person who taking MDMA is less than 10% of parent drug.[3]

Table 2: Analysis of stimulants in human biological sample using LC-MS/MS

No	Analyte	Sensitivity	Sample	Preparation	References
1	Stimulants	LOQ : 0.025-0.1 ng/mL	Urine	SPE	(5)
2	Stimulants	LOQ : 2.03–2.48 ng/mL	Plasma	Protein Precipitation	(6)
3	MDMA, MDA	LOQ : 100 ng/mL	Urine	SPE	(1)
4	Stimulants	LOQ : 2.5 ug/L	Blood, Urine	SPE	(7)

Materials and methods

Materials and standards

The Metamphetamine, MDEA, MDA, MDMA, and IS (Methamphetamine-d5, MDA-d5, and MDMA-d5) standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents and reagents used were of HPLC grade and purchased from Merck (Darmstadt, Germany). Control plasma samples were obtained from the UMMC blood bank Malaysia**.**

Samples

A total of 100 blood samples were obtained from drug addiction that consenting to participation in the study. Demographic details such as gender, age, residential area, and ethnicity data were recorded. The inclusion criteria were 18 years of age or older, fully conscious, physically healthy, and no serious current psychiatric symptoms (i.e. psychotic episode). The exclusion criteria were refusal to participate, requiring advanced medical attention for a serious illness, and requiring psychiatric care for psychiatric symptoms. Institutional approval for the analysis of human samples was obtained from the Ethical Committee of University Malaya Medical Centre. The collection sample method used the method was published before.[8]

Extraction of plasma

Sample preparation was carried out using a protein precipitation extraction method**.** The frozen plasma was thawed at room temperature (25 ± 1°C). The thawed plasma was vortexed to ensure the sample was homogenous. To each 100 µL plasma sample, 50 µL of internal standard (IS) (containing 120 ng/mL of IS) was added, followed by the addition of 250 µL of acetonitrile (ACN). The mixture was vortexed, shaken for 5 s, and centrifuged for 2 min at 14800 rpm. The supernatant was filtered with a 0.2 µm syringe filter, then transferred to new a vial. Eight microliters were then injected into the LC-MS/MS system.

Liquid chromatography apparatus and conditions

In this research, the LC system consisted of an LC-20AD XR UFLC system with a SIL-HT automatic sample injector (Shimadzu, Kyoto Japan) were used. The analytical column used was a Agilent, Eclipse Plus Phenyl-Hexyl (150 mm length x 2.1 mm ID, particle size 5µm). Column temperature was 40°C with total running time 10 min. Mobile phase used were 10 mM ammonium formate in water (pH 6.6) in pump A and 0.1% FA (in ACN) in pump B. The flow rate was set at 0.3 mL/min and a gradient elution was used at room temperature. The gradient program began with 5% B, then ramped to 40% B at 3.00 min, and held until 4.00 min. The gradient was ramped to 100% B at 6.00 min and held until 8.00 min.The gradient then returned to 5% B at 8.01 min and this condition was held for a further 10.00 min. Sample injection volume was 8 µL.

Mass spectrometry parameters

A Linear Ion Trap Quadrupole LC-MS/MS Spectrometer, QTRAP 5500, fitted with an ESI probe, and operated in the negative ionization mode was used to perform mass spectral analysis. The LC-MS/MS system was controlled by the Analyst software, version 1.6.3 (Applied Biosystems). Nitrogen was used as the nebulizer, auxiliary, collision, and curtain gas. Analytes were then quantified by multiple reactions monitoring (MRM). The optimal conditions were as follows: ion source temperature of 450°C, ion spray voltage of 5500 V, curtain gas of 20.0 psi, collision gas of medium, ion source gas 1 of 35.0 psi, and ion source gas 2 of 35.0 psi. Analyst 1.6.3 software was used for system control and data quantification.

Results

Linearity, sensitivity, and specificity

No significant interfering peaks were observed from the retention time corresponding to stimulants and the internal standards (Fig. 1-2). Seven calibration points were used to evaluate the linearity of the standard calibration curve. The standard curve was linear with 1/X as weighing factor and reached good linearity ($r > 0.997$). The signal to noise ratio which was greater than 10. The LOD for MDMA and methamphetamine was established at 0.25 ng/mL; MDA and MDEA was 1.25 ng/mL.

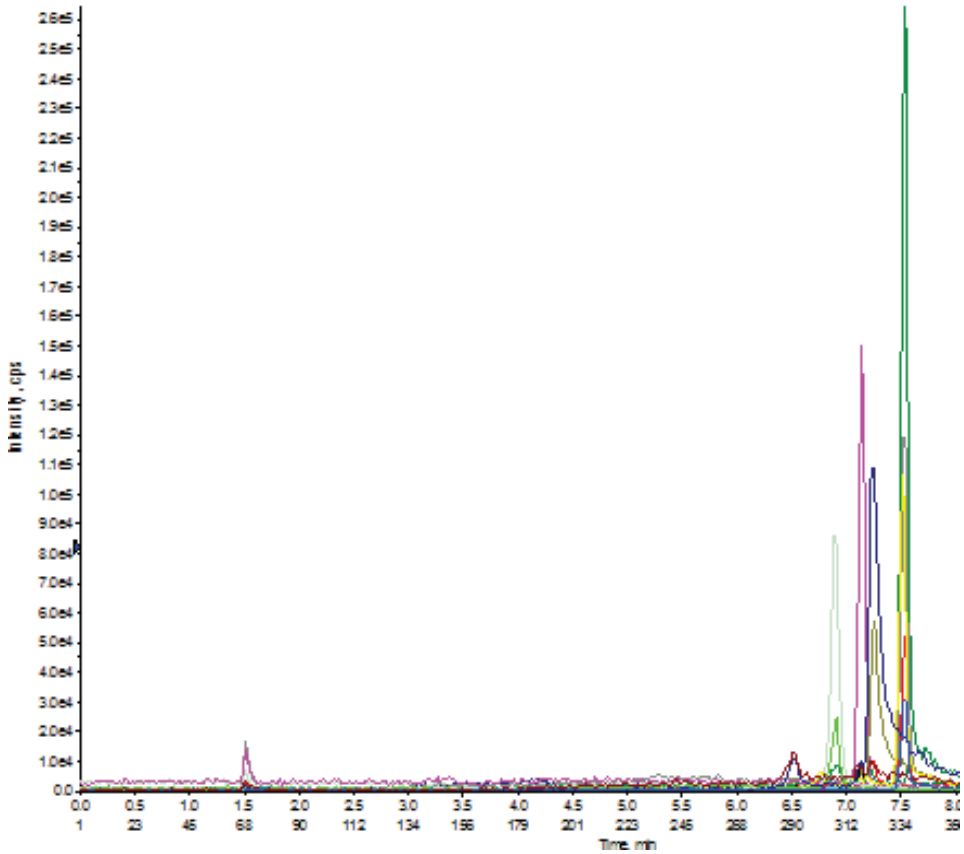


Fig. 1. Representative MRM chromatogram of blank.

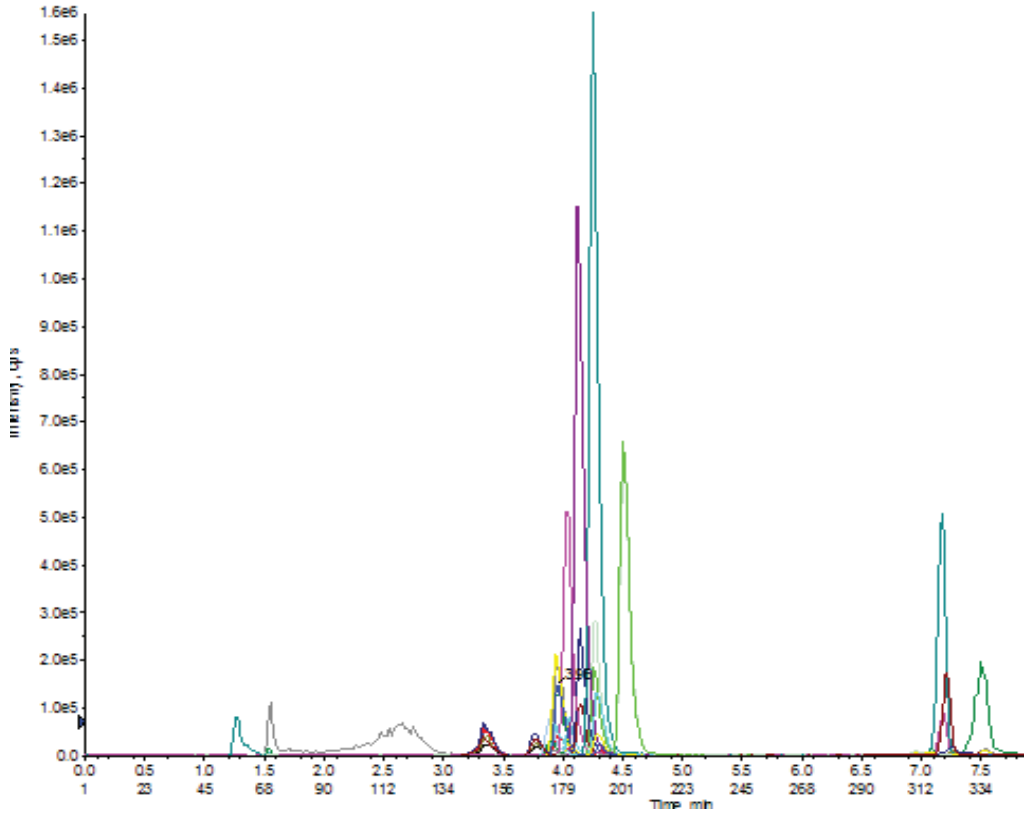


Fig. 2. Representative MRM chromatogram of stimulants in human plasma (QCL:1.5e6)

Precision and accuracy

Based on the mean percentage of coefficient of variation (%CV) for five quality control samples, the intra-day precision of plasma sample ranged from 0.41% to 9.93%. When assessed by means of the five quality control samples the accuracy for LLOQ intra-day plasma sample ranged from 98.16% to 118.4%, then for others ranged from 89.22% to 114%. The inter-day precision and accuracy were 7.17% to 9.97% and 95.4% to 113.12%. The results from this data clearly showed that the method that was developed has good accuracy, precision, and reproducibility for the quantification of stimulants from human plasma.

Recovery

The recovery of stimulants was tested at 0.5 and 5 ng/mL (Table 8). These results indicated that the extraction efficiency of stimulants using protein precipitation was quite good.

Table 3: Recoveries for DoA in human plasma

Compound	0.5 ppb	5 ppb
MDA	100%	91.9%
MDMA	97.5%	96.6%
Methamphetamine	96.8%	89.3%
MDEA	92.4%	88.1%

Stability testing

The stimulants precision ranged from 0.41% to 9.72% and accuracy ranged from 88.22% to 114% for freeze and thaw stability in plasma. The results indicated that the stimulants was stable in plasma for two cycles when stored at -20ºC and thawed to room temperature. The precision and accuracy for stimulants bench top 4 hours stability in plasma ranged from 1.35% to 9.72% and from 95.08% to 114% respectively. The results indicated that the stimulants was stable in plasma at room temperature up to 4 hours when put on the bench. The precision and accuracy for stimulants autosampler stability in plasma ranged from 1.75% to 9.51% and from 98.54% to 114.2% respectively. The stimulants in plasma sample could be analyzed over 24 h in an autosampler at 20±1^{0}C with satisfactory precision and accuracy. The precision and accuracy for stimulants long term (2 months) stability in plasma ranged from 1.59% to 8.87% and from 91.16% to 108.2% respectively.

Discussion

Analysis of plasma samples

This method was applied to the screening of stimulants levels in human blood plasma that were taken from 100 drug addicts of Malaysia (Table 4). Mostly participant from Kuala Lumpur and Selangor. Research subjects were 46.03±10.31 years old in average, with the youngest was 21 and the oldest was 72 years old. The mean of height and weight was 164.42±7.48 cm and 61.43±13.03 kg respectively.

Table 4. Baseline characteristics of participants.

Variable	**N (%)**	**Variable**	N (%)
Gender		Job Scope	
-Male	95 (95)	-Jobless	26(26)
-Female	5 (5)	-Private sector employee	74(74)
Age Group		Types of Food	
-18-30 yo	7 (7)	-Boiled	16 (16)
-31-40 yo	26 (26)	-Fried	84 (84)
-41-50 yo	34 (34)	-Raw	0 (0)
-51-60 yo -61 yo and abobe	24 (24) 9 (9)	Source of Food -Home cooking	68 (68)
BMI		-Dining out	23 (23)

Table 4. Baseline characteristics of participants.

-Under weight	19 (19)	-Take away	9 (9)
-Normal weight	57 (57)	Smoking	
-Pre obesity	12 (12)	-Yes	85 (85)
-Obesity class 1	12 (12)	-No	15 (15)
Hometown		Source of Drinking	
-West Malaysia	149 (149)	-Treated water	72 (72)
-South Malaysia	1(1)	-Bottle water	28(28)
Race			
-Malaysian	80 (80)		
-Chinese	9 (9)		
-Indian	11 (11)		

In this study the majority of drug addicts were men. More addicts are men because they are braver and more easily influenced by the environment than women.. There are addicts who started taking drugs because of curiosity to try it. There are also those who use drugs to get recognition of their social status, also to feel the same experience with their friends. The results of this study are consistent with several other studies that show more male addicts than female. A study in Pakistan involving 150 respondents, was 100% male.[9,10]

In this study most addicts were of working age. The possible cause is the heavy workload that exists and life style factor. Heavy workload, will be a heavy stressor too, so there are workers who are looking for pleasure by consuming drugs. According to combined 2008 to 2012 National Survey on Drug Use and Health (NSDUH), 113.1 million persons of US Population are full-time workers. In the other hand, some of the addicts don't even have a job. This is consistent with NSDUH, 13 million addicts in the USA do not work. There are some of the addicts who lost their jobs, because of using drugs.[11]

Drug abusers tend to lose weight. This is because immunity in general decreases, so it often suffers from illness both mild illness and long-term pain, such as HIV and hepatitis. HIV and hepatitis are diseases that can be transmitted through sharing needles and free sex between drug addicts. In 2007, the number of HIV in Russia among drug addicts reached 1.7 million, while the injecting drug user (IDU) with HIV reached 1.2 million. In 2002, the number of HIV in the USA among drug addicts reached 1.9 million, while the injecting drug user (IDU) with HIV reached 1.7 million. In 2005, the number of HIV in China among drug addicts reached 2.3 million, while the injecting drug user (IDU) with HIV reached 2.1 million.[12]

Chronic diseases will stimulate the body's immunity to be more active, and to stimulate metabolism faster. This can reduce the amount of fat in the body, if it continues continuously the addict will become underweight. In this study, the majority of addicts had a normal body mass index. The second place is underweight with some suffering from chronic diseases.[13]

Almost all addicts in this study were active smokers. This is consistent with studies in Pakistan, that the majority of addicts are active smokers. In drug addicts, the number of cigarettes smoked was affected by the type of drug consumed. Heroin users, had the highest cigarette consumption compared to other drugs.[14] This can cause various respiratory illnesses. In addition, smoking will also put dozens of poisons into the body.[10]

From blood screening, we detected 47 cases with positive methamphetamine. Methamphetamine use, can be via oral, intranasal, smoking, and intravenous. The largest intravenous bioavailability, which reached 100%, while for smoking and oral about 67% and 79% intra nasal. Methamphetamine is metabolized in the liver by a process: N-demethylation to produce amphetamine, (ii) aromatic hydroxylation to produce primarily 4-hydroxymethamphetamine; and (iii) β-hydroxylation to produce norephedrine. Polymorphic cytochrome P450 2D6 causes different metabolisms in methamphetamine. Methamphetamine in the body has a different detection time, in the blood 24-48 hours, in saliva 24 hours, in urine up to 87 hours after taking 10 mg of methamphetamine tablets. Around 70% of a methamphetamine dose is excreted in the urine (within 24 hours) : 30-50% as methamphetamine, 10% as amphetamine, and up to 15% as 4-hydroxymethamphetamine. Acute effects will appear for more than 8 hours.[15]

Conclusions

This study successfully employed an inexpensive, rapid, and simple procedure for stimulants extraction and subsequent detection from human plasma by LC-MS/MS. The plasma extraction consisted of a simple protein precipitation method that consumes a low volume of organic solvent and trace volume of sample (100 µl). The method was suitable for identifying and effectively separating stimulants with good precision and accuracy. This pilot study enrolled 100 drug addicts to evaluate stimulants testing from plasma. Our findings found 47 cases with positive methamphetamine. Determination of stimulants human plasma concentrations from the subjects could be satisfactorily performed by this proposed method.

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Prevalence and Injury Risk Factors based on Sleman Health and Demographic Surveillance System (HDSS) Data

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Abstract

Background: Injuries can occur in various groups of individuals with various risk factors and complications. Health problems due to injuries will put a burden on the victims' individuals and families, health services, health insurance, even various parties related to the work productivity of victims. The analysis of injury risk factors is an initial effort for injury prevention efforts which are expected to avoid reducing productivity and quality of life of individuals. Problem: Need to explore and analyze risk factors for injury, including demographic profile. Objective: The research to be carried out to determine the prevalence of injury cases and risk factors. Methods: This study used a cross-sectional observational design. The data used is secondary data from HDSS Sleman. Research subjects were all injury cases recorded in the Sleman HDSS document. Statistical analysis was performed to calculate prevalence and see the strength of the relationship between risk factors and the incidence of injury. Results: Males had an accident 54%, in 2017, it rose to 56%, and in 2019 it dropped to 55%. In 2016, adults suffered 48% injuries, then decreased to 38% and 36% in the next cycle. In 2016, the number of bruises and abrasions was 72%, and decreased in the next two cycles of 67% and 58%. Part of the body that was most often affected by injury during three cycles was the upper limb. Conclussion : Continued research on injuries and associated risk factors, needs to be done to create health surveillance data.

Key word : *injury, risk factor, prevalence, accident, trauma, surveillance, HDSS*

Background

Injury is an event that can befall anyone in a variety of situations and places, both with deliberate and accidental elements. Injury events are caused and influenced by many factors, external and internal. The consequences also vary.[1] Drug and alcohol consumption can also influence a person to get involved in a fight. Involvement in gang activities and drug trafficking also increases the incidence of murder. Regions with high unemployment have a tendency for homicides to be high.[1]

Intentions cause injury, can also originate from within the individual. Attempted suicide is one of them. Countries with lower middle-income countries (or low-and-middle income countries or LMIC) in Southeast Asia in 2012 had the highest suicide rates among other countries with lower middle income. Globally suicide rates in LMIC occur mostly in the 30-49 year age group, whereas in countries with high incomes the most are at the age of 15-29 years. The incidence of suicide increases with increasing age in the elderly group.[2] The incidence of suicide is more dominated by men than women.[2–4]

Accidents are the cause of injury cases. The causes of accidents differ between age groups. Accidents are even the most unnatural method of death in the elderly.[4,5] Elderly people are more prone to injury because of declining health and dependence on others. Most cases of injury causing death in the elderly are traffic accidents, followed by falls, and poisoning.[6] The most common cause of injury due to accidents in children is falls and is followed by bites.[7] Accidents also differ in pattern between urban and urban areas. The risk of suicide in urban areas is influenced by marital status, income, ethnicity, and psychiatric status.[8] Based on the

level of education, the higher the level of education the lower the suicide rate.[2]

A cohort study by Fukuchi et al. (2013) in Japan showed that individuals who committed suicide mostly married marital status, although after hazard hazard calculation was performed, unmarried marital status had the highest HR. Research Ponnudurai and Jeyakar (1980) getting married is not a protective factor for women. Getting married makes even women more vulnerable to suicidal behavior in Asia. The underlying stress is associated with young marriages, low socioeconomic status, domestic violence, and economic dependence.[9]

The use of alcohol and narcotics such as cocaine can increase the risk of death from injury in accidents.[10] Fracture is one of the factors that increase mortality due to falls, the prevalence is different between urban and rural areas. Findings of fracture cases in urban areas show lower bone density in the elderly compared to rural areas.[11] Urban areas have higher traffic accident rates than rural areas.[12,13] Research Malik et al. (2017) found traffic accident as one of the highest contributors to injury rates with a proportion of 25% occurring in rural areas and 74% in urban areas.[14] Indonesia is one of the countries that has the highest number of two-wheeled accidents. Accidents are often found in middle and low income countries because the high number of vehicles is not followed by firmness in enforcing policies regarding vehicle and driving standards, including the lack of infrastructure devoted to cyclists and pedestrians.[16]

Subjects who work have a higher risk of having an accident while working and also an accident while traveling to and from work. According to Hartley and Arnold (1996) individuals who work and engage in traffic accident have a history of work-related fatigue.[17] Research Fazel et al. (2012) found that individuals who did not work spent more time at home, so that they were more likely to have an accident at home.[18] Housewives have a higher risk of injury and death due to accidents at home.[15]

Research Method

This study uses an observational cross-sectional design. The data used is secondary data from HDSS Sleman. Research subjects were all injury cases recorded in the Sleman HDSS document. Sampling in this study took all existing cases (total sampling). The inclusion criteria in this study were injury cases recorded in the Sleman HDSS document. Exclusion criteria are subject data with incomplete risk factors. The sample size of this study was 1676 people and was followed up in three years. The duration of this study was 3 cycle (2016, 2017, and 2019).

Result

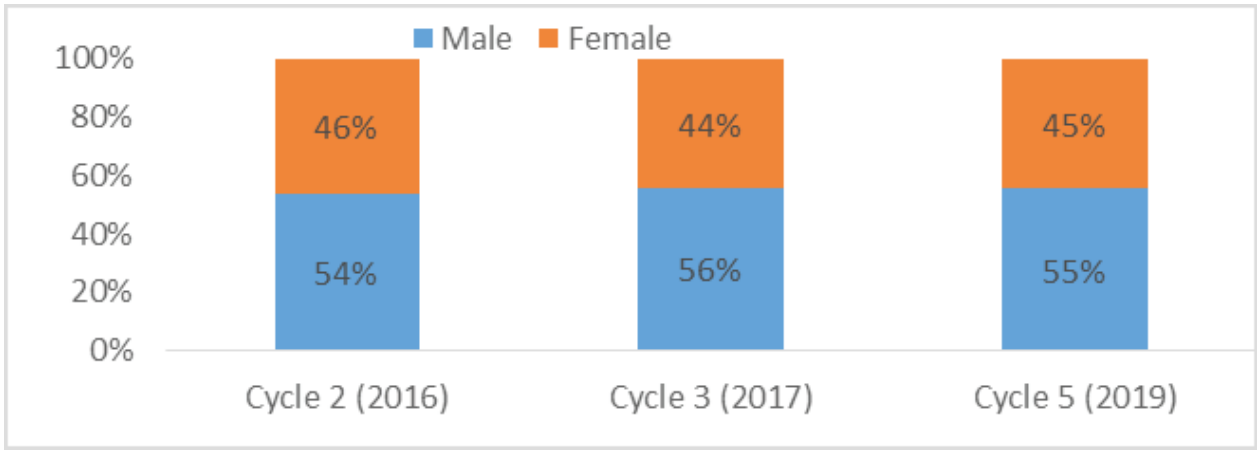


Figure 1. Injury by Gender

Based on Figure 1, in 2016, males had an accident 54%, in 2017, it rose to 56%, and in 2019 it dropped to 55%. Whereas women, in 2016 had accidents by 46%, then dropped to 44% in 2017, and rose again in 2019 by 55%.

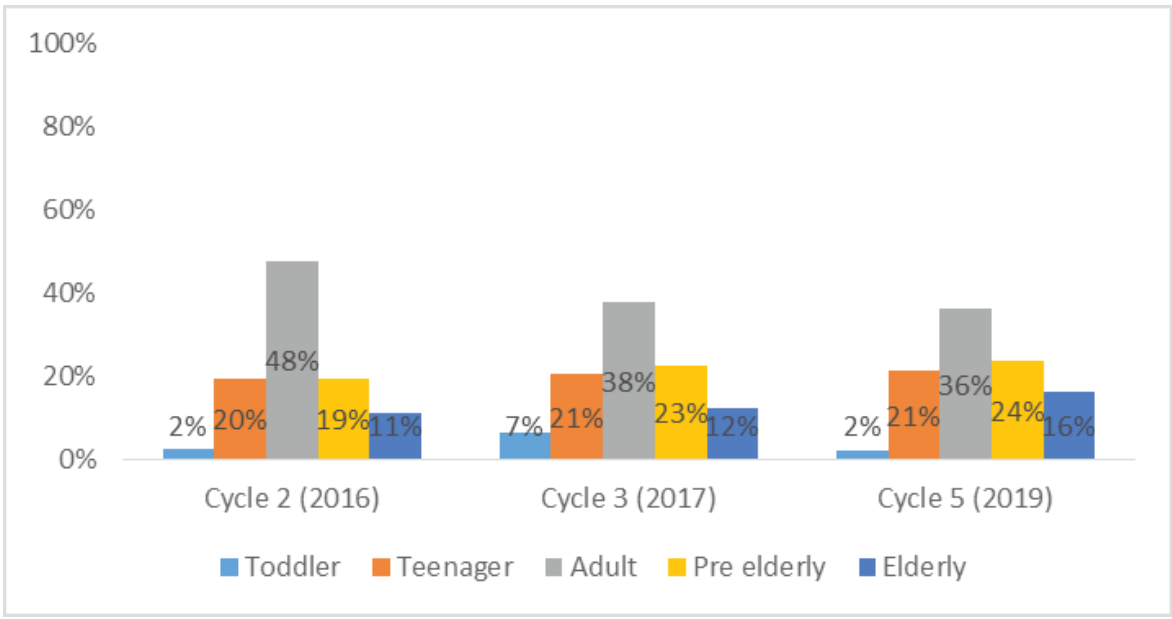


Figure 2. Injury by Age

Based on Figure 2, from year to year, adulthood has the highest frequency of injury. In 2016, adults suffered 48% injuries, then decreased to 38% and 36% in the next cycle. In 2016, teenager were ranked second highest after adulthood. Whereas in 2017 and 2019, pre-elderly were ranked 2. In the third year, the next highest frequency was pre-elderly, and the least was toddler.

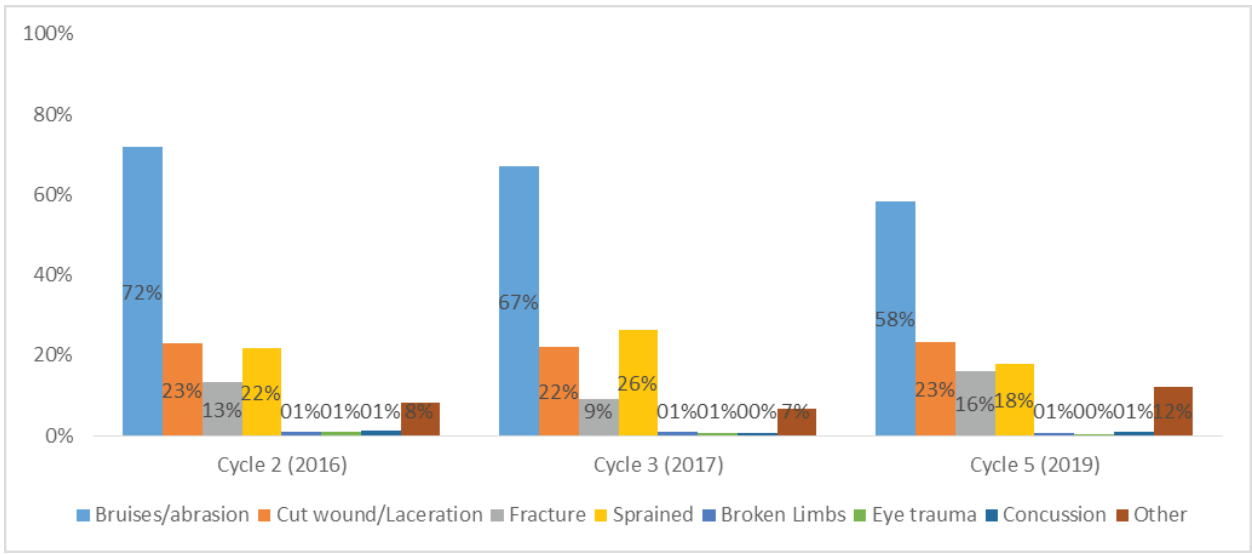


Figure 3. Injury by Kind of Injury

Bruises and abrasion ranks highest in this type of injury. In 2016, the number of bruises and abrasions was 72%, and decreased in the next two cycles of 67% and 58%. In 2016 and 2019, cut wound and laceration occupy the second highest place. Whereas in 2017, sprained occupies the second highest place. At all years, the third most frequent frequency is fracture, followed by other causes.

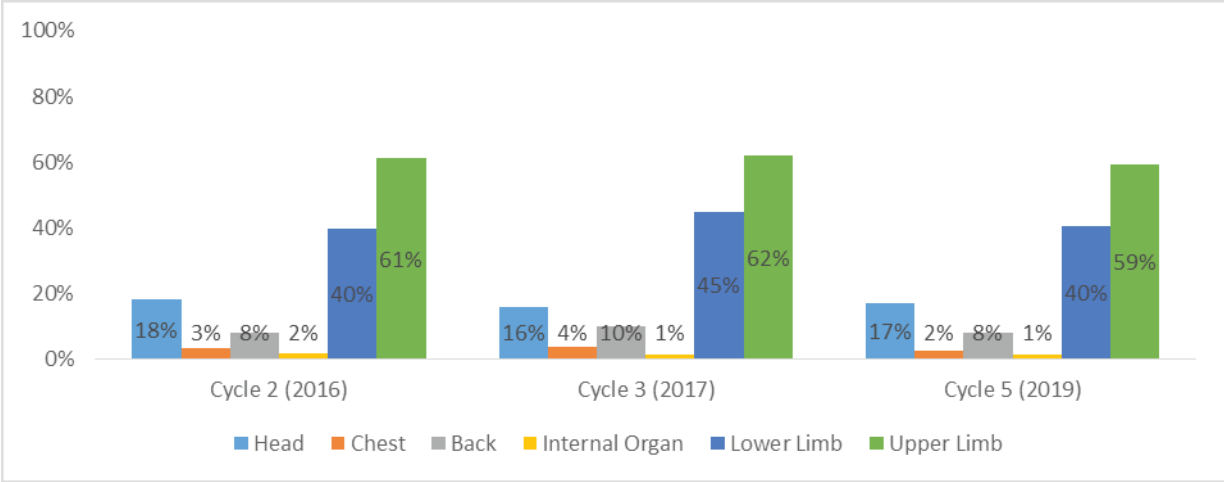


Figure 4 Injury by Organ Damage

Part of the body that was most often affected by injury during three cycles was the upper limb. The frequency in 2016 was 61%, then it increased to 62% in the following year, and in 2019 it fell to 59%. The second highest limb to be injured was lower limb, followed by the head, and other limbs. The frequency of injuries in the lower limb in 2016, as much as 40%, in the following year to 45% and down again to 40%.

Discussion

Based on the the data of Sleman HDSS, prevalence of injury is 3.8% in 2016, 5.1% in 2017 and 3.5% in 2019. Higher prevalence was found in 2017. Extreme weather in 2017 influenced some disasters was noted in Province Disaster Management Team. Injury in men is higher than women (Figure 1). This study is in line with research in Germany, which states that men have a risk of 77.5% for injury.[18] This is because men experience more traffic accidents, exercise, and do heavy work compared to women. In traffic accidents, the classification includes pedestrians (14.37%), vehicles (38.36%), motorcyclists (43.37%), and 3.9% of users injured with other vehicles. men drive vehicles more than women.[13]

Many adults experience injuries, due to high work mobility, and high risk of injury in terms of work (Figure 2). Research in Germany states that the productive age of 40.1 to 49.5 years has the most injuries.[18] While research in Iran, the peak of accidents at the age of 33 years, this is consistent with the results of the study.[13] After adult, in the age group of pre-elderly and also teenagers there are many accidents. Pre-elderly is associated with a high risk of falling due to bodily imbalance, whereas in adolescents due to high carelessness in driving a vehicle.[12]

Based on the the data of Sleman HDSS, in 2016, 2017 and 2019 (Figure 3), abrasion and bruises was the most common injury caused by trauma. These results were in line with a study in Klaten, Central Java, showing that blunt trauma was the highest trauma. abrasion and bruises was the most common type of injury in a traffic accident. Abrasion and bruises is a type of injury that often arises because the force required to cause the injury is weak to moderate energy.[19]

Based on HDSS data from the three cycles (Figure 4), the limb with the most injuries is the upper limb followed by the lower limb. This study is the same as the results of research conducted in Yemen, which states the upper and lower limbs of the most common sites of injury.[20] Another study, stated that injuries that occur most in the upper limb followed by lower limb and facial. This is because the part is the part most exposed to the outside so it is most at risk for trauma.[21]

Based on the the data of Sleman HDSS, in 2016, 2017 and 2019, it has been discussed that most injuries occur in men and in their productive age. However, we can see that old age has a 0.18 times greater risk of injury, and this is statistically significant (p=0.02). Physiological response in the elderly has been reduced at this time, in addition to the strength of the tissue in holding the force that comes also decreases. This is what causes elderly requires intensive monitoring and

aggressive management in response to injury.[22]

Lower education experiences a higher incidence of injury. This is due to lack of adequate education, resulting in a lack of knowledge. Educational interventions need to be done to increase knowledge, so that it will improve human compliance in using self-protection tools. In addition, education is also important to improve the ability to identify the sources of injury and increase alertness to avoid it.[23]

Among all the jobs available, private sector is the highest frequency of injury among others. Private sector is private industries and services are owned or controlled by an individual person or a commercial company, rather than by the state or an official organization. The private sector covers a wide range of work fields, including work that takes a lot of energy in the field, for example in the field of building construction. Work in the field carries a high risk of injury. The use of adequate personal protective equipment is very important in the context of preventing injury.[24]

Road accidents are the most frequent accidents, and motorbikes are the types of vehicles that have the most accidents. The use of helmets that are not standardized increases the risk of accidents. The results of this study are in line with the results of studies in India, which states the motorbike is the vehicle with the most accidents.[21]

Conclussion

Based on HDSS data, most injuries occur in men and in their productive age. Many adults experience injuries, then abrasion and bruises was the most common injury caused by trauma. The limb with the most injuries is the upper limb followed by the lower limb.Lower education experiences a higher incidence of injury. Private sector is the highest frequency of injury among others. Road accidents are the most frequent accidents, and motorbikes are the types of vehicles that have the most accidents. The use of helmets that are not standardized increases the risk of accident. Continued research on injuries and associated risk factors, needs to be done to create health surveillance data.

Conflict of Interest NIL

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Emerging Premarital Sexual Behavior among Adolescent in Indonesia: The impact of Knowledge, Experience, and Media Use to Attitudes

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Abstract

The number of premarital sexual behavior among adolescent is increasing around the globe. Research showed attitudes influence the increasing number of premarital sexual behavior among adolescent. However, there is a gap in understanding factors influencing the positive attitudes of adolescent. Therefore, this study aims to determine the three factors affecting attitudes (knowledge, experience, and access to media) to prevent premarital sex behavior among adolescent. This study was a quantitative research with cross-sectional design in determining the sample. The simple random sampling method was used and data were collected from 75 high school students of first, second, and third grade (15-19 years old). The knowledge, experience, and the use of media is helpful for adolescent but only to a certain extent. Their positive attitude formed due to other factors that is beyond the scope of this research. This study showed that positive attitudes of adolescent is necessary to help them preventing premarital sex behavior that leads to many health problems. Therefore, we need to revisit existing health programs for adolescent to reinforce their positive attitudes to help them making healthy life choices.

Keywords: *knowledge, experience, media, attitude, premarital sex*

Introduction

Adolescence is the time when an individual starts showing secondary sexual signs until he/she reaches sexual maturity, this group includes individuals from the age range 10-19 years[(1)]. The development of significant sexual and physical changes in adolescents results in sexual attraction or encouragement towards the opposite sex. Premarital sex is a common issue of the current era among various sexual problems related to teenagers in Indonesia. Approximately 21 million girls aged 15-19 years in developing countries experience pregnancy every year, and nearly half of these pregnancies (49%) are not desirable. Approximately 38 million girls aged 15-19 years are at risk of pregnancy and do not want children within the next two years[(2)]. In the year of 2012, Indonesia has a 14% unexpected pregnancy rate. This figure is greater when compared with the data from 2010 which is approximately 5.8%[(3)]. In 2008, Indonesia recorded 2.4 million abortions and among them, 21 % was performed in adolescence[(4)].

The high number of unintended pregnancy events can be triggered by pre-marital sex behavior. The presumption that sex as taboo causes reluctance or embarrassment among adolescents in discussing reproductive health or sexual education with elders, especially with their family members. This Lack of sexual education cause adolescents to seek sexual knowledge from other sources such as peers, the internet, and

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media. This results in an unsafe sexual practice among teenagers, ultimately causing unwanted pregnancy leading to abortions[5]. Premarital sex that occurs in adolescents can be influenced by internal and external factors such as adolescent knowledge about reproductive health, emotional state, peer groups, and media influence. Sexual knowledge has a significant effect on premarital sexual behavior, lack of knowledge and vulnerable emotional conditions can increase chances of premarital sex which ultimately poses a threat to reproductive health and can even cause mortality[6]. Internal factors include knowledge level and education, while external factors include peer pressure and media influence. These factors have shown a significant relationship with adolescent sexual behavior in Indonesia[7]. In Addition, there was a significant influence on media, counselors, adolescent knowledge, courtship status, and peer influences in premarital sexual behavior[8]. The major external factors affecting sexual behavior are cell phones, media usage, internet, and pornographic videos[9,10]. In Indonesia adolescents who have watched pornographic films, 62.7% of them admitted losing virginity and 21.2% had an abortion. These figures indicated the crucial role of the internet in increasing premarital sex and unwanted pregnancy[11]. East Java is the province of Indonesia, which is among the top three largest cities with the highest number of adolescent issues such as HIV and AIDS, drugs, and unwanted pregnancies[12]. Also, premarital sex was one of the reasons teenagers get married early[13]. Therefore, this study aimed to understand the influence of knowledge, experiences, and media factors on the attitude of adolescents to prevent premarital sex behavior.

Materials and Methods

Data for this article come from a cross-sectional study of 182 unmarried high school students. Due to the sensitive issue of this study, out of 182 participants, 75 were willing to participate in this study.

Data was collected using a preapproved questionnaire. Informed consent for participation in this study was obtained by the participant's teacher because the participants are students (15-19 years old). The consent was written by the teacher as the participant's willingness to join this study.

Questionnaires used in this study were taken from validated questions regarding attitudes from the Theory of Planned behavior. In this study, we have measured variables that are directly affecting pre-marital sex behavior such as sexual knowledge, experience, and media use. Data analysis was done by using the Gamma correlation test with a significance level (α) of 0.05.

Results

Table 1 showed high school students have sufficient knowledge about the prevention of premarital sexual behavior. Out of 75 students, 71 (94.7%) reported understand premarital sex behavior and its prevention. Further, only 49 students (65.3%) reported having the premarital sexual behavior experience. Additionally, Out of 75 students, only 50.7 percent of them reported using media to look for information regarding the premarital sex behavior and its prevention.

Table 1 Distribution level of independent variables in high school students of 2017.

No	Variables	Categories						Total	
		Good		Enough		Less Good			
		n	%	n	%	n	%	N	%
1	Knowledge	71	94.7	4	5.3	0	0.0	75	100.0
2	Experience	49	65.3	8	10.7	18	24.0	75	100.0
3	Media Use	38	50.7	22	29.3	15	20.0	75	100.0

Table 2. Attitude level of high school students on prevention of premarital sex behavior

No	Attitude level	n	%
1	Very positive	74	98.7
2	Positive	1	1.3
3	Less positive	0	0.0
	Total	75	100.0

Table 3. Statistic Test Results of Knowledge, Experiences, Media Usage on Adolescent Attitudes to Prevent Premarital Sex Behavior in 2017.

Variabel	Attitude						Total		T	p-value
	Less Positive		Positive		Very Positive					
	n	%	n	%	n	%	N	%		
Knowledge										
Good	0	0	1	1,4	70	93,3	71	94,7	1,000	0,361
Enough	0	0	0	0	0	0	0	0,0		
Less Good	0	0	0	0	4	5,3	4	5,3		
Experience										
Good	0	0	1	1,4	48	64,0	49	65,4	1,000	0,314
Enough	0	0	0	0	8	10,6	8	10,6		
Less Good	0	0	0	0	18	24,0	18	24,0		
Media Usage										
Good/frequently	0	0	0	0	38	100	38	50,7	-1,000	0,142
Enough	0	0	0	0	22	100	22	29,3		
Less	0	0	1	1,4	18,6	93,3	15	20,0		

Furthermore, Table 2 indicates most students possessed positive attitude toward preventing premarital sex behavior. Table 3 is an extended version of table 1, in which we have categorized each independent variable into three different levels. Based on the statistical tests, the previous experience of the students has no impact to their attitude to prevent premarital sex. The previous experience of these students were mainly holding hands, hugging, and kissing. In regards to the use of media, result showed that it did not affect adolescents' attitudes to prevent premarital sex behavior.

Discussions

Characteristics of participants by age indicate that most of them were in early adolescence (15-18 years old) of 98.6% and 1.4% were in late adolescence. The participants were from grades 10, 11, and 12. In the middle adolescent phase teens began to test new things as a form of self-discovery[14]. Adolescent age will affect a person's maturity either physically, sexually, mentally, or socially[15]. The characteristics of participants based on the level of education in this study indicate that the majority of participants were from grade 10. At this level, girls still experience the transition from junior to senior high school. This is the phase when a child makes a new connection apart from family members and this sudden change in environment can push them into the dark side of their life. In Africa, lots common reasons for having premarital sex are peer groups pressures of 50.0% and monetary gains of 27.5%[16].

Based on Table 3, the results of statistical tests show that there is no influence between knowledge and adolescent attitudes in preventing premarital sex behavior. Previous study states that there is no relationship between pre-marital sex knowledge level and adolescent attitudes with premarital sex behavior in the high school students[17]. However, the results of this study do not line up with other studies, which states a significant relationship between knowledge and premarital sexual attitudes of adolescents. These studies show adolescents who have good knowledge are more likely to avoid premarital sexual behavior[18]. Based on research results in Kenya, teenagers in schools have a conservative attitude towards premarital sex[19].

Students lacked true information related to premarital sex, so they still thought that premarital sex was a pre-marital relationship[20]. From our results, it can be seen that the problem of premarital sex, is influenced by the attitude or behavior of adolescents, and our study does not account for that. The factors which affect individual are mainly parental negligence, parental divorce, bad behavior of guardians, and any rift among family members. Further, other affecting factors might be an association with sexually active peers and pornographic contents[21]. Previous experience will have a direct effect on an individual's behavior. Besides, someone who is considered important or someone who means special will greatly influence the formation of individual attitudes toward something[22]. The influence of culture has a major influence on the formation of one's attitude and behavior actions. In Indonesia, sex was considered taboo for ages, however, there has been a revolution that leading the free attitude and behavior of adolescents and promoting premarital sexual intercourse[23]. Based on the results of statistical tests in Table 3, it is known that the experience of the students did not prevent premarital sex. Experience widely gained from the sense of sight and hearing and can affect a person's knowledge by motivating or demotivating her/her for specific actions[24]. One of the major pathways of getting experience would be in communication with others. However, in this study, we did not investigate adolescent communication with any reference groups that potentially influence their health.

Teens' communication with medical or health workers regarding sexual behavior, sexuality, and protective behavior were essential to support the prevention of sexually transmitted infections and unwanted pregnancies. The presence of such communications may enhance the correct knowledge of sexuality[25]. Besides, the quality of parent-child relationships with children has a meaningful effect on reducing teenage intentions to have sex[26]. Quality of parental communication allows as an effective target for family-based interventions aimed at fostering adolescent sexual health[27]. The level of religiosity plays a powerful role in one's life. This is related to the dimension of experience related to the consequences of beliefs, ritual practices, knowledge, and experience of the religion held. The higher a person's religiosity, the lower the risk of premarital sex[28]. Attitudes gained through experience will have a direct bearing on behavior.

Based on the results of statistical tests in Table 3, it is known that the use of media does not affect adolescent attitudes to prevent premarital sex behavior. The results of this study were lined up with other studies that found no relationship between information about premarital sex and adolescent attitudes toward premarital sex[29]. Students who use media to obtain information about premarital sex can increase their chances to prevent premarital sex. Whereas, students who use media for watching pornography content can increase the risk of premarital sexual behavior. A total of 70% of female students, said that they often use the media to obtain information or for entertainment. However, very few students use the media to obtain information about premarital sex. The majority of students in our study were smartphone users so they can easily access all the information from the internet. As the technological sophistication goes on, it is necessary to supervise and limit the use of media by adolescents, so that we can direct them towards the positive side of media. Therefore, in this parental supervision could play a major role and ultimately can decrease the case of premarital pregnancies[27]. Based on research results in Nigeria, it is known that 84% think that adolescents should be given sexuality education. Sexuality education must be provided for adolescents in schools through their choices and a reliable source of information[30].

Efforts to prevent premarital sex need to be done consistently and comprehensively by various parties. This effort should be carried out simultaneously from the regional to the national level[31]. The development of adolescent health programs must be carried out comprehensively and implemented by all levels of stakeholders ranging from decision making, provider, user, and representative[32]. The results of research on stakeholder mapping showed that not all stakeholders had an attitude of support and active involvement[33].

Conclusions

Knowledge, experience, and media use does not affect adolescent attitudes to prevent premarital sex, knowing that there may be other more influential. These findings are expected to contribute to reducing a variety of adolescent problems caused by premarital sex behavior in Surabaya in Indonesia. In addition, we must analyze adolescent needs in efforts to prevent premarital sex and analyze the role of various stakeholders in the implementation of adolescent health programs. Therefore, we need to revisit existing health programs for adolescent to reinforce their positive attitudes to help them making healthy life choices.

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Decreasing and Determining Child Mortality in Indonesia

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Abstract

Child mortality is very important to be considered by policymakers, especially for developing countries, like Indonesia. Child mortality rates reflect the level of health development of a country and the quality of life of its people. The Government of Indonesia through the 2015-2019 National Medium-Term Development Plan (RPJMN) has set a target of achieving a reduction in infant mortality of 24 per 1000 births in 2019. Achievement targets that have been made, are intended to follow the major goals of the Sustainable development goals (SDGs) in health and welfare. The government has issued a policy to reduce child mortality through programs such as Healthy Indonesia with a family approach through Permenkes no.39 of 2016. Based on data obtained by the Indonesian Central Statistics Agency (BPS), it was found that neonatal mortality, infant mortality, and infant mortality from 1991 to 2017 there was a decline which meant that there was progress in efforts to reduce child mortality. The success of the Government of Indonesia in reducing child mortality can not be separated from the use of expert theory well. the theories used as references are those of Mosley and Chen, and Filmer who states that children's survival is influenced by social, economic, and biomedical factors.

Keyword: *Decreasing, Determining, Child Mortality*

Introduction

Child mortality rates in Indonesia are still of concern, with the highest rate of infant mortality in 1991 of 97 deaths per 1000 live births, this rate is higher than the average ASEAN under-five mortality rate in the same year[(1)]. Child mortality rates not only reflect the magnitude of health problems that have a direct impact on child mortality, such as diarrhea, infections, respiratory tract, or prenatal conditions, but also reflect the level of maternal health, environmental health conditions, and the level of general socioeconomic development of the community [(2)]. Every child has the right to survival, growth, and development and is entitled to protection from violence and discrimination [(3)]. Children have the right to care and protection, both during the womb and after birth [(4)]. In 1990 the Indonesian government approved the Convention on the Rights of the Child which was also approved by the United Nations in 1989 [(5)]. Indonesia itself raised the theory put forward by Mosley and Chen, as well as Filmer which was then used as an evaluation of sectoral development [(6)]. In the 2015-2019 RPJMN, the Indonesian government wants development that can improve the welfare of children that have been carried out in the previous year such as improving education, improving malnutrition, and health development, and gender equality in Indonesia [(7)]. The Government of Indonesia has now made a program called the Community Movement (GERMAS) which aims to improve the environment and change behavior towards a healthy direction, by increasing awareness, willingness and the ability of people to behave healthily to improve their quality of life [(8)]. The BKKBN has formulated KKBPK's policies and program strategies aimed at improving maternal and child health, reducing maternal mortality, and infant mortality, and improving community nutrition is the main focus of BKKBN [(9)].

Methods

The method used in this study is a qualitative descriptive of child mortality in Indonesia. The secondary data obtained will be further discussed with theories related to the chosen topic.

Result

Cases of Complications and Contraceptive Failure in East Java in 2019

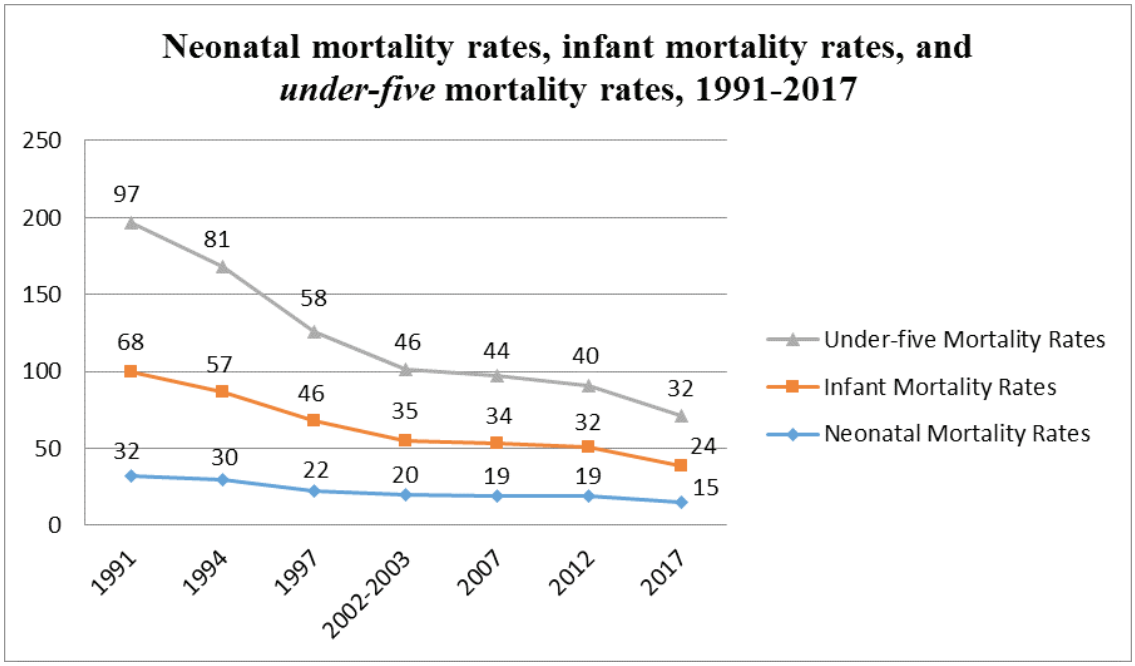
Tabel1.Underfive Mortality Rate in ASEAN Countries, 1990 - 2015

Country	Years					
	1990	1995	2000	2005	2010	2015
Brunei Darussalam	10	10	10	10	7	10
Cambodia	181	135	124	83	54	35
Indonesia	97	81	55	45	42	26
Laos	170	150	131	98	76	86
Malaysia	17	13	8	9	8	8
Myanmar	130	82	76	71	35	52
Phillipinnes	80	67	49	34	36	31
Singapora	8	5	4	3	3	3
Thailand	13	12	12	11	10	9
Vietnam	58	52	45	27	24	22

Source:Indonesian Children's Profile Book2018

During the period 1990-2015. Singapore has the lowest child mortality rate. Data in 2015 showed that under-five deaths in Singapore were only 3 deaths per 1,000 live births. The country that has the highest child mortality rate in Southeast Asia is Laos. In 2015 the under-five mortality rate in Laos was 86 deaths per 1000 live births.

Figure 1.Neonatal mortality rates, infant mortality rates, and under-five mortality rates, 1991-2017



Source:Indonesian Demographic and Health Survey 2018, Central bureau of Statistic

Discussion

Based on data obtained by the World Health Organization (WHO) the main causes of death among children in 2017 are complications of preterm birth, acute respiratory infections, intrapartum-related complications, congenital anomalies, and diarrhea. Neonatal deaths accounted for around half of under-five deaths in the world in 2017. Ending preventable child deaths can be done by giving early and exclusive breastfeeding, increasing access to skilled health workers for prenatal care, birth, and post-birth, increasing access for nutrition, promoting family awareness of danger signs, increasing access to clean water, sanitation, hygiene, and also providing immunization.

In terms of the causes of child mortality, it can be seen from an endogenous and exogenous perspective. Endogenous causes are causes that are carried from birth and are inherited from parents at the time of conception or from the mother during pregnancy. Exogenous causes are causes related to external environmental factors. The more the baby's age increases, the endogenous causes will decrease and exogenous causes increase. Endogenous causes play a role in infants under one month of age (neonatal). While exogenous causes are responsible for most infant deaths over the age of 1 month and child deaths. The quality of the environment, including hygiene, sanitation and socioeconomic conditions, is crucial for the level of child mortality. It also explains that the state of environmental health greatly influences the child mortality rate [10].

Poverty is still a major problem in Indonesia, which then has an impact on the welfare of children's lives. In addition to poverty, nutritional problems are still found in Indonesia, toddlers with stunting, and malnutrition [11]. Usually the problems of poverty and stunting and malnutrition are encountered simultaneously, babies who are malnourished and stunted can come from families with economic abilities that are less capable so that the nutritional needs of children cannot be fulfilled. Lack of nutritional intake of children will inhibit growth and development. Poverty is also synonymous with inadequate housing, lack of sanitation and densely populated settlements will facilitate infectious diseases spread quickly, infectious diseases such as diarrhea and typhus are one of the factors that can increase the mortality rate of children themselves.

In March 2019 there were approximately four million people in East Java who were classified as poor. Based on data obtained by the Central Statistics Agency, the poor population in the province of East Java is more common in rural populations with around two million residents, and food is the commodity that contributes the most to the poverty line compared to other commodities. This shows that poverty is one of the factors that makes fulfilling nutritious food difficult to achieve. Difficult fulfillment of nutritious food will impact on children's growth. Lack of nutritious food intake in pregnant women will impact on pregnancy outcomes, a lack of chronic energy can cause low birth weight in children which will then affect the child's growth and development.

Childbirth assistance is also a factor that can affect childmortality. If childbirth is assisted by health workers, it can be ensured that the delivery process is carried out according to safety procedures and with good equipment so that the possibility of infection due to unsterile equipment and complications due to erroneous labor procedures. Based on data obtained by the Central Statistics Agency of East Java, in 2017 and 2018 there were still some laboratories that were not accompanied by health workers in some areas. Delivery by other than professional health such as unprofessional midwives must be avoided. Because unprofessional midwives are not trained to deliver and handle complications during delivery. Usually, maternal and child deaths occur during complications during childbirth and the unprofessional midwives cannot handle it so that mothers who experience complications are late to be referred to the hospital so that eventually death of both mother and child in the womb. Delivery must be carried out by trained health workers such as professional midwives because they have been trained to handle the delivery process and have knowledge related to the delivery process so that if complications occur that can not be handled correctly and referrals can be made directly so that mothers who experience complications during labor are not late to be carried to the hospital and can be saved from the risk of maternal death and child death can be avoided.

In developing countries, there are about 50,000 adolescent girls aged 15-19 years who die each year

during pregnancy or labor. About one million babies born to adolescent girls also die before they reach one year of age. Babies of mothers who give birth under the age of 18 are more at risk of dying before reaching the age of one year. Mothers who are pregnant at the age of risk will carry the risk of death of children born, women who are pregnant at too young or too old have the risk of miscarriage due to weak physical condition. Certainly, the risk to the mother will also carry the risk to the child being born. Therefore, pregnancy at risk of age should be avoided because it will increase the risk of child death.

The Indonesian government has made several programs that can reduce child mortality, for example, family planning programs, the creation of a National Health Insurance (JKN), the provision of pregnancy examinations, the provision of sexual education for married couples, requiring childbirth to be carried out by health workers trained. The program was made by the Indonesian government to accelerate the reduction in maternal and child mortality in Indonesia. Based on data obtained by the Central Statistics Agency in 2018, the delivery process has been handled by many trained health workers such as doctors and midwives.

Reducing child mortality is not only done by the government through its policies but also requires the role of the community who must be aware of the importance of children's survival. Policies made by the government will not run well if people do not obey them. Policies are made so that various sectors can comply with the hope that the child mortality rate can be accelerated. Health workers act as educators and also implement health efforts, while the community plays a role in implementing various efforts to improve health status based on expert recommendations. Synergies from various sectors will facilitate the government in reducing child mortality.

If the family already has good health awareness then it is not difficult for the community to pursue maternal and child health, the community will remind each other to seek maternal health and especially children's health, government programs are no longer considered coercive but can be considered as a standard if at The community already believes that recommendations from experts and government programs are something good. Efforts to promote health-related to child death prevention efforts need to be intensified in giving to the community, health workers must be able to embrace community leaders in health promotion efforts undertaken so that people can more easily receive information provided by health workers. If health workers are not able to embrace community leaders in this health promotion effort, then the provision of health information will also be difficult to convey, because the community has their respective cultures and norms that have been trusted and applied in the community.

Determinants Of Child Deaths According To Mosley And Chen

According to Mosley and Chen's theory, all social and economic determinants must go through intermediate variables to be able to influence children's survival. The intermediate variables are categorized into 5 categories by Mosley and Chen namely maternal factors, environmental pollution, malnutrition, injury, control of individual diseases [12].

Mother factor

Maternal factors include age, parity, and birth spacing. Each of these factors has an influence on pregnancy outcomes and infant survival. For example, the age of the mother who is too young to make the condition of pregnant women become dangerous, reproductive organs that have not fully grown to the maximum will make the process of childbirth into a process that is full of risk, can even cause death.

Environmental pollution

Environmental pollution is related to disease transmission to children and mothers.Four categories that describe the spread of disease in the community which includes air, food, or water that transmits digestive diseases such as diarrhea and typhus, then the skin that spreads infectious diseases of the skin, and the last category is insects that transmit diseases through parasites or viruses. carried by insects.

Malnutrition

Malnutrition can affect the survival of children and mothers. Because the adequacy of maternal nutrition during pregnancy will affect the outcome of pregnancy, and during breastfeeding affects the amount and quality of mother's milk.

Injury

The injury category includes injuries that attack physically that can cause disability or even cause death. Although often regarded as something unintentional, there are times when these injuries are intentionally inflicted, for example, an attempted murder aimed at an infant can cause physical injury to the baby. In the wound category, the wound variable can be measured by looking at wounds related to disability.

Control of Individual Diseases

One component in controlling individual diseases is preventive actions taken by healthy people to prevent disease. This includes traditional behaviors such as adhering to traditional believes and modern practices such as immunization or prevention of pregnancy by taking antenatal care. Individual disease control factors influence the level of disease through prevention and cure rates through treatment.

Determinants of child deaths According to Filmer

Filmer's theory explains socioeconomic factors as causes of child death. Child mortality and child nutrition are influenced by the demand and supply sides. The demand side referred to by Filmer is the behavior of household and individual characteristics such as sanitation, preventive measures in the family, income, education, and parental knowledge. The better sanitation, disease prevention measures, family income, education and parental knowledge, the lower the child mortality and the better the child's nutrition. The supply side according to Filmer is that government policies both at the micro and macro levels as well as the implementation of its policies, the capabilities of local governments, and infrastructure as well as access and quality of health services are very important and affect the health of children themselves. Difficult access and poor quality health services will increase mortality. Government policies must be able to guarantee in terms of supply regarding services and health insurance, especially for people classified as poor, so that the quality of life of children can be increased and child mortality can be reduced [13].

Conclusion

Based on the data obtained it can be seen that the child mortality rate from 1991 - 2017 has decreased

· The main causes of child death are preventable causes such as complications of pregnancy in the mother and diarrhea in the child.

· Based on Mosley and Chen's theory, 5 factors that could affect child survivalwere maternal factors, environmental pollution, malnutrition, injury, control of individual diseases.

· According to Filmer's theory, there are two factors that influence children's survival, these two factors are supply factors which are government policies that will impact on the quality of health services and demand factors which are characteristics of households in seeking personal health, education, parental knowledge, and sanitation of the home environment.

Recommendation

Suggestions that can be given are as follows:

a. Need to be given knowledge related to reproductive organs, and knowledge related to nutrition, as well as good parenting to brides and women of childbearing age.

b. The need for strengthening by the government related to efforts that have been made previously to strive for children's health such as family planning, giving additional food to pregnant women, and also giving supplementary food for nursing mothers.

c. Preventive health programs need to be promoted and accelerated. This will require the promotion of a range of services from adolescence and pre-pregnancy and continuing through pregnancy, childbirth, and childhood.

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Effect of *Parkia speciosa* Hassk Peels Extract on Total Cholesterol Levels of Hypercholesterolemia Rats

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Abstract

Background: This research aimed to examine the secondary metabolites in the ethanol extract of *Parkia speciosa* Hassk peels and determine the effect of the *P. speciosa* Hassk peels on the reduction in total cholesterol levels of male hypercholesterolemia white rats.

Methods: Thirty white male rats were divided into six treatment groups (n=5): Group I: normal control (0.5% CMC Na suspension), Group II: negative control (high cholesterol feed), Group III: positive control (suspension simvastatin), Group IV, V, VI: hypercholesterolemia rat received *P. speciosa* Hassk shell at dose of 300, 400, and 500 mg/kg BW orally for 14 days. The total cholesterol levels were measured on days 0, 14, 28, 35.

Results: Quantitative test showed that the ethanol extract of *P. speciosa* peels contained 0.21% alkaloids (w/w), 8.34% flavonoids (w/w), 4.21% tannins (w/w) and 0.18% saponins (w/w). The ethanol extract of *P. speciosa* peels at a dose of 300 mg/kg BW was able to reduce total cholesterol levels in hypercholesterolemia white mice (52 mg/dL).

Conclusion: The ethanol extract of *P. speciosa* peels can be considered as an effective treatment for hyperlipidemia disease.

Keywords*: Parkia speciosa Hassk shell; hypercholesterolemia; high cholesterol feed.*

Introduction

Hypercholesterolemia is indicated by an increase in total cholesterol, especially LDL, and followed by a decrease in blood HDL levels. Hypercholesterolemia is considered a high risk factor for coronary heart disease if an increase of cholesterol level reach to ≥ 240 mg/dL.[1] *Parkia speciosa* Hassk is a plant that contains alkaloids, polyphenols, flavonoids, saponins and tannins. This plant has the benefit to prevent and overcome several diseases such as anemia, high blood pressure, diabetes, cholesterol, constipation, reducing the activity of free radicals, and serves to capture superoxide anion and superoxide lipid radicals. *Parkia speciosa* pells has several nutrient such as phosphorus, vitamin C, vitamin A, protein, carbohydrate, mineral and iron content.[2,3]

Angelina et al.[3] reported that the ethanol extract of *Parkia speciosa* peel exhibited antioxidant activity and contained several metabolite compounds such as phenolic, flavonoid and tannin contents. Previous research by Tandi et al.[4] showed that the ethanol extract of purple eggplant peels at dose of 50 mg/kg BW was effective to reduce total cholesterol levels of 140.8 mg/dL. Tandi[5] reported the effects of ethanol extract of pumpkin seeds could reduce total cholesterol levels at dose 360 mg/kg BW.

Based on the above explanation, researchers are interested in conducting further research on the effectiveness of *Parkia speciosa* peels ethanol extract on reducing cholesterol levels of male white rats (*Rattus norvegicus*) hypercholesterolemia with different dose.

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Materials and Method

Preparation of *Parkia speciosa* peels extract

The extraction *Parkia speciosa* peel extract was carried out by maceration method using 96% ethanol. 1000 grams of powdered samples was extracted with 6 L ethanol for 3x24 hours in dark place while occasionally stirring. The vessels used were 4 maceration vessels. The maserate obtained was then evaporated using a Rotary Vaccum Evaporator at 60°C and continued with thickening carried out using a waterbath at 60°C.

Preparation of simvastatin suspension

Ten mg of simvastatin tablet were crushed. 1.8 mg of simvastatin were put into mortar by adding 0.5% Na CMC suspension and crushed until homogeneous.

High cholesterol induction

The component of high cholesterol feed was pig oil and quail egg yolks. The lard were melted by heating. The eggs are separated from the yolk and the egg whites, taken the yolk and mixed with pig oil until homogeneous, given for 14 days orally.

Experimental design and treatment

Thirty white male rats were divided into six treatment groups (n=5): Group I: normal control (0.5% CMC Na suspension), Group II: negative control (high cholesterol feed), Group III: positive control (suspension simvastatin), Group IV, V, VI: hypercholesterolemia rat received *P. speciosa* Hassk shell at dose of 300, 400, and 500 mg/kg BW orally for 14 days. The total cholesterol levels were measured on days 0, 14, 28, 35.

Determination of the total alkaloid

A total of 100 mg sample was added with 5 mL of 2N HCl. Then, it were washed with 10 ml of Cloroform 3 times in a separating funnel and discarded the Cloroform phase. The solution was neutralized by adding 0.1 n NaOH and then added with 5 mL of BCG solution and 5 mL of Phosphate Buffer. The solution was extracted with 5 mL Cloroform and stirred with a magnetic stirrer at 500 rpm for 15 min. The extraction was repeat with Cloroform 2 times. The evaporated chloroform phase was collected with nitrogen gas and added the chloroform to 10 mL. The absorption levels was measured at 470 nm.

Determination of total flavonoid

Briefly, 0.10 g of samples was added with 2 ml of HCl 4 N and heated for 2 h at 110°C. Then, samples were cooled and extracted with ether. Ether solvent was steamed, then dried with N_2. 0.3 ml of 5% sodium nitrite was added. After 5 min, samples were added with 0.6 ml of 10% aluminum chloride, left for 5 min. Samples were added with 2 ml of 1 M NaOH and distilled water. Samples were diluted 25 times and moved into the cuvette. Then, the absorption levels were measured at 510 nm.

Determination of total saponin

Ten hundred mg of sample was added with 2 ml of 25% H_2SO_4. Samples was incubated for 120 min at 110^0C. Then, samples were extracted with ether and dried the filtrate. 1 ml of water was added and extracted for 5 min. Samples were then added with 50 µl of anisaldehyde and left for 10 min. Samples were added with 2 ml of 50% sulfuric acid and heated at 60°C for 10 min. Then, samples were added with water to a volume of 10 ml. As much as 10x dilution and the absorption was measured at 435 nm.

Determination of total tannin

Ten hundred mg of samples were added with 10 ml of methanol for 20 h. Samples were evaporated and then added with aquadest up to 10 ml volume. Then, 1 ml sample solution was added with 0.1 ml of Folin Ciocalteu reagent and incubated for 5 min. Samples were then added with 2 ml of 20% Sodium Carbonate and incubated for 5 min. Samples were stirred with aquadest up to a volume of 10 ml and diluted 5 times. After incubating for 30 min at room temperature, the absorbance was measured at 760 nm.

Data Analysis

Data were statistically analyzed using One Way ANOVA. Post Hoc Least Significant Difference (LSD) test was performed to determine significant differences between treatments ($p < 0.05$). Data analysis was done by SPSS v.23 for Windows.

Results and Discussion

A preliminary test was carried out to determine the secondary metabolite compounds from the peels of *Parkia speciosa*. Determination of the total quinine equivalent alkaloid content obtained by the regression equation Y = 1.88853e-004 - 3.95444e-004 and the correlation coefficient (r2) = 0.99926 (Table 1). Based on the regression equation, total alkaloid level of the ethanol extract of *P. speciosa* peels was 0.21% (w/w). The total levels of equivalent flavonoids quercetin was obtained from the regression equation Y = 0.00404347 x- 0.00637338 and the correlation coefficient (r2) = 0.99786. Based on the regression equation, total flavonoid levels of the ethanol extract of *P. speciosa* peels was 8.34% (b/b). The total saponin content obtained by the regression equation y = 9.44156e-004 - 0.00380439 and the correlation coefficient (r2) = 0.99959. Based on the regression equation, total saponin content was calculated in the sample to obtain the total saponin content the ethanol extract of *P. speciosa* peels was 0.18% (w/w). The total tannin content equivalent obtained regression equationy = 0.0116490 x + 2.92199e-004 and the correlation coefficient (r2) = 0.99996. Total tannin content of the ethanol extract of *P. speciosa* peels was 4.21% (b/b).

Table 1: Determination of total flavonoid, alkaloid, saponin, and tannin

Parameter	Result	Unit	Regression Equation	Method
Total Flavonoid quercetin	8.34	% b/b	y = 0.00404347 x- 0.00637338 Correlation Coefficient r2 = 0.99786	UV-Vis
Total Quinine Alkaloids	0.21	% b/b	Y = 1.88853e-004 - 3.95444e-004 Correlation Coefficient r2 = 0.99926	UV-Vis
Total Saponins	0.18	% b/b	y = 9.44156e-004 - 0.00380439 Correlation Coefficient r2 = 0.99959	UV-Vis
Total Tannin	4.21	% b/b	y = 0.0116490 x + 2,92199e-004 Correlation Coefficient r2 = 0.99996	UV-Vis

The results showed that all animals before treatment (day-0) have a normal total cholesterol levels (Table 2) range of 10-54 mg/dL.[6] The total cholesterol levels on the day-14 had a significant difference between normal control and all treatment groups (Figure 1). It was indicated that there was an effect due to high-cholesterol feed containing 844 mg/dL of quail eggs and pig oil containing 25% saturated fat, which could increase triglyceride levels and affect the viscosity of red blood cells and reduce HDL.[7]

Table 2: Total cholesterol levels after treatment with the ethanol extract of *P. speciosa* peels

Total blood cholesterol levels (mg/dL)							
Day	**Normal Control**	**Negative Control**	**Positive Control**	**300 mg/kg BW**	**400 mg/kg BW**	**500 mg/kg BW**	**P value**
0	35 ± 9.2	45 ± 2.3	36 ± 8.6	39 ± 12	43 ± 13.1	44 ± 9.2	0.464
14	41 ± 5.8	150 ± 16.5	152 ± 20	160 ± 30.4	150 ± 17.5	171 ± 27.3	0.000
28	51 ± 7.3	165 ± 48.3	84 ±28.5	146 ± 60.4	118 ± 30.6	110 ± 27.4	0.004
35	50 ± 10.2	110 ± 38.2	47 ±23.2	52 ± 31.7	71 ± 19.2	87 ± 35	0.023

On day-28th day, treatment with *P. speciosa* peels extract could not reduce the total cholesterol of hypercolesterolemia mice. However, on day-35, the total cholesterol of hypercolesterolemia mice was decreased after *P. speciosa* peels extract treatment. Only dose of 300 mg/kg BW of *P. speciosa* peels extract could reduce the total cholesterol of hypercolesterolemia mice compared to other dose treatment. The decreasing of total cholesterol in hypercolesterolemia mice was also found in simvastatin group treatment. This study also showed that *P. speciosa* peels extract at dose 300 mg/kg BW exhibited the same effect as simvastatin group to decrease total cholesterol reach to normal levels. Simvastatin is a drug which act as HMGCo-A inhibitors and the most effective drug for reducing cholesterol levels.[8,9]

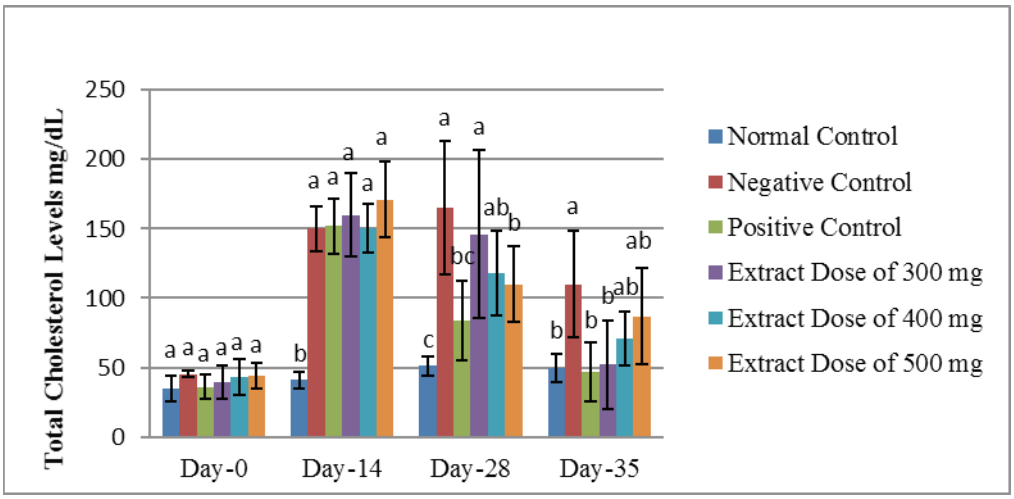


Figure 1: The total cholesterol levels after *P. speciosa* peels extract treatment

Alkaloid compounds could prevent the increase in levels of total cholesterol, triglycerides, LDL, and atherogenic indices, and also significantly increase HDL. Flavonoid compounds and tannins act as antioxidants by reducing triglycerides and LDL, so it can inhibit the accumulation of LDL in the blood vessel wall. Saponins can affect the biosynthesis of cholesterol in the liver.

Previous study showed that the ethanol extract of purple eggplant skin at a dose of 50 mg/kg BB with an average reduction of 140.8 mg/dL to reduce total cholesterol levels,[10] then ethanol extract of yellow pumpkin seeds at a dose of 360 mg/kg body weight with an average average reduction of 159 mg/dL to reduce total cholesterol in male white rats[11] and ethanol extract of kenikir leaves (*Cosmos caudatus*) dose of 400 mg /

kg BW with an average decrease of 209.9 mg / dL to reduce total cholesterol levels.[12] Based on current study, the peels of *Parkia speciosa* Hassk was more effective to reduce total cholesterol levels with an average reduction of 52 mg/dL.

Conclusion

In conclusion, the ethanol extract of *Parkia speciosa* Hassk peels contains several secondary metabolites include 0.21% alkaloids (w/w), 8.34% flavonoids (w/w), 4.21% tannins (w/w) and 0.18% saponins (w/w). The administration of 300 mg/kg BW of *Parkia speciosa* peels extract was an effective dose to reduce total cholesterol levels in hypercholesterolemia rats.

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Histopathological Evaluation of Non-Obstructive Azoospermic Males Using Testicular aspirate (TESA) biopsy

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Abstract

Background: Male infertility considers a real diagnostic challenge because of several factors have been involved in its pathogenesis. Histopathology of the testicular biopsy consider the corner stone in the diagnosis. The aim of this study is to report the utility of using of Testicular aspirate biopsy (TESA) for the evaluation of the male infertility & compare the outcomes with different parameters.

Materials & methods: we design prospective study for 205 infertile males with Non -Obstructive azoospermia who referred to the Teeba IVF & genetic center for the period from December 2016 to December 2018, they underwent bilateral TESA for histopathological evaluation & 45 patients out of 205 male had previous open testicular biopsy. The hormonal profile for all patient. were done. **Results:** among 205 patients underwent TESA procedure the commonest histopathological pattern was spermatocyte maturation arrest, present in 113 patients (55.1%) followed by round maturation arrest in 41 (20%). There was a good degree of agreement between the spermatogenesis scoring according to TESA and open surgical biopsy (P=< 0.001). The plasma levels for both LH & FSH were statistically showing significant differences in their levels according to the TESA spermatogenesis scoring. (P<0,001).**Conclusion:** the utility of TESA biopsy in the evaluation of testicular biopsy from Azoospermic males is significantly associated with the histopathological pattern of spermatogenesis & hormonal profile.

Key words: *TESA, Non -obstructive azoospermia (NOA), histopathology, microscopic testicular sperm extraction (TESE), spermatogenesis score.*

Introduction

Infertility in general can be defined as failure to achieve conception after twelve months of sexual unprotected intercourses.[1] Approximately 10% of couples unable to conceive and asked treatment for primary infertility with equal percentage unable to achieve a second conception . [2,3]

Male infertility causes can be subdivided into three main categories; pretesticular, testicular & post testicular.[4]

Azoospermia simply defined as complete absence of spermatozoa in the ejaculated semen for at least two times & its account for about 1% of male population & 10 % of infertile male.[5]

For male infertility evaluation, it's very important to have a comprehensive clinical history & complete physical examination along with hormonal profile and semen analysis [3], however different testicular phenotype changes can't be predicted accurately through these parameters mentioned above only, so clinicians recommended testicular biopsy and / or testicular sperm aspiration (TESA) to the accurate evaluation of the spermatogenesis and any pathological conditions.[6,7]

The aim of the present study is to evaluate a sample of Iraqi azoospermic males clinically & pathologically & to compare the efficacy of TESA biopsy versus open surgical testicular biopsy in evaluating the process of

spermatogenesis.

Materials and Methods

Between the period from December 2016 to December 2018 a prospective study for205 infertile azospermic men was done, a detailed history & physical examination for all patients were done to exclude obstructive azoospermic ones by specialist in the center, and hormonal assessment for testosterone, LH, and FSH were performed.

All patients then were subjected to bilateral testicular sperm aspirate (TESA) procedure under general anesthesia, the samples that obtained were divided into two parts; one to be fixed in liquid nitrogen when it was positive for mature sperms, the other part preserved into Boun's fixative for histopathological assessment.

Only 45 men who undergone TESA have pervious open testicular biopsies, the slides for those biopsies together with those from TESA biopsies were examined by two expert pathologists and the scoring for spermatogenesis was performed according to Johnsen 1970 modified by Holestein et. al. [8] And then the patients were subdivided into six groups according to the histopathological patterns.

Statistical analysis: Statistical analysis was carried out using SPSS version 20. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as (Means ± SD). ANOVA test was used to compare means between three groups or more. Kappa statistics was used to assess the degree of agreement between paired categorical readings. A p-value of ≤ 0.05 was considered as significant.

Results

The histopathology of 205 TESA biopsies were reviewed retrospectively with reviewing the results of open testicular biopsies for 45 patients. the age range of the patients from 19-60 years, 195 of patients were of primary infertility (95.1%), 10 patients were of secondary infertility (4.9%), with 76.6% of the total patients being with elevated FSH, 64.9% elevated LH and 89.3% with low testosterone (table 1).

Table 1: The Distribution of patients according to study variables

Study variables			
Age (years)		(36.89± 7.64)	(19-60)
Type of infertility	**Primary**	195	95.1%
	Secondary	10	4.9%
	Total	205	100.0%
FSH (IU/L)	**Normal**	48	23.4%
	Elevated	157	76.6%
	Total	205	100.0%
Testosterone (ng/dl)	**Normal**	15	7.3%
	Low	183	89.3%
	Elevated	7	3.4%
	Total	205	100.0%
LH (IU/L)	**Normal**	133	64.9%
	Low	2	1.0%
	Elevated	70	34.1%
	Total	205	100.0%

In this study, the most common pattern of spermatogenesis, according to TESA scoring was the spermatocyte maturation arrest, present in 113 patients (55.1%) fallowed by round maturation arrest in 41 (20%) (table 2) and according to testicular biopsy the spermatocyte maturation arrest pattern of spermatogenesis is present in 18 patients (40.0%) (table.3).

Pattern			
	Spermatogenesis score	**No.**	**%**
Normal spermatogensis	10	0	0%
Hypospermatogensis	9 and 8	8	3.9%
Round spermatid Maturation arrest	6 and 7	41	20%
Spermatocytes maturation arrest	3, 4 and 5	113	55.1%
Sertoli cell only	2	19	9.3%
No germ cells and no sertoli cells	1	24	11.7%
Total		205	100.0%

Table 2: distribution of Azoospermic males according to histopathological pattern in TESA biopsies. (n=205)

Table 3: The classification of patients according to open biopsy score (n=45)

Pattern	**Histopathological classification of testicular biopsies**		
	spermatogenesis score	**No.**	**%**
Normal spermatogenesis	10	0	0%
Hypospermatogensis	9 and 8	3	6.7%
Round spermatid Maturation arrest	6 and 7	11	24.4%
Spermatocytes maturation arrest	3, 4 and 5	18	40.0%
Sertoli cell only	2	6	13.3%
No germ cells and no sertoli cells	1	7	15.6%
Total		45	100.0%

Table 4 & Fig. 1 show the degree of agreement between the spermatogenesis scoring according to TESA and open biopsy. The value of Kappa equal (0.701) which indicate good agreement between two types of score. (N=45, P=< 0.001*).

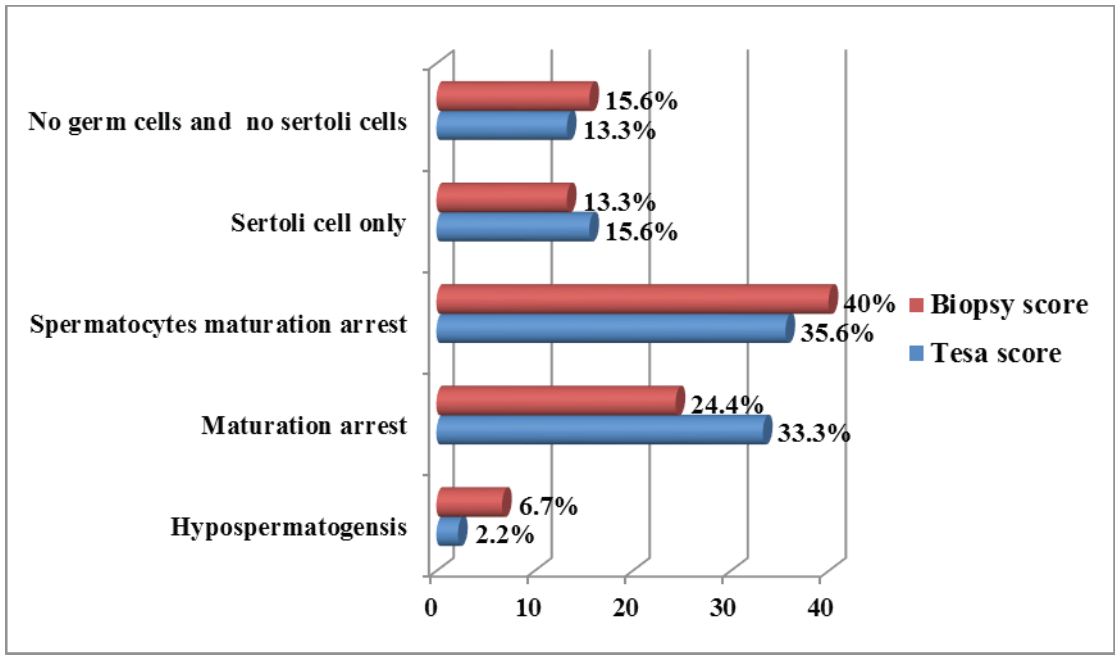


Figure 1: Comparison between spermatogenesis scoring using TESA biopsy & open surgical biopsy (n=45)

Table 4: Degree of agreement between diagnosis of patients according to TESA and open surgical biopsy scores.

Results according to TESA score	Results according to surgical biopsy score		Total	Proportion in agreement	Kappa	P-value
	6-9	1-5				
6-9	12 (26.7%)	4 (8.9%)	16 (35.6%)	0.867	0.701	< 0.001*
1-5	2 (4.4%)	27 (60.0)	29 (64.4)			
Total	14 (31.1%)	31 (68.9%)	45 (100.0%)			

The mean plasma levels for all patients for testosterone, LH and FSH were 360 ng /dl, 8.18mIU/L and 24.20mIU/L respectively. There were no statistically significant differences among the groups of spermatogenesis scoring according to the plasma levels of testosterone, while the plasma levels for both LH & FSH were statistically showing significant differences in their levels according to the TESA spermatogenesis scoring. (P<0,001) as shown in Table 5.

Table 5: The mean differences of testosterone, LH and FSH according to TESA biopsy score.

Pattern (according to TESA score)	No.	Testosterone ng /dl		LH mIU/L		FSH mIU/L	
		Mean	SD	Mean	SD	Mean	SD
Hypospermatogensis	8	362.00	154.22	7.4250	6.48311	20.52	16.25
Round spermatid Maturation arrest	41	395.21	187.85	5.2976	3.40533	13.80	10.03
Spermatocytes maturation arrest	113	360.04	158.58	7.8628	4.39440	23.52	11.69
Sertoli cell only	19	357.47	184.20	11.7263	7.21563	38.37	14.78
No germ cells and no sertoli cells	24	301.04	184.04	12.0417	7.63151	35.18	20.38
Total	205	360.00	170.33	8.1800	5.49107	24.20	15.01
P - value	0.329			<0.001*		<0.001*	
F- value	1.162			9.172		16.284	

Discussion

Infertility is not uncommon health problem in multiple societies & male infertility account for about quarter of the causes of infertility. [9]

In this study evaluation of non - obstructive azoospermic males were done by TESA mainly & in 45 cases the TESA were preceded by open surgical biopsy, the most common pattern for spermatogenesis was spermatocytes maturation arrest similar to the results obtained by several studies although none of them use TESA like Al- Dabbgh et al [10] that is carried in Iraq Using testicular FNA for assessment of spermatogenesis & also Khalifa et al [11] , Seo and Ko [12], Amer et al [13], and Tusjimura et al [14]. while other studies reported lower frequency of spermatocytes maturation arrest like those carried in Saudi Arabia by Molham M et al [15] & in Jordan by Haddad et al [16] revealed that the most prevalent pattern of spermatogenesis among azoospermic males was normal spermatogenesis followed by hypospermatogenesis putting in our minds that those studies were not excluding patients with Azoospermia due to obstructive causes as in ours, Beside that germ cell maturation arrest may be due to variety of causes like genetic factors or secondary causes such as toxic substances (chemotherapy or radiotherapy), or due to certain chronic renal or liver diseases.

In this study there was good agreement in spermatogenic scoring between TESA and open biopsy, this result indicate that TESA can be used as a substitute to open biopsy because its less invasive and eliminate the need for incision, it's also simple & cost effective than those of TESE & open surgical biopsy which report higher incidence of intra- testicular hemorrhage, a more important advantage of TESA procedure is low complication rate when it compared with other procedures.

However, the testicular architecture is well preserved in TESA biopsy than in testicular FNA .

These results agrees with Abraham Kurien et al [17] Sridevi et al[18], who found no significant differences between spermatogenic patterns in testicular biopsy and

testicular FNA, this result indicates that TESA can be used as a substitute to FNA which is less representative.

Our present study revealed that most of the patients were of primary infertility type (95.1%) with 76.6% of them being elevated FSH, 64.9% elevated LH and 89.3% with low testosterone, these results agree with Ramesh Babu et al[19] , and Accordingly, most of the patients in the current study are with hypergonadotrophic hypogonadism which may be due to different etiologies ; congenital or acquired causes (due to damage to or dysfunction of the gonads) include orchitis, trauma, surgery, autoimmunity, chemotherapy, radiation, infections (e.g., sexually-transmitted diseases), toxins (e.g., endocrine disruptors), and drugs (e.g., antiandrogens, opioids, alcohol). [20,21,22]

Preoperative assessment for FSH levels in azoospermic men has long been used as an indicator for prognosis & to correlate with spermatogenesis score as established by several studies using different methods like Ezeh et al[23] showed that there were significant differences between means of FSH level and pattern of spermatogenesis according to testicular biopsy and similar results obtained by Tunc L et al [24] with TESE biopsy, our study revealed similar outcomes with significant differences between FSH levels & spermatogenesis score (P<000.1). according to these results, FSH level can be used as indicator or predictor for patients pattern according to TESA scores.

Our study also revealed the same correlation between LH levels & TESA scores with the highest levels being with those of spermatogenesis score one & two , in contrast to other studies carried by Elchanan Bar-On et al [25] and Tomomoto Ishikawa et al [26] , who revealed that serum LH level appears to have no significant association with spermatogenic pattern in testicular biopsy, while our results were in agreement with the results obtained by Ramesh Babu et al [19] and Micic S et al [27] who revealed that mean serum LH levels were significantly associated with spermatogenesis score with the highest level in Sertoli- only categories (score 2).

Similar to several studies carried worldwide, we didn't notice significant differences between spermatogenesis scoring obtained by TESA scoring & serum testosterone level: Elchanan Bar-On et al [24] showed that plasma testosterone level is of no diagnostic value in predicting any specific spermatogenic pattern in testicular biopsy. Sultan A. Althakafi et al [28] and Reifsnyder JE et al [29] showed that serum testosterone level appears to have no significant association with microdissection testicular sperm extraction (microTESE) outcomes in non-obstructive azoospermia. Tomomoto Ishikawa et al [26] concluded that testosterone level was unrelated to the stage of maturation arrest in testicular biopsy .

In summary, We demonstrate that TESA biopsy is an efficient method for assessment of spermatogenesis in NOA males, its valuable surgical method as its faster, less invasive &less coasty than TESE, and can cover several areas of the testis more than those covered by open surgical biopsy, with keeping the testicular architecture well preserved if it compared with testicular FNA.

Ethical Clearance: the research was prepared and done according to standardized national law of research committee of ministry of higher education and scientific research of Iraq.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Affirmation-Tapping to Reduce Pain Perception and Glutamate Serum Levels of Post-cesarean Patients

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Abstract

Introduction: Affirmation, by praying and positive evaluation accompanied by acupoint stimulation, has been shown to reduce pain in postoperative patients. In other studies, affirmations can reduce chronic pain due to the down-regulation performance of Glutamate receptors. Acupuncture can reduce pain complaints by modulating Glutamate at the spinal level. So it is suspected that affirmation-tapping can reduce post-surgical pain due to modulation of Glutamate; however clinical studies have not been conducted. The aim is to compare the pain perception of postoperative patients given affirmation-tapping therapy with other treatment patients as complementary nursing interventions. This is to see if the modulation of the performance of serum Glutamate levels is different from other treatments. **Methods:** We used a randomized post-test control group design that was performed parallel in post-cesarean patients. A sample of 40 patients was divided into four groups (10 in affirmations, 10 in tapping, 10 in affirmation-tapping and 10 in controls). They were obtained through simple random sampling. The instruments included affirmation-tapping guides, Elisa kits and McGill Melzack Pain's short questionnaire form (MPQ-sf). The independent variable was the affirmation-tapping intervention and the dependent variable was the perception of pain and Glutamate serum level. Data were analyzed using simple linear regression. **Results:** The average of Glutamate levels in the Affirmation-tapping treatment group was lower (0. 034±0. 004) pg/mL and significantly different (Sig=0.00) from other groups (0. 056±0. 011) pg/mL. **Conclusion:** Affirmation-tapping as a complementary nursing intervention decreases pain perception and Glutamate serum levels in patients post-cesarean section that supports conventional treatment. Affirmation-tapping is recommended as an intervention to overcome pain perception in postoperative nursing patients who support conventional treatment.

Keywords*: affirmation-tapping, complementary, pain, glutamate serum*

Introduction

Untreated acute postoperative pain results in impaired wound healing, even chronic if it is not treated immediately. The activation process of N-Methyl-D Aspartate (NMDA) increases central and peripheral nerve sensitivity, produces long-term potentiation (LTP), and pain complaints last longer[(1)]. Even though they have been treated, there are still many post-operative pain complaints felt by the client. Severe pain after heart surgery was reported by 28% of patients[(2)], severe pain after thoracic surgery was reported by 25% of patients, even chronic. Moderate post-sectional pain was reported by 48.2% of patients, and pain incidence was 92.7% (IC 95%: 90.9-94.2). Average pain intensity at worst 6.6 (dp = 2.2)[(3)]. Complaints of pain produce disturbances in the process of recovery, wound healing[(1)] and impaired

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productivity[4]. An incomplete pain intervention will reduce a person's quality of life[5]. Affirmation-tapping has been proven successful in overcoming pain complaints, but scientific evidence and linkages with Glutamate have not been studied.

Post-operative pain from moderate to severe is still felt by more than half of all patients who have undergone surgery, despite receiving care as a standard postoperative patient[6,7]. Recommendations for postoperative pain management with drugs and non-drugs[8,9] and a complementary approach with Affirmation-tapping[10,11]. Postoperative nursing care using the Spiritual Emotion Freedom Technique (SEFT) method has been performed on postoperative patients and it provides good benefits[10,11]. Reading the verses of the Koran as a prayer has also been proven to overcome the pain of post-operative patients[12]. The complementary nursing approach has consistently been in line with nursing care policies, specifically for managing pain. This is still rarely done because of limited scientific support regarding the performance and effectiveness of complementary affirmative nursing interventions[8,9,13]. The aim is to compare the perception of average pain reported by postoperative patients given Affirmation-tapping with other treatments as complementary nursing interventions. It is proven that the performance of Glutamate serum levels is different from how it is in other treatments, where affirmative sentences are used as prayers. They take verses from the Koran and this is still combined with the stimulation of several acupoints simultaneously.

Materials and Methods

This study used a Randomized Controlled Post-test Design group in parallel for all treatment groups. The study sample was a partial postoperative cesarean section taken by simple random sampling of 40 respondents[14]. Random assignments were then carried out so there were ten respondents for each affirmation, tapping, affirmation-tapping, and control group+. The criteria for inclusion in the sample were: 1) Moslem patients after cesarean section who volunteered to participate after getting an explanation and 2) they were recruited on the first day of the 4th hour after surgery, 3) they were 18-41 years old, 4) they did not have complications outside of pregnancy and childbirth and 5) they receive anti-pain treatment according to hospital standards

The independent variable was affirmation-tapping intervention and the dependent variable was pain perception and Glutamate level. Data collection tools were 1) affirmation-tapping procedure guidelines, 2) McGill Melzack's short pain questionnaire with permission from Prof. Melzack, with adjusted language[15,16] and 3) equipment used to take venous blood specimens[17] Glutamate levels were analyzed using the ELISA kit[18], conducted by the Institute of Tropical Diseases (ITD) Airlangga University. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8 °C. Add 50µl standard to standard well. Note: Don't add antibody to a standard well because the standard solution contains a biotinylated antibody. Add 40µl of sample to sample wells and then add 10µl of anti-GLM antibody to sample wells, then add 50µl of streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37 °C. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37 ° C in the dark. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution[18]. All groups received standard treatment, with the affirmation treatment group getting additional affirmations, praying with the Qur'an surah Al-Fatihah for 10 minutes. The tapping group added knock for 5 minutes while the "affirmation-tapping" group added affirmations and knocked at the same time for 10 minutes. The control group only received standard care four hours after the end of anesthesia. The distance between treatments was 8 hours, and they were given four treatments. Then, 10 minutes after the last treatment, pain perception data were collected using MPQ-sf.

Venous blood specimens are then taken for examination of Glutamate levels using the ELISA method. Data analysis was directed to examine the different effects of Glutamate on pain perception for affirmation. Data processing of pain perception and Gutamate from four groups was carried out through simple linear regression with a significance level of 95%.

Findings

The characteristics of participants from all groups were listed in Table 1. The mean age was 32.1 (± 5.8) years. Bodyweight was 56.5 (± 7.8) kg and height was 164.5 (± 8.8) cm. Systolic pressure is 127.9 (± 4.3) mmHg, diastolic pressure is 84.0 (± 7.0) mmHg. The pulse frequency is 88.2 (± 0.6) bpm, the respiratory frequency is 21.2 (± 2.1) per minute. All data are normally distributed (-2 <Skewness Ratio <2), so comparative analysis can be carried out between groups.

Table 1. The characteristics of respondent (n=40)

Variables	Group							
	Statement (No.10)		Tapping (n=10)		Affirmation-tapping (n=10)		Control+ (n=10)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (year)	32.1	19.12	31.1	6.4	31.4	5.1	29.3	6.1
Weight (kg)	55.7	4.9	54.4	7.9	56.5	7.8	52.9	6.4
Systolic	126.8	4.7	127.9	4.3	125.9	6.5	125.5	5.0
Diastolic	79.6	1.3	78.6	5.1	81.5	6.9	84.0	7.0
Pulse	88.2	0.6	88.2	3.8	84.6	3.8	87.2	2.7
Respiratory	21.0	3.0	20.8	2.5	20.4	2.1	21.2	2.1

SD = Standard Deviation

Table 2. Pain perception and glutamate serum levels (pg/mL) per group (n=40)

Variables	Group							
	Statement (No.10)		Tapping (n=10)		Affirmation-tapping (n=10)		Control + (n=10)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pain perception	3.927	0.586	4.795	0.598	3.197	0.835	4.919	0.522
Glutamate	0.042	0.001	0.040	0.001	0.034	0.004	0.056	0.011

SD = Standard Deviation

Table 2, it can be seen that the highest average pain perception was found in the control group+ (4.919±0.522) and the lowest in the "affirmation-tapping" group (3. 197±0.835). Glutamate levels were highest in the control group+ (0.056±0.011) pg / mL and lowest in the Affirmation-tapping group (0.034±0.004) pg / mL. To test the effects of pain perception treatment related to serum Glutamate levels, a simple linear regression analysis was performed. Regression requirements ie normality of residual data normally distributed, fulfilled (Sig residual data = 0.072 >0.05). The result of simple linear regression analysis obtained Sig=0.00 (<0.05) means that there is an influence between Glutamate on pain perception due to affirmation-tapping treatment.

Discussion

Data processing proves that affirmation-tapping reduces the patient's pain perception after a cesarean. Clinical trial studies conducted in hospitals prove that affirmation-tapping as a complementary approach has been able to reduce patient pain in accordance with integrative treatment recommendations for the treatment of postoperative pain patients (19). Research related to affirmation-tapping also helps reduce the pain of dysmenorrhoea in adolescents[20].

Research conducted by Wijiyanti has also proven the affirmation-tapping approach can reduce pain after a cesarean section[11]. The affirmation-tapping approach has also been shown to reduce the pain suffered by cancer patients[21], control complaints of fibromyalgia pain[22] and control pain and depression complaints of war veterans[12,23].

Biological perception of pain is the result of the accumulation of stimulus and response performance that is consciously or unconsciously controlled by the brain, especially the forebrain and central nervous system including the spinal cord[24-26]. By utilizing descendent pain control pathways, the course of stimulus and pain response can be controlled using neurotransmitter media through the forebrain and amygdaloid brain[27,28]. Empowerment of the forebrain by affirmation can eliminate the default-inhibition (inhibition of functional work as a necessity) of the amygdaloid[27] to activate the descendent pathway that blocks pain signals that lead to the spinal cord. This means that the transduction of pain through the ascendent pathway to the central nerve and brain can be prevented[29]. Praying with attention, focusing and thinking about Allah SWT followed by acupoint stimulation through affirmation-taping will inhibit Glutamate[30]. This will modulate their pain perception as a result of the inhibition of pain transduction[32].

Affirmations using the Surah Al-Fatihah prayers that are sincerely and sincerely hope Allah's help can multiply empowerment in the forebrain and amygdaloid, improve its performance so that the pain control function becomes better and more effective[12,29,33,34]. Tapping as a form of acupoint stimulation can inhibit the transduction of pain from various areas of the body to the center, thereby stimulating pain from surgical wounds can be inhibited. As a result, the participants did not suffer pain[30].

Affirmation-tapping lowers Glutamate levels, thereby strengthening the performance of descendent pain inhibitors, thereby inhibiting peripheral to central pain transduction, thereby overcoming pain complaints[35-37]. The limitation of this study is that there is no screening for diabetic participants. Affirmations are made using prayers from the Surah Al-Fatihah followed by knocking on several acupoints to reduce postoperative pain complaints. Affirmation-tapping interventions can be continued and recommended by nurses who have been trained and licensed to do so.

Conclusion

Participant pain complaints were lowest in the affirmation-tapping group with low Glutamate levels, this was very different from the control group. Affirmation-tapping has been shown to have a therapeutic effect in the context of overcoming complaints of pain after a cesarean section. The novelty of this study is the affirmation-tapping performance of reducing pain complaints associated with Glutamate. Affirmation-tapping with Al-Fatihah prayer can be recommended to overcome pain complaints as a nursing approach.

Conflict of Interest: No

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Prevalence of Cryptosporidium Infection in Calves with Effect on Biochemical Parameters

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Abstract

This study was conducted throughout the year on 93 calves from different regions of Baghdad province. Three age groups included; Below 1 month, 1-2 months and above 6 months. A changed aim-the technique of stain Neelsen (MZN) has been used for the identification of the development of cryptosporidium and fecal diarrheic samples.

In biochemical research, blood samples have been obtained. Cryptosporidium occurrence was much higher in spring season (56%), followed by the winter (52%), the allergy season (46%), and lowermost in a season of summer (40%).The highest rate of infection in animals which was given was the age group of the animals that under the age of 30 days (71%), 2-6 months were (59%) and (43%) were over 6 months old. The animals concerned reported substantial rises in plasma, urea and creatinine relative to stable animals in the management of biochemical changes. Finally, Age and seasonal changes were associated with cryptosporidiosis. The biochemical factors affected by the calves also have a negative effect.

Key words: *Calves, Cryptosporidium, Age, Season.*

Introduction

Cryptosporidium is one of the most military personnel responsible for many outbreaks over the worlds. They often participated in military exercises in pathogens, especially in calves until the age of one month [(1)]. There was a link between the era of animals and animals [(2)].

Most cryptosporidium, *C. parvum* is the main parasite and later calves found in the genus Epicomplexan and *C. bovis*. The causes of the rash are determined. C. Androsi was the largest number of host species, including birds and mammals, is found most frequently in livestock over one year of age [(3)]. Fish and fecal reptiles [(4)]. The infection was spread mainly by calf samples, as 19.2% of the germs passed through calves infected with Cryptosporidium [(5)].

Economic losses due to infection were in 2 weeks of age when 29 were not the only result of illness due to death, 30 calves stimulated the eye. On a dairy farm, but due to the slow growth of livestock, in Maryland, the United States cost medications that were widespread before birth and veterinary aid and labor growth was higher in calves (1-8 weeks old) (45.8%) was. Later included [(6)]. Cryptosporidia is a clinical disease, in which calves (3 to 12 months) (18.5%) and wheels (12 to 24 are usually the most common symptoms of gastroenteritis) (2.2%) [(7)]. The signs of cryptosporidiosis starting with the mild and severe symptoms of a large proportion of diarrheal disease among American hosts [(8)]. Many studies done in iraq to investigate the prevalence of Cryptosporidium infection in man animals [(9; 10)].

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This study was designed to investigate the prevalence of cryptosporidiosis in calves at different ages and seasons of Baghdad province.

Materials and Methods

Animals:

During a year, 93 calves were obtained from various area in Baghdad province. There are three age groups: less than a month,1-2 months and above 6 months.

Sample Collection:

In a clean container with the labels stool samples have been taken out of the animal's rectum. Samples were formed and skewed. Blood samples from all animal tested in a sterile tube, and made centrifuge in 3,000 rpm, were collected for biochemical analysis and separated into smaller, dated and keep tubes at _20 ◦C. Biochemical tests investigated in this study include creatinine and urea which tested by using commercial kits of Biolabo company.

Detection of Oocysts:

Soft smear stains with a Modified Zeihl - Nielsen Stein (MZN) adjustment. Eggs were measured with the help of a stage micrometer connected to an optical microscope at 10x and 100 x on the target. All measurements for about 20-50 eggs per micrometer (nanometers) [11].

Statistical Analysis:

This is done by using a Software (SPSS).

Results

Oocysts:

Individuals who discovered Cryptosporidium in the fecal calf with the Ziehl-Nielsen technique were of the same size as Cryptosporidium perfume tissue in an oval shape on a smooth acid-fast wall (pinkish red). 50 eggs measure with an average of 4.4 to 5.8 x 4.3-4.9m (5.1 x 4.6) and index 1.0–1.2 with average (1.1) (Fig. 1).

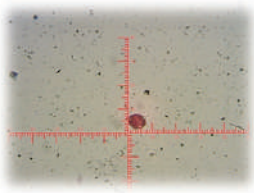


Figure 1. Cryptosporidium

Cryptosporidium Infection prevalence in calves at different seasons, age ranges and fecal quality:

As for the seasonal frequency, the occurrence was much higher in spring season (56%), followed by the winter (52%), the Autumn season (46%), and lowermost in a season of summer (40%).The highest rate of infection in animals which was given was the age group of the animals that under the age of 30 days (71%), 2-6 months were (59%) and (43%) were over 6 months old.

Some Biochemical indices alteration in relation with Cryptosporidiosis:

The biochemical study of infected calves has shown that blood urea and creatinine rates have risen significantly increase than non-infected calves. In the other hand, there was a significant rise in blood urea rates with an increase of blood creatinine.

Discussion

It was observed that in this study, a higher prevalence of cryptosporidium was found in calves under a month, followed by 30-60 days, whereas animals over six months old had the lowest incidence of Cryptosporidium. These findings were in agreement with other study showed that the infection rate among individuals is high (11,7 to 15,6 %) younger than one month and then reached 0% in individuals older than 4 months [12,13]. Many researches have shown that pre-calf calves are only significant sources of animal origin [14], which are of animal origin in calves less than two months old.

There may be several factors explaining the difference in the prevalence of these studies from the current study, such as severity of infection, health measures, foot multiplication, and screening season. Any of these factors may increase the risk of cryptosporidium transmission or spread among calves individually or collectively [15].

It has been found that cryptosporidiosis is more prevalent on oblique calves than on non-skewed animals, as these findings were accepted during the spring and in the summer at the lowest levels, It was in agreement with these findings [16] and other investigator [17] found that the most widespread of cryptosporidiosis was in spring season then winter, autumn, and summer. The disparity between regions and weather (humidity and temperature) can be attributed to these variations. If the precipitation or amount of births in several seasons has

risen, seasonal effects can only be calculated accurately if many successive years of studies are repeated.

The study showed a large rise in serum urea and creatinine relative to healthy animals in infected animals. The results were consistent with L-Desocki and L-Massey [18], who reported that serum urea and creatinine levels increased significantly in infected calves. Also, [19] found a marked increase in serum urea levels with a slight increase in creatinine after infection with cryptosporidiosis. A large rise in blood urea levels in calves and renal insufficiency may be attributed to the fluid loss in order to retain fluid in the body [20]. However, the high rates of all metabolic waste may be attributed to a significant raise in serum creatinine levels in diseased calves, including creatinine as a result of the reduction in the urine for fluids in the body [21].

Conclusion

Cryptosporidium is a parasite that causes calves diarrhea, which slowly decreases with age, and adults are unable to disrupt the parasite. Moreover, disease management is strongly influenced by the system and associated with seasonal changes and negatively affects the biochemical factors of the affected calves.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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Evaluation of Lifestyle among Patients with Irritable Bowel Syndrome in Kirkuk City

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Abstract

Background: Irritable Bowel Syndrome (IBS) is one of the utilitarian gastrointestinal disorders regularly found in the all inclusive community

Aims: To evaluation the lifestyle among patients with Irritable Bowel Syndrome according to physical, psychosocial and independence areas.

Methodology: The samples purposively collected from the patients who referred in Azadi teaching hospital and Kirkuk general hospital in kirkuk city for period from 21th October in 2018 to 28th of April in 2019.The study sampled comprise of (101) patients with IBS diseases were chosen (male & female). A questionnaire was developed for purposive of study & included 2 parts were demographic data and lifestyle of irritable bowel syndrome. The data were arranged for, age, gender, residence and the results of the procedure.

Results: The age group (20-30) years was represent (26.5%) of the sample, the female represent (48.5%) from the sample. And findings of the study indicated that the physical domain, also the psychosocial domain of the lifestyle for patients had greatly affected.

Conclusion: The study highlights that is most of the patients of IBS to have constipation. Also in a physical domain with good quality of life. Thus, the socio-demographic factors that appear to be most significant in this concomitant with chronic disease condition.

Recommendation: The study recommended future studies that include measurement of dietary factors, habits, exercise and psycho-social factors are needed to understanding of the scope and dimensions of IBS in lifestyle.

Key Ward: *Lifestyle, Irritable Bowel Syndrome.*

Introduction

Irritable Bowel Syndrome (IBS) is one of the practical gastrointestinal issue regularly found in the general population [1]. It is described by intermittent abdominal pain associated with change in bowel habits, diarrhea and/or constipation and swelling. Based on these manifestations, three subgroups have been differentiated: constipation-predominant(IBS-C), diarrhea-predominant(IBS-D)and those with substituting bowel movements[2].The commonness of IBS in the all inclusive community in the western nations is 15-24% independent of age and ethnicity, and the male to female proportion is 1:1.5. In any case, a lower prevalence of 5-10% has been found in Asian countries while considers from Africa have detailed prevalence of between 8-30%[3]. The exact an etiology of this condition is yet to be established but, several mechanisms have been proposed as the hidden pathogenesis and these incorporate visceral hypersensitivity, change in intestinal motility, psychological status and abnormal bowel barrier[4]. Psychological distress and stressful life occasions have been found to impact clinical introduction and results of IBS[5]. Irritable bowel syndrome (IBS) is characterized as bowel pain and inconvenience with

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changed intestinal habits in the absence of any other mechanical, inflammatory, or biochemical clarification for these manifestation. [6].It is the most widely recognized digestive disorders. The prevalence of IBS is gone between 10-20 % worldwide. It influences the lifestyle of those who suffer from it, and records for large amounts of health care resources, both in essential and expert consideration[7].

Methodology

Design: To achieve the objectives of the study, a descriptive study was carried out from the 21th October in 2018 to 28th of April in 2019

Sampling: A purposive sample that comprise of (101) patients, (35) males and (66) females chosen according to the following criteria:- Age ranges between (20 -80) years and definitely diagnosed with Irritable bowel syndrome patients were included from the present study.

Setting: The present study was conducted at azadi teaching hospital and Kirkuk general hospital which receiving large number of patient who have IBS done for them.

Tools: A questionnaire was adopted and developed for the purpose of the study, it comprises of [2]parts, part (I) included demographic characteristic of patients which consists of [8] items. It includes (age, gender, marital status, educational Level, occupation , Types of IBS, Concomitant with chronic disease, Common food source). Part (II) lifestyle of irritable bowel syndrome(IBS) It consist of [3] domains They are: Physical domains, psychosocial domains and level of independence domains: The overall items were (38) items, All items were measured by using 3-likert scale option were used in the rating scale as (Always, Sometime, Never) . The data were collected through the utilization of constructed questionnaire, interview technique with the patients in hospital. The data was collected between (29th November in 2018 to 28th February in 2019).

Data analysis: Data were organized and coded into the computer files; Statistical Package for Social Science (SPSS) version (24) that was used for data analysis while the significance level depended was (P. value < 0.05). Descriptive statistical methods (Frequency, percentage, Mean,) and Inferential statistical methods (t-test, and ANOVA)were used to analyzed the data.

Results

Table (1): Distribution of the Socio-Demographic Data of the Study Sample (No=101).

Variables	Frequency	percentage
Age		
20-30 years	36	26.5
31-40 years	18	13.2
41-50 years	13	9.6
51-60 years	22	16.2
61-70 years	10	7.4
71-80 years	2	1.5
Total	101	74.3
Gender		
Male	35	25.7
Female	66	48.5
Total	101	74.3
Marital status		
Single	32	23.5
Married	46	33.8

Cont... **Table (1): Distribution of the Socio-Demographic Data of the Study Sample (No=101).**

Divorced	4	2.9
Widowed	19	14.0
Total	101	74.3
Level of education		
Unable to read and write	17	12.5
Read and write	9	6.6
Primary school graduate	16	11.8
Intermediate school graduate	7	5.1
Secondary school graduate	13	9.6
Institution graduate	7	5.1
College graduate	32	23.5
Total	101	74.3
Occupation		
retired	4	2.9
jobless	7	5.1
employee	25	18.4
house wife	41	30.1
student	24	17.6
Total	101	74.3
Type of IBS		
constipation- predominant	55	40.4
diarrhea- predominant	13	9.6
mixed IBS	33	24.3
Total	101	74.3
Concomitant with chronic disease		
diabetes	15	11.0
hypertension	13	9.6
neuron	11	8.1
arthritis	7	5.1
digestive	16	11.8
pulmonary	4	2.9
diabetes and hypertension	35	25.7
Total	101	74.3
Common food source		
home	64	47.1
Restaurant and fast food	37	27.2
Total	101	74.3

Table(1) indicates the socio-demographic characteristics of the whole study sample. The it shows that the highest percentage of age groups is between(20-30years) and constituted(26.5%), and according to gender, the high percent was of females and constituted(48.5%). Most of the patients were married and constituted(33.8%).(23.5%)of the sample were college, As regard to occupation, (30.1%) patients were housewives. With relation to type of IBS, the majority of the subjects (40.4%) were constipation. The high percent in concomitant with chronic disease and constituted of (25.7%),were diabetes and hypertension. Finally, in respect to the food source the majority of the sample (47.1%)were scheduled for food intake at home.

Table(2)One-way Analysis of Variance for IBS Patient's Lifestyle Domains in respect to their Age.

Domains	Groups	S.O.S	Degree Freedom	M.S	Frequency	Significant
Physical domain	Between Groups	128.119	5	25.624	2.543	.033
	Within Groups	957.208	95	10.076		
	Total	1085.327	100			
Psycho-Social domains	Between Groups	64.677	5	12.935	.546	.741
	Within Groups	2251.164	95	23.696		
	Total	2315.842	100			
Level of independence domains	Between Groups	52.850	5	10.570	.643	.667
	Within Groups	1561.725	95	16.439		
	Total	1614.574	100			

Table(2)demonstrates that there is significant difference in physical domain in respect to the patient's age.

Table(3): Association of Lifestyle Domains of IBS Patients in regard to their Gender .

Domains	Mean Difference	t-value	df	Sig.	95% of C.I.	
					Lower	Upper
Physical domain	1.72727	2.577	99	.678	.39741	3.05713
Psycho-Social domains	.23550	.233	99	.072	-1.77064-	2.24164
Level of independence domains	1.36234	1.635	99	.055	-.29103-	3.01571

Table(3)indicates that there are significant differences in independence domains in respect to the patient's gender.

Table(4)One-way Analysis of Variance for IBS Patient's Lifestyle Domains in regard to their Marital status .

Domains	Groups	S.O.S	Degree Freedom	M.S	Frequency	Significant
Physical domain	Between Groups	64.274	3	21.425	2.035	.114
	Within Groups	1021.052	97	10.526		
	Total	1085.327	100			
Psychosocial domain	Between Groups	150.587	3	47.862	2.137	.050
	Within Groups	2172.255	97	22.394		
	Total	2315.842	100			
Level of Independence domain	Between Groups	48.658	3	16.219	1.005	.394
	Within Groups	1565.916	97	16.143		
	Total	1614.574	100			

Table(4) indicates that there are a significant differences in psychosocial domains in regard to the patient's marital status.

The IBS patients depicts that there are no significant differences in this domains in regard to the patient's level of education. The results for IBS Patients Lifestyle Domains in respect to Patients Occupation, it reveals that there is a significant difference in independence domain in regard to the patient's occupation. For IBS Patients Lifestyle Domains in respect to Patients Concomitant with chronic disease, the results demonstrates that there is a significant difference in physical and Psychosocial domain in respect to the patient's concomitant with chronic disease. The current results also reveals that there is a significant difference in physical domain only in regard to the patient's food source.

Discussion

Table (1) Our results demonstrate that socio-demographic data of the whole study sample. The it shows that the highest percentage of age groups is between(20-30 years) and established (26.5%). IBS is found much of the time in all age gatherings. [8] However, the prevalence of IBS is higher in the more youthful age group than the older age group in numerous studies.[9] In agreement with that, our examination indicated that most of the IBS cases were under 30 years. As indicated by gender, the high percent was of women and comprised (48.5%). Indicated that IBS has been more predominant in females than males, and it was additionally noticed that females announced less fortunate IBS-lifestyle scores than males [10]. Most of the patients were married and constituted (33.8%). (23.5%) of the sample were college, As regard to occupation, (30.1%) patients were housewives[11]. With relation to type of IBS, the majority of the subjects (40.4%) were constipation. I disagree with discoveries were quite to have been accounted by Wilson S et al, 2004 [12]. Nonetheless, others have detailed various figures where cases were divided similarly between IBS with constipation, irritable bowel syndrome within diarrhea and irritable bowel syndrome rotating among looseness of the bowels and abstraction bowel[13]. Whereas others have suggested that diarrhea IBS-D, may have a lower lifestyle than constipation IBS-C or mingled sort IBS-M, or that patients with IBS-C have a lower lifestyle than those with IBS-D. However, such examinations may be difficult to decipher, as patients with IBS regularly travel between the deferent subtypes if they are followed up for extensive stretches[14]. The high percent in associative with chronic disease and established of (25.7%) were diabetes and hypertension. Another study which utilized "WHO lifestyle and "Bradley well-being lifestyle" measures demonstrated that lifestyle was not influenced by socio-demographic parameters in diabetic patients[15]. the regard to the

nourishment source, most of the example (47.1%) were planned for nourishment admission at home. the respect to the food source, the majority of the sample (47.1%) were scheduled for food intake at home. The decent eating routine is mostly focused on increased fiber admission, improving nourishment habits in patients who are accustomed to skipping breakfast and snacks, and furthermore, on redistributing meals, calories over the 24 hr time frame. The improvement that was also observed among patients who routinely devoured breakfast, morning nibble, lunch, afternoon snack and dinner, could get from the redistribution of nourishment more than five meals[16]. Although this causative relationship is hard to demonstrate, most of patients limit or prohibit food things from their eating regimen and a little however important proportion of them have a poor reaction and an a lacking diet[17]. To find out the nonappearance or nearness of relationship between socio-demographic characteristics of IBS patients and the lifestyle among them, the tables(2– 8) shows numerous significant statistical differences in certain domains of the lifestyle among IBS patients in regard to certain characteristics of the patients; In this study, lifestyle was independently associated with age, gender, depression symptom, IBS subtypes, educational, marital status and Concomitant with chronic disease were related to lifestyle. The patients were older than in most different studies. The age has in many studies has a restricted impact on lifestyle in patients with IBS[18]. Concentrates on the impact of age, occupation, training, marriage and socio-economic status have yielded differing results on lifestyle in patients with IBS[19]. Our study finds that people's order report conflicting impacts of their IBS on their lifestyle. It is misty why women report the greatest debilitation of their lifestyle more than men. We presume that psychosocial, social and medicinal services looking for practices are potential supporters to this result[8]. Other study work of Toner et al. has given bits of knowledge into gender-related contrasts in how symptoms might be experienced and deciphered. While perceiving that studies have predominantly centered on the biologic sex contrasts among male and female illness in IBS, gender-specific subjects such as conceivable sexual and physical abuse, gender role socialization, public open shame and humiliation because of GI symptoms[20]. Kanasawa etal found that lifestyle was not identified with gender, IBS subtypes, instructive, and marital status[21]. Simren et al revealed that IBS subtypes didn't influence lifestyle[10]. Although food shirking and social response scores were few in females. However, irritable bowel syndrome in lifestyle scores were similar in males and females in most Asian study[22]. Lifestyle had a lower in patients with a lower instruction and socio-economic status is uncovered in Egypt study[23].

Conclusion

This study was showed the high percentage in the age ranging beginning(20-30)years, with gender is female. Although in high percent of type in constipation-predominant in patients. Further, the home food significantly correlate with a higher rate of IBS-lifestyle. Thus, physical domain were the most aspects of life among irritable bowel syndrome patients that were associated with many socio-demographic variables. Concomitant with chronic disease are the most socio-demographic variables that were associated with many aspects of life among irritable bowel syndrome patients.

Recommendation

1- Creating modern centers of irritable bowel syndrome to deal with physical, psycho-social problems of IBS patients because suspecting increasing number of patients will affected in future.

2- Providing patients with booklets containing information about irritable bowel syndrome, guidance's, to optimize quality of life.

3- More studies are needed to fully exploration the factors related to lifestyle of IBS in Kirkuk.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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Factors Contributed of Breast Cancer for Women in Iraq

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Abstract

Background and aim: Breast cancer is abnormal growth of breast cells, result from spurts or abnormal changes genetic that responsible for cell growth and maintain its health. It is one of the most dangerous cancers that affect woman and reports high level of morbidity yearly if not treat early.

The study aimed to assess the Factors that Contributed of Breast Cancer for Women.

Materials and method: A descriptive design was employed through the present study from November 1st 2017 to March 20th 2018, which carried out on four Health Directorates of different Governorates; (Salah Alden, Kirkuk, Diyala and Sulaymaniyah).

Assessment questioner consist of two parts contains of women socio-demographical characteristic, the other part concerning of the assessment the risk factors that contribute breast cancer. A non-probability sample (purposive approach) is selected for the study which includes (88) women diagnosed with breast cancer were treated in Hospital or Nuclear centers, or the patients who visited the outpatient clinic in the same hospital or specialist center for medical follow–up and treatment. Data were gathered through the patients` interviewed. Content validity of the questionnaire was done through eliciting the opinions of a panel of (10) expert, while the reliability of this tool is determined through application of a pilot study by using internal consistency through the computation of the Cronbach alpha correlation coefficient of the scale on data gathered from patients.

Results: The overall results revealed that most women were adult (42.0%) married (72.7%) with low educational level and they lives in urban (64.8%) with sufficient income (40.9%). Most sample`s work was house wife (64.7 %), and about half of sample were feed his children natural feed. (35. 2 %) were normal body weight and (21.5 %) of high weight.

Conclusion: Most women depended on a style of food contain :(fat, kidneys, red meat, dairy product and salt) that will be contributed to breast cancer development. Greatest women experience of psychological problems and don't compliance of breast self-examination every month.

Keywords: *Breast, Cancer, Factor, Women*

Introduction

Breast cancer starts when cells in the breast begin to grow out of control. These cells usually form a tumor that can often be seen on an x-ray or felt as a lump. The tumor is malignant (cancerous) if the cells can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. Breast cancer occurs almost entirely in women, but men can get it, too [(1).] Assessing women's risk for the development of breast cancer is important in providing primary and secondary preventive strategies such as chemoprevention, life style changes, and enhanced surveillance that may reduce a woman's risk for developing the disease [(2).] Risk Factor of Breast Cancer can be anything from a lifestyle choice (ex. diet) to a personal characteristic (ex. menarche [age when menstruation started]) to an environmental

exposure (ex. radiation) that can influence a person's risk of developing a certain disease [3]. Breast cancer incidence increases with age. It is rare before the age of 25, then incidence rises, increasing steeply with age 30–49. After age 50, breast cancer incidence continues to increase, although more slowly, to the oldest ages. In contrast the incidence of other adult and does not show the same dampening of increase in mid-life [3].

Globally, over one million women are diagnosed with breast cancer every year [4]. It is the most frequently diagnosed cancer in women and the leading cause of cancer death in women, every year more than 500,000 women die from the disease [5]. In Europe over 400,000 women are diagnosed with breast cancer every year and 120,000 women die from the disease. It accounts for 17% of female cancer deaths in this region [6].

In Iraq, breast cancer ranks the first among the commonest malignancies among all the population and accounts for approximately one-third of the registered female cancers according to the latest Iraqi cancer Registry which shows a trend for the disease to affect younger women [7].

Materials and Method

A descriptive design is adapted throughout the present study from November 1st 2017 to September 20th 2018 in order to assess the Factors that Contributed of Breast Cancer for Women.

A non-probability (purposive) sample is selected for the purpose of study which includes (88) woman patient diagnosis of breast cancer which carried out on four Health Directorates of different Governorates that collecting from it: Salah Alden: (8) patient, Kirkuk: (22) patient, Diyala: (28) patient, and Sulaymaniyah: (30) patient.

Throughout comprehensive review of relevant literature, a questionnaire is constructed by the researchers in order to achieve the objectives of the study which comprised of the following: - Part I: Socio-Demographical Data; This part includes; Governorate, age, Occupation, Educational level, Feeding type if you have child, Weight by BMI, Monthly Income, Marital status, if she was marred, widow, or divorced; (time of married), and Residence.

Part II: Genetic and Biological domain: that include (9) items. Part III: Food style and nutrition domain: That include (6) items. Part IV: Work environment and housing domain: This part consist of (5) items. Part V: Psychological and Social domain: It consist of (6) items. Part VI: Personal and family organization domain: This part include (7) items, each items which are measured on 2 levels scale of Yes, and No and rated as Yes=2, and No=1.

Reliability and validity of this tool is determined through application of a pilot study and panel of experts. The data are collected through the interview technique as a means of data collection. Each interview takes approximately (15-20 min). Data are analyzed through the application of statistical procedures and by using (SPSS) version (25) which assist to determine the study results.

Results

Table 1: Frequencies and Percentages of Socio-Demographical Characteristics (n=88)

Items Characteristics	Groups	F	%
Governorate	Salah Alden	8	9.0
	Kirkuk	22	25.0
	Diyala	28	31.8
	Sulaymaniyah	30	34.0
Age	20-29 Y	8	9.0
	30-39 Y	10	11.3
	40-49 Y	37	42.0
	50-59 Y	23	26.1
	60 y and more	10	11.3

Cont... **Table 1: Frequencies and Percentages of Socio-Demographical Characteristics (n=88)**

Occupation	Don't work	3	3.4
	House wife	57	64.7
	Farmer	11	12.5
	Private works	2	2.2
	Employed	11	12.5
	Student	4	4.5
Education Level	Read & write	4	1.21
	Primary	38	11.51
	Intermediate	27	8.18
	Secondary	159	48.18
	Institution	46	13.93
	College	56	16.96

Continued

Items Characteristics	**Groups**	**F**	**%**
Feeding type if you have child	Breastfeeding	49	69.0
	Artificial	4	6.0
	Both	18	25.0
Body Mass Index (BMI)	Low weight	12	13.6
	Normal weight	31	35.2
	High weight	19	21.5
	Low obese	12	13.6
	Middle obese	11	12.5
	High obese	3	3.4
Monthly income	Sufficient	36	40.9
	Barely Sufficient	30	34.0
	Insufficient	22	25.0
Marital Status	Single	10	11.3
	Married	64	72.7
	Widow	4	4.5
	Divorce	10	11.3
If you married or widow or divorce: Time when married	Less than 15 year	3	4.0
	15-19 year	28	36.0
	20-24 year	23	30.0
	25-29 year	16	21.0
	30-34 year	7	9.0
	35 year and more	3	4.0
Residence	Urban	57	64.8
	Rural	31	35.2

Table 2: Mean Score with Standard Deviation for women`s genetic and biological domains.

List	Item	Yes		No		M.S	SD±	LF
		F	%	F	%			
1	None of my relations has breast cancer.	48	54.5	40	45.5	1.54	0.50	M
2	My relations do not suffer from any type of cancers.	44	50.0	44	50.0	1.50	0.50	M
3	I did not get any cancers in my body places other than my breast.	74	84.1	14	15.9	1.84	0.36	M
4	There are not tumors in the breast or other places in the body.	40	45.5	47	53.4	1.48	0.56	L
5	I was not exposed to any infection or inflammation in the breast.	53	60.2	35	39.8	1.60	0.49	M
6	Absence of abnormal secretion from breast such as blood and others.	63	71.6	25	28.4	1.71	0.45	M
7	The glands and hormones of the body were normal.	70	79.5	18	20.5	1.79	0.40	M
8	The menstrual cycle was regular.	64	72.7	24	27.3	1.72	0.44	M
9	My heart is healthy and does not suffer from any disturbance or constant acceleration in the pulse.	67	76.1	21	23.9	1.76	0.42	M
10	No medical laser was used.	76	86.4	12	13.6	1.86	0.34	M

Table 3: Mean Score with Standard Deviation for women food style and Nutrition domain.

List	Item	Yes		No		M.S	SD±	LF
		F	%	F	%			
1	Do not contain foods such as fat, kidneys, red meat, dairy product and salt.	39	44.3	49	55.7	1.44	0.49	L
2	Avoid eating fruits and vegetables that are genetically modified and immature.	55	62.5	33	37.5	1.62	0.48	M
3	My food does not contain artificial foodstuff.	64	72.7	24	27.3	1.72	0.44	M
4	I have not had shortage of vitamins over the last five years.	59	67.0	29	33.0	1.67	0.47	M
5	I did not smoker.	73	83.0	15	17.0	1.82	0.37	M
6	I did not drink alcohol.	79	89.8	9	10.2	1.89	0.30	M

Table 4: Mean Score with Standard Deviation for women`s work environment and housing domain.

List	Item	Yes		No		M.S	SD±	LF
		F	%	F	%			
1	Work environment and housing free of pollutants such as smoke and chemicals.	55	62.5	33	37.5	1.62	0.48	M
2	The absence of housing and work place from the accumulated of all waste type.	54	61.4	34	38.6	1.61	0.48	M
3	The water used for drinking and washing was healthy and periodically checked.	65	73.9	23	26.1	1.73	0.44	M
4	The absence of weapons and radioactive contaminants.	53	60.2	35	39.8	1.60	0.49	M
5	Use of safe energy in cooking, healthy and domestic uses.	82	93.2	6	6.8	1.93	0.25	M

Table 5: Mean Score with Standard Deviation for women`s Psychological and Social domain

List	Item	Yes		No		M.S	SD±	LF
		F	%	F	%			
1	Do not experience psychological or social shock.	42	47.7	46	52.3	1.47	0.50	L
2	The state of mental, emotional and mood stable.	47	53.4	41	46.6	1.53	0.50	M
3	Stability of relation and dealing with families and society.	77	87.5	11	12.5	1.87	0.33	M
4	I feel good about myself and deal with the community.	83	94.3	5	5.7	1.94	0.23	M
5	Do not worry about thinking about the future and fearing it.	47	46.6	41	46.6	1.53	0.50	M
6	I was not subjected to family violence.	68	77.3	20	22.7	1.77	0.42	M

Table 6: Mean of Score with Standard Deviation for women Personal and family planning domain.

List	Item	Yes		No		M.S	SD±	LF
		F	%	F	%			
1	Do not use anti axillary sweeting drugs.	42	47.7	46	52.3	1.47	0.50	L
2	Avoid using tight bras.	47	53.4	41	46.6	1.53	0.50	M
3	I did not use birth control pills.	77	87.5	11	12.5	1.87	0.33	M
4	Refrain from the use of contraceptive such as uterine coil, condom, plastic ring and others.	83	94.3	5	5.7	1.94	0.23	M
5	Keep away from using chemicals cream.	47	46.6	41	46.6	1.53	0.50	M
6	Use electronic devices in limited way.	68	77.3	20	22.7	1.77	0.42	M
7	Obligate of breast self-examination every month.	43	48.9	45	51.1	1.48	0.50	L

Frequency MS: Mean Score (LF): Level of Risk Factor (H): High (<2.5) MS (M): Moderate (1.5 – 2.5) MS (L): **Low (> 1.5) MS.**

Discussion

Table (1) throughout the data analysis, the sample socio-demographic characteristics had showed that more samples were collected from Sulaymaniyah Governorate (34.0%) where the lowest on Salah Alden one (9.0%), this due to Sulaymaniyah Governorate contain more than a specialized center for tumors and cancer diseases in contrast of Salah al-Din Governorate, which does not contain any one.

With respect to women age, highest percentage (42.0%) was between (40-49) years. Due to the exposure of women in this period of age to menopause and stop the menstrual cycle, which is characterized by hormonal fluctuations.

According to women occupation; majority sample (64.7%) housewives and more than quadrant of women (28.4%) have primary school level of education if the study, this reflect the lack awareness toward health and how to an attempt to avoid the risk factor of the disease.

Most women (69.0%) feed her child a breastfeeding.

Normal weight (35,2%) followed by high weight (21,5%) present the large portion of the women, the reason of incident women with the ideal weight probability back to attempting to get the ideal weight through the diet food led to the gastric breast cancer. The women with overweight the reason goes back to the large number of fat in their bodies and the lack of their movement led to increase diagnosed breast cancer. Being overweight having fatter tissue can increase your chance of getting breast cancer by raising estrogen levels, that increase diagnosed breast cancer [(8).]

The highest percentage were married (72, 7%) and have sufficient income (40.0%). Perhaps, effectively hormone prolactin which secreted from the pituitary gland in the blood more effective than not married when uses the breastfeeding incorrectly [(9).]

Portion of (64.8%) were live in urban place, this conducted that lived of cities are more prone to breast cancer, lack of oxygen the city for rural making the cell growth weak because of lack of oxygen that's what makes it more prone to injury breast cancer for the villages[(10).]

Discussion the women`s genetic and biological domains (Table 2): Low Mean Score for item 4 (MS=1.48, SD±0.56) which concerned for patient have previous detection of any tumors in breast and another area in the body. Other items of this domain have moderate Mean Score one. Women who have previously suffered with benign breast cancer are at greater risk of developing breast cancer in the future [(4)].

Discussion the women food style and Nutrition domain (Table 3): demonstrations that a large proportion of the women with breast cancer has a lot of fat this foods item (1) (MS=1.44, SD±0.9), and most him food contain (kidneys, red meat, dairy product and salt). because of the eating food and many fat and its effect fatty tissue on the physiological fat cell claim the immune in important role of metabolism endocrine [(11) (12).]

Discussion the women`s work environment and housing domain. (Table 4): indicates that majority percent (MS=1.93, SD±0.25) of women used safety and sanitation water for drinking, cooking, washing and daily uses (MS=1.73, SD± 0.44). This due to water added to substances such as chlorine has an effect on the risk of breast cancer; some of the substances suspected to cause breast cancer are organ chlorine, a chemical industry that is manufactured from chlorine and other organic substances that contain carbon [(13).]

Discussion women`s Psychological and Social domain (Table 5): shows that a large proportion of women with breast cancer experienced of psychological and social shock (MS=1.47 SD±0.50). The psychological shock therefore double the immune system and the percentage of lumpy cells responsible for providing protection of the body cancer, and the trauma also affect the endocrine increasing or reduce the secretion hormone body suddenly for shock[(14).] The chronic stress works on activate the nervous system know system killer or and or escape influential significantly job in lymph functions bland found in breast helping the spread of cancer cells [(15).]

Discussion the women Personal and family planning domain (Table 6): illustrate that the majority women were not Obligate of breast self-examination every month (MS=1.47, SD±0.50).

Conclusion

The overall results revealed that most women were

adult married with low educational level and they lives in urban with sufficient income, also the result indicated that majority patients that most women have previous breast tumor and they depended on a style of food contain :(fat, kidneys, red meat, dairy product and salt) that will be contribute to breast cancer development. Most women experience of psychological problems and don't compliance of breast self-examination every month.

Recommendations

The study recommended of adopted a special health education programs for all women to learn them with the risk factors that may contribute of breast cancer development, as well as teach them the right procedure of breast self-checkup and encourage them to it every month.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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The Relationship between Believing in Resurrection (Ma'ad in Islam) and Self-efficacy in Mothers of Children with Thalassemia

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Introduction

Thalassemia creates many limitations and problems for patients and their families, which decease their self-efficacy, but believing in resurrection can help to alleviate stressful conditions in the life of effected people. This study was designed to investigate the relationship between believing in resurrection and self-efficacy in mothers of children with thalassemia.

Method: This descriptive correlational study was performed on 155 parents of adolescents with thalassemia in Golestan province of Iran in 2017, who had been selected by convenience sampling method. Data collection tools included; believing in resurrection questionnaire and Domka's parental self-efficacy questionnaire. Data were analyzed by descriptive statistics (mean, standard deviation, tables) and statistical tests (correlation coefficient of Spearman, U-Mann Whitney and Kruskal Wallis) using SPSS software version 21.

Results: The mean score of believing in resurrection was 33.11 ± 6.25 and the mean score of mothers' self-efficacy was 41.44 ± 6.55. Also, Spearman's correlation coefficient did not show any significant relationship with parental self-efficacy and believing in resurrection (r = 0.1 and p = 0.17).

Conclusion: Believing in resurrection shapes the behavior and performance of people in using all their capacities in difficult situations. Therefore, health managers and practitioners need to pay more attention to the importance of believing in resurrection and the concept of self-efficacy.

Keywords: *Resurrection belief, Self-efficacy, Mothers, Thalassemia*

Introduction

It is important to take care of the health of the children and their careers[1, 2]. This condition is inherited from parents and can lead to abnormal hemoglobin formation [3]. The World Health Organization (WHO) has identified thalassemia as the most common chronic genetic disorder in the world, which affects the lives of about 100,000 children annually. In Iran, about 4% of people carry the thalassemic gene and there are over 18,000 cases of thalassemia in the country that are dispersed in different provinces [4]. Thalassemia, like other chronic diseases, causes many problems for patients and their families despite the fact that, the combination of transfusion and complex therapy has dramatically increased the life span of these patients [5]. The life of people with thalassemia is fraught with limitations and problems such as changing life style, frequent absence from school, limited social activities, low self-esteem, sense of helplessness and out of touch with others, depression and fear of early death [6]. On the other hand, repeated blood transfusion that leads to iron overload, and chronic nature of the disease can cause a wide range of complications for adolescents and

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young people with thalassemia major [5]. In addition, they may face problems such as heart failure, pulmonary hypertension, bleeding, osteoporosis, infection, and thrombosis [7]. A family with a child with chronic illness is more likely to experience stress, and mental and financial problems than other families. Studies indicate that, the presence of a disabled or sick child in the family creates psychological pressure for parents, especially the mother [6]. In thalassemia, parents in addition to dealing with such pressure, are faced with reduced self-efficacy [8]. Self-efficacy as self-esteem is the ability to successfully perform specific tasks or health-related behaviors in different circumstances [9]. It also affects the behavior and quality of one's performance, and how long can he or she tolerate the difficult situations and carry on (10). With self-efficacy we can undertake a behavior even in unpredictable and stressful circumstances [9]. Self-efficacy includes believing in future actions and not past performances, and focuses on one's abilities instead of performance outcomes [11]. It is also the basis of motivation and effectiveness in different activities [11, 12]. The sources of self-efficacy include family and peers' processes and own experiences that create the ability to meet the challenges ahead and succeed in overcoming them [13]. People with thalassemia have different needs in the face of their illness, one of the most important of which is the belief in a world full of moderation [14]. Believing in resurrection and God, who oversees all things and helps man at all times, enhances the physical health and mental strength of patients and increases their suffering tolerance and self-esteem of their parents to care for them [15, 16]. It also has the potential to greatly reduce the anxiety associated with stressful situations and pressures in life [17], and plays an important role in coping with problems and increasing mental health [18]. Therefore, it can be argued that believing in God and after life wholeheartedly causes people to rely on God in the face of difficulties and experience less anxiety and negative emotions [19]. Studies show that people use the belief in after life as a moderator of stressful life conditions [14]. Therefore, considering the importance of thalassemia that affects all aspects of life in patients and their caregivers, this study was conducted to determine the relationship between believing in resurrection and self-efficacy in parents of adolescents with thalassemia, especially the mothers.

Materials and Methods:

This descriptive correlational study was performed on parents of adolescents with thalassemia in Golestan province of Iran in 2017, who had been selected by convenience sampling method. The study settings included hospitals of Gorgan, Aliabad Kotool, Gonbad, Aqh'qhala, Minoodasht, and Kordkouy that had thalassemia center. The inclusion criteria were; being parents of a thalassemic child whose thalassemia has been diagnosed and confirmed by a physician, having a medical record in the Thalassemia Association of Golestan province, having the ability to read and write (for parents), being aware of time and place, and having no chronic mental illness and history of psychiatric hospitalization. Exclusion criteria included; not completing the questionnaire fully and withdrawal from the study.

The sample size in this study was calculated to be 155 individuals based on Mashayekhi et al (1396) study [20] with the correlation coefficient of r = 0.22 and test power of 80% at the significance level of 0.05. Data collection tools in this study include: 1) Demographic information form (gender, age, educational level, birth rank, place of residence, history of previous illness, and parents' age, sex, marital status, education level, economic status, relationship with patient, and job status, having the support of institutions or organizations, number of family members, having another child with thalassemia in the family, and parents' family history of thalassemia). 2) Believing in afterlife questionnaire, containing 8 questions in 6-point Likert scale from strongly disagree (score 1) to strongly agree (score 6). Total score of the questionnaire is obtained by summing up the scores of all questions, which range from 8 to 48 with the higher score indicating a higher degree of belief in resurrection and afterlife and lower score referring to a lower degree of belief. The validity of this questionnaire was confirmed by Golparvar et al (21), and its reliability was calculated to be 0.81 by Cronbach's alpha.

Domka's parental self-efficacy questionnaire [22] was also used to assess the self-efficacy of parents. This questionnaire consists of 10 questions in seven-point Likert scale. In this questionnaire, each individual could obtain a score between 10 and 70, with higher score indicating higher self-efficacy. Also, the score of between 10 and 20 indicates low level of parental self-efficacy, the score of between 20 and 40 indicates moderate level of parental self-efficacy, and the score of higher than 40 representes high level of parental self-efficacy. Talei (2010) estimated the Cronbach's alpha coefficient of self-efficacy questionnaire to be 70% (23). In this study, after the approval of project

by University's Research Council and receiving a code of ethics, the researcher began to select the samples by convenience method. After the sampling, the participants were provided with necessary explanations about the objectives and method of study, and they were assured about the confidentiality of their information and the possibility of withdrawal from the study at any time with any reason without any consequences. Then, an informed consent was obtained from all of them. The same questionnaires were used for all subjects. After obtaining consent from the participants and explaining how to fill the questionnaire, the questionnaires were distributed among them and no time limit was applied to the compilation of questionnaires. During this time, the researcher was available to the participants and answered their questions. The data were analyzed by SPSS-21 software using Kolmogorov-Smirnov test for data normality. Then, the data were analyzed by descriptive statistics (mean and standard deviation, tables) and statistical tests (the correlation coefficient of Spearman and U-Mann Whitney and Kruskal Wallis) at the significant level of 0.05.

Findings

The mean age of the subjects was 40.06 ± 9.41 years and the mean age of thalassemic children was 13.88 ± 4.77 years. Most parents in this study (61.4%, 105 persons) were female and 38.6% of them (66 persons) were male. Majority of patients 73.7% (126 persons) were not covered by supportive organizations and 26.3% of them (45 persons) were covered. Most children with thalassemia 60.8% (104 persons) in this study were female and 39.3% of them (67 persons) were male. In terms of education, most parents 38.6% (n = 16) had secondary school education, 36.8% of them (n = 63) had high school education, 16.4% (n = 28) had high school diploma, and 8.2% of them (n = 14) had university education.

Results showed that, the mean score of believing in resurrection was 33.11 ± 6.25 and the mean of mothers' self-efficacy was 41.44 ± 6.55. Also, the result of Spearman's correlation coefficient showed no significant relationship between believing in resurrection and self-efficacy of mothers ($r = 0.1$ and $p = 0.17$).

Kruskal-Wallis test showed a significant relationship between believing in resurrection and parents' age ($p = 0.001$), parents' education ($p = 0.004$) and child's age ($p < 0.001$). So that, parents under 30 years of age had a higher belief in resurrection, and parents with university education had lower belief in resurrection. Also parents with children under the age of 6 had a higher belief in resurrection.

The U-Mann Whitney test showed a significant relationship between believing in resurrection and parent's gender ($p < 0.01$) and parental support system ($p < 0.002$), so that mothers had higher belief in resurrection. Also parents who had a support system from an organization had a higher belief in resurrection. However, this test did not show any significant relationship between believing in resurrection and the gender of children with thalassemia ($p = 0.06$). Kruskal-Wallis test did not show any significant relationship between parents' self-efficacy, parents' education ($p = 0.19$) and parent's age ($p = 0.19$), but it showed a significant relationship with the child's age ($p = 0.04$). Self-efficacy was lower in parents who had children under 6 years of age. Also, U-Mann Whitney test did not show a significant relationship between parental self-efficacy, social support organizations ($p = 0.79$) and child's sex, but it showed a significant relationship between parental self-efficacy and parent's gender ($p = 0.008$), so that self-efficacy was higher in men.

Table 1: Relationship between believing in resurrection and demographic characteristics of parents of thalassemic children

Believing in resurrection Demography			**Mean + SD**	**P-value**
Parent's gender	Female	105	34.53 ± 5.74	P < 0.01
	Male	66	30.88 ± 6.39	
Parents' age	Under 30 years	26	38.65 ± 6.21	P = 0.001
	30-40 years	52	33.32 ± 5.85	
	Over 40 years	93	32.01 ± 6.16	
Parents' education	Secondary school	66	32.06 ± 4.46	P = 0.004
	High school	63	34.8 ± 6.03	
	High school diploma	28	33.85 ± 4.76	
	University education	14	29 ± 6.48	
Supportive organization	Have	45	35.55 ± 5.38	P = 0.002
	Not have	128	32.24 ± 6.32	
Child's sex	Female	104	41.66 ± 7.01	P = 0.66
	Male	67	41.11 ± 5.81	
Child's age	Under 6 years	15	35.6 ± 6.11	P < 0.01
	6-12 years	37	32.89 ± 5.47	
	Over 12 years	59	31.45 ± 6.54	

Table 2: Relationship between self-efficacy and demographic characteristics of parents of thalassemic children

Self-efficacy / Demography			Mean + SD	P-value
Parent's gender	Female	105	40.41 ± 6.43	P = 0.008
	Male	66	43.07 ± 6.41	
Parents' age	Under 30 years	26	39.8 ± 8.05	P = 0.17
	30-40 years	52	42.15 ± 6.07	
	Over 40 years	93	41.5 ± 6.29	
Parents' education	Secondary school	66	41.96 ± 6.33	P = 0.19
	High school	63	39.96 ± 6.27	
	High school diploma	28	43.82 ± 7.1	
	University education	14	40.85 ± 6.63	
Supportive organization	Have	45	41.4 ± 6.52	P = 0.79
	Not have	128	41.46 ± 6.58	
Child's sex	Female	104	41.66 ± 7.01	P = 0.66
	Male	67	41.11 ± 5.81	
Child's age	Under 6 years	15	37.26 ± 5.9	P = 0.04
	6-12 years	37	41.62 ± 5.82	
	Over 12 years	59	40.89 ± 5.88	

Discussion

The results of this study showed that participants had a moderate belief in resurrection. The study of Madmali et al (2018) found a moderate level of spiritual well-being in people with β-thalassemia major [24]. Babaei Menghari (2016) also found a moderate level of spiritual health in thalassemic patients [25]. Zeigami Mohammadi (2014) study showed that, young people with thalassemia major had moderate level of spiritual health (26). Study of Fooladvand et al (2015) however, showed a high level of spiritual health and attitude in hemodialysis patients [27]. Also in the study of Hojati et al (2011), the frequency of prayer was high in hemodialysis patients [28]. The findings of this study and comparing them with other similar studies indicate that, spiritual attitude is institutionalized in most human beings, and as a common coping strategy, it becomes more pronounced at the times of illness [27, 29]. Spiritual needs and religious beliefs are also increasing in the later stages of illness [30]. Spiritual attitude helps patients to better cope with the suffering [31]. Studies have shown that spirituality and religious beliefs serve as a coping strategy during hardships and give meaning to life [32].

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Assessment of Family Knowledge toward their Children with Autism Spectrum Disorder at Al-Hilla City, Iraq

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Abstract

Background: The parents lay the foundations for the lives of their children. When a child is born with a developmental disorder, parents also need support in maintaining normal developmental abilities. There's no way of predicting when a child can master self-help skills. Parents should be assisted, including the child's primary caregiver and teacher, and must also include sufficient details on the treatment, disease state and commercially accessible tools that can help their children gain independence.

Aims: This study aims to assess the family knowledge toward their children With Autism Spectrum Disorder at Al-Hilla City, Iraq

Method: This quantitative study using a descriptive correlational design to study the assessment of Family Knowledge toward their children With Autism at Al-Hilla City. A convenience sample of (n=50) consisting of families. Data were selected over two months from the first of September 2019 to the first of November 2019.

Results: showed significant differences in autism disorder knowledge between age group.

Conclusion: Knowledge of the family of Autism spectrum disorder at Hila is on the moderate and differs significantly by age, education, and location. **Consequently, it is recommended** that autism disorder education should be used to improve their knowledge of the disorder, especially for those with non-formal education, older parents and those in the rural areas, through intervention by government and non-government agencies.

Keywords: *Assessment, Family Knowledge, Autism Spectrum Disorder*

Introduction

Autism Spectrum Disorder (ASD), also called pervasive developmental disorder [(1)]. Autism is a common disorder of childhood and complex neurodevelopmental disorders of unknown etiology composed of qualitative alterations in social interaction and verbal impairment with repetitive, restricted, and stereotype behavioral patterns[(2)]. The number of children with autism has been rapidly rising in the past decade in us; the number of cases increased by 123% from 2002 to 2010, with an estimated prevalence of 1in 68 children aged eight years[(3)].

Around four times more prevalent in boys than girls (although girls are more seriously affected[(4)].

The degree can vary from moderate to very extreme and is often referred to as Autism Spectrum Disorder. It is known as Classic Autism at the lower end and is called Asperger Syndrome at the higher end. The UN General Assembly proclaimed on 18 December 2007 World Autism Awareness Day. ASD is a category of neuropsychiatric disorders with specific delays and variations in social, communicative and cognitive development. ASD encompasses autism, Asperger syndrome, and omnipresent developmental disorder — not otherwise specified; autistic children often tend to be completely normal. However, invest time in puzzling and confusing behaviors that differ markedly from normal children [(5)].

Although the studies have provided to ASD etiology is established, the dramatic rise in ASD prevalence cannot be attributed entirely to genetics alone. An analysis of twin concordance found that common environmental variables account for 58 percent of the variation in ASD liability[1]. In addition, prenatal and early childhood cycles are considered to be crucial growth stages, during which infants are especially vulnerable to the adverse effects of environmental hazards that can contribute to infant diseases. The aspect of environmental factors in the onset of ASD, however, is still largely unknown and is not related to socioeconomic, cultural, parenting style [2]. Nevertheless, it still remains unrecognized and undiagnosed before or after late pre-school age, usually between the ages of 18 and 36 months, as sufficient resources for regular developmental screening and autism-specific screening were not available[6].. Early detection of children with autism and intense, early intervention during infancy and pre-school years (improves outcome for children with autism[8].

This practice parameter reviews the scientific evidence available and offers detailed suggestions for children with autism.

This approach requires a dual process:

1) Routine developmental evaluation and screening of autism common to all children, first identifying those at risk for some form of developmental delay and identifying those clearly at risk for autism

2) Diagnosing and assessing autism, separating autism from other developmental conditions[2]. The parental burden of raising an autistic child affects both the psychological well-being and the functioning of the parents; parenting a child with autism may often present additional stressors linked to the communication difficulties of children with challenging behaviors in self-care [8]. When a child is born with developmental disorders, parents often need help promoting normal developmental competencies. There's no way of predicting when a child will master self-help skills. Parents must be assisted, including the child's primary caregiver and teacher, and must also provide sufficient details on the treatment, disease condition and commercially accessible tools that can help their children gain independence[9].

Children with autism dream about killing themselves and dying horrifyingly from suicide. Limited studies have found that 20 percent – 40 percent of autistic adults considered killing themselves [10],which at least one attempt was made by 15 percent[11]. A major analysis of the Swedish population found that people with autism are nine times more likely than others to die from suicide [12].There is no way to avoid autism disorder, but treatment options exist. Early detection and intervention is very helpful and can enhance behavior, competencies and language development[7].

Methodology

Study design:

A descriptive correlation design study was done to assess the family knowledge toward their children with Autism Spectrum Disorder at Al-Hilla City, Iraq. The study started from the first of November 2019 to 30 January 2020

Study Setting:

The study has been conducted in Al Hussein Center, Wesam AlRuhma Center, Mazaya Center at Al-Hilla city, Iraq

Sample of the study

A convenience sample has been selected through a non-probability technique. The total sample included (50) family, which Assessment the family knowledge toward their children with Autism Spectrum Disorder at Al-Hilla City, Iraq, invalid for research criteria.

Methods of Data collection

Data was collected using the study instrument (questionnaire), and The study started from first of November 2019 to 30 January 2020. After obtaining permission from the Al-Hussein center, Wesam AlRuhma center, Mazaya center so, the researcher used a direct interview with patients who admission to fill the questionnaire.

Statistical Data Analysis

The researchers used appropriate statistical methods in the data analysis using (SPSS) version 2010, which includes the following statistical methods:

a. Frequency

b. Percentage

Results

Table(1): demographic characteristics of parents:

Demographic data	Rating	Frequency	Percentage
Gender	Male	27	54
	Female	23	46
Age/ year	22-30 years old	10	20
	31-40 years old	24	48
	41-50 years old	13	26
	51-60 years old	3	6
Parent's education level	Primary degree	9	18
	Secondary degree	5	10
	Bachelor degree	35	70
	Postgraduate degree	1	2
Marital status	Married	48	96
	Divorced	2	4
Occupation	Not work	11	22
	Work	7	14
	Employer	32	64
Monthly income	Sufficient	20	40
	Insufficient	12	24
	Barely sufficient	18	36
Living location	Urban	44	88
	Rural	6	12

Table (1) indicates that (54%) of fathers were aged between (31_40) years old =(27) Concerning the patient's educational level (70%) of fathers were at bachelor degree. (96%) of this is marriage more than half of the father were employed(64%). Regards to family income (40%) of families reported sufficient monthly income. (44%) of this family were from the country.

Table (2): demographic characteristics of child

Demographic data	Rating	Frequency	Percentage
Sex child	Male	37	74
	Female	13	26
Age/ year	2/5-3 years old	3	6
	3/5-4 years old	12	24
	4/5-5 years old	35	70
Age diagnosis of disease	1years	1	2
	2years	20	40
	3years	24	48
	4years	5	10

Table (2): indicates (74%) of a male were aged between (3/5-4 years old) is a diagnosis of autism higher than female was (26%) and(48%) from child in age (3 years)is a diagnosis with autism

Items	Responses	Freq.	%
1. Autism is a lifelong developmental disorder	Yes	42	21
	No	24	12
	Don't know	34	17
2. The smallest age at which autism can be recognized in children is 3 years	Yes	50	25
	No	40	20
	Don't know	10	5
3. Children develop autism as a result of watching television and using electronic devices in abundance	Yes	64	32
	No	22	11
	Don't know	14	7
4. The abuse of parents by children is one of the causes of autism	Yes	38	19
	No	46	23
	Don't know	16	8
5. Vaccines are the cause of autism	Yes	12	6
	No	56	28
	Don't know	32	16
6. Most autistic children have a mental disability	Yes	40	20
	No	44	22
	Don't know	16	8
7. The majority of autistic children are male	Yes	28	14
	No	48	24
	Don't know	24	12

Cont... Table(3): knowledge of autism disorder

Items	Responses	Freq.	%
8. Children with autism do not make any visual contact while talking to others Yes	Yes	78	39
	No	18	9
	Don't know	4	2
9. Children usually show weak language interaction and communication in order to be diagnosed with autism	Yes	84	42
	No	12	6
	Don't know	4	2
10. Shows autistic child stereotypes (repetitive)	Yes	86	43
	No	8	4
	Don't know	6	3
11. A child with autism is seen as not responding to his name when he is called	Yes	76	38
	No	24	12
	Don't know	0	0
12. Autistic children need lifelong care for their families	Yes	74	37
	No	16	8
	Don't know	10	5
13. Some individuals with autism show great abilities and very high skills (drawing, music)	Yes	58	29
	No	8	4
	Don't know	34	17
14. Did you know that centers designated for autism have a good effect on the health of your child?	Yes	90	45
	No	2	1
	Don't know	8	4
15. Children with autism can be integrated into schools with their regular peers when receiving appropriate educational support	Yes	76	38
	No	8	4
	Don't know	16	8
16. There is currently no treatment for autism, but it has been shown that rapid and intensive intervention can reduce symptoms of the disease	Yes	84	42
	No	6	3
	Don't know	10	5

Cont... **Table(3): knowledge of autism disorder**

17. Children with autism suffer from other disorders such as (epilepsy, depression, anxiety, hyperactivity)	Yes	76	38
	No	8	4
	Don't know	16	8
18. The high percentage of psychological and social burdens suffered by the autistic child's brothers	Yes	70	35
	No	14	7
	Don't know	16	8
19. The high percentage of families with a child with autism (difficult family problems and disorders) may lead to the separation of parents	Yes	36	18
	No	34	17
	Don't know	30	15
20. Autism has a relationship with aggression	Yes	62	31
	No	22	11
	Don't know	16	8

Table(3): distribution the percentage of items responses about knowledge of family toward autism disorder

Table (4): Knowledge level on ASD among parents

Knowledge level	Frequency	Percent
Low	26	13
Moderate	60	30
High	14	7

Table (4) shows that most of the parents have moderate knowledge level about the syndrome

The relationship between parentage and level of knowledge, this part shows that 60% of the parents have a moderate level of knowledge about syndrome. The age group 51-60 has only one sample at low-level .the age group 31-40 most of them with a moderate level of knowledge .20-30 age group have a most higher level of knowledge.

Discussion

The purpose of this questioner study was to assess the level of knowledge regarding ASD among families. The study revealed that both the rural and urban family's knowledge levels of autism disorder were generally moderate. This ratio is almost closer to reality and is rather good because of the lack of educational programs on Autism disorder presented by the health care provider and caregiver. However, studies were done in France [13],and study in China shows only 33.1% and 57.8% of the respondents have good knowledge on autism, respectively[14]. The study was done in Ireland reports (69%) of family knowledge[15]. These are consistent with our finding where the majority(60%) of the respondents who were aware of autism, has moderate knowledge. A similar result for our study was obtained

in an India study where the family's knowledge about autism disorder was moderate[16]

The results showed significant differences in autism disorder knowledge between age group, 60% of a parent have a moderate level of knowledge about syndrome .the age group(51-60) have only one sample at low-level .The age group (31-40) most of them with a moderate level of knowledge, about (20-30) age group have a most higher level of knowledge. The difference in knowledge level could be because (20-30) and (31-40) age group parents have easier access to information than their other age group. 84% of families showed that rapid and intensive intervention could reduce symptoms of the disease, and 90% know that centers designated for autism have a good effect on the child's health. Therefore, signs and symptoms of autism will ease improvement. The child can break his concerns and can improve communication with others.

Conclusion

The knowledge of family for Autism spectrum disorder in al- Hilla city is on the moderate and differed significantly by age, education, and location. Consequently, it is recommended that autism disorder education should be used to improve their knowledge of the disorder, especially for those with non-formal education, older parents and those in the rural areas, through intervention by government and non-government agencies.

Recommendations:

The study recommends the following:

1. Intervention programs on autism understanding should be conducted for the community, specifically among housewives who take care of their children most of the time and, at the same time, spread their clear understanding of autism to their friends and family.

2. The government should also implement a special program to encourage its employees to provide health information on autism and be role models

3. Further study is required to determine the effects of early intervention on autistic children's language skills, as language skills can enhance social interaction.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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The Effect of Warm Red Ginger Compress Therapy on the Decrease in Rheumatoid Arthritis Pain in the Elderly at the Social Institution Tresna Werdha Pagar Dewa Bengkulu

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Abstract

Background: This study aims to determine the effect of compress therapy using warm red ginger to reduce the scale of rheumatoid arthritis pain in the elderly.

Study Design: This study used a one-group pretest-posttest pre-experimental research design, with a total sample of 15 respondents.

Results: Based on the results of the bivariate analysis using the Dependent T test, it was found that there was an effect between compresses using warm red ginger with a value (p value = 0, 000) on the decrease in the scale of rheumatoid arthritis pain in the elderly at the Tresna Werdha Pagar Dewa Bengkulu Home. The provision of non-pharmacological management in the form of compressing warm red ginger will provide a heat effect so that it can eliminate inflammation and acidity in the fluid content in the joints, besides that there is also gingerol content in the area around the ginger rhizome which has an analgesic effect with techniques to create vasodilation conditions in vessels. blood resulting in decreased pain. Therefore, giving this therapy is very beneficial and helps the elderly who suffer from rheumatoid arthritis pain.

ConclusionThe role of the orphanage will be very helpful in informing and facilitating the elderly in reducing the scale of rheumatoid arthritis pain through non-pharmacological management in the form of warm red ginger compress therapy with the hope of improving the health status of the elderly at the social institution Tresna Werdha Pagar Dewa Bengkulu Home.

Keywords: *Red Ginger, Pain, Rheumatoid Artritis*

Introduction

Rheumatoid arthritis is a chronic, systemic inflammation that can attack various tissues, but the most important thing is the joint which produces nonsupurative proliferative synovitis which can damage the cartilage of the joints and, if allowed to become disabled[1].

According to the World Health Organization, the prevalence of rheumatoid arthritis (RA) in the world is vulnerable between 0.3 to 1%, with a greater incidence of attacking the female sex. Data from Riskesdas in 2018 results from interviews with ≥ 15 years of age, the average prevalence of joint disease / rheumatism is 7.3% of the Indonesian population. The province with the highest prevalence rate is occupied by Aceh province, which is around 13.3%, while Bengkulu Province is the province with the second highest prevalence after Aceh province[2]. Rheumatoid arthritis is also the second most common chronic disease after hypertension affecting the elderly with a proportion of 48% of rheumatoid arthritis[3].

Rheumatoid arthritis is characterized by damage and proliferation of the synovial membrane, resulting in damage to bones, joints and deformities that occur in the elderly. Elderly is a condition where there is an increase in age along with a decrease in physiological function, so that the elderly often have a higher risk of experiencing pain.This is also caused by several pathological things such as decreased function of the musculoskeletal system due to changes in the composition of the fluid solution

in the cartilage will overload the joints to work even harder which can trigger pain[4]. Rheumatoid arthritis also produces constitutional symptoms such as low-grade fever, where these manifestations are caused by the same mediators as joint inflammation. This disease also appears at the same time as it begins with stiffness and pain in the joints, especially in the morning[1]..

Pain is a complaint of people with rheumatoid arthritis to health workers when doing medication or treatment. There are several pain management that can be done both pharmacologically and non-pharmacologically. To reduce the pain of rheumatoid arthritis through non-pharmacological management, it is carried out through warm compresses, ergonomic exercises, warm ginger compresses. One of the pain management is compresses using warm red ginger, while the advantage compared to other non-pharmacological therapies is that red ginger contains the enzyme cyclo-oxygenase which can reduce the feeling of inflammation in people with rheumatoid arthritis, besides also having a burning effect, and contains a functioning analgesic. to reduce pain[5].

A survey conducted by researchers at the Tresna Werdha Pagar Dewa Social Institution, Bengkulu on April 4, 2019, there were 67 respondents with the incidence of rheumatoid arthritis 20 people. Then the results of the interviews conducted show that so far, there has never been any non-pharmacological treatment to reduce pain, such as a warm red ginger compress. Based on the above background, the researcher was interested in taking a study entitled "The Effect of a Warm Red Ginger Compress on the Decrease in Rheumatoid Arthritis Pain in the Elderly at the Tresna Werdha Pagar Dewa Social Institution Bengkulu.

Method and Method

This research is a quantitative research with the type of pre-experimental research one-group pretest-posttest. This research was conducted in June 2020 which was conducted at the Tresna Werdha Pagar Dewa Social Institution, Bengkulu. The population in this study were all elderly suffering from rheumatoid arthritis. The sampling technique in the study used non-probability sampling techniques, namely consecutive sampling. Sample selection was carried out by establishing inclusion and exclusion criteria.

This research data collection procedure starts from administrative procedures where getting a letter of passing the ethical test, processing a research permit to the Tresna Werdha Pagar Dewa Social Institution, Bengkulu. Furthermore, selecting potential respondents based on the inclusion criteria, asking the elderly to become respondents and explaining the purpose of the study. Then the researcher asked the respondent's identity, asked the pre-pain scale, explained the procedure or technique for warm red ginger compress, positioned the respondent as comfortable as possible, gave the compress intervention to the pain section for 60 minutes with a temperature of 45-50.5 ^{o}C. After being compressed for 60 minutes the post intervention pain scale was measured again. This action was carried out for 5 interventions.

Result

Univariate Analysis

The results of the univariate analysis of this study were used to see the description of the frequency distribution of clients based on the pain scale before intervention, the pain scale after the intervention in elderly people suffering from rheumatoid arthritis at the Tresna Werdha Pagar Dewa Bengkulu Social Institution:

Table 1: Distribution of Respondents Based on Pre-Intervention Pain Scale

Rheumatoid Arthritis Elderly At The Social Institution Tresna Werdha Pagar Dewa Bengkulu

Pain	N	Percentage (%)
Mild Pain	2	13,3
Moderate Pain	13	86,7
Total	15	100

Based on table 1, the frequency distribution of the pain scale before the intervention of elderly respondents with rheumatoid arthritis at the Tresna Werdha Pagar Dewa Social Institution, Bengkulu, most of them fell into the moderate pain scale category, namely 13 (86.7%) while those in the mild pain scale category 2 (13.3%)

Table 2 : Distribution of Respondents Based on Post Intervention Pain Scale Rheumatoid Arthritis Elderly At The Social Institution Tresna Werdha Pagar Dewa Bengkulu

Pain	N	Percentage (%)
Mild Pain	8	53,3
Moderate Pain	7	46,7
Total	15	100

Based on table 2, the frequency distribution of the pain scale after the intervention of elderly respondents with rheumatoid arthritis at the Tresna Werdha Pagar Dewa Bengkulu Social Institution, most of them fell into the mild pain scale category, namely 8 (53.3%) while those in the moderate pain scale category were 7 (46.7%).

Bivariate Analysis

The results of the bivariate analysis were used to see the effect of warm red ginger compresses on reducing rheumatoid arthritis pain in the elderly at the Tresna Werdha Pagar Dewa Social Institution, Bengkulu. The results of these researchers themselves can be seen in the following table 3:

Table 3: Distribution of Average Pain Scale of Respondents Before and After the Intervention in the Form of Warm Red Ginger Compress Rheumatoid Arthritis Elderly At The Social Institution Tresna Werdha Pagar Dewa Bengkulu

Variable	Mean	SD	P Value	N
Pain Scale			0,000	15
Pre Intervention	5,22	0,94		
Post Intervention	3,89	1,01		

Based on table 3, the results of the analysis of the average pain scale pre intervention warm red ginger compress is 5.22 with a standard deviation of 0.94. After giving the warm red ginger compress the average pain scale was 3.89 with a standard deviation of 1.01. The results of the statistical test with a P value of 0.000, it was concluded that there was a significant influence between the pain scale before and after the intervention of warm red ginger compresses in the elderly with rheumatoid arthritis at the Tresna Werdha Pagar Dewa Social Institution, Bengkulu.

Discussion

Univariate Analysis

1. Distribution of Respondents Based on Pain Scale Pre Intervention

Based on table 1, it is known that the pain scale before being given a warm ginger compress was 13 (86.7%) experiencing moderate pain and 2 (13.3%) experiencing mild pain. The results of this study are one of the manifestations that most of the elderly experience moderate pain. This is also based on changes in the function of the musculoskeletal system such as decreased muscle mass and strength, bone decalcification, degenerative joint changes that can lead to rheumatoid arthritis in the elderly[3].

The results of this study are in line with research conducted by Hamdana (2018) note that the intensity of pain before compressing is 9 respondents (60%)[6]. These data are the same as a study that is currently being carried out where the pain is the mildest by elderly people with arthritis who have moderate pain. This pain is a form of subjective and emotional sensory experience that is unpleasant tissue damage, especially in the joints of the elderly with rheumatoid arthritis.

According to researchers, if this is allowed to prolong it will increase the increase in intention. This increase can be characterized as a result of synovial damage which will lead to infection. This infection can attack the synovial area so that joint damage with the end result appears pain intensity with an increasingly bad category

2. Distribution of Respondents Based on Pain Scale Post Intervention

Based on table 2, it is known that the scale of pain in the elderly with rheumathoid arthritis after the intervention of warm ginger compress therapy at the Tresna Werdha Social Home is as many as 8 (53.3) experienced mild pain and 7 (46.7%) experienced moderate pain. Based on the results of this study, it can be seen that there is a decrease in the pain scale after the intervention of the red ginger warm compress.

The results of this study are in line with the research of Rahayu (2017) where the pain scale after the intervention of red ginger is mostly on a scale of 2 and 3 with (mean 2.5) these results indicate a decrease in pain scale after this intervention because of red ginger[7]. has a higher oil and oleoresin content than other types. The results of the study also said that after the ginger compress was carried out, the pain scale of the respondents was mostly in the mild category according to 9 respondents (60%) or experienced a decrease in the pain scale in the elderly with rheumatoid arthritis [6].

While the results of this study are also in line with the research of Hemmati 2016) which presents herbal remedies which contain ginger which can help improve the condition of patients with rheumatoid arthritis[8]. These improvements take the form of minimal reduction of inflammation and pain. The hope will eventually be to apply the dosage to chemical management uses

According to researchers the cause of reduced pain intensity also occurs due to the presence of the enzyme cyclo-oxygenase found in red ginger which has an impact on reducing inflammatory conditions. In addition, the content of red ginger, which can release a hot and spicy sensation, can cause vasodilation of blood vessels, especially those in the area where the compress is applied so that this condition will inhibit the release of pain receptors with the manifestation that the elderly will feel more comfortable than the previous condition.

Bivariate Analysis

Based on table 3 the average pain scale before the intervention was 5.22 with a standard deviation of 0.94. After giving the warm red ginger compress intervention, the mean pain scale was 3.89 with a standard deviation of 1.01. The statistical test results obtained a P value of 0.000, it can be concluded that there is a significant influence between the pain scale before and after the intervention of warm red ginger compresses in the elderly with rheumathoid arthritis at the Tresna Werdha Pagar Dewa Social Institution, Bengkulu.

The results of this study are in line with the research of Rahayu (2017) where it was found that there was a significant effect of giving red ginger compress therapy in reducing joint pain in elderly people with rheumatoid arthritis with a p value. 0.037 ($p < 0.05$)[7]. Ginger rhizome has the same pharmacological properties as NSAID drugs which can reduce inflammation and pain in the elderly with fewer or no side effects compared to pharmacological management[9]. This is also based on the nature of ginger which has pharmacological and physiological effects where ginger is able to release heat compounds, as anti-inflammatory, anti-tumor, antioxidant, helps lose weight and as a treatment for the digestive tract[10].

Ginger is a good therapy for pharmacological side therapies such as NSAIDs and DMRAD which are given orally for the treatment of arthritis, both pain and inflammation. benefits or good responses to reduce inflammation and pain[11]. This is in line with the opinion of Therkleson (2010) that ginger compresses are able to create a feeling of warmth in the body so that it makes the client relax which results in increasing the opportunity to rest the client and feel calmer, more comfortable and reducing body tension because ginger contains heat, anti- inflammation and analgesics that can reduce pain in clients with musculoskeletal problems including rheumatoid arthritis[12].

According to researchers the activeness of the

elderly to follow the process of giving intervention in the form of warm red ginger compresses will increase maximum results related to reducing the scale of rheumatoid arthritis pain which is based on the content of the compounds in red ginger which also has an important role in reducing the pain scale. in the elderly because red ginger has the effect of being able to dilate blood vessels around the compressed area so that pain receptors such as prostaglandins, histamine and bradykinin can be inhibited, so that the pain scale decreases.

Conclusion

The scale of pain in the elderly with rheumatoid arthritis before being given warm ginger compress therapy at the Tresna Werdha Pagar Dewa Bengkulu Social Institution, as many as 13 respondents (86.7%) experienced moderate pain and 2 respondents (13.3%) experienced mild pain

The scale of pain in the elderly with rheumatoid arthritis after being given warm ginger compress therapy at the Tresna Werdha Pagar Dewa Bengkulu Social Institution, as many as 8 respondents (53.3%) experienced mild pain and 7 respondents (46.7%) experienced moderate pain

There is an effect of a warm red ginger compress on the reduction of rheumatoid arthritis pain at the Tresna Werdha Pagar Dewa Social Institution, Bengkulu.

Conflict of Interest: The authors declare that there is no conflict of interest.

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Ethical Clearence: Health Research Ethics Committee, Health Polytechnic of Health Ministry Bengkulu

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Legal Philosophy about the Performance of the Indonesian National Police

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Abstract

This study is intended to absorb various aspirations and information about all aspects of police performances. It is also projected so that the ranks of the police can guarantee openness and accessibility to comprehensive information about their performance. Through this study, the impact of the implementation of the police programs and activities which are then realized through their performances can thus be examined carefully. With this in mind, police legitimacy and community support are expected to flourish. It can be said, the study is actually a kind of 'tool' that is important in the effort of raising partnerships as well as synergistic joint problem solving among the police, stakeholders, and the public. Through this study, meaningful dialogue in order to hear, understand, accept, solve problems, and then meet the needs of the police, can take place.

Keywords: *police, performance, Indonesia*

Introduction

Philosophically, the study of an understanding of a case, in this case let's say about the performance of the police can actually be considered like a 'consultation'. In summary, 'consultation' can be understood as a process of finding information from a person, group of people, institutions, and other sources of reference. Furthermore, consultation on the cross is a process of dialogue that ultimately leads to a decision making[1]. In relation to the police, consultation provides a way for users of police services, stakeholders, and the public in general, to understand and influence the decisions and policies regarding them[2]. Like a consultation, this research can be said to be an embodiment of Good Governance, whose main pillars are transparency, public participation, and public accountability.

Within the framework of police relations and performance, the study as a consultation is intended to absorb various aspirations and information about all aspects of police performance. On the other hand, this study is also intended so that the ranks of the police can guarantee openness and accessibility to comprehensive information about their performance[3]. Through this research, the impact of the implementation of programs and activities which are then realized through the performance of the police ranks can thus be carefully examined. With this in mind, police legitimacy and community support are expected to be built. Consultation for police is actually a kind of 'tool' that is important in the effort of raising partnerships, between as well as synergistic joint problem solving by the police, stakeholders, and the public. Through studies/research as public consultation, meaningful dialogue in order to hear, understand, accept, solve problems, and then meet the needs of the police, can take place.

This research on the performance of the Indonesian National Police was built by 5 components, which included mutual understanding, handling, barriers and support, linkages, and satisfaction level. Starting from the five components of the study, this study then traced 5 areas of study, namely abuse of authority, including the abuse of rights and power to act for inappropriate purposes; allegations of corruption, which is an act in the form of requesting compensation for money or in other forms for work that should have been done by the police; poor service can be in the form of protracted delays, improper handling, conspiracy, procedural irregularities, and embezzlement of evidence; discriminatory treatment, i.e. services that are biased or biased or give importance to one party, without regard to the applicable provisions so that it harms the other party; and incorrect use of discretion, which is the free use of authority or authority that is unwise and lacks consideration, which results in wrong or harmful decisions or actions. Furthermore, the five fields of study 'collide' with what is referred to in this

paper as the 5 pillars of the development of the police, namely professionalism development, consolidation of independence, budget planning, development of human resources, and provision of facilities and infrastructure,

Methodology

Working in the realm of legal philosophy with a comparative model, this research on police and performance in other words about Polri's performance is carried out as a literature study supported first by Field Research, and then also by Survey, and guided by a constructivism paradigm[4,5]. The Methodology of Philosophy Research Methodology is followed because the Philosophy of Law in its cross is more a part of Philosophy[6]. This research was conducted for approximately 4 months in various institutions with the potential availability of informants and/or respondents who were adequate and had a treasury of literature on policing in general, as well as literature on police performance in particular. These institutions include the National Police Headquarters, National Police Education and Training Institutions such as the Police College of Education (STIK), National Police Commission (Kompolnas), and so on.

Results

Understanding and Handling

In the current reform era, openness or transparency has indeed become our daily life, including within the scope of the police. Therefore, it is not surprising that Polri's performance is reviewed through transparency including in the handling of the five fields of study. It is seen that the majority of informants/respondents who stated that the handling of alleged corruption in this case in terms of transparency turned out to be going very well. This finding was followed by the handling of poor service issues, and then followed by the handling of cases of discriminatory treatment, and then behind it was a case of abuse of authority. What is unfortunate is that there are not many informants/respondents who see that handling the problem of using the wrong discretion is very good. It can be suspected that the abuse of authority and discretion which is one form of authority is still a sensitive issue within the National Police.

Barriers and Support

The results showed that handling the issue of using the wrong discretion faces the highest enormous obstacles. The next very big obstacle relates to handling abuse of authority. The findings showed that the highest enormous obstacle that must be overcome in handling cases is incorrect use of discretion. Consistent with the foregoing description, this finding makes it clear that the problems that underlie the misuse of discretion are indeed significant. This problem stems from the still weak understanding of discretion and its use. Therefore, it requires commitment from all related parties to make it sturdy and strong. In this study it was revealed, the highest level of support was apparently given to the handling of suspected corruption. This proves that the enthusiasm of all levels of society to eradicate corruption, including those that are contagious within the National Police, has indeed reached a proud level. Critical demands from the community for good police services were also raised here. On the other hand, the new atmosphere covers, firstly the problem of the use of erroneous discretion and secondly the problem of abuse of authority. Previously each was shown to face the highest obstacle and the second highest.

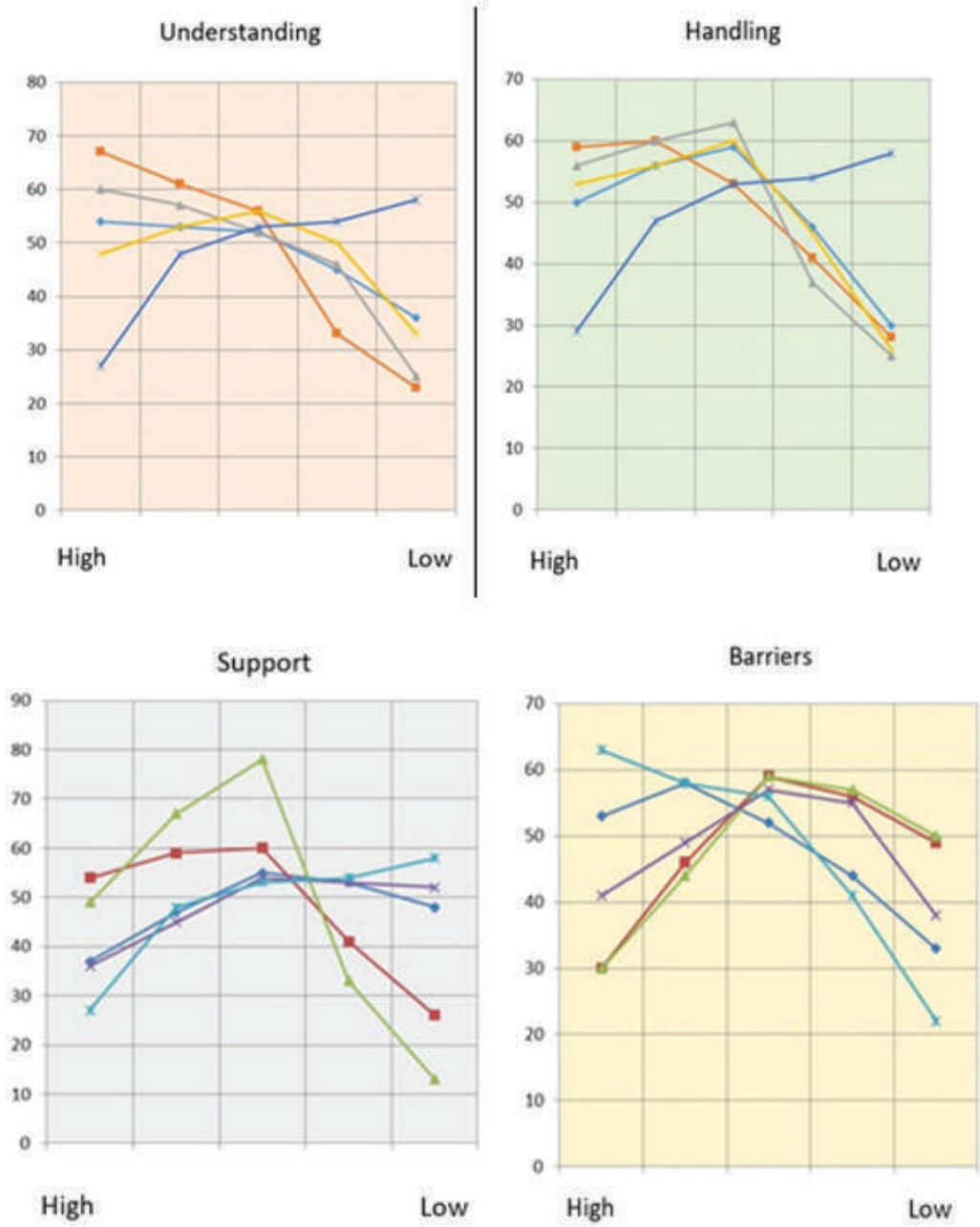


Figure 1. Understanding, Handling, Support and Barriers of Police

Linkages

The linkage is intended to examine how each of the National Police Development Pillars are: budget planning; human resource development; provision of facilities and infrastructure; professionalism development; and strengthening the independence of the National Police will affect the Field of Study and its handling, which has already known consists of: abuse of authority; alleged corruption; poor service; discriminatory treatment; and incorrect use of discretion. The findings show how professionalism is really related to handling police performance issues in a number of ways. First, the wrong use of discretion, only the second, abuse of authority. This is understandable given that as a profession, the police are characterized by certain specifications relating to intellectual or theoretical expertise as well as technical, training and education, competence, organization, discipline and code of ethics, as well as commitment to altruistic services (public services)[7]. Only by mastering these specifications in other words only by professionalism, discretion and authority of the police in general can be understood and used properly and correctly.

Independence

In general, the findings show that independence has a high relationship with all categories or fields of study, along with its handling. In particular, independence has a very high relationship with: first, the alleged corruption and second, the abuse of authority. This proves that the handling of the alleged corruption requires the party assigned to avoid 'intervention' from all directions, both from the internal and external sides of the National Police. Independence is very much needed here, precisely because of the nature of the case of the alleged corruption itself which castrated independence through the flow of collusion and nepotism, but it needs to be distinguished between 'intervention' and 'participation'. The term 'participation' does not deny independence at all, it actually strengthens independence in the context of synergistic partnerships, because only those who take part, not those who depend, can partner and work together in a healthy manner.

Budget

Similar to the previous description, in general the budget has a high average relationship with all categories or fields of study and their handling. In particular the budget has a very high connection with alleged corruption and poor service. This makes sense because only with an adequate budget, corruption, especially the element of corruption is expected to be prevented. Also with an adequate budget, service for the community is expected to be improved. Good and correct budget planning is thus expected to be able to control the creativity 'of police officers in the field in order to find additional costs to cover the shortfall in the cost of carrying out their duties and to boost efforts to improve community service standards. Among all categories, the very high linkage between the budget and the use of erroneous discretion is the lowest. This relation should not be read as if handling the case of the use of the wrong discretion does not require a budget. For the wrong use of discretion, so far the relationship has focused on professionalism.

Human Resources

The findings are in line with the previous exposure, in general the relationship between the human resources of the National Police with the overall categories or fields of study and their handling is quite high. But this time what happened to professionalism turned out to be repeated again. In particular, the very high linkages of the Indonesian Police Human Resources are by handling the problem of using the wrong discretion and handling the field of abuse of authority. This is understandable because discretion can be used appropriately and authority is not misused only by qualified police human resources, within the scope of the police, this means professional police human resources. It is at this point that professionalism and HR work in handling cases of misuse of discretion and handling abuse of authority reaches a meeting point. In other words professionalism is apparently identified with HR: talking about professionalism means talking about HR and professionalism.

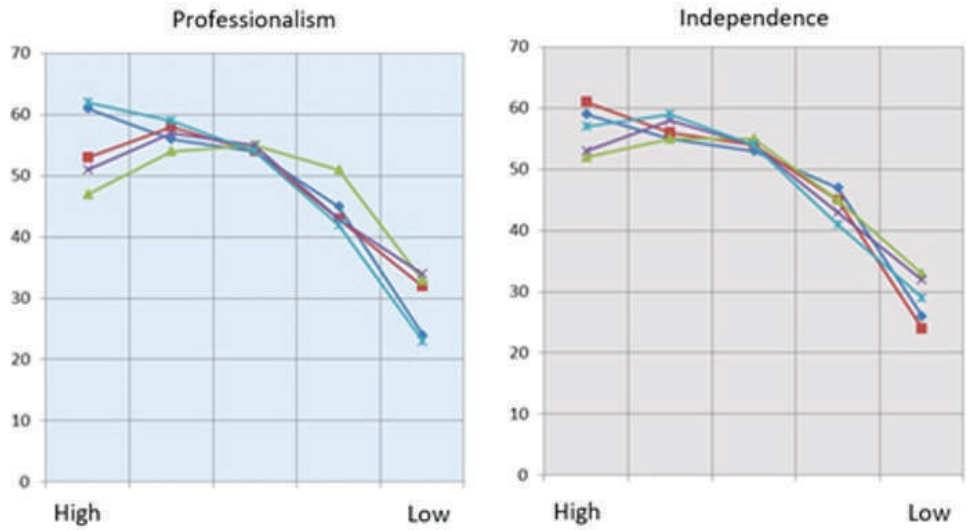
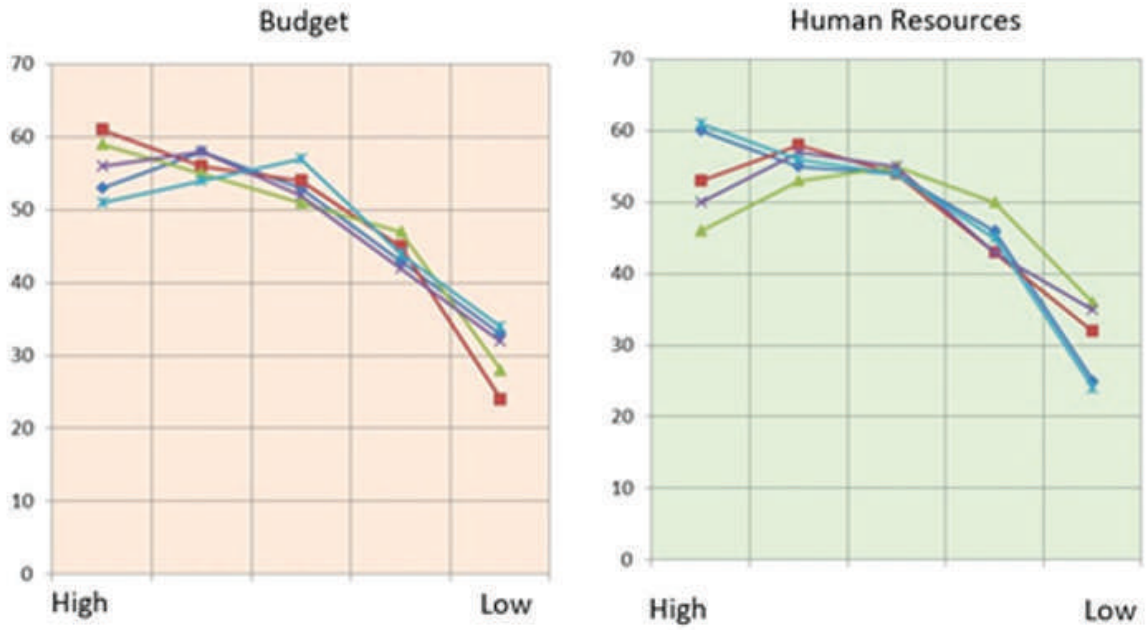


Figure 2. Professionalism, Independence, Budget and HR

Cont... **Figure 2. Professionalism, Independence, Budget and HR**



Facilities and Infrastructure

The finding shows that Polri's facilities and infrastructure are generally related to all categories or fields of study, as well as handling them at a high enough to high level. But specifically, the facilities and infrastructure of the National Police are related to very high levels first of all with the poor service category. The informant/respondent seems to rationally associate poor service with the availability of adequate police infrastructure and facilities.

The point is, if the problem of police performance in the field of poor services is raised, then the solution should be sought first through efforts to provide adequate facilities and infrastructure for the Police. It is common knowledge that so far the poor service of the National Police has largely passed away to the pillars of the development of the National Police's infrastructure and facilities, in this case being limited to other pillars.

The study also revealed that there were actually some informants/respondents who did not know how the actual linkages between the National Police development pillars were professionalism, independence, budget, human resources, as well as Polri facilities and infrastructure by handling police performance issues. However, there are some informants/respondents who have been able to see the relationship between the two. Others understand that the pillars of the development of the National Police are not only related, but also affect the handling of police performance issues.

Most informants/respondents then came to the understanding that independence was ranked third, professionalism was ranked second, and police HR was ranked first, in terms of the most related or most influential, both the process and the results of handling. On the other hand there are some informants/respondents who judge that all the same are related or influence, the process and/or the results of treatment. The findings at least illustrate that the police performance problems that have been complained by the public, namely the abuse of authority, suspected corruption, poor service, discriminatory treatment, and the use of erroneous discretion following the process and handling do have a common thread with the pillars of the development of the National Police, namely development professionalism, stabilization of independence, budget planning, human resource development, and the provision of facilities and infrastructure of the Indonesian National Police. From one side to be able to resolve the policing issues, the National Police development pillar needs to be upheld. From the reverse side, it can also be said to be able to uphold the development pillars of the National Police, the problem of police performance needs to be resolved.

Thus, the relationship between the performance of the police and the development pillars of the Indonesian National Police is essentially reciprocal. The answer to the question about which direction to go is based on the needs that exist, according to the context of space and time. The task of all parties is to make the relationship optimal for the progress of the police in particular and for the welfare, justice and prosperity of the Indonesian people in general.

Satisfaction

In general, the level of satisfaction of informants/ respondents towards the overall handling of police performance issues is actually quite high to high. But in particular, the respondents' very high level of satisfaction with the handling of police performance problems in the field of alleged corruption was the highest, followed by poor service and discriminatory treatment. This could mean that the handling of the problem of police performance in these fields really meets the expectations of the community. One component that contributes to the achievement referred to may be intense promotion, both from the National Police itself and from other parties regarding the need for efforts to improve the handling of police performance problems in these three categories. The weakest point lies precisely in the wrong use of discretion which is then followed by abuse of authority. Once again, these two categories indeed need deeper and broader attention from all parties for their improvement.

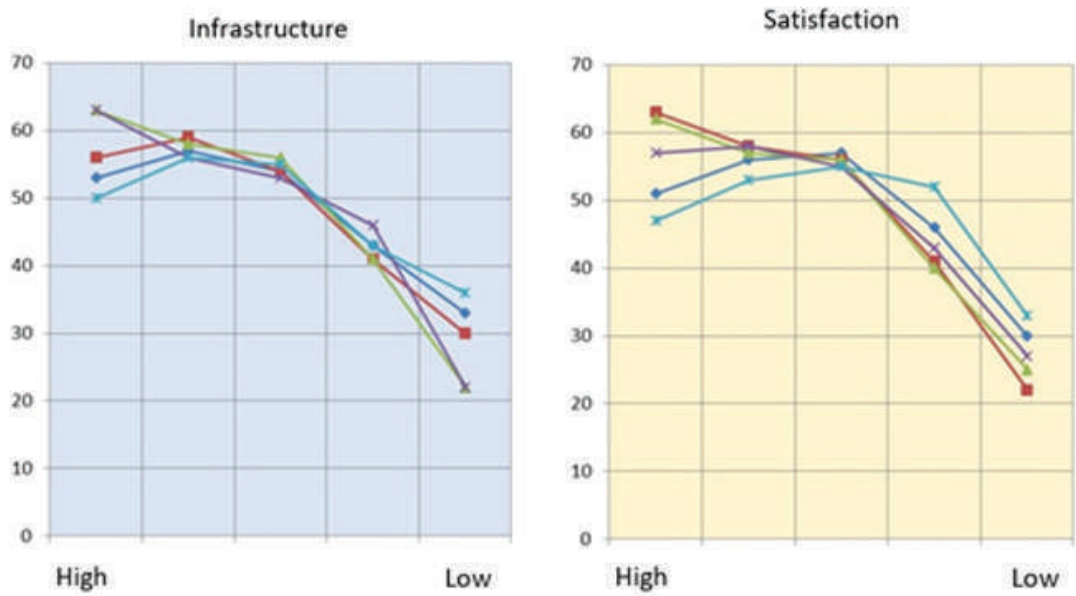


Figure 3. Infrastructure and Satisfaction

Conclusion

This study shows that there are at most informants/ respondents who are well aware of what is meant by police performance problems in the field of alleged corruption, followed by poor service. This can be understood because since the reformation of corruption and poor service has been a major issue that has surfaced in all public institutions, including the National Police. What is least understood is the area of incorrect use of discretion. Even if they understand, discretion is usually considered as an abuse of authority.

In this case, the handling of alleged corruption was stated very well by the most informants/respondents. Handling the problem of using the wrong discretion was declared very good by at least informants/ respondents. This finding seems to preach that all parties' understanding of police discretion and its application is apparently far from expectations. Even though the police leadership itself has underlined on various occasions that police members are essentially bearers of discretion. This study also revealed that the highest obstacle was actually faced in the handling of the problem of using the wrong discretion, while the highest very large support was apparently given to the

handling of the alleged corruption. The issue of police performance and its handling has a common thread with the pillars of the development of the Police. In this case, if professionalism is really related to handling the use of incorrect discretion, then independence and budget have a very high relationship with the alleged corruption. Overall independence ranks third, professionalism ranks second, and human resources ranks first, in terms of the most related, or most influential, processes and results of handling of police performance problems. Thus, the very high level of satisfaction of informants/respondents regarding the handling of police performance problems in the field of alleged corruption is the highest and the weakest point lies precisely in the wrong use of discretion. In this regard, the very high need to improve the handling of police performance problems is related to the field of incorrect use of discretion.

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Efforts to Prevent Criminal Acts of Money Laundering Using Penal Policy Measures

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Abstract

The context of enforcement of money laundering is not a simple concept that is very complicated because it is so complex that it is very difficult to formulate criminal offenses in an objective and effective manner. This can be seen from countries that regulate the problem of money laundering. Money laundering is already a phenomenon of cross-border crime and organized crime which requires special law enforcement as well, in this case one of the means used is a means of punishment. The modus operandi of money laundering crime continues to change from time to time in line with the advancement of the era and the technology of the community. To that end, increasing the ability of law enforcement officers in the field of enforcement of money laundering crimes must always be improved, one of the efforts to overcome and prevent money laundering by means of a penal policy. In this study, the data used are secondary data with a normative juridical approach and normative descriptive analysis reported in a research report. From the results of the research that has been carried out, it has been concluded that the means of reasoning are useful in efforts to prevent and deal with money laundering.

Keywords: *crime, money laundering, penal policy.*

Introduction

Indonesia's economic development aims to create a just and prosperous society based on Pancasila and the 1945 Constitution as reflected in good economic growth and controlled inflation. Some principles that must be considered and applied in bank management so that the banking system can be healthy can be done, among others, by the principle of prudential, safety, profitability, and efficiency that can support the strength and growth of the banking system and can accommodate the development needs of government and society. Like other countries, Indonesia also pays special attention to the problem of money laundering (Law No. 25/2003) and bringing abroad considered transnational organized crime (Transnational Organized Crime/TOC).

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In further development, with the increasingly sophisticated forms of crime that have international networks and use financial institutions, especially banks as targets and facilities, for example, it can be stated as white-collar crime or money laundering, banks must more alert. In some countries, banks are required to have and implement a Know Your Customer Principle policy and system, so that bank management and banking authorities can have legal consequences, both civil and criminal sanctions against the bank. The involvement of banks in money laundering is due to the ease of the process to manage the proceeds of crime in various bank businesses, including in the form of deposits such as deposits, demand deposits, and can also be placed in the form of other financial instruments such as Bank Indonesia Certificates, the use of safe deposit boxes and so. The use of banks as mentioned above is an indispensable thing in money laundering because criminal organizations need to manage financial cash flow by placing their funds in banking business activities so that they do not need to reinvest their funds in criminal activities.

The process of money laundering, there is always a connection with financial service providers. Money laundering is a crime that harms the interests of the community, and can lead to economic instability in a country and economically not beneficial to the country.

Banks conduct their business on the basis of economic democracy using the precautionary principle, with the main function being to collect and distribute funds to the public. Thus, banking is a very fertile place to disguise the proceeds of crime in the form of laundering the proceeds of crime that enter the financial system as if it became lawful property (clean money). For this reason, criminal sanctions need to be formulated in the policy of handling and preventing money laundering. The problems raised in this study are to explore the criminal sanction policy is formulated in or specifically the issues raised whether criminal sanctions (imprisonment) can be used as a means to prevent and eradicate non-criminal money laundering.

Research Methods

The research approach is preferred through the normative juridical approach and supported by a sociological juridical approach. The normative juridical approach relies on secondary data sources in the form of criminal law regulations, or other supporting materials. The sociological juridical approach relies on primary data/materials, which is the source of the data that is directly found. The data obtained will be analyzed and presented qualitatively using discrete normative analysis.

Money Laundering Practice

In practice, money laundering almost always involves banks because of the globalization of the banking system, so that through payment systems, especially those that are electronic in nature, large proceeds of crime will flow or move beyond the jurisdiction of a country by utilizing the bank secrecy factor that is generally upheld high by banks[1,2]. After the ratification of the Law of the Republic of Indonesia Number 15 of 2002, which has been amended by the Law of the Republic of Indonesia Number 25 of 2003, concerning Criminal Acts of Money Laundering, and finally with the enactment of Law Number 8 of 2010 concerning Prevention and Eradication of Criminal Acts Money Laundering which is expected to be a crime of money laundering can be prevented or eradicated, where the form includes criminalization of all acts in each stage of the money laundering processes.

In Law Number 8 of 2010 described in Article 3, Article 4, Article 5 regarding money laundering stated that laundering, transferring, donating, exchanging, or other acts of assets that are known or reasonably suspected are the results of criminal acts with the intention to conceal, or disguise the origin of assets so that they appear to be legitimate assets. The formulation in the article above can be used as a picture of the public about what is meant by money laundering activities, which is a crime where by putting cash into a financial system, transferring assets that originate from criminal acts, using assets or money illegal, which originates from criminal offenses that successfully enter the financial system which is motivated to pursue profit and has a negative influence or can be harmful or detrimental to national and even international interests. Another instrument which is an institution to prevent and eradicate money laundering is the establishment of the Financial Transaction Reports and Analysis Center (PPATK) by the government as mandate for the enactment of Law Number 8 of 2010 concerning Prevention and Eradication of Money Laundering Crimes. The Financial Transaction Reports and Analysis Center (PPATK) is tasked with storing and evaluating suspicious transaction information and reporting it to the police and prosecutors if there are elements that fulfill the crime of money laundering[3]. Besides that, PPATK is an independent institution formed in order to prevent money laundering.

In international practice in the field of money laundering, the same institution as PPATK is called the Financial Intelligence Unit (FUI), which is implicitly regulated for the first time in forty recommendations, from the Financial Action Task Force on money laundering (FATF). The Financial Intelligence Unit is a harvesting agency that specializes in handling money laundering. This institution is one of the most important structures in money laundering efforts in each country.

Politics of Law of Criminal Policy in Money Laundering

From the perspective of politics of criminal law, the use of a legal means cannot be a priori or absolutely stated as a necessity or otherwise stated as something that must be rejected or abolished altogether. This means, seen from the criminal point of view of politics of criminal law, the main problem does not lie in the pro or contra problem of the use of criminal sanctions,

but the most important is the lines of policy or approach which should be taken in using criminal sanctions. One of the sounding considerations of the Caracas Declaration of the Sixth UN Congress in 1980 consider that the phenomenon of crime, through its influence on society, disrupts the entire development of nations, damages the welfare of the people both spiritually and materially, endangers human dignity and creates an atmosphere of fear and violence that encourages quality of life. Therefore, planning policies to improve social welfare must also be accompanied by a policy of social protection planning. In fact, in determining social policy, which is rational efforts to improve the welfare of the community, it must also include a policy regarding social protection planning, called "politics of criminal law"[5]. The ultimate goal of criminal policy is "community protection" to achieve the main goal which is often referred to by various terms, for example "happiness of the citizens", "a healthy and refreshing cultural life" (a wholesome and cultural living), "Social welfare" or to achieve "equality". Thus, politics of criminal law which are part of the planning of community protection are also part of the overall social policy.

In connection with this concept of thought,[6] once stated that if criminal law is to be involved in efforts to overcome the negative aspects of community development, then it should be seen in the overall relationship of politics of criminal law or "social defense planning". It was also stated later, that even "social defense planning" must be an integral part of the national development plan. This integrated conception of thought and policy also underlies the discussion of the issue of "Crime and development" which became the central theme of the fourth 1970 United Nations Congress on Prevention of Crime and The Treatment of Offenders. Therefore it is also natural that in one report on the issue of "Social defense policies in relation to development", it was stated among other things: "every dichotomy between state policy for the protection of society and planning for national development is something that is not according to its definition" (Fourth United Nations Congress). Thoughts of this integral policy were put forward by W. Clifford at the 32nd International Seminar Course on Reform in Criminal Justice in Japan in 1973[7].

From the concept of thought and the integral policy, there are two things that need to be considered in the crime prevention policy using criminal sanctions. First, there needs to be an integral approach between penal and non-penal policies. Second, policy approach and value approach are needed in the use of criminal sanctions. Efforts to tackle crime problems have been carried out in various ways, but the results have not been satisfactory. It is very interesting what was stated by Habib-Ur-Rahman Khan in his article entitled "Prevention of Crime - It Is Society Which Needs the Treatment" and Not the Criminal"[8], that criminal policy as an inherent part of general political and social policy. One of the efforts to tackle crime is to use criminal law with criminal sanctions. However, even this business is often questioned. The debate over the role of the criminal in dealing with this crime, according to Inkeri Anttila[9], has been going on for hundreds of years and according to Herbert L. Parker[10], efforts to control anti-social acts by wearing criminal someone who is guilty of violating a crime is "a social problem that has an important legal dimension"[11].

The use of legal measures, including criminal law as one of the efforts to overcome social problems, including in the field of law enforcement policies, in addition because it aims to achieve the welfare of society in general, the law enforcement policy is also included in the field of social policy, namely all rational efforts to achieve community welfare. As an issue that is a policy issue, the use of criminal law is not a necessity. There is no absolute in the field of policy because in essence in the policy problem people are faced with the problem of policy assessment and selection of various alternatives[10]. Thus, the problem of controlling and overcoming crime by using criminal law is not only a social problem as stated by Parker[10], but also the problem of policy.

Policy formulation can be said as a stage of functionalization, in this case the functionalization of criminal sanctions of prisons related to the problem of prevention and eradication of the crime of money laundering. Broadly speaking, the functionality of criminal sanctions against money laundering prevention and eradication can be divided in some ways. First, the functionalization of criminal law can be interpreted as an effort to make the criminal law can function, operate or work and be realized concretely. The term functionalization of criminal law can be identified with the term operationalization or concretization of criminal law which is essentially the same as the definition of criminal law enforcement. Contrary to this understanding, the functionalization of criminal law, such as the functionalization or law enforcement process in general, involves a minimum of three interrelated factors, namely legal factors, law enforcement agencies/

factors and legal awareness factors. The division of these three factors can be related to three components of the legal system, namely the legal substance, legal structure and legal culture.

Second, legislative factors that should be reviewed are legislative policy factors relating to the problem of preventing and eradicating money laundering. Reviewing this issue is very important because legislative policy is basically the most strategic initial stage of the overall planning of the legal functionalization process during or criminal law enforcement process. In other words, the legislative policy stage is the most strategic stage for crime prevention efforts. This stage is the formulation stage that forms the basis, basis and guidelines for the next functionalization stages, namely the application stage and the execution stage[12]. Hence, crime prevention planning or policy as outlined in the legislation, in general covers planning or policies regarding what prohibited acts will be dealt with because they are considered dangerous or harmful; planning or policy on what sanctions are imposed on the perpetrators of the prohibited conduct (whether in the form of a criminal or act) and the system of its application; planning or policy regarding procedures or mechanisms of the criminal justice system in the framework of criminal law enforcement processes.

Therefore, a review of legislative or formulative policies relating to money laundering should also be focused on the three policy areas (policy or plan of action) above.

Conclusion

Efforts are made in preventing and overcoming the Crimes of Money Laundering there are two suggestions that can be chosen: firstly, using the means of punishment (criminal sanctions), and secondly, using non-penal means (other than criminal sanctions), such as social sanctions, economic sanctions, and so on. Basically, the means of punishment are retaliatory/ retributive of crimes committed by perpetrators, while non-penal means are essentially meaning to prevent the occurrence of criminal acts or commonly referred to as a means of incentive. Perpetrators of money laundering can be classified into two namely active and passive perpetrators. The two perpetrators can be criminally liable, Money Laundering Crime Countermeasures conducted by the Financial Transaction Analysis Center must pay attention to: alert principles and reporting system, implementation of financial reporting systems, the application of the principle of getting to know customers, cooperating with related parties. The modus operandi of money laundering often uses bank secrecy, but by Bank Indonesia confidentiality can be excluded for checking customer accounts. The use of penal means is intended to deter and act against any act that can harm the public or state finances. For suggestions, it is necessary to increase bank supervision by the PPATK in particular for suspicious transactions and the enforcement of the principle of knowing customers as a means of preventing money laundering. It is also suggested to uphold the principle of state financial protection in the criminal justice system relating to the practice (legal case) of money laundering. Then hold relations between countries as an effort to prevent and prosecute cases of money laundering and finally the imposition of maximum penalties as a means of law enforcement.

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Fiduciary Security Execution after Constitutional Court Decision

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Abstract

Fiduciary collateral is one of the material guarantees, thus giving a fiduciary recipient a strong position. This is the main principle of fiduciary collateral, one of which is if the debtor defaults then the fiduciary collateral object can be executed. With the decision of the Constitutional Court No. 18/PUU-XVII/2019, there are various conflicting opinions. This study aims to find evidence and analyze with legal studies relating to the execution of fiduciary guarantees after the existence of the decision. The research method used is socio legal. From the results of the study it can be seen that the existence of the Constitutional Court decision lead to a variety of different interpretations. It is assumed that fiduciary recipients can no longer carry out executions based on their own power because they have to go through court. On the other hand, it is of the view that if the fiduciary recipient does not have the right to carry out executions based on the power of the fiduciary recipient himself and must go through a court of law then this is contrary to the principle of material security. The difference in interpretation can lead to legal uncertainty and lack of legal protection for fiduciary recipients who should have a strong position. Among other things in the case of debtor defaults, execution should be carried out without having to go through court.

Keywords: *execution, guarantee, fiduciary, constitutional court decision, default*

Introduction

As a material guarantee, fiduciary security is a strong guarantee and easy to implement. Therefore, if the debtor defaults, execution can be carried out on the object of fiduciary security in accordance with Article 29 of Law Number 42 of 1999 concerning Fiduciary Guarantee. The granting of fiduciary guarantees is done through a process called the Constitutum Procedure (the transfer of ownership of objects without giving up the physical object). In connection with this fiduciary guarantee, the physical object remains in the hands of the owner or debtor[1]. Article 1 number 1 number 2 Law No. 42 of 1999 concerning Fiduciary Security states that Fiduciary is the transfer of ownership rights of an object on the basis of trust provided that the object whose ownership rights are transferred remain in the possession of the object owner. Based on the provisions of Article 1 number 2 of Law No. 42 of 1999, fiduciary guarantee is the right of guarantee for movable objects both tangible and intangible and immovable objects, especially buildings which cannot be encumbered by mortgage rights as referred to in Law No. 4 of 1999 concerning Mortgage Rights which remain in the possession of the Fiduciary Giver, as collateral for the settlement of the debt, which gives priority position to the Fiduciary Recipient of other creditors. The emergence of the fiduciary guarantee institution as it is known today in the form of fiduciare eigendomsoverdracht or FEO is related to the provisions in Article 1152 paragraph (2) Civil Code that require that the authority over the mortgaged object may not be in the hands of the pledge (inbezitstelling requirements). The birth of a fiduciary guarantee was basically born as an effort to anticipate a deadlock from having to move the collateral object in a pawn[2].

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For fiduciary creditors, fiduciary guarantees benefit because they can guarantee the security of their receivables. For fiduciary givers, this fiduciary guarantee is beneficial because the object can still be used for daily needs, especially if the object is an item for the purposes of running a business[3,4]. The benefits of fiduciary guarantees for the development of the business world cannot be denied. However, on the other hand there are often various problems that arise in the regulation, implementation and enforcement of fiduciary guarantee laws, including the issue of execution of fiduciary guarantees[5]. As one of the material guarantees, it gives authority to creditors to carry out the execution of fiduciary guarantees in accordance with what is regulated in Article 29 of Law Number 42 of 1999 concerning Fiduciary Guarantees which covers 3 ways of execution. With regard to the right to execute in the case of debtor default, these problems often arise, even though they are explicitly regulated in the Fiduciary Security Act. One of them is related to Article 15 Paragraph (2) Fiduciary Guarantee Certificates stating that fiduciary guarantees have the same executorial power as court decisions that have permanent legal force. This even caused a dispute between the parties that ended in the process in court. There is a judicial review of the Article to the Constitutional Court, which in the end the Constitutional Court makes a decision namely decision No. 18/PUU-XVII/2019. This decision raises pros and cons in their implementation and various interpretations in society.

This study uses the socio legal research method with library research to obtain secondary data relating to the regulation of fiduciary guarantee execution and field research to obtain primary data by examining the implementation of fiduciary guarantee in public life after the Constitutional Court Decision Number 18/PUU-XVII/2019. The concepts and theories that will be used as analysis knives are the Fiduciary Guarantee concept, the concept of Execution of material guarantees, the concept of material rights, and the concept of property rights. This study raised the issue of legal study of the Constitutional Court's decision on the implementation of fiduciary guarantee execution.This study is aimed at uncovering and analyzing the legal consequences of the Constitutional Court decision on the implementation of fiduciary guarantees.

Literature Review

Collateral is everything that is received by creditors and handed over by the debtor to guarantee a debt receivable in the community or guarantee the security of creditors' debts. Thus in principle the guarantee is an accessoir because its main function is to guarantee the fulfillment of the achievements that arise from an engagement[6]. In Article 1131 Civil Code stated that all debtors, both movable and immovable, both existing and new will later become guarantees for all debtor's personal commitments the. The civil code can be seen that everyone is responsible for his debt, this responsibility in the form of providing his wealth both movable and immovable objects, if necessary sold to pay off his debts (the principle of schuld and haftung)[7].

In Article 1131 Civil Code laid the general principle of a creditor's rights to his debtor[8]. Thus, all debtors' assets are collateral for debtors' debts to creditors. General principles contained in Article 1131 The Civil Code is further described in Article 1132 Civil Code, which states that the material preformance in Article 1131 is a joint guarantee for creditors, and the results of the auction of the material are divided between creditors balanced according to the size of their respective receivables unless there are valid reasons to put the receivables from one another.

From the provisions of Article 1132 of Civil Code can be seen that in the event that a creditor has several creditors, the position of the creditors is the same (the principle of creditorium parity). If there is not enough wealth to pay off his debts, the creditors get paid based on the principle of balance/equality, that is, each gets a payment proportionally according to the size of the credit of each creditor. Thus contained a general principle that is the equality of the rights of creditors to the assets of the debtor, these legal reasons as referred to in Article 1133 Civil Code, if there are debts with privileges, liens and mortgages. These debts must be paid in full[3].

Receivables whose repayment must take precedence over other receivables are called preferred receivables, while receivables which are settled according to the principle of balance are called concurrent receivables[9]. Fiduciary guarantee is one of the special guarantees which are material guarantees. The Fiduciary Guarantee Institute was actually very old and was well known and used in Roman legal society. In Roman law, this guarantee institution is known as the fiduciary cum creditore contracta (that is, a promise of trust made with a creditor). The contents of the promise made by the debtor with his creditor is that the debtor will surrender ownership rights to an object to his creditor as collateral

for his debt with an agreement that the creditor will transfer the ownership back to the debtor when the debt has been paid in full. Such relationships are called fides or fiduciary relationships[10].

In addition to the guarantee agency, in Roman law there is also a depositary institution known as fiducia cum amico contracta (that is, a promise of trust made with a friend). With the two fiduciary institutions mentioned above, to avoid misunderstanding in the Fiduciary Guarantee Act it is emphasized that what is regulated is a fiduciary guarantee institution, so the title of the law is "Fiduciary Guarantee", while in the brief title it is sufficient to mention the Law about Fiduciary. Usually guarantees in business relationships, especially in financial institutions are made in the form of fiduciary transfer of ownership[11]. The granting of fiduciary guarantees is done through a process called the Constitutum Procedure (transfer of ownership of objects without giving up the physical object)[1]. In connection with this fiduciary guarantee, the physical object remains in the hands of the owner or debtor.

Fiduciary security institutions have characteristics[12]. First, fiduciary guarantees have the nature of accessoir, meaning that fiduciary guarantees are not independent rights but the birth of their existence or removal depends on the principal agreement. Second, giving a preferential position to the fiduciary creditor recipient over other creditors (Article 27 of the Fiduciary Guarantee Act), meaning that the creditor has the right to take the settlement of his receivables over the results of the execution of objects which become fiduciary objects take precedence over other creditors. Third, it always follows the object guaranteed in the hands of whoever that object is (droit de suite) (Article 20 of the Fiduciary Guarantee Law). Execution of the Fiduciary Guarantee is regulated in Articles 29-34 of the Fiduciary Guarantee Act.

Method

This study uses the socio legal research method with library research to obtain secondary data relating to the regulation of fiduciary guarantee execution and field research to obtain primary data by examining the implementation of fiduciary guarantee in public life after the Constitutional Court Decision Number 18/PUU-XVII/2019. The concepts and theories that will be used as analysis knives are the Fiduciary Guarantee concept, the concept of Execution of material guarantees, the concept of material rights, and the concept of property rights.

Decision of the Constitutional Court Relating to the Execution of Fiduciary Guarantees

The Constitutional Court on January 6, 2020 decided in its ruling that among other things stated that "Article 15 paragraph (2) of the Fiduciary Security Law as long as the phrase" executive power "and the phrase" equals a court decision with permanent legal force "contradicts the Law Basic of the Republic of Indonesia in 1945 and does not have binding legal force insofar as it is not interpreted "to fiduciary guarantees that there is no agreement on breach of contract (default) and debtors object to voluntary submission of objects that become fiduciary guarantees, then all legal mechanisms and procedures in the execution of execution Fiduciary Security Certificates must be carried out and apply the same as the execution of court decisions that have permanent legal force.

In practice there are various interpretations of the Constitutional Court's ruling which ultimately results in legal uncertainty in its implementation. One of the reasons for the presence of the concept of seizure executives against moving material security was because of the uncertainty in the execution of civil decisions in Indonesia. The number of financial institutions that are not small in Indonesia will certainly give rise to potential disputes in the courts arising from the decision of the Constitutional Court so that it will cause new problems. It will also cause problems related to the efficiency of dispute handling which is likely to occur in court between creditors and debtors if the value of the fiduciary collateral is not so great if it must be done through the realm of the Court which will take up to three months which of course will require a lot of bailiffs to that the Constitutional Court Ruling above mentioned that the Act No. 42 of 1999 concerning Fiduciary Guarantee becomes raw again, because the spirit of this fiduciary law was revoked. According to Kelvin Wiratno, Chairperson of the Indonesian Financial Services Association (APPI), the Constitutional Court's Decision clarifies Article 15 of Law No. 42/1999 concerning Default or Injury Promise between Debtor and Creditors. Hence, the leasing can still pull the vehicle from the defaulted debtor that was previously warned, provided the procedure has been carried out.

It should be based on the legal principles of the agreement, if the debtor does not carry out the obligations stipulated in the agreement, there will be a default. Therefore, even though the default clause was not formulated, the debtor had failed to fulfill the promise (default). With the debtor default, the fiduciary recipient should be executed based on the legal principle of the fiduciary material security of the recipient according to Article 29 Paragraph (1) stating that "if the debtor or Fiduciary Giver fails the promise, the execution of the object which becomes the object of the Fiduciary Guarantee can be carried out by implementation of the executorial title referred to in article 15 paragraph (2) by the Fiduciary Recipient; sale of objects which become the object of the Fiduciary Guarantee at the authority of the Fiduciary recipient himself through a public auction and take the payment of the receivables from the proceeds of the sale; underhanded sales made under the agreement of the Fiduciary Giver and Receiver if in this way the highest price is obtained that benefits the parties".

Furthermore Article 29 Paragraph (2) states that the sale as referred to in paragraph (1) letter c is conducted after 1 month has passed since written notice by the Fiduciary Giver and or Recipient to the parties concerned and announced at least in 2 newspapers circulating in the relevant region. Article 30 of the Fiduciary Guarantee Law states that fiduciary givers must submit objects that are objects of Fiduciary Security in the context of carrying out the execution of Fiduciary Guarantees. Decision of the Constitutional Court relating to the default clause must be formulated in the deed in advance is actually not quite right and does not provide legal protection to the creditor receiving the material guarantee, in this case the fiduciary guarantee. In addition, relating to the execution of fiduciary guarantees also gives rise to various interpretations. Some argue that the execution of fiduciary guarantees must be carried out through the mediation of the court. On the other hand, there are those who argue that they can still execute without the mediation of the court. This makes legal uncertainty in the implementation of fiduciary guarantees.

Conclusion

Decision of the Constitutional Court Number 18/PUU-XVII/2019 gives rise to different interpretations in the implementation of the execution of fiduciary guarantees. On the one hand there is an assumption that after the decision of the Constitutional Court, the fiduciary creditor can no longer carry out his own execution, but must go through the court. On the other hand, not all executions of fiduciary guarantees must go through court. This creates legal uncertainty and lack of legal protection for fiduciary recipients. The difference in interpretation can lead to legal uncertainty and lack of legal protection for fiduciary recipients who should have a strong position. Among other things in the case of debtor defaults, execution should be carried out without having to go through court. Therefore, execution should be carried out based on the authority of the fiduciary recipient by public auction. Therefore, there are still 3 ways of execution as regulated in Article 29 of the Fiduciary Security Act.

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The Anti-Proliferative Activity of Ephedra Aphylla Methanol Extract Against Liver Cancer Cell Line

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Abstract

This study aimed to investigate the anti-proliferation activities of methanol extract *ephedra aphylla* against (HC) liver cancer cell line in *vitro and to identify the possible mechanism of action*. Anti-proliferative activity of the plant extracts against the cancerous cell lines was tested by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay on liver cancer cell line (HC). Gene expression studies using qPCR for the proapoptotic gene P53 were also carried out. The methanol extract of *ephedra aphylla* exhibited promising anti-proliferative activity. Dose response relationship was shown after 72hr incubation. Significant cytotoxic activity of methanol extracts has identified on HC, IC50 was (6.79 µg/ml). Furthermore, methanol extract significantly up-regulated the expression of P53. Thus, these findings suggest potential utilization of *ephedra aphylla* as new source in researching for new anticancer agents.

Keywords: *ephedra aphylla, HC, antiproliferative, p53*

Introduction

Liver cancer is the second leading cause of cancer mortality worldwide and fifth most common cancer [(1)], Beside that a 5-year survival rate is only 8.9% even with intensive conventional therapy, the burden of this aggressive malignancy is growing globally, and it could be 1 million cases by 2030 [(2,3)]. lethality of liver cancer mainly due to its resistance to the current anticancer agents, a lack of biomarkers that can detect early stage of disease, and underlying liver disease that restrict the use of chemotherapeutic drugs [(4)] . Recently, in attempt to enhance patient's life style, and to increase efficacy of convention chemotherapy, the Plants became effective source of complex chemical compound that could be used as a good candidate for the development of such novel anticancer agents [(5)]. Many plants and natural agents have been investigated through in vitro and in vivo as potential an anti-HCC agent for several years [(6)].

Ephedra aphylla is amember of Ephedraceae family that known for its medicinal value in the Mediterranean area because of the ephedrine alkaloid and other chemicals in the stems of most members of this genus [(7)]. Even though there are very few content of alkaloids in *ephedra aphylla* but there are many on flavonoids and condensed tannins, triterpenes, and cardiac glycosides; compounds that were reported to have medicinal efficacy against numerous diseases [(8)]. Some studies have reported that many of phytochemical compounds that had isolated from Arial parts of *ephedra aphylla* had chemo preventive potential as it inhibits in vitro chemical carcinogenesis and exerts pro-apoptotic and anti-proliferative effects in different tissues [(9,10)].

The study of gene expression profile of cancer cells has become an important tool to understand the biological modifications involved in disease development, to generate personalized pharmacological therapies for patients, and to explore the molecular effects of drug exposure with the purpose of improving the effectiveness of treatment [(11)]. A p53 protein an important candidate target against which new anticancer treatments could be developed [(12)] as the p53 transcription factor stands out as a key tumor suppressor and a master regulator of various signaling pathways involved in this process. The many roles of p53 as a tumor suppressor include the ability to induce cell cycle arrest, DNA repair, and apoptosis [(13)] .in liver cancer , Inactivation of this multiple tumor suppressor gene plays an significant role in the progression of chronic liver damage to hepatocellular carcinoma by directly or indirectly

inducing chromosome instability, cell proliferation and neovascularization [14,15,].

Materials and Methods

Preparation of Extract

ephedra aphylla arial parts (300 g), the stems were dried in the open air and away from light and moisture then powdered, sieved (60mesh) size and stored in a well closed container.

Preparation of Methanol Crude Extract

300 g powder *ephedra aphylla* stems was divided into three parts and extracted with methanol for for 8 hours,in shaking water bath at 40c with stirring, The extraction ratio of each solvent was 4:1 W/V (100gm of powder/400ml of solvent). and then the mixture filtered, the filtrate kept in umber bottle and the residue dried. Extraction process repeated three times .the extract was concentrated using a rotary-evaporator under vacuum. The extract was then kept in desiccators at room temperature.

Serial Dilution of Methanol Extract

A 10 mg of the crude extracts were dissolved in 1 ml dimethyl sulfoxide (DMSO) to get 10mg/ml as stock solutions. Form stock solutions of each extract 5 different concentration were prepared (100, 50, 25, 12.5, 6.25 µg/ml).

Routine cell culture

HC cell lines were obtained from the Iraqi Center for Cancer and Medical Genetic Research (ICCMGR) Cell Bank Unit, Cancer cell lines were maintained in RPMI-1640 supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. Cells were passaged using Trypsin-EDTA reseeded at 80% confluence twice a week, and incubated at 37 °C in a humidified 5% CO2 atmosphere, The cells used for the experiments were between passage 4 and 7[16].

Cell viability assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used as a measurement of cell proliferation in accordance with **Mosmann method** 1983[17]. The principle of this assay is based on the ability of living cells to change the yellow water-soluble substrate MTT into dark blue formazan product (insoluble in water) by mitochondrial succinate dehydrogenase enzymes. Only viable cell are able to reduce the yellow MTT while cells being dead following a toxic damage cannot. The precipitates can be dissolved after cell lysis, this product is proportion to the viable cell number and inversely proportional to the degree of cytotoxicity [18].

Cell lines were seeded at 1 × 104 cells/well After 24 hrs and when a confluent monolayer was achieved, cells were treated with serial concentrations of *ephedra aphylla* covering the range of 6.25–100 µg/µl while the untreated cells received only DMSO as a control. After 72 hrs, the medium had been eliminated and, and for each well added 28 µL of MTT solution which prepared by added 2 mg/mL of phosphate buffer saline (PBS). The plates were incubated for 2.5 h at 37 °C, in 5% CO2. The plates were taken out from the incubator and the supernatant layer was removed. After removing the MTT solution, the crystals residual in the wells were solubilized by the addition of 130 µl of DMSO (Dimethyl Sulphoxide) followed by 37 °C incubation for 15 min with shaking. The optical density reading (OD) was recorded using a micro plate reader at 492 nm for absorbance and 650 nm as reference, the assay was performed in triplicate. The percentage of cell growth inhibition was determined as a mean ± SD, using the following equation was calculated as the following equation: **Inhibition %= 1- cell viability%** [17].

Cancer cells collecting

Hc cell lines in T75 flasks, were treated with methanol extracts of *ephedra aphylla* at concentrations equivalent to the calculated IC_{50} earlier obtained. The cells were incubated for 48 hrs. After which the media was decanted and cells washed in phosphate buffer solution (PBS) to remove any debris. Trypsinization of the cells was done.

RNA extraction

The Total RNA was extracted from the samples using Bioneer Kit following the manufacturer's instruction (AccuZol™ Total RNA Extraction Solution, Bioneer®, Australia). After followed the steps of RNA extraction according to manufacturer s instructions,

the RNA pellet was dissolved in RNase-free water by passing the solution a few times through a pipette tip. The extracted mRNA was stored at -80℃.

Real-time PCR

Real-time PCR test was used to define the effect of the *ephedra aphylla* extract on the degree of gene expressionof p53 , Real time Polymerase chain reaction was carried out in AriaMx Real-Time PCR (qPCR) Instrument (agilent technologies, United States) using one-step method .This method uses a One-Step EvaGreen qRT-PCR® Kit (abm good)that contain reverse-transcriptase (RT) enzyme which convert mRNA to complementary single stranded DNA (cDNA) and also contain a DNA polymerase enzyme that convert cDNA to double stranded DNA. All these reactions take place in the same reaction tube at the same time in a reaction mixture volume of 20 µL. GAPDH was used as a housekeeping gene. The sequence of used primers is indicated in Table 1.

TABLE (1) PRIMERS SEQUENCE USED IN THE PCR REACTION

Gene	Forward primer	Reverse primer
P53	5'GATCTGTTGCTGCCCCAGGAT3'	5'AGATGACAGGGGCCATGGAGT
GAPDH	5'TGGCCGTATTGGGCGCCT3'	5'TCTCCATGGTGGTGAAGA3

Statistics

The statistic design for this study was presented as mean ±SD (standard deviation). The value of groups were compared by the one-way ANOVA then by means of Tukey Post-hoc test (t-test) and measured significance at $p<0.05$,0.01 and 0.001 by using the GraphPad Prism software, version 8.2.1 for Windows 10**.** the concentration that inhibits 50% from the cell growth (IC50) was analyzed and calculated for *ephedra aphylla* extracts by linear regression equation: y=mx+b, where y is the percentage of inhibition and is set to be 50%, m is the slope of the standard curve, x is the concentration of the compound tested in µg/mL, and b is the y-intercept of the line of standard curve.

Results

In order to determine the effect of *ephedra aphylla* extract on the growth of HC liver cancer cell lines proliferation, MTT assay was used at 72 h after incubation with different doses of *ephedra aphylla* methanol extract. The survival curve shows that the cytotoxic effects of *ephedra aphylla* methanol extract on treated cells are dose dependent. There was a significant decrease in the viability of cells incubated for 72 h. The data suggests that the *ephedra aphylla* extract significantly inhibited HC cell proliferation in dose -dependent manners. Cell growth was inhibited considerably in 100 µg/ml and 72 h compared with control groups as shown in Figure 1, cell proliferation was decreased to 64.35 % ($P<0.05$) The IC50 value was yield from the graph for extract of *ephedra aphylla* by using the linear regression equations where (x) is the concentration Y= the percentage of inhibition The IC50 value ME was 6.79 µg/ml.

The effects of *ephedra aphylla* extract on p53 expression

The mRNA levels of p53 gene were analyzed. The genes CT values were normalized against the mRNA level of GAPDH as a housekeeping gene and the relative expression for each group was measured. Figure 2 shows that, there is a significant fold incensement in the level of P53 expression by 3.53 when compared with the control group.

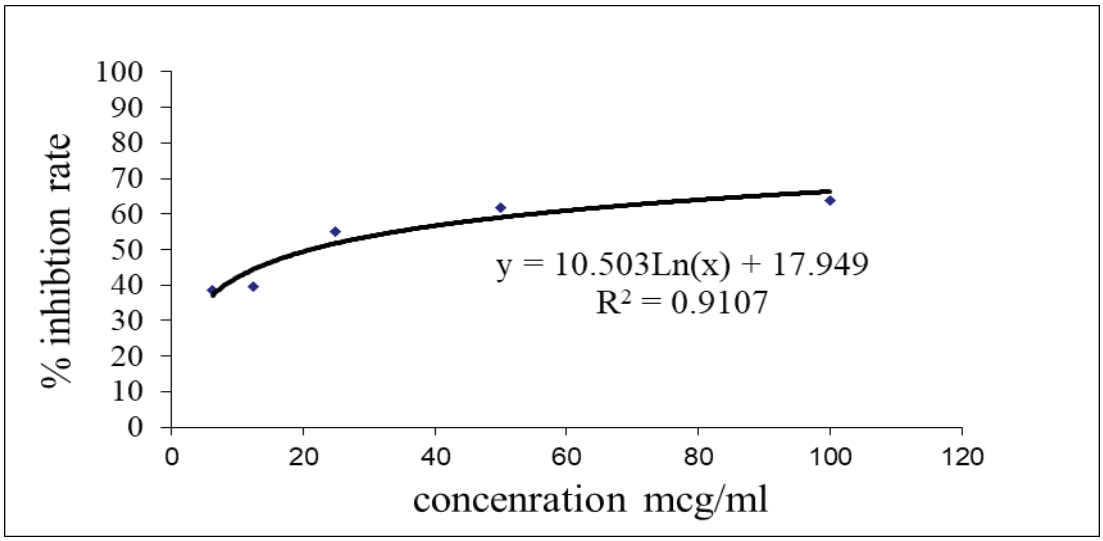


Figure (1) Dose response curve of methanol extract of *ephedra aphylla* against HC cancer cell line.

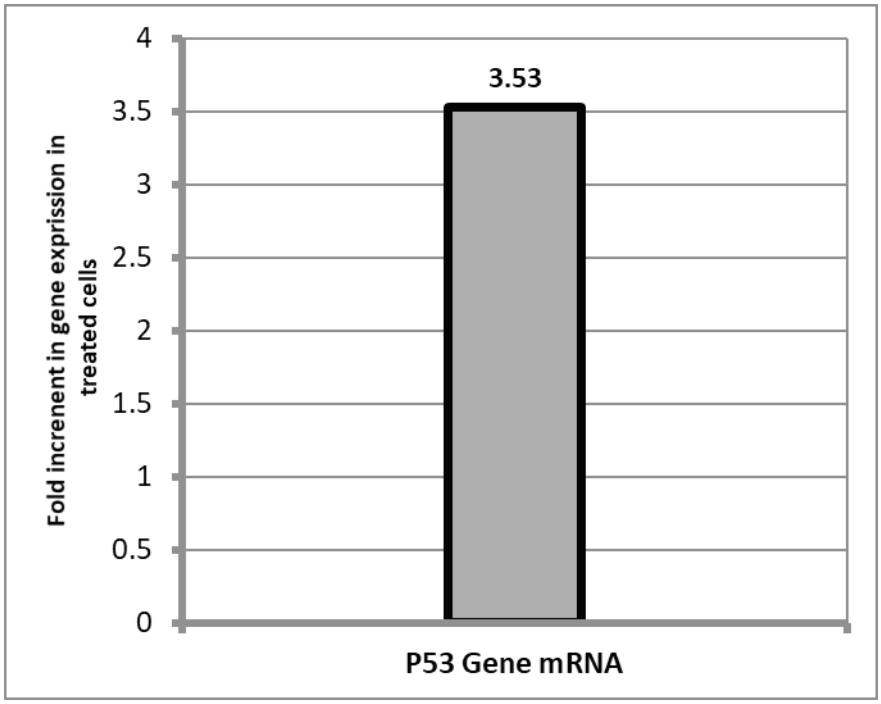


Figure (2): Fold increment in P53 gene as a result of treating the mice hepato-carcinoma cells (HC) with the plant extract.

Discussion

Methanol extract of *ephedra aphylla* has been tested against (HC) cell line to analyzed it's anti proliferative activity and measured it by using MTT assay which is one of the simple and rapid colorimetric assess and yields quantitative data [19] . The extracts showed significant dose dependent inhibition of liver cancer cell line while compared to the control (DMSO 1%) ($P<0.05$) after 72 hr. with IC50 (6.79 µg/ml) which suggesting that this sample has significant cytotoxic effect as it was lower than 20µg/ml, This is a well-recognized criterion for a compound to be judged as cytotoxic, as defined by the National Cancer Institute (NCI) [20] .

the methanol extract of *epedra aphylla* contain a wide spectrum of polyphenol and flavonoid compounds that may responsible for the anti-proliferative potential of that plant against HC cell line either by direct effect or synergistic boosting of the anticancer activities between it [21] and that suggestion support by the results of Jaradat

et al. found Methanol to be the best solvent in relation to the amount of polyphenol and flavonoid that detected in it [22].

In this study, the significant up-regulation of the P53 gene by the methanol extract of *ephedra aphylla* is an indication that it one of its way to induces apoptosis in the cancer cells (HC) via P53 dependent pathways. The activation of p53 trigger the expression of downstream genes leading to either cell cycle arrest (to allow repair and survival of the cell) at the G2/M phases [23] or it initiates apoptosis to dispose of the damaged cell through multiple mechanisms, including transactivation of specific target genes, down regulation of a distinct set of genes, and transcription-independent mechanisms [24]. Mounting evidence has confirmed that p53 is a potent activator of the caspase cascade by stimulating pro-apoptotic proteins (Bid and Bax) and stimulating the release of apoptogenic actors (cytochrome c), which lead to caspase-9 activation and in turn cleave effector caspases such as caspase-3 [25], p53 protein also activates extrinsic signaling cascades of apoptosis, that belong to tumor necrosis factor (TNF) superfamily with main signaling protein caspase 8 [26].

Conclusion

The methanol extract from *ephedra aphylla* have been demonstrated to possess significant anti-proliferative activity. The mechanism of action of the anti-proliferative activity of *ephedra aphylla* can be linked to its up-regulation of the pro-apoptotic gene p53. These findings suggest potential utilization of *ephedra aphylla* as good candidate in developing new anticancer agents.

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Histomorphological Studies of the Organs of Malaria Mice Model After Administration Combination of Leaf and Stem Bark Extract of *Syzygium cumini* with Chloroquine

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Abstract

This study aims to assess the histomorphological finding of the in spleen, brain, kidneys and lungs organ in malaria mice model after administration combination of Leaf and Stem bark extract of *Syzygium cumini* with chloroquine. Thirty-five male albino mice were randomly assigned into 7 treatment groups. K0: not infected, K1: infected. K2: infected+chloroquine. P1: infected+*Syzygium cumini* leaf extract. P2 : infected+chloroquine+*Syzygium cumini* leaf extract. P3: infected +*Syzygium cumini* stem bark extract. P4: infected +chloroquine+ *Syzygium cumini* stem bark extract. Therapy was given once a day for 4 days after 24 hours post infection. The 21stday, mice were euthanasia. The organs were taken in order to make histopathology preparations, stained with hematoxylin and eosin. The combination of *Syzygium cumini* leaf extract and chloroquine in mice infected with *P. berghei* can reduce cerebrum and cerebellum microglia cell counts, white pulp diameter size, haemorrhages damage, alveolar congestion, edema and thickened alveolar septa of lung, decreased tubular necrosis, and interstitial nephritis of renal.

Keywords: *chloroquine, histomorphological, mice, P. berghei, Syzygium cumini*

Introduction

In 2018, an estimated 228 million cases of malaria occurred worldwide (95% confidence interval [CI]: 206–258 million), there were an estimated 405 000 deaths from malaria globally[1]. Malaria infection in erythrocytes causes clinical symptoms of fever and anemia. Then, complications in severe malaria can cause multisystem disorders that can affect different organs (brain, lung, liver, kidney and spleen).In patients with childhood malaria, clinical complications are showed by cerebral malaria, severe anemia and metabolic acidosis. While in adults sufferers are shown to have cerebral malaria, metabolic acidosis, acute kidney failure, jaundice and acute respiratory disorders [2].

The pathogenesis mechanism of severe malaria is associated with immune cell activation and upregulation of pro inflammatory cytokine, endothelial dysfunction, dysregulation of coagulation pathways and microvascular obstruction mechanisms by the sequestration of infected erytrocytes.In erythrocytic phase, hemoglobin degradation by parasites causes free heme (Fe^{3+}), this molecule plays a role in inducing oxidative stress. Oxidative stress has implications for lipoprotein oxidation and serious damage to different organs through the formation of reactive oxygen

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intermediates and nitrogen intermediates (ROI and NO) by host cells. LDL oxidation will upregulate the expression of adhesion molecules, and also sequestration and adhesion of infected red blood cells in endothelial cells which endanger the permeability of blood vessels in vital organs [3].

Therapy for malaria is not enough to only provide antimalarial primary drugs, but also the addition of therapeutic adjuvants such as antioxidants is needed to increase efficacy and reduce complications that directly action specific biological pathways[4]. Leaf and stem bark extract of *Syzygium cumini* contains flavonoids, terpenoids, tannins and polyphenols which have scavenging antioxidant activity to reduce the effects of free radicals [5], so it is expected from this study that the combination of *Syzygium cumini* leaf and stem bark extract with primary antimalarial drugs as an adjuvant therapy can reduce the damage to mice organs in the malaria model.

Materials and Methods

Ethical approval

This study has obtained approval by certificate no 722-KE from Animal Care and Use Committee on Veterinary Medicine Universitas Airlangga Surabaya Indonesia.

Materials

Plasmodium berghei parasite strain ANKA from Institute of Tropical Diseases Universitas Airlangga, male albino swiss mice with 20g-30g and aged 2.5 months from the Veterinaria Farma Center in Surabaya. Chloroquine Pro Analysis (PA) from Sigma Chemical Co dose 25 mg /kgBW. The leaf and stem bark of *Syzygium cumini* from Kediri, East Java and then identified in the laboratory of Purwodadi botanical garden Pasuruan dose of 600mg / kgBB.

Dose of *Plasmodium berghei* infection in mice

The dose of *Plasmodium berghei* which is infected in mice were $1x10^6$ in 0.2 ml, and given intraperitoneally. Infection that occurred in mice was examined with thin blood smears taken from veins tail and stained with 25% Giemsa for 30 minutes then examined with a microscope using 400 x magnifications.

Preparation of leaf and stem bark of *Syzygium cumini*

The leaf and stem bark of *Syzygium cumini* are cleaned, aerated until dry and pounded in order to make fine simplicia.500 grams of fine simplicia from the leaves and stem bark of *Syzygium cumini*, each soaked with 2 L 96% ethanol solvent, then ultrasonic for 6 minutes. After that, it was filtered using a Buchner funnel, the filtrate was evaporated until a thick extract was formed and then freez dried into a dry extract.

Treatment of the experimental animals

Mice were adapted for two weeks, given feed and distilled water in ad libitum.Mice were randomly divided into 7 treatment groups.The details of the treatment group are as follows, control group: K0: mice were not infected, K1: mice were infected. K2:mice were infected and treated chloroquine25 mg/kgBW. Treatment groups P1: mice were infected and treated *Syzygium cumini* leaf extract 600mg/kgBW. P2 :mice were infected treated chloroquine 25 mg/kgBW and *Syzygium cumini* leaf extract 600mg/kgBW. P3: mice were infected treated Syzygium *cumini* stem bark extract 600mg/kgBW. P4: mice were infected treated chloroquine 25 mg/kgBW and *Syzygium cumini* stem bark extract600mg/kgBW. Therapy is given once a day for 4 days after 24 hours post infection. The 21stday, mice are euthanasia in order to take the organ.

Organ collection to make histopathology preparation

Mice were anesthetized with ketamine 40 mg/kgBW intra muscular, the four legs were fixed in the supine position, thoracotomy was performed until the viscera organs were seen.Perfusion of the heart at the end of left ventricular using PBS buffer by cutting the right auricle until clean blood fluid is replaced with PBS buffer.The taken organ was put into the specimen pot fixed with 10% formalin, then alcohol dehydration followed by xylol clearing, after that the paraffin organ infiltration stage was soaked in paraffin liquid, then cut with a thickness of 3-4 μm, deparafinization in xylol then stained with hematoxylin and eosin.Assessment of the histopathology examination results is using microscope with scoring system.

Data Analysis

Data of histopathology changes of brain, lung, spleen and kidney organs were analyzed using *Kruskal Wallis Test* then proceed with *Mann-Whitney Test*. Statistical analysis in this study using SPSS (*Statistical Program of Social Science*) program.

Results

The results of scoring the number of microglia cells in the cerebrum and cerebellum can be seen in table 1, white pulp diameter spleen in table 2, the lungs histopathological changes in table 3 and renal

Table 1 Number of microglia cells in the cerebrum and cerebellum in the control and treatment groups

Groups	Cerebrum (Mean ± SD)	Cerebellum (Mean ± SD)
K0	6.20a ± 0.84	6.80a ± 0.84
K1	11.80c ± 0.84	9.60b ± 2.30
K2	6.60a ± 0.55	6.80a ± 0.84
P1	6.60a ± 0.89	6.60a ± 0.89
P2	6.40a ± 0.55	6.40a ± 0.55
P3	8.20b ± 0.45	7.60ab ± 1.52
P4	7.40ab ± 0.89	7.40ab ± 0.55

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%

Table 2 White pulp diameter of spleen in the control and treatment groups

Groups	Mean ± SD
K0	271.82a±0,26
K1	458.21b±0,37
K2	339.75c±0,09
P1	446.18b± 0,35
P2	387.22c± 0,11
P3	405.27b± 0,26
P4	393.34c± 0,18

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%

Table 3. Lungs histopathological changes of mice (*Mus musculus*) of control and treatment group

Groups	Hemorrhage	Alveolar congestion	Edema	Thickened Alveolar septa
K0	0.40d ±0.14	0.44d ±0.09	0.52f ±0.11	0.28e ±0.18
K1	1.40a ±0.24	1.44a ± 1.67	1.52a ± 0.39	1.36a ± 0.17
K2	1.24a ±0.38	1.32b ± 1.18	1.24abc ± 0.36	1.24a ± 0.41
P1	1.28a ± 0.27	1.36a ± 0.26	1.32ab ± 0.27	1.32a ± 0.23
P2	0.68c ± 0.11	0.72c ± 0.18	0.80de ± 0.20	0.56cd ± 0.09
P3	1.16a ± 0.33	1.40a ± 0.51	0.96cd ± 0.17	1.16ab ± 0.17
P4	1.28a ± 0.27	1.48a ± 0.3	1.12bc ± 0.18	0.92b ± 0.41

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%

Table 4. Kidney histopathological changes of mice (*Mus musculus*) of control and treatment group

Groups	Tubular Necrosis	Glomerulonephritis	Interstitial Nephritis
K0	0.12c ± 0.11	0.52b ± 0.23	0.00c ± 0.00
K1	1.80a ± 0.45	1.08a ± 0.30	1.32a ± 0.33
K2	1.00b ± 0.24	0.64a ± 0.22	0.64b ± 0.33
P1	1.08b ± 0.30	0.60a ± 0.14	0.76b ± 0.26
P2	0.96b ± 0.09	0.67a ± 0.29	0.68b ± 0.11
P3	1.00b ± 0.50	0.96a ± 0.26	0.88b ± 0.30
P4	1.16b ± 0.48	1.04a ± 0.44	1.08b ± 0.44

Note: Different superscripts on the same column show a significant difference at significant level of 0.05%

Heritability of Ambulatory Blood Pressure in Population of Western Region of Iraq histopathology changes in table 4.

Discussion

During the infection of *Plasmodium sp*, the microglia is activated and spreads to the brain of malaria sufferers [6]. Activated microglia cells release proinflammatory cytokines and trigger free radical formation [7]. Active ingredients in *Syzygium* contains of tannins, alkaloids, steroids, flavonoids, terpenoid, fatty acids and vitamins. The flavonoids contained in *Syzygium cumini* have pharmacological activities as antioxidants that able to capture free radicals and provide a protective effect on tissues and organs by inhibiting lipid peroxidation [8]. The combination of *Syzygium cumini* leaf and stem

bark extract with chloroquine can reduce the number of microglia cells. The use of antioxidants as adjuvant therapy combined with antimalarials is important to accelerate the healing process and reduce more severe tissue damage, prevent and inhibit the occurrence of more severe complications by providing slow release which stimulates the immune system, decreases clinical symptoms and decreases disease severity [9].

Malaria infection is a foreign body if it enters the body and its immediately recognized by the specific immune system so that there is sensitivity of immune cells that causes proliferation of white pulp and causes expansion of the white pulp diameter [10], because of the increased development of lymphocytes, macrophages and reticular cells in the white pulp [11]. Expansion of the white pulp diameter of spleen causes enlarged spleen size in malaria infections due to increased erythropoiesis and hematopoiesis [12]. Increase of hyperphagocytotic activity of macrophages and cytotoxic substances accumulation to fight against the infection [13]. The administration of chloroquine, combination of *Syzygium cumini* leaf and stem bark extract and chloroquine can reduce the size of the white pulp diameter. This is because chloroquine as an antimalarial and antioxidant content in *Syzygium cumini* leaf and stem bark extract can improve the host's immune system so that it increases phagocyte cell activity which can reduce the number of plasmodium parasites so that white pulp diameter decreases, can reduce the courses of inflammation that occurred in malaria infected-spleen and also cell swelling reduction, likely that VitC alone does not completely help reducing the damages in both morphology and histology of liver and spleen but should be combination with antimalarial drugs [14].

Malaria caused by *Plasmodium berghei* can infect red blood cells causing microvascular obstruction due to parasite sequestration. The increase in free radicals in malaria infection causes cell membrane lipid peroxidation to trigger an inflammatory response and pathological changes in the lung organ [15].The treatment of *Plasmodium berghei* infection treated with *Syzygium cumini* leaf extract and chloroquine showed a decrease in lung organ damage, namely haemorrhages, alveolar congestion, edema, wall thickening of the alveoli septa. This occurs because of barriers to the reactive oxygen species formation. Malaria therapy is not enough just by giving antimalarial drugs, however adjuvant therapy is needed by giving antioxidants from the content of *Syzygium cumini* leaf extract, such as flavonoids and polyphenols[16]. Flavonoids as antioxidants can inhibit lipid peroxidation. Antioxidants are important in supporting the immune system and reducing oxidative stress. Flavonoids can inhibit cyclooxygenase or lipooxygenase and inhibit leukocyte accumulation which can reduce damage to the lung [17].

Chloroquine administration in malaria infection can reduce the percentage of parasitemia so that it can prevent cytoadhrenece parasite which causes a decrease in tubular renal hypoxia so that it can reduce changes in tubular necrosis and interstitial nephritis. Then, chloroquine combination therapy with *Syzygium cumini* leaf and stem bark extract can protect kidney damage caused by free radicals [18]. Adjuvant therapy can work directly on factors that are being a key to the pathophysiology of malaria such as increased TNF alpha, free radicals and low levels of NO [19].

Conclusion

The combination of Syzygium cumini leaf extract and chloroquine in mice infected with P. berghei can reduce cerebrum and cerebellum microglia cell counts, white pulp diameter size, haemorrhages damage, alveolar congestion, edema and thickened alveolar septa of lung, decreased tubular necrosis, and interstitial nephritis of renal.

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Conflict of Interest : The authors declare that they have no competing interests.

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Heritability of Ambulatory Blood Pressure in Population of Western Region of Iraq

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Abstract

Heritability of ambulatory blood pressure parameters in western population has been well described, there is no information on Iraqi population. Therefore, the aim of the present study is to investigate genetic influences on intra-individual variation, by performing heritability analysis on an Iraqi population (Anbar province).

A total of 389 individuals, constituting 93 families were ascertained. Most of the parents included in the study were in their 5th and 6the decade of life, and the siblings included in the study were in their 2nd and 3rd decade of life.

To define the portion of difference of variables that is due to genetic and non-genetic influences, full sib analysis was used to estimate heritabilities of blood pressure parameters systolic, diastolic, mean and pulse pressure were estimated during the morning and evening. Heritabilities were estimated in narrow sense which were ranged from 28 % for morning systolic blood pressure to 84% for morning systemic pulse pressure.

The finding of modest narrow sense heritability indicates the significant role of non-genetic factors affecting morning systolic, evening systolic and diastolic, and mean blood pressure, whereas the high estimate of heritability for morning diastolic and pulse pressure indicate the significant effect of genetic.

Key words: Heritability, Blood pressure, systolic, diastolic

Introduction

Hypertension, which has an impact onabout1/3 of the old age people of the population in Western countries, is currently becoming a considerable problem in Middle Eastern region too, with incidence ranging from 16% to 32% amongst adults[1-3].Globally, by 2025, it is expected that there will be 1.5 billion people with systemic arterial hypertension[4]. An elevated blood pressure measurement is an important risk factors in 49% of coronary artery disease and 69% of stroke, and lead to half of all cardiovascular mortality [5,6].

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An increasing number of studies exhibit the presence of significant genetic difference in natural populations. The amount of genetic difference for phenotypic characters that is exist in a population is the key element of their potential for reacting to different aspects. The impact of genetic difference on a character might be very subtle to diverse contributions an individual receives from its environment. It is very inspiring to reveal genetic variations that require this things. In human being population genetics studies, there is no opportunity to governor the environmental exposures. These can be examined by intensive subject profiling. Though, it might be essential to study populations having individuals with significantly diverse environmental exposures to detect the major effects, and the price and complication shaped by this kind of researches is an obstacle[1,7,8].

The first step in unscrambling the genetic sources of an illness or character is the heritability estimate which is usually reflected the first pace in revealing the genetic bases of a disease or trait and numerous studies existing or heritability estimates for different characters or diseases. Following step is publishing the finding results later these results to assist in determining the necessary sample size in order for the study to have a positive influence to recognize the vulnerable gene[1]. Highly heritable traits could be correlated with important or complex traits, and these interactions very important in any program to understand the effects of genetic and environment factors that effect on blood pressure parameters[9]. Although heritability of ambulatory blood pressure in many populations such western population and east African population have been well described[10,11], there is no information in Iraqi population. Therefore, the purpose of the present study is to investigate genetic influences on intra-individual variation of ambulatory blood pressure parameters, by performing full-sib heritability analysis on a sample of Iraqi population reside in the western region of Iraq.

Materials and Methods

Subjects

Ninety three families were included in this study. The total number of studied population was 389 individuals, consisting of 18- to 61-year-old healthy subjects. All subjects provided informed consent.

Measurements of Anthropometric

Sex, waist circumference, height and weight were measured at home using standard methods. Body mass index (BMI) was measured as weight/height^2 (Kg/m^2).

Blood pressure

Measurements of ambulatory blood pressure were documented throughout the period of the study. Blood pressure was documented by the average of two measurements taken at specified times in the morning and afternoon. The blood pressure was measured after the subjects have been seated for 5 min, using a standard mercury sphygmomanometer. The subjects were in a supine position. A first reading was taken followed by a second reading after a 2 min delay. The mean of two consecutive measurements which were less than 10 mmHg apart was used. Siblings included in the study were in their 2nd and 3rd decade of life, most of them were having a normal body mass index (BMI), and a waist circumference (WC) below 80 cm. The measured phenotypes of blood parameters were morning systolic blood pressure(MSBP), morning diastolic blood pressure (MDBP),evening systolic blood pressure(ESBP), evening diastolic blood pressure (EDBP) , mean of morning systolic blood pressure(MMSBP), mean of morning diastolic blood pressure (MDBP), mean of evening systolic blood pressure(MESBP), mean of evening diastolic blood pressure (MEDBP) , morning pulse pressure (MPP), evening pulse pressure (EPP)

Mean BP (MBP) was estimated as :

$$MBP = DBP+[(SBP-DBP)/3] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1).$$

The difference between the systolic and diastolic pressure define as pulse pressure. It is reading in millimeters of mercury (mmHg). It shows the force that the heart produces each time it contracts. was calculated by the following formula.

$$\text{Systemic pulse pressure} = P_{systolic} - P_{diastolic} \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Also, Uric acid (UR), cholesterol (CH), and triglycerides (TRI) were measured for each individual under investigation.

Statistics

Descriptive statistics were accomplished by SPSS software package (IBM Corporation New York, USA) [12].

Heritability

Heritabilities estimate accomplished by utilizing the maximum-likelihood-grounded on variance breakdown technique executed by the SAS program[13].

To define the portion of variance of the variables that is due to genetic influences, the full-sib method was used to estimate the heritability for different characters under investigation. In order to reduce the inflation of genetic variation estimate that may arise from similarity of a number of environmental effects, therefore the potential effect of all main effects that were included in this analysis were statistically factored out. Those factors were age, body mass index and waist circumference.

A P-value less than 0.05 were considered statistically significant. Estimated heritability near (0) imply that there are no genetic variation whereas values close to (1) imply strong genetic variation under the assumption of an underlying multi factorial model[9]. All analyses were performed using SAS[13].

Results and Discussion

Blood pressure is a complex genetic character with heritability estimation between 30 to 70% of the character difference which is due to genetic difference. The advancement of genetic exploration to recognize the genes that cover this difference and thus has impact on blood pressure regulation and effect the risk of hypertension has gotten a new level with large number of publication of wide scale of population genetic researches. Those have studied very large numbers of genetic markers in an effort to link single markers with blood pressure and hypertension [1,3,14,15].

Heritability values are helpful in predicting the expected genetic and environmental variations for any traits under investigation for specific population. Estimates of heritability in narrow sense ranged from 28 % for MSBP to 84% for morning systemic pulse pressure (Table 1). The estimate of heritability in table (1) of MSBP, EDBP , ESBP, MMBP, MEBP, Cholesterol, Uric acid were low category and other traits were moderately to very high. This study was conducted to estimate heritability by full-sib method. Whereas, greatest heritability researches on ambulatory BP were conducted in twins and have stated big value of heritability estimates. Researches in diverse cohorts of twins of Caucasian and African backgrounds stated heritability value for systolic BP (SBP) and diastolic BP (DBP) throughout day and night (sleep) time reaching 0.44 to 0.75 [11,14,16].

Table (1) Heritabilities and means for different characters under investigation with their corresponding standard errors

Characters	No.	h2 ± S.E	Mean ± S.E
Morning DBP	389	0.70 ± 0.24	78.14 ±7.16
Morning SBP	389	0.28 ± 0.22	120.22 ± 9.650
Evening DBP	389	0.36 ± 0.23	77.47 ± 7.72
Evening SBP	389	0.34 ± 0.23	119.49 ± 9.53
Mean Morning BP	389	0.39 ± 0.21	91.74 ± 6.94
Mean Evening BP	389	0.39 ± 0.20	91.01 ± 7.40
Morning Systemic pulse pressure	389	0.84 ± 0.22	42.08 ± 8.35
Evening Systemic pulse pressure	389	0.28 ± 0.21	41.69 ± 7.97
Cholesterol	389	0.31 ± 0.22	180.88 ± 25.56
Triglyceride	389	0.53 ± 0.21	196.22 ± 100.02
Uric acid	389	0.39 ± 0.19	6.86 ± 16.914
Age	389		28.13 ± 9.93
BMI	389		24.05 ± 6.65
WC	389		85.10 ± 16.39

The characters having low heritability indicated relative small contribution of the genetic factors and large contribution of environmental factors to the phenotype and such characters could be fairly easy manipulate by modifying environmental factors like medicine , smoking and diet due to low additive effect and large environment effects. In addition to that, heritability could be altered over time since the difference in genetic variance can change, the difference due to environmental elements alteration, or the association between genetic factor and environment can altered. Genetic variation can modified if allele occurrences altered might be due to consanguinity, novel variations obtained by the population by mutation or immigration which will add to the genetic difference resulting from the change in genetic bases or the environment. Similar character estimated throughout an individual's lifespan might have diverse environmental and genetic influencing it, like the difference sturn out to be a role of age. The results are in conformity with heritability estimation of blood pressure in people descended from east African [10,17,18].

Table 2 The correlation between different characters under investigation.

	MSBP	**MDBP**	**ESBP**	**EDBP**	**CH.**	**UR**	**TG**	**MMBP**	**MEBP**	**MPP**	**EPP**	**WC**	**Age**
MSBP	1												
MDBP	.54**	1											
ESBP	.62**	.43**	1										
EDBP	.36**	.67**	.62**	1									
CH	.13	.03	.09	.06	1								
UR	.00	.031	.02	.04	.03	1							
TG	.18	.08	.15	.04	.24*	-.06	1						
MMBP	.80**	.92**	.54**	.62**	.07	.02	.14	1					
MEBP	.50**	.62**	.83**	.93**	.07	.04	.10	.65**	1				
MPP	.69**	-.23*	.39**	-.16	.12	-.01	.14	.13	.05	1			
EPP	.37**	-.17	.61**	-.24*	.03	-.01	.15	.04	.09	.58**	1		
WC	.37**	.04	.34**	-.05	.19	-.02	.04	.19	.10	.40**	.47**	1	
Age	.35**	.28**	.42**	.28**	-.02	-.07	.04	.34**	.33**	.16	.16	.38**	1
BMI	.55**	.13	.44**	.15	-.23*	.002	.18	.32**	-.29**	.53**	.35**	.59**	.44**

**P≤0.01

*P≤0.05

The degree of association among the characters is an significant factor particularly in vital and complex character as blood pressure. Steel and Torrie [19] showed that correlations are measures of the intensity of association between traits. The positively correlated characters resulted in progress all characters and retrogress for characters that are adversely correlated.

The result of correlation investigation as presented by their coefficients (Table 2) showed that MSBP, displays significantly positive correlation with the MDBP, ESBP, EDBP,MMBP, MEBP, MPP and EPP which were 0.54^{**},0.62^{**},0.36^{**},0.80^{**},0.50^{**},0.69** and 0.37**respectively.

Throughout the day, BP had no significant differences in SBP and DBP between morning and evening measurements. There is significant correlation between MSBP and ESBP which was 0.54**. Age was significantly correlated with MSBP, MDBP, ESBP, EDBP, MMBP, MEBP,MPP and EPP which was 0.35^{**} , 0.28**, 0.42** , 0.28**, 0.34**, 0.33**, 0.16, and 0.16 respectively. Sex, age, BMI and waist circumference were kept as covariates in all model-fitting analyses. Body mass index correlated significantly with MSBP (0.55**) but not with MDBP (0.13). The correlation between blood pressure characters and cholesterol, triglycerides and uric acid showed no significant correlation. While, the correlation between cholesterol and BMI was significantly negative -0.23**[20].

These results confirm that the assortative mating and environmental influences have shown to be an effective factors which effect heritability estimation, in addition to the familial aggregation of cardiovascular risk factors happens and that much of this aggregation has a genetic background. Since the study was carried out in very much consanguineous marriages, and multigenerational Arab individuals, with an average age of 28.13 years,90% of whom were younger than30years.Those people were more similar environmental exposure with a like social and economic status and similar in habits related health like habitual physical activity; diet, smoking and the strict religious abstinence from alcohol [7,21,22,23].

Conclusion

The results showed that there is sufficient genetic variation existing in the material studied. The narrow sense heritability analysis of the study revealed that the morning diastolic blood pressure and morning systemic pulse pressure were the most traits with genetic variation components. The other characters also showed moderate to low heritability and genetic variation. Therefore, the results suggest that morning diastolic blood pressure and morning systemic pulse pressure are important characters contributing in the phenotype of blood pressure and based on these traits would be most effective in any program to control blood pressure.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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Ischemic Stroke Caused by Metabolite Factors in Indonesia (GBD Study 2017)

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Abstract

Ischemic stroke was one of the leading causes of death. There are 2 risk factors, namely can and cannot be modified. Metabolite factors can be modified. This article analyzes the 2017 GBD study data in Indonesia. Quantitative analysis of secondary data from the 2017 GBD. The dependent variables are DALYs, Death, YLL to YLD ratios. The independent variable is gender and 4 metabolite factors. Data were analyzed using descriptive and inferential statistics. The overall average value of ischemic stroke DALYs was 11334.26 (143440.73 - 145.95). The average ischemic stroke mortality rate is 489.03 (3.23 - 7170.45). The average ratio of YLL to YLD is 4.74 (0.86 - 7.70). YLL / YLD ratio values are greater than 1 for all metabolite factors. This ratio indicates that the YLL value dominates the DALYs value. The value of ischemic stroke burden in men is greater than in women. The highest value of stroke for metabolic factors in a row are high systolic, high fasting, high LDL and high Body Mass Index. The difference in sex does not affect the value of DALYs. High metabolite factors affect the value of DALYs. The YLL value dominates the DALYs value.

Key words: *Ischemic Stroke, Metabolite Factors, Global Burden of Diseases 2017*

Introduction

Ischemic stroke or commonly called a non-hemorrhagic stroke is the death of brain tissue due to disruption of blood flow to the brain region. This is due to cerebral or cervical artery blockages or cerebral veins that are less likely to be blocked.[1] Classification of ischemic stroke subtypes that are often used in studies is the Trial of ORG 10172 classification in Acute Stroke Treatment (TOAST), namely atherosclerosis of large blood vessels, cardioembolic , lacunar, other causes, and unknown causes.[2]

Stroke is the cause of death among 240 types of causes of death in the world and stroke is among the second highest types of causes of death after ischemic heart disease,[3] and is projected to remain so in 2030.[4] Patient with stroke generally suffer from disabilities, need temporary or lifelong assistance. Stroke can be a burden for the family who cared for it and stroke including catastrophic diseases or diseases that require very expensive healing costs.

Stroke is caused by 2 different risk factors, namely risk factors that can be modified and factors that cannot be modified. The main risk factors for stroke include age, history of cerebrovascular events, smoking, alcohol consumption, physical activity, hypertension, dyslipidemia, diabetes mellitus, cardiovascular disease, obesity, metabolic syndrome, diet, nutrition, and genetic risk factors.[5-6] In addition, the higher incidence of ischemic stroke shows that ischemic stroke patients have a large exposure to modifiable risk factors whose control through lifestyle modification can prevent most of these incidents.[7] In general, a strong association between several risk factors (such as hypertension, diabetes mellitus, dyslipidemia, smoking, and age) and the incidence of stroke have been reported in literatue.[8]

Based on the information above, shows that ischemic stroke is still a serious problem that contributes to the highest cause of death in the world, therefore information related to ischemic structure is very important to be analyzed. This article will present information on the results of ischemic stroke analysis specifically in 34 provinces in Indonesia. The purpose of this study was to analyze GBD 2018 Ischemic Stroke research data in

Indonesia. Data analyzed were deaths, DALYs, YLL and YLD due to ischemic stroke. Analysis aims to determine the value of death, YLL and YLD ischemic stroke due to metabolic factors and the influence of sex and metabolic factors on the value of DALYs Ischemic Stroke.

Material and Methode

Study Design

This type of research is quantitative with cross-sectional design. The data analyzed were sourced from secondary data from Global Burden of Disease research provided by Health Metric International. This study was conducted in 2018 with Ethical Clearance no: LB.02.01/2/KE.086/ 2018.

Data Source

To understand the epidemiological aspects of disease in various parts of the world, including Indonesia, GBD researchers developed statistical tests to estimate the global burden of 291 diseases in all countries divided into 21 groups.[10] The GBD database is managed by the Institute for Health Metrics and Evaluation at the University of Washington in Seattle, Washington, USA (http://www.healthdata.org).

Data sources used to build the GBD database include representative national surveys, cancer registration, vital records systems, and health service usage data and covering 291 diseases in all countries in the world separated into 21 groups.[10] Information about GBD data can be found at GBD Data Input Source Tool at GHDx (http://ghdx.healthdata.org/gbd-results-tool). This tool allows users to explore input sources to GBD based on various criteria, and to export and explore the results. The GBD database is managed by the Institute for Health Metrics and Evaluation at the University of Washington in Seattle, Washington, USA (http: //www.healthdata .org). Therefore, this database provides annual assessments of deaths, years of life lost (YLL) due to premature mortality, years of life with disabilities (YLD), years of life adjusted for disability (DALY).

The GBD study uses a rigorous method to remove bias in the sources and models used to produce estimates based on the epidemiological complexity of the disease. In addition, sophisticated statistical methodologies are used to estimate regional results, thereby reducing sampling bias. The results are updated annually for the entire time series and these results replace the previous version of the GBD study.

Variables studied

The dependent variables examined for ischemic stroke cases were Death, YLL, YLD, and DALYs as a whole in 2017. The independent variables used were sex and metabolite factors, such as high fasting, high systolic, high LDL and high body mass index (BMI).

Data Analysis

Data analyzed were GBD research data in 2018. Subjects in this analysis were men and women who suffered ischemic stroke with 4 metabolic factors. All causes are analyzed from the DALYs, death, YLL/YLD ratio. DALYs were generated by summing YLLs and YLDs. Data were analyzed in addition to descriptively in the form of tables and graphs, an analysis was also conducted to determine the effect between variables using the KrussKall-Walles test. The basis for using the KrussKall-Walles test is because the independent variable is categorized as a scale (gender or type of metabolic disorder) and one dependent variable is on a ratio scale, in addition to that the data between variants of non-homogeneous data groups with homogeneity test results p value <0.05 . The indicators studied are presented in Table 1.

Table 1 Epidemiological indicators, calculations, and interpretation of the results of the indicators[10]

No	Indicator	Calculation	Interpretation
1	Death	No of Death with Specific Case	Proportion of people who die of a Stroke Iskhemik
2	DALYs	Disability Adjusted Life Years adalah YLL (kematian prematur) + YLD (tahun hidup dengan kondisi disabilitas).	Years of healthy life lost
3	YLL	Year life Loss adalah Number of deaths x standard life expectancy at the age of death	The number of years a person could have lived if the illness that killed him had not occurred
4	YLD	Year Life With Disability adalah Number of incident cases x weight of disability x average duration until remission or death	The number of years that an individual lives with a functional impairment caused by an illness

Results

The results of Ischemic Stroke analysis in 34 provinces in Indonesia, according to the results of the 2018 GBD research, were analyzed based on sex and metabolite factors. Four metabolic factors analyzed were high fasting, high systolic, high LDL and high BMI.

Table 2. Descriptive Statistics
DALYS Ischemic Stroke Cause by Metabolite Factors11

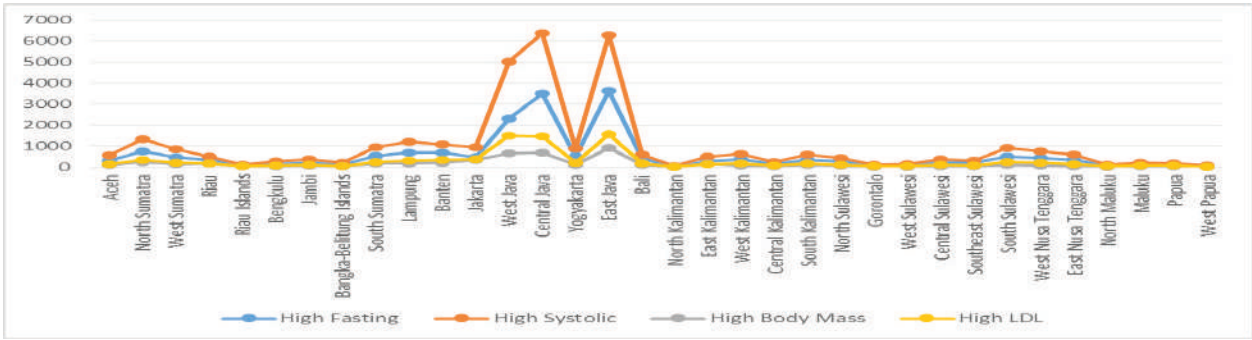
Gender	Factors Metabolite	Mean	Std. Deviation	95% Confidence Interval	
				Lower Bound	Upper Bound
Male	DALYs High Fasting	12269.57	18642.33	408.88	81434.71
	DALYs High Systolic	22718.19	35527.78	765.71	143440.73
	DALYs High BMI	4810.13	6638.33	305.33	28987.46
	DALYs High LDL	7232.70	10850.80	254.5	42249.72
Female	DALYs High Fasting	10386.29	14912.19	206.49	62030.5
	DALYs High Systolic	21078.25	32385.62	365.28	130306.99
	DALYs High BMI	5230.10	6885.30	175.23	27867.73
	DALYs High LDL	6948.81	10007.43	145.95	38185.21

The average value of DALYs in men is greater than women. The mean value of DALYs high systolic is the highest. This can be interpreted as ischemic stroke sufferers who have high systolic have a measure of the loss of life years due to the inability to move to early death due to illness which is the biggest among the 3 other metabolite factors. The next metabolite factor with a high DALYs value are high fasting, high LDL and high BMI.

The Kruskal-Wallis test results has no gender effect on the value of DALYs for Ischemic Stroke. This is known by the value of p = 0.845> 0.05. The high metabolite factors influence DALYs ischemic stroke. This is known by the value of p = 0,000 <0.05. The average mortality rate with metabolite factors is 489.03 (3.23 - 7170.45). The average YLL value is 9226.00 (71.32 - 120615.36). The average YLD is 2108.30 (57.98 - 26868.13). The average ratio of YLL to YLD is 4.74 (0.86 - 7.70).

Graph 1. Death in Men Ischemic Stroke

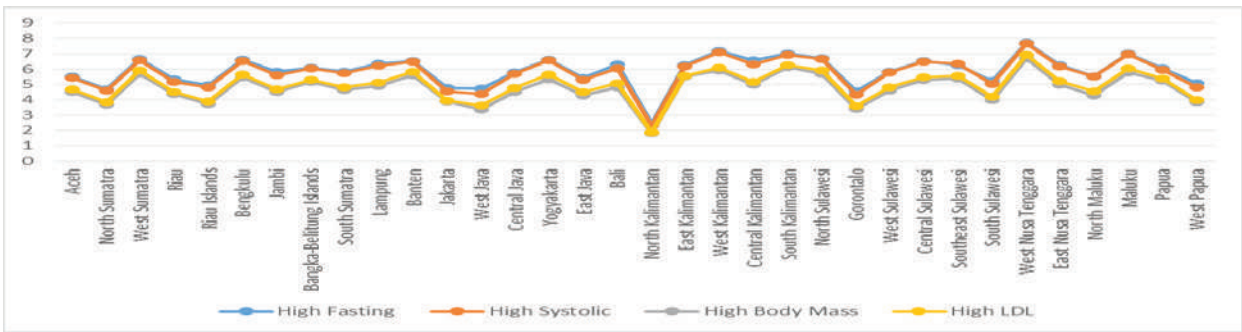
Causes by High Metabolites in 34 Provinces of Indonesia[11]



Based on graph 1, the three highest provinces of ischemic stroke mortality in men population were West Java, Central Java and East Java Provinces. Three provinces of Gorontalo, West Papua, North Kalimantan have the smallest ischemic stroke mortality due to high fasting, high systolic, high LDL. Whereas the 3 provinces that have the smallest ischemic stroke mortality with the causes of high body mass index were Gorontalo, North Kalimantan and North Maluku.

Graph 2. YLL/YLD Ratio in Men with Ischemic Stroke

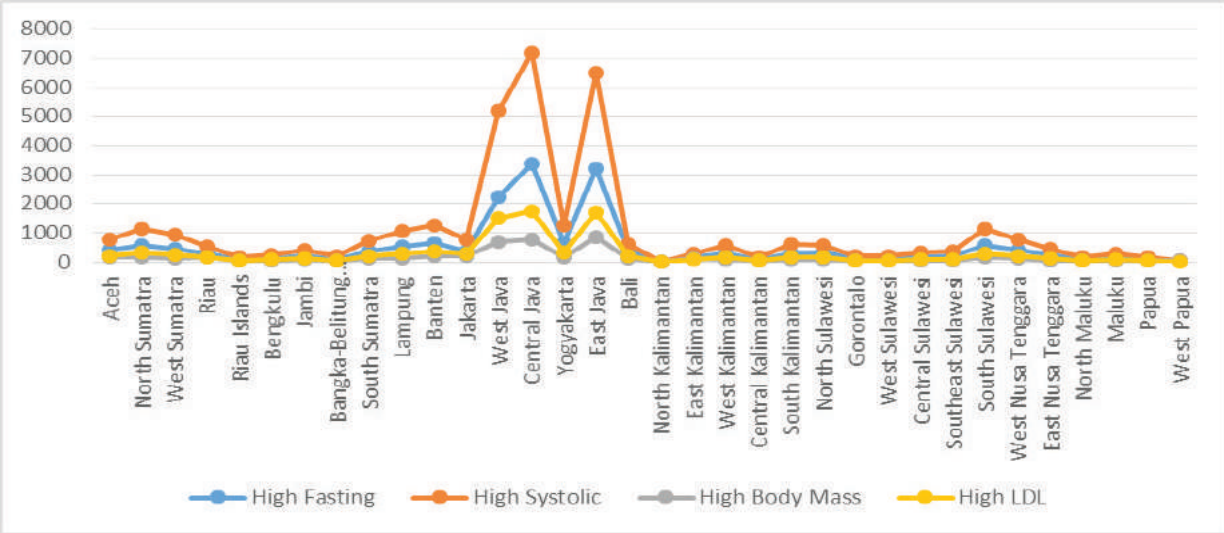
Causes by High Metabolites in 34 Provinces of Indonesia[11]



Based on chart 2, the three highest provinces of the YLL/YLD ratio of ischemic stroke in men population with high fasting and high systolic factors were West Nusa Tenggara, West Kalimantan, Maluku Province. The three provinces with the highest YLL/YLD ratio of ischemic stroke in men with factors that cause high body mass index and high LDL are West Nusa Tenggara, West Kalimantan, South Kalimantan. While the three provinces with the lowest YLL/YLD ratio of ischemic stroke in men with factors that cause high Body Mass Index, high systolic, high LDL are West Java, Gorontalo, North Kalimantan. The three provinces with the lowest YLL/YLD ratio of ischemic stroke in men with high fasting factors are Gorontalo, North Kalimantan, North

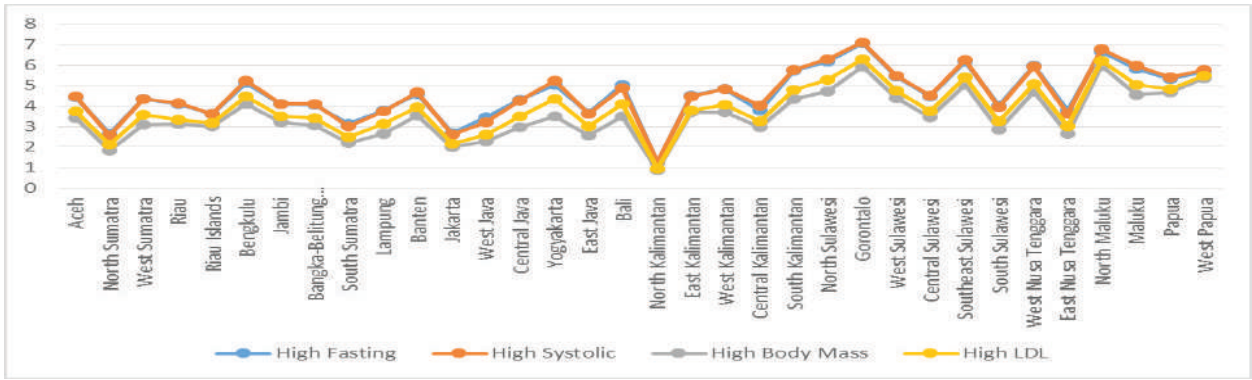
Sumatra.

Graph 3. Death in Women with Ischemic Stroke Causes by High Metabolites in 34 Provinces of Indonesia[11]



Based on chart 3, the three highest provinces of ischemic stroke mortality in women in all (4) high metabolite factors are West Java, Central Java and East Java Province. Three provinces of Riau Island, West Papua, North Kalimantan have the smallest ischemic stroke mortality due to high fasting, high systolic, high LDL. Whereas the 3 provinces that have the smallest ischemic stroke mortality with the causes of high BMI are West Papua, North Kalimantan and North Maluku Provinces.

Graph 4. YLL/YLD Ratio in Women with Ischemic Stroke Causes by High Metabolites in 34 Provinces of Indonesia[11]



Based on graph 4, the three provinces with the highest YLL/YLD ratio of ischemic stroke in women with factors that cause high Body Mass Index and high LDL are Gorontalo, West Papua, North Maluku provinces. The three provinces with the highest YLL/YLD ratio of ischemic stroke in women with the factors that cause high fasting are Gorontalo, Southeast Sulawesi, North Maluku. The three provinces with the highest YLL/YLD ratio of ischemic stroke in women with the factors that cause high fasting are Gorontalo, North Sulawesi, North Maluku. Whereas the three provinces with the lowest YLL/YLD ratio of ischemic stroke in men in all (4) high metabolite factors are the provinces of Jakarta, North Sumatra, North Kalimantan.

Discussion

This study resulted in the value of DALYs ischemic stroke in men is higher than women but statistically there was no effect of sex on the value of ischemic stroke DALYs. Different results based on the 2013 GBD

study which identified a significant difference in stroke burden between men and women.[12] Stroke burden by sex and stroke type, higher epidemiological indicators were observed between men compared to women.[10] Other studies conducted by Jhonson, et al also stated the DALYs value of female strokes was lower than men.[13] This study also produced higher ischemic stroke mortality in men. Other studies in line suggest fewer women die from stroke (2.6 million [2.5-2.7] deaths) than men (2.9 million [2.8 - 3.0] deaths).[13] A study at 2013 stated that the proportionate contribution (%) of stroke-related deaths to deaths from all causes in women was greater than in men.[12]

Based on 2017 GBD data processing, ischemic stroke was significantly influenced by 4 metabolite factors. Other research also states that the majority of stroke DALYs (88.8% [95% UI 86.5 - 90.9]) can be associated with risk factors measured in GBD; this percentage is similar for both types of stroke. Metabolic risk (high systolic blood pressure, high body mass index, high fasting plasma glucose, high total cholesterol, and low glomerular filtration rate) accounts for 72.1% (66.4 - 77.3) DALYs stroke.[13]

Another important finding is the positive and significant relationship between diabetes mellitus and the incidence of stroke, especially the ischemic subtype.[17] According this, 36% of all stroke patients are diabetic. Also, the risk of ischemic stroke is 47% higher than hemorrhagic stroke in diabetic patients.[14] Hyperglycemia is positively related to the functional outcome of acute ischemic stroke.[15] The prevalence of MetS as well as hyperglycemia and dyslipidemia in the infarction group are significantly higher than in the non-stroke group.[16] Mortality Diabetes and global glucose intolerance are the third critical risk factors for stroke mortality in 2017.[17]

The highest value of DALYs ischemic stroke was high systolic. Other studies also state that hypertension is a major risk factor for stroke, both ischemic and hemorrhagic.[18] Other studies state that although the most frequent risk factors in both subtypes stroke were hypertension, the chi-square test shows that this result is not statistically significant (P> 0.05).[14] This study also found that the number of ischemic stroke mortality with the highest causes was high systolic. Recently, a prospective study of 50,000 adults in Golestan showed a positive relationship between hypertension and stroke mortality.[19] In 2017, high systolic blood pressure and dietary risk are the leading causes of death from stroke and world burden.[17]

The effect of high BMI on ischemic stroke was also found in research conducted by Bal SS, et al. States that central obesity and dyslipidemia (increased TG, decreased HDL-C and increased LDL-C) will increase the development and progress of cerebrovascular atherosclerosis, especially diseases occlusive large arteries.[20] Takahashi, et al. stated that there was a significant correlation between increased waist circumference of stroke patients and the prevalence of risk factors for hypertension, hyperglycemia and dyslipidemia.[16] Mortality due to high BMI in ischemic stroke patients ranks fourth among 4 metabolic causes. Other studies have also found obesity globally to be the fourth most influential indicator of stroke mortality.[17]

Research that also states the relationship of dyslipidemia with stroke was carried out by Habibie, et al. Their study found that dyslipidemia was strongly associated with the incidence of total stroke (P <0.05).[14] Recently, the Strong Heart Study (2017) revealed that low density lipoprotein cholesterol levels equal to or greater than 130 mg / dl provided a risk higher ischemic stroke, but interestingly, individuals with high triglycerides and low density high lipoprotein levels who also suffer from diabetes show an incidence of ischemic stroke more than 2 times higher.[21] The mortality rate due to high LDL in ischemic stroke patients ranks third among 4 metabolic causes. Other research also states Hypercholesterolemia, specifically high LDL-C, globally being the eighth most important indicator of death from stroke in 2017.[17]

YLL/YLD ratio values are greater than 1 for all metabolite factors. This ratio indicates that the YLL value dominates the DALYs value. This can be interpreted that the year of life lost due to ischemic stroke is greater than the value of the year of life lost due to disability. The value of the ratio of men is greater than in women. This study is in line with the results in the GBD1990 study and the GBD2000 study, which is about four-fifths of DALY because of YLLs.[22] In this study the YLL number in ischemic stroke men is higher than in

women. Consistent research conducted by Matos, et al who stated that the YLL number strengthens the thesis that men still lose many years of life due to ischemic stroke.[4]

Nearly half of stroke-related deaths may be caused by modifiable risk factors (ie hypertension, diabetes, dietary risk, impaired glucose intolerance, obesity, smoking, air pollution, alcohol use, hypercholesterolemia, and lack of physical activity), which partly large is the result of poor clinical management, limited access to health care, and late detection of the underlying risk factors.[17] The high burden of stroke worldwide indicates that primary prevention strategies are not widely applied or are not effective enough. In addition to targeting behavioral risk factors, effective screening for conditions that increase stroke risk, such as hypertension, atrial fibrillation, and diabetes mellitus, is very important.[13] Stroke prevention is a complex medical and political problem, there is strong evidence that stroke prevention needs to be done. The need to increase action is very urgent. Stroke prevention has entered a new era and is one of the priority NCDs in WHO.[12]

Conclusion

Kruskal-Wallis test results on gender showed no effect on DALYs of Ischemic Stroke but four factor of high metabolites affected to DALYs. The value of the burden (DALYs, Death, YLL/YLD ratio) ischemic stroke in men is greater than women. This occurs in all metabolite factors.

Metabolite factors with the highest ischemic stroke load values of the highest are high systolic, high fasting, high LDL and high Body Mass Index. The high death value in an area does not make the area will have the highest ratio YLL/YLD. The ratio are greater than 1 for all metabolite factors. It indicates that the YLL value dominates the DALYs value. It can be interpreted that the year of life lost due to ischemic stroke is greater than the value of the year of life lost due to disability. The value of the ratio of men is greater than in women.

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Genotyping of Exon 2 BRCA1for Breast Cancer in Iraqi Women by Sanger Sequencing

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Abstract

Background: Sporadic, familial and hereditary kinds account for approximately (70-75%), (10-15%) and (five-10%) of all breast cancers respectively. Although numerous chance factors predispose to hereditary breast cancer, the maximum mighty is mutations within the breast cancer susceptibility genes, BRCA1.An important example for tumor suppressor genes are BRCA1 .

Material and methods: We examined the frequency of the BRCA1 Exon 2 in 80Iraqi breast cancer (BC) patients, and 60 controls and then the previously genotyped variants in ages between 25 to 45 and also 45 to 70,who attended the Oncology Hospital in the City of Medicine during the period from June 2018 to April 2019, The products of PCR from amplification of BRCA gene analyzed by PCR-sequencing method.

Result: Our study refer to the genotyping of BRCA1 that distributed to (GG and TT genotyping while TG equal zero.). A significant change was observed in the comparison between the newly diagnosis group and the control group. T allele was associated with 3.0 fold higher risk for cancer than the G allele (OR=2.38; p=0.041). In BRCA-1 Polymorphism, the odd ratio for the mutant GG genotype was 2.12 with p=0.0141 indicating that Patients with an allele GG genotype a higher risk factor of newly diagnosis group compared to patients who carry allele TT genotype and control group. Also BRCA have a positive correlation with age and BMI in newly and treatment group also with some biomarkers.

Conclusion:SNP variants and different genotypes of BRCA1 have important roles in the development of cancer. Their application on such patients would discriminate those with high susceptibility to DNA damage from those with low susceptibility. This is specially applied for the GG and TT variants of BRAC1 Exon 2.

Key words: *breast cancer , BRCA1 gene , sanger sequencing, polymorphism.*

Introduction

Breast cancer is a multi-factorial ailment with contributions from both genetic and environmental elements. Worldwide, it's far the most typical malignancy in girls accounting for 23% of all cancers [1][2]. In Sri Lanka, breast cancer in ladies diagnosed at a mean age of 50 years, approximately 27% of all woman cancers [3]. Sporadic, familial and hereditary kinds account for approximately (70-75%), (10-15%) and (five-10%) of all breast cancers respectively. Although numerous chance factors predispose to hereditary breast cancer, the maximum mighty is mutations within the breast cancer susceptibility genes, BRCA1[4].

Molecular profiling of different subtypes of breast cancer has discovered that TNBC subtype is substantially related with basal-like functions with the aid of expressing basal cytokeratin markers like CK-5, [5,6]. Furthermore, subsequent-technology sequencing studies have shown that notably high mutational fee in both BRCA1 and BRCA2 genes and a defective DNA repair mechanisms are hallmarks of a TNBC subtype of breast cancer [7,8,9,10].

An important example for tumor suppressor genes are BRCA1 and BRCA2 genes . though BRCA gene mutations are inherited in an autosomal dominant manner, their expression relies upon on acquiring a 2nd

mutation within the wild kind allele in somatic cells. According to Knudson's ("Two Hit") speculation, each alleles of a tumor suppresser gene need to be mutated so as for a tumor to expand, therefore a patient who manifests a tumor, inherits one mutation from a parent, and develops the second mutation inside the same gene in the affected organ as a somatic mutation, at which factor the tumor starts off evolved to appear. Even though children of mutation companies are at 50% risk of inheriting the mutation, the age of onset in their cancer is hard to are expecting[11,12].

Material and Methods

This study designs as prospective study, One hundred and forty (140) Iraqi women subject were participated in this study in ages between 25 to 45 and also 45 to 70, who attended the Oncology Hospital in the City of Medicine, present studyweredivided into two groups patients with breast cancer (80) blood samples and sixty (60) blood samples are collected from healthy women as a control group.

BRCA-1 gene polymorphisms were genotyped with a PCR method by confronting primers briefly, genotyping was also performed by PCR-sequencing . Genomic DNA was amplified by up strand, 5'- GAA GTT GTC ATT TTA TAA ACC TTT- 3' and down strand, 5'- TGT CTT TTC TTC CCT AGT ATG T- 3' .PCR contained 2µl genomic DNA, 5µl Go Pre Master Mix,1µl of Primer Forward , 1µl of Primer Reverse and 16µl distilled water. Thermal cycling condition for the BRCA1 were: initial denaturation 1 step for 3 minutes at95°C, followed by 1 cycles and {denaturation 2 step for 45 seconds at 95°C , Annealing step for 45 seconds at 56°C and extension 1 step for 45 seconds at 72 °C} followed by 35 cycle. The final extension 2 step was performed at 72°C for 7 minutes. The PCR products (amplicon) were sequencing successfully via Macrogen Corporation/Korea (Sanger sequencing method). Homology search was conducted by using BLAST option, which is available online in NCBI By using BioEditprogramand NCBI, the SNPs were determined. The data were tested for normality, homogeneity and normal distribution. Also, it was expressed as median ± SD, the probability was tested using IBM SPSS version 25.0. While, the genotyping and alleles frequencies were tested through Hardy-Weinberg equilibrium calculator. Such, the Odd ratio and Fisher exacts' probability were tested by using WinPepi version 11.65. The probability was significant when it $p< 0.05$.

Results

The result of this research represented that two grouped (newly diagnosis of breast cancer and control group) showed the compatibility 99% with gene band under number MH043259.1, score 389 and expected 1e-105, Sequencing study of our groups showed a Transversion substitution in location 203 and Nucleotide T>G especially in newly diagnosis group. While control grouped showed no change in sequencing of BRCA1 Exon 2 its remain TT.

Group newly diagnosis breast cancer Figure (1): Matching of the primers sequences on the bioinformatics programs/ NCBI blast sequence were matched by the bioinformatics programs NCBI,

Sequence ID: MH043259.1

Score	Expect	Identities	Gaps	Strand
389 bits(431)	1e-105	217/218(99%)	0/218(0%)	Plus/Plus

Query 190 GAACAGAATTGACCTTACATAC**G**AGGGAAGAAAAGACA 227

||||||||||||||||||||||||||||||||||||||

Sbjct 181 GAACAGAATTGACCTTACATAC**T**AGGGAAGAAAAGACA 218

Group newly diagnosis breast cancer and control group Figure (2): **Homo sapiens clone I103_P-10_EXON2-F_D04 breast cancer 1 (BRCA1) gene, partial cds**

Sequence ID: MH043259.1

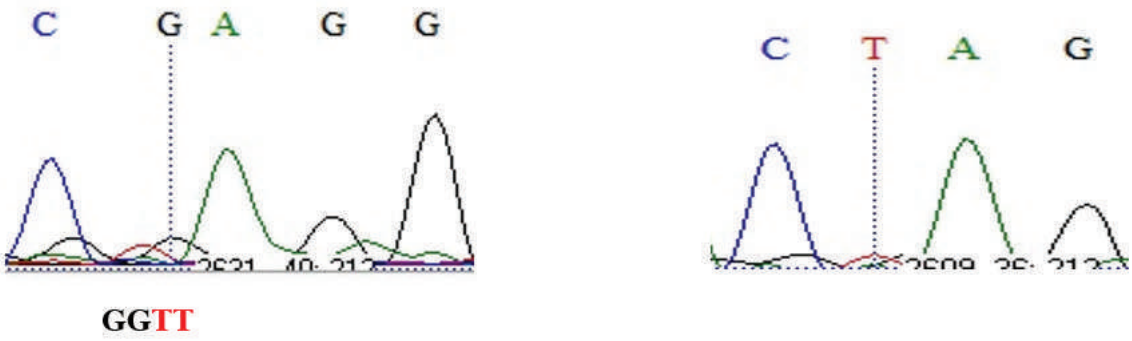
Score	Expect	Identities	Gaps	Strand
374 bits(414)	2e-101	207/207(100%)	0/207(0%)	Plus/Plus

Query 187 GAACAGAATTGACCTTACA**T**ACTAGGG 213

||||||||||||||||||||||||||

Sbjct 181 GAACAGAATTGACCTTACA**T**ACTAGGG

The genotyping by graphic of sequencing Microgen of BRCA1 illustrate below figure and illustrated the GG and TT genotyping while TG equal zero.



The distribution of genotype and allele frequencies among newly diagnosed and control groups is shown in table (1) . The genotypic frequencies of cancer patients were 72% (n=58) normal TT and 0.0 % (n=0) heterozygous TG. Mutant homozygous was found in GG 27% (n=22).In controls, the results demonstrate 77.97% (n=46) wild type TT, 0.0 %(n=0) heterozygous TG and mutant homozygous GG 22.0% (n=13).In BRCA-1 Polymorphism, the odd ratio for the mutant GG genotype was 2.34 with p=0.055 indicating that Patients with an allele GG (homozygous) genotype a higher risk factor of a newly diagnosis group compared to patients who carry allele TT (homozygous) genotype, and control group. Total statistical manual allele frequency. The ratio was allele T for the patients 72%, while allele G 28%, whilst control allele T 78 %, allele G 22 %.A significant change was observed in the comparison between the newly diagnosis group and the control group. T allele was associated with 3.0 fold higher risk for cancer than the T allele (OR=2.38; p=0.041).

Table (1): genotyping and allele frequency of BRCA1 gene between newly diagnosed and control groups.

Genotypes	Hardy-Weinberg frequency								OR	P
	Newly diagnosed				Control					
	Observed		Expected		Observed		Observed			
	No.	%	No.	%	No.	%	No.	%		
TT	58.0	72.50	42.05	52.56	46.0	77.97	35.86	60.79	0.75	0.554
TG	0.0	0.00	31.90	39.88	0	0.00	20.27	34.36	-	-
GG	22.0	27.50	6.05	7.56	13.0	22.03	2.86	4.85	2.34	0.0554
Total	80.0	100.00	80.00	100.00	59.0	100.00	59.00	100.00		
P-HWE	4 x 10-19				2 x 10-14					
Allele frequency										
T	0.72				0.78				2.38	0.041
G	0.28				0.22				0.73	4.414

Our study assessed whether BRCA-1 gene and associated with an increased risk of breast cancer, especially Iraqi women patient with various types of breast cancer (newly malignant diagnosis, and control). The genotyping of BRCA-1genes were classified by PCR- and the results showed a no significant change when comparing control group with newly diagnosis according to risk factor especially in age table (2).

Table 2-: The age median distribution according to the genotyping for BRCA-1 of the studied groups.

	Newly diagnosed 2			P value	Control 3			P value
	TT	TG	GG		TT	TG	GG	
AGE	54.71 ± 10.70a		50.07 ± 15.07a	NS	48.30 ± 10.75b		49.92 ± 8.43a	NS
BMI	33.29 ± 3.95b	-	25.68 ± 6.0a	0.019	27.57 ± 3.67a		23.77 ± 2.83a	0.007⁎⁎

While a positive correlation was found between TT and GG in control group (p value = 0.007) and newly diagnosis (p value = 0.019) while no significant in another group according to BMI.

Discussion

Complete sequencing of the BRCA1 is a favored procedure in hereditary directing of a breast cancer patient's sufferers in various Western European nations, what's more in the US, Australia, and Japan. In any case, these investigations are cost and work expending; thus, it is exceptional that clinicians routinely utilize screening for known harmful germline changes. Under this study, it is critical to distinguish transformations of the best an incentive informative value for diagnostics, just as to build up the methods for their proficient detection our examination refers to a change in BRCA1 and agreement with numerous investigations that the effect of genotype on relative malignancy hazard has been evaluated in a few investigations up to now [13].

This was seen in the frequency of TT genotype and T allele in disease understanding remembered for the present examination in correlation with the control gathering. It is obvious that the polymorphic allele and genotype prevailed in malignant growth patients gathering. It appears that the search for such a polymorphism is a delicate test for scanning for DNA damage by radiation particularly in the heterozygote group GG in the two both patients and radiation groups. In any case, it is specific for malignancy patients whereby both GG and TT genotype were related with significant differences.[[14, 15].

Also contrasting the frequencies of BRCA1-TT genotype in this examination with different investigations, result discovered TT homozygosity by and large much the same as with Nigeria (YRI) (1.8%), Kenya (LWK) (1.1%), Japanese (JPT) (7%), Tuscan (TSI) (11%), (Kinyawa, Kenya) MKK (2.8%) and, (Mexican heritage in Los Angeles) MEX (8.2) (Brewster BL et al.,2012 [15]. This information proposes that the BRCA1- variation G>T presents an expanded danger of creating malignancy in our gathering of patients. One could think from these detection that the BRCA1 -variation works likewise to that of standard BRCA1 open-perusing outline variations, which are all the more usually connected with advancement of the disease rather than the different subtypes. [16].

BRCA-1 gene and related with an expanded danger of disease, particularly Iraqi ladies quiet with different sorts of malignant growth (newly malignant diagnosis and control) The genotyping of BRCA-1genes were characterized by PCR-and the outcomes demonstrated a no noteworthy change when contrasting control group and newly diagnosis as indicated by the risk factor particularly in age, this investigation agreement with many examination [17]. They no found a critical or we found no distinction in mean age among ladies with a BRCA1 transformation in general just as for cases analyzed under age 45. Furthermore, disagreement with [18], as of late studied shown a positive relation of an age on disease in BRCA change transporters analyzed under age 50. In their examination which included 2424 BRCA mutation carrier , Britain with 990 episode malignancies, the auther detailed breast cancer rates as indicated by age bunch at disease analysis [overall (20–49), 20–29, 30–39, 40–49] by age from the outset full-term birth class (nulliparous, < 21, 21–29, 31–39, >39) additionally our investigation disagreement with women who carry the BRCA1 transformation have a more serious danger of creating malignancy before they arrive at 40 years old when contrasted with women having the BRCA2 transformation. In ladies beyond 55 years old years the ladies carry the BRCA2 transformation who stay at higher danger of creating malignant growth while ladies that have the BRCA1 change have a lesser hazard [19].

This examination pointed that a positive connection be tween's a BRCA1 genotyping with BMI especially in newly diagnosis and control group and this outcome agreement with numerous investigations in different populace Ricks-Santi, L. J. 2013 [20]. Because the rate of transformation increase with increment BMI that influenced were related with DNA repair limit in ladies with additionally refer to build danger of BRCA1 transformation with increasing 5Kg in corresponding. This examination demonstrated that the danger of a gene change in BRCA1 expanded by 25% when the weight was expanded to 5 Kg from the ordinary weight limit BMI>25 [20] .

Conclusion :SNP variants and different genotypes of BRCA1 have important roles in the development of cancer. Their application on such patients would discriminate those with high susceptibility to DNA damage from those with low susceptibility. This is specially applied for the GG and TT variants of BRAC1 Exon 2.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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Importance of FoxO3 Level in Progression of Nodal Osteoarthritis Disease

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Abstract

Objective : To explain a important of Forkhead box O3(FoxO3) protein level in progression of Nodal osteoarthritis (NOA) disease , the location of study at fallujah – Iraq at February 2020 .

Method: Collection of samples take for patients with NOA and controls at Rheumatology & Rehabilitation Consultation Unit in Al-fallujah Teaching Hospital / Iraq .The Diagnosis NOA patients depended on American College of Rheumatology (ACR) criteria and used technique of ELISA to measure FoxO3 protein level.

Results: This study show NOA patient grade 1 have non significant decrease when compare with control group in FoxO3 protein level ,but observed significant decrease when compare with NOA patient grade 4. Also show significant decrease when compare NOA patient grade 4 with control group .

Conclusion: This study show that level of FoxO3 protein decrease in NOA cartilage, this lead to reduce of autophayg and antioxidant proteins .This decrease can result progression of NOA disease .

Key words / *Nodal osteoarthritis (NOA) ,autophayg process and Forkhead box O3(FoxO3).*

Introduction

Nodal osteoarthritis (NOA) is the disease occur in the hand finger's joints,principally interphalangeal and first carpometacarpal joints. It's degradation arthritis in the cartilage of finger's joints , this lead to cartilage loss. NOA is one of the arthritis forms , the females are more affected than male at age more than 45 years , therefore NOA is within geratric disease [(1)]. Chondrocytes affected by arthritis generate reactive oxygen species (ROS) as response to generate cytokines and stress on cartilage ,this lead to support development and progression of NOA[(2)]

Forkhead box O3(FoxO3) is protein responsible to transcription action therefore consider as transcription factor ,product by FoxO gene that have many biological pathways for example :-

-Phosphatidylinositol-3 kinase (PI3K)/Akt signal transduction pathway -Autophagy process that act to reduce effect of geriatric disease [(3)].

Reduced antioxidant factor are also shared in aged cartilage with NOA , this process can results different in chondrocyte phenotype , senescence , and lead to cell death , key mechanisms involved in disease onset and progression. Increased ROS in aged chondrocyte can induced cell , this related to decrease of antioxidants factors [(4)]. Autophagy is mechanism to save protein quality under conditions of oxidative stress, and its activity decreases with NOA patients[(5)] .

FoxO3 proteins in geriatric stage become dys-regulated this can result NOA development due to loss of cartilage. Therefore FoxO3 decrease with age lead to increase FoxO3 phosphorylation (inactive form) in cartilage with NOA[(4)].

Materials and Methods

Study design: The study designed regarded to agree of Institutional Ethics Committee in Medical College /Al-Faluja University - Iraq and it done in Rheumatology & Rehabilitation Consultation Unit in Al- fallujah Teaching Hospital / Iraq .The patients and healthy individuals that included in this study were obtained consent .Patients with NOA diagnosed regard to American College of Rheumatology (ACR). ACR depend on clinical examination ,symptoms and X-ray for diagnosis NOA patients (6).This study included NOA patients 50 grade1(G1) and 50 grade 4(G4) cases and 50 controls ,The age were more than 40 years for cases and controls .

Collection and sample : 5 milliliters were aspirated from arm venous blood by using syringes . The collection time between 09.00-12.00 am. , blood sample allowed in tube for 10min. to clot ,then transferred to centrifuge to separate and obtain serum and frozen it at (-20) C°.

FoxO3 proteins measurement :The Enzyme immunoassay kit used to determine the quantitative of FoxO3 protein in humane serum. This kit designed speclist to use in vitro diagnostic only. The FoxO3 protein measured by solid phase enzyme-linked immune-sorbent assay (ELISA) based at the sandwich method .This study used My BioSource company / USA FoxO3 ELIZA kit to assay , with standard curve .Figure 1 .

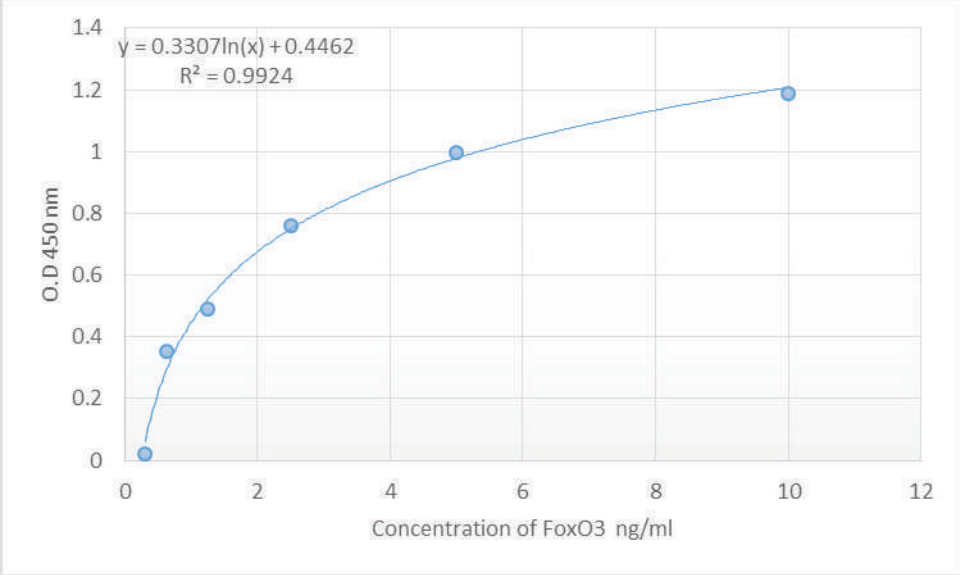


Figure1/ FoxO3 protein Standard curve

Statistic analysis : This study used style mean ± standard deviation (SD) to show the results , and compare between cases and control groups by use The GraphPad PRISMstatistical software (version6 .0, San Diego, CA, USA). The P-value < 0.05 consider as significant different .

Results

This study used mean ±standard deviation (SD) ,standard error (SE) and P-value to show significant correlation for compare between groups G1 NOA ,G4 NOA and control according to FoxO3level .There are significant differences in this study when compare G4with control and G1 with G4 ,but not significant when compare G1 with control .Table 1.

Table 1/ Comparsation between G1 NOA, G4 NOA and control groups according to FoxO3 level

	G1	Controls	P-value
Mean +SD	3.63+ 1.34	3.69+1.32	0.89 (non S)
SE	0.19	0.19	

	G4	Controls	P-value
Mean +SD	1.27+ 1.242	3.68+1.41	<0.05(S)
SE	1.10	0.28	

	G1	G4	P-value
Mean +SD	3.63+ 1.34	1.27+ 1.242	<0.05(S)
SE	0.19	1.1	

*SD : standard deviation , SE : standard error .

Discussion

The NOA can define as loss of finger's joints cartilage by degradation process , this process always coupled with inflammatory factors as mediators and the age consider risk factor to progression OA as general [7].The oxidative condition is more likely with age ,therefore oxidative stress also consider risk factor to develop OA as general due to oxidative condition can result elevate reaction oxygen species (ROS) level ,for example hydrogen peroxide, with age[8]. . In normal condition the chondrocyte can convert ROS to water by catalase and GPX1 and save the cartilage from harmful of ROS factors. [4].

Autophagy process activated by any damage occur in chondryocyte due to ROS increase ,this process lead to damage remove in cartilage cells . Mechanism of autophagy lead to safety of protein homeostasis in cartilage cell under effect oxidative stress ,this mechanism included up regulation of Beclin1 and LC3 that consider as autophagy proteins [9].

FoxO3 function is various regulations in cell such as cell cycle regulation due to FoxO3 consider as transcription factor , so FoxO3 protein regulate the error that result from stress in cell . The Oxidative stress that generate with age can result FoxO3 deacetylation via SIRT-1 after transfer it from cytoplasm to nucleus (FoxO3 become inactive form)[4]

This study explain reduced level of FoxO3 in G4 NOA group more than G1 NOA and control groups .This reduced can lead to loss regulation and homeostasis of anti oxidants and autophagy proteins transcription , this condition can promote progression and develop cartilage loss by degradation process in finger's joints to result NOA disease [10] . ALMEIDA, Maria; PORTER and et al (2019) agree with our study via stabled decrease FoxO3 level in patients with OA disease[11]

This study demonstrate that FoxO3 protein decrease lead to NOA development via decrease of antioxidant factors and autophagy process . Also that decrease the regulation of FoxO3 transcription in human cartilage cells promote some genes encoding pro-inflammatory factors such as matrix-degrading enzymes and cytokines in chondrocytes [4].

Conclusion

FoxO3 protein is transcription factors, in cartilage

with NOA the FoxO3 become dys-regulation this result decrease of autophagy and antioxidant proteins .This reduction can promote chondrocyte degradation process that leads to cartilage loss and NOA development.

- -The ethical clearance of my article taken from the Institutional Ethics Committee in Medical College / fallujah University / Iraq ,this ethical clearance Installed and written in materials and methods of my article .

-The Source of funding (self).

-Conflicts of interest (Not found) .

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The Role of Anti-interleukin-4 in BALB/c Mice Infected with Visceral Leishmaniasis

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Abstract

Leishmaniasis is endemic in Iraq with the visceral form of the disease, caused by the *L. donovani* complex, is principally endemic in the central part of the country. 45 mice (male) were divided into three groups, the first was injected with ($3x10^6$) promastigote as control positive, the second was injected with ($3x10^6$) promastigote and treated with anti-interleukin 4 and the third was injected with PBS as control negative. After 30, 45 and 60 days the serum was taken and examined by the ELISA test to find out the level of interferon gamma (IFN-γ).

The present study revealed that the level of (IFN-γ) at day 30, was higher in group 2 (539.45±2.352), than in group 1 (173.26±1.21), and group 3 (109.69 ± 0.25). At day 45, the level recorded in group 2 (393.89±2.012), than group 1 (286.6±1.521), and group 3 (109.93±0.22). At day 60, the level was in group 2 (259.48±1.841), than group 1 (310.22±1.632), and group 3 (109.69±0.23). The aim of present study was to determine the efficient role of anti IL-4 on IFN-γ activity in mice infected with *L. donovani*. We concluded our results that the level of interferon gamma (IFN-γ) was higher in group 2 at all periods.

Keywords: *Visceral leishmaniasis, interferon gamma, anti-interleukin 4, ELISA, mice.*

Introduction

Visceral leishmaniasis consider as a vital community health problem in Iraq and the foremost lethal form, classically which is, known as Kala-azar or black fever [(1)], caused by *Leishmani donovani* complex that include three specie; *L. infantum, L. chagasi* and *L. donovani*. This form of the infection is the majority severe shape, it is progressive and can be deadly if not treated will typically result in death, and the fatality rate can be as high as 100% [(2)].

The life cycle of *Leishmania* parasites is distinguished by its alternating generation have, and exist in two particular shapes: the amastigote in human host and the promastigote in sand fly vector [(3)]. During a blood feeding, motile *Leishmania* promastigotes are exchanged from the sand fly into a human or animal host where they are phagocytized by neutrophils, macrophages, or dendritic cells; *Leishmania* parasites distinguish into amastigotes and reproduce inside phagolysosomes, finally rupturing the host cell and releasing the parasites [(4)].

The classical symptom of visceral leishmaniasis includes; delayed fever, serious weight loss, hepatosplenomegaly, anemia, leucopenia, and hypergammaglobulinemia [(5)]. Immunity against *leishmaniasis* requires Th1 induced IFN-γ for activation of infected macrophages to produce nitric oxide (NO), antileishmanial immunity is mediated through both innate (macrophages, dendritic cells (DCs) and neutrophils), and adaptive (T cells) immunity, but the CD4+ T cell subset is crucial for resistance [(6)]. IL-2 and IFN-γ were related to Th1 activation and disease healing, whereas IL-4 and IL-5 were related to Th2 activation and disease progression [(7)].

Most of the drugs available to treat *leishmaniasis* are not able to reliably remove the infection, but they are able to reduce the load of parasites [(8)]. For this time, no

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effective vaccine has been found against at all form of *leishmaniasis* for general human use [9].

Material and Methods

In total, group of (45) BALB/c mice, males, (6-8) weeks old, and weight range about 25-30g, divided to three subgroup (15 mice of each group).

Group 1 injected ($3x10^6$) promastigotes as control +, group 2 Injected ($3x10^6$) promastigotes and treated (50) µg of anti IL-4 intraperitoneally, twice weekly after the first week of infection for four connection weeks post- infection and group 3 Injected (0.25) ml phosphate buffer saline only as control-. Blood samples were collected from five mice of each group for (30 – 45 – 60) days post-infection to determine the level of interferon gamma (IFN-γ), by ELISA test.

Results

A total of 45 sample of serum taken from mice was examined by ELISA and detected the level of INF-γ. In our study the result indicated that, the level of interferon gamma INF- γ during time periods (30 ,45 and 60) days in three groups ,in group (3) negative control there is no significant difference in level of INF- γ during time periods, in group (2) infected mice and treated with anti IL-4 enhanced to INF- γ production and peak at 30^{th} day that help the mice to control infection then began to decrease until 60^{th} day, which lead to progressive the disease, while in group (1) infected mice observed the level of INF-γ was low, this associated with parasites multiply rapidly during the first weeks, after four weeks recover their ability to produce IFN-γ , reach to the peak at 60^{th} day when parasite growth was controlled.

Table.1 Distribution the level of INF-γ in 30 days

Groups	Mean ± SEM	LSD
Group1 (control+) **	173.26±1.21	0.564
Group2(Anti-IL4) ***	539.45±2.352	2.251
Group 3 (control -) *	109.69 ± 0.25	0.025

Table.2 Distribution the level of INF-γ in 45 days

Groups	Mean ± SEM	LSD
Group1 (control+) **	286.6±1.521	1.521
Group 2 (Anti-IL4) ***	393.89±2.012	1.652
Group3 (control -) *	109.93±0.22	0.024

Groups	Mean ± SEM	LSD
Group1 (control+) **	310.22±1.632	2.312
Group 2 (Anti-IL4)***	259.48±1.841	1.325
Group 3 (control -) *	109.69±0.23	0.025

Table.3 Distribution the level of INF-γ in 60 days

Discussion

Leishmaniasis is endemic in 98 countries, and 350 million people are at risk of contracting this disease, according to the World Health Organization. Each year, approximately 58,000 cases of visceral leishmaniasis are diagnosed. It is usually, accepted that IFN- γ is required for the management of and therefore protection from *Leishmania* infections, IFN-γ is one of the Th1 prototype cytokines responsible for activating macrophages, resulting to an efficient killing of the parasites [10]. The mechanisms of control of *Leishmania* parasites in mice are very clear and require the activation of Th1 responses, which leads to iNOS up regulation by phagocytic cells [11]. The low level of IFN-γ in group 1 (Table. 1), agreement with [12] through first 4 weeks after starting infection, parasite replication is associated with, the immune cell inability to produce IFN-γ and IL2 (macrophage-activating cytokines), whereas production of IL4 or IL5 is preserved. Treatment with anti IL-4 induces a resistance phenotype with matching induction of INF- γ [13], this agreed with our results that appeared the level of interferon gamma significant increased in group (2) in comparative with group (1) control + and group (3) control – in (Table. 1).

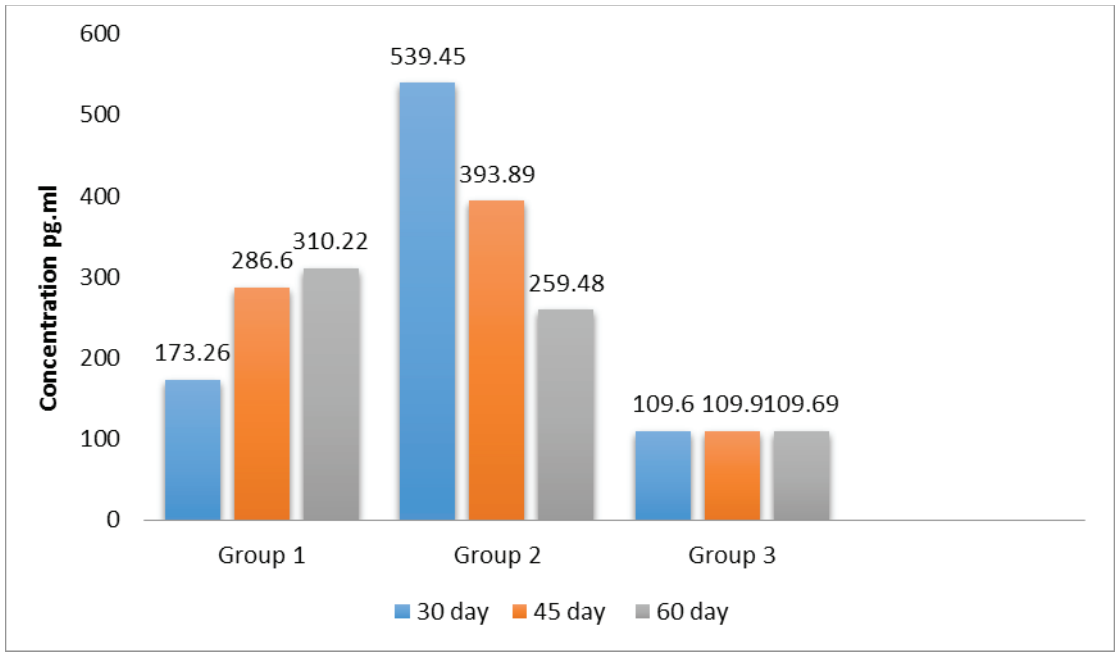
After the first 4 weeks of infection, CD4+ T, CD8+ T and natural killer (NK) cells recover their capacity to produce IFN-γ, thus promoting the macrophage microbicidal activity with (NO) synthesis, this observed in group (1) (Table. 2)[12].

Mice treated with anti-IL-4 antibody, demonstrating that they heal infection. This explains the significant increase in the level of interferon gamma in group (2) in comparative with other groups [14].

During experimental VL in genetically susceptible mice, parasite growth was controlled after 6–8 weeks post infection [15], this suggested agree with our result, that observed in (Table. 2) group (1) the level of INF-γ increased in compare with first 4 weeks.

In visceral *leishmaniasis*, the infection will be resolved within 6 to 8 weeks due to development of Th1, then granuloma response will occur, which is caused by the production of IFN-γ [16], this suggested agree with our result, that observed in (Table. 3) group (1).

The anti-IL-4 treated mice eventually developed progressive infection, as evidenced by the increase in tissue parasites at 8 wk.[17], this agreement with our result, that observed in (Table. 3) group (2) the level of INF-γ decreased in day 60.



Level of interferon gamma in 30, 45 and 60 days

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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Predictors of Malaria Incidence in Rural Eastern Indonesia

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Abstract

In Indonesia, the largest number of malaria cases was contributed to by Eastern Indonesia. The study aimed to analyze the predictors of malaria incidence in rural Eastern Indonesia. This study analyzes the 2018 Indonesia Basic Health Survey data. In the final stage binary logistic regression was used to determine the incidence of malaria incidence in 86,382 respondents. Variables analyzed included malaria incidence, regional/province, age, gender, educational level, work type, marital status, wealth status, and health insurance. The analysis found that people who lived in rural Maluku and rural North Maluku had a lower risk of experiencing malaria than those who lived in rural areas of East Nusa Tenggara. People in rural West Papua and rural Papua have a higher risk than those who live in rural East Nusa Tenggara. Men were 1.107 times more likely than women to have malaria incidence.People with primary school education were 1.237 times more likely to develop malaria than those without education. People with a college education were 1.440 times more likely to develop malaria than those without education. People who have a wealth of poorer status are 0.804 times more likely to have malaria than those who are poorest. People who have the richest wealth status are 0.851 times more likely to have malaria than those who are the poorest. There were 4 variables that have proven to be significant as predictors of incidence in rural Eastern Indonesia, namely regional/province, gender, education, and wealth.

Keywords: *malaria incidence, Eastern Indonesia, Indonesian basic health survey.*

Background

The Indonesian Ministry of Health at the end of 2018 reported that confirmed malaria incidence reached 180 thousand cases. This figure is the incidence in 26 districts from all regions in Indonesia[1]. This malaria incidence rate is dominated by reports from Eastern Indonesia. There are at least 5 provinces which are malaria-endemic areas, namely Maluku, North Maluku, East Nusa Tenggara, West Papua, and Papua[2].

In addition to environmental or ecosystem factors, several studies have tried to link malaria incidence with individual characteristics. Environmental factors found to be associated with malaria incidence are often related to water location and climate change. This is related to the Plasmodium ecosystem as parasites, Anopheles mosquitoes as vectors, and humans as hosts[3,4,5]. Ecosystem risks of this kind are found in many rural areas[6,7,8].

Individual characteristics and household characteristics also did not escape the attention of the researchers. This relates to individual behavior in responding to environmental conditions, including individual knowledge and behavior towards the prevention of malaria transmission, for example, the use of insecticide-treated bed nets[9,10].

Based on the background, the purpose of this study is to analyze the predictors of malaria incidence in rural Eastern Indonesia. The results of this study will be input for the Ministry of Health in determining the right target

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in malaria endemic areas in rural Indonesia.

Materials and Methods

This study analyzes secondary data from the 2018 Indonesia Basic Health Survey (Riskesdas). The survey conducted by the National Institute of Health Research and Development, the Indonesia Ministry of Health was a five-year cross-sectional survey.

The population in this study was all population> 10 years old in rural Eastern Indonesia covering 5 provinces, namely East Nusa Landmark Province, Maluku, North Maluku, West Papua, and Papua. The sampling method uses multi-stage cluster random sampling to determine samples. 86,382 respondents were found to be aged >10 years. Respondents of this age were considered mature enough to be able to answer the questions in the questionnaire submitted[2].

This study specifically analyzes respondents who live in rural Eastern Indonesia. Malaria incidence was the respondent's acknowledgment of the official diagnosis after a blood test. The variables analyzed included regional/province, age group, gender, education level, work type, marital status, wealth status, and health insurance.

A correlation test was performed using Chi-square on dichotomous variables, and T-test for continuous variables. This test was to assess whether there are statistically significant differences between regions. Estimates using Binary Logistic Regression were conducted to study disparities in malaria incidence across regions. All statistical accounts were carried out using SPSS 21 software.

Findings

Before conducting further regression tests, the co-linearity test was first performed. The results of the co-linearity test can be seen in Table 1. The test results show that there is no co-linearity between the dependent and independent variables. Table 1 shows that the tolerance value of all variables is greater than 0.10. While the VIF value for all variables is less than 10.00. Then referring to the basis of decision making in the multicollinearity test it can be concluded that there are no symptoms of multicollinearity in the regression model.

Table 1. Results for the co-linearity test of malaria incidence in rural Eastern Indonesia (n=86,382)

Variables	Collinearity Statistics	
	Tolerance	VIF
Regional/Province	0.896	1.116
Age groups	0.529	1.890
Gender	0.920	1.087
Education level	0.891	1.123
Work type	0.835	1.197
Marital status	0.538	1.859
Wealth status	0.828	1.208
Health insurance	0.982	1.018

*Dependent Variable: Malaria incidence

Table 2 is a descriptive statistics of respondent characteristics. Table 2 shows that all rural areas in the regional/province are dominated by those who have malaria. In both categories, malaria incidence is dominated by those in the 11-20 age group.

Table 2 informs that those who experience malaria incidence are dominated by men, while those without malaria are dominated by women. Based on the education level, both categories (experiencing malaria incidence or not) are dominated by those with primary school education. Based on work type, both categories (experiencing malaria incidence or not) are dominated by those who work as farmer/fisherman/labor/driver/maid.

Table 2 shows that both categories (experiencing malaria incidence or not) were dominated by those who were married. Based on wealth status, both categories (experiencing malaria incidence or not) are dominated by those who are the poorest. While based on ownership of health insurance, both categories (experiencing malaria incidence or not) are dominated by those who have health insurance.

Table 3 displays the results of the binary logistic regression test to illustrate the predictors of malaria incidence cases in rural Eastern Indonesia. As a reference, the chosen category is experiencing "malaria incidence".

Table 3 shows that people living in rural Maluku had 0.513 times the possibility of malaria compared to people living in rural East Nusa Tenggara (OR 0.513; 95% CI 0.410-0.643). People who live in rural North Maluku are 0.497 times more likely to have malaria than people who live in rural East Nusa Tenggara (OR 0.497; 95% CI 0.386-0.639). This means that people who live in rural Maluku and rural North Maluku have a lower risk of experiencing malaria than those who live in rural areas of East Nusa Tenggara.

Table 2. Descriptive Statistic of Respondent Characteristics on Malaria Incidence in Rural Eastern Indonesia (n=86,382)

Characteristics	Malaria Incidence				P
	No		Yes		
	n	%	n	%	
Regional/Province					***0.000
· East Nusa Tenggara	32572	39.3%	720	20.3%	
· Maluku	13898	16.8%	112	3.2%	
· North Maluku	11451	13.8%	93	2.6%	
· West Papua	9274	11.2%	421	11.8%	
· Papua	15634	18.9%	2207	62.1%	
Age Groups					***0.000
· 11-20	22362	27.0%	881	24.8%	
· 21-30	13414	16.2%	579	16.3%	
· 31-40	16328	19.7%	785	22.1%	
· 41-50	13743	16.6%	666	18.7%	
· 51-60	9377	11.3%	427	12.0%	
· 61-70	4911	5.9%	153	4.3%	
· > 70	2694	3.3%	62	1.7%	
Gender					***0.000

Cont... **Table 2. Descriptive Statistic of Respondent Characteristics on Malaria Incidence in Rural Eastern Indonesia (n=86,382)**

· Male (ref.)	39727	48.0%	1878	52.9%	
· Female	43102	52.0%	1675	47.1%	
Education level					***0.000
· No education (ref.)	7157	8.6%	376	10.6%	
· Primary School	38332	46.3%	1494	42.0%	
· Junior High School	14035	16.9%	559	15.7%	
· Senior High Scool	16686	20.1%	850	23.9%	
· College	6619	8.0%	274	7.7%	
Work Type					***0.000
· No work (ref.)	19112	23.1%	811	22.8%	
· Schooling	16082	19.4%	635	17.9%	
· Public servant/army/ police	4119	5.0%	225	6.3%	
· Private Employee	2932	3.5%	153	4.3%	
· Entrepreneur	4927	5.9%	253	7.1%	
· Farmer/ Fisherman/ Labor/Driver/Maid	30543	36.9%	1308	36.8%	
· Others	5114	6.2%	168	4.7%	
Marital status					***0.000
· Never married (ref.)	28051	33.9%	1078	30.3%	
· Married	49220	59.4%	2287	64.4%	
· Divorced/Widowed	5558	6.7%	188	5.3%	
Wealth status					***0.000
· Poorest (ref.)	19672	36.8%	1057	34.7%	
· Poorer	10663	19.9%	403	13.2%	
· Middle	9565	17.9%	489	16.1%	
· Richer	7206	13.5%	485	15.9%	
· Richest	6370	11.9%	611	20.1%	
Health insurance					0.052
· No (ref.)	2681	3.2%	136	3.8%	
· Yes	80148	96.8%	3417	96.2%	

Note: Note: Chi-Square test was used for dichotomous variables, and T-test for continuous variables; * $p < 0.05$; ** $p < 0.01$; * * *$p < 0.001$.

Table 3. Binary Logistic Regression of Malaria Incidence in Rural Eastern Indonesia (n=86,382)

Predictor	Malaria Incidence			
	Sig.	OR	Lower Bound	Upper Bound
Regional/Province: East Nusa Tenggara	-	-	-	-
Regional/Province: Maluku	***0.000	0.513	0.410	0.643
Regional/Province: North Maluku	***0.000	0.497	0.386	0.639
Regional/Province: West Papua	***0.000	2.885	2.509	3.318
Regional/Province: Papua	***0.000	7.745	7.026	8.536
Age Groups: 11-20	-	-	-	-
Age Groups: 21-30	0.817	0.978	0.811	1.180
Age Groups: 31-40	0.557	1.062	0.868	1.300
Age Groups: 41-50	0.131	1.173	0.954	1.443
Age Groups: 51-60	0.072	1.220	0.983	1.515
Age Groups: 61-70	0.413	1.114	0.861	1.441
Age Groups: >70	0.626	1.087	0.778	1.518
Gender: Male	*0.014	1.107	1.020	1.202
Gender: Female	-	-	-	-
Education level: No education	-	-	-	-
Education level: Primary school	**0.002	1.237	1.084	1.411
Education level: Junior High School	*0.018	1.203	1.032	1.402
Education level: Senior high school	***0.000	1.644	1.414	1.912
Education level: College	**0.001	1.440	1.158	1.792
Work Type: No work	-	-	-	-
Work Type: Schooling	0.180	1.121	0.949	1.324
Work Type: Public servant/army/police	0.454	1.084	0.878	1.338
Work Type: Private Employee	0.684	0.956	0.772	1.185
Work Type: Entrepreneur	0.271	1.102	0.927	1.310
Work Type: Farmer/Fisherman/ Labor/Driver/ Maid	0.932	1.005	0.895	1.129
Work Type: Others	0.773	0.971	0.796	1.185
Marital status: Never married	-	-	-	-
Marital status: Married	0.609	0.960	0.823	1.121
Marital status: Divorced/Widowed	0.201	0.863	0.688	1.082
Wealth status: Poorest	-	-	-	-
Wealth status: Poorer	***0.000	0.804	0.712	0.907
Wealth status: Middle	0.319	0.943	0.841	1.058
Wealth status: Richer	**0.008	0.852	0.757	0.959
Wealth status: Richest	**0.007	0.851	0.758	0.956

* $p < 0.05$; ** $p < 0.01$; ***$p < 0.001$.

Table 3 informs that people living in rural West Papua are 2.885 times more likely to have malaria than people living in rural East Nusa Tenggara (OR 2.885; 95% CI 2.509-3.318). People who live in rural Papua have a 7.745 times chance of having malaria compared to people living in rural East Nusa Tenggara (OR 7.745; 95% CI 7.026-8.536). This information shows that people in rural West Papua and rural Papua have a higher risk than those who live in rural East Nusa Tenggara.

Consistent research results were reported from a study in Kenya, Nigeria, and Ghana. The difference in malaria incidence spatially is influenced by several variables, including rainfall, proximity to water, vegetation, and population density. The differences in these variables show different effects on malaria incidence[5,7,11].

Table 3 shows that men were 1.107 times more likely than women to have malaria incidence (OR 1.107; 95% CI 1.020-1.202). Table 3 shows that people with primary school education were 1.237 times more likely to develop malaria than those without education (OR 1.237; 95% CI 1.084-1.411). People who have junior high school education are 1,203 times more likely to develop malaria than those with no education (OR 1.203; 95% CI 1.032-1.402). people with senior high school education were 1.644 times more likely to develop malaria than those with no education (OR 1.644; 95% CI 1.414-1.912). People with a college education were 1,440 times more likely to develop malaria than those with no education (OR 1.440; 95% CI 1.158-1.792).

A better level of education has a negative relationship with malaria incidence. Because those who are educated have better possibilities to understand malaria prevention behavior[12,13]. A study in Nigeria found that education level was a determinant of ownership of mosquito nets. The use of mosquito nets is one of the behaviors to prevent malaria[14].

Table 3 informs that people who have wealthy poorer status are 0.804 times more likely to have malaria than those who are poorest (OR 0.804; 95% CI 0.712-0.907). People who have wealth richer status are 0.852 times more likely to have malaria than those who are poorest (OR 0.852; 95% CI 0.757-0.959). People who have the richest wealth status are 0.851 times more likely to have malaria than those who are poorest (OR 851; 95% CI 0.758-0.956).

Similar research results were found in Rwanda. People who have lower wealth status have a higher risk of experiencing malaria incidence[15]. A study in Zambia with pregnant women subjects also found the same results. Poor pregnant women have a greater risk than pregnant women who are rich in experiencing malaria incidence[13].

Conclusions

Based on the results of the study it could be concluded that there were 3 variables that have proven to be significant as predictors of incidence in rural Eastern Indonesia. The three determinants were regional/ province, education level, and wealth status.

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Relationship between Benzene Concentrations with Erythrocyte, Hemoglobin, and Health Complaints of Workers in Surabaya Printing Industry

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Abstract

Printing industry uses a lot of raw materials that contain benzene in the production process. Exposure to benzene can cause haematological effects that affect the production of blood cells. The purpose of this study was to determine the relationship between benzene concentrations with erythrocytes, hemoglobin, and health complaints among workers in Surabaya Printing Industry. This research was conducted in Printing X Surabaya. This research is observational, quantitative approach, and cross-sectional. The respondents in this study were 19 people. The variables studied were the concentration of benzene in the air, erythrocytes, hemoglobin, and health complaints among workers at Printing X Surabaya. Data analysis using Spearman rank correlation test. Measurement of the concentration of benzene in the air exceeds the Threshold Limit Value. There were 5 respondents had erythrocyte level above the normal and 1 respondent had hemoglobin level below the normal. There was a quite significant and positive relationship between the concentration of benzene in the air and erythrocytes (p=0.033). But there was no significant relationship between the concentration of benzene in the air and hemoglobin (p=0.158). Health complaints experienced by workers in Printing X Surabaya included coughing, headaches, and shortness of breath. There is a fairly strong relationship between benzene concentrations and erythrocytes but there is no relationship between benzene concentration and hemoglobin.

Keywords: *Benzene, erythrocytes, hemoglobin, health complaints, printing*

Introduction

Printing industry is one of the industries that in the production process uses many raw materials such as ink, solvents, and cleaning agents. These raw materials contain substances that can interfere with the health of workers, one of which is benzene. Benzene is a colorless liquid with a sweet aroma. It can evaporate very quickly, is slightly soluble in water, and flammable. In addition, it has lipophilic property that it is a good solvent in ink, paint, glue, and thinner. Steam or gas from benzene-containing materials such as paint, glue, furniture wax can be a source of exposure for workers in the workplace. Workers who have the possibility of

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being exposed to benzene include printing workers, rubber workers, shoe makers, laboratory technicians, firefighters, and gas station workers[2]. According to the Regulation of the Minister of Manpower of the Republic of Indonesia Number 5 of 2018, benzene is categorized as A-1 category, which is proven to be a carcinogen for humans[15]. The Environmental Protection Agency (EPA) classifies benzene as a known human carcinogen for all routes of exposure namely inhalation, ingestion, and skin[2].

In Indonesia, the benzene TLV in the environment is 0.5 ppm and the permitted short exposure (PSD) of benzene is 2.5 ppm[15]. ACGIH regulates the benzene TLV in the air to be 0.5 ppm[1]. NIOSH stipulates that benzene exposure at work for 8 working hours per day and 40 hours per week is 0.1 ppm[12]. A research conducted by Nikmah in the Semarang X printing industry shows that the results of the measurement of benzene level in the environment exceeded the exposure limit set by NIOSH by 0.132 ppm[11].

Benzene can enter the body through the respiratory tract, digestive tract, and skin. At high concentrations, approximately half of the benzene that is inhaled enters the lungs, then enters the bloodstream and spreads throughout the body[2]. Benzene can cause cells to not work properly. The severity caused by benzene exposure depends on the amount/concentration, route of exposure, length of time of exposure, age, and preexisting medical conditions of the person exposed. Long-term exposure to benzene has a major effect on blood. Benzene causes harmful effects on the bone marrow and can cause a decrease in erythrocytes which can lead to anemia. Benzene can also cause excessive bleeding, affect the immune system, increase the chance of infection and cause leukemia[4].

Workers exposed to high levels of benzene vapor in the printing industry exhibit severe haematological system effects[2]. Research conducted by Parinduri in the printing industry in Medan, North Sumatra found that the reduction in hemoglobin levels was affected by the length of exposure and length of service among printing industry workers[14].

Setiowati in his research got the result that the most common health complaints experienced by workers exposed to benzene were the nervous system and haematological complaints. Complaints of the nervous system that are often experienced include pain in the feet, hands, shoulders and dizziness. Haematological complaints that are often felt by workers are easily tired and sleepy[16]. Research by Yuniati showed that most workers who were exposed to benzene complained of dermatitis[20].

The purpose of this study was to determine the relationship between benzene concentrations with erythrocytes, hemoglobin, and health complaints among workers in Surabaya Printing Industry.

Materials and Methods

This research is observational, quantitative approach, and cross-sectional. The research was conducted in December 2019 at Printing X Surabaya. Respondents in this study used a population of workers in Printing X Surabaya with a total 19 people. The variables to be examined are the concentration of benzene in the air, erythrocytes, hemoglobin, and worker health complaints. Respondent characteristic data including age, sex, and years of service were taken using a questionnaire. The concentration of benzene in the air was measured using the NIOSH 1501 method with the gas chromatography technique carried out by the Technical Implementation Unit for Occupational Safety and Health (UPTK3) Surabaya. Erythrocytes and hemoglobin measurements were carried out by taking a sample of the respondent's blood and then analyzing it at the Nutrition Laboratory of Airlangga University. Data analysis using the Spearman rank correlation test.

Result

Characteristics of Respondents

Table 1. Frequency Distribution of Worker's Characteristics

Respondents Characteristics	Frequency	Percentage
Age (year)		
16-25	2	10,5%
26-35	4	21,1%
36-45	8	42,1%
46-55	3	15,8%
56-65	2	10,5%
Gender		
Male	15	78,9%
Female	4	21,1%
Years of Service		
< 10 years	10	52,6%
≥ 10 years	9	47,4%

Table 1 shows that the majority of workers in Printing X Surabaya were 36-45 years old (42.1%), were male (78.9%) and had a working period of <10 years (52.6%).

Benzene concentration

Table 2. Distribution of Benzene Concentrations in Printing X Surabaya

Respondent	Benzene Concentration (ppm)	Benzene TLV (Permenaker RI No. 5 / 2018)
1	15,6418	0,5 ppm
2	1,9267	
3	15,6418	
4	15,6418	
5	1,9267	
6	15,6418	
7	0,9695	
8	0,9695	
9	15,6418	
10	15,6418	
11	1,9267	
12	1,9267	
13	15,6418	
14	15,6418	
15	1,9267	
16	1,9267	
17	1,9267	
18	1,9267	
19	15,6418	

Table 2 shows the measurement of benzene concentration in the air at Printing X Surabaya was carried out at three points with the highest concentration of benzene being 15.6418 ppm. The results of measurements of the concentration of benzene in the air show that the concentration of benzene in the air at work has exceeded the TLV set by Permenakertrans No. 5 of 2018 at 0.5 ppm.

Erythrocyte and Hemoglobin Levels

Table 3. Erythrocyte and Hemoglobin Levels of Printing Workers X Surabaya

Respondent	Erythrocytes (106/mL)	Haemoglobin (g/dL)
1	6,09	16,3
2	5,31	15,3
3	5,52	15,0
4	5,60	16,2
5	4,57	12,4
6	5,65	16,2
7	5,37	15,1
8	5,16	16,7
9	5,64	16,9
10	5,02	15,4
11	4,20	12,0
12	5,28	15,8
13	4,91	15,0
14	5,40	15,2
15	5,57	16,0
16	5,50	13,6
17	4,98	14,0
18	4,92	13,6
19	5,84	16,4

Table 3 shows that the majority of erythrocyte levels and hemoglobin level of Printing X Surabaya workers are normal. But there are five respondents with erythrocyte levels above normal and one respondent with a hemoglobin level below normal. There is one respondent with a minimum level of normal hemoglobin. Normal erythrocyte level in men is $4.4x10^6$ - $5.6x10^6$ cells/mm^3 and in women is $3.8x10^6$ - $5.0x10^6$ cells/mm^3. Normal hemoglobin levels in men are 13-18 gr/dL and in women is 12-16 gr/dL[7].

Relationship between Benzene Concentration, Erythrocyte and Hemoglobin

Table 4. Test Results for Benzene Concentration, Erythrocyte and Hemoglobin

Independent Variable	Dependent Variable	P value	Correlation coefficient	N
The concentration of benzene in the air	Erythrocytes	0,033	0,490	19
	Hemoglobin	0,158	0,338	19

The relationship between benzene concentration, erythrocyte and hemoglobin was obtained through the Spearman rank test. Table 4 shows that there is a significant relationship between the concentration of benzene in the air and erythrocytes ($p=0.033$; $p<0.05$) with a correlation coefficient of 0.490, which means the relationship between the two variables is quite strong and has a positive direction. There was no significant relationship between the concentration of benzene in the air and hemoglobin ($p=0.158$; $p>0.05$).

Health Complaints

Table 5. Health Complaints Experienced by Printing X Workers in Surabaya

Health Complaints	Total	Percentage
Headache	12	63,2%
Tottering	1	5,3%
Out of breath	5	26,3%
Cough	14	73,7%
Low Appetite	1	5,3%
Sore eyes	2	10,5%
High tempered	1	5,3%
Nausea	2	10,5%
Itchy skin	1	5,3%

Table 5 shows that the most common health complaints experienced by workers were coughing (73.7%), headache (63.2%), and shortness of breath (26.3%).

Discussion

Measurement of the concentration of benzene in the air at Surabaya Printing X was carried out at three points with the highest benzene concentration was 15.6418 ppm and the lowest concentration was 0.9695 ppm. The benzene threshold value (TLV) in the environment determined by Permenaker Number 5 of 2018 is 0.5 ppm[15]. Thus the concentration of benzene in the air at Surabaya X Printing has exceeded the specified TLV. Another research conducted by Setiowati shows that the production room for making sandals has levels of benzene in the air that exceeds TLV that is equal to 2.97 ppm[16]. Other studies have found that there are 2 measurement points where the concentration of benzene in the air exceeds TLV that is equal to 0.9129 ppm and 2.333 ppm[18]. The results of measurements of benzene

concentrations in the air at Printing X Surabaya have a higher average than the two studies.

Exposure to benzene can result cells in the body not working normally. The severity of the effects of benzene exposure depends on several factors including the amount of exposure, the route of exposure, duration of exposure, age, and pre-existing health conditions in exposed people[4]. As many as 52.6% of workers had ten years of service <10 years and 47.4% of workers had ≥ 10 years of service. The longest working period is 24 years while the shortest working period is 1 year. Prolonged work period can be a risk factor for health effects due to benzene exposure. According to the results of research by Triyadi, the duration of work (years) can increase the level of risk due to benzene exposure. The longer the duration of work, the greater the health risk due to benzene exposure[17]. Other studies have also found that there is a significant relationship between the length of work experience (years) and the effect of benzene on the peripheral blood of exposed workers. The longer the duration of work, the higher the effect of benzene exposure on the blood[10].

The majority of the effects of long-term benzene exposure (exposure to benzene for a year or more) occur in the blood[4]. Some more recent epidemiological studies have shown haematological effects (including significant reductions in the number of erythrocytes, leukocytes, and platelets) in workers who are chronically exposed to benzene with concentrations below 10 ppm and even as low as 1 ppm or less[2]. The results of blood tests showed that there were 5 out of 19 respondents in Printing X Surabaya who had erythrocyte level above the normal. The results of the examination of erythrocyte levels differed in studies conducted by Lazarevic that there was a decrease in erythrocyte levels in workers exposed to benzene[3]. This difference in results can be due to different individual characteristics, different research sites, and different sources of benzene exposure. But the results of erythrocyte levels both in this study and Lazarevic's research showed abnormalities in the body's erythrocyte levels. This health condition needs to be considered in order to be able to prevent the appearance of haematological effects caused by benzene exposure.

The results of blood tests also showed that 18 workers had normal hemoglobin levels. Although the majority is normal, one respondent has a hemoglobin level below normal and 1 other respondent has a minimum hemoglobin level normal. Similar examination results for hemoglobin levels were found in Tualeka's study that 95% of respondents had normal hemoglobin levels[18]. In addition, Nikmah in her research explained that although the results of examining blood profiles (hemoglobin, erythrocytes, leukocytes, platelets, hematocrit, MCV, MCH, and MCHC) workers in the Printing X industry in Semarang City were still in the normal category, but the numbers tended to be low or close to lower limit of normal standards[11].

The test results of the relationship between the concentration of benzene and erythrocyte showed that there was a significant relationship between the concentration of benzene in the air with erythrocytes with p=0.033. This relationship is quite strong and has a positive direction, which means that the higher levels of benzene in the air, the higher the level of erythrocytes. The results of this study are in line with the results of Haen's research that there is a relationship between the concentration of benzene in the breathing zone with the number of erythrocytes[6]. Mohamed's research to assess the haematological effects on benzene exposure stated that there were significant differences in the levels of erythrocytes and leukocytes between the benzene and non-exposed groups. Erythrocyte and leukocyte levels decreased in the group exposed to benzene[10]. The direction of the relationship in Mohamed's research results is not in line with this study where a positive direction relationship was found which means that the level of erythrocytes has increased along with the higher levels of benzene in the air. Benzene can interfere with the system in the bone marrow so that the process of blood formation cannot work normally[4]. Hemoglobin is a protein used by red blood cells to distribute oxygen to other tissues and cells in the body[9]. Low hemoglobin levels cause anemia while high hemoglobin levels will cause erythrocytosis as a result of too many red blood cells[19].

There was no significant relationship between the concentration of benzene in the air and hemoglobin with p=0.158. The results of this relationship test are in line with a research in Printing X, Semarang that there was no significant correlation between benzene exposure and hemoglobin[11]. Mohamed in his research explained that

there was no difference in hemoglobin levels between the benzene exposed group and the unexposed group[10].

Printing X Surabaya workers experienced several health complaints including cough (73.7%), headache (63.2%) and shortness of breath (26.3%). Similar health complaints were also found in a study at Printing X in Semarang City in 2017, that one of the health complaints experienced by workers is a headache[5]. Other studies have found that there are a number of workers who experience health complaints such as headaches, nausea, and shortness of breath[13]. According to the CDC, individuals who inhale benzene in high levels can experience signs and symptoms such as drowsiness, dizziness, rapid and irregular heartbeat, headaches, tremors, confusion, unconsciousness, and even death (at very high levels). The exposure of benzene that occurs directly to the eyes, skin, or lungs can cause injury to the tissues and irritation[4]. According to the Leukemia & Lymphoma Society, symptoms of Polycythemia or erythrocytosis include headaches, dizziness or vertigo, excessive sweating, impaired vision, fatigue, and prutitus or itching of the skin[8]. Worker health complaints are signs that benzene exposure is starting to have an effect on health.

Conclusion

The concentration of benzene in the air at Surabaya X Printing has exceeded the TLV set by Pemenakertrans No. 5 of 2018. The majority of Surabaya Printing X workers have erythrocyte levels and hemoglobin levels that are classified as normal. There are 5 respondents with erythrocyte levels above normal and one respondent has hemoglobin levels below normal. There is a significant relationship between benzene concentration and erythrocytes. The two variables are strongly related and in the same direction the higher the levels of benzene, the higher the level of erythrocytes. There is no significant relationship between benzene concentration and hemoglobin. Many health complaints experienced by workers in this study included coughing, headaches and shortness of breath.

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Original article

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mRNA Expression of PITX3 gene in Congenital Cataract Caused by Rubella Virus Infection

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Abstract

Objective: To analyze mRNA expression of PITX3 gene in congenital cataract caused by rubella virus infection

Methods: Patients under the age of 18 who were diagnosed with congenital cataracts, were tested for anti rubella antibodies, toxoplasma and cytomegalovirus and then underwent cataract surgery, and lens capsule and lens mass samples were taken. Samples taken will be examined for mutation and expression of the PITX3 mRNA gene.

Results: 15 eye samples were included in the study. there was no relationship with rubella infection with PITX-3 gene mRNA expression in the capsule sample and lens mass ($p > 0.05$). However, in terms of average PITX-3 mRNA gene expression was found to be higher in rubella infection compared to non-rubella infection. Based on the examination site, there was no significant difference in expression of PITX-3 mRNA gene between the lens capsule sample and the lens mass ($p > 0.05$). Although there was no statistically significant difference, in terms of the average, the PITX-3 Gen mRNA expression was found to be higher in the lens capsule compared to the lens mass.

Conclusions: Rubella virus infection was the most common cause in the sample, which indicated the need for prevention of rubella infection in pregnant women to reduce the adverse effects of rubella virus infection. PITX3 gene mRNA expression was higher in the Rubella infection group, indicating the possible role of the PITX3 gene in the pathogenesis of congenital cataracts due to rubella virus infection. The average mRNA expression of the PITX3 gene is higher in the lens capsule compared to the lens mass although it does not significantly indicate the important role of the gene in the process of regulating epithelial cells and lens equatorials.

Keywords: *Congenital cataract, mRNA expression, PITX3 gene, Rubella virus infection*

Introduction

Congenital cataracts are the most common cause of preventable blindness in children throughout the world. The global prevalence of pediatric cataracts ranges from 0.32 to 22.9 / 10000 children and congenital cataracts range from 0.63 to 9.74 / 1000 children, with an incidence between 1.8 and 3.6 / 10000 per year[1].

Hereditary, metabolic, ocular or systemic abnormalities and trauma are factors that are known to play a role in the pathogenesis of cataracts in children. Among the causes that can be prevented are infections[2]. According to reported the incidence of congenital rubella syndrome in Indonesia with hospital-based surveillance and obtained 6% laboratory-confirmed cases and 21.4% clinically compatible cases from 201

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suspected CRS cases[3,4,5]. Among these cases, 66.7% suffered from congenital cataracts. The administration of rubella vaccine in several developing countries has not been widely implemented, causing a high incidence of CRS, especially in Indonesia[6,7].

The exact molecular mechanism of how the virus causes cataracts is also not clearly known. Previous studies have shown that rubella virus interferes with cellular proliferation pathways, changes cytoskeletal structures and induces mitochondrial changes and gives rise to the hypothesis that apoptosis induced by viruses contributes to the teratogenic effects of Rubella virus[8]. The Research conducted by George S, et all prove that the replication of the rubella virus in fetal endothelial cells causes downregulation of genes needed for ear and eye development. It is still not known with certainty which viral gene products and which cellular transcription factors are responsible for the downregulation of these genes[9]. Based on previous studies it can be suspected that the virus can indirectly cause interference with the genes involved in the process of lens formation and development[10].

Genetically hereditary or sporadic congenital cataracts are also very heterogeneous. This is because different mutations in the same gene can cause the same cataract pattern, while the morphology of cataracts varies greatly, where in several families can show the same mutation in a single gene and can cause different phenotypes[11]. Identification of genes that cause congenital cataracts from several causes, whether hereditary, sporadic or infection can help explain the etiology of some cataracts[12].

Some of the genes involved in cataractogenesis include genes that encode crsyallin (CRYA, CRYB, and CRYG), certain lens connexins (Cx43, Cx46, and Cx50) (22), major intrinsic proteins (MIP) or aquaporine, cytoskeletal structural proteins, paired-such as homeodomain transcription factor 3 (PITX3), avian musculoaponeurotic fibrosarcoma (MAF), and transcription factor 4.

One of the genes involved in the process of cataractogenesis is the transcription factor gene. Mutations in this gene have caused abnormalities in the development of the anterior segment, but only three genes, namely PITX3, MAF and HSF4, cause isolated cataracts7. PITX3 is a very important factor for lens formation[4]. Deletions in the gene cause interference with the formation of the lens. Disturbances in these genes can cause lens cells to not differentiate, decrease cell proliferation and increase apoptosis, which will cause interference with lens development[8].

To the best of researchers, there are no studies that report the expression of the PITX3 mRNA gene in patients with congenital cataracts with causes of rubella infection and rubella non-infection by comparing samples from lens lens capsules and lens mass.

Materials and Methods

Subjects and sample collections

15 congenital cataract patients of less than 18 years old who underwent cataract surgery were enrolled in this study. Ethical approval for this study was obtained from the Human Research Ethic Committees of Hasanuddin University. Samples were derived from the lens capsule and lens mass during cataract surgery.

Patients selected were tested for IgM and IgG antibody for rubella, toxoplasmosis and cytomegalovirus. Patients with positive IgM or IgG antibodies for rubella were included in rubella infection group, whereas patients with negative IgM or IgG antibodies for rubella were included in non-rubella infection group.

All patients underwent cataract extraction surgery under general anaesthesia. During surgery, lens capsule and lens mass were collected to be examined for mRNA expression of PITX3 using real time PCR test.The capsules and lens masses specimens were frozen and stored at -80^{o} C until its subjected for RT-PCR analysis.

RNA's Extraction and cDNA Synthesis

Total RNA was extracted from the capsules and lens masses samples using Qiagen RNA extraction kit according to the manufacturer's instructions. The RNAs were reverse-transcribed using Superscript III (invitro gen) .The integrity of RNA and adequate cDNA synthesis were confirmed using beta actin primers. PCR-reaction were initially heated to 65 ^{o} C for 5 seconds for denaturation

Real time PCR examination

For the estimation of PITX3 gene mRNA expression level, real time PCR was performed on SYBR green dye in cDNA derived from total RNA extracted from the capsules and lens masses speciment. Total PCR volume was 20 µl Prior to the amplification, the master mix was prepared by adding 12.5 µl SYBR green mix (Thermo), 0.5 µl cDNA, 1 µl 5 pmol/□l primers targeted PITX3 gene (F: 5' - GCC ACC CTT AGT CCG TG -3 '; R: 5' - GCA AGC CAG TCA AAT G - 3'). 40 times (95°C for 1 minute, 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds) and ended with final extension at 72°C for 10 minutes. The relative expression of targeted gene was calculated as follows: 'D'Ct = Ct (targeted gene) – Ct (housekeeping gene), $R = 2^{-('D'Ct)}$.

Result

Table 1: The Characteristics of Respondents and Congenital Cataract Causes (n = 15)

Characteristic	n	%	Mean±SD
Age (Year)			4,4±2,9
Type of Cataract			
Membranous	3	20,0	
Nuclear	8	53,3	
Total	4	26,7	
Etiology of Catarac			
Rubella Infection	11	73,4	
Non-Rubella Infection	4	26,6	

The results of the analysis of the characteristics and causes of cataracts showed an average age of respondents 4.4 ± 2.9 years, more than half of respondents experienced membranous cataracts (53.3%), congenital cataracts were mostly caused by rubella infection (73.4%) (Table 1)

Table 2: Results of examination mRNA expression of PITX-3 gene in patients with congenital cataracts

Location	mRNA Expression of PITX3 gene (Mean±SD)
Lens Capsul	0,015±0,034
Lens Mass	0,005±0,009

The Results of examination mRNA expression of PITX-3 gene in patients with congenital cataracts were found in lens capsules on average 0.015 ± 0.034 and on lens mass on average 0.005 ± 0.009.(Table 2).

Table 3: Relationship of rubella infection with mRNA expression of PITX-3 gene In Congenital Cataract Patients

Caused infection	n	Location Ekspresi mRNA Gen PITX-3			
		Capsul Lens		Mass Lens	
		Mean±SD	p	Mean±SD	p
Infection Rubella	11	0,019±0,039	0,602	0,007±0,010	0,695
Non-Infection Rubella	4	0,003±0,003		0,0001±0,002	

Mann Whitney Test

The Results that about" analysis of the relationship of rubella infection with mRNA expression of PITX-3 gene in Congenital Cataract Patients showed no relationship with rubella infection with expression of PITX-3 gene mRNA in capsule and lens mass (p> 0.05) (Table 3).

Table 4: The Differences in mRNA expression of PITX-3 gene in Congenital Cataract Patients based on examination site (n = 15)

Variable	Location	Result	
		Mean±SD	p
mRNA Expression of PITX-3 Gene	Lens Capsul Lens Mass	0,015±0,034 0,005±0,009	0,178

Uji: Mann Whitney Test

The Results of analysis of differences in PITX-3 gene mRNA expression in Congenital Cataract Patients based on the examination site found that there was no significant difference in mRNA expression of PITX-3 gene between capsule and lens mass (p> 0.05). (Table 4)

Discussion

According to the results, the average mRNA expression of the PITX3 gene in the lens capsule is higher than in the lens mass. This can be explained by the fact that the PITX3 gene has a greater role in controlling the lens epithelial cells and at an advanced fetal stage after the lens is formed, the PITX3 protein is found in epithelial cells and lens equators that will control the cell cycle and differentiation of fiber cells[13].

PITX3 gene mRNA expression in Congenital Cataract Patients found no relationship between rubella infection and non rubella infection groups both expression in the capsule and lens mass. The expression of the PITX3 mRNA gene in both rubella and non-rubella infection cases indicates the importance of this gene in the lens formation process that occurs from the embryo until after birth and even for life in both groups[14].

The absence of differences in expression levels is probably due to the involvement of other genes or environmental factors that play a role in the process of cataracts in both groups. Although there were no significant differences, the expression of PITX3 gene mRNA was more in the Rubella infection group, indicating the possible role of the PITX3 gene in the pathogenesis of congenital cataracts due to rubella virus infection. PITX3 gene clearly proves to be very important for normal lens development and differentiation. In aphakia (*ak*) mutant, the developing lens does not develop and differentiate, and the lens cells do not form[10]. In addition, lens proliferation is reduced and apoptosis is increased. Based on these studies it was concluded that the abnormal phenotype of the mutant lens is not just one molecular pathway or a specific interaction and is not solely due to the absence of PITX3 expression so that apoptosis, proliferation and differentiation of lens cells are greatly disrupted

Conclusion

Infection by rubella virus was the most commonly found in this study, which signifies the need for prevention of rubella infection in pregnancy to suppress the adverse effects of rubella virus infection. Although statistically it was insignificantly different, PITX3 gene mRNA expression was higher in the rubella infection group, indicating a possible role of the PITX3 gene in the pathogenesis of congenital cataracts caused by rubella virus infection. Increase on average PITX3 gene mRNA expression in the lens capsule compared to the lens mass indicates the important role of the gene in the process of regulating epithelial cells and lens equatorials.

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Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 267/ UN4.6.4.5.31//PP36/2020), and all research subjects give written informed consent.

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Determination of Sex Using Humeral Dimensions and Radiographic Measurements of the Humerus in Egyptian Population, Fayoum Governorate

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Abstract

Background: Sex determination of unidentified skeletal stays from crime scenes or uncovering destinations is a significant part in the improvement of the natural profile in criminology and human science.

Aim of work: The purpose of this research was to determine if sexing of obscure grown-up human humerus bones can be done by applying estimations of morphometric parameters produced by present investigation on these bones of grown up humans of known sex. Also, it aimed to discover the best parameters for sex determination.

Method: This study was conducted on 52 humeri of 28 males and 24 females and 98 radiographs of patients (48 males and 50 females) from the population of Fayoum governorate in Egypt. Humeri bones used in this study were collected from the Anatomy Department, Faculty of Medicine, Fayoum University.

Results: The study illustrated that no statistically significant differences with p-value >0.05 were found between bone and X-ray findings as regards age and sex distribution. Also, no statistically significant differences with p-value >0.05 were reported between both findings regarding other measures including TDMS, UEB, VDH, and ad ML measures. On the other hand, statistically significant differences with p-value <0.05 were reported between different genders regarding EP, TDMS, UEB, VDH, ML, and CT with higher mean values noticed among males.

Conclusion: It is presumed that the distal humerus is a viable bone for sex estimation, even if the bone is in a fragmentary state.

Keywords: *Sex determination, Humerus, Radiograph, Egypt*

Introduction

Sex determination is the initial phase in the identification procedure of a person in legal assessment. This phase is of a very high importance. Age, race, and stature, when identified from the different human remains, are among the few key parameters which should be quickly determined and incorporated in the organic profile. [1, 2] Previous studies reported that the humerus bone can be utilized in sex determination.

The sexing accuracy for various bones reported in literature is 80–92% for cranium, 90% for skull and mandible, 95–98% for pelvis and 80% for long bones. [3,4] Larger limb bones may provide clear evidence of sex particularly if other individuals of same race and of both sexes are available for comparison. Marked sexual dimorphism is exhibited by several bones and they are suitable for sexing skeletons with high accuracy.[5]

Populations contrast in size and extent of human bones; these differences influence the metric assessment of sex[6]. The degree of sexual varieties among Egyptians has not been evaluated and no measurement guidelines have been presented for the distal humerus bones.

The humerus is one of the important long bones of the skeleton because of its quality, even in a divided state, in addition to being conceivable to be recuperated in a measurable case. Old style osteometric methods have been utilized to estimate the humerus length from its sections [7, 8] and affirm the presence of sexual dimorphism in this bone. [9, 10] In anthropometric examinations, the humerus is a tolerably-contemplated bone. It has a fundamental job in sex distinguishing proof, stature estimation, scientific examinations, etc. [11]

This is the first study in the Egyptian governorate, Fayoum, designed to assess the accuracy of sex determination from the length of right and left humeri by evaluating normal anatomy in adults to establish metric standards for sex assessment from the X- ray radiographs of the distal humerus in the population of Fayoum.

Methods

This study was conducted on 52 humeri of 28 males and 24 females and 98 radiographs of patients (48 males and 50 females) from the population of Fayoum. Humeri bones used in this study were collected from the Anatomy Department, Faculty of Medicine, Fayoum University. These bones were selected in a dried and fully-ossified state. The humeri having deformity atrophy or pathological lesions were excluded from the study. The age for both sexes included in this study ranged from 20 to 70 years. The present study was done on dry human bones, so ethical issues were not raised. Radiographs used in this study were taken from an anteroposterior view and collected from a private radiology centre in Fayoum. X-rays with deformities, fractures, recent surgery, or bone tumours were excluded from this study. The age range of patients whose X-rays were used in this study was from 16 to 66 years.

Right and left humeri from bones [11, 12] and radiographs [13] were subjected to the following measurements (in centimetres):

1. Maximum length (ML): Straight distance from the most superior point on the head of the humerus to the most inferior point on the trochlea.

2. Vertical diameter of the head (VDH): Direct distance between the highest and the lowest point on the articular margin of the head measured.

3. Transverse diameter at the middle of the shaft (TDMS): Distance between the medial and lateral margins of the humeral shaft at the middle.

4. Upper epiphyseal breadth (UEB): Obtainable distance between the medial-most point on the articular surface of the head and the lateral-most point on the greater tubercle.

5. Epicondylar breadth (EB): Distance between the most lateral point on the lateral epicondyle and the tip of medial epicondyle.

The maximum length of each humerus was measured roughly using a metal ruler, while other measurements were taken by digital caliper. The digital Vernier caliper provides precision readings from 0.01 mm and 0.000500 through a clear liquid–crystal dis-play. [11] All radiographic measurements were taken by a metal ruler

Results

Table (1) illustrated that there were no statistically significant differences with p-value >0.05 between bone and X-ray findings as regards age and sex distribution. While in **figure (1), it is** demonstrated that there was a statistically significant difference with P value <0.05 between X-ray and bone measurements as regards EP with higher mean value observed among bones. On the other hand, no statistically significant differences with p-value >0.05 were reported between bone and X-ray measurements as regards other measures such as TDMS, UEB, VDH, and ad ML measures. Moreover, statistically significant differences with p-value <0.05 were reported in **table (2)** between different genders as regards EP, TDMS, UEB, VDH, ML, and CT with higher mean values noticed among males. No statistically significant differences with p-value >0.05 were found between bones and radiographs as regards other measures (such as MT).

Moreover, statistically significant differences with p-value <0.05 were reported between males and females among the cases assessed by X-rays as regards EP, TDMS, UEB, VDH, ML, and CT with higher mean values noticed among males, while no statistically significant differences with p-value >0.05 were reported between males and females among these

cases as regards other measures (MT) as shown in **Table (3)**. **Table (4)** illustrated that there were statistically significant differences with p-value <0.05 between males and females among the cases assessed by bone measurements as regards EP, TDMS, UEB, VDH, and ML with higher mean values among males. In addition, statistically significant differences with p-value <0.05 were reported between right and left sides among case regarding TDMS, UEB, VDH, MT, and CT with higher mean values noticed among the right side. On the other hand, no statistically significant differences with p-value > 0.05 were found between the right and left sides as regards other measures (EP and ML) as shown in **table (5).**

Table (6) documented that there were statistically significant differences with p-value <0.05 between the right and left sides among cases assessed by X-rays as regards TDMS, UEB, VDH, MT, and CT, with higher mean values noticed among the right side. On the other hand, no statistically significant differences with p-value >0.05 were reported as regards other measurements including EP and ML.

Table (1): Comparisons of demographic characters in different study groups.

Variables	X -ray (N=98)		Bone (N=52)		p-value	Sig.
Age (years)						
Mean /SD	39.5	16.3	41.8	17.8	0.4	NS
Sex						
Male	48	49%	28	53.8%	0.6	NS
Female	50	51%	24	46.2%		

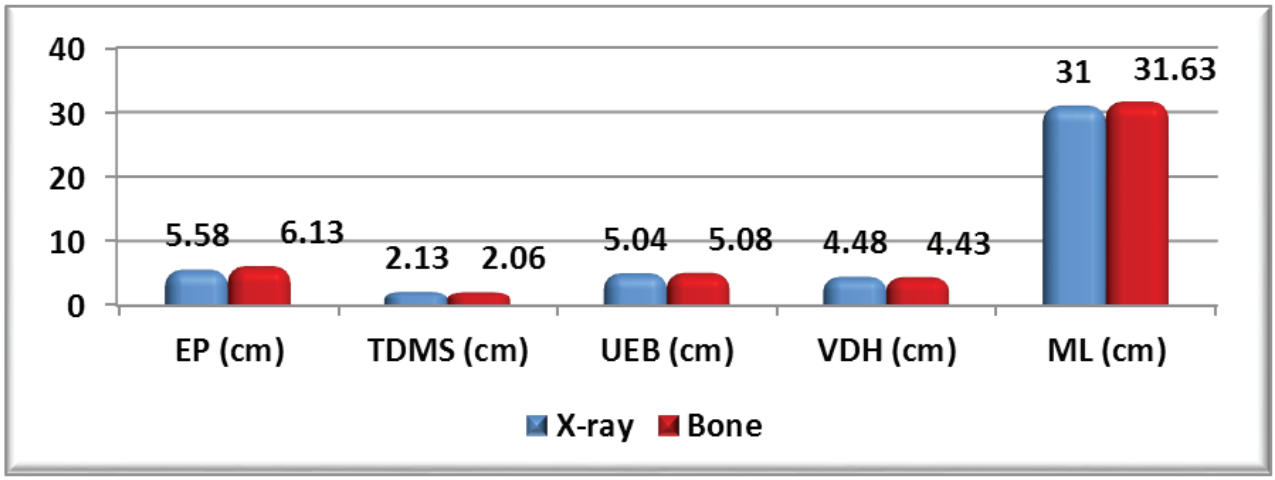


Figure (1): Comparisons of different variables in different study groups.

Table (2): Comparisons of different variables in different gender.

Variables	Males (N=76)		Females (N=74)		p-value	Sig.
	Mean	SD	Mean	SD		
EP (cm)	6.06	0.56	5.48	0.43	<0.001	HS
TDMS (cm)	2.21	0.24	2	0.21	<0.001	HS
UEB (cm)	5.35	0.36	4.76	0.36	<0.001	HS
VDH (cm)	4.72	0.33	4.19	0.31	<0.001	HS
ML (cm)	32.23	1.86	30.20	1.69	<0.001	HS
MT (ml)	7.29	1.22	6.92	1.17	0.1	NS
CT (ml)	3.71	0.54	3.24	0.59	<0.001	HS

Table (3): Comparisons of different variables in different gender assessed by x-ray.

Variables	Males (N=48)		Females (N=50)		p-value	Sig.
	Mean	SD	Mean	SD		
EP (cm)	5.72	0.38	5.44	0.41	0.001	HS
TDMS (cm)	2.2	0.25	2.1	0.19	0.01	S
UEB (cm)	5.3	0.38	4.8	0.36	<0.001	HS
VDH (cm)	4.69	0.39	4.3	0.28	<0.001	HS
ML (cm)	31.6	1.6	30.4	1.5	<0.001	HS
MT (ml)	7.3	1.2	6.9	1.2	0.1	NS
CT (ml)	3.7	0.54	3.24	0.59	<0.001	HS

Table (4): Comparisons of different variables in different gender assessed on bone.

Variables	Males (N=28)		Females (N=24)		p-value	Sig.
	Mean	SD	Mean	SD		
EP (cm)	6.63	0.27	5.56	0.45	<0.001	HS
TDMS (cm)	2.23	0.21	1.85	0.15	<0.001	HS
UEB (cm)	5.5	0.24	4.6	0.31	<0.001	HS
VDH (cm)	4.77	0.16	4	0.30	<0.001	HS
ML (cm)	33.2	0.19	29.7	2	<0.001	HS

Table (5): Comparisons of different variables in different sides.

Variables	Right (N=98)		Left (N=52)		p-value	Sig.
	Mean	SD	Mean	SD		
EP (cm)	5.7	0.55	5.9	0.62	0.1	NS
TDMS (cm)	**2.2**	0.25	2	0.21	<0.001	HS
UEB (cm)	**5.1**	0.42	4.9	0.51	0.004	HS
VDH (cm)	**4.5**	0.40	4.3	0.4	0.004	HS
ML (cm)	31.4	2.1	30.9	1.9	0.1	NS
MT (ml)	7.3	1.1	6.7	1.3	0.04	S
CT (ml)	**3.6**	0.6	3.2	0.58	0.02	S

Table (6): Comparisons of different variables in different sides assessed by x-ray.

Variables	Right (N=72)		Left (N=26)		p-value	Sig.
	Mean	SD	Mean	SD		
EP (cm)	5.55	0.41	5.67	0.45	0.2	NS
TDMS (cm)	2.18	0.23	1.98	0.17	<0.001	HS
UEB (cm)	5.13	0.39	4.8	0.43	0.001	HS
VDH (cm)	4.5	0.41	4.31	0.35	0.01	S
ML (cm)	31.2	1.8	30.6	1.4	0.1	NS
MT (ml)	7.3	1.1	6.7	1.3	0.04	S
CT (ml)	3.6	0.61	3.2	0.58	0.02	S

Discussion

The estimations of long bones' length are crucial for evaluating an individual's stature. These estimations have an important role in medico-lawful examinations for recognition of missing people. Because the humerus is the longest, biggest, and most grounded (even in a divided state) bone of the chest area, stature estimation should be done from this bone without other appropriate long bones such as the femur bone. [14]

In this research, our results revealed that the mean humeral length was of statistically significant difference (p-value <0.05) between males and females among cases assessed by X-rays as regards EP, TDMS, UEB, VDH, ML, and CT where higher mean values were noticed among males compared to females. This finding agrees with the results of other studies in which all

measurements were significantly higher in males than females. Also, our findings are in conformity with the findings reported by Singh [15, 13].

This research also documented statistically significant differences between males and females among the cases assessed by X-rays as regards EP, TDMS, UEB, VDH, ML, and CT, with higher mean values noticed among males. This is in agreement with other researches [16, 17] that illustrated that the following parameters: ML, VDH, circumference of the head, transverse diameter at the middle of the shaft [TDMS], TDUS, TDLS, upper shaft circumference, MSC, lower shaft circumference, upper epiphyseal breadth, and EB were inspected and statistically significant differences were reported, with the mean values of these parameters higher in males compared to females.[18]

This study demonstrated that there were statistically significant differences between right and left sides among cases assessed by bone measurements as regards TDMS, UEB, VDH, MT, and CT, with higher mean values noticed among the right side. Also, statistically significant differences were observed among cases assessed by X-rays as regards TDMS, UEB, VDH, MT, and CT, with higher mean values noticed among the right side. These findings disagree with other studies which reported no statistically significant differences regarding all the investigated values in the comparison carried out between the right and left humera. [19]

Conclusion

the estimations of the humerus seem, by all accounts, to be acceptable discriminators of sex right now by stepwise and coordinate discriminant work investigation.

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Study the Effect of Chronic Toxoplasmosis on Serum Testosterone Level in Men

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Abstract

Toxoplasma gondii (*T.gondii*) is an obligate intracellular protozoon which infects both human and animals. *T. gondii* parasitize in different organs even in testes and it was isolated from semen. The level of testosterone in the serum was evaluated for men with chronic toxoplasmosis.

One hundred twenty-one blood samples were collected from apparently healthy men with different age groups they were attending the teaching hospital in Al-Diwaniyah province. Three milliliters of blood was drawn from each man, and then sera were separated by centrifugation. Anti-Toxoplasma IgG antibody was detected. Same numbers of positive and negative sera were included in cases and controls groups respectively, and then the quantitative determination of testosterone was analyzed in the serum of both groups.

Thirty eight (31.4%) out of 121 examined serum samples were given positive results for *T.gondii* IgG antibodies. The concentration of testosterone, in case group was relatively more than in the control group with no statistical differences ($p>0.05$). According to age, the comparison of testosterone in two groups appeared that testosterone level in 15-29 age in case group was with significant differences ($P<0.05$).

From the current study we concluded that the infection with *T.gondii* can affect the serum testosterone level in men.

Keywords: *Toxoplasma gondii, Toxoplasmosis, Testosterone.*

Introduction

Toxoplasmosis is a zoonotic disease, caused by *T.gondii*, a protozoan of widespread in the world [(1)]. The infection with *T. gondii* is mainly obtained by the ingestion of tissue cysts within raw or undercooked meat or the consumption of contaminated food or water with sporulated oocysts [(2-3)]. Generally, this disease is without clinical signs, but it may lead to local lymphadenopathy disorders, hydrocephalus, and calcification of intracranial, choriorenitis, microcephaly and deafness in newborns and death of immunodificient patients [(4-5)]. Many obstetrical problems may be associated with toxoplasmosis, as it leads to miscarriage of pregnant women or the birth of children with congenital defects, especially if the parasite infection occurs in the first trimester of pregnancy [(6)]. In males *T.gondii* also invade the reproductive system and cause defect in testicular functions [(7)], so the chronic phase showed changes in the men generative parameters [(8)].

Many studies were conducted around the world related to men toxoplasmosis which concentrated on the effect of infection on the parameters of male hormones [(9)]. In Iraq few or absent like studies regarded to the effect of *T.gondii* infection on level of serum testosterone in

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men, so for this reason the aim of this study was develop.

Materials and Methods

One hundred twenty-one blood samples were collected from apparently healthy men, their ages ranged between 15 - 80 years who were attending the teaching hospital in Al-Diwaniyah province. The ages were category into four categories (15-29, 30-44, 45-59 and ≥ 60 years) and some information about housing (rural or city) was taken first and they were raised by cats.

Three milliliters of venous blood was drawn from each man and collected in serum gel tubes, then sera was separated by centrifugation (3000 cycles/minutes for 3 minutes) at room temperature. The sera were kept at (-20˚C) till used. The sera were analyzed to detect the anti *T. gondii* antibodies by used ELIZA technique (*T.gondii* IgG EIA kit; Foresight, Germany). Next, two groups were used in the comparisons, the case group which includes the positive sera and control group which included the equal number of negative sera. Testosterone ELIZA kit (Vida Lab, France), was utilized for quantitative determination of testosterone in cases and controls, where duplicate of all standards, samples, and controls were done. The standard curve was used to calculate the testosterone concentrations in samples. For both *T. gndii* IgG and testosterone evaluating, procedures have been implemented, according to the manufacturer's directions. Then necessary statistical analysis was done.

Results

The result of the current study revealed that 38 (31.4%) out of 121 examined serum samples were given anti *T. gondii* IgG antibodies positive results**.** The 30-44years age group had the highest infection rate (47.37%) compared to other groups with significant differences ($p<0.05$). In spite of the incidence in the urban was higher (39.14%) than in rural areas, also the people who had contact with cats were revealed higher infection rate (35.71%) than those who had no contact with them, but there were no significant differences ($p>0.05$) showed among these comparisons (Table 1).

Table (1): The IgG seropositive of *T.gondii* infection according to the age, residence and cat contact.

Age (year)	No. of examine	No. of +ve IgG	%
15-29	57	14	24.56
30-44	23	11	47.37*
45-59	22	8	36.36
≥60	19	5	26.31
Total	121	38	31.4
X2:- 9.869 p-value:0.019 *:significant differences(p<0.05)			
Residence	No. of examine	No. of +ve IgG	%
Urban	82	28	39.14
Rural	39	10	25.64
Total	121	38	31.4
X2:-2.813 p-value:0.093			
Cat contact	No. of examine	No. of +ve IgG	%
Yes	28	10	35.71
N0	93	28	30.1
Total	121	38	31.4
X2:-0.478 p-value:0.489			

Regarding to the quantitative determination of testosterone concentration, the results revealed that this hormone concentration in case group (1.1497 ng/ml) was relatively higher than in the control group (1.1387 ng/ml), with non-significant differences (p>0.05) between them (Table 2).

Table (2): Testosterone concentration in case and control groups

Group	number	Testosterone concentration (ng/ml) Mean	Std. D	P-value
Case	38	1.1497	0.00006	1.00
Control	38	1.1387	0.00006	1.00
Non-significant differences (p >0.05)				

According to age, the testosterone in two compared groups appeared that it's level in 15-29 age in case group was statistically differences (P<0.05). (Table 3).

Table 3: Comparison among concentrations of testosterone in case and control groups according to age

Age (year)	Mean of testosterone concentration in case group (IU/ml)	Mean of testosterone concentration in control group (IU/ml)	P-value
15-29	1.143 ±0.0011	1.139 ±0.0015	0.048*
30-44	1.140 ±0.0014	1.138 ±0.0008	0.244
45-59	1.142 ±0.0022	1.137 ±0.0015	0.322
≥60	1.145± 0.0036	1.142 ± 0.0017	0.457
*significant differences(p<0.05)			

Regarding to the habitat, there was statically non-significant (p >0.05) in comparison of testosterone concentration level in both urban and rural habitant in case group and control groups. (Table 4).

Table (4): Comparison among concentrations of testosterone in case and control groups according to habitat

Habitat	Mean of testosterone concentration in case group (IU/ml)	Mean of testosterone concentration in control group (IU/ml)	P-value
Urban	1.140 ±0.0015	1.139 ±0.0012	0.566
Rural	1.142 ±0.0019	1.138 ±0.0013	0.133
Non-significant differences (p >0.05)			

Discussion

Toxoplasma gondii is consider as an important protozoa pathogens that infect both human and animals, causing many severe affects which can develop to death in immunocompromised individuals [10]. The results indicated a high rate of infection among males in different age groups, especially in the 30-44 years group with significant differences ($p<0.05$). This result come to an agreement with Tabbara *et al.,* [11] in Saudi Arabia, Al- Rubaia, [12] in Iraq and Shin *et al.*,[13] in Korea, where all these studies referred to increase of infection rates among patients of 20-40 years. The rises of infection in this age stage may be due to the cumulative exposure to it with the advancement of age, in addition to this stage is a childbearing in women, in which it is exposed to stress factors due to pregnancy, which negatively affects the level of the body's immunity, so the work of an early screening of antibodies against *T. gondii* at Pregnant women are important to control abortions or fetus congenital defects caused by an infection Han *et al.,* [14].

With reference to the residence, the urban areas showed high infections in compare with rural (39.14% and 25.64% respectively), in spite of there is no statistical difference ($P>0.05$). This finding is in consistent with the previous study performed by Razzak, [15], whereas do not match what came in some other studies which confirmed that the infection of women in rural were more than in urban areas [16-17]. These differences in infection rates may be attributed to factors related to the way of feeding and contact with cats, as well as other factors related to the level of health services and environmental conditions [18].

In other hand, testosterone concentration mean of both case and control groups was in normal range and without significant difference ($P>0.05$). A comparison of testosterone levels appeared that the hormone concentration was relatively higher in the case group than in the control group at ages 15-29 years with significant differences, while no significant difference was observed when comparing the case group and the control group in the urban and rural. This result come to an agreement with the result of Lim *et al*., [19] who referred to that the infection with *T. gondii* leading to a long-term increase of testosterone, where experimentally a *Toxoplasma gondii* attack the testes in laboratory rat [20-21] and making changes in the synthesis of testosterone and nucleus accumbal dopamine content in this animal model [19- 22]. Zouei *et al.,*[23] had reported elevated of testosterone concentration in study group as compared to control group.

A significant relation had been reported between IgG anti Toxoplasma antibodies and testosterone [24], and the results are differ according to the protozoan strain type [25].

Most testosterone is synthesis in testicular Leydig cells and luteinizing hormone receptor (LHR) is play an important role as regulator of steroidogenesis [26-27]. *T.gondii* infected animals showed with high mRNA expression of luteinizing hormone receptor that will lead to high testosterone production (LHR) [28-29-19].

Many studies that have been emphasize a presence of correlation between infection with chronic toxoplasmosis and disorders in male's reproductive parameters [30-31-25]. Terpsidis *et al.*[31]showed rising of sperm abnormalities in infected mice. Some other studies referred to decrease the hormone level in both sexes of mice infected with virulent strain T38 of *T. gondii* [25], while others didn't determine if *Toxoplasma* can affect the testosterone level or no [32-33-34].

The level of hormones can change in some conditions, and alter effects on the immune system may cause resistance stimulation or exposition to different parasitic attacks. Toxoplasmosis can increase in elevated acute sexual steroids, mainly by inhibiting the immune endocrine network (IEN) and provided that parasite replication [35].

Although it has been thought that the *T. gondii* infection is asymptomatic in immune-competent individuals and they seem apparently healthy, indeed a hidden infection is present lead to change and raise the level of testosterone hormone [34-36-37].

Conclusion

Toxoplasmosis is more affective and can cause increasing in testosterone level during the active period of maturity in men.

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Curcumin vs Chloroquine in Coronavirus Global Pandemic: Trend analysis Study in Google

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Abstract

COVID-19 is an infectious disease caused by the newly discovered corona virus. Curcumin and Chloroquine are one of the medications to combat against the pandemic. This paper has objective to analyze the trends inside the use of Chloroquine and Curcumin as medical treatment against corona in global big data. This study uses quantitative method of data mining design in collecting the data. This study uses simple random sampling to determine the number of the population needed for this study. This study uses Google trends to gather the statistical result based on the keyword. The result shows that curcumin is more popular to be searched as information in bigdata. USA is the country with the biggest demand of information about curcumin, as seen on the number of queries variation as well as the relevance score. It can be concluded that information about curcumin has demanded more because of low side effect than chloroquine.

Keywords: *Chloroquine, curcumin, coronavirus, Google Trends*

Background

COVID-19 is an infectious disease caused by the newly discovered corona virus. Most of the patients who are the container of the COVID-19 virus will show the symptom from light to moderate respiratory illness and need special care. Moreover, this virus is more vulnerable to attack Elderly people who are older than 60 years and have basic medical problems such as cardiovascular disease, diabetes, chronic respiratory illnesses, and cancer [1]. The case of COVID-19 is also called the severe acute respiratory syndrome Corona Virus 2 (SARS CoV-2). This is a new type of corona virus that has not been previously identified in human body [2].

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The vaccine to combat for this outbreak is currently under developed by experts of medical world across the globe. However, it is estimated that the vaccine will only be completed next year. Vaccines are antigenic substances used to produce immunity to a disease [3]. To resolve this problem, drug testing is being carried out, and one of them is Chloroquine. Chloroquine phosphate is a drug for treating malarial and Amebicides. This drug has been used to prevent and treat malaria for around 70 years [4]. President of USA, Donald J Trump states that the drug had been used and showed satisfactory results. He also mentioned that the US Food and Drug Administration (FDA) have approved the use of chloroquine. Moreover, he said that the drug may circulate with a doctor's prescription [5].

Another medicine to treat the symptom is curcuma. Curcuma is a natural antioxidant and anti-inflammatory agent that can help increase cell resistance to damage, infection, inflammation, and prevent some degenerative diseases. Curcumin substance in curcuma is very good for consumption every day, to improve the body's immune system, especially for people who are active every day [6]. Curcumin has many health benefits for the body. In addition to overcoming the problems of the digestive

system, ginger can also be an anti-inflammatory drug and wound healer. This plant can also prevent cancer, as an antibacterial and antifungal, and maintain liver health. This herb is safe for consumption on a regular basis, because it is able to maintain the immunity system of the body [7].

The fourth industrial revolution has been changing the medical system and workplace. Albeit most of the data in the health service sector is currently stored in print, the trend towards digitalization is happening fast [8]. The emergence of many standards, regulations and incentives for digitizing data and sharing data in the health sector, and reducing the cost of data storage and processing technology are the sign of digital revolution in medical world. When the covid-19 outbreak began at 2019, AI and big data were calculated the scope of the outbreak and the number of victims [9].

AI and big data technology have been used in many hospitals in many provinces and cities, and continues to be applied in more hospitals throughout China. AI and big data also recorded the most frequent use of drug and herb for medication treatment of this outbreak [11]. Based on the background above, this study has purpose to observe the trends inside the use of Chloroquine and Curcumin as medical treatment against corona in global big data. The theoretical implication of this study is to persuade society to learn more about big data to treat symptom caused by Covid-19.

Method of the Study

This study uses quantitative method of data mining design in collecting the data. This study uses simple random sampling to determine the number of the population needed for this study. This study uses Google trends to gather the statistical result based on the keyword. The inclusion criteria of the sample are the country with high number of death and active cases frequently uses either Chloroquine or Curcumin as medication treatment, and modern country using big data in medical treatment [12].

There are 3 samples of this study based on the keywords typed in Google Trends, which are *chloroquine* and *curcumin,* USA, UK, and Italy. The period of the observation is December 2019 to March 2020 when the outbreak started actively across the globe. The statistical data are related queries of those two keywords in three countries. The data are measured the occurrence and relevance score based on how many the information available during that period [13].

Occurrence score shows how many the keywords appeared in the data, while relevance score based on how relevant some information with the keywords that are being observed. The relevance score measured from 1 to 10 from the scale. Data is downloaded in CSV file from Google trends. Data analysis was performed using Vosviewer version 1.6.14 for windows [14].

Result of the Study

Table 1 The relevance and occurrence point of Curcumin in USA bigdata

Term	Occurences	Relevance
Benefit	1	4.00
Best curcumin supplement	1	4.00
Breakout	2	4.00
Curcumin	4	4.00
curcumin3	1	4.00
Ginger	1	4.00
Turmeric curcumin	2	4.00
United States	1	4.00

Table above shows that curcumin information is quite popular in USA. There are various of related queries about curcumin, when the outbreak is happened. There are 8 queries that has relevance score 4.00 means that some information about curcumin is not really connected to each other. It can be seen from the table above that although there are many queries contains information about curcumin, the relevance score are low or has no relevance at all.

Table 2 The relevance and occurrence point of Chloroquine in USA big data

Term	Occurences	Relevance
Chloroquine phosphate breakout	1	6.05
Chloroquine phosphate1	1	6.05
Couple	2	6.05
Breakout	3	3.71
Chloroquine phosphate	7	3.71
Sale	1	3.71
United States	1	3.71

Table above shows that the information search of chloroquine has higher relevance point than curcumin. The infrmation about chloroquine phsophate in breakout has highest relevance point because the high usage of chloroquine in medical treatment in hospital. There are 3 queries related to the usga eof chloroquine has relevance point higher than 6 and 4 queries has higher than 3. USA seems more believe in chloroqune phospahte rather than curcumin in treating the outbreak.

Table 3 The relevance and occurrence point of Curcumin in Italy bigdata

Term	Occurences	Relevance
Curcumin	2	3.50
Italy	1	3.50

Table above shows that information search about curcumin is really low in Italy. Table above also shwos that there is a variant of curcumin usage such as curcumina piperina or curcumn powder. It means that the extract from curcumin is dried into powder to prolong the expiration date.

Table 4 The relevance and occurrence point of Chloroquine in Italy big data

Term	Occurences	Relevance
Chloroquine phosphate	1	3.00
Italy	1	3.00

Tabel above shows that the information search of chloroquine also low both in varieties as wel as the frequency in Italy. It is similar with curcumin, there is only few people interested in chloroquine as the way to fight the covid 19 pandemic. There are only 6 varieties of queries related to chloroquine, fewer than curcumin. The relevnace score of information is also lower too than curcumin. It can be concluded that Italian are not relly care to figure out the best way to cope with the pandemic.

Table 5 The relevance and occurrence point of Curcumin in UK bigdata

Term	Occurences	Relevance
Barret	1	3.83
Benefit	1	3.83
Curcumin	2	3.83
Curcumin3	1	3.83
Holland	1	3.83
United Kingdom	1	3.83

Table above shows that there is a high demand of information about curcumin in UK. It can be seen that there are many queries related to curcumin. There are 18 queries related to curcumin, 4 of them are the varieties of curcumin product such as tablet, vapsule, powder, and supplement. There is also curcumin information about side effect to know the negative side of this substance. The highest relevance score is 3.83 with more than 6 queries.

Table 6 The relevance and occurrence point of Chloroquine in UK big data

Term	Occurences	Relevance
Chloroquine phosphate	1	4.0
United Kingdom	1	4.0

Table above shows that chloroquine information is not as popular as curcumin. There are only 8 queries but the highest relevance score is 4.00, a bit higher than curcumin. It can be concluded that information aboutchloroqune is less popular than curcumin.

Discusssion of the Result

Based on the result above, it can be concluded that curcumin is more popular to be searched as information in bigdata. USA is the country with the biggest demand of information about curcumin, as seen on the number of queries variation as well as the relevance score. United States of America the biggest number of covid-19 suspect than the rest of the world. As a result, the demand of information about the medicine for covid-19 is also high.

Study by Mounce et al states that curcumin preserve the liver by stopping myocardial fibrosis through modulation of the renin-angiotensin enzyme system and increasing ACE2 pressure which can help repair fibrosis that cannot be tolerated to use ACEis drugs. Curcumin also has effect of inhibiting the process of viral growth, both directly by damaging the physical virus and by suppressing cellular signaling pathways that are important in the process of virus replication [6].

Curcumin is a polyphenol compound which can befound in turmeric and ginger. Curcumin is also contained in several species of Curcuma from the Zingiberaceae family Curcumin has a yellow color cannot be dissolved easily in water but, in organic solvents. Curcumin is non toxic to normal cells and very efficacious in the treatment of cancer therapy. Thus it can become as an antioxidantgood because of its phenolic compounds which acts as an antioxidant and inhibitor melanogenesis [15].

Tables above shows that the demand of information about curcumin in USA bigdata is a bit lesser than chloroquine. One of the reason because Food and Drug Administration in USA has been permitted in using hcloroquine and hydroxychloroquine as the medication

againts coronavirus. Moreover, President Trump pushed for federal bureaucracy to cut through bureaucracy and move faster to find ways to combat the COVID-19 virus. Trump said he wanted to speed up vaccine approval and treatment to combat the COVID-19 pandemic [16]. Trump claims, the US Food and Drug Administration (FDA) has approved the use of chloroquine for the treatment of corona patients. Trump expressed his appreciation for the drug chloroquine which calls it a potential game-changer drug, while apparently stumbling on the regulatory pathway needed to give it to a wider set of patients [17].

Chloroquine is a drug used to prevent or treat malaria. The drug is also used to treat autoimmune disorders, rheumatoid arthritis and lupus. It is also used to treat amebiasis or parasitic infections of Entamoebae histolytica (E. histolytica) in human intestine. But there is also a risk of using this anti-malaria drug to treat patients with COVID-19, a disease that attacks the respiratory system [18]. Side effects ranging from mild such as headaches, stomach cramps, and nausea, to severe ones, namely heart rhythm disorders. In some cases, administration of chloroquine phosphate can cause QT intervals to elongate at which the heart rhythm becomes abnormal [19].

Tables above show that Italy is the country with least demand of information about curcumin and chloroquine. Different from Italy, UK has bigger demand of information about curcumin. Moreover, the number of varieties of queires and relevance point of curcumin are far higher than information about chloroquine. Thus, there is also some of the derivative product of curcumin for public, while chloroquine only has one.

Mounce et al state that curcumin natural supplements are as effective as drugs, such as antidepressants and without significant side effects too the body [6]. Antidepressants which is consumed for a long time can cause a chronic disease, while curcumin is very safe and there is no toxicity, even when you consume curcumin up to 12 grams a day for six months, it has no toxicity [7]. In the global pandemic where people must isolated themselves at home as a result of lockdown, antidepresant tend to be used against solicitude feeling and depression. Moreover, the massive lost of job and lack of food supply also causes massive depression in society which led to the usage of antidepressant to calm down the mind [1].

Conclusion

Based on the result above, it can be concluded that the demand of information of curcumin is highe than chloroquine in UK and Italy, while USA as a result of bureaucration emphasize on the use of chloroquine more than curcumin to cope againts the global pandemic. USA has the hihgest demand of information either in curcumin or chloroquine. Italy has the least information demand about both of the medication. Curcumin has demanded more because of low side effect than chloroquine. Moreover, curucumin which has effect as strong as antidepressant without significant side effect really helpful for people who are isolated themselves because of the lockdown.

Ethical Clearance : This research does not involve any participants. Instead it is used big data as subject of research that was accordant with the principle of research ethic in collecting the data. The paper has never been submitted in anyone else and declared as original paper. the paper is free from any plagiarism and maintain the high standard of research ethic.

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Influence of the Work Units' Psychological Ownership and Intention in Establishing the Patient Safety Program in Hospitals in Probolinggo, East Java

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Abstract

Introduction: There is high rate of patient safety incidents in the hospitals, which reaches 1217 incidents (21,31%) from 2016 until July 2017. There are some factors which cause the high rate of the patient safety incidents which come from the hospitals. The aim of this research is to analyze the effects of the psychological ownership and intention towards the work units' output performance in carrying out the patient safety program in the hospitals.

Materials and Method: This research uses the survey research method with the cross-sectional approach. It was carried out from October 2018 until January 2019. The research samples are the service unit, supporting unit, management and general sector in the work units 4 hospital ini Probolinggo City, Indonesia. The samples are obtained using the simple random sampling method. The instruments used are questionnaires and observation sheets. The analysis is carried out using linear regression tests.

Result: The work unit's psychological ownership in carrying out the patients' safety program is regarded as good, with the rate of 46,7%. The work unit's intention in carrying out the patients' safety program is also regarded as good, with the rate of 41,1%. The work unit's output in carrying out the patients' safety program in Probolinggo Regency is regarded as bad, with the rate of 64,5%. There is a significant influence of the psychological ownership variable (p-value=0,02) and intention (p-value=0,00) towards the output's work performance in carrying out the patients' safety program in the hospitals of Probolinggo Regency.

Conclusion: The work unit's psychological ownership and intention have a significant effect towards the work unit's work performance in in carrying out the patients' safety program in the hospitals of Probolinggo Regency on 2019. The hospitals should carry out work unit approaches instead of individual approaches to increase the report of the patient safety incidents.

Keyword: *psychological ownership, intention, output performance, patient safety*

Introduction

The patients' safety is currently a significant global issue, where there are many reports of the patients' demands or medical errors. As one of the organizations which provide health facilities, hospitals are part of the health resources which are crucial in establishing the efforts of patient safety program. Thus, there needs to be a safe system to avoid the risks of errors in applying the health technologies[1].

From WHO's publication on 2004, the rate of adverse events in various countries such as the United States, England, Denmark was around 3,2–16,6%. The

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IOM report concluded four principle things: a) the accidental injury is a serious case, b) the cause is not individual error, but the system's error, c) there needs to be a redesign of the service system, d) patient safety must become a national priority[2].

The implementation of the patient safety is all practices which decrease the chance of side effects as a result of the health service system. The implementation of the patient safety is an activity which is carried out to ensure the safety of patients, such as hand hygiene, patient identification, safety of medicine, and effective communication. They are nursing activities which are applied daily by the nurses. The patient safety is further encouraged to minimize the number of adverse events which may happen in the hospitals. Even so, based on the researchers' observation, not all adverse events have been documented in the adverse event documentation and report filing system in the hospitals[3].

The psychological ownership is the work unit's feeling of belonging towards the organization and the work which overshadow them. The psychological ownership usually come from the learning process in the organization. This has a positive effect towards the organization, which include the increase of motivation, commitment, and loyalty. The psychological ownership may be built in an organization by actively involving the work units to contribute in developing the organization actively and creatively[4].

Based on the report of the Patient Safety Committee of Rizani Hospital on 2017, the number of adverse events were 176 cases. There were 7 cases of incident without injury and 1036 cases of potentially-injuring incident. These cases include numbers of phlebitis, incompletion of medical reports, errors in giving diets, errors in prescribing medicines, and the non-compliance rates of patient identification. The problems which currently exist have not been carried out well according to the standard operational procedures and the hospital safety guidelines, thus there needs to be an analysis.

The problem of this research is the high rate of the patient safety incidents in hospitals, which is 1217 cases (21,31%) from 2016 until June 2012. There are some factors which cause the high rate of the Patient Safety Incidents which come from the factor of the hospitals. The aim of this research is to analyze the effect of the psychological ownership and the intention towards the work unit's work performance in carrying out the patient safety program in the hospitals of Probolinggo Regency on 2019.

Method

This research uses the survey research method using the cross-sectional approach. The research was carried out from October 2018 to January 2019. The research samples are the work units, supporting unit, management and general sector in the work unites of Rizani Hospital Paiton, Waluyojati Regional Public Hospital Kraksaan, dr. Shaleh Regional Public Hospital Probolinggo, and Tongas Regional Public Hospital. The samples are collected using the simple random sampling technique. The informants are chosen from these criteria: They are the Head of the Unit or the Person in Charge of the Unit analyzed and the Champion or the Person in Charge of the safety program in the work unit. The instruments used are questionnaires and observation sheets. The analysis is carried out using linear regression tests. The questionnaires have been validated. One of the requirements of the multiple linear regression is the passing of the classical assumption test. The classical assumption test results carried out towards this study's data show that there are three independent variables which show the existence of multicollinearity Thus, it can be concluded that the standard error value is low and the multicollinearity is not detected. This means that it is distributed normally.

Result

Descriptive Results of Psychological ownership, Intention and Output Performance

Table 1. The work units' Psychological ownership in carrying out the patient safety program in the hospitals of Probolinggo Regency on 2019

No	Psychological ownership	Frequency	%
1	Very Bad	5	4,7
2	Bad	37	34,6
3	Good	50	46,7
4	Very Good	15	14,0
	Total	107	100,0

Based on table 1, it can be seen that most of the work units' psychological ownership in carrying out the patient safety program in the hospitals of Probolinggo Regency on 2019 is regarded as good, with the rate of 46,7%.

Table 2. The work units' Intention in carrying out the patient safety program in the hospitals of Probolinggo Regency on 2019

No	Intention	Frequency	%
1	Very Bad	0	0,0
2	Bad	19	17,8
3	Good	44	41,1
4	Very Good	44	41,1
	Total	107	100,0

Based on table 2, it can be seen that most of the work units' intention in carrying out the patient safety program in the hospitals of Probolinggo Regency on 2019 is regarded as good and very good, with the rate of 46,7%.

Table 3. The work units' output performance in carrying out the patient safety program in the hospitals of Probolinggo Regency on 2019

No	Output Performance	Frequency	%
1	Very Bad	9	8,4
2	Bad	69	64,5
3	Good	21	19,6
4	Very Good	8	7,5
	Total	107	100,0

Based on table 3, it can be seen that most of the work units' output performance in carrying out the patient safety program in the hospitals of Probolinggo Regency on 2019 is regarded as bad, with the rate of 64,5%.

Statistic Test Results of the psychological ownership and intention towards the output performance

Table 4. Effect of Work Units' Performance Management, Learning Organization, Knowledge, Psychological Ownership and Intention Towards the Output Performance in Carrying Out the Patient Safety Program in the Hospitals of Probolinggo Regency on 2019

Independent Variables	Dependent Variable	p-value	B	Significance
Performance management	Output Performance	0,37	-0,18	Not Significant
Learning organization		0,78	0,04	Not Significant
Knowledge		0,09	0,43	Not Significant
Psychological ownership		0,02	-0,20	Significant
Intention		0,00	0,58	Significant

Table 4 shows the information that the variables which has the p-value (0,00) less than 0,05 are the psychological ownership p-value (0,02) and the intention p-value (0,00) variables. This means that there is the significant influence between the psychological ownership and intention variables towards the output performance in the establishment of the patient safety program in the hospitals of Probolinggo Regency. performance management, learning organization, and knowledge do not have the direct influence towards the units' output performance. Yet, it must go through the intention unit so that it has direct influence towards the work units' output performance in the establishment of the patient safety program in the hospitals of Probolinggo Regency. The variables which have influence towards the work units' output performance are psychological ownership and intention.

Discussion

The psychological ownership of an employee may grow if he/she feels comfortable and he/she feels that all aspects of the company or that of the organization is also part of him/herself[5]. This may encourage the employees to work better for the company. The employees who have a sense of belonging towards the organization will have better satisfaction and work performance. If the employees are satisfied and they have good work performance, there is a greater chance that they will stay longer in the company[6].

Based on the research's results, it is shown that the majority of the work units' psychological ownership in the establishment of the patient safety program in the hospitals of Probolinggo Regency of 2019 is regarded as good, with the rate of 46,7%. Yet, 34,6% of the work units have bad psychological ownership. This is because of the work units' low awareness, thoughts, and certainty on the importance of the patient safety program establishment in the hospitals. Psychological ownership also has an inversely proportional influence towards the work units' output performance in the establishment of the patient safety program (negative b value, -0,20). The negative b value shows that the psychological ownership variable has negative influence towards the work units' output performance. This means that the higher the employees' psychological ownership is, the lower the

work units' output performances are in carrying out the patient safety program in the hospital.

The condition of the psychological ownership is influenced by the situations and the individual factors which direct the individual behaviors[5]. It is true that the individual ownership cognition or perception are proven to influence the behaviors in managing teamwork[7]. The employees' Knowledge have an important role in increasing their psychological ownership in implementing the patient safety program in the hospitals in Probolinggo. The psychological ownership studies are especially aimed to know how far an individual perceives some of the knowledge on the organization[8]. Only some researches have analyzed the relation between knowledge and psychological ownership. The researchers have identified the preparedness for knowledge as one of the impacts of that person's psychological ownership[7].

Psychological ownership plays a crucial role in an individual's process in increasing knowledge by sharing extra role behaviors which are usually motivated by strong intrinsic motivations[9]. There is a relatively small attention given to relate the human resource management with the organizational knowledge management[10].

Meanwhile, the performance management, learning organization, and knowledge variables do not have a significant influence towards the output's performances in the establishment of the patient safety program in the hospitals of Probolinggo Regency. Yet, the variable which has the most dominant influence towards the work units' output performance is intention, with P=0,00 and B=0,58, which has a positive relation towards the increase of the work unit's output performance. This is because intention has a crucial role in influencing one's behavior. It indicates how big their intentions are in carrying out the patient safety program in the hospitals.

One of the behavioral determinants in carrying out the patient safety program in the hospitals of Probolinggo Regency is intention. The intention to carry out a certain behavior is one's tendency to choose whether or not he/she will carry out a particular activity[11]. Most of the employees' intention in the work unit level in carrying out the patient safety program is good. The behavioral intention is the willingness to carry out a particular behavior, the behavioral intention is not yet in the form of the behavior itself. Intentions are not always static and it may change from time to time. The longer the time interval is, the higher the chance of change in intention[12].

The research results show that the work units' intention in carrying out the patient safety program in the hospitals in Probolinggo Regency on 2019 is regarded as good and very good, with the rate of 41,1%. Based on the research results, it is shown that the units' intention has an influence towards the unit's performance output in carrying out the patient safety program with the significance value of (0,01) less than α (0,05).

The results of this research are parallel to the research carried out by Ekayani, Wardhani, and Rachmi which stated that the intention has an influence towards the performance of nurses in reporting adverse events ($r = 0.031$; p value = 0.783)[13]. This finding shows that the nurses' intention in reporting adverse events are influenced by behaviors, not subjective norms. Inversely, intention does not have influence towards the behavior in reporting an adverse event. The factors which influence the intention to carry out the patient safety program in the Oncology Hospital Surabaya are knowledge, awareness, commitment, motivation, perceived behavioral control on policies, patient safety culture, the existence of the hospital patient safety committee and standard operational procedures of the patient safety in that hospital[14].

In the organizational factor, partially, perception, knowledge, and patient safety culture have significant influences towards the intention to report patient safety incident. This is in parallel with and strengthens the researches of Hwang, et al. which stated that knowledge, patient safety culture, and performance management and the parties related influence the intention to report[15].

Conclusion

Psychological ownership and intention work units have significant influence towards the work units' output performance in the establishment of the patient safety program in the hospitals in Probolinggo Regency on 2019. Hospitals should carry out work unit approaches instead of individual approaches to increase the reports of the patient safety incident. These efforts may be carried out by increasing the employees' knowledge on the patient safety incident reporting process, eliminating the fear regarding the impact of the reports, and developing

the culture of patient safety. Another effort which may be carried out is by making a good reporting system and giving a fast report response. The punishments given do not blame, nor should they punish individuals.

Conflict of Interest: Authors declare that the information above is correct and the manuscript submitted by us is original. We have no conflict of interest to declare.

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The Effect of Estrogen, Progesterone, and Its Combination on The Expression of Brain-Derived Neurotrophic Factor Medula Spinalis in Regeneration Process of Peripheral Nerve

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Abstract

Background: The presence of modulation factors in the regeneration process of peripheral nerve cells influenced by estrogen and progesterone. Peripheral nerve injury is a fairly common case of trauma. Brain-derived neurotrophic factor (BDNF) is the most active group of neurotrophins in stimulating neurogenesis. **Objectives:** To proves the effect of estrogen and progesterone on the expression of BDNF on the spinal cord in peripheral nerve regeneration process. **Methods:** Laboratory experimental study with the completely randomized design. Total transitory nerve ischiadicus was performed on four groups of rats. Each group received hormone therapy according to the group. Hormone therapy is administered every 3 days for 28 days, and on the 29th day is termination, spinal cord sampling, and followed by BDNF expression examination by Immunohistochemistry method. **Results:** In the control group, BDNF expression of spinal cord neuron cells was 93.0±14.0. In the treatment group, BDNF expression was obtained after estrogen therapy was 77.25±19.19, progesterone was 84.5±20.61, and in a combination of estrogen and progesterone was 77.75±16.54. After statistical tests, no significant differences were found between the treatment groups ($p = 0.316$). **Conclusion:** The administration of estrogen, progesterone, or a combination of both did not significantly increase BDNF expression when compared to the control group.

Keywords: *Peripheral nerves, Ischiadicus nerve, Estrogen, Progesterone, Neurotrophin, Brain-derived Neurotrophic Factor*

Introduction

Peripheral nerve injury is a fairly common case. The incidence rate of peripheral nerve injury in general is covering 2% of all cases of trauma[1]. A study in 2011 of 16,753 trauma patients, 219 (1.3%) of whom suffered peripheral nerve injury; 182 patients (83.1%) were men, where the most cause of peripheral nerve injury was laceration from sharps (61%), followed by second by traffic accident (22%)[2]. Peripheral nerve damage also happened in infection cases, such as leprosy[3]. In the United States, 11,000 cases of paralysis occur each year. This case costs up to seven billion US dollars each year. Until 1995, more than 50,000 surgical procedures were performed to repair peripheral nerve injuries[4]. Injury to peripheral nerves can cause severe functional disturbances that will greatly disrupt the daily and professional activities[5].

The treatment of peripheral nerve injuries is a difficult clinical problem. Because nerve cells are unique cells compared to other cells, their inability to proliferate and only regenerate their axons. Epineurial neurorrhaphy has been widely practiced as a procedure to repair peripheral nerve injury, with the ultimate goal of improving functional conditions such as the time

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before the injury is still unsatisfactory[6]. Unlike injury to the central nervous system, if the axons in the peripheral nerves are injured, regenerative responses may produce a good functional outcome. However, the results are not very satisfactory in cases of proximal nerve injury, due to slower regeneration and the occurrence of changes caused by chronic denervation. Strategies to increase axon growth can provide benefits in peripheral nerve regeneration[7].

Neurotrophin is a growth factor group consisting of Nerve Growth Factor (NGF), Brain-derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3), NT-4/5, and NT-6 [8]. Neurotrophin serves to protect nerve cells from degeneration and trigger nerve regeneration of the injury and increase the differentiation of nerve cell stem[9]. Based on another study, it was found that functional improvement was associated with increased levels of neurotrophin in muscle, serum, and nerves[10]. BDNF is a protein found in the central and peripheral nervous system. Part of the group Neurotrophin is most active in stimulating neurogenesis, and the most widely distributed in the nerve system[11,12].

Previous research found that prednisolon administration could improve nerve function impairment[11]. In the study, it was found that endogenous BDNF is absolutely necessary for the regeneration and remyelination process after peripheral nerve injury. BDNF is strongly expressed in peripheral nerves injured and also in denatured muscles. Provision of exogenous BDNF therapy actually lowered the expression of BDNF on peripheral nerves and resulted in inhibition of regeneration and re-myelination process after significant peripheral nerve injury[12]. In some other studies found the existence of modulation factor in the regeneration process of peripheral nerve cells, which is influenced by estrogen and progesterone hormones. In the study, it was found that administration of estradiol preparations in female rats post-treatment of crush injury on the ischiadicus nerve modulated the process of nerve cell regeneration[13].

Progesterone has functions other than the reproductive organs, also functions on other target organs such as the central nervous system and the peripheral nervous system. In the study found progesterone and its derivatives can cause the remyelination of axons by oligodendrocyte[14]. The effect of progesterone in the nervous system involves various signaling mechanisms. The identification of classic intracellular progesterone receptors as a therapeutic target for myelin repair demonstrates new health benefits for synthetic progestins, designed specifically for contraceptive use and replacement hormone replacement therapy. There is a great advantage in the use of natural progesterone in myelin improvement strategies because progesterone is converted into biologically active metabolites in neural networks and interacts with some target proteins. The formation of endogenous progesterone is currently explored as an alternative strategy of neuroprotection, axonal regeneration, and myelin repair. Progesterone modulates the regeneration process of peripheral nerve cells post crush injury. In the treatment group, rats' motor results returned after 21 days post-treatment, while the control group took longer time[15]. Therefore, in this study we measured the effect of estrogen and progesterone on the expression of BDNF as the peripheral nerve regeneration process in the spinal cord.

Methods

Laboratory experimental research with the completely randomized design of a factorial pattern were used in this study. The research was conducted in sample animal at Laboratory Department of Phytochemistry, Universitas Airlangga. The data were taken at Pathology Anatomy Faculty of Medicine, Universitas Airlangga - Dr.Soetomo Teaching Hospital during September 2014 to February 2015. The subjects were divided into four groups: control group, treatment group 1, treatment group 2, and treatment group 3. The experimental unit was a 2-3-month-old Wistar male rats that weigh 200-300 grams and in a good health. Animal health can be observed from the movement that quite agile, not lethargic, clean skin and no injuries, bright eyes and not wistful. Total sample was 12 rats.

The study procedure consisted of acclimation, peripheral nerve lesion model, estrogen preparation test, progesterone, and estrogen-progesterone combination and examination of BDNF spinal cord expression. In the acclimation of weighted, included in a complete randomized treatment group, acclimatized for seven days were fed and drank ad libitum; Furthermore, in peripheral nerve model models using a total transect

model, starting with a skin incision as high as the major trochanter, then the incision is extended in the distal direction, followed by a muscle-splitting incision. After nerve immobilization, the transaction model (neurotmesis) is performed using scissors, at the lowest possible level, just above the terminal nerve branching. The next step, Proximal and distal from the neural pieces was then reconnected end to end with simple interrupted suture stitches of two stitches on the epineural layer. After that, the skin was closed and stitched, in the control group, the rats was operated on the same part but not administered the drug preparation.

The examination of estrogen preparations, progesterone, and a combination of estrogen and progesterone. The administration of the drug was administered over a period of 28 days with a pause between the administration of the drug preparation for three days. The experimental group 1 (P1) was injected with an estrogen preparation, the treatment group 2 (P2) was injected with progesterone preparation, and treatment group 3 (P3) was injected with estrogen and progesterone preparations parallel to the leg then pushed through the abdominal wall into the peritoneal cavity. While the control group injected 0.9% NaCl and treated for 28 days; subsequent examination of BDNF expression of the spinal cord on the 29th day was taken by a sample of spinal cord tissue. Rats were given anesthesia using eter before L4 - L6 intumesensia lumbosakral tissues were taken. These area consists of ischiadicus neuron. These tissues were send to the Pathology Anatomy Faculty of Medicine, Universitas Airlangga - Dr.Soetomo Teaching Hospital for further examination of the BDNF expression of spinal cord using IHC. This study has been approved ethically by the Medical Research Ethics Committee of Faculty of Medicine, Universitas Airlangga Surabaya (177/EC/KEPK/FKUA/2017).

The collected data is analyzed by using the Shapiro-Wilk test. When using normal data distribution, the data were analyzed by parametric test, i.e., the factorial variance analysis (ANOVA) to determine the potency of estrogen, progesterone, and the combination of both, by comparing each estrogen, progesterone and combination group with the control group. The results are said to be significant if the obtained price $p < 0.05$, however, if obtained abnormal data distribution, then the data will be analyzed by non-parametric test Kruskal-Wallis.

Results

The total number of samples included in this study were sixteen rats which then divided into four groups. So for each group both treatment and control consists of four rats. After treatment and painting, an evaluation is performed on all preparations and the whole preparation is well-grounded, neuron cells can be well differentiated, whether expressed either BDNF or non-BDNF.

In this study, a spinal medallion preparation was performed using IHC technique to assess BDNF expression in neurons of the ischial nerve cells found in the spinal cord. Assessment of BDNF expression was done on the gray matter of the spinal cord, using a microscope with 400 times magnification. BDNF expression was examined in three fields of view, calculated in the percentage of the number of neuron cells that are colored by the total number of neuron cells in each field of view. The results of BDNF IHC dye preparation can be seen in Figures 1.

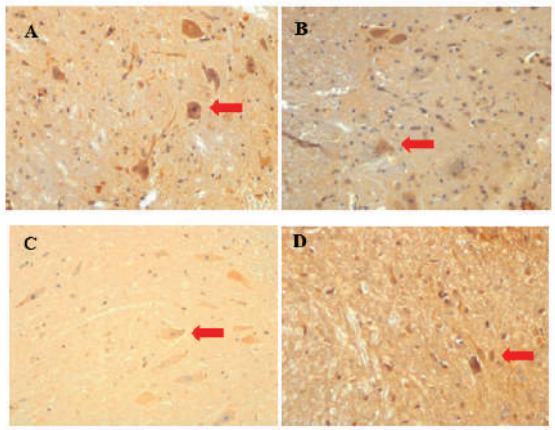


Figure 1. The results of colored IHC BDNF in rat's spinal cord: (A) control treatment; (B) estrogen therapy; (C) progesteron therapy; (D) estrogen and progesteron combination therapy

After the painting and interpretation of BDNF expression results in spinal cord rats try. In the control group, BDNF expression of spinal cord neuron cells was 93.0±14.0. In the treatment group, BDNF expression was obtained after estrogen therapy was 77.25±19.19, progesterone was 84.5±20.61, and in the combination of estrogen and progesterone was 77.75±16.54. The first data analysis was performed to test the normality of data distribution by using Shapiro-Wilk test, control group

obtained p = 0.001. Thus it can be concluded that the data has abnormal distribution, and continued with the non-parametric test.

Comparison of BDNF expressions between groups

Table 1 showed the results of the BDNF expression assessment of each group. To compare inter-group BDNF expression, Kruskal-Wallis non-parametric test was used to determine the effect of estrogen, progesterone, or estrogen and progesterone combination compared with the control group on BDNF expression on neuron cells in the spinal cord. From the result of Kruskal-Wallis test obtained p-value equal to 0.316, meaning there is no significant difference between treatment group. Thus it can be concluded from the test, that statistically no effect of giving estrogen, progesterone, or combination of estrogen and progesterone to the expression of BDNF cells of spinal cord neurons in this study.

Table 1. Comparison of BDNF expressions between groups

Groups	n	BDNF Expression (%)					P
		Mean	SD	Medium	Min	Max	
Control	4	93.0	14.0	100	72	100	0.316
Estrogen	4	77.25	19.19	81	54	93	
Progesterone	4	84.50	20.61	93	54	98	
Combination	4	77.75	16.54	75	60	100	

BDNF: Brain-derived neurotrophic factor

Discussion

Based on data obtained from the results of this study, it was obtained that giving estrogen, progesterone, or combination therapy did not statistically increase the expression of BDNF cells of spinal cord neurons. In the control group, BDNF expression of spinal cord neuron cells was quite high (93.0±14.0%), while in the treatment group, BDNF expression was obtained after estrogen therapy, progesterone therapy, and combination therapy showed lower number. From these data, it can be seen that on estrogen therapy, progesterone, or combination of both the relatively lower BDNF expression, although not statistically significant.

Previous research on the effect of estrogen and progesterone therapy on mRNA and BDNF protein levels in rat brain, in which we found the significant increase in mRNA and BDNF protein levels in some areas of the rat brain after estrogen and progesterone hormone therapy[15]. Low level of plasma estrogen also increases Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) and Osteoprogeterin (OPG)[16]. In this study, BDNF was evaluated by quantitative method by measuring BDNF mRNA by Reverse Transcription Polymerase Chain Reaction (RT-PCR) method, and BDNF protein content by Enzyme-linked Immunosorbent Assay (ELISA) method, while in this study, BDNF was evaluated using anatomical pathology examination with IHC painting, which was more subjective.

There was a significant increase in mDNA and BDNF levels after estrogen and progesterone hormone therapy in the hippocampus and piriformis cortex areas, whereas, for the frontal and olfactory cortical area, the study itself found no significant effect[17]. It can be concluded that the effect of estrogen and progesterone hormone therapy on elevated levels of mRNA and BDNF protein was specific depending on which area being examined. This may also apply to peripheral nerves, although in other studies estrogen and progesterone therapy has been shown to increase levels of mRNA and BDNF proteins in certain areas of the brain, but not necessarily when applied to the spinal cord.

Expression of BDNF, NT3, and NT4 on nerves and muscles, after being treated with various ischiadic nerve injuries (neuropraxia, axonotmesis, and neurotmesis). From the research, it was found that in the treatment of neurotmesis BDNF mRNA levels of iskiadicus nerve increased significantly more than 10-fold on days 7 to 28 when compared with the control group that was not given treatment[18]. From these findings, it can be concluded that BDNF levels will increase in peripheral nerve injury conditions, in this case, neurotmesis even without therapy. This may explain the findings of this study in the control group where only given peripheral nerve treatment (neurotmesis) without hormonal therapy, on day 29 after treatment was obtained results of high BDNF expression of 93.0 ±14.0 %, in which the results were not statistically significant when compared with the group with estrogen, progesterone, or combination therapy. As with other studies also using quantitative methods with RT-PCR with more measurable results when compared with IHC method used in this study[17,18].

Conclusion

The administration of estrogen, progesterone, or combination of both did not increase BDNF expression. The effect of estrogen and progesterone hormone therapy on elevated levels of mRNA and BDNF protein was specific depending on which area being examined. The BDNF levels will increase in peripheral nerve injury conditions such as neurotmesis.

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Conflict of Interest: There is no conflict of interest.

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In Silico Study : *Phyllanthus Niruri L* as Immunomodulator Against Covid-19

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Abstract

In December 2019, a mysterious case of pneumonia was first reported in Wuhan, Hubei Province. The source of transmission of this case is still unknown, but the first case was linked to the fish market in Wuhan. From 18 December to 29 December 2019, there were five patients treated with Acute Respiratory Distress Syndrome (ARDS). 2 From 31 December 2019 to 3 January 2020 this case increased rapidly, marked by 44 cases reported. In less than a month, the disease has spread to other provinces in China, Thailand, Japan and South Korea. This virus can be transmitted from human to human and has spread widely in China and more than 190 other countries and territories. On 12 March 2020, WHO announced COVID-19 as a pandemic. As of March 29, 2020, there were 634,835 cases and 33,106 deaths worldwide. While in Indonesia, 1,528 cases were confirmed with COVID-19 and 136 deaths. Phyllanthus niruri L (meniran) is one type of immunostimulator that can improve the immune system in animal experiments and humans. This study aims to determine Phyllanthus niruri L as an immunomodulator for Covid-19. From the results of research conducted that Phyllanthus niruri L can improve the activities and functions of several non-specific immune system components and specific immune systems, both soluble and cell-related forms. The conclusion from the results of this study is Phyllanthus niruri L as an immunomodulator for Covid-19.

Keywords: *Phyllanthus niruri* L, Immunomodulator, Covid-19

Introduction

In December 2019, a mysterious case of pneumonia was first reported in Wuhan, Hubei Province. The source of transmission of this case is still unknown, but the first case was linked to the fish market in Wuhan. From 18 December to 29 December 2019, there were five patients treated with Acute Respiratory Distress Syndrome (ARDS). 2 From 31 December 2019 to 3 January 2020 this case increased rapidly, marked by 44 cases reported. In less than a month, the disease has spread to other provinces in China, Thailand, Japan and South Korea [1,2].

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The sample studied shows the etiology of a new coronavirus. Initially, the disease was temporarily named as the 2019 novel coronavirus (2019-nCoV), then WHO announced a new name on February 11, 2020 namely Coronavirus Disease (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) virus.). This virus can be transmitted from human to human and has spread widely in China and more than 190 other countries and territories. On 12 March 2020, WHO announced COVID-19 as a pandemic. As of March 29, 2020, there were 634,835 cases and 33,106 deaths worldwide. While in Indonesia there have been 1,528 cases tested positive for COVID-19 and 136 cases of death[3,4,5].

Phyllanthus niruri L (meniran) is one type of immunostimulator that can improve the immune system in animal experiments and humans. The administration of Phyllanthus niruri L extract can increase the activity

and function of several components of the nonspecific immune system and specific immune system, both dissolved (humorous) and cell-related forms. Effects of nonspecific immune responses include increased phagocytosis and macrophage and neutrophil chemotaxis movements, NK cell cytotoxicity and complement hemolysis activities. Cellular immunity can increase the proliferation of T lymphocytes by increasing the secretion of TNFa, IFNg and IL-4, and decreasing the secretion of IL-2 and IL-10, whereas in humoral immunity, this drug can increase the synthesis of immunoglobulin M (IgM) and IgG, play a role in against infection[6,7,8,9,10,11] This study aims to determine Phyllanthus niruri L as an immunomodulator for Covid-19.

Materials and Methods

Phyllantus niruri L. Compound and Protein Target Preparation

The Bioactive component of Phyllantus niruri L. was obtained from a literature review and the component that could be identified was Cathechin, and Quercetin was then downloaded via pubchem in .sdf format, then changed the extension to .pdb using Discovery Studio Visualizer and the energy was minimized using Autodock.

The crystal structure of the target protein is TNF-alpha (PDB ID: 2AZ5), IL-1 (PDB ID: 2NVH), IL-6 (PDB ID: 1P9M) and iNOS (PDB ID: 1NS1). The protein is the main protein that plays a role in the signaling of inflammation that occurs in the case of Covid-19 and causes cytokine storm, causing symptoms of Acute Respiratory Distress Syndrome. The things that can interfere with docking results are water molecules because they can increase resolution and must be eliminated by using Discovery Studio Visualizer.

Molecular Docking Analysis

The docking was carried out by Pryx and the results of docking is in Kcal/mol. Docking is performed on all protein targets with different ligands. Then the results of Molecular Docking are analyzed and visualized the results through PyMOL and LigPlot+ to determine binding pocket and residual amino acids that bind into ligand.

Results

Molecular docking was carried out using PyRx software on TNF-alpha, IL-1, IL-6 and iNOS proteins that had been prepared before and using Phyllantus niruri L. bioactive components, namely Cathecin and Quercetin which had also been prepared before. Molecular docking is carried out in certain grid boxes with different sizes for each protein. For TNF-α use a gridbox (x: y: z) with centers -14,286: 65,875: 26,349, ntps 126: 126: 126, and spacing 0.644. For IL-1 center 46,251: 14,257: 69,836, ntps 100: 114: 124, and spacing 0.481. In IL-6 protein center -57,152 175,358 45,212, ntps 126 126 126 and spacing 1,000 and finally in iNOS Protein using center 52,683 47,596 52,073, ntps 126 126 126 126, and spacing 1,000.

The strength of molecular docking can be seen from the value of binding affinity expressed by Gibbs free energy (ΔG). The binding energy is strongly influenced by the amino acid residues of the target protein which bind and react hydrophobically to the ligand and follow the energy equation as follows:

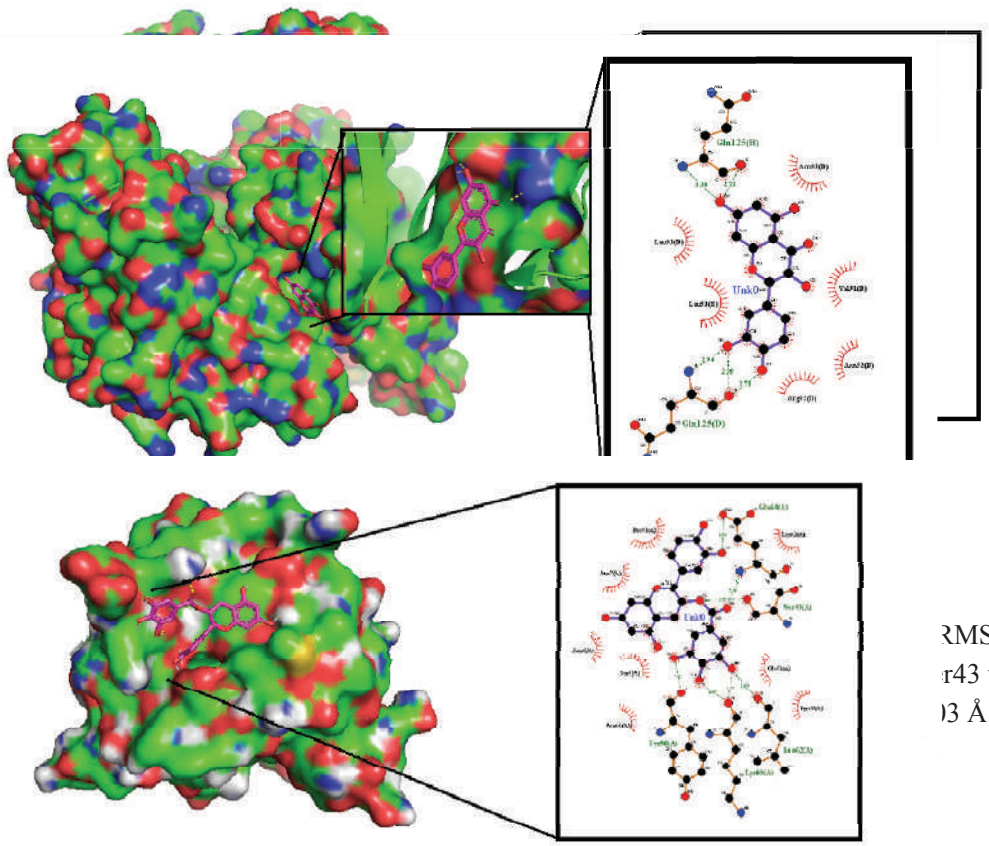
$$\Delta G_{binding} = \Delta G_{Gauss} + \Delta G_{Repulsi} + \Delta G_{HBond} + \Delta G_{Hydrophobic} + \Delta G_{Torsion}$$ [12].

The more amino acid residues that interact with the ligands, the smaller the value of ΔG will be (negative) and the reaction will take place spontaneously and bind very strongly so that the inhibitory effect it creates will also be stronger [13].

To see the validity of the docking results, the value of the root mean square deviation (RMSD) can be used to assess the error rate or linearity of a compound to be tested with a compound that is made as a standard. The smaller the RMSD value, the less error is considered. So the RMSD value <2.00 Å is considered the validation measurement standard value [14].

Table 1. The binding affinity of molecular docking between the bioactive compound Phyllantus niruri against the proinflammatory cytokine target protein.

Bioactive Compound	Target protein	ΔG (Kcal/mol)	RMSD (Å)
Cathecin	TNF-α	-8,3	2,383 Å
	IL-1	-7,3	1,682 Å
	IL-6	-8,6	2,014 Å
	iNOS	-10	1,037 Å
	TNF-α	-8,3	1,067 Å
			1,869 Å
			1,729 Å
			1,861 Å



l with RMSD 2,383
ıd forms a hydrogen
125 (B) with a bond
SD 1.067 Å with the

RMSD of 1.682 Å and for
r43 with bond distances of
)3 Å and in quercetin binds

Figure 2. The docking result of Cathecin (Top) and Quercetin (Bottom) with IL-1

From the IL-6 obtained the binding affinity for Cathecin was -8.6 Kcal/mol with RMSD 2.014 Å and -8.1 Kcal/mol for Quercetin with RMSD 1.729 Å. The cathecin have hydrogen bonds with Phe168 with a distance of 3.09 Å, Phe134 with 2.81 Å, 3.16 Å, 3.12 Å, and 2.85 Å. Gln135 with 2.76 Å, Leu64 with 3.13 Å and Leu62 with 2.76 Å. Where the quercetin binds to Phe136 with a bond distance of 2.97 Å, Ala152 with 2.71 Å and 2.93 Å, Gly127 with 3.14 Å, Arg128 with 2.84 Å and Thr130 with 2.98 Å..

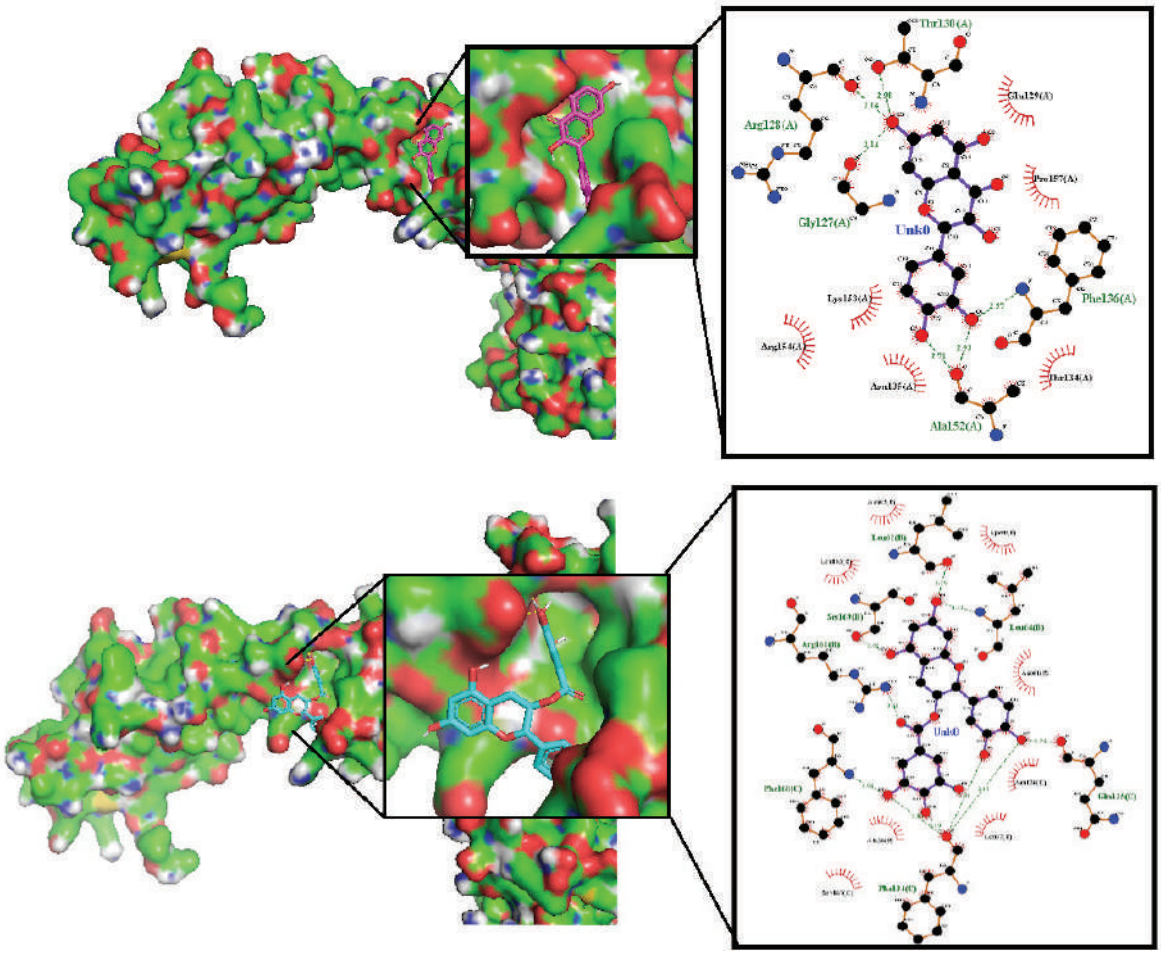
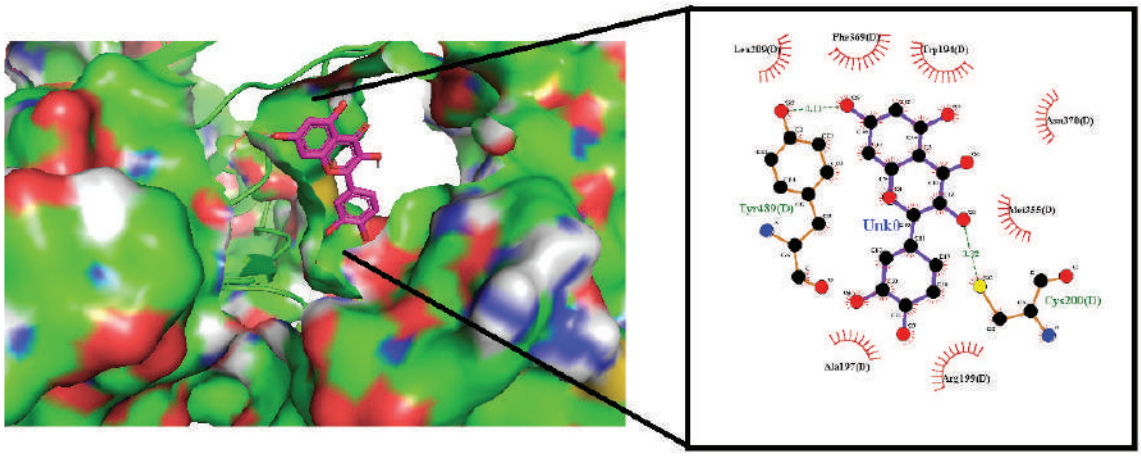


Figure 3. The docking result of Cathecin (Top) and Quercetin (Bottom) with IL-6

In iNOS, the binding affinity of -10 Kcal /mol was obtained for Cathecin with RMSD 1,037 Å and -8.3 Kcal / mol with RMSD 1,861 Å. For cathecin it have hydrogen bonds with Trp372 with a bond distance of 3.02 Å, Asn370 with 2.75 Å and Tyr489 with 2.70 Å. In quercetin it binds with Cys200 with 3.32 Å and Tyr489 with distance 3.13 Å.



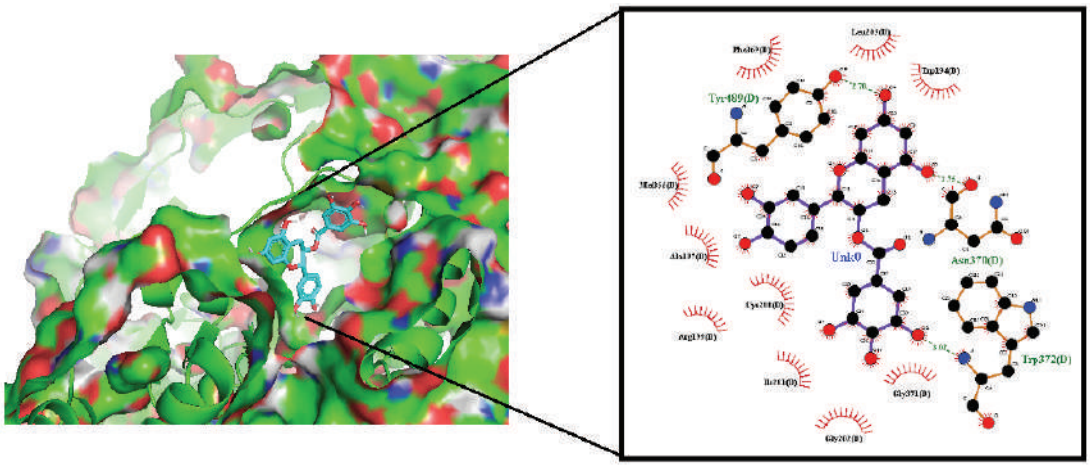


Figure 4. Hasil docking Cathecin (Atas) dan Quercetin (Bawah) dengan iNOS

Discussion

In the activation of the immune system, macrophages and monocytes produce Tumor Necrosis Factor-Alpha (TNF-α) and will bind to TNFR1 or TNFR2 and will stimulate the formation of degenerative protein caspase 3 and cause apoptosis of cells because they have death domains in the carboxyl terminal[15]. Overexpression of TNF-α will cause excessive cell apoptosis and stimulate the process of excessive inflammation [15]. Cathechin and quercetin bind to TNF-α with binding energy -8.3 Kcal / mol and inhibit the expression of TNF-α and inhibit excessive apoptosis of cells.

Cytokines produced by Presenting Cell Antigens such as macrophages are very important in the stimulation of T cells and B lymphocytes. Helper T cells (Th) will produce cytokines IL-2, IL-6 and IL-1 which are important in celullar immunity and humoral immunity [16]. IL-1 as a proinflammatory cytokine that plays a role in acute and chronic inflammation. Responsible for the febrile process and also sepsis and activation of neutrophils in the non-specific immune system [16]. Cathecin and quercetin in Phyllantus niruri bind to IL-1 with binding energy -7.3 Kcal / mol and -7.1 Kcal / mol. Both of these bioactive compounds inhibit the overexpression of IL-1 and have anti-inflammatory effects.

Activation of IL-6 as a proinflammatory cytokine will activate the inflammatory process and autoimmune response and generally occurs in the pathogenesis of immune mediated arthritis [17]. Cathechin and quercetin bind to IL-6 with binding energy -8.6 Kcal / mol and -8.1 Kcal / mol and have anti-inflammatory effects.

Inducible nitric oxide synthase (iNOS) functions to synthesize Nictric Oxide (NO) from L-Arginine. iNOS plays a role in NO biosynthesis preventing bacterial growth and inhibiting inflammation by inhibiting T cells [18]. Excessive NO production will induce inflammatory diseases [18]. Cathecin and quercetin bind to iNOS with bond energies of -10 Kcal / mol and -8.3 Kcal / mol. These compounds will inhibit the production of NO thereby preventing the effects of excessive inflammation.

Conclusion

Catechin and Quercetin in Phyllanthus niruri can inhibit the expression of TNF-α, IL-1, IL-6, and iNOS thus inhibiting the process of excessive inflammation and functioning as an immunomodulator

Conflict of Interests: The authors declare that they have no conflict of interest.

Source of Funding : Self (the research with independent costs)

Ethical Approval : This study was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia (No. 2.KE.142. 08. 2018).

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Characterization of Aflatoxin B1 Biomarkers as Preference for Antitoxin Candidates for Biosensors for Halal Food Supply

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Abstract

The fungus grows on various types of food and feed, its growth will cause damage, including damage to flavor, color, softening, and the formation of toxic compounds. The damage is because the fungus produces extracellular enzymes that break down certain compounds in food and feed, and produce toxic secondary metabolites, called mycotoxins. Fungal contamination requires serious attention, not only because it causes damage to food and feed but is related to the potential of the fungus to produce mycotoxins and form conidia that are pathogenic or allergic, mycotoxins produced by various types of fungi, each of which has varying toxicity, in general chronic causes mycotoxicity. Mycotoxins have caused several types of diseases in humans and animals. Aflatoxin B1 (AFB1) is a potential toxin as a hepatocarcinogen. Eating foods tainted with AFB1 can cause acute (short term) and chronic (moderate or long term) poisoning; chronic disorders such as disorders of the central nervous system, cardiovascular and lung systems, and digestive tract. Some mycotoxins are carcinogenic, estrogenic, mutagenic, teratogenic, and immunosuppressive. The purpose of this study is the characterization of Aflatoxin B1 Biomarkers as Preference for Antitoxin Candidates for Biosensors for Halal Food Supply. The method of this research is AFB1 induction in mice, observed for 14 days, on the 14th day liver organs were taken. The liver is prepared into organ extracts, analyzed using IHC. From the research results obtained protein expression in the liver organs of experimental animals by the IHC method. Further research is needed as an antitoxin and biosensor.

Keyword: *Aflatoxin B1, MAPK1, AKT1, Antitoxin.*

Introduction

Pathophysiologically Aflatoxin B1 (AFB1) as a carcinogenic material arises because of chronic exposure to hepatocyte cells that cause epigenetic and genetic changes that lead to the induction of oncogenic proteins and/or activation of tumor suppressor genes. This pathogenesis occurs in four important signaling pathways (WNT, TGFß, PI3K / Akt, and RAF / MEK / ERK)[1].

The RAS / RAF / MEK / ERK pathway plays an important role in the development of liver cancer. Similar to many signaling networks, this pathway is activated through a variety of mechanisms. RAS activation is initiated by the binding of extracellular signaling compounds such as hormones and growth factors with VEGFR and PDGFR. The bond between signaling compounds will cause a phosphorylation reaction in the RAS and initiate a series of cascades that lead to the activation of ERK for proliferation and differentiation and angiogenesis. Certain mutated components or Ras-Raf-MEK-ERK / MAPK overexpression are increasingly being studied in carcinogenesis. The abnormal target protein signaling pathway contributes to cell proliferation, differentiation, survival, and uncontrolled cell apoptosis is a biomarker of carcinogenic processes [2].

The PI3K pathway is activated through various mechanisms such as the binding of IGF-1 to IGFR so that it starts the P13K signaling series and activates AKT which directly influences mTOR which will regulate proliferation and angiogenesis[1].

Hepatocellular carcinoma (HCC) is a malignant

tumor that attacks the liver. The prevalence of malignant tumors is very high, ranked fourth globally as cancer with an incidence rate of 5.3% compared to other cancers[3]. Barriers from HCC signaling compounds such as MAPK1 (ERK) and AKT using special interventions can inhibit the proliferation and angiogenesis of HCC and reduce the concentration of these compounds due to excessive expression due to genetic and epigenetic factors[4].

Based on the results of in silico research, there is a super-expression of AKT-1 and MAPK-1 evidence as a molecular mechanism pathway for AFB-1 carcinogenic material in hepatocyte cells. Analysis of amino acid residues, against AKT-1 and MAPK-1, have residues at the Binding site that affect receptors on oncogenic proteins[5].

From this background, this study aims to test the exposure of AFB1 to the expression of AKT1 and MAPK1 target proteins using the Immunohistochemistry (IHC) method to determine the reaction between receptor-ligand (antigen-antibody) complexes formed in vivo tests.

Material and Methods

This study used an experimental laboratory research design with experimental animals (In vivo) with an examination of the immunohistochemical method.

Results and Discussion

Immunohistochemistry (IHC).

Immunohistochemical examination is intended to determine the expression of AKT 1 and MAPK1 (ERK) on cells of mice hepatocytes (Mus muscullus). AKT 1 and MAPK 1 (ERK) expression score data were obtained using the modified Remmele method[6], Remmele scale index (Immuno Reactive Score / IRS) is the result of multiplying the percentage score of immunoreactive cells with the color intensity score on immunoreactive cells. Data for each sample is the average value of the IRS observed in 5 (five) Field View (LP) at 400x magnification.

Comparison of AKT 1 expression (chromogen brown) in hepatocyte (arrow) cells between treatment groups. AKT1 is expressed in both the cytoplasm and the nucleus of hepatocyte cells.

The results of the examination showed that the expression of AKT1 in the treatment group giving AFB1 (3 mg / Kg BW) (P1) seemed stronger than the control treatment group (P0).

Comparison of MAPK1 (brown chromogen) expression in hepatocyte (arrow) cells between treatment groups. MAPK1 is expressed in both the cytoplasm and the hepatocyte cell nucleus. The results of the examination showed that the expression of MAPK1 in the treatment group Giving AFB1 (3 mg / Kg BW) (P1) seemed stronger than the control treatment group (P0).

The data obtained in the form of a Remmele Scale Index score (Immuno Reactive Score / IRS) results from the multiplication score of immunoreactive cells with a color intensity score, were analyzed by Kruskal Wallis followed by the Z Test, statistical analysis using the IBM SPSS Corp. computer statistical program. Real 21.

Statistical analysis, data are presented as mean (mean) ± standard error. Differences between groups were assessed for statistical significance using the Kruskal-Wallis test or the Multiple comparison test with the Z test (Multiple comparisons by Z test), depending on data distribution. P values <0.05 were considered statistically significant.

The data obtained indicate the number of assessment scores with the Immuno Reactive Score (IRS) on the expression of AKT 1 under each treatment condition (mean ± SEM of six replications). Statistically significant difference from controls (* $p<0.05$; ** $p<0.01$).

The data obtained shows the number of assessment scores with Immuno Reactive Score (IRS) on the MAPK 1 expression under each treatment condition (mean ± SEM of six replications). Statistically significant difference from controls (* $p<0.05$; ** $p<0.01$).

The highest MAPK1 expression in AFB1 (P1) treatment, with a mean rank of 18.41 ± 1.4108 showed a significant difference ($p<0.05$) with a control treatment (P0) with a mean rank of 8.75 ± 3.5137. It was also found that the treatment was significantly different ($p<0.05$).

Based on the results of testing using Kruskal Wallis can provide information that the administration of

AFB1 can increase the expression of AKT 1 and MAPK 1 on mouse hepatocytes induced by AFB1 in super expressions Vivo administration so that it can be used as individual biomarkers exposed to AFB1.

The immunohistochemistry technique is a method that aims to identify specific cells based on antigenic components or cellular products with complex antigen-antibody reactions. Immunohistochemistry is used as a basis for diagnosis and identification of cell types based on cytomorphology. This examination is often done in cases of tumors or malignancies[7,8]. Also, immunohistochemistry is often used for research to determine the distribution and location of biomarkers or expressed proteins in various body tissues[9].

Immunohistochemistry is a combination of histological or cytological examination with immunology. The method of coloring substances or active ingredients in tissues uses the basic principle of immunology, namely by binding the active ingredients or antigens on the specific active side by the active ingredients or antibodies. The results of this reaction can be identified in the specimen if the antibodies are bound by a marker that can be in the form of fluorescein, enzymes, particulate matter, or isotopes that can be visualized, to indicate the presence of active ingredients in the tissue. Active ingredients can be in the form of proteins, carbohydrates, nucleic acids, fats, other natural ingredients and synthetic materials[10,11].

The immunohistochemical examination has a high ability to separate, select, and be specific. This examination is to detect the presence of antigens, this is due to the presence of specific bonds between antigens and antibodies [12,13,14.]

The results of this study obtained a reaction to AKT1 and MAPK-1 based on immunohistochemistry. Also, based on the results of Scoring with Immuno Reactive Score / IRS showed the expression of MAPK 1 in each treatment.

This shows that if AFB1 enters the body it is predicted to potentially influence the activation of the AKT1 and MAPK1 oncogenic protein activation, so that it triggers the regulation of the underlying process (down-regulation) so that it can trigger cancer cells in the hepatocellular carcinoma (HCC). Capsaicinoids include nordihydrocapsaicin, capsaicin, dihydrocapsaicin, nor capsaicin, homodihydrocapsaicin, homocapsaicin, nonivamide[15]. One of the most important compounds in chili is capsaicin, capsaicin is a secondary metabolite of the chili plant. In the pharmaceutical field in addition to relieving pain or pain, capsaicin is also known to have anticancer activity because it inhibits certain oncogenic proteins[16]. AFB1 has binding energy that is more stable compared to other proteins so it allows the formation of the AFB1-AKT1 molecule complex. It is predicted that AKT1 is one of the oncoproteins so that there is an AFB1 barrier indirectly interfering with activation of the AKT1 and MAPK1 proteins.

Based on the results of in silico research, super-expression of AKT-1 and MAPK-1 occurs in AFB1-induced cells, this shows evidence as a molecular pathway mechanism and can be used as a marker as making antitoxin (anti-AFB1) against AFB-1 as an effort in searching biosensors in the context of halal and thoyib food products.

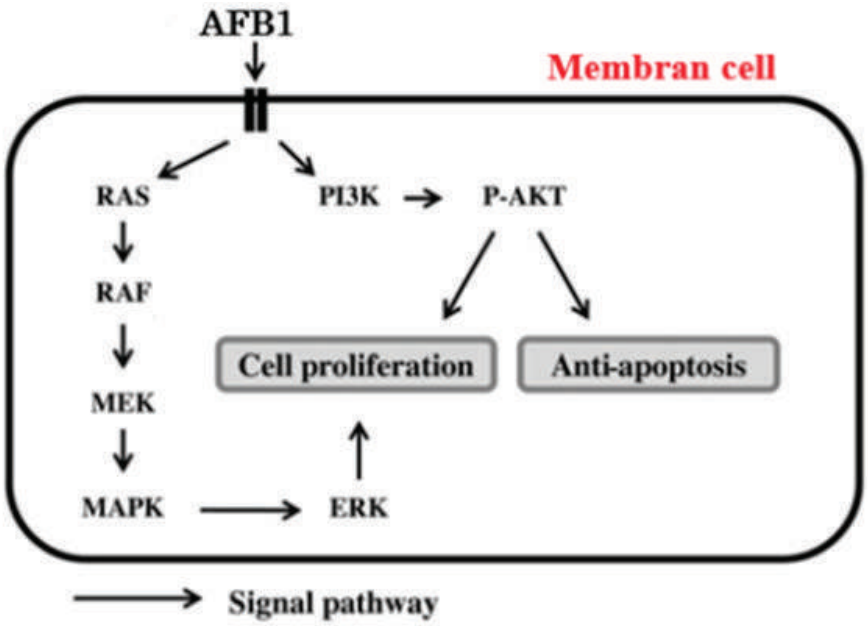


Figure 1. Schematic diagram of the cellular mechanism AFB1 in the RAS / RAF / MAPK pathway and the PI3K-AKT pathway that occurs super expression[17] (*modification*).

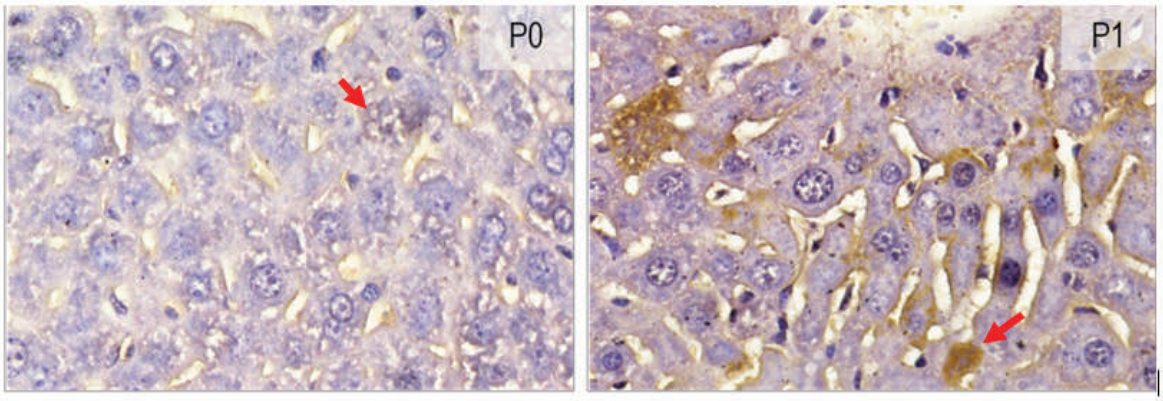


Figure 2. Immunohistochemical staining, 1000x magnification; microscope Nikon H600L; DS Fi2 300 megapixel camera.

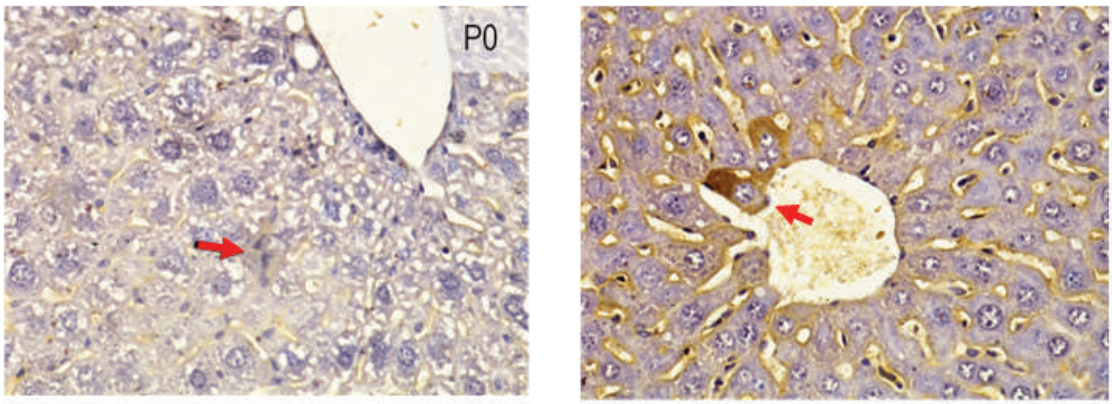
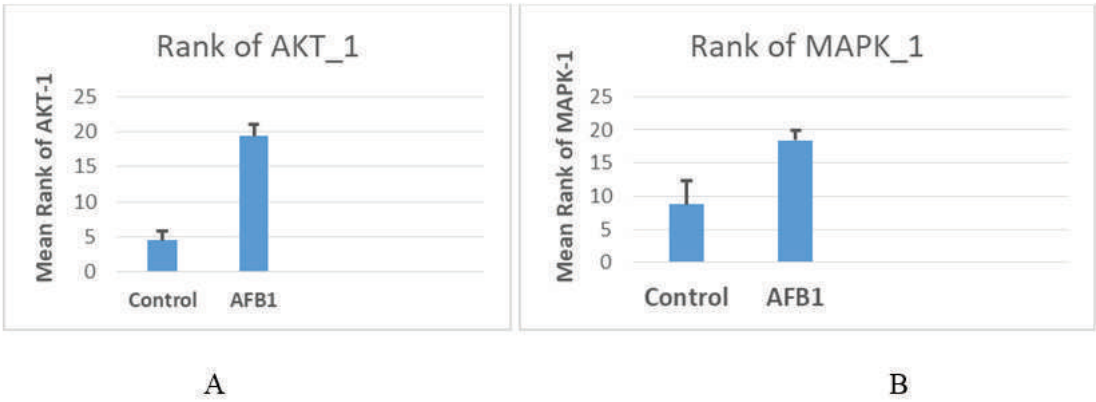


Figure 3. Immunohistochemical staining, 1000x magnification; Nikon H600L microscope; DS Fi2 300 megapixel camera.

Table 1. AKT-1 and MAPK-1 Rank Table

				AKT_1	MAPK_1	Rank of AKT_1	Rank of MAPK_1
P	Kontrol	1		2.2	4.6	2.0	6.0
		2		1.6	4.4	1.0	4.5
		3		4.0	2.6	10.0	2.0
		4		2.6	2.4	4.0	1.0
		5		2.4	8.0	3.0	21.5
		6		3.6	6.8	7.0	17.5
			Mean	2.733	4.800	4.500	8.750
		Total	Std. Error of Mean	.3676	.9136	1.3844	3.5137
	AFB1	1		6.4	6.4	20.0	15.5
		2		7.8	6.2	23.5	14.0
		3		6.0	8.2	19.0	23.0
		4		5.8	6.8	18.0	17.5
		5		4.2	7.0	12.0	19.0
		6		7.8	8.0	23.5	21.5
			Mean	6.333	7.100	19.333	18.417
		Total	Std. Error of Mean	.5554	.3376	1.7401	1.4108



A B

Figure 4. Average data diagram (mean) ± standard error AKT 1 (A) and average data (mean) ± standard error MAPK 1 (B) with group differences for significance statistics.

Conclusion

In conclusion, our data show that AFB1 can synergistically react through the anti-apoptotic AKT signaling pathway and MAPK 1 to potentiate antiproliferative actions that can promote apoptosis, so that it is suspected to have potential as a marker in the manufacture of antitoxin candidates in the search for biosensors in the framework of biosensor food products Halal and Thoyib, a finding that demands further clinical testing.

Suggestion: Conducting further tests and standardization of formulations in the manufacture of antitoxin as a biosensor against AFB1.

Ethics and Consent: All applicable institutional guidelines for the care and use of animals were followed. This research received ethical clearance approval from Animal Care and Use Commitee, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya, East Java, Indonesia (No. 1.KE..198. 12. 2019).

Confilct of Interests: The authors declare that they have no conflict interests

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Testing the New Parameters affecting The Outcome of Extracorporeal Shockwave Lithotripsy for Upper Ureteric Stones

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Abstract

Background: Extracorporeal Shock wave lithotripsy (ESWL) is widely used in treating patients with ureteral stones because it is effective, safe, and noninvasive. Based on factors such as size and the location of stones, there is a significant variation in the overall stone-free rate (SFR).

Aim of the study: To evaluate the effect of ureteral wall thickness (UWT), stone attenuation, the time from first attack of pain till first session of ESWL and stone/ rib density on the outcome of SWL in the treatment of upper ureteral stones (UUS).

Patient and methods: A prospective study when 127 patients with radio-opaque UUS ranging from 7 to 20 mm and treated by ESWL were included in this study. The effect of (stone/ 12th rib) density by KUB, ureteral wall thickness by NCCT and the time from first attack of pain till first ESWL session was studied.

Results: The overall successful fragmentation was 75.5%, with the overall success rates in the low density (LD) and high density (HD) groups were 83.8% and 52.94%, respectively. The average number of SWL sessions needed in the two groups for success was 1.9 compared with 2.7 sessions ($p<0.05$). For stones < 10 mm; those with ureteral wall thickness <3.25 mm have success rate about 90.3% VS 69.7% with ureteric wall thickness > 3.25 mm which is highly significant. Early ESWL within the first 24 hours of acute attack of first pain has successful fragmentation of 85.45%. With significant effect on number of ESWL sessions. The stone free rate reaches 91.1% for stones <10 mm.

Conclusions: The stone free rate is inversely affected by stone /12th rib density ; ureteral wall thickness and the time from first attack of pain till first session of ESWL, were important predictors of successful ESWL.

***Keywords**: **ESWL** Extracorporeal Shockwave Lithotripsy, **SSD** skin to stone distance, **MAV** mean attenuation value, **NCCT** non contrast CT scan, ureteral wall thickness **UWT**, stone /12th rib density (**LD** low density and **HD** high density).*

Introduction

Urolithiasis is a common disease with an increasing prevalence. In the general population, its lifetime risks for men and women are 13% and 7% respectively. After being formed in the renal collecting system, ureteric stones move downward into the urerer. The physiologic narrowings (pelviureteric junction, crossing over the iliac vessels, and the ureteric meatus) of the ureter are areas where stones tend to lodge. [(1)]

The degree of obstruction caused by urinary lithiasis depends on the size of the calculus, urothelial edema, location and the level of impaction where an urgent instrumental method of treatment is sometimes needed. There is still a debate on the best treatment method for ureteral calculi. Some of the varying treatment options include passage of ureteral stent, extracorporeal shockwave lithotripsy (ESWL), expectant management, open ureterolithotomy and ureteroscopy with basket extraction or intracorporeal lithotripsy [(2, 3)].

For ureteric stones that are uncomplicated and moderately sized, ESWL can be used as the first choice of treatment. ESWL is a safe, simple and robust procedure and for cases where stones are resistant to medical treatment without total indication of ureteral drainage, it is usually recommended [4-6]. In addition, ESWL cannot be used to fragment all urinary stones easily because its success rate depends on factors such as stone size, location and composition [7]. Using radiological tools such as kidney-ureter-bladder (KUB) x-ray and non-contrast computed tomography (NCCT), diverse studies have examined how to predict successful treatment by ESWL. Some studies have recently tried to correlate NCCT findings with the treatment success of ESWL.

Patients and Methods

Study design and setting:

This is a prospective study that included 127 patients with radio-opaque upper ureteral stones who referred to lithotripsy unit in Al-Shaheedd Ghazi al Hariri surgical specialties teaching hospital in Baghdad from August 2015 – October 2016, 78 males (average 40 years) and 49 females (average 48years), treated with extracorporeal shock wave lithotripsy (ESWL). All patients included in the study underwent: Ultrasonographic study, kidney-ureter-bladder (KUB) x-ray, computed tomogram (CT) without contrast, coagulation profile, urinalysis and culture, serum creatinine and fasting blood sugar. The night before ESWL, patients were directed to take laxative to help lower the gases in the intestine and promote the localization of stones. Also all patients were given analgesia before lithotripsy and those with positive urine culture treated with antibiotic according to the culture and sensitivity.

Inclusion criteria:

1. Single upper ureteric stone within size (≥7--≤20 mm)
2. First attack of pain.

Exclusion criteria:

1. Previous surgical intervention
2. Any contraindication for ESWL.
3. Stones that pushed back to the kidney when DJ insertion needed.
4. Bilateral or multiple ureteral stones.
5. Structural urinary tract abnormality

The new parameters that was studied in this study including the following:

A- Stone density relative to that of the 12th rib: Based on visual criteria, a density less than or equal to that of the 12th rib was assigned to the lower-density (LD) group while a density greater than that of the 12th rib was assigned to the higher-density (HD) group [8].

B- Ureteral wall thicknessUWT; was measured by NCCT in addition to other parameters like size, skin-to-stone distance (SSD), and the mean attenuation value [10].

C- Time from first attack of pain till first ESWL session: to evaluate the role of early intervention on stone free rate and treatment outcome; with early intervention means less than or equals to 24 hours.

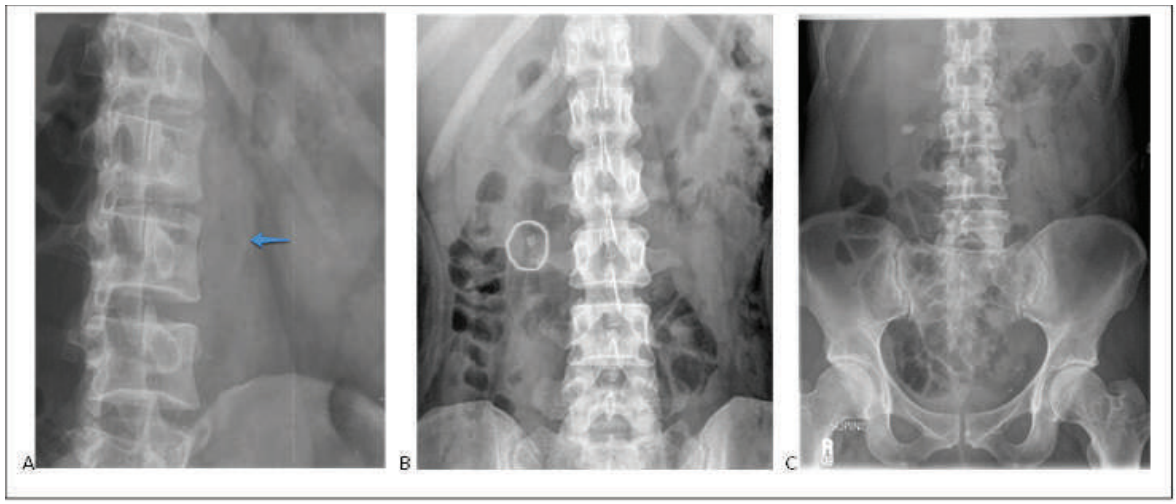


Fig 1: Classification of stone density: lower (A), equal (B), and higher density (C).

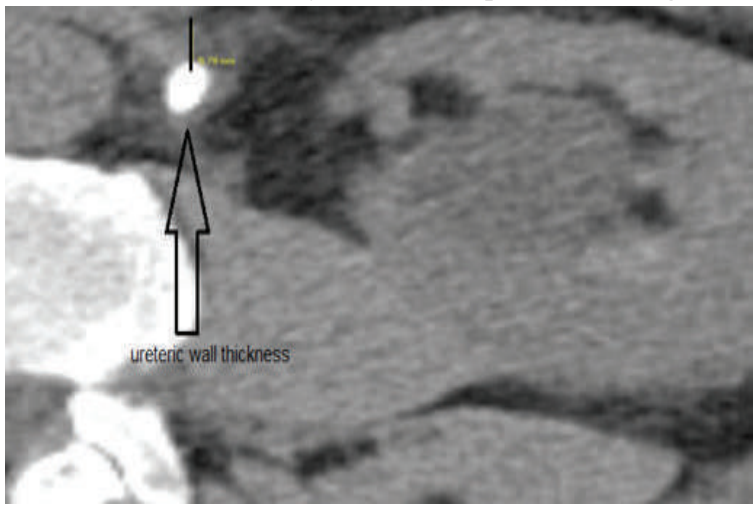


Fig 2: NCCT showing evident increase in ureteral wall thickness (UWT) around the large proximal ureteral stone.

ESWL technique:

After explaining the different treatment modalities and complications, informed consent was taken from the patient.The procedure was done on an outpatient basis. Using **Siemens Lithoskop** lithotripter .The number of shock waves ranges from 2500-3000 shocks in 15.5-16.7 k.v. according to patient tolerance and stone disintegration, with a frequency 2 Hz = 120 pulse/min. **(9)**

The patients were requested to return for follow up with plain film as well as an ultrasound examination after two weeks. This is to enable the detection of stone clearance and fragmentation along with the effect of treatment and upper urinary tract obstruction. A second session is scheduled after 2 weeks if significant (>4mm) fragments were still seen.

Statistical Analysis

Multiple t-test and Fisher's exact tests with two tailed p-value were used to evaluate stone characteristics, NCCT signs and KUB in order to predict the success rate of ESWL. A p-value less than 0.05 was considered to indicate statistical significance. The program used is SPSS version 24.

Results

The overall mean patient age out of the 127 patients evaluated was 42.3 years (24 - 63 years). The overall mean attenuation value (MAV) was 849.72 HU (460-1346) while the ureteral wall thickness (UWT) was 3.25 mm (0.9– 6.6 mm). No significant difference were found

regarding SSD while highly significant for the rest of variables (table 1).

Table 1: Baseline parameters using univariate analysis.

Characteristics	Successful fragmentation	Unsuccessful fragmentation	p-value
Time from 1stpain till 1st ESWL (days) (mean± SD).	3.5± 1.7	4.8±2.1	<0.001
SSD (mean ± SD) cm	11.1±1.8	11.6±1.4	0.160
Mean Attenuation value (HU) (mean, ± SD)	715 ± 132	989 ± 144	<0.001
Stone size, mm(mean± SD)	10.2 ± 2.67	12.5 ± 3.65	<0.001
Stone /12 rib density LD (N, %) / HD (N, %)	78(81.2%)/18(18.8%)	15(48%)/16(52%)	<0.001
Ureteral wall thickness mm.	2.98 ± 0.75	4.21 ± 0.90	<0.001

A total of 96 (75.5%) patients became completely SF after 3 months of follow up with regards to ESWL success rates. The mean stone size and mean attenuation value were 10.2± 2.67 mm and 715±132 **HU** respectively. Of these patients with successful fragmentation, the Low stone/12th rib density was found to have higher stone free rate than High stone /12th rib density stones, (78) patients (81.2%) **LD** versus (18) patients (18.8%) **HD** group respectively with (p=<0.001). Significant differences in the SFR and the number of ESWL sessions in patients with stone size less than or equal to 10 mm were found between both groups in the analysis of the subgroups divided by stone size (Table 2). In the two groups, the mean of ESWL sessions required for success were 1.5±0.7 in the L.D compared with 2± 1in the **HD** group.

Table 2: Comparison of treatment outcomes between low density and high density groups in accordance with stone size.

Stone Size	LD (n=93)	HD (n=34)	P value
Total treatment success	78 (83.87%)	18 (52.94%)	<0.001
≤ 10 mm (n=105)	81	24	
Ø treatment success	70 (86.4%)	16 (58.3%)	0.0368
Ø no. of ESWL session	1.50±0.7	2±1	0.0339
>10 mm (n=22)	12	10	
Ø Treatment success	8 (58.3%)	2 (20%)	0.04271
Ø no. of ESWL sessions	2.50 ±0.75	3.50±0.5	0.1185

Evaluation of the Ureteral wall thickness **(UWT)** and time from the first attack of pain was found to be significantly different in patients with successful fragmentation from that in the failed group; with UWT (2.89±0.75 vs. 4.2±0.9 mm) and time till first ESWL session (3.5±1.75 days vs. 4.8±2.1) respectively, with significant difference in the number of ESWL sessions needed especially in stones ≤ 10 mm as in tables (3 and 4).

Table 3: **Distribution of stones according to their size and the time from first attack of pain until the first session of ESWL**.

	≤ 24 hrs from onset of 1st attack of pain	**>24 hrs after onset of 1st attack of pain**	**P - value**
≤10 mm (n=105)	45	60	
Successful (total 86)	41 (91%)	45 (75%)	0.0415
NO. of ESWL sessions	1.75±0.5	2±0.5	0.0230
> 10 mm (n=22)	10	12	
Successful (total 10)	6 (60%)	4 (33.3%)	0.3913
NO. of ESWL sessions	2±0.5	2±0.75	1.0000

The effect of time and ureteral wall thickness (**UWT**) on stone fragmentation declines as the stone size increases so that there is less significant effect on the stone free rates and the number of sessions of ESWL required when the stone size is more than 10 mm. table (4).

Table 4: **Distribution of stone fragmentation outcome according to stone size and ureteral wall thickness.**

Stone size	**UWT≤ 3.25 mm**	**UWT> 3.25**	**P value**
≤10 mm (n=105)	62	43	
Treatment success (n=86)	56(90.3%)	30(69.7%)	0.0098
ESWL Sessions	1.5± 0.75	2±0.25	0.0007
> 10 mm (n=22)	12	10	
Treatment success(n=10)	5(41.6%)	5(50%)	1.000
ESWL Sessions	2.5±0.5	2.75±0.75	0.5524

Discussion

Stone fragility has been found to be correlated with effective shockwave energy transmission and stone composition. Also, brushite stones, cysteine, and calcium oxalate monohydrate are well known to be hard and resistant to ESWL. In radiological modalities, stone composition and attenuation value are related. Many studies have tried to establish that stone fragility can be predicted by the attenuation value of calculi. HU density by NCCT and radio density by KUB are the ways attenuation value can be measured [8].

Lim KH et al, [8] found a correlation between stone density on plain x-ray and HU density on CT. As a result, stone density on plain x-ray may be regarded as one of the factors acting alone that can be used to predict the success of ESWL.

Based on radiographic patterns, **Dretler SP,** [9] proposed the ability of a plain KUB to show stone fragility.

In this study, when SFR values of stone/12rib density stratified according to stone size, which is the most important independent factor, and a confounding parameter we found that ESWL failure can be predicted independently by using a stone density that is relative to that of bone (12^{th} rib) on KUB.

Sarica K et al, in another study [10] found that at the impacted stone site, ureteric wall **(UWT)** diameter is highly predictive. They therefore strongly recommend that in all such cases, before selecting between SWL and URS as treatment options, the calculation of the UWT diameter should be taken into consideration seriously.

In this study it is found that ureteric wall thickness UWT at the site of impacted stone is an important independant predictor for success of ESWL, and a value of less than 3.25 mm has significant success rate about 90.3% in stones less than 10mm.

In ER setting, the use of ESWL as a prompt therapeutic tool has not yet been given the deserved attention, although, in the treatment of ureteral stones, it is globally considered as one of the treatment options.

Ghalayini IF [11] in his study found that Emergency ESWL for obstructing ureteral stones has a satisfactory success rate and very low morbidity.

The study by **Tombal B et al, in 2005** [12] demonstrated that the proportion of patients who were stone-free (SF) after 48 hours and the proportion of patients discharged from the hospital after 72 hours were increased by 40% and 25% respectively in emergency ESWL when the stone was proximally located and less than 5 mm. They suggested that in these cases, it should be strongly recommended. Their study showed that emergency ESWL can be used to improve the removal of ureteral stones and reduce the duration of hospital stay if it can be proven that the location of the stone is proximal to the iliac vessels.

In the present study we found that doing ESWL within 24 hours from the first attack of pain felt by the patient and confirmed diagnosis of ureteral stone, this will limit the degree of inflammation and impaction and has great role in increasing stone free rates and the number of ESWL sessions especially for stones less than 10 mm.

In stone size more than 10 mm it is found than there is still effect of the time on the stone free rate and number of ESWL sessions but has no statistical significance .

Conclusions

The risk of ESWL failure is higher for a stone with a density greater than that of the 12th rib compared to that with a lower density.

Radiological findings on the preoperative KUB may be helpful for predicting ESWL outcomes.

Ureteral wall thickness as a secondary characteristic of Non-contrast CT scan is an important predictor of ESWL outcome for ureteric stones less than 1cm.

Ureteral wall thickness is correlated to the degree of inflammation and impaction of ureteral stone.

Early intervention using ESWL within <24 hours from first attack of pain is important predictor for ESWL Success

Recommendation:

Regular and precise calculation of these studied parameters will help us to reduce the cost of treatment

but most importantly limit the adverse effects of treatment intervals that are longer than usual, repeated SWL sessions and additional procedures on the QOL of the treated patients.

No **Conflicts of Interest**

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Ethical clearance: was taken from the scientific committee of the Iraqi Ministry of health

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Changes of Anterior Chamber Biometry and Relationship to Intraocular Pressure Changes after Phacoemulsification Emulsification Surgery in Non-Glaucomatous Eyes

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Abstract

Fifty percent of blindness worldwide is attributed to cataract, and cataract surgery is the most common surgery performed by ophthalmologist. Cataract surgery has its effects on the structures of the anterior segment, and hence the aqueous humor outflow, these may include more space in anterior chamber, wider angles, as the thick aging lens would be replaced by the thinner intraocular lens. So this study to investigate the changes in anterior segment parameters and its correlation to IOP changes after phacoemulsification. A prospective Cohert study that was done at Ibn Al-Haitham Teaching Eye Hospital for patients planned to have phacoemulsification, for 55 patients during 8 months, from the 1st of July/2018 until the 28th of February/2019. The data included full preoperative and postoperative assessment, key parameters were: axial length measurement, intraocular lens, anterior chamber depth, anterior chamber volume, anterior chamber angle for the four quadrants and their mean value was calculated. It was done before the operations and after two months from the operation. Findings were the mean age of the study group was 56.9years, with 30 (54.5%) males and 29 (52.7%) right eyes,. The mean axial length was 23.12±1.11 mm. There were statistically significant differences in preoperative compared to postoperative values of IOP (decreased by 4.55 mmHg), anterior chamber depth (increased by 0.74 mm), volume (increased by 32.75 µl), and mean angle (increased by11.78 degree) maximum inferiorly (12.31 degree) and minimum superiorly. There were no statistically significant correlation between IOP changes with anterior chamber depth, volume, or angles, but there was a statistically significant correlation with preoperative IOP .Conclusions cataract surgery significantly decreases the IOP, and increase anterior chamber depth, volume, and angle. The decrease in IOP does not correlate with anterior chamber depth, volume, and angle. The decrease in IOP was significantly higher in patients with higher preoperative values of IOP.

Keywords *: cataract; phacoemulsification: anterior segment parameters pre and post -surgery ; IOP*

Introduction

A cataract is a degradation of the optical quality of the crystalline lens that affects vision. Mostly related to aging. They can occur in one or both eyes .the changes that affect the quality of lens optics with subsequent change of vision known as cataract[(1)] ;still the world wide main cause of blindness and also in Iraq[(2)].cataract may affect vision from mild decline in early stages to clinically blind (VA 3/60), and is may be unilateral or bilateral [(2)]

The cataract surgery rate is known as correlation between number of surgically treated cataract to millions of country pupation, that previous study state that Iraq occupied the 30rd position to 50 countries involved[(3,4)]

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Histologically, the crystalline lens consisted of following structures a cellular capsule ,epithelium ,cortex and nucleus and provide one third of refractive index power of the eye, in the un-accommodated state, the average adult lens parameters are about 4–5mm thickness, with a 10mm anterior radius of curvature, a 6mm posterior radius of curvature, a refractive index of 1.386 (1.406 centrally), with the dioptric power of 18D [5] .The other important parameters of anterior chamber is the follow of aqueous humor that occur through Trabecular Outflow and uveoscleral pathway [6]. It is known that trabecular meshwork is pressure sensitive site of aqueous outflow and act as one way valve i,e without energy demand aqueous get out of the anterior chamber ,but there is no return . in the Schlemm's canal the path of aqueous not well clear and presence of intracellular and intercellular pores might cause bulk flow , the direct connection of inter trabecular spaces (giant vacuole) suggested active transport[7]. Mathematically The facility of outflow (C in the Goldmann equation;) is the inverse of outflow resistance and varies widely in normal eyes, with mean value ranging from 0.22 to 0.30 µL/min/mm Hg. it's supposed that Outflow facility decline with age and is affected by several factors like surgery, trauma, medications, and endocrine factors. Also the glaucomatous or ocular hypertension Patients IOP typically have decreased outflow facility [8]

the remaining outflow by non-trabecular pathway is by uveoscleral outflow , start from anterior chamber into the ciliary muscle and then into the supraciliary and suprachoroidal spaces then exits the eye through the intact sclera along the nerves and the vessels that penetrate it, some evidence that outflow via the uveoscleral pathway is has important roles in human eyes, about to 45% of total aqueous outflow ,also there is significant effect of aging process on this pathway, that cause decline of its and also in glaucomas patients. This pathway known to be pressure insensitive (independent) path and increased by some pharmacological agents like prostaglandin analogues ,while miotics cause reduction in it . The exact calculation cannot be done in non -invasive method and calculated by the Goldmann equation [9] as it is known that cataract surgical interversion is most common surgery in the world that aiming to achieve best correct vision, and become direct from large incision (10 mm) to very small wound less than (3mm) with better and faster visual outcome by using phacoemulsification[10].The effect of cataract surgery on IOP had been studied in several studies With different period of fellow up ranged from 6 month until 3 years , and conclude that the phacoemulsification had generally IOP lowering effect that fall in a range 1.5 to 9 mmHg. The explained effect is by the mechanical influence of the lens removal, also increment uveoscleral outflow and increased trabecular outflow [11, 12]. So this reduction in IOP may be useful in patient with glaucoma or ocular hypertension[13].The suggested mechanism for surgery effect on IOP not well understood that might be change of anatomical structures of AC especially in angle closure glaucoma ,also the effect that obtained by lens removal would lead to high movement of the posterior capsule to posterior that displacing the zonula over the ciliary body, and subsequent facilitates more aqueous outflow as a result of Schlemm's canal expansion[13,14], the role of inflammatory mediators IL-1 that released by phacoemulsification and IOP sudden rising during surgery will cause more aqueous outflow[15,16]. There are published studies for evaluating the relationship between ocular biometric parameters and post-op IOP reduction in normal eye patients [17], anterior chamber volume (the area that extend from the anterior iris plane to the endothelium) [18], axial length , angle opening distance, anterior chamber area, central corneal thickness, lens thickness, iris thickness, and pupil diameter [19, 20]. By any means, exact explanation of those changes still not understood well, but there is an increasing fact that the greatest factor among them is the level of the preoperative IOP. Also the axial length, "the distance from the anterior corneal surface to the retinal-pigmented epithelium" would contribute a significant role in predicting IOP changes following cataract surgery [21]. In the end the previous researches showed that there is a potential association between the observed reduction in IOP and axial length of the eye [22]

Aim of the study: To investigate the changes in anterior segment parameters and there correlation to IOP changes after phacoemulsification.

2. Subjects and methods:

Cohert prospective study that was done at Ibn Al-Haitham Teaching Eye Hospital in Baghdad for patients planned to have phacoemulsification, the data was collected from 55 patients by the researchers, from

the 1st of July/2018 until the 28th of February/2019. The data included both preoperative and postoperative assessment: VA, slit-lamp examination, routine fundus examination, Gonioscopy(grade2 angles and below were excluded), and IOP measured by Goldmann applanation tonometer. The fundus was examined by using condensing lens, some cases the posterior segment was assessed by B-scan ultrasonography.

1. Axial length measurement and the intraocular lens (IOL) power calculation was done using IOL MASTER (Zeiss, Jena, Germany)

2.Anterior segment imaged by a Pentacam (PTC, Oculus Inc., Wetzlar, Germany) for anterior chamber depth (ACD) from the corneal epithelium to the anterior lens capsule [23] (or anterior IOL surface postoperatively), anterior chamber volume (ACV), anterior chamber angle (ACA) for the four quadrants (Inferior, Superior, Nasal, and Temporal) and their mean values were calculated. It was done before the operations and after two months from the operation, in standard setting (dimly illuminated room) by experienced technician. Selection was Adult patients older than 18 years, Patients' acceptance, no other ocular pathologies or surgery also surgical and post-surgical complications persons excluded.

The collected data was handled and analyzed by IBM© SPSS© Statistics Version 23. Independent samples T-test was used for numerical and normally distributed data. Pearson Correlation was used to identify the possible linear correlation between the study variables. All analyses were done with 95% confidence intervals (CI). P-values less than 0.05 were considered statistically significant throughout this study.

Findings

In the current study, the mean age was (56.9±9.9) years; the commonest age group was (60 – 70) years (50.9%) , very close gender distribution , this applied also to laterality as there were 29 (52.7%) right eyes, compared to 26 (47.3%) left eyes. The mean axial length was 23.12±1.11 mm.

Table (1): baseline characteristics of patients included in this study

Variables		Number	%
Age groups	30-49	12	21.8
	50-59	15	27.3
	60-70	28	50.9
Age (mean ±SD)	56.96±9.99		
Gender	Male	30	54.5
	Female	25	45.5
Laterality	Right eye	29	52.7
	Left eye	26	47.3
Axial length (mean±SD)	23.12±1.11		

There were statistically significant differences in IOP (decreased by 4.55 mmHg), ACD (increased by 0.74 mm), ACV (increased by 32.75 µl), and mean AC angle (11.78 degree) maximum inferiorly (12.31 degree) and minimum superiorly (8.29 degree).

Table (2): comparison between preoperative and postoperative IOP and

Variables	Preoperative	Postoperative	Difference	P-value
IOP	18.65± 2.84	14.11±2.61	4.55	<0.001
ACD	2.62±0.43	3.36±0.71	0.74	<0.001
ACV	139.84±37.20	172.58±35.97	32.75	<0.001
Anterior chamber angle width table (3)				
Superior	32.09±7.06	40.38±6.54	8.29	<0.001
Inferior	31.71±6.60	44.02±5.84	12.31	<0.001
Temporal	34.95±7.58	44.72±6.29	9.77	<0.001
Nasal	34.04±6.19	45.93±4.87	11.89	<0.001
Mean angles	32.80±4.92	44.58±4.32	11.78	<0.001
Paired samples T-test				

There were no statistically significant correlation between IOP changes with ACD, volume, or angles, but there was a statistically significant correlation with preoperative IOP (correlation coefficient 0.513), as showed in Table (4) and Figure (1).

Table (4): correlation of IOP changes with axial length and changes of other parameters

Changes	Correlation coefficient	P-value
AL	-0.174	0.204
ACD	-0.019	0.888
ACV	-0.133	0.332
Superior angle	0.030	0.830
Inferior angle	-0.058	0.675
Temporal angle	-0.012	0.933
Nasal angle	-0.073	0.597
Mean of angles	-0.035	0.798
Preoperative IOP	0.513	<0.001
Pearson correlation		

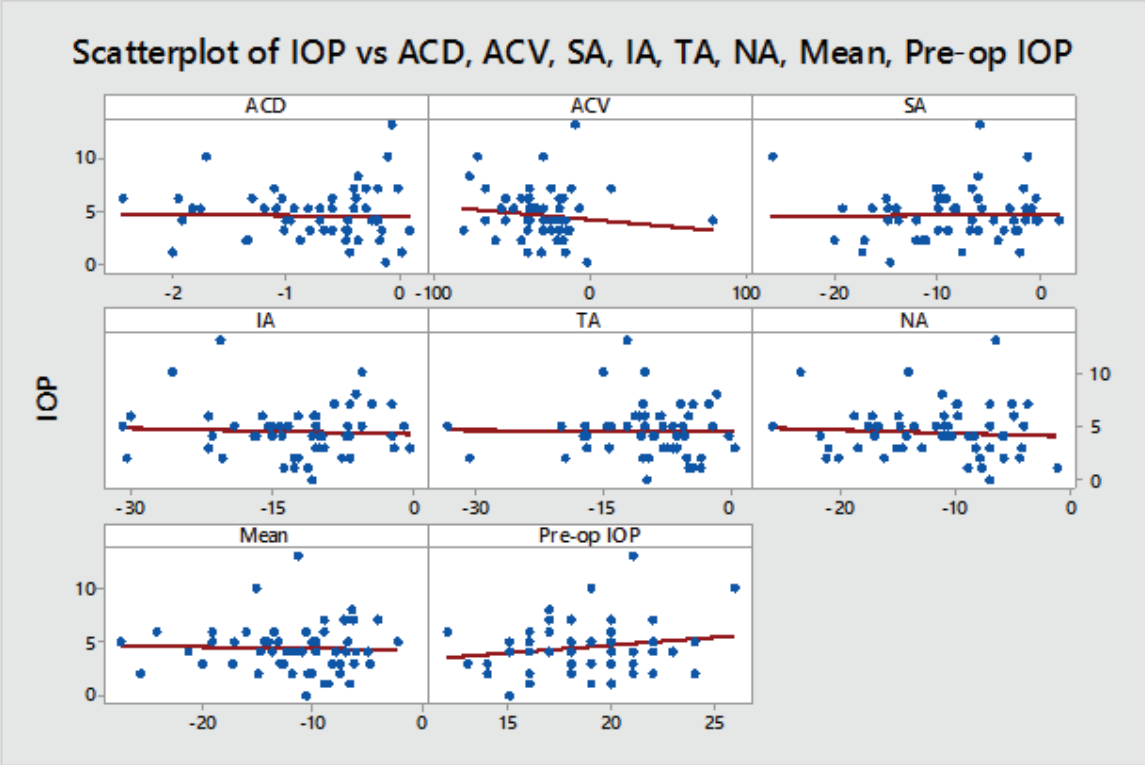


Figure (1): Scatterplot of IOP changes versus changes in ACD, ACV, SA, IA, TA, NA, mean chamber angle, and preoperative IOP.

Discussion

This study showed a significant decrease in IOP post cataract surgery in non-glaucomatous eye with significant increase in ACD, ACV and ACA. Although ; there is no relationship between IOP and ACD, ACV, and ACA.

In the current study, there was a significant decrease in mean IOP preoperative and postoperative, with a statistically significant mean difference of 4.55 mmHg. This was comparable to the results reported by Sengupta et al [24],also Yang et al [25], reported significant IOP reduction postoperatively. This could be explained by the different proposed mechanisms, which could be an inflammatory response that may lead to increase in uveoscleral outflow, blood-aqueous barrier changes, effects on the ciliary body, in addition to anatomical alterations in the anterior segment which could be the pivotal factor explaining aqueous humor dynamics post cataract surgery.

In the current study, there was a statistically significant increment by 0.74 mm in ACD and by 32.75 mm^3 in ACV postoperatively. This was lower than the results of Huang etal [26]. While our results were close to result of Simsek et al [27] . In the current study, the mean increase in ACA was maximum inferiorly and minimum superiorly. This was comparable to results of Dooley [28], also Huang et al [29] and Simsek et al [27]. The crystalline lens continue to grow throughout life, and when doing so, it's pushing the iris anteriorly, shortening the AC and decreasing the angle opening, with lens removal and the smaller size of the implanted PC IOL, there won't be a structure that pushes the iris, which may be related to IOP decrement postoperatively, together with dysregulation of pre-inflammatory mediators after cataract extraction [30].

The study showed no statistically significant correlation between IOP changes with ACD, ACV, or ACA, but there was a statistically significant correlation with preoperative IOP. This was in agreement with the results of Huang etal [31]. Also our results were similar to results of Sengupta etal [24]. These differences might

be attributed to patients' selection, that was whether they had open or closed angle, and considering the proposed relationship between the ACD and angle closure, as suggested by Pakravan etal[32], there will be a greater proportion of patients that will be considered to have narrow angles, with significant high risk for developing angle closure (ACD ≤2.1 mm). Another factor might be related to the way of measuring the ACD (AC OCT in Huang etal [31] and Sengupta etal [24], and Pentacam in our study.

Conclusions

1. Phacoemulsification in Iraqi patients , get lower IOP, and increase ACD, ACV, and ACA.

2. The decrease in IOP does not correlate with ACD, ACV, and angle. . So IOP seems not related to anatomical changes directly but to facility of drainage change after the surgery or uveoscleral outflow increase.

3. The decrease in IOP was significantly higher in patients with higher preoperative values of IOP.

List of abbreviations

Abbreviation	Explanation
ACA	Anterior chamber angle
AC	Anterior chamber
ACD	Anterior chamber depth
ACV	Anterior chamber volume
AL	Axial length
CCT	Central corneal thickness
IA	Inferior angle
IOP	Intraocular pressure
IOL	Intraocular lens
PA	Posterior angle
SA	Superior angle
TA	Temporal angle

Recommendation

Further studies should be done measuring the changes of IOP in patients with open, narrow, and closed angle glaucoma as a therapeutic action to decrease need for multiple anti-glaucoma medications.

Conflict of Interested: Nil

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Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in.

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Bacterial Contamination between Computers in Computer Labs

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Abstract

This study was conducted to identify the role of computers in transmitting bacterial infections to users of these devices. The bacteria on the surface of computers (mouse and keyboard) were isolated to identify their genotypes and their effects on the health of their users. The study sample included 100 samples divided as follows: 10 computers for each section of the Technical Institute in Baquba. Wipes grew on the following circles: Macconkey agar, Nutrient agar, Blood agar for its study and recognition of its shape and was dyed with chrom. The most common species in the studied samples were Staphylococcus spp. The prevalence of Gram-positive spherical bacteria on the surface of computers (mouse and keyboard) was higher than that of Bacillus and Gram negative bacteria. (192) isolates of computers and the percentage of contamination of the computer keyboard higher than the percentage of contamination of the mouse as the number of isolates of the phenomenon of swabs taken from the keyboard (117) and the proportion of about (60.9%) while the number of isolates visible from swabs taken from the mouse (75%). The percentage of bacteria found on the surface of the keyboard is more numerous than the bacteria on the mouse surface. The results of the antibiotic sensitivity test showed that all of these species were sensitive to antibiotics but to varying degrees.

Keyword: *Bacterial contamination, computer labs, computers.*

Introduction

The use of computers has spread to include all age groups including children. This may pose a risk to their health, for their weak immunity if the computer proves to be a catalyst for the transfer of microbes. Computers are becoming necessary for every kind of work and their use is becoming part of the lives of people of all ages. The study of different university environments indicates that students who use computers (100%) and the number of students who use the Internet in an organized manner (92.1% And (73.3%) use the email[1].

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The capability of a pc to act as fomites has been formerly documented in healthcare[2] And sanatorium Environment[3]. In art work place, infection of the office environment Which encompass the laptop(keyboard and mouse) with bacteria is likewise recognized[4]. Universities have developed a multi-user (pc lab) on college computers for general scholar access[5]. The availability of multi-user computers in many numbers at the university or institute and that these gadgets are treated through many customers on a each day foundation and given that the computer systems aren't robotically cleared (periodic), the hazard of transmission of polluting microbe is widespread no matter our information For the abundance of microorganisms within the surroundings develops but the threat or gravity of the pollution this is formed via computer and mouse keyboards has now not yet been completely understood and no clear policies or even widely identified hints had been formulated for the dangers brought on via computer components (keyboards, Mouse), this isn't in the hobby of campus

students mainly that computer keyboards and mouse may be the cause of many diseases spread[1]. Surface infection of public user interface systems enables spread disease, raising many questions. Although the studies effects have shown many proof in guide Of their lively role in this regard, common purchaser interfaces such as laptop keyboard and mouse (being confined in near contact) And ordinary hands) as reservoirs for the transport of microorganisms[6,7]. The purpose of this observe become to have a look at the microbial infection of the computer keyboard and the mouse, computer components that serve as popular interfaces for users within the Internet cafe[8].

Materials and Methods

Collection of samples

During the study period, 100 computer labs were collected in the following sections (community health department, pathological analysis department, computer systems department, electricity department, survey department, nursing department, materials management department, accounting department, mechanics department, department). Swabs were implanted on the McConkey agar medium and the blood-containing center containing 5% of the sheep's blood 5% blood agar. In plate streaking method, dishes were incubated aerodynamically and at 35-37 ° C for 24-48 hours.

Central collection of the sample : This medium consists of the use of Normal saline to collect swabs and bacteria before isolation and technology.

Culture and isolation

Nutrient agar:14gm was dissolved in 500 ml of distilled water, and the environment was sterilized at 121 ° C for 15 minutes.6 This medium was used for the development and isolation of different species of bacteria as well as for the study of morphological characteristics of bacterial colonies and for the purpose of purification of plantations. **Blood agar:**10.5gm was dissolved in 500 ml of distilled water and 5 ml sterile blood was added. The nutrient environment was first fed, then sterilized and then sterilized. Sterilized blood was then added to the study of blood decomposition by the bacteria[9]. Macconkey agar**:** Prepare as in previous seasons and use this environment to isolate negative bacteria and to ferment fermented from non-fermented to lactose sugar[9,10]. Pigmentation: dyed by chromium dye and used to determine the type of pigment and form of bacteria[11].

Sensitivity Test

Use five types of antibiotics to check the sensitivity or resistance of bacteria to these antibiotics**: (Amoxcillin ,Aztreonam ,Imipenem ,Gentamycin ,Levofloxacin).**

Antibodies were then distributed to the incubated environment for 18-24 hours at 37 ° C and the incubation period was measured in millimeters (mm).



analysis

Statistical evaluation of the results of the current have a look at the use of the Statistical Package for Social Sciences conducted (SPSS) metadata. Test was used T-test, variance analysis ANOVA, Chi-square, percentage, standard deviation and standard error in the present study to find a moral differences and their relationship to cutaneous leishmaniasis infections and immunological and compared the moral differences between the mean values depending on the testing Game-Howell and there were significant differences (P<

0.05).

Results and Discussion

The results indicated that the percentage of pollution in the computers (keyboard, mouse) in the computer labs in the sections of the technical institute in Baquba under study was uneven.

Table (1): Percentage of contamination of computers (keyboard, mouse) in the sections of the Technical Institute Baquba.

Place of collection	Number of samples	type of isolates				Total isolates
		Keyboard	%	Mouse	%	
community health techniques	10	15	12.821	8	10.667	23
Nursing techniques	10	18	15.385	9	12	27
Techniques for pathological analysis	10	7	7.983	6	8	13
Electricity technologies	10	8	6.838	6	8	14
Accounting Techniques	10	11	9.402	8	10.667	19
Space techniques	10	6	5.128	7	9.333	13
Computer Systems Technologies	10	20	17.094	11	14.667	31
Techniques of machinery and equipment Materials	10	11	9.402	7	9.333	18
Techniques Management	10	13	11.111	8	10.667	21
Mechanics techniques	10	8	6.838	5	6.667	13
Total	100	117	100.000	75	100.000	192

The percentage of contamination of the computer keyboard was higher than the percentage of mouse contamination. The number of isolated isolates from the swabs taken from the keyboard was (117) and the percentage was about (60.9%) while the number of isolated isolates from the swabs taken from the mouse (75) The percentage of micro organism on the surface of the keyboard is more than the number of bacteria on the surface of the mouse and this may be due to the area of the computer keyboard larger than the mouse area, making it more susceptible to microbial contamination[12].

We note from the first table that the highest percentage of pollution was inside the computer labs of the computer systems department followed by the nursing department and the community health department.

Table (2): Distribution of microbial isolates taken from the keyboard and mouse from computer labs

Place of collection	Community health		Nursing		pathological analysis		Electricity		Accounting		Space technique		Computer Systems		machinery and equipment Materials		Techniques Management		C.	
Isolates	keyboard	Mouse	Keyboard	mouse	Keyboard	mouse	Keyboard	Mouse	keyboard	mouse	keyboard	Mouse	keyboard	mouse	keyboard	Mouse	keyboard	mouse	Keyboard	Mouse
Staphylococcus spp.	+	+	+	+	-	-	-	-	+	+	+	-	+	+	-	+	+	-	-	+
Bacillus spp.	+	-	+	-	+	-	+	-	+	-	-	-	+	+	+	-	+	-	-	-
Streptococcs spp.	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-
Escherichia coli	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	+	-	+	-
Fungi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The table 2: shows that most of the isolates were *Staphylococcus spp,*[13] which may be due to normal appearance on the skin and nose and are found in people with skin injuries because they are opportunistic bacteria[14]. followed by bacteria *Bacillus spp* bacteria through the exposure of keyboards and mouse to environmental pollution from the air or dust or water when washing the hands after eating food or baths in addition to the emergence of isolates from bacteria *Streptococcs spp, Escherichia coli* while all isolates showed the presence of fungi on the surface Keyboard and mouse for computer.

Table (3): Distribution of the effectiveness of antibiotics on isolates taken from computer (keyboard, mouse).

Isolates	Genes	Amoxicillin	Aztreonam	Imipenem	Gentamycin	Levofloxacin	p-value
Staphylococcus spp.	12	25mm	20mm	27mm	27mm	29mm	
Bacillus spp.	9	27mm	20mm	25mm	15mm	21mm	
Streptococcs spp.	5	27mm	20mm	22mm	21mm	20mm	
Escherichia coli	5	15mm	21mm	28mm	20mm	15mm	
Mean ±ED	2.500±0.645	23.500±2.872	20.250±0.250	25.500±1.323	20.750±2.462	23.333±2.848	0.0088
SD	1.291	5.872	0.500	2.646	4.924	4.933	
Lower	0.446	14.359	19.454	21.290	12.290	11.079	
Upper	4.554	32.641	21.046	29.710	28.586	35.587	

Table (3):indicates the effectiveness of antibiotics for microbal in the present study that all isolates were sensitive to antibiotics but there was a difference for each bacterial genotype.*Staphylococcus spp* ranged from 29 to 20 mm, The *Bacillus spp* ranged from 27 to 15 mm, *Streptococcs spp.* ranged from 27-20 mm and *coli Escherichia* ranged from 28 to 15 ml . There were significant differences at the the probability level ($P < 0.05$).

Conclutions

The study conducted on the computer surfaces concluded that:

1-Computer surfaces (keyboard, mouse) are the source of different types of bacteria, including pathogens.

2- Computer keyboard is more polluted than the mouse.

3-The micro-computer pollution varies from one section to another.

There are a number of tips that if applied, it is possible to reduce the risk of bacteria on the surface of the computer are:

1-Wash hands frequently and regularly.

2-Clean the computer regularly and repeatedly.

3-Do not eat and drink in computer laboratories.

4-In the case of personal computer not give it to children because this increases the likelihood of infection with bacterial.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: Self

Ethical Clearance: There is no violation of human rights and environmental pollution

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Allele Frequencies of 13 Chromosome X STR in Arab Iraqi Population

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Abstract

Short tandem repeat (STR) markers are highly being used for identification of human being in addition to paternity and other forensic cases.The X-chromosome STR (X-STR) markers are a powerful supplementary system particularly in deficiency paternity analysis. Several X-linked microsatellites have been assessed but more studies are necessary to verify the population statistics. In our study, we report allele frequencies of 13 X-linked microsatellites (DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5, DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807, and DXS7424) in the Iraqi arab population. fifty Blood samples were collected from healthy unrelated males. A total number of alleles were detected for for all 13 X-STR loci were (83) and the related frequencies ranging from 0.0200 to 0.5000. Heterozygosity ranged from 0.652 to 0.858. Large values were observed, at least 0.999999994, in combined powers of discrimination. The results powerfully suggest that all the X-linked microsatellites defined here can possibly support the autosomal systems that used in parentage analysis and different forensic case work.

Keywords: *X-chromosome STR, Allele frequency, Forensic parameters, X-STR database, Haplotype.*

Introduction

Armed war and other cases of aggression in Iraq since the 1980s have culminated in the killings of thousands of civilians. Series of events from the Iran -Iraq conflect to newer massacres committed by the Islamic State of Iraq and the Levant (ISIL). Large numbers of people are missing, and their bodies were never identified and returned to their families [1][2]. To try and tackle this problem, Mass Graves Depart-ment in the Iraqi Medico-Legal directorate that was established in 2010 to deal with the missing individuals and it has increased its capability in forensic anthropology and forensic genetics to retrieve and identify the large number of missing people. Recently, The Mass Graves Department has adopted the X-chromosomal STR (XSTR) markers as a one of the important tools that used in human identification purposes [3]. Because of its pattern of inheritance the X- chromosomal short tandem repeat (XSTR) has potential applications for forensic casework and kinship analysis as compared to autosomal STR (A-STR), Y-chromosomal STR (Y-STR), and mitochondrial DNA (mtDNA), [4]. Males usually have one X-chromosome transmitted as haplotypes from their mothers to their daughters, whereas females have two Xchromosomes transmitted from both parents to their offspring in the same manner as autosomes [5]. XSTR analysis can be helpful in trace identification with female minor component and male major component, and in complicated cases of kinship, such as cases of deficiency (father-daughter or mother-son), grandparent-grandchild links, half sister analysis, incest paternity testing, and so on [6] [7].

hile X-Plex PCR Amplification Kit is a short tandem repeat (STR) multiplex assay that used to amplifies 13 X-STR loci and an amelogenin locus located on human chromosome X in a single PCR reaction that is DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5. DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807 and DXS7424 [8] (Table 1).

Table 1: Allelic Ladder of GenePhile X-Plex Kit with its loci, the relating dyes used, alleles contained in the allelic ladder and the genotype of the Control DNA 9947A and K01 are registered in the table [8].

Locus	Designation Alleles Included in X-Plex	Dye Label	9947A Genotype	Control DNA K01 Genotype
DXS8378	8-14	6-FAM™	10,11	11
DXS9898	8.3-16		12,15	12
DSX8377	33-60		45,47	51
HPRTB	6-17		14,14	15
GATA172D05	5-12	VIC®	10,10	11
DXS7423	12-18		14,15	14
DXS6809	27-40		31,34	34
DXS7132	8-19	NED™	12,12	16
DXS101	14-32		24,26	25
DXS6789	13-25		21,22	20
AMEL	X, Y	PET	X,X	X/Y
DXS9902	6-13		11,11	11
DXS6807	11-17		12,14	15
DXS7424	9-18		14,16	16

Autosomal markers are useful for the resolution of most forensic DNA analysis. Some of them, however, require implementing STRs on the sex chromosomes (gonosomes) [9]. Gonosomal STR markers are useful when investigating relationships between individuals of different generations, mainly when key pedigree persons are missing. In addition, the use of gonosomal STRs in forensic analysis of DNA traces is increasing sharply [10]. The development of population studies would improve our understanding of the associated polymorphism and allellic distribution of these loci. As there is a connection between the level of polymorphism at a given locus and the rate of mutation [11].

In this study, we started to create an X-STR database on the population of Iraqi arabs and investigate the polymorphisms and haplotypes of the investigator GenePhile X-Plex loci kit and evaluated their effectiveness to be helpful not only in criminal investigation such as personal identification and kinship testing but for other anthropological purposes as well [12].

Materials and methods

Blood samples were collected from 50 healthy, unrelated and consenting males who were identified themselves as Iraqi Arabs. The consent form were signed by the donor people during the collection of the samples in medico-legal directorate / Baghdad. Finger lancets were used to collecte the samples of blood and then stored on FTA ® Classic Card (GE Healthcare, Pittsburgh, USA). For each sample a 1.2 mm punch was directly amplified following the manufacturer's recommended instruction. PCR using the GenePhile X-Plex Amplification Kit System (GenePhile Bioscience Co., Ltd TAIWAN). with an Applied Biosystems 9700 thermocycler was carried out using the standard manufacturer's recommended instruction: this co-amplified the amelogenin sex marker and 13 STR loci (DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5. DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807 and DXS7424). Capillary electrophoresis was accomplished using a 3500 Genetic Analyzer (Applied Biosystems, Foster City, USA) in accordance with the manufacturer's instructions; ILS-500 (Promega) was used as an internal size standard. Genotyping was carried out using GeneMapper ID-X v1.4 software (Applied

Biosystems). Alleles were designated depending on the published nomenclature; off ladders were termed if they fell into a virtual bin as long as the profile as a whole was judged to be of high quality, before designation, re-run at least once if alleles that were not in a bin or virtual bin.

Statistical Analysis

Once the frequencies achieved, important statistical tests are performed on the data to assess whether the database will be helpful when used in human identification. The allele frequencies were calculated manually for each locus. Match probability (PM), heterozygosities (HET), homozygosities(h), polymorphism information content (PIC), power of discrimination (PD) and power of exclusion (PE) were calculated with ChrX-STR.org 2.0 software web site.

Results and Discussion

Allele frequencies for 13 X-linked microsatellites

(DXS8378, DXS9898, DXS8377, HPRTB, GATA172DO5. DXS7423, DXS6809, DXS7132, DXS101, DXS6789, DXS9902, DXS6807 and DXS7424) and amelogenin were reported in the Iraqi Arab population. GenePhile X-Plex PCR amplification kit showed that all X-linked markers were highly polymorphic with a high discrimination power.50 (males) samples were collected. Such X-STR markers cover the entire X-chromosome and represent all 4-linkage group previously classified by Szibor

[10]. Supplementary (Table 2) shows allelic frequencies for 13 STR markers. The highest allele frequencies (0.5000) were found in the locus DXS7132, (allele 14).

The minimum number was (3) alleles and the maximum number was (11) alleles were observed per locus, and a total of 83 alleles were observed for all 13 X-STR loci. Heterozygosity varied between 0652.–0.858. Such findings found that DXS8377 is the most informative marker with heterozygosity at 0.844, where it is the least informative as DXS7423 and DXS7132, with heterozygosity at 0.652. DXS8377 was the most polymorphic locus (with 11 alleles, PIC = 0.845), while DXS9902 had the lowest values (with 3 alleles, PIC 0.583). (Figure 1). Power of exclusion (PE) varied from 0.359 (DXS7423,DXS7132) to 0.711 (DXS8377) (Figure 2). Power of discrimination (PD) varied from 0.652 (DXS7423,DXS7132) to 0.858 (DXS8377) (Figure 3). X-STR genotyping can complement the analysis of autosomal, mitochondrial, and Y-chromosomal markers in a complex kinship test. Combined Power of Discrimination (CDP)=0.999999994, and Probability of Combined Exclusion (CEP)=0.9998578. These values mean that the loci can be used safely to establish a database based on DNA for Iraqi populations.

Table 2. Allele frequencies at thirteen X-STR loci of 50 Iraqi unrelated males.

Allele	DXS8378	DXS9898	DXS8377	HPRTB	GATA172D05	DXS7423	DXS6809	DXS7132	DXS101	DXS6789	DXS9902	DXS6807	DXS7424
6					0.12								
8					0.12								
8.3		0.1											
8.5		0.02											
9					0.02								
10	0.28				0.38						0.26		
11	0.46	0.3		0.08	0.26						0.36	0.32	
12	0.2	0.3		0.12	0.1			0.02			0.38		0.02
13	0.06	0.18		0.48				0.16				0.02	0.06
14		0.08		0.26		0.4		0.5				0.18	0.08
15		0.02		0.04		0.4		0.26		0.06		0.42	0.2
16				0.02		0.16		0.06				0.02	0.42
17						0.04						0.04	0.16
18									0.06	0.02			0.06
19										0.04			
20									0.02	0.48			
21									0.06	0.2			
22									0.04	0.18			

Cont... **Table 2. Allele frequencies at thirteen X-STR loci of 50 Iraqi unrelated males.**

24								0.24	0.02				
25								0.14					
26								0.22					
27								0.14					
28								0.06					
29								0.02					
31						0.08							
32						0.12							
33						0.32							
34						0.2							
35						0.16							
36						0.1							
38						0.02							
42		0.06											
45		0.04											
46		0.06											
47		0.12											
48		0.06											
49		0.12											
50		0.28											
51		0.1											
52		0.1											
53		0.02											
54		0.04											

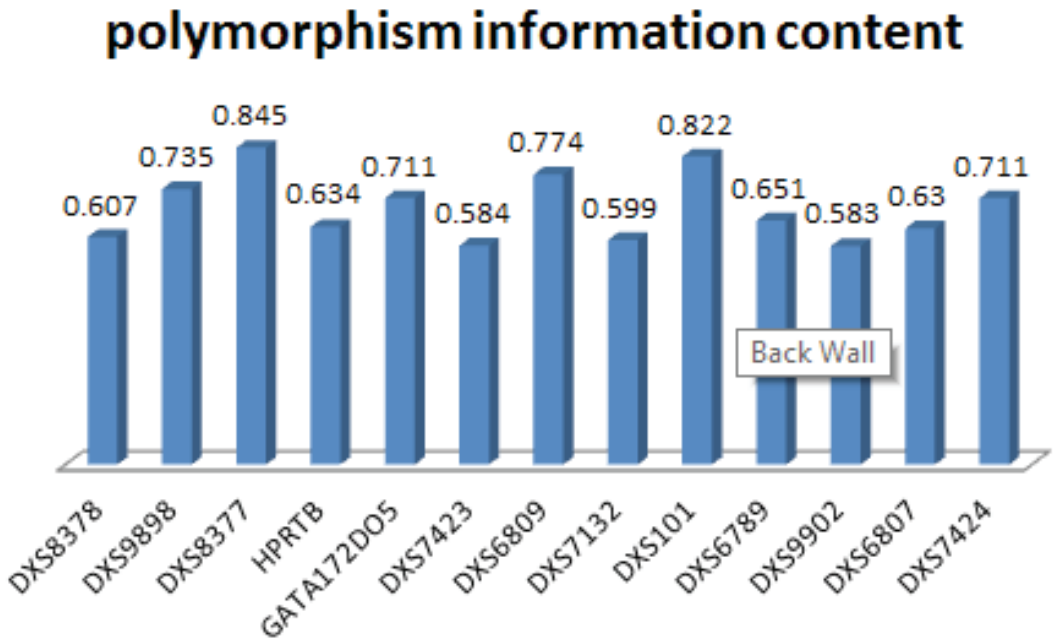


Figure 1. Forensic efficiency parameters: Polymorphism information content (PIC)

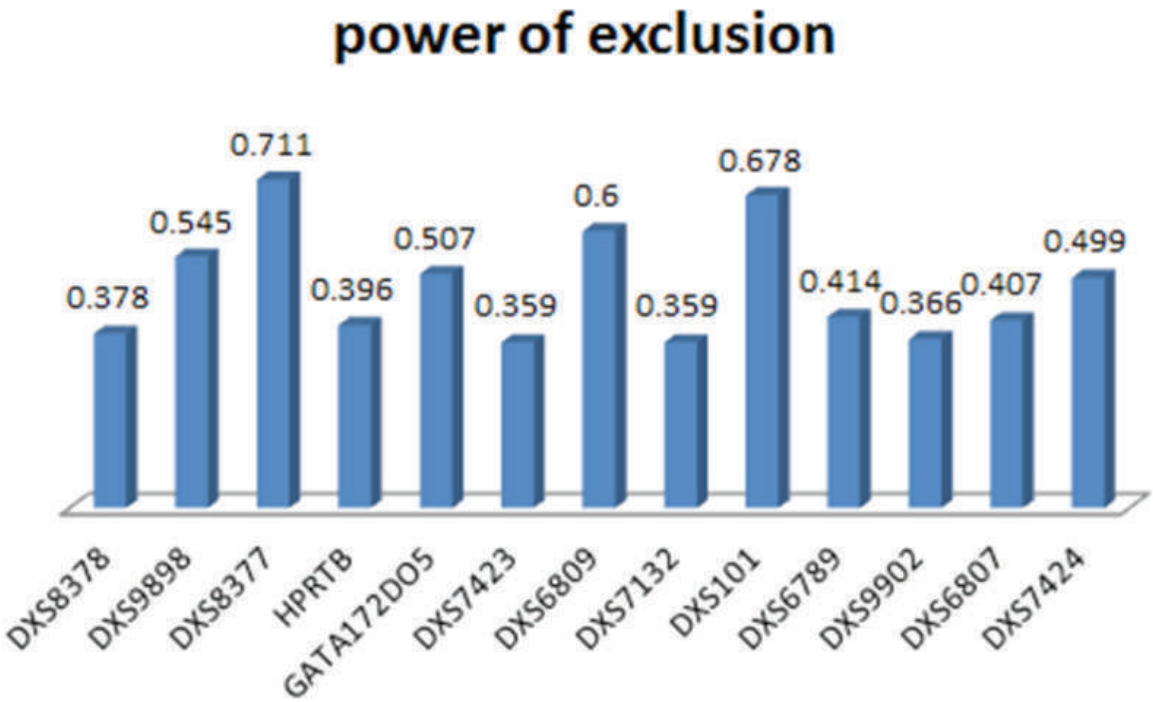


Figure 2. Forensic efficiency parameters: Power of exclusion (PE).

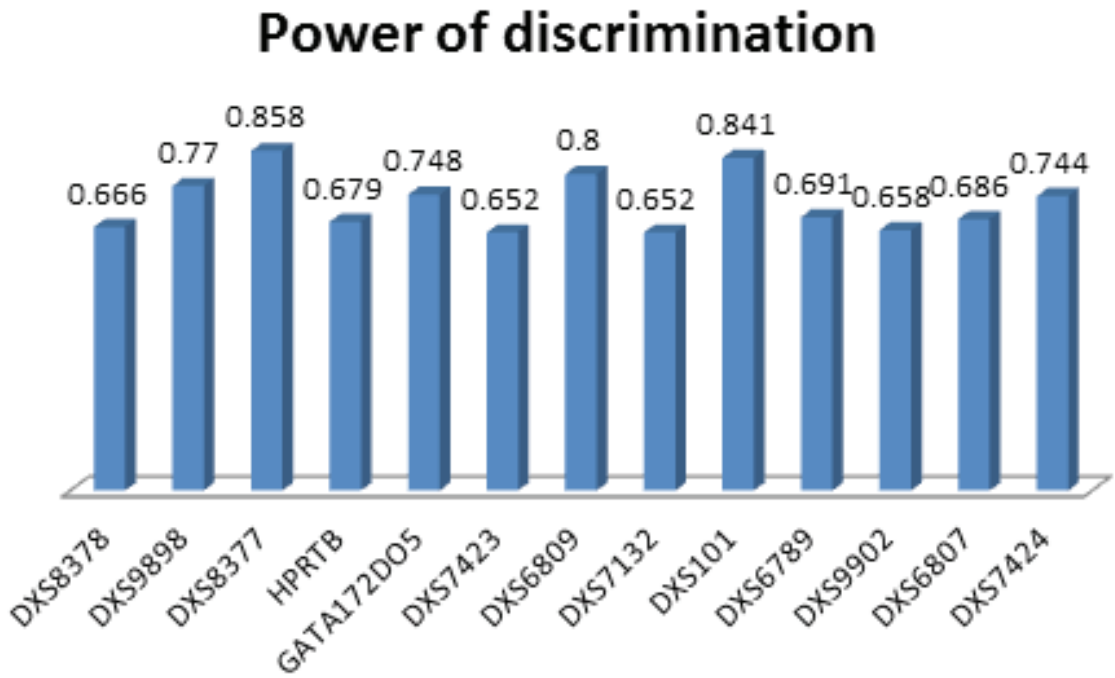


Figure 3. Forensic efficiency parameters: Power of discrimination (PD).

More studies are expected to get a general idea of the X-STR variability in all Iraqi people, and there are plans for inclusion of kurd and turkman in the database.

More studies are expected to provide an understanding of the X-STR variability among all Iraqi populations, and the future plans to inclusion Kurd and Turkman in this database.

Conclusion

The population database created permits for the application of the STR markers included in the GenePhile X-Plex 13 PCR amplification kit to be used in routine case work. High forensic statistical parameter values combined with the studied loci 's in dependent inheritance make them a very useful marker collection for the investigation of forensic genetics. Application of X-STR loci can be a useful addition to autosomal markers, particularly in complex cases of kinship and paternity, e.g. when his mother (i.e. the child's putative grandmother) is the source of the reference DNA sample, instead of the alleged parent.

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Ethical Clearance : The principles and the experimental protocol in this study was approved by the Medico-Legal Directorate, Ministry of Health, Baghdad, Iraq.

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Conflict of Interest : Nil.

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The Biophysical Efficacy of Smart Phones on the Eyes of Children from 3-12 Years

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Abstract

The modern day electronic gadget like laptops, smart phones (which offers advanced Technologies' with functionality similar as a personal computer), TV, computer games that held by hand, … etc. that completely influence the vision, a books or a newspaper that read is healthier than reading taken away the electronic devices due to the printing materials are typically great in divergence while the electronic screen contentment approach with drop contrast and thus needs further optical focus as well as added strain to the eye. In the current study , we assess the biophysical effectiveness of mobile phone on children up to 12 years, the current study was enrolled fifty (50) children, the information were taken as (age, gender, time of using mobile phone in hours, at which time the child use mobile phone day or night, visual acuity, family history, symptoms). Many optical devices used to obtain precise measurements from the patients which included (Snellen chart, autorefractometer, retinoscope, trial case, E card test, finger cube test, hand card test, catford drum test, Sheridan gardiner test and prism bar).

After conducting the necessary statistical methods and tests, it revealed that the most factor has more effect is the number of hours of using mobile phone devices and the children between (6-8) years were the most used mobile phone devices.

Keywords: *biophysical efficacy; smart phones; eyes of children.*

Introduction

The modern day electronic gadget like laptops, smart phones (which offers advanced technologies with functionality similar as a personal computer), TV, computer games that held by hand, … etc. that completely influence the vision, a books or a newspaper that read is healthier than reading taken away the electronic devices due to the printing materials are typically great in divergence while the electronic screen contentment approach with drop contrast and thus needs further optical focus as well as added strain to the eye. During working on the smart device, most individuals do not blink their eyes, in addition, they working for long time e.g., many hours incessantly evaporates the drops of tear, then, dryness in the eye such as blurring of vision, eyes painful, , inadequate blinking that causing eyes dryness as well as headaches can result due to the eyes focusing on the smart device screen for longer time interval [1].

Video games that held by hand that children played for long time duration without giving break to their eyes, continuously straining the eyes on the small screen of the video games for a longer duration of time can cause trouble, eye irritation with objects focusing at different distances, high or low radiation energy that produce from almost all electronic devices that involve internal part of human such as" brain "screens are below planes that can cause damage of eye such as cataract [2]. Using of mobile devices excessively can cause problems of eyes such as myopia condition (short-sightedness) and amblyopia (eye lazy) in children in younger age [3].

Rays enter the lens by passing through the pupil then iris then lens, rays pass through the vitreous humor, fovea; the area of visual acuity, that is a measure of the vision clarity, then retina(when the rods and cones) are react to ranges of light upon a great energetic level, in short ranges of light simply just the rods are activated. The inability to differentiate the visible colors at nighttime

is due to the rods inability to discriminate between the colors. A fully adapt to low light is up to 35 minutes as a time taking by the eyes that result in a monochromatic mode called "scotopic vision". While, in brighter light, the active parts are cones, therefore, the adaptation to this trichromatic mode is called "photopic vision", may take up to 10 minutes [4]. The (reading and writing) of video display terminals and learning that using for long hours has been accompanied with a reduction in maximum blink interval, since the symptoms of dry eye are progressing [5][6][7]. After persistent work with video display terminals sustainly, the ocular fatigue was reported by many people [8]. Ordinary cellular phones affect health of human as well as daily life that reported by previous studies, also cellular phone has associates with many problems of health like; disorders of sleep; headaches; leukemia; brain tumors as well as eyes malignant melanoma [9][10] An association between smart phone using and ocular health have reported by many recent studies, two cases of transient monocular vision loss as a study reported due to smart phone using [11]. The rate and mean of time spending due to smart phones using were higher in a study reporting and including subjects with pediatric dry eye disease (DED) than the non-DED group [12]. When smart phones using excessively, in addition, an increasing in using time of smart phone is related to (DED) these may affect the ocular surface and film of tear. Symptoms of severe ocular as the results of watching visual display terminal (VDT) by office workers who spent more than 4 hours, similarly, symptoms of multiple ocular are associated to the intensive using of smart phone [13] .One of the studies indicated as smart phone screen was emitted the blue light that had adverse effect on the human eyes especially in corneal epithelial cells [14]. At the ocular surface of mice, deterioration of the tear film and induced planes of inflammatory markers and production of reactive oxygen species (ROS) as the results of over exposure to the blue light [15].

Where the parallel rays approaching from the distance, respecting the refraction through the cornea and the lens, myopia as a refractional result anomaly, passing through the vitreous humor and focusing to the retina. Before the focus in divergence state of focusing, they initiate retinal dispersive circles with blurring image of the subject that is placed in infinity [16]. Heredity and environmental factors; endocrine disorders; obesity; malnutrition, obesity; chemical deficits (calcium, vitamin deficit); excess activities (working conditions) during near-sighted as well as intense or insufficient using of glasses are the main causes of myopia that elaborated by several issues [17] [18].

The blue light can causes the oxidative damage was shown to be inhibited by effective antioxidant extract associated-free radical elimination, hence improving the clinical symptoms of the eye surface in a dry eye mouse model[13]. Also the additional established that the formation of dry eye is associated with the blue eye.

Aim of Study

to evaluate the biophysical effect of mobile phones on the eyes during working a longtime front of the mobile phones.

Patients and Methods

The study was attended in the Ghazy Al-hariri hospital, Al-Yarmouk hospital and from learning clinic for optical techniques department. The study includes fifty subjects in order to assess the biophysical effect of mobile phones on children up to 12 years. The data were collected from the patients themselves and their files, the patients were examined by using Snellen chart to detect the visual acuity and by retinoscopy and autorefractometer to detect the type of refractive error and its degree measurement.

Snellen chart: Used to examine the visual acuity of both patient's eyes .so **Autorefractometer :** Used to measurement of refractive error and the curvature of the cornea. and **Retinoscope** Used to examine the refraction of the eye for prescription evaluation.**Trial case :**Used to corrective for any type of the refractive error in the eye. **E test:** The E's may be printed on cards or on the sides of a cube the child may be asked to communicate his answer by pointing his flingers in the direction of the limbs of the E or by holding up a wooden E to correspond with the test E [33].**Hand test:** using cards which is held up with the fingers open, it is a good method where we can examine very small children & they co- operate with us, if it used as a game [33].**Finger cube test:** using cubes with different sizes of finger picture.**Sheridan-Gardiner test:** this uses the principle of matching letters, the child is given a card with seven letters printed on it and another card is held up at the standard distance

with one letter printed on it and the child is asked to point to the corresponding one on his card [33].**Catford drum test:** is a rotational device to vision test, since the individuals are accommodated facing the drum wall, the internal apparent surface of the drum is typically striped, as the drum rotates, the eyes of subject are submit to a movable vision field , since the subject rests stationary, this phenomenon is called optokinetic nystagmus, test duration and drum speed may be varied, after exposure to the rotating drum, subjects are surveyed to regulate their susceptibility to motion sickness[34]. **Prism bar:** used with cover-uncover test to determine the angle of deviation of squint.

Finding

In the current study, a total 50 patients included 25 males and 25 females, the smaller age of group in the samples was between (3-5) years in percentage 30%, (6-8) years in percentage 40% and more than 8 up to 12 years in percentage 30%.as the in table.

Table (1): The relation between age(years) & gender.

Items			Gender		Total
			Male	Female	
Age	3-5	Count	7	8	15
		% within Gender	28.0%	32.0%	30.0%
	6-8	Count	11	9	20
		% within Gender	44.0%	36.0%	40.0%
	more than 8 up to 12	Count	7	8	15
		% within Gender	28.0%	32.0%	30.0%
Total		Count	25	25	50
		% within Gender	100.0%	100.0%	100.0%

In the table (2), it was found that 68.4% who use mobile phone for (1-3)hours daily was between age (3-5)years while those who use mobile phone more than 7 hours daily in that age was 0 percent, 55% who use mobile phone for (4-6) hours daily was between age (6-8) years and 54.5% who use mobile phone for more than 7 hours daily was more than 8 up to 10 years age.

Table (2): The relation between age(years) & time of using mobile phone.

Items		Time of using mobile phone			Total
		1-3 hour	4-6 hour	>7 hours	
Age 3-5	Count	13	2	0	15
	% within time of using mobile phone	68.4%	10.0%	.0%	30.0%
6-8	Count	4	11	5	20
	% within time of using mobile phone	21.1%	55.0%	45.5%	40.0%
more than 8up to 12	Count	2	7	6	15
	% within time of using mobile phone	10.5%	35.0%	54.5%	30.0%
Total	Count	19	20	11	50
	% within time of using mobile phone	100.0%	100.0%	100.0%	100.0%

It was observed that who use mobile phone at day time their percentage was 61.9% in the age between (3-5) years while 19% was in the age between (6-8)years and also for the age more than 8 up to 10 years, This means that as the children's age increase they become using mobile phone more time during the day and night, table (3).

Table (3): The relation between age(years) & interval of using mobile phone.

Items		Interval of using mobile phone			Total
		Night	Day	Both	
Age 3-5	Count	0	13	2	15
	% within interval of using mobile phone	.0%	61.9%	7.1%	30.0%
6-8	Count	0	4	16	20
	% within interval of using mobile phone	.0%	19.0%	57.1%	40.0%
more than 8 up to12	Count	1	4	10	15
	% within interval of using mobile phone	100.0%	19.0%	35.7%	30.0%
Total	Count	1	21	28	50

Table (4) : The relation between age(years) & V.A without glasses.

Items	V.A (R or L)					
Age(years)	6/6-6/12		6/18-6/36		less than 6/60	
	Right	Left	Right	Left	Right	Left
3-5	12	11	2	3	1	1
% within V.A (R or L)	36.4%	40.7%	16.7%	15.8%	20.0%	25.0%
6-8	12	10	5	8	3	2
% within V.A (R or L)	36.4%	37.0%	41.7%	42.1%	60.0%	50.0%
More than 8 up to 10	9	6	5	8	1	1

Cont... **Table (4) : The relation between age(years) & V.A without glasses.**

% within V.A (R or L)	27.3%	22.2%	41.7%	42.1%	20.0%	25.0%
total	33	27	12	19	5	4
% within V.A (R or L)	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

In table (4), it was found that most of the samples between (3-5)years have visual acuity without glasses (6/6-6/12) for R.E. 12 samples in percentage (36.4%) and for L.E. 11 samples in percentage (40.7%) , most of the (6-8) years age have visual acuity without glasses (6/6-6/12) for R.E. 12 samples in percentage 36.4% .

This means that most of children's have visual acuity within the range between (6/6-6/36) and only 5 cases have V.A less than 6/60 for the right eye and 4 cases for the left eye.

Table (5): The relation between age(years) & V. A With glasses.

Items	V.A (R or L)					
Age(years)	6/6-6/12		6/18-6/36		less than 6/60	
	Right	Left	Right	Left	Right	Left
3-5	14	14	1	1	0	0
% within V.A (R or L)	30.4%	30.4%	25.0%	25.0%	0%	0%
6-8	18	18	2	2	0	0
% within V.A (R or L)	39.1%	39.1%	50.0%	50.0%	0%	0%
More than 8 up to 12	14	14	1	1	0	0
% within V.A (R or L)	30.4%	30.4%	25.0%	25.0%	0%	0%
total	46	46	4	4	0	0
% within V.A (R or L)	100.0%	100.0%	100.0%	100.0%	0%	0%

It was found that most of the samples between (3-5) years age have visual acuity with glasses (6/6-6/12) for R.E. 14 samples in percentage 30.4% and also for L.E., most of the (6-8)years age have visual acuity with glasses (6/6-6/12) for R.E. 18 samples in percentage 39.1% and also for L.E. , and more than 8 up to 12 years age have visual acuity with glasses (6/6-6/12) for R.E. 14 samples in percentage 30.4% and also for L.E. , it was observed

that samples with less than 6/60 are (0) while there was in total 5 samples for R.E. and 4 samples for L.E. in cases of without glasses, table (5). That is means that all the children with V.A less than 6/60 and some with V.A (6/18-6/36) their vision have corrected with eye glasses.

Conclusion

We conclude that most of children play smart mobile phone devices between age (6-8) years and the most of the children use mobile phone at the day and night time between age (6-8) years, as well as most of the children have visual acuity (6/6-6/12) for both eyes in cases of with glasses and without glasses.

Conflict of Interest: non

Source of Findings: self-findings.

Ethical Clearance: This research was carried out with the patient's verbal and hospital approval before the cases was taken.

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The Effect of 2-Thioxo Imidazolidin-4-Ones (2- Thiohydantion) on Anticancer Activity: An in Vitro Study

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Abstract

Background and Purpose: Cancer is one of the most common factors of death in society today. Despite much research into cancer and its treatment, it is still one of the greatest health problems. The biggest obstacle to cancer treatment is that they are inherently resistant to chemotherapy or become drug resistant during treatment. For this reason, researchers around the world have made great efforts to identify new natural or synthetic compounds with anticancer properties. So the aim of this study is to find out the effect of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) on anticancer activity.

Materials and Methods: Imidazoles are compounds that have antibacterial, antifungal and antitumor properties in many studies. Through the results of this study, we discovered effectiveness of the new compound of 2-Thioxo imidazolidin-4-ones (2 Thiohydantion) as an anticancer agent against a cell line that is a cancer model system. To determine the IC_{50} of this compound, we treated the cancer cells by different concentrations (10, 20, 30, 40, 80 and 160 µg / ml) and then the cells were evaluated by MTT assay after 24 and 48 hour intervals.

Results: After preparing the required compound, MTT assay showed that the IC_{50} of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) compound on MCF7 cells, 135 and 40 µg / ml, respectively, after 24 and 48 h, respectively. In addition, the toxicity of these compounds on normal cells was evaluated, which is very different from their toxicity for cancer cells, and this is a very important feature. 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) has the ability to stimulate cell death in cancer cells, and these results provide new insights into the use of these compounds in cancer treatment.

Conclusion: So 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) at different concentrations significantly reduced the growth of cancer cells compared to the control group (50% inhibition of cell growth). Future studies will need to investigate the mechanism of this cytotoxicity as well as the possible anticancer effectiveness of this compound in vitro and in vivo.

***Keywords**: 2-Thioxo imidazolidin-4-ones (2-Thiohydantion), cytotoxicity, cancer cells*

Introduction

Cancer is the second leading cause of death worldwide.[1-4] Cancer is a disease caused by proliferation and growth without cell control.[5-7] Cancer cells create a tumor that in some cases can invade and metastasize to other parts of the body.[8] Methods of treatment for this disease are often ineffective and, despite the available therapeutic methods, there is still a need for research to discover new and targeted ways to treat the disease.[9] Most types of tumors can be treated with surgery, chemotherapy or radiation, but most metastatic cancers, such as breast cancer, cannot be treated with chemotherapy or other methods. The biggest obstacle to treating these cancers is that they are inherently

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resistant to chemotherapy or become drug resistant during treatment.[10] For this reason, researchers around the world have made great efforts to identify new natural or synthetic compounds with anti-cancer properties. Among the compounds considered for the treatment of malignancies are heterocyclic organic compounds due to their presence in the reaction groups and their cytotoxic properties.[11]

The thiohydantoin heterocycles are appear in a broad field of biologically active compounds consisting therapeutic drugs for the treatment of anti-tumor compounds. Thiohydantoins have also been applied as anti-cancer agents. However, the principal current interest comes from the application of thiohydantoins for the treatment of cancers. Structural characterization of thiohydantoin is important to comprehend their effect mechanisms because of their considerable biological effects.[12]

Studies have shown that heterocyclic, in addition to its antioxidant and anti-inflammatory effects, also has anti-cancer effects and cancer-preventing properties.[13] It is used in the treatment of various diseases , including diabetes, rheumatoid arthritis, Alzheimer's and cancer. Imidazoles are among the heterocyclic compounds.[14-17] The most important therapeutic feature of imidazoles is in relation to the drugs used to synthesize chemotherapy drugs.[18] Imidazoles are an important class of heterocycles that contain nitrogen and are currently under great attention due to their wide range of pharmacological effects.[19] Given the importance of imidazoles and their derivatives and their use in the medical and pharmaceutical industry, there are always many efforts to centrally design new imidazole derivatives or provide improved methods for the synthesis of these important heterocyclic compounds with minimal disadvantages.[20]

Imidazole, a five-membered heterocyclic ring containing two nitrogen and three carbon atoms at positions 1 and 2, is found in many natural compounds and biologically active compounds.[21]

Imidazole is found in the structure of many important biological constituents of the body such as histidine, histamine, as well as a number of pharmacologically active compounds.[22]

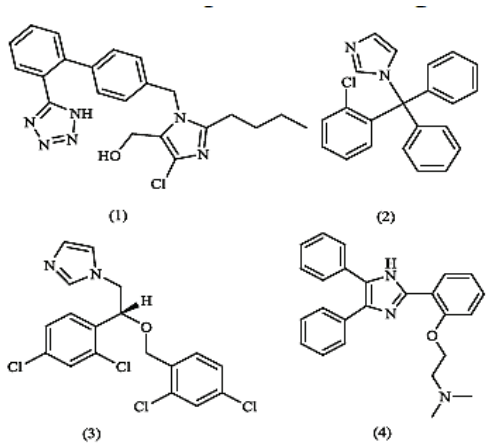


Figure 1. Structure of a number of imidazole drugs.[23]

In current study, we investigated the effect of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) on MCF cancer cells activity.

Materials and Methods

The combination of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) was synthesized from the reaction of orthophenylene diamine with 4-methoxybenzaldehyde in the presence of ferric hydrogen sulfate (FHS) catalyst at room temperature. This reaction was performed in a mixture of water and ethanol solvents. The reaction scheme is as follows:

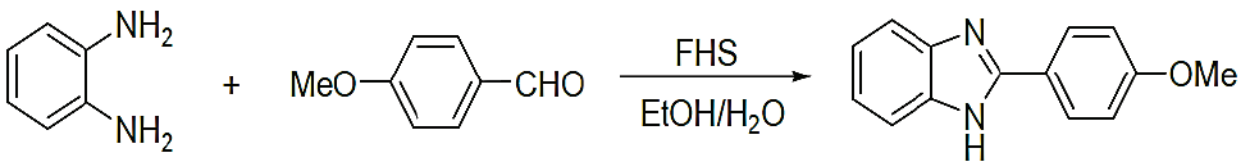


Figure 2. Reaction scheme of study

To investigate the cytotoxicity of the above compound, 8000 MCF 7 cells were cultured in each 96-well flask in DMEM cell culture medium have 10% serum of bovine then incubated in 37 ° C and CO_2 (10%) for 48 hours. To determine the concentration of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) that causes half of the cells to die (IC_{50}) MCF7 cells with different concentrations of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) (10 , 20, 30, 40, 80 and 160 µg/ml) and were treated with the same amount of DMSO used to dissolve the desired compound. Cell viability was evaluated by MTT assay at 24 and 48 h intervals.

For the MTT assay, the tetrazolium solution was prepared in a concentration of 5 mg/ml and after filtration in each well, a 20-ml volume of 96-well flask was added and kept for 4 hours in the dark at 37 °C. After the passage of time, during which the metabolism of tetrazolium was formed in the living cells of formazan purple crystals, the contents of each house were replaced with 150 µl of DMSO and colored violet solutions of varying color intensities were created. The amount of light absorbed by each cell, which is a measure of cell viability, was recorded by an ELISA reader at 545 nm.

Results and Discussion

In this study, we investigated the potential of the new compound of 2-Thioxo imidazolidin-4-ones (2 Thiohydantion) as an anticancer agent against a cell line that is a cancer model system. To determine the IC_{50} of this compound, cancer cells were treated with different concentrations (10, 20, 30, 40, 80 and 160 µg / ml) and then the cells were evaluated by MTT assay at 24 and 48 hour intervals.

After synthesis of the desired compound, MTT assay showed that the IC_{50} of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) compound on MCF7 cells, 135 and 40 µg / ml, respectively, after 24 and 48 h, respectively. In addition, the toxicity of these compounds on normal cells was evaluated, which is very different from their toxicity for cancer cells, and this is a very important feature. The 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) compound has the potential to induce cell death on cancer cells, and the results revealed new insights into the use of these compounds in the treatment of cancerous tumors.

Morphological observations have also shown that it is able to exert its toxic effects in the form of cytoplasmic granules, especially after 48 h. According to the results, 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) can be considered as a substance that has the potential to kill cancer cells and given that it retains its effect for a long time, further studies could potentially be used as an anticancer compound (Figure 3).

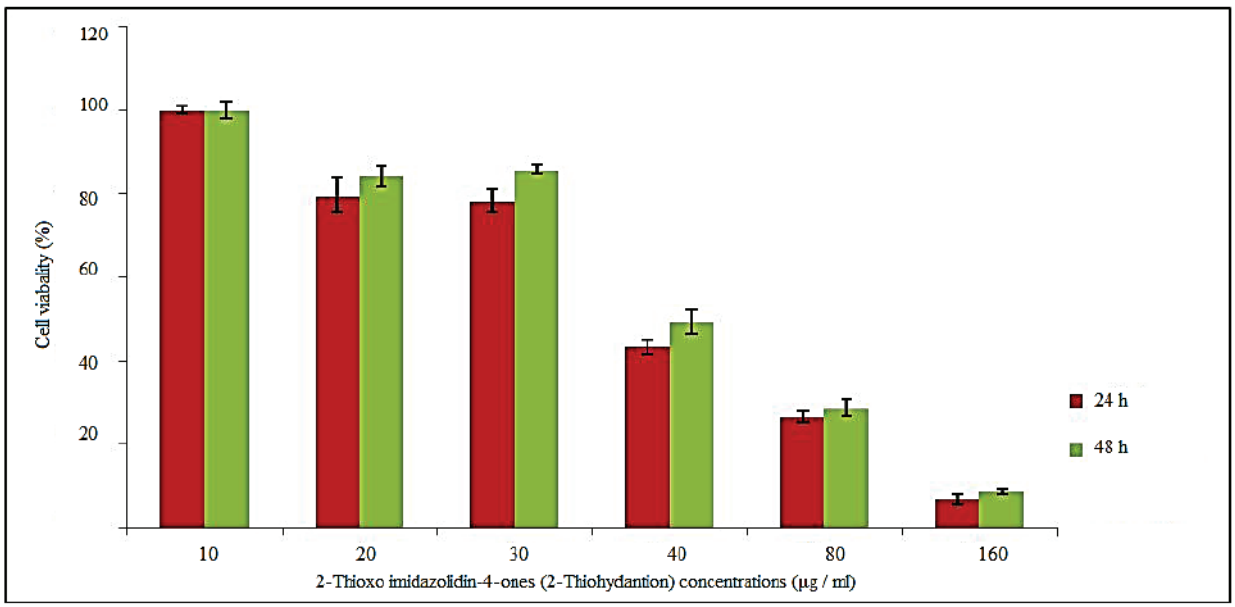


Figure 3. cells viability percent in different concentrations of 2-Thioxo imidazolidin-4-ones

(2-Thiohydantion) at time 24 and 48 hours.

Figure 3 shows that viability rates for 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) were dependent on concentration and time by analogy with the control group, the effect of this compound on the cells increased with increasing concentration and time, thus decreasing viability rate.

Repeated measurement statistical test showed that the effect of concentration increase on viability was statistically significant ($p < 0.05$). In addition, the same test to examine the effect of time showed that increasing time also had a significant effect on decreasing survival rate ($p < 0.05$). The 50 IC for 24 hours was 135 µg/ ml and for 48 hours was 40 µg/ ml.

Considering the IC_{50} of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) for various cancer cells is different and also due to the high prevalence of breast cancer in Iran, the present study was conducted. In current study, we assessed the toxicity of this compound on the cancer cell by MTT method and determined the IC_{50} level for it. The results also showed that the viability rate for the compound 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) decreased significantly with increasing time, so that the viability rate at 24 was greater than 48.

Therefore, this substance has been considered as an effective compound for the treatment of cancer. Some studies confirm significant association between benzimidazole derivatives and reduction in cardiovascular disease and cancer and they are used to make antibacterial, antiviral, antifungal and anti-tumor drugs. This derivative of benzimidazole has anticancer properties.

Several studies have been conducted to determine the therapeutic dose and also to determine their IC_{50} on a variety of cancer cells.[24]

In this study, we determined viability rates and IC_{50} on cancer cells. The results of these study showed that the viability rate of the cells in the control group was highest, which is 100%. But after 24 and 48 hours exposure to 2-Thioxo imidazolidin-4-ones (2-Thiohydantion), at two time points, it was shown that the decrease in viability was dependent on both concentration and time and with both factors increased. The survival rate of the cells was significantly decreased. MTT assay showed that the IC_{50} of 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) compound on MCF7 cells, 135 and 40 µg / ml, respectively, after 24 and 48 h, respectively. However, the principal current interest comes from the application of thiohydantoins for the treatment of prostate cancers.[25-26] The anti-cancer effects of all synthesized compounds have been evaluated in compared with two cell lines HepG-2 cells (human hepatocellular cancer cell line), and MCF-7 (breast carcinoma cell line).[27-28]

Conclusion

The final results showed that cell viability depended on the concentration of 2-Thioxo Imidazolidin-4-Ones (2-Thiohydantion) and the incubation time. As the concentration of the solution increased, the toxicity increased and the cell viability decreased 48 hours compared to 24 hours.

So 2-Thioxo imidazolidin-4-ones (2-Thiohydantion) at different concentrations significantly inhibition the growth of cancer cells compared to the control group (50% reduce of cell growth). Future studies will need to investigate the mechanism of this cytotoxicity as well as the possible anticancer effects of this compound in vitro and in vivo.

Conflict of Interest: Nil/The authors declare no conflicts of interest.

Source of funding: The study was self funded.

Ethical Clearance: Obtained from the Institutional Ethics Committee of Wasit University of Iraq.

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Determination of Probiotic Potential and Anticancer Activity of *Lactobacillus* Isolated from Cheese and Yogurt

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Abstract

In this study, microorganism was isolated from cheese and yogurt, cultured on specific culture media, then recognized molecularly through 16S rDNA f quality sequencing. The supernatant of *Lactobacillus* strains contained probiotic activity such as anticancer effects against cancer cell lines such as HeLa, RD and normal cell line (REF)cells and antibacterial. The cytotoxicity effects were assessed by MTT stain and apoptosis in mitochondria. The supernatant showed had anticancer activity against the tested cell lines ($P< 0.05$) with no significant cytotoxic effects against normal cell line .

Key words*: lactobacillus, cheese, yogurt, anticancer, apoptosis*

Introduction

Probiotic microorganisms consider fermented food products and are known to offer functional effects on mucosal damages, specifically preventing the effects of cancer on the digestive tract [1,2]. Probiotic microbes, which are known to lead to the generation of therapeutic anti-carcinogenic compounds, are primarily dependent on determination the relationship between probiotics and colorectal cancer [3,4].The present examination was planned to survey the fprobiotic and fanticancer exercises of *Lactobacillus*f.

Material and Methods

Isolation of *Lactobacillus*

42 isolates **of *Lactobacillus*** were gatheredf from dairy items which were gathered from various markets in Baghdad incorporate new drain of bovinesf, imported yogurtf (fActiviaf), localf yogurtf (fcanonf) and cheese f. For all isolates, about 1 gm or 1 ml was taken from source and was grown in 9 ml MRS broth (Himedia-India), and subcultures were done to get pure isolates by streaking petri dishs. The culture was incubated anaerobically at 37 °C for 24- 48 hrs. Depending on the cultural, microscopical examinations and biochemical tests, 42 strains were identified as *Lactobacillus* spp. [5].

preparation of Cell- Free culture supernatant (CFCS):

The isolates were inoculated in MRS broth for 24 hrs. at 37ºC . Bacterial cells were evacuated by centrifuging the culture at 8000 rpm for 15 min at 4ºC. pH of fsupernatant was adjusted at 6.5-7.0 by the faddition of 1N NaOHf and supernatants were filtrated through milliporef 0.22 µmf [6,7].

DNA extraction and Identification by PCR

DNA was extracted from *Lactobacillus* strains. DNA mini kit Geneaid, (Korea) was used according to the manufacturer's instructions [20]. PCRf intensificationf conditions were fperformed by [8,9].

Antibiotic Tests

The antibiotic susceptibility of the strains was examined by disc diffusion method against some antibiotics such as: Amoxicillin Clavulanic acid, Erythromycin, Imipenem, Ceftazidime, Vancomycin, Clindamycin, Chloramphenicol and Cefotaxime. Inhibition zones were compared with Standards of Antimicrobial Disk Susceptibility tests [10,11].

Probiotic antimicrobial assay

The diffusion method was used to determine inhibition effects for bacterial extraction toward many pathogenic

bacteria such as *Escherichia coli, Streptococcus spp, Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Candida albicans* isolates which were obtained from lab of graduate studies in Biology Department /Sciences Collage / Baghdad University/ Iraq. Culture media were used to inoculate pathogenic bacteria and fungi. Wells were made in the media to inoculate 50 µL of CFCS and incubated at 37°C for 18 hrs.. After that measurement of inhibition zones was performed [12].

Cytotoxicity by MTT assay

Cytotoxic impact of the **CFCS** on proliferation of the adherent cells in 96-well microtiter plate had been performed by [13,14].

Apoptotic cell detection

The dye of Mitocapture reagent kit would be concentrated in the mitochondria. The assay was carried out according to [15].

Statistical Analysis

One route of analysis of ANOVA was performed to test whether group variance was significant or not. Statistical analysis was performed by utilizing the program SPSS. Differences were considered significant at $P< 0.05$. **[16]**

Results and Discussion

Isolation and identification

Results of current study demonstrated that 42 isolates which were isolated from cheese and yogurt confined Gram-positive, bacilli. Catalase, gelatinase, and indole tests were negative, whereas, both Arginine and Skim milk coagulation tests were positive (table1). Molecular identification was depended on 16S rDNA sequencing, belong to *lactobacillus spp*. figure (1)

Antibiotic susceptibility

This examination revealed that this bacteria which was isolated from cheese and yogurt, was sensitive to vancomycin, erythromycin, amoxicillin clavulanic acid, clindamycin, and Imipenem. However, it was resistant to chloramphenicol and with intermediate susceptibility to cefotaxime, ceftazidine (table 2) . Here, it was affirmed that tested isolate can be considered as probiotics, possesses strong antibacterial and anticancer properties.[17].

Probiotic antimicrobial activity

Antimicrobial activity of CFCS of the *lactobacillus spp* isolates was tested against some pathogenic microorganisms, and showed the capacity to have abroad range of inhibition activities against isolates *E coli, Streptococcus spp, S aureus, P aeruginosa*, and *Candida albicans* (table 3). CFCS demonstrated inhibition zones which were greater than 18 ,13,10 mm against *E. coli*, *S. aureus* and *Streptococcus spp.* respectively, but did not affect *candida albicans* and *P. aeroginosa*. Lactobacilli can inhibit pathogen colonization and consequently prevent contamination. Present investigation fexhibited that the chosen isolates of *lactobacillus* are great probiotic strains, which concurred with previous study [18].

Cell viability assay

cytotoxicity was performed by utilizing MTT technique. Dose and time were depended on the cytotoxic impacts of *lactobacillus spp* against HeLa, RD and normal cell line (REF) cells and was carried out in three periods (24, 48 and 72 hrs. as showed in figure (2). Cancer cells viability was significantly inhibited according to time and dose treatment. It was shown that increasing of incubation time and dose of CFCS led to decrease the Hela and RD cancer cells viability and inhibition percentage were 48% and 36% respectively after 24hrs. of incubation. Then again, it was shown that in every incubation period and concentration that were used in this study, important inhibition effect on HeLa ($P<0.05$) was appeared, and the highest dose (70µg/mL) in the greatest time (72hrs.) gave the best inhibition effect against HeLa cells.

The antiproliferative impact of the CFCS was more noteworthy against HeLa than against RD cells. Additionally, the effect of **CFCS** against REF normal cells was determined , and it was indicated that CFCS had the important effect on cancer cells but no effect against REF normal cells. Therefore, over 90% of REF cells were viable . Production of pro-inflammatory cytokines, in addition to oxidative stress achieved by MTT was thought to have responsibility to cause cells death [19].

Apoptosis

The results showed that there is a significant effect of **CFCS** on cell proliferation in cancer cell lines (Hela and RD) and in normal cell line (REF) by using mitochondrial kit which was chosen to detect its antiapoptotic effects. The apoptotic cells appeared green regions and healthy cells were red regions. Both treated and untreated (control) cells have been delineated and computed under the fluorescent microscope using cationic fluorescence dye (mitocapture reagent). The percentage of apoptosis was determined as a mean of five fields for each test which was clearly showed apoptosis induction in treated groups in comparison to their control. The different concentrations of **CFCS** showed significant differences at $P \leq 0.05$ in comparison to control, and the high concentration 1000µg/ml gave 84% and 69% of apoptosis in Hela, and RD cells respectively. In living cells, mitochondria continuously divide and combine with each other [20] and Bcl-2 family members are engaged with these procedures. As a conclusion, it was discovered that **CFCS** has a cytotoxic activity, and confirmed that the tested isolates had anticancer activity impacts against some cancer cell lines (table 4**).**

Table (1): Result of Biochemical tests of *Lactobacillus spp*

Biochemical Tests	Result
Catalase test	–
Oxidase test	–
Indol test	–
Gelatinase test	–
Arginine test	+
Skim milk coagulation	+

Table 2: Antibiotic discs used in the present work

Id	Antibiotic discs	Disc potency(µg/ disc)	Zone diameter (mm)	Susceptibility
1	Vancomycin	30	21	S
2	Ceftazidime	10	16	I
3	Imipenem	10	23	S
4	Clindamycin	2	23	S
5	chloramphenicol	30	7	R
6	Erythromycin	15	25	S
7	Cefotaxime	10	16	I
8	Amoxicillin Clavulanic acid	10/20	18	S

S= sensitive ; R= resistant ; I= intermediate.

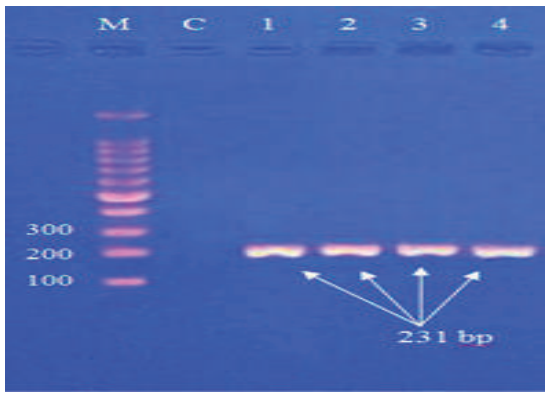
Table 3: The inhibitory effect of *Lactobacillus ssp* against pathogenic bacteria

Pathogenic bacteria	Growth	Susceptibility
Escherichia coli		ES
S. aureus		S
Streptococcus spp		I
P. aeruginosa		R
Candida albicans		R

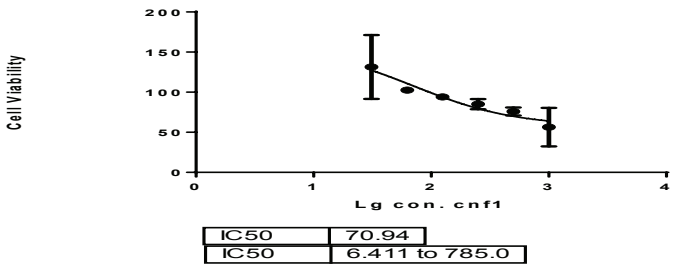
R=Resistant ,S=Sensitive (11-14) , Intermediate (6-10) ,ER=Extra sensitive >14

Table(4) Apoptosis ratio of cell lines induced by significant concentrations of CFCS

CFCS concentration(µg/ml)	Hela cell	RD cell	REF cell
1000	84	69	11%
500	78	61	6%
250	56	47	3%
125	46	32	0%



Figure(1) Gel electrophoresis for amplification genes encoded to 16SrRNA in Lactobacillus isolates. Electrophoresis was performed on 1% agarose gel and run with **at 5 V/cm for 1 hour, stained with ethidium bromide and visualized on a UV transiluminator documentation system. The size of amplified gene at 231pb**



Figure(2) Cytotoxicity effect of partial purified CFCS on Hela cells after 72 hours of incubation at 37ºC

Conclusion: The supernatant of *Lactobacillus* strains contained probiotic activity (antibacterial and anticancer) such as apoptosis in mitochondria.

Conflict of Interest: No conflict of Interest

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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The Protective Effect of Annona Extracts on Renal Histopathology Induced by Ethylene Glycol in Male Rabbits

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Abstract

Annona is one of the many plant extracts that have been explored owing to their anti-inflammatory and anticancer effects. Male locale rabbit were separated into five groups, Group I consider as control, The 4th other groups were treated orally with 0.75% ethylene glycol (EG) to induce renal damage this treatment done till 30th days. Group III to V animals were served as curative regimen and received flavonoids, glycosides and alkaloids extract of the plant of Annona at a dose of 100 mg/kg body weight from 15th day to 30th day. The extracts was administered twice daily by oral route. significant kidney injury in EG animals as demonstrated by a significant elevation (P= 0.05) in serum activities urea, creatinine, total protein and calculated globulin levels. Meanwhile there was significant drop (P=0.05) in albumin, and A/G ratio compared to the control and treated group , The levels of lipid peroxidation were significantly higher in the Serum of the groups of animals treated with EG , while the levels of SOD, CAT and GPx were significantly lower than animals were treatment of EG group with Annona significantly elevated the antioxidant mechanisms differ with that before treatment. Renal Histopathological changes includes deposition of oxalates in the renal tubules lumen, degeneration and necrosis of epithelium of renal tubules , congestion of blood cells , infiltration of inflammatory cells and atrophy in glomerular tuft leading to expansion of bowmans spaces. While Histopathological changes in animals group treated with Annona extract and ethylene glycol were limited dilatation of renal tubules with vascular changes with blood vessels congestion and interstitial hemorrhage , reduced renal damage revealed by less degenerative and necrotic lesion in tubular epithelium and no inflammatory reaction and the glomeruli appeared normal compared with ethylene glycol sections.

Keyword: *Anonna extract , Ethylene glycol, Antioxidant , Kidney, Histopathology*

Introduction

Ethylene glycol is a colorless , odorless and sweet testing synthetic organic chemical substance , which is used in different consumer and industrial application (Staples et al., 2001) , further more ethylene glycol used anti freezer , poly ester fiber and poly ester resins , heat transfer agent and as solvent, manufactured of asphalt emulsion paints (song et al., 2017). As time progresses, we become more aware of what nature contains as beneficial to our health, and with it our desire to learn more about these elements, One of these ancient elements in its existence, which has been talked about in recent research is the fruit of the cream (Annona), where research on the components of this fruit and on its health benefits (Asma et al., 2020 (. Annona is one of the many fruit extracts that have been explored due to their anti-inflammatory and anticancer properties. Extensive phytochemical evaluations on different parts of the Annona plant have shown that the presence of different compounds and phytoconstituents which include alkaloids, megastigmanes , flavonol,triglycosides, phenolics , cyclopeptides and essential oils(Coria-Téllez et al., 2018). The barks, seeds , leaves and fruit which taken from Annona have been used for various ethnomedicinal purposes by traditional healers to treat several diseases including cancer, inflammation, diabetes, liver diseases, and abscesses , Also It is well known that fruits are rich in various antioxidants, including ascorbic

acid, carotenoids, and phenolics(Yajid et al., 2018). Antioxidants are substances present at low concentration that protect cells components from damage caused by unstable molecules known reactive oxygen species. (Gyesi eal.,2019 ; Manochai et al., 2018).

Material and Methods

Annona was purchased from the local market. It was classified according to plant classification references related to medicinal plants (10). Also a vouchers specimens of the plant was identified and authenticated at the herbariums of the College of Education, University of Mosul.

- Preparation of extracts

Preparation of flavonoids, glycosides and alkaloids extracts of Annona were done according to the method described by(11).

- Animal grouping

Male locale rabbit's weightings between 700-800 gm were used , rabbits were separated into 5 groups, each containing 3 animals. The first group consider as control and treated on normal laboratory diet and water ad labium. The second group till to 5th animals were treated with 0.75% ethylene glycol (EG) in water to induce renal damage for 30th day (12). Group III to V group were served as curative regimen and received flavonoids, glycosides and alkaloids extract of the Annona at a dose of 100 mg/kg B.W. from 15th day to 30th day. The extracts was administered twice daily by oral ingestion.

- Kidney function tests

The serum was collected from the blood sample for the determination levels of total protein & albumin by using (17). The estimation of globulin levels and Albumin/globulin ratios, urea(18) and creatinine (19).

- Estimation Lipid peroxidation and antioxidant enzyme in serum.

Estimation of malondialdehyde (MDA) levels via Thiobarbituric acid reactions technique. Thiobarbituric acid (TBARS) in the serum was measured by the technique of(20)& estimating the GSH by the method of (21).Antioxidant enzyme activities (CAT, SOD and GPx), the activities were estimated by the methods of (22,23) .

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- Histopathology

Tissue samples , were collected from the kidney and fixed in Neutral Buffered formalin 10 % solution for 3-5 days then trimmed to suitable sizes , washed with tap water, dehydrated in ascending concentration of ethyl alcohol (70 ,90, 100%) , cleared with xylene , Embedded by paraffine wax, Sectioned by microtome at 5-6 μM , stained with routine hematoxylin & Eosin and finally examined under a light microscope (24).

Finding

Table 1 showed that rabbits exposed to the EG alone developing significant kidney injury as demonstrated by a significant elevation (P= 0.05) in serum activities urea, creatinine, total protein and calculated globulin levels. Meanwhile there was significant drop (P=0.05) in albumin, and A/G ratio compared to the control and treated group. Table 2 revealed levels of lipid peroxidation were significantly elevated in Serum of EG group comparing with control animals . Also the result showed a significantly elevation in GSH level in the serum of rabbits. While the level of the SOD, CAT and GPx were significantly lower than the normal animlas , were EG animals which treated with Annona significantly elevated the antioxidant protection mechanisms compared with animals treated with EG.

Table1: Changes in kidney function tests ethylene glycol group and treated ethylene glycol group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
urea(mg/dl)	18.95±0.805	38.17±1.02*	21.61±0. 19	31.55±2.325 *	30.01±1.14*
creatinine(mg/dl)	2.283±0.08	4.133±0.16*	2.13±0.13	4.33±0.20 *	2.113±0.29
Total Protein (g/dL)	10.72±0.03	12.46±0.11*	11.89±0.11	12.11±0.10	10.79±0.11
Albumin (g/dL)	6.35±0.03	5.79±0.55*	7.17±0.61	7.77±0.42	6.47±0.42
Globulin (g/dL)	3.57±0.05	4.44±0.23*	4.54±0.12*	4.44±0.12*	4.21±0.11*
A/G Ratio	1.78±0.04	1.31 ±0.23*	1.57±0.20	1.75±0.12	1.53±0.12*

Blood samples were taken after 30 days of oral administration, number of rabbit each group = 5 Values are given as mean± SD

. * means P value <0.05 = significant level

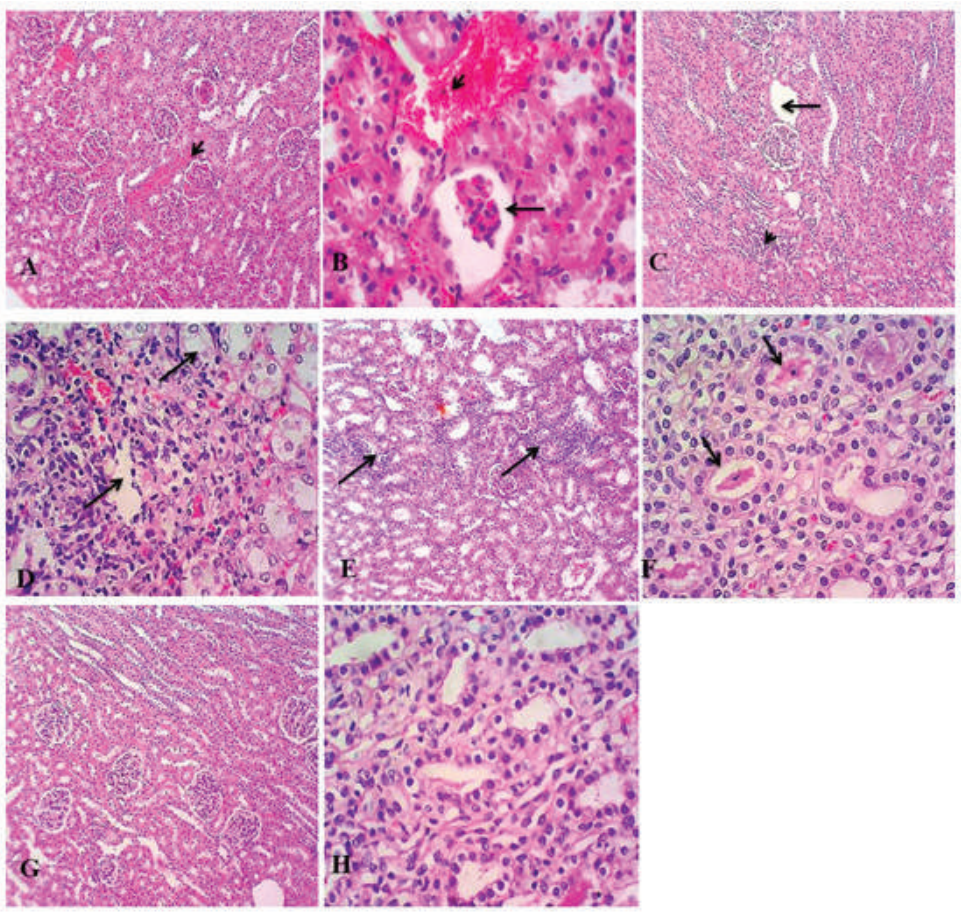
Table 2: Changes in kidney lipid peroxidation and enzymatic antioxidants of ethylene glycol group and treated ethylene glycol group as compared to control.

Parameters	Control	EG group	EG group with flavonoid	EG group with glycoside	EG group with alkaloids
MDA (mM/100 g tissue)	0.52±0.02	0.92±0.02	0.95±0.03	0.87±0.03	0.58±0.02
GSH (mg/100mg tissue)	85±3.21	47±2.8	78±3.3	79±3.3	81±2.9
CAT (mmol/l)	0.170±0.01	0.09±0.01	0.132±0.02	0.169±0.02	0.136±0.02
GPx (U/mg protein)	6.80±0.51	3.43±0.32	6.55±0.42	5.43±0.49	6.98±0.49
SOD	2.30±0.2	3.23±0.2	2.07±0.12	2.01±0.13	2.06±0.12

MDA = Malondialdehyde; GSH = glutathione; CAT= Catalase; GPx = glutathione peroxdiase; SOD = superoxide dismutase, tissue samples were taken after 30 days of oral administration, number of rabbit each group = 5 Values are given as mean± SD. * means P value <0.05 = significant level.

Histopathological finding:

The histopathological examination of renal sections taken from kidney specimens of animals treated with ethylene glycol indicated that the administration of ethylene glycol for thirty days lesions involved the cortical renal tubules which characterized by deposition of oxalate crystals in the lumen of renal tubules , degeneration and necrosis of the epithelial cells lining the renal tubules as well as tubular dilatation with cystic formation in renal paranchyma , Other sections revealed infiltration of inflammatory cells in the interstitial parenchyma , vascular changes also observed which characterized by generalized blood vessels congestion with vasculitis. The histopathological lesions in renal glomeruli characterized by expansion of the bowman's space which appear as a result of atrophy and shrinkage of the glomerular tuft. While the group of animals which treated with ethylene glycol and flavonoid showed improvement in the histopathology of the kidney , these histopathological changes were limited dilatation of renal tubules with vascular changes which were blood vessels congestion and interstitial hemorrhage , with reduced renal damage revealed by less degenerative and necrotic lesion in tubular epithelium and no inflammatory reaction and the glomeruli appeared normal compared with ethylene glycol sections (Figure 1).



Discussion

This study, showed that Annona extract have an ameliorative effect and reduction on kidney damage caused by ethylene glycol. Ethylene glycol cause damage in the renal functional capacity , kidney fail to carry out their normal metabolic and endocrine functions , this will cause alteration in tubular function or structure affect glomerular function and vice versa (Tsujihata, 2008). Also this occur due to deposition of oxalates in the lumen of the renal tubules which induced inflammatory reaction then leads to increase in the concentration of proteins (globulins and albumins) in the blood

(Tugcu et al., 2008). Annona is a species of the Annonaceae family that has been commonly studied in the last periods due to its therapeutic actions. Table No. (1) illustrates kidney function in rabbit treated with ethylene glycol and Annona extract as demonstrated that decreasing in serum levels , urea, creatinine, total protein and calculated globulin levels , compare with ethylene glycol and control rabbits , While table No. (2). Natural antioxidants from plant species have gained interest due to their protective effect against oxygen-derived from free radicals involved in the development of many diseases . This result agreement with that of Abdul Wahab (2018) and Correa-Gordillo et al. (2012) were domenstrated that the Annona extract have an antioxidant protective and improve organs properties. A histopathological study of kidney showed severe damage and crystal formation occur by ethylene glycol treated animals as compare with animals treated with flavonoid of Annona extract that reverted and ameliorative the renal damage , and these results were agreement with (Abdul Wahab ,2018) That demonstrated the organ protection , antioxidant and anti inflammatory properoties of Annona extract. Histological alteration of renal tissue reported in this study occur because the kidney is a critical organ for the toxicity of ethylene glycol in both human and experimental animals. Consistently cause metabolic acidosis , degeneration and necrosis and deposition of calcium oxalates in the lumen of the renal tubules , these changes have been observed in our study. (Song et al., 2017 ; Khatib et al., 2010 ; Thapa and Walia , 2007). The treatment with ethylene glycol cause deposition Ca+ oxalates because the end product of ethylene glycol metabolism is oxalates which cause histological changes in the renal tubules and glomeruli causing degeneration and necrosis (Thamilselvan and Khan, 1998 ; Hadjzadhe et al., 2007). The protection against free radicals are done by preventing their formation , by blocking the chain reaction or by repairing the oxidative damage of these biomolecules (Gyesi et al., 2018) .

Conclusion

The conclusion of this study showed that Annona extracts have ameliorative effect of renal histopathological changes and biochemical changes induced by ethylene glycol.

Conflict of Interest: non

Source of Findings: was done by the researcher himself and without any government funding

Ethical Clearance: non

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The Prevalence of Illicit Drugs and Alcohol in Road Traffic Accident Fatalities in the Eastern Region of Saudi Arabia

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Abstract

The study aims to investigate the prevalence of alcohol and illicit drugs in Road Traffic Accident fatalities from January 2015 to December 2019 in Eastern region – Saudi Arabia**.** From a total of 1939 deaths investigated in the Dammam Poison Control Center, only 57 of them were related to Road Traffic Accidents (RTA), of which 96.4% were males, 36.8% were in the age group between 18–30 years. The highest incidence was among Saudi nationals (82.5%). 70.2% of the investigated cases tested positive for ethanol either alone or with other illicit drugs, cannabis ranked the second (38.6%), followed by amphetamine (17%) and heroin (10%). Ethanol was the sole toxicological finding in (57.5%). The single drug–abusing pattern (50.9%) exceeded bi-and triple drug–abusing pattern (31.5 %). None of the investigated samples showed a positive result for illicit cocaine or benzodiazepines. In Conclusion, ethanol identified as a key element, being the highest frequently detected substance in the blood samples of RTA victims which mandates the need for planning appropriate interventions and more strict application of Saudi national program for drug prevention together with wide community action.

Keywords: *Blood Alcohol; illicit drugs; Patterns of Abuse; RTA; Saudi Arabia*

Introduction

Road fatalities in KSA have increased over the last decade from 17.4 to 24 per 100000 individuals compared with 10 in the USA and 5 in the UK, respectively, where road safety rules are being meticulously applied and all primary and secondary preventive measures are being applied strictly [(1)].

Alcohol and illicit drugs have a strong association with the increased incidence of RTA fatalities, which is considered a cause of concern and an alarming problem across the world. Driving under the Influence of Drugs (DUID) and its inhibitory effect on the central nervous system increases the risk of road accidents [(2)].

For instance, alcohol significantly diminishes individuals' capabilities and driving skills due to its effect on coordination, alertness, and reflexes of the user [(3)]. In South Africa and Slovenia, alcohol was the main cause of road accidents causing 58% and 42.4% pedestrian mortalities from 2001 to 2004 and 1999 to 2006, respectively [(4,5)].

This study aims to investigate the prevalence of illicit drugs and alcohol in Road Traffic Accidents (RTA) fatalities from January 2015 to December 2019 in the eastern region of Saudi Arabia**.**

Methods

Collection of Data

Data of RTA deaths was collected by referring to postmortem toxicology and autopsy reports. Data analysis concerning demographics, victim status, state of putrefaction, types of the collected samples, toxicology findings, and pattern of drug abusing were taken into consideration.

Analytical Methods

Blood samples were collected from the femoral vein (being the most reliable sample for toxicology analysis) in containers with sodium fluoride as a preservative (1–

2%, v/v) to determine ethanol level. A vitreous sample (whenever submitted) was used to confirm the level of ethanol in the samples. Urine was initially screened for ethanol and illicit drugs (amphetamine, barbiturates, benzodiazepine, cannabis, cocaine, and opiates) by Fluorescence Polarization Immunoassay (FPIA on ARCHITECT system i1000 SR and c4000, model by Abbott laboratories). Quantification and confirmation of positive screening results were conducted by liquid-chromatography mass-spectrometry after liquid–liquid extraction [6]. Detection of cannabis was based on the presence of Tetrahydrocannabinol (THC) using a method of a solid-phase extraction. [7]. Samples were tested for all volatiles including ethanol using gas chromatography–headspace GC ultra-model K0C33B730000000, Milano, Italy. Other drugs were analyzed by a gas chromatography mass-spectrometer GC– MS–QP2010, Shimadzu.

Blood and urine samples were tested for Ethyl Glucuronide and Ethyl Sulphate (EtG and EtS) by liquid chromatography-tandem mass spectrometry by using the methodology approved by Helander et al. [8].To distinguish between antemortem and postmortem blood alcohol source, only cases with EtG levels ≥ 0.5 µg/mL (positive cut-off for EtG) and EtS ≥ 0.1 µg /mL (positive cut-off for EtS) were included in the study.

Statistical Analysis

Data of the investigated cases were submitted for statistical evaluation by SPSS version 25 and the results were compared with other similar studies.

Results

From a total of 1939 deaths investigated in Dammam Poison Control Center, only 57 RTA–related deaths were identified. As shown in table 1, the highest number of RTA fatalities was found in males (96.4%) and the commonly affected age group was 18–30 years old. Saudi nationals represented the dominant nationality 82.5% while non-Saudi people represented 17.5 %.

70.2% of the studied samples yielded ethanol in their analytical results. Cannabis ranked second (38.6%), followed by amphetamine (17%) and heroin (10%); samples were reported positive for heroin after the detection of 6-mono acetyl morphine in the urine samples as shown in figure 1.

The analytical findings showed that 82.4% (n=47) of the investigated fatally injured RTA victims had one or more substances detected in their specimens. While 17.5 % had negative analytical results. The pattern of abusing single drugs predominated (n=29 ,50.8 %) over those of bi-and triple drug abusing (n= 18,31.5 %) (figure 2).

Of the total ethanol positive samples (n = 40), ethanol was the sole toxicological finding in 57.5% and combined with other substances in 42.9 %. The combination of ethanol with cannabis was the most commonly detected combination (4.8 %) (figure 3).

Distribution of BAC means showed that the highest mean (270 mg/dl) was found in the 18–30 years old age group, while the lowest mean (114 mg/dl) was observed in the 51–60 years old age group (Figure4).

Most of the victims were drivers (56%), others were pedestrians and car passengers (21% and 14%). However, the categorization of other RTA fatalities (8.7%) was not evident due to the lack required data. Variable ranges of alcohol concentration were detected among different victim categories tested positive for ethanol (Table 2).

Table (1): Socio-demographic characteristics of RTA victims included in the study.

Age group	Sex		Total No (%)
	Males No (%)	Females No (%)	
< 18 yrs	1 (100%)	0(0.00%)	1 (1.8%)
18-30 yrs	21(100%)	0. (0.00%)	21(36.8%)
31-40 yrs	14 (93.33%)	1(6.67%)	15 (26.3%)
41-50 yrs	16 (94.12%)	1(5.88%)	17(29.8%)
51-60 yrs	2(100%)	0.0(0.0%)	2 (3.5%)

Cont... **Table (1): Socio-demographic characteristics of RTA victims included in the study.**

>60 yrs		1(100%)	0.0(0.0%)	1(1.8%)
Total		55(96.4%)	2 (3.5%)	57 (100%)
Nationality	Saudi	45 (95.74%)	2(4.26%)	47(82.5%)
	Non-Saudi	10(100%)	0(0.00%)	10(17.5%)

Table (2): Summary of alcohol results in blood and urine showing the range of concentrations and the percentage number above the current KSA legal driving limit among different victim categories

		Alcohol concentration ranges mg/dl					**Overall ranges mg/dl**	**% No above legal driving limit**
Victim Category	Samples no	< 50	51-100	101-200	201-300	>300		a Blood > 0.00 mg/dl
Drivers= 23	Blood 35 Urine 11	4 1	8 3	15 5	5 2	3 0	30-410 15–290	100%
Pedestrians=7	Blood 9 Urine 5	1 2	2 0	4 3	0 0	2 0	13-516 40-165	100%
Car passengers=5	Blood 7 Urine 3	1 1	3 0	2 2	1 0	0 0	24-282 28-166	100%
Unknown =5	Blood 8 Urine 2	1 0	1 1	5 1	1 0	0 0	42-287 78-160	100%

[a] Saudi Arabia has a strict, zero-tolerance policy for alcohol

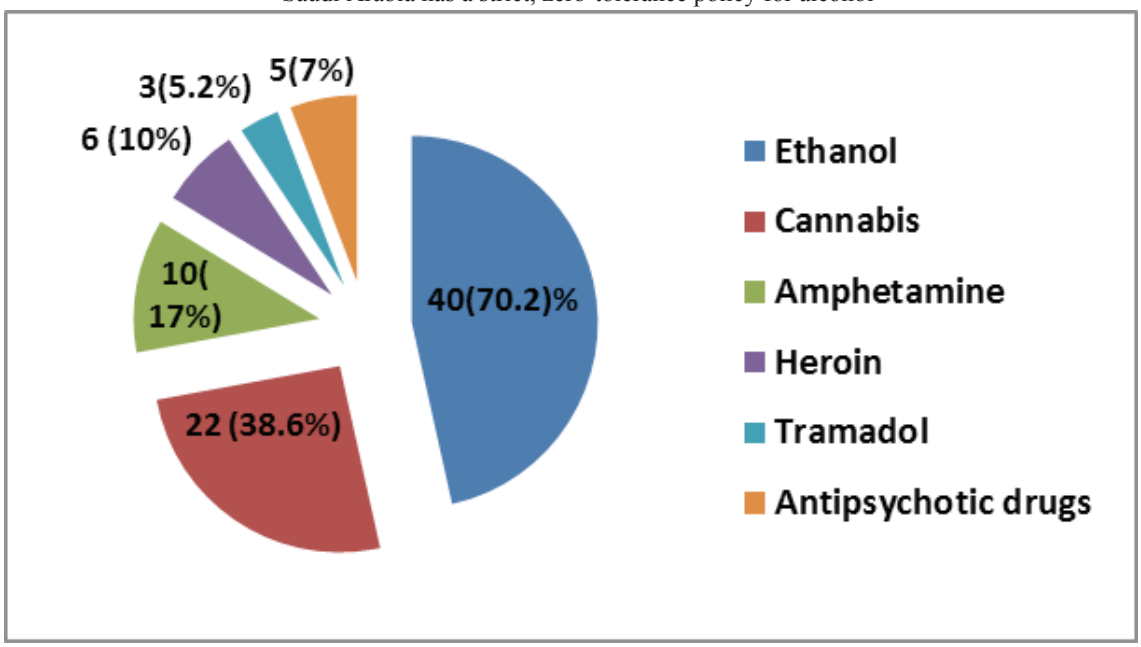


Figure (1): Identified drugs in the studied postmortem samples

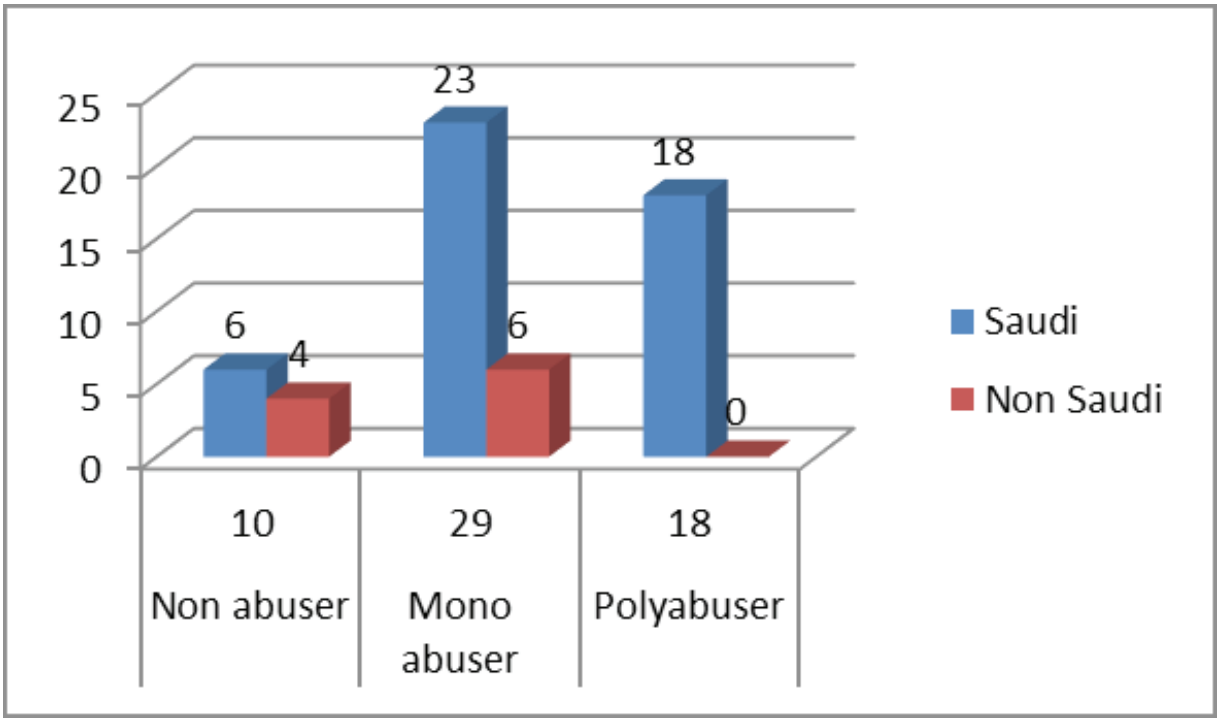


Figure (2): pattern of drug and substance abusing according to victim's nationality

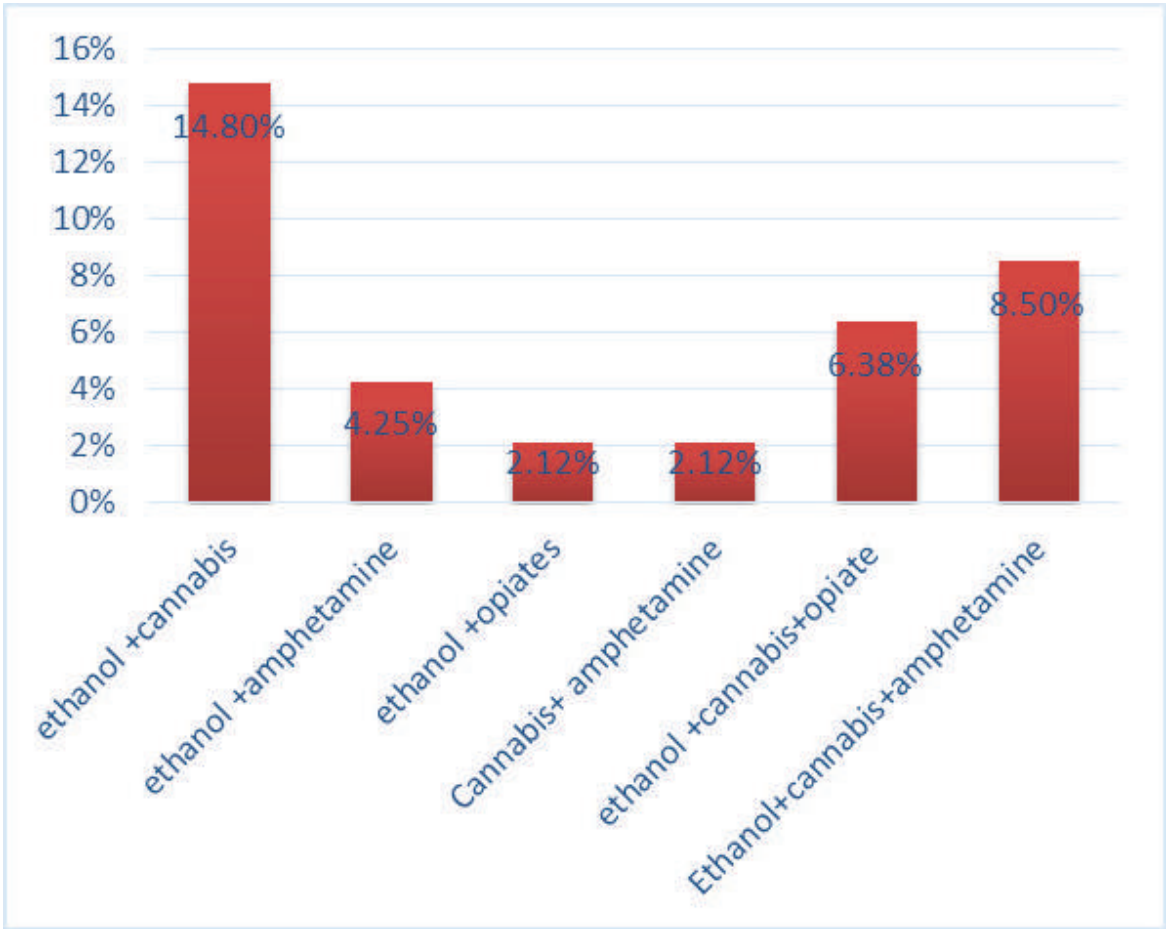


Figure (3): incidence of drug combinations detected in postmortem samples.

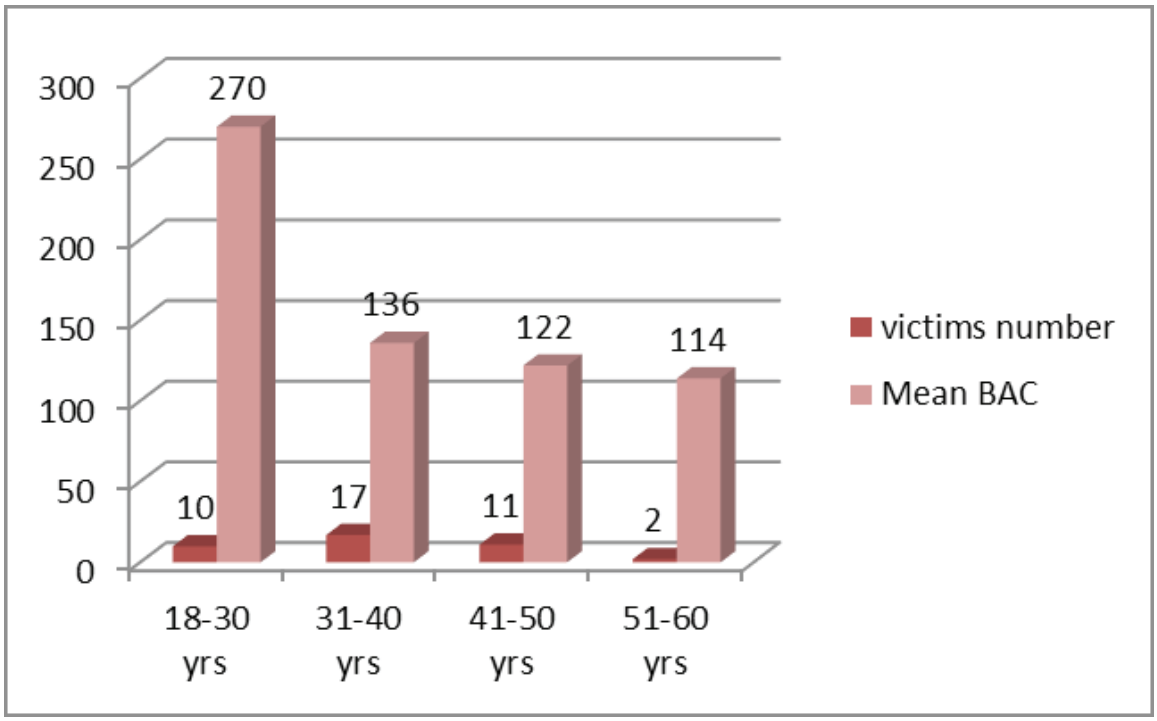


Figure (4): Distribution of BAC means in relation to age groups.

Discussion

There is a growing concern regarding the correlation between driving under the influence and RTA, where alcohol and abused drugs can affect the behavior and cause perception alteration for those who consume it [9]. The results of the study showed that males were dominantly present in fatal road traffic accidents and represented 96.4%, which concurs with statements by other authors [10].This prominent sex factor could be attributed to the Saudi culture of limited social and professional activities of females with no permission for driving license during the study period.

The majority of involved males were in the age group from 18 to 30 years old, whether being directly related to the accident as in driving or indirectly as in being car passengers or pedestrians. These results were similar to those obtained from the studies conducted in Amman and The Center of Portugal [11,12] and could be explained by the risk-taking attitude of young males; thereby, it affects the driving behavior besides their lack of driving experience. However, young drivers, contrary to their older peers, maintain better reaction response and reflexes.

As stated by other authors and in consistency with our results, the majorities of the victims were drivers followed by pedestrians [13]. On the contrary, pedestrians were the most frequently affected category as reported by other authors [11].

In many countries such as Australia and Slovenia [13,14] and consistent with our results, ethanol was the most frequently detected component either solely or combined with other substances. Our findings were also in line with other relevant studies conducted on Saudi population and revealed that ethanol was the main analytical finding detected in 49% of postmortem specimens and considered the most common substance linked to road accident deaths (41%) [15].

Many studies are confirming that alcohol level as low as < 0.02 g/100 ml can impair driving performance [16]. However, currently, there is no clear approved threshold ethanol level below which impairment could be excluded and there is no agreed definition of the victim who will be excluded from being under alcohol influence [17]. Considering the transgression of driving under the influence, alcohol is completely banned in Saudi Arabia with a strict zero-tolerance policy for alcohol and drugs [18].

It is vital to consider the factors contributing to the interpretation of postmortem and antemortem BAC or drug levels and its effect on the state of conscious impairment at the time of death due to its crucial role for legal and insurance purposes such as putrefaction degree, postmortem interval time, types of the collected samples for analysis, and the environmental conditions. However, in our study, bodies showed signs of putrefaction or postmortem changes were excluded [19].

An important issue is to differentiate between postmortem ethanol synthesis and antemortem ingestion. This could be avoided by extracting the blood samples from the femoral vein, which is the least prone to postmortem changes. Also, Ethyl Sulphate (EtS) and Ethyl Glucuronide (EtG) were utilized to eliminate the potential of false-positive or negative results, as it is not vulnerable to the Escherichia coli bacteria by creation or degradation. EtS has proven to be more accurate and a reliable biomarker of ethanol exposure that is quickly replacing EtG as the gold standard for alcohol [8].

The consumption of recreational drugs varies among countries which reflected in the postmortem toxicology findings of victims killed in road-traffic crashes [20-21]. This study illustrated that cannabinoids were the most frequently found illegal drug, followed by amphetamine and heroin. However, one notable result is the absence of cocaine in all investigated postmortem samples due to its relative scarcity and high cost. In line with our results, previous studies documented that the pertinence of illegal drugs was variable: in an Australian study, cannabinoids were found in 13.5% of postmortem samples, opioids ranked second with a percentage of 4.9% and stimulants were observed in a percentage of 4.1% [13].

Another recent Swedish study confirmed that illegal drugs were found in 7.2% of all victims with the predominance of amphetamine and cannabis and rare frequency of cocaine [22].

Conclusion

The results of the present study strongly prove the relation between driving under the influence and the increasing incidence of fatal RTA. Ethanol was identified as a key factor in traffic accidents' fatalities, being the most commonly detected substance in the blood samples. Furthermore, cannabis ranked the second, necessitating the enforcement of specific legislations, and a stricter application of the Saudi National Program for Drug Prevention alongside community-wide action to increase the public awareness about the negative effect of these substances and their contribution for the surging incidence of RTA fatalities.

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Ethical Approval: Obtained from the institute ethics committee.

Conflict of Interest: None.

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Identify and Prioritize Criteria for Efficient Allocation of Donation Resources

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Abstract

Introduction: Using civil society's and donors' resources can be helpful options to strengthen financing of the Iranian Health System to reduce the Out-of-Pocket Expenditures. Although local and public funds can address many problems of health systems, but the main concern is allocation of resources mobilized by donors.

Objective: the current study aims to identify and prioritize effective factors on allocation of resources mobilized by donors in the Iranian Health System context.

Methods: The current study comprises of three parts. The first part was a qualitative study to identify factors that influence allocation of resources mobilized by donors. At the second part, Delphi method was used to evaluate experts' consensus on allocation criteria. In the second part, identified criteria were prioritized. Hierarchical analysis was used to prioritize criteria.

Results: The results of the analysis of interviews were divided into 3 groups: Financial Sustainability Framework, Corporate Funding Organization, Optimum Funding Allocation and 6 main categories including (Recruitment, Maintenance and Active Participation, Organizing and Planning, Executive factors, method of investment, identification of real need, need-based decision criteria) and 36 sub-categories were extracted.

Conclusion: Policy-makers and managers of the health system must channel the mobilized resources through appropriate planning, organizing, and monitoring. Furthermore, parallel activities must be avoided. Lack of appropriate management and good organization of donations result in waste of resources, which distract donors' attention from the health system

Keywords: *resources allocation, financing, health donors*

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Introduction

Health systems mobilize resources from different sources such as taxes, social health insurance contributions, private insurance, direct payments, and donors(1). Health financing is defined as management and allocation of incomes to purchase health services(2). Due to factors such as scarcity of resources, rising health

expenditures, and inefficiency of health insurances, Out-of-Pocket Expenditures (OoPE) have increased in the Iranian Health System (IHS). Therefore, families are faced with catastrophic health expenditure. Hence, using civil society's and donors' resources can be helpful options to strengthen financing of the IHS to reduce the OOP (3). Donation can either be local or international, and is an important source of financing in health systems of low income countries (4). Although local and public funds can address many problems of health systems, but the main concern is allocation of resources mobilized by donors(5). Studies on financing health systems by donors have revealed that in Iran most of resources mobilized by donors are allocated to build and expand physical infrastructures of, charity-based healthcare facilities as well as covering health expenditures of poor patients(3, 4, 6). Given that one of the most important tasks of managers is optimal allocation of financial resources and maximizing efficiency in using available resources(7), it seems that lack of clear criteria on allocation of such resources has led into challenges for health policy-makers. Therefore, the current study aims to identify and prioritize effective factors on allocation of resources mobilized by donors in the IHS context. It worth noting that according to the best knowledge of the authors no study has been conducted on allocation of donations in the IHS.

Methodology

The current study comprises of three parts. The first part was a qualitative study to identify factors that influence allocation of resources mobilized by donors. Study context was the MoH, MU, donor institutions, and Non-Governmental Organizations (NGOs). Research community also comprised of MoH's and MU's experts, faculty members of universities, social health professional, public health professional, and health donors. Semi-structured interviews were used to collect data. Interviewees were informed about data collection methods, recording, confidentiality, and study's objectives. Purposive and snowball sampling methods were used. Interviews continued to the saturation point. In total 41 interviews were conducted. Interviews took 30 to 40 minutes.

At the second part, Delphi method was used to evaluate experts' consensus on allocation criteria. Research community was experts, university professors, health system researchers, and sociologists with sufficient knowledge and experience (those whom were willing to participate and had enough time). Experts of this step were health policy-makers and health system's experts. The Delphi technique was applied for two rounds. To collect data a questionnaire comprised of two parts (demographic and allocation criteria) was used. To answer the questions, a Likert Scale ranging from 1 to 9 was used (i.e. 1 was equal to lowest level of consensus and 9 the highest). To sample the participants, purposive and snowball sampling methods were employed. The sample size was 23, and the questionnaire was electronically sent to all participants. Descriptive statistics was used to analyse the data. Answers were categorized into three categories: weak consensus (1-3); moderate consensus (4-6); and high consensus (7-9). Consensus was defined as at least 70% of agreement. Such criteria were selected as final criteria, others were removed.

In the second part, identified criteria were prioritized. Hierarchical analysis was used to prioritize criteria. Research community of this part comprised of experts and policy-makers. After identifying allocation criteria, in order to prioritize criteria, a questionnaire on comparing pairs was sent to the research community. The study validity was evaluated by experts and pundits. Reliability of the questionnaire was evaluated by using consistency rate. To prioritize the criteria, data were entered into the Expert Choice software, and criteria were mutually evaluated. After data entry, weight of each factor of the questionnaire was determined for data analysis (i.e. based on the results of the comparisons (which could be very important, important or equally important) a weight equal to 9, 7, 5, 3 and 1 was given to each factor). Finally, based on the scores and geometric mean, the criteria were prioritized according to the obtained weights.

Findings

In the current study, to conduct interviews, interviewees were categorized into three categories: faculty members of Departments of Health Economics, donors, and managers (policy-makers) of the IHS. 71 and 19% of interviewees were males and females, respectively. Interviews took between 30 to 45 minutes. 36 criteria on allocation of resources mobilized by

donors were extracted (table 1).

Table 1 Effective factor (criteria) for allocation of resources mobilized by donors

Category	Sub-themes	Main themes
Creating infrastructures of sustainable financing	Attraction, maintenance, and active participation of donors	1. Attraction, maintenance, and active participation of donors; 2. Providing feedbacks to donors; 3. Increasing public trust in governmental organizations; 4. Culture building among donors; 5. Increasing trust among donors; 6. Facilitation and encouragement of investment; 7. Direct monitoring of projects by donors;
Lay the ground for organizing participations and financial resources	Organization and planning	8. Coordination of governmental organizations; 9. Establishing committees and instructions; 10. Organizing collection of donations; 11. Establishing a financial fund; 12. Establishing targeted centres; 13. Establishing hospital donors' assembly.
	Operational issues	14. Investor-management split; 15. Emphasizing on social responsibility of organizations, by donors, and reducing negative consequences of Social Determinants of Health (SDH); 16. Using operational structures of leading countries; 17. Providing technical and scientific consultations to donors.

Cont... **Table 1 Effective factor (criteria) for allocation of resources mobilized by donors**

Optimal allocation of financial resources	Investment method	18. Donation to training and research areas; 19. Donation to knowledge based organizations; 20. Investing in infrastructure, based on levelling rules; 21. Investing in hospital and clinical equipment, based on levelling rules; 22. Helping to the poor; 23. Providing c of hard to cure diseases;
	Identifying real needs	24. Identifying local needs by investigating incidence and prevalence of different diseases; 25. Investigating macro and micro indicators of health system as well as allocation of resources by the MoH and other organizations; 26. Conducting research to identify real needs and to perform need assessment; 27. Managing tendencies of donors toward short-term projects; 28. Identifying socio-economic status and demographic characteristics of the area.
	Need-based criteria to make decisions	29. Prioritizing preventive services, instead of treatment and universal health coverage; 30. Aligning donors' desire with identified needs; 31. Anticipating regional needs; 32. Prioritizing long-term projects over short-term ones; 33. Prioritizing deprived areas; 34. Investigating cost-effectiveness; 35. Access to health services and health equity; 36. Tendency toward services/areas which private sector is not willing to participate.

Level of consensus among experts (over identified criteria) was investigated by employing the Delphi technique. 23 policy-maker, manager, and health expert participated at the first round. Response rate was 100%. Most of the participants of the first round of the Delphi were married, male experts, with a M.Sc. degree and/or managers of the health system. Aged between 36 to 45.

for 15 criteria consensus was around 50 to 70. Therefore, at the second round of the Delphi, 18 experts and policy-makers were asked to rate their answers on the Likert scale (Most of the participants were married, male experts, with a B.Sc. degree and/or health system managers. Aged from 46 to 55.At the next step, criteria that were identified through Delphi were prioritized by using a paired comparison questionnaire. Hierarchical

analysis was used to prioritize, in a sample of 18 participants. At this step, most of participants were married females with a professional degree. As shown in table 2, identifying local needs by investigating incidence and prevalence of different diseases and using operational structures of leading countries, obtained the highest and lowest weights, respectively.

Table 2 Final weights of criteria to effectively allocate resources that are mobilized by donors

Row	Theme	Sub-theme	Weight
1	Attraction, maintenance, and active participation of donors	Providing feedbacks to donors;	0.071
		Culture building between donors and the public.	0.046
2	Organization and Planning	Establishing a special fund;	0.069
		Coordination of governmental organizations;	0.063
		Organizing collection of donations;	0.021
		Establishing hospital donors' assembly.	0.003
3	Operational issues	Using operational structures of leading countries;	0.055
		Providing technical and scientific consultations to donors;	0.005
4	Investment method	Providing care of hard to cure diseases;	0.075
		Helping to the poor;	0.067
		Investing in hospital and clinical equipment, based on levelling rules.	0.066
5	Identifying real needs	Identifying local needs by investigating incidence and prevalence of different diseases;	0.09
		Conducting research to identify real needs;	0.082
		Identifying socio-economic status and demographic characteristics of the area.	0.016
6	Need-based criteria to make decisions	Prioritizing preventive services, instead of treatment and universal health coverage;	0.084
		Prioritizing deprived areas;	0.079
		Anticipating regional needs;	0.077
		Aligning donors' desire with identified needs	0.031

Discussion

According to findings of the current study, the first step of optimal allocation of resources mobilized by donors is attraction, maintenance, and active participation of donors through providing feedback to them and building an appropriate culture between donors and the public. In this line, lack of appropriate feedback as well as appropriate informing of donors are among the most important reasons of donors' distrust to healthcare centers (8, 9). Ward and Mackillop (2011) in a study in Ireland found that management of resources mobilized by donors and its continuation can be achieved through providing appropriate feedbacks(10). In other words, providing quality healthcare services and providing appropriate feedbacks to donors about the impacts of their donations on patients' health, have a significant impact over continuation of donations(9). Previous studies also mentioned to reputation and credibility of NGOs as important factors to obtain donors' trust(11, 12).

As participating experts in the current study mentioned, establishing a special fund can be a great step for sustainability of financial resources provided by donors. As well, it is necessary to monitor the whole process of the project, both in terms of occurred costs and progress. A study in Cameroon and Chad reported that applying appropriate management and good leadership along with effective use of human resources can potentially improve efficiency of donations made to health systems of these two countries(13).

Findings of the current study show that in investing resources from donor's partnerships Consideration should be given to the providing care of hard to cure diseases; Helping to the poor and Investing in hospital and clinical equipment, based on levelling rules. Donors pay a fraction or total treatment expenditures of poor patients(3). Based on the experts' opinions, inappropriate management of resources leads into waste, and real needs of hospitals and patients will not be met. Therefore, if this method is selected as a way to allocate resources mobilized by donors, it will require exact monitoring of health facilities' needs. Yousefi et al. (2010) in a study in Iran found that the most important indicators to estimate resources required by health facilities are: catchment population; mortality; socioeconomic indicators; costs related to common diseases; excessive costs related to rural and remote areas; historical expenditures; family physician services; and drugs expenditures(14).

All around the world policy-makers consider incidence and severity of diseases as important criteria to allocate resources. The current study also revealed the importance of investigating incidence and prevalence of different diseases in the catchment area and diseases severity as important factors. Patients with severe diseases need immediate medical interventions, therefore high priority must be given to such patients (6, 15, 16). Various studies emphasized on prioritizing patients based on the disease severity(17, 18).

Finally, based on the findings of the current study, prioritizing preventive services to treatment and universal health coverage, prioritizing deprived areas, anticipating regional needs, and aligning donors' desire with identified needs are mentioned as criteria for need-based decision making. Financial resources mobilized by donors must be allocated based on the society's priorities and needs. Mosadeghrad (2019) also reported that in Iran resources mobilized by donors are spending for expansion of physical infrastructures, supplying equipment and supplies, paying salaries and wages of health personnel, and for utilities(3). Nekoee Mogadam also noted that donors tend to concentrate their donations on treatment costs, instead of preventive and public health services(4), which is different from findings of the current study. Identifying deprived areas can be a great help to allocate resources based on the needs. Rice & Smith showed that deprived areas, disability, rural areas, lack of shelter are macro indicators of resource allocation.

Conclusion

The current study employed a qualitative approach to collect opinion's of managers, policy-makers, and experts of the IHS about donors' participation in the financing of IHS and allocation method of such resources. Policy-makers and managers of the health system must channel the mobilized resources through appropriate planning, organizing, and monitoring. Furthermore, parallel activities must be avoided. Lack of appropriate management and good organization of donations result in waste of resources, which distract donors' attention from the health system. Policy-makers

and managers of the health system must prioritize real needs of the health system and plan mobilized resources regarding such needs.

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Ethical Clearance- Taken from North Tehran Branch, Islamic Azad University, ethics committee

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The Utilization of Sugarcane Bagasse to Remove Cr (VI) Heavy Metal Waste with Adsorption

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Abstract

Sugarcane bagasse obtained from sugar factories is the residue that is usually left behind and considered useless. In fact, sugarcane bagasse can be made nanopore-activated carbon in the form of powder and tablet, which is effective as adsorbent of Cr (VI) in heavy metal waste pollution. The production in this study started from drying using sunlight, crushing sugarcane bagasse into powder, carbonizing the powder at 300^0 C, activating it using HCL5N, sieving with 200 mesh and sonicating in 15 minutes with 40% amplitude. After accomplishing those steps, nanopore powder was obtained and then pressed into a tablet. Absorption capacity testing of Cr (VI) heavy metal was performed with an artificial sample using K2Cr2O7 solution. Sugarcane bagasse nanopore powder was tested for its contact time by adding 0.1 gram of nanopore powder into the sample solution, leaving the sample for a while, and analyzing the sample using spectrophotometry. The results show that the optimum contact time of nanopore powder was six hours to reduce 91.80% of Cr (VI) level and the optimum contact time of the tablet was five hours to remove 56.35% of Cr (VI). Testing on a fixed variable of 105 rpm stirring speed and various contact times produced an optimum result with 90 minutes stirring duration and adsorption capacity of 91.80% for removing Cr (VI). Examination on fixed variable of stirring time and independent variable of stirring speed resulted in an optimum adsorption capacity of 90.07% in Cr (VI) removal, with 105 rpm stirring speed. This study concludes that powder had a greater adsorption capacity than a tablet, while the tablet was more practical for usage.

Keywords*: sugarcane bagasse, nanopore powder and tablet, adsorption, chromium heavy metal.*

Introduction

The environmental order system needs to be preserved and maintained well so as not to be damaged by various pollutions caused by hazardous and toxic waste that is included in the category of Toxic and Hazardous Material, which will disturb the environmental balance, such as heavy metal pollution. Some heavy metals obtained from industrial byproducts that have polluted the environment and are poisonous are lead (Pb), chromium (Cr), cadmium (Cd), mercury (Hg), silver (Ag), nickel (Ni), zinc (Zn), cobalt (Co), lead (Sn), copper (Cu), iron (Fe), molybdate (Mo) and several other metal substances. Heavy metals are metallic elements that have a specific gravity higher than 5 or 6 g/cm^3 . Heavy metal pollution has become a serious problem that will threaten the global environment [(1)].

Chromium (Cr VI) is a heavy metal with an atomic weight of 7.19 g/cm^3, which is potential to contaminate the environment and harmful. In the natural environment, Cr (III) metal is the least active, less soluble and stable, but Cr (VI) is very active, easily dissolved and biologically available (Choppala et al., 2013). The heavy metal pollution of chromium or Cr (VI) has a high toxicity level and it can poison and damage human organs such as the liver, kidneys, and others so that it can interfere with human health [(2)]. Chromium Cr (VI) enters

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the environment from various sources, but the common sources that are supposed to be the most influential are industry, mining, household activities, and combustion residue and fuel mobility [3].

Sugarcane bagasse is waste from a sugar factory that has a sucrose content in sugarcane stems of merely about 8-16%, while the remaining 84% is bagasse, water and other solids [4]. Sugarcane bagasse can also be used to reduce heavy metal content, including chromium Cr (VI), in waste pollution by changing it first into an adsorbent due to the large carbon content in it [5]. The function and economic value of sugarcane bagasse are still low nowadays, and these become the reasons to utilize it as the absorbent Cr (VI), which is extremely dangerous.

The production of nanopore-activated carbon from bagasse is completed through four stages, namely dehydration process, carbonization process, activation process, and sonication process. The quality of bagasse nanopore-activated carbon is largely determined by the type of raw material, processing technology, working method, and accuracy of usage.

The large surface area due to the change to nanomaterial today becomes interesting and has been widely used for adsorption to remove heavy metal content in waste because it produces high adsorption efficiency [6]. The area of nanoparticles is controlled through the material size, chemical composition, surface modification, and control of particle interaction [7].

The adsorbent is produced in the form of powder and tablet, for comparing the effectiveness of Cr (VI) adsorbing activity. Tablet is practical but it needs the addition of other materials such as binders in this case Polyvinyl alcohol (PVA) so that it can be granulated and then compressed or formed. The binder can also improve the fragility and strength of the granules so that the tablet is not easily cracked and broken [8,9].

Methodology

Instruments and Materials

The equipment used in this research includes crusher, analytical balance, furnace, oven, shaker, porcelain cup, desiccator, funnel, clamp, retort stand, 200 mesh sifter, 1-liter glass beaker, pH meter, burette, condenser, water heater, Erlenmeyer, pipette, sonicator, tablet press machine, Atomic Absorption Spectrophotometer, BET.

The materials used consist of sugarcane bagasse from sugar factories, $K_2Cr_2O_7$ solution, HCl, aquades, standard Na2S2O3 solution, standard iodine, standard KIO3, 10% KI, 2N H2SO4, 1% amylum indicator, and lead filter paper.



Figure 1. Dried and crushed sugarcane bagasse

The Production of Sugarcane Bagasse Charcoal begins some following steps. Sugarcane bagasse that had been dried and crushed was put into a furnace for 30 minutes at the temperature of 300^0 C. After charcoal was made, it was frozen in a desiccator. For the chemical activation, the results of carbonization from furnace in the form of charcoal were activated using chemical activation in a 0.5 N HCl solution. This was carried out in reflux for two hours and then filtered and cooled in the form of a slurry. This was, afterward, synthesized using ultrasonic irradiation using a sonication device for making nanopores.The Production of Sugarcane Bagasse Nanopore-Activated Carbon is executed by using this pattern. Activated carbon, obtained from sugarcane bagasse charcoal activation in the form of a slurry, was sonicated for 15 minutes with a 40% amplitude without breaking.



Figure 2. Carbon bagasse after carbonization and irradiation of ultrasonic waves

The results of sonication in the form of activated charcoal were filtered, rinsed with aquades, and dried with an oven to remove the water at a temperature of 105^0C. The produced powder was pressed using a tablet press machine. The cavity in the activated carbon was checked using Brunaeur-Emmet-Teller (BET) to determine the surface area and pore radius of sugarcane bagasse [10].



Figure 3.Sugarcane bagasse nanopore tablets

The adsorption ability of nanopore powder and tablet from Bagasse activated to Cr (VI) heavy metal was tested on artificial samples of $K_2Cr_2O_7$ solution. The test was carried out by weighing 0.1 gram of bagasse nanopore powder, putting it into an Erlenmeyer that contained 25 ml of $K_2Cr_2O_7$ solution with a concentration of 100 mg/L and leaving it for a certain time. The variable of contact time of sugarcane bagasse nanopore-activated carbon and sample included one hour, two hours, three hours, 4 hours, and five hours. The same treatment was also carried out to tablet, weighing 1.32 grams. Other variables, stirring speed and duration, were also examined. The stirring speed was measured using a shaker, by placing the powder into Erlenmeyer and shaking it with various stirring speeds, 55 rpm, 60 rpm, 75 rpm, 90 rpm, and 105 rpm. Meanwhile, the variations of contact time and stirring duration were 15 minutes, 30 minutes, 45 minutes, 60 minutes, 75 minutes and 90 minutes. The filtrates obtained were checked using Atomic Absorption Spectrophotometer.

Finding and Discussion

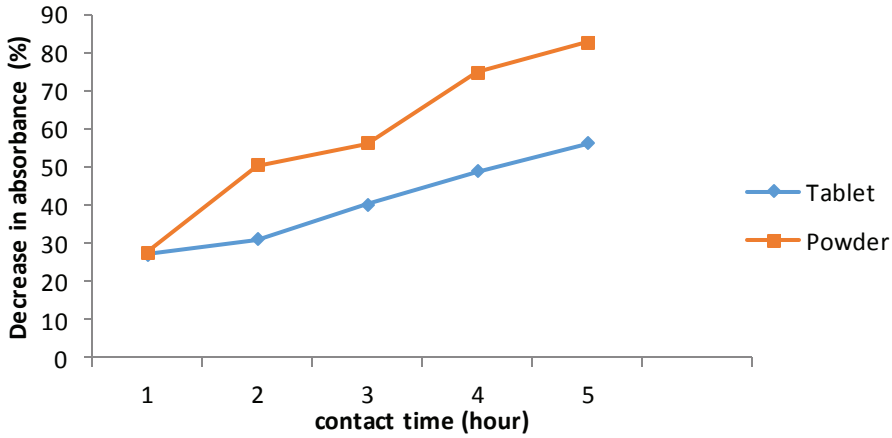


Figure 4. Adsorption of nanopore-activated carbon powder and tablet made of sugarcane bagasse with various contact times

This research was carried out at the Industrial Chemistry Laboratory and the Pharmacy Technology Laboratory of Universitas Setia Budi, Surakarta. The material used in this research, sugarcane bagasse, was obtained from the Sugar Factory in Sragen, and the artificial sample in the form of a $K_2Cr_2O_7$ solution was obtained from the Industrial Chemistry Laboratory. The results of bagasse nanopore carbon powder analysis using Brunaeur-Emmet-Teller (BET) indicate that the pore diameter of bagasse nanopore-activated carbon was 4.10264+01A^{O} [14].

The results of the analysis on the adsorption capacity of nanopore-activated carbon powder and tablet 1,32 grams made of sugarcane bagasse for removing Cr (VI) using $K_2Cr_2O_7$ solution with contact time variations are as follows. Adsorbent dissolved in sample solution without stirring had optimum adsorption capacity 56.35% in a contact time of five hours. The optimum adsorption capacity of powder 82.90% was also achieved in time contact variation of five hours [11].

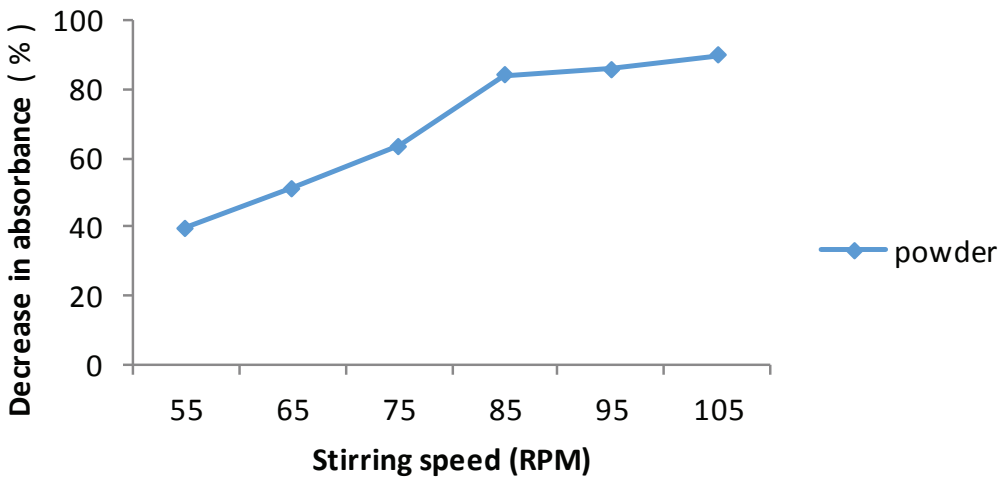


Figure 5. Graph of adsorption capacity of sugarcane bagasse nanopore-activated carbon powder with various stirring speeds.

The analysis of the adsorption process of sugarcane bagasse nanopore powder with various stirring speeds on a heavy metal sample of $K_2Cr_2O_7$ solution reveals that the maximum result was obtained at stirring speed of 105 rpm with an adsorption capacity of 90.07%.

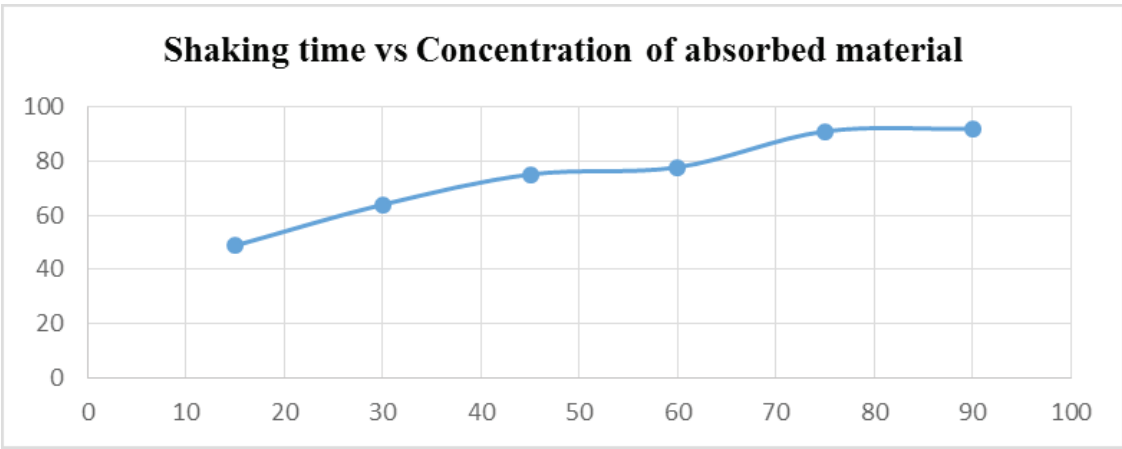


Figure 6. The adsorption capacity of sugarcane bagasse nanopore-activated carbon powder in various stirring time duration

The analysis on the adsorption process of sugarcane bagasse nanopore powder with variations of stirring time duration and fixed stirring speed of 105 rpm on a heavy metal sample of $K_2Cr_2O_7$ solution shows that the maximum result was obtained when the stirring duration of 90 minutes, the adsorption capacity of 91.80%, although the addition of time duration would cause an increase in adsorption capacity, the increase was not significant [12,13].

Conclusion

In conclusion, analysis using Brunaeur-Emmet-Teller (BET) results in the pore diameter of bagasse nanopore-activated carbon of 4.10264 + 01 A^O. The highest adsorption capacity of sugarcane bagasse nanopore tablet on Cr (VI) heavy metal removal 56.35% with the sample of $K_2Cr_2O_7$ solution was in a five-hour stirring duration. The highest adsorption capacity of powder 82.90% also occurred in a five-hour stirring duration. The powder had a greater adsorption capacity, while the tablet was more practical to be used. The adsorption capacity of sugarcane bagasse nanopore powder in removing Cr (VI) was optimum, reaching 90.07%, with a 105-rpm stirring speed. The adsorption of sugarcane bagasse nanopore powder was optimized with a stirring duration of 90 minutes, which resulted in an adsorption capacity of 91.80% [15].

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Biological Hazard on Multidrug Resistance (MDR) of Escherichia Coli Collected From Cloacal Swab of Broiler Chicken on Wet Markets Surabaya

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Abstract

Objective: The purpose of this study was to determine the multidrug resistance (MDR) profile of Escherichia coli from broiler chicken from Wonokromo Market and Tambahrejo Market Surabaya to some antibiotic groups, namely streptomycin, levofloxacin, cefotaxime, trimethoprim and chloramphenicol.

Materials and Methods: Seventy samples were taken from Wonokromo market and Tambahrejo market, each of which had thirty-five samples. Sampling was used cloaca swab technique. Then inoculated on Eosin Methylene Blue Agar (EMBA) media, purification of bacteria on EMBA media, and tested biochemically with Sulfide Indol Motility (SIM) and Triple Sugar Iron Agar (TSIA) media.

Antibiotic sensitivity test was used the Kirby-Bauer method. Muller-Hinton Agar (MHA) media incubation. Then the inhibition zone was measured according to the Clinical and Labortary Standard Institute (CLSI) standards. Detect positive multidrug resistance (MDR) bacteria characterized by resistance to ≥3 types of antibiotics.

Results: Detection results of Escherichia coli multidrug resistance (MDR) at Wonokromo market was 85.7%, and at Tambahrejo market was 51.4%. Presumptive Extended Spectrum Beta Lactamase (ESBL) producing E. coli at Wonokromo market was 14.3% and at Tambahrejo market was 2.9%.

Conclusion: The high level of MDR of Escherichia coli from cloacal swabs of broiler chicken was a threat to public health and the environment, and is an important concern to reduce the rate of its spread.

Keywords: *Broiler chicken, Multidrug resistance, Escherichia coli, Cloacal swab, Public health*

Introduction

Chicken is one of the sources of animal protein needed by Indonesian people[1]. Some of the products produced by chickens and used by the community include meat and eggs. Increased population growth affects the food needs of the community as seen from the data consumption of chicken meat each year[2]. Chicken production industry is inseparable from the use of antibiotics. Antibiotics are used as treatments and growth triggers[3]. The reason for the use of antibiotics is because chickens are very susceptible to pathogenic diseases due to the high density of cages which causes the chickens to become stressed. Uncontrolled use of antibiotics will leave residues and cause pathogenic microbes to become resistant to antibiotics.

Escherichia coli can also act as a reservoir for the spread of antibiotic resistance because it can easily move

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resistance genes to other bacteria. Multidrug resistance (MDR) Escherichia coli has been isolated from food from animals, hospital environments, plants, and feces. Some studies also report the high prevalence of MDR Escherichia coli in food-consuming animals, food products, and the environment. Escherichia coli is a contaminant bacterium commonly found in meat[4].

Based on this background, a study was conducted on the detection of multidrug-resistant (MDR) Escherichia coli in broilers isolated from cloacal swabs of broiler chicken in Wonokromo Market and Tambahrejo Market Surabaya.

Materials and Methods

Sampling

Sampling by cloacal swab was 70 broilers taken from Wonokromo Market and Tambahrejo Market. Samples were taken using a sterile cotton swab with a cloaca swab and then put in a tube filled with 0.9% Sodium Chloride solution. Sampling must be aseptic and the samples were taken to the Bacteriology and Mycology Laboratory of the Faculty of Veterinary Medicine, Airlangga University using a cool box for further research.

Bacteria Isolation and Identification

Samples inoculated on EMBA media were incubated by the streak method at 37^0C for 24 hours and their growth was observed. On EMBA selective media, E. coli bacteria which grow their colonies are metallic green[5]. After that proceed with the purification process. Purification is done by scraping using heated ose that has been preheated until it is red and left to stand for a while then used to extract bacterial colonies from inoculation EMBA media, streaking the EMBA media for purification[6].

Planting in Triple Sugar Iron Agar (TSIA) media is carried out by stabbing to the bottom of the tube and streaking on the oblique portion of the media using an ose needle. Positive sample E. coli on TSIA is marked by the change in color at the base of the media from orange to yellow on the slant. Planting in Sulfide Indol Motility (SIM) media, bacterial inoculation was carried out by means of a single prick in the center of the media using an ose needle. Positive samples of E. coli bacteria will form indole and positive motility[7].

Bacteria Sensitivity Test

Bacterial sensitivity testing was used by the Kirby-Bauer diffusion method using Mueller-Hinton Agar (MHA) media. The E. coli suspension was taken using a sterile cotton swab and rubbed onto the surface of the Mueller Hinton Agar (MHA) medium and allowed to stick on. Then put a 10µg streptomycin antibiotic disc, levofloxacin 5µg, cefotaxime 30µg, trimethoprim 5µg and chloramphenicol 30µg placed on Muller Hinton Agar (MHA) media using tweezers and incubated at 37^0C for 24 hours. The clear area formed around the antibiotic disc after incubation, measured in diameter and referred to as the diameter of the antibiotic inhibitory zone against the growth of test bacteria[8,9].

Results and Discussion

The results of isolation and identification with biochemical tests of 70 samples tested showed that all positive samples were identified as E. coli bacteria which can be seen in Table 1. The diameter of the inhibition zone results of the bacterial sensitivity test on 70 samples taken from Wonokromo Market and Tambahrejo market are shown in Table. 1 grouped into three categories namely Resistant, Intermediate, and Sensitive according to the standards of the Clinical and Laboratory Standards Institute[8].

Table. 1. Antibiotics sensitivity test on 70 samples of *E. coli*

No	Antibiotic	R (%)	I (%)	S (%)
1.	Streptomycin (10µg)	52 (74%)	7 (10%)	11 (16%)
2.	Levofloxacin (5µg)	42 (60%)	9 (13%)	19 (27%)
3.	Cefotaxime (30µg)	6 (9%)	3 (4%)	61 (87%)
4.	Trimethoprim (5µg)	55 (79%)	1 (1%)	14 (20%)
5.	Chloramphenicol (30µg)	30 (43%)	3 (4%)	37 (53%)

Legend : R= Resistant, I= Intermediate, S= Sensitive

Table 1. showed that E.coli bacteria obtained from Wonokromo market and Tambahrejo market have the highest resistance levels in Trimethoprim antibiotics in 55 samples (79%), Streptomycin in 52 samples (74%), Levofloxacin in 42 samples (60%), Chloramphenicol in 30 samples (43%) and Cefotaxime in 6 samples (9%).

Table. 2. Multidrug Resistnace (MDR) and Presumtive ESBL on Wet Markets Surabaya

Wet market	Positive MDR	Presumtive ESBL	Non MDR
	n (%)	n (%)	n (%)
Wonokromo	30 (85.7%)	5 (14.3%)	5 (14,3%)
Tambahrejo	18 (51.4%)	1 (2.9%)	17 (48.6%)
TOTAL	48 (68.6%)	5 (9%)	22 (31%)

Determination of multidrug resistance (MDR) if there is resistance to ≥3 classes of antibiotics[10], as shown on Fig. 2. Bacteria that are not resistant> 3 antibiotic groups are categorized as MDR negative bacteria. Determination of MDR presumptive Extended Spectrum Beta Lactamase (ESBL) if the bacteria are resistant to ≥3 classes of antibiotics and third-generation sephalosporin (Cefotaxime). Bacteria that are resistant to ≥3 classes of antibiotics but are not resistant to cefotaxime, bacteria including non presumptive MDR ESBL. Bacterial sensitivity test results can be obtained by E. coli MDR positive bacteria, MDR negative bacteria, and also presumptive ESBL E. coli bacteria, as shown on Fig.1.

Table 2. shows the highest levels of E. coli-positive MDR bacteria found in the Wonokromo Market of 30 out of 35 samples or 85.7%. Whereas in the Tambahrejo Market it was found that E. coli had positive MDR of 18 out of 35 samples or 48.6%. The results of detection of E. coli MDR, presumptive ESBL, and MDR negative are made in the diagram in Figure 1. to facilitate the reading of research results.

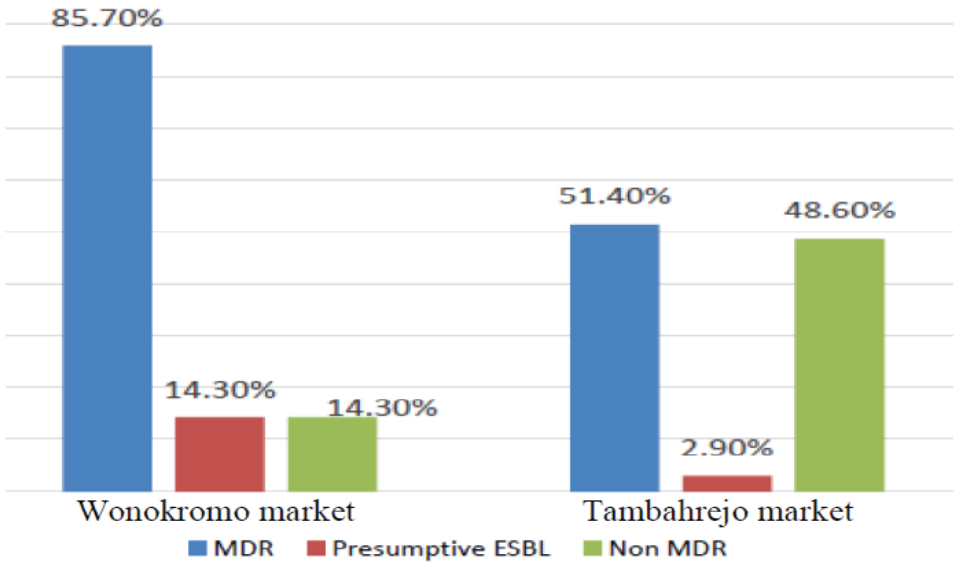


Figure 1. Diagram of percentage of MDR, presumptive ESBL, and Non MDR on two markets.

Bacteria that are resistant indicate that bacteria can still grow even though they are exposed to these antibiotics. Bacteria that are still sensitive to antibiotics indicate that antibiotics can still inhibit bacterial growth and the possibility of therapeutic success is still high[11].

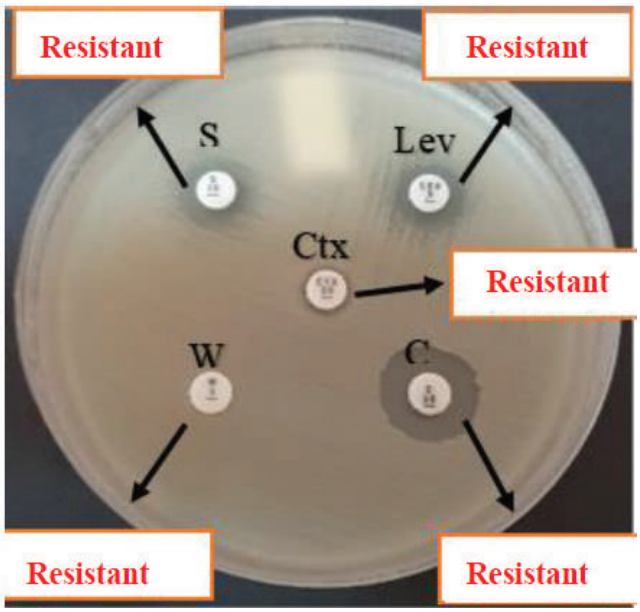


Figure 2. Result of MDR E. coli from wet markets, Surabaya

Legend : S = Streptomycin; Lev= Levofloxacin; Ctx= Cefotaxime; W= Trimethoprim; C= Chloramphenicol

The level of sensitivity of bacteria that experienced the greatest resistance was trimethoprim as many as 55 of 70 samples or 79%, intermediates as much as 1 of 70 samples or by 1% and sensitive as many as 14 of 70 samples or by 20%. Trimethoprim is an antibiotic that works by inhibiting dihydrofolic acid to become tetrahydrofolic acid so that there is an inhibition of bacterial growth[12]. The cause of resistance to trimethoprim modification in the target enzyme dihydrofolate reductase[13].

The antibiotics that experienced the second most resistance were streptomycin found in 52 of 70 samples or 74%, intermediates in 7 out of 70 samples or 10% and sensitive in 11 out of 70 samples or 16%. Streptomycin is an aminoglycidid class of antibiotics which means it works by modifying or inhibiting protein synthesis. The cause of resistance to streptomycin is due to mutations in the S12 protein ribosome gene[14].

Antibiotics that experienced the third largest resistance were Levofloxacin in 42 out of 70 samples or 60%, intermediates in 9 out of 70 samples or 13% and sensitive in 19 out of 70 samples or 27%. Levofloxacin is a quinolone class of antibiotics that works to influence the gyrase enzyme and IV topoisomeration enzymes which become toxic enzymes on bacterial chromosomes. The cause of resistance to levofloxacin is the mutation of these two enzymes so that the interaction between quinolones and enzymes is reduced[15].

E. coli bacteria isolated from Wonokromo Market and Pasar Rejo are still sensitive to cefotaxime antibiotics with data obtained 61 of 70 samples or 87% are still sensitive, intermediates are 3 out of 70 samples or 4% and are resistant as many as 6 out of 70 samples or 9%. Cefotaxime is a third generation sephalosporin that is widely used for Gram negative bacterial infections. Resistance to third generation sephalosporin is often associated with extended spectrum β-lactamase[16, 17, 18].

The isolated E. coli bacteria were still sensitive to the chloramphenicol antibiotic found 37 out of 70 samples or 53% were still sensitive, intermediates were 3 out of 70 samples or 4% and resistant were 30 out of 70 samples or 43%. Chloramphenicol is an antibiotic that inhibits or modifies bacterial protein synthesis. Chloramphenicol inhibits protein synthesis by binding to the 50S ribosomal subunit and inhibiting the extension of the peptide chain. Resistance to chloramphenicol is caused by bacteria that produce chloramphenicol acetyltransferase that damage drug activity[19].

The results of the detection of Escherichia coli multidrug resistance were most commonly found in the Wonokromo Market, 30 of 35 samples were taken or 85.7%. At Tambahrejo market found 18 out of 35 samples taken or 51.4%. In total, 48 out of 70 samples were E. coli multidrug resistance, or 68.6%. E. coli presumptive ESBL in Wonokromo Market was 5 out of 35 samples or 14.3% while in Tambahrejo market there were 1 in 35 samples or 2.9%. Overall in the two markets it was found that E. coli presumptive ESBL bacteria were as many as 6 out of 35 samples or 9%.

The high level of antibiotic resistance or multidrug resistance is caused by the use of antibiotics that are not the right dose, not the right diagnostic, and not the right bacteria causing[18]. Antibiotics also have a minimum dose to achieve the therapeutic effect, the use of antibiotics above the dose gives a stronger selection pressure against bacteria so that they can mutate and eventually become resistant[20].

The discovery of multidrug resistance bacteria isolated from the cloaca swab at Wonokromo Market and Tambahrejo market indicates that bacteria originated

from individual chickens and is thought to originate from farms that still use antibiotics. On farms, antibiotics are given as feed additives, to spur growth (growth promoters) and increase feed efficiency[21, 22, 23]. The use of antibiotics as feed additives and growth promoters kills some bacteria, but there are also those that survive and experience mutations and resistance. This situation is a serious problem and can endanger humans. Bacteria that are already immune to several antibiotics will continue to replicate, spread and contaminate water, food, soil and the environment. Humans are exposed to resistant bacteria through food consumed (foodborne) and contaminated environment.

Conclusion

The conclusion from the results of this study is that as many as 70 out of 70 samples can be isolated Escherichia coli or as much as 100% through broilers cloacal swabs in Wonokromo market and Tambahrejo market, Surabaya. Multidrug resistance (MDR) E. coli can be found in Wonokromo market was 85.7% and in Tambahrejo market was 51.4%. It can be concluded that the isolates were biological hazard for spreading MDR to human health

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Designation :

Nenny Harijani as a researcher on Universitas Airlangga**,**

Surya Jovinson Tansil Oetama as an undergraduate student on Universitas Airlangga**, Koesnoto Soepranianondo** as a professor on Universitas Airlangga**,**

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Wiwiek Tyasningsih as an associate professor on Universitas Airlangga

Ethical Clearance: Cloacal swabs were used in this study, hence ethical clearance was not necessary. Cloacal swab samples were collected from wet markets in Surabaya, East Java province, Indonesia.

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Conflict of Interest: Nil

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Analysis of Human Decomposition Effect on DNA Quality with Short Tandem Repeats [STRS] Combined Index DNA System [CODIS]

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Abstract

Introduction: Personal identification is a problem in criminal or civil cases. Exact determination of personal identity is very important in investigation as any error can be fatal in the judicial process. The process of identification that is often used is through DNA analysis. The problem that often becomes a serious problem for both forensic DNA expert and other DNA expert is the condition of degraded DNA.

Method and Materials: This study was conducted by analysing DNA damage patterns using CODIS STR (Short Tandem Repeat) markers to effect the decay process. The type of research used by experimental laboratories, with research design used is time series.

Results and Discussion: The result of DNA sample extraction from Psoas and Masseter muscle samples shows the average minimum range of DNA levels for DNA typing is 0.25 ng with purity of 1.8 – 2(1). The result of this study prove the effect of decomposition on DNA concentration on each muscle. This study is also showed a decrease in levels and purity in the samples of Psoas and Masseter muscle tissue buried in soil, sea/salt water and river/fresh water from day 1, 7, 14, 20 and 40.

Conclusion: Psoas and Masseter muscle can be alternative material for forensic identification. The success of sequence mapping pattern from STR loci are TPOX, THO1, and CS1PO according to each GC content. GC content of THO1 and TPOX has the same relative value of 0,48, compared to CSF1PO value of 0,33.

Keywords**:** *Decomposed, Identification, STR CODIS*

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Introduction

Identification is to determine the truth or error of an accusation, including in criminal cases: identification of criminals, murderers, perpetrators in torture, rape and others. Civil matters involve insurance, inheritance rights and the alleged father of a child. Identification is generally divided into namely identification for victims of life and identification for people who have died either known or unknown.[1,2]

Identification through DNA analysis methods is an accurate and stable diagnostic tool. Individual genetic information has similarities to the whole cell and will remain unchanged even after death.[3]

In its development examination using DNA analysis method is not without obstacles. One obstacle that is often a serious problem for both forensic DNA expert and other DNA expert is the condition degraded DNA.[3,4]

Muscle tissue is an organ that has enough cell content so that there is DNA in the cell nucleus. One type of the muscle that is often used/recommendations in DNA analysis is the Psoas muscle and the Masseter muscle. Psoas muscle is a muscle located in the formation of the lower abdominal wall or posterior abdominal wall behind the diaphragm, associated with Quadratus lumborum in which there are arteries, veins and lymph nodes. While the Masseter muscle is a chewing muscle with vertical fibre model so that it will strengthen the direction of the bite/chew movement.[1,5]

Decomposition on the body is one of the obstacle in the DNA analysis method. Although DNA degraded is considered as a frightening thing for forensic DNA expert, forensic DNA experts overcome this situation by conducting research o research to create a way of examining DNA that under certain conditions. So far the effect of the decaying process of the body on the quality of DNA undergoing fragmentation in the examination of forensic identification are well known.[6,7]

In DNA analysis with a repetition unit length of less than 1 kbp is called a Short Tandem Repeat (STR). This STR is popular because it has a small allele size (less than 1 kbp) so it can be easily amplified by PCR and even degraded samples can be analysed. The FBI recommendations for identification are 13 STR-CODIS loci. The study used 3 STR loci [CSF1PO, THO1, TPOX] which have high discriminant power for Asian continental populations.[4]

Materials and Methods

This was an experimental laboratory with a time series research design. The sample came from psoas and masseter muscle. The used body was not identified in 2 x 24 hours [according to article 133 Law of Criminal Procedure]. Psoas and masseter muscles were stored in soil, sea/saltwater, river/fresh water for 1,7,14,20, and 40 day. The sample size is 24 psoas and 24 masseter muscle samples. This research have been approved by the ethical commite 33/hrecc.fodm/IV/2019.

Research material: psoas and masseter muscles, material for DNA extraction: DNAzol Reagent, 100% solution & 70% ethanol. Material for PCR: PCR mis (12.5 ml) consisting of dNTP (ATP< CTP< TTP< GTP), $MgCl_2$ and Taq Polymerase, DW sigma (Nuclease Free water), CSF1PO promer [Gen Bank Accession X14720]: 5'-AACCTGAGTCTGCCAAGGACTAGC-3' &5'-TTCCACACACCACTGGCCATCTTC-3', THO1 [Gen Bank Accession D00269]: 5'-CTGGGCACGTGAGGGCAGCGTCT-3' & 5'-TGCCGGAAGTCCATCCGCCAGC-CCC [CCG-CCG] ACCG-3 [CC-CCG]]' &5'-GGAGGACTGGGAACCACACAGGT-3'

Result and Discussion

Analysis of decomposition effect on DNA concentration

This study begins with the exposing samples with soil, sea water/saltwater, river water/fresh water within 1,7,14,20, and 40-day period. The exposed sample extracted with DNAzol method and were measured of DNA levels and DNA purity of the sample using UV-Visible Spectrophotometer.

The result of DNA extraction/isolation obtained DNA levels of Psoas muscle samples with average DNA levels of 572.89 ± 5.71 mg/ml with average DNA purity of 1.09 ± 0.21. Whereas DNA samples from the Masseter muscle produced an average DNA level of 589.19 ± 5.58 mg/ml with mean DNA purity of 1.29 ± 0.26. All of the average DNA samples were still in the minimum range of DNA levels for DNA typing, ie 0.25 hg with a purity of 1.8-2.[1]

From the results of this study prove the effect of the decomposition process on the DNA levels. It can be seen from the measurements of DNA levels through a UV spectrophotometer showing decreased of DNA levels of psoas muscle tissue samples that are buried in the soil and submerged in seawater where from days 1, 7, 14, 20 and 40.[1]

However, with the decreasing of DNA level, is not a

major problem since the remaining DNA levels are still possible to do a DNA profiling (minimum of 50 ng).

Water quality is tested by several parameters, such as physical parameters which are temperature, turbidity, dissolved solids, etc. Chemical parameters are pH, dissolved oxygen, BOD, metal content, and biological parameters namely the presence of plankton and bacteria. The pH and NaCl tests resultfrom two media (sea water and fresh water) at the Surabaya Health Laboratory Center stated that sea water media has high levels of NaCl which is 5.50 and 1,652.93 mg / L, compared to regosol soil media has a pH content at on 7.00 is 8.75 and NaCl 314.20 mg / L.[8,9,10]

DNA degradation of the human body can be caused by 2 factors, specifically endogenous and exogenous. Endogenous factors originate in the cell itself, which are also known as spontaneous degradation. Exogenous factors come from the environment. Postmortem decomposition of the human body is a very complex process, beginning with autolysis and decay and followed by aerobic and bacterial decomposition of organic matter. Environmental factors such as humidity and environmental temperature are very significant on the condition of DNA used as DNA identification material in the forensic field, as in DNA analysis in other fields. In general, forensic samples carried out by DNA testing, 40% have experienced degradation or contamination, so the STR analysis which has a core sequences of less than 1 kb (kilobases) is very effective and the success rate is high, especially on degraded DNA by producing short fragments.[4,11,12]

DNA level is an important factor in the DNA analysis, especially on the success of amplification of DNA samples. A decrease in DNA levels up to 1 ng has the potential to reduce the STR's detection ability to 95%. DNA integrity is a major concern for DNA forensic examination. This implies that even though the examination of DNA levels obtained is relatively high, if DNA has undergone fragmentation or degradation, then high DNA levels, become less meaningful.[2,9,13,14]

In addition to DNA levels, it is also necessary to have sufficient DNA quality, in other word, the degradation of DNA must be minimum. If the DNA degradation is severe, the primer cannot stick to the selected target DNA to be duplicated.[1,15]

Analysis of Visualized Amplification at loci: CSF1PO, THO1 and TPOX

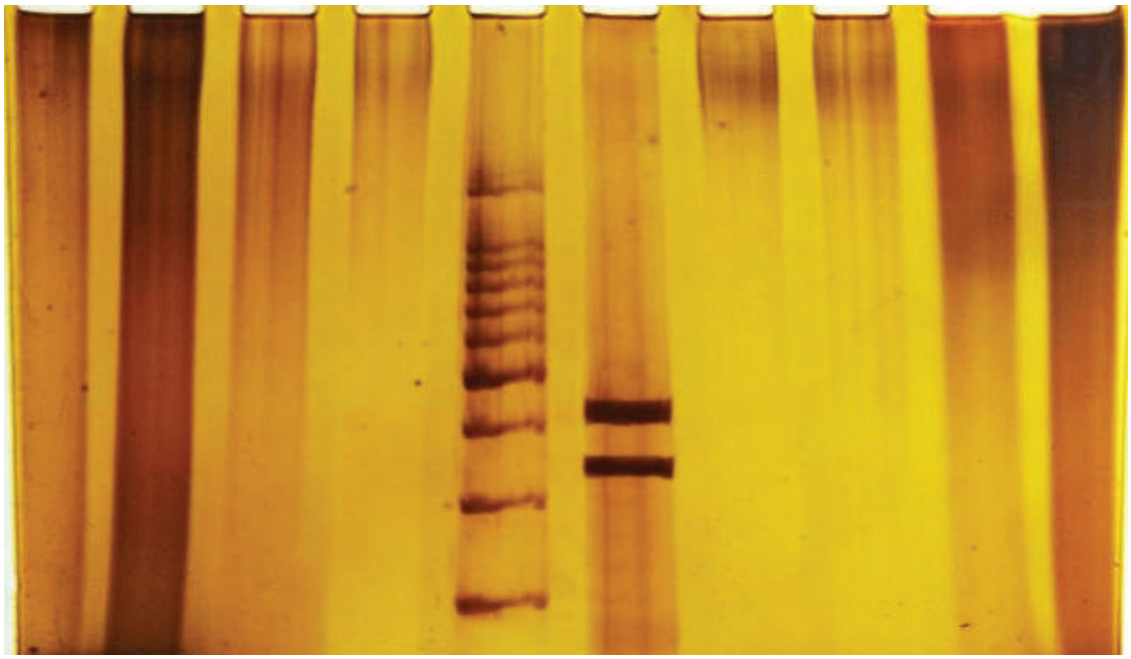


Figure 1. Results of PCR STR CODIS visualization locus CSF1PO [321bp - 357bp],Ma [100bp marker], Psoas [P} muscle &Masetter [M] muscle, on day 7,14,20 on sea water media.

The amplification process begins with the preparation of the DNA template through the extraction and isolation process using an extraction kit (DNAzol). The PCR process uses primers at the STR CODIS locus [CSF1PO, THO1, TPOX] as shown by Figure 1, Figure 2, and Figure 3:

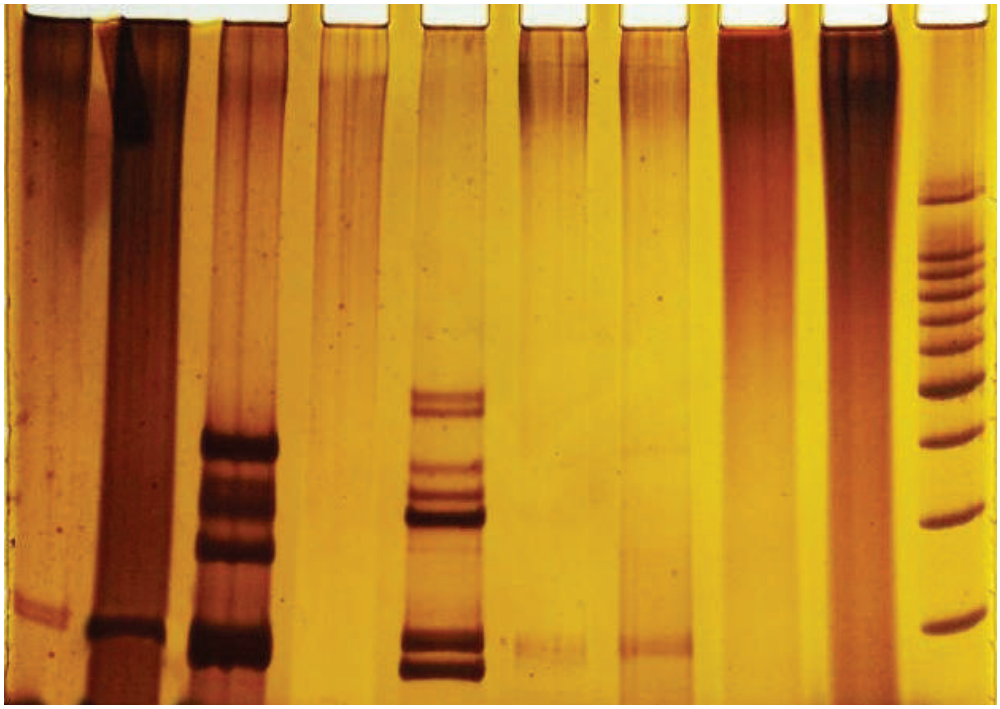


Figure 2. Results of PCR STR CODIS visualization locus CSF1PO [321bp - 357bp],Ma [100bp marker], Psoas [P} muscle &Masetter [M] muscle, on day 1in the media Land, Sea Water and River Water.

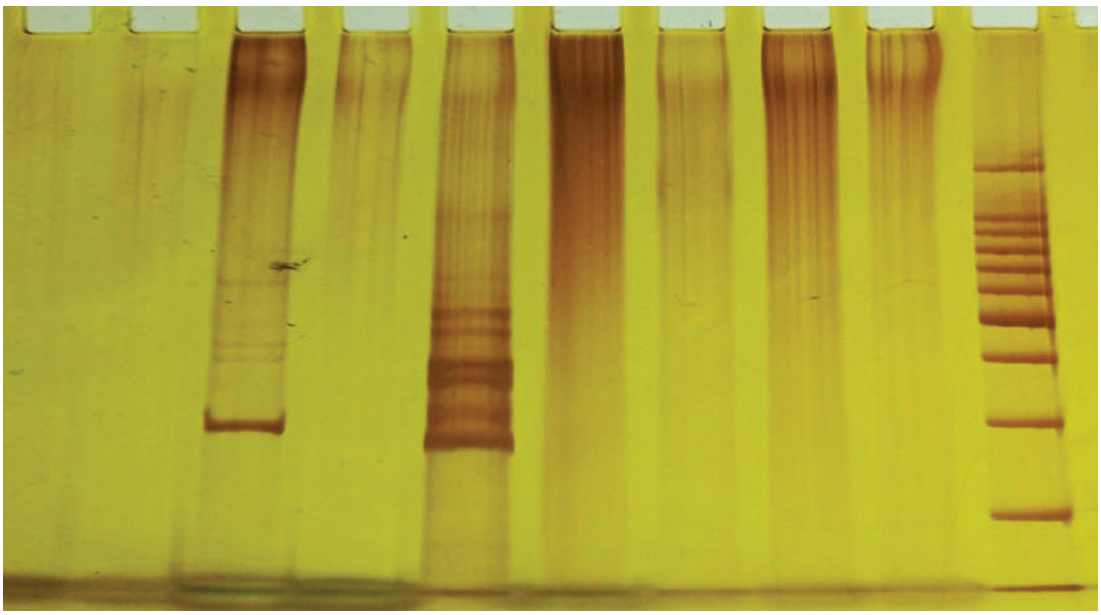


Figure 3. Results of PCR STR CODIS visualization of the TPOX locus [262bp - 290bp],Ma [100bp marker], Psoas [P} muscle &Masetter [M] muscle,on day 7,14,20,40 River Water media

Table 1. Detection results of STR CODIS loci: CSF1PO, TPOX, THO1 on Media Land

SAMPEL	Loci	CSF1PO					THOI					TPOX				
	Day	1	7	14	20	40	1	7	14	20	40	1	7	14	20	40
Psoas Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%
Masetter Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	4/ 100%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%	0%	0%	4/ 100%	4/ 100%	4/ 100%

Table 2. Detection results of STR CODIS loci: CSF1PO, TPOX, THO1 on Sea Water Media

SAMPEL	Loci	CSF1PO					THOI					TPOX				
	Day	1	7	14	20	40	1	7	14	20	40	1	7	14	20	40
Psoas Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	4/ 100%	4/ 100%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%	4/ 100%	0%	0%	4/ 100%	4/ 100%	4/ 100%
Masetter Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	0%	4/ 100%	0%	4/ 100%	4/ 100%	4/ 100%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	0%	4/ 100%	0%	0%	0%	4/ 100%	4/ 100%

Table 3. Detection results of STR CODIS loci: CSF1PO, TPOX, THO1 on River Water Media

SAMPEL	Loci	CSF1PO					THOI					TPOX				
	Day	1	7	14	20	40	1	7	14	20	40	1	7	14	20	40
Psoas Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	0%	0%	0%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%
Masetter Muscle	+	4/ 100%	0%	0%	0%	0%	4/ 100%	4/ 100%	0%	4/ 100%	0%	4/ 100%	0%	0%	0%	0%
	—	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%	0%	0%	4/ 100%	0%	4/ 100%	0%	4/ 100%	4/ 100%	4/ 100%	4/ 100%

The table 1,2, and 3 above show negative detection results [-] on average from day 20 almost all STR CODIS

loci: THOI, TPOX detected negative, except STR CODIS locus: CSF1PO detected negative [-] starting on day 7 on soil media, sea water and River water.

Study on STR CODIS as a general has not been reported, only a few primers. The results of related study of STR CODIS is solitary the pattern of mapping sequence of the STR locus are TPOX, THO1 and CSF1PO, based on GC content ratio. The calculation of the GC ratio of THO1 and TPOX content having the same relative value of 0.48, compared to CSF1PO of 0.33. Several studies have been conducted from several STR CODIS focus areas. The accuracy of research at the CSF1PO, THO1, TPOX locus has been reported in several studies including: chromosome populations and allele sequences at the THO1 locus, populations in Thailand with 8 STR loci including THO1, TPOX, CSF1PO and vWA and study of Chinese populations in Taiwan with STR. Genetic variation in Caucasia, examined genetic variation in population Filipinos and Thais living in Taiwan, use 9 STR loci. Whereas in Indonesia, THO1 allele pattern in the Batak population in Surabaya and D1S80 and D17S5 locus populations in Surabaya. The method of typing STR loci especially THO1 is a method that seem sensible, is convincing and efficient so that it is a useful method in forensic cases. 5 to 6 STR loci have a ratio of 1: 100 billion. In principle regarding the number of loci examined is that the more loci used for examination the better the accuracy.[16,17,18]

Associated with the the success of the detected loci, it is because by differences in amplification products and the presence of GC content or guanine and cytosine bonds at each locus. GC content or guanine cytosine bonds have a high level of stability against denaturation factors compared to the bonds between Adenine and Thymine.[19,20,21,22]

Conclusion

Psoas and Masseter muscles can be an alternative component in forensic identification. The success pattern of mapping the sequence of the STR locus is TPOX, THO1 and CSF1PO. According to its GC content ratio. This is consistent with the calculation of GC THO1 and TPOX content ratios that have the same relative value of 0.48, compared to CSF1PO of 0.33.

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Ethics Approval and Consent to Participate

This study has been agreed by ethical commite which number 33/hrecc.fodm/IV/2019

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Association between The Study of Nerve Conductivity and The Medianus Nerve Cross-Sectional Area in Carpal Tunnel Syndrome

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Abstract

Background: Carpal tunnel syndrome (CTS) is a collection of symptoms caused by clasps on median nerve. CTS diagnosis is based on anamnesis, physical examination and investigation. Medianus nerve electrodiagnostic examination is the gold standard of CTS diagnosis.

Objective: To determine the association between the study of nerve conductivity measured by electrodiagnostic and the median nerve cross-sectional area measured by musculoskeletal USG in CTS patients.

Methods: This study was conducted at medical rehabilitation installation of Dr. Soetomo Teaching Hospital Surabaya, Indonesia. The samples were 15 hand that obtained from 13 females with CTS.

Results: The Pearson Association analysis showed a significant positive correlation between the the cross-sectional area and the motor distal latency of medianus nerve ($r = 0.625$; $p = 0.013$). There was no significant correlation between the cross-sectional area with sensory distal latency, sensory amplitude, motor amplitude, sensory nerve conductivity velocity and motor nerve conductivity velocity of median nerve.

Conclusion: Electrodiagnostic and musculoskeletal USG have different roles in the diagnosis of CTS. Electrodiagnostics as the gold standard provided information about the level and severity of lesions n. Medianus. Musculoskeletal ultrasound could be used as an additional examination that provided information on the anatomy of median nerve and other structures located in the carpal tunnel.

Keywords: *nerve conductivity, cross section, median nerve, carpal tunnel syndrome.*

Introduction

Carpal tunnel syndrome (CTS) is a syndrome caused by the compression of the median nerve along the carpal tunnel [1]. This diseases occurs because gap in bottom arm to wrist the narrowing of causing can limit to function of the wrist [2]. CTS is the most common compression neuropathy [3] in the upper limb [4–6].

CTS release operations cost an approximately of 2 million dollars annually in the United States [4,7,8]. CTS diagnosis is based on anamnesis, physical examination and supported by an investigation. A frequent investigation of CTS is a neurophysiologic examination of median nerve using electromyography, meanwhile, structural examination of the median nerve and surrounding soft tissues using musculoskeletal ultrasonography (USG) to confirm the diagnosis of CTS and exclude other conditions [9]. Electrodiagnostics is still the only method available to assess the physiological

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changes that occur in CTS. Although electrodiagnostic examination had high sensitivity, however, false negative incidence was still reported to be about 8-12%, even a literature mentioned about 10-20% [10,11]. This examination might provide information about the level of the lesion, however, the information of median nerve spatial or surrounding tissue was not enough [10].

Musculoskeletal USG usage for the diagnosis of CTS is rare. Whereas the use of musculoskeletal USG can provide additional information that complements the electrodiagnostic results, thus it increases the diagnostic strength and management of CTS patients. The presence of additional information on the anatomy of the median nerve and surrounding structures may help to avoid misdiagnosis such as tenosynovitis or lesion due to tumors in median nerve, ganglion, fibroma [12].

Methods

This research was an observational analytic research with cross-sectional approach. The subjects of this study were CTS patients who visited the rehabilitation unit at Dr. Soetomo Teaching Hospital Surabaya from July to September 2014. CTS patients who fulfilled the inclusion criteria were performed electrodiagnostic and musculoskeletal USG examinations. All these examination were conducted within three days. The protocol of this study was approved by Dr. Soetomo Teaching Hospital Surabaya [13]. Data analysis was performed using SPSS 17. The data in this study was quantitative data, thus the analysis test used was Pearson correlation test.

Results

Subjects were 13 females, the mean age of the subjects was 45.8 ± 10.9 years, the youngest was 33 years old and the oldest was 60 years old. The mean body mass index (BMI) of subjects was 25.68 ± 3.4 kg/m^2 with the lowest BMI of 18.34 kg/m2 and the highest BMI of 31.53 kg/m2. One subjects had BMI less than 18.5 kg/m2, six subjects had BMI of 18.5-24.9 kg/m2, five subjects had BMI of 25-29.9 kg/m2, and a subject with BMI >30 kg/m2.

Two subjects had the occupation history of factory labour, office employees of 8 people, and housewife as many as 3 people. All subjects were right-hand dominance. Subjects with bilateral CTS was 8 patients and subjects with unilateral CTS was 5 patients (CTS on the right side was 4 people and CTS on the left side was 1 person). The mean duration of CTS symptoms in this subjects was 4.5 ± 2.4 months, the fastest duration was 1 month and the longest was 10 months (Table 1).

Results of Nerve Conductivity Study

Electrodiagnostic in 15 subjects obtained abnormal nerve in 5 subjects (Table 2).

Musculoskeletal USG Examination Results

All samples were performed an examination of muskuloskeletal USG. (table 3).

Correlation between The Study of Nerve Conductivity and The Median Nerve Cross-Sectional Area

There was no significant correlation was found between cross-sectional area with sensory KHS of median nerve (r = -0.291, p = 0.293), and between the cross-sectional area with motor amplitudes of median nerve (r = 0.385; p = 0.156) (Table 4).

Table 1. Subjects Characteristics

Variables	Frequency	Mean	Minimum	Maximum
Age (years) 31-40 41-50 51-60	 5 (38.4%) 3 (23.2%) 5 (38.4%)	45,8 ± 10,9	33	60
Sex				

Cont... **Table 1. Subjects Characteristics**

Male Female	0 (0%) 13 (100.0%)			
Occupation				
Laborer Office workers Housewives	2(15.3%) 8(61.5%) 3(23.2%)			
BMI: Body Mass Indeks (kg/m2) <18.5 18.5-24.9 25-29.9 >30	 1 (7.7%) 6 (46.1%) 5 (38.5%) 1 (7.7%)	25.68 ± 3.4	18.34	31.53
Hand dominance				
Right Left	13(100%) 0(0%)			
CTS symptom duration (months)		4.5 ± 2.4	1	10
Clinical CTS				
Bilateral Unilateral Only Left Only Right	8 (61.5%) 5 (38.5%) 4 (30.8%) 1(7.7%)			

Table 2. Results of Nerve Conductivity Study

Variables	Frequency	Mean	Minimum	Maximum
Sensoric-Distal Latency Abnormal Normal	2(13.33%) 13(86.67%)	2.90 ± 0.55	2.25	4.34
Sensoric-Amplitude Abnormal Normal	0(0%) 15(100%)	33.57± 13.91	8.40	66.50
Motors- Distal Latency Abnormal Normal	4(26.67%) 11(73.33%)	3.90±0.57	2.88	4.79
Motors-Amplitude Abnormal Normal	0 (0%) 15(100%)	7.01±1.68	4.30	10.30
Motors- KHS Abnormal Normal	0(0%) 15(100%)	57.97±4.48	51.40	65.10

Tabel 3. Muskuloskeletal USG (Ultrasonografi)

Variables	Frequency	Mean	Minimum	Maximum
Cross-section area of median nerve (mm2) Normal Abnormal	15 3(20%) 12 (80%)	12.87±3.26	5.67	19.00
Tendon flexor digitorum muscle				
Hyperechoic Normoechoic Hypoechoic	4 (26.67%) 8(53.33%) 3(20%)			
Tenosynovitis				
Yes No	0(0%) 15(100%)			
Lesions urge space				
Yes No	0(0%) 15(100%)			
Other discoveries				
Bifid median nerve	1			

Table 4. Correlation between Nerve Conductivity Study and Cross-Section Area of Median Nerve

		Cross-Section Area of Median Nerve
Sensoric-Distal Latency	r p N	0.099 0.725 15
Sensoric-Amplitude	r p N	-0.68 0.808 15
Sensoric-KHS	r p N	-0.291 0.293 15
Motors- Distal Latency	r p N	0.625* 0.013 15
Motors-Amplitude	r p N	0.385 0.156 15
Motors-KHS	r p N	0.142 0.614 15

Description:

r: correlation coefficient

p: p value indicates probability or level of significance.

*significant correlation at p <0.05 (2-tailed)

Discussion

Thirteen females were enrolled in this study in order to avoid the influence of sex on the results of the examination. Carpal Tunnel Syndrome is more common in female than male [11]. The prevalence of CTS in the general population was about 5.3% in female and 2.1% in male [8]. The median nerve cross section area at the proximal level of the carpal tunnel was greater in male than female, although it was statistically insignificant [14]. The results of a nerve conductivity study at median nerve and ulnaris showed that female have higher sensory amplitude, shorter distal latency and faster KHS than male [15].

The mean age of the subjects was 45.8 ± 10.9 years, the youngest was 33 years old and the oldest was 60 years old. Brault (2007) [4] stated that female at the age of 40-60 years old were more likely to experience CTS. The most findings was found in the subjects at the age group of 31-40 years and 51-60 years. This showed that age was not the only factor for CTS.

Three groups of occupation were obtained namely labour factory, office workers and housewives. Huisstede et al., stated that CTS was related to the frequency of hand-arm vibrations, working with the wrists on long flexion or extension positions, working on continuous hand forces, with repetitive motions or combinations of all the mentioned items [5].

One subjects had BMI less than 18.5 kg/m2, six subjects had BMI of 18.5-24.9 kg/m2, five subjects had BMI of 25-29.9 kg/m2, and a subject with BMI >30 kg/m2. Data from the literature on the effect of BMI on the tendency of CTS incidence showed variant results. Some literature suggested a correlation between BMI and the tendency of CTS [16,17] however other stated they were unrelated [18]. This might be due to BMI calculations did not include the ratio of fat and muscle [19].

All subjects of this study were right-handed. Subjects with bilateral CTS were 8 people and unilateral CTS of 5 people (CTS on the right side as many as 4 people and CTS on the left side of 1 person). This was consistent with the literature which stated the hands on the dominant side were at greater risk of CTS due to greater physical activity [19].

Electrodiagnostic results in 15 sample units using clinical diagnosis of CTS obtained 5 samples (33.33%) with abnormal nerve. Two hands with extended sensory distal latency (13.33%) meanwhile, the normal sensory amplitudes were in all samples. The number of samples with extended distal motor latency extends was 4 hands (26.67%) while the motor amplitudes were normal in all samples. The extended distal latency and decreased of nerve conduction velocity indicated the demyelination of the nerve fibers, whereas the axon abnormality would result in a decrease in the amplitude of the nerve action potential [20].

There were 5 samples who had lesions of demyelination and no samples had axonal lesions. The results of a normal nerve conductivity study with a clinical diagnosis of CTS obtained 9 samples. This might be due to a nerve conductivity study using Johnson's cut-off value [21]. Electrodiagnostic examination was the best and most reliable examination for confirmation of CTS diagnosis that had high sensitivity and specificity [6].

The mean of median nerve cross-sectional area was 12.87 ± 3.26 mm2, ranging from 5.67 mm2 to 19.00 mm2. The number of samples with the abnormal cross-sectional area was 12 hands (80%) with a cut-off value of 10 mm2. This result was consistent with the literature that musculoskeletal USG examination in CTS showed a magnification of median nerve [22]. Mechanical compression and local ischemia in median nerve on the carpal tunnel caused the onset of CTS symptoms. The continuous increase in pressure and long duration caused a change in function and structure of the median nerve. It resulted in a intraneural edema that might progress into fibrotic scars. This change appeared to be a median nerve enlargement on USG examination [9].

There were no lesions on space for all examined hands. CTS due to the lesions urge space was rare, however, complications were often more severe. Chen et al., documented 23 of the 779 patients who performed

CTS release had lesions urged space due to gout arthritis, lipoma, fibroma and ganglion cyst [23]. Ozden et al, [24] also documented that 12 of 410 CTS patients were caused by space-induced lesions. Lesions urged space were identified from USG, MRI and CT scans [24]. Information on the existence of lesions urged space were used as a consideration in the determination of therapy about the possibility of surgery [24,25].

We obtained one hand sample with variation anatomy of bifid median nerve. The bifid median nerve was also obtained in several studies. Padua et al. found two patients with a bifid median nerve from 54 CTS patients. Bifid median nerve using USG examination in 32 of 170 CTS cases and 11 of 120 controls. Cross section area of bifid median nerve was relatively larger than non-bifid [26]. Incident of bifid median nerve in CTS patients was about 0.8-2.8% and it was often accompanied by persistent median arteries. The bifid median nerve was often associated with compression median nerve in the carpal tunnel because it was relatively larger in size compared to the normal median nerve [26–28]. The presence of bifid median nerve was considered in the determination of therapy, especially surgical therapy because surgeons will consider the possibility of a persistent median artery. The presence of a two-nerve compartment considered other actions except for decompression action; it might be necessary to have epineurectomy [28].

This study found a significant positive correlation between the cross-sectional area with a motor distal latency of median nerve. Extended motor distal latency, the cross-section area of median nerve was getting bigger. This might be due to the motor nerve fibers had a larger size than the sensory nerve fibers. Large nerve fibers were more susceptible to ischemia and compression than small nerve fibers [29]. Large fascicides and wrapped in thin epineurium layers would experience heavier compression than small fascicides wrapped in thick epineurium [29]. Increased carpal tunnel pressure caused compression in all the components inside, including compression in the median nerve. Repeated compression of the median nerve caused a focal demyelination. This initial demyelination occurred in areas that compressed and it spread throughout the internodal segments that caused obstacles to neural transmission (neuropraxia) [9,30]. Continuous compression resulted in a disruption of the endoneurial blood flow of the endoneurial capillary system and caused endoneurium edema. Venous congestion, ischemia and local metabolic changes caused the inflammatory process to continue and axonal degeneration occurred. Chronic inflammation caused fibrosis in the advanced stage that would inhibit nerve gliding, it caused trauma and the formation of a scar on the mesoneurium median nerve. These changes were seen as median nerve enlargement on USG examination [9,30].

There were several potentially confounding factors in determining the cut-off value of the cross-sectional area of abnormal median nerve. The reason was the cross-section area of median nerve might be affected by race, sex and BMI. USG could not determine the severity of neuropathy median nerve as electrodiagnostic examination. The cross-section area of median nerve with a demyelinating lesion was sometimes greater than those with severe axonal lesions [31].

Conclusion

There was a correlation between motor distal latency and cross-sectional area in CTS patients, however, no correlation between sensory distal latency and cross-sectional area of median nerve was found in this study.

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Ethical Clearance : This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga

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Relationships of Service Response with Service Quality in Emergency Department of Rsud Dr. Loekmono Hadi, Kudus, Indonesia

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Abstract

Emergency Department is a service installation in a hospital that provides the first service for 24 hours to patients with the threat of death and disability in an integrated manner by involving various multidisciplinary sciences and multi professions including nursing services. The quality of service in IGD can be assessed based on 2 (two) things, namely service quality and minimum service standards (SPM). The main factor in evaluating service quality in the emergency room is the speed of service response time or response time. The purpose of service response time is the delivery of services that are fast, responsive and able to save emergency patients who need help. This research was conducted in January 2020 in the Emergency Room at Dr. Loekmono Hadi Kudus. It aims to analyze the relationship between service response time and service quality in the ED. This is a descriptive quantitative study carried out through analytic observation with a cross sectional approach. The population in this study was the visit of all patients who entered and received services by the guardian in the emergency room as much as 25-35 patients per shift per day, while the number of samples in this study were 117 patients. The independent variable in this study is service response time and the dependent variable is service quality. Data were analyzed by bivariate test using Kendall Tau. The p-value is 0.963, thus, there was no relationship between service response time and service quality in the Emergency Room Dr. Loekmono Hadi Kudus. Further research is needed because the limited understanding and knowledge of the sample influences the evaluation of service response time to service quality becomes irrelevant.

Keywords: *Emergency Room, Response Time, Service Quality*

Introduction

Emergency Department (IGD) is a service installation in a hospital (RS) that provides the first service for 24 hours to patients with the threat of death and disability in an integrated manner by involving various multidisciplinary sciences and multi professions including nursing services[(1).] General principles of emergency services in hospitals are regulated in the Republic of Indonesia Minister of Health Regulation No. 47 of 2018, as follows: every hospital is required to have emergency services with the ability to conduct initial examinations of emergency cases and conduct resuscitation and stabilization (lifesaving). Hospital EDs must provide services 24 hours a day and seven days a week. Hospitals must not ask for advances when handling emergency cases, emergency patients must be treated no later than 5 (five) minutes after arriving at the ER, IGD organizations are based on multi-disciplinary, multi-professional organizations and integrated functional organizational structures (elements leaders and executors) who are responsible for implementing

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services to emergency patients in the emergency room with full authority led by doctors, and each hospital must try to adjust its emergency services to a minimum according to classification[1]. The services provided must be in accordance with the standard operating procedures set by each hospital[2]. Policies regarding hospital ED standards are issued to improve the quality of services running well and optimally.

The quality of service in ED can be assessed based on 2 (two) points including service quality and minimum service standards (SPM). Service quality is not only related to human resource issues but also includes service flow, rules and policies governing services and infrastructure facilities in the IGD[3]. This minimum service standard is intended to be a guide for a service unit that includes planning for implementation, control and supervision and accountability of services, especially in hospitals[4]. The minimum service standard in the ED is an instrument for maintaining service quality.

The main factor in evaluating service quality in the emergency room is the speed of service response time. Service response time is the speed of patient handling starting from the patient admission to the emergency room until the patient gets the initial action due to health problems experienced[5]. The purpose of service response time is the delivery of services that are fast, responsive and able to save emergency patients who need help[2]. The standard time for service response is stated in the Republic of Indonesia Minister of Health Regulation No. 47 of 2018 which states that emergency patients must be served no later than 5 (five) minutes after arriving at the Emergency Department[1]. There are several factors that affect the response time of services in the emergency room. This include the number of health workers or emergency staff, high workload, suitability of staff competence and the number of patient visits[6].

This study aims to analyze the relationship between service response time and service quality in the ED. The procedure for measuring service response time was conducted by observation. In this procedure, the researcher calculated the time it takes the nurse to perform the initial action or anamnesa since the patient enters the emergency room by using a stopwatch (watch). This is calculated from the patient opening the IGD entrance to get the first treatment based on Triage[7].

Method

This is a descriptive quantitative study carried out through analytic observation with a cross sectional approach to the predetermined characteristic status of the research subjects. The study began in January 2020. The population in this study was the visit of all patients who entered and received services by a guardian in the emergency room of Dr. Loekmono Hadi Kudus with an average population of 1800-2000 patients per month or 25-35 patients per shift per day. Determination of the study sample was carried out using propulsive samples which produced a total sample of 117 patients.

The variables in this study consisted of independent variables and dependent variables. The independent variable in this study is the response time of emergency services. The dependent variable in this study is the quality of service in the emergency room of Dr. Loekmono Hadi Kudus. The measuring instrument used in this study was the observation sheet. After the data was collected, the analysis test was performed using SPSS XI with bivariate test using Kendall Tau.

Finding

Characteristics of respondents in this study include gender, age, education, occupation, financing and triage category. Here is a table that explains the distribution of respondents based on gender characteristics in the Emergency Room Dr. Loekmono Hadi Kudus.

Table 1. Frequency Distribution of Respondents by Gender in ED of RSUD Dr. Loekmono Hadi Kudus.

Variable	Clasification	Frequency	Percentage
Gender	Female	61	52,1%
	Male	56	47,9%
Total		117	100%

Source: 2020 primary data

Based on table 1, female respondents constituted the largest sample with 61 people (52.1%) while there were only 56 (47.9%) male respondents.

Table 2. Distribution of Frequency of Respondents by Age, Education, Occupation, Financing and Triage Category in ED of RSUD Dr. Loekmono Hadi Kudus.

Variable	Average	Min	Max	Standard of Deviation
Age	39,7	> 1 year	< 51 year	12,07
Education	2,44	None	Undergraduate	12,49
Occupation	2,19	Unemployed	Civil Servant	13,45
Payment method	2,88	Free	BPJS	3,26
Triage	2,54	P1	P4	7,60

Source: 2020 primary data

Based on table 2 above, the average age of respondents was 39.7 years, the youngest age was <1 year while the oldest age was 80 years with a standard deviation of 12.7. A total of 2.44 respondents were elementary school (SD) graduates. The standard deviation of the level of education not schooling until undergraduate education is 12.49. The average occupation was farmers (2.19). The standard deviation of the type of work from unemployed to Civil Servants (PNS) was 13.45.

The table also shows the average patient financing where 2.88 were BPJS insurance users. The standard deviation of the type of financing from free to patient using BPJS insurance was 3.26. The average triage category of patients who entered the emergency room of Dr. Loekmono Hadi Kudus is a patient with a priority triage 3 (P3) of 2.54. The standard deviations for the types of triage categories of P1 through P5 is 7.60.

Table 3. Distribution of Response Time in the Emergency Room of RSUD Dr. Loekmono Hadi Kudus

Response Time	Frequency	Percentage
Not good	61	52,1
Good	56	47,9
Total	117	100,0

Source: 2020 primary data

Based on the results of the analysis in table 3 the highest response time distribution in services in the Emergency Room Dr. Loekmono Hadi Kudus was not good (52.1%).

Table 4. Distribution of Service Quality in Emergency Room Dr. Loekmono Hadi Kudus

Service quality	Frequency	Percentage
Achieved	98	83,8%
Not achieved	19	16,2%
Total	117	100%

Source: 2020 primary data

Analysis of the data in table 4 shows the distribution of service quality in the Emergency Room Dr. Loekmono Hadi Kudus was achieved (83.8%).

Table 5. Relationship of Response Time and Service Quality in the Emergency Room Dr. Loekmono Hadi Kudus

Variable	Correlation coefficient	P value
Service response time	0,004	0,963
Service quality		

Source: 2020 primary data

Based on the results of the Kendall Tau analysis in table 5 the p value is 0.963. This shows that there is no relationship between service response time and service quality in the emergency room of RSUD Dr. Loekmono Hadi Kudus.

Discussion

The results of the study of service response time variables to the quality of service obtained the results there is no significant relationship between service response time and service quality in IGD RSUD Dr. Loekmono Hadi Kudus. The response time of service to patient acceptance even though in the results stated that there was no effect on the quality of service, the researchers saw that the results of service response time were not according to the standard, which exceeded 5 minutes. This is influenced by an imbalance between the ratio of the number of guard officers (nurses and doctors) and the number of patient visits that enter the emergency room. Patient's evaluation of service response time to service quality is also influenced by patient and family understanding of low service standards. This may due to patient education most of which is primary education that created limited understanding and knowledge of patients making irrelevant judgments.

According to hospital emergency services standards issued by the Nursing and Medical Services Development the availability of the number of medical personnel to patients is 1: 7 and the ratio of nurses to patients is 1: 4. However, the number of patient visits that enter the emergency room needs to be considered in order to avoid inequality which results in a decrease in the quality of service in the emergency room[8]. Officers on duty every shift at the IGD RSUD Dr. Loekmono Hadi Kudus consisted of one emergency doctor and four nurses. This is not in accordance with the rules of the Ministry of Health which determines the ratio of 1:7 doctors on duty, which means 6-7 nurses are needed with an average number of visits per shift of 25-35 patients.

The results of previous studies regarding the achievement of the standard of service quality evaluation of the Hardjono Ponorogo Hospital on the competence variable and the number of IGD guard staff stated that there would be a decrease in the quality of service in the emergency room if the adequacy of the number of personnel was not properly calculated compared to the large number of patients who entered[9]. Another different research result states that service response time seen from the variable number of workers does not significantly influence the quality of service in the ED[10]. The results of the study have shown that high workload has a significant effect on service quality. High workload caused by the inability of some professions that eventually carried out by other professions. Therefore, even though the number of staff is sufficient, if the workload is not in accordance with their respective professionalism, it will have an impact in the process of providing services to patients.

Patient's evaluation of the variable elements of service response time did not significantly influence the quality of services provided by the IGD emergency department attendant Dr. Loekmono Hadi Kudus. This is most likely due to the patient's understanding and knowledge of hospital emergency procedures and standards. This is evidenced from the results of the study of educational averages of elementary school (SD) graduates from the lowest education level to the highest education level. It is very possible that the limitations

of understanding and knowledge of patients affect the evaluation of service response time to the quality of service to be irrelevant. Calculation of the number of ratios between staff and the number of patient visits that should also be a concern for the emergency room management because it will be a factor that can affect the response time of officers to patient acceptance.

Conclusion

The researchers concluded that based on the results of the analysis of the relationship between the response time of service to the quality of services performed in the scope of services of the Emergency Room Dr. Loekmono Hadi Kudus p value is 0.963 which is greater than the value of p value 0.05. This explains that there was no relationship between service response time and service quality. Based on this, the researcher suggests the need to calculate the ratio of the number of watchmen both doctors and nurses with the number of patient visits according to the standards set by the Indonesian Ministry of Health so that it is expected that the response time for services can be carried out properly.

Conflict of Interest: All authors have no conflicts of interest to declare.

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Study of Adult Injury Fatality in Nigeria

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Abstract

Background: Although Injury is a major cause of death in low and middle income countries, its demographic characteristics in such regions is poorly characterized.

Aims and Objective: The study aims at determining the demographic pattern of adult injury-related deaths in a metropolitan City in Nigeria.

Materials and Methods: This is a 14-year (2003-2016) descriptive retrospective autopsy study of adult injury fatalities in Warri, Nigeria. The gender, age, and causes of death analyzed using SPSS version 22 and the results presented in tables and figures.

Results: Of the 975 medico-legal deaths in this city from 2003 to 2016, 860(88.2%) deaths were attributed to adult injury; among 778 (90.5%) males and 82 (9.5%) females. The highest and lowest incidence were among the 21-30 years (39.8%) and 51-60 years (6.6%) age brackets respectively. Homicide, accident and suicide contributed 639 (74.3%), 205 (23.8%) and 16 (1.9%) of the fatalities respectively. Firearm injury (47.4%), road injury (11.7%), sharp weapon injury (18.1%), blunt injury (8.1%), drowning (3.5%), burns injury (2.9%), electrocution (2.3%), hanging (1.5%) and petrochemical gas inhalation (1.3%) and falls (0.9%) were the major causes of death.

Conclusion: Adult injury fatality is poorly addressed public health problem in Nigeria. The study showed high vulnerability of victims in the 3rd decade of life and a disproportionately higher male fatality. Homicide is the most common manner of death while is very rare. Proper understanding of causes and manners of these preventable deaths is necessary to public health interventions and policies.

Keyword: *Unintentional injuries, Adult, Autopsy, Homicide, Road Traffic Injuries*

Introduction

Injury refers to "physical damage that results when a human body is suddenly or briefly subjected to intolerable levels of energy".[1] The energy may be mechanical/kinetic, electrical, thermal or chemical as seen in road traffic injury, gun-shot injury and suffocation; electrocution; burns; and gas inhalation and poisoning respectively.[1] Each year, injury contributes about 16% of the global burden of diseases,[2] and 9% of all deaths.[3]

The impact of injury is immense and can be measured in terms of mortality, morbidity, disability, and economic loss. While the public health implication of injuries are well documented in developing countries, the situation is different in sub-Sahara Africa which are continually burdened with economic crisis and other competing health challenges.[4]

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The age-group 15-60 years (adult) is a critical period in human lifespan as it represents the reproductive and most productive age range.[5] According to world health organization, the global mean adult(15-60 years) mortality is about 142 deaths per 100,000 population, with the higher rates encountered among low income countries.[6]

The study hopes to evaluate the epidemiological profile of adult fatal injuries among medicolegal deaths in Warri, Nigeria with the hope of providing relevant information for policy makers in this region.

Materials and Method

Warri, Nigeria is located 5.52 latitude and 5.75 longitude and situated 6 meters above sea level. It has high density of natural water bodies, with a population of 536, 023 inhabitants. Its location is shown in figure I.

This is a 14-year descriptive retrospective study of adult injury related deaths among medico-legal deaths in this City. The information used for this study was extracted from the testimonies of the investigating police officers, the eye-witness account, the scene of crime observations and the written autopsy reports. The date, sex, age and manner of death for these cases were used for this study.

Data were analyzed using Statistical Package for the Social Sciences version 21(Released 2012, Armonk, New York: IBM Corporation), and presented in tables and figures.

All death confirmed as primarily due to homicide, suicide or accidents within the geographical region after autopsy studies were included in the study, while deaths primarily of natural causes or of inconclusive diagnosis were excluded. Fifteen cases with inconclusive diagnosis were excluded from the study.

Result

We encountered 860 adult injury fatalities, representing 83.7% of medicolegal deaths. The gender and manner of death is shown in table 1, with the victims composed of 778 males and 82 females, which gave a male to female ratio of 9.5:1. Homicide, accident and suicide contributed 639(74.3%), 205(23.8%) and 16(1.9%) of the death burden respectively. Of the 778 males fatalities, the leading causes were intentional firearm injury(48.6%), Sharp weapon injury(18.8%),road injury(10.5%), blunt weapon injury(8.1%), drowning(3.7%), electrocution(2.6%), burns(1.7%), gaseous chemical inhalation(1.4%), suicidal hanging(1.3%) and accidental fall(1.0%). Of the 82 adult female fatalities, leading contributors were intentional firearm injury (30%), road injury (23.2%), burn (14.6%), sharp weapon (12.2% and blunt weapon injury (8.5%).

The yearly distribution of adult injury fatality is shown in Figure II. The peak and trough corresponded to 2014 and 2006 respectively.

The relationship between the causes of fatality and the ages of the victims is shown in table 2. The highest mortality was in the 3rd decade and a continuous decline noted until the 6th decade.



FIGURE I: LOCATION OF WARRI, NIGERIA

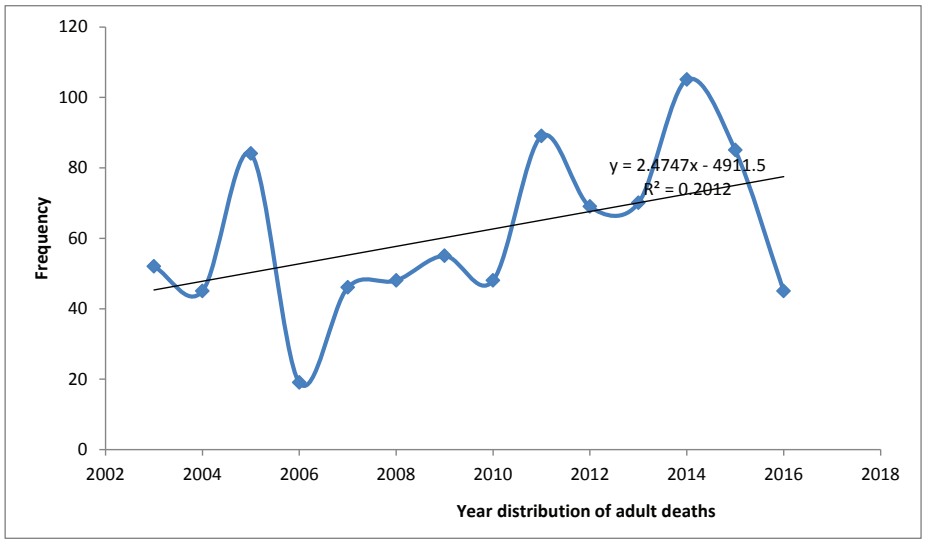


FIGURE II: YEARLY DISTRIBUTION OF ADULT INJURY FATALITY IN WARRI, NIGERIA

TABLE I: SEX DISTRIBUTION OF CAUSES OF INJURY FATALITY IN NIGERIA

Manner of death	No of cases	Cause of Death	Sex		Total
			Male	Female	
Homicide	639(74.3%)	Blunt weapon injury	63(8.1)	7(8.5)	70(8.1)
		Firearm injury	378(48.6)	25(30.5)	403(46.9)
		Sharp weapon injury	146(18.8)	10(12.2)	156(18.1)
		Suffocation	9(1.2)	1(1.2)	10(1.2)
Accidental	205(23.8%)	Accidental poisoning	1(0.1)	1(1.2)	2(0.2)
		Burn injury	13(1.7)	12(14.6)	25(2.9)
		Carbon monoxide Poisoning	2(0.3)	1(1.2)	3(0.4)
		Drowning	29(3.7)	1(1.2)	30(3.5)
		Electrocution	20(2.6)		20(2.3)
		Fall from height	8(1.0)		8(0.9)
		Firearm injury	5(0.6)		5(0.7)
		Road injury	82(10.5)	19(23.2)	101(11.7)
		Gas/chemical inhalation	11(1.4)		11(1.3)
Suicide	16(1.9%)	Hanging	10(1.3)	3(3.7)	13(1.5)
		Chemical/drug ingestion	1(0.1)	2(2.4)	3(0.4)
Total	860(100%)		778(100.0)	82(100.0)	860(100.0)

$c^2 = 83.506$ df = 13 $p < 0.001$

Table II: AGE-WISE DISTRIBUTION OF CAUSES OF ADULT INJURY FATALITY

Manner of death	Mechanism of death	Age in years					No of cases (%)
		≤20	21-30	31-40	41-50	51-60	
Homicide	Blunt weapon	7(7.7)	21(6.1)	22(9.3)	11(8.2)	9(15.8)	70(8.1)
	Firearm	28(30.8)	168(49.1)	118(50.0)	64(47.8)	25(43.9)	403(47.4)
	Sharp Weapon	34(37.4)	71(20.8)	31(13.1)	17(12.7)	3(5.3)	156(18.1)
	Suffocation	1(1.1)	1(0.3)	1(0.4)	3(2.2)	4(7.0)	10(1.2)
Accidental	Accidental poisoning	1(1.1)			1(0.8)		2(0.2)
	Burn	4(4.4)	6(1.8)	8(3.4)	7(5.2)		25(2.9)
	CO poisoning	2(2.2)		1(0.4)			3(0.4)
	Drowning	2(2.2)	11(3.2)	12(5.1)	3(2.2)	2(3.5)	30(3.5)
	Electrocution	2(2.2)	7(2.1)	7(3.0)	4(3.0)		20(2.3)
	Fall	2(2.2)	1(0.3)	1(0.4)	3(2.2)	1(1.8)	8(0.9)
	Firearm		2(0.6)	3(1.3)			5(0.6)
	Road injury	4(4.4)	45(13.2)	24(10.2)	18(13.4)	10(17.5)	101(11.7)
	Gas inhalation		5(1.5)	3(1.3)	1(0.7)	2(3.5)	11(1.3)
Suicide	Chemical/drug Injection	2(2.2)	0(0.0)	1(0.4)			3(0.3)
	Hanging	2(2.2)	4(1.2)	4(1.7)	2(1.5)	1(1.8)	13(1.5)
	Total	91(100.0)	342(100.0)	236(100.0)	134(100.0)	57(100.0)	860(100.0)

Discussion

This is the first study of injury fatality among adults in this region. Unlike in developed countries, the vital registration in Nigeria is a perennial failure.[6] Information from medico-legal autopsy data has advantage of capturing unnatural deaths in the community, irrespective of where the death occurred. This is corroborated by the high rate of unnatural deaths in autopsy-based mortality studies in Nigeria.[7] It is also a general observation that injury fatality represents the tip of a pyramid whose base is composed of non-recorded, non-treated and treated non-fatal injuries.[8] There is therefore need for improved surveillance system for more holistic retrieval of data on injury mortality of the population to guide health intervention and policies.

In developing countries, injuries have remained a poorly-addressed public health concern. Poverty, non-existence of preventive measures and the poorly equipped health-care system remain major draw-backs in low and middle income countries (LMIC). This corroborated by the report that more than 90% of injury fatality occur in LMIC. [9]

In this study, we noted a preponderance of male injury fatality, which is consistent with other reports.[10] The gender disparity on adult fatal injuries is attributed to multiple factors, including male masculinity, gender role in the society, occupational choices, sociocultural and behavioral factors.[10,11] Generally, relative to women, men have higher propensity to risk taking.[11] Males are more involved in outdoor occupations associated with high risk of injuries such as electrification, welding, boat-driving, haulage services, and automobile services. Alcohol and substance abuse, a proven risk factor for injury is also more prevalent among males.[12] Women's role in childbearing increases their cost of risk-taking since offspring survival depends on them, making them to be more prudent in their actions.[13] Although, injury fatality among adult females is relatively lower, injuries are also an important cause of morbidity and mortality among this group.[14] There is need for public health intervention that do not only advocate for behavioral change but also societal changes, especially as it relates to gender perceived roles in the society.

Our report shows that mortality rate increased with age and peaked in the 3rd decade and declined gradually thereafter. This finding is corroborated by reports from WHO, supporting a relatively high injury fatality among the 15-29 years age group, mostly attributed to homicide, road injuries and war.[15] High risk-taking behavior, peer influence and delinquent behavior are the major triggers of such high fatality among young adults.[15] Older adults are more prudent hence they are less adventurous which reflects in low injury rates in the older age group.

In relation to manner of death, homicide was the most common with suicide being the least. The high rate of homicide in this region may be attributed to multiple factors including the age-long militancy, cultism, long-standing inter-ethnic crisis, unemployment, ignorance, inequality, poverty and the ineffective health system.[16] The low level of suicide death is expected in our environment where suicide is seen as a taboo and a desecration of the land.[17] Earlier studies across the globe also showed that suicide rate is lowest in Africa.[18] It could be postulated that accidental deaths are under-reported due to laxity of law-enforcement agencies, who would not be bothered over accidental deaths as they are over homicide cases.

In this study, fatal intentional firearm injuries ranked topmost among causes of fatality with 70% of cases encountered in the 21-40 years age-group. There was also a marked gender disparity with 93.8% of the victims being males. This is not surprising as activities associated with use of firearm such as interpersonal violence, armed robbery, communal crashes, religious violence, militancy, cultism and political vendettas are common in this age group.[19] Also corroborating this fact is the report of influx of illicit firearms into this region[20] despite the law that restricts ownership of firearms in the country.[21] There is therefore need for the law enforcement agencies to ensure enforcement of this law.

Road injury is a serious problem in developing countries, resulting in increasing death, disability and economic loss.[22] In this study, it accounted for 11.7% of fatality, 82% were among males, and 70% occurred among adults of 21-40 years. This is attributable to the explosive economic and population growth in a backdrop of non-existing railway system and unaffordable air transport. Road safety is determined by a combination of human behavior and structural factors. Better road design that accommodates footpath for pedestrians, street lighting, proper road maintenance, use of road worthy vehicles, enforcement of road safety rules, banning of motor cycle in major roads and provision of surveillance ambulance services are ways of effectively reducing road injury rate.[22,23]

Fatal drowning in this study is low in view of the WHO report that drowning claimed 372,000 lives in 2012 and that most of these cases occurred in LIMC.[24] *The cases in our study therefore represent a tip of the iceberg with a base composed of unreported fatal drowning. High gender disparity may be attributed to greater involvement of adult males in water-related leisure and occupations. There is need for individuals, community and policy makers to adopt preventive measures to tackle this menace.*

The electrocution fatalities in this report were all unintentional deaths and exclusively among males, with 70% affecting the 21-40 years age group. There is need for proper electric safety training both at home and workplace to reduce the risk of electrocution injuries.

Flame burn injury was encountered in 2.9% of fatality in this study with a fairly equal gender distribution. Most studies in Nigeria are based on in-hospital patients or autopsy studies and may not give a clear picture of the incidence. Studies have shown that 4.8% of trauma deaths in Nigeria result from burn injuries.[25] The rising incidence of burns injury are due to pilfering and vandalization of petrochemical pipelines, kerosene adulteration, poor understanding of burns prevention strategy, poverty and ignorance. [26] There is need to focus on burn prevention strategies, and to strengthen the health sector on management of burn injuries.

Cases of gas explosion related deaths were also reported in this study. These are attributed to poor maintenance culture of the multi-national companies, and the lingering pipeline vandalization that frequently occur in this region.[27]

With the available weight of evidence, there is an urgent need for all stake holders to ensure reversal of this death toll. If the law enforcement agencies are better equipped to persecute criminals, it will discourage prospective homicide offenders. Educating the society on injury prevention strategies will go a long way in reducing accident related injuries. There is no better time than now for the government to invest in road rehabilitation, considering the present state of the roads. Above all, developing a low cost but effective trauma care service within the state will no doubt reduce the mortality rate of injured victims.

In sum, adult injury fatality is poorly addressed public health problem in Nigeria. The study showed high vulnerability of victims in the 3rd decade of life and a disproportionately higher male fatality. Homicides appeared relatively high while suicides was rare. Proper understanding of causes and manners of these preventable deaths is necessary to guide design of public health interventions and policies.

Limitation of the study: The study is an autopsy study and is based on cases referred to us by the law enforcement agencies. Some cases may not be reported to the Police and some cases, the people concerned may avert the course of justice through illegal means. Therefore, the autopsy rate may only cover a fraction of injury related deaths. There is therefore need for greater surveillance and enforcement of autopsy on all coroner cases.

Conflicts of Interest: Nil

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Determination and Correlation of Electrolytes and Trace Elements from Vitreous Humour and Synovial Fluid by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for Estimation of Post-Mortem Interval (PMI): Experimental Study in an Animal Model

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Abstract

The estimation of post-mortem interval (PMI) procedures seem to subjectivity in assessment and less sensitivity and specificity. Biochemical determination of trace elements from closed-system samples, such as vitreous humour (VH) and synovial fluid (SF) is an alternative laboratory investigation for PMI estimation. The inductively coupled plasma-mass spectrometry (ICP-MS) provides more rapid, precise and sensitive analysis of elements samples. However, determination of electrolytes and trace elements in VH and SF by ICP-MS, and application for PMI has not been intensively investigated. In this study, VH and SF from twenty adult domesticated pig carcasses were collected at 1, 3, 6, 12, 24, 36, and 48-hour after death. Samples were analysed for total protein concentration, Ca, Cu, Fe, K and, Na using ICP-MS. The results showed that the protein concentration in VH was significantly increased in time dependent manner, but not for SF. The level of K and Fe in both VH and SF increased in time dependent manner, with a good correlation between both sample sources. The calcium concentration of both VH and SF increased in time dependent manner, but there was no correlation between both sample sources. Sodium was significantly reduced at 36-48 h, with a correlation between both sample sources. However, the copper level in VH was increased at 36-48 h but reduced in SF. In conclusion, this study showed *for the first time* that multiple trace elements and electrolytes could be detected, using the ICP-MS, in both of VH and SF simultaneously and additionally its correlation.

Keywords: *time since death; post-mortem interval; inductively coupled plasma-mass spectrometry; vitreous humour **;** synovial fluid*

Introduction

The time since death or post-mortem interval (PMI) estimation through the analysis of post-mortem biochemistry was first investigated in the mid-twentieth century [1]. Biochemical markers are examined to predict time since death [2-4]. The majority of the PMI parameters utilize the ante-mortem and post-mortem concentration discrepancies of biochemical substances in blood, which occur rapidly and are also influenced by several factors and become limitation of interpretation [5]. A "closed compartments" such as cerebrospinal fluid, synovial fluid, and vitreous humour could be more suitable for the assessment of post-mortem changes.

The vitreous humour is one of the preferred mediums for post-mortem electrolyte concentrations [6, 7]. Several organic and inorganic analytes have been investigated [3,

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[8]. Alternatively, synovial fluid could also be a source for PMI estimation [9]. The electrolyte analytical technique that is most widely used for potassium and sodium ions in vitreous humour and synovial fluid by means of ion-selective electrodes (ISE) and flame photometry. This process utilises commercial automated or manually operated analytical instruments which are available in most hospitals and laboratories. [8, 10-14].

Inductively coupled plasma-mass spectrometry (ICP-MS) provides more rapid, precise, and sensitive analysis of very low concentrations of elements in liquid samples. However, the application of ICP-MS in the determination of electrolytes and trace elements in vitreous humour and synovial fluid has not been intensively investigated. The purpose of this study is to integrate ICP-MS into the investigation of multiple electrolytes and trace elements in vitreous humour and synovial fluid for PMI estimation.

Materials and Methods

Chemical and reagents

The internal standards for ICP-MS; Ca, Cu, Fe, K and, Na were supplied by SCP Science (Quebec, Canada). Ultrapure water was purchased from Agilent (California, United States). Other chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri, United States).

Materials

Twenty adult domesticated male and female pig (*Sus domesticus*) carcasses of varied ages were selected as the animal model. Each pig carcass weighed ≥ 45 kgs. The pig carcasses were purchased from a standard abattoir, Pratumthani province, Thailand. Immediately after death, the animal carcasses were immediately transported to an open field research site, where they were placed on the grass under natural shade and ambient conditions. The ambient and pig carcass temperature was recorded simultaneously in accordance with sample collection time.

Vitreous humour collection

Approximately 100 µl of vitreous humour was collected from both eyes of each pig carcass using a 21G needle with a 1 ml plastic syringe. Vitreous humour samples were collected 1, 3, 6, 12, 24, 36, and 48-hour intervals from the same animal carcasses. The same needle and syringe were used to collect vitreous humour from both eyes, stored at -80°C until further analysis.

Synovial fluid collection

Approximately 100 µl of synovial fluid was collected from each pig carcass using a 21G needle attached with 1 ml plastic syringe. The same needle and syringe were used to collect synovial fluid from all 4 joints at 1, 3, 6, 12, 24, 36, and 48-hour intervals from the same animal carcasses. The samples were stored at -80°C until further analysis.

Sample Preparation

An aliquot part of 50 µl of thawed vitreous humour sample was transferred into 15-ml plastic tubes. Ultrapure nitric acid 5% (v/v) was then slowly added to the sample to give a final volume of 3 ml. The samples were centrifuged at 500 x g at room temperature for 15 minutes. The supernatant was then transferred to pre-washed 15-ml plastic tubes with natural caps for multi-element analysis.

Determination of protein concentration by Bradford protein assay

Fifty microliters of the protein samples were added to 2.5 ml of Bradford reagent (BIO-RAD, USA) and incubated at room temperature. Sample absorbance was measured using a spectrophotometer at 595 nm.

Determination of electrolytes and trace elements by inductively coupled plasma-mass spectrometry (ICP-MS)

All samples were analysed three times for Ca, Cu, Fe, K and, Na using inductively coupled plasma-mass spectrometry (ICP-MS) (7500 Series, Agilent). The samples and standards were aspirated and nebulized using a quartz Scott-type spray chamber into the argon plasma through a peristaltic pump operated at a flow rate of around 0.9-1.0 ml/min. The elements were analysed with a quadruple mass analyser.

Statistical Analysis

All values were expressed as mean ± standard deviation (S.D.). All comparisons were assessed by one-

way analysis of variance (ANOVA), followed by the Tukey-Kramer test. A statistical value of less than 0.05 was considered significant. Correlation analysis was calculated using Pearson's correlation or Spearman's rank correlation.

Results

1. Alteration of carcass body temperature

The pig carcasses were placed on the grass under natural shade and ambient conditions (Figure 1A). The body temperature dropped to 28.5 °C at 12 h after death which corresponded to the ambient temperature (Figure 1B). The carcass temperature was then stable until the end of the data recording period, regardless of fluctuating ambient temperatures.

2. Determination of total protein content from vitreous humour and synovial fluid

The results showed that vitreous humour protein collected at 6 hours after death was significantly higher than at 0-3 hours. The vitreous humour protein peaked at 36 hours after death. (Figure 1C), which significantly different when compared to other periods. In contrast, the synovial fluid results showed no significant change in protein concentration.(Figure 1D).

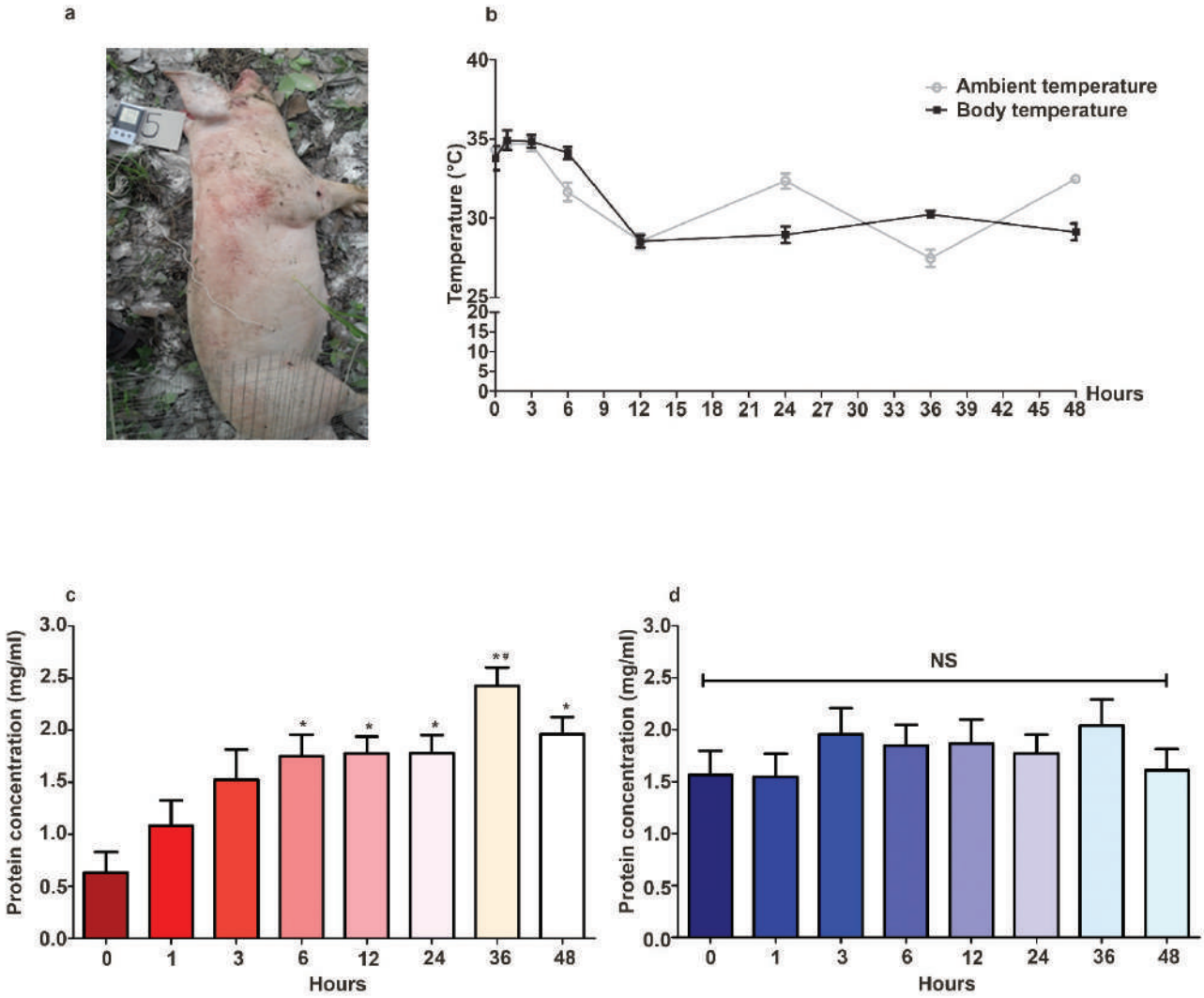


Figure 1. The animal carcasses were placed on the grass under natural shade and ambient conditions

(a). The ambient and pig carcass temperatures were recorded over 48 hours (b). The protein concentration of vitreous humour (c) and synovial fluid (d) was determined by Bradford assay.

3. Determination of sodium ion from vitreous humour and synovial fluid

The sodium ion concentration in vitreous humour and synovial fluid changed at 36 hours after death. However, the sodium ion concentration in vitreous humour at 48 hours after death was significantly lower than at earlier time checks (Figure 2A). Whereas, the sodium ion in synovial fluid at 36 hours and 48 hours after death were significantly lower than at earlier time points (Figure 2B). Interestingly, the results showed a correlation of sodium ion concentration, at 48 hours after death between vitreous humour and synovial fluid ($r = -0.529$, $p = 0.02$) (Figure 2C).

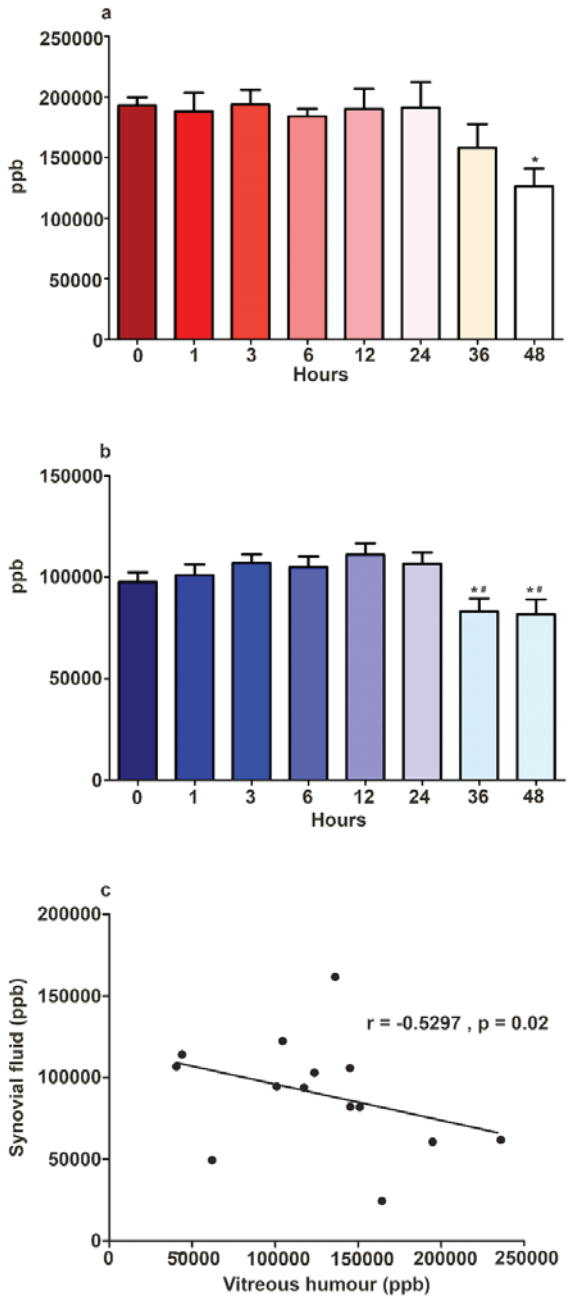


Figure 2. Determination of sodium ion from vitreous humour (a) and synovial fluid (b) and correlation of sodium ion at 48 hours after death (c)

4. Determination of potassium ion from vitreous humour and synovial fluid

The results showed that the concentration of potassium ion in both vitreous humour and synovial fluid continuously increased over the data collection period. The potassium ion concentration in vitreous humour increased significantly at 12 hours after death (Figure 3A), whereas the synovial fluid potassium ion concentration increased significantly at 6 hours after death (Figure 3B). However, there was no correlation in potassium ion concentration at 48 hours after death between vitreous humour and synovial fluid (r = -0.433, p = 0.06) (Figure 3C).

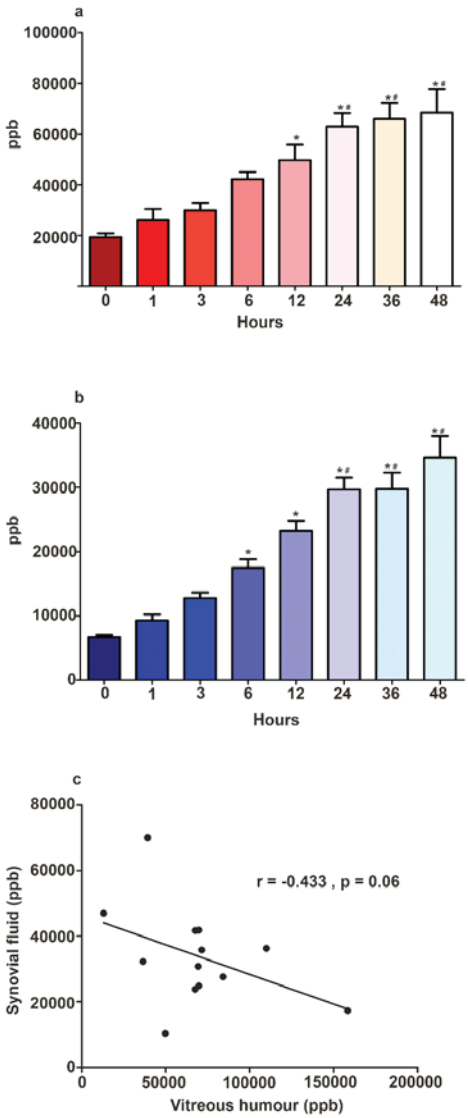


Figure 3. Determination of potassium ion from vitreous humour (a) and synovial fluid (b) and correlation of potassium ion at 48 hours after death (c)

5. Determination of calcium ion from vitreous humour and synovial fluid

The calcium ion concentration in vitreous humour increased significantly at 24 hours after death (Figure 4A), whereas the calcium ion concentration in synovial fluid was significant at 12 hours after death. (Figure 4B). There was no correlation of calcium ion concentration at 48 hours after death between vitreous humour and synovial fluid (r= 0.233, p = 0.212) (Figure 4C).

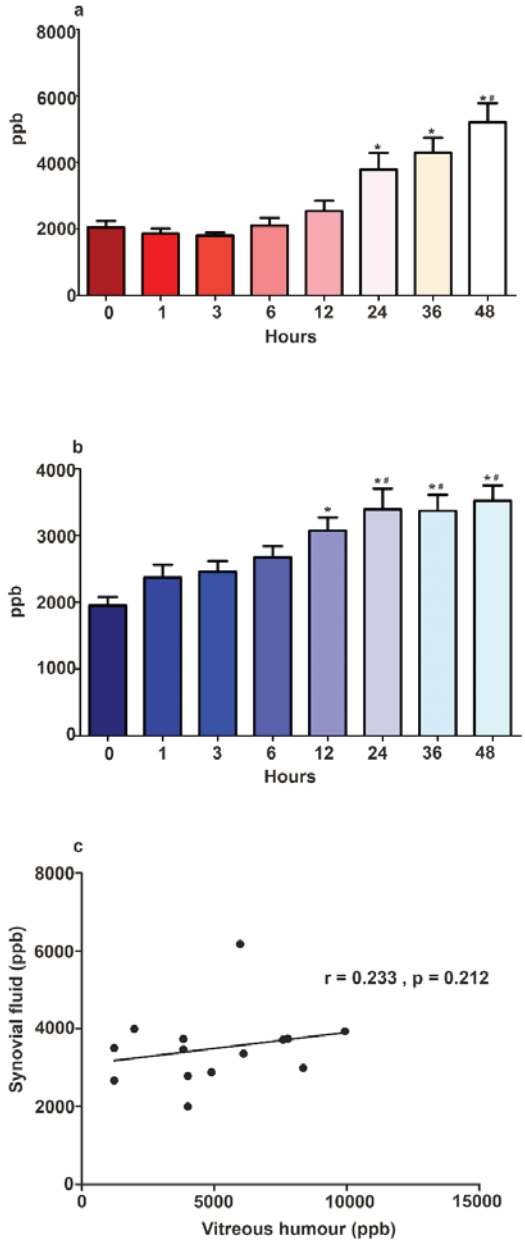


Figure 4. Determination of calcium ion from vitreous humour (a) and synovial fluid (b) and correlation of calcium ion at 48 hours after death (c)

6. Determination of iron ion from vitreous humour and synovial fluid

The results showed a continuous increase in iron concentration in vitreous humour throughout the data collection period. At 36-48 hours after death the level of iron ion concentration in vitreous humour was significantly higher than earlier time points (Figure 5A). In contrast, the iron concentration in synovial fluid increased 1 hour after death and remained unchanged thereafter. However, statistical analysis showed that at 48 hours after death the level of iron ion concentration in vitreous humour was significantly higher when compared to 0 hours (Figure 5B). Interestingly, there was a positive correlation in iron ion concentration, at 48 hours after death, between vitreous humour and synovial fluid ($r = 0.610$, $p = 0.02$) (Figure 5C).

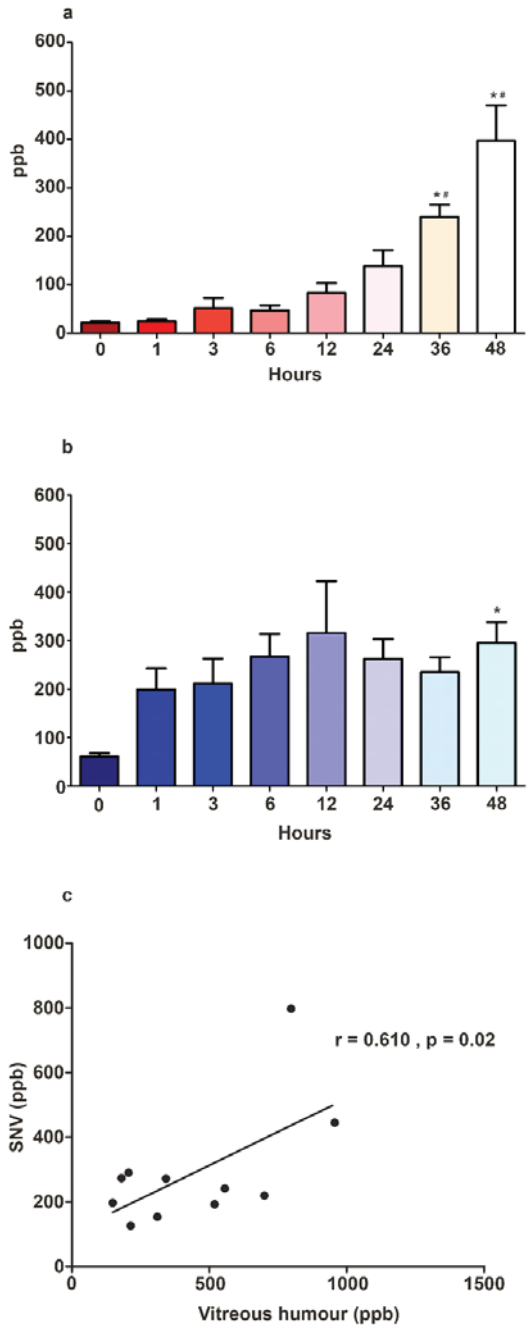


Figure 5. Determination of iron ion from vitreous humour (a) and synovial fluid (b) and correlation of iron ion at 48 hours after death (c)

7. Determination of copper ion from vitreous humour and synovial fluid

At 24-48 hours after death the level of copper ion concentration in vitreous humour was significantly higher from other time points (Figure 6A). In contrast, the copper ion concentration in synovial fluid was unchanged until 36-48 hours after death. The level of copper ion concentration in synovial fluid was significantly lower from 36 hours onwards when compared to the previous time points (Figure 6B). Interestingly, there was a significant correlation of the copper ion concentration, at 48 hours after death, between vitreous humour and synovial fluid ($r = 0.517$, $p = 0.02$) (Figure 6C).

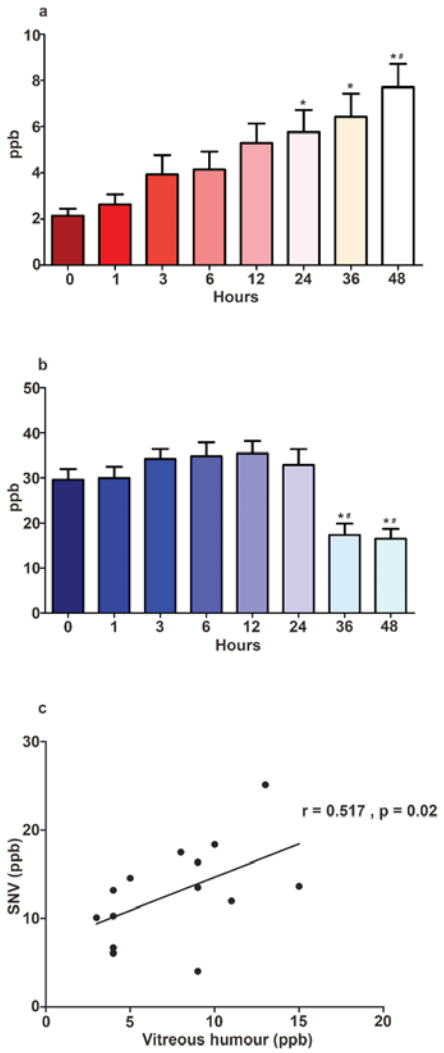


Figure 6. Determination of copper ion from vitreous humour (a) and synovial fluid (b) and correlation of copper ion at 48 hours after death (c)

Discussion

The majority of post-mortem interval (PMI) studies concentrate on ^{12} in vitreous humour and there is insufficient information available on other electrolytes and minerals beyond K, Na, Cl and Ca. Moreover, studies on the correlation of various electrolyte and mineral concentrations between different body fluid compartments are limited [15].

Vitreous humour, potassium ion ^{12} is the most extensively investigated biochemical marker as its concentration increases after death [15]. Vitreous humour is one of the most suitable mediums for investigation because, firstly, electrolytes are secure and isolated in the eyeball. The extreme density and toughness of the sclera offers significant protection and stabilization of internal content. In which the electrolyte concentrations in vitreous humour can be stable for up to 120 hours post-mortem [3]. Therefore, vitreous humour is considered ideal for the study of PMI, which firstly reported in 1959 [16]. However, there was some discrepancy in conclusions due to the estimated intercepts and gradients or slopes of regression line(s), the reliability and confidence interval and the standard error or deviation [12, 17].

Alternatively, synovial fluid is also considered as medium for PMI estimation. The protein and large biochemical constituents in synovial fluid are well established in rheumatology [18]. The joint capsule (stratum fibrosum) and the inner layer comprises a unique cellular lining of synoviocytes creating the synovial membrane [19]. A study by Ropes *et al*. [20] found the presumptive absence of fibrinogen in human synovial fluid in the absence of significant pathology, which leads to a probable advantage of synovial fluid over other mediums for PMI.

The results from the current study demonstrated that all measurable elements displayed significant changes over the data collection period. Interestingly, three of the eight elements; sodium, iron and copper ions revealed significant correlations between vitreous humour and synovial fluid. The study found that there was no single element that could serve as a marker for universal PMI estimation. The pattern of alteration did not clearly indicate a process of diffusion of some intracellular elements within vitreous body or synovial fluid.

A study by Tumram et. al. [14] also exhibited significant changes in potassium and glucose concentration in both vitreous humour and synovial fluid in cadavers.

It was apparent that different elements displayed different relationships with PMI. Thus, it was feasible to obtain a high degree of accuracy of PMI estimation using multiple vitreous elements as predictor variables. To obtain multi-elemental analysis simultaneously, the ICP-MS appeared to be the most appropriate technique and its potential has not been sufficiently explored .

Conclusions

This study is *the first* to quantitatively determine multiple elements in vitreous humour and synovial fluid simultaneously and additionally its correlation using the ICP-MS. Moreover, utilizing alterations and correlations of multiple elements in the vitreous humour and synovial fluid tends to increase the accuracy of post-mortem interval estimation. The latter has a potential use as an alternative medium for the estimation of time since death.

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Conflicts of Interest: The authors declare that they have no competing interests

Ethical Clearance: The ethical Clearance was proved by National Research Council of Thailand.

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Comparison of Surgically Induced Corneal Astigmatism in Cataract Patients after Phacoemulsification Versus Manual Small-Incision Cataract Surgery

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Abstract

The advances in cataract surgery are aimed at finding a surgical method capable of providing accurate and predictable results in terms of outcome. Surgically Induced Astigmatism (SIA) is one of the causes of the poor quality of vision post-operatively because of the blurring of images. SIA in turn, depends on the type, length and the position of the incision.so this study to compare the mean keratometeric reading preoperative and one month postoperative in phacoemulsification versus manual small-incision cataract surgery and to find which produce less surgically induced astigmatism. This was a prospective, observational, cohort study on patient with cataract carried out from April 2018 to September 2018. This study conducted on (206) patients attending to ophthalmic department in Najaf. The cases were divided into two groups. Each group had 103 patients. One group had undergone phacoemulsification and the other group had undergone superior scleral tunnel incision and follow up after one month to find which procedure less surgically induced astigmatism. Preoperative and postoperative keratometeric was performed after one month. According to this study mean keratometeric reading in the Phaco group preoperative k1= 43. 80, k 2= 44.69 and postoperative k1 = 43.71and K2 =44.62.While Mean keratometeric reading in the SSTI group preoperative k1=44.00, K2 = 45.02 and postoperative k1 =43.91 and K2= 45.41.

Mean astigmatism at one month postoperative in patient undergoing Phaco was 1.3 ±0.9 and undergoing SSTI was 1.3± 0.7, there was no statistical difference in astigmatism at one month postoperative (P=0.5).

This study reveals that superior approach in PHACO and MSICS produced similar astigmatic outcome, there is no significant difference in the mean surgically induced astigmatism in both types of surgery.

Keyword: *cataract surgery, Phacoemulsification, manual small-incision cataract surgery, Surgically Induced Astigmatism, incision site*

Introduction

The sclera forms the posterior five-sixths of the connective tissue coat of the globe. The sclera maintains the shape of the globe, offering resistance to internal and external forces, and provides an attachment for the extraocular muscle insertions. [1] The transition zone between the peripheral cornea and the anterior sclera, known as the limbus. The surgical limbus can be divided conceptually into two equal zones: anterior bluish-gray zone and posterior white zone. [2] The cornea is a transparent structure at the front of the eye. It is a powerful refractive surface and a robust barrier that protects the ocular contents. Peripheral corneal asphericity reduces optical blur from spherical aberration [3] The crystalline lens is biconvex, avascular, transparent structure enclosed by capsule, which is basement membrane secreted by lens epithelium. The normal lens transparent any congenital or acquired opacity in the lens capsule or substance, irrespective of the effect on vision, is a cataract. [4]

Cataract poses both a significant socioeconomic burden and a public health concern, as it is the leading cause of blindness worldwide. [5]. By far the most

important risk factor for cataract is age; ageing-related cataract constitutes the great majority of all cataracts and is a major public health problem worldwide. In developing countries, where the availability of surgical facilities is limited, ageing-related cataract is the leading cause of blindness. Because at present there is no efficacious non-surgical therapy for cataract, the problem is expected to increase in magnitude in coming decades as the world population becomes progressively older. [6] The change of surgical techniques from large corneal incision to phaco has great effect on SIA. [7] The current treatment for cataract is surgery while phaco remains the more advanced and technically superior method of cataract surgery; manual small incision cataract surgery (MSICS) is the most popular surgical management option for cataracts in developing countries. [8] The first, phaco, involves using a high frequency ultrasound probe to fragment the lens, and this machine also removes the lens fragments from the eye. The second, called MSICS involves using instruments to remove the lens from the eye through a small incision. [9] Advantage of phaco versus MSICS was smaller wound size, faster healing time, fewer wound problems (wound leak and iris prolapse) less astigmatism, less risk of expulsive hemorrhage; operation can perform under topical anesthesia and conjunctival sparing. Disadvantage of phaco versus MSICS was machine dependent, longer learning curve and higher complication rate during learning curve. [10] At present time, cataract surgeries by phaco through clear corneal incision have become principal method for cataract surgery because of its bloodless and fast approach. The postoperative SIA has always been concern to most surgeons. [11] Post cataract surgery astigmatism causes a delayed in visual rehabilitation, limiting the visual outcome and reduces the effectiveness of the surgical procedure. [12] Factor like location, direction, length, width, depth, and shape of incision, types of suture and suture material have been found to influence the astigmatism.[13] Low induced astigmatism, faster healing of corneal wound and no direct damage to cornea justify the usage of small self-sealing sclera-cornea tunnel incision.[14] With reference to the location of the incision, placing the incision on the steeper corneal meridian based on the preoperative keratometeric reading has been recommended [15] .The idea is that because of the one-to-one coupling from corneal incisions, there is flattening of the corneal curvature in the meridian on which the incision is placed, with a corresponding steepening to the same degree of the orthogonal meridian [16]. Thus, there will be a reduction in the corneal power of the steeper meridian when an incision is placed on that meridian, with a corresponding steepening to the same degree of the flat orthogonal meridian. The difference in corneal powers between the flattened steeper meridian (meridian on which the incision was placed) and the steepened flatter meridian will then be reduced postoperatively leading to minimal postoperative corneal astigmatism. [17]

Aim of Study

1- To compare the mean keratometeric reading preoperative and one month postoperative in phacoemulsification versus manual small-incision cataract surgery

2- To find which produce less surgically induced astigmatism.

Patients and Methods

This was a prospective, observational, cohort study in patients with cataract carried out from April 2018 to September 2018. This study conducted on (206) patients attending to Al-Sadder medical city in Najaf.

The cases were divided into two groups. Each group had 103 patients. One group had undergone phacoemulsification and the other group had undergone MSICS with one month for follow up.

Inclusion Criteria

Patient having cataract of any grade affect the vision

Exclusion Criteria

1. Patients with additional visual comorbidity, which may influence the visual outcome after surgery, glaucoma, and corneal pathology.

2. Patient having temporal incision.

3. Patients with traumatic, subluxated cataract.

Preoperative ophthalmological examinations consisted of the best-corrected visual acuity using the Snellen metric chart, slit-lamp examination to detect the type of cataract, intraocular pressure measurements and

fundus examination if possible.

Preoperative keratometeric reading was performed using autokeratometer A-scan was used to measure the axial length and to calculate the power of intraocular lens (IOL) aiming for emmetropia. B-Scan (Ophthalmic Ultrasonography Scanner was used to check the posterior segment and postoperative keratometry and auto refraction was performed using autorefractokeratometer after one month.

Surgical procedure:

After pupil dilation, under aseptic approach ,local anesthesia was given, In the manual small-incision cataract surgery steps[9] And in phaco group steps [9]

Data collection:

Name, Age, sex, side of eye, k-reading (k1, K2) preoperative, after one-month postoperative k- reading and autorefraction.

Patient name **Age** **Sex**		
Eye	**Right**	**left**
k-reading	K1	K2
Preoperative		
Postoperative (After one month)		
Auto-refraction		

Data of the studied group were entered and analyzed using the statistical package for social sciences, SPSS, version 20. Analytic statistics presented as mean, standard deviation (for the age, keratometeric reading and astigmatism), and proportions (for categorical variables). Chi square test was used to compare patients with regard to age group and gender. Data were analyzed using Student's t-test for means difference of two groups. Qualitative data were presented as numbers and percentages and compared using the $\chi 2$-test Level of significance, P. value of less than or equal 0.05 was considered as significant. Results presented in tables.

Findings

Table 1 Gender, side and age distribution of the studied group (N =206)

Characteristics		**Type of operation**		**Total**	**P**
		Phaco n =103 No. (%)	**MSICS n=103 No. (%)**		
Gender	Female	61 (59.2)	55 (53.4)	116 (56.3)	0.4
	Male	42 (40.8)	48 (46.6)	90 (43.7)	
Side	Rt	51 (49.5)	44 (42.7)	95 (46.1)	0.3
	Lt	52 (50.5)	59 (57.3)	111 (53.9)	
Age (Mean ± SD)		58.69±10.4	62.56±7.9		0.003

Table 2 Preoperative and postoperative mean keratometeric reading

Reading	Phaco N=103 Mean± SD	MSICS N=103 Mean± SD	p
Pre k1	43.80 ± 1.905	44.00 ± 1.757	0.4
Pre k2	44.69±1.764	45.0 ±1.725	0.2
Post k1	43.71±1.757	43.91±1.766	0.4
Post k2	44.62±1.714	45.14±1.856	0.04

Table 3 Distribution of SIA after one month in the patients according type and study group

Type of astigmatism	Type of operation		Total	P
	Phaco n=103No. (%)	MSICS n=103No. (%)		
With rule astigmatism"	21 (20.4)	20 (19.4)	41 (19.9)	0.9
"Against rule astigmatism"	64 (62.1)	65 (63.1)	129 (62.6)	
"Oblique astigmatism"	16 (15.5)	16 (15.5)	32 (15.5)	
"No astigmatism"	2 (1.9)	2 (1.9)	4 (1.9)	
Total	103 (100)	103 (100)	206 (100)	

Table 4 Postoperative autorefractive reading of astigmatism and mean SIA in the patient according type and study group

Postoperative autorefraction cylindrical	Type of operation		Total	P
	Phaco n=103 No. (%)	MSICS n=103 No. (%)		
0-0.50 D	31(30.3)	17 (16.5)	48(23.4)	0.07
0.51-1.00 D	27 (26.2)	38 (36.9)	65 (31.5)	
1.01-1.50 D	22 (21.3)	18 (17.5)	40 (19.4)	
1.51-2.00 D	9 (8.7)	17 (16.5)	26 (12.6)	
more tnan2.01 D	14 (13.5)	13 (12.6)	27 (13.1)	
Total	103 (100)	103 (100)	206 (100)	
Mean SIA± SD	1.3±0.9	1.3±0.7		0.5

Discussion

This study was comparative prospective cohort study that compares SAI between phaco and MSIC surgery. In the phaco group difference keratometeric reading between preoperative and postoperative K1 = 0.09 and K2 = 0.07. In the MSICS group difference keratometeric reading between preoperative and postoperative K1= 0.09 and K2 = -0.14. There was no significant difference between preoperative and postoperative mean k1 reading in both phaco and MSICS group and slight change in mean K2 reading. The distribution of postoperative astigmatism in Phaco was higher incidence of against rule astigmatism 62.1%, with rule astigmatism 20.4%, oblique astigmatism 15.5% and no astigmatism 1.9% among of that 103 patients with shifted toward against rule astigmatism. The distribution of postoperative astigmatism in the group MSICS was higher incidence of against rule astigmatism 63.1%, with rule astigmatism 19.4%, oblique astigmatism 15.5% and no astigmatism 1.9% among of that 103 patients with shifted toward against rule astigmatism. Generally, the corneal astigmatism seen is with-the-rule in young patients and there is a shift towards against-the rule astigmatism as age advances. With the rule, astigmatism in younger age can be due to the upper and lower lids compressing a portion of the cornea and causing steepening of the vertical curvature. However, as age advances - the compressive effect of the lids is lessened by lid laxity, decreased tone of Muller's muscle and increased corneal rigidity. [18] Our study correlates with the observation made by Reddy B et al[19] concluded that significantly against the rule shift in astigmatism in the phacoemulsification group and the MSICS superior incision group. Like our study with observation made by Renu M Magdum et al[20] where Post operatively at the end of 3rd month in superior MSICS group, majority of the patients (74%) had ATR astigmatism and only 8 (16%) of the patients had WTR astigmatism. This is because incision on the vertical meridian causes flattening of the vertical meridian and steepening of the horizontal meridian leading to ATR shift. In temporal incision group, majority of the patients (56%) had WTR astigmatism as the incision on the horizontal meridian n causes flattening in that meridian and steepening along the vertical meridian leading to WTR shift. Unlike our study with observation made by Latha N. et al [21] comparing the Superior corneo-scleral incision and clear corneal incision, ATR was found to be 60% in Superior corneo-scleral and 32% in Superior clear corneal incision. Superior clear corneal incision tends to produce less ATR astigmatism than corneo-scleral incision while in our study was no significant differences, both surgeries produced ATR astigmatism. Mean astigmatism at one month postoperatively in patients undergoing phaco was 1.3D. Mean astigmatism at one month postoperatively in patients undergoing MSICS was 1.3 degree. There was no statistical difference in astigmatism at one month postoperative (p =0.5) .Our study correlates with the observation made by Kalaf M. et al .[22] the mean SIA was 2.08 in the PHACO group, whereas in the MSICS group it was 2.96. There was no statistically significant difference in the amount (P=0.166) of SIA between patients treated with phaco and patients treated with MSICS. The same results were obtained by Ruit et al. [23] The result obtained by Kağnici1 D et al [24] ;mean SIA was less in the group with limbal incision than in the group with corneal incision on postoperative first day, first week and first month. However, this difference was not statistically significant. Different results were obtained by both Venkatesh et al. [25] and George et al. [26]; they reported that phaco caused significantly lesser SIA compared with MSICS at 6 weeks postoperatively.

Conclusions

This study reveals that superior approach in PHACO and MSICS produced similar astigmatic outcome, there is no difference in the mean surgically induced astigmatism in both type of surgery.

Recommendations:

In both type of surgeries had similar results, MSICS can be consider as alternative to PHACO in very hard cataract cases , in which the cornea may be comprised by excessive uses of ultrasound power and affect the outcome .

Conflict of Interested: Nil

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Ethical committee :

1. The council of Faculty of Medicine / Kufa University and the Department of ophthalmology approved the study protocol.

2. Official agreements were obtained.

3. Patient's agreements were obtained.

Data and information of the participants were kept confidentially and not disclosed to unauthorized personal

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The Improvements in the Prolactin, Thyroid Hormones and Lipid Profiles that are Observed During Three Pregnancy Periods

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Abstract

This research was to explore improvements in lipid profile, TSH, T3, T4 and prolactin during three stage of the progress. Pregnancy leads to development in both hormones and biochemistries of the maternal blood. The study involved a control group of 20 non-pregnant women and 30 pregnant and. Automatic hormone analyzer[1] and biochemistry automatic analyzer measured hormones, with the lipid profile[2]. Although T3, T4, TC and TG showed no significant increases in first ($p \geq 0.05$) compared with the control group, TC and TG increased significantly in the second phase and third stage of pregnancy trimesters and T3 and T4 significantly in the third phase of pregnancy quarter ($p \geq 0.05$) compared with the control team ($p \geq 0.05$). The results demonstrate a significant increase in prolactin in three stage of pregnancy ($p \geq 0.05$). No significant increase ($p \geq 0.05$) in comparison with control groups was shown in LDL-C and HDL -C over all phases of the prolactin. Increased prolactin, thyroid hormones and the rise in lipid fractions follow normal pregnancy.

Keywords*: LDL-C, HDL-C, TC, TG, T_3, T4, Prolactin, Stage, pregnancy*

Introduction

Pregnancy is the time when one or more offspring evolves inside the womb of a woman. Several gestations, including twins or triplets, may take place during pregnancy. Childbornness usually takes place after the birth about 38 weeks. For women with 4 weeks of a menstrual cycle, it is about 40 weeks after the last regular menstrual period[3]. During the pregnancy, there are great modifications in the physiology of a mother to provide the requisite fetal nutrients and the mother's additional work resources (before fetal needs occur). The first half (up to 13 weeks after conception) starts, when fetus is about 13 g and can be as long as 8 cm. These changes are not recognized. Rapid fetal growth occurs during the second trimester (13 to 26 weeks), and by the end of the second trimester, the fetus weighs around 70 g and is 30 cm long, within which the fetal organs would have begun to develop[4]. The fetal organs complete maturation during the third trimester (26–40 weeks)[5].Pregnancy is affected by a number of factors, including culture, the environment, social and economic circumstances and medical access[6]. Therefore, the prevention of adverse outcomes in pregnancy by identifying causal factors for these outcomes is of clinical and economic importance. Unfavorable outcomes of pregnancy have serious short-term consequences, such as increased perinatal morbidity and mortality of mother and child[7]. Women show an increase in lipid levels during normal pregnancy, including triglycerides (TG) and total cholesterol (TC) at gestational age [8-11].Pregnant complications including hypertension, preterm birth, low birth weight, placental abruption, and fetal death have been linked with clinical thyroid dysphunction. The relation between subclinical and pregnant hypothyroidism has not been well studied. In order to assess the outcome of pregnancy of women with high levels of thyroid thyrotropin (TSH) and normal free thiroxin levels, we carried out the prospective thyroid screening

test [12-15].Maybe prolactin's [PRL] most important traditional role is to encourage milk production in women following childbirth. Increases the concentrations of prolactin during pregnancy contributing to the enlargement and secretion of colostrums in mamma glands following delivery in preparation for breastfeeding [16-18].In this study we investigate the changes in lipids profile, T3, T4, TSH, and prolactin in a group of pregnant women to determine the changing these parameters during normal pregnancy[19-21].

Material & Methods

This study was conducted in AL-Batol Learning Hospital, Diyala Governorate, Iraq from May to October 2018. A total of 50 women aged between 20 and 41 volunteer for the study. This group consisted of 50 volunteers, 30 pregnant women and 20 non-pregnant women. In about the 32 weeks of pregnancy, 30 pregnant women were studied according to pregnancy period. Blood samples from all participating women were taken after having done quick 8-12 hours of blood overnight. Growing woman was collected with 5ml disposable syringes and transferred to the flat tube 5ml of fasting vein blood.

For half an hour, the blood was left to clotted, then centrifuged for 5 minutes at 4000 rpm andused to check the parameters tested. The study was performed by an automated to analyze total cholesterol (TC), triglycerides (TG) and cholesterol (HDL-C) in serum. Hormones were measured by hormones automated analyzer[1]. The Friede Wald method was used to estimate the low density of lipoprotein-cholesterol (LDL-C) = Total cholesterol - (TG/2.2 - HDL-Cholesterol) (mg / dl).The statistical software SPSS data analysis was submitted as a mean ± Standard Deviation (M±SD). The results are presented. The t-test student was used for data analyzes and $p \leq 0.05$ is statistically significant.

Result

Table 1 displays the mean ± standard (M±SD) values for the control blood parameters and pregnant women that studied in three stages.

Table 1: The table illustrates (M ± SD) values of studied parameters in control and the three trimesters of pregnancy.

Parameters	control	1st trimester	2nd trimester	3rd trimester
TSH mIu/l	3.50±1.08	2.90 ± 1.23	3.40±0.69	3.50±1.15
T3 nmol/l	2.00±0.30	3.20 ± 0.44	2.90 ±0.96	2.60±0.47
T4 nmol/l	90.00±12.58	138 ± 22	140 ± 34	150.0±62.31
PRl ug/l	15.00±3.01	34.0 ± 32.7	100.0±33.45	131.1±31.44
TC mg/l	150.4±17.4	166.4±28.1	180.0±23.4	207.3±32.2
TG mg/l	80.7±6.6	108.7±13.1	126.5 ± 18.4	130.7 ± 20.8
HDL-C mg/l	43.1±5.4	44.7±4.2	48.0±6.3	52.0 ± 5.8
LDL-C mg/l	81.8 ± 14.6	90.3 ± 9.6	123.7 ± 10.9	159.5 ± 22.4

As shown in table 1 there has been no significant differences between the blood levels of these parameters, with the exception of prolactin, showing a significant increase in the first quarter when comparing the blood levels of these parameters with control groups ($p \leq 0.05$) [20-21].

Table 2: The relation with control of the studied parameters showed in the first trimester.

Parameters	Control	1st trimester	P-value
TSH	3.50±1.08	2.90±1.23	0.784
T3	2.00±0.30	3.20±0.44	0.234
T4	90.00±12.58	138.0±22.05	0.439
Prolactin	15.00±3.01	34.00±32.75	0.01*
TC	150.4±17.4	166.4±28.1	0.195
TG	80.7±6.6	108.7±13.1	0.125
HDL-C	43.1±5.4	44.7±4.2	0.254
LDL-C	81.8±14.6	90.3±9.6	0.321

The result showed an elevation in the levels of the parameters tested ($p \geq 0.05$), without noticeable difference, with the exception of prolactin, TC and TG, which showed a small elevation ($p \geq 0.05$), of the presence in the second quarter relative to control and TSH showed almost normalization.

Table 3: Comparison of studied parameters with control demonstrated in the second trimester.

Parameters	**Control**	**2nd trimester**	**P-value**
TSH	3.50±1.08	3.40±0.69	0.72
T3	2.00±0.30	2.90±0.96	0.089
T4	90.00±12.58	140.0±34.22	0.077
Prolactin	15.00±3.01	100.0±33.45	0.001*
TC	150.4±17.4	180.0±23.4	0.049*
TG	80.7±6.6	126.5±18.4	0.045*
HDL-C	43.1±5.4	48.0±6.3	0.231
LDL-C	81.8±14.6	123.7±10.9	0.063

The findings from Table3 show that there is a substantial increase of the number of parameters ($p \geq 0.05$) in most of the parameters, except for HDL and LDL rates showing a high presence of the three without significant differences($p \geq 0.05$) , and TSH showed a standardization as shown in Table 3.

Table 4: The Comparison of the parameters measured with control showed in the third trimester.

Parameters	**Control**	**3rd trimester**	**P-value**
TSH	3.50±1.08	3.50±1.15	0.733
T3	2.00±0.30	2.60±0.47	0.023*
T4	90.00±12.58	150.0±62.31	0.017*
Prolactin	15.00±3.01	131.1±31.44	0.001*
TC	150.4±17.4	207.3±32.2	0.042*
TG	80.7±6.6	130.7±20.8	0.044*
HDL-C	43.1±5.4	52.0±5.8	0.09
LDL-C	81.8±14.6	159.5±22.4	0.051

Table 4 explain the result that showed an increase of the number of parameters tested ($p \geq 0.05$), with noticeable difference, with all and only exception of HDL-C and LDL-C, which showed a small elevation ($p \geq 0.05$), of the presence in the third trimester relative to control and TSH showed almost normalization.

Discussion

The results showed an increase in T3 and T4 thyroid hormone levels, especially in the third stage of the trimester in pregnant women. This finding is consistent with the scientific evidence that the thyroid gland states during pregnancy increases and hyper-stimulate thyroid hormone levels, which will improve the basal metabolism rate appropriate for the mother and fetus. Therefore, a thyroid function assessment is important for thyroid hormone change during pregnancy. The predicted normal increase in thyroid hormone levels during pregnancy allows individualized supplementation when required [22-25]. Thyroid stimulation hormone (TSH) concentrations are small with standardization in the second and third trimesters in the first trimester [26-28]. Consequently, during pregnancy the average TSH level is lower or less than normal in contrast to non-pregnancy. TSH values vary with the mother going from the first to the third quarter [29].Prolactin concentration in this study revealed significant elevation in the first trimester when compared to control group and also increased progressively with high significant elevation in second and third stages of pregnancy. This elevation is to preparation and induces the mammalian gland for secretion of milk for feeding the neonate. This result agreement with some previous studies[30].

The current study revealed increases of lipid fractions (TC, TG, HDL-C and LDL-C) [31]. The growth and development of the fetus and support its structures during pregnancy period demand increases the metabolic fuels and lipids are high energy food [32-33].

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Correlation between Seasons and the Prevalence of Preeclampsia at Tertiary Hospital, Indonesia

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Abstract

Background: Preeclampsia is the second most common cause of maternal mortality following postpartum hemorrhage. In East Java alone the maternal mortality rate due to preeclampsia was 31%. Some studies explain that the incidence of preeclampsia can be caused by seasonal variations, whereas other studies say that seasonal factors can be a protective factor for preeclampsia.

Objective: To find out how seasons relate to the prevalence of preeclampsia.

Method: A descriptive analytic study using medical record data during. The sample meets the inclusion criteria, namely pregnant women with preeclampsia, severe preeclampsia, chronic hypertension with superimposed severe preeclampsia, or eclampsia. The exclusion criteria were pregnant women with chronic hypertension or gestational hypertension. The sampling technique used was total sampling and cross-sectional design with observation method using secondary data from medical records. Data was processed using IBM SPSS Statistics and presented narratively.

Results: Respondents who experienced preeclampsia in the dry season were 558 people or 19.3% and those who did not experience preeclampsia were 844 people or 29.2% The results of data analysis with statistical tests using Chi-Square obtained a significance value or p-value of 0.091 (p> 0.05).

Conclusion: No correlation between seasonality and the prevalence of preeclampsia.

Keywords*: preeclampsia, rainy season, dry season*

Introduction

Pregnancy Induced Hypertension (PIH), including gestational hypertension, preeclampsia and eclampsia, is a complication in pregnancy that makes an important contribution to maternal and perinatal morbidity and mortality worldwide[1,2]. The pathophysiology of PIH is not fully understood, but several risk factors are known, including primipaternity, multifetal pregnancy, chronic hypertension, a history of previous preeclampsia and family history, gestational diabetes mellitus and thrombophilia. Environmental factors also appear to have a role in the pathogenesis of PIH[3].

The effect of environmental conditions such as temperature, relative humidity of the air and the seasons of the year on the incidence of diseases has been recognized since the time of Hippocrates and is easily perceptible in infectious diseases[4]. In recent years, observations on the effect of environmental conditions on the incidence of preeclampsia have been documented. Although these remain controversial, recent systematic review on the subject found mostly consistent evidence of seasonal variation in the occurrence of the hypertensive disorders of pregnancy in both tropical and non-tropical regions[5].

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The pathogenesis, pathophysiology and pathological changes in the function of organs in preeclampsia have been widely discussed, but none have satisfactory results. There are many factors that explain the occurrence of PIH and several studies include seasonal trends as factors that can contribute to environmental risk factors for PIH. From research that study how seasonal trends can affect the occurrence of PIH, results are obtained with considerable diversity. This diversity is partly due to differences between populations, regions of the world and climate in which immunological factors also have a role5[5,6]. This research to find out the relationship between the seasons in Indonesia (rainy and dry seasons) and the prevalence of preeclampsia (preeclampsia, severe preeclampsia, chronic hypertension with severe preeclampsia and eclampsia).

Method

This research is a descriptive analytic study which uses medical record data at Dr. Soetomo Teaching Hospital from January 2017 to December 2018. The research design was cross-sectional design using observation method, which was collecting secondary data in the form of medical records (MR) that was processed using IBM SPSS Statistics Version 20.

The population of this study were women who underwent delivery (vaginally or abdominally) at Dr. Soetomo Teaching Hospital in 2017-2018. The inclusion criteria include pregnant women diagnosed with (1) preeclampsia, (2) severe preeclampsia, (3) chronic hypertension with superimposed severe preeclampsia, or (4) eclampsia. The exclusion criteria in this study were pregnant women diagnosed with chronic hypertension and gestational hypertension.

The instrument used in this study was the medical record of all pregnancies with preeclampsia, severe preeclampsia, chronic hypertension with superimposed severe preeclampsia and eclampsia. Total sampling of pregnant women with a diagnosis of preeclampsia was recorded in 2017-2018. The collected data was processed and presented in tabular and narrative form.

Results and Discussion

Distribution of season frequency and respondent characteristics

The total of samples in this study was 2887 respondents, 1485 respondents or 51.4% of which were from the rainy season and in the dry season were 1402 respondents or 48.6%. And then it was found that respondents who experienced preeclampsia were 1195 people or 41.4% and respondents who did not experience preeclampsia were 1692 people or 58.6%.

The Correlations between seasons and preeclampsia

Based on the results of the cross tabulation in the table, it can be seen that number of respondents who experienced preeclampsia in the rainy season were 637 people or 22.1%, while those who did not experience preeclampsia were 848 people or 29.4%. The results of data analysis with statistical tests using Chi-Square obtained a significance value or p-value of 0.091 ($p>0.05$), meaning that there is no significant relationship between seasons and the incidence of preeclampsia.

Table 1: The Correlations between seasons and preeclampsia

			Preeclampsia		Total	P
			Yes	No		
Season	Rain	Count	637	848	1485	0.091*
		%	22.1%	29.4%	51.4%	
	Dry	Count	558	844	1402	
		%	19.3%	29.2%	48.6%	
Total		Count	1195	1692	2887	
		%	41.4%	58.6%	100.0%	

Characteristics of respondents by preeclampsia cases based on age in rainy season

Based on age group, it is known that from a total of 2887 respondents, respondents aged less than or equal to 16 years were 23 people or 0.8%, respondents aged 17-34 years were 1992 people or 69.0%, and respondents aged over or equal to 35 years were 872 people or 30.2%.

Preeclampsia cases based on age in dry season

Based on the SPSS calculation results, it is known that from a total of 558 respondents with preeclampsia in the dry season, 4 respondents or 0.3% were aged below or equal to 16 years, respondents aged 17-34 years were 351 people or 25.0% and respondents aged over or equal to 35 years were 203 people or 14.5%.

In this age category, the results show that most women with the preeclampsia were aged 17-34 years. These results were not in accordance with studies that were carried out in various countries, which reported that maternal age at high risk for preeclampsia is the older or equal to 35 years old because this age is more likely to develop atherosclerosis, which affects small arteries, such as those in the kidneys and uterus, and causes hypertension[7,8]. Because of this, older pregnant women can easily develop preeclampsia. Maternal age may correlate with aging of the placenta. Duckitt reported an almost 2-fold increased risk of preeclampsia in pregnant women aged 40 years or more. The difference in results obtained can be due to the age distribution of the population, which is different for each country. However with the theories that support the occurrence of preeclampsia, it is still important to be vigilant and conduct strict screening of risk factors that exist in each pregnant woman[9,10].

Characteristics of respondents based on parity

It resulted that from a total of 2887 respondents, the number of respondents included in the primigravida category were 845 people or 29.3% and respondents belonging to the multigravida category were 2042 people or 70.7%. From total number of the respondents, those who were diagnosed with preeclampsia as many as 1195 respondents, whereas the primigravida was counted to be 344 respondents (28.79%) and multigravida as many as 851 (71.21%).

According to season with each parity category, it can be seen that number of labor for primigravida is obtained more in the rainy season, which is as much as 53.48% of the number of deliveries in primigravida in both years. For multigravida deliveries also found more in the rainy season (53.23%) than the number of deliveries in multigravida in 2017-2018. To see the relationship between season and parity in the two years, a cross-test with Chi-Square was carried out and the result showed p = 0.936. This figure explains that there was no significant difference between primiparous and multiparous patients with preeclampsia during dry and rainy seasons.

Table 2: The relationship between season and parity in 2017 and 2018

Parity	PE	Season				Total		p
		Rainy		Dry				
Primigravida	Yes	184	53.48%	160	46.52%	344	28.79%	0.936*
Multigravida	Yes	453	53.23%	398	46.77%	851	71.21%	
Total		637	53.31%	558	46.69%	1195	100.0%	

*Chi-Square test

Preeclampsia generally occurs in the first pregnancy, so a history of previous normal pregnancy is associated with a decrease in the frequency of preeclampsia. Therefore, nulliparity is a significant risk factor for preeclampsia. The relative risk (RR) of preeclampsia among women with nulliparity is reported to be 2.1 (95% CI, 1.9-2.4)[11]. Based on the season and parity relationship tests conducted in this study, there were no significant results. Pregnant women with preeclampsia are also mostly classified as multigravida parity. This might occur due to the limitation factor of this study itself, which was not enough data available about other risk factors for preeclampsia associated with parity, such as primipaternity, which is the impact of partner turnover on the incidence of preeclampsia in multiparity[12,13].

Distribution of preeclampsia per month

The distribution of the number of cases of preeclampsia per month each year in this study as well as in the two years can be seen in figures 1, 2 and 3. The highest number of cases of preeclampsia per month in 2017 (Figure 1) were obtained in August and October, with 4 cases each, or 14% of the total number of deliveries in that year. In accordance with Indonesian Meteorology, Climatology, and Geophysical Agency (BMKG) operational definition, for the 2017 season zone, August was included in the dry season and October was in the rainy season.

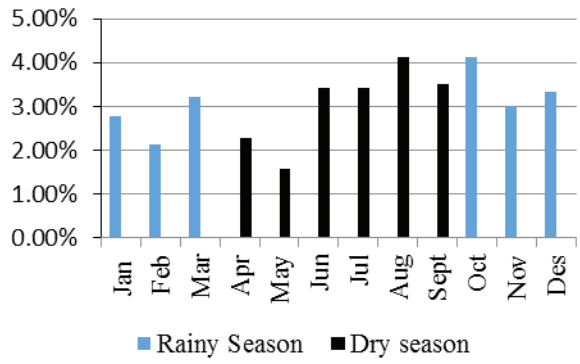


Figure 1: Chart of the number of preeclampsia per month in 2017

Similar to the data obtained in 2018 (Figure 2), incidence of preeclampsia was also obtained in September and October (respectively 4.65%), with being included in the dry season and October the rainy season. As explained previously, there is no difference in the incidence of preeclampsia between the two seasons because Indonesia is a tropical country, so differences such as humidity and temperature are not too significant[14].

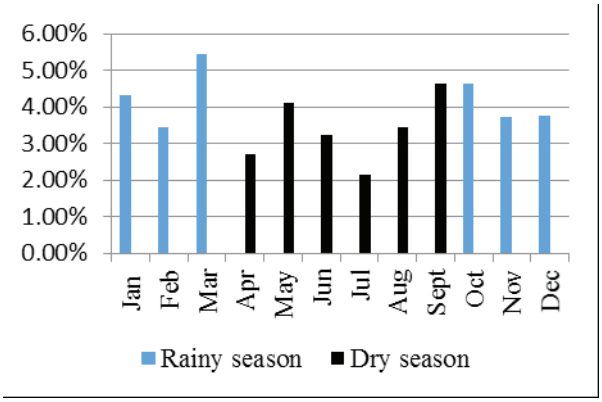


Figure 2: Chart of the number of preeclampsia per month in 2018

Based on the number of cases of preeclampsia in 2017-2018 (Figure 4), the highest incidence of preeclampsia was found in October, which was 10.63% of the total number of women with preeclampsia in both years. As has been explained above, according to BMKG's operational definition, October is included in the rainy season[15]. It was also found that March (the rainy season) is the second month with the most cases of preeclampsia. From these two data, it can be concluded that the distribution of the most prevalence of preeclampsia in Dr. Soetomo Teaching Hospital Surabaya for the period of 2017 - 2018 was mostly obtained in the rainy season and at least in April (dry season).

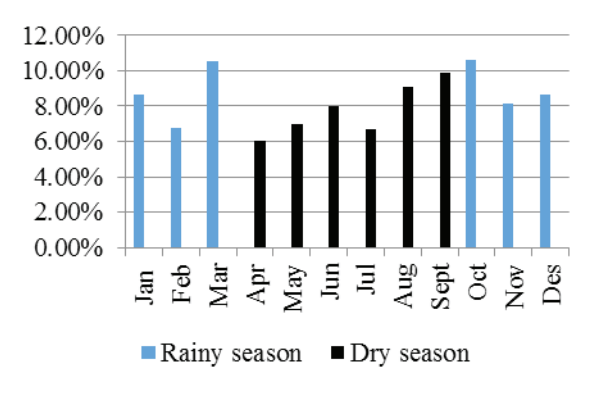
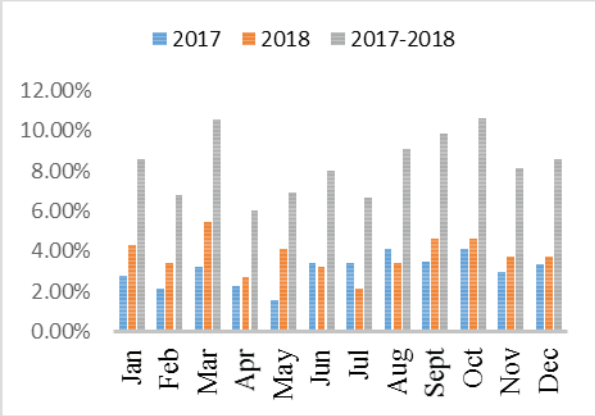


Figure 3: Chart of the number of preeclampsia per month in 2017-2018

These results are in agreement with some researchers who argue that low temperatures cause vasospasm which is part of the pathogenesis of preeclampsia. This is analogous to cold air which causes ischemia

which is strongly associated with the incidence of myocardial infarction; preeclampsia is thought to have a predisposing factor (cold air)[14]. Elongi et al. (2011) also found that preeclampsia might occur due to vasoconstriction, because the incidence increased during months with lower temperatures. Subramaniam (2007) further proposed that dehydration protects the brain from seizures that are usually experienced with eclampsia and that warmer temperatures result in increased fluid loss[9].

Figure 4: Comparison of the number of preeclampsia per month in 2017-2018



Further research is needed to examine more extensive cases of preeclampsia (preeclampsia, severe preeclampsia, chronic hypertension, superimposed severe preeclampsia and eclampsia), especially regarding other risk factors that can affect the prevalence of preeclampsia when associated with or not associated with seasons. This study does not display data on other preeclampsia risk factors, for example primipaternity factor; whether a woman's pregnancy with preeclampsia is a single or twin pregnancy; whether or not pregnant women with preeclampsia are patients who routinely perform antenatal care at Dr. Soetomo Teaching Hospital Surabaya; the patient's residence in which by knowing the patient's residence, information is obtained about how the humidity or the estimated temperature of the region might affect the prevalence preeclampsia when related to seasonal factors; and other risk factors that can affect the validity of the results of this study[16,17].

These other risk factors might be considered as exclusion criteria if the researcher really wants to know the depth or extension of the influence of the season on the incidence of preeclampsia, or as a multivariate analysis to determine the characteristics that influence the incidence of preeclampsia in this particular season in Indonesia, especially the city of Surabaya. The authors also suggest adding data on the highest rainfall in the year according to BMKG to find out the number of preeclampsia cases in the months with the highest rainfall, whether it increases or remains, and can be related to the results of cross-tests between seasons and preeclampsia[18,19]. Because of the limitations of this study, the authors call for further research to discuss the characteristics of other risk factors for preeclampsia in order to find out more about the things that influence the occurrence of preeclampsia, especially in patients of Dr. Soetomo Teaching Hospital Surabaya. By doing this, future research is expected to be able to produce data that can be useful in the prevention and better management of preeclampsia in each season in Indonesia, so that maternal and infant morbidity and mortality rate can be reduced.

Conclusion

From the data that has been collected and processed, it can be concluded that there was no significant correlation between seasons and the incidence of preeclampsia (preeclampsia, severe preeclampsia, chronic hypertension superimposed severe preeclampsia and eclampsia) in labor cases in Dr. Soetomo Teaching Hospital Surabaya for the period of 2017 - 2018.

Conflict of Interest: None

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Ethical Clearance: Taken from Health Research Ethics Committee of Dr. Soetomo Teaching Hospital Surabaya with number 1969/KEPK/IV/2020.

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Construction of a New Scale for Biological Estimation of Public Speaking Stress

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Abstract

Stress is a psychophysiological phenomenon that is needed to be quantified from biological aspect for further studies and clinical applications .The objectives of the current study was to construct a valid and reliable scale for providing a quantitative estimate of the public speaking stress among academic staff .A methodological design was applied to achieve the objectives of the study.The study was conducted at the University of Kufa in the city of Najaf. A purposive sample of 200 academic staff was used the present study. A scale of 17 items for public speaking stress have been generated . Content validity was quantified throughout the measurement of the following values : item content validity index (I-CVI) and scale content validity index average (S-CVI/Ave) and content validity ratio (CVR). Two forms of reliability have been used in the current study : stability (test-retest reliability) and internal consistency . The result of the study revealed that the majority of items have been accepted (I-CVI $\geq$ 0.8) and the average of all items is accepted (S-CVI/Ave = 0.88). All items have shown acceptable level of reliability between test and retest of the sample (r=0.99). Test of internal consistency (Cronbach's Alpha reliability) for the overall public speaking stress scale (PSSS) is acceptable (> 0.7). It was concluded that the suggested public speaking stress scale can be accepted as a valid and reliable scale for quantification of public speaking stress phenomenon.

Keywords : *Scale , Public Speaking, Stress. Validity , Reliability*

Introduction

Psychosocial stress is a well-investigated phenomenon that comes across different social situations; one of most stressfull situations is public speaking [(1)] . Public speaking is speaking in front of an audience in order to affect them ; whivh may include the negative experience of stress while speaking and the detrimental effects on speech performance [(2)] . The source of psychosocial stress can be explained by the task of standing and speaking in front of an large strange professional audience; this situation is stressful enough to trigger wide neural and hormonal responses throughout activation of the hypothalamic pituitary adrenal axis [(3)] . The stressor elicit the activation of the sympathetic autonomic nervous system, which promotes the release of adrenaline (epinephrine) and noradrenaline (norepinephrine) from the adrenal medulla. Secretion of adrenaline and noradrenaline leads to increase respiratory rate, heartbeat, blood concentration of glucose and the blood flow to skeletal muscles; this fast response is primarily related to survival [(4)] . Stress is a risk factor for several different diseases, including hypertension, inflammatory disease, cardiovascular disease, obesity, diabetes mellitus , atherosclerosis, and cancer, as well as neurodegenerative diseases [(5)] . Therefore, stress can influence individuals in every age, sex, race, education and situation, and can result in both physical and psychological health consequences [(6)] . Stress may be either external with environmental source, or caused by internal perceptions of the individual. The second form can, in turn, produce anxiety, and/or other negative emotions , thought and feelings such as press, pain, sadness which may result in depression and panic

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[7] . Nowadays, Stress may be categorized as the problem of the millennium, since the recent life is mixed up with stress in all its aspects [8] . Low levels of controlled stress could be desired, useful, and even healthy. Stress, in its useful form, can enhance psychosocial health and improve performance [9] .

Fear of public speaking (glossophobia) may lead to communication problems with an impact on the individual's social, and emotional life [10] . Many scales have been developed to measure several phenomena related to public speaking such as : anxiety and fear [11,12] . However, because stress is a physiological phenomenon, we did not find any scale to measure public speaking stress [13] . The new scale focuses on the biological aspects of the scale related to sympathetic and HPA axis activation, so that any signs associated with excessive fear, phobia and anxiety have been excluded.

Methodology

Subjects of the Study

The study was conducted at the University of Kufa in the city of Najaf. It was achieved by selecting a typical form of non-probability (purposive) sample. A size of sample of 200 academic staff was included in the study, which is an accepted sample size in the construction of scale [14] .

Ethical Consideration

The participants were fully acquainted of the current study and its aims and then a voluntary verbal consent was obtained in order to participate in the study, besides, the confidentiality of information obtained from academic staff has been taken into account and used only for the research purpose. The protocol was approved by the ethical committee in the university of Kufa .

Study Instrument

A methodological study was used to design a scale to meet the objectives of the study. It is suggested by the teamwork including the researcher and her supervisors. This scale includes seventeen items , with three responses (0= never, 1= sometimes, 2= always) .

Steps of Scale Construction

Content Validity

Content validity was quantified throughout the measurement of the following values : item content validity index (I-CVI) and scale content validity index average (S-CVI/Ave) and content validity ratio (CVR) .

Item Content Validity Index (I-CVI)

Item content validity index (I- CVI) was calculated by the following equation, values above 0.79 were considered accepted [15] :

$$I-CVI = \frac{\text{No. of expert voted (strongly related and related)}}{\text{Total No. of experts}}$$

If the I- CVI is higher than 0.79,the item is acceptable; between 0.7-0.79,the item is needs revision; and in the I- CVI is less than 0.7, the item is omitted.

Scale Content Validity Index Average (S-CVI/Ave)

Scale content validity index average (S-CVI/Ave) was calculated by having the average score of the of all items' I-CVI scores [15] .

Content Validity Ratio (CVR)

The following formula was used to calculate CVR, CVR value bigger than 0.49 is considered acceptable [15] :

CVR=(Ne - N/2)/(N/2)

Where :

Ne : is the number of expert determining "essential"

N ; total number of expert

Reliability

Two forms of reliability have been used in the current study : stability (test-retest reliability) and internal consistency .

Stability (Test-Retest Reliability)

Stability (test-retest reliability) has been quantified by calculating the Pearson correlation Coefficient (r) between test and retest scores of public speaking stress [15] .

Internal Consistency

Internal Consistency is a measure for the

homogeneity of scale items , it has been quantified by calculating Cronbach's Alpha. Values of Cronbach's Alpha equal or above 0.7 have been considered accepted threshold for internal consistency reliability; while high quality psychological scales are preferred to have of Cronbach's Alpha between 0.8-0.9 [14 , 16] .

Statistical Analysis:

Data of studied sample were entered and analyzed using the statistical package for social sciences (SPSS) version 25. Descriptive statistics presented as mean, standard deviation, frequencies and percentages. Chi-square test was used to compare frequencies. Pearson's correlation test was used to assess the correlations. Level of significance of ≤ 0.05 was considered as significant difference or correlation.

Results

The current study examinded the content validity in terms of item content validity index (I-CVI) and content validity ratio (CVR) for the items of the suggested public speaking stress scale (PSSS). The findings indicated that most items have been accepted ; some items may need to be modified (items numbered 3, 6,13,15) ; and the only item that was to be deleted according to the content validity evaluation was item number 7 as it had I- CVI less than 0.7 (table 1) .

Table (2) is about test of stability (test-retest reliability and internal consistency) for the overall public speaking stress scale (PSSS), it explains an acceptable level of reliability was seen between test and retest of the sample (r= 0.99 ; p value < 0.0001) and acceptable Cronbach's Alpha score (=0.87) .

Table (1): Content validity in terms of item content validity index (I-CVI) and content validity ratio (CVR) for the items of the public speaking stress scale (PSSS)

Items	I-CVI	CVR	Assessment
1. On presentation day, I wish to cancel my presentation	0.88	0.75	Accepted
2. On presentation day, I have increased concern about my performance in the speech	0.94	0.88	Accepted
3. Before giving a speech, I need to drink water .	0.75	0.50	Modified
4. Giving a speech behind a platform is better for me than speaking on a stage	0.94	0.88	Accepted
5. When I begin to speak , my heart beats more rapidly and/or intensively	1.00	1.00	Accepted
6. While giving a speech , I suffer from dry mouth	0.75	0.50	Modified
7. While giving a speech , I have GIT cramps	0.69	0.38	Deleted
8. It is hard for me to concentrate in the beginning of my speech	0.94	0.88	Accepted
9. Fear from failure makes me more tense before giving a speech	1.00	1.00	Accepted
10. While giving a speech, I feel sweating	0.88	0.75	Accepted
11. During my speech, I forgot some information	0.94	0.88	Accepted
12. It is difficult for me to look at the audiences' eyes	0.94	0.88	Accepted
13. While giving a speech , my legs tremble and my posture disturb	0.75	0.50	Modified
14. While giving a speech, my voice changes (high pitch)	0.94	0.88	Accepted
15. When facing the audience, I feel difficult breathing	0.75	0.50	Modified
16. I become more confused if the audience is large and professional	1.00	1.00	Accepted
17. While giving a speech , it is difficult to use body language and voice intonation	0.94	0.88	Accepted
Overall Public Speaking Stress Scale (PSSS)	S-CVI/Ave= 0.88		Accepted

Table (2): Test of stability (test-retest reliability and internal consistency) for the overall public speaking stress scale (PSSS)

Items	Test-retest reliability	Cronbach's Alpha	Assessment
Overall Public Speaking Stress Scale (PSSS)	r = 0.99 (p value = 0.000)	0.87	Accepted

Discussion

According to table (1), the item content validity index (I-CVI) has been calculated according to the opinion of experts about the degree of relevance of each item to the concept of the phenomenon that is to be measured . Previous studies reported that I-CVI scores ranges between 0 and 1, and each item with I-CVI more than 0.79 is considered (accepted), those with I-CVI score between 0.7-0.79 need to be revised or modified, while those I-CVI level lower than 0.7 should be deleted [15] . In the current study, the majority of items have been accepted (I-CVI ≥ 0.8), it mean that experts considered these items relevant and valid to measure the stress of public speaking . The other indicator of content validity that has been used in this study is content validity ratio (CVR), in which the experts were asked to determine whether an item is essential for measuring the construct (public speaking stress) or not [17] ; each item with CVR higher than 0.49 is accepted (18) . According to the result of the present study, some of the items may need revision as their I-CVI is between 0.7-0.79 and their CVR is lower than 0.49 ; these are items numbered 3 , 6 , 13 , and 15 , while item seven has failed to reach the minimal level of I-CVI (I-CVI = 0.69) and CVR (CVR=0.38) indicated that expert did not accept it as relevant or essential to measure the public speaking stress. Through qualitative methods, items were improved until saturation was achieved [17] .One limitation of content validity is the subjectivity ; the scale and its items are evaluated according to the opinions of the expert, subjecting them to bias that may be present among the experts [15] ; to avoid such bias, the current study have used qualitative face validity and interrater agreement to further refine the items and the whole scale.

According to Table (3): Test of stability (test-retest reliability) for the overall public speaking stress scale (PSSS) ,it explains an acceptable level of reliability between test and retest of the sample (0.99). The test retest reliability, also known as the coe□cient of stability, is used to assess the degree to which the participants' performance is repeatable, i.e., how consistent their sum scores are across time. Researchers vary in how they assess test– retest reliability. While some prefer to use intra class correlation coe□cient, others use the Pearson product-moment correlation. In both cases, the higher the correlation, the higher the test–retest reliability, with values close to zero indicating low reliability. In addition, study conditions could change values on the construct being measured over time (as in an intervention study, for example), which could lower the test-retest reliability [14] .

Table (2) is about test of internal consistency (Cronbach's Alpha) for the overall public speaking stress scale (PSSS), it explains an acceptable level of internal consistency reliability (= 0.87) was recorded for overall public speaking stress scale (PSSS). Cronbach's alpha assesses the internal consistency of the scale items, i.e., the degree to which the set of items in the scale co-vary, relative to their sum score. An alpha coe□cient of 0.70 has often been regarded as an acceptable threshold for reliability; however, 0.80 and 0.95 is preferred for the psychometric quality of scales. Cronbach's alpha has been the most common and seems to have received general approval [14] .

The researchers concluded that the scales of anxiety and fear of public speaking combined psychological and physiological indicators, and it talks about what happens before, during, and after the day of the speech, and our scale only talks about the day of the speech.

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Conflict of Interest : Nil

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Larvicidal Activity of *Aedes Aegypti* from a Simple Preparation of Cashew (*Anacardium occidentale* L.) Nut Shell Extract for Community Level Use

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Abstract

Dengue fever is a major risk to public health due to the recent worldwide spread. Chemical vector control are usually considered, therefore, negative consequences are commonly occurred due to chemical resistance and environmental effects. Volume of cashew nut shell is become increasing as by-product after nut processing. This study was aimed to evaluate larvicial activity and toxicity of cashew shell extract with simple preparation for community use. Fresh cashew nut shell was dried and grinded in powder form. Material was immersed in water tank for 72 h and ratio of flavor and material and water ratio was 100 g/L (10% W/V). The supernatant of stock solution was diluted to 1:5, 1:10, 1:20, 1:50 and 1:100 of larvicidal solution for phenolic content measurement, analysis of alkyl phenols by gas chromatography-mass spectrometry (GC/MS) and larvicial activity test. Larvicidal activity was effective at LC_{50} = 3.8 mg of GAE/ml; LC_{90} = 11.5 mg of gallic acid equivalent (GAE) /ml. The solution was contained five alkyl phenols, which included saturated cardol, monounsaturated cardol, anacardic acid, diunsaturated cardanol and monounsaturated cardanol. Anacardic acid was major active compound, which was correlated to mass spectra. This larvicidal solution preparation was simple and cost effectiveness, which may useful in community level especially in cashew cultivating area. Field application and public training on this study will be conduct on controlling of mosquito-borne diseases.

Keywords*: Aedes aegypti, Anacardium occidentale, cashew, lavicidal activity, arthropod-borne disease, vector control,*

Introduction

Dengue fever is a major risk to public health due to the recent spread of the virus worldwide, which caused by dengue virus [1, 2]. The most important vector-borne disease transmitted by *Aedes* spp. (Diptera: Culicidae). Southeast Asia, including Thailand, the primary vector for dengue virus is *Aedes aegypti* and the secondary vector is *A. albopictus* [3]. *Aedes* spp. is also vectors of chikungunya virus [4] and Zika virus [5]. The vector control is one of most health policy for prevention of mosquitoes borne diseases, including larva and adult, which may controlled by include elimination of breeding sites, use of chemical control, genetic and biological control [4]. Chemical control are usually considered as the first method for mosquito control, therefore, negative

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consequences are commonly occurred due to chemical resistance and environmental effects [6, 7]. Plant origin is currently attention larvicides and consider because of harmless and biodegrade characteristics. larvicides of plant origin are also currently receiving considerable attention due to relatively harmless biodegradable properties [8]. Interactions between plants and insects are revealed the potential use of plants for fundamental pest control programs [9]. The various forms of natural products in folk medicinal treatments are more attend and consider for vector control [10, 11]. Green larvicides are now being considered because most plants are said to be nontoxic and biodegradable. Unlike the conventional insecticide which is based on single active ingredient, green insecticides comprise of bioactive chemical variations which have behavioral and physiological activities [11, 12]. Cashew (*Anacardium occidentale* L.) is well-known specie belongs to the *Anacardiaceae* family, which is a perennial-flowering plant and originate from Brazil. The pseudo fruit (cashew apple) can be used to make juice, jelly, jam, wine and syrup [13, 14], and its waste has been proposed as a feedstock for protein-enriched animal feed or as a high fructose source [15]. Different parts of the cashew tree have traditionally been used across the world to treat various diseases. Leaves or nut shell extracts from cashew, commonly known as the cashew tree, have long been used to treat inflammation and other conditions, including asthma, ulcers, and cancer [16]. In addition, cashew tree bark and leaves have been used in ancient medication for toothache and malaria [17]. Currently, Brazil, India, Vietnam, Tanzania and also Thailand are the main producers of cashew nuts. The nut is considered as more important in international market due to its widespread acceptance and demand [13]. Cashew nut shell is produces a heavy vesicant liquid called cashew nut shell liquid (CNSL) and contains high concentration of unsaturated long chain phenols, such as anacardic acid, cardanol, cardol and their derivatives [18, 19]. Recently, insecticidal action of cashew nut shell liquid (CNSL) on larvae of *A. aegypti* which is safe to mammals [20]. The remarkable toxicity effects exhibited by the solvent fractions of the ethanol extract of cashew shell wastes against the third and fourth instars larvae of *A. aegypti* [21]. Southern Thailand is major region of cashew nut production, which located in tropical zone and characterized by high humidity and rain throughout the year [22]. In addition, cashew nut is one major of agriculture product in Ranong province and after nut processing; the volume of cashew nut shell is become increasing as by-product. Hence, this study was aimed to evaluate larvicial activity and toxicity of cashew shell extract with simple preparation for community use.

Materials and Methods

Sample collection and preparation of larvicidal solution

Cashew nut shell waste was collected on March, 2019 from local agricultural group nearby Suan Sunandha Rajabhat University, Ranong academic campus, Thailand. Cashew tree were botanically identified as Phayam variety, which most common cultivated in this area. Fresh cashew nut shell (5 kg) was sundried and then dried material (~3 kg) was grinded in powder form. Grinded material was immersed in water tank for 72 h and ratio of flavor and material and water ratio was 100 g/L (10% W/V). The supernatant of stock solution was diluted to 1:5, 1:10, 1:20, 1:50 and 1:100 of larvicidal solution for phenolic content measurement, analysis of alkyl phenols by gas chromatography-mass spectrometry (GC/MS) and larvicial activity test.

Total phenolic content

In this step, each diluted larvicidal solution (0.1 ml) was added to 4.6 ml of distilled water and 1 ml of Folin-Ciocalteu reagent, and mixed by vortex mixer. After that, mixture was left in room temperature for 3 minutes. Next, 3 ml of 2% Na_2CO_3 (w/v) was filled into the tube, and shaken with the speed of 150 RpM for 2 hours. Then, the extract was measured to find out the light absorbance at 760 nm by comparing with the gallic acid at the intensity of 1, 0.875, 0.75, 0.625, 0.5, 0.375, 0.25 and 0.125 mg/ml. The total phenolic content was calculated into mg of gallic acid per g of the extract [23].

Analysis alkyl phenols in larvicidal solution

Extraction of alkyl phenols in larvicidal solution was modified according from previous study, which was extracted active of constituents in cashew nut liquid shell (CNLS) [24]. GC/MS was performed on a Hewlett-Packard 5971 instrument with the following components and conditions: dimethylpolysiloxane DB-1 coat-fused silica capillary column (30 m x 0.25 mm); He carrier gas (1 mL/min); injector temperature and detector temperature

were 250 and 200 °C, respectively. Programming of column temperature was 35-180 °C, at 4 °C/min; and 180-250 °C, at 10 °C/min. Mass spectrometer operating conditions were 70 eV of ionization energy. Mass spectra were recorded from 40 to 450 m/z. Percentage of peak area was obtained electronically from GC/MS response without the use of an internal standard or correction factors; and *m/z* of individual compounds were evaluate based on previous study [25].

Larvae rearing and larvicidal assay

A. aegypti eggs were reared in Laboratory of Faculty of Public Health, Western University, Kanchanaburi, Thailand. The egg rafts were kept in the basin containing tap water that served as the culture medium at 28±2 °C and a photoperiod of 12-h light followed by 12-h dark (12L:12D). Appropriate amount of biscuit powder were added to enhance the growth of the larvae. The third and fourth instar larvae were used in the study. The late third or early fourth instar larvae were used for bioassay test following the WHO standard larvicidal bioassay method. The procedures was followed briefly, 25 larvae in 249 ml of declorinated-tap water with 1 ml of each concentration of larvicidal solution was tested and repeated at least 4 times. Mortality counts were made after 24 hours. The results were analysed to obtain the LC_{50} by probity analysis using a computer program. 1% (w/v) Temephos (Abate, Cyanamid, USA) was used for positive control [26, 27].

Results and Discussion

The concentration of stock and diluted larvicidal solutions were represent as total phenolic content (mg of gallic acid equivalent/ml); and larva mortality of each solution was shown in Table 1. Chromatographic analysis of solution was contained five alkyl phenols, which included saturated cardol, monounsaturated cardol, anacardic acid, diunsaturated cardanol and monounsaturated cardanol (Fig. 1). Anacardic acid was major active compound, which was correlated to mass spectra (Fig. 2). This finding was corresponded to previous studies, which were applied on mosquito control with safe to mammals [20, 21]. Our result was less effective than previous studies; however, active alkyl phenols were still remained in appropriate amount. This larvicidal solution was simpler to prepare and cost effectiveness was considered by use of water as solvent and container, which may useful in community level especially in cashew cultivating area. Field application and public training on this study will be conduct on controlling of mosquito-borne diseases.

Table 1: Total phenolic content and activity from each dilution of larvicidal solution*

Larvicidal solution	Stock (10% W/V)	1:5	1:10	1:20	1:50	1:100
Total phenolic content (mg of GAE/ml)	180.1 ± 9.5	35.6 ± 0.5	15.1 ± 1.2	7.2 ± 2.0	4.0 ± 1.7	3.1 ± 0.4
Larva mortality (%)	100	100	92	65	51	30

* LC_{50} = 3.8 mg of GAE/ml; LC_{90} = 11.5 mg of GAE /ml; GAE = gallic acid equivalent; positive control = 1% temephos

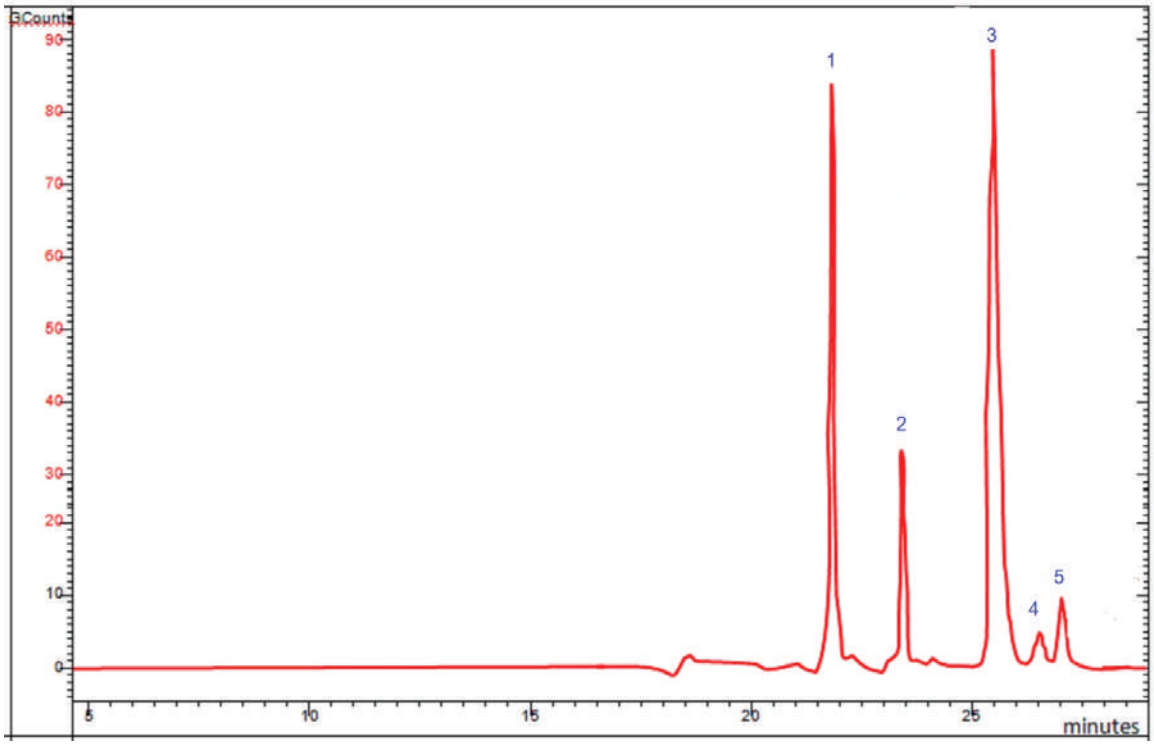


Figure 1: Chromatographic separation: (1) saturated cardol; monounsaturated cardol (2); anacardic acid (3); diunsaturated cardanol (4); monounsaturated cardanol (5)

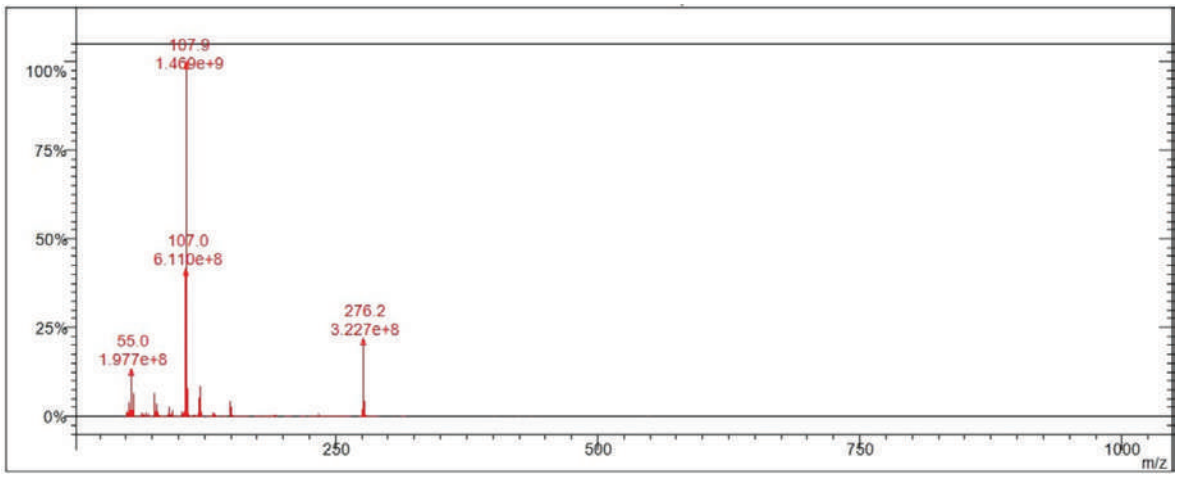


Figure 2: Mass spectra of anacardic acid as main constituent in larvicidal solution

Conclusion

Simple cashew nut shell preparation of larvicidal solution may useful on controlling mosquito-borne diseases, such as, dengue fever. Anacardic acid was active alkyl phenol, which was remained in preferable concentration.

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Ethical Clearance: Ethics Committee of Suan Sunanha Rajabhat University was exempted in consideration of research protocol (COE. 1-053/2018).

Conflicts of Interest: The authors confirm that there are no conflicts of interest.

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Analysis of the Mean Gray Scale and the Mandibular Thickness (Micro-CT) To Mandibular bone Density in Synthetic SCAFFOLD-hADMSC

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Abstract

Introduction. The concept of biofactor transplantation in porous and degradable materials as scaffolds is a place for regenerating new cell and bone tissue growth in the application of tissue engineering. Human Adipose Derived Mesenchymal Stem Cells (hADMSC) is a multipotent cell which has osteogenic, chondrogenic, and adipogenic potential. It also has somany similar characteristics with Bone Marrow stem cells (BMSCs) but hADMSC has higher proliferation rate compared to BMSCs.Inspite of this, another ability of biofactors is gene engineering. **Aim.** This study aimed to analyze mandibular bone density from the synthesis of scaffold-hADMSC with the mean gray scale and the mandibular bone thickness (Micro-CT).**Material and Methods.** This research involved several processes, namely synthetic scaffold manufacturing process, differentiation and characterization of hADMSC, and Micro-CT. **Results.** The results of the marked expression of the characterization of hADMSCand analysis of the mean gray scale and mandibular thickness (Micro-CT) to mandibular bone density in synthetic scaffold-hADMSC showed to be significant. **Conclusions.** Synthetic scaffold-hADMSC can increase mandibular bone density after Micro-CT analysis.

Keywords: *Micro-CT,Mesenchymal Stem Cell, syntheticscaffold*

Introduction

The concept of transplantation is biofactors (cells, genes and or proteins) which is an porous and degradable materials as scaffold.[1] Scaffold is a place for regenerating new cell and bone tissue growth in tissue engineering applications.[2] Human Adipose Derived Mesenchymal Stem Cells (hADMSC) is a multipotent cell which can differentiate into osteogenic, chondrogenic, and adipogenic. hADMSC has many similar characteristics of Bone Marrow stem cells (BMSCs) but it has a higher proliferation rate compared to BMSCs.[3] The development of bioceramic scaffold has so many benefits onits high porosity interconnection which the place for the growing of the new tissue enters and binds.[4] The process of bone regeneration and cartilage utilization of hADMSCare still less effective and its process must be combined with the using of scaffold and growth factors.[5] Bioceramic as a scaffold synthesis seeded with hADMSC is a concept of bone regeneration process in tissue engineering.

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Material and Methods

Animal Selection and Criterion Procedure

This research obtained using rat (Rattus norvegicus) weighing 200-300 gram, male rats, and aging 2-3 months years old. These rats were divided into five groups, such

as: the first and third groups were given scaffold Y-TZP, the second and fourth groups were given both scaffold Y-TZP and hADMSC, and the fifth group was a control group. There were two kinds of measurements in this research, the first was the looking time and the second was the variables that would be looked for.

Adipose Sampling Procedure

Adipose sampling was performed with an oval slice on the upper surface, remaining adipose on the inner surface together with muscles and other tissues, with an area of approximately $5cm^3$.

Isolation, Culture, Characterization, Differentiation Procedure, and Flowcytometry hADMSC

Isolation and Culture Expansion Procedure of hADMSC according to (Banyard et al., 2015) without modification until passage 4.[6] Differentiation of hADMSC to determine adipogenic and osteogenic according to (Jeon et al. 2016) without modification.[8] Flowcytometry is carried out according to the procedure from (Van Pham et al, 2016) without modification.[7,8,9]

The procedure for implanting synthetic scaffold in the mandibular bone of rat

The anesthesia procedure is carried out by administering ketamine HCL injection intramuscularly at a dose of 20 mg per kg body weight and xylazin premedication 3 mg per kg body weight intramuscularly and then wait until the anesthetic effect works.

Disinfection of the mandibular extra oral with 10% povidone iodine in the work area and covered with sterile cloth. Mandibular incisions on the left side and flap to muscle and periosteum are released from the mandibular bone surface. After that, drilling is done laterally in the ramus mandibular area with a diamond wheel contra angle low speed handpiece. Furthermore, implant material is synthetic scaffold, both with seeding hADMSC and without seeding hADMSC on the mandibular bone, then suturing using gut 5/0 atraumatic silk cut thread. For experimental animals in the control group the treatment of the mandibular bone only defects the bone by drilling. The experimental animals in normal controls were not treated and terminated at 8th week in experimental animals. After treatment, each experimental animal is given antibiotics and analgesics to avoid secondary infections, pain, and death. Post-implantation wounds are controlled every day. Similarly, daily control is carried out on the condition of the cage and food.

Procedure for taking research samples

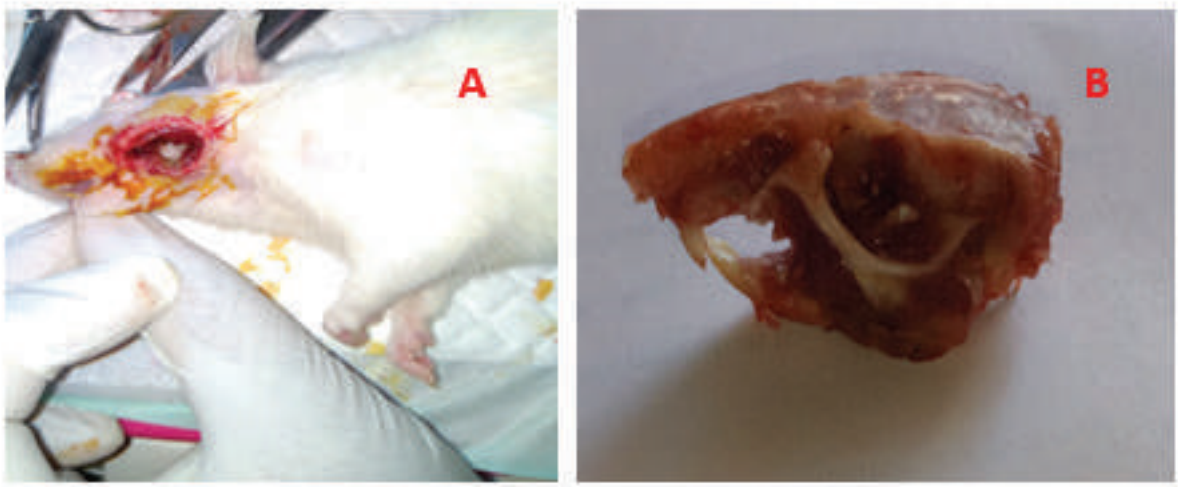


Figure 1.Procedure for sampling and treatment. A) Sacrificed methode and implantation of synthetic scaffold. B)Treatment sample.

After synthetic scaffold implantation at the end of the first week and 8th week of each treatment group and treatment control and normal control were taken 4 rats were sacrificed for the procedure of taking the research specimens. After decaputating process, the mandibular rat were cut in the control group and treatment with synthetics caffold implantation by cutting laterally included synthetic and soaked in a fixation solution of 10% buffered formaldehyde for a minimum of 3 days. The sample was then examined with Micro CT and incinerated at the the Faculty of Veterinary Medicine, Airlangga University.

Micro-CT examination procedure

The scanning procedure is carried out using a Micro-Computed Tomography (Micro-CT) device Bruker SkyScan 1173 High Energy Micro-CT.

Reconstruction image is a collection of 2D images in the lateral slice plane (x-y plane). Reconstruction is done by using the isotropic spatial resolution mode where the image voxel has a resolution of 28.15 microns, which also means that the thickness of the 2D image resulting from the reconstruction is 28.15 microns. This image stack contains complete information on the structure of 3D space, which can be visualized in the form of orthoslice or 3D volumetric visuals. Giving color codes can be done to reinforce differences in structure that are very useful for qualitative analysis. The analysis was then carried out on the sample by calculating the Mean of Grayscale Index, and the trabecular thickness according to the operational definition described above.[10]

Ethics

This study has been evaluated and approved by Committee Ethics Airlangga University Hospital with Number: 107/KEH/2018.

Results

Isolation, Culture Expansion, Characterization, Differentiation, and Flowcytometry of hADMSC phenotype

Based on the characterization analysis of hADMSC, MSCs on the expressions of CD 90 (97.61%), CD 73 (99.56%) and CD 105 (97.41%) were above 95%, while they on CD 14 (0.74%), CD 19 (0.83%), CD 34 (1.45%), CD 45 (1.42%) and also HLA-DR (0.84%) were below 2%.

The Micro-CT analysis

Figure 2.The Micro-CT analysis. A)2D image of Micro-CT orthoslice. B) 2,5D image of Micro-CT orthoslice. C) 3D image of Micro-CT orthoslice. D) analysis of MGI statistics between treatment groups in termination of the same week. E) MGI statistical analysis between groups 1 and 8 weeks. F) analysis of mandibular thicknessstatistics between treatment groups in termination of the same week. G) mandibular thickness statistical analysis between groups 1 and 8 weeks.

Table 1. Statistical Analysis of The Mean Grayscale Index (MGI)

Group	n	MGI			P value
		Mean±SD	Median	Min-Max	
Normal (KN)	4	88.5 ± 5.31	90.67b	80.60 - 92.07	
Defect One (K1)	4	56.69 ± 2.34	56.21a	54.55 - 59.77	
Defect + Scaffold 1 (P1)		85.63 ± 1.45	86.16b	83.50 - 86.70	
Defect + Scaffold + Stem Cell 1 (P2)	4	87.09 ± 2.31	87.55b	84.20 - 89.07	0.011*
Defect Eight (K2)	4	62.76 ± 4.88	65.25a	57.14 - 65.89	
Defect + Scaffold 8 (P3)	4	88.02 ± 0.88	88.37b	87.03 - 88.68	
Defect + Scaffold + SC 8 (P4)	4	88.39 ± 3.53	87.48b	85.60 - 93.00	

*Kruskal-Wallis test showed that P2 has a significant value with p=0.011 (α=0.05)

[a,b] denotes from Wilcoxon-Mann Whitney Test which [a,b] showed that there was no significant differences (α=0.05)

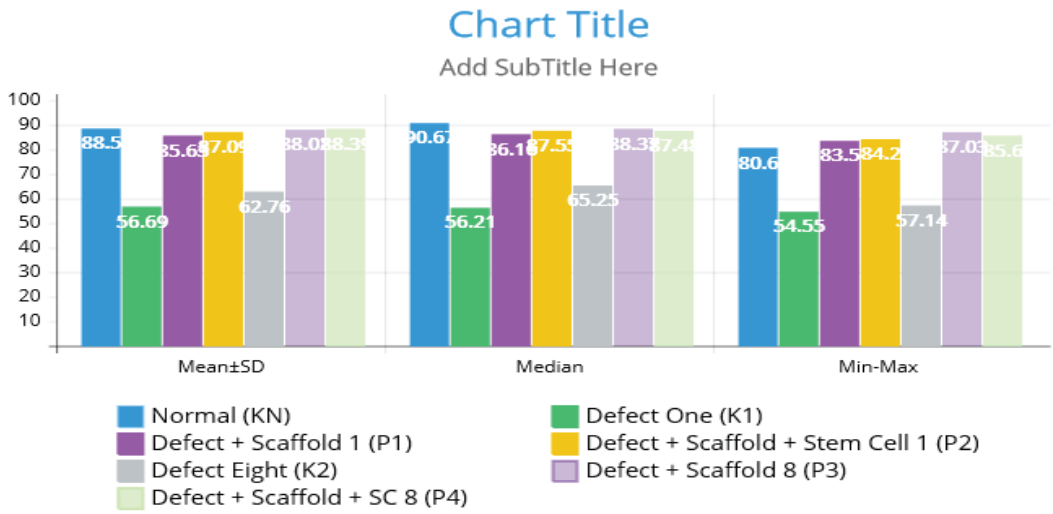


Figure 3.The graphic of descriptive statistic MGI

Table 1 and Figure 3 showed the data of Mean Grayscale Index (MGI) of each group was checked by normality test and the distribution of the data was not normal, so it must be analyzed using non-parametric test. From these non-parametric test, between the P1, P2, P3, and P4 there was no significant difference with KN, but the group K1 and K2 there was significant difference with KN. Between each group, K1 K2 K3 and K4 there was significant difference with K1 and K2.

Table 2. Statistical Analysis of Mandibular Thickness

Group	n	Trabecula Thickness			P value
		Mean±SD		Min-Max	
Normal (KN)	4	0.38 ± 0.07^{c}		0.31-0.45	
Defect One (K1)	4	0.08 ± 0.01^{a}		0.07-0.09	
Defect + Scaffold 1 (P1)		0.16 ± 0.06^{ab}		0.12-0.23	
Defect + Scaffold + Stem Cell 1 (P2)	4	0.18 ± 0.04^{ab}		0.15-0.24	0.001*
Defect Eight (K2)	4	0.20 ± 0.11^{abc}		0.13-0.32	
Defect + Scaffold 8 (P3)	4	0.21 ± 0.05^{abc}		0.17-0.27	
Defect + Scaffold + SC 8 (P4)	4	0.18 ± 0.03^{b}		0.14-0.20	

*Statistic Welch showed that P2 has a significant value with p=0.001 (α=0.05)

[a,b,c] denotes from multiple comparisons Games-Howell which [a,b,c] showed that there was no significant differences (α=0.05)

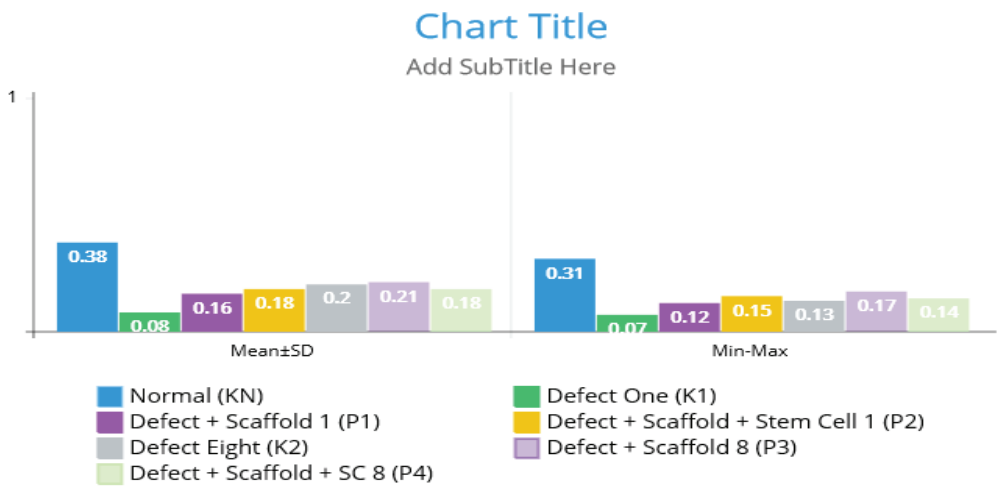


Figure 4.The graphic of descriptive statistic mandibular thickness

Table 2 and Figure 4 showed the statistical analysis of mandibular thickness using both Statistic Welch and multiple comparisons Games-Howell. The result of the mandibular thickness was normally distributed, so that it can be analyzed using parametric test using both Statistic Welch and multiple comparisons Games-Howell. After being tested using both parameters, it can be asked that between group K2 and P3 there was no significant difference, but for the group P1, P2, P3, and K2 there was no difference with groups P4, K1, and KN. But, there was significant difference between with groups KN, K1, and P4.

Discussion

The main problems that affect the process of success in an implant process consists of: 1. Inflammation, 2. Osteogenic, and 3. Osseointegration. In the first stage is the process of inflammation or inflammation considering the implant process is a process that is invasive so it may cause tissue damage that will spur the body's immune system to conduct repair or regeneration. One form of immunity is the release of cytokines due to the formation of Danger Associated Molecular Patterns (DAMPs). DAMPs can consist of 2 types, namely: High Mobility Group Boxes-1 (HMGB-1) protein and Adenosine Tri Phosphate (ATP). DAMPs are released and formed by degenerating body cells, causing sterile inflammation in the vertebrates and triggering the activation of the immune system and transcription factors. Activated immune cells are macrophages and neutrophils which further stimulate the formation of inflammatory factors (IL-1β and TNF-α). In addition to the inflammatory factors, the presence of proteases and free radicals also contribute to the formation of a secondary inflammation system in tissues facilitated by NF-KB which was initially in an inactive state to become active. Activation of NF-KB is caused by the synthesis of IKB, and activation of NF-KB in the next stage will trigger a bond with Toll Like Receptor-4 (TLR-4) so that there is a regulation of multiple-level network transcription factors, such as: IL-1β, TNF-α, IL-6, the enzyme Cyclooxygenase 2/COX-2, Matrix Metalloproteinase, and transcription factors and other signals related to osteoblast synthesis (Runx2 / Run-Related Factor Transcription-2, Osterix, Alkali Phosphatase / ALP enzymes, Ephrine specifically Ephrine 4, Caderin specifically CDH2 and CDH10b, Osteoprotegerin / OPG, Wnt LPR5 / 6). Inflammatory factors that are formed in the initial phase are also related to the stem cell material used in this study, namely: by using human Adipose Mesenchymal Stem Cells (hADMSCs) which have a common pathway in the activation process, namely through the CXCR 4 chemokine with the CXCR 4 also present in the inflammatory phase which is influenced by the chemokine CXCL12. The CXCR4 chemokine is also related to one of stem cell characterization, which is homing characterization on stem cells.

After dealing with inflammatory phase, there are several types of transcription factors which can originate from the transcription factors themselves and from the transcription factor signals which further enhance osteoblast synthesis. Increased osteoblast activity will cause an acceleration of bone formation, which is in accordance with the results of statistics conducted on animal experiments that have a significant value with a p value of 0.001 which turned out to be smaller than the α value of the statistical test 0.05. After a lot of osteoblast synthesis, both from the conduction of osteogenic transcription factors and from the HADMSCs stem cells, there is further collagen synthesis, specifically type I collagen which functions in the process of biomineralisation. This biomineralization process is an important process which is controlling and forming Hydroxy Apatite.

The final stage of the implantation process is the osseointegration process which means that the process of integration between the implant material and surrounding bone tissue. This is controlled by caderin which is a protein located at the adhesive junction between cells involved in the process of morphogenesis, tissue formation, and tissue integrity. Caderin works in two ways, namely: in the osteogenic process and endosteal niche which has the same end result, namely the increase in osteoblast cells caused by caderin, specifically CDH-2, CDH-10b will increase the occurrence of Wnt complex with β-catenin and related with differentiation of hAMDSCs into osteoblast cells.

The next step is to examine the thickness of the bone with a device called a Micro-CT which provides information about terminology for the measured parameters and related units, and further analysis has the potential to be done with this technology. Shortly, qualitative and quantitativeanalysis, including basic image processing (selection and VOI threshold) and measuring of several morphometric variables (total VOI volume, object volume, percentage of total volume, total VOI surface, object surface, object surface / volume ratio, object density surface , structure thickness, structure separation, total porosity).[10] The micro-CT observation was carried out by 2D image analysis; 2.5D; 3D. In 2D and 2.5D images there are noise / artifacts (radioopaqe spans) in the area around synthetic scaffold in all treatments. This is caused by the density of material from synthetic scaffold after sterilization using gamma rays. Synthetic scaffold shrinks from its original form before sterilization using gamma rays. Sterilization is needed to avoid contamination of synthetic scaffold especially for preparing seeding with hADMSC.At MGI it was found that mandibular bone density was significantly greater in the treatment of scaffold-hADMSC synthesis compared to the treatment of scaffold synthesis without hADMSC with reference to normal control. At the mandibular bone thickness it was also significantly clear that the mandibular bone density was greater in the treatment of scaffold – hADMSC synthesis compared to the treatment of scaffold synthesis without hADMSC, the normal control group as standartization of mandibular bone density.The hypothesis with sintesis scaffold-hADMSC in tissue engineering is that it has a marked osseointegration capabilities comparedsintesis scaffold withouth ADMSC. High power and osseointegration acceleration can reduce implantation failure. In the future, sintesis scaffold-hADMSC made a tissue engineering in dental implantation and orthopedics.

Conclusion

In conclusion,synthetic scaffold-hADMSC can increase mandibular bone density after Micro-CT analysis.

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The Effect of Trivalent Influenza Vaccines on Immunoglobulin G and Interleukin 4 in Ferret (*Mustela putorius furo*)

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Abstract

The seasonal influenza virus has infected 5-15% of the human population every year, resulting in 250,000-500,000 deaths worldwide. The seasonal influenza epidemic that occurs every year is caused by the continuous evolution of seasonal influenza viruses, which allows them to avoid body immunity due to previous infection or vaccination, and the ability of these viruses that can be transmitted efficiently from human to human through breathing, direct contact, and through items that have been touched by sufferers of seasonal influenza. Vaccination is the most effective method for controlling the infection of seasonal influenza and the most important strategy for pandemic prevention and control. The ideal vaccine must improve the humoral and cellular immune response to reduce morbidity and mortality. This study was conducted to determine the role of trivalent influenza vaccination against the response of ferret antibodies by testing IgG levels and IL-4 levels. IgG is an antibody formed in response to vaccination, whereas IL-4 is a cytokine that stimulates B cells to differentiate into plasma cells and produce antibodies. The method of this study is an experimental laboratory. Twenty five ferrets separated into 5 group, control, trivalent vaccine dose 3.8 µg, dose 7.5 µg, dose 15 µg, and dose 30 µg. Ferrets were vaccinated with trivalent vaccine, intramuscular, 3 weeks later, that were challenge by H1N1, H3N2 and by influenza H1, H3 dan B (wild subtype) virus. On day 35th serum was taken and examined for IgG and IL-4. The level of IgG and IL-4 was measured by Elisa. We used SPSS 23 for data analysis. From the results of the research conducted enhancement in specific levels of IgG was obtained against H1 and H3 antigen at dose of 3.8 µg and 7.5 µg. There is no change in specific levels of IgG against By antigen. There is no change in IL-4 levels. Therefore, we conclude that IgG and IL-4 values can be used as biomarkers in testing influenza vaccines.

Keywords: *immunoglobulin G, influenza vaccine, interleukin-4, Mustela putorius furo*

Introduction

Seasonal viral influenza infections cause annual epidemics which are a significant source of public health burden throughout the world[1]. Influenza also resulted in significant economic losses. Influenza virus infections cause respiratory diseases that are highly contagious. The symptoms may be mild to severe, often leading to hospitalization and death[2]. Pneumonia is a complication that most often causes death during influenza outbreaks[3]. Influenza viruses include Orthomyxoviridae family and have genomes containing eight single stranded, negative sense RNA segments. Three types of influenza viruses A, B, and C have the ability to infect humans, although A and B are the most common circulating strains. Influenza viruses are classified based on antigenic differences in viral nucleoproteins (NP) and matrix proteins (M).

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Influenza type A viruses are further classified into subtypes by a combination of two different proteins, hemagglutinin (HA) and neuraminidase (NA), which are found on the surface of the virus. The influenza A virus subtype currently circulating among humans as seasonal influenza is influenza A (H1N1) and A (H3N2)[1,2]. Influenza B viruses can be divided into 2 main groups or lineages, B/Yamagata and B/Victoria[2].

Influenza viruses have a high evolutionary rate, and this allows the virus to mutate rapidly leading to antigenic shift and antigenic drift. The seasonal influenza epidemic that occurs every year is caused by the continuous evolution of seasonal influenza viruses, which allows them to avoid body immunity due to previous infection or vaccination, and the ability of these viruses that can be transmitted efficiently from human to human through breathing , direct contact, and through items that have been touched by sufferers of seasonal influenza[4]. The diversity of influenza virus species providing viruses has many opportunities for reassortment between subtypes, and the natural reservoir of influenza A makes elimination of disease impossible[2]. Therefore, antiviral drugs and vaccinations are more prospective solutions for controlling influenza outbreaks[5].

Vaccination is the most effective method for controlling seasonal influenza infection and the most important strategy for pandemic prevention and control[6]. Prophylactic vaccine is a tool that plays a role in preventing various infectious diseases[7]. The ideal vaccine must improve the humoral and cellular immune responses to reduce morbidity and mortality[8]. Effective vaccination in human diseases depends on the capacity of the vaccine to stimulate a protective immune response to pathogens and can produce humoral immunity and cellular immunity. In humoral immunity, B lymphocytes secrete antibodies responsible for specific recognition and neutralization pathogens derived from antigens. In addition, IL-4 is one of the most influential cytokines of the most studied immune system. The initial function of IL-4 is a B cell stimulation factor, but it is now known that these cytokines regulate a myriad of immune functions including switching immunoglobulin isotype, MHC class II expression by B cells, and the role of T cell subset differentiation from Th to Th 2[9]. In addition, ferrets and humans share similar lung physiology, and human and avian influenza viruses exhibit similar patterns of binding to sialic acids (the receptor for influenza viruses), which are distributed throughout the respiratory tract in both species. Hence, This study was conducted to determine the role of trivalent influenza vaccination against the response of ferret antibodies by testing IgG and IL-4 levels.

Materials and Methods

Experimental Materials and Ethical Statement

This study is a laboratory experimental study with the design of pre and post test, which was conducted at Animal Biosafety Level-3, Universitas Airlangga, Surabaya, Indonesia in January 2017 - June 2017 and the Institute Medical Sciences, the University of Tokyo, Japan, in September 2017. The experimental animal treatment was carried out in Animal Biosafety Level-3, Universitas Airlangga has obtained the Animal Welfare Assurance A5966-01 for foreign institutes from the National Institutes of Health's Office Laboratory Animal Welfare (OLAW). The experimental animals used were ferret (Mustela putorius furo), female, aged 4-6 months, weight 800-900 grams. The minimum sample size used 5 ferret for each treatment group, and there were 5 treatment groups. The total sample size is 25.

Study Design

The experimental animals are divided into 5 treatment groups as follows:

- Group A: Trivalent vaccine dose of 3.8 µg
- Group B: trivalent vaccine dose of 7.5 µg
- Group C: trivalent vaccine dose of 15 µg
- Group D: trivalent vaccine dose of 30 µg
- Group E: without vaccine

Ferret trial animals of 25 tails were adapted for 1 week (seven days) before treatment. Day -1: Taking 3 mL of blood sample for pre-test, followed by vaccination I with trivalent vaccine, 0.5 mL with A1 dose, 3.8 µg; B1 7.5 µg; C1 15 µg; D1 30 µg; E1 with PBS by intramuscular injection in the left femur. On the 22nd day a challenge was tested for H1N1, H3N2, and viruses. On the 36th day: Euthanasia with intramuscular ketamine injection, then surgery and intracardiac blood were taken with a 10

cc syringe. Then, we were measure IgG levels with the Elisa method (using Ferrero IgG heavy and light chain Antibody, A140-108P) according to the manufacturer's instructions. The the measurement of IL-4 levels with the ELISA method (Antiferret IL-4 Ab paint No. 111132 Company; antibody research corporation) according to the manufacturer's instructions.

Processing and analysis of data

Data on IgG levels and IL-4 levels were analyzed statistically using IBM SPSS 23.

Results and Discussion

The results of measuring levels of IgG in ferret induced by trivalent vaccine

Measuring IgG levels is done before and after vaccination. Each serum sample was tested for IgG levels 3 times, namely against H1N1 antigens, H3N2 antigens and antigens.

IgG levels against H1NI antigens before and after trivalent vaccination

IgG levels before and after trivalent vaccination with H1N1 antigens can be seen in Table 1. From the statistical test with SPSS 23, it was found that after vaccination, in testing with H1N1 antigens in groups A, B, and C there was an increase in IgG levels that were significantly different from IgG levels before vaccination. Whereas in groups D and E, there was an increase in IgG levels which were not significantly different from IgG levels before vaccination. There are differences in IgG levels between groups in measuring IgG levels with H1N1 antigens.

Table 1. Levels of IgG against H1 antigens before and after treatment.

Group	n	IgG Levels on H1NI Antigens (IU/mL)			p (before after)
		Before treatment	After treatment	Difference	
A	5	2.07±0.29	3.03±0.06	0.96±0.28a	0.002
B	5	2.01±0.64	2.98±0.09	0.98±0.57a	0.018
C	5	2.32±0.44	0.07±0.05	-2.25±0.49b	0.000
D	5	2.41±0.29	1.37±0.91	-1.04±1.00b	0.081
E	5	2.41±0.17	0.18±0.15	-2.234±0.23b	0.000

Description: * significant at $\alpha = 0.05$

IgG levels against H3N2 antigens before and after trivalent vaccination

The results of measuring IgG levels with H3N2 antigens can be seen in Table 2. Testing with H3N2 antigens in groups A and B showed an increase in IgG levels that were significantly different from IgG levels before vaccination. Whereas in groups C, D, and E, there was an increase in IgG levels which were not significantly different from IgG levels before vaccination. There were differences in IgG levels between groups in measuring IgG levels with H3N2 antigens.

Table 2. Levels of IgG against H3 antigens before and after treatment.

Group	n	IgG Levels on H3N2 Antigens (IU/mL)			*p* (before after)
		Before treatment	After treatment	Difference	
A	5	1.66±0.27	2.96±0.13	1.30±0.22[a]	0.000
B	5	1.52±0.49	2.91±0.14	1.40±0.42[a]	0.002
C	5	1.90±0.22	0.06±0.01	-1.84±0.23[c]	0.000
D	5	1.93±0.40	0.95±0.98	-0.984±0.97[c]	0.080
E	5	2.11±0.14	0.11±0.07	-1.99±0.16[b]	0.000

Description: * significant at α = 0.05

Level of IgG against B antigen (yamagata) before and after trivalent vaccination

The results of measuring IgG levels with antigen By can be seen in Table 3 below. Testing with antigen By in group B there was an increase in IgG levels that were significantly different from IgG levels before vaccination. Whereas in groups A, C, D, and E, there was an increase in IgG levels which did not differ significantly from IgG levels before vaccination. There was no difference in IgG levels between groups in measuring IgG levels with H3N2 antigens.

Table 3. Levels of IgG against antigen By before and after treatment.

Group	n	IgG Levels on ByAntigens (IU/mL)			*p* (before after)
		Before treatment	After treatment	Difference	
A	5	1.73±0.47	0.98±0.54	-0.75±0.81[a]	0.108
B	5	1.64±0.42	2.01±0.10	0.37±0.44[a]	0.131
C	5	1.45±0.28	1.83±0.24	0.38±0.45[a]	0.133
D	5	1.66±0.34	1.96±0.21	0.30±0.37[a]	0.141
E	5	1.79±0.17	1.75±0.17	-0.04±0.13[a]	0.508

Description: * significant at α = 0.05

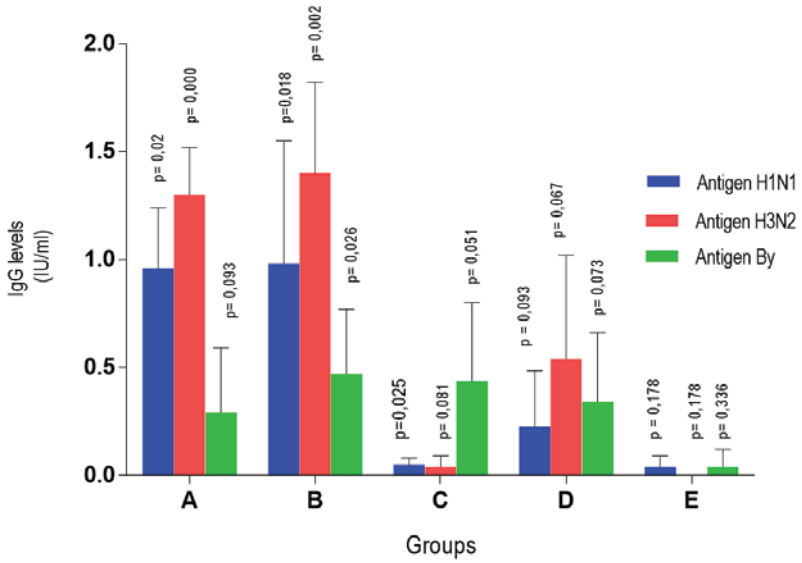


Figure 1. IgG levels of each group against H1N1, H3N2 and By.

Based on Figure 1 above, trivalent vaccines of 7.5 µg (group B) and 3.8 µg (group A) doses can induce high levels of IgG in all three antigens.

IL-4 levels after trivalent vaccination in ferret

The results of measurement of IL-4 levels can be seen in table 4 below. IL-4 levels in groups A, B, C, and D showed a significantly different increase than before vaccination. In group E, there was no increase in IL 4 levels which was not significantly different from before vaccination. There were differences in IL4 levels between groups after trivalent vaccination.

Table 4. Levels of IL-4 before and after trivalent vaccination.

Groups	n	IL-4 (pg/mL)			
		Before treatment	After treatment	Difference	p (before after)
A1	5	0.047±0.003	0.048±0.007	0.0016±0.006a	0.304
B1	5	0.050±0.005	0.063±0.042	0.013±0.040a	0.515
C1	5	0.084±0.083	0.095±0.081	0.011±0.010a	0.08
D1	5	0.046±0.008	0.052±0.005	0.005±0.010a	0.329
E1	5	0.049±0.007	0.050±0.006	0.001±0.010a	0.871

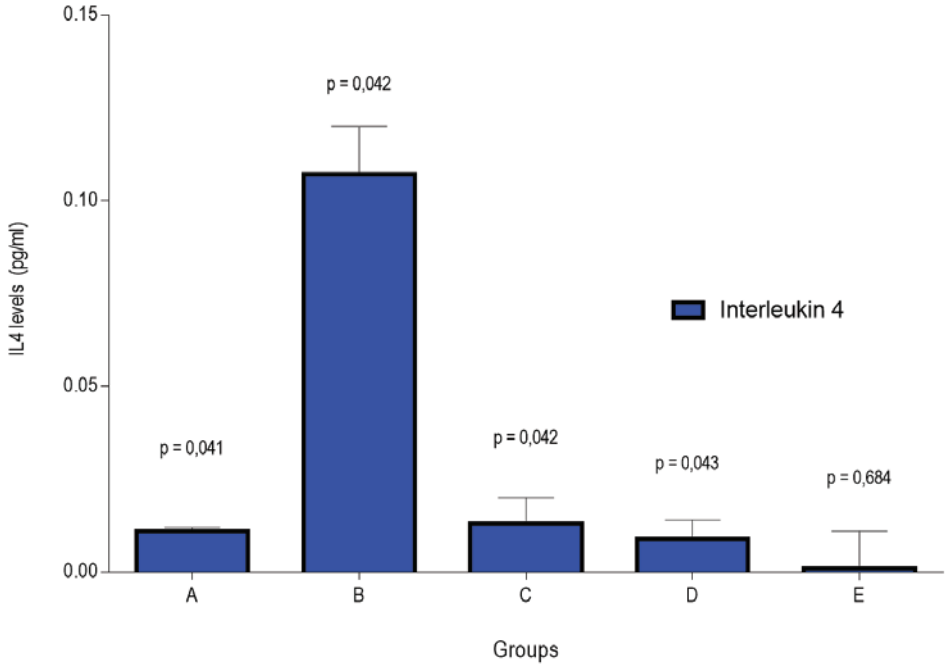


Figure 2. Increased levels of IL4 from each group after trivalent vaccination.

From Figure 2, the experimental group that received a dose of 7.5 µg, had the highest increase in IL4 levels after trivalent vaccination. Based on the results of research that has been done, before trivalent vaccination, the serum already contains IgG antibody titers. The response was due to differences in pre-vaccination memory deposits among subjects[10]. In measuring IgG levels with all three antigens, group B who received vaccinations at a dose of 7.5 µg showed being able to induce ferret to produce the highest levels of IgG from the five groups, and the IgG levels differed significantly from IgG levels before vaccination. There is an increase in IgG levels in all groups, and in all antigens, but not all are significantly different.

In group E, who did not get vaccinations, but received a challenge test with trivalent influenza virus, showed a slight increase in IgG levels, and did not differ significantly from IgG levels before treatment. The main function of B lymphocytes is producing antibodies. Another name for antibodies is immunoglobulin, which is a glycosylated protein molecule found on the surface of B cells, functions as an antigen receptor, or secreted also to the extracellular space so that it can bind and neutralize the antigen. A single antibody molecule consists of four protein chains, 2 heav chain, 2 light chains. Antibodies consist of 5 isotypes or classes, namely IgM, IgD, IgA, IG and IgE[11]. Anti-influenza antibody titers and cross reactivity with new viruses are routinely used to assess the potential for protection against new influenza virus infections. When combined with the characteristics of viral genome diversity, serological tests can provide insight into immunity stimulated by the evolution of the virus.

The results of measurements of IL-4 levels showed that there were differences in IL 4 levels between groups after trivalent influenza vaccination. The dose groups 3.8 µg, 7.5 µg, 15 µg, and 30 µg had increased IL4 levels after vaccination and differed significantly compared to group E who did not get vaccinations. Group B with a dose of 7.5 µg had the highest IL4 level. Based on the type of cytokines produced, Thelper lymphocytes are divided into Th1 which produces proinflammatory cytokines TNF-α and TNF-β which are now called lymphotoxins, IFN-γ, IL-1, IL-6, IL-8, IL-12, which function to activate cellular immunity and non specific. Besides that there is Th2 which produces anti-inflammatory cytokines, namely IL-4 and IL-10 which activate humoral immunity[12].

IL-4 is one of the most influential cytokines of the most studied immune system. Initially as a B cell stimulation factor, it is now known that this cytokine

regulates a myriad of immune functions including switching Ig isotype, class II MHC expression by B cells, and the role of T cell subset differentiation from Th to Th 2. The presence of IL-4 in the early stages of the immune response has an important effect on the natural immune response. IL-4 supports the further production of IL-4 by T cells, stimulates antibody formation, and it inhibits IFN-γ production, thus inhibiting hypersensitivity reactions. IL-4 is a cytokine released by various types of cells including T cells, eosinophils, basophils, mast cells, natural killer cells (NK) and several antigen presenting cells (APC)[6].

Conclusion

In summary, we conclude that IgG and IL-4 values can be used as biomarkers in testing influenza vaccines.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Ethical Approval: The experimental animal treatment was carried out in Animal Biosafety Level-3, Universitas Airlangga has obtained the Animal Welfare Assurance A5966-01 for foreign institutes from the National Institutes of Health's Office Laboratory Animal Welfare (OLAW).

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The Possible Nephroprotective Effect of Roflumilast Ameliorating Glycerol -Induced Myoglobinuric Acute Renal Injury in Rabbits Model

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Abstract

Glycerol encouraged kidney damage through originating an oxidative injury to renal tubular cells which further leads to tissue necrosis and inflammation. While, roflumilast N-oxide is an active metabolite of roflumilast that rising the cyclic adenosine-3′, 5′-monophosphate by inhibiting PDE4.

Thirty rabbits were divided into three groups, control group was received normal saline intra-muscularly and drived from water for eighteen hours before. Second group was received a single dose glycerol injected intra-muscularly after drived from water for eighteen hours .Third group was received roflumilast intraperitoneal daily for three days after received a single dose of glycerol .

All animals were sacrificed after 72 hour, and then blood collected by cardiac puncture for evaluation the changes in the levels of renal function tests and histopathological study of rabbit's kidneys tissues are evaluated evaluation and scoring the changes of tested agents. The numerical data are expressed as means ± standard error means and analyzed by using SPSS version 20.

The results of the levels serum urea and creatinine found to be significantly reduced by roflumilast using ANOVA test. Besides, the histopathological changes showed ameliorative effects of roflumilast in compared to glycerol group. From this study, one could be concluded the protective effect of roflumilast in case of renal failure induction by glycerol.

Key words: *nephroprotective, roflumilast, glycerol*

Introduction

Acute kidney encompasses smaller changes in the renal function without obvious failure that could be resulted in significant worse consequences and the rate of disease in a population, moreover may be ended to death [(1).] The incidence rate of these emergent cases that is needed to hospitalization reached to 2-5%. Acute kidney impairment developed within one month postoperatively in rate 1 of 100 patients of general surgery cases and arises in up to near 70 % of intensive care unit patients [(2).] Nephrotoxicity induced by drug overdose or time dependent is a frequent problem in nephritic-medicine and the incidence of drug-accompanying acute kidney impairment may be as high as sixty percent[(3)] . The etiology of pathological condition associated with acute renal failure is septic shock at 47.5 % of total patients that needed hospitalization. The percent of death to total hospital mortality was 60.3%[(4)] . Other causative condition of acute renal function deterioration is a myoglobinuric case, which is uremic syndrome followed rhabdomyolysis and muscle destruction with releasing of intracellular elements such as myoglobin and other intracellular constituents into blood stream

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[5]. The myoglobin casts producing its effects by tubular obstruction or damaging of tubular epithelium with leakage of filtrate across it after reducing glomerular filtration rate [6]. Acute nephrotic injury given that an increase in serum creatinine often lags two and three days behind the onset of injury that may reflect the damage might be included two third parts of kidney were included [7] . Indication of roflumilast in case of chronic obstructive respiratory disease, it is effective in reducing the frequency of respiratory disease exacerbations[8]. The current study aimed to evaluate and investigate the potential nephroprotective effect of roflumilast against nephrotoxicity induced by glycerol in rabbits.

Experimental Design and Method

Rabbits (albino & domestic) of different sexes were weighing (1- 2 Kg) were acclimated for new animal house for ten days before any experimental procedures and fed with commercial pellets and allowed water *ad libitum*. They were reserved under dominated condition of temperature (23 ± 2 °C) and humidness (55 ± 15%). Also, lighting was systemized lighten to half day then to darken later. All methods performed in this current study were in accordance with regulatory content on the maintenance and practice of lab animals and reported to ethical committee approval of college of pharmacy. In the present study, after 72 hour of water deprivation, three groups of rabbits (n = 10 for each group involved control group was received intraperitoneal (5 ml) of normal saline (0.9 % of sodium chloride); group 2 (induction group) was only received given dose of diluted glycerol (50 %; 8 ml/ kg , i.m.), the total dose was divided equally, then administered in each hind limb muscle (18). Group 3 was given glycerol intramuscularly as single dose plus roflumilast that was given intraperitoneal after 60 minutes of the glycerol injections(1 mg/kg)[9] .Blood samples are collected by cardiac puncture to prepare serum, and then kidneys tissues were harvested for further biochemical and histopathologic analyses.

Measurement of serum urea concentration involved the usage of a commercially available kit (Biosystems) R [10]. The urea levels in the samples react with the kit's reagent components [11]. Measurement of serum creatinine concentrations were done by using a commercially available kit was used (Biosystems)R . The auto- analyzer apparatus is used to measure the absorbance of standard and samples after 30 and 90 second based on spectrophotometric measurement at 505nm[12, 13].

Histopathological assessment involved the macroscopic examination of the kidneys, then microscopic evaluation [14] [15]. A pathologist, who was unaware of the group assignments, performed a semiquantitative analysis of the kidney sections using a light microscope[16]. The statistical analyses were performed by using statistical package of social science (SPSS version 20). All numerical data are expressed as mean ± standard error of the mean (SEM). The data were evaluated by one-way analysis of variance [17] and Mann Whitney U test. *P* value is considered significant when its value equal or less than 0.05[18].

Findings

Induction was done by glycerol-received group and it showed a significant elevation of urea level compared to control group. While, treatment group (roflumilast-treated group) displayed a significance reduction of urea level in comparison with induction group (shown in table 1). In addition, induction group exhibited there is significance elevations of serum creatinine level compared to control one. While the treatment group (roflumilast treated group) offered a significance reduction of creatinine level in comparison with induction group as shown in table (1(.

Sections of kidneys of control group revealed normal appearance of renal tubules in both cortex and medulla (figure1). Glycerol treated group showed area of interstitial inflammation and others changes discussed in scoring system (figure 2, table 2). While, roflumilast treated group showed as clearly improvement of histopathological changes (figure 3). Kidneys in the induction group showed moderate to severe lesions. Lesions were included deposition of hyaline material in the tubules of cortex and medulla, marked dilation of renal tubules, vacuolization of the renal tubular epithelium particularly in the medullary region, this group showed marked epithelial vacuolation to obtained score 4 in statistical significant level of $P \leq 0.01$ than control group (displayed in table 2). Some of the epithelial cells revealed statistically significant level of necrosis ($P \leq 0.05$) than both G1 and G3, this change was indicated by fragmentation of cell membrane and absence of

nuclear staining to get score 2 as showed in table 2. Some apoptotic cells were also observed indicated by fragmentation of nuclei with intact cell membrane to give score 1 but it was statistically non-significant ($P \geq 0.05$) in comparison with both G1 and G3 (table 2). Marked (score 2) interstitial lymphocytic infiltration was noted in this group, it was significant in comparison with G1 and G3 (table 2) but no significant glomerular changes had been detected in the level of light microscopic inspection (table 2). Complete absence of hyaline casts in the renal tubules, no necrosis and apoptosis in the renal tubular cells ((figures 3), (tables 2)). As G1 and G2, there were no obvious glomerular changes at the level of light microscope illustrated table2.

Table (1) Effect of roflumilast on level of serum creatinine against glycerol- induced acute renal failure

Group	Urea level (mg16)	Serum creatinine
Control	43.58	0.79
Glycerol	61.43*	1.25*
Roflumilast	31.80*#	1 *#

* means p ≤ 0.05 when compared to control group; # means p ≤ 0.05 when compared to glycerol (induction) group

Table (2) represent the scores of interstitial inflammation, evacuolation, necrosis, apoptosis and epithelial cell glomerular injury in the groups of study

S	Group	Score of interstitial inflammation	Score of epithelial cell vacuolation	Score of epithelial cell necrosis
1	G1	00 ± 00 A score 0	00 ± 00 A score 0	00 ± 00 A score 0
2	G2	40% ± 0.32 B score 2	84% ± 0.2 B score 4	25% ± 0.14 B score 2
3	G3	10 % ± 0.11 A score 1	37% ± 00 C score 3	00 ± 00 A score 0
P Value		P≤ 0.01	P≤ 0.01	P ≤ 0.05

S	Group	Score of epithelial cell apoptosis	Score of epithelial cell glomerular injury
1	G1	00 ± 00 A score 0	00 ± 00 score 0
2	G2	13 % ± 00 B score 1	00 ± 00 score 0
3	G3	00 ± 00 A score 0	00 ± 00 score 0
P Value		P ≤ 0.05	P ≥ 0.05

- These notes applied for all tables
- Values were expressed as a mean ± standard error.
- P values ≤ 0.01 was considered as significant.
- different capital letters refer to the significance among the groups.

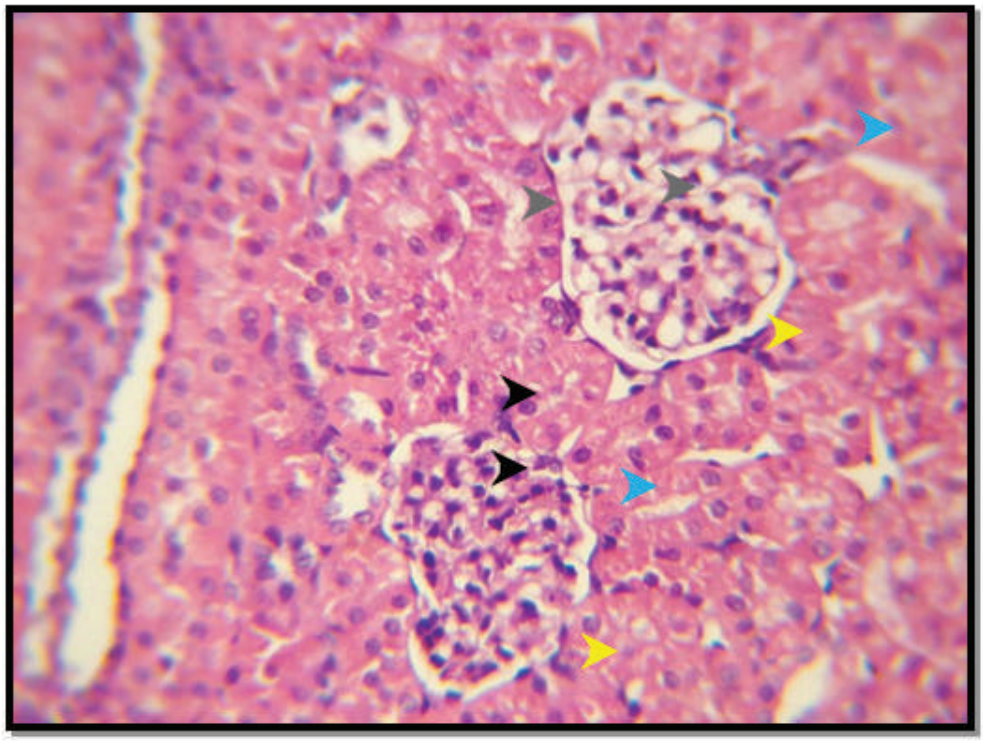


Figure (1) Kidney of control group shows normal proximal renal tubules (white arrow head), distal tubules (black arrow head), glomerular tuft (blue arrow head) and bowman's space (yellow arrow head) in the renal cortex (3) H&E 500X

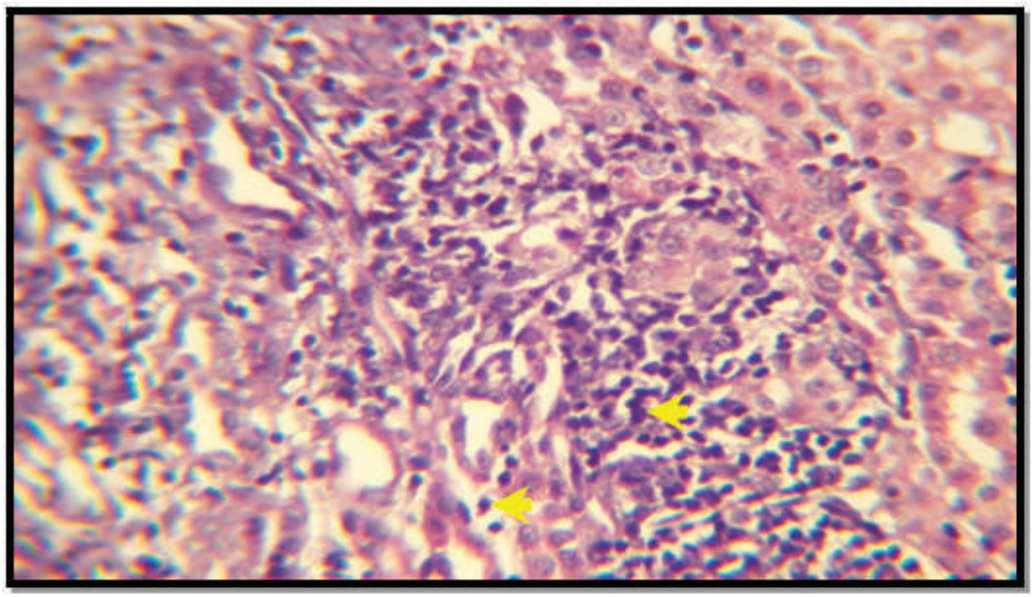


Figure (2) Kidney of Glycerol treated group shows area of interstitial inflammation (yellow arrow head) H&E 500X

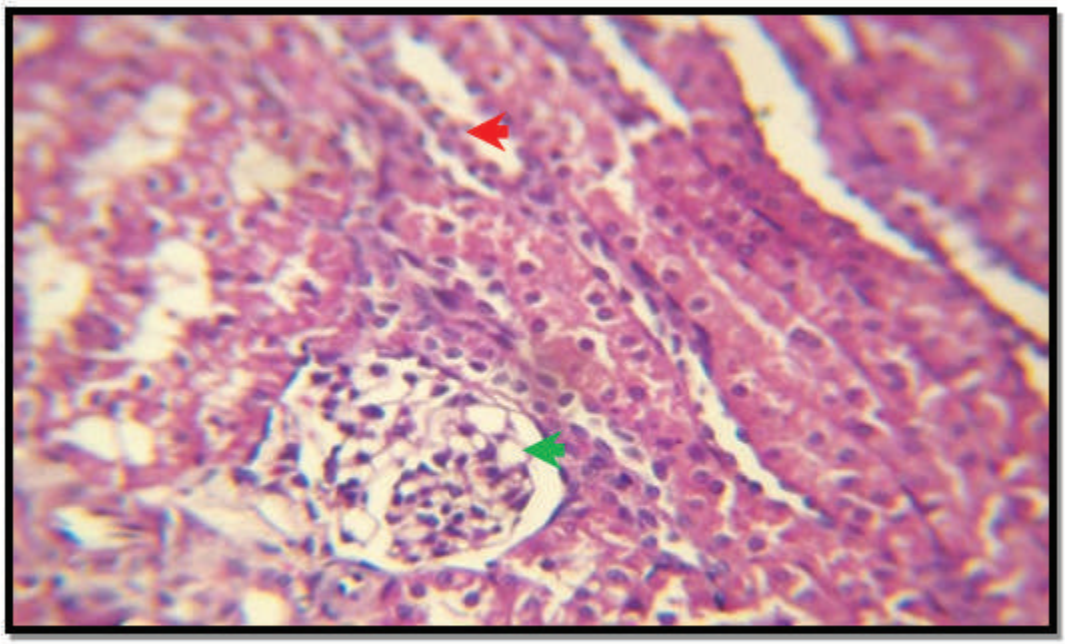


Figure (3) Kidney of glycerol + roflumilast treated group shows normal renal tubules (red arrow head) with normal glomerulus (green arrow head) in the cortex H&E 500X

Discussion

Glycerol-induced kidney impairment is cardinal of the most remarkably used model of experimental rhabdomyolysis inducing renal failure and is reflected as an experimental similarity of human myoglobinuric renal failure that resulted from transfusion problems or crush injury or random traffic accident[19] .

Myoglobin-induced tubular damage; or by renal actions of cytokines released[20]. Glycerol mediated nephrotoxicity by different mechanisms including a uncorrected renal ischemia of renal blood vessels , whereby blood flow ceases or arterial or arteriolar constriction[21] . This may be interpreted to increment of high proteins level in the blood leading to elevation of osmotic pressure and reduction of hydrostatic pressure , then both of them leading to reduced glomerular filtration pressure and reduced filtration process through glomerulus to Bowman's capsule , leading to elevation levels of both serum urea and serum creatinine[22] .The reduction of blood flow leading to persistence ischemia and free radical formation ended with glomerulopathy terminated with allow high molecular weight molecules such as protein to penetrate into tubules and elevate intratubular pressure that may equilibrate with oncotic pressure and diminished the glomerular filtration rate. In contrast , when intratubular oncotic pressure increased leading to loss of diffusion process so loss of kidney filtration function[17]. Glycerol treated group showed epithelial cells vacuolation and apoptotic cells besides the deposition of hyaline casts in the renal tubule[20]. Roflumilast is mainly effective through selective PDE 4 inhibitors , pleotropic effects including anti-inflammatory, anti-emphysema, and antibiotic[23] . Also, the beneficial effects were including pulmonary hypertension and hypersecretion of mucus [24]. The pharmacological actions behind nephroprotective effects attributed to inhibition of reactive molecules formation epithelial cells besides inhibition of neutrophils recruitment. Furthermore, it modulate vascular remodeling due to endothelial cells and probably some inflammatory cells[25]. Roflumilast is the one of drugs that could be item for further researches for improving these beneficial effects through improving effects on levels of kidney function biomarkers and ameliorating histopathological change[26]. Inhibition of glomerular vasoconstriction one of the other two mechanisms participating in the development of acute renal failure

is the decrease in blood flow in the glomerulus[27]. The roflumilast advantageous role of specific diesterase inhibition may be through blocking signaling pathways of inflammation and refreshing cell survival[28]. These results informed the advantageous role of PDE4 suppression in treating of nephrotoxicity, possibly through histopathological changes amelioration[28]. Also , The vasodilator effect may be considerable effect for roflumilast shared with another drug of PDEs inhibitor on reversing nephrotoxicity[29].

Conclusion

Roflumilast had a therapeutic effect against glycerol induced renal failure and ameliorating the renal impairment by rendering the level of urea, creatinine and histopathological tissue feautres.

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Diagnosis of Empathic Abilities of Medical Students

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Abstract

The authors of the article focus on empathy as one of the dispositions related to the emotional functioning in the process of communication, which plays an important role in determining success in a profession. It is believed that people with higher empathy tend to solve professional, personal problems more readily in subject-subject relationships. The technique for diagnosing the empathic abilities of medical students was based on a questionnaire developed by V.V. Boyko. Methods of analytical and synthetic study of scientific and methodological literature, questioning are applied. For empirical data percent distribution and ranking are used. As a result of the study, the following levels of empathic abilities in medical students were evaluated: rational, intuitive, attitudes, penetration, identification. The authors of the article came to the conclusion that the empathic abilities of medical students are important and influential factors of communication not only in the doctor-patient model, but also in their interpersonal communication, academic performance in educational activities.

Keywords: *communication; communicative competence; medical students; levels of empathic abilities; empathy channels; penetrating ability.*

Introduction

Numerous world studies conducted by scientists in various medical institutions have revealed differences in the results of treatment of doctors with high communication skills and those doctors who did not attach much importance to their professional activities. The research emphasizes that on average, for the entire period of his work, the doctor accepts a fair number of patients, about 200,000, and that, the key to the correct diagnosis and treatment of the patient is precisely an effective process of communication with him. It is also noted that the more experience of a doctor, the more attention he pays to verbal and nonverbal means of communication to improve professional and interpersonal interactions with colleagues, patients, their relatives.[1-12] Accordingly, in the context of modern medical education, the importance and appropriateness of the formation and development of the communicative competence of students is updated. By the definition of scientists, communicative competence is an open-type phenomenon that is constantly increasing as it progresses in professional development, a measure of human inclusion in communicative activities, in the process of communication.[13,14]

Communication, by its very nature, is a communication process for the exchange of information between two or more individuals. The exchange of any information is possible only through verbal and non-verbal communication (signs, speeches, gestures, facial expressions, etc.). Every communication is carried out simultaneously at the level of content of information and at the level of relationships, and the level of relations determines a meaningful level. Accordingly, there is a relationship between them: the verbal channel transmits speech, verbal information in its pure form, and the non-verbal ratio to the interlocutor is transmitted.[15-17]

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The level of communication in the communication process determines empathy. Empathy is two-part. First, it is sensitivity to the emotional needs of a person, the ability to understand verbal and nonverbal manifestations of feelings (words, touches, flirting, gestures), willingness to share these feelings. "89 s. Secondly, it is the ability to respond to emotional need in a way that will be appropriate and effective for the interlocutor.[18-21]

Material and Methods

The sample for the study consisted of first- and second-year medical students. 288. The authors of the article chose the method of V.V. Boyko[22] to diagnose students' empathic abilities. Subjects for assessing the level of empathic abilities were generated around six areas of the empathic capacity scale: the rational channel of empathy; the emotional channel of empathy; the intuitive channel of empathy; Women who promote empathy; Penetrating ability in empathy; In empathy. Consider the general characteristics of empathy channels.

The rational channel of empathy characterizes the focus of attention, perception and thinking of the empathizer on the essence of any other person – on his condition, problems, behavior. Emotional channel of empathy. The ability of the empathizer to enter into an emotional resonance with others is to empathize, to participate is fixed. The intuitive channel of empathy indicates the ability of the Respondent to see the behavior of partners, to act in the conditions of a lack of initial information about them, based on the experience stored in the subconscious. Attitudes that promote or hinder empathy that facilitate or hinder the operation of all empathic channels. Penetrating ability in empathy is regarded as an important communicative property of a person, which allows creating an atmosphere of openness, trust, and intimacy. Identification the ability to understand the other based on empathy, putting yourself in the place of a partner.[22]

The total score can theoretically vary from 0 to 36 points. According to preliminary data of V. V. Boyko, we can consider: 30 points or higher – a very high level of empathy; 29-22-average; 21-15-low; less than 14 points is very low.[22]

Results and Discussion

As the results of our research, we give an example of the scale of assessments of empathic abilities of medical students (Table 1)

Table 1. The scale of assessments of empathic abilities of medical students

Scale of the coefficient of empathic abilities	Percentage of respondents, %	Points	Ability level
Rational empathy channel 0.1 to 0.35	76,58	17	Low
Emotional empathy channel 0.36 to 0.45	25,34	20	Below average
Intuitive Empathy Channel 0, 46 to 0, 55	17,72	26	Average
Installations that promote empathy from 0.56 to 0.65	10,10	37	High
Empathy pervasiveness from 0.66 to 0.75	4, 65	41	Very tall
Empathy identification from 0.76 to 1.00	1,76	58	High

Source: compiled by authors

The distribution of medical students by the levels of manifestation of their empathic abilities was investigated. Spontaneous interest in the interlocutor, opening the emotional reflection of the partner are characterized by a low level of manifestation of rational channel empathy more than 76% of respondents. In 25.34% of respondents, theempathy empathy ratio is within the boundaries of 0.36 to 0.45, which states that the average level of emotional responsiveness in the partner's field, i.e. in understanding of the inner world, complicity and empathy for him. 10. 10. 10% of respondents have a high level of emotional responsiveness and empathic perception in settings that promote or inhibit empathy.

According to our study, the most advanced channels of empathic abilities of medical students are: installations that promote empathy; penetrating ability in empathy; empathy.

Conclusions

The questionnaire of V.V. Boyko has a simplicity in understanding the performance of tasks, is focused on a wide age range of respondents, which does not involve significant derivatives of questions on the part of researchers, does not distinguish the gender of respondents. The results of The Diagnosis of V.V. Boyko can be used by both teachers and students themselves to reflect on their emotional functioning in order to identify their strengths and weaknesses and increase their emotional awareness and adaptability to effectively work with emotionally stressful situations during daily communication with patients, peers and other medical professionals. Also, given the age-related psychological characteristics of young people between the ages of 16 and 22, suffering from excessive emotionality, youthful maximalism, students in the medical field of training can use this knowledge to improve their interpersonal skills and academic performance in educational activities, including group and team work.

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Conflict of Interest: There is not conflict of interest.

Ethical Clearance: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. A study was approved by Ethics Committee of the Surgut State University, November 27, 2019, No 1459-A.

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Structures Development of Master of Public Health Program and Doctor of Philosophy Program in Public Health Innovation Research and Development in Thailand

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Abstract

Aim: To study the need about the Master of Public Health Program (MPH) and Doctor of Philosophy Program (PhD) in Public Health Innovation Research and Development (PHIRD), to analyze factors components of the institute, and to analyze components of the MPH and PhD.

Methods: 304 samples were selected by stratifying random sampling. Data were analyzed by mean, standard deviation, and priority needs index with Modified Priority Needs Indexing technique ($PNI_{Modified}$), the exploratory factor analysis (EFA), and the second-order confirmatory factor analysis (the 2nd CFA).

Results: The research showed that samples have the level of necessity related to the programs. Regarding the appropriateness of the draft of the programs, the expected level was at a high level = 4.31/5 and 4.33/5, the $PNI_{Modified}$ = 3.31/5 and 3.3/5, respectively. Models of factors were composed of consistent with empirical data and were based on Financial (1.00), Accept (1.00), and Progress (1.00). Models of structures are composed of consistent with empirical data and were based on Professional (1.00) and Potential (1.00).

Conclusion: The observed variables and latent variables of the four models had high construct validity by EFA and 2nd CFA that can be used for the development of programs.

Keywords: *need; structures of the program; master of public health; doctor of philosophy program; public health innovation research and development*

Introduction

Meaning of "innovation" is the ability to do new something to use knowledge, creativity, skills, and experience in technology or management to develop and produce new products new production process or new service which meets the needs of the market. In a part of "economic innovation" is the introduction of new concepts or uses[1] and of "Public Health Innovation" is the ability to use knowledge, creativity, skills, and experience in technology or management public health [Health Promotion, Prevention, Treatment, and Rehabilitation] to develop and manufacture new tools (Machine, Material, Tool, etc.), new services (Curriculum, Program, Activity, etc.) or new processes that solve the cause of the problem and meet the needs of customers.[1]

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The Praboromarajchanok Institute (PI), Established by the Ministry of Public Health since 1993 to be the main duty of the production and development of public health personnel.[2] Researchers, therefore, saw the need to develop the structure MPH and PhD in PHIRD (new program) to produce researchers and develop public health innovations that can research in creating a new body of knowledge for the program and use the research process to improve the public health develop a public health management system develop the capacity of public health personnel and develop the supervision system monitor and evaluate the management of public health to further improve the quality of public health of the nation.

Objectives

To study the need for the MPH and PhD, analyze factors components of the institute, and components of the MPH and PhD.

Methodology

This research survey is the first step in research and development.[1,3] The population is 4th-year students and graduates in health science in 2019, which is the case of the unknown population or infinite numbers (Infinite Population). Determination of sample size if the population size is not known to use a 95 percent confidence level by using the following formula[4], number of samples needed=384 records. The data has collected a questionnaire divided into 5 parts by checking content validity with Index of Item Objective Congruence (IOC) and selected items with a consistency index of 0.50 or more, try out the questionnaire with 30 4th-year students at a College in Phitsanulok Province of Thailand to improve the quality by the Cronbach method[5] by finding the alpha coefficient (α- coefficient), the reliability of the four types of questionnaires (Part 2-5) were 0.93, 0.83, 0.98, and 0.95, respectively by sending online questionnaires to the 4th-year students and graduates in the health sciences, 2019, as the respondents and compile the returned online questionnaires then used in the analysis of 304 data, accounting for 79.17 percent and analyzed with basic statistical, $PNI_{Modified}$[6], EFA, and the 2nd CFA.

Results

1. Results of needs analysis found that the expected level was at a high level (= 4.31/5 and 4.33/5), the $PNI_{Modified}$ of MPH = 3.31/5 and $PNI_{Modified}$ of PhD = 3.33/5.

2. The results of the analysis of the factors that influence the decision to study in the MPH and PhD in PHIRD

2.1 The EFA that were studied by analysis Kaiser-Meyer-Olkin Measure of Sampling Adequacy found a KMO or MSA equal to 0.801 and 0.881, which are greater than 0.80, very good level, according to the criteria of Kim and Mueller[7]and the results of Bartlett's Test of Sphericity[8] found that the variables were statistically significant at the level of 0.001, could be used to analyze the components.

For component extraction of each variable used in analyzing the factors, A total of 12 elements, between 0.461 - 0.795 and 0.508 - 0.807, are medium-high and have Cronbach's Alpha = 0.826 and 0.868. Orthogonal rotation using the varimax method found that all 12 variables used in the analysis can be grouped into 3 components with eigenvalues more than 1.00, which showed that each element could explain the variance of all 12 variables more than 1.00 were 59.733% and 66.682%. When considering to put each variable into one single element in which the variables have the highest factor loading found that all 3 elements can be classified into 12 elements, with each element consisting of variables 3-6 numbers, each variable that has been arranged in the composition weights elements from 0.569 to 0.862 and 0.556 to 0.834. The compositions of 3 components have been named a meaningful as detailed in Table 1.

Table-1: The structural adjustment of economic, social, and institutional factors that influence the decision to study in the MPH and PhD in PHIRD

Side/ Core Component	Sub-Component
MPH	
1) Financial	1. Scholarships (Giving Fund)
	2. Expense per term (Expense)
	3. Own income (Self Income)
2) Accept	4. Proud of the family (Proud)
	5. Need acceptance (Acceptance)
	6. Increase qualifications (Add Academic)
	7. The famous reputation of course instructors (Famous)
	8. Interesting course (Interest)
	9. The convenience of study locations (Convenience)
3) Progress	10. The income of parents (Parent Income)
	11. Career advancement (Occupation Progress)
	12. Increase career paths (Add Occupation)
PhD	
1) Financial	1. Expense per term (Expense)
	2. Scholarships (GivingF)
	3. The convenience of study locations (Con)
	4. Own income (SelfIn)
	5. The famous reputation of course instructors (Famous)
	6. Interesting course (Interest)
2) Accept	7. The income of parents (ParentIn)
	8. Proud of the family (Proud)
	9. Need acceptance (Acceptance)
3) Progress	10. Increase career paths (AddOcc)
	11. Increase qualifications (AddAcad)
	12. Career advancement (OccPro)

2.2. Results of the 2nd CFA showed that the 12 elements are considered to be a subset of the 3 main components. Overall the field of research and innovation in public health is real as detailed in Figure 1-2 as follows

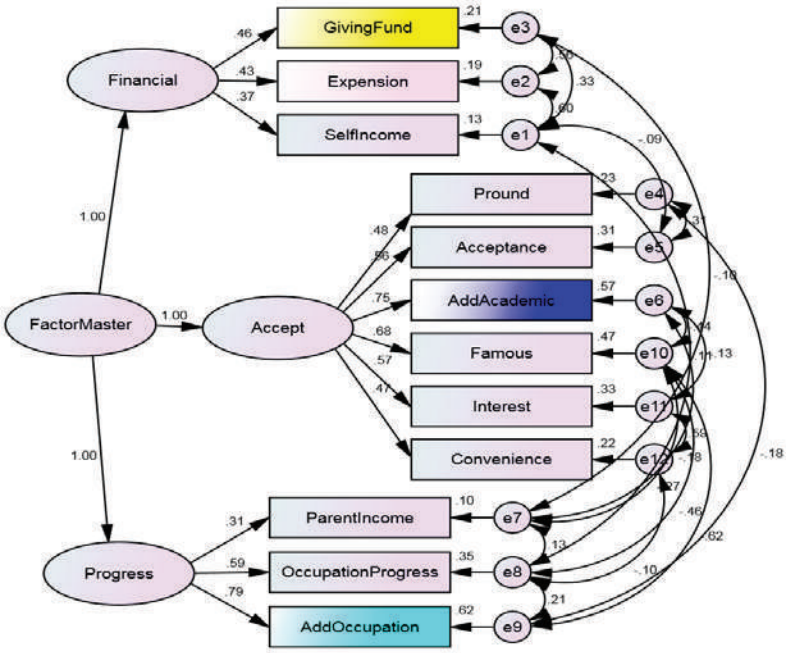


Figure-1: Diagram of the 2nd CFA of economic, social, and institutional factors that influence the decision to study in the MPH in PHIRD

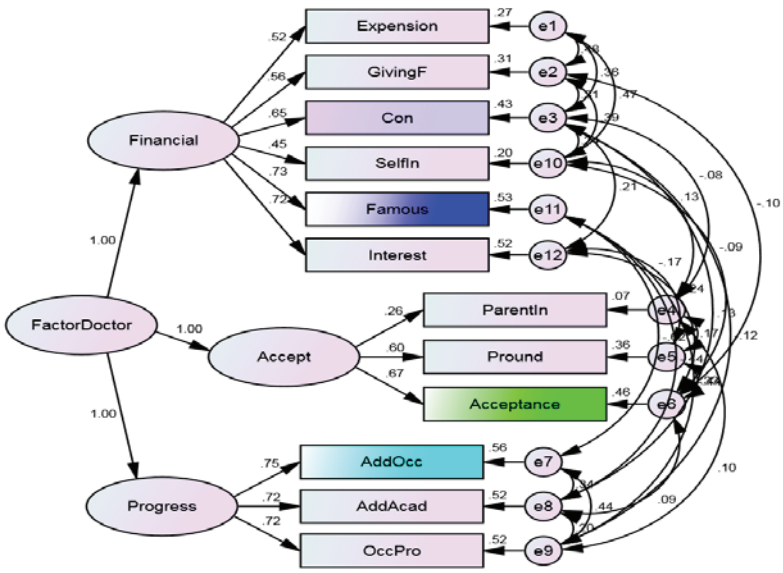


Figure-2: The 2nd CFA of the economic, social, and institutional factors that influence the decision to study in the PhD in PHIRD

3. Results of the Composition Analysis of the MPH and PhD in PHIRD

3.1. Results of the EFA found a KMO or MSA equal to 0.955 and 0.938, which are greater than 0.80, very good level, according to the criteria of Kim and Mueller[7] and the results of Bartlett's Test of Sphericity[8] found that the variables were statistically significant at the level of 0.001, could be used to analyze the components. The results of component extraction of each variable used in analyzing, A total of 20 numbers, between 0.345 - 0.712 and 0.583 - 0.838 are medium-high and have Cronbach's Alpha = 0.952 and 0.977. The result of variable arrangement into the structure, adjusting structure, and name the elements with Orthogonal Rotation found that all 20 variables used in the analysis can be grouped into 2 components with eigenvalues greater than 1.00, indicating that each element can explain the variance of all 20 variables more than 1.00. Two elements could explain the variance of the total of 20 variables were 58.697% and 75.739 %. When considering to put each variable into one element, only that variable has the highest weight (Factor Loading) found that 2 elements could be classified into 20 elements, with each element consisting of variables 3-17 characters, each variable that has been arranged in the composition weights elements from 0.514 to 0.843 and 0.623 to 0.853. The element 2 elements have been named so that they can be interpreted clearly as detailed in Table 2.

Table-2: Restructuring the Structure of the MPH and PhD in PHIRD

Side/ Core Component	Sub-Component	
MPH		
1) Professional	1.	Seminar in Public Health Management following the sufficiency economy (Seminar)
	2.	Integrated research methods in public health (Research)
	3.	Public health development theory (Theory)
	4.	Theory and design of public health innovations (Design)
	5.	Seminar on research and innovation in public health (SeminarResearch)
	6.	Public Health Thinking Strategy (Strategy)
	7.	Public health supervision strategies (Supervisor)
	8.	Information technology for the development of public health innovations (Technology)
	9.	Public health analysis and evaluation (Evaluation)
	10.	Techniques for creating public health gauges (Measurement)
	11.	Public health measurement and evaluation innovation (Innovation)
	12.	Qualitative research in public health (Qualitative)
	13.	Quantitative research in public health (Quantitative)
	14.	Knowledge management and learning organization (KM)
	15.	Development of public health innovations (DevelopmentInnovation)
	16.	Action research on innovation in public health (ActionResearch)
	17.	Seminar in Information Technology in Public Health (Information)

Cont... **Table-2: Restructuring the Structure of the MPH and PhD in PHIRD**

2) Potential	18. English for Master (Eng)
	19. Thesis (Thesis)
	20. Multivariate statistical analysis (multilevel) (Statistics)
PhD	
1) Professional	1. Advanced statistics in public health (AdvancedStat)
	2. Advanced research methods in public health (AdvancedR)
	3. Theory and design of advanced innovations in public health (AdvancedDesign)
	4. Seminar in Advanced Research and Innovation in Public Health (AdvancedSeminar)
	5. Seminar in Advanced Information Technology in Public Health (AdvancedTechSem)
	6. Advanced analysis and assessment in public health (AdvancedEval)
	7. Innovative measurement and evaluation in public health (AdvancedTechMea)
	8. Techniques for creating advanced metrics in public health (AdvancedMea)
	9. Advanced qualitative research in public health (AdvancedQualitative)
	10. Advanced quantitative research in public health (AdvancedQuantitative)
	11. Advanced integrated research in public health (AdvancedMMethods)
	12. Advanced Health Economics Analysis (AdvancedEconomic)
	13. Development of future innovations (InnovationFuture)
	14. Dissertation (Dissertation)
	15. Development of advanced innovations in public health (DevelopmentAdvInno)
	16. Action research, Advanced Innovation in Public Health (AdvancedActResearch)
	17. Advanced information technology for the development of public health innovations (AdvancedInformation)
3) Potential	18. Seminar in Public Health Management following the guidelines of the World Health Organization (AdvancedSeWHO)
	19. English for Doctorate Degree (EngDissertation)
	20. Research and development of public health innovations at the international level (RandD)

3.2. Results of the 2nd CFA showed that the 20 elements are two sub-components. Overall the field of research and innovation in public health is real as detailed in Figure 3-4 as follows.

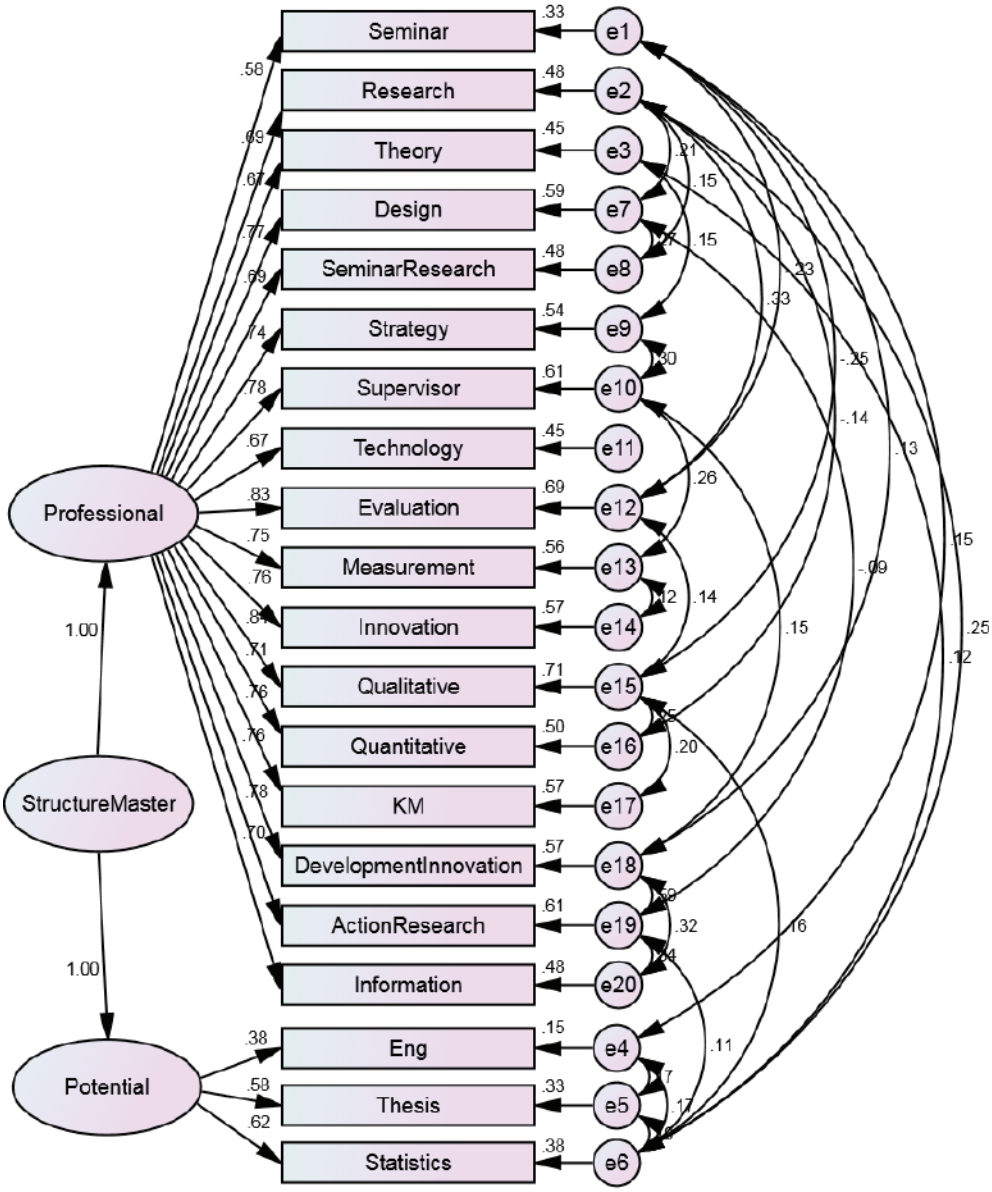


Figure-3: Diagram of the 2nd CFA of the components of the structure of the MPH in PHIRD

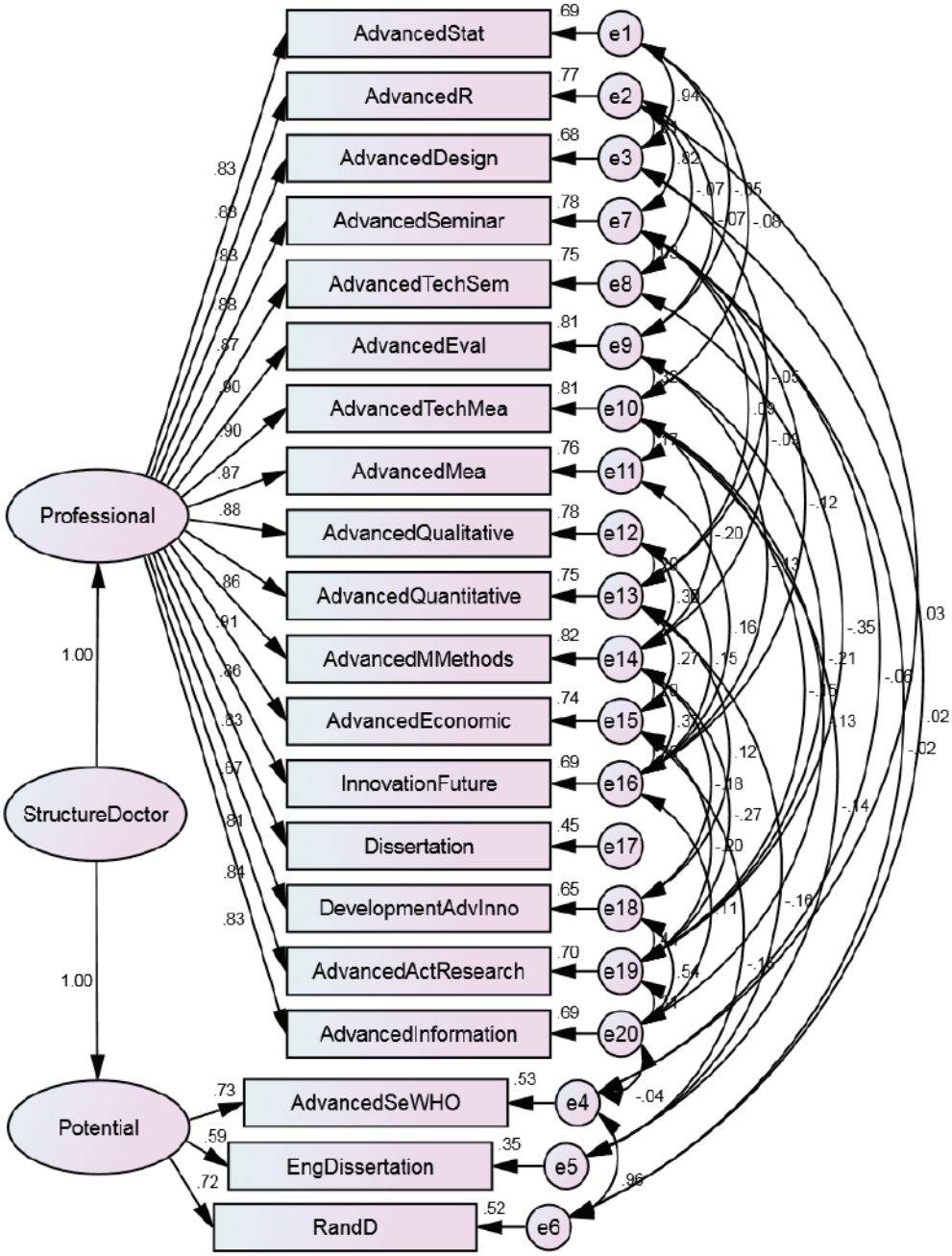


Figure-4: Diagram of the 2nd CFA of the structure of the PhD in PHIRD

Discussions

Needs of 4th-year students and graduates in health sciences regarding curriculum MPH and PhD in PHIRD. The expected level was at a high level (= 4.31/5 and 4.33/5), the $PNI_{Modified}$ of MPH = 3.31/5, and $PNI_{Modified}$ of PhD = 3.33/5.

1. 2. 3. 3.1. The analysis of the factors found that all 12 studied variables can be grouped into 3 components, with each element having a value of more than 1.00 and all the components could explain the variance of all variables 59.733 percent and 66.682%. The weight of each variable have valued from 0.569 to 0.862 and 0.556 to 0.834 showed that the variables analyzed can be used to study the factors and the decision to study further because these variables are documented and research supported by the researcher who has studied the related documents and research and therefore has important variables that are consistent and cover the large structure of the three aspects, namely

finance, acceptance side, and progress and then to check the structural accuracy again by analyzing the 2nd CFA and found that the factors consists of 3 main components and 12 elements in which the model is consistent with the empirical data were good, with a statistical measure of harmonization, passing all good criteria, have good structural validity and can be used to study the factors and found that the model has good accuracy because the variables and components used in the analysis have relevant documents and research and have been through the 2nd CFA. The structures are good which should be suitable for importing data in the design of programs.

The analysis of the structure found that the 20 studied variables can be grouped into 2 components, each element has a greater than 1.00 value, and all the components could explain 58.697% and 75.739% of the variance of all variables. The weight of each variable have valued from 0.514 to 0.843 and 0.623 to 0.853 showed that the variable analyzed can be used to study because these variables are documented and supported by the researcher and the relevant researches and documents have important variables that are consistent and cover a large structure of the two aspects are professional and potential, and then to check the structural accuracy again by analyzing the second confirmatory element. The 2nd CFA of this format showed that the structure consists of 2 main components and 20 sub-elements that the model consistent with empirical data, were satisfied with the statistical value of harmonizing through all criteria and showed that the 2nd CFA developed has good structural accuracy and can be used to study the structures because the variables and components used in the analysis have relevant documents and research and have passed the 2nd CFA and found that structural precision is in good shape which should be suitable for importing data into the design of programs and the results from this research resulting in participation and coordination among graduate students, public health officers, and the health network. In the development of the programs as input for curriculum development and course opening to produce public health innovations that can be used to solve health problems

In this study found that needed in the further studies. Therefore, the person responsible for the program and the Faculty of Public Health and Allied Health Sciences of Royal Institute should focus on curriculum design and course opening and found that the components have good structural accuracy because the model is consistent with the empirical data with good statistics, with the statistical measurements of conformity passing almost all good criteria in all 4 cases except the curriculum structure of PhD at $\chi 2$ with statistical significance p-value = 0.001[8], CMIN / DF> 2 (2.257)[9], AGFI <0.90 (0.853)[10] and RMSEA> 0.05 (0.064)[11,12]. The desired composition of the model must be $\chi 2$ with no statistically significant p-value> 0.05[8] CMIN. / DF <2[9,13] GFI values> 0.90, AGFI values> 0.90[10] NFI values> 0.90[11] CFI values> 0.90[11] RMR <0.05[14,15] and RMSEA <0.05[11,12] by the importance of components of financial, acceptance and the progress and components of professionalism and potential are equally important, 4 models of these elements appropriate in designing by experimenting and can be used more completely**.** Suggestions for further research, the expert's perspective on the curriculum structure should be studied and other issues related to developing and requesting to open a course to make the course more complete to be consistent with the context and social conditions allowing graduates to have the capacity to produce public health innovations**.**

Ethical Clearance- Taken from Sirindhorn College of Public Health Phitsanulok committee

Source of Funding- Self

Conflict of Interest- Nil

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Hyperbaric Oxygen Exposure Reduces ICAM-1 And HIF-1α Expression in Brain Endothelial Cells from Experimental Cerebral Malaria Mice

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Abstract

This study aimed to reveal the role of ICAM-1 and HIF-1α in brain endothelial cell of cerebral malaria mice model after exposure to 2.4 absolute atmospheres (ATA) hyperbaric oxygen (HBO). Thirty-nine C57BL/6 mice were divided into three groups: control negative (normal mice without any exposure), control positive (*Plasmodium berghei* ANKA [PbA] infection without HBO exposure), and treatment (PbA infection and exposed to HBO for 10 sessions after the parasite grew). Parasitemia and clinical symptoms were observed every day. Brain tissues were isolated on day 13 post-infection for histopathological and immunohistochemical examination (observed at 400x magnification in 10 visual fields). HBO decreased HIF-1α and ICAM-1 expression in endothelial cells. There was a moderate correlation between HIF-1α and ICAM-1 expression. Ten HBO sessions prevented cerebral malaria, as denoted by the decreased expression of ICAM-1 and HIF-1α in brain vascular endothelial cells from the experimental mice.

Keywords: *Plasmodium berghei ANKA, hyperbaric oxygen/HBO, ICAM-1, HIF-1α.*

Introduction

Malaria is an infectious disease caused by the parasite *Plasmodium* sp. Based on a 2017 World Health Organization (WHO) report, there were 219 million malaria cases that caused 435,000 deaths worldwide[1]. Millions of malaria cases are caused by *P. falciparum*, and approximately 1% of cases will develop into cerebral malaria. Even with adequate antimalarial therapy and intensive therapy, malaria mortality rates still reach 15-20%, and there are long-term neurological deficits in patients who have been declared cured[2]. Cerebral malaria is characterized by neurological abnormalities, seizures, decreased consciousness, and coma. The pathogenesis of this disease is still unclear. However, previous studies showed that one of the causes of brain damage is brain vascular endothelial damage triggered by hypoxic conditions due to vascular obstruction, sequestration of parasitized red blood cells (pRBCs) using vascular endothelial receptors, and excessive inflammation[3].

The main regulator that participates in cell adaptation and survival in hypoxic conditions is hypoxia-inducible factor 1α (HIF-1α)[4]. There is cross-talk between hypoxia and inflammation, where HIF-1α activation becomes a signaling pathway that initiates transcription factors, namely nuclear factor kappa B (NF-κB), which then increases inflammation. Hypoxic conditions during malaria infection will increase HIF-1α expression, leading to NF-κB activation and increased intercellular adhesion molecule-1 (ICAM-1) expression[5]. Various studies suggest that ICAM-1 is the main endothelial receptor in the brain, and it plays a crucial role in the endothelial sequestration process by binding to *P.*

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falciparum erythrocyte membrane protein-1 (PfEMP-1), which is expressed by pRBCs[6].

HBO therapy is the systemic exposure to 100% oxygen in a closed room with a pressure greater than 1 absolute atmosphere[7]. Research on the effect of HBO on malaria infection is very limited. HBO exposure directly increases dissolved oxygen in the blood plasma, a change that can reduce HIF-1α[8] and modulate the inflammatory response, namely by reducing ICAM-1 expression[5]. The purpose of this study was to examine the effect of exposure to 10 sessions of 2.4 ATA and 100% O_2 HBO three times for 30 min on the expression of ICAM-1 and HIF-1α in C57BL/6 mice infected with *P. berghei* ANKA (PbA).

Materials and Methods

This study used a randomized post-test-only control group design. This study was performed at the Clinical Parasitology Laboratory, Faculty of Medicine, Universitas Brawijaya (for infecting the experimental mice), the Hyperbaric Laboratory, Faculty of Medicine, the University of Hang Tuah (for the HBO treatment and euthanasia), and the Anatomic Pathology Department, Faculty of Medicine, Universitas Airlangga (for hematoxylin & eosin [H&E] and immunohistochemistry [IHC] preparation, staining, and observation). The experimental subjects were 39 pathogen-free 7-to-10-week-old C57BL/6 female mice. They were divided into three groups: Group 1 (G1) or control negative (mice were not infected with PbA and did not receive HBO treatment), Group 2 (G2) or control positive (mice were infected with PbA without receiving HBO exposure), and Group 3 (G3) or treatment (mice were infected with PbA and subjected to HBO exposure). PbA isolates were obtained from the Clinical Parasitology Laboratory, Faculty of Medicine, Universitas Brawijaya.

The experimental mice (15-20 g) were obtained from Indoanilab, Bogor, Indonesia. The adaptation and acclimation phases were performed for 1 week in a room that was sterilized one day before conducting the research using ultraviolet light for 2 h. The mice were given sterilized mineral water and food pellets *ad libitum*. The study was approved by the Ethics Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya (No.2KE.070.04.2018).

HBO exposure

G3 mice were subjected to HBO exposure with a pressure of 2.4 ATA and 100% O_2 three times for 30 min with a 5 min resting phase between each exposure[9]. HBO exposure was performed in an animal chamber every morning before examination of parasitemia and the clinical manifestations of cerebral malaria. The temperature during HBO exposure was adjusted to 25ºC. Each G3 mouse was subjected to 10 exposure sessions, beginning on day 3 post-infection (when parasitemia was apparent) until day 12.

Parasitemia examination

Observation of the parasitemia level was performed serially from day 1 to day 12 post-infection by microscopic examination of a blood smear aseptically taken from the tail and stained with Giemsa. Observations and calculations were repeated twice each day and performed by two different observers. The percentage of parasitemia was calculated using the following formula: pRBC/1000 RBC x 100%[10].

Brain tissue Immunohistochemical Stainning

Brain tissues were incised with a 4-µm-thick incision, with the direction of the incision in the coronal field, and stained with hematoxylin-eosin[12]. The immunohistochemical staining process commenced by washing slides with phosphate-buffered saline (PBS, pH 7.4) for 5 min, 3% H_2O_2 for 20 min (endogenous peroxide block), and PBS 3 x 5 min. Non-specific protein binding was blocked using 5% fetal bovine serum (FBS) and 0.25% Triton X-100, and sections were washed with PBS for 5 min. Sections were incubated with monoclonal HIF-1a antibody (Santa Cruz Biotechnology, catalog number: sc-53546) and ICAM-1 (Santa Cruz Biotechnology, catalog number: sc-107) for 60 min, and then washed with PBS 3 x 5 min. Sections were incubated with the chromogen diaminobenzidine (DMB) at 25 °C for 10 min, followed by H&E solution for 3 min, and finally rinsed in running water. HIF-1a and ICAM-1 expression in the brain vascular endothelium was observed in the cerebrum and cerebellum at 400x magnification.

Statistical Analysis

Average values and standard deviations were

determined for all data, and normality and homogeneity were examined using the Shapiro-Wilk and Levene tests, respectively. Multivariate analysis of variance (MANOVA), with post-hoc analysis, was used to determine differences between groups (for normally distributed data). Pearson's correlation tests were conducted to determine the correlation between two variables of normally distributed data. Data that were not normally distributed or homogeneous were analyzed using the Kruskal-Wallis or Mann-Whitney U test to determine differences among groups. All *p*-values <0.05 were considered to be significant, and there was a correlation between the studied variables.

Results and Discussion

Parasitemia appeared on day 2 post-PbA infection. The peak parasitemia level occurred on day 7 post-infection, and it decreased on days 8–12. This phenomenon is probably due to the increased pRBC circulation in the tissue due to the sequestration process or pRBC trapping in capillaries[14]. G3 mice, which received HBO treatment, showed negative parasitemia on days 10-12, while G2 (control positive mice) showed a positive parasitemia level until the last day of observation.

There was a significant difference in HIF-1a expression in the brain endothelial cells between G3 and G2 mice (p = 0.001), but there was no significant difference between G1 and G3 mice (p = 0.258; Figure 1). The average ICAM-1 expression in vascular brain endothelial cells in G3 mice was significantly lower than in G2 mice (p = 0.005). ICAM-1 expression in G3 brain was higher than in G1 brain, but this difference was not statistically significant (p = 0.059; Figure 2). Representative micrographs of HIF-1α and ICAM-1 staining appear in Figures 3 and 4, respectively. Correlation analysis showed a moderate correlation between HIF-1a and ICAM-1 expression in brain endothelial cells (r = 0.507; p = 0.001).

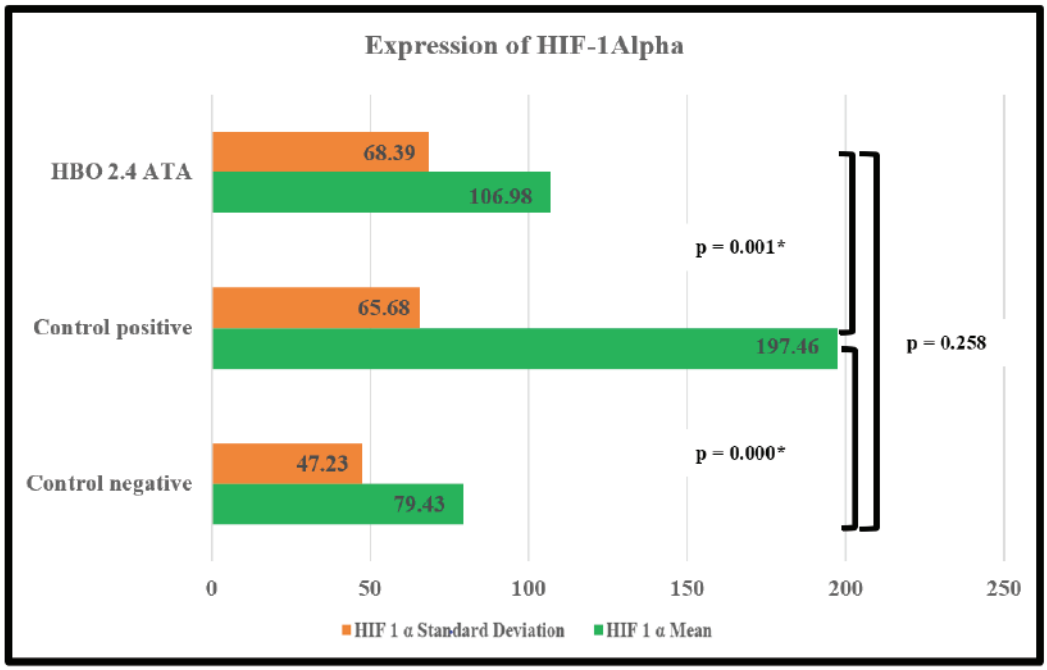


Figure 1. The avarage HIF-1α expression in the control negative (G1), control positive (G2), and HBO treatment (G3) groups. *MANOVA test and post-hoc least significant difference (LSD) test showed a significant difference; $p < 0.05$.

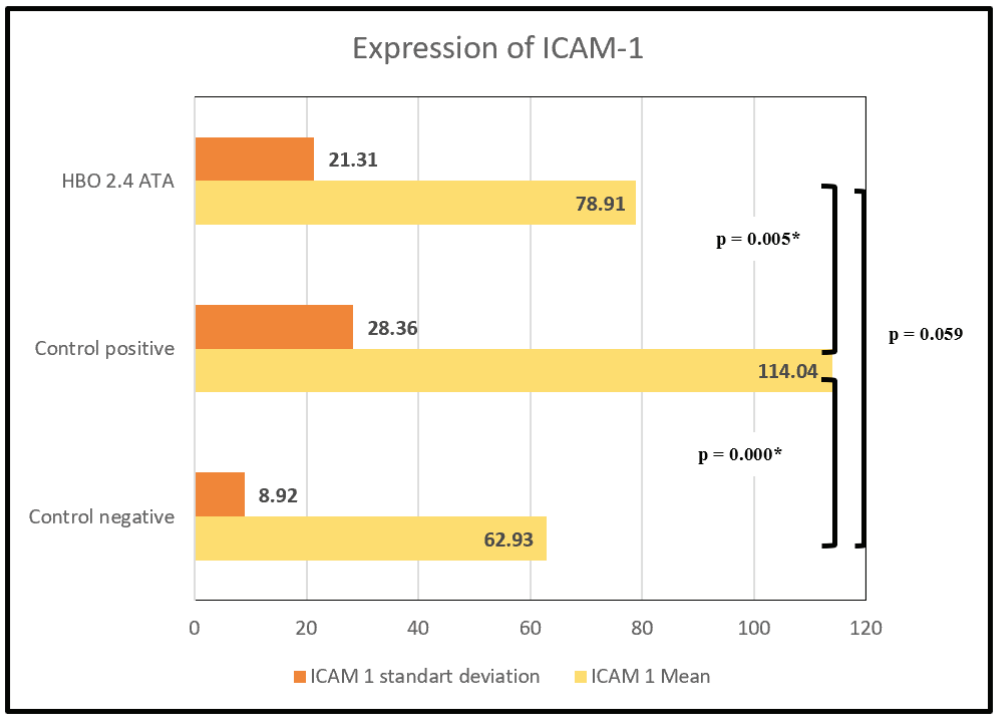


Figure 2. The avarage ICAM-1 expression in the control negative (G1), control positive (G2), and HBO treatment (G3) groups. *MANOVA test and post-hoc Games Howell test showed significant differences; $p < 0.05$.

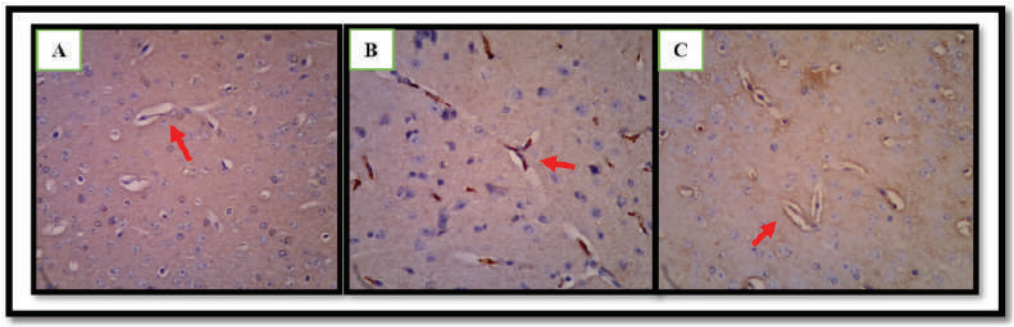


Figure 3. HIF-1α expression in the (A) control negative (G1), (B) control positive (G2), and (C) HBO treatment (G3) groups. 400x magnification.

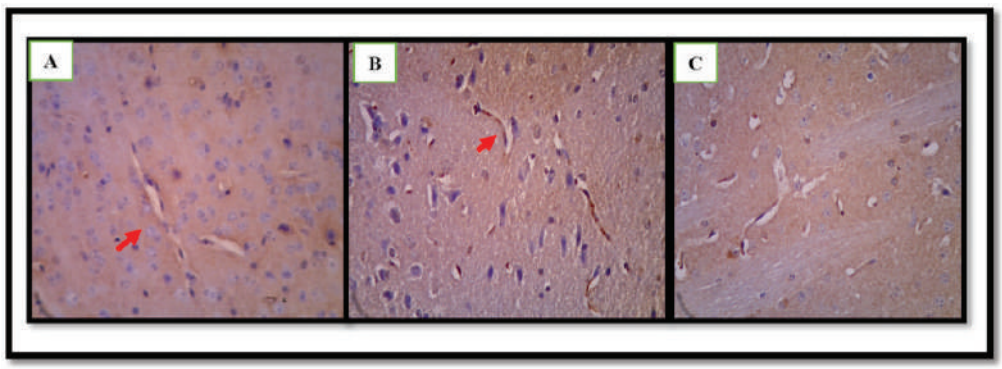


Figure 4. ICAM-1 expression in the (A) control negative (G1), (B) control positive (G2), and (C) HBO treatment (G3) groups. 400x magnification.

pRBC cytoadherence and sequestration play an important role in the pathogenesis of cerebral malaria in humans; both damage capillary blood vessel walls, inhibit blood flow to capillary blood vessels and cause hypoxia and dysfunction in some vital organs, especially the brain. Infected erythrocytes will express PfEMP-1 protein in knobs on the membrane surface with vascular endothelial receptors such as CD-36, ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), platelet endothelial cell adhesion molecule-1 (PECAM-1), E selectin, and thrombospondin. There are differences in the histopathological profile and cerebral malaria manifestations between human and mouse brains, where erythrocyte sequestration in vascular endothelium is relatively infrequent in mice. Nevertheless, in PbA-infected mice, leukocytes especially T lymphocyte cells accumulate in the vascular endothelium like what is observed in humans with cerebral malaria[15]. Various endothelial receptors that play a role in the process of sequestration of PRBC in humans are not all expressed in mice. ICAM-1 is considered the most important role in the pathogenesis of cerebral malaria[16]. High expression of ICAM-1 facilitate sequestration of T lymphocyte cells through LFA 1 integrin protein, causing obstruction and circulatory disorders in the tissues, especially the brain. ICAM-1 expression is enhanced when the body experiences inflammation[17]. ICAM-1 expression in G3 brain endothelial cells was significantly lower than in G2 ($p = 0.005$) and no different from G1 ($p=0.059$). This result showed that administration of HBO was able to regulate inflammatory response accompanied by decreased ICAM 1 expression.

Hypoxia in malaria, especially cerebral malaria, is caused by O_2 transport deficiency, a condition that leads to hemolysis, pRBC sequestration, and infiltration and activation of various immune cells that can obstruct the brain micro-vascularity[18]. Hypoxia occurs when the amount of oxygen is insufficient for cellular processes. HIF senses low oxygen concentrations; it is a transcription factor that serves as a key regulator in cell adaptation and survival in hypoxic conditions through increased angiogenesis and erythropoietin production and cell proliferation. On the other hand, high HIF-1α expression stimulates inflammatory activity through crosstalk with NF-κB. Indeed, elevated HIF-1α causes lung injury in severe malaria[4], so its expression must be suppressed to reduce excessive inflammatory processes and tissue damage. In this study, HIF-1α expression in G3 brain endothelial cells was significantly lower than in G2 brain ($p = 0.001$) and no different from G1 mice ($p = 0.258$). This finding indicates that HBO significantly reduced HIF-1α expression in brain vascular endothelial cells, likely because HBO above 1 ATA increases the O_2 concentration and solubility in blood plasma. The O_2 enters cells directly through diffusion, and so it is more quickly used by the cells compared to O_2 bound by hemoglobin. Increased tissue oxygenation will attenuate ubiquitous HIF-1α transcription via von Hippel Lindau protein, and so HIF-1α will be degraded in proteasomes[19].

Conclusion

In summary, our study showed a moderate correlation between HIF-1α and ICAM-1 expression in brain endothelial cells, between ICAM-1 expression and the histopathological profile, and between HIF-1α expression and the brain histopathological profile.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Ethical Approval: This study was approved by the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Correlation of Safe Benzene Duration (Hours/Day) and Blood Profile (Leukocytes, Hematocrit, Hemoglobin) in the Osowilangun Shoe Home Industry

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Abstract

Introduction: Benzene is a volatile organic solvent that easily enters the body when inhaled. Continuous exposure to benzene can cause interference with blood profiles such as leukocytes, hematocrit and hemoglobin. The Threshold Limit Value for benzene exposure is 8 hours /day, but the safe duration in each person is different in hours / day. One industry that uses benzene solvent is the home shoe industry in Osowilangun, Surabaya. The purpose of this study was to determine the relationship between the safe duration of benzene (t_E) in hours / day with blood profiles (leukocytes, hematocrit and hemoglobin).

Methods: This research was conducted at the Osowilangun shoe industry in Surabaya. This research is observational using bivariate analysis using Pearson correlation test. The population in this study amounted to 38 people and used accidental sampling method with 12 samples. The variables studied included the safe duration of toluene (hours/day) and blood profile (leukocytes, hematocrit and hemoglobin).

Result: The average safe duration (t_E) of workers in the Osiwilangun shoe industry in hours / days is 0.0418. The majority of blood profiles (leukocytes, hematocrit and hemoglobin) are normal. Through Pearson correlation, the safe duration (hours/day) of benzene are leukocyte levels (p = 0.933), hematocrit levels (p = 0.119) and hemoglobin levels (p = 0,000).

Conclusion : There was no significant relationship between safe duration (hours/day) of benzene with leukocyte and hematocrit levels. However, there was a significant relationship between safe duration (hours/day) of benzene and hemoglobin levels.

Key words: *Hemoglobin, Leukocyte, Hematocrit, Safe Duration, Benzene*

Introduction

One of the fast growing informal shoe industries in Surabaya is located in the Osowilangun area. A total of 40% of Indonesian shoe exports are sent to the United States market, while 33% are sent to Europe and the rest are exported to African countries, the Middle East and South America. Despite a decline in exports due to the economic crisis, shoe production remains one of the main industries in Indonesia[1]. Every year there are more than 250 million accidents at work and more than 160 million workers become sick because of hazards at

work. In addition, 1.2 million workers died as a result of accidents and illness at work[2]. In the process of making shoes, there are several stages in the shoe production process. One of the steps in the shoe making process is the process of installing the upper part of the shoe and the bottom of the shoe using glue. The glue used contains hazardous chemicals that can affect health. One of the dangerous chemicals in glue is benzene.

Benzene is an organic solvent that is very volatile and is toxic. The impact of chronic benzene exposure is damage to the hematopoiesis system in the bone marrow. The impact of this bone marrow damage is a progressive decrease in the number of blood cell elements[3]. The results of research that states that there is a decrease in hematological parameters (total white blood cells, red blood cells, platelets, and hematocrit) during workers exposed to benzene that exceeds normal limits[4]. China, the largest shoe manufacturer in the world, reported that there were health problems due to benzene exposure. This study shows that workers exposed to benzene were more at risk of death from leukemia with a relative risk of 2.3 compared to those who are not exposed to benzene[5].

The chemicals that are in the glue during the shoe-making process are a risk hazard. In the process there is exposure to organic solvent vapor contained in the glue and is very likely to have an impact on health if inhaled (Lu, 2006). The path of exposure can show differences in the way to enter the body. The benzene exposure pathway can be through the respiratory tract, digestive tract and through the skin. Benzene entering through the respiratory tract and skin is a major concern with several exposure scenarios[6]. Exposure to benzene with low concentrations and in a long time continuously can cause interference with the formation of blood cells such as decreased red blood cells, white blood, platelets which can cause blood cancer (leukemia). Organic solvent vapors that enter the human body can cause a variety of reactions, such as mild irritation, addiction, kidney disorders, pulmonary odema reactions to disorders of the central nervous system[7]. Exposure to benzene in the body can result in damage to the bone marrow so as to make the production of blood cells disrupted, including disrupting the function of hemoglobin in the body, thus hemoglobin level in the blood will decrease[8].

To see the high content of organic solvents in the form of benzene contained in the glue used, an analysis of benzene exposure to blood profiles is needed. Benzene levels in the blood can be used to assess low exposure to benzene as a sensitive method. However, this method also has technical constraints i.e reduced levels due to volatile benzene and contamination in sampling. A trial conducted by Brugnone et al (1992) the half-life of benzene in the blood is estimated to be 8 hours[9]. The amount of exposure allowed by the NIOSH (National Institute for Occupational Health and Safety) for 8 working hours is only 0.1 ppm for benzene. In Indonesia the Minister of Health Regulation No. 70 of 2016 concerning the Standards for Industrial Health Environmental Requirements stipulates that the threshold value of benzene in the air is 0.5 ppm[10].

Materials and Methods

This is an observational study with no special treatment given to respondents. This research was conducted at the shoe home industry in Osowilangun, Surabaya, which uses benzene as a solvent in its production process. The population in this study were all 38 workers exposed to benzene in the home shoe industry in Osowilangun, Surabaya. The research sample was taken using the accidental sampling method for 12 respondents. The variables studied in this study were safe duration (t_E) and blood profile (hemoglobin, hematocrit, leukocytes). Analysis of the data used is bivariate analysis using Pearson correlation test. To find out the safe duration in hours per day of benzene exposure in certain concentrations, the following formula is used[1].

$$t_E\ (jam/hari) = \frac{W_b \times t_{avg} \times \mathrm{RfC}}{\mathrm{C} \times \mathrm{R} \times f_E \times D_t}$$

t_E = safe daily exposure time (safe duration (hours / day))

W_b = weight (kg)

T_{avg} = average time period (30 years × 365 day/year)

RfC= benzene reference concentration = 0,03 mg/ m^3

C = concentration of risk agent (mg/m3)

R = concentration of risk agent (mg / m3)

f_E = annual exposure frequency (day/year)

D_t = duration of exposure (years)

The safe duration of benzene is obtained through calculations proposed by Tualeka (2015). Data on the characteristics of respondents such as age, sex and education level were taken through a questionnaire. The concentration of benzene in the air was measured using the NIOSH 1501 method with the gas chromatography technique carried out by the Work Safety and Health Technical Implementation Unit (UPTK3) Surabaya. Blood samples are taken by doctors who are experts in their fields and then analyzed in the Nutrition Laboratory of Airlangga University.

Result

Distribution of Characteristics of Respondents to Workers Exposed to Benzene

Respondent characteristics include age, sex, level of education, and work area. The following is a distribution table of the characteristics of workers exposed to Benzene in shoe home industry.

Table 1. Distribution of Characteristics of Workers Exposed to Benzene

Characteristics of Workers	Frequency	Percentage
Age		
16-25	1	0,083%
26-35	0	0%
36-45	4	0,33%
46-55	3	0,25%
56-65	4	0,33%
Gender		
Male	6	50%
Female	6	50%
Level of Education		
Primary	1	0,083%
Junior High	5	0,42%
Senior High	6	50%

Source: primary data

Based on table 1, most industrial workers are aged 36-45 years (0.33%) with male and female sexes having the same percentage and level of education and most are high school (50%).

Safe Duration (t_E)

Table 2. Distribution of Safe Duration of Benzene Exposed Workers

No. Res	Wb (kg)	Tavg	RfC (mg/kg/hari)	C (ppm)	C (mg/m3)	R (m3/hour)	fE (day/year)	Dt (year)	tE safe (hour/day)
1.	60	10950	0.0003	0.4	0.000308	0.62	364	25	0.0283
2.	100	10950	0.0002	0.2	0.000219	0.73	364	15	0.0943
3.	55	10950	0.0003	19.40	0.000326	0.60	312	10	0.0017
4.	69	10950	0.0002	19.40	0.000282	0.65	312	25	0.0007
5.	41	10950	0.0003	8.50	0.000390	0.53	312	22	0.0017
6.	46	10950	0.0003	0.06	0.000364	0.56	364	30	0.1572
7.	84	10950	0.0002	53.80	0.000247	0.69	364	20	0.0002
8.	53	10950	0.0003	0.40	0.000334	0.59	312	25	0.033
9.	70	10950	0.0002	8.50	0.000279	0.65	364	7	0.0047
10.	65	10950	0.0002	0.20	0.000308	0.62	364	30	0.0471
11.	75	10950	0.0003	0.20	0.000293	0.63	360	17	0.1165
12.	55	10950	0.0003	0.20	0.000326	0.60	364	40	0.0177

Based on table the highest concentration of benzene in the air is 53.8 ppm and the lowest is 0.06 ppm with an average value of 9.2 ppm.

$$t_E\ (jam/hari) = \frac{W_b \times t_{avg} \times \text{RfC}}{\text{C} \times \text{R} \times f_E \times D_t}$$ the safe duration in hours / day using the following formula.

Based on the calculation of safe duration in hours/day the highest is 0.1572 hours and the lowest is 0.0002 hours. The average safe duration in hours/day is 0.0418.

Blood Profile

Table 3. Blood Profile of Workers Exposed to Benzene

Blood Profile	Frequency	Percent	Average
Leukocytes (103/ µL)			
Abnormal	1	0,083%	8425
Normal	11	0,92%	
Hematocrit (%)			
Abnormal	2	0,17%	41.76
Normal	10	0,83%	
Hemoglobin (gr/dL)			
Abnormal	1	0,083%	15.81
Normal	11	0,92%	

The blood profile examined in this study included the number of leukocytes, hemoglobin and hematocrit. This is then compared with the existing TLV and is categorized into 2 types ie not normal and normal. Normal leukocyte counts generally range from 3200 - 10,000 / mm3 or 3.2 - 10.0 x 109 / L. The normal amount of hemoglobin in women is 12-16 gr/dL and in men 13-18 gr / dL. For the normal amount of hematocrit ranges from 32-36 gr/ dL[12]. Based on the table, the majority of workers have leukocyte, hemoglobin and hematocrit levels under normal conditions. In sum, the average value of workers blood profiles is in normal category.

Correlation of Safe Benzene Duration and Blood Profile

Table 4. Statistical Test Results of Safe Benzene Duration and Blood Profile

Variables	P-Value	Correlation coefficient	N
tE Benzene	0,933	-0,027	12
Leukocytes			
tE Benzene	0,119	-0,474	12
Hematocrit			
tE Benzene	0,000	-0,975	12
Hemoglobin			

Based on the statistical test results in table 6, there is no significant relationship between the safe duration (t_E) of benzene and leukocytes, hematocrit in workers in the home shoe industry with a p value> 0.05. Whereas in the benzene safe duration (t_E) statistical test with hemoglobin there was a significant relationship in workers in the home shoe industry with a p value <0.05.

Discussion

Based on the results of the study, the highest concentration of benzene in the air was 53.8 ppm and the lowest was 0.06 ppm with an average value of 9.2 ppm. Regulation of the Minister of Health of the Republic of Indonesia Number 70 Year 2016 regarding the Standards for Health Standards for the Industry Work Environment states that benzene has a TLV TWA of 0.5 ppm[16]. Based on the measurement results there are 5 measurement points that have benzene levels above TLV that exceed 0.5 ppm. The results of the average measurement of the concentration of benzene are higher when compared to other studies which state that the average concentration of benzene in the breathing zone of respondents (sol workers) within the safe limit below TLV is 0.238 ppm[13]. Other research also shows that the measurement results of benzene concentration in the Semarang City X Printing Industry are below the quality standard with the average concentration of inhaled benzene being 0.422 mg / m3 or 0.13 ppm[14]. This is still below average as compared to research conducted in the Home Shoe Industry.

Based on the results of the study there was no significant relationship between the safe duration (t_E) of benzene, the number of erythrocytes and hematocrit in workers with a p value> 0.05. However, there was a significant relationship between safe duration (t_E) of benzene and hemoglobin levels in workers with a p value <0.05. The results also showed that there was no dose effect on response to most of the hematologic parameters examined. Another study in the printing industry X in Semarang city concluded that there was no significant relationship between benzene exposure and levels of erythrocytes, leukocytes, platelets, hematocrit, MCH and MCHC among workers with a value of p> 0.05. This is possible because the level of benzene exposure experienced by workers is in a mild level, with an average benzene level of 0.132 ppm (SD ± 0.1742) and an average duration of exposure for 7.5 years (SD ± 6.642)[15]. Other related research is the level of benzene with acute exposure in humans with a duration of 1 day with a time of 5-10 minutes with exposure up to 20,000 ppm can cause death, for a duration of 1-21 days with a time of 2.5–8 hours / day with exposure to 60 ppm can cause respiratory system disorders such as mucus, membrane irritation and dyspnea), haematological system disorders such as leukopenia, anemia, thrombocytopenia, increased MCV, and disorders of the skin system[16]. When an increase in the safe duration of benzene (hours / day) there is also an increase in blood profile levels. However, there are several aspects that can influence the increase in blood profile when there is an increase in exposure to safe duration of benzene such as equipping workers with PPE (Personal Protective Equipment), nutritious supplementary feeding, adequate ventilation, smoking habits, and personal hygiene[17]. This is also in line with other studies which state that there are other factors such as length of work, nutritional status and others that cause no relationship with blood profile[18]. According to Minister of Health Regulation R1 No. 1077/ MENKES/PER/ 2011 concerning guidelines for indoor air sanitation, good ventilation is to meet the criteria for ventilation area> 10% of workplace floor area[19].

Conclusion

There are 5 measurement points with benzene content above TLV (0.5 ppm) and there are 7 measurement points with benzene content below TLV. The results of the mean benzene levels are above the TLV that has been set by ACGIH. A total of 41.67% of workers are in locations with Benzene concentrations above the threshold value. The majority of workers had leukocyte, hematocrit and hemoglobin levels in the normal category. There was no significant relationship between benzene safe duration (t_E) with leukocyte and hematocrit levels in workers with a p value> 0.05. There was a significant relationship between safe duration (t_E) of benzene and hemoglobin levels in workers with a p value <0.05.

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A Five Years Retrospective Study of Incidental Findings in Medico-Legal Autopsies

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Abstract

Aim of the study: The aim of the study is to find out the spectrum of incidental pathological lesions in the deceased during medico legal autopsy which are not directly related to the cause of death. This study emphasizes the incidence of various incidental lesions which otherwise would have been unnoticed during a person's life.

Materials and method: A five years retrospective study was conducted from 2012 to 2017 at the department of Forensic medicine Assam Medical College and hospital,. The organs where the pathological lesions were detected mostly comprise of lungs, liver, kidneys and heart. The sections from organs were preserved in 10% formalin and prepared for histopathological examination. The gross and histopathological findings were noted.

Results: 75 cases of cirrhosis of liver, 62 cases TB lung,93 cases of coronary artery disease and 18 cases of cystic lesions of kidney were detected where the cause of death is not directly related with these lesions.

Key words: *Incidental findings, autopsy, histopathology, cause of death, cirrhosis of liver, coronary artery disease, TB lung, cystic lesions of kidney*

Introduction

A fistful of pathological findings unrelated to the cause of death are noticed in routine gross and histopathological examination of medico legal autopsies. These findings will be proved to be of great academic value and will help us to find out the relation between the disease and injury. As we know that an underlying disease which was unnoticed or undiagnosed during the life time of an individual may have contributory factor or may have precipitating fraction towards the cause of death [1].These pathological findings may serve as a gain for the defense in the court of law. The medicolegal autopsy provides an opportunity for studying not only medically diagnosed and treated lesions but also the natural evolution of untreated disease [2]. Autopsy also helps in the diagnosis of undiagnosed or misdiagnosed pathological lesions irrespective of underlying cause of death, which may or may not be related to malignancy [3]. But many incidental findings have been highlighted on histopathological examinations which have proven to be great learning tools for the pathologists as well as the forensic medicine expert.[4]

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Materials and Method

Total 5449 numbers of medico legal autopsies were performed during that period of five years from 1st June 2012 to 31st May 2017. These cases were brought to the

mortuary of Department of Forensic Medicine, Assam Medical College & Hospital, Dibrugarh by investigating authority. Out of these cases histopathological examination was done for the cases where the autopsy surgeon have noticed gross pathological leisons in the organs viz. liver, lungs, heart and kidney. Total number of cases where histopathological examination done was 291.Out of these 291 cases ,in 75 cases cirrhosis of liver, in 62 cases TB lung, in 93 cases of coronary artery disease and in 18 cases of cystic lesions of kidney were detected i.e. positive histopathological finding was found in248 (75+62+93+18) number of cases. Out of these 248 cases, in 182 cases the cause of death is not directly related with these pathological lesions. In the present study highly decomposed bodies were not included. The present study has been undertaken on the basis of external and internal postmortem examinations with special reference to gross and histopathological examination of lungs, liver,heart and kidneys. In the cases where death of the victim occurred in hospital, the treatment records available were also studied. A detailed, careful and thorough postmortem examination was done on every case.

Methods

After gross findings are noted, the tissues from the viscera are preserved in 10% formalin and tissue blocks were prepared in histopathological laboratory of Department of Forensic Medicine, Assam Medical College & Hospital, Dibrugarh.

Table1. Table showing the number of autopsies with positive Histopathological findings:

Total number of autopsies	No of autopsies where Histopathological examination done	No of autopsies with positive Histopathological findings
5449	291	248

Table 2: Table showing type of histopathological lesion:

No of autopsies with positive Histopathological findings	No of autopsies with cirrhosis of liver	No of autopsies with coronary heart disease	No of autopsies with TB lungs	No of autopsies with cystic lesions of kidney
248	75	93	62	18

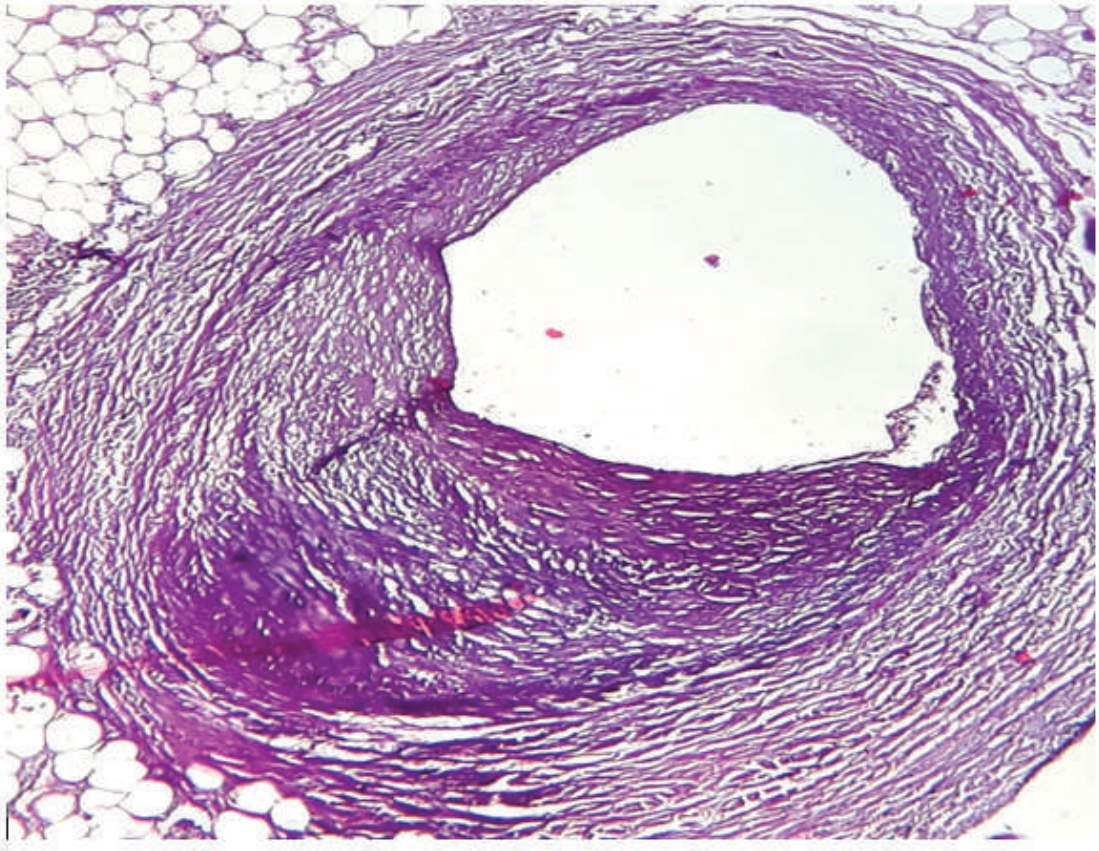
Table 3: Table showing number autopsies with incidental findings(No of autopsies with positive Histopathological findings where cause of death is not directly related to the pathological lesion)**:**

No of autopsies with positive Histopathological findings	No of autopsies with incidental findings
248	182

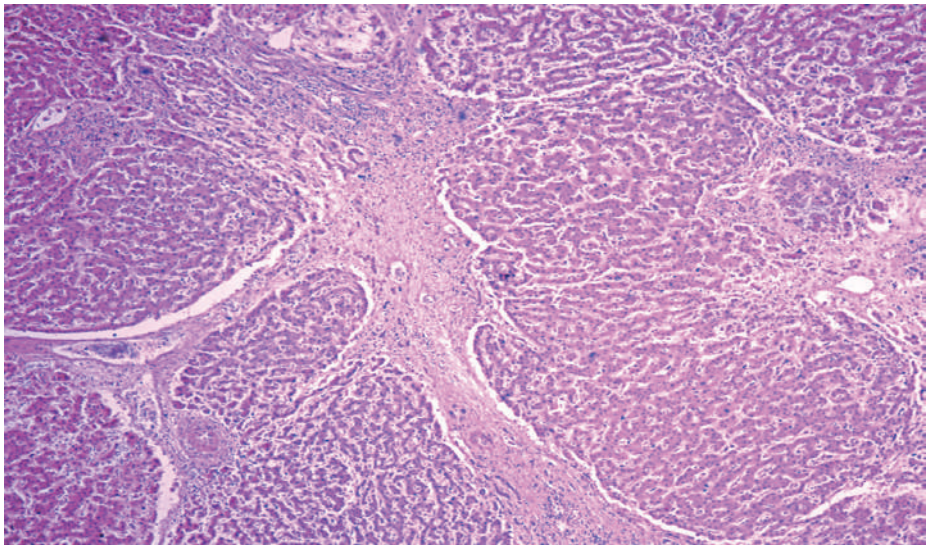
Table 4: Table showing the autopsies having different of causes of death with incidental findings:

Total Number of autopsies with incidental findings	Coma due to Head injury	Coma due Cerebrovascular accident	Asphyxial death (hanging)	Asphyxial death(drowning)	Hemorrhage and shock	Burn	Poisoning
182	38	14	26	7	27	40	30

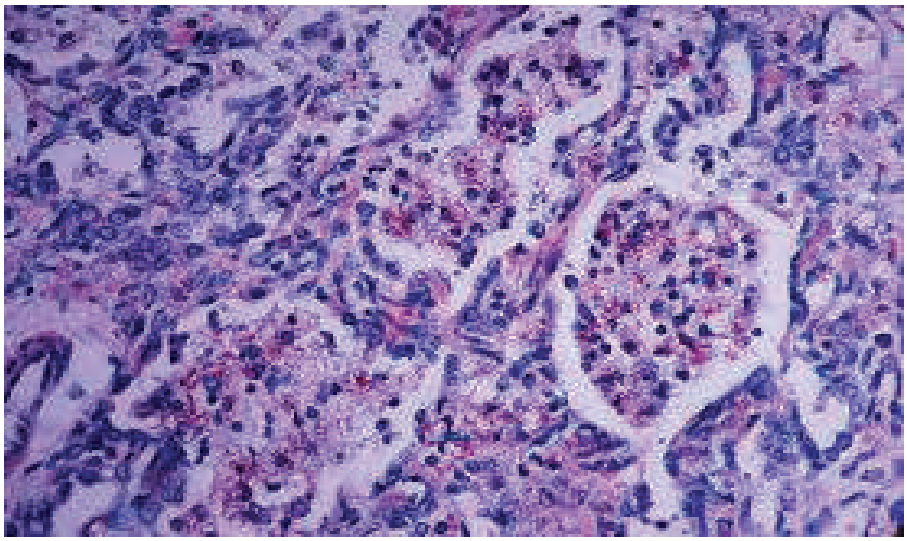
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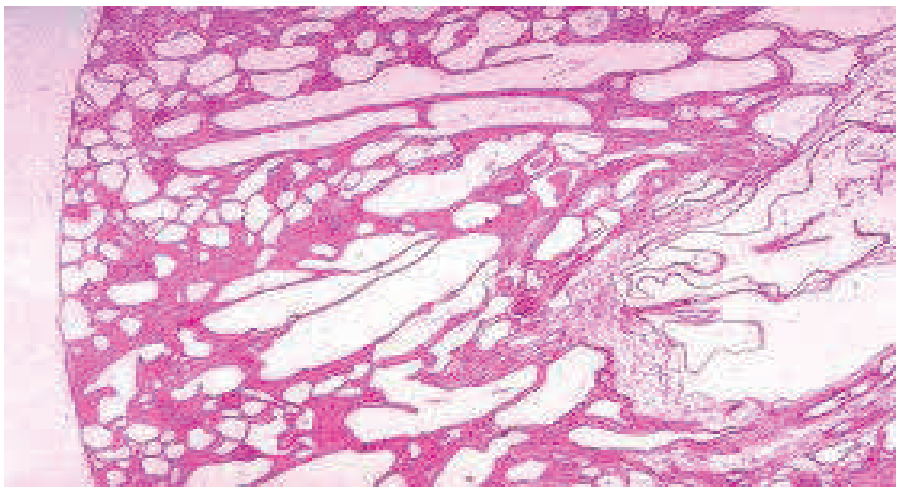
PICTURE 1.ATHEROSCELORIS WITH PARTIAL OCCLUSION OF CORONARY ARTERY



PICTURE 2. CIRRHOSIS OF LIVER



PICTURE 3.TB LUNG



PICTURE 4. POLYCYSTIC KIDNEY DISEASE

Discussion

The results of the study showed that renal cystic lesion cases, this is comparable to the study by Sulegaon et al [6] which showed cystic lesion in 38 cases in Medicolegal autopsies. Regarding cirrhosis of liver study of Alagarsamy et al [7] shows similar statistics with our study. From our study we have seen that that Coronary artery was the commonest histopathological finding followed by Cirrhosis of liver which is similar to the study done by Patel S et.al [5]. Histopathology in autopsy plays a vital role in the study of some of the rare neoplastic lesions contributing to the knowledge of pathology. This study highlights the various incidental unexpected rare cases in medico-legal autopsies, which are imperative in academic and research purposes. Histopathology would not have been necessary for some of these conditions during a life time but an incidental finding in autopsy has unveiled the histo-pathological changes that help in the understanding of disease processes which are otherwise rare for a pathologist to encounter in the day to day specimens. Such retrospective and prospective studies also provide an insight into the true prevalence of diseases or lesions. Tuberculosis (TB) has been described as a 'global emergency' by WHO and is still considered as a major cause of morbidity and mortality worldwide. The poor socio-economic condition is one of the precipitating factor for this and often both the pulmonary and extra pulmonary Tubercular lesion remain undiagnosed during life time in a large number of cases unless the patients access the health care facility.

Conclusion

From our autopsy study, we would like to conclude that, though incidental findings in autopsies may not be the cause of death but gives many information related to epidemiology of a disease. In our study, we found atherosclerosis to be the commonest incidental finding which led to IHD, LVH and sudden death in the 20-40 years age group in males. This was associated with history of alcohol consumption and smoking can lead to quarrels, both of which are high risk factors contributing to development of IHD. A detailed and both, prospective as well as retrospectives, studies on prevalence of certain diseases in the community might help to find out actual prevalence figures as well as a useful data in controlling/ monitoring certain disease processes.

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Ethical Clearance: Taken

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Study the Incidence, Types of Anemia and Associated Risk Factors in Pregnant Women

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Abstract

Background: Anemia is a global public health problem affecting both developing and developed countries and more prevalent among pregnant women. Worldwide the main cause of anemia during pregnancy is iron deficiency, if remain untreated it might leads to increased risk for perinatal morbidity and mortality.

Aim of the study: is to find an incidence of anemia among pregnant women along with types and severity to assess the association of various maternal characteristics with anemia forms.

Materials and methods: One hundred twenty eight pregnant women were enrolled in this study. Seventy five pregnant women were diagnosed with anemia while fifty three pregnant women without anemia included in this study as a control subjects. Blood samples were collected to determine hemoglobin level and complete blood counts (CBC).

Results: The mean age was 27.76 years for 75 anemia pregnant women and mean of 26.08 years for 53 control non- anemic pregnant women with no statically significant differences (P>0.05). Most of anemic pregnant women had a pregnancy gap ≤ 1 year (51%) while most of non-anemic pregnant women had a gap of > 3 years (62%) with highly significant differences between both groups (P<0.01). Approximately half of anemic pregnant women surveyed found to had a moderate anemia (Hb 7- 9.9 g/dl), one third (32%) of pregnant women is multigravida with moderate anemia type and (62%) of anemic pregnant women found to had Microcytic& Hypochromic anemia. In the present study (80%) of pregnant women receiving iron supplementation, also (73%) receiving folic acid supplement. Only (20%) receiving blood transfusion for severe anemia type.

Conclusion: The results of this research support the idea that the incidence of anemia is still high among pregnant women and iron deficiency anemia is the most prevalent type with anemia became more pronounced at third trimester of pregnancy and mostly with moderate severity type.

Keywords: *Anemia, Pregnant women, Incidence, Types, Risk factors.*

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Introduction

Anemia is a global public health problem affecting both developing and developed countries and more prevalent among pregnant women, young females in reproductive age and young children during the phase of rapid growth [(1)].

Anemia is defined as insufficient number of red blood cells to meet the body's physiologic needs which

vary with age, gender, smoking behavior, and different stages of pregnancy [2].

Worldwide the main cause of anemia during pregnancy is iron deficiency due to inadequate dietary intake, aggravated by the fact of increase demands of the fetus and maternal blood volume expansion [3, 4]. Another cause is genetic predisposition and poor hygiene which might lead to infections during pregnancy in addition to depleted iron stores from too early, too many and too frequent pregnancies [5, 6].

Outcome of anemia during pregnancy includes increased risk for perinatal morbidity and mortality due to the impairment of oxygen delivery to placenta along with increasing risk of preterm deliveries and low birth Weights [7].

The aim of this study to find an incidence of anemia among pregnant women along with types and severity to assess the association of various maternal characteristics with anemia forms.

Materials and Methods

Study design: Cross sectional study conducted during the period from April 2019 until January 2020.

Study population

One hundred twenty eight pregnant women were enrolled in this study. Seventy five pregnant women were diagnosed with anemia during their admission to Al- Yarmouk teaching hospital, in Baghdad. Also Fifty three pregnant women without anemia included in this study as a control subjects.

Blood samples were collected from the pregnant women and hematological investigations were carried out to determine hemoglobin level and complete blood counts (CBC). Questionnaires were also used to obtain demographic information. Gestational age was estimated based on the date of last menstruation and ultrasonography (U/S).

Inclusion and exclusion criteria:

Inclusion criteria include pregnant women irrespective to age, had normal pregnancy, anemia with accidental or symptomatic discovery whatever the term of pregnancy while the exclusion criteria includes: refused to participate, history of anemia due to chronic blood loss, abortion or stopped pregnancy whatever the term.

Hemoglobin levels to diagnose anemia

Anemia severity was classified according to world health organization (WHO) into mild, moderate, severe anemia according to hemoglobin level. For pregnant women non- anemia is considered if hemoglobin level is 110 g/l or higher (11g/dl or higher), mild anemia if hemoglobin level 100-109 g/l (10- 10.9 g/dl), moderate anemia if hemoglobin level is 70-99 g/l (7- 9.9 g/dl), and severe anemia if hemoglobin level lower than 70 g/l (<7g/dl) [2].

Classification of anemia

Anemia is classified into:

1- Microcytic& Hypochromic anemia: mean corpuscular volume (MCV) <80 FL, mean corpuscular hemoglobin (MCH) < 27 pg which occur in iron deficiency anemia, thalassemia, anemia of chronic disease (some cases), lead poisoning, siderobastic anemia (some cases).

2-Normocytic & Normochromic anemia: MCV 80-95 fL MCH ≥ 27 pg which occur in in hemolytic anemia, anemia of chronic disease (some cases), after acute blood loss, renal disease, mixed deficiencies, bone marrow failure (e.g. post chemotherapy, infiltration by carcinoma, etc.).

3- Macrocytic anemia MCV> 95fL occur in megaloblastic: vitamin B12 of folate deficiency, non-megaloblastic: alcohol, liver disease, myelodysplasia, aplastic anemia etc [8].

Statistical Analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1, 2010). Independent t-test was used to assess the differences between patients and control. The percentages were compared by using Chi-square test. $P < 0.05$ is considered statistically significant.

NS: No significant differences ($P>0.05$), (*) Significant difference ($P<0.05$), (**) Highly Significant difference ($P<0.01$).

Results

Table (1) showed the patient demographic and disease characteristics of 128 pregnant women include 75 anemic pregnant women and 53 non -anemic pregnant women with age range 13-44 years and mean age of 27.76 years for anemia pregnant women and age range of 16-45 years and mean of 26.08 years for control non-anemic pregnant women and most of pregnant women in both groups were non-employed 73% and 68% respectively with no statically significant differences (P>0.05).

It can be seen from the data in Table (1) that there is significant differences of education level among studied parameter (P<0.05), even though most of studied population were at university level 39 % and 62 % respectively it was found that 28% for anemic pregnant women were complete only primary school education which might be one of the risk factors for developing anemia among pregnant women.

Interestingly, the present work showed that most of anemic pregnant women had a pregnancy gap ≤ 1 year (51%) while most of non-anemic pregnant women had a gap of > 3 years (62%) with highly significant differences between both groups (P<0.01), table (1).

Table (1): Distribution of pregnant women by their socio-demographic characteristics

Variable	Groups		P- value
	Anemic pregnant women	Non-anemic pregnant women	
Age (year)	27.76± 6.71	26.08± 5.73	0.14 NS
Occupation	n (%)	n (%)	0.50 NS
Employed	20 (27)	17 (32)	
Non-employed	55 (73)	36 (68)	
Education	n (%)	n (%)	0.04*
Illiteracy	4 (5)	0 (0)	
Read and write	3 (4)	1 (2)	
Primary	21 (28)	6 (11)	
Secondary	12 (16)	10 (19)	
University	29 (39)	33 (62)	
Higher	6 (8)	3 (6)	
Pregnancy gap	n (%)	n (%)	<0.0001**
≤ 1 year	38 (51)	6 (12)	
2 years	14 (19)	8 (15)	
3 years	7 (9)	6 (11)	
> 3 years	16 (21)	33 (62)	

Data presented as number (Percentage), mean ± SD

NS: No significant differences (P>0.05), (*) Significant difference (P<0.05), (**) Highly Significant difference (P<0.01).

Approximately half of anemic pregnant women surveyed found to had a moderate anemia (Hb 7- 9.9 g/dl) with highly significant differences (P<0.01), table (2).

Table (2): Classification of anemia by degree of severity among pregnant woman according to world health organization (WHO)

Anemia	n (%)
Mild anemia (Hb 10-10.9 g/dl)	28 (37)
Moderate anemia (Hb 7- 9.9 g/dl)	41 (55)
Severe anemia (Hb <7 g/dl)	6 (8)
P-value	<0.0001**

Data presented as number (Percentage), Hb: hemoglobin, (**) Highly Significant difference (P<0.01).

If we now turn to comparison of anemia classification with gravidity, one third (32%) of pregnant women is multigravida with moderate anemia type.

Higher percentage (52%) of anemic pregnant women found that they were at third trimester of pregnancy; most of them had moderate type (27%) as the demand of iron increased on advanced pregnancy, table (3).

Table (3): Severity of anemia in relation to gravidity and trimester of pregnancy among pregnant women with anemia

Gravidity	Anemia classification			P-value
	Mild	Moderate	Severe	
Primgravida	8 (11)	17 (23)	1 (1)	0.34 NS
Multigravida	20 (27)	24 (32)	5 (6)	
Trimester of pregnancy	Mild	Moderate	Severe	P-value
First trimester (1-13 week)	3 (4)	3 (4)	0 (0)	0.56 NS
Second trimester (14-27 week)	11 (15)	18 (24)	1 (1)	
Third trimester (28-42 week)	14 (19)	20 (27)	5 (6)	

Data presented as number (Percentage), NS: No significant differences (P>0.05).

The most striking result to emerge from the present data is that out of 75 anemic pregnant women 37 had performed complete blood counts and highly significant differences (P<0.01) found as (62%) of them found to had Microcytic& Hypochromic anemia, table (4).

Table (4) Type of anemia among pregnant women with anemia

Type of anemia	Anemic pregnant women	P-value
Microcytic& Hypochromic	23 (62)	0.0002**
Normocytic & Normochromic	11 (30)	
Macrocytic	3 (8)	
Total	37 (100)	

Data presented as number (Percentage), (**) Highly Significant difference (P<0.01).

Table (5) provides an overview of supplementations and treatment intake for anemia throughout the pregnancy, higher frequency (80%) of pregnant women receiving iron supplementation, also (73%) receiving folic acid supplement (knowing that many patients from those percentage receiving both iron and folic acid supplementations) with highly significant differences (P<0.01). Only (20%) receiving blood transfusion for severe anemia type.

Table (5) Supplementations and treatment intake for anemia throughout the pregnancy

Iron supplementation throughout the pregnancy	**n(%)**	***P*- value**
Yes	60 (80)	<0.0001**
No	15 (20)	
Folic acid supplementation throughout the pregnancy	**n(%)**	***P*- value**
Yes	55 (73)	<0.0001**
No	20 (27)	
Blood transfusion	**n(%)**	***P*- value**
Yes	15 (20)	<0.0001**
No	60 (80)	

Data presented as number (Percentage), (**) Highly Significant difference (P<0.01).

Discussion

Approximately one-fourth of the world's population affected by anemia, iron deficiency is the leading cause of anemia allover countries and in both sexes, with women being more affected than man [9, 10].

The present study highlights the incidence of anemia among pregnant women along with risk factors that might predispose pregnant women to anemia.

The education about anemia prevalence and its risk factors to pregnant women is very important. Although in the present work most of pregnant women in both groups found to complete university level, (28%) of anemic pregnant women were found to complete only primary school education which might be one of the risk factors for developing anemia in those patients.

Alem *et al* found that the risk of anemia is (8.8) higher for illiterate pregnant women when compared

with women > 12 educational level [11].

Also a study in China found that the prevalence of anemia among pregnant women was significantly different according to educational level: for college, secondary and primary education the percentage was 52.9, 62.4 and 66.5%, respectively [12].

Also in Jordan a study revealed that only one third of pregnant women had educational level higher than high school [13].

Interestingly, the present work showed that the pregnancy gap might also play a role in developing anemia among studied groups as most of anemic pregnant women showed that had a pregnancy gap ≤ 1 year (51%) while most of non-anemic pregnant women had a gap of > 3 years (62%) with highly significant differences between both groups (P<0.01).

Agreement reported in a study found that (27.6%) of anemic pregnant woman have pregnancy gap of 2 year, (12.3%) have a gap of 3 years and (8.6%) have gap more than 3 years[11].

In this present work (65%) of anemic pregnant women was multigravida. Iron reserves were depleted and anemia occurs in pregnancy with rising number of children. On the other hand, higher percentage (57%) of non- anemic pregnant women also was multigravida and can be illustrated by increased knowledge about anemia prevalence and consequences gained by previous pregnancy experience along with good diet and regular antenatal visit, might neutralize its effect [14].

The present findings seem to be consistent with other research that also conducted in Baghdad which found that the number of anemic pregnant women who primigravida (9.6%) lower than that of pregnant women multipart gravida (45.8%) [15]. About (52%) of anemic pregnant women were in third trimester of pregnancy and (27%) of them had a moderate anemia type while (40%) were in second trimester an only (8%) were in first trimester of pregnancy. The findings of the current study are consistent with those of Alem *et al* and Al –Mehaisen *et al* which found that the prevalence of anemia is higher in third trimester of pregnancy [11,13].

While previous study in Iraq which found that the prevalence of anemia was the highest among pregnant women in 2nd trimester (50.4%) compared to those in the 1st trimester (27.1%) and 3rd trimester (22.5%) and (38.8%) of them had moderate anemia type [16].

The most important clinically relevant finding was that (62%) of anemic pregnant women found to had Microcytic& Hypochromic anemia which is the most common type in pregnancy that occur mostly due to iron deficiency.

This finding confirms the association between the current study and another study at the same region as in Baghdad the majority of anemic pregnant women found to have microcytic &hypochromic anemia [15].

It is somewhat surprising that the majority of pregnant women in the present work found to had a folic acid and iron supplementations for prophylaxis or treatment for already exciting anemia with highly significant differences among study group, despite that they diagnosed with anemia at the time of the study it might be because of most of women enter pregnancy with seriously depleted iron stores [16]. So if there is low iron stores before pregnancy it is difficult to replenish iron stores by diet alone or even with supplementation especially by the last half of pregnancy where the demands increased by expanding blood volume of the mother and the rapidly growing fetus and placenta in addition to low compliance to iron supplementation (gastrointestinal side-effects are believed to be the main reason for limited compliance) all these factors lead to iron deficiency anemia during pregnancy [17-21].

Conclusion

The results of this research support the idea that the incidence of anemia is still high among pregnant women and iron deficiency anemia is the most prevalent type with anemia became more pronounced at third trimester of pregnancy and mostly with moderate severity type.

These findings suggest several courses of action represented by enhanced the education of pregnant women about the risk factors of anemia and possible avoidance and encourage women with childbearing age or desire to became pregnant to had a food rich in iron along with prescreening of hemoglobin and iron before pregnancy to prevent anemia as possible as they can.

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Are Problems During Pregnancy a Predictor of Childbirth in The Hospital?: Determinants Analysis of Hospital Childbirth in Urban Poor Communities in Indonesia

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Abstract

Although hospital services were available, urban poor people often have limited access to hospitals when needed. This study was conducted to analyze the determinants of childbirth in a hospital in urban poor women in Indonesia. The study uses raw data from the 2017 IDHS. With stratification and multistage random sampling, 7,891 women aged 15-49 years old in urban poor communities with live births in the last 5 years were sampled. Data were analyzed using a Binary Logistic Regression test. The results of the study found that "problems during pregnancy" was not proven as a determinant of the hospital use for delivery in urban poor women in Indonesia. Age was proven to be one of the determinants, while in the education level category, urban poor women with tertiary education were 2.506 times more likely to use hospitals for delivery than urban poor women who were not in school. Parity was significantly proven as one of the determinants that influence the use of hospitals for delivery in urban poor women in Indonesia. Urban poor women who were covered by health insurance have 1.933 times more possibility of utilizing hospital for delivery than those who do not have health insurance. It could be concluded that the "problem during pregnancy" variable was not a predictor of the hospital use for childbirth in the urban poor community in Indonesia. The determinants or variables that influence, on the hospital use for childbirth were age, education, parity, and health insurance.

Keywords: *childbirth, hospital, pregnancy, wealth, poor, urban.*

Introduction

Maternal Mortality Rate (MMR) in Indonesia is still quite high, even when compared to countries in the Southeast Asia region. In 2015 MMR was recorded at 305 per 100,000 live births. This achievement has decreased compared to MMR in 2012 which was in the range of 359 per 100,000 live births[1]. This milestone is documented in the 2016 Annual Report of Directorate of Family Health[2].

The 2017 Indonesian Demographic Data Survey (IDHS) results found that 74% of women in Indonesia gave birth in health care facilities. As many as 42% deliver at first-level health service facilities, which include the Health Center (Puskesmas) and its network, clinics, and practices of health workers. While there are 32% who deliver at the advanced level of referral service facilities, namely hospitals[3].

Urban poor people like living in a vicious circle. Between poor, uneducated, and sick, like a chain that is difficult to break[4,5]. Urban poor people tend to form their own colonies in urban slums. This condition is often due to high housing and land prices in urban areas[6]. Slum housing conditions actually increase the risk of the urban poor to fall ill[7]. The government must pay serious attention to the urban poor. This group of people tends to neglect health issues because they have little education. The urban poor tend to prioritize work matters rather than school and health matters[8].

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Although hospital services were available, urban poor people often have limited access to hospitals when needed[9,10]. This situation could occur because of their ignorance, or because they did not have health insurance[11].

Based on the background, this article was prepared to analyze the determinants of childbirth hospitals in urban poor communities in Indonesia. The analysis in this study is useful for policymakers in the field of maternal health to formulate more detailed policies according to the targets identified in the study results. This step is necessary to ensure the reduction of obstacles for urban poor women to access maternity services in hospitals.

Materials and Methods

Secondary data from the 2017 Indonesian Demographic Data Survey (IDHS) was used as an analysis material in this study. The IDHS was part of the international Demographic and Health Survey (DHS) program conducted by the Inner City Fund. In Indonesia context, the 2017 IDHS was carried out by the Central Statistics Agency, in collaboration with the National Population and Family Planning Board and the Ministry of Health.

The 2017 IDHS sample was determined through stratification and multistage random sampling. The unit of analysis in this study was women aged 15-49 years old who had given birth in the last 5 years. The sample size of the 2017 IDHS used in this analysis was 7,891 women.

Problems during pregnancy were the respondent's acknowledgment of problems experienced during pregnancy until delivery. These problems consist of: prolonged labor, vaginal bleeding, fever, convulsions, baby in the wrong position, swollen limbs, faint, breathlessness, tiredness, and others[3]. Socioeconomics was the wealth status of respondent compiled based on index of goods ownership quintile stated by the respondent. The five categories were poorest (quintile 1), poorer (quintile 2), middle (quintile 3), richer (quintile 4), and richest (quintile 5). The urban poor category used in this study was people who live in urban areas and with wealth status in quintiles 1 and 2.

The 2017 IDHS data was obtained through a structured questionnaire. Variables analyzed included, age, education level, work status, marital status, parity, health insurance, and antenatal care. Statistical analysis using chi-square was carried out for dichotomous variables and t-test for continuous variables. This statistical analysis was conducted to assess whether there were differences in childbirth facility that were statistically significant between the category of wealth status. Estimates were performed using Binary Logistic Regression because of the nature of the dependent variable. All statistical analyses were carried out using SPSS 22 software.

Result and Discussion

Table 1 informs descriptive statistics on the category of "problems during pregnancy" in urban poor women who have given birth in the last five years in Indonesia. Table 1 shows that there are significant differences in urban poor women who have given birth in the last five years in the use of hospitals for delivery in Indonesia. This difference is statistically significant for all observed characteristics. Table 1 informs that urban poor women who have delivered in the last five years have more dominant non-hospital deliveries.

Table 1 shows that urban poor women who gave birth in the last five years had a slightly older average age for those who did not have problems during pregnancy. Urban poor women who have given birth in the last five years predominantly have secondary education and are unemployed. The urban poor women who gave birth in the last five years were dominated by women with marital status married/living with partners. Urban poor women who have given birth in the last five years have a slightly higher average parity in those who have no problems during pregnancy. Moreover, the urban poor women who gave birth in the last five years were dominated by women covered by health insurance. Urban poor women who have given birth in the past five years have been dominant for antenatal care more than 4 times before giving birth.

Table 1. Descriptive Statistics of Problems During Pregnancy in Urban Poor Women and Related Factors in Indonesia (n=7891)

Characteristics		Problems during pregnancy		Sig.
		No	Yes	
Hospital childbirth*				p<0.001
-	No	1341(61.68%)	3063(53.58%)	
-	Yes	833(38.32%)	2654(46.42%)	
Age (mean)**		2174(31.36)	5717(31.30)	p<0.001
Education level*				p<0.001
-	No education (ref.)	18(0.83%)	7(0.12%)	
-	Primary	483(22.22%)	600(10.50%)	
-	Secondary	1410(64.86%)	3436(60.10%)	
-	Higher	263(12.10%)	1674(29.28%)	
Work status*				p<0.001
-	No work (ref.)	1208(55.57%)	2879(50.36%)	
-	Work	966(44.43%)	2838(49.64%)	
Marital status*				p<0.001
-	Never married (ref.)	0(0.00%)	3(0.05%)	
-	Married/Living with Partner	2079(95.63%)	5588(97.74%)	
-	Divorced/Widowed	95(4.37%)	126(2.20%)	
Parity (mean)**		2174(2,47)	5717(2.27)	p<0.001
Health insurance*				p<0.001
-	No (ref.)	904(41.58%)	1714(29.98%)	
-	Yes	1270(58.42%)	4003(70.02%)	
Antenatal care*				p<0.001
-	< 4 times (ref.)	513(23.60%)	994(17.39%)	
-	≥ 4 times	1661(76.40%)	4723(82.61%)	

*Chi-Square test was used for dichotomous variables; **T-test for continuous variables.

Table 2. Binary Logistic Regression of Hospital Childbirth in Urban Poor Communities in Indonesia (n=7891)

Predictor	Childbirth in Hospital		
	OR	Lower Bound	Upper Bound
Problems during pregnancy:Yes	1.056	0.948	1.177
Age	1.049***	1.039	1.059
Education level:Primary	0.765	0.323	1.815
Education level:Secondary	1.255	0.533	2.955
Education level:Tertiary	2.506*	1.059	5.929
Work status:work	1.021	0.927	1.125
Marital status: Married/Living with Partner	0.000	0.000	0.000
Marital status: Divorced/Widowed	0.000	0.000	0.000
Parity	0.816***	0.777	0.857
Health insurance:Yes	1.933***	1.744	2.143
Antenatal care:≥4 times	1.059	0.934	1.201

*p<0.05; **p<0.01; ***p<0.001.

Table 2 presents the results of the binary logistic regression test to illustrate the results of the analysis of determinants of the use of hospitals for delivery in urban poor women in Indonesia. As a reference, the chosen category is "nonhospital childbirth".

Table 2 shows that "problems during pregnancy" were not proven as a determinant of the use of hospital for delivery in poor women in Indonesia. There is no significant difference in the category of "problems during pregnancy" in the use of hospital for delivery in urban poor women in Indonesia. This means that urban poor women, even though they are experiencing problems with their pregnancy, cannot access hospitals for childbirth. The results of this study reaffirm that there are problems in the urban poor in accessing hospitals. Several studies have found that the cost factor is the dominant factor that is a barrier in the urban poor community[10,12].

The age is proven to be one of the determinants of hospital use for poor urban women in Indonesia. While in the education level category, urban poor women with tertiary education are 2.506 times more likely to use hospitals for delivery than urban poor women who are not in school (OR 2.506; 95% CI 1.059-5.929). The low level of education makes the ability of the urban poor community to communicate and access information about health encounters obstacles and is limited. The relationship between low levels of education as a barrier to access to health care facilities is in line with the results of research in several countries[13–15]. Education level is often found as a positive determinant of health program output[16–18].

Meanwhile the marital status is not a determinant of the use of hospital for delivery in urban poor women in Indonesia. While parity is significantly proven as one of the determinants that influence the use of hospital for delivery in urban poor women in Indonesia. Table 2 informs that urban poor women covered by health insurance are 1.933 times more likely to use the hospital for delivery than those without health insurance (OR 1.933; 95% CI 1.744-2.143). While the frequency of antenatal care by urban poor women is not proven as a determinant of the use of hospitals for delivery in Indonesia.

There are at least two cost factors found in several studies that have become a barrier for people to access health services in various countries. First is the cost to

get health services[19,20], and second, the costs required for transportation[21]. The combination of the two types of barrier cost can make urban poor women increasingly have no access to deliver to the hospital, even though they feel they have a disruption in their pregnancy.

Health insurance ownership was found to be a predictor of the use of hospitals for delivery in urban poor women in Indonesia. This condition is consistent with the results of previous studies on health costs as a barrier to access to health services. Insurance ownership is one of the solutions to improve urban poor people's access to health care facilities[22,23].

In the Indonesian context, the government has provided subsidies by including membership contribution assistance mechanisms for the poor in the National Health Insurance. But some of the results of research that conduct evaluations, in general, still show that the health service system that runs in Indonesia is still pro-rich for public access to health services in hospitals[24,25]. While access to services found to be pro-poor is only demonstrated in health services at the primary level, or at the Health Center[26,27].

Conclusions

Based on the findings of the study it could be concluded that problem during pregnancy was not a predictor of the use of hospital for delivery in urban poor women in Indonesia. The determinants or variables that influence, on the use of hospital for delivery were age, level of education, parity, and health insurance.

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Blood Cells Depletion after Chemotherapy in Iraqi Women with Breast Cancer

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Abstract

The research was aimed to study the effect of chemotherapy on white blood cells especially lymphocytes, and the relationship between chemotherapy and red blood cells, platelets in women with breast cancer. Our results showed that the mean ± SE of White Blood Cells (WBCs) and lymphocytes before treatment was significantly higher than the mean ± SE of lymphocytes and WBCs after treatment at (P<0. 01), the mean ± SE of Red Blood Cells (RBCs) and platelets before treatment was higher than the mean ± SE of RBCs and lymphocyte after treatment at (P<0. 01). The reason probably due to the poor nutrition and the effect of cancer of the breast on erythropoiesis on the cases, or metastasis to the bone marrow from breast cancer can be associated with suppression of blood cells synthesis. We concluded that chemotherapy for breast cancer is in association with long-term changing in immune system parameters and other cells during clinical management.

Key words: *chemotherapy, breast cancer, Blood cells depletion*

Introduction

Breast cancers are the most common in women and causes more than half a million deaths in the world [1]. Treatment is surgical tumour resection in combination with endocrine therapy, radiotherapy and using of chemotherapy. Chemotherapy is a therapy of choice in thirty percent of patients [2], and is recommended when cancer shows poor diagnosis features, including enlargement in nodal size, high grade, and lack of expression of progesterone and estrogen receptors [3]. Chemotherapy also reduces the rates of metastatic recovery and increasing in survival rates [4, 5]. Treatment of chemotherapy is already in association with a wide effects on tissues with non-target, for example, immune system substantial impacts. Neutrophils reduction is often regarded as the most dangerous haematological toxicity and can be associated with infections that may force chemotherapy dose reduction and delays may compromise treatment [6, 7]. The level of blood neutrophil is known to recover after chemotherapy with agood management and this transient reduction in the number of neutrophils is not have common persistent results. Treatment with chemotherapy also cause problems in the adaptive immune system overall the body. Number of research have reported effects of chemotherapy on the number of lymphocytes in breast cancers women during the period of therapy or more than three months after the last dose of chemotherapy and cause reduction in the number of lymphocyte levels in the blood[12]. Reduction in the number of lymphocytes shortly following chemotherapy for other types of cancers is also well established. Depression in the number of T and B cells for more than 12 months after chemotherapy have been

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reported, while others have found all lymphocyte cells except T- helper cells to recover to its pre-treatment levels at the same time-point, even after a particularly dose intense chemotherapy regimen, while T- cytotoxic lymphocyte cells levels recover more rapidly after chemotherapy than T-helper cells [9,11] and that cause reduction in the number of naïve cells, and in contrast increasing in memory cells population [12]. In addition to lymphocyte reduction, chemotherapy may reduce other types of blood cells as red blood cells and thrombocytes.

Materials and Methods

The experimental groups: The experimental groups used consist of 250 women with breast cancer.

Blood samples collection: Five millileter (ml) of blood samples were aspirated from each woman patient and control cubitus vein to test the hematological parameters. Complete Blood count (CBC) was measured by (CBC) method (Rubby from abbut, USA).

Statistical analysis: The Statistical Analysis System [13] program was used to compare between control and patient groups in study parameters. T- test was used to significant compare between means in this study.

Results

Table (1) showed the mean ± SE of WBCs and lymphocytes before treatment was significantly higher than the mean ± SE of WBCs and lymphocytes after treatment at (P<0. 01). Table (1) was also showed the T-test for lymphocytes was significantly higher than T-test for WBCs.

Table (1): Comparision between before and after treatment in WBC and Lymphocytes

Treatment	Mean ± SE	
	WBC	Lymphocyte (%)
Before	7.80 ± 0.41	33.33 ± 2.95
After	4.06 ± 0.22	14.33 ± 1.51
T-test	0.929 **	6.596 **
P-value	0.0001	0.0001
** (P<0.01).		

Table (2) showed the mean ± SE of RBCs and PLT before treatment was significantly higher than the mean ± SE of RBCs and PLT after treatment at (P<0. 01). Table (2) also showed the T-test for PLT was significantly higher than T-test for RBCs at (P<0. 01).

Table (2): Comparision between before and after treatment in RBC and PLT

Treatment	Mean ± SE	
	RBC	PLT
After	4.79 ± 0.12	3.19 ± 0.15
Before	315.11 ± 10.48	165.46 ± 7.92
T-test	0.398 **	26.091 **
P-value	0.0001	0.0001
** (P<0.01).		

Discussion

In the recent study, our results showed that the level of all blood cells types before treatment was higher than the level of these cells after treatment, this finding is similar to the finding of [14,15] who find that chemotherapy cause declining in blood cells, also to the finding of [16] find similar result in chinese patients. The mean ±SE of WBCs Before and after chemotherapy was lower than the Mean ±SE of lymphocyte ($p<0.01$), the mean ±SE of WBCs Before and after chemotherapy was higher than the Mean ±SE of lymphocyte ($p<0.01$). This result is in agreement with the result of [17]. The mean ± SE of WBCs and lymphocytes before treatment was significantly higher than the mean ± SE of WBCs and lymphocytes after treatment at (P<0. 01). The mean ± SE of RBCs and PLT before treatment was significantly higher than the mean ± SE of RBCs and PLT after treatment at (P<0. 01). A higher percentage of anemia reported in this study after chemotherapy compared with the lower Caucasian values in the study of [18] and the control group in this study, the reason probably due to the poor nutrition of Iraqi patients and the effect of cancer of the beast on erythropoiesis on the cases compared with the control group, or metastasis to the bone marrow from breast cancer can be associated with suppression of erythropoiesis. Bone marrow metastasis also may be associated with defective thrombopoiesis causing malignancy induce thrombocytopoiesis.

Conclusion

Chemotherapy of breast cancer is associated with changing in immune system parameters and red blood cells that should be considered during clinical management.

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The Organizational Learning Implementation to Improve Accreditation Performance at the Gayungan Health Center, Surabaya, Indonesia

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Abstract

This study aims were to determine the application of organizational learning to improve accreditation performance. Descriptive research method with a cross-sectional approach. The population in this study was all employees of Gayungan Health Center with a purposive sampling technique consisting of 13 respondents. Based on the survey results using a questionnaire on the Gayungan Health Center accreditation team; it was found that 75% of respondents stated the organizational learning capability and organizational learning process were in a good category. The implementation of organizational learning in Gayungan Health Center where employees were directed to continually learn through every activity create innovations in problem-solving and form good teamwork were proven could improve accreditation performance. On the last re-accreditation, Gayungan Health Center got the best accreditation status.

Keywords*: performance, accreditation, organizational learning, performance improvement*

Introduction

Gayungan Health Center as one of the health service organizations providing services directly to the community must be able to provide the best quality and acceptable health services. Through Regulation of Minister of Health No. 46, 2015, the Ministry of Health sets accreditation standards for Gayungan Health Center aimed at improving the quality of services, patient safety, as well as increasing protection for human resources in health, society, and the environment[1,2]. Besides, the Gayungan Health Center works as an institution to improve performance in providing individual health services and/or public health. With accreditation, Gayungan Health Center may encourage organizations to improve their performance and is expected to meet the requirements as intended for the implementation of accreditation[3,4]. This effort needs to be done to improve Gayungan Health Center access which is still low. Previous research still found disparities between urban-rural, socioeconomic, and between regions[5–7].

According to Ilyas, performance is the work of personnel both quality and quantity in an organization. Performance is the result of individual and group work. Performance results are not limited to individuals who hold functional or structural positions, but also to all members of the organization [8]. To improve performance, the Gayungan Health Center must develop organizational learning both for individuals in the organization and for the organization itself. The results showed organizational learning could lead to an increase in public and team learning in an organization, as well as increase organizational activities, improve individual and group behavior and eventually rise efficiency, effectiveness, and productivity[9].

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Organizational learning is the capability to develop thinking and productivity through commitments purposely to make continuous improvements in the organization [10]. Organizational Learning is the deliberate use of learning processes at the individual, group, and system levels to transform the organization in a sustainable manner, which leads to increasing stakeholder satisfaction [11]. Organizational learning is the process of finding mistakes and problems, then completing and fixing them. Organizational learning is a process occurred by applying science and improving performance over time. The benefits of organizational learning are improving organizational performance, especially for the long-term empowering human resources, increasing creativity and innovation, and accelerating the process of organizational change towards organizational learning[12,13].

The main purpose of organizational learning is to improve the quality and quantity of performance, which allows companies to develop and increase the number of sales, find support, create, retain, and grow new customers. Organizational learning can enhance strategic capabilities and enable companies to strengthen competitive advantage and advance performance. The concept of organizational learning is so essential to foster the long-term performance of an organization [14,15]. The knowledge accumulated through organizational learning produces a basis for knowledge at a higher level associated with high performances at the organizational level [16]. The relationship between Organizational Learning and organizational performance is complex, but using a good management system, it may produce better performance.

This study aimed to find out how the implementation of organizational learning in Gayungan Health Center, as an accredited health service provider in 2015. Moreover, this study also looked for the organization's efforts in improving the accreditation status of the Gayungan Health Center, so it could achieve a better level of accreditation by applying organizational learning to all members of the organization.

Materials and Methods

This study was a descriptive quantitative research with a cross-sectional approach. The research variable was organizational learning capability consisted of dimensions of experimentation, risk-taking, interaction with the external environment, participative decision-making, and organizational learning process. The organizational learning process consisted of the dimensions of knowledge acquisition, information distribution, information interpretation, and organizational memory. The study used surveys and questionnaire methods. Organizational Learning Capability utilized a standardized questionnaire developed by Chiva[17]. The variable Organizational Learning Process was measured with a questionnaire developed by the researcher. The researcher firstly modified the theoretical basis as presented in Huber's study[18].

The populations in this study were employees of Accredited Gayungan Health Center in Surabaya. The sampling was performed using a purposive sampling method in each accreditation-working group consisting of one head of Gayungan Health Center, 3 heads of working groups, and 12 team members.

Findings

Table 1 displays the characteristics of respondents by gender, age group, education level, and work tenure. Based on gender, it appears to be dominated by women. Based on the age group, it was dominated by respondents who entered the age group of 36-50 years. Table 1 informs that almost all respondents had an undergraduate education. Based on work tenure, respondents are dominated by respondents who have work tenures> 10 years.

Table 1. Overview of Respondents by Gender, Age Group, Education Level, and Work Tenure

Variables	n	%
Gender		
Male	1	7
Female	14	93
Age Group		
20 – 35	5	7
36 – 50	9	60
>50	1	33
Education level		
Diploma	4	27
Undergraduate	10	67
Postgraduate	1	7
Work tenure		
<5 years	4	27
5 – 10 years	5	33
> 10 years	6	40

Figure 1 shows organization learning capability at Surabaya Gayungan Health Center indicates 100% of respondents stated that the conditions of experimentation, interaction with the external environment, and participatory decision making are in a good category. In the risk-taking dimension, there are still 13% of respondents declared the risk-taking condition is sufficient.

The research study shows emphasize organizational learning-capability influences innovation and organizational performance. Organizational learning capability plays an essential role especially in the process of optimizing innovation in improving organizational performance[19]. Another study conducted by Khadra Dahou proves that knowledge management using the knowledge conversion process significantly alters the development of organizational learning capability[20].

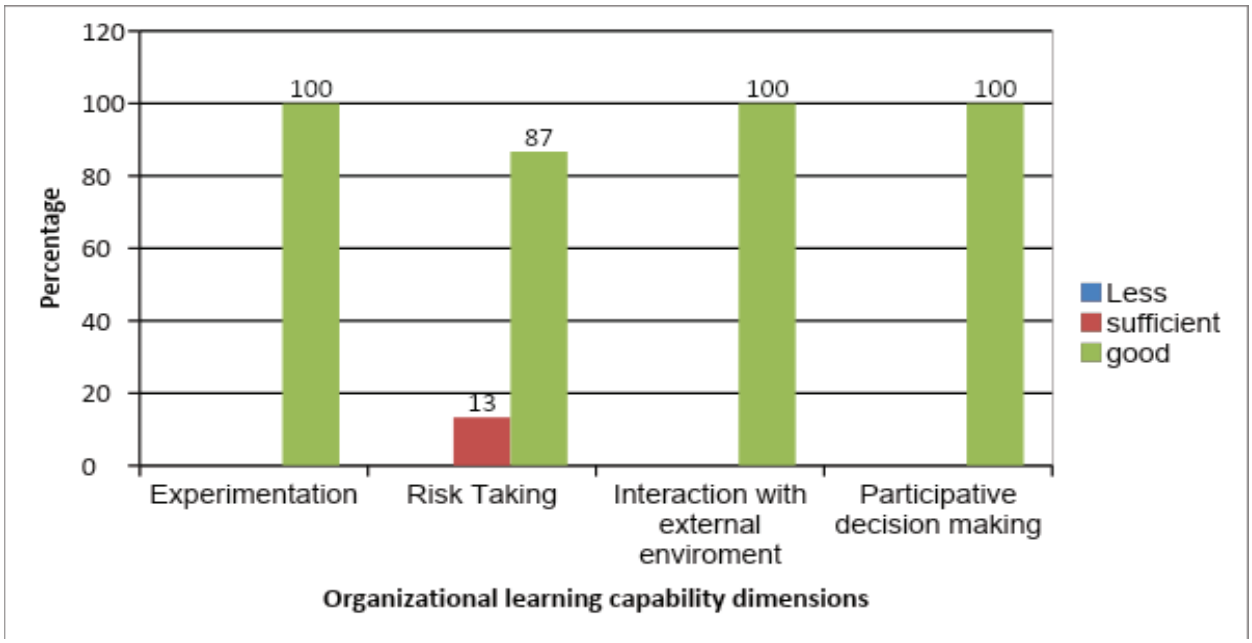


Figure 1. Overview of organizational learning capability at Gayungan Health Center in 2019

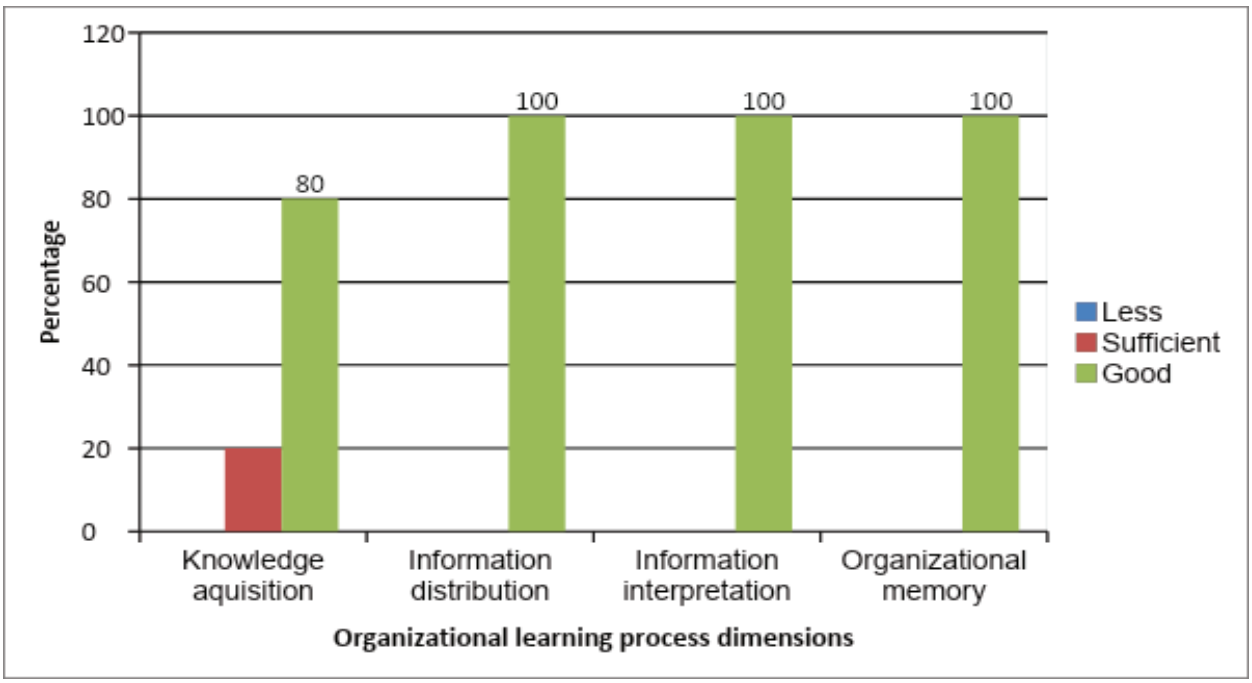


Figure 2. Overview of the organizational learning process in Public Health Office X in 2019

The figure above indicates the condition of the organizational learning process in Gayungan Health Center as a whole has been going well. From each dimension measured, 100% of respondents stated the condition of the organizational learning process was good, while the dimension of knowledge acquisition, 80% of respondents stated well; another 20% said the organizational learning process condition was still in the fair category. The survey results above showed, in general, the condition of the organizational learning process at Gayungan Health Center was good.

According to research conducted by Shien Ping Huang, the better the learning process of an organization, the greater the amount of knowledge gained[21]. Zheng et al, explain knowledge management plays an important role in cultural relations, structure, and organizational

strategy with organizational commitment[22]. Other research on the organizational learning process describes organizational learning processes (information acquisition, information dissemination, shared interpretation, declarative memory, and procedural memory) that have a positive effect on organizational performance[23].

In organizational learning, leaders must create an environment that supports all members of the organization as learners, teachers, and leaders to improve the progress of what they choose; they do that to develop the ability of employees[10,24]. A previous study revealed that there is a positive relationship between organizational learning capability elements and organizational performance[25,26].

To build awareness of learning, organizations must focus on four important things. Leaders must strive for organizational learning and foster a culture of continuous improvement that prioritizes organizational learning. Besides, organizations must define learning structures that determine the people responsible for acquiring, filtering, applying, and sharing knowledge and the organization must design intuitive knowledge processes aligned with work procedures[27,28].

Conclusions

Based on the results the implementation of organizational learning in both capability and process was included in both categories. This condition shows that in the implementation of accreditation, all officers have been involved in the process of preparing the re-accreditation assessment. Improvements shall be made by the Gayungan Health Center starting from the results of learning for the application of daily tasks as well as from the input of the accreditation assessment team and companion accreditation from the Health Service.

From the implementation of organizational learning, it is expected that Gayungan Health Center can continue to improve organizational performance in the next accreditation assessment. Implementation of organizational learning is expected not only in the aspect of accreditation but also in every program run by the Gayungan Health Center, to increase productivity and overall performance of the Gayungan Health Center.

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Relationship between Toluene Concentration, Malondialdehyde (MDA) Level and Health Complaints in Workers of Surabaya Printing Industry

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Abstract

Introduction: Toluene is one of the chemical compounds used in the production process in the printing industry. Exposure toluene that exceeds the threshold value continuously can result in increased levels of malondialdehyde and the possibility of experiencing various health complaints. The purpose of this study was to determine the relationship between toluene concentrations, malondialdehyde levels and health complaints in workers at X Surabaya printing.

Methods: This research is observational with quantitative approach and cross sectional design. The study was conducted on workers in X Surabaya printing. The number of respondents in this study were 19 people. The variables studied were toluene concentration, malondialdehyde levels, and health complaints. Data were analyzed using Pearson correlation test.

Result: The average concentration of toluene was 1.2311 ppm and the average concentration of malondialdehyde was 8,323 MU. Pearson correlation test results showed the relationship of toluene concentration with malondialdehyde obtained p value = 0.508 (p> 0.05). While the results of health complaints that were most experienced were coughing (74%), headaches (63%) and shortness of breath (26%).

Conclusion: There is no significant relationship between toluene concentration and malondialdehyde level. While the most commonly experienced health complaints are coughing, headaches, and shortness of breath.

Key Word**:** *Malondialdehyde levels, health complaints, toluene concentration*

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Introduction

Informal industrial sector is frequently found in developing countries such as Indonesia. One industry that is developing both on a small and large scale is the printing industry. Each industrial sector has certain risks and potential hazards. In the production process in the printing industry is inseparable from the use of

chemicals. One of the chemicals used is volatile organic compounds or Volatile Organic Compounds (VOCs) which are usually used as solvents. Organic solvents are widely used in printing activities such as tin, copper, and other metals[1]. Materials that are often used in general are toluene. Toluene is widely used in printing activities. Toluene is used in 75% of work activities in printing. The use of toluene dominates the printing ink components around 60-75%[2].

Toluene is a chemical compound that belongs to the aromatic hydrocarbon group. In the printing industry thinner is used as a solvent, printing ink and glue that causes the risk of exposure to toluene vapor due to its volatile nature. Thinner has a strong and pungent odor so that it can irritate the eyes and breathing[3]. These toxic chemicals can spread during the production process and affect the health of exposed workers. ACGIH (2019) recommends the toluene threshold (TLV) value at work is 20 parts per million (ppm) with a dose of toxic gas based on 8 hours / day 40 hours / week of work time[4]. Toluene can enter the body through three pathways namely inhalation, ingestion and skin contact. The volatile nature of toluene causes the easy entry of these substances through the respiratory tract. Toluene that is absorbed is distributed throughout the body, especially in tissues that contain lots of fat such as adipose tissue, brain bone marrow, kidneys and liver. Toluene is detoxified in the liver before it is excreted out of the body through urine in the form of hipuric acid[5].

Toluene metabolism can also produce toluene epoxide which is very reactive and is a free radical. If there is an imbalance in the amount of oxidants and antioxidants in the body, conditions of oxidative stress can occur. One of the biomarkers of oxidative stress is the activity of lipid peroxidation that can be measured from the levels of malondialdehyde (MDA) in organs. Malondialdehyde is a compound that is used as one of the instructions for oxidative stress because it can describe the activity of free radicals in cells[6]. Lipid peroxidation is a continuous reaction of free radical oxidation to lipids. Oxidation of free radicals to lipids produces malondialdehyde compounds. An increase in malondialdehyde levels indicates an increase in free radicals or oxidants in the blood. If there is an increase in free radicals continuously, then lipid peroxidation will produce more free radicals that damage tissue. High MDA levels indicate the oxidation process in the cell membrane, conversely a decrease in MDA levels is generally followed by an increase in high antioxidant status[7].

Continuous exposure toluene can have adverse effects on health. Workers who use toluene as a solvent can be affected by health problems such as dizziness, vertigo, eye irritation, skin irritation, breathing, liver, kidney, and central nervous system disorders[5]. Exposure toluene can have acute and chronic effects on the human body system. Workers who are exposed to this substance can experience neuropsychological symptoms, such as mild headaches, fatigue, and cognitive impairment. The effects of solvents on psychological conditions and emotional disorders include depression, headaches, fatigue, anxiety, feelings of motion sickness, and insomnia. Other disorders may include difficulty concentrating or remembering, fatigue and drowsiness. Low toluene exposure to the nerves can cause neurological disorders[8]. There are many factors in the use of solvents related to neurological disorders including personal factors such as age and gender, inappropriate behavior such as smoking and alcohol consumption, not using personal protective equipment such as masks and gloves, improper working conditions, working hours long, and also exposure pathways to the body such as the respiratory tract and skin[9]. A serious health problem with toluene is its effect on the central nervous system which can occur temporarily, such as headaches, dizziness or unconsciousness[10].

Workers who are exposed to toluene are more likely to experience complaints of short or forgetful memory, often feel irritated for no particular reason, often feel depressed for no particular reason, have abnormal heart beats, often sweat for no particular reason, often headaches, weakness in the arms or legs and trembling in the hands[11]. Based on preliminary studies, the room where the respondent works has a poor ventilation system. This causes toluene vapor does not circulate properly in the room and can be inhaled by workers. Most workers rarely use Personal Protective Equipment (PPE) such as masks because they feel uncomfortable and sometimes forget to wear while doing their work.

Methods

This research is observational with quantitative approach and cross sectional design. The study was conducted on workers in X Surabaya printing. The number of respondents in this study were 19 people. The variables studied were toluene concentration, malondialdehyde levels, and health complaints. Data

were analyzed using Pearson correlation test.

Data on the characteristics of respondents were obtained through a questionnaire. The concentration of toluene in the air was measured at three points using the NIOSH 1501 method with the Gas Chromatography Technique[12]. The measurement was carried out by the Work Safety and Health Technical Implementation Unit (UPTK3) Surabaya. Measurement of malondialdehyde (MDA) levels was carried out by taking respondent's blood samples and then analyzed at the Nutrition Laboratory of Airlangga University. Health complaints experienced by respondents were obtained through a questionnaire. Testing and data analysis using Pearson correlation test.

Result

Characteristics of Respondents

Table 1. Characteristics of Respondents

Characteristics of Respondents	Frequency	Percentage
Gender		
Male	15	79%
Female	4	21%
Age		
16-25	2	11%
26-35	4	21%
36-45	8	42%
46-55	3	15%
56-65	2	11%
Years of Service		
< 10 years	10	53%
≥ 10 years	9	47%

Based on table 1, most workers were male (79%), aged 36-45 years (42%), had ten years of service <10 years (53%).

Toluene Concentration

Table 2. Distribution of toluene concentrations in X Surabaya printing

Measurement Point	Toluene Concentration (ppm)
Point 1	0,3050
Point 2	2,1516
Point 3	0,4271

Based on table 2, the measurement results show the concentration of toluene at point one in the bending part is the lowest (0.3050 ppm). The second point on the printing press is the highest (2.1516 ppm). The third point in the sorting and finishing (0.4271 ppm). The average measurement of toluene concentration in the air was 1.2311 ppm. The measurement results of toluene concentration in Surabaya X printing air show that the concentration of toluene in the workplace air has not exceeded the TLV set in Indonesian Ministry of Manpower and Transmigration Regulation no. 05 in 2018 (20 ppm).

Malondialdehyde levels

Table 3. Distribution of malondialdehyde levels in X Surabaya printing workers

Respondent number	MDA (MU) Level
1	5.279
2	9.680
3	7.340
4	7.710
5	5.397
6	10.735
7	4.351
8	12.168
9	11.120
10	5.232
11	5.139
12	5.589
13	7.426
14	8.915
15	12.863
16	14.402
17	10.214
18	7.926
19	6.657

Based on table 3 of the 19 respondents who had taken blood samples the highest levels of malondialdehyde were 14.402 MU and the lowest was 4.351 MU. The average yield of malondialdehyde workers in X Surabaya printing was 8,323 MU.

Relationship of Toluene Concentration and Malondialdehyde Level

Table 4. Statistical Test Results between Toluene Concentration and Malondialdehyde Level

Dependent Variable	Independent Variable	P value	Correlation coefficient
Toluene concentration in the air	Malondialdehyde levels	0,508	-0,162

Based on Pearson correlation test results there is no significant relationship between toluene concentration and malondialdehyde levels with a value of p = 0.508 (p> 0.05).

Health Complaints

Table 5. Distribution of health complaints of printing workers X Surabaya

Health Complaints	Yes		No	
	n	%	n	%
Headache	12	63%	7	37%
Tottering	1	5%	18	95%
Breathing problem	5	26%	14	74%
Cough	14	74%	5	26%
Decreased appetite	1	5%	18	95%
Sore eyes	2	10%	17	90%
High tempered	1	5%	18	95%
Nausea	2	10%	17	90%
Itchy Skin	1	5%	18	95%

Based on table 5, most respondents experienced health complaints due to exposure toluene at work, including coughing (74%), headaches (63%), and shortness of breath (26%).

Discussion

Of the three measurement points is the bending, printing, sorting and finishing the highest was 2.1516 ppm and the lowest was 0.3050 ppm with the average of 1.2311 ppm. From these results value of toluene concentration in printing air X did not exceed the toluene Threshold Value (TLV) determined by Indonesian Ministry of Manpower and Transmigration Regulation Number 05 of 2018 (20 ppm)[13]. Although the concentration of toluene is below the normal limit, continuous inhalation this can affect the amount of toluene that enters the body[14]. Workers exposed to toluene in the long term can potentially have a negative impact on the health of X Surabaya printing workers.

In this study no significant relationship was found between the concentration of toluene in the air and MDA levels in Surabaya X printing workers. However, the longer workers are exposed to toluene compounds, the more and accumulates in the body resulting in an increase in free radicals and lipid peroxidation. Lipid peroxidation subsequently results in MDA. Subandrate & Syafudin (2016) found that the average level of MDA in gas station employees was higher than the

average level of MDA control[15]. This is because the oxidant compounds in the gas station environment are metabolized into free radicals which oxidize lipids and form MDA. This is in line with Giyanti's research (2018) which states that MDA levels in the group exposed to shoe craftsmen are significantly higher than MDA levels in the group not exposed to toluene[16]. According to Moro et. al (2012), the levels of malondialdehyde were significantly higher in painters compared to controls with MDA levels relatively nearly as high as those of controls ($p < 0.001$)[17]. Research by Dewi & Ayuniastuti (2019) also shows that there are significant differences between the MDA levels of gas station attendants who are higher compared to the control group[18].

From the results of interviews with the questionnaire, health complaints experienced by Surabaya X printing workers were quite varied. The results of research on health complaints due to toluene exposure showed that the most common health complaints experienced by workers include coughing, headaches and shortness of breath. Based on research conducted in Magelang, complaints felt due to toluene exposure such as shortness of breath, dizziness, weakness and nausea[19]. Other research conducted in the Ciomas shoe industry, Bogor, there were complaints or health problems that were felt more than 50% were colds, fatigue, headaches, and tingling. The complaint is thought to be due to acute exposure to toluene[20].

Haen & Oginawati's research (2012) conducted in the shoe industry shows that many respondents have health complaints that are identical to the symptoms of BTX exposure, including dizziness, nausea, weakness and shortness of breath[21]. A study in Thailand described psychosomatic symptoms including headaches, shortness of breath, lethargy, fatigue, nausea, vomiting, and loss of appetite in workers exposed toluene[9]. Not using PPE when working can increase exposure to toluene that enters the body through inhalation and results in an increased risk of health complaints experienced by workers. This is most likely caused by risky behavior of workers, where most workers do not wear masks when working.

Conclusion

The results of this study indicate that there was no significant relationship between the concentration of toluene in the air and malondialdehyde levels in X Surabaya printing workers. The concentration of toluene in the air in Surabaya X printing is safe because it is under the TLV recommended by Indonesian Ministry of Manpower and Transmigration Regulation No. 5 of 2018. The higher the toluene concentration, the higher the concentration of malondialdehyde in workers. Health complaints due to toluene exposure are most numerous and often experienced by workers including coughing, headaches, and shortness of breath.

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The Relationship among Technology Utilization, Obedience to Law and Smoking Culture In Community with Smoking Behavior in Male Adolescents in Semarang Indonesia

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Abstract

Background*:* Smoking behavior in adolescent is still a problem recently. The number continue to increase. This study purpose was to investigate the connectedness of technology utilization, obedience to law, and smoking culture in community with smoking behavior in adolescent in Semarang.

Method: This study used cross sectional method. The sample was 104 adolescents with range age 15-17 years old. The sample was chosen using purposive sampling. Data collection using a questionnaire. Four aspects namely demographic data, technology utilization, obedience to law, and smoking culture in community were measured. Those variables the compared to smoking behavior of adolescents. The data then analyzed with Spearman's Rho ($\alpha = 0.05$).

Conlusion: This study found that technology has no significant correlation with smoking behavior (r=0.017; p=0.864). There is significant correlation between obedience to law (r=0.284; p=0.003), and smoking culture in community (r=0.251; p=0.014) with smoking behavior in adolescences. It is necessary for efforts from various parties to overcome smoking behavior in adolescents such as the need for socialization in the community to participate in preventing adolescents who smoke in their environment.

Keyword: *adolescence; obedience to law; smoking behavior; smoking culture; technology utilization.*

Introduction

Smoking for teens becomes a symbol of maturity [1]. Smoking behavior in adolescents generally starts because of following an adult, following a trend, having a heavy smoker friend, making a means to escape from problems until unwittingly smoking becomes a habit in adolescents [2]. Smoking is a risk factor for early death. The World Health Organization (WHO) in 2014 mentioned smoking has killed around 6 million people per year [3]. The percentage of deaths due to smoking in ASEAN countries in Indonesia is in the first rank (46.16%) with the age trend of starting smoking is in age group of 15-19 years (55.4%), [4]. Based on preliminary studies at one of senior high school in Semarang Regency, it is found that 45% of male students are active smokers. Previous research [5] found that the higher the knowledge of adolescents about smoking, the lower the tendency to smoke.

Leininger's Sunrise Model Theory suggests that technological factors; religious and philosophical factors; kinship and social factors; cultural values and lifestyle; legal and political factors; economic factors; and educational factors influence behavior [6]. Different technology usage, state of law dan culture might be have a different influence on adolescent\nt smoking behavior [5,7,8].

The technological factor is the use of technology to find information about health problems, sick health perceptions, or medical habits [9]. Previous study showed that 91.7% of teenage boys aged 15 years and over accessed information about cigarettes on television, roadside billboards, and other sponsor media [10,11]. One of the efforts made by the government is the Directorate General of Primary and Secondary Education of the Ministry of Education and Culture, concerning prohibitions on students carrying cigarettes. Legal regulations related to cigarettes such as Government Regulation of the Republic of Indonesia Number 109 of 2012 concerning Safeguarding Cigarettes for

Health.

Smoking has become a deeply entrenched culture in Indonesia, as in meetings or gatherings it will not feel familiar if you have not offered cigarettes [12]. Culture is everything that is learned and experienced socially by community members. Ethnic and cultural variations in smoking reflect the multiple interactions between income, cigarette prices, culture, stress, heredity, age, and type of cigarette advertisements [13]. However, the inculcation of smoking culture cannot be explained in the age group under 18 years in Semarang.

Based on the above background the researcher intends to explain about the "Relationship between Technology Factors, Legal Factors, and Culture with Smoking Behavior in Adolescent Boys Based on Transcultural Nursing Theory in SMA Negeri 1 Bergas". Explaining the relationship of technology, law, and culture with smoking behavior in adolescent boys based on the theory of Transcultural Nursing in SMA Negeri 1 Bergas.

Method

This study used a cross sectional design.

The population in this study were male students who smoked or had smoked in Senior High Schoo of Bergas totaling 142 students. The sampling technique in this study sampling is done by using purposive sampling.

The questionnaire used was based on transcultural nursing theory which has been modified. This questionnaire was tested for its validity and reliability toward 12 respondents r=0.576 is obtained. Based on the analysis results, it can be concluded that the instrument is valid.

Study permit were obtained from school. Then, respondents were chosen through puposive sampling, after obtaining the research subject by giving an informed consent letter and the respondent was collected at one time in one designated place for research data collection. The questionnaire was distributed to respondents after study. Subjects were asked to fill out biodata and questionnaires provided to determine their smoking behavior. After the questionnaire was collected and completed, health education related to the study were delivered to provide positive health benefits for students.

This study was conducted in line with ethical *standards such as anonymity, informed consent,* voluntary participation, privacy and confidentiality, justice and beneficence, and right to review.

Results

The majority of respondents aged 17 years, consuming 1-4 cigarettes per day. The majority of respondents want to quit smoking, have been smoking for 1-5 years, almost all respondents have smoker friends as much as 99%, majority of respondents have parents smokers as much as 54.8%, the reason smoking shows that the majority of respondents smoke because they join friends / invite friends as much as 44.2%, the majority of respondents receive pocket money in range of 10.000 idr-15.000 idr, the majority of respondents living with parents as much as 86.5, the majority of respondents fill their leisure time with sports activities as much as 60.6%. (table 1)

Table 1 Demography data of respondents

Variable	n(%)
Age (years)	
15	12 (11.5)
16	25 (24)
17	67 (64.4)
Cigarette consumed in a day	
1-4	80 (76.9)
5-14	20 (20)
>15	4 (3.8)
Desire to stop smoking	
Yes	95 (91.3)

No	9 (8.7)
Length of smoking behavior (year)	
<6 month	35 (33.7)
6 month-1 year	18 (17.3)
1-5 year	42 (40.4)
>5 year	9 (8.7)
Friend who are smoking	
Yes	103 (99)
No	1 (1)
Parent who are smoking	
yes	57 (54.8)
No	47 (45.2)
Reason of smoking	
Going along with friends / friend invitation	46 (44.2)
Parents are smoking	2 (1.9)
Relieve stress because of many problems	28 (26.9)
It has become a habit due to nicotine dependence	28 (26.9)
Pocket money	
10.000-15.000 idr	77 (74)
16.000-20.000 idr	17 (16.3)
21.000-25.000 idr	4 (3.8)
>25.000 idr	6 (5.8)
Living place	
With parents	90 (86.5)
Dormitory	5 (4.8)
Other Family house	8 (7.7)
Friend's house	1 (1)
Spare time activity	
Enjoying smoking with friends	28 (26.9)
Reading book, magazine, etc	13 (12.5)
Exercising futsal, badminton, etc	63 (60.6)

There is no significant correlation between technology and smoking behavior (r=0.017; p=0.864). There is significant correlation between obedience to law (r=0.284; p=0.003), and smoking culture (r=0.251; p=0.014) with smoking behavior in adolescences (table 2).

Table 2 The relationship of technology, law and culture toward smoking behavior in adolescent

Variable	n(%)	p-value	r
Smoking behavior			
Mild	73 (70.2)		
Moderate	30 (28.8)		
Severe	1 (1)		
Technology utilization		0,864	0,017
Underutilize	7 (6.7)		
Enough utilize	91 (87.5)		
Good utilize	6 (5.8)		
Obedience to law		0,003	0.284
Less	2 (1.9)		
Moderate	53 (51)		
Good	49 (47.1)		
Smoking culture in society		0,014	0,251
Less	16 (15.4)		
Moderate	82 (78.8)		
High	6 (5.8)		

Disussion

This study result showed that there is no correlation between technology utilization with smoking behavior. Adolescent have a good behavior to utilize technology to find information about smoking behavior but has nothing to do with their smoking behavior. Health seeking behavior of information is influenced by health workers' attittude, the needs, and interests of the community (Leininger in Loedin, 2003). Smoking behavior advertisement that delivered through technology media could influence the smoking behavior in adolescences [14–16]. Technology utilization such as mass media and electronic media could be an exposure to adolescent about smoking behavior through its advertisement. Adolescents knowledge about smoking effect also can not prevent of their smoking behavior [17]. In this study, this technology usage is not correlate with smoking behavior. Eventhough adolescent in this study often watch advertisement about smoking, they said that those advertisement is not the main reason of their smoking behavior.

Based on Leininger theory, the use of technology can be influenced by the specialization of the individual itself. so it can be said even though the individual uses technology to find information about cigarettes quite well but not necessarily these individuals engage in smoking [18]. Adolescents in this study agreed that the use of technology with cell phones is easier to find information about cigarettes. It can be concluded that although respondents agreed that cellphones usage can foster the information finding, but it depends on adolescent interest about what information that they are searching for.

This study showed that there was a significant correlation between law obedience with smoking behavior in adolescents. The less obedience towards law, the more severe adolescents' smoking behavior. Policies and regulations will affect individual activities in cross-cultural nursing care (Leininger in Andrew and Boyle, 1995). Regulations related to cigarettes in adolescents is concerning prohibitions on students carrying cigarettes. Adolescent agreed about prohibition in students carrying cigarettes but they still smoking. Smet (1994) explained that political and legal factors are protective for non-smokers and efforts to reduce smoking behavior. Although there are regulations in the school regarding smoking bans but are not deterred for adolescent smoking behavior. A study found that teacher role model was correlated with their students smoking behavior [19].

Many adolescents reported that they want to stop smoking. This intention to stop a smoking behavior need to be supported by adolescents environment such as school regulation, teacher behavior as role model and regulation of smoking in community [19]. Regulations will affect a person's attitude to act as Leininger said, rules can affect individual activities, so depending on the attitude taken by the respondent if the respondent takes a positive attitude then it will obey the rules, if the respondent takes a negative attitude then will violate it. So it can be said that the more respondents have low legal violations, the more types of mild smoking behavior, while the less severe violations of the law, the less the type of heavy smoking behavior.

This study found that there was significant correlation between Smoking culture in society with smoking behavior in adolescents. Culture is the norm or rules in the actions of group members who are studied and shared and give instructions in thinking, acting and making decisions [20]. The smoking culture is included in a main concepts of transculturalism, which means the perspective of an individual or group in viewing their lives, giving rise to beliefs and values (Leininger 1984 in Pratiwi 2011). Community culture is a factor that influence smoking behavior, one of them is socio-cultural factors which say that cultural habits and prestige will influence smoking behavior in individuals [21].

Most of adolescents reported that the have a lot of smokers friends and their parents also smoking. The existence of a cultural relationship with smoking behavior can occur because as explained above that adolescents smoke because of teachings in the family, belief in the benefits of cigarettes as traditional medicine, and smoking habits in daily life which is reinforced by Leininger's theory that says culture can occur if the individual's perspective give rise to belief and value [22]. There are 3 strategies in transcultural nursing namely maintaining culture, cultural negotiation, and changing culture (Leininger in Pratiwi 2011). It can be said that smoking culture is detrimental to health status, which can use a strategy of changing the culture from smoking to non-smoking with a life plan that is in accordance with the individual's belief (Pratiwi, 2011). The more respondents do not have a smoking culture, the more types of mild smoking behavior and the fewer respondents who have a culture the less the type of heavy smoking behavior.

Conclusion

The results of this study indicate that the use of technology is not related to smoking behavior in adolescents. Although there is a lot of information about smoking behavior obtained from the internet as well as many advertisements about smoking, it does not change the views of adolescents about their smoking behavior. Compliance with the law can prevent adolescents from engaging in smoking even though adolescents continue to smoke but not in the school environment. Smoking culture in the community can be a trigger for smoking behavior in adolescents. from this conclusion it is necessary for efforts from various parties to overcome smoking behavior in adolescents such as the need for socialization in the community to participate in preventing adolescents who smoke in their environment. in addition, the participation of parents and teachers is also important to instill perceptions about smoking in adolescents.

Conflict of Interest: None

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Detection of Multidrug-Resistant (MDR) *Escherichia coli* Isolated from Raw Milk in East Java Province, Indonesia

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Abstract

The aim of this study is to figure out the profile of antibiotic resistance in *E. coli*, sourced from raw milk, obtained from five districts in East Java Province, Indonesia. A total of 250 samples were tested using the Kirby-Bauer Test method. The result revealed that the highest percentage of antibiotic resistance was Tetracycline (17.05%), followed by Streptomycin (14.2%), Trimethoprim (9.66%), Chloramphenicol (7.95%) and Aztreonam (1.7%). A total of 16 *E. coli* isolates (9.1%) were detected as MDR, and 3 *E. coli* isolates (1.7%) were suspected as presumptive ESBL. Thus, threat of multidrug resistance *E. coli* possibly sourced from the milk.

Keywords: *Escherichia coli*, multidrug resistant, raw milk, antibiotic susceptibility test.

Introduction

Contamination of milk with microorganisms is something that cannot be completely avoided, therefore microbial contamination in milk is an important point in measuring its quality[1]. Some microorganisms can be found in milk and other dairy products, one of which is *E. coli*. Pathogenic *E. coli* infections in humans, which originate from milk, are widely reported in several cases in developing countries[2].

The case of *E. coli* contamination that is resistant to various antibiotics has been widely studied. At present, a focus on public health is being developed regarding antibiotic resistance due to its use in the treatment of livestock, following the conditions of human diseases in developing countri[3]. Antibiotics are used as prevention and treatment of various cases of *E. coli* bacterial infections that attack livestock[4]. However, inappropriate and irrational administration of antibiotics results in the emergence of antibiotic resistance in *E. coli* bacteria[5].

E. coli can be a reservoir of various antibiotic resistance genes, which can be transmitted to other bacteria. Raw milk, including one of the foodstuffs of animal origin which is a potential source in the spread of multidrug-resistant bacteria, which impacts on human health[6]. Therefore, the objective of this study was to detect the presence of multidrug resistant *E. coli* from raw milk in East Java Province, Indonesia.

Materials and Methods

Raw milks were taken from dairy farm with a total of 250 samples, located in Kediri, Probolinggo, Pasuruan, Batu and Blitar Region, East Java Province, Indonesia, in the period between August-November 2019. Raw milk samples contained in wrapped sterile plastic and were taken to the laboratory using cool box container at 4˚C.

Each sample were cultured into enrichment media Brilliant Green Bile Lactose Broth (Merck, 105454) and incubated at 37ºC for 18-24 hours. The positive results characterized by change of media from green colour to cloudy green and gas presence in Durham tube[7]. Then were streaked onto Eosin Methylene Blue (EMB)

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agar (Merck, 101347) and incubated at 37 °C for 18-24 hours[8]. Colonies that showed metallic green colour (Figure. 1) was purified and continued to identification with IMViC test.

Antibiotic sensitivity testing was done using Kirby-Bauer disc diffusion assay on medium Mueller-Hinton agar (Oxoid, CM0337). Antibiotics disks used was Tetracycline 30 µg (Oxoid, CT0054), Streptomycin 10 µg (Oxoid, CT0047), Chloramphenicol 30 µg (Oxoid, CT0013), Trimethoprim 5 µg (Oxoid, CT0057), Aztreonam 30 µg (Oxoid, CT0264) (Fig. 2 and 3). Interpretation of results by measuring the diameter of the inhibitory zone formed, based on Clinical and Laboratory Standards Institutions[9].

Table 1. Antimicrobial Resistance Profile

Location (Code)	Sample Size	Confirmed E. coli	Resistant to					Multidrug Resistant	Presumptive ESBL
			TE	S	W	C	ATM		
Kediri (K)	50	35	7	5	1	4	0	3	0
Probolinggo (A)	50	36	3	2	3	2	0	2	0
Pasuruan (G)	50	30	7	5	8	1	1	4	1
Batu (H)	50	38	4	5	1	1	2	1	2
Blitar (S)	50	37	9	8	4	6	0	6	0
Total	250	176	30	25	17	14	3	16	3
Percentage (%)	100	70.4	17.05	14.2	9.66	7.95	1.7	9.1	1.7

Info: TE: Tetracycline, S: Streptomycin, W: Trimethoprim, C: Chloramphenicol, ATM: Aztreonam

Table 2. Measurement of inhibition zone on antibiotic sensitivity test of Multidrug Resistant and Presumptive ESBL *E. coli*

No.	Sample Code	Diameter of inhibition zone of antibiotics in mm					MDR	Presumptive ESBL
		TE	S	W	C	ATM		
		30 µg	10 µg	5 µg	30 µg	30 µg		
1.	K-03	6 (R)	6 (R)	6 (R)	6 (R)	36 (S)	+	-
2.	K-04	6 (R)	10 (R)	30 (S)	6 (R)	33 (S)	+	-
3.	K-36	6 (R)	6 (R)	30 (S)	6 (R)	34 (S)	+	-
4.	A-26	6 (R)	6 (R)	6 (R)	6 (R)	31 (S)	+	-
5.	A-44	16 (S)	9 (R)	6 (R)	6 (R)	40 (S)	+	-
6.	G-12	6 (R)	6 (R)	6 (R)	22 (S)	30 (S)	+	-
7.	G-31	17 (S)	11 (R)	7 (R)	16 (I)	6 (R)	+	+
8.	G-35	6 (R)	6 (R)	6 (R)	23 (S)	31 (S)	+	-
9.	G-43	8 (R)	6 (R)	26 (S)	6 (R)	30 (S)	+	-
10.	H-11	6 (R)	6 (R)	6 (R)	6 (R)	30 (S)	+	-
11.	H-15	9 (R)	6 (R)	6 (R)	28 (S)	34 (S)	+	-
12.	H-28	10 (R)	6 (R)	25 (S)	7 (R)	28 (S)	+	-
13.	H-37	9 (R)	9 (R)	22 (S)	6 (R)	27 (S)	+	-
14.	H-44	7 (R)	10 (R)	30 (S)	6 (R)	29 (S)	+	-
15.	H-45	7 (R)	10 (R)	31 (S)	6 (R)	30 (S)	+	-
16.	S-25	7 (R)	16 (S)	24 (S)	7 (R)	15 (R)	+	+
17.	S-38	18 (S)	11 (R)	27 (S)	30 (S)	10 (R)	-	+

Info: R: Resistant, I: Intermediate, S: Sensitive.



Figure 1. E coli in EMB Agar

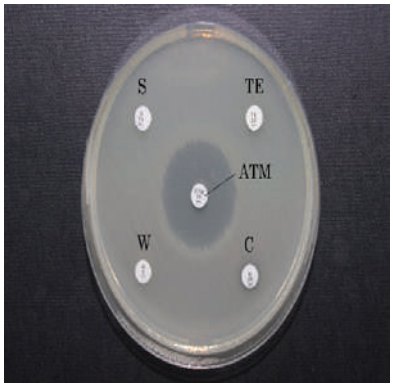


Figure 2. Isolate resistant to 4 antibiotic discs (K-03)

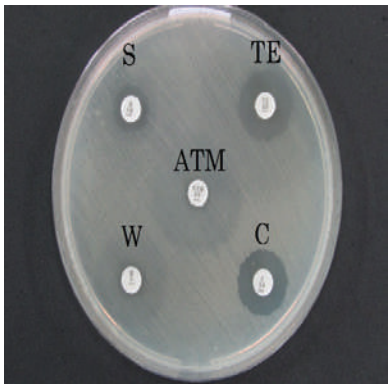


Figure 3. Isolate of Presumptive ESBL (G-31)

Results and Discussion

Among the total 250 raw cow milk samples collected from different dairy farms, 176 samples (70.4%) were found to be positive for *E. coli* (Figure 1). The antimicrobial resistance profiles of the bacterial isolates were summarised in Table 1. *E. coli* showed resistance to antibiotics like Tetracycline (17.05%), Streptomycin (14.2%), Trimethoprim (9.66%), Chloramphenicol (7.95%) and Aztreonam (1.7%). A total of 16 multiple drug resistance (MDR) isolates were found (8.52%) with six different patterns (Figure 2.). The highest MDR patterns noted was Tetracycline, Streptomycin, and Chloramphenicol in 7 out of 16 MDR isolates (Table 2). Three presumptive ESBL isolate detected from Pasuruan and Blitar, which resistant to Aztreonam disc (Figure 3).

The result of this study showing high level for *E. coli* found in milk indicating a poor sanitation practices of farmers during milking process[10]. This number has the same level reported by Chey et al.[11] in Malaysia, stated that the highest prevalence (72.2%) of *E. coli* originated from the Eastern zone of the research location. Compared to other developing countries, namely Bangladesh, as much as 75% of milk samples studied contain E coli bacteria[12].

Tetracycline has the highest antibiotic resistance because it is commonly used in the veterinary field, as well as other antibiotics used in this study such as aminoglycoside (Streptomycin), macrolide (Chloramphenicol) and sulphonamide (Trimethoprim)[13]. The use of broad-spectrum antibiotics such as the tetracycline and beta-lactam classes is more common in clinical mastitis cases of dairy cows in Europe, due to their effective treatment results. For respiratory and digestive tract problems, the tetracycline and aminoglycoside groups are the first choice antibiotics, while the second choice is the macrolide group and the combination of sulfonamid-trimethoprim drugs which have a significant impact on microbial activity in the rumen, and the last choice is the cephalosporin class three and four antibiotics (the sulfonamide-trimethoprim drug combination which has a significant impact on microbial activity in the rumen, and the final choice is the cephalosporin class three[14].

Seventeen MDR and Presumptive ESBL *E. coli* isolates that have been studied in this study, have almost the same pattern of resistance to one antibiotic, that is resistant to aminoglycoside class drugs, (Streptomycin), except for S-38 isolates which are still intermediate status. This resistance can be attributed to the effectiveness of the drug streptomycin in the treatment of dairy cows affected by mastitis in Indonesia. According to Riyanto et al.[15], a good treatment for dairy cows suffering from mastitis is a combination of the Penicillin-Streptomycin antibiotic. Although in this study the level of resistance of the drug Streptomycin is quite low (14.2%), but there is a need to monitor the use of antibiotics Streptomycin in dairy cows in Indonesia.

Based on this study, Trimethoprim and Aztreonam antibiotics may still be used to treat MDR bacteria that are found because there are still several isolates of *E. coli*

MDR that are sensitive to them. Trimethoprim is usually combined with Sulfonamide which works synergistically with broad spectrum activity. In the case of urinary tract infections, Trimethoprim has been shown to be effective in killing infections caused by *E. coli* bacteria[16].

While monobactam (Aztreonam) has not been defined for veterinary applications, the present of resistant isolates has the possibility of ESBL producing *E. coli* found in raw milk. The finding of ESBL Enterobacteriaceae sourced from milk, indicates the presence of environmental contamination, and the lack of environmental sanitation when milking is carried out[17]. *E. coli* is a bacterium that can be a reservoir of various antibiotic resistance genes, including the beta lactam antibiotic resistance gene, which makes *E. coli* capable of producing the β-lactamase enzyme[7,18]. Cage sanitation, enclosure base, and drainage of the cage need to be considered by breeders to prevent contamination of milk by the ESBL *E. coli* presumptive bacteria.

The occurrence of antibiotic resistance is known to originate from the bacterial plasmid which is able to decrease the resistance gene and spread it to other bacteria around it. *E. coli* is a bacterium that is well known as the owner of resistance genes for various antibiotics[19]. Various resistance genes can accumulate in bacterial plasmids, usually in plasmid R (resistance), which is the cause of the discovery of bacterial isolates that are resistant to various antibiotics. In the R plasmid, there is an integron sequence, which is able to add various kinds of resistance coding genes to the base arrangement in the plasmid, so that a series of resistance genes is created in a bacterium[20, 21].

E. coli MDR isolates are very common in various countries and are responsible for a series of infections with high severity and difficult to treat. In Canada, there are studies on cases of urinary tract infections due to *E. coli* bacteria, of which 60% are cases of *E. coli* infections with resistance to more than 3 classes of antibiotics. Consumption of food from raw uncooked animals, inter-regional travel habits, and contact with reservoir animals is associated with an increased risk of urinary tract infections caused by *E. coli* MDR[22].

The multidrug resistance reported in the current study might be due to high and irrational antimicrobial use in individual cows to treat various diseases affecting the dairy sector. As a presence of resistance to many antimicrobial drugs has become a public health problem according to the fact that food borne outbreaks would be difficult to treat and this findings of MDR *E. coli* in food supply establish a source for communicable resistant genes[23].

Conclusion

Multidrug resistant E coli is a public health threat that can be sourced from the consumption of foods derived from animals, one of which is raw milk. From this study it was concluded that E. coli is prevalent in raw milk and has multidrug resistance (MDR) properties from East Java Province, Indonesia. E. coli found in milk is expected to increase public awareness of the dangers it poses. In this way, the application of sanitation and monitoring of antibiotic use must continue to be promoted.

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Ethical Clearance: Raw milk were used in this study, hence ethical clearance was not necessary. Raw milk samples were collected from dairy farms East Java province, Indonesia.

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First Report of Protein and Fat Level of Alabio Duck (*Anas platyrhynchos* Borneo) Eggs in Hulu Sungai Utara, Indonesia for Improving Human Health

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Abstract

Indonesian local ducks are native germplasm of Indonesia which has the genetic quality and has the potential to be developed as a productive egg producer. Each type of duck has a different chemical composition in their eggs. The purpose of this study is to find out the levels of protein and fat in Alabio duck (*Anas platyrhynchos* Borneo) eggs in Hambuku Raya Village and Putat Atas Village, Hulu Sungai Utara, South Kalimantan, Indonesia. This study used a completely randomized design (CRD). Interestingly, we showed that the level of protein and fat in Alabio duck eggs was relatively good. Therefore, this finding is the basic data for further research on Alabio duck in the future.

Keywords*: Anas platyrhynchos Borneo, fat level, protein level, eggs.*

Introduction

Eggs are known as one of the nutrition sources of an animal which is highly nutritious. Egg contains a high-quality protein because it has a complete composition of essential amino acids that are used as a marker for determining the protein quality from other foods[1]. In addition, duck eggs may be eaten as table eggs where they can be scrambled, fried, poached, and boiled, or they can substitute for chicken eggs as an ingredient in quiche or baking, for example[2].

Duck poultry is one of the poultry business has been developed a lot in Indonesia, although it is not as popular as chicken. Ducks have great potential as eggs and meat producers. Compared to other poultry animals, ducks have good immunity to disease. The duck business has relatively smaller interests, so it has the potential to be developed[3]. Ducks donated more than 18,000 tons of eggs, around 16% of national egg production in 2005[4]. According to the Directorate General of Livestock and Animal Health of Indonesia, national duck egg production in 2011-2015 was relatively stable. The growth increase from 2014 to 2015 was 3.49%.

According to Muharlien (2010), Alabio duck eggs are very popular especially for the people of South Kalimantan, who value duck eggs more than chicken eggs. In the market, Alabio duck eggs are classified as pond duck eggs and beach duck eggs, which are distinguished by the size and degree of yolk, where the pond duck eggs are larger and have a reddish-yellow yolk[5]. The nutritional content of duck eggs is strongly influenced by the food consumed by these ducks[6]. One duck egg contains several components, including 66% water and 34% dry contents which are distributed into 12% protein, 10% fat, 1% carbohydrate and 11% ash. Egg yolks contain about 48% water and 33% fat. Egg yolks also contain vitamins, minerals, pigments and cholesterol. Egg white consists of protein, especially lysozyme which has the anti-bacterial ability[7].

Andriani *et al*. (2015) explained that the proximate levels are the results of chemical analysis to identify the nutritional content of food ingredients such as protein and fat content. The chemical composition of eggs is one of the important factors in poultry nutrition. Therefore,

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the selection of food has to focus on the content as well so that it can support healthy living. Factors that determine egg production are genetic or nationality, nutrition, age, age of production, type of cage, maintenance system (extensive, semi-intensive, and intensive), and temperature[7].

Egg white protein in the form of ovalbumin, ovotransferrin, ovomucoid and lysozyme is synthesized by tubular glands, while avidin and ovomucin are synthesized by goblet cells. Protein in eggs will increase if the level of protein in the feed is adequate. The determination of protein needs is always associated with the energy level in the feed because protein can be used as an energy source and is needed in the formation of protein. Jaya *et al*. (2019) stated that laying eggs period requires amino acids supplement from the feed, especially essential amino acids. Thus, a study on analyzing the level of protein and fat in Alabio duck egg is necessary in order to provide information to the public about the nutrient content of the Alabio duck egg[8].

Materials and Methods

Time and Location:

The study was conducted in May 2018. The study locations were Hambuku Raya Village, and Putat Atas Village, Sungai Pandan District, Hulu Sungai Utara, South Kalimantan, Indonesia. Hulu Sungai Utara is located between 2°-3° South Latitude and 115°-116° East Longitude, with a height of 0-7 meters above sea level.

Experimental Animal:

This study consisted of three groups. Each group was located in a different cage and not close to one cage with another. The distance between one cage and another was approximately 1 km. Each group consisted of 200 female Alabio ducks. This 200 population was then divided into 10 flocks with a size of 1.5 m×1.0 m×0.5 m. Each flock had one place to eat and one place to drink made of plastic material. For the lighting, one lamp was placed in each cage with 25 Watt of power. Ten eggs per group were tested to determine the quality of protein and fat levels. The examination was conducted at the Post Harvest Laboratory, Faculty of Agriculture and Animal Husbandry, Lambung Mangkurat University, Banjarmasin, Indonesia.

Experimental Design:

This study used a complete randomized design (CRD) with unidirectional patterns. It consisted of three groups with the same treatment. The study was exploratory or non-experimental because researchers did not perform different treatments.

Data Analysis

Egg production, feed conversion, and egg quality data were analyzed by using analysis of variance (ANOVA) and continued with LSD (if the result is significant). Kruskal-Wallis test was used for data that were not normal, then continued with the Wilcoxon-Mann-Whitney test (if it is significant).

Result and Discussion

The test results are significant if *p*-value is less than 0.05[9]. The average level of protein of Alabio duck eggs in each group was almost the same. Group 1 showed an average of 11.13%, followed by group 2 of 11.06% and group 3 by 11.03%. The fat level of Alabio duck egg in each group was no different. The highest level of fat was experienced by Putat Atas group (group 3). It had 33.34% fat, while group 1 had 33.67% and group 2 had 34.34 % of fat.

Table 1: Protein and Fat Level of Alabio Duck in Putat Atas (PA), Hambuku Raya 1 (HR 1), and Hambuku Raya 2 (HR 2), Hulu Sungai Utara, South Kalimantan, Indonesia.

Variables	N	Groups		
		PA	HR 1	HR 2
Protein Level (%)	10	11.13±0.52	11.06±0.57	11.03±0.67
Fat Level (%)	10	33.34±1.50	33.67±1.04	34.34±1.47

Fats, proteins, and carbohydrates are a group of macronutrients. Protein and fat content in food are very important for animals' and humans' life, because it has inorganic compound needed by the body, namely: C, H, O and N. The roles of protein in the body, among others, are as a mold in the manufacturing process (in chromosomes), antibodies, and to replace damaged tissue cells. While fat plays a role as a constituent of cell walls and biomolecular materials. Fat is also the source of bio-calories, essential sources of unsaturated fatty acids are linoleic and linolenic, and natural sources of oil-soluble vitamins which are vitamins A, D, E, and K[1,4].

Fat has many functions in the body. It plays role as a source of energy after carbohydrates, as energy stored from fat tissue, the fat under the skin works as an isolator to maintain body's temperature, to protect vital organs such as bones and eyeballs, and source of vitamins A, D, E, and K. Some examples of fat sources are meat, oil, and cholesterol, including eggs, and dairy products[1,4].

In this study, the highest egg protein levels were 11.13±0.52. There were no significant differences between each group. Egg protein decreases during the storage period. The egg protein level after 28 days of storage was 9.85%-10.4%. Meanwhile, the protein level of a fresh egg was 12.8%-13.4%[9,10]. Protein is one of the main sources of egg formation. The formation of vitellogenin (vitellogenesis) is the synthesis of lipoprotein in the liver which is controlled by estrogen. Then, vitellogenin is accumulated by blood in the follicles which will then develop into yolk (egg yolk). The follicle is surrounded by blood vessels, when the oocyte is mature, the stigma will tear and ovulation will occur[11,12].

On the other hand, the highest level of duck egg fat in this study was 34.34±1.50. However, there were no differences between each group. This condition is different from Sari *et al*. (2015), that the yolk content in the Rantau Karau Hilir and Hambuku Baru was 34.05-35.95%. This shows the concentration of egg yolk can be influenced by several factors such as the nutritional content of the feed[13].

Conclusion

In sum, the study present that the quality of protein and fat level of Alabio duck eggs was relatively good. Therefore, this finding is the basic data for further research on Alabio duck (*Anas platyrhynchos* Borneo) in the future.

Conflict of Interest : The authors declare that they have no conflict of interest.

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Anxiety Level Assessment to Primigravidae Women (28-40 week) with Hamilton Anxiety Rating Scale (HARS) Method

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Abstract

Background: the prevalence of pregnancy anxiety varies in the different trimesters of pregnancy with high levels in the first and third trimesters. Anxiety in pregnancy is a strong predictor of poor birth processes and infant outcomes.

Methods: This study uses quantitative research with quasi experimental / quasi experimental approaches and one group design pre and post test design with group control. This research will use the Hamilton Anxiety Rating Scale (HARS) method.

Results: There is a significant difference ($p = 0.004 < \propto$) the average level of anxiety in the control group before (16.5 ± 0.80) and after observation (18.17 ± 1.03) this means that Yoga exercises can reduce anxiety levels in third trimester pregnant women. In the control group ($n = 12$) there was no negative rank anxiety level which meant that there was no person with an anxiety level before greater than after. Furthermore, a positive rank indicates 10 which means there are 10 pregnant women with anxiety levels after being higher than before or in other words there are 10 pregnant women who have increased anxiety in the third trimester.

Conclusion: There is a significant difference ($p = 0.004 < \propto$) the average level of anxiety in the control group before (16.5 ± 0.80) and after observation (18.17 ± 1.03), so it can be concluded that prenatal yoga reduces anxiety levels before and after treatment.

Keywords: *Anxiety, Method HARS , Primigravida, Yoga*

Introduction

The period of pregnancy starts from the end of the menstrual period until the birth of the baby, around 266-280 days or 37-40 weeks[1], which consists of three trimesters. Stress and anxiety during pregnancy have been linked to premature babies and low birth weight, possibly through fetuses exposed to glucocorticoids. Anxiety is an uncertain feeling that results from anticipating a state of danger. Someone will feel anxious when faced with change and the need to take different actions. Anxiety is a condition when someone experiences uneasy feelings of concern and worry because of a threat to a person's value or security pattern[2,3]. Anxiety as a concern that is not clear and spread, and associated with uncertain feelings. This emotional state basically has no specific object, is experienced subjectively and communicated interpersonal[4,5]

A prospective study among 160 Iranian third trimester pregnant women, shows a significant relationship between general anxiety and fear of childbirth. Nulliparous women reported higher levels of anxiety at 28 and 38 weeks multiparous gestational age[6,7]. Studies among 660 low-risk pregnant women

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in the third trimester revealed a significant relationship between childbirth fears and general anxiety and higher scores of childbirth fear in nulliparous women compared to multiparous women[8,9,10.]

Yoga has several advantages because it is more affordable, can be learned and practiced using digital video discs (DVD). Yoga is a comprehensive system between physical (asanas), breathing exercises (pranayama), concentration and meditation (dharana and dhyana) and contemplative practice[11.] Yoga can also be interpreted as a holistic approach in the form of physical, mental and spiritual. Physiologically, yoga exercises will reverse the effects of stress involving the parasympathetic part of the central nervous system.[12,13]

Several studies have proven the benefits of yoga in pregnancy. Hope to mediate the reduction in abnormal activity of the maternal sympathetic adrenal medullary system (SAM) and hypothalamic pituitary adrenocortical axis (HPA-axis)[14,15]. Several studies have reported positive effects of yoga on depression and anxiety positive effects of yoga on the well-being of pregnant women and reducing stress, anxiety and reducing labor pain, the effect of yoga in overcoming discomfort in pregnant women reducing pain during labor [16.,17]

In this study, the Hamilton Anxiety Rating Scale (HARS) method will be used to measure anxiety levels so that overall anxiety levels are known. The HARS scale has been proven to have a high enough validity and reliability to measure anxiety in trial clinical studies of 0.93 and 0.97. This condition indicates that the measurement of anxiety using the HARS scale will obtain valid and reliable results[18,19]. Therefore most interested researchers look at anxiety levels of pregnant women and the effects of yoga exercises on third trimester pregnant women.

MATERIALS AND METHODS

This study used quasi experimental / quasi experimental approaches and one group design pre and post test design with group control. In this study the researchers' treatment or intervention was prenatal Yoga exercises in third trimester pregnant women (UK 28-40 weeks),

The sample in this study were 11 respondents who will be intervened. Calculation of sample size needs to be considered the existence of a sample drop out then corrected by 10% the number of samples as many as 12x2 = 24 respondent.

Result

Table 1 Test results for normality of age data

Group	p-value	distribusi
Control	0.038	Tidak normal
Treatment	0.417	Normal

In Table 1 based on Shapiro-Wilk test results obtained that the age data in the control group showed a p-value = 0.038, this value is smaller than the significance level ∝ = 0.05 which means the control group is not normally distributed. While the age data in the treatment group showed a p-value = 0.417

Tabel 2 The Age characteristic

Group	rerata ± stan.dev.	p-value
Control	22.83 ± 2.86	0.177
Treatment	24.50± 3.37	

Based on Table 2 the Mann-Whitney test results in pregnant women showed no significant difference (p = 0.177> ∝) between the control group and the treatment group. In the control group the mean ± stan.dev value was 22.83 ± 2.86 years. While the mean value ± stan.dev in the treatment group was 24.50 ± 3.37 years.

Table 3 The Result Anxiety Scale Before And After Yoga Treatment

Group observation	Before Rerata ± SD	After Rerata ± SD	p-value
Control	16.5±0.80	18.17±1.03	0.004
Treatment Yoga	16.5±0.67	11.75±3.49	0.002

In Table 3 explains that there is a significant difference (p = 0.004 <∝) the average level of anxiety in the control group before observation (16.5 ± 0.80) with observations after (18.17 ± 1.03) Yoga exercises can reduce anxiety levels in third trimester pregnant women.

Tabel 4 The result comparison Anxiety before anda After in group observation

Group observation	Post-pre	N	Rank rerata	Jmh rank
Control	Rank negative	0a	.00	0.00
	Rank positif	10b	5.50	55
	Seri	2c		
	Total	12		
Treatment Yoga	Rank negative	12d	6.50	78
	Rank positif	0e	.00	0.00
	Seri	0f		
	Total	12		

In Table 4 the explains that there is a significant difference (p = 0.004 <∝) the average level of anxiety in the control group before observation (16.5 ± 0.80) with observations after (18.17 ± 1.03) Yoga exercises can reduce anxiety levels in the third trimester pregnant women.

Discussion

There is now plenty of evidence to suggest that in utero experience is an important determinant of fetal outcomes, One factor that has been widely studied in this regard is the adverse effect of maternal prenatal pressure on birth outcomes. The term 'prenatal pressure' is often used to collectively refer to negative psychological well-being, and includes stress, anxiety, and depression. The prevalence of prenatal distress is estimated at 31%, 28% and 12% respectively for stress, anxiety and depression[20,21.]

Based on the results of data analysis in shows that there are significant differences (p = 0.004 <∝) the average level of anxiety in the control group before observation (16.5 ± 0.80) with observations after (18.17 ± 1.03). The average anxiety level after, it appears, is greater than the average anxiety level before. So this means that in the control group anxiety levels of third trimester pregnant women will be a significant increase.

In the treatment group showed a significant difference (p = 0.002 <∝) the average level of anxiety before being treated (16.5 ± 0.67) and after being treated (11.75 ± 3.49). The average anxiety level after the value is smaller than the average anxiety level before. This means that in the treatment group the anxiety level of trimester III pregnant women experienced a significant decrease. In other words there is the effect of Yoga exercise treatment on the anxiety level of trimester III pregnant women, namely Yoga exercises can reduce the anxiety level of trimester III pregnant women. So the first minor hypothesis has been proven, namely prenatal Yoga exercises affect the level of anxiety in primigravida trimester III.

Prenatal yoga interventions in the treatment group and in the control group only did standard antenatal exercises, exercises were carried out in an integrated manner 1 hour / day in the 20-36 week gestational age range, the results showed respondents in the yoga group experienced a 15.65% reduction in anxiety, while in the control group that only did standard antenatal exercises with anxiety increased by 13.76%, so it can be concluded that regular integrated yoga exercises were more effective than antenatal exercises in reducing anxiety [22,23].

The effect of yoga 20 minutes per day for 12 weeks, compared with social support therapy, the results showed a decrease in anxiety levels in each group. So it can be recommended that yoga can be an effective intervention to reduce anxiety. Increased vagal activity after yoga can explain the observed effect. In another yoga study, prenatal stress decreased 32% in the yoga group and vagal activity increased in the yoga group from baseline by 64% in the 20th week of pregnancy and 150% in the

36th week, indicating increased relaxation. Increased vagal activity indicates that stress related to pregnancy can be significantly reduced by yoga[4,18].

Prenatal yoga reduces anxiety in pregnant women. A number of successful preventive intervention efforts targeting psychosocial and physiological risk factors for perinatal depression have utilized mind-body practices, which embody the idea that the mind interacts with the body to influence physical function, improve symptoms, and improve health[7]. Yoga has sparked a special interest because of its increased acceptance in the Western world and more evidence of its relationship with increased influence, decreased depressive symptoms and reduced cortisol in nonpregnant populations, and currently remembers the many benefits of yoga on psychophysiology so that the requester extends into pregnancy[24,25].

Stress during pregnancy is a unique condition, which requires physical, mental and social adaptation. Animal experiments and human studies have shown that prenatal maternal stress is associated with an increased risk of spontaneous abortion, preterm labor, fetal malformations, and asymmetrical growth retardation. Evidence of long-term functional impairment in offspring after prenatal exposure to stress is limited. Retrospective study. two prospective studies support this effect. on behavioral development with attention deficits, anxiety and disturbed social behavior.

Relationship between maternal psychological health (trait anxiety, perceived stress, and depressive symptoms) during pregnancy or postpartum and baby's visual, language, motor, and overall cognitive development. Results no significant negative consequences on cognitive development in infants from maternal anxiety, antepartum and postpartum depression symptoms, and stress[4,26.]

Prenatal depression contributes to the incidence of prematurity, developmental delay, behavior problems in childhood and adolescence, highlighting the need for prenatal intervention. Pharmacological and non pharmacological therapy. Non-pharmacological therapy, in the form of massage and yoga therapy can be used as an alternative therapy that is also very effective [6,14,18]. For example, moderate pressure massage therapy has been shown to reduce prenatal depression and prematurity. Yoga has the advantage of being more affordable, can be learned and practiced using DVDs.

In yoga pregnancy exercise movements contained relaxation effects that can stabilize the emotions of pregnant women, because the yoga movement focuses attention on the rhythm of breathing, prioritizing comfort and safety in practice so as to provide many benefits Yoga practice is a physical treatment that can also provide psychological effects because it provides a relaxing effect on a person's body and affects several psychological aspects of people who do it, so it is said to help reduce anxiety[10,16,27]

Conclusion

There was a significant difference ($p = 0.004 <\propto$) the average level of anxiety in the control group before (16.5 ± 0.80) and after observation (18.17 ± 1.03), while the treatment group showed a significant difference ($p = 0.002 <\propto$) the mean level anxiety before being treated (16.5 ± 0.67) with after being treated (11.75 ± 3.49), it can be concluded that prenatal yoga reduces the level of anxiety before and after treatment.

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Conflict of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 839/UN4.6.4.5.31//PP36/2019), and all research subjects gave written informed consent.

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Validation of Horizontal Genes Transfer Using Selected Clinical Strain of *P. Mirabsilis* for Swarming Activity

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Abstract

Introduction: *Proteus mirabilis* expresses several virulence factor which enable them to colonize, survive and grow in the host organism. This study was to investigate whether *P. mirabilis* could deliver a spontaneous plasmids to competent strains, and to investigate whether *P. mirabilis* can be as a donor strains to delivery their virulent such as adhesion and swarming activity.

Methodology: PCR was used to detect the motility genes that encoded in *P. mirabilis.* Using also transformation assay to transfer these genes of *Proteus* to standard strain. PCR technique used again to determine the whether genes were transfer or not after transformation.

The results: the findings show that 9 (*fil*D, *flh*C, *flh*D, *mot*A, *mot*B, *fli*L, *fla*A, *flg*M,) genes were encoded on chromosomal DNA of *P. mirabilis*. Interestingly data, only 3 (*fla*A, *flg*M and *fil*D) genes were carried on different plasmids of *P. mirabilis.* The results also show that spontaneous plasmids of *P. mirabilis* can transfer to standard strains of *E. coli*. We found one gene transfer to *E. coli* S17λ *fil*D. The gene(*fil*D) has a role in the *P. mirabilis* swarming motility.

Conclusions: we demonstrated that these genes can carry not only on chromosomal DNA but also in Plasmidal DNA and transferring genes associated-characteristics, swarming motility

Key words*: filD ,flhC, flhD, motA, motB, flaA, Proteus mirabilis, swarming motility.*

Introduction

One of opportunistic pathogen is *Proteus mirabilis* which is can cause severe invasive diseases, in critically ill and patients who have immunocompromised [1]. It is an important source of hospital-acquired infections [2]. *P. mirabilis* is the main cause of urinary tract, respiratory tract and wounds infections, burns, digestive tract infections, ear infection and otitis [3]. *P. mirabilis* has also a wide virulence factors which enable them to colonize, survive and grow in the host organism such as swarming, fimbriae, urease, hemolysin, protease and Lipopolysaccharides (LPS) [4]. Furthermore, *P. mirabilis* is well known for its ability to form biofilms, and thereby resists antibiotic treatment [5].

Swarming motility of the *Proteus* is made up of circular waves on solid media [6]. These waves include consolidation and swarming rings, during the consolidation phase, the cells are in short form, and in the swarming phase, they are in long form [6].

P. mirabilis have many virulence factors. Some of these virulence factors encoded in plasmids could be acquired from hospitals or environments. We try to demonstrate whether clinical strains have acquired some genes related with swarming activity and can transfer these genes to standard bacteria strains, which is *E. coli* S17λ.

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Methodology

Bacterial strains:

We isolated 40 samples of *P. mirabilis* from different site of infection, and we selected (P23) which has plasmids and a strongly swarming. *P. mirabilis* (P23) was grown in lysogeny agar. It was also grown an overnight culture and were cultured at 37 °C to reach OD_{600} 0.4. It was equal $1x10^8$. *E. coli* S17λ was provided from Life technologies New England Biolabs®.

PCR detection of genes encoded in chromosomal or plasmidal DNA

Isolation of total DNA

The Genomic DNA Extraction Kit was used for DNA extraction by manufacturer's protocol performed according to (Intron\ Korea).

Isolation of total Plasmid

DNA plasmid were extracted from all bacteria isolates using QIAprep spin miniprep kit (Qiagen) according to the manufacturer's instructions.

Gel electrophoresis

PCR product was separated based on their size, using gel electrophoresis. 2g/100mL of agarose from Fisher Scientific was dissolved in tris base, acetic acid and EDTA (TAE) buffer by boiling. The agarose solution was left in 25˚C to allow cooling around 55 °C, and then 5µL of 0.5 mg/ml of ethidium bromide to stain the DNA fragments. Typically, 1 µL of 5X DNA gel loading dye (Thermo) and 5µL of samples were mixed before loading in the gel. The UV transilluminator was used and images was captured using an EDAS 290 imaging system (Kodak) to visualise the DNA pieces.

Table 1 primers quotation

Gene type	Primer Sequence (5'-3')		Amplicon size (bp)	Reference
flhD	F	AAGGCTTCCGCAATGTTTAGAC	190	[7]
	R	GTTGCAAATCATCCACTCTGGA		
mot A	F	GATGGTGACGGGGAATATGAA	215	[8]
	R	CCATTTCCCCAGCAGGTCTA		
mot B	F	GCGTTACGTCCACATCTCAA	200	[9]
	R	ATGTCGCGCATATAGGGTTC		
fliL	F	GGTGATCGCCATTATTGCAG	220	[8]
	R	AGCGTAACGTGATCCCTATG		
flhC	F	GCCAGTGAAAAAAGCATTGTTCA	500	GenBank: O34202
	R	CCAACAGCCTGTACTCTCTGTTC		
flgM	F	CGCACAAATCCACTTATCCC	180	[8]
	R	GGGCAACTTTTTCGACATTG		
flaA	F	TGCTGGTGCAACTTCATACG	200	[9]
	R	TTTGTCAGCACCTTCCAGTG		
fliD	F	GCCGCAAGTATTTCATCGCTGGG	650	GenBank: P42274
	R	CCTTTGGAATTACTGTTGTTTTC		

Statistical Analyses

Statistical analyses are performed using GraphPad Prism.8. Significance was determined using ozone correlation test. Data are presented as mean ± standard error of mean.

The Results and Discussion

Detection of swarming genes

Nine virulence genes *rpo*A, *flh*C, *flh*D, *mot*A, *mot*B, *fli*L, *fla*A, *flg*M,*fil*D that carried on *P. mirabilis* DNA were studied. These genes have a role to induce many infections [8] [9] [10]. The aim of this section was to quantify *P. mirabilis* genes whether were encoded in chromosomal and/or plasmid.

The *mot*A, *mot*B and *fli*L genes play a role in the rotary motor of flagella [11]. Two *mot*A and *mot*B genes are required for swimming and twitching motility of *Proteus mirabilis*. The stator complexes are not only encoded in *P. mirabilis* but also in most pathogenic bacteria that need to swarm in over surfaces [12]. All isolates 40 (100%) have *mot*A and *fli*L genes ,while *mot*B was detected in 37 isolates (92.5%), **Fig1**. These results were in consistent with result of [13] [14]. This findings were similar with other researchers [15] [16]. **As shown in Fig 2**, From 8 genes, which were encoded in DNA chromosome, only 3 genes were carrying on DNA plasmid of *P. mirabilis*. The percentage of genes *fil*D , *fla*A and *flg*M, were accounted in 3(60%),3(60%) and 2(40%) isolates respectively. These results in regards to swarming activity genes were similar with findings obtained by [17, 18]. [15] [16]. They demonstrated that the virulence genes are encoded in DNA plasmodial or chromosomal by using plasmid curing, which is clean the bacteria from any plasmid.

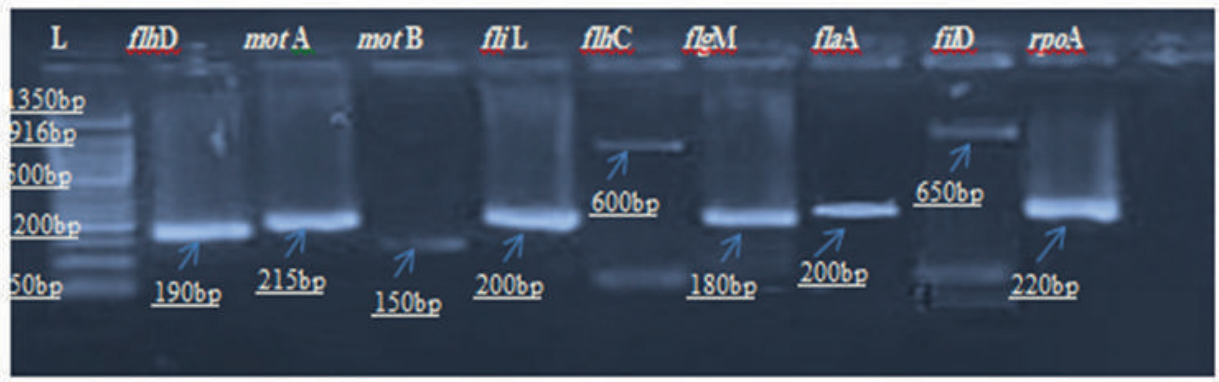


Figure (1) Agarose gel electrophoresis of PCR amplified products of chromosomal DNA genes using species-specific PCR primer sets interested in this study. Lanes of genes are examined *P. mirabilis* isolate NO.23 . Lane L, 1350 bp (ladder).

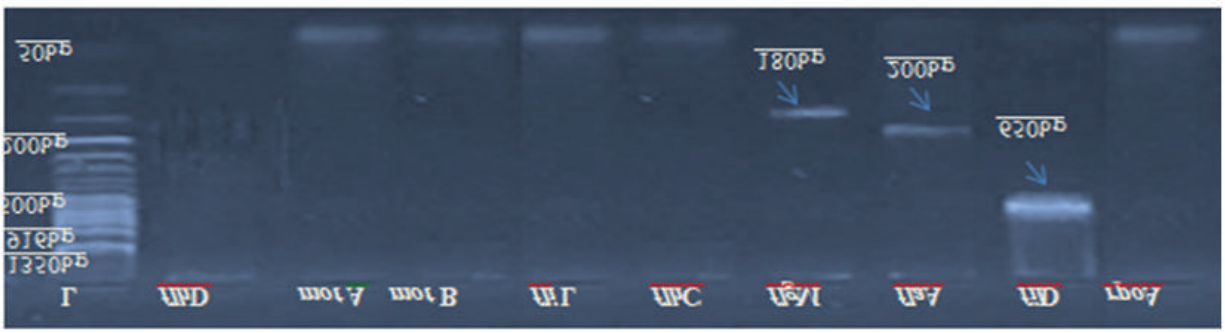
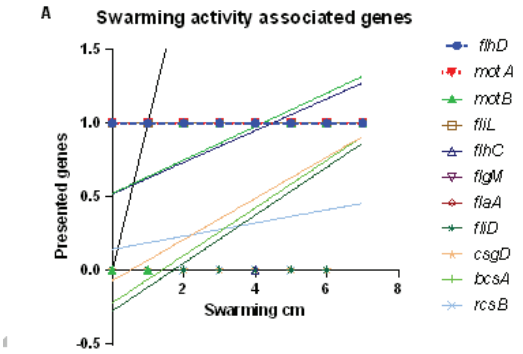


Figure (2) Agarose gel electrophoresis of PCR amplified products of plasmidial DNA genes using species-specific PCR primer sets interested in this study. Lanes of genes are examined P. mirabilis isolate NO.23 . Lane L, 1350 bp (ladder).

Correlation of ***P. mirabilis*** swarming activity and 8 genes

The relation between swarming activity and *flhC, flhD, motA, motB, fliL, flgM, flaA& fliD* genes were conducted in different isolates of *P. mirabilis*. The results showed there are a good correlation between *flhC, flhD, motA, motB, fliL, flgM, flaAa&fliD* with swarming activity. as demonstrated in Figs (3A and B). Many studies are investigated the correlations between virulence genes and swarming. For example, study has been shown that *fliF, motA, motB, flhC, fliG,* and *fliM* genes of *Salmonella enterica* were related with swarming [19]. However, not only these genes have a role in swarming, but also *wosA , flaA, flhD, flgM* and *fliL* genes have a role in this phenomenon [20]



Genes	R-square	P-value	
flhD	1	0.000	*****
mot A	1	0.000	*****
motB	0.3552	0.0011	**
fliL	1	0.000	*****
flhC	0.2461	0.0089	**
flgM	1	0.000	*****
flaA	1	0.000	*****
fliD	0.2412	0.0004	**

Figure (3 A and B) Relationship between swarming activity and associated genes.

Swarming is totally positively correlated with *fihD, motA, flgM* and *flaA* (R-square 1, Kruskal–Wallis). Swarming is also positively correlated with *motB, flhC* and *fliD* genes (P values were between 0.001-0.01) . The significant differences between all genes were tested by using ozone correlation test in Graphpad prism-8 program. Bule means direct role in swarming, green means indirect role in swarming activity.

Genes transferring

This step was established to detect which gene can transfer from *P. mirabilis* to competent bacterial cells using the horizontal gene transfer method, transformation. It was accomplished using *E. coli* S17λ competent cells and isolates *P.mirabilis* donor cells. *E. coli* S17λ competent cells because one of them only used in transformation based on the gene profile of these competent bacterial cells. Briefly, for transformation, we used heat shock method based on antibiotics resistance marker. It was purified the *E. coli* S17λ based on whether successfully resisted to antibiotics and received the plasmid from *P. mirabilis*. The results appear that both methods were shown that only 1 gene are transferred to *E. coli* S17λ from the DNA plasmid extracted from *P. mirabilis*. In addition *P. mirabilis* gene can also transfer to *E. coli* S17λ during transformation process, which were included: *fil*D. **Fig (4)**.

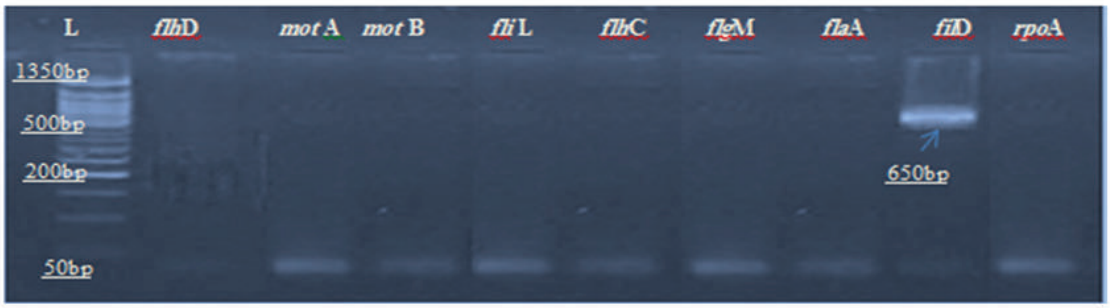


Figure (4) Agarose gel electrophoresis of PCR amplified products of plasmidial DNA gene using species-specific PCR primer sets interested in this study. Lanes of genes are examined *E.coli* S17λ competent strain after transformation . Lane L, 1350 bp (ladder).

Conclusions

P. mirabilis can form swarming and attach to Uroepithelial cells strongly. From 8 different genes, which have a role in swarming, only 1 gene could be expressed in plasmid profile, and transfer to standard strain.

Declaration: The authors have no conflict of interest to declare.

Ethical statement: This article does not contain any studies with human participants or animals performed by any of the authors.

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Awareness of Patient Rights and Practice at Fayoum University Hospital

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Abstract

Background: Patient rights are essential pillars to provide good health care and to promote ethical medical practices. **Aim of work:** to understand the perspectives of Fayoum patients' on patients' rights in the government-run teaching hospitals of Fayoum University of Medical Sciences **Method:** A cross-sectional descriptive study was carried out at the inpatient wards of Fayoum University Hospital, Fayoum Governorate, Egypt. The study was conducted over four months from July to November 2019 **Results:** Only (21.8%) heard about patients' rights. The knowledge score was significantly higher among; the working patient, those who heard or read about the patients' rights and patients admitted in surgical sections (p-value =0000). **Conclusion:** Regarding the awareness and practice of the patients' rights among the patients in Fayoum university hospital there was a lake of knowledge. Only 42.34% of the patients were of good knowledge.

Keywords; *Patient's Rights, Awareness, Egypt.*

Introduction

Human beings have physical, mental, social and spiritual dimensions, from which they arise certain rights, whether they are in good or bad health. [(1)] These rights cannot be achieved, defended and supported without assistance. [(2)] Contemporaneous ethical thinking on "human rights" places increasing emphasis on the concept of "ethics" within different professions, especially those that directly involve human beings, and forces the authorities in these professions to take fundamental human rights into consideration to achieve citizenship rights, consider patients as vulnerable human beings with special needs, and stress their rights more seriously than before [(3)]

Patient rights are a pivotal human right since patients are one of the most helpless gatherings in the general public. [(4)] Patient rights are basic columns to give decent social insurance and to advance moral medicinal practices. [(5)]

Finally, it should be stated that the principles of humanity and respect for human rights apply to the entire world. In Egypt, due to the moral lessons of Islam and the setting in cultural and human terms, special attention is paid to human rights, in general, and patients' rights, in particular. This study aimed to understand the perspectives of Fayoum patients' on patients' rights in the government-run teaching hospitals of Fayoum University of Medical Sciences.

Methodology

1) Study design and setting:-

A cross-sectional descriptive study was carried out at the inpatient wards of Fayoum University Hospital, Fayoum Governorate, Egypt. Fayoum University Hospital is the only university hospital with multi-specialty departments in the governorate. The study was conducted over four months from July to November 2019.

2) Study population

A convenience sample of 418 patients admitted to the hospital was enrolled for this study. The inclusion criteria for the study were a fully conscious patient more than 18 years old or the relatives for the pediatric patients, able to give consent. Participants were aware of the aim of the study and they were informed that the participation is optional the critically ill patients were excluded.

3) Study tools

An interview structured questionnaire was designed to collect data for this study. The data collected by the authors. The questionnaire was developed based on the Egyptian Hospital Accreditation standards [6, 7], and questionnaires applied in previous studies [4, 8].

The questionnaire consisted of two parts: The first part for the patients' demographic characteristics, such as age, gender, residence education, marital status, working status, the admitted section and the source of the subject's information about their rights.

The second part consisted of scales designed to assess the patients' knowledge and practice about the patients' rights. the knowledge section included 18 questions to assess patient awareness about different aspects of the patients' rights such as: Health care and respect as human being, adequate information and, give written informed consent, hygienic environment and health education, Choice of care and participation and representation while, the practice section of patients' rights covered by 17 questions .The questionnaire was scored and the total score was computed for each subject. The knowledge score for each question was based on either 0 if the subject was not aware of this specific right and 1 if the subject was aware of the right. The lowest score would be 0 points while the highest score would be 18 points. A cut-off point was determined based on the median value of the total knowledge score (12) at or above the median value were classified as good knowledge, whereas those having a knowledge score less than the median value classified as having poor knowledge [9]

For the practice questions (done was scored 1 and not done was scored 0) with maximum total score 17, the practice score was considered poor if the score was less than or equal 8 (less than 50%), moderate if the score was 9 to 12 (50% to 70%) and good if the score was 13 to 17 (71% to 100%) [10].

4- Sample Size Calculation:

The sample size was determined for the study using Open Epi, Version 3, It was calculated based on the proportion of patients who know about the patients' rights 22.7 % [8].Awareness and practice of patient rights from a patient perspective: an insight from Upper Egypt, International Journal for Quality in Health Care, 2017, 1–7) and a design effect of 2 and confidence limit 90% ±5%. Thus the least required sample size was 380 increased by 10% to avoid unresponsive rate 418. Frequencies and percentages for qualitative .All data were coded, entered, and analysed using Statistical Package for Social Sciences Program (SPSS, version 21, SPSS, Chicago, IL,U.S.A.). Mean and SD or median and interquartile range (IQR) were calculated for quantitative variables in the form of simple descriptive analysis. Independent test; Mann-Whitney, or Kruskal-Wallis test were used as a test of significant; a p-value of ≤ 0.05 was considered statistically significant. Categorical data were analyzed by computing numbers and percentages.

Results

The data was collected from 418 subjects; 65.1 % of them were males, and 34.1% were females. More than one-third of them (32.8) less than 30 years old, (44%) of them were illiterate. They were admitted at the internal medicine sections by (45%) and surgical sections (55%) Fayoum University Hospital. Most of them (75.6%) were working. Only (21.8%) heard about patient rights from those patients the mean source of the hearing was the mass media while only 11.2% of the patients read about patients' rights most of those through the hospital posters (95.7%) **(Table1)**. More than half (57.66%) of the patients have poor knowledge **(figure1)**

The majority of our patients were aware of the aspects related to the items of health care and respect as a human being, the knowledge score was ranged from 364/418(87.1 %) aware about the right to receive respectful care at all times to 378/418(90 %) knowledgeable patients about the right to privacy during the medical examination. The patients were aware of the right to health care and respect regardless of the age, gender, color or religion by 362/418 (86.6%) **(figure 2),** while in relation to other items, 262/418(62.7%) mentioned that they have the right to accept or refuse to participate in any medical research that may adversely affect them and 206/418 (49.3%) and the patient were aware, about the right to give a written and informed consent before any medical procedure. Only 200/418 (47.8%) of patients mentioned that they should participate in making decisions related to their medical care plan .The lowest knowledge score was reported regarding the awareness about the financial policy 112/418 (26.8%) of the patient knew about the policy of dealing with financial costs and health insurance and only 22/418(5.3%) mentioned that they knew about the compensation methods on exposure

to injury, disease or complication during medical and nursing malpractice **(figure 3).**

Regarding the practice of the medical team towards the patients, only 2 patients (0.5%) received a copy of the patient rights charter. However, the majority (95.7%) and (96.7%) mentioned that they got health care at all times and emergency and first aid services whenever necessary. Regarding the curative services 24.6% could choose between different pharmaceutical products, 42.8% of them got a second opinion consultation from another specialist and 42.6% received a copy of the medical reports and the results of the tests. As regarding the consent form, 56.5% of the patient mentioned that they signed an informed consent form before any medical result and 44.5% received all necessary information before signing the consent form. Only 7.7 % of the patient informed how to complain about any concerns related to the quality of health care **(figure 4).** Good practice represents 7.42% of the practice score, poor practice 24.16% and moderate practice 58.42% .

The knowledge score was significantly higher among the working patient, among those who heard or read about the patients' rights and among patients admitted in surgical sections (p-value =0000). The awareness was significantly higher among the younger patients (p-value=0.01) post-hoc test demonstrates difference was observed between the patient aged less than 30 to 49 compared by those aged 50-59 and those ≥60 years but not in-between the first three age groups. By post hoc tests between groups, this difference was significantly higher for the patient who heard from the physicians followed by the nurses. The knowledge score was significantly higher among the secondary and higher education compared by the other groups (p- value=0.000) There was no significant difference in the practice of the patients' rights towards the patients **(Table2).**

Table (1): Basal characteristics of study participants and their source of knowledge about patient rights, Fayoum, Egypt, 2019

Variables	Number (418)	Percent
Age of less than 30	137	32.8
30-39	107	25.6
40-49	55	13.2
50-59	48	11.5
≥60	71	17.0
Mean ±SD	39.24±16.27	
Sex		
Male	146	65.1
Female	272	34.9
Residence		
Urban	243	58.1
rural	175	41.9
Marital state		
Married	303	72.5
unmarried	115	27.5
Education		
Illiterate	184	44
Read and write	35	8.4
Primary education	53	12.7
Secondary education	111	26.6
High education	35	8.4

Cont... **Table (1): Basal characteristics of study participants and their source of knowledge about patient rights, Fayoum, Egypt, 2019**

Working		
Working	316	75.6
Not	102	24.4
Inpatient admission		
Internal medicine	188	45
Surgery	230	55
Hearing about PR		
Yes	91	21.8
No	327	78.2
Source of information (91)		
Doctor	5	5.5
Nurse	12	13.2
Relatives	5	5.5
Educational seminar	8	8.8
Mass media	61	67
Reading about PR		
Yes	47	11.2
No	371	88.8
Source of reading (47)		
Hospital posters	45	95.7
Book	2	4.3

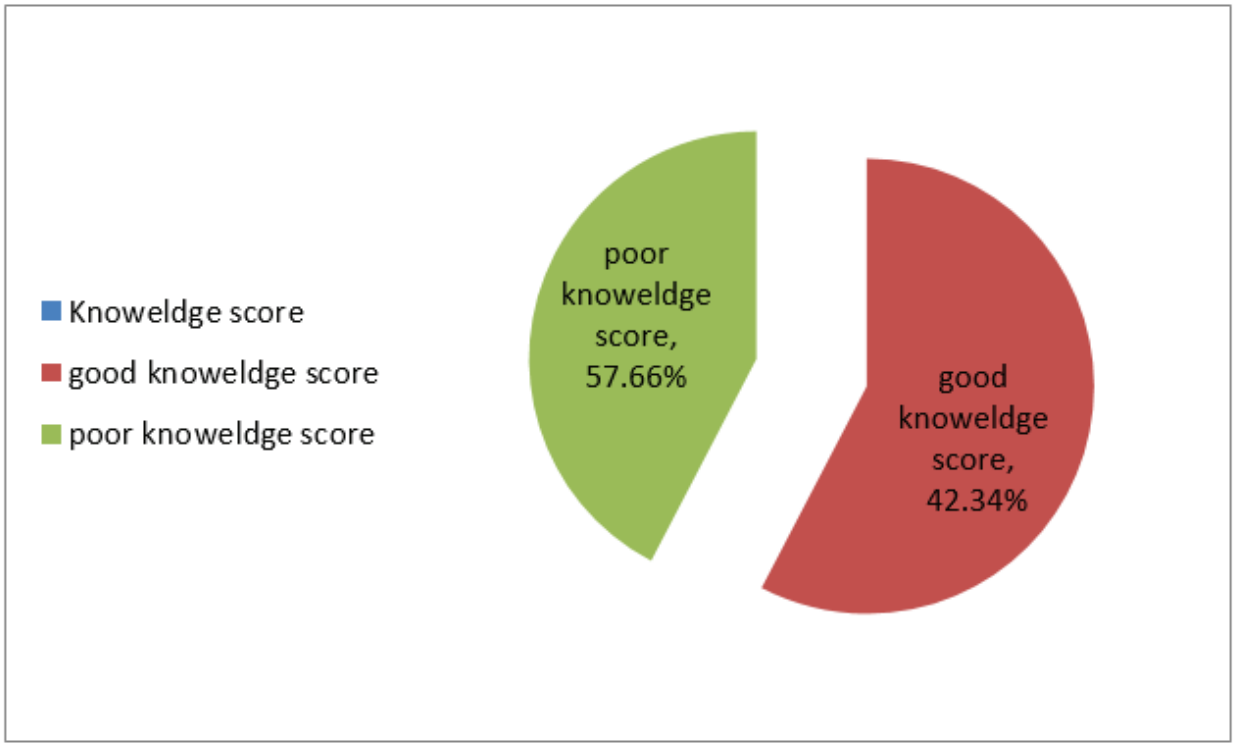


Figure (1): Distribution of knowledge score of the patient rights

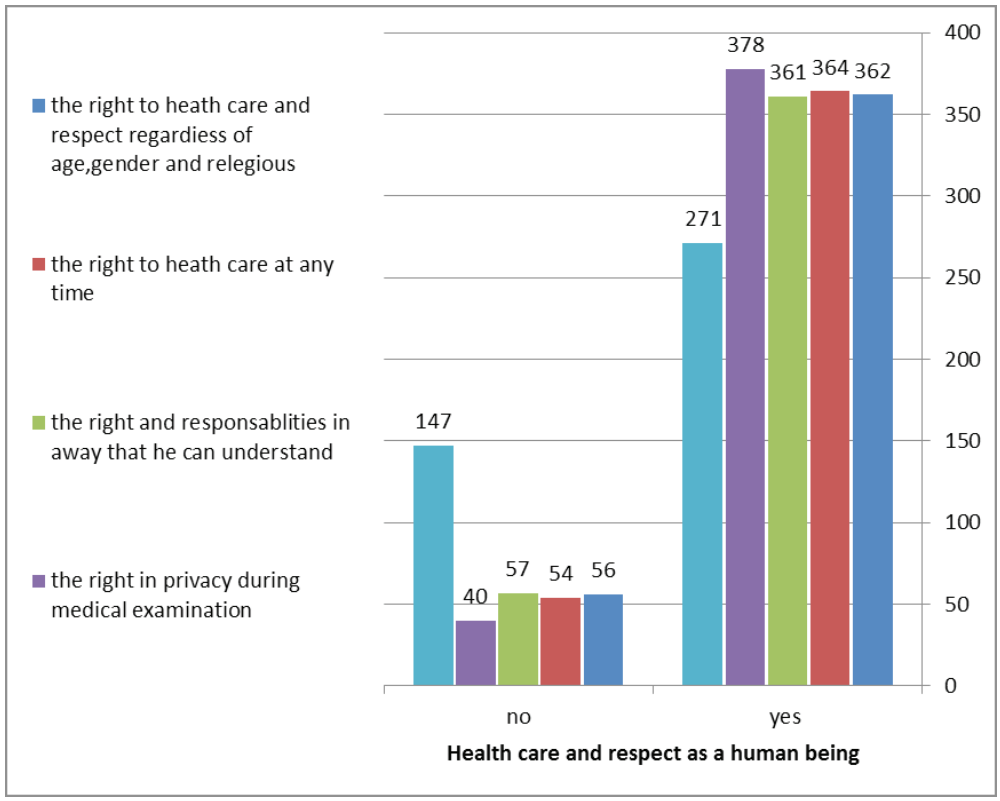


Figure (2): Knowledge of the participants about health care and respect as human being at Fayoum University Hospital (FUH).

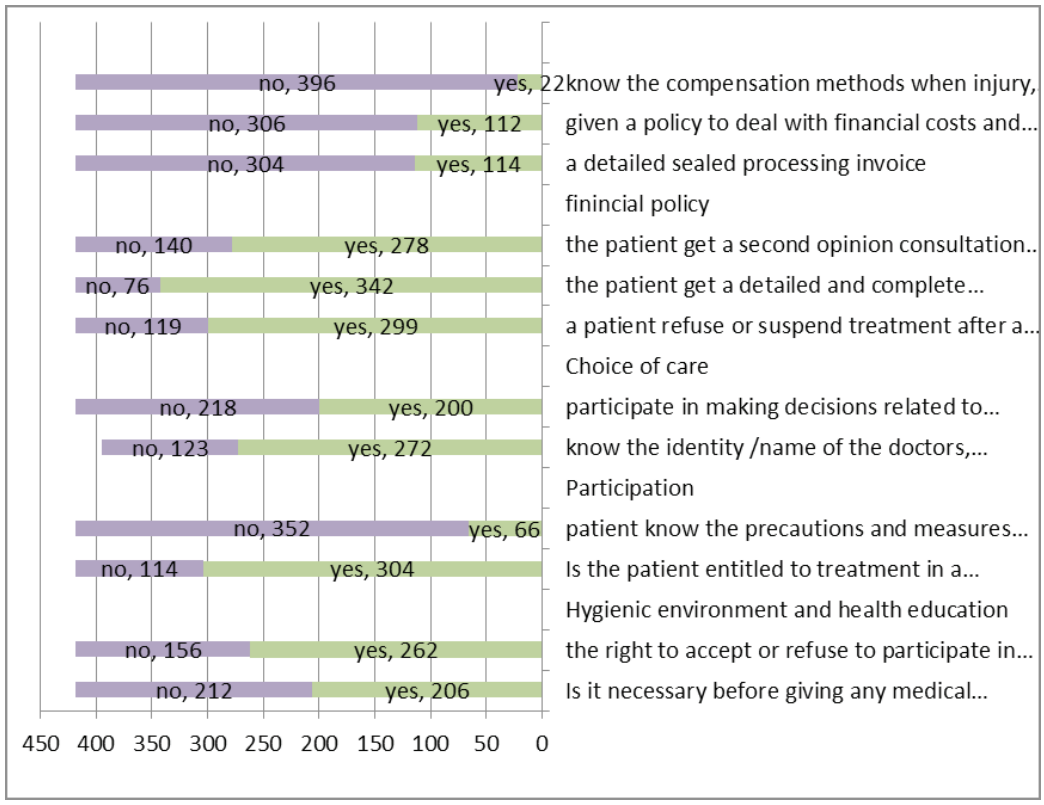


Figure (3) : Knowledge of the participants about their rights of adequate information and given written consent of hygienic environment, participation, choice of care and financial policy at Fayoum at Fayoum University Hospital (FUH).

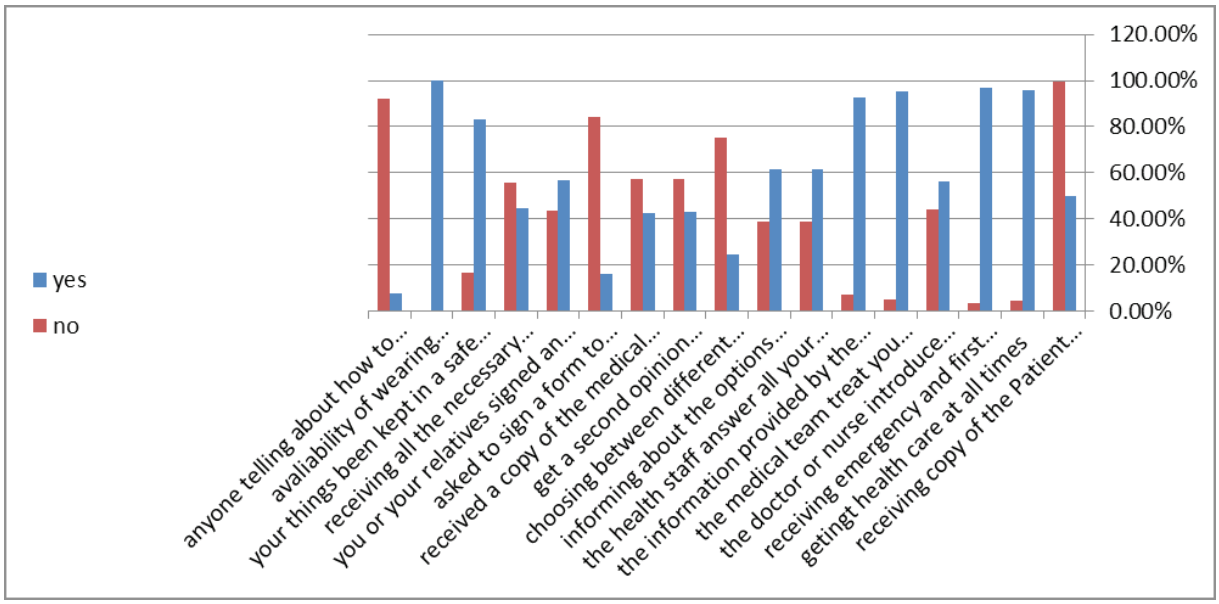


Figure (4): Practice of the medical team towards patients in Fayoum University Hospital (FUH).

Table (2): Knowledge and practice scores of the patients' rights according to socio-demographic and source of knowledge.

Characteristics	knowledge score median (IQ)	P- value	Practice score Median (IQ)	P- value
Sex Male females	 12 (14-8) 11 (14-8)	0.728	 10(11-8) 10(11-9)	0.063
Marriage Yes No	 12 (14-8) 11 (14-7)	0.616	 10 (11-9) 10 (11-8)	0.056
Residence Urban rural	 12 (14-8) 11(14-7)	0.295	 10(11-8) 10(11-9)	0.558
Working Yes No	 14(15.5-10) 11(13-7)	0.000*	 10(11-9) 10(11-8)	0.303
Hearing Yes No	 14(16-13) 10(13-7)	0.000*	 10(11-8) 10 (11-9)	0.388

Cont... **Table (2): Knowledge and practice scores of the patients' rights according to socio-demographic and source of knowledge.**

Source of hearing -physician -nurse -relatives -educational seminar -mass media	 18(18-16) 15 (16-13) 8 (14-4) 14.5 (15-13) 14 (16-13)	0.005	 9 (11-7) 10.5(11-7.25) 11(11-9) 9(10-9) 10(11-9)	0.599
Reading Yes No	 14(16-13) 11 (13-7)	0.000*	 10(11-9) 10(11-9)	0.931
Department Surgical sections Internal medicine	 12 (14-9.75) 10 (13-5)	0.000*	 10(11-8) 10(11-9)	0.851
Education Illiterate and read and write Primary education Moderate and high education	 9(12-5) 12(13-8) 15(16-13)	0.000	 10(11-8) 10(11-9) 10(11-9)	0.247
Age of less than 30 30-39 40-49 50-59 ≥60	12(15-8) 12 (15-9) 12(14-8) 9 (13-7) 11 (13-6)	0.001	10(11-8) 10(11-9) 10(11-9) 10(11.75-8) 10(12-9)	0.299

$P \leq 0.05$

Discussion

Health care organizations have established characters for the patients' rights to achieve patient satisfaction and ethical health care. The results of this research indicate that, despite the introduction of specific legislation, hospital patients are not yet aware of their legal rights. As the majority of the patients (78.1 5%) did not hear about the character of patient rights this was similar to a study conducted in Minia University Hospital, Egypt [8] and Beni-Suef University Hospital, Egypt [11], but was higher than that reported by Ghanem et al. [8], who found that 27% of patients in Alexandria Main University Hospital and 53% of patients in Matrouh General Hospital were not knowledgeable about the charter of patient rights. The main source of information (hearing) in our study was the mass media (67%) followed by the nurse (13.2%) also; reading posters and brochures in the hospitals this was approximately similar to Zeina et al. [10]

In the current study, Most of the patient more than 85% were aware of the right to health care, respect and at any time, the right to privacy during the medical examination, the right of the patient to get a detailed and complete explanation of any unexpected thing during the care and treatments, these results were in agreement with Mohammed et al [9],to a study conducted in Saudia Arabia [11] on the other hand the lowest score was about the awareness of financial policy ranged from 5.3% to 27.3 % while others reported the lowest score of the awareness about the rights of the patient in the participation in the treatment plan and choice of care. [12,13,14]

The knowledge score was significantly higher among the working patients , educated patient (secondary and high education) , among who heard or read about the patient rights p value=0.000, although the mass media was the mean source of knowledge however the score

was significantly higher among the patients provided the information by the physician followed by the nurse this pointed the important role of the medical team in giving the confidential information and the importance of direct communication between the medical team and with the patients in improving the awareness of the patients also, the knowledge score was significantly higher among the age group from 30 to 39 years old (p value=0.01) and among the patient admitted to the surgical section (p value=0.000) this may explain the difference in the treatment process and fair of surgical procedures. Other studies found that there was an inverse relation between awareness score and age [9,15], there was a positive association between the knowledge score and higher levels of education. [16, 17]

Conclusion

Regarding the awareness and practice of the patients' rights among the patients in Fayoum university hospital, there was a lake of knowledge. Only 42.34% of the patients were of good knowledge and 21.8% heard about patient rights character.

Funding: No source of funding

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Carbon Dioxide Laser in the Treatment of Oral and Craniofacial Soft Tissue Lesions, Pros and Cons

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Abstract

Background and objectives: Whether to use a cold scalpel or laser surgery to remove a lesion in the skin of the craniofacial area is the main question the surgeon asks him- or herself to do. The study tried to extend the literature with data that may help the surgeons to choose the right method.

Methods: Thirty patients with intra- and extraoral craniofacial skin lesions managed by Carbone dioxide (CO2) laser surgery.

Results: The most common type of lesion treated was melanocytic nevi (15 patients; 50%).

Conclusion: The main complication of CO2 laser surgery is the remaining permanent hypopigmentation of the treated area; however, the CO2 laser has many advantages (especially at the time of surgery) making it a good choice for the management of these lesions.

Keywords: *CO_2 laser; skin resurfacing; melanocytic nevi; maxillofacial surgery.*

Introduction

When the patients have an intraoral lesion, they are afraid of the dental needle and the pain during the surgical procedure to remove that lesion, the laser here plays a role in decreasing these terrifying feeling for the patients. Not only the oral cavity lesions, the face is also the first site to take an impression upon anyone, if this area has a disfiguring lesion such as nevi, skin tags and other skin lesions, they should be removed.

The correct diagnosis of the lesion is essential before deciding which type of laser used. This is depending on surgical skills and experience to differentiate between benign and malignant lesions clinically or even by using dermoscopy [(1)].

In 1964, Patel published an article describing the first use of carbon dioxide (CO_2) laser [(2)]. Carbone dioxide laser has been used medically for different indications in oral and maxillofacial surgery like skin resurfacing in burns or any pathological skin lesions, management of oral mucosal lesions as in leukoplakia, pre-prosthetic surgery as in vestibuloplasty, facelift, intraoral vascular malformation and many other applications [(3-8)].

The wound resulted from a laser is different from that of burn, although it will vaporize the fluid inside the cells causing disintegration of the cell structures but without releasing of the inflammatory mediators in such a way that occurred in burns. Intraoral wounds after laser surgery usually healed without any scar; rapid healing without suturing or packing, likewise with some facial benign lesions like skin tags or commonly acquired moles [(9)].

In general, the energy exerted by the laser depends on the chosen power, time of laser application and the characteristics of the laser used. The laser can be used in two modes, a non- contact laser scalpel in which, the laser is focused on a small area (0.1-0.5mm), used for cutting, while the other mode is defocused laser, to be applied over a large surface area (1-5mm) which is

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usually useful in cases of lesions spread superficially; the lesion is removed by layers [10].

The aim of the study was to evaluate the CO_2 laser in the management of oral and craniofacial soft tissue lesions.

Methods

This study was conducted in accordance with the World Medical Association Declaration of Helsinki. All the procedures performed in the study were in accordance with institutional ethical standards.

Thirty patients presented with intraoral (Fig. 1) as well as extraoral craniofacial soft tissue lesions (Fig. 2-5) managed by ablation using CO_2 laser. The selected patients were otherwise healthy with no history of systemic disease that may alter the healing process after laser surgery.



Figure 1 Intraoral lesions ablated by CO_2 laser (A,B,C; mucocele and D,E,F; pyogenic granuloma). (A) Mucocele on the lower lip. (B) Healing with coagulum. (C) Very well healing on follow-up. (D) Pyogenic granuloma on the upper alveolus. (E) Postoperative result. (F) Very well healing on follow-up.



Figure 2 Seborrheic wart on the nose ablated by CO_2 laser. (A) Preoperative. (B) Postoperative result. (C) Pitting scar (two years follow-up).

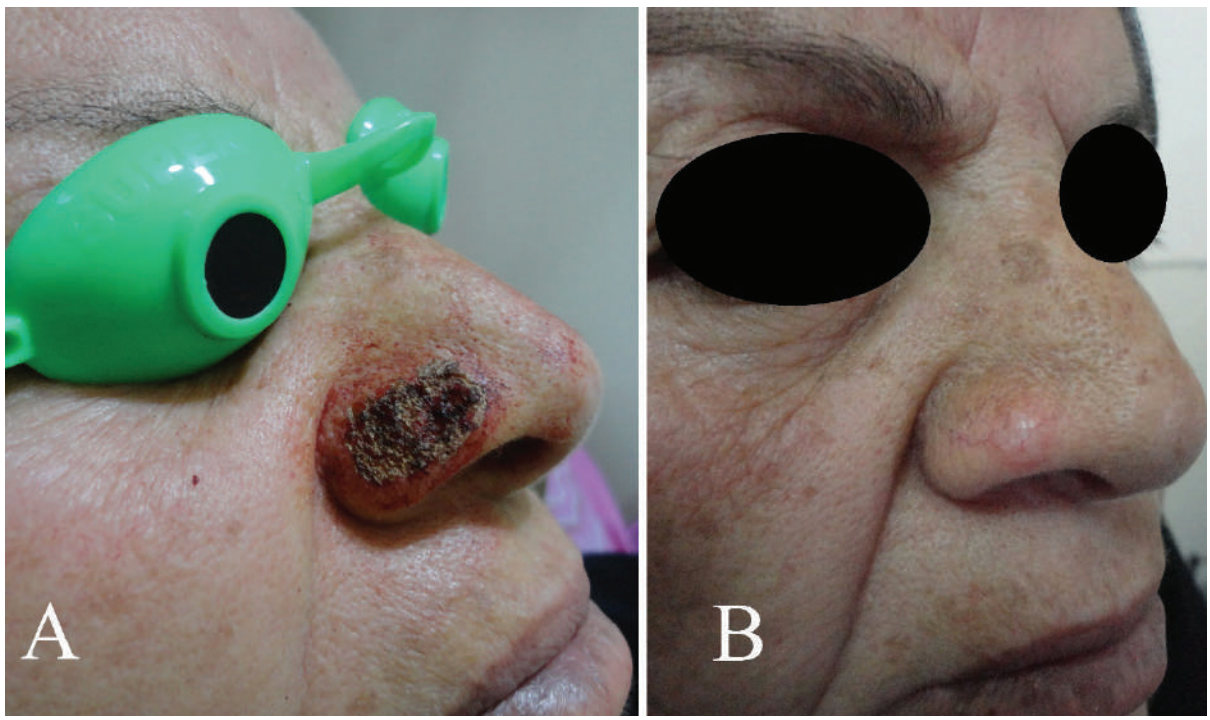


Figure 3 Basal cell carcinoma on the nose ablated by CO_2 laser. (A) Preoperative. (B) Very well healing on follow-up.

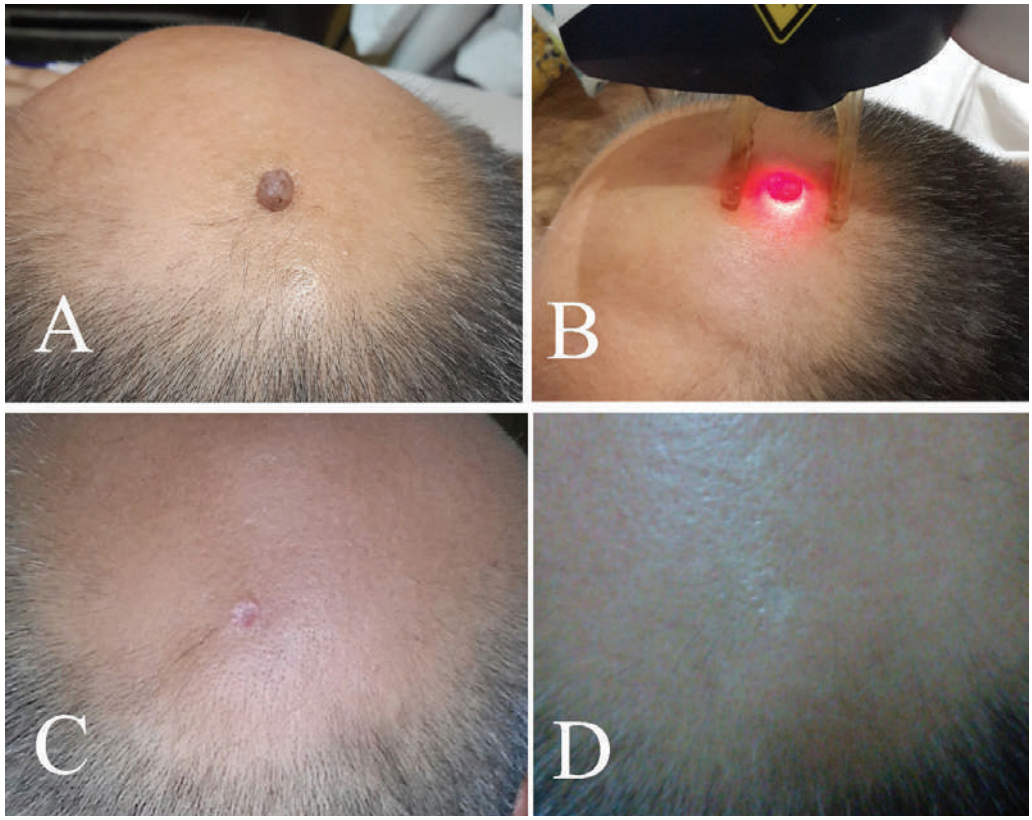


Figure 4. Nevus on the scalp ablated by CO_2 laser. (A) Preoperative. (B) Intraoperative. (C) One day postoperative. (D) Two years of follow-up (hypopigmentation).

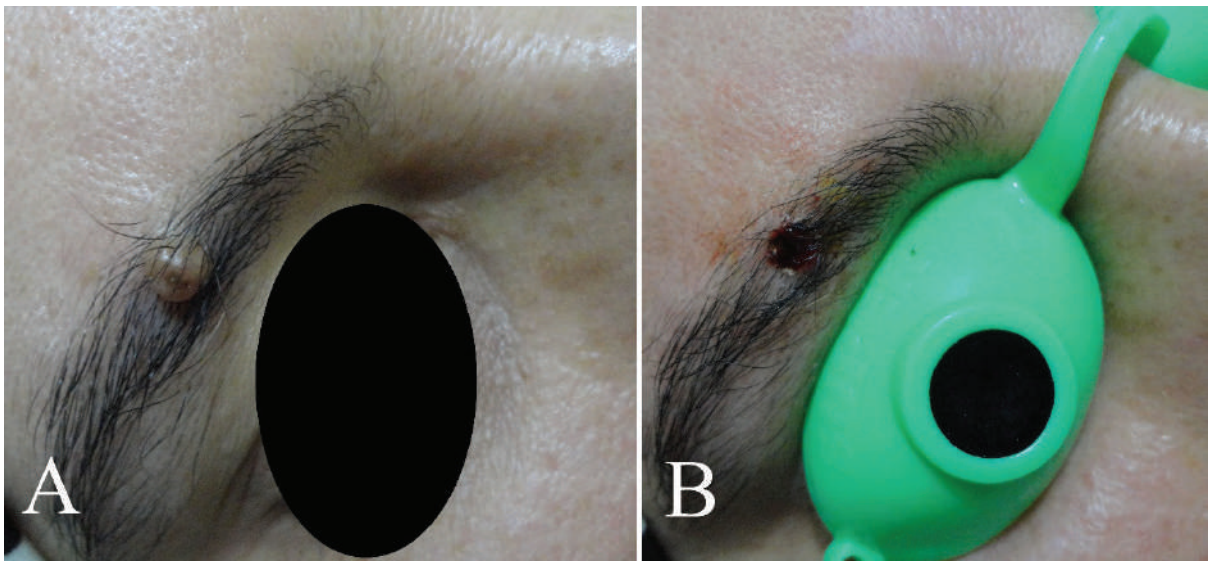


Figure 5 Nevus on the right eyebrow ablated by CO_2 laser. (A) Preoperative. (B) Postoperative result.

Either of two techniques of local anesthesia was used preoperatively; infiltration with the lidocaine 2% or topical anesthesia by eutectic mixture of local anesthetics (EMLA), depending on the nature, size and the site of the lesion.

CO_2 Laser

All the precautions regarding laser use were followed. The wavelength of the CO_2 laser was 10,600 nm, which is absorbed very well by water, with the power depends on the type and the site of the lesion.

Postoperative Care

For the extraoral lesions, a closed wound dressing was applied for the first 24 hours. As a protocol, no postoperative analgesia given to the patients unless needed (mefenamic acid tablets / 500mg).

Follow-up

The follow-ups were scheduled at ten days, one month, one year and as required after that or between these periods. The parameters to be assessed during follow-up were a pain, edema, scar formation, functional disturbance, aesthetic satisfaction as well as recurrence of the lesions or any other related complications.

Results

The study included 30 patients (20 females; 66.7%) and (10 males; 33.3%). The age range was 13-70 years; the mean age was 30.4 years. The most common lesion treated was extraoral melanocytic nevi (15 patients; 50%) (Fig. 4) (Table 1).

Table 1 Distribution of the lesions treated by Co_2 laser

Site	Lesion	N	Percentage
Extraoral	Acquired Melanocytic Nevi	15	50%
	Sebaceous Hyperplasia	2	6.7%
	Seborrheic Warts	1	3.3%
	Basal Cell Carcinoma	1	3.3%
Intraoral	Pyogenic Granuloma	5	16.7%
	Tongue Papilloma	3	10%
	Mucocele in the Lower Lip	3	10%
Total		30	

N: Number of cases

There was no pain during procedures; however, there was a minimal postoperative pain in all the patients at the site of operation. There was no functional disturbance, edema or recurrence of the lesions. Two of the lesions healed by a scar (Fig. 2C). Twenty-four patients (80%) were satisfied esthetically, while the other six patients (20%) were not satisfied.

Discussion

Carbon dioxide laser has been proved effective in the excision of soft tissue lesions with less morbidity and excellent pain control. It is characterized by being an effective photo-thermal ablation device with efficient coagulation capabilities [11, 12].

Although the laser works with minimal pain, local anesthesia is required to finish the work, however, the amount of local anesthesia used with the surgical laser is usually less than that required with the cold scalpel [13]. In our study, we used two techniques of local anesthesia; eutectic mixture of local anesthetics (EMLA) as topical anesthesia and the traditional lidocaine infiltration.

There were difficulties in the diagnosis of a solitary basal cell carcinoma (BCC) (Fig. 3) on the face because it could have the same clinical features of seborrheic keratosis (Fig. 2), malignant melanoma, melanocytic nevi or even hypertrophic scar. Carbone dioxide laser ablation of BCC gives superior results than the traditional invasive surgical excision. Of all the types of BCC, superficial and nodular types are the most types responsive to CO_2 laser therapy. However, in this study, a pigmented BCC has been treated by CO_2 laser with very well healing and with no recurrence [14-17].

Wound care after laser therapy is essential for better healing. The moist dressing is superior to the dry dressing by gauze alone. Some authors prefer the open dressing by applying ointment alone, others, prefer the moist closed dressing, which is associated with a low infection rate. In our study, a moist closed dressing was used for the first 24 hours after extraoral laser surgery, no more than that (keeping the dressing for a long time may increase the risk of infection) [18-21].

The effect of laser on tissue depends on the wavelength of the light-emitting which could be reflected absorbed or scattered. Carbon dioxide laser is absorbed by water (vaporization) with minimal penetration. A thin layer of denatured proteins (coagulum) (Fig. 1B) cover the wound after the excision of the lesions by the laser acting as a natural wound dressing, protecting the wound from irritation especially in the oral cavity [9].

The common consequences of using CO_2 laser are erythema due to vascular reaction to the healing process and hypopigmentation (Fig. 4D), which is occurred due to the effect of laser on the melanocytes in the treatment area; it is either replaced by peripheral melanocyte or remain deficient permanently. Textural alteration occurred due to deep vaporization and destruction of the deep skin layers [16,17]. In this study, six of the patients (20%) were not satisfied esthetically because of the visible permanent hypopigmentation of the area treated by laser.

Carbone dioxide lasers worked in a continuous mode affecting also the adjacent surrounding healthy tissues causing more scar possibilities. Recent CO_2 lasers have short-pulsed high energy mode making them more precise to target the lesion only, sparing the healthy tissue [22].

Conclusion

While the cold scalpel is still convenient for use by many surgeons, the laser is increasingly replacing it. The advantages of CO_2 laser are being safe, provide bloodless surgical field, less operative time, no need for suturing, deferred acute inflammatory reaction, cause less damage to the surrounding tissues and less scarring (inhibit myofibroblast activity). While the disadvantages of CO_2 laser can be summarised as expensive to buy and expensive to maintain, need high power consumption and need very well training.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: This research has exemption as it a routine treatment (no new materials were used).

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Profile of Multidrug Resistance (MDR) and Methicillin Resistant *Staphylococcus aureus* (MRSA) on Dairy Farms in East Java Province, Indonesia

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Abstract

Staphylococcus aureus is one of the causes in the case of Foodborne Disease, with the resulting toxin triggering the occurrence of Foodborne Intoxication. The purpose of this study was to identify multidrug resistant patterns and detect Methicillin Resistance Staphylococcus aureus (MRSA) in cow's milk in East Java, Indonesia. A total of 170 cow's milk samples were taken from dairy farms on 3 Districts, then the bacteria were cultured and purified using Mannitol Salt Agar media. Resistance tests were carried out with 5 antibiotics on Mueller Hinton Agar media and Methicillin Resistant Staphylococcus aureus (MRSA) by Oxacillin Resistance Screen Agar Base (ORSAB). The results showed there were 76 (44.7%) S. aureus, then antibiotic testing showed 13 (17.1%) S. aureus isolates had multidrug resistant (MDR) properties on the antibiotics tested and 84.6% isolates with MDR confirmed MRSA properties by ORSAB. It was concluded that the raw milk can be a potential reservoir for MDR and MRSA strains to threat public health.

Keywords: *Foodborne Disease, Raw Milk, MDR, MRSA, Public health.*

Introduction

Foodborne disease is a major public health problem throughout the world and is defined as an infectious or toxic disease due to consuming contaminated food or water[1]. Staphylococcus aureus (*S. aureus*) is an opportunistic pathogen and one of the causes of foodborne intoxication / poisonning which has a high incidence rate related to consumption of raw milk and milk products[2], it is caused by ingestion of food contaminated by Staphylococcal enterotoxins (SE) strains of Staphylococcus[3]. The enterotoxins produced by S.aureus can cause poisoning even at very low doses, which are 20 ng -1 µg / ml[4]. SE is very stable to heat, resistant to environmental conditions such as freezing, drying and resistant to proteolytic enzymes such as pepsin or trypsin and low pH, enabling them to function fully in the digestive tract after consumption[5].

The main treatment for staphylococcal infection is oxacillin preparations, but the development of antibiotics and misuse in agriculture, stock farming, veterinary medicine, and human diseases treatment increases the potential for antibiotic resistance by bacteria[6, 7]. This study aimed to evaluate the antibiotic resistance from raw milk on dairy farms in East Java. The antibiotic resistance that have impact in human health as well as in dairy cows health as such a potential transmission of MRSA strains with MDR properties in several

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antibiotics.

Material and Methods

The total sample of 170 dairy cows were taken on 3 dairy farms in the districts of Kediri, Probolinggo and Blitar during September - November 2019. Dairy Cows milk was taken at milkcan deposited by farmers at 25 ml milk storage posts, put in a 50 ml centrifuge tube setril (Biologix, BD-T0034). A total of 1 ml of each sample was taken aseptically using Syringe 3CC (AKD 20902900277) and placed in a 10 ml vaculab containing 4 ml of media broth Buffered Peptone Water (Oxoid, CM0509) referring to the enrichment method from research in *Veterinary World* and *Journal of Veterinary Medicine* which was modified[8, 9]. Vaculab containing enrichment media was incubated in an incubator (Isuzu Model 2-2195, Jica) at 37ºC for 24 hours. Samples were cultured and purified using Mannitol Salt Agar (HiMedia Pvt. Ltd, M118) and then incubated at 37ºC for 24 hours.

Microscopic examination of the colony was carried out by Gram's method of staining to produce a display of Gram positive bacteria in the form of coccus and clustered. Biochemical examination was carried out by Catalase test and Coagulase test. Catalase tests were carried out by dripping hydrogen peroxide (HO) 3% on clean glass objects[10, 11]. Coagulase tests were carried out by modifying the method from *Journal of Clinical Microbiology*[12], Coagulase slide test / clumping factor gave 50 µl rabbit blood plasma dripped on a glass object, then mixed with 1 ose of bacterial colony, Coagulase tube test using 200 µl blood plasma was added with as many as 3-4 isolate colonies then incubated 37ºC for 24 hours.

The resistance pattern of S. aureus was determined by Disk-diffusion test[13]. 5 antibiotics such as Tetracycline 30 µg, Erithromycin 15 µg, Gentamicin 10 µg, Cefoxitin 30 µg and Oxacillin 30 µg (Oxoid) were selected based on various data on the use of antibiotics in human medicine, national veterinary therapy and various epidemiological purposes studies. Isolates that are resistant to 30 µg Oxacillin (Oxoid) will be confirmed with Oxacillin Screen Agar Base (HiMedia Pvt. Ltd., M1415) added Oxacillin Resistance Selective Supplement (HiMedia Pvt. Ltd., FD191) to detect MRSA strains.

Results and Discussion

Sample test results showed that from 170 milk samples taken in 3 districs, there were 76 samples (44.7%) positive for of S. aureus based on morphological cultural characteristics and biochemical tests. This number is distributed with the number of different S.aureus positive samples in each location, such as in Kediri found 20 (40%) S.aureus positive isolates from 50 samples, while in Probolinggo found 30 (60%) S.aureus positive isolates from 50 samples and Blitar 26 (37%) positive isolates of S. aureus from 70 (Table 1). Basically S. aureus can be transmitted to humans through contamination of milk or milk products, but also milk contamination can be sourced from humans[14]. The positive amount of S. aureus which is more than 40% can be caused by a variety of factors, which are mainly due to the hygine milking factor played by the milker. A researcher from *International journal food contamination* states that contamination in milk can be sourced from post-harvest handling which is categorized into 3 main sources including udder health and udder surfaces to teat, milk handling and storage equipment[15].

Table 1. Isolation of *S. aureus* according to sampling location

Location	Location Code	Sample size	Positive *S. aureus* (%)
Kediri	Kr	50	20 (40%)
Probolinggo	Pb	50	30 (60%)
Blitar	Bl	70	26 (37%)
Total		170	76 (44.7%)

Note : % (Percentage of *Staphylococcus aureus* in all milk samples).

Profile of antibiotic resistance from antibiotic susceptibility test it was found that as many as 25 (32.9%) S.aureus isolates had resistance to 1 class of antibiotics tested, while 21 (27.6%) isolates were resistant to 2 classes

of antibiotics and 13 (17.1%) isolates were confirmed to be multidrug resistant (MDR) because it has resistance to ≥3 classes of antibiotics (Fig 1.). There are 5 MDR S.aureus patterns in which predominantly have 3 (3.9%) isolate patterns of Cefoxitin, Oxacillin, Tetracyclin, and Erythromicin, followed by a pattern of resistance to Cefoxitin, Oxacillin, Tetracyclin, Erythromicin and Gentryn in a number of 3 (3.9%) isolate. While those who have resistant patterns of Cefoxitin, Oxacillin, Tetracyclin and Gentamicin are 3 (3.9%) isolates (Table 2).

Table 2. Resistance profile of isolated *S. aureus* by Group of Antibiotic.

Group of Antibiotic	Resistance profile	Number of *S. aureus* isolates (n = 76)	Total Number of Isolates (%)
		Resistant Isolates (%)	
1	OX	2 (2.6)	25 (32.9)
	TE	16 (21.1)	
	E	6 (7.9)	
	CN	1 (1.3)	
2	FOX, OX, TE	3 (3.9)	21 (27.6)
	OX, TE	5 (6.6)	
	OX, E	2 (2.6)	
	TE, E	10 (13.2)	
	TE, CN	1 (1.3)	
≥3	FOX, OX, TE, E	3 (3.9)	13 (17.1)
	FOX, OX, TE, CN	3 (3.9)	
	FOX, OX, TE, E, CN	4 (5.3)	
	OX, TE, E	2 (2.6)	
	TE, E, CN	1 (1.3)	

Note : FOX=Cefoxitin 30 µg, OX= Oxacillin 30 µg, TE=Tetracycline 30 µg, E=Erythromycin 15 µg, CN=Gentamicin 10 µg, % (percentage).

Info: Total 13 isolate Resistance with ≥3 Group of Antibiotic are Multidrug resistant (MDR).

S.aureus actually has intrinsic susceptibility to antibiotics, but has tremendous potential to develop, or obtain resistance to almost all antimicrobials exposed to these bacteria[16]. Actually the resistance of pathogenic bacteria to antibiotic agents is a natural mechanism for survival[17], but its presence has an adverse effect on public health.

Table 3. Profile Multidrug Resistance of *S. aureus* isolates by location.

Location	Sample code	Resistance profile				
		FOX 30 µg	OX 30 µg	TE 30 µg	E 15 µg	CN 10 µg
Kediri	Kr 05	✓	✓	✓	✓	-
	Kr 37	✓	✓	✓	-	✓
Probolinggo	Pb 04	✓	✓	✓	✓	✓
	Pb 07	✓	✓	✓	✓	✓
	Pb 17	✓	✓	✓	-	✓
	Pb 21	✓	✓	✓	✓	✓
	Pb 31	✓	✓	✓	-	✓
	Pb 35	✓	✓	✓	✓	-
	Pb 45	✓	✓	✓	✓	✓
Blitar	Bl 11	-	✓	✓	✓	-
	Bl 30	-	-	✓	✓	✓
	Bl 31	✓	✓	✓	✓	-
	Bl 46	-	✓	✓	✓	-

Note : ü= Resistant, FOX= Cefoxitin 30 µg, OX= Oxacillin 30 µg,

TE= Tetracycline 30 µg, E= Erythromycin 15 µg, CN= Gentamicin 10 µg.

Researcher from *BMC Microbiol* believes that the presence of multidrug resistant (MDR) by S.aureus is due to genomic integrated genetic transfer from its main source, Coagulase negative staphylococcus (CNS)[18]. Where CNS itself has a low pathogenetic level compared to S. aureus but has a high tendency to have MDR properties[19]. S.aureus positive isolates that are MDR are found most at Probolinggo location, which is 7 isolates or 53.8% of S.aureus isolates that are MDR (Table 3) and shown in Fig. 2. The most isolated isolates found by S.aurus with MDR characteristics were at the location of Kediri, namely 2 isolates or 15.4% of S.aureus isolates that were MDR. This can be caused by differences in the management of post-harvest handling and post-mastitis treatment in each region. Agree with this *Food Control Journal* states that the percentage of S.aureus resistance to several antibiotics tested can result from misuse and miss control of antibiotic use for the treatment of infections in dairy farming areas, where risk factors can be sourced from the farmer, veterinarian in question[20].

Table 4. Total Number Confirmed MRSA by ORSAB

Location	Resistance Group of Antibiotics	Positive ORSAB Test	Number of MRSA by location
Kediri	1	1	5
	2	2	
	≥3	2	
Probolinggo	1	-	8
	2	2	
	≥3	6	
Blitar	1	1	6
	2	2	
	≥3	3	
Total Number of MRSA			19

Info : Total number MRSA isolates were 19 isolates.

Testing for MRSA was carried out on oxacillin resistant isolates, of which a total of 24 oxacillin resistant isolates contained 19 (79.2%) strains of MRSA confirmed by ORSAB (Table 4) and shown in Fig. 2. Not all MRSA strains in this study were MDR on the antibiotics tested, where there were 11 MRSA isolates that were MDR while 8 other MRSA isolates were not MDR.

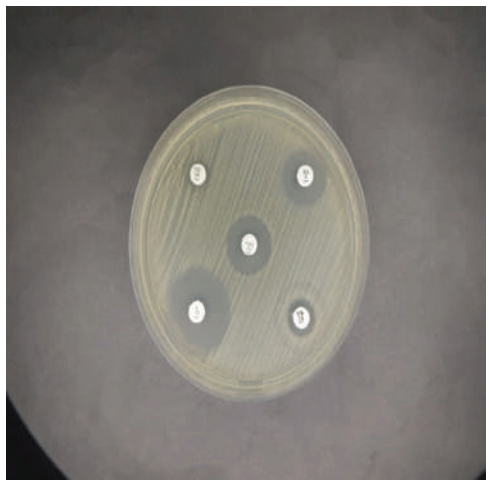


Figure 1. Antibiotic susceptibility test of Staphylococcus aureus isolate of MDR in Mueller Hinton Agar Media.

Legend : FOX=Cefoxitin, OX= Oxacillin, TE=Tetracycline, E=Erythromycin, CN=Gentamicin (Cannon 600D DSLR).

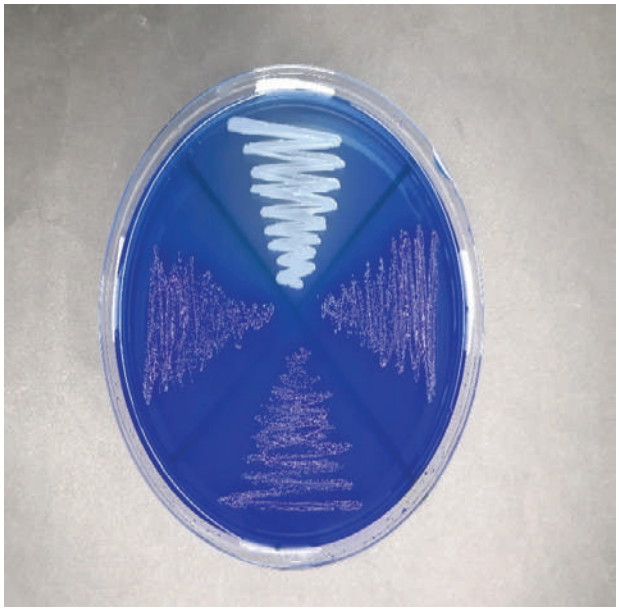


Figure 2. *Confirmation for MRSA by* **Oxacillin Screen Agar Base (ORSAB) (HiMedia Pvt. Ltd, M1415). Blue color shows positive confirmation results, white results show negative confirmation results. (Cannon 600D DSLR).**

Detection of MRSA resistance can be achieved by using oxacillin disk or cefoxitin folowed by ORSAB test[11]. Oxacillin is a class of lactam antibiotics and is the first choice drug in the treatment of mastitis infections caused by S. aureus bacteria. The mechanism of action of penicillin inhibits bacterial growth by interfering with the transpeptidation reaction in cell wall synthesis[8]. There are mechanisms of resistance to beta-lactam namely: inactivation of antibiotics with beta-lactamase; modification of the Penicillin-Binding-Protein (PBP) target; impaired drug penetration to achieve PBP goals[21].

We found that cefoxitin discs, as recommended by *Journal of Medical Microbiology*, is a good method for detecting MRSA by combining oxacillin discs to confirm MRSA presence[22]. It is always recommended to combine the two methods, oxacillin with high sensitivity and the cefoxitin with high specificity. According to the present results, 19 (25%) included MRSA of S. aureus isolates based ORSAB test. It is concluded that the ORSAB test is more sensitive but less spesific compared with the cefoxitin test. This finding is important to confirm the existence of MRSA and advice the government to take steps to control MRSA sourced from raw milk.

Conclusion

This is the high incidence of MDR and MRSA from Staphylococcus aureus in dairy cows. ORSAB test showed the MRSA were higher compared to the MDR cases. The MRSA and MDR of Staphylococcus aureus showed the potential for rapid and wider dissemination and poses a threat to animal health and public health. This is an alarming high prevalence of MRSA from dairy farms in East Java Province, Indonesia.

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Designation :

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Ethical Clearance: Raw milk were used in this study, hence ethical clearance was not necessary. Raw milk samples were collected from dairy farms in East Java province, Indonesia.

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Conflict of Interest: Nil

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Questionnaire Study on Knowledge, Attitude and Practice on Factors Associated with Initiation and Cessation of Tobacco Among Patients Using Tobacco Products

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Abstract

A study was conducted to assess the knowledge, attitude and practice (KAP) of factors associated with initiation and cessation of tobacco among patients using tobacco products. A KAP study was conducted among patients using tobacco products of various age groups attending the outpatient department in a dental college in Chennai, Tamil Nadu. A self-constructed questionnaire by our team was framed and distributed to 100 subjects. The data collected was then analysed with SPSS software (version 21) statistically to obtain the results.

In this study, predominantly 69% of the subjects started the habit of using tobacco products in the age group of 15-25 years. Most commonly used tobacco product was cigarette (56%) and the frequency of tobacco usage was 5-10 times per day (50%). About 68% of the questioned subjects are aware of passive smoking and 94% are aware of the consequences of using tobacco products. High prevalence of tobacco usage was observed among younger age group. This calls for the need that educational institutions mandate the inclusion of awareness of health hazards of cigarette smoking in their curriculums. This is a much needed intervention to extricate them from this habit.

Keywords: *Tobacco, smoking, cessation, awareness, attitude.*

Introduction

Out of 5 million deaths annually, tobacco smoking is one of the prominent causes of preventable deaths [1]. More than 1 billion people of current population smoke, with prevalence of 80% of people living in low and middle-income countries [2]. Compared with those who continue to smoke, adults who quit smoking gain 6 to 10 years of life. Furthermore, the mortality risk is decreased by approximately 90% when smoking is quit before the age of 40 years [3]. To describe the course of alcohol and illicit drug use from adolescence to young adulthood, a maturation process has been proposed, whereby transitions in adult social roles such as marriage, parenthood, and employment, factor significantly in the cessation process [4].

Due to change attributed to the growing awareness of smoking-related illnesses with advancing age, older smokers – over 40 years of age, are more likely to quit than younger smokers [5]. Evidentially, age modifies the relationship between heavy-smoking and quitting, thus making it nonlinear. History of childhood conduct problems and a lifetime history of major depression are other potential factors that could affect cessation of smoking [6]. Development of nicotine dependence has been related to early smoking initiation. Nevertheless, this relationship can be nullified in the age group with a history of conduct problems [7].

Recently, the Centers for Disease Control and Prevention (CDC, USA) found that, only 6.2% of all smokers quit smoking 1 year later, while more than half of adult smokers reported past-year quit attempts [8], with the majority of relapse having occurred within the first

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week after quitting the habit [9]. Effective intervention is one of the challenges that medical researchers and practitioners face. This endeavour, however, provides valuable information on components to consider at a nation's, state's, and local levels for effective tobacco control programs.

Our aim is to assess the knowledge, attitude, and practice (KAP) of factors associated with initiation and cessation of tobacco and its products among patients.

Materials and Methods

- A KAP study was conducted among patients using tobacco products of various age groups attending the outpatient in a dental college in Chennai, Tamil Nadu.

- A self-constructed questionnaire of 10 questions (Table – 1) were framed and distributed to 100 subjects.

- The questionnaire included information related to the patient's name, age and gender.

- The data collected was then statistically analysed with SPSS software (version 21) to obtain the results.

Table – 1 : Questionaire

S.NO	QUESTIONS
1.	WHAT MADE YOU TO INITIATE THE HABIT OF TOBACCO?
2.	AT WHAT AGE DID YOU START USING TOBACCO?
3.	WHAT KIND OF TOBACCO PRODUCTS DO YOU USE?
4.	HOW MANY TIMES DO YOU USE TOBACCO PER DAY?
5.	HOW SOON AFTER YOU WAKE UP DO YOU SMOKE YOUR FIRST CIGARETTE
6.	ARE YOU AWARE THAT PASSIVE SMOKING KILLS MILLIONS OF PEOPLE?
7.	ARE YOU AWARE OF THE CONSEQUENCES OF TOBACCO USAGE?
8.	HOW MANY TIMES HAVE YOU TRIED TO QUIT THE HABIT IN THE PAST?
9.	WHAT IS THE LONGEST TIME YOU HAVE GONE WITHOUT USING TOBACCO?
1O.	WHAT IS ONE OF THE MOST IMPORTANT REASONS YOU WANT TO QUIT THE HABIT?

Results

The questions in Table – 1 were presented to the test subjects and the responses have been statistically illustrated in this section. The response of the study was 100% out of which 56% of the subjects reported that they started the habit of using tobacco after having been influenced by their favourite actors in entertainment shows, predominantly movies, by fraternizing with friends and social circle which transcend the factors of using tobacco as a coping mechanism and also that initiated due to peer pressure, grouping to 13% and 25% respectively.

Figure 1 represents the age of commencement of smoking cited by the subjects. 69% of the subjects have reported that they commenced the habit of tobacco usage between the ages of 15 and 25 years, whereas 15% of them started before the age of 15 years, 14% in the age group of 25-45 years and the least of 2% of the subjects initiated the habit above 40 years of age (fig 1).

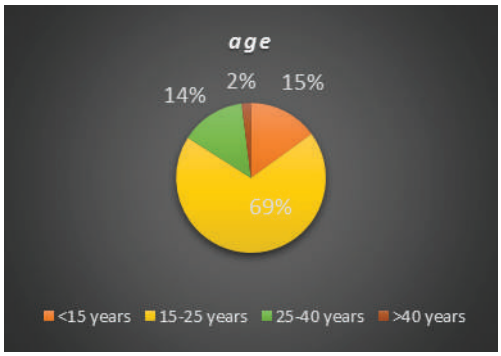


Fig 1

Figures 2 illustrates the type of tobacco product which the subjects used. It was found that cigarette was the most frequently used tobacco product by majority of the patients leading to about 56% of the subjects, usage of beedi was about 27%, gutka was consumed by 13% and snuff by about 4%.

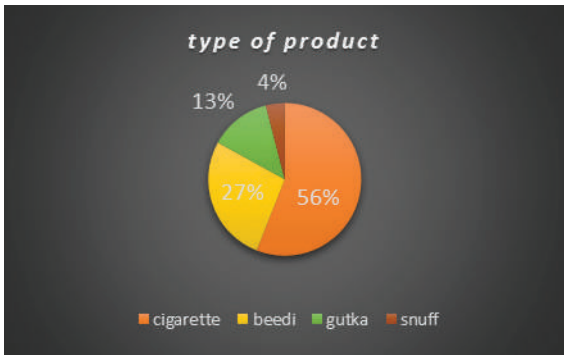


Fig 2

Figure 3 represents the frequency of smoking on a daily basis. Nearly half (50%) of the patients have delineated that they smoke 5-10 times per day, 28% smoke less than 5 times per day, 10-15 times by 17% of the victims and 5% smoke for more than 15 times per day. These details were described by the subjects upon inquiring specific times of using tobacco per day with respect to their hourly activities.

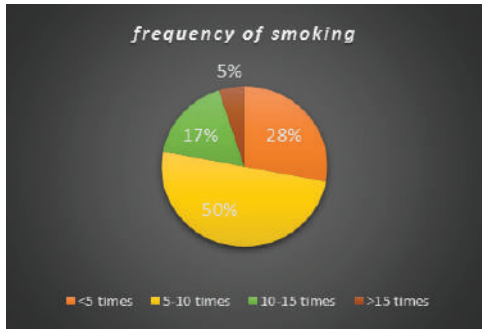


Fig 3

Categorizing the subjects based on the duration between the time they wake up and the first time they consume their tobacco product, majority of 36% of the reported subjects smoke their first cigarette in a day after 1 hour in the morning, 35% smoke after 30 minutes, 16% smoke within the first 30 minutes, whereas 13% smoke after 2 hours of waking up. Figure 4 denotes percentage of the subjects who are aware of the fact that passive smoking kills millions of people, and surprisingly 68% of them are aware of the effects of passive smoking and the rest are unaware of it.

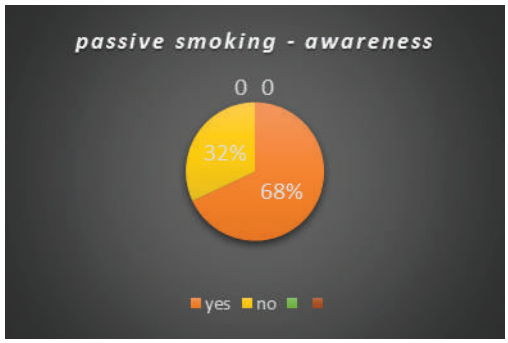


Fig 4

All the subjects were posed with the question if they are aware of the consequences of smoking and reportedly 94% are aware while the rest of the subjects have rarely thought of it as shown in Figure 5.

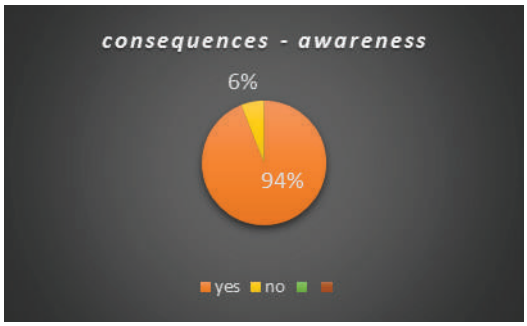


Fig 5

In order to quantitatively measure the persistence of attempts to quit the habit of using tobacco, inquiry of the subjects revealed that 40% of them have tried quitting the habit during some times and later stopped attempting, among others who are still trying to quit (29%), have tried many times (19%), or never tried to quit (12%).

The longest periods of abstinence lasted mostly for days and months, compassing about 40% and 35% of the questioned subjects respectively, compared to periods lasting for several hours or years grouping around 21% and 4% of the subjects respectively. The final and most significant question factors the motivation to quit use of tobacco, whereby the reason for quitting in 36% of the subjects is health issues, while 30% wanted to quit the habit bearing social acceptability in mind, 25% wanted to quit due to pressure or obligations due to family circumstances and 9% citing excessive and saveable expenditure towards purchase of tobacco.

Discussion

Transitions are complex between smoking states and increasingly unstable, that ultimately dictate the public health impact of cigarette smoking behaviour requiring a holistic, population-based perspective to understand the stocks and flows of smoking [10].

This study presents information regarding the knowledge, attitude and practice of initiation and cessation of tobacco among patients who use tobacco products. More than half of the respondents of this study reported that they had initiated smoking of cigarettes predominantly due to the influence of friends/peers and/or their favourite contemporary film stars' ostentatious smoking movie scenes. This result indicates that cigarette smoking is mostly induced by socialization. More than half of the participants (69%) in our study have also stated that they succumbed to this habit in the age group of 15-25 years. Similar results were seen in a study done by Kelkar et al where the prevalence increased with rise in age from 2.1% at <14 years to 9.8% at 18-20 years of age [11]. This probably is due to the encouragement from other adolescents to smoke as they grow older.

Our study shows that the most commonly used tobacco product is cigarettes (56%). Similar study conducted by Helena Koprivnikar et al in which 15-year-old students reported cigarette (93%) was the main tobacco product used among their fellow group [12].

The frequency of using tobacco was 50% in our study which was 5-10 times per day, whereas a study conducted by Meysami et al have shown that the frequency of using tobacco was 95% among their participants [13].

Non-users of tobacco products are also affected via passive smoking. Existing studies suggest that active and passive smoking might equally increase the risk of certain diseases, such as allergic rhinitis, allergic dermatitis, food allergies [14], breast cancer in females [15], to name a few. Smoke is considered a toxic agent to the foetus during pregnancy and an established, important and independent risk factor for low birth weight which is associated with neonatal and foetal mortality and morbidity, cognitive development and inhibited growth, and risk of chronic diseases in later life [16].

More than half of the participants (68%) of our study are aware of passive smoking. Similarly, a study conducted by Savvas et al [17] according to The International Tobacco Control Four Country Survey has reported that 40% of the participants have rarely thought of it.

Majority of the participants (94%) in this study are aware of the consequences of tobacco smoking. Similarly, a study conducted by Muhammad Ashraf Nazir et al states more than 69.9% of participants were aware of the adverse effects of tobacco consumption on oral health which included poor oral hygiene, bad taste and breath, periodontal disease, dental caries, oral ulcers, and oral cancers [18].

Young adolescents tend to take up smoking influenced by closely associated older relatives such as siblings or older students in schools, colleges, sport clubs, or neighbourhood residential communities. To refrain from picking up the habit, repeated educative sessions in different creative forms might help adolescents.

Reinforcing the necessity of quitting smoking and repeated consultation is important at every clinical visit [19]. Furthermore, counselling provided by health workers increases quit rates [20]. This intervention is relatively cost-effective, which is used by the majority of smokers as it is part of the existing health care services.

The habit of using tobacco products is increasing rapidly as a fashion and a way of socialization among young generation. Clinical and social intervention should be organized keeping in mind about the toxic and addictive behaviour of using tobacco products.

Conclusion

From a study of tobacco initiation and cessation among 100 subjects, statistics show that high prevalence of tobacco usage was observed among the younger age group – attending an educational institution. This demonstrates that the curriculum in school and college should include the awareness of health hazards of cigarette smoking which is needed as an intervention to extricate them from this habit. Dental professionals should be trained in tobacco cessation intervention to treat tobacco dependence.

Conflict of Interest:– Nil –

Source of Funding: Self

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The Perspective of Puritan Moslem on the Family Planning Program: The Case of Salafimovement in Semarang, Central Java, Indonesia

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Abstract

Family Planning Program is one of the policies in Indonesia related to the issue of population and family development that has been widely considered as successful. However, some obstacles emerged during the process of policy implementation, one of which was related to religious believes on family planning. An Islamic group called the Salafi Movement, for example, believes in pro-natalism and forbids the use of contraception. One of the sub-districts in the City of Semarang, called Banyumanik, has experienced a significant growth in the number of this group's members. Using a qualitative approach with in-depth interviews to collect data. Most members of this group have more than two children. Interestingly, many of them hope to have many more children in the future. Most informants considered the Family Planning program is limited to control or restrict births using particular contraceptives. The value of the Family Planning program challenged by the Salafi movement is related to the issue of limiting the number of children. The government should extend the scope of the campaign, especially dialogues with them.

Keywords: *Perspective, Puritan Muslims, Salafi Movement, Family Planning Program*

Introduction

Indonesia is the world's 4th most populous country after China, India and the United States [(1)]. According to the Indonesia's Central Bureau of Statistics, the population in Indonesia is about 255,461,707 [(2)]. One of the problems with this huge population is its low quality. In 2015, the Human Development Index (HDI) of Indonesia rank 110th out of 188 countries [(3)]. To overcome the problem of population, since the 1970s, the Indonesian government issued a policy called *Keluarga Berencana* (KB) or Family Planning program. In general, the goal of the Family Planning program is to achieve a balanced population growth and to improve the quality of population and families in order to realize a more advanced and independent community, and to accelerate the realization of sustainable development [(4)]. The success of the Family Planning program is generally measured by the declining *Total Fertility Rate* (TFR). In recent years, the TFR in Indonesia has been stagnant 2.6 from 2002 until 2012 and slightly decreased to 2.4 in 2017.

Widowati *et al* explain that the external obstacles in the implementation of the Family Planning program could include knowledge/education of the community, cultural values, religion, and socio-economic conditions[(5)].

The performance of family planning services since decentralization has stagnated and some has declined. Considering their belief on and views that contradict the Family Planning program, this study suggests that the Salafi movement contribute the declining trend in practice of the Family Planning program. For example, in terms of fertility, the average couples in reproductive age have more child between 5-6 children per family [(6)]. The main reason behind the

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high fertility is the belief embraced by the pronatalist Salafist movement stating that having more children will increase the number of people who are ready to face the external challenges.

The Salafist movement has developed massively in Indonesia. The estimated number of Salafists in 2013 was only 1% of the total Indonesian population – that is, 240 million. The number has increased into 1.5% to 2%. The increase in the number of the Salafi members creates more challenges for the implementation of the Family Planning program. As the number of the Salafists is about 1.5% of the total population, it means that there are at least 3.5 million Salafistsin Indonesia, not to mention the inclusion of the new members of this group.

Table 1. The Estimated Member Salafi 2010 - 2035

No	Year	The number of Indonesians based on IBS projection (Bappenas, et al , 2013)	Estimated Percentage of number of Salafist members	Estimated number of Salafist members in Indonesia
1	2010	238.518.800	1%	2.3 million
2	2015	255.461.700	1.5% - 2%	4 - 5 million
3	2020	271.066.400	2.5% - 3%	6 - 8 million
4	2025	284,829,000	3.5% - 4%	9 - 11 million
5	2035	305.652.400	4.5 - 5%	13 - 15 million

In Semarang, according to Rokhmad (2012), the Salafi movement can been identified using these characteristics: Middle Eastern styles, the *letterlijk* or literal understanding of Islam, and the use of Arabic terms such as *daurah, halaqah,* and *mabit* for their group activities. This Salafist movement's pattern of dissemination includes the introduction of religious teachings to students at school, madrasah, and college levels, which then continues to spread to the members of general community[7].

In Banyumanik, a sub-district in the City of Semarang, whose Islamic population is about 108,343 people out of 132,508 [8], we can spot a significant development of the Salafist movement. This is evidenced from our field observations that many Holy Qur'an studies or *halaqah* are frequently held in several mosques in this sub-district. Because of this frequent activities of *halaqah* held by the Salafist movement in several Banyumanik mosques, this subdistrict was selected as the area of this study. In addition, the Banyumanik area is located in the area adjacent to Semarang regency (semi-urban area), which allows us to have a variation of interviewees based on their occupation and education.

Methods

This study is a qualitative descriptive study using a case study [9]. This study was conducted in Banyumanik, Semarang. This location was selected because of a significant development of the Salafist movement in the area.

This study was used a primary and secondary data. The data were analyzed using an interactive technique as mentioned by Miles & Huber man [10]. In this interactive analysis model, data analysis is divided into three components: data reduction, data presentation, and conclusion. The data triangulation by Patton is an attempt to test the validity of the data, by comparing the same data from different sources, which is expected to minimize the errors that may occur [9].

Results

The Salafist movement in Banyumanik, Semarang,

tends to become an accommodative movement, carrying puritan ideology disseminated to society through religious preaching and sermons (*halaqoh* and *dauroh*).

In addition, the dissemination of the Salafist ideology is also conducted through their own online media. They disseminated their ideology on social media. They also spread their teachings through a radio, which is managed by the center of the Salafi movement. Furthermore, the Salafi movement also disseminated their ideology and teachings by making flyers and publishing religious magazines and books. They put the flyers and make it available in several mosques so everyone in the mosques can freely take the flyer and read it. Meanwhile, the magazines and books were circulated through some bookstores around Banyumanik area. The ideology of the Salafi movement in Banyumanik spread through the hands of religious teachers (*ustadz*).

On average, the informants of this study have a high educational background, although some of the informants have not graduated yet from the university. It is one of the strengths of the Salafi movement that the average members of this group are people considered intellectuals with a high educational background. In terms of employment, most members of the Salafi movement prefer to do a freelance work or business, although some of the members work formally as civil servants and private employees. It is important to note that most members of this group use their occupations as a means of *da'wah* (a means to spread their teachings). They sell an Islamic style of herbal medicines such as honey and *habbatus sauda* (black cumin), and offer Cupping therapy, which they believe as an Islamic treatment according to the tradition of the prophet (*thibbunnabawiyah*).

Most members of this group have more than two children. Interestingly, many of them hope to have many more children in the future. An informant has expressed his dream to have more than five children as he believed that it is suggested by the Quran and Sunah. When he was asked about the target of the number of children that he expected, he said that the target is twelve children. He said, "*The prophet loved to have a lot of followers"*.

This dogmatic belief is an absolute conviction of the Salafist members, which is quite difficult to change. They consider that that the value of this belief is final. We will give the explanation about this in more detail in the later section of this paper on the conflict between the Salafi movement and the Family Planning program.

Most informants considered the Family Planning program is limited to control or restrict births using particular contraceptives. This view seems to ignore a more substantive objective of this program – that is, reproduction health. As far the educational background is concerned, the informants of this study have various educational background, from high school to college and postgraduate levels. However, the meaning of the Family Planning program to the Salafists' members is almost the same. They think that Family Planning program is merely a program to limit the number of children or to regulate births using certain contraceptives.

When we investigate more on this view, we found that most information was obtained from the secondary sources, such as from religious teachers, advertisement, and other secondary information. The information on Family Planning program was not obtained from official sources such as from government officers, health workers, or community members who have received training on Family Planning programs.

A similar opinion was expressed by several Salafist online media. Badri (2009) argues that the Family Planning program, which encourages a restriction of children, is considered a program belongs to the enemies of Islam and is deviated far from the truth and guidance of Islam [(11)]. Furthermore, the program is against the reality and the needs of human nature. Moreover, limiting and preventing births, according to him, may damage economic, social, physical, spiritual and other aspects of human life.

Other online site said that Family Planning program is exactly the government's efforts to limit children [(12)]. Islam teaches its followers to have children through legitimate marriage. It is a mistake for people who do not want to have children. The Family Planning program, which has an agenda to limit the number of children. Accordingly, Muslims are not allowed to participate in such a child restriction program. The Salafist movement argues Muslims should not be afraid of poverty, because fortune is controlled by Allah, the God.

The informants' responses and rejection on the Family Planning programs are similar to the statement in religious decree made by the religious leaders of the Kingdom of Saudi Arabia, which states that Muslims should not refuse pregnancy for fear of poverty because Allah provides fortune to all of His creatures (Hayati,

2015: 79-80). According to Hisham (2010), the Salafi movement tends to be oriented towards the Saudi and Middle Eastern scholars [13]. Therefore, the decision taken by the Salafist movement tend to be resemble to that of the Saudi Arabia and the Middle East.

One of articles, argues that the FP program is illegal so that every Muslim should not join the program for fear of poverty. Hakim (2007) argues that preventing births for the fear of poverty is the belief of ignorant people in the pre-Islamic Arab community and is the belief of *kuffar* (non-believers) [14]. These ignorant people and the *kuffar* thought that having many children will lead to poverty. They attempt to prevent children from various methods ranging from *azl* (coitus interruptus) to using contraceptives. Such an action should not be performed by Muslims.

An article states that someone joining the family planning programs for fear of poverty has a bad prejudice to Allah [15]. He argues that the fear of poverty is just like the acts of the infidels, who killed their children because they were afraid of not being able to provide a good living for their children. Muslims should need afraid of becoming poor because their lives and fortunes are set by Allah.

Conclusions

This study comes to these four conclusions:

1. Salafi movement's perspectives on family planning programs include:

a. The knowledge of the Salafi members on the benefits of the family planning program tends to be very technical and implementation. Their knowledge is limited to the technical nature of the family planning program, including its implementation to control the births and the use of contraception, without their understanding of the more substantive objectives of the policy – that is, to improve the women's reproductive health.

b. The positive transformation to create more prosperous families in Indonesia, as the core objective of the family planning program, are not of the concern of the Salafi movement.

c. The influence of technical and implementation knowledge of the Salafi movement leads to the assumption that the family planning program run by the Indonesian Government is an instrument of authoritarian regime, and it is influenced by the other nations' and the other religious policies.

d. The Salafi movement considers the family planning program forbidden. However, there are certain religious considerations that allow the implementation of the family planning program under certain conditions. Explicitly those considerations of the permissibility of family planning programs are education and welfare of children and the maternal safety and health.

2. The value of the Family Planning program challenged by the Salafi movement is related to the issue of limiting the number of children.

3. Salafi movement will act according to their beliefs in opposing the family planning program by having more children and campaigning the rejection of family planning programs through their medias.

4. Involving the Salafi movement on the family planning programs can be achieved by the participatory strategy.

Recommendation

Based on the conclusion, this study suggests these recommendations:

1. The government should extend the scope of the campaign on the family planning program to cover not only the technical aspects of implementation, such as the use of contraceptives and children's restrictions, but also the substance of the program.

2. The government should open the media of dialogue on the internet as a consultation media.

3. The government should facilitate a dialogue on the family planning program from the perspective of Islamic law (*fiqh*).

4. The government should facilitate a study discussing about the sharia clinics serving the family planning program to provide services for those who do not allow the touch between non-*mahram* men and women.

Ethical Clearance: NILL. This research was conducted without treating informants

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Forensic Application of ATP6 Gene Haplogroups in a Cohort of Obese Saudis

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Abstract

Blood samples from 108 obese individuals (73 males and 35 females) and 106 healthy ones (79 males and 27 females) were collected from a cohort of Saudis inhabiting Riyadh province. ATP6 mitochondrial gene was amplified and sequenced to identify obesity-related SNPs and the mitochondrial DNA haplogroups for forensic purposes. A novel site (A8660G) was found while C8655T and T9103C were obesity- related sites. The haplogroups H2a and H1a were common as they were found in 70.4% and 66% of obese and non-obese individuals, respectively. L2 was recorded in the obese samples only and H21 was recorded in the obese males only. The J2a haplohroup was more common in obese individuals. Constructing mtDNA database for people inhabiting different regions of Saudi Arabia for forensic purpose and to affirm the utility of H21 and L2 in identifiying obesity is necessary.

Keywords: *haplogroups, obesity, ATP6 gene, Saudi Arabian*

Introduction

The mitochondrial genome (mtDNA) plays an important role in determining human haplotype as its SNPs could determine the population pattern of ethnic groups[1,2]. It can also be used to calibrate the migration events of human ancestors and their origins as it expresses their maternal inheritance[1]. MtDNA might be, therefore, used to determine the population structure of a particular society.

MtDNA played an important role in identifying obesity as mitochodria plays a key role in cellular energy metabolism. ATP6, cytochrome b (cytb) genes and d-loop are the most effective mtDNA segments in this perspective. ATP6 gene controls the production of ATP molecules while cytb gene plays a role in electron transport during the oxidative phosphorylation and d-loop controls the heavy strand replication of the mtDNA. Researchers used these fragments in identifying obesity from differnt ages and sexes[3-7].

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Studies investigated the association of obesity and metabolic disorderes to the mtDNA haplogroups[8-11]. Demir et al.[6] found obesity-related SNPs in ATP6 and cytb genes in a cohort of obese Turkish children. Knoll et al.[5] revealed a significant correlation of ATP6 gene and d-loop SNPs to obesity in W haplogroup. The d-loop acquired 5 SNPs related to abdominal fat accumulations and diabetes and blood pressure[12]. Similar investigations have been conducted for Turkish, Caucasian and Arabian ethnic populations[7, 13-15].

Few studies have been conducted so far on mtDNA of Arabian populations[2,7,10]. The mitogenome of over 500 random individuals from different 5 geographical Saudi Arabian regions have been targeted and showed that Saudi Arabians are belonging to 5 main haplogroups L, M1 U6, U, M[10]. Amer et al.[7] used cytb gene for a population from the western Saudi Arabia and found 4 haplogroups H2a, JT, U5a and R0a. Eaaswarkhanth et al. [2] detected d-loop variants and haplogroups identifiable to obesity in a Kuwait population. The present study amplified and sequenced ATP6 gene to identify obesity-related SNPs and haplogroups for a cohort of Saudis inhabiting Riyadh city for forensic purposes.

Materilas and Methods

Sampling

Blood samples of 110 obese (75 males and 35 females) and 104 normal (74 males and 30 females) adults (age > 21 and < 52 years) were collected from randomly selected Saudis inhabiting Riyadh city under the supervision of a specialized clinician. According to WHO[16], obese inidivduals acquired BMI >30 kg/m^2 and the normal ones acquired BMI between 20 kg/m^2 and 25 kg/m^2. Individuals with chronic diseases were excluded. Those who had BMI >25 ~ <30 or BMI <19 kg/m^2 and those with age <18 years were also excluded. Demographic data and family history were collected from donors in a consented questionnaire.

DNA extraction, PCR and sequencing

300 µl blood samples were pippetted into 1.5 ml sterilized tube. DNA extraction was conducted using Qiagen DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Polymerase chain reaction was conducted in 50 µl reaction mixtures containing 2 µl DNA template, 25 µl GoTaq Green Master Mix (Promega Corporation, Madison, WI 53711-5399, USA), 22.6 µl nuclease free water and 20 pmoles of each primer (forward primer: 5'-TCT GTT CGC TTC ATT CAT TG -3' and the reverse primer: 5'- TGA AAA CGT AGG CTT GGA-3'). The PCR conditions were according to Amer et al.[17]. Purified products were sequenced from both strands using The Koryean Macrogen company facilities.

Statistical Analysis

Sequnces were prepared by DNASIS v. 3.5 and MacClade V. 4.10 software[18] and were aligned with the revised Cambridge Reference Sequence[19] (RSRS). Data were deposited in DDBJ/NCBI Genbank database (MN427654 - MN427867). ATP6 haploroups were predicted by using MitoMaster program using HaploGrep2 with Phylotree 17 for haplogroup determination (Topic revision: r3 -02. May2017, MarieLott).https://www.mitomap.org/foswiki/bin/view/MITOMASTER/WebHome).

Results and Discussion

ATP6 gene variants of 214 adult indivduals from both sexes were investigated by direct sequencing of 531-bp fragment between nucleotide position 8615 and 9145. Polymorphisms with a frequency 5³% in controls or obese cases were assumed to be relevant and further analyzed[14]. Table 1 shows the ratios of 7 recorded variants, 5 of which were between pyurines and 2 were between pyrimidines. They were C8655T, A8664G, G8697A, A8701G, A8860G, G8994A and T9103C. The variant C8655T was recorded in 5.6% of obese patients, while it was recorded in 3.8% controls. The two variants A8664G (2%) and G8994A (0.9%) were recorded in the patients only in spite of being irrelevant (below polymorphism threshold). The frequencies of the G8697A (8.3% vs 8.5%), A8701G (25% vs 22.6%) and A8860G (100% vs 100%) were high in both obese and controls, respectively. The last variant was considered to be a novel as it did not appear in the reference sequence. The variant T9103C was recorded in 7.4% patients and in 4.7% controls. Among the 7 variants, 3 were non-synonymous substitutions and the other 4 were silent (C8655T, A8664G, G8697A and G8994A). The variants A8701G and A8860G exhibited exchange of thrionine to alanine at the potions Thr^{59}Ala and Thr^{112}Ala, respectively. At T9103C, the amino acid changed from phenylalanine to leucine at the position Phe^{193}Leu.

Haplogroups were predicted using HaploGrep2[20] with PhyloTree mtDNA tree Build 17[21]. Seven haplogroups (H2a, H1a, H21, T, J2a, L0 and L2) were identified, most of which are belonging to three macro-haplogroups H, T, L and J. Table 2 and Table 3 show haplogroup percentages and their prevalence. H2a was common exhibiting 51.9% in the obese samples and 46.2% in the controls. H1a was prevalent in 18.5% patients and 18.8% in the controls. The haplogroup T was equally prevalent (7.5%) in obese and controls. J2a was found in obese samples with 8.3% and in controls with 4.7% being higher in obese females (14.2%). In obese males, the haplougroup H21 appeared with 1.9%, while L2 appeared in 2.8%. The predicted haplogroups refelected the results shown by Abu-Amero et al.[10] that the Arabian Peninsula has received substantial gene flow from Africa, detected by the presence of a major L lineage (62%) being migrated from the Northern hemisphere (H, T and J).

By comparing this study to the previous ones (Table 4), 5 novel variants associated with obesity were found (8655C>T, 8664A>G, 8697G>A, 8994G>A and 9103C>T). The two variants 8701A>G, 8860A>G were recorded also in obese Turkish[8] and Japanese[3,4] juveniles. However, these two variants could be associated to ethnicity rather than obesity since they appeared in a larger healthy Saudi population[10] as well.

It could be therefore possible to use this gene in forensic purposes.

An individual mtDNA haplogroup can be used as a reference when analyzing the mitochondrial genome data of an unknown sample since a human race to which this sample belongs can be predicted. Rodriguez-Flores et al.[22] suggested that the difference in mtDNA haplogroups may be also due to the heterogeneous marriage from outside and inside the population. The present study, therefore, used random samples from both sexes to excute a preliminary mtDNA database for an ethnic group inhabiting Riyah city. H, R[23] and L[24] are associated with some genetic disorders. In the present study, the sub-haplogroup H21 (2.7%) was associated with obesity in males, while J2a was associated with obesity in females (14.2%).

The 7 predicted sub-haplogroups herein belong to 4 main haplgroups (H, T, J, L) and all of which were recoreded in Sadui Arabian populations inhabiting diffrerent geographical regions[7,25,26]. By sharing haplogroups (H, T and L) with those predicted by Abu-Amero et al.[10], the present study might confirm that Arabian Pinnensula received human races from diffrernt ethnic groups of Africa, Asia and Europe. Meanwhile, the haplogroup H (H2a, H1a and H21) was dominant in the studied population as it showed high prevalence in both pateints (71.2% ♂ and 74.2% ♀) and controls (63.2% ♂ and 74% ♀). In spite of its low prevalence among Saudis[10], H haplogroup is common in the middle region (Riyadh) from which the samples of the present study were collected. Roostalu et al.[25] also recorded the major prevalence of H2a followed by H1a in the middle region of Arabian Pinnensula. Khubrani et al.[27] predicted the Y-chromosome haplogroups for 500 Saudis inhabiting 5 distant geographical regions and found that the haplogroup J is common in the midde region. The present study recorded this haplogroup too.

Table 1. Synonymous ATP6 gene SNPs and their frequency % in obese and control Saudis.

Synonymous substitution	Gender %					
	Control=106			Obese =108		
	F	M	∑	F	M	∑
C8655T	3.3	2.7	6.0	5.7	5.3	5.5
A8664G	0	2.7	2.7	0	1.3	1.3
G8697A	6.6	9.5	16.1	2.9	10.7	13.6
G8865A	0	0	0	0	2.7	2.7
G8994A	0	0	0	0	2.7	2.7
T9042C	2.9	1.4	4.3	6.7	4	10.7

Table 2. Non-synonymous ATP6 gene SNPs and their frequency % in obese and control Saudis.

Nonsynonymous substitution	Amino acid subsitition	Gender %					
		Control =106			Obese=108		
		F	M	∑	F	M	∑
A8701G	Thr59Ala	30	22.9	52.9	22.9	29.3	52.2
A8836G	Ala104Val	0	2.8	2.8	0	2.8	2.8
A8860G	Thr112Ala	100	100	100	100	100	100
A8887G	Ile121Val	0	2.7	2.7	0	0	0
G9055A	Ala177Thr	0	4.1	4.1	0	4	4
T9103C	Phe193Leu	3.3	5.3	8.6	14.3	6.6	20.9
T9116C	Ile193Thr	0	0	0	0	2.7	2.7

Table 3. Prevalence % of the predicted ATP6 gene haplogroups.

Predicted haplogroup	Gender				Total		Variants
	Female		Male				
	control	obese	control	obese	obese	control	
H2a	48	60	45.5	48	51.9	46.2	A8860G
H1a	26	14.2	17.7	20.5	18.5	19.8	A8701G/A8860G
H21	0	0	0	2.7	1.9	0	A8860G/G8994A
T	3.7	2.8	8.8	9.5	7.4	7.5	G8697A/A8860G
J2a	3.7	14.2	5	5.5	8.3	4.7	A8860G/T9103C
L0	3.7	5.7	2.5	1.4	2.8	2.8	A8860G/A8701G/C8655T/ C9042T
L2	0	2.8	0	2.8	2.8	0	A8860G/A8701G/C8655T

Table 4. Comparison between the present and previous studies for the recorded SNPs.

Ethnic group	Sample size	Number of detected substitutions	non-synonymous variations
Japan (Fuku et al., 2002)	Young obese (n=96) Controls (n=0)	n=26 novel=17	8584G>A, 8563A>G, 8701A>G, 8764G>A, 8950G>A
Japan (Guo et al., 2005)	Young obese n=96 Type 2 diabetes (n=96)	obese group: n=26	8584G>A, 8563A>G, 8701A>G, 8764G>A, 8950G>A
Saudi Arabia (Abu-Amero et al., 2008)	553 healthy unrelated individuals	n=2	8557G>A,8860A>G
Turkey (Demir et al., 2014)	Obese children (n=100) Controls (n=100)	obese group: n=39 novel=1	8557G>A, 8584G>A, 8684C>T, 8701A>G, 8764G>A, 8812A>G, 8836A>G, 8843T>C, 8860A>G, 8867T>C, 8869A>G, 8950G>A, 8962A>G, 8966T>C, 8975T>C, 9007A>G, 9055G>A
This study	Obese Adult (n= 108) Controls Adult (n= 106)	n= 7 novel= 5	8655C>T, 8664A>G, 8697G>A, 8701A>G, 8860A>G, 8994G>A, 9103C>T

Conclusions

Seven ATP6 gene variants were recorded in the studied samples among which, 5 were related to obesity and 2 were related to ethnicity and therfore can be used for forensic purpose. Building mtDNA database for human population inhabiting a specific gegraphic area could be used as a forensic reference since a human race to which this sample belongs can be predicted.

Ethical Clearance: 214 Saudi individuals gave written informed consent before participating in this

study. Ethical clearance was also obtained from Internal Security Forces Hospital in Riyadh.

Conflicts of Interest : Authors declare that there are no conflicts of interest regarding the publication of this paper.

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Bidan Lewu as Local Determinant Factors in Pregnancy and Childbirth Care

(Study in the Dayak Ngaju Tribe in Kotawaringin Timur District)

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Abstract

Background: he coverage of antenatal care was low in Kotawaringin Timur in 2019. The first visit during pregnancy (K1) was 81.20%, and the fourth visit during pregnancy (K4) was 74.50%, with a national visit standard of 95%. Kotawaringin Timur's adolescent pregnancy rates reached 66.89%, higher than the other districts' average rate in Central Kalimantan Province. The adolescent pregnant women had a low intention of antenatal care as much as 84.48% in 2019.

Objective: This study aimed to analyze the local determinant factors that contribute to adolescent pregnant women's intention in carrying out antenatal care.

Methods: This study was qualitative research. The data were collected by a focus group discussion (FGD) method and in-depth interviews in four sub-districts of Kotawaringin Timur. The informant of this study was 45 people consisting of 44 adolescent pregnant women and one traditional leader (Damang). Data were processed using the self-view value domain method.

Result: Adolescent pregnant women's intentions during antenatal care were influenced by their responses to the availability and ability of people who help or assist the process of pregnancy and childbirth care. Adolescent pregnant women prefer traditional birth attendants or bidan lewu as their pregnancy nurses and childbirth assistants. It was triggered by the trust of adolescent pregnant women towards bidan lewu because they were more experienced, attentive, and patient in serving. The involvement of bidan lewu during pregnancy and childbirth has been used for generations.

Conclusion: Some of the Local determinant factors related to pregnancy and childbirth care are the presence of bidan lewu who have been used for generations to treat pregnancy and assist in the childbirth process.

Keyword: *determinant factors, pregnant women's intention, antenatal care*

Introduction

Health services in the pregnancy phase are known as antenatal care (ANC). Antenatal care is a health service provided by health workers to maintain pregnant women and their babies' health during pregnancy[1]. This service is essential for pregnant women to maintain pregnant women's health so that the pregnancy process of the mother runs normally, and ANC services are expected to detect pregnancy problems early[2].

Indonesia's Health Ministry shows that there has been an increase in K4 coverage (fourth visit during pregnancy) in Indonesia from 87.30% in 2017 to 88.03% in 2018[3]. The targeted strategic plan of K4 coverage by the Ministry of Health in 2018 is 78%. When compared

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with the realization of K4 coverage in 2018, Indonesia has reached the target. While the coverage of K4 services in Central Kalimantan in 2018 was 84.79%. The K4 service achievement is higher than in 2017, with K4 service coverage of 83.88%[4]. While the examination of pregnancy in Kotawaringin Timur in K1 (first visit during pregnancy) was 81.20%, and K4 was 74.50%, including low[5].

Adolescent pregnancy is a pregnancy that occurs in women aged 10-19 years. Teenage girls who become pregnant at the age of 10-19 years have a higher risk because the reproductive organs are not mature enough to perform their functions[6]. Law Number 35 the Year 2014 article 26 paragraph 1c regarding child protection states that parents are obliged and responsible for prohibiting child marriage[7]. In Indonesia, there are 37.91% of married children under the age of 16[8]. Meanwhile, around 16 million women aged 15 to 19 years, and 2 million women aged less than 15 years give birth each year[9].

Based on the Unicef report, Indonesia is a country with the seventh-highest child marriage rate, amounting to 457.6 thousand women aged 20-24 years old married before 16[8]. Badan Pusat Statistik stated that there were 20 provinces in Indonesia with a higher prevalence of adolescent marriages than the national figure of 22.82% in 2015. The highest percentage of adolescent married women is Central Kalimantan Province, with a rate of 33.56%[10]. The percentage of women who had given birth or were pregnant with their first child in Central Kalimantan in 2017 was 13.8%, this figure is above the national average of 7.1%11. Kotawaringin Timur has a relatively high early pregnancy rate compared to other regions[12].

Mortality of pregnant women, childbirth, and puerperal are significant problems in developing countries. The process of pregnancy and birth in adolescence is one of the factors that contribute to maternal mortality. Women who are married in their teens have a higher risk of pregnancy, the younger the age of a woman having a pregnancy, the higher the risk for complications[13]. Complications during pregnancy and childbirth are common causes of death in adolescents aged 15-19 years. Toddlers born to mothers with teenage pregnancies have a higher risk of experiencing low birth weight (LBW), preterm delivery, fetal growth restriction (FGR), and fetal distress[14].

It is known that 80% of maternal mortality can be prevented if pregnant women have access to health services to conduct regular antenatal care[15]. Low antenatal care caused by individuals, families, communities, and health institutions' problems[16]. Constraints faced in implementing health services for pregnant women are the lack of access of pregnant women to health services provided by health workers and the quality of services that must be further improved[17]. Besides, the intention of prenatal care for pregnant women in Kotawaringin Timur also tends to be low, namely 81.4%[5]. This study aimed to analyze the local determinant factors that contribute to adolescent pregnant women's intention in carrying out antenatal care.

Methods

This study used a qualitative research method, conducted in October 2019, and located in four sub-districts of Kotawaringin Timur district. Data were collected from the results of focus group discussions (FGD) and in-depth interviews. The informant of this study was 44 adolescent pregnant women and a traditional leader (Damang). Data collected from the results of FGD and in-depth interviews are processed using the self-view value domain method.

Result

The low antenatal care for adolescent pregnant women can be caused by the mother's intention to have her pregnancy checked by health workers who are still low. Adolescent pregnant women's intentions during the process of pregnancy care are influenced by several things, such as the availability and ability of people who help or assist the process of pregnancy and childbirth care. Based on the results of FGDs and in-depth interviews, the informants revealed that the obstacles during antenatal care because of the distance to a distant health facility, and no one accompanies them. Mrs. RN is a 19-year-old pregnant mother who said that she rarely checked her pregnancy there because no one was accompanied her, and the distance of the health facility was also far from home.

"...Kalau kami disini jarang memeriksakan kehamilan karena tidak ada yang mengantar lalu jarak Puskesmas juga jauh..."

"...We rarely check our pregnancy here because there is no one to accompany, so the distance of the Puskesmas is also far..." (Mrs. RN, 19 yo)

Based on the FGDs and in-depth interviews, adolescent pregnant women also revealed that bidan lewu was more attentive, patient, and experienced in handling her patients and had managed many childbirths. In contrast to midwives who are less familiar, less alert, and less skilled in dealing with their patients. Mrs. SY is a 16-year-old pregnant mother who said that she believed that the bidan lewu is more experienced, has dealt dozens of people, and if their pregnancy got a problem, it will be fixed by bidan lewu.

"...Menurut saya, dukun kampung lebih berpengalaman, puluhan orang sudah ditangani dan kalau perut kita turun maka akan dibetulkan posisinya..."

"...In my opinion, traditional birth attendants are more experienced, have dealt dozens of people and if our stomach falls then its position will be fixed..." (Mrs. SY, 16 yo)

The local traditional leaders stated that the bidan lewu would immediately come if asked to help. Therefore, pregnant women still entrust their pregnancy to bidan lewu because they are considered influential people in the community. The traditional leader (Damang) also said that ancestors already used the services from bidan lewu for over the years. In the past, the people didn't have midwives as health workers, so they came to the bidan lewu. Bidan lewu serves the care for pregnant women, such as stomach massage, that hopefully will help giving birth comfortably.

"...Kalau orang dulu itu kan bilangnya kalau enggak diurut perutnya tuh... Orang dulu kan enggak ada segala bidan... Nah datangnya itu ke dukun kampung supaya enak melahirkannya, gitu bilangnya..."

"...The ancestors told me that if they didn't massage their stomach... They didn't have midwives... So they came to the traditional birth attendant in order to give birth comfortably, so they said..." (Mr. X, 57 yo)

The perceptions and beliefs of adolescent pregnant women in bidan lewu have been going on for generations and still very strong. The cultural factor put forward by traditional leaders is that bidan lewu not only deals with the childbirth process, but they also understand traditions. They also can deal with complaints of adolescent pregnant women. There is a culture of "Bapalas Midwife" in which midwives or people who help pregnant women during childbirth will be given food as a thank you and "redeem" the baby. Bidan lewu is believed to hold tightly still and understand the meaning of these traditions. Therefore adolescent pregnant women tend to come to bidan lewu to help respect them.

Discussion

The results of this study were in line with research conducted by Ye et al. (2010)[18] and Gross et al. (2012)[19]. They showed that education, income, knowledge, attitudes, distance, and the cost of antenatal care services could influence the use of antenatal care services[18]. According to Gross et al. (2012)[19], the low quality of service and awareness about the benefits of antenatal care, late knowing pregnancy, and social and economic factors can influence the timeliness in antenatal care examination. In this study, pregnant women prefer bidan lewu as pregnancy caretakers and childbirth helpers because they feel the distance to health facilities is quite far, and no one takes them there. Besides, they doubt the competence and availability of health workers when needed, unlike the bidan lewu available near them. Pregnant women assume bidan lewu has sufficient experience in dealing with pregnant women.

Depkes (2008)[17] stated that the percentage of birth attendants provided by non-health workers is quite high, with 80% of mothers giving birth in Indonesia prefer to give birth to a traditional birth attendant rather than to a health facility. Meanwhile, Nurhidayanti's research (2018)[20] showed that pregnant women are more comfortable giving birth at home and assisted by a traditional birth attendant. This result is caused by giving birth in a health facility that requires many costs. Public trust in bidan lewu and the comfort of giving birth at home were higher than those at health facilities.

Trust is a belief in individual behavior that expects someone who is trusted will show positive behavior[21]. This research is in line with the theory of Mayer (1995)[21] which showed that traditional birth attendant gives more attention, patience, friendliness, obeying the requests of pregnant women and mutual trust that makes pregnant women choose traditional birth attendant for antenatal care. This study's results were also in line with research conducted by Nuraeni (2012)[22], which showed that the feeling of safety and comfort is also felt by pregnant women assisted by a traditional birth attendant. Similar studies showed that pregnant women have trust in bidan lewu. Apart from the limitations and doubts about health workers' ability, bidan lewu are considered to be more

prepared if needed and experienced in handling pregnant women so that pregnant women are more confident to go to the bidan lewu during pregnancy care and childbirth assistance. This belief can encourage the intention of pregnant women to check their pregnancy.

Conclusion

Various factors influence the intention of antenatal care of adolescent pregnant women in Kotawaringin Timur. One of them is the existence of local determinants that develop in the culture area of Dayak Ngaju. The local determinant factor contributing to the adolescent pregnant women's intention of antenatal care in Dayak Ngaju is the involvement of bidan lewu. Bidan Lewu has traditionally been used by the local community to treat pregnancy and assist the childbirth of pregnant women. Local people still believe that bidan lewu has more experience, attention, and patience in serving them.

Conflict of Interest: Nil

Ethical Clearance: This research has been proved by Health Research Ethics Committee, Faculty of Public Health universitas Airlangga

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The Effects of Prenatal Yoga for Primagravida with Gen Expression mRNA FKBP5 (FK506-binding Protein 51)

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Abstract

Background: FKBP5 protein plays an important role in determining sensitivity to negative glucocorticoid feedback, a key mechanism for stopping the HPA axis response in stressful episodes.

Methods: The design of this study is a quasi-experimental approach / quasi experimental and one group design pre and post test design with group control. The sample in this study amounted to each group of 12 respondents as control and treatment of yoga.

Results: There was a significant difference ($p = 0.000 <\propto$) on average FKBP5 gene expression in the control group before (7.1 ± 0.59) and after observation (9.43 ± 0.68), meaning that there was an increase in cortisol levels in the control group in the control group before and after observation, while the treatment group showed a significant difference ($p = 0.001 <\propto$), the average expression of FKBP5 gene before being treated (7.52 ± 0.49) and after being treated (6.88 ± 0.54),

Conclusion: There was a significant difference ($p = 0.000 <\propto$) on average FKBP5 gene expression in the control group, which means that prenatal yoga decreased FKBP5 gene expression before and after treatment.

Keywords: *MRNA expression, FKBP5 gene, Yoga, Trimester III*

Introduction

During pregnancy there is a period of physiological, psychological and environmental transition that results in varying degrees of difficulty for women. 1,2 bio-physio-psycho-social changes during pregnancy can cause stress. especially in the third trimester, because in this period is (waiting period) where there is growth and development of the fetus that increases dramatically. Research using ultrasound Doppler flow velocimetry shows high resistance of uterine arteries in women with high anxiety scores in the third trimester[1,2.] Psychological burden on pregnant women is more common in the third trimester of pregnancy than in the first and second trimester, so that in the third trimester mothers need calm and support from their husbands, families and health workers, with the expectation that mothers can face pregnancy and preparation calm labor, without complication[s3,4,5].

When psychological problems arise during pregnancy, a risk assessment of treatment for the mother and fetus must be carried out, because pharmacological therapies such as antidepressants and benzodiazepines are known to cross the placental barrier. Therefore, non-pharmacological therapies must be considered as possible options for pregnant women in an effort to avoid side effects dru[g6]. Some non-pharmacological therapies that can be considered include psychotherapy, massage therapy, yoga, and listening to music. Yoga has sparked a special interest as an alternative therapy, because more and more evidence has to do with the increasing effect of decreasing depressive symptoms and reducing cortisol in some populations7,8

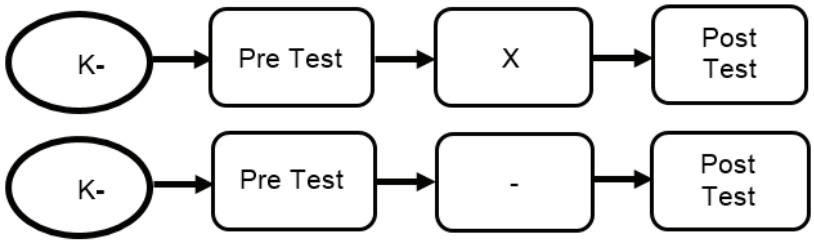
The FKBP5 gene is located on chromosome 6p21.31 and has 13 exons. Overexpression of FKBP5 can reduce the affinity of hormone binding and GR nuclear translocation, thereby reducing GR sensitivity Decreased GR sensitivity can cause interference with negative feedback on the HPA axis and can cause HPA axis dysfunction. Many studies show that the interaction between FKBP5 polymorphisms and childhood trauma is associated with stress-related psychiatric conditions [910,11].

The importance of FKBP5 in GR signaling was initially discovered in New World Monkey which showed an increase in plasma cortisol levels compared to other apes and primates, including humans. In squirrel monkeys for example, free cortisol levels are 50-100 times that of humans but these animals do not show signs of hypercortisolism due to GR resistance in the target organ. It has been found that this GR resistance is partly conferred by overexpression of FKBP5.[12,13].

FKBP5 was chosen in this study because it is based on some evidence. FKBP5 protein plays an important role in determining sensitivity to negative glucocorticoid feedback, a key mechanism for stopping the HPA axis response in stressful episodes[14,15.] Changes in FKBP5 expression or function can increase cortisol load and contribute to allostatic changes in cortisol regulation that occur with repeated stress exposure FKBP5 which is a co-chaperone of heterocomplex GR, part of negative feedback that regulates GR activity. When cortisol binds to GR, FKBP5 is replaced by a positive regulator signaling GR, FKBP4, and the complex is inserted into the nucleus for transcriptional regulation activity. Overexpression of FKBP5 reduces nuclear translocation from the GR complex by isolating it in the cytosol. Interestingly, glucocorticoid exposure increases FKBP5 expression, reduces glucocorticoid negative feedback and allows cortisol to participate in its own regulation [16,17].

Materials and Methods

This study uses quantitative research with quasi experimental / quasi experimental approaches and one group design pre and post test design with group control. The research design can be seen in the research design scheme.



Bag 31 Reseach Design

Ket

K-1	:	Group intervensi
K-2	:	Group control
Pre test	:	Test Conducted before treatment /intervention
X	:	Prenatal Yoga intervention
Post test	:	Test conducted after treatment/ ntervention

The population in this study were all trimester III primigravida. The sample in this study amounted to 24 respondents. The inclusion criteria used as research subjects include the following: Primigravida trimester III (UK> 28 weeks), with a planned pregnancy and willing to become a respondent and sign an informed consent. Ages 20 to 35 years, Normal pregnancy., Fetus alive, single. Criteria for moderate anxiety

Data Analysis

Normality test of sample data with the Shapiro-Wilk test, this test is subject to the FKBP5 content ratio scale data

Result

In the comparison test FKBP5 gene expression in the control group and the treatment group between observations before and after paired sample t test (paired sample t test) was used.

Table 1 Results Of Comparison Tests Before FKBP5 Gene Expression and After Treatment

Group treatment	Before Rerata ± SD	After Rerata ± SD	p-value
Control	7.1±0.59	9.43±0.68	0.000
Treatment Yoga	7.52±0.49	6.88±0.54	0.001

Table 1 shows that there is a significant difference (p = 0,000 <∝) on average FKBP5 gene expression. In other words there is the effect of the treatment of Yoga exercises on the expression of FKBP5 gene in third trimester pregnant women, namely Yoga exercises can reduce FKBP5 gene expression in third trimester pregnant women. So the second minor hypothesis has been proven, that prenatal Yoga exercises affect FKBP5 gene expression in trimester III primigravida.

In the results of the comparative test of FKBP5 gene expression between the control group and the treatment group, the independent sample t test was explained and shown concisely as shown in table 2 below.

Table 2 Test results for comparison of FKBP5 gene expression groups control with the treatment group

Observation	Control Rerata ± SD	Treatment Rerata ± SD	p-value
Before	7.1±0.58	7.52±0.49	0.072
After	9.43±0.68	6.88±0.54	0.000

Table 2 shows that there was no significant difference (p = 0.072> ∝) of average FKBP5 gene expression between the control group (before) (7.1 ± 0.58) and the treatment group (before) (7.52 ± 0.49). This means that FKBP5 gene expression between the control group and the treatment group before observation was the same or there was no significant difference.

Appear in Table 2 shows that there is a significant between non-action and prenatal yoga exercises on FKBP5 gene expression in primigravida trimester III.

Table 3 Correlation test results between variables in groups

Corelasi variable	n	Koefisien korelasi (r)	p-value
Worred with FKBP5	12	0.819	0.001
cortisol with FKBP5	12	0.901	0.000
CRHR1 With FKBP5	12	0.846	0.001

Discussion

Table 1 shows that there was no significant difference (p = 0.072> ∝) on average FKBP5 gene expression at 27-28 weeks gestation between the control group (before) (7.1 ± 0.58) and the treatment group (before) (7.52 ± 0.49) . This means that FKBP5 gene expression between the control group and the treatment group at 27-28

weeks gestation is the same or no significant difference. The results of this study are in line with this study of two European cohorts characterized by both middle-aged and elderly adults using candidate genes and broad genome approaches to identify genes associated with diurnal cortisol secretion and their relationship with depressive symptoms.

The results of the study obtained evidence of the relationship between FKBP5 SNP rs9470080 with salivary cortisol concentration and depressive symptoms. FKBP5 is a co-chaperone of hsp90, which is part of a receptor complex that regulates glucocorticoid receptor sensitivity (GR). The increase in FKBP5 gene expression causes an increase in GR resistance to cortisol, which can cause hypercortisolism[18,19].

The examined the relationship of three HPA glucocorticoid receptor (NR3C1) -related genes, mineralocorticoid receptors (NR3C2), and FK506 binding protein 5 (FKBP5) with suicide in populations in Japan. The results showed that the haplotype in the FKBP5 gene was associated with suicide between the observed suicide group and the control group. FK506 binding protein 5 (FKBP5), which acts as a co-chaperone of GR, is associated with GR sensitivity to cortisol and SNP in the FKBP5 gene has been shown to be associated with depression treatment response and recurrence of depressive episodes) proved that SNP FKBP5 has been associated with depression.

In the literature review, there are several studies related to the FKBP5 gene polymorphism associated with suicide. Among them are analyzing the American population, where it was found that the polymorphism of rs1360780 and rs3800373 in FKBP5 was related to suicide attempts ($p < 0.01$). Likewise, those who analyzed the African population and found that carriers of polymorphism rs3800373 had a high rate of suicide attempts (0.35), compared with patients exposed to life difficulties. On the other hand, there was a significant relationship between the hslotype rs3800373TT and rs1360780TC in FKBP5 with suicidal behavior in the Japanese population ($p < 0.05$ for each haplotype it can be concluded that prenatal yoga decreases FKBP5 gene expression before and after treatment [20, 21].

The study analyzed FKBP5 and glucocorticoid receptor (GR) genes and protein expression in amygdala suicides, without a history of clinical psychiatry and was not treated with anxiolytic or antidepressant drugs and as appropriate. The results showed that FKBP5 and GR gene expression was significantly reduced in AMY (−38% and −48%, respectively) of suicide victims compared with controls. Interestingly, FKBP5 and GR protein expression also decreased significantly (−41% and −42%, respectively) in AMY of suicide victims compared to controls. In line with the research conducted it was proven that no relationship was found between FKBP5 gene polymorphisms and bipolar disorder with psychotic characteristics, such as melancholic depression[22].

Carriers of the rs9470080 genetic variant in the FKBP5 gene have lower cortisol and an increased risk of depressive symptoms compared to non-variant carriers. Observations that FKBP5 rs9470080 carriers are more likely to report clinically relevant depressive symptoms and are also more likely to use antidepressants or antipsychotics further indicate that depressive symptoms are good markers of severe psychiatric disorders [8,16,20]. The results of this study assume that the relationship between FKBP5 variants and depression cannot be explained by cortisol parameters because there is no relationship between cortisol and depressive symptoms, this reflects a lack of power, but can also indicate that other mechanisms besides the regulation of basic cortisol underlie the observed relationship[1,7,9].

FKBP51 levels can reach 13 times higher in Bolivian squirrel lymphocytes compared with humans, which leads to heterocomplex GR and reduced affinity for GC ligands. In humans, evidence for GC resistance mediated by FKBP51 can now be found. High levels of FKBP51 correlate with resistance to GC therapy for asthma. This finding suggests that the genetic variant of FKBP5 might play an important role in the chronic stress response rather than the acute stress response[23].

The increase in FKBP5 activity is also induced by stress hormones such as glucocorticoids, thus affecting the subsequent transcription of the FKBP5 gene and, consequently, GR sensitivity. FKBP5 induction by elevated cortisol levels can lead to regulation of disturbed negative feedback from the HPA axis, thereby extending cortisol levels which increase in response to stressors and thus increase susceptibility to psychiatric illness. Several SNPs in FKBP5 have been identified as interesting, including rs1360780 proven to have functionality. The rs1360780 minor allele has been associated with twice the amount of FKPB5 protein in lymphocytes relative to other genotypes [4,9,16].

In vitro experiments have shown that overexpression of humans FKBP5 also reduces the binding affinity of the hormone and GR nuclear translocation. it makes sense that changes in FKBP5 expression can also affect human GR function in vivo. FKBP5 expression is also induced by steroids, including glucocorticoids as part of an intracellular ultra-short negative feedback loop for GR activity. Increased transcription and translation of FKBP5 after activation of steroid receptors will then reduce the sensitivity of GR[11,13,]

Conclusion

There was a significant difference ($p = 0.000 < \propto$) on average FKBP5 gene expression in the control group before (7.1 ± 0.59) and after observation (9.43 ± 0.68), it could be interpreted to mean an increase in cortisol levels in the control group in the control group before and after observation , it can be concluded that prenatal yoga decreases FKBP5 gene expression before and after treatment.

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Conflict of Interest- None of the authors has competing interests

Ethical Clearance- This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 839/ UN4.6.4.5.31//PP36/2019), and all research subjects gave written informed consent.

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Ecological Analysis of Preeclampsia/Eclampsia Case in Sidoarjo Regency, Indonesia, 2015-2019

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Abstract

Preeclampsia is hypertension that arises after 20 weeks of pregnancy accompanied by proteinuria, the symptoms are divided into mild and severe preeclampsia. Preeclampsia with a coma is called eclampsia. Globally 80% of maternal deaths are classified as direct death. Hypertension is directly responsible for approximately 20% of maternal deaths in the United States. Supas in 2015 showed maternal mortality rate in Indonesia 305/100,000 live births and the highest in Southeast Asian countries which only 40-60/100,000 live births. One of the main causes is preeclampsia/eclampsia. In Sidoarjo Regency in the last 5 years, the most common cause of maternal death was preeclampsia/eclampsia by 58%. The purpose of this study was to determine the description of the incidence of preeclampsia/eclampsia in the last 5 years in the Sidoarjo Regency. This research is a descriptive study with a quantitative approach using secondary data from the Sidoarjo District Health Profile in 2015-2019. The results found that the highest incidence of preeclampsia/eclampsia in the Sidoarjo Regency was Temple Health Center and Taman Health Center (5.74%), and Buduran Health Center (4.10%). The trend in the incidence of preeclampsia/eclampsia in the Sidoarjo Regency over the past 5 years has fluctuated and has not shown a better change. It could be concluded that there are 3 Health Centers that have the highest percentage of preeclampsia/eclampsia due to lack of knowledge about maternal health. This shows that the program carried out by the Government to reduce the incidence of preeclampsia/eclampsia in the Sidoarjo Regency has not been effective so further intervention is needed.

Keywords*: preeclampsia, maternal health, ecological analysis.*

Introduction

Preeclampsia/eclampsia is a collection of symptoms that occur in pregnant women, childbirth, and the puerperium consisting of the tree: hypertension, proteinuria, and edema, which usually occurs at more than 20 weeks' gestation, sometimes accompanied by convulsions to coma. Although pregnant women do not show signs of vascular or hypertension before [1]. Eclampsia is divided into three, namely antepartum eclampsia, intrapartum eclampsia, postpartum eclampsia and may occur in the last trimester and increase as labor approaches [2].

Risk factors for preeclampsia and eclampsia include primigravida, primiparity, age, history of preeclampsia or eclampsia, kidney disease, and pre-existing hypertension before pregnancy, multiple pregnancies, and obesity [3–5]. However, from these risk factors, it is still difficult to determine the most dominant factor [5].

Hypertension in pregnancy makes up 5-15% of pregnancy complications and is one of the three highest causes of morbidity and mortality besides bleeding and infection. Globally 80% of maternal deaths are classified

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as direct maternal mortality. Hypertension is directly responsible for approximately 20% of maternal deaths in the United States, some cases of eclampsia occur within 24 hours after delivery but about 3% of cases are diagnosed between 2-10 days postpartum.

According to the World Health Organization in 2008, that every year more than 500,000 maternal women were dying, one of the causes of maternal and fetal morbidity and mortality was preeclampsia, the incidence rate was between 0.51%-38.4%. In developed countries, the incidence of preeclampsia ranges from 6-7%, and eclampsia 0.1-0.7%. While the maternal mortality rate due to preeclampsia and eclampsia in developing countries is still high. Preeclampsia, one of the syndromes found in pregnant women over 20 weeks, consists of eclampsia and proteinuria with or without edema [6].

A study found that there is a relationship between age and the incidence of pre-eclampsia, there is a relationship between knowledge and the incidence of pre-eclampsia. This finding is strengthened by other research which states that there is a relationship between knowledge of the incidence of preeclampsia/eclampsia [7]. Based on the background description, the study aimed to find out the description of the incidence of preeclampsia/ eclampsia during the last 5 years in Sidoarjo Regency [8].

Methods

This research was a descriptive study with a non-reactive quantitative approach. The study used ecological analysis was conducted by utilizing secondary data on the Health Profile of Sidoarjo Regency from 2015 to 2019 [9]. The ecological analysis is one way for researchers to see the large-scale impact of a specific policy or intervention on the health of the population in an area [10]. Descriptive analysis was conducted in the form of narratives by utilizing previously published literature.

Findings

The trend of the number of live births in the Sidoarjo Regency in the last five years (2015-2019) is presented in the form of a bar diagram which can be seen in Figure 1. Live births in Sidoarjo Regency if in the past 5 years amounted to 179,609 people. Health Centers that had the highest live births were Waru Health Center (7.54%), Taman Health Center (6.79%) and Candi Health Center (6.75%), while those who had the lowest live births were Porong Health Center (1.4%), Kepadangan Health Center (1.66%) and Sekardangan Health Center (1.87%)[11–15].

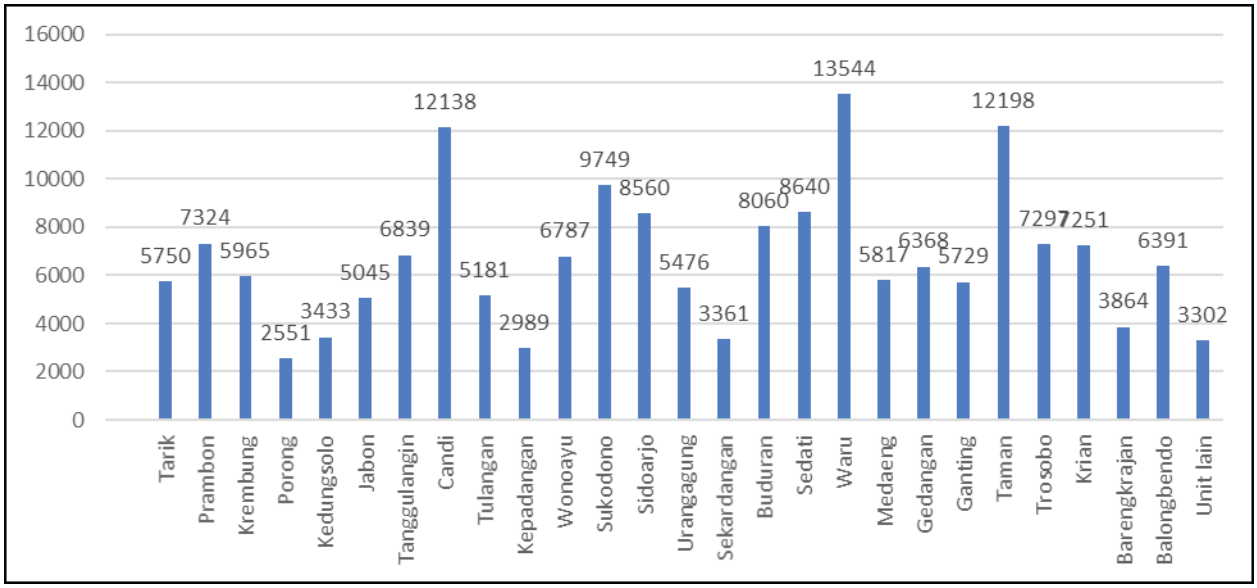


Figure 1. The trend of Live Birth in the Last 5 Years in Sidoarjo Regency in 2015-2019

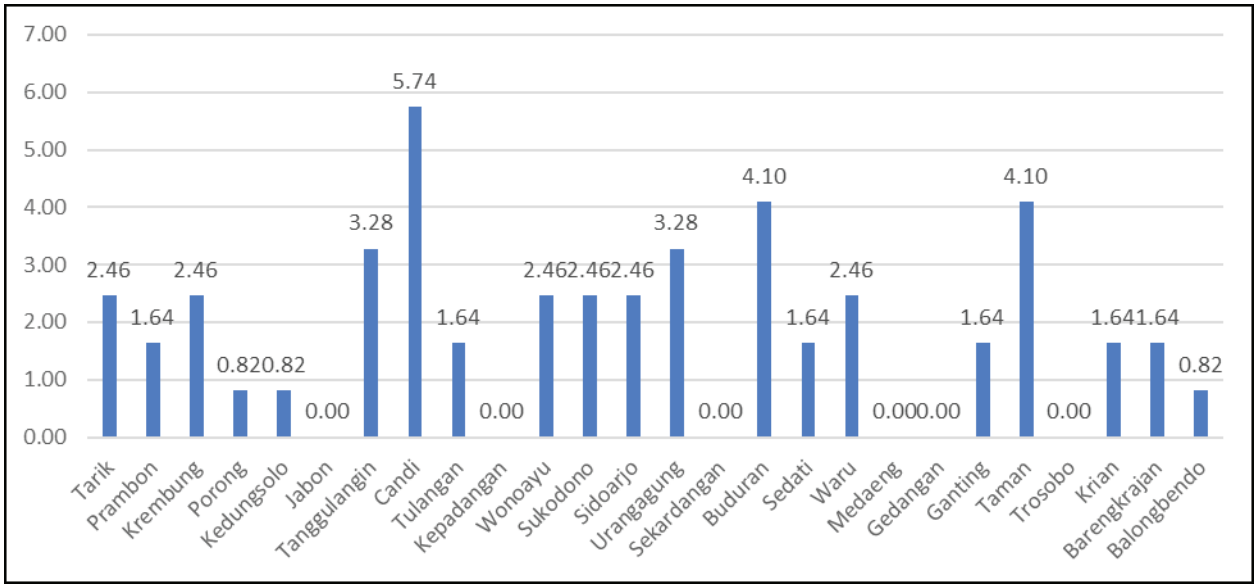


Figure 2. Preeclampsia/Eclampsia Occurrence in Sidoarjo Regency in 2015-2019

The incidence of pre-eclampsia/eclampsia in the Sidoarjo Regency in the last five years (2015-2019) is presented in the form of a bar chart and can be seen in Figure 2. The incidence of preeclampsia/eclampsia has been added up over the past 5 years at 47.54%. Health Centers that have the highest cases are Candi Health Center (5.74%), Taman Health Center and Buduran Health Center (4.10%), Urangagung Health Center and Tanggulangin Health Center (3.28%), while Health Centers which have the lowest cases were Balongbendo Health Center, Porong Health Center, Kedungsolo Health Center (0.81%), Prambon Health Center, Sedati Health Center, Tulangan Health Center, Ganting Health Center, Krian Health Center, Barengkrajan Health Center (1.64%) and Prambon Health Center, Krembung Health Center, Wonoayu Health Center, Sukodono Health Center, Sidoarjo Health Center (2.46%)[11–15].

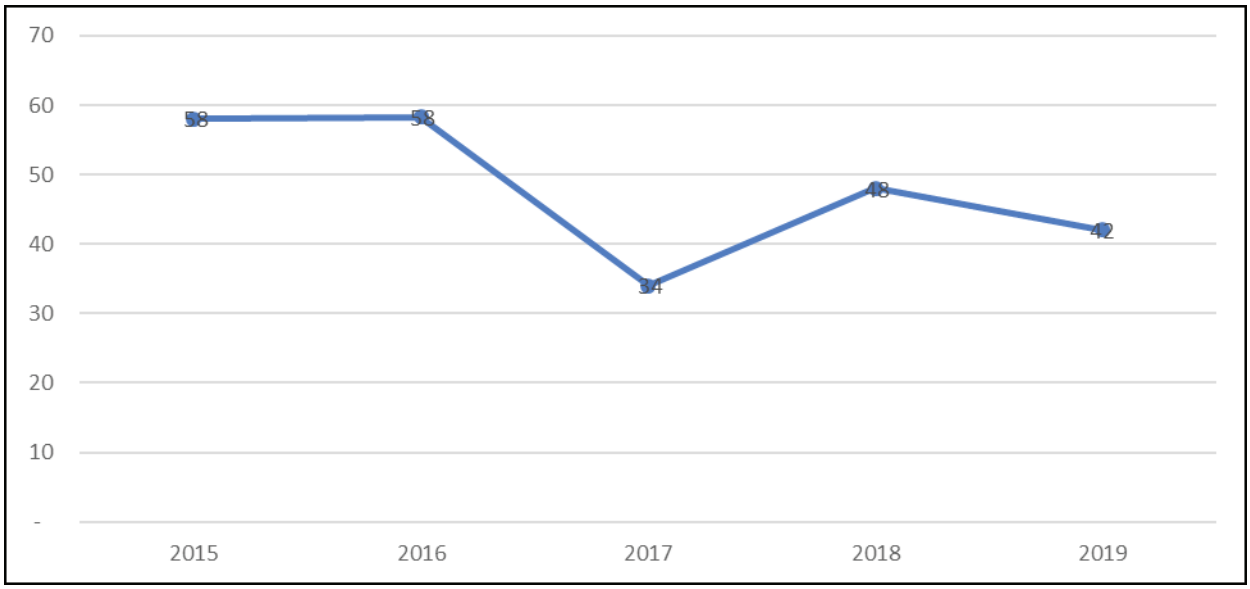


Figure 3. Trends in Pre-Eclampsia/Eclampsia Cases in Sidoarjo Regency in 2015-2019

The results of this study illustrate that the incidence of preeclampsia/eclampsia in the Sidoarjo Regency in the last five years is almost evenly distributed in all regions of the Sidoarjo Regency. The impact that can affect the mother and baby where during preeclampsia in pregnant women is reduced blood flow to the placenta that can cause impaired fetal growth, premature birth until the death of the fetus and the placenta can be released prematurely. In more extreme conditions, the occurrence of eclampsia can cause bodily damage to maternal death. Based on these reasons, a breakthrough is needed in its management in overcoming problems related to obstetric issues as stipulated in the Decree of the Minister of Health of the Republic of Indonesia Number 369/MENKES/SK/III/2007 concerning Midwifery Service Standards following Standard 3, namely midwives providing high-quality antenatal care to optimize health during pregnancy which includes early detection, treatment or referral of certain complications [16].

Early recognition of the diagnosis and perfect handling needs to be done to avoid eclampsia because the diagnosis of preeclampsia with chronic hypertension is not uncommon to cause difficulties. In chronic hypertension, elevated blood pressure before pregnancy in a young pregnancy, or at 6 months postpartum, will be useful for making a diagnosis. Increased blood pressure can be prevented by resting and eating a diet. Rest can be done by reducing daily work, and it is recommended to sit and lie more, a diet high in protein and low in fat, carbohydrates, salt, and unnecessary excessive weight gain should be recommended [17].

Primigravida women are also more at risk of dying from preeclampsia/eclampsia compared to multigravida mothers. The results of this study are also consistent with previous research which states that women who experience first pregnancy have a risk of severe preeclampsia 2.2 times compared to a pregnant woman who is more than 1 time [18]. Preeclampsia is considered a disease in the first pregnancy long ago. The incidence of preeclampsia in primigravida and nullipara is higher than multigravida/multipara. Generally, pregnancy with preeclampsia occurs in primigravida because immunologically the formation of blocking antibodies against placental antigens in the first pregnancy is incomplete, resulting in an unfavorable immune response to histoincompability of the placenta [19].

Based on the data contained in the Sidoarjo District Health Profile report, the author shows the trend of preeclampsia/eclampsia in Sidoarjo regency from 2015 to 2019 as presented in Figure 3. Figure 3 shows the trend of preeclampsia/eclampsia occurrence fluctuating and decreasing significant in 2016 (58%) to (34%) in 2017, then increased in 2018 (48%), then decreased again in 2019 (42%)[11–15].

The data display showing the trend of fluctuating preeclampsia/eclampsia gives a picture of the impact on increasing morbidity and mortality in mothers and infants in the Sidoarjo Regency. This situation shows that the existing programs so far have not been effective. The obstacles found in the field to reduce the incidence of preeclampsia/eclampsia are the knowledge and behavior of the community itself that is less supportive. For example, the description of the 4th antenatal care visit that is only 97.8% of the target set 100%. Antenatal care plays an important role in preventing and detecting obstetric complications, antenatal care services are one of the maternal and child health service programs [15,20].

The results of this study are in line with the results of previous studies which inform that there is a relationship between the mother's knowledge and the incidence of preeclampsia [21]. Knowledge is the result of knowing and occurring after people have sensed a certain object. The level of knowledge is influenced by several factors such as education, age, information, social culture, environment, and experience [22,23]. The more dominant factor of the results of this study is information where the more information obtained, the higher the level of knowledge about preeclampsia [21].

Policies on maternal health in the last five years need to be reviewed, community support for maternal and child health service programs needs to be encouraged again, especially for antenatal care [20,24]. The government needs to promote counseling about pregnancy and childbirth with complications, especially for pregnant women who are at risk. Especially in primigravida mothers, have a history of hypertension and have hereditary preeclampsia/eclampsia, and pregnancy checks at least 4 times during pregnancy by health professionals for early detection of pregnancy complications [22,25,26]. Midwives as health workers who play a role in services are expected to be able to carry out optimal supervision if finding pregnant women with age and gravida who are at risk, especially at vulnerable times [27,28].

The Health Office is expected to be more selective in monitoring the completeness of the recording and

reporting system in each health service, such as the Health Center and its network, and Private Practice Midwives, so it is necessary to conduct an audit with the Head of Health Center, Doctors and Midwives in all Health Centers in the work area that aims as learning so that cases of death do not recur and increase the competency standard of health workers by conducting training and a Memorandum of Understanding with the medical services in the Hospital [29,30]. Advocacy is needed in the field of budgeting for training for health workers, especially midwives, as the spearhead of maternal and child health services at the Health Center. Training and refreshing are needed, especially for competencies in obstetric emergencies.

Conclusions

The incidence of preeclampsia/eclampsia in the Sidoarjo Regency in the last five years was 48%. Health Centers with above-average preeclampsia/eclampsia were 11 Health Centers including Tarik Health Center, Krembung Health Center, Wonoayu Health Center, Sukodono Health Center, Sidoarjo Health Center, Waru Health Center (2.46%), Tanggulangin Health Center, Urangagung Health Center (3.28%), Buduran Health Center, Taman Health Center (4.10%) and Candi Health Center (5.74%). Based on the Sidoarjo District Health Profile Report states that the prevalence of preeclampsia/eclampsia which was evenly distributed and quite high was caused by people's behavior that is less supportive of antenatal care services.

Trends in the incidence of preeclampsia/eclampsia in the Sidoarjo Regency in the past five years have fluctuated and have not shown any changes for the better. There was an increase in 2018 where the percentage of the incidence of preeclampsia/eclampsia was 34% to 48% in 2018.

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Pharmacological Study of Weight Losing of Liraglutide Loaded Within Layered Double Hydroxide Carrier

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Abstract

The anti-diabetic drug liraglutide was loaded within LDH nanoparticles using the Fe^{+3}, Al^{+3}, Fe^{+2} and Ni^{+2} ions, the preparation of LDH was carried out via the titration of 2M of NaOH with trivalent and divalent ions in presence 0.5M HCl, the prepared compounds of liraglutide with LDH were tasted with selective groups of rats.

The compounds were characterized with Fourier transform infra-red (FTIR), X-ray diffraction (XRD), Atomic force microscopy (AFM), Zeta potential and Scanning electron microscopy (SEM).

The animals that injected with weekly dose of the prepared compounds shows the weight reduction comparative with that of the animals injected daily dose of pure liraglutide.

Keyword; *Liraglutide, double layered hydroxide, nanoparticles, antidiabetic drug.*

Introduction

Liraglutide is a fatty acid derivative of glucagon like peptide-1 [GLP-1]. It is formed by attaching a 16-carbon fatty acid molecule at position Lys26 and making an Arg34Lys substitution on GLP-1, and shares approximately 97% sequence homology with GLP-1 so retains the physiological activities of GLP-1[1, 2]. These structural modification of liraglutide increase chain aggregation, promote reversible non-covalent binding to other molecules, such as albumin, and resist dipeptidyl peptidase degradation. Liraglutide marketed as Victoza®, is a 1.8 mg daily subcutaneous injection that was initially approved by the FDA in 2010 as an adjunct therapy to diet and exercise for management of type 2 diabetes. Appetite suppression and delayed gastric emptying are thought to be responsible for the weight lowering effects of GLP-1. As a result, liraglutide was also developed as a weight loss agent [3].

Layered double hydroxides (LDHs), also known as hydrotalcite-like compounds, represented by the general formula$[MII_{1-x}MIII_x(OH)_2]^{x+}[(A^{n-})_{x/n} \cdot yH_2O]^{x-}$, where M^{II} and M^{III}, di-and tri-valent metal cations, respectively, are capable of occupying the octahedral interspaces of brucite-like sheet. A is anions between the layers can compensate the positive charges of the layer structures and these interlayer regions may contain water molecules[4]. Recently, particular attention has been focused on the LDH-based controlled release systems. Although many biomolecules/LDH hybrid complexes have been reported, only a few examples have been studied as drug delivery carriers. In the present study, liraglutide drug was selected as a model drug and intercalated into Fe^{+2}/ Fe^{+3} LDH, Fe^{+2}/Al^{+3}-LDH and Ni^{+2}/Fe^{+3}- LDH successfully by coprecipitation technique. This work focus on the structure and slow/ controlled release property of a synthesized drug–LDHs composite intended for providing basic data for organic–inorganic LDH hybrids[5].

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Materials and Methods

1. Preparation of precursor and hybrids

Synthesis of Fe^{+2}/Fe^{+3} layered double hydroxides nanoparticles

The synthesis of Fe^{+2}/Fe^{+3} LDHs NPs was done by

mixing 1:2 molar ratio of Fe^{+2}/Fe^{+3} (25 mL of 0.02 M of $FeSO_4.7H_2O$ solution and 25 mL of 0.04 M of $FeCl_3$ solution addition 2.1 mL 0.5 N HCl. Then a drop wise titration is done with 2 M NaOH with vigorous magnetic stirring at 80 ˚C until pH elevated from 2 to 9 with change in color of the solution from clear yellow to dark brown suspension which is then left at room temperature for 24 hours. The suspension is filtered and continuously washed with deionized water until the filtrate became neutral. The obtained solid residue was dried at 50˚C in oven for 4 hours [6].

Synthesis of Fe^{+2}/Al^{+3} Layered Double Hydroxides nanoparticles

In the present work Fe^{+2}/Al^{+3} LDHs NPs was synthesized by mixing 25 mL of 0.06 M of $Al(NO_3)_3$ solution and 25 mL of 0.02 M of $FeCl_3$ (3:1 ratio) respectively. To which 2.1 mL of 0.5 N HCl was added, then titrated drop by drop with 2 M NaOH with vigorous magnetic stirring at 80˚C until the pH elevated from 2 to 9 with change in the color of the solution from clear yellow to light brown suspension which is then left at room temperature for 24 hours. The resultant suspension filtered and washed with deionized water several times, until the filtrate became neutral [7].

Synthesis of Ni^{+2}/Fe^{+3} Layered Double Hydroxides nanoparticles

Ni^{+2}/Fe^{+3} LDHs NPs was synthesized by mixing 25 mL of 0.75 M of $Ni(NO_3)_2$ solution and 25 mL of 0.25 M of $FeCl_3$ (3:1 ratio) respectively. To which 2.1 mL of 0.5 N HCl was added, then titrated drop by drop with 2 M NaOH with vigorous magnetic stirring at 80˚C until the pH elevated from 2 to 9 with change in the color of the solution from clear yellow to brown suspension which is then left at room temperature for 24 hours. The resultant suspension filtered and washed with deionized water several times, until the filtrate became neutral [8].

Synthesis of Fe^{+2}/Fe^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml (0.00121g, 0.0008 M) of $FeSO_4.7H_2O$ solution mixed with 10 mL (0.00064g, 0.0004 M) $FeCl_3$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from yellow to dark brown suspension [9].

Synthesis of Fe^{+2}/Al^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml(0.0017g, 0.0008M) of Al $(NO_3)_3$ solution add to 10 ml of (0.00064g, 0.0004M) $FeSO_4$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from pale yellow to light brown suspension [10].

Synthesis of Ni^{+2}/Fe^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml (0.00146g, 0.0008M) of $Ni(NO_3)_2$ solution mixed with 10 mL (0.00064g, 0.0004M) of $FeCl_3$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from yellow to brown suspension [9].

Animals and study design:

Thirty adult's male wistar rats (weighing 220-248 gm) were utilized in this study. Animals were attained and placed in animal house of College of Pharmacy/ Mustinsiryiah University, they received water and ordinary pellets for one week for acclimatization, after that they feeding lard [11] for 12 weeks to develop obesity were divided into 5 groups:

· (Group A) was receive 0.5ml/kg/week normal saline.

· (Group B) was receive 600µg/kg/day of pure liraglutide.

· (Group C) was receive 650µg/kg/week IP of Fe^{+2}/Fe^{+3} LDH loaded liraglutide.

· (Group D) was receive 655µg/kg/week IP of Ni^{+2}/Fe^{+3} LDH loaded liraglutide.

· (Group E) was receive 658µg/kg/week IP of

Fe^{+2}/Al^{+3} LDH loaded liraglutide.

Animals were kept in plastic cages each with dimension (20x25x35 cm) that harbor three rats, animals within same cage were discriminated by back fur marking by using waterproof colored marker. The animals were placed under controlled condition of room temperature (25 $\pm 1^0$C) and light stream of 12 hours light/ 12 hours dark cycles. Diet and water were attainable easily to animals. Animals study design started in February /2019 and ended in Jun /2019. Ethics committee of the College of Pharmacy/ Mustansiriyah University was given their approval to begin the study.

Results and Discussion

Characterization of prepared compounds

FT-IR spectra for LDHs and LDHs loaded liraglutide

The FT-IR spectra of the synthesized compounds displays the characteristic absorption bands by which the functional groups were identified [12]. The assigned bands of LDHs spectra and LDHs loaded drug spectra refer to the type of binding between the molecules as hydrogen bonding only as a physical tied and easy liberate in target site[13].

X-ray diffraction (X-RD)

A general analysis of these patterns shows that the LDHs loaded liraglutide shows the peaks at 33 two theta assigned to the dihedral geometry as amorphous structure with small difference between LDHs types. Such difference is attributed to the higher similarity of ionic radii for the ion pair.

Atomic force microscopy (AFM) Results:

Results of the AFM images of the LDHs typical surface (in three and two dimensions), the spectrum of AFM pictures of prepared compounds before and after loading the liraglutide since the difference between the arrangements of layered after loading is very clear refer to the affecting of binding of drug with the LDH, from the study of surface and layered of nanocompounds were arrange as vertical cylinders this shape make the molecules easy to liberate the liraglutide.

Zeta potential measurement

Zeta potential measurement for the prepared LDHs loaded liraglutide. The criteria of stability of NPs are measured when the values of zeta potential ranged from higher than +20 mV to lower than -20 mV. The values of the zeta potential of the prepared compounds are listed in table-1 provided satisfactory evidence about their little tendency towards aggregation when its zeta potential in the negative scale and below -20 mV. These results suggested that the prepared LDHs loaded drug particles were stable with no tendency to aggregates and this in agreement with the results reported for colloidal nanoparticles behavior [14].

Table 1: Zeta potential values for the prepared LDHs NPs loaded liraglutide

LDHs NPs Type	Zeta Potential (mV) ± SD	Mobility (µ/s)/(V/cm) ±SD
Fe+2/Fe+3 LDHs loaded liraglutide	-32.82	-2.56
Al+3/Fe+2 LDHs loaded liraglutide	-26.45	-2.07
Ni+2/Fe+3 LDHs loaded liraglutide	-28.00	-2.19

Scanning electron microscopy (SEM)

The pictures shown in figure-1 represented the SEM images of LDHs loaded liraglutide. The images confirmed the layered shape of LDHS with multiple agglomeration areas, due to the hydrogen bonding attraction between multiple hydroxyl groups those had not been hydrolyzed by acidic hydrolysis [15].

In vivo

The change in the initial and final mean of body weight of group A (0.5ml/kg/week) normal saline intraperitoneal after four weeks was small that indicate there is no significant difference ($p = 0.07$). While body weight of group B was significantly decreased ($p < 0.05$) after daily administration of 600µg/kg liraglutide plus 0.5ml/kg normal saline intraperitoneal for four weeks. This result in weight reduction compatible with result that reported by Astrup *et al*. [16]. Liraglutide lead to slows gastric emptying time and decrease food intake, so cause weight reduction [17].

The body weight of prepared compounds groups C, D and E was significantly decreased ($p< 0.05$) after weekly administration of prepared LDHs loaded liraglutide plus 0.5ml/kg normal saline intraperitoneal for four weeks.

Reduction in the weight of groups C and E about 18% of body weight after four weeks this results very close to results of group B (daily dose of pure liraglutide) that mean Fe^{+2}/Fe^{+3} LDH and Fe^{+2}/Al^{+3} LDH are more suitable for loading drugs because these compounds have higher loading capacity than group D as shown in (figure-2). The statistical analysis of rat ´s body weight represented as mean ± standard error of mean in groups (n=6 for each group).

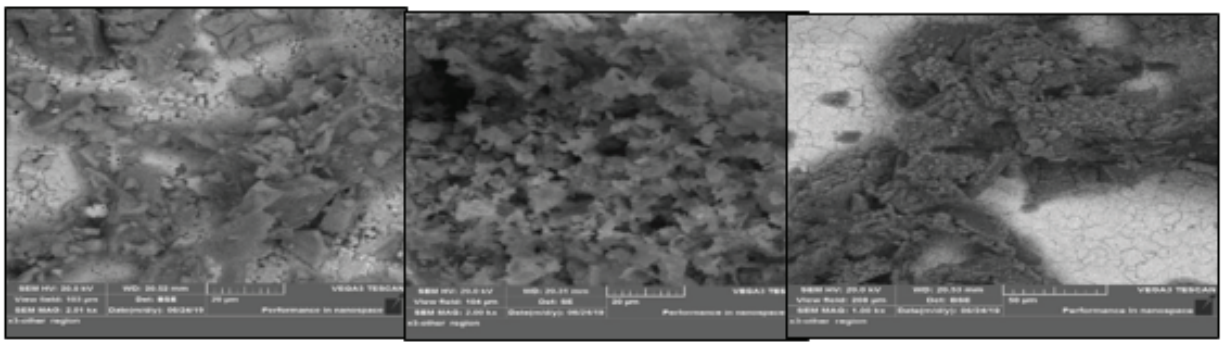


Figure 1: SEM images of the prepared LDHs loaded liraglutide in different measurements.

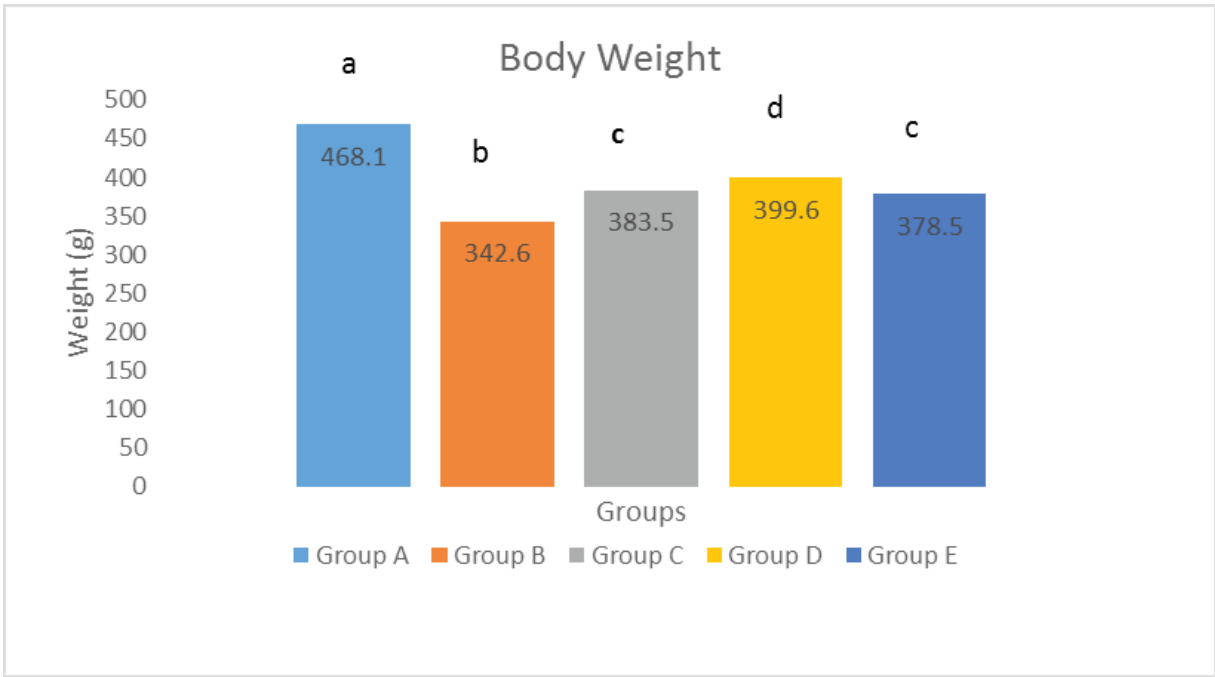


Figure 2: Bar chart illustrate the changes in body weight after four weeks of receiving these prepared compounds:

Different small letters indicate significant differences among the treatments group $p< 0.05$.

Conclusion

1. Characterization and identification of the synthesized compounds were confirmed by determination of FT-IR spectroscopy.
2. The size of the nanocarrier was successfully confirmed by AFM, SEM and XRD.
3. The zeta potential analysis provided a satisfactory evidence about the prepared LDHs little tendency towards aggregation.
4. The Preliminary study of the weight reduction activity by intercalation of liraglutide drug within LDHs NPs via co-precipitation method provide a promising nanocarriers to prolong duration of action of liraglutide.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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The Relationships of Temperature and Humidity in Air-Conditioned Room to the Occurrences of Sick Building Syndrome

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Abstract

High rates of *Sick Building Syndrome* in other countries where pollution levels are far lower than Indonesia, it is suspected that *Sick Building Syndrome* in Indonesia is already worrying and that pollution levels are very high. This syndrome can cause respiratory infections and can worsen people with asthma and allergies due to dirty air. The main objective of this study is to analyze the relationship of temperature and humidity in air-conditioned rooms to the emergence of *Sick Building Syndrome* in UPT PSMB-LT Surabaya. This research is a descriptive study using an observational and approach *cross-sectional*. The population in this study were all employees who worked at UPT PSMB-LT Surabaya, as many as 35 people, using *Non-Probability Sampling* namely *techniques, total sampling*. Data collection is done by interview and observation. Data will be processed and presented in the form of frequency tabulation and described using a contingency coefficient test. The results of this study indicate that the measured air temperature meets the standards except for the physics laboratory room and the air humidity measured mostly does not meet the standards except the leadership room, treasurer room, quality assurance room, physics laboratory room and cation room. Symptoms of *Sick Building Syndrome* most commonly complain of symptoms on the skin. There is a moderate relationship between the incidence of *Sick Building Syndrome* and age. Suggestions for companies are to pay attention to the temperature of the air inside and outside the room as well as the humidity of the air, SBS complaints need to be aware of early on, use a lotion before entering an air-conditioned room.

Keywords: physical quality of the air, air-conditioned rooms, *Sick Building Syndrome*

Introduction

The development of population and the world economy is driving rapid urbanization from rural to urban areas, almost every major city in the world stands tall buildings soaring into the sky which is a result of limited land for settlements, offices and centres of economic activity.

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Tall buildings are built with closed structures and are equipped with air circulation and artificial cooling systems to create comfortable working environment conditions. The outside air entering the building's ventilation system will be reduced to even zero, only recirculating air is used for breathing. This causes poor *indoor air quality* (IAQ) and many free radicals originate from cigarette smoke, ozone from copiers and *printers*, furniture, paints and cleaning materials [1].

The United States Agency or the *Occupational Safety and Health National Institute for Occupational Safety and Health* (NIOSH) conducted a study of 446 buildings in the United States and found that there were six main sources of air pollution in the building. The six main sources of air pollution are the causes of

air pollution in buildings due to pollution from tools and or materials inside the building (17%), pollution entering from outside the building (11%), pollution from building materials and office equipment (3%), microbial pollution (5%), inadequate ventilation (52%), and 12% from unknown sources [2].

The physical quality of the air such as air temperature and humidity are important things to consider. Research in air-conditioned rooms shows air temperature and humidity significantly influence *Sick Building Syndrome*. The study was a study of [3] which showed that there was a significant relationship between temperature (OR = 3.363) and humidity (OR = 2.923) with the incidence of *Sick Building Syndrome*.

Sick Building Syndrome (SBS) has been known since 1970. Occupational medicine in 1980 introduced the concept of SBS as a health problem due to the work environment related to air pollution, IAQ and poor ventilation of office buildings. *World Health the Organization* (WHO) reported in 1984 that 30% of new buildings around the world complained to workers linked to IAQ [4]. Regionally, low and middle-income countries in Southeast Asia and the West Pacific had the highest levels of air pollution in 2012, with a total of 3.3 million deaths related to indoor air pollution and 2.6 million deaths related to outdoor air pollution 5.

The high rate of morbidity *Sick Building Syndrome* (SBS)in other countries whose pollution levels are much lower than Indonesia raises the suspicion that *Sick Building Syndrome* (SBS) in Indonesia has also been alarming, especially in Surabaya where pollution rates are very high. UPT PSMB-LT Surabaya is deemed necessary to be investigated because its location is located in an area with quite high pollution levels and the use of a system *Air Conditioning* that has the potential to cause *Sick Building Syndrome* (SBS).

The number of activities in the building can increase the number of pollutants in the room. In closed buildings that use air conditioners or air conditioners, the design of air conditioners that are used to regulate room temperature continuously can remove some of the pollutant material, but the air circulation only revolves around the same place and added to the presence of pollutants in the same air. This can lead to *Sick Building Syndrome* (SBS). This syndrome can cause respiratory infections and can worsen people with asthma and allergies due to dirty air. Therefore, an occupational health effort is needed so that no more employees are complaining about the symptoms of *Sick Building Syndrome* (SBS). One effort that can be done is to get to know and know SBS complaints early which is common in offices with the use of AC.

This study aims to analyze the relationship of air temperature in air-conditioned rooms to the emergence of *Sick Building Syndrome* in UPT PSMB-LT Surabaya.

Methods

type of research is a descriptive study with a study design *cross-sectional*. The population is all employees who work at UPT PSMB-LT Surabaya as many as 35 people. The sampling method is done by using the technique, *Non-Probability Sampling* which is a total sampling of 35 people.

The research variables include the independent variable (free) in the form of physical quality of air in the room which includes air temperature and the dependent variable (bound) in the form of complaints *Sick Building Syndrome* that is felt by employees due to air exposure while in the air-conditioned room during working hours.

Data collection techniques include primary and secondary data collection using data collection instruments in the form of observation sheets and questionnaires. Primary data collection is done by field observations, interviews and the results of measurements of air temperature, while secondary data is obtained from data that is already in the office.

Results

Relationship of Physical Air Quality toe vents *Sick Building Syndrome*

Relationship of Air Temperature events The *Sick Building Syndrome* following is a cross-tabulation data of *Sick Building Syndrome in* terms of air temperature in UPT PSMB-LT Surabaya. The table explains the relationship between air temperature and the incidence of *Sick Building Syndrome.*

Table 1: Relationship between Air Temperature and the Occurrence of *Sick Building Syndrome*

Temperature	SBS				Total	
	Yes		No			
	n	%	n	%	n	%
18-28˚C	15	44.1	19	55.9	34	100.0
<18˚C or> 28˚C	1	100, 0	0	0.0	1	100.0
Total	16	45.7	19	54.3	35	100.0

Based on the cross-tabulation above it can be seen that respondents who experienced SBS at 18-28˚C air temperature were 15 people (44.1 %) of the total respondents who worked in the room with the temperature as many as 34 people, while the respondents who experienced SBS at air temperature <18˚C or> 28˚C as many as 1 people (100.0%) of the total respondents who worked in the room with the temperature is 1 person.

Based on the test results using a contingency coefficient, the associational value is 0.184. When viewed from the level of relationship, the value of the association is 0.184 in the range of values from 0.00 to 0.25, which means it has a weak relationship level. This shows that there is no relationship between air temperature and the incidence of *Sick Building Syndrome*.

Relationship of Air Humidity toe vent The *Sick Building Syndrome*

following is a cross-tabulation data of *Sick Building Syndrome in* terms of air humidity in UPT PSMB-LT Surabaya. The table explains the relationship between humidity and the incidence of *Sick Building Syndrome.*

Table 2: Relationship of Air Humidity to The Occurrences of *Sick Building Syndrome*

Air Humidity	SBS				Total	
	Yes		No			
	n	%	n	%	n	%
40-60%	7	63.6	4	36.4	11	100.0
<40% or> 60%	9	37.5	15	62.5	24	100.0
Total	16	45, 7	19	54.3	35	100.0

Based on the cross-tabulation above it can be seen that respondents who experienced SBS in air humidity 40-60% were 7 people (63.6%) of the total respondents who worked in a room with that temperature as many as 11 people, while respondents who experienced SBS in humidity <40% or> 60% were 9 people (37.5%) of the total respondents who worked in the room with the temperature as many as 24 people.

Based on the results of testing using the contingency coefficient, the associational value is 0.237. When viewed from the level of relationship, the association

value of 0.237 is in the range of values from 0.00 to 0.25, which means it has a weak relationship level. This shows that there is no relationship between air humidity with the incidence of *Sick Building Syndrome*.

Discussion

Relationship between Physical Air Quality and occurrence of *Sick Building Syndrome*

The temperature that is too high or too low can affect one's concentration and workability. Too high a temperature causes a person to lose fluids faster and in extreme conditions can cause a *heat stroke*. Conversely at low temperatures force a person to work harder to maintain body temperature remains in normal conditions. In extreme conditions temperatures that are too cold can cause *frostbites*. In both above conditions either the temperature is too high or too low the body can feel fatigued faster than normal and experience a variety of symptoms including SBS symptoms [4]. According to the Standards of Quality following [6], the temperature in the workspace is 18-28˚C.

Based on the results of the study note that most respondents who experienced SBS at temperatures 18-28˚C, as many as 15 people (44.1%). The results of temperature measurements show that most of the temperature rooms still meet the standards, only that there are rooms that exceed the standard temperature that is considered comfortable for the working atmosphere, namely the physics laboratory room with a temperature of 29.4 ° C.

Analysis of these two variables uses a contingency coefficient and the associated value is 0.184. When viewed from the level of relationship, the value of the association is 0.184 in the range of values from 0.00 to 0.25, which means it has a weak relationship level. This shows that there is no relationship between air temperature and the incidence of *Sick Building Syndrome*. These results are in line with research conducted by [7] and [8] that the temperature in the work environment is not a trigger for SBS complaints. This can occur because the temperature is still within normal limits for the work environment for employees. Because with these temperatures, body temperature does not waste too much energy to adapt to the surrounding environment. This is what might cause in this study, the temperature has no relationship with SBS.

Relationship of Air Humidity to occurrence *Sick Building Syndrome*

Relatively low air humidity of less than 20% can cause dryness of the membrane mucous membranes, whereas high humidity will increase the growth of microorganisms [9]. According to the Standards of Quality following [6], the humidity in the workspace is 40-60%.

Based on the results of the study note that the majority of respondents who experienced SBS in humidity <40% or> 60%, as many as 9 people (37.5%). Meanwhile, the results of humidity measurements show that most of the humidity rooms do not meet the standards, there are 7 rooms with humidity not meeting the standards of the total rooms that are as many as 12 rooms. Humidity in 7 rooms has exceeded the existing standard that is more than 60% to increase the growth of microorganisms following the opinion [10] that lower humidity of 70% can cause dryness of the mucous membrane while high humidity can increase the growth of microorganisms.

According to [9], room air humidity is one of the factors that influence room temperature so that if humidity is high, the air temperature will drop, conversely if humidity is low, the air temperature will rise. This can affect the health of the occupants of the room, so it needs attention.

Analysis of these two variables uses a contingency coefficient and the associated value is 0.237. When viewed from the level of relationship, the association value of 0.237 is in the range of values from 0.00 to 0.25, which means it has a weak relationship level. This shows that there is no relationship between air humidity with the incidence of *Sick Building Syndrome*. These results are in line with research conducted by [7] and [8] that humidity in the work environment is not a trigger for SBS complaints. This can occur because there are still other factors that influence the onset of SBS symptoms, for example, the quality of air chemistry and air microbiology.

Conclusion

The results of physical air quality measurements include air temperature and humidity. Most of the measured temperatures have met the standard, except

for the physics laboratory room where the temperature exceeds the specified standard of 29.4 ° C. The measured air humidity mostly does not meet the standard with the number of rooms where the humidity does not meet the standard that is 7 rooms out of a total of 12 rooms, except the leadership room, treasurer room, quality assurance room, physics laboratory room and cation room that have met the standard.

The symptoms of *Sick Building Syndrome* most complained are symptoms on the skin of 21 people (21.7%), respondents experiencing SBS as many as 16 people (45.7%).

From the results of testing using the contingency coefficient, it is found that there is a moderate relationship between the incidence of *Sick Building Syndrome* and age, but there is a weak relationship between *Sick Building Syndrome* with gender, length of work and length of service.

From the test results using a contingency coefficient, it is found that there is a weak relationship between the incidence of *Sick Building Syndrome* and the physical quality of the air which includes the temperature and humidity of the air.

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Sambiloto (*Andrographis paniculata*) Extract Improves the Performance of Animal Model Infected with *Escherichia coli*

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Abstract

This study aims to understand the effect of natural antibiotics from sambiloto (*Andrographis paniculata*) extract to laying hens infected with Avian Pathogenic *Escherichia coli* (APEC). The activation test of sambiloto was performed by using a dilution method included a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC). Laying hens were distributed into five groups: normal group, APEC group with no treatment, and APEC group given 10%, 20%, 30% sambiloto extract for two weeks. The data were analyzed using ANOVA and continued with the F test. Based on the results, the 30% extract has improved the hen day production (HDP) of laying hens infected with APEC.

Keywords*: Andrographis paniculata, Avian Pathogenic Escherichia coli, Antibiotic, Hen Day Production*

Introduction

Poultry farming has increased significantly every year, both in production and consumption. But its development has become difficult, one of which is caused by APEC. The decline in production caused by colibacillosis is quite alarming. This condition leads to high morbidity and mortality[1].

APEC attacks the reproductive system, affecting daily egg production or HDP. Laying hens exposed to APEC cause a 60% decrease in HDP. Antibiotic is commonly used as a treatment for colibacillosis[2]. The administration of synthetic antibiotics was performed as an effort to eliminate APEC. However, drug administration causes antibiotic residues in organs and bacterial resistance, creating a lower ability for the drug to tackle the disease. APEC has a good ability to adapt to drugs so that the continuous use of antibiotics, such as enrofloxacin, oxytetracycline, and sulfadimethoxine, would not overcome the APEC[3].

Antibiotic resistance affects the hen's performance. An alternative treatment is needed, that has a similar function as synthetic antibiotics but fewer side effects. One of which is by using medicinal plants. Indonesia has a very high diversity of medicinal plants[4,5]. A potential medicinal plant that can be used as a substitute for antibiotics is sambiloto. Sambiloto leaves possess analgesic, anti-inflammatory, antimalarial, antiviral, immunostimulator, antibacterial, hepatoprotective, anticancer performance.

Materials and Methods

Plant Identification

Taxonomic identification of the sambiloto (*Andrographis paniculata*) was carried out by the Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia.

Ethical Clearance: This study was approved by

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the Committee of Animal Care and Use, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

Sambilito Extract Preparation

This study conducted at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. Sambiloto extract was made into several doses, to know which dose gave the best effect against APEC. Around 1000 grams of sambiloto were smoothed into powder and two liters of 95% ethanol was added. The mixture was macerated for three days. The supernatant was evaporated and 50 grams of crude extract were collected.

Activity Test of Sambiloto Extract against APEC

The bacterial activation test was carried out using the diffusion method to determine the sensitivity of the sambiloto extract to APEC. The dilution method included a minimum inhibitory concentration (MIC) and a minimum bactericidal concentration (MBC). The MIC method was done by adding 1 mL APEC suspension (3×10^8) to each concentration then incubated at 37 ºC for 24 hours. The MBC method was carried out using 6 plates of EMBA. One media was distributed into eight groups (7+1 controls) and then each was labeled. The MIC test results were planted in EMBA by streak method and incubated at 37 ºC for 24 hours.

Administration of Sambiloto Extract in APEC Laying Hens

The sample used in this study was 25 laying hens, strain Isa Brown, 20 weeks old. Animals were divided into five groups: normal group, APEC group with no treatment, and APEC group given 10%, 20%, 30% sambiloto extract for two weeks. All animals were acclimatized for one week. Laying hens aged 21 weeks were infected with 1 ml APEC (10^6 cells/kg BW) intramuscularly and then clinical symptoms were observed for three days. Treatment began at the age of 26 weeks and kept for four weeks until they are 30 weeks old. Data collected were feed consumption, egg production, feed conversion, and the amount of egg production.

Data Analysis

Data were analyzed by Anova (analysis of variance) and the F test. If there were significant differences ($p<0.05$), the statistical test was continued with the least significant difference (LSD) test.

Result and Discussion

The activity test result of sambiloto extract toward APEC shows the zone of inhibition at the concentration of 30%.

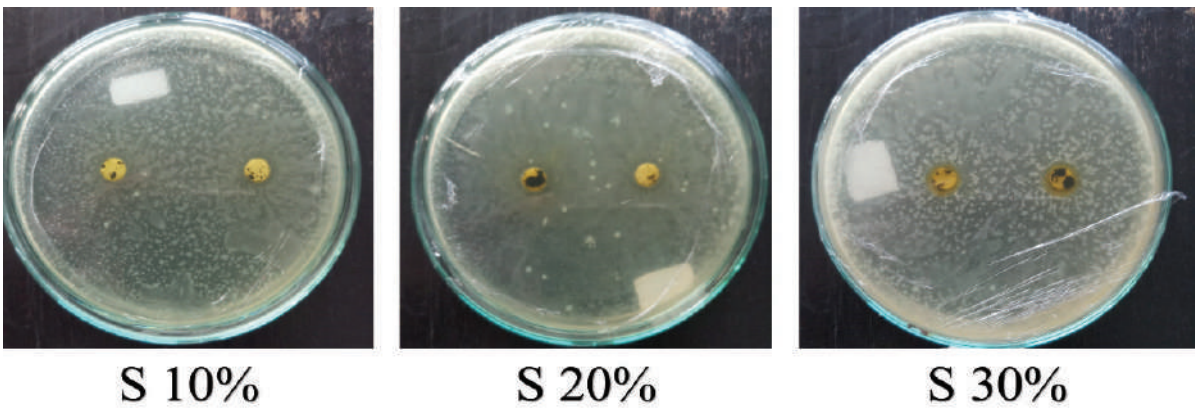


Figure 1. The MIC results using the diffusion method of sambiloto extract to APEC in different concentrations.

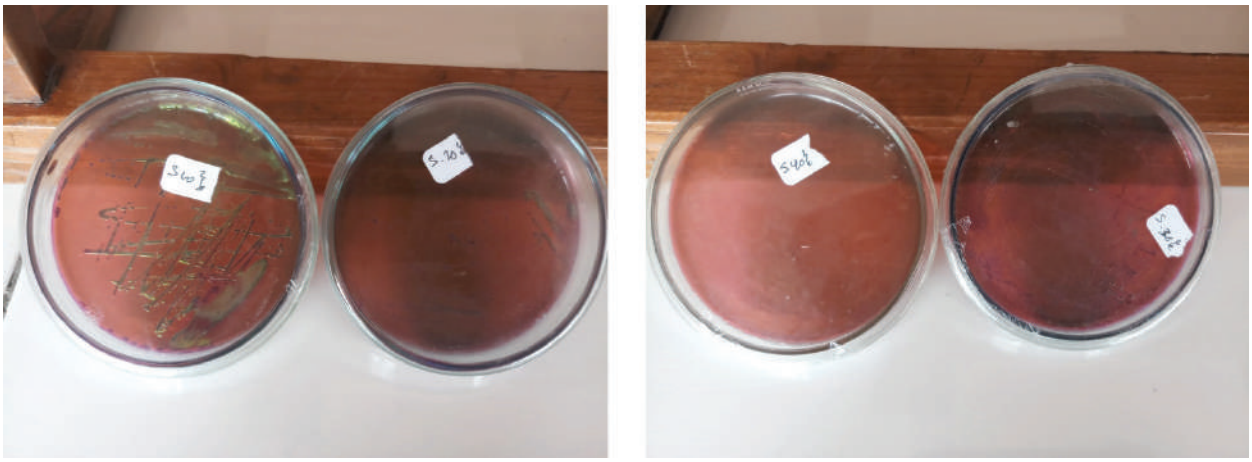


Figure 2: The MBC results of sambiloto extract to APEC in EMBA.

There was no APEC growth at 30% concentration in the MIC method. The MBC test is the continuation test of MIC to prove the MIC test visually. The growth or absence of bacteria at each concentration can be caused by different antibacterial levels in each concentration. The more dilute the MIC test means that the amount of solvent is greater than the amount of the sambiloto extract.

Based on the results of this study, the higher the concentration of sambiloto extract, the better its ability to inhibit APEC development. This was due to the higher the concentration, the higher the active substance contained in the extract. At the opposite, the lower the concentration, the antibacterial ability of the extract will also be lower. In general, high concentrations of antibacterial agents are considered as bactericidal while at low concentrations it is considered as bacteriostatic[6].

Table 1. The Performance of Laying Hens: Food Consumption, Egg Production, Food Conversion, and Total HDP.

Treatments	Food Consumption (X±SD)	Egg Production (X±SD)	Food Conversion (X±SD)	HDP (X±SD)
P0-	92.42b ± 3.64	65.40d ± 2.33	1.41b ± 0.07	97.5c±5
P0+	115.85e ± 2.74	52.40a ± 1.87	2.21e ± 0.09	82.5a±5
P1	108.30d ± 2.77	56.50b ± 1.19	1.91d ± 0.07	87.5ab±5
P2	100.32c ± 2.59	62.20c ± 1.96	1.61c ± 0.06	95bc±5.77
P3	85.60a ± 3.79	65.87d ± 2.00	1.29a ± 0.05	97.5c±5

Indonesia has a high biodiversity with may natural medicine sources. It has around 30,000 different plant species, of which 7,000 are known to possess medicinal properties[7,8,9,10]. Sambiloto is a medicinal herb that is cultivated in Southeast Asia. This plant is widely used as a traditional medicine in Taiwan, China, India, and Thailand to treat infections, colds, fevers, inflammation, and diarrhea. Andrographolide is the major lactone diterpene from sambiloto which contains 1.7% of the dried leaves and 0.8% of the stem. Sambiloto and andrographolide have recently got considerable interest because of their diverse physiological functions and therapeutic potential, including antioxidants, anti-inflammatory, antiapoptotic, antiatherosclerotic, anticancer, antiviral, antiviral and hypoglycemic effects[11].

Tannin is one of the polyphenol compounds which has antibacterial properties. Tannins can inhibit the adhesion of bacterial cells attached to the host cell, inhibit enzymes and interfere with protein transport in the cell layer. Tannins cause lysis in bacterial cells through osmotic pressure and physical stress. Flavonoids in sambiloto can act as antioxidants, antibacterial, anti-inflammatory, anti-allergic, and anti-thrombosis[12]. Our previous study found that *Phyllanthus niruri* L. extract can increase the immune response in laying hens infected by *E. coli*[13].

Food consumption in P0+ treatment was the highest, with a value of 115.85 grams/hen/day. While the lowest was P3 treatment with a value of 85.60 grams/hen/day. Increased food consumption in the APEC group with no treatment was presumably because the feed could not be absorbed properly by the infected intestine and caused much-wasted food. The food consumption in P1 and P2 groups was decreased because the infected hens had been treated with sambiloto extract with concentrations of 10% and 20%, respectively. Sambiloto contains tannin compounds that can coat the intestinal mucosa so that food absorption is inhibited. Tannin compounds that are contained in sambiloto plants are able to reduce food absorption by depositing mucous protein in the intestine, making it possible to reduce food consumption. Food consumption in the P0- group shows higher results than the P3 group, this is due to the presence of APEC in the intestine.

The Anova test, followed by the Duncan test, showed that there was a significant difference ($p<0.05$) on egg production among groups. The P0- group was not significantly different from P3 but it was significantly different from P0+, P1, and P2. The P1 group was significantly different from all groups. The P2 group was significantly different from all groups. The P3 group was not significantly different from P0- but significantly different from P0+, P1, and P2. The egg production of P0- and P3 groups showed similar results which were 65.40 grams/hen/day and 65.87 grams/hen/day, respectively. The egg production of P1 group (56.50 grams / hen / day) was lower compared to P2 (62.20 grams/hen/day). The lowest egg production was experienced by the P0+ group with 52.40 grams/hen/day. This was caused by the presence of APEC that attacks the reproductive system in laying hens, especially in the infundibulum. Increased egg production is also affected by andrographolide contained in sambiloto extract which functioned as antibacterial. Andrographolide is the most abundant substance in sambiloto. Besides its antibacterial property, andrographolide can also activate B lymphocyte cells to produce antibodies[11]. As an antibacterial, andrographolide can inhibit *E. coli* that have been infected in these laying hens. The number of doses of sambiloto extract also affects the amount of andrographolide content in the extract and influences the bacterial inhibition.

The food conversion data shows that the P0+ group was significantly different from P0-, P1, P2, and P3. The P0- group was significantly different from P0+, P1, P2, and P3. The P1 group was significantly different from P0+, P0, P2, and P3. The P2 group was significantly different from P0 +, P0-, P1, and P3. The P3 group was significantly different from P0+, P0-, P1, and P2. The P0+ and P1 groups had the highest food conversion with a value of 1.66 while P3 showed the lowest results with a value of 1.62. The P0 was not significantly different from the P2 group, with a conversion value of 1.63. Based on these results, the administration of sambiloto extract can reduce food conversion rates on laying hens infected with APEC. The higher the sambiloto extract dose, the lower the feed conversion rate. This is due to the flavonoid content in the extract. Flavonoids can inhibit bacterial growth by damaging the permeability of bacterial cell walls, microsomes, and lysosomes. It is the result of interactions between flavonoids and bacterial DNA[6]. The flavonoid content in sambiloto extract is what caused the inhibition of *E. coli* and the hens continued to form good antibodies and produce high egg production and low food consumption. Food conversion is closely related to food consumption and egg production. Low food consumption and high egg production can reduce food conversion while high food consumption and low egg production can increase food conversion which will be detrimental to farmers.

Duncan test results on HDP showed that the P0 group was significantly different from P0+ and P1 whereas it was not significant from P2 and P3. The P0+ group was significantly different from P0, P2 and P3 groups whereas it was not significant from P1. The P1 group was significantly different from P0 and P3 groups whereas it was not significant from P0+ and P2 groups.

The P2 group was significantly different from P0+, whereas it was not significant from P0, P1, and P3. The P3 group was significantly different from P1 and P0+ whereas it was not significant from P0 and P2. Based on these results, it was confirmed that the administration of sambiloto extract can increase the HDP of laying hens. It was due to the antibacterial and the antidiarrheal properties of the extract so that egg production is not inhibited by *E. coli*. The administration of sambiloto extract healed the hen's infundibulum infected with *E. coli*. This healing process normalizes the egg formation and production[14].

Conclusion

In sum, the study present that the administration of sambiloto extract at a 30% dose can improve the performance of laying hens infected with APEC.

Conflict of Interest: The authors declare that they have no conflict of interest.

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The Effect of Roasting Temperature on Ferulic Acid Levels of Robusta Coffee Bean with Thin Layer Chromatography (TLC)-Densitometry

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Abstract

The purpose of this study was to quantitatively analyze ferulic acid levels of Robusta coffee bean extracts from two samples from different regions (Argopuro and Kaliwining, Jember District), with different roasting temperatures (180 °C and 200 °C for 10 minutes) using Thin Layer Chromatography (TLC)-Densitometry method. The analytical method used was the TLC-Densitometry, which utilized methanol solvent p.a; stationary phase: Merck silica Gel 60 F254 plate; chloroform mobile phase: methanol: formic acid (v/v/v) = (85:15:1) in a saturated chromatographic chamber; the test concentration of 50 ppm and the plates were analyzed with the Densitometry winCATS Camag scanner using a UV-Vis detector at 254 nm wavelength and blue fluorescence at 366 nm wavelength. The results showed that the standard correlation coefficient (R) of ferulic acid was 0.9947. Quantitative analysis of ferulic acid levels obtained on the average results of Argupuro coffee bean extract at roasting temperatures 180 °C and 200 °C amounted to 1.935598±0.020671 mg/g and 1.419963±0.051633 mg/g, while Robusta Kaliwining coffee bean extract at 180°C and 200°C roasting temperature were 3.18087±0.06738 mg/g and 2.420237±0.1989 mg/g. Statistical analysis with One-Way Anova obtained *p* value <0.005. Least Significant Different Test (LSD) obtained *p* value <0.005 in each group. Based on the statistical analysis it was concluded that there was an effect of roasting temperature on ferulic acid levels in Robusta coffee bean extract, with the highest ferulic acid content obtained in Robusta Kaliwining coffee bean extract in Jember Regency at roasting temperature of 180 °C was 3.180875±0.020671 mg/g.

Keywords: *ferulic acid, Robusta coffee bean, roasting, TLC-Densitometry.*

Introduction

Coffee is one of the most consumed drinks in the whole world. Robusta coffee comes from the equator forests in Africa, from the west coast to Uganda. Coffee plants can start to produce coffee fruit after the age of 4-5 years depending on local maintenance and climate condition. Since 1900, Robusta coffee has been widespread throughout the tropical island. Robusta coffee can grow better in areas with an altitude of 0-1000 meters asl, where it is not suitable for Arabica coffee that requires a height of more than 1000 meters above sea level to avoid pest Hemelia vastatrix (HV) attacks. This causes more robusta coffee to be cultivated in Indonesia where the area is dominated by lowlands[1].

Jember Regency is one of the biggest coffee manufacturer regions in East Java. The total coffee plantations in Jember are 16,882 ha with the production of 4,911 ha of community coffee spread across 27 sub-districts in Jember. Furthermore, there are 14 gardens managed by PT. Perkebunan XII (6,009 ha), 7 estates of

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2,22267 ha (PDP) and 10 estates of 3,695 are managed by the other private sector[2]. Kaliwining Plantation (45 masl) and Durjo Plantation Sumber Kembang Argopuro village (450-650 masl) are plantation areas in Jember with commodities such as Robusta coffee.

Coffee is rich in compounds with high antioxidant activity such as polyphenols, especially chlorogenic acid and its degradation products (ferulic, cucumber, and caffeic acid), and melanoidin compounds derived from the Maillard reaction[3]. Coffee bean extract is one of the most popular drinks in the whole world. Only a small proportion of coffee consumers drink coffee extracts made from green coffee beans. Most of them prepare a coffee drinks from roasted and ground coffee beans or from instant coffee, form of coffee that has been obtained in a high temperature process. Roasting causes changes in the coffee bean content through modification or degradation. During the roasting process, high temperatures will result in degradation of polyphenols[4]. Chlorogenic acid, malic and citric acid levels decrease, while ferulic acid and quinic acid increase due to degradation of chlorogenic acid[5]. The roasting temperature used, ranges from 160 °C to 240 °C for 8 to 24 minutes[6]. The optimal roasting time for Robusta coffee is around 10 minutes[7].

One of coffee polyphenols degradation is ferulic acid[8]. Ferulic acid is a type of phenolic acid found in components of cell walls, leaves and coffee beans. Ferulic acid is produced from the metabolism of phenylalanine and tyrosine. This acid has an important role for plants because of its ability to inhibit free radicals. Recent evidence shows that ferulic acid has anti-inflammatory, anti-diabetic, anticancer, and cardioprotective properties. Some investigations have also shown that foods rich in ferulic acid can prevent hypertension[9].

Ferulic acid can be quantified by many analytical methods, such as High-Performance Liquid Chromatography (HPLC) combined with photodiode array (PDA) wavelength detectors or UV-Vis detectors[10,11], UV-Vis spectroscopy[12,13], TLC[14], thin layer chromatography high performance (HP-TLC) [15], gas chromatography[16], chemiluminescence[17], and capillary electrophoresis[18]. Rapid quantitative and qualitative analysis with TLC of crude extracts will give practical and simple results[14]. The purpose of this study was to quantitatively analyze ferulic acid levels of Robusta coffee bean extracts from two samples with different regions namely Robusta Argopuro coffee and Robusta Kaliwining coffee from Jember Regency with different roasting roasting temperatures (180 °C and 200 °C for 10 minutes) using valid TLC-Densitometry method.

Materials and Methods

Chemical and Reagen

Coffee roaster, Coffee grinder, Coffee filter, Aquadest, Freeze dryer, Analytical scales, measuring flask, TLC silica gel 60 F254 plate (20 cm×20 cm), chloroform (Merck), methanol (Merck), formic acid (Merck), standard ferulic acid (Sigma-Aldrich), Camag's TLC Scanner, winCAT software.

Robusta Coffee Bean Sample

Dried green coffee beans obtained from two places, Robusta Argopuro green coffee beans Jember obtained at the State Polytechnic of Jember, Department of Agricultural Technology, whereas Robusta Kaliwining coffee beans obtained from the Central Java Coffee and Cocoa Kaliwining Research Center Jember East Java.

Roasting Procedures

A 500 gram sample was roasted using a drum coffee roaster. The initial roasting temperature is 180 °C, after that the roasting temperature is divided into two namely 180 °C and 200 °C for 10 minutes[19]. All roasting samples are ground using an electric grinder and ground coffee is filtered using a standard filter to remove large particles 440 grams of ground coffee are produced.

Extraction and freeze Dryer Procedures

Samples of 5 grams of ground coffee were transferred to 100 ml flasks and 60 mL of distilled water was added at 100 °C, and then stirred until the solution became homogenous[20]. After it reach homogeneous condition, the extracted water was filtered and the freeze drying of the filtered supernatant was carried out. The supernatant was put in a freeze dryer at -72 °C for 17 hours.

Ferulic Acid Analysis with TLC-Densitometry

Thin Layer Chromatography (TLC) method is used

to determine the level of ferulic acid. The stationary phase used is the 60 F254 silica gel plate (20 cm × 20 cm). The mobile phase in the system is consisted of chloroform: methanol: formic acid (85: 15: 1). Standard 1 µL ferulic acid is bottled using Camag's linomat at 15 mm from the bottom of the plate, the spot is dried. The plate is then inserted into the Wheaton chamber which is saturated with the mobile phase. Elution of the mobile phase is carried out along the 100 mm. The plate is then dried until all the mobile phases evaporate at room temperature and scanning is done using Camag's TLC Scanner which has been equipped with winCAT software. Detection was carried out by modify of the method[21]. Ferulic acid absorbs strongly at 254 nm wavelength while the blue fluorescence at 366 nm. Quantification of ferulic acid was obtained using a standard curve (Figure 1).

Quantitative analysis of ferulic acid in Robusta coffee bean extract.

Argopuro and Kaliwing Robusta coffee bean extract solution was prepared in methanol solution. The sample was applied to a 60 F254 silica gel plate, developed and dried as described above. Plate/plate TLC that has been eluted with mobile phase is observed under UV lamps. Standard color stains and coffee samples were observed under UV light which is dark blue fluorescence. Then the TLC plate that has been eluted is paid with a densitometer. Ferulic acid levels in Robusta coffee bean extract samples can be calculated based on standard curric acid curves (Table 1).

Statistical Analysis

All values are expressed as mean±SD. Data were analyzed using analysis of variance for each group (One-Way ANOVA). When a significant F value (p <0.05) is obtained, an LSD test is performed for post hoc analysis. Previously, Shapiro-Wilk Normality Test was carried out with a significance level of 5% to determine the normality distribution of data. In addition, the normality test is carried out as a condition in determining parametric or non-parametric tests. Furthermore, the Levene homogeneity test is performed to determine whether the data is homogeneously distributed or not.

Results

Linearity was obtained using five standard concentrations of ferulic acid. Concentration is 100; 200; 300; 400; and 500 ng. The results of linearity are shown in Fig. 1. Based on the results obtained by calculate the equation of linear regression of the calibration curve y = 4.171x+66.56 and the correlation coefficient (R2) = 0.9947. This correlation coefficient shows a linear result, because it fulfill the acceptance criteria, namely the value of the correlation coefficient (r) ≤ 1[22]. The equation of the line will then be used to determine the ferric acid content of the sample (Table 1).

Table 1 showed the results of Robusta Arguporo and Kaliwining coffee bean extracts with roasting temperatures of 180 °C and 200 °C, where the average of ferulic acid level from Robusta Argopuro coffee bean extract with 180 °C and 200 °C roasting temperatures are 1.935598±0.020671 mg/g and 1.419963±0.051633 mg/g.

The data obtained were then tested for Normality and Homogeneity. The Shapiro-Wilk normality test showed p >0.05, as of the data distribution was normally distributed and continued with the parametric test. The Levene homogeneity test showed p >0.05. This shows that the data was homogeneous. Data were then analyzed using analysis of variance for each group (One-Way ANOVA) and continued by LSD test. The p value <0.05, as of it can be concluded that there is a significant difference between the roasting temperature and ferulic acid levels. LSD test found there were significant differences between groups of Robusta coffee extracts against ferulic acid levels.

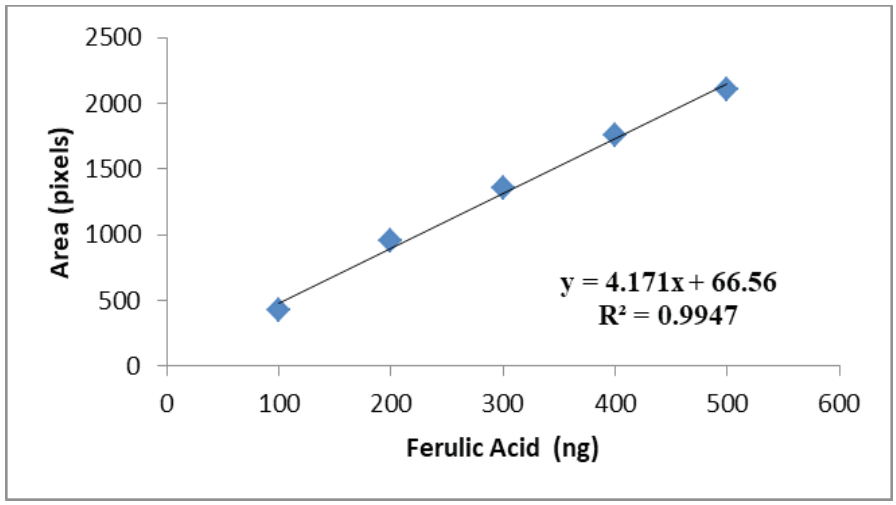


Figure 1. Standard curve of ferulic acid level.

Table 1. Ferulic Acid Level of Robusta Coffee Bean Extract.

Sample	Area	vol (µL)	Level (ng)	Level (ng/1000 µL)	Level (ng/g)	Level (mg/g)	Mean	SD
A	1210.1	14	274.1645	19583.17635	1958317.635	1.958317635	1.935598	0.020671
	1193.9	14	270.2805	19305.75059	1930575.059	1.930575059		
	1186.5	14	268.5064	19179.02524	1917902.524	1.917902524		
B	908.5	14	201.8557	14418.26215	1441826.215	1.441826215	1.419963	0.051633
	917.4	14	203.9895	14570.67507	1457067.507	1.457067507		
	861.3	14	190.5394	13609.95993	1360995.993	1.360995993		
C	1885.8	14	436.164	31154.57068	3115457.068	3.115457068	3.180875	0.06738
	1921.8	14	444.795	31771.07237	3177107.237	3.177107237		
	1964.4	14	455.0084	32500.59938	3250059.938	3.250059938		
D	1376.8	14	314.1309	22437.9217	2243792.17	2.24379217	2.420237	0.1989
	1457	14	333.3589	23811.35048	2381135.048	2.381135048		
	1605.7	14	369.0098	26357.84498	2635784.498	2.635784498		

Note: A) Robusta Argopuro coffee bean extract 180 °C roasting temperature; B) Robusta Argopuro coffee bean extract roasting temperature 200 °C; C) Robusta coffee bean extract Kaliwining roasting temperature 180 °C; D) extract Robusta Kaliwining coffee beans roasting temperature 200 °C.

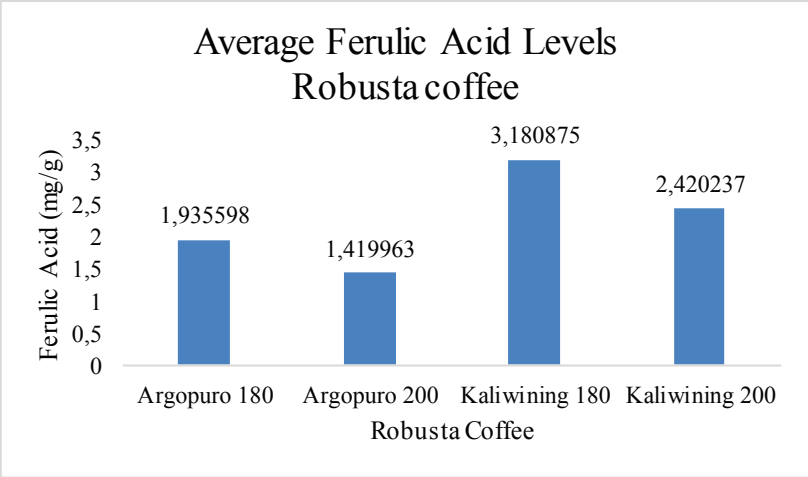


Figure 2. Average of Argopuro and Kaliwining Robusta Coffee's Ferulic Acid Levels (mg/g) at 180 ºC and 200 ºC roasting temperatures.

Discussion

Roasting is an important processing step for developing the unique chemical, physical and sensory characteristics of roasted coffee[23]. During the roasting process, coffee beans experience high temperatures, resulting in physical-chemical changes in their structure. Roasting from 180 °C, coffee beans begin to form compounds that are responsible for the color, taste and aroma of roasted coffee beans[24].

Ferulic acid is defined as the result of the coffee chlorogenic acid degradation due to the roasting process. Ferulic acid is also found in food, especially its free form, has an important function to protect human health[25]. Roasting will increase the amount of free ferulic acid and reduce the ferric acid bounding form. It is estimated that the increase in the amount of free ferulic acid due to some part of the ferulic acid which is bound in food will be in its free form due to the roasting effect[26].

In Argopuro and Kaliwining Robusta coffee beans, ferulic acid levels reach a peak at roasting temperatures of 180 °C and decrease at 200 °C. This is because of ferulic acid experiencing degradation in the temperature above of 180 °C[27].

Ferulic acid levels of Kaliwining Robusta coffee bean extract is greater than in Argopuro Robusta coffee bean extract. Robusta coffee grows optimally in the lowlands, hence, the ferric acid levels is greater in Robusta coffee in the Kaliwining area which is lower than the Argopuro area.

Conclusion

In sum, there is an effect of roasting temperature on ferulic acid level in Robusta coffee bean extract with the highest ferulic acid level obtained in Kaliwining Robusta coffee bean extract at Jember Regency at 180 °C roasting temperature is 3.18087±0.06738 mg/g.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Ethical Approval: This study was approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

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Antibodies of Double Stranded Deoxyribonucleic Acid and Antinuclear in Patients with Rheumatoid Arthritis: Comparison Study of Seropositive and Seronegative

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Abstract

Rheumatoid arthritis is a heterogeneous disease, which can be, based on data combining genetic risk factors and autoantibodies, sub-classified into positive and negative RA. The present study aims to establish a comparative analysis between seronegative and seropositive rheumatoid arthritis (RA), with regard to autoantibodies. A total of 151 patients with rheumatoid arthritis divided into two groups, Group A, 78 patients with seropositive RA and Group B, 73 patients with seronegative RA. The measurements included ESR, CRP, RF, anti CCP,ANA, and anti dsDNA. Highly significant differences ($p \leq 0.01$) in ESR (31.4 vs 52.8 ml/hr), CRP (10.85 vs 23.59 IU/L), RF (8.34 vs 47.67 IU/L), anti CCP (21.72 vs 41.17IU/L), ANA(0.92 vs 2.3 IU/L), and anti dsDNA(13.01 vs 29.49 IU/L) levels. Significant correlation of anti dsDNA were observed with CRP, anti CCP (and ANA in seronegative RA. Meanwhile highly significant correlation were observed with ESR, CRP, and ANA in seropositive RA. Many of RA-associated autoantibody systems have been identified and recognize post translationally modified proteins, indicating the immunogenicity of such proteins for human B cells. Among theme is anti dsDNA which may have a role of the inflammatory process of RA.

Keyword: *Rheumatoid arthritis, Anti-cyclic citrullinated peptide, antinuclear antibodies, Autoimmunity, Rheumatoid factor, Anti-double-stranded (ds) DNA.*

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune, inflammatory disease, affecting 0.5 to 1% of adults worldwide that may cause progressive joint damage and deformity, leading to functional disability and reduced quality of life [1,2]. Rheumatoid arthritis is a heterogeneous disease, which can be, based on data combining genetic risk factors and autoantibodies, sub-classified into positive and negative RA [3]. Various biomarkers can be used to identify subjects susceptible to the disease and those with pre-clinical rheumatoid arthritis before the onset of symptoms such as rheumatoid factor and anti-citrullinated protein antibodies (anti-CCP) [4] . The presence of rheumatoid factors is used as a marker for RA. However, rheumatoid factors have modest specificity (~70%) for the disease [5]. Citrullination is one of many types of posttranslation modification undergoes protein, where an arginine amino acid is converted to a citrulline amino acid. Citrullination is a normal reaction during cell death. Apoptosis is usually accompanied with a clearance process through scavenger cells. A defect in the clearance system either in terms of efficiency or capacity may occur due to massive cell death, which may result in the accumulation and leakage of peptidyl arginine-deiminase (PAD) enzymes and the citrullinated peptide from the necrotized cell which could be recognized by the immune system, where the immunological tolerance will be avoided and the autoimmune disorders will be

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subsequently triggered [6]. The citrullination process is implicated in many human diseases and inflammations that induce autoimmunity responses against citrullinated proteins such as rheumatoid arthritis [7]. Both rheumatoid factor and anti-citrullinated protein antibodies can be correlated with a risk of developing rheumatoid arthritis and can predict more bone erosions and severe disease progression. Biomarkers such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels provide information about disease activity, while predictive biomarkers allow clinicians to assess the probability of a treatment response before starting a particular therapy particularly in the era of biological drugs[8]. Autoantibodies are proven beneficial diagnostic tools for a diversity of rheumatic and non-rheumatic autoimmune disorders. Antinuclear antibodies (ANAs) are a diverse group of autoantibodies that recognize nuclear macromolecules and their complexes. ANAs represent key biomarkers in the evaluation of rheumatic diseases [9]. It is important to highlight that certain ANAs have such high specificity that are considered predictive for certain autoimmune diseases, even before developing signs or symptoms [10]. Disease modifying antirheumatic drugs slow disease progression and can induce disease remission in some patients. Methotrexate is the first line therapy Additional disease-modifying antirheumatic drugs or biologic agents should be added if patients become intolerant to this drug [11]. Patients with rheumatoid arthritis can develop anti –double strand DNA (anti-dsDNA) antibodies, however they are usually treatment related. They are a type of *autoantibody* usually low avidity and are only detectable transiently after treatment. Anti-dsDNA specifically targets the genetic material. The presence of these antibodies can induce a lupus-like syndrome in some cases [12]. The present study aims to establish a comparative analysis between seronegative and seropositive rheumatoid arthritis (RA), with regard to autoantibodies.

Methods

A total of 151 patients with rheumatoid diagnosed according to the 1987 American College of Rheumatology (ACR) criteria [13] were conducted .They were between 25-60 years of age with duration of disease (1-5 year) and all were undergo MTX therapy .They were divided into two groups, Group A, 78 patients with seropositive RA and Group B, 73 patients with seronegative RA. ESR was measure indirectly in a sample of blood that has been placed into a tall, thin, vertical tube. Results are reported as the millimeters of plasma that are present at the top portion of the tube after one hour. Serum levels of CRP, RF and anti CCP were measured by using Nephelometry assay kits. ANA and, anti-ds DNA were measured by using coated magnetic microparticles based chemiluminescence immunoassay**.**

Statistical Analysis

Data were statistically analyzed through statistical package for social sciences software version 19.0 (SPSS 19.0, 2010, IBM Corp., NY, USA). Data were presented as mean ± standard deviation and comparisons between groups were undertaken using 2-tailed independent t-tests test which determined the significance among groups considering $P < 0.05$ statistically significant. Relationship between the variables was measured by Pearson linear correlation.

Findings

The results presented in Table 1 indicated matching in age (50.29 &47.62 year) for both groups ($p \geq 0.05$). In comparison of the results of group A (seronegative) to that of group B (seropositive) it has been observed highly significant differences ($p \leq 0.01$) in ESR (31.4 vs 52.8 ml/hr), CRP (10.85 vs 23.59 IU/L), RF (8.34 vs 47.67 IU/L), anti CCP(21.72 vs 41.17IU/L), ANA(0.92 vs 2.3 IU/L), and anti dsDNA(13.01 vs 29.49 IU/L) levels.

Table 1: Clinical Data of patientis with seropositive Rheumatoid arthritis (group A) and patientis with seronegative Rheumatoid arthritis (group B)

Parameters	Group A N=78	Group B N=73	P value
Age (year)	50.29 ±13.21	47.62 ± 14.68	0.24
ESR (mm/hr)	31.42 ± 20.15	52.85 ± 20.39	0.00
CRP (mg/dl)	10.85 ± 9.32	23.59 ± 12.06	0.00

Cont... **Table 1: Clinical Data of patientis with seropositive Rheumatoid arthritis (group A) and patientis with seronegative Rheumatoid arthritis (group B)**

RF (IU/L)	8.34 ± 5.37	47.67 ± 8.61	0.00
Anti CCP (IU/L)	21.72 ± 15.64	41.17 ± 10.88	0.00
ANA (IU/L)	0.917 ±. 0.635	2.3240 ± 1.0782	0.00
Anti dsDNA (IU/L)	13.01 ± 9.32	29.49 ± 10.26	0.00

All data are presented as the means ± SD. ESR: erythrocyte sedimentation rate; CRP C-reactive protein; RF: Rheumatoid factor; Anti CCP: anti-citrullinated protein antibodies; ANA: Antinuclear antibodies; Anti dsDNA: anti–double strand DNA. The *P* value between two groups was analyzed using the t-test.

**P* < 0.05,

***P* < 0.01.

The correlation study of anti dsDNA with diagnostic marker of rheumatoid arthritis in seronegative patients (group A) (Table2) indicated no significant correlation with ESR and RF. In contrast, a significant correlation was observed with CRP (Figure 1).Mean while highly significant correlation were observed with anti CCP(Figure 2) and ANA (Figure 3)

The correlation study of anti dsDNA with diagnostic marker of rheumatoid arthritis in seropositive patients (group B) (Taple 2) indicated no significant correlation with RF and anti CCP. In contrast, highly significant correlation were observed with ESR. , CRP and (ANA Figure 6).

Table 2: Person correlation Anti–double strand DNA with diagnosis parameters of rheumatoid arthritis

Parameter	Group A N=78	Group B N=73
ESR (mm/h)	0.19	0.372**
CRP (mg/dl)	0.245*	0.375**
RF (IU/L)	0.092	0.182
Anti CCP (IU/L)	0.56**	-0.084
ANA (IU/L)	0.36**	0.348**

**Correlation is significant at the level 0.01(2-tailed)

* Correlation is significant at the level 0.05(2-tailed)

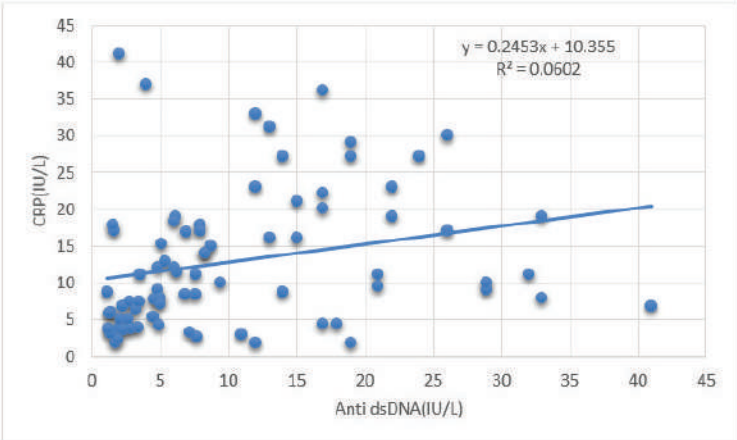


Figure 1: correlation between serum Anti ds DNA and CRP in seronegative rheumatoid arthritis patients

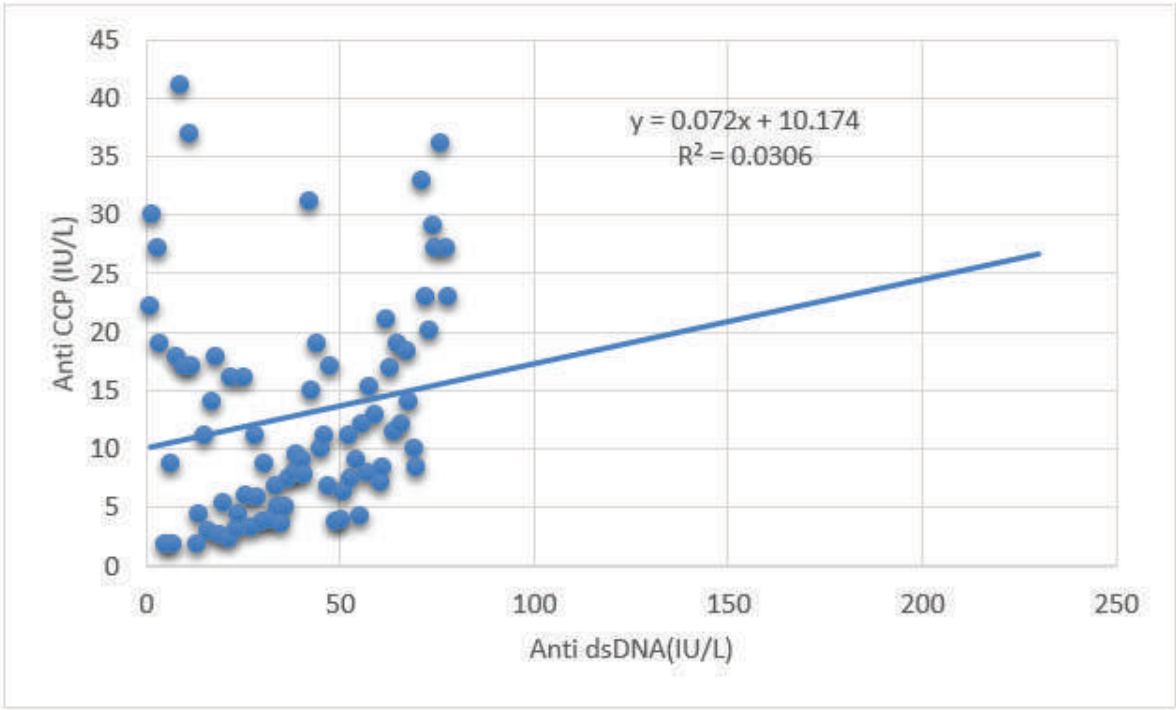


Figure 2: correlation between serum Anti ds DNA and Anti CCP in seronegative rheumatoid arthritis patients

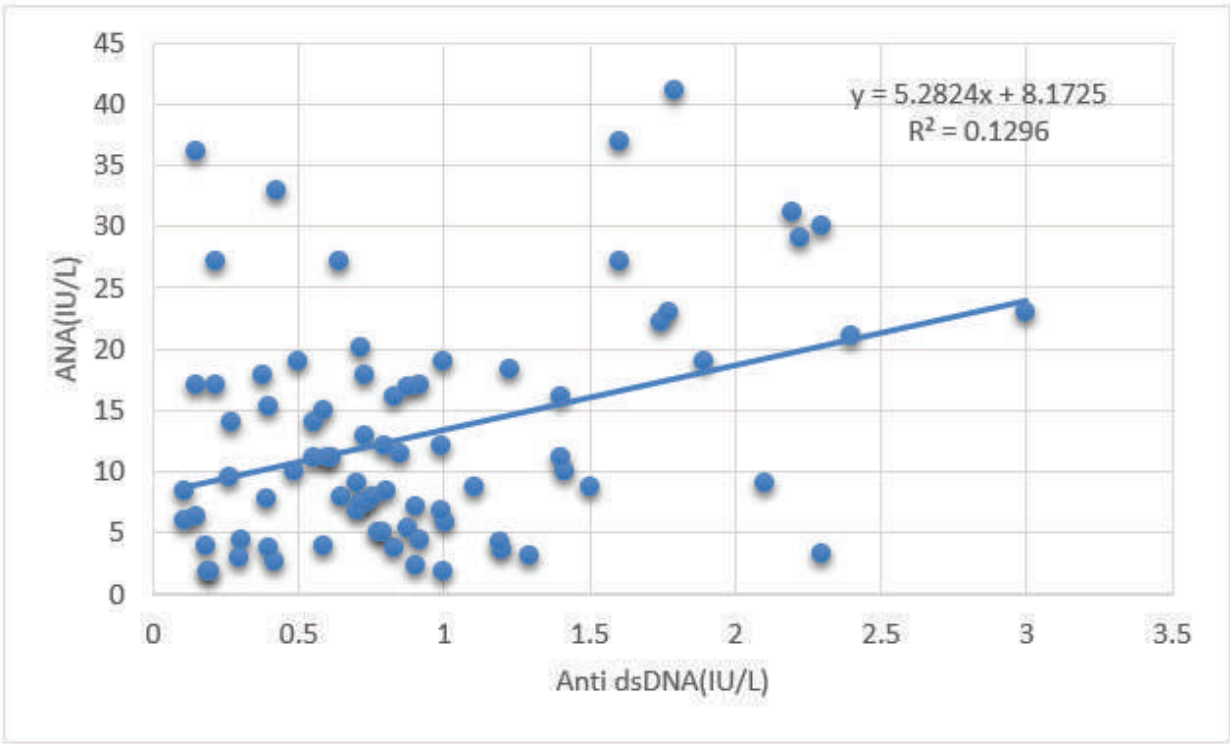


Figure 3: correlation between serum Anti ds DNA and ANA in seronegative rheumatoid arthritis patients

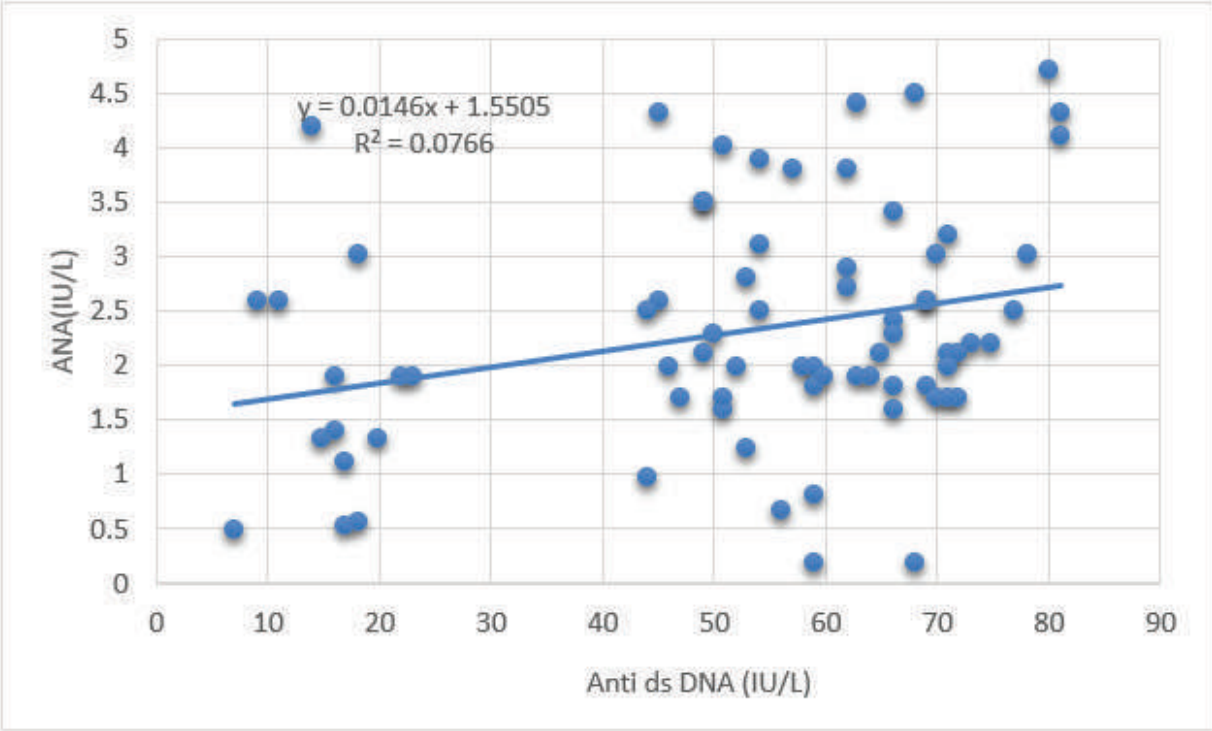


Figure 4: correlation between serum Anti ds DNA and ANA in seropositive rheumatoid arthritis patients

Discussion

The associations of RA with advancing age is consistent with prior studies [14, 15, 16]. The association of RA with age is well-known, with a peak onset among adults in their sixties [14]. The development of RA related to aging is unclear, however Weyand CM et al reported that immunosenescence occurs with aging may lead to chronic inflammation and immune-mediated tissue damage [17].In the current research there was matching in age for both studied groups which helps to eliminate differences related age in parameters results. The major role of biomarkers can be objectified by comparing the diagnostic criteria. Rheumatoid factor is the only ACR 1987 criteria biomarker for rheumatoid arthritis. Currently, the ACR/ EULAR 2010 criteria for the RA diagnosis use the rheumatoid factor (RF) and antibodies against cyclic citrullinated proteins (anti-CCP)[8] .The current research indicated significant differences in ESR and CRP levels between seronegative RA and seropositive RA. This results were agreement with Shin YS et al study who's reported that the ESR at diagnosis was 49.1 ± 36.8 mm/hr and 26.6 ± 24.3 mm/hr (p<0.01) in seropositive and seronegative groups, respectively and, the same trend was noted in CRP levels; 2.9 ± 4.1 mg/dL and 0.8 ± 1.2 mg/dL (p<0.01) [18] . In contrast Sahatçiu-Meka V, & Anton K reported that elevated average values of ERS, CRP were found in seropositive patients, but they did not present statistically significant difference with regard to sero-status [19]. Also in previous study, it has been reported that ESR was enhanced in seropositive RA patients compared to seronegative RA patients while CRP was not statistically different [20]. CRP is a sensitive index for RA disease activity and changes in CRP can predict treatment response of patients where, autoantibodies might be relevant for characterizing distinct phenotypes of RA patients [21,22]. Autoantibodies not merely provide useful information on disease outcome but also offer insights into the development of RA [23].Highly significant increase of anti-citrullinated protein, Antinuclear antibodies and Anti dsDNA in seropositive RA in comparison to that of seronegative RA which mean these test results frequently affect the course of patients' evaluations, diagnosis, and treatment. The results of correlation study showed that there were differences between seronegative RA and seropositive RA especially the relation of anti ds DNA with anti CCP

which indicated difference in the pathophysiology of the two groups. Previously it was reported that not clear whether these autoimmune responses play a pathogenetic role in the development of RA or are a consequence of the chronic inflammatory process of RA, even when the autoantibodies precede the manifestation of clinical symptoms [24,25].However the present results showed a significant correlation between anti dsDNA and ESR which suggested impossible role of the inflammatory process of RA.

Conclusion

Many of RA-associated autoantibody systems have been identified and recognize post translationally modified proteins, indicating the immunogenicity of such proteins for human B cells. Among theme is anti dsDNA which may have a role of the inflammatory process of RA

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Source of Funding: Self.

Ethical Clearance: All patients were agree befor included in this study.

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Model Mentoring Teachers and Parents as an Efforts for Brushing Teeth Behavior in Preschool Children

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Abstract

The dental health problems of preschool children are higher compared to primary schools, this is due to children not being able to do independent activities in brushing their teeth. The teacher and parent mentoring model is a model of learning to brush teeth in an effort to form the behavior of preschool brushing teeth with a period of 10 days. Methods: This study is the Research and Development (R & D) and test the model using experimental quasy pretest and posttest with control group design. Preschoolers research subjects were divided into 2 groups: 1. Intervention model of mentoring teachers and parents brushing teeth 2. Model 21 days as the control. Data were tested using intraclass correlation coefficient tests, repeated measures anova, friedman, t-test and mann whitney. Results: The application of the teacher and parent mentoring model was equally effective with the 21-day brushing model for improved tooth-brushing skills ($p > 0.304$) and equally effective for increasing plaque-free scores ($p < 0.788$) compared to the control group. Conclusion: The application of the teacher and parents mentoring model effectively as efforts to establish the behavior of preschool children brushing teeth.

Keywords: *Mentoring; teachers and parents; behavior of brushing teeth; preschoolers*

Introduction

Dental caries in childhood can interfere with mastication system and interfere with the digestive system that can be detrimental to health and child development. Riskesdas in 2018 proved that the prevalence of dental health of children aged 5-6 years by 93% with def-t index of 8.43. Such conditions do not meet the WHO target and FDI is 50% of children aged 5-6 years free of dental caries.[1, 2] One cause of the high prevalence of dental caries for dental health maintenance behaviors are less than the maximum, this is evidenced Indonesia's population had brushed his teeth with the categories of behavior really only reached 2.8% and by 2.8 West Java Province.[2, 3]

Efforts to prevent the occurrence of dental caries can be done through the behavior of most major dental maintenance and recommended by way of brushing your teeth. That is the simple act of brushing teeth to remove plaque and food debris with a toothbrush and toothpaste, because plaque and leftover food is a major cause of dental caries, therefore, required the establishment of maintenance behavior of oral health from an early age.[4]

Early childhood is a "golden age period", meaning the golden period for all aspects of human development, whether physical, emotional and social cognition, where the development of intelligence in this period increased by 50%. Early childhood is the ideal time for a child's motor skills, including brushing teeth, so that will cause a sense of responsibility for the cleanliness of himself.[6]

Changes in a child's behavior depends on the ability of adaptation to the stimulus response beyond himself. It fits in the Roy adaptation theory (Sari et al, 2012) suggests that changes in a person's behavior depends on the incoming stimulus and adaptability of the person, that is to say through the right stimulus and the appropriate development of children, will help in entering the next phase of development is well. It is also affecting the child's behavior change is a stimulus

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from the environment, namely the involvement of members of the family and the school. Through the active participation of parents it will be better the child's behavior in brushing teeth. In addition teachers who can provide guidance brush after lunch to reduce the number of plaques students.[5, 7, 8]

According to the theory of behavior change (Maher, 2014) states that a person is required to change the habit of a constant period and conditioned for 21 days in order to change the habit.[9] as innovation researchers to model the formation of the behavior of brushing teeth with a time of intervention 10 days through the approach of the teachers and parents in phases: first 2 days of the formation of consciousness which aims to create knowledge, this phase teachers provide stimulus in the form of counseling, simulation and practice brushing teeth consistently using a variety of instructional media video brushing and parents accompany brushing teeth at night before bed, 2 days arouse interest in the second stage with the aim of having the ability to identify the child; 2 days of the third stage of formation the ability to assess (evaluate) aimed at children have ability compare; 2 The fourth day is awaken the ability to practice (try) with the purpose of the child has the ability to practice.

Based on this background, researchers are interested to prove that the application of the model and the teacher mentoring can shape the behavior of brushing teeth in 10 days, especially preschoolers.

Method

The method used in this research is the Research and Development (R & D) with quasy experiment (pre and post-test with control group design). This study aims to develop a learning model of oral health in preschool children. The procedure of research and development include the 5 main steps, as follows: 1) the collection of information, 2) design of products/ models, 3) expert validation and revision, 4) testing products/ models, and 5) the product.

The sample used in this study amounted to 123 people divided into 2 groups: Sample I: the sample for the information gathering stage with purposive sampling amounted to 9 people. Sample II: sample for model testing of teachers and parents with purposive sampling, 6 teachers, 54 parents and 54 preschool children. Data collection on the measurement of tooth brushing skills using an observation sheet to brush teeth and a plaque-free score using a free plaque examination sheet for preschoolers scores. The statistical test at the expert validation stage uses the intraclass corelation coefficient while the test phase is analyzed using repeated measure anova/friedman test and independent t-test/mann whitney test.

Result

A. Information collection

The results of the information gathering concluded that to shape the behavior of preschoolers in brushing their teeth, it is necessary to provide education on dental and oral health by providing education and practice of brushing their teeth through the assistance of parents and school teachers, who have previously been given training by dental health workers.

B. Product/ Model Design

The result data from information gathering is used to design teacher and parent mentoring models as a model for the formation of tooth brushing behavior for preschool children.

C. Validation Expert

Table 1. Statistical test the validity of the expert

Validity Expert *			
	n	**f (%)**	**p-value**
Relevant	10	100	0,000
Irrelevant	0	0	

* Correlation coefficient intraclass

The results of the validity of the experts pointed out that the p-value = 0.000, which means mentoring models relevant teachers and parents as a model of dental health education in preschool children.

D. Trial Product / Model

Table 2. Test the data pairs in the intervention group and the control group

Group Mean ± SD		Skills brushing teeth*		Free score plaque**	
		p-value	Mean ± SD	p-value	
Intervention	Pre-test	28.63 + 13.63	0.000	60.33 + 10:41	0.000
	Post-test1	98.33 + 2,304		91.70 + 4,331	
	Post-test2	99.63 + 0688		93.00 + 2,961	
Control	Pre-test	32.19 + 17.61	0.000	56.77 + 7334	0.000
	Post-test	96.26 + 3108		86.85 + 4.120	
	Post-test2	98.74 + 2.330		90.11 + 3,683	

** Friedman ** Repeated Measure Anova*

Table 2 shows the results of the effectiveness of paired data on tooth brushing skills and plaque-free scores showed the p-value of the intervention and control group was 0.000 ($p < 0.05$), meaning that the 10-day brushing model was accompanied by teachers and parents and the 21-day brushing model was equally effective Improve teeth brushing skills and plaque-free scores for preschoolers.

Table 3. Test data is unpaired intervention group and the control group

Group ΔMean + SD		Skills brushing teeth *		Free score plaque**	
		p-value	ΔMean + SD	p-value	
Intervention	Pre-test		0.304		0.788
	Post-test1	70.81 + 13.70		32.96 + 9460	
	Post-test2				
Control	Pre-test				
	Post-test	66.37 + 17:49		33.59 + 7526	
	Post-test2				

** Mann Whitney ** Indepenedent t-test*

Table 3 shows the results of the effectiveness of unpaired data on tooth brushing skills and plaque-free scores between the intervention group and the control group showed a p-value ($p > 0.05$), meaning that the model of assisting brushing teeth by teachers and parents 10 days and brushing models 21 days the same- is equally effective at improving preschool brushing skills and plaque-free scores.

F. **Product Results**

Products such as training curriculum and monitoring sheets brushing teeth is the output from the learning model development and media for the dental health of preschool children. Implementation of this model by providing a stimulus undertaken by school teachers and parents.

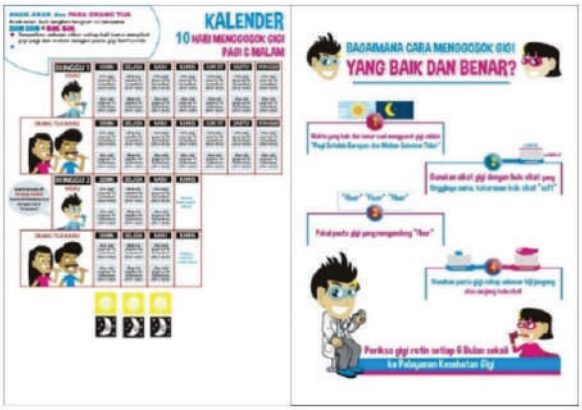


Figure 1. Monitoring sheets brushing teeth mentoring teachers and parents

Discusision

The collected information can be concluded that to establish the independence of preschool children in brushing teeth need for efforts to provide education

methods are appropriate and supported a variety of media that can attract attention so that children are able to carry it out and giving education/ stimulation in early childhood should be appropriate aspects of child development. The learning model brushing teeth suitable to realize it is a model of mentoring teachers and parents. The results of the validity of the experts pointed out that the p-value = 0.000, which means mentoring models relevant teachers and parents as a model of dental health education in preschool children.

The validation process is essential expert in the development of products / models in order to produce a product / model useful in improving the quality of education.[10] Based on the characteristics of preschool children have not been able to do their own hygiene activities including brushing their teeth so they still need help from others both teachers and parents. Teachers and parents have a role in their dental health maintenance behaviors, besides that they play an important role in a child's learning process such as learning to brush their teeth so that a school-based dental health program involving teachers and parents is effective in brushing children's teeth. Teachers and parents are given training aimed at improving knowledge, attitudes, actions for maintaining dental health towards the implementation of the model, so that it is expected to be able to transfer knowledge of skills to children. In accordance with the research of Gao et al. (2013) the model of dental health promotion to preschool children should emphasize promotive and preventive involvement by involving the role of parents and teachers in their implementation children.[11] School teacher for 10 days interventions such as counseling with simulation methods brushed his teeth using multiple media learning. To create awareness of children, the first 2 days every day a children is given counseling by watching the video brushing teeth followed by a simulation using pillow books and puzzles. Next go 2 the second day up to 2 days five children with a given extension by using a simulation method pillow books and puzzles as well as the practice of brushing your teeth.

The initial phase of school teachers do themed film screenings brushed his teeth using audio-visual media. The response of samples in initiating more passion and enthusiasm, which means that the stimulus given to the child succeed. This video media will make the child to concentrate to follow the activities because the two senses are used simultaneously view and hear. Children will understand the messages conveyed through animation moves seen concretely in the video that encourages the birth of a child's emotional response that ultimately children become enthusiastic or motivated to follow the next activity. This is consistent with the theory of behavioral change Stimulus-Organism-Response (SOR) which says that the different causes behavior changes depending on a given stimulus or stimuli.[12]

The results of the paired variable data effectiveness test showed that the intervention group's p-value was 0,000 ($p < 0.05$) meaning that the teacher and parent mentoring model was effective in improving the teeth brushing skills of preschool children. Teeth brushing skills in the intervention group increased because intervention was provided with simulations using pillow book and puzzle media, demonstration methods using phantom and brushing practice methods. Using multiple methods can improve a child's ability to brush his teeth

according to the objective indicators that researchers set. This is in line with research by Ruhaena (2015) using various methods to improve the skills of preschoolers.[13] In addition to using various methods, the implementation of the model also uses learning media so that children are directly involved in the use of media such as pillow books and puzzles and it is possible for many senses to play a role.

The more the five senses that are used will facilitate understanding of the material, someone who has an understanding of a concept, that person will easily do the practice. The practice of brushing teeth for 10 days consistently brushing their teeth in the morning after rest accompanied by the teacher and the night before going to bed with the assistance of parents has brought significant changes to the intervention group.

According to the theory of behavior change revealed to change a person's habits, it requires a constant period.[9]

The success of this model is also seen an increase in plaque-free score preschoolers. The test results showed that the effectiveness of the data pairs p-value intervention group was 0.000 ($p < 0.05$) means that the model mentoring teachers and parents to parents and teachers free of plaque effectively improve the scores of children. Score plaque free preschool children had increased because the sample had been taught to understand the practice to brush their teeth. Practice brush their teeth will be able to remove plaque. Research Raj (2013), proving that brushing your teeth with the correct technique will improve oral hygiene preschoolers.[14]

The results of the effectiveness of the unpaired test of tooth brushing skills in the intervention and control group were 0.304 ($p < 0.05$), meaning that the teacher and parent mentoring model was equally effective in increasing the brushing skills of preschool children compared to the brushing model for 21 days.

In the intervention group for 10 days effectively increasing the skills of brushing teeth of preschool children due to the advantages of mentoring models of teachers and parents provide a lot of stimulus in the learning process, the implementation is carried out by the teacher and parents and children are directly involved in demonstrating themselves how to brush teeth properly and correctly. Also supported by the toothbrush storage model facilities found in the intervention group, each child has a toothbrush and toothpaste as well as a storage mouth rinse for personal storage so that children find it easier to collect and store toothbrushes without the help of others. Facility support is one of the successes of the dental health program at school. Hayat Research (2013) factors that influence the success of UKGS are health service facilities.[15]

Conclusion

The results showed that the teacher and parent mentoring model had been shown to be effective in increasing the teeth-brushing skills of preschool children.

Ethical Clearance: This research was approved by the Health Research Ethics Commission of the Faculty of Dentistry of Sultan Agung Islamic University, Semarang, Indonesia under number 048/B.1-KEPK/ SA-FKG/ III/ 2019. We explained the objectives to the participants and received their written.

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Conflict of Interest: The authors declare that they have no conflict interests.

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The Relationship between Housing Condition and Pulmonary Tuberculosis Disease in Baraya Village Bontoala Makassar, Indonesia

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Abstract

Objective: This study aims to determine the relationship between the incidence of pulmonary tuberculosis with housing conditions consist of several factors, such as occupancy density, roomization, ventilation, temperature, humidity, and lighting.

Method: This study was observational with a cross-sectional approach. The population in this study was the patient's home with pulmonary TB disease in Baraya village, Bontoala sub-district, Makassar. The samples were selected by simple random sampling involved 65 houses. Data were analyzed using SPSS for Windows with the Chi-square test.

Result: This research found a significant relationship between pulmonary tuberculosis with occupant density (p=0.000), roomization (p=0.000), ventilation (p=0.000), temperature (p=0.000), and humidity (p=0.001). There is no relationship between lighting exposure (p=0.757) to the incidence of pulmonary tuberculosis.

Conclusion: The house condition primarily occupant density, roomization, ventilation, temperature, and humidity has strongly influenced the occurrence of Tuberculosis in the community, especially in Indonesia, even though lighting exposure statistically not significant.

Keywords: *housing condition, tuberculosis, density, roomization, temperature.*

Introduction

Tuberculosis is a transmitted disease caused by infection with *Mycobacterium tuberculosis*. Generally, after entering the body through the respiratory cavity, these bacteria will go to the lung. Not only in the lung, but these bacteria can also go to other organs, such as the kidneys, spleen, bones, and brain. Someone who is infected with Tuberculosis will cause various impacts on his life, both physically, mentally, and socially. Physically, someone who has been infected with pulmonary tuberculosis will often cough, shortness of breath, chest pain, weight loss, decreased appetite, and sweating at night. All these things will certainly cause someone to become weak. Mentally, someone who has been infected with tuberculosis will generally feel a variety of fears in him, such as fear of death, long time treatment, side effects in doing the treatment, loss of work, the possibility of transmitting the disease to others, and fear of being rejected and discriminated against by people around them.[1]

South Sulawesi health profile report 2015 recorded the most positive smear, pulmonary TB patients, in Makassar at 1,982 cases. The increase in the incidence of pulmonary tuberculosis in Makassar is related to the high population density. Population density causes more house had not met healthy housing standards.[2]

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At the Malimongan Baru Community Health Center in the city of Makassar based on data obtained in 2017, pulmonary tuberculosis still included the top ten diseases with a total of 224 cases. Malimongan Baru Health Center covered five villages area, namely the Wajo Baru, Baraya, Tompo Balang, Timungan Lampoa, and Malimongan Baru.[3]

Based on the initial observation in the Baraya village, Bontoala, there were 1,123 houses with densely populated conditions, dense environmental conditions for houses become factors causing diseases due to unhealthy environments. In the Baraya, there were 78 residents positive with pulmonary TB disease.

Method

This study was conducted in the Baraya village, the Bontoala sub-district, Makassar. The independent variables identified in this study were occupant density, roomization, ventilation, temperature, humidity, and lighting. The dependent variable in this research was the incidence of pulmonary TB disease in the Baraya village, the Bontoala sub-district, Makassar.

The population in this study was the patient's house with pulmonary TB disease located in the Baraya village, totally was 78 houses. Research samples were taken randomly from the population, the number of samples as many as 65 houses.

Data collection was taken by a questionnaire developed by the researcher based on the literature. The questionnaire was consist of one question about density, five questions about roomization, one question about ventilation, one question about humidity, one question abought lighting, and followed by home physical sanitation measurement for humidity, lighting, and temperature.

Processing and analysis of data in this study were performed by using the SPSS for Windows carried out in several stages. Correlation between variables analyzed by the Chi-square test and presented using tables and described in narrative form.

Results

The density of residents with pulmonary TB disease in the Baraya village, Bontoala sub-district, Makassar detailed, as shown in Table 1 presented below:

Table 1. Relationship between Occupancy Density and the incidence of Tuberculosis in Baraya Village, Bontoala sub-district, Makassar in 2019

Occupation density	Pulmonary TB				Total	%	Statistic
	TB Cases		Non-TB				
	n	%	n	%			
Fullfilled	11	44.0	14	56.0	25	100.0	p=0.000
Not fullfilled	39	97.5	1	2.5	40	100.0	

Roomization was related to the incidence of pulmonary TB cases in Baraya Village, the Bontoala sub-district, Makassar.

Table 2. Relationship between Roomization and the incidence of Tuberculosis in Baraya Village, Bontoala sub-district, Makassar in 2019

Roomization	Pulmonary TB				Total	%	Statistic
	TB Cases		Non-TB				
	n	%	n	%			
Qualified	12	44.4	15	55.6	27	100.0	p=0.000
Not qualified	38	100.0	0	100.0	38	100.0	

Ventilation was related to the incidence of pulmonary TB cases in Baraya village, Bontoala sub-district, Makassar

Table 3. Relationship between Ventilation and the incidence of Tuberculosis in Baraya Village, Bontoala sub-district, Makassar in 2019

Ventilation	Pulmonary TB				Total	%	Statistic
	TB Cases		Non-TB				
	n	%	n	%			
Adequate	15	51.7	14	48.3	29	100.0	p=0.000
Inadequate	35	97.2	1	2.8	36	100.0	

The temperature was related to the incidence of pulmonary TB cases in Baraya village, Bontoala sub-district, Makassar

Table 4. Relationship between Temperature and the incidence of Tuberculosis in Baraya village, Bontoala sub-district, Makassar Tahun 2019

Temperature	Pulmonary TB				Total	%	Statistic
	TB Cases		Non-TB				
	n	%	n	%			
Qualified	15	51.7	14	48.3	29	100.0	p=0.000
Not qualified	35	97.2	1	2.8	36	100.0	

Humidity was related to the incidence of pulmonary TB cases in Baraya village, Bontoala sub-district, Makassar

Table 5. Relationship between Humidity and the incidence of Tuberculosis in Baraya village, Bontoala sub-district, Makassar Tahun 2019

Humidity	Pulmonary TB				Total	%	Statistic
	TB Cases		Non-TB				
	n	%	n	%			
Qualified	22	61.1	14	38.9	36	100.0	p=0.001
Not qualified	28	96.6	1	3.4	29	100.0	

Lighting was related to the incidence of pulmonary TB cases in Baraya village, Bontoala sub-district, Makassar

Table 6. Relationship between Humidity and the incidence of Tuberculosis in Baraya village, Bontoala sub-district, Makassar Tahun 2019

Lighting	Pulmonary TB				Total	%	Statistic
	TB Cases		Non-TB				
	n	%	n	%			
Qualified	17	81.0	4	19.0	21	100.0	p=0.757
Not qualified	33	75.0	11	25.0	44	100.0	

Discussion

The relationship between occupancy density and the incidence of tuberculosis in this research showed significantly related. Statistic analysis with the chi-square test resulted in significant value ($p = 0.000 < \alpha = 0.005$), which meant H0 was rejected, implied that there was a relationship between the occupant's density and the incidence of pulmonary tuberculosis in the Baraya village, Bontoala sub-district, Makassar. This result suggested that the occupant's density who met the requirements and TB Cases indicated as many as 11 houses (44.0%), and did not fulfill the criteria as much as 39 homes with TB Cases (78%).

The high density of occupants in a house affects the health status of occupants. Occupancy density can also affect the quality of air in the home, where the more number of occupants, the faster quality of air decreased in the house because of oxygen decreases. This poor quality of air could affect the development of microorganisms if there is a carrier or person as a source of infection living in the house; it will easily be transmitted to others directly or indirectly.

In another study[4] found that there was a significant relationship between room occupancy and the incidence of pulmonary tuberculosis ($p = 0.010$, OR = 7.000). According to government regulation,[5] the total sleeping area should be at least $8m^2$ and not recommended occupying by more than two people. Buildings that are narrow and not suitable for the number of occupants will cause a lack of oxygen in the room and lately decrease the resilience of the inhabitants.

Roomization in this study has a significant correlation with the incidence of pulmonary tuberculosis in Baraya village, Bontoala sub-district, Makassar. Statistic analysis result using the Chi-square test found p-value ($0.000 < \alpha = 0.005$) confirmed that H0 was rejected and Ha was accepted.

Based on the results, it was shown that qualified roomization with TB cases found in 12 cases while not qualified roomization with TB cases found in 38 houses. The condition of a house without roomization does not meet the requirements of the healthy house due to this will facilitate easily the transmission of disease in the home, especially respiratory infections and other diseases that spread through the air. The transmission facilitated by the absence of room wall that separates occupants from the air droplet and other residents, especially a family member suffering from the disease.

The results of this study support previously study[6] that there was a relationship between roomization and the incidence of pulmonary tuberculosis. Another finding in the Malawei Village, Sorong also found that roomization (p=0.029) had a significant correlation with the occurrence of Pulmonary Tuberculosis.

In preventing the transmission of Tuberculosis, the house needs to be arranged for fulfilling a qualified room. The aim of this act to avoid infection contact between family members. Besides that, roomization also serves as a place of isolation to avoid direct contact or disease transmission from family members who suffer from illness to other family members, especially children under five who are still vulnerable to the disease.

This study found the relationship between ventilation with the incidence of pulmonary tuberculosis in the village of Baraya, Bontoala sub-district, Makassar. Analysis statistics using the Chi-square test found p-value ($p=0.000<\alpha=0.005$), concluded that ventilation has a significant relation with the occurrence of pulmonary tuberculosis.

In this study, the proportion of TB cases related to adequate and Inadequate ventilation approximately 1: 2 cases. TB cases with adequate ventilation found 15 cases while 35 cases found in the house with inadequate ventilation.

Ventilation greatly determines adequate air quality in the house because adequate ventilation will allow the flowing circulation of air and the entry of sunlight that can kill bacteria. Ventilation inadequate of a healthy home is less than 10% of the floor area. The air quality in the house become worse due to a lack of fresh air supply from outside into the house. The expenditure of dirty air out of the house is also not optimal.

In another study[7] stated an association between home ventilation and the incidence of pulmonary TB. This study found risk being exposed to pulmonary TB 1.314 times in occupants living in a home with inadequate ventilation compared to adequate ventilation and met healthy ventilation requirements.

This condition certainly has the potential to increase the risk of pulmonary TB disease to others. Besides, home conditions do not meet the requirements, such ventilation less than 10% of the floor area, one of the consequences is the lack of sunlight entering the house so that microorganism like bacteria can multiply.

This study found a significant relationship between temperature and the incidence of pulmonary tuberculosis in Baraya village, the Bontoala sub-district, Makassar. From the results of the chi-square test results obtained ($p = 0.000 <\alpha = 0.005$), Based on the results show that the temperature met the requirements and be founded occupant suffer from Tuberculosis as many as 15 houses (51.7%), while in the house with temperature not qualified be founded 35 occupants suffer from tuberculosis (97.2%).

One of the conditions of indoor air is that the temperature and humidity requirements are fulfilled the requirements, so bacteria growth can occur and the number of germs in the air increases.

The results of this study are following the previous studies[8] found that the temperature of the bedroom (p=0,000) with OR=0.036 has a significant relationship with the incidence of pulmonary tuberculosis. The research also found that the temperature of the bedroom based on multivariate analysis becomes a risk factor for pulmonary tuberculosis.

This is because the house is not getting enough sunlight and the air circulation is not flowing. So that the increase in indoor temperature causes the growth of bacteria that can cause disease. Besides, there is a lack of respondents' houses that have ceilings so that during the day it feels hot inside the house and vice versa if the night will feel cold. This can provoke an illness.

The relationship between humidity and the incidence of tuberculosis in this research showed significantly

related. Statistic analysis with the chi-square test resulted in significant value ($p = 0.001 < \alpha = 0.005$), it mean H0 was rejected, implied that there was a relationship between humidity and the incidence of pulmonary tuberculosis in the Baraya village, Bontoala sub-district, Makassar. This result suggested that the humidity who met the requirements and TB Cases indicated as many as 22 houses (61.1%), and did not fulfill the criteria as much as 28 homes with TB Cases (96.6%).

High humidity can affect a person's immune system and increase the body's susceptibility to diseases, especially pulmonary tuberculosis. Moisture can also increase the survival of bacteria.

The results obtained are in line with the previously research[9] found the relationship between home humidity and the risk of pulmonary tuberculosis (OR=1.3; 95%Ci=1.1 to 1.5).

The natural humidity of the house was closely related to temperature and ventilation that does not meet health requirements. Humid air will interfere with the health of its inhabitants. The air humidity in the home is a good medium for the growth of bacteria that cause pulmonary tuberculosis.

Relationship of lighting with the incidence of pulmonary TB disease in the Baraya village, Bontoala sub-district, Makassar. From the results of the chi-square test results were obtained ($p = 0.757 > \alpha = 0.005$). Thus it can be concluded that there was no relationship between lighting and the incidence of pulmonary tuberculosis in the Baraya village, Bontoala sub-district, Makassar. This showed that other factors cause pulmonary tuberculosis. These factors could come from the home environment and behavior.

A healthy home requires sufficient light, not less, and not too much. Lack of light entering the house, especially sunlight, besides being uncomfortable, is also a medium or a place that is not good for life is also the development of seeds of disease. Conversely too much light in the house will cause glare that can damage our vision.

The results of another study also stated that people who live in homes whose lighting does not meet the requirements are at risk of developing pulmonary tuberculosis based on statistical tests obtained ($p = 0.128 < \alpha = 0.005$).[10]

Many respondents suffered from tuberculosis because the room in the house received less sunlight in the morning. The respondent's habit not used to open the window in the early morning for lighting. This enables an appropriate environment for viruses and pathogenic bacteria to live and multiply.

Conclusion

Based on the results of research in the Baraya village, Bontoala sub-district, Makassar in 2019, found that there was a relationship between the density of occupants of the house, roomization, ventilation, air temperature, humidity and the incidence of pulmonary TB disease in the Baraya village, Makassar while no relation found between home lighting and the incidence of pulmonary TB disease in the Baraya village, Makassar.

More attention needed to ventilation, roomization, occupant density, temperature and humidity of the house for preventing tuberculosis occurrence by always keeping the house clean from dust, keep the windows of the house open in the appropriate time so that sunlight can go straight into the house and the air circulation in the house is well maintained. Preventive and promotive efforts in the context of controlling infectious diseases need to improve by the government of Indonesia.

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Ethical Clearance: This study has been ethically approved and allowed by the Regional Investment and Coordination Board of South Sulawesi in Makassar.

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CYP2D6 (G1934A) Gene Polymorphism in Rice Farmers with Long-term Pesticide Exposure, Suphan Buri, Thailand

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Abstract

CYP2D6 G1934A (rs 3892097) gene polymorphism in coding region is leads to catalytic rate of cytochrome P450 enzyme activity toward xenobiotic, including insecticide. Study was aimed to investigate CYP2D6 (G1934A) gene polymorphism in rice farmers along with occupational health risks and behaviors; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels. Cross-sectional study was carried out from June 2019 to February 2020. Data had collected from health service program by U-Thong district health promoting hospital. Gathered information concerning of pesticide exposures from 50 rice farmers and 50 control respondents was conducted by questionnaire interviewing. Each serum sample was determined SChE activity test by paper test and automatic analyzer; and EDTA blood was analyzed genotyping by PCR-RFLP. Chi-square was used to analyze on the different of personal information; and of polymorphism. Person correlation was evaluated relationship between SChE level and genotypes. 60% of rice farmers were used pesticide over 10 years. Means of SChE level of both groups were within reference value and significantly different ($p = 0.033$). Polymorphisms were included wild type (GG), heterozygote (GA) and homozygote (AA); and were significantly different between rice farmers and control ($p = 0.0001$). GA genotype in rice farmers was more frequent and AA genotype was not detected. Relationship of polymorphism and SChE level were negative correlated ($r = -0.258$; $p = 0.009$). CYP2D6 gene polymorphism may useful biomarker for duties assignments in agricultural workers especially risk group (GA genotype).

Keywords*: CYP2D6 gene polymorphism, cholinesterase inhibitors, chronic pesticide exposure, cytochrome P450, organophosphate, serum cholinesterase*

Introduction

Pesticides are substances that are meant to control pests, including weeds. The most common type of pesticides imported in Thailand is herbicides, followed by insecticides and fungicides [1] (Ministry of Agricultural and Cooperatives Office of Agricultural Economics, 2014). Thai agricultural workers report they typically use a variety of pesticides, applying them an average of three to four times a month [2, 3]. Suphanburi province is located in central plain, which is a member of "rice bowl" of Thailand. Because of enough water supplies in this cultivation area, two or three rice growing cycles can be done rather than Northeast area. Rice growing cycle is consisting of land preparation, seeding, planting and harvesting. Agrochemicals for

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seeding and planting period are more use in Central area than in Northeast area, thus, farmers may expose to pesticides more frequent rather than other area also. Moreover, agropesticide application in Central of Thailand is on intensively cultivated rice crop land when water conditions allow had been occurred [4]. This is a significant factor in allowing many farmers to continue growing rice, but it has also created health hazards for those who took up agropesticide spraying as a profession [5]. Agricultural workers in Suphanburi had reported using several brands of insecticides, plant hormones, and chemicals for "control of plant diseases". Most of active insecticide substances in commercial products are abamectin, chloropyrifos, carbofuran, and cypermethrin [6, 7].

Chloropyrifos and carbofuran are common members of organophosphate (OP) and carbamates (CB), respectively. OP and CB act as acetyl cholinesterase (AChE) inhibitors, which are affecting to several organs, such as peripheral and central nervous systems, muscles, liver and pancreas. Impairment of enzymatic pathways modulated biochemical metabolism within cytosol, mitochondria, and peroxisomes may cause by AChE inhibition at target organs [8-10]. Biological effects of pesticide are involved by oxidative stress, epigenetic controls and gut microbiological digestion and modulation of genetic polymorphisms [11]. Organophosphate pesticides (OPs) are primarily metabolized by several xenobiotic metabolizing enzymes, which are including cytochrome P450 enzymes. Cytochrome P450 polymorphisms are common biomarkers for evaluation of susceptibility in pesticide exposure [12]. CYP2D6 G1934A (rs 3892097) gene polymorphism is leads to catalytic rate of enzyme activity [13]. CYP2D6 1934A allele is related to the susceptibility to organophosphate chronic toxicity in Egyptians [14]. This study was aimed to investigate CYP2D6 G1934A gene polymorphism in Thai rice farmers along with occupational health risks and behaviors; and to evaluate the relationship of gene polymorphisms and serum cholinesterase (SChE) levels. This genotypic polymorphism may help to identify the risk of occupational exposure especially management of chronic pesticide toxicity.

Materials and Methods

Subject recruitment and data collection

The study was conducted in U-Thong district, Suphan Buri province, about 150 km from West of Bangkok, where rice is main harvesting product. Main of herbicide use is paraquat and glyphosate. Outsource pesticide sprayers were common finding in rice field. Cross-sectional study was carried out from June 2019 to February 2020 on data had collected from annually health service program by health promoting hospital, This study was recruited 100 respondents included 1) 50 rice farmers (risk group) were aged 18-65 yr who lived in this area, which had handle pesticide regularly or work in paddy field at least three years or more 2) The control group was included 50 respondents who lived nearby field area and listed in house registration, had non-related professional for farm workers. Respondents with a history of serious conditions, such as, liver diseases, severe cardiovascular diseases, cancer were excluded. Questionnaire interviewing and blood collection were conducted by well-trained research assistants and medical technologists, respectively. Gathered information concerning of long-term pesticide exposures including personnel information, adverse health symptoms, personal protective equipment (PPE) used, and practical knowledge of pesticide use were recorded from questionnaires by personnel interviewing. The sample size was estimated using the single proportion formula with 95% confidence interval and based on percentage of abnormal SChE level in previous study [3]. The Ethics Committee of Thammasat University was approved this research protocol (COA No. 085/2562). The director of U-Thong district ′s health promoting hospital, Suphan Buri province gave permission to conduct on this study. All participants gave informed consented.

Blood collection, preparation and storage

Each 5 ml of blood sample was obtained by venepuncture from median cubital vein during morning (7-9 a.m.); and drawn into clotting blood and EDTA tubes for 3 ml and 2 ml, respectively. Clotting blood tube was further centrifuged; and serum was separated within 2 h after phlebotomy and stored at -20 °C [15] for SChE activity test. Whole blood contained in EDTA tubes were prepared for genomic DNA extraction by using the QIAamp blood DNA mini kit (QIAGEN Thailand,

Bangkok, Thailand) and genomic DNA was stored at -20 °C.

SChE activity test

Screening of SChE level by paper test and confirming of SChE level was done by automatic analyser. The paper test kit was developed and manufactured by Government Pharmaceutical Organization (GPO), Thailand. The efficiency of test including sensitivity, specificity and positive predictive values were 77, 90 and 85%, respectively [16]. The quantitative for SChE level was conducted by automatic analyzer, COBAS c501 (Roche-diagnostics, Rotkreuz, Switzerland), which were performed in certified clinical laboratories. SChE level were interpreted by reference values according by instruction of manufacturer.

CYP2D6 G1934A polymorphisms by PCR-RFLP

DNA template was amplified by polymerase chain reaction (PCR) using forward 5'-GCT TCG CCA ACC ACT CCG-3' and reverse 5'-AAA TCC TGC TCT TCC GAG GC-3' primers, which were corresponded to CYP2D6 G1934A region [17]. PCR was performed in 25 µl of total volume, which was contained 1 µl of DNA template, 0.5 µl of each primer (20 pmol/µL), 5 µl of 10X PCR buffer (1.5 mM Mg^{2+}) include dNTP mixture and 0.2 µl of 1 U AmpliTaq polymerase (Thermo Fisher Scientific, USA). PCR was performed with initial denaturation at 94°C for 5 min. followed by 35 cycles consisting of denaturation at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72 °C for 1 min followed by final extension at 72 °C for 7 min by using Thermal cycler (Applied Biosystems, USA). Restriction fragment length polymorphism (RFLP) was performed: DNA product was digested by *BstNI* Restriction Enzyme (New England Bio Labs, Cambridge, UK). Digested DNA fragments was separated on 2% of agarose gel electrophoresis apparatus then stained with ethidium bromide. DNA electrophorogram was read by using ultraviolet transillumination (Promega, USA). CYP2D6 G1934A single nucleotide polymorphisms (SNPs) were 1) 104- and 230-bp fragments for the 1934G allele and 2) 334-bp fragment (undigested) for the 1934A allele. The interpretation of CYP2D6 G1934A genotypes were represented 104, 230-bp fragment for wild type (GG); 104, 230, 334-bp fragments for heterozygous (GA); and 334-bp fragments for homozygous (AA) [14]. Quality control of test was done by DNA sequencing, which was randomized from 15% of samples.

Statistical Analysis

Descriptive data was explained by using mean and standard deviation; and frequency. The Kolmogorov-Smirnov test was used to test for normal distribution of data. Chi-square was used to analyze on the different of personal information and CYP2D6 G1934A genotypes between rice farmer and controls groups. Independent *t*-test was used for comparison of SChE level between rice farmer and controls groups. Person correlation was evaluated between SChE level and PON1 Q192R genotypes. The statistical significance was judged at $p < 0.05$. SPSS 21.0 software was used for statistical analysis (SPSS, Chicago, Illinois, USA).

Results and Discussion

Chronic pesticide exposure in rice farmers

No statistical significance different between rice farmers and controls for gender and risked behavior, such as alcoholic consumption. All of rice farmers were long-term pesticide exposure and 60% of rice farmers were used pesticide over 10 years; and rate of pesticide exposure was mainly for 1-2 time/week. The related pesticide used symptoms were rarely occurred and almost of them had health education for awareness of pesticide uses (Table 1). However, unexpected finding may due to unspecific symptoms, imprecisely explain by personal interviewing and tolerance of frequent exposed farmers. Means of SChE level were significantly different between rice farmers and controls ($p = 0.033$), however, there were within reference value (Table 1).

Some of rice farmers had SChE level lower than reference value (data not show), which was implied that SChE may not be a good marker for quantifying exposure to pesticide among sprayers, especially during spraying season. In previous study, agriculturists can be exposed to pesticides divided into sprayers, agriculturists and other professions, however, the SChE levels among them were not significantly difference and level was still within reference range [18]. Most of studies on Thai pesticide exposure had reported exposure of single type of pesticide; however, mixed pesticide uses are more common for multi-crop cultivation. Thus, screening of other biomarkers for evaluation of long-

term pesticide exposure rather than serum cholinesterase such as, alkyl phosphate metabolites (DAPs), urinary 3-phenoxybenzoic acid (3-PBA) and urinary glyphosate, is still necessary for public provider [19].

CYP2D6 (G1934A) gene polymorphism in rice farmers

The DNA fragments electrophoresis of CYP2D6 (G1934A) gene polymorphism in rice farmers were included wild type (GG), heterozygote (GA) and homozygote (AA) (Fig. 1). The genotypic polymorphisms of rice farmers and control were significantly different (p=0.0001); rice farmers were more frequent RR genotype rather than control (Table 2), however AA genotype was not detected from both groups. The relationship of CYP2D6 (G1934A) gene and SChE level were negative correlated (r = -0.258) with statistically significant (p = 0.009) (Table 3). Six of rice farmers were low SChE level (<5,500 U/L) and GA genotype polymorphism (data not show).

CYP2D6 is important member of cytochrome P450 based on various xenobiotic substrates, which are shows a very high degree of variability due to the extensive genetic polymorphism that influences gene expression and enzymatic function [20]. CYP2D6 gene is involved in the secondary metabolic pathway of desulphuration of isocarbophos (ICP). ICP enantiomers and its oxidative desulphuration metabolite, isocarbophos oxon (ICPO) has been reported to be inhibitors of acetylcholinesterase [21, 22]. The CYP2D6 inhibitors are resulted in 50% inhibition of cholinesterase activity for parathion, 38% diazinon and 30% chlorpyrifos as compared to control [23] and SChE is significantly reduced in chronic organophosphate exposures [14]. The variants of CYP2D6 alleles at third intron and four exons are resulted in missing of transcription and single based deletion of mRNA [24]. Thus, this finding was corresponded to previous study by reduction of cholinesterase activity and GA genotype was more susceptible to pesticide rather than wild type in Thai population, which was sparely reported. Large scale and seasonal variation studies on gene polymorphism and environmental expose will conduct to confirming the finding. Genotyping on polymorphism may also useful biomarker for agricultural workers to assignment of appropriate duties, especially high risk group.

Table 1: The frequency of personal data and pesticide-exposing factors from rice farmers and controls

Personal data/ Exposing factors	Rice farmer (%)	Control (%)	p-value
Gender : Male	34 (68)	30 (60)	0.405
Female	16 (32)	20 (40)	
Age : < 40 years	22 (44)	40 (80)	0.0001*
≥ 40 years	28 (56)	10 (20)	
Alcohol intake: none	36 (72)	33 (66)	0.517
drinking	14 (28)	17 (34)	
Duration of pesticide use: 4-9 years	18 (36)	-	
> 10 years	32 (64)	-	
Frequency of exposure: 1-2 days/week	30 (60)	-	
3-4 days/week	4 (8)	-	
5-6 days/week	16 (32)	-	

Cont... **Table 1: The frequency of personal data and pesticide-exposing factors from rice farmers and controls**

Clinical symptoms: None	36 (72)	-	
Headache/vertigo	11 (22)	-	
Abdominal cramp	3 (6)	-	
Health education: none	13 (26)	-	
educated	37 (74)	-	
Serum cholinesterase (U/L) **	7247.2 ± 1293.3	7775.9 ± 1152.8	0.033*

* Statistically significant at $p<0.05$; ** Serum cholinesterase (SChE) was screened by paper test before tested with automatic analyzer and represented as mean ±SD (reference value = 5,500-13,000 U/L).

Table 2: Genotypic frequency of CYP2D6 (G1934A) in rice farmer and control groups

Genotype	Control		Farmer		p-value
	N	%	N	%	
Wild Type (GG genotype)	44	88	22	44	0.0001*
Polymorphism (GA genotype) a	6	12	28	56	

* Statistically significant at $p<0.05$

[a] Homozygous polymorphism (AA genotype) was not detected

Table 3: Relationship of CYP2D6 (G1934A) genotypes and SChE level

Genotype	N	SChE (U/L)	r	p-value
Wild Type	66	7820.14 ± 1103.25	-0.258	0.009*
GA Polymorphism	34	7278.89 ± 1307.70		

* Statistically significant at $p<0.05$; r = Pearson correlation coefficient



Figure 1: DNA fragment separation. First Lane (from left): DNA ladder; Lane 2, 6, 9, 11, 12, 17, 23, 50 and 59: 104, 230-bp for GA genotype (polymorphism); and Lane 15, 16, 30, 32 and 42: 334-bp for GG genotype (wild type).

Conclusion

CYP2D6 (G1934A) polymorphism may useful biomarker combining with SChE activity test for duties assignments in agricultural workers especially risk group (QR genotype).

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Ethical Clearance: Ethics Committee of Thammasat University was approved this research protocol (COA No. 085/2562).

Conflicts of Interest: The authors confirm that there are no conflicts of interest.

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National Health Insurance Participants Satisfaction in General Polyclinic Services at First Level Healthcare Facilities

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Abstract

The need of health services, especially at first level healthcare facilities (FKTP) for the community to access basic health services has increased in Indonesia. This condition related to the regulation for the entire citizen in Indonesia to become a National Health Insurance (JKN) participant. Some problems related to the services still occur, such as queues length and non-optimal examination process. This research aimed to analyze patients' satisfaction in general polyclinic services differences in FKTP. This was an observational research with a cross-sectional study using primary data from both types of FKTP (Puskesmas and Non-Puskesmas) as the healthcare provider in two cities of East Java. The number of research respondents was 219 in 20 FKTP. The sampling method used was proportional random sampling technique. Patients' satisfaction scores obtained from the two types of FKTP were analyzed using independent sample T Test. The results obtained was the satisfaction score in Puskesmas and Non-Puskesmas exceeded 95%. Patients' satisfaction in Puskesmas was significantly higher than Non-Puskesmas (p = 0.01). Out of the six dimensions, dimensions of service ability and aesthetics in exception, the other four dimensions had significant differences. The results provide recommendation that FKTP should improve their services from various dimensions.

Keywords: *patient satisfaction, first level healthcare facilities, National Health Insurance*

Introduction

Healthcare providers need to build patients' satisfaction to measure the quality of their services, including the participants of National Health Insurance (JKN). Healthcare provider in JKN program implementation consists of first and advanced level healthcare facility (FKTP and FKTL). Most first-level healthcare facilities (FKTP) which are public health centers (Puskesmas) and primary clinics (Non-puskesmas) in Indonesia have already cooperated with Healthcare and Social Security Agency (BPJS Kesehatan) in JKN program. Analysis of patients' satisfaction in FKTP that cooperated with BPJS Kesehatan is still limited.

Satisfaction is one of the main indicators that determine the standard of a health facility. Knowing the expectations and opinions of patients about the services obtained is an important step to find out the quality of health services provided. Patient satisfaction of health services in certain FKTP will make an impact on the reuse of these health services.[1] Satisfaction is a function of the difference between the perceived and expected performance. Patient satisfaction as a measure of healthcare quality level is a typical and complicated phenomenon. This can be in line with and also not in line with the professional code of ethics and quality standards set by the government.[2]

According to Hidayah (2015)[3] there are some problems related to JKN services. One of them is the

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large number of patients that must be served causing long queues. The examination process of patients tends to be done quickly and seems rushed so that patients feel there is not enough time to consult with the doctor. The limited administration of drugs makes the patient uncomfortable because the participants must return back and forth to queue for treatment again. By using a tiered referral system, patients feel the referral process is taking longer. The limited-service time at family physicians also raises complaints from patients.

Royanah (2015)[4] stated that several aspects that resulted in patient dissatisfaction related to JKN participants in FKTP were aspects of technical competency of puskesmas staff, the number of human resources, and human relations. Some patients are also dissatisfied with the problem of long waiting duration, and the facilities provided are still not as expected.

Garvin (1984)[5] stated that that product quality evaluates from 8 factors, namely performance, features, reliability, durability, aesthetic, conformance, service ability, and perceived quality. Adopting 8 dimensions of product quality, this research uses 6 dimensions that considered can be applied in the health services area, namely performance, features, reliability, conformance to specification, service abilities, and aesthetics.

East Java Province is the second-most populous province in Indonesia after West Java, amounting to 39,7 million in 2019.[6] This condition has an impact on the increasing population in urban areas. The demand for health services and healthcare providers in cities also increased. The purpose of this research was to analyze the satisfaction score of patients in FKTP (Puskesmas with Non-Puskesmas) that cooperated with BPJS Kesehatan.

Method

This research was an observational research with cross-sectional study approach using primary data from FKTP that cooperated with BPJS Kesehatan in 2 cities of East Java. The selection of the sample was done randomly by proportional random sampling with 20% of the total FKTP, amounted to 20 organizations and 219 respondents. The satisfaction in general polyclinic services assessed from 6 dimensions of service quality namely performance, features, reliability, conformance to specification, service ability, and aesthetics. The satisfaction score is obtained by comparing the patient's assessments and expectations. If the result obtained is greater than 95%, then the patient is considered satisfied with the services. Patients' satisfaction differences in each dimension of service quality were compared between Puskesmas and Non-Puskesmas using an independent sample T-test.

Results and Discussion

Respondents Characteristics

The number of samples was 219 respondents, according to gender, the latest education level, and JKN Participant Class in each FKTP types can be seen in Table 1.

Table 1. Distribution of Respondents' Characteristics Based on Gender, The Latest Education Level, and JKN Participant Class in both FKTP types in 2 cities in East Java (n=219)

Respondents' Characteristics	FKTP Type				Total	
	Non-Puskesmas		Puskesmas			
	n	%	n	%	n	%
Gender						
Male	48	42.11%	32	30.48%	80	36.53%
Female	66	57.89%	73	69.52%	139	63.47%

Cont... **Table 1. Distribution of Respondents' Characteristics Based on Gender, The Latest Education Level, and JKN Participant Class in both FKTP types in 2 cities in East Java (n=219)**

Total	114	100.00%	105	100.00%	219	100.00%
The latest education level						
None	1	0.88%	4	3.81%	5	2.28%
Primary school	8	7.02%	29	27.62%	37	16.89%
Junior high school	14	12.28%	25	23.81%	39	17.81%
Senior high school	69	60.53%	36	34.29%	105	47.95%
College	22	19.30%	11	10.48%	33	15.07%
Total	114	100.00%	105	100.00%	219	100.00%
JKN participant class						
I	17	14.91%	9	8.57%	26	11.87%
II	79	69.30%	20	19.05%	99	45.21%
III	18	15.79%	76	72.38%	94	42.92%
Total	114	100.00%	105	100.00%	219	100.00%

The majority of respondents were female in both Puskesmas and Non-Puskesmas. Male respondents were only one third out of the total respondents. Respondents have varied levels of education and the majority of the latest education was senior high school. Most of the respondents were registered as class III in Puskesmas while the majority of Non-Puskesmas respondents were registered as class II.

Patient Satisfaction Score

The patient satisfaction score in general polyclinic services for all dimensions exceeds 95%, as shown in Table 2.

Table 2. Patient Satisfaction Score in General Polyclinic Evaluate From 6 Dimension in FKTP (Puskesmas and Non-Puskesmas) in 2 cities in East Java (n=219)

No	Dimensions and Indicators	Satisfaction Score				p
		Non-Puskesmas (n=114)		Puskesmas (n=105)		
		Mean	*SD*	*Mean*	*SD*	
	Performance					
1	Medical records and patient identity are suitable	98.14%	9.45%	101.08%	4.28%	0.01
2	Confidentiality of patient information is well-secured	97.67%	8.44%	101.69%	4.82%	0.01
3	Given service and complaints are suitable	98.76%	9.57%	102.07%	4.48%	0.01

Cont ... **Table 2. Patient Satisfaction Score in General Polyclinic Evaluate From 6 Dimension in FKTP (Puskesmas and Non-Puskesmas) in 2 cities in East Java (n=219)**

No	Dimensions and Indicators	Satisfaction Score				p
		Non-Puskesmas (n=114)		Puskesmas (n=105)		
4	Service given by medical personnal and priority of treatment are suitable (not biased)	98.23%	10.31%	101.42%	5.31%	0.01
5	Medical equipments are well equipped (examination bed, stethoscope, consultation table, et al.)	97.86%	10.56%	102.19%	5.87%	0.01
6	Medical personnel's expertise in providing services is suitable	98.65%	9.64%	101.12%	4.44%	0.01
	Performance satisfaction score	98.22%	8.73%	101.59%	3.41%	0.01
	Features					
1	Direction to general polyclinic is clear	100.71%	6.86%	101.88%	5.00%	0.15
2	General polyclinic is easy to access	100.61%	6.06%	101.38%	4.54%	0.29
3	Waiting room for general polyclinic is available	99.84%	6.48%	101.63%	5.12%	0.03
4	Queues are well ordered	99.69%	7.76%	101.85%	6.43%	0.03
	Features satisfaction score	100.21%	6.00%	101.69%	4.34%	0.04
	Reliability					
1	Services hours and schedule are suitable	97.62%	10.36%	100.71%	3.59%	0.01
2	Medical personnels provide explanations related to patients' disease properly	98.73%	9.77%	101.65%	5.26%	0.01
3	Medical personnels are well trained in treating patients	98.63%	10.69%	100.80%	5.46%	0.06
4	Medical personnels are accurate in handling patient's complaints	100.22%	10.70%	100.68%	4.95%	0.68
5	Medical personnels are calm in providing services	98.99%	9.60%	100.89%	4.89%	0.06
6	Medical personnels are able to conduct a credible examination	99.44%	10.54%	103.10%	7.40%	0.01
	Reliability satisfaction score	98.94%	9.30%	101.30%	4.12%	0.02
	Conformance to specification					
1	Service steps or mechanism with procedure or SOP is suitable	97.81%	7.70%	100.98%	5.08%	0.01
	Conformance to specification satisfaction score	97.81%	7.70%	100.98%	5.08%	0.01
	Service ability					
1	Queue waiting duration to general polyclinics is brief	97.56%	12.19%	100.40%	5.80%	0.03
2	Treatments in general polyclinic are spry and quick	98.86%	11.81%	100.91%	5.63%	0.10
3	Medical personnels are polite and friendly in giving treatment	100.93%	11.61%	101.82%	7.16%	0.49
4	Medical personnels look confident in giving treatment	100.91%	10.81%	103.08%	7.11%	0.08
5	Medical personnels are proper in asking patients' complaint	100.71%	10.94%	102.09%	6.21%	0.25

ont ... **Table 2. Patient Satisfaction Score in General Polyclinic Evaluate From 6 Dimension in FKTP (Puskesmas and Non-Puskesmas) in 2 cities in East Java (n=219)**

No	Dimensions and Indicators	Satisfaction Score				p
		Non-Puskesmas (n=114)		Puskesmas (n=105)		
6	Medical personnels are able to respond to patients' complaints properly	100.49%	10.60%	102.40%	6.31%	0.11
7	Medical personnels provide explanations regarding patient's disease properly	99.73%	11.47%	102.76%	6.90%	0.02
8	Medical personnels provide sufficient opportunity to ask regarding patients' disease	100.53%	11.22%	101.78%	6.12%	0.30
9	Medical personnels raise sense of safety	99.99%	9.81%	101.61%	6.30%	0.14
	Service ability satisfaction score	99.97%	10.17%	101.87%	4.49%	0.07
	Aesthetics					
1	Medical personnels' appearance are clean and tidy	100.00%	6.44%	100.94%	4.02%	0.20
2	General polyclinics examination room is clean and tidy	98.88%	7.63%	100.67%	7.32%	0.08
3	General polyclinics examination room is comfortable and in order (not noisy)	99.22%	7.31%	99.86%	6.95%	0.51
	Aesthetics satisfaction score	99.37%	6.38%	100.49%	4.95%	0.15
	Total satisfaction score	99.08%	6.74%	101.32%	3.37%	0.01

The results showed that patients feel satisfied in services given by FKTP (Puskesmas and Non-Puskesmas). It can be seen from all satisfaction scores that exceed 95%. Out of the six dimensions assessed, Puskesmas patients had a higher satisfaction score in all dimensions compared to Non-Puskesmas patients. Patient satisfaction at Puskesmas was significantly higher than Non-Puskesmas ($p = 0.01$). Ouf the six dimensions, except the dimensions of service ability and aesthetics, the other four dimensions had significant differences.

In the performance dimension, Puskesmas had a higher satisfaction score than Non-Puskesmas. The lowest satisfaction score in Non-Puskesmas FKTP was in statement number two, which related to the confidentiality of patients' information. This result is in line with research by Sani, Rantetampang, and Zainuri (2017)[7] about JKN patients' satisfaction in Papua which states that the confidentiality of patients' data greatly influences satisfaction. Well-maintained confidentiality of patients' data will make patients feel a lot safer.

Non-Puskesmas had a lower satisfaction score regarding features dimension in the general polyclinic. The availability of facilities such as the waiting room is still not properly provided. This condition can be caused by several FKTP that still use the same waiting room for registration, general polyclinic, and pharmacy. These results are supported by Wildan's research (2017)[8] regarding JKN patients' satisfaction which states that the lowest satisfaction score in the health service dimension is the physical facility. The physical facility in FKTP is still considered to be lacking in many ways, such as lack of waiting rooms, toilet cleanliness, and parking lots. The availability of good physical facilities can certainly increase patients' satisfaction in FKTP services.

The satisfaction score of reliability dimension in Non-Puskesmas was lower than Puskesmas. Punctuality of operational hours and schedule statement had the lowest satisfaction score in Non-Puskesmas FKTP. These results are supported by Arbitera, Sjaaf, and Sulistiadi's research (2016)[9] which states the variables that influence patients' satisfaction the most are

punctuality, easiness, and coziness in getting treatment. Punctuality of operational hours in Non-Puskesmas is still considered lacking compared to Puskesmas. The punctuality of operational hours in health services is an important aspect of customer satisfaction. One of the statements with a high score of satisfaction is the ability of medical personnel to provide credible treatment. According to Pramayanti dan Arnaya (2019)[10], the competence of medical personnel has a significant effect on patients' satisfaction. A great competence of medical personnel will affect the quality of health services.

Puskesmas had a higher satisfaction score in conformance to specification dimension. This means that in Puskesmas, the medical personnel is seen to be more in order, following the standard operating procedure in providing treatments to patients. This result is in line with Wildan's research (2017)[8] about JKN patients' satisfaction which states that the ability of medical personnel to provide services following the standard of procedure in FKTP is very important. According to Patandianan, Ratnawati, dan Sardjono (2018)[11], a doctor must place patients' interests above theirs, set and maintain integrity and standards of competence, also provide solutions to public health problems.

Based on the dimension of service ability, it can be seen that Puskesmas and Non-Puskesmas had a relatively similar satisfaction score. The statement with the lowest satisfaction score in both types of FKTP was statement number one, which related to queue waiting duration in general polyclinics. Meanwhile one of the statements that had a high satisfaction score was politeness and hospitality of medical personnel. These results are supported by the research of Arbitera, Sjaaf, dan Sulistiadi (2016)[9] in East Jakarta Puskesmas which states that politeness and hospitality of medical personnel affect patients' satisfaction. The easiness and coziness in getting treatment also have a significant influence on patients' satisfaction.

In the aesthetic dimension, Puskesmas and Non-Puskesmas had a similar score of satisfaction. According to most respondents, medical personnel's appearance and environmental conditions of health facilities were great and comfortable. This result is supported by the research of Sani, Rantetampang, dan Zainuri (2017)[7] regarding JKN patients' satisfaction in Papua. The research mentions that the clean and neat appearance of medical personnel affects patients' satisfaction. Environmental conditions also have an important influence on the aesthetic dimension.

Conclusions and Suggestions

Conclusion

1. JKN patients in FKTP (Puskesmas and Non-Puskesmas) tend to have a high satisfaction score which exceeds 95%.

2. Patients in Puskesmas have a higher satisfaction score compared to Non-Puskesmas patients in all dimensions.

3. Out of the six dimensions assessed, the dimension of service ability and aesthetics in exception, the other four dimensions have significant differences.

Suggestion

Some suggestions for FKTP to improve patients' satisfaction in general are:

1. Pay attention to 6 dimensions of patients' satisfaction in providing services.

2. Patients' information needs to get more attention to maintain well-secured confidentiality. Punctuality of operational hours needs to be improved and queue waiting time must get more attention.

Conflict of Interest: Nil

Ethical Clearance: This research has been proved by Health Research Ethics Committee, Faculty of Public Health universitas Airlangga

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Identification of DNA Corpse in Ordo Diptera Familia Calliphoridae Larvae in CSF1PO, TH01, and TPOX Locus Using Polymerase Chain Reaction (PCR) Methods

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Abstract

Introduction: Biomolecular techniques using DNA are known to have high accuracy in the identification of decaying bodies. In the process of identification, larvae found in corpses that have undergone decay can be used as alternative biological evidence to estimate the time of death.

Aim: To identify the transfer of body DNA to the larvae of the fly ordo Diptera family Calliphoridae and the process of DNA transfer of decomposed bodies.

Method: blood from the postmortem corpse was taken as a control, then soft tissue was taken as a decomposition product for the breeding of Dipdo family Calliphoridae larvae. The protocol used is DNA profiling using the Short Tandem Repeat (STR) locus such as CSF1PO, TH01, and TPOX. The results of DNA extraction from the postmortem body and DNA extraction results obtained from the skin of the larvae and the digestive system of the larvae are then matched.

Result: The results of this study found that there was DNA transfer in larvae proved by the matching of DNA at the STR loci on the skin and the digestive system of larvae with the STR locus in blood taken from the postmortem body

Conclusion: the presence of decomposed corpse DNA decomposition to the larvae of members of the order called Calliphorideae family which can be detected through the results of nuclear DNA visualization at the CSF1PO, TPOX, and TH01 loci using the PCR method

Keywords: *corpse DNA, Diptera, Calliphoridae, Short Tandem Repeat (STR), polymerase chain reaction (PCR)*

Introduction

Death is the final stage of the life of every living thing including humans. In the world of forensic medicine, it is known that death can take place naturally or unnaturally (natural and unnatural death). Both natural death and unnatural death will undergo a process of decomposition (decomposition)[1]. The decay process is caused by the effect of proteolytic enzymes and microorganisms. Generally, the decay process starts 18 to 24 hours after someone dies[2].

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In the process of identifying corpses that have undergone decay, conventional methods such as visual methods and fingerprints cannot be used, so using DNA biomolecular analysis is a fast and appropriate method to use. The biomolecular method itself was only demonstrated by scientists in the early 1980s, by Jeffreys with DNA technology successfully demonstrated that parts of DNA can be used as a means of specific (personal) identification of a person so that since then various kinds of methods have begun to be applied to the investigation of cases forensics[3]. Compared to conventional methods that rely on serology and electrophoresis technology, DNA technology has advantages, especially in its potential for criminalization and sensitivity[4].

Identification towards the use of molecular forensics is increasingly needed. This method was first introduced by Sir Alex Jeffreys in 1985, who utilized medical and biological knowledge at the molecular or DNA level (deoxyribonucleic acid)[3]. Especially in cases of mutilation murders, victims of airplane accidents, suicide bombings that cause the victim's body to break into pieces, scorched and often found decomposed. These parts are difficult to recognize visually, so DNA analysis is an alternative method that can be used in the situation above because of its high level of accuracy[5].

The decayed products eaten by flies contain the nucleus of human cells[6]. The larvae found at the crime scene are one alternative biological evidence that needs to be analyzed. Considering the information obtained on the body of a flight will help police and investigators in determining the estimated time of death (estimation time of death) and help provide information on the possibility of fabricating a crime scene. Meanwhile, Welles and his colleagues in their research found that mitochondrial DNA (mtDNA) sequence data can be obtained from the intestine dissected from maggots that have been given to human tissue. This data can be used to identify both the human corpse upon which the maggot has eaten and the species of the maggot itself[7,8].

This study will use larva specimens (maggots) from the fly family of the Diptera Ordo results of postmortem soft tissue cultivation, with a view to knowing the process of transferring human DNA to fly larvae. In addition to knowing that the process of transferring human DNA into the larvae of the fly passes through the digestive system (migration occurs because the larvae feed on the corpse's soft tissue), or through the skin of the larvae in contact with the decomposed corpse's soft tissue. To be able to know the existence of decomposed human DNA and decomposed processes, it is necessary to extract the adult larvae in full and extract the inside (intrainstestinal digestion) rather than flies that are cultured from the soft tissue of decomposed human bodies. Considering that corpse cases that have undergone decay are very rarely found in the Department of Forensic Medicine/Soetomo Teaching Hospital, Surabaya. So that the cultivation of larvae is carried out by taking soft tissue samples of fresh human remains and allowed to rot to invite flies to lay their eggs. The larvae of the fly will live and eat the soft tissue of deceased human bodies.

The locus that will be taken for analysis is the Short Tandem Repeat (STR) locus: CSF1PO, TH01, TPOX which are some of the 13 loci determined by the Federal Bureau of Investigation (FBI). Examination using the PCR method because this method can multiply millions of DNA to billions of Kalli so that it is possible to conduct an analysis with a very minimal number of samples[9,10]. In addition to the PCR method is also able to analyze materials that have been partially degraded. This is important because DNA samples in the skin or in the digestive system of larvae will experience degradation due to temperature, PH, larval metabolic system, and contamination with other objects around the body[8]. The principle of PCR is selective exponential amplification of certain DNA fragments. The purpose of this study was to identify the transfer of body DNA to the larvae of the fly ordo Diptera family Calliphoridae and the process of DNA transfer of decomposed bodies.

Method

This type of research used in this study is an observational laboratory with a longitudinal study approach to analyzing the STR locus: CSF1PO, TPOX, and TH01. Some of these loci include loci recommended by the FBI (Federal Bureau of Investigation) with DNAzol extraction techniques. The results of this study are presented descriptively. The population in this study is the remains of humans who have undergone a process of decay. To obtain larvae, researchers still need to breed fresh bodies by autopsy. Whereas for the breeding of the order larvae, the Calliphoroda family is labeled to take part in soft tissue that is still fresh in the same body.

DNA samples were obtained from blood spots that would be used as a comparison material, and the larvae of the order were listed as the Calliphoroda family that grew in the soft tissue of the decomposed corpse. A sampling of research from the corpse has received permission from Forensic Medicine and Medicolegal Department/ Soetomo Teaching Hospital and thi ethical clearance permit from the ethics committee at Soetomo Teaching Hospital Surabaya, Indonesia. The variables of this study consisted of the independent variable (bodies that have undergone decay, fly larvae, larval exposure system) and the dependent variable (DNA degradation (core DNA) and DNA transfer to larvae).

Samples taken were larvae obtained from corpse soft tissue (postmortem) that had not been decomposed and decomposed, adult larvae of the order flies were put on the Calliphoridae family that were exposed to the corpse or soft tissue used as media (breeding) larvae. Sampling was conducted in the mortuary of the Soetomo Teaching Hospital Forensic and Medicolegal Department and sample examinations were carried out at the Human Genetic Study Group Laboratory, Institute of Tropical Disease (ITD) Universitas Airlangga.

Result

DNA Levels and Purity Measurement

DNA levels obtained in the skin and digestive tract of larvae were respectively: 0.2181 µg / ml and 0.0870 µg / ml, and the purity of DNA was 1.34 µg / ml and 1.02 µg / ml.

Table 1: DNA Content and Purity of Larval Isolation

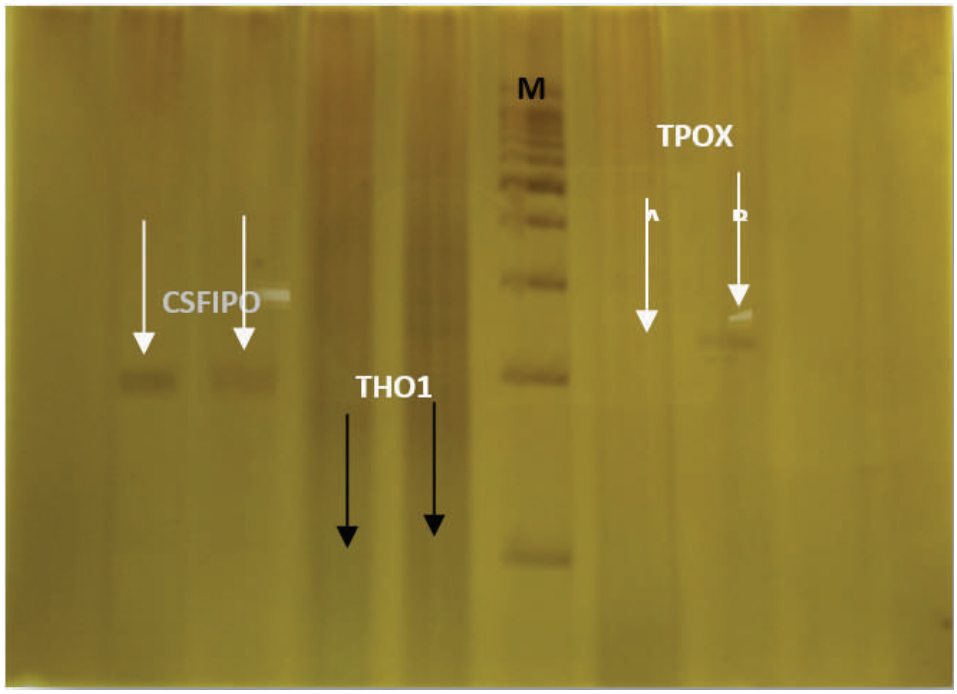
Sample	Absorbance		DNA levels (µg/ml) Absλ260x dilution x 50)	Purity of DNA (Abs λ 260 / Abs λ 280)
	λ 260	λ 280		
Fresh tissues	0.451	0.449	0.3268	1.46
Larvae skin	0.301	0,225	0.2181	1.34
Gastrointestinal tract	0.120	0.082	0.0870	1.02

DNA Visualization

After being colored, a picture of bands or ribbons from each locus will be examined with a marker tape.

Picture 1: Visualization of CSFIPO, THO1 & TPOX loci

A: Larvae skin, B: Gastrointestinal tract M: Marker ladder 100 bp



In electrophoresis visualization using polyacrylamide agarose gel composites in the form of a ribbon, it is determined whether the TH01 locus appears, by pulling the tape line from the sample towards the 100 bp marker, where the TH01 locus is between 179 - 203 bp. Then the tape is pulled towards the control (K562 marker as a positive amplication control) to determine the location of the allele whether under or over the control. The K562 marker is the allele marker of the CTT multiplex from Promega Corporation 2001. The TH01 locus of the multiplex CTT marker with alleles between 5-11 (K562 is located at alleles 9.3, 9.3). Then the larval skin bands and larvae are compared whether they are parallel (identical / consistent / matching) at the TH01 locus. Whereas the TPOX locus is between 224 to 252 basepair with allele variations ranging from 6 to 13 and CSF1PO locus is between 295 - 327 basepair, and contains T-A-G-A core sequence repeat with an allele range of 7 to 15 bp.

Table 2: Results of reading of electrophoresis visualization between larval skin and larvae with blood samples at the CSF1PO, THO1, and TPOX

No	Sample		THO1 Locus	TPOX Locus	CSF1PO Locus
1	1	Skin larvae	Identical	Identical	Identical
		Blood			
2	2	Gastrointestinal larvae	Identical	Identical	Identical
		Blood			

Discussion

DNA levels and purity are important stages in the PCR process as we know that the purity of DNA samples will affect amplification. The purity of DNA depends very much on the quality of the DNA samples taken, DNA purity 1-2 (ideally 1.8-2)[11]. DNA levels are an important factor in forensic DNA testing, which influences the success of STR and PCR on DNA samples. Decreasing levels up to 1 µg has the potential to decrease the STR's detection ability by 95%[12]. For obtaining adequate visualization results it requires adequate DNA purity and adequate DNA levels so that it can be used as a DNA examination material[13]. The amount of DNA needed in forensic DNA analysis varies depending on the needs and type of examination. The STR only requires a minimum DNA concentration of 1-25 µg. In addition to depending on DNA levels, the examination material also requires sufficient DNA quality that is DNA that is used in degraded conditions to a minimum[14]. If DNA is severely degraded, it will cause the primer to not be able to stick to the target DNA that will be duplicated[15].

To eliminate contamination from the outside attached to the skin of larvae, then to find out the transfer of DNA in the digestion of larvae, the skin of the larvae is rinsed with distilled water twice and sonified[16]. In this study using larvae as personal identification material, the results of this study found that larvae can be used as an alternative material in personal identification. The existence of the method of amplification of Polymerase Chain Reaction (PCR) with minimal DNA levels can be used typing checks. From various studies on the use of PCR in forensic medicine in amplifying target DNA, it is reported that the PCR technique has a very high success rate, because it requires relatively little DNA and relatively low 'freshness' levels compared to Restriction Fragment Length Polymorphisms (RFLP) techniques. The use of the TH01, TPOX and CSF1PO loci given that they were one of the first loci developed by the Forensic Science Service, and they have a probability of matching with a ratio of 1 in 50 million.

The results of the lottery or visualization of DNA eaten by larvae of members of the order listed Calliphoridae family were assessed (suitable) both on the skin and on the digestive system at the loci TH01, CSF1PO, and TPOX. The ribbon images in agarose determined at each locus differ in the thickness of the ribbon, this is related to several factors, according to the quality of the sample, the method of selecting larvae,

the technique of making extraction[20]. The results of the visualization or summary of DNA bands in agarose also show a difference, in which there is more band larvae than in the digestive tract. This is because in the stomach (plant) larvae of the fly, temporary liquid food and primary digestion do not occur in the stomach because proteolytic enzymes are not excreted in this area.

The results of this study can identify the three loci selected because they are part of the 13 STR loci that are used as a CODIS (Combined DNA Index System) data base identification system, and are the first loci available. These three loci rarely experience mutations because of the possibility of mutations of 1: 500, in the caucasian population these three loci have the least discrimination[1,12]. Thus it can be seen that the isolation of DNA from the larvae of members of the order family called the Calliphoridae family can still be an alternative material in forensic identification. Generally, DNA isolation from the larval digestive tract gets DNA levels or quantities low or even less, but DNA bands are still visible on the results of electrophoresis visualization. From these low levels, the identification test through the digestive tract of larvae has a low effectiveness value when compared with larval skin samples. However, DNA in the digestive tract can still be an alternative material for examination.

Conclusion

After analysis, the transfer of corpse's DNA to the larvae of members of the order is called the Calliphorideae family where it can be directly contacted by decaying results into the skin of the larvae or through the digestive tract.

Conflict of Interest: None

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Children Death with Child Abuse: A Case Report at Bhayangkara Hospital, Kediri Indonesia

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Abstract

Background: Child abuse has negative impact for children including emotional performance, mental dysfunction, and decrease intelligence.

Case report: A boy went to the emergency room at Bhayangkara Kediri Hospital. There was swelling on the left head, expanding on the forehead and, bruising on the left ear. Also found signs of sodomy marks in the form of abrasions around the anus. There are also bruises on the back and neck of the end.

Discussion: For a definitive diagnosis, the results of the CT scan were that the patient obtained extensive cerebral contusion and caused the deviation of the midline structure to the right as far as ± 0.9 cm with subgaleal hematoma and the dilation of cerebral blood vessels.

Conclusion: The body's exact death is expected to blunt force in the head, resulting in a skull fracture and bleeding of the brain.

Keywords*: children abuse, violence, fracture skull*

Introduction

The number of acts in children abuse is increasing every year. This can be seen from the data reported by KPAI (Indonesian Child Protection Commission) that from 2010 to 2014, there were 21,869,797 cases spread throughout the province. 42-58% of violations of children's rights are sexual crimes against children, and the rest are cases of physical violence and child neglect[1,2].

Acts of children abuse can occur in various forms and range from physically seen to unknowingly violent forms of treatment. In general, acts of violence can be grouped into three categories: physical, verbal, and emotional violence or psychological maltreatment. Acts of physical violence include pinching, tweaking, touching, touching or holding (with the intention of sexual harassment), beating, strangling, elbowing, punching, kicking, biting, clawing, and spitting on a bullied child, pushing it to a painful, destructive position and destroy, or seize the property of the oppressed child. Verbal violence includes nicknames, reproaches, slanders, destructive criticisms, insults (whether personal, group or racial), statements about sexual harassment, terror, letters, e-mails, intimidating texts, and allegations not true, cruel and false gossip, and others. Emotional violence (neglect) includes the treatment of alienating or rejecting a friend or even damaging a friendly relationship, systematically weakening the victim's self-esteem through neglect, exclusion, exclusion or avoidance, hidden attitudes such as aggressive views (glares), eye glances, eyebrow movements, head nods, sighs, shaking shoulders, sneers, mocking laughter and rough body language[3,4]. Child abuse harms adult emotional performance, presents signs of anxiety dysfunction, reduces intelligence and memory, risks social delay, loss of empathy, and avoids social involvement[5]. The case report is to report one case of children abuse at East Java, Indonesia.

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Case Report

A boy went to the emergency room at Bhayangkara Kediri Hospital on June 27, 2016, at 13.16 with complaints of unconsciousness. Previously the patient was found in a cellphone shop in an unconscious state. From the witness' testimony, the patient previously played at his uncle's house (Mr. S) with his 8-year-old brother. When it came to the emergency room, there was swelling on the left head measuring 10cm x 20cm widening to the back, expanding on the forehead measuring 5cm x 3cm, bruising on the left ear. Also found signs of sodomy marks in the form of abrasions around the anus. There are also bruises on the back and neck of the end. According to the description of the patient's grandmother, abrasions on the victim's anus had existed since one week before the incident.

Examination Result

External Inspection:

1. The body of a boy is between one and five years old, body length eighty-seven centimeters, brown skin color, and adequate nutritional status.

2. The body was wrapped in three sheets of green linen and one purple sheet.

3. The remains of the body: the veil attached to the left side covers the face.

4. Body labeled, not sealed, the body's identity on the label matches the personality of the body on the SPVR.

5. Found bruised corpses in the back area, found corpse nails in all joints. No signs of further decay were found.

6. Head: asymmetrical shape, straight black hair, hair length of an average of one centimeter. Bluish red bruises were found on the left head of twelve centimeters and nine centimeters, reddish-colored blisters on the left side of the head shaped like a line.

7. Forehead: found several bruises, round shape, purplish blue, an average size of one centimeter

8. Eyes: Right: bleeding spots on the upper and lower eyelid mucous membranes. Left: no bleeding spots found on the upper and lower eyelid mucous membranes, white eyeball and brown rainbow mucous membrane.

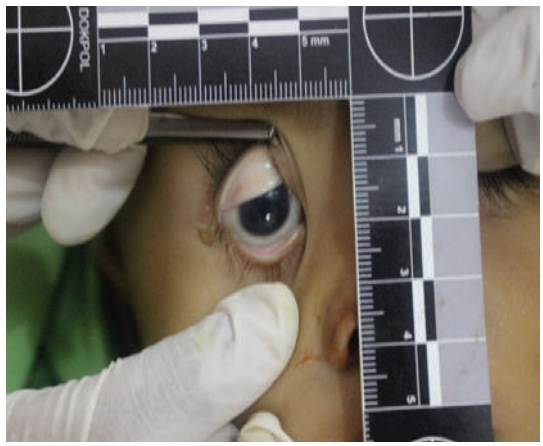


Figure 1: Mucous membrane of the upper eyelid pales

9. Ears: no abnormalities and signs of violence were found.

10. Mouth: pale lips and lower mucous membranes

11. Chin: no abnormalities and signs of congestion were found with red blisters of zero point, five centimeters times zero location, and five centimeters.

12. Teeth: right and left upper jaw: back three molars have not yet grown. Furthermore, right and left lower jaw: back three molars have not yet grown.

13. Neck: found extended bruises on the back horizontally, purplish blue, located five centimeters below the hairline, measuring eight centimeters times one-half centimeter.

14. Chest: no abnormalities or signs of violence were found.

15. Stomach: bruises found, greenish-blue, located five centimeters to the left of the midline of the body and one centimeter above the left intestinal bone, measuring one and a half centimeters by one centimeter.

16. Back: bruises found in the upper right back, greenish-blue, located three centimeters to the right of the midline and four centimeters below the right shoulder, five centimeters, and four centimeters in diameter. The first bruise was found in the middle back area, purplish blue, three centimeters by two and a half centimeters in size. A second bruise was found in the middle back

area, purplish blue, measuring ten centimeters by nine centimeters.

17. Upper limb: Right: Pale fingernails, pale palms, bruises found on the back of the hand, bluish-red, located one centimeter below the wrist, measuring one and a half centimeter and one and a half centimeters, found in brownish-black abrasions on the elbow area, two centimeters and one centimeter in size. Left: Pale fingernails, bruised bruises found in the elbow field, a purplish-blue, three centimeters, and two centimeters in size.

18. Right and left lower limbs: pale fingernails, bruised bruises found on the left instep region, round, purplish in blue, measuring two centimeters and one centimeter.

19. Genitals: Male sex is not circumcised. No signs of violence were found.

20. Anal: the shaped funnel, irregular edges, with a diameter of seven centimeters, found open wound uneven edges, blunt angles, muscle base, and reddish color at six o'clock.

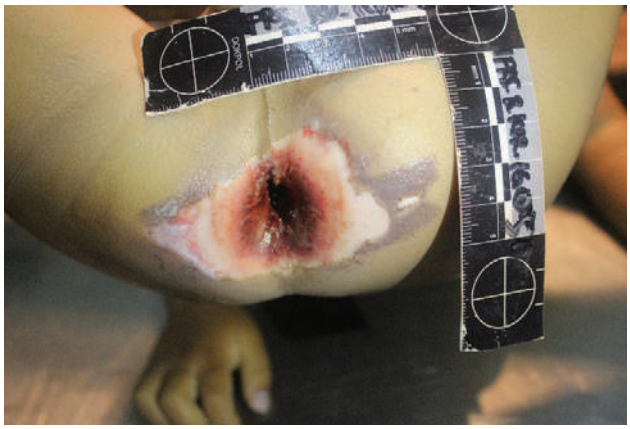


Figure 2: Abrasions around the red anus

Internal Inspection:

1. Chest cavity: symmetrical shape, thick chest fat zero point five centimeters. Found in red chest muscles with thick chest muscles zero point three centimeters. No blood absorption was found in the subcutaneous tissue.

a. There is no abnormality and signs of violence in the sternum and ribs

b. Fill the chest cavity: Right: The lungs fill the chest cavity. Left: deflated lungs.

c. Heart: No fluid is found in the peritoneum, at the solid touch of springy fifty grams, brownish-red. Heart length of eight centimeters, the width of six centimeters, no attachment was found between the heart sacs with the right and left lungs. An incision in the main artery of the heart found one hundred percent open.

d. Lung: Right: lung color brownish red, palpable palpation of air, blunt edges, smooth surface. Pulmonary weight of fifty grams. Left: Reddish-brown deflated lung color, attached to the back of the chest cavity, no fluid, palpable palpation of sharp edge air, smooth surface. One hundred gram lung weight.

2. Stomach: The subcutaneous tissue of the stomach: found thick belly fat three centimeters. No liquid was found. In the abdominal cavity, the intestinal curtain is brownish yellow. Fill the abdominal cavity: there is a blood absorption in the inner abdominal wall measuring twice one centimeter, one centimeter left of the spine, and is a continuation of a bruise on the left-back.

a. Heart: brownish red, flat surface, touch chewy, sharp edges. Weight of three hundred and seventy-five grams. Measuring eighteen by fifteen centimeters. In the incision, not found abnormalities.

b. Spleen: not found abnormalities.

c. Stomach: filled with liquid chocolate, smooth mucous membranes.

d. Intestine and umbai worm: no abnormalities were found.

e. Kidney: Right: not found abnormalities. Left: There is blood absorption in the muscles (spinal cord just below the left kidney).

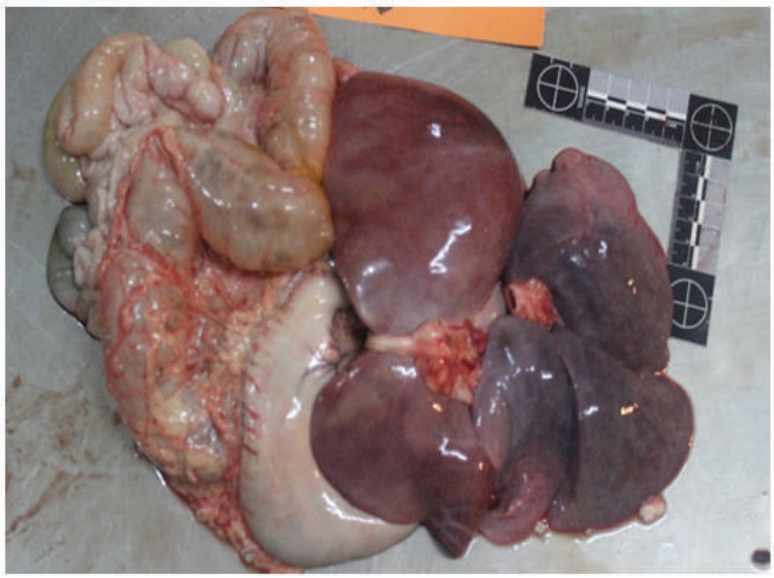


Figure 3: Internal Inspection

3. Head :

a. Under-scalp tissue: there is blood absorption in the inner skin measuring fourteen centimeters long, twelve centimeters wide.

b. Skull: Roof: on the left side of the head there are two skull fracture lines, the first fracture measuring seven centimeters extending from top to bottom is located eight centimeters above the left ear hole, the second fracture is situated to the left of the first fracture in a horizontal direction from left to right measuring fourteen centimeters is located below the tip of the first fault. Found an inverted U-shaped skull roof fracture located at the lower end of the first fracture. Bone fracture inside. Baseline: no abnormalities and signs of violence were found.

c. Brain: It was found bleeding and blood clots between the thick membrane and brain tissue in the left brain measuring an area of eight centimetres by six centimetres with a weight of ten grams. There is bleeding in the spider membrane eight centimeters by four centimeters. At the incision of the cerebellum, there were no signs of abnormality or violence. There is a widening of the bulge in the left brain.

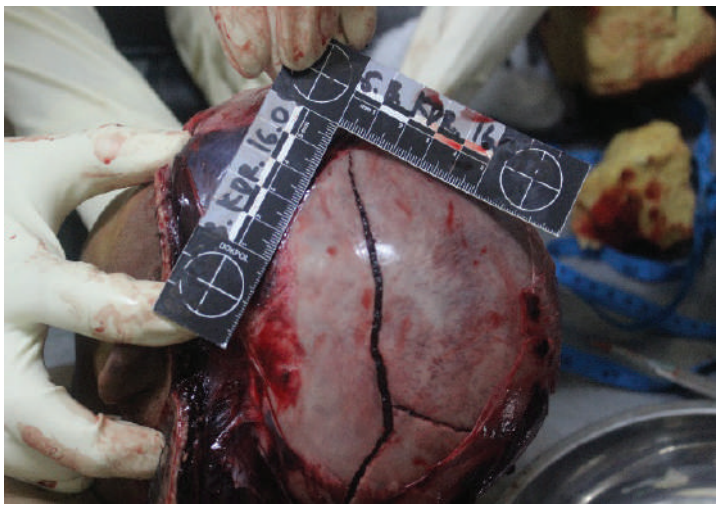


Figure 4: Skull Fracture Lines

4. Neck :

No signs of violence were found, no abnormalities were found on the incision. Including issues under the neck skin, neck muscles, thyroid gland, tongue bone, large neck blood vessels, tongue, and throat.

Discussion

One of the roles of forensic medicine is in the form of making a visum et repertum at the request of the investigator, the police, which is useful in assisting law enforcement in a crime involving human life. Visum et repertum is made based on medical examinations carried out on living victims, dead victims, or material that is thought to originate from the human body[6,7]. For a definitive diagnosis of patient, it can be seen from the results of the CT scan where the CT scan results of the patient obtained extensive cerebral contusion in the frontal D / S lobe, the entire parietal S lobe, and part of the D parietal lobe, temporal lobe, and S occipitalis, pressing the ventricle lateral to the right and causes the deviation of the midline structure to the right as far as ± 0.9 cm, cerebral edema, os parietal D fracture, os frontalis S, dorsum sella with subgaleal hematoma of the right fronto-parieto-occipital region and from the results of an in-depth examination of the brain found dilation of cerebral blood vessels. It was found bleeding and blood clots between the thick membrane and brain tissue in the left brain measuring an area of eight centimeters by six centimeters thick with a ten-gram diameter. There is bleeding in the spider membrane eight centimeters by four centimeters[7–9].

On an in-depth examination found in the tissue under the scalp, there is blood absorption in the inner skin measuring fourteen centimeters long, twelve centimeters wide. Upon checking in the brain, it was found that the cerebral blood vessels were widening, and bleeding and blood clots between the thick membranes and the brain tissue in the left brain were large, eight centimeters by six centimeters, with ten grams of a beret. There is bleeding in the spider membrane eight centimeters by four centimeters. The weight of the cerebrum is one thousand grams, and the cerebellum is one hundred grams. At the incision of the cerebellum, there are no signs of abnormality or violence. There is a widening of the bulge in the left brain[10].

In subdural hematomas, this condition arises after severe head trauma, such as contusional bleeding resulting in a venous rupture in the subdural space. Brain shifts in acceleration and de-acceleration can attract and sever veins. When acceleration occurs, there are two events, namely the acceleration of the skull toward the impact and shifting the brain in the direction opposite the primary impact direction. Head acceleration and brain shifts are linear. Therefore, lesions that can occur are called contusion lesions. Contusional injuries under impact are called “coup” contusion lesions where there is no compression force, so there are no lesions. If there are lesions, they are called “countercoup” contusion lesions[8].

The patient also obtained signs of sodomized scars in the form of a red wound around the diameter of seven centimeters, found uneven edge open wounds, blunt angles, muscular base, reddish color in the direction of the six-hour anus and the results of the external examination post-mortem obtained a funnel-shaped rectum. The reddish color indicates a sign of inflammation, which means the wound is still new. Found abrasions around the anus and burns with bullets that have broken the base of the epidermis[9,11]. It is suspected that the patient experienced sexual violence. From the results of calculations based on the process of the occurrence of rigid corpses, the estimated time of death of the patient is on June 28, 2016, at 01:00 to 13:00. Because the patient’s death is unreasonable, the manner of death is caused by blunt force violence seen from the type of wound found on the external examination of the patient’s body in the form of bruises and blisters. The mechanism of death due to a brain hemorrhage that causes brain volume to increase so that intracranial pressure increases and herniation occurs, which creates a decrease in consciousness. If the brain volume continues to increase, the brain cannot compensate anymore, causing brain blood flow to decrease, tissue hypoxia occurs, and cause death[11]. Wounds obtained in the anus of the patient are new wounds marked with a reddish color, can be caused by the hardness of blunt objects or objects that can cause burns, injuries can cause bleeding, but seen from the extent of the wound it is not a cause of death[12]. Violence against children is an intentional act that causes harm or harm to children (both physically and emotionally). This act is carried out using force, threats, bribes, deception, and even pressure. Activities of sexual

violence against children do not involve physical contact between the perpetrator and the child as a victim. These forms of sexual violence can be in the form of rape or sexual abuse[1,13].

Conclusion

The boy body's exact death is expected to blunt force in the head, resulting in a skull fracture and bleeding under the lining of the brain. It occurred after he got physical and sexual assault.

Conflict of Interest: None

Source of Funding: The research was self-funded

Ethical Clearance: Taken from ethic committee of Bhayangkara Hospital, Kediri.

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Effecttiveness of WhatsApp Group on Breastfeeding Practices

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Abstract

Background: Breastmilk is the ideal food for newborn, but in the first days of birth are vulnerable for babies to be given prelactal food by mother and families. The Successful of exclusive breastfeeding related to various factors including media exposure. Mothers who are exposed to the media have more opportunities to give exclusive breastfeeding. WhatsApp in a new trend in communication method for giving information to the public.

Objective: This study aims to determine the Effectiveness of WhatsApp Group Towards Pregnant woman in The Practice of First Week Breastfeeding in Palangkaraya

Methods: This study is a quasi-experimental study with posttest only with control group design. Purposive sampling technique was used in this study, consisted 36 respondent who were divided into two groups. Analysis were performed by chi-square test.

Result: The results showed the average age of respondents was 26.94 years. Most of the respondents were housewives or unemployed (63.9%), had high and middle education (88.9%) and had high family income (77.7%). Based on statistical test results, WhatsApp Group is effectively used to improve breastfeeding behavior of mothers in the first week after birth ($p < 0.05$).

Conclusion: Health workers are expected to be able to use WhatsApp Group as a health education media on breastfeeding to increase the scope of exclusive breast milk.

Keywords *: Breastmilk, Exclusive breastfeeding; Pregnant woman;WhatsApp Group*

Introduction

Breastmilk is the ideal food for newborn. Breastmilk is safe, clean and contains antibodies that can protect children from many common illness. Exclusive breastfeeding is given breastmilk to the baby from birth until six months, without adding and/or replacing with other food or drinks (except drugs, vitamins, and minerals). Globally, 3 of 5 babies are not breastfed in the first hour of their life and nearly 2 of 3 infant not exclusively breastfed. [1]

In Indonesia, the babies who had received exclusive breast milk in 2016 was 29.5% of 80% exclusive breast milk targets.[2] In 2017, exclusive breast milk coverage was 61.33%. This coverage has exceeded the strategic plan target of exclusive breast milk that were 44% in 2017. [3] Exclusive breast milk coverages in Central Kalimantan Province in 2017 is only 11.1%.[4] This coverage has decreased by almost 50% when compared to the 2016 coverage, and making Central Kalimantan Province one of the three provinces that did not reach the 2016 exclusive breast milk target. This decline occurred for two consecutive years after the previously exclusive breast milk coverage in 2015 were 27.58%.[5] Menteng Health Center is one of the health centers in Palangka Raya City with the second lowest exclusive breast milk achievement in 2017 with. The coverage is just 6,3% in 2017, this number is decreased from 2016, which was 9.5%. This coverage is under the exclusive breast milk coverage of Palangka Raya city.[6]

In the first days of birth, breast milk is usually not released. This period is a vulnerable period for babies to be given prelactal feeds by the mother, families and health workers. Prelactal feeding is a barrier for exclusive breastfeeding and increases neonatal morbidity and mortality. [7,8] Exclusive breastfeeding is also associated

to various factors such as education, employment, mother's knowledge, mother's attitude, the role of the officer, the role of the husband, the role of parents and media exposure.[9–13] Mothers who are exposed to the media have 9.64 times the opportunity to give exclusive breastfeeding. [14] Social media group also influence breastfeeding behavior as well as to longer duration of breastfeeding.[9]

Social media is a new trends in terms of increasingly intensive means of communication in utilizing technological advancements. All fields are indirectly forced to follow this trend if they do not want to be left behind, especially in the health sector which is mostly related to communication or giving information to the general public. [15] Previous studies identified different effect of social media use by patient for health related reasons. Social media can serve as an aid to patient.[16] Whatsapp is one of the social media under Facebook Inc., which was introduced in 2010. Some literature has published about Whatsapp Utilization in the health field.[17]

This study aims to determine the effectiveness of WhatsApp Group in Pregnant Women Toward Breastfeeding Practices in the First Week in Menteng Health Center, Palangka Raya.

Method

This is quasi-experimental study with post test only design with nonequivalent group.[18] This research was conducted in March-September 2019 at Menteng Health Center in Palangka Raya. The Population in this study were all pregnant women in third trimester who performed antenatal care at the Menteng Public Health Center that met the inclusion and exclusion criteria. Purposive Sampling Technique was used in this study. Thirty Six pregnant woman were divided into two groups: the intervention group and the control group.

The inclusion criteria in this study are pregnant women who live in the Menteng Health Center area, have smartphones, do not have severe comorbidities during pregnancy, have no complications during pregnancy and are willing to participate in research by filling out informed consent. The exclusion criterion in this study was to have complications during delivery and stillbirths.

In the Intervention Group, respondents were given health education through WhatsApp Group while the control group was given through leaflets. After providing health education to both groups, respondents were followed up to seven days after birth to find out about the respondent's breastfeeding behavior. The instruments used in this study were the filling sheet and observation. Data analysis was performed univariately to find out the characteristics of respondents and bivariate with chi square test to find out the Effectiveness of WhatsApp Group ASI.

Results

From total 36 respondents, the ages of respondents in Intervention group, maternal ages (Mean ± SD) was 28,44 ± 4, 718, and in control group was 25,44 ± 3,959.

Table 1. Ages of respondents

Variable	n	Mean	Min-Max	SD
Age				
Control Group	18	25,44	21-38	3,959
Intervention Group	18	28,44	18-34	4,718

Table 2. Mothers Education, Mothers Occupation and Family Income

No	Variable	Group				Total	
		Control		Intervention			
		n	%	n	%	n	%
1.	Mothers Education						
a.	Primary	3	8,3	1	2,8	4	11,1
b.	Middle and Higher	15	41,7	17	47,2	32	88,9
2.	Mother's Occupation						
a.	Unempoyed	12	33,3	11	30,6	23	63,9
b.	Employed	6	16,7	7	19,4	13	36,1
3.	Family Income						
a.	<UMR	6	16,7	2	5,6	8	22,3
b.	≥UMR	12	33,3	16	44,4	28	77,7

Table 2 showed that most of respondents both in the control and intervention groups were had midlle and higher education level, unemployed and have good family income

Table 3. Practice of First Week Breastfeeding

Group	First Week Breastfeeding				p value	OR	CI (95%)
	Non Exclusive		Exclusive				
	n	%	n	%			
Control	13	36,1	5	13,9	0,019	5,200	1,253 – 21,572
Intervention	6	16,7	12	33,3			

Table 3 showed that were differences in mothers practices of first week breastfeeding (p< 0,05). Mothers from intervention group had 5,2 times greater chance to breastfeed their babies.

Discussion

Table 1 showed that both in intervention and control group, maternal ages mostly on 20-35 years old. There is no significant differences in maternal ages in both grup. Previous study mentioned that older mothers had a lower likelihood of breastfeeding their babies than younger mothers.[19] But in other studies found that there is no relationship between mothers age and exclusive breastfeeding practice. [10,11] At the education level, the results of the study showed that most respondents in both groups had middle and higher education levels. High levels of education should have a positive relationship with breastfeeding because education can influence knowledge. Mothers who have higher education would probably breastfeed their babies exclusively.[20] However, several studies mentions that maternal education is not related to exclusive breastfeeding and related to failure of exclusive breastfeeding. [10,11,13]

Most of the mothers in both the intervention and control groups were housewives or unemployed. Work is one of the triggering factors that is often considered to prevent mothers from giving breast milk. Mothers who work find it difficult to give maximum milk to the baby, for various reasons such as the child does not want to suckle, breast milk is reduced, there are no nursing room facilities at work, and so forth. Raheel and Tharkarin their research concluded that working mothers who have information about the benefits of breastfeeding are lacking which is an obstacle to breastfeeding.[21] Another study by Al-Ruzaihan et al states that the mother's work is not a barrier for mothers to breastfeed but it will only affect the duration and frequency of breastfeeding every day. [22]

The results also showed that most mothers had family incomes above the UMR Central Kalimanta Province both in the intervention group and the control group. Based on the literature, income does not significantly influence the breastfeeding process.[23,24]

Effectiveness of Whatsapp Group in the Practice of First Week Breastfeeding

Breastfeeding is an ideal way of feeding babies for optimals growth and development. Breastfeeding also has many health benefits for mother's health.[25,26] However, lack of guidance and support can result in women often stop breastfeeding in the early weeks when they face or see problems related to breastfeeding.[27] In this study, mothers who joined the WhatsApp Group (Intervention Group) gave more exclusive breastfeeding in the first week to their babies than mothers in the control group. It can be concluded that WhatsApp Group as a medium of health education and communication as well as breastfeeding counseling that were effective in improving the behavior of the mother to breastfeed her baby exclusively in the first week of baby's life.

This study found that mothers who failed to give breast milk exclusively in the first week gave formula milk and some food like bananas to stop the baby crying because they thought the babies were hungry. These results are in line with research on the administration of prelactal intake as a failure factor for exclusive breast milk. Their research states that giving prelactal intake is given by the mother on the grounds of the baby's thirst, so that the baby stops crying and breastfeeding has not come out but the mother does not know the adverse effects of giving prelactal care and the respondent is not aware if the intake of food is a failure factor in exclusive breastfeeding. The types of intake provided are coconut water, starch, honey, formula milk and bananas.[28]

Another study in the results of his study said that most babies (72%) get food / drink other than breast milk in the age range of 0-7 days. This is due to breast milk that has not come out in the early days after delivery, the habit of washing the baby's mouth with water and the suggestion of formula feeding by health workers.[29]

One obstacle to breastfeeding behavior is the lack of communication and lactation counseling services. Providing health education can increase the duration of exclusive breastfeedin.[24]

Mother's behavior in breastfeeding is the result of health knowledge, in this case, knowledge of exclusive breast milk. Currently, the method commonly used in providing health education about breastfeeding is by giving leaflets or direct counseling to patients. In the use of social media such as WhatsApp, exposure, feed back, connecting and sharing provided, it creates a big change for communication. The flow of information is no longer considered one-way but is an interactive process that allows the exchange of information at the same level between the recipient and the messenger, creating a long-lasting feedback communication process, and increasing the total involvement of the recipient of the message. In addition, the effectiveness of social media that is able to reach thousands and even millions of targets in a short time makes this media a new prima donna for every health promoter.[15]

Before using WhatsApp, research on the use of social media as a support group for breastfeeding was also carried out by Robinson et al. The results show that the use of Facebook as social support for nursing mothers is significantly associated with an increase in the duration of breastfeeding.

Research on the use of WhatsApp in health services was also conducted by Dewi and obtained results that are in line with this study. The study states that health promotion and the use of WhatsApp reminders can increase self efficacy in adherence to taking Fe tablets in pregnant women.[30]

Conclusions and Recommendation

WhatsApp Group is considered effective in improving breastfeeding behavior in the first week of baby's birth. Health workers are expected to be able to use WhatsApp Group as a health education media on breastfeeding to increase the scope of exclusive breast milk and try to use another media social.

Confict of Interest : Nil

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The Effect of the Blood Cupping Therapy on High Density Lipoprotein (HDL) and Low Density Lipoprotein (LDL) in Hypercholesterol Patients

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Abstract

Introduction: Hypercholesterol is a high cholesterol level in the blood. Blood cupping therapy is a technique of excreting metabolic waste in the blood through the skin. The study aimed to measure any effect of blood cupping therapy to HDL and LDL cholesterol. **Method**: It was a Quasi-Experimental study using humans as research subjects. Non random consecutive sampling. Patiens with hypercholesterol after 12 hours of fasting were treated with blood cupping, 9 points. Research subjects were 51 men divided into three groups, the cupping group only consisted of 17 men, the drug group without cupping consisted of 17 men, and the cupping and drug consisted 17 men. Collecting data from third of the groups as pre and post data. The data were analyzed by Mann Whitney, Wilcoxon and Kruskal Wallis. The research took place at the Laboratory of Biochemistry, Universitas Jember. **Results**: Measurement of HDL cholesterol on the cupping group was pretest=33.03±3.73; posttest=37.58±6.54; p=0.000. The drug group was pretest=33.40±3.18; posttest=33.56±3.50; p=0.788. The cupping and drug group was pretest=34.32±3.38; posttest=37.61±2.01; p=0.002. Kruskal Wallis test on pretest group was 0.534 and on posttest group was 0.002. Measurement of LDL cholesterol on the cupping group was pretest=154.70±39.68; posttest=123.89±41.86; p=0.000. The drug group was pretest=151.24±44.17; posttest=151.24±44.17; p=0.019. The cupping and drug group was pretest=147.48±62.66; posttest=105.57±57.94; p=0.001. Kruskal Wallis test on pretest group was 0.439 and on posttest group was 0.082. This means that there was no difference in the average pretest and posttest LDL levels in the cupping, drug, cupping and drug groups. Conclusion: The intervention of blood cupping therapy can increase HDL cholesterol level, and reduce LDL cholesterol level. Further research needs to be done to measure the potential prevention of atherosclerosis.

***Keywords**: blood cupping; HDL; LDL; cholesterol*

Introduction

Blood cupping therapy or the wet cupping therapy has been used for medication since ancient times. Even Hipocrates used blood cupping in cases of internal drug.[(1)] The long history of blood cupping therapy proves that the blood cupping therapy when performed correctly is a safe and effective method.

There is a misperception in interpreting blood cupping therapy. The depth of the needle into the skin is only 0.05mm. Injuries with such needles do not cause blood flow. Blood comes out after being pulled with a pump of negative strength -200 mmHg.[(2,3)] Actually, blood cupping therapy is not a treatment for removing blood circulation but for removing metabolic waste or

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the so-called causative pathological substances.[4] In other words, blood cupping therapy does not reduce circulating blood volume. The bloodlike substance that comes out of the injury is cholesterol metabolism wastes, old erythrocytes, etc. The blood amount due to the conduct of a correctly performed blood cupping therapy does not reduce hemoglobin.[5]

We have understood that high levels of total blood cholesterol, especially in the form of LDL cholesterol, are a major risk factor for developing coronary heart diseases.[6] Some patients who receive LDL cholesterol reduction therapy still have complaints of coronary heart disease.[7] The aim of the study was to measure effect of blood cupping therapy to HDL and LDL cholesterol. The existence of HDL cholesterol in the reserve cholesterol transport is a mechanism to protect endothelium against the risk of atherosclerosis. HDL has anti-inflammatory, antioxidant, antithrombotic properties. It is also antiaterogenic.[8]

Method

It was a Quasi-Experimental study using humans as research subjects. Non random consecutive sampling. Patiens with hypercholesterol after 12 hours of fasting were treated with blood cupping, 9 points. Research subjects were 51 men divided into three groups, the cupping group only consisted of 17 men, the drug group without cupping consisted of 17 men, and the cupping and drug consisted 17 men. Collecting data from third of the groups as pretest and posttest. The research subjects were selected based on sample inclusion criteria, such as being aged between 45-55 years old, and having total cholesterol> 160mg/dl. After fasting for 12 hours and still consuming statin anti-cholesterol drugs, blood is taken through the cubiti vein as much as 5ml. To measure the HDL and LDL cholesterol, the enzymatic colourimetry method, diasys reagent, Biolyzer100 spectrophotometric device, in units of mg/dl were implemented. Data analysis was performed with the Wilcoxon, Mann Whitney, Kruskall wallis test with a significance level of 5%, in which the data were pretest-posttest and compared fom thid groups. The research was carried out at the Laboratory of Biochemistry, Universitas Jember.

Findings and Discussion

Table 1. Result of analysis HDL level on the cupping, drug, cupping and drug group

Groups	n	Levels HDL Mean±SD Median ± IQR (Min-Max)		p
		Pretest	Posttest	
Cupping	17	33.03 ± 3.73 32.49 ± 5.54 (27.81-39.97)	37.58 ± 6.54 35.64 ± 4.34ab (31.59-55.35)	0.000
Drug	17	33.40 ± 3.18 32.67 ± 5.26 (29.27-39.96)	33.56 ± 3.50 34.56 ± 5.13a (25.92-38.61)	0.788
Cupping and drug	17	34.32 ± 3.38 34.84 ± 5.20 (26.19-39.16)	37.61 ± 2.01 37.57 ± 3.25b (35.11-42.17)	0.002
p		0,534	0.002	

Add : * significan on a = 0,05

IQR = Inter Quartile Range

Measurement of HDL cholesterol on the cupping group was pretest= 33.03±3.73; posttest=37.58±6.54; p=0.000. The drug group was pretest=33.40±3.18; posttest=33.56±3.50; p=0.788. The cupping and drug group was pretest=34.32±3.38; posttest 37.61±2.01; p=0.002. Kruskal Wallis test on pretest group was 0.534 and on posttest group was 0.002.

This data shows an average increase in HDL levels from 33.03 to 37.58. There was an increase of 4.55 point (13.8%).

Measurement of LDL on the cupping group was pretest=154.70±39.68; posttest=123.89±41.86; p=0.000. The drug group was pretest=151.24±44.17; posttest=151.24±44.17; p=0.019. The cupping and drug group was pretest=147.48±62.66; posttest=105.57±57.94 ; p=0.001. Kruskal Wallis test on pretest was 0.439 and on posttest was 0.082. This means that there was no difference in the average pretest and posttest LDL levels in the cupping, drug, cupping and drug groups.

Table 2. Result of analysis LDL Level on the cupping, drug, cupping and drug group

Groups	n	LDL level Mean±SD Median ± IQR (Min-Maks)		p
		Pretest	Posttest	
Cupping	17	154.70 ± 39.68 151.03 ± 79.77 (94.34-214.92)	123.89 ± 41.86 116.45 ± 81.13 (67.45-193.18)	0.000
Drug	17	151.24 ± 44.17 138.34 ± 40.19 (112.16-284.03)	136.40 ± 42.12 130.71 ± 42.40 (83.08-264.89)	0.019
Cupping and Drug	17	147.48 ± 62.66 136.96 ± 28.65 (74.84-337.66)	105.57 ± 57.94 95.41 ± 74.37 (38.08-256.47)	0.001
p		0,439	0.082	

Add : * significan on a = 0,05

IQR = Inter Quartile Range

This data shows an a decrease in LDL levels from an average of 154 to 123. There was a decrease of 31 point (20%).

The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) guidelines recommend checking non-HDL cholesterol levels containing apolipoprotein B when TG levels elevated.[9]

The main action of statin anti cholesterol drugs in reducing blood cholesterol is inhibiting HMG CoA reductase, which reduces the concentration of hepatocyte cholesterol and stimulates absorption of liver LDL cholesterol from the circulation.[10] However, there is an increasing evidence stating that statins can also have pleiotropic effects. Statins can alter the synthesis and metabolism of lipoproteins and accelerate the degradation of intracellular apolipoprotein B, resulting in a reduction in apopoprotein B secretion.[11] The Atorvastatin Comparative Cholesterol Efficacy and Safety Study

(ACCESS) in patients with hypercholesterolaemia reported reductions in plasma apopoprotein B levels following statin therapy.[12]

This measurement follows the research conducted by Saryono[13], that blood cupping therapy can reduce cholesterol levels. As a result of keratinocyte enrichment in the skin the body can experience hypoxia and induce Hipoxia Inducible Factor (HIF-1α) as a self-defense effort.[14] HIF-1α will activate macrophages in the skin which subsequently induce proinflammatory genes such as IL-1. IL-4, IL-6 and TNF-α.[3] Interleukin-6 which is secreted by macrophages acts to stimulate the body's immune response, for example after trauma or tissue damage that leads to inflammation. The release of IL-6 stimulates young macrophage cells to mature and be able to do phagocytosis more efficiently. IL-6 also stimulates monocytes to produce inflammatory cytokines that play a role in local and systemic inflammation, resulting in accelerated proliferation and differentiation of macrophages.[1]

LDL is a cholesterol source for extrahepatic tissue. When LDL is very excessive, its capture system will be saturated so that excessive LDL can be taken by macrophages. Some LDL cholesterol is captured by macrophages before being oxidized. The more LDL cholesterol levels exist in the plasma, the more cells will be captured by macrophages. Furthermore, macrophages will experience efflux and nascent HDL will approach the macrophage to take LDL cholesterol. Then, nascent HDL becomes adult HDL. After taking cholesterol free from macrophage cells, free cholesterol will be esterified to cholesterol ester by the enzyme Lechitin Cholesterol Acyl Tranferase (LCAT). So HDL here serves as an absorber of LDL cholesterol from macrophages and as a carrier of LDL cholesterol back to the liver so that cholesterol levels in plasma decrease.[7]

According to El-Sayed et al.[4], blood cupping therapy is an excretory minor surgical procedure that has medical and scientific basis for cleaning blood and interstitial space from cholesterol causative pathological substanses (CPS) as metabolic waste production. Many studies have reported that blood cupping therapy can reduce LDL cholesterol. HDL cholesterol functions as an absorber of LDL cholesterol from macrophages and as an LDL cholesterol carrier back to the liver with the help of pre-HDL.[15] Pre-HDL has a role in the reverse cholesterol transport to increase excess cholesterol efflux from peripheral tissue back to the liver to be excreted through bile. The acceleration of migration of macrophages also increases due to stimulation of IL-6.[16,17]

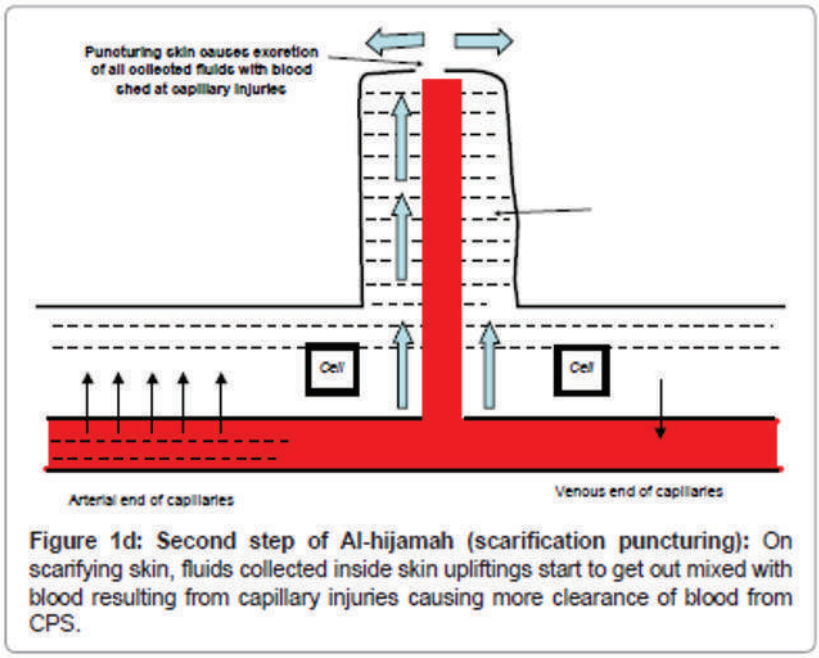
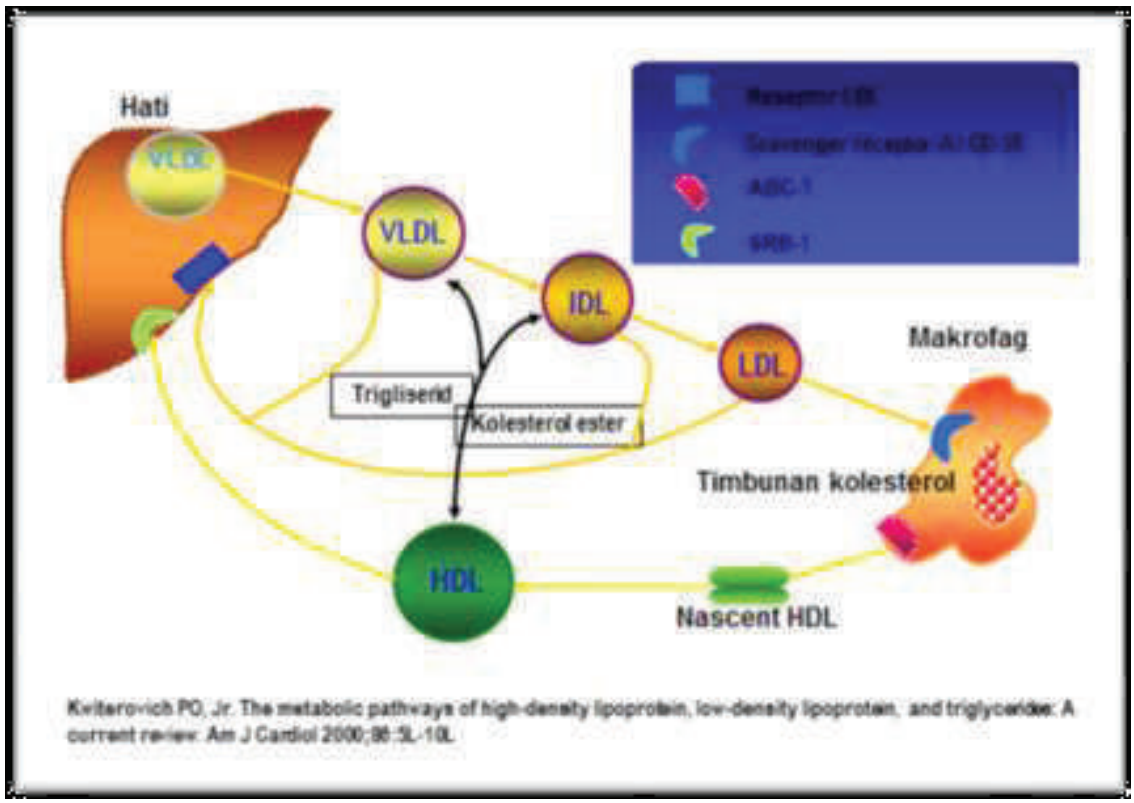


Figure 1d: Second step of Al-hijamah (scarification puncturing): On scarifying skin, fluids collected inside skin upliftings start to get out mixed with blood resulting from capillary injuries causing more clearance of blood from CPS.

Figure 1. The process of taking out metabolic waste from the blood

Source: Elsayed, 2013[4]

HDL is released as small cholesterol-poor particles containing apolipoprotein (apo) A, C, and E, and is called HDL nascent. Nascent HDL comes from the small intestine and liver, has a flat shape and contains apolipoprotein A1. Nascent HDL will approach macrophages to take cholesterol stored in macrophages. After taking cholesterol in macrophages, the nascent HDL turns into a rounded adult HDL. In order to be retrieved by nascent HDL, cholesterol (free cholesterol) on the inside of the macrophage must be brought to the surface of the macrophage cell membrane by a transporter called Adenosine Triphosphate-Binding Cassette Transorter-1 (ABC-1). After taking free cholesterol from macrophage cells, free cholesterol is esterified into cholesterol ester by the enzyme Lechitin Cholesterol Acyl Tranferase (LCAT). Furthermore, some cholesterol esters carried by HDL take two pathways. The first path is to the heart and is captured by a class B type 1 receptor known as SR-B1. The second pathway is the cholesterol esters in HDL will be exchanged with TG from VLDL and IDL with the help of Cholesterol Esters Transfer Protein (CETP). Thus the function of HDL as a cholesterol absorber from macrophages has two pathways namely direct to the liver and indirect pathways through VLDL and IDL to carry cholesterol back to the liver.



Source: Kwiterovich PO, 2000[18]

Figure 2. Reverse Cholesterot Transport Pathway

Metabolism of apolipoprotein is related to the development of atherosclerosis. Increased levels of apolipoprotein B or low levels of apolipoprotein A-I related to cardiovascular disease. Because apolipoprotein B and apolipoprotein A-I have opposite effects namely atherogenic and atheroprotective.[19]

Currently, the inflammatory mediators implicated in the pathogenesis of atherosclerosis include cytokines, chemokines, vasoactive molecules and growth factors. The anti-inflammatory effects of statins are attributed to multifaceted mechanisms including inhibition of cell cycle progression, induction of apoptosis, reduction of cyclooxygenase-2 activity and a biphasic, dose-dependent effect on angiogenesis.[1]

Cholesterol in nascent HDL is then esterified as fatty acids derived from lecithin by lecithin acyl cholesterol

transferase (LCAT) and its co-factor, apo A-1, producing spherical, mature HDL particles. Cholesterol esters in the HDL nucleus are then returned to the liver, either through the interaction of HDL with SR-B1 receptors, or transferred to lipoproteins containing B apo by cholesterol transfer proteins (CETP). (Reproduced with permission from H. Bryan Brewer Jr., personal communication, National Heart, Lung, and Blood Institute.[18]

Conclusion

The intervention of blood cupping therapy therapy has the potential to reduce levels of Apolipoprotein-B and total cholesterol in the blood. Bloodcupping therapy can be considered as an intervention that can reduce cholesterol, in addition to the use of anti-cholesterol drugs. Further research needs to be done to measure the potential prevention of atherosclerosis.

The main effect of statins decreasing the serum level of low-density lipoprotein (LDL) cholesterol, due to the inhibition of intracellular cholesterol biosynthesis. A minor effect is the decrease of serum triglycerides. Statins inhibit HMG-CoA reductase and decrease the production of mevalonate, geranyl pyrophosphate, and farnesyl pyrophosphate, and subsequent products on the way to construction of the cholesterol molecule. Thus, statins could inhibit inflammation, by inhibition of the cholesterol pathway and intracellularly interfering with Ras superfamily protein function. Mutations in transporter molecules or receptors can lead to an accumulation of cholesterol and a breakdown in the normal process of reverse cholesterol transport and cholesterol metabolism.

Ethical Clearance: The research ethical approval was obtained from the Ethics Committee of the University of Jember in Agustus 2019

Source of Funding: Authors

Conflict of Interest: No

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Impact of High Fidelity Simulation Training on the Quality of Cardiopulmonary Resuscitation Performance among Emergency Medical Services (EMS) Professionals in Pune, India

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Abstract

Introduction: The provision of high quality cardio pulmonary resuscitation (CPR) is a key modifiable factor associated with survival in cardiac arrest. Management of a patient with cardiac arrest is a dynamic and high-pressure challenge requiring critical decision making in a short period of time. Numerous studies emphasize that quality of chest compressions directly influences survival rate from cardiac arrest. Hence, improving CPR quality is of paramount importance. This can be achieved by accurate measurement of various CPR metrics and provision of real time feedback to the rescuer.

Objective: The study aims to assess impact of high fidelity simulation training on quality of CPR performance of EMS Professionals.

Methods: A high fidelity manikin was utilized to create four unique healthcare simulation scenarios based on cardiac arrest. The manikin software was capable of capturing real-time CPR data and measured parameters like chest compression depth (CCD), chest compression rate (CCR), chest recoil (CR) and chest compression fraction (CCF). The participants were initially taught the assessment and management of cardiac arrest. The participants underwent a pre-intervention test conducted on high fidelity manikin. The CPR metrics were recorded during the session. The participants were then imparted training with real time CPR feedback for a period of two months. This was followed by a post-intervention test and the CPR metrics of both tests were compared.

Discussion: The participants showed improvement in chest compression depth and chest compression rate but the chest recoil frequency and chest compression fraction decreased after the two-month intervention period. The overemphasis on achieving target depth and rate led to ignorance of the other two parameters affecting overall quality of CPR.

Conclusion: Maintaining adequate attention on all aspects of CPR metrics is a complex challenge even for trained professionals. Utilizing technology for real-time feedback is a reliable method to gauge improvement in quality of CPR during training sessions of EMS professionals.

Keywords*: Cardio Pulmonary Resuscitation(CPR), Chest compression rate (CCR), chest compression fraction (CCF), Emergency Medical Services Professionals.*

Introduction

The provision of high quality cardio pulmonary resuscitation (CPR) is a key modifiable factor associated with survival in cardiac arrest. Despite its significance, the sustained delivery of high quality CPR is not achieved in regular clinical practice.[1] Management of a patient with cardiac arrest is a dynamic and high-pressure challenge requiring critical decision making in a short period of time. A study conducted to evaluate

the impact of comprehensive effort to improve trauma care through multidisciplinary education showed that improvements in team performance using innovative technology can translate into more efficient care with fewer errors.[2] There has been an increased focus on high quality of CPR with greater emphasis on the quality of chest compressions.[3] Numerous studies emphasize that quality of chest compressions directly influences survival rate from cardiac arrest.[4-7] The various parameters in CPR associated with improved survival include greater CPR fraction, greater depth of compression, optimum compression rate and briefer peri-shock pauses.[8-12] Hence, improving CPR quality is of paramount importance. This can be achieved by accurate measurement of various CPR metrics and provision of real time feedback to the rescuer.[13-14]

Current systems used to train, assess and provide performance feedback reveal poor performance in CPR.[15-18] Very few studies have focused on improving CPR quality using high fidelity simulation and real time feedback.[19] Yet, distributed CPR training with real time feedback has shown to improve compliance to American Heart Association (AHA) guidelines of quality of CPR.[20]

Objective: To assess impact of high fidelity simulation training on quality of CPR performance among EMS Professionals.

Methods

A high fidelity manikin was utilized to create four unique healthcare simulation scenarios based on cardiac arrest i.e. Ventricular Fibrillation (VF), pulseless Ventricular Tachycardia (pVT), Asystole, Pulseless Electrical Activity (PEA). The manikin software was capable of capturing real-time CPR data and measured parameters like chest compression depth (CCD), chest compression rate (CCR), chest recoil (CR) and chest compression fraction (CCF) using sensors and potentiometer present in manikin chest. The scenarios were dry run by facilitators before the student sessions. 50 EMS professionals participated in the study. The participants were initially taught the assessment and management of cardiac arrest through didactic lecture method and case study discussions. For the pre-intervention test, the participants were divided into four groups and prebriefed on the features of high fidelity simulation manikin and the background of their respective cases. Informed consent was taken from participants to record the sessions. Each simulation session lasted for approximately 10 minutes followed by structured debriefing lasting for 20 minutes. The CPR metrics were recorded for the sessions. Over the next 8 weeks, participants were trained on various cardiac arrest scenarios using High fidelity simulation. Each simulation training session lasted for four hours and the participants were provided real time feedback on their performance on various CPR metrics. At the end of 8 week period, the participants underwent post intervention test structured in the same format as the pre-intervention test. CPR metrics were again recorded for the 4 case scenarios during the test. The pre-intervention vs post-intervention data was tabulated and analyzed for difference in means.

Results

The study focused on major four key metrics to determine the quality of CPR i.e. chest compression depth, chest compression rate, chest recoil and chest compression fraction.

The pre and post intervention values have been tabulated below:

Table 1: Mean CPR Metrics (Pre-intervention vs Post-intervention)

Parameters	Pre-intervention Means	Post-intervention Means
CCD (% of time)	55.25	61
CCR (per min)	95.77	112.50
CR (% of time)	94.75	91.25
CCF (% of time)	56.75	51.50

Discussion

Chest compressions create blood flow by increasing intrathoracic pressure and directly compressing the heart which in turn results in critical blood flow and oxygen delivery to the heart and brain. Sufficient chest compression depth (about 5cm as per AHA 2015 guidelines) is essential for effective CPR. The participants could achieve optimal chest compression depth for only 55% of time during the CPR in the pre-intervention test. This improved to 61% in the post intervention test. Although reasonable improvement was recorded, yet the values show that greater practice is required to reach optimal compression depths which can be sustained throughout the CPR process.

Apart from depth of compression, AHA 2015 guidelines recommend maintaining a steady chest compression rate of atleast 100-120/minute is crucial for the effectiveness of CPR. The participants achieved a mean rate of 95.77/min during the pre-intervention test which improved significantly to 112.50/min i.e. ideal range in the post intervention phase.

Chest compression recoil is vital during CPR as it allows for maximum possible output with each compression during CPR. Interestingly, the chest recoil was achieved for 94.75 % of the time in the pre intervention phase but it reduced to 91.25 % of the time in the post intervention phase. This finding lays emphasis on conveying the significance of complete chest recoil during training. The participants are pre – occupied with achieving target chest compression rates thereby, not fulfilling requirement of complete chest recoil during all compressions.

Chest compression fraction is the percentage of time in which chest compression are done by rescuers during a cardiac arrest. An increased CCF is independently predictive of better survival. The AHA 2015 guidelines recommend that the CCF should be maintained at atleast 60%. In the pre-intervention phase, the recorded fraction was 56.75% which surprisingly decreased to 51.50 % in post intervention phase. This could be attributed to greater time spent in checking pulse and rhythm analysis during the scenario. This could have occurred due to greater awareness of the medical conditions learnt during the intervention leading to more inquisitiveness on the cause and diagnosis during the scenario.

The study shows that greater focus and attention is paid to CCD and CCR whereas CR and CCF are ignored, thereby affecting the overall quality of CPR. A wholesome approach on all metrics during training can help tide over this problem.

Technology allows for real-time monitoring recording and feedback on CPR performance metrics. This data can be used during debriefing after resuscitation to improve the quality of CPR among EMS Professionals.

Conclusion

Maintaining adequate attention on all aspects of CPR metrics is a complex challenge even for trained professionals. Utilizing technology for real-time feedback is a reliable method to gauge improvement in quality of CPR during training sessions of EMS professionals. The study is limited by its sample size and short intervention period, yet throws light on importance of key CPR performance metrics through objective data.

Source of Funding: Self

Conflict of Interest: Nil

Ethical Clearance: Taken from IEC, SIU

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Relationship of Smartphone Usage and Academic Performance among Undergraduate students – A Systematic Review

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Abstract

Background: Smartphones have become the new normal of 21st century. With growing need to become digitally competent each individual is trying to own one smartphone or a similar gadget.

Methodology: PRISMA guidelines were used to conduct a systematic review of Literature. 98 research articles were reviewed by the team using specific key words. They were scrutinized for their eligibility to get selected for further in-depth review. Articles were selected based on the appropriateness of the titles, abstracts, and results. After a thorough check only 06 reviews were analyzed and paraphrased.

Results: smartphone addiction is the major reason for poor academic performance of the students.

Conclusion: The smartphones must be assessed for their usability and suitability to be used by the students. The phones maybe of a great help to a student if he sees learning opportunities in it.

Keywords*: smartphone usage, academic performance, Undergraduate students*

Introduction

Smartphone addiction has become a problem that needs an immediate attention. This habit initially develops as a liking and later conquers young minds.[1] Present study is an effort to understand relationship of smartphone usage and academic performance among undergraduate students.

Materials & Methods

This review is conducted by reviewing articles using the selection criteria from Pubmed, ScienceDirect, and CINAHL databases from January 2016 to August 2020. The dependent variable in this study is academic performance and independent variable is smart phone usage. Using the online search engine the data was collected. The articles were limited to full text articles. The search was conducted by using key words like smartphone, academic performance and Undergraduate students. PRISMA Guidelines were used for data analysis.

Inclusion criteria:

- Articles from Peer-reviewed journal
- Publications from January 2016 to August 2020
- Cross-sectional and Survey designs were included

The filtered articles were then reviewed by the reviewers for their content especially with the results. The Studies with irrelevant titles, unrelated abstracts and study designs were removed. Data was analyzed and results were reported using a narrative synthesis.

Result

Total 98 articles were identified by the review team using PRISMA Guidelines. A systematic review was conducted to find out the relationship between smartphone usage and academic performance of under graduate students. After a thorough review, six studies were extracted from the irrelevant studies related to smartphone usage and its effect on academic performance. These studies were analyzed using

narrative synthesis and interpreted in the following table.

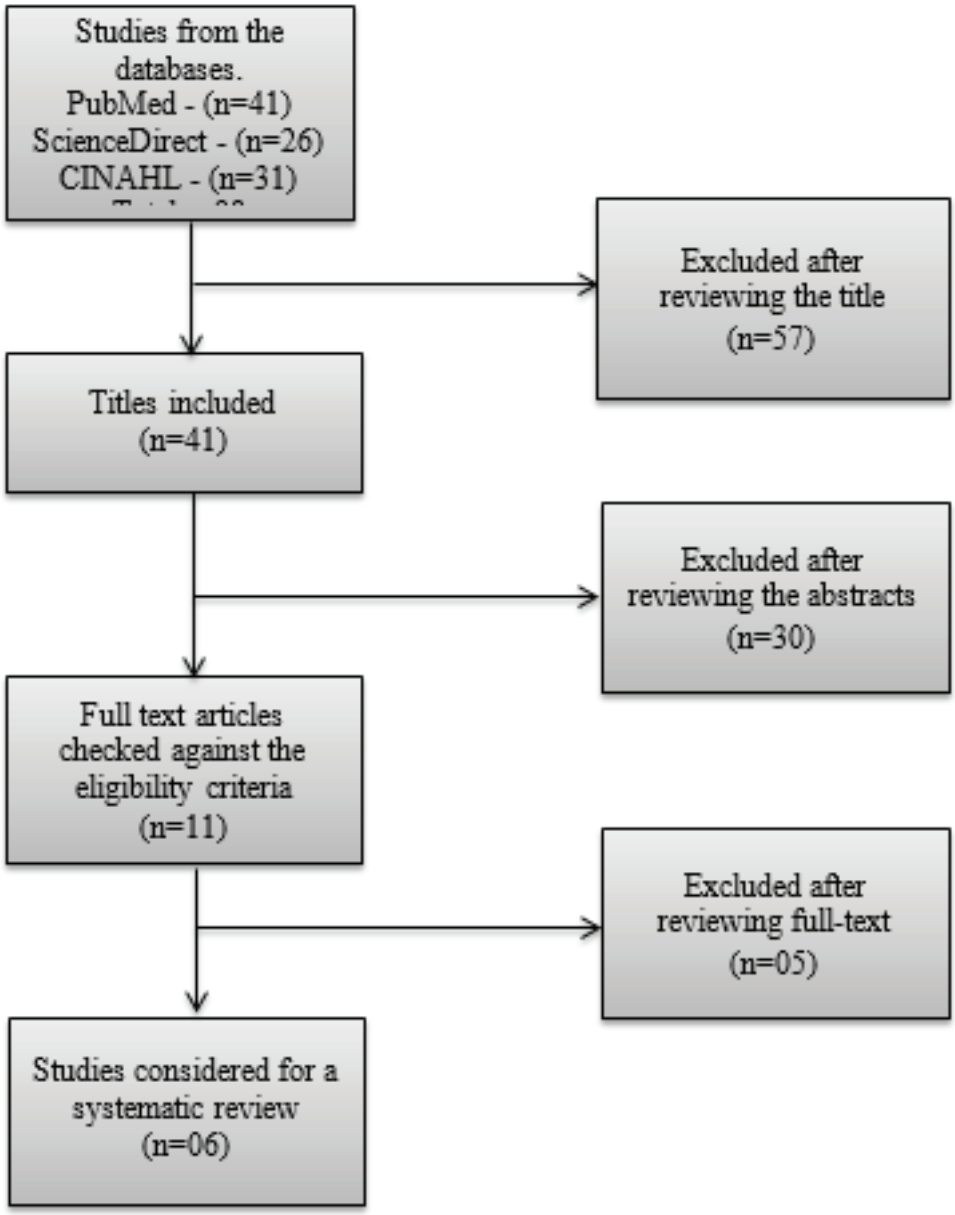


Fig 1: Prisma guidelines

Table 1: Systematic Review on relationship of smartphone usage and academic performance among Undergraduate students

SN	Authors/ Years	Sample size	Outcome
1.	Nayak, J. K. (2018).	429	Usage of smart phone was seen more in females than males. This also affected the academic performance of male students specifically.[2]
2.	Felisoni, D. D., & Godoi, A. S. (2018).	100	The academic performance in terms of rank corresponded to every 100 minutes spent on use of smart phones. The students' grades fell down with 6.3 points.[3]
3.	Giunchiglia, F., Zeni, M., Gobbi, E., Bignotti, E., & Bison, I. (2018).	72	There was a negative effect of long term usage of social media sites that affected the academic credits of the student.[4]
4.	Samaha, M., & Hawi, N. S. (2016).	300	Students' usage of smart phones was positively related to perception of stress and negatively related to satisfaction with life and academic performance.[5]
5.	Ahmed, R. R., Salman, F., Malik, S. A., Streimikiene, D., Soomro, R. H., & Pahi, M. H. (2020).	684	High level addiction of smart phone has strong influence on the students. There are positive as well as diverse effects and must be weighed with the type of features and use of technology to assess the students' performance.[6]
6.	Grant, J. E., Lust, K., & Chamberlain, S. R. (2019).	31,425	20.1% of the students revealed that they their low grades and alcohol use were only due to smartphone addiction. This also is a major factor for diseases like ADHD, Depression, and anxiety.[7]

Looking at the above table it is clear that smartphones have affected the students predominantly with their poor academic performances. Their low grades have pushed them to alcohol addiction too. However, there are evidences that smartphones have been a boon to some students to gain additional knowledge on their subject aspects and has improved their grades and perceptions.

Discussion

Similar study was conducted on 62,276 participants in Korea. Smartphone usage was one of the major reasons for conflicts in family & friends, poor academic performance, and suicidal attempts.[8]

Conclusion

Smartphones have been a boon as a result of digitalization and modernization. Everything is available on the click of a button. Hence, this gadget must be handled with great responsibility.

Conflict of Interest –Nil

Source of Finding -Self

Ethical Clearance – ethical clearance was obtained from IRC of Symbiosis College of nursing.

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A Study to Assess the Effectiveness of Mindful Meditation on Health and Psychological Adjustment Faced by Nursing Students in Selected Colleges of Pune City

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Abstract

Nursing students experiences more stress in their early academic period. Psychological adjustment becomes difficult for them due to many challenges. It is assumed that mindful meditation will helpful for them to cope up with adjustment problems. A study to assess the effectiveness of mindful meditation on health and psychological adjustment faced by first year B.Sc nursing students in selected nursing college of Pune city. Purpose of the study to assess the level of health and psychological adjustment and evaluation of mindful meditation on their adjustment. Pre-experimental study was conducted to find out the adjustment problems. The research design was one group pre-test post-test experimental design. The sample size was 40. Purposive sampling technique was used to select the students. The result showed that first year B Sc Nursing students faces multiple adjustment problems related to their health and psychological adjustments. Mindful Meditation in early academic life was proved very useful in adjusting the new world of academic environment.

Keywords – *Plastics, Harmful effects, Mindful Meditation, Psychological adjustments, Nursing students*

Introduction

Entering into a new situation or new profession leads the person into anxiety. It is a signal of our mind and body gives us some changes. The change may come from internal or external sources. When feeling overburdened, excessive stress and Anxiety can lead to lowered coping and adjustment.[1]

Nursing students, an important part of college students, must not only abide burdensome theoretical learning but also experience clinical practice. The clinical environment may cause them to experience stress and anxiety, and the initial clinical practice may be the most stressful period in students' education.[2,3]

Nursing students by virtue of their professional activities like clinical work, shift duties may experience stress in clinical practice, which include social relationships, morals, ethics, values, and academic problems.[4] Reasons for the increased negative emotions and stress associated with this aspect of an educational program include lack of knowledge and experience, feeling unprepared for clinical work..[5,6]

Mindfulness is regarded not as something to get or to acquire, but as an internal resource that already exists, patiently awaiting to be reawakened.[7] The study had found that increased mindfulness can improve psychological functions, reduce in suffering, and has been proved to be an important predictor of depression in nursing students.[8] Mindfulness meditation (MM) is originated from Vipassana meditation in Buddhism, which is defined as a process of focusing one's mind in the present moment, maintaining a non-judgmental attitude, and detaching from destructive thoughts and feelings.[9] MM is a group of meditation practice centered on mindfulness techniques. Through relaxation and concentration exercises, people may improve consciousness in focusing on a particular object, exercising emotions, relaxing the role of the body.[10]

Methodology

Study design, setting and sample size

A pre-experimental study was conducted on effectiveness of Mindful Meditation on health and adjustment problem among first year B.Sc. Nursing students in selected colleges of Pune city. The pre-experimental one group pre-test post-test design was adopted and 60 samples were selected through purposive sampling technique. Recruitment of the samples was done as per laid down criteria. Study was conducted 4 nursing colleges of Pune city after permission from the Principals of Nursing Colleges of Pune city and ethical clearance from ethics committee of the institution.

Objectives of the study

1. To assess the level of health and Psychological adjustment problem among 1st year B. Sc. Nursing students in selected college.

2. To evaluate the effectiveness of mindful meditation on health and Psychological adjustment problem among first year B. Sc. nursing students in selected college

Research Hypothesis

There is no significant difference in the level of adjustment before and after administration of Mindful meditation among first year B. Sc. Nursing students.

Data collection method

The tool had been prepared in two sections which consist of the socio demographic variable and 20 closed ended questions related to mindful meditation on Health and Psychological adjustment. Every right answer gained one (1) point and wrong answer gained zero (0). The overall score ranged from 1-20 from each component, participants with reasonably adequate adjustment considered 14-20 score, adequate adjustment considered 8-13 score and poor adjustment considered 1-7 score. Content validity of the tool was done with experts from the field. Reliability was calculated and found 0.84 by test-retest method.

The questionnaires were administered in English and Marathi language. The same questionnaires were used before and after the providing session on mindful meditation programme.

Data Analysis: The data had been analyzed through descriptive and inferential statistical analysis.

Result

Section I: Description of samples based on personal characteristics

Table No.1 Distribution of participants as per demographic variables

N=60

Demographic variable	Frequency (f)	Percentage (%)
Gender:		
· Male	22	36.67
· Female	38	63.33
Residency		
· Hosteller	46	76.67
· Day-scholars	14	23.33
Religion		
· Hindu	46	76.67
· Muslim	4	6.67
· Christian	10	16.67
Locality		

Cont... **Table No.1 Distribution of participants as per demographic variables**

N=60

· Urban	49	81.67
· Rural	11	18.33
Parents' Education		
· Graduate	36	60.00
· High school	24	40.00
Family Income		
· 11000-20000	8	13.33
· 21000-30000	22	36.67
· 31000-40000	30	50.00

Table 1: Illustrate the distribution of demographic variables of 60 students; most of the participants 38 (63.33%) were female and male were 22 (36.67%).

Majority subjects 46 (76.67%) resides in hostels and few were 14 (23.33%) day-scholars.

As per religion, 46 (76.67%) subjects belong to Hindu religion as compare to Muslim and Christian were 4 (6.67%) and 10 (16.67%) respectively.

The most of the participants 49 (81.67%) from Urban locality where as only few 11 (18.33%) from rural locality.

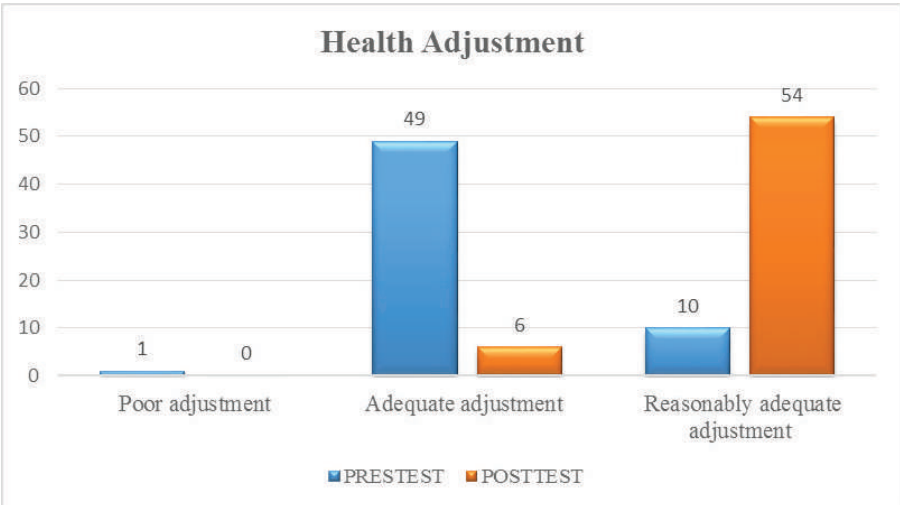
36 (60%) parents had completed their education whereas 24 (40%) completed high school.

As per family Income wise, 30 (50%) subjects were having monthly income of Rs.31000-40000 and 8 (13.33%) were having Rs. 11000-20000.

Section II: Effectiveness of Mindful meditation on health and Psychological adjustment problem

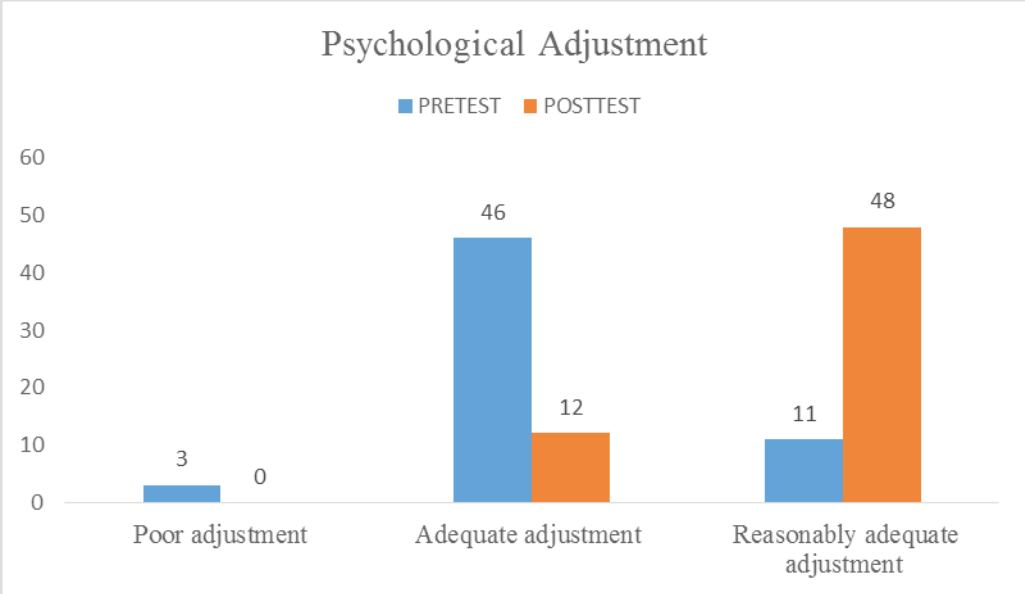
Figure 1 - Figure shows the Health Adjustment level of the first year students on Pre-test and Post-test

N-60



From the above figure it shows that on Pre-test least participant 1 (1.67%) having poor health adjustment and most of them have 49 (81.67%) adequate health adjustment and few participants 10 (16.67%) are having reasonably

adequate health adjustment. Post-test very few samples 6 (10%) having adequate health adjustment and 54 (90%) participants are having reasonably adequate health adjustment. It shows there is an improvement in the Health aspect after the education on Mindful meditation.



From the above figure it shows that on Pre-test, few participants 3 (5%) have inadequate psychological adjustment and most of the samples 46 (76.67%) have adequate psychological adjustment and 11 (18.33%) samples have reasonably adequate psychological adjustment. Post-test 12 (20%) participants have adequate psychological adjustment and most of them 48 (80%) are having reasonably adequate psychological adjustment. It shows there is an improvement in the Psychological aspect after the education regarding the mindful meditation

Statistical

Comparison of mean, standard deviation and paired 't' test showing effectiveness of mindful meditation and their in health and psychological adjustment.

S.No.	Assessment	Mean	S.D	Mean difference	't' value
1	Pre-test	12.04	2.28	3.76	19.33
2	Post-test	15.80	1.89		

Comparison of mean and standard deviation on health and psychological adjustment after administration of mindful meditation among first year B Sc Nursing students found that pre-test mean was 12.04 with Standard deviation 2.28 and post-test level adjustment increased to 15.80 with SD 1.89. The mean difference was found to be 3.76. The paired't' test was applied with df 59 and value found 19.33 at significant level of $p<0.05$. It proves that mindful meditation is effective tool to improve the health and psychological adjustment among the first year B Sc Nursing students

Discussion

A pre-experimental one group pre-test post-test design was adopted for this study. First year nursing students from different 4 colleges were selected with

purposive sampling method and as per laid down inclusion and exclusion criteria. Sample size was 60. Demographic characteristics includes age, sex, residence, income of the family members etc. independent variable was mindful meditation session and dependent variables were health and psychological adjustment problems. Questionnaire were prepared regarding mindful mediation and health and psychological adjustment problems. Total 20 questions were asked before pre-test, session on mindful meditation was conducted followed by post-test after gap of 7 days. Result showed that mindful meditation is useful tool for the higher education students to improve on psychological adjustment and promote health of the students. Paired 't' test was applied for verification of the result at level of significance <0.05. This proves that mindful meditation is significantly improves the psychological adjustment, health and reduces stress among students. Similar study was conducted by K Uthramani, Kerala state and she also proved that mindful meditation is useful in reducing stress and improving academic adjustments among students[11].

Conclusion

Adjustment to nursing college life immediately after higher secondary becomes a difficult transition for many students. Going to nursing college gives a chance to the students to learn new things, meet new people, to deal effectively with clinical experiences and challenges which eventually help them in their personal growth and development. Adjustment difficulties arise from the differences between the expectations of the students and realities of college life and yoga helps students to deal with adjustment problems in smooth manner.

Ethical Clearance: Permission obtained from Ethics Committee of Institution.

Source of Funding: Nil

Conflict of Interest: Nil

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Health and Lifestyle Practices of Under Graduate Students Studying in Selected Nursing College

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Abstract

Life styles of the people is decided as early in early childhood period. School and family practices are essential in deciding healthy and lifestyle practices in adulthood. Sometimes, peer pressure changes the individuals' lifestyles. The study aimed to assess the healthy and lifestyles practices of undergraduate students studying in selected nursing college. **Methodology**: A Non-Experimental Descriptive Research approach used in various nursing colleges in Pune city. The selection of the sample was based on easy geographical accessibility, cooperation and availability of samples .The sample size for the study was 96,selected by using non probability convenience sampling technique who met the inclusion criteria. A self-structured questionnaire was developed based on the research problem, to get the information from the samples.

Result : As for the nutrition the mean score is 48. 64% of adolescents replied that they will avoid half cooked food, 56% replied they avoid fast foods and 59% replied they prefer healthy eating.91% replied that they don't have any problem in paying attention. 75% doesn't have any problem for sleep. 62% replied that they have troubles in relaxing and 44 % replied they usually get into the arguments. 55% involved in activities like sports, games and dance. 70% feels healthy and 68% have complaints of stomachaches, headaches, or other aches and pains. 96% have no problems in handling life. Around 93 % satisfied with their family . Around 93% have the support with the family and friends. 57.29 % replied that they don't have computer in their home. 61.46 % students live with their parents. 96.88% has personal mobile. 73% regularly visit social networking sites. 96% of adolescents wash their hands before eating foods and 83% wash their hands with soap or hand wash. 16.67% have habits of cigarettes/chewing Pan/Guthka but 945 aware about the harmful effects of smoking. 13% replied they tried alcohol but they believe that 41% of their friends has alcohol drinking habits. 86% students feel getting good score in college is important to family. 94% feel safe in college and only 4% says that their behavior influenced by peer pressure. Others 96% doesn't agree with it.

Conclusion: Diet practices, concentration in work , family connectedness scored less compared to other components. Very few students have the habits of alcohol, smoking etc. Personal hygiene was practiced well in adolescents. Decision is taken by themselves more than the peer pressure. Majority of the adolescents knows the important of their education and they feel safe and good in college.

Key Words: *Lifestyle practices, health, nursing students*

Introduction

Around 1.2 billon people (1 in 6 of the world's population) are adolescents 10 to 19 years. Most of the adolescents groups were healthy but some face premature death due to diseases, injury and accidents. Many adolescents are habituated towards un healthy lifestyle practices like alcohol or tobacco use, less physical activity, unprotected sex and exposure to violence. This habits not only threat current health of adolescents, but also their future health as adults.[2] However, parents should encourage healthy behaviours during adolescence, and should take steps to protect young people from risks which are critical for the individuals' future health.[3]

This study assessed changes in students' health and lifestyle behaviours[4] . In this study health status means the measurement of health and lifestyle of nursing

students studying in nursing college of Pune city and Lifestyle refers to the ways in which individuals live that could affect their health. Health promoting lifestyle refers to actions that individuals take the initiative to pursue that could benefit their health[5,6].

The 9 components of health-promoting behaviours included were nutrition, mental health, physical health, Social connectedness, relationship with others, family environment, personal habits, personal hygiene and social environment. This study aimed to assess the health and lifestyle practices of nursing students studying in nursing college of Pune city.

Objectives of the study : 1) To assess the Health practices of students studying in selected nursing college of Pune city.**2)**To assess the lifestyle practices of students studying in selected nursing college of Pune city.

Research design: non-experimental descriptive design used to find out the health and lifestyle practices of nursing students studying in nursing college of Pune city. In the present study, the population is comprised of the School students studying in different School across Pune. In this study, non –probability convenience sampling method was used. Sample size consists of 96 adolescent students studying in under graduate nursing colleges of Pune city. Undergraduate nursing college students those who are willing to participate are included in the study. Students those who are absent during the data collection excluded the study.

To collect the data for present study , self-structured questionnaire was constructed to assess the health and lifestyle practices of undergraduate students studying in nursing college.The tool was developed based on related review of literature guidelines, journals, reports and articles published and unpublished studies were reviewed and used to develp the tool. **A tool consist of three sections as follows**: **Section 1** consist of demographic variables (age, gender, education, monthly income) **Section 2** consist of self-structured questionnaire to assess the health status of the nursing students(nutrition, mental health, physical health, Social connectedness and relationship with others**)** and **Section 3** consist of self-structured questionnaire to assess the knowledge of nursing students regarding health status (family environment, personal habits, personal hygiene and social environment)

The tool was given to 8 experts in the field of nursing student's lifestyle for content validity. All the comments and suggestions given by the experts were duly considered and corrections were made after discussions with research guide. Reliability was assessed using Cronbach's α coefficient method used to check the internal consistency and the value is 0.76 and the instrument is highly reliable.

Data collection: On the day of the study, the study was explained to nursing students and ensured about the confidentiality of their response. Total time given to fill the checklist was 30 minutes.

I . Findings related to Scio-demographic details

Table1: Description of socio -demographic variables n=96

Sr.No	Demographic variable		Frequency	Percentage %
1	Age	17 to 20 years	34	35.42
		21 to 24 years	50	52.08
		25 years and above	12	12.50
2	Parents Income:	Rs10,000 – 20,000	29	30.21
		Rs21,000 – 30,000	24	25.00
		Rs31,000 – 40,000	26	27.08
		Rs41,000 and above	17	17.71
3	Academic level:	1st year B.Sc. Nursing	22	22.92
		2nd year B.Sc. Nursing	14	14.58
		3rd year B.Sc. Nursing	46	47.92
		4th year B.Sc. Nursing	12	12.50

Cont... **Table1: Description of socio -demographic variables** **n=96**

4	Parent's education:	No formal education	14	14.58
		High school	52	54.17
		Graduate	23	23.96
		Postgraduate and above	7	7.29

Majority of the students age group is 21- 24 years, 47 % of third year nursing students participated enthusiastically. A Majority of the parent's monthly income in Rs. 10,000 to 20,000 Parents education was reported 54% by majority of the women. Parent's income of about equally distributed.

II. Findings related to healthy status of nursing students

Table 2: Description of health status of nursing students studying in selected nursing college.

I. Nutrition		**Yes**		**No**	
		F	**%**	**F**	**%**
1	Benefits of healthy eating	57	59.38	39	40.63
2	Avoid fast foods	54	56.25	42	43.75
3	Avoid half cooked food	62	64.58	34	35.42
4	Have you include protein rich food in your daily diet?	86	89.58	10	10.42
	II. Mental health				
5	Did you have a hard time paying attention?	88	91.67	8	8.33
6	Did you have trouble falling asleep or staying asleep?	72	75.00	22	22.92
7	Did you have trouble relaxing?	60	62.50	34	35.42
10	Did you get in arguments or fights?	43	44.79	53	55.21
	III. Physical Health				
11	Are you involved in any sports/games/dance?	53	55.21	43	44.79
12	Did you have stomachaches, headaches, or other aches and pains?	66	68.75	30	31.25
13	Are you feel healthy?	68	70.83	28	29.17
	IV. Social connectedness				
14	I am able to handle daily life.	92	95.83	4	4.17
15	I get along with family members.	62	64.58	34	35.42
16	I am satisfied with our family life right now.	90	93.75	6	6.25
17	I am doing well in college.	66	68.75	30	31.25
	V. Relationship with others:				
18	I know people who will listen and understand me	79	82.29	17	17.71
19	I am comfortable talking my child's problems	89	92.71	7	7.29
20	In a crisis, I have the support I need from family or friends.	90	93.75	6	6.25

As for the nutrition the mean score is 48. 64% of adolescents replied that they will avoid half cooked food, 56% replied they avoid fast foods and 59% replied they prefer healthy eating.91% replied that they don't have any problem in paying attention. 75% doesn't have any problem for sleep. 62% replied that they have troubles in relaxing and 44 % replied they usually get into the arguments. 55% involved in activities like sports, games and dance. 70% feels healthy and 68% have complaints of stomachaches, headaches, or other aches and pains. 96% have no problems in handling life. Around 93 % satisfied with their family . Around 93% have the support with the family and friends.

III. Findings related to lifestyles

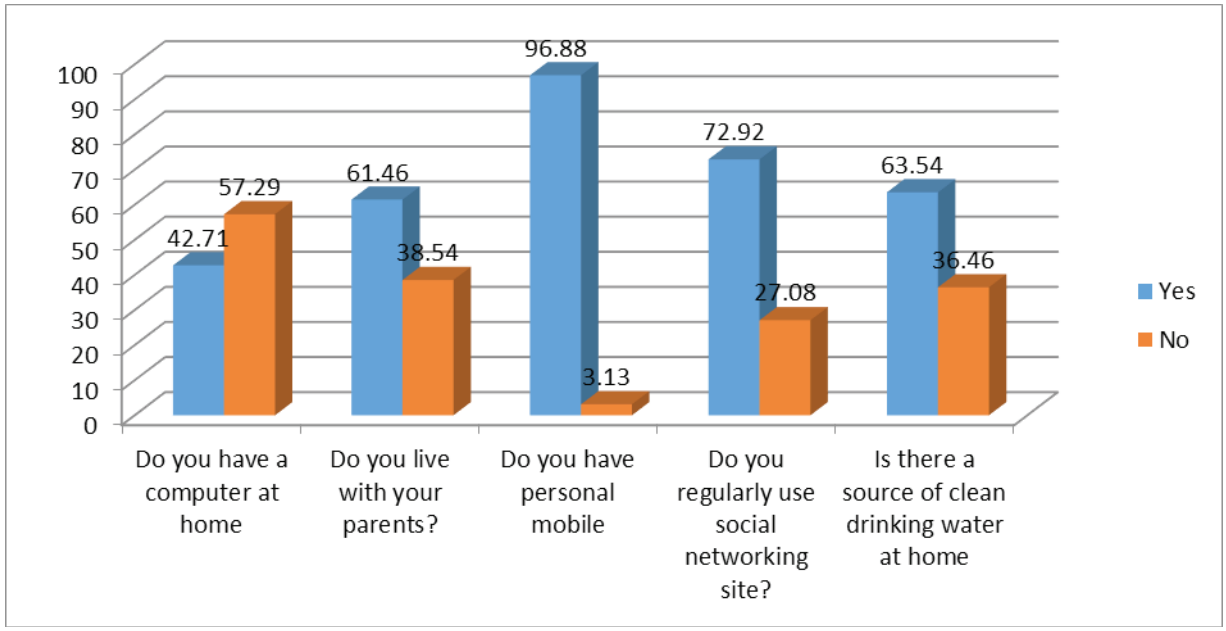


Figure 1: Family and home environment in adolescents 57.29 % replied that they don't have computer in their home. 61.46 % students live with their parents. 96.88% has personal mobile. 73% regularly visit social networking sites.

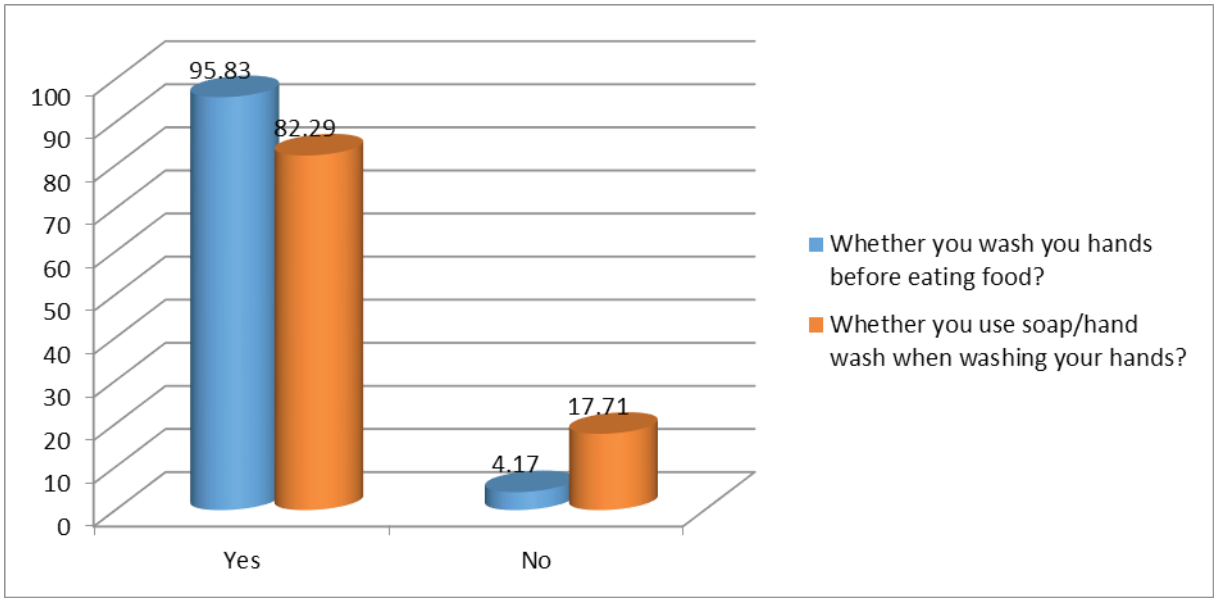


Figure 2 : Personal hygiene practices of adolescents 96% of adolescents wash their hands before eating foods and 83% wash their hands with soap or hand wash.

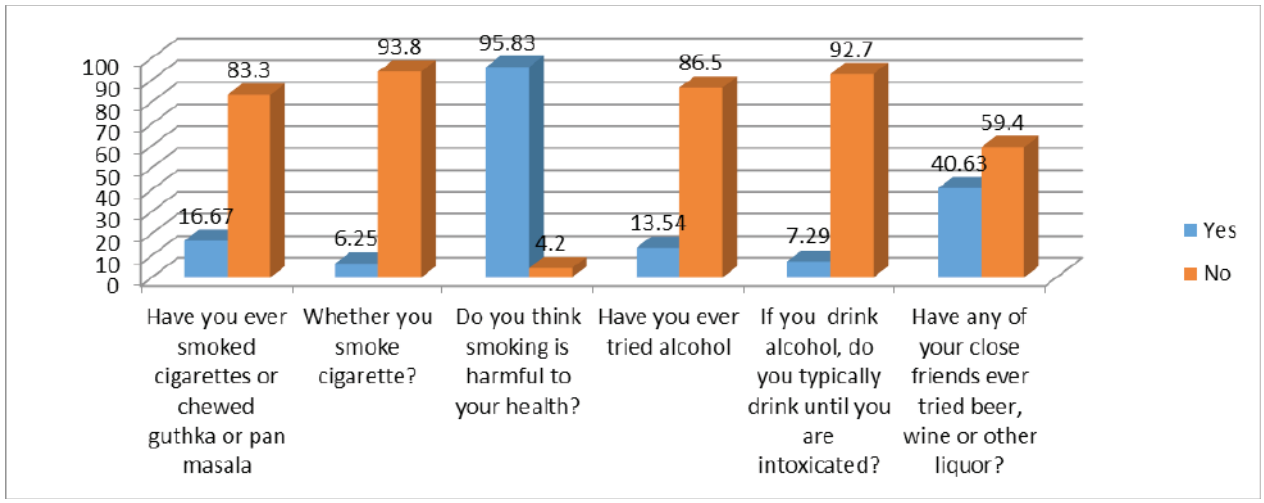


Figure 3 : Personal habits of adolescents

16.67% have habits of cigarettes/chewing Pan/Guthka but 945 aware about the harmful effects of smoking. 13% replied they tried alcohol but they believe that 41% of their friends has alcohol drinking habits.

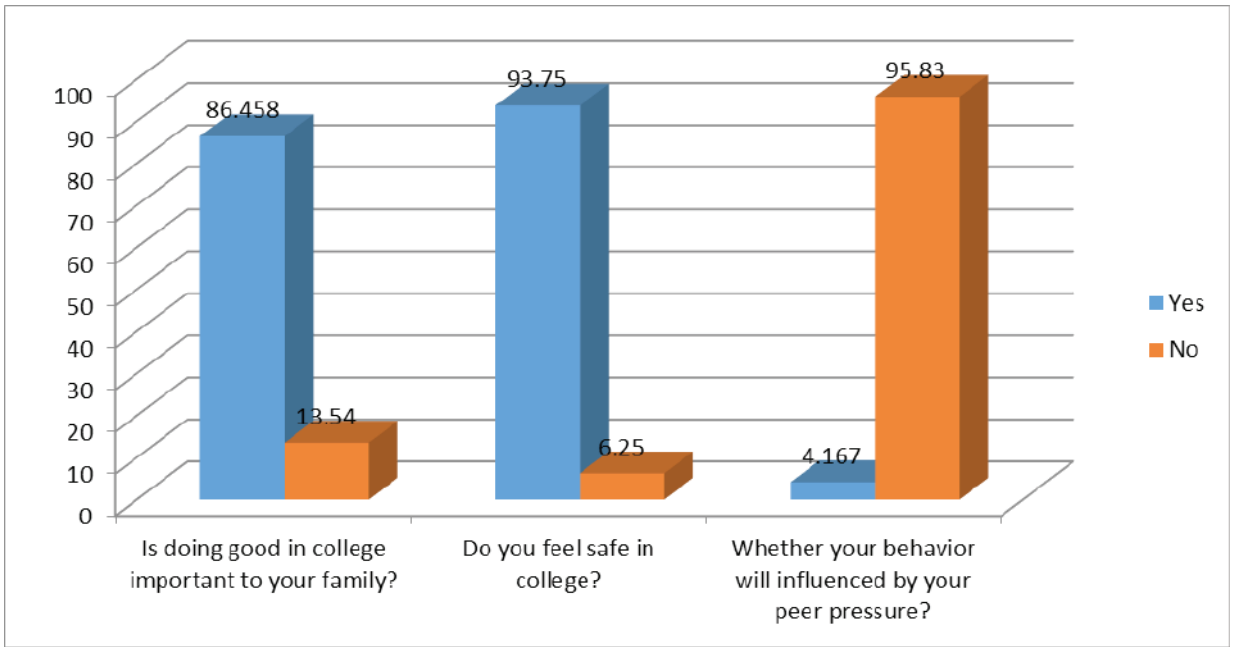


Figure 4 : Social environment of adolescents

86% students feel getting good score in college is important to family. 94% feel safe in college and only 4% says that their behavior influenced by peer pressure. Others 96% doesn't agree with it. Diet practices , concentration in work , family connectedness scored less compared to other components. Very few students have the habits of alcohol, smoking etc. Personal hygiene was practiced well in adolescents. Decision is taken by themselves more than the peer pressure. Majority of the adolescents knows the important of their education and they feel safe and good in college. Evidence shows that nursing students who engage in healthy lifestyle practices are more likely to counsel patients about healthy behaviors [7,8].

Ethical Consideration: Ethical approval of the study taken from Symbiosis College of nursing ethical committee. Informed consent was taken from the student nurses in different colleges of Pune city. Informed the responders regarding the data collection procedure. The collected data was used only for research purposes and kept confidential.

Funding : Authors have no financial support to this project. The study was fully funded by authors.

Conflict of Interest: The author has no conflict of interest

Conclusion

A similar study may be replicated on large samples; thereby findings can be generalized for a large population, A comparative study may be conducted using different lifestyle practices of nursing students, A similar study may be conducted to find out the knowledge regarding effect of health and lifestyle practices and A similar study may be conducted to find out the lifestyle of nursing students.

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Osteoporosis Risk Analysis in Women Residing in Selected Urban Areas

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Abstract

Osteoporosis is a bone disorder with significant changes in bone biologic material and consequent bone structural disruption, affecting millions of people around the world from diverse ethnic groups. The number of osteoporotic fractures increases in both men and women (by more than 3-fold over the next 50 years) as a result of the ageing population particularly in Asia and Latin America.

Methodology: Results: Majority of the women are in the age group of 40 to 50 years. Majority of the women around 38% doesn't have any formal education. Majority of the women (20%) were home makers. Very few women completed post-graduation. Around 36% working in private institution. Around 40 % women monthly family income is 60,000-80,000. Around 40% women were in moderate risk of getting osteoporosis and 2 women has a high risk and 29% doesn't have any risk towards osteoporosis. None of the variables are associated with the risk analysis of the women anyhow age of the women is near by the association as the value is 0.05.

Conclusion: Previous studies suggested that osteoporosis is related to age, weight, intake of corticosteroid tablets, history of fracture, history of thyroid diseases, menstrual history and Vitamin D deficiency[7]. The tool is prepared to assess the risk factors. And it found that majority of the women are in moderate risk

Keywords: *Osteoporosis, Women, Urban areas, Vitamin D*

Introduction

In current era, Osteoporosis is a major health problem faced by people in older age. This leads a morbidity and mortality in older age especially to women. Osteoporosis is characterized by low bone mass and degeneration problems leads to brittleness of the bones. Early identification leads to prevention of fractures[2] Osteoporosis is a significant problem in Indian postmenopausal women with significant increase in morbidity and mortality following fragility fractures[3]

Methodology

Research methodology used for this study is non-experimental, descriptive design was used. In the present study, the population is comprised of the women in different urban areas across the Pune city. Sample size consist of 45 women of selected urban area of Pune city .Inclusion criterion included the women from selected urban areas of Pune and those who were willing to participate. Women above 40 years till 60 years were included in the study

To collect the data for present study, self-assessment screening questionnaire was prepared based on the old literature and studies. The tool consists of two sections as follows. Section1 consist of Demographic variables (age, education, occupation and monthly family income). Section 2: consists of questionnaire to find the risk of osteoporosis in women. The tool was given to 8 experts in the field of osteoporosis for content validity. All the comments and suggestions given by the experts were duly considered and corrections were made after discussions with research guide. Pearson Test-retest was used to establish a reliability of structure questionnaire

and it was satisfactory, value is 0.80 and the tool is highly reliable.

Results

Table 1: Section A: Frequency distribution table of demographic variables. n=45

Demographic variables		Frequency	Percentage %
1.	Age Group		
a.	40-45	11	24.44
b.	46-50	11	24.44
c.	51- 55	14	31.11
d.	56- 60	9	20.00
2.	Education		
a.	Post graduate	3	6.67
b.	Diploma &Graduate	12	26.67
c.	High School	13	28.89
d.	No formal education	17	37.78
3.	Occupation		
a.	Private	16	35.56
b.	Government	7	15.56
c.	Semi-skilled worker	2	4.44
d.	House wife	20	44.44
4.	Monthly family income		
a. 20,000 -40,000		9	20.00
b. 40,000 -60,000		18	40.00
c. 60,000 -80,000		12	26.67
d. 80,000 -1,00,000		6	13.33

Majority of the women are in the age group of 40 to 50 years. Majority of the women around 38% doesn't have any formal education. Majority of the women (20%) were home makers. Very few women completed post-graduation. Around 36% working in private companies. Around 40 % women monthly family income is 60,000-80,000.

Table 2 : Section B : Risk analysis in osteoporosis women n=45

Sr. No	Risk score of osteoporosis	Frequency	Percentage
1	Lower risk	13	28.88
2	Moderate	18	40
3	High risk	2	4.44

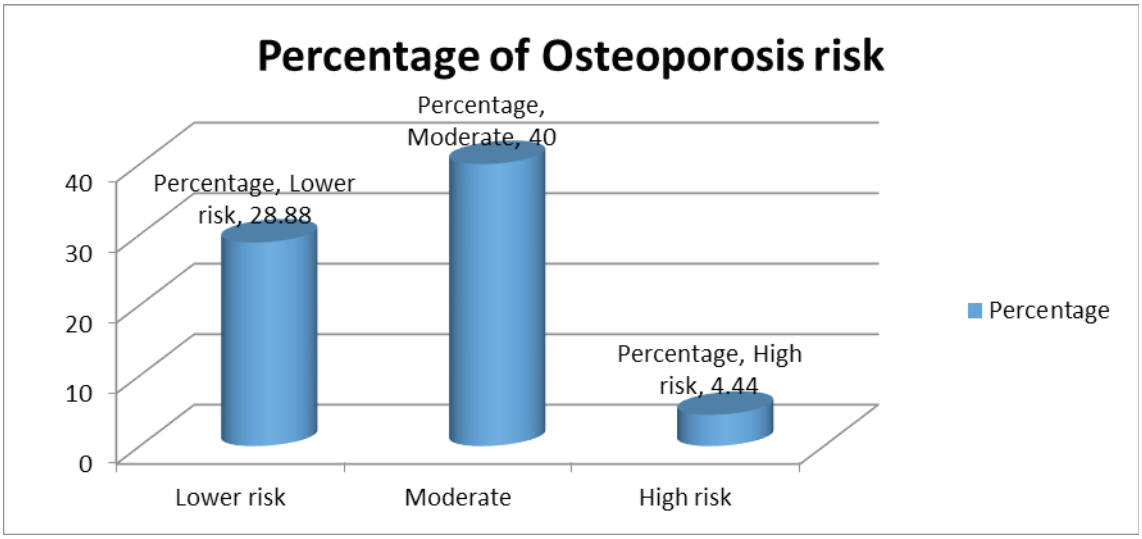


Figure 1 : Risk analysis in women for Osteoporosis

Around 40% women were in moderate risk of getting osteoporosis and 2 women has a high risk and 29% doesn't have any risk towards osteoporosis.

Table 3 : Association between the samples demographical details and osteoporosis risk score

Demographic variables	Lower risk	Moderate risk	High risk	Probability
Age Group				
40-45	5	6	0	0.05
46-50	10	1	0	
51- 55	5	8	1	
56- 60	5	3	1	
Education				
Post graduate	1	2	0	0.57
Diploma &Graduate	8	4	0	
High School	5	7	1	
No formal education	11	5	1	
Occupation				
Private	11	5	0	0.52
Government	5	2	0	
Semi-skilled worker	1	1	0	
House wife	8	10	2	

Cont... **Table 3 : Association between the samples demographical details and osteoporosis risk score**

Monthly family income				
20,000 -40,000	5	4	0	0.07
40,000 -60,000	7	10	1	
60,000 -80,000	9	3	0	
80,000 -1,00,000	5	0	1	

None of the variables are associated with the risk analysis of the women anyhow age of the women is near by the association as the value is 0.05.

Previous studies suggested that osteoporosis is related to age, weight, intake of corticosteroid tablets, history of fracture, history of thyroid diseases, menstrual history and Vitamin D deficiency[7]. The tool is prepared to assess the risk factors. And it found that majority of the women are in moderate risk. Urgent considerations need to be taken in women are calcium and Vitamin D supplementation. This can help the women to reduce the risk of osteoporosis. Anyhow further researches on this topic need to be evaluated.

Ethical Consideration: Ethical approval of the study taken from Symbiosis College of nursing ethical committee. Informed consent was taken from the women in different urban areas of Pune city. Informed the responders regarding the data collection procedure. The collected data was used only for research purposes and kept confidential.

Funding: Authors have no financial support to this project. The study was fully funded by authors.

Conflict of Interest: No conflict of interest

Conclusion

Further researches have to be encouraged on large samples so findings can be generalized for a large population, A similar study may be conducted to find out the knowledge regarding Osteoporosis effect of health and lifestyle practices and a similar study may be conducted to find out the lifestyle of women who are risk for osteoporosis.

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A Study to Assess the use of Body Mechanics Practices among Nursing Students in Selected Colleges of Nursing in Pune City

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Abstract

Mechanics is concerned with the analysis of the action of forces on object. Body mechanics is the term used to describe the efficient, coordinated and safe use of the body to move objects and carryout the activities of daily living. The major purpose of body mechanics is to facilitate the safe and efficient use of appropriate muscle groups to maintain balance, reduce the energy required, reduce fatigue and decrease the risk of injury. Good body mechanics is very much essential for the nurses. When a person moves, the balance of that person depends on the interrelationship of the center of gravity and the base of the support. The closer the line of gravity is to the center of base of support, the greater the person's stability. Appropriate preparation prevents potential falls and injury and safeguards the person and equipment[1]. Descriptive research approach was used with Convenience sampling technique to select 300 nursing students. The results revealed that 61.52% of correct body mechanic techniques were used by study subjects. Whereas only 38.4% were using incorrect body mechanic techniques. Most of correct body mechanic practices were following by subjects who were worked in CCU/ICU.

Keywords: *Body Mechanics, Nursing Students, Practice.*

Introduction

Body mechanics is a term used to describe the ways we move as we go about our daily lives. It includes how we hold our bodies when we sit, stand, lift, carry; bend and sleep. Poor body mechanics are often the cause of back problems. When we don't move correctly and safely, the spine is subjected to abnormal stresses that overtime can lead to degeneration of spinal structures like discs and joints, injury and unnecessary wear and tear .That is why it is so important to learn the principles of proper body mechanics. Proper body mechanics are vitally important for keeping our spine healthy. And it's easy to incorporate these principles into our daily life[2].

There must be proper alignment in order to have proper movement. Some of the most common injuries sustained by members of the health care team are severe musculoskeletal strains. Many injuries can be avoided by the conscious use of proper body mechanics when performing physical labor[1].

Body mechanics is the efficient use of the body as a machine and as a means of locomotion. Body mechanics is directly related to the effective functioning of the body. The correct use of body mechanics should be evident of every activity and even during rest periods because correct use of body mechanics is another phase of illness prevention and health promotion. Correct body alignment reduces the strain on musculoskeletal structures. Body alignment means positioning of the joints, tendons, ligaments and muscles while in standing, sitting and lying position. Body balance is achieved when center of gravity is balanced over a wide stable of supports. The student nurse can increase body balance when working by spreading their feet apart and by flexing their hips and knees[3].

Faulty body mechanics is a contributing factor in most back disorder. Lifting with the back in a flexed posture, especially repetitive lifting is one of the leading causes of back disorders. Injury to the disc occurs gradually as a result of perhaps hundreds of thousands of repeated forward bends and lifts. This forward bending and lifting is especially stressful on the lower back when done with the legs straight. If the legs are straight the trunk acts as a lever arm and increases the compressive

load on the back by seven to ten times.

Compared to other occupations nursing personnel are among the highest at risk for musculoskeletal disorders. The bureau of Labor Statistics lists registered nurses 6th in a list of at risk occupations for strains and sprains. Research on the impact of musculoskeletal injuries among nurses in US showed that 52% of nurses complain of back pain, 12% of nurses 'leaving for good' because of back pain, 20% transferred to different unit or employment and 38% suffered occupational related back pain severe enough to require leave from work and 6%, 8% and 11% of registered nurses reported even changing jobs for neck, shoulder and back problems respectively.[4]

Objectives

To assess the use of body mechanic practices among Nursing Students.

Material & Methods

RESEARCH APPROACH: Quantitative approach (Descriptive)

RESEARCH DESIGN: Pre-experimental Research design

RESEARCH SETTING: College of Nursing in Pune city

STUDY POPULATION: All nursing students

TARGET POPULATION: Nursing students who were undergoing ANM, GNM, B.Sc. Nursing and Post Basic courses.

SAMPLE AND SAMPLING TECHNIQUE:

Size: 300 nursing students were taken as sample for the study.

Sampling Technique: Convenience sampling technique.

Criteria

Inclusion Criteria:

Nursing students who were:

} Undergoing nursing courses in the selected institute will be included.

} Nursing students who were working in selected hospitals.

} Both male and female nursing students.

Exclusion Criteria:

Nursing students who were absent during data collection.

Development and Description of tool

The tool consists of basically 2 parts:-

TOOL-1: It was comprised of five items of demographic variables for observe the body mechanic practice i.e. Gender, Grade, Working area, Working hours/day and Footwear used.

TOOL-2: It was comprised of 34 items .Each item was having two options.

Scoring Procedure: There were 34 items pertaining to the practice of body mechanic. Each item was having two options with one appropriate answer. The maximum score for correct response to each item was given '1' and for the wrong response '0'. The level of practice was categorized based on the percentage of scores obtained.

Ethical consideration:

ü IRC approval has been obtained from Symbiosis College of Nursing, Pune

ü Confidentiality and anonymity of subjects was maintained throughout study.

Results

Table 1: Distribution of Socio-demographic variables according to their mean score regarding body mechanic practices.

Socio demographic Variables	N	Mean Score	Mean %
Gender Female	300	20.92± 3.36	51.52%
Class ANM GNM B.Sc	012 159 129	19.66± 3.20 21.13± 3.82 20.78± 2.76	57.82% 62.14% 61.11%
Working History ICU CCU Emergency Unit General Ward Other	008 017 063 144 068	24.50 ± 1.06 23.00 ± 2.42 20.71 ± 3.98 20.50 ± 3.22 21.05 ± 3.11	72.05% 67.60% 60.88% 60.29% 61.91%
Working hours/Day <8hrs >8hrs	293 007	20.93± 3.41 20.57± 2.07	61.55% 60.40%
Footwear Used Flat Heel Shoes Medium Heel shoes	296 004	20.94 ± 3.39 19.00 ± 1.41	61.50% 55.80%

The above table depicts that the distribution of Socio-demographic variables according to their mean score regarding body mechanic practices.

Table 2: Mean Percentage score of subjects regarding body mechanic practices.

N= 300

N	Mean Score	Mean%
300	20.92 ± 3.36	61.52%

The above table showed that overall mean percentage score of correct body mechanic practices were used by study subjects 61.52% and mean score was 20.92 ± 3.36. Less than half 39.48% of incorrect body mechanic techniques were used by study subjects while giving care to immobilized patient, performing procedure, sitting , standing and also while moving the patient to the side of the bed without an assistant. So the result is maximum of correct body mechanic techniques were using by study subjects while only few were using less accurate technique.

Conclusion

The findings of the study revealed that 61.52% of correct body mechanic techniques were used by study subjects. Whereas only 38.4% of incorrect body mechanic techniques were used by nursing students. Most of correct body mechanic practices were following by subjects who were worked in CCU/ICU.

Source of Funding: Self

Conflict of Interest: Nil

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A Descriptive Study to Assess the Stress and Coping Mechanism Due to Lockdown among School Going Children

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Abstract

Background: Corona virus is an infectious disease came in December 2019 in China. People with infection get the respiratory problem but doesn't show any symptoms and not required any treatment. But those are having underlying problems like diabetes mellitus, pregnant women and children are having risk of getting complication with corona virus infection. The government announced immediate lockdown in country for the prevention of spread of infection and has change life of million children into complete confusion. Many are starving for the foods and calling for the help as the country shuts down to battle the pandemic. The largest child population is in India with 472 million, and Promoters say due to this immediate lockdown many children has disturbed. A assessment survey was done among parents has shown that children's are having more anxiety, increased use of TV and Social media and changed eating pattern. Another study shows that 37% of the children are psychological disturb and 88% spent most of the time on using social networking sites

Objective: To assess stress and coping mechanism due to lockdown among school going children. **Methodology:** A Descriptive Research Approach is used. 60 samples were selected by using Non Probability Purposive Sampling Technique. Data was taken using Stress and coping mechanism questinnarrie through online Google form. **Result:** The study findings show that majority (41%) of the children answered were from 10^{th} standard ,30% from 9^{th} standard ,17% from 8^{th} standard and 12% from 7^{th} standard. It also shows that 58% were male and 42% were girl children. The assessment of stress is done by using the questionnaire based on the stress parameter like physical, psychological, family, financial burden, academics, quarrel with siblings, food .The rating were selected through 5 point likert scale from strongly disagree to Strongly agree. The result of stress due to lockdown findings shows that in majority of the children are strongly agree and agree with the physical stress, psychological stress ,family stress, stress due to financial burden, Academics stress, quareel with sibling and food due to lockdown. The children were using the many coping mechanism to relive this stress and majority of the children were using the coping mechanisms like using social media, exercise, doing study, doing household work, entertainment.

Conclusion: The findings indicate that the questionnaire was effective in assessing the stress and coping mechanism among school going children. The study also concluded that majority of the children are having the stress due to lockdown but they are also using the coping mechanism to reduce this stress

Keywords: *Assess, Stress, Lockdown, Coping Mechanism, COVID 19,Children*

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Introduction and Background

Corona virus is an infectious disease came in December 2019 in China. People with infection get the respiratory problem but doesn't show any symptoms and not required any treatment. But those are having underlying problems like diabetes mellitus,

pregnant women and children are having risk of getting complication with corona virus infection[1].

The government announced immediate lockdown in country for the prevention of spread of infection and has change life of million children into complete confusion. Many are starving for the foods and calling for the help as the country shuts down to battle the pandemic. The largest child population is in India with 472 million, and Promoters say due to this immediate lockdown many children has disturbed.[2]

A assessment survey was done among parents has shown that children's are having more anxiety, increased use of TV and Social media and changed eating pattern. Another study shows that 37% of the children are psychological disturb and 88% spent most of the time on using social networking sites [3]

Though the stress is the risk factor for mental health problem in children and teenager which include the depression, anxiety, eating disorder etc. But to reduce this stress the coping mechanism and positive thinking help to reduce the stress. Compass says "the good news is the brain is changeable .If the coping strategies are used it can reduce the stress.[4]

The children living under lockdown, social restriction and closure of the school. are suffering with feelings of anxiety, stress. The survey done by Save the Children shows result that 65% of the children are getting with boredom and feel isolated.[5]

During the lockdown with limitation on all the movement, social distancing made the children emotionally and psychological disturb. The routine of the children has changed drastically .The talks they used make with schoolmate has stopped .The school activites has stopped. These all things made the children disturbed. So to come out of this stress they need to use the coping mechanism which will help them to have normal life style[6].

Objectives: "To assess the stress and coping mechanism due to lockdown among school going children"

Material and Methods:

A Non Experimental Research design with quantitative approach was used. 60 samples of secondary school going children in selected by Non Probability Purposive sampling technique. The study was conducted online through the assessment Google form of stress and coping mechanism. The tool included three sections; the first section included demographic data the second section included a stress assessment like physical, psychological ,family, financial, academics, quarrel with siblings, food due to lockdown among school going children and third section include the coping mechanism like Use of social media, study, exercise, household work, and entertainment due to lockdown among school going children.

Result of the Study

Section- I

Table 1: Frequency and Percentage of Demographic Data

Demographic Variable Standard		
	Frequency	(Percentage)%
Standard		
7th	7	12%
8th	10	17%
9th	18	30%
10th	25	41%

Cont... **Table 1: Frequency and Percentage of Demographic Data**

Gender		
Male	35	58%
Female	25	42%

Section-II

Analysis of data on stress due to lockdown among the school going children

Table 2: Data related to physical stress among the school children.

Physical Stress	**Strongly Disagree**	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
Not able to do exercise	16%	20%	17%	22%	25%
Going out for sports and play is impossible	20%	17%	17%	17%	30%
Sitting home 24/7 makes me feel lethargic	12%	16%	22%	23%	27%
Physical activity has become minimal	10%	17%	20%	25%	28%
I am facing physical problems due to weight Gain	11%	20%	17%	25%	27%

Table no 3: Psychological stress due to lockdown among school going children

Psychological Stress	**Strongly Disagree**	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
Feel scary due to increasing number of COVID-19 cases	17	17	20	20	26
I feel locked in my house	17	23	12	20	28

Table 4: Stress related family due to lockdown among school going children

Family Stress	**Strongly Disagree**	**Disagree**	**Neutral**	**Agree**	**Strongly Agree**
Family is also suffering due to situation	8%	20%	12%	25%	35%
I am not able to help my family	13%	25%	12%	22%	28%

Cont... **Table 4: Stress related family due to lockdown among school going children**

I am worried about the health of my family member	10%	17%	12%	26%	35%
My family members are tensed about the job	13%	17%	20%	27%	23%

Table 5 : Stress of Financial burden due to lockdown among school going children

Stress of Financial Burden	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Due to lockdown our financial condition got disturb	17%	17%	23%	23%	20%
All are getting worried about the financial management	15%	14%	23%	30%	18%
Our shop is closed since locked down so our income source is stopeed	17%	27%	12%	25%	25%
My parents are worried about payment of my tution fees	10%	25%	15%	25%	25%
My parents are also worried about the payment of bank loan	12%	18%	10%	30%	30%
All are tensed about the post COVID financial management	2%	5%	20%	38%	35%

Table 6: Stress related to academics due to lockdown among school going children

Stress of Academices	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
I am very tensed about my studies	8%	5%	13%	32%	42%
Its really very hectic to submit the assignemnt online	2%	13%	20%	17%	48
I am not able to get the book to read	8%	20%	10%	33%	28%
I am missing the classroom teaching	10%	12%	13%	30%	35%
I am very tensed about the decision of final exam and my result	12%	15%	8%	33%	32%
I am worried about the school reopening	5%	3%	12%	37%	43%

Table 7: Stress related Quarrel with Sibling due to lockdown among school going children.

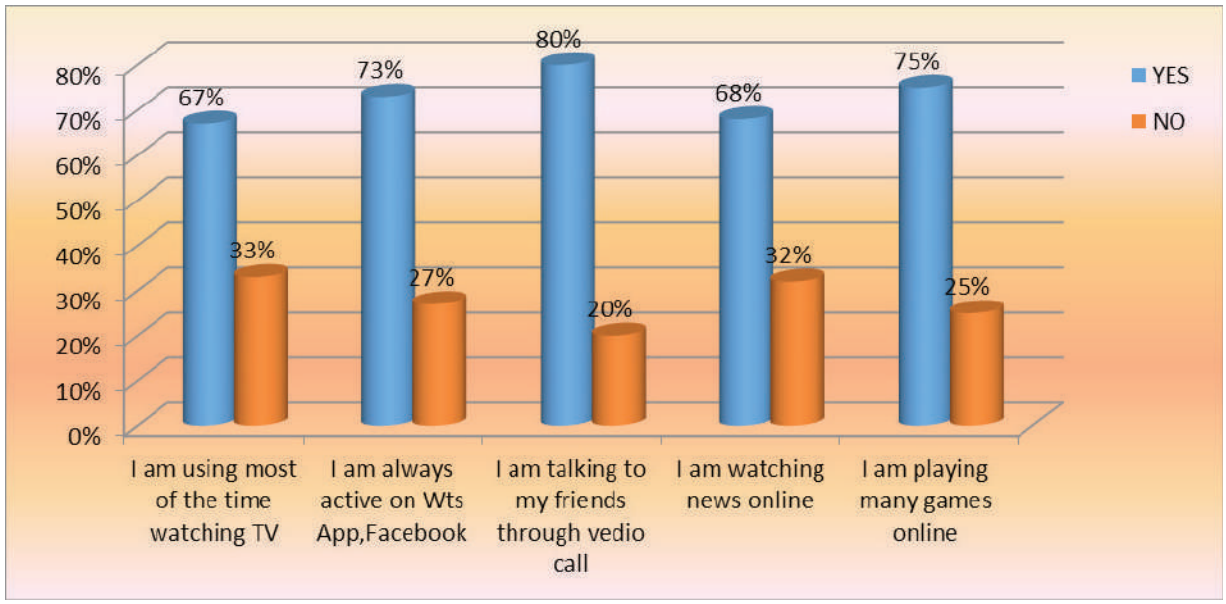
Stress on Quarrel with Sibling	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
I am fighting with my siblings most of the time	5%	15%	15%	33%	48%
My brother/sister dont help in work	7%	23%	12%	28%	30%
My brother/sister troubles me during my study time	10%	23%	3%	30%	33%

Table 8: Stress related to food due to lockdown among school going children.

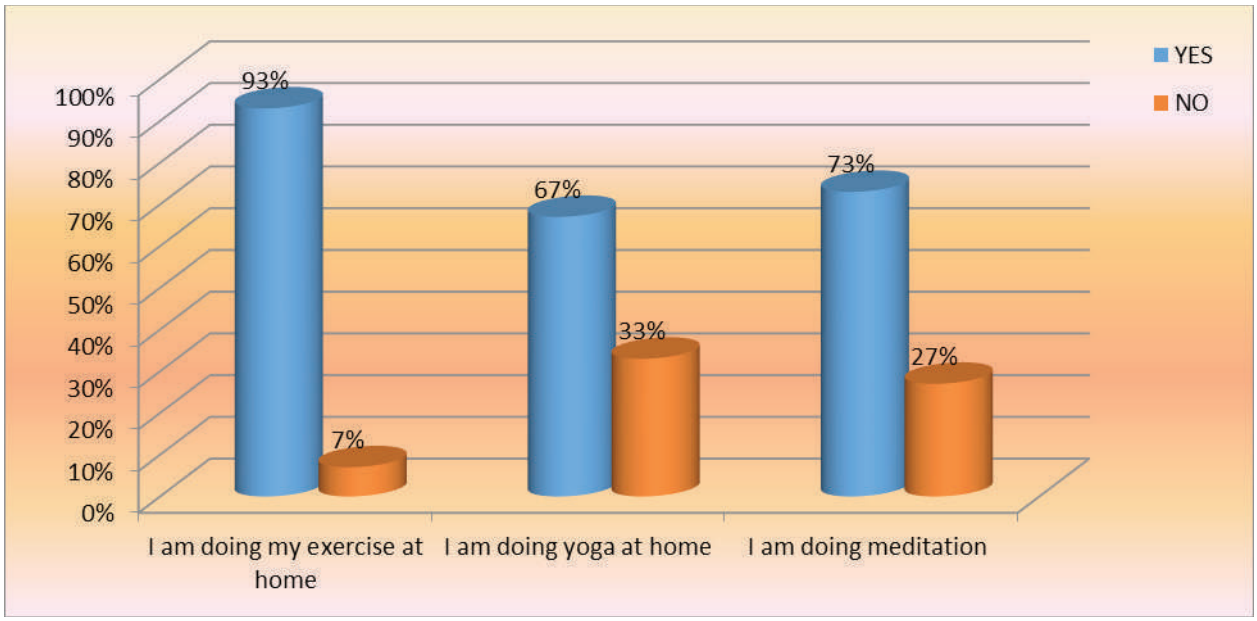
Stress about Food	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
I am not able to eat outside food	10%	12%	8%	28%	42%
I am missing my family dinner in hotels	3%	2%	3%	47%	45%
I am not able to go with my friends for having junk food like pani puri,Pizza,Burger	3%	7%	10%	38%	42%

Section-III

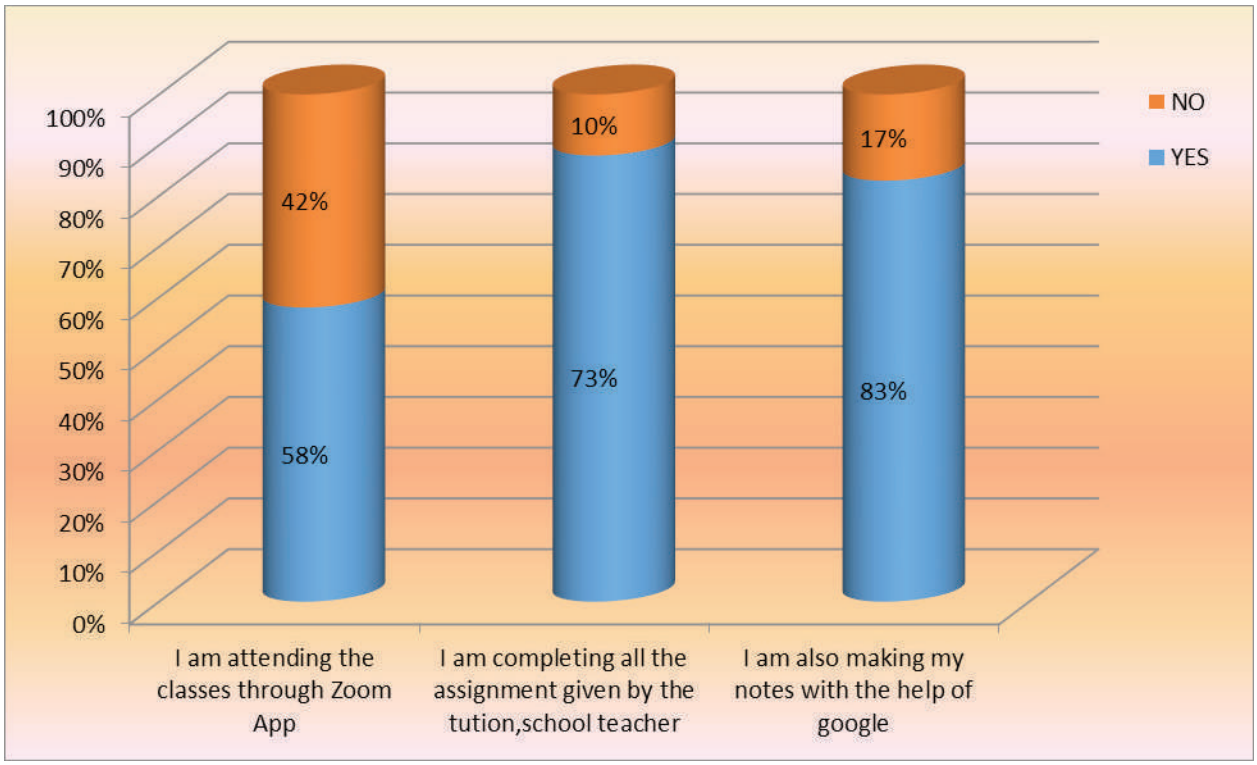
Analysis of data on coping mechanism among the school children



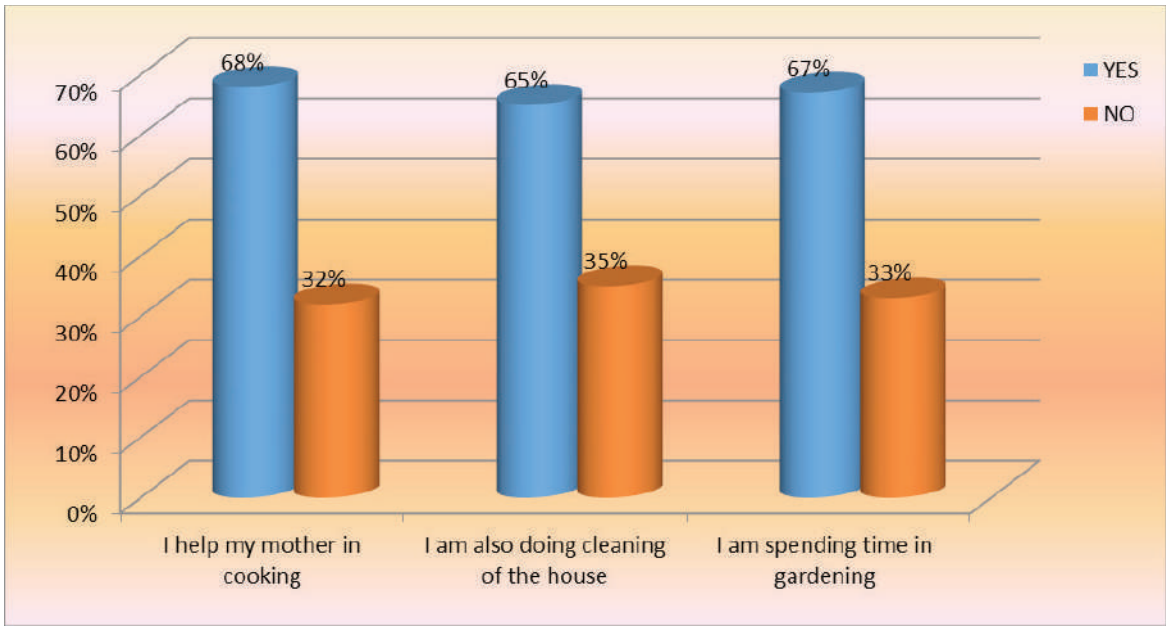
Graph 1: Use of Social Media as coping Mechanism in lockdown among school going children.



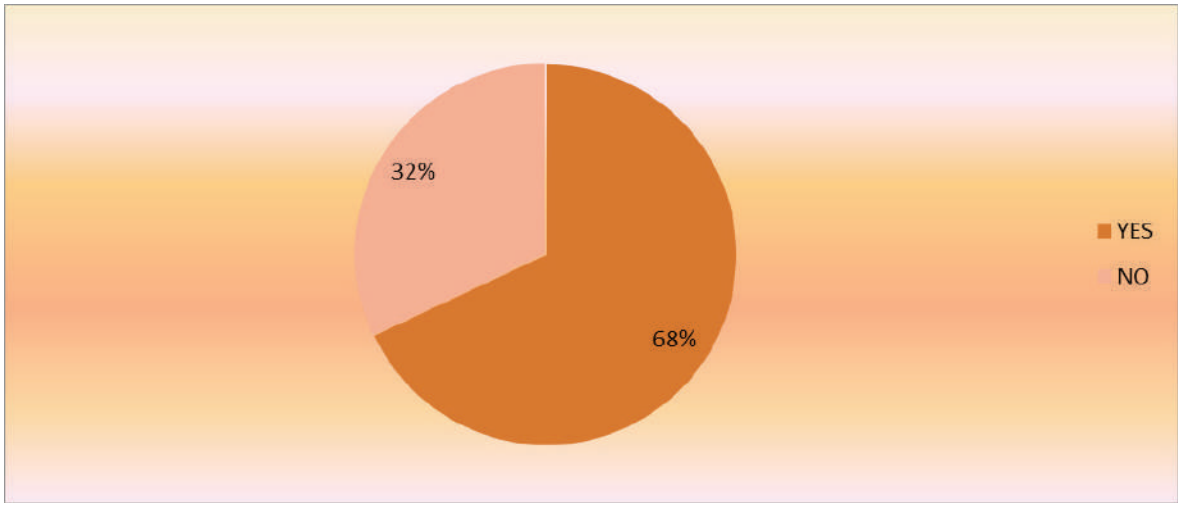
Graph 2: Doing exercise as coping Mechanism in lockdown among school going children.



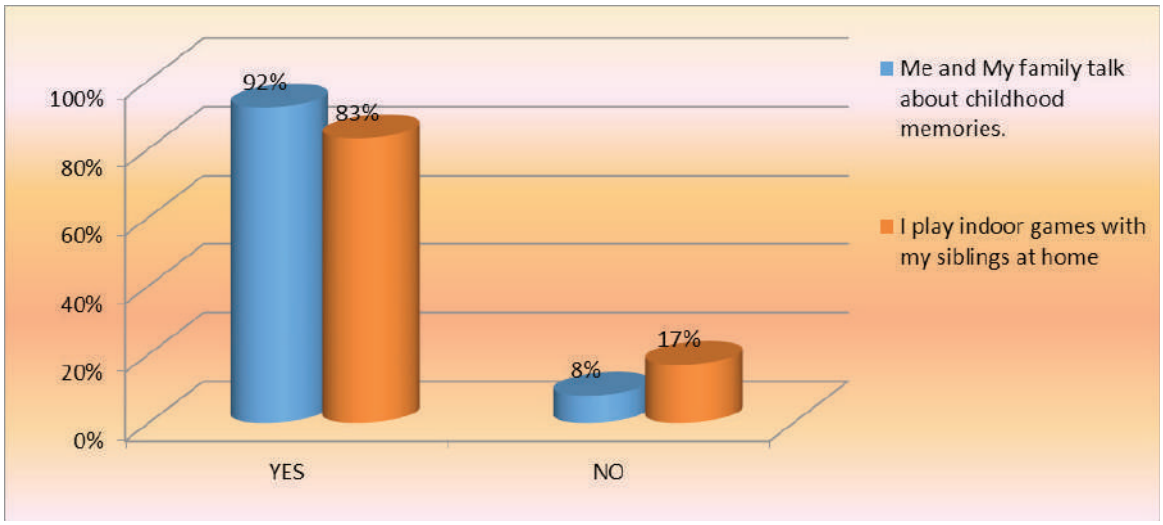
Graph 3: Doing study as coping Mechanism in lockdown among school going children.



Graph 4: Doing study as coping Mechanism in lockdown among school going children



Graph 5: Rest and Sleep as coping Mechanism in lockdown among school going children



Graph 6: Entertainment as coping Mechanism in lockdown among school going children

Discussion

In this present study a Non Experimental Descriptive research design is been used. Non Probability Purposive Sampling Technique was used for selecting 60 samples.Data collected through online Google form from school going children. The tool included three section; the first section had demographic data and the second section included assessment of stress due to lockdown among school going children, Third section included the assessment of coping mechanism due to lockdown. The data was analysed further which revealed that The study findings show that majority (41%) of the children answered were from 10^{th} standard ,30% from 9^{th} standard ,17% from 8^{th} standard and 12% from 7^{th} standard. It also shows that 58% were male and 42% were girl children. The assessment of stress is done by using the questionnaire based on the stress parameter like physical, psychological, family, financial burden,academics,quarrel with siblings, food .The rating were selected from strongly disagree,Disgaree,Neutral,Agree and Strongly agree. The assessment result of stress due to lockdown findings shows that in majority of the children are strongly agree and agree with the physical stress, psychological stress ,family stress, stress due to financial burden, Academics stress, quarrel with sibling and food due to lockdown. The children were using the many coping mechanism to relive this stress and majority of the children were using the coping mechanisms like using social media, exercise, doing study, doing household work, entertainment.

Conclusion

The findings indicate that the questionnaire was an effective strategy in assessing the stress and coping mechanism due to lockdown among the school going children.and it shows that childrens are having stress due to lockdown but they are using the coping mechanism also to get relief from stress.

Conflict of Interest -Nil

Source of Funding- Funded by self

Ethical Clearance-: Obtained from Institutional Research Committee .

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Knowledge and Practices on Intra Venous Cannulation among Student Nurses

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Abstract

Back ground: The study is generally concerned about the care and maintenance of peripheral intravenous (IV) cannulation and to assess the knowledge and practice of student nurses towards care and maintenance of IV cannula. Intravenous cannulation is a commonest procedure performed by student nurses and staff nurses in hospitals and the procedure is closely associated with the risk of nosocomial infections if standard care is not provided. **Design**: Non experimental descriptive study design was carried out and non-probability convenience sampling method used for data collection. **Methods**: Nurses knowledge and practice towards intravenous cannulation were assessed using a validated semi-structured self-administered questionnaire. Reliability was assessed using Cronbach's α coefficient method.Data were analysed through SPSS program and Microsoft excel. **Result** :The findings revealed that 54% respondents were doing correct practices and only 6% shown poor practices in IV cannulation. Most student nurses (68%) has good subject knowledge of caring and maintaining peripheral intravenous cannulation but there were some(8%) without proper knowledge. None of the demographic variables and scores of knowledge and practice has an association except education and the practice score. Education and practice score showed the significant relation. Conclusion: Only 6-8% of total sample expressed poor practices and knowledge . Anyhow this can be dangerous also as student nurses are dealing with patients in hospital. Further education need to be given to the student nurses before practicing on patients.

Key words: *IV cannula, Nursing, Intravenous*

Introduction

Intravenous (IV) cannula support to distribute IV fluids directly into a vein to maintain normal fluid or electrolyte balance.[1]Intravenous Cannula means a tube that can be inserted into the body, often for the delivery or for the gathering of samples.[2]

The procedure of establishing peripheral venous access carries the risk to both the patient and the practitioner. Complications includeinfection, phlebitis and thrombophlebitis, emboli, pain, haematoma, extravasation, arterial cannulation and needle stick injuries[4].Nurses play a vital role in the prevention of such complications like infections. Most of the interventions and prevention strategies such as insertion and assessing intra venous catheter site are part of routine care .The nurse should have accurate knowledge of the preparation and administration of the IV Infusion and IV device. In addition, they should also know about the prevention, treatment and management of local and systematic complications supported by dynamic evidence-based practice guidelines. One of the major risks for phlebitis occurrence is due to the placement and maintenance of IV cannulation by insufficiently trained staff.[5]previous studies suggested that phlebitis is one of the commonest complication and around 31% observer in hospitals however monitoring IV fluids in general admission and ward areas of hospitals is often left to staff nurses and sometimes to student nurses who may lack in required competence so the present study aimed to assess the knowledge and practice towards care of Intra venous cannula among student nurses in selected colleges of Thiruvallur City.

Objectives of Study include 1)To assess the knowledge on Intra venous cannula among student nurses in selected nursing colleges at Thiruvallur District. 2) To assess the practiceson Intra venous Cannula among student nurses in selected nursing colleges at Thiruvallur

District. 3)To associate the demographic variables with the knowledge score 4)To associate the demographic variables with the practice score

Methodology

Non experimental descriptive research approach used for the study .non–probability convenience sampling method was used. Sample size consists of 50 adolescent students studying in under graduate nursing colleges .Student nurses who are willing to participate and Student nurses who are doing second and third year B.Sc. Nursing degree programme were included in the study. Student nurses who are doing GNM, P.B.B.Sc. nursing, M.Sc. Nursing and first year B.Sc. nursing degree were excluded in the study. Sampling technique applied for the study was Purposive sampling technique.

Development and Description of Tool: Part I consist of selected demographic variables questionnaire consist of age, sex, education, and area of residence and year of studya and Part II:Consists of structured questionnaire on knowledge regarding care and maintenance of intravenous cannula like indication, contraindication, risk factors, and complication.

The tool was given to 8 experts in the field of health sciences and content validity checked for the self-structured questionnaire . All the comments and suggestions given by the experts were duly considered and corrections were made. Reliability was assessed using Cronbach's α coefficient method used to check the internal consistency and the value is 0.76 and the instrument is highly reliable.

Result & Discussion

Table 1 :Section I: Frequency and percentage distribution n=50

Sr.No	Demographic Variables	Frequency	Percentage (%)
1	Age		
	17-19 years	13	26.0
	20-22 years	33	66.0
	23- 25 years	4	8.0
2	Gender		
	Male	15	30.0
	Female	35	70.0
3	Education		
	BSc Nursing second year	20	40.0
	BSc Nursing third year	8	16.0
	BSc Nursing fourth year	22	44.0
4	Residence		
	Urban	45	90.0
	Rural	5	10.0

In present study, 26% students were in 17-19 years and 66% were in 20-22 years while 8% were 23-24 years age.30% of samples were male and 70% of samples were female student nurses.40% of students were studying in BSc nursing second year , 16% in BSc nursing third year and 44% in BSc Nursing fourth year respectively. BSc First year students were excluded from the study, as they do not have enough experience in the clinical area .90% student nurses residing in urban area and 10% coming from rural area

Table 2 Section B : Frequency and percentage distribution of level of Knowledge score on Intra venous cannula among student nurses n=50

Sr.no	Level of Knowledge	Frequency	Percentage(%)
1	Good	34	68
2	Average	12	24
3	Poor	4	8

The overall level of knowledge revealed that majority 68% had good knowledge, 24% had average knowledge and 8% had poor knowledge among student nurses.

Table 3:Section B :Frequency and percentage distribution of level of Practice score on Intra venous cannula among student nurses n=50

Sr.no	Level of Practice	Frequency	Percentage
1	Best	27	54
2	Good	16	32
3	Average	7	14

The overall practices score revealed that majority of 54% had best practices, 32% had good practices and 7% shown poor practices towards care of Intra venous cannula among student nurses.

Section C : Association of selected demographic variables with level of knowledge on Intra venous cannula care among student nurses n=50

Sr.No	Demographic variables	Level of significance
1.	Age * knowledge score	.163
2	Gender * knowledge score	.186
3	Education * knowledge score	.197
4	Residence * knowledge score	.101

None of the variables (like age, gender, education, residence) showed the association between the knowledge score.

Section D : Association of selected demographic variables with level of practice on Intra venous cannula care among student nurses n=50

Sr.No	Demographic Variable	Level of significance
1.	Age * Practice score	.045
2.	Gender * Practice score	.046
3.	Education * Practice score	.003
4.	Residence * Practice score	.250

Education of the studentsi.e year of the students and knowledge showed the correlation between the practice. In this study second and third year B.Sc nursing students participated in the study. So level of education have significant association with practice score.

Discussion

The study revealed that majority of student nurses had good knowledge, 24% had average knowledge and 4% had poor knowledge among student nurses. The overall practices score revealed that majority of 54% had best practices, 32% had good practices and 7% shown poor practices towards care of Intra venous cannula among student nurses .The demographic variable of age, gender and education had shown statically significant association with the level of practice towards care of Intra venous cannula among student nurses. There is no association between the demographic variables and knowledge score towards care of Intra venous cannula among student nurses[6]

Student and staff nurses need to reread the IV cannulation steps and maintenance algorithm to empower the knowledge in them. The clinical standard should be improved with the help of clinical IV maintenance algorithms or defined care protocols for IV assessment monitoring and/or management of IV fluid[7].

Conclusion

The findings revealed that 54% respondents were doing correct practices and only 6% shown poor practices in IV cannulation. Most student nurses (68%) has good knowledge of caring and maintaining IV cannulation but there were some (8%) without proper knowledge. None of the demographic variables and scores of knowledge and practice has an association except education and the practice score. Education and practice score showed the significant relation. 6-8% of total sample expressed poor practices and knowledge . Anyhow this can be dangerous also as student nurses are dealing with patients in hospital. Further education need to be given to the student nurses before practicing on patients[8].

Funding: No funding sources

Conflict of Interest: None declared

Ethical considerations: Approval was obtained from Institution Research Ethical and approval Committee. Formal Permission was obtained from the Principal. Indira College of Nursing and also permission was obtained from the selected Hospital of Thiruvallur. Informed written consent was obtained from the student nurses that confidentially, privacy would be maintain, no harm will be inflicted on them, and they can drop out the study anytime if they feel so.

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Effect of lockdown on Students Studying in Various Colleges of Nursing

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Abstract

The pandemic has driven many countries in the world to freeze its economic, social and physical developmental activities, thus has affected the world in every possible ways. India is also imprison under the tight lockdown. Government of India announced curfew on 22nd March, 2020 followed by 1st very uncompromising lockdown phase led the country to put up the shutter in all sector. Additionally, it has also hit the educational sector. Many countries have decided close down institutes, this will definitely have consequences on skill growth. Considering the effects, the current study aims to assess effects of lockdown on nursing students and to find out the association of demographic details with effects of lockdown.

Methodology: The study was conducted among the nursing college of Pune city and non-experimental descriptive research approach was done. The sample size for study was 60, selected by using non probability convenience sampling technique from various students studying in nursing college in Pune city who met the inclusion criteria. A structured modified questionnaire was developed based on the research problem, to get the information from the samples. It was divided in 2 sections; **Section I** consisted of items related to demographic variables of the study. **Section II:** Consisted of 15 questions to assess the effects of lockdown on various aspects i.e social, emotional, financial and academics in students studying in nursing colleges.

Result: The analysis shows that 55% students were somewhat affected and 45% students were very much affected by the lockdown effects. 11.67% students were found to be not at all affected emotionally, 21.67% students were somewhat affected and 66.67% were found to be very much affected emotionally during lockdown. 1.67% of students were not at all affected financially, whereas 66.67% students were somewhat affected and 31.67% students were very much affected by this crisis. Additionally, considering academics 26.67% students were somewhat affected and 73.33% students were very much academically affected due to this COVID-19 pandemic crisis. Since p-values corresponding only to year of programme is found to be less than 0.05, it shows association with lockdown effects.

Conclusion: The current study concludes that COVID-19 has immensely affected Emotional, Financial and academicals aspects of nursing students.

Keywords: *Effects, Lockdown, Students, Nursing College*

Introduction and Background

While the world was running swiftly in the name of progress and economic gain, the COVID-19 has led the world to stop because of its deadly outcome. With the COVID-19 pandemic, most of the countries have announced lockdown and advised people to stay indoors so as to prevent transmission of disease. This has resulted many instances, which were impossible to witness in usual life. The Novel Coronavirus has set a pause button on the world. All over the world pharmaceutical companies and other are battling to find a way of this pandemic but looks like it will take a while to regain its normal life. This lockdown has affected the world economic, social, physical, psychological and moreover educational aspect. It has been more than a month that all the educational institutes are been closed

and we have no idea when it will be reopen. While we are missing out daily routine and outing there is serious issue raising each new day.[1] This is the crucial time for educational sector- board exams, school admissions and entrance tests of various universities are on hold during this period. With no immediate solution to outbreak of COVID-19 the educational institutions will not only have short-term effects but also affect the future economic and societal consequences.

The pandemic has certainly affected the higher education sector as well, which is critical determinant of countries future. Talking about Nursing colleges across the country, the students studying in nursing colleges are getting ready to be the future worriers in healthcare sector but seen to be academically weak with lot many challenges due to the lockdown. Certainly, many nursing institutes have found alternatives for continuing education programmes.[2] Overcoming the challenge in healthcare sector, institutes have started e-learning activity to bridge a gap created by the pandemic. Though the academics are not much affected by the online pedagogies the students studying in various nursing colleges in different year of programme are affected socially, financially and emotionally too. Taking in consideration all the social, financial and emotional aspects the current study was conducted to find out the effects of lockdown on students studying in various colleges of nursing.

Objective:

1. To assess the effects of lockdown on students.

2. To find association of selected demographic variables with lockdown effects

Methodology

Research Design: The researchers have used A Non-Experimental Descriptive Survey. The study was conducted in the selected nursing colleges of Pune City.

Sampling techniques: Non Probability Purposive sampling technique was used and all students from various nursing colleges of Pune City were selected

Subject Size: 60 students; willing to participate and studying in the nursing colleges of Pune city were selected. These participants were clearly informed about the study and its purpose.

Tools of data collection: In the present study the below scales were used to collect the data.

Structured Questionnaire to assess the effects of lockdown on students studying in various colleges of Pune city. It has two sections: **Section I:** Consisted of items related to demographic variables of the study. **Section II:** Consisted of 15 questions to assess the effects of lockdown on students studying in nursing colleges. It was divided in four parts Part A: Effects of Social aspects of lockdown on students. Part B: Effects of Emotional aspects of lockdown on students. Part C: Effects of Financial aspects of lockdown on students. Part D: Effects of Academics aspects of lockdown on students.

Validity and Reliability of tools: Tool was validated by the 5 experts and the reliability was done by Cronbach's Alpha coefficient test. Cronbach's alphas was r=0.76 for assessing the effects of lockdown on nursing students.

Findings

The analysis and interpretation of the data collected to assess the effect of lockdown on various students studying colleges of nursing was done keeping the objective of the study in consideration. 1. To assess the effects of lockdown on students. 2. To find association of selected demographic variables with lockdown effects. The result was divided in III sections

Section I: Description of demographic details. **Section II:** Description of effects of lockdown on students studying nursing colleges. **Section III:** Association of selected demographic variables with lockdown effects.

Section I: Description of demographic details.

Describes the demographic details of Nursing Students to assess the effects of lockdown. 21.7% of students belong to 18-20 age group, 40% belong to age group of 21-23 years, 28.3% were between the age group of 24-26 year and 10% were 27 years and above. Among all the participated students 36.7% were male and 63.3% were female. 15% of students were studying General Nursing Midwifery, 36.67% students were

studying B.Sc. Nursing, 40% were under P.B.B.Sc. Nursing and 8.33% were studying M.Sc. Nursing. Considering year of programme about 41.67% of students were in First Year, 38.33% were in second year, 13.33% were in third year and 6.67% in fourth year.

Section II: Effects of lockdown on students N= 60

Part: A

Social Aspects	Not at all		Somewhat		Very Much	
	Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
	0	0	33	55	27	45

Table 2: Described the Social aspects related to effect of lockdown on students studying in various college of Nursing

Part: B

N=60

Emotional Aspects	Not at all		Somewhat		Very Much	
	Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
	7	11.67	13	21.67	40	66.67

Table 3: Described the Emotional aspects related to effect of lockdown on students studying in various college of Nursing

Part: C

N=60

Financial Aspects	Not at all		Somewhat		Very Much	
	Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
	1	1.67	40	66.67	19	31.67

Table 4: Described the Financial aspects related to effect of lockdown on students studying in various college of Nursing

Part: D **N=60**

Academics	Not at all		Somewhat		Very Much	
	Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
	0	0.00	16	26.67	44	73.33

Table 5: Described the Academic aspects related to effect of lockdown on students studying in various college of Nursing

Section III: Association of demographic details with lockdown effects N=60

Age	**Not at all**	**Somewhat**	**Very Much**	**Chi Square Value**	**P value**	**Remark**
18 - 20	4	4	7	1.67	0.947	Non-Significant
21 – 23	3	3	7			
24 – 26	4	5	6			
27 and above	3	7	7			
Gender	Not at all	Somewhat	Very Much	Chi Square Value	P value	Remark
Male	5	5	8	0.25	0.7	Non-Significant
Female	13	15	14			
Programme	Not at all	Somewhat	Very Much	Chi Square Value	P value	Remark
GNM Nursing	4	6	4	1.66	0.94	Non-Significant
B.Sc. Nursing	3	4	7			
P.B.B.Sc. Nursing	4	5	8			
M.Sc. Nursing	4	5	6			
Year of Programme	Not at all	Somewhat	Very Much	Chi Square Value	P value	Remark
First Year	3	4	6	1.79	0.032	Significant
Second Year	4	7	4			
Third Year	3	8	5			
Fourth Year	4	7	5			

Table 6: Describes Association of demographic details i.e. Age, Gender, Programme and year of programme with lockdown effects.

Discussion

This study was aimed to assess effects of lockdown on students studying various colleges of nursing in Pune city. Hence the data was gathered from the participants and analysis was done to find out the result.

Findings related to effects of lockdown on students studying nursing college

Part A: Social Aspects

Data describes the effects of lockdown on social aspects in students studying nursing college. The parameters measured to assess the effects were: Did the students spend quality time with their family members during this lockdown? Were students able to connect themselves to their loved ones loved ones, did they miss their daily travelling and weekend outings, were the basic necessities of food-grains, fruits, vegetables easily available to students. The analysis says that the social effects towards the above aspects was found to be somewhat affected to 55% students and very much affected to 45% nursing students.

Part B: Emotional Aspects

Data describes the effects of lockdown on emotional aspects in students studying nursing college. The parameters measured to assess the effects were: How much did the fear of getting infected and the associated physical suffering worry the students, How much does the fear of death worries the students, How much does the fear of spreading infection to near and dear ones worries the students. The analysis says that the emotional effects towards the above aspects was found to be not at all affected to 11.67% students, somewhat affected to 21.67% students and very much affected to 66.67% nursing students.

Part C: Financial Aspects

Data describes the effects of lockdown on financial aspects in students studying nursing college. The parameters measured to assess the effects were: Were you facing any financial burden during this lockdown? Was procuring daily supplies challenging to you? Other than basic necessities, were you able to procure other required materials from the market? Were you satisfied with whatever things were available to you? The analysis says that the financial effects towards the above aspects was found to not at all to 1.67% students, somewhat affected to 66.67% students and very affected to 31.67% Nursing students.

Part D: Academic Aspects

Data describes the effects of lockdown on academicals aspect in students studying nursing college. The parameters measured to assess the effects were: Were you studying during this lockdown? Did your academic classes continued during lockdown? Did you face any challenges during online classes/study? Will it be a good decision to cancel your exam and promote to next class? The analysis says that the academic effects towards the above aspects was found to be somewhat affected to 26.67% students and very affected to 73.33% students.

Findings related to association of demographic details with ffects of lockdown on students studying nursing college

Table 6 represents that since the p value corresponding to age, gender and programme are large (more than 0.05) it has no significant association. Whereas p-value corresponding to year of programme is less than 0.05 (0.032) shows significant association with lockdown effects this may be result of fresher students are less affected compared to the final year students, as they may be affected by their final year exam, passing out, placements, etc.

Conclusion

The overall study results indicates that among the social, emotional, financial and academic aspects, the majority students were emotionally, financially and academically affected as a result of lack of connectivity to their loved ones, financial support and worry about the academics, exams and placements. Also the students may be worried about the fear of being jobless.

Source of Funding: Authors have no financial support to this project. The study was fully funded by authors.

Ethical Clearance: Ethical approval of the study taken from Symbiosis College of Nursing ethical committee. Informed consent was taken from the

students studying various colleges of nursing in Pune city. Information given to the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential.

Conflict of Interest: Nil

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A Study to Assess the Effectiveness of Pranic Healing Therapy on Backache among Adults At Pranic Healing Centre in Pune City

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Abstract

Introduction: Pranic healing is a energy based therapy . It activates the healing meachanism in the body **Objective :** To assess the degree of pain among the adult on back pain.,to assess the effectiveness of Pranic healing therapy on backache among adults. And to determine the association between backpain and Pranic Healing therapy with selected demographic variables variables **Methods:**. A quantitative approach and Pre-experimental one-group pretest-posttest research design was used to assess the effectiveness of pranic healing therapy among adults group. study was conducted in a selected Pranic healing therapy centre in Pune city more than 200 adult came for the therapy. Amongst them 40 samples were selected for the study who fulfills the inclusion criteria.. Convenience sampling techniques was used for the selection of samples in this study.Tool included three Sections . Part I – Demographic variable . Part II - modified Bayerchiro's pain scale to assess the symptoms of pain, stiffness, and physical function in patients with back pain. The samples were given a pretest , followed by Pranic healing therapy was given and post test is given. **Result:** Average pain score in pretest was 33.1 which decreased to 29.3 in posttest. T-value for this test was 9.3 with 39 degrees of freedom. Corresponding p-value was small (less than 0.05), null hypothesis is rejected. This is evident that the Pranic healing therapy improved the backache among adults significantly **Conclusion:** After the therapy given to the adult age group with backpain , pranic healing therapy is one of the most important aspect to decrease pain without any side effects

Key words: *Pranic healing; Backache; Effectiveness :Adults*

Introduction

Pranic healing is a healing system considering no touch . Findings show that t Pranic Healing fit the sociodemographic profile of CAM users in the United States, some respondents are experiencing positive outcomes attributed to this modality. [1]

Pranic healing is a energy based therapy . It activates the healing meachanism in the body.[2]

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The study established that pranic healing was effective in enhancing the quality of life of working women employees.[3] Significant change were observed in six domains after the pranic healing , which were Physical fitness , Feelings , Change in Health , Overall Health, Paiand Sleep. [4]

Statement of the Problem

A Study To Assess The Effectiveness Of Pranic Healing Therapy On Backache Among Adults At Pranic Healing Centre In Pune City

Objective of the study

- To assess the degree of pain among the adult on back pain.
- To assess the effectiveness of Pranic healing

therapy on backache among adults.

· To determine the association between backpain and Pranic Healing therapy with selected demographic variables

Hypothesis

· H0: There is no significant difference between mean pre-test and post-test score on pain among adults

· H 0.1: There is significant difference between mean pre-test and post-test score on pain among adults

Material and Method

A quantitative approach was used to describe the existing practices on Pranic healing therapy. The evaluative approach helped the investigator to find out the effect of Pranic healing therapy. Study adopted the Pre-experimental one-group pretest-posttest research design was used to assess the effectiveness of pranic healing therapy among adults group. Inndependent variable is Pranic healing therapy. This study was conducted in a selected Pranic healing therapy centre in pune city more than 200 adult came for the therapy. Amongst them 40 samples were selected for the study who fulfills the inclusion criteria , The accessible population consisted of adult age group who are having back pain and who are available at the time of the study at pranic healing centre. Convenience sampling techniques was used .

Tool included three Sections . Part I – Demographic variable . Part II - modified Bayerchiro's pain scale The samples were given a pretest , followed by Pranic healing therapy was given and post test is given.

Findings

Section I

Table 1: Data of demographic variables

n= 40

Variable	Freq	%
Age		
21-35 years	21	52.5%
36-50 years	12	30.0%
51 and above	7	17.5%
Gender		
Male	29	72.5%
Female	11	27.5%
Employment		
Employed	7	17.5%
Unemployed	33	82.5%
Religion		
Hindu	31	77.5%
Muslim	1	2.5%
Christianity	2	5.0%
Other	6	15.0%
Qualification		
10th standard	1	2.5%
12th standard	1	2.5%

Cont... **Table 1: Data of demographic variables**

Undergraduate	19	47.5%
Postgraduate and above	19	47.5%
Income		
Not applicable	8	20.0%
Upto Rs. 30,000	16	40.0%
Rs. 30,001-50,000	6	15.0%
Above Rs. 50,001	10	25.0%

Section II

Table 2: Description of pain among the adult group by using pain scale **n= 40**

Pain	**Pretest**	
	Freq	**%**
Fairly Severe (Score 54 or higher)	0	0.0%
Moderate (Score 43-53)	5	12.5%
Some problem (Score 30-42)	22	55.0%
Relatively mild (Score 18-29)	13	32.5%

Section III: Data in regards to the effectiveness of Pranic healing therapy on backache

Table 3: shows effectiveness of Pranic healing therapy on backache

n= 40

	Mean	**SD**	**t**	**df**	**p-value**
Pretest	33.1	7.8	9.3	39	0.000
Posttest	29.3	7.3			

Researcher applied paired t-test for the evaluating the efficacy of the Pranic healing therapy on backache p-value was small , null hypothesis is rejected. This is evident that the Pranic healing therapy improved the backache among adults significantly.

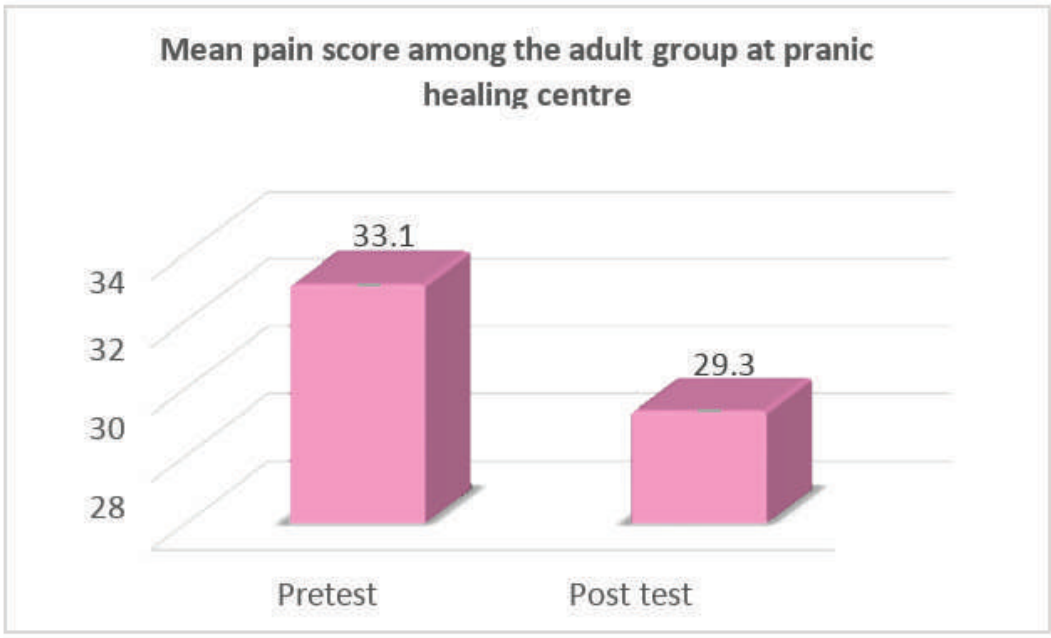


Figure 1: Bar chart representing the distribution of samples according to their average degree of pain before and after intervention of Pranic healing therapy

Section IV : Data in regards to the association between degree of pain and demographic variables

Fisher's test used to determine the association . Since p-value corresponding to demographic variable age is small (less than 0.05), age (p-0.003) shows significant association

Discussion

Study findings revealed that Pranic Healing Therapy is effective in reducing level of backpain. In this study it is noted that Pranic Healing Therapy is effective in reducing level of backpain among adults undergoing the treatment.it is also be effectively used to control acute pain.

Similar findings been seen in study findings where it showed significant decrease in pain level and sympathetic activity in pranic healing group and non-significant change in placebo group.[5]

Conclusion

After the therapy given to the adult age group with backpain , pranic healing therapy is one of the most important aspect to decrease pain without any side effects. This chapter deals with the entire summarization of the search under study in comparision with findings of the present study with available research literatures. It also depicts the suggestions and recommendations that could be necessarily taken in further studies.

Conflict of Interest : Nil

Finding : Self funding

Ethical Consideration: The researcher gained the Institute research Committee approval and Study was approved at Institute Ethical subcommittee. Informed consent was obtained from each sample. Privacy and confidentiality maintained

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Relationship of Environmental Hygiene Practices with Perception of Health among People in Rural Community – A Systematic Review

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Abstract

Background: Environmental sanitation is highly preferred intervention over medical treatment of nutritional deficiencies; respiratory diseases; gastro-intestinal and dermatological issues. This study was an attempt to understand the relationship of Environmental hygiene practices with perception of health among people in rural community. **Methodology**: A systematic review was conducted using the popular PRISMA guidelines that allow us to categorically filter out the research articles giving us a clear picture of the issue under study. 138 research articles were selected out of which various filtering techniques were used and reduced to 6 articles. These articles were analyzed, paraphrased, and reported. **Results**: The studies revealed that environmental sanitation must be given utmost importance as it is the only way out to improve our health and well-being. Good sanitation practices are very much necessary in household, schools, neighboring areas, and all our surroundings. **Conclusion**: Environmental sanitation plays a very important role in uplifting our health status to a higher level. Each individual is responsible for his healthy practices to ensure cleanliness around him.

***Keywords**: Environmental sanitation, perception, health, rural community, systematic review.*

Introduction

Environmental sanitation is the integral part of a healthy living. Healthy environment is possible only after proper planning and implementation of improved environmental sanitation practices. Poor practices have resulted in the development of vector born disease that become a hindrance to health and well-being.[1]

Methodology

This review is conducted by reviewing articles using the selection criteria from Pubmed database from 2015 to 2020 on relationship of Environmental hygiene practices with perception of health among people in rural community. Data was collected using the online search engine. The articles were limited to full text articles. The search was conducted by using key words like Environmental sanitation, perception, health, and rural community. PRISMA Guidelines were used for analysis of data.

Inclusion criteria:

- Articles from Peer-reviewed journal
- Publications from 2015 to 2020
- Cross-sectional designs were included

The filtered articles were then reviewed by the reviewers for their content especially with the results. The Studies with irrelevant titles, unrelated abstracts and study designs were removed. Data was analyzed and results were reported using a narrative synthesis.

Result

Total 138 articles were identified by the review team using PRISMA Guidelines. A systematic review was conducted to find out the relationship of Environmental sanitation practices with perception of health among people in rural community. After a thorough review, six studies were extracted from the selected studies related

to Environmental sanitation practices with perception of health among people in rural community. These results of this study were reported using narrative synthesis and interpreted in the following table.

Fig 1: PRISMA guidelines

Table 1: Systematic Review on relationship of Environmental hygiene practices with perception of health among people in rural community

SN	Authors/ Years	Sample size	Outcome
7.	Andrade, E. L., Bingenheimer, J. B., Edberg, M. C., Zoerhoff, K. L., & Putzer, E. M. (2019).	480	A strong change in behavior is a must to embrace the positive attitude towards good sanitation practices like hand washing, personal hygiene and food hygiene. [2]
8.	Dey, N. C., Parvez, M., Islam, M. R., Mistry, S. K., & Levine, D. I. (2019).	4775	Unclean habits of use of latrine, unsafe disposal of faeces in nearby habitats has endangered the lives of small children and proper monitoring and training of people is much needed to improve the behavior of the people. [3]
9.	Gebremariam, B., Hagos, G., & Abay, M. (2018).	388	Community led total sanitation and hygiene had a positive impact on the behavior of the individuals in the community. Government has a major role to play in the upliftment of the rural community and to empower the people with right information and tight regulations.[4]
10.	Grover, E., Hossain, M. K., Uddin, S., Venkatesh, M., Ram, P. K., & Dreibelbis, R. (2018).	450	Environmental nudges like intense hygiene training has shown an outstanding result and also encouraging.[5]
11.	Shohel, M. M. C., & Howes, A. J. (2018).	70	Many students drop from the schools due to poor sanitation facilities like unhygienic drinking water and unclean toilets.[6]
12.	Lawrence, J. J., Yeboah-Antwi, K., Biemba, G., Ram, P. K., Osbert, N., Sabin, L. L., & Hamer, D. H. (2016).	67	Social taboos proved to be hindrances to use the toilets. Community empowerment is very essential to educate and improve the environmental sanitation.[7]

Looking at the above table it is clear that a set rules and regulations by the local and state governments must be led in order to control the unhealthy practices of the rural population. Over and above this, a reward for good habits can create a win-win situation for both the inhabitants as well as the government.

Discussion

According to a study, community led sanitation initiatives get affected by perceptions of rural population on use of latrine, beliefs of household cleanliness practices over community rather than practicing what is good.[8]

Conclusion

Despite of many benefits of good sanitation, the perceptions of various communities and their beliefs overpower the actual good thing about good sanitation practices. A detailed educational approach may be very much essential to refine the practices of rural population.

Conflict of Interest –Nil

Source of Finding -Self

Ethical Clearance – ethical clearance was obtained from IRC of Symbiosis College of nursing.

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Attitude & Practices of Hospital Staff Towards Safety In A Tertiary Care Hospital, Pune – A Compilation of Safety Experiments

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Abstract

Introduction -Safety is a critical component to the quality health care. The culture of safety extends to patients, staff, caregivers, and attendants and to anyone visiting the hospital. As health care organizations endeavor to improve their quality of care, there is growing recognition of the importance of establishing a culture of safety. With growing awareness of accreditations like NABH and JCI, the focus on safety culture is rising. Safety culture plays a vital role and it comes with hospital team effort, at protecting patients and staff from harm and empowered to do the right thing. The study observes attitudes and practices towards safety culture.

Aim & Objectives – The study aims to conduct various safety experiments to assess the attitudes and observe the practices towards the safety culture. The study also identifies areas for potential safety improvements.

Methodology – The study was an observational one where the response of the staff of the hospital was noted towards a particular pre-planned event leading to breach of safety or deviation from the safety norms. The response of the staff members who have witnessed deviations were noted.

Results & Conclusion - The results showed that most of the hospital staff of this hospital feel positively toward patient safety culture in their organization. The training needs and areas of improvement were identified.. Such safety experiments should be conducted from time to time to know error and event reporting

Key words – *Safety culture, Hospital safety, incident reporting*

Introduction

Safety culture plays a vital role in a hospital and it comes with hospital team effort, at protecting patients and staff from harm and empowered to do the right thing. Essential practices of healthcare employees are often invisible. Also, there is the need for major changes in individual behaviour to shift from the culture of 'blame' to an objective response to errors. This means shifting the blame toward the systems that allow such errors, rather than blaming the individual. Thirdly, healthcare staff's fear of losing others' confidence and trust, and thus, fear of damage to personal reputation, need to also be alleviated.[1]

Patient safety, if not assessed, cannot be improved. Changing the culture, or even a few practices and policies, requires health-care professionals and especially the top-level administrators and their employees to share a common vision. A good safety system depends on having a culture that supports and encourages employees to report their errors and near misses.[2]

Measuring the safety culture will help managers understand its impact on the occurrence of errors. It will help to identify the relative contribution of causal factors

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to errors.[3-4] All staff in hospitals should consider the importance of encouraging and supporting the people they supervise when errors are reported.[5,6]

Promoting a culture of reporting errors is applicable to the patient care environment so that staff members learn from each other and help to avoid the occurrence of future errors.[7,8,9] When addressing safety within one particular health care organization, measuring the existing safety culture may help enlighten management regarding issues that impede making progress in improving safety.[10]

Assessment of the safety culture has been carried out in several ways, one of which is the use of observational experiments. Some experiments used have assessed patient-safety features and evaluate the health-professional's perceptions of actions and behaviours in their work environment. The results will help to determine which actions and behaviours, relative to safety requirements and regulatory goals, should be prioritized for process improvement. Knowledge will help only when put into practice. So there is a dire need to study and observe the practices exhibited by the healthcare staff to create a safety culture.

Aim & Objectives – The study aims to conduct various safety experiments to assess the attitudes and observe the practices towards the safety culture. The study also identifies areas for potential safety improvements. This will help to develop a positive attitude towards safety culture.

Methodology – The study was an observational one where the response of the staff of the hospital was noted towards a particular pre-planned event leading to breach of safety or deviation from the safety norms. The response of the staff members who have witnessed deviations or breach in safety were noted.

Experiments performed to assess attitude and practices towards safety culture

The following experiments were leading to error in the safety protocol were performed.





1. PATIENT FILE LYING ON THE CHAIR AT OPD WAITING AREA

- Experiment duration: 2 hours. One hour at the OPD waiting area.This experiment resulted in the positivity towards the Patient Safety Culture.
- Ten Housekeeping staff noticed the unknown file and then six of them submitted the file at the nearby reception. Four of them reported about the unknown file at the nearby reception area.
- Seven Doctors had noticed the unknown file and then they informed the nearby reception to pick the file and look for the respective person of that file.
- Few patients, relatives and some staff went unobserved. Some staff noticed but ignored.
- This shows areas of weaknesses and improvement scope



2. CIGARETTE BUDS AT SECIRITY CABIN NEAR ATTENDANCE MACHINE

- Experiment duration: 1 hour.
- The security staff who were present at the site informed their supervisor and informed Housekeeping staff to clean the area and then started searching for the person who did the incident.
- Each and every staff who was coming at the site for punching their attendance started informing security about the presence of the cigarette buds at the site.



3. UNKNOWN BAG ON THE PATHWAY NEARBY TO SECURITY CABIN

- Experiment duration: 1 hour.
- Five people form security noticed the unknown bag and by making sure that the bag does not contain any harmful material in it, stored bag at the security office and was searching for the owner of the bag.
- Ten Housekeeping staff noticed the unknown bag at the site and then informed at security cabin about the bag. Seven family members showed the response towards the unknown bag and informed security person at the cabin about the bag.



4. DANGLING WIRE AT ATM SITUATED NEAR HOSPITAL ENTRANCE

- Experiment duration: 1 hour.
- One of the security person noticed the dangling wire and was asking people who were wandering form that area to stay away from that wire.
- Three of the Housekeeping staff shown the response towards that wire by informing security.
- Five of the security staff shown the response by calling the electrician.



5.CARDBOARDS LYING ON THE STAIRS (2nd FLOOR)

- Experiment duration: 1 hour.
- The two-executive people from Housekeeping department who were passing from this site shown the response towards the cardboards and informed Housekeeping person first to pick them up and was interrogating about the incident.
- Ten Housekeeping staff exhibited the response towards the cardboards by lifting them up.
- Five doctors noticed the cardboards and informed security of that floor.

	**6. LAMP POST WIRE LYING OPEN AT STAFF PARKING AREA** ▪ Experiment duration: 2 hours. ▪ The respective security of that parking area noticed the wire and asking staff to walk away from that wire and then he called electrician to disconnect the wire connection and remove that wire. ▪ Each and every staff coming across the respective area shown the response towards it by informing the security about the incident.
	**7. BROKEN TILE KEPT OUTSIDE THE ICU WAITING AREA NEARBY AT CORRIDOOR** ▪ Experiment duration: 1 hour. ▪ Each and every Housekeeping Staff who were shifting patients on wheelchairs etc., shown the response on the broken tiles by picking them up. ▪ Ten consultants and 8 Resident Doctors who came across that area informed security to ask Housekeeping Staff to pick it up. ▪ Some of the Patient relatives informed security about the broken tiles.
	**8. BLOOD STAINED LINEN DUMPED NEAR THE LIFT** ▪ Experiment duration: 2 hours. ▪ At every floor each Housekeeping staff who came over the linen shown the response on that by asking the security at respective floor. ▪ At two of the floors the Housekeeping staff started picking it up by wearing the hand gloves. ▪ Ten patient relatives coming across the linen shown response by informing the security. ▪ Twelve doctors informed the security about the linen. ▪ This portrays the presence of Patient Safety Culture within the Hospital Staff.
	**9. BLOOD STAINED SWABS NEAR BED AT DRESSING ROOM** ▪ Experiment duration: 1 hour.. ▪ The Consultants whoever came in that room asked Nursing Staff to pick them up. Each and every Resident Doctor entered the room responded on this incident by asking Nursing Staff to pick the swabs. ▪ The other Nursing Staff from other departments who visited that room was asking the respective Nursing Staff of that dressing room that why the blood stained swabs are on the floor. ▪ These responses proved the existence of patient Safety Culture within the Hospital Staff.

Conclusion

In general, hospital staff in this hospital feel positively toward patient safety culture within their organization and immediate response was seen to the errors and preplanned events. There were some areas of improvement where staff needs to be more proactive and more alert. Such safety experiments should be conducted from time to time to know the attitudes and practices of staff during error and event reporting.

Ethical Clearance- IEC of Symbiosis International (Deemed University)

Source of Funding- NIL

Conflict of Interest-None

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Students Perception on the Effectiveness of Online Teaching

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Abstract

With the prevalence of online teaching, it's vital to convey student's quality education. Institutes are utilizing online teaching to extend effectiveness furthermore as accessibility of learning throughout this pandemic. Online teaching may be a great platform that provides learners to realize skills and strengthen their knowledge at their own place. [1]

As teachers and support systems to students we would like to provide services which are beneficial as well as useful for the learners and that capture their interest towards online learning. So, to do so it's important to understand students perception regarding online teaching that will lead to student's satisfaction. [2]

The result shows that 73 % of students remained neutral towards satisfaction on instructional design and delivery of a course compared to classroom teaching, 53% of students believe on the importance of their roles and responsibilities to own a positive expertise with an online class and 76% of students agree that teachers and support systems is effective in providing services as required.

Keywords: *Online Teaching, Perception, Effectiveness, Student Satisfaction*

Introduction

A decade past, it absolutely was common to listen that "online learning is the future!" Indeed, it became the future and is now very much the present especially during this pandemic. Online learning is a powerful, versatile medium which will provide transformative learning experiences to our students. [3]

An online classroom is naturally a lot of sophisticated than a face-to-face classroom, and to prevent at the rudimentary basics of a face-to-face course born – again to an internet atmosphere creates disconnect with student expectations of an internet learning atmosphere. [4]

This research aims to explore the undergraduate student's perception on the effectiveness of online experiences at Symbiosis College of Nursing and therefore, the results of this study will be used to develop simpler and more effective ways of online teaching. [5]

Statement of The Problem

'' A study to assess undergraduate students perception on the effectiveness of online teaching at Symbiosis College of Nursing, Pune, Maharashtra''

Objectives of The Study

- To investigate the perception of undergraduate students on online teaching.

- To explore the effectiveness of online teaching.

- To find the association between selected demographic details and students perception on

Online- training.

Methodology

Research approach

- The research approach adopted for this study was a qualitative approach to assess undergraduate student's perception on the effectiveness of online teaching.

Research design

- The research design used in this study was Non-Experimental Descriptive survey.

- In Non-Experimental Descriptive survey the investigator selected the Non - Probability convenience

sampling technique.

- The tool will comprise of following parts

1. Demographic variables

2. Objective type questionnaire.

The tool will be a structured objective type questionnaire in English.

Variables

Dependent variable: - Student perception

Independent variable: - Effectiveness of online teaching

Extraneous variables: -The demographic variables of the student such as age, gender and different batch.

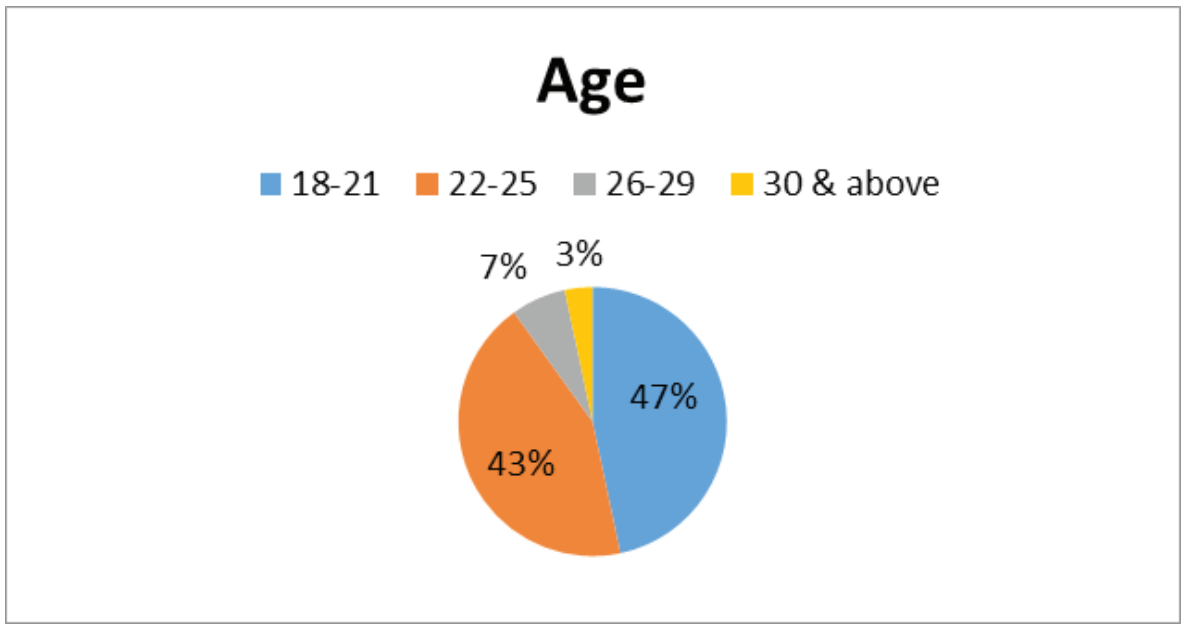


FIGURE 1: Percentage distribution of undergraduate students according to age.

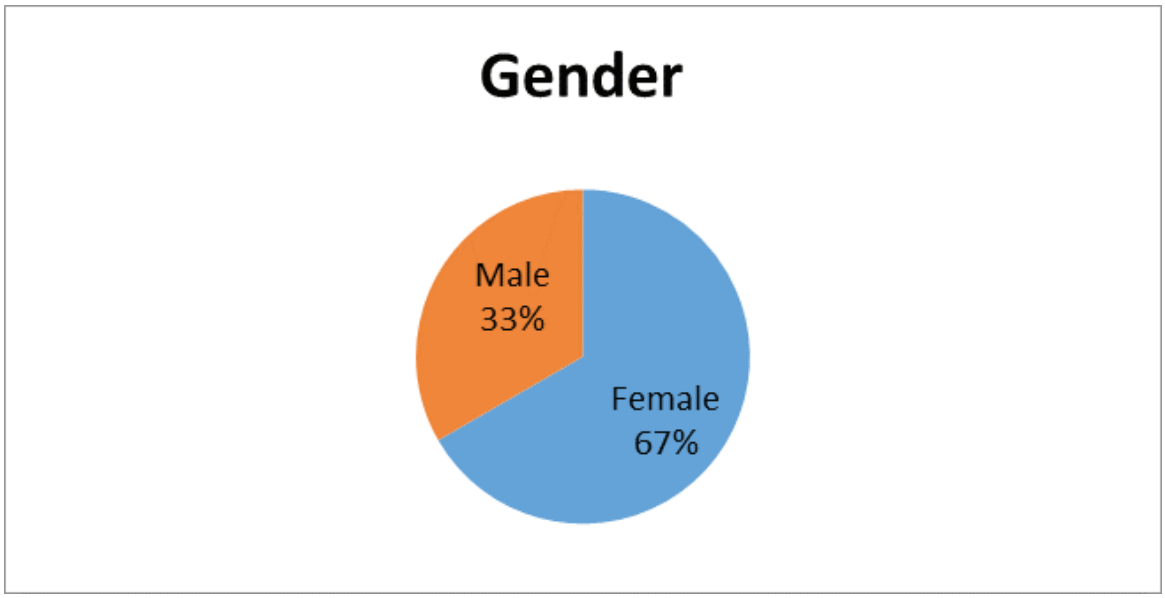


FIGURE 2: Percentage distribution of undergraduate students according to their gender.

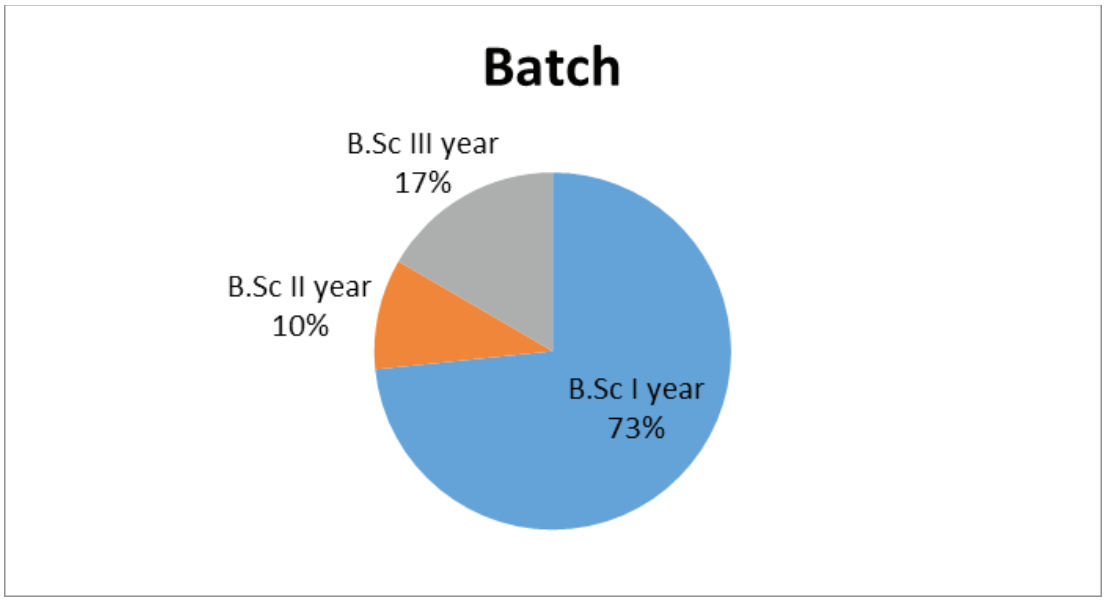


FIGURE 3: Percentage distribution of undergraduate students according to their batch

Setting of the study

The setting was chosen on the basis of the feasibility in terms of availability of the subjects, who were the undergraduate students at Symbiosis College of Nursing.

Sample and sampling technique

60 students were selected by using the Non - Probability convenience sampling technique.

Sample size

Sample sizes of 60 students were selected based on the inclusion and exclusion criteria.

Inclusion criteria:

The study includes undergraduate students who are:

- Present in Symbiosis College of nursing, Pune.

- Willing to participate in the study.

- Able to communicate freely in English.

Exclusion criteria:

The study excludes undergraduate students who are:

- Does not include minimum outcomes.

- Not available during data collection.

Development and description of tool

In this study, structured objective type questionnaire was prepared to assess undergraduate student's perception on the effectiveness of online teaching. A structured objective type questionnaire was developed by the investigator in order to obtain exact and complete response from the students.

The following steps were carried out while preparing the tool.

• Review of literature

• Based on expert opinion

Description of the tool

The tool consists of four sections:

Section A- Demographic data. It includes items of demographic variables (age, gender, and batch)

Section B- Structured objective type questionnaire to describe the student's satisfaction on instructional design and delivery of a course.

Section C- Structured objective type questionnaire to describe the importance of student's roles and responsibilities to have a positive experience with an online class.

Section D- Structured objective type questionnaire to describe teachers and support systems is effective in providing services as needed.

Aspect	Number of items
Demographic data	3
To describe the students satisfaction on instructional design and delivery of a course.	8
To describe the importance of students roles and responsibilities to have a positive experience with an online class.	4
To describe teachers and support systems is effective in providing services as needed.	4

- There were totally 19 items of objective type questions. Every response if 'strongly agree' is given score of 5, if 'agree' score of 4, if 'neutral' score of 3, if 'disagree' score of 2 and if 'strongly disagree' score of 1.

Result And Interpretation:

Section A: Description of the demographic Variables of samples.

Table 1: frequency and distribution of sample according to demographic variable (n=60)

Demographic variable		Freq	%
Age	Between 18-21	28	46.6
	Between 22-25	26	43.3
	Between 26-29	4	6.6
	30&above	2	3.33
Gender	Female	40	66.6
	Male	20	33.3
Batch	B.Sc I year	44	73.3
	B.Sc II year	6	10
	B.Sc III year	10	16.6

Table 1: shows that maximum percentage (46.6%) of subjects were from the age group of 18-21 years wherein (3.3%) of them were in the age group of 30 & above years. Maximum (66.6%) students were female whereas (33.3%) were males. Majority (73.3%) of subjects are from B.Sc I year, (10%) were from B.Sc II year and (16.6%) from B.Sc III year.

Descriptive statistics

• The data was organized in master coding sheet. Frequency and percentage distribution was used to analyze the demographic variables of students such as age, gender and their batch.

• Frequency, percentage distribution, mean and standard deviation were used to analyze the student's perception on the effectiveness of online teaching.

Result

- Majority of the student's satisfaction on instructional design and delivery of a course were neutral (73.3%).

- Maximum students (58.33%) have a neutral opinion regarding importance of student's roles and responsibilities to have a positive experience with an online class.

- Results show that majority of students (76.6%) agree that teachers and support systems are effective in providing services as needed.

Section B: Description of perception of undergraduate students on online teaching.

Table 2: Describes the students satisfaction on instructional design and delivery of a course (n=60)

Item	SA(33-40)		A(29-32)		N(17-28)		D(9-16)		SD(0-8)	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Students satisfaction on instructional design and delivery of a course	4	6.667	12	20	44	73.33	0	0	0	0

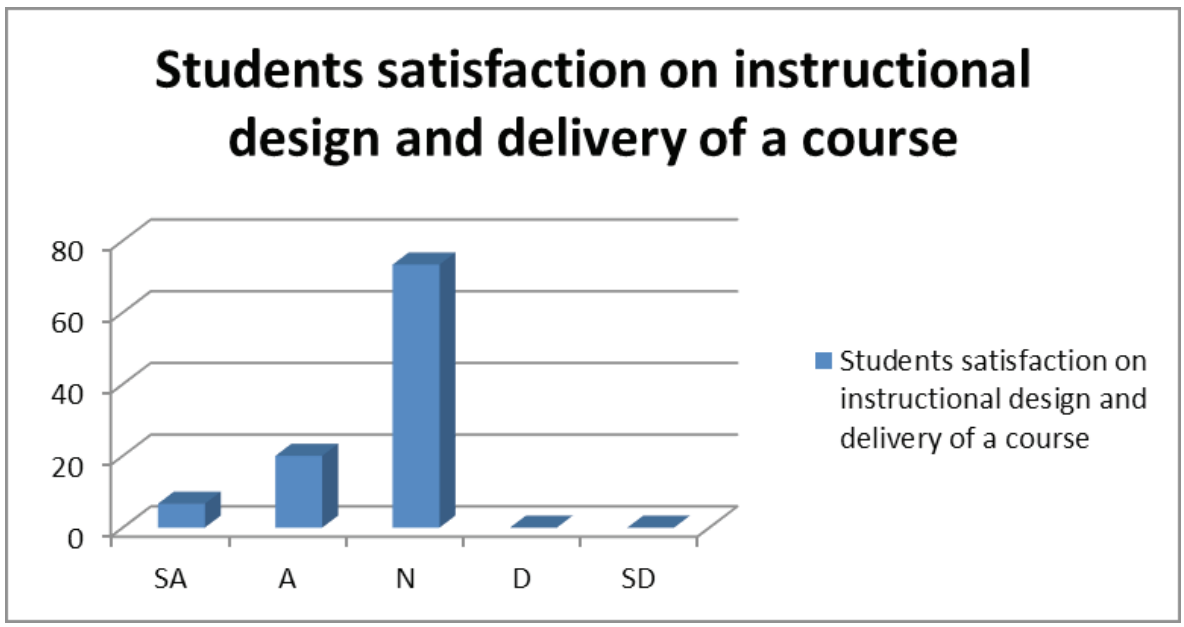


FIGURE 4: Percentage distribution of undergraduate students according to their satisfaction on instructional design and delivery of a course.

Table 3: Describes Importance of student's roles and responsibilities to have a positive experience with an online class. (n= 60)

Item	SA(17-20)		A(13-16)		N(9-12)		D(5-8)		SD(0-4)	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Importance of students roles and responsibilities to have a positive experience with an online class.	2	3.333	20	33.33	35	58.33	2	3.333	0	0

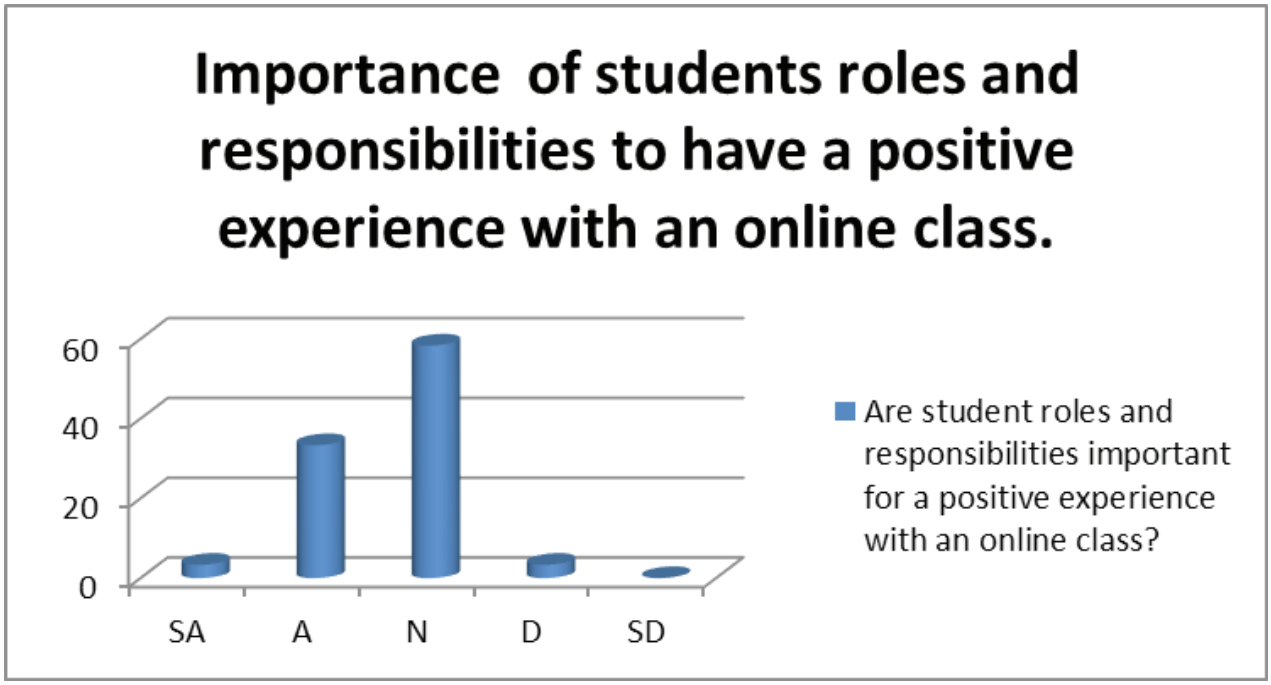


FIGURE 5: Percentage distribution of undergraduate students according to the importance of student's roles and responsibilities to have a positive experience with an online class.

Table 3: Describes Teachers and support systems is effective in providing services as needed. (n= 60)

Item	SA(17-20)		A(13-16)		N(9-12)		D(5-8)		SD(0-4)	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Teachers and support systems is effective in providing services as needed.	0	0	46	76.67	14	23.33	0	0	0	0

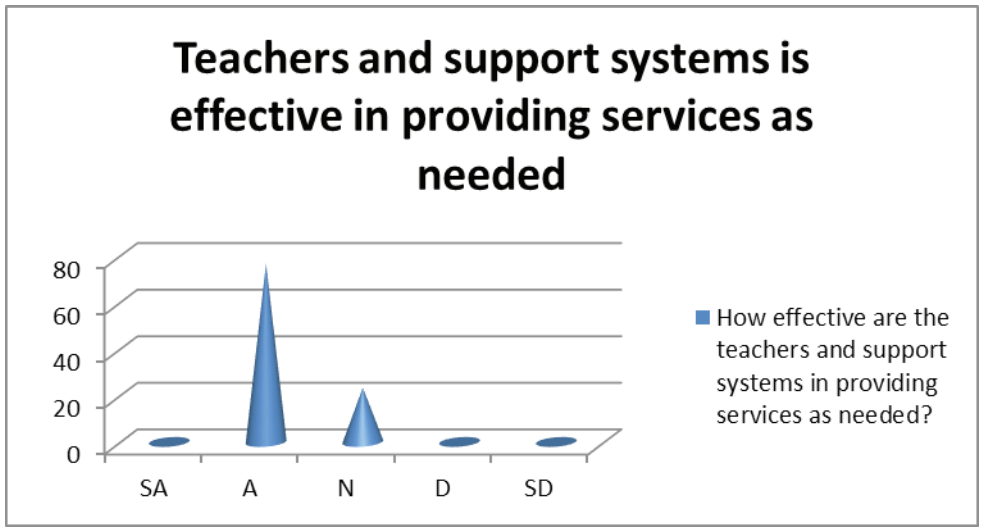


FIGURE 6: Percentage distribution of undergraduate students according to the teacher and support systems is effective in providing services as needed.

Section C: Association between selected demographic details and students perception on online training. (n= 60)

Demographic variables		SA	A	N	D	SD	P value	Remark
Age	Between 18-21	4	3	4	3	3	0.999377	Non -Significant
	Between 22-25	3	2	4	5	3		
	Between 26-29	2	3	3	3	2		
	30&above	3	2	3	2	3		
Gender	Female	6	5	7	6	5	0.990867	Non -Significant
	Male	7	6	7	5	6		
Batch	B.Sc –I year	4	5	5	3	5	0.994065	Non -Significant
	B.Sc –II year	4	4	5	4	4		
	B.Sc –III year	5	3	4	5	4		

Since the P value > 0.05.It says that the demographic variable age, gender and batch does not have any association with the students perception on online training.

Discussion

This current study investigated student's perception on the effectiveness of online teaching. As indicated, solely truthful results were achieved however slightly positive response towards teacher and support systems in providing online services looked as if it would be effective. However, it's vital to lift vital problems with students and supply them insight concerning the importance similarly as implementation of online learning throughout and after this pandemic is foremost important. It suggests that lecturers ought to build an endeavor to integrate and promote larger levels of interaction to create digital platforms fortunate all told means that for college student's satisfaction equally as exploring its full potential. [6]

The problem explicit is "A study to assess undergraduate student's perception on the effectiveness of online teaching at Symbiosis College of Nursing, Pune, and Maharashtra''

The data was collected from 60 students by structured objective type questionnaire.

The findings of the study are mentioned with relation to the objectives, hypothesis.

Conclusion

The COVID-19 pandemic has modified education dramatically, whereby giving rise to E-learning. Students and academics can be confused to handle this situation which they weren't ready for. [8]

However, as state of affairs obtaining worse, it's vital for online teachers to facilitate, act and perceive students perception for the betterment of their future to guide them towards their skilled role. [9]

Non - Probability convenience sampling technique was used to select the samples. The info was collected from 60 students with the assistance of structured objective type questionnaire. On the idea of the findings the subsequent conclusions were created. The findings of the study revealed that Majority of the scholars were neutral on satisfaction on instructional design and delivery of a course (73.3%) and relating to importance of scholars roles and responsibilities to possess a positive expertise with a web category (58.3%). Also, show that bulk of scholars (76.6%) agree that teachers and support systems are effective in providing services pro re nata. Moreover, as students become more experienced towards online learning may be their perception and approaches towards modification may change in future.

Ethical Consideration : For the present study, the investigator took in to consideration the ethical issues. There were no ethical issues confronted while conducting the study.

Conflict of Interest: Nil

Source of Funding: Self

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Perception of Counselors Regarding the Contribution to Patients Care by Student Nurses

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Abstract

Nursing, a profession that recalls the name of the Florence Nightingale is indeed one of the noblest professions in the world that requires lots of love, care and compassion. However availability of nursing professionals in this area is below accepted norms per population. [1]

This study enables the nurses to know how nurses themselves perceive their own profession and see how others perceive their profession. It is also important to encourage positive attitude of nurses or non-nursing professionals towards nursing profession. [2]

On the opposite side it is important to extend patient satisfaction on medical care. The study result helps to general policy makers, authorities in health care system and especially nurses to figure on bringing up nursing as a valued profession so that image of nursing are going to be positively changed in several professional, communities and families. [3]

Keywords: *Nursing profession, patient satisfaction*

Introduction

Nursing education is becoming more demanding each day. Future nurses need to consider theoretical, practical, ethical and legal aspects of their careers especially in the area of rights of the patients. They also have to cater their professional skills to the large number of the needy and the sick. [4]

They have to re-enforce their skills within the practical activity, complementing it with theoretical background and wish to be updated in their knowledge in the era of information technology. Indeed nursing isn't only employment, but a career, profession and also service to mankind. [5]

As professional perception features great influence on the event of nursing student, and thus the longer term quality of nursing, exploring factors that influence this perception are vital. [6]

Therefore, we must endeavor to maintain update knowledge and skills, to enable us render quality care and love to the patient wherever they might be found whether in the hospital, home or community because lack of skill makes the patient lose their trust on the student nurses. [7]

Hence, this study explored counselors' perception of nursing care rendered by student nurses.

Statement of the Problem

"A study to assess the perception of counselors regarding the contribution to patients care by student nurses at Chaitanya Rehabilitation Centre, Pune.

Objectives of the Study

1. To identify the contribution to patient's care by student nurses.
2. To assess the perception of counselors towards the contribution of student nurses to patient's care.
3. To find out the association between perception and demographic variable regarding contribution of student nurses to patient's care.

Methodology

Research design:

Research design used in this study was **non-experimental descriptive design** to find out the perception of counselors regarding the contribution towards patients care of student nurses.

Research approach:

In this study **Explorative research approach** was used.

Variables:

The variables included in this study are:

· Dependent variable **:** The perception of counselors**,** the contribution towards patients' care by student nurses

Setting of the study:

This study was undertaken at the Chaitanya Rehabilitation Centre, Pune city.

Sample and Sampling technique:

In this study, **non-probability convenience** sampling method was used.

Sample size:

Sample size consists of 30 counselors at the Chaitanya Rehabilitation Centre, Pune city.

Criteria for selection of samples:-

Inclusion criteria:

ü Counselors present in Chaitanya Rehabilitation Centre.

ü Those who are willing to participate.

ü Those who understand English.

Exclusion criteria:

ü Other staff members working in Chaitanya Rehabilitation Centre.

ü Those who are not willing to participate.

ü Those who do not understand English.

Tool preparation:

To collect the data for present study, self-structured questionnaire was selected as constructed.

Development of tool:

The tool was developed based on:

ü Related review of literature (NACO guidelines books, journals, reports, and articles, published and unpublished studies) were reviewed and used to develop the tool.

ü Based on expert opinion.

ü Objectives of the study.

A self-structured questionnaire was developed to assess the perception of counselors.

Description of the tool:

A tool consists of two sections as follows:

Section I: Demographic variables (age, gender, education, experience, position, ward).

Section II: Self-Structured questionnaire to assess the perception of counselors regarding the contribution of student nurses towards patient's care.

Blueprint

Table 1.1 depicts the blueprint of the self-structured questionnaire to assess the perception of the counselors.

Sr. No.	Questionnaire	Items No.	Percentage
1.	Punctuality	4	20
2.	Personality	4	20
3.	Discipline	4	20
4.	Patients' care.	8	40

Section III: Association between perceptions with selected demographic variable.

Result and Interpretation

Organisation of the Findings

Presentation of data is organized in two sections.

Section I: Demographic variables (age, gender, education, experience, position and ward).

Section II: Analysis of the data related to assessment of the perception of counselors regarding the contribution of student nurses towards patient's care.

Section III: Analysis of association of perceptions with selected demographic variables.

SECTION I

DEMOGRAPHICAL DATA

Sr. No.	Demographic variable	Frequency	Percentage
1	AGE		
	20-25	10	45.0
	26-30	7	40.0
	31 & above	13	15.0
2	GENDER		
	Male	10	90
	Female	20	10
3	EDUCATION		
	GNM	5	10.0
	B.SC. Nursing	15	70.0
	PB.B.Sc. Nursing	10	20.0
4	EXPERIENCE		
	1 yr	3	10.0
	2 yrs	5	25.0
	3 yrs	7	35.0
	4 yrs	5	25.0
	≥ 5 yrs	10	5.0
5	POSITION		
	counselors	27	90.0
	Incharge	3	10.0
6	WARD		
	Geriatric ward	11	80.0
	Male ward	9	15.0
	Female ward	10	5.0

Analysis of Data related to assessment of the perception of the counselors towards the contribution to patients care by the student nurses.

PUNCTUALITY

Sr. No.	Item	Category	Frequency	Percentage
1.	Morning Reporting Time	Never	0	0
		Rarely	0	0
		Sometimes	5	5
		Most of the times	20	20
		Always	10	10
2.	Completion of assigned work	Never	0	0
		Rarely	0	0
		Sometimes	4	4
		Most of the times	10	10
		Always	16	16
3.	Reporting time after the break.	Never	2	2
		Rarely	2	2
		Sometimes	4	4
		Most of the times	15	15
		Always	7	7
4.	Leaving the ward after the duty.	Never	0	0
		Rarely	0	0
		Sometimes	8	8
		Most of the times	10	10
		Always	12	12

(n= 30)

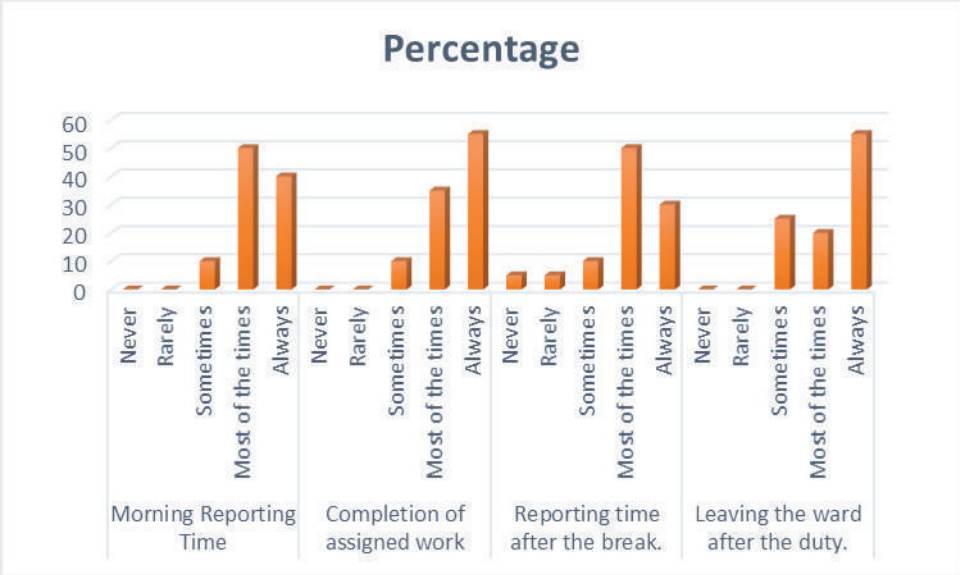


Table 1.3 & Fig 2 depicts the analysis of the perception of the counselors regarding the punctuality of student nurses to the clinical area.

PERSONALITY

Sr. No.	Item	Category	Frequency	Percentage
1.	Properly Groomed	Never	0	0
		Rarely	0	0
		Sometimes	5	5
		Most of the times	10	10
		Always	20	20
2.	Providing help to the nursing officers.	Never	0	0
		Rarely	0	0
		Sometimes	8	8
		Most of the times	15	15
		Always	12	12
3.	Willing to learn new procedures.	Never	0	0
		Rarely	0	0
		Sometimes	9	9
		Most of the times	10	10
		Always	11	11
4.	Maintain good rapport with ward nurses.	Never	0	0
		Rarely	0	0
		Sometimes	5	5
		Most of the times	18	18
		Always	7	7

Table 1.4 depicts the analysis of the perception of the counselors regarding the personality of student nurses in the clinical area.

DISCIPLINE

Sr. No.	Item	Category	Frequency	Percentage
1.	Following instructions given by Nursing Officers.	Never	2	2
		Rarely	3	3
		Sometimes	5	5
		Most of the times	5	5
		Always	15	15
2.	Wishing the Nursing Officers.	Never	5	5
		Rarely	13	13
		Sometimes	5	5
		Most of the times	0	0
		Always	8	8
3.	Behave well in the hospital premises	Never	0	0
		Rarely	0	0
		Sometimes	6	6
		Most of the times	15	15
		Always	9	9
4.	Use of mobiles during clinical hours.	Never	2	2
		Rarely	3	3
		Sometimes	8	8
		Most of the times	10	10
		Always	7	7

Table 1.5 depicts the analysis of the perception of the counselors regarding the discipline of student nurses in the clinical area.

PATIENT CARE.

Sr. No.	Item	Category	Frequency	Percentage
1.	Respond to patients when needed.	Never	0	0
		Rarely	3	3
		Sometimes	8	8
		Most of the times	11	11
		Always	8	8
2.	Aware of their responsibilities in the hospital.	Never	0	0
		Rarely	5	5
		Sometimes	16	16
		Most of the times	7	7
		Always	7	7
3.	Have knowledge about basic procedures.	Never	0	0
		Rarely	0	0
		Sometimes	6	6
		Most of the times	12	12
		Always	12	12
4.	Provide comprehensive patient care	Never	3	3
		Rarely	3	3
		Sometimes	6	6
		Most of the times	6	6
		Always	12	12
5.	Provide atraumatic care to the patients	Never	0	0
		Rarely	5	5
		Sometimes	6	6
		Most of the times	13	13
		Always	6	6
6.	Maintaining confidentiality of patients' details.	Never	0	0
		Rarely	0	0
		Sometimes	18	18
		Most of the times	10	10
		Always	2	2
7.	Conduct procedures skillfully.	Never	3	3
		Rarely	0	0
		Sometimes	6	6
		Most of the times	8	8
		Always	13	13
8.	Maintain sterility when performing procedures	Never	0	0
		Rarely	3	3
		Sometimes	5	5
		Most of the times	13	13
		Always	9	9

Table 1.6 depicts the analysis of the perception of the counselors regarding the patient care provided by student nurses in the clinical area.

Discussion

In the present study, it was based on the clinical learning environment of the student nurses. This is a quantitative study. The numbers of participants or samples used are 30. The data collection method used is the self-structured questionnaire. In this research, the counselors have a positive perception regarding the communication skills, theoretical knowledge and practical skills of the student nurses. The counselors have a positive perception of the contribution of the student nurses irrespective of the challenges faced by the student nurses in the clinical practice environment.

According to the students, the hospital setting provided direct contact with routine nursing care and patients. The students appreciate learning from professional practice combined with scientific knowledge, which can be reverted to change their future practice." and "The teaching and learning process: strategies used.

Conclusion

The study revealed the importance of integrating students within the hospital context to reinforce learning and permit greater approximation with the truth of the nursing profession. The partnership between education and repair was also highlighted, which stresses the necessity for permanent education that serves as a tool of reflection for professors and nursing professionals, and enhances curricular reorganization. [8]

In present study, exploratory research design with quantitative approach was used. All the data in this study was collected from counselors. This study has also revealed the importance of the integration of the student nurses in the hospitals as the counselors have positive perceptions regarding their contribution towards patients care. The immersion of the student nurses in the hospital learning has also affected their contribution to patients care.

Ethical Consideration: For the present study, the investigator took in to consideration the ethical issues. There were no ethical issues confronted while conducting the study.

Conflict of Interest: Nil

Source of Funding: Self

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Effectiveness of Instructive Bookleton Knowledge About Organ Donation among GNM Nurses Working at Selected PHCs of Pune

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Abstract

Background: At the point when an individual permits their very own organ to be evacuated and relocated to someone else, legitimately, either by assent while the benefactor is alive or dead intends to organ gift. To comprehend Organ Donation, it is first basic to comprehend organ Transplantation. An exchange is a clinical technique where one individual's broken organ or tissue is supplanted by that of a solid individual; in this way re-establish its capacity. The organ gift and transplantation framework endeavour to respect the endowment of gave organs by completely utilizing those organs to spare or improve the greatness of the lives of relocate beneficiaries.

Objectives: 1. Toassess the knowledge about organ donation among GNM nurses working at selected PHCs of Pune, 2. To evaluate the effectiveness of instructive booklet on knowledge about organ donation, amongstGNM nurses working at certain PHCs of Pune.

Methodology: Study is quantitative-one group pre-test post-test design. Collected information by self-structured questionnaire by purposive sampling method. The questionnaire had two sections, to gather information regarding demographic variables, knowledge on organ donation.

Result: Assessed the knowledge regarding organ donation and result were found as: 56.4% of the GNM nurses had poor knowledge and 43.6% of them had average knowledge. In posttest, 14.5% of the GNM nurses had poor knowledge, 25.5% of them middlinginformation and 60% of them had superiorunderstandingon the subject of organ endowment.

Conclusion: Study revealed that instructive booklet is effective to increase the knowledge on organ donation among GNM nurses.

Key Words: *Organ Donation, knowledge, informative leaflet, GNM nurses.*

Background

Yearly musings of lives are lost in India from heart and liver disappointment since transplantation of unpaired organs like heart, liver and pancreas is either troublesome or outlandish from living contributors.This is just conceivable for an enormous scope if these organs are accessible from corpse benefactors.[1]

While passing removes you, your organ can remain and spare another life. The requirement for organ gift is rarely more noteworthy. In India the greater part a million Indians are evaluated to be in require of an organ relocate.[1]

In any case, not exactly a thousand transfers from expired givers are played out every year an extraordinarily little and insignificant number contrasted with the figures far and wide. A portion of these individuals will locate a

living giver who will give an organ to them. The rest will most likely die hanging tight for an organ.[2]

The organ gift and transplantation framework endeavours to respect the blessing of gave organs by completely utilizing those organs to aggregate the nature of the lives of relocate beneficiaries. Because of advances accomplished through fundamental and clinical examination in the course of recent decades, organ transplantation has become the ideal treatment for some, end-stage organ-explicit sicknesses.[2]

Advance in clinical science have made transplantation of essential human organ potential a great many individuals in India lose then lives on the grounds that the givers are not accessible in satisfactory numbers. In excess of one lakh fifty thousand individuals are determined to have end point kidney illness consistently in India.[3]

Subsequently basic activity is required on a few fronts if the intense inadequacy of human organs in India must be met. Readiness ought to be expanded about the advantages of organ gifts through the methods for mass correspondence and odd notions among individuals ought to be battled by the method of strict orders.[4]

Objective

1. To assess the knowledge about organ donation among GNM nurses working at selected PHCs of Pune.

2. To evaluate the effectiveness of instructive booklet on knowledge about organ donation, amongst GNM nurses working at certain PHCs of Pune

Methodology

Design of the study is quasi- experimental research. A total 55 participants were selected for the study to find out the effectiveness of instructive bookleton knowledge about organ donation among GNM nurses working at selected PHCs of Pune. The data of this study were collected by self-structured questionnaire by purposive sampling method. The questionnaire had two sections, to gather information regarding demographic variables, knowledge on organ donation.

Analysis and Interpretation

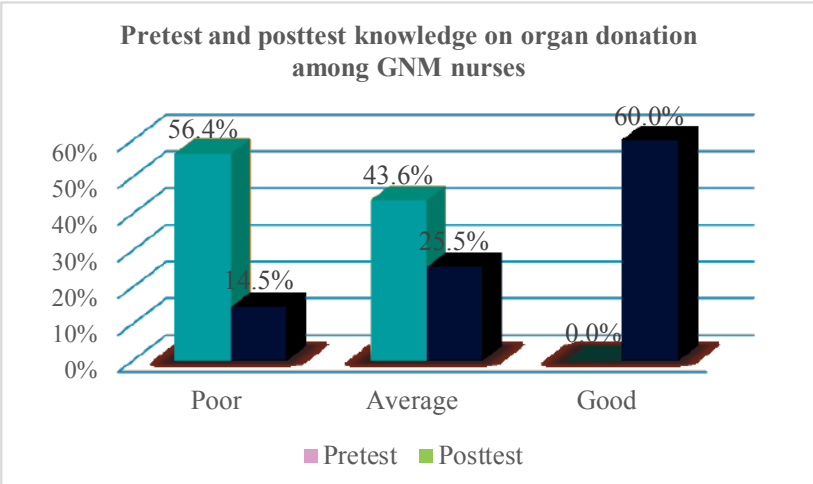
In this study demographic data was described like, 17 (30.9%) samples in the age group of 20 to 30 years, 26 (47.28%) samples in the age group of 31 to 40 years and 12 (21.82%) samples in the age group of 41 to 50 years.

Regarding gender all GNM nurses was females. Regarding religion, 33 (60%) were Hindu, 15 (27.27%) were in other religion, 04 (7.28%) were Christian, 03 (5.45%) were Muslim.

Regarding year of experience 23 (41.82%) were having less than 2 years working experience, 19 (34.54%) were having less than 2.1 to 5 years working experience and 13 (23.4%) were having more than 5.1 years working experience.

Before administering instructive booklet of organ donation 56.4% of the GNM nurses had poor knowledge and 43.6% of them had average knowledge.

Effect of instructive bookleton knowledge about organ donation among GNM nurses



At baseline, 56.4% of the GNM nurses had poor knowledge and 43.6% of them had average knowledge. In posttest, 14.5% of the GNM nurses had poor knowledge, 25.5% of them middling information and 60% of them had superior understanding on the subject of organ endowment.This shows that after administering instructive booklet,there is remarkable improvement in the knowledge of the GNM nurses regarding organ donation.

Paired t-test for the effect of instructive bookleton knowledge about organ donation among GNM nurses

N=55

	Mean	SD	t	df	p-value
Pretest	7.9	2.3			
Posttest	17.2	5.0	14.7	54	0.000

Researcher applied paired t-test for the effect of instructive bookleton knowledge about organ donation among GNM nurses. Average knowledge score in pre-test was 7.9 which increased to 17.2 in post-test. T-value 14.7 with 54 degrees of freedom. Subsequent p-value was small and order of 0.00 its less than 0.05, the null hypothesis abandoned. Instructive bookletwasconsiderable effective in improving the knowledge of the GNM nurses regarding organ donation.

Discussion

The post-test knowledge, 14.5% of the GNM nurses had poor knowledge, 25.5% of them middling information and 60% of them had superior understanding on the subject of organ endowment.Same investigation got contrasted and these examination which was distributed in 2017, Apoorva Sindhu and et. al has directed the investigation to evaluate the information on clinical understudies with respect to organ gift in a chose school of western Maharashtra and the outcomes indicated that 87% of the understudies had right information in regards to cerebrum passing happening after street car crash. Fifty-four percent (54.45%) of the understudies didn't know that an individual experiencing irresistible infection can't give organs. 61% of the understudies didn't know that a National Network for organ gift exists in their city. 48.5% of understudies didn't have a clue how to enroll oneself as organ giver. The aftereffects of the examination uncovered that there exists an information hole among the clinical understudies in regards to organ gift and there is a critical requirement for tending to this information hole which help in improving the organ gift rate in our nation.[5]

So as to discover the critical distinction between the mean of pre-test and post-test information scores of the GNM attendants in regards to organ gift, combined 't'- test was registered. Normal information score in pre-test was 7.9 which expanded to 17.2 in post-test. T-esteem for this test was 14.7 with 54 degrees of opportunity. Comparing p-esteem was little and request of 0.000 (under 0.05), the invalid speculation is dismissed. Informational booklet was critical compelling in improving the information on the GNM medical attendants with respect to organ gift. Same investigation got contrasted and these examination which was distributed in April (2015) S. Pauline Sheela Priya and et. all subsequent to dissecting the information they found that there was noteworthy ($p< 0.001$) improvement in post-tests information scores. The information improvement mean score was 6.2 with the't' esteem 17.2 which shows the viability of organized educating program.[6]

Conclusion

The instructive bookletwas found to be very effective in improving the knowledge among GNMnurses regarding organ donation. The knowledge regarding organ donation was improved by education, as it is found that post-test score is higher than pre-test score. Being a nursing profession it's our responsibility to try to make each citizen of India aware of organ donation by organizing various means of health education programme.

Conflict of Interest – Nil

Source of Funding - Self

Ethical Clearance – Obtained from Institutional Research Committee of Symbiosis College of Nursing

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Knowledge Regarding IMNCI among Health Workers

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Abstract

Background: Successful approaches and projects are critical to the venture of solid propensities and the alleviation of hazard factors for ailment. The Integrated Management of Child Illness (IMCI) system was presented in the principle focal point of the system was on improving clinical consideration given at wellbeing offices to outpatient debilitated kids. IMCI expects to decrease demise, sickness and inability. IMCI incorporates both preventive and remedial components that are actualized by families and networks just as by wellbeing offices.

Objectives: 1. To assess the knowledge of health workers regarding IMNCI, 2. To evaluate the effectiveness of Structured teaching programme on IMNCI, 3. To discover a relationship between pre-test information scores and chose segment factors.

Methodology: Evaluative research approach used for this study. Pre- experimental (One group Pretest – posttest) design carried out for the Study. Collected information by self-structured questionnaire by purposive sampling method.

Result: Assessed the knowledge regarding IMNCI among health workers and result were found as: pre-test majority of the health workers 60% common knowledge, 26% poor knowledge and 14% good knowledge. Whereas, in post-test 68% of usual knowledge, 24% had good information & 8% poor knowledge on the subject of IMNCI among health workers.

Conclusion: Study revealed that effectiveness of structured teaching programme is effective to increase the knowledge on IMNCI among health workers.

Key Words: *IMNCI, knowledge, structured teaching programme, Health workers.*

Introduction

The wellbeing of the kid populace is intelligent of the to a great extent soundness of a Nation, and has numerous ramifications for the Nation's future as these kids develop into grown-ups. Physical, mental, and enthusiastic wellbeing influence for all intents and purposes each aspect of life, for example, learning, interest in relaxation exercises and so forth.[1] Wellbeing propensities perceived in youth regularly proceed all through the life expectancy, and numerous medical issues in adolescence, for example, weight and helpless oral wellbeing, impact wellbeing into adulthood.[2] Successful approaches and projects are critical to the venture of solid propensities and the alleviation of hazard factors for ailment.[3] All things considered, the wellbeing and medicinal services needs of kids change after some time, and current information on these issues is fundamentally significant as strategy creators and program organizers try to misuse the strength of kids later on.[3]

The Integrated Management of Child Illness (IMCI) system was presented in the Eastern Mediterranean Region in 1996. At first, the principle focal point of the system was on improving clinical consideration given at wellbeing offices to outpatient debilitated kids.[4] Throughout the years, more consideration has step by step been given to completely incorporated childcare, tending to wellbeing, development and advancement of any little youngster, regardless of whether debilitated or sound, whether taken to wellbeing offices or thought

about in the home. The methodology has then expanded its degree in this Region and changed into the Integrated Management of Child wellbeing, while as yet holding its unique abbreviation "IMCI".[5] More accentuation is likewise being put on advancing great youngster care rehearses at home and the network. IMCI is a coordinated way to deal with youngster wellbeing that centers on the prosperity of the entire kid. IMCI expects to decrease demise, sickness and inability. IMCI incorporates both preventive and remedial components that are actualized by families and networks just as by wellbeing offices.[6]

Statement of Problem:

"Effectiveness of structured teaching programme on knowledge regarding Integrated Management of Neonatal and Childhood Illness among health workers working for PHCs of Pune

Objectives of The Study

1. To assess the knowledge of health workers regarding IMNCI

2. To evaluate the effectiveness of Structured teaching programme on IMNCI

3. To discover a relationship between pre-test information scores and chose segment factors.

Hypothesis:

Ø H1- The mean post-test information scores will be higher than the mean pre-test information scores of health workers with respect to coordinated administration of neonatal and youth ailment at the 0.05 degree of significance.

Ø H2- There will be critical relationship between pretest information scores and chose segment factors.

Research Methodology

Research Approach:

Evaluative approach used for this study.

Research Design

Pre- experimental (One group Pretest – posttest) design carried out.

Results

· Findings related to socio-demographic variables.

The majority of health workers23 (46 %) were in the age group of 30-35, 36(72%)health workers were female. Majority of health workers27(54%)were studied ANM. Majority of health workers 36 (72%) had 3-6 years of work experience. 70% of health workers had previous source of information on IMNCI.

· **Health workers frequency and percentage distribution regarding knowledge scores of IMNCI.**

n = 50

	PRE-TEST		**POST-TEST**	
	Frequency(f)	**Percentage**	**Frequency(f)**	**Percentage**
Good	7	14%	12	24%
Average	30	60%	34	68%
Poor	13	26%	4	8%

Above table reveals that in pre-test majority of the health workers 30 (60%) common knowledge, 13 (26%) poor knowledge and 7 (14%) good knowledge. Whereas, in post-test 34(68%) of usual knowledge, 12(24%) had good information & 4 (8%) poor knowledge.

Mean difference, Standard Error of Difference, hypothesis and paired't' values of knowledge score on IMNCI. n=50

Mean Difference (d)	Standard Error Difference (SEd)	Paired 't' Values	
		Calculated	Tabulated value
22.38	0.62	39.79*	1.96

Above table uncovers, that determined paired't' esteem (t=39.79) is more noteworthy than organized worth (t=1.96). Thus H1 - The mean post test information scores was higher than the mean pre test information scores of wellbeing laborers with respect to coordinated administration of neonatal and youth disease at the 0.05 degree of essentialness was acknowledged.

Association between the existing pre-test knowledge of health workers on IMNCI and selected demographic variables.

It was evidenced a statistically significant association involving year experience as calculated value 13.9 is greater than table value 12.5, and previous sources of information as calculated value 41.6 was greater than table value 21.02, with knowledge score regarding IMNCI at the 0.05 level of significance.

Discussion

knowledge scores of health workers regarding IMNCI present examination it shows that pre-test lion's share of the wellbeing workers 30 (60%) had normal information, 13 (26%) had helpless information and 7 (14%) great information. While, in post test 34 (68%) of had normal information, 12 (24%) had great information and 4 (8%) helpless information.

Similar study was conducted by Sruthi Kanuganti 2015, in that study it was seen that, majority of Health Care Providers 26 (86.66%) were Inadequate Knowledge whereas in Post-test majority 22 (73.3 %) were Adequate Knowledge regarding IMNCI among health care providers.

Effectiveness of structured teaching programme INMCI.

Present investigation it shows that post test mean estimation of information was 34.08 with SD of 3.16, which was higher than the pretest estimation of information was about 11.7 with SD of 3.6. The mean distinction among pretest and post test information was 22.38 and the got 't' esteem was 39.79 which was noteworthy at (p=0.05). The determined 't' esteem is more than the table worth, which was critical at 0.05 level.

Similar study was conducted by Sruthi Kanuganti 2017, The Paired 't' test value for Knowledge between Pre and Post-test was 18.293, when compare to table value 2.05, it was high. There was highly significant effectiveness on Knowledge regarding IMNCI among Health Care Providers.

One more comparable investigation was directed by Pooja jishi et al 2018, in that review it uncovered that, that the mean pre test information on nursing understudies was 11.05 and the mean post test information on the nursing understudies was 25.19. The 't' esteem for complete pre test and post test was 42.22. There was huge contrast between pre test information score and post test information scores. Mean change in pretest and post test was 14.14. Examination of studies it demonstrates that organized encouraging project was exceptionally successful in expanding the information on with respect to INMCI.

Conclusion

The structured teaching programme was found to be very effective in improving knowledge among health workers regarding IMNCI. The knowledge regarding

IMNCI was improved by education, as it is found that the posttest score is higher than pretest score. Being a health profession, our responsibility is to try to make each citizen of India aware of IMNCI by organizing various means of the health education program.

Source of Funding: Self

Conflict of Interest : Nil

Ethical Clearance: Institutional Research committee

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A Study to Assess the Effectiveness of Structured Education on Prevention of Human Papillomavirus Infection among Adolescent Girls in a Selected School at Pune City

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Abstract

***Objective*:** "A study to assess the effectiveness of structured education on prevention of human papillomavirus infection among adolescent girls in a selected school at Pune city"

***Methods*:** A non-experimental descriptive design and quantitative approach was carried on 64 adolescent girls in a selected school at Pune city

***Results*:** In this study, it revealed that with regard to the pre-test knowledge assessment the mean percentage scores was 61.75 with mean and 24.6 ±2.94. Area wise mean percentage of knowledge scores was 70.27% in the area of "General concept regarding reproductive organs" With the mean of 7.73 ± 0.61. In the area of "knowledge regarding cervical cancer" the mean percentage was 54.75 with mean 8.76 ± 2.67 and in the area of "knowledge regarding HPV prevention" the mean percentage was 63.15 with mean 8.21 ±2.57. The analysis of the study revealed a highly significant difference in the post test knowledge scores and the mean gain 93.65%.

***Conclusion*:** Considering above findings the study showed that structured education was effective in improving knowledge of adolescent girls on prevention of human papillomavirus infection.

***Keywords*:** *Effectiveness, structured education, human papillomavirus infection, adolescent girls.*

Introduction

Cancer is one of the second leading cause of death in all the countries especially the developing one after the cardiovascular diseases.Talking about the European and American Countries almost every fifth death is due to cancer.[1] As per World Health Organization, approximately 50 million mortality rate counts with cancer annually. With the increase in the life span of human beings in the demography of India, more than 5 lakh newly emerged cases of cancer occur every year.[2]

Indian cancer society stated in their report about the point prevalence of cancer which is crossing over 1.5 million. In India, access to screening is limited due to lack of wakefulness of women on HPV vaccination in preventing cervical cancer. One of the major reason of increase is cervical cancer which can be usually treated by hysterectomy but if it evades beyond the uterus, then continuous chemotherapy is the way forward.[3]

But situation is not so easy, as even despite chemotherapy more than 33% of womenwho already got treated showed relapse will relapse and increased mortality graph within 5 years after taking the treatment.[4]

Human papillomavirus is commonest causes of the sexually transmitted infections. Therefore Cervical cancer is considered and leading the board with second most leading cancer among women. Situation is even worse in case of sexually active women where more than 50% of them are exposed to at least one HPV type.This infections peaks at within 5–10 years of the first sexual experience and the highest rates seen between the agesof 20–24 years.[5]

Adolescent period is considered as one of the crucial phase of human development. They will be the parents of near future. The health of adolescents develops based on already existing family norms and conditions, schoolenvironment,existing community, and recreational activities. They need to be educated about these crucial subjects to create an awareness among Otherwise If their needs are not addressed properly it will landed up in the troublesome conditions.[6]

Objective of the study:

- To assess the knowledge regarding prevention of Human Papilloma Virus among adolescent girls
- To assess the effectiveness of structured education on prevention of Human Papilloma Virus among adolescent girls

Methodology

The design of the study is a quantitative one group pretest and posttest design. A total of 50 participants were selected for the study to find out the effectiveness of structured education program on knowledge about prevention of Human Papilloma Virus among adolescent girls in Pune city. The data of this study were collected by self structured questionnaire using the convenient sampling method. The questionnaire had two sections, namely to gather information regarding demographic variables and knowledge on organ donation.

Analysis and interpretation:

Percentage distribution of demographic variables of adolescent girls:

Percentage distribution of adolescent girls according to their age group incompleted years shows the highest percentage 70% of the respondents were inbetween the age 12-14 years and 30% of respondents were in 15-17 years. Percentage distribution of adolescent girls in relation to the course of study shows that 60% were studying 9th standard and 40% were studying 10th standard. Percentage distribution of adolescent girls in relation to the course of study shows the majority were from 44% Muslim, 38% were from Hindu and remaining 18% were from Christian religion.

Comparison of the pretest and posttest knowledge score about prevention of Human Papilloma Virus of adolescent girls:

56% of adolescent girls were having inadequate knowledge, 38% of adolescent girls were having moderately adequate knowledge and 6% of adolescent girls were having adequate knowledge about prevention of Human Papilloma Virus. 4% of adolescent girls were having inadequate knowledge, 62% were having moderately adequate knowledge and 34% were having adequate knowledge about prevention of Human Papilloma Virus. After the analysis of data, the mean pretest knowledge score was found to be 6.06 and after giving structured education program, the mean posttest knowledge score was found to be 9.64.

Discussion

56% of adolescent girls were having inadequate knowledge, 38% of adolescent girls were having moderately adequate knowledge and 6% of adolescent girls were having adequate knowledge about prevention of Human Papilloma Virus. Similar study Arunachalam D et al 2019, data was collected from adolescent girls in the age group of 13-18 years among selected school students. The results found that majority of adolescent girls having inadequate knowledge (56.7%). There are 36.7% of adolescent girls having moderately adequate knowledge and only 6.6% of adolescent girls are having adequate knowledge on HPV.[7] On the basis of above studies it is found that knowledge level of adolescent girls need to improve. So this data revel that, the mean pretest knowledge score was found to be 6.06 and after giving structured education program, the mean posttest knowledge score was found to be 9.64. It had been observed that there is an increase in the knowledge score of participants after the administration of structured education program.

Conclusion

It concluded that the administration of structured education program was found to be very effective in improving knowledge of adolescent girl about prevention of Human Papilloma Virus. The knowledge regarding prevention of Human Papilloma Virus was improved by education, as it is found that the posttest score is higher than pretest score. This study suggested that awareness is cost effective in improving the knowledge on Human Papilloma Virus and can be implemented in daily living

to have a healthy life of adolescent girls.

Source of Funding: Self

Conflict of Interest : Nil

Ethical Clearance: Institutional Research committee

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To Evaluate the Knowledge and Practice Regarding Home Care Management among Patient on Hemodialysis with Information Education Communication (IEC) Module in Selected Hospitals of Pune City

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Abstract

Background: Hemodialysis is the medical procedure to remove extra fluid and harmful waste products from blood and to correct electrolyte imbalance of patient whose kidney can no longer do it. It is done using a hemodialysis machine and dialyzer also called as "artificial kidney. Haemodialysis is one of three renal replacement therapies. The important role play by the kidneys in the body are in the regulation of body electrolytes; controlling the acid base balance. The primary function of excretory system is to maintain body's state of homeostasis by carefully regulating the fluid and electrolytes, removing wastes. Irregularity in the functioning of kidneys and lower urinary tract is common and may occur at any age with varying degrees of severity**.** Dialysis is needed if kidneys no longer remove enough wastes and fluid from your blood to keep healthy.

Purpose and Objective: The purpose of the study was to evaluate effectiveness of information education communication module on knowledge and practice regarding home care management among patient on hemodialysis. 1. To assesses the level of knowledge and practice of hemodialysis patient regarding home care management. 2. To evaluate the effectiveness of information education communication (IEC) module regarding home care management of hemodialysis patient. 3. To associate the knowledge and practice of patient on hemodialysis regarding home care management with the selected demographic variables.

Methodology: A quasi-experimental non-randomized pre-post-test control group design was used involving 40 patients of haemodialysis with AV fistula. After obtaining informed and written consent from the patient, samples were selected using Non-Probability Purposive sampling technique and allocated into two groups of 20 patients each in experimental and control group. Self-Structured questioner was used for data collection. The data was analysed by using descriptive and inferential statistics. The statistics used were frequency, mean, percentage, standard deviation and Fishers exact statistic.

Results: The descriptive analysis was done to evaluate effectiveness of information education communication on knowledge and practice regarding home care management among patient on hemodialysis which shows that information education communication module is effective for enhancement of knowledge and practice regarding home care management in experimental group than in control group. In experimental group, the mean knowledge score was from 8.35(pre-test) to 13.15(post-test) with SD from 1.42(pre-test) to 0.99(post-test) and The mean practice score was from 12.15(pre-test) to 13.8(post-test) with SD from 1.04(pre-test) to 0.52(post-test).The demographic variable for knowledge and practice regarding home care management among patient on hemodialysis are not significantly associated.

Conclusion: This concluded from the result of the study that information education communication (IEC) module is having significant effect on knowledge and practice regarding home care management. The demographic variable for knowledge and practice regarding home care management among patient on hemodialysis are not significantly associated.

Key words: *Haemodialysis, IEC module, Knowledge, Practice, Home Care Management.*

Introduction or Back Ground

The major organs of the urinary system and chief function that regulate the volume and composition of extra cellular fluid (ECF) and excess products from the body is kidney. The important role plays by the kidneys in the body are in the regulation of body electrolytes; controlling the acid base balance. The kidneys also operate endocrine functions. [1] Healthy kidneys filter blood and remove extra fluid in the form of urine. It also makes substances that keep body healthy. Dialysis carries out some of these functions when kidneys no longer work. There are two different types of dialysis - haemodialysis and peritoneal dialysis. Dialysis is needed if kidneys no longer remove enough wastes and fluid from your blood to keep healthy. This commonly happens when we have only 10 to 15 per cent of kidney function left. [2] Chronic renal failure (CRF) is the progressive and irreversible destruction process of renal function in which body's ability to maintain water and electrolytes and keep their balance would be devastated and uremia would occur consequently. The end stage renal disease (ESRD) treatment is kidney transplantation. However, considering that accessibility of renal transplantation is not easy in most parts of the world, the patient should be treated with dialysis until kidney transplantation time. Annually, more than 60 thousand people in the world loss their life due to renal failure. [3] As part of its agenda to achieve Universal Health Coverage (UHC) by 2022, the Indian government has committed to establishing at least one eight-station dialysis unit in each of its 688 districts, and is contributing free haemodialysis to people living below the poverty threshold. The government's ability to meet this commitment will depend not only on increasing its financial capacity, but also on the implementation of frugal innovations, enhanced early screening interventions and better access to home-based peritoneal dialysis. Better access to peritoneal dialysis would probably mitigate the substantial non-medical costs associated with travel and lost productivity to attend haemodialysis units. [4] Home treatment of hemodialysis requires that patient and family must have training and on-going support from health care providers who are master in treating patients with home hemodialysis. This usually includes a nephrologist (kidney specialist) and specially trained nurses. Patients treated with home hemodialysis can often empower to live independent lives and may have improved survival outcomes compared with those treated in a dialysis centre. This is due, in part, to home hemodialysis patients having more frequent or longer dialysis treatments than those treated in a dialysis centre. [5]

Material and Methods

A Quasi Experimental study design with quantitative approach was used, as this study was aimed, the approach was found to be most appropriate. Non probability purposive sampling technique was use. An experimental group received intervention (IEC Module) and control group was given no intervention. This study was conducted in selected hospital of Pune city which consist 40 samples. The selection was based on easy accessibility, cooperation and availability of samples. Total 40 haemodialysis patients with AV fistula of selected hospital, Pune city who met the inclusion criteria were selected. A Self-Structured questioner was prepared as tool for the collection of data to evaluate knowledge and practice regarding haemodialysis home care management. The content validity was determined by the experts from Medical Surgical Nursing, Nephrology, dialysis technician, dialysis critical care specialist. The reliability of the tool was done by test-retest method and was found to be 0.89 by using Karl Pearson's correlation coefficient formula and the test was reliable and the tool was relevant. Pilot study was conducted on 10 samples at Nephrocare Health Service Private Limited (Sahyadri Super Specialty Hospital), Pune to fulfil the criteria. And it was found feasible to conduct as no major problem faced.

For the main study the formal permission was obtained by writing an application to the Hospital, at Nephrocare Health Service Private Limited (AEC Hospital) Pune. The actual study was conducted on 40 sample Data collection was done from 21st December 2019 to 29th December 2020.

Results

Findings: The analysis and interpretation of the data collected to determine the Effectiveness of IEC Module on knowledge and practice among patients with haemodialysis is carried out based on objectives set by the researcher taking the level of significance as 0.05. In experimental group, the mean knowledge score was from 8.35(pre-test) to 13.15(post-test) with SD from 1.42(pre-test) to 0.99(post-test) and The mean practice

score was from 12.15(pre-test) to 13.8(post-test) with SD from 1.04(pre-test) to 0.52(post-test).The demographic variable for knowledge and practice regarding home care management among patient on hemodialysis are not significantly associated.

DESCRIPTION ON FINDINGS OF THE EFFECTIVENESS OF IEC MODULE ON KNOWLEDGE OF HEMODIALYSIS PATIENT REGARDING HOME CARE MANAGEMENT FOR EXPERIMENTAL GROUP.

N=20

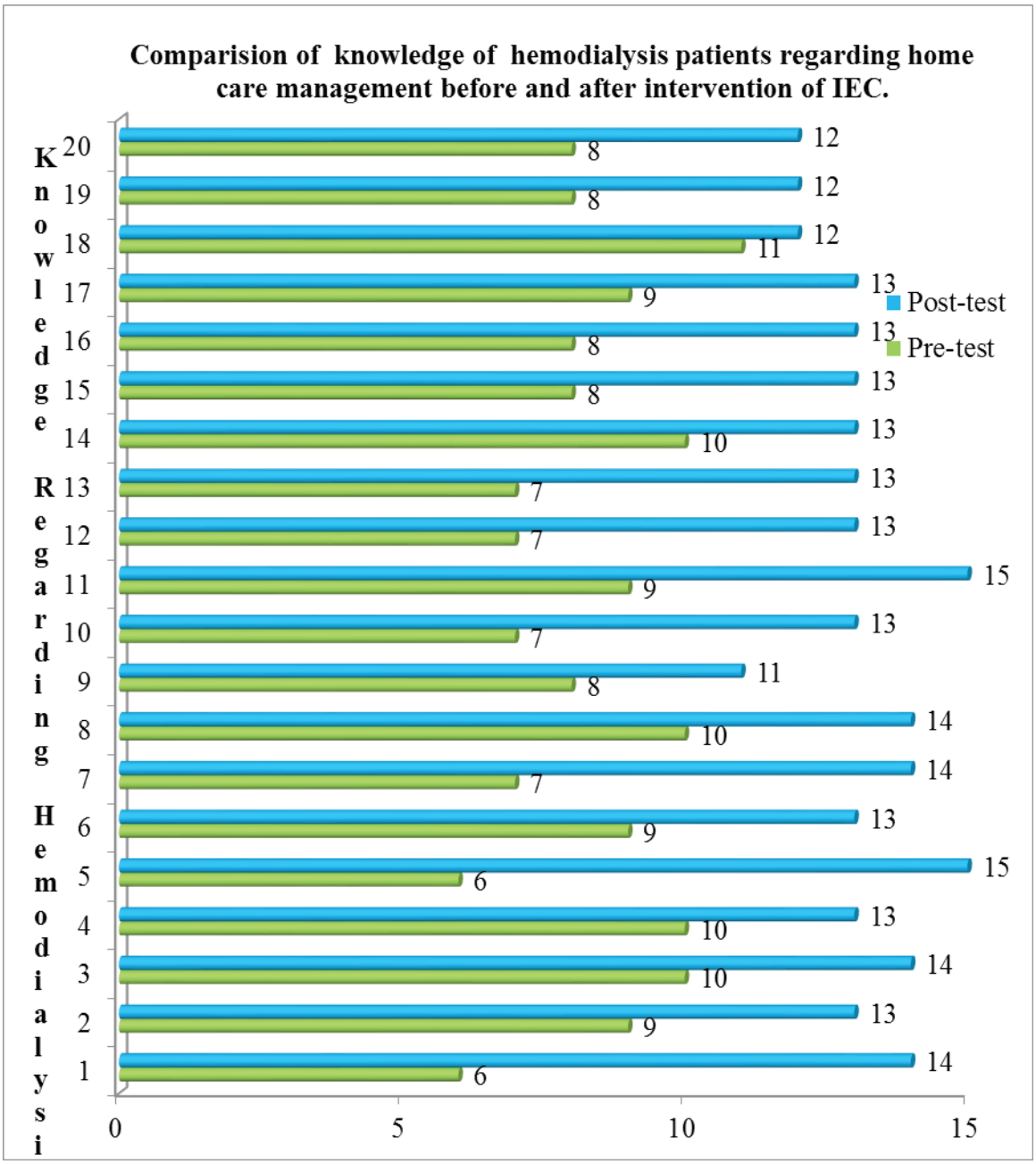


Figure 1 : Bar diagram showing Pre-test and post-test knowledge comparison of hemodialysis patients regarding home care management for experimental group.

Table No: 1 Descriptive statistics for effectiveness of IEC Module on knowledge of hemodialysis patients regarding home care management for experimental group.

N=20

Knowledge level	Frequency		Percentage		Calculated t-value	p-value
	Pre-test	Post-test	Pre-test	Post-test		
Poor	0	0	0	0		
Average	20	0	100	0		
Good	0	20	0	100	11.37	0.00001
	Pre-test		Post-test			
Mean	8.35		13.15			
SD	1.42		0.99			

The above table provides data about the effectiveness of IEC Module on knowledge of hemodialysis patients regarding home care management. The mean knowledge level was from 8.35(pre-test) to 13.15(post-test) with SD from 1.42(pre-test) to 0.99(post-test). As the calculated t-value is 11.37 and p-value= 0.00001, where $p<0.05$, Significant at 0.05 level of significance. Hence, statistically there is effectiveness of IEC Module on knowledge of hemodialysis patients regarding home care management.

N=20

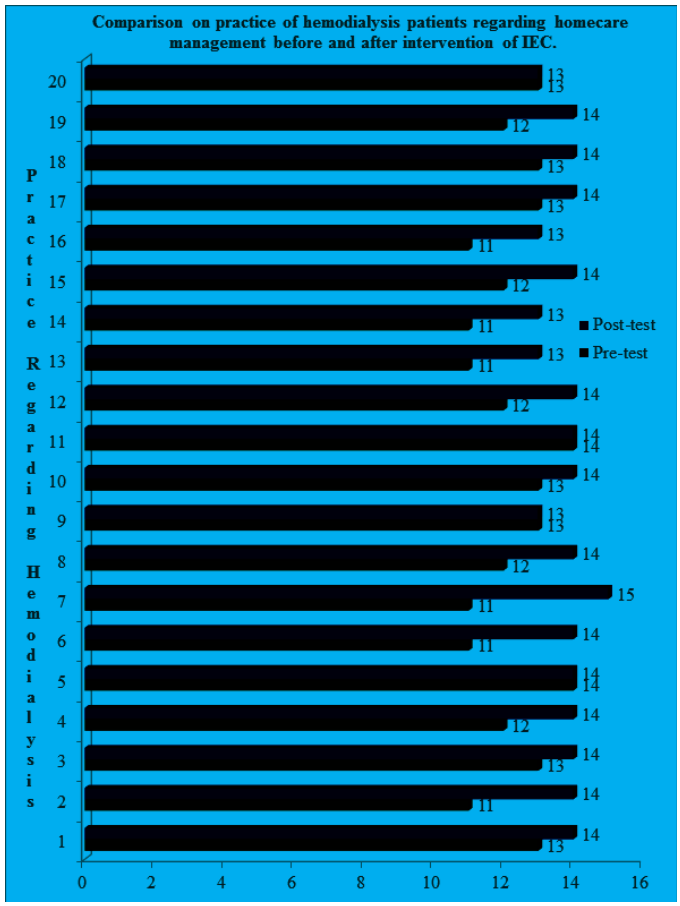


Figure 2 : Bar diagram showing Pre-test and post-test practice comparison of hemodialysis patients

regarding home care management for experimental group.

Table No: 2 Descriptive statistics for effectiveness of IEC Module on practice of hemodialysis patients regarding home care management for experimental group

N= 20

Practice level	Frequency		Percentage		Calculated t-value	p-value
	Pre-test	Post-test	Pre-test	Post-test		
Poor	0	0	0	0		
Average	0	0	0	0		
Good	20	20	100	100	8.39	0.00001
	Pre-test		Post-test			
Mean	12.2		13.8			
SD	1.04		0.52			

The above table provides data about the effectiveness of IEC Module on practice of hemodialysis patients regarding home care management. The mean practice level was from 112.2 (pre-test) to 13.8(post-test) with SD from 1.04(pre-test) to 0.52(post-test). As the calculated t-value is 8.39 and p-value= 0.00001, where $p<0.05$, Significant at 0.05 level of significance. Hence, statistically there is effectiveness of IEC Module on practice of hemodialysis patients regarding home care management.

Discussion

The above finding of the study is supported by a similar study was conducted by Hepsiba Beula Rajam on to evaluate the effectiveness of information education and communication on knowledge regarding management of dialysis among patients with chronic renal failure published in 2016. A quasi experimental design with pre-test post-test used to collect data. The sample size was 60 among which 30 samples for experimental group and 30 samples for control group. Samples were selected by using convenient sampling technique. To assess the knowledge of the patient structured interview questionnaire was prepared. Pre-test was conducted then as intervention information, education and communication was used for management of dialysis for 45 minutes. After intervention post-test was conducted. The result of the study shows that among experimental group, the pre-test mean score of knowledge was 11.5. Standard deviation 3.6 and post-test mean was 25.6, standard deviation was 2.5 and the mean difference was 14.5. The obtained 't' value 18.35 was significant at $p<0.001$ level. Among control group, the pre-test mean score of knowledge was 8.5, standard deviation 2.8 and post-test mean was 10.6, standard deviation 2.8 and the mean difference was 2.1. the obtained 't' value 2 was not significant. Hence the hypothesis was not accepted. The researcher concluded that IEC Module are effective and shows that knowledge regarding management of dialysis among patients with chronic renal failure is improving. **(6)**

Conclusion

The findings of the study show that there is significant difference between pre-test and post-test score of knowledge and practice in the experimental group. The finding reveals that the Information Education Communication (IEC) Module brought a significantly effect on knowledge and practice regarding hemodialysis home care management.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Obtained from Institutional Research Committee of Symbiosis College of Nursing

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A Descriptive Study to Assess the Preferred Leadership Styles among Nursing Inchrages

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Abstract

Background: Many hospital are confronted by the challenges faced in the workplace, demands and expectations of the consumers, issues related to quality and safety of health care due to lack of effective leadership technique .According to the survey, The nursing in charges around the world practice different leadership style which and the leadership style of each nursing in charge differ from each other or it may be same . Hence This study enables to understand the preference to various leadership styles by nursing in charges with their subordinates **. Objectives:**1.To assess the Leadership Style among Nursing in charges with their subordinates of selected hospitals of Pune city.2.To find out association of selected demographic variables to the leadership styles. **Methodology:** A Non-Experimental Descriptive Research Approach The study was conducted among various nursing in charges working in various hospitals of Pune City. The selection of sample was based on easy geographical accessibility, cooperation and availability of samples .The sample size for study was 60,selected by using Non Probability Convenience Sampling Technique from various hospitals of Pune City who met the inclusion criteria. **Results:** Majority of them 55% have preferred to have transformational leadership styles , 15 % of them have preferred to have transactional leadership styles , and only few preferred to have autocratic and democratic learning styles 10% and 1 % respectively. and when we compare the selection of two leadership styles at a time we found that (7%) of them preferred to have autocratic and transactional leadership style together as a leadership styles ,and 7% of them had preferred to have transformational and transactional leadership style together as a leadership style. To find out the association of demographic data with leadership style data was analyzed and found that there is no significant association between any of the demographic data with leadership style as the P value is more than 0.05.**Conclusion:** The findings indicated that questionnaire was an effective strategy in bringing the preferred leadership styles. The structured questionnaire was acceptable and useful method of assessing the preferred leadership styles and can be used in future to identify preferred leadership styles used by Nursing in charges and Also leadership style has no association with the demographic profile.

Keywords: *assess , leadership styles, nursing in charges .*

Introduction or Background

"Leaders become great not because of their power but because of their ability to empower others"

- John Max well

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There are some various ways to steer, and each leaders will demonstrate their leadership style.Amoung all the leadership style we have 2 main leadership style that are transformational and transactional leadership styles.It is said that effective leaders will be engaged in professional leadership behaviors and also in private leadership behaviors. Were the professional leadership behaviors is to seek advice from setting a vision, creating a path for achieving the set goals, Prioritizing the processes to be carried out and procedures, etc., and often these activities are translated as per the organizational strategies and practices.(1)The non-public leadership are involved in building trust also for caring of people and to act morally etc. Hence they , often try to take advice from the various leadership styles .leadership style represents as very crucial as it also consider for the

determination of the success or failure of organizations and the institutes.[2]Many researches on leadership has said thet, leadership is known as an inherently gained moral endeavor as it involves a relationship to influence and power between the followers. Personality of a leader is also to be considers as an effective important quality of a leader as the follower of the leaders will debit the personality traits of the leader they are following [3][6][7][8]. Characteristics like demographics, personality traits, skills and skills are all essential components which helps to predict the leadership style .If the organizations study about these important traits of leadership with any sort of training and learning methods they can develop good leaders in their organizations and institutes .As we have lack of transformational leader which look for vision, passion. An effective leader should be praised for the improvement of their personal, professional and social life ,Hence to bring out a positive environment and to changes the negative atmosphere to appositive it needs an important and meaningful response. In nursing we need very dynamic leaders to lead were leadership is and essential quality to be acquired by all the nursing staffs and leaders which is need to ensure the safety of patients and to execute positive staff development.The nurse leaders are the main who plays and vital role towards excellent patient care. It promotes Frankel's affirmation that it's important to possess dynamic clinical leaders and supportive clinical environment between the event and achievement of best practice chart.[12]

Objectives of the study:

1.To assess the Leadership Style among Nursing in charges with their subordinates of selected hospitals of Pune city.

2.To find out association of selected demographic variables to the leadership styles.

Material and Methods

Research Design:The researchers have used a A Non-Experimental Descriptive Research.The study was conducted in the selected hospitals.

Sampling techniques: Non Probability convenient sampling technique was used and selected all nursing supervisors and in-charges of the maternity wards**Subject Size**:Nursing supervisors and in-charges altogether was 60 who are willing to participate and working in the maternity wards. These participants have informed clearly about the study and its purpose.

Tools of data collection: In the present study the below scales were used to collect the dataStructured Questionnaire on preference of leadership style which was developed by the researchers based on the review of the literature. It has two sections; **Section I**: Consisted of items related to demographic variables of the subjects of the study, such as age, sex, department, qualification, years of experience**Section II**: Consisted of 20 questions regarding preference of leadership style **Validity and Reliability of tools**: Tools were validated by the experts and the reliability was done by Cronbach's Alpha coefficient test. Cronbach's alphas were r= 0.86, & 0.9 for preference of leadership style .

Findings

The analysis and interpretation of the data collected to determine the leadership styles preferred among nursing in charges during her work process between here subordinates was done keeping the objectives of the study in consideration

The result was divided in III Sections

Section- I

Description of samples characteristics in frequency and percentage.

Table 1: Frequency and Percentage distribution of demographic data

N=60

Sr.No	ITEMS	FREQUENCY	PERCENTAGE (%)
1	AGE		
	20-30 yrs.	49	82.4 %
	31-40yrs	11	17.6 %

Cont... **Table 1: Frequency and Percentage distribution of demographic data**

N=60

2	**GENDER**		
	Male	**22**	**35.3%**
	Female	**38**	**64.7%**
3	**RELIGION**		
	Hindu	**41**	**68.8%**
	Christian	**6**	**12.5%**
	Other	**6**	**12.5%**
4	**EDUCATION**		
	B,Sc	**19**	**31%**
	P,B.Sc	**1**	**1.3%**
	M.Sc	**30**	**50.1%**
	Other	**10**	**17.6 %**
5	**Occupation**		
	Govt. Sector	**49**	**82.4 %**
	Pvt. Sector	**11**	**17.6 %**
6	**Marital status**		
	Married	**22**	**35.3 %**
	Unmarried	**38**	**64.7 %**
7	**Duration of practice**		
	<10 year	**36**	**60 %**
	>10 years	**24**	**40 %**

Section- II

Description of data related to preferences of leadership styles

Table 2: Data related to preferences of leadership styles **N=60**

SR/N	PREFERED LEADERSHIP STYLE	FREQ	%
1	AUTOCRATIC LEADERSHIP	6	10%
2	TRANSACTIONAL LEADERSHIP	9	15 %
3	TRANSFORMATIONAL LEADERSHIP	33	55 %
4	DEMOCRATIC LEADERSHIP	1	1 %
5	TRANSACTIONAL LEADERSHIP/TRANSFORMATIONAL LEADERSHIP	4	7 %
6	AUTOCRATIC AND TRANSACTIONAL LEADERSHIP	4	7 %
7	AUTOCRATIC AND TRANS FORMATIONAL LEADERSHIP	3	5%

Section- III

Association between demographic details and Leadership styles

Table 3: Describes Association between demographic details and Leadership styles

N=60

SR NO	Demographic variable		Autocratic	Demo-cratic	Transac-tional	Transfor-mational	p-value	Remarks
1	Age	20-30 years	9	10	12	13	0.084	Non Significant
		31-40 years	6	5	3	2		
		>41years	0	0	0	0		
2	Gender	Male	4	3	0	3	1	Non Significant
		Female	10	12	13	15		
3	Education	B.Sc	3	2	0	13	0.072	Non Significant
		P.B.Sc	3	0	0	14		
		M.Sc	2	5	8	10		
		Oher	1	1	1	1		
4	Occupation	Govt. Sector	3	2	2	10	0.075	Non Significant
		Pvt. Sector	3	5	10	25		
5	Religion	Hindu	6	11	10	10	0.247	Non Significant
		Muslim	6	3	4	0		
		Christian	3	1	2	3		
		Other	0	0	1	1		
6	Marital status	Married	4	6	5	15	0.404	Non Significant
		Unmarried	8	7	5	10		
7	Duration of practice	<10 year	3	2	2	10	1	Non Significant
		>10 years	3	5	10	25		

Discussion

This study has been undertaken to assess the popular leadership style and associate the training nursing in charges from various hospitals of Pune city. This study fortunately reproduce , makes various inputs to leadership studies . the overall objective of this research was to go looking out various leadership style preferred by nursing in charges and also if there's significant association between demographic factors and leadership styles .

Table:1 Shows In that majority of samples that is 82% were from the age group 20 to 30 years, 17.1

% samples were from the age group of 31 to 40 years .In gender description it was found that Majority of the samples (64.7%) were females and Only 35.3 % samples were Male. In religion description it was noted that Maximum 68.8% were Hindu, 12.5% were Christian , only 12.5% were belonging from other religions were .When we look upon the education status 50.1 % of total students have completed M.Sc, 31% have done B.Sc , 17.6% have done other educational course of nursing .and only 1.3 % have done P.B.BSc Nursing .When we look upon their occupation status 82.4 % were working in government hospitals and only 17.6 % were working in private hospitals. Only 35.4 % were married and Majority of them 64.7 % were unmarried. Majority of them (60%)had less than 10 years of practice in the profession and only 40% had more than 10 years of practice in the profession **Fig 2**: Describes data related to preferences of leadership styles it was found that among nursing in charges. Majority of them 55% have preferred to have transformational leadership styles , 15 % of them have preferred to have transactional leadership styles , and only few preferred to have autocratic and democratic learning styles 10% and 1 % respectively. and when we compare the selection of two leadership styles at a time we found that (7%) of them preferred to have autocratic and transactional leadership style together as a leadership styles ,and 7% of them had preferred to have transformational and transactional leadership style together as a leadership style.Table 3: Describes the association of demographic variables with leadership style. In proceeding To find out the association of demographic data with leadership style data was analyzed and found that there is no significant association between any of the demographic data with leadership style as the P value is more than 0.05.

The result of the current study taken for comparison says that there is no significant difference between the educational qualification and leadership style. Which tells us that whatever kind of educational level and certification you have it will not affect your kind of leadership style of practice or follow. Because leadership work towards motivating your subordinates to fulfill the set organizational goals.These results argument are for those other researchers, who are indicating that leadership styles don't essentially depends on any social demographic factors. As stated by Burns (1978) that transformational leadership can be a process of engendering higher levels of motivation and commitment among followers. The main strain is on generating a vision and values of subordinates for creating a way of justice, loyalty and trust.[13]

Conclusion

The findings indicated that questionnaire was an effective strategy in bringing the preferred leadership styles. The structured questionnaire was acceptable and useful method of assessing the preferred leadership styles and can be used in future to identify preferred leadership styles used by Nursing in charges and Also leadership style has no association with the demographic profile.

Conflict of Interest – Nil

Source of Funding- Authors have no financial support to this project. The study was fully funded by authors.

Ethical Clearance – Ethical approval of the study taken from Symbiosis College of nursing ethical committee. Informed consent was taken from the Nursing In charges working in different hospitals of Pune city. Informed the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential

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Nurses Opinions about the Transformational Leadership in Hospitals of Pune City

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Abstract

Transformational leadership in nursing is different from other industries. Nurses working pattern is different with different shift and many times they go through a lot of emotional changes with the patient care. Effective nursing leadership also engenders staff retention, job satisfaction, commitment, work unit climate and client satisfaction with the nursing care services provided to them .Transformational leadership globally plays a vital role in the health care system especially for patient care by nurses, because the performance of the staff nurses is directly linked to the leadership styles. Hence this study is aimed to assess the of nurses on transformational leadership style

Methodology: ANon-Experimental Descriptive Research approach used. The study was conducted among various Hospitals of Pune City. The selection of the sample was based on easy geographical accessibility, cooperation and availability of samples .The sample size for the study was 95,selected by using non probability convenience sampling technique from various Hospitals of Pune City who met the inclusion criteria. A structured modified questionnaire was developed based on the research problem, to get the information from the samples.

Result: The result of current study says 54.74%staff nurses given the opinion that their nurse supervisors are having average transformational leadership. 38.95% of staff nurses perception was their nurse supervisors are having good transformational leadership and 6. 32 % of staff nurses perception was their nurse managers are having poor transformational leadership qualities.The average score of staff nurses regarding their supervisor's transformational leadership is 3.1 to 4.2. Out of the four domains Individualism got higher points (79.72%) than other and improvements of subordinates got the lowest (64.65%).

Conclusion: The nursing leader transformational qualities in four main domain is analysed in this study. The overall competencies in nurse leaders analysed were knowledge, innovativeness, self-confidence, good communication skill, adaptability, teamwork, organisational skill etc. The result says that nurse managers are having average transformational leadership qualities.

Key words: *Nurses ,opinions ,transformational leadership*

Introduction

Leadership is not new, indeed Plato refers it to a higher function in humans, who can lead others to enlightenment and represents an ideal. However, the ways the individuals have influenced and directed the ideas have built in the 18th and 19th centuries. Leadership style is the manner and approach of providing direction and motivating people[1].

The term 'Transformational Leadership' has undergone a lot of investigations and discussions at various stages. James MacGregor Burns, an authority on leadership studies defined transformational leadership in 1978 as a process by which leaders raise the aspirations and motivations of others to higher levels by appealing to higher ideals and values" (Burns, 1978).[2]Increasing followers performance by motivating them to transcend

self-interest may not be sufficient in transformational leadership. Transformational leadership is most effective when the leader identifies the need in the subordinates and guide them to achieve the changes through inspiration.[3]

Nurse leaders have an important role in hospital .Staff nurses turnover is a the biggest problem faced by the nurse leader's worldwide. If nurse leaders follow the transformational leadership, staff nurses will get more satisfaction towards the job and staff retention will increase. The nursing administrators, supervisors, the head nurses and nursing staff in team nursing all are the nursing leaders. Even student nurses were considered as leader for the patients[4].

In transformational leadership, the head nurses need to be more visionary and with an inspirational approach. They tend to communicate a clear and acceptable vision and goals[5]. Head nurses have the key role in making the staff nurses capable of decision making, gratitude, Individuality and growth of them and the unit. Many hospitals need this type of adaptive and flexible leaders.[6.7]

This study aims to find the opinion of the staff nurses regarding transformational leadership in their higher authorities.

Objectives:

1. To assess the staff nurses opinions regarding their nurse supervisors about the transformational leadership

2. To assess different qualities of transformational leadership like decision making, gratitude, individuality and attitude towards subordinates improvements.

3. To associate the transformational leadership score with selected demographic variables of staff nurses in hospitals of Pune city.

Tool : Tool consists of two sections. Section A consist of 5 demographic questions and the section B consist of 22 items of self-structured rating scaleto assess transformationalleadership. It has 4 sub items including decision making, gratitude, improvement, and individualism.

Table 1 : Blue Print of the tool:

Sr. No	Items	Total questions	Score - maximum	Score - minimum
1	Decision making	5	25	5
2	Gratitude	4	20	4
3	Improvement of subordinates	8	40	8
4	Individualism	5	25	5
	Total	22	110	22

Total score was evaluated as 110 and the minimum score is 22 and the result choices are as followsGood transformational leader qualities – 80 to 110 , average transformational Leader qualities - 50-79 and poor transformational leader qualities – 22 to 49

Variables: Demographic variables used in this study were gender, marital status, education, position in hospital and employment type.

Inclusion criteria for nursing staff include: 1.Staff nurses above 21 years, 2.Staff nurses who can read and write English3. Staff nurses who are willing to participate and 4. Staff nurses with minimum one yearexperience in hospitals after their education.

Research Methodology: A non-experimental, descriptive study was conducted in the staff nurses working in general hospitals of Pune city. The participants were working in various hospitals in Pune

city. Around 12 hospitals the participants responded to the study. Consent was taken from all the participants. A convenience sampling method used and 95 nurses participated in the study.

Analysis of Data:

Section A: Demographic details of staff nurses who participated in the study .

Table 2: Distribution of demographic data in terms of frequency and percentage. n=95

Sr. no	Demographic variable	Frequency	Percentage (%)
Gender			
1	Male	35	36.84
2	Female	60	63.16
Designation			
1	Head nurse	14	14.74
2	Senior nurse	62	65.26
3	Junior nurse	19	20
Type of employment			
1	Permanent	64	67.37
2	Temporary	31	32.63
Marital status			
1	Married	75	78.95
2	Unmarried	17	17.89
3	Divorced	2	2.11
4	Widowed	1	1.05
Educational Level			
1	Diploma	28	29.47
2	Bachelor's	60	63.16
3	Master's	7	7.37

Section B : Opinion of staff nurses regarding their nurse supervisors on transformational leadership

n=95

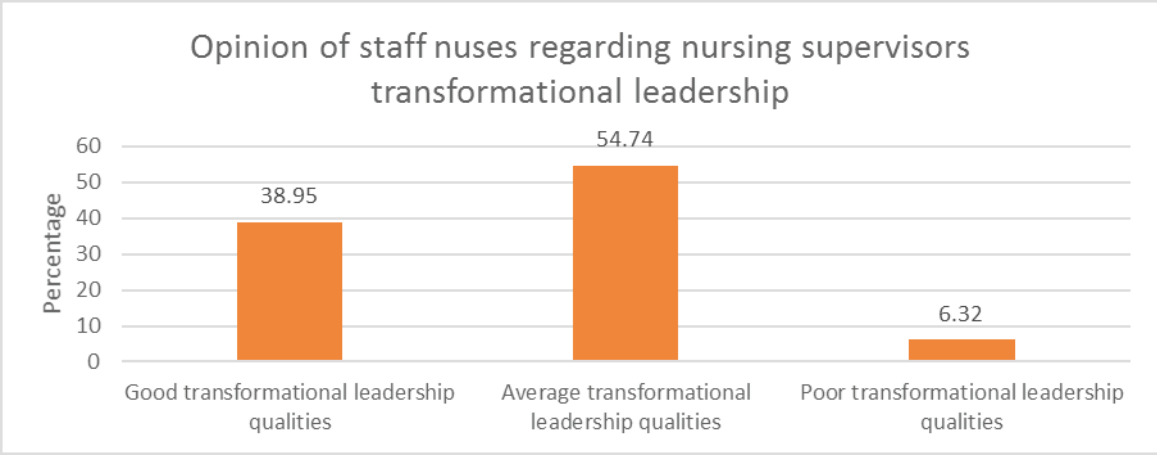


Figure 1: opinions of staff nurses regarding nursing supervisors transformational leadership.

54.74%of staff nurses given the opinion that their nurse supervisors are having average transformational leadership. 38.95% of staff nurses perception was their nurse supervisors are having good transformational leadership and 6. 32 % of staff nurses perception was their nurse managers are having poor transformational leadership qualities.

Table 3: Staff nurses opinion regarding nurse supervisors of transformational leadership qualities

n=95

Sr. No	Items	Score - maximum	Average Score Obtained	Percentage (%)
1	Decision making	25	19.35	77.4
2	Gratitude	20	13.74	68.7
3	Improvement of subordinates	40	25.86	64.65
4	Individualism	25	19.93	79.72

The average score of staff nurses regarding their supervisor's transformational leadership is 3.1 to 4.2. Out of the four domains Individualism got higher points (79.72%) than others and improvements of subordinates got the lowest (64.65%).

Section D : Association between Staff nurses opinion and demographic variables

Table 4: Describes the data related to association of Staff nurses opinion with selected demographic variables

n=95

Sr. no	Demographic variable	Good TL	Average TL	Poor TL	Probability
A	Gender				
1	Male	12	21	2	0.79
2	Female	25	31	4	
B	Position				
1	Head nurse	10	4	0	0.049
2	Senior nurse	18	39	5	
3	Junior nurse	9	9	1	
C	Type of employment				
1	Permanent	21	39	4	0.19
2	Temporary	16	13	2	
D	Marital status				
1	Married	28	44	3	0.09
2	Unmarried	8	6	3	
3	Divorced	0	2	0	
4	Widowed	1	0	0	
E	Educational Level				
1	Diploma	8	17	3	0.199
2	Bachelor's	25	33	2	
3	Master's	4	2	1	

The Freeman-Halton extension of the Fisher exact probability was used in this study. None of the demographic variables are significant with the findings except the position . It signifies that opinions will get change according to the position. It also explains that throw the experience of being in higher position, helps the staff nurses to understand the transformational leadership.

The study result shows that individuality and decision making skills are good in the leaders. Nurses today work in the environment, where they need to face a lot of challenges and obstacles. Nursing leaders need to train the staff nurses and should provide a supportive environment where there is growth is notified in junior level nurses also.

Ethical consideration: Ethical approval of the study taken from Symbiosis College of nursing ethical committee. Informed consent was taken from the staff nurses working in different hospitals of Pune city. Informed the responders regarding the data collection procedure. The collected data was used only for research purposes and kept confidential.

Funding : Authors have no financial support to this project. The study was fully funded by authors.

Conflict of Interest: The author has no conflict of interest.

Conclusion

The nursing leader transformational qualities in four main domain is checked in this study . The overall competencies of nurse leaders need to be checked are knowledge, innovativeness, self-confidence, good communication skill, adaptability, teamwork, organisational skill etc. These can be checked in future studies.

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Recalling the Definitions with a Mind Mapping and Self-Learning Technique: A Comparative Study in Undergraduate Students

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Abstract

Around 13.34% weightage is given to the definitions of university exams in nursing.[1] Nursing educators need to help the students in memorizing terms, so that students can write the correct concept in the exam effectively.**Aim:** To assess the comparison of mind mapping and self-learning techniques in recalling the definition. **Design** : A comparative approach was used to understand the difference between the knowledge of self-learning group and mind mapping group . Total 5 definitions were used from the nursing foundation textbook .To obtain baseline data, subjects completed a short test and it is randomly allocated to two groups: 'self -learning' and 'mind map'. After a one hour of study period short test was taken. Recalling was measured after a week later. Setting: Symbiosis College of Nursing, Symbiosis International University, India. **Subjects** : 48 First year Bachelor Nursing Students. **Results:** Mean score in Mind mapping group were 5.87 and 5 in session one and two . For self-learning group, the mean effect was 6.21and 4.67 in the first and second sessions. Hence Mind mapping technique proven to be more effective than Self learning method in recalling the definition. Two sample t-test for comparison of effect of Mind mapping vs self-learning method.t-values for this comparison were –0.76 and 0.62 . Corresponding p-values were 0.45 and 0.54 and the p-value < 0.05 , so the null hypothesis is rejected. **Conclusion**: mind maps were proved affectivity as a way of recalling the definitions in the second session. Students need to be encouraged to adopt this approach more than the other conventional learning techniques.

Keywords: *,Nursing , Mind mapping, Self leraning*

Introduction

Patient care has taken a courageous change in recent years but teaching and learning styles are almost the same in students and teachers. Nursing teachers who use active learning approaches in their nursing classes can teach to a range of students: visual learners, auditory learners, tactile learners[2]. Lifelong learning is anticipation in the professional performance of nurses, which is directly related to the success of students in nursing schools and colleges.

A mind map can be expressed as a central image with sub themes radiating from the central image, branches with key images and key words and branches forming a connected nodal structure [3]. Mapping procedures have been initiated to motivate students to represent ideas visually, thus causing them to analyze, evaluate, and think critically [4]. The goal of this strategy is for the students to learn through actively connecting new concepts to existing concepts[5].

Self-learning effects active learning and students achieve more thoughtful points of view about the topics instead of having a superficial attitude and come to a deep understanding of their learning materials. Moreover, independent learning lets the students take the responsibility of their thought process and participate actively in the learning process. This increases the sense of belonging to learning and enhances the students' positive motivation [6].Self-directed learners are usually characterized as being independent and positive towards learning [7]. It is also believed that they can plan, manage, and evaluate their own learning [8].

In this competitive era, it is essential to transform our nursing students from textbook muggers and encourage for critical thinking. The teaching and learning strategy to support the capacious amount of course material, needs to be completed in the four years[9].Nursing syllabus always allows various teaching pedagogies including lecture cum discussion, Project work, seminar, case study, presentations, demonstration, role play, group discussion etc.[1]Simply teaching a concept to a student or learning a theory will not help the students. Student Nurses need to engage in the activity of learning to understand it and use that knowledge in life science. Around 13.34% weightage is given to the definitions of university exams. Student nurses are forced to learn the definitions which is difficult in many times . Teachers need to help them in memorizing terms, so they can spit that concept in the exam effectively. To promote recall and memorization students require active teaching strategies to promote meaningful learning, instead of relying on traditional methods. The present study was aimed to assess the comparison of mind mapping and self-learning technique in recalling the definition.

Objectives:

1. To assess the effect of the mind mapping technique to recall the definitions for nursing students.

2. To assess the effect of the self-learning method to recall the definitions for nursing students.

3. To compare the effect of mind mapping technique and self learning method to recall the definitions for nursing student

4. To associate the demographic details with the selected findings of the mind mapping technique and self-learning technique.

Method and design:

The researcher adopted Pre experimental two group comparison design among first year BSc nursing students in Pune city. The tool was validated with 6 experts, who all are the teaching faculty of the various nursing institutes in Pune. Face validity and content validity checked. The total batch strength is 50 at Symbiosis College of Nursing during November 2019. Convenience sampling technique was used to select the samples. All students who are willing to participate in the study were included in this study and the students who all are absent and not ready to participate in the study were excluded from the study. Total 48 students participated in the study . 2 students were absent during the data collection.

The tool consist of 2 sections. Section i: Socio demographic profile which consist of age, higher education, and gender. Section ii: Self structured questionnaire. (5 definitions are asked to the students. They are **health, hospital, communication, nursing and infection**)

Informed written consent was taken from all the students. Total 2 hours utilized by the researcher. Total participants (n=48) distributed into 2 groups with the lottery method.24 students in mind mapping group and 24 students in self-learning group. Each group was sitting in a different class room .The researcher took 15 minutes to explain the session to the students. Five definitions were taken from the subject nursing foundation. health, nursing, infection, hospital and communication were given to students of self-learning group. At the same time in the mind mapping group mind mapping method was explained and given the same definitions like self-learning group. For mind mapping group pencil, scale, crayons and sketch pens are distributed by the researcher. Both group students were supervised during this activity. The questionnaire had given to the students after one hour . Each right response carried 2 marks. The knowledge of the students was assessed on Day 0 (immediately after intervention) and on Day7.During the second session after 7 days students got 5 minutes to recall the definition. There were no students dropped out of any of the sessions.

Variables :Independent variables in this study were mind mapping and self- learning and dependent variable is level of knowledge.

Hypothesis : Ho=There will be no significant change in the level of knowledge and the type of learning.

Results

A total of 48 participants were recruited and randomized into two groups of 24 each.

Table 1 : Demographic details of mind mapping and self-learning group n=48

Sr. no	Demographic variable			Frequency	
				(Mind Mapping Method)	(Self-learning Method)
1	Age	a.	Below 18years	9	10
		b.	18years- 19years	8	9
		c.	19years- 20years	4	3
		d.	20years and above	3	2
2	Education	a.	CBC board	14	8
		b.	State board	8	12
		c.	International	2	4
3	Gender	a.	Male	7	6
		b.	Female	17	18

Both the groups, there were (n = 12) males and (n = 35) females. 19 students were below 18 years, 17 students are between the age group of 18to 19 years . 7 students were 19 years to 20 years and 5 students above 20 years. 22 students were passed from CBC board, 20 students were from the state board and 6 students completed their studies from the international board

Table 2 : Comparison of Mind mapping and self-learning session n=48

		Mind mapping				Self-learning method			
Sr. no	Knowledge Score	1st session		2nd session		1st session		2nd session	
		Frequency	%	Frequency	%	Frequency	%	Frequency	%
1	Good	15	31.25%	9	18.75%	16	33.34%	5	10.41%
2	Average	6	12.50%	9	18.75%	7	14.58%	13	27.08%
3	Poor	3	06.25%	6	12.50%	1	2.08%	6	12.50%

The table 2 shows the comparison of the rating of the two learning methods given by the students on the knowledge score. Total two sessions were taken . In the first session , on the day of intervention self learning group scored well. Good knowledge score achieved by 16 students (33.34%). Poor score taken by one student (2.08%). In second session (after 7 days), 5 students achieved a good score while 13 students scored averaged and 6 students taken poor scores. In the mind mapping session first session 15 students taken a good scores (31.25%) and 6.25 % (n=3) received poor scores. In the second session 18.75 % students received good scores and 12.50 % students received poor scores. But the second session Mind mapping group showed improvements than the self- learning group and the mean score difference is 0.33.

Table 3: Comparison of t score in mind mapping and self-learning group

Sr. no	Session	Mind mapping		Self-learning Method		T	p-value
		Mean(μ)	SD(σ)	Mean(μ)	SD(σ)		
1.	First session	5.87	1.69	6.21	1.25	-0.76	0 .45
2.	Second session	5	1.93	4.67	1.72	0.62	0.54

Mean score in Mind mapping group were 5.87 and 5 in session one and two . For self learning group, the mean effect was 6.21and 4.67 in first and second sessions. Hence Mind mapping technique proven to be more effective than Self learning method in recalling the definition.

Two sample t-test for comparison of effect of Mind mapping vs self-learning method.t-values for this comparison were –0.76 and 0.62 . Corresponding p-values were 0.45 and 054 and the p-value < 0.05 , so the null hypothesis is accepted

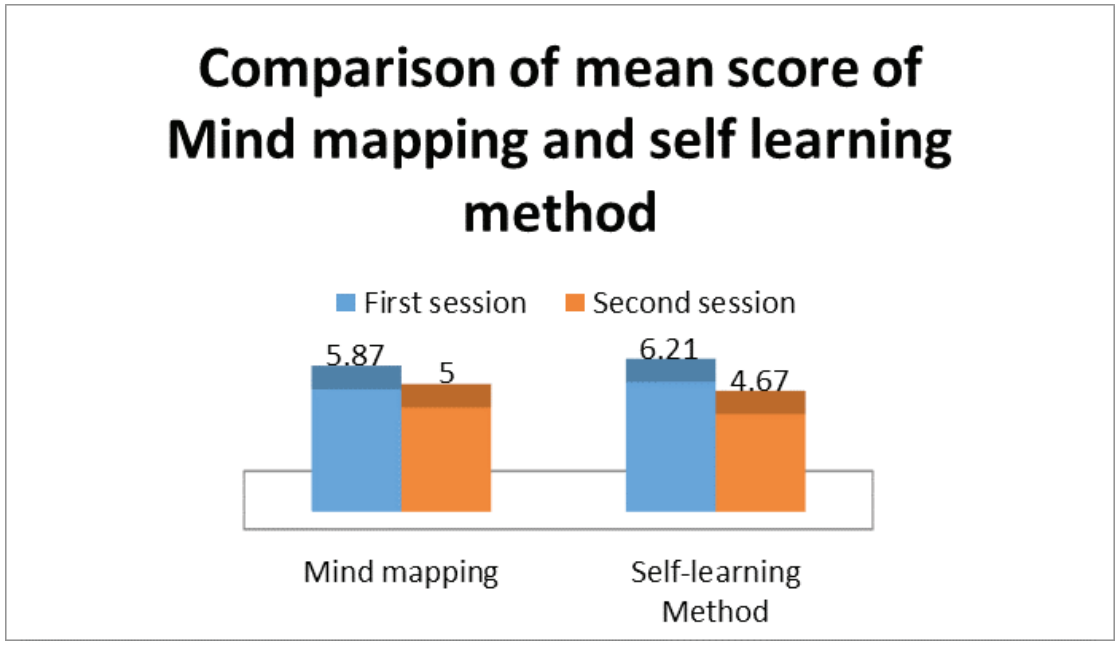


Figure 1 Comparism of mean score of mind mapping and self-learning method.

Table 4 : Association between the Self learning and selected demographic variables

Sr. No	Demographic variables for Self learning method group in the first session			Good	Average	Poor	p-value
1	Age	Below 18 years	9	7	1	1	0.75
		18years- 19years	8	4	2	2	
		19years-20years	4	2	2	0	
		20years and above	3	2	1	0	
2	Higher education	CBC board	14	9	3	2	0.87
		State board	8	4	3	1	
		International	2	2	0	0	
3	Gender	Male	7	6	1	0	0.53
		Female	17	9	5	3	

Table 5 : Association between the Mind mapping and selected demographic variables

Sr. No	Demographic variables for Mind mapping group in the first session			Good	Average	Poor	p-value
1	Age	Below 18 years	10	8	2	1	0.92
		18years- 19years	9	6	3	0	
		19years-20years	3	2	1	0	
		20years and above	2	1	1	0	
2	Higher education	CBC board	8	6	1	1	0.45
		State board	12	8	4	0	
		International	4	2	2	0	
3	Gender	Male	6	5	0	1	0.10
		Female	18	11	7	0	

None of the demographic variables was found to have a significant association with mind mapping and self-learning technique knowledge score and demographic variables.

A surprising finding was that enthusiasm in the self-selected study technique group was significantly higher than that in the mind map group in the first session. But in the second session recalling was upright with mind mapping group. That improvements in recall at a week re-test remained evident with just a single exposure to the mind map technique suggests a real benefit of this study technique compared with other memory strategies where benefits have been confined only to tests of immediate recall . Study techniques which have separately incorporated imagery, colour or the visual-spatial arrangement of keywords have each been

reported to significantly improve recall when compared with simple note taking or rote rehearsal[9].

Summary :Mind maps are an effective study technique when used to improve factual recall from written material. Mind mapping facilitates the development of reflective thinking and deeper understanding and it helps learners to focus on a topic and solve a difficult topic in fun and creative way. More effort should be spent to support different approaches of learning compared to the traditional education strategies, especially here in India where lecture based teaching method predominates. So as both the teacher and the student have a better appreciative subject knowledge gained by this innovative teaching and learning method.

Recommendation

According to Blooms taxonomy, the order of thinking skills in increasing order (lower to higher)[3]. This study examined the remembering (Lower order thinking skills) part only. There is a need for advance studies to check the effect of the mind map strategy on higher order thinking skills like creative thinking

Ethics: This present study got approval from Symbiosis College of Nursing ethics committee. All participants who participated in this study was well informed about the study and a written consent is taken from them.

Contribution Details: Dr. Sharadha Ramesh and Dr. Seeta Devi contributed in manuscript editing and manuscript review. Ms. Leeja Rajan And Ms. Diana Prasad contributed in the data collection and review of literature Part. Ms. Merlin Suji contributed in statistical analysis, research concept, and overall integration of work .

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Conflicts of Interest/ Competing Interests : There are no conflicts with the publication of the manuscript or an institution with the current study.

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Effectiveness of Structured Teaching Programme on Hazards of Water Pollution

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Abstract

Water pollution occurs when unwanted material enter into water bodies (eg: lakes, rivers, sea, ponds, oceans) and contaminate the standard or quality of water. Humans and every one living species within the world are facing worst hazards of contaminated water. Raising awareness and knowledge regarding this issue is of great importance. At this point, environmental education on hazards of water pollution plays a key role. This study aims to assess the effectiveness of structured teaching program on knowledge regarding hazards of water pollution among undergraduate nursing students of Symbiosis College of nursing, Pune city. A pre-experimental one group pre-test post-test research study was conducted to assess the effectiveness of teaching. A structured questionnaire and checklist consists of total 30 question was the tool of the study to conduct the pre-test and post-test to the sample. From the results of pre-test it is evident that undergraduate students had average knowledge on hazards of water pollution and after the structured teaching program the results of post-test it is evident that students gained excellent knowledge regarding hazards of water pollution after teaching program. The main focus was to develop knowledge, skills and ways to shield the water through structured teaching program and absolutely was achieved.

Keywords: *water pollution, hazards, structured teaching programme, effectiveness, knowledge.*

Introduction

Water is that the essential element that creates life on earth possible. Without water there would be no life. Although 71% of the earth surface is covered with water only a tiny fraction of this water is available to us as fresh water. About 97% of the entire water available on earth is found in oceans and is just too salty for drinking or irrigation. The remaining 3% is fresh is fresh water. Of this 2.997% is locked in ice caps. Thus only 0.003% of the earth's total volume of water is easily available to us as lakes, groundwater, rivers, streams and wetlands. [1]

When the standard or composition of water changes directly or indirectly as a results of man's activities such it becomes unfit for any useful purpose is claimed to be polluted. Source of pollution can be readily identified and cannot be identified which is said to be as point and non-point sources of pollution. [2]

The major cause of water pollution are urban runoff, excess pesticides usage, industrial waste, oil spillage, sewage water and many improper way of using water in household. This lead to major hazards of water pollution like water borne disease, increase in mortality rate of under five children due to water borne disease, unfit drinking water, Arsenic poisonings, danger to marine life.While the foremost necessity is prevention, fixing effluent treatment plans and treating waste through these can reduce the pollution load within the recipient water. There is need for awareness of water pollution and its effects are essential so that we can act responsibly to protect and conserve water for future generations. [3]

Statement

A study to assess effectiveness of structured teaching program on knowledge regarding hazards of water pollution among undergraduate students of Symbiosis College of Nursing, Pune city.

Objectives

1) To assess the pre-test knowledge regarding hazards of water pollution among under-graduate students of Symbiosis College of Nursing of Pune City.

2) To assess the post-test knowledge regarding hazards of water pollution among under-graduate students of Symbiosis College of Nursing Pune City.

3) To determine the effectiveness of Structured Teaching Programme regarding hazards of water pollution among under-graduate students of Symbiosis College of Nursing Pune City.

4) To find out the association pre-test level of knowledge with their selected demographic variables.

Operational Defintion

- **Assess**

In this study it means the assessment of the effectiveness of Structured Teaching Program amongst under-graduate students of Symbiosis College of Nursing Pune City.

- **Effectiveness**

In this study effectiveness means improving the knowledge and practice regarding hazards of water pollution among under-graduate students of Symbiosis College of Nursing of Pune City.

- **STRUCTURED TEACHING PROGRAMME**

In this study it refers to systematically planned teaching programme designed to provide knowledge regarding hazards of water pollutions among under-graduated students of Symbiosis College of Nursing Pune City.

- Knowledge

In this study, knowledge means to understand the level of information, understanding and skills gained by the under-graduate students of Symbiosis College Nursing Pune City regarding the hazards of water pollutions.

- Hazards

In this study, hazards means those dangerous things that likely cause water pollution which knowledge is to be assessed among under-graduate students of Symbiosis College of Nursing Pune City.

- Water Pollution

In this study, it is referred to the assessment of the effectiveness of Structured Teaching Programme on knowledge regarding substances that are harmful to water among the under-graduate students of Symbiosis College of Nursing Pune City.

- Under-Graduate Students

In this study, under-graduate students are the students who the research will be carried out on regarding their knowledge on hazards of water pollution in Symbiosis College of Nursing Pune City.

ASSUMPTION:

- The structured teaching programme may have effectiveness on knowledge regarding hazards of water pollution among undergraduate students.

- The teaching activities may show them to protect and conserve water for future generations.

Research Design:

The selection of research design is the most important step as it provides the framework for the study.

Experiment Group	Pre-test	Teaching Program	Post test

Keeping in view the objective of the study, the research design selected for the study is pre-experimental one group pre-test post-test design. In this present study we have a one experiment group. We will assess the previous knowledge by conducting pre-test and provide a teaching program. After that we will conduct the post-test to assess the effectiveness of our structured teaching program.

In this study independent variable is structured teaching program and dependent variable is knowledge regarding hazards of water pollution among undergraduate students.

Setting of The Study

Symbiosis College of nursing which is located in Senapati Bapat road of Pune city is the setting of the study.

POPULATION:

The population of this study comprises of undergraduate students of Symbiosis College of nursing**,** Pune city.

SAMPLE

The sample of our study is undergraduate student of Symbiosis College of nursing.

Sample size:-

Sample size consists of 60 nursing students.

Criteria for selection of sample

The criteria that specify population characteristics are referred to as inclusion and exclusion criteria.

INCLUSION CRITERIA

- Nursing students of symbiosis college of nursing
- Those who understands English

EXCLUSION CRITERIA

- Those who are not willing to participate
- Those who were absent during data collection.

SAMPLING TECHNIQUE

Non-probability convenience sampling technique was used for selecting the sample for the research study.

DATA COLLECTION TECHNIQUE AND INSTRUMENTS:

This study is aimed at assessing the knowledge regarding hazards of water pollution among undergraduate nursing students through structured teaching program. Hence a self-structured questionnaire and checklist tool was prepared to collect the data.

DEVELOPMENT OF TOOL

Self-structured questionnaire and checklist was prepared to assess the effectiveness of structured teaching program regarding hazards of water pollution among undergraduate nursing students.

DECRIPTION OF TOOL

SECTION 1 - Demographic variable which comprised of name of the student, age, gender, marital status, Education, and household annual income and email-id of the student.

SECTION 2- self structured questionnaire consists of 20 question regarding hazards of water pollution.

SECTION 3- Checklist to know the views on hazards of water pollution.

CONTENT VALIDITY

To check the content validity of the tool it was submitted to seven experts. The experts were experience in clinical expertise, interest in the problem being studied. They were requested to give their opinion on the appropriateness and relevance of items in the tool. As a whole the suggestions and comments of experts included was to add demographic variables and grammatical corrections. The necessary modifications were made as per as the expert advice and tool was found to be relevant.

RELIABILITY:

Reliability is a degree to which an assessment tool produces stable and consistent results. An instrument is reliable to the extent that its measure reflect true scores that is, to the extent that measurement errors are absent from obtained scores.

Karl Pearson test-retest method was used to establish a reliability of structured questionnaire and checklist. Reliability was satisfactory and the value of knowledge obtained is 0.80.

DATA COLLECTION:

On day one we explained the purpose of our research

study to each students and instructions regarding the pre-test was given. Confidentiality of their response was assured. Pre-test response was received on the same day using the tool.

On day two we provided them a video presentation of our teaching program and a power point presentation file and made sure they watched the video and file.

On day three, post-test was taken using the same tool.

Analysis of Data

SECTION – I

Table - 1 Frequency and percentage distribution of samples on selected demographic variables.

n=60

Sr. no.	Demographic data	Frequency	Percentage (%)
1.	Age		
	● Less than 17	-	-
	● 17-20 year	32	53.3%
	● 20years and above	28	46.7%
2.	Gender		
	● Male	15	25%
	● Female	45	75%
	● Other	-	-
3.	Marital status		
	● Married	3	5%
	● Unmarried	57	95%
4.	Education		
	● Secondary school	1	1.7%
	● Higher secondary	9	15%
	● Undergraduate	50	83.3%
5.	Household annual income		
	● 50000-100000	27	45%
	● 100000-300000	18	30%
	● 300000 and above	15	25%

The data presented in the above table shows, no samples were found in the age group of less than 17, 32(53.3%) samples were in the age group of 17-20 years, and 28(46.7%) samples were in the age of 20years and above.

The above table shows, 15(25%) were male students, 45(75%) were female students and the other students were not there.

The above table demonstrates that 3(5%) of the students are married and 57(95%) of the students are unmarried.

Related to education, 1(1.7%) of the Nursing students were secondary school qualified, 9(15%) were higher secondary qualified, and 50(83.3%) were undergraduate.

The above table shows that 27(45%) of the samples household annual income is between 50,000-1,00,000, 18(30%) of the samples household annual income is between 1,00,000-3,00,0000, and 15(25%) of the samples household annual income is 3,00,000 and above.

SECTION- II

Table- 2 Distribution of samples according to the pretest knowledge scores of the nursing students regarding hazards of water pollution. (n=60)

	Pre-test	
Level of knowledge	**Frequency**	**Percentage (%)**
Excellent	20	33.33%
Good	38	63.33%
Poor	2	3.34%

Table- 2 shows the frequency and percentage distribution of samples according to the pre-test knowledge score of the nursing students regarding hazards of water pollution. It revealed that 20(33.33%) of the students have excellent knowledge, 38(63.33%) have good knowledge and 2(3.34%) of the Nursing students have poor knowledge regarding hazards of water pollution.

SECTION-III

Table- 3 Distribution of samples according to the post-test knowledge scores of the nursing students regarding hazards of water pollution. (n=60)

	Post-test	
Level of knowledge	**Frequency**	**Percentage (%)**
Excellent	34	56.66%
Good	26	43.34%
Poor	0	0

Table- 3 shows the frequency and percentage distribution of samples according to the post-test knowledge scores of the Nursing students regarding hazards of water pollution. It revealed that 34(56.66%) of the Nursing students have excellent knowledge and 26(43.34%) of the Nursing students have good knowledge.

SECTION-IV

Table-4 Comparison of the pre-test and post-test knowledge scores of the nursing students regarding hazards of water pollution.

To test the statistically significant difference between the mean pre-test and post-test knowledge scores of the Nursing students regarding hazards of water pollution. the following null hypothesis was stated.

Hypothesis-01

The mean post-test knowledge score is higher than the mean pre-test knowledge scores of the nursing students regarding hazards of water pollution. (n=60)

Knowledge	Mean	Mean difference	T-test value	Probability value
Pre-test	18.767	1.783	2.1845	P = 0.0107
Post test	20.55			

Table- 4 shows that, mean post-test knowledge score of the nursing students regarding hazards of water pollution are significantly higher than their mean pre-test knowledge scores. In order to find out the significant difference between the mean score of pre and post-test knowledge score of the nursing students regarding hazards of water pollution paired't' test was computed. The calculated value is higher than the table value (T-test value 2.1845), the null hypothesis was rejected, and the research hypothesis was accepted. Hence the researcher concluded that gain in knowledge is not by chance but by structured teaching program on hazards of water pollution.

SECTION V

Association between the pre- test knowledge and practice scores of the nursing students regarding hazards of water pollution and demographic variables

To identify the association between the pre-test knowledge scores on Nursing students and the selected demographic variables. The following null hypothesis was stated

HYPOTHESIS-02

There will be no significant association between the pre-test knowledge scores of Nursing student's knowledge and selected demographic variables.

Table-5 Association between the pre- test knowledge score of the nursing students regarding hazards of water pollution with the demographic variable (n=60)

Demographic Variable	Frequency numbers	Excellent	Good	Poor	Fisher's exact test
Age Less than 17 17-20 year 20years and above	 00 32 28	 00 11 09	 00 20 18	 00 01 01	1.0

Cont... **Table-5 Association between the pre- test knowledge score of the nursing students regarding hazards of water pollution with the demographic variable (**n=60)

Gender Male Female Other	 15 45 00	 05 15 00	 09 29 00	 01 01 00	 0.634
Marital status Married Unmarried	 03 57	 02 18	 01 37	 00 02	 0.346
Education Secondary school Higher secondary Undergraduate	 01 09 50	 00 03 17	 01 06 31	 00 00 02	 0.999
Household annual income 50000-100000 100000- 300000 300000 and above	 27 18 15	 10 05 05	 16 13 09	 01 00 01	 0.836

In the table-5 shown above, fisher's exact test is carried to find out the association between the knowledge of nursing students regarding hazards of water pollution and demographic variables. The result shows there is no significance association between age, gender, marital status, education, household annual income of the student.

Recommendation:

- A study can be conducted to assess the awareness of environmental issues among undergraduate students.

- A study can be conducted to assess the effectiveness of environmental science on school students.

Conclusion

The structured teaching program through presentation found to be effective in improving the knowledge regarding hazards of water pollution among undergraduate nursing students. Through this study, researchers have come to the statement of conclusion that nursing students had average knowledge before structured teaching programme regarding hazards of water pollution. Thereafter, the raised post test score gained from the structured teaching programme suggested effective in upgrading their knowledge about water pollution. Education activities on the hazards of water pollution is extremely important as it helps students to apply right attitudes when dealing with the environment.

Ethical Clearance:

- The study proposal was sanctioned by the ethical committee of Symbiosis college of Nursing.

- The study was explained to participants and informed consent was taken from the participants.

- Confidentiality of data collected was maintained.

Source of Funding: Self

Conflict of Interest:

There is a need to have teaching activities or raising awareness program regarding the environmental issue like water pollution among undergraduate students. Upgrading knowledge helps students understand

how their decisions and actions affect the renewable sources. The students will seek more knowledge about environment issue and this will help to conserve renewable resources, it also teaches about ways we can take action to keep our environment healthy and sustainable for the future. The need for sustainable development is a key to future mankind. This will empower them to protect and act responsibly towards environment.

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To Assess the Awareness, Beliefs and Practice Patterns Regarding Oxygen Therapy in Prehospital Management of Respiratory Emergencies amongst EMS Professionals in Pune, India

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Abstract-

Background: Respiratory emergencies is one of the life threatening conditions that quickly develop into respiratory failure landing into cardiac arrest hence EMS professional should posses updated knowledge regarding oxygen therapy and same should reflect in their beliefs and practice patterns.

Aim: To assess the Awareness, Beliefs and Practice patterns regarding Oxygen therapy in prehospital management of respiratory emergencies amongst EMS professionals in Pune, India

Methodology: The study was conducted among 100 EMS professionals at Symbiosis Center for health Skills, Pune, India. A pretested and validated questionnaire developed by Aloushan et al[3] was circulated among EMS professionals. The original 25 items questionnaire was utilized for the study, 9 items pertaining to awareness, 7 items pertaining to belief and 9 items pertaining to practice patterns. EMS professionals were given 1 day time limit to submit the completed questionnaire. The data collected was tabulated and statistically analyzed using SPSS version 23.0.

Result: The mean awareness score of the participants in the study was found to be 4.67 and S.D is 1.63 whereas the mean Practice pattern score of the participants in the study was found to be 3.44 and S.D is 1.44. Majority of respondents agreed that oxygen should be given under medical supervision and that humidified oxygen prevented dryness of upper respiratory tract. Greater emphasis needs to be laid on practical sessions demonstrating oxygen therapy in the EMS programs.

Conclusion: It is essential that EMS professionals undergo regular skill upgradation courses and are evaluated at the end of each course. This shall ensure appropriate utilization of the oxygen therapy and minimization of medical errors thereby saving more lives.

Keywords: *Awareness, beliefs, practice patterns*

Background

Respiratory emergencies are one of the most common life threatening conditions encountered by Emergency Medical Services (EMS) professionals. Most patients present with respiratory distress which can quickly develop into respiratory failure and associated cardiac arrest.

A study by Ghosal et al[1] (2016) stated that prevalence of Asthma in India range between 2.05% to 3.5%, while

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COPD prevalence in India varied from 3% to 8%.

Oxygen is the drug of choice in life threatening conditions like Acute Myocardial infarction, Stroke, Cardiac Arrest and some life compromising Respiratory diseases like COPD, Asthma, Congestive Heart Failure, Pulmonary edema etc.[2] Oxygen is listed as one of the most effective and safe drug in these conditions.[3]

The rationale behind utilizing oxygen therapy is that it compensates for the oxygen burden and helps maintain optimum levels of oxygen in blood thereby reducing breathing efforts.[3]

In prehospital scenarios oxygen is commonly delivered without any particular indication and in unknown concentration to treat hypoxemia, however administering higher concentration of oxygen can lead to a state of hyperoxia. The amount of oxygen to be delivered and the method of delivering oxygen depends on underlying medical conditions as well as acute and chronic stage of the particular disease.

Accurate knowledge of these principles is essential for all EMS professionals. Inappropriate use of oxygen can cause major harm to the patient and lead to rapid deterioration.[4]

A study by Kondo et al[5] (2019) concluded that prehospital oxygenation can improve the mortality rate among trauma patients but it should be administered and monitored by only trained staff.[6]

A study by Ghebremicheal et al[7] (2019) emphasized on formulating a protocol for appropriate oxygen delivery.

Hence EMS professionals should possess updated knowledge regarding oxygen therapy and the same should reflect in their beliefs and practice patterns.

Aim

This study intends to assess the Awareness, Beliefs and Practice patterns regarding Oxygen therapy in prehospital management of respiratory emergencies amongst EMS professionals in Pune, India

Methodology

The study was conducted among 100 EMS professionals at Symbiosis Center for health Skills, Pune, India. The EMS professionals were previously given training in their academic curriculum.

A pretested and validated questionnaire developed by Aloushan et al[3] was circulated among EMS professionals. The original 25 items questionnaire was utilized for the study, 9 items pertaining to awareness, 7 items pertaining to belief and 9 items pertaining to practice patterns.

The 9 items pertaining to awareness and 9 items pertaining to practice patterns are in form of multiple choice questionnaire where the participants were supposed to select single best option out of 4 options. The 7 items pertaining to belief required response on a 5 point Likert scale ranging from strongly agree to strongly disagree.

Informed and written consent was taken from all the participants prior to the administration of the questionnaire. The participants were asked to respond to the questionnaire in 1 day. Responding to all items in the questionnaire were mandatory. All 100 participants submitted the completed questionnaire. The data collected was tabulated and statistically analyzed using SPSS version 23.0.

Result

Table 1. Demographic data distribution

SEX	PERCENTAGE
MALE	60%
FEMALE	40%
AGE IN YEARS	
25-30 YEARS	85%
31- 35 YEARS	10%
36-40 YEARS	3%
ABOVE 40 YEARS	2%
EDUCATION	
B.A.M.S	30%
B.H.M.S	40%
B.U.M.S	20%
Other allied medical degree	10%

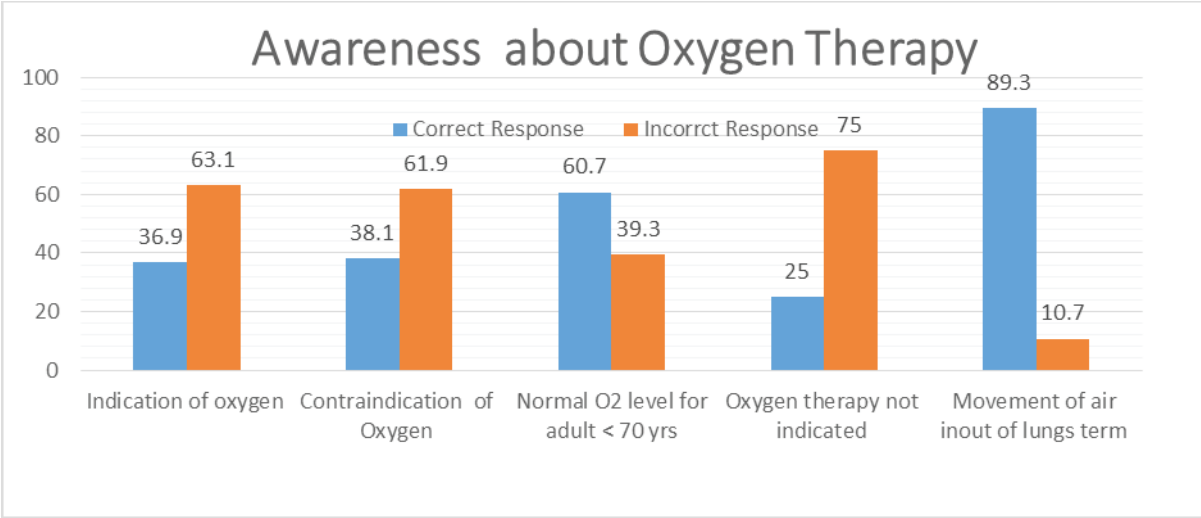


Fig 1. Frequency graph distribution on Awareness among EMS Professionals about Oxygen Therapy

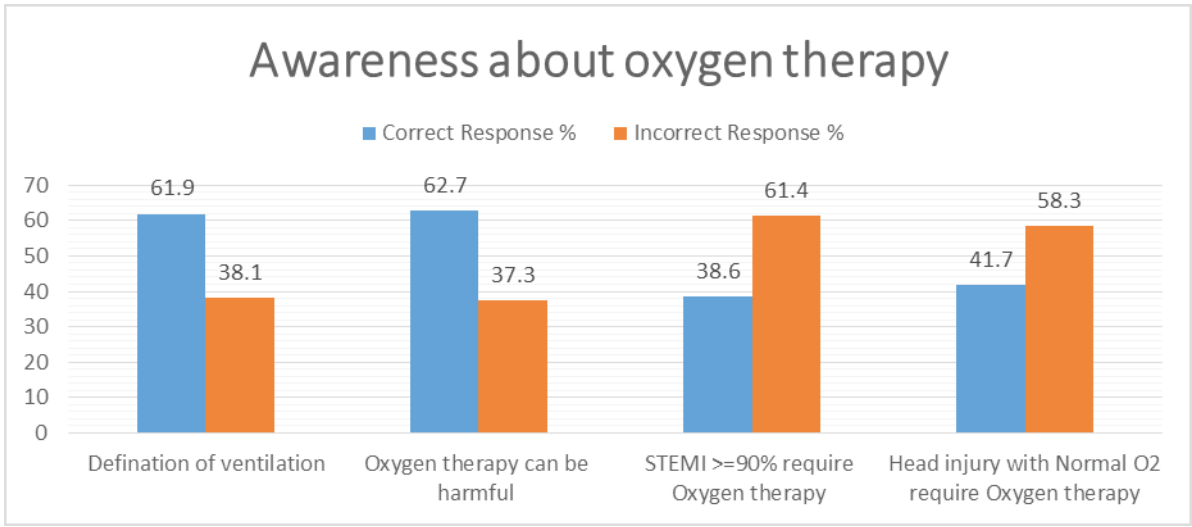


Fig 2. Frequency graph distribution on Awareness among EMS Professionals about Oxygen Therapy

As shown in Fig 1 a significant majority of EMS professionals responded inaccurately on the items pertaining to indication and contraindications of oxygen therapy. Majority of respondents were aware of the correct definition of ventilation and normal oxygen level.

As depicted in Fig 2, the respondents were well aware of the harms of oxygen therapy but responded incorrectly on the utilization of oxygen therapy in patients of ST Elevation Myocardial Infarction (STEMI) and head injury with normal oxygen saturation.

The mean awareness score of the participants in the study was found to be 4.67 and S.D is 1.63

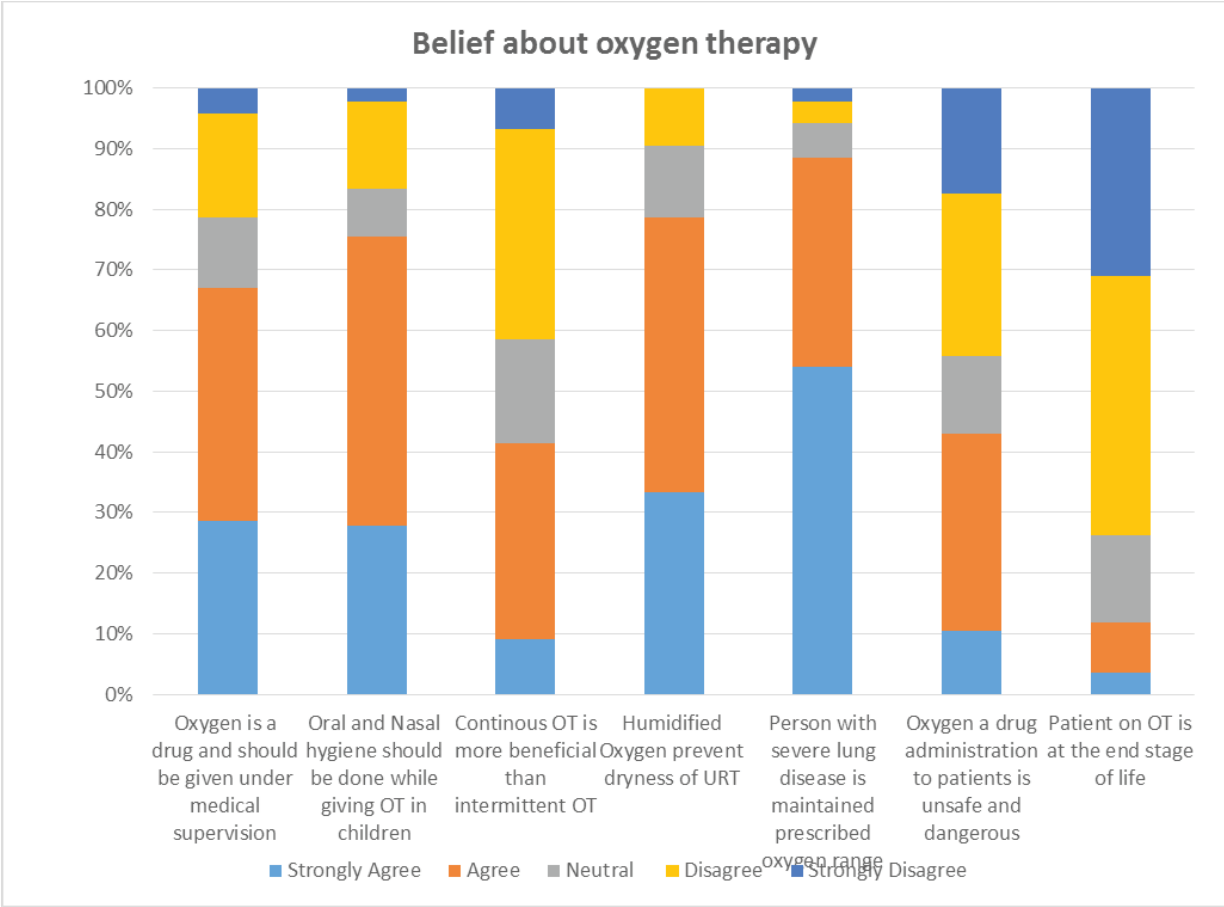


Fig 3. Table showing perception level among Health care professionals about Oxygen therapy

As seen in Fig 3. majority of respondents agreed that oxygen should be given under medical supervision and that humidified oxygen prevented dryness of upper respiratory tract.

There was consensus amongst respondents that person with severe lung disease should be maintained in a prescribed oxygen range

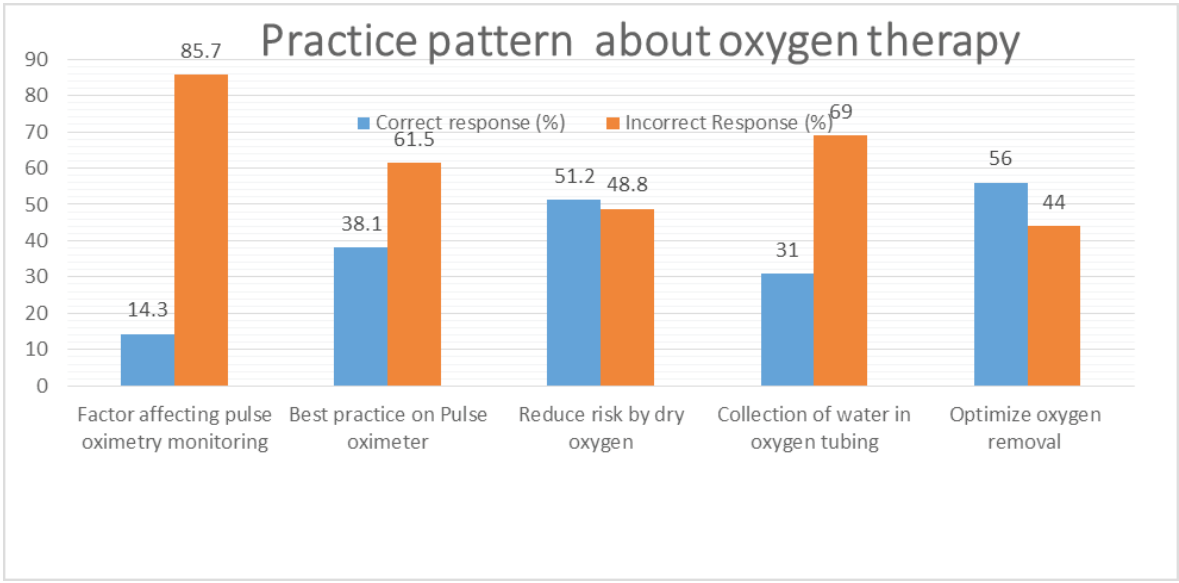


Fig 4. Frequency Graph distribution on Practice Pattern among EMS professionals about Oxygen Therapy

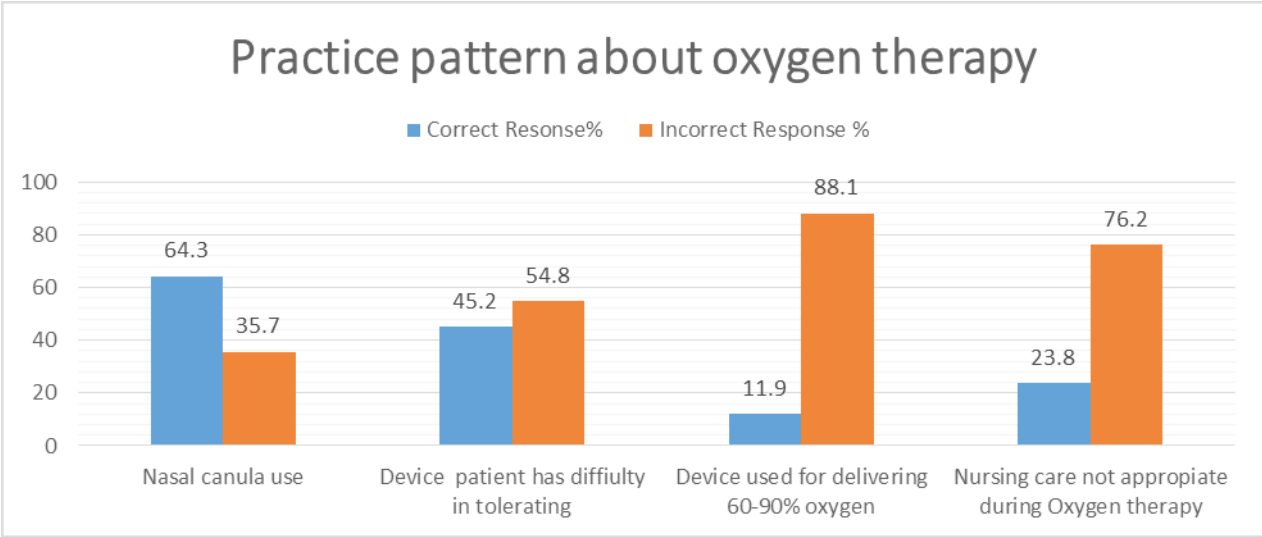


Fig 5. Frequency Graph distribution on Practice Pattern among EMS professionals about Oxygen Therapy

Fig 4 the majority of respondents were not aware of the factors affecting pulse oximetry monitoring and collection of water in the oxygen tubing. Significant number of respondents responded incorrectly on the items pertaining to optimization of oxygen removal.

As shown in Fig 5 most participants responded incorrectly on the items pertaining to devices used in Oxygen delivery.

The mean Practice pattern score of the participants in the study was found to be 3.44 and S.D is 1.44.

Discussion

Judicious use of oxygen therapy in critical emergencies can significantly impact the survival rate of patients. Unlike common knowledge that oxygen therapy is always beneficial, numerous studies have shown that unnecessary oxygen therapy can cause severe harm to the patient.

The respondents being EMS professionals trained in oxygen therapy were expected to score better on the awareness items , but the mean score 4.67 is below par and points to the urgent need of continued education in the form of workshops and seminars.

Majority of the respondents were unaware of the role of oxygen therapy in non-respiratory emergencies like ST Elevation myocardial Infarction (STEMI) and Head injury.

The opinion of EMS professionals on continuous oxygen therapy versus intermittent oxygen therapy was deeply divided similarly there was no consensus on whether administration of oxygen as a drug is unsafe and dangerous .However majority of the respondents appreciated the importance of the oral and nasal hygiene while giving oxygen therapy to children.

The scores obtained on the correct practice pattern in oxygen therapy were abysmal. This clearly shows that EMS professionals require more hands on training in real clinical situations. Most of the respondents were unclear about the methodology of oxygen delivery, monitoring and nursing care during oxygen therapy.

Greater emphasis needs to be laid on practical sessions demonstrating oxygen therapy in the EMS courses.

Conclusion

The study attempts to edge the awareness, beliefs and practice patterns with regards to oxygen therapy amongst actively working EMS professionals. Although the sample size is limited, result points towards a vast scope of improving the knowledge and skill set of EMS professionals.

It is essential that EMS professionals undergo regular skill upgradation courses and are evaluated at the end of each course. This shall ensure appropriate utilization of the oxygen therapy and minimization of

medical errors thereby saving more lives.

Acknowledgement: I pay my immense gratitude and token of thanks to Dr. Amairah Fahad Aloushan for granting permission to use the questionnaire for assessing awareness, beliefs and practice patterns among EMS professionals regarding oxygen therapy in India.

Source of Funding: Self

Conflict of Interest: None

Ethical Clearance: Obtained from IEC, SIU

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Simulation based Healthcare Education- Barriers in Initiation

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Abstract

Introduction: This paper summarizes a structured expert panel session on the subject of barriers in initiating Simulation based andragogy in Healthcare Education in India. The expert panel consisted of members of academia in Medicine, Nursing, paramedical and representatives from the healthcare simulation technology industry.

The global burden of unsafe medical care is significant and remains a cause of concern. Studies have shown that majority of medical errors do not result from individual negligence. Majority of the medical errors have been found to be preventable. Simulation Based Medical Education is the way forward in achieving greater patient safety and improving patient outcomes.

Hence there is an urgent need to incorporate Simulation into the healthcare education system in our country.

Objective: To discuss the current scenario of healthcare education, the role of Simulation and experience sharing on the barriers in its large scale initiation.

Result: The key barriers that emerged during the discussion in utilization of Simulation based medical education were capital investment, psychological resistance, difficulty in integrating curriculum and lack of trained man power.

Conclusion: This paper attempts to offer pragmatic solutions to the existing barriers and help mitigate medical errors and improve patient safety.

Keywords: *Simulation, challenges, healthcare education*

Introduction

This paper summarizes a structured expert panel session on the subject of barriers in initiating Simulation based andragogy in Healthcare Education in India. The panel session was conducted on the first World Patient Safety Day on September 17, 2019. The expert panel consisted of members of academia in Medicine, Nursing, paramedical and representatives from the healthcare simulation technology industry. The objective of the session was to encourage a free willing discussion on the current scenario of healthcare education, the role of Simulation and experience sharing on the barriers in its large scale initiation.

The global burden of unsafe medical care is significant and remains a cause of concern[1]. Although comprehensive and precise data on medical errors is not available in India, studies conducted in the states of Uttarakhand and Karnataka estimate the rate of medical errors in tertiary hospitals to be as high as 25.7 % and 15.3% respectively[2,3.] The landmark paper, "To err is human: Building a safer health system" in 2000 estimated that between 44000 to 98000 people die in

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hospital each year as a result of medical error. Studies have shown that majority of medical errors do not result from individual negligence. Errors are commonly caused due to faulty systems and processes that make people prone to commit error[4].

Majority of the medical errors have been found to be preventable. A Harvard medical practice study noted that 69% of the errors occurring in hospitalized patients are preventable[5]. Patient safety emphasizes the reporting, analysis and prevention of medical errors that lead to adverse health events[6]. The Government of India has taken a number of steps to improve the quality of healthcare services and strengthen patient safety[7,8,9]. The Government of India has collaborated with the Global Patient Safety Collaborative (GPSC). Under this initiative, India is the only country in the Southeast Asian region to receive technical support to strengthen education training capacity, research and leadership in patient safety[10]. Simulation Based Medical Education is the way forward in achieving greater patient safety and improving patient outcomes[11].

Hence there is an urgent need to incorporate Simulation into the healthcare education system in our country. Elaborated below are the broad themes that emerged during the expert panel discussion which focused on barriers in Simulation integration.

Key Barriers in Integration of Simulation Based Medical Education-

Capital Investment:

The teaching methodology in Healthcare Education has slowly evolved in the past few decades and has witnessed subtle transitions from the archaic "chalk and talk" to usage of posters, slides and power-point presentations. To make learning more practical, case based methodology was introduced into the curriculum. All these sessons remained didactic and were completely centered around the instructor, and the learners acting as mere spectators. This form of learning failed to engage the learner and could not perform a platform for team based experiential learning.

Simulation experiences across the globe have validated the efficacy of this methodology in improving the knowledge and practice of healthcare concepts, through numerous studies. Although the data available is limited in India, there is no reason to believe that Simulation wouldn't positively impact Healthcare Education. In interaction with management representatives of various medical and paramedical colleges, a clear resistance to adoption of this andragogy has emerged.

A key reason attributed, has been lack of funds and resources to introduce this technology. Although it is true that High Fidelity Simulation is a capital intensive investment where returns cannot be measured in real economic terms. The ultimate purpose of Simulation is to reduce medical error and patient safety, hence the outcome analysis should focus on the decrease in errors and complications in real healthcare settings. The skills learnt in a Simulation lab are applied by the learner, during his entire lifetime and hence the impact assessment in tangible terms during the learners training period is not practically possible.

Even though, cost concerns are genuine, a resource crunched country like ours can explore the 'Hub and Spoke' model wherein one Simulation Centre in a geographical area can cater to all institutes in its radius. This shall allow extremely limited encounters per student but it would definitely help in providing a first-hand experience to maximum learners and facilitators. The experiences gained through this model can be utilized for a slow phasic expansion, based on the specific needs of each institute. This shall help in avoiding unnecessary investment on costly technology. Institutes shall be able to take decisions on their individualized requirements and opt for appropriate technology.

Psychological Resistance:

Traditionally, medicine has been learnt by bedside and clinical skills learnt on a live patient, still remain the gold standard. Yet, in the past few decades the opportunities of learning in the Medical ward are steadily shrinking. This is partly due to the increasing empowerment of patients leading to refusal of repeated examinations done by students on them. This has resulted in greater dependence on theory and lack of confidence among newly graduated doctors and nurses due to insufficient practice. When these healthcare professionals join a hospital or nursing home they are unable to perform clinical examination and invasive

procedures independently.

Simulation offers an alternative for repetitive practice which can help boost the skill set of the learners. Simulation allows the learners to commit mistakes without causing harm to anyone and in many cases provide objective data for clinical skill performance. There is a widespread misconception that adoption of Simulation shall completely obliterate bed side teaching on live patients, but Simulation at its best is an adjunct and not a substitute. This false impression is responsible for psychological resistance amongst decision makers in healthcare institutions. This issue can be resolved by increasing advocacy and awareness created by existing simulation users. Existing users can share their experiences with application of Simulation, thereby allaying the fears of non-adopters.

Simulation allows the teaming of rare clinical cases as well as deliberate practice of difficult clinical procedures. Both of these objectives cannot be achieved by bedside teaching alone. Hence, rather than shunning simulation, healthcare institutions should increasingly adopt this methodology for achieving better patient outcomes.

Challenges In Integration:

It emerged during the discussion that, for eminent institutions which have initiated Simulation based education, comprehensive integration into the curriculum remains a challenge. The vastness in the curriculum leads to paucity of time for both the instructors and learners and restricts the use of Simulation. Simulation sessions require elaborate preparation as well as background training n the methodology. This causes resistance among instructors to teach clinical cases on Simulators. They continue to prefer didactic teaching method because they are conditioned with this methodology for many years.

Even guidelines for Introduction of Simulation into Curriculum and focus on skill learning have been emphasized by various medical and nursing councils very recently. These guidelines shall definitely boost the adoption but Healthcare Education Institutes shall require time and effort to implement the guidelines. Simulation is generally misinterpreted as only being the usage of high end technology manikins. High Fidelity Simulation is better for clinical scenario and team based learning, and hence should be utilized only for this purpose. Part task trainers constitute low fidelity simulation and can be used for effectively teaching a gamut of basic clinical skills. They require very less capital investment and can drastically improve the skillset of learners.

Utilizing standardized patient for teaching case history taking and clinical examination is another modality of Simulation which is largely being unexplored in our country. Most international certifying examinations utilize standardized patients for student assessment. Hence a variety of simulation modalities can be used based on appropriate clinical requirement.

Lack of Trained Man Power:

Although the technology has gradually trickled into Indian institutions, providing qualified man power undoubtedly remains a daunting task. Simulation based education requires a radically different approach compared to didactic teaching. Also, the instructors need to be trained on high-end softwares for programming clinical cases. Faculty Development Programme have been designed and implemented in a few institutions but the frequency of such training sessions remains abysmally low. This can be attributed to the presence of only handful of certified Simulation educators in the country. For Simulation to be truly a pan India phenomenon, there is pressing need to develop a dedicated cadre of Simulation educators across the country. Train the trainer programs should be conducted at regular intervals by pioneering institutions to create a sizeable number of trainers well versed in Simulation Based Education modalities.

Conclusion

Every new technology has faced varied kinds of barriers in its large scale implementation. Simulation Based Medical Education is a valuable addition to the healthcare education system in our country. The benefits of Simulation Based Medical Education far outway the limitations associated with it. This paper attempts to summarize the current simulation environment in our country. It also offers pragmatic solutions to the existing barriers in a hope that Simulation shall be adopted across the length and breadth of the country's healthcare institutes thereby mitigating medical errors

and improving patient safety.

Source of Funding: Self

Conflict of Interest: Nil

Ethical Clearance: Taken from IEC, SIDU committee

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To Evaluate the Knowledge and Attitude Regarding Management of Acute Poisoning amongst the Emergency Medical Professionals in Pune, India

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Abstract

Introduction: Poison is any substance, which causes ill effects, or death when inhaled, ingested or injected in living cells. Emergency Medical Professionals working in Ambulances play a very important role in initial management of poisoning. Hence, it is imperative that the EMS professionals should have requisite knowledge and possess the right attitude to be effective caregivers in cases of poisoning.

Objective: To evaluate the Knowledge and attitude regarding management of Acute Poisoning amongst the Emergency Medical Professional in Pune, India.

Methodology:

The study was conducted amongst 170 Emergency Medical Professionals in Pune, India. The eighteen-item questionnaire comprised of two key parameters – eight items testing Knowledge and ten items measuring Attitude. Each item on the questionnaire required a binary response. All items were required to be mandatorily filled.

Discussion: The study evaluated the knowledge and attitude of the EMS professionals in initial management of cases of acute poisoning. Majority of the respondents possessed the right general knowledge about poisoning except that most felt that it was more important to treat the poison rather than patient but could not correctly differentiate between the early stage and late stage symptom of poisoning.

Conclusion: The study attempts to evaluate the knowledge and attitude of the EMS responders in acute poisoning and provides with significant areas with scope of improvement that can guide educators to accordingly tailor the teaching curriculum of the subject of poisoning.

Keywords: *Acute Poisoning, Knowledge, Attitude and Practices (KAP), Prehospital Management, Emergency Medical Services Professionals*

Introduction

Poison is any substance, which causes ill effects, or death when inhaled, ingested or injected in living cells.[1]Poisoning can be differentiated into three types viz. Suicide, Homicide or Accidental.

As per study conducted by Raut Asawari et *al*[2], *India* has the highest incidence of poisoning in the world with more than 50,000 people dying every year due to toxin exposure. The National Poisons Centre (NPIC) at the All India Institute of Medical Sciences, New Delhi, India has held household products, followed by pharmaceuticals, agricultural pesticides and industrial chemicals, responsible for high incidence of poisoning.[3]

Rakesh Sharma *et.al*[4] in a study conducted in 2019, highlighted that the morbidity and mortality of poisoning can be reduced by development and implementation of effective intervention at primary level by health care providers and strategies on preventive aspect.

Emergency Medical Professionals working in Ambulances play a very important role in initial

management of poisoning. A study conducted by Fridtjof Heyerdahl *et al*[5] observed that majority of the poisoned patients who were treated in prehospital settings were discharged without any requirement of hospital transfer and thus deemed prehospital treatment of poisoning as safe.

Being first responders, the EMS professionals have a crucial role to play in detection and management of ill effects of poisoning. Hence, it is imperative that the EMS professionals should have requisite knowledge and possess the right attitude to be effective caregivers in cases of poisoning.

Objective

To evaluate the Knowledge and attitude regarding management of Acute Poisoning amongst the Emergency Medical Professional in Pune, India

Methodology

The study was conducted amongst 170 Emergency Medical Professionals in Pune, India. A previously tested and validated questionnaire by Fatmah Ali Abdullah Hakam *et.al*[6] was utilized for the study. Majority of the respondents possessed the right general knowledge about poisoning except that most felt that it was more important to treat the poison rather than patient.

The eighteen-item questionnaire comprised of two key parameters – eight items testing Knowledge and ten items measuring Attitude. Each item on the questionnaire required a binary response. All items were required to be mandatorily filled.

The questionnaire was administered through online mode after obtaining informed consent from the respondents. The respondents were given two days to fill the questionnaire and revert. Any queries pertaining to questionnaire was clarified during data collection.

All 170 respondents reverted with completely filled questionnaire. The data was tabulated and statistically analysed with the help SPSS version 23.

RESULTS:

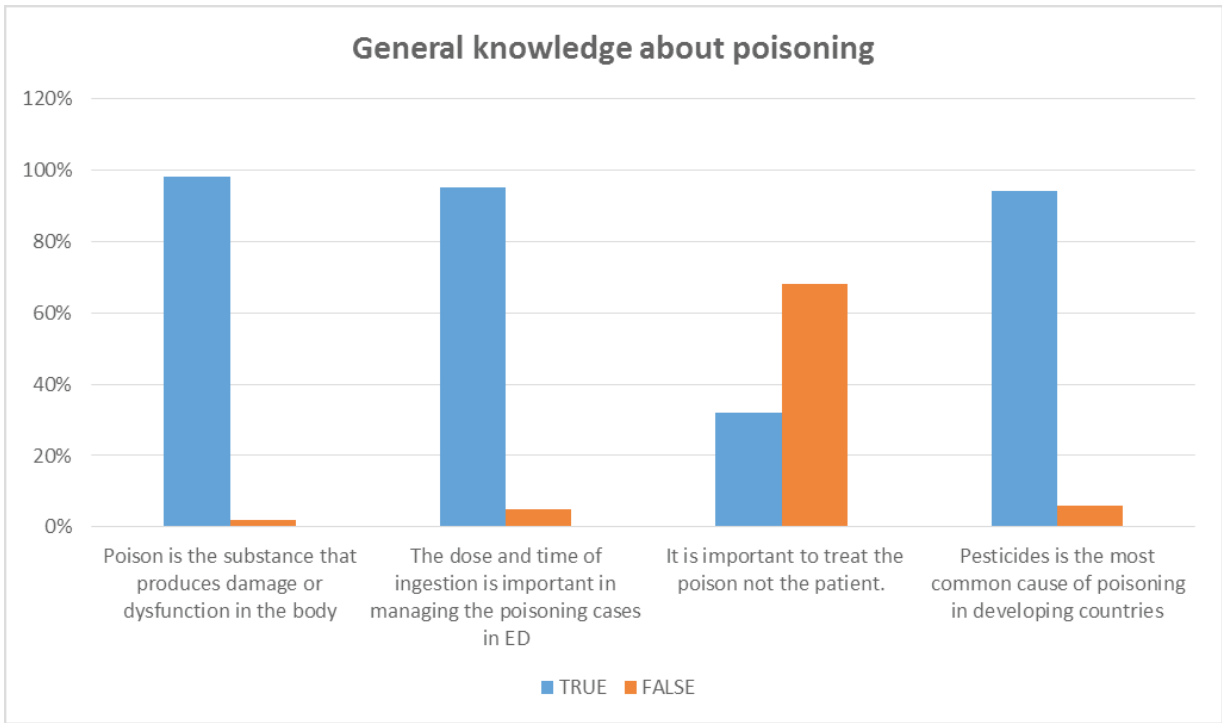


Figure 1.

As seen in figure 1. More than 95% of the respondents had the knowledge of the definition of poison and the importance of the dose and time of ingestion. 94% were aware that pesticides are the most common cause of

poisoning in developing countries. However as many as 32 % of the respondents felt it more important to treat the poison rather than patient.

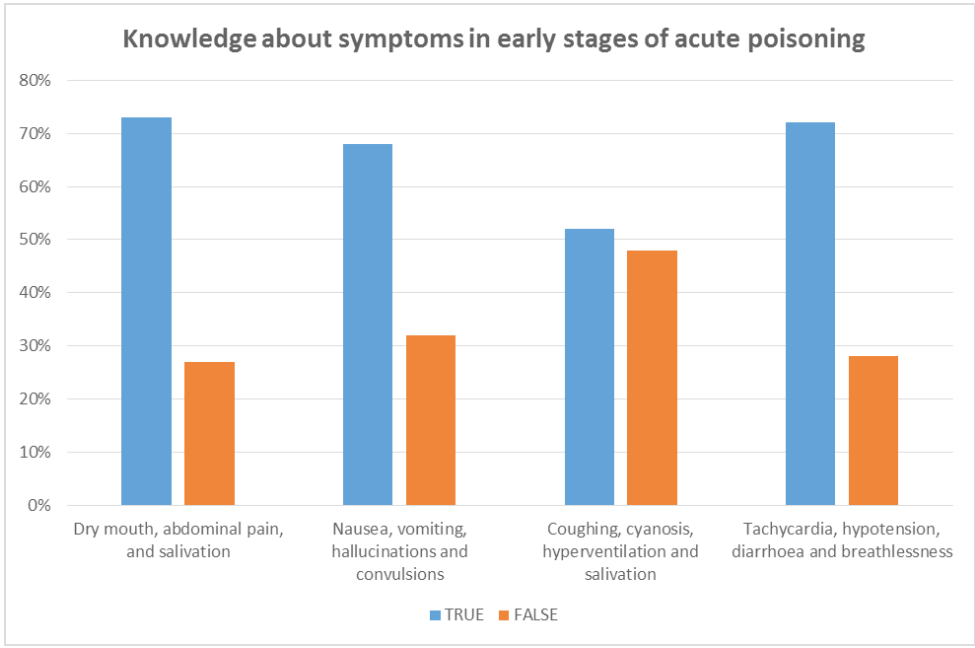


Figure 2.

As seen in figure 2. 73% of the respondents could identify Dry Mouth, Abdominal Pain and Salivation as the early symptoms of poisoning.

However majority of respondents incorrectly classified the late stage symptoms into early stages of acute poisoning.

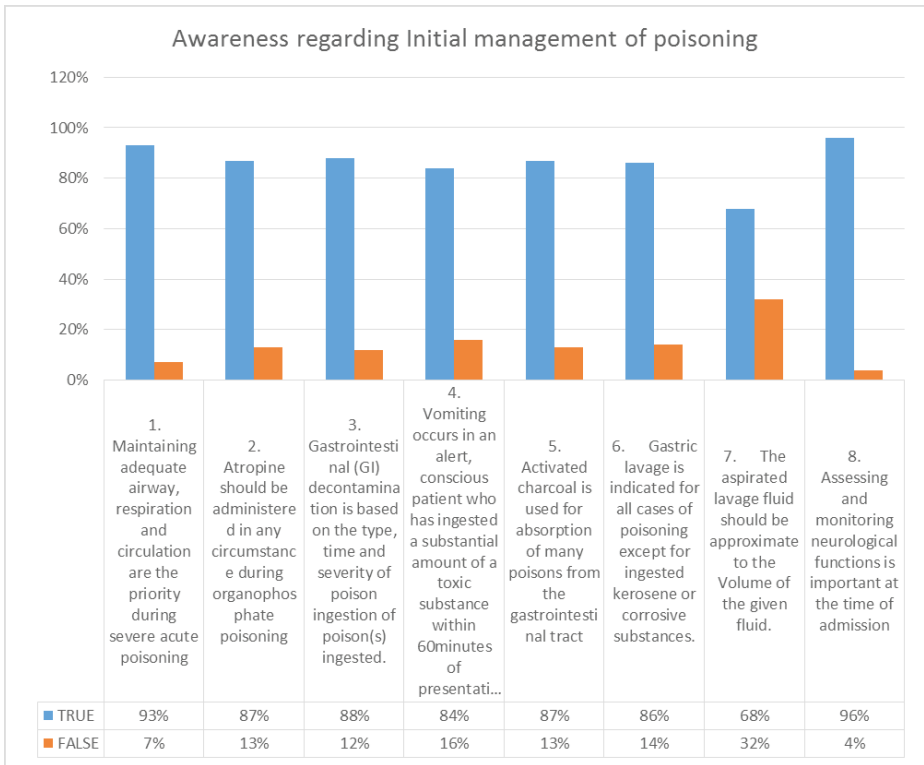


Figure 3.: As seen in figure 3. More than 80 % of the respondents correctly answered on the various initial

management strategy of poisoning.

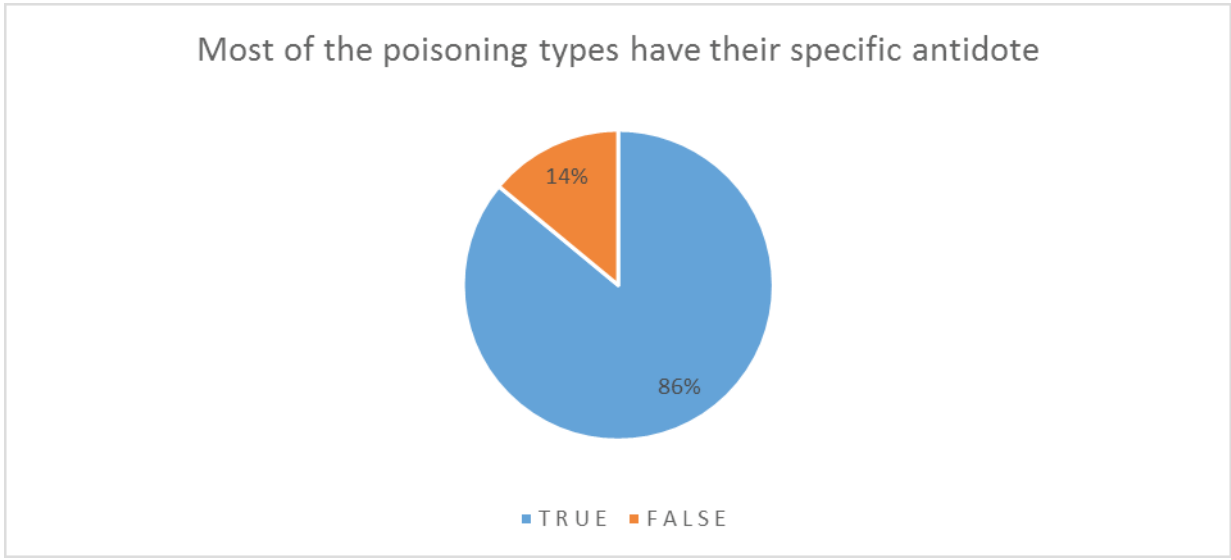


Figure 4: As seen in Figure 4. As many as 86% respondents felt that most poisoning type have their specific antidotes.

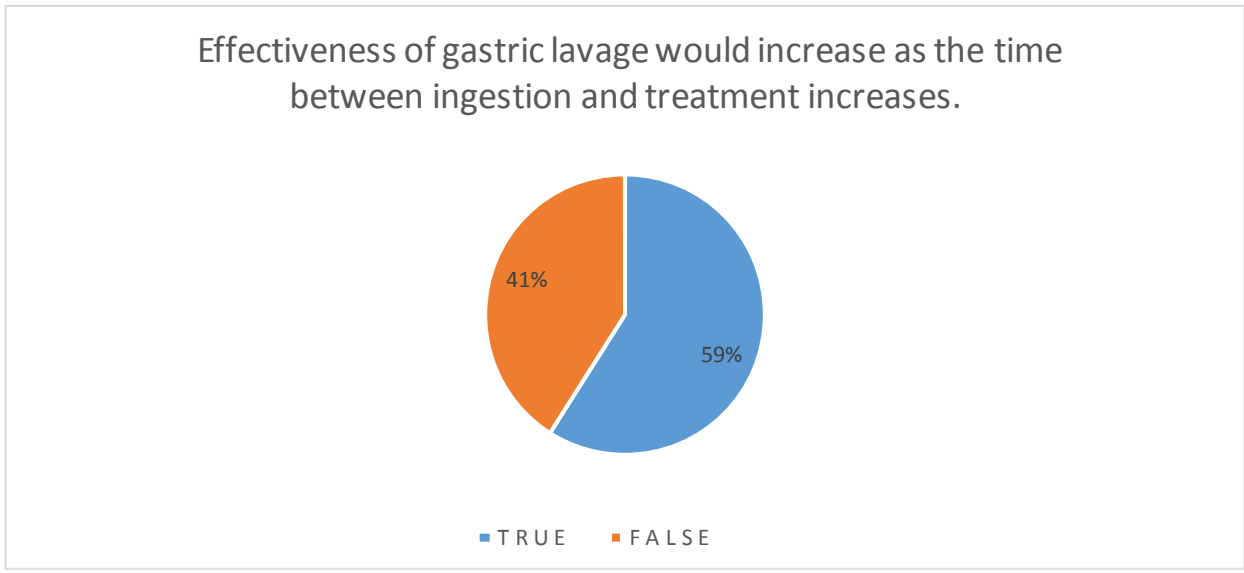


Figure 5.

As seen in Figure 5. 59% of the respondents felt that effectiveness of gastric lavage would increase as the time between ingestion and treatment increases.

DISCUSSION

The study evaluated the knowledge and attitude of the EMS professionals in initial management of cases of acute poisoning.

Majority of the respondents possessed the right general knowledge about poisoning except that most felt that it was more important to treat the poison rather than patient. This approach can adversely affect the initial management of the patient because the EMS respondents should focus on stabilizing the patient and providing symptomatic relief rather than finding the exact poison.

Majority of the respondents could not correctly differentiate between the early stage and late stage symptom of poisoning. This could be due to lack of practical experience and can be corrected by increasing the exposure of the Emergency Medical Professionals to real world cases. The respondents scored well on all aspects of awareness about initial management of poisoning. This is significant because it showed the preparedness of the EMS Professionals in providing standard care in the initial management of poisoning. Surprisingly, only 14 % of the respondents were aware that most poisons do not have a specific antidote. Management of most poisoning cases is based on general principles and these needs to be effectively communicated to the Emergency Medical Professionals. This will help them prioritize the management of poisoning effectively rather than waste precious time in searching specific antidotes. Another noteworthy point that needs to be emphasized is that majority of respondents that effectiveness of gastric lavage would increase as the time between ingestion and treatment increases. This is incorrect, as this would allow the poison to be absorbed into bloodstream thereby drastically reducing the effectiveness of gastric lavage.

Conclusion

The study attempts to evaluate the knowledge and attitude of the EMS responders in acute poisoning and provides with significant areas with scope of improvement that can guide educators to accordingly tailor the teaching curriculum of the subject of poisoning. It throws light that there should be clarity regarding application of theoretical knowledge to the practical management of acute poisoning.

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Source of Funding: Self

Conflictof Interest: None

Ethical Clearance: Obtained from IEC, SIU

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Assessing the Effectiveness of Online Teaching Methodology among Emergency Medical Professionals in Pune, India

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Abstract

Introduction: With rapid evolution of information and research in Healthcare, students find it very challenging to keep pace with the latest knowledge. To overcome this challenge, Healthcare Information Technology should be implemented at multiple levels i.e. during their Under Graduate and Post-Graduate training which shall make them well versed with online learning. During the COVID-19 pandemic many healthcare institutions have adapted to online teaching methodology, to continue with the academic year but it is necessary to assess the effectiveness and impact on students.

In developing countries, where efficient educators in Healthcare are less in number, adapting to online based curriculum shall increase the reach of Healthcare Education to maximum learners.

Objective: To assess the effectiveness of online teaching methodology among Emergency Medical Professionals

Methodology: The study was conducted amongst 100 Emergency Medical Professionals in Pune, India. An online session on Basic Life Support and Emergency Medical Services protocols was conducted via Zoom application. Participants were asked to respond to a pretested and validated tool, the Student Evaluation of Online Teaching Effectiveness (SEOTE).

Result and Discussion: Online teaching was introduced in most of the universities as an alternative to classroom training due to the COVID 19 pandemic. The study is an unique attempt to assess the participant feedback on the effectiveness of a single online session. The results clearly demonstrate that majority of the participants provided a very positive feedback of the online teaching session.

Conclusion: The participants' feedback with regard to online teaching was found to be highly encouraging. Further longitudinal studies are essential to gauge the impactfulness of online teaching as an alternative modality to classroom instruction.

***Keywords**:* Online teaching, effectiveness, healthcare education,

Introduction

With rapid evolution of information and research in Healthcare, students find it very challenging to keep pace with the latest knowledge. To overcome this challenge , Triola *et al* suggested that Healthcare Information Technology should be implemented at multiple levels i.e. during their Under Graduate and Post-Graduate training which shall make them well versed with online learning.[1] Due to the COVID -19 pandemic, various healthcare institutions have adapted their teaching methods to online format instead of classroom training. This has been implemented to ensure continuity in the student academic year. Although online learning is a safe method of educational instruction, the online teaching

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methods used during this period need to be assessed for its effectiveness and impact on students.[2,3]

The current COVID -19 pandemic situation should be used as an opportunity to introduce newer modalities of teaching and provide better solutions in Healthcare Education.[4] A research study conducted by Peia *et al* recommends that there should be evaluation for assessing the effectiveness of online teaching method and it should be considered as a potential teaching method.[5] The objective of online teaching is to give optimum training to students, to enhance their knowledge and skills.

Aminizadeh, *et al*, in a study conducted in 2017 concluded that online teaching methods can be recommended for theoretical aspects and the traditional classroom methods can be used for teaching subjects which are more practical and skill based.[6] Redding *et al* compared online teaching with traditional classroom teaching among pre–licensing insurance students and found that online courses were more effective. They suggested that similar research should be conducted in other fields to determine the utility of online teaching.[7]

Mahadevan *et al* in a study concluded that online courses for emergency medicine can be effective as an add on for classroom-based programs. In developing countries, where efficient educators in Healthcare are less in number, adapting to online based curriculum shall increase the reach of Healthcare Education to maximum learners.[8]

Objective:

To assess the effectiveness of online teaching methodology among Emergency Medical Professionals

Methodology

The study was conducted amongst 100 Emergency Medical Professionals in Pune, India. An online session on Basic Life Support and Emergency Medical Services protocols was conducted via Zoom application. The session covered topics on Activating Emergency Medical Services (EMS), Patient Assessment, Acute Poisoning, High Quality Cardiopulmonary Resuscitation, Rapid Defibrillation, Early transport to Hospital and Post Cardiac Arrest Care. The session lasted for four hours and the participants interacted verbally, by unmuting themselves and via chat method in the zoom application. Videos depicting scenarios, skills and protocols alongwith power point presentations explaining the theoretical aspects of Basic Life Support were shared. Course assignments were provided to the participants on completion of the session.

As soon as the session was completed, participants were asked to respond to a pretested and validated tool, the Student Evaluation of Online Teaching Effectiveness (SEOTE). This tool developed by Bangert (2004) to assess constructivist-compatible online teaching practices recommended by Chickering and Gamson's Seven Principles of Effective Teaching (1987).[9] The questionnaire tool was further validated by Bangert in 2008 recommending that it is best suited and appropriate tool for assessing the quality of online instructional effectiveness of higher education.[10]

Out of the original twenty-three item questionnaire, five items based on Web based Course development Tools (WebCT) were excluded from the present study as they were considered inapplicable and eighteen items were retained and comprised of seven key parameters – four items testing Student Faculty Interaction (SF), four items testing Cooperation Among Students (CAS), three for testing Time on Task (TT), two for Prompt Feedback (PF), two for Diverse Talent and Ways of Learning (DTWL), two for High Expectations (HE) and one for Active Learning (AL).

Participant responses were elicited using a six-point Likert scale which ranged from strongly agree (6) to strongly disagree (1) (i.e., strongly agree (6), agree (5), mildly agree (4), mildly disagree (3), disagree (2), strongly disagree (1)). All items were required to be mandatorily filled. The questionnaire was administered through online mode after obtaining informed consent from the respondents. The respondents were given one day deadline to fill the questionnaire and revert. Any queries pertaining to the questionnaire were clarified during data collection.

85 respondents out of 100 participants reverted with completely filled questionnaire. The data was tabulated and statistically analysed with the help of SPSS version 23.

Result

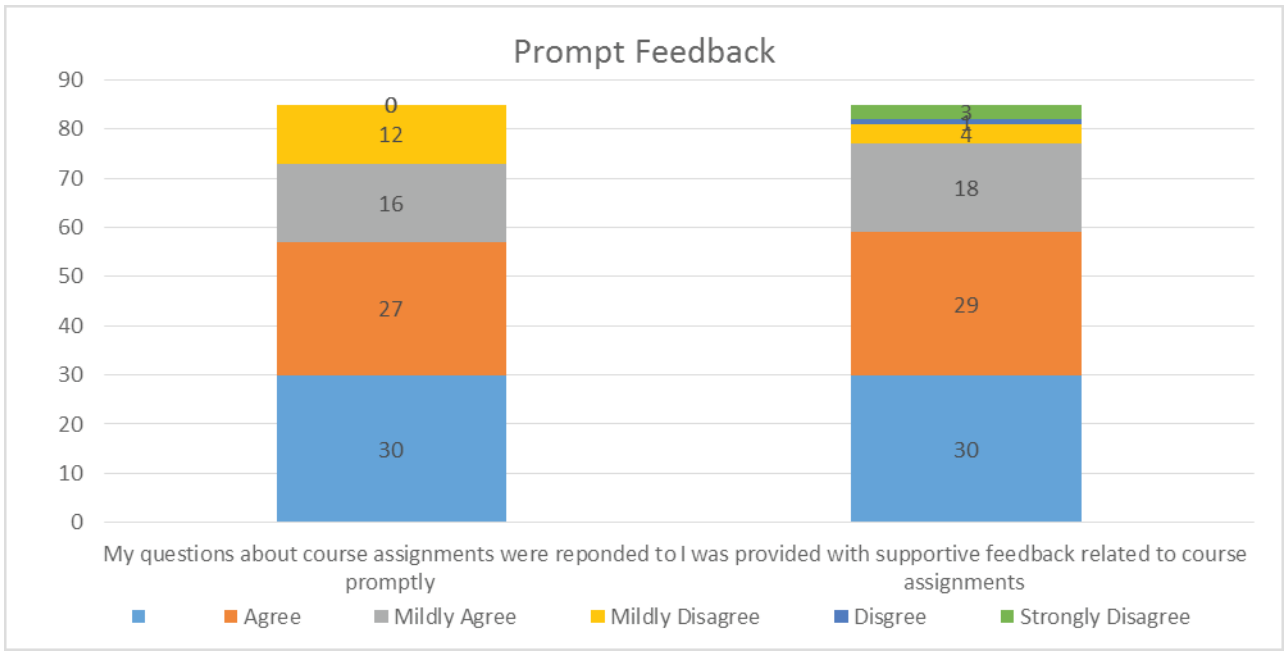


Figure 1: Responses for Prompt Feedback

As seen in Figure 1 majority of the participants were satisfied that their questions about course assignments were promptly responded and they were provided with supportive feedback related to course.

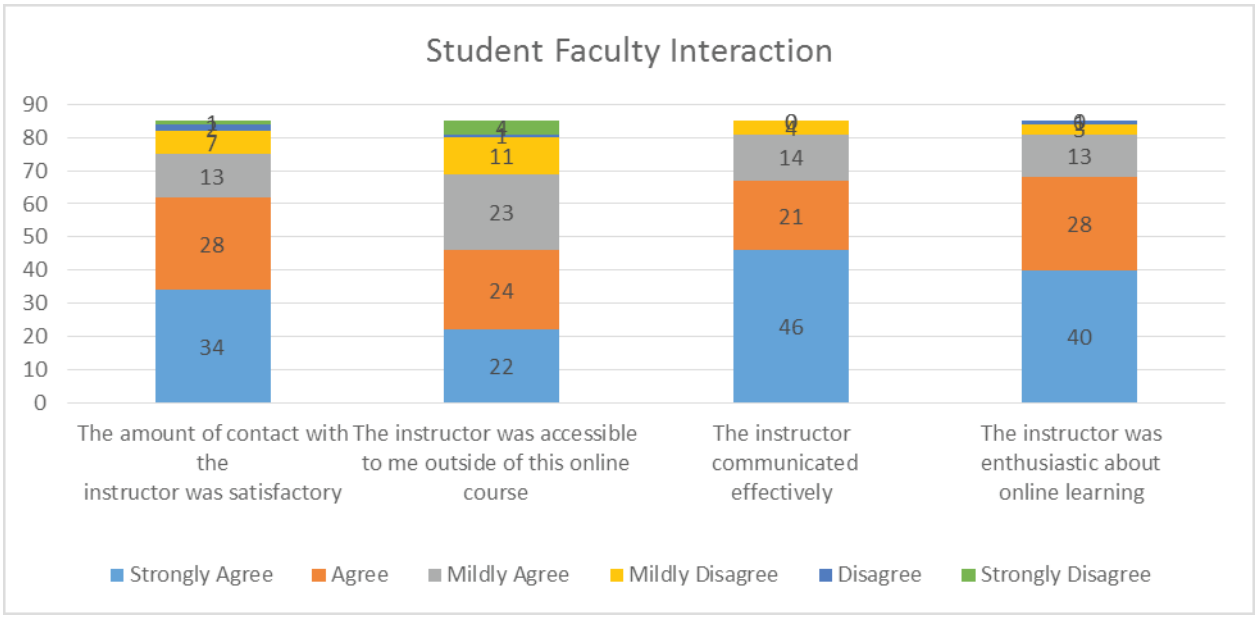


Figure 2: Student Faculty Interaction

As seen in Figure 2 almost all participant felt that the instructor communicated effectively and was enthusiastic about online learning.

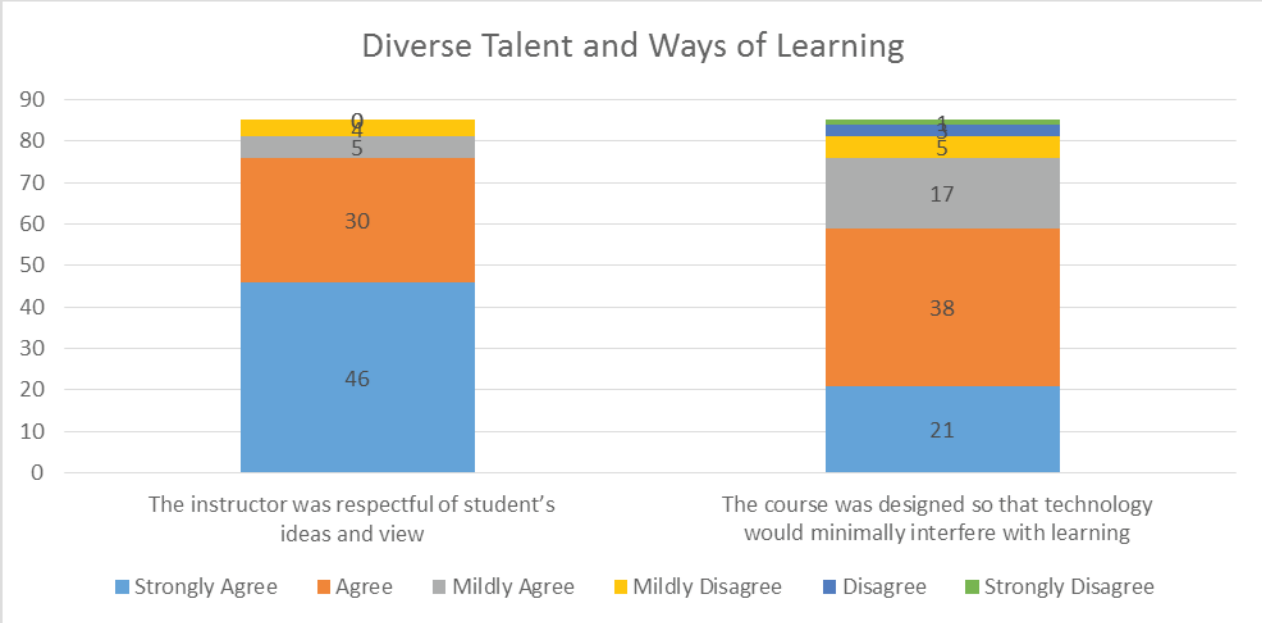


Figure 3: Diverse talents and ways of learning

As seen in Figure 3, most students agreed that the instructor was respectful to students' ideas and views as well as the technology did not interfere with learning.

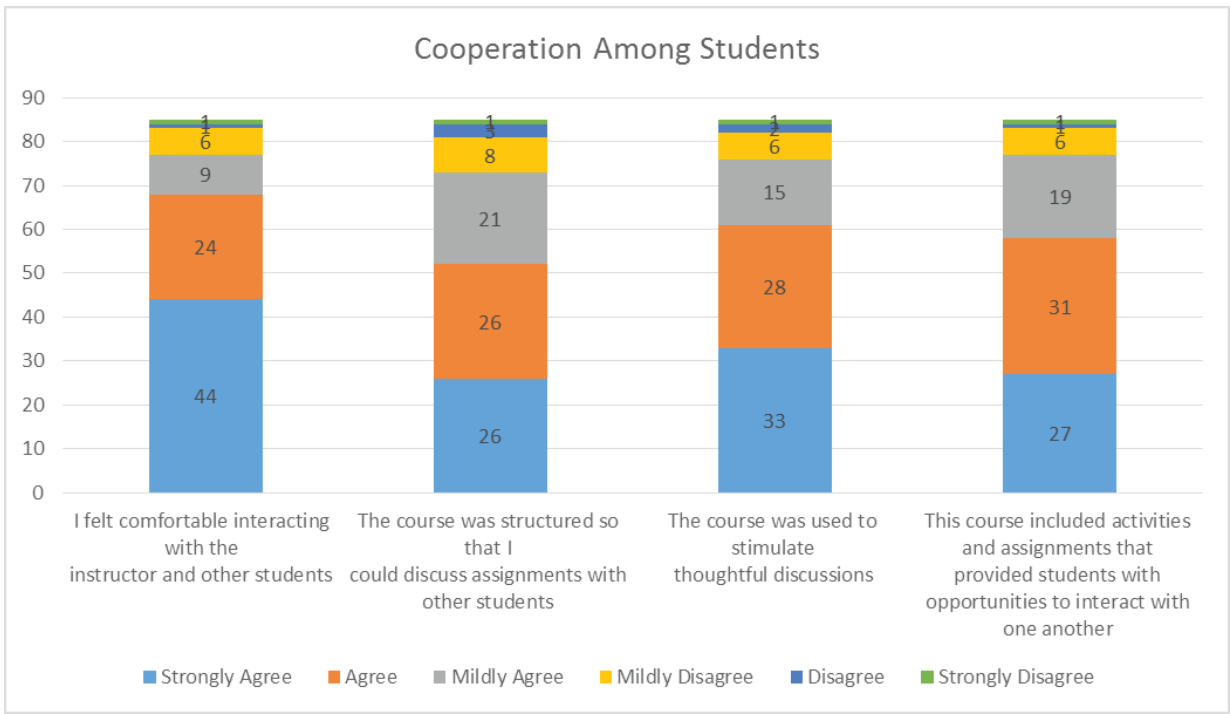


Figure 4: Cooperation among participants

Figure 4 shows almost all participants felt comfortable interacting with the instructor and other participants as well as the course design stimulated thoughtful discussions.

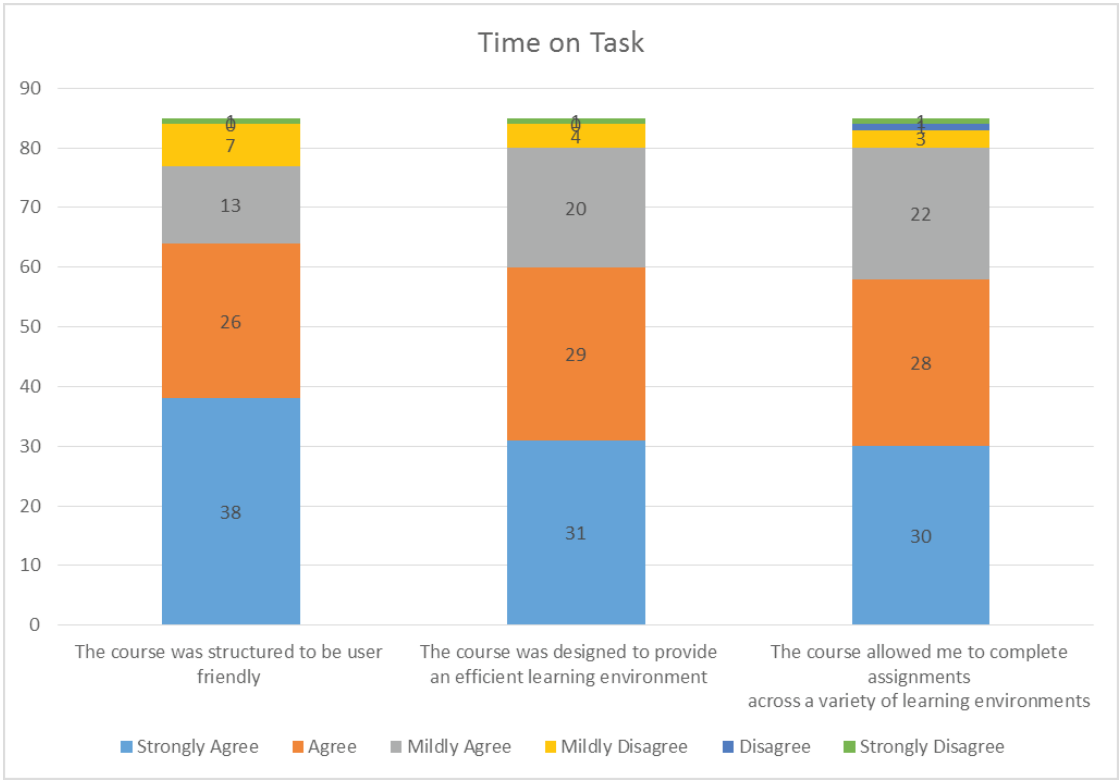
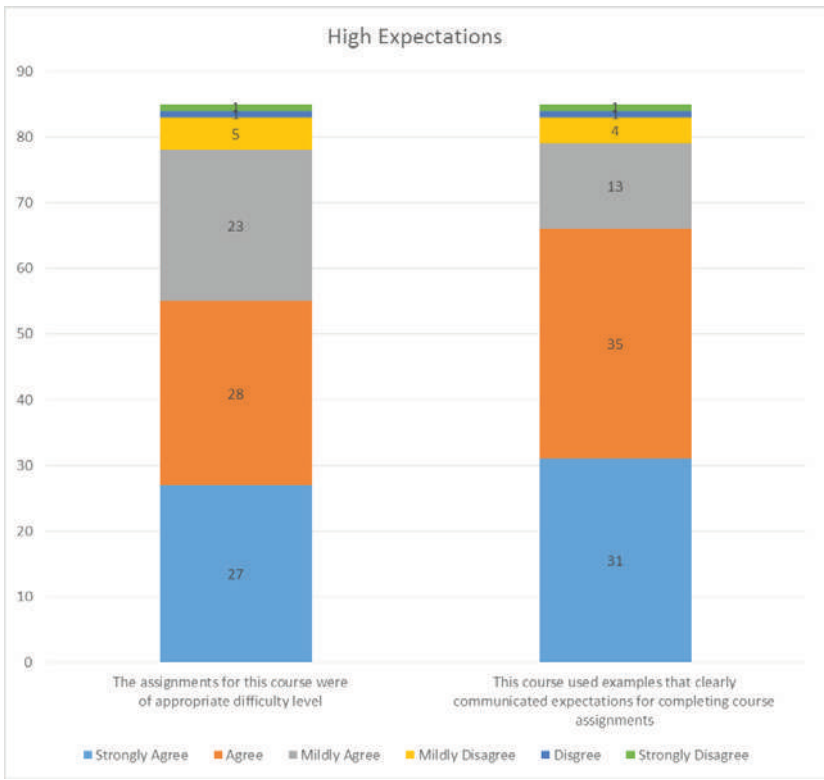


Figure 5: Time on Task

Figure 5 shows that the participants found the course to be user friendly and allowed them to complete assignments across a variety of learning environments



As seen in Figure 6, participants found the assignments for the course to be of appropriate difficulty level.

Discussion

Online teaching was introduced in most of the universities as an alternative to classroom training due to the COVID 19 pandemic. As the pandemic continues to be a global healthcare challenge, novel modalities are essential to ensure the continuity of student education. Online teaching should just not remain a formality to complete the curriculum instead it should be an effective and impactful teaching tool.

The study is an unique attempt to assess the participant feedback on the effectiveness of a single online session. The results clearly demonstrate that majority of the participants provided a very positive feedback of the online teaching session. The participants found the instructor to be accessible even outside of the online course which is essential because it is difficult to communicate all aspects of a subject within the limited time available online.

Technology at times can be a barrier in effective communication of the subject but if the course is well designed, technology doesn't interfere with the learning process as was found in this study.

Classroom teaching allows a free flow of interaction between the instructor and students as well as within themselves. The study found that participants experienced similar satisfaction with regards to interaction even during an online session.

Majority participants agreed that the course used examples that clearly communicate expectations for completing online assignments. This is crucial because the assignments are utilized to judge the level of understanding of the students with regard to the course subject.

Conclusion

The students' feedback with regard to online teaching was found to be highly encouraging. The current study is limited by its sample size as well as the feedback being collected only for a single online session. Further longitudinal studies are essential to gauge the impactfulness of online teaching as an alternative modality to classroom instruction. Repetitive practice with hands on training for skills based courses is very essential.

Source of Funding: Self

Conflict of Interest: Nil

Ethical Clearance: Taken from IEC, SIDU committee

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Opportunities and Obstacles Faced During Work From Home in Teaching Faculties

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Abstract

Introduction- The lockdown period amid Covid -19 has given the opportunity to the teaching faculties to work from home and to practise teaching learning activities using various online pedagogies (Zoom, WebEx, Google classroom, Google meet etc) to fill the knowledge gap which may transpire if the teaching learning activities are ignored and not taken earnestly. Hence taking this in consideration and prioritizing the need of learning activities to be carried in the virtual methods mostly all teaching faculties in various institutes have planned itinerary with the online teaching learning session hour by hour and day by day and also assigned assignments to the students to evaluate the effectiveness of their teaching. **Methodology**:A Non-Experimental Descriptive Research Approach ". The study was conducted among various teaching institutes of Pune City. The selection of sample was based on easy geographical accessibility, cooperation, and availability of samples.The sample size for study was 100,selected by using Non Probability Convenience Sampling Technique from various teaching institutes of Pune City who met the inclusion criteria.. **Objective:**1.To assess the opportunities with work from home in teaching faculties from selected institutes of Pune City 2.To assess the obstacles faced with work from home in teaching faculties from selected institutes of Pune City3. To associate the demographic variables with the opportunities and obstacles of work from home in teaching faculties from selected institutes of Pune City.**Result :**The analysis asserts that the opportunities related to personal growth were sometimes applicable for 83% and was often applicable for 17 % of the teaching faculties, work opportunities for majority of the teaching faculty's i.e. 82 % were often applicable and for only 18% of them these opportunities were applicable some times. Work obstacles were often applicable for 53 % of teaching faculties and sometimes applicable for 47 % of teaching faculties. Technological obstacles were often applicable to 88% of teaching faculties and for 12 % it was applicable sometimes. **Conclusion:** Work from home related to teaching faculties is associated with various opportunities and obstacles however it proposes that work from home can be given to teaching faculties also as it is more opportunistic to the teachers as well to the institutes.

Key words: *Opportunities,Obstacles work from home, teaching,faculties.*

Introduction and Background

Is It Time to Let Teaching faculties to work from home?

There is a prediction given by Bill Gates that till the year 2050, It is been assumed that 50 % of the staff working may move toward work from home by choosing home as workplace. Anyhow we don't know these employees will work from home extensively or not, they have given this estimation, based on the current situation. There was a study done in august 2019 saying that work from home performance becomes more commonplace .There is evolution emerging for work performing anywhere, During which the workers can live and work from any were .There was a study in 2017 that says that the many workers are willing to just accept 8% less choice to work from and also with the work from home strategy ,the organizations can even add more importance to the workers and slo can grant area flexibility ,with this they might have a peaceful environment which will help them to maintain work life balances and can lower their travel expenses [1,4]The importance of work-life balance

has significantly increased in recent years and though work from home is often more common in IT sector however the teaching faculties amid covid-19 lockdown have gained the opportunity to figure from home and to practice teaching learning activities using various online pedagogies (Zoom, WebEx, Google classroom, Google meet etc) to fill the knowledge gap which may result if the teaching learning activities are overlooked. Hence taking this in consideration and prioritizing the requirement of learning activities to be carried within the virtual methods mostly all teaching faculties in various institutes have planned agenda with the web teaching learning session hour by hour and day by day and also assign assignments to the scholars to referee the effectiveness of their teaching and the evidences say that work life balance is decisive involvement within human resources altogether organizations..There is a research which says that remote working is beneficial to learn the institutes also. The increased productivity at the extent of enterprises, increased retention and access to cost-saving are documented well.[3] With all the above evidences and identifying the current need of work from home in teaching faculties. The researchersproposed the need to conduct the current research to identify opportunities and obstacles related to work from in teaching faculties. The current research will help to identify the obstacles faced by the teaching faculties and further strategies can be developed to overcome the obstacles and facilitate work from home in teaching smoothly and effectively.

Objective:

1.To assess the opportunities with work from home in teaching faculties from selected institutes of Pune City

2.To assess the obstacles faced with work from home in teaching faculties from selected institutes of Pune City

3.To associate the demographic variables with the opportunities and obstacles of work from home in teaching faculties from selected institutes of Pune City

Methodology:

Research Design:The researchers have used A Non-Experimental Descriptive Research.The study was conducted in the selected teaching institutes.

Sampling techniques: NonProbabilityconvenient sampling technique was used and all teaching faculties from various institutes of Pune City were selected

Subject Size:100 Teaching faculties; willing to participate and working in the various teaching institutes of Pune city were selected. These participants were clearly informed about the study and its purpose.

Tools of data collection: In the present study the below scales were used to collect the data.

Structured Questionnaire to assess the opportunities and obstacles with work from home in teaching faculties from selected institutes of Pune City.It has two sections:**Section I**: Consisted of items related to demographic variables of the subjects of the study. **Section II**: Consisted of 15questions regarding opportunities with work from home in teaching faculties.It is divided in 2 Parts Part A-Questions related to Personal growth opportunities Part B- Questions related to Work opportunities**Section III**: Consisted of 15 questions regarding Obstacles faced with work from home in teaching faculties.It is divided in 2 Parts Part A- Questions related to work obstaclesPart B- Questions related to technological obstacles

Validity and Reliability of tools: Tools were validated by the experts and the reliability was done by Cronbach's Alpha coefficient test. Cronbach's alphas were r= 0.86, & 0.9 for assessing the opportunities and obstacles with work from home in teaching faculties from selected institutes

Findings

The analysis and interpretation of the data collected to determine the opportunities and obstacles with work from home in teaching faculties from selected institutes was done keeping the objectives of the study in consideration.

1. To assess the opportunities with work from home in teaching faculties from selected institutes of Pune City.2.To assess the obstacles faced with work from home in teaching faculties from selected institutes of Pune City.3.To associate the demographic variables with the opportunities and obstacles of work from home in teaching faculties from selected institutes of Pune City.

The result was divided in IV Sections

Section –I: Description of Distribution of Demographic Variables.Section – II: Description of Opportunities' of Work from home in teaching faculties.Section – III: Description of obstacles faced with work from home in teaching faculties.Section –IV: Association of demographic variables with the opportunities and obstacles of work from home in teaching faculties.

Section – II

Description of Opportunities' of Work from home in Teaching faculties

Part-A Opportunities -Personal Growth

N=100

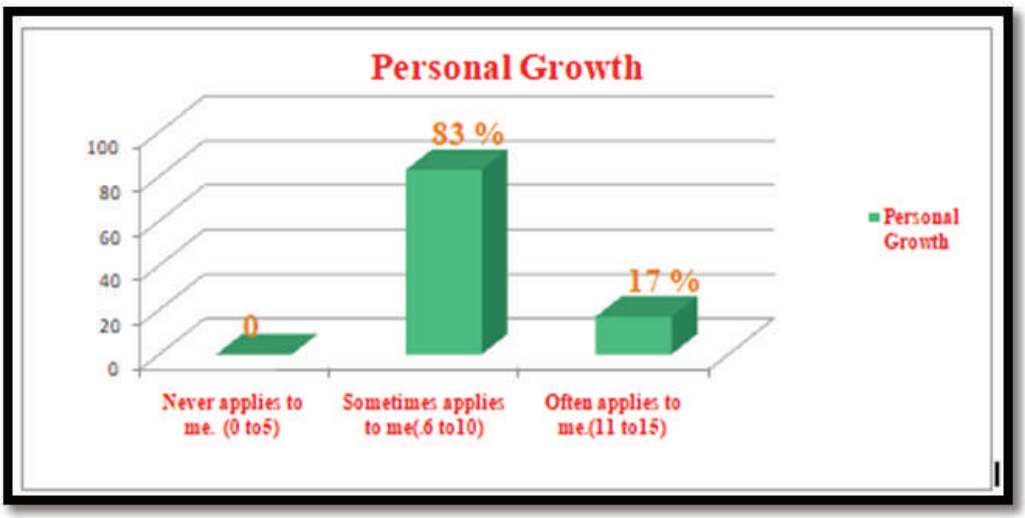


Fig 1 : Describes the data related to opportunities related to personal growth in teaching faculties.

Part B -Work opportunities

N=100

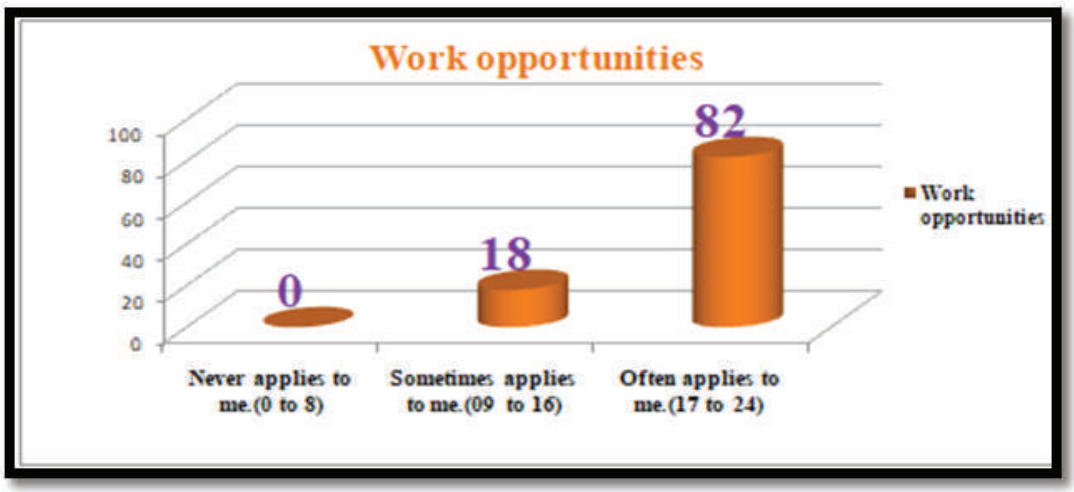


Fig 2: Describes the data related to Work opportunities in teaching faculties.

Section – III

Description of obstacles faced with work from home in teaching faculties

Part-A Work obstacles

N=100

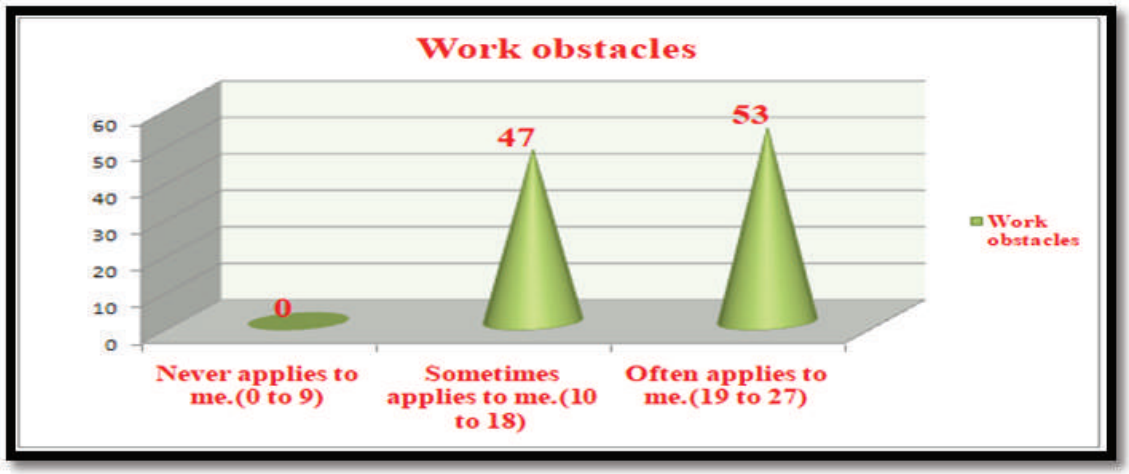


Fig 3: Describes the Work Obstacles during work from home in the teaching faculties

Part-B Technological obstacles

N=100

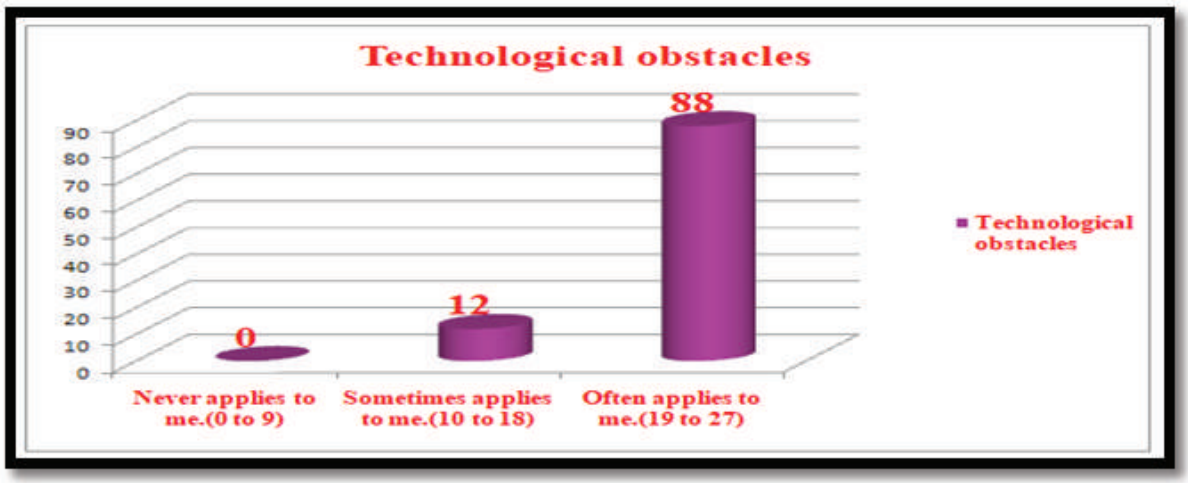


Fig 4 : Describes the technological Obstacles during work from home in the teaching faculties

Section –IV

Association of demographic variables with the opportunities and obstacles of work from home in teaching faculties

Table 1: Describes the data related to association of demographic variables with the opportunities and obstacles of work from home in teaching faculties.

N=100

Demographic variable		Never applies to me.	Sometimes applies to me.	Often applies to me.	P Value
Age	21-30	7	15	15	0.593439
	31-40	8	18	14	
	41 and above	8	7	8	
Gender	Female	10	20	16	0.590949
	Male	10	29	15	
Marital Status	Unmarried	10	10	35	0.07503
	Married	8	17	20	
Syllabus allocated for which class	All	3	3	8	0.99187
	B.Sc	6	3	10	
	M.Sc	5	2	12	
	B.Sc	3	2	11	
	P.B.BSc and B.Sc	5	3	9	
	P.B.BSc and M.Sc	5	2	8	
Number of children's You have	1	8	8	15	0.223479
	2	14	8	18	
	Not Applicable	15	7	7	
Age of your Children's	0- 1 Year	8	10	9	0.985481
	2-5 Years	7	8	10	
	6 years and above	8	9	7	
	No applicable	8	7	9	
Type of Family	Nuclear family	35	10	10	0.07503
	Extended/Joint Family	20	8	17	

Discussion

This study was aimed to assess the opportunities and obstacles faced during work from home teaching faculties working in various institutes of Pune City. Hence the data was gathered from the participants and analysis was done to find the result.

Findings related to Opportunities of Work from home in teaching faculties

Opportunities related to personal growth in the teaching faculties

Figure 2 : Data describes the Part A of opportunities related to work from home in teaching faculties which deals with the opportunities related to personal growth The parameters measured related to personal growth were - Are the teaching faculties able to master various online training session, getting opportunity for execution of existing technical skills one step ahead, Able to enhance their skills on online teaching applications and technologies, Acquiring time to work on new research, obtaining chance to mould virtual classroom more productive and useful for students. The analysis says that the above points were sometimes applicable for 83% and was often applicable for 17 % of the teaching faculties.Also majority of them i.e. 58 % of them able to enhance their skills on online teaching applications and technologies and 50 % of them were getting opportunity for execution of existing technical skills one step ahead.

Work Opportunities in the teaching faculties

Figure 3 describes the Part B of opportunities related to work from home in teaching faculties which deals with the work opportunities. The parameters measured related to work opportunities were –They have feasibility to work more in most productive time, Increased Possibility to take care of family members by giving more attention and time ,They can also work in case of sickness, They can conserve and save traveling expenses, It has also advanced their time planning and management skills, They are able to give preference for quality time with family, Able to attain slot to de stress themselves by yoga and medication, Also able to enhance quality in teaching students via virtual teaching and now they are confident in online teaching. The result says that the above work opportunities for majority of the teaching faculties i.e. 82 % these work opportunities were often applicable and for only 18% of them these opportunities were applicable some times.

Findings related to description of obstacles faced with work from home in teaching faculties

Work Obstacles during work from home in the teaching faculties

Figure 4 describes the Part A of obstacles related to work from home in teaching faculties which deals with the work obstacles .The parameters measured related to work obstacles were –There were hinders in exchange of information ,coordinating in team work and communication gap with co-workers, Faced difficulty to access organization documents from home(Eg : Books and various reading materials), Faced plagiarism issues in students assignments, Lack of continuity in work , Students face issues of lack of attention and concentration ,Unable to maintain inter personal relationships with students and social interaction within the class ,Course quality is not up to the mark, Difficulty in accessibility of laptop or PC. Hence the result says that these obstacles were often applicable for 53 % of teaching faculties and sometimes applicable for 47 % of teaching faculties.

Technological Obstacles during work from home in the teaching faculties

Figure 5 describes the Part B of obstacles related to work from home in teaching faculties which deals with the technological obstacles .The parameters measured related to technological obstacles were –difficulty in accessibility of various online pedagogies, network issues which effects the work, difficulty in student accessibility for applications and software, inadequate technology support, frequent technology failures, the software's rapidly change or update due to hanging issues, difficulty in maintaining privacy and confidentiality as software's gets hacked, increased workload due to network issues and other technology failures, teaching is becoming technological centered then student centered .Hence the result for these parameters says that these obstacles were often applicable to 88% of teaching faculties and foe 12 % it was applicable sometimes.

Findings related to Association of demographic variables with the opportunities and obstacles of work from home in teaching faculties

Table 7 Represents that since all the p-values corresponding to age, gender, marital status ,syllabus allocated ,number of children's ,age of children's and type of family ,are large (more than 0.05).Hence age, gender, marital status, syllabus allocated ,number of children's ,age of children's and type of family were found to have no significant association with the opportunities and obstacles of work from home in teaching faculties.

Similar to current study findings there was study done on the tele working the basic an assessment of the benefits and drawbacks. In their result the researcher had said that though there's a crucial variation to form and there were several drawbacks which will make the performance during telworking to decline, these problems and work are different from the obstacles that can be prevent teleworking if they are implemented in the institutes. Several employees have told that they had problem to get the office documents and information while teleworking[1].

A study on teaching professionals for their work life balance was conducted. The study result the researcher has said that the 9.467 which is the result value using chi square test for age of the participants and also their level of attitude they have for work life balance. Since the p value was 0.304 and 0.595 that is larger than 0.005. Hence the research has accepted null hypothesis stating that no significant relation between the participants's age, gender with their level of attitude towards their work life balancing [4].

In an another study result which is done on the suitability of the working place reception and decreased time for communication within the co-workers are the some of the most important factors which are badly affected via tele working. In this study the research has also said there is no significant effect on the work outcome of tele working with their time planning skills and lowering travel expenses also there difficult o acquires documents from office. However, reduced time for communication within the co-workers has increased the productivity of tele-workers and may be seen as a contra argument to the social isolation of tele-workers.

Conclusion: The study result has shown various opportunities and obstacles faced by teaching faculties to render here knowledge towards students,Work from home if often very common in IT sector but know after knowing the opportunities related to work from home in teaching faculties. It can be concluded that teaching faculties can also work from home effectively and also gain various opportunities related to their work, fortheir personal growth and also for the institutes, Though working from home by teaching faculties also associated to have various obstacles, But if the institutes resolve their obstacles then they can work more productively.

Conflict of Interest: Nil

Source of Funding: Authors have no financial support to this project. The study was fully funded by authors.

Ethical Clearance: Ethical approval of the study taken from Symbiosis College of Nursing ethical committee. Informed consent was taken from the teaching faculties working in various teaching institutes of Pune city. Information given to the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential.

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Assessment of Selected Practices of Menstrual Hygiene among Rural Women at Pune City

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Abstract

Menstruation: Despite the irrational taboos related to it, menstruation is a natural physiological method that each healthy girl undergoes. Though every girl experiences it on a regular basis, not everyone seems to be intelligent about the necessity to confirm hygiene. Menstruating girls ought to pay explicit attention to private hygiene. During this time, a woman's vulnerability towards major ailments will increase. Poor expelling hygiene will result in several problems, such as fungal or microorganism infections of the reproductive tract and the urinary tract.

The result shows during assessment of selected practices of menstrual hygiene among rural women 97% wash hands after changing pad, 98% women take bath during their menstruation, 38% women's family keeps them and their things separate during menstruation, 5% women have financial crisis/problem for buying sanitary napkins and also 57% women avoid travelling to menstrual hygiene

Key words: *Women, menstrual hygiene practices, rural community*

Introduction

The challenge, of addressing the socio-cultural taboos and beliefs in menstruation, is further compounded by the fact the girls' knowledge levels and understandings of puberty, menstruation, and reproductive health are very low. [1]

In India, still women of rural areas are suffering from reproductive tract infection due to poor management of menstrual hygiene. [2]

Causes of poor menstrual hygiene in rural area in India are due to believe myths and taboo like using of cotton cloths during menstruation and lack of money. This study finding says that a lot of attention is needed for the practices of menstrual hygiene among rural women. [3]

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Materials and Methods

Research design

- Research design used in this study was Non-Experimental descriptive design to find out the assessment of selected practices of menstrual hygiene.

Research approach:

-In this study Quantitative approach was used.

Setting of the study:

-This study was conducted in selected rural area of Pune city.

Sample and Sampling technique:

-Non-probability convenience sampling technique was adopted.

Tool preparation:

-To collect the data for present study a structured questionnaire schedule was developed for the Assessment of selected practices of menstrual hygiene among rural women at Pune city.

Development of tool:

A tool consists of two sections as follows:

Section I: Demographic variables (Marital status, Religion, Education, Income, Duration of menses).

Section II: Self-Structured questionnaire to assess selected practices of menstrual hygiene among rural women.

Sample size:

-Sample size consists of 100 women at selected rural area of Pune city.

FINDINGS:

SECTION I: Distribution of the samples based on the demographic variables in terms of frequency and percentage.

Table no. 1 Description of demographic variables

n=100

Demographic variable		Frequency	Percentage
Marital Status	Married	82	82
	Unmarried	18	18
	Separated	0	0
	Divorced	0	0
Religion	Hindu	98	98
	Muslim	1	1
	Christian	1	1
	Any Other	0	0
Education	Illiterate	18	18
	SSC	41	41
	HSC	32	32
	Graduate & Above	9	9
Income	Below 10,000	36	36
	10,001 - 20,000	37	37
	20,001 - 40,000	15	15
	Above 40,000	12	12
	Extended Family	14	14
	Single Parent	2	2
Duration of menses?	2 - 3 days	32	32
	4 - 5 days	52	52
	5 - 7 days	16	16
	More than 7 days	0	0

Section II: Analysis and Interpretation of data collected from the women residing in rural area (Susgaon) of Pune city.

Table no. 2: Analysis and Interpretation of data collected from the women on menstrual hygiene practice.

Self-administered practice variables		Frequency (f)	Percentage (%)
Do you wash your hands after changing pad?	No	3	3
	If yes, (specify)…	97	97
Do women take bath during your menstruation?	If Yes, how many times do you take bath? (……..	98	98
	No	2	2
Does women family keeps them and their things separate during menstruation?	Yes	38	38
	No	62	62
Do women have financial crisis/problem for buying sanitary napkins?	Yes	5	5
	No	95	95
	No	4	4
While travelling how do you maintain your menstrual hygiene?	Avoid Travelling	57	57
	Using Public Washroom	43	43

Data collected from women on menstrual hygiene shows that the majority of women (97%) does not use natural material like sand, leaves, cotton, ash etc. during menstruation. It's also seen that majority of women (79%) do not use cloth during menstruation but if they use, they prefer washing cloth of period stain with soap and water (15%) and they use natural light to dry it (15%). Majority of women (96%) shave their pubic area and also, (74%) of rural area women uses Indian commode present at home.

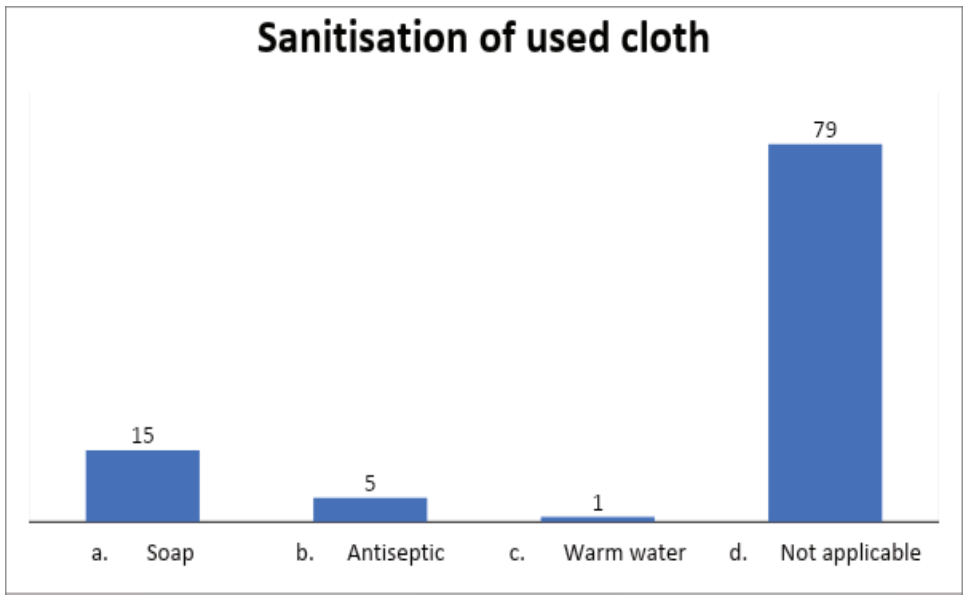


Fig. 1. Bar diagram shows sanitization of used cloth by women during menstruation.

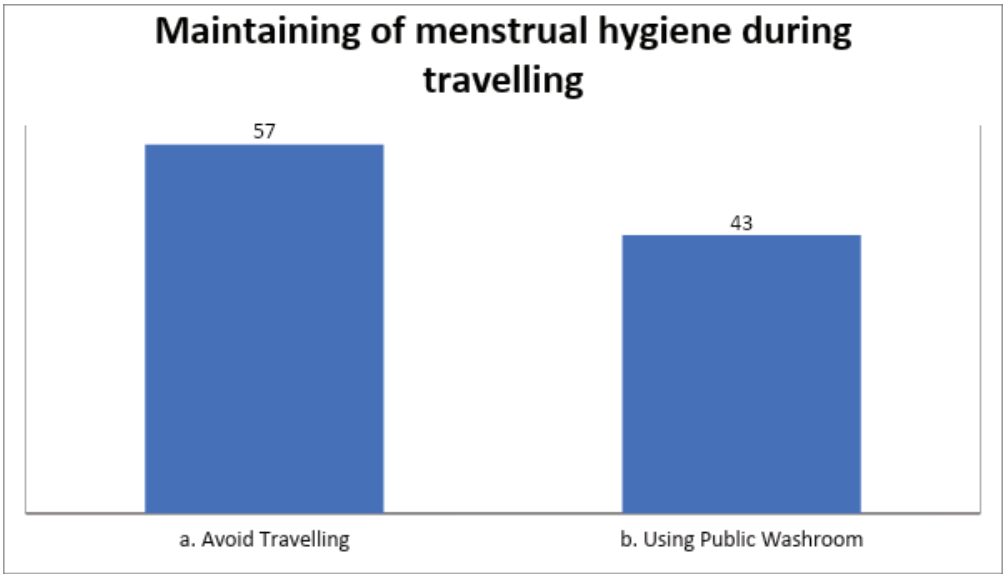


Fig. 2. Bar diagram shows how women maintain menstrual hygiene during traveling.

Discussion

This current study investigated the assessment of selected practices of menstrual hygiene among rural women at Pune city.

As indicated, the data was collected from 100 women from the rural area by a structured objective type questionnaire and solely truthful results were achieved.

However, it's important to educate about the importance of the use of sanitary pads and the harms of using cloths.

The findings of the study are mentioned in relation to the objectives.

Conclusion

Menstruation is a normal biological function in females and in the society, the practices followed are highly variable across different regions.

The analysis of data reveals that menstrual hygiene practices plays a very important role to prevent women from various deadly diseases and infections.

57% of the respondents avoid traveling which indicate their perception towards good hygiene practice.

As a result, we found that majority of the women menstrual hygiene practices were found to be satisfactory among the rural women but few restrictions were practiced during menstruation.

Ethical Consideration: The Study was approved by Institute research Committee & Institute Ethical subcommittee.

For the present study, the investigator took in to consideration the ethical issues. There were no ethical issues confronted while conducting the study.

Conflict of Interests: Nil

Source of Funding: Self

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Comprehensive Study on Medication Error to Improve Patient Safety

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Abstract

Despite of the increasing advancement in the healthcare system, medication errors continue to exist. These errors are an increasing burden in modern healthcare practice in terms of both human sufferings and financial costs. Classification, Preventive Measures and the methods of Incident Reporting are the crucial elements to be considered while determining medication errors. The data available for same is vast and widely distributed. So an effort is made to compile the data available and provide a learning platform to improve understanding regarding medication errors among trainees as well as all healthcare professionals in order to upgrade the existing healthcare facilities.

***Key words**: Crucial elements, incident reporting, medication errors, patient safety, preventive measures, healthcare providers.*

Introduction

Medication errors leave remarkable impact on both known and unknown subject. Neglected, unnoticed or non- documented errors can prove to be fatal. Medication errors have also increased burden in modern healthcare practice in terms of both human sufferings and financial cost. Drug- related errors cause an estimated 7000 deaths per year in USA [1]. Also, it has been found that adverse drug event cost a single teaching hospital $5.6 million of which $2.8 million was preventable [2]. Adverse drug event (ADE) is serious type of medication errors. ADE's are defined as injuries resulting from medical interventions related to drugs [3]. Among these ADE's, the preventable ones are medication errors [2–5]. At least 1.5 million preventable ADE's occur every year in the United States [3]. Preventable ADE's costs over $4685 in tertiary hospitals [6] and $3511 in community hospitals [7]. The field of anaesthesia and critical care medicine are even at a higher risk for medication errors due to increased used of highly potent fast acting narrow dose range drugs in a relatively short time period. Medication errors have a large impact on healthcare cost and patient safety as well [6,7]. Numerous studies have been conducted by various authors in the developing countries to evaluate the cause and determine the preventive measures to reduce the same. But the data regarding same is vast and widely distributed. The purpose of this review is to provide a platform to improve understanding regarding medication errors among undergraduates, interns, trainee as well as all healthcare professionals and take a step ahead in reducing the burden of medication error and improve patient safety in the future.

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Methods

The online data sources Scopus, Web of science, PubMed, JSTOR, Science direct, MESH, google scholar and bibliographic databases were searched for key terms like incident reporting, medication errors, patient safety, preventive measures. Total 980 papers published from

1980 till 2019 were screened. Out of which 33 papers written in an English language, focusing on classification and cause of medication error, preventive measure were included for narrative review.

Definition of Medication errors:

The most compressive and widely accepted definition was proposed by Ferner and Aronson [8].

They defined medication error as a 'failure in the treatment process that lead to or has the potential to lead to harm to patients'. The 'treatment process' also known as the 'medication use process' is collectively the prescribing, compounding, dispensing, drug administration and monitoring processes, which are carried out after the decision for treatment has been made by the doctor. A 'failure' is the inability to attain a specified standard during the course of these processes.

The United States National Coordinating Council for Medication Error Reporting and Prevention defines a medication error as "any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the healthcare professional, patient, or consumer. Such events may be related to professional practice, health care products, procedures, and systems, including prescribing, order communication, product labelling, packaging, and nomenclature, compounding, dispensing, distribution, administration, education, monitoring, and use"[9].

Classification of Medication Errors

Medication error can be classified in many ways some of the commonly used methods of classifying medication errors are as follows.

I. According to their cause [8]

a. Mistakes: happen when an error is made in planned action. Further subdivided as

i. Knowledge- based errors: are due to lack of knowledge

E.g. Doctor prescribing wrong dose of drug due to unfamiliarity.

ii. Rule- based errors: are due to misapplication of a good rule or application of a bad rule.

E.g. prescribing penicillin-related drug to patient with known drug allergy to penicillin despite a system warning.

b. Skill- based errors: are committed when executing correctly planned actions. Could be

i. Slip (action based) E.g. pharmacy technician intends to dispense amoxicillin but picks wrong bottle and dispenses ampicillin.

ii. Lapse (memory based) E.g. nurse intends, but forgets to administer the evening dose of drug to a patient.

II. According to the stage in the process [8]

a. Prescribing errors: further subdivided as

i. Irrational and inappropriate prescribing: occur if drug is prescribed based on missing or incorrect information and also if drug is prescribed without understanding the relation between the pathophysiology of the problem and the mechanism of action of the drug.

ii. Ineffective prescribing: is prescribing a drug that is not effective for the indication in general or for the specific patient.

iii. Under prescribing; is failure to prescribe a drug that is indicated and appropriate, or the use of too low a dose of an appropriate drug.

iv. Overprescribing: is prescribing a drug in too high a dosage.

b. Transcription errors: specific type of data entry error that is commonly made by human operators

c. Dispensing errors

d. Drug administration errors

Dispensing errors and drug administration errors are further subdivided as

i. Wrong drug: is giving totally different drug than that is prescribed

ii. Wrong dose: giving high or low dose

iii. Wrong frequency: advising to take a particular drug once rather than twice.

iv. Wrong route: giving oral dose rather than intravenous/intramuscular.

v. Wrong patient: giving a drug not intended for that patient.

III. According to the severity of outcome [10]

They are classified as per the degree of severity as

a. A- Potentially serious error that can cause permanent harm to patient, may increase hospitalization or need of additional treatment.

b. B- Clinically significant error can increase need for patient monitoring.

c. C- Clinically non- significant error that does not harm the patient.

IV. According to medication error severity index [9].

The most widely used severity scoring system for medication errors was introduced by the National Coordination Council For Medication Error Reporting And Prevention (NCC MERP) of United States.

Is based on severity reporting program and is effective in identifying problem areas and trends so that quality assurance and medical committees can implement measures to improve drug use.

But this system underestimates the actual number of medication errors.

The categories of medication errors as per severity index are:

Category A: circumstances or events that have the capacity to cause errors.

Category B: An error occurred but the medication did not reach the patient.

Category C: An error occured that reached the patient but did not cause patient harm.

Category D: An error occurred that resulted in the need for increased patient monitoring, but no patient harm.

Category E: An error occurred that resulted in the need for treatment or intervention and caused temporary patient harm

Category F:An error occurred that resulted in initial or prolonged hospitalization and caused temporary patient harm.

Category G: An error occurred that resulted in permanent patient harm.

Category H: An error occurred that resulted in near- death event.

Category I: An error occurred that resulted in patient death.

V. According to the nature of onset [11]

They are distinguished by the length of time taken for a bad outcome to occur and the place in the organizational hierarchy where the errors originate.

a. Active errors: have an immediate effect; active errors are committed by people who are in direct contact with patients

b. Latent errors: have delayed effects; latent errors occur due to failure in strategic decision making in the system that takes place higher up in the organizational hierarchy. They do not have immediate bad outcomes, and may lie dormant for a long time until they combine with an active failure to allow an error to happen.

Preventive Measures:

According to Reason's theory, medication errors occur as a result of combination of both active and latent failure[12,13].

Active failures occurs due to human negligence, carelessness and forgetfulness [13]. But human errors are inevitable.

On the other hand, medication errors can be easily reduced by stopping latent failure by improving the system in a way that error cannot occur[13].

Broad approaches that can be used to combat medication errors are as follows –

A. Technological Interventions

a. Computerized Prescription Order Entry [14]

Refers to a process of medical professional entering and sending medication orders, test results and treatment instructions electronically via a computer application instead of on paper charts directly to the pharmacy or other healthcare provider to administer to patient.

b. Computerized decision support system [15,16]**.**

Is defined as "any software designed to directly aid in clinical decision- making in which characteristics of individual patients are matched to a computerized knowledge base for the purpose of generating patient-specific assessments or recommendations that are then presented to clinicians for consideration."

c. The Prescribing Information & Communication Systems [17]

Is an evolving system developed in Birmingham, England. It combines electronic prescribing facilities together with patient history and reports and contains algorithms to prompt the prescriber.

d. Personal Digital Assistants [18,19]

Also known as Handheld Computers, Pocket Personal Computers and Palm Pilots.

They are pocket sized computers that are capable of accessing the internet, sending and receiving data and storing textbooks worth of information.

They provide immediate access to vital and clinically relevant disease information at the point of care.

Several infectious diseases applications are available that provide information on pathogens, diagnosis, medication and treatment.

e. Mobile Clinical Assistants (MCA) [20]

These are smart phones and tablets containing medical software for use by Health Care Providers.

Smartphones and tablets combine both computing and communication features in a single device that can be held in hand or stored in a pocket, allowing easy access and use at the point of care. With the advancements, MCA's have become handheld computers.

f. Automated Dispensing Machines [21]

Are decentralized medication distribution systems that provide computer-controlled storage, dispensing, and tracking of medications.

g. Smart Devices [22]

Smart devices have built-in drug libraries and dose error reduction system (DERS), which allow the users to choose the desired medication from an approved list and input the required patient information, after which the smart devices calculate the infusion rate. Drug libraries contain the most commonly used IV medications and the DERS alerts the user if the calculated infusion rate exceeds the normally accepted dosing limits. These dosing limits can be expressed as either hard dose limits (i.e. cannot be bypassed by the users thereby preventing users from starting the programmed infusion) or soft dose limits (which provide a warning that the dose may be too high but will still allow users to start the infusion as programmed after the limits are acknowledged).

h. Bar-code Assisted Drug Dispensing (BCDD) System [23]

The BCDD System consists of a barcode printer, a barcode reader, a mobile computer (with Wi-Fi), a computer server and software. Each drug in the hospital is labelled with a unique barcode. When a patient is prescribed medication it is faxed, sent electronically or hand delivered to the hospital's pharmacy and entered into the computer system by a pharmacist. The pharmacist dispenses the barcoded dose of the drug to the patient's floor. When it's time for the clinician to administer the medication, he uses handled device to scan the barcodes on his identification badge, the patient's wristband and the drug. If the barcode point-of-care system (BPOC) cannot match the drug to be given with the order in the system, it alerts the clinician with a visual warning. Each patient's barcode holds all the information about the patient and his medication. The BPOC system is designed to make sure that the right drug is given to the right patient via the right route in the right amount and at the right time. This is referred to as the "Five Rights."

Advantages of Technological Interventions[14–18,20–24]

- Standardizes the medication order.

· Eliminates the illegibility of hand written prescriptions.

· Eliminates verbal orders.

· Minimizes human errors.

· Increases evidence- based practice.

· Helps in decision making and selecting drugs as per patient's needs.

· Decrease Medication error.

Limitations of Technological Interventions

· Large capital is required for installation of the systems.

· Successful implementation depends on user attitudes and acceptance of technology.

B. Improving the Quality of Hand-Written Prescriptions

Following Strategies should be incorporated to improve the quality of Hand-Written Prescriptions

i. Using Standard Prescription Formats [25].

j. Using 'one write' non-carbon prescription forms that generate an instant copy [26].

k. Using standard approved abbreviations [25,26].

l. Introduction of 'Do Not Use' list of error-prone abbreviations [27–29].

m. Teaching medical undergraduates universal prescribing standards [30].

C. Marking Or Labelling Of Storage Facility [31,32]

All Drug Storage facilities (small or large) should have proper markings or labels; whether it is related to Pharmacy, Nursing Station, Operation theatre, Wards, emergency drug trays, etc, this will help in avoiding medication errors while handling look-alike medicine ampoules, vials or drug packagings. Unique identification marks or colour codes can be used.

Syringes in anaesthesia departments also can be labelled or different colour codes can be used to prevent medication errors.

D. Preparing Check List And Judicious Follow Up By Second Person [31]

Standard check list should be prepared and judiciously followed by the healthcare provider team. For e.g. in operation theatre before starting any procedure, the operation theatre technician should check operation theatre against standard check list and emphasize on issues related to technical details, after that sister in-charge should do the same and emphasize on issues related to her services, later junior doctor and senior doctor of respective specialty should do the same before starting the procedure. This helps in eliminating errors at each level.

E. Detailed Referral Notes

All referral cases should have comprehensive referral note. The note should contain essential information in relation to patient's past history, as well as medication administered and procedures carried out at primary level. This will alarm the treating doctor regarding the patient's condition and prevent repetition of any error if at all it has occurred.

Incident reporting:

With the advancements in the field of education and technology, patient's awareness and knowledge about diseases and their management is also increasing, so healthcare providers need to be more vigilant to avoid unfortunate outcomes and medico-legal claims. All efforts should be invested in reporting and prevention of medication errors.

Annie et al in their survey in India found that fear of medico legal issues (43.3%), fear of judgment from colleagues (23%) and unavailability of a system or an authorized person to report (20.2%) were reasons for not reporting errors[32].

"Culture of blame" and a notion that an error can be a sign of inability on the part of the physician were identified as barriers to incident reporting in various studies [33,34]. Without reporting there can be no data and without data there can be no analysis and without analysis active and latent conditions will remain undiscovered and may influence future occasions.

Data regarding incident reporting in the developing world is scare which is indirectly increasing burden on healthcare providers; so following strategies should be incorporated.

1. Establishment of blame free, non-punitive environment where errors are openly reported and discussed without fear of retribution, penalties or loss of employment.

2. Empowerment of all medical personnel to voice concerns when they believe actions may lead to patient harm. This technique is known as a "hard stop" where any healthcare professional can call for an immediate cessation of the process if they believe harm could result to the patient.

3. Quadruple learning. Means using feedback to learn local lessons and make local changes at the level of individual practitioner or team; using that information to also make changes at level of institutions or hospitals, while simultaneously feeding the reports into a national system and being able to share the national information at international level.

Conclusion

Healthcare providers being humans make errors from time to time. Medication error being 14th cause of death all over the globe becomes concern for healthcare fraternity. There are various causes of medication errors and numerous preventive measures to avoid them. It is of utmost importance to accept that error can occur at any and every stage of drug administration and the healthcare providers should be vigilant enough to identify the error, report them in a blame free environment, analyse the root cause and take measures to change the procedures as per the lessons learnt and continue the monitoring process. All the healthcare organizations should be vigilant to implement the technological advancements as well as training of professionals for same to ameliorate the existing facilities and improve patient safety.

Ethical Approval – Not applicable

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A study to Assess the Student's Anxiety Level about Examination During Lock Down in Selected Colleges of Pune City

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Abstract

While whole world is facing the outbreak of COVID-19, universities are crucially affected at their core: The students. For them, the period is undoubtedly very stressful: classes are moved online, semesters abroad are postponed, examinations and how they are conducted remain uncertain. As universities have gone virtual within a matter of days, how have these affected students? For the students the stress and anxiety level is very high due to lock down. [1]

Due to COVID-19 student's anxiety level about examination has become very serious issue now days. College students are experiencing very serve anxiety level due to the lock down. . When stress is perceived negatively or becomes excessive, it leads to anxiety before and during examinations and ultimately affects their academic achievement. As due to the pandemic situation many classes were conducted online mode of teaching but as the

COVID -19situation is not yet under control students anxiety level is increasing day by day about their examination[2]. The present study was conducted to know the examinations anxiety felt by college students due to lock down. The sample consisted of 60 college students drawn using Non Probability convenience sampling method from selected colleges of city studying in Professional Courses (Including first year, Second year, Third year and Fourth year respectively) . A questionnaire was developed by the researcher to access examination anxiety among college students. The result shows correlation between examination and anxiety of college students due to lock down. **Design -** The present study is a descriptive research. The data was collected from the primary sources. **Results-** Non Probability convenience sampling technique used to collect information from 60 participants studying in colleges of Pune city. And found that out of 60 participants who participated in the current study the results were 50% students have very severe anxiety level, 20% have severe anxiety, 20% moderate level of anxiety and 5% have Mild anxiety and reaming 5% no anxiety level about their upcoming examinations **Conclusion-** This study was mainly conducted to assess the students anxiety level about examination during lock down .and it is understood that students are suffering with anxiety level. .

Keywords *– Examination, Anxiety, College students, COVID-19, lock down*

Introduction

In response to the COVID-19 pandemic facing the world it is affecting more on students. When we left, there was not so much fright about COVID-19, as it had just started. It started sinking in gradually.[3] But now, there is a lot of anxiety among students about their examination. As how examination will go on. **Universities** and schools across the country have been closed and exams postponed since March 16, 2020 when the Centre announced a countrywide classroom lock down as one of the measures to contain the COVID-19 outbreak. Later, a nationwide lockdown was announced on March 24, 2020 which has now been extended till 31st June 2020, The COVID situation is not in control and examination schedule is not clear for students [4].Exactly this is creating lot of anxiety among the students.[5] As students left for their native places before the lock

down announced and if university will make sudden anncounmets for examination leaving them no time to prepare for examination as well many students have left their books at hostels left hurry at their native places and many students are staying at very remote place where they cannot reliable completely on online mode of study.[6,7] This all creating many more anxiousness among the students. As well many students are worried about as the syllabus is not complete and assignments are yet and how the mode of examination will be there, ? As COVID -19 cases are higher day by day and there is no clear about examination schedule this all have impact on students psychology. There is lot of disturbances to the mental health due to this lock down. As well many students facing that without library and other resources it is impossible to study

This Lock down is having great impact on students' academic performance.

Objectives of The Study

1. To assess the anxiety level of students on examination during lock down.

Methodology

The non-experimental descriptive survey study was conducted in the various cities where youth were participated through non probability convenience sampling technique. The self-administered structured questionnaires and check list were used. The collected data were analyzed by using descriptive and inferential statistics

Analysis and Interpretation

Description of subject characteristics

A total sample of 60 college students was selected by using non Stratified random sampling technique. The professional college going students have participated in the study (Including first year, second ,third year fourth year and other respectively) out of which the from first year5%, second year 10% ,third year 14% ,fourth year 11% and other 20%) accordingly

Male participants were least in numbers i.e. 12% as compare to female participant's i.e.48% Out of 60 participants selected for the study majority the age group was 21 -22 years of age that is 25% , 18-19 years of age was 3% , 19-20 years of age was 8% and 20-21 years of age 10% and remaining were from other that is 14% have participated in the study.

Discussion

The study was conducted to check the student's anxiety on examination during lock down. ". Total 60 participants have participated in the study. Out of which five age group was selected from 18-19yrs, 19-20, 20-21years and 21-22 years and others yes and 20-21 years the majority of the participants were from the age group of 21-22 yrs. and majority of female participants have participated in the study.

The **HAMILTON ANXIETY** scale was used to assess the data. After obtaining the data the result was found that And found that out of 60 participants who participated in the current study the results were 50% students have very severe anxiety level, 20% have severe anxiety, 20% moderate level of anxiety and 5% have mild anxiety and reaming 5% no anxiety lev

The research found that. The same study was conducted with the title "EXAMINATION STRESS AND ANXIETY: A STUDY OF COLLEGE STUDENTS" shows that the correlation between examination stress and anxiety is highly significant so the, states that there is no relationship between examination stress and anxiety among college students. Students of college feel stress before examination and it leads to anxiety. High aspirations, poor study habits, more study problems, change in medium of instructions and low socio economic conditions, test anxiety, fear of failure, competition for grades, excessive academic load and concentration and memory are the factors responsible for examination.

Conclusion

This study was mainly conducted to assess the "student's anxiety on examination during lock down" The present study highlights the perceived stress by college students who already lead a hectic routine and lifestyle.. We believe that providing students psychological support and assurance can help the students to overcome the anxiety in the lockdown and perform better in their studies. As well we should provide periodic counselling to the students and encourage them to study on daily basis during lock down .Hence the students will have

less fear for upcoming examination. As well be should help the students to check various coping strategies which they can adopt to come out of this anxiety level and this will help them to concentrate their studies and have better academic performance .

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Prevalence of Childhood Obesity and its Prevention- Systematic Review

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Abstract

Introduction: Childhood obesity is one of the most serious public health challenges of the 21st century and prevalent global public health issue as children. The number of overweight or obese infants and young children (aged 0 to 5 years) increased from 32 million globally in 1990 to 41 million in 2016. Overweight and obese children are likely to stay obese into adulthood and more likely to develop non communicable diseases like diabetes and cardiovascular diseases at a younger age. Therefore Prevention of childhood needs high priority. **Material & Methods:** 125 citations in PubMed & ISI Web of science found by electronic search and finally 19 articles were selected for writing systemic review. The search strategy consisted of search strings composed of terms targeting: prevalence, childhood obesity, prevention we restricted the search to articles published since 2012 to appropriate balances feasibility of implementation and the validity of the resulting information. **Result:** The prevalence of overweight and obesity among children and adolescents aged 5-19 has risen dramatically from just 4% in 1975 to just over 18% in 2016. (WHO, 2017). Somewhere between 5.74 percent and 8.82 percent of school children in India are obese. In China, the overweight and obesity rate in children aged 7 and over was 12.2% and 7.3%, respectively. Four different studies were screened which was focused on at least one components like physical activity, physical fitness, fruit consumption. Significant changes observed on sedentary time (Watching TV and studying), active play time and fruit consumption but there was no effect on BMI or the prevalence of Overweight/ Obesity. A family Based Obesity prevention programme is effective strategy to prevent childhood obesity by providing physical activity classes to improve behavior and skills of the children. **Conclusion**: The strength of evidence is stated that family based intervention plays pivotal role in preventing Childhood obesity

Key words- *Childhood Obesity, prevention, prevalence, family based intervention*

Introduction

Childhood obesity is one of the most serious public health challenges of the 21st century and prevalent global public health issue as children. The problem is global and it has been seen in both developed and developing countries, with recent prevalence estimates of 23 and 13%, respectively. It is steadily affecting many low and middle-income countries, particularly in urban settings. (1)

The World Health Organization (WHO) has stated that the child population is more in developing countries, so childhood obesity rapidly increasing in developing countries than in developed countries.(2) The number of overweight or obese infants and young children (aged 0 to 5 years) increased from 32 million globally in 1990 to 41 million in 2016. In the WHO African Region alone the number of overweight or obese children increased from 4 to 9 million over the same period. (3)

An estimated 170 million children under 18 years of age were overweight or obese in 2008, and it has been projected that approximately 30% of all children will be affected by these conditions by 2030 (4, 5)

Childhood obesity has reached epidemic levels in the republic of India. India has the second-largest number of childhood obesity patients in the world, next to China. It has been estimated that 14.4 million children in India are affected by obesity, and its occurrence is increasing rapidly. (6)

Overweight and obese children are likely to stay obese into adulthood and more likely to develop non communicable diseases like diabetes and cardiovascular diseases at a younger age. Therefore Prevention of childhood needs high priority

Childhood Obesity prevention training is the strategy to halt obesity problem in childhood. A WHO (World Health Organization) classification distinguishes between general, selective and targeted intervention. Prevention programs can be classified as behavior-oriented (individual-based intervention) or community-/ environment-based (context-related intervention). Families have the ability to influence and shape child behaviors on a daily basis. Therefore, experts suggest that family involvement in the prevention of childhood obesity may provide greater behavior changes and sustainable weight loss over time compared to interventions without parental involvement. (7, 8)

Family-based interventions have grown considerably in recent years to prevent childhood obesity. Parents are described as integral targets in interventions, given their highly influential role in supporting and managing the four behaviors that affect children's energy balance (diet, physical activity, media use, and sleep). (9, 10, 11)

School based interventions include actions taken in schools to assist children in the prevention or treatment of obesity independent from family. Family based interventions include actions taken with the child's parents or caregiver in the home to help prevent or treat obesity. The purpose of this review is to identify, review, and critically appraise the evidence from studies examining the effect of family based intervention and school-based interventions on childhood obesity. (12)

A less active childhood is one of the most important factors contributing to childhood obesity. It has been reported that childhood obesity tracks into adulthood and is associated with an increased risk of mortality& morbidity, independent of weight loss later in life. Various health hazards may arise as complications of obesity like Coronary heart disease, Stroke, Type 2 diabetes, Hypertension, Hyperlipidemia etc.(2)

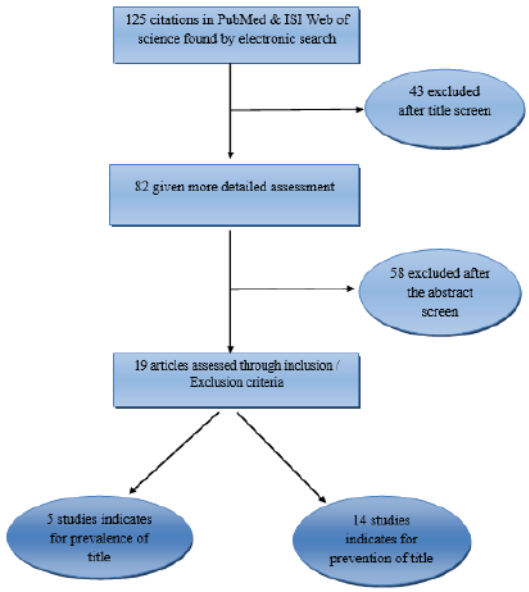
Objectives

This study systematically reviewed the prevalence of developing and developed nation and preventive strategies for Childhood obesity.

Search strategy and initial screening

With the help of a research librarian, most common databases used (PubMed, PsycINFO, and CINAHL) in recent systematic reviews and using individually tailored search strategies most appropriate for each database. The search strategy consisted of search strings composed of terms targeting: prevalence, childhood obesity, prevention we restricted the search to articles published since 2012 to appropriate balances feasibility of implementation and the validity of the resulting information.

Material & Methods



Result

The prevalence of overweight and obesity among children and adolescents aged 5-19 has risen dramatically from just 4% in 1975 to just over 18% in 2016. (WHO, 2017). The vast majority of overweight or obese children live in developing countries, where the rate of increase has been more than 30% higher than that of developed countries. (13)

Prevalence

Childhood obesity is highly prevalence in the United States and it is risk for poor health in children and adolescents. 18.5 % (13.7 million) children and

adolescents were obese and it is higher in Non hispanic blacks (22%) than non-Hispanic whites (14.1%).[14] In developing countries, the prevalence of Childhood Obesity is rapidly changing due to dietary practices and sedentary lifestyle. Somewhere between 5.74 percent and 8.82 percent of school children in India are obese. In urban south India, 21.4 percent boys and 18.5 percent girls aged 13-18 are either overweight or obese.[15] The study was conducted in Nepal where 575 students were included, 107 (18.6%) were overweight and 41 (7.1%) were obese. Out of that 328 male children, 62 (19.0%) were overweight and 35 (10.6%) were obese. Likewise, among 247 female children, 45 (18.2%) were overweight and 6 (2.4%) were obese.[16] In China, the overweight and obesity rate in children aged 7 and over was 12.2% and 7.3%, respectively. The corresponding number of overweight and obesity increased to 34.96 million.[17]

Prevention

School based interventions include actions taken in schools to assist children in the prevention or treatment of obesity independent from family. Family based interventions include actions taken with the child's parents or caregiver in home to help prevent or treat obesity.

The 3 studies focused on lifestyle i.e. incorporated nutrition, physical activity and behavioral components. Many parents stated that they did not want their child to '*be put on a diet* 'whereas more interested on holistic approach for weight management of the child. Motivating factors about parents that they had opportunities to adopt new skills and improve their knowledge on lifestyle related behaviors.[18, 19, 20] A family Based Obesity prevention programme is effective strategy to prevent childhood obesity by providing physical activity classes to improve behavior and skills of the children.[21]

Parents plays important role in childhood obesity prevention programs, to make them engage in weight loss management because they help their children to adopt healthy behavior.[22] Most of the parents do not understand the importance of Obesity prevention as they do not accept that their child has excess weight and adverse effect would happen due to diet consumed by child. Families are able to construct children's lifestyle habits, perhaps through their "parenting style" and management of "family functioning."[23] After attending of Parents in this prevention programmes, their children have to have more fiber diet and reduce sedentary lifestyle. Most of these programs were successful in decreasing body mass index (BMI) z-score and some health consequences of overweight In some cases, significant decrease in fat mass is documented, as well. [24]

School-based interventions programme plays an important role on promoting physical activity as well as preventing obesity. Four different studies were screened which was focused on at least one components like physical activity, physical fitness, fruit consumption. Significant changes observed on sedentary time (Watching TV and studying), active play time and fruit consumption but there was no effect on BMI or the prevalence of Overweight/ Obesity. [25, 26]

BMI z-score decreased with statistical significance for the students who received School-based intervention as compare to who did not receive the intervention.[27, 28, 29] Also decrease in their percentage of body fat with statistically significant for children who received school based interventions. [28, 30, 31]

Conclusion

The strength of evidence is stated that family based intervention plays pivotal role in preventing Childhood obesity. Nutrition, physical activity, reduce screen time intervention with behavioral modification are more effective strategies to maintain children's weight. Parental lifestyle influences the dietary pattern and behavioral changes in the child. More research and consistent methods are needed to understand the comparative effectiveness of childhood obesity prevention programs in the community setting.

Conflict of Interest – Nil

Source of Funding- self

Ethical Clearance – Ethical clearance taken from Institutional Research Committee of Symbiosis College of Nursing

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A Study to Assess the Effectiveness of Information Booklet on Selected Behavioral Problems among School Going Children in Terms of Knowledge of Selected Schools of Primary Schools Teachers of Pune City

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Abstract

Behavioural problems are refers to as the abnormal behaviour or abnormality of emotions, behaviour or relationship. The problems may be sever or mild. But the behavioural problems are sufficiently sever & persistent to handicap the child in his social or personal functioning & to cause distress to the child.

The present study assessed the effectiveness of Information Booklet on selected Behavioural problems among school going children in terms of knowledge of selected schools of primary schools teachers. Majority of subjects were (20%) were in age group of 25-30 years and 13.3% samples are from age group of 36-40 years. 33.33% sample's educational qualification was post graduate, 20.06% sample's educational qualification was graduate and 13.33% sample's educational qualification were higher secondary. 66.6% sample's work experience was above 12 years, 6.66% sample's work experience was 7-12 years, 16.60% sample's work experience was 4-6 years, and only 10% sample's work experience was 0-3 years. On the basis of gender, 96.66% of samples were females and 3.34% were male. T-test was used to assess the effectiveness of informational booklet regarding behaviour problems and p-value was found to be less than α (p= .031).

Key words: *Behavioural problems & School going children*

Introduction

Childhood is the period of dependency. Gradually children learn to just in the environment. Although it is difficult to get accurate estimate of child mental disorders, the few available epidemiological data indicate that 12-51%; with average around 29% of the world's children suffer from emotional & other mental problems that warrant mental health treatment.

Recent evidence indicates that emotional and behavioural disorders frequently lead to poor school performance and to dropping out of school. Primary school age children can cause significant difficulties in children's healthy development some children shows symptoms that are consistent with diagnosis of anxiety, ADHD, autism, dyslexia, etc. The school teachers are in a unique position to observe the child both at work & play and their role in detection of early symptoms cannot be overlooked. However, we need to advocate more active involvement of parents with the teachers.

The principal aim of the study is to enrich the physical & social environment of school children & has been an effective means of providing health care to these children.

A two stage epidemiological study of psychological disturbance among 5 to 8 years old school going children was conducted in Bangalore city. A sample of 1535 children were selected using a purposive sampling procedure and were assessed using the children behaviour questionnaire. The finding indicated that 18.31% of the children were found to be disturbed,

with boys having a significantly higher prevalence than the girls. In additional 'antisocial' problems were more commonly found among boys than girls more often manifested 'neurotic' problems[1] A study was done in Australia among the school teachers showed the mean number of children presenting with persistent behaviour difficulties in the classroom was reported to be 2.45. The most commonly occurring behaviour problems (presented as percentages), addressed at least once a day, were calling out (41%), disobedience (30%), not listening (33%) and poor concentration (25%)[3] . The total sample of recent graduate and student teachers were moderately confident in their behaviour management and discipline abilities and were moderately prepared to teach. Graduate and student teachers did not differ significantly in their perceived level of experience with children who showed persistent behaviour problems, rating themselves on a 5-point Liker scale, as having little experience, with a mean of 2.35(SD=1.05) and 2.60 (SD=.71), respectively. The primary aim of this study was to assess the importance of preparedness[4]

and classroom experiences as factors that may contribute to the development and maintenance of teacher self-efficacy in classroom behaviour management[2]

Problem Statement

"A study to assess the effectiveness of information booklet on selected behavioural problems among school going children in terms of knowledge of selected schools of primary schools teachers of Pune city."

Objectives

1) To develop an Information Booklet regarding selected Behavioral problem at school children.

2) To assess the knowledge of primary school teacher before & after administration of information booklet.

Hypothesis:

H0: There is no change in knowledge of school teachers after administration of information booklet on behavioural problems.

Methodology

Ø **Research approach:** Quantitative Research approach

Ø **Research design:** Pre-experimental design

Ø Variables:

Independent variable: informational booklet on behavioral disorder of school going children.

Dependent variable: Knowledge of primary school teacher**.**

Ø **Research setting:** Primary schools

Ø **Target population:** School teachers

Ø **Sampling:** Non-probability sampling

Results

Table 1: Frequency Percentage Distribution of sample by their characteristics of age.

Sr. no	Variables	Frequency	Percentage
1	Age 25-30 years	06	20.00
	31-35 years	03	10.00
	36-40 years	04	13.33
	Above 40 years	17	56.66
Total		30	100%

Table 1, reveals that total 30 samples under final data collection. In that 56.66% samples from age group of above 40 years, 13.3% samples are from age group of 36-40 years, 10.00% samples from age group of 31-35 years and 20.00% samples from age group of 25-30 years.

TABLE 2: Frequency Percentage Distribution of sample by their characteristics of Educational Qualification.

Sr. no	Variables	Frequency	Percentage
1	Educational		
	qualification		
	Higher secondary	04	13.33
	Graduate	06	20.06
	Post graduate	10	33.33
	Other(P.T.C.)	10	33.33
Total		30	100%

Table 2, reveals that total 30 samples under final data collection. In that 33.33% sample's educational qualification is other (P.T.C.), 33.33% sample's educational qualification is post graduate, 20.06% sample's educational qualification is graduate and 13.33% sample's educational qualification is higher secondary.

Table 3: Frequency Percentage Distribution of sample by their by their characteristics of work experience.

1	Experience		
	0-3 years	03	10.00%
	4-6 years	05	16.60%
	7-12 years	02	06.66%
	Above 12 years	20	66.66%
Total		30	100%

Table 3, reveals that, 30 samples under final data collection. In that 66.66% sample's work experience is above 12 years, 06.66% sample's work experience is 7-12 years, 16.60% sample's work experience is 4-6 years, and 10.00% sample's work experience is 0-3 years

Table 4: Frequency Percentage Distribution of sample by their characteristics of gender

Sr. no	Variables	Frequency	Percentage
1	Sex or Gender		
	Male	01	03.34%
	Female	29	96.66%
Total		30	100%

Table 4, reveals that, 96.66% of samples are female and 03.34% samples are male.

Table 5: Frequency Percentage Distribution of sample by their characteristics of previous knowledge.

Sr. no	Variables	Frequency	Percentage
1	Previous knowledge		
	No		
	Yes	01	03.34%
		29	96.66%
Total		30	100%

Table 4, reveals that, 96.66% samples having previous knowledge regarding topic, while 03.34% samples having no previous knowledge regarding topic.

Table 5: "t" value of Pre-test and Post-test knowledge scores of samples on behavioural problems of school going children.

Knowledge scores	Mean	MeanD	S.D	"t"Test calculated	"t"Test tabulated
Pre –Test	11.36	5.51	2.76	1.65	.031
Post-test	16.87		2.51		

(* "t" significant at 0.001 and DF=29)

Table 5, presented the data which showed that Mean post-test knowledge score 16.87 was higher than the Mean pre-test knowledge score 11.36 with Mean Difference of 5.51 which was found to be statistically significant as evident from "t" value for DF (29) at 0.031 level of significance, less than α= .05).

This indicate that the difference obtained in the Mean pre-test and post-test knowledge score was a real difference and not by chance. Hence, the null Hypothesis was rejected and research Hypothesis (H1) was accepted.

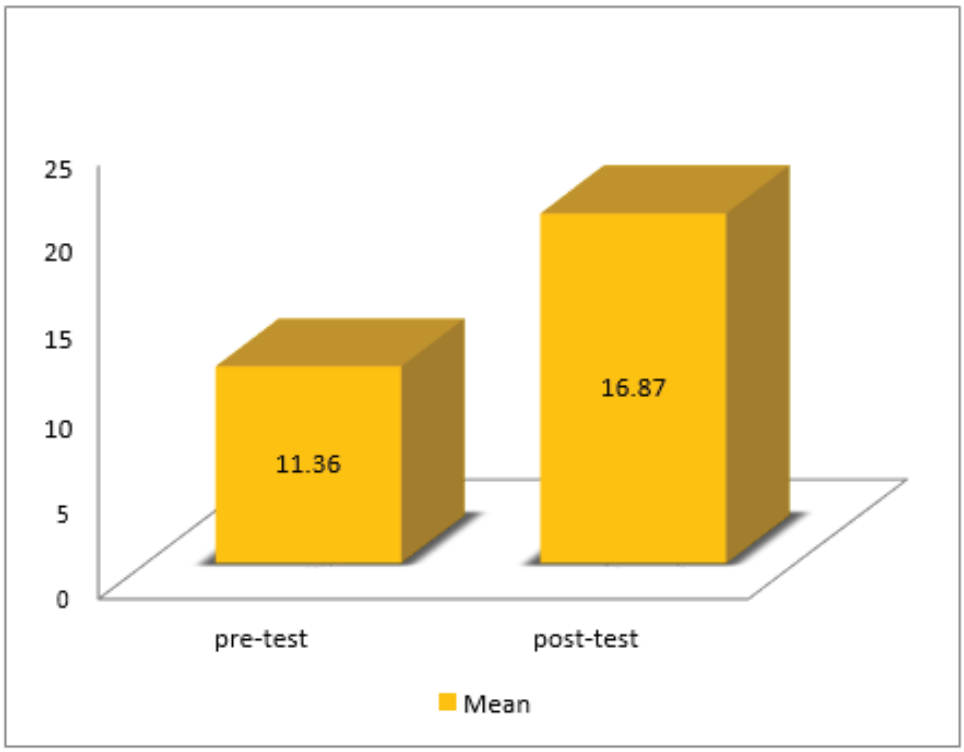


Figure 1 shows the comparison of Mean pre-test and post-test knowledge scores of primary school teachers regarding behavioural problems of school going children.

Ethical consideration: There is no Ethical issues came in the study, consent was taken from all the participants.

Funding: The study was self-funded.

Conclusion

From all the above findings, it can be concluded that the samples had poor knowledge regarding behavioural problems of school going children in the pre-test. so sample need to improve knowledge regarding this problems.

Finding shows that the Mean knowledge scores obtained by the primary school teachers in the pre-test phase was less than the Mean post - test. So this clearly indicates that the information booklet was effective in increasing the knowledge of primary school teachers. And in future also this type of information booklet will be helpful to improve the knowledge of primary school teachers regarding behavioural problems of school going children.

Conflict of Interest: Nil

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A Study to Assess the Eating Habits, Physical Activity, and Knowledge Regarding the Prevention of Obesity among School Children in a Selected Residential Area of Pune City

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Abstract

Background: India stands at second position in possessing a large number of children with obesity after China. Worldwide, millions of children are suffering from health problems related to obesity. In addition to faulty eating habits, obesity among children also caused by genetic factors and lack of physical activities.

Methodology: In the present study, researchers have executed a descriptive research design with a sample of 60. The samples were of school-going children with age group between 9-16 years and they were nominated in the study using convenient sampling design. The parameters include height, weight, and BMI of the school children to estimate the obesity of the children. This study also had focused to assess the physical activity and eating habits of subjects by assessing the knowledge regarding the prevention of obesity assessed by administering using a checklist.

Results: 65% of students had average knowledge on the prevention of obesity. 11.7% of the students have a long-term illness which is limiting their daily activity. The majority of the subjects preferred outdoor games while some subjects wished some sort of motivation to engage in sports activities. 61.7% of students liked to eat junk foods at least once a day. 16.7% of students fall under the category of overweight with BMI 24.6-30 kg/m2.

Conclusion: School going children need to be highly educated regarding the benefits of eating a healthy diet and they need to be discouraged for favoring junk foods by quoting results of health issues after eating junk food items.

Keywords: *Eating habits, physical activity, knowledge, prevention, obesity school children*

Background

India stands at second position in possessing a large number of children with obesity after China. Worldwide, millions of children are suffering from a health problem related to obesity. In addition to faulty eating habits, obesity among children also caused by genetic factors and lack of physical activities.

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In addition to the imparting education of school-going children regarding the prevention of obesity, parents also need to be educated about the healthy diet, and the importance of engaging the child in physical activities. They have to be well informed about the health hazards if the child develops obesity. Many research findings revealed that the parents giving a healthy diet to their children found less obesity among their children than others. (1)

Maintenance and leading a healthy lifestyle among the school-going children is very essential as they are the future citizens. A healthy lifestyle among the children includes having a highly nutritious diet, regular exercise

or any physical activities, and adequate sleep. According to the centers for disease control, 18.8% of school-aged children were obese who did have a healthy lifestyle.

The deficiency of adequate nutrition leads to disorders in mental and physical growth. Environmental problems such as lifestyle performance and the cultural environment of the child can play an important role in raising the problem of obesity worldwide. The classification and diagnosis of obesity can be detected by knowing the BMI of the person and it is calculated as

BMI = Weight (kg) / height (m2)

The obesity in children is calculated through BMI for age percentile charts for male and female gender of children. To estimate the categories of obesity, Body mass index (BMI) calculation is broadly used. The values of BMI profoundly changed with age, gender, and climate. [2]

Operational Definitions:

Assess: - In this study, researchers assessed the eating habits, physical activity of the children and assessed their knowledge regarding the prevention of obesity.

Knowledge:-In this study knowledge about obesity prevention was assessed

Prevention: In this study, the information regarding the prevention of obesity was collected from school children.

Obesity:- In this study, BMI levels of the school children checked.

Objectives of the study:

1. To assess the knowledge regarding the prevention of obesity among school children.
2. To assess the physical activity of the school children.
3. To assess the eating habits of school children.
4. To assess the BMI levels of the school children.

Methodology:

In the present study, researchers have executed a descriptive research design with a sample of 60. The samples were of school-going children with age group between 9-16 years and they were nominated in the study using convenient sampling design. The parameters included in the study were, height, weight, and BMI of the school children to estimate the obesity of the children. This study also had focused to assess the physical activity and eating habits of subjects by assessing the knowledge regarding the prevention of obesity assessed by administering using a checklist.

The procedure of conduct of the study:

In this study the data collection procedure was consist of the following steps;

Step 1: Formal permission was obtained from the authorities corporate

Step 2: Explained the participants about the study.

Step 3: Assessed knowledge and enquired about the eating habits and physical activity.

Step 4: Checked the height, weight, waist circumference, hip circumference, and calculating BMI of school children.

Results

Table 1: Distribution of the school children based on their characteristics in terms of frequency and percentages

n=60

Demographic variable	Frequency	Percentage
Age		
9-11 years	12	20.0
12-14 years	36	60.0
15-16 years	12	20.0
Gender		
Male	41	68.3
Female	19	31.7
Education of the student		
4 std. – 6 std	14	23.3
7 std. – 10 std	46	76.7
Educational qualification of mother		
Profession or Honors	3	5.0
Graduate or postgraduate	5	8.3
Intermediate or post-high school diploma	7	11.7
High school certificate	32	53.3
Middle school certificate	1	1.7
Primary school certificate	1	1.7
Illiterate	11	18.3
Occupation of mother		
Profession	8	13.3
Semi-Profession	5	8.3
Clerical, Shop-owner, Farmer	1	1.7
Skilled worker	9	15.0
Semi-skilled worker	27	45.0
Unskilled worker	10	16.7
Family Income per month		
≥36017	1	1.7
18000-36016	16	26.7
13495–17999	6	10.0
5387–8988	10	16.7
1803–5386	27	45.0

Table 2: Distribution of the school children based on their physical parameters in terms of frequency and percentages

Physical parameter	Frequency	Percentage
Waist circumference (cm)		
40-60	20	33.3
61-80	32	53.3
81 & above	8	13.3
Hip circumference (cm)		
45-65	23	38.3
66-85	30	50.0
86 & above	7	11.7
Weight (kg)		
25-35	21	35.0
36-45	18	30.0
46-55	15	25.0
56 & above	6	10.0
Height (cm)		
110-130	7	11.7
131-150	43	71.7
151-170	9	15.0
171 & above	1	1.7
BMI (kg/m2)		
12.5-18.5	20	33.3
18.6-24.5	25	41.7
24.6-30	10	16.7
31 & above	5	8.3

Table 3: Distribution of school children based on their knowledge regarding the prevention of obesity.

n=60

Knowledge	Frequency	Percentage
Poor (Score 0-5)	8	13.3
Average (Score 6-10)	39	65.0
Good (Score 11-16)	13	21.7

Table 4: Distribution of school children based on their physical activity

n=60

Physical activity	Frequency	Percentage
Presence of long-term illness or disability which limits the daily activity		
Yes	7	11.7
No	52	86.7
Not sure	1	1.7
What would help you to take part in more sport/physical activity?		
Insight of health-conscious	14	23.3
Motivation	28	46.7
Interest for maintaining	18	30.0
What has previously stopped from taking part in sport/physical activity?		
Lack of motivation to take part	17	28.3
Health issue concerns	3	5.0
Lack of child-friendly activities	20	33.3
Lack of childcare	1	1.7
Transport issues	5	8.3
Not interested in sport and physical activity	14	23.3
How often do you participate in sport and physical activity?		
Daily	30	50.0
Weekly	18	30.0
Monthly	6	10.0
Never	6	10.0

Table 5: Distribution of school children based on their physical activity

n=60

Physical activity	Frequency	Percentage
Would you like to participate in sports at school?		
Yes	51	85.0
No	9	15.0
Which sport do you like to play?		
Cricket	37	61.7
Football	12	20.0
Other (dancing, rope climbing, etc.)	11	18.3
What would you like to do in your leisure time?		
Watching TV	30	50.0
Listening to music	2	3.3
Other	28	46.7
Would you like to go to morning run/walk?		
Yes	39	65.0
No	21	35.0
Which game do you prefer indoor or outdoor?		
Indoor	25	41.7
Outdoor	35	58.3
Which one will you prefer among these?		
Going out for a run	30	50.0
Playing video games	30	50.0

Table 6: Distribution of children based on their eating habits.

n=60

Eating habits	**Frequency**	**Percentage**
What all are the food items you get in lunch		
Chapatibhaji	52	86.7
Dal rice	4	6.7
Pizza, burger	4	6.7
Which food items you like the most		
Chapatibhaji Dal rice	37	61.7
Pizza, burger, junk foods	23	38.3
How frequently you consume the food		
After every 2 hours	8	13.3
After every 3 hours	33	55.0
After every 4 hours	11	18.3
After every 5 hours	8	13.3
How many times you eat junk food in a week		
Twice a week	16	26.7
Thrice a week	8	13.3
Sometimes	28	46.7
Never	2	3.3
Daily	6	10.0
Which food items does your mother often cook		
Chapatibhaji	46	76.7
Daal rice	7	11.7
Paratha	1	1.7
Others	6	10.0
Why do you prefer fast food		
Status symbol	14	23.3
Just for taste	36	60.0
As a normal meal	10	16.7
In a day how many meals you replace with fast food		
Once	37	61.7
Twice	11	18.3
More than that	12	20.0
At what time you prefer to eat fast food		
Morning	13	21.7
Afternoon	8	13.3
Evening	21	35.0
All	18	30.0

Discussion and Conclusion

The main purpose of the study is to assess the physical activity, eating habits of the school children, and their knowledge regarding the prevention of obesity. The data was collected with the help of a self-administered questionnaire. Height, weight, and BMI were the main physical parameter checked for the school children. The students were very active and cooperative in giving the data for our study. The evaluation was made based on scoring.

The study revealed that a large number of the students have average knowledge regarding the prevention of obesity while the result of other related studies in the relevant literature reflects that knowledge about childhood obesity among primary school children was moderate. (3) The study on eating habits of the school children revealed that there is an impact on parents' education and their socioeconomic status on their dietary patterns and habits that make the difference to select the food. This study has indicated that the parent's education on the right dietary habits is a more essential component, particularly the middle-income population. Whereas other related studies in the related reviews replicate that frequencies of avoiding meals, food selections as well as socio-demographic features of subjects affect their food habits. (4) other related studies in the relevant literature reflect that physical activity was significantly associated with socioeconomic status and consumption of fruits and vegetables, whereas this study shows that the children are not motivated to play and has a lack of child-friendly activities. Also, the family's socioeconomic status plays an important role in allowing children to perform physical activities such as playing outside which excuses the urge to watch television more. (5)

Conflict of Interest – Nil

Source of Funding- self

Ethical Clearance – Ethical clearance was obtained from the ethical committee of Symbiosis College of Nursing.

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Assess the Preparedness Regarding Triage Skills Amongst Emergency Medical Professionals in Pune, India

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Abstract

Background: The word Triage means to sort or sieve and has originated from the French word "trier". The concept of triage was first applied in the military to evaluate and categorize wounded soldiers quickly during the battle probably around 1792. Emergency medical services (EMS) professionals are the first responders during mass casualty incidents. Successful application of triage requires continued education and practice in real clinical situations. Hence, it is essential to assess the adequate preparedness with regards to triage among EMS Professionals on a regular basis.

Objective: To assess the preparedness regarding triage skills amongst Emergency Medical Professionals in Pune, India.

Methodology: The study was conducted amongst 120 Emergency Medical Professionals in Pune, India. A

previously tested and validated Triage Skill questionnaire designed by Sitotaw Kerie *etal* and published in 2018 was utilized for the study. Participants were asked to respond to each item using 1-5 Likert scale. Questionnaire was administered to the participants through online mode after obtaining informed consent.

Discussion: The results indicate that participants tended to over prioritize indicating lack of knowledge of degree of care required by different patients. Majority of the participants scored between 146 to 180 corresponding to high degree of confidence in their skills regarding triage and management of patient. The scores obtained in the scenarios do not correlate with the self-perceived confidence.

Conclusion: The study throws light on the over prioritization bias amongst EMS professionals. Although the study has been conducted amongst 120 EMS Professionals and is limited, yet it provides insight on the various aspects of triage assessment. Regular hands on training is required to improve the knowledge and skill set of the EMS professionals with respect to triage that can be extremely useful in emergency situation.

Keywords: *Emergency Medical Services Professional, Triage Skills, Preparedness, Prioritize, Self-Perceived*

Background

The word Triage means to sort or sieve and has originated from the French word "trier"[1]. The triage sieve is a method where patient is sorted into immediate, urgent and non-urgent depending upon his/her current condition and has immense utility in mass casualty incidents. This method is widely used across the globe and in our country[1].

The concept of triage was first applied in the military to evaluate and categorize wounded soldiers quickly during the battle probably around 1792. It was later adapted by the healthcare community to categorize patients based on the severity of their injuries and to segregate them in the order of priority of care and monitoring.[2]

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Triage being a dynamic process requires regular review depending on the emergent state of the patient [3].

Emergency medical services (EMS) professionals are the first responders during mass casualty incidents. Timely provision of the required medical aid by EMS professionals can significantly impact the survival of patients.[4]

Kelly AM *etal*[5] in a study in 2001 emphasized that triage skills if not carried out in a standardized way can compromise the clinical outcome of the patients. Robert B Dunne *etal* [6] in 2003 in his study further reiterated this where he emphatically stated that EMS personnel unaided by triage protocols or specific training could not reliably identify those patients in need of emergency medical treatment.

Successful application of triage requires continued education and practice in real clinical situations. In this regard, a study by J E Pointer *etal* [7]in 2001 holds significance where he concluded that using written guidelines by Paramedics affects triage accuracy. Hence, it is essential to assess the adequate preparedness with regards to triage among EMS Professionals on a regular basis.

Objective:

To assess the preparedness regarding triage skills amongst Emergency Medical Professionals in Pune, India.

Methodology

The study was conducted amongst 120 Emergency Medical Professionals in Pune, India. A previously tested and validated Triage Skill questionnaire designed by Sitotaw Kerie *etal*[8] and published in 2018 was utilized for the study. The original sixty two item questionnaire consisted of 2 items assessing the Knowledge , 8 items in the form of Scenario assessing the attitude, 11 items denoted to the practices with regards to assessing the triage , 4 items pertaining to review of equipment required and 37 items pertaining to self-perceived triage skills was meant for nurses. The eighteen items that were exclusively pertaining to nursing were left out.

The 44 items questionnaire consisted of 2 parts. First part consisted of eight items in the form of MCQ pertaining to the attitude of the EMS Professionals related to situations requiring triage where participants were supposed to mark the single best option. A score of 1 was given to every correct option and a 36-item questionnaire with three dimensions, including rapid assessment, patient categorization, and patient allocation. Participants were asked to respond to each item using 1-5 likert scale: (1 = need improvement, 2 = poor, 3 = fair, 4 =good, and 5 = very good).

Questionnaire was administered to the participants through online mode after obtaining informed consent. Responding to all questions were mandatory. The participants were given two days to fill the questionnaire. Any queries pertaining to questionnaire was clarified during data collection.

All 120 participants reverted with filled questionnaire. The data was tabulated and statistically analyzed with the help SPSS version 23.

Result

Table 1: Demographic Data

Demographics	
Age	21- 25 Years: 69% 26- 30 Years: 23% More than 30:8%
Sex	Male: 23% Female: 77%
Qualification	BHMS: 52% BAMS: 33% BUMS: 11% Others: 3%

Cont... **Table 1: Demographic Data**

Work Experience	1-5 Years: 57% 6-10 Years: 33% 11-15 Years: 10%
Do you currently work in triage room	Yes: 72% No: 24% Maybe: 4%
Did you attend any training, course or workshop during the past three years	Yes: 53% No: 47%
Type of Training Attended	Basic Life Support(BLS): 78% Advanced Cardiac Life Support (ACLS): 2% Emergency Care: 46% Disaster Management: 15% Triage Course: 16%

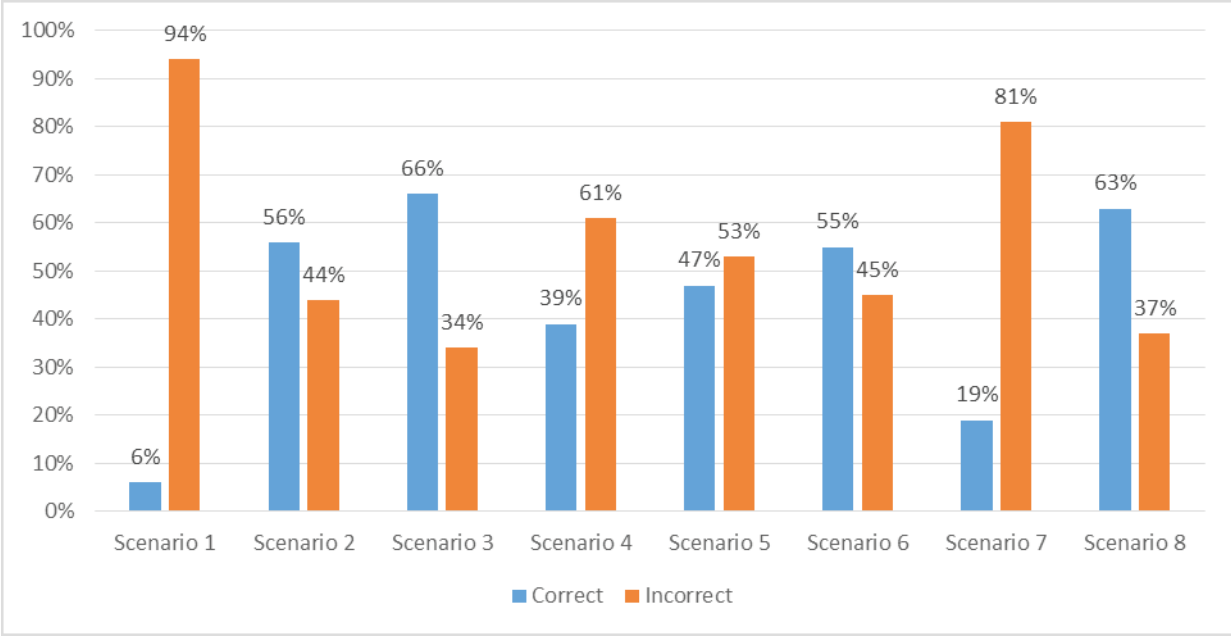


Figure 1.

As shown in figure 1, majority of the participants could not correctly prioritize the patients in triage situations in 4 out of 8 scenarios. A mean score of 3.48 with standard deviation of 1.25 was obtained in the triage prioritization of scenarios.

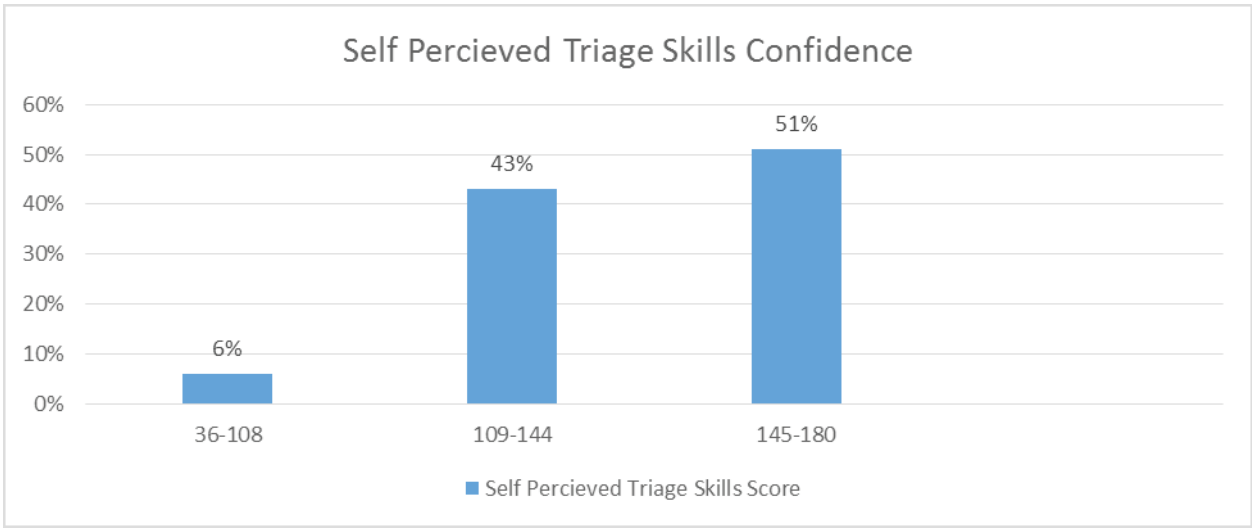


Figure 2.

In Figure 2, Score of 36-108 corresponds to scale of 1-3, 109- 144 corresponds to scale of 3-4 and 145 to 180 corresponds to scale of 4-5. As many as 51% of participants had scored well in the self-perceived triage skills confidence whereas 6% scored extremely low with 36 as the lowest and 180 as the highest score.

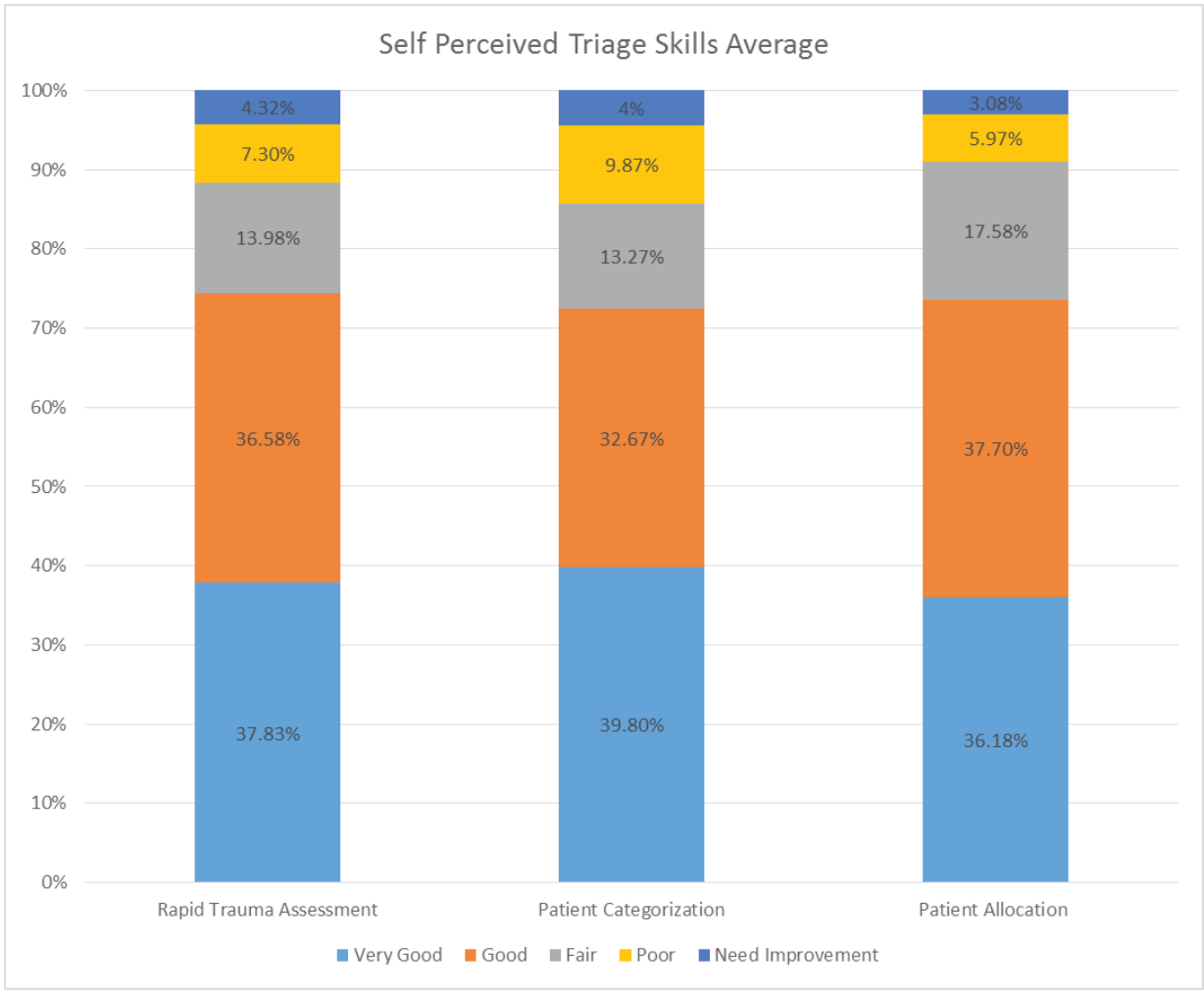


Figure 3.

As shown in figure 3, pertaining to self- perceived triage skills, majority of the participants showed high degree of confidence in their skills regarding triage and management of patient.

Discussion

Accurate prioritization of patients based on the severity of conditions play a significant role in allocation of limited resources in an emergency situation. EMS professionals are therefore expected to be well versed and possess a high degree of confidence in classifying the patient and allocating them the available resources.

The first part of the questionnaire consisted of 8 short scenarios describing different clinical pictures and the participants were expected to prioritize them into P1(immediate attention), **P2(intermediate or urgent care needed) and P3(delayed care).The results indicate that participants tended to over prioritize indicating lack of knowledge of degree of care required by different patients. In real clinical situation over prioritization of relatively stable cases shall lead to resource depletion and impact the survival of highly unstable patients. This tendency of over prioritizing can be corrected by subjecting the participants to regular case based scenario and simulation teaching.**

In second part of questionnaire, the participants had to self-assess their skills pertaining to rapid Patient assessment, patient Categorization and Patient Allocation with respect to triage. Self-perceived assessment is an accurate indicator of self-efficacy of the EMS professional. Majority of the participants scored between 146 to 180 corresponding to high degree of confidence in their skills regarding triage and management of patient.

Rapid Patient Assessment comprises Head to Toe assessment of the patient, identifying the life-threatening situations and managing them immediately so that the patient's situation do not deteriorate. Airway, Breathing, Circulation and Cervical Spinal Immobilization form the cornerstone of prehospital patient assessment and play an especially important role in determining the patient outcome. Majority of the participants were confident on most parameters of Rapid Patient assessment except low score was obtained on assessment of patency of airway by means of feeling for air. This could be attributed to the change in guidelines which suggest looking for chest rise instead of feeling for air entry to confirm the patency of airway.

In terms of Patient Categorization, majority of the participants were confident about their ability to categorize the patient, accord priority and identify immediate life-threatening injuries and managing them. However, the scores obtained in the scenarios do not correlate with the self-perceived confidence. This indicates need for more hands-on training with components of feedback and evaluation which shall clarify their concepts.

In terms of Patient Allocation, more than half of the participants were highly confident that they shall be able to allocate the patient to the right level of care after categorization. However correct patient allocation is the function of accurate prioritization.

Conclusion

The study was intended to gather perspectives on the triage skills of EMS professionals. The study throws light on the over prioritization bias amongst EMS professionals. Although the study has been conducted amongst 120 EMS Professionals and is limited yet it provides insight on the various aspects of triage assessment. Regular hands on training is required to improve the knowledge and skill set of the EMS professionals with respect to triage that can be extremely useful in emergency situation.

Acknowledgement: We thank Sitotaw Kerie *etal* for granting permission to use the questionnaire for the research study.

Source of Funding: Self

Conflict of Interest: None

Ethical Clearance: Obtained from IEC, SIU

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Challenges Faced by Working Population During Lockdown in Response to Corona Virus Outbreak

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Abstract

COVID-19 pandemic has created an unprecedented social and health challenges on the working-class population in India and across the globe. To mitigate the spread of the virus all the state and central government in India imposed lockdown in the society over the weeks or, in some cases months. This lockdown has made a severe impact on the physical, psychological, financial, and social conditions of the people. The aim of this study is to understand the impact of lockdown amongst people on their personal life, work life and health conditions. Independent survey was conducted to collect the descriptive statistics from 120 participants. Majority of the participants expressed that their life become difficult in lockdown. There is big challenge observed for women to manage children under 10 years of age. Work from home pattern was found favourable for those who are using computer and internet. Many participants were stressful and fearful as their job is at stake due to heavy financial loss to their employing organization. We have applied chi square test to find out association and it was found that age and gender is not significantly associated with facing challenges while other demographic factors are significantly associated.

Key words *– Corona, Covid-19, challenges, stress, lock down, social distancing*

Introduction

CORONA – Epidemics History

Covid-19 is a communicable disease caused by coronavirus. First case of covid-19 was found in Wuhan, China during December 2019. Covid-19 has now become a pandemic and has cases all over the world. Coronavirus is known to cause respiratory infections ranging from common cold to severe acute respiratory syndrome (SARS)[1]. The most common symptoms are headache, cold, conjunctivitis, sore throat, diarrhoea etc. some people may have mild symptoms. Older people with co-morbid conditions like hypertension, diabetes, heart disease, lung disease are at higher risk of developing serious illnesses even death[2]

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The pandemic of COVID-19 is causing a worldwide crisis in 2020. Many countries have closed schools and daycare centers, canceled events and closed borders. Under these circumstances, working population must work from home and complete their tasks. In this unique situation, research nest group is conducting an nationwide study to learn more about the challenges working population have to face and their way of coping with these[3]

Local weekly markets, ATMs peoples were advised to follow social distancing norms, however, it has been observed due diligence in most of the places. Migrant Labourers are suffering without earnings, facing serious problems due to hunger, health, and hygiene during this lockdown[4]

The current crisis is different because it is a pandemic. Nationwide lock down happened first time in Indian history. It has got impact on every citizen in the country. Poor peoples are suffering most as their livelihood is

depends upon daily work. Bigger corporate companies are pressurizing people to complete their tasks by work from home. This working class are directly affected by it. Most of them may not suffer too much from the virus itself, but they must understand why they must give up their daily routines, why they should not meet friends or pursue their hobbies etc. Events and vacations, they have been looking forward to having to be canceled. Many things that offered them opportunities for growth and for dealing with all the personal issues, an altruistic reason not easy to understand. If they stay at home, their parents or other adults may be in the same house at the same time, but they are not able to professionally replace the educator, as they have their own work responsibilities as well as dealing with the general stressful situation[5]

Television, streaming, and computer games offer entertainment and help to cope with emotions like boredom, anxiety, and loneliness. On the other hand, it may also contribute to negative effects such as emotional stress when they are used too long, when being exposed to information that may foster emotional uncertainty, or to pictures (e.g., on Instagram), that foster lower self-esteem and body image. Additionally, it is often a reason for arguments in families, who are under stress anyway because of this special situation of being trapped together[6].

Looking into intensity of the disease and fatality of the cases, Government of India announced total lockdown from March 23, 2020 onwards to reduce infection among population. Infections and death were increasing. The situation of India and other countries pushed economy into crisis and unemployment increased for daily wage labourers. The lockdown situation posed many problems for working population as well as non-working groups. All working and productive population is away from work due to slow down. Entire lock down population facing challenges related to finance, social withdrawal, shut down of schools, colleges, industries, etc.

Hence researchers have decided to conduct the study titled - challenges faced by working population at home in response to corona outbreak.

Material and Methods

Study on the Covid-19 crisis 2020 is exploratory descriptive study aims at identifying the challenges of working population and its association with demographic variables. Online survey was conducted, and nationwide data was collected. The target group are working population from 25 to 60 years who are willing to participate and questionnaire were prepared, validated, and distributed to participants. Most of the questions were closed questions (28) but some will be open ended (2). Each state must organize minimum n=20 working peoples, maximum N=120.

The questionnaire was developed in cooperation with national experts; a snowball system was used, and the questionnaire was circulated one to one using online mode. The field time was March 31st to April 30th.

Objectives

1) To determine the challenges faced by working population in response to Corona outbreak.

2) To find the association between the demographic variables and the challenges faced.

Results

Table No 1. Demographic Characteristics of Participants (N=120)

Socio Demographic Variable	No.	Percentage
Gender		
Male	69	57.5
Female	51	42.5
Age group (years)		
21 - 30	48	40
31 - 40	40	33.3
41 - 50	13	10.8
more than 50	19	15.8

Cont... **Table No 1. Demographic Characteristics of Participants (N=120)**

Religion		
Hindu	110	91.7
Muslim	1	0.8
Christian	8	6.7
Other	1	0.8
Educational Level		
Primary	0	0.0
Secondary	4	3.3
Higher Secondary	10	8.3
Bachelor or above	106	88.3
Occupation		
Business	22	18.3
Service	80	66.7
Agriculture	5	0.8
Housewife	13	2.5
Source of Information		
Mass Media (TV, Internet, Radio)	93	77.5
Family / Relatives	8	6.7
Health Professionals	16	13.3
Friends	3	2.5
Media Used to get Updates on Corona		
TV News	69	57.5
Internet	19	15.8
Radio	1	0.8
Facebook	2	1.7
Instagram	14	11.7
Mobile Phone	15	12.5
Others	0	0.0

Table 1: Demographic Characteristics of Participants (N=120). (a) Gender, (b) Age Group, (c) Religion, (d) Occupation, (e) Education Level, (f) Source of Information, (g) Media

Table 1, shows total 120 participants were participated in online survey. 69 (57 %) of the respondents were male and 51 (43 %) of respondents were female. Respondents were from 35 different cities in India. Maximum respondents (39%) were from Pune followed by (28%) respondents from Mumbai. Maximum number of respondents i.e. 40 % belongs to Age group 21 – 30 Years, followed by 33 % respondents belong to age group 31 – 40, followed by 16% of respondents belonging to age group 50 and above and least number 11% of respondent belonging to Age group 41 0 50 Years. Above table shows that 110 respondents belong to Hindu Religion, 8 belong to Christian, 1 belong to Muslim and 1 respondent belong to other religion. All respondentsfound Literate. It was found that 80 respondents belong to Service Sector, 22 belongs to Business sector, 3 respondents were housewife and 1 respondent was from Agriculture sector. Findings shows that 93 respondents received the information from source like Mass Media, 16 respondents received information from Health Personnel, 8 respondents received information from Family members and Relatives and 3 respondents received information from Friends. 69 respondents received information from TV New, 19 respondents received information from Internet, 15 respondents received information from Mobile, 14 respondents received information from Instagram, 2 respondents received information from Facebook and 1 respondent received information from Radio.

Section I

CHALLENGES FACED BY WORKING POPULATION AT HOME IN RESPONSE TO CORONA OUTBREAK

Table No. 2 Personal Life Challenges

Questions	Strongly agree	Agree	Disagree	Strongly Disagree
This Lockdown has brought a big change in the daily routine	48%	42%	5%	5%
Managing work from home and household responsibility is making life miserable	12%	56%	24%	8%
Personal Life equilibrium is disturbed	28%	48%	20%	4%
Children health during this Pandemic is a big worry	36%	53%	8%	3%
Being at home for almost more than 20 days is making me feel sick	29%	35%	30%	6%
Not able to meet friends and relatives	39%	47%	10%	4%
Worried about the health of old aged parents	53%	41%	3%	3%
Lockdown had made it difficult to buy items of daily needs	29%	36%	30%	5%
Managing daily requirements of the family is becoming difficult	22%	57%	16%	5%
Managing small children along with work is difficult	23%	53%	16%	8%
Mean %	32	47	16	5

Table 2 shows depicts that maximum of 47% people Agree that their Personal life was very much affected due to lockdown, whereas 32 % people strongly have an opinion that Lockdown have affected their Personal life and routine at a great extent. Table No 2, shows that 56 % participants agreed that their life has become miserable whereas 53% are at opinion that they are worried about their children for obvious reason. 57% participants found difficult to fulfil the requirement of family members.

Table No. 3 Work Life Challenges

Questions	Strongly agree	Agree	Disagree	Strongly Disagree
Working from home is difficult during this phase of lockdown	24%	41%	30%	5%
Lot of communication and collaboration gaps during work is being faced	28%	52%	18%	3%
Quality and Quantity of work is affected	30%	52%	13%	5%
Face lot of distraction during work at home	19%	51%	26%	4%
Getting internet access is also a big challenged during this lockdown phase	31%	38%	25%	7%
Working efficiently at home along with household work is difficult	23%	50%	23%	3%
Team working is impossible due to lack of control on the team	42%	33%	23%	3%
Creating work environment at home is impossible	15%	52%	28%	5%
Lack of motivation and energy to work	20%	48%	26%	6%
Task completion and deadlines of work are affected	33%	43%	19%	5%
Mean %	27	46	23	5

Table 3 depicts that maximum of 46% people agree that working from home during lockdown was a stressful situation, whereas 27 % people strongly agree with the fact the Work from home affected their work culture a lot. Surprisingly an approximately same number of people i.e. 23 % of people have an opinion that lockdown have not at all affected their work life and culture. Table No 3 indicates that 52% responded that their quality and quantity of work is affected due to lockdown. More than half participants agreed that creating work environment is impossible.

Table No 4 Physical Fitness Challenges

Questions	Strongly agree	Agree	Disagree	Strongly Disagree
My daily physical fitness routine is disturbed	27%	47%	19%	8%
Going out for sports and Gym is impossible	41%	51%	3%	5%
Sitting home 24/7 makes me feel lethargic	21%	48%	25%	6%
Physical activity has become minimal	31%	57%	10%	3%
I am facing physical problems due to weight Gain	21%	33%	31%	16%
I enjoy household work	13%	53%	26%	9%
I tried to be friendly with my family members	20%	66%	9%	5%
I watch my favourite TV programmes	26%	41%	18%	15%
Mean %	25	49	14	6

Table 4 depicts that maximum of 49% people Agree that their physical health was affected during the lockdown, their physical activity has become minimal. Whereas 25% people strongly agree that their physical fitness routines are disturbed to a great extent. As per the open ended views of participants about how does they utilize the time at home during lockdown, views expressed were that 40 % sample manage the situation by using the internet, 31% are playing with kids and family member, 25 % are doing the house work and 4% are not doing as such activities.

When asked about ways to manage their stress during lockdown, responses were 33 % sample manage the situation by doing housework, 29% are playing indoor games, 25 % are doing the exercise and 13% mentioned they do activities as per situation.

Section II

Association of findings with demographic variables

SN	Variable	Chi^2	P Value	Result
1	Gender	43.55	0.2125	Reject H0
2	Literacy	80.00	0.0001	Accept H0
3	Age	140.00	0.0298	Accept H0
4	Religion	192.00	0.0001	Accept H0
5	Education	57.00	0.9225	Reject H0

$p < 0.05$ significant *

There is association between the demographic variable and challenges faced by working people. The data presented in the table shows that there is significant association with Literacy of the participants, age, and religion. Whereas gender and educational level there is no association.

Discussion

Covid-19 corona virus disease outbreak has been spreading all over the world. In view to prevent further spread and to protect Indian population from getting affected Government of India announced total lockdown from March 23, 2020. Due to prolong confinement of people in home during this outbreak work life, personal life and physical health of the working population is affected[7]. conducted to study to find out during lockdown physical as well as mental health of the people is at stake. A study was conducted to assess challenges faced by working population across the country during March 31 to April 30, 2020; online cross-sectional observational study conducted among 120 participants recruited by snowball technique. Demographic characteristics shows that 57.5 % male and 47.5 % female participants. Majority (40%) participants were from age group of 21-30 years whereas 33.3 % from age group of 31-40 years of age. All participants were literate and 66 % participants were working in office, industries and other places whereas 22 % participants were from business sector. All participants included in the study was working from home and adopted total lock down. Observations were made for work life, personal life and health challenges. It was observed that working population found personal life challenges found more intensive and people faced less health challenges. This observation was supported by study conducted by Mahendra kumar -2020 he found that social life challenges were 52 % while 79% people were worried about family and friends. He also found that 67 % people had altered sleep pattern during lock down[8]. It is also observed that working people with advanced age are more concerned about their health and they take rich food, do regular exercise and yoga practices while younger population found less attentive to health matter.

Conclusion

Corona virus pandemic affected working population worldwide, working population facing major issues related to the work life, personal life and health challenges. One needs to prepare themselves to tackle all these challenges. Personal life challenges are more intense than any other challenges. Not only working population but also non-working population, old age peoples, housewives, children needs to adopt themselves for this changing pattern of life. Use of mask, social distancing, personal hygiene and use of sanitizer should become part of life of everyone.

Ethical Clearance- Institutional Ethics Committee approved the research study

Source of Funding- Self funded

Conflict of Interest – Nil

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Assess the Knowledge Regarding Food and Vegetable Sanitation among Housewives in Selected Areas of Pune City

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Abstract

In order to preserve our health and remain healthy, it is of upmost importance to maintain the quality of food we intake and avoid contamination. To achieve this, proper sanitation of the food and vegetables we ingest is required. The poor knowledge of food sanitation causes several food-borne disease and various health issues and in turn puts extra strain on our Health Sector. Hence, it is important to prevent the food from getting contaminated with microbes or toxic chemicals, and follow healthy food sanitation practices. To achieve this, food safety at the household level and knowledge regarding handling food is essential. The aim of this study was to gain and scrutinize the knowledge related to food and vegetable sanitation among housewives who cook the food at home. **Objective:** 1) To assess knowledge regarding food sanitation among housewives in selected areas of Pune city. 2) To assess knowledge regarding vegetable sanitation among housewives in the selected areas of Pune city. **Result:** The analysis shows that 7.94% of housewives have excellent knowledge regarding cleaning of vegetables and fruits, 23% of housewives have very good knowledge, 23% of housewives have good knowledge while 44.4% of housewives have average knowledge. Also 15.87% of housewives have excellent knowledge regarding separation (keep-apart) of food & vegetables, 7.6% of housewives have very good knowledge, 28.57% housewives have good knowledge while, 47.6% of housewives have average knowledge. Also 7.94% of housewives have excellent knowledge regarding cooking at safe temperatures, 15.87% of housewives have very good knowledge, 25.39% housewives have good knowledge while, 50% of housewives have average knowledge. And 6.35% of housewives have excellent knowledge regarding chilling i.e refrigerating the food items and vegetables, 15.87% of housewives have very good knowledge, 31.7% housewives have good knowledge while, 46.0% of housewives have average knowledge. **Conclusion:** The housewives have satisfactory knowledge regarding separation and cleaning of food and vegetables, also they have less knowledge regarding cooking at safe temperature and the refrigerating of the food and vegetables. Hence, they need to be motivated to follow good food and vegetable practices in order to prevent contamination of food as well as to reduce the rate of food-borne illnesses.

Key words: *Knowledge, Food, Vegetable, Sanitation and Housewives.*

Introduction and Background

It is time to boost our immunity.

Good food hygiene describes how to avoid the spread of bacteria when cooking, washing, preparing, and storing food items on a daily basis. As we all know, if the food is not cooked, stored and handled correctly, it can cause food poisoning and other health issues. Most of the people assume that food consumed from roadside stalls or from food hawkers causes sickness and stomach upset, but it may not always be the result of outside food; it could also be the result of poor kitchen hygiene. Mainly, the germs get transferred to other food items or to the kitchen surfaces while preparing and cooking from raw

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foods like chicken, eggs, meat, poultry, sea-food, raw fruits and vegetables[1]. There are chances that such food gets spoilt immediately and the person who consumes such kind of food or vegetables, can fall ill. Especially, people with low immunity like small children, pregnant women and elderly adults and others who are suffering from HIV/AIDS, kidney issues, cancer, diabetes, and other chronically ill diseases are at more risk[2]. Also in a country like India, the housewives are mostly responsible to handle the kitchen and food needs of the family in order to meet the nutritional requirements. Hence, taking this into consideration, the researchers proposed the need to conduct the current research to assess the knowledge regarding food and vegetable sanitation among housewives. This research helps to gauge the knowledge of the housewives regarding food and vegetable sanitation and also to develop strategies to fill the knowledge gap by different methodologies.

Objective:

1. To assess knowledge regarding food sanitation among housewives in selected area of Pune city.

2. To assess knowledge regarding vegetable sanitation among housewives in selected area of Pune city.

Methodology

Research Design: The researchers have used A Non-Experimental Descriptive Research. The study was conducted on the housewives of selected areas of Pune city.

Sampling techniques: Convenient sampling technique was used.

Subject Size: 126 homemakers who are staying in different areas of Pune city and willing to participate. These participants were informed clearly about the study and its purpose.

Tools of data collection: In the present study, the below scales were used to collect the data. Structured questionnaire to assess the knowledge regarding food and vegetable sanitation among the housewives staying in different areas of Pune city. It has two sections; **Section I**: Consisted of items related to demographic variables of the subjects of the study. **Section II**: Consisted of 20 questions regarding food and vegetable sanitation. It was divided in 4 parts; Part A- Questions on knowledge related to cleaning of food & vegetables. Part B- Questions on knowledge related to separation (keep-apart) of food & vegetables. Part C- Questions on knowledge related to cooking at safe temperature, which kills germs. Part –D Questions on knowledge related to chilling i.e refrigerating the food items and vegetables.

Validity and Reliability of tools: The experts validated tools and the reliability was done by Cronbach's Alpha Coefficient Test. Cronbach's Alphas were r= 0.86 & 0.9 for assessing the knowledge regarding food and vegetable sanitation among housewives in selected areas of Pune city.

Findings: The analysis and interpretation of the data collected to determine the knowledge regarding food and vegetable sanitation in housewives from selected areas was done keeping the objectives of the study in consideration. 1. To assess knowledge regarding food sanitation among housewives in selected areas of Pune city. 2. To assess knowledge regarding vegetable sanitation among housewives in selected areas of Pune city.

The results were divided in two Sections: Section – I: Description of Distribution of Demographic Variables. Section – II Part A: Description of practices related to cleaning. Section – II Part B: Description of practices related to Separation (Keep Apart) of food items. Section –II Part C: Description of practices related to cooking at safe temperatures which kills germs. Section –II Part D: Description of practices related to Chilling i.e Refrigerating the food items.

SECTION I

Description of demographic details

Table 1: Represents the data related to description of demographic variables.

Demographic variable		Freq	%
Age	21-30	20	15.9
	31-40	60	47.6
	41 and above	46	36.5
Type of Family	Nuclear family	80	63.5
	Extended/Joint Family	46	36.5
Education	Graduate	81	64.3
	Post Graduate	10	7.9
	Higher Secondary	20	15.9
	Illiterate	15	11.9
Years of Marriage	0-3 Years	26	20.6
	4-6 Years	40	31.7
	6 Years	60	47.6
No of family members	0- 2 Member	60	47.6
	3-5 Member	20	15.9
	More than 5 Members	46	36.5
Type of food habits	Vegetarian	70	55.6
	Non – Vegetarian	56	44.4

SECTION II

Part A: Knowledge related to Cleaning of food & vegetables

Table 2: Represents the data related to knowledge of cleaning the food and vegetables

N=126

Cleaning	Average		Good		Very Good		Excellent	
	Freq	%	Freq	%	Freq	%	Freq	%
	56	44.4	30	23	30	23	10	7.94

Part B: Knowledge related to separation (keep-apart) of food & vegetables

Table 3: Represents the data related to knowledge of separating of food and vegetables.

N=126

	Average		Good		Very Good		Excellent	
Separation (Keep Apart)	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
	60	47.6	36	28.5714	10	7.93651	20	15.87

Part C: Practices related to cooking at safe temperature, which kills germs.

Table 4: Represents the data related to knowledge regarding cooking at safe temperature, which kills germs.

N=126

	Average		Good		Very Good		Excellent	
Cooking to safe temperatures kills germs	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
	64	50.8	32	25.3968	20	15.873	10	7.94

Part D: Practices related to Chilling i.e Refrigerating the food items and vegetables.

Table 5: Represents the data related to knowledge regarding Chilling i.e Refrigerating the food items and vegetables.

N=126

	Average		Good		Very Good		Excellent	
Chilling (Refrigerating) t	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**
	58	46.0	40	31.746	20	15.873	8	6.35

Discussion

This study was aimed to assess the knowledge regarding food and vegetable sanitation among housewives in selected areas of Pune city. Hence, the data was gathered from the participants and analysis was done to find the results.

Findings related to the knowledge regarding Cleaning of food & vegetables.

Table 2: Data describes the Part A which deals with the knowledge related to cleaning of food & vegetables among housewives, and the parameters measured related to cleaning were: Wash hands in warm soapy water for at least 20 seconds before and after touching food;

washing of cutting boards, dishes, forks, spoons, knives, and counter tops with hot soapy water; cleaning of the lids on canned goods before opening; cleaning of all the fruits and vegetable purchased from the market rinsed in running water followed by rubbing with hands; if damage or bruising occurs before eating or handling, it is to be cut away the damaged areas before preparing or eating. The analysis says that only 7.94% of housewives have excellent knowledge regarding cleaning of vegetables and fruits, while 23% of housewives have very good knowledge, 23% of housewives have good knowledge and 44.4% of housewives have average knowledge.

Findings related to the knowledge regarding separation (keep-apart) of food & vegetables.

Table 3: Data describes the Part B which deals with the knowledge related to separation (keep-apart) of food & vegetables to prevent contamination among housewives, and the parameters measured related to separation (keep-apart) were: Keeping of raw meat, poultry, seafood, and eggs away from other foods while shopping and while keeping in fridge; boiling of the marinades of raw foods if need to use second time; using of a special cutting board or plate for raw foods only; cooking of non-vegetarian foods in separate utensils. The analysis says that 15.87% of housewives have excellent knowledge regarding separation (keep-apart) of food & vegetables, 7.6% of housewives have very good knowledge, 28.57% housewives have good knowledge while and majority that is, 47.6% of housewives have average knowledge.

Findings related to the knowledge regarding cooking at safe temperature, which kills germs.

Table 4: Data describes the Part C which deals with the knowledge related to cooking at safe temperature to kills germs among housewives, and the parameters measured related to cooking at safe temperature were: Cooking fish and sea-foods, chicken, finely chopped (ground) chicken, vegetables at appropriate temperatures to maintain its nutritive value as well as kill the germs. The analysis says that 7.94% of housewives have excellent knowledge regarding cooking at safe temperatures, 15.87% of housewives have very good knowledge, 25.39% housewives have good knowledge while 50% of housewives have average knowledge.

Findings related to the knowledge regarding Chilling i.e Refrigerating the food items and vegetables.

Table 5: Data describes the Part D which deals with the knowledge related to chilling i.e refrigerating the food items and vegetables which helps prevent food from spoiling or getting contaminated with germs, as low temperature prevents growth of microbes, and the parameters measured related to chilling i.e refrigerating the food items and vegetables were: Storing foods in the fridge or freezer within 2 hours after cooking; after purchasing from the store; within 1 hour if it is 90 degrees or hotter outside; and marinating foods in the fridge. The analysis says that 6.35% of housewives have excellent knowledge regarding chilling i.e refrigerating the food items and vegetables, 15.87% of housewives have very good knowledge, 31.7% housewives have good knowledge while, 46.0% of housewives have average knowledge.

Similar to current study findings, there was a study done on Food Hygiene, Knowledge, Attitudes and Practices of Food Handlers in Food Businesses in Accra, Ghana, where the researcher had said that the responses regarding food storage of the food handlers from the different hotels varied in their response, where only 42.9% knew that the correct temperature for refrigeration was 1˚C - 5˚C also even though the respondents food hygiene attitudes were found to be satisfactory, the microbial analysis results have proved that, strict food hygiene practices were lacking in them. Thus, food handlers must strictly follow food hygiene practices and the managers should motivate them for the same, to prevent from serving cross-contaminations of foods to the consumers[3].

In an another study, result which was done on food safety policies and practices in public spaces done on vendors of fresh fish sellers in Mzuzu, Malawi, where the researcher found that hand washing with soap and water by vendors while at work and keeping food at a proper temperature appears to be the most effective ways of reducing *E. coli* and *Salmonella* spp. Also, the vendor's knowledge regarding food-borne diseases is low, and that a change in the behavior and facilitation of practices is needed through proper infrastructure along with more training[4].

Conclusion

The study results have shown that the housewives have adequate knowledge regarding cleaning as well as separation of food and vegetables as it is their daily routine although they need to be motivated to improve their habits related to refrigeration and cooking at safe temperatures, which will increase their knowledge as well will help in reducing food contamination which will further prevent food-borne illnesses.

Conflict of Interest: Nil

Source of Funding: The study was fully funded by authors and had no financial support to this project.

Ethical Clearance: Ethical approval of the study taken from Symbiosis College of Nursing's ethical committee also, Informed consent was taken from the housewives living at Pune city. Information was given to the housewives regarding the data collection procedure. The data collected was kept confidential and used only for research purposes.

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"Social Impact of Pandemic disease (COVID-19) in General Population

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Abstract: The COVID-19 pandemic has swept the entire world off its feet by its deadly outcomes and India is no exception to it. The pandemic has affected our country in many different terms that has caused different issues for the general public and rendering many jobless. There were many problems cause like economic loss, loss of life, business, jobs etc[2]. Considering this current situation, this research is be based on highlighting the social impact of the pandemic disease on the general population. **Methodology**: A Non-Experimental Descriptive Research Approach "Quantitative research which involves the collection and collation of numerical information under considerable control." The study was conducted among the general population of Pune City. The selection of sample was based on easy geographical accessibility, willingness of the samples and availability of samples. The sample size for study was 100, selected by using Non Probability Convenience Sampling Technique from various residents living in Pune City who met the inclusion criteria. A structured modified questionnaire was developed based on the research problem, to get the information from the samples. **Objective:** 1) To assess the effect of lockdown on social life in general population in selected area of Pune city. 2) To assess the obstacles faced by general public during the Covid-19 pandemic. 3) To associate the demographic variables with the lockdown effect and obstacles in selected areas of Pune City. **Result:** The analysis shows that the family relations of 28.6% population were severely affected by the pandemic situation and 69.84% of population were less affected by it. The social relations and recreation of 76.19% population were severely affected by the pandemic situation and 15.9% of population were less affected by it. The 74.6% population were severely affected psychologically due to the pandemic situation and 14% of population were less affected psychologically. Also 79.37% population were faced severely difficulties in getting the basic necessities during the pandemic situation while 12.7% of population faced less problems in procuring the basic necessities. Also 36.51% population faced sever obstacles related to work while 52.4% of population faced less obstacles by it. Since all the p-values corresponding to gender is less (less than 0.05). Hence gender is to have significant association with the lockdown effect and obstacles. **Conclusion:** This research concludes that COVID-19 has had a great effect on social relations, psychological aspect

Key words: *Social impact, Pandemic, disease (COVID-19) general population.*

Introduction and Background

When there is any type of problem or crises our leaders have the main responsibility of solving the arrived problem and prevent it from occurring again. The current situation of COVID-19 pandemic is one of them[1]. As we, all know about this Novel Corona virus, which first made its presence in Wuhan, China and then rapidly spread across the globe causing huge economic, social, health, psychological issues, and eventually loss of lives. A similar study was conducted in 2017 on the impact of Ebola virus outbreak[3]. Another study, which was a quantitative approach survey, conducted in 2008 to identify the long-term effect specially socioeconomic impact due to Nipah virus. The researcher in there result found that the urgent cost of management was not and over burden to the middle class home makers .Most of them i.e. 92 % were treated via Inpatient care and

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were treated freely in the available public hospitals[4]. An African swine fever caused in 2017 sept That study result concludes that the effect of wine flu in that year of swine flu outbreak was complex [5].

All the countries tried curbing the spread of COVID-19 using their best possible efforts and India chose to battle it by imposing a complete lockdown. Thereafter, the Indian government put in huge efforts in bringing up new makeshift hospitals, testing units, making PPEs available for healthcare team, and disinfecting areas most affected. Apart from this, the government has also taken efforts to provide essentials to the needy citizens i.e. providing food-grains and other essentials by launching different relief schemes. Help reached the poorer sections of the society not only through the government, but through also many social and private organizations who did their best to make sure that help reaches to the maximum needy around by providing food packets and other essentials through contributions. We also should remember the contribution made by the working class people all over India by contributing a portion of their salaries towards the relief funds.

Objective

2. To assess the effect of lockdown on social life in general population in selected area of Pune city.

3. To assess the obstacles faced by general public during the Covid-19 pandemic.

4. To associate the demographic variables with the lockdown effect and obstacles faced in selected areas of Pune City.

Methodology

Research Design: The researchers have used A Non-Experimental Descriptive Research. The study was conducted on the general population of selected areas of pune city.

Sampling techniques: Convenient sampling technique was used.

Subject Size The general population altogether were 63 who are staying in different areas of pune city and willing to participate. These participants were informed clearly about the study and its purpose.

Tools of data collection: In the present study the below scales were used to collect the data. Structured Questionnaire to assess the impact and obstacles during the COVID-19 pandemic situation and lockdown in the different areas of pune city. It has two sections; **Section I**: Consisted of items related to demographic variables of the subjects of the study. **Section II**: Consisted of 16 questions regarding social impact related. It is divided in 3 Parts Part A- Questions on impact related family relations. Part B- Questions on impact social relation & recreation. Part C- Questions related to psychological impact. **Section III**: Consisted of 12 questions regarding Obstacles related to work and basic necessities faced by general population. It is divided in 2 Parts Part A- Questions on obstacles related to basic necessities. Part B- Questions on obstacles related to work.

Validity and Reliability of tools: Tools were validated by the experts and the reliability was done by Cronbach's Alpha coefficient test. Cronbach's alphas were r= 0.86, & 0.9 for assessing the impact and obstacles during the COVID-19 pandemic in selected areas.

Findings

The analysis and interpretation of the data collected to determine the social impact of pandemic disease COVID-19 on general population was done keeping the objectives of the study in consideration, To assess the effect of lockdown on social life in general population in selected area of Pune city, To assess the obstacles faced by general public during the Covid-19 pandemic, To associate the demographic variables with the lockdown effect and obstacles faced in selected areas of Pune City.

The result was divided in IV Sections Section –I: Description of Distribution of Demographic Variables, Section – II: Description of impact on general population Section – III: Description of obstacles faced with basic necessities & work. Section –IV: Association of demographic variables with the impact and obstacles in getting the basic necessities work related in general population.

Section-I

Table 1: Description of Distribution of Demographic Variables N=63

Demographic variables	Freq	%
1. Gender		
Female	45	71.42
Male	18	28.57
2. Age		
Less than 20 years	2	3.17
21 to 40 years	54	85.7
41 to 60 years	4	6.34
61 to 80 years	2	3.17
81 years and above	1	1.58
3. Occupation		
Agriculture	3	4.7
Business	2	3.17
Employee	26	41.26
Homemaker	16	25.39
Retired	5	7.9
Student	8	12.6
Other	3	4.76
4. Education		
Less than graduation	22	34.9
Graduate	30	47.61
Post graduate	11	17.4
5. During lock down staying with:		
Family	48	76.1
Friends	4	6.34
Colleagues	6	9.5
Alone	5	7.93

Table no 1 : Shows the frequency & percentage distribution of samples regarding the Demographic Variables were 71.42% were female and 28.57% were male. 85.7% were between the age group of 20-40 yrs. Majority of them were employees and homemakers, 47.61% were graduate and majority of them were staying with their family.

SECTION-II

Table 2: Description of impact on general population

Part-A Family relations n-63

Item	Not at all related to me		Somewhat related to me		Extremely related to me		Mean	SD
Impact related to family relations	Freq	%	Freq	%	Freq	%	21	26.6
	1	1.59	44	69.84	18	28.6		

Table 2 : Shows the frequency & percentage distribution of samples regarding the family relations which reveals that the family relations of 28.6% population were severely affected by the pandemic situation and 69.84% of population were less affected while 1.59% were hardly affected.

SECTION-II

Table 3: Description of impact on general population

Part-B Social relations and Recreation n-63

Item	Not at all related to me		Somewhat related to me		Extremely related to me		Mean	SD
Impact related to social relations and recreation	Freq	%	Freq	%	Freq	%		
	5	7.93651	10	15.9	48	76.19	21	23.5

Table 3: Shows the frequency & percentage distribution of samples regarding the social relations and recreation where 76.19% population were severely impacted during the pandemic situation and 15.9% of population were moderately impacted while 7.93% population were scarcely impacted by it.

SECTION-II

Table 4: Description of psychological impact on general population

Part-C Psyhological Impact n-63

Item	Not at all related to me		Somewhat related to me		Extremely related to me		Mean	SD
Psychological Impact	**Freq**	**%**	**Freq**	**%**	**Freq**	**%**		
	3	3.22581	13	14	47	74.6	21	23

Table4 : Shows the frequency & percentage distribution of samples regarding psychological Impact on general population where 74.6% population were severely impacted psychologically due to the pandemic situation and 14% of population were moderately impacted while 3.2% population was scarcely impacted psychologically during the pandemic situation.

SECTION-III

Table 5: Description of Obstacles related to basic necessities in general population

Part-A Obstacles related to basic necessities n-63

Item	Not at all related to me		Somewhat related to me		Extremely related to me		Mean	SD
Obstacles related to Basic necessities	Freq	%	Freq	%	Freq	%		
	5	7.94	8	12.7	50	79.37	21	25.16

Table 5 : Shows the frequency & percentage distribution of samples regarding obstacles related to basic necessities where 79.37% population have faced severely difficulties in getting the basic necessities during the pandemic situation, 12.7% of population faced less problems in procuring the basic necessities while 7.9% population have hardly faced any difficulties.

SECTION-III

Table 6: Description of Obstacles related to work in general population

Part-B Obstacles related to work n-63

Item	Not at all related to me		Somewhat related to me		Extremely related to me		Mean	SD
Obstacles related to Work	Freq	%	Freq	%	Freq	%		
	7	11.11	33	52.4	23	36.51	21	13.11

Table 6 : Shows the frequency & percentage distribution of samples regarding obstacles related to work where 36.51% population faced sever obstacles related to work, 52.4% of population faced moderate obstacles while 11.11% population faced less obstacles related to work.

Discussion

This study was aimed to assess the social impact of pandemic disease COVID -19 on general population. The samples were taken from different areas of pune city.

Findings related to the impact on general population.

Impact on family relation.

Table 2 :Data describes the Part A of the impact on family relations. The parameter measured were- Is the relationship good with the family members. Able to spend quality time with the family. Experience any emotional violence, physical violence during this lockdown.

Impact related to social relation and recreation.

Table 3 :Data describes the Part B of the impact related to social relation and recreation.. The parameter measured were- Able to connect to loved ones. Able to play games with family and loved ones. Prefer spending time in reading, writing, watching television, cooking, exercise, yogasana. Missed the daily travelling and outing.

Psychological Impact.

Table 4 :Data describes the Part C of the psychological impact. The parameter measured were- any feelings of low in energy and downhearted during

the lockdown. Anxiousness about the implications of COVID-19 in life. Fear and worry of getting infected and the associated physical suffering. Fear of death. Fear of spreading infection to others.

Findings of the obstacles faced by general population

Obstacles related to basic necessities.

Table 5 :Data describes the Part A of the Obstacles related to basic necessities. The parameter measured are- where procuring daily supplies challenging. Were the basic necessities of food-grains, fruits, vegetables easily available. Able to procure other required materials from the market. Satisfied with whatever things were available. Any anxious to visit ATM amidst this lockdown. Cooking gas easily available during lockdown period.

Obstacles related to work.

Table 6 :Data describes the Part B of the Obstacles related to work. The parameter measured were- whether working from home during lockdown. Whether getting paid adequately during this lockdown period. Faced any financial burden during this lockdown. Whether had to work for long hours. Was any loss of business.

Conclusion: The study result shows that the general population's social relations, recreation were more impacted along with their psychology and faced more obstacles to procure the basic necessities while faced moderate obstacles related to work as everyone had to work from home. It can be concluded that due to lockdown people faced more problems related to social relation, recreation and procuring the necessary materials required, as they were not allowed to make social gatherings.

Conflict of Interest: Nil

Funding: The study was funded by authors.

Ethical Clearance: Ethical approval was taken from Symbiosis College of Nursing's ethical committee. The informed consent was taken from the general population living in different areas of Pune city. Information was given to the participants regarding the data collection procedure. The confidentiality was maintained and the data collected was used only for research purpose.

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Study to Evaluate Level of Job Satisfaction among Health Care Personnel

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Abstract

Health care Industry has been getting growing significance in economy development and overall wellbeing of nation. This segment has government, public owned and private operators working to cater to the needs of the society. Health care and hospitals are considered to be very critical and stress associated functions unlike other work or business. The reasons are obvious the life and death scenario, patients treatment and recovery process, complex instruments, relatives and friends stress , sometimes lack of medicines or treatment and add to it emergency situations. The employees working in this industry are doing noble job and have noble reasons to make them feel special about contribution they give to organisation.

The purpose of the study was to evaluate the level of job satisfaction among the health personnel of Hospital and to describe variables related to their job satisfaction. The opinions of health personnel regarding satisfaction with their jobs were studied using a Job Satisfaction questionnaire. Two levels of analyses were carried out. Job satisfaction item and a four items construct called nature of job satisfaction were selected as dependent variables. Pearson correlations were used to determine whether the independent variables were significantly correlated with job satisfaction.

The key results were that overall job satisfaction was moderately high (2.23 on a scale of 3); however there are differences in satisfaction models between categories of personnel. In addition, there are significant differences in the correlations between job satisfaction and the independent variables among the groups of personnel.

Key Words: *Job Satisfaction, Health Care , Personnel/ Human Resource, Hospitals*

Introduction

Role of Health care personnel is critical to any hospital, society and nation. They struggle to meet their needs and at the same time they are responsible for meeting needs of their patients Hospital managers have responsibilities to both staff and patients. The health care industry will grow multi folds and time to come and will be in need of staff. The industry should be capable of attracting and retaining the staff. WHO report[1] has indicate short fall of health care staff by approximately 40 %.

However, despite importance of human factors in the quality of care and despite great changes in the work environment that is expected to increase stress among hospital staffs there are no systematic efforts to measure staff satisfaction in many of the hospitals. The human resource link is week however many of private sector hospitals are doing remarkable job by having world class human resource practices.[2] Still most of the hospitals are in public sector and hardly any practices or provision of taking care of their own people exists. National Health Mission[2] has also emphasised on raising the satisfaction level of health care workers. This was with directions to improve working conditions, reduction of work load and stress.[3]

The purpose of the study was to evaluate the level of job satisfaction among the staff of hospital and to describe variables related to the job satisfaction of staff. Also study aims establish a system of indicators and reference levels for measurement of job satisfaction as a component of human resources development.

Objective-

· To understand what variables are associated with a certain level of job satisfaction of health care personnel.

· To assess if there is a difference in the level of job satisfaction between the professional groups.

· To demonstrate if the variables of job satisfaction vary by different professional groups.

· To find out satisfaction level in hospital staff (physicians, nurses, Paramedical Staff, Non Medical staff) with respect to their jobs.

Hypothesis-

There is a positive correlation between 6 variables (relationship with superior, knowledge of the work place, work stress, Sense of belongingness, unrealistic expectation and recognition) and job satisfaction.

Research Methodology

A analytical survey was conducted in Hospital where the opinions of staff regarding satisfaction with their jobs were studied using a questionnaire. A quantitative approach with analytical survey design for this study was preferred because questionnaires provide anonymity, is fairly economical, and a large number of staff members can be involved.

Selection of Sample

The survey was conducted on the Staff members of the Hospital. The sample selected was by the method convenience sampling among the 4 groups. Sample size was of 100, 25 from each group

Nurse / Attendants	25
Physician/ Doctors	25
Paramedical Staff (physiotherapist, Nursing assistants, x-ray , laboratory assistant etc.)	25
Non-medical profession (psychologist, patient insurance team , Administration and support staff, clerks, social workers etc.)	25

Data Collection

Primary data was collected through questionnaires.

Job satisfaction questionnaire consists of two sections. The first section includes demographic data (sex, age, profession). The second section included independent variables consisted of constructs measuring relationship with hospital (4 items) relationship with superior (6 items), strategic knowledge of workplace (5 items), work stress (5 items), unrealistic expectations (5 items), recognition (3 items) and sense of belonging (single item), Overall job satisfaction (single item). Job satisfaction is measured using the responses to a single item on the questionnaire using a 5-point likert scale (1-very dissatisfied to 5-very satisfied). The job satisfaction questionnaire developed by the foundation for Health Services Research was used for the study.

Result

Section I : Variables (Relationship with Superiors, Knowledge of Workplace, Work stress, Unrealistic Expectation, Sense of Belongingness, Recognition) with Job Satisfaction

Table.1 Variables and Job Satisfaction

Variables * Job Satisfaction Ranking Crosstabulation

Count

		Job Satisfaction Ranking			
		Low	Medium	High	Total
Variables	Relationship with superiors	30	40	30	100
	Knowledge of workplace	25	53	22	100
	Work Stress	26	38	36	100
	Unrealistic Expectation	9	23	68	100
	Sense of Belongingness	17	42	41	100
	Recognition	42	42	16	100
Total		149	238	213	600

The table **1** shows the fact that the Mean Job satisfaction ranking out of 3 is maximum (2.59) for the variable Unrealistic Expectations, Which means that all the 4 groups rated this variable as least bothering factor and had high Job satisfaction. However on the contrary all the groups rated the Recognition Factor as least satisfactory variable out of all the 6 variables (rating of 1.74/3). That means recognition play crucial role in job satisfaction of health care workers. "There is a positive correlation between Overall Job satisfaction and Variables, (Relationship with superiors, Knowledge of the workplace, Recognition, Sense of Belongingness)"**.**

Section II Relationship with superiors and Professional Groups

Table 2.Ranking of Relationship with superiors and Professional group

Professional Group	Respondents	Mean Ratings (3)	ANOVA (F Value)	P - Value	Correlation value
Nurses	25	2.36	9.95	0	0.253
Physicians	25	1.48			
Paramedical	25	2.36			
Non -Medical	25	1.8			
Total	100	2.0			

Table 2 shows that some groups reported better relationship with their superiors than others. Physicians reported the lowest mean score (1.48/3). Nurses and Paramedical scored 2.39/3 in their relationship with the superiors. Nonmedical staff report it 1.8 close to average. Physicians are usually operating independently and have rarely any one above them keeping in constant touch. However other staff have hierarchy and perhaps they have better connection with the superiors. The P Value is 0 suggesting that the groups have impact on the variable mentioned above (Relationship with superiors). There is 25% positive correlation between the factor and

the variable.

Section III : Knowledge of Workplace for the professional groups

Table 3. Knowledge of workplace for the professional groups

Professional Group	Respondents	Mean Ratings (3)	ANOVA (F Value)	P - Value	Correlation value
Nurses	25	2.08	3.741	0.014	- 0.015
Physicians	25	2.28			
Paramedical	25	1.76			
Non Medical	25	1.76			
Total	100	1.96			

Table shows Physicians reported significantly higher mean scores in knowledge of the hospital goals, plans, budget, results and staffing problems compared with Nurses (2.08), 1.76 for both Paramedical and Non medical group. P Value (0.014) suggests high impact of both the factor and variable. However there is no or poor correlation of the job satisfaction with the variable, Suggesting that high knowledge of workplace cannot guarantee job Satisfaction.

Section IV: Nature of Satisfaction and professional groups

Table 4:Nature of Satisfaction and Professional Groups

Professional Group	Respondents	Mean Ratings (3)	ANOVA (F Value)	P - Value	Correlation value
Nurses	25	2.44	5.07	0.003	0.11
Physicians	25	2.80			
Paramedical	25	2.60			
Non Medical	25	2.24			
Total	100	2.52			

The table above shows that the Nature of Satisfaction is highest among the physicians (2.80/3) and lowest among the Non Medical personnel (2.24/3). P Value indicates that the professional groups have high impact on the nature of satisfaction. However the correlation is very low at 0.11 for all the groups with the Nature of Satisfaction , probably due to low predictability.

Section V : Unrealistic Expectations and Professional groups

Table 5: Unrealistic Expectations and Professional Groups

Professional Group	Respondents	Mean Ratings (3)	ANOVA (F Value)	P - Value	Correlation value
Nurses	25	2.48	0.897	0.414	- 0.19
Physicians	25	2.76			
Paramedical	25	2.60			
Non Medical	25	2.52			
Total	100	2.59			

None of the categories of the hospital staff reported that their superiors, patients/patients` families or colleagues presented unrealistic expectations that was stressful. The 'unrealistic expectations' scores were all relatively lowest for Nurses (2.48) and highest for Physicians (2.76),out of the possible Ranking of 3, with no significant differences between categories. The Job satisfaction does have negative impact on the variable (Unrealistic Expectation), Pearson's Correlation is negative in this case. P Value (.414) also suggests that the Null Hypothesis is proved.

Section VI Recognition at work and professional groups

Table 6: Recognition at work and professional groups

Professional Group	Respondents	Mean Ratings (3)	ANOVA (F Value)	P - Value	Correlation value
Nurses	25	1.76	7.72	0.0	0.006
Physicians	25	2.24			
Paramedical	25	1.56			
Non Medical	25	1.40			
Total	100	1.74			

The Recognition is highest for Physicians with the value of (2.24/3) and lowest for nonmedical staff with a value of (1.4/3). The work of Nonmedical staff is not directly with the client care so they feel left out in the hospital as an organisation. The P Value (0.0) shows that there is significant impact of professional groups with the variable. However there is no high positive correlation (0.006) to conclude that this variable will lead to high satisfaction.

Section VII Sense of Belongingness and professional Groups

Table 7:Sense of Belongingness and professional Groups

Professional Group	Respondents	Mean Ratings (3)	ANOVA (F Value)	P - Value	Correlation value
Nurses	25	2.24	5.625	0.001	0.12
Physicians	25	2.64			
Paramedical	25	2.20			
Non Medical	25	1.84			
Total	100	2.23			

Discussions

Feeling a part of the organization was significantly correlated with satisfaction by all categories of staff. The sense of belongingness is high among the Physicians (2.64/3) and lowest among the Non Medical Staff (1.84). P Value suggests that the 4 groups have high impact on the variable (Sense of Belongingness).

Section VIII : Work Stress and professional groups

Work Stress overall seems to be moderately high with highest stress reported by the Physicians (2.2/3) and lowest level seen among the Non Medical Staff (1.48/3). Nurses reported average (1.76/3) and paramedical (2.16/3). Despite the patient rush and workload the individual coping of stress played a major role among the staff members as the hospital as an organization has neither any means to measure stress nor provides any mitigating solutions. P Value (0.312) suggests that high stress has negative impact on the Job Satisfaction. Correlation is negative (-0.212) suggesting the two factors cannot be correlated. Work Stress is critical component of job satisfaction for health care employees.

Section IX: Gender with Job Satisfaction

Impact of gender on job satisfaction was measured in this study to make out the difference both gender working in health care services have on job satisfaction. In the study the ratio of women were slightly higher than the men 58:42. The score for satisfaction seems to be almost equal for Men (2.21/3) and Women (2.24/3). The P Value(0.82) suggests that there is no significant impact of gender on Job Satisfaction. The correlation has not been established with a negative value of (-0.842).

Section X : Age with Job satisfaction

Correlation of age to job satisfaction was studied and it was found that age is not affecting job satisfaction. The Age group 36-45 years (2.30/3) and 56-65 years (2.8/3) have reported high satisfaction mean ranking. The lowest satisfaction was reported by the age group of 25-35 years(2.12/3). 46-55 have reported (2.18/3). The P Value does not support the impact of age with satisfaction in these age groups. There is poor correlation between the two factors (Age and Job Satisfaction) with value of (0.156)

Section XI: Demography (Professional Groups) And Job Satisfaction

From the analysis it was comprehended that Average rating of Job Satisfaction is 2.23 out of 3 points with low significant difference between the four categories. Furthermore, a significant difference in job satisfaction ratings was revealed between Physicians (2.56 ± 0.57) and the Non Medical staff (2.04 ± 0.79). Nurses had rating of (2.24 ± 0.66) and Paramedical staff (2.08 ± 0.57) P-Value of 0.021 suggests significant impact of Professional group on the factor of Overall Job

Satisfaction. The correlation Value is between (+1 to -1) ie 0.069 suggesting a low level of correlation of these two factors.

The percentage of data collected from the 4 Professional Group was kept equal in order to compare the results, i.e 25% for Professional Groups (Nurses, Physicians, Paramedical and Non Medical) each.

Discussions

Every organisation has different category of staff working with them. The structure of organisation is always such that various levels of people are required to achieve the goals of organisation. Hospitals and health care organisations also have these categories and each has its own role which is critical to the success of hospital and service to patients and their cure. Each category has its own earmarked duties surrounding around patient care and treatment. [4]

Doctors

Doctors and physicians form most critical part of health care organisations. Contrasted to different other positions, doctors have extent of freedom in their work arrangement this involves a further higher extent of duty. With empowerment doctors also need information on monetory situations or the organisation. This helps them plan ideal treatment and care for their patients. New technologies can be adopted for treatment purposes. Insufficient resources available to a doctor may be one of the variables causing disappointment. The empowerment in decision making and information sharing is most important factor for the job satisfaction of doctors and physicians. This information enables them to take decision in favour of patient and also make them aware of compensation that can be taken away by them.

Attendants

Doctors hold authority over analysis and treatment of the patients inspite of that nurses and attendants hold critical position. [6]They provide support for all encompassing consideration, including nutrition, sustenance, physical solace, individual cleanliness, hygiene, preparation of treatment, passionate consideration, adhere to schedule of medicine and social help, persistent effort lies with the medical attendant and, somewhat, with other clinic staff. Besides, different investigations of carried out by researchers on medical attendants have demonstrated that sporadic working hours, frequently including extra time is the principle cause of stress and that exorbitant worry at work might be one of the components causing disappointment or dissatisfaction.

Paramedical Staff

The tasks in the hands of paramedical staff is tiring and huge. The demanding aspect of nature of job impacts the satisfaction levels from work place[7]. Contribution given by nursing collaborators isn't viewed as significant, and isn't given the gratefulness as is expected. When fundamental job stability and pay issues are settled, the most significant reason of satisfaction for workers are associations with colleagues and bosses.

Non Medical Staff

The expert obligations of those working in authoritative and bolster positions, (for example, bookkeepers, secretaries, representatives and tasks faculty) are very not the same as those in direct health care positions. At the point when the managers and support staff has clear objective roles and can elevate these to the staff, the job satisfaction among this set of non-clinical staff increments. Taking care of the requirements of the authoritative and care staff for acknowledgment and settling their work related issues is the most significant errand for the supervisors of those professional who are not directly associated in the provision of direct care to patients.

Conclusions and Recommendation

The degrees of work stress revealed in this study were at moderate levels for all classifications of jobs understudy. Levels of job satisfaction were moderately high. Additionally, unnecessary occupation requests or unreasonable expectations from bosses, patients/ patients' families, and associates were not found to be at high levels.

The study propose that heads of health care organization/ hospitals that are educated about the desires and day by day work related issues of their workers are better ready to comprehend the requirements of their staff. Administrators should: 1) develop successful

connections among supervisors and staff; 2) recognize negative working conditions which influence staff; 3) suitably delegate position to people, and consider them responsible for the work done in their hierarchical units; 4) keep staff educated about changes that will influence them; and 5) express gratefulness and acknowledgment for the endeavors of staff individuals in achieving tasks of health care unit. This will make a great workplace for the health care staff. The outcome of this discussion can be utilized as a lot of reference levels and pointers for the HR improvement part of the quality administration arrangement of Hospital.

Conflict of Interest: NIL

Source of Funding: Self

Ethical Clearance: Institutional Research Committee

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Effectiveness of Selected Yogasanas on Practice of Coping Strategies among Women with Premenopausal Symptoms Residing in Rural area of Pune

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Abstract

Background Study: The premenopause is experienced by 1.5 million women each year and often involves symptoms and it divided in to three categories somatic and vasomotor , psychological , and urogenital sexual symptoms. In the rural area women are not aware about those symptoms and coping strategies to minimize the symptoms by practicing of Yogasanas.

Methodology: In the present study quantitative research approach used and one group pretest post-test Pre-experimental research design was executed. Total sample was 50; they were selected using non probability purposive sampling technique. The setting of the study was rural area of the Pune. The questionnaire and observational checklist was used in this study to collect the data. The independent variables were Premenopausal symptoms while the dependent variables were practice of selected Yogasanas on coping strategies. The group was taught about Yogasanas and explained them how to adopt coping strategies with premenopausal symptoms. The researchers have supervised and follow up was done for 20 days after intervention. In order to establish the reliability of the tool inter rated method was used. The scores were calculated and the reliability was established by Cohen's Kappa 0.86.

Result: In this study paired t-test was used to check the significance between pretest and post test results, and statistical significant result was p-value is less than 0.05, it suggests that women with premenopausal symptoms are minimized after the practice of Yoga asana on coping strategies.

Conclusion: It is concluded that practice of selected Yogasanas on coping strategies are effective and helps to minimize the premenopausal symptoms.

Keywords: *Effectiveness, Practice, Yogasanas, Coping strategies, Premenopausal symptoms.*

Introduction and Background

Women health is differ from men due to unique conditions that are biological, social and behavioral aspects, according to WHO health is defined as " a state of complete physical, mental and social well -being and not merely absence of disease or infirmity" . Women health is an example of group of people living in geographical area. By this women's health has been explained about issues of women health related to their reproductive health (maternal and child), genital health and breast health, endocrine health including menstruation, birth control and menopause. [1]

During the life cycle of women, they go through many reproductive stages, there will be a lot many changes observed in women's life, these changes effect physical and mental health of the women. The

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stages of reproductive life of women are Puberty or menarche; it usually starts from 13-15 year due to fluctuated hormonal influence. In this stage, girl starts menstrual cycle. Second stage of women's life is sexual maturation. In this stage women are able to conceive and produce a new life that is pregnancy. Third stage of reproductive life of a woman is menopausal stage, in this cessation of menstruation starts, women faces the sub categories that are perimenopause, premenopause and post menopause due to hormonal changes, these changes have a huge iimpact on women's physical and mental health. Menopause is defined as a permanent cessation of menstruation at the end of reproductive life due to ovarian follicular inactivity. Before starting the menopause women go through the stages of menopause which is premenopause, perimenopause and post-menopause. Premenopause refers to the period before getting menopause, post menopause refers to period after entering into menopause and perimenopause refers to period around the menopause that is 40-55 years. In premenopausal stage, estrogen and progesterone levels in blood will be decreased. The menopausal symptoms divided in to three phases vasomotor and somatic symptoms include hot flush, discomfort, sleeping problem, joint and muscular discomfort. Second sub aspect of symptoms is psychological symptoms are depressive mood, irritability, anxiety, physical and mental exhaustion. Last one is urogenital sexual symptoms such as sexual problem, bladder problem, dryness of vagina. [2]

In India the premenopause is experienced by 1.5 million women each year and often involves severe symptoms which including vasomotor, vaginal dryness, sleeping disturbance, lethargy[3]

Many studies have stated that, coping strategies help the women to cope up with menopausal symptoms and some of the coping strategies such as practicing yoga minimize the symptoms of premenopause. There are some of the self -coping strategies useful to control the symptoms, in case of hot flushes, sweating, we can advise the woman to sit in a cooler environment, take shower, cold drink, wear sweat absorbing, non-nylon under wear, avoid over sleeping, not having tea or coffee in the evening, have milk yogurt, crying to get relax, etc.[4]

Yoga is Vedic, original and ancient holistic art of living which includes Physical, mental, moral and spiritual spheres. Yogasanas has a great effect in keeping the body and mind peace , which helps to bring an energy and balance the hormonal levels which are fluctuating rapidly in premenopausal period. Yoga calms the nervous system, minimizes stress and aggregation of hot flush. Yoga therapy is weight bearing exercise, strengthening our bones and preventing osteoporosis. Pranayam helps to reduce the hot flush because it minimize the body temperature naturally. [5]

Hormonal levels and which are natural consequences of aging so it is essential to know the coping strategies, alternative therapies and Yoga asana practice with or without medical treatment. [6,7]

Prevalence of women with menopausal symptoms among midlife women with aging of the world wide population in the coming decades, estimated 1.2 billion women by 2030. [8,9]

Based on the review of literature, researcher has decided to execute a research to assess the effectiveness of selected Yogasanas on practice of coping strategies among women with premenopausal symptom residing in rural area of Pune.

Objective of the study

1. To assess the premenopausal symptoms among women.

2. To assess the practice of selected Yogasanas and coping strategies among women with premenopausal symptoms after intervention.

3. To associate the ipractice and coping strategies with the demographic variables

Hypothesis

H0-There is no significant effect between pretest and post practice of Yogasanas and coping strategies on women with premenopausal symptoms

Methodology

Research Approach and research Design

The researchers have used Quantitative research approach and I pre- experimental pretest and posttest

research design was executed in the present study.

Setting: The study was conducted in rural area of Pune district.

Sampling techniques: iA non probability purposive sampling technique was used.

Sample size: 50 women with premenopausal symptoms, who are willing to participate in this study. These participants have informed about the study and its purpose.

Tools of data collection: In the present study 5 sections were used to collect data

Section I: Demographic data:

The first part of the tool consists of 9 items for obtaining information about the selected background factors such as age in years, marital status, education qualification**,** employment status, socio economic status, family income per month, family support, religion, and type of family, the researcher collected the information using interview method.

Section II: iAssess the level of premenopausal symptoms before and after intervention

12 symptoms were assessed by rating scale. The scores were based on the severity of symptoms, such as mild 1-4, moderate 5-8, and severe 9-12

Section III: iCoping strategies

Coping strategies includes self-coping and alternative strategies adopted by the women as per the symptoms. Self-coping strategies are sitting in cooler environment, taking two time shower, sharing problem with loved ones etc which is listed to assess. And alternative strategies are Yoga, exercise, mediation, consuming more vitamin content fruits, use of garlic in food etc

The coping strategies assessed by structured questionnaire. They responded for questions

Section IV: Practice of Yogasanas

Asanas are Anulom- vilom Pranayam, Vajrasanas, Badhakonasana (butterfly), Padmasana, Shavasanas, and Dhyana. Women had practiced of these Asanas every day for 20 days at least 30 min.

The investigator observed the practice on daily bases for 20 days, once a day

Section V: Consists of observational checklist to measure practice of Yogasanas on coping strategies .The practice of Yogasanas was observed by follow up.

Women were selected, who were having premenopausal symptoms and willing to adopt coping strategies and practice of Yogasanas. The group was taught about selected Yogasanas and adopted coping strategies. In order to establish the reliability of the tool inter rated method was used. The score were calculated and the reliability was established by Cohen's Kappa 0.86. i

Results

Table 1: Premenopausal symptom before and after intervention among women in premenopausal period

N=50

Premenopausal symptoms	Pretest		Posttest	
	Frequency	Percentage %	Frequency	Percentage %
Mild (Score 0-4)	16	32.0%	50	100.0%
Moderate (Score 5-8)	34	68.0%	0	0.0%
Severe (Score 9-12)	0	0.0%	0	0.0%

Table 2: Paired t-test for the effect of Yogasanas on Premenopausal symptom

n=50

	Mean	SD	t	Df	p-value
Pretest	5.34	1.24	25.3	49	0.000
Posttest	0.68	0.87			

Table 3: Self-coping strategies before and after intervention

n=50

Self-coping	Pretest		Posttest	
	Freq	%	Freq	%
Good (score 25-36)	0	0%	50	100%
Average (score 13-24)	6	12%	0	0%
Poor (score 0-12)	44	88%	0	0%

Table 4: Alternative coping strategies before and after intervention

N=50

Alternative coping	Pretest		Posttest	
	Frequency	Percentage %	Frequency	Percentage %
Good (score 17-25)	0	0%	50	100%
Average (score 9-16)	3	6%	0	0%
Poor (score 0-8)	47	94%	0	0%

Table 5: Yogasanas Practice before and after intervention

n=50

Practice	Pretest		Posttest	
	Frequency	Percentage %	Frequency	Percentage %
Inadequate (Score 0-19)	49	98%	0	0%
Adequate (Score >19)	1	2%	50	100%

Table 6: Fisher's exact test for association of the premenopausal symptoms among women with the demographic variables

n=50

Demographic variable		Premenopausal symptoms		p-value
		Mild	Moderate	
Age in year	30-35	12	12	0.024
	36-40	3	10	
	41-45	1	12	
Educational qualification	High school certificate	4	11	0.171
	Middle school certificate	3	8	
	Primary school certificate	7	5	
	Illiterate	2	10	
Employment status	Semiskilled worker	5	15	0.538
	Unemployed	11	19	
Socio economic status	Lower middle class	16	25	0.043
	Upper lower class	0	9	
Family income month	Rs. 13495-17999	5	9	0.746
	Rs. 8989-13494	11	25	
Physical and psychological support	Yes	1	3	1.000
	No	15	31	
Religion	Hindu	16	31	0.542
	Muslim	0	3	
Type of family	Joint family	6	3	0.021
	Nuclear family	10	31	

Discussion

In this study pre experimental one group pretest posttest design was adopted to determine the effectiveness of selected Yogasanas on coping strategies among women with premenopausal symptoms.

The mean posttest 0.68 was lesser than the mean pretest score 5.34, computed t-test statistic value is 25.3 with 49 degree of freedom. Corresponding p- value was 0.000 (less than 0.05). it shows that the selected Yogasanas on coping strategies was effective to minimize the premenopausal symptoms. We reject the null hypothesis stating that will be a significant effect of selected Yogasanas on coping strategies to minimize the premenopausal symptoms.

In the present study, majority 34(68%) of the women had moderate premenopausal symptoms (score 5-8), and 16 (32%) of them had mild premenopausal symptoms (Score 0-4). i

In pretest severity of women with premenopausal symptoms had 34(68%) of moderate symptoms, and 16(32%) women had mild symptoms but in posttest showed minimized premenopause symptoms after practicing Yogasanas and coping strategies.

In this study Self coping strategies adopted by women with premenopausal symptoms that in pre-test, 44 (88%) of the women had poor self-coping (score 0-12) and 12% of them had average coping (score 13-24). In post-test, all the women had good self-coping (score 25-36). It indicates that the self-coping strategies of women improved remarkably after the selected yoga asana.

Alternative coping strategies shows that in pre-test, 47 (94%) of the women had poor alternative-coping strategies (score 0-8) and 3 (6%) of them had average alternative coping strategies (score 9-16). But In post-test, all the women had adopted alternative-coping strategies (score 25-36). And they have adopted alternative coping strategies in good manner that indicate that the alternative-coping of women has been improved remarkably after the selected yoga asana.

In current study practice of selected Yogasanas by women in pre-test, none of the women were doing Yogaasana Vajrasanas and Badhakonasana. Only 1 (2%) of them were practicing yoga asana that are anulom-vilom Pranayam, Padmasana, Shavasanas and Dhyana. In post-test, 43 (86%) of them were doing all the yoga asana once a day every day and 7 (14%) of them were doing all the yoga asana once a week.

The above findings of the study are similar to this study. A cross sectional study was conducted to assess menopausal symptoms and coping strategies adopted are exercise, yoga, and relaxation breathing technique, ifocus on diet. The result shown that there is an association between menopausal symptoms and nature of exercise. These findings can be used to plan minimize the premenopausal symptoms by adopting coping strategies. [12]

Another study reveals that prevalence, severity and quality of premenopausal symptoms was assessed and results shown that mental exhaustion (49.5%), joint and muscular discomfort (48.5%) and irritability (41.3%) frequently noticed. And quality evaluated by socioeconomic status, physical activity and body mass index.[13]

A study was done on prevalence and predictor of depressive symptoms assessed in older premenopausal symptoms, they have used CES-D (Center for Epidemiologic Studies Depression Scale) and score was 16. They have discussed those women who have 1-2 children or more that this they don't suffer from depression but women who is widow or nulliparity is more likely risk having depression. In present study researchers have used structured questionnaire method i to assess depressive mood and they have shared problem with their loved ones. [14]

A study conducted on effect of Yoga therapy and periodical physical activities on control of FBS, Glycated hemoglobin and TSH level in women with premenopause. They have divided the sample in two groups, one is experimental (Hatha yoga) used to relieve stress and enhance health and other is control group (physical exercise). iPractice of yoga therapy was given for 45 minutes, daily for 12 weeks. The result shown that the practice of Yoga therapy was significantly ieffective in controlling FBS hto calm and relieve the stress.[15]

A cross sectional study conducted to assess the incidence of intensity of menopausal features and their

impact on value of living style among middle aged women. Study conducted over 8373 women aged 40-50 years. The menopausal rating scale was executed to find the menopausal features such as personal characteristics, joints discomfort and psychological symptoms . 77 % women noted with premenopausal symptoms .[16]

A retrospective study was conducted to assess the climacteric complaints in association with development of menopausal problems. In their study they have found an association between sociodemographic data and premenopausal symptoms. They have checked about the regularity of menstrual cycle, use of HRT and socio demographic data. Results revealed that tiredness, irritability and unhappiness were powerfully related with socioeconomic characteristics. Life events were only occasionally associated with prevalence of the studied complaints. In current study have shown that socioeconomic status is associate with premenopausal symptoms. [17]

Conflict of Interest – Nil

Source of Funding- self

Ethical Clearance – Ethical clearance was obtained from ethical committee of Symbiosis College of nursing.

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Effectiveness of Video-Assisted Teaching Program Regarding Risk Factors of Cardiovascular Diseases on Knowledge, Attitude, and Practice among IT Professionals

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Abstract

Background: Hypertension is one of the primarily responsible factions for causing cardiovascular diseases, significantly, which results in heart diseases such as stroke, myocardial infarction, and finally leads to death.

Objective: To assess the effectiveness of video-assisted intervention regarding risk factors of cardiovascular diseases on knowledge, attitude and practice among IT professionals

Methodology: A quantitative research approach was executed in the current study. The preexperimental, one group pretest posttest design was used in the study. Experts of medical surgical nursing and physician's opinion were obtained for the content validity of the tool and interventions. The effectiveness was evaluated by a self-structured questionnaire for knowledge, attitude, and practice before and after the intervention. Data was compiled and analysis was done by using inferential and descriptive statistics.

Results: In the pretest, no participant had good knowledge, whereas 92 % of the participants had gained well in posttest regarding risk factors of cardiovascular diseases. In the pretest, 88% of participants had a poor attitude and it stooped down to 12 % in posttest regarding CVD. In the pretest, none of the participants had good practices in view to prevent cardiovascular diseases.

Conclusion: The results show that educational interventions with video were significantly effective in refining the awareness, attitude, and practices about risk factors of cardiovascular diseases.

Keywords: *Video-assisted teaching, risk factors of cardiovascular diseases, knowledge, attitude, practice, IT professionals.*

Introduction

Hypertension is one of the most responsible factors for causing a stroke, heart diseases, and other coronary artery diseases. The diseases related to renal system and cerebrovascular system also, caused by hypertension. [1]

In the case of uncontrolled blood pressure, development of the conditions such as encephalopathy, eye complications, acute cardiac problems, aortic dismemberment, and acute renal injuries are common. Worldwide, approximately, 13% of patients are dying with coronary artery diseases. Around 15 % of deaths from stroke, 45 % of the deaths from heart diseases, and 4% of patients become disabled due to hypertension and CVD. Thus the awareness on prevention and control of hypertension and CVD among the population is very important.[3] About 151,377 million people are disabled due to CVD.[4]

The number of cases with CVD raising day by within the past 20 years. India stands in the first position by possessing a high number of CVD cases. Approximately 25 % of cases are affected by myocardial infarction

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(MI) [5]. Men are at high risk at the development of CVD comparing with women.

52 % of men and 28% of women are at risk for heart diseases due to sedentary work. Daily exercise and the physical activities keep the arteries, patent, softens the joints, and helps the individual feel healthy. [6.]

The unhealthy food habits had a great impact on causing coronary artery diseases.[7] Present researcher has mainly focussed to educate the sedentary workers especially working in IT companies about risk factors that may cause coronary artery diseases.

Materials and Methods

The research approach of this was quantitative research. The research design was pre-experimental, one group pretest-posttest. Experts of medical surgical nursing and physician's opinion were obtained for the content validity of the tool and interventions. The tool reliability was 0.8. The sample size was 100 IT professionals who work in IT departments. The 25 subjects were randomly divided into four groups. Each experimental group has received a video-assisted teaching program twice a week for 2 weeks. The effectiveness was evaluated by a self-structured questionnaire for knowledge, attitude, and practice before and after the intervention. Data was compiled and analysis was done by using inferential and descriptive statistics.

Results

Table - 1: Distribution of participants based knowledge in pre-test and post-test

n=100

Knowledge	Pretest		Posttest	
	f	%	f	%
Poor (0-4)	22	22	0.0	0
Average (5-9)	78	78	8	8
Good (10-14)	0	0%	92	92

In the post-test majority of the participants (92%) had gained good knowledge from 0 % to 92%.

Table 2: Overall Mean knowledge score before and after the intervention

n=100

	Mean	SD	t	Df	p-value
Pretest	5.6	1.2	31.9	99	0.000
Posttest	11.1	1.2			

The mean score also improved from 5.6 to 11.1 from pre-test to post-test. Since the p-value represents the chances of rejection of the null hypothesis as observed based on given data, we can be fairly sure that the data supports the alternative hypothesis. In other words, in all three educations, it proves to be efficient in refining the attitude scores of participants, and the improvement is statistically significant.

Table 3: Distribution of participants based attitude in pre-test and post-test

n=100

Score	Pretest		Posttest	
	Freq	%	Freq	%
Poor (score 16-28)	88	88%	0	0%
Average (score 29-40)	12	12%	0	0%
Good (score 41-52)	0	0%	53	53%
Very Good (score 53-64)	0	0%	47	47%

In the post-test majority of the participants (53%) had gained good knowledge from 0 % to 53%.

Fig 1: Overall Mean attitude score before and after the intervention

n=100

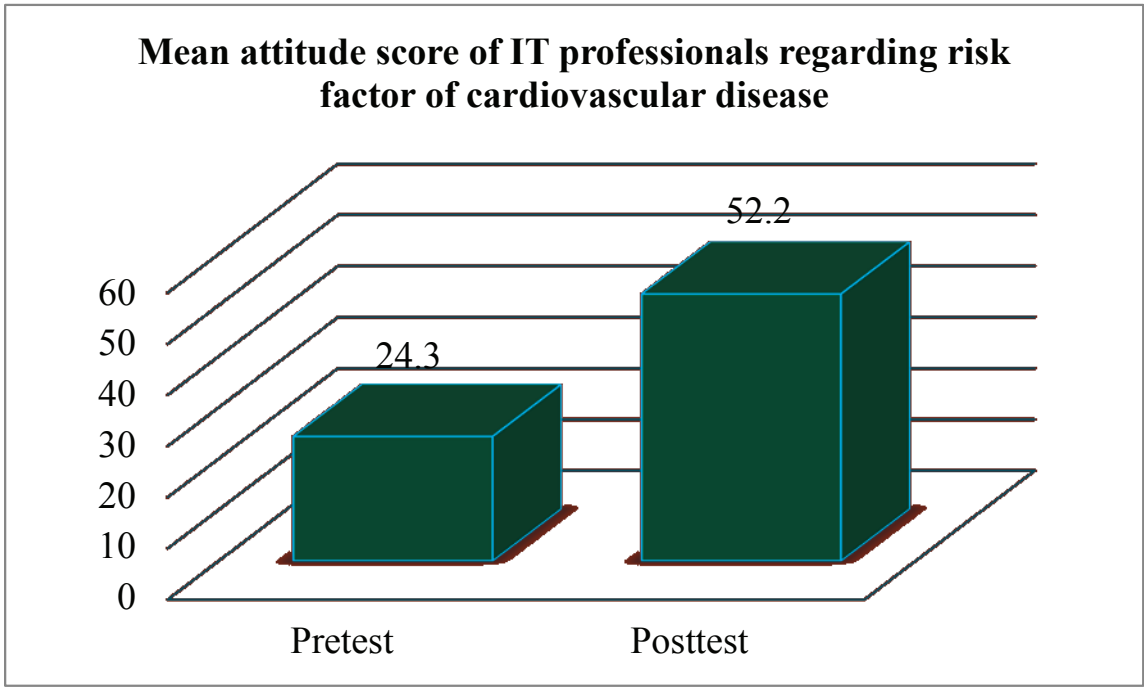


Fig. 2: Distribution of participants based on mean of the practice before and after intervention

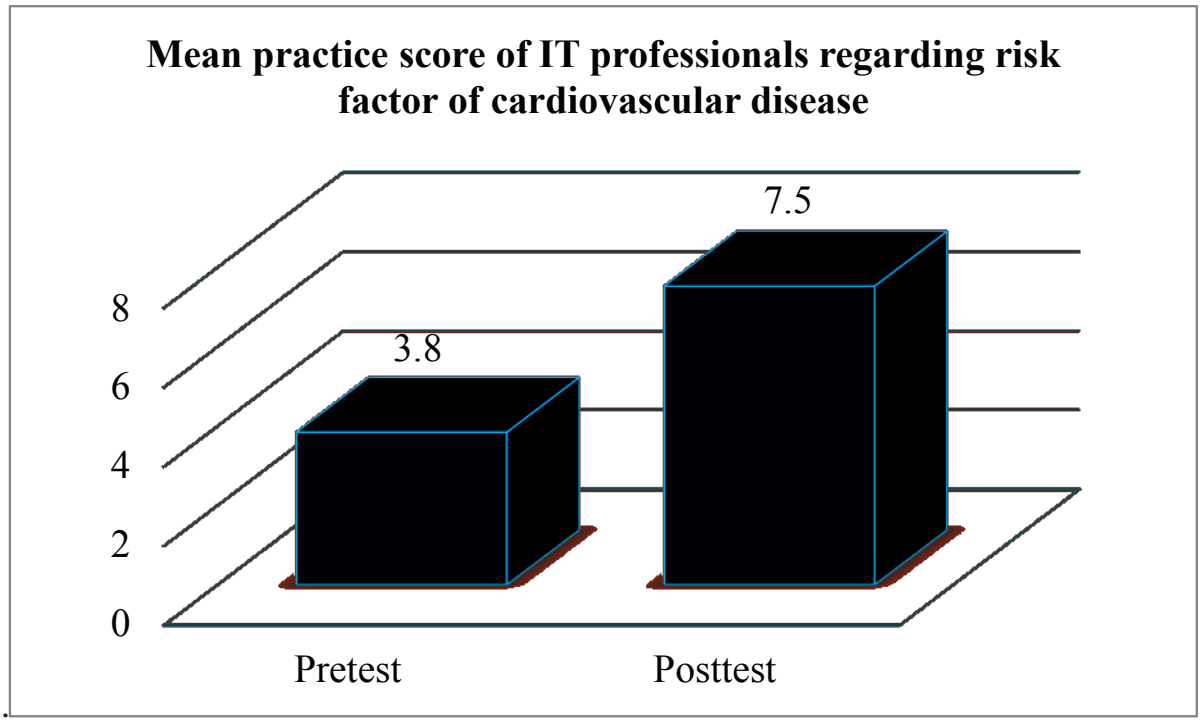


Table 4: Impact of Video Assisted Teaching education regarding risk factors of Cardio Vascular Diseases on practices among IT professionals

n=100

	Mean	SD	T	df	p-value
Pretest	3.8	0.6	35.3	99	0.000
Posttest	7.5	0.9			

The investigator has applied the t-test to check the sigcance of Video Assisted Teaching education The average practice score in the pretest was 3.8 which was improved to 7.5 after intervention. The the result t values was 35.3 at 99 degrees of freedom. The p value is matching to t value was 0.000, which is lesser than less than 0.05. It shows that the educational intervention has improved the practice of IT employees to reduce the risk of CAD.

Table 5: Association of knowledge with personal characteristics of subjects.

n=100

personal characteristics		Awareness		p-value
		Average	Poor	
	41-45 years	14	5	

Cont... **Table 5: Association of knowledge with personal characteristics of subjects.**

n=100

Gender	Male	51	14	1.000
	Female	27	8	
	Never	16	3	
Diet/Nutrition	Sodium rich diet	0	3	0.005
	Fiber-rich diet	2	0	
	Lots of fruits & vegetables	37	5	
	Non- vegetarian	39	14	
BMI	<18.5	3	3	0.023
	>=30	0	1	
	18-5-24.99	59	17	
	25-29.99	16	1	

Discussion

In the present study, the sample size was 100. Video-assisted teaching was given 45 min to each group. The knowledge, attitude, and practice regarding risk factors of CVD were assessed by a self-structured questionnaire. The mean knowledge score by self-structured questionnaires in the group was 5.6 + 11.1 followed by 24.3 +52.2 and 3.8 +7.5 which is significant as $p < 0.05$. So the study is concluded that video-assisted teaching program is effective among IT professionals. This shows that eduvational intervention had good impact in improving knowledge, practice, and attitude regarding risk factors of cardiovascular disease among IT professionals.

Jaana Keto et al executed a study to assess the awareness and who were at high risk of developing cardiovascular diseases. The sample size was 5038, they belonged to the age group between 31 to 46 years ago. The majority of the subjects were at high risk of developing CAD.

Conclusion

Video-assisted teaching, risk factors of cardiovascular diseases, knowledge, attitude, practice, IT professionals.

Ethical Clearance: The Symbiosis College of Nursing, has given ethical clearance for the conduct of this study.

Conflict of Interest: Nil

Source of Funding: Self

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Impact of Transformational Leadership on Patient Safety & Outcome– A Systematic Review

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Abstract

Background: Patient safety in healthcare is a worldwide issue. A lot of studies have depicted that nursing leadership has significant impact on patient safety exclusively in the developed world. Leadership and Management in healthcare profession are very important. A good leader can bring about surprising development in the way patient care is rendered. Nursing leaders' amplified focus on their own roles in healthcare system as well as on nursing conditions could decrease adverse patient outcomes and improve patient safety. This study was conducted to understand the Impact of Transformational Leadership on safety of patients.

Methodology: A systematic review using the (PRISMA) Published Guidelines for Reporting Systematic Reviews and Meta-Analyses guidelines was conducted. 121 research articles were reviewed using the key words. They were thoroughly read and understood for being eligible to be included in the systematic review. Finally after a series of review, 06 articles were analyzed, paraphrased and results were reported.

Results: The studies depicted that there was a very strong relationship between workplace implementation and patient safety. Transformational leadership was positively related to nursing unit organization.

Conclusion: Leadership styles play a very important role in enhancing quality in patient safety.

Keywords: *Transformational leadership styles, Patient Safety, Patient Outcome.*

Introduction

Transformational leadership is a theory of leadership wherein the leader creates an environment that is feasible to work and will improve the output of the committed members of his group. This type of leadership is strongly associated with improved patient care with high-performing teams.[1-2]

Methodology

A review was conducted using the PRISMA guidelines on patient safety through Transformational leadership.[3] This research was conducted by reviewing articles through Pubmed and Web of Science databases from January 2015 to April 2020. The variables in this study consisted of the dependent variable patient safety and the independent variable Transformational leadership style. The online search engine was used in collecting data. Data collected is limited only to English articles and free full-text articles. The search was done in the title and abstract by entering keywords: Transformational leadership, nursing and patient safety. Data analysis using PRISMA Guidelines (Figure 1).[4-5]

Five major inclusion criteria were adopted:

- Peer-reviewed journal
- Papers published from January 2015 to April 2020
- Cross-sectional and Survey designs were included
- Studies on patient safety

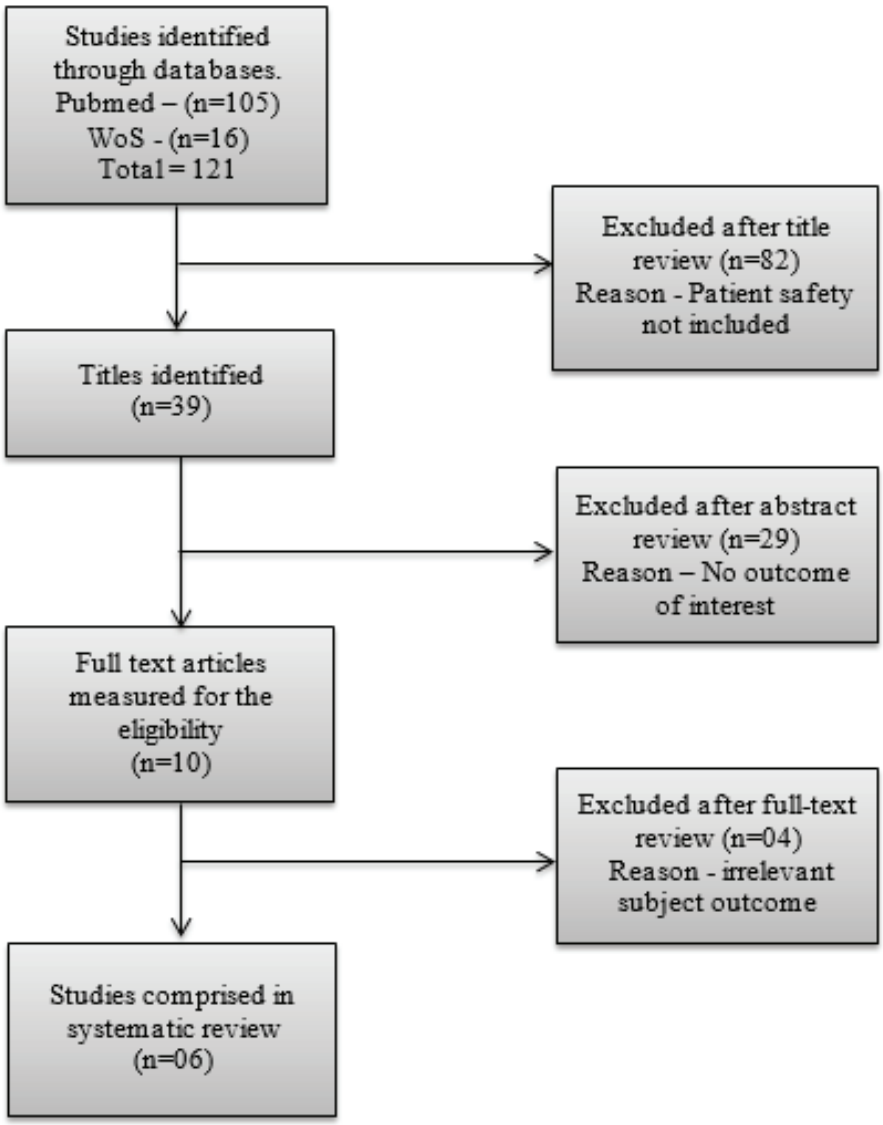


Fig 1: Prisma guidelines

The selected articles were then filtered. The studies with irrelevant titles and unrelated abstracts and study designs like experimental studies and qualitative studies were excluded. All the abstracts and full text articles were read carefully by the authors. At the end, six studies were tabulated based on authors, year of publication, study design, sample size and results. Data were analyzed by using a narrative synthesis of which articles were compared to each other. Then the conclusion was drawn out of the text and discussed critically.

Result

Identification of 121 articles was done by reviewing the title of the articles, and then the reviewed abstracts were reviewed in full text form. Studies were extracted from other irrelevant studies to obtain six studies related to patient safety & outcome and Transformational leadership styles. These six studies were analyzed and reviewed using narrative synthesis with interpretations (Table 1).

Table 1: Systematic Review of impact of transformational leadership on Patient safety and outcome.

SN	Authors/ Years	Study Design	Sample size	Outcome
1.	Ree, E., & Wiig, S. (2020).[6]	Survey	139	Results revealed that the Transformational leadership affects the patient safety in a big way. Patient engagement, perceptions regarding job, demands, and facilities at the workplace influence patient safety predominantly.
2.	Lappalainen, M., Härkänen, M., & Kvist, T. (2019). [7]	Electronic survey	161	Results revealed that there was moderate but statistically significant correlation between Transformational leadership and medication safety and positively affect their perceptions of medication safety.
3.	Liukka, M., Hupli, M., & Turunen, H. (2018).[8]	Survey	11	Patient centered care, skills to reduce undue mishaps, constructive discussion and pro-activeness in patient safety are very important for the nurse managers.
4.	Boamah, S. A., et al (2018) [9]	cross-sectional survey	378	The results revealed that Transformational leadership eventually increased the job satisfaction and gave patients a safe environment.
5.	Merrill, K. C. (2015).[10]	descriptive correlational study	41	Results revealed that Transformational leadership style established a positive association with patient safety climate.
6.	McFadden, K. L., et al (2015). [11]	Survey	204	The results depicted the importance of using quality indicators to efficiently improve the quality and patient safety climate to improve patient safety outcomes.

Based on these six studies, it is clear that, Transformational leadership is associated with behavior of the worker in any healthcare organization.[12] An appropriate practicing atmosphere is utmost essential to improve the patient's care.[13]

Discussion

Studies assessed the impact of leadership styles in healthcare Industry were neglected, while a few studies have adopted qualitative approach. One of the studies potrayed that Transformational leadership style established a positive contributor to patient safety climate.[14-16]

Since the period of review was very small we could get few studies that spoke about adverse effects of particular leadership styles on patient outcome. That never stopped the researcher to explore more studies. Few studies reported that the administrators must look into the need for a change in the Health care administrators need to consider change of policies to make better environment that will build leaders. This not only creates new leaders but also can improve the structure of patient safety gimmicks.

Conclusion

Health care organizations need to provide regular training to develop skills, build capacities & organizational culture. The transformational leadership of a good head nurse will have a 6.2 times chance to implement a good safety environment. It needs an awareness of the head as a first-line nursing manager in improving knowledge and skills of her team supported by the Director of the Hospital and the nursing fraternity through defensible and regular mentoring/in-house

training sessions.

Conflict of Interest - Nil

Source of Finding - Self

Ethical Clearance- Study was approved at Institute Research Committee. Informed concerned from each sample been taken for the study.

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Knowledge and Attitude Towards First Aid Management in School Teachers

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Abstract

First aid comes in the picture because it gives us first and foremost action required to prevent any complication. Compared to all other age group the school going children in a country are exposed to various kinds of health related incidents like, bleeding, Fracture, skin cuts, burn, Heatstroke, etc. which may contribute to higher mortality rate if not treated or not handled initially with appropriate care. Most of these incidents occur when the child is in school, hence it is essential for all the primary school teaches to be trained to handle emergencies in school to prevent further various kinds of life threatening complications. Present study aims to assess the level of knowledge regarding First Aid Management among selected school teachers of Pune City; to assess the Attitude regarding First Aid Management among selected school teachers of Pune City; to find out association between Knowledge score and selected demographic variables and to find out association between Attitude score and selected demographic variables.[1]

Methodology: A Non-Experimental Descriptive Research Approach was conducted among various schools of Pune City. The selection of sample was based on easy geographical accessibility, cooperation, and availability of samples. The sample size for study was 60, selected by using Non Probability Convenience Sampling Technique from various school of Pune City who met the inclusion criteria.

Result: The analysis asserts that 82.66% of the school teachers shows Good knowledge regarding First Aid Management and 13.33% of the school teachers shows average knowledge regarding First Management. About 6.66% of total school teachers were found to have positive attitude towards First aid Management, whereas 88.33% of school teachers shown neutral reaction towards First Aid Management and 5% of the teachers were found to have Negative attitude towards First Aid Management.

Conclusion: Knowledge related to First Aid Management among school teachers is found to be good and Attitude towards the same is Neutral. However the study proposes that First Aid Management training should be given to all the school teachers to make school a safer place for children and prevent all the life threatening complication.

Key words: *Knowledge, Attitude, First Aid, School Teachers*

Introduction and Background

First Aid is the immediate help provided to a victim of any kind of accident. Firs Aid is provided to a victim with the aim to save life, promote good health and to prevent any serious life threatening complication. First aid is the most common thing any layman can perform to prevent further complication with the utmost information procured by any channel. Anyone who is present during the time of accident and who can provide help is a First Aider. But for further medical emergencies required one should need to update themselves to provide exact care for serious casualties. First aid is a complex situation where specific care is required which should be given by well trained professionals so as to preserve lives. Unintentional accidents or injuries contributes the major part of our daily living where most of the population is exposed to health related damage. Besides, school children are at most risk. These children are exposed to

various incidents in their everyday journey to school. Unintentional injuries in playgrounds such as bleeding, head injuries, fracture, etc. which requires sudden attention and curative measure by a trained professionals. Unfortunately it is been ignored all over which results in decreased awareness regarding knowledge towards first aid. School life is vital part of children's life time which directly affects their physical and mental health, thus school health services should be considered as seriously. Children spends most of their time in school under the teacher's supervision. The teachers are the first line and main care giver to school children during the school hours. Thus teachers should be well trained and capable to handle casualties so as to preserve life and promote health until the medical help arrives. Also teacher's attitude towards the same plays an important role. [2]

A cross-sectional descriptive research was conducted at the primary school in Kerbala city in Iraq, during the period from November 2018 to December 2018 which shows out of 200 teachers, About half of primary school teachers' did have low level of knowledge concerning First aid, most of the primary school teachers not participant in any training program about First aid , and difference in gender has effect on their knowledge. Another cross sectional study done to assess knowledge, attitude, and practice about first aid among male school teachers and administrators in Riyadh, Saudi Arabia shows the mean score of knowledge about the first aid of the population was found to be 10.36. It was found that teachers who have good knowledge of first aid (≥15 out of 25 points) are 14.9% (N = 65). Also, it was found that first-aid scores of knowledge showed a significant difference in age and having received first-aid training.[1]

This study aims to assess the knowledge and attitude of school teachers towards first aid of Pune City also to identify the association between knowledge and attitude score towards First Aid management and selected demographic Characteristics. [2]

Objective

1. To assess the level of knowledge regarding First Aid Management among selected school teachers of Pune City.

2. To assess the Attitude regarding First Aid Management among selected school teachers of Pune City

3. To find out the association between Attitude score and selected demographic variables

4. To find out the association between Knowledge score and selected demographic variables.

Methodology

Research Design: The researchers have used A Non-Experimental Descriptive Survey. The study was conducted in the selected Schools of Pune City.

Sampling techniques: Non Probability Purposive sampling technique was used and all teachers from various schools of Pune City were selected

Subject Size: 60 Teachers; willing to participate and working in the schools of Pune city were selected. These participants were clearly informed about the study and its purpose.

Tools of data collection: In the present study the below scales were used to collect the data.

Structured Questionnaire to assess the knowledge and attitude regarding First Aid Management in school teachers from selected schools of Pune City. It has three sections: **Section I:** Consisted of items related to demographic variables of the study. **Section II:** Consisted of 25 questions to assess the teachers knowledge regarding first aid management. **Section III:** Consisted of 10 questions to assess the attitude of school teachers towards first aid management.

Validity and Reliability of tools: Tools were validated by the 5 experts and the reliability was done by Cronbach's Alpha coefficient test. Cronbach's alphas were r=0.86, & 0.76 for assessing the knowledge and attitude with first aid management in teachers from selected schools.

Findings

The analysis and interpretation of the data collected to assess the knowledge and attitude with first aid management in teachers from selected schools was done keeping the objective of the study in consideration. 1. To assess the level of knowledge regarding First Aid Management among selected school teachers of Pune

City. 2. To assess the Attitude regarding First Aid Management among selected school teachers of Pune City. 3. To find out association between Knowledge score and selected demographic variables. 4. To find out association between Attitude score and selected demographic variables. The result was divided in IV Sections

Section – I: Description of Demographic details **Section II:** Description of Knowledge of First Aid Management in school teachers. **Section III:** Description of Attitude towards First Aid Management in school teachers. **Section IV:** Association of demographic variables with Knowledge of First Aid Management in school teachers. **Section V:** Association of demographic variables with Attitude of First Aid Management in school teachers.

Section – I: Description of Demographic details

Describes demographic details related to assessment of knowledge and attitude towards first aid management in school teachers. 50% of teachers belong to age group from19 to 25. 40% of teachers belong to age group of 26-35 and 10% teachers were between the age group of 36-50. Among all the teachers that participated in study 35% were male and 65% were female. About 35% of teachers were found to have 0-2 year of experience, 35% were found to have 3-5 year of experience, 21.66% were found to have 6-9 years of experience and about 8.33% were found to have experience of 10 year and above. Educational qualification of the teachers that were participated in study was 8.33% of Diploma, 80% of Graduates and 11.66% of teachers were post graduates and above. Among all the teachers that participated in the study 75% were trained for First Aid management and 25% did not undergo any training related to First Aid Management.

Section II: Description of Knowledge of First Aid Management in school teachers. N=60

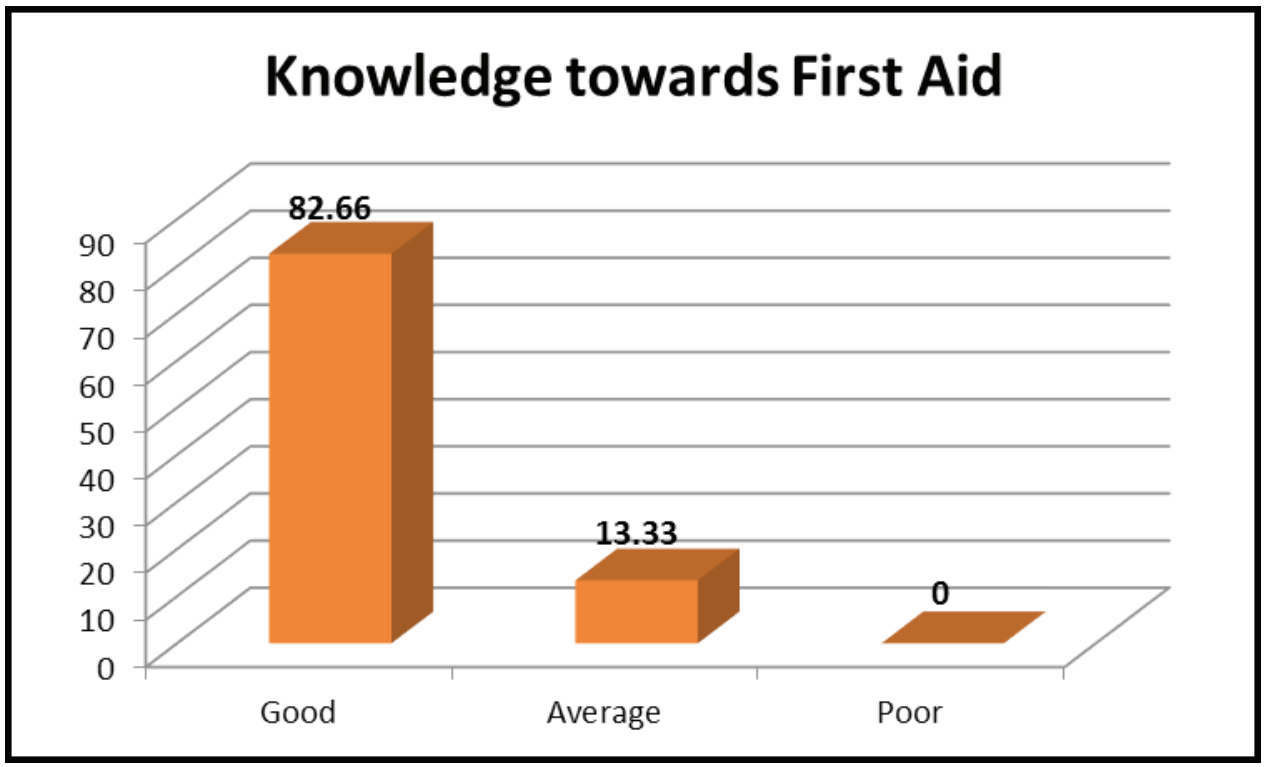


Fig 1 : Describes the data related to knowledge towards first aid management in school teachers. 82.66% of teachers were found to have good knowledge and 13.33% of teachers were found to have average knowledge towards first aid management in school teachers.

Section – III

Description of Attitude towards First Aid Management in school teachers N=60

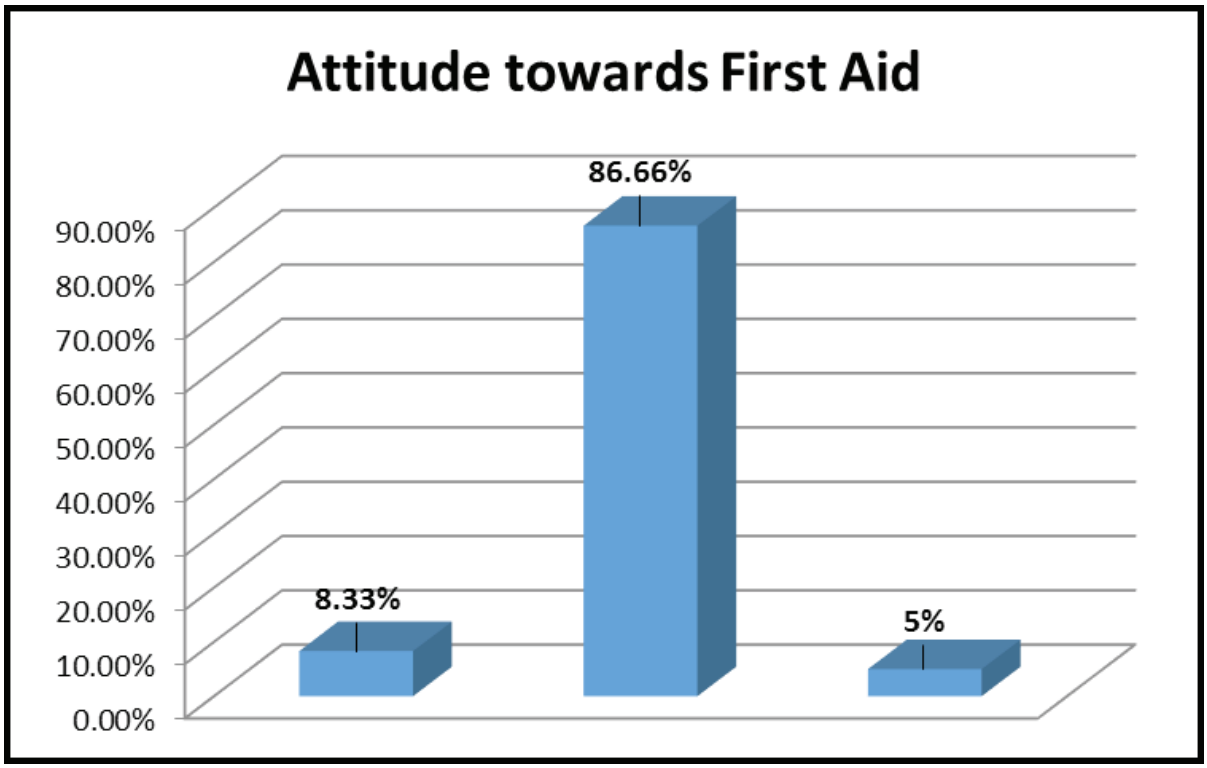


Fig 2: Describes the data related to Attitude towards First Aid Management in school teachers. 8.33% teachers were found to have positive attitude towards first aid, 86.66% were found to be neutral and 5% were shown negative attitude.

Section IV

Association of demographic variables with Knowledge of First Aid Management in school teachers N=60

Table 1: Describes the data about Association of demographic variables with Knowledge of First Aid Management.

Sr.No	Demographic variables	Level of significance
1.	Age * knowledge score	0.643
2	Gender * knowledge score	0.042
3	Experience * knowledge score	0.826
	Education *knowledge score	1
4	Training of first aid* knowledge score	1

Section –V

Association of demographic variables with Attitude of First Aid Management in school teachers.

N=60

Table 2: Describes the data about Association of demographic variables with Attitude score of First Aid Management.

Sr.No	Demographic Variable	Level of significance
1.	Age * attitude score	0.08
2.	Gender * attitude score	0.84
3.	Experience * attitude score	0.006
4.	Education *attitude score	0.501
5.	Training of first aid* knowledge score	0.235

Discussion

This study was aimed to assess the knowledge and attitude towards first aid management in school teachers in selected schools of Pune city. Hence the data was gathered from the participants and analysis was done to find the result.

Findings related to description of Knowledge towards First Aid Management in School Teachers.

Figure 1: Data describes the knowledge of school teachers towards first aid management. The parameters measured to assess the knowledge were: Are the school teachers aware about the basic concepts of first aid, are the school teachers aware about their role and responsibility towards first aid management, specified care for different case situations, how to maintain official records of first aid. The analysis says that the knowledge towards above points were found to be good in 82.66% school teachers and average in 13.33% school teachers.

Findings related to description of Attitude towards First Aid Management in School Teachers.

Figure 2: Data describes the attitude of school teachers towards first aid management. The parameters measured to assess the attitude were: is the child's responsibility to be handled by teachers when the child is in school, there should be a training session for teachers regarding First Aid, first aid is not important for school teachers, there should be a discussion regarding health update during parents meeting, it is un pleasant for me to give first aid, First aid should be given in health care setting, There is no need to maintain a special register regarding the health updates, There should be health check-up in school on regular basis, All the health emergencies should be informed directly to parents, teachers need not to take part in it, First aid is only effective for adults. The analysis says that the attitude towards above points were found to be positive in 8.33% , neutral in 86.66% and negative in 5 % of school teachers.

Findings related to Association of demographic variables with Knowledge regarding First Aid Management in school teachers.

Table 1 represents that since the p-values corresponding to age, experience, education and training of first aid are large (more than 0.05). Hence age, experience, education and training of first aid were found to have no significant association with knowledge regarding first aid management in school teachers. Whereas, the p- value corresponding to gender is less than 0.05 and thus, has significant association with knowledge regarding first aid management in school teachers.

Findings related to Association of demographic variables with Attitude regarding First Aid Management in school teachers.

Table 2 represents that since the p-values corresponding to age, gender, education and training of first aid are large (more than 0.05) except for experience. The p-value for demographic variable experience was found to be less than 0.05 and has significant association with attitude regarding first aid management. Hence age, gender, education and training of first aid were found to have no significant association with attitude.

Conclusion

The overall study result indicates that among all the school teachers participated in study majority of them have requisite knowledge about first aid by procured information and training. Whereas remaining others indicates paucity of knowledge as a result of lack of awareness regarding the same which can be overcome by emphasizing on the importance of first training in teachers. Additionally the overall teacher's attitude towards first was neutral, while some of the negative attitude towards providing medical care at this level can be explained as due to lack of training about health care and first aid management. The current study recommends to establish the mandatory first aid training courses, initially and also periodically for all the school teachers ensuring the safety of children to handle all casualties.

Conflict of Interest: Nil

Source of Funding: Authors have no financial support to this project. The study was fully funded by authors.

Ethical Clearance: Ethical approval of the study taken from Symbiosis College of Nursing ethical committee. Informed consent was taken from the teaching faculties working in various schools of Pune city. Information given to the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential.

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Effectiveness of Education Program on Knowledge Regarding Gestational Diabetes among Nurses

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Abstract

Background: India has got highest number of the cases with gestational diabetes mellitus (GDM), In India, prevalence of GDM is about 16.55%, common among young girls.

Objectives: The main objective of this study was to assess the effect of education programme on gestational diabetes among nurses by assessing the nurse's knowledge before and after educational programme.

Methods and materials: One group pre and pre experimental one group pre and post-test research design was developed and implemented. Setting of the study was municipal corporate and private hospitals of Pune city was randomly selected. The total sample size was 160 nurses, they were selected by using simple random sampling technique. A self-administered structured on knowledge regarding gestational diabetes was developed and administered.

Results: Mean values of pre-test regarding GDM among nurses was 14 and the score was enhanced to 15.4 in post-test. In this study, t-value was 3.1 with 159 degrees of freedom. P-value matching to this evaluation was 0.00, it was less than 0.05. The awareness program on GDM was proved to be meaningfully effective in refining the awareness of nurses regarding gestational diabetes.

Key words: *Education program, knowledge, gestational diabetes*

Introduction

Diabetes mellitus is one of the important metabolic disorder, in this condition blood glucose levels are high from normal values, it happens due to the deficiency of insulin in blood. The complications of diabetes mellitus are enormous, which damage the vital organs such as hear, nerves, eyes, kidneys and blood vessels. [1.2.]

During pregnancy, remarkable metabolic changes occur, especially in fat and carbohydrate metabolisms. Low fasting plasma levels and high postprandial levels are noticed in glucose mentalism during antenatal period. [3]

In India approximately 50.8 million people are living with DM, it is considered to be India is the capital for DM [4]

Gestational diabetes mellitus (GDM) is the branch of Diabetes, cases with GDM consistently high in India. GDM is mainly diagnosed by glucose tolerance test (GTT) in antenatal period..[5] GDM is associated with an increased risk of pre-eclampsia for mothers in the antepartum period. The conditions includes large for gestational age, jaundice, RDS, premature destruction of RBC are common in new-born of mother with GDM. [6]

During post-delivery, the blood glucose levels may come to normal values, however some women might develop type 2 Diabetes Mellitus and the new-born baby of GDM mother is prone to develop metabolic

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syndrome.[6]

The complications related to GDM can retain under control with proper monitoring of blood glucose levels, dietary modifications, exercise, and good insulin regime.

Nursing officers are the front line workers, who regularly meet the antenatal women in antenatal OPDs, and even in community. The have huge opportunity to expand the knowledge on gestational diabetes.[7]

Awareness of gestational diabetes, or diabetes induced by pregnancy among nurses is less than what is necessary. Therefore researcher has taken special interest to impart the education to the staff nurses by conducting this research study.

Materials and Methods

The study was intended to evaluate the outcome of education programme on awareness regarding Gestational diabetes. In this study, researchers have used evaluative research method. Pre experimental research design was applied to for the conduct this study.

Educational programme was independent variable and knowledge of Nurses on Gestational diabetes was dependent variable. Setting of the Study was Municipal corporate hospitals and private hospitals of Pune, Maharashtra. Samples were Nurses of these hospitals. Sample size was 160. These samples were selected by applying sampling random sampling method. A self-administered planned knowledge questionnaire administered and it consisted of 2 divisions. Part I contains of personal characteristics of subjects and part II had knowledge on Gestational Diabetes Mellitus. This training programme conducted in the form of workshops covered the topics of meaning of diabetes and gestational diabetes, pathophysiology, impact of diabetes on pregnancy and fetus, care of pregnant women with GDM, complications of GDM and newborn care of mother with GDM. Pre test was administered after obtaining consent from the participants. Post test also was administered after 7 days of two hours of education on Gestational diabetes.

Results

Table I: Description of demographic variables in terms of frequency and percentage

n=160

Personal characteristics of subjects	Freq	%
Age		
20-25 years	28	17.5%
26-30 years	11	6.9%
31-35 years	16	10.0%
More than 35 years	105	65.6%
Gender		
Male	7	4.4%
Female	153	95.6%
Qualification		
GNM	137	85.6%
BSC/PBBN	21	13.1%

Cont... **Table I: Description of demographic variables in terms of frequency and percentage**

n=160

MSC	2	1.3%
Experience		
Less than 2 years	27	16.9%
2-5 years	19	11.9%
5-10 years	11	6.9%
More than 10 years	103	64.4%
Training attended		
Yes	24	15.0%
No	136	85.0%

Table 2: Pre-test and post-test knowledge scores of nursing officers regarding Gestational Diabetes

n=160

Knowledge	**Pretest**		**Posttest**	
	Freq	**%**	**Freq**	**%**
Poor knowledge (Score 0-8)	62	38.75	10	6.3%
Good knowledge(Score 9-17)	90	56.25	99	61.9%
Very good knowledge (Score 18-25)	8	5	51	31.9%

In pretest, 30.75 % of the nursing officers had poor knowledge, with the scores of 0-8, only 5 % of nursing officers had good knowledge with the scores of 18-25. In post-test 31.9% of the nursing officers had acquired the very good knowledge with the score of 18-25.

Table 3: pre-test and post-test Mean knowledge scores of nursing officers regarding gestational diabetes

n=160

	Mean	**SD**	**T**	**df**	**p-value**
Pretest	12.0	5.2	3.1	159	0.001
Posttest	15.4	4.3			

Researcher has administered paired t-test to check the association between pre-test and post-test knowledge values of nursing officers. In pre-test, mean score was 12.0, it was increased to 15.4 in post-test. t value was 3.1 at 159 degrees of freedom. Matching P value was 0.001, which is smaller than 0.05 that means hypothesis is rejected. It is said to be the awareness programme has a greater impact in refining the knowledge of nurses regarding gestational diabetes.

Table 4: Association between knowledge scores and personal characteristics of nursing officers

n=160

Personal characteristics of subjects		Knowledge			p-value
		Poor	Good	Very good	
Age	20-25 years	10	1	17	0.011
	26-30 years	0	4	7	
	31-35 years	4	2	10	
	More than 35 years	29	14	62	
Gender	Female	40	20	93	0.013
	Male	3	1	3	
Qualification	GNM	36	20	81	0.043
	BSC/PBBN	7	1	13	
	MSC	0	0	2	
Experience	Less than 2 years	6	2	19	0.165
	2-5 years	7	2	10	
	5-10 years	2	2	7	
	More than 10 years	28	15	60	
Training attended	No	34	19	84	0.013
	Yes	9	2	12	

Subsequently p-values matching to personal characteristics includes age, gender, qualification and training attended were showed less than 0.05 that means null hypothesis is rejected. Above mentioned characteristics were found to be significant association between knowledge of nursing officers about gestational diabetes.

Discussion

In current research , the mean knowledge values of pretest and post-test was 12 and 15. The teaching package on gestational diabetes was showed to be meaningfully had impact in enhancing the knowledge of nursing officers regarding gestational diabetes.

A comparable results are identified in study of Upendra Sheela, [8] in which mean knowledge scores of nursing students in pretest test poor (15.5)and it has increased good knowledge (34.3) in post test results. Matching p - value was 0.000 which is lesser < 0.05. The workshop has upgraded the awareness on diabetes care among the nursing students.

Training module on diabetes care for nurses was showed to be suggestively effective in refining the knowledge of nurses concerning to diabetes care[9]

Similar findings were seen in Kaur Sukhpals [10] study where the mean knowledge score of the nurses before starting the workshop was 21.9±3.37 and it improved to 35.5±3.78 at the end of the workshop

The other similar study in Mandelson, [11] in this study researchers have checked the maternal health, control of blood glucose levels, and neonatal outcome after administering educational interventions. In the post test results showed that the educational programme was significantly effective in controlling the blood glues levels.

Conclusion

The educational intervention has showed that the present awareness programme helped in refining the knowledge of staff nurses regarding gestational diabetes. This study also suggests that periodical educational interventions need to be conducted for staff nurses to strengthen their knowledge and skills in taking care of the patients with GDM.

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Conflict of Interest: None

Ethical Clearance- Taken from Symbiosis College of Nursing, SIU, Pune

Source of Funding- Self

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Influencing Reasons for Participation and Nonparticipation of Women in Cervical Screening

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Abstract

Introduction and background: Cervical cancer is one of the leading responsible condition for causing high death rate among women after breast cancer. It is preventable and curable disorder, but the prevalence of cervical cancer remaining too high in India. Majority of women do not aware of cervical screening procedures. Maharashtra is standing in second position in possessing more number of cervical cancer cases.

Methodology: A quasi- experimental pretest and post research design was used to execute the study. This study was conducted in selected hospitals of PCMC. The samples were the women age group between 30-60 years attending Gynec OPD. The systemic random sampling techniques was used to select the samples. Total sample size was 501. A self-administered tool was administered to find the reasons for participation and nonparticipation in cervical screening. Tool and content validity was done. The reliability of the tool was done r value is = 0.88. The study proposal has been approved by the Ethical Committee, MGM Institute of Health Sciences, Kamothe, Navi Mumbai. Permission has been taken from the concerned authority of the selected urban health care centers for data collection. The content was prepared on prevention and early detection of cervical screening in the form of video and modules in their regional languages.

Results: Majority of participants among three experimental groups, about 38.32 % in verbal group, 41.91% in written group and 40.71 % in video group have responded that they could not take part in the cervical screening programme as they are very embarrassed with cervical screening procedure. While others had responded that, I am healthy I do not have to go for this test, some women said, I have only one sexual partner so I don't require this test. A few women have assumed that the test is very expensive, thinking that not required the Pap test as no symptoms of cancer observed and other have stated that don't know the frequency of test.

Conclusion: A key strategy to improve the attendance of the women for cervical screening is to spread the awareness on prevention and early detection of cervical cancer by using various educational methods.

Key Words: *Influencing reasons, participation, non-participation, women, cervical screening*

Introduction and Background

Cervical cancer is one of the extreme cancers among the Indian women, it is one of the leading cancers among the women after the breast cancer. Cervical cancer is noticeably preventable and curable but in some regions terrifyingly very high. Statistics shows that, nearly 90% of deaths due to cervical cancer had occurred in low and middle income nations. The incidence of cervical cancer cases in India accounts for 23 % (1,22,844) in worldwide and India is the second positioned populated country in the world. There is a great burden for developing and middle income nations with high frequency of cervical cancer due to absence of screening services to detect precancerous lesions at an initial stage.[1]

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In India, The proportion of the range is raised up from 15 % to 55 % female cancers from diverse parts of India. About 80% of the women with cervical cancers reported in the clinics in advanced stage of cancer. More than 200 women die every day, eight women die every hour and one woman die every 8 minutes. As per 2012 statistics, after Uttar Pradesh, Maharashtra has shown the increasing trend in the sum of cases with cervical cancer. [2]

Primary prevention of cervical cancer is likely to be achieved by administering two doses of HPV vaccine. It is a quadrivalent vaccine protect the women from HPV 6, 11, 16 and 18 and binovalent vaccine, protect the women from HPV 16 and 18. The action of this vaccine is more efficacious, if it is administered before the sexual exposure, between the age group of 9 -13 years.[3]

Another important strategy of prevention of cervical cancer is secondary preventive approach, it is the systematic screening of women through an organized program along with treatment and follow-up of the abnormal pap test. The national guideline for cervical cancer screening in India advocates screening of women between 30 to 59 years of age.[4]

Though women were given adequate education on prevention of cervical cancer, and importance of cervical screening, majorly women are not convinced for the test. Many times the women who were taken for the particular study, they only take part in the cervical screening, but not others. The women those who have participated also do not follow the next periodical schedule of the screening programme.[5]

The reasons for not participating in the cervical screening have many reasons such as lack of money to reach the private clinics, less pubic clinics available to provide screening at free of cost, difficult in attending various appointments, lack of resources at public health clinics, family members do not allow for the test, lack of time, fear, embarrassment etc.[6]

In previous studies, the present researcher has organized cervical screening programmes, during initial interview, it was revealed that 99 % of the women have never undergone for cervical screening. Researcher has given education on prevention of cervical cancer for those women who have never undergone through Pap test, after giving education also some of the women have not participated in cervical screening. Then the researcher has developed interest to find the reasons for non-attendance in screening. This study results showed the reasons for non-participation of women in cervical screening

Objectives of the Study

1. To find influencing reasons for participation and nonparticipation for cervical screening among three experimental groups.

2. To find the views of women regarding verbal, written and video based health education on prevention and early detection of cervical cancer.

Methodology

Research Design: A quasi- experimental pretest and post research design used to execute the study.

Setting: This study was conducted in selected hospitals of PCMC.

Sample type: The women age group between 30-60 years attending Gynec OPD recruited as a sample. The systemic random sampling techniques was used to select the samples.

Subject Size: Total sample size was 501.

Sampling criteria: Sample includes women who were;

- age group between 30-60 years
- Seeking help for reproductive related health services at the Gynec OPD
- resident of Pune Chinchwad Municipal Corporation
- able to read and understand Marathi /English

Tools of Data Collection

The tool consisted of three sections;

Section 1 contains the reasons for non-participation in cervical screening before attending the health education. This scale included 15 items to find out the reasons why women have not participated in cervical

screening. These 15 statements had two options namely; Yes or No.

Section 2 contains the reasons for non-participation in cervical screening even after attending the health education. This scale included 8 items to find out the reasons why have not participated for cervical screening even after attending the intervention. These 8 statements had two options namely; Yes or No.

Section 3 of the tool is on reasons for participation in cervical screening among three experimental groups after attending health education. This scale included 8 items to find out the reasons why have they participated for cervical screening after attending the health education. These 8 statements had two options namely; Yes or No.

Tool validity and reliability:

Content validity and face validity of tool for conduct of study was done by the experts of tool validation committee of Mahatma Gandhi Memorial Institute of Health Sciences. The content validity of three interventions (verbal, written and video) was done by experts of FOGSI, POGS and PCMC. The reliability of the tool was calculated using value of Cronbach's Alpha (α) method for reasons checklist. It was found reliable that is r = 0.88.

Ethical consideration:

The study proposal has been approved by the Ethical Committee, MGM Institute of Health Sciences, Kamothe, Navi Mumbai. Permission has been taken from the concerned authority of the selected urban health care centers for data collection. Participants were explicated regarding the study and then informed consent was taken before the data collection

Educational Program

The content was prepared on prevention and early detection of cervical screening in the form of videos and modules in their regional languages. Content has covered the definition, mode of transmission of cancer, HPV vaccines and screening tests such as (Pap test, VIA, VILI and HPV DNA.

Statistical Analysis

Descriptive and inferential statistics were applied for the present study. The collected data was arranged in tables, figures, numbers and graphs. Various tests like Mean, Median, frequency distribution, tests were applied.

Results

Table No 1: Responses on reasons for non-participation in cervical screening before educational intervention **n=167 in each group**

Reasons	Verbal		Written		Video	
	f	%	f	%	f	%
Not aware of cervical cancer screening	89	53.29	97	58.08	90	53.89
This test make me worry and fear of exam	52	31.13	54	32.33	53	31.73
Fear of test results.	22	13.17	14	8.38	26	15.56
It is test is too painful.	26	15.56	38	22.75	34	20.35
It is too embarrassing	64	38.32	70	41.91	68	40.71
Male examiner may discourage me	12	7.18	32	19.16	37	22.15
This test is too expensive	52	31.13	63	37.72	55	32.93
I am healthy, so not required the test	71	42.51	70	41.91	74	44.31

Cont... **Table No 1: Responses on reasons for non-participation in cervical screening before educational intervention n=167 in each group**

This test is not important for my age.	32	19.16	32	19.16	36	21.55
I have only one sexual partner	70	41.91	71	42.51	73	43.71
I am not aware of the place of Pap test.	50	29.94	50	29.94	51	30.53
My partner does not allow for test	22	13.17	33	19.76	26	15.56
PAP test results cannot be trusted	16	9.58	8	4.79	13	7.78
I do not know who will perform test	34	20.35	43	25.74	38	22.75
Don't know the frequency of test	75	44.91	71	42.51	73	43.71

In all three study groups majority of women has never participated in the cervical screening in their life as they have no adequate knowledge and their responses to the reasons for non-participation were: absence of information, it was too embarrassing to have a test like this, Pap test may be expensive, I am healthy so I don't require pap test, and do not know how frequently I have has to be screened

Table No 2: Responses on reasons for non-participation even after attending educational intervention among three study groups n=-104

Reasons	**Verbal**		**Written**		**Video**	
	23		64		17	
	f	%	f	%	f	%
I feel Pap smear test is painful.	14	60.86	59	92.18	14	82.35
It is too embarrassing to have a test	20	86.95	61	95.31	2	11.76
I do not need test as I have only one sexual partner	4	17.39	54	84.37	4	23.52
My husband does not like	21	91.30	60	93.75	12	70.58
I don't have any discomfort or pain , healthy	21	91.30	62	96.87	15	88.23
Fear of test results.	8	34.78	9	14.06	6	35.29
I did not find time to go for Pap smear test	4	17.39	4	6.25	15	88.23
I could not come as I go for job in week days	2	8.69	4	6.25	14	82.35
Clinic is far away from my home	21	91.30	60	93.75	14	82.35
Appointment timings are not suitable	3	13.04	8	12.5	1	5.88
Waiting list is too long	1	4.34	2	3.12	0	0
Forgotten screening dates	5	21.73	58	90.62	15	88.23
Did not understand the concept and confused about Pap smear test	2	8.69	51	79.68	3	17.64

Across three experimental groups, a few of the participants had not participated in the screening even after attending the educational programme , their responses for non-participation were, this test is painful, husband did not like for these kind of investigations , the clinic is far away from my home and don't require Pap smear test, as I am healthy.

In written and video based group, majority of participants said that we have forgotten the dates for screening. 88.23% of women from video group said that, they could not participate in the screening as did not find time to go for Pap smear test.

Table No 3: Responses on reasons for participation after educational intervention among three study groups

n=354

Reasons	**Verbal**		**Written**		**Video**	
	n=135		n=89		n=130	
	f	%	f	%	f	%
I realized that this test can find cervical changes before they become cancer	70	78.65	63	70.78	121	93.07
I understood that cervical screening is simple procedure.	72	80.89	53	59.55	128	98.46
I realized that it is too cheap to have a Pap smear test in future.	41	46.06	50	56.17	129	99.23
Educator encouraged me to participate in test	80	89.88	41	46.06	124	95.38
I had no fear and worries about Pap smear test and its results	12	13.48	41	46.06	45	34.61
I did not feel embarrassing after attend the health education	12	13.48	41	46.06	17	13.07
It was clear about the clinic and doctor	10	11.23	20	22.47	124	95.38
My partner and other family members also well understood about importance of test	19	21.34	25	28.08	65	50

Across all three study groups, there were four main reasons and responsible factors, motivated them to participate in cervical screening programme, they are , they have realized that a Pap smear test can detect the pre-cancerous lesions, adequately understood that cervical screening is simple test, realization that it is not too inexpensive to test in future and being educated and examined by a health educator encouraged me to participate in a Pap smear test examination.

Table 4: Views of women regarding educational programmes

View of women	Verbal		Witten		Video		Chi-square
	f	%	f	%	f	%	
Opportunity to express doubts	125	79.11	127	83.01	121	77.07	1.74 P=0.42
Feel comfortable during education	124	78.48	134	87.58	124	78.98	5.39 P=0.067
Like to attend such education programme	136	86.08	130	84.97	130	82.80	0.67 P=0.71
Motivated for further screening	122	77.21	131	85.62	118	75.16	8.03 P=0.018

In all three study groups Majority of participants expressed the views that , they have got an opportunity to express their doubts regarding cervical cancer during education, they were comfortable during education, willing to attend such health education programs in the future and they were motivated for regular screenings in future.

Table 5: Cumulative index of reasons for nonparticipation in cervical screening before health education among three experimental groups: n=501

Reasons	95% CI					
	Verbal		Written		Video	
I am not aware of cervical cancer and its screening procedures	45.74	60.87	50.61	65.58	46.34	61.47
This test make me worry and fear of exam	24.12	38.17	25.25	39.44	24.69	38.8
I don't want to get a Pap smear test because of fear of test results.	8.05	18.32	7.56	17.61	10.08	21.07
It is test is too painful.	10.08	21.07	11.11	22.44	9.57	20.4
It is too embarrassing to have a Pap smear test	45.74	60.87	35.61	50.64	39.15	54.28
Being examined by a male provider would discourage me from getting a Pap smear test.	8.05	18.32	7.56	17.61	7.06	16.91
This test is too expensive	29.23	43.84	30.38	45.09	28.09	42.58
I don't need a Pap smear test, as I don't have any discomfort or pain	46.34	61.47	36.79	51.86	42.13	57.3
This test is not important for my age.	40.93	56.1	39.74	54.89	39.74	54.89

Cont... **Table 5: Cumulative index of reasons for nonparticipation in cervical screening before health education among three experimental groups: n=501**

I do not need a Pap smear test as I have only one sexual partner	25.82	40.07	24.12	38.17	24.69	38.8
I don't know where I must go for Pap smear test.	37.96	53.07	36.79	51.86	35.61	50.64
My partner does not want me to have a Pap smear test	41.53	56.7	39.15	54.28	39.74	54.89
PAP test results cannot be trusted	8.05	18.32	6.57	16.2	6.57	16.2
I do not know who will perform Pap smear test	37.96	53.07	36.79	51.86	36.2	51.25
I do not know how often I need to get a Pap smear screening.	37.96	53.07	36.79	51.86	36.2	51.25

Table 6: Cumulative index of reasons for nonparticipation in cervical screening after health education among three experimental groups:

Verbal: Total No.of participants in screening=**135** Not Participated **=23**

Written: Total No.of participants in screening= **89** Not Participated **-64**

Video: Total No.of participants in screening**: 130** Not Participated **– 17**

Reasons	**95% CI**					
	Verbal		**Written**		**Video**	
I feel Pap smear test is painful.	53.47	68.28	88.13	96.26	76.58	88.15
It is too embarrassing to have a Pap smear test	81.86	92.07	92.12	98.53	6.89	16.66
I do not need a Pap smear test as I have only one sexual partner	11.66	23.15	78.88	89.89	17.1	29.97
My husband does not want me to get a Pap smear test	87.04	95.59	90.08	97.43	63.68	77.51
I don't need a Pap smear test, as I don't have any discomfort or pain , healthy	87.04	95.59	94.25	99.52	83.36	93.13
I don't want to get a Pap smear test because of fear of test results.	27.57	42.02	8.8	19.35	28.06	42.55
I did not find time to go for Pap smear test	11.66	23.15	2.58	9.93	83.36	93.13
I could not come as I go for job in week days	4.43	12.98	2.58	9.93	76.58	88.15
Clinic is far away from my home	87.04	95.59	90.08	97.43	76.58	88.15

Cont... **Table 6: Cumulative index of reasons for nonparticipation in cervical screening after health education among three experimental groups:**

Appointment timings are not suitable	7.95	18.16	7.49	17.52	2.32	9.47
Waiting list is too long	1.26	7.45	0.49	5.78	0	0
Forgotten screening dates	15.49	28	86.22	95.05	83.36	93.13
Did not understand the concept and confused about Pap smear test	4.43	12.98	73.59	85.8	11.87	23.44

Discussion

In current study majority of participants among three experimental groups, about 38.32 % in verbal group, 41.91% in written group and 40.71 % in video group have responded that they could not take part in the cervical screening programme as they are very embarrassed with cervical screening procedure. Similar findings are seen in the study conducted by Augusto EF (2013), results shown that embarrassment was the supreme obstacle to seek care from health care professionals, which was conveyed by all subjects, irrespective of educational qualification. [7]

In the current study, across three experimental groups, the responses of women for not attending the cervical screening as follows; absence of knowledge, embarrassment, assuming that the test is very expensive, thinking that not required the Pap test as no symptoms of cancer observed and don't know the frequency of test. This study is supported by other study conducted by Marie G, results showed that, I feel healthy (55%) , feel discomfort to be confronted with gynecologic examination (29%), fear that they discover something abnormal e.g., cancer (14), embarrassed to show myself naked (12%) and it is too expensive (8%). [8]

In the present study, majority of the participants, had responded that, it is too embarrassing to have the test like this, I am healthy I do not have to go for this test, some women said, I have only one sexual partner so I don't require this test . Similar responses are observed in a study conducted by Laura Conde-Ferráez1 (2012), investigator had interviewed the participants to find the reasons, responses were ignorance about cervical screening, lack of time, recent sexual exposure, embarrassment and scary of test and outcomes. Only 38.9% know regarding cervical cancer and 25% knew about Pap smear [9]

Conclusion:

Cervical cancer has become one of the most important heath issues in the public health. According to the review of literature and results of the current study, awareness regarding cervical cancer amongst the women found was very poor even in the urban community. A key strategy to improve the attendance of the women for cervical screening is to spread the awareness on prevention and early detection of cervical cancer by using various educational methods.

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Transformational Leadership Educational Bundle for Nursing Supervisors and Nurses in-Charges and its Impact on Clinical Performances of the Nurses in the Labour Room

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Abstract

Background: In the process of transformational leadership, leaders and followers have mutual understanding and help each other to promote a higher levels of morale and motivation among them. Employees in the organization take ownership of their roles and responsibilities and perform the work activities out of expectation of the leaders, leaders describe only the goals and objectives of the organization.

Methodology: In this study quantitative research approach was used and one group pretest posttest quasi experimental research design was used. The study conducted in the labour rooms of selected maternity hospitals. The samples were nursing supervisors, nursing in-charges and nursing officers of the maternity wards and participants were chosen by adopting convenient sampling technique. Structured questionnaire was used, one tool consisted of 20 questions regarding knowledge on transformational leadership and another tool comprised of clinical performance evolution check list. Tools were validated by the experts and reliability values includes Cronbach's alphas were r= 0.88, & 0.96 for knowledge transformational leadership and nurses' job performance evaluation respectively.

Results: Majority of the nursing officers were falling under the category of age between 26 to 30 years. Around 62 % of the nursing officers have an experience of 6 to 10 years. Majority of the nursing officers have completed GNM qualification. About 40 % of the nursing supervisors and nursing in charges have completed BSc Nursing qualification. In pretest, majority of supervisors and in charges have poor knowledge regarding transformational leadership while in post test77 % of subjects gained the good knowledge. In posttest majority of the nursing officers were competent in establishing the expected behavior of leaders, maintaining nursing process model, providing quality of the care and adequate information and education to the patients and relatives as per the requirement.

Conclusion: It is concluded that, as transformational leadership is successful in many domains of health care delivery system, and this study results showed that, using transformational is effectives labour room and other maternity wards for successful maternal and newborn outcome.

Keywords*: Transformational Leadership, Educational Bundle , nursing supervisors, nurses in-charges , clinical performances, labour room*

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Introduction and background

Leadership is one of the most essential roles in each and every level of nursing cadres in the hospital. At the same time, effective leadership is required as per the situation demands. Nursing leaders always should remember that, the role of leadership is important to

provide the quality of the nursing care at the bedside in a safe and effective manner by influencing their subordinates. Nursing leaders need to focus to improve the work process on the subordinates as per the institutional and policy-making boards.[1]

Transformational leadership is a leadership style, in which employees take ownership of their roles and responsibilities and perform the work activities out of expectation of the leaders. In this style of leadership, leaders describe only the goals and objectives of the organization. Employees find better ways to achieve to reach the goals instead of executing the work that was assigned from the top. In this leadership style, leaders inspire the nurses to mobilize themselves into the groups, raise morale, and wellbeing to attain common aims of the organization. The characteristics, model of transformational leadership is; each and every employee should frame their own goals based on organizational goals, it is a model for integrity and fairness, the performance of the workers perform work beyond the expectations, employees stand supportive pillar for leadership, leadership is able to stimulate the people's emotions and these leaders always motivate and inspire employees. [2]

Need for transformational leadership in Labour room

Nurses who work in the labour shall have a lot of patience, compassion, and empathy on maternal women as well as on family members. If a nurse is taking care of a primigravida woman who is in the labour, the nurse needs to stay with that woman for almost 14 to 16 hours. During this time nurses need to perform so many roles to keep the women and relatives supported psychologically and physically. Even nurses also require immense support from the women and relatives for the successful outcome of the pregnancy. Nurses have to perform numerous roles in the labour room such as Caregiver: this role includes the activities to support the women physically and mentally while conserving the dignity of the women during labour. Communicator: Nurses have significant role in nursing care such as communication with women and relatives, about the progress of the labour. .Teacher: Nurses need to perform the role of an educator, so that she can give the education on different aspects of the nursing care during the progress of labour. Client advocate: She has to be an advocate, timely progress of the labour need to be informed to her physician. Counselor: This is one of the important roles to counsel the women about family planning methods.

In the labour room, transformational leadership is very useful as it allows the nurses to take ownership in regarding patient care as per the policy and guidelines given by the organization. This leadership style is more applicable in the labour room, as it creates an environment where nursing officers shall provide the care to the parturient women and newborn beyond the expectation of leaders and organizational goals, by devouring higher levels of morale, job satisfaction, and work performance. In other domains, it was proved that transformational leadership has a greater impact in gaining the staff nurses satisfaction towards the work and reduced medication errors, improved relative satisfaction, improved medical records quality and improved the retention of staff nurses.

Therefore present researcher had applied the transformational leadership in the labour room and checked its effect on nursing officer's clinical performance in labour room.

Significance of the Study in the labour room

Nursing supervisors and nurse in charge practice the transformational leadership to empower the nursing officers to establish a good relationship with senior staff, junior staff, patients and their relatives and to meet the organizational objectives and it improves the morale, abilities, and interest of the nursing officers in the labour room. In the labour room, certain protocols and standards are already given by the Govt. of India, in which nursing officers can perform some defined nursing skills, when the patient is in a critical situation or in a condition where nursing care is essential in the labour room. In addition to this, if the transformational leadership is added, nurses are enhanced with self-esteem and motivate them to give their best nursing services in the labour room. Thereby it results into positive clinical outcome by reducing morbidity and mortality rate in the labour room. [3]

Objectives of the Study

1. To assess knowledge of nursing supervisors and nursing in-charges regarding transformational leadership

2. To assess the clinical performance of nursing officers after implementation of education bundle regarding transformational leadership on nursing supervisors and nurses in-charges

Null Hypothesis

1. There will not be significant improvement in the knowledge of nursing supervisors and nurse in charges related to transformational leadership after implementation of educational bundle.

2. There will not be a significant enhancement in the Nurses' clinical performances after implementation of educational bundle

Methods and materials:

Research Design

The researchers have used a quasi-experimental pretest and posttest research design to execute the present study

Setting

The study was accompanied in the labour rooms of designated maternity hospitals.

Sampling techniques: A convenient sampling technique was used and selected all nursing supervisors and in-charges of the maternity wards

Subject Size

Nursing supervisors and in-charges altogether was 26 and nursing officers altogether was 52 who are willing to participate and working in the maternity wards. These participants have informed clearly regarding the study and its purpose.

Tools of data collection: In the present study three scales were used to collect the data

Structured Questionnaire on knowledge regarding transformational Leadership, which was designed by the researchers based on the material observed during review literature. It has two sections;

Section I: Consisted of items related to demographic variables of the participants of the study, such as age, sex, department, qualification, years of experience

Section II: Consisted of 20 questions regarding knowledge on transformational leadership

Knowledge was checked on below four components.



Fig 1: Components of the transformational leadership

Scoring Pattern was followed as below;

The one mark was given for the correct answer, and "zero was given for wrong answer.

Scores were given as below

- Excellent knowledge with scores >70%
- Moderate knowledge with scores between 50-70%
- Good knowledge with scores between 40-50%
- Poor knowledge with scores <40 %

Nursing officers' Clinical Performance Evaluation was assessed by observational check list. It was comprised of two sections;

Section I: Consisted of demographic variables such as age, sex, section, qualification, years of experience, and marital status of the Nursing officers working in the labour room

Section II: Consisted of four components namely,

i. Expected hospital behavior such as respect towards seniors and stakeholder, communication, coordination and comfort

ii. Maintaining Nursing process model ; consisting

of assessment, diagnosis, implementation and evaluation

iii. Providing quality of the care

iv. Education to the patients and family members

Scoring pattern

The answers relating to performance component had arranged in using the 3 point Likerts scale, consisted as follows below.

Choices: Three for competent, two for needs improvement, and one for unacceptable.

Nursing officers are considered to be competent if the scores on this scale is exceed 68% and incompetent otherwise

Validity and Reliability of tools: Tools were validated by the experts and Cronbach's alphas score were r= 0.88, & 0.96 for knowledge transformational leadership and nurses' job performance evaluation respectively.

Ethical Considerations: Nursing supervisors, nursing in-charges, nursing officers informed regarding the study purpose. Official consent was acquired from the hospital

Results

Table - 1: Distribution of nursing supervisors, nursing in-charges and staff nurses based on demographic characteristics

Variables		Nursing Supervisors		Nursing in-charges		Nursing Officers	
		n=8		n=18		52	
		f	%	f	%	f	%
Age in years	<25	0	0	0	0	12	23.08
	26 – 30	0	0	7	38.89	30	57.69
	31- 35	3	37.5	6	33.33	5	9.62
	Above 35	5	62.5	5	27.78	5	9.62
Sex	Male	0	0	3	16.67	12	23.08
	Female	8	100	15	83.33	40	76.92
Qualification,	GNM	7	87.5	13	72.22	44	84.62
	BSc Nursing	1	12.5	5	27.78	8	15.38
	MSc Nursing	0	0	0	0	0	0
Number of years of Experience	<5	0	0	0	0	22	42.31
	6-10 years	0	0	2	11.11	32	61.54
	Above 10	8	100	16	88.89	0	0

Table 2 -: Distribution of Nursing survivors and nursing in-charges based on their knowledge regarding Transformational leadership

n=26

Knowledge score	Pretest		Post test	
	f	%	F	%
0 – 5 (Poor)	16	61.54	1	3.85
6 – 10 (Average)	6	23.08	2	7.69
11 – 15 (Good)	4	15.38	20	76.92
16 – 20 (Excellent)	0	0	3	11.54

Table 3: Transformational Leadership Educational bundle for nursing supervisors and nurses in-charges and it's impact on clinical performances

I. **Expected hospital behavior**

S.No	Parameters	Competent 3	%	Needs improvement 2	%	Unacceptable 1	%
1.	Positive attitude towards seniors, junior staff and patients	44	84.62	4	7.69	4	7.69
2.	Patient satisfaction and comfort	42	80.77	4	7.69	6	11.54
3.	Appropriate communication and coordination with staff and others	40	76.92	6	11.54	6	11.54

II. Maintaining Nursing process model

S.No	Parameters	Competent 3	%	Needs improvement 2	%	Unacceptable 1	%
1.	Assessment	40	76.92	6	11.54	6	11.54
2.	Diagnosis	36	69.23	10	19.23	6	11.54
3.	Planning	36	69.23	10	19.23	6	11.54
4.	Implementation	30	57.69	10	19.23	12	23.08
5.	Evaluation	28	53.85	12	23.08	12	23.08

III. Providing quality of the care

S.No	Parameters	Competent 3	%	Needs improvement 2	%	Unacceptable 1	%
1.	Hand washing before after procedure	44	84.62	6	11.54	2	3.85
2.	PV examination as per the WHO protocol	40	76.92	10	19.23	2	3.85
3.	Use of Sterile techniques in labour room	44	84.62	6	11.54	2	3.85
4.	Maintained partograph	30	57.69	10	19.23	12	23.08
5.	Administered oxytocin	44	84.62	6	11.54	2	3.85
6.	Provided privacy to the patients	40	76.92	10	19.23	2	3.85

IV. Education to the patients and family members

S.No	Parameters	Competent 3	%	Needs improvement 2	%	Unacceptable 1	%
1.	Preparation for childbirth	46	88.46	6	11.54	0	0.00
2.	family planning methods	40	76.92	6	11.54	6	11.54
3.	Postnatal care	40	76.92	6	11.54	6	11.54
4.	Newborn care	39	75.00	7	13.46	6	11.54
5.	Breast-feeding	39	75.00	7	13.46	6	11.54
6.	Postnatal visits	40	76.92	10	19.23	2	3.85
7.	Immunization	46	88.46	6	11.54	0	0.00

Discussion

Transformational leadership is one of the numerous current management classes; in this leadership style, leaders have idealistic behavior by motivating the subordinates to take over the greater ownership in their clinical performance and execute the work in an oganisation better than organissation's expectations. [4]

In the present study the results of posttest showed that, the knowledge of nursing supervisors and nursing in-charges regarding transformational leadership improved. Even the clinical performance of the nursing officers in the labor room improved in the posttest. Same findings found in one of famous journals published regarding effect of educational programme on leadership among head nurses [5]

In the present study, knowledge regarding transformational leadership among Nursing supervisors and in charges, had poor knowledge in pretest and in posttest remarkably knowledge levels increased. In this study four components of leadership styles were used. First one is to find out the expected hospital behavior of the nursing in charges working in labour ward the impact was observed for Positive attitude towards seniors, junior staff and patients in the labour ward, Patient satisfaction and comfort coming for labour process and Also appropriate communication and coordination with staff and others while working in the wards. Results revealed that 84.62 % were competent to practice a positive attitude towards seniors, junior staff and patients. [6]

The second component was on maintaining the nursing process model, results revealed that 76.92 % of nurses were competent to do an assessment of the patient, 69.23 % were able to identify a nursing diagnosis for the mothers in the labour ward, 69 % were able to plan nursing care as per the identified nursing diagnosis, 57.69 % had implemented planned nursing care on their patient to improve the health of the mother and baby. [7]

The third component was on providing quality of care. Providing quality of care in their patients admitted in the labour room is very important to improve the health of their patients which can help to obtain an early discharge if the health of the client is improved early and the client recovers at earliest and also to improve the standards of nursing care provided to patients admitted in labour ward. The nurses were examined for certain parameters that are practiced to improve their quality of care in the labour and to reduce infection among patients. These parameters were hand washing before and after the procedure, PV examination as per the WHO protocol, Use of Sterile techniques in the labour room, Maintained partograph, Administered oxytocin, provided privacy to the patients. It was observed 84.62 % of nurses were competent to perform hand washing have practiced hand washing before and after the nursing procedure, they performed in their patients while delivering care in the labour ward were infection control measures are important to maintain a healthy environment for the mother and baby. Per vaginal examination procedure which is performed in the labour room and is covered under the umbrella of different indications were as contraindications. It is one of the important procedures and comes in essential care during the nursing care of the mother. A nurse working in the labour room should be very well confident and skillful to perform PV examination and also should strictly abide by the WHO protocols for the PV examination procedure. 76.92 nurses were competent to perform PV examination as per WHO protocols and followed all the guidelines to perform the procedure.[7] Similarly, sterile techniques in labour rooms is an important point to be considered by the nurses which will reduce infection as well as will improve the effectiveness of care and procedure.84.62% of the nurses were identified who use sterile techniques in implementing nursing care while caring for their patients to provide them with quality care and recover soon return back home early with good health.11.54 % of the nurses were lacking in the uses of Sterile techniques in the labour room and needed improvement.

The role of a nurse working in the labour room is very important and with that maintaining partograph comes into action. As she has to maintain the Partograph for all her patients to monitor their progress towards successful labour and also to maintain the health of mother and baby to reduce all the complications which can be prevented if the nurse in the labour room maintains the partograph on time and accurately. When the researcher investigated among the sample of this study about the recording of partograph, it was identified that 57.69 % of the nurses were competent to maintain the partograph and 19.23% needed improvement to maintain the partograph. [9]

Oxytocics are the drugs of varying chemical nature that have the power to excite contractions of the uterine muscles. Among a large number of drugs belonging to this group, Oxytocin is an important one and is extensively used in clinical practice. Hence, a nurse working in a labour ward should be completely well versed with the complete knowledge about oxytocin

its uses, indications, contraindications, side effects and appropriate administration process. Knowledge about the oxytocin administration in the nurses working in the labour room is tested and the results say that 84.62 % of the nurses were competent in administrating oxytocin with an appropriate method to their patients and 11.54% of the nurses needed improvement in the method of oxytocin administration to their patients.

The fourth component is education to the patients and family members. Educating patients is the process by which health professionals impart information to patients about the care and all-important what they should know about their health and medical conditions also their caregivers that will alter their health behaviors or improve their health status. The health education helps patients make informed decisions about their future care. Results reveal that 88.4 % of them had gained an education in preparation for childbirth. This study results in education on family planning methods among the patients and their family member revel that 76.9 % had complete education on family planning and 11.54 % of them needed improvement in their education on family planning.

Newborn care is a part of postnatal care to be given to the mother and baby after delivery. Mother and Family members should be well educated about the essential newborn care and complication to be prevented. The study result on the education of patients and family members on newborn care says that 75% of them had education on Newborn care and 13.46 % of them needed improvement in their education on Newborn care.

Education on Breastfeeding, Postnatal visits, and Immunization should be given to all mothers before their discharge from the hospital by the nurses which will help them to keep track of their future visits, understand the importance of Breastfeeding for their newborn and Advantages of timely immunization to be given. This study results say that 75 % of them had an education on breastfeeding and 13.46 % needed improvement in their education about Breastfeeding. Also results say that 76.9 % of them had education on Postnatal visits and 19.23 % needed improvement in their education about postnatal visits. The study results also say that 88.4 % had complete education of immunization and 11.54 % needed improvement in the education about Immunization.

Conclusion

It is concluded that, as transformational leadership is successful in many domains of health care delivery system, and this study results showed that, using transformational is effectives labour room and other maternity wards for successful maternal and newborn outcome.

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Effectiveness of Structured Teaching Programme on the Level of Knowledge about Psychological First Aid among Psychiatric Nurses Working in Selected Psychiatric Nursing Homes in Pune City

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Abstract

Introduction: Psychological first aid (PFA) is an important procedure which is designed and practiced with an intention to reduce the incidence of disorders related to stress. The use of STP was effective to enhance the level of knowledge regarding the use of psychological first aid, thereby helped in improving the quality of life among people. **Method:** Research approach used for the study is Quantitative research and the research design used is Pre-experimental research design. 60 samples were selected for the study using Probability sampling method. Pre-test was administered to subjects, followed by structured teaching Programme through AV aid on psychological first aid was given, followed by post-test, which was carried after two weeks. **Result: Findings** revealed in Pre-test, average knowledge score was 12.66 with standard deviation of 1.78 whereas in post-test average knowledge score was 19.99 with standard deviation of 3.26. This indicates that the structure-teaching programme was effective to enhance the level of knowledge about the psychological first aid among the psychiatric Nurses. **Conclusion:** The present study enhanced the knowledge on the psychological first aid in psychiatric Nurses. The study found that majority of Nurses have average knowledge towards psychological first aid

Keywords: *Effectiveness; Structured Teaching Program; Knowledge; Psychological First Aid; Psychiatric Nurses*

Introduction

Psychological first aid (PFA) is a skill aimed to decrease the stress due to any of the crisis event happening[1]. Psychological first Aid was established by the National Centre for Post-Traumatic Stress Disorder and was first announced theoretically in the mid-Twentieth Century. Psychological first aid appeared as the important factor after the 9/11 incidence, it was of very much help for the survivors of Manmade disasters and life-threatening events[2]. PFA is an evidence based integrated method to help families, societies and communities in the post disaster and terrorism events. Psychological First Aid intends to lessen the initial suffering caused by disturbing events and to adapt to the ongoing situation and helps in coping[3].

Psychological First aid Training helps to increase the knowledge to deliver the needed psychological first aid, and promotes confidence for providing PFA[4]. As a result of few reviews, researchers have suggested that medical colleges, nursing colleges, and other healthcare programmes related to direct care of patients should implement PFA training in their core curricula. It was also discussed that Healthcare organization's management and higherups should promote the application of PFA in hospitals, work settings with both colleagues and patients. PFA training should be promoted same as the training for CPR as it is of similar importance and healthcare organizations needs to focus on this aspect too[5].

The current study aims to, assess the effectiveness of STP on the knowledge level regarding psychological first aid among psychiatric nurses working in selected

psychiatric nursing homes in Pune city.

Hypothesis

Ho: There will be no significant change in the knowledge of the Psychiatric Nurses regarding psychological first aid.

Materials and Methods

For the current study research approach adopted was Quantitative approach. The research design used was Pre experimental research design. 60 samples were selected for the study using non-probability purpose sampling technique.

Tool for the study has sections as follows:

Section I – demographic baseline data. Section II - Structured questionnaire with thirty items. Permission to conduct the main study was taken from the organization's authority. Data for the study was gathered from Feb 05, 2019 to Feb 26, 2019. Data was collected after taking consent from the participants. Pre-test was conducted on the first day followed by a structured teaching programme on psychological first aid again followed by post-test.

Results

Section I Demographic Data Analysis:

An overall of 60 participants were selected from Psychiatric Nursing Homes located in Pune, Maharashtra. Questionnaires were circulated to all the participants. Out of the 60 participants 32.5% of samples falls in Age in 21-25 years. 35.5 % of samples falls in an age 26-30. 10.5% falls in 31-35 years and 21.5% of samples falls above 35 years age group.

Section II Pre interventional Knowledge among the Psychiatric nurses regarding psychological first aid.

As per the data obtained during pre-test 72.5%, subjects had poor knowledge and 18.5% subjects had average knowledge and 9% had scored good knowledge level about psychological first aid.

Table.1 representing the Pre-test Knowledge

N = 60

	Good	Average	Poor
Frequency	5	11	44
Percentage	9%	18.5	72.5

Section III - Effectiveness of structured teaching program on knowledge about PFA among psychiatric Nurses.

Paired t-test was applied to analyse the effect of STP on knowledge about Psychological First Aid. Mean score during pre-test was 18.5 which has increased to 27.5 during the post-test. 21.31 was the T-value with 39 degree of freedom. 2.730 was the corresponding p-value which was small, therefore the Alternative hypothesis was accepted. The results of the study reveal that the STP on Psychological First Aid has a significant positive effect on the knowledge level of the psychiatric Nurses.

Discussion

The objective of the study was assessing the effectiveness of STP on the knowledge level regarding psychological first aid among psychiatric nurses working in selected psychiatric nursing homes in Pune city.

The study showed that during pre-test 72.5%, subjects had poor knowledge and 18.5% subjects had average knowledge and 9% had scored good knowledge level about psychological first aid. Similar study was conducted by (Jong-Sun Lee, Sungeun You etal.) which shows that the PFA training significantly enhanced students' knowledge and perceived skill in PFA skill[6].

Conclusion

As per the findings of the research study it was found that approximately 80% Psychiatric nurses were positively affected by the training on Psychological First Aid. So, to conclude, this study that more trainings on psychological first aid should be conducted for all the healthcare professionals. It was felt that more in-depth understanding of Psychological First Aid can be provided and the benefits of the approach could be demonstrated to the Nurses in India.

Ethical Clearance: Clearance obtained from the Institutional Research Committee.

Conflict of Interest: None

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Effectiveness of antenatal IEC Package On Knowledge and Attitude of Antenatal Mother's Spouses

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Abstract

Background: In a family, a spouse is the most important and liable family member to take care of the family members. He is the one who might take care of his wife in pregnancy period. Therefore, he must be knowledgeable regarding antenatal care of his wife in pregnancy, but in India, alas! There is no much involvement of husband in taking care of an antenatal women during pregnancy. Moreover, pregnant woman is a care taker of a family and they are majorly neglected, it is happening since decades due to lack of respect for a daughter-in –law in family. **OBJECTIVES:** 1. to evaluate the knowledge of pregnant woman's spouse about antenatal care. 2. To assess the attitude of antenatal mother's spouse regarding Antenatal care. 3. To assess effectiveness of antenatal care on IEC package the knowledge and attitude among the spouse of antenatal mother. **Methodology:** In this study researchers have adopted pre experimental one group Pre- test post-test design. It evaluates and assess the effectiveness of knowledge and attitude of antenatal care IEC package among antenatal mother's spouses. The researchers also described the association among level of knowledge as well as attitude using personal characteristics included in this study was age, religion, education, family income and no of children. The sample size is 100. Non probability purposive sample technique is used in present study. **RESULT:** In this section the mainconclusions of the present study have been discuss as follows. The posttest knowledge score showed at higher level than the pretest score. The mean post test score was greater than the mean pretest score. Thecalculated t-test statistic value and Corresponding p-value are small and the posttest attitude score was greater than the mean pretest score. **Conclusion:** It indicates that the IEC package was effective method for enlightening the knowledge and attitude of mother's spouses about antenatal care.

Keywords*: Antenatal IEC package, effectiveness, knowledge and attitude, spouses of antenatal mother.*

Introduction

The WHO has taken a keen interest and delivered recommendations to increase excellence of antenatal care and to decrease the complications in pregnancies. They focused on safe deliveries and provided provision for extensive antenatal care.[1,2]

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Care during pregnancy for primi mothers is challenging task for mother and even for her family members.[3] The women one who is in pregnancy, high for many obstetrical complications. These complication can be prevented by applicable care and treatment during pregnancy by identifying the symptoms at an early stages of pregnancy. Nevertheless, the objective of antenatal care is to provide structured education to women and their family members about prenatal care, which includes early antenatal registration, regular antenatal visits, antenatal diet, antenatal physical activities and identification of warning sign of pregnancy. [5] There are some of the pregnancy related complications such as anaemia, preeclampsia and eclampsia, breeched presentation, and some other infections can threaten

the life of the mother as well as the foetus. Effective interventions are required to prevent and treat such complications.[6]

Husband is a key person in the family and he is the responsible figure to take of his children, wife and other family members. As per Indian culture, husband is always there to protect and safeguard the family. There are many studies revealed that, husband who took care of woman during pregnancy, prevented complications during pregnancy and maternal mortality rate was also less. [7] The maternal mortality ratio is a measure of the risk of death a woman faces every time shebecomes pregnant.[7]Norms value tradition, these are the essential factors in Indian culture. Though for women. Reproductive and child health also important factor. Males are not involved much more in it, but though they have more authority in mother and child related decision. In household pattern female has limited roles taking care of the family and rearing children. where each and every ANC mother and New-born obtains excellent care during the prenatal period, during childbirth and the postnatalperiod. Reproductive health care, pregnancy care (ANC) deliverssignificant health-care functions, with health promotion, screening and analysis, and illness prevention. It documented that by executing appropriate evidence-based practices, ANC can save lives. [8]

Problem Statement:

"A study to assess effectiveness of antenatal care IEC package on the knowledge and attitude of antenatal mother's spouse attending the selected hospital of Pune city."

Objectives:

1. To evaluate the awareness of antenatal mother's spouse regarding Antenatal care.

2. To assess the attitude of antenatal mother's spouse regarding Antenatal care.

3. To assess effectiveness of antenatal care on IEC package the knowledge and attitude among the spouse of antenatal mother.

Materials and Methods

In this study researchers have adopted pre experimental one group Pre- test post-test design. Knowledge and practice regarding antenatal care is taken as dependent variables. Antenatal care on IEC package as independent variables. This study was conducted in ANC clinics of selected hospitals in Pune city. In this study the target population was spouses of antenatal mother. 100 women were selected by using non probability test. Antenatal mother's spouses who attended, willing to take part in data collection included in this study. Thus a questionnaire is constructed to assess the knowledge and attitude of antenatal mother spouses. The questionnaire consisted of three sections , part I consisted of demographic variables such as age, religion, , education, no.of children , family income, part II consisted of a self-structured questionnaire to assess the knowledge on IEC package and part III consisted of a self-structured questionnaire to assess the attitude on IEC package. Tool validity and reliability was done. . In order to establish the reliability of the tool inter rated method was used. The score were calculated and the reliability was established by Cohen's Kappa 0.86. i

Results

Table 1: Distribution of participants based on age , educational qualification , occupation, income and religion:

Table 1 : Distribution of participants based on type of the family and other obstetrical information :

S.No	Demographic Variables	f	%
1	Types of family		
a	Joint family	56	56
b	Nuclear family	44	44

Table 1 : Distribution of participants based on type of the family and other obstetrical information :

2	Gestational age		
a	1-3 month	14	14
b	4-6month	46	46
c	7-9month	40	40
3	Initiation of ANC visit		
a	1st trimester	63	63
b	2nd trimester	17	17
c	3rd trimester	20	20
4	Number of antenatal visits		
a	0-1	33	33
b	2-4	38	38
c	More than 4	29	29
5	Number of children		
a	< 1	33	33
b	>1	25	25
c	one child	42	42
6	Is the present pregnancy planned ?		
a	Yes	40	40
b	No	60	60

Table 2: Distribution of participants based on knowledge regarding antenatal care

n=100

Level of Knowledge	Pre test		Post Test	
	f	%	f	%
Good Knowledge (11-15)	16	16	78	78
Average Knowledge (6-10)	61	61	19	19
Poor Knowledge (1-5)	23	23	3	3
Mean	8.04		12.07	
SD	2.96		2.79	

Table 3: Distribution of participants based on attitude regarding antenatal care n=100

Attitude	Pre Test		Post Test	
	f	%	f	%
Positive Attitude (34-75)	31	31	71	71
Negative Attitude (1-33)	69	69	29	29
Mean	28.49		33.36	
SD	9.09		6.04	

Table 3: Effectiveness of IEC regarding knowledge on antenatal care among antenatal mother's spouses.

n=100

Level of Knowledge	Pre test	Post Test	t-value	p-value	Remark
Mean	8.04	12.07	9.621305	<0.00001	significant
SD	2.96	2.79			

Table 4: Effectiveness of IEC regarding attitude on antenatal care among antenatal mother's spouse n=100

Level of Attitude	Pre test	Post Test	t-value	p-value	Remark
Mean	28.49	33.36	4.559696	0.00001	significant
SD	9.09	6.04			

Disccusion

In pre-test majority 61(61%) of the spouses had average knowledge, and 23(23%) had poor knowledge regarding antenatal care. Whereas in post-test, majority 78 (78%) mother's spouse had good knowledge, while 19(19%) had average knowledge regarding antenatal care. A similar results found in a study conducted by Barun Bhai Eram U, et al [9], in pre-test 63% had good knowledge , 32(31%) had positive and 69(69%)of husbands had negative attitude regarding antenatal care. Where as in post-test majority 71(71%) has positive attitude and 78 % of husbands had good knowledge about the antenatal care. In another study also similar outcome was shown, Mean knowledge regarding antenatal care has increased from 33.36% to 42 %, this study was conducted by Shora T et al [10]. In the current study, in pretest mean values attitude has increased from 28.49 to 33.36 among the spouses.

The mean post-test knowledge score 12±2 of mothers was significantly higher than their pre-test

knowledge score 8±3. The calculated t-test statistic value is 9.6, matching p-value was 0.000, which is small (less than 0.05), and the null hypothesis is rejected, it shows that the IEC was effective method for improving the knowledge of antenatal care among mother's spouse.

Conclusion

Majority of the mother's spouse had average knowledge and attitude score before the administration of the IEC package. After administering IEC package it was showed improved the scores . Statistical findings showed that the IEC package was effective in improving the knowledge and attitude of the mother spouse's regarding antenatal care.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Ethical clearance was obtained from ethical committee of Symbiosis College of Nursing.

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Smartphone Usage and Its Addiction among Undergraduate Nursing Students

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Abstract

Introduction: The mobile phone is becoming an integral part of students concerning managing the critical situation and maintaining a social relationship. This behavior may reduce thinking capabilities, affect Cognitive function. The sciences of Smartphone addiction are constantly checking the phone for no reason, feeling anxious, or restless without the phone, waking up in the middle of the night to check the mobile and communication update, delay in academic performance as a result of prolonged phone activities and distracted with smartphone applications.

Aim: Most of the global population, especially college and university students uses a Smartphone. Due to its wide range of applications, while beneficial in numerous ways, Smartphone has disadvantages such as a reduction in academic performance, psychological addiction.

Materials and Methods: In the present study, a Quantitative Research approach is used. The sample comprised of 100 students, consisting of both male and female students. The convenient sample technique was used to collect the data from Students. Students were requested to fill a specially designed, self-Administered, questionnaire which comprised the Socio-demographic characteristics, Frequency and pattern of mobile-phone usage, questions related to physical behavior, psycho-social behavior. Regarding their health, they were asked whether they felt eye pain, thumb pain, a backache, neck pain and tiredness, and total time spent on a mobile phone.

Results: 73% of the students aged 18-21 years, 23% of them had age 22-25 Years and 4% of them had age 26-29 years.98% of them had prepaid SIM card, and 2% of them had postpaid SIM.97% of them had the android, and 3% of them had IOS. 96% of them responded, that excessive use of the mobile phone can cause harm to health. All of them play PUBG on their Phone. 61% of them were using the mobile up for to 6 hours, 31% of them were using the mobile for 7-12 hours, 2% of them were using the mobile for 13-18 hours and 6% of them were using mobile for 19-24 hours. Majority of 79% of students used both internet and talk-time plan, 17% of them had internet plan and 4% of them use only the talk-time plan. The majority of 77% of them feel that the use of a mobile phone affects the students in their academic performances. 38% of them use a mobile-phone while lecture going in the class.48% of them often feel that your phone is vibrating, even when it is not.96% of them responded that excessive use of the mobile phone can cause harm to health.11% of them had pain in the thumb. 9% of them had a backache after long time use of the mobile phone, 35% of them have a headache and 56% of them had pain in eyes after having used the mobile phone for a long time.

Conclusion: Addiction of Smartphone among students has seen that its effect on academic performance and 77% of them feel that the use of the mobile phone affects physical behavior.

***Keywords**: The smartphone, addiction, nursing, student.*

Introduction and background

"The telephone has become the young adult's transitional object, replacing the toddler's teddy for comfort and a way of belonging." Now a day's people use the mobile not only for talking, especially chatting, but also use for browsing the internet or taking a picture, shopping and net-banking. The ministry of gender

equality and family implemented research on the actual condition of media usage rate among teenagers and reported that the middle schools 86.2% of students, high School 84.9% Students are Smartphone users.[1] In this study, Smartphone use has adversely affected learning in the classroom, has affected driving safety, and has negatively affected the Academic Performance of the students. Over usages of the mobile phone may cause psychological illness such as dry eyes, computer vision syndrome, weakness of thumb and wrist, neck pain and rigidity, increased harmful radiation, nomophobia, insecurity, delusion, auditory sleep disturbance, insomnia, hallucinations, lower self-confidence and mobile addiction disorder. The mobile phone is becoming an integral part of students concerning managing critical situations and maintaining the social relationship. This behavior may reduce thinking capabilities, affect cognitive function. [1]

Objectives:

1. To assess the addiction of Smartphone among student.

2. To assess the effect of addiction of mobile phone on the psycho-social and physical behavior of the students.

Methodology

The quantitative approach is the structure of the study to provide a framework that supports the study and holds it together.[2] The present study were aimed to assess Smartphone usage and its addiction among students. This approach was considered to be the most suitable one to conduct the study because the samples were not aware of the ill effects of excessive use of mobile phones on their health. Students were requested to fill a specially designed, self-administered questionnaire which comprised the social demographic characteristics, frequency and pattern of mobile-phone usage,[3] Questions related to physical behavior, psycho-social behavior. The population is the entire set of individuals who meet the sampling criteria.[4] A group of students using Smartphone are addicted, among students. The sample comprised of 100 students, consisting of both male and female students.

Section I

Description of samples (UG students) based on their personal characteristics

Table 1: Description of samples (UG students) based on their personal Characteristics in terms of frequency and percentages

n = 100

Demographic variable	f	%
Duration of use of smartphone		
Upto 6 hours	61	61%
7-12 hours	31	31%
13-18 hours	2	2%
19-24 hours	6	6%
Recharge plan in INR		
199	10	10%
299	34	34%
399	37	37%
499	3	3%
500	1	1%
599	9	9%
2000	6	6%
SIM card		
Prepaid	98	98%
Postpaid	2	2%

Section II

Analysis of data related to the addiction of Smartphone among student

Table 2: Addiction of Smartphone among student

n =100

Item	**f**	**%**
How often do you recharge your phone		
Every month	63	63%
Weekly	1	1%
Quarterly	31	31%
Yearly	5	5%
Which internet recharge do you prefer		
1.5GB/ day	76	76%
3GB / day	19	19%
500 MB/ day	4	4%
Only talk time	1	1%
What is the primary purpose for using internet on your mobile phone		
How much time do you spend on your mobile phone on an average in a day		
30 min-1 hour	3	3%
1-3 hours	5	5%
More than 3 hours	97	97%

Section III

Analysis of Psycho-social effect of addiction of mobile phone

Table 3: Psycho- social effect of addiction of mobile phone

n =100

Psycho-social Item	**f**	**%**
Do you think Mobile Phones are the best companion for youngsters		
Yes	62	62%
No	38	38%
Did you ever use mobile phone while lecture was going on in the class		
Yes	38	38%
No	63	63%
Do you often feel that your phone is vibrating, even when it is not		
Yes	48	48%
No	53	53%

Section IV

Physical behavior effect of addiction of mobile phone

Table 4 : Physical effect of addiction of mobile phone

n =100

Physical behavior item	f	%
How do you feel when your mobile internet connection stops working		
I wait for some time	38	38%
Throw my phone in anger	2	2%
Get irritated	28	28%
Check& recheck my phone	36	36%
How much of time do you keep mobile phone near your body without using it		
1 hour	21	21%
2 hours	10	10%
3-5 hours	25	25%
More than 5 hours	44	44%

Results

The sample comprised of 100 students, consisting of both male and female students. Only UG students were selected. 73% of the students had age 18-21 years, 23% of them had age 22-25 Years and 4% of them had age 26-29 years. The study depicted70% of UG students expressed that they are addicted to the Smartphone's in their day-to-day life.65% of students expressed that they are psycho-socially addicted to Smartphones even while spending time with their family and friends.85% of UG students expressed that they are addicted to the Smartphone's in their day to day life which is affecting their physical behavior.

Majority (77%) of them felt that use of mobile phone affects the students in their academic performances.96% of them responded that excessive use of mobile phone can cause harm to health. 61% of them were using mobile for up to 6 hours, 31% of them were using mobile for 7-12 hours, 2% of them were using mobile for 13-18 hours and 6% of them were using mobile for 19-24 hours. 48% of them often feel that your phone is vibrating, even when it is not.11% of them had pain in thumb, 9% of them had backache after long time use of the mobile phone, 35% of them have a headache and 56% of them had pain in eyes after having used the mobile phone for a long time. 23% of them responded that the use of mobile phones may lead to Migraine, 13% of them responded that use of mobile phones may lead to neck pain, 75% of them responded it may lead to vision problems in later life.

Discussion

The main purpose of the study is to assess the Addiction of smartphones among students and to assess the effect of addiction on mobile Phones on Psycho-social and physical behavior of the student. The age group of the data was collected with the help of a questionnaire which was having certain scoring for a particular section. Questions related to physical behavior and psycho-social behavior included their health accessibility stress, anxiety, or whether they have ever thrown their phone because of Internet issues. Regarding their health, they were asked whether they felt eye pain, thumb pain, backache, neck pain and tiredness, and total time spent on mobile phones.

A similar study was conducted by Arpita Kumari et al (2013) on "Severity of mobile phone and internet use among 114 B.Sc. Nursing students. Results revealed that 43.9% of the students belonged to 19-20 years of age. 1.8% subjects reported of having mild addiction to smartphone use. 2.6% of subjects had moderate internet addiction and 35 (30.7%) subjects reported of getting mild internet and mobile addiction. The 'r' value ($r=0.610$, $p=\leq 0.001$) showed that there's a positive relationship between use.[5]

A study was conducted by Shailesh Rai et al 2014 on the Effect of excessive use of smartphones among 300 professional college-going students Indore India. Results the maximum students used smartphones around 4 hours daily the most use if smartphones for communication and social networking. They felt uncomfortable without their smartphone, got irritated by frequent notification, and got angry when someone interrupts them while using smartphones. Finally, most of the students felt that overuse of smartphones effect like, dry eyes and headache disturbance in sleep. Also they spend excess money on mobile recharge.[6]

According to a study conducted on 187 students, one-fourths (25.1%) of the nursing students had a moderate level of mobile dependency as compared to mild (64.7%) mobile dependent. And no one was found in severe level of mobile dependency.[7]

A study conducted by Seong-soo cha and Bo-Kyung Seo. (2018)" a study of Smartphone use and Smartphone addiction in middle school students in Korea Results depicted that according to Smartphone addiction proneness scale scores 563(30.9%) were as a risk group for addiction, 1261 (69.1%) were normal user group. Final results the adolescents used mobile messenger long time followed by internet surfing, gaming, social networking use.[8]

A similar study was conducted by Lubna Ahmed Soomro, Shabana Tunio 2004 "Impact of usage of the cellular phone on study habits of 100 students and their psychosocial development Sindh University. Results revealed that the majority of students spend more time on the cell phone which have an effect on their psychosocial development.[9] Student keep their smartphones switched on for 24 hours, it was also significantly tested true that the students who spend more time using cell phone were more vulnerable to Psychological disturbance attachment towards the cell phones which is caused the student to experience symptoms such as anxiety, depression and lack of sleep which affected their overall psychological function.

Conclusion

The study results suggests that majority no. of students had android phone and 3% IOS, use both internet and talk time plan. They used their mobile phone more than 3 hours. All of them feel that use of mobile phone affects on their academic performance. It's also affects on the psycho-social behaviors and physical behavior such as thumb pain, backache, headache, eyes pain, it also find that most of them keep mobile phone near their body more than 5 hours. One fourth of participant responded for migraine, neck pain, and most of them responded for vision problem.

Recommendation:

1. A similar study can be done using a large sample size.

2. A study can be conducted using qualitative approach.

Conflict of Interest –Nil

Source of Finding -Self

Ethical Clearance – ethical clearance was obtained from ethical committee of Symbiosis College of nursing.

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A Study to Assess the Usage of Personal Protective Equipment among Health Care Providers Working in Covid-19 Wards in Pune City, Maharashtra

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Abstract

Background: The Protective equipment consists of garments placed to protect the health care workers or any other persons to get infected. All personal protective equipment should be safely designed and constructed, and should be maintained in a clean and reliable fashion. It should fit comfortably, encouraging worker use. If the personal protective equipment does not fit properly, it can make the difference between being safely covered or dangerously exposed. Personal protective equipment (PPE) limits the health care workers contact with all secretions or biological products. This study was planned to find gaps between use of PPE among the health care providers (HCPs) in Pune city.

Methods: A cross sectional design was used to assess the usage of PPE among health care workers selected by purposive sampling. The Data were collected by pretested structured questionnaire. Data analysis was done using SPSS 20 version at 5% of level of significance.

Results: 60 Health care workers were participated in study. Among them, there were 38.9% doctors, 51.9% nurses and 9.2% were technicians. 18.1% health care workers who work outside the ICU had appropriate uses of PPE. It was high among doctors 31.5% followed by nurses 9.3% and technicians 8.2%. which was statistically significant p=0.001.

Conclusions: The study showed inappropriate use and poor usage and practice of PPE among HCWs.

Keywords: *Personal protective equipment, Health care providers, Infection control*

Introduction

Personal protective equipment is designed to protect HCWs from serious workplace injuries or illnesses.[1] PPE provides a barrier between microorganism like Bactria, virus etc. and health care workers. It offers protection by preventing microorganism from contaminating hands, eyes, clothing, hair and shoes.[2] A violation in infection control practices increases the chances of transmission of infection from patients to health care workers.

Nosocomial infections spread by direct-contact can be avoided by adapting standard precautions guidelines. Proper use of PPE is the easiest way to prevent contact from secretions and transfer of pathogens. It's needed to assess the level of contour with use of PPE by the various HCWs who make direct contact with client, Hence this study was conducted.

PPE includes gloves, protective eye wear, mask, apron, gown, shoe cover & hair cover. PPE should be used by all health care providers and family members who provide care to patients in hospitals.[3]

The disclosure of life-threatening infections such as severe acute respiratory syndrome and re-emerging infectious diseases had spotlighted the urge for efficient infection control programs in all health care settings[3] and research into standard precautions has been adopted out in many countries.[4,5]

Methodology

Research approach: Quantitative approach

Research design: One shot case study (cross sectional design)

Sampling technique: Convenience sampling

Sample size: 50

Data collection method: Pretested structured questionnaire

Setting: Various hospitals of Pune city

Analysis: SPSS 20v and summarized by descriptive statistics.

Results

The health care providers included in the study was 50. Among them, there were 38.9% doctors,

51.9% nurses and 98 9.2% technicians.

Among 50 health care providers who worked outside the Critical area, appropriate use of PPE among were only 18.1%.

Table 1: Appropriate PPE use among health care providers outside OT and ICU.

Health care providers	Appropriate use n (%)	Inappropriate use n (%)	Total
Doctors	31.5	68.5	12
Nurses	9.3	90.7	30
Technicians	8.2	91.8	8

Table 1 describes that; appropriate use of PPE was high among the doctors 31.5% followed by nurses

9.3% and technicians 8.2% which was statistically significant ($\chi 2=56.82$, $p=0.001$).

Table 2: Appropriate PPE use among health care providers inside OT and ICU.

PPE	CCU (n=22) (%)	ICU (n=8) (%)
Gloves	100	100
Mask	100	95.6
Goggles	7.3	4.4
Apron	100	62.3
Gown	100	44.3
Hair cover	100	57.9
Shoe cover	8.3	27.3

Table 2 shows that Health care providers working in CCU and ICU were 22 and 8 respectively. Among the HCPs working in CCU, appropriate use of gloves, mask, apron, gown and hair cover was 100%. But the use of goggles and shoe cover was very low. This was attributed to the fact that goggles and shoe cover was not available.

While doing procedures in Intensive care unit, use of gloves was 100%, mask was 95.6%. but the use of other PPE was very less to the extent that the use of goggles 4.4%, apron 62.3%, gown 44.3%, hair cover 57.9% and shoe cover 27.3%. This was attributed to the fact that the unavailability of PPE and unaware of the importance of goggles.

Fig 1 shows then various reasons given by the health care providers for use of PPE, around 78% Health care workers mentioned the PPE are not Available in their hospitals. 11% of Health care workers were not aware of PPE followed by only 4% of Health care workers did not use PPE because they did not expect any spill in ICU, which makes them more prone to get infection. 2% Health care workers said they only use PPE in case of HIV and Hepatitis B patients.

Discussion

Akbar Ziauddin et al, showed in their study that the knowledge and attitude towards PPE, among the health care workers were 75% to 97% only. But, in practice, it is observed that only up to 80% responded positively [6]

Jeong et al The goggles use was 5% % in this study which was comparable with the study done by 2% and much lower than the study done by Kermode (32%) and Wilson (43.5%).[6,8,9]

The reason for inaccurate use of PPE was non availability of PPE 88% which was similar to the other studies. But the study done by Sandeep et al described that the reasons were busy schedule, non-use of PPE by co- workers, emergencies, risk that patients may get offended and discomfort while using PPE.

Conclusion

The present study showed accurate use of PPE was very low among health care workers specially nurses.

A violation in infection control practices increases the chances of transmission of infection from patients to health care workers. Steps should be taken to ensure adequate availability and strict infection control guidelines should be followed to improve the same.

Funding: Self-funding

Conflict of Interest: There is no conflict of interest in the study

Ethical Approval: The study was approved by the Institutional Ethics Sub-Committee

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A Study to Assess the Menstrual Hygiene Practices among the Women of Rural Community of Pune City

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Abstract

Background and objective: Poor menstrual hygiene is most common etiology in women for gynecological problems especially in rural area where they cannot afford sanitary napkins or they don't use it because of beliefs in myths and taboo. **Methods:** A Quantitative Approach: Non-Experimental descriptive design was adopted to assess the knowledge on menstrual hygiene practices and demographic variables, which included 100 samples from selected areas. Non-probability convenience sampling technique was adopted in this study. The final draft of the tool consisted of 05 items on structured questionnaire to collect demographic data and 10 items on self-administered practice questionnaire. After obtaining the consent form, the data was collected from 100 women using structured questionnaire, which was prepared in Marathi language. **Result:** During assessment of knowledge on practice the findings were 58% of the study participants avoid religious things, 88% do not avoid going to school/college/office during menstruation, 44% women do not avoid any events or family functions, 85% women use disposal sanitary pads and 10% use old wash cloth, 39% women change their napkins or cloth twice a day, 44% change their panties during menstruation more than twice a day, 56% dispose used napkins in a paper wrap, 84% dispose used napkins or cloth in dustbin or PNC waste collection and 13% burn it , 36% wash their perineum 3 to 4 times and 34% washes 1 to 2 times in day, 38% use soap and water to wash genital area, 36% use antiseptic and 24% use only water, **Conclusion :** Majority of the women have not attended the awareness programme in spite of that still majority of the women maintain good menstrual hygiene practice, it can be possible that they get information through various sources. It is found that women in rural area still avoid the religious things during menstrual period.

Key words: *Women, menstrual hygiene practices, rural community*

Introduction

It is been observed that majority of girls has less knowledge on menstruation till their first experience as topic of menstruation is that is not often discuss at homes [1].

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The study findings says that there is poor knowledge and poor personal sanitary practices during menstruation followed by girls which is associated with genital tract infections, urinary tract infections, and bad odor[2]

Puberty and menarche, or the onset of menstruation, provide a window of opportunity to not only teach young adolescent girls and boys about the changes occurring in their body[3]

Consequence of greater attention to menstrual hygiene management (MHM) over the past few years in India [4]

Materials and Methods: The present study is a Non-Experimental descriptive research design, which was adopted to assess the menstrual hygiene practices

among women of rural community in Pune city. The target population comprises of women staying in selected rural areas. Sample consisted of women in selected at rural areas of Pune City and sample size was 100 women resides in Susgaon, Pune. Non-probability convenience sampling technique was adopted. A structured questionnaire schedule was developed to assess the menstrual hygiene practices among the women of rural community. Tool had two sections. Section 1: -included demographic variable and section II was self-administered practice questionnaire. Tool was given to eight experts from the department of Nursing for validity. Reliability of tool was 0.8996.

After obtaining the written consent from samples tool were administered to participants.

Findings

SECTION I: Distribution of the samples based on the demographic variables in terms of frequency and percentage

Table no. 1 Demographic variables

n=100

Demographic variable		Frequency	Percentage
Age (Years)	11-17	10	10
	17.1-23	23	23
	23.1-29	39	39
	29.1 & above	28	28
Age of Menarche (In years)	8 – 10	5	5
	10.1 - 13	34	34
	13.1 – 16	49	49
	After 16	12	12
Employment status	Self Employed	18	18
	Unemployed	7	7
	House maker	61	61
	Any other	14	14
Family type	Joint Family	52	52
	Nuclear Family	32	32
	Extended Family	14	14
	Single Parent	2	2
Attended any awareness programmed?	Yes	25	25
	No	75	75

Section II : Analysis and Interpretation of menstrual Hygiene practice data

Table no. 2 : Analysis and Interpretation of data collected from the women on menstrual hygiene practice.

Self-administered practice variables		Frequency (f)	Percentage (%)
Restriction's/ during menstruation?	Avoid certain food	0	0
	Avoid religious things	58	58
	Avoid housework	7	7
	Any other (specify)…	1	1
	None	34	34
Avoid going to school/ college/ office?	Yes	12	12
	No	88	88
Avoiding of any events/family function during menstruation period?	Yes	44	44
	No	66	66
Absorbent used during your menstruation?	Disposal sanitary pads	85	85
	New clothes	4	4
	Old washed clothes	10	10
	Any other (specify) …	1	1
Frequently change in napkins/cloth in a day?	Once	26	26
	Twice	39	39
	After every time you go to toilet	20	20
	As and when require	15	15
How many times in a day women wash the perineum during menstruation?	1 – 2 times	34	34
	3 – 4 times	36	36
	5 – 6 times	21	21
	More than 6 times	9	9

For Changing of panties during menstruation in a day? Majority of respondenst (44%) preferred for twice and more than two times . For How women disposed of the used napkins? Majority of respondents (56%) answered chose for use of paper for wrapping selected. For where women dispose the used napkins/cloth? Majority of respondents

preferred use of dustbin/PMC waste collection (84%) For how many times in a day women wash the perineum during menstruation? Majority of respondents (36%) selected 3-4 times in a day. For what women prefer to wash their genital area with during menstruation? Majority of respondents(38%) selected Soap and water

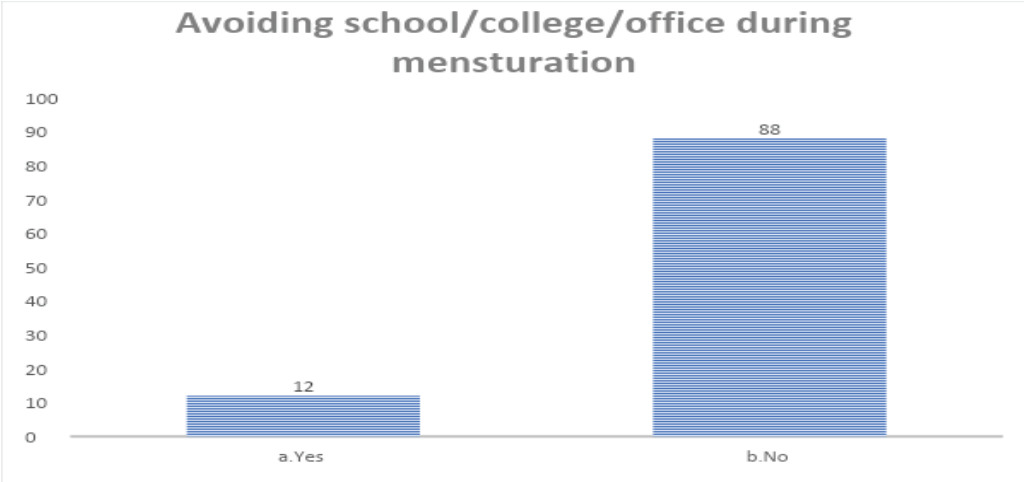


Fig. 1 Bar diagram shows the avoiding of going to school/ office/ college during menstruation.

Discussion

The present study reveals that out of 100 study participants 14% (14 out of 100) use cloth as absorbent during menses, out of that 15% use soap for washing cloth having period stain.

5% use antiseptic, 1% use water**.** Study of Deshpande selected cross-sectional study, which was conducted in urban slum area. Seventy six percentage had no knowledge of menses before menarche. Sixty percentage girls used sanitary pad and the rest used cloth pieces. About twenty two percentage used water and no soap for hand washing [5].

Conclusion

The analysis of data reveals that menstrual hygiene practices are very important in order to prevent women from disease. As a result, we found that majority of the women have not attended the awareness programme in spite of that still majority of the women maintain good menstrual hygiene practice, it can be possible that they get information through various sources. It is found that women in rural area still avoid the religious things. 88% of the respondents do not avoid going to school/ college/office which indicate their perception towards good hygiene practice. Our study reveals that majority of participant uses sanitary napkins but there are few participants who use cloth.

Recommendations:

· A similar study can be conducted in the schools for adolescent girls who have attended menarche.

· A similar study can be conducted for the women who have attended menarche in urban community.

· A similar stud can be conducted in the hospitals for the female patients during their menstruation.

Ethical Consideration: The researcher gained the Institute research Committee approval and Study was approved at Institute Ethical subcommittee. Informed consent was obtained from each sample. Privacy and confidentiality maintained

Conflict of Interests: Nil

Source of Funding: Self

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Impact of Information Booklet on Harmful Effect of Use of Plastics in Relation to Practices among Urban Resident of Pune

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Abstract

Introduction: Plastics are a wide variety of combinations of properties when viewed as a whole. They are used for variety of items and the list could go on and on and it is obvious that much of what we have today would not be possible without plastics

Material and Methods: An experimental study was conducted on practice of community regarding harmful effects of plastics among urban residents of Pune city. The pre-experimental design was adopted and 40 samples were selected through simple random sampling technique. The data was obtained from the participants using an interview method which included details on socio-demographics of the participants, practice of plastics. The same Questionnaire were administered after providing informational booklet as well.

Result: The findings revealed that practice of people regarding the harmful effect of the use of plastics was 45.65 % whereas Post –test score of the practice was 55.11 %, the practices in t-Test at 0.05 level hence the booklet in improving practice needs more hammering among the urban residents.

***Key words**: Plastics, environmental pollution, harmful effect, bio-degradable, practice.*

Introduction

Plastic is material which is used worldwide and plastic wastages do not get decompose easily,resulting in one of our planet's greatest environmental challenges.[1] The production of plastic with lower number of microns is very cheap and people uses plastic due to its durability, low cost and its ease availability.[2]

Plastic bag wastes pose serious environmental pollutions and health problems in humans and animals. Disposal of plastic waste which is a major cause of environment pollution becomes carcinogenic to human, birth defects, impaired immunity, endocrine disruption, development and reproductive effect.[3]

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Plastic material consists of synthetic and semi-synthetic compounds which can be used to manufacture solid material

India consumes an estimated 16.5 million tonnes, about 1.6 million truck-full, of plastic annually, as per the June 2018 report.[4]

Styrofoam products used in single use plastic, contain carcinogenic chemicals like styrene and benzene, which are extremely toxic if ingested and cause damage to the nervous system, lungs and reproductive organs. Disposing of plastic waste by burning it in open-air pits releases harmful gases like furan and dioxin, which are harmful to the environment and living organisms including man.[5]

The plastic has unique feature to prepare well-designed and aesthetic products from very low cost material, which are useful for common people. In the plastic and polymer technology there is huge innovations

to shape the plastic into various useful material.[6]

Background

Plastic wastages do not get decompose easily, settles down in the earth that leads to increase in the mass of waste in the Earth. It affects the water cycle by disturbing the rainwater to enter into the land as the water and air cannot pass through it. Initially it show decrease in ground water level and it leads to so many environmental issues. Even it sometimes causes death of animals due to the intake of plastic. The cost for recycling the plastic is very high and almost it is a loss process. The half-life period for plastic is too long that it takes some millions of years to decay.[7]

So this study conducted to have the baseline information regarding the practice of using plastic among community.

Methodology

Study design, setting and sample size

An experimental study was conducted on practice of community regarding harmful effects of plastics among urban residents of Pune city. The pre-experimental one group pre-test post-test design was adopted and 40 samples were selected through simple random sampling technique. The permission was obtained from the Pune Municipal Corporation to conduct study.

Objectives of the study

1. To assess the practices regarding the harmful effects of use of plastics.

2. To determine the effect of informational booklet on practices regarding the harmful effect of use of plastics

Data collection method

The tool had been prepared in four section which consists of the socio demographic variable, assessment of practice (23 items) and information booklet on prevention of hazards of plastics

The interview method was adopted to collect the information. The questionnaires were administered in English and Marathi language. The same questionnaires wereused before and after the providing informational booklet. The participants had to reply 23 items to assess the practice of harmful effects of plastics. The favorable reply, considered one (1) point and unfavorable reply, considered zero (0) point.

Data Analysis: The data had been analyzed through descriptive analysis and statistical analysis.

Result

Section I: Description of samples based on personal characteristics

Table No.1 Distribution of participants as per demographic variables (N=40)

Demographic variable	Frequency (f)	Percentage (%)
Age		
· 15-30years	25	62.5%
· 31-45years	7	17.5%
· 46-60years	8	20%
Sex		
· Male	19	47.5%
· Female	21	52.5%
· Other	0	0%
Education		
· Primary	7	17.5%
· Secondary	13	32.5%
· Graduate	20	50%

Cont... **Table No.1 Distribution of participants as per demographic variables (N=40)**

Occupation		
· service	14	35%
· business	10	25%
· unemployed	16	40%
Marital status		
· Married	20	50%
· Unmarried	19	47.5%
· Widow	1	2.5%

Table no. 1 depicts that 25 (62.5%) of the participants are in age group of 15-30 years and 8 (20%) of them are in age group of 46-60years. Most of subjects 21 (52.5%) were females and 19 (47.5%) were male. 20 (50 %) of participants had completed their graduation.14 (35%) of the participants were doing service and 16 (40%) of them were unemployed. Most of the participants 20 (50%) were married.

Section II – Analysis of data related to the practices of harmful effect on use of plastic

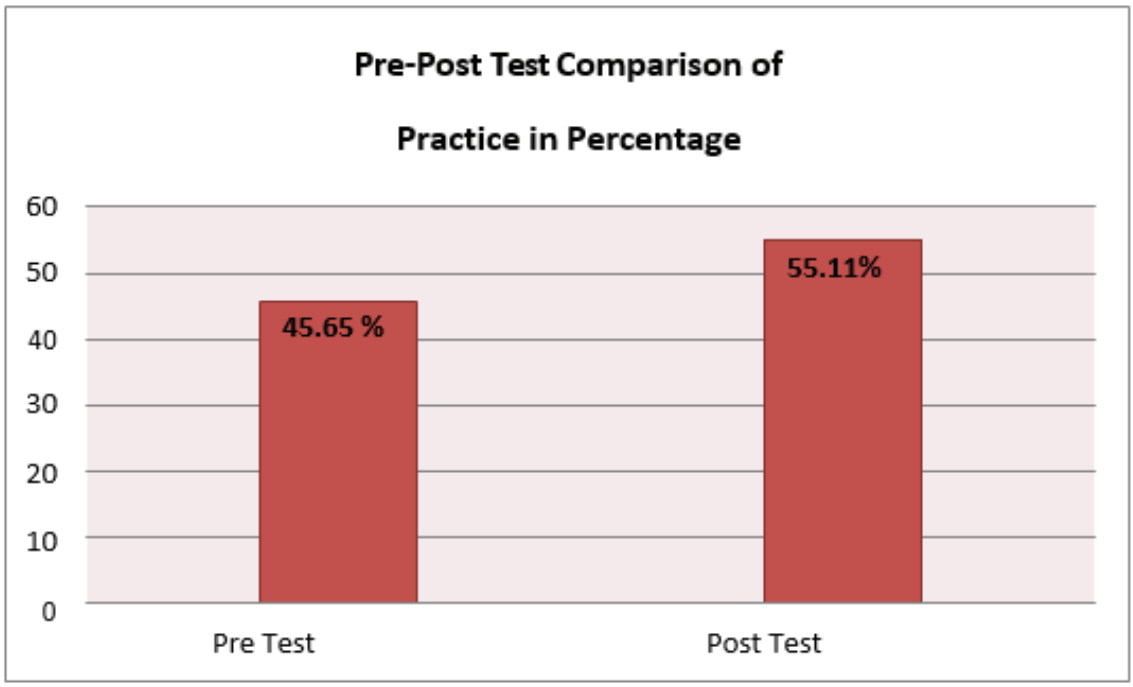


Fig No. 1 Pre and Post Test Comparison of Practice in Percentage

Fig no.1 shows that participants were replied (23)questions regarding the harmful effect of the plastics, the favorable reply before intervention was 45.65% and 55.11 % was the favorable reply after providing informational booklet. Statistics shows that calculated value was greater than table value, hence the informational booklet was effective in improving the favorable practice of use of plastics among the urban residents

Table no.2: Effectiveness of the informational booklet on practices regarding recycling of plastics

Sr. No	Practice Based Questionnaire	Pretest Score frequency (n)	Pretest Score Percentage (%)	Posttest Score frequency (n)	Posttest Score percentage (%)
1	Do you keep on changing your pen made of plastic instead of changing Its refill	14	35%	15	37.5%
2	Do you get your own cloth paper bag for buying groceries	22	55%	28	70%
3	Do you ask your vendor to provideplastic bags	19	47.5%	18	45%
4	Do you use plastic bags or plastic containers to buy parcels	13	32.5%	12	30%
5	Do you try to reuseplastics	20	50%	28	70%
6	How often do you tryto reuse plastics	2	5%	4	10%
7	How often do youbuy plasticproducts	28	70%	35	87.5%
8	Do you advicepeople to reduce the use of plastics	17	42.5%	21	52.5%
9	Do you think we can live without plastics	25	62.5%	34	85%
10	Do you useplasticgarbage bags	8	20%	7	17.5%
11	How often do youuse plastic garbage bags	9	22.5%	12	30%

Table no.2 stated that practice of participants has been improved in most of the aspect after giving intervention about recycling of plastics.

Table No.3:Effectiveness of the informational booklet on practices regarding the discarding and replacing of plastics

Sr. No	Practice Based Questionnaire	Pretest Score frequency (n)	Pretest Score percentage (%)	Posttest Score frequency (n)	Posttest Score percentage (%)
1	How do you discard plastics'waste at your home	12	30%	19	47.5%
2	Should we start usingproductsmade up of papers instead of plastics	26	65%	32	80%

Table no.3stated that practice of participants has been improved after giving intervention about discarding and replacing of plastics.

Table No.4: Effectiveness of the informational booklet on practices regarding the harmful effect of plastics related to food

Sr. No	Practice Based Questionnaire	Pretest Score frequency (n)	Pretest Score percentage (%)	Posttest Score frequency (n)	Posttest Score percentage (%)
1	Do you use plastic containers to keep your food	17	42.5%	20	50%
2	Do you consume food in plastic plates and bowls	22	55%	24	60%
3	Do you drink tea or coffee in plastic cups	21	52.5%	15	37.5%
4	How often do youhave tea or coffee in plastic cups	30	75%	34	85%
5	How often do you use plastic products	16	40%	26	65%
6	Do you use polythene bags to keep yourvegetables and fruits fresh inside the refrigerator	15	37.5%	23	37.5%
7	Which type of buckets do you use at home	18	45%	25	62.5%

Table no. 4 depicts that practice of participants has been improved in most of the aspects after giving information about harmful effect of plastics related to food.

Table No.5:Effectiveness of the informational booklet on practices regarding the segregation of plastics

Sr. No	Practice Based Questionnaire	Pretest Score frequency (n)	Pretest Score Percentage (%)	Posttest Score frequency (n)	Posttest Score percentage (%)
1	Do you segregate plastics ‘drywastes fromwet wastes	16	40%	18	45%
2	Do you burn plastics	22	55%	26	65%
3	Where do youthrow the chocolate plastic wrappers	28	70%	35	87.5%

Table no. 5 described about practice of participants has been improved after providing information about segregation of plastics

Discussion

People should be educated and get aware of harmful effect of plastics. Pre-experimental study was conducted on 40 participants, which was selected by simple random sampling. One group pre-test post-test design was used. The findings of the study is organized as per objectives of the study. Researcher found out that pre-test practice score of the participant was 45.65%. Information booklet was administered to the participants, post-test was conducted, results were recorded, and it was found that practice score increased to 55%. Hence, it is proved that information booklet on prevention of hazards of plastics among residents of Pune city.it was found that 95% peoples are using plastics in day-to-day practices starting from using plastic bags to plastic cups to drink hot beverages.

The similar study conducted in Gujrat in 2019, the post test knowledge score of high school students regarding hazards of plastic use was significantly higher at 0.05 level of significance of paired' test. calculated value (16.42) was higher than paired 't'test table value (1.74) at 0.05 level of significance.[8]

Conclusion

Findings of the study showed that information booklet on harmful effect of plastics in regards to practices of residents of Pune city found effective

Ethical Clearance- Ethical approval taken from ethics committee of the institute

Source of Funding- Self funded

Conflict of Interest – Nil

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A Study to Assess the Effectiveness of Information, Education and communication on Knowledge and Practice regarding MR Vaccination among the Mothers in Selected Slum Areas of Pune City

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Abstract

Measles is a very contagious disease which continues to become the killer disease for most of the children and infants. On the other side Rubella Infection also is deadly dangerous especially in the vulnerable population of pregnant women where it disables a child for life with congenital rubella syndrome[1]. This may leads blindness, deafness, and other congenital cardiac defects. Bothe the diseases, however, can be prevented with a safe and effective vaccination of the long term immunity[2]. This immunization directly contributes to the reduction of under-five child deaths across the country population, thereby reducing the disabilities. The present study title: "A Study to Assess the Effectiveness of Information, Education and communication on Knowledge and Practice regarding MR Vaccination among the Mothers in Selected Slum Areas of Pune City." The main purpose of the study was to assess the knowledge, practice and to evaluate the effectiveness of IEC regarding MR vaccination among mothers of selected slum areas of Pune city and to determine associations of the findings with socio demographic variable. **Material and Methods**: A Pre experimental, one group-Pre- test post-test design was adopted for study . Study carried out on 80 samples as below 15yrs. children in selected slum area. The non-probability convenience sampling technique was used to collect the data through structured questionnaire and observational checklist. Ethical clearance was Obtained from Institutional ethics committee. Data analysis was done mainly using descriptive and infrantial statistics. **Result**: The mean post test score 14.27(SD=1.64) was higher than the mean pretest score 8.48(SD=2.9) .The computed t-test statistic value is 15.124733, Corresponding p-value was 0.000, which is small (less than 0.05)and for the practice mean post test score 38.5(SD=7.63)was higher than the mean pretest 26.36(SD=4.25). As the calculated t-value is 12.725452 and p- value was 0.00, which small(less than 0.05.**Conclusion:**Study conclude that the knowledge and practice of mothers was inadequate in pre-test but then after the administration of IEC there was an increase in the knowledge and practices about MR vaccination, so IEC was effective in increasing the knowledge and practice of mothers regarding MR vaccination**.Recommendation:** A similar study can be conducted in pediatric hospital settings, in PHS and CHC center in rural areas, and carried out to evaluate the efficiency of various teaching strategies like self-instruction module, pamphlets, leaflets, on measles and Immunization.

Keywords:*(Assess, Effectiveness, Measles Rubella Vaccine, Mother, Knowledge, Practice, Information, Education, Communication)*

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Introduction

Measles is a very contagious disease which continues to become the killer disease for most of the children and infants. On the other side Rubella Infection also is deadly dangerous especially in the vulnerable population

of pregnant women where it disables a child for life with congenital rubella syndrome.[1] This may leads blindness, deafness, and other congenital cardiac defects. Both the diseases, however, can be prevented with a safe and effective vaccination of the long term immunity[2]. This immunization directly contributes to the reduction of under-five child deaths across the country population, thereby reducing the disabilities.[3] Indian Government has already inculcated measles vaccine in the Universal Immunization Programme.[1] Ever Since the vaccine has been introduced the burden of the measles in India has reduced considerably. The further drop was accelerated by giving a second chance for measles vaccination a mass round happened in the year 2010 in the country.[4] On the basis of the expert committee recommendations, the measles-rubella vaccine is being introduced in our country through a phased MR vaccination campaign in all the states covering 9 month to 15 yr year old children and consecutively introduction of MR vaccine in the national immunization schedule.[5]

Need For The Study

Ministry of Health and Family Welfare, Government of India introduced Immunization schedule and program in India and furher modified as 'Universal Immunization Programme.[2]It initiated in phased manner to cover all districts in the country . It is considered as one of largest health programme in the world.[5] Ministry provides several vaccines to infants, children and pregnant women through that Programme. There are many vaccine included in Immunization Programme to prevent children from many killing diseases.[6]

The researcher felt need of this study is because of poor knowledge and practices of mothers about immunization especially in slum or rural areas of India. Poor knowledge and care less attitude can leads to incomplete vaccination and that can leads to many diseases in children which are preventable. Vaccination is the responsibility of a family or parent however mother is always considered primary person to play role in child care starting from birth of child to growing up in a favorable environment.[7,8] It's because of attitude of society towards role and responsibility of male & female. Indian society still thinks that women are the primary responder to look after everything in family like child care, growth, schooling, handling of old age family members etc. weather they are working or house wife[8] . However Indian societies not digest the role of male in all activities where women look after. That's by father do not aware much about immunization of children as they think not a part of this.

Objectives of This Study

1. To assess the knowledge regarding MR vaccination among mothers of selected slum areas of Pune city.

2. To determine the practice regarding MR vaccination among mothers of selected slum areas of Pune city.

3. To evaluate the effectiveness of IEC regarding MR vaccination among mothers of selected slum areas of Pune city.

4. To determine associations of the findings with socio demographic variable regarding MR vaccination among mothers of selected slum areas of Pune city

Material and Method

In present study, researcher adopted pre experimental one group-Pre- test post-test design. Study carried out on 80 samples as 15yr children in selected slum area. The non-probability convenience sampling technique was used to data was collected using the structured questionnaire and observational checklist. Data was analyzed statistically. Ethical clearance was taken from Institutional ethics committee. Data analysis was done mainly using descriptive statistics

Description of Tool:

The tool includes three sections:

Section A: Part I:- Demographic variables such as age, area of residence, educational status, occupation, family income, religion, type of family and source of information.

Section B: A Self Structured Questionnaire to assess the Structured questionnaire (knowledge assessment).

Section C: Observational checklist (practice assessment) Plan for Data Analysis:

Data analysis was done by using descriptive and

inferential statistics based on objectives of study.

Result and Discussion

SECTION I: Analysis and interpretation of the data are based on data collected from 80samples.

The above table shows that maximum mothers 46.25% are from the age group 28-38 years, majority 35% of the mothers have studied from 8th -10th standard, maximum 41.25% of mothers have family income of 5000-10000. 56.25% gender of the child are male with maximum 42.5% age group from 1-5 years of age. Majority 53.75% of the children have been administered with MR vaccine at school.

Table1: Effectiveness of IEC on Knowledge

SECTION II: FINDINGS RELATED TO EFFECTIVENESS OF IEC ON KNOWLEDGE OF MOTHERS REGARDING MR VACCINATION.

n=80

Knowledge level	**Pre-test**		**Post-test**		**Calculated t-value**	**p-value**
	Frequency	Percentage	Frequency	Percentage		
Good (12-17)	15	18.75	76	95	15.124733	<0.00001
Average (6-11)	49	61.25	4	5		
Poor (1-5)	16	20	0	0		
Mean	8.48		14.27			
SD	2.9		1.64			

The above table provides data about the effectiveness of Information Education Communication (IEC) on knowledge regarding MR vaccination among mothers. There is increase in the mean knowledge of the nursing officers from 8.48(pre-test) to 14.27 (post-test), and standard deviations from 2.9 (pre-test) to 1.64 (post-test). As the calculated t-value is 15.124733 and p-value= <0.00001, where $p<0.05$, Significant at 0.05 level of significance. Hence, statistically there is effectiveness of Information Education Communication (IEC) on knowledge MR vaccination among mothers in selected slum area of Pune city.

Cont... **Table1: Effectiveness of IEC on Knowledge**

Section Iii: Findings Related to Effectiveness of Iec on Practice of Mothers Regarding Mr Vaccination

Practice Score	Pre-test		Post-test		Calculated t-value	p-value
	Frequency	Percentage	Frequency	Percentage		
Good (12-17)	0	0	39	48.75	12.725452	<0.00001
Average (6-11)	74	92.5	41	51.25		
Poor (1-5)	6	7.5	0	0		
Mean	26.36		38.5			
SD	4.27		7.63			

n=80

Table 2: Effectiveness of IEC on Practices

The above table provides data about the effectiveness of Information Education Communication (IEC) on practice regarding MR vaccination among mothers. There is increase in the mean knowledge of the nursing officers from 26.36 (pre-test) to 38.5 (post-test), and standard deviations from 4.27 (pre-test) to 7.63 (post-test). As the calculated t-value is 12.725452 and p-value= <0.00001, where p<0.05, Significant at 0.05 level of significance. Hence, statistically there is effectiveness of Information Education Communication (IEC) on practice of MR vaccination among mothers in selected slum area of Pune city.

SECTION IV: FINDINGS RELATED TO CORRELATION BETWEEN KNOWLEDGE AND PRACTICE OF MR VACCINATION AMONG MOTHERS

n=80

Table 3: Correlation between Knowledge and practices

Research Variables	Knowledge Post-test	Practice Post-test	r- value	Remark
Mean	14.27	38.5	-0.0685	Negative correlation
SD	1.64	8.63		

The above table shows the correlation between knowledge and practice of MR vaccination among mothers. The value of r is -0.0685. Although technically a negative correlation, the relationship between the variables is only weak (nearer the value is to zero, the weaker the relationship). Therefore there is no any correlation between knowledge and practice of MR vaccination among mothers in selected slum are of Pune city.

SECTION V: Findings on Association of knowledge and demographic variables.

The result shows the association of obtained scores on knowledge regarding MR vaccination with selected demographic variables of the mothers. The chi square calculated values of the demographic variables are less than the table values and value of $p<0.05$, at 0.05 level of significance. So, statistically there is no association between the selected demographic variables with the knowledge regarding MR vaccination among mothers in selected slum area of Pune city.

n=80

Sr.No	DEMOGRAPHIC	POOR	AVERAGE	GOOD	P Value
1	AGE OF THE MOTHER				
a	18-28	8	21	7	0.956
b	28-38	6	23	8	0.987
c	38-48	2	5	0	
d	48 and above	0	0	0	
2	EDUCATION OF THE MOTHER				
a	Illerate	1	3	0	
b	1st std -7th std.	2	11	5	8.423
c	8th std – 10th std	4	21	3	0.588
d	11th std- 12th std	6	7	5	
e	Graduation	3	6	2	
f	Post graduation	0	1	0	
3	MONTHLY FAMILY INCOME				
a	Less then 5000.	5	5	5	10.198
b	5000 -10000	8	19	6	0.117
c	10000-15000	2	19	2	
d	15000-20000	1	6	2	
4	GENDER OF THE CHILD				2.821
a	Male	7	27	11	0.244
b	Female	9	22	4	
5	AGE GROUP OF A CHILD				
a	9 months – 1 year	4	10	5	3.688
b	1 year – 5 years	8	21	5	0.719
c	6 years- 12 years	4	13	2	
d	15 year and below	0	5	3	
6	Whether MR vaccine is administered in school?				
a	Yes	5	31	7	5.346
b	No	11	18	8	0.069

Table 4: Asociation of knowledge with demographic variables

SECTION VI: FINDINGS ON ASSOCIATION OF OBTAINED SCORES ON EFFECTIVENESS OF IEC ON PRACTICE REGARDING MR VACCINATION AMONG MOTHERS

Table 5 : Association between Practices and demographic variables n=80

Sr.No	DEMOGRAPHIC	POOR	AVERAGE	GOOD	P Value
1	AGE OF THE MOTHER				
a	18-28	4	32	0	0.953
b	28-38	2	35	0	0.987
c	38-48	0	7	0	
d	48 and above	0	0	0	
2	EDUCATION OF THE MOTHER				
a	Illerate	0	4	0	
b	1st std -7th std.	1	17	0	0.512
c	8th std – 10th std	2	26	0	1.000
d	11th std- 12th std	2	16	0	
e	Graduation	1	10	0	
f	Post graduation	0	1	0	
3	MONTHLY FAMILY INCOME				
a	Less then 5000.	1	14	0	0.634
b	5000 -10000	3	30	0	0.996
c	10000-15000	1	22	0	
d	15000-20000	1	8	0	
4	GENDER OF THE CHILD				1.933
a	male	5	40	0	0.380
b	female	1	34	0	
5	AGE GROUP OF A CHILD				
a	9 months – 1 year	2	17	0	0.741
b	1 year – 5 years	3	31	0	0.994
c	6 years- 12 years	0	19	0	
d	15 year and below	1	7	0	
6	Whether MR vaccine is administered in school?				
a	Yes	3	40	0	0.037
b	No	3	34	0	0.982

The result shows the association of obtained scores on practice regarding MR vaccination with selected demographic variables of the mothers. The chi square calculated values of the demographic variables are less than the table values and value of $p<0.05$, at 0.05 level of significance. So, statistically there is no association between the selected demographic variables with the practice regarding MR vaccination among mothers in selected slum area of Pune city.

Discussion

This study was a quasi-experimental study, pretest-posttest design without control group approach was used to assess the to assess the effectiveness of IEC on knowledge and practice on MR vaccination among the mothers of selected slum area of Pune city. The mean post test score 14.27(SD=1.64) was higher than the mean pretest score 8.48(SD=2.9) .The computed t-test statistic value is 15.124733, Corresponding p-value was 0.000, which is small (less than 0.05)and for the practice mean post test score 38.5(SD=7.63)was higher than the mean pretest 26.36(SD=4.25). As the calculated t-value is 12.725452 and p- value was 0.00, which small(less than 0.05). It shows that the IEC was effective method for improving the knowledge and practice of mothers regarding MR vaccination.

Ethical Clearance: Institutional Ethics Committee

Source of Funding: Self

Conflict of Interest: Nil

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A Study to Assess the Awareness and Attitude Towards maintaining Social Distancing during Corona virus Disease (COVID 19) Pandemic among Residents of Selected Area of Pune City

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Abstract

There is a public health alert as a huge pandemic situation has emerged in the country and across the globe regarding the novel corona virus outbreak. India is on high alert as total cases crossed one lakh.[1] Maharashtra is leading the scoreboard with 33000 plus cases and number is increasing day by day. Social Distancing plays important role in combating the situation but depends on the attitude and awareness of the community[2]

Objective: The main aim of the study is to identify the attitude and level of awareness among the community regarding social distancing in pandemic situation

Methods: A cross sectional design was used along with structured questionnaire tool for data collection. A snowball sampling method was adopted where a goggle form was developed which was sent through various online modes. The study mode was purely online. Participants were from the age of 18 and above who knows English and were willing to participate in the study.

Results: The results showed that People have partial awareness related to the pandemic situation of corona virus spread. Surprisingly 33.1% people think that there is treatment of COVID 19. They showed good interest and positive attitude towards hand washing and maintain social distancing but for maintaining protocols and guidelines to follow for social distancing they were not comfortable.

***Key words**: Corona, COVID 19, Attitude, Awareness, Pandemic, Social Distancing*

Background and need of the study

There is a public health alert as a huge pandemic situation has emerged in the country and across the globe regarding the novel corona virus outbreak. India is on high alert as total cases crossed one lakh.[1] Maharashtra is leading the scoreboard with 33000 plus cases and number is increasing day by day. The entire government, health administration officials at various levels are constantly monitoring the situation.[2] The unstable situation is being closely monitored by the Ministry of Health and Family Welfare. The Joint Monitoring Group (JMG) convened under the Chairmanship of Director General of Health Services (DGHS) with representations from various stakeholders (health and non-health sectors) and WHO are instrumental in bringing about the responsiveness in the health systems.

However, the incident and contingency measures undertaken on a war footing after the identification of the Novel corona virus. Social distancing is considered as one of the method adopted to survive in this situation. It is only method to maintain the quarantine declared by the government of India.

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The Covid-19 pandemic situation possibly may lead to huge morbidity and mortality for millions of population globally in current year.[3] As per Mathematical institute at Imperial College London stated that even if community tries to initiate social contacts and reduce the common meetings the mortality and morbidity still will be out of control. They also suggested that if no social distancing ways were adopted at all, the disease could leads to a big disaster and will kill million people worldwide.[4] suggestively they also emphasised that reduction and total cutting of their social gatherings may help to halved the diseases spread.

The key measures adopted by various governments at various levels for social distancing to alleviate the spread of COVID-19. It is very much challenging task to implement the strict measures for social distancing all over the world. The biggest reason is Industrialisation and level of urbanization, norms and culture of particular country especially related to religious gatherings and high economic visibility international gatherings. Many countries has started introducing significant social distancing measures as a precautionary aspect even before the eruption of first case of COVID-19 confirmed.[5] These decisions ranges from deferment or withheld the social and religious gatherings , stopping entertainment and multiplex resources, temporary closure of education organisations postponing of all non-essential gatherings, and imposing legal curfews . The effect of these actions on the epidemic curve is a big anxiety state to deal with. However, as per present COVID-19 situation, many countries may take courageous and probably out of favour measures for the prevention of mortality and morbidity[2]

The condition of lock-down due to pandemic across the globe, has halted the global economy because of non-participation in services and products. This has shattered the global supply chains and therefore impacted brutally on the global economy[3]

Transport, Import and export of material across the countries has been revoltingly affected. Even at national levels Transport business has ceased due to lock-down in different countries. Maximum employees are working from own residences, which further provoking financial advantages and disadvantages.[6] Educational organisations have been put the lid down. Economy has a huge set back. In this situation it is bit obvious to get panic but to maintain the social distancing is the necessity of survival. The attitude plays a very important role in this situation

There are certain groups who are still not following the social distancing. This disease and the response of the public towards the disease is so novice that nobody can predict the factors to accept or neglect the lockdown situation. The awareness plays very important role to maintain a positive lifesaving attitude towards the disease. The awareness and attitudes towards maintain the social distancing of the public is largely influencing the level of devotion to the personal protective measures and ultimately the expected outcomes.

In Maharashtra, Mumbai and pune are the areas where maximum cases corona are reported. Various factors plays role to understand the reason behind that. Awareness and attitude are one of those factors to assess. Therefore the researcher as a community health nurse wants to study the level of awareness and attitude towards the social distancing among the residents of pune.

Objectives

1. To assess the level of awareness towards maintaining Social distancing during Corona virus disease (COVID 19) Pandemic among residents of pune city

2. To determine the attitude towards maintaining Social distancing during Corona virus disease (COVID 19) Pandemic among residents of pune city

Research Methodology

A cross sectional design was used. A structured questionnaire was developed for data collection. A snowball sampling method was used with due consent. A goggle form was developed which was sent through various online modes. The study mode was purely online. Participants were from the age of 18 and above who knows English and were willing to participate in the study. The answers were obtained in goggle form as a result in two parts part 1 depicted awareness regarding COVID 19 and social distancing and Part 2 represented attitude towards social distancing.

Results

The online survey, was conducted related to awareness and attitude regarding COVID 19 and social distancing among community during the COVID 19 pandemic, in the pune population. A total of 662 responses were recorded. Total 158 samples were covered. All the participants were above the age of 18 years. The study included only those participants who can understand English and had access to the internet. The study results showed that average mean age was 28.7. Maximum participants (48%) were from the business category followed by Housemakers (27%) college students (12%) farming (7%) and 6% from other families.

Part 1: Awareness Regarding COVID 19 and Social Distancing

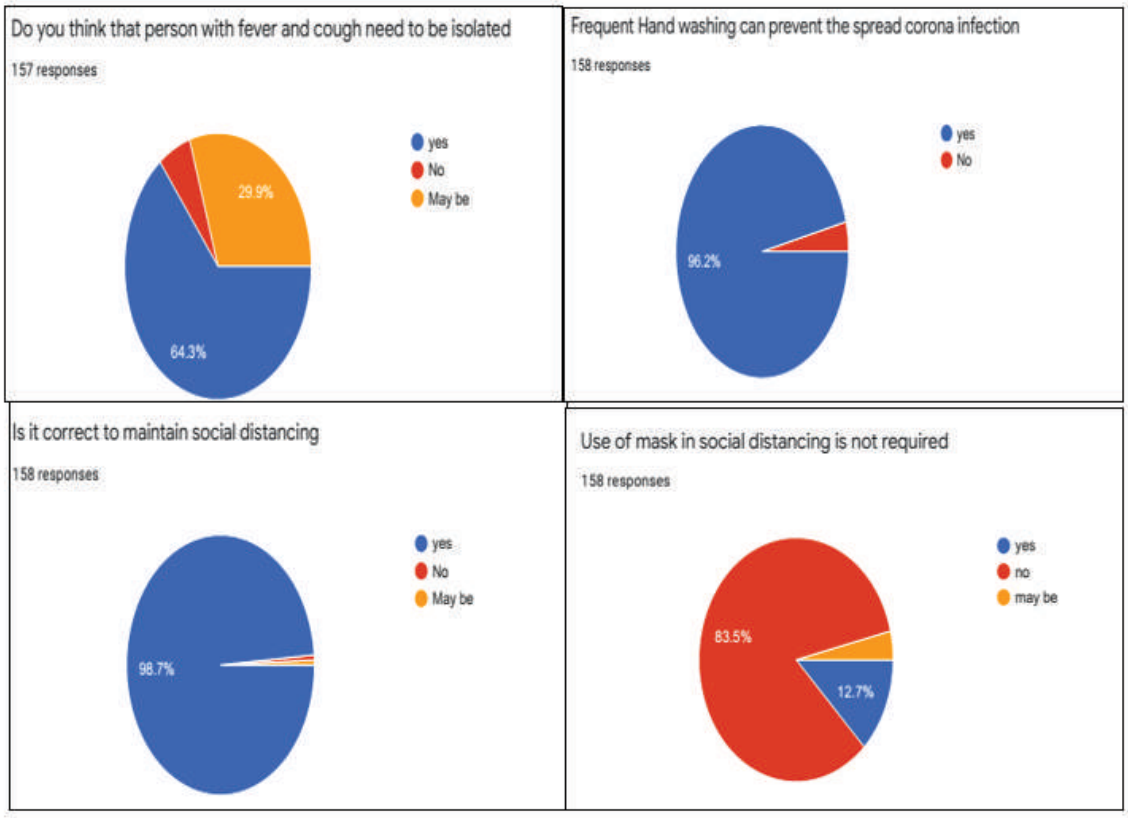
The results were obtained through the structured google form only and analysis was obtained through the received answers. The results showed that 98.7% of people have knowledge about the corona. 80.9% knows about the spread of disease. 93% knows the symptoms very well. Surprisingly only 33.1 % people gave answer that there is no treatment for COVID 19. Maximum people are aware that the virus is contagious. The results are depicted in fig. No 1



Fig 1: Awareness of People about COVID 19

Part 2. Attitude regarding measures and social distancing

Maximum people that is 96.2% showed positive attitude towards hand washing. 35.7% of people were not favouring isolation if person has symptoms. They said it either no or may be. Almost 12 % shows the negative attitude towards rules of social distancing. 10% showed negative attitude and think that it is safe to travel in lock down period. Almost 16.5 % of people don't prefer use of mask in social distancing and 20% are still favouring the small gatherings in societies. Almost 30% of people have negative attitude towards the social distancing. 50% of public are having negative attitude towards corona patients.



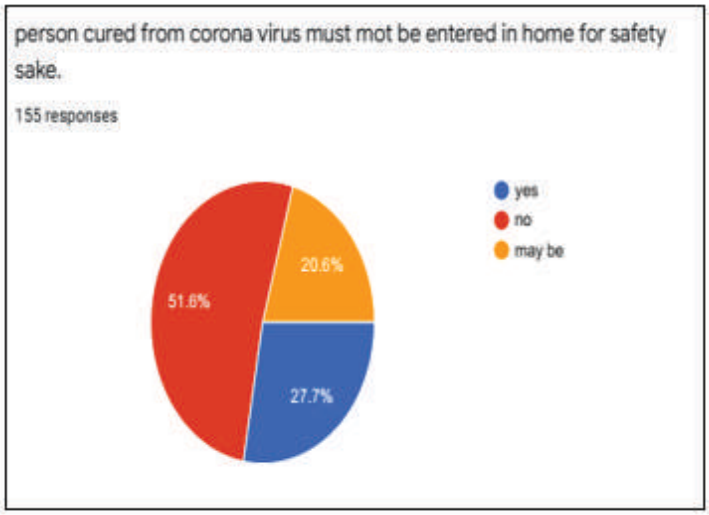


Fig No 2: Attitude of People regarding measures and Social Distancing

Discussion and Conclusion

The results clearly showed that people have good awareness about COVID 19 but still the attitude towards the measures and social distancing is not very much positive . They are not favouring it. This shows that there is strong need to change the attitude as it may not fetch the results. The study participants showed good interest and positive attitude towards hand washing and maintain social distancing but for maintaining protocols and guidelines to follow for social distancing they were not comfortable. It may be possible as people may face many challenges in the lock down period like food, financial dearth etc which may force them to exploit the protocols. More factors need to be ruled out and to intensify the awareness along with available resources so that attitude should not hinder the expected Outcome.

Conflict of Interest: Nil

Ethical Clearance: Institutional Ethics Committee

Source of Funding: Self

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Hurdles and Possible Solutions in Development and Implementation of Online Learning in Nursing Education- an Integrative Review

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Abstract

Background of the study: Presently, there are more than 3 million cases and one lakh deaths reported, and still counting.[1]This has fetched essential changes in all aspects of our lives. Social distancing and preventive movement procedures have noticeably unbalanced traditional educational practices. The time course of these changes due to instability of pandemic is unspecified. The changeover from conventional to online learning is full of challenges. Escalating issues of time constraints and load are constantly to be found in almost all students and educators. It is really motivating the educational organizations to search new ideas of implementing custom-made, own-governing learning.

Objective: The purpose of the study is to appraise the available literature on perceived hurdles and possible solutions that are encountered by the teachers while executing e-learning for nursing students.

Material and Methods: An integrative review of the literature was performed keeping in mind the inclusion criteria of studies with varied methodologies and designs. A search strategy was devised and data was searched in the following databases like, Medline (Ebsco), Medline, Scopus, Science Direct, Biomedical Central, and Google Scholar. Boolean operators were used for searching the terms. Critical Appraisal Skill was used for data appraisal.

Results: A total of 56 articles were screened with 23 articles found appropriate for further review. Data was segregated as per the objectives of each study, sample characteristics, data collection methods and adopted approach for the analysis. Results suggested that the major constraints that affect the execution of e- learning include lack of skills, inadequate infrastructure, poor communication and negative attitude. A way forwarded solutions those include improved training and continuous commitment, redefining the cost, Team collaboration and developing positive attitude in all those who are stakeholders of the online content.

Conclusion: This is very much important that educational institutes, Nursing institutions and their stakeholders having insight of hurdles and barriers as well as solutions to the way it has to be developed and implement the kind of e-learning. There is the need for a culture to be in executed which should support and promote the use of online learning

***Key words**: Educators, Online learning, digital learning, Barriers, solution*

Background of the Study

Presently, there are more than 3 million cases and one lakh deaths reported, and still counting.[1] This has fetched essential changes in all aspects of our lives. Social distancing and preventive movement procedures have noticeably unbalanced traditional educational practices. The time course of these changes due to instability of pandemic is unspecified. These have affected conventional in-person Nursing education and training patterns.

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Before the pandemic of COVID-19, various kind of teaching pedagogical tools had been used in institutes. During COVID-19, the need has been accelerated for more for innovative approaches to meet the educational requirements. Many programs tried to get along with new technologies like Cisco webex, zoom. Microsoft team google meets etc. From few last years there has been a shift in nursing education from conservative teaching to various other methods and ways for imparting online learning.[3]Many education institutes adopted various electronic resources and strategies to sustain academics during this pandemic. The arranged necessary information Technology support for the execution of the online teaching methods. Whereas others deny the execution and showed a negative attitude towards the varieties. With adoption of innovative virtual ways, many technical issues are bit obvious expected but those can be easily handled with a due course of time as the students and teachers becomes more recognizable. The primary challenge of this approach depends on the motivation and

Willingness to accept the technology.[2]

Aims and Objectives: The purpose of this study is to analyze the existing literature on perceived hurdles and the solutions that are encountered by the teachers while executing online learning for nursing students.

Material and Methods: An exhaustive review of the literature was performed keeping in mind the inclusion criteria of studies with varied methodologies and designs

Search Strategies: A search technique was adopted and data was searched in the following databases like, Medline (Ebsco), Medline, Scopus, Science Direct, Biomedical Central, and Google Scholar. Boolean operators were used for searching the terms. Critical Appraisal Skill was used for data appraisal. . Boolean operators search terms included "online learning", "Online teaching strategies, distance learning", "Nursing educators" and "digital learning".

This review was finished in a two stages with a review of abstracts and titles. All full text articles which meets inclusion criteria and exclusion criteria were reviewed separately.

Inclusion and exclusion criteria

All research articles that from peer reviewed journals in last 10 year and targeted nursing online learning were part of study. Studies that displayed implementations with online learning, online content development and implementation were included.

Studies that were pertaining to online learning other than nursing educators were excluded.

Data appraisal

Critical Appraisal Skill was used for data appraisal, Qualitative Checklist for qualitative studies and quantitative research Quantitative and mixed method studies were ranked as Excellent, Good and Satisfactory.

Data analysis

Thematic analysis was the done for the data analysis under following headings.

1. Hurdles in the online learning
2. Solutions for the hurdles in e-learning.

Results

A total of 56 articles were screened with 23 articles found appropriate for further review. Data was segregated as per the objectives of each study, sample characteristics, data collection methods and adopted approach for the analysis. Results showed that the major constraints which affect the execution of online learning include lack of skills, inadequate infrastructure, poor communication and negative attitude.

Coding

After the exhaustive analysis process four main themes emerged. The following hurdles and solutions are identified.

Hurdles – lack of skill for online teaching

Lack of technical skills, was found to be one of the hitch faced by the users, developing and implementing the content, inadequate technical skills a feeling of fear or phobia to engage with the implementation of content.

Solution – training and continuous Commitment

To attain the adequate skills, it was suggested that engagement with online learning, and the development of content and trying to implement as practice sessions can be done to gain skills.

"I participated so that I could learn a little bit more about teaching in an electronic environment. I think I learned quite a bit" [6]

Perlman et al.[7] emphasized on the value and strong need to provide the faculty with training skills to gain confidence .Workshops must be arranges which help the teachers to learn and practice this new skill and gain acquaintance with these kind of tool

Hurdle – Logistics infrastructure

In many cases and scenarios, lack of logistic facilities, inadequate technology were acted as hurdle especially in the under developed countries [8]. They lack technological support like continuous web and internet, and other e resources. These limitations are the hurdles for online learning. Attardi & Rogers [9] found technical lacking like lack of internet supply for broadcasting lectures.

Solution – cost redefining

Maloney et al.[10] found that there is need to redefine and establish the cost of online education'. Though it seems very tough in case of medical and nursing, it is still suggested as one of the idea where one can relook the cost of correct infrastructure as a hurdle as well as possible solution.

Hurdle –poor communication

Very less or no institutional handholding, lack of guidance regarding implementation of the specific online seems to be the reason of unsuccessful approach [11]

"I have found it difficult at times to have a 'discussion' online as you are never quite sure about the exact meaning of what people are saying." [12]

Solution –Team collaboration

Bediang et al.[13] stated one significant way for the successful implementation of online learning is to include all concerned faculty to facilitate collaboration. They also suggested to appoint online learning managers to manage the system according to the needs and requirements of novice faculty'.

Hurdle – Attitude

Negative and passive attitude in the faculty in adapting the new ways and e- technologies was seen as hurdle. Faculty was feeling besieged with the adaptation and engagement with new tools and have very less endurance for solving even very minute technical problems [14]

"If you ask me to peer-review something that I have no expertise in I'm reluctant to do that" [15]

Solution – Positive culture

A culture need to be developed to attain the positive attitude for managing and using e-learning tools and technologies. It was significant to have a positive attitude and endurance [16]

"Try to maintain a positive attitude and assume that any slights or overly harsh criticism is due to the asynchronous communication and to not take it personally" [16]

Discussion

This study has come up with facts of major hurdles and solutions towards the development and implementation of online learning from the Nursing faculty viewpoint. These were skills, resources, and support and attitude and institutional strategies which found common in many studies. There was positive experiences with many faculty, where they found it enjoyable to adopt new tools and technologies [8,16] There was a strong need for institutional support to help the faculty technically . In cases where there is no institutional support and guidance regarding the use of tools and other intricacies the programs were quite unsuccessful or rarely successful [17] There is a need of clear ins for online learning with proper guidelines. [8] There is also a well-built cross collaboration to make sure that consistent education for the e- learners [8, 12] Many of the codes identified in this review referred the old studies in nursing profession education. Childs et al. [18] focused on the hurdles and solutions as successful online learning skills as both a problem as well as solution. Lack of teaching skills was

seen as one of the problem er by Pettersson&Olofsson[19] One of the solutions to the lack of training was proposed by Childs et al. [18]is good quality based trainings.

Conclusion

Online education in nursing is a comparatively new idea and is expanding very quickly. Therefore It is important that Nursing training institutes and colleges should be aware of the problems seems to be encountered and solutions towards those in the process of online learning. There is strong need for a culture to be in place which motivates the use of online learning among faculty. That is the only way to stand steadily in the nursing education to face the challenges in this digital era.

Source of Funding: Self

Ethical Clearance: Institutional Research Committee

Conflict of Interest: Nil

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Effectiveness of Community Education Programme on Knowledge and Attitude Regarding Prevention and Management of Selected Non-Communicable Diseases among Urban Adults in Pune City

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Abstract

Non-communicable diseases (NCDs) accounted for two thirds of deaths (34.5 million of a total 52.8 million) worldwide in 2010. In the same year, cancer was responsible for 8 million deaths, ischemic heart disease and stroke collectively for 12.9 million deaths, and diabetes for 1.3 million deaths, with all showing significant rising trends. Non-communicable diseases (NCDs) have emerged as serious public health problem worldwide affecting all the populations across the globe in general but low- and middle-income populations in particular. **The present study title:** "A study to assess the effectiveness of community education programmeon knowledge and attitude regarding prevention and management of selected non-communicable diseases among urban adults in Pune city." The **objective** of the study was to assess the knowledge, attitude effectiveness of community education programme and finding with selected demographic variables regarding prevention and management of selected non-communicable disease among urban adults in Pune city. **Material and Methods:** In present study, researcher adopted pre-experimental one group pre-test post-test research design. Study carried out on 60 samples with adult age group 36-55years residing in selected urban areas. The non-probability convenient sampling technique was used to data was collected using the self-structured questionnaire. Data was analyzed statistically. Ethical clearance was taken from Institutional ethics committee. Data analysis was done mainly using descriptive statistics. **Result:** The mean posttest score9.6 (SD=3.14) was higher than the mean pretest score 5.25(SD=2.34 .The computed t-test statistic value is7.59029, Corresponding p-value was 0.000, which is small (less than 0.05)and for the attitude mean post test score 32.86(SD=9.28)was higher than the mean pretest 21.9(SD=7.33). As the calculated t-value is 7.196212 and p- value was 0.00, which small(less than 0.05). **Conclusion:** Study conclude that It shows that the effectiveness of community education programme was effective method for improving the knowledge and attitude regarding prevention and management of selected non communicable disease. **Recommendation:** A similar study can be conducted in hospital settings and rural areas also carried out to evaluate the efficiency of various teaching strategies like self-instruction module, pamphlets, leaflets, on non-communicable diseased.

Keywords: *Effectiveness,Community Education Programme,Knowledge, Attitude, Prevention, Management, Non-Communicable Disease, Adults*

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Introduction

Non-communicable diseases (NCDs) accounted for two thirds of deaths (34.5 million of a total 52.8 million) worldwide in 2010. In the same year, cancer was responsible for 8 million deaths, ischemic heart

disease and stroke collectively for 12.9 million deaths, and diabetes for 1.3 million deaths, with all showing significant rising trends.[1]The government of India launched a revamped NCD control program in 2016, which aims to screen men and women for hypertension, diabetes, and oral cancer and women for breast and cervical cancers.[2]Great inequity exists in the delivery of health care in India, particularly within the rural population.Women in developing countries like India are victims of the worse deprivation as a consequence of poor empowerment and discriminatory beliefs and practices.[3]Non-communicable diseases (NCDs) have emerged as serious public health problem worldwide affecting all the populations across the globe in general but low- and middle-income populations in particular. The trend of urbanization is increasing all over the world leading to an economic transition. Among the consequences of this economic transition has been a shift in the diseases spectrum from communicable diseases to non-communicable diseases (NCDs). Cardiovascular diseases, diabetes mellitus, and stroke have emerged as major NCDs of public health importance in India, with morbidity and mortality in the most economically productive years of life posing a challenge to society as well as the economy of the nation[4]

Need of the study: About 87 percent of the panchayat members were also aware that healthy diet can help to prevent diabetes. However only around three fourths of them were aware about the benefits of physical activity in preventing diabetes; which is lower than that reported by a recent study in Kerala among adults(Kurian, 2016).[5]Rajiv Kumar Gupta et al (2018) conducted a study to assess the awareness among rural adolescents about NCDs and their risk factors. A cross-sectional study was conducted in Miran Sahib Zone of RS Pura Block. Students from intermediate classes from ten intermediate level schools, five governments, and five private setup were selected for inclusion in the study. A predesigned, pretested, and self-admissible questionnaire was developed by three public health experts for use as a tool to collect the data. Male respondents had better awareness about NCDs ($P < 0.0001$). A higher percentage of male students thought that lifestyle changes could prevent NCDs ($P < 0.005$). The study concluded that there is a need to reinforce the knowledge among adolescents in those areas about NCDs where they are lacking some knowledge.[6]

Objectives of Study

1. To assess the knowledge regarding prevention and management of selected non-communicable disease.

2. To assess the attitude regarding prevention and management of selected non-communicable disease.

3. To assess the effectiveness of community education programmeregarding prevention and management of selected non-communicable disease among urban adults in Pune city.

4. To associate the finding with selected demographic variables on knowledge and attitude regarding prevention and management of selected non-communicable diseases in Pune city.

Material and Method

In present study, researcher adopted pre-experimental one group pre-test post-test research design. Study carried out on 60 samples with adult age group 36-55years residing in selected urban areas. The non-probability convenient sampling technique was used to data was collected using the self-structured questionnaire. Data was analyzed statistically. Ethical clearance was taken from Institutional ethics committee. Data analysis was done mainly using descriptive statistics

Result

Table 1: findings related to demographic distribution of urban adults

Table 1 : shows that maximum adult 56.67% are from age group of 35-40 years.,63.33% are female, majority 40% of the adult have a graduat, 46.67% are in private sector.76.67% are living in joint family, 41.67% are having mix and vegetarian diet ,85% are not suffering from any disease.28.33% are consuming tobacco .80% adult are not taking any medication.

n=60

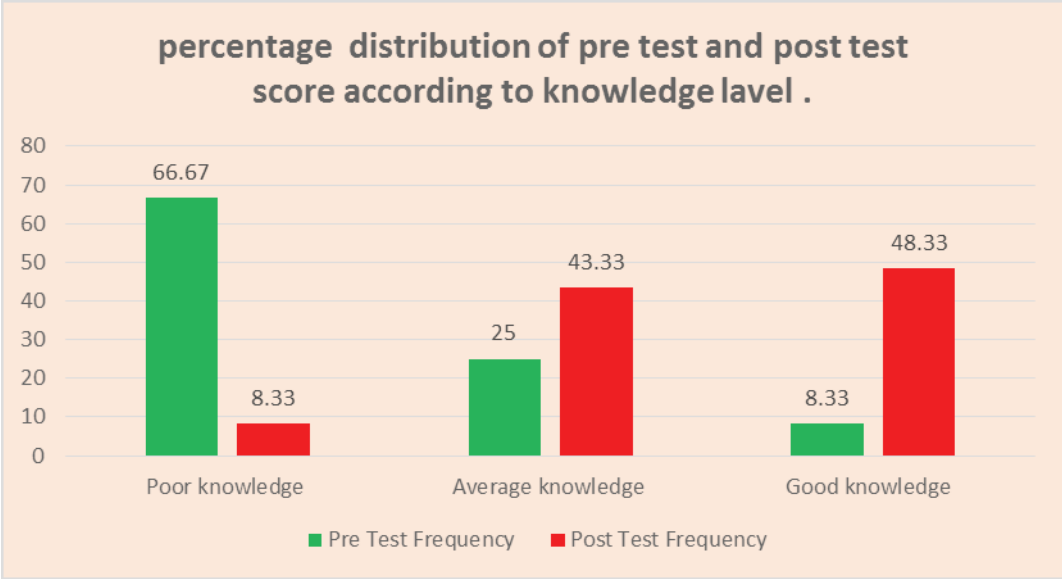


Figure 1: Findings related to comparison of pre test and post test score of knowledge regarding prevention and management of selected noncommunicable disease after intervention of community educationprogramme.(Distribution of adults according to the level of knowledge on non-communicable disease).

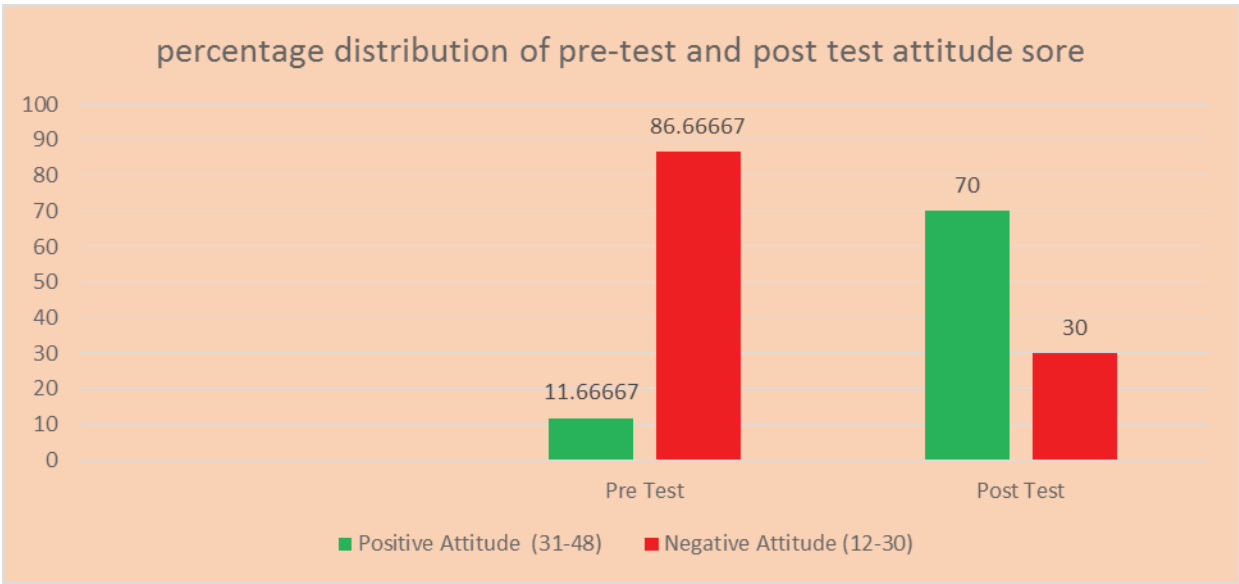


Figure 2 :Findings Related To Comparison Of Pre Test And Post Test Score Of Attitude Regarding Prevention And Management Of Selected Noncommunicable Disease After Intervention Of Community Education Programme Among Urban Adults.

Table 2: findings related to the effectiveness of community educationprogramme on knowledge regarding prevention and management of selected noncommunicable

knowledge	Mean	SD	t-test	P-Value	Significant
Pre Test	5.25	2.34	7.59029	<0.00001	Significant
Post Test	9.16	3.14			

Table 2: The table shows that there was a significant increase in post-test scores of adults. The mean post-test knowledge score 9.16±3.14 of adults was significantly higher than their pre-test knowledge score 5.25±2.34 The computed t-test statistic value is 7.59029, Corresponding p-value was 0.000, which is small (less than 0.05), and the null hypothesis is rejected. It shows that the effectiveness of community educationprogramme was effective method for improving the knowledge regarding prevention and management of selected non communicable disease.

Table 3: findings related to the effectiveness of community education programme on attitude regarding prevention and management of selected noncommunicable disease after intervention of community education programme among urban adults.

Attitude	Mean	SD	t-test	P-Value	Significant
Pre Test	21.95	7.33	7.196212	<0.00001	significant
Post Test	32.86	9.28			

Table 3: shows that there was a significant increase in post-test scores of adults. The mean post-test positive attitude score 32.86±9.28 of adults .in pre-test attitude score was 21.9±7.33. The computed t-test statistic value is 7.196212 , Corresponding p-value was 0.000, which is small (less than 0.05), and the null hypothesis is rejected. It shows that the effectiveness of community educationprogramme was effective method for improving the positive attitude regarding prevention and management of selected non communicable disease.

Table 4: Findings related to associate the finding with selected demographic variables regarding

Table 4: shows the association of obtained scores on knowledge regarding prevention and management of selected no communicable diseases with selected demographical variable of the adults. The chi square calculated values of the demographic variables are less than the table values and value of $p<0.05$, at 0.05 level of significance. So, statistically there is no association between the selected demographic variables with the knowledge regarding prevention and management of selected non communicable disease.

Table 5: findings related to associate the finding with selected demographic variables regarding attitude on prevention and management of selected noncommunicable diseases.

Table 5: shows the association of obtained scores on attitude regarding prevention and management of selected no communicable diseases with selected demographical variable of the adults. The chi square calculated values of the demographic variables are less than the table values and value of $p<0.05$, at 0.05 level of significance. So, statistically there is no association

between the selected demographic variables with the attitude regarding prevention and management of selected non communicable disease.

Discussion

This study was pre experimental one group designed to determine the effect of community education programme on attitude regarding prevention and management of selected non communicable disease after intervention of community education programme among urban adults. The mean posttest score9.6 (SD=3.14) was higher than the mean pretest score 5.25(SD=2.34 .The computed t-test statistic value is7.59029, Corresponding p-value was 0.000, which is small (less than 0.05)and for the attitude mean post test score 32.86(SD=9.28) was higher than the mean pretest 21.9(SD=7.33). As the calculated t-value is 7.196212 and p- value was 0.00, which small(less than 0.05). It shows that the effectiveness of community educationprogramme was effective method for improving the knowledge and attitude regarding prevention and management of selected non communicable disease.

Ethical Clearance: Permission obtained from ethical committee of the institute

Source of Funding: Nil

Conflict of Interest: Nil

Conclusion

It shows that the effectiveness of community educationprogramme was effective method for improving the knowledge and attitude regarding prevention and management of selected non communicable disease.

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Impact of Information Booklet on Harmful Effect of Use of Plastics in Relation to Knowledge among Urban Resident of Pune

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Abstract

Introduction: Plastics are a wide variety of combinations of properties when viewed as a whole. They are used for variety of items and the list could go on and on and it is obvious that much of what we have today would not be possible without plastics. Material and Methods: An experimental study was conducted on knowledge of community regarding harmful effects of plastics among urban residents of Pune city. The pre-experimental design was adopted and 40 samples were selected through simple random sampling technique. The data was obtained from the participants using an interview method which included details on socio-demographics of the participants, knowledge of plastics. The same Questionnaire were administered after providing informational booklet as well. Result: The findings revealed that pre-test score of the knowledge of participants was 53.92 % whereas 87.83 % of post test score, to test the effectiveness paired 't' was applied at the significance level 0.05 and result shows the booklet on harmful effects of plastics was effective.

***Key words**: Plastics, environmental pollution, harmful effect, bio-degradable, knowledge*

Introduction

Plastics are a widely used across the globe and substantial use found in developing countries due to its low cost. They are used for variety of items, the list could go on and on, and it is obvious that much of what we have today would not be possible without plastics[1]. It was so much cheaper to manufacture than other materials and the various ways it could be used was staggering[2].

Plastic material consists of synthetic and semi-synthetic compounds which can be used to manufacture solid material

Plastic changed the world and had a great role in advancement of technologies as plastic were cheaper to be manufactured than other materials and hence it was used very often used in all type of products available today. Because of this reason plastics became part and parcels of our life[3].

The plastic has unique feature to prepare well-designed and aesthetic products from very low cost material, which are useful for common people. In the plastic and polymer technology there is huge innovations to shape the plastic into various useful material[4].

Background

Plastics have been with us for more than a century, and by now they are everywhere, for good and for ill. Plastic contains compounds called phthalates that have been implicated in male reproductive disorders. Studies have also shown that childhood exposure to environmental pollutants can have significant negative effects later in life, including reduced labor force participation and even earnings[5]. Since local residents of the Pune city residing in low cost dwelling and less literate people are not aware of the hazardous effects of the plastics. Education and training methods can be used to educate them and to create awareness among them

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regarding ill effect of the plastics[6].-

Methodology

Study design, setting and sample size

An experimental study was conducted on knowledge of community regarding harmful effects of plastics among urban residents of Pune city. The pre-experimental one group pre-test post-test design was adopted and 40 samples were selected through simple random sampling technique. The permission was obtained from the Pune Municipal Corporation to conduct study.

Objectives of the Study

3. To assess the knowledge regarding the harmful effect of use of plastics.

4. To determine the effect of informational booklet on knowledge regarding the harmful effect of use of plastics

Data collection method

The tool had been prepared in four section which consists of the socio demographic variable, assessment of knowledge (30 items) and information booklet on prevention of hazards of plastics

The interview method was adopted to collect the information. The questionnaires were administered in English and Marathi language. The same questionnaires were used before and after the providing informational booklet. The participants had to answer 26 items to assess the knowledge of harmful effects of plastics. Every right answer gained one (1) point and wrong answer gained zero (0). The overall score ranged from 1-26, participants with good score considered 1-8 score, average score considered 9-17 score and poor score considered 18-26 score.

Data Analysis: The data had been analyzed through descriptive analysis and statistical analysis.

Result

Section I: Description of samples based on personal characteristics

Table No.1 Distribution of participants as per demographic variables (N=40)

Demographic variable	Frequency (f)	Percentage (%)
Age		
· 15-30years	25	62.5%
· 31-45years	7	17.5%
· 46-60years	8	20%
Sex		
· Male	19	47.5%
· Female	21	52.5%
· Other	0	0%
Education		
· Primary	7	17.5%
· Secondary	13	32.5%
· Graduate	20	50%
Occupation		
· service	14	35%
· business	10	25%
· unemployed	16	40%
Marital status		
· Married	20	50%
· Unmarried	19	47.5%
· Widow	1	2.5%

Table no. 1 depicts that 25 (62.5%) of the participants are in age group of 15-30 years and 8 (20%) of them are in age group of 46-60years. Most of subjects 21 (52.5%) were females and 19 (47.5%) were male. 20 (50 %) of participants had completed their graduation.14 (35%) of the participants were doing service and 16 (40%) of them were unemployed. Most of the participants 20 (50%) were married.

Section II – Analysis of data related to the knowledge of harmful effect of plastics

Fig No. 1 Pre & Post Test comparison of Knowledge Score

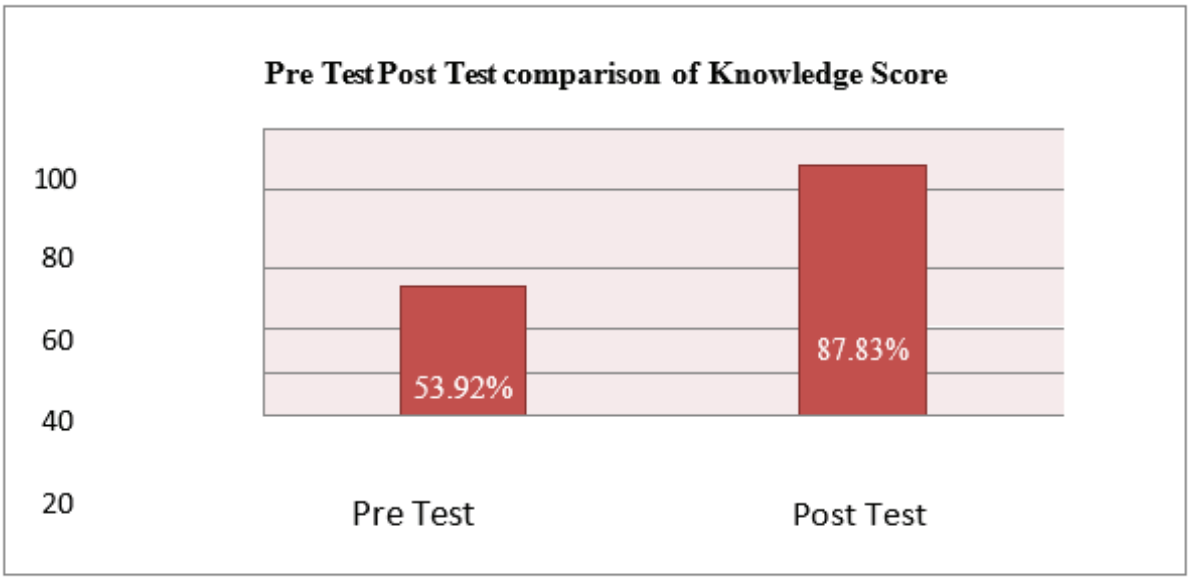


Fig no. 1 shows that participants were asked (26)questions, the Pre-test score of the knowledge of subjects regarding the harmful effect of the plastics was 53.92 % and Post test score of the knowledge was 87.83 %. Hence the informational booklet was effective in improving the knowledge of use of plastics among the urban residents

Table No. 2 Effectiveness of the informational booklet on basic knowledge regarding the harmful effect of the use of plastics

Sr. No	Knowledge Questions	Pretest score		Posttest score	
		Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
1	Plastics are made up of	34	85	40	100
2	Are there any types of plastics	17	42.5	30	75
3	When did dominancy of plastics start	10	25	35	87.5
4	Which among the following is not a property of plastics	9	22.5	23	57.5

Cont... **Table No. 2 Effectiveness of the informational booklet on basic knowledge regarding the harmful effect of the use of plastics**

5	Do plastics make up a large part of municipal solid waste	30	75	36	90
6	Do electronic gadgets contain plastics substances	25	62.5	36	90
7	Does plastics improve durability of goods	25	62.5	37	92.5
8	Are toxic chemicals included in the plastic products we buy	26	65	36	90
9	Which toxins are released when biodegradable plastics break down	8	20	24	60
10	Which size (thickness) of plastic is more harmful	14	35	39	97.5

Table no. 2 depicts that knowledge of the participants has been increased in all the aspects after giving intervention about harmful effect of the use of plastics among residents.

Table No. 3: Effectiveness of the informational booklet on knowledge regarding effect on human health, animals and environment on use of plastics

Sr. No	Knowledge Based Questionnaire	Pretest Score Frequency (n)	Pretest Score Percentage (%)	Posttest Score Frequency (n)	Posttest Score percentage (%)
1	Do you think plastics are harmful for human	32	80%	38	95%
2	Do you think plastics can harm the surrounding environment	37	92.5%	40	100%
3	Do plastics play a role in soil erosion	17	42.5%	37	92.5%
4	Will plastic cause acidic rain	15	37.5%	36	90%
5	Do you think plastics can harm animals	34	85%	39	97.5%
6	Do plastics harm the marine life	28	70%	39	97.5%
7	Do you think plastic burning may cause diseases	31	77.5%	39	97.5%
8	What can be the major diseases of burning plastics	14	35%	36	90%
9	Can plastics cause cancer	23	57.5%	38	95%

Cont... **Table No. 3: Effectiveness of the informational booklet on knowledge regarding effect on human health, animals and environment on use of plastics**

10	Are plastics hazardous when buried in land	26	65%	37	92.5%
11	Does burning of plastics creates plastics toxic fume	30	75%	39	97.5%
12	Which type of plastics can release more amount of harmful chemical into the surrounding soil ,and seep into the ground water and contaminating it	9	22.5%	32	80%

Table no. 3 depicts that knowledge of the participants has been increased in all the aspects after giving intervention regarding effect on human health, animals and environment on use of plastics

Table no.4: Effectiveness of the informational booklet on knowledge regarding recycling and regeneration of plastics

Sr. No	Knowledge Based Questionnaire	Pretest Score frequency (n)	Pretest Score percentage (%)	Posttest Score frequency (n)	Posttest Score percentage (%)
1	Do you think plastics are degenerative	21	52.5	36	90
2	Do you think all the types of plastics are degenerating	21	52.5	27	67.5
3	How much time is required for degeneration of plastics	9	22.5	31	77.5
4	Can plastics be recycled	22	55	36	90

Table no. 4 stated that knowledge of the participants has been increased after giving intervention about recycling and regeneration of plastic

Statistical Analysis

The dependent t test, also called paired t test is used for testing values associated to have some connectivity. Knowledge score of the participants is compared with mean of the pre-test with mean of the post-test using paired t test. Post intervention to pre intervention comparison indicated higher overall knowledge score at significance level ($p<0.05$) and concluded that booklet on harmful effect of plastics proved effective.

Discussion

Plastic is not only harmful to human health but environment and entire ecosystem is affected[7]. People should be educated and get aware of harmful effect of plastics. Pre-experimental study was conducted on 40 participants, which was selected by simple random sampling. One group pre-test post-test design was used. The findings of the study is organized as per objectives of the study. Researcher found out that pre-test knowledge score of the participants was 53%. Information booklet was administered to the participants, post-test was

conducted, results were recorded, and it was found that knowledge score increased to 88%. Hence, it is proved that information booklet on prevention of hazards of plastics among residents of Pune city.it was found that 95% peoples are using plastics in day-to-day practices starting from using plastic bags to plastic cups to drink hot beverages. This study found that education and creating awareness is utmost important among the people to avoid further damage to the health as well as environment. This study justified by the action of state government to ban on plastic bags and it is implemented in most of the states of India, which is supported by the article published in Economic Times in June 2018, which explains in detail hazards of the plastic on environment ultimately human beings are sufferers. The study is supported by the survey conducted by Chitrakshi Khairnar and Sujita Devi -2019 awareness regarding hazards of plastics bag use among adults and she found that majority of the participants (95%) were aware of plastic use is hazardous to health[8].

Conclusion

Findings of the study showed that information booklet on harmful effect of plastics in regards to knowledge and practices of residents of Pune city found effective

Ethical Clearance- Taken from Ethics Committee of the Institute

Source of Funding- Self funded project.

Conflict of Interest – Nil

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A Study to Assess the Knowledge Regarding Air Pollution and Its Prevention People Residing in Selected Urban Areas of Pune City

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Abstract

Background: World Health Organization, around 4.2 million individuals kick the bucket rashly from the impacts of contaminated, open air each and every year, while a further 3.8 million pass on because of messy indoor air.[4,5] Air contamination is a tremendous issue and not only for individuals living in brown haze gagged urban areas: through such things as an unnatural weather change and harm to the ozone layer, it can possibly influence every one of us. So what precisely aims this major natural issue ? Air contamination counteraction is a financial weight to an individual and to a country on a worldwide scale.[1] Air contamination is a danger to human and condition; along these lines, it is critical to comprehend key sources, causes, wellbeing impacts related with air contamination. This monograph gives a review about air contamination and proposes the appropriate preventive measures to lessen air contamination. [2]

Methods: A non-experimental descriptive design with quantitative approach, was adopted to assess the knowledge on air pollution and its prevention and Air Quality index among people residing in urban areas of Pune city. It includes 250 samples from selected areas .Non probability convenience sampling technique was adopted in this study.

Result: Based on the objectives of the study which is to assess the knowledge of the people and the association between the demographic variables, the following result has been identified.

The knowledge level was identified as; 20.8% of the people have a poor knowledge level on air pollution, its prevention and air quality index; 76% of the people have an average knowledge regarding air pollution, its prevention and air quality index and only 3.2% of the people have good knowledge regarding air pollution, its prevention and air quality index. By using the paired t test formula, the t value is 0.05. The demographic variable of education alone has an association with 0.001 as the closest value to the average. The other demographic variables of gender, age and employment do not have an association with the t value, as they are more than the average with findings of 0.119, 0.643 and 0.481 respectively.

Key words: *Air Quality Index, Air pollution, Awareness and knowledge.*

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Introduction

Air contamination might be portrayed as pollution of the climate by vaporous, fluid, or strong squanders or results that can imperil human wellbeing and government assistance of plants and creatures, assault materials, diminish deceivability.[1] The indoor air

contamination became obvious during 80's while outside air contamination has been around for quite a while. Air contamination happens when hurtful or inordinate amounts of substances are brought into Earth's climate. Air contamination is a huge hazard factor for various contamination related maladies.[2] An air contamination is a material noticeable all around that can effects affect people and the environment. The substance can be strong particles, fluid beads, or gases. A contamination can be of characteristic starting point or man-made. Air contamination chance is a component of the peril of the poison and the introduction to that toxin. [3]Air contamination introduction can be communicated for a person, for specific gatherings. An absence of ventilation inside concentrates air contamination where individuals regularly invest most of their energy. India has the most noteworthy demise rate because of air contamination. India additionally has a bigger number of passing from asthma than some other country as per the World Health Organization. There is a positive connection between pneumonia-related passing and air contamination from engine vehicle emanations. [4,5]

Statement of the Problem

"A Study to Assess the Knowledge Regarding Air Pollution and Its Prevention among People Residing in Selected Urban Areas of Pune City."

Objectives of the Study

 To determine the level of knowledge among the people regarding air pollution and its prevention in selected urban areas of Pune city.

 To determine the association between knowledge and socio demographic variables.

Assumption

Assessment will give data on level of awareness and knowledge of people regarding air pollution and prevention measures taken in selected urban areas of Pune.

Research Design

The present study is non experimental descriptive design, which was adopted to assess the knowledge regarding air pollution and its prevention and air quality index among the people residing in selected urban areas of Pune city.

Population

The term population is the entire set of individuals or objects having common characteristics that meet certain criteria for inclusion in the study.

In the present study, the target population comprises people from selected urban areas of Pune city.

Sample

Sample refers to the portion of the population, which represents the entire population.

In this present study, a sample is considered of random people selected in urban areas of Pune city.

Sample Size

Sample size refers to the numbers of subjects needed for the study. The total sample sizes of the study were 250 people from selected urban areas of Pune city.

Sampling Technique

A non probability convenience sampling technique was adopted.

Criteria for Selection of Sample

Inclusion Criteria

People who reside in urban areas.

Specifically living in Pune city.

Those who knows reading and writing English Language

Exclusion Criteria

People who don't show willingness to participate in study

Physically and psychologically disabled.

Analysis

Section l: Distribution of samples with regard to demographic data.

Distribution of demographic data with regard to

gender.

The data that is presented shows that 48% of the samples were from male and 52% of the sample were from female.

Distribution of demographic data of sample with regard to age.

The data that is presented shows that 80% of the samples were from age group 21-30 years, 5% of them were from the age group 31-40 years, 4% of them were from age group 41-50 years and the remaining 11% of them were from age group 51-60 years.

Distribution of demographic data of samples with regard to Education.

The data presented shows that 2.8% of the samples have less than high school degrees, 26% of the samples have high school degrees, 49.6% of the samples have graduate degrees and 21.6% of samples have post graduate degrees.

Distribution of demographic data of samples with regard to employment.

The data present shows that 20.8% of the sample are government employees, 44.4% of the samples are private employees, 16.8% of the samples are household makers and the remaining 18% of the samples are in business.

Section II: Distribution of knowledge among people regarding air pollution, its prevention and air quality index in selected urban areas of Pune city.

N=250

KNOWLEDGE LEVEL	FREQUENCY	PERCENTAGE
Poor	70	28%
Average	170	68%
Good	10	4%

From the above table, it shows that 28% of the people have a poor knowledge level on air pollution, its prevention and air quality index; 68% of the people have an average knowledge regarding air pollution, its prevention and air quality index and only 4% of the people have good knowledge regarding air pollution, its prevention and air quality index.

Section III: Association between demographic variables.

N=250

Demographic Variables	Frequency	Poor Knowledge	Average Knowledge	Good Knowledge	Paired T Test
Gender : Male Female	 110 140	 30 20	 78 116	 02 04	0.119

Cont... **Section III: Association between demographic variables.**

N=250

Age :					
21-30 years	220	40	174	06	0.643
31-40 years	10	03	07	00	
41-50 years	10	02	08	00	
51 and above	10	02	08	00	
Education :					
Less than High school	10	06	04	00	0.001
High school	60	22	37	01	
Graduate	120	10	106	04	
Post Graduate	60	15	44	01	
Employment :					
Private	100	15	82	03	0.481
Government	50	10	38	02	
Business	40	11	29	00	
Household	60	18	41	01	

The association between the demographic variables of gender, age, education and occupation was found by using the paired t test method of calculation. The demographic variable of education alone has an association, having an average of 0.001; which is the closest to 0.05. The other demographic variables of gender, age and employment do not have an association with the t value, as they are more than the average with findings of 0.119, 0.643 and 0.481 respectively.

Ethical Clearance : The study proposal was sanctioned by the ethical committee of Symbiosis College of nursing. This study was explained to participants and informed consent was taken from the participants. Confidentiality of data collected was maintained.

Conclusion

The present study concluded that there is a difference between the demographic variables of gender, age and employment with findings of 0.119, 0.43 and 0.481 respectively. It does not have any association as the values are greater than 0.05. Yet, there is an association between education and knowledge with the value of 0.001 as the closest to 0.05.

The similar research results can be seen in the study conducted by Stephen T. Odonkor and Tahiru Mahami on Knowledge, Attitudes, and Perceptions of Air Pollution in Accra, Ghana: where it has been found that air pollution knowledge increases with the level of education being 2.3 times more likely to be aware of air pollution.

Source of Funding: Self

Conflict of Interest : Nil

Ethical Clearance: Institutional research Committee

Recommendations

1. A similar study can be conducted in the schools for our younger generation who will take over in the future. To make them aware and raise concern and knowledge on air pollution.
2. In order to raise awareness among people various awareness programs and campaigns should be

initiated and conducted by the government.

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A Study to Assess the Knowledge Regarding Air Quality Index among People Residing in Selected Urban Areas of Pune City

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Abstract

Background and Objective: Air pollution is the most common cause of global warming, it has become a major contributor in these past few years due to lack of awareness and control of pollution among people.[1] Most of the people in India are unaware of what Air Quality Index is either. In urban areas excess use of fuels and wastage of resources has caused massive increase in air pollution which lead to decrease in average life span of human beings.[2]

Our objective was to assess the level of awareness and knowledge among the people regarding air pollution and its prevention in selected urban areas of Pune.

As well to find the level of knowledge on AQI and to find the association between knowledge among the people regarding air pollution and its prevention in selected urban areas of Pune city

Methods: A non-experimental descriptive design with quantitative approach, was adopted to assess the knowledge on air pollution and its prevention and Air Quality index among people residing in urban areas of Pune city. It includes 250 samples from selected areas .Non probability convenience sampling technique was adopted in this study.

Result: Based on the objectives of the study which is to assess the knowledge of the people and the association between the demographic variables, the following result has been identified.

The knowledge level was identified as; 20.8% of the people have a poor knowledge level on air pollution, its prevention and air quality index; 76% of the people have an average knowledge regarding air pollution, its prevention and air quality index and only 3.2% of the people have good knowledge regarding air pollution, its prevention and air quality index. By using the paired t test formula, the t value is 0.05. The demographic variable of education alone has an association with 0.001 as the closest value to the average. The other demographic variables of gender, age and employment do not have an association with the t value, as they are more than the average with findings of 0.119, 0.643 and 0.481 respectively.

Key words: *Air Quality Index, Air pollution, Awareness and knowledge.*

Introduction

The environment involves the animate and inanimate surroundings and their interactions making them to coexist, the balance between interaction and coexistence lead to ecological balance. Natural and human activities influence the balance, which is manifested through changes occurring in air.[1] Any disturbances or changes in air is reflected in the deviation from natural in living beings. Air pollution is any atmospheric conditions in which substances are present at high concentration and above their normal ambient level to produce a measurable effect on man and animals[2]. The causes of air pollution are both natural as well as man-made. The pollution generated by natural processes is controlled by natural cycles themselves. In man-made, the human activities such as modern life style, vehicles pollution, thermal generation of power, automobiles, metallurgy and nuclear activities. [3]The causes in community areas

are agriculture side effects, forest fire, tobacco smoke, CFCs, Industrial pollutants, burning fossil fuels including coal, oil and natural gas.[4] These activities change the composition of Pune air by adding other unwanted gases. The air prevention and control of pollution act 181 the air act is an act to provide for the prevention, control and abatement of air pollution and for the establishment of boards at the central and state levels with a view to carrying out the aforesaid purposes.[5]

As we all know, the atmosphere is the life blanket of the earth. It is therefore essential that we know more about the atmosphere and the way in which it is polluted. Air is considered safe when it contains no harmful dust and gases.

Statement of the Problem

"A Study to Assess the Knowledge Regarding Air Pollution and Its Prevention and Air Quality Index among People Residing in Selected Urban Areas of Pune City."

Objectives of The Study

- To determine the level of knowledge among the people regarding air quality index in selected urban areas of Pune city.
- To determine the association between knowledge and socio demographic variables.

Assumption

Poor air quality leads to Health concerns and causes serious health problems related to it.

Absence of knowledge and awareness about air pollution and air quality index challenges and affects air quality of a geographical area.

Research Design

The present study is non experimental descriptive research design, which was adopted to assess the knowledge regarding air air quality index among the people residing in selected urban areas of Pune city.

Population

The term population is the entire set of individuals or objects having common characteristics that meet certain criteria for inclusion in the study.

In the present study, the target population comprises people from selected urban areas of Pune city.

Sample

Sample refers to the portion of the population, which represents the entire population.

In this present study, a sample is considered of random people selected in urban areas of Pune city

Sample Size

Sample size refers to the numbers of subjects needed for the study. The total sample sizes of the study were 250 people from selected urban areas of Pune city.

Sampling Technique

A non probability convenience sampling technique was adopted for this study.

Criteria for Selection of Sample

Inclusion Criteria

People who reside in urban areas.

Specifically living in Pune city.

Exclusion Criteria

People who were not willing to participate in the study.

Physically and psychologically disabled.

Analysis

Section l: Distribution of samples with regard to demographic data.

Distribution of demographic data with regard to gender.

The data that is presented shows that 46% of the samples were from male and 54% of the sample were from female.

Distribution of demographic data of sample with regard to age.

The data that is presented shows that 88.8% of the samples were from age group 21-30 years, 5.6% of them were from the age group 31-40 years, 4% of them were from age group 41-50 years and the remaining 1.6% of them were from age group 51-60 years.

Distribution of demographic data of samples with regard to Education.

The data presented shows that 2.8% of the samples have less than high school degrees, 26% of the samples have high school degrees, 49.6% of the samples have graduate degrees and 21.6% of samples have post graduate degrees.

Distribution of demographic data of samples with regard to employment.

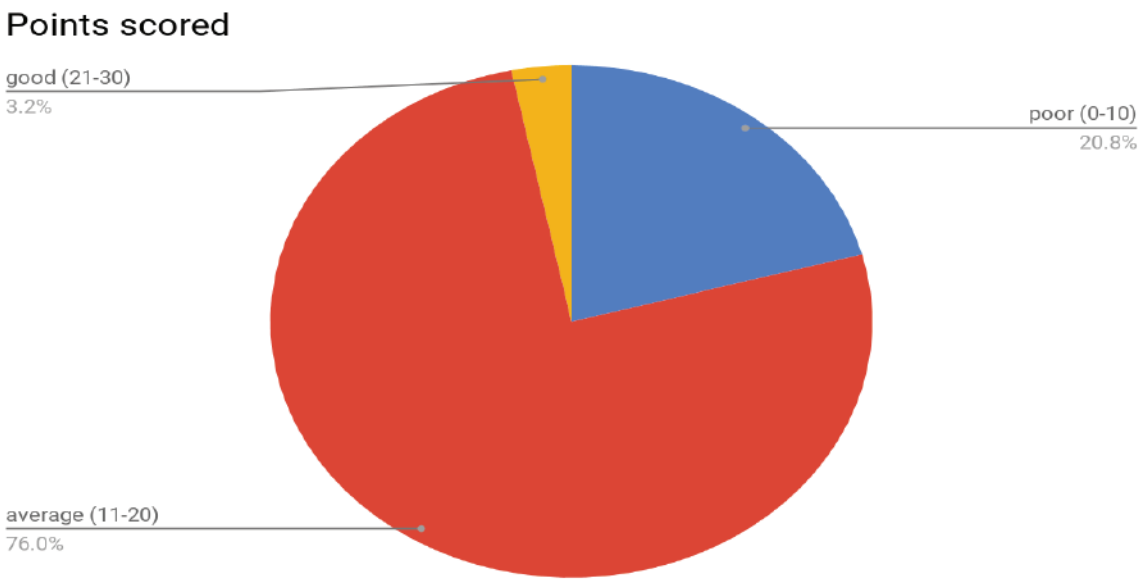
The data present shows that 20.8% of the sample are government employees, 44.4% of the samples are private employees, 16.8% of the samples are household makers and the remaining 18% of the samples are in business.

Section II: Distribution of knowledge among people regarding air pollution, its prevention and air quality index in selected urban areas of Pune city.

N=250

KNOWLEDGE LEVEL	FREQUENCY	PERCENTAGE
Poor	52	20.8
Average	190	76
Good	8	3.2

From the above table, it shows that 20.8% of the people have a poor knowledge level on air pollution, its prevention and air quality index; 76% of the people have an average knowledge regarding air pollution, its prevention and air quality index and only 3.2% of the people have good knowledge regarding air pollution, its prevention and air quality index.



Pie diagram showing categorization of knowledge level.

Section III: Association between demographic variables.

N=250

Demographic Variables	Frequency	Poor Knowledge	Average Knowledge	Good Knowledge	Paired T Test
Gender : Male Female	 114 136	 27 19	 85 113	 02 04	0.119
Age : 21-30 years 31-40 years 41-50 years 51 and above	 222 14 10 04	 39 03 02 02	 177 11 08 02	 06 00 00 00	0.643
Education : Less than High school High school Graduate Post Graduate	 07 66 123 54	 06 22 09 09	 01 43 110 44	 00 01 04 01	0.001
Employment : Private Government Business Household	 110 53 45 42	 15 10 11 10	 92 41 34 31	 03 02 00 01	0.481

The association between the demographic variables of gender, age, education and occupation was found by using the paired t test method of calculation. The demographic variable of education alone has an association, having an average of 0.001; which is the closest to 0.05. The other demographic variables of gender, age and employment do not have an association with the t value, as they are more than the average with findings of 0.119, 0.643 and 0.481 respectively.

Ethical Clearance : The study proposal was sanctioned by the ethical committee of Symbiosis College of nursing. This study was explained to participants and informed consent was taken from the participants. Confidentiality of data collected was maintained.

Conclusion

In conclusion it has been observed that there is a significant difference between the demographic variables of gender, age and employment with findings of 0.119, 0.43 and 0.481 respectively. It does not have an association as the values are greater than 0.05. Yet, there is an association between education and knowledge with the value of 0.001 as the closest to 0.05.

People have partial knowledge regarding Air quality index. Similar result can be seen in the study conducted by Lagorio S, , et al. on Air pollution and lung function among adult . They recommended that community should be more aware about the air pollution effects and value of air quality index

Source of Funding: Self

Conflict of Interest : Nil

Ethical Clearance: Institutional Research committee

Recommendations

1. Air quality index should be an important topic of discussion during primary as well as secondary education

2. The constitution of India must take serious and strict measures in enforcement of laws developed for controlling air pollution to the violators.

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Knowledge and Attitude on Hazards of Passive Smoking among Women of Pune City

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Abstract

Passive smoking has become a serious health issue in the recent times. World is facing serious public health problem known as Second Hand Smoke (SHS). Globally, it is estimated that one-third of the population is frequently exposed to Second Hand Smoke, with 0.6 million individuals dying each year from SHS exposure. This paper was in response to the rising trend of active smoking where the nearby people are affected more than the actual smokers. This study was conducted with objectives to assess the knowledge, attitude and association between knowledge and attitude. Quantitative research approach with descriptive research design was used on a sample size 50 women with age group of 12 years and above using non-probability purposive sampling. Analysis of data was done by using descriptive and inferential statistics.

Keywords: *Women, Hazards, Passive Smoking, Knowledge and Attitude*

Introduction

Passive smoking is the inhalation of smoke, called second-hand smoke (SHS). Persons other than the intended "active" smoker also sometimes refer it to environmental tobacco smoke (ETS). It occurs when tobacco smoke permeates any environment, causing its inhalation by people within that environment. Second-hand smoke exposes a healthy individual to disease, disability, and death.[1] Adolescents are the vulnerable population to the adverse effects of environmental tobacco smoke. This smoke induces Asthma, exacerbation, as well as acute respiratory infections.[2]

Tobacco smoke reduces the lung functions, and increased bouts of exacerbations in both adults and adolescents.[3] Smoking rates among women are growing in developing countries which were hard to find sometimes ago. The researcher wants to understand the magnitude of exposure to secondhand smoke among antenatal mothers in an urban slum. "Smoking is injurious to health".[4]

Nicotine, carbon monoxide and tar are the main components of Cigarette smoke leading to a disease and affect the individual's health drastically. It can lead to tachycardia and hypertension by releasing hormones like adrenaline that leads to constriction of blood vessels.

NEED FOR THE STUDY

Smoking and passive smoking are harmful to one's health: they increase the risk or exacerbate the severity of cancer, respiratory diseases, and cardiovascular diseases.[6,7,8]

Research Methodology

A quantitative research approach with descriptive design was used. 50 women whose age is 12 yrs and above were taken from selected urban community by using non-probability purposive sampling technique. Data was analyzed using descriptive and inferential statistics.

Findings

Descriptive Analysis

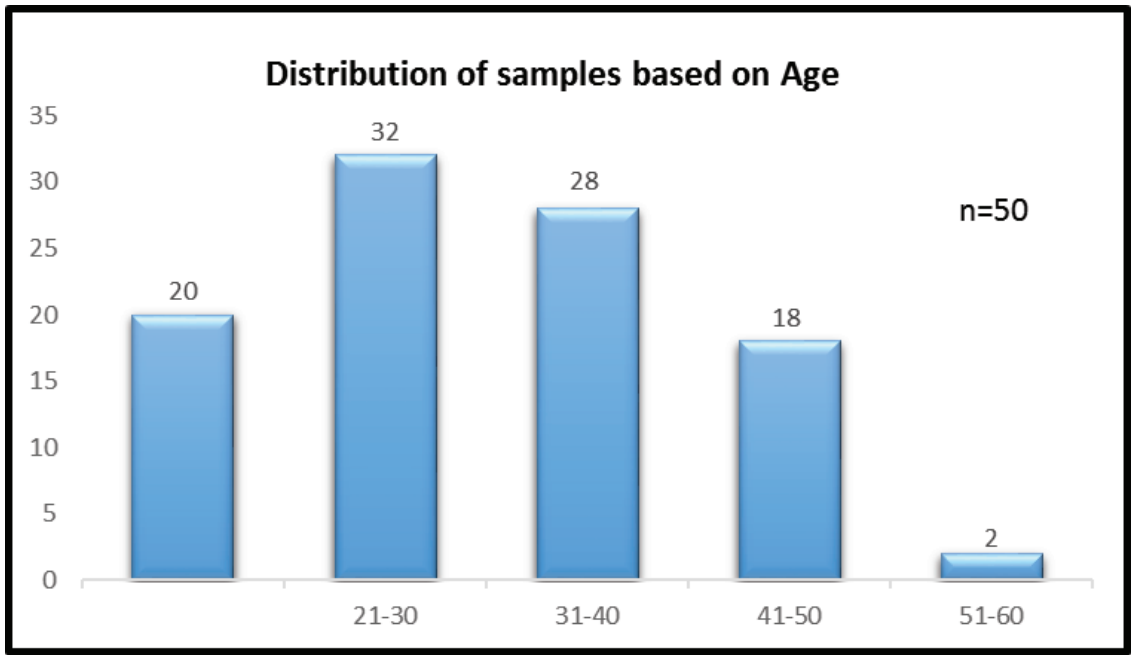


Fig 1: Distribution of sample based on their age

Fig 1 depicts that 20% of women were between 12-20 years of age in the experimental group, 32% of women were from 21-30 years, 28% of women were from 31-40 years, 18% of women were from 41-50 years and 2% of women were from 51-60 years.

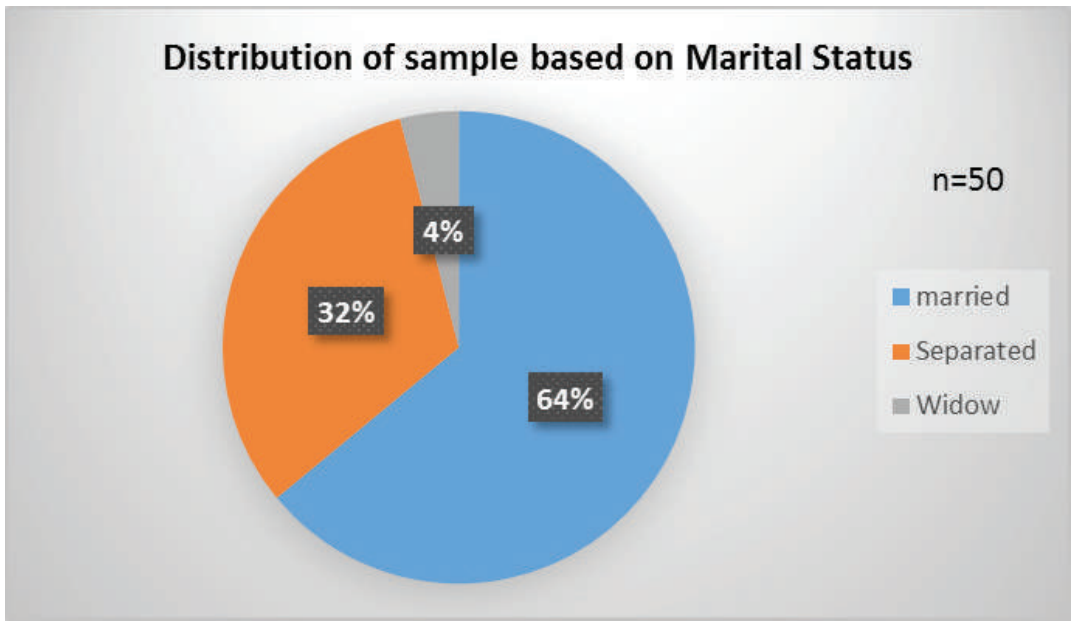


Fig 2: Distribution of sample depending on their Marital Status

Fig 2 depicts that 64% of women were married, 32% of women were separated and 4% of women were widows.

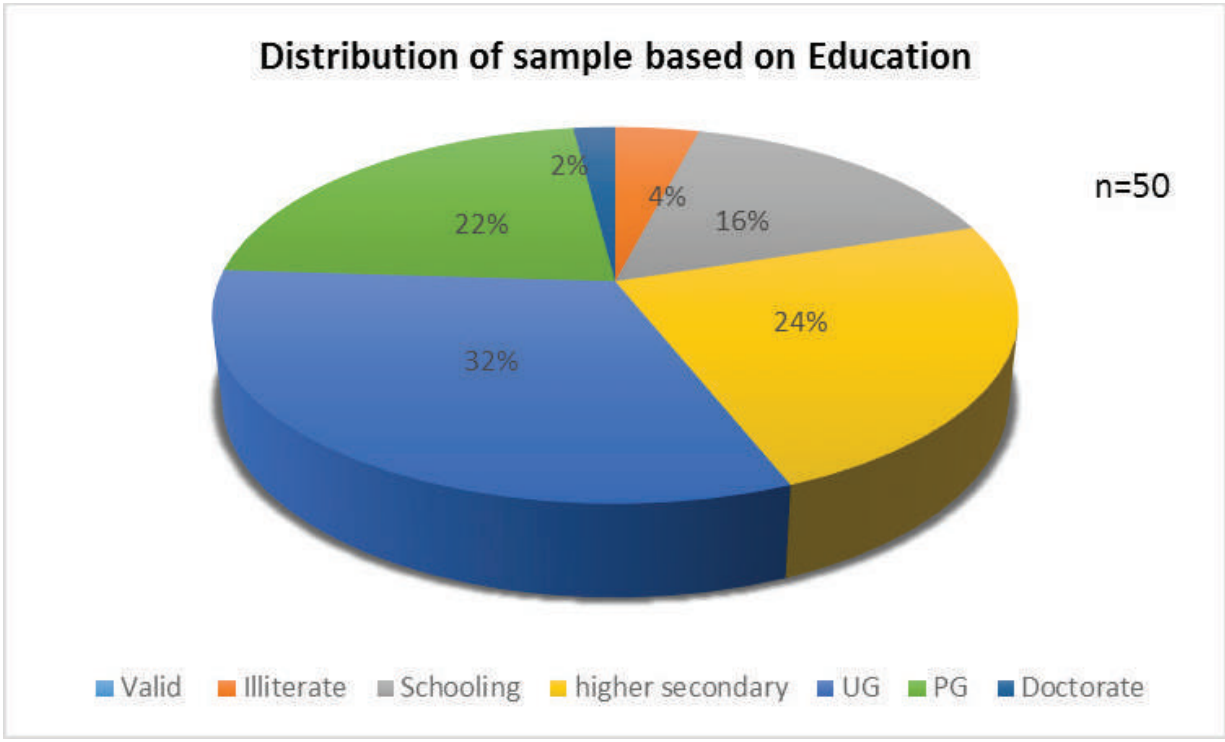


Fig 3: Distribution of sample depending on education

Fig 3 depicts that 4% of women were illiterate, 16% of women were schooling, 24% of women were higher secondary, 32%of women were UG, 22% of women were PG, 2% of women were Doctorate.

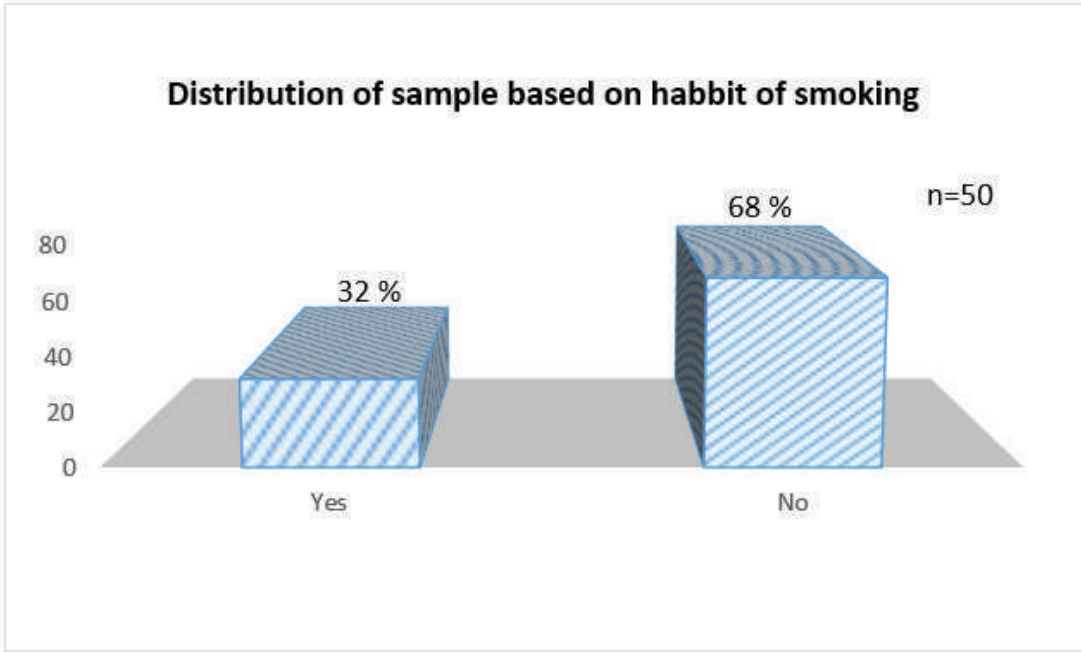


Fig 4: Distribution of samples depending on habit of smoking

Fig 4 depicts that 32% of women are exposed to tobacco smoke, and 68% of women are not exposed to tobacco smoke.

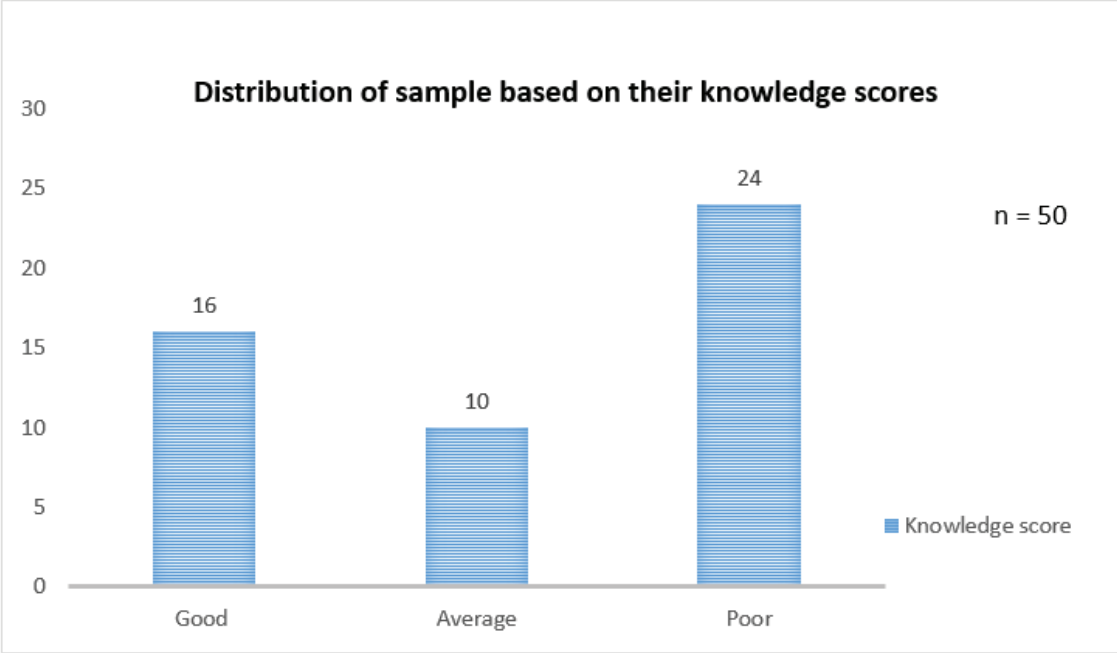


Fig 5: Distribution of sample depending on their knowledge score

Fig 5 depicts that 24% of women are having poor knowledge, 16% of women are having good knowledge and 10%of women are having average knowledge.

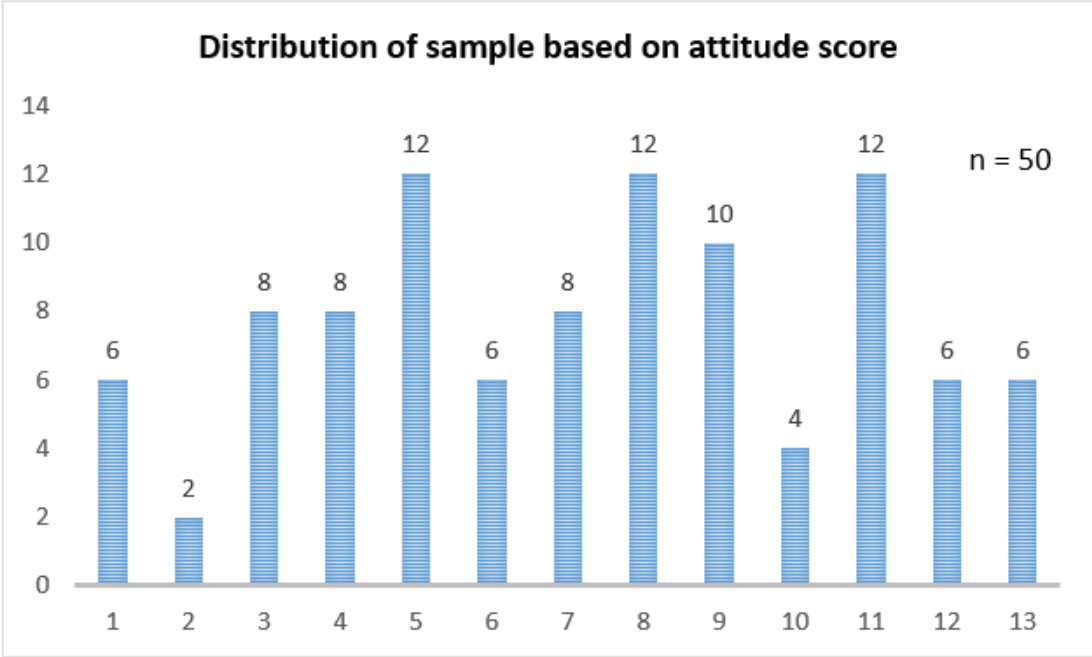


Fig 6: Distribution of sample depending on their attitude score

Fig 6 depicts that the attitude score maximum score is 45% of women are having good attitude and 25%of women are having minimum score.

Table 1: Association between Knowledge and Attitude Score

Chi-Square Tests				
Test	**Value**	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**
Pearson Chi-Square	191.111a	204	.732	.000
Likelihood Ratio	135.234	204	1.000	.000
N of Valid Cases	50			

Table 1 depicts that there is an association between knowledge and attitude score, the attitude and knowledge score are highly associated with each other ($p<0.05$)

Results

Results revealed that 24% of women had poor knowledge, 16% of women had good knowledge and 10% of women had average knowledge. The maximum attitude score was 45% who had good attitude and 25% of women had minimum score. There was an association between knowledge and attitude score, the attitude and knowledge score are highly associated with each other ($p < 0.05$). The demographic variables showed dynamic results. 20 % of women were between 12-20 years of age, 32% of women were from 21-30 years of age, 28% of women were from 31-40 years of age, 18% of women were from 41-50 year of age and 2% of women were from 51-60 year of age. 64% of women were married, 32% of women were separated, 4 % of women were widows. 4 % of women were illiterate, 16% of women had completed their schooling, 24% of women completed their higher secondary, 32% of women were UG, 22% of women were PG, and 2% of women were Doctorate. 36% of women were self-employed, 40% of women were employed, and 24 % of women were homemakers. The income per month of 12 % of women was <10000, 58% of women earned 10001-30000, 26% of women earned 30001- 60000, and 4% of women earned >60000. 32% of women are exposed to tobacco smoke.

Discussion

The study was conducted on a group of 50 women about knowledge and attitude regarding hazards of passive smoking among women. Results revealed that 24% of women had poor knowledge, 16% of women had good knowledge and 10% of women had average knowledge. The maximum attitude score was 45% who had good attitude and 25% of women had minimum score. There was an association between knowledge and attitude score, the attitude and knowledge score are highly associated with each other ($p < 0.05$).[9]

Implications

Nursing Practice

The findings of the study regarding knowledge and attitude about hazards of passive smoking indicate need to assess early and deal with it effectively. The nurse as a caretaker should promote healthy behavior, prevent risk through lifestyle changes. The cases found positive for respiratory problems may be promptly taken care in hospital settings as nurses will be competent enough to manage such cases.

Nursing Education

The nursing curriculum should cover broader aspects of study with objectives to identify the risk behavior, adopt preventive, control measures, and combat the ill effects due to passive smoking. In-service education must be an integral part of Nursing education. Nurses should be encouraged to provide health teaching as and when required and thereby promote good health. Nurses play a pivotal role in educating common man in community settings. Discharge planning and educating

the patients will also help in reducing the incidences and relapses of respiratory illnesses caused due to passive smoking.

Nursing Research

Nursing research uplifts the nursing profession, thus research studies can develop new strategies and further studies can be carried out based on present findings. Similar studies can be done to find the effects of smoke / passive smoke on growth & development of small children.

Limitations

Ø The study is limited to a sample of 50 women from community of Pune City.

Ø The responses of questions may be based on their nature towards subjectivity.

Recommendations

Ø It is suggested that a larger sample may be used to conduct a similar study .

Ø It is recommended that further study can be conducted to identify the hazards of passive smoking among under-five children.

Ø A qualitative study also can be conducted on the lived experiences of patients who have quit smoking in selected rehabilitation centers of the state.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: This article is cleared from ethical committee of Symbiosis College of Nursing

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COVID – 19 Awareness among Adolescents in Pune City: A Survey Study

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Abstract

Introduction: Coronavirus 2019 (COVID -19) is an infectious disease caused by a newly discovered novel coronavirus caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The new virus was first discovered in December 2019 in Wuhan, Hubei province of China. **Background and Objectives:** The Covid-19 pandemic has rapidly affected the world around us and the society which we belong to. The Aim of this study is to assess the awareness of COVID-19 disease among adolescents and to find out the association between the awareness and selected demographic variables. **Materials and Methods: Quantitative** non experimental descriptive survey research design was used with sample size of 108 adolescents of Pune city. Non probability convenience sampling technique was used for the study. Reliability of the tool and was 0.81. The tool was divided in two sections. Section I included demographic proforma of the sample. Section II was about self-administered structured knowledge questionnaire comprised of twenty questions (Multiple choice questions). Each correct item was given 01 mark and wrong response received 0 marks. After taking the consent from each samples tool was administered to participants. **Results**: the awareness on the basis of frequency and percentage among adolescents of Pune city that majority of respondents have good knowledge (52.8%), average knowledge (43.50%) and 3.7% respondents had poor knowledge. **Conclusion:** Awareness in terms of knowledge among adolescents are good followed by average score. It is very crucial and essential to maintain and update self with the current scenario related to COVD 19 disease

Key words: *Knowledge, Covid-19, Adolescents, infectious disease, Coronavirus*

Introduction

The Covid-19 virus was initially called as 2019-nCoV but later the International Committee on Taxonomy of viruses termed it as syndrome coronavirus 2 (SARS-CoV-2) []

Novel coronavirus is found to be new type of strain which was not found in humans earlier [2]. It has found out that MERS-CoV was being transmitted from the camels to humans whereas SARS-CoV was being transmitted from civet cats to the human beings [3]. Coronavirus disease was declared as a Pandemic disease by The World Health Organization (WHO) on March 11, 2020[4]. The source of SARS CoV-2 is still to be found out, although many investigations are still going on to find out the exact zoonotic source of the outbreak [5].

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Statement of the Problem

"COVID – 19 Awareness among Adolescents in Pune city: A Survey Study"

Objectives of the study

§ To describe the Socio demographic characteristics of adolescents.

§ To assess the awareness of COVID-19 disease among adolescents

Assumptions

Adolescents of the Urban Community of Pune city may have awareness about the COVIF-19 disease.

Operational Definition

Awareness: refers to the knowledge among the adolescents of Urban Community of Pune city as measured by the self-structured questionnaire-based survey on COVID-19.

Covid-19: - Covid-19 disease refers to a mild to severe respiratory illness which is caused by a novel coronavirus

Adolescents: - Young adults who are residents of Urban Community of Pune city and are between the age group of 16-18 years.

Infectious Disease: - refers to a disease which is caused by the entry of the pathogenic microorganisms into the body where they grow and multiply.

Coronavirus: - refers to a larger group of viruses that causes a variety of illness in human beings and various animals.

Method and Material

Study used the quantitative non experimental descriptive survey research design. Sample size was comprised of 108 adolescents of Pune city. Non probability convenience sampling technique was used for the study. Urban community of Pune city was the setting of the study. Cronbach's alpha method was used for the reliability of the tool and was 0.81, which is highly reliable. A self-administered structured knowledge questionnaire on COVID-19 disease was used. The tool was divided in two sections. Section I included demographic proforma of the sample. Section II was about self-administered structured knowledge questionnaire comprised of twenty questions (Multiple choice questions). Each correct item was given 01 mark and wrong response received 0 marks. After taking the consent from each samples tool was administered to participants.

Findings

Section – I: Description of Demographic variable

Table 1: Distribution of demographic variable

n=108

Variable		Frequency (f)	Percentage (n)
Age (In Years)	16	4	3.7%
	17	10	9.3%
	18	94	87%
Gender	Male	52	48.1%
	Female	56	51.9%
During lockdown staying with	Family	87	80.6%
	Friends	7	6.5%
	Colleagues	4	3.7%
	Alone	3	2.8%
	Away from home	7	6.5%

Cont... **Table 1: Distribution of demographic variable**

n=108

Religion	Hindu	87	80.6%
	Muslim	17	15.7%
	Christian	04	3.7%
	Sikh	0	Nil
	Any other (Specify)	0	Nil

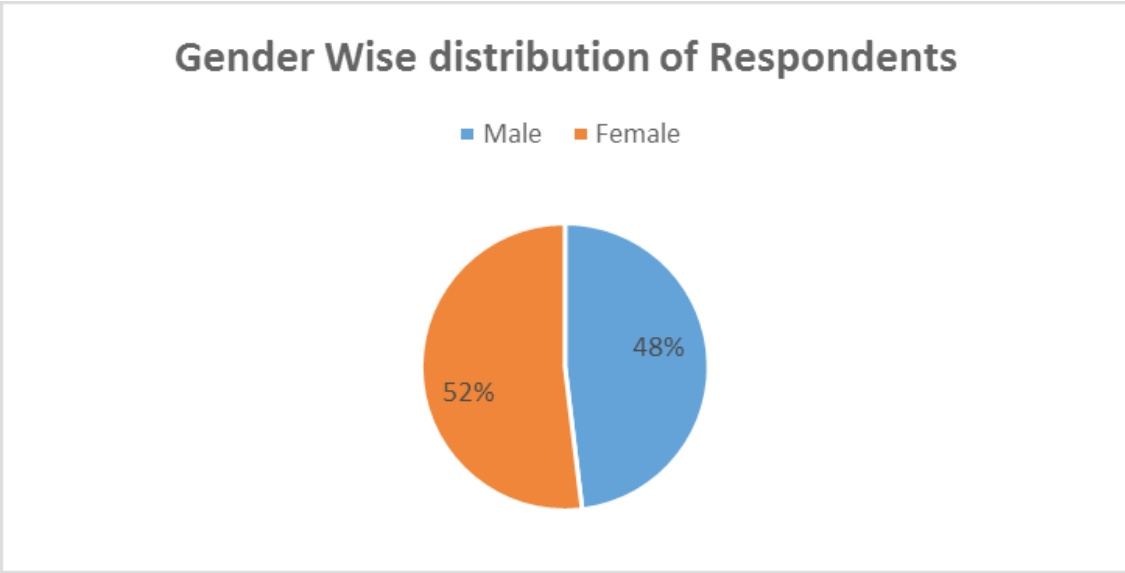


Fig 1. Gender wise distribution of Respondents

Section – II

Assessment of Awareness of COVID – 19 among Adolescents in Pune city

Table 2: Knowledge score of COVID – 19 among Adolescents

Knowledge	**Frequency**	**Percentage**
Good (14-20)	57	52.8%
Average (7-13)	47	43.5
Poor (0-6)	04	3.7

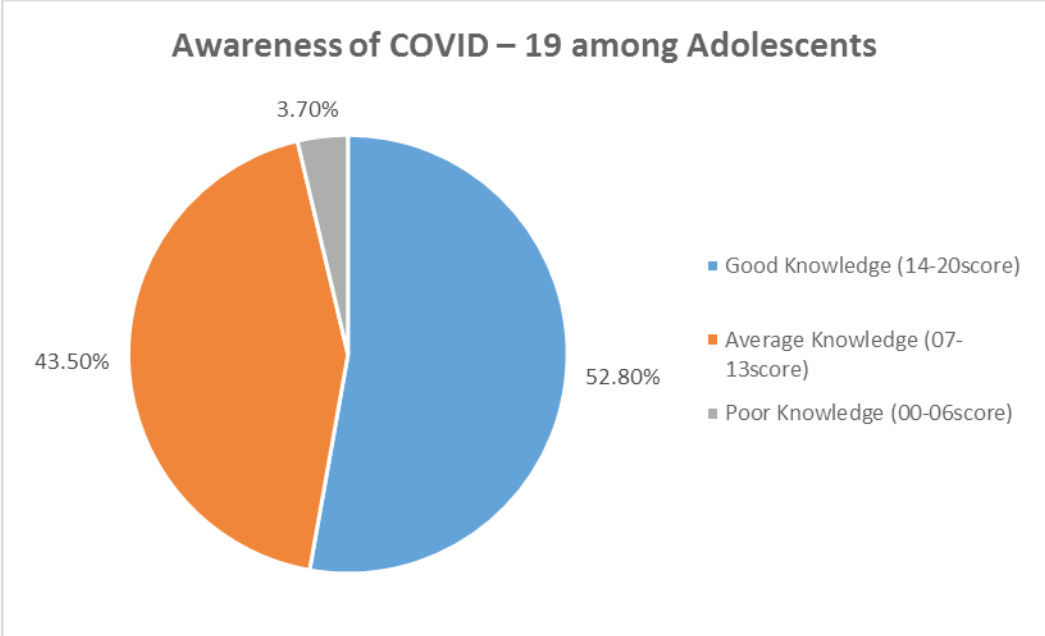


Fig 2: Awareness of COVID – 19 among Adolescents in Pune city

Fig 2 shows the awareness on the basis of frequency and percentage among adolescents of Pune city that majority of respondents have good knowledge (52.8%), average knowledge (43.50%) and 3.7% respondents had poor knowledge

Discussion

Study findings of present study revealed that Knowledge score of adolescents on COVID 19 is good. Majority of respondents have good knowledge (52.8%), average knowledge (43.50%) and 3.7% respondents had poor knowledge.

Study findings emphasized that the most common sources of COVID-19 information and considered trustworthy but were less likely to reach less educated households.[6]

Conclusion: Awareness in terms of knowledge among adolescents are good followed by average score. It is very crucial and essential to maintain and update self with the current scenario related to COVD 19 disease.

Conflict of Interest: Nil

Source of Fund: Self-funded

Ethical approval: Study was approved at Institute Ethical subcommittee. Informed consent was obtained from each sample. Privacy and confidentiality maintained

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A Descriptive Study to Assess on Knowledge and Attitude of Nurses Regarding Prevention of Pressure Ulcers in Selected Hospitals

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Abstract

Background: - Inspite of the fact that decubitus ulcer progress is normally viewed as a sign for standard of the care provided by the nurse, queries and anxiety about the condition during which they are inevitable still persists. **Objective:** To assess the knowledge and attitude of the nurses regarding safeguard of the pressure ulcers in selected hospitals. **Methodology:** A Non-Experimental Descriptive Research Approach is used. 60 samples were selected by using Nonprobability Purposive Sampling Technique. Investigator used structured questionnaire to assess the knowledge, 5-point Likert's scale to assess the attitude. **Result:** Majority of the subjects (67%) were between the age group 21-25 years while the remaining (33%) subjects were of the age group of 26 -30 years. Majority of the subjects (57%) were females while (43%) were males. Majority of the subjects (98.3%) were B.Sc. nurses while (17%) Post Basic B.Sc. qualified. Majority of the subjects (64%) had 2.1 - 4 years of experience while the remaining (36%) had 1- 2 years of experience. Majority of the subjects (75%) had good knowledge score and the remaining (25%) had average knowledge score. All of the subjects (100%) had a positive view score regarding anticipation of the pressure ulcers. **Conclusion:** Findings indicate that the questionnaire and Likert attitude scale was effective in assessing the knowledge and point of view of the nurses regarding the prevention of Bed sore. Study also concluded that majority of the nurses had good awareness and positive approach towards the prevention of pressure ulcer.

Keywords: *Assess, Knowledge, Attitude, Nurses, Pressure ulcer.*

Introduction

Pressure Ulcer is defined as a localized vandalization to the skin or to the primary tissues generally above the skeletal prominence as a consequence of continuous pressure or abrasion [1]. The persons mostly at the possibility of acquiring pressure ulcers are the ones who has impaired mobility and bed ridden patients [2]. In Jordanian, a study was carried out which stated that the nurses possess inadequate knowledge related to prevention of pressure ulcer in contrast with the national advisory pressure ulcer council guidelines. Hence it is essential for all the nurses to be acquainted with the guidelines which are standardize to prohibit any problems related with bed sores to foster patient safety and greater results [3]. The four mechanism which contribute towards the development of pressure ulcers are Moisture, Friction, External Pressure and Shearing. Pressure ulcers are caused due to inadequate blood supply to the tissues. The most common sites of Bed sores are ischial tuberosity, sacrum, Heels, buttocks, shoulder blade, ball of foot, elbow and back of head [4].

Various reasons add on to the occurrence of Pressure ulcers which includes poor nutrition, ageing, abrasion, previous ulcers, smoking, restricted mobility, disease of the vascular periphery, diabetic mellitus and moisture. The attitude and knowledge of the nurses are seen as an extrinsic factor towards the formation of pressure ulcer [5].

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Awareness and a positive attitude to prevent the Bed sores and adequate knowledge are key factors to prevent the pressure ulcers effectively.

Statement of the Problem:

"A descriptive study to assess on knowledge and attitude of nurses regarding prevention of pressure ulcers in selected hospitals."

Objectives of the Study:

1. To Assess the knowledge of the nurses regarding prevention of pressure ulcers in selected hospitals.

2. To Assess the attitude of nurses to prevent pressure ulcers in selected hospitals.

Assumptions:

Nurses in the selected hospitals may have awareness and a positive approach towards prevention of Bed sores.

Operational Definitions:

Assess: Assess refers to the make a judgement related to Pressure ulcer.

Knowledge: Knowledge refers to the understanding and awareness of nurses related to prevention of pressure ulcers.

Attitude: Attitude refers to the point of view of nurses towards the anticipation of decubitus ulcers.

Nurses: A person who is trained to provide care for the sick by preventing the ulceration.

Pressure Ulcers: Refers to the injury caused to the skin or underlying tissues by continuous pressure over it.

Material and Methods

A Non-Experimental Research design with quantitative approach was used. 60 samples selected by Non-Probability Purposive sampling technique. The study was conducted among the selected hospital of Pune city. The tool included two sections; the first section included demographic data the second section included a knowledge questionnaire and Likert scale for measuring the attitude related the precaution of pressure ulcer.

Findings

Section- I

DISTRIBUTION OF SUBJECTS IN RELATION TO DEMOGRAPHIC DATA

Table no.1: Distribution of staff nurses working in selected hospitals in relation to demographic data

n=60

Parameters		No of nurses	Percentage (%)
Age of nurses (Years)	21 – 25	40	67
	26 – 30	20	33
Gender	Male	26	43
	Female	34	57
Education	B Sc	50	83
	PB B Sc	10	17
Total years of experience (Years)	1 – 2	22	36
	2.1 – 4	38	64

SECTION - II

ANALYSIS OF LEVEL OF AWARENESS AND VIEWPOINT OF NURSES REGARDING PREVENTION OF DECUBITUS ULCERS

Table no.2: Distribution of level of knowledge score of nurses related to avoidance of ulcers

	Poor (0-8)	Average (9-17)	Good (18-25)
Knowledge score	0%	25%	75%

Table no.3: Distribution of attitude of the nurses regarding avoidance of Bed sores.

Attitude of nurses regarding avoidance of Bed sores	Positive Attitude	Negative Attitude
Attitude Score	100%	0%

Discussion

In this present study a Non-Experimental Descriptive research design is been used. Non-Probability Purposive Sampling Technique was used for selecting 60 samples. The patient answered and the researcher recorded the answer. The tool included two section; the first section had demographic data and the second section included the knowledge questionnaire and Likert attitude scale. The data was analysed further which revealed Majority of the subjects 88.3% belonged to the age group of 21-25 years, 81.7% were females, 51.7% of the subjects had 1-2 years of experience, none of the subjects received in service education. Also, majority of the subjects 58.33% had average knowledge and the remaining 41.67% had good knowledge in the pretest while in the post test all of the subjects 100% had a good knowledge score. 90% of the subjects had good practice score and the remaining subjects 10% had an average practice score in the pretest; while all the subjects 100% had a good practice score in the post test.

Conclusion

The findings indicate that the questionnaire and Likert attitude scale was effective in assessing the knowledge and attitude of nurses related to the anticipation of pressure ulcer. Study also concluded that majority of the nurses had good cognition and positive beliefs towards the elimination of pressure ulcer.

Conflict of Interest: - Nil

Source of Funding- Funded by self

Ethical Clearance-: Obtained from Institutional Research Committee and Hospital. Privacy and confidentiality maintained.

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A Study to Assess the Impact of Social Media on Psychological Health During Lock Down among Adolescents in the Selected Colleges of Pune City

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Abstract

Introduction -Research focuses on the use of social media on psychological health during lock down among adolescents .Technology is having highly command on human beings. As it is found that adolescents has become the victim of using excessive social media. It is extremely necessary to educate young population regarding the correct utilization of social media to uphold in their personal as well as professional growth. Technology has significant impact on our body as well on mind. As our lifestyle is also changing hence social media is having lot of impact in our mind. [1] During complete lock down it is observed that social media has influence the young minds a lot. The (COVID19) epidemic is a public health emergency of international concern, it is decision of governmental bodies that to stop the spread the corona virus .Hence the lock down was announced to spread of this virus. The present pandemic situation is rapidly affecting the population and having the negative impact on the whole population not only on physically but mentally also. [2]As when the complete lock down was announced it was extremely difficult to an individual to cope up with that changing life style. The purpose of study was to check the impact of social media on adolescents during lock down and it was found that the adolescents had major negative impact of excess use of social media and affected not only physically but emotionally also . **Design** - Non-Experimental Descriptive survey. **Results**- Non Probability convenience sampling technique used to collect information from 100 participants studying in schools and colleges. And found that 68% adolescents have negative impact and 32% have positive impact of social media on mental health during lock down among adolescent age group **Conclusion**- The research was emphasis on the effect of utilization of social media on mental health of young generation during lock down. . It is concluded that they are misusing their valuable time instead of giving focus on study which is leading to not only physical damage but also have impact on their psychological health

Keywords – *social media, lock down, psychological health, adolescents*

Introduction

Adolescent is very crucial phase of Human life because it's a transformation from child into an adult. Now a day due to changing lifestyle the adolescent's behaviour is also changed. This age group has become more attracted towards social media. And we have seen many parents have complaining that their child is using social media without any limit. This is not only affected physical health but psychological health of the child. [3] As during lock down it was found that social media had tremendous impact on young generation as well distracted their mind from studies which is further affected their personal and professional growth.

Objectives of the Study

1. To know the effect of social media towards adolescents

2. To assess the influence of social media on psychological health of adolescents

3. To find association between influence of social media and mental health among adolescent

Methodology

The non-experimental descriptive survey study was conducted in the Pune city where adolescents

were participated through non probability convenience sampling technique. The self-administered structured questionnaires and check list were used. The collected data were analyzed by using descriptive and inferential statistics

Analysis and Interpretation

Description of subject characteristics

It is observed that the majority of the participants (67%) were under the age group of 20-21, years, (18 %) were from 18-19 yrs. And the remaining (15%) were comes under 16 -17 yrs. as of age group.

Male participants were least in numbers i.e. 25% as compare to female participant's i.e.

75% %

Out of 100 participants selected for the study majority i.e. 1% from 10^{th} std , 12% from 12^{th} std and remaining were from 87% were from other educational background

It was observed that during lock down their stay along with family members were 91%, alone 8% and 1% with others.

Discussion

The study was conducted to find out the impact of social media on psychological health during lock down among adolescents in the selected colleges of Pune city". Total 100 participants have participated in the study. [4] Out of which three age group was selected from 16-17yrs, 18-19 yrs and 20-21 years the majority of the participants were from the age group of 20-21 yrs. and majority of female participants have participated in the study.

The liker scale was used to assess the data. After obtaining the data the result was found that

The research found that 68% have negative impact and 32% had positive influence of social media on mental health of during lock down among adolescents. The same study was conducted with the title [5] "A Study on Impact of COVID-19 Lockdown on Psychological Health, Economy and Social Life of People in Kashmir" and they have found that (67.5%) reported that if this lock down will continue which will affect not only physical health of an individual but also psychological health will disturbed where, as (23.5%) of the participants says that the lockdown will not have much impact on the mental health as people are in the awareness of the fact that lockdown is extremely needed for their protection .

Conclusion

This Research was giving focus on the effect of social media on psychological health among adolescents during lock down .The researcher found that due to lock down the adolescent's age group have been affected and have negative impact of use of social media and affected their psychological health also. [6] It is found that they are wasting their valuable time instead of focusing on studies. The current COVID-19 pandemic situation which is going out of control is causing widespread concern to public health.. The awareness needs to be created among youth of safely and carefully handling the social media so it will have balance and healthy life and will not affect the psychological health of an individual.

Acknowledgement- The researcher thanks the participants who actively participated in this study. The main objective of writing this paper was to assess that how social media had influence the youth generation and affected not only their physical but psychological health also during lock down.

Ethical Clearance-Institutional Research committee

Source of Funding- Self

Conflict of Interest – Nil

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Association of Knowledge on Environmental Hygiene With Demographic Variables of Rural Population in the Community

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Abstract

Background: Environmental hygiene is a major concern in developing countries. The major factors responsible for poor domestic as well as environmental hygiene are poverty and population explosion. Practices of solid waste disposal, water waste disposal and human habitat have been contributing factors for poor environmental hygiene. **Objective**: To find out the association of knowledge on environmental hygiene with demographic variables of rural population in the community. **Methodology:** Pre-experimental (pretest posttest one group design) was used to find out the association of knowledge on environmental hygiene with demographic variables of rural population in the community. 150 samples were selected using convenience sampling technique. **Results:** Chi-square test results revealed that education (Chi-Square = 9.309, P-Value = 0.015), family type (Chi-Square = 4.299, P-Value = 0.011) and nearby water bodies (Chi-Square = 11.750, P-Value = 0.019) were strongly associated with the knowledge on environmental hygiene. **Conclusion:** Knowledge on environmental hygiene is achieved only by better education and family values in the community.

Keywords: *Association, environmental hygiene, rural population, Community*

Introduction

Population growth in developing countries is a contributing factor in imbalance of environmental hygiene. Every development that is happening due to urbanization is affecting the health particularly the rural population.[1] Lack of good sanitation practices is a leading cause for disease spurt in rural population. Lack of personal hygiene, poor management of solid wastes and lack of proper drainage system has paved way to health issues that are serious in nature.[2]

Objective

To find out the association of knowledge on environmental hygiene with demographic variables of rural population in the community

Research Methodology

The researcher used pre-experimental one group pretest and posttest research design. Sampling technique used was convenience sampling on 150 samples in Mutha Village. Self -structured questionnaire were used to assess the knowledge regarding environmental hygiene. Association of knowledge with demographic variables was conducted using Chi-square test.[3]

Findings

Association of knowledge on environmental hygiene with demographic variables of rural population in the community

Association of Knowledge with level of Education

n = 150

	A	B	C	D	All
HIGH	3	1	3	4	11
	2.933	3.740	2.127	2.200	
LOW	10	7	3	2	22
	5.867	7.480	4.253	4.400	
MODERATE	27	43	23	24	117
	31.200	39.780	22.620	23.400	
All	40	51	29	30	150

Pearson Chi-Square = 9.309, DF = 6, P-Value = 0.015

Likelihood Ratio Chi-Square = 9.701, DF = 6, P-Value = 0.013

Association of Knowledge Type of family

n = 150

	A	B	All
HIGH	6	5	11
	5.79	5.21	
LOW	16	6	22
	11.59	10.41	
MODERATE	57	60	117
	61.62	55.38	
All	79	71	150

Pearson Chi-Square = 4.299, DF = 2, P-Value = 0.011

Likelihood Ratio Chi-Square = 4.458, DF = 2, P-Value = 0.010

Chi-square test was used to find association between the knowledge and Demographic variables. The p-values for education and family types are less than 0.05, rejecting H_0 (null hypothesis) hence shows that level of knowledge is significantly associated with Education and Family Type of samples.

Discussion

According to a study conducted by K J Nath on environmental sanitation. Results revealed that the poverty was the main cause for poor hygiene. Public Institutions need to work in congruence with the local community leaders to contribute in the improvement environmental sanitation.[4]

Conclusion

Study findings revealed that there is a significant association of knowledge with selected demographic variables. The p-values for education and family types are less than 0.05, rejecting H_0 (null hypothesis) hence

shows that level of knowledge is significantly associated with Education and Family Type of samples. This shows that proper education necessarily will improve the mindsets of people dwelling in the rural community. Family values play a very important role in developing self as well as the community.[5]

Limitation

The study is limited to only the rural community of Pune City.

Conflict of Interest - Nil

Source of Finding - Self

Ethical Clearance- Study was approved at Institute Research Committee. Informed concerned from each sample been taken for the study.

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A study to Assess the Effectiveness of Mindful Meditation on Health and Academic Adjustment Problem Faced by Nursing Students in Selected Colleges of Pune City

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Abstract

Stress is one of the common problem among students of higher education institutes, it affects health and academic performance as well. Mindful meditation is one of the useful strategy to reduce stress and improves health and academic performance among young population. The objective of the study is assess the effectiveness of mindful meditation on health and academic adjustment problems among 1st year B Sc Nursing students in selected nursing colleges of Pune city. The research design adopted for this study pre-experimental, pre-test post-test design. The study was conducted at selected nursing schools of Pune. Total 60 students selected by purposive sampling. The data collected were analysed by descriptive and inferential statistics. The result showed that there are multiple health problems student faces during their first year of college life. The stress which occurs in early academic life causes low academic performances. Mindful meditation found effective on health and academic performances during early academic life hence college should exercise mindful meditation during their induction and orientation period.

Keywords *– Mindful Meditation, Academic adjustments, Nursing students, Health*

Introduction

Adjustment is a process by which a living organism maintains a balance between the needs and the circumstances. An individual feels a sort of emotional tension, uneasiness and restlessness when he does not or cannot adjust himself with social environment. Good (1959) states that adjustment is the process of adopting modes of behavior suitable to the environment or the changes in the environment. Unless a person is not able to adjust himself to the environment, he/ she cannot develop his/her wholesome personality.[1]

Stress is a natural body reaction to difficult or challenging situations. Clinical practice has been identified as one of the most anxiety producing components in nursing programs. Lack of experience, fear of making mistakes, discomfort at being evaluated by faculty members, worrying about giving patients the wrong information or medication and concern about possibly harming a patient are just a few of the stressors for the beginning student nurse.[2]

The researches shows that the stress among nursing students is high and the investigator herself has undergone a lot of emotional stress during the 1st year of B.Sc. Nursing course due to new environment, parental separation, work load and new peer groups.[3]

Mindfulness meditation has been proven effective for reducing stress. Recently healthcare providers of multiple disciplines are overwhelmingly turning to the practice of mindfulness meditation as a useful tool in building a self-care routine.[4] Meditation has several benefits such as deeper level of relaxation, builds self-confidence, helps to control own thought, Improve learning ability and memory, increase emotional stability, increase productivity, develop will power,

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react more quickly and more effectively to a stressful event, more sociable behavior, increases listening skills and empathy, helps make more accurate judgments, greater tolerance, more balanced personality, develops emotional maturity experience an inner sense of "assurance or knowingness", increases the synchronicity in your life.[5]

Methodology

Study design, setting and sample size

A pre-experimental study was conducted on effectiveness of Mindful Meditation on adjustment problem among first year B.Sc. Nursing students in selected colleges of Pune city. The pre-experimental one group pre-test post-test design was adopted and 60 samples were selected through purposive sampling technique. The permission was obtained from the Principal of Nursing Colleges of Pune city.

Objectives of the Study

1. To assess the level of health and academic adjustment problem among 1st year B. Sc. Nursing students in selected college.

2. To evaluate the effectiveness of mindful meditation on health and academic adjustment problem among first year B. Sc. nursing students in selected college

Data collection method

The tool had been prepared in two sections which consist of the socio demographic variable and 20 closed ended questions related to mindful meditation on Health and academic adjustment. Every right answer gained one (1) point and wrong answer gained zero (0). The overall score ranged from 1-10 from each component, participants with reasonably adequate adjustment considered 7-10 score, adequate adjustment considered 4-6 score and poor adjustment considered 1-3 score.

The questionnaires were administered in English and Marathi language. The same questionnaires were used before and after the providing education on mindful meditation programme.

Data Analysis: The data had been analyzed through descriptive analysis and statistical analysis.

Result

Section I: Description of samples based on personal characteristics

Table No.1 Distribution of participants as per demographic variables

(N=60)

Demographic variable	Frequency (f)	Percentage (%)
Gender:		
· Male	22	36.67
· Female	38	63.33
Residency		
· Hosteller	46	76.67
· Non-Hosteller	14	23.33
Religion		
· Hindu	46	76.67
· Muslim	4	6.67
· Christian	10	16.67
Locality		
· Urban	49	81.67
· Rural	11	18.33

Cont... **Table No.1 Distribution of participants as per demographic variables**

(N=60)

Parents' Education		
· Graduate	36	60.00
· High school	24	40.00
Family Income		
· 11000-20000	8	13.33
· 21000-30000	22	36.67
· 31000-40000	30	50.00

Table 1: Illustrate the distribution of demographic variables of 60 students; most of the participants 38 (63.33%) were female and male were 22 (36.67%).

Majority subjects 46 (76.67%) resides in hostels and few were 14 (23.33%) Non-hosteller.

As per religion, 46 (76.67%) subjects belong to Hindu religion as compare to Muslim and Christian were 4 (6.67%) and 10 (16.67%) respectively.

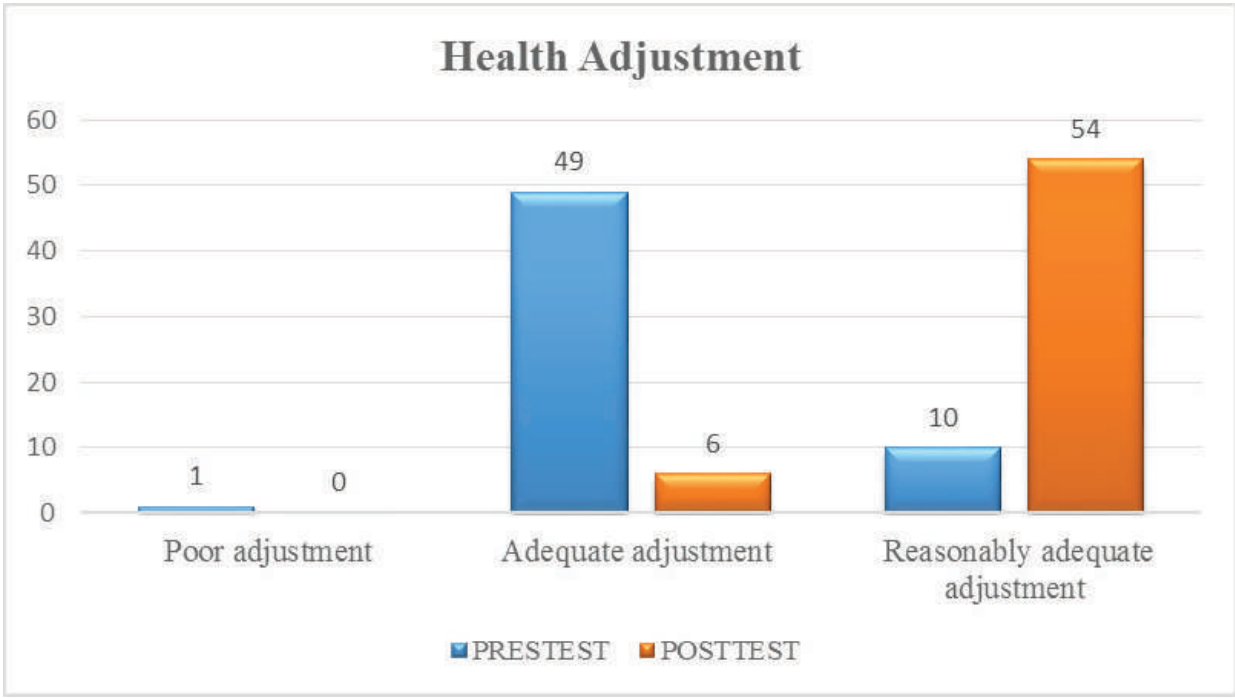
The most of the participants 49 (81.67%) from Urban locality where as only few 11 (18.33%) from rural locality.

36 (60%) parents had completed their education whereas 24 (40%) completed high school.

As per family Income wise, 30 (50%) subjects were having monthly income of Rs.31000-40000 and 8 (13.33%) were having Rs. 11000-20000.

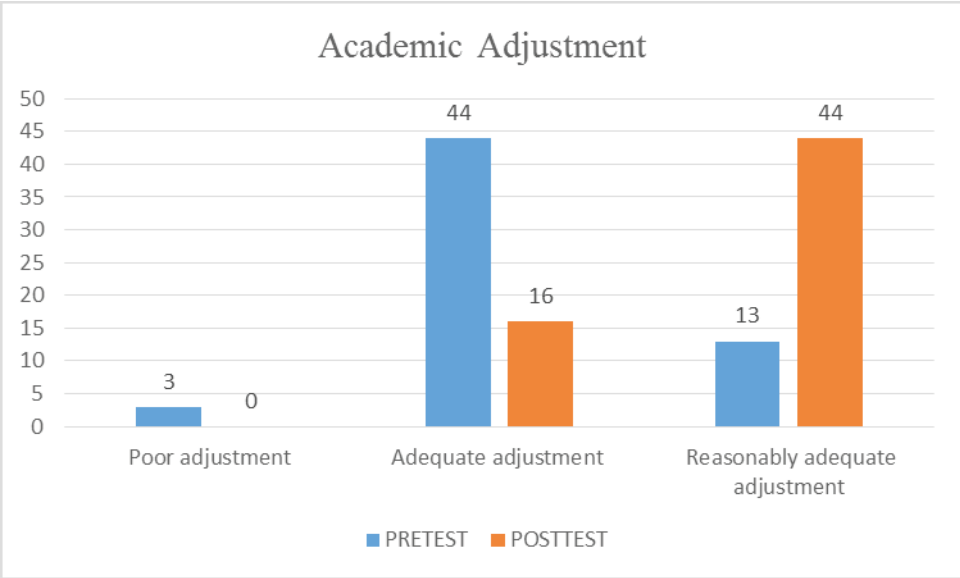
Section II: Effectiveness of Mindful meditation on health and Psychological adjustment problem

Figure 1 - Figure shows the Health Adjustment level of the first year students on Pre-test and Post-test N-60



From the above figure it shows that on Pre-test least participant 1 (1.67%) having poor health adjustment and most of them have 49 (81.67%) adequate health adjustment and few participants 10 (16.67%) are having reasonably adequate health adjustment. Post-test very few samples 6 (10%) having adequate health adjustment and 54 (90%) participants are having reasonably adequate health adjustment. It shows there is an improvement in the Health aspect after the education on Mindful meditation.

Figure: 1 - Figure shows the Academic Adjustment levels of the first year students on Pretest and Post-test N-60



From the above figure it shows that on Pre-test, few participants 3 (5%) have poor academic adjustment and most of the samples 44 (73.33%) have adequate psychological adjustment and 13 (21.67%) samples have reasonably adequate academic adjustment. Post-test 16 (26.67%) participants have adequate academic adjustment and most of them 44 (73.33%) are having reasonably adequate academic adjustment. It shows there is an improvement in the academic aspect after the education regarding the mindful meditation

Statistical

Comparison of mean, standard deviation and paired 't' test showing effectiveness of mindful meditation and their in health and psychological adjustment.

S.No.	Assessment	Mean	S.D	Mean difference	't' value
1	Pre-test	12.05	2.57	4.05	17.28
2	Post-test	16.10	2.09		

Comparison of mean and standard deviation on health and academicl adjustment after administration of mindful meditation among first year B Sc Nursing students found that pre-test mean was 12.05 with Standard deviation 2.57 and post-test level adjustment increased to 16.10 with SD 2.09. The mean difference was found to be 4.05. The paired't' test was applied with df 59 and value found 17.28 at significant level of

$p<0.05$. It proves that mindful meditation is effective tool to improve the health and academic adjustment among the first year B Sc Nursing students

Discussion

A study was conducted to assess the effect of mindful meditation on health and academic adjustment among first year B Sc Nursing students in selected nursing colleges of Pune city. 20 questionnaire was asked to 60 participants who were selected through purposive sampling. Sessions on mindful meditation was given to the participants which was selected as per laid down criteria. Post-test was conducted and same questionnaire was used for data collection. Result revealed that mindful meditation is useful means to reduce stress and improves health and academic performance of the students. Paired 't' test was applied to confirm the effectiveness of the meditation sessions at <0.05 level of significance and it was confirmed that mindful meditation is useful in health and academic adjustments among first year B Sc Nursing students

Conclusion

Mindfulness is regarded not as something to get or to acquire, but as an internal resource that already exists, patiently awaiting to be reawakened. The study had found that increased mindfulness can improve psychological functions, reduce in suffering, and has been proved to be an important predictor of depression in nursing students

Ethical Clearance- Ethical clearance was taken from Institutional Research Ethics Committee

Source of Funding- Self-Funding Project.

Conflict of Interest – There is no any conflict of interest.

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A study to Assess Anxiety and Coping Strategies Adopted by the Students Regarding Education During COVID-19 Lockdown

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Abstract

Background: COVID-19 is world-wide concern affecting the educational institute (EIs). This widespread drive to a strong reaction among the student involvement facing anxiety.

Objectives: This cross- sectional study is designed to inspect student's anxiety and coping strategies during the COVID-19 pandemic.

Results: Results displays that students are retained with anxiety and consciousness. Nontherapeutic preventive measures were discern as efficient method. Students were pleased with the government's response to alleviate dilemmas. Although, a reluctant view with online- teaching learning access was noted.

Conclusions: Students used enormous ways to adopt the situation mentally and physically. It's important to abide student's insanity during this COVID -19 Widespread.

Key words: *Anxiety, Monetary, Fear, Coping Strategies, Insanity, COVID- 19 Widespread*

Background and need of the study

Anxiety is very common in students even during normal event. In pune during widespread and lockdown period students have come across monetary, fear, anxiety and challenges of correspondence instructions. The objective of this study was to assess levels of anxiety and ways of coping among students.[1]Around the month of January, the World health organization declared the eruption of Novel Coronavirus infection COVID- 19, as societal climax among a globe.[2] The first documented death outside the Wuhan China, was in Karnataka on March 13, 2020 as a travel history. WHO also declared that there high drive of spreading COVID-19 in others countries around the globe? According to WHO hypotheses COVID-19 is diagnosed as highly widespread disease across the centuries.[3] Moreover, Quarantine term had come into existence by the official authority to help people and to bifurcate the adverse effects of COVID-19 infection. However, the crises have knock out in lower socioeconomic group than higher society people. [4] Consequently, there is no valid evidence, of using antiviral drugs alone or combination of corticosteroids can improve the out coming result among patients. No specific treatment or vaccinations are available for the disease. [5]Since than established of COVID-19 Widespread, the psychological and physical challenges are recorded as proof. The spread of disease has created fear and anxiety among people. These widespread has impacted the educational institute and several other universities. [6] The curriculum was stopped during lockdown period. Majority of educational institutes and universities have encounter the threat with synchronous E-learning source. [7] Universities and educational institutes are convinced to manage situation regarding widespread to inculcate healthy habits among students. Scrutinizing the approach of above all constrain, the study steered to assess the level of anxiety and assess the level of coping strategies adopted by the students regarding managing education in COVID – 19

Widespread.[8]

Research Methodology

A survey designed was conducted during May-June 2020, whereas adolescent (≥25 years old) the students residing in Pune city were included in the study Nonprobability convenience sampling technique was used. Data was collected by structured questionnaire. Data was processed and analyzed in descriptive and inferential way.

Table 1. Demographic characteristics of survey respondents.

SR.N	Demographic variables	Frequency	Percentage %
1	Gender		
a	Male	31	48
b	Female	38	76
2	Age		
a	14-18 years	0	0
b	18-20years	0	16
c	20-30years	30	79
d	30-40years	0	0
3	Educational Level		
a	Primary	0	0
b	Secondary	5	10
c	Graduate	42	84
d	Post-Graduate	0	0
4	Residence		
a	Urban	32	64
b	Rural	0	0
c	Tropical Areas	0	0
d	None of these	0	0
5	Occupation		
a	Business	0	0
b	Services	34	68
c	Agriculture	0	0
d	Housewives	0	0
6	Marital Status		
a	Married	6	12
b	Single	43	86
c	Divorcee	0	0
d	Widow	0	0

Table 1: Shows maximum number of females 76% was included in study from the age group between the 20-30 years and they were from graduate level 84% all of them were from urban city.

Figure 1: Education Challenges faced during COVID-19.

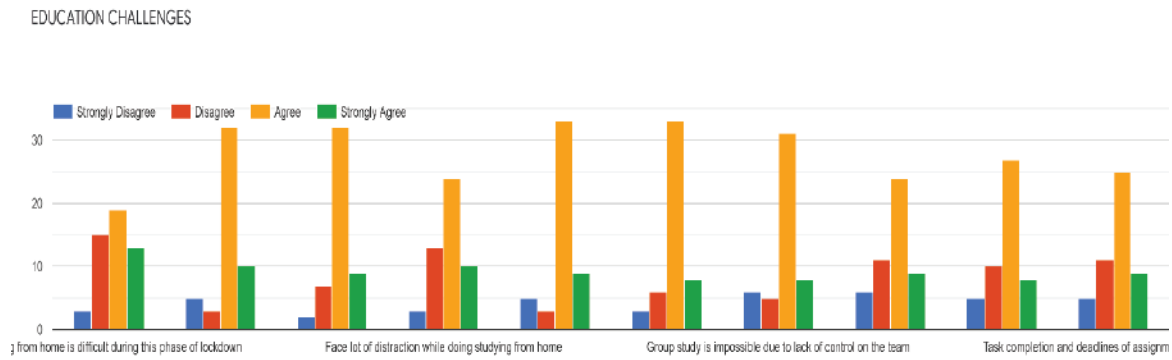


Figure 1: shows lots of communication gaps 64% and quantity and quality is affected 64%. Also, internet accesses are big challenged 66% in managing the education during Widespread of COVID-19.

Figure 2: PERSONAL COPING STATERGIES. (During community quarantine)

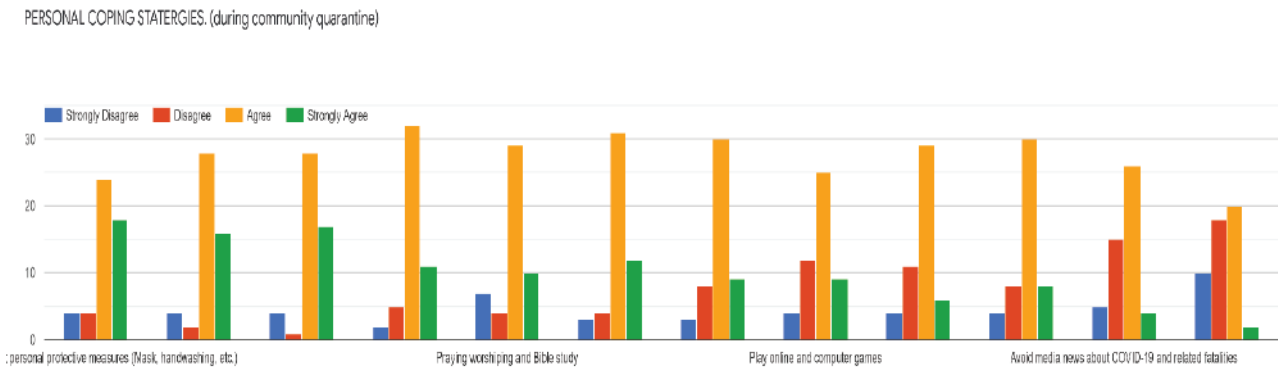


Figure 2: Shows relaxation therapy 64% is used as coping strategy to overcome widespread

Ethical Approval: Ethical approval was obtained from IRC Committee of College.

Source of Funding: Nil

Conflict of Interest: Nil

Conclusion: Shows sample faced communication gap and internet accessibility during this widespread. Thus group formation is necessary to avoid communication gap and provide common website for E-learning.

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A Study to Assess the Knowledge Regarding Corona Virus and Its Management among the People Residing in Pune City

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Abstract

Background: Assessment of public general knowledge on coronavirus and its management is essential to develop health policies for segregating the spread of disease.

Objectives: To assess the level of knowledge regarding Coronavirus among the people residing in Pune city. To identify its predicators Respiratory Syndrome due to Coronavirus**.**

Methods: A survey designed was conducted during April-May 2020, whereas adult (≥ 18 years old) the people residing in pune city were included in the study. Non probablity convience sampling technique was used. Data was collected by structured questionnaire. Data was processed and analyzed in descriptive and infrential way.

Results: A total 60 samples were recruited (age range18-30 years) the majorities were male and 45% were university graduate students. Among all interviewees 35% believed that the disease transmission can occurs through infected birds and animals. As a result the disease has no specific treatment was known by 45% and 20% of them denied any role of traditional medicines in preventions and treatment of disease? Participants had satisfactory knowledge about the disease and its management were.**Conclusions:** Although effort done by health professionals in city to educate the people and to expand their awareness about disease, there is knowledge deficit among local public. Future planning and educational interventions should focus on people and those who have educational background below the university level.

Key words*: Knowledge, Corona Virus, Management, Respiratory syndrome*

Background and Need of the study

The last two durationhave viewed the emergence of advance respiratory tract disease which has threatened the global health around the world [1]. Examples of diseases are contributed in form SARS (severe acute respiratory syndrome) Also, Middle East respiratory syndrome (MERS) is a viral respiratory disease caused by a novel coronavirus it is symbolized as (Middle East respiratory syndrome coronavirus, or MERS-CoV)[2]. Coronavirus is group of large viruses consists of common cold to severe acute respiratory tract infections [3]. Occurrence of disease and death are higher among men as compared to woman, among age between 20 to 25 years there is higher mortality rate among the younger people with less immunity [4]. In Pune since 2019 a total of 1233 cases are confirmed with diseases, of which 979 are taking treatment 79 persons have died and of which 182 cases have discharged from the hospital after effective recovery and 45 people are in crucial stage [5] Knowledge about disease and its transmission is significant to developed effective control. The transmission of respiratory syndrome due coronavirus is frequent, among family members, mostly occurs in health care settings and people coming around close contact with infected person [6]. The disease mainly affects the lower respiratory tract muscles following with clinical features: Fever, cough, difficulty in breathing, Pneumonia, which can increase to acute respiratory distress syndrome, if the threshold progress then death is confirmed as per health professionals [7].However, there is no valid evidence, of using antiviral drugs alone or combination of corticosteroids can improve the out coming result among patients. No specific treatment or vaccinations are available for the disease [8].

Heath professionals in Pune have taken measurable efforts in order to control the spread of disease [9]. Health education plays an important role in controlling the infectious disease [10]. Few studies were conducted among the city and health care workers to assess the knowledge about spread of disease and its management [11]. Assessment among the public general knowledge on respiratory syndrome due to coronavirus is delicate to develop the health policy in regulation of the disease. As a result, this study was conducted to rule out the following

Objectives

1. To assess the level of knowledge on Respiratory Syndrome and to identify arithmeticalvariables associated with adequate level of knowledge if any.

Research Methodology

A survey designed was conducted during April-May 2020, whereas adult (≥ 18 years old) the people residing in pune city were included in the study. Non probablity convience sampling technique was used. Data was collected by structured questionnaire. Data was processed and analyzed in descriptive and infrential way

Results

Table 1. Demographic characteristics of survey respondents.

SR.N	Demographic varaibles	Frequency	Percentage %
1	Gender		
a	Male	31	52
b	Female	29	48
2	Age		
a	14-18 years	0	0
b	18-20years	21	35
c	20-30years	8	13
d	30-40years	31	52
3	Educational Level		
a	Primary	0	0
b	Secondary	38	63
c	Graduate	22	37
d	Post-Graduate	0	0
4	Residence		
a	Urban	60	100
b	Rural	0	0
c	Tropical Areas	0	0
d	None of these	0	0
5	Occupation		
a	Business	0	0
b	Services	18	30
c	Agriculture	0	0
d	Housewives	42	70
6	Marital Status		
a	Married	42	70
b	Single	18	30
c	Divorcee	0	0
d	Widow	0	0

Table 1: Shows Maximum number of males 52% was included in the stduy whearas from the age group between 30 -40 years and they were from secondary educal level 63% all of them was from urban city

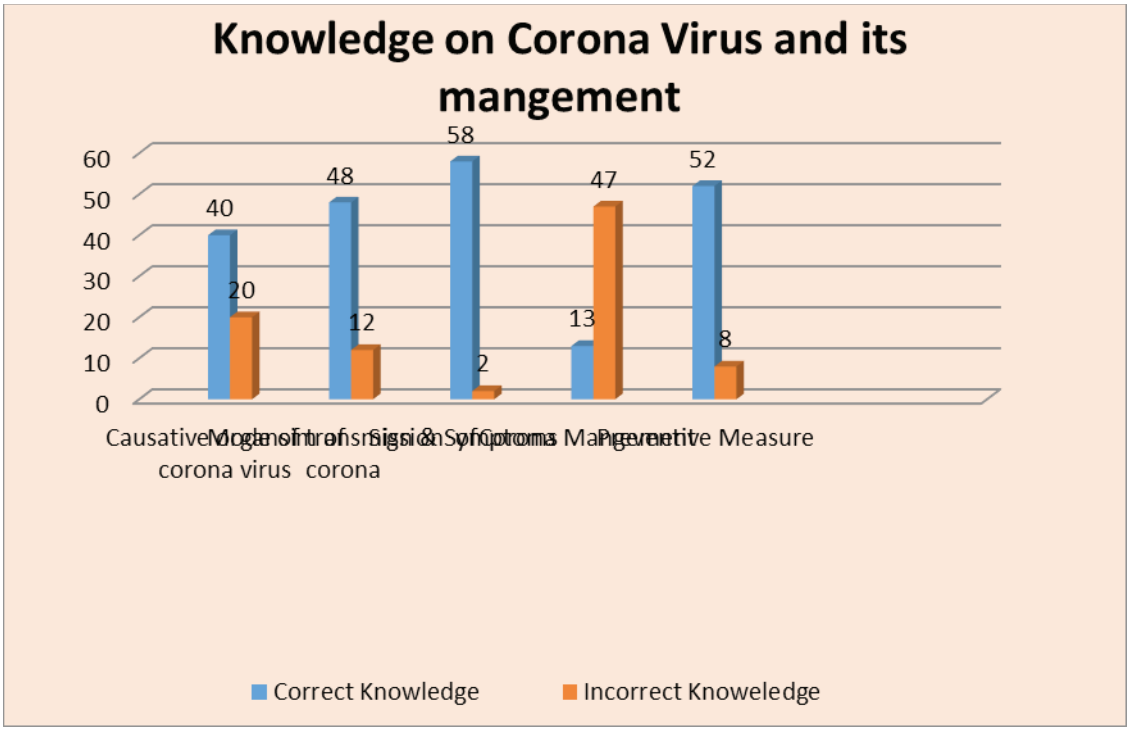


Figure 1: Knowledge related to cornona virus and its mangement

Figure 1: Shows samples had more knoweldge 58% on sign and symptoms of corna and its prevention but leass knowledge 13% about its mangement.

Ethical Approval: Ethical approval was obtained from IRC Committee of College.

Source of Funding: Nil

Conflict of Interest: Nil

Conclusion

Shows samples had more knoweldge on sign and symptoms of corna and its prevention but leass knowledge about its mangement.Thus more awareness must be spread on its management also.

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A Descriptive study on Knowledge of Environmental Hygiene Among Rural Residents in Rural Community

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Abstract

Introduction : In communicable diseases, one of the important and prime aspect is practice of hygiene for individual's health and well-being. These communicable diseases are considered to be contribute **Objective**: To assess the existing level of knowledge regarding environmental hygiene in rural community **Materials and methods:** Study has adopted Non experimental research approach and descriptive survey research design. Self administered structured questionnaire were used to assess the knowledge regarding environmental hygiene. After obtaining the permission from each respondents the questionnaire was administered and survey form was collected. Written consent was taken from each sample prior to pretest. Sample size was 150 . Setting was Mutha Village of Pune city. and samples were from population from rural community **Results** Majority of respondents has poor knowledge (47.7%), 40% of respondents has average knowledge and only 13.3% respondents has poor knowledge regarding environmental hygiene **Conclusion:**. Study concluded that residents of rural community has poor knowledge regarding environmental hygiene.

Keywords: *Descriptive , Rural Residents, Knowledge, Environmental hygiene.*

Introduction

There is an unwillingness to recognize and admit the home as a setting of equal status along with the community institutions for transmits of spreading of different types of disease in the community [1]. In communicable diseases, one of the important and prime aspect is practice of hygiene for individual's health and well-being. These communicable diseases are considered to be contribute 80% of the illnesses together with other infectious diseases and for malnutrition[2]

Out of important factors, few factors like sanitation, hygiene and water is considered important and , lower and avoid the burden of communicable diseases significantly if measures been taken [3] There are factors like sanitary material , water and facility of sanitation can bound expressively hygiene practice that if a discrepancy and inequality between knowledge or attitude and practice can be expected·

Study findings shown that washing hands with soap and water can reduce diarrheal disease by 35% or more[4]

Objectives

· To assess the existing level of knowledge regarding environmental hygiene in rural community.

Research Metholodogy

Study has adopted Non experimental research approach and descriptive survey research design. Self administered structured questionnaire were used to assess the knowledge regarding environmental hygiene. After obtaining the permission from each respondents the questionnaire was administered and survey form was collected. Written consent was taken from each sample prior to pretest. Sample size was 150 . Setting was Mutha Village of Pune city. and samples were from population from rural community

Findings

Section I

Table 1: Demographic variables in terms of frequency and percentage

n=150

Variables		Frequency (f)	Percentage%
Gender	Male	75	50
	Female	75	50
Marital status	Married	66	44
	Unmarried	42	28
	Widow	42	28
Nearby health facility	PHC	150	100
	CHC	0	0
	District hospital	0	0
	Other (specify)	0	0
Kitchen	Separate room	130	86.7
	Combination room for kitchen	20	13.3
How do you dispose sanitary pads?	Through in field	44	29.33
	Through with other domestic waste	40	26.67
	Burn them	33	22
	In separate pouch with red mark	33	22
Do you have access to personal toilet?	Yes	150	100
	No	0	0
	Common toilet	0	0
	Open defecation	0	0

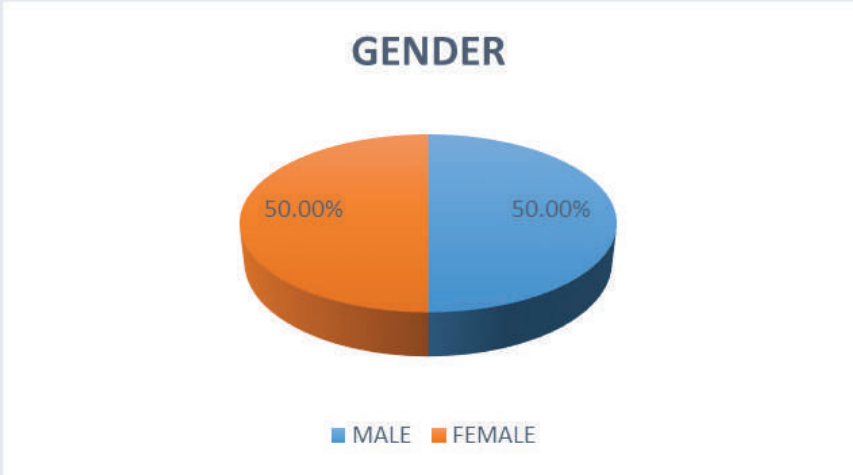
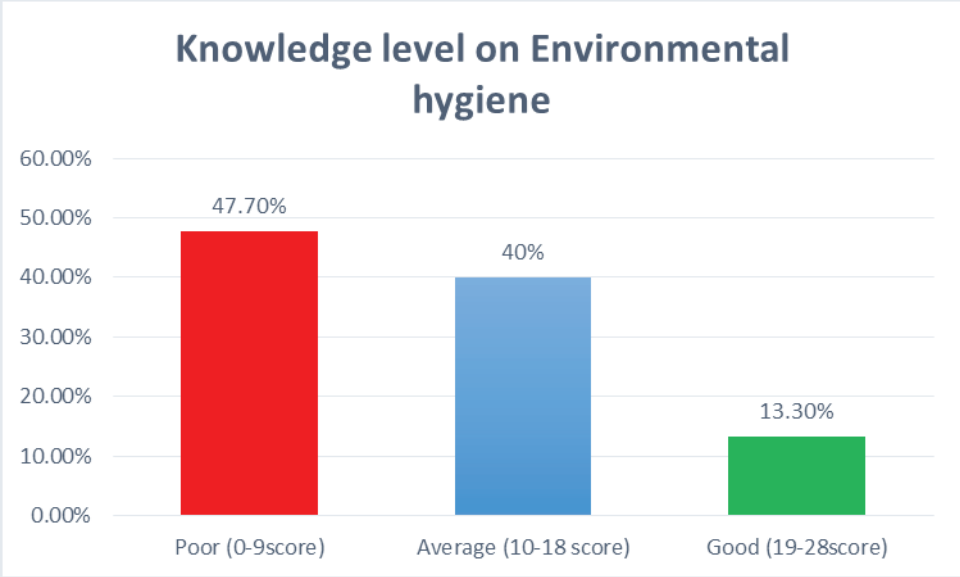


Fig 1 : Pie diagram showing distribution of samples as per gender wise

SECTION II

Data related to the knowledge on environmental hygiene among rural community

Table 2: Knowledge level about environmental hygiene



Majority of respondents has poor knowledge (47.7%), 40% of respondents has average knowledge and only 13.3% respondents has poor knowledge regarding environmental hygiene.

Discussion

Present study findings shown there regarding environmental hygiene the knowledge level is poor (13.3%) among residents of rural community

Study findings regarding the hygiene knowledge of the respondents shown 150 (44%) were knowledgeable. [5]

Conclusion

Study concluded that residents of rural community has poor knowledge regarding environmental hygiene. Timely education on awareness programme to be arranged where all members of the family and all sectors from community individual to be involved in the programme educating the people on environmental hygiene which is highly recommended to prevent disease and to promote healthy environment.

Conflict of Interest - Nil

Source of Finding - Self

Ethical Clearance- Study was approved at Institute Ethical subcommittee. Informed concerned from each sample been taken for the study.

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Correlational Study to Assess the Musculoskeletal Quandry with the Bag Carried by Children Studying at Selected School of Pune

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Abstract

Introduction and objective: The prevalence rate of musculoskeletal pain among Indian school-going children is 55% to 85% in between and the recommended weight is given to school children should be less than 10% of the body weight but children carry bag weight on the body i.e. 15% of the body weight. Hence the main **objective** of this study was to correlate the weight of the student with bag weight and musculoskeletal quandry. **Methodology:** Quantitative approach with non-experimental correlational design used in this study to assess the musculoskeletal quandry with the bag carried by children studying at selected school of Pune". Musculoskeletal pain was assessed with the help of standardized tool which was Modified cornell musculoskeletal discomfort questionnaire, Wongs baker pain rating scale. 200 school children were selected by using Non-probability convenient sampling technique. In this study the data was analysed using Pearson coefficient correlation test, and Fishers' exact test. **Result:** The following observation were made from the study. The p-values for this test were large for these parameters, which is evident that though there is a negative correlation of weight of students with Pain frequency (0.490), Discomfort (0.422), Interference (0.432), and Pain (0.486), the correlation is not significant Pearson's correlation coefficient between the weight of school children and bag weight was 0.12 which is positive. Fisher's exact test was applied to find the association and the result shows that the p-value is less than 0.05 it indicates that there is a significant association with the musculoskeletal quandry. **Conclusion:** Hence, the significance of this study indicates of evidence for the null hypothesis and analysis and results show that there is a negative correlation between the weight of the student with the bag weight.

Keywords: *Assess, Musculoskeletal Quandry, Heavy bag pack, School children.*

Introduction

School children have been estimated that 40 million students across the overall United States carry their stuff or material in their school backpacks and there is a very serious concern about how the heavy backpack gives the harmful effect on their spine. The multiple professional associations have given the guidelines for safe backpack e.g. American Academy of Orthopedic Surgeon, American Occupational Therapy (1).

The prevalence rate of musculoskeletal pain among Indian school-going children is approximate 55% to 85% in between and the recommended weight is given to school children should less 10% of the bodyweight but children carry bag weight on the body i.e. 15% of the body weight.[2]

The parents had risen their voice against the organization and representative who are present in the schools and the teachers, health professionals, school authorities, students themselves have risen the voice against the heavy loads which must be carried by students.[4] There are many pieces of research which have proven the strong correlation between heavy school bag and physical problem in all students that can result in severe complications.[5]

Objectives

- To assess the musculoskeletal quandry of the students.
- To correlate the weight of the student with bag weight and musculoskeletal quandry.
- To associate the selected demographic variables with the musculoskeletal quandry

Material and Method

It is a quantitative study; Non-experimental correlation design has been used. Study conducted in selected schools of Pune. 200 school children were included in the study using non-probability convenience sampling technique. The study participants were given proper information about the study and consent obtained from their parents. Their confidentiality and anonymity have been maintained and the data was obtained with the help of standardized tool which was Modified cornell musculoskeletal discomfort questionnaire, Wongs baker pain rating scale. **Findings:** The following observation were made from the study based on description of samples (students) based on their personal characteristics in terms of frequency and percentages. In this study, the sample consist of 200 school children in which 55.5% were male and 44.5% were female. Majority of school children fall in the age group from 13-14 years whereas 27% school children in age group from 11-12 years and the minimum children fall in the age group of 15 years. In this study data were collected from 6^{th}, 7^{th}, 8^{th} and 9^{th} standard student in which maximum number of students were studying in the 9^{th} standard i.e.30.5%. Among 111 male students, 45.9% had weight ranging from 25.9-54.8 kg, on other hand among 89 girls 43.8% of the girls had weight ranging from 26.7-53.4 kg. 55% of the male children had height ranging from 133.0-158.6 cm whereas majority of the female students i.e.49.4% fall in the shortest height group ranging from 133.7-157.5 cm. Maximum number of the student had school bus as their mode of transport. Maximum number of students had their classroom on 3^{rd} floor whereas 2^{nd} and 4^{th} floor had 30.5% students each.

Table 1: Frequency of Pain, ache experience among the students based on Modified Cornell Musculoskeletal scale

N=200

Frequency of pain	Freq	%
Slight frequent	131	65.5%
Moderate frequent	68	34.0%
Very frequent	1	0.5%

The data in table1. Shows that majority of 65.5% of the students had slight frequent pain ache whereas 34% of them had moderate frequency of pain, ache and 0.5% of them had very high frequency of pain and ache.

Table 2: Discomfort experienced due to ache, pain among the students based on Modified Cornell Musculoskeletal scale

N=200

Discomfort due to pain, ache	Freq	%
Slight discomfort	189	94.5%
Moderate discomfort	10	5.0%
Very much discomfort able	1	0.5%

The data in table 2. Shows that maximum of 94.5% of the students had slight discomfort due to pain, ache, 5% of them had moderate discomfort and 0.5% of them had very much discomfort able due to pain and ache.

Table 3: Interference experienced due to ache, pain among the students based on Modified Cornell Musculoskeletal scale

N=200

Interference due to pain, ache	Freq	%
Slight interference	180	90.0%
Moderate interference	20	10.0%
Very much interference	0	0.0%

The data in table 3. Describes that among 200 school children in which 90% of the students had slight interference due to pain, ache whereas 10% of them had moderate interference due to pain and ache.

Table 4: Pain among the students based on Wong Baker Faces Pain rating scale

N=200

Pain	Freq	%
No hurt (0)	14	7.0%
Hurts little bit (2)	63	31.5%
Hurts little more (4)	67	33.5%
Hurts even more (6)	43	21.5%
Hurts whole lot (8)	12	6.0%
Hurts worst (10)	1	0.5%

The data in table 4. Shows that 7% of the school students had no hurt, for 31.5% of them had little bit hurt, for 33.5% of them, hurt little more, for 21.5% of them had hurt even more, for 6% of them it hurt whole lot and for 0.5% of them hurt worst.

Table 5: Correlation with the weight of the student with bag weight and musculoskeletal Quandry

N = 200

Parameter	r	t	p-value
Pain frequency score	-0.04	0.02	0.490
Discomfort score	-0.12	0.20	0.422
Interference score	-0.11	0.17	0.432
Pain score	-0.05	0.03	0.486
School bag weight	0.12	0.21	0.415

Researcher used Pearson's correlation coefficient to assess the correlation between weight of the student with bag weight and musculoskeletal quandry (Pain frequency, Discomfort, Interference and Pain). Correlation coefficient of weight with musculoskeletal quandry were negative, which indicates that less the weight of the student, more are the pain frequency, discomfort due to pain & ache, interference due to pain & ache. The significance of this relationship was tested using t-test for significance of correlation coefficient. The p-values for this test were large for these parameters, which is evident that though there is negative correlation of weight of students with musculoskeletal quandry. Pearson's correlation coefficient between weight of school children and bag weight was 0.12 which is positive. The strength of this positive correlation was tested using t-test for significance of correlation coefficient. P-value for this test was large (greater than 0.05), the correlation between weight of school children and weight of bag though positive is not significant.

Fisher's exact test was used for association between frequency of pain, interference due to pain, Wong baker pain scale and demographic variables of school children: All the p-value corresponding to class, gender, weight of the female child, floor of classroom, height of the male child is small (less than 0.05), demographic variables are found to have significant association with frequency of pain of school children. Hence, the significance of this study shows that the p-values are greater than 0.05 it indicates evidence for the null hypothesis this means the researcher retains the null hypothesis and rejects the alternative hypothesis.

Discussion

Similar results were seen in a study the researcher did the study on effect of weight of backpack and physiological stress among school going children as the study result say that in their study the p-value was higher than 0.05 level of significant which showed that the null hypothesis was accepted and there were no significant differences between the comparison of backpack weight and recommended backpack weight and physiological stress due to carrying heavy backpacks [(2)] .

Ethical Clearance: Ethical clearance was taken from Symbiosis College of Nursing Institutional Research Committee authorities before conducting the study. Informed consent was taken from the parents. Information given to the responders regarding the data collection procedure. The collected data was used only for research purpose and kept confidential.

Conflict of Interest: Nil

Source of Funding: Self

Recommendation

· It is suggested that the study may be replicated using a larger population of samples, to know the relationship heavy bag weight with a musculoskeletal problem.

· It is also suggested that the researcher can focus on the calcium and other micronutrient assessment with quandry issues.

· A further research study should focus on lifestyle and endurance and psychological domain causing fatigue and pain cannot be overlooked.

Conclusion

School attendance is a daily routine for school children but with that carrying heavy backpack is also the same as a daily routine for students. The improper handling of school bags with excessive bag weight can lead to back pain in schoolchildren. It is recommended that the children carrying school backpack not more than 10% of their body weight but it is a concern for school children since it increases the risk of chronic back pain in their adulthood [3].

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A Study to Assess The Incidence of Suspect Cases of Leprosy and Tuberculosis at Selected Urban Slum of Pune City

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Abstract

Background: Communicable diseases are those that spread by an infectious agent, such as bacteria, viruses, fungi or parasites. Leprosy was once feared as a highly contagious and devastating disease, but it doesn't spread easily and treatment is very effective.[1] However, if left untreated, the nerve damage can result in crippling of hands and feet, paralysis, and blindness. Leprosy and Tuberculosis, is been a threatening issue all over the world. It's been hugely spreading, since it caused by air-borne organisms and is highly contagious. It's been observed that 1/3rd population of the world have latent tuberculosis, which simply means, if not taken care, they can develop higher stages and get ill. [2]There is a strong need to identify the cases at early stages so that mortality and morbidity can be prevented

Aim and Objective: This study aims to identify the suspects cases of leprosy and tuberculosis based on tentative sign and symptoms and its association with the gender so that early detection can be done to reduce the mortality and morbidity rates.

Research Methodology: The quantitative approach is used for the Study. A descriptive survey design is adopted on 300 samples which are chosen by the convenience sampling method. An assessment protocol has been prepared to assess the sign and symptoms of leprosy and Tuberculosis. The suspect cases then referred to the Urban Primary Health centres for the confirmation of the diagnosis so that treatment can be initiated at the earliest.

Results and conclusion : The study results showed,almost 13 to 15 suspects found in the Urban slum with the surveyed population of 300. The associated factors with selected demographic variables were lesion and eye pain in leprosy cases and cough in Tuberculosis Cases .The suspect cases are referred to UPH where 13 cases of Leprosy and 14 cases of Tuberculosis were confirmed. It is recommended that the associated factors need to be explored further to rule out the other factors relate to incidence of diseases.

Keywords*: leprosy, Tuberculosis, Suspect Cases, Urban Slums*

Background

"Prevention is always better than cure. When Something with the passage of time might become dangerous, then it is better to take a precautionary step to combat the danger. In fact, "Prevention is better than cure" is one of the most common and popular proverbs.[1] Prevention means to avoid and cure means to correct anything that is troublesome or detrimental. "**Early diagnosis and its treatment "play** a role to prevent many communicable diseases. And identifying High risk cases and their management is one of the challenges in community.[2]

Communicable diseases are those that spread by an infectious agent, such as bacteria, viruses, fungi or parasites. Most of these diseases can be passed from person to person so the words "contagious" or "infectious" are often used when talking about communicable diseases. Some communicable disease spread through the air. [3]Others require direct contact with a contaminated surface, food or beverage, blood or other bodily fluids. In some cases, a bite from an infected animal or insect is also capable of spreading the disease. Some diseases can be transmitted in more than one way.[4]

Leprosy also known as H. disease **(also known as leprosy**) is an infection caused by slow-growing bacteria called *Mycobacterium leprae*. It can affect the nerves, skin, eyes, and lining of the nose (nasal mucosa). With early diagnosis and treatment, the disease can be cured. People with Hansen's disease can continue to work and lead an active life during and after treatment.[4]

Leprosy was once feared as a highly contagious and devastating disease, but it doesn't spread easily and treatment is very effective. However, if left untreated, the nerve damage can result in crippling of hands and feet, paralysis, and blindness.[4]

Tuberculosis (TB) is caused by a bacterium called *Mycobacterium **tuberculi.*** The bacteria usually attack the lungs, but TB bacteria can attack any part of the body such as the kidney, spine, and brain. Not everyone infected with TB bacteria becomes sick. As a result, two TB-related conditions exist: latent TB infection (LTBI) and TB disease. If not treated properly, TB disease can be fatal.[3,5]

Leprosy and Tuberculosis, is been a threatening issue all over the world. It's been hugely spreading, since it is transmitted through air-borne route organisms and is highly contagious. It's been observed that 1/3rd population of the world have latent tuberculosis, which simply means, if not taken care, they can develop higher stages and get ill. A person who is been treated with tuberculosis has to be conscious towards hygiene. Since, with closer contacts it will spread over. It is always better to prevent this spreading rather than curing it later.

But now it's time to act and need to focus on prevention of the communicable disease by public awareness by educating the community people. Early diagnosis /High risk cases in the community and need to take prompt action against this communicable disease by educating general public.

Aim and Objective: This study aims to identify the suspects cases of leprosy and tuberculosis based on tentative sign and symptoms so that early detection can be done to reduce the mortality and morbidity rates.

Research Methodology: The quantitaive approach is used for the Study. A descriptive survey design is adopted on 300 samples which are chosen by the convenience sampling method. An assessment protocol was prepared to assess the sign and symptoms of leprosy and Tuberculosis. The suspect cases then referred to the Urban Primary Health centres for the confirmation of the diagnosis so that treatment can be initiated at the earliest.

Results

Section I: Demographic characterstics of the samples

Majority of subjects were female (78%) and (22%) were male. Majority of the subjects were living in a joint family in (68%) whereas (32%) were living in a nuclear family.

Section II:Distribution of Clinical Manifestation of Leprosy

Majority of subjects (95.7%) had no lesions over the body surface and (4.3%) had lesions over the body surface. Majority of subjects (95.3%) had no ulceration and (4.7%) had ulceration over the body surface area.8.3% had numbness or tingling sensation over the body surface area.8% of subject had the muscle pain along with the other clinical manifestation and only.(13.7%) had presence of eye pain as clinical manifestation. 30% had change in vision.

Section III:Distribution of Clinical Manifestation of Tuberculosis

According to the presence of chest pain. Majority of subjects (87.3%) had no presence of chest pain and (12.7%) had the presence of chest pain.Majority of subjects (87.3%) had no complain of blood in sputum and 12.7% subject had sputum production with blood. 30% of cases showed Anorexia Symptoms. 100% samples shows the symptoms of weakness. Majority of subjects (94%) had no symptoms of night sweat and only 6% had night sweat complain.

Section IV: Association between gender and presence of symptoms of Leprosy

Non parametric Chi square test was used to identify the association between gender and Clinical manifestation of Leprosy. No clinical manifestation seems associated except Presence of Lesion and eye pain. The standardized statistic is 3.335.in case of lesion and .257 in case of Eye pain. The expected count is less than 5.

Table 1: Association of Presence of lesion in leprosy with gender

N=300						
	Value	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**	**Point Probability**
Pearson Chi-Square	11.370a	2	.003	.037		
Likelihood Ratio	4.786	2	.091	.053		
Fisher's Exact Test	7.174			.024		
Linear-by-Linear Association	11.120b	1	.001	.020	.020	.013
N of Valid Cases	300					

Table 2: Association of Presence of eye pain in leprosy with gender

	Value	**Df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**	**Point Probability**
Pearson Chi-Square	.370a	2	.831	.872		
Likelihood Ratio	.369	2	.832	.872		
Fisher's Exact Test	.649			.872		
Linear-by-Linear Association	.066b	1	.797	.880	.492	.05
N of Valid Cases	300					

Section V: Association between gender and presence of symptoms of Tubeculosis

Non parametric Chi square test was used to identify the association between gender and Clinical manifestation of Tuberculosis. No clinical manifestation seems associated except Cough. The standardized statistic is 1.491. 2 cells (33.3%) have expected count less than 5.

Table 3: Association of Presence of Cough in Tuberculosis with gender

	Value	**df**	**Asymp. Sig. (2-sided)**	**Exact Sig. (2-sided)**	**Exact Sig. (1-sided)**	**Point Probability**
Pearson Chi-Square	8.393a	2	.015	.010		
Likelihood Ratio	9.488	2	.009	.006		
Fisher's Exact Test	8.990			.007		
Linear-by-Linear Association	2.222b	1	.136	.170	.002	.001
N of Valid Cases	300					

Conclusion

The study concluded that there are almost 13 to 15 suspects found in the Urban slum with the surveyed population of 300. The associated factors seem to be lesions and eye pain and cough in case of tuberculosis. The suspect cases are referred to UPH where 13 cases of Leprosy and 14 cases of Tuberculosis were confirmed. It is recommended that the associated factors need to be explored further to rule out the associated reason and causes so that mortality and morbidity can be prevented.

Ethical Clearance: Taken from Institutional research Committee

Conflict of Interest: Nil

Source of Funding: Self

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A Phenomenological Study of Pregnant Women's Experience : COVID-19 Lockdown Period

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Abstract

Introduction and objective: Pregnant women are facing challenges with the restrictions during the lockdown period. Advices/instructions from husband and support system contribute to anxiety. The objective of the study includes describing pregnant women's experiences during COVID -19 lockdown period. **Material and Method**: Qualitative Research Approach and Descriptive Phenomenological as research Design were used. Sample size was four Pregnant women from Urban community during COVID -19 lockdown period .The investigator collected the data on lived experiences of pregnant women through in-depth interview, which was lasted for 45-60 minutes. Qualitative rigor was done, researcher 'Bracketed' her own feelings and experiences about the content under investigation. Triangulation was applied and member checking was done. To ensure credibility the investigator had co researcher review the data. Dependability is achieved by clearly laid down the steps and also in analysis process to ensure that anyone wishing to conduct study can easily follow and carry out the steps. Transferability of the study was ensured by the exhibition of a dense narration of the samples research context and setting. The quality of data collected was significant. **Finding**: Four themes were emerged: Unknown fear, Anxiety, inclination towards Spirituality and State of Dilemma. **Conclusion**: Most of the participant reported a negative overall experience but very much appreciated the support of husband and other family members. Shown strong peer relationships creating an environment which is supportive in nature. An unknown fear and pressure is prevailing which needs to be addressed

***Key words** : Phenomenological Study, Pregnant Women , Experience , COVID-19, Lockdown Period*

Introduction

Pregnant women are facing challenges with the restrictions during the lockdown period. Advices and instructions from husband and support system contribute to anxiety.

As per Indian Council of Medical Research, that transmission of coronavirus from mother to baby before birth or during delivery is possible although the proportion of pregnancies affected and the significance to the neonate is yet to be determined [1]

Study of Psychological Assessment journal shows the that pregnant women's feelings about their changing bodies could help predict how well the mother might bond with her unborn baby and her emotional well-being after childbirth [2]

Marie Health , spokes person fro Midwives Australia says "The impact of those restrictions during labor is going to be very traumatic for many women. the resulting anxieties of expecting parents -- are about to get worse[3] .Pregnant women have a lot of queries about whether coronavirus poses a threat to self and her baby[4]. Pregnant women are more vulnerable to infection with COVID-19 compared to the general population[5]

Purpose: The purpose of this phenomenological study was to describe lived experience in COVID -19 lockdown period from the perspective of pregnant women.

Problem statement: "A phenomenological study of pregnant women's experience: COVID-19 Lockdown period"

Objective: To describe Pregnant women's experiences during COVID -19 lockdown period.

Significance of Study: It was the purpose of this study to contribute to the knowledge base from unique perspective of pregnant women during lockdown period. The respondents in this phenomenological study were able to contribute to the knowledge base in a way that could beneficial to other pregnant women in providing the beat psychological environment possible. As pregnant women share the lived experience, they can offer a unique perspective for pregnancy period.

Research Questions:

How do pregnant women describe their experience during COVID -19 lockdown period?

Operational definitions:

Phenomenology: The study of pregnant women's experience perceived as phenomena shared during COVD- 19 lockdown period

Pregnant Women: Women who is pregnant (In any trimester) from Pune city during COVD- 19 lockdown period.

Experience: Pregnant women of urban community express the feeling about pregnancy during COVID -19 Lockdown period.

Inclusion Criteria:

- Pregnant women in any trimester.
- Pregnant women of urban community.
- Pregnant women who will explore all answers genuinely and with openness.

Exclusion Criteria:

- Pregnant women who will be unwilling to participate

Material and Method

Research Approach used for the study was Qualitative Research Approach. Descriptive Phenomenology as research Design was used. This design helps the investigator to have equal value and allowed to bracket the phenomenon so that the focus of study remains clear. Data source was interviewing the participants. Sample size was four Pregnant women from Urban community during COVID -19 lockdown period . Data saturation was reached after the fourth interview as no new themes emerged from the interviews. Samples were from the urban community and purposive sampling techniques was used for the study.

Before Interview consents been taken from each participants for the study. The investigator collected the data on lived experiences of pregnant women through in-depth interview, which was lasted for 45-60 minutes. The investigator maintained the notes encompassing of observations made during the interview and due importance was given to voice, pitch, tome of speech. An interview guide with open-ended questions was used. The women were asked to share their experiences without disruption and with openness. Each Pregnant women were given time to speak comprehensively. Probing questions were imposed, to obtain clarity or redirect the interview.

Qualitative rigor was done, researcher 'Bracketed' her own feelings and experiences about the content under investigation and through. Triangulation was applied and member checking was done.

The investigator maintained the objectivity using member review process for trustworthiness. The Interview discussion was transcribed and reviewed by co researchers. To ensure credibility the investigator had co researcher review the data. Dependability is achieved by clearly laid down the steps and also in analysis process to ensure that anyone wishing to conduct study can easily follow and carry out the steps. Transferability of the study was ensured by the exhibition of a dense narration of the samples research context and setting. The quality of data collected was significant.

Seven steps of Colaizzi's phenomenological method of data analysis were monitored for data analysis.

Findings

Description of Demographic Variables

All women were Pregnant. The ages of the participants were ranged between 24-29 years. Two women were in second trimester and two were in third trimester. Four women were from urban community of

Pune city.

Four main themes emerged after the analysis of data.

Themes That Emerged

Theme 1: Unknown Fear

Under this theme Women develops feeling of knowing that something of tragic is likely to happen. An impending sense of uncertainty arises at times.

…….. *I feel insecure due to unknown factor and feel something will happen to my baby* (P 03)

I experienced numbed when I heard about lockdown and the rules of lockdown. I consider but could not stop myself , A fear on unknown always around me….(P 01)

Theme 2: Anxiety

This theme refers to the apprehension expressed by the participants. At times, it is very difficult to handle the tension. It is risky to go out especially during routine hospital check-up and follow-up. Women are anxious about the delivery which has to take place in hospital. Anxiety tend to see the situation a threatening and sometimes dangerous.

…… *things are not going to work out as I planned* (P-04)

*What if something bad happens to my child …….(*P 03)

Theme 3: Inclination towards Spirituality

Apart from support from husband and other family members, all women acknowledged the significance of spirituality as a source of personal strength in lockdown situation. Spirituality guides them, show the way and give source of security.

"*God play important part in my life; he is my strength and believe and he will take away my all worries and tension"*(P 01)

Theme 4: State of Dilemma

Women being having experience of anxiety about the delivery in hospital environment. If she comes in contact with infection, whether baby will be infected.

I am in a dilemma. I may not be able to visit the hospital for follow up. I might get infected with infection. (P 04)

During the delivery process how the situation will impact my baby….(P-02)

Discussion

Present Study findings shows that expecting mothers are anxious, state of mixed feeling and unknown fears prevail.

Finding of the study showed that pregnant women who felt more positively about their body changes were more likely to have better relationships with their husbands, depression and anxiety scores are low. These women also showed a more positive attachment to their unborn child. Rather, pregnant women who had negative feelings about their appearance during pregnancy desirable additional emotional support during pregnancy [2].

Conclusion

Most of the participant reported a negative overall experience but very much appreciated the support of husband and other family members. Shown strong peer relationships creating an environment which is supportive in nature. An unknown fear and pressure is prevailing which needs to be addressed.

Limitations:

- The study was limited to four participants
- The study was limited to Phenomenological research design.
- The findings could also not be generalized, as the study was conducted with a limited sample, which is not representative of all pregnant women
- The study population was purposively selected to fulfill inclusion criteria and which resulted in a small number of samples

Ethical Consideration: The researcher gained the Institute research Committee approval and Study was approved at Institute Ethical subcommittee. Informed consent was obtained from each sample. Confidentiality was maintained throughout the study by concealing the

names of the samples, as different codes were allotted to each sample during the interviews and when the results were made. All transcripts, notes and data were stored with researcher and it will be shredded once the study ends and result have been documented.

Conflict of Interest: Nil

Source of Funding: Self-Funded

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Awareness on Immunity Boosting Measures for Self-care among Residents of Urban Community of Pune City

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Abstract

Introduction: Ministry of Ayush applauds guidelines for immunity boosting measures for self-care to enhance immunity with special reference to respiratory health of individual. These guidelines are supported by Ayurveda literature and scientific publications. To maintain the optimum health it is very important to enhance the body's natural defense system i.e immunity of individual **Objective :**To assess the Immunity boosting measures for self-care among residents of Urban community and to determine the association of awareness with selected demographic variables **Methods:** Quantitative non experimental survey research design was used for the study. Sample size comprised of 100. Non probability convenience sampling technique was used for the study. Reliability of the tool was calculated by cronbach's alpha method and was 0.802.Tool includes Two sections. Section I included demographic proforma of the sample. It consisted of information on variables like age, gender, Occupation. Education and Religion. Section II was about self-administered structured knowledge questionnaire comprised of twenty one questions .After taking the consent from each samples tool was administered to participants. **Result:** Majority of respondents have average knowledge (49.7%) , good knowledge (04%) and 46.3% respondents had poor knowledge . Since all the p-values are large (greater than 0.05), there is no evidence against null hypothesis. None of the demographic variable was found to have significant associated with knowledge of the urban community residents regarding immunity boosting measures for self care **Conclusion:** The residents had considerable average knowledge regarding Immunity boosting measures for self care. The enhancement in knowledge is greatly required on Immunity boosting measures for self care

Key words: *Awareness; Immunity boosting measures; self care:Urban Community*

Introduction

Ministry of Ayush applauds guidelines for immunity boosting measures for self-care to enhance immunity with special reference to respiratory health of individual. These guidelines are supported by Ayurveda literature and scientific publications. During the COVID -19 pandemic it is good and advisable to take preventive measures which boost body's immunity in such crises time. To maintain the optimum health it is very important to enhance the body's natural defense system i.e immunity of individual [1]

Nature's most captivating invention is the immune system.It is quite possible to intervene in this process and make the immune system stronger using immune boosters. Immune boosters work in many ways [2]

Numerous studies have reported functional foods could provide a wide range of benefits to immune health. [3] There are diversity of methods to measure variation in immune function in a field setting [4]. The immune system shows a role in every human condition safeguarding successful reproduction or responding to internal stressors. [5]

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The study is about to assess the knowledge on immunity boosting measures for self-care, as prevention is better than cure. It is advisable to take preventive measures, which boost our immunity.

The study is about to assess the knowledge on immunity boosting measures for self-care, as prevention is better than cure. It is advisable to take preventive measures which boost our immunity.

Statement of the Problem

"Awareness on Immunity Boosting Measures for Self-care among Residents of Urban Community of Pune city"

Objective of the Study

· To describe the socio demographic characteristics of residents of urban community of Pune city.

· To assess the Immunity boosting measures for self-care among residents of Urban community

· To determine the association of awareness with selected demographic variables

Assumptions

Residents of selected urban community of Pune city may have awareness about Immunity boosting measures for self-care.

Operational Definitions

Knowledge: knowledge refers to the verbal responses of residents of Urban Community of Pune city as measured by the knowledge score of structured questionnaire on Immunity boosting measures for self-care.

Immunity boosting measures: Practice or activity which enhance the body's natural defense mechanism

Residents: People who are residents of urban community of Pune city and are between the age of 18 years or above.

Inclusion Criteria

· Residents of Urban community

· 18 and above years of age of residents of Urban community, both male and female.

Exclusion Criteria:

· Residents of Urban community who are not willing to participant

Material and Method

Quantitative non experimental descriptive survey research design was used for the study. Sample size comprised of 100 residents of selected Urban Community of Pune city. Non probability convenience sampling technique was used. Urban community of Pune city was the setting of the study .Reliability of the tool was calculated by cronbach's alpha method and was 0.802. A self-administered structured knowledge questionnaire on hazards of Immunity boosting measures was used. The tool was divided in two sections. Section I included demographic proforma of the sample. It consisted of information on variables like age, gender, Occupation. Education and Religion. Section II was about self-administered structured knowledge questionnaire comprised of twenty one questions (Multiple choice questions). Each correct item was given 01 mark and wrong response received 0 marks. After taking the consent from each samples tool was administered to participants.

Findings

The data was analyzed using both descriptive and inferential statistics.

Section I : Description of demographic variables in terms of frequency and percentage

Table 1: Distribution of demographic variable

n= 100

Variables		Frequency (f)	Percentage (n)
Age (In years)	19-25	24	24%
	25.1-35	20	20%
	35.1-50	48	48%
	50.1and above	08	08%
Gender	Male	18	18%
	Female	82	82%
Education	Illiterate	0	0%
	Higher secondary	07	07%
	Graduate	48	48%
	Postgraduate and above	45	45%
Religion	Hindu	77	77%
	Muslim	08	08%
	Christian	13	13%
	Sikh	02	02%
	Others	0	0%
Occupation	Government servant	12	12%
	Private Job	59	59%
	Service	10	10%
	House wife	11	11%
	Others	08	08%

Table 1- shows maximum 48% of sample was in the age in the age group of 35.1-50 years whereas minimum(8%) in the age group of 50.1 and above age group. Majority of respondents were female (82%) and 18% was males. Majority of respondents were graduate (48%) and 45% were highly qualified as postgraduate and above. Majority (59%) were doing private job whereas 11% were house wife.

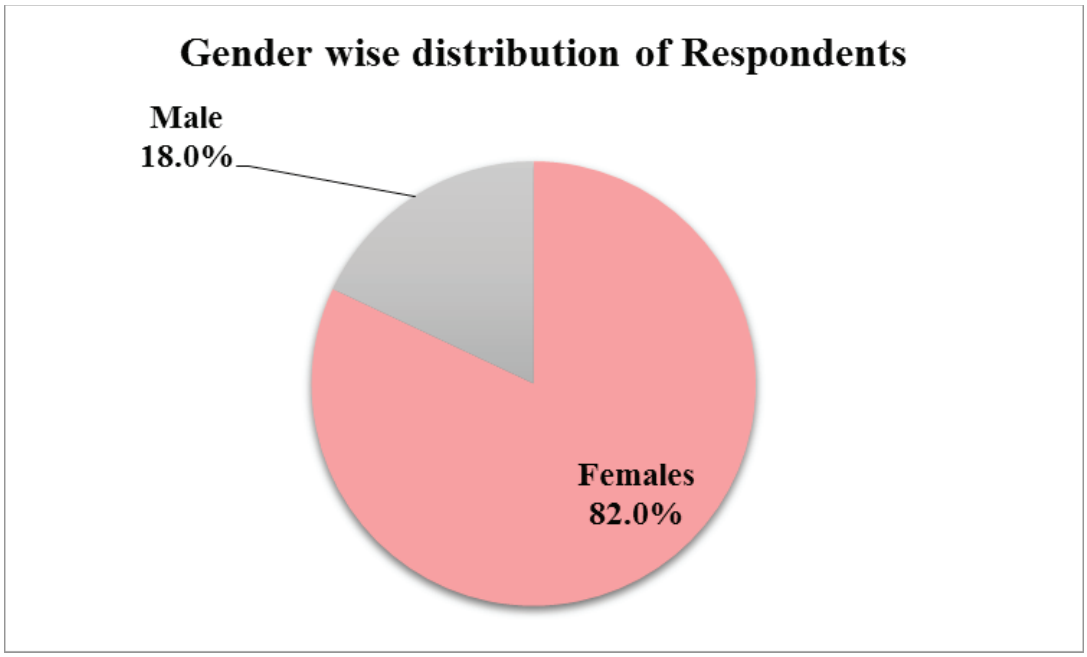


Fig 1. Gender wise distribution of Respondents

Section –II –Assessment of Awareness on Immunity boosting measures for self-care among residents of urban community.

n= 100

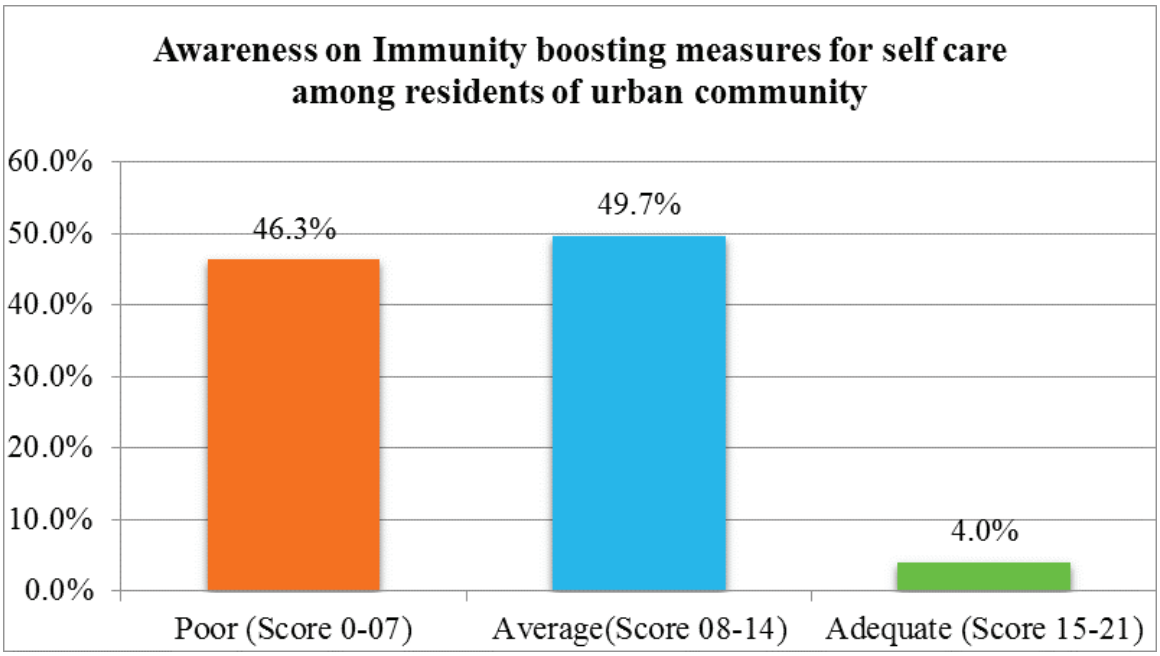


Fig 2: Awareness on Immunity boosting measures for self-care among residents of urban community

Fig 2 shows the awareness on the basis of frequency and percentage among residents of urban community of Pune city that majority of respondents have average knowledge (49.7%) , good knowledge (04%) and 46.3% respondents had poor knowledge

Section III

Table 2: Association of awareness with demographic variable

n= 100

Demographic variable		p-value
Age(years)	19-25	0.321
	25.1-35	
	35.1-50	
	50.1and above	
Gender	Female	0.301
	Male	
Education	Illiterate	0.501
	Undergraduate	
	Graduate	
	Postgraduate and above	
Religion	Hindu	0.643
	Muslim	
	Christian	
	Sikh	
	Others	
Occupation	Government servant	1.000
	Private Job	
	Service	
	House wife	
	Others	

Since all the p-values are large (greater than 0.05), there is no evidence against null hypothesis. None of the demographic variable was found to have significant association with knowledge of the urban community residents regarding immunity boosting measures for self care .

Discussion

Present study findings revealed that there is average knowledge among the residents of Urban community though they are educated , updates with current information but awareness is not adequate**.**

It is recommended by Ministry of Ayush to follow the guidelines of immunity booting measures for self care to enhance one's immunity to fight against the infection[1]

Conclusion The present study revealed the residents had considerable average knowledge regarding Immunity boosting measures for self care. The enhancement in knowledge is greatly required on Immunity boosting measures for self care to enhance the natural defense system of individual.

Conflict of Interest : Nil

Finding : Self funding

Ethical Consideration: The researcher gained the Institute research Committee approval and Study was approved at Institute Ethical subcommittee. Informed consent was obtained from each sample. Privacy and confidentiality maintained

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Effectiveness of Video Teaching Programme on Knowledge Regarding Impact of Smartphone Dependence on Physical Health, Academics and Behavior among Adolescents Studying in Selected in Junior Colleges

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Abstract

Mobile phone use has become very common in today's world. Though it is very handy and we can connect in any part of the world there are also number of health issues. The study purposes to ascertain the knowledge level about smartphone's dependence impact on physical health academics and behavior among adolescents studying in junior colleges. Design used for the purpose of the study was Single set pre-test post-test. Sampling technique used for the selection of participants was Probability Random. To select the sample Random sampling technique was used. After the pre-test, intervention was planned followed by post-test was done after 1 week. Result shows that there was effectiveness of video teaching Programme on knowledge.

***Key Words**: Smartphone, video teaching programme, Knowledge, Effectiveness, Adolescent population.*

Introduction

Mobile are one of the important forms of communication these days. With the use of mobile we can connect to people living at any corner of the world not only that it is also very handy for young generation people to study as well as to carry their work by side[2]. Impacts of smartphone are mendable if the adolescents have adequate knowledge regarding the impacts of smartphone dependence on physical health, academic and behavior of the adolescents. We reside in a sphere that is abutting by description as well as deliberation of latest details and presenting communication technology as it is the key to future possessions , the promise of diminishing the hard work ,overcrowding the difficulty of lengthy lectures, and dishearten educators of permitting access to up to date the worldwide details, news and also in giving consistently new exterior of amusement and freedom[1]. Through new space education mechanics, digital athenaeum, computerized voting, video conferencing, e-mail, teleshopping, mobile telephone, online chatting. Multiple users of mobile phones experience feeling of some symptoms during and after using mobile phones such as giddiness, insomnia, flickering and tingling sensation, headache restlessness, forgetting, tachycardia and disturbance in digestive systems[4]. It also has been declared some parts of the human skull are more prone to destruction due to heat, bodily formation with faulty vascular system such as nerve fibers[5].

Statement of The Problem

Effectiveness of video teaching Programme on knowledge regarding smartphone dependence on physical health, academics, and behavior among adolescents studying in selected junior colleges in Pune city.

Methodology

Safeguarding the study purposes, experimental research design single group pre-test and post-test (P1XP2) was selected by the investigator. The population of this study includes both male and female adolescents studying in Junior colleges in Pune city. Probability sampling method was used for selecting 100 adolescents who fits in the criteria. In this study the investigator assessed the knowledge level regarding impact of smartphone dependence on physical health,

academics and behavior.

Content validity

The tool was validated by submitting to 11 professionals, for their valuable view on the accuracy and relevancy of tool. The experts were basically selected on clinical expertise, interest and experience. Suggestions and comments of experts included grammatical error of the sentences.

Reliability

Reliability was evaluated using test retest method. The tool was reliable as Cronbach's alpha was found to be 0.4610.

Data Collection:

During the data collection time the main motive of the study was explained and confidentiality of the reply was assured to the adolescents. After the pre-test on the same day video teaching programme was given to samples, post-test was done on the day 7 using the same tool.

Results

Table No 1: Description of samples (adolescents studying in Junior colleges)

Sr. No.	Demographic variable	Knowledge	
		Frequency	%
1.	Age		
a.	14-16 years	0	0.0%
b.	16-18 years	74	74.0%
c.	18-20 years	26	26.0%
d.	20 and above	0	0.0%
2.	Gender		
a.	Male	49	49.0%
b.	Female	51	51.0%
3.	Education		
a	XI	0	0.0%
b.	XII	100	100.0%
4	Place of resident		
a.	Home	100	100.0%
b.	Hosteller	0	0.0%
c.	Paying guest	0	0.0%
d.	Relatives	0	0.0%
5.	Type of family		
a.	Nuclear	74	74.0%
b	Joint	15	15.0%
c.	Extended	0	0%
d.	Single parent	11	11.0%2

Cont.. **Table No 1: Description of samples (adolescents studying in Junior colleges)**

6	**Use of smartphone for**		
a.	Messenger /WhatsApp	24	24.0%
b.	Entertainment	20	20.0%
c	Web surfing	31	31.0%
d	Calls	25	25.0%
e.	Others	0	0.0%
7	**How long you have been using smartphone?**		
a.	Less than a year	9	9.0%
b.	1-3 year	67	67.0%
c.	3-5 years	14	14.0%
d.	more than 5 years	10	10.0%
8	**Time spend on smartphone usage in a day**		
a.	4 hours	66	66.0%
b.	6 hours	6	6.0%
c.	8 hours	19	19.0%
d.	more than 8 hours	9	9.0%
9	**Family income**		
a.	1-3 lakhs	26	26.0%
b.	3-5 lakhs	9	9.0%
c.	5-7 lakhs	26	26.0%
d.	7 lakhs and above	39	39.0%

The above table shows that experimental samples which are included were of age 16 and above. In which 74% were of 16-18 years, 26% of them were 18-20 years. It consists 100 samples in which 49% were male and 51% were female,100% were from standard XII and 100% of them used to stay at home. It also shows that 74% belong to nuclear family, 15% belong to joint family, 11% were single parent. The experimental sample also shows that 25% used smartphone for calls ,31% for web surfing ,20% for entertainment while 24% for WhatsApp /messenger and also that 9% have been using smartphone for less than a year,67% for 1-3 years ,14% for 3-5 years and 10 5 for more than 5 years. The study shows that 66% spend 4 hours,6% for 6 hours,19% for 8 hours and 9 % for more than 8 hours on their smartphone and 26% family income was from 1-3 lakhs ,9% was 3-5 lakhs while 39 % was 7 lakhs and above.

Table 2: Baseline level of knowledge in adolescents in pre-test

Knowledge	Pre-Test	
	Frequency	%
Fair (score 0-8)	13	13.0%
Good (score 9-16)	87	87.0%
Excellent (score 17-24)	0	0.0%

The overhead table indicate the level of knowledge among adolescent in pre-test was 87.0% of the adolescent had good knowledge (score 9-16) and 13.0% of them had fair knowledge (Score 0-8).

TABLE 3: Level of knowledge post intervention of the adolescents

Knowledge	Post-Test	
	Frequency	%
Fair (0-8)	0	0%
Good (9-17)	83	83.0%
Excellent (18-24)	17	17.0%

The overhead table indicates the level of knowledge among adolescent in pre-test was 17.0% of the adolescent had excellent knowledge (score 18-24) and 83.0% of them had good knowledge (Score 9-17).

Table 4: Effectiveness of video teaching programme on the level of knowledge in adolescent

Knowledge	Pre-Test		Post-Test	
Fair (score 0-8)	13	13%	0	0%
Good (score 9-17)	87	87%	83	83%
Excellent (score 18-24)	0	0%	17	17%

The overhead table indicates that in pre-test, majority of 87% of the adolescent population had good knowledge (score 9-17) and 13% of them had fair knowledge (score 0-8) In post-test, 83% of the adolescent population had good knowledge (score 9-17) and 17% of them had excellent knowledge (Score 18-24). This indicates that the knowledge among adolescent population improved after the video teaching programme.

Table 5: Effectiveness of video teaching on the level of knowledge among adolescent using paired t-test

	Mean	SD	p-value
Pre-Test	12	2.44	0.000
Post-Test	15	23.33	

Investigator has applied paired t-test for effectiveness of video teaching programme on the level of knowledge among adolescent studying in selected junior colleges in Pune city. Average knowledge score is 12 in pre-test while in post-test it is 15. Hence null hypothesis is rejected as the corresponding p-value was small (less than 0.05). So, it is apparent that the intervention is significantly productive in improving the knowledge among adolescent population studying in junior colleges in Pune city.

Table 6: Wilcoxon sign rank test for association between selected demographic variable and the finding among adolescent studying in junior colleges during pre-test

DEMOGRAPHIC VARIABLE		KNOWLEDGE		p value
		Excellent	Fair	
Age	16-18 years	50	24	
	18-20 years	17	9	
Gender	Male	32	17	0.724
	Female	35	16	
Maximum use of phone for	Messenger/WhatsApp	50	24	0.778
	Entertainment	9	6	
	Web surfing	8	3	
How long you have been using smartphone?	Less than a year	17	7	0.515
	1-3 years	13	7	
	3-5 years	23	8	
	More than 5 years	14	11	
Time spends on smartphone	4 hours	6	3	0.462
	6 hours	48	19	
	8 hours	8	6	
	More than 8 hours	5	5	
Type of family	Nuclear	45	21	0.746
	Joint	4	2	
	Extended	11	8	
	Single parent	7	2	
Family income	1- 3 lakhs	19	7	0.159
	3- 5 lakhs	5	4	
	5-7 lakhs	21	5	
	7 lakhs and above	22	17	

Hence, entire p-values are higher than 0.05, so we accept H_0 (null hypothesis) so that we can declare that there is no relationship among the response of pre-test score in section II and the demographic variables.

Recommendations:

· A homogenous investigation can be done on immense samples, so that detection can observed for larger samples.

· A relative research can be conducted on the level of knowledge

Summary:

In the present study, 100 adolescents studying in junior colleges were selected through random sampling technique. Single group pre-test post-test method was the study design. The samples who participated in the study comprises 74% in the age group of 16-18 years. Hence, it reveals that among the samples 4% of them were male while 51% of them were females.

The pre-test knowledge regarding impact of smartphone dependence was assessed using self-structured questionnaire. The intervention used was the video teaching programme after pre-test post-test was done 1 week later. The mean score of knowledge in pre-test was 12 and in post-test it was 15. Here the null hypothesis is rejected as the p value is less than 0.05. Accordingly, it shows that video teaching programme was effective in upgrading the knowledge of adolescents studying in junior colleges.

Conclusion

Mobile are one of the important forms of communication these days as well its multiple function is replacing laptop and computers day by day. We can see mostly the youngsters /teenagers engaging in mobile phone and spending most of their time. Mobile has replaced almost everything. There are various impacts of using mobile phones and people are unaware about it. Many researches have been conducted to see for the impacts caused by smartphone among certain population. Proper knowledge is required in order to minimize the impacts caused by smartphones. The findings of the study proved that there is noticeable efficiency of video teaching Programme on knowledge regarding impact of smartphone on physical health, academics and behavior among adolescents studying in junior colleges.

Ethical Clearance- Ethical clearance taken from Institutional Research committee.

Conflicts of Interest: All authors have no conflicts of interest to declare.

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Nursing Clinical Preceptorship: Knowledge and Attitude of Nurse Educators Working in Teaching Institutes in India

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Abstract

Background: Nursing is a field of clinical expertise, which deals with caring for the patients with illness and providing them a quality nursing care. To provide a good quality Nursing care, it is very important that the Nurses should be properly trained and expert in providing care. A clinical educator plays a very important in training and updating nurses with the updated and current trends in Healthcare sector. Clinical preceptorship is one of the best methods used to train the nurses in Clinical setting. Clinical preceptorship is a method of teaching and guiding students by a knowledgeable nurse in medical area to train them in clinical Skills and techniques.

Clinical preceptorship is a concept which is mostly used in western countries. In India clinical preceptorship is not practiced.

Since it is a new concept in India scenario, this study evaluated the knowledge and attitude of Nurse Educators towards clinical preceptorship.

Methods: The study was conducted amongst Nursing Educators through online survey method.

Results: More than 50 % of the Nursing professors does not have any knowledge about Nursing preceptorship.

Out the educators having knowledge about Clinical preceptorship maximum had a positive attitude towards it.

Conclusion: The Nursing Educators in India are very much favourable with the concept of Clinical preceptorship, whilst there is knowledge gap present.

Keywords: *Nursing, Preceptorship, Educators, Knowledge, Attitude, Healthcare, Clinical, India*

Introduction

Nursing is a profession having both the flavours of Arts and Science which resembles heart and mind[1]. Nursing at its heart depicts the two important aspects of Nursing profession that is Respect for Human Dignity and understanding patients need. Whereas when it comes at mind, it supports the heart in the form of rigorous core learning. Nursing is a dynamic field having many specializations and which is practice based and stands on theoretical knowledge and practical skills. All nurses gain these knowledge and skills after undergoing a comprehensive Nursing Education programme[2].

To provide quality nursing care a nurse must be skill full and competent. This skills and competency are gained by nursing students during their clinical practice. Significantly the clinical learning is the most important and fundamental core area.

As nursing profession is all about skills and competency, preceptorship plays a very important role in training the nursing students during their training period. Preceptorship is precisely pairing a trained teacher with learner to achieve the desired goal. Preceptorship is an effective way of training students in clinical area. It enables the student to learn and get trained by a clinical

nurse or educator in the clinical area. The overall aim is to teach supervise and evaluate the student's clinical performance and give them a constructive feedback.

Preceptorship is a vital part of nursing education. It supports to fill the gap between the theory and practice. It provides students with an educative and nurturing environment within the clinical area, thereby allowing the students to apply the principles of nursing profession[3].

In India, nursing teachers are only allocated as the clinical instructors for the undergraduate students, from the first year of the programme. This clinical Instructor is either a BSc. Nursing graduate or a Post Graduate teacher in Nursing. This Instructors are usually assigned for a large group of students[4]. As per the Nursing Statutory Body in India (INC) the students to teacher ratio has to be 10:1. As per the ratio a single clinical instructor has to look after at least 10 students, which makes it quite difficult for her to provide on to one teaching and demonstration of procedures in the clinical area. The current trend of teaching and learning in clinical nursing is not allowing the Instructor to provide individual focus on the students. This might be the reason between the huge gap between the Theory and practice.

In India, preceptorship is a totally unknown concept. Nursing colleges in India follow the traditional way of mentoring the students by the clinical instructors in the clinicals. These Clinical Instructors are basically Graduates or Post Graduates in Nursing and are handling both the theory and the practical part of Nursing Education. Currently, the quality of care given by Nurses in India is much compromised and needs a lot of revision and analysis, as most of the newly graduated nurses lack the clinical skills. Therefore, applying preceptorship concept as a part of teaching learning process in the hospitals and Healthcare organizations can be the useful and fruitful strategy in order to improve the quality of care and thereby improving the Nursing profession status[5].

In India as preceptorship is a new and never used concept. Therefore, the researcher felt to conduct a study to assess the knowledge and attitude of Nursing Educators towards Nursing clinical Preceptorship.

Methods

This study is an exploratory descriptive study aims to assess the knowledge and attitude of Nursing Educators regarding Clinical preceptorship. The study was conducted from January 2018 to February 2018 in Pune city. Nursing educators from all the Nursing Institutes having Bachelors Nursing programme were approached to participate in the study. Convenient sampling technique was used to select the participants. Nursing educators having minimum 1 year of teaching experience were included in the study.

Data was collected using a semi- structured questionnaire. The tools were made in English language. The study tool was divided into 3 parts:

- Demographic Questions
- Questionnaire to assess the knowledge regarding Clinical preceptorship
- Questionnaire to assess the attitude regarding Clinical preceptorship

An informed consent was taken from the participants and they were assured and informed about the aim of the study.

Results

Demographic Data:

A overall of 60 teachers were selected from teaching organizations located in Pune, Maharashtra. 60 questionnaires were distributed out of which 10 Educators did not respond back. Therefore, 50 questionnaires were used in the data analysis. **(See Table 1)**

Table No 1. Demographic Characteristics of Participants (N=50)

Socio Demographic Variable	Frequency	Percentage
Gender		
Male	18	36
Female	32	64
Age group (years)		
21 - 30	10	20
31 - 40	22	44
41 - 50	14	28
more than 50	4	8
Qualification		
Bachelor's Degree in Nursing	14	28
Master's Degree in Nursing	29	58
Doctorate in Nursing	7	14
Years of Experience		
1 – 3 Years	12	24
3 – 5 Years	9	18
5 – 7 Years	15	30
7 years and Above	23	46

Analysis shows that when the participants were asked whether they know about clinical preceptorship (66%) expressed that they do not know and only (44%) were knowing about clinical preceptorship. About practicing Clinical preceptorship at the institutions (92%) participants said that they do not practice and (8%) participants were not sure about it. (64%) of educators said the clinical preceptorship is conducted in hospitals whereas, (36%) educators said that it is conducted in classrooms. The table shows that over all the Maximum Nursing educators were having an idea and superficial knowledge about Clinical Preceptorship. (See Table 2)

Table 2: Baseline level of knowledge Nursing Educators regarding Clinical Preceptorship

(N= 50)

Q. no.	Questions	Frequency	Percentage
1.	Do you know about nursing clinical preceptorship?		
2.	1. Yes	17	34
3.	2. No	33	66
4.	Does your institution use nursing clinical preceptorship programs?		
5.	1. Yes	0	0
6.	2. No	46	92
7.	3. I am not sure	4	8
8.	Clinical preceptorship programs mostly take place in?		
9.	1. Hospitals	32	64
10.	2. Classrooms	18	36
11.	The key intent of clinical preceptorship to students is to?		
12.	1. Develop clinical skills	26	52
13.	2. Ease transition from being a student to being a professional	12	24
14.	3. Both	12	24
15.	Who is a Preceptor?		
16.	1. A student	12	24
17.	2. Teacher	29	58
18.	3. Other Professional	9	18
19.	Perfect preceptor to student's ratio is?		
20.	1. 1:1	6	12
21.	2. 1:10	29	58
22.	3. 1:5	5	10
23.	4. 1:20	10	20
24.	Does the teaching faculty designate or assign a preceptor for each student?		
25.	1. Yes	24	48
26.	2. No	26	52
27.	Roles of preceptors in the medical set?		
28.	1. Medical skill teaching	9	18
29.	2. Training nursing ethics to students	12	24
30.	3. Effective training in mixing knowledge and training	4	8
31.	4. All of the above	25	50
32.	Qualities that a Clinical preceptor should demonstration		
33.	1. Knowledge	4	8
34.	2. Real communication skills and association	6	12
35.	3. Cognitive and problem-solving skills	12	24
36.	4. 1,2 & 3	28	56
37.	Can a preceptor prove real problem-solving skills and show compassion and care in specialized practice?		
38.	1. Yes	37	74
39.	2. No	13	26
40.	Is Clinical preceptorship a kind of adult education system?		
41.	1. Yes	42	84
42.	2. No	08	16

Cont... **Table 2: Baseline level of knowledge Nursing Educators regarding Clinical Preceptorship**

43.	Communicating effectively is a significant way to effective preceptor faculty relations?		
44.	1. Yes	38	76
45.	2. No	12	24
46.	Which of the following can be encouraging outcome of preceptorship programs towards health care establishments?		
47.	1. Improved employment of new nurses	10	20
48.	2. Improved retaining of already working force	11	22
49.	3. Enhanced results for patients	25	50
50.	4. None	4	8

Usefulness of the Clinical preceptorship was agreed by maximum (58%) of the nursing Educators. Maximum number of educators (52%) does not support the idea of implementing Nursing clinical preceptorship programme. (60% of the educators disagree for integrating the Clinical preceptorship programme in Nursing curriculum. Over all a mix of attitude was found among the Nursing Educators regarding Clinical Preceptorship, its implementation in Indian Scenario and the benefits of the program. (See Table 3)

Table 3: Attitude of Nursing Educators towards clinical preceptorship

(N= 50)

Q. no.	Questions	Frequency	Percentage
1.	Do you think that clinical preceptorship programs are beneficial to students?		
2.	1. Yes	29	58
3.	2. No	21	42
4.	Do you believe that clinical preceptorship programs will improve students' hands-on skills and nursing ethics?		
5.	1. Yes	20	40
6.	2. No	26	52
7.	3. I am not sure	4	8
8.	Should clinical preceptorship programs be integrated in the nursing curricula and applied nationwide?		
9.	1. Yes	12	24
10.	2. No	30	60
	3. I am not sure	8	16
11.	Do you think that teaching ethical principles is important during clinical preceptorship?		
12.	1. Yes	39	78
13.	2. No	6	12
14.	3. I am not sure	5	10

Cont... **Table 3: Attitude of Nursing Educators towards clinical preceptorship**

(N= 50)

15. 16. 17. 18. 19.	In your view, to accept the position of a preceptor, the nurse should have an educational qualification of?		
	1. A Diploma	7	14
	2. A Bachelor's Degree	15	30
	3. A Master's Degree	28	56
	4. No Qualification needed	0	0
20. 21. 22. 23.	In your view, to accept the position of a preceptor, the nurse should have a work/clinical experience of?		
	1. No experience needed	13	26
	2. 6 months	11	22
	3. One year and more	26	52
24. 25. 26.	Other than their benefits for the students, do you think that clinical preceptorship programs are beneficial for educationalists and preceptors?		
	1. Yes	22	44
	2. No	28	56
27. 28. 29. 30.	To accept the position of a preceptor, is academic and logical knowledge important?		
	1. Very important	32	64
	2. Important	18	36
	3. Not important	0	0
31. 32. 33.	Do you believe that using preceptors with no nursing background, is suitable?		
	1. Yes	0	0
	2. No	50	100
34. 35. 36.	For being respectable Preceptor are teaching skills necessary?		
	1. Yes	23	46
	2. No	27	54
37. 38. 39.	Should preceptors provide evaluation and response to students?		
	1. Yes	32	64
	2. No	18	36

***Cont.*. Table 3: Attitude of Nursing Educators towards clinical preceptorship**

(N= 50)

40. 41. 42.	Who do you think should take the upper hand(s) in the execution of clinical preceptorship programs?		
	1.Teaching institutions	28	56
	2. Hospitals	12	24
	3.Government	10	20
13. 51. 52. 53. 54.	What do you think will be the challenge/s for implementing nursing clinical preceptorship in India?		
	1. Shortage of Nurses	12	24
	2. Inability or unwillingness to apply	15	30
	3. Lack of funding or incentive for preceptors	23	46
	4. None	0	0

Discussion

The objective of the study was to assess the knowledge and attitude of the nursing educators regarding Clinical preceptorship. Attitude and viewpoint of Nursing Professors towards Clinical preceptorship was also assessed.

This study showed that (66%) of respondents were aware about clinical preceptorship and however it was found that maximum number of respondents were aware about Clinical preceptorship and the roles of a Clinical preceptor. Similar study was conducted by (Andreas A. Teferra, Daniel Mengistu) which shows that (82.6%) respondents were familiar with Clinical Preceptorship and they found that (43.1 %) of respondents were having adequate knowledge about clinical preceptorship[1].

Regarding the attitude towards clinical Preceptorship, it was found that (58%) of the respondents feel that it is useful for Nursing students. And there was an approximately equal kind of attitude towards implementing the clinical preceptorship in nursing in Indian scenario. Similar study conducted showed that (96%) of the respondents have a positive attitude towards clinical preceptorship.

Conclusion

As per the findings of the research study it was found that approximately 60% nursing educators who took part in study were familiar to clinical preceptorship. However, the attitude towards clinical preceptorship was equally distributed. It was felt that more in-depth understanding of clinical preceptorship can be provided and the benefits of the approach could be demonstrated to the educators in India.

Ethical Clearance: Ethical clearance taken from Institutional Research committee.

Conflict of Interest: None

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Effect of Group Therapy Promotion on of Psychological well-being among Elderly at Selected Old Age Home

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Abstract

This study aimed to assess the effect of Group therapy on promotion of psychological well-being among elderly at selected old age homes in Pune. The investigators adopted the Experimental design for the study. The participants were 60 elderly from selected Old Age Home from Pune**.** Random Assignment technique was used to select the sample. The instrument consisted of two sections. Study has shown significance at 0.05 levels and found group therapy is effective for improving Psychological wellbeing among elderly.

Key words*: Group therapy, Psychological wellbeing, Elderly.*

Introduction

The term psychological wellbeing (PWB) is a similar as positive mental states, such as cheerfulness or contentment[1].

Psychotherapy is indeed effective not just because of the theoretic base but because of the therapist's strong faith in the effectiveness of the practice[2].

Various forms of group therapy or psychotherapy can be used to manage some problems related to mental health like Elderly with anxiety, and it can also be useful in preventing the relapse of such conditions[3].

Group therapy has been a core aspect of drug and improving Psychological wellbeing for several decades[4].

In Group therapy one or more therapists treat a small group of clients together as a group. Group therapy and group feeling encourages the expression of affect, tension and fear. So, Group therapy has been considered as the most prevalent treatment modality for improving Psychological wellbeing[5].

Problem Statement: "Effect of Group therapy on promotion of psychological well-being among elderly at selected old age homes in Pune."

Hypothesis:

Ho: There will be a no significant difference in the level of Psychological wellbeing after Group therapy in the experimental group.

H1: There will be a significant difference in the level of Psychological wellbeing after Group therapy in the experimental group.

Research Methodology

A pre-test & post-test experimental and control group design was used. The study was conducted at Old Age Homes on 60 Elderly. Samples were selected through random assignment. Pretest was taken followed by Group therapy for ten days. On 11th day Post-test was taken using same instrument.

Tool

The tool used in the present study were as follows

· Baseline Proforma of the samples.

· Questionnaire for assessing Psychological wellbeing.

Data Collection:

After obtaining permission from the authorities, written consent was taken from the participants. Pretest was conducted on the first day. !0 day Group therapy sessions were conducted as planned below followed by Post-test on the 11th day.

Group Therapy Schedule

Sr. No	Day	Intervention
1.	Day 1	Introduction of Group members and Researcher and introduction of the study.
2.	Day 2	Deep breathing exercises and brief history of group members regarding their drinking habit.
3.	Day 3	Deep breathing and anger management techniques
4.	Day 4	Deep breathing and introduction to Psychological wellbeing
5.	Day 5	Deep breathing and facts related to Psychological wellbeing
6.	Day 6	Deep breathing and how to stay positive and Happy
7.	Day 7	Deep breathing and other problems related to staying away from family
8.	Day 8	Deep breathing and psychological damage caused by situations around
9.	Day 9	Deep breathing and ventilation of self-ideas
10.	Day 10	Deep breathing and healthy living

Major findings

Table 1: Frequency and percentage distribution of Elderly according to their age, religion, Education and duration of stay (N= 60).

Parameters		Experimental Group	Control Group
		Percentage	Percentage
Age	50-55 Years 56 – 60 Years 61- 65 Years 66 and above	10% 42% 36% 12%	14% 38% 22% 26%
Religion	Hindu Muslim Christian Others	86% 6% 4% 4%	72% 16% 6% 6%
Educational status	Graduation Graduation and above	92% 8%	82% 18%
Years of stay in Old Age home	Less than 1 year More than one year	88% 12%	94% 6%

Table 2: Comparison of pre and post-test value before and after group therapy in experimental group

	Experimental group		't' value
	Mean	SD	
Pre-test	38.0	6.3%	**14.55**
Post test	78.9	13.7%	

The mean value of experimental group in pre-test (before group therapy) was 38.0, SD 6.3, where as in the post-test (after group therapy) was 78.93, SD 13.7, thus the calculated 't' test value was14.55 (P=0.05). Which was significant at 0.05 levels.

Table 3: Comparison of level of psychological wellbeing among the experimental and control group

	Sample Size	Experimental group		't' value
		Mean	SD	
Experimental Group	30	78.9	13.7%	**16.62**
Control Group	30	34.4	5.2%	

While comparing the scorings of psychological wellbeing of elderly before and after group therapy in the experimental group, the mean score were increased after group therapy. Results paraded that there was improvement in the psychological wellbeing.

Discussion

The findings of the study also revealed that there was statistical difference in psychological wellbeing between the experimental group and control group. The frequency of psychological wellbeing for 100% of elderly before group therapy was poor psychological wellbeing. Whereas after group therapy 66.7% of elderly showed high psychological wellbeing and 33.3% of elderly showed moderate psychological wellbeing. While comparing psychological wellbeing of Elderly before and after group therapy, the psychological wellbeing was increased after group therapy.

Similar findings were seen in the study conducted by Monras M, Ortega L, Mondon S, Gual A, Med Clin Barc (2000), which also showed that the alcoholic dependents exhibited positive interpreters are high self-efficacy and confidence in high risk situation, positive outcome expectancies, sufficient coping skills and low craving after group therapy[6].

Conclusion

The findings of the study revealed that there was statistical difference in psychological wellbeing between the experimental group and control group. The frequency of psychological wellbeing for 100% of elderly before group therapy was poor. Whereas after group therapy 66.7% of elderly showed high psychological wellbeing and 33.3% of elderly showed moderate psychological wellbeing. The investigators been concluded that Group therapy has promoted the psychological wellbeing among Elderly.

Recommendations

· It is recommended that larger sample can be studied for making generalization.

· It is recommended that interview schedule can be developed for assessing the psychological wellbeing for further refinement and standardization on a large scale.

· It is further recommended that study can be done using teaching strategies like, making

informational booklet for health care team members and self-instruction module.

· It is also recommended to study the effect of various modalities for promotion of psychological wellbeing in elderly.

Ethical Clearance- Ethical clearance taken from Institutional Research committee.

Source of Funding- Self.

Conflict of Interest - Nil.

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Quality of Sleep among the Patients in High Dependency Unit (Hdu)- A Descriptive Study

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Abstract

Background: Sleep is a vital physiologic process, serious outcomes results due to lack of sleep. The circadian rhythm plays an important role in our biologic function. Various studies have recorded the function of circadian rhythm in our body and the various environmental parameters which affect the sleep of the patients. Along with other factors critical illness also contributes to the vulnerability of the patient.The recovery of the patients depends on the quality of the sleep. Hence this study assess the quality of sleep among the patients in HDU.**Objective:**To assess the quality of sleep among the patients in HDU.**Methodology:** A Non-Experimental Descriptive Research Approach is used. 30 samples who had three days of HDU stay were selected by using NonProbability Purposive Sampling Technique. Data was congregated using Modified Pittsburgh sleep Quality Index.In order to establish the reliability of the tool test-retest method was used and the pearson's correlation coefficient was found to be 0.98. **Result:** Majority of them, 50% had age group of 41-50 years. 23% had age group of 51-60 years and 13.33% has 31-40 years and above 60 years age group. Majority 66.67% were male and few 33.33% were female. Majority 63.33% had three days of HDU stay whereas 36.67% had 4 days of HDU stay. 33.33% had diagnosis related to cardiovascular, 30% had respiratory related diagnosis, 20% were related to gastrointestinal, 13.34 % were from endocrine and few 3.33% were from other systems. The quality of sleep reveals that majority 40% of them had poor sleep (score 30-44), 26.66% had average sleep (score 15-29), 20% had good sleep (score 1-14), 13.33% had very good sleep (score 0)**Conclusion:** The findings indicate that the questionnaire was effective in assessing the quality of sleep among the patients in HDU. The study also concluded that majority of the patients in HDU had poor quality of sleep.

Keywords: *Assess, Quality of Sleep, High Dependency Unit (HDU).*

Introduction and Background

Sleep is a process which is clout by the one's own biologic and environmental aspect. The increasing evidence suggest that sleep disturbances are combined with inimical outcomes. Sleep is a composite biological rhythm. A person is said to be in a circadian synchronization,

when one's biological clock coincides with sleep wake cycles. This broach that when the persons body temperature is high the person is said to be awake and when body temperature is lowest the person is said to be asleep. Sleep anomaly occur intermittently in the critical care unit. Studies have shown that patients in Intensive Care Unit (ICU) have sleep latency, sleep fragmentation, numerous arousals. Day time sleep is been reported with a noticeable shift towards light stages of sleep. The recent studies have directed on actuating the best method to score the sleep in critically ill patients. Major aspect

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for deprivation of sleep includes the underlying causes such as pain, stress, anxiety.[1][2][3]

Sleep disturbances in the intensive care unit sounds development of delirium. This confess that satisfactory sleep is necessary for positive result. Various studies acknowledge pathological sleep patterns in critically ill patients havinghigh arousal and awakening index,abnormal circadianrhythm, REM sleep.[7]

Studies also report the environmental factors in the critical care unit contribute more to the sleep disruption. Noise is one of the aspect among the environmental factors which includes multiple sources such as alarms, staff conversations, pagers, mechanical ventilators. The nocturnal lights in the critical care unit accord for sleep disruption. The nocturnal light affect the melatonin secretion in the body which again causes sleep deprivation. The other aspect such as patient care activity such as monitoring vitals, collection of labs, and other care activity brings about sleep disturbance among the patients. Some studies have reported mechanical ventilation responsible for fragmented sleep among critically ill patients.[1][6] Lack of sleep affect the immune system, hormone levels, neurocognition and pulmonary mechanics. Changes in the sleep Structure flourished during a stay in the critical care unit may compellingly add to sleep disorders among patients.[6] There are various questionnaires which help to identify the sleep among the patients in critical care unit. The patients in critical care report poor sleep quality.[1][2]

Objectives

"To assess the quality of sleep among the patient's in HDU"

Material and Methods:

A NonExperimental Research design with quantitative approach was used. 30sampleswho had three days of HDU stay and consciouswere selected by NonProbability Purposive sampling technique. The tool included two sections; the first section; demographic data included age, gender, length of stay in HDU, Diagnosis and any invasive lines.The second section included a modified scale to assess sleep. The Modified Pittsburgh Sleep Quality Index consisted of 11 questions and 4 columns; the patient answered and the researcher marked the answer. The scoring embodied; if the score is 0 the sleep quality is opined to be very good, if the score is between 01-14 the sleep quality is good, Average quality of sleep suggests that the score is between 15-29 and the score between 30-44 conjecture a bad sleep quality. Experts validated the tool, the reliability was assessed using test retest method, and the correlation coefficient was found to be 0.98.

Findings

Section- I

Description of samples characteristics in frequency and percentage.

Table 1: Distribution of demographic data in Frequency and Percentage

n=30

DEMOGRAPHIC VARIABLE		
	F	**%**
AGE		
31-40 years	4	13.4%
41-50 years	15	50.0%
51-60 years	7	23.3%
Above 60 years	4	13.4%
GENDER		

Cont... **Table 1: Distribution of demographic data in Frequency and Percentage**

n=30

Male	20	66.6%
Female	10	33.3%
Length of stay in HDU		
3 days	19	63.3%
4 days	11	36.6%
5 days	0	00.0%
DIAGNOSIS		
Endocrine	4	13.4%
Respiratory	9	30.0%
Gastrointestinal	6	20.0%
Cardiovascular System	10	33.3%
Any other	1	3.3%
ANY INVASIVE LINES		
Foleys catheter	1	3.3%
IV Cannula	28	93.3%
Fistula	1	3.3%

Section-II

Analysis of data on Quality of sleep among the patients in HDU

Table 2: Data related to quality of sleep among the patients in HDU.

n= 30

Quality of sleep	F	%
Very good (score 0)	4	13.3
Good (Score 1-14)	6	20
Average (Score 15-29)	8	26.6
Poor (Score 30-44)	12	40

Discussion

In this present study a Non Experimental Descriptive research design is been used. Non Probability Purposive Sampling Technique was used for selecting 30 samples who had three days of HDU stay and who were conscious. A written and informed consent was obtained. The patient answered and the researcher recorded the answer. The tool included two section; the first section had demographic data and the second section included the Modified Pittsburgh Sleep Quality Index. The study found that maximum 50% patients were from 41-50 years age group, 23.3% patients were from 51-60 years age group, few 13.4% patients were from 31-40 and above

60 years age group. Frequency of Males were more than that of the females. The further the demographic data was studied and it was found that maximum 63.3% people had 3 days of HDU stay whereas 36.6% patients had 4 days of HDU stay. Maximum 30% Patients were with respiratory disease. Most of the patients had IV cannula.Thedata was analysed further which revealed that maximum 40% of the patient had poor sleep (score 30-44), 26.6% of the patient had average sleep (score 15-29), 20% of the patient had good sleep (score 1-14), 13.3% of the patient had very good sleep (score 0). This suggest that the patients in HDU possess poor sleep quality. Further studies are recommended to find out the affected intervention to improve the quality of sleep among the patient in HDU

The above findings of the study are supported by a similarstudy;In this descriptive study102 samples were selected who met the inclusion criteria. Data was congregated using Pittsburgh Sleep Quality Index. The data revealed that 49% patient were found to have poor sleep quality. The study concluded with varied degrees of quality of sleep; the personal and environmental factors affects the quality of the sleep. The study suggested strategies to enhance physical and mental comfort and minimum sleep disruption to enhance the quality of sleep.(4)

A descriptive cross- sectional study was lugged out on 150 patients. The data was mustered using Pittsburgh Sleep Quality Index. The data was evaluated in percentage, mean, independent group test, one way ANOVA test, chi square test. The study found that the patients had poor sleep quality. The study suggested more supportive nursing care.(5)

A study was conducted on sleep deprivation. It studied the impacts of sleep deprivation on health and recovery from illness. The study unfolds that sleep deprived patients suffer from immune system dysfunction, impaired wound healing and behavioural change. The factors responsible for this are fear, anxiety, pain, noise, light exposure, and frequent awakening from caregivers. It also added that the underlying medical illness and medications dramatically affect patient's sleep. The suggested therapy along with minimum disruption from care givers during night. Also, certain protocols to reduce sleep deprivation should be used.(3)

Conclusion

The findings indicate that the questionnaire was an efficacious strategy in assessing the quality of sleep among the patient in HDU. The structured questionnaire was acceptable and appropriate method for assessment of the quality of sleep among the HDU patients.

Conflict of Interest -Nil

Source of Funding- Funded by self

Ethical Clearance-: Obtained from Institutional Research Committee and Hospital.

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Nurses' Preparedness regarding Emerging Infectious Diseases (EID) in Selected Hospitals of Maharashtra (India)

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Abstracts

Covid-19 outbreak is spread all over the world. It is important that all nurses including healthcare professional be prepared to care for people affected by such emerging Infectious Diseases (EID. Research approach adopted was exploratory survey method. Research hypothesis (Ho) is healthcare preparedness about Emerging Infectious Diseases (EID is independent of cadre of nurses. Convenient sampling method was used. The study was conducted during May 2020 across (Maharashtra). The total 180 nurses of various cadres were participated in online survey. Semi structured questionnaire was prepared with 30 questions on preparedness of nurses. However, overall preparedness level was found satisfactory, still there was gap in preparedness. Preparedness level of Emerging Infectious Diseases (EID is independent of cadre of nurses. (Ho) To test this we applied standard F test for collective comparison of means using ANOVA table. Since the computed F ratio is less than the table value the mean, preparedness level of four cadres does not differ significantly accepting null hypothesis (Ho).

Key Words: *Nurses, Pandemic COVID-19, Biological disasters, Preparedness, Abilities*

Background

Infectious Diseases are occurring more frequently in India and around the world[1]. Severe Acute Respiratory Syndrome (SARS), Swine Flu outbreak, Pandemic Influenza and now COVID-19 these emerging infectious diseases (EID) becoming threats to mankind across the globe. In the corona virus pandemic health care workers became warriors to help the patients suffering from acute infectious diseases. Nurses play significant role in caring the patients suffering from acute exacerbation of infectious diseases. **EID** have devastating results due to inadequate preparation, the latest being the Covid-19 corona virus outbreak[2]. In India particularly Maharashtra it is observed that, there is shortage of equipment, and supplies, poor infection control guidelines, shortage of nurses. Since calamities are unavoidable. Efforts have to be made to withstand the aftermath of catastrophes. Recent corona virus outbreak have shown how unprepared we are to tackle extreme **Emerging Infectious Diseases**. According to study done especially on Indian conditions, epidemics have killed millions of people. Nurses' preparedness is highly important in reducing the ill effects of infections and improves the recovery of the patient[3]

During Emerging Infectious Diseases (EID) outbreak, health care workers and nurses can play a significant role in caring for people. Thus, preparedness is important for nurses. Nurses' working in clinical areas should acquire knowledge and practices skills needed to respond to such situations[4]. Based on all above facts the investigator felt the need of exploring the healthcare preparedness in regards to their knowledge and self-expressed practices among the nurses regarding Emerging Infectious Diseases[5]

A researcher was interested in finding related to nurses and health care preparedness in relation to their knowledge, practices to tackle infectious disease outbreak. Researcher was interested in finding the answers to the following[6]

1. Do nurses adequately prepared in regards to knowledge and self-expressed practices related to Emerging Infectious Diseases

2. Do their knowledge and self-expressed practices regarding preparedness is associated with socio-demographic factors

Methodology

Research approach adopted was online exploratory survey method. Convenient Sampling method was used. The study was conducted during May 2020 across state of Maharashtra. The total 180 nurses of various cadres (Nurse Educator, Head Nurses, Senior staff Nurses and Junior staff nurses) were participated from more than 10 cities of Maharashtra. Semi structured questionnaire was prepared with 30 questions regarding knowledge and self-expressed practices about emerging infectious diseases, Infectious disease. Questionnaire includes knowledge related to prevention, causes, transmission, government policies, adequacy of the equipment, training related to EID, future practices and resources available in healthcare organization related to patient care.

Questionnaire

Emerging Infectious Disease (EID) questionnaire was prepared to measure nurses' knowledge and self-expressed practices. Questionnaire was duly validated by experts in the field including nursing and public health. Questionnaire included 30 items A close-ended schedule was administered. Tool consists of two sections. Section I: Demographic Profile Form Section II: **Emerging Infectious Diseases (EID**) Knowledge and Self-Expressed practices Semi structured questionnaire duly validated by experts. Each correct answer to the question was given one mark and incorrect answer was given zero mark. The level of preparedness was classified based on correct responses viz. Poor (0-10), Good (11-20), Excellent (21-30). Data were analyzed by using standard statistical techniques of analysis.

Results and Discussion

According to Table 1. The majority of the respondents were female 120 (67%) as nursing profession consist of majority female population, Mean age of Junior Nurses was 21 years, and mean age of staff nurse were 28 yrs. Mean age of Head Nurses and Nurse Educator was 36 years. The majority of the nurses 120 (67%) was working in a medical surgical setting. and 100% respondents were working currently in clinical area.

Table – 1 : Distribution of Subjects

Subjects	Male	Female	Total	%
Nurse Educators	27	18	45	25
Head Nurses	0	45	45	25
Senior Staff Nurses	18	27	45	25
Junior Staff Nurses	15	30	45	25
Total	60	120	180	100
%	33.33	66.67	100	

Section I : Level of Preparedness – Total 30 questions were asked to the 180 participants through online survey method following are the observations

Table 2 : Frequency distribution and percentage of samples according to level of preparedness

Preparedness Score	Nurse Educators	Head Nurses	Senior Staff Nurses	Junior Staff Nurses	Total	%
Poor (0-10)	6	9	9	9	33	18
Good (11-20)	6	12	12	6	36	20
Excellent (21-30)	33	24	24	30	111	62
	45	45	45	45	180	100

Fig. 1 Percentage of samples according to level of preparedness

According to table 2 and Fig1 : Overall preparedness level of Nurses is good but looking at table 2 it is seen that 62 % nurses are in excellent category of preparedness while 38 % nurses are yet to prepare for infectious diseases. It is interpreted that though their level of preparedness is satisfactory but there are gaps in their level of preparedness.

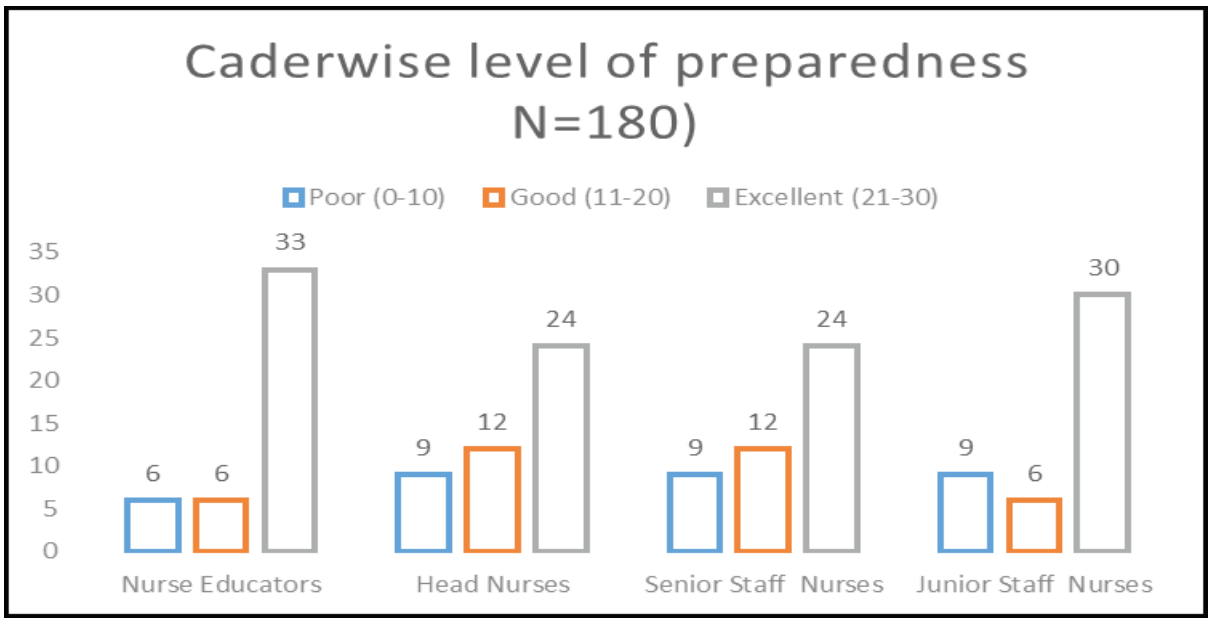
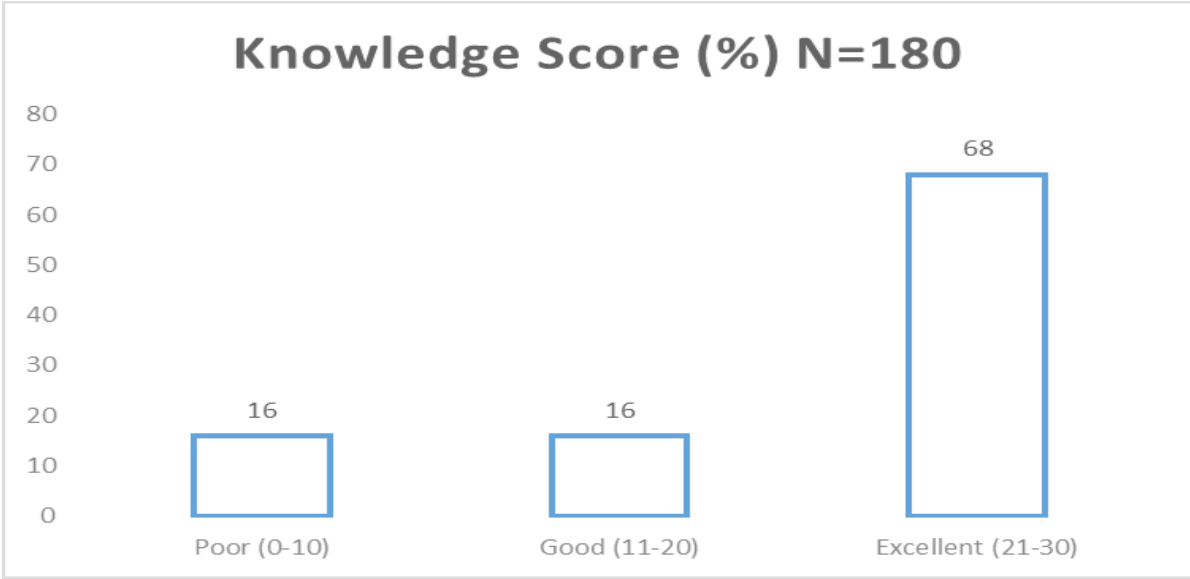


Fig. 2 Caderwise level of preparedness among nurses

The Fig. 2 depicts that Nurse Educator's and Junior Staff nurses' preparedness level is better than head nurse and senior staff nurses. However, nurse educator's preparedness is at optimum, followed by junior staff nurse and senior staff nurses

Section II : Knowledge level of nurses

The knowledge and self-expressed practices were assessed using semi-structured questionnaire and following were observations



TFig. 3. Knowledge level of Nurses

The 15 questions were asked regarding knowledge and it was found that only 68% nurses identified in excellent category while 16 % nurses in were in poor and good category of level of knowledge.

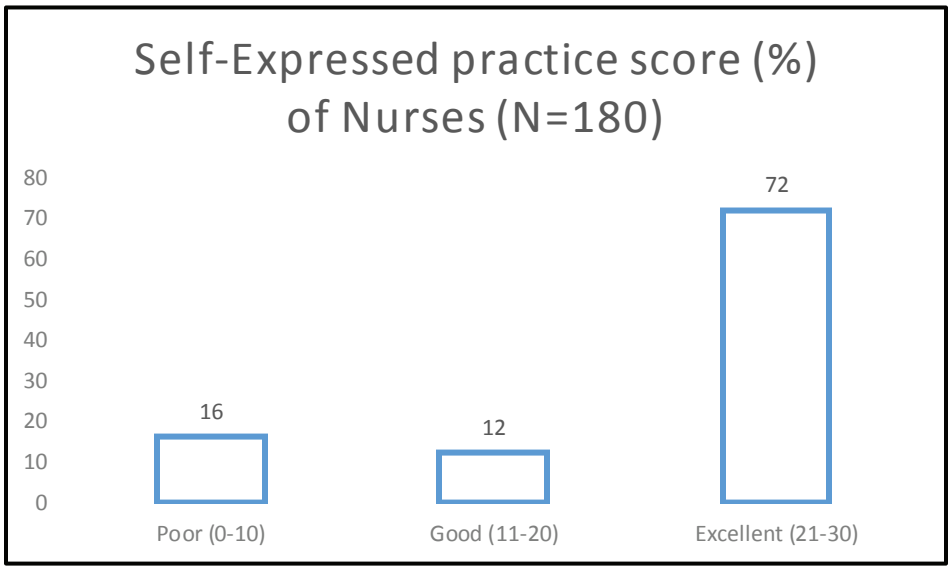


Fig. 4 Self-expressed practice score of Nurses

Simultaneously in self-expressed practices 72 % nurses found in excellent category and only 12 % found in Good and 16 % in poor category

Overall knowledge and practices of nurses found satisfactory but researcher found following major gaps in their knowledge and practices

1. The question was asked regarding training related to infection control guidelines, procedure or policies for the training schedule only 66% of the nurses stated that their hospital has written policies for the training.

2. Core capabilities includes bio-safety, infection control guidelines, practices about PPE, bio-waste management, management of Isolation, Immunization, standard of care guidelines, emergency preparedness unfortunately nobody could answer all these components of capabilities.

3. Infection control committee is one of the important committee in hospitals but only 54% nurses answered presence of ICC.

4. Only 66% participants told that their hospital has testing services for selected communicable diseases.

5. Out of all 30 hospitals from where 180 participants responded from Maharashtra it was found that only 11 hospitals were accredited by NABH.

Section III : Association of findings with demographic variable

We tried to test the hypothesis (Ho) that preparedness about **Infectious Diseases (EID** is independent of cadre of nurses. To test this we applied standard F test for collective comparison of means using ANOVA table.

Table 5 : Analysis of variance (ANOVA) table

Variance (square)	Degree of freedom	Sum of Square	Mean Sum of Square	F - Ratio
Between the cadre	4-1=3	39	13	0.68
Error	59-3=56	1066	19.04	

Computed 'F' ratio = 13 / 19.04 = 0.68 table F value for 3 df and 56 df at 5 % level of significance = 2.76. Since the computed F ratio is less than the table value the mean preparedness level of four cadres does not differ significantly accepting our hypothesis (Ho).

On looking at the means for four cadres we find that preparedness level of nurse educators, head nurses, senior staff nurses and junior staff nurses does not differ significantly.

Discussion

Recent covid-19 outbreak across the world and in India made devastating effect on lives of the people recent statistics shown that there are more than 16 lakh people infected in India and 25000 died as on July 20, 2020[7] This study was conducted to measure nurses knowledge and self-expressed practices for EID among nurses of Maharashtra State. The influx of the corona virus infected patients are visiting hospitals in large numbers[8]. The facilities, equipment, manpower, their skill was found below the expected level as evidenced by increasing death rates in the hospitals[9]. Since this kind of outbreak is found for the first time, healthcare workers and nurses are not prepared to tackle such a crisis[10]. Research found that knowledge of nurses regarding management of **Infectious Diseases (EID)** was reasonably good as 68 % found in excellent category but researcher found huge gap in the knowledge of nurses similar finding is observed in self-expressed practices.

In this study it was revealed that the level of preparedness of nurses is good, their knowledge level also found reasonable but their self-expressed practice was found below average. This study is supported by study conducted by Kalanlar and found that nurses and health care workers are poorly prepared to manage emerging infectious diseases[12]. Hence it is suggested that every healthcare organization, government health agencies to identify the areas of capability buildings and enhance nurses knowledge and practices to manage the emerging infectious diseases[13].

Implications to Nursing

1. Principles of Quarantine, identification of infected clients, Isolation, Notification, Drugs availability, Immunization, Diet, Basic Life Support (BLS) and Advance Cardiac life Support (ACLS) training programme may be included in each nursing programme.
2. INC recognized Disaster Nursing programme should be encouraged to vulnerable regions like Gujarat, Maharashtra, NE states etc.
3. Present curricula needs to be reformed looking into current emerging diseases.

Ethical Clearance- Taken from Ethics Committee of the Institution.

Source of Funding- Self funded.

Conflict of Interest –Nil

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A Study to Assess Knowledge and Attitude Towards HIV among Students from Pune City

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Abstract

Background: India has the third largest HIV epidemic in the world. The Indian epidemic is characterized by low levels in the general population and elevated concentrations among high-risk groups. The present study was planned to determine the awareness of HIV among students from Pune city.

Methods: A cross-sectional study was carried out among students from Pune city, India during March, 2020. 50 students were approached to participate in the study (males: 32; females: 18). Pretested questionnaire was distributed and collected data was analyzed using SPSS version 16.

Results: Study participants had high knowledge (86%) and attitude score (87%). There was no significant difference between males and female participants for attitude and knowledge, except for one question regarding knowledge about HIV transmission via breastfeeding to child.

Conclusions: Present study showed that there are no misconceptions or negative attitudes regarding HIV among students.

Keywords: *AIDS, Attitude, HIV, Knowledge*

Introduction

HIV/AIDS has become one of the most devastating diseases humanity has ever faced. It has become a major public health concern with about half of new infections occurring in young people[1]. Sub-Saharan Africa, which has just over 10% of the world's population, remains the most seriously affected region [1]. The impact of HIV/AIDS has caused much consternation among policy-makers as it threatens to erode socio-economic through its associated increase in morbidity and mortality of people in the productive age group [2].

A more serious challenge today, is the growing infection rates among the adolescents in sub-Saharan Africa. Research has shown that the highest group found to be infected with the virus is the age group 15 to 24 [3]. This adolescent high-risk group accounts for 60 percent of all new infections in many countries4. Transactional sex, very common among young adults in Africa has been identified as one of the critical pathways for the transfer of the virus in Africa[5]. It is not surprising that sexual intercourse has become the most predominant mode of transmission of HIV in sub-Saharan Africa, accounting for approximately 90% of all infections[6].

Young people are particularly vulnerable to HIV infection because of the physical, psychological, social and economic attributes of adolescence[7]. Young adults are also at risk as a result of high risky sexual behaviors, attitudes, and constraints of the societies in which they grow up[8]. Qualitative and quantitative studies conducted in Ghana suggest that the lifestyles of university students are placing them at risk of contracting the HIV [9, 10]. Moreover peer pressure to obtain luxury items, such as expensive clothing, jewelry, fashionable hairstyles, accessories, and makeup, motivates young women to engage in transactional sex [11–13].

It has also been demonstrated that increased knowledge about AIDS is not a predictor for behavioural change 14–17, although knowledge about the disease is a prerequisite for change 18. Knowledge of HIV and other STIs have been lacking among adolescents in

Ghana, with sparse and limited literature in this area as previous studies conducted have focused on public universities [10] with little or no data on private university students. According to the 2008 Ghana Demographic Health Survey (GDHS) 98% of women and 99% of men were aware of HIV in Ghana. However, this has not translated into comprehensive knowledge and safe sexual behaviour as only 25% of females and 33% of males aged 15 – 24 years had comprehensive knowledge of HIV[1]. This has health implications for propagation of the HIV/AJDS disease prevention and policy formulation.

In essence, there is a need to gather information on the basic knowledge of private university college students about HIV/AIDS, etiology, transmission, methods of prevention as well as their sexual practices since almost 45% of Ghana's population of about 24 million is aged 15 years or less.

Methodology

Research approach- Descriptive

Research design- One shot case study (Pre-Experimental)

Sampling technique- Convenience sampling

Sample size- 50

Data collection method- Survey questionnaire was prepared in English

Data entry and analysis: Data were analyzed by using descriptive statistical methods and a bivariate analysis was conducted with all relevant independent variables, p- value ≤0.05 was considered as significant. SPSS version 16 was used for statistical analysis.

Data entry and analysis: Data were analyzed by using descriptive statistical methods and a bivariate analysis was conducted with all relevant independent variables, p- value ≤0.05 was considered as significant. SPSS version 16 was used for statistical analysis.

Results

Table 1 shows participants knowledge and attitude towards HIV as well as bivariate analysis to determine differences between male and female responses. There were 50 participants consisting of 32 males and 18 females. Participants had overall good knowledge and attitude as seen by average knowledge score of 86% and average attitude score of 87%. Out of 15 questions regarding knowledge about HIV other than 3 questions regarding transmission through breastfeeding, kissing/ hugging, and insect bite, more than 80% of the participants gave right answers. Only 48% of the participants gave correct response "yes" to HIV transmission through breastfeeding. Similarly, only 62% and 72% participants gave correct response "no" to HIV transmission through kissing/hugging and insect bite respectively. Out of 11 questions focused on attitude towards HIV, other than only 1 question focusing on secrecy about family member's HIV, more than 80% of the participants gave the right answers. Thirty-five percent of the participants felt the need to keep secrecy if family member becomes infected with HIV.

Table 1: Knowledge and attitude towards HIV

Variable	Expected answer	Male n %)	Female n (%)		□2 value	p-value
Gender		**32**	**18**		**NA**	**NA**
Education						
12TH		14	9			
UG		10	5		4.13	0.13
PG		8	4			
HIV Knowledge						
HIV can be transmitted through contaminated syringes.	Yes	94%	95%		0.21	0.64

HIV can be transmitted through blood or blood product transfusion.	Yes	97%	100%		2.07	0.15
HIV is a sexually transmitted disease.	Yes	98%	100%		1.03	0.31
HIV testing before marriage decreases the spread of disease.	Yes	89%	91%		0.13	0.71
A pregnant woman can transmit HIV to her fetus.	Yes	86%	90%		0.41	0.52
HIV can be transmitted by sharing public telephone.	No	97%	94%		1.79	0.18
HIV can be transmitted through shaking hands.	No	95%	95%		0.0	0.98
Healthy-looking individuals can be living with HIV.	Yes	86%	82%		0.42	0.52
People can protect themselves from contracting HIV by using condoms.	Yes	92%	91%		0.11	0.74
HIV can be transmitted by sharing a meal with a person living with HIV.	No	92%	88%		0.67	0.41
HIV can be transmitted to a child through breastfeeding.	Yes	43%	58%		4.02	0.04
HIV can be transmitted by swimming in a public pool.	No	92%	94%		0.36	0.55
HIV can be transmitted by using a public toilet.	No	89%	93%		0.51	0.48
HIV can be transmitted through kissing/hugging a person living with HIV.	No	73%	70%		0.25	0.62
HIV can be transmitted through mosquito or other insect bites.	No	62%	63%		0.01	0.94
HIV attitude						
Would go to a restaurant if knew that the owner is living with HIV?	Yes	83%	72%		3.71	0.05
I am willing to share a meal with a person living with HIV.	Yes	85%	84%		0.05	0.82
Individuals living with HIV should be quarantined.	No	83%	81%		0.12	0.73
A female /male teacher living with HIV should be allowed to continue teaching.	Yes	96%	96%		0.05	0.82
Will host an individual living with HIV at home?	Yes	89%	82%		2.09	0.15
Member of my family can become friend with an individual living with HIV.	Yes	91%	93%		0.15	0.7
I am willing to work in an institution that tends to individuals living with HIV.	Yes	88%	94%		1.86	0.17
Will volunteer in an institution that works for people with HIV?	Yes	87%	96%		3.47	0.06
Want to remain secret if family member becomes infected with HIV.	No	64%	69%		0.5	0.48
An individual living with HIV can go to school and earn a degree.	Yes	98%	97%		0.49	0.48
Will maintain friendship if a friend becomes infected with HIV?	Yes	97%	97%		0.0	0.99

Conclusion

Present study showed that there are no myths or negative attitudes regarding HIV/AIDS among students of Pune city. Knowledge levels of students about basics of transmission, prevention and diagnosis and their attitude towards HIV positive clients are important. Many migrants are travelling worldwide and experiencing rapid changes in cultural as well as religious changes contributing to heightened risk of HIV/AIDS On this background it is extremely important to have adequate knowledge about the disease so that they can help to protect themselves and others against possible risks. The generalizability of study results is limited due to the sample size, differences in the level of education, and cultural values among people from different states and cities.

Ethical Issues: There was no ethical issues in the study. However, the study has undergone the Institutional Ethics Sub-committee at Symbiosis College of Nursing (SCON), Pune, Maharashtra India.

Funding: Self-funding

Conflict of Interest: Nil

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A Study to Assess the Effectiveness of Video Assisted Teaching (VAT) on Knowledge of Environmental Hygiene in Rural Community

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Abstract

Introduction : Environmental hygiene is a major public health issue in Indian community. Knowledge on environmental hygiene in rural community is one of the most effective ways to solve this public health issues. The aim of this the study is to assess the effectiveness of video assisting teaching (VAT) on knowledge of environmental hygiene among rural community This paper discusses teaching intervention and implementation strategies related to environmental hygiene in Indian emphasizes to prioritize it according to the need of the country. **Aim**: To assess the effectiveness of video assisting teaching (VAT) on knowledge of environmental hygiene among rural community **Materials and methods:** Quantitative experimental approach (pretest posttest one group design) was applied to find out the effectiveness of video assisted teaching (VAT) on knowledge of environmental hygiene in rural community. Convenience sampling technique used to select the 150 sample for the study. **Results:** Average pretest score was 12.45 and posttest score was 20.54 where the standard deviation of pretest is 3.30 and posttest is 2.53. Range of pretest score is 4 – 20. After video assisted teaching, the range of posttest is 11 – 26 (median of pretest is 13 and posttest median is 21). There was a significant difference between pretest and posttest knowledge score of 8.9, so we support and accept alternate hypothesis. **Conclusion:** Video Assisting Teaching (VAT) is an effective method to teach the people of rural community.

Keywords: *Effectiveness, Video Assisted Teaching (VAT), Rural community, Knowledge, Environmental hygiene*.

Introduction

Environmental hygiene is aimed at developing and maintaining a clean, safe, and pleasant physical well-being of all section of the population[1].

Most diarrhea deaths in the world are caused by unsafe water, sanitation or hygiene[2]

Hygiene is important for all helping to maintain health and increase life spans[3]. Improvement in sanitation requires newer strategies and targeted intervention with follow up evaluation[4] In absence of hygiene it is considered that stored water supply is also source of infection[5].

Objectives

To evaluate the effectiveness of video assisted teaching program regarding environmental hygiene

Research Methodology

The investigators used pre experimental one group pretest and posttest research design. Self -structured questionnaire were used to assess the knowledge regarding environmental hygiene. Video Prepared on environmental hygiene of 8 minutes. After administer the pretest from the samples, the video was shown to the sample followed by posttest with same samples. Written consent was taken from each sample prior to pretest.

Findings

Section I

Table 1: description of the samples based on their demographic variables in terms of frequency and percentage

n=150

Variables		Frequency (f)	Percentage%
Age (in years)	18 - 24	37	24.7%
	24.1 - 34	43	28.7
	34.1 - 44	37	24.7
	44.1 - 54	33	22
Education	Middle class degree (< S.S.C)	40	26.7
	High school degree (10 to 12 std)	51	34
	Bachelor degree (more than 12 std)	29	19.33
	Other (specify)	30	20
Religion	Hindu	53	35.33
	Muslim	36	24
	Christian	32	21.33
	Other specify	29	19.33
Income level	< Rs. 5,000 (low income)	27	18
	Rs.5001 - Rs.10000 (lower middle income)	34	22.7
	Rs.10001- Rs.20,000 (middle income)	33	22
	Rs.20001- Rs.30,000 (middle income)	23	15.33
	Rs.30,001 & above	33	22
Family type	Nuclear family	79	52.7
	Joint family	71	47.3
Number of children	One	46	30.7
	Two	53	35.33
	Three and above	51	34
Type of housing	Hut	38	25.33
	Mud house (kaccha)	39	26
	Pakka (well-constructed)	44	29.33
	Metal-steel house	29	19.33
Water supply in area	Municipality water supply	34	22.7
	Harvester rain-water	45	30
	Tap water (bore water)	40	26.7
	Other specify	31	20.7
Nearby water bodies	River	43	28.7
	Ponds	61	40.7
	Other (specify)	46	30.7

Section II

Data related effectiveness of video assisted teaching Program regarding environmental hygiene.

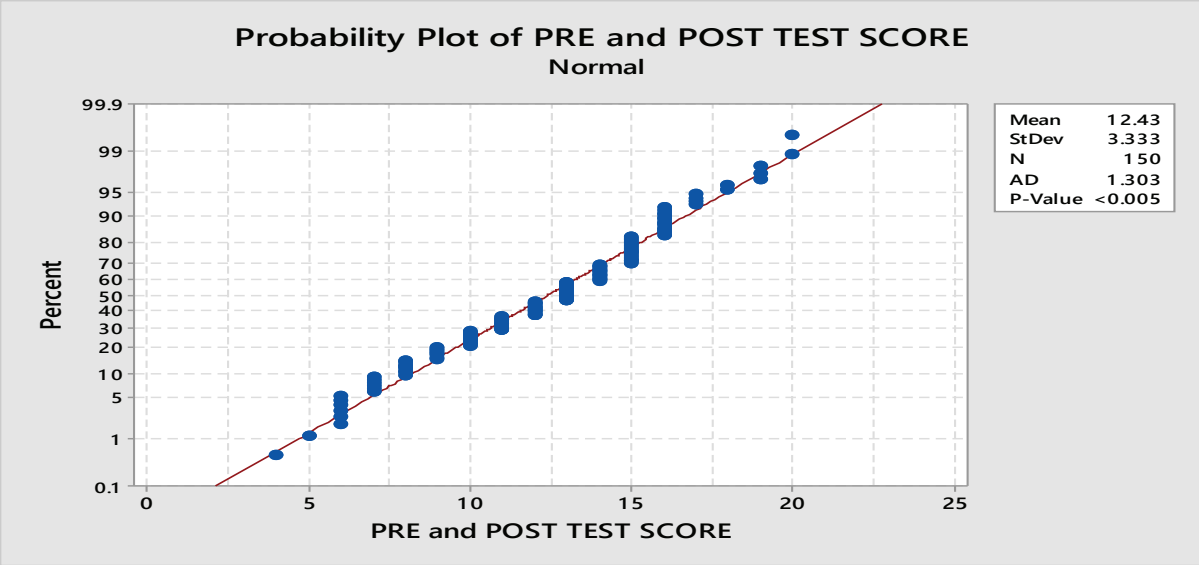


Fig: 01 : Probability plot for pre-test and posttest knowledge score

The p- value is very small so that data is non-normal. Hence, nonparametric version of Pair t test, which is Wilcoxon sign rank test, is used.

There is no sufficient evidence to accept the null hypothesis ($p < 0.05$). The sample median is statistically different from zero. Here estimated median is – 8

Discussion

Video Assisted Teaching is one of the most effective ways to increase knowledge. It was based on the need of the community and it has helped the individuals to understand the concept of environmental hygiene.

Similar results were found in a study conducted significant association with demographic variables age, educational status and socioeconomic status with the level of health beliefs among women in study group[6].

Similar study was conducted the t- test revealed 26.50 i.e. video-assisted teaching programme was found effective in increasing the knowledge of the children regarding benefits outdoor play[7].

Conclusion

Video assisted teaching was very effective in providing knowledge to the rural residents regarding environmental hygiene. Different strategies and method to be adopted to provide continues education and awareness related to environmental hygiene

Limitation

The study is limited to only the rural community of Pune City.

Conflict of Interest - Nil

Source of Finding - Self

Ethical Clearance- Study was approved at Institute Ethical subcommittee. Informed concerned from each sample been taken for the study.

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Systematic Literature Review: "Effect of Hypoglycemia on Quality of Life among Patient with Diabetes mellitus

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Abstract

Background: According to the International Diabetes federation 8.8%, adults were having diabetes mellitus, which is the chronic disease. The pervasiveness of Diabetes mellitus is the basic interminable infections, with rising worldwide prevalence. Anxiety of hypoglycemia may add to an absence of adherence to treatment, hypoglycemia is one of the primary restricting components for controlling glycaemia in diabetes mellitus. The goals of this survey are to decide, utilizing the best degree of proof accessible, the impact of hypoglycemia on personal satisfaction among patients with diabetes mellitus. Selection of the investigations is finished by screening dependent on the screening based on title, unique, catchphrases and finding. The analyst and sentiment from two specialists taken for the determination of studies finish choice procedure. In view of this, all out, 25 investigations were chosen, out of that with incorporation and prohibition rules dependent on the PRISMA agenda 10 were chosen, and 15 were terminated. In all the examinations people with a background marked by hypoglycemia encountered a significant and more prominent weight of burdensome side effects and more unfortunate rest contrasted with those without a past filled with hypoglycemia. As helpless rest and gloom may bring about more prominent glycemic variety, clinical screening for these issues ought to be considered extensively in populaces with type 2 diabetes, particularly among those with hypoglycemia. It likewise shows critical assortment of proof that hypoglycemic scenes detrimentally affect Quality of life and treatment fulfillment in patients with diabetes. To comprehend these overwhelming connections further, exploration could incorporate distant observing of glucose levels, rest, and natural flashing appraisal. Understanding with diabetes mellitus should urged to take brief treatment for the counteraction of hypoglycemia. Further properly well-designed randomized controlled trials are required to add evidence-based information to the current knowledge.

Keywords: *Hypoglycemia, Diabetes Mellitus, Quality of life, Depression, Dementia, Cognitive impairment*

Background

Severe hypoglycemia make the financial impact and also affect the quality of life among patients with diabetes mellitus (1) found in their study that continues hypoglycemia increases the risk of cardiovascular diseases and also morbidity and mortality in the diabetes patients. Another study found severe episode of hypoglycemia increase the risk of death and financial burden. 2) In the study done on a Korean population, the cost of medical facilities for treatment of severe hypoglycemia is high. Prospective Diabetes Study (1998) shows that diabetes patients having more episode of hypoglycemia have the lower quality of life compare to others .The episode of hypoglycemia gives an negative impact on the health of the individual with diabetes in longer period.3) Diabetes patient always lives with the fear and anxiety for getting the episode of hypoglycemia anytime4)Therefore, it is clearly understood diabetes and the episode of hypoglycemia affect the quality of life of persons with diabetes mellitus.Insulin and a number of other glucose-lowering drugs may cause hypoglycaemia.(5)

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Objectives

The objectives of this systemic review are to find out the effect of hypoglycemia on quality of life among patients with diabetes mellitus by using the review articles

Material and Methods

Search strategy and selection criteria

We have selected, PubMed, Science Direct, and Scopus databases sources (2009-2019) for studies that evaluated the related effect of hypoglycemia on quality of life among the diabetes patients quality of life. QOL from October 2018 to March 2019.

All standardized and organized arrangements of words and phrases and provide a consistent way to describe data and free text terms were used to find the review. MeSH terms used were “diabetes mellitus”, “Hypoglycemia”, “Quality of Life”. Searches were limited to literatures available in full text and humans within a valid limit within the database. The suitable articles were further review by performing the screening os abstract and the full text article. Eligibility appraisement and data separation were done independently in an pure standardized mannered by researcher.

Selection criteria and data extraction

Selection of the studies is done by screening based on the screening on the basis of title, abstract, keywords and finding. Selection process is done by the researcher and opinion from two experts taken for the selection of studies.Based on this total 25 studies were selected and out of that with inclusion and exclusion criteria based on the PRISMA checklist 10 were selected and 15 were rejected.

The inclusion criteria for the article was if the studies were cross-sectional, case–control, or cohort; studies. The adult populations studies based on, intensity impact, risk and effect of hypoglycemia on quality of life among patients with Diabetes mellitus. Articles were removed incase letters, abstracts, conference proceedings, reviews, and meta-analysis.

Systemic Review of the Analysis

Result

Study Characteristics:

As is shown in above , all of the 10 studies included were cross-sectional studies, Prospective cohort, longitudinal cohort with a complete sample size of 127,006. The following different scales were used to research quality of life of type 2 diabetes patients: Case report, Questionnaire meausres,patient reported sympos,visual analog scale for quality of life, internet based survey, population based survey, self-reported history, web based survey. All the study shows that there is impact of hypoglycemia on quality of life like impair cognitive ability, Dementia, Depression, Fear, Absenteeism in work place, Insomnia.

Conclusion

The present systemic review on the effect of hypoglycemia on quality of life among patient with Diabetes mellitus. The systemic review was done on the 10 studies based on the selection criteria. The findings of the study show that the episode of hypoglycemia has Hyperglycemia Medical treatment, increased caloric intake, Fear, Absenteeism in job, Quality of sleep Depression. It shows that it has negative impact on quality of life among patient with Diabetes mellitus.

Discussion

In all the studies individuals with episode of hypoglycemia had symptoms of depression and insomnia compare with those who have no episode of hypoglycemia. The insomnia and depression symptoms have the impact on changes in blood glucose level of the patient so it is important to do the regular check up of the diabetes is patient is required mainly among those with hypoglycemia. It also shows significant body of evidence that hypoglycemic episodes have a detrimental effect on quality of life and treatment satisfaction in patients with diabetes. To know more about the hypoglycemia and quality of life further more research studies need to be conducted. Patient with diabetes mellitus should encouraged taking prompt treatment for the prevention of hypoglycemia. More well designed randomized controlled trials studies are required to get evidence-based information for the current knowledge.

These studies should help clinically for treatment on hypoglycemia and will produce more data to be use into a future update of this review.

Conflict of Interest -Nil

Source of Funding- Funded by self

Ethical Clearance-: Obtained from Institutional Research Committee.

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A Study to Assess the Effectiveness of Structured Teaching Programme on Knowledge Regarding Updated National Immunization Schedule among the Postnatal Mothers Visiting Health Centre

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Background

Youngsters are an epitome of our fantasy. They are the most defenceless gathering in the general public. So kids require unique consideration to have a great wellbeing. Thus youngsters require good attention to have a better well-being. $_1$Immunization, specifically has spared a huge number of youthful lives and with appropriate use.[1] The IAP committee on immunization has classified some vaccine as optional vaccine e.g. Hepatitis A, Hb vaccine, varicella, Pneumococcal vaccine, typhoid vaccine, IPV. Optional vaccine for personal use depends on occurrence of the contamination and illness, age, sickness and possibility of illness in vulnerable person when receiving of vaccine prompted immunity and the result of immunisation is assessed on volunteered immunization in the national immunization programme. Now hepatitis B vaccine is only the one which is considered.[2]

Methods: A true experimental study design using pre-test as well as post-test with no control group. The data composed with 30 postnatal mothers under non probability convenient sampling technique to know the efficiency of structured teaching plan on knowledge about Updated National Immunization Schedule.

Pre-test was done among postnatal mother with closed ended survey questions. Soon after pre-test structured teaching programme on awareness about Updated National Immunization Schedule was given to the postnatal mothers. Evaluation was be done by conducting post-test on the same day of the operation by using same closed ended questionnaire which was used for pre-test.

Results: Findings reflected, many of the postnatal women (43.33%) were in 20-25 years. Highest percentage 70% of postnatal mother was housewife. 66.67% of postnatal mother were completed primary education. 70% postnatal mothers are from middle class family. During pre-test 30% postnatal mother had moderately adequate knowledge and 70% postnatal mother had inadequate knowledge whereas, during post-test 73.33% postnatal mother had adequate knowledge, 26.67% postnatal mother had moderate adequate knowledge.

Conclusion: The study demonstrated that the structure teaching program about Updated National Immunization programme in the postnatal mothers going to wellness centre, was effective to improve the knowledge of postnatal mothers.

***Keywords**: Knowledge, updated national immunization schedule, postnatal mothers.*

Introduction

Immunization has protected many children. At present just the hepatitis B antibody can be considered for such incorporation. Antibodies are essentially added to the overall decreases in mortality and mortality by lessening the occurrence of genuine irresistible illness. The present individuals everywhere throughout the world experience the advantages of in inoculations starting in outset.$_3$ Generally grown-up in the United State have not seen direct the stripping disease getting with immunizations after securities for the E.g. Polio,

Diphtheria and Haemophilus Influenza, meningitis. An immunization is an antigenic readiness used to create dynamic resistance to illness.[3]

Methodology

Research approach: Quantitative

Research design: True -Experimental

Sampling technique: Convenience sampling

Sample size: 60

Data collection method: structured questionnaire

Analysis: Descriptive and inferential statistics

Result and Findings

Demographic characteristics by frequency with percentage in pre-test, post-test.

Description of samples according to Demographic characteristics by frequency and percentage in pre-test.

Demographic Data

Demographic details show that 44% of postnatal mothers were from age group of 20-25 years, 33% were from 25-30 years and 23% were from 30-35 age group. 24 respondents belong to Hindu Religion, 4 belong to Muslim and only 1 respondent is Christian. Besides this 21 postnatal mothers were housewife, 6 belong to Service Sector, and 2 were from government Sector. 65% of postnatal mothers had completed primary education, 26% completed college education, whereas 3 % were with secondary education and 6 % were from other educational background. 21 were from middle class, 7 were from lower middle class and only 2 were from upper economic class**.**

SECTION II

Analysis of data of knowledge about updated national immunization schedule.

Knowledge of postnatal mothers regarding updated national immunization schedule in pre-test.

In pre-test, 21 mothers had inadequate knowledge (score below 10) [70%]. And 9 mothers had Moderate knowledge (score 10-15) [30%] regarding updated national immunization schedule.

SECTION III

Knowledge of postnatal mothers regarding updated national immunization schedule in post-test.

In post-test, 22 mothers had Adequate knowledge (score more than 15) [73.33%]. And 08 mothers had Moderate knowledge (score 10-15) [26.67%] regarding updated national immunization schedule.

SECTION IV

Assessment effectiveness of structured teaching programme

To assess the efficiency of structured training plan regarding knowledge on updated national immunization schedule the formula E=Y–X was used,

Where Y = Post-test, X = Pre-test and E= Effectiveness.

Association of pre-test, post-test level of knowledge about updated national immunization schedule.

Table No. 1 Shows region wise association of mean, SD, and mean percentage of pre-test and post- test knowledge scores about updated National immunization schedule.

Region	Highest No.	Pre test scores			Post test score			Difference (%)
		Mean	SD	%	Mean	SD	Mean %	
Knowledge on introduction, definition, incidence	10	3.67	1.39	36.7	8.2	1.41	82	45.3
Knowledge on principles.	2	0.83	1.01	41.5	1.6	0.49	80	38.5
Knowledge on old immunization	4	1.77	1.17	44.25	3.1	0.64	77.5	33.25
Knowledge on new immunization	2	0.5	0.61	25	1.23	0.8	61.5	36.5
Knowledge on effects and management	3	1.5	0.89	50	2.28	0.81	76	26
Overall	**21**	**8.27**	**5.07**	**39.38**	**16.4**	**4.13**	**78.1**	**38.72**

Association of pre-test and post-test knowledge about updated national immunization schedule.

COMPARISON OF KNOWLEDGE SCORE

In post-test, 22 mothers had adequate knowledge about updated national immunization schedule.73.33%. And 08 mothers had Moderate knowledge, where score was between10-15, 26.67% regarding updated national immunization schedule.

SECTION V

Testing of hypotheses

To assess the efficiency of structure teaching plan on knowledge about national immunization programme in postnatal women, hypotheses testing was done using 't' test and chi-square.

I) **Comparison between differences of pre-test, post-test totals about updated national immunization programme in postnatal women.**

Table No.2

Sr. No	Region	't'	Level of Significance
1	Overview, definition, incidence	-12.58	HS
2	Principles of vaccination	-3.35	HS
3	Old immunization	-6.04	HS
4	New immunization	-4.29	HS
5	Effects and management	-3.66	HS

Table. Shows that there is greater significance alteration in the region wise total of pre-test and post-test. So, the mentioned null hypothesis is rejected and statistical is acknowledged. Thus difference observed in mean score value of pre-test and post-test were real difference.

I) **Association between pre-test score on updated national immunization schedule among postnatal mother with demographic variable.**

Table No. 3

Sr. No	Demographic variables	Df	Table value	χ2 value	Level of significant
1	Age	3	7.82	1.27	Not significant
2	Religion	3	7.82	2.32	Not significant
3	Economic class	3	7.82	2.16	Not significant
4	Mothers education	3	7.82	7.4	Not significant
5	Mother occupation	3	7.82	1.04	Not significant

Table displays no major link among pre-test knowledge numbers of postnatal women when matched with demographic variables. So, null hypotheses of association among pre-test knowledge scores and demographic variables are accepted.

Table No. 4. **Association between post-test score on updated national immunization schedule among postnatal mother with demographic variable.**

Sr. No	Demographic variables	Df	Table value	χ2value	Level of significant
1	Age	3	7.82	6.36	Not significant
2	Religion	3	7.82	3.08	Not significant
3	Economic class	3	7.82	3.9	Not significant
4	Mothers education	3	7.82	7.1	Not significant
5	Mother occupation	3	7.82	1.22	Not significant

Table shows that there was not major association among post-test scores of postnatal females when matched with demographic variables. So, null hypotheses of association in the middle of post-test knowledge, demographic variables are accepted.

It can be understood that structure teaching programme was in effect for all postnatal women regardless of alteration in demographic variables.

Funding: Self-funding

Conflict of interest: no conflict of interest in research.

Ethical approval: The study was permitted by the Institutional Ethics Sub-Committee.

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A Study to Assess the Impact of Lockdown on Physical and Psychosocial Health among Senior Citizen in the Selected Areas of Pune City

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Background

Over the previous few weeks, the COVID pandemic has impacted lives across the world in unimaginable ways, and quite ever at now like this, it's essential we concentrate to our elderly who are the foremost vulnerable segment of the population. With aging, immunity decreases making elderly more prone to the virus. Additionally, the general public above the age of 60 have some or the opposite medical condition like diabetes, hypertension or asthma; all of which are risk factors.[1] The demographic pyramid of India is shifting fast with a more number of within the elderly population. It appears that the socio-economic and socio cultural transformation to higher individual freedom, hedonism, family unit, feminism, materialism etc. is catching older generation unaware. Aims: within the above context, the target of the study was to spot the themes that are related to the standard of life of home-dwelling elderly. In fact, the time of the 2011 census there have been around 104 million people above the age of 60, which implies that a staggering 9 percent of our population fall within this high risk group. But besides being most in danger, social distancing and therefore the nationwide lockdown even have implications on mental state of the elderly, which simply shouldn't be ignored.[2]

Methods: A non-experimental descriptive survey method was used accustomed assess the impact of lockdown on physical and psychological health of grownup selected by purposive sampling. The information was composed by pretested questionnaire. Data analysis was carried out with level of significance.

Results: 60 senior citizen participated in the study.58% were not satisfied with lock down as it is affecting their physical and mental health. Impact is more on age group of 60 years and above.

Conclusions: The present study showed unsatisfied impact of lockdown among senior citizens. Lockdown has affected them in all physical, social and psychological way. It is necessary to take measures to relieve their stress due to lockdown.

Keywords*: Lockdown, senior citizen, psychological and physical health.*

Introduction And Background

More adults are progressively plausible to have just got basic conditions like diabetes and respiratory ailment comorbidities that we presently perceive increment the threat of serious COVID-19 and COVID-19 related demise. Conceivable more fragile framework makes it harder for more established grown-ups to restrict contamination. Therefore, the effect on more established grown-ups is remarkable.[3]

Material and Methods:

Research approach: Quantitative

Research design: The researchers have used A Non-Experimental Descriptive Survey.

Sampling technique: Convenience sampling technique was used.

Sample size: 60

Data collection method: structured questionnaire

Analysis: Descriptive and inferential statistics

Objectives

1. To assess the effect of lockdown among senior citizen living selected areas of Pune city.
2. To assess the impact of lockdown on physical health among senior citizen of Pune city.
3. To find the association between the effects of lockdown and physical wellbeing among selected demographic variables.
4. To find the association between the effects of lockdown and psychosocial health among senior citizen.

Result & Discussion

Description of demographic details-

Table 1: Section I: Frequency and percentage distribution n=60

Sr. No.	Demographic variable	Frequency	(%)
1	Gender		
	Female	33	55
	Male	27	45
2	Age (Years)		
	60 to 70	38	63
	70 and above	22	37
3	Literacy 1.		
	Literate	50	83
	Illiterate	10	17
4	Educational Level		
	Primary	19	32
	Secondary	19	32
	Higher Secondary and above	22	37
5	Occupation		
	Business	24	40
	Retired serviceman	20	33
	Agriculture	16	27
6	Covid-19 zone		
	Red	40	67
	Orange	12	20
	Green	8	13

Cont... **.Table 1: Section I: Frequency and percentage distribution n=60**

7	During lock down staying with:		
	Family	21	35
	Alone	24	40
	Away from home	15	25
8	Chronic illness		
	Yes	41	68
	No	19	31

Table shows total 60 participants participated in online survey. 27 (45 %) of the respondents were male and 33 (55 %) of respondents were female. Maximum number of respondents i.e. 63 % belongs to Age group 60 – 70 Years, followed by 37 % respondents belong to age group above 70 years. Above table shows that 83% respondents found Literate whereas 17 were illiterate. It was found that 40% respondents belong to Business sector, 33% Service Sector, and 27 respondent was from Agriculture sector. Findings shows that 40 respondents were from Covid-19 red zone, 12 respondents belongs to orange zone and 13 were from green zone. 40 participants were staying alone during lockdown, 35 participants were staying with their family and 25 were with their friends. Out of 60 total 41 participants were suffering from chronic illness and other 19 were healthy.

Section I

CHALLENGES FACED BY WORKING POPULATION

Table 2 Section B: Frequency and percentage distribution of impact of lockdown n=60

Sr.no	Score	Frequency	Percentage(%)
1	Satisfied	5	8
2	Moderately satisfied	21	35
3	Dissatisfied	34	58

The table shows that 58 % sample are not satisfied with the lockdown and 35% are moderately satisfied where as 08% are satisfied with lockdown as it has no impact on their routine life.

Figure 1: Impact score of lockdown

Section C: Association of selected demographic variables with impact of lockdown

n=60

Sr. No	Demographic variables Table	Probability p
1	Gender	
	Male	0.7771
	Female	
2	Age (Years)	
	60 to 70	0.9656
	70 to 80	
3	Literacy	
	Literate	0.5521
	Illiterate	
4	Educational Level	
	Primary	0.9771
	Secondary	
	Higher Secondary and above	
5	Occupation	
	Business	0.9078
	Retired serviceman	
	Agriculture	
6	Which zone you belong to	

Cont ... **Section C: Association of selected demographic variables with impact of lockdown**

	Red	0.9212
	Orange	
	Green	
7	During lock down staying with:	
	Family	0. 7833
	Alone	
	Away from home	
8	Any chronic illness?	
	Yes	0.8777
	No	

The lockdown has dealt a body blow to the ageing across the nation. Even as aged people say they are missing the healing touch of their doctor at this time, the lockdown has caused in worsening social relationships, leaving many disheartened, anxious and offended.

More than 58 per cent the senior population participants who joined in a survey claimed their relation with family members, mostly their own children, further deteriorated during the lockdown. Hardly any communication with children at this time, ego clashes as reasons for the failure in relationships.

The survey, conducted by to assess the impact of the lockdown on their lives. Around 58 per cent declared that the lockdown was disturbing their health condition, while 38 per cent said they were adjusting with the situation, however they are finding it difficult and only 5 percent were satisfied with it.

Conclusion

The present study displayed older people were not satisfied. They had disturbed physical and mental health. Hence the study reflected more unsatisfied and disturbed adults in lock down.

Funding: Self-funding.

Conflict of interest: There is no conflict of interest in the study.

Ethical Clearance: The study was affirmed by the Institutional Ethics Sub-Committee.

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Quality of Life in Children with Type 1 Diabetic – A Systematic Review

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Abstract

Background : Quality of life (QOL) is commonly used in the health care industry to assess the general well-being of the individual. Assessing QOL among children with diabetes, is completed with a disease specific approach.[1] The main aim of the present study was to systematically review the characteristics of published studies in children with type 1 diabetic Quality of life and examine the quality of their findings.

Method and Material: The 141 research articles searched in (PubMed, Web of Science, CINAHL, Scopus, Academia ,open access) databases using the following search terms: "type 1 diabetic" and "quality of life" . Depending on the inclusion and exclusion criteria studies were filtered and 12 studies were chosen for the final analysis. This systematic review was designed and conducted with the Published Guidelines for Reporting Systematic Reviews and Meta-Analyses (PRISMA)

Result : The studies showed that sampling size differs in each study from 20 till 3005 it selected . but in many studies sample size is below 100. Validity and reliability were not done in many studies. For reliability al studies who mentioned about validity used Cronbach's α coefficient to check the tool efficiency. Studies showed that people with diabetes had lower QoL . however the Quality of Life is directly related to the disease condition, HbA1 c and glycaemic control of children . Better control of Diabetic Mellitus is associated with better health related QoL.

Key words : *quality of life, children, type 1 diabetic* .

Introduction

Diabetes is demanding disease. Sometimes children with type 1 diabetes will manage their diabetes effectively and manage to live their normal life, but other times it can powerfully affect their lives. Many children feel burdened by the demands of the disease. Quality of life is a multidimensional construct incorporating an individual's subjective perception of physical, emotional, and social well-being, including both a cognitive component (satisfaction) and an emotional component (happiness)[1].

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According to International Diabetes Federation (IDF), Diabetes mellitus has been affecting 425 million people globally and as per the estimations the number of people with diabetes may increase to 629 million in 2045.1 Out of the total, one-third of these diabetics are elderly; people older than 65 years of age. In addition to a high prevalence of DM in adults and aged the estimates of children and adolescents with T1DM is on the rising trend.[2]

It is estimated that the incidence of T1DM among children and adolescents is increasing in many countries particularly in children and adolescents under the age of 15 years, and the overall annual increase is estimated to be around 3% with strong indications of geographic differences. More than 96,000 children and adolescents under 15 years are estimated to be diagnosed with T1DM annually and the number is estimated to be more

than 132,600 when the age range extends to 20 years. The treatment for type 1 diabetes, its evaluation and the approach to the disease are different between children and adults.[3]

The high prevalence of diabetes and its related complications have attracted the researchers. In response to this, considerable research published in journals to evaluate the quality of life of diabetic and its determinants in diabetic patients.[4] These studies aimed to improve QoL in children with diabetes and provide interventions.

The current systematic review was conducted

1. to describe the general characteristics of the latest available information about QoL in people with type 1 diabetes.

2. to understand the main methodological flaws of the studies

3. to investigate how QoL was measured in Type 1 diabetic population

4. to understand the factors that were mainly associated with QoL in people with type 1 diabetes and their interventions.

Method and Materials

Literature search

A systematic literature search was autonomously conducted from October 2019 to April 2020. The researcher selected studies which assessed the Quality of Life (QoL) among children with type 1 diabetic mellitus. The results of this literature search were independently verified and updated in May 2020. Studies published up to May 2020 were included in this review. National and international databases (Pubmed, Web of Science, CINAHL, Academia) were searched for the following key terms "type 1 diabetic" and "quality of life". Other than this "type 1 diabetes, "IDDM", "QoL", "Child" , "children", "paediatric" and "pediatric" terms were used . Moreover, researcher searched the Google database for extra open access publications. Researcher followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Selection of studies

Six major inclusion criteria were adopted:

- Papers published in peer-reviewed journal (pubmed, Cochrane library, Academia.edu, open access journals)
- Papers are written in the English language
- Papers published from January 2015 to May 2020 (focus on more recent knowledge)
- Children with type 1 diabetic (0 years to 18 years)
- Cross sectional studies
- Quantitative studies

Five exclusion criteria were applied:

- QOL in people with type 2 diabetes
- Above 18 years with type 1 diabetes
- Systematic a review article

The initial search resulted in 114 documents. After excluding duplicates and non-relevant studies, 25 articles were selected for full text examination. The reference lists of these 25 documents were manually searched. In total, 12 studies were included in the review .Data extracted from the selected studies are included like a year of publication, journal name ,aim of the study, Methodology adopted in the study, the age range for the sample, sample size, a setting of the data collection, data collection tool and the result of the study.

Systematic Review of Quality of Life in type 1 diabetic children (2015-2020)

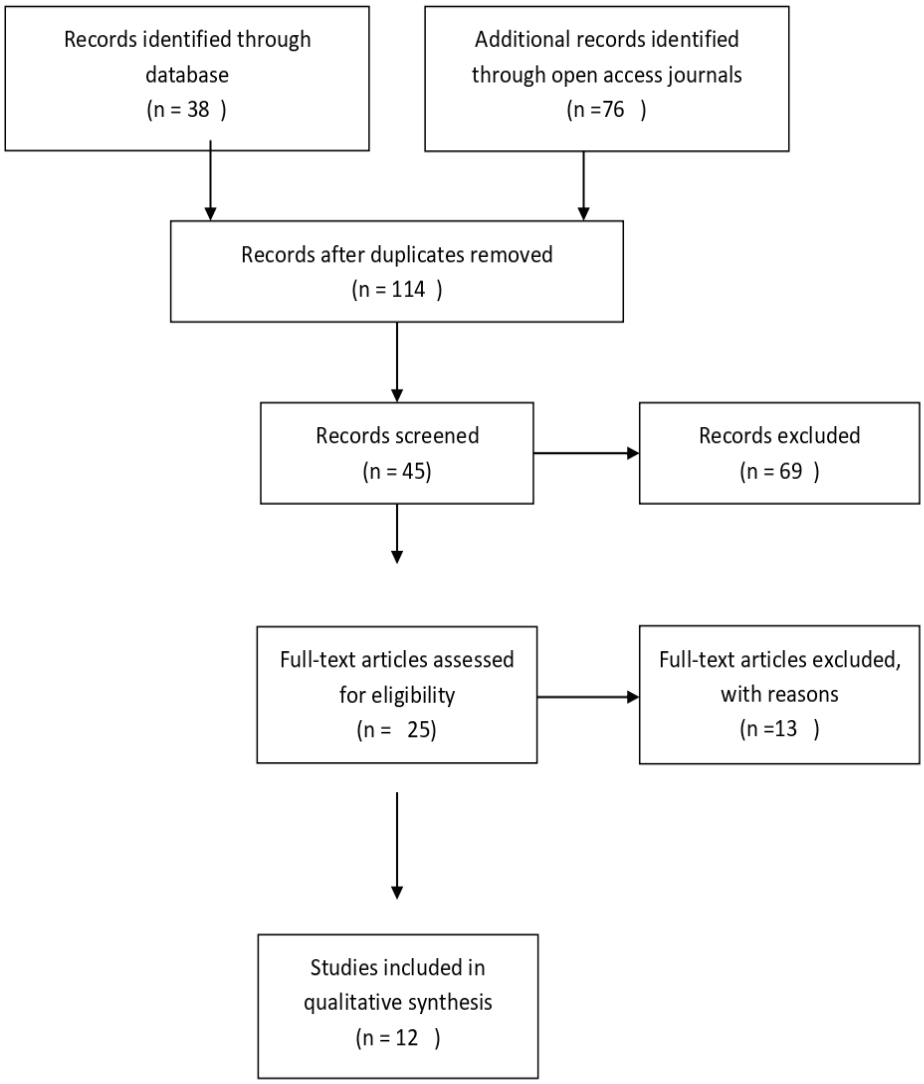


Figure 1 : Systematic review flow diagram

Table 1: Characteristics of the Systematic review studies (2015-2020)

Sr.No	Published year	Journal name	First author	Age range	Sample size	setting	Reliability & validity	Data collection year	Data collection tool	Intervention	Outcome
1	2015	Diabetology and metabolic syndrome. 5	Joao Soares Felici	10 to 19	3005	clinics	NA	2008-2010	EuroQol	NA	compared the QoL with different regions
2	2015	SCIELO analytics.6	Luce Marina F.C. da Costa	10 to 19	96	clinics	NA	2012-2014	DQOLY	NA	good quality of life
3	2016	diabetes research Aand Clinical Practice.7	Fadia AlBuhairan	12 to 18 years	315	hospitals	Cronbach's α & validaton done	NA	Peds QL™ Diabetes Module 3.0	NA	parents score is less than the adolescents
4	2016	Mindfulness.8	Inge J. P. Serkel-Schrama	12 to 18	129	NA	NA	NA	PedsQL3.2™	Mindful Parenting	positive QoL
5	2017	Journal of Diabetes & Metabolism .9	Basma AbdelMoez Ali	7-18 years	72	OPD	NA	2015	Peds QL 3.0, DM Module, CDI	NA	Poor QoL related with Depression
6	2017	International Journal of Nursing Sciences 10	Nurcan Ozyazıcıoglu	8-12 & 12-18	64	Clinic, OPD	validity and reliabilty done	2012	PedsQL	NA	no difference in parents and child views about QoL
Sr.No	Published year	Journal name	First author	Age range	Sample size	setting	Reliability & validity	Data collection year	Data collection tool	Intervention	result
7	2018	Open Access Journal of Obesity and Metabolic Disorders 11	Azimova Ozoda	8-12 &13-18	20	dispensary	high reliabilty & validity	NA	DQOLY-SF	Pump Insulin therapy	QoL improved
8	2018	Diabetes Care 12	Marie-Anne Burckhardt	2-12	49	hospital	Validity done	NA	PedsQL 4.0 , 3.0 , 2.0 DM Module	Dexcom G5 Mobile CGM	positive QoL with CGM
9	2019	Journal of Postgraduate Medicine, Education and Research 13	Rakesh Kumar	4 to 15 years	97	Clinic, hospital	Cronbach's α	2012	QOLID questionnaire	NA	QoL related to glycemic control
10	2019	Current Medical research and Opinion 14	Julio López-Bastida	under 18	275	pediatric centers	NA	2014	EQ-5D and PedsQL	NA	HbA1c related to HRQOL.
11	2020	International Journal of Health Sciences and Research [2]	Niraj Kumar	6−18	140	hospital	NA		DAWN QolY, DTSQ, DSQoL,SDQ	NA	depend on the disease condition
12	2020	Diabetes technology & Therapeutics[15]	Manuela Sinisterra,	2 to 5	46	hospital	NA	NA	HRQOL	CGM	sleep improved

Results and Discussion

General characteristics

The first objective was to describe the general characteristics of the latest available information about QoL in people with type 1 diabetes. The characteristics of the 12 eligible studies for this systematic review are shown in Table 1. The first 2 studies were published in the year 2015 and since then there were few publications per year on QoL of children with type 1 diabetes. In the year 2015 to 2020 the publications are distributed twice per year except in 2019, where no publication on the topic found relevant. All studies were published in different countries with 2 Indian publications on the topic of Quality of life in children with type 1 diabetes. Sample sizes across the studies ranged from 20 to 3005 participants. All the studies published in the English language . In all the studies, participants age group is below 18 years that is, children and adolescents. Two studies compared the QoL of Children with Adolescents . In all the studies ,children below 12 years were participated and their parents marked the QoL for children.

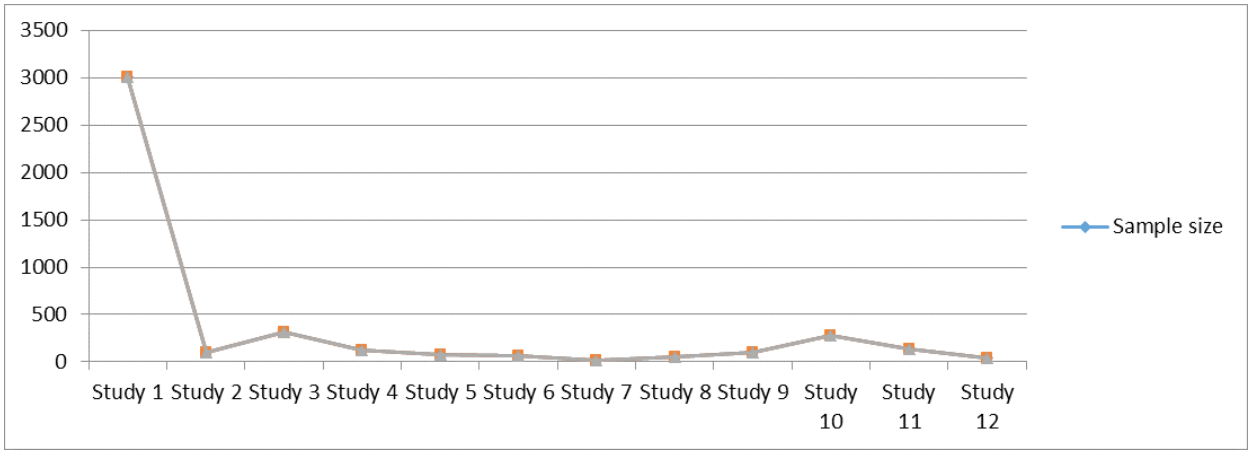


Figure 2 : Sample size distributions in the QoL of type 1 diabetic children from 2015-2020

The second objective was to understand the main methodological approach of studies .The data was collected from the samples mostly from the hospital, OPD, Clinic, Pediatric centers and in one study data collected in endocrinology dispensary also. In 7 studies reliability details weren't stated. However, in two studies type of reliability (Cronbach's α) mentioned clearly .Also, from all of the 12 studies only one study mentioned about the validity and it stated that 10 experts performed the validity of the tool. Around, 8 studies doesn't mentioned about the validity and other 4 studies declared that validity and reliability done.

The third objective was to investigate how QoL was measured in Type 1 diabetic population .In 6 studies data collection year is not mentioned. The other 6 studies data collection year ranges from 2008-2015. Furthermore, 6 studies used PedsQL for data collection. Different module of PedQL is used for the data collection like of PedQL 2.0, of PedQL3, of PedQL 3.2 module was used to check the Quality of life in children. Others used QOLID questionnaire, EuroQol , DQOLY-SF, DAWN QolY, and DQOLY.Apart from this, studies evaluated QoL as a main variable.Also, 4 studies checked the other variables like depression, sleep and other variables that affect the diabetic children. They have used other tools like DTSQ, DSQoL,SDQ and CDC to check the above mentioned variables.

The effects of interventions

The fourth objective was to understand the factors that were mainly associated with QoL in people with type 1 diabetes and their interventions .A total of 12 studies examined the effects of an intervention on QoL in people with type 1 diabetes. Eight studies doesn't have any interventions as it checked only the quality of life in children. Two studies procured continuous glucose monitoring (CGM) as an intervention . In that one study used Dexcom G5 mobile for CGM monitoring. One

study used the Pump insulin to improve the Quality of Life and other study used mindfulness practices to improve QoL. However,it is observed that the majority of the studies preferred to do surveys and 3 studies used invasive practices to improve the Qol while one study used non-invasive supportive measurement to improve QoL that is mindfulness practices.

The researchers who focused on the QoL with interventions especially with Continuous Glucose Monitoring(CGM) showed slight improvement or higher level of improvement in part of Quality of Life. Research with mindfulness practices showed the improved QoL after the practice. The researchers Who compared the QoL in both groups like adolescents and children showed not much different in their QoL.One study compared the Qol with glycemic control and it proved that QoL is depend on the glycemic control. According to the stability of the glycemic control the QoL will change. Other research studies evaluated the HbA1c with QoL and it also proved that enhanced QoL with reduced HbA1c level. One study which was published by Niraj Kumar in 2020 states that depend on the severity of the disease condition QoL will change. One study which is published by Basma AbdelMoez Ali explained that parent's predition of QoL is less than that of adolescents score about their conditions. Other studies showed the comparison of QoL in different aspects like with regions of the country , with depression level of the children, with other age group and with sleep also.

Clinical Implications

Overall, many studies showed improvement in QoL of children especially after the glycemic control and Continues Glucose monitoring. These type practices should be improved in children with type 1 diabetes. The Psychological impact is also beneficial to the children like mindfulness practices. None of the studies evaluated about the educational interventions which can be a helpful for children, adolescents and anxious parents. Technological innovations like mobile applications and active monitoring need to be used for these children and they must be practiced by parents , children and health care workers. Only one study used a mobile application to monitor CGM and proved the effect in QoL.

Conclusion

This present review of the literature showed that people with diabetes had lower QoL however the Quality of Life is directly related to the disease condition, HbA1 c and glycaemic control of children . Better control of diabetes mellitus is associated with better health related QoL. Paediatricians, nurses, parents and other health care workers should continue their researches with long term interventions to improve quality of life the children.

Ethical Consideration: Ethical approval of the study taken from Bharati College of Nursing ethical committee.

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Conflict of Interest: The author has no conflict of interest

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An implementation study on Hazard Identification and Risk Assessment (HIRA) technique in the Critical Care Unit of a Tertiary Care Hospital

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Abstract

In the studied hospital an increased number of adverse events were noted in the critical care unit (CCU) department of the tertiary care hospital in the last three months. Hence, a risk management technique, Hazard Identification and Risk Assessment (HIRA) is applied to identify the hazards and to reduce the risk of the identified hazards by administering suitable mitigation and the control programs. The study was carried out for three months i.e from May 2019 to July 2019 and all the staff's responses of the CCU department were captured to prepare the list of the perceived hazards. Moreover, the risk matrix is prepared for the identified hazards by the responses of the CCU staff captured under the categories probability and severity. The findings of the study conclude that the risk associated with hazards H12 (Hindrance in the movement of patients, staff and utilities due to obstructed corridors), H2 (Malfunctioning of Life-saving equipment or patient monitoring Biomedical Equipment) and H3 (The broken cable of equipment results in power leakage, short circuit and the trip of electricity supply in the department). The suitable interventions for these can be increased frequency of safety rounds, widening of the corridors and restricting access to the patient movement areas, and buffer stock for the life-saving devices.

Keywords: *Hazard, Risk Assessment, HIRA Plan, Hospital, Risk Reduction*

Introduction

In the various healthcare systems, clinical threats have attracted the attention of authorities and regulating bodies. In the United States itself around 3. 7 per cent of the hospital patients are at the risk of adverse events in their stay at the hospital.(1) However, the clinical risk can be defined as the "probability of a patient being subject to an adverse event (i.e., an unintended injury or complication that results in disability at the time of discharge, death or prolonged hospital stay) caused by health care management rather than by the patient's underlying disease process".(2) Moreover, the adverse events can be understood as those incidents in which a patient is unintentionally harmed by medical treatment. (3) However, as per the earlier studies, the adverse events affects around 10 per cent of hospital patients and around 50 per cent of them are preventable.(4) Different areas of the hospitals have different susceptibility towards adverse events. Among all the areas the Intensive care units (ICU's) are one of the areas with the highest probabilities for adverse events.(5) For instance, in the United States of America, only around 10 per cent of the patients admitted in the intensive care units suffer from nosocomial infections, moreover, the rate is 21-25 infections per 1000 ICU days.(6) Intensive care units also have a significant number of workplace violence and patient falls.(7) This study attempts to check the status of implementation of all clinical and non-clinical risk measures with the application of hazard identification and Risk assessment (HIRA) in the Critical Care Unit department of a tertiary care hospital of Delhi in India.

Literature Review

Risk can be defined as potential losses and rewards resulting from exposure to a hazard or as a result of the risk event. Similarly, hazards can be explained as something which can lead to an undesired outcome in the process of meeting an objective.(8) HIRA can

be understood as a process which starts with the identification of hazards and then assigning risk values to the identified hazards with the help of risk assessment techniques to take precautionary measures to minimize the impact of the identified hazards. The HIRA technique has been applied in the past in Thermal Power Plants [9], Construction based organizations ([10]), and iron ore pelletizing organizations [11]. The results were satisfactory and the risk ratings were reduced to as low as reasonably practicable (ALARP) levels. There is another version of HIRA which is called as HIRAC " Hazard Identification Risk Assessment and Control."[11] However, in the healthcare organizations, the application of HIRA is limited as some of the studies have carried out risk management initiatives in the hospital operation theatre areas[12] and for the home healthcare nurses working in the metro cities of India.[13]However, the objectives of the HIRA technique can be summarized as:

1. To carry out a systematic, crucial appraisal of all capacity hazards involving personnel, plant, services and operation methods

2. Identify the prevailing safeguards to be had to manipulate the dangers due to the hazards

3. Suggest additional control measures to reduce the hazard to an applicable level.

4. Prepare a Risk Register to assist in continuously monitoring the dangers, locate any changes and make certain the controls are effective.

Methods

The study design is cross-sectional and descriptive. The study setting is the critical care unit of a tertiary care hospital in the Delhi City of India. The study period is of three months ie from May 2019 to July 2019. The data collection technique is unstructured interview technique. The Sampling frame is the medical and paramedical staff working in the 12-bed critical care unit data that includes a survey research design. The exclusion criteria were the staff who have worked for more than one month in the department as a duration less than that will limit their ability to identify the perceived hazards. The staffs were interviewed to identify the hazards in the second stage of the HIRA procedure. Moreover, in the next phase of the study, the identified hazards were ranked by the CCU staff in terms of their probability of occurrence and severity of impact on human beings in the CCU area, impact on hospital property and lastly impact on the business as a whole as depicted in figure 1, See figure 1.

Analysis

The demographic data of the participants revealed that around 36 per cent of the respondents were male and the majority were females with 64 per cent. In terms of age distribution, the majority of the staff were in the age group 20-30 years with 40 per cent, followed by the age group 40 years and above with 35 per cent. The occupation data revealed that around 57 per cent of the respondents were nursing staff, 12 per cent were medical practitioners, 18 per cent of the respondents were housekeeping staff and lastly, 13 per cent of the respondents were technicians, see table 1.

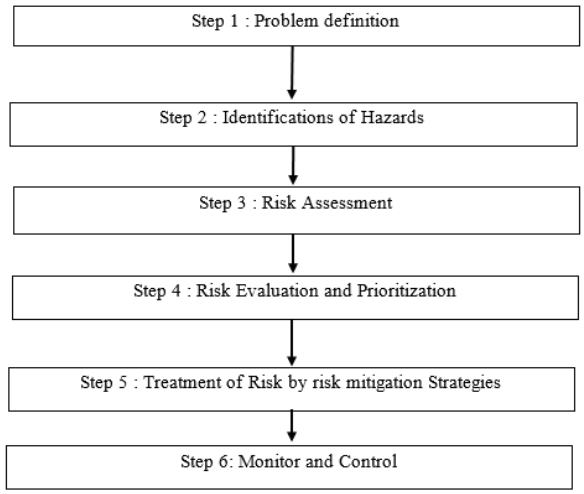


Table 1: Demographic Profile of the respondents.

Gender (Percentage)	
Male	36%
Male	64%
Age (years)	
0-20	0
20-30	40%
31-40	25%
40 & above	35%
Occupation (Percentage)	
Medical Professionals	12 %
Nursing	57 %
Housekeeping Staff	18 %
Technicians	13 %

In the interview content analysis the identified hazards were majorly associated with the following process categories :

1.Major spill management

2.Biomedical Equipment Safety

3.Declaration of patient death to patient attendant or relatives

4.Facility Management

5.Patient safety

6.Infection Control

7.Medication safety

As highlighted in figure 1, in the third stage the risk assessment is carried out by multiplying the probability of the occurrence with the severity of impact in the categories such as the impact on humans, impact on property and lastly impact on business. The scoring was done in a scale of 1-3, where 1 = low probability, 2 = medium probability and 3 = high probability. Similarly for severity impact 1 = low impact, 2= medium impact and 3= high impact. The summarized ratings based on the mode method is mentioned in table 2, See Table 2

Table-2: Risk Matrix With probability and severity impact on Human, Property and Business

Hazard Number	Hazard Description	Probability	Impact On Human	Impact On Property	Impact on Business
H1	A chemical spill inwards and CCU resulting into slip and fall of patient, employees and visitor	3	2	2	1
H2	Malfunctioning of Life-saving equipment or patient monitoring Biomedical Equipment	3	3	3	2
H3	The broken cable of equipment results in power leakage, short circuit and trip of electricity supply in the department	3	3	3	2
H4	Workplace violence by a patient attendant or patient relatives	3	2	3	2
H5	Patient fall from bed	3	3	1	3
H6	Patient fall or slip in the toilet due to the absence of handrails in the toilet or wet surface	3	3	1	3
H7	Patient fall during mobilization inwards	3	3	1	3
H8	High-risk medication errors	2	3	1	3
H9	Lookalike sound-alike medication errors while giving medications to patients	2	3	1	3
H10	The higher dose of narcotics leads to severe injury or critical illness to the patient.	2	3	1	3
H11	Hospital-Acquired infections to patients or the staff due to lack of signages or avoid the use of PPE while visiting patients in the Isolation room.	2	3	1	3
H12	Hindrance in the movement of patients, staff and utilities due to obstructed corridors	3	3	3	3

Results

The results are summarized in all the three categories of impact on human beings, impact on property and impact on business. The findings from the perspective of impact on humans highlight that the hazards, H2 (Malfunctioning of Life-saving equipment or patient monitoring Biomedical Equipment), H3 (The broken cable of equipment results in power leakage, short circuit and trip of electricity supply in the department), H5 (Patient fall from bed), H6 (Patient fall or slip in the toilet due to the absence of handrails in the toilet or wet surface), H7 (Patient fall during mobilization inwards), H12 (Hindrance in the movement of patients, staff and utilities due to obstructed corridors) fell into high priority category denoted by red colour in figure 2 and needs to be addressed first, see figure 2.

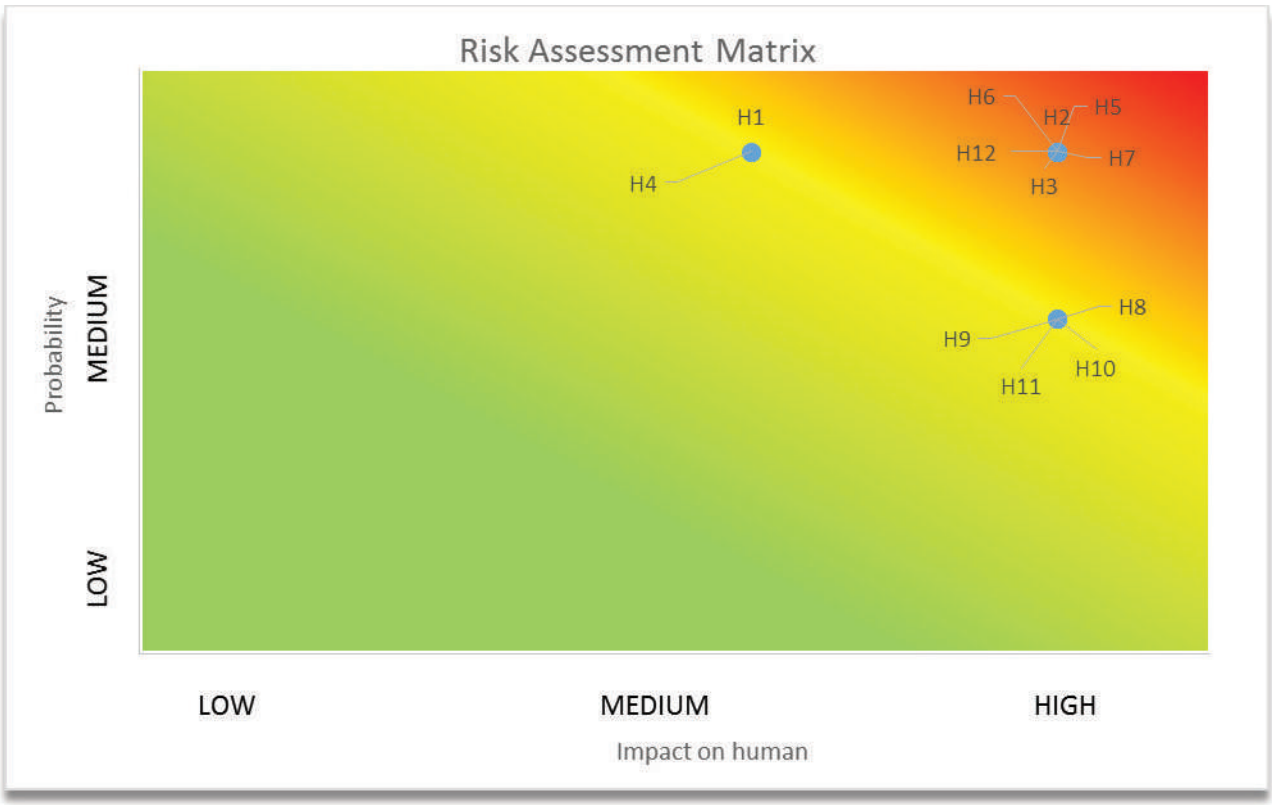


Figure 2: Risk Assessment of Different Hazard Categories and its impact on Human

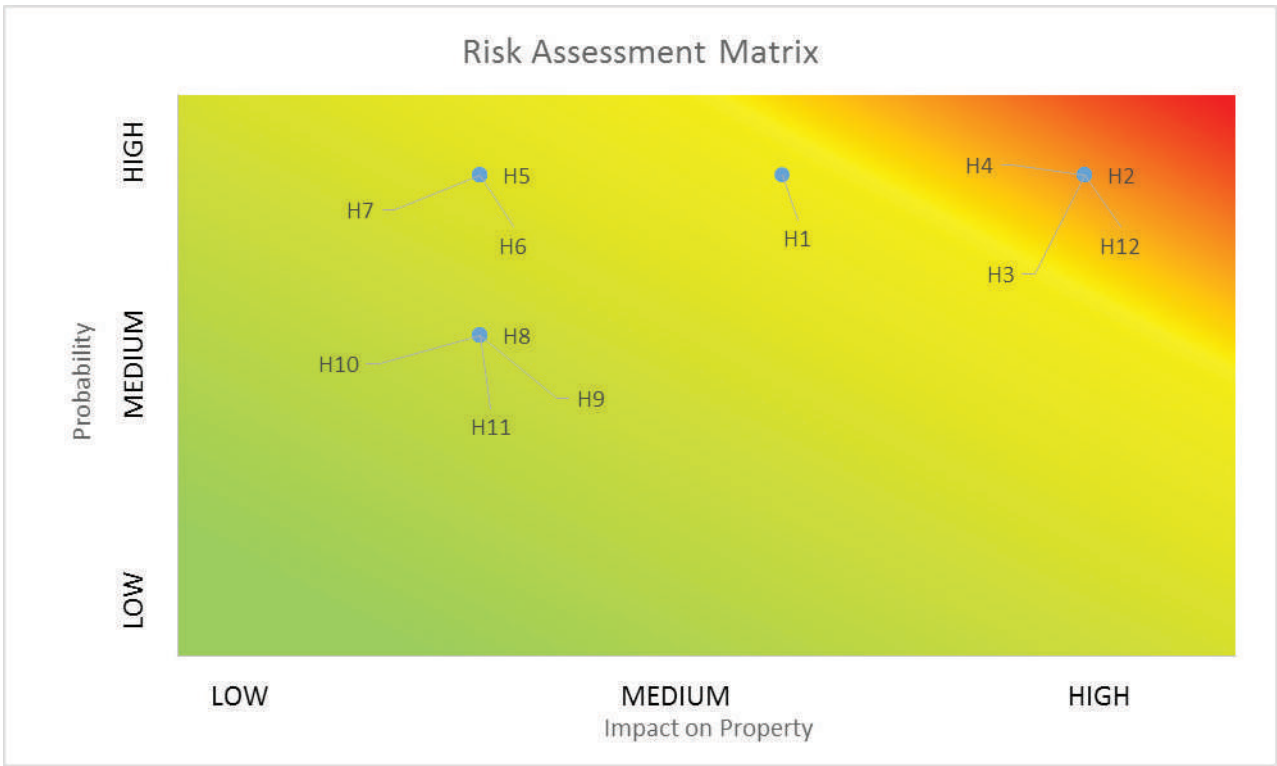


Figure 3: Risk Assessment of Different Process Categories and their impact on Property

Similarly, the findings from the perspective of impact on the property revealed that the hazards such as H2 (Malfunctioning of Life-saving equipment or patient monitoring Biomedical Equipment), H3 (The broken cable of equipment results in power leakage, short circuit and the trip of electricity supply in the department), H12 (Hindrance in the movement of patients, staff and utilities due to obstructed corridors), and H4 (Workplace violence by a patient

attendant or patient relatives) fell into the red zone denoting priority should be given to these hazards for addressal.

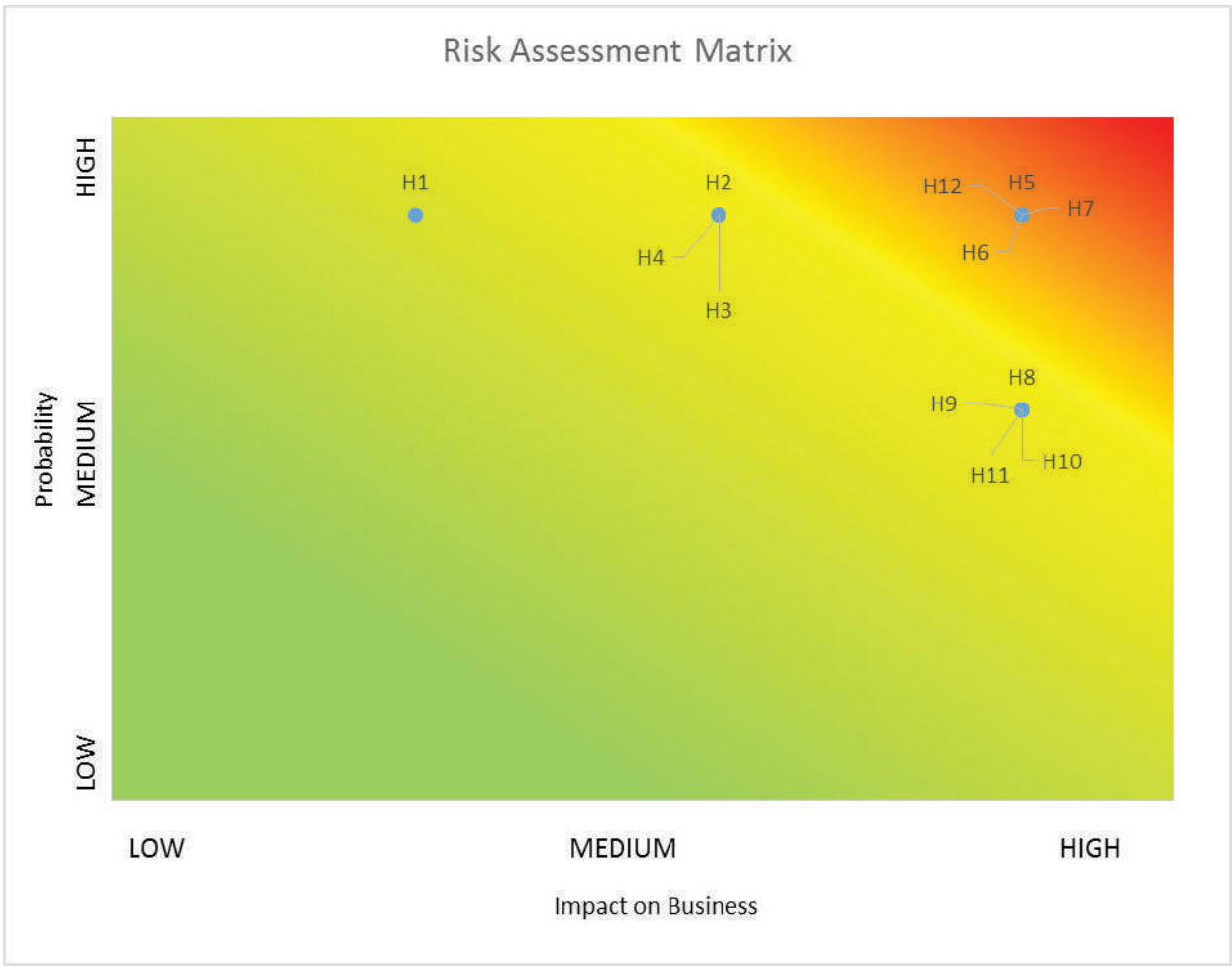


Figure 3: Risk Assessment of Different Process Categories and its impact on Business

Lastly, In the category of impact on business the hazards such as H12 (Hindrance in the movement of patients, staff and utilities due to obstructed corridors), H6 (Patient fall during mobilization inwards), H7 (Patient fall or slip in the toilet due to the absence of handrails in the toilet or wet surface) and H5 (Patient fall from bed) were the one with high probability and having a high impact on the business. Hence these should be taken on priority for risk mitigation.

Discussion

The findings of the study revealed that H12 (Hindrance in the movement of patients, staff and utilities due to obstructed corridors) had a high impact from both the perspectives of impact on property and impact on business. This finding is consistent with earlier studies where hindrance with the fire doors is accepted as a significant barrier for patient movements.[14] Furthermore, hazards such as H2 (Malfunctioning of Life-saving equipment or patient monitoring Biomedical Equipment) and H3 (The broken cable of equipment results in power leakage, short circuit and the trip of electricity supply in the department) were the common priority hazards in the category of impact on humans and impact on the property. This finding is also similar with the earlier study which has highlighted that the patients in the intensive care units are at the higher risk of adverse events as they utilize more biomedical equipment in comparison to the patient admitted in the wards.[15] However, the control measures for risk mitigation for the three categories are discussed as below mentioned.

Control Measures to mitigate the risk of Human-

- Physical Audit of the condition of all biomedical

equipment cables and all default cables to be removed and discarded.

· Periodic maintenance of biomedical equipment and its peripherals.

· Biomedical engineers training in electrical safety and importance of checked and dressed cables with emphasis on safe work practices

· Patient assessment using the Morse Fall Scale. Bedside rails, Identification band of fall risk. Patient call bell for assistance.

· Handrails in staircase & toilet.

· Assistance to the patient while immobilization inward or ICU.

· Wet floor signage to alert patient walking in corridors.

· Continuous training of HK staff to use the wet floor signage.

· Identification of fall risk patients.

· Response to patient call bell.

· Administration: putting rules, policy and signages for awareness.

· Redesign: Making the utilities maintained and safer.

Control Measures to mitigate the risk impact on the property-

· Physical Audit of the condition of all biomedical equipment cables and all default cables to be removed and discarded.

· Periodic maintenance of biomedical equipment and its peripherals.

· Regular conduct of the patient family meeting and its record-keeping.

· Consent of patient relatives in treatment planning.

· Effective communication by all health care workers.

· Elimination of hazard completely from the workplace.

· Redesign the process of handling death cases

· Continuous training of all health care workers ineffective communication and priority with a sequence of activity to be done for the smooth handling of deceased patient relatives.

· All corridors and evacuation corridors must be obstruction-free, for free movement of patient, employee, fire safety & evacuation teams.

· Administration: putting rules, policy and signage's for awareness.

· Continuous training on evacuation corridors in the hospital building and the importance of clear passage during situations such as fire, electricity shut down etc.

Control Measures to mitigate the Risk Impact on Business-

· Patient assessment using the Morse Fall Scale. Bedside rails, Identification band of fall risk. Patient call bell for assistance.

· Handrails in staircase & toilet.

· Assistance to the patient while immobilization inward or ICU.

· Wet floor signage to alert patient walking in corridors.

· Continuous training of HK staff to use the wet floor signage.

· Identification of fall risk patients.

· Response to patient call bell.

· Administration: putting rules, policy and signage's for awareness.

· Redesign: Making the utilities maintained and safer.

· Barrier nursing, hand washing by all health workers, adherence to care bundles, sterilized linen, stringent facility upkeep, 1:1 nursing ratio.

· Adherence to medication safety policy and controls.

· Maker checker process before administrating high-risk medications.

· Medication safety audit.

· Elimination of hazard completely from the workplace.

· Adherence to medication safety policy and controls.

· Maker checker process before administrating high-risk medications.

· Medication safety audit.

· Training on infection control practices.

· Doctor, nursing & pharmacy staff training on medication safety and management of high-risk medications.

Other control measures

· Careful handling of chemical containers. In case of a major spill of hazardous material, use a major spill kit to control the hazards of slip and falls.

· Activate code grey: Major spill.

· Training in handling major spill code grey. And safe handling of hazmat containers.

· Administration: putting rules, policy and signage's for awareness.

· Personal protective equipment shall be used during a major spill of hazardous material.

Conclusion

This study concludes that the HIRA (Hazard Identification and Risk assessment) technique is an effective strategy to identify the hazards related to the complex healthcare providing areas of the hospitals. Moreover, in the studied hospital the hazards such as H12 (Hindrance in the movement of patients, staff and utilities due to obstructed corridors), H2 (Malfunctioning of Life-saving equipment or patient monitoring Biomedical Equipment) and H3 (The broken cable of equipment results in power leakage, short circuit and the trip of electricity supply in the department) needs to be addressed on priority. The suitable interventions for these can be increased frequency of safety rounds, widening of the corridors and restricting access to the patient movement areas, and buffer stock for the life-saving devices.

Limitations: The study was carried out for three months due to shortage of time, hence the after-effects of the intervention couldn't be captured.

Ethical Clearance- IEC of Symbiosis International (Deemed University)

Source of funding- NIL

Conflict of Interest-None

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A study to Assess the Awareness And Practice of Fast Food Consumption and Hazards on Health among Adolescents in Urban Community Area of Pune City

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Abstract

Introduction: Food is something everyone needs, every day. Life can be sustained only with adequate nourishment and it requires for growth, development and to lead an active and healthy life. Fast food refers to fast foods which are easy to make, and easy to consume. They have only fats lying in it causing ill effects on the health of the adolescents. The present study was done to assess the knowledge and practice of fast food consumption and hazards on health among adolescents.

Materials and methods: The study design adopted was descriptive in the colleges of urban area, where 100 college students were participated through convenience sampling technique. Semi-structured self-administered questionnaire were used to collect the data.

Result: The findings revealed that10 % of the participants had inadequate knowledge, 28% of the participants had moderate knowledge and 62% of the participants had adequate knowledge about fast food consumption. Respondents stated that that majority of participants (85%) eat restaurant fast food regularly and 51 % of subjects frequently eat fast food. The main reason for having fast food was its Flavourful taste, as per 64% respondents had replied.

Conclusion: Though fast foods are tasty they have low nutritive value and high calories. Awareness regarding the significance of a balanced diet and harmful effects of fast foods may help to reduce the fast food addiction.

Key words- *fast food, adolescents, consumption, awareness*

Introduction

Food is something everyone needs, every day. Life can be sustained only with adequate nourishment and it requires for growth, development and to lead an active and healthy life.[2]Fast foods are easily available and delicious to have it but it contains lot of fats which is causing bad effects on the adolescents' health.[1]

Proper nutrition is important for growth, developments of young adults and it promotes eating behavior as well.[5]They miss an increasing number of meals at home as they get older. Teenagers identify time as the biggest barrier to eating properly. They form positive associations with junk foods. Other factors includes mood, body image, concerns, habits, media influences and life style choices.[2]

As per compound annual growth rate (CAGR), fast food market of India is expected to grow 18% by 2020 due to lifestyle and demography. At present India ranks in 10th position in the per capita expenditure consumption with spending per annum 2.1% on fast food. The rising rate is 40% per year of the fast-food sector in India.Several harmful effects on young adults due to adaptation of universal model for fast food consumption.[3]

Obesity is the major health problems in adolescents and consumption of fast food is one of the root cause

of it.During the17-year period between 1998 and 2015, low and middle-income countries has more prevalence rate as compare to developed countries. Their salt content is also very high which not only increases the risk of elevated blood pressure but also aggravates the pre-existing metabolic syndrome and insulin resistance. Increasedintake of fast food raises the risk of metabolic syndrome in consumers up to 150%, that of diabetes mellitus up to 68% and of cardiovascular mortality by 162%.[4]

Material and Methods

A descriptive study was conducted among adolescents in urban community area of Pune city. In this study total 100 subjects were participatedthrough convenience sampling technique.

Structured self-administered questionnaire were used to collect data from participants. The questionnaire were prepared in 4 section which included socio demographic information, knowledge based questions about fast food consumption, and practice based questions about fast food consumption and its hazards on health.

Every right answer gives 1 point and wrong answer gives zero. Total 12 items were included in knowledge based questionnaire, subjects with the score 0-4 considered inadequate knowledge, 5-8 considered moderate and 9-12 considered adequate knowledge.

Result

(N- 100)

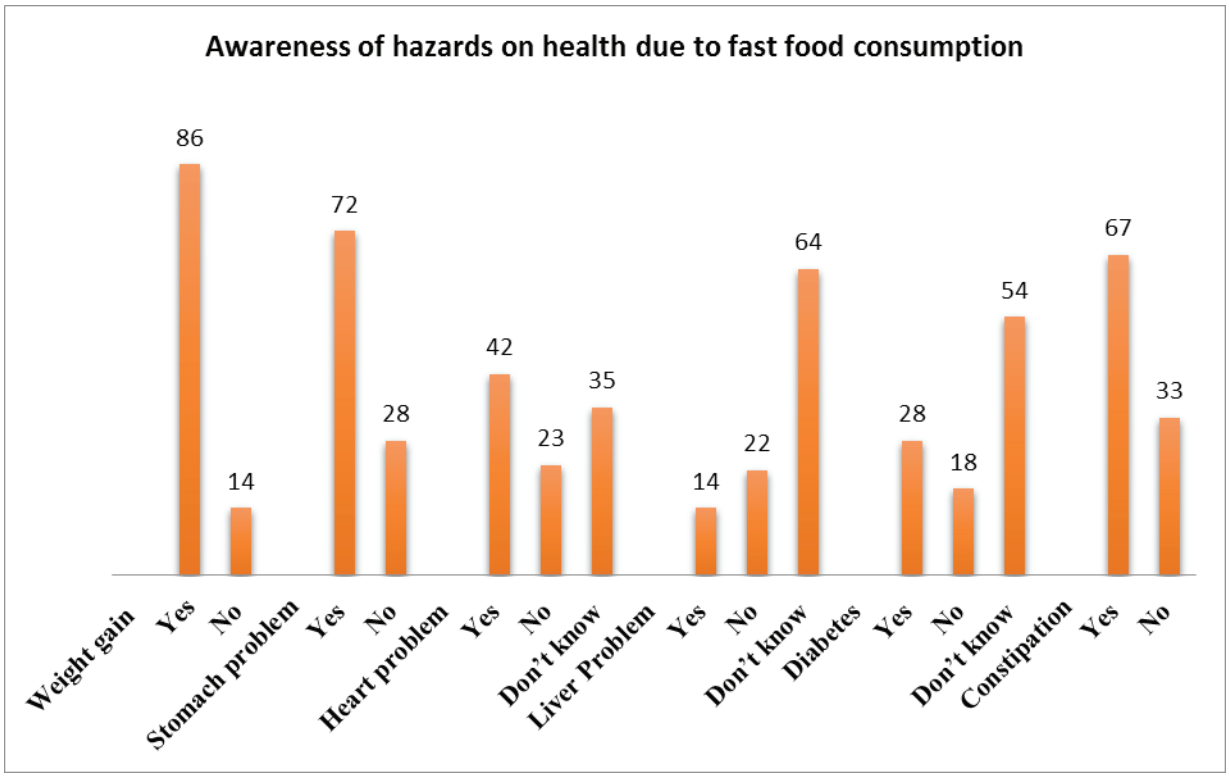


Fig No.1 Awareness of hazards on health due to fast food consumption

In fig no.1 stated that 86 participants pointed out weight gain as hazard on health due to fast food consumption and 67 participants mentioned constipation. Whereas 72 % participants stated that stomach problem. 23 % & 22% of the participants are not aware about heart problems and liver problems occur due to fast food consumption respectively.

Table No. 2: Distribution of Participants on the basis of Level of Knowledge

(N- 100)

Sr. No.	Level of Knowledge	frequency (n)	Percentage (%)
1.	Adequate knowledge	62	62
2.	Moderate Knowledge	28	28
3.	Inadequate Knowledge	10	10

Table no. 2 depicts that majority of study subjects 62 % samples had average knowledge while 28 % samples having Good knowledge, & 10 % samples having Poor knowledge regarding the health hazards of fast foods.

Table no. 3 Practice based items of Fast Food consumption

(N- 100)

Sr. No	Items		frequency (n)	Percentage (%)
1. 2.	Fast food consumption regularly	Yes	85	85
		No	15	15
3. 4. 5. 6.	Pattern of fast food consumption	Frequently	51	51
		Occasionally	26	26
		Monthly	13	13
		Weekly	10	10
7. 8. 9. 10.	Type of fast foods	French fries	19	19
		Potato chips	27	27
		Pizza /Burger	18	18
		Chinese food	36	36
11. 12. 13.	Different types of drinks with fast food	Cold drinks	51	51
		Coffee/Tea	35	35
		Juice or shake	14	14
14.	Why do you eat fast food	Flavourful taste	64	64
		Easily available	28	28
		Inexpensive	8	8

Table no. 3 depicts that majority of participants (85%) eat restaurant fast food regularly and 51 % of subjects frequently eat fast food. Most (36%) of them likes to eat Chinese food whereas 19 % had French fries. 51 % participants likes to drink soft drink whereas 14 % only have fresh fruit juice. The main reason for having fast food was its Flavourful taste, as per 64% respondents had replied.

Discussion

The Similar Study Was Conducted On The Knowledge And Practice Of Fast Food Consumption Among Pre-University Students In Udupi Taluka, Karnataka, India. The Findings Of Awareness About The Effect Of Fast Food Consumption Was The Subjects With Inadequate Knowledge Were 51(31.87%) And 42(26.25%) Of The Subjects Had Adequate Knowledge. based On The Practice, 15(9.4%) Of Theparticipants Regularly Eat Fast Food And Sometimes 115(71.8%) Subjects Had. Whereas Majorityof The Participants 100 (62.5%) Likes To Have Chinese Food And Monthly 67 (41.9%) Participants Visited To Restaurant To Have Fast Food. Most Of The Participants 83 (51.9%) In Favour To Drink Soft Drink And The Main Reason To Have Fast Food Was Its Deliciousstaste, As Per Reply Given By 116 (72.5%) Participants.[6]

Whereas In Current Study Majority Of Subjects 62 % Samples Had Average Knowledge While 10 % Samples Having Poor Knowledge About Hazards On Health Due To Fast Foodsconsumption. Based On Practice About Fast Food Consumption, Majority Of Participants (85%) Eat Restaurant Fast Food Regularly And 51 % Of Subjects Frequently Eat Fast Food. Most (36%) Of Them Likes To Eat Chinese Food Whereas 19 % Had French Fries. 51 % Participants Likes To Drink Soft Drink Whereas 14 % Only Have Fresh Fruit Juice. The Main Reason For Having Fast Food Was Its Flavourful Taste, As Per 64% Respondents Had Replied.

The Similar Study Was Conducted On Health Problems Due To Junk Food, The Findings Revealed That129(34.9%) Participants Had Oralhygiene Problem, 62 (16.8%) Had Gastroenteritis Problems And18(4.9%) Had Constipation. The Participants Had Complained About Stomach Upset And Bloatinghealth Issues In 44.6% (165) And 11.9% (44) Respectively.[7]

In Present Study Participants (85%) Eat Restaurant Fast Food Regularly And 51 % Of Subjects Frequently Eat Fast Food. Most (36%) Of Them Likes To Eat Chinese Food Whereas 19 % Had French Fries. 51 % Participants Likes To Drink Soft Drink Whereas 14 % Only Have Fresh Fruit Juice. The Main Reason For Having Fast Food Was Its Flavourful Taste, As Per 64% Respondents Had Replied.

Conclusion

Balanced Diet Is The Essential For Growth And Development Of The Adolescents. Most Of The Adolescents Are Getting Addicted For Fast Food Due To Taste, Attractive Advertisements And Easily Available. The Young Adults Are Ignoring The Hazards Of Fast Food On Health Though They Are Aware Of It. The Proper Guidance And Nutrition Counselling About Nutritious Food Is Required To Halt Habituated Of Fast Food Consumption And Can Prevent Hazards On Health.

Conflict of Interest – Nil

Source of Funding- Self

Ethical Clearance – Ethical Clearance Taken From Institutional Research Committee Of Symbiosis College Of Nursing

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An Exploratory Study on Opinion of Tracking System For Patient with Dementia among Mental Health Team Member of Pune City

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Abstract

Introduction and objective: **Patient with dementia who wander are at high risk of harm self and pose threat to self and stress and worry for care givers too. Care givers feel under stress and worry due to this symptoms**. The objective of this study was to to determine the opinion from mental health care team member or tracking system for patient with Dementia and to find association between opinions of mental health team members and selected demographic variable. **Material and Method:** Quantitative Non-Experimental research approach was used and research design was Descriptive Exploratory Research Design to explore on opinion of tracking system for patient with dementia among mental health team member of Pune city. Sample size was 30 and non-probability convenience sampling technique. Tool had two sections. Part A involved the demographic variables age, gender, marital status, designation, and years of experience and part B was structured opinionnair 0 to 2 numerical rating scale which is further divided into 3 categories Yes, No, Don't Know. **Result:** Mental health team members is in opine for demand of tracking system for patient with dementia. ANOVA test used to check the association between opinion of mental health team member and selected demographic variable. The p-values are less than 0.05, there is a significant association between opinion of mental health team member and years of experience of mental health team members and with other demographic variables there is no any significant association.

Key Words: *Tracking system, Dementia, Opinion, Mental health Team Member*

Introduction

Dementia is characterized by deterioration in memory, decision making, behaviour, language and ability to do daily activitiess. Cognitive function declines like forgetfulness. Monitoring is very important, it is demanding and crucial.

Patient with dementia who wander are at high risk of harm self and pose threat to self and stress and worry for care givers too. Care givers feel under stress and worry due to this symptoms. Though tracking system are available of different variety and very costly this helps in locating the patient and to minimise risk and security to patient.

Study said that the sample emphasized malfunctions and usage difficulties of the device, which made that, not appropriate to her needs [1].

A Study focused on various factor like network signal strength, weather condition, and location, in testing for the parameters set [2]

For monitoring tracking system would more easy and comfortable for care givers. Felt need by researcher for the tracking system which would cost effective and the present study focused on the opinions from the mental health team for the tracking system for patient

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with Dementia to minimise the burden on caregivers

Objective :

- To determine the opinion from mental health care team member or tracking system for dementia patient.

- To find association between opinion of mental health team member and selected demographic variable.

Hypothesis:

H_0: There is no relationship between tracking system with dementia patient among mental health team member of Pune city.

H_1: There is relationship between tracking system with dementia patient among mental health team member of Pune city.

Material and Method

Quantitative Non-Experimental research approach was used and research design was Descriptive Exploratory Research Design to explore on opinion of tracking system for patient with dementia among mental health team member of Pune city. Tool had two sections. Part A involved the demographic variables age, gender, marital status, designation, and years of experience and part B was structured opinionnaire 0 to 2 numerical rating scale which is further divided into 3 categories Yes, No, Don't Know.

To ensure the content validity of tool, it is validated from experts from field of Psychiatrist, Psychiatric nurse, Counsellor, Psychologist, Researcher, mental health professor and statisticians. Suggestions given by them were taken under consideration and modification of the tool was done as per the recommendations. Reliability was established by Cronbach's alpha and it was 0.806. Thus the tool was found to be highly reliable.

After obtaining the written consent from each sample, opinionnaire was administered.

Findings: The data was analysed using descriptive and inferential statistics.

Sections 1: Description of demographic variables

Table 1.1: Demographic variables n=30

Variables		Frequency(n)	Percentage (%)
Age (In years)	25-30	17	56.7
	30.1-35	7	23.3
	35.1-40	3	10.0
	40.1-above	3	10.0
Gender	Male	19	63.3
	Female	11	36.7
Marital Status	Married	13	43.3
	Unmarried	17	56.7
	Divorced	0	0.0

Cont... **Table 1.1: Demographic variables n=30**

Designation	Psychiatrist	3	10.0
	Psychologist	4	13.3
	Psychiatric clinical nurse	3	10.0
	Psychiatric social worker	10	33.3
	Counsellor	10	33.3
Years of Experience	2-5 years	14	46.7
	5.1-15 years	13	43.3
	15.1 and above	3	10.0

Section II: Analysis of Data related to the opinion of mental health team members on tracking system for Patient with Dementia

Majority (92%)of Mental health team members opine for the use of tracking system for Patient with dementia and (8%) of the members are not in opinion of use of tracking system for patient with Dementia

Section III: Analysis of data on association between opinion of mental health team member and selected demographic variable.

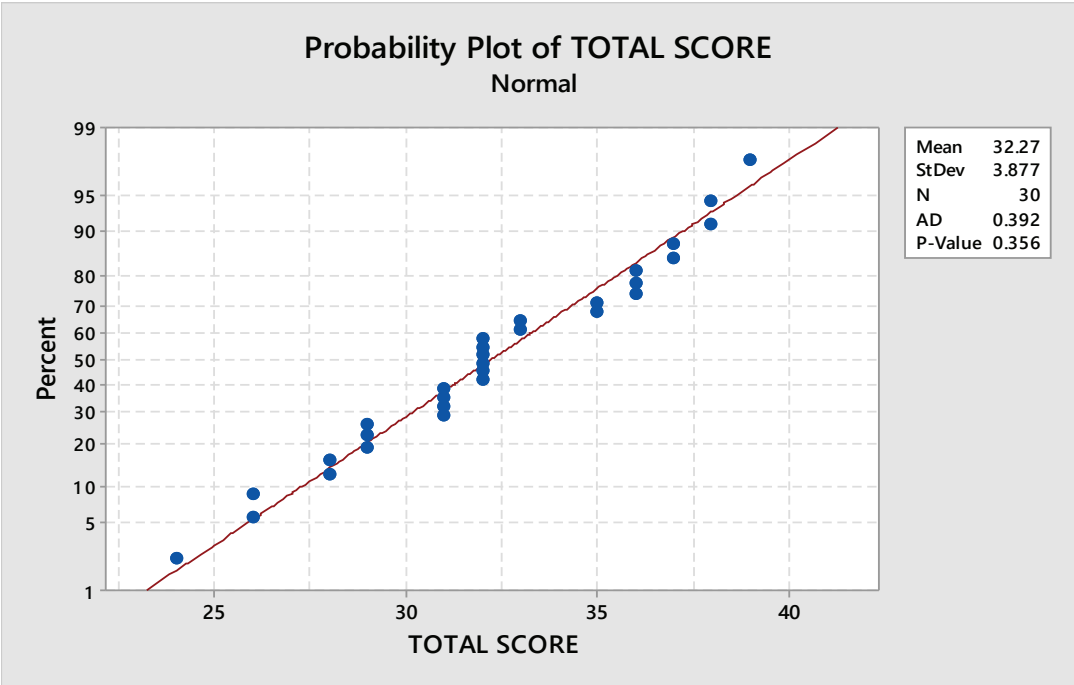


Fig.No 1.1: shows that data is normal and used ANOVA test to check the association between opinion of mental health team member and selected demographic variable..

p- Value is 0.028, less than 0.05 rejecting H_0 (Null Hypothesis) . It shows there is significant association opinion of mental health team member and years of experience. In other demographic variable the p-Value is greater than 0.05 so there is no significant association between opinion of mental health team member and other demographic variables.

Discussion

Present study findings shows Mental health team members opine for the use of tracking system for Patient with dementia and so there is no significant association between opinion of mental health team member and other demographic variables

The caregivers provide fondness to patients' safety more than autonomy when they are responsible for the patients [3]

Findings highlight the current lack of high-quality evidence to determine whether Assistive technology is effective in supporting people with dementia to manage their memory problems [4].

Conclusion: Mental health team members are in favour to for use of tracking system for patient with dementia. The health care professional and caregivers knows that there is a hope of Patient with Dementia care which can improve their condition, quality of life, daily activities.

Conflict of Interest: Nil

Source of Funding: None

Ethical Consideration: Study was approved at Institute Ethical sub committee.Informaed concerned from each sample been taken for the study. Privacy and confidentiality maintained

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Knowledge Assessment on Hazards of Plastic Use among Residents of Selected Urban Community of Pune City

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Abstract

Introduction: Plastic products have become integral part of our daily lives. Due to which the production of Plastic have gone up worldwide. Plastic consumption in India increased from 61 thousand tons in 1996 to 1.78 million tons in 2017, which is a huge increase. **Objective** of the study is to assess the knowledge about hazards of Plastic use among selected areas of Pune city. **Methods:** Non experimental descriptive survey research design for the study. Sample comprised of 400 residents of selected urban community of Pune city. Non probability convenience sampling technique was used for the study. A self-administered structured knowledge questionnaire on hazards of Plastic use was used. The tool is divided in two sections. Section I is dealt with demographic proforma of the sample. Section II was self-administered structured knowledge questionnaire comprised of thirty questions (Multiple choice questions) in four domains on knowledge of hazards of plastic use, after permission from the concerned authority and informed consent from each sample, the investigator has administered the tool. **Result:** Majority of respondents have average knowledge (46.75%) and 3.5% respondents had poor knowledge on hazards of Plastic use. **Conclusion:** The present study revealed the residents had considerable average knowledge regarding hazards of plastic use. The enhancement in knowledge is greatly required on all aspects of hazards of plastic use.

Key words: *Knowledge; Plastic; Hazards: Community*

Introduction

Plastic are the group of material either synthetic or naturally occurring, that may be shaped when soft and then hardened to retain the given shape. The development of plastic has evolved from the use of Natural Plastic material to the use of chemically modified natural material and finally to completely Synthetic Materials. Most plastic are durable and degrade very slowly as their chemical structure renders them resistance to many natural processes of degradation. [1]

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Plastic products have become integral part of our daily lives. Due to which the production of Plastic have gone up worldwide. On an average 150 million tons of Plastic is produced globally. Once Plastic is discarded is termed as Plastic Waste and it is a fact that plastic waste never degrades and remains in landscape for several years. With the largest population, China produce the largest quantity of Plastic at nearly 60 million tons. It is followed by United States and Germany. [2]

Whereas India generates 9.4 million tons of Plastic Waste per year out of which 5.6 million tons per year is recycled and 3.4 million tons per year is left and collected or littered. [3]

North America is the highest consumer of Plastic at 90kg/ person and India it is 5kg/person. (4)

Plastic consumption in India increased from 61 thousand tons in 1996 to 1.78 million tons in 2017,

which is a huge increase. [3]

Need for the study

Plastic waste possess a threat to environment and health problem in human and animal. Due to the non-biodegradable nature of plastic there is negative impact. The negative effect in human beings include birth defect, impaired immunity, endocrine dysfunction, etc.

Not only is human being the Plastic Waste known to be a threat to marine organism as well. In the late 20's it has become to light micro plastics also disrupts the function of living organism.

India recycles 60% of its Plastic Waste, which is highest in world. Maharashtra tops in production in Plastic Waste followed by Gujarat.

Government of India put forward Plastic Waste Management and rule in 2016 which was reinstalled in 2018 which is applicable to all states. According to which, the most favored option is reduce and reuse and least favored option is recovery and landfill. Public awareness and participation is the way forward for curbing the negative impact of Plastic Pollution. [3]

A study conducted in Tamil Nadu among Professional students on Knowledge and Practice regarding plastic pointed out that 46% of students had good knowledge but 27.6% only followed good practices. Though knowledge was good their practice and terms of disposal was less. [4]

A study conducted in Delhi that 76.4% of individual considered of plastic as a threat, but only 40.3 % considered paper bag as an alternative. It also remarked that only 48% housewives had attended some sort of sensitization programme related to plastic waste management.[5]

A descriptive study conducted in 2012 among homemakers of urban area in Mangalore, India found that 71% had inadequate knowledge about plastic waste management, also least profession of knowledge was in the area of use and reuse of plastic.[6] .

A study conducted on hospital based survey, in order to reduce plastic waste, the popularity of plastic waste management among the public has to be changed. These researches show that imparting knowledge and creating positive and long lasting behavioral change among the public. As Maharashtra is the top most contributor of plastic waste and since pune which has population of more than 5 million persons. It becomes a major contributor in the state. Pune produces 166 tons of plastic waste per day. out of which 12.5% of plastic waste leaks into environment and rest are recycled and reused. Approximately 70% of plastic waste in packaging. This indicates that main source of plastic waste is related to consumption of packed products. Branded packaging waste, 86% is from food product followed by 8% from personal care product and 6% from household product. Plastic are also used in growing range of building and construction applications which are another large source.[7]

A case study on Pune plastic waste disposal pointed out that increased public awareness and providing education to residents on sorting and segregating is important. [7] Public awareness and education can be undertaken only after accessing the level understanding among the population. This study undertaken to find out the knowledge about the plastic waste management among residents of Pune.

Statement of the Problem

"Knowledge assessment on hazards of plastic use among residents of selected Urban Community of Pune city"

Objective of the study

- To describe the socio demographic characteristics of residents of urban community of Pune city.
- To assess the Knowledge on hazards of Plastic use among residents of selected urban community of Pune city.

Assumptions

Residents of selected urban community of Pune city may have knowledge about hazards of Plastic use

Operational Definitions

Knowledge : knowledge refers to the verbal responses of residents of Pune city as measured by the knowledge part of structured questionnaire on hazards

of plastic use.

Plastic : Any item made of synthetic or semi synthetic organic compounds that are malleable and is harmful in nature.

Residents : People who are residing in urban community and are between the age of 18 years or above of Pune city.

Material and Method

The researcher has adopted the Non experimental descriptive survey research design for the study. Sample comprised of 400 residents of selected Urban Community of Pune city. Non probability convenience sampling technique was used for the study.

Inclusion Criteria

- Residents of Urban community
- Age of residents to be above 18 years of age.
- Residents who can read and understand English and Hindi

Exclusion Criteria:

- Residents of Urban community who are not willing to participant

Reliability was done by using test re test method and reliability coefficient obtained by pearson correlation formula was found reliable 0.808 . Validity of tool was done by giving it to experts in following field Educator, researcher, statistician and Physician

A self-administered structured knowledge questionnaire on hazards of Plastic use was used. The areas has selected after extensive literature review and consultations with expert of concerned discipline. The tool is divided in two sections. Section I is dealt with demographic proforma of the sample. It consisted of information on selected demographic variables like age, gender, educational qualifications and occupation. Section II was self administered structured knowledge questionnaire comprised of thirty questions (Multiple choice questions)in four domains on knowledge of hazards of plastic use. Each correct item was given 01 mark and wrong response received 0 marks..

After permission from the concerned authority and informed consent from each samples, the investigator has administered the tool.

Findings

Section 1: Sample characteristics

Table 1: Frequency and percentage distribution of demographic variable

n= 400

Variables		Frequency	Percentage
Age (In years)	19-25	117	29%
	26-35	182	45%
	36-50	82	21%
	51 and above	19	5%
Gender	Male	185	46%
	Female	215	54%

Cont... **Table 1: Frequency and percentage distribution of demographic variable**

Educational Status	Illiterate	12	3%
	Higher secondary	180	45%
	Graduate	182	45.5%
	Postgraduate and above	26	6.5%
Occupation	Students	38	10%
	Skilled worker	70	18%
	Un -skilled worker	129	32%
	Professional	160	40%

Table 1- shows maximum 45% of sample was in the age in the age group of 26-35 years whereas minimum in the age group of 51 and above age group. Majority of respondents were female (54%) and 46% was males. Majority of respondents were graduate and undergraduate (45.5% and 45%) and 6.5% were highly qualified as postgraduate and above. Majority (40%)were professionals and 32% were un-skilled workers whereas 18% were skilled workers and only 10% were students.

Fig 1. Age wise distribution of Respondents

Section –II –Assessment of level of Knowledge of the residents of selected area of pune city on hazards of plastic use.

n= 400

S.No	Level of Knowledge	Frequency	Percentage
1	**Very poor (1-6)**	**0**	**0%**
2	Poor (7-12)	14	3.50%
3	Average (13-18)	187	46.75%
4	Good (19-24)	150	37.50%
5	Excellent (25-30)	49	12.25%

Table 2 : Shows level of knowledge of residents of Urban community on Hazards of plastic use

Fig 2: Level of knowledge on hazards of plastic use among residents of urban community

Table 2 and fig 2 shows the level of knowledge on the basis of frequency and percentage among residents of urban community of Pune city that majority of respondents have average knowledge (46.75%) and 3.5% respondents had poor knowledge

Discussion

In present study shows that the urban community residents have average level of knowledge related to hazards of plastic use .

Similar findings been revealed in the study of Malathi on knowledge assessment among adolescents on hazards of plastic use .Their study findings says that adolescence has average knowledge on hazards of plastic use.[8]

Another study also has similar findings on awareness of hazards of plastic bag usage. Study findings show the participant in the settings had the awareness of hazards of plastic bag usage. Still need for spreading the awareness of using alternative strategies and effective implementation of legislation in order to minimize the usage of plastics in the community. [9]

Conclusion The present study revealed the residents had considerable average knowledge regarding hazards of plastic use. The enhancement in knowledge is greatly required on all aspects of hazards of plastic use.

Conflict of Interest : Nil

Source of Funding : Self funding

Ethical Consideration: The researcher gained the Institute research Committee approval and Study was approved at Institute Ethical subcommittee. Informed consent was obtained from each sample. Privacy and confidentiality maintained

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Molecular Mechanism of Caffeine-Aspirin Interaction in Kopi Balur 1 as Anti-Inflammatory Agent: A Computational Study

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Abstract

Balur treatment (BT) is a method that works to remove toxins in the form of free radicals released via the skin. One of the remedies used is Kopi Balur 1 (KB1) which contains aspirin and caffeine used during the BT, so that KB1 is believed to have the potential to reduce or inhibit the occurrence of inflammatory processes in the body. Therefore, we investigate the interactions that occur between ligands and proteins, predict the action mode of caffeine-aspirin contained in KB1 as an anti-inflammatory agent, and modeling the 3D structure of pro-inflammatory proteins in the human body. The target compound, aspirin-caffein, was obtained from a database, then used to identify the pathway on the STITCH webserver, then the target protein was obtained and modeled in 3D using the SWISS-MODEL webserver, and the structures obtained were represented in PyMol software. In sum, the aspirin-caffeine ligand complex contained in KB1 has the potential as an anti-inflammatory agent in the human body via the molecular mechanism of binding and inhibiting the biological activity of proinflammatory proteins, i.e. PTGS1, PTGS2, and ADOR2A.

***Keywords**: Anti-inflammatory agent, computational study, kopi balur 1, molecular mechanism.*

Introduction

Balur treatment (BT) is a method used to remove toxins in the form of free radicals contained in the human body. Balur is a treatment method from the Javanese society that has been developed to improve the quality of life. BT uses natural ingredients as free radical scavenging and components of aromatic chemical compounds such as amino acids and other aromatic compounds. The body of a person undergoing BT must lie on a copper plate and this therapy is believed to have the potential to reduce or inhibit the effects of inflammation in the body[1].

Caffeine is a type of heterocyclic alkaloid in the methylxanthine group, and by definition means organic compounds containing nirogens with a two-ring structure. This molecule naturally occurs in many types of plants as secondary metabolic. Caffeine is a compound belonging to the xanthine derivative alkaloids that contain a methyl group. Caffeine has long been obtained from plant extracts such as coffee beans, tea, and chocolate[2]. In addition, Indonesia possesses a vast biodiversity with a high number of potential natural medicinal sources[3,4].

On the other hand, aspirin or acetylsalicylic acid (acetosal) is a type of drug that is often used as an analgesic, antipyretic, and anti-inflammatory. Aspirin can also be used in low doses for a long period of time to prevent heart attacks. Aspirin is made by esterification, in which the active ingredient of aspirin is reacted with anhydrous acetic acid and glacial acetic acid. In the process of making this esterification reaction, it is assisted by an acid catalyst to speed up the reaction[5].

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Inflammation is an immune response to the local tissue caused by injury or damage caused by certain factors, which function to destroy, reduce infectious agents that damage the tissue or play a role in the regeneration process of the injured tissue[6,7]. Therefore, we investigate the interactions that occur between ligands and proteins, predict the action mode of caffeine-aspirin contained in KB1 as an anti-inflammatory agent, and modeling the 3D structure of proinflammatory proteins in the human body.

Materials and Methods

Sample Preparation

This study conducted at Department of Biology, Faculty of Mathematic and Natural Sciences, Universitas Brawijaya, Indonesia. This study used aspirin (ID 2244) and caffeine (ID 2519) in the form of 3D structures (structure data format or sdf) and canonical SMILES obtained from the PubChem database (pubchem.ncbi.nlm.nih.gov). Pubchem is a specific database that stores information related to organic chemical compounds, synthesis, and certain substances and is a branch of NCBI. A sample of chemical compounds originating from PubChem are in the form of 3D and 2D structures[8].

Drug-Likeness Prediction

Samples of target compounds were tested for their potential as drug molecules by checking their accuracy according to Lipinski's rules[9,10]. The structure of 3D compounds in the sdf format was analyzed on the Lipinski webserver (scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) and the results were identified which consisted of molecular weight, hydrogen acceptor donors, lipophilicity, and molar refractivity.

Pathway Analysis

The interaction between aspirin-caffeine and the target protein in the human body can be identified on STITCH (stitch.embl.de). STITCH is a webserver whose role is to predict the interaction of a chemical compound in the target organism, which will get stronger association scores based on a database or evidence from experiments that have been carried out[11].

Protein Modeling and Validation

Protein sequences contributing to the inflammatory response were obtained from the Uniprot database (uniprot.org), then modeling via the SWISS MODEL webserver (swissmodel.expasy.org), and the results of the modeling were identified by Ramachandran plot. The modeling used in this research is homology and works based on the availability of templates in the database to produce a target protein model[8,12]. The quality of the model is identified via a plot that works based on the position of specific amino acids that will determine the favored score on the model[13].

Result and Discussion

Molecular Interaction of Caffein-Aspirin with Proteins in Human Body

Based on the results of *in silico* analysis of the interaction between ligand-protein on the STITCH webserver, caffeine (ID 2519) have a canonical SMILES CN1C=NC2=C1C(=O)N(C(=O)N2C)C and aspirin (ID 2244) have a canonical SMILES CC(=O)OC1=CC= CC=C1C(=O)O, most likely interact with 10 proteins inside the body of *Homo sapiens*. The results of identifying the interaction of aspirin-caffeine with 10 proteins in the body of *Homo sapiens* showed that all of them are the highest evidence because they have a score of above 0.900 or 90.0%. In STITCH, there are a number of edge convention scores starting from low (0.150), medium (0.400), high (0.700), and highest confidence (0.900)[11]. So, the greater the score, the stronger of the suspicion of interaction occurs when the caffeine aspirin ligand complex is in the body of *Homo sapiens*.

Based on the identification of the interaction between the aspirin-caffeine ligand complexes with the target protein, there are also target proteins that contribute to the inflammatory mechanism in the human body, such as PTGS1 and PTGS2 (interact with aspirin) and ADORA2A (interact with caffeine). Carrol *et al*. have explained that PTGS1 can increase the production of mucus layers on the inside of the stomach, making other contributions such as reducing stomach acid levels[14]. PTGS1 is often found in areas where there are inflammatory mechanisms, although digestive tract organs are also found[15]. Consequently, most likely when aspirin is in the human body, it will interact with the PTGS1 receptor.

ADORA2A is a type of adenosine receptor[16,17]. A2A receptors can be found throughout the body and are present on the surface of immunocompetence cells. These receptors have an important role in the inflammatory process (anti-inflammatory agent)[6]. Thus, when caffeine is in the human body, it is likely that caffeine will interact with the ADORA2A receptor. The results of the STITCH analysis in the form of a complex interaction of aspirin-caffeine molecules with target proteins in the human body, are displayed in the form of shells which have several lines connecting between one another. Proteins with colored boxes are proinflammatory agents in the human body (Figure 1).

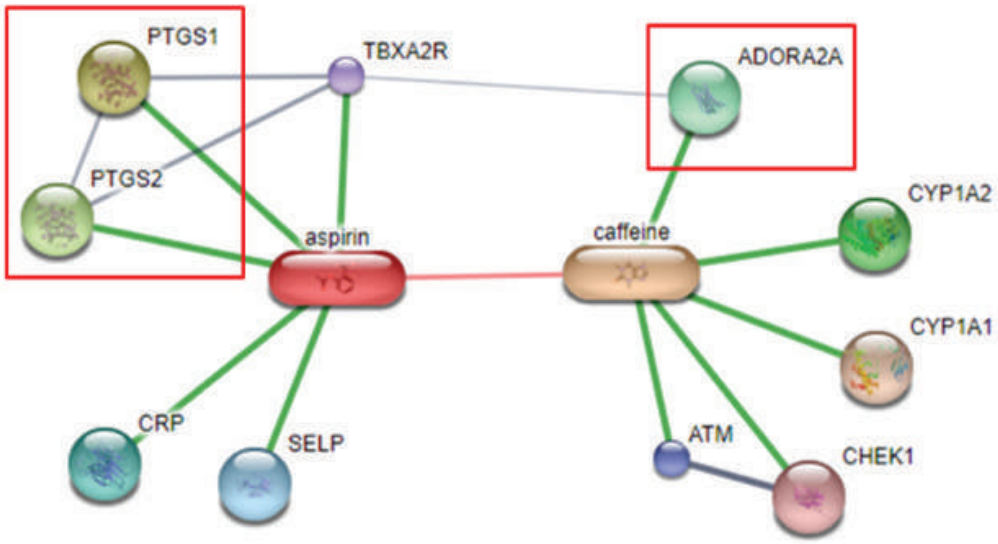


Figure 1. The molecular pathway of the aspirin-caffeine complex with proteins in the human body. The lines show the interactions of proteins (gray), ligands (green), and ligands (red).

Action Mode of Aspirin-Caffein as Antiinflammatory Agent

Aspirin and caffeine are identified in relation to drug-likeness to determine whether the compound can be categorized as a drug molecule that refers to the five Lipinski rules. We predicted that the aspirin compound has a molecular weight of 180.000 D, high lipophilicity 1.310, one hydrogen bond donor, four hydrogen bond acceptors, molar refractivity 44.710, whereas for caffeine it has a molecular weight of 194.000 D, high lipophilicity 0.061, 0 hydrogen bond donor, five hydrogen bond acceptor, and molar refractivity 49.100. Lipinski's rules explain that a compound can be categorized as a drug molecule because it fulfills at least two rules, here are the rules of molecular weight ≤500 D, high lipophilicity ≤5, hydrogen bond donors ≤5, hydrogen bond acceptors ≤10, and molar refractivity 40-130[9,10]. Thus, aspirin and caffeine are categorized as drugs because they meet all five Lipinski's rules.

Table 1. Drug-likeness test of aspirin and caffeine.

Compound	Lipinski Rules				
	MM (D)	LOGP	HBD	HBA	MR
Aspirin	180.000	1.310	1	4	44.710
Caffein	194.000	0.061	0	5	49.100

Note: MM: Molecular mass; LOGP: High lipophilicity; HBD: Hydrogen bond donors; HBA: Hydrogen bond acceptors; MR: Molar refractivity.

After obtaining Lipinski results, aspirin and caffeine compounds were re-identified, the prediction of the action or the complex interaction of the ligand when inside the human body via STITCH webserver. This can produce information related to the nature of the molecular interactions that occur in the query ligand with the target protein, usually in the form of information on binding, activation, expression, and inhibition[11,18]. Thus, it can be seen the type of action mode of the two query compounds, with the detected properties marked using the sign (√). There are five target proteins whose activity can be inhibited by the aspirin-caffeine ligand complex using direct binding, these proteins include PTGS1, PTGS2, CYPA2, ADORA2A, and ATM (Table 2). In this study, we revealed that the aspirin-caffeine ligand complex has an action mode to be an anti-inflammatory agent by binding directly to the target proteins such as PTGS1, PTGS2, and PTGS3.

Table 2. The interaction of aspirin-caffeine ligand complex in the target protein.

Compound	Target Protein	Binding	Inhibition
Aspirin-Caffeine	PTGS1	√	√
	PTGS2	√	√
	CYP1A2	√	√
	ADORA2A	√	√
	CRP	-	√
	SELP	-	√
	ATM	√	√
	TBXA2R	-	√
	CHEK1	-	√
	CYP1A1	√	-

The 3D Model of Pro-Inflammatory Agent

Based on the analysis of previous stages, it is known that there are three proteins that play an important role in the occurrence of inflammatory mechanisms in the human body, PTGS1, PTGS2, and ADORA2A. The structure of three target proteins is modeled on the SWISS-MODEL webserver using a homology modeling method that has the principle of modeling based on query sequence alignment with a template in the database[19]. If there are more templates available in the database, the resulting model is increasingly homologous, the modeling results also show a score, which must be above 20% in order to be called identical to the template[12,20]. After obtaining the protein structure of the model, the quality of the model is identified again, which refers to a minimum favored region score of 80%, so that the model can be said to be valid[13]. Before doing the modeling, the sequences of the three target proteins in the FASTA format that were successfully obtained from the Uniprot database can be seen in Figure 2.

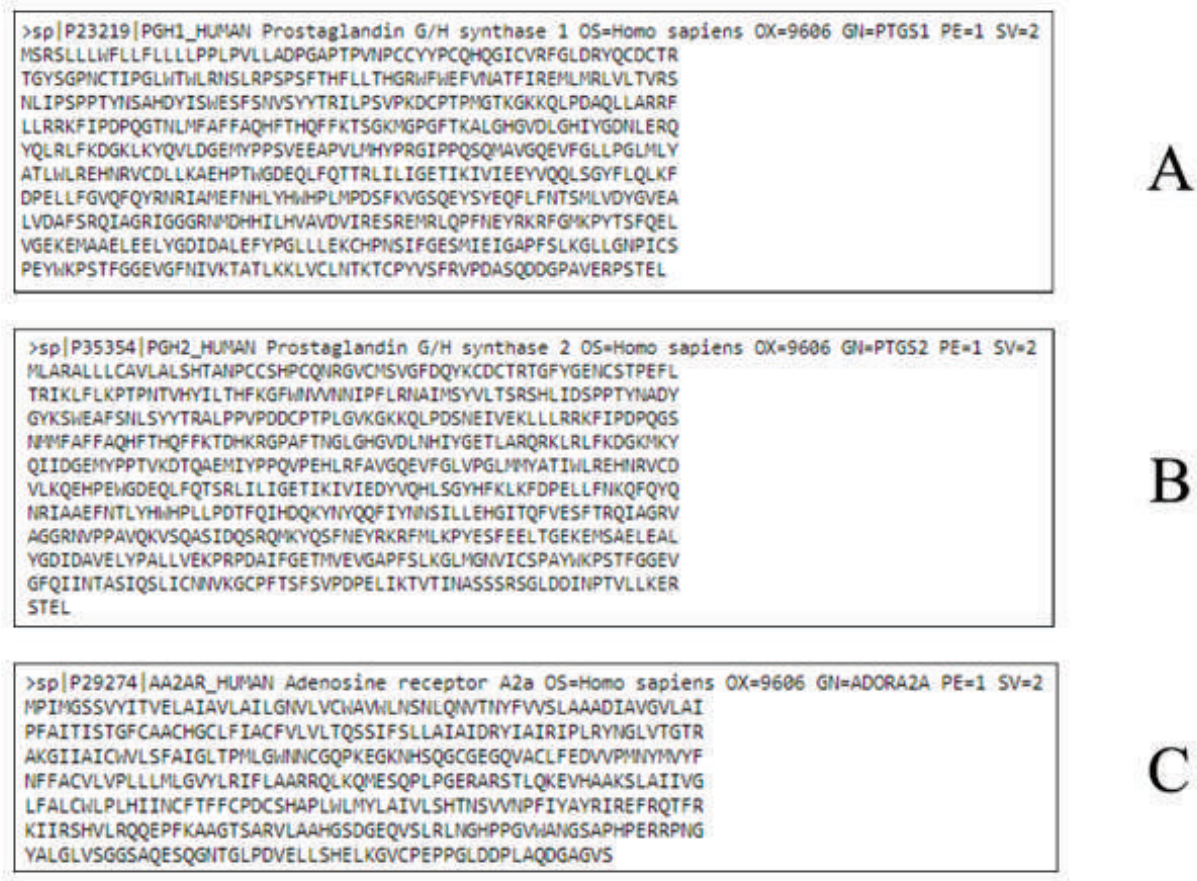


Figure 2. The target protein sequences were obtained from the Uniprot database in FASTA format. (A) PTGS1, (B) PTGS2, and (C) ADORA2A.

PTGS1 protein sequence sample (ID P23219) has a length of around 599-mer, 92.88% identified as similar to the template, and 96.64% favored region, PTGS2 protein sequence (ID P35354) has a length of around 604-mer, 100% identified as similar with a template, and 97.09% favored region, the ADORA2A protein sequence has a sequence length of around 412-mer, 98.42% identified as similar to the template, and 98.39% favored region. So the results of modeling the protein structure of the three target proteins produce a structure that is identical to the template because everything is similar to the template and the results of the quality test show that the favored region score of the three target proteins is above 80% so that the protein structure produced in this study is valid. The structure of the modeling results is displayed in the form of transparent surface, cartoon, and coloring selection based on the chain of constituent proteins in the PyMol8 software[8]. The target protein that has two chains namely A (green) and B (blue) are PTGS1 and PTGS2, while ADORA2A has only one chain, visualizing the results of modeling also displays the results of the Ramachandran plot (Figure 3).

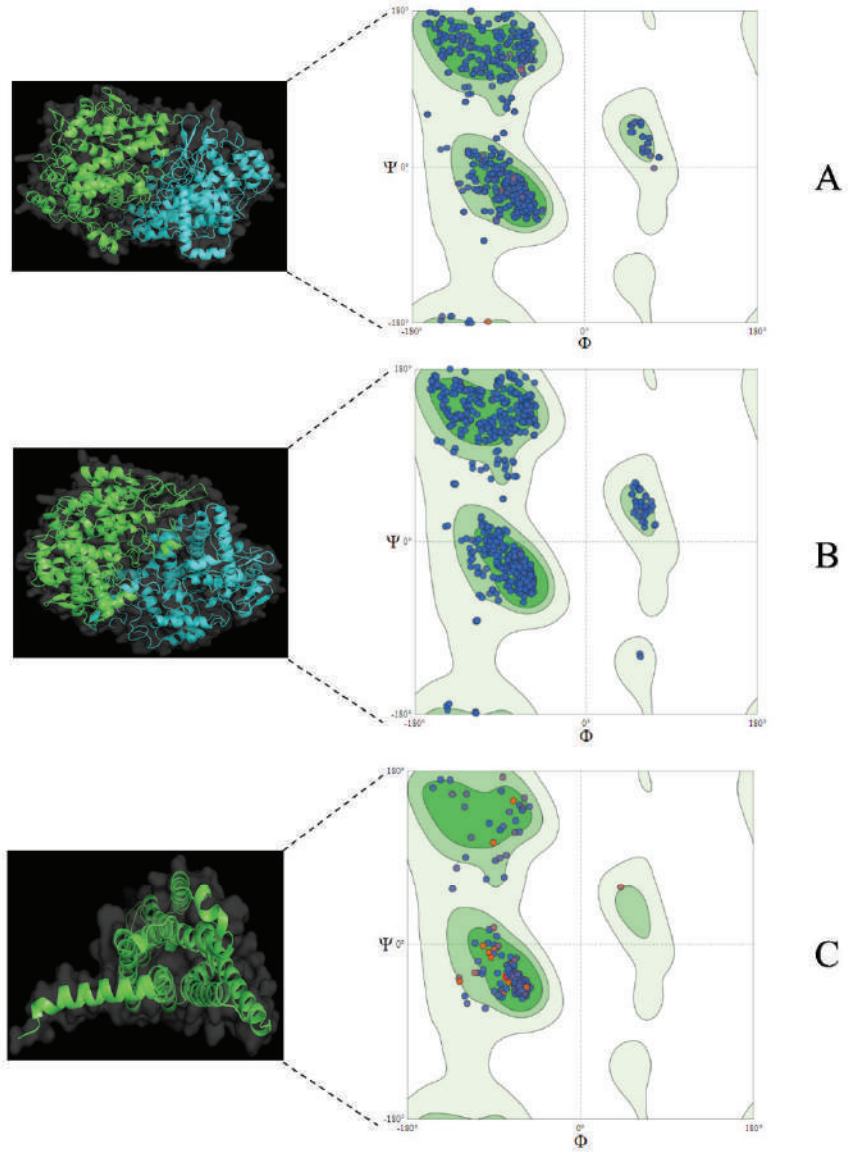


Figure 3. Results of protein modeling and Ramachandran plots. (A) PTGS1, (B) PTGS2, and (C) ADORA2A.

Conclusion

In sum, the aspirin-caffeine ligand complex contained in KB1 has the potential as an antiinflamatory agent in the human body via the molecular mechanism of binding and inhibiting the biological activity of proinflammatory proteins, i.e. PTGS1, PTGS2, and ADOR2A.

Conflict of Interest : The authors declare that they have no conflict of interest.

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Ethical Approval: Ethical approval is not required for this study.

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Literacy Level on Dental and Oral Health in Health Students (Non Dentisty)

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Abstract

Background: Dental and oral disease is one of the most common health problems in Indonesia with a prevalence of 57.6% and only 10.2% who receive treatment. The level of oral health literacy in health students needs to be known to carry out this role based on interprofessional education (IPE) in improving dental and oral health services to the community. **Purpose:** To determine the level of dental and oral health literacy in health students (fields other than dental health) in Surabaya. **Methods:** Using a research design is an observational analytic conducted on health (non-dentistry) students at universities in Surabaya. Using the questionnaire there were 7 questions answered on a scale of 0-4 (very not-very agree), categorization using a score of T. Where T> 50 is a good category, and T <50 is a poor category and then analyzed the data using the Chi-square method. **Results:** 51.32% of non health students were categorized as having dental health literacy levels. Based on the study program group, 58.2% of the students of the Faculty of Medicine and 47.54% of the students of health (non-dentistry) were categorized as good. Based on sex 44.12% male health students and 52.9% female health students were categorized as good. **Conclusion:** The level of dental and oral health literacy in health students (non dentistry) in Surabaya as a whole does not show a significant comparison with the level of literacy in medicine students better than health students (non dentistry).

Keywords*: Literacy level, dental and oral health Health major students, interprofessional education, public health services*

Introduction

Dental and oral health is one thing that needs attention[1]. Because, the oral cavity is a port of the entry that allows all kinds of diseases to infect the body. The Basic Health Research Data in 2018 recorded the percentage of dental and mouth problems at 57.6%, and only 10.2% were treated for these problems. Whereas the percentage of people who brush their teeth properly is only 2.8%. There are still many dental and oral health problems in Indonesia and a good strategy is needed to reduce this prevalence[2]. Not only dentists, health professionals also have a role in socializing education about oral health[3]. Collaboration or collaboration between professionals especially in the health sector is needed in building strong clinical teams and improving patient care and reducing the incidence of health problems.

Literacy is defined as the ability of readers to understand the contents of reading, knowledge in a particular field, and individual knowledge in survival. In addition, literacy also has a broader meaning, covering several processes namely reading, writing, speaking, listening, imagining, and seeing.

Oral health defined according to FDI includes the ability to speak, smile, smell, feel, touch, chew, swallow, and convey various feelings through facial expressions with confidence and without pain, discomfort, and craniofacial complex disease[4]. It shows physical and mental health and well-being[5], which reflect physiological, social, and psychological attributes that are essential for survival. Several risk factors are known to cause oral diseases such as smoking, alcohol consumption, and unhealthy eating patterns[6]. A person‘s

dental health is influenced by good habits such as brushing teeth, avoiding cigarettes, visiting the dentist[7,8], and using dental floss[9], which is a good example above[10].

According to WHO, health literacy includes knowledge and skills in the community that determine the ability of everyone to obtain knowledge and information in maintaining good health[11,12]. The purpose of oral health literacy is to improve the prevention of dental and oral diseases in the community[13].

Teamwork is needed in achieving adequate levels of oral health literacy. Each member takes an important role in order to create a synchronous relationship to achieve good literacy[13]. In addition, the level of poor oral health literacy can also cause caries, dental plaque, temporo mandibular joint disorder (TMJD), mucosal lesions, fluorosis, and other dental and oral problems[14].

The implementation of Interprofessional Education (IPE) for health students is a method which is quite relevant in the case of high dental and oral health problems with the aim of improving health services to the community. According to WHO, interprofessional education is an effective collaboration of students from two or more professions learning about, from and with each other to improve health status. Interprofessional collaboration is the process of professionals developing ways of practice by providing integrated and cohesive answers to the needs of clients / families / populations involving continuous interaction and knowledge sharing between professionals, organized to solve various problems of education and care while trying to optimize patient participation. Collaboration enhances predictors of intermediary quality such as knowledge transfer, information sharing, and improving decision making. Dental and oral interprofessional education methods begin by increasing collaboration among various members of the dental health team (such as dentists, dental health experts, dental therapists, dental technicians, and dental assistants) or expanding to cover various health professions (such as medical, nursing, pharmacy, and health related)[15]. This study aims to determine the level of literacy in health students (non dentistry) by comparing the students of the Faculty of Medicine and health students (non medicine).

Methods

This study was an observational analytic study conducted on a sample of 189 non-dentistry students in health, including 67 medical faculty students, 5 nursing students, 68 pharmacy students, 21 public health students, 9 nutrition science students, 7 students veterinary medicine, and 12 health students other than the above study programs at public and private universities in the city of Surabaya, namely Universitas Airlangga, Hang Tuah University, Surabaya Muhammadiyah University, Surabaya Pharmacy Academy, Nahdlatul Ulama University Surabaya, Surabaya University, Wijaya Kusuma University, and Widya Mandala University. Of the several study programs, grouped into 2 groups as variables to be observed, namely medical students (ie respondents who take medical studies) as many as 67 people and non-medical health students (ie respondents who take courses other than medicine) as many as 122 people.

This study uses a questionnaire instrument with 7 statements, namely: (1) I often use the information presented by the dentist to make decisions in dental and oral care; (2) I care about oral health; (3) I often take the time to take care of my teeth and mouth; (4) I often read brochures about oral health in clinics or public places; (5) I often apply instructions given by the dentist; (6) I often consider the advice of the dentist in making decisions in caring for my teeth and mouth; (7) I often ask someone to accompany me to the dentist. The statements on the questionnaire were then assessed by respondents based on their individual behavior with answers on a scale of 0 - 4 (strongly disagree - disagree - doubt doubt - agree - strongly agree), in this questionnaire there were no right and wrong answers. Then the sample answers will be scored with a minimum score of 0 and a maximum of 28. The level of literacy about dental and oral health will be categorized into 2 groups: good and not good.

Determination of the category of dental and oral health literacy level uses a T score. Where T> 50 is a good category, and T <50 is a poor category. This study uses Chi-square data analysis to determine whether there are differences in the level of dental health literacy in non-dentistry health students, based on the division of medical students and non-medical health students.

Result

Table 1. Comparison of dental and oral health literacy levels in (non-dentistry) health students

Variable	n	Good litercacy level in Dental Health (%)	Poor literacy level in dental health (%)	p value	Contingency Coefficient	PR
Field of Study						
Medicine	67	39 (58,2%)	28 (41,8%)	0,160	0,160	1,224
Non-Medicine	122	58 (47,54%)	64 (52,46%)			
Gender						
Male	34	15 (44,12%)	19 (55,88%)	0,353	0,353	0,834
Female	155	82 (52,9%)	73 (47,1%)			
Class of Study						
1	24	15 (62,5%)	9 (37,5%)			
3	33	15 (45,45%)	18 (54,55%)	0,434	0,434	1,357 0,996 0,834
5	57	26 (45,61%)	31 (54,39%)			
7	75	41 (54,67%)	34 (45,33%)			

In women, the level of literacy in oral health is better than in men, with a literacy rate in men 0.834 times that of women. The percentage level of dental and oral health literacy in women is 52.9%, while in men 44.12%. However, the comparison of dental and oral health literacy levels in women and men has no significant difference. This is because the p value owned is 0.353 which is smaller than the α standard of 0.05.

The level of dental and oral health literacy in semester 1 students has the highest rate with a percentage of 62.5%, followed by students in semester 7 with a percentage of 54.67%. 5th semester students are ranked third with a percentage of 45.61%, while the percentage of 3rd semester students is 45.45%. The percentage comparison does not have a significant difference because it has a p value of 0.434 which is less than the α standard of 0.05.

Based on calculations, contingency coefficients are obtained based on study programs (medical and non-medical) of 0.160; based on sex by 0,353; based on the semester level traveled by 0.434. This result is greater than 0.05. This shows that there is no significant relationship between the study program, gender, and the level of semester that is lived with the level of oral health literacy.

Discussion

Dental and oral health literacy within the scope of health students needs to be considered to improve the degree of oral health in the community. Health literacy itself can be defined as the process of obtaining, processing, and understanding basic health information and services that are important to be implemented in the environment. Therefore, oral and oral health literacy can be interpreted as the process of obtaining, processing, and understanding basic dental and oral health information that is important[16].

The level of dental and oral health literacy in medical students and non-medical health students found no significant differences. This can be caused by the two groups having similarities, namely as health students who have insights on basic health even though the two groups study different disciplines. The insignificant comparison can also be influenced by the habits of each student. Medical students and non-medical health students have good habits related to oral health[16,17]. The level of dental and oral health literacy in medical students is better than non-medical health students. This is because medical students get better education about dental awareness. Dental awareness can be linked to oral health literacy because awareness of dental health can trigger an increase in someone‘s literacy on oral health[18].

In male and female students, literacy levels were obtained with insignificant differences. Although the data scores obtained by female students tend to be higher than male students. This is also supported by the results of a study which states that the results of oral hygiene preventive literacy levels in men and women are not significant differences even though 60% of female correspondents have higher literacy rates than men[19]. In other studies, it shows that the level of women‘s health literacy tends to be higher than that of men. This is due to the tendency of women to be more susceptible to disease so women tend to have the opportunity to know the basics of health concepts compared to men[20].

In the results of the study the relationship between dental and oral health literacy and class of study (semester) of students shows that semester 1 students have the highest grades, these results are similar to the results of studies that have been conducted previously using cross sectional methods to identify the level of health literacy in students faculty of medicine at the University of South Bohemia[21]. According to a study in 2018 in Jakarta, the oral health literacy rate decreases with age. This is influenced by several factors, such as mental health, reading ability, health status, and vision[22]. From the results of the study, 7th semester students have the second highest grades, then followed by 5th semester and 3rd semester students. There is no certain reason why this can occur so further research is still needed.

The study semester taken in this study also did not have a significant relationship with the level of oral health literacy. The same previous study, using the Health Literacy Questionnaire (HLQ) in health and non-health faculty students at the University of Indonesia showed similar results[23]. Further studies are needed to find out the specific reasons for the results of this study.

Conclusion

The level of dental and oral health literacy in non-dentistry health students in Surabaya as a whole did not show a significant comparison. Health student literacy level is needed because health students are expected to be able to help and play a role in improving the level of dental and oral health in the community as an implementation of interprofessional education.

Conflicts of Interest: There are no conflicts of interest.

Source of Funding: Self-Funding

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Assessments Self –Care of Sickle- Cell Patients at Baghdad Teaching Hospitals

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Abstract

Sickle-cell disease (**SCD**)) is a group of genetic disorders that is characterized by the development of abnormal hemoglobin (Hgb S), abnormal red blood cells, and the resultant complications include vaso-occlusive pain crises, acute chest syndrome, acute splenic sequestration& pneumococcal disease.

Objectives of the study: To assess the self efficacy of patients with sickle – cell anemia at blood disease ward in Baghdad Teaching hospital and to find out the association of some demographical characteristics of the sample such as (age , gender, level of education & years of disease affected) with self efficacy. **Methodology**: A descriptive design of study was carried out, for the period 1st Oct, 2017 – 15th of May, 2018, to identify the self – efficacy of sickle cell patients, A 40 patients was participate at the study. The setting was Baghdad Teaching Hospital, Ghazi –Al Hariri Teaching Hospital IBN-Al Belady teaching hospital. **Results:** Most of study participants are females (60.0%) , More than a third are within the age group of (22-25) years-old (35.0%),they were at high school graduate (27.0%), followed by those who are middle school graduates (25.0%), those who are both elementary school graduate and hold a bachelor's degree (15.0%), and those who are unable to read and write (7.5%). More than a half are married (55.0%), followed by those who are married (30.0%), those who are divorced (12.5%). Less than a third are students (n = 12; 30.0%), followed by those who are self-employed (27.5%), those who are out of work (20.0%), those who are officers (12.5%), and those who are housewives .Less than a half reported that their father is the affected person (45.0%), followed by mothers (27.5%), relatives (15.0%), and both parents (12.5%). Regarding health status, the health of the majority are fair (80.0%), followed by those who have poor health (n = 6; 15.0%), and those who have good health (5.0%). More than two-fifth reported that they are hospitalized (4-6) times per year. All patients reported that they have been treated for more than 10 years (100.0%). **Conclusions:** findings of study shows that ,male participants have a better self-efficacy of activity of daily living than female participants of activity of daily living, level of education, age, family ranking & financial social status.

Key words: *Assessments, self -care, Sickle- cell, Patients*

Introduction

Sickle cell disease (SCD) is an inherited blood disorder affecting predominantly persons of African descent [(1).] Diagnosis of SCD is typically made at birth during newborn screening. Disease management focuses on pain, hydration, and preventing infections and other complications that result in vaso-occlusive crises [(2).]Sickle cell disease (SCD) represents a group of serious inherited blood disorders associated with acute and chronic morbidity, recurrent unpredictable and unrelenting episodes of pain, increased risk of infection, stroke, organ damage and other debilitating complications [(3).] The Sickling of the cells causes impaired blood circulation which results in pain. This is the most common complication of sickle cell disease. It can begin as early as infancy and can happen unpredictably throughout life [(4).]Hematopoietic stem cell transplantation has shown promising outcomes, particularly when donors are human leukocyte

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antigen (HLA) matched siblings; however, older age (transplantation has optimal outcomes during childhood) and advanced disease are associated with poor transplant outcomes [5].

Methodology

A descriptive analytic design of study was carried out, for the period 1st Oct, 2017 – 15th of May, 2018, to assess the self – efficacy(care) of sickle cell patients, A 40 patients was participate at the study. The setting was Baghdad Teaching Hospital, Ghazi –Al Hariri Teaching Hospital IBN-Al beldy teaching hospital. Administrative and ethical agreements in order to conduct the study, the researcher first must get the approval of the council of Nursing College for the study and submitted a detailed description including the objectives of the study to the Ministry of Health in Iraq (Department of Planning / Health research section) and the obtain an official permission to carry out the study , The consent form facilitated having access to the hospital facilities, as well as meeting, once written permission was giving by the patients for their participation in the study. The study instrument was composed of two parts, the first part was socio- demographical information& clinical characteristics consist of 15 item which include gender, age, level of education, occupation, health Status & other items. The second part was the scale to measure the self- efficacy with some modification to be adapted the sickle- cell Iraq- patients & it was consisted of 31 items.

The self –efficacy scale was rated & scored by assigning each sickle- cell adult patients response an ordinal value, three levels options (sure, some sure never). The validity of the questionnaire was determined through a panel of (15) expert. The reliability of the present study instrument was determined by Test-Retest, the result was coefficients for the patients (r=86). Data were analyzed through the application of descriptive & inferential data analysis approach by using SPSS version 20.

Results

Table(1) :Distribution of Participants' Self-care responses.

List	Items	Always	Sometimes	Never	Mean (SD)	Ass.
1	How sure are you that you can do something to cut down on most of the pain?	(7.5%)	(82.5%)	(10.0)	1.98 ± .42	Fair
2	How sure are you that you can keep doing most of the things you do day-to-day?	(37.5%)	(57.5%)	(5.0%)	2.32 ± .57	Fair
3	How sure are you that you can keep sickle cell disease pain from interfering with your sleep	(22.5%)	(70.0%)	(7.5%)	2.15 ± .53	Fair
4	How sure are you that you can reduce your sickle cell disease pain by using methods other than taking medication	(15.0%)	(72.5%)	(12.5%)	2.02 ± .53	Fair
5	How sure are you that you can control how often or when you get tired?	(17.5%)	(80.0%)	(2.5%)	2.15 ± .42	Fair
6	How sure are you that you can do something to help yourself feel better if you are feeling sad or blue?	(32.5%)	(60.0%)	(7.5%)	2.25 ± .58	Fair
7	As compared with other people with sickle cell disease, how sure are you that you can manage your life from day-to-day?	(52.5%)	(47.5%)	(0.0%)	2.52 ± .50	Good
8	How sure are you that you can manage your sickle cell disease symptoms so that you can do the things you enjoy doing?	(27.5%)	(70.0%)	(2.5%)	2.25 ± .49	Fair
9	How sure are you that you can deal with the frustration of having sickle cell disease?	(20.0%)	(72.5%)	(7.5%)	2.12 ± .51	Fair
10	How sure are you that you can marry and make a family?	(67.5%)	(32.5%)	(0.0%)	2.68 ± .47	Good
11	How sure are you that you can get a job?	(27.5%)	(67.5%)	(5.0%)	2.22 ± .53	Fair
12	How sure are you that you can be in high places?	(12.5%)	(32.5%)	(55.0%)	1.58 ± .71	Poor

Cont... **Table(1) :Distribution of Participants' Self-care responses.**

13	How sure are you that you can keep a balanced nutrition system?	(47.5%)	(40.0%)	(12.5%)	2.35 ± .70	Good
14	How sure are you that you can be away of dehydration?	(82.5%)	(17.5%)	(0.0%)	2.82 ± .38	Good
15	How sure are you that you can keep yourself calm (not feel with cold)?	(87.5%)	(12.5%)	(0.0%)	2.88 ± .33	Good
16	How sure are you that you Can walk?	(100.0%)	(0.0%)	(0.0%)	3.00 ± .00	Good
17	How sure are you that you can run?	(27.5%)	(67.5%)	(5.0%)	2.22 ± .53	Fair
18	How sure are you that you can do sports or exercise?	(15.%)	(60.0%)	(25.0%)	1.90 ± .63	Fair
19	How sure are you that you can left heavy things?	(7.5%)	(22.5%)	(70.%)	1.38 ± .62	Poor
20	How sure are you that you can take bath or shower?	38 (95.0%)	(5.0%)	(0.0%)	2.95 ± .22	Good
21	How sure are you that you can do chores around house?	(37.5%)	(60.0%)	(2.5%)	2.35 ±.53	Good
22	How sure are you that you can doing things other peers do?	(32.5%)	(67.5%)	(0.0%)	2.32 ± .47	Fair
23	How sure are you that you Have low energy?	(7.5%)	(80.%)	(12.5%)	1.95 ± .45	Fair
24	How sure are you that you Feel afraid or scared?	(0.0%)	(17.5%)	(82.5%)	1.18 ± .38	Poor
25	How sure are you that you Feel sad or blue?	(17.5%)	(52.5%)	(30.0%)	1.88 ± .68	Fair
26	How sure are you that you Feel angry?	(75.0%)	(22.5%)	(2.5%)	2.72 ± .50	Good
27	How sure are you that you have Trouble sleeping?	(5.0%)	(72.5%)	(22. 5%)	1.82 ± .50	Fair
28	How sure are you that you Worry about what will happen?	(82.5%)	(10.0%)	(7.5%)	2.75 ± .58	Good
29	How sure are you that you Hard to concentrate?	(40.0%)	(50.0)	(10.0%)	2.30 ± .64	Fair
30	How sure are you that you Forget things?	(13.5%)	(55.0%)	(32.5%)	1.80 ± .64	Fair
31	How sure are you that you Hard to keep up when play with others?	(22.5%)	(62.5%)	(15.0%)	2.08 ± .61	Fair
					68.90 ± 7.96	

Cut-off-point: Poor = 1-1.66, Fair = 1.67-2.33, Good = 2.34-3.00

Table (1) shown that most participants experience very poor self-care in the items 24, 19, 12 ,30 ,27 ,25 ,18 ,23 ,1 ,4 (Mean [SD] = 1.18 ± .38, 1.38 ± .62, 1.58 ± .71, 1.80 ± .64, 1.82 ± .50, 1.88 ± .68, 1.90 ± .63, 1.95 ± .45, 1.98 ± .42, 2.02 ± .53) respectively.

Table (2) : Association of sample self-care response & gender groups.

Ranks					Mann-Whitney U	Asymp. Sig.
	Gender	N	Mean Rank	Sum of Ranks		
Self-care	Male	16	32.47	519.50	.500	.000
	Female	24	12.52	300.50		
	Total	40				

Table (3): Association of sample response of self-care among age groups.

Ranks				Chi-Square	Df	Asymp. Sig.
	Age	N	Mean Rank			
Self-care	18-21 years	8	36.50	33.016	3	.000
	22-25 years	14	24.32			
	26-29 years	12	13.75			
	30-33 years	6	3.75			
	Total	40				

Table (4) : Difference in self-care among the occupation groups .

Ranks				Chi-Square	df	Exact. Sig.
	Occupation	N	Mean Rank			
Self-care	student	12	34.46	34.470	4	.000
	officer	5	26.10			
	gainer	11	15.82			
	jobless	8	11.50			
	housewife	4	2.50			
	Total	40				

Table (5) : Difference in self-care among the family members groups .

Ranks				Chi-Square	df	Exact. Sig.
	Family Members	N	Mean Rank			
Self-care	three	4	37.50	34.312	5	.000
	four	11	31.36			
	five	14	17.54			
	six	7	9.93			
	seven	3	3.00			
	eight	1	1.00			
	Total	40				

Discussion

The result of the study demonstrated that majority of the sample were female at age group of (22-25) years-old, level of education graduated from elementary school, self-employee,

More than two-fifth reported that they are hospitalized (4-6) times per year (42.5%), followed by those who are hospitalized (1-3) times (35.0%), those who are hospitalized (4-6) times (17.5%), and those who are hospitalized (10-12) times (5.0%). All patients reported that they have been treated for more than 10 years (100.0%). The majority reported that they are not alcoholics 90.0%), most are non-smokers (n = 27; 67.5%), and most have fair self-care (67.5%).

Sickle cell disease (SCD) represents a group of serious inherited blood disorders associated with acute and chronic morbidity, recurrent unpredictable and unrelenting episodes of pain, increased risk of infection, stroke, organ damage and other debilitating complications [3,4] .

Distribution of sickle- cell **self- care participant responses,** participants experience very poor self-efficacy in the items 24, 19, 12 ,30 ,27 ,25 ,18 ,23 ,1 ,4 (Mean [SD] = 1.18 ± .38, 1.38 ± .62, 1.58 ± .71, 1.80 ± .64, 1.82 ± .50, 1.88 ± .68, 1.90 ± .63, 1.95 ± .45, 1.98 ± .42, 2.02 ± .53) respectively, moreover,70%of the study sample presents fair for item how sure you that you can reduce pain by using methods other than taking medication, Adults with Sickle Cell Disease (SCD) are a significant population to study when considering self-medication behaviors, as they utilize multiple prescription drugs for management of symptoms related to their disease. Adults living with SCD will demonstrate adverse self-medication practices as recorded on the Actual Meds™ Medication Management System via use of Apple iPad® digital mobile device during a single interview Beyers, Courtney Marie.

Table(3) presented that There is a statistically significant difference between Participants who were within the age group of (18-21) years-old have a better self-care than those who were within the age group of (22-25) years-old, participants' self-efficacy of activity of daily living among age groups (Chi-square = 33.016, df = 3, p-value = .000).

Life expectancy for persons with SCD has recently increased from age fourteen in 1973 to the

mid to late forties in 2004, transforming SCD into a long-term chronic illness. This chronic disorder may result in a lifetime of pain experiences and frequent hospitalizations [7] .There is a statistically significant difference in participants' ,who were unable to read and write have a better self-care than those who were elementary school graduates, those who were middle school graduates, those who were high school graduates, those who hold a bachelor's degree, and those who have other educational qualification. self-efficacy of activity of daily living among the level of education groups (Chi-square = 36.481, df = 5, p-value = .000). Finding showed the Participants who were students have a better self-efficacy than those who were officers, those who were gainers, those who were jobless,. and those who were housewives. There is a statistically significant difference in participants' self-efficacy of activity of daily living among the occupation groups (Chi-square = 34.470 ,df = 4, p-value = .000).Sickle cell disease (SCD) represents a group of serious inherited blood disorders associated with acute and chronic morbidity, recurrent unpredictable and unrelenting episodes ofpain, increased risk of infection, stroke, organ damage and other debilitating complications [3,4].Participants who were 4-6 times have a better self-efficacy than those who were 1-3 times, those who were 7-9 times, and those who were 10-12 times. There is a statistically significant difference in participants' self-efficacy of activity of daily living among the Hospitalization during a Year groups (Chi-square = 33.428 ,df = 3, p-value = .000).In the U.S., this disease primarily affects African Americans. Within this group, there is an incidence of one in 500 births and the sickle cell trait occurs in about one in 12. Sickle cell disease is anautosomal recessive disorder, and males and females are affected equally (Pack-Mabien& Haynes, 2009)[9]. Diagnosis of SCD is typically made at birth during newborn screening. Disease management focuses on pain, hydration, and preventing infections and other complications the result in vaso-occlusive crises [2].

Conclusion

The study concluded that participant of the research experience very poor self- care at most of the items, while there was statistical significant association between gender, which present Male participants have a better self-care of activity of daily living than female participants of activity of daily living, level of education, age, family ranking &financial social status. The researcher recommended to initiated a specialized center for providing medication, care, educational programs, fellow up & financial for sickle cell patients.

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Adverse Effects of Mercury Exposure in DDW Strain Mice during Organogenesis

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Abstract

Mercury (Hg) was known as a teratogenic which is distributed in tissue. This study aims to determine the retention and embryotoxicity of Hg-exposed pregnant mice. Thirty female mice was treated with $HgCl_2$ (mercuric chloride). $HgCl_2$ (5 and 6 kg/mg BW) was inducted in pregnant mice at 9 and 11 gestational days. Hg levels were measured in hair, uterus, liver, kidney, brain, blood, placenta, visceral fetus, and fetus brain on the 18th day of gestation using the atomic absorption spectrophotometry (AAS) method. Embryotoxicity test on the fetus was carried out after the surgery took place. Scales and calipers are used to calculate fetal weight and crown rump length. Statistical tests were analyzed using the SPSS 21 program. The results showed that the liver, kidney, brain, visceral fetal, and fetal brain were significantly increased ($P < 0.05$) in the treatment group at pregnant mice. Hg also produced a significant difference ($P < 0.05$) on the decrease in live fetuses, fetuses, body weight, and crown rump length and an increase in resorbed fetuses. Hg accumulation in the body apparently can cause adverse effects in pregnant mice.

Key words: *Fetus, Mercury, Mice, Organogenesis, Retention, Toxicity.*

Introduction

Hg is a very toxic heavy metal whose very high quantity in the environment. Humans can be exposed to Hg from dental amalgams, drug treatments, and food sources[1,2]. The effects of Hg toxicity in humans depend on its composition, transportation pathway, and the length of Hg accumulated in the body. In the blood, Hg will undergo an oxidation process by catalase enzymes so that it turns into Hg^{2+} ions, then through the blood circulation will be accumulated in the liver and kidneys[3]. Furthermore, microorganisms also support the conversion of Hg to Hg^{2+} and MeHg[4]. Hg has a limited ability to penetrate biological membranes, but it can pass the placenta after dissolving in the blood and will be distributed to the body. Heavy metal Hg enters the placenta along with food that is carried through blood circulation and then enters the fetus so that it can cause fetal disability[2]. Hg has a long retention time in the body so it requires a long process to eliminate it. That is because this heavy metal has a half-life of about 70 days in the body[5].

One of the developmental processes is organogenesis which in this process occurs the formation of organs in the fetus. The developmental process in mice begins at 9 days of gestation with the appearance of the forelimb bud and its differentiation continues until 18 days of gestation[6]. Based on this description, Hg can be distributed into tissues that are very vulnerable and sensitive so that it is teratogenic. Since the environment has been exposed to toxic metals such as Hg, knowledge of its adverse effects on organs is the most important global health problem. This study aims to determine the retention and embryotoxicity effect in fetuses after Hg exposure.

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Materials and Methods

The study was conducted at the Animal Laboratory of the Department of Biology, Faculty of Science and Technology, Airlangga University. The experimental animal used in this study was female mice (*Mus musculus*) DDW strain with 6-8 weeks of age and bodyweight around 25-30 g. Thirty female mice were divided randomly into six groups, with a total of 5 mice for each group. Group I (control group) was pregnant mice with aquades at 9 days; Group II (control group) was pregnant mice with aquades at 11 days; Group III (treatment group) was pregnant mice with $HgCl_2$ (5 mg/kg BW; i.p) at 9 days of gestation; Group IV (treatment group) was pregnant mice with $HgCl_2$ (6 mg/kg BW; i.p) at 9 days gestation; Group V (treatment group) was pregnant mice with $HgCl_2$ (5 mg/kg BW; i.p) at 11 days of pregnancy; Group VI (treatment group) was pregnant mice with $HgCl_2$ (6 mg/kg BW; i.p) at gestational age 11 days. On the 18^{th} day of pregnancy, all of the groups were anesthetized with chloroform and then dissected to get the organs and fetuses. The sample that had been obtained was then inserted into a tube that contains a neutral buffer of 10% formalin solution for analysis. Furthermore, embryotoxicity testing is done by observing the condition of the fetus after surgery such as the percentage of the number of implants, number of live fetuses, number of dead fetuses, and the number of fetuses absorbed based on differences in fetal movement, shape, and color. Meanwhile, the growth retardation test uses a digital balance to measure fetal body weight and calipers to measure the length of crown rump fetuses.

Measurement of Hg levels in samples using the AAS method based on Keil et al. (2011) method[7]. Firstly, the use of AAS is to prepare samples at a temperature of 300-400°C for 1 hour, then the sample was mashed using a mortar. Standard solutions were prepared and then put into a 50 mL volumetric flask to be diluted. The Hg standard solution was analyzed by AAS. A total sample was added with HNO_3 and H_2SO_4 then heated at 300°C for 3 hours using reflux. H_2O_2 was added until the clear solution. Then cooled and filtered using Whatman paper. The sample that had been destroyed was put in a bottle. Samples were analyzed by AAS type flame with a wavelength of 254 nm, so the levels of the samples were obtained. Existing data were analyzed using SPSS 21. It will proceed using the Two Way Anova test. A Duncan test was performed to determine significant differences between each group ($P < 0.05$).

Results

Hg concentration in pregnant mice could be seen in Table 1. Based on statistical tests, there were significant differences ($P < 0.05$) between the control group and treatment group in the liver, kidney, brain, fetal brain, and visceral fetal. However, the placenta, uterine, and hair showed insignificant differences ($P > 0.05$) in the treatment group. The embryotoxicity test showed a significant decrease ($P < 0.05$) in the treatment group compared to the control group in the percentage of live fetuses. Conversely, a significant increase ($P < 0.05$) occurred in the percentage of resorb fetuses in the treatment group compared to the control group. Meanwhile the percentage of implantance and dead fetus did not show any significance ($P > 0.05$) between the treatment group and the control group. The results of the overall embryotoxicity test in pregnant mice can be seen in Table 2. The results of the embryotoxicity test also showed a visual difference in the fetal shape of the mice. Fetal resorb is evident with the loss of limbs from the fetus of mice, even there are also fetal mice that do not have limbs after being dissected from their mothers. Results of visual embryotoxicity test can be seen in Figure 1. Growth retardation test showed a significant difference ($P < 0.05$) in the treatment group where Hg caused a significant decrease in fetuses body weight. In addition, Hg also caused a significant reduction in crown rump length ($P < 0.05$) in the control group. The results of the growth retardation test can be seen in Table 3.

Table-1: Toxicities of Hg concentration from various organs in pregnant mice

Organs	Gestational days	Hg Concentration (ppm)		
		Control	5 mg/kg BW	6 mg/kg BW
Placenta	9	0.0008±0.0001a	0.0063±0.0039a	0.0028±0.0010a
	11	0.0007±0.0001a	0.0022±0.0010a	0.0017±0.0010a
Uterus	9	0.0003±0.0001a	0.0021±0.0009a	0.0023±0.0014 a
	11	0.0002±0.0002a	0.0032±0.0024a	0.0019±0.0009a
Hair	9	0.0011± 0.0001a	0.0024±0.0015a	0.0048±0.0036a
	11	0.0006±0.0002a	0.0044±0.0023a	0.0089±0.0066a
Liver	9	0.0007±0.0002a	0.0020±0.001b	0.0022±0.0002b
	11	0.0002±0.0001a	0.0019±0.0007b	0.0020±0.0001b
Kidney	9	0.0004± 0.0001a	0.0020±0.001b	0.0024±0.0006b
	11	0.0003± 0.0001a	0.0026±0.0003b	0.0029±0.0004b
Brain	9	0.0003±0.0001a	0.0016±0.0005b	0.0022± 0.0008b
	11	0.0003±0.0001a	0.0023±0.0004 b	0.0026±0.0006b
Fetal Visceral	9	0.0003± 0.0001a	0.0021±0.0009b	0.0015±0.0004b
	11	0.0001± 0.0001a	0.0024±0.0008 b	0.0022±0.0005b
Fetal Brain	9	0.0002±0.0001a	0.0010±0.0001b	0.0015±0.0006b
	11	0.0001±0.0001a	0.0020±0.0005b	0.0019±0.0002b

Means bearing different superscripts (a,b,c) in a column within the period indicate significant difference in values ($P < 0.05$).

Table-2: Embryotoxicity of mice exposed to Hg at 9 and 11 days of gestation

Parameters	Gestational days	Hg Concentration		
		Control	5 mg/kg BW	6 mg/kg BW
Fetuses implantations (%)	9	9.20±0.58a	8.80±2.08a	10.20±0.66a
	11	9.20±0.58a	10.80±1.02a	11.40±0.93a
Live fetuses (%)	9	100.00±0.00a	54.00±22.27b	20.00±20.00c
	11	100.00±0.00a	90.10±3.00b	21.76±16.60c
Dead fetuses (%)	9	0.00±0.00a	4.00±4.00a	0.00±0.00a
	11	0.00±0.00a	6.54±2.72a	26.92±11.25a
Resorbed fetuses (%)	9	0.00±0.00a	42.00±23.75a	80.00±20.00b
	11	0.00±0.00a	3.36±2.07a	51.30±14.96b

Means bearing different superscripts (a,b) in a column within the period indicate significant difference in values ($P < 0.05$).



Figure-1. Embryotoxic effects on mice fetuses exposed to Hg dose of 6 mg/kg BW on gestational days 11. Dead fetuses (A, F); live fetuses (B, C, D, E); resorbed fetuses (G, H, I, J, K, L, M).

Table-3: The impact of developing fetal mice after Hg exposure.

Parameters	Gestational days	Hg Concentration		
		Control	5 mg/kg BW	6 mg/kg BW
Fetuses body weight (g)	9	1.41±0.05a	1.19±0.11b	0.93±0.01b
	11	1.41±0.05a	1.14±0.11b	0.87±0.19b
Crown rump length (cm)	9	2.42±0.04a	2.19±0.04b	1.97±0.00a,b
	11	2.42±0.04a	2.14±0.09b	1.97±0.17a,b

Means bearing different superscripts (a,b) in a column within the period indicate significant difference in values ($P < 0.05$).

Discussion

Placenta did not show a significant increase in the treatment group. This is due to the different response in Hg exposure to intrauterine which is also caused by the role of sex steroids in immune function[8]. However, uterine Hg levels can be transferred to the placenta so they can have a direct impact on the fetus[9]. The developing fetus has a high sensitivity to heavy metal toxicity. Fetal visceral organs showed significant differences in all treatment groups. This indicates the existence of a protective system in the fetus against Hg. According to Yoshida (2002), Hg that successfully crosses the placenta will be oxidized by fetal liver and bound by fetal hepatic methallotionein[10]. It shows that this protein plays an important role in protecting the fetus from Hg toxicity. Meanwhile, Oliveira et al. (2015) state that Hg accumulation can also occur in fetal brain, especially in brain motor nerve cells[11]. In addition, Hg can also accumulate in the hippocampus and fetal cerebellum[12]. Thus, the development of fetal brain is considered to have a high sensitivity to Hg[13]. However, the concentration of Hg in the parent brain is still higher than in the fetal brain. The high concentration of Hg in the brain is caused by the presence of inorganic Hg such as MeHg which can penetrate the brain barrier through diffusion and active transport[14,15,16]. The liver showed a significant difference in the treatment group. Sharma et al. (2005) revealed that exposure to Hg caused a significant increase in lipid peroxidation, ALT, and AST levels[17]. The main toxicity in Hg is related to a number of interactions in various cell metabolic processes such as in the formation of thiol complexes which can increase oxidative stress[18]. Hg^{2+} and MeHg will form covalent bonds with GSH and cysteine after they are absorbed in the cell. GSH serves as the mainline of cellular defense against Hg. However, GSH may experience a decrease in function after fighting Hg toxicity. It has been proven that Hg can reduce the antioxidant system and produce oxidative damage through the emergence of H_2O_2 and can trigger lipid peroxidation[19]. Generally, liver cells have higher GSH levels than kidney cells so that the Hg levels in the liver show lower concentrations compared to the kidneys[20,21]. In the kidney, proximal tubules are the main target tissue of Hg. Meanwhile, the half-life of Hg concentration in the kidney is around 60 days[22]. Meanwhile, the concentration of inorganic Hg has always increased since the first day until the 16th day of exposure to the Hg[23].

Significant reduction in the percentage of live fetuses indicates a high embryotoxic exposure of Hg. Moreover, the existence of fetus live after exposure because it has a higher body resistance to outside material exposure compared to other fetuses and also can begin recovery process by forming new cells. Meanwhile, Hg can cause severe damage that makes fetuses do not have time to recover[21]. Fetuses that are exposed to Hg can significantly increase the percentage of fetal resorption. This condition occurs because of fetus inability to carry out repairing damaged cells process into normal cells. In addition, fetal death that occurs can trigger the mother's body to reabsorb the dead fetus. Fetal resorption occurs in fetuses that have the lowest immune system against teratogenic substances compared to other fetuses in one parent[22]. In this study, measurements of fetal body weight and length of crown rump could not be measured in fetuses undergoing resorption because their death occurs before the organogenesis process ends where the organs of the body have not formed completely. Hg exposure caused a significant decrease in fetal body weight compared to controls. This induction can cause obstacles to fetal development by reducing the length of the crown rump. In addition, a fetus that succeeds after being exposed to teratogenic material still compete with other fetuses to obtain nutrients for its body. The mother can not provide optimal nutrition so that it causes the fetus to be smaller in size. Weight loss and fetal crown rump length are the mildest effects of the effects of teratogenic substances[24].

Fetal development and growth begin with an increase in cell number followed by the differentiation of various organ systems. The development is also influenced by several factors such as nutrition from the parent and genetics. Furthermore, fetal nutrition comes from the mother through the placenta[21]. Hong et al. (2012) states that the presence of toxic substances that enter the body causes mice require a large amount of energy to neutralize these toxic substances[25]. Thus, resulting in less than optimal parent body in providing growth nutrition for the fetus. High toxic substances

in the body can increase cell damage resulting in cell death. The number of dead cells causes disruption of the body's metabolic processes, so that it can disrupt the normal functioning of organs, and reduce growth, body weight, and body length. Low body weight in the fetus is closely related to intrauterine growth restriction (IUGR). The factor that influence IUGR include placental dysfunction, malnutrition, developmental abnormalities, chromosomal disorders, disorders of the parent metabolism, toxic substances, and drugs[11]. According Saini et al. (2013), fetal weight loss is an appropriate and sensitive indicator of developmental obstacles[26]. Interference with blood flow to the uterus due to reduction in vascularization of the uterus can cause developmental obstacles in the fetus. Fetal weight loss accompanied by a delay in ossification in the fetal skeleton can be a factor in the development of the fetus. In addition, Hg can also inhibit the transmembrane transport of nutrients in the placenta and cause an obstacle to fetal development[2].

Conclusion

Hg can be distributed and accumulates for a long time in experimental animals in the pregnant mice. The differences of concentration in Hg due to the ability of the defense mechanism in each of these organs to be exposed with it or the ability of mercury to pass through the organ's defense mechanism. This study also reveals that the administration of teratogen material Hg causes teratogenic effects in the form of embryotoxicity and growth retardation. This teratogen when exposed to pregnant mice will cause teratogenic effects that can inhibit fetal development and cause an abnormal condition in the fetus.

Conflict of Interest : The authors declare that they have no conflict of interest.

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Bioremediation of Crude Oil-Contaminated Water and Sediments from the Shatt Al-Arab River (SAR)

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Abstract

In the SAR, the oil-degrading bacteria (O-DB) are widespread, forming 8 % to 14 % of heterotrophic bacteria (HB). The O-DB numbers and biodegradability of petroleum hydrocarbons (PHY) are important in summer (SU) and sediments (SE) compared to winter (WI) and water (WA). The common O-DB retrieved from the SAR are *Pseudomonas* sp. (*PS*), *Pseudomonas putida* (*PP*), *Pseudomonas fluorescens* (*PF*), *Pseudomonas aeruginosa* (*PA*), *Pseudomonas cepacia* (*PC*), *Corynebactrium* sp. (*CO*), *Bacillus* sp. (*BA*), *Bacillus cereus* (*BC*), *Bacillus subtilis* (*BS*), *Flavobacterium* sp. (*FL*), *Aeromonas* sp. (*AE*), *Arthrobacter* sp. (*AR*), *Vibrio* sp. (*VI*), *Nocardia* sp. (*NO*), *Acintobacter* sp. (*AC*), *Micrococcus* sp. (*MI*), and *Staphylococcus* sp (*ST*). The most effective O-DB utilized more than 52 % of oil in 21-days. A mixed culture made of mutant *PP+AR* utilized 93 % of oil during the same period. The oil biodegradation rates in the SAR have been restricted by biotic and abiotic factors. By providing these factors to the oil-contaminated sites would improve the degradation rates. The biodegradation of n-alkanes was much faster than polycyclic aromatic compounds (PAC).

Keywords: *Bacteria, Crude oil, Hydrocarbons, Bioremediation*

Introduction

Petroleum production leads to hydrocarbons (HY) pollution, which has become one of the most important problems facing the environment today. Oils may contaminate WA, SE, soil and air, menacing human health and other organisms, and causing the deterioration of the ecosystem[1]. Nevertheless, most of the oil released into the environment is largely harbored as a result of the ability of microorganisms to degrade HY[2]. These HY degraders are ubiquitous in the ecosystem, and their PHY degradation rates depend on the environmental factors. The stability of oil pollutants in the environment relies on the HY quality and quantity that make up the crude oil and on the influencing environmental properties[1].

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The current research will demonstrate the SAR ability to biodegrade the PHY. The SAR is one of the economic, social and environmental rivers in Iraq and the prime fresh WA source in its southern part and Arabian Gulf (AG). The WA of SAR has been diagnosed as containing oil pollutants due to various industrial processes and aqueous activities[3]. PHY can be introduced to the

SAR from transportation operations, fixed structures, naturalistic inputs etc. A few is known on the HY biodegradation in the SAR. Most of the former studies on this topic focused on HY degraders isolation.

There is a necessity to comprehend the PHY behavior and fate in the SAR. Therefore, this study was accomplished. The aim of study is to estimate the O-DB and HB numbers and isolate O-DB from SAR, determine the crude oil biodegradability in SAR WA, SE and bacterial isolates, know the seasons, nutrients and external O-DB effects on the bioremediation, and

create a mutation in O-DB and make a high ability mixed bacterial cultures to degrade oil.

Materials & Methods

Three sites on SAR were selected for the study (Abu-Al-Kahaseeb (30°27'44.5'' N-48°00'06.0'' E), Basrah (30°33'00.0" N-47°47'10.0" E) and Karmat-Ali (30°48'10.6'' N-47°45'03.8'' E)). The WA and SE samples were taken from the sites during the SU and WI of 2019. The samples were transported to the laboratory and their temperature (TE), salinity (SA) and pH were determined.

The HB were counted by mixing WA (1 mL) or SE (1 g) with normal saline (9 ml). The suspension was then left to 10 minutes. Six dilutions were intended and 0.1 ml of suitable dilution was spread over the nutrient agar (Difco), incubated at 37 ° C for 48 hours.

The O-DB were counted by adding WA (1 mL) or SE (1 g) to a Erlenmeyer flasks (250 ml) containing liquid mineral media (LMM) of Chaineau et al[4]. Samples were not added to some flasks (control). The flasks were incubated for 30 days at 37 °C in shaker incubator (155 rpm). Bacterial growth in liquid cultures was observed at 7, 14, 21, and 28 days by planting 0.1 mL of suitable dilution on the nutrient agar (Difco), incubated for 48 hours at 37 °C. Each type of colonies on the media were registered and selected. The colonies were purified, counted and examined. Stock cultures were made. The bacteria were identified based on morphological and biochemical parameters.

Various methods have been utilized to estimate the bacterial degradation ability in samples. These included subjecting the WA and SE to crude oil and then determining the O_2 consumption[5], the production of CO_2[6], and the alternation that occur in the PHY weight and concentration, every 7 days, up to 21 days. The PHY weight alterations were determined by gravimetric method[7]. To determine the PHY concentration, the HY were extracted from the liquid cultures according to Pourbabaee et al[8]. The extract was then analyzed by a spectrofluro-meter **(**Shimadzu RF-540).

The ability of each bacterial isolate was examined for PHY degradation in LMM. The flasks placed in shaker incubator (155 rpm) at 37 °C for 21 days. Bacterial growth were observed and the oil concentration percentages loss were determined (spectrofluro-meter).

To mutate the bacteria (*PA*, *PP*, *PC*, *PF*, *BC* and *BS*) were subcultured on the nutrient agar, incubated for 8 or 14 hours, and suspended in the phosphate buffer (20 mL), and exposed to ultraviolet light (UVL) for 6, 35 or 65 minutes. The various radiation rates and stages were destined to involve different exponential bacterial growth phases to stimulate an effective mutation. A germicidal-lamp (254 nm) was the source of UVL with output was 10 erg/mm/second. Irradiation was only practiced while the lamp fluency was at a constant maximum. The morphology of each bacteria growing on the nutrient agar for phenotypic expression was observed, and its ability to degrade PHY was examined. After that, mixed cultures were made from mutated and non-mutated bacteria and their ability to biodegrade PHY in LMM was examined.

In situ, biological remediation was studied by designing four experimental WA ponds (2×2 ×1 m) in site 2 within the SU. The first pond was equipped with 1L of crude oil. The second pond was supplied with crude oil, nutrients (350 g of $(NH_4)_2SO_4$ and K_2HPO_4) and mixed O-DB (*MI*, *CO*, *BA*, *PS*, *AR*, and mutant *PP*). The third one was supplied with crude oil and O-DB. The fourth pond was without treatment (control). The WA samples were collected from ponds every 7 days to 21 days. The O-DB were counted on a solid mineral medium (it is LMM with 20 g of agar), incubated in 37 °C. The PHY were extracted and fractionated[9] (n-alkanes and PAC) from samples, and the PHY concentration percentages loss were estimated by analyzing them in gas chromatography (GC) (Allegent, USA). The data were statistically analyzed by ANOVA. The means were compared through Duncan-test.

Results

Average TE, pH, and SA of SAR WA were 16 °C, 7.5 and 4.0 ‰, in WI, and 37 °C, 7.6 and 5.4 ‰, in SU, respectively.

The O-DB makes up from 8 % to 14 % of HB. The SE have more bacteria than WA. The bacteria in SU samples were high than WI. The highest bacterial numbers and percentages were in site 2 than others (Table 1).

The O-DB growth in SE was higher than WA and in SU was more than WI in LMM. The highest O-DB growth was in site 2 samples. The growth generally increased with the incubation period. The O-DB numbers in WA and SE on 21-days were high than 7-days of incubation. The O-DB growth declined on 28-days of incubation (Table 1).

The SE showed the highest rates of O_2 consumption and CO_2 production and crude oil weight and concentration percentages loss

Table 1: Bacterial numbers CFU/ml WA or g SE in SAR, LMM and ponds

Sample	**Bacteria**	**WI**			**SU**			
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	
WA	HB	7.73x104	7.96x104	7.25x104	5.12x106	7.65x106	2.68x106	
	O-DB	6.44x103	8.34x103	5.83x103	4.14x105	8.31x105	2.47x105	
	O-DB/HB%	8	10	8	8	10	9	
		LMM						Control
	O-DB 1st week	2.74×102	3.28×102	2.01×102	1.23×104	1.96×104	2.14×103	0
	O-DB 2nd week	5.25×102	7.69×102	4.03×102	2.89×104	4.17×104	4.13×103	0
	O-DB 3rd week	6.26×102	8.88×102	4.28×102	4.97×104	6.32×104	4.16×103	0
	O-DB 4th week	4.62×102	6.75×102	2.84×102	2.36×104	3.19×104	0.55×103	0
		Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	
SE	HB	2.86x105	3.89x105	2.24x105	5.29x107	5.46x107	2.87x107	
	O-DB	2.42x104	5.72x104	2.17x104	4.74x106	6.23x106	2.79x106	
	O-DB/HB%	8	14	9	8	11	9	
		LMM						Control
	O-DB, 1st week	6.57×102	9.15×102	5.08×102	7.16×104	9.76×104	4.94×104	0
	O-DB, 2nd week	1.32×103	2.85×103	1.09×103	1.47×105	2.11×105	1.12×105	0
	O-DB, 3rd week	2.47×103	3.04×103	2.07×103	2.35×105	3.15x105	1.89×105	0
	O-DB, 4th week	0.86×103	1.22×103	0.54×103	1.44×105	2.17×105	1.23×105	0
					Pond-1	Pond-2	Pond-3	Pond-4
WA	O-DB, 1st week				3.1x104	7.3x109	2.3x106	3.8x102
	O-DB, 2nd week				4.3x104	8.2x109	4.4X106	4.3x102
	O-DB, 3rd week				6.2X104	9.9x109	5.4x106	4.6X102

compared to WA. These rates and percentages were generally higher in the SU than WI. In site 2 samples, the O_2 consumption and CO_2 production rates and oil weight and concentration percentages loss were higher than another sites. An increase in the rates and percentages were observed with an increase in the incubation period up to 21-days (Fig. 1, 2, and 3).

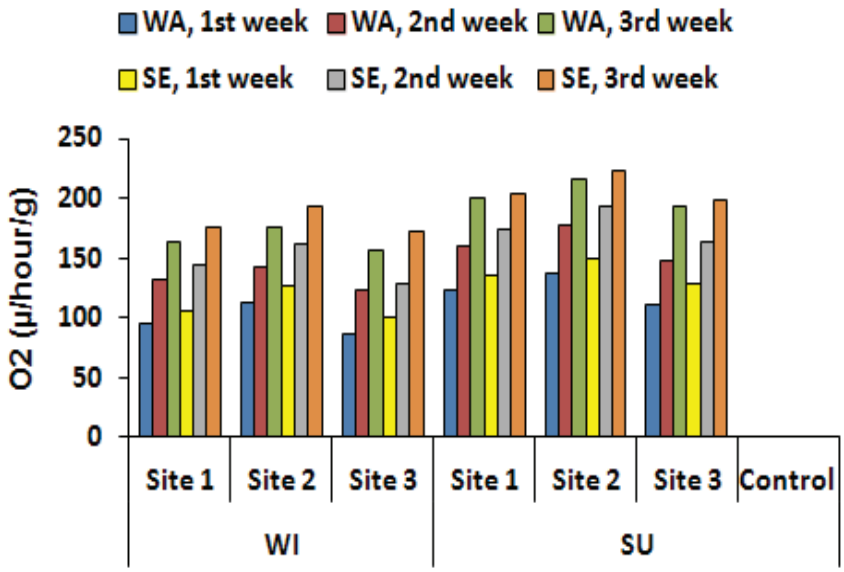


Fig. 1: O_2 consumption rates in oil-exposed SAR samples

Various species of O-DB were identified from WA and SE of the SAR, including *PS*, *PP*, *PF*, *PA*, *PC*, *CO*, *BA*, *BC*, *BS*, *FL*, *AE*, *AR*, *VI*, *NO*, *AC*, *MI* and *ST*. Elements of the same bacteria were observed in WA and SE.

The oil biodegradation rates of O-DB were varied (Table 2). The oil concentration percentages loss ranged from 65% (*PS*) to 33% (*VI*). The most potent bacteria in the oil degradation was *PS*, *PP*, *PA*, *AR*, *CO* and *MI* (% concentration loss < 52%).

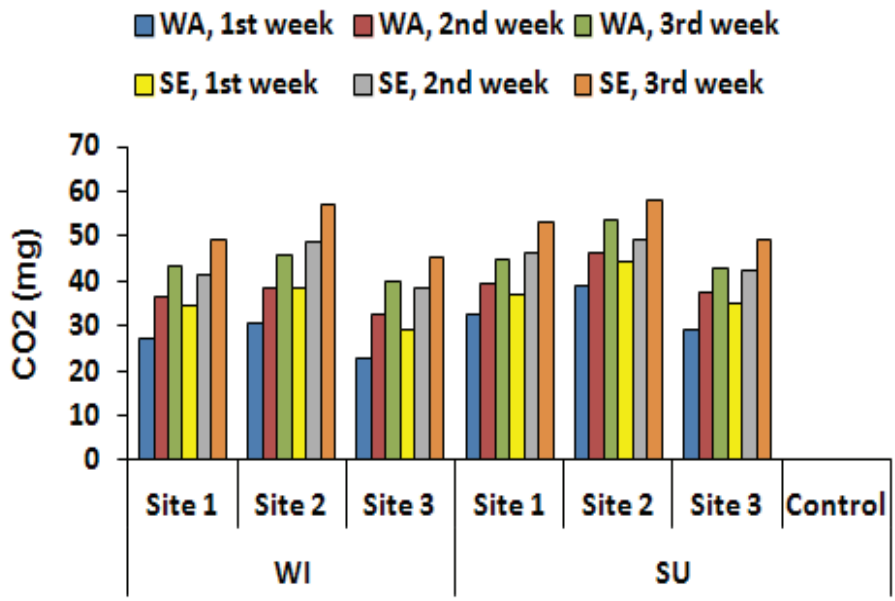


Fig. 2: CO_2 emancipated from SAR oiled samples

Mutation experiments have shown that only two bacteria (*PP* and *PA*) have mutated among six bacteria (*PP*, *PA*,

PF, *BS* and *BC*) exposed to UVL. The *PP* demonstrated a high ability to break down crude oil. The oil concentration percentage loss by mutant bacteria was 67 %. The mixed culture (mutant *PP+AR*) utilized the oil (93 % during 21-days) best than the other mixed cultures produced (Table 2).

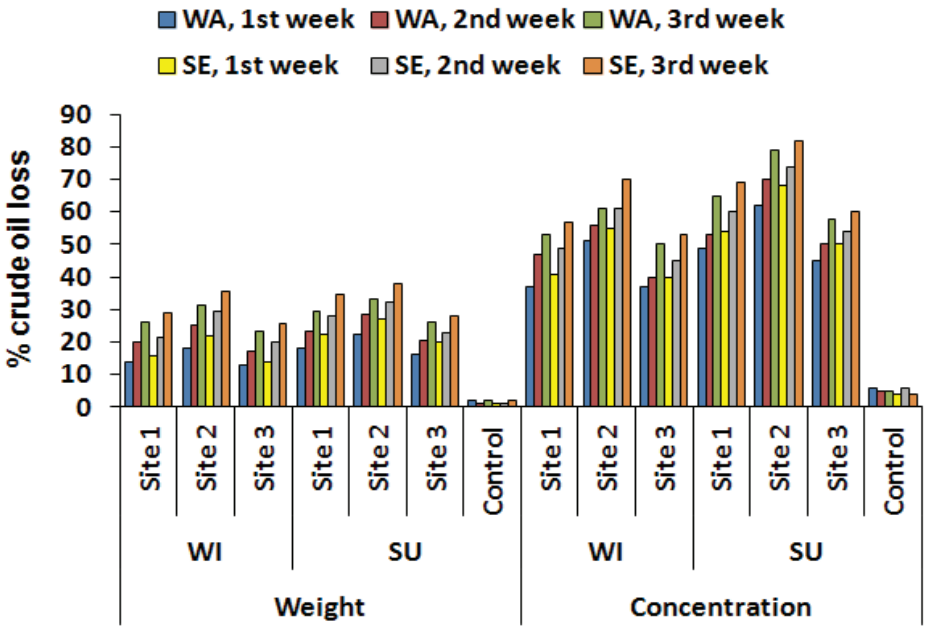


Fig. 3: Oil weight and concentration percentage loss in SAR samples

Table 2: Bacterial degradability on 21-days of incubation

O-DB	Oil concentration loss %
MI	58
VI	33
FL	36
CO	61
PS	65
PP	55
PA	56
PC	47
PF	38
AR	60
BA	52
BS	40
BC	44
ST	37
NO	47
AC	37
AE	41
PP (mutant)	67
PP (mutant)+AR	93
PP+AR	67
PS+AR	77
PS+CO	77
AR+CO	71
PS+BC	67

Table 4 shows that the bacteria numbers in the WA of four pond were significantly different. The largest number was

in the oiled pond, equipped with nutrients and external bacteria. Fig. 4 illustrate changes in the petroleum composition in oiled ponds. GC analysis evaluated the n-alkanes of C_{14} to C_{30} in addition to pristane (PI) and phytane (PH). Low molecular weight n-alkanes (C_{14} to C_{16}) were almost completely utilized by O-DB. Other n-alkanes (C_{17} to C_{30}) were less biodegraded. Higher molecular weight PAC (floranthene (FE), pyrene (PE), benzo [a] anthracene (BAE), chrysene (CE), benzo [b] fluoranthene (BBF), benzo [k] fluoranthene (BKF), benzo [a] pyrene (BAP), benzo [ghi] perylene (BGP) and indino [1, 2, 3 ed] pyrene (IP)) were more resistant to biodegradation than lower molecular weight PAC (fluorine (FO) and anthracene (AE)). An increase in the HY degradation rates was observed in the pond with nutrients and exogenous bacteria. The n-and branched-alkanes degraded faster than PAC.

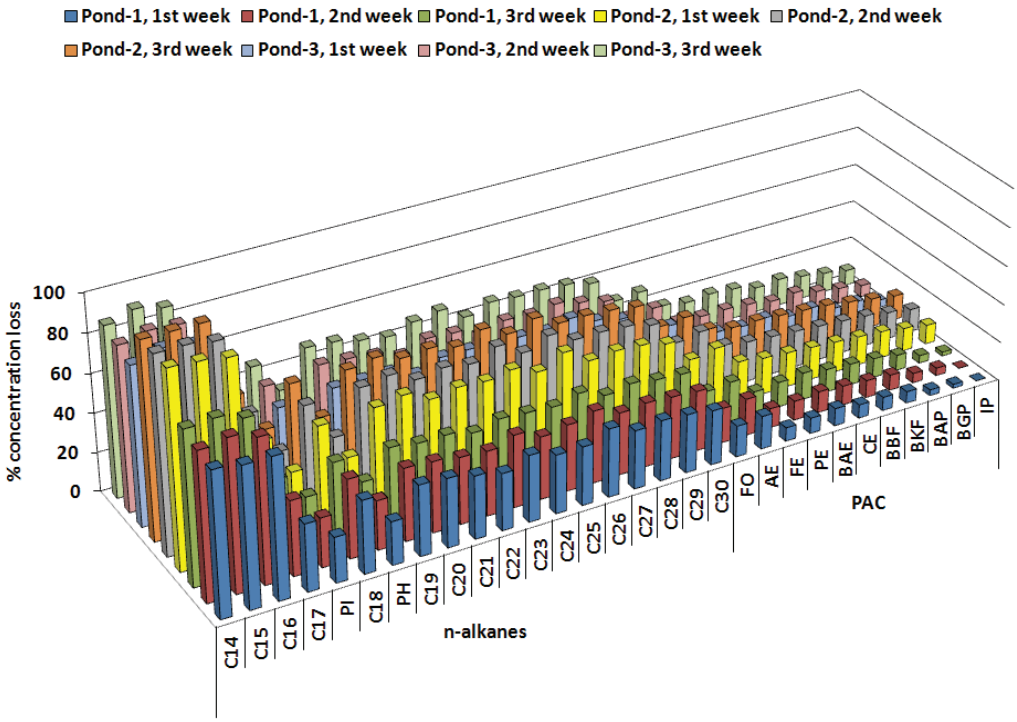


Fig. 4: n-alkanes and aromatics bioremediation in oiled-pond at 21-days

Discussion

The SAR has the potential for biodegradation of PHY due to the wide distribution of O-DB in SAR. Atlas et al[5] showed that the oil degraders numbers in a given ecosystem partially determine the system's ability to degrade oil pollutants. The O-DB numbers in the SAR vary according to TE. At high TE during the SU, O-DB numbers were much higher than in low TE during WI. Chaineau et al[4] previously reported the relationship between the change in O-DB numbers and the environment TE. The difference in O-DB numbers in the SAR sites depend on the differences in nutrients available and oil pollutants level[10]. A large numbers and percentages of ODB at site 2 may indicate that this site may has previously been exposed to PHY[5].

A significant changes in O-DB numbers of oil-exposed samples in LMM within 21-days of incubation indicates the rapid adaptation of O-DB to the PHY degradation[11]. However, on 28-days, O-DB numbers start to decline. The O-DB response to the oil was immediate without time delay associated with a prolonged period of enzymatic adaptation. After the depletion of biodegradable HY, the residual PHY

resist the degradation, thus O-DB numbers decreased. Accumulation of some microbial metabolites may also inhibit the PHY degradation in media[10]. This may partly explain the decrease occurred in the O-DB numbers after a certain period.

The WA and SE samples are able to metabolize PHY. A rapid increase in the O_2 consumption rates when adding oil indicates that samples are effective in PHY degradation when collected and are able to immediately initiate the degradation[1]. The CO_2 production suggests that samples are able to convert PHY to CO_2 and WA as a result of biodegradation[5]. A significant decrease in the oil weight and concentration in the samples refers that these samples suffer from biodegradation. The loss of oil weight also indicates that non-biological factors may impact on the biodegradation[11].

The degradation effectiveness in SE was higher than WA[5]. Biodegradation rates in SU were generally greater than WI, possibly due to differences in oil degraders levels and non-biological factors especially TE[2]. The site 2 shows higher biodegradation than other sites due to the fact that this location is characterized by a large O-DB numbers able to utilize PHY.

Some current bacterial isolates were Gram-negative (*PS*, *PP*, *PF*, *PA*, *PC*, *AE*, *VI*, *FL*, and *AC*) and others were Gram-positive (*AR*, *BA*, *BC*, *BS*, *ST*, *CO*, and *MI*). The bacteria were able to utilize oil at different rates. These bacteria were also isolated and characterized as O-DB by other authers[4 and 11].

The UVL are often used to produce mutant in microorganisms. DNA and amino acids prefer to absorb the wavelength of this beam, and therefore this radiation has great genetic and biological effects[12]. The mutated bacteria (*PP*) and the mixed culture (mutant *PP+AR*) showed high oil degradation capacity.

The biological degradation of oil in the ponds was limited by the nutrients availability (N and P). By providing nutrients and oil degraders to the oily ponds, biodegradation is enhanced[2]. The crude oil really biodegrade in the ponds. The biodegradation of n-alkanes and PAC is inversely proportional to the increase in molecular weight. The PI and PH (isoprenoids) and PAC were resistant to degradation, due to their molecular construction[10].

Conclusions

The ability of the SAR to biodegrade the petroleum is largely due to its ownership of the oil degraders, The spatial and seasonal changes of the SAR affect the biodegradation. By adding nutrients and oil degraders to environment, the petroleum biodegradation rates stimulated. Some n-alkanes are completely degraded, while others are slowly utilized. Branched alkanes are relatively more resistant to degradation than linear alkanes. PAC are the most rebellious to biodegradation.

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Conflict of Interest- Nil

Source of Funding- Self

Ethical Clearance- From the College

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Contamination of Polynuclear Aromatic Hydrocarbons-(PNAH) in Sediments: Identification And Distribution in the River of Shatt Al-Arab-(ROSA)

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Abstract

The sediments were analyzed for total organic carbon content-(TOCC), grain size-(GS), and PNAH levels and origins. The sediments were taken from 9 stations-(ST) on ROSA. Gas chromatography-(GC) analysis showed that the PNAH level in ROSA sediments was comparatively low to medium compared to other world locations. The PNAH total levels varied from 31.86 ng/g dry weight-(DW)-(ST 9) to 88.99 ng/g DW-(ST 6). The highest PNAH levels were close to oil pollution sources at ST 2, 4, 6, and 8. The TOCC ranged from 0.32 % to 1.64 %. The data suggested that the PNAH levels in the ROSA were linked to TOCC and GS. The PNAH compounds and ratios and analysis of principal components-(AOPC) indicated that the PNAH sources in the river were biogenic-(organisms), pyrogenic-(combustion), and petrogenic-(petroleum). Ecological risk evaluation showed that PNAH did not cause any deleterious impacts on the ROSA.

Keywords**:** *Water pollution, aromatic hydrocarbons, organic carbon, Shatt Al-Arab.*

Introduction

The PNAH are a widespread and stable group of pollutants in the aquatic environment. They have long transportation capacity and causes harmful environmental impacts[1]. There are many PNAH in nature but 16 compounds of them are usually studied, classified by the US-EPA as highly toxic. PNAH are generally hydrophobic and slightly soluble in water. PNAH of high-molecular-weight-(POHMW)-(≤ 4 rings) are slightly soluble in water, low evaporation and highly lipophilic than PNAH of low-molecular-weight-(POLMW)-(≥ 3 rings)[2].

Because of the hydrophobic nature of PNAH, tend to correlate with organic materials in the aquatic environment, so sediments are the ultimate PNAH stores in the water and are a good tools for monitoring PNAH[3]. The PNAH accumulation in sediments is due to the industrial and nature releases of them[4]. PNAH industrial emissions include petrogenic and pyrogenic sources. Pyrogenic sources include fossil fuel combustion, trees and weeds burning, and volcanic eruptions, etc. Whereas, PNAH petrogenic sources are linked to the spills of oils[4]. The PNAH from petrogenic or pyrogenic sources show different behaviors and distribution. Pyrogenic PNAH are more closely associated with sediments and resistant to biodegradation than petrogenic[2].

Exposure to hydrocarbons has many health-damaging effects such as reproductive disorders, mutations in DNA, and cancer. Several guidelines for estimating PNAH damage to aquatic organisms have been documented by authors and environmental concern international organizations[1].

The employ PNAH ratios having the same molecular mass enables us to explain the PNAH formation and sources. Various criteria have been established to

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demonstrate the PNAH origin in aquatic sediments such as phenanthrene/anthracene-(PH/AN), fluoranthene/pyrene-(FL/PY), anthracene/anthracene+phenanthrene-(AN/AN+PH), benzo[a]anthracene/benzo[a]anthracene+chrysene-(BA/BA+CH), and fluoranthene/fluoranthene+pyrene-(FL/FL+PY)[6].

Despite great global interest in the PNAH effects on the ecosystem. Little is known about their concentrations and sources in some world countries. Iraq has many industries, but it has not focused much on monitoring PNAH in its environment. There is also restricted knowledge about the range to which different combustion processes attribute to PNAH releases.

This paper aims to study the PNAH existence, distribution and sources in the ROSA sediments. The ROSA is an important and unique ecosystem due to the presence of many aquatic habitats and species that live in a completely different environment (Arabian Gulf). However, it is described as having considerable inputs of various pollutants that come from urban areas and industrial locations. This ecosystem, over the years, due to its proximity to many villages, towns and various human activities such as agricultural, industrial and municipal efficacies, possesses the PNAH accumulation and other pollutants in its sediments[7].

The study area is located in the city of Basrah, between longitudes, 29°56'33.8'' N and 30°48'10.6'' N and latitudes, 47°45'03.8'' E and 48°34'37.4'' E in southern Iraq. The region consists of a number of urban, suburban, and rural settlements surrounding the ROSA. Many lands in the area are used to grow different crops and trees. Solid waste disposal and wastewater treatment sites are also part of land use. Moreover, the area is distinguished for containing some heavy and light industries, in addition to power stations and oil refineries scattered along the ROSA banks. The area also has sites for burning municipal, agricultural and household waste. The average annual temperature is 25 °C, ranging from 13 °C-(January) to 33 °C-(July). The average annual downpour is 141 mm, and its duration is from October-(1 mm) to December-(29 mm).

Materials and Methods

The used glassware has been completely cleaned out. All solvents were of an analytical degree and distilled before work. Anhydrous sodium sulfate-(Na_2SO_4) ashed in 450 °C. Silica and alumina were solvents washed and dried prior to use.

Forty-five samples of superficie sediment were taken at a depth of 10-30 cm from 9 ST located along the ROSA during 2019. The ST were Karmatt-Ali-(ST 1)-(30°48'10.6" N-47°45'03.8" E), Nahran-Amar-(ST 2)-(30°45'05.0" N-47°39'53.0" E), Al-Hurtha-(ST 3)-(30°39'48.0" N-47°44'41.0" E), Basrah-Center-(ST 4)-(30°33'00.0" N-47°47'10.0" E), Abu-Al-Khaseeib-(ST 5)-(30°27'44.5" N-48°00'06.0" E), Abadon-(ST 6)-(30°03'42.0" N-48°27'03.0" E), Al-Sibea-(ST 7)-(30°20'16.5" N-48°15'34.5" E), Al-Faw-(ST 8)-(29°58'28.6" N-48°29'09.5" E) and Ras-Al-Beshea-(ST 9)-(29°56'33.8" N-48°34'37.4" E).The samples were then transferred to the laboratory with plastic bag and stored at 3.5 °C. The sediments were exsiccated, homogenized, grinded with mortar and maul, and sifted with 400 µm sieve before analysis.

PNAH was extracted from sediment samples depending on the procedure of Jiao et al.[2]. About 30 g of sediment was placed in Soxhlet and extracted for 18 hours using hexane and methylene chloride (250 ml, 1:1). The extract was reduced to 2.5 ml by nitrogen gas. The extract was then cleaned up with a chromatographic column containing activated silica and alumina (10 g, 100-200 mesh) and Na_2SO_4 (1 g). PNAH was eluted with benzene (20 ml). Benzene was removed and the remaining sample was analyzed by GC.

To validate the sediment samples, they were spiked with a standard mixture (1 µl, 100 mg/l) of 16 PNAH to distilled water (500 ml) pre-extracted with solvent (30 ml) (blank). After that, the spiked sediments were extracted and analyzed. The PNAH standard were employed to calibrate the GC.

The PNAH were analyzed by using GC-(Agilent-USA) equipped with flame ionization detector-(FID) (Agilent-USA). The detector and injector running temperatures were 300 °C and 320 °C. The column temperature was set at 50 °C to 8 minute and then 8 °C/minute for 350°C. The rate of PNAH standards recoveries ranged of 87.6 % to 96.8 %. The limit of detection varied of 0.16 to 0.81 ng/g DW.

The sediment GS analysis was performed based on the traditional procedure of Folk[8]. The TOCC were estimated by titration technique of Walkey's and Black[9].

PNAH concentrations were statistically analyzed. The standard error-(SE) for replications was calculated. One-way analysis of variance-(ANOVA) was used to compare between samples multiple groups. Statistical significance was determined as $P < 0.05$. The AOPC was employed to identify different PNAH sources. The data on the correlation matrix was analyzed by SPSS-(version16) of Windows-(I.B.M., Armonk, USA).

Result and Discussion

PNAH are introduced to the aquatic environment through natural and industrial operations[4]. The PNAH estimation in the ROSA sediments reflected their emission sources (pyrogenic or petrogenic). The total PNAH (naphthalene-(NA), acenaphthylene-(AC), acenaphthene-(AE), fluorene-(FU), phenanthrene-(PH), anthracene-(AN), fluoranthene-(FL), pyrene-(PY), benzo[a]anthracene-(BA), chrysene-(CH), benzo[b] fluoranthene-(BF), benzo[k]fluoranthene-(BK), perylene-(PE), benzo[a]pyrene-(BP=), indeno[1,2,3-cd]pyrene-(IP) and benzo[g,h,i]perylene-(BG)) concentrations ranged from 31.86 ng/g DW-(ST 9) to 88.99 ng/g DW-(ST 6) with high concentrations in the ROSA sediments close to the locations of high pollution sources (ST 2, 4, 6 and 7) (Fig. 1). The ROSA is habitat of numerous industries and human events, which may illustrate the marked variation in PNAH concentrations in the different ST sediments. The ROSA is exposed to PNAH pollution from various sources, including sewage waste, the waste of industrial, agricultural and household and the oil disposal from petroleum refineries and tankers, as well as the oil natural leakage from the ROSA oil production sites[7]. By comparing of the PNAH levels in the ROSA sediments with other world sites, the PNAH levels are high compared to studies of Wang et al.[10] and Zrafi et al.[6], and lower than those reported by Shilla and Routh[1] and Adeniji et al.[11].

The PNAH determination origin depends on the aromatics identification of particular sources. The FL and PY existence refers the pyrolysis importance because these components consist of condensing POLMW at elevated temperatures[6]. The CH is an ideal aromatic that indicates to petroleum origin because of its resistance to biodegradation and non-biological factors[12]. Therefore, the high CH concentration in the ROSA sediments (ST 2, 4, 6 and 7) suggest a petrogenic origin. The PE origin remains dialectical. Lin et al.[3] reported the PE origin in the marine environment sediments from terrestrial organisms. While, Wang et al.[11] showed that the PE origin is more from the aquatic environment. Shilla and Routh (2018) indicated that the PE source in the marine sediment is organic detritus. Likewise, small PE concentrations may come from industrial activities (Zrafi *et al*., 2013). In current sediments, PE is present in all ST (5.57 - 8.78 ng/g) with high concentrations at ST 1, 3, 5, 8 and 9, a large part of which is reckoned to the terrestrial origin.

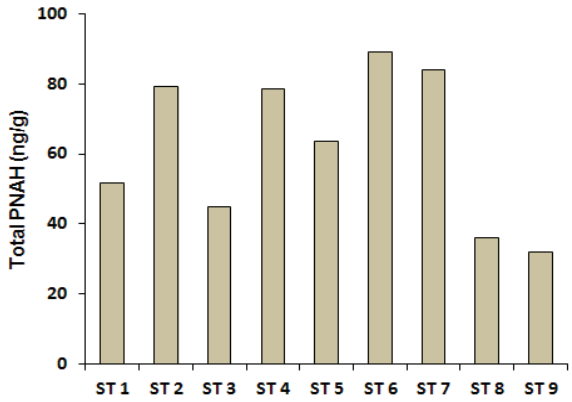


Fig. 1: PNAH spatial profiles in the ROSA sediments.

POLMW and POHMW identification is a valuable tool in determining oil pollution sources. Petroleum sources have comparatively high levels of POLMW-(2 and 3-rings), while POHMW-(≤ 4-rings) is an ideal feature of pyrolysis sources[1]. In the ROSA sediments, POHMW-(4 to 6-rings) predominated over POLMW (Fig. 2). This may be due to the high POHMW adsorption by sediments and POLMW constantly subjected to the biodegradation[10]. POHMW has been found to be environmentally stable caused carcinogenicity and genotoxicity, and these effects increase as their molecular weight increases[11].

With the geochemistry advancement, some ratios were developed to distinguish between PNAH sources. PH/AN <3 denotes pyrogenic source and >3 indicates petrogenic source[3]. FL/PY ratio of less than 1 indicates a petroleum source, while a ratio greater than 1 refers pyrolysis[6]. AN/AN+PH ratio of less than 0.1 indicates petroleum and diagenesis origin, while more than 0.1

are properties of combustion operations[4]. FL/FL+PY less than 0.4 denotes crude oil, diesel and gasoline combustion, and between 0.4 to 0.5 refers crude oil burning and more than 0.5 indicates to pyrogenic sources[1]. BA/BA+CH <0.20 is the petroleum characteristic, from 0.20 to 0.35 indicates crude oil burning, and more than 0.35 means combustion processes[11]. The current ratios indicated that the sediments PNAH in the ST 2, 4, 6 and 7 are from petrogenic and combustion sources. While the PNAH source in the ST 1, 3, 5, 8, and 9 may be from the pyrolysis origin (Table 1), however, the last ST cannot be excluded from the petroleum inputs due to the presence of many oil effectiveness in the ROSA attributed to PNAH.

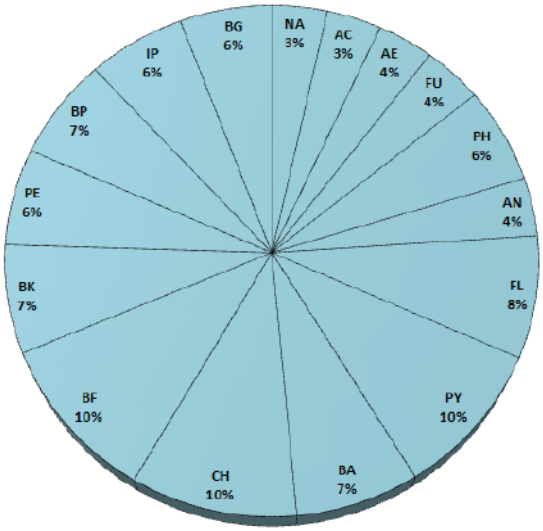


Fig. 2: PNAH distribution in the ROSA sediments.

PNAH contamination sources were identified in the ROSA sediments using AOPC. The major components in AOPC results formed 41.3%, 18.9% and 12.1% of the total variance (Table 2). PC-1 was highly loaded on POLMW, and identified as petrogenic. PC-2 was strongly loaded on parent-PNAH, and could be identified as a pyrogenic. PC-3 was heavily loaded on none of the PNAH, which was determined as an unknown source. The AOPC data showed that there are various PNAH sources in the sediments.

Several factors affecting PNAH levels have been found in sediments. Among them, TOCC and GS are examined in this study. TOCC % and GS for the sediments are shown in Table (3). TOCC % ranged from 0.33 % (ST 9) to 1.63% (ST 6). The difference in TOCC between ST sediments may be due to the variation in environments and organic matter sources. A direct relationship has been found between PNAH and TOCC % (r= 0.99). The organic matters significance for sediments in the PNAH partitioning demonstrated by[4].

Guidelines of sediment quality-(GOSQ) are a good instrument for assessing the harmful effects of pollution in aquatic sediments[11]. The range of low impacts-(ROLI), range of medium impacts-(ROMI), level of threshold impacts-(LOTI), and level of probable impacts-(LOPI) are employed to estimate the PNAH environmental toxicity in sediments (Fig, 3). In the following chemical concentrations ranges, the harmful bio-influences anticipated scarcely (<ROLI/LOTI), occasionally (≥ROLI/LOTI and <ROMI/LOPI) and repeatedly (≥ROMI/LOPI) (Olayinka *et al*., 2019). The estimations exhibited that PNAH concentrations were lower than the values of ROLI and LOPI at all investigating ST by comparing pollutant levels with GOSQ. It can be concluded that PNAH does not cause any harmful bio-effects in the ROSA sediment.

Conclusions

Sediment analysis provides worthy data on the PNAH levels and origins in the environment. This investigate demonstrated the dominance of POHMW over POLMW. Comparing with other world sediments, PNAH contamination was moderate. The most polluted ST with PNAH are close to heavy oil pollution sources (ST 2, 4, 6 and 7). The PNAH distribution in sediments shows a direct relationship with TOCC and GS. PNAH environmental risk assessment indicated that PNAH levels do not produce any instant bio-effects on the ROSA. Depending on PNAH profiles, markers and ratios and AOPC can be deduced that the PNAH origin in the ROSA was biogenic-(biosynthesis and biodegradation), pyrogenic-(combustion of fossil fuel, coal, wood, etc.), and petrogenic-(oils). Further investigates should be done on PNAH to detect their impacts and control their elevated levels harmful to mans, animals and plants in the ROSA.

Table 1: PNAH ratio in the ROSA sediments.

Ratio	ST 1	ST 2	ST 3	ST 4	ST 5	ST 6	ST 7	ST 8	ST 9
PH/AN	0.660	9.085	0.841	7.719	1.093	7.918	8.730	0.527	0.480
FL/PY	3.708	0.311	2.801	0.707	3.459	0.521	0.292	3.155	3.069
AN/AN+PH	0.468	0.080	0.374	0.208	0.355	0.130	0.087	0.514	0.624
BA/BA+CH	0.294	0.242	0.768	0.396	0.282	0.295	0.461	0.531	0.659
FL/FL+PY	0.637	0.291	0.555	0.367	0.602	0.286	0.291	0.610	0.574

Table 2. PNAH principal components-(PC) factor loading in the ROSA sediments.

PNAH	PC-1	PC-2	PC-3
NA	0.94	0.24	0.27
AC	0.92	0.43	0.17
AE	0.85	0.32	0.52
FU	0.93	0.18	0.19
PH	0.87	0.29	0.55
AN	0.74	0.26	0.21
FL	0.37	0.78	0.27
PY	0.14	0.96	0.31
BA	0.35	0.85	0.55
CH	0.37	0.94	0.06
BF	0.33	0.84	0.35
BK	0.25	0.78	0.36
PE	0.43	0.88	0.15
BP	0.24	0.83	0.24
IP	0.18	0.77	0.14
BG	0.33	0.84	0.08
Variance (%)	18.9	41.3	12.1

Table 3. Sedimentary properties of the ROSA.

ST	Sediment kind	TOCC (%)
1	Puddly-Sand	0.68
2	Sandy-Slime	0.96
3	Slime	0.61
4	Sandy-Slime	0.82
5	Sandy-Slime	0.73
6	Sandy-Slime	1.63
7	Sandy-Slime	1.01
8	Sandy-Cay	0.56
9	Sandy-Slime	0.33

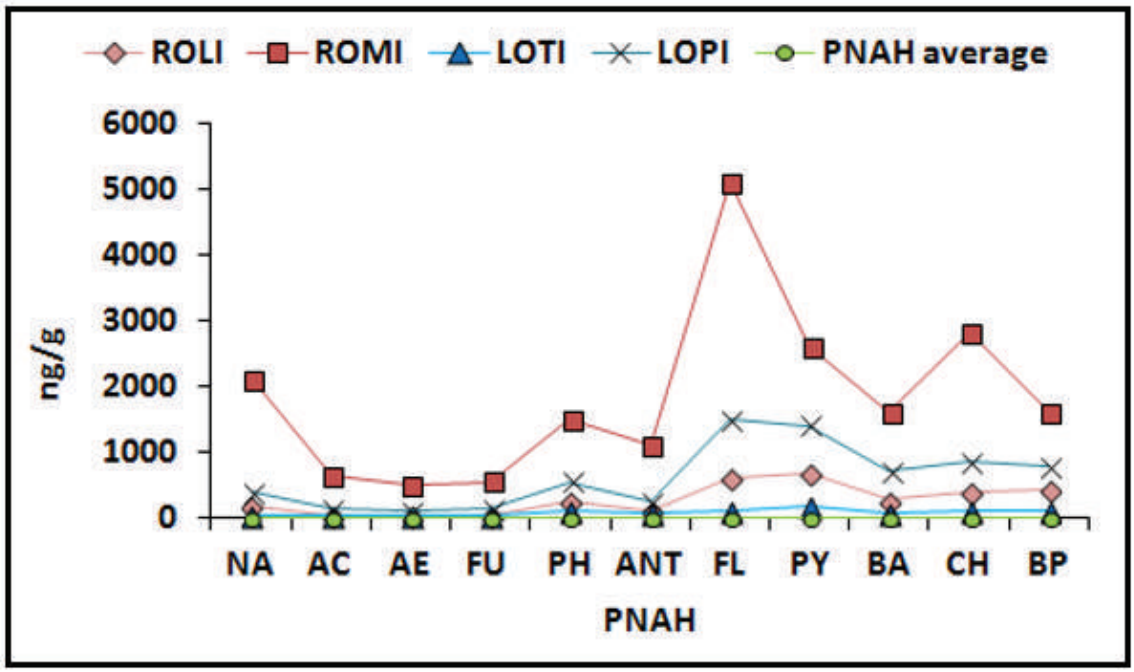


Fig. 3: PNAH average in the ROSA sediments relative to ROLI, ROMI, LOTI, and LOPI.

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Source of Funding- Self

Ethical Clearance- College

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Community Awareness and Participation in Handling Covid-19 in Surabaya, Indonesia

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Abstract

This study aimed to determine the level of public awareness and participation in handling Covid-19 in Surabaya, Indonesia. Surabaya was one of the second cities with the largest covid-19 events in Indonesia, after Jakarta. This research used survey method. The sample was determined by the simple random sampling method. The sample size in this study was 251 respondents. Research data were collected using a questionnaire, and analyzed with descriptive analysis**.** The results showed: an average awareness value of 3.43 (very high) and participation of 1.72 (very high), high business initiatives by 55%, a correlation value between awareness and participation of 0.25 included in the weak category. The results of this study can provide significant benefits in the disciplines of education and health psychology. The mayors can use the findings in this study to overcome the problem of Covid-19 in the community. It turns out that to reduce the spread of covid-19 is not only enough to do with health education, but increasing awareness and participation in the community is very important and significant in overcoming the spread of covid-19 due to lack of awareness in the application of physical and social distancing, the use of masks, hand washing.

Keywords: *Awareness; Participation; Community; Handling; Covid-19*

Introduction

The Covid-19 outbreak has spread to almost all corners of the countries in the world, including Indonesia. According to the report of the Task Force for the Acceleration of Management of Covid-19 sourced from WHO as of May 3, 2020, the Corona Virus (SARS-Cov-2) outbreak has hit 215 countries with 3,356,205 confirmed and 238,730 dead [1-3]. Meanwhile in Indonesia, there were 11,192 people tested positive, 1,876 people were declared cured, and 845 people died[1]. Predictions of some experts that the disease will run full time for six to seven months since it was first reported to WHO [4-6]. Covid-19 as a national disaster [7-9]. Various attempts have been made by the Indonesian government to prevent and control the spread of the Covid-19 outbreak, by implementing management strategies such as which is done in other countries, China, Thailand, Japan, the Republic of South Korea [7, 8, 10, 11].

Keeping the social distance (social distancing) and distant (physical distancing) are the two actions carried out by all countries in the world in preventing the spread of Covid-19 wider. It is, according to the management strategy of preventing transmission Covid-19, namely: cut off the transmission (blocking transmission), isolation, protection, and alternative medicine. The experience that occurred in China that quarantine, social distancing, and the isolation of infected populations can withstand an epidemic [12]. In accordance with the nature

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of the spread of the 2019-nov virus that occurs through droplets when coughing or sneezing from one person to another [13, 14]

Currently Indonesia has implemented a large-scale social restriction policy, in various provinces and municipal districts, but in a few weeks there are new cases that continue to increase and there are also those whose cases have decreased after the policy. Specifically in the city of Surabaya, the data still shows an ever-increasing curve despite large-scale social restrictions. Based on this the article aims to analyze the level of awareness and participation in handling co-19 in the city of Surabaya

Material and Method

This research used survey research methods. A large sample of 251 respondents was taken by simple random sampling. The research data were collected using an awareness questionnaire of 6 questions with answers using a Likert scale with a score assessment using 1 = strongly disagree; 2 = disagree; 3 = agree; 4 = totally agree. While the participation questionnaire consists of 15 questions with answers 1= no, 2 = yes. For assessing the level of awareness and participation with a range of values 1.75-2.00 (very high). 1.5-0-1.74 (high), 1.25-1.49 (moderate), 1.00-1.24 (low). Data analysis was used to analyze the level of awareness and participation in handling covid-19 by using descriptive analysis with percentage values, while to analyze the relationship between level of awareness and participation with the Pearson Correlation test with The level of significance was set at $p < 0.05$.

Result

Table 1. Profile of Respondents in the awareness and participation of Covid-19 prevention in Surabaya (n = 251)

No	Respondent's Identity	N	Percentage (%)
1.	**Gender**		
	- Men	99	39.40
	- Female	152	60.60
2.	**Age (Year):**		
	- ≤ 25	120	47.81
	- 26 - 35	49	19.52
	- 36 - 45	42	16.73
	- 46 - 55	30	11.95
	- ≥ 56	10	3.98
3.	**Occupation**		
	- Student / College Student	101	40.24
	- Teacher / Lecturer	66	26.29
	- City Government/Regency Government Employee.	3	1.20
	- BUMN employee	2	0.80
	- Private Employee	50	19.92

Cont... **Table 1. Profile of Respondents in the awareness and participation of Covid-19 prevention in Surabaya (n = 251)**

	-	Street Vendors / Markets / Shops	4	1.59
	-	Housewife	15	5.98
	-	Others	10	3.98
4.	City / Regency of Residence			
	-	Surabaya	96	38.25
	-	Regency. Gresik	74	29.48
	-	Regency. Sidoarjo	81	32.27

Table 2 Community Awareness Levels in Covid-19 prevention in Surabaya city (n = 251)

	Social Awareness Variables	**Strongly Agree n (%)**	**Agree n (%)**	**Disagree n (%)**	**Strongly Disagree n (%)**
1	Realizing that the Corona Virus (Covid-19) outbreak originally originated from Wuhan China, but now is a threat to the Indonesian, especially East Java	137 (54,6)	106 (42,4)	3 (1,2)	5 (1,9)
2	Realizing that the Corona Virus outbreak (Covid-19) is very dangerous for the safety of the lives of all citizens.	147 (58,6)	95 (37,9)	5 (1,9)	4 (1,6)
3	Recognizing that many victims died as a result of the Covid-19 Plague attack, and worried that the victims will continue to grow and afflict themselves and family members	110 (43,8)	115 (45,8)	18 (7,2)	8 (3,2)
4	Realizing that all government efforts and the task force for accelerating to handle Corona Virus Disease 2019–planned (Covid-19) throughout Indonesia,	127 (50,6)	115 (45,8)	8 (3,2)	1 (0,4)
5	Realizing that the accelerated handling of Corona Virus Disease 2019 Being (Covid-19) needs to be done, such as washing hands, spraying the environment with disinfectant, (social distancing), (physical distancing), using masks, and prohibiting returning to birthplace	151 (60,2)	94 (37,5)	5 (1,9)	1 (0,4)
6	Realizing that the Large-Scale Social Limitation policy is the most appropriate way to accelerate the handling of Corona Virus Disease 2019 Being (Covid-19) taken by the government	81 (32,3)	142 (56,6)	24 (9,6)	4 (1,6)

Mean = 3,43

Table 3: Level of Community Participation in Covid-19 prevention in Surabaya (n = 251)

	Variable Community Participation	Yes N (%)	No N (%)
1	Variable Community Participation	242 (96,4)	9 (3,6)
2	Following the conditions set by the government, namely large-scale social restrictions policy	226 (90,0)	25 (10,0)
3	Participation in activities, such as Work From Home (WFH), Learning From Home (LFH) and Pray From Home (PFH)	242 (96,4)	9 (3,6)
4	Participation in wearing masks every time you leave the house	226 (90,1)	25 (9,9)
5	Participation in washing hands with soap or hand sanitizer.	235 (93,6)	16 (6,4)
6	Participation not to engage in worship activities in the mosque/ mosque/ temple/temple/temple or attend meetings that involve many people (the crowd), even though it is not included in the red zone	185 (73,7)	66 (26,3)
7	Participation in driving by not carrying passengers other than family members and not exceeding the stipulations.	222 (88,4)	29 (11,6)
8	Participation not returning to the village of birth on Eid al-Fitr, or visiting relatives in other cities	233 (92,8)	18 (7,2)
9	Participation in reporting to the local government If I am a newcomer from another city, and follow the rules of self-isolation or isolation on orders	234 (93,2)	17 (6,8)
10	Participation in reminding family members to wash their hands with soap, wear masks when going out, not play in a group, exercise independently, and stay at home.	201 (80,1)	50 (19,9)
11	Participation invites neighbors around the house not to return to their hometowns during the holidays	157 (62,6)	94 (37,4)
12	Participation Together with the community management in mutual cooperation spraying the environment with disinfectants	171 (68,1)	80 (31,9)
13	Participation in handling Covid-19 as a volunteer.	47 (18,7)	204 (81,3)
14	Participation in donating part of the income to be donated by the existing Covid-19 aid institutions	122 (48,6)	129 (51,4)
15	Participation in helping neighbors and neighbors affected by Covid-19 economically by distributingfood packages	127 (50,6)	124 (49,4)
Mean = 1.72, Pearson Correlation test p = 0.000, with Pearson Correlation value = 0.254			

Discussion

The prediction of a Covid-19 pandemic outbreak in Indonesia would end in July 2020 and depended on 3 things, namely the conditions and efforts to change the speed of transmission and even sever the chain of transmission of the disease; the phenomenon of returning to the area during the May religious holidays on a massive scale or other forms of migration from the central area of distribution, especially the red zone area that has the potential to be ridden by a virus; and related to future conditions related to the consistency of government regulations [7, 15, 16]

Termination of the transmission chain Covid-19 by keeping social distancing and physical distancing are government regulations that must be complied with. Data from this study proved that there were a large number of people surveyed in the Greater Surabaya Region having a high level of awareness (94%) and participation (55%) in tackling and preventing the spread of the Covid-19 outbreak.

Awareness and participation during Social distancing and physical distancing can be done in 2 types, namely: individually: isolating from cases, quarantining from contacts, and encouraging staying at home, involving many people: closing Education institutions, closing workplaces, closing for special populations, such as orphanages, boarding schools, prisons, psychiatric services, cancellation of mass meetings, quarantine orders for buildings or residential areas [17, 18].

During the implementation of social distancing and physical distancing. Everyone is required individually to do the following: washing hands using running water and soap or *hand sanitizers*, clean hygiene behaviors, wearing masks, keeping a distance *(physical distancing)* at least within the range of 1 (one) meters when outside the home, and carry out independent isolation both in the house and/or isolation space; recommended staying at home [17, 19].

In fact, most of the people have already had awareness and participation in handling Covid-19. The results of this study indicated that almost all respondents had very high awareness. The problem was that the culture of the community was still communal and quite strong, so it tent to get along with communal. Therefore Indonesian live with communal relations and strong social interaction. Therefore, in dealing with Covid-19 community responses vary greatly according to the understanding and awareness of each individual. So, it is natural if there are people who do not obey the rules, but on the other hand not a few people who comply with government regulations in accordance with the provisions, including stay at home.

Many factors affected one's awareness in enforcing rules, including: encouragement from within, and encouragement from outside the self. Public awareness in handling Covid-19 was in accordance with the beliefs, hopes, worries, and desires that exist in each individual community. Self-awareness was the driving force for discipline [20-23].

The non-compliance of some people in enforcing social barriers also depended on the community's knowledge of Covid-19. Behavior displayed by people who did not comply with government appeals was based on cognitive bias [24]. Cognitive bias was a systematic error in thinking that affected the decisions and judgments a person makes. Building community awareness must be done by providing education related to the risk of Covid-19 transmission and also emphasizing to live a healthy life, wearing masks, and keeping a distance of [18, 21, 25].

Conclusion

Most people who have already had awareness and participation in handling Covid-19 Surabaya with a very high level of awareness and participation, and there was a relationship between awareness and participation in the prevention of Covid-19 in Surabaya. For this reason, it is necessary to build a joint awareness and participation of the city community continuously in preventing Covid-19, through communication, information and education among the community, Covid-19 arrest officers, volunteers and social groups, including religious groups.

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Ethical Clearance – Taken from Institutional Ethical committee

Conflict of Interest - Nil

Source of Findings: Self

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Patterns of Antimicrobial Resistance on *Staphylococcus Aureus* Isolated From Raw Milk from Dairy Farms in Surabaya

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Abstract

The aim of this research was to show the pattern of *S. aureus* bacteria isolated from raw milk from two dairy farms in Surabaya against antibiotics oxacillin, penicillin, amphicillin, chloramphenicol, and gentamicin. The study used purposive sampling method. About 72 samples isolated on *Mannitol Salt Agar* media (MSA) from the result which identified as *Staphylococcus sp* was 14 (19.44%) showed the positive samples on coagulase test. Antibiotic disk used i.e oxacillin, penicillin, amphicillin, chloramphenicol, gentamicin by using Kirby Bauer method. Inhibiton diameter zone measured at millimeter to determined a sensitivity level of antibiotic. The result showed about 14 (100%) of samples was resistant to Antibiotic oxacillin type, 14 (100%) of samples was resistant to penicillin, 9 (64,2%) samples was resistant, and 5 (35,7%) of samples was sensitive to amphicillin, 14 (100%) of samples was sensitive to chloramphenicol, and 14 (100%) of samples was sensitive to gentamicin. These results will open up our view that cooking milk is important to eliminate the danger of S. aureus isolates which present in milk and have potential hazards to public health.

Key words *: Dairy farms, S. aureus, Antimicrobial Resistance, Raw milk.*

Introduction

Staphylococcus aureus is a pathogenic bacteria that usually causes mastitis in dairy cows, can cause economic losses for dairy farmers [(1)]. The incidence of mastitis caused by S. aureus bacteria is the highest incidence rate[(2, 3)]. For a long time S. aureus has been a causative agent of mastitis, besides that the bacteria can also cause food borne disease (FBD), because these bacteria can contaminate processed dairy products during processing, it is caused by hygiene factors from workers, equipment that used less sterile, the environment also affects the sanitation of the cage that is not good [(1)].

The use of antibiotics is a step of choice in treatment in cases of infections caused by bacteria known as the drug of choice, because one mechanism of action of antibiotics is to damage the bacterial cell wall, so that the bacteria can die. Recorded from some documentation about S. aureus for antibiotics has two relevant effects, the first is the reduction in cure rates after treatment, and the second is the potential impact of transmission of resistant bacteria to humans through the food chain [(3, 4)].

Antibiotics that are usually recommended in animal husbandry include β-lactam, tetracycline, and macrolide groups. However, the β-lactam group is most often used in the treatment of mastitis [(5)], due to S. aureus bacteria can produce β-lactamase. Therefore, almost all isolates of S. aureus bacteria are resistant to penicillin class antibiotics, it is caused by β-lactamase which can break the β-lactam ring found in penicillin group [(6, 7)].

In Surabaya, the number of cases of subclinical mastitis required treatment of antibiotic treatment, so we need data on antimicrobial patterns, especially from beta lactam antibiotics as the main antibiotics for the treatment of mastitis. Therefore, this study was to identify S. aureus antimicrobial resistance isolated from raw cow

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milk samples from two dairy farms in Surabaya, East Java, Indonesia based on antibiotic sensitivity testing and to understand patterns of antimicrobial resistance.

Materials and Methods

Sample collection

Thirty eight milk samples were taken from Kaliwaron Farm, and thirty four milk samples were taken Bendul Merisi Farm during the morning milking time. 10 ml of milk samples in test tubes collected from different farms were used for isolation of *S. aureus* by streaking on the MSA isolation media were incubated 37ºC for 24 hrs. The appearances of yellow coloured colonies were subjected to grams staining [8].

Identification of Staphylococcus aureus

The presence of MSA was assessed by Catalase test by addition of 3% hydrogen peroxide on the yellow coloured colonies. The appearance of gas bubbles revealed the presence of *S. aureus* (Fig. 1). The catalase test was performed by inoculating the yellowish colonies in nutrient brooth, incubated at 37ºC for 24 hrs followed by addition of 1 ml rabbit plasma and evenly mixed and incubated for 24 hrs. The clotting of plasma confirms the presence of S. aureus, as shown Fig.2 [9].

Antibiotic sensitivity test

The antibiotic sensitivity test for *S.aureus* was carried out as per Kirby-Baurer method using Oxacillin, Penicillin, Amphicillin, Chloramphenicol and Gentamicin (Fig. 3) [10].

Results and Discussion

The results of isolation and identification on 72 samples of raw milk from 2 dairy farms in Surabaya revealed at 14 (19.45%) positive samples of Staphylococcus aureus (Tabel 1.). Fourteen positive samples for Staphylococcus aureus were subjected to antibiotic sensitivity test using five antibiotics and the results are presented in Table 2.

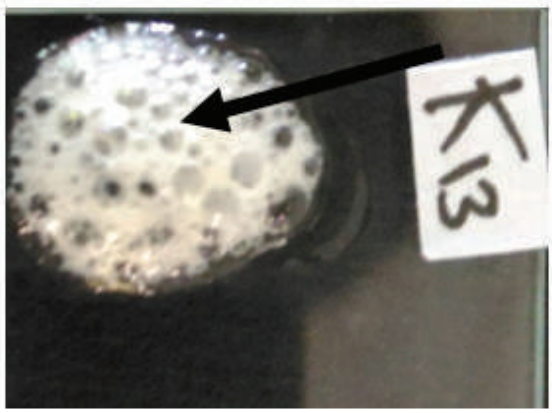


Figure 1. S. aureus catalase test shows positive presence of bubbles

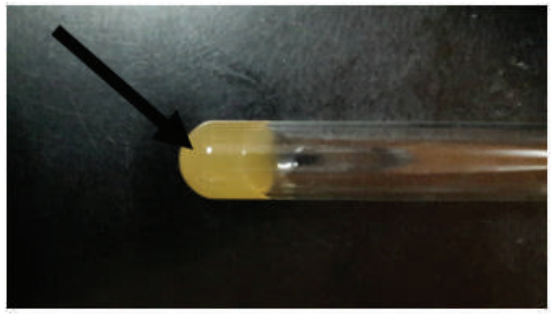


Figure 2. The positive S. aureus coagulase test has a lump in plasma

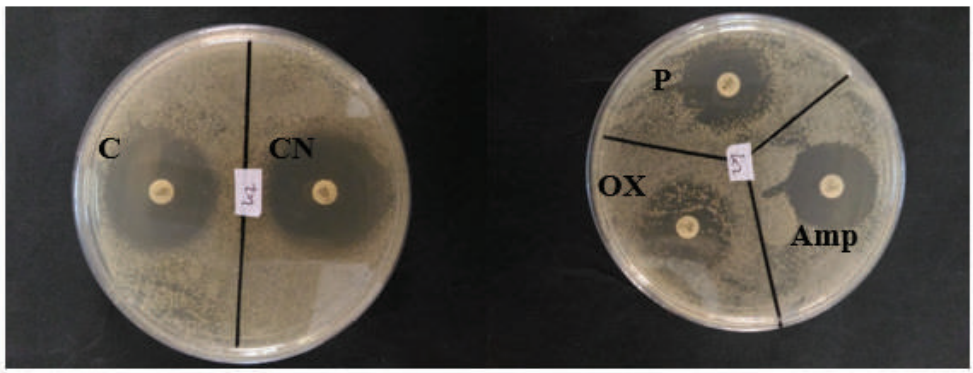


Figure 3. Zones of inhibition of antibiotic sensitivity test on the K2 sample

Note : OX= Oxacillin; P= Penicillin; AMP= Amphicillin; C= Chloramphenicol;

CN= Gentamicin

Table 1. Presence of Staphylococcus aureus from milk samples in Surabaya, Indonesia.

Location of farm	Sample size	(+) S. aureus
Kaliwaron (K)	38	7
Bendul Merisi (B)	34	7
Total	72	14

Table 2. Results of measurement of antibiotic sensitivity test on S. aureus

Sample code	Antibiotic disk inhibition zone diameter in mm				
	Oxacillin	Penicillin	Amphicillin	Chloramphenicol	Gentamicin
K2	28(R)	18(R)	29(S)	31(S)	30(S)
K3	27(R)	16(R)	29(S)	27(S)	26(S)
K13	19(R)	19(R)	18(R)	24(S)	22(S)
K16	0(R)	18(R)	20(R)	28(S)	30(S)
K28	0(R)	0(R)	19(R)	26(S)	18(S)
K29	0(R)	0(R)	19(R)	28(S)	27(S)
K33	27(R)	21(R)	16(R)	31(S)	19(S)
B1	26(R)	0(R)	21(R)	30(S)	27(S)
B12	22(R)	18(R)	18(R)	28(S)	31(S)
B13	27(R)	26(R)	19(R)	30(S)	26(S)
B17	26(R)	17(R)	42(S)	26(S)	30(S)
B19	26(R)	19(R)	40(S)	27(S)	29(S)
B25	22(R)	18(R)	12(R)	27(S)	30(S)
B27	28(R)	18(R)	40(S)	29(S)	28(S)

Note : R = resistant; S = sensitive

Based on the results of the S. aureus resistance test against several antibiotics, there were 14 (100%) samples resistant to oxacillin antibiotics, 14 (100%) samples were resistant to penicillin, 9 (64.2%) samples were resistant and 5 (35.7%) samples sensitive to amphicillin, 14 (100%) samples were sensitive to chloramphenicol, and 14 (100%) samples were sensitive to gentamicin.

Oxacillin, penicillin, and amphicillin belong to the β-lactam antibiotic class. B-lactam antibiotics are antibiotics that are often used in the treatment of mastitis in dairy cows. This antibiotic has activity in S. aureus through the interaction of three heavy molecules and one light molecule in penicillin binding proteins. The function of penicillin binding protein has an effect on the synthesis of peptidoglycan cell walls and cell growth. B-lactam antibiotics bind to and inhibit penicillin binding protein (PBPs) which is an enzyme for Peptidoglycan synthesis [6, 11].

According to Quinn et al., (2002) stated that beta-lactam antibiotics have the ability to inhibit bacterial

growth by influencing the process of bacterial cell wall synthesis[12]. The mechanism of action of β-lactam antibiotics starts with penicillin binding protein (PBPs) in bacteria. Obstruction of bacterial cell wall synthesis occurs because the process of transpeptidation between the peptidoglycan chains is disrupted, then the activation of proteolytic enzymes in cell walls [13].

Resistance that occurs with oxacillin, penicillin and amphicillin antibiotics in S. aureus from fresh cow's milk in Surabaya is probably due to the presence of PBP 4 gene. PBP 4 gene is a PBP group that has a low BM and is associated with β-lactam antibiotic resistance, and possibly PBP 4 causes the ability to tolerate β-lactam antibiotics. The nature of PBP 4 causes penicillin deacylates about 10-20 faster than PBPs with high BM. This is possible because PBP 4 binds oxacilin, penicillin and amphicillin at higher concentrations. Some PBP 4 molecules that are not bound to the concentration level that has been binding to PBP 2a molecules will tolerate β-lactam antibiotics given [6, 14]. Resistance to β-lactams that occur can be caused by S. aureus being able to produce β-lactamases which can break down β-lactam rings or the expression of PBP 2a which has a low affinity for oxacillin or other β-lactams. The resistance of S. aureus bacteria to lactam β group antibiotics is caused by this broad-spectrum antibiotic, both in vivo and in vitro and against a large number of Gram positive and Gram negative bacteria [15, 16].

B-lactam antibiotics in this study showed that Oxacillin and penicillin were no longer effective for the treatment of S. aureus infections because based on the results of the study 14 samples were resistant to these antibiotics. Whereas amphicillin also showed the same thing even though there were 5 samples that were still sensitive in samples B17, B19, B27, K2, and K3. Samples that are sensitive to β-lactam show that β-lactamase is still able to hydrolyze the β-lactam ring which can cause sensitivity [17].

Gentamicin antibiotics are very well used because 14 samples are sensitive with 100% effectiveness for S. aureus. Gentamicin is an aminoglycoside class of antibiotics. Gentamicin has a sugar group (glycoside) that is bound to the NH2 (amino) group by a mechanism of action that binds irreversibly to the ribosome 30s sub unit of bacteria, namely by inhibiting or preventing cells from making proteins properly and causing errors in the translocation of the genetic code [18, 19] .

The results also showed that 14 samples were 100% sensitive to the antibiotic Chloramphenicol, making it very effective for S. aureus. Chloramphenicol is an antibiotic group that can inhibit the growth of bacteria with a broad spectrum of work against S. aureus bacteria. Chloramphenicol works by breaking the binding of new amino acids in the peptide chains that begin to arise, largely because Chloramphenicol inhibits peptidyl transferase in the process of protein production. Chloramphenicol is primarily bacteriostatic and the growth of microorganisms begins again when drug administration is stopped [20, 21].

The overall discussion of this study is that β-lactam antibiotics can still be the first choice for the treatment of S. aureus infections, but it is necessary to be aware of the resistance and transfer of antibiotic resistance that occurs in bacteria. The use of alternatives for treatment using Chloramphenicol and Gentamicin is highly recommended because it is very sensitive and effective for treatment, also provides information that in animal products namely raw cow's milk in Surabaya has the potential for food borne disease (FBD) through S. aureus bacteria.

Conclusion

Based on the research results it can be concluded that 14 (100%) samples are resistant to oxacillin, 14 (100%) penicillin resistant samples, 9 (64.2%) resistant samples and 5 (35.7%) amphicillin sensitive samples, 14 (100%) chloramphenicol sensitive samples, and 14 (100%) gentamicin sensitive samples. These results will open up our view that cooking milk is important to eliminate the danger of S. aureus isolates present in milk and have potential hazards to public health.

Designation :

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Ethical Clearance: Raw milk were used in this study, hence ethical clearance was not necessary. Raw milk samples were collected from two dairy farms in Surabaya, East Java province, Indonesia.

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Conflict of Interest: Nil

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Spatial Analysis of Pneumonia in Toddlers Based on Environmental, Individual, and Behavior Factors in Pelalawan Regency, Riau Province

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Abstract

Pelalawan Regency is one of twelve districts in Riau Province with pneumonia cases in toddlers is quite high, namely in 2016 there were 155.7% of pneumonia cases in toddlers. Based on these cases, there is no clear information on how the cases were spread related to environmental risk factors. The research aims to conduct spatial pneumonia analysis in toddlers based on environmental, individual and behavioral factors. By knowing the pattern of disease distribution and its possible causes, it will be easier for officers to plan pneumonia prevention efforts and prevention more effectively, community-based or make it easier to design the development of further prevention programs. This research is a survey research with quantitative analytic methods conducted in 12 Districts of Pelalawan Regency, Riau Province. The results showed the highest distribution of pneumonia frequency in toddlers by region in 12 Districts of Pelalawan District was the highest in Districts of Pelalawan.

Keywords*: Pneumonia, Toddler, Spatial Analysis, Environmental Risk Factors, Individuals, Behavior*

Introduction

Pneumonia is a disease that attacks the lungs and is characterized by coughing and breathing difficulties[(1)]. Pneumonia is one of the types of lower respiratory tract infections and has become a serious concern, because it is a major cause of infant mortality, especially in developing countries with 3 million deaths each year. Pneumonia in toddlers is most often caused by respiratory viruses and most often caused by the bacterium Mycoplasma Pneumoniae[(2),(3)]. Globally, pneumonia is the 7th largest cause of death related to the environment. [(4)].

Toddlers are the most vulnerable group to pneumonia. In 2015, the World Health Organization (WHO) reported nearly 6 million toddlers died, 16% of this number was caused by pneumonia as the number 1 killer of toddlers in the world. Based on data from the United Nations Agency for Children (UNICEF), in 2015 there were approximately 14 percent of 147,000 children under the age of 5 in Indonesia died of pneumonia. From these statistics, it can be interpreted as many as 2-3 children under the age of 5 years die from pneumonia every hour. This causes pneumonia as the main cause of death for children under 5 years of age in Indonesia [(5)].

The prevalence of pneumonia in toddlers in Indonesia in 2016 has reached 25% and increased in 2017 by 46.34% (447,431 cases). The number of underfive deaths due to pneumonia in 2017 amounted to 1,351 people with a Case Fatality Rate (CFR) of 0.3%[(6)].

The number of cases of finding and handling pneumonia in toddlers in Riau Province in 2014 was 10,367 cases, in 2015 9,552 cases and an increase in 2016 amounted to 11,250 cases. From reports of health problems due to smoke and land fires mentioned from 6 Provinces (Riau, Jambi, South Sumatra, Central Kalimantan, West Kalimantan and South Kalimantan) monitored by the Ministry of Health, pneumonia cases in Riau Province are quite high and tend to increase. One indicator to be achieved in the Millennium Development Goals (MDGs) is to reduce infant and toddler mortality[(7)].

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Some research results reveal that environmental conditions influence the occurrence of pneumonia in toddlers. Poor air quality caused by the presence of air pollution sources such as land / forest fires, motor vehicle and industrial exhaust gases containing SO2, NO and NOx pollutants [8]. Waste disposal, the presence of livestock around the residence is a factor associated with pneumonia [9]. Environmental factors in the home such as inadequate ventilation area [10], inadequate floor and wall conditions of the house [11][12], temperature and humidity air in the house that is too low or high has a big role in the pathogenesis of pneumonia [9]. The density of residential in the house, lighting that does not meet the requirements to increase the growth of gram-positive bacteria [13] and the use of mosquito coils and the presence of smokers in the home can be a cause of transmission of pneumonia in toddlers [14][29].

Socio-economic factors also play a role in the occurrence of pneumonia such as working mothers and low family incomes in meeting the living needs of family members. Children who have poor nutritional status, do not get exclusive breastfeeding and are not immunized include factors that influence the onset of pneumonia in toddlers [15][4]. Pneumonia is also influenced by climatic conditions and seasons. Parasites and disease vectors are very sensitive to climatic factors, especially temperature, humidity, and rainfall [16]. Climate change has direct and indirect effects on human health, the direct effects of extreme cold and heat. Extreme rainfall can increase pneumonia cases [17]. Further, to see a picture of the situation of pneumonia along with the factors that influence it, then spatial analysis can help map the distribution of a disease.

Pelalawan Regency is one of twelve districts in Riau Province with pneumonia cases in toddlers is quite high, namely in 2016 there were 155.7% of pneumonia cases in toddlers. Topographically Pelalawan Regency has a low and hilly land structure. Physically part of this area is a conservation area with soil characteristics in certain parts are acidic and is organic soil, brackish ground water, humidity and air temperatures are rather high. The difference in height of an area will affect the differences in climate conditions [16]. Based on the background above, it is necessary to conduct research on the spatial analysis of pneumonia in toddlers based on environmental, individual and behavioral factors in Pelalawan Regency, Riau Province.

Method

This research is a survey research with quantitative analytic methods. This research was conducted in 12 Districts of Pelalawan Regency, Riau Province began in 2019. The population in this study was households with children under the age of 1 to 5 years who lived in Pelalawan Regency, Riau Province. The minimum sample size in this study was 394 respondents, this was calculated using the lemes how formula. The sampling technique in quantitative research is Probability sampling with cluster random sampling technique. The distribution of pneumonia will be analyzed using spatial analysis and the data used consists of spatial and non-spatial data. The statistical method used to analyze data by calculating spatial factors is Geographically Weighted Logistic Regression (GWLR).

Findings and Discussion

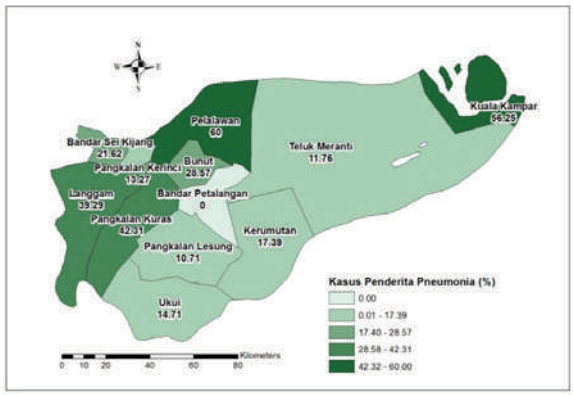


Figure 1. Percentage of pneumonia distribution in toddlers in Pelalawan District

Figure 1 above shows the spread of cases of pneumonia in toddlers in each district. The full model and the reduction model produce the significance of different variables. To compare the best model, the AIC (Akaike Information Criterion) value is used for each model. The model that has the smallest AIC value is the best model. The full model has a value of AIC = 170.21 while the reduction model has a value of AIC = 157,644. The reduction model has a smaller AIC value than the full model so the reduction model is the best model.

Table 1. Estimating the parameters of the final logistic regression model

Variable	Parameter Coefficient Value	Standard Error	p-value
(Intercept)	-11.61	1.979	0.000**
Wall	2.977	0.5279	0.000**
Lighting	1.304	0.4792	0.0065**
Humidity	1.845	0.5007	0.000**
The existence of smokers	2.112	0.6591	0.0013**
Birth weight	0.00259	0.000616	0.000**
Immunization	1.716	0.5025	0.000**
Pseudo R-Square		0.70	
AIC		145	

Note: * = significant at the level 10%, ** = significant at the level 5%

Model Comparison

Table 2. Comparison between models

Model	AIC
Binary Logistics Regression	145.00
GWLR	142.92

The model comparison test in this study was conducted by comparing the binary logistic regression model with the GWLR model based on the AIC value. So, it can be concluded that the GWLR model is the best model.

Table 3. Summary of GWLR Model Parameter Coefficients

Independent Variable	Parameter coefficient value		
	Minimum	Average	Maximum
Intercept	-12.389	-11.6893	-9.4711
Wall	2.55457	2.906877	2.9965
Lighting	1.15773	1.225492	1.5164
Humidity	1.63911	1.887415	1.9591
The existence of smokers	1.94845	2.174809	2.305
Birth weight	0.00199	0.002642	0.0029
Immunization	1.65114	1.715248	1.7968
Pseudo R-Square	0.717		

Table 3 shows the interval coefficient values for each independent variable based on the minimum, average, and maximum values. In addition, the Pseudo R-Square value of 0.717 indicates that the independent variables are able to explain the diversity of pneumonia status by 71.7% while the rest are explained by other variables outside the model.

Map of Pneumonia Distribution in Toddlers Based on Factors Affecting it

The following is a map of the distribution of pneumonia in toddlers based on the type of wall of the house, lighting, humidity, the presence of smokers in the house, birth weight and immunization status in Pelalawan District.

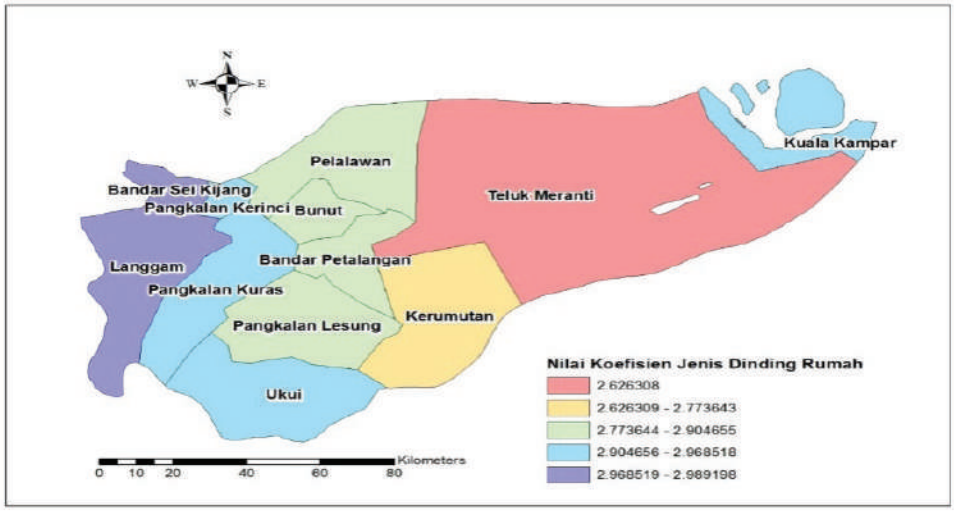


Figure 2. Distribution of Pneumonia in toddlers based on the type of wall of the house

The results of research Li and Sun (2014) said the use of wood in the house can increase the prevalence of respiratory infections in children, especially wood whose quality is not good and thin because it can remove volatile organic compounds in the room. Compared to cement, construction materials including, laminated wood, real wood, and wallpaper positively increase pneumonia risk factors. Composite wood materials emit formaldehyde and have been identified as a source of emissions that worsen indoor air quality [18].

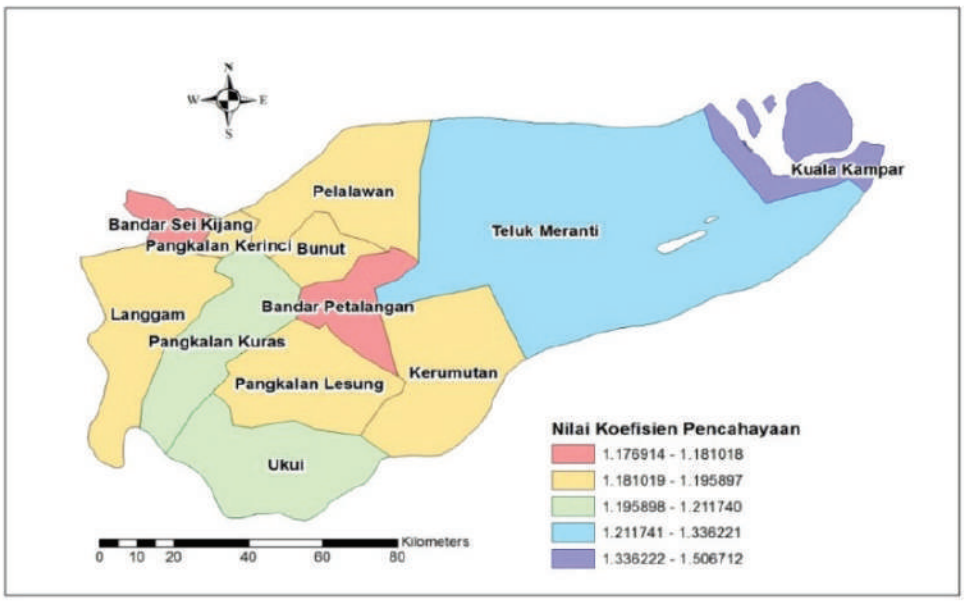


Figure 3. Distribution of Pneumonia in toddlers based on lighting

Rahmiza [18] states that there is a significant relationship between lighting and pneumonia genesis in toddlers. Toddlers who live in homes with inadequate light have a 4.117 times greater risk of developing pneumonia compared to toddlers who live in homes with lighting that meets the requirements. Dust mites and molds support the humid environment. Many mushrooms that produce toxins and irritants that affect respiratory health [20].

A research done by Bornehag et al. mention the problems associated with humidity in the building is strongly associated with respiratory infections in children and adults. Humidity and fungi at home are significant with increased respiratory infections [21]. Measures against housing risk factors can help prevent pneumonia in children [18]. A study of children under 5 years at a teaching hospital in Nepal found that the incidence of pneumonia was four times greater in children living in households with smoking parents [22].

Exposure to indoor air pollution increases the risk of pneumonia in children, which causes around one million deaths globally. Exposure to solid fuels, carbon monoxide (CO) shows a significant relationship with pneumonia in children under 5 years [23]. Studies in Vietnam show that children in Vietnam are exposed to ETC (Environmental Tobacco Smoking) which results in 44,000 excess hospitalizations due to pneumonia each year among children <5 years old [24].

The results of the study Supriatin [25] states there is a significant relationship between birth weight with the incidence of ARI in toddlers. LBW toddlers do not have enough nutrition and protein for the formation of the immune system, so if a toddler breathe unhealthy air will be susceptible to respiratory infections. The results of the study by Ramazeni [26] estimated that 19% of children born with low birth weight in developing countries have pneumonia.

Economic and educational characteristics are also found to be lower in rural areas. The effect of measles immunization on pneumonia based on economic status can be seen that children who get measles immunization and middle-income family members are better protected from pneumonia than children who are immunized against measles and belong to low-income families [27, 28].

Conclusion

1. The highest frequency of pneumonia distribution in toddlers by region in 12 Districts of Pelalawan Regency is the highest in the District of Pelalawan.
2. The spread of pneumonia in toddlers is based on the type of wall of the house, areas with high risk are Meranti Bay District and Crowds Sub-District.
3. The spread of pneumonia in toddlers based on lighting does not meet the requirements is the District of Bandar Seikijang and Bandar Petalangan.
4. The spread of pneumonia in toddlers based on house humidity that does not meet the requirements is Meranti Bay and Kuala Kampar Districts.
5. The spread of pneumonia in toddlers based on the presence of smoker behavior in the house is Kuala Kampar District.
6. The spread of pneumonia in toddlers based on a history of low birth weight is in the Subdistrict of Meranti
7. The spread of pneumonia in toddlers based on incomplete immunization status is Kuala Kampar District

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Forensic Medicine in Indonesia: The Application of Visum ET Repertum in Case Resolution

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Abstract

This research aimed at explaining important comprehension concerning with Forensic medicine in Indonesia: the application of visum et repertum in case resolution. As we know that, the community still have not known and comprehended about how important *visum et repertum* in law is. Moreover, it is often occurred uncompleted law well as the impact of not being supported by *visum et repertum*. This research utilized normative research method, which studied about medicine forensic and visum et repertum. Main material that was used in this research was data in form of health laws and regulations and also other data which were contained in books, journals and various scientific works and they related to the research theme. Furthermore, *visum et repertum* is one of important elements that cannot be abandoned in medical forensic process.

Keywords*: Forensic, Medicine, visum et repertum, Indonesia*

Introduction

Various problems which are occurred in legal field require to the role of medical field for participating in giving consideration. It can be seen from the existence of *visum et repertum* in legal process to stipulate physical and mental condition from either victims or suspects. (1) Various cases which become public debate in Indonesia can be resolved through *visum et repertum.* As in 2018 political year, the case of "Ratna sarumpait" who was sympathizer of one of the presidential candidates claimed to have been persecuted by strangers. Initially, the case was considered to have a correlation with politics, but after the *visum et repertum* was held, it was only a facial operation that was made up as if there had been a persecution. In 2019, there was also a murder case of "Jamalludin" who was a judge in Medan District Court. Initially, it was considered as a case of car accident because the car that he drove plunged into the river. But after being conducted *visum et repertum*, it was discovered that there was a case of premeditated murder that was done by the judge's wife who was helped by two assassins by putting the judge's body in the car to be plunged into the river.

People in Indonesia consider *visum et repertum* is less important in law enforcement process. Whereas, if it is studied more deeply, the *visum et repertum* is an inseparable part from the process in stipulating formal truth and legal material. In evidentiary process at trial court, *visum et repertum* becomes a valid evidence to stipulate clearly and objectively about violation of law that is occurred toward victim or perpetrator. Moreover, it is like stipulating the normal death that is different from the death which is caused by violation of law. Besides, the perpetrator's motive can also be stipulated by *visum et repertum* psychologically regarding why the perpetrator committed murder.

Research Method

This study used normative legal research methods that comprehended law as a positive legal system in laws and regulations. (2) (3) The approach that was used in this study was the philosophy of law. The philosophical

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approach studied the object of this research deeply. Therefore, it could be found the comprehension of fundamental laws. [4] Meanwhile, philosophical studies could provide information, verification, correction, complements and explanations in more detail. [5] The analysis was also conducted descriptively in order to obtain general description of the explanations which were related to visum et repertum. [6]

Discussion

1. Comprehension of *visum et repertum* based on legal practice in Indonesia

Definition of *visum et repertum* in Indonesia actually is not explained clearly in a certain definition. As in Criminal Code (*Kitab Undang-Undang Hukum Pidana (KUHP)*) of Indonesia that relates directly with the use of *visum et repertum*, due to becoming main legal basis in its use, *visum et repertum* does not state that there is a term of *visum et repertum*. Haroen Atmodirono and Njowito Hamdani who defined *visum et repertum* based on Stbl. of Hindi Number 350 Year 1937, which was a written report for justification that was made by a doctor based on an oath about everything that was seen and found on examined object according to the best possible knowledge. [7] Similar thing was also stated according to Abdul Mun'im Idris, *visum et repertum* was a written report from a doctor who had been sworn about what was seen and found in the evidence that was examined and it also included conclusion of the examination for the interests of justice [8].

For clearer comprehension about *visum et repertum,* it is important to know the content of what *visum et repertum* is. The characteristic that is contained in *visum et repertum* that is commonly used in Indonesia is the word "pro justitia" in the upper left corner of paper, which is a juridical requirement as a substitute for stamp duty. [9]

The use of *visum et repertum* usually aims at making clearer incident of a criminal act that is occurred [10]. Therefore, law enforcement officers in their written request toward the doctor state the type of *visum et repertum* that is needed through using format in accordance with the case that is being handled. Several types of *visum et repertum* according to Criminal Code which are commonly used as a proofing tool of criminal cases in Indonesia are:

1) *Visum et repertum* for alive victims. In visum et repertum, there are three divisions of circumstances that need to be explained.

a. Visum et repertum that is given at once. This means that making *visum et repertum* is committed if the person that is asked for *visum et repertum* does not need further treatment for the condition of injuries which are caused by a criminal act. In the context of criminal law, it relates to criminal act of mild persecution as what is regulated in KUHP Article 352.

b. Temporary *visum et repertum*. This means that it is necessary if the person who is requested for *visum et repertum* requires further treatment that relates to injuries which is caused by criminal acts. In the context of criminal law, it relates to criminal acts of ordinary persecution as what is regulated in KUHP Article 351 paragraph (1).

c. Advanced *visum et repertum*. This means that *visum et repertum* is given if the person who is asked for *visum et repertum* wants to leave the hospital due to being healed, forced to go home, moved out from a hospital or die. *Visum et repertum* is usually used for third degree injuries or class A injuries. In criminal law context, it relates to serious criminal persecution as what is regulated in Criminal Code Article 351 paragraph (2) and Article 354 paragraph (1).

2) Visum et repertum for human corpses. *Visum et repertum* is committed for people who have died whose death are suspected due to criminal incidents. Examination way against the human corpses must be conducted by forensic autopsy in order to investigate the exact cause of the death from the person. [11]

3) Visum et repertum for digging corpse. *Visum et repertum* is conducted by digging a buried body whose death is assumed due to criminal act. *Visum et repertum* is conducted against corpses that have or have not been given a *visum et repertum*. Moreover, it is enabled to be conducted *visum et repertum* repeatedly if the results of previous *visum et repertum* is in doubt.

4) Visum et repertum about age. This *visum et repertum* aims at investigating the certainty of a person's age as a victim or a perpetrator. The interest

in determining the certainty of person's age that relates to the victims of a criminal act usually relates to the offenses of morality or other criminal acts whose victims are children as what is stipulated in Law of Indonesia Number 35 Year 2014 concerning the protection of children in Criminal Code.

5) Psychiatric *visum et repertum* which is a report of autopsy that relates to the perpetrator of a criminal act whose soul is suspected of being deformed in their growth or disruption due to illness. This visa is usually given to criminal perpetrator who did criminal acts in out of the limits of normal human beings, such as murder by mutilating victims or criminal acts which are considered sadistic that cannot be done by perpetrators in normal mental conditions.

6) Visum et repertum for the victims of illegal intercourse or morality is a *visum et repertum* that is given for criminal acts in intercourse field that violates law or morality (sexual abuse, sexual assault, and sexual violence).

Visum et repertum actually can not only be used in criminal cases, but also can be used for civil cases, such as; sex change, insurance claims, proof of one's physical and mental health, proof of status of children and offspring. Therefore, *visum et repertum* is an information that cannot be underestimated or ignored in the effort to overcome legal problems. Civil matters which are personally also need valid medical evidence according to applicable laws and regulations. As an example of sex change case is "Lucinta Luna" case which is from man who changes to be woman is a horrendous case in Indonesia in 2019 through *visum et repertum*. Thus, legal certainty that is obtained from the results of medical examination that is conducted by medical experts can be achieved and accounted for its truth.

In other fields, *visum et repertum* can be used to resolve cases or make policies, such as case in state administration field in determining health status of a candidate for President and Vice President, DPR, DPD, Regional Head and DPRD. Although it is government or politic field, if it relates to the matters that explain person's physical and mental state, *visum et repertum* is very required.

2. Legal Basis in implementing *visum et repertum* di Indonesia

Although *visum et repertum* term is not explicitly mentioned in laws and regulations in Indonesia, the matters that relate to the existence of *visum et repertum* have been more talked about in law enforcement process. This can be seen in *staatsblad* (*Stbl*) of Republic of Indonesia State Number 350 Year 1937 concerning "*visa reperta*". According to Latin language, *"visa"* means witness or confession has seen something, while *"reperta"* means report. Hence, if it is explained, it is a report that is made based on witnessing or recognition in seeing something. In STBL of Republic of Indonesia Number 350 Year 1937, it is stated that *"visa reperta"* from doctors is made based on the doctor's oath which was stated when they completed studies in Indonesia, as well as special oaths as what is stated in *staatsblad* (STBL) of Republic of Indonesia State Number 350 Year 1937.

Government Regulation of Republic of Indonesia Number 26 / 1969 concerning with doctor's oath which states that medical record that is made by doctors in writing can be used as evidence because it has legal force as what is explained in Article 187 of Criminal Code. Both doctor's statements in medical records are valid evidences which relate to doctor's role in uncovering criminal cases.

Criminal Code is the most important legal basis in the implementation of the use of *visum et repertum* because criminal cases which are occurred strongly require the existence of *visum et repertum* as a formal condition and also as material evidence. Furthermore, there are various Articles in Criminal Code which closely relate to visum et repertum, [12] such as:

1) Article 44 of Criminal Code concerning with the need for *visum et repertum* against criminal perpetrators who are suspected of suffering from mental illness or having mental disability in their growth and development.

2) Articles 133, 134, 135, and 179 of Criminal Code concerning with the role of doctors as experts in examining criminal cases.

3) Articles 186 and 187 of Criminal Code

concerning with expert's (doctor's) statement as legal evidence.

4) Articles 284, 285, 286, 287, 289, 290, 292, 293 and 294 of Criminal Code concerning with morality which need to be proven by doctor's statement.

5) Articles 287, 288, 290, 291,292,293, 294, 295, 300 and 301 of Criminal Code concerning with Children and Law Number 35 Year 2014 concerning with the protection of children that is associated with children as victims of criminal act in morality field and the Law of Republic of Indonesia Number 11 Year 2012 concerning with Children juvenile justice system which relate to criminal perpetrator toward children. [13]

6) Articles 338, 339, 340, 341, 342, 343, 344, 345, 345, 346, 347 and 348 of Criminal Code concerning with crimes against lives.

7) Articles 351, 352, 353, 354 and 355 of Criminal Code concerning with persecution.

8) Articles 359 and 360 of Criminal Code concerning with negligent acts that cause death or injury for others as what is regulated in Law of the Republic of Indonesia Number 22 Year 2009 concerning with traffic and road transportation.

Legal basis in the implementation of *visum et repertum* that relates to Criminal Code (KUHP) is Code of Criminal Procedures (*KUHAP (Kitab Undang-Undang Hukum Acara Pidana*) which is:

1) Article 133 paragraph (1) in Code of Criminal Procedure which stipulates that juctice expert or doctor and / or other experts for the purposes of investigation and justice provide expert's statements (report) in conducting examination against victims of criminal acts who are injured, poisoned, or dead.

2) Article 133 paragraph (2) in Code of Criminal Procedure also describes the information (report) that is given by judicial medical experts which is known as expert's statements and it is a legal evidence in law.

3) Article 134 in Code of Criminal Procedures explains the need for post-mortem. In post-mortem process, it involves a doctor, thus, the doctor's statement from the results of the post-mortem can be used by investigators to conduct further legal process in accordance with Article 133 in Code of Criminal Procedure.

4) Article 135 in Code of Criminal Procedure stipulates that investigators for legal purposes can conduct digging corpses as what is stated in Article 133 paragraph (2) and Article 134 paragraph (1).

Ministerial Decree of Justice in Republic of Indonesia Number M.01.PW.07.03 Year 1982 becomes guidelines for the implementation of Article 133 paragraph (2) in Code of Criminal Procedure concerning with statements from judicial medical experts and doctor's statements. In that regulation, actually it implies the importance of doctor's statements, both statements from judicial medical experts and from doctor's statement which is not from judicial medical experts. According to Article 10 in Letter of Decree of the Minister of Justice in Republic of Indonesia Number Mo4.UM.01.06 Year 1983 states that examination result from the science of Medical jurisprudence is known as *visum et repertum*. Thus, referring to the Decree of the Minister of Justice, the examination of Medical jurisprudence science by doctors which is usually in writing form is known as *visum et repertum*.

Regulation from the Minister of Health in Republic of Indonesia Number 749a / Men.Kes / Per / XII / 1989 concerning with medical records also can be used as a legal basis for the implementation of *visum et repertum.* This is because in medical record, it contains files that contain notes and documents about the patient's identity, examination, treatment, actions, and other services toward the patients in health care facilities. The medical records that contain notes about patient's condition when accepting health services or certain medical actions, can function as evidence in the examination of criminal cases. Although medical record cannot replace the position of *visum et repertum*, the medical record can be used as an evidence of expert certificate. This function is almost similar to *visum et repertum*. Medical record which is used as evidence can be categorized as a letter of evidence. There are several articles in Regulation of Minister of Health in Republic of Indonesia Number 749a / Men.Kes / Per / XII / 1989 concerning medical records that closely relate to *visum et repertum,* such as:

1) Article 13 letter (b) which states that medical records can be used as evidence tool in legal cases.

2) Article 11 paragraph (2) which states that chief of health service facility can explain clearly the contents of medical record without the patient's permission based on applicable laws and regulations.

3) Article 9 which states that medical record file belongs to the health service facility, while, the content of medical record belongs to the patient. The investigator can request a copy of the medical record to complete the evidence that is needed in legal case (criminal case).

Visum et repertum is also regulated in Regulation of the Chief of Indonesian National Police Number 10 / 2009 concerning with the procedures and requirements for requesting an inspection of criminal technical of the crime scene and criminal laboratory for the evidence to forensic laboratory of the Indonesian National Police. In this regulation, there are provisions that regulate forensic field in assessing evidence of a criminal act [(14)], which are:

1) Article 1. Police has a criminal forensic laboratory to conduct investigation that relates to evidence for the criminal acts. In conducting activities in forensics fields, the police can involve experts from doctors.

2) Article 2 which relates to forensic investigations aims to conduct scientific proof regarding the criminal act.

Regulation from Chief of Indonesian National Police Number 12 Year 2011 concerning police medicine also explains matters that relate to *visum et repertum*, which are:

1) Article 1 relates to police forensic medicine in conducting investigations against evidence for the interest of law and justice.

2) Article 5 relates to police medical activities which include forensic medicine.

3) Article 6 relates to physical and mental identification of humans in forensic medical activities.

4) Article 10 relates to the ability of police medicine in conducting forensic medical activities.

Conclusion

Visum et repertum is an important thing in law enforcement process because it stipulates legal and formal truth requirements. Without any statement letter of *visum et repertum*, legal case cannot be processed legally because there are no elements which are suspected of violating law. Through *visum et repertum*, the cause of law violation that is committed to the victim by the perpetrator can be known because *visum et repertum* is a written report from a doctor who has been sworn about what is seen and found in the evidence that is examined and it also includes the conclusion of the examination for the interest of justice. The implementation of *visum et repertum* has been regulated in several laws and regulations in Indonesia, thus, the legal basis is strong. Even, *visum et repertum* itself becomes a formal and material legal requirement, which means that the use of *visum et repertum* in legal process does not violate the rules, even, it becomes a legal obligation.

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A Root Cause Analysis of Prolonged Waiting Times for Compounded Prescriptiobs at an Indonesian Private Hospital

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Abstract

Background: Waiting times have been shown to contribute to patient satisfaction with hospital services. Previous studies investigated long waiting times in the hospital, one of which was the time it takes to prepare compounded prescriptions in pharmacy unit. However, few studies have explored the main causes of prolonged waiting times for compounded medications in the hospital. This study aimed to understand the causal factors that influence prolonged waiting times for compounded prescriptions in Indonesian private hospital. **Methods:** A root cause analysis was used to identify the root cause of a reoccurring issue in pharmacy unit. Data were collected from hospital documents, observations, and interviews of 15 representatives from the hospital directors, head of pharmacy department, purchasing staff, pharmacy storage manager, other pharmacy staff, and service quality manager. **Results and Discussions:** This study identified possible causes of prolonged waiting times for compounded prescriptions which include drug shortage, human resources shortage, waiting times for drug preparations, and duration of health insurance coverage confirmation. In addition, this study determined the actual causes of the problem, such as managerial, and health insurance factors which in turn provide suitable recommendations and solutions. **Conclusion:** It can be concluded that there were four key factors to the success of hospital pharmacy services: drug and human resources management, waiting times for drug preparations, and duration of health insurance coverage confirmation. It is recommended that hospital management could optimize drug formulary management, improve coordination in drug distribution system to patients, and redesign waiting room in the hospital.

Keywords: *Root Cause Analysis, Waiting Times, Compounded Prescriptions, Private Hospital*

Introduction

Pharmaceutical services play an important role on preventing medication errors in the hospitals by preparing drug prescriptions, monitoring, and evaluating side effects of drugs[(1)]. Many factors influenced the quality of hospital services including prolonged waiting time when pharmacists and pharmacy technicians prepare medications. A study by Alam *et al*. revealed a variety of factors related to prolonged waiting time in the pharmacy unit, such as employee management system, employee workflows, available resources, and pharmacy unit design[(2)]. Competent pharmacists, drug insurance coverage, heavy workloads, and pharmasicts' skills in managing prescriptions were also main problems affecting the quality of pharmacy services[(3)].

There were four major big issues that pharmacists face right now: (1) difficulties in reading medical doctors' prescriptions as they are manually written, (2) unavailability of patient classification and coordinating care for patients with complex needs, (3) the pharmacy unit need to provide services for all patients in the hospital, (4) a lot of unclaimed prescriptions which might affect waiting time, and (5) poor pharmacy unit

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design[4]. In addition, Fauzia *et al.* stated that heavy workloads in pharmacy unit influenced patient waiting time[5]. Besides that, prolonged waiting time happened due to the fact that there were incomplete information with regards to drug prescriptions and drug shortage (70%), incorrect drug doseages (7.61%), and inadequate drug instruction (8%)[6]. Therefore, a better quality management tools is needed to improve the efficiency of pharmacy services.

Waiting times have been shown to contribute to patient satisfaction with hospital services. Previous studies investigated long waiting times in the hospital, one of which was the time it takes to prepare compounded prescriptions in pharmacy unit[5],[7]. However, few studies have explored the main causes of prolonged waiting times for compounded medications in the hospital. This study aimed to understand the causal factors that influence prolonged waiting times for compounded prescriptions in Indonesian private hospital.

Materials and Methods

The pharmacy unit provides pharmaceutical services for inpatients and outpatients in the hospital. A root cause analysis was used to identify the root cause of a reoccurring issue in pharmacy unit. Additionally, a root cause analysis is a strategic approach to understand the causal factors and determine potential solutions[8]. Data were collected from hospital documents, observations, and interviews in a face-to-face setting of 15 representatives from the hospital directors, head of pharmacy department, purchasing staff, pharmacy storage manager, other pharmacy staff, and service quality manager. Interviews and observations were conducted as an approach to understand the causal factors that influence prolonged waiting times for compounded prescriptions in Indonesian private hospital. Data were then extracted, summarized, and analyzed to identify the key points. Hospital documents were other forms of data which provided factual information as a basis for reasoning in root cause analysis.

Results and Discussion

The results of the study explained the pharmaceutical care services which include the management of pharmaceutical preparations (selection, planning of needs, procurement, reception, storage, distribution, destruction and withdrawal, control, and administration), and clinical pharmacy services (assessment and prescription services, tracking drug use history, drug reconciliation, drug information services, counseling, patient visits, drug therapy monitoring, drug side effects monitoring, drug use evaluation, sterile dispensing supplies, and monitoring blood drug levels). Some types of services developed in accordance with quality management were new inpatients visits, Dose Dispensing Unit (DDU), and Pharmacy Intravenous Admixture Service (PIVAS).

This study identified possible causes of prolonged waiting times for compounded prescriptions which include drug shortage, human resources shortage, waiting times for drug preparations, and duration of health insurance coverage confirmation. In addition, this study determined the actual causes of the problem, such as managerial, and health insurance factors which in turn provide suitable recommendations and solutions.

Table 1 showed the average number of patients per day within the last five years from 2015-2019, in which both outpatient and inpatient service users continue to increase. Pediatric Clinic accounts for the highest number of patients among all clinics, and the number was relatively stable. Growth and Development Clinic which provides services for children also continues to increase. Those who visited these two clinics were generally private health insurance customers. Hospital management maintained patient loyalty by developing image of the hospital, so various efforts were made to increase patient satisfaction, including waiting time in compounding prescriptions. Doctors in the Neurology and Medical Rehabilitation Clinic also often prescribe drugs in the form of capsules, because most of the patients were elderly who need dosage adjustments, either due to decreased organ function or due to polypharmacy tendencies.

Table 1: Average Number of Patients Per Day

Year	Obstetric Gynecology Clinics	Pediatric Clinic	Dental Clinic	Medical Rehab Clinic	Growth & Development Clinic	Neurology Clinic	Other Types of Clinics	Emergency Department
2015	53.9	97.39	19.38	16.92	11.71	0.8	42.38	50.45
2016	53.96	105.75	28.13	17.4	13.79	0.85	41.34	60.78
2017	62.07	108.56	32.8	40.76	20.37	1.81	49.93	65.55
2018	69.59	104.57	41.44	56.74	29.74	13.84	125.43	70.44
2019	63.81	110.85	45.29	95.41	39.72	46.36	258.31	85.78

Table 2: Indicators of Hospital Service Quality

Year	Categories				
	Number of Beds	Bed Occupancy Ratio (%)	Turn Over Interval	Average Length of Stay	Net Death Rate (%)
2015	69	52.38	3.31	3.6	2.8
2016	69	67.94	1.76	3.7	4.3
2017	85	50.56	3.37	3.35	2.86
2018	95	51.96	3.02	3.05	3.62
2019	95	62.71	1.71	2.87	1.39

As can be seen in Table 2, the number of beds provided by the hospital and the level of hospital service quality also continues to improve. With the increase in the number of patients undergoing hospitalization, the pharmacy staff workload also increased, especially for patient visits, DDU, and PIVAS. Along with the increased utilization of hospital services, the quantity of activities in this pharmacy unit has also increased.

Table 3: Productivity Growth of Pharmacy Unit in 2015-2019

Year	Number of Clinical Pharmacists	Number of Pharmacist Technicians	Average Number of Prescriptions Per Day	Private Health Insurance Prescrip-tions	Public Health insurance Prescrip-tions	Inpatient DDU/ PIVA* (item/ day)	New Inpatient Visits Per Day
2015	3.5	12	546	-	-	11.87	2.9
2016	4.5	14	642	-	-	13	3.76
2017	5.5	17	659	1.02	17.25	16.13	3.9
2018	5.5	21	930	6.54	116.73	17.67	5.3
2019	6.5	21	1,459	4.12	193.04	20.63	10.72

*DDU/PIVA: Dose Dispensing Unit /Pharmacy Intra Venous Admixture Service

Table 3 illustrated productivity growth of pharmacy unit in 2015-2019 and several other pharmaceutical activities. In 2015, pharmacists were responsible for managing 546 prescriptions per day, in 2019 increased to 1459 prescriptions per day. In addition, there are currently additional tasks in the form of inpatient visits, UDD, and PIVAS.

A root-cause analysis in Malaysia showed several root problems that result in prolonged waiting times in pharmacy services: (1) the number of inexperienced human resources who do not understand the workflow, (2) lack of human resources, (3) the number of clinics that operating at the same time, (4) handling prescription errors, (5) poor steps in the dispensing procedure to ensure its safety, and (6) the presence of drugs that require special treatment, such as psychotropics that require separate records[9]. Meanwhile, a study in Indonesian hospital showed that the process that required adequate human resources in pharmaceutical services was drug preparation. The lack of available human resources causes medication errors and extends queue time[10].

Meeting the needs for facilities and infrastructure at the X Hospital Pharmacy Installation, such as the expansion of the compounding area and Pharmacy Warehouse, also requires a large amount of cost. Preparation in this direction has been carried out by the Management of RS X, and will be completed in 2020. Because of that, now another alternative solution is needed to overcome the problem of not achieving the standard time for the provision of this powder.

Arrangement of human resources work schedules, electronic prescribing, redesign of service workflows were carried out in several countries in an effort to reduce waiting times for pharmaceutical services. In Malaysia, Value Added Service (VAS) has been proven to increase the number of patients receiving services in less than 30 minutes, from 83.2% to 90.3% after being promoted massively within 6 months. VAS is a collection of innovative dispensing systems that provide options for patients to take their medicine. Broadly speaking, this consists of the Integrated Drug Dispensing System (SPUB), Drive Through Pharmacy, Courier Service, and SMS and Take Service[9]. Reducing pharmacists workload and enhancing doctors' manual prescriptions could considerably decrease medication errors as well as increase the quality of drug prescriptions[11-13]. Managing heavy workload in pharmacy unit is not easy, therefore a multidisciplinary teamwork is important to manage the issues. We need competent pharmacists to perform specific tasks and manage the complex process in the pharmacy unit[14-19]. Redesign waiting room in the pharmacy unit could affect overall patient experience in the hospital. The entire space in this room should incorporate comfort components such as sunny and clean rooms, comfort furniture and layout, visual and audio stimulation to calm patients and families[20].

Various things above can be considered by hospital management tpolicies and procedures to improve patient satisfaction in relation to pharmaceutical services. Based on the existing situation and conditions, it can be recommended several solution options that can be further studied: drug formulary management, improve coordination in drug distribution system to patients, and redesign waiting room in the hospital. After implementing potential solutions, it is suggested that the hospital management should make an analysis of its impact on the hospital standards to be achieved. This will be a documentation of best practices that can be used by other hospitals that face similar problems.

Conclusion and Acknowledgement

It can be concluded that there were four key factors to the success of hospital pharmacy services: drug and human resources management, waiting times for drug preparations, and duration of health insurance coverage confirmation. It is recommended that hospital management could optimize drug formulary management, improve coordination in drug distribution system to patients, and redesign waiting room in the hospital. We would like to express our gratitude to Master Program of Hospital Management, Faculty of Medicine, University of Brawijaya, and Dr. Radjiman Wediodiningrat Lawang Psychiatric Hospital for their support during the study.

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Rehabilitation Policy for Drugs Abuse in Indonesia

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Abstract

Narcotics Abuse and Distribution are the main causes of the continuing number of narcotics addicts in Indonesia. Therefore, the Indonesian government has formulated a policy to deal with narcotics crimes, which will allow the handling of narcotics addicts and non-narcotics addicts. This article discusses the rehabilitation policy for narcotics addicts in Indonesia. This research is a normative juridical research. Designed using the statute approach and conceptual approach. The results of the research show that: first, the issue and distribution of narcotics are narcotics crimes; Second, Indonesia makes a policy regarding the handling of narcotics addicts and non-narcotics addicts; Third, the means to determine someone in narcotics or not is done through an assessment that is governed by an assessment made through a Joint Regulation of the relevant state institution.

Keywords: *Indonesian Drugs Policy, Rehabilitation, Drugs Abuse*

Introduction

Problems in society that require special attention are the abuse and illicit trafficking of narcotics. Narcotics are used in a limited way in the medical world, but recently there has been a deviation in their function and use. The use of various types of narcotics and other addictive substances is currently increasing, especially among the younger generation.[1]

Abuse and illicit trafficking of narcotics is an extraordinary crime. Crimes that are qualified as extra ordinary crimes are then the responsibility of the state (orga omnes obligation) to try them, because extra ordinary crimes fall into the domain of humanist generic hosts or enemies of all humanity. The Head of the National Narcotics Agency (BNN) in the End of 2016 Press Release said that the abuse and illicit trafficking of narcotics is an extraordinary crime that threatens the world and can be used as one of the weapons in the proxy war to paralyze the power of the nation. Therefore, this crime must be eradicated. Narcotics crime is a crime that has a large and multi-dimensional impact on social, cultural, economic and political as well as the enormity of the negative impact caused. [2]

The sanction provisions stipulated in Law Number 35 Year 2009 concerning Narcotics adhere to a double track system in the form of criminal sanctions and sanctions for actions. Rehabilitation is a form of action sanction. However, based on empirical facts on the ground it shows that judges tend to impose sanctions on prison addicts. As a result, narcotics addicts languish in the Penitentiary (Lapas) without being given the opportunity to be rehabilitated, so that the implementation of rehabilitation has not been running optimally. At present the number of prisoners in Indonesia reaches 23,779 people who are drug abusers undergoing imprisonment in correctional facilities.[3]

This condition results in other problems such as the burden of correctional institutions, hereinafter abbreviated as Lapas to over capacity,[4] Lapas actually

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becomes a safe place for drug abuse and the emergence of other criminal acts caused by drug abuse in Lapas, besides that drug trafficking is also rife in Lapas and even several times drug production is found in Lapas.

Therefore there must be a new paradigm in dealing with drug addicts. In dealing with drug addicts, law enforcement officials must be oriented to sanctions in the form of rehabilitation to save their future. To be able to function the role of judges in deciding or establishing rehabilitation, it needs support from other law enforcement officials. Of course this must be based on mutual understanding and agreement that drug abuse is a serious problem for the nation and the enemy of the nation. The government and law enforcement officials must unite to unite the vision and mission to tackle drug abuse in order to realize the noble ideals of the nation to make a healthy generation of the nation.

Research Method

The research method used in this study is a normative juridical, statutory approach, conceptual approach, and historical approach. The specification of this research is descriptive research. The data used in the form of secondary data with the main material in the form of primary legal material (legislation) and secondary legal material in the form of literature textbooks and scientific journals. The data obtained were analyzed using qualitative analysis.

Result and Discussion

Criminal Policy Against Narcotics Crimes in Indonesia

The problem of overcoming crime in the community cannot be separated from the context of the discussion regarding criminal politics. The meaning or definition of criminal politics itself, according to Sudarto, is a rational effort by the community in tackling crime. Efforts to tackle these crimes can be both penal and nonpenal in nature.[5]

Criminalization policy is a policy in determining an act that was not a criminal offense (not convicted) into a criminal act (an act that can be convicted). So in essence the criminalization policy is part of the criminal policy by using the means of criminal law (penal) so that it is part of the penal policy.[6]

The use of legal remedies, including criminal law, as an effort to overcome social problems, including in the field of law enforcement policies. In addition, because the aim is to achieve public welfare in general, this law enforcement policy is also included in the field of social policy, namely all rational efforts to achieve public welfare. As an issue that is a policy issue, the use of criminal law is not a necessity. There is no absolute in the field of policy, because in essence in the policy problem people are faced with the problem of policy assessment and selection of various alternatives.[7]

Criminal law policy is essentially also a policy of criminal law enforcement. Criminal law enforcement policy is a series of processes that consist of three policy stages. First, the stage of formulative policy or legislative policy stage, namely the stage of formulation / formulation of criminal law. The second stage, the judicial / applicative policy stage, namely the stage of applying criminal law. The third stage, the stage of administrative / executive policy, namely the stage of implementation / execution of criminal law.[8]

M. Cherif Bassiouni called the three stages in terms of: the stage of formulation (the legislative process), the application stage (the judicial process) and the execution stage (the administrative process). The first stage (legislative policy) which is part of the author's current research study is the "in abstracto" law enforcement stage, while the second and third stages (the judicial and executive policy stage) are the "in concreto" law enforcement stage.[13]

The three stages of the criminal law enforcement policy mentioned above contain three legislative powers or authorities that formulate or determine actions as acts that can be convicted (criminal acts) and criminal sanctions, powers / authorities of law application by law enforcement officials, and power / authority to execute or carry out concretely law by the authorized apparatus / agency. These three powers / authority are similar to the term used by Masaki Hamano when describing the scope of jurisdiction.[9]

An integral legislative policy in the field of criminal law enforcement does not mean that it must be set forth in one book of the law. Various laws such as the present (there are material criminal laws inside and outside the Criminal Procedure Code; and there are criminal

implementing laws). Errors / weaknesses at the legislative / formulation policy stage are strategic mistakes that can become obstacles to "in concreto" law enforcement efforts. Strategic policies provide the basis, direction, substance, and limits of authority in law enforcement to be carried out by the judiciary and executive authorities. The strategic position has the consequence that the weakness of the criminal law formulation policy will affect the criminal law enforcement policy and crime prevention policy.[14]

Narcotics crime needs special countermeasures. Drug abuse and drugs (drugs) are closely related to illicit trafficking as part of the world of international criminal acts. The illicit mafia supplies drugs so that people are dependent so that the amount of supply increases. Establishing a relationship between the dealer / dealer and the victim makes it difficult for the victim to break away from the dealer / dealer, not infrequently the victim is also involved in illicit trafficking due to the increasing need and their dependence on drugs.[14]

Seeing the rampant abuse of narcotics in the community, it is feared that it will hamper national development with material-spiritual aspects. The dangers of drug use have a profound effect on the country, if there is massive drug use in the community, then the Indonesian nation will become a sick nation, if so the country will be fragile from the inside because national security is declining.[10]

The use of criminal law as a means of preventing and overcoming the abuse of narcotics is actually a last resort (ultimum remedium) given the nature of criminal law that can cause misery or suffering. In the framework of criminal law, Law No. 35 of 2009 concerning Narcotics as a means of punishment in the effort. Some actions related to narcotics abuse have been criminalized as a criminal act with various criminal threats attached to them. However, there is one interesting thing about this issue is the opportunity for law enforcers (judges) to order narcotics addicts to undergo treatment and / or treatment through rehabilitation (for those who are guilty or innocent), and for those who are guilty the treatment period is counted as a period serving a sentence (Article 103). Article 103. The article stipulates that:

(1) Judges examining Narcotics addicts cases can:

a. decide to order the person to undergo medication and / or treatment through rehabilitation if the Narcotics addict is proven guilty of Narcotics crime; or

b. determine to order the person to undergo medication and/or treatment through rehabilita-tion if the Narcotics addict is not proven guilty of Narcotics crime.

(2) (2) The period of treatment and / or treatment for Narcotics Addicts as referred to in paragraph (1) letter a is calculated as the period of serving sentence.

Judges are given full authority to determine whether an offender is an addict or not, and are given the freedom to make a decision to provide rehabilitation or not. This authority actually began at the time the case reached the level of investigation, but based on this law, only the judge has the right to decide. The problem that arises is who is referred to as an addict.[11]

Article 1 number 13 determines that what is meant by a narcotics addict is a person who uses or abuses narcotics and is in a state of dependence on narcotics, both physically and psychologically. This definition gives rise to a broad interpretation, because every person who is arrested will always put forward the argument that he is an addict, not a narcotics criminal to get treatment or decision as determined in Article 103. Law enforcement (police, prosecutors, judges) must be observant in seeing things This is so that it is not deceived by narcotics offenders.

Joint Regulation: Determining Instrument for the Narcotics Addict Rehabilitation in Indonesia

The 1988 Vienna Convention produced a political declaration on narcotics disaster management with a balanced approach between the legal approach and the health approach. The Narcotics Law which is in effect when balancing these two aspects. In addition to increasing criminal threats, the Narcotics Act also emphasizes the rehabilitation of addicts and / or narcotics abusers. In order to optimize the efforts of the legal approach and the health approach in a law enforcement process, a Joint Regulation was born in handling drug addicts and drug abusers to be rehabilitated.

This understanding and agreement from the government and law enforcement officers was then realized through a Joint Regulation of the Chairperson of the Supreme Court of the Republic of Indonesia, Minister of Law and Human Rights of the Republic of Indonesia, Minister of Health of the Republic of Indonesia, Minister of Social Affairs of the Republic of Indonesia, Attorney General of the Republic of Indonesia, Chief of Police of the Republic of Indonesia, Head of the National Narcotics Agency of the Republic of Indonesia Number: 01 / PB / MA / III / 2014, Number: 03 of 2014, Number: 11 of 2014, Number: 03 of 2014, Number: PER-005/A/ JA / 03/2014, Number: 1 of 2014, Number: PERBER/01/ III/2014 / BNN concerning the Handling of Narcotics Addicts and Victims of Narcotics Abuse into the Rehabilitation Institution, which is referred to hereinafter referred to as the Joint Regulation. Thus drug addicts no longer lead to sanctions imprisonment but rather lead to rehabilitation, because sanctions for addicts agreed in the form of rehabilitation.

Based on the Joint Regulation, an integrated assessment team was formed at the central, provincial, district/ city levels consisting of a team of doctors and a legal team. The team then carried out legal analysis, medical analysis and psychosocial analysis and made a rehabilitation plan that included how long rehabilitation was needed.

The results of the assessment as a complete case file function as information such as visum et repertum. The establishment of this Joint Regulation, among others, aims to be a technical guide in the handling of narcotics addicts as suspects, defendants, or prisoners in undergoing medical rehabilitation and / or social rehabilitation. Besides aiming also that the process of medical rehabilitation and social rehabilitation at the level of investigation, prosecution, and trials can be carried out synergistically and integrated.

Basically the act of using and abusing narcotics is a criminal act, so that the perpetrators should have carried out a legal process as is the case for law enforcement against other criminal cases. But for now law enforcement against addicts does not always use a means of punishment, because there is a need for rehabilitation for addicts who report themselves to certain agencies the recipient must report, as determined in Article 54 of Law Number 35 Year 2009 concerning Narcotics.

According to Mudzakir[14], shifting the form of punishment from corporal punishment to action punishment is a process of depenalization. Depalization is due to the development or shift in the value of law in people's lives that affects the development of legal value in the norms of criminal law. The act is still a despicable act, but does not deserve to be subjected to severe criminal sanctions, more precisely subject to minor criminal sanctions or actions.

With the Joint Regulation, the process of investigation of addicts and drug abuse has changed. For addicts and narcotics abuse caught or caught red-handed, an assessment will be carried out by an integrated assessment team consisting of a team of doctors consisting of doctors and psychologists, as well as a legal team consisting of elements of the National Police, BNN, Attorney General and Kemenkumham. The task of the integrated assessment team is to stop the illicit trafficking of narcotics and narcotics abuse and conduct medical, psychosocial assessment and analysis and recommend a therapeutic and rehabilitation plan for drug addicts and drug abuse.

The integrated assessment team also has several authorities which include several things such as requests from investigators to analyze the role of a person who is captured or caught red-handed; the role of someone as a victim of abuse, addicts or narcotics dealers; determine the criteria for the severity of narcotics users according to the type of content consumed; recommend treatment and rehabilitation plans for addicts and victims of narcotics abuse.

Conclusion

Drug addicts must be rehabilitated. Narcotics addicts who report voluntarily, will be directed to the IPWL for an assessment to determine whether or not they can be rehabilitated. Handling of suspected addicts arrested is to conduct an assessment by the integrated assessment team, the case will still be further processed by law. However, in the case of a suspected addict who also doubles as a BNN dealer will not provide an opportunity for assessment.

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Effect of Acridine Orange on Pathogenicity of *E. coli* Isolated from Urinary Tract Infection Patients

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Abstract

A total of (108) isolate from Urinary tract infection (UTI) patients, only (47) isolate gave a positive result (43.51%) after a number of morphological and biochemical characterization. Our result showed only 28 isolate have the ability for protease production and haemolysin production (59.5%), and *E. coli* no.6 was the highest protease production isolate (24mm of lysis area) while the *E. coli* no.18 was lowest protease production (12 mm of lysis area). After incubtion on skim milk agar medium at 37°C for 18h and with (100%) haemolysin activity for all 28 isolates after growing on blood agar medium. In our study, first attempt made on the effect of acridine orange (0.1%) as a curing agent on virulence factors of pathogenic bacteria (both protease and haemolysin productin) by highly producer *E. coli* no.6 at concentrations (0, 10^{-1} to 10^{-10}) and the results showed that *E. coli* no.6 loss the protease and haemolysin productin at concentration of (10^{-1} to 10^{-4}) while little and normal activities were observed at concentration of (10^{-5} to 10^{-10}) of acridine orange. Also, this study was investigate the agarose - gel electrophoresis of both cured and normal cells and the results showed the presence of both chromosomal and plasmid bands in the normal cells and only the presence of chromosomal band for the cured cells *E. coli* treated with acridine orange at concentrations of (10^{-2} to 10^{-4}).

Key words: *Acridine orange, E. coli, Protease, Haemolysin*

Introduction

Urinary tract infection (UTI) could be defined as the presence of microorganism in property collected specimen of Urine bacteriuria more than 10^5 bacteria per ml of urine [1]. The symptoms of UTI are frequent urination, flank, pain, dysuria, bumming while urination and some time fever In general bacterial infection of urinary tract are the commonest cause of both community acquired and nosocomial infection in patients admitted to hospital occurred Secondly after respiratory tract infection [2].

The infection are a common source of morbidity as much as twelve per 1000 consolation in general practice are on account of them [3]

The Genus *Esherichia*

The genus Escherichia is related to the family Enterobacteriacae, is a gram-negative, facultative anaerobic , rod-shaped , coli form bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms) *E. coli* and other facultative anaerobes constitute about of gut flora, [4] and fecal oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination. A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for extended periods outside of a host.

Uropathogenic *E. coli* (UPEC) is one of the main causes of urinary tract infections.[5] It is part of the normal flora in the gut and can be introduced in many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices. Anal intercourse can also introduce this bacterium into the male urethra, and in switching from anal to vaginal intercourse, the male can also introduce UPEC to the female urogenital system [5].

Hemolysis can be identified by their ability to lyse red blood cells in vitro [6]. One way hemolysin lyses erythrocytes is by forming pores in phospholipids bilayer. Other hemolysis lyse erythrocytes by hydrolyzing the phospholipids in the bilayer. Hemolysin is normally secreted by the bacteria in a water-resoluble way. These monomers diffuse to the target cells and are attached to them by specific receivers. After this is already done, they oligomerize, creating ring-shaped heptamer complexes. Hemolysin can be segregated by many different kinds of bacteria such as *Staphylococcus aureus*, *Escherichia coli* or *Vibrio parahemolyticus* among other pathogens. We can take a look at the bacterium *Staphylococcus aureus* to study more precisely the formation of these pores. *Staphylococcus aureus* is a pathogen that causes many infectious diseases such as pneumonia and sepsis. This ring-shaped complex we'll take a look at, is called staphylococcal alpha-hemolysin pore. In nature, what happens with these pathogens is that, in its fight for resources, bacterium.pores. *Staphylococcus aureus* is a pathogen that causes many infectious diseases such as pneumonia and sepsis. This ring-shaped complex we'll take a look at, is called staphylococcal alpha-hemolysin pore [6].

Protease

Are extracellular proteolytic enzyme produced by several species 01 pathogenic bacteria, both gram positive and gram negative. Several line of evidence suggest that the enzymes play a role in bacterial virulence Especially, by *E.coli*, gonorrhea , bacterial meningitis ,and upper respiratory infections this enzyme can be detected quantitatively by ELISA and by measuring the lysis aria after plating on skim milk agar media [7].

Acridine Orange

Acridine Orange (AO) is an organic compound used in nucleic acid selective fluorescent cationic dye useful for cell cycle determination at 502nm &emission maximum shifts to 460nm (blue). A.O will also enter acidic compartment such as lysosomes & become pronated & sequestered in this low pH conditions . the dye will emit orange light Thus Acridine orange can be used to identify engulfed apoptotic cell , because it will fleuoresce up on engulfment the dye is often used in epifluorescence microscopy & cytometry analysis of cellular physiology &cell cycle status [8].

Materials and Methods

Sample collection

Fourty seven bacterial samples used in this study were obtained from midstream urine of U.T.I patients.

Bacterial Diagnosis

Initial diagnosis depending on gram reaction and morphological characteristics of the colonies based on bacterial growth on macConkey agar , blood agar and EMB (Eosine methylene blue medium). , as well as the number of biochemical test and VITEK2 compact system)(Biomeriex –France).

Acridine orange A.O.

In this part of study , the highly *E. coli* protease producer were used for studying the effect of curing agent A.O. concentration of (0, 10^{-1} to 10^{-10}) by dissolving 0.1 mg of acridine orange in 10ml D.W. then the above concentration were prepared and added to a plate of skim milk agar and blood agar seeded with alawn of nutrient broth(18-24 h) culture of *E. coli* no.6. Then the protease production and haemolysin production were measured at each concentration [9].

Protease production [10]

Protease activity were measured before and after acridine orange by measuring the diameter of lysis area(in mm) after growing of isolates of (18-36h) of incubation at 37°C on skim milk agar media of all isolates using well methods.

Haemolysin production [10]

Haemolysin production were measured before and after acridine orange by measuring the lysis activity of isolate after growing on blood agar medium of (18-36h) of incubation at 37°C by spreading method.

DNA profile by gel electrophoresis

Bacterial plasmid DNA was extracted from cultured cells using the alkaline SDS method with promega DNA kit described by as follow [11]:

25ml culture was centrifuged, resuspended the pellet in 0.5ml of lysozyme solution containing (0.3M NaOH, 2% SDS) mix and incubate at 70°C for 15min,

add 0.08ml of acid phenol/chloroform (1:1) and mix gently, separate phases by centrifugation at 1000xg for 10 min and transfer the upper aquous phase to a new Eppendroff tube containing 0.07M sodium acetate and 0.7ml isopropanol, centrifuge again for 2min, the pellet dissolve in 0.05ml TE buffer, Stored at 4°C and samples were electrophorated using 0.7% agarose with 5V/cm for 2h, after ending of electrophoresis process, the gel was exposed to U.V light with 340nm to observe plasmid bands.

Results and Discussion

The present study included (47) samples from U.T.I. patients with different ages and sexes from different hospitals even laboratories. Bacterial isolates were identified to the level of subspecies using the traditional biochemical and morphological test described by [10] and then confirmed by VITEK2 compact system. So, the results revealed that all (47) isolates were belonged to *E.coli* and only(28) isolate showed the ability for protease and haemolysin production(59.5%).

Protease production and Haemolysin Formation by *E. coli* isolates

The production of extracellular protease from a number of pathogenic bacteria represent one of the most important virulence factors have a wide spread of interesting field for studying, the results of this study showed that *E. coli* no.6 was the highest activity (24 mm in diameter) by measuring the diameter of lysis area on skim milk agar media (or by wells method) while *E. coli* no.18 the was the lowest protease activity (12mm in diameter) as shown in table (1) and figure (1), figure (2).



Figure 1: The haemolysin production by *E.coli* on blood agar media

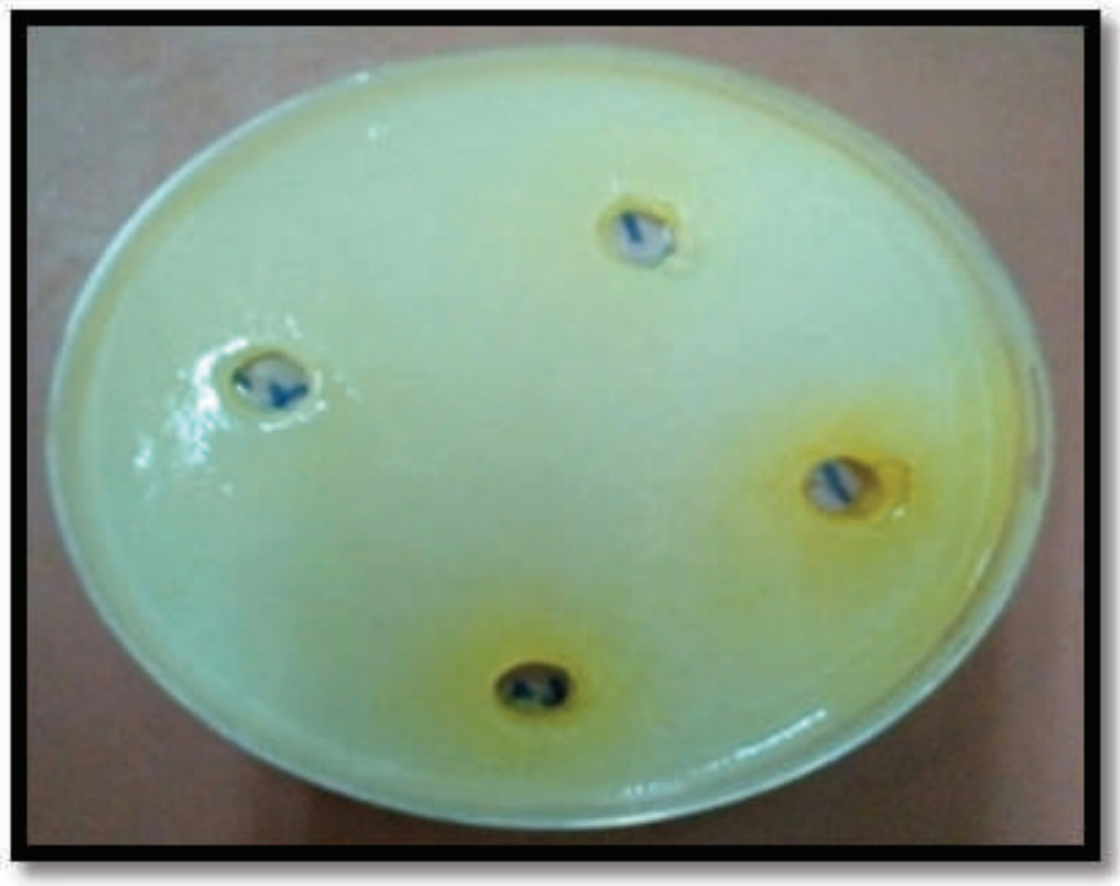


Figure 2: The protease production by *E.coli* on skim milk agar media

These findings are in agreement with results obtained by Taher (2003) who found similar and higher results of protease from *Proteus mirabilis*, in some genetic studies of protease production from different bacteria found that it is chromosomally determined [12,13]. There are many factors affect protease production such as the time of incubation, the presence of enhancers, metals. in addition, protease can cleavage the immunoglobulin and many immune cells [14].

In contrast, our results showed that all the 28 *E. coli* isolates showed complete haemolysin formation (100%) as in table (1) and figure (2). These results are agreement with that of Podschan *et al* (2000) which they can isolate a high frequency of Germany clinical isolates that have similar virulence factors activity like capsule production, siderophores, resistance to serum and protease production [15]. Also a high Beta-TEM-59-lactamaseresistance and others including protease and haemolysin production by K. Oxytoca were isolated at (26%) from different European clinical sources [16].

Table 1: Showed both the protease activity and haemolysin production of 28 *E. coli* isolates

Activity Isolation No.	Protease activity diameter of lysis area (mm)	haemolysin production
1	18	100%
2	16	100%
3	16	100%

Cont... **Table 1: Showed both the protease activity and haemolysin production of 28 *E. coli* isolates**

4	14	100%
5	16	100%
6	24	100%
7	18	100%
8	20	100%
9	16	100%
10	16	100%
11	20	100%
12	20	100%
13	14	100%
14	14	100%
15	16	100%
16	18	100%
17	16	100%
18	12	100%
19	14	100%
20	14	100%
21	14	100%
22	16	100%
23	16	100%
24	16	100%
25	18	100%
26	18	100%
27	20	100%
28	16	100%

The Effect of acridine-orange on both Protease and haemolysin production by *E. coli* isolates

The results were shown in table (2) represent that the mutagenic agent (acridine-orange) have an effect at concentrations (stock solution, to 10^6) at which all 28 isolates loss the protease activity and haemolysin production, while little and normal activities were observed at concentrations (10^{-5} to 10^{-10}) as in table (2). These findings are in agreement with the results obtained by many researchers [17] who found many of antibiotic resistance were plasmid determined and affected after curing experiments. In contrast, many results revealed the chromosomally determined abilities such as Actinorhodin-like substance production by *Streptomyces* IQ45 [17]. Jones *et al* (1990) isolate many *Proteus mirabilus* mutants affect their virulence factors [18].

Table 2: Show the effect of acridine-orange concentration on both protease and haemolysin production by *E.coli* isolates

Bacterial Activivty Acridine orange solution	Protease production	haemolysin production
Stock solution	No activity	No activity
10-1	No activity	No activity
10-2	No activity	No activity
10-3	No activity	No activity
10-4	No activity	No activity
10-5	Little (10mm)	100%
10-6	Normal(14mm)	100%
10-7	Normal 18mm	100%
10-8	Normal 22mm	100%
10-9	Normal 24mm	100%
10-10	Normal 24mm	100%

DNA profile by gel-electrophoresis

The results of DNA profile by gel-electrophoresis of both normal and cured isolates showed the prescence of chromosomal and plasmids DNA bands (520 & 950 bp) in (6, 7) isolates respectively in (normal case) while only chromosomal bands observed in *E. coli* isolates 8 which treated with acridine-orange at concentrations (10^{-2} and 10^{-4}) as in figure (3), the absence of plasmids was correlated with the absence of protease production and haemolysin formation by *E.coli* isolates as mentioned below which explain the fact of their genetics, they may be, in most probable plasmids determined neither than chromosome.

Finally, the obtained results in this research showed that acridine-orange have an effect on the virulence factors especially protease and haemolysin formation due to the mutagenic effect on the specific genes of their production by *E. coli* isolates under this study.

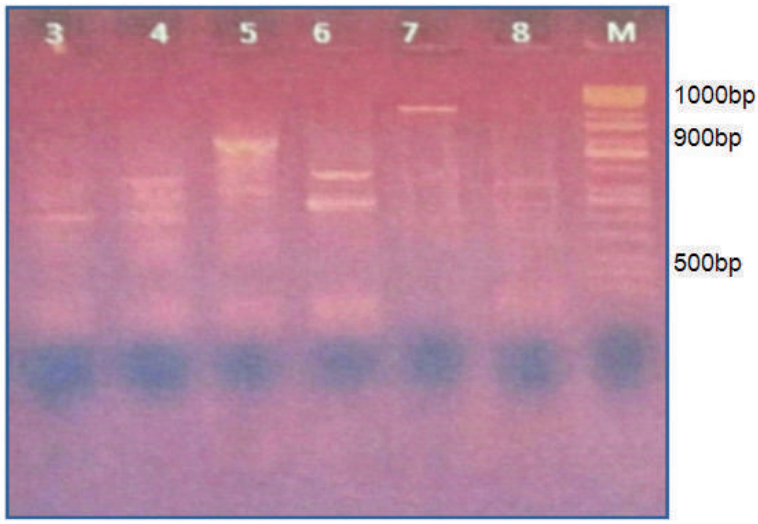


Figure 3: Agarose gel electrophoresis of both chromosomal and plasmid DNA isolated from *E.*coli iraqi isolates.lane 6,7 represent normal isolate, lane 8 represent treated isolate with acridine orange at concentration (10^{-2} and 10^{-4}) , M represent a marker.

Another study showed the presence of one mega plasmid band of *Bacillus thuringenisis* after 1.30h of electrophoresis while, there is a complex plasmid profile with different molecular weight occure within the same species, This reason may related to the differences of plasmid properties between strains related to the same species or even subspecies level [19].

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Ethical Clearance: The researchers already have ethical clearance from [2]Biology Department, College of Science, Mustansiriyah University, Baghdad, Iraq

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Evaluation of HE4 and IGFBPs as Novel Biomarkers of Systemic Lupus Erythematosus with Lupus Nephritis

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Abstract

Background: many organs of the body such as kidneys, skin, joints, nervous system, blood cells, blood vessels and serous membranes can be affected by systemic lupus erythematosus (SLE). A wide range of this disease effects may be attributed to its behavior as an autoimmune disease; this means, numerous complications of SLE with many organs can be results. In this study, levels of serum human epdidymis secretory protein4 (HE4) and insulin like growth factor binding protein-2 (IGFBP-2) for Iraqi patients in SLE with and without lupus nephritis (LN) were investigated to knowledge their ability to be useful markers for identification of kidney diseases, like lupus nephritis and chronic kidney disease in patients who suffering from SLE.

Methods: one hundred twenty subjects from both sexes were enrolled in this study. They have been classified into two patients groups together with the control group. Forty patients of SLE with LN (8male-32female) with age range (19-44) years, represent the first patients group, the second patients group includes forty patients suffer from SLE without LN at (16-45) years age range and the control group which consists of 40 healthy subjects with age range (19-62) years. HE4 and IGFBP were estimated by ELISA method.

Results: results show high level of HE4 and IGFBP in both patient groups with SLE in comparison with control group. At the same time, results confirmed a high positive correlation between HE4 and IGFBP ($r = 0.85$, $p<0.01$), ROC analysis data revealed that HE4 is the best parameters for predicting development lupus nephritis as a main complication of SLE disease.

Conclusion: both patient groups revealed a significantly increase in levels of HE4 and IGFBP compared with control group. High positive correlation coefficient between HE4 and IGFBP is obtained. Data of ROC analysis confirmed that HE4 represents a better biomarker for diagnosis of LN in patients with SLE disease.

Keywords: *SLE, LN, HE4, IGFBP-2, CKD*

Introduction

Systemic lupus erythematosus (SLE) complications include many organs; lupus nephritis represents the major one of these complications that consists about 50% of patients with SLE disease [(1)]. Previous studies were referred to the association between hyperglycemia and nephropathy [(2, 3)], neuropathy [(4, 5)], osteoporosis [(6, 7)] and the treatment by plant extracts [(8, 9)]. In spite of the early diagnosis and fast treatment of renal disease course, which attributed to the extensive of treatment, it is very necessary to determine a suitable bio marker to predict lupus nephritis in patients with SLE at early time [(10, 11)].

The identification and characterization of HE4 was conducted in previous study [(12)], it is widely detected in different organs that include salivary glands, kidney, respiratory tract and nasopharynx [(13-15)], while numerous studies were reported that HE4 coordinated

or no coordinated with the tumor marker (cancer antigen 125) to follow the development of ovarian cancer [16-17]. Lately, considerable augmentation of HE4 level was found in different kidney disorders [18-19].

Proteins that binding with insulin growth factor, which are abbreviated as (IGFBPs) form a high affinity secreted proteins group that serves for transport and regulating bioavailability and function of IGFs. Family of IGFBPs consists of six types of IGFBP, specifically IGFBP1 - IGFBP6. On the other hand, some proteins with little binding attraction to IGFs were mistakenly named as IGFBP7, IGFBP8 and IGFBP9 [20-21]. Depending on the protein composition conservation and the rising binding similarity, just the six types of IGFBPs are true binding proteins to the IGFs. Accordingly, in the case of cancer status "through IGF-independent pathways'' and autoimmune diseases "via IGF dependent and IGF-independent pathologies" IGFBPs participate in development of SLE complications disorders [22-23].

This study aimed to estimate of HE4 and IGFBPs levels in sera of patients with SLE disease and its complication of LN, to knowledge their ability, whether be useful as biomarkers that can applied for early predicting for this complicated disease.

Materials and Methods

One hundred twenty persons from both genders were enrolled in this study, who attended to Baghdad Teaching Hospital/Medical City, Iraq, for a period (December 2018 to May 2019). They are classified into patients group and control group. Patients group is subdivided into two groups: first patients group include 40 patients who suffering from SLE with LN (8male-32female) with age scope (19-44) years, second patients group consist of 40 patients in SLE without LN with age variety (16-45) and 40 hygienic subjects as control group with age range (19-62) years. Screening markers that include dsDNA and ANA together with C3 and C4 were used in clinical diagnosis for patients group without LN, whereas patients with renal nephritis analyzed by biopsy. Venous blood (5 ml) was drawn from each subject into a suitable syringe at the period of 8.30-11 AM. An adequate amount (3 ml) was transferred into sterile tubes. Three ml of each sample transferred to sterile plain tubes and permitted to coagulate at room temperature, then centrifuged for 15 min at 3000 RPM. The obtained serum were divided into numerous aliquots and directly frozen at -20 °c until tested. The remaining amount (2 ml) of the blood specimens were transferred into EDTA tubes for hematological analysis. HE4 and IGFBP analyzed by ELISA kit using sandwich method.

Statistical Analysis

Statistical programs include ANOVA, LSD, ROC, and Chi square were applied in this study to distinguish of results differences factors, probability, correlation coefficient and the best biochemical factors that can be represent a better biomarker for early prediction of the studied disorders[24] .

Results

Values of several factors that include age, BMI, duration of disease, urea, creatinine, total protein, Albumin C3, C4, ANA, Anti ds DNA, ESR and h-CRP for all the studied groups were recorded in table (1). The results exhibit a significance differences in age between patients and healthy groups ($p<0.01$), a significant increase was shown in body mass index (BMI), blood urea (BU), creatinine (Cr) , ANA, Anti dsDNA, ESR and h-CRP in patient groups (SLE with LN ,SLE without LN) in comparison with control group. Whereas, a significant decrease was found in total protein, Albumin C3, C4 in patient groups than healthy subjects.

Table (1): Demographic and clinical characteristics

Factors	Mean ± SE			LSD value
	SLE without nephrites	SLE with nephrites	Control	
Age (year)	29.37 ± 1.25 b	31.05 ± 1.33 b	41.02 ± 2.01 a	4.400 **

Cont... **Table (1): Demographic and clinical characteristics**

BMI (kg/m2)	28.54 ± 0.49 a	29.56 ± 0.61 a	26.94 ± 0.40 b	1.438 **
Duration (year)	3.63 ± 0.19 b	6.11 ± 0.26 a	---	0.657 **
Urea (mg/dl)	31.08 ± 0.63 b	57.98 ± 1.06 a	23.47 ± 0.88 c	2.45 **
Total protein (mg/dl)	6.66 ± 0.08 b	6.50 ± 0.08 b	7.05 ± 0.17 a	0.348 **
Creatinine (mg/dl)	0.887 ± 0.03 b	1.785 ± 0.06 a	0.815 ± 0.04 b	0.139 **
Albumin (mg/dl)	3.55 ± 0.07 b	2.96 ± 0.34 b	4.21 ± 0.08 a	0.592 **
C3 (mg/dl)	90.81 ± 1.38 b	80.55 ± 1.33 c	161.56 ±5.13 a	8.86 **
C4 (mg/dl)	23.23 ± 0.46 b	25.03 ± 0.78 b	47.96 ± 3.10 a	5.23 **
AnitdsDNA (IU/ml)	60.23 ± 1.39 b	75.48 ± 2.37 a	10.66 ± 0.90 c	4.68 **
ANA (IU/ml)	7.45 ± 0.34 b	19.01 ± 1.07 a	1.264 ± 0.80 c	2.24 **
h-CRP 9(mg/l)	9.32 ± 0.22 b	15.14 ± 0.60 a	1.800 ± 0.15 c	1.077 **
ESR (mm/h)	47.09 ± 0.85 b	57.84 ± 1.22 a	4.88 ± 0.21 c	2.44 **
** (P<0.01). differed in significant values of the measured factors in this table are represented by different letters in the same row				

Table 2 shows mean± SE values of HE4 and IGFBP-2. The results referred to present a significant increase in these parameters within patient groups (SLE with LN and SLE without LN) than control group.

Table -2: Mean ± SE of HE4 and IGFBP-2 levels in all the studied groups

parameters	**Mean ± SE**			**LSD value**
	SLE without nephritis	**SLE without nephritis**	**control**	
HE4 (pmol/l)	5.34 ± 0.30 b	24.51 ± 2.63 a	3.94 ± 0.87 b	4.52 **
IGFBP-2 (ng/ml)	5.34 ± 0.41 b	39.08 ± 3.22 a	1.17 ± 0.12 b	5.26 **
** (P<0.01). differed in significant values of the measured factors in this table are represented by different letters in the same row				

The correlation between HE4 and IGFBP-2 was conducted. The results revealed very strong correlation coefficient (0.85) between HE4 and IGFBP-2 as shown in figure 1.

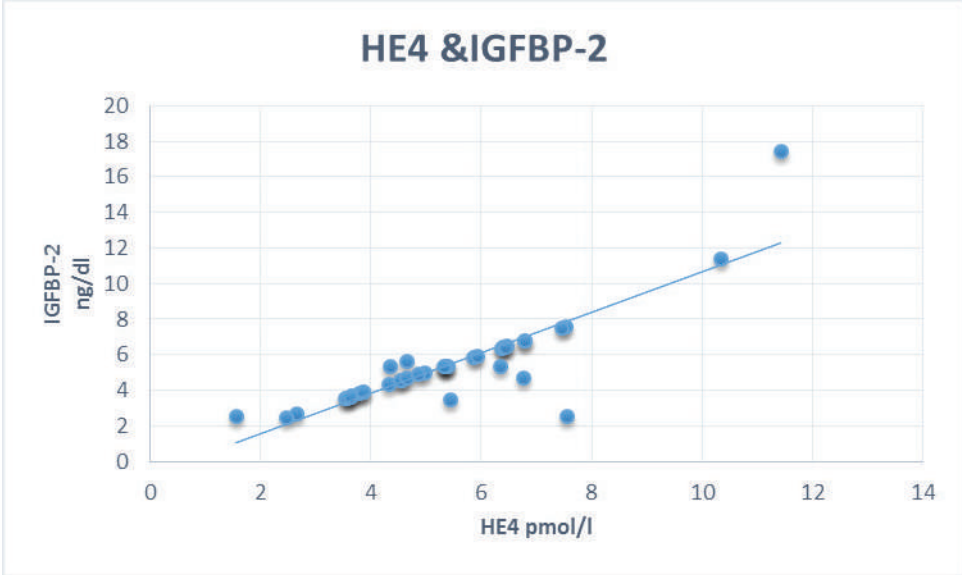


Figure (1): The correlation between HE4and IGFBP-2

Correlation between each of HE4and IGFBP-2 and other parameters in this study was studied. Correlation study was included two cases, SLE with nephritis and without nephritis. The results confirmed that no correlation occurred between these parameters as shown in tables 3.

Table 3 shows correlation between HE4, IGFBP-2 in SLE patients group without nephrites and other relevant biochemical parameters.

parameters	HE4		IGFBP-2	
	r	p	r	p
Age	-0.26	0.103	-0.27	0.086
BMI	0.03	0.810	-0.08	0.585
Creatinine	-0.21	0.188	-0.17	0.283
Total protein	-0.13	0.393	-0.03	0.847
Albumin	0.07	0.661	0.08	0.595
h-CRP	-0.06	0.668	-0.04	0.798
ESR	-0.17	0.283	-0.09	0.547
C3	-0.06	0.681	-0.04	0.771
C4	-0.04	0.780	-0.01	0.968
Anti ds DNA	0.23	0.139	0.14	0.360
ANA	0.05	0.741	-0.01	0.930
* (P: 0.01-0.05), ** (P: less than 0.01), NS (P: More than 0.05).				

ROC analysis has been applied in this study; the analysis reveals that both HE4 and IGFBP-2 are increased significantly. The level of HE4 considers a strong parameter to diagnose SLE (P<0.0001). Also, the results of ROC analysis showed that both sensitivity (98.7%) and specificity (97.5%) recorded at a value of ≤ 2.41(pmol/l).

Consequently, the test value below 2.41 (pmol/l) considers abnormal case (disease condition) while, the value above 2.41(pmol/l) represents the healthy condition. As shown in figure (2).

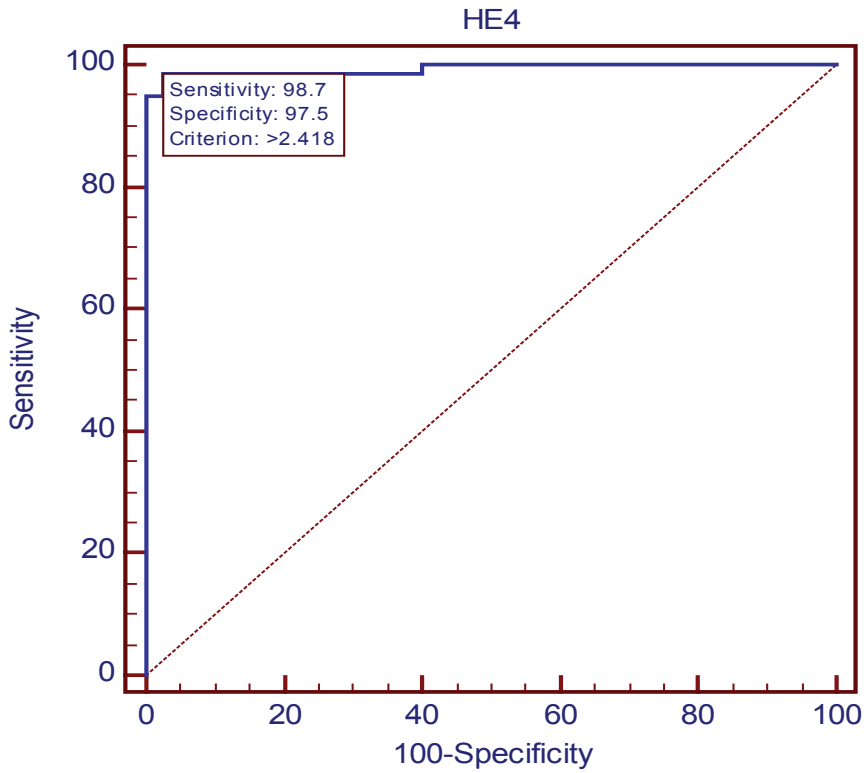


Figure (2) shows the ROC curve data for HE4

ROC analysis for IGFBP-2 also drawn, the AUC was found to be (0.994). The best cut-off point derived from the ROC curve shows a sensitivity of (93.7%) and specificity of (100.0%), it is found to be of≤2.81(ng/ml). That means, the test value less than 2.81(ng/ml) represents the abnormal case (disorders condition), whereas the value more than 2.81 (ng/ml) refers to the healthy case, the significance level is obtained at (P<0.001).

Discussion

The investigation of association of both HE4 and IGFBP-2 with SLE complications like LN patients considers the first attempt that conducted for Iraqi patients. HE4 and IGFBP-2 levels were found to be increased in disease case than healthy condition. On the other hand, all the screen factors that mentioned in this study have been useful to follow the development the complications for this type of diseases, but they represent a modest predictive value [25].

Lupus nephritis identification can be conducted by invasive test. This test is not accepted by patients. Moreover this invasive test considers unreliable test due to the global kidney status. Thus, noninvasive biomarker that can early discriminate LN before kidney injure in the case of SLE disease. Consequently, HE4 level in lupus nephritis is found to be higher than in patients without LN, and higher than control. The mechanism of HE4 elevation is not clear yet. The suggested mechanism in previous study and our study can be summarized on the basis of the dysfunction of kidney may let to escape HE4 out of kidney which leads to elevation of its level in

blood especially in the fibrosis case. Thus, the elevation of HE4 and renal disorder may be an interactive loop [26].

In spite of approved HE4 to follow the growth of ovarian cancer, several attempts were conducted to apply its medical values to in other diseases as reported in previous studies [27].

It was reported that IGF-1 IGFBP4 and IGFBP6 involved in tumor growth and some other disease such as SLE and LN. In other studies IGFBP2 was investigated in SLE disease, the studies referred to that IGFBP2 as a best predictor for kidney histopathology and biological changes in lupus nephritis[28].

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Antimicrobial Resistance on Escherichia coli from Poultry Production on Blitar, Indonesia

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Abstract

The use of antibiotics in poultry can encourage antibiotic resistance, one of which is *Escherichia coli* which produces Extended Spectrum Beta-Lactamase. Extended Spectrum Beta-Lactamase is most produced by *Escherichia coli*. This study was carried out in a cross sectional descriptive manner aimed to determine the prevalence of ESBL in *Escherichia coli* bacteria in layer and broiler chicken farms in Talun and Kademangan subdistricts, Blitar district. Samples of 76 cloacal swabs were taken from laying hens and broilers. Positive isolates of *Escherichia coli* were tested for antibiotic sensitivity on agar-resistant Mueller-Hinton agar and *Escherichia coli*-resistant beta lactam (ampicillin) groups and then confirmed using the Double Disc Synergy Test, to confirm as *Escherichia coli* producing ESBL. The results showed a high prevalence of MDR in commercial chicken farms in Blitar district. *Escherichia coli* has MDR resistance properties in Layer Chicken in Talun sub-district by 88.23%, and in Kademangan sub-district by 63.16%, while for Broiler chickens in Talun sub-district by 90% and in Kademangan sub-district by 100%. The prevalence of *Escherichia coli* producing ESBL in Kademangan Sub-district was 15.79% and in Talun sub-district by 5.88%, while in Broiler chickens in Kademangan sub-district was 85%, and in Talun sub-district the *Escherichia coli* producing ESBL was not detected.

Keywords*: Broilers, Escherichia coli, Extended spectrum beta-lactamase, Layer, Multidrug resistance*

Introduction

The use of antimicrobials in the field of animal husbandry provides benefits for animals and breeders, but can pose a risk of antimicrobial resistance if the use is not appropriate to the dose or time of use. Animal husbandry is one source of antimicrobial resistance [(1,2)]. The consumption of antibiotics medically in the animal sector reaches around 80%, mostly to promote growth in healthy animals. The World Health Organization (WHO) recommends that farmers stop using antibiotics regularly to promote the growth and prevention of diseases in healthy animals. WHO recommendations aim to help maintain the effectiveness of antibiotics that are important for human medicine by reducing unnecessary use in animals [(3)]. The use of antibiotics in animals can encourage antibiotic resistance, one of which is *Escherichia coli* which produces Extended Spectrum Beta-Lactamase (ESBL) [(1,2,4)].

Extended Spectrum Beta-Lactamase (ESBL) is most commonly produced by *Enterobacteriaceae*, especially *Escherichia coli* and *Klebsiella pneumoniae* [(5)]. ESBL is derived from the mutated beta-lactamase enzyme. This mutation causes an increase in the enzymatic activity of beta-lactamase [(6)]. Beta-lactamase is an enzyme

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produced by bacteria that functions against beta-lactam antibiotics [7].

The main bacteria that produce ESBL are *Escherichia coli* and *Klebsiella species* [5,8]. ESBL-producing bacteria can also be resistant to the antibiotics class of aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol, and sulfamethoxazole-trimethoprim [9]. The multidrug nature of resistance to third generation cephalosporin and other class antibiotics is often found in ESBL-producing bacteria [10]. Infections involving ESBL become an outbreak that exacerbates the infection of other diseases [1]. The existence of ESBL-producing *Escherichia coli* in poultry in Indonesia has been reported in broiler feces in chicken slaughterhouses in Bogor by molecular detection (genotypic) examination using a PCR of 6% [11] and clinical microbiology (phenotypic) examination by the double disc method of 25% [10]. Chicken livestock and egg production are important economic activities in Blitar District, with the second largest level in Indonesia involving thousands of workers [12]. Poultry is one of the important reservoirs of zoonotic bacterial agents [2].

Materials and Methods

Research design

This research is a descriptive study conducted in the form of cross sectional observation with the aim to see the relationship of the prevalence of *Escherichia coli* producing ESBL and its relationship with laying hens and broilers as carrier birds in Kademangan and Talun subdistricts, Blitar District.

Sampling and bacterial isolation

Samples of 76 cloacal swabs were taken from laying hens and broiler chickens in Talun and Kademangan subdistricts, Blitar. Random sampling of chicken cloaca swabs using sterile cotton swabs Amies Viscosa transport media (deltalab, Spain). And stored in a coller box before being taken to the laboratory [13]. Samples are stored at cold temperatures and taken to the laboratory for the isolation of *Escherichia coli* bacteria. Bacterial isolation on MacConkey selective media 3 (Oxoid, England) incubated at 35-37 ℃ for 20-24 hours, then biochemical identification of bacteria was carried out with the IMVIC and TSIA tests [14,15].

Antibiotic sensitivity test and ESBL-producing *Escherichia coli* confirmation test

Positive isolates of *Escherichia coli* were tested for antibiotic sensitivity tests on Mueller-Hinton agar (Merck, Germany), [16] using an antibiotic disk, namely the antibiotic ampicillin 10µg (Oxoid, England), streptomycin 10µg (Oxoid, England), erythromycin 15µg (Oxoid , England), tetracyicline 30 µg (Oxoid, England), sulphamethoxazole-trimethoprim 25µg (Oxoid, England). Bacterial isolates were determined to be sensitive to antibiotics is determined through the size of the diameter of the light zone or inhibition formed based on CLSI standard recommendations [14,16,17]. *Escherichia coli* which is resistant to beta lactam group is then confirmed by Double Disc Synergy Test (DDST), to confirm it as an ESBL-producing bacteria. This confirmation test used the Amoxycillin-clavulanic 30µg antibiotic disc (Oxoid, England), Cefotaxime 30µg (Oxoid, England), Ceftazidime 30µg (Becton Dickinson, USA), and Aztreonam 30µg (Oxoid, England) [14,16]. The results of the evaluation after incubation showed that the inhibition zone that appeared in the cup was measured based on CLSI guidelines [16,18,19].

Results and Discussion

Results of Isolation identification of a total of 76 layer cloaca swabs and broiler chickens in Talun and Kademangan sub-districts, Blitar District, as many as 76 (95%) positive isolates of *Escherichia coli*. The existence of *Escherichia coli* in the chicken cloaca swab respectively 36 (90%) isolates in laying hens and 40 (100%) *Escherichia coli* isolates in broiler chicken cloaca swabs in Talun and Kademangan subdistricts, Blitar. This result was in line with research which states that the presence of *Escherichia coli* in broilers cloaca is 68.75% [20].

The *Escherichia coli* resistance pattern (Figure 1) in the chicken cloacal swab in Talun sub-district (Table 1) showed the results in the laying hens, there is resistance to the antibiotic Ampicillin by 88.24%; in Streptomycin antibiotics resistance of 70.58%; Erithromycin antibiotic resistance was 88.24%; in Tetracyclin antibiotics resistance is 29.41%; in sulphamethoxazole-trimethoprim antibiotic resistance was 94.12%. *Escherichia coli* resistance patterns in broiler chickens, there was resistance to ampicillin antibiotics by 95%;

the antibiotic Streptomycin resistance is 80%; the antibiotic Erithromycin resistance is 80%; in Tetracyclin antibiotics resistance of 70%; in sulphamethoxazole-trimethoprim antibiotic resistance by 75%.

The resistance pattern of *Escherichia coli* in chicken cloaca swabs in Kademangan subdistrict (Table 1) showed that in laying hens, there is resistance to Ampicillin antibiotics at 68.43%; in Streptomycin antibiotics resistance of 68.43%; the antibiotic Erithromycin resistance was 73.68%; the Tetracyclin antibiotic resistance was 42.12%; in sulphamethoxazole-trimethoprim antibiotic resistance was 52.64%. The resistance pattern of *Escherichia coli* in broiler chickens, there was resistance to the antibiotic Ampicillin by 100%; the antibiotic Streptomycin resistance wss 95%; the antibiotic Erithromycin resistance wais 80%; in Tetracyclin antibiotics resistance of 15%; in sulphamethoxazole-trimethoprim antibiotic resistance by 90%.

The high level of resistance occurrence in laying hens in Blitar district is in line with the results of the sensitivity test in laying hens in Bogor, which shows the level of resistance in Amoxicillin (84.62%), Kanamycin (69.24%), Colistin (63.75%), Doxycycline (53.75%) %), Neomycin, and 100% in Tetracycline, Ciprofloxacin, Enrofloxacin (10). Other studies stated that the level of resistance of *Escherichia coli* bacteria in broilers in Bogor Regency to ampicillin (89.5%), streptomycin (84.2%), erythromycin (86.8%), tetracycline (89.5%), and trimethoprim-sulfamethoxazole (76.3%) [21]. High antibiotic resistance in broiler chickens was also reported in Jordan, where the highest resistance level in sulphamethoxazole-trimethoprim, was 95.5% [22].

Escherichia coli which has MDR resistance (Figure 2) properties in laying hens on Talun sub-district is 88.23% (15/17), and in Kademangan sub-district is 63.16%. A large number of antibiotics used in layer chicken farms are also used for human therapy, resulting in selection of pathogenic bacteria that are resistant to several kinds of drugs. Infection by *Escherichia coli* can be treated using sulfonamides, ampicillin, cephalosporins, chloramphenicol, tetracycline and aminoglycosides. Aminoglycosides are poorly absorbed by the gastrointestinal tract, and have toxic effects on the kidneys. The most commonly used type of antibiotic is ampicillin [23].

The prevalence of Multi-Drug Resistant (MDR) *Escherichia coli* bacteria in Broiler chickens in Blitar District is quite high (Figure 2), among others in Talun sub-district by 90% (18/20) and in Kademangan sub-district by 100% (20/20). Antibiotic resistance to *Escherichia coli* from broiler cloaca swabs on some antibiotics is a result of the continuous use of antibiotics by farmers to prevent and treat disease. Silbergerd et al. (2008) states that the addition of antibiotics in feed is a major factor in the increase in the prevalence of antibiotic resistance. Feed in the business of broiler chicken farming is a major component that reaches 60%, so that if the food circulating on the farm contains antibiotics can be a source of resistance [23].

The prevalence of antibiotic resistance tends to increase, one of which is the Extended Spectrum Beta Lactamases (ESBL) producing bacteria a group of enzyme-producing bacteria that can hydrolyze beta lactam antibiotics containing oxyimino groups such as cephalosporins one to third generation and aztreonam. This research was carried out in a cross sectional descriptive manner aimed to determine the prevalence of ESBL in *Escherichia coli* bacteria (Figure 3) in layer and broiler chicken farms in Talun sub-district and Kademangan sub-district, Blitar district.

The prevalence of ESBL-producing *Escherichia coli* (Table 1) in Kademangan District was 15.79% and in Talun sub-district it was 5.88%. The prevalence rate of *Escherichia coli* producing ESBL in the laying hens cloacal swab was the same as the results of the *Escherichia coli* study in the laying hens cloacal swab in Thailand by 6.7% [24], but this result is much smaller than the rate of *Escherichia coli* producing layer ESBL in Thailand. in India in chicken layers around 42% [25]. The prevalence of *Escherichia coli* producing ESBL in Broiler chickens (Table 2) in Kademangan sub-district was quite high (Figure 3), including 85% (17/20), while in Talun sub-district *Escherichia coli* producing ESBL was not detected. The prevalence rate of *Escherichia coli* producing ESBL in cloaca swabs in broiler chickens is consistent with the prevalence of *Escherichia coli* producing ESBL in India in broiler chickens around 87% [25].

Table 1. Percentage of ESBL-producing *Escherichia*

***coli* resistance from layer and broiler farms on Kademangan and Talun sub-districts, Blitar District.**

Location	Poultry	AMP	S	E	TE	SXT	MDR positive	ESBL positive
Talun	Layer (n=17)	15 88.24 %	12 70.58 %	15 88.24 %	5 29.41 %	16 94.12 %	15 88.23%	1 5.88%
	Broiler (n=20)	19 95 %	16 80 %	16 80 %	14 70 %	15 75 %	18 (90 %)	0 0 %
Kademangan	Layer (n=19)	13 68.43 %	13 68.42 %	14 73.68 %	8 42.12 %	10 52.64 %	12 63.16%	3 15.79%
	Broiler (n=20)	20 100 %	19 95 %	16 80 %	3 15 %	18 90 %	20 100 %	17 85 %

Note : AMP = ampicillin , S= Streptomycin , E= Erythromicin , TE= Tetracycline , SXT= Sulfamethoksazol-Trimetropin

Figure 1. (A) Patterns of *Escherichia coli* resistance to several classes of antibiotics (1) Ampicillin, (2) Streptomycin, (3) Erythromicin, (4) Tetracycline, and (5) Sulfamethoxazole-Trimethropin

(B) *Escherichia coli* producing positive ESBL by the method Double Disc Synergy Test (DDST). Arrows: Synergy forms, ATM: Aztreonam, CAZ: Ceftasidime, AMC: Amoxycillin clavulanic, and CTX: Cefotaxime.

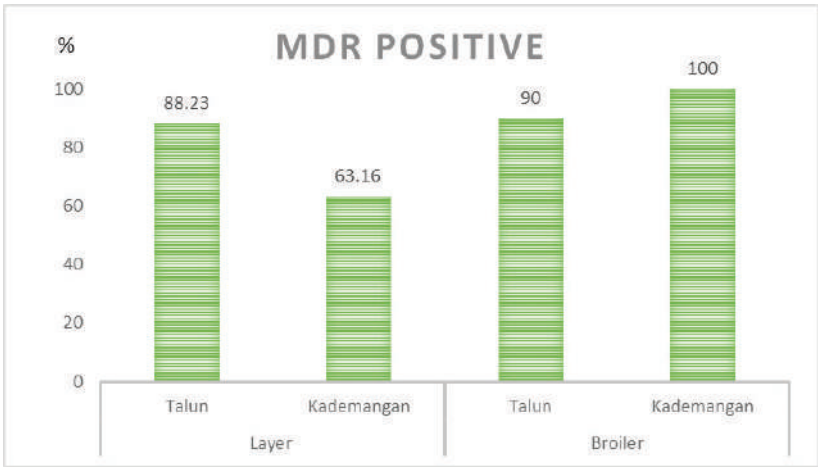


Figure 2. Multidrug resistant (MDR) *Escherichia coli* from layer and broiler farms on Kademangan and Talun sub-districts, Blitar District

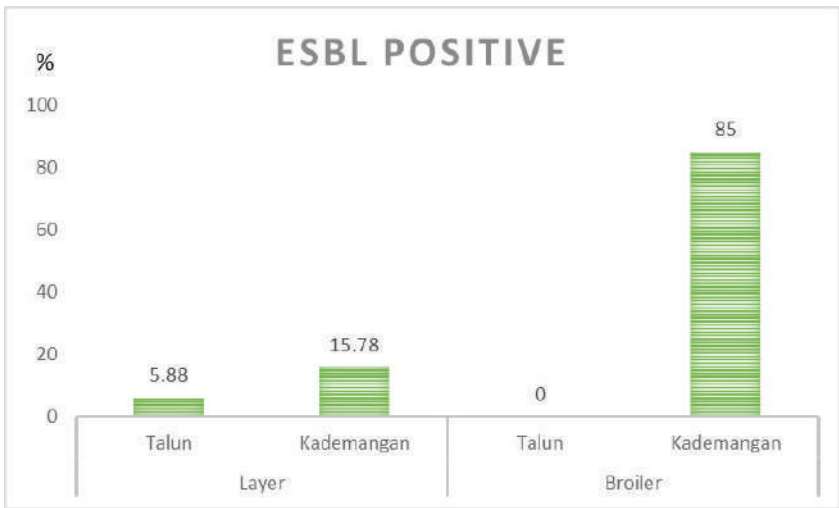


Figure 3. *Escherichia coli* producing ESBL in layer and broiler chickens in Kademangan and Talun sub-districts, Blitar District

Conclusion

Escherichia coli which has MDR resistance properties in layer and broiler chickens in Talun and Kademangan sub-districts is quite high, around 60-100%. The prevalence of *Escherichia coli* producing ESBL in laying hens in Kademangan Sub-district was 15.79% and in Talun sub-district by 5.88%, while the prevalence of *Escherichia coli* producing ESBL in Broiler chickens in Kademangan sub-district was 85% (17/20), and in Talun sub-district it was not detected. the prevalence of *Escherichia coli* producing ESBL.

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Designation : Freshinta Jellia Wibisono as a Doctoral candidate on Universitas Gadjah Mada, Bambang Sumiarto as a professor on Universitas Gadjah Mada, Tri Untari as an associate professor on Universitas Gadjah Mada, Mustofa Helmi Effendi as an associate professor on Universitas Airlangga, Dian Ayu Permatasari as a researcher on Universitas Airlangga, Adiana Mutamsari Witaningrum as a researcher on Universitas Airlangga

Ethical Clearence: Cloacal swabs were used in this study, hence ethical approval was not necessary. Cloacal swab samples were collected from two subdistrict on Blitar poultry farms in East Java province, Indonesia.

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Conflict of Interest : Nil

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Adherence and Satisfaction of Deferasiroxversus Deferoxamine in Transfusion-Dependent Beta-Thalassemia

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Abstract

Objectives: The present study was aimed to compare the adherence and satisfaction with deferoxamine (Desferal®) versus deferasirox (Exjade®), a novel oral iron chelator, in patients with transfusion-dependent beta-thalassemia.

Patients and Methods: In this cross-sectional, single-center study, 108 homozygous βthalassemia major patients aged between 3-21 years old were enrolled. They were on regular blood transfusions and iron chelators (either deferoxamine or deferasirox) for about three months. Another fifty-six apparently healthy non-thalassemic subjects were enrolled as a control group. **Results:** Adherence and satisfaction wereassessed byusing a simplequestionnaire establishedduring an interviewwithpatientsortheirparents.The serumferritin level was measuredbythe ELISAmethod**.**

Our findings showed that adherence and satisfaction in the deferasirox group were more than that with deferoxamine group patients. Serum ferritin level in the deferasirox group was significantly lower than in the deferoxamine group at ($p<0.001$). while the control group was significantly lower than both patients groups at ($p<0.001$). Patients on deferasirox had lower adverse effects than the deferoxamine group since deferoxamine produce irritation and pain at the injection site for the patients.

Conclusion: Our findings concluded that deferasirox is an effective iron chelator as deferoxamine but with better adherence and satisfaction than deferoxamine. Deferasirox can be used as a preferred iron chelator therapy in iron-overload patients with beta-thalassemia.

Key Words: *BetaThalassemia, Satisfaction, adherence, Iron Chelators, Deferoxamine, Deferasirox.*

Introduction

Beta-thalassemia may be a severe inherited blood disease that has been known to be caused by a mutation within the β-globin gene resulting in the excessive destruction of red blood cells1,2. it's been estimated that over 42000 newborns are suffering from β-thalassemia per annum worldwide. Without transfusion, β- Cooley's anemia causes death amongst infected children before the age of three years old 2. Thalassemia may be a disease status during which patients need continuous control and management, regular blood transfusions can prevent death and reduce mortality. However, excessive iron accumulated from transfused red blood cells can cause organ failure3,4. Therefore, iron chelation treatment is important to scale back the iron store within the body and improve the long-term survival rate of patients with thalassemia, which is taken into account important adjuvant therapy.4

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Currently, the most iron chelators available for clinical use in Iraq are deferoxamine and deferasirox. Deferoxamine, which needs routine intravenous or subcutaneous injections from 5 to 7 days weekly, has been considered a typical therapy for hemochromatosis over the past four decades.However, the treatment with deferoxamine for about 8 to 12 hours daily may lead to poor compliance and a few negative impacts like skin rash, hematological toxicity, and heart problems 5-7.

Deferasirox, a once-daily oral iron chelator, was introduced in 2005 as first-line therapy for patients over 2 years aged with chronic hemochromatosis thanks to blood transfusions5. Although Deferasirox has some mild adverse events, some studies indicated that it's a positive effect on lowering liver iron and producing high patient compliance8,9,10.

Similar to normal individuals, Thalassemic patients should have normal life status, however, continuous transfusion, iron chelation, and clinic visits have an excellent psychological impact leading to rejection of taking their medical management at the proper way, which assessed by satisfaction and compliance.

The present study was aimed to match the satisfaction and adherence with the deferoxamine group versus the deferasirox group. to verify the efficacy of both chelators; serum ferritin was also measured to match patients groups and with the control group.

Materials and Methods

A cross-sectional, single-center investigation study was carried out with the cooperation of the medical staff of Thalassemic Center of Ibn Al-Atheer Teaching Hospital in Nineveh Province, Mosul, Iraq. Ethical clearance was obtained from the Research Board Committee after approval of the protocol.

One hundred and eight patients of 3 to 21 years old of age with a diagnosis of β-thalassemia and having a chronic iron overload from regular blood transfusions as assessed by serum ferritin of > 600 ng/ml were divided into, firstly deferoxamine group treated by 20-50 mg/kg/day (n = 54, 30 male & 24 female) with age mean (13.2±4.03 years), second deferasirox group treated by 10-30 mg/kg/day (n = 54, 31 male & 23 female) with age mean (9.65±3.32 years), and the third control group of apparently health subject (n = 56, 36 male & 20 female) with age means (11.8±5.3 years). In this study satisfaction and adherence was assessed by using a questionnaire which usually done during an interview and reporting notes for each patient

Informed consent was taken from the patients or their relatives, a questionnaire regarding the personal and treatment details was obtained as name, age, sex, frequency of transfusion, type of chelation used, satisfaction, and adherence.

Patient compliance depended on the patient's use of deferoxamine. Five to seven times/week were regarded as compliance, while for Deferasirox patients, six to seven times/week were regarded as compliance. The iron overload was assessed by serum ferritin determination by using Ferritin AccuBind® ELISA - Monobind Inc. (Lake Forest, California, USA) commercial kit, depending on

Chemwell 2910 fully automated devices for enzyme-linked assay methods.

Data are presented as mean ± S.D. and were analyzed by using one way ANOVA test and nonpaired t-test were used to compare between the groups. RResults were considered statistically significant at $p \leq 0.05$. The correlation was done using Pearson and spearman's rho depending on the type of data (parametric or nonparametric).

Results

Table 1 shows that patients treated with deferasirox significantly were more satisfied in comparison with those treated with deferoxamine (90.7% vs 66.7%, respectively). Among patients who had previously taken deferoxamine then received deferasirox before the study 77% were reported a preference for deferasirox than deferoxamine.

The proportion of patients' compliance was greater in those receiving deferasirox than in those receiving deferoxamine (90.7% vs 61.1%), as shown in Table 1.

In this study serum ferritin levels within and between groups shows a wide range of variation.

There is a significant difference ($p<0.001$) in the serum level of ferritin between the deferoxamine group

(3891.2±1845.6 ng/ml) as compared with the deferasirox group (2212±1485 ng/ml).

Also, there are high significant variations ($p<0.001$) in each of the deferoxamine group and deferasirox group when compared with the Control group with (26.4±19.7 ng/ml) as shown in Table 2. The importance of adherence can be determined by serum ferritin level mean for all patients (N=108, 3051±1868 ng/ml) and when grouped as adherence patients (N=82,2620.4±1713.2 ng/ml) and non-compliance patients (N=26, 4411.6±1704 ng/ml), a significant difference ($p<0.001$) presented as shown in Figure 1.

Figure 2 represents the distribution of ferritin level data frequency for non- adhered patients that appear after the mean of all patients (3051ng/ml) especially for deferoxamine treated patients, while the distribution for compliance patients before the mean of all patient especially for deferasirox treated patients.

By determining correlations for all patients' data (N=108), the Pearson correlation showed a significant positive correlation ($p<0.01$) between age and ferritin level ($r = 0.374$), and negative correlation ($p<0.01$) between serum ferritin level and adherence ($r = -0.412$). Moreover, there is a significant positive correlation ($p<0.01$) between compliance and satisfaction ($r = 0.342$) by using Spearman's rho correlation (nonparametric correlation) these correlations are shown in Table 3.

Table 1. Percentage of satisfaction and adherence for deferoxamine and deferasirox

	Parameters	Deferoxamine Group (N=54)	DeferasiroxGroup (N=54)
Satisfaction	Satisfied	66.7%	90.7%
	Non Satisfied	33.3%	9.3%
adherence	adhered	61.1%	90.7%
	Non adhered	38.9%	9.3%

Table 2. Comparison of serum ferritin between deferoxamine group and deferasirox group and each group with controls group

Parameters	Control Group	Deferoxamine	Deferasirox Group
Serum ferritin Level	26.4±19.7	3891±1845a	2212±1485b,c

a $p<0.001$ vs control; b $p<0.001$ vs control; c $p<0.001$ vs deferoxamine group **Table 3. Relationship between studied parameters for all patients**

Correlations	R value	p-value		
Serum ferritin level	Age	N=108	r = 0.374	p<0.01
Serum ferritin level	Adherance	N=108	r = -0.412	p<0.01
Adherance	Satisfaction	N=108	r = 0.342	p<0.01

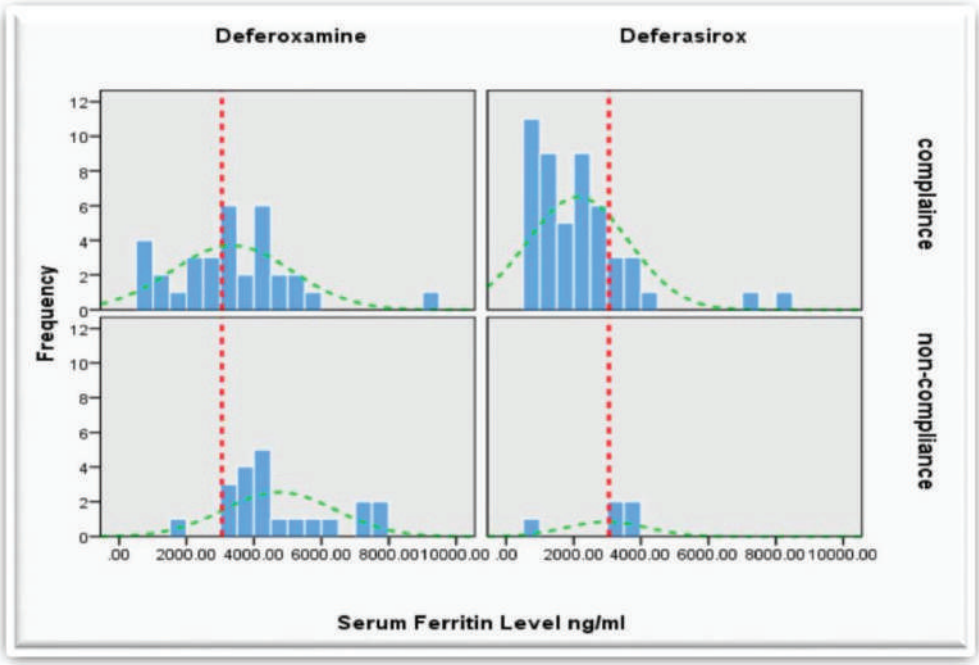


Figure 1. Frequency of Distribution of serum ferritin level among cmplianed and non-complianed patients for deferoxamine group and deferasirox group

Discussion

Iron chelators act by removing transiently available (labile) iron pools, either in plasma or within cells, because cellular storage iron (present as ferritin and hemosiderin) are not directly accessible for chelation11-13. Chelate iron is derived from two main sources: (a) iron released from macrophages after the catabolism of red cells and (b) iron released within cells after the catabolism of cellular ferritin and hemosiderin. For transfusion-dependent thalassemia major patients, it is important to choose an appropriate iron chelator to reduce iron burden in the body to prolong and improve the quality of life [14,15].

This study showed that Deferasirox had a rational better satisfaction and compliance than deferoxamine, especially for younger ages. Some older ages prefer deferoxamine because they are adapted on deferoxamine as part of their life or they cannot tolerate deferasirox. From another view no one of the chelation has very satisfied chelator due to limitation of normal life, so patients opinions about deferoxamine come from problem of subcutaneous injection for long period (8-12 hours per day) with painful and ulceration at injection sites, these agree with (Rofail et al, 2009) who showed administration of deferoxamine slow subcutaneous infusion negatively impacts on satisfaction which was shown to be a determinant of adherence[16]. While Deferasirox has taken orally as an effervescent tablet once daily is more suitable, but have the problem of bitter taste with slight large particle size cause irritation to the larynx and induce nausea, that agrees with (Trachtenberg et al, 2011) when showed patients with poor adherence to Deferasirox noted stomach pain and bad taste [17]. Deferasirox is preferable by 77% of patients who had previously been treated with deferoxamine. The reasons for preferring Deferasirox were, more convenient to be taken, less disruptive to their day or their sleep, and to their families, that mentioned by (Cappellini et al,2007)[5].

Serum ferritin is used as an indirect measure of iron overload, it is not an accurate assessment but gives a fairly good idea of iron overload [18,19]. In this study, serum ferritin at the Deferasirox group was lower than the deferoxamine group.

Pennel et al, (2014) when compared between deferoxamine versus Deferasirox showed a small difference of serum ferritin level means at the end their study 3129 ng/ml versus 3375 ng/ml respectively [20], that disagree with the present study which had significant

means variation p<0.001 between groups 3891 ng/ml versus 2212 ng/ml respectively these may be due to more compliance for Deferasirox than deferoxamine that leads to decrease ferritin level more than non-compliance while the result of this study agree with (Bashir and Sadoon, 2010) as they concluded that serum ferritin is higher in an older patient, the higher rate of blood transfusion and poor compliance [21].

Also (Mahmoud and Aziz, 2012) showed significant variations p<0.001 between serum ferritin levels of patients on deferoxamine and patients on Deferasirox and also for good compliance and poor compliance[22].

Improved satisfaction and convenience of a once-daily oral dose of Deferasirox compared to deferoxamine has been shown in other studies10,23 which may be translated into increased compliance as showed in this study that leads to improving health outcomes and quality of life.

The minor adverse effects with ease of use of deferoxamine or Deferasirox act as a choice factor for patients to determine satisfaction and compliance that in turn decrease ferritin level and consequently decrease complications, morbidity, and mortality.

Conclusion

It has been concluded that deferasirox is an effective iron chelator as deferoxamine but with a better satisfaction and compliance and lowered serum ferritin level than that of deferoxamine, in mild to moderate iron overloaded patients.

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Forensic Odontology-A Review

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Abstract

Forensic dentistry may be defined as that branch of forensic medicine that applies dental knowledge to civil and criminal problems. It is the branch of Forensic sciences which utilizes the skill of the dentist in the processing, review, evaluation and presentation of dental evidence with the purpose of contributing scientific and objective data in legal processes. The methods of collecting the data utilizes the developed technologies and have undergone significant transformation. The methods used include, dental imaging techniques, bite-mark analysis, DNA analysis using oral tissues, cheiloscopy, and rugoscopy in addition to facial reconstruction, denture identification, comparison microscopes, and tongue prints which are the recent inputs in the field of forensic odontology. This article provides an overview of the recent trends in conventional forensic methods and also provides an insight into the recent concepts used in this field.

Keywords: *Forensic odontology, DNA Analysis, Bite Marks*

Introduction

The word forensic is derived from the ancient Roman "forum" the home of the law courts – and means "relating to the law." Forensic dentists are involved in assisting investigative agencies to identify recovered human remains in addition to the identification of whole or fragmented bodies; forensic dentists may also be asked to assist in determining age, race, occupation, previous dental history and socioeconomic status of unidentified human beings. So a forensic dentist requires knowledge encompassing a number of disciplines, since the dental records obtained can identify an individual and can contribute to the information needed by the government to establish neglect, fraud or abuse. The role and importance of forensic dentistry in the judiciary is fast growing and hence knowledge in this field is needed by the general practitioner[1].

It is difficult to establish the identification of a dead person, when the body is disfigured or mutilated beyond recognition as a result of criminal activities, accidents, natural disasters, fire, and when the body is in unrecognizable, decomposed state. It is in these situations forensic dentists play a major role. Forensic anthropology, fingerprinting, forensic odontology (FO), radiology and DNA typing can be used for identification of the victims. The identification of a person by means of dental identification is one of the most reliable methods. Dental identification has always played a key role in natural and manmade disaster situations[1].

Dental identification

The common methods used for individual identification are using visual identification, personal information (such as height, build, age, presence or

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absence of hair), medical information (such as scars, tattoos, birthmarks, implants, amputations, prosthesis), footprint records from a chiropodist/podiatrist, clothing, personal effects, fingerprints, DNA profiling and dental identification. Dental ID, like fingerprint ID, can be used as a definitive means to identify human remains, as and when required. It has several significant advantages, when used properly. The dental evidence tends to survive much better than does soft tissue evidence as the teeth are the hardest substance in the human body and are even harder than bone. They are resistant to the environmental effects that can easily damage the soft tissue evidence, as they are calcified.It should be noted that teeth are not destroyed by immersion in water, by desiccation, fire or by decomposition. In general teeth are destroyed by heat, if the temperatures increase more than 1000°F. As the roots of the teeth are encased in the alveolar bone, it provides an additional layer of protection[2].

Traditionally, comparisons have been made between postmortem dental records and the antemortem (living) records for the presence of dental fillings, endodontic treatments, crowns or bridges, radiological studies to verify the clinical findings, the presence of malocclusions or dental fractures to determine whether both records correspond to the same individual. Several identification techniques are used by forensic dentists, including rugoscopy, cheiloscopy (lip prints), the obtainment of imprints, or the use of molecular techniques such as polymerase chain reaction (PCR) for analyzing the DNA contained in dental pulp tissue.Dental identification can be performed by examining the previous dental records of the deceased person to identify the similarities and confirm the same[1,2].

Problems in dental identification[3]

The dentist may encounter problems with dental identification because of the lack of an organized structure and adequate knowledge. They may use multiple systems to identify the procedure provided for a patient and to indicate the nature of the treatment. The forensic dentist should be familiar with all the types of common treatments and materials including the charting and numbering system. They should be able to differentiate the normal functional wear and other age related changes from the abnormalities. In general, the changes are not distinct over a short period of time, but over an extended time period the small changes can accumulate to produce significant differences[3].

Dental identification can have three different applications:

(a) Comparative identification, in which the postmortem dental records are compared with the antemortem records of an individual in order to establish whether both records correspond to the same person.

(b) The obtainment of dental information to narrow the search for an individual when the antemortem records are not available and there are no possible data referred to the identity of the subject.

(c) Identification of victims following mass disasters or catastrophes.

In general forensic odontology can be used in three areas as follows: (1) diagnostic and therapeutic examination and evaluation (2) The identification of individuals, (3) Identification, examination, and evaluation of bite marks. They can also be used to determine age, sex, and ethnicity of the person of interest.

According to American board of forensic odontology dental identification can be divided into four types[4]:

1. Positive identification: The ante-mortem and postmortem data match to establish that it is from same individual;

2. Possible identification: The ante-mortem and postmortem data have few consistent features, but because of quality of the records it is difficulty to establish the identity;

3. Insufficient evidence: The data is not enough to from the conclusion;

4. Exclusion: The ante-mortem and postmortem data clearly inconsistent.

Bite mark analysis

The forensic dentist should document the available bite marks and its characteristics to correlate with the suspect. When the marks are analyzed, all the possible characteristics which may influence the bite including fractures, shape of the teeth, congenital malformations,

should be accounted for. The anatomical location, severity, and quality of the bite marks play a significant role in the identification of the individual. It should be noted that the biting surfaces of the individual groups of teeth are unique and related to the function. As the bite mark findings may deteriorate from the site of the actual bite, it should be documented as quickly as possible. In case of bites which are not visible to the naked eye, it should be identified using ultraviolet light illumination and should be recorded. It is advised to get an second precise impression of the bite surface to register all the irregularities produced by the teeth upon the skin. This can be done with the help of vinyl polysiloxane, polyether or other impression materials recommended for the obtainment of imprints for fixed prostheses. As dry saliva is hard to detect the amylase test is needed to identify its presence. Before the evidence evidence from the bite suspect is collected, a proper consent should be obtained. A detailed history, photographs, the details of extra- and intra-oral examination along with high-quality impressions of the upper and lower arches should be collected along the impression.Bite marks are compared by measurement of size, shape, and position of the individual teeth. Overlays aremost used for this purpose. In addition to all these methods, salivary DNA recovery and bacterial genotyping from the bite marks are the most recent ones and have become the backbone of forensic investigation[5].

DNA analysis

DNA analysis is a new tool used in the field of forensic odontology, gains importance when conventional identification methods fail due to the effects of heat, traumatism or autolytic processes, distortions, and difficulties in analysis. There are many biological materials such as blood, semen, bones, teeth, hair, and saliva that can be used to accomplish DNA typing. With the advent of polymerase chain reaction which allows enzymatic amplification of a specific DNA sequence even in a negligible amount of source material, forensic identification using DNA analysis becomes increasingly popular with investigators.

The oral cavity is a useful source of DNA. The latter is obtained from saliva, the oral mucosal cells and the teeth. The main DNA source is blood, though in some situations this type of sample is not available for analysis. In teeth, DNA is found in the pulp tissue, dentin, cement, periodontal ligament and alveolar bone. Forensic dentists should incorporate these new technologies, since a number of methods are available for the extraction of DNA from biological samples, though no standardized protocols for their use have been established to date[4,5].

Cheiloscopy

Cheiloscopy is a forensic investigation technique which deals with identification of humans based on their lip traces. Like the prints present in the finger, palm and foot, and lip prints are also unique and do not change during the life of a person. Lip prints provide enough information for forensic investigations as the lips also possess furrows and grooves. Lip prints can be obtained at the crime scene either directly from the lips of the deceased or from the clothing, cups, glasses, cigarettes, windows, or doors. Lip prints have to be obtained within 24 h of death to prevent erroneous data that would result from postmortem alterations of lip. Lip print pattern depends on whether mouth is opened or closed. In closed mouth position, lip shows well-defined grooves; whereas in open mouth position, the grooves are relatively ill defined and hard to interpret[6].

Recent studies have proven lip prints as a superior tool compared to fingerprints and mandibular canine index in gender determination. Lip prints can be obtained using cellophane tape or a scotch tape which are pressure sensitive.

Rugoscopy

In case of teeth loss, due to reasons such as trauma, palatal rugae pattern serves as an alternative method for identification because of its uniqueness. As rugae is internally placed in the oral cavity and is protected by tongue and buccal pad of fat, it remains undisturbed from heat and other assaults. Rugae patterns change with age and other environmental influences such as orthodontic movements, tooth extraction, cleft palate surgery, periodontal surgery, and impacted canine eruption[7,8].

Materials and methods used to analyze the rugae patterns includes, photographs and impression of maxillary arch, computer software programs (for e.g., RUGFP-

ID), calcorrugoscopy or overlay print, stereoscopy (through which three-dimensional [3D] image of palatal rugae can be made), stereophotogrammetry (which is comparatively accurate)[5,8].

Facial reconstruction

Face is crucial for human identity and is a boon to the humankind. Forensic dentistry functions only in cases where the face of the person is destroyed by some means. Skulls can remain unaltered even for millions of years and can provide an inimitable means of identification. The cranial appearance is very much helpful in determining the sex of the individual. Computerized facial reconstruction method uses a laser video camera interfaced with a computer or with CT scanning. The face can be drawn with the help of computer software (for e.g., Vitrea 2.3 version volumetric visualization software). Although exact picture of the face may not be made, this method helps in identifying the individual[5,9].

Comparison microscopes

Examination of teeth under microscope can confirm sex by the presence or absence of Y-chromatin.The phase contrast microscope is useful in analyzing the cemental annulations for age estimation. A prototype Virtual Comparison Microscope (VCM).is a device which helps in analyzing the specimens simultaneously and utilizes images of deformed bullets, bullet fragments, and various types of rifling from the company's BulletTrax-3D system. With the VCM, it is easy to find significant markings in any direction while maintaining a consistent appearance[5].

Tongue prints

Tongue is unique to each person in its shape and surface textures and is the only Use of tongue prints for forensic identification is at budding stage now. For this technique to be successful, the antemortem photograph or impression of the tongue should be available. The lingual morphological aspects can be preserved using the alginate molding technique for duplicating the minute details which are unique for each individual. The lingual impression, together with its photographic image, may constitute secure methods for forensic dentistry identification.Tongue biometric template can be made using three views such as left lateral view, right lateral view, and profile view[5,10].

Conclusion

Forensic odontology is an upcoming branch of dentistry with a lot of scope for development. At the crime scene, the forensic odontologists play a major role in investigating and interpreting the dental evidence. The unique nature of the dental anatomy and the custom restorations ensure accuracy when the techniques are appropriately employed[5]. A consistent effort has to be made to computerize all the data available to facilitate comparison. Efforts have to be made to maintain the dental records which will serve as antemortem data. Each dental professional has a responsibility to understand the forensic involvements associated with their dental practice. The practicing dentists and the dental students should be made aware of the available technologies and its use in forensic dentistry. New researches have to be encouraged in the field of forensic dentistry which will pave way for incorporating newer technologies in establishing the human identity[4].

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Study of Violent Asphyxial Deaths in Raichur District of Karnataka

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Abstract

On taking the consent from Head of the Department of Forensic Medicine, a retrospective study was conducted by analysing post mortem reports maintained in record section. Reports of post mortem examination done from 1st Jan 2017 to 31st Dec 2018 were analysed. Out of 520 cases done during this period, 105 (20.19%) were death due to asphyxia. In the same time data was also analysed with age of deceased, sex of deceased, season and manner of death. Among 105 cases of asphyxial deaths, males were more, accounting for 61 %(64) of study cases and females were 41(39%) cases. In the present study, commonest method of asphyxial deaths was by hanging (75.1%), followed by ligature strangulation (12.3%) and 1 case each of postural and sexual asphyxia was the cause of death, accounting as a least method of asphyxia deaths. When age wise assessment done, asphyxial deaths were most common in second and third decade of life, constituting most of the suicidal cases. Asphyxial deaths were almost equally distributed among all seasons.

Key Words: *Asphyxia, Hanging, Strangulation, Drowning, Ligature.*

Introduction

Asphyxia is a condition in which body tissues suffers lack of oxygen supply by some interference in breathing process[1]. Among all asphyxia deaths, hanging produces painless death for the victim and involves only a ligature material to be put around the neck and suspend the body, which produces unconsciousness within 15 seconds[2]. Suicidal asphyxia deaths are more common than homicidal. Hanging and drowning are commonest methods adopted in suicidal asphyxia were as ligature or manual strangulation are commonest methods in homicidal mechanical asphyxia[3]. In addition to these accidental mechanical asphyxia by traumatic compression of the chest and sexual erotic asphyxia deaths, also accounts for very minimum cases[4].

Suicides and homicides by mechanical asphyxia are commonly seen, because if its painless, easiest and cost effective method of ending life. So many parameters play a role in increase of suicides and homicides like poverty, failures, marital conflicts, professional conflicts and population explosion. The aim of this study is to analyse some of the parameters those determine suicides and homicides by mechanical asphyxia.

Methodology

After obtaining permission from Head of the Department Forensic Medicine and Toxicology, a retrospective analysis of post mortem reports, maintained in Forensic Medicine Department of RIMS, Raichure, was done from 1st Jan 2017 to 31st Dec 2018 for a period of 3 years. During this period, a total of 520 cases of autopsies were done. Data analysis was restricted only to asphyxia deaths. Among 520 cases, 105 cases were death due to asphyxia. Hanging, Drowning, Strangulation, Postural and sexual asphyxia were included. Relationship of asphyxial deaths with the age, sex of the deceased, season, timing and marital

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status was analyzed.

Results

Total number of post mortem examination done during the study period were 520, of which 105 cases were of asphyxia deaths. The incidence of asphyxia deaths was more in males, i,e, 64 (61%) cases and females were 41 (39%) cases. The asphyxia deaths were more in third and fourth decades of life accounting 64 cases in theses age groups fallowed by 12 (11.4%) in the age group of 41 to 50 years. Surprisingly asphyxia deaths were more in Hindu religion (62.8%). Marrital conflicts may be the important reason for ending their life by mechanical asphyxia, since asphyxia deaths were more in married individuals (63.8). Asphyxial deaths were almost equally distributed in all seasons with mere increase in the summer. Most of the suicides and homicides by mechanical asphyxia were in the afternoon between 12 pm to 6 pm (57.1%)

Table No. 01: Profile of Asphyxial Deaths

Type of Asphyxia		Female		Male		Total	
		No	%	No	%	No	%
Drowning		02	1.3	03	2.8	05	4.7
Hanging	Complete	23	21.9	49	46.6	72	68.5
	Partial	05	4.7	02	1.3	07	6.6
Traumatic asphyxia		00	00	03	2.8	03	2.8
Postural asphyxia		00	00	01	0.9	01	0.9
Sexual asphyxia		00	00	01	0.9	01	0.9
Strangulation	Ligature	09	8.5	04	3.8	13	12.3
	Manual	02	1.3	01	0.9	03	2.8
Total		41	39	64	61	105	100

Table No.0 2: Age and Sex Wise Distribution of Asphyxial Deaths

Age in years	Female		Male		Total	
	No	%	No	%	No	%
0 – 10	02	1.3	01	0.9	03	2.8
11 – 20	05	4.7	03	2.8	08	7.6
21 – 30	17	16.1	15	14.2	32	30.4
31 – 40	08	7.6	24	22.8	32	30.4
41 – 50	03	2.8	09	8.5	12	11.4
51 – 60	00	00	04	3.8	04	3.8
61 – 70	04	3.8	04	3.8	08	7.6
71 – 80	02	1.3	03	2.8	05	4.7
81 – 90	00	00	01	0.9	01	0.9
91 - 100	00	00	00	00	00	00
Total	41	39	64	61	105	100

Table No. 03: Religion Wise Distribution of Asphyxial Deaths

Religion	Female		Male		Total	
	No	%	No	%	No	%
Hindu	23	21.9	43	40.9	66	62.8
Muslim	15	14.2	19	18	34	32.3
Christian	03	2.8	02	1.9	05	4.7
Total	41	39	64	61	105	100

Table No 04: Distribution of Asphyxial Death Cases according to marital status

Marital status	Female		Male		Total	
	No	%	No	%	No	%
Married	29	27.6	38	36.1	67	63.8
Unmarried	12	11.4	26	24.7	38	36.1
Total	41	39	64	61	105	100

Table No. 05: Season Wise Distribution of Asphyxial Deaths

Season	Female		Male		Total	
	No	%	No	%	No	%
Rainy	11	10.4	23	21.9	34	32.3
Summer	18	17.1	31	29.5	49	46.6
Winter	12	11.4	10	9.5	22	20.9
Total	41	39	64	61	105	100

Table No. 07: Distribution of asphyxial deaths on different timings

Timings	Female		Male		Total	
	No	%	No	%	No	%
Morning (6 am To 12 Pm)	04	3.8	07	6.6	11	10.4
Afternoon (12 pm To 6 pm)	21	20	39	37.1	60	57.1
Evening (6 pm To 12 am)	10	9.5	14	13.3	24	22.8
Night (12 am To 6 am)	06	5.7	04	3.8	10	9.5
Total	41	39	64	61	105	100

Discussion

The incidence of mechanical asphyxia was 20.19% was more when compared to other studies.[5,6] and in few studies[7,8] the incidence was lower. The incidence difference may be because of different geographical area of population.

In the present study, the commonest type of asphyxia deaths are due to hanging, followed by strangulation, were as drowning was second commonest method in some of the studies[5,6,7] but in one of the study[6] drowning was most common method (59.40%) which could be due to the presence of water reserves in the region of the study. Postural and sexual asphyxia are very rare accounting only 1 (0.9%) case each respectively.

Asphyxial deaths were more in second (30.4%) and third (30.4%) decade of life, which is similarly observed in some of other studies[6,9,10]. On analysing sex wise, it was noticed that males were more in number who ended their life by means of mechanical asphyxia (61%) which is similar to observations reported by other studies[7,9]. This high incidence in this age group and in males clearly suggests that adult males are one who are exposed to more stress and strain and are more sensitive for bad incidences occurring in their life.

High incidence of suicide by mechanical asphyxia is more common in Hindus (62.8%) which is consistent with other study reports[6]. This could be because of religious belief in Muslim and Christian community, that life is a gift of god and should be taken back by him and not to end it by our self.

Incidence of asphyxia deaths were more (63.8%) in married persons when compared to unmarried (36.1%). On season wise analysis, asphyxia deaths occurred at the higher rates in summer (46.6%) which is similar to other studies[11].All hanging cases were suicidal in nature, which is similar to observations of other studies[12] and is the most commonest method to commit suicide.

Conclusion

Among 520 cases of autopsies done during study period 105 (20.19%) cases were deaths due to mechanical asphyxia. Most frequent method of asphyxia is by hanging (75.1%) followed by strangulation (15.1%). All hanging cases were suicidal in nature and strangulation cases were homicidal in nature, suggesting, that commonest method to end life either by suicide or homicide is, cost effective mechanical asphyxia. Sexual asphyxia and postural asphyxia were least accounting only 1 case of each. Adult male victims were more in number, who died because of committing suicide by hanging, hence a special counselling should be given to adults to tackle problems in their life.

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Post Mortem Organ Weight at Bhayangkara Pekanbaru Polda Riau Hospital, Indonesia

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Abstract

Post mortem organ weight is one of indicator that serves as supporting evidence in determining the cause of death, because if there is a difference between the post mortem organ weight with normal organ weight indicates the occurrence of pathological conditions that may be related to the cause of death. The objective of this study was to determine post mortem organ weight in autopsy cases at Bhayangkara Pekanbaru Polda Riau Hospital in 2017-2018. This research is a retrospective descriptive study using secondary data. Data were obtained from autopsy data in the forensic section of Bhayangkara Pekanbaru Polda Riau Hospital in 2017-2018. Data were analyzed by using univariate analysis carried out on each variable of the research results. The results of this study were obtained from twenty one autopsy cases with a mean value of organ weight: the brain (1334.29 grams), liver (1130.90 grams), right lung (370.48 grams), left lung (311.24 grams) gram), heart (275.33 grams), left kidney (114.52 grams), right kidney (114.00 grams), and spleen (113.43 grams). Organs in men are heavier than women, except the liver. Organ weight has decreased in old age. People with short bodies tend to have lighter organ weights than people with tall bodies. The conclusion of this study is the average value of post mortem organ weight in this study is different from the weight value of organs found in forensic textbooks.

Keywords: *autopsy, post mortem, organ weight*

Introduction

Autopsy is one of the important parts in medical science, because in addition to establish the final diagnosis, the function of autopsy also to find the relationship between the cause of death with abnormalities in the organs that cause death, and can explain the relationship between these. An autopsy is done in suspicious death case, death due to murder, and sudden death without apparent cause.[1]

Autopsy consists of 2 types examination, external and internal examination. On internal examination, one of the examinations is weighing post mortem organ weight.[2] It plays an important role as supporting evidence in determining the cause of death, because if there is a difference between the weight of a post mortem organ with the average weight of the post organ mortem is normal, it indicates a change in the organ caused by a pathological state in the organ that is likely related to the cause of the death.[3]

Standard values of post mortem organ weights are needed to determine whether a person's organs are normal or not, such as those found in forensic books and studies that have been conducted in other countries, like South Africa, India, Korea, Thailand, Iran. Research by Vadgama et al. in 2014 in Jamnagar region, India, stated that the standard weight of organs contained in forensic books written by authors from other countries is not appropriate if used in populations in India.[4] It shows that the standard weight of organs using samples in other

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countries may not necessarily be used as a reference to determine Indonesian organs are normal or not.

The value of organ weight obtained using samples in other countries may not be appropriate if it is used as a standard for assessing the weight of Indonesian, because between one country and another country has different races and ethnicities and it is known that both of these are some factors that influence weight post mortem organ. These racial and ethnic differences affect organ weight due to differences in the type of food consumed, the amount of water consumed, climatic conditions, and genetic variations in various ethnicities in various countries in the world.[5] In addition to race and ethnicity, there are other factors that may influence weight post mortem organs, namely sex, age, body weight, body length, and body mass index.[3]

Based on that problems that have been described, the author wants to know the average value of post mortem organ weight in autopsy cases that performed at Bhayangkara Pekanbaru Polda Riau Hospital, Indonesia and the average value of post mortem organ weight based on age, gender, and body length.

Methods

This research is a retrospective descriptive study using secondary data. This research was conducted in the forensic department of the Bhayangkara Pekanbaru Polda Riau Hospital from August 2018 to May 2019.

The population in this study were all of the autopsy data in the forensic section of Bhayangkara Pekanbaru Polda Riau Hospital in 2017-2018. The sample studied in this study was taken from autopsy data in the forensic section at the Bhayangkara Pekanbaru Polda Riau Hospital in 2017-2018. The sample is part of the population that meets the inclusion and exclusion criteria.

Data was collected from the results of postmortem examinations at Bhayangkara Pekanbaru Polda Riau Hospital in 2017-2018 which were included in the inclusion and exclusion criteria. Data that has been obtained from the data collection process will be changed in the form of tables, then the data is processed using computer software.

Results

Table 1 Characteristics of Research Subjects

	f	%
Gender		
Men	16	76,2%
Women	5	23,8%
Total	21	100%
Age		
21-28 year	7	33,3%
29-36 year	5	23,8%
37-44 year	3	14,3%
45-52 year	2	9,5%
>52 year	4	19,1%
Total	**21**	**100%**
Body Length		
142-149 cm	1	4,8%
150-157 cm	3	14,3%
158-165 cm	8	38,1%
166-173 cm	7	33,3%
>173 cm	2	9,5%
Total	**21**	**100%**

Based on the characteristics table of the research subjects it is known that there are more men subjects than women subjects, 76.2%. The highest number of cadavers is found in the 21-28 year age group, which is 33.3%. Meanwhile, the highest number of cadavers was found in the 158-165 cm body length group, which was 38.1%.

Tabel 2 Post Mortem Organ Weight (gram)

Organ	Range	Mean	Deviation Standard
Brain	1070-1783	1334,29	161,84
Heart	185-420	275,33	68,17
Right Lung	100-665	370,48	153,18
Left Lung	100-535	311,24	122,95
Liver	858-1670	1130,90	187,24
Spleen	40-421	113,43	90,46
Right Kidney	50-200	114,00	41,97
Left Kidney	40-200	114,52	40,15

Based on table 2, it is known that the brain is an organ that has an average value of the heaviest organ weight (1334.29 grams), while the spleen is the lightest organ (113.43 grams) compared to other organs. Comparison of weight between the same organs with different position, such as the lungs and kidneys shows different results, namely the right lung is heavier (370.48 grams) compared to the left lung (311.24 grams), while the right kidney weight (114.00 grams) lighter than the left kidney weight (114.52 grams).

Tabel 3 Post Mortem Organ Weight (gram) depend on Gender

Organ Weight	Gender					
	Men (n=16)			Women (n=5)		
	Range	Mean	S.D	Range	Mean	S.D
Brain	1070-1783	1350	175,9	1180-1440	1284,0	103,3
Heart	185-420	284,1	66,33	185-350	247,0	73,62
Right Lung	160-665	385,6	152,3	100-500	322,0	162,5
Left Lung	120-535	320,9	127,3	100-400	280,2	155,0
Liver	656-1600	1130,8	180,9	875-1480	1131,0	229,2
Spleen	40-421	120,3	103,0	60-100	91,2	17,5
Right Kidney	50-200	116,5	42,7	60-150	105,8	42,9
Left kidney	40-200	115,6	40,8	60-150	110,8	42,1

The post mortem organ weight table by sex shows that almost all men organs are heavier than women organs, except that the liver in men (1130.8 grams) has an average weight value that is almost equal to the weight of the liver in women (1131.0 grams).

Tabel 4 Post Mortem Organ Weight (gram) depend on Age (year)

Organ	21-28	29-36	37-44	45-52	>52
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Brain	1297,8 ± 84,8	1456,4 ± 214,5	1195,6 ± 192,2	1367,00 ± 188,0	1333,0 ± 120,5
Heart	214,8 ± 31,4	265,2 ± 63,4	292,3 ± 13,0	362,5 ± 17,6	337,5 ± 64.8

Cont... **Tabel 4 Post Mortem Organ Weight (gram) depend on Age (year)**

Right Lung	342,1 ± 148,8	404,4 ± 179,0	230,0 ± 88,8	497,5 ± 215,6	419,5 ± 105,4
Left Lung	301,2 ± 137,2	388,8 ± 103,2	171,0 ± 67,8	430,0 ± 98,9	277,5 ± 41.7
Liver	1020,2 ± 118,5	1119,8 ± 250,8	1140,6 ± 53,2	1374,0 ± 319,6	1209.5 ± 96.3
Spleen	166,2 ± 126,1	102,8 ± 65,4	54,6 ± 11,5	139,5 ± 85,5	65.2 ± 27.6
Right Kidney	118,4 ± 49,5	100,6± 45,9	90,0 ± .00	155,5 ± 62,9	120.2 ± 24.0
Left Kidney	115,00 ± 45,78	100,60 ± 52,23	113,00 ± .000	135,0 ± 21,21	106.2 ± 48.8

Table 4 show that almost all organs in the 45-52 years age group have an average value of maximum organ weight when compared with other age groups, except the brain (1367.0 grams) and spleen (139.5 grams).

Tabel 5 Post Mortem Organ Weight (gram) depend on Body Length (cm)

Organ	**142-149**	**150-157**	**158-165**	**166-173**	**>173**
	Mean ± S.D	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**	**Mean ± S.D**
Brain	1180	1266,6 ± 57,7	1376,0 ± 144,3	1290,8 ± 122,9	1498,0 ± 403,0
Heart	185	250,0 ± 86,6	297,8 ± 62,0	257,1 ± 67,6	332,0 ± 25,4
Right Lung	210	300,0 ± 173,2	406,8 ± 156,8	352,8 ± 121,5	472,5 ± 272,2
Left Lung	251	250,0 ± 132,2	323,5 ± 116,8	312,4 ± 127,8	380,0 ± 219,2
Liver	875	1100,0 ± 100,0	1258,1 ± 180,8	1041,0 ± 126,9	1111,0 ± 316,7
Spleen	96	100,0 ± .00	87,1 ± 51,5	148,4 ± 138,8	125,0 ± 118,7
Right Kidney	69	120,0 ± 51,9	108,2 ± 42,6	115,7 ± 43,9	144,5 ± 34,6
Left Kidney	74	120,0 ± 51,9	99,5 ± 32,8	120,1 ± 36,4	167,0 ± 45,2

In this study, it was obtained that most organs increased according to a person's height. In the 150-157 cm body length group, brain, heart, right lung, left lung, and liver are lighter than the 158-165 cm body length group. Organs in the body length group > 173 cm are heaviest compared to organs in other body length groups.

Discussion

In this study, the average value of post mortem organ weight obtained from 21 autopsy cases started from the organ with the heaviest to the lightest organ weight, namely the brain (1334.2 grams), liver (1130.9 grams), right lung (370.4 grams), left lung (311.2 grams), heart (275.3 grams), left kidney (114.5 grams), right kidney (114.0 grams), and spleen (113.4 gram). Research conducted by Yosiati et al in 2012 obtained the results of the average weight value of organs, namely the brain (1218.9 grams), heart (312.2 grams), right lung (477.4 grams), left lung (417.0 grams), liver (1252.9 grams), spleen (162.8 grams), right kidney (150.6 grams), and left kidney (151.0 grams). The average value of organ weight obtained from research conducted by Yosiati et al. is not much different from the average weight of organs obtained from this study.[8] The results of this study are also not much different from the research conducted by Govender et al. in the Mongoloid race and the Negroid race. The weight of post mortem organs in the colored skin in Mongoloid race obtained by Govender et al., heart (353.6 grams), right lung (613.0 grams), left lung (486.9 grams), liver (1384.3 grams), spleen (120.8 grams), right kidney (125.0 grams), and left kidney (136.3 grams). Meanwhile, the weight of organs in the Negroid race are the heart (319.7 grams), right lung (571.3 grams), left lung (478.7 grams), liver (1333.5 grams), spleen (145.8 grams) , right kidney (145.0 gram), and left kidney (152.1 gram).[9]

Govender et al. in 2017 conducted research on three different races, namely the Negroid race, the Mongoloid race (colored and Indian skin), and the Caucasoid race showed differences in organ weight in each race. In the research of Govender et al. conducted on the Caucasoid race, there is a very clear difference in the value of organ weight when compared with the value of organ weight in this study which examined the Mongoloid race. The value of organ weight in the study of Govender et al. performed on the Caucasoid race that is the heart (433.4 grams), right lung (753.4 grams), left lung (628.7 grams), liver (1843.8 grams), spleen (233.0 grams), right kidney (164.9 grams), as well as the left kidney (179.3 grams). Based on the results of research by Govender et al. carried out in this Caucasian race, it seems clear that the Caucasoid race has heavier organs than the Mongoloid race.[9] This is due to differences in the type of food consumed, the amount of water consumed, climatic conditions, and genetic variation.[5]

The average weight of post mortem organs by sex in this study differed between men and women. In men, the average weight of post mortem organs were obtained, namely the brain (1350.0 grams), the heart (284.1 grams), the right lung (385.6 grams), the left lung (320.9 grams), the liver (1130.8 grams), spleen (120.3 grams), right kidney (116.5 grams), and left kidney (115.6 grams). Meanwhile, the average weight of post mortem organs in women are the brain (1284.0 grams), heart (247.0 grams), right lung (322.0 grams), left lung (280.2 grams), liver (1131.0 grams), spleen (91.2 grams), right kidney (105.8 grams), and left kidney (110.8 grams). Based on these results, it is known that almost all men organs have an average weight value that is more compared to women, except that the liver in men has a weight that is almost equal to the weight of the liver in women. This is in line with the research of Kim et al. which shows that the liver has almost the same weight in both men and women.[10] Men have organs heavier than women due to the influence of physical posture, ie men tend to be taller and heavier than women. This physical posture affects the weight of the organ.

In the study conducted by Peddle and Kirk in 2017 with Negroid race samples also obtained results that are in line with this study, namely the average value of men organs is heavier than women organs.[3] Similarly, research conducted by Yosiati et al who obtained the result of organ weight in men is heavier than organs in women.[8]

The results of this study are in line with research conducted by Grandmaison et al. in 2000 which showed that men had heavier organs than women. The weight of men organs are heart (365 grams), right lung (663 grams), left lung (583 grams), liver (1677 grams), spleen (156 grams), right kidney (162 grams), and left kidney (160 gram), while the weight of women organs are the heart (312 grams), right lung (546 grams), left lung (467 grams), liver (1475 grams), spleen (140 grams), right kidney (135 grams), and left kidney (136 grams) .[11]

Nonetheless, the average value of post mortem organ weight obtained from this study was very different from

the study conducted by Grandmaison et al., because the average value of organ weight obtained from the study of Grandmaison et al. is heavier than the average value of organ weight in this study. This is likely due to the differences in the sample races studied.

In this study, the weight of the heart organ in the 21-28 year age group was not much different from the weight value of the heart organ in the 21-30 year age group obtained by Deepika et al., but there was a considerable difference in the value of the weight of the heart organ . Deepika et al. obtained 280 grams for heart weight and 1311.03 for liver weight, while this study obtained a weight value of 214.86 for the heart and 1020.29 grams for the liver weight.[5] Meanwhile, the value of organ weight in the age group 37-44 years and 45-52 years in this study were not much different from the weight value of organs obtained from studies conducted by Sheikhazadi et al. in India in 2009.[12]

In this study, it is known that the value of organ weight has increased if the weight of organs in the age group 21-28 years compared with the age group 45-52 years, then experienced a decrease in organ weight in the age group > 52 years. The results of this study are in line with organ weight values contained in Knight's Forensic Pathology, which shows that the weight of organs in the youngest age group is heavier than the oldest age.[13] This is due to the reduced amount of potassium in the aging process. Potassium is a component that plays a role in the body's metabolic function, so it is an indirect indicator of muscle mass. Reduced muscle mass causes reduced organ mass.[14,15]

In addition, almost all of the organs that studied had the heaviest organ weights in the age group of 45-52 years, except for the brains that had the maximum weight in the 29-36 year of age group. The results of this study are in line with the results of research conducted by Singh et al. in 2004. Research Singh et al. states that the brain and liver have a maximum weight in their 20s and 40s compared to other ages, while the lungs and spleen have a maximum weight at 45-50 years of age.[16] Research conducted by Kim et al. also in line with the results of this study, where the weight of the kidneys and liver reaches a maximum weight in their 40s.[10] The heart becomes heavier with age and most organs (spleen, liver, and kidneys) are heavier in individuals in their 40s.[17] After reaching maximum weight, the weight of the organs will decrease due to the aging process which can cause the size of these organs to decrease. In the heart, when middle age, blood pressure will increase, so that the heart's work increases and causes an increase in heart mass.[14] Meanwhile, brain weight tends to increase until adulthood in line with the growth of neurons and glia cells as its supporters, then after through adulthood the brain's weight has decreased.[17]

In this study, it was found that almost the entire weight of the organ increased in accordance with an increase in a person's body length, except in the body length group of 166-173 cm. In the 150-157 cm body length group, the weight of the brain, heart, right lung, left lung and liver organs is lighter than the 158-165 cm body length group. In addition, in this study it appears that all the organs studied have maximum weight in the highest body length group, which is > 173 cm. Based on the results obtained from this study it can be interpreted that people with shorter bodies tend to have lighter organ weights than people with higher bodies. This is because the growth of body organs goes hand in hand with human physical growth.[18] Body proportions will determine the body's metabolism, so the size of the organs must adjust to body proportions.[8]

The results of this study are in line with research conducted by Sheikhazadi et al. in 2009, which found that the weight of organs in both men and women increased with increasing body length. Organs in the body length group 176-192 cm heavier than the body length group 166-175 cm and organs in the body length group 166-175 cm heavier than weight than organs in the body length group 151-165.[12]

Organ weight in this study in each group of body length has a weight value that is very different from research conducted by Grandmaison et al. 2001 in the Caucasoid race. In this study, the organ weight of body length groups of 166-173 cm namely heart (257.1 grams), right lung (352.8 grams), left lung (312.4 grams), liver (1041.0 grams), spleen (148.4 grams), right kidney (115.7 grams), and left kidney (120.1 grams). The weight of organs at body length intervals of 165-175 cm, including the heart (360 grams), right lung (625 grams), left lung (551 grams), liver (1637 grams), spleen (150 grams), right kidney (157 grams) , and the left kidney (175 grams) had very large differences in organ weight and organ weights of 166-173 cm in the length of the body in this study. This very large difference in organ weight values is likely due to differences in race in the sample studied, ie the race studied in this study is the Mongoloid race, while the race in the study of

Grandmaison et al. is a Caucasoid race, because racial differences cause differences in genetic variation. In addition, the Caucasoid race also has a longer body length interval than the Mongoloid race.[11]

Conclusion

Based on research that has been done at Bhayangkara Pekanbaru Polda Riau Hospital, the average value of post mortem organ weight, namely the brain (1334.29 grams), liver (1130.90 grams), right lung (370.48 grams), left lung (311.24 grams), heart (275.33 grams), left kidney (114.52 grams), right kidney (114.00 grams), and spleen (113.43 grams). The organs in men are heavier than in women, except the liver. Organ weight has decreased in old age. People with shorter bodies tend to have lighter organs than taller people.

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The Profile of Laryngopharyngeal Reflux Patients at Dr. Soetomo Teaching Hospital, Surabaya Indonesia

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Abstract

Introduction: Laryngopharyngeal reflux (LPR) is a collection of symptoms due to gastric contents or gastroduodenal backflow reflux fluid. The prevalence of LPR is very difficult to determine because of the limitations of the gold standard and the considerable variation in LPR symptoms.

Aim: to determine the profile of laryngopharyngeal reflection patients

Method: This research used analytic retrospectively, the data obtained from the medical record of outpatient unit of the Otolaryngology-Head and Neck Surgery Outpatient Unit, Dr. Soetomo Teaching Hospital. The data obtained in the study are displayed in tables and analyzed descriptively. The research sample was taken according total sampling from January 2017 to December 2018 who met the inclusion criteria.

Result: 42 samples met the requirements. 64.29% patient were female and 35.71% were male with the majority of patient was patient was 41-50 years old (26.19%). Most of the comorbidities had a history of GERD about 76.2%. The mean of the reflux symptom index score performed was 18.38, with a standard deviation of ± 8.01 and the reflux findings scores was 9.11, with a standard deviation of ± 4.25. The most frequent complaints experience was frequent mucus or clearing of the throat (90.48%) and the most frequent finding in laryngeal endoscopy was erythema/hyperemia (88.1%).

Conclusion: Profiles of patients with laryngopharyngeal reflux were mostly female with age between 41 and 50 years old. The main complaint of the throat felt blocked, and the most comorbidities were GERD.

Keywords: *patient profile, laryngopharyngeal reflux, LPR, GERD, extra-esophageal reflux*

Introduction

Laryngopharyngeal reflux (LPR) is a collection of symptoms due to gastric contents or gastroduodenal backflow reflux fluid. It contains acid, pepsin, and other digestive enzymes to the esophagus, larynx, and hypopharynx, causing contact and injury to the tissue in the upper aerodigestive tract. Laryngopharyngeal reflux has hoarseness, throat clearing, chronic cough, globus sensation, postnasal drip, dysphagia, and sore throat.[1-3] Laryngopharyngeal reflux has no specific or pathognomonic symptoms.[1,2] Laryngopharyngeal reflux may be a symptom continuation of gastroesophageal reflux.[2,4]

Gastroesophageal reflux disease (GERD) is a condition that can be differentiated and separated from LPR. [1,3] The most prominent clinical symptoms of GERD are heartburn, regurgitation and difficulty swallowing. [2] The prevalence of LPR is not known with certainty, but it is estimated that 20-30% of patients with laryngeal complaints are LPR patients. The incidence of the population with symptoms of LPR in the UK studied

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by Kamani et al. was 34.4%. Research conducted by Koufman was cited by Kamani showing symptoms and signs of LPR in about 4-10% of patients coming to Otolaryngology-Head and Neck Surgery doctors.[5] The prevalence in America in 2016 was 9.7% of all those who went to otolaryngology doctors. The prevalence of LPR is very difficult to determine because of the limitations of the gold standard and the considerable variation in LPR symptoms. [3]

The diagnosis of LPR is based on history, clinical symptoms and laryngoscope examination.[3] Belafsky, et al., Developed the Reflux Symptom Index (RSI) and Reflux Finding Score (RFS) to simplify the diagnosis of LPR. The RSI questionnaire consisted of nine items to assess various symptoms associated with LPR. Each piece has a scale ranging from zero (no complaints) to five (severe complaints), with a maximum of 45 indicating the most severe symptoms. An RSI score higher than 13 is considered abnormal and shows an LPR.[6] The RFS score uses an endoscopic examination of the larynx with eight criteria. An RFS score more magnificent than seven means an LPR. [7] Based on the description above, this study aims to determine the profile of patients with laryngopharyngeal reflux who are examined at the Otolaryngology-Head and Neck Surgery Outpatient Unit of Dr. Soetomo Teaching Hospital, Surabaya.

Methods

This study is a retrospective study using secondary data from the Otolaryngology-Head and Neck Surgery Outpatient Unit (URJ), Dr. Soetomo Teaching Hospital, Surabaya Indonesia. The research data were taken from the medical records of LPR patients examined from January 2017 to December 2018.

The research sample was of a population that met the inclusion and exclusion criteria. The inclusion criteria in this study were laryngopharyngeal reflux patients over 18 years of age who had undergone RSI and RFS examinations at the Otolaryngology-Head and Neck Surgery Outpatient Unit of Dr. Soetomo Teaching Hospital, Surabaya. The exclusion criteria in this study were age less than 18 years and abnormalities in the larynx.

Results

LPR patients who checked at the Otolaryngology-Head and Neck Surgery Outpatient Unit, Dr. Soetomo Teaching Hospitals from January 2017 to December 2018 showed 73 patients, 31 patients did not meet the inclusion and exclusion criteria, so only 42 samples met the requirements. A total of 27 LPR patients (64.29%) were female, and 15 (35.71%) were male.

Distribution of Age

The youngest age who experienced LPR was 20 years old, while the oldest age who experienced LPR was ≥ 71 years.

Table 1. Distribution of Age

Age (year)	Number	Percentage (%)
20 – 30	9	21,43
31 – 40	7	16,67
41 – 50	11	26,19
51 – 60	8	19,05
61 – 70	4	9,52
≥ 71	3	7,14
Total	42	100

Distribution of Comorbidities

Most of the comorbidities had a history of GERD as many as 32 patients (76.2%), followed by hypertension in 8 patients (19.04%). Two patients (4.76%) had no comorbidities.

Table 2. Distribution of Comorbidities

Comorbidities	Number	Percentage (%)
GERD	32	76,20
Hypertension	8	19,04
No comorbidities	2	4,76
Total	42	100

Major Complaints

The main complaint that was rarely felt was lumpy throat in 15 patients (35.71%).

Table 3. Distribution of Major Complaints

Major Complaints	Number	Percentage (%)
Throat feels lumpy	15	35,71
Hoarse voice	12	38,58
Swallowing pain	6	14,29
Difficulty swallowing	4	9,52
Throat feels dry	3	7,14
Heartburn	2	4,76
Total	42	100

RSI and RSF Score

The mean of the RSI score performed on LPR patients was 18.38, with a standard deviation of ± 8.01. The mean RFS score in LPR patients was 9.11, with a standard deviation of ± 4.25.

Table 4. RSI and RSF Score

Assessment	Mean	Standard Deviation (SD)
RSI	18,38	± 8,01
RFS	9,11	± 4,25

Reflux Symptom Index

Frequent mucus or clearing of the throat in 38 patients (90.48%) of the total sample of 42 were the most frequent complaints experienced by LPR sufferers.

Table 5. Distribution of Reflux Symptom Index

RSI	Number	Percentage (%)
Hoarseness	28	66,67
Frequent mucus	38	90,48
Excessive mucus / PND (post nasal drip)	35	83,34
Trouble swallowing	23	54,76
Cough after eating / lying down	18	42,86
Difficulty breathing / choking	17	40,48
Annoying cough	22	52,38
A lump in the throat	37	88,09
Heart burn, chest pain, indigestion, acid regurgitation	33	78,57

Distribution of Reflux Findings Scores

Erythema/hyperemia of the larynx in 37 patients (88.1%) from 42 total samples was the most frequent finding in laryngeal endoscopy.

Table 6. Distribution of Reflux Findings Scores

Finding	Number	Percentage (%)
Subglottic Udim	24	57,14
Ventricular obliteration	25	59,52
Erythema/hyperemia of the larynx	37	88,10
Udim the vocal cords	36	85,71
Udim the larynx thoroughly	34	81,00
Posterior commissural hypertrophy	23	54,76
Granuloma / granulation tissue	6	14,29
Thick endolaryngeal mucus	17	40,48

Discussion

The ratio between men and women is one to three. A similar study was conducted by Kesari et al. On 200 LPR patients consisting of 123 (61.5%) women and 77 (38.5%) men.[8] Another study conducted by Andriani, et al., for 51 patients with LPR, there were female (62.75%) and male (37.25%) patients.[9] The youngest age of LPR patients who sought treatment was 20 years old, and the oldest was more than 71 years. The largest age group with LPR was 41 to 50 years old, with 11 patients (26.19%) (Table 1). The results of this study were the same as those conducted by Ratunanda, et al., with the largest age group 40-49 years as many as 41 patients (47.6%). [11]

Over 40 years of age, there has been a change in the laryngeal mucosa, namely the superficial layer of edema in the lamina propria, especially in women after menopause. Mucus production is reduced due to changes in the glands in the larynx. Old age histologically, the endoplasmic granular reticulum and Golgi apparatus in the mucus and serosa of the larynx are small so that the quality and quantity of secretions are reduced. Other changes occur in the epithelial mucosa of the vocal cords, which becomes thinner so that at the age of over 40, the larynx is susceptible to acidic substances.

The comorbidities of LPR sufferers in this study were GERD totaling 32 patients (76.2%) (Table 3). This study follows Nennstiel et al., where most comorbidities were GERD in 12 patients (57%). [13] Reflux gastroesophageal disease is a physiological backflow of gastric content into the esophagus, which can occur 50 times a day, especially after eating. Inflammation of the larynx can be a cause of GERD. Laryngopharyngeal reflux is a supraesophageal manifestation of GERD caused by gastric contents' backflow into the laryngopharynx, thus showing a significant correlation between GERD and LPR. The backflow of acid in LPR is acted by GERD.[14] Patients who have GERD and LPR are related to each other.

LPR sufferers found that the main complaints that caused patients to go to the hospital were 15 patients (35.71%) of the throat felt blocked (35.71%) and the hoarse voice of 12 patients (28.58%) (Table 3). This study follows the one conducted by Lechien et al., The main complaint that many people feel is a lump in the throat and hoarseness of 16 patients (20%). 12 Putri conducted a similar study, et al., The main complaint was the feeling of a lump in the throat of 43 patients (91.5%).[15] Some of the clinical symptoms of LPR sufferers can include irritation of the throat, changes in voice, and trouble swallowing. Symptoms of throat irritation can include a dry or itchy feeling in the throat, throat clearing or clearing the throat, a sensation of mucus, chronic cough, globus sensation, and sore throat.[16]

Reflux causes the mucosal barrier to be damaged, causing trauma, inflammation, and dysfunction of the respiratory cilia, causing clinical symptoms of a blocked throat, pharyngeal globus, and throat clearing. Reflux also increases nasal secretions and the sensation of secretions in the back of the nose.[15] The mean RSI score in this study was 18.38, with SD ± 8.01 (Table 5). This study follows the one conducted by Karakaya et al., The mean RSI score was 18.3, with SD ± 4.4.17. Another study conducted by Asyari, et al., obtained a mean RSI score of 18.53 with an SD 4.46.10 Mean RFS score at This study was 9,11 with SD ± 4,25 (Table 5). Following the research conducted by Nunes et al., the mean RFS score was 9.53, with SD ± 2.64.14. This indicated that the patient who came to the Dr. Soetomo hospital was an LPR.

RSI complaints most often experienced by patients with LPR in this study often had mucus or cleared their throat in 38 patients (90.48%) and a lump in the throat of 36 patients (76.19%) (Table 5). This study was similar to that conducted by Lechien, et al., Complaints according to the RSI score were the throat-clearing of 38 patients (92.68%).[12] This study follows the one conducted by Karakaya et al., According to the RSI score, 48 patients (92.7%) and a feeling of blockage in the throat of 37 patients (71.3%) .[17] A similar study conducted by Asyari, et al., stated that the most frequent complaints of the clearing were experienced by 30 patients (100%) and 27 patients (90%).[10] The study's results are similar to the study by Ratunanda, et al., Showing that the complaints according to the RSI score were clearing 86 patients (100%) and a lump in the throat of 86 patients (100%).[11]

There are two theories about the mechanism by which stomach acid can provoke clinical signs and symptoms of LPR disorder. The first theory is due to the direct trauma of pepsin acid to the larynx and surrounding tissue. The second theory is that acid in the distal esophagus stimulates reflex mediated by the vagus nerve resulting in bronchoconstriction, which results in clearing and coughing, resulting in mucosal lesions. Symptoms develop due to direct mucosal trauma or damage to the cilia, resulting in mucus stasis, clearing,

and coughing.

Complaints of dysphagia, globus sensation, and odynophagia can cause mucosal inflammation of the upper aerodynamic tract.[16,19] Hypersecretion of thick mucus causes mucosal irritation because pepsin reduces mucin expression and bicarbonate secretion. These complaints can also be caused by the stimulation of the lower aerodigestive chemoreceptors by refluxate. Symptoms of postnasal drip, throat clearing, globus, and cough are caused by mucus accumulation. Symptoms of dysphonia are a more difficult mechanism and undergo macroscopic and microscopic changes in the mucosa of the vocal cords.

The findings of the RFS score in this study were erythema or laryngeal hyperemia of 37 patients (88.1%) and edema of the vocal cords of 36 patients (85.71%) (Table 6). This study is following that conducted by Andriani et al., The findings of the most RFS scores were 51 patients (100%) of laryngeal erythema, 34 patients (65.38%) of the vocal cords, 9 This study was similar to that conducted by Asyari, et al., the findings of the most RFS scores were laryngeal erythema in 30 patients (100%) and udim of the vocal cords as many as 26 patients (96.67%).

Belafsky et al. Said that RFS is a method that can be used to diagnose and evaluate LPR therapy.[7] The severity of inflammation and the presence or absence of lesions on the RFS score are associated with LPR. An RFS score is easy to do with a score above seven, having a 94% probability of experiencing LPR. Subglottic edema is associated with non-acid reflux. Posterior commissural hypertrophy and ventricular obliteration were associated with reflux exposure time but not the number of reflux events.[16]

Conclusion

Profiles of patients with laryngopharyngeal reflux were mostly female, the most common age was between 41 and 50, the main complaint of the throat felt blocked, and the most comorbidities were GERD. According to the RSI score, the most common complaint of patients was the frequent clearing of the throat, and endoscopic findings with the RFS score were erythema or laryngeal hyperemia.

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The Association between Sex, Knowledge, and Education Level of Evidence-Based Dentistry Behavior among Dentistry Students

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Abstract

Background: Almost all faculties of dentistry have implemented Evidence Based Dentistry (EBD) curriculum to improve students ability to provide the best treatment for patients supported by the best research evidence, but there is still little data to show evaluations in their use. **Objective**: The aim of this study was to determine the association between sex, knowledge, and education level of evidence-based dentistry behavior among dentistry students. **Method:** The study was a cross sectional survey and has received ethical approval. Self-administrated questionnaires distributed to second, third and fourth year students of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya. Contingency Correlation analysis was performed to determine the association between sex and behavior, Spearman's correlation analysis was performed to determine the association between knowledge, education level and behavior. All data were analyzed with a significance level of 95%. **Result**: A total of respondents filled out and returned the questionnaire were 221. Eighty five percent of respondents were females. Based on statistical analysis, there is association between sex and behavior of the implementation of EBD with 0,000 significance value, there is association between the knowledge and behavior of the implementation of EBD with 0.008 significance value, and there is no association between the level of education and behavior of the implementation of EBD with a significance value of 0.458. **Conclusion:** There is association between sex and with behavior, but there is no association between the level of education of dentistry students with evidence-based dentistry behavior.

Key Words: *evidence-based dentistry, dentistry students*

Introduction

In the current 4.0 revolution era, most fields of human life are supported[1] by digitalization manufacturing and exploitation of the potential of new technologies[2], including in the health sector[3], peoples will find out about their health conditions through digital references[4]. The health workers can also take advantage of this technology to always find out about the latest and best treatment developments[5], but not all sources we get through digital technology can be trusted[6]. Important to close the gap between research results and dentist practices to optimize the information available so that it can provide the best care[7]. This gap can be closed by formulating evidence-based clinical guidelines for the best treatment that can be performed by dentists and can be conveyed in simpler language that can be easily accepted by patients[8].

Evidence-based medicine were introduced in the 19th century and are referred to the best use of evidence in making decisions about patient care for each individual carefully, clearly, and wisely[9]. With the evidence-based application[10], it is expected that there will be continuity between patient needs and a good treatment plan from the dentist, so they can provide best treatment[11]. According to the American Dental Association (ADA)[12], Evidence-Based Dentistry (EBD)

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is an approach in the field of dental and oral health that requires a systematic overall understanding of clinically relevant scientific evidence, relating to the condition and health history of the whole body, teeth and the patient's mouth, with the clinical condition discovered by the dentist, as well as the patient's care needs and preferences. Not all reference can be evidence-based for treatment or decision making[13]. Among the sequence of evidence-based results that we can make reference are systematic reviews and meta-analysis which occupies the top position which is considered as the highest level of evidence-based, then followed by randomized clinical trials (RCT). After that there are non-RCT studies, cohort studies, case-control studies, cross-over studies, cross-sectional studies, case studies or case reports, and expert opinions[6].

Many dentists believe they practice evidence-based dentistry (EBD), but in reality the dental profession lags behind many other health professionals in making evidence-based treatment decisions[14]. In 2013, The US Institute of Medicine report that available evidence-based information was rarely applied to improve the quality of treatment, especially when evidence-based treatments for prevention and management were disseminated[15]. Almost all faculty of dentistry in Indonesia as an initial line to produce the next generation of dentists, have implemented evidence-based in the curriculum as a mechanism to educate students to be able provide best treatment to patients supported by research evidence, not just based on experience[16]. Survey conducted on dental students in Saudi Arabia showed that only 6.3% of students routinely read journals and 85% did not apply evidence-based in patient care[17].

If knowledge is an important factor but the supporting factors are not enough to understand and apply evidence-based dentistry, then we need to conduct research to find out the application of evidence-based behaviour to improve patient treatment. Therefore, we first need to understand the association between sex, knowledge, and education level of evidence-based dentistry behavior among dentistry students.

Methods

The study was a cross sectional survey[18]. The questionnaire already used in a previous study carried out by Nazir et al.[19] was selected to obtain responses from the participants. The questionnaire included questions which enquired demographic information and level of education of the respondents. Respondents were asked about knowledge including critical appraisal and access of practicing EBD, awareness about three components of EBD, selection of best evidence, and capability of patients in clinical decision making process.

Simple random sampling technique was employed to collect data. Ethical approval was obtained from respective institutes. Self-administrated questionnaires distributed to second, third and fourth year students of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya. Informed consent was distributed and filled out by respondents before completing the questionnaire. The respondents were assured about their confidentially during collection, analysis and reporting of research findings. The distribution and collection of questionnaires were carried out during the months of September – December 2019.

Contingency Correlation analysis was performed to determine the association between sex and behavior, Spearman's correlation analysis was performed to determine the association between knowledge, education level and behavior. All data were analyzed with a significance level of 95%.

Result

A total of respondents filled out and returned the questionnaire were 221 from 456. Consists of 120 second year student, 40 third year student, and 61 fourth year students.Eighty five percent of respondents were females. From the table above, it can be seen that female students who implement good behavior of EBD 167 respondents (89%) and not good 35 respondents (11%). While male students who implement good behavior of EBD 17 respondents (42%) and not good 16 respondents (48%). Total students who implement good behavior of EBD 184 respondents (83%) and those who implement not good behavior of EBD 37 respondents (17%). Total respondents analyzed were 221 respondents. The contingency correlation coefficient shows a value of 0.266. This means that the level of strength of the relationship (correlation) between sex and the behavior of the implementation of EBD is 0.266 or weak. The

contingency correlation coefficient has a positive value of 0.266 so that the association between the two variables is in the same direction. Based on the above results, it is known that the significance is 0.000, that is ≤ 0.05, it means that there is an association between sex and the implementation behavior of EBD.

From the table above, it can be seen that the 2nd year students who implemented good behavior of EBD 94 respondents (78%) and not good 26 respondents (22%). The 3rd year students who implemented good behavior of EBD 35 respondents (88%) and not good 5 respondents (12%). The 4th year students who implemented good behavior of EBD 55 respondents (91%) and not good 6 respondents (9%). Total students who implemented good behavior of EBD 184 respondents (83%) and those who not good 37 respondents (17%). Total respondents analyzed were 221 respondents. The Spearman correlation coefficient shows a value of 0.05 means the level of strength of the association (correlation) between education level and behavior of the Implementation of EBD is 0.05 or very weak. The Spearman correlation coefficient has a positive value of 0.05 so that the two variables are in the same direction. Based on the above results, it is known that the significance is 0.458, that is ≥ 0.05, it means there is no association between the level of education and the behavior of the implementation of EBD

From the table above, it can be seen that students who have high knowledge and implement good behavior of EBD 176 respondents (79%) and not good 26 respondents (12%). Students who have low knowledge and implement good behavior of EBD 8 respondents (4%) and not good 11 respondents (5%). Total students who implement good behavior of EBD 184 respondents (83%) and those who not good in implementing EBD 37 respondents (17%). Total respondents analyzed were 221 respondents. The Spearman correlation coefficient shows a value of 0.177. This means that the level of strength of the association (correlation) between knowledge and behavior of the application of EBD is 0.177 or very weak. The correlation coefficient value is positive that is 0.177 so the two variables are in the same direction. Based on the above results it is known that the significance of 0.008 is ≤ 0.05, that meaning there is an association between knowledge and behavior of the implementation of EBD.

Table 1. The association between sex, knowledge, and education level of evidence-based dentistry behavior among dentistry students

Behavior	Sex		Education Level			Knowledge	
	Women	Men	2nd	3rd	4th	High	Low
Not Good	21	167	26	5	6	26	11
Good	16	17	94	35	55	176	8
Total	33	188	120	40	61	202	19
Sig.	.000		.458			.008	
Correlation Coefficient	.266		.050			.177	

Discussion

In this study, it was found that 89% of female students implement good behavior of EBD while only 42% of male students implement good behavior of EBD. A significance of 0.000 is known that is ≤ 0.05, meaning that there is an association between sex and the implementation behavior of EBD. These results are consistent with research conducted by Fedorowicz et al (2004)[20]. A woman has a broader orientation towards information and depends on compromise in meeting her needs so that it is possible to implement EBD well because

the EBD concept is to find the best research evidence to provide maximum care for patients[20]. Whereas men are more aggressive, independent, and competitive in meeting their needs, so they tend to choose a faster path with a minimum analysis according to EBD. But this result is the opposite of the research conducted by Nazir al. that is, men implement EBD better than women[19]. A man spends less time accessing the internet, but a large proportion of his internet access is used to search various information, in contrast to women who spend more time on internet access but most of the proportion is used to socialize[19].

In this study, it was found that the results of more than 75% of the 2nd,3rd,4th years students implement good behavior of EBD. A significance of 0.458 is known as ≥ 0.05, meaning that there is no association between the level of education and the behavior of the implementation of EBD. These results contradict the research conducted by Nazir et al. (2015) which states that the higher the level of education the better it will be in implementing EBD. This is because the higher the level of education it is possible to have more experience in implementing EBD so that it can be more confident in implementing EBD than the lower level of education[19]. This shows at any y years level, when they have received EBD learning, students can be implement good behavior of EBD.

From the above data, it can be seen that students who have high knowledge and implement good behavior of EBD 176 people (79%) and not good 26 people (12%). Students who have low knowledge and implement good behavior of EBD 8 people (4%) and not good 11 people (5%). Based on the above results it is known that the significance of 0.008 is ≤ 0.05, it means that there is a significant association between the variable level of knowledge and behavior of the implementation of EBD. These results suitable with Fedorowicz et al (2004) and Nazir et al. (2015)[19,20]. Knowledge is a repository of information in one's mind. Student knowledge of EBD learning concepts will help the success, completeness of learning material in a more complex and detailed manner so that it can improve the application of EBD well[19]. Then the higher the knowledge of someone, the better the implementation of EBD.

Conclusion

There is association between sex and with behavior, but there is no association between the level of education of dentistry students with evidence-based dentistry behavior.

Conflict of Interest : None

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Ethical Clearance : This study was approved by the Universitas Airlangga, Faculty of Dental Medicine Health Research Ethical Clearance Commission

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Knowledge, Attitude and Practices Regarding Epilepsy among Nurses in a Tertiary Care Hospital in South India

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Abstract

Background: In a resource constraint nation like India, nurses play an important role in bridging the epilepsy treatment gap. They are not only caregivers but also opinion leaders. So this study has been undertaken to study the knowledge, attitude and practices regarding epilepsy among the nursing staff.

Material and Methods: A hospital based cross-sectional study was conducted among 213 staff nurses in South India for a period of four months using a 50 item questionnaire.

Results: In our study very few nurses (8%) had never witnessed an epileptic seizure .Most of them believed to have insufficient experience in taking care of such patients (86%) and were also reluctant to take care of them (28%). Certain negative beliefs like epileptics are sinners, contagious and under the influence of supernatural power were still prevailing among them. They practiced administration of oxygen and giving a metallic object in the hands of the patient during an epileptic seizure. Half of them had average to poor knowledge, attitude and practice regarding epilepsy.

Conclusion: The study findings show that knowledge, attitude and practice regarding epilepsy is low. This calls for urgent need to include practical as well as theoritical aspects of epilepsy care in the nursing curriculum and at the same time address the misconceptions linked to it.

Keywords: *Knowledge, attitude, practice, epilepsy, nurses, India*

Introduction

Globally epilepsy is one of the most frequently occurring neurological disorder affecting more than 60 million people irrespective of their age.[1,2] About 80% of them are residents of middle and low income countries. [3] Mortality risk in people with epilepsy is double than the general population [4] Epilepsy is a social disease accompanied by a stigma that is more debilitating than the disease itself .It creates distress among even those whose seizures are under control as they are constantly subjected to rejection, isolation and discrimination.[5,6,7]

In India, epilepsy is often neglected due to lack of knowledge as well as the stigma attached to it .In addition to this, the extreme shortage of neurologists with most of them being available only in the cities has

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resulted in epilepsy treatment gap of 80% -90%.[8,9] In such resource poor settings nurses can play a pivotal role in plugging the lacunae by providing continuum of care between settings . They are an essential, economic and rational asset who provide information, support, counselling and care to people with epilepsy.[1,10]

Poor knowledge, negative attitude and stereotyped beliefs about epilepsy among them results in the tendency to discriminate against the people with epilepsy thereby affecting the quality of care.[11] Therefore we have undertaken this study to assess the knowledge, attitude and practice regarding epilepsy among staff nurses in a tertiary care hospital in South India

Methodology

Study population

An institutional based cross sectional study was conducted from June 2018 to September 2018 among 213 staff nurses of a tertiary care teaching hospital in South India after obtaining consent from the Institutional Ethics Committee. All the staff nurses willing to participate and were present at the time of visit were recruited for the study.

Data collection:

The study participants were interviewed using a predesigned, pretested semi structured 50 items questionnaire after obtaining consent from them .They were assured that their anonymity and confidentiality would be maintained.

The questionnaire was devised based on the previous survey tools utilized to assess the knowledge attitude and practice towards epilepsy among the health staff. The content validity of the questionnaire was tested by dispensing the questionnaire to three specialist working in the department of Neurology. To devise the final questionnaire a pilot study was initially carried out among 20 staff nurses working in the same institute and their feedback was pursued which helped us to rephrase certain questions

The questionnaire consisted of four sections namely: profile of study participants (5 questions), their knowledge (23 questions), attitude (15 questions) and practice (7 questions) regarding epilepsy . In each section, the correct responses were given a score of '1' and '0' was allotted for all incorrect responses. The final outcome scores were divided into tertiles and classified as poor, fair and good knowledge , attitude and practice respectively.

Data Analysis

The data was tabulated and analyzed by using SPSS version 15.0 software. Results were expressed as percentages and proportions. Continuous data are summarized using mean and standard deviation.

Results

Socio-demographic profile and familiarity with epilepsy

The present study was conducted among 213 staff nurses in the age group of 20-58 years with a mean age of 30 ± 8 years. Majority of them were females (96%) and were predominantly hindus (83%). Half of them were married (51%). Equal number of them had completed general nursing and diploma nursing course (40% and 42% respectively). Very few of them had a family member suffering from epilepsy (8%). Training of more than half of them (55%) involved both theoretical as well as practical aspects of epilepsy. Most of them believed that they had inadequate experience in taking care of a patient with epilepsy (86%) and wished for additional training in the field of epilepsy(91.5%)

Knowledge regarding epilepsy

All the study participants had heard about epilepsy mostly from their nursing curriculum (84.6%) followed by books (32.7%), internet (15.4%), newspaper (8.4%) and television(1.4%). Majority of them (91.5%) had witnessed an epileptic seizure.

More than three fourth of them opined that organic disease was the cause of epilepsy (76.5%) followed by hereditary disease (58.2%), birth defect (40%), head trauma (35.2%) and mental illness (29%). One fourth of them (25%) believed epilepsy to be a result of sins committed past life.

Very few of them (6.5%) correctly knew all the clinical manifestations of epilepsy. About half of them were of the opinion that epilepsy can be prevented by ketogenic diet (48%) .Most of them (63%) were familiar

with the antiepileptic drugs and their side effects (58%). (Table 1). Half of them had poor knowledge (50.7%), whereas 36.2% and 13% had good and fair knowledge regarding epilepsy respectively.

Table 1: Knowledge about epilepsy among study participants

Knowledge about epilepsy (N=213)	Frequency(%)
1. Epilepsy is a contagious disease	38(18)
2. A patient with epilepsy is possessed by supernatural powers	39(18)
3. Cited manifestations of epilepsy	
Convulsions	186(87.3)
Loss of consciousness	148(69.5)
Change in behavior	60(28.2)
Period of amnesia	66(31)
Screaming	29(13.6)
Foam in mouth	122(57.3)
4. Every person who has convulsions has epilepsy	71(33.3)
5. Epilepsy is a public health problem	47(22)
6. There is a specific diet for patients with epilepsy	23(11)
7. There is a specific diet to prevent epilepsy	15(7)
8. Recommended place of treatment	
Hospital	205(96)
Traditional healer	5(2.3)
Ayurvedic /homeopathic practitioner	8(3.7)
9. Epilepsy can be cured	117(54.7)
10. Epilepsy requires chronic treatment	160(74.8)
11. Familiarity with antiepileptic drugs	135(63.4)
12. Familiarity with side effects of these drugs	124(58)

Attitude towards epilepsy

Majority of the study participants had a positive attitude towards a person with epilepsy attending school and having a job. Though their attitude towards a person with epilepsy getting married and having children was positive (67%) only 48% were willing to get their child married to an epileptic. (Table 2) On the whole half of the study participants had a positive attitude towards epilepsy (50.2%) whereas 30% and 35.7% had neural

and negative attitude respectively.

Table 2: Attitude of study participants towards epilepsy

Attitude about epilepsy		Frequency(%)
1.	Patients with epilepsy can attend school	177(83)
2.	Patients with epilepsy can marry	143(67)
3.	Patients with epilepsy can have children	143(67)
4.	Patients with epilepsy can breastfeed	131(61.5)
5.	Patients with epilepsy can participate in sports	66(31)
6.	Patients with epilepsy can travel alone	35(16.4)
7.	Patients with epilepsy can swim	30(14)
8.	Patients with epilepsy can have a job	132(62)
9.	Patients with epilepsy can drive	36(17)
10.	Willingness to shake hands with a person with epilepsy	184(86.4)
11.	Willingness to let their child to play with someone with epilepsy	186(87.3)
12.	Willingness to allow their child to marry someone with epilepsy	102(48)
13.	Willingness to work with someone with epilepsy	186(87)
14.	Society should behave differently with person with epilepsy	53(25)
15.	Willingness to take care of a person with epilepsy	154(72)

The study participants followed certain faulty practices like administering oxygen (67%) and placing a metallic object in the hands of the patient (44%) during an epileptic seizure.(Table 3) Overall more than half of the participants followed a good practice (53%) during an epileptic seizure

Table 3: Study participant's practice when someone has seizures:

Practice when someone has seizures		yes	no
1.	Stand back away from the patient	29(14)	184(86)
2.	Give oxygen	143(67)	70 (33)
3.	Turn patient to side	208(98)	5(2)
4.	Move objects such as furniture away from the person	198(93)	15(7)
5.	Move him from dangerous place such as road or top of staircase	198(93)	15 (7)
6.	Put metallic object in their hand	93(44)	120 (56)
7.	Avoid putting anything in his mouth	179(84)	34 (16)

Discussion

The health care system in India shows a glaring disparity in the availability of specialized health services due to the urban – rural divide. [8,9,11] In such a scenario nurses can play a crucial role as they can work in multiple settings where one can find people with epilepsy right from schools to specialized centers. A well trained nurse can relieve the burden on the health system by shouldering the responsibility of providing care and support to people with epilepsy thereby reducing the rates of readmission. They are closely associated with the community and act as a torchbearer in elucidating various social and educational concepts regarding epilepsy .[12] This study explored the knowledge, attitude and practice regarding epilepsy among the staff nurses in a tertiary care hospital in South India.

In the present study all the study participants were aware about epilepsy which was analogous to the previous studies conducted in developing African countries. [14,15] In our study 91.5% of the staff nurses had witnessed epileptic seizures . Similar findings were found in other studies conducted by among staff nurses[14,15,16] in Brazil and African countries. In the present study the familiarity of epilepsy among nurses is high as epilepsy is a part of the nursing curriculum and by the very nature of nursing profession they are more likely to witness seizures and epilepsy.

In our study 81% of the staff nurses knew someone suffering from epilepsy which was contradictory to the study conducted by RK Surekha et. al (20%) [17] in north India. A greater proportion of study participants knew someone with epilepsy in a study conducted by Njamnshi AK et al(86.5%).[14]

In the present study hardly 8% of the study participants had a family member suffering from epilepsy . This finding was much lower than the studies conducted by Chomba EN et al in Zambia (33.2%) , Vancini RL et al among nurses in Brazil(38%) and Dayapoglu N et al among clinical nurses in Turkey (23.5%). [13,15,16]

In our study when asked about the causes of epilepsy 76.5% of staff nurses stated that epilepsy is caused by organic brain disease which was much higher than a study conducted by Surekha RK et. al (43.47%) .[17] Also, 58.2% of our study participants stated that epilepsy is a hereditary disease .These responses were much greater than studies conducted by Surekha RK et. al (34.78%) and Njanmshi et al (16%), but lower than the study conducted by Vancini RL et al (86.2%).[14,17] Only 35.2% of our study participants responded that head trauma could be a cause for epilepsy. This response was much lower than a study conducted by Vancini RL (95.9%) in Brazil and Nishina Y in Japan (85.3)[1,16]

In this study the nurses had certain fallacies in knowledge like epilepsy is a mental illness (29%) which is caused due to past sins (25%) or due to possession by supernatural powers (18%). This shows that ancient beliefs are still deep rooted in spite of the information about epilepsy obtained from the training school. Similar misconceptions that epilepsy is a mental illness, were also noted in studies conducted in African countries , India and China.[2,14,15,17,,18]

In our study 18% of the respondents had a misconception that epilepsy is contagious. This is an alarming number as it will provide hindrance in the quality of care rendered by them. A comparatively lesser number of study participants had the same opinion in a studies conducted in African countries and one in Northern India. [14,15,17]

In the present study 33.3% believed that all types of seizures are due to epilepsy .These findings were higher than those of Chomba et.al (2.9%) . [15] In an Indian setting nurses tend to spend more time with the patient ,so they can help in fastening the diagnostic process if they are aware of are some medical conditions which present with seizures.

In the present study only 22% of study participants felt that epilepsy is a public health problem as compared to those conducted in Zambia. [15]This means that they are still unaware of the magnitude of the problem.

In the present study 57% responded that epilepsy can be cured which was similar to studies conducted in Turkey (36.5%) [13] This shows that more than half of them are not aware that most forms of epilepsy are incurable but can be well controlled by antiepileptic drugs.

In our study 83% of study participants felt that epileptics can attend school. Similar findings were seen

in a studies across Africa, Guinea-Bissau, china and east Asia. [13,15,18]

In our study 62% of the respondents were of the opinion that epileptics can have a job and were also willing to work with them (87%). Similar positive findings were seen in studies conducted across Asia, Africa and Europe.[1,2,13,14,18,19,20]

In the present study, although 67% of the study participants felt that epileptics can marry and have children. But only 48% of them were willing to let their children marry epileptics. This is in accordance with the age old belief that all types of epilepsy are genetic .So although they knew that there was nothing wrong in marrying a person with epilepsy they preferred their child to marry healthy individuals so as to avoid the possibility of having a child with epilepsy. These findings were in par with the study conducted by Otte WM et al.[2]

In the present study 69% of the respondents were of the opinion that epileptics cannot participate in sports. Similar findings were seen in a study conducted by Chomba et. al (66.4%)[15]

In the present study 83.6% were of the participants were of the opinion that epileptics cannot travel alone . This was in contrast to the earlier studies in Japan and Zambia where a higher percentage believed otherwise. [1,15]

In the current study 86% of the staff nurses believed that epileptics should not swim. These findings were analogous with the Zambian study (85.1%) [15]

In our study 83% of the study participants were of the opinion that epileptics should not drive. This shows that they are aware of the Indian law which states that people with epilepsy are prohibited from driving.These findings go in line with those of Yuko Nishina (83%) and Chomba et. al (65.4%).[1, 15]

In the present study around 13% of study participants were not willing to shake hands or work with or let their child play with someone with epilepsy. This may be because of their erroneous belief that epilepsy is contagious. Similar findings were found in studies conducted in North India and Guinea Bissau.[2,17] Whereas a greater proportion of participants were not willing to work with epileptics in a study conducted in Japan (38.2%) [1]

In the current study 25% of the staff nurses were of the opinion that society should behave differently with a person with epilepsy. Similar findings were seen in a study conducted by Surekha RK et. al in North India(26.08%). [17]

In our study 28% of the staff nurses were not willing to take care of epileptic patients which is a big number in case of a health care professional as this kind of negative attitude will directly hamper the quality of care provided by them. Almost similar findings were found in the study conducted by Y Nishina et.al (37.9%) [1] unlike the study conducted by Chomba et.al (5%)[15]

In this study, the nurses followed faulty practices like administering oxygen and placing a metallic object in the hand of the patient during a seizure which were similar to those seen in a study conducted by Chomba et. al. [15]

Study limitations:

As it is a single center study the results cannot be generalized. The quantitative nature of the study restricted the elucidative power of the study. Also there is a likelihood that the questions were answered in a manner considered socially acceptable by them

Conclusion and recommendation:

The study concludes that the knowledge regarding epilepsy is inadequate among the study participants Their attitude is prejudiced by the myths, taboos and misinformation accompanying epilepsy which are entrenched in the Indian society. Most of them lack awareness about appropriate first aid measures to be taken at the time of an epileptic seizure. This calls for an urgent need to conduct timely workshops to provide skill based epilepsy care education in addition to modification in the course content of nursing curriculum. There is a dire need for global standardization of nursing competencies, thereby intensifying role of nurses in epilepsy care. Further qualitative studies should be conducted to explore the reasons behind the negative beliefs among these nurses.

1. Declaration of competing interest

There was no conflict of interest between the authors

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Pre –treatment implant CBCT Analysis of Anterior Mental Loop Length, Lingual Concavity and Inclination of Alveolar Ridge in the Mandible – A UAE Study

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Abstract

Aims: This study aims to use 3D imaging (CBCT) in order to identify the presence of anterior mental loop, know its dimensions and variation in anatomy, lingual concavity and the angle of tilt in the mandibular ridge aiding in better treatment planning for surgical approaches.

Method: Forty CBCT images of patients scanned by Planmeca Romexis for different diagnostic purposes, were analyzed using the Romexis software.

Results: The study results showed that mental loop was present in 62.5% of patients on the right side and 70% of patients on the left side, with a mean length ranging from 0.30 mm to 3.12 mm. The presence of anterior canal was observed in 5 patients (2 Females and 3 Males) 12.5% of the cases examined. As mentioned in methodology lingual concavity was classified based on its morphology into three classifications: A (Acute undercut) B (stream line) C (Kidney shape), with class C (Kidney shape) amounting up to 42.5% of the cases while the least common was class B (stream line). In 40% of the cases, deepest lingual concavity was in the region of the second molar.

Conclusions: Placement of implants in the molar region of the mandible may result in perforation of the lingual cortex due to severely inclined ridge or deep lingual concavity. Presence of mental loop and anterior mandibular canal in the region of mandibular region needs careful evaluation using different tomographic slices.

***Key words**: Mental loop, Lingual concavity, CBCT, Mandibular ridge inclination.*

Introduction

With the latest advancement in the dental imaging, it is legally mandatory to have a complete knowledge about the anatomy of the ridge prior to performing an implant surgery or any surgical procedure. Thorough information about the location, tilt type of the lingual concavity and the mental loop is a boon for the clinicians to avoid any surgical mishaps.

The inferior alveolar nerve curves up and in most of the cases traverses for some millimeters anterior to the mental foramen and then return back to exit as the mental foramen, which is called as the anterior loop.[7] Bifid canals and anterior extension of the loops of this pathway or route are commonly seen. In such variations procedures like osteotomy or implant placement might result in paresthesia and hemorrhage due to violation of critical anatomical structures.[8]

The information about the safe distance, the location, and the amount of looping of inferior alveolar canal is quite critical and needs detailed investigations. Due to the limitations of conventional examination methods like clinical examination such as probing or 2D image radiography, cone beam computed tomography is one of the most accurate methods to have such detailed information and readings. CBCT helps in preoperative investigation and surgical planning. It is even used in intraoperative observation for more successful results and avoidance of many complications postoperatively.[2]

It was observed that 50% of the radiographically detected anterior loops of the mental canal were misread with panoramic radiograph, and 62% of the anatomically identified loops were not detected radiographically. It was concluded that panoramic radiograph images are undependable and have high numbers of false-positives and false-negative results in identifying the anterior loop.[4]

It is really important to know the angulation of the hand piece during osteotomy to avoid perforation of the lingual concavity and any violation to the mental foramen. The use of CBCT as a preoperative investigation aids in minimizing the complications as it helps in knowing the degree of angulation needed while performing the osteotomy.[3]

In a study conducted by Sahman et al it was shown that almost in 30% of the patients were detected with anterior loop of the mental nerve. The study also concluded that CBCT image prior to surgical implants procedure is a mandatory pre-operative examination in order to identify the presence of the anterior mental loop.[9]. In another study, conducted in Saudi Arabia, results have shown that more than half of mental foramens studied were located below the apex of second premolar while only 30% were between the first and the second premolar's apices.[1] The morphology of the lingual concavity was classified into 3 types: Convex ©, Parallel (P) and Undercut (U). the classification was done based on the shape of the mandible ridge and the presence of the concavity. The undercut shape was found in more than half of the study samples. Planning for implants in the mandibular first molar region was reinforced by the detection of the lingual concavity, its location and shape.[3]

In another retrospective study, both dentulous and edentulous cross-sectioned 3D images of patients were analyzed to see the morphology of lingual undercuts classifying it into U (undercut), P (parallel), and C (Convex). Their results showed that more than half of the cases presented with lingual concavity and 90% of it was in the second molar area.[5]

2. Materials and methods.

This retrospective cross-sectional study was conducted on 40 CBCT scans of patients treated at or referred to Ajman university clinics, Fujairah campus. All images were analyzed using the same CBCT Planmeca Romexis 3D software version 4.2.0 in High resolution mode. The exposure settings: 90 KVp, 6.3 mA, 12 seconds time, 8x8 cm field of view, and 0.200 mm voxel size. Population of the study was CBCT archived Images of 40 patients. Ethical committee approval was obtained from Ajman University ethical committee.

2.1 Inclusion criteria:

1.All the CBCT images in Ajman university server with good quality and good radiographic contrast, and density.

2.Images of dentate patients (18 to 60 years old) without any signs of bone loss in the crestal area

2.2 Exclusion criteria:

1. Any CBCT image involving bone loss in the mental foramen region.

2. Any CBCT image of patient with pathology causing bone loss in the region.

3. Any CBCT image where the mental region is not clearly defined due to metal artifacts or noise from the fillings.

Descriptive Statistical analysis was performed on 40 patients in both right and left sides of lower jaw (80 nos: in total) using IBM SPSS statistics viewer. Chi square test and cross tabulation test was done at 0.1 significant level to check the correlation of anterior loop length with age and gender of the patients on both right and left side.

Same tests were used to check for the correlation between lingual concavity with gender and age of the patients. The procedure was performed as follows: CBCT images are evaluated from the software. Axial, coronal and sagittal sections are evaluated for mental loop location. Distance between convexity of the mental loop and anterior border of the mental foramen is measured in axial view (Figure No. 1). Most concaved area in the lingual plate of the mandible is identified in implants view of Romexis software. The degree of the ridge inclination to the labial cortex is measured in the most concaved coronal section in implants view. The degree of concavity angle was measured using implant coronal view. (Figure No. 2) The morphology of lingual concavity was traced using trace paper on an image of sectioned implant view with the most concaved region. This is to classify different types of concavity, lingual concavity and are classified into three classifications: (Figure 3)

Class A: Acute undercut Class B: Stream line Class C: Kidney shape.

We analyzed the presence of anterior extension of the canal in axial sections. (Figure 4) Measuring the angle of ridge inclination was done, using the flush terminal on the buccal shelf and the axis of inclination of the alveolar ridge. Grading of the mandibular ridge inclination done based on the degree of angulations as follows:

Grade I: 5° - 10°

Grade II: 11° - 15°

Grade III: 16° - 20°

Grade IV: 21° - 25°

Grade V: 26° - 30°

Measuring the angle of the depth of lingual concavity Grading of the degree of lingual concavity done based on the angle of depth of the concavity as follows and it was done measuring the flush terminal plane producing the depth of lingual concavity.

Grade I: 100° - 120 °

Grade II: 121° - 140 °

Grade III: 141° - 160°

Grade IV: 161° - 180°

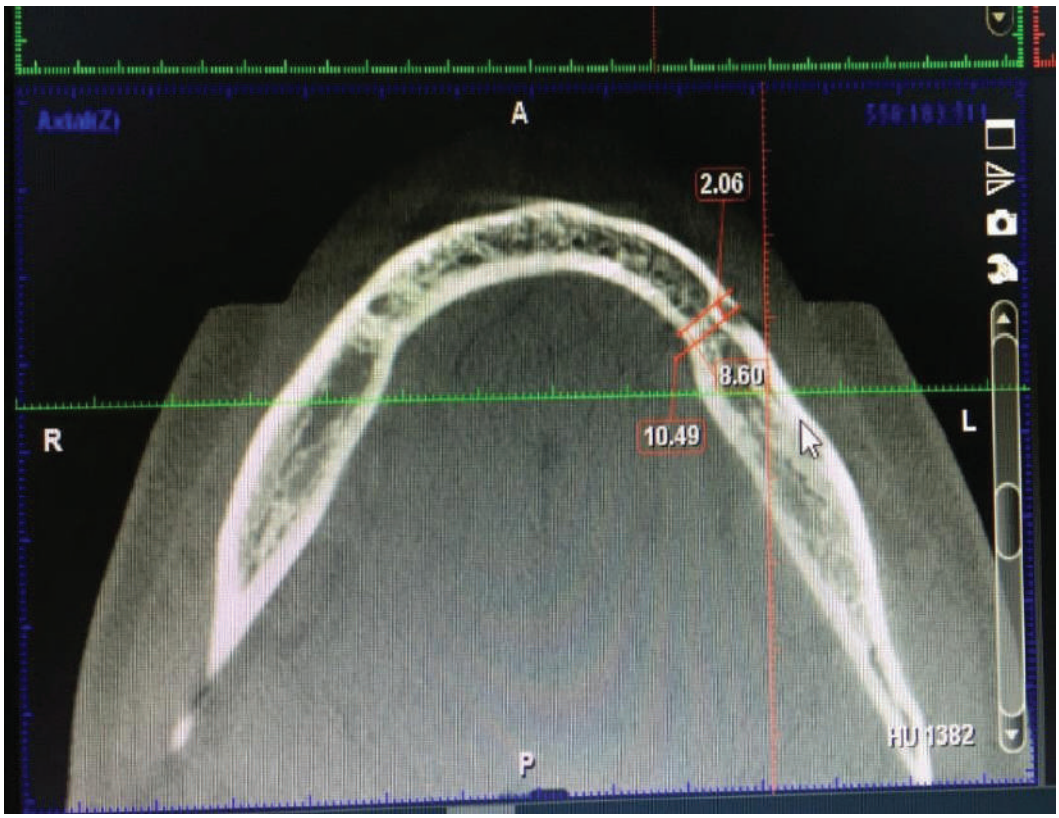
Results

The mental loop length of the mental foramen was measured in all 40 patients on both right and left sides, and in those patients evaluated, mental loop was identified in 62.5% of patients on the right side and 70% of patients on the left side. Mean length of mental loop in females is 0.871mm with SD of 0.54642 while in Males is 1.1034 mm with SD of 0.76803. It clearly shows that male patients are having a higher mean mental loop distance measurements compared to female patients. In the right side 2.5 % of the cases present with 1 mm length of anterior loop and 5 % of the cases with 2 mm anterior loop length, while on the left side anterior loop length of 1 mm was present in 7.5% of the cases and 12.5 % of them present with 2 mm loop length. The longest mental loop length observed was 3.12 mm in length and was seen in a male patient. In 20% of the cases the absence of the anterior mental loop was bilateral, unilateral absence of the loop on the right side is 37.5% of the cases, while on the left side unilateral absence of the loop was in 30% of the cases. Thus clinically 1/3rd of the cases were showing either right or left unilateral absence of mental loop.At $\alpha = 0.1$ There is no statically significant correlation between the presence and absence of the mental loop and gender or age of the patients. Association between the length of the loop with either the age of the patient or the gender of the patients was not statistically significant (P value = 0.536) as shown in (Graph 1, Table 1)

As mentioned in methodology lingual concavity was classified based on its morphology into three classifications: A (Acute undercut) B (stream line) C (Kidney shape), the most common class was class C (Kidney shape) in both right and left sides represented in 42.5% of the cases while the least common was class B (stream line) expressed in 17.5% on the right side and 20% on the left side. At $\alpha = 0.1$ It was observed that there is significant correlation between lingual concavity on the right side and left side for both females and males with age. (F: r = 0.44, P value = 0.087) (M: r = 0.494, P value = 0.019). (Graph 2 & 3) Maximal depth of the lingual concavity was commonly seen in the Mandibular second molar region (40% on the right and 47.5% on the

left side), while the least common region to have lingual concavity was observed in the third molar region. Angle of the depth of lingual concavity was commonly ranging from 141°- 160° (Grade III) in 60% of cases on the right side and in 65% on the left side. 45 % of the total cases on the right side present with inclination of the alveolar ridge with reference to buccal cortical plate in a range from 11° to 15° (Grade II) and 40 % of the cases on the left side present with ridge inclination with reference to buccal cortical plate from 16 to 20°(Grade III).

		Mean ALL	**Age**
	Pearson	1	.101
	Correlation		
Mean ALL	Sig. (2-tailed)		.536
	N	40	40
	Pearson	.101	1
	Correlation		
Age	Sig. (2-tailed)	.536	
	N	40	40



Figures 1 Measurement of mental loop in the axial section

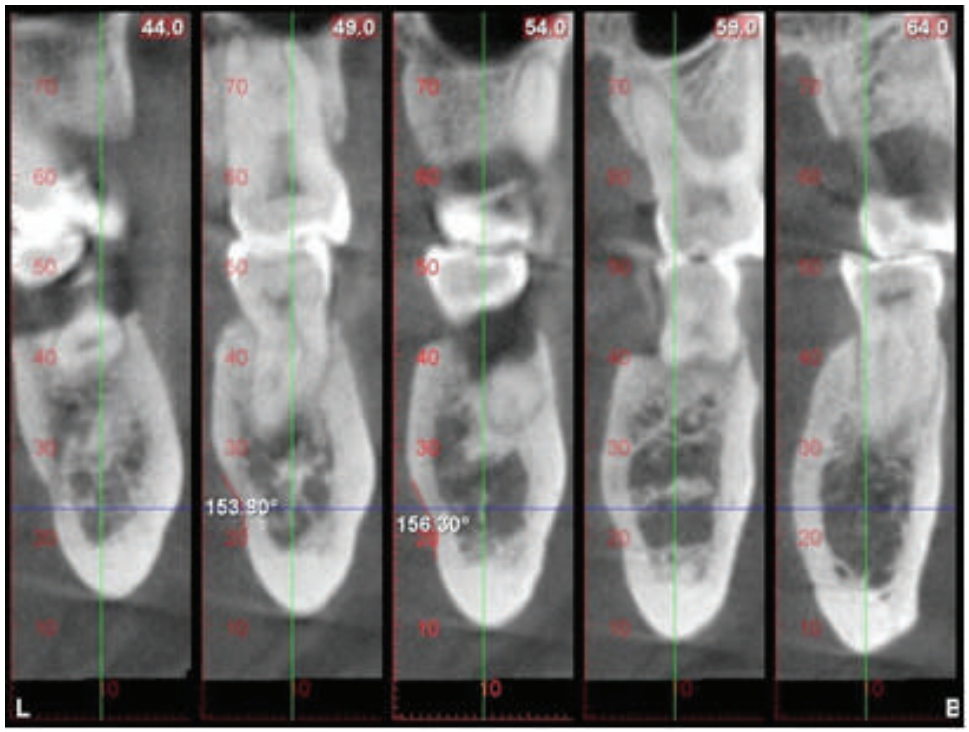


Figure 2 Lingual concavity measurement in the coronal implant view.

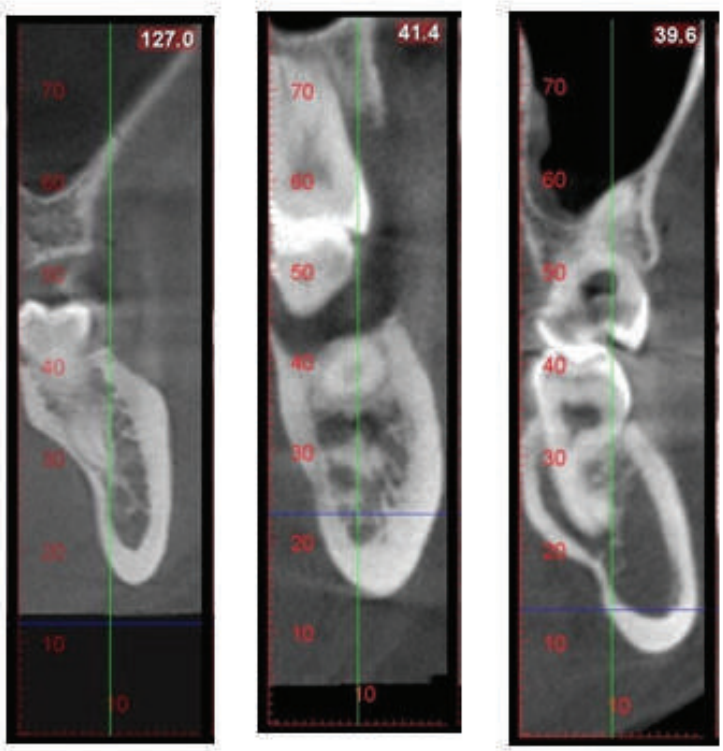


Figure No. 3 Morphological classification /Biotypes.

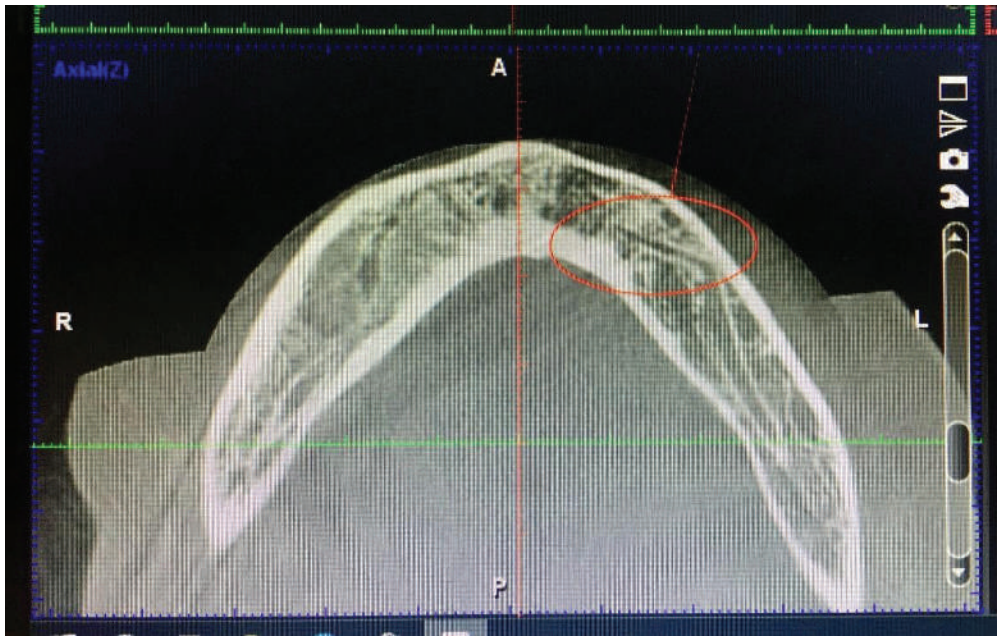
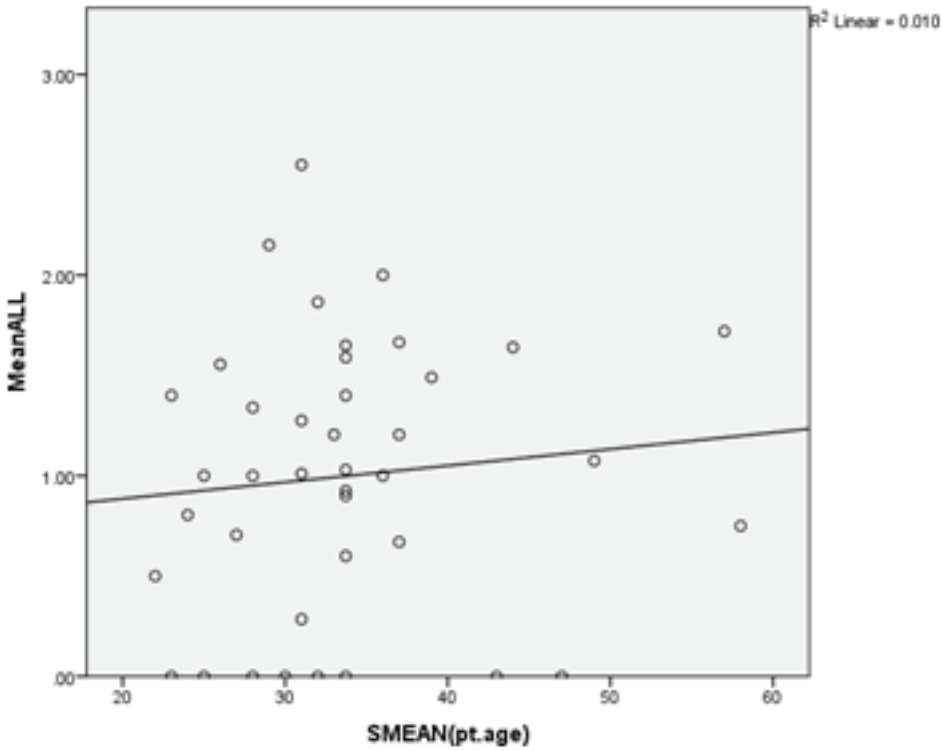
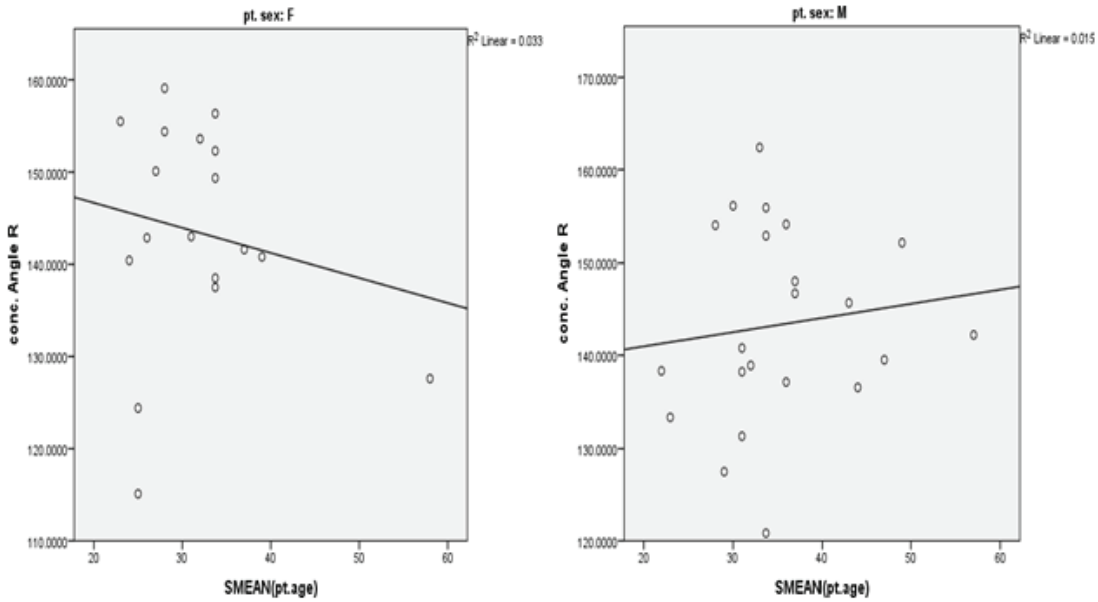


Figure 4 Presence of anterior canal (axial section)

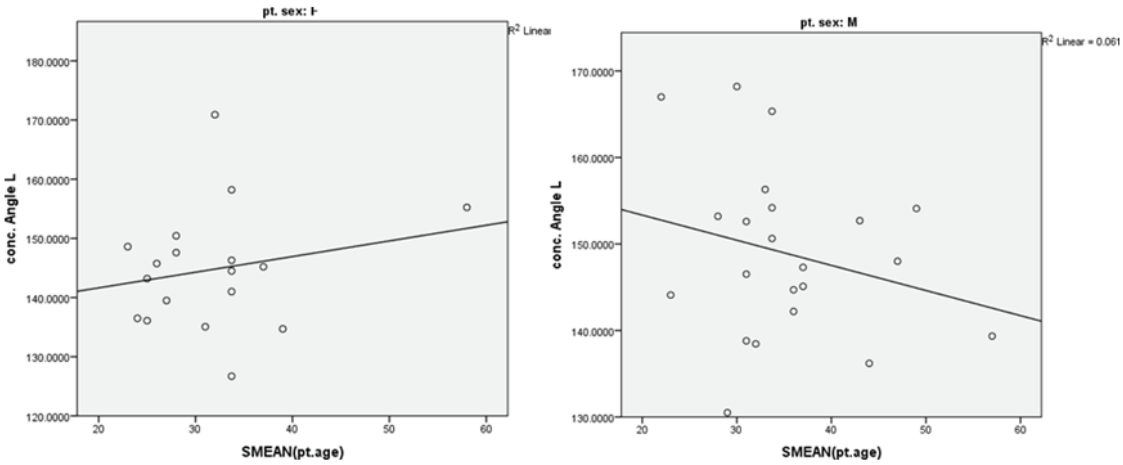
GRAPHS



Graph No. 1: Correlation of age with mean anterior loop length



Graph 2 Lingual concavity angle and correlation with gender



Graph3 Lingual concavity angle and correlation with left side age

Discussion

In the current study evaluation of the mental loop and lingual concavity was done on CBCT images, and the study has found that the mean length of anterior loop was 0.9989 mm with a standard deviation of 0. 67932. These results suggest a possibility of trauma to the nerve in case of performing an osteotomy for implant placement or any surgical approach in the premolar area. In one of the previous studies their results showed a mean length of anterior loop length was ranging between 0.21 and 2.71 mm.[4] The difference in the results between our study and theirs can be due to different sample size, different age of examined patients, race, or dental status. In some studies, correlation between anterior loop length and the gender of the patient was significant.[9] While in this study, there is no significant correlation between anterior loop length and Gender. Some studies were observing the correlation between right and lefts side of the anterior loop length[2] while in our study our focus was on the correlation of the Anterior loop length with gender and age. In another study anterior loop length has shown a significant correlation among

different age groups, but in the current study there is no correlation found with age of the patients.[4] Chan et al in their study has classified lingual concavity into C (convex) P (parallel), and U (undercut) while in our study lingual concavity was classified into A (acute undercut) B (stream line), and C (kidney shape) which is more clinically useful in implant planning.[3] In one of the studies reviewed previously they did lingual concavity analysis in the canine area also not only in the posterior region. However, they also concluded that most of the cases observed the presence of lingual concavity is in the second molar region which is the same result derived and in agreement with the study. In one of the studies, a positive relationship between mandibular lingual concavity with age and gender was observed which was endorsed in our study.[6] The following conclusions were drawn from the study are given below with the use of CBCT as a preoperative investigation aids in minimizing the complications and it helps in knowing the degree of angulation of hand piece needed while performing the osteotomy.

In conclusion the ridge inclination, (with reference to buccal cortical plate) was ranging from 11-20 degrees in majority of patients. It also sheds light on the accurate safe distance needed to avoid any damage to adjacent mental nerve and inferior alveolar nerve. Angle of the depth of lingual concavity was commonly ranging from 141°- 160° (Grade III) in 60% of the right side samples and 65% of the left side samples examined. Avoidance of post-surgical complications such as perforation of the lingual concavity or violation to the mental nerve can be achieved by the use of accurate investigation methods like CBCT. Improper placement of implants without cone beam CT image analysis in the molar region of the mandible may result in perforation of the lingual cortex due to severely inclined ridge or deep lingual concavity especially in the 2nd mandibular molar region. Mental loop varies in length so much between the patients as observed in this study so CBCT investigation is recommended to know the exact safe distance in implant placement as there was one male patient showing length exceeding 3mm, more than the expected anterior loop length. There is also presence of anterior canal as an extension of mental loop in 12.5% of patients examined. In this study, we devised new clinically useful morphological classification of the lingual concavity. I would recommend the future studies to have larger sample size and observation within family members, racial groups in order to evaluate the relation of anterior loop and lingual concavity with hereditary or genetic factors.

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Conflict of Interest - Nil

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A Multicentral Quasi experimental Study on Impact of Neuromuscular Taping over Neck Pain

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Abstract

Introduction: Neck pain in particular is considered to one of the major health problem in modern societies. It is also increasing in intensity, frequency, and severity because of more stress and strain on neck region. Neck pain is assumed to be multi factorial in origin, implying that several risk factors can contribute to its development. **Objective:** This study conducted to check the effectiveness of Neuromuscular Taping over Neck Pain in collaboration with Aimst University (Malaysia), University of Indonesia (Indonesia), NMT Institute Italy & USA, Mekelle University Ethiopia (East Africa) & Insitute of Paramedical Sciences, Kannur, India,. **Methodology:** A qualitative research model in the form of a prospective type quasi experimental study design was carried out in this study. The study was conducted in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy & USA, Insitute of Paramedical Sciences, Kannur, India, and Mekelle University Ethiopia. In this study all Academic and non-academic professionals those who diagnosed with neck pain for at least for 6 month duration, were included as the Population of this study. Conveniently 800 participants were selected in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy & USA, Mekelle University Ethiopia & Insitute of Paramedical Sciences, Kannur, India. **Result:** All Selected Participants with neck pain symptoms were assessed for Neck pain with neck pain index (NPI) respectively before and after intervention with neuromuscular taping. The results from most of the participants showed significantly improvement after intervention. **Conclusion:** As the pain factor is reducing, considering the fact NMT can be included as one among the modalities used for the treatment of neck Pain. Introduction of new intervention such as neuromuscular taping in terms of neck pain management itself is a very challenging. The investigation of such studies and their result will help for further research prospective.

Key Words: *Multicentral, Quasi experimental, Neuromuscular, Taping, Neck Pain.*

Introduction

Musculoskeletal disorders became increasingly common worldwide during the past few decades. It is common cause of work-related disability, among workers with substantial financial consequences due to workers compensation (Ariens, et, al, 2011)[1]**.** Neck pain in particular is considered to one of the major health problem in modern societies. It is also increasing in intensity, frequency, and severity because of more stress and strain on neck region**.** Neck pain is assumed to be multi factorial in origin, implying that several risk factors can contribute to its development[2]. Chronic neck pain patient uses the health care system twice as often as the rest of the population (Bongers, et, al, 2016)**.**Over a decade ago, the national Institute for Occupational Safety and Health, estimated that the cost associated with work related Musculoskeletal Disorder was $13 billion annually; more recently, this was projected to be between $45-54 billion[3]. With children being exposed to computer – related activities at even earlier ages, the health of the future work force deserves

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contemplation (Bovimet, al, 2014)[4].The prospective studies on prevalence of neck pain are important to study the size and extent of this problem that would facilitate accurate prediction of the need for preventive measures[5]. It has also been suggested that cognitive or emotional processes play a strong role in the genesis. Neck -related disability, such as depression and anxiety or pain catastrophizing which may intervene in the relationship between structural pathology and disability (Chiu, et, al, 2012) [6]. Since higher educated population generally performs more reading tasks, higher education level may be one of the risk factors for neck. The past two decades showed rapid developments in technology which resulted in the increased use and requirement of computers at the workplace (Fejer, et, al, 2014)[7]. Varying requirements and demands at each profession require these skilled staff to use computers more in order to be able to perform their tasks efficiently (Fine, 2009)[7]. immobilization and static work lead to reduced blood circulation, which prevents the proper supply of nutrients to the muscles, and an accumulation of waste products, causing fatigue and pain and that on a daily basis, persistence of these conditions could be resulting chronic musculoskeletal disorders work related neck pain amongst university administrative staff [8]. Neck pain is one of the most common musculoskeletal complaints that affect about half of the adult population during a 1-year period (Grant,et,al., 2014)[9].It is considered as a major public health problem, both in terms of health and overall well-being of the person and the society. It has imposed relatively high direct and indirect costs, and may affect the quality of life and working condition of patients (Guez, et, al, 2012)[10]. They also correct the alignment of joints, support muscles during movement, and improve stability and posture. The NMT concept has five major functions: Removes congestion of body fluids activates endogenous analgesic systems, corrects joint problems, muscle support, and scar treatments [11]. Neuromuscular Taping often provides a solution in difficult situations, in acute stages, and in functional rehabilitation to reduce pain and inflammation. Results can be seen in a wider range of motion, with less pain and discomfort, facilitating the rehabilitation of patients [12.] Over the last 5 years in Europe proprioceptive NMT technique has become a mainstream treatment protocol in post-operative, oncology, neurological care of patients and in sports medicine. It is commonly used in the sports traumatology as well as patients with Multiple Sclerosis [13]. These study specific application processes of a NMT in specific therapeutic areas it has already been shown a certain improvement in mobility and lymphatic drainage[14]. The study was focused on neck pain magament, quantifying the efficacy of Neuromuscular Taping as a treatment method [15]. Lack of knowledge about NMT directly affects access.

Objectives of the Study

This study conducted to check the effectiveness of Neuromuscular Taping over Neck Pain in collaboration with Aimst University (Malaysia), University of Indonesia (Indonesia), NMT Institute Italy & USA, Mekelle University Ethiopia (East Africa) & Insitute of Paramedical Sciences, Kannur, India,.

Methods and Subjects

A qualitative research model in the form of a prospective type quasi experimental study design was carried out in this study. The study was conducted in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy & USA, Insitute of Paramedical Sciences, Kannur, India, and Mekelle University Ethiopia. In this study all Academic and non-academic professionals those who diagnosed with neck pain for at least for 6 month duration, were included as the population of this study. Conveniently 800 participants were selected in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy & USA, Mekelle University Ethiopia & Insitute of Paramedical Sciences, Kannur, India.

Treatment Procedures

Features: Width Cm 5 and Length 30 cm.

Reference points: Cervical-vertebra C1 –C7

Standard Application procedure (Cervical): Patient standing with hands resting on a table, anterior cervical flexion of 45 °. Apply the base of the tape on the C1 and laterally 2 cm from the vertebral column. Remove the paper leaving only a small piece to hold the strip. Ask the patient to round the cervical section (the skin over the cervical area must be stretched). The tape is applied with absolutely no tension. The tape is applied parallel to the column and must end at exactly the same level. The treatment was given twice per week for 6 weeks (8-10 sittings). The cure tape is approved by the FDA and the product name is Tape and bandage, adhesive. The route of administration is over the skin to achieve biomechanical therapy method using decompressive and compressive stimuli to obtain positive effects in the

musculoskeletal, vascular, lymphatic and neurological systems. Before the primary neuromuscular taping application VAS (visual analogue scale for pain scaling) were collected and this procedure was continuing till the end treatment sessions.

Work Plan & Contributions

No	Activities	Responsible Persons
1	Proposal development & draft submission	1Assoc.Prof.Dr.yu Chye Wah /2Kshtrashal Singh(AIMST University,Malaysia)
2	Ethical clearance	1Ms. Kavitha Shetty(AIMST University,Malaysia)
3	Select & training of data collectors	1Kshtrashal Singh (AIMST University,Malaysia)
4	Data collection	1Susmitha Govind/2Kasmalina 3Mr.Mahesh Hegde/4Ms.komala/5Ms.Shankari/6Ms. Malisha/7Mr. Nazri/8Mr.Goh/9Aditya Denny Pratama / 10Rahul Krishnan Kutty (MAHASA University , Malaysia)/ 11Saurabh Singh/ 12Khushboo Gupta/ 13Sivakumar Pendyala/ 14Kameswari Kondreddy/ 15Saurabh Shekhar.(1-8 & 11-15 AIMST University ,Malaysia)
5	Pilot Report	1Kshtrashal Singh(AIMST University,Malaysia) / 2Dr. Alemayehu Berhe(Mekelle University, Ethiopia) /3David Blow(NMT Italy & USA)/ 4Aditya Denny Pratama(University of Indonesia) / 5Rahul Krishnan Kutty(MAHASA University, Malaysia)
6	Data entry & analysis	Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)
7	First draft	Kshtrashal Singh/(AIMST University,Malaysia) Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)/ Dr. Alemayehu Berhe(Mekelle University, Ethiopia)/ David Blow(NMT Italy & USA)/ Aditya Denny Pratama(University of Malaysia) / Rahul Krishnan Kutty(MAHASA University Malaysia)/Heera S(Institute of Paramedical Sciences, Kerala, India)/Kamaraj B(AKG Institute, Kerala India)
8	Second draft	Kshtrashal Singh(AIMST University,Malaysia)/ Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)/ Dr. Alemayehu Berhe (Mekelle University, Ethiopia)
9	Final report	Kshtrashal Singh(AIMST University,Malaysia)/ Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)/ Dr. Alemayehu Berhe(Mekelle University, Malaysia)/ David Blow(NMT Italy & USA)/ Aditya Denny Pratama(University of Indonesia) / Rahul Krishnan Kutty(MAHASA University Malaysia)/Heera S(Institute of Paramedical Sciences, Kerala, India)//Kamaraj B. (AKG Institute, Kerala, India)

Results

A total of 800 subjects were selected in collaboration with Insitute of Paramedical Sciences, Kannur, India, Mekelle University, Ethiopia, NMT Institute Italy & USA, University of Indonesia and AIMST University Malaysia.

Table 1: Distribution of study samples by location

Centre/Country	Frequency	Percentage
India	200	25%
Ethiopia	200	25%
Indonesia	200	25%
Malaysia	200	25%

All selected participants with neck pain symptoms from respective centre were assessed for neck pain with neck pain index (NPI) respectively before and after intervention with neuromuscular taping. The results from most of the centres showed significantly improvement after intervention (Table 2). In India, there was a significant difference in neck pain reduction before and after intervention ($t(199)=34.290$, $p<0.001$).In Ethiopia, there was a significant difference in reduction of neck pain ($t(199)=33.604$, $p<0.001$).Similarly, a significantly reduction in neck pain ($t(199)=37.810$, $p<0.01$)were found in Indonesia. For Malaysia, there was a significant difference in reduction of neck pain ($t(199)=9.539$, $p<0.001$).Between pre and post intervention with neuromuscular taping (Table 2).

Table 2: Neck pain intensity index during pre and post intervention by centre/country

Centre	NPI-Pre It (mean±SD)	NPI-Post It (mean±SD)	t (df)	pa
India	3.69±1.32	2.16±1.360	34.290(199)	0.000
Ethiopia	4.00±1.22	2.62±1.278	33.604 (199)	0.000
Indonesia	3.69±1.32	1.81±1.14	37.810(199)	0.000
Malaysia	2.11±1.45	1.73±1.04	9.539 (199)	0.000

Note: NPI: Neck pain index; It: Intervention; SD: Standard deviation, DF: Degree of freedom; Paired t-test

The results from comparison of overall neck pain intensity index between pre and post intervention among all participants showed significant effect ($Z=-22.520$, $p<0.001$) (Table 3).

Table 3: Effect of overall neck pain after intervention

	Mean	SEM	Z	p[a]
Overall neck pain index Pre-intervention Post-intervention	 3.2261 2.0555	 0.5299 0.4095	-22.520	0.000

Note: SEM: Standard error of mean, a: Wilcoxon signed rank test

The results comparing the mean difference in reduction of neck pain revealed significant difference among the centres ($\chi 2$ (3) =508.742, $p<0.001$).

Table 4: Comparison of improvement between neck pain index and low back pain index among university groups

Outcome Difference	Centre-Country	Mean	SE	95% CI		χ^2	p[a]
				Lower	Upper		
Neck pain index	ku-India mu-Ethiopia Ui-Indonesia au-Malaysia	1.343 1.669 1.819 0.014	0.032 0.032 0.032 0.032	1.280 1.605 1.756 -0.049	1.405 1.731 1.880 0.076	508.742	0.000

Note: SE: Standard errors; CI: Confidence interval; a: Kruskal-Wallis test

To illustrate the magnitude of reduction of neck pain index during pre and post intervention. Figure 1 below showed participants from Indonesia recorded the highest reduction in neck pain index which achieved 50.9% from 3.39 to 1.81. The second highest reduction in neck pain index was participants from India which recorded 41.5% reduction in neck pain index from 3.69 to 2.16. The third highest reduction in neck pain index was participants from Ethiopia which achieved 34.5% from 4.00 to 2.62. The least reduction of neck pain index was reported from participants from Malaysia which recorded 18% from 2.11 to 1.73

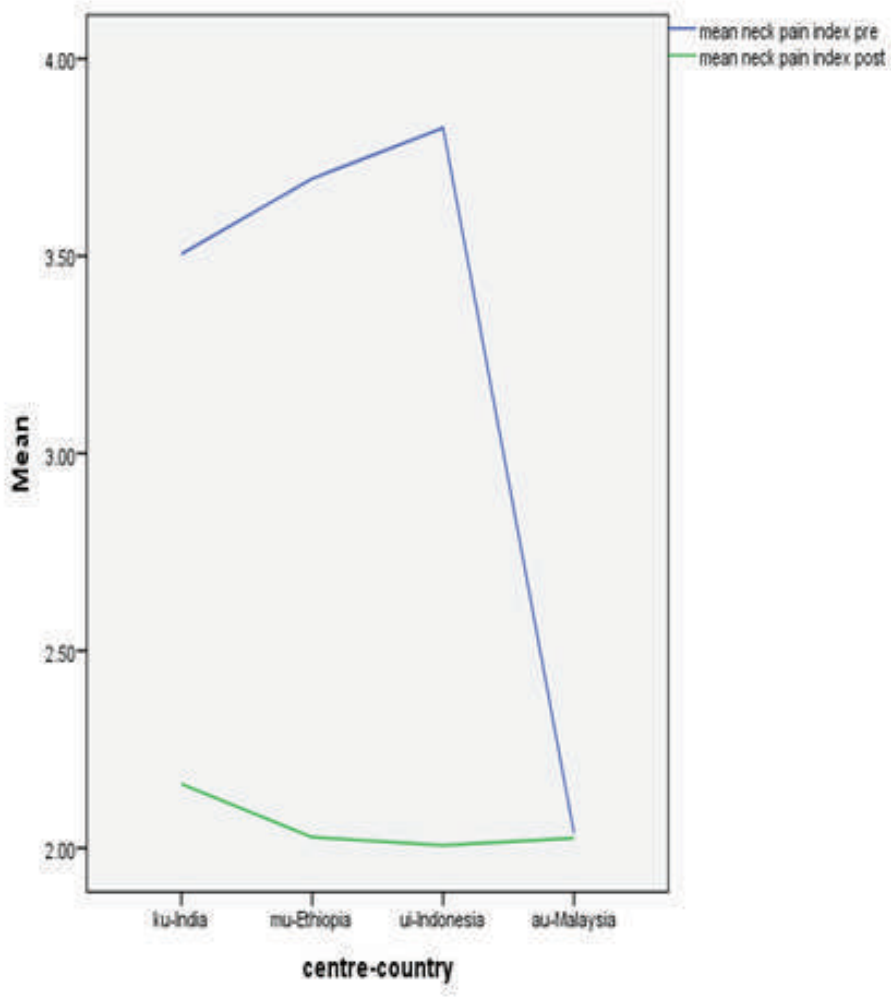


Figure 1: Neck pain intensity index pre and post intervention in 4 different countries

Discussion

Neuromuscular taping has been used as a therapeutic material in more developed countries; so many pilot studies have already been done on various clinical conditions like pain multiple sclerosis, phantom pain and other systemic problems. In this study, subjects were treated for neck pain and followed up for a period of 6-8 weeks [16]. With wide ranges of limitations, the result after series of statistical process shows evidence of reduction of parameters pain (VAS) in single blinded comparative study. Here by based on the result it can be concluded that the neuromuscular tapping is more effective in reduction of neck pain and can be considered as a treatment protocol for neck pain [17]. This data report could be considered an observational pilot study with the prospect to inform clinical practice [18], [19].

Conclusion

As the pain factor is reducing, considering the fact NMT can be included as one among the modalities used for the treatment of neck Pain. Introduction of new intervention such as neuromuscular taping in terms of neck pain management itself is a very challenging. The investigation of such studies and their result will help for further research prospective.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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A Multicentral Quasi experimental Study on Impact of Neuromuscular Taping over Low Back Pain

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Abstract

Introduction: Low back pain in particular is considered to one of the major health problem in modern societies. It is also increasing in intensity, frequency, and severity because of more stress and strain on the back and. chronic lower back pain patient uses the health care system twice as often as the rest of the population. **Objective:** This study is done to check the effectiveness of Neuromuscular Taping over Low Back Pain in collaboration with Aimst University (Malaysia), NMT Institute Italy & USA, University of Indonesia, Mekelle University Ethiopia & Institute of Paramedical Sciences, Kannur, India. **Methodology:** A qualitative research model in the form of a prospective type quasi experimental study design is carried out in this study. The study is conducted in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy & USA, Institute of Paramedical Sciences, Kannur, India and Mekelle University Ethiopia. Conveniently 800 Participants were selected in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy & USA, Mekelle University Ethiopia & Institute of Paramedical Sciences, Kannur, India. **Result:** All selected Participants with Low back pain symptoms were assessed for low back pain index (LBPI) respectively before and after intervention with neuromuscular taping. The results from most of the participants showed significantly improvement after intervention. **Conclusion:** In this study pain factor is reducing, considering the fact NMT can be included as one among the modality used for the Treatment of Back Pain. Introduction of new intervention such as neuromuscular taping in terms of back pain Management itself is a very challenging. The investigation of such studies and their result will help for further research prospective.

Key Words: *Multicentral, Quasi experimental, Neuromuscular, Taping, Low Back Pain.*

Introduction

Musculoskeletal disorders became increasingly common worldwide during the past few decades [1]. It is common cause of work related disability, among workers with substantial financial consequences due to workers compensation (Ariens, et, al, 2011) [2]**.** Low back pain in particular is considered to one of the major health problem in modern societies. It is also increasing in intensity, frequency, and severity because of more stress and strain on the back and**.** Chronic lower back pain patient uses the health care system twice as often as the rest of the population (Bongers, et, al, 2016) [3]**.**Over a decade ago, the national Institute for Occupational Safety and Health, estimated that the cost associated with work related Musculoskeletal Disorder was $13 billion annually; more recently, this was projected to be between $45-54 billion. With children being exposed to computer – related activities at even earlier ages, the health of the future work force deserves contemplation (Bovimet, al, 2014) [4].The prospective studies on prevalence of low back pain are important to study the size and extent of this problem that would facilitate accurate prediction of the need for preventive measures.lower back -related disability, such as depression and anxiety or pain catastrophizing which may intervene in the relationship between structural pathology and disability (Chiu, et, al, 2012) [5]. Not the least of which is the patient's own perspective of their condition. Low back pain is costly in

terms of treatment, individual suffering and time lost due to work absenteeism. Many studies have been conducted to investigate the relationship between lower back and occupation (Coft, et, al, 2011) [6]. Factory workers as well as office workers have been mentioned as risky groups. Machine operating and carpentry compared to sedentary work were found to be risk indicators for lower back (Evans, et, al 2010)[7].Found that lower back pain was a prevalent problem among managers and professionals also reported that university academic staff had higher prevalence of lower back pain and they were found to be a high-risk group of occupational related low back pain, Since posture and monotonous work have been pointed out as important factors in the development of occupational lower back disorders (Falla, et, al, 2011)[8]. Subjects with low back pain spent significantly more time in reading. Since higher educated population generally performs more reading tasks, higher education level may be one of the risk factors for low back pain [9]. The past two decades showed rapid developments in technology which resulted in the increased use and requirement of computers at the workplace (Fejer, et, al, 2014).Varying requirements and demands at each profession require these skilled staff to use computers more in order to be able to perform their tasks efficiently (Fine, 2009)[10]. immobilization and static work lead to reduced blood circulation, which prevents the proper supply of nutrients to the muscles, and an accumulation of waste products, causing fatigue and pain and that on a daily basis, persistence of these conditions could be resulting chronic musculoskeletal disorders work related lower back pain amongst university administrative staff[11] . lower back pain is one of the most common musculoskeletal complaints that affect about half of the adult population during a 1-year period (Grant,et,al., 2014).It is considered as a major public health problem, both in terms of health and overall well-being of the person and the society[12]. It has imposed relatively high direct and indirect costs, and may affect the quality of life and working condition of patients (Guez, et, al, 2012) [13]. Among occupational groups, office workers, especially intensive computer users, are at high risk. The ethology of work-related low back pain disorders seems to be multidimensional and is associated with physical and psychosocial factors (Grandjean, et al., 2012) [14]. A number of factors, including both individual factors (e.g. gender and age), and work related factors (e.g. repetitive work, prolonged sitting, and static posture), and also psychosocial factors, have been discussed as risk factors (Haartz, et al., 2015) [15]. They also correct the alignment of joints, support muscles during movement, and improve stability and posture[16]. The NMT concept has five major functions: Removes congestion of body fluids activates endogenous analgesic systems, corrects joint problems, muscle support, and scar treatments. Neuromuscular Taping often provides a solution in difficult situations, in acute stages, and in functional rehabilitation to reduce pain and inflammation [17]. Results can be seen in a wider range of motion, with less pain and discomfort, facilitating the rehabilitation of patients. Over the last 5 years in Europe proprioceptive NMT technique has become a mainstream treatment protocol in post-operative, oncology, neurological care of patients and in sports medicine [18]. It is estimated that 70% to 80% of the world's population has at least one episode of back pain in their life time [19]. This condition may cause a decrease in the quality of life of individuals, as well as deterioration in physical activity [20]. Generally incidents of back pain most commonly occur in between ages 25 and 50 years. Lack of knowledge about NMT directly affects access [21].

Methods and Subjects

A qualitative research model in the form of a prospective type quasi experimental study design is carried out in this study.

Standard Application procedure:

Patients diagnosed by Low back and neck pain for at least 6 months,

Features: Width Cm 5 and Length 30 cm.

Reference points: - Intergluteal line - Vertebral column.

Patient standing with hands resting on a table, anterior lumbar flexion of 45 °. Apply the base of the tape on the interglutea line and laterally 2 cm from the vertebral column. Remove the paper leaving only a small piece to hold the strip. Ask the patient to round the lumbar section (ask to "pull in the belly", the skin over the lumbar area must be stretched). The tape is applied with absolutely no tension. The tape is applied parallel to the column and must end at exactly the same level.

The treatment was given twice per week for 6 weeks (8-10 sittings). The cure tape is approved by the FDA and the product name is Tape and bandage, adhesive. Before the primary neuromuscular taping application VAS (visual analogue scale for pain scaling) and back pain index (Oswestry Low Back Pain Disability Questionnaire) were collected and this procedure was continuing till the end treatment sessions.

Work Plan & Contributions

No	Activities	Responsible Persons
1	Proposal development & draft submission	1Assoc.Prof.Dr.yu Chye Wah /2Kshtrashal Singh(AIMST University,Malaysia)
2	Ethical clearance	1Ms. Kavitha Shetty(AIMST University,Malaysia)
3	Select & training of data collectors	1Kshtrashal Singh (AIMST University,Malaysia)
4	Data collection	1Susmitha Govind/2Kasmalina 3Mr.Mahesh Hegde/4Ms.komala/5Ms.Shankari/6Ms. Malisha/7Mr. Nazri/8Mr.Goh/9Aditya Denny Pratama / 10Rahul Krishnan Kutty (MAHASA University , Malaysia)/ 11Saurabh Singh/ 12Khushboo Gupta/ 13Sivakumar Pendyala/ 14Kameswari Kondreddy/ 15Saurabh Shekhar.(1-8 & 11-15 AIMST University ,Malaysia)
5	Pilot Report	1Kshtrashal Singh(AIMST University,Malaysia) / 2Dr. Haftu Berhe Gebru (Mekelle University, Ethiopia) /3David Blow(NMT Italy & USA)/ 4Aditya Denny Pratama(University of Indonesia) / 5Rahul Krishnan Kutty(MAHASA University, Malaysia)
6	Data entry & analysis	Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)
7	First draft	Kshtrashal Singh/(AIMST University,Malaysia) Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)/ Dr. Haftu Berhe Gebru (Mekelle University, Ethiopia)/ David Blow(NMT Italy & USA)/ Aditya Denny Pratama(University of Malaysia) / Rahul Krishnan Kutty(MAHASA University Malaysia)/Heera S(Institute of Paramedical Sciences, Kerala, India)/Kamaraj B(AKG Institute, Kerala India)
8	Second draft	Kshtrashal Singh(AIMST University,Malaysia)/ Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)/ Dr. Haftu Berhe Gebru (Mekelle University, Ethiopia)
9	Final report	Kshtrashal Singh(AIMST University,Malaysia)/ Assoc.Prof.Dr.yu Chye Wah(AIMST University,Malaysia)/ Dr. Haftu Berhe Gebru (Mekelle University, Malaysia)/ David Blow(NMT Italy & USA)/ Aditya Denny Pratama(University of Indonesia) / Rahul Krishnan Kutty(MAHASA University Malaysia)/Heera S(Institute of Paramedical Sciences, Kerala, India)//Kamaraj B. (AKG Institute, Kerala, India)

Results

A total of 800 academic and non-academic professionals are selected in collaboration with Institute of paramedical science, kannur, India Mekelle University, Ethiopia; University of Indonesia, Indonesia and AIMST University, Malaysia.

Table 1: Distribution of study samples by location

Centre/Country	Frequency	Percentage
India	200	25%
Ethiopia	200	25%
Indonesia	200	25%
Malaysia	200	25%

All selected participants with low back pain symptoms from respective centre were assessed for low back pain index (LBPI) respectively before and after intervention with neuromuscular taping. The results from most of the centres showed significantly improvement after intervention (Table 2). In India, there was a significant difference in reduction of low back pain reduction (t (199) =21.175), p<0.001). In Ethiopia, there was a significant difference in reduction of low back pain (t (199) =10.436, p<0.001). Similarly, a significantly reduction in low back pain (t (199) =37.546, p<0.01) were found in Indonesia. For Malaysia, however no significant difference was found in reduction of low back pain (t (199) =0.277, p=0.782) between pre and post intervention with neuromuscular taping (Table 2).

Table 2: low back pain index during pre and post intervention by centre/country

Centre	LBPI-Pre It(mean±SD)	LBPI-Post It(mean±SD)	t (df)	p[a]
India	2.99±1.43	1.88±1.291	21.175(199)	0.000
Ethiopia	2.73±1.39	2.35±1.40	10.436(199)	0.000
Indonesia	3.85±1.30	2.03±1.17	37.546(199)	0.000
Malaysia	1.99±1.27	1.98±1.32	0.277 (199)	0.782

Note: NPI: Neck pain index; LBPI: Low back pain index; It: Intervention; SD: Standard deviation, df: Degree of freedom; [a] Paired t-test

The results from comparison of overall low back pain index between pre and post intervention also depicts significant effect (Z=-21.501, p<0.001) (Table 3).

Table 3: Effect of overall low back pain index after intervention

	Mean	SEM	Z	pa
Overall low back pain index Pre-intervention Post-intervention	 3.1844 2.1511	 0.5304 0.4378	-21.501	0.000

Note: SEM: Standard error of mean, a: Wilcoxon signed rank test

The results comparing the mean in reduction of low back pain depicted significant difference among the centres (Table 4).

Table 4: improvement of low back pain index among university groups

Outcome Difference	Centre-Country	Mean	SE	95% CI		$\chi 2$	pa
				Lower	Upper		
Low back pain index	India Ethiopia Indonesia Malaysia	1.660 1.353 1.232 -0.112	0.030 0.030 0.003 0.030	1.600 1.293 1.172 -0.171	1.720 1.413 1.292 -0.052	498.02	0.000

Note: SE: Standard errors; CI: Confidence interval; a: Kruskal-Wallis test

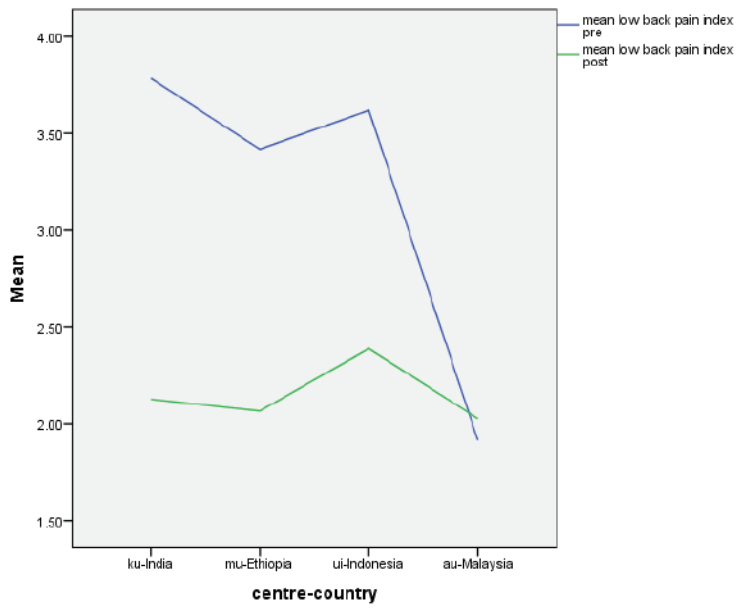


Figure 1: Low back pain index PR

The magnitude of reduction of low back pain index was depicted in Figure 1. The participants from Indonesia recorded the highest reduction of low back pain index which achieved 47.3% from 3.85 to 2.03. The second highest reduction in low back pain index was participants from India which reported 37.1% from 2.99 to 1.88. The third highest reduction in low back pain index was participants from Ethiopia which achieved 13.9% from 2.73 to 2.35. The least reduction in low back pain index was participants from Malaysia which merely achieved 0.5% from 1.99 to 1.98

Discussion

As back pain can cause temporary physical disabilities to understand the pattern of disabilities post back pain, Oswestry Low Back Pain Disability index (ODI) was included in this study. ODI values were expressed in Mean ±SD. The result shows a marked variation between the pretest ODI value and posttest ODI value. Here by based on the result it can be concluded that neuromuscular tapping is more effective in reduction of back pain and can be considered as a treatment protocol for back pain. In contrast with NMT application was also shown to be effective so based on these facts that NMT has some superficial placebo effect on pain reduction in case of back pain. This data report could be considered an observational pilot study with the prospect to inform clinical practice.

Conclusion

In this study pain factor is reducing, considering the fact NMT can be included as one among the modality used for the treatment of Back Pain. Introduction of new intervention such as neuromuscular taping in terms of back pain management itself is a very challenging. The investigation of such studies and their result will help for further research prospective.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Effect of Cytokines and CD Markers in Patients Infected with COVID- 19

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Abstract

Study aimed to show the effect of inflammatory mediators (IL-6 and gamma interferon) and molecules CD54 & CD2 in patients infected with COVID-19

A overall of (100) positive patients for COVID-19 were selected for project .Patients attended general lab. With sings of COVID-19 then select to expression 0f gamma interferon & IL-6 in serum of patient and indication expression of CD54 and CD2in serum of patients. Results indicated that gamma interferon & IL-6 highly important increases ($p<0.001$) in serum level of covid- 19 patients as matched with healthy control groups , acute covid 19 exposed great as well as , rises in serum near of gamma interferon & IL-6 pointedly($p<0.001$) ,although modest cases of disease show great proliferation in serum level of IL-6 expressively($p<0.001$).immune molecules showed more expression of CD2 & CD54 in covid- 19 patients as matched with healthy normal groups.

Key word: *Inflammatory, immune reaction and COVID-19*

Introduction

Corona virus infected patient have several communal types such as fever, cough, and fatigue while diarrhea and dyspnea were initiate to be as unusual chin([1]). Numerous of them patient described bilateral abnormalities

Main case of corona virus was advised as cold in 1960([2]).

Original coronavirus-induced pneumonia, which was named as coronavirus disease 2019 (COVID-19) by the WHO on the February 11, 2020, has speedily improved in epidemic measure since it first appeared inWuhan, China, in December 2019([3]).

Coronaviruses are enveloped viruses with a positive sense single-stranded RNA genome (26e32 kb)([4]). Four coronavirus genera (a, b, g, d) have been recognized up to now([5])

IL -6 is noticed at a few management level during acute viral infection , the most patent increase in I L-6 is sensed in patients with a higher degree of neutrophil infiltration. The level of cytokines such as IF N-y , TN F-α , I L-6 and I L-8 are amplified in persons with chronic viral infection and some authors have revealed that this rise is virtual to the grade of the harm histologically in COVID-19 patient ([6]).

Cytokines expression due to pathogenesis of COVID-19 infection is relatively due to the immunologic response , plenty of interleukins & chemokines are elaborate in the progress of the inflammatory process by altering the evolution of the inflammatory via changing the native T h0 response to a T h1 ,T h2 or mixed T h1 & T h2 response. Both responses have been revealed to relate in a viral disease ([7]) and the inequality among them prefer HIR and depressed adjust CMI, that is essential for immunity beside diseases ([8]).

Materials & Methods

1- Patients

The study registered (100) COVID 19 patient , admitted at the public health laboratory and with fever, nonproductive cough, dyspnea, myalgia, fatigue,

normal or decreased leukocyte counts, and radiographic evidence of pneumonia .

Samples Collection

blood samples (5-10) ml was obtained from patients(COVID -19). then the blood samples were centrifuged to get blood serum

Serum cytokine

Sizes of cytokines in the serum were done by ELISA test (R&D Systems). The last concentration was expressed in pg/ml.

Statistical Analysis

Statistical analysis was showed by using Chi-square ($\chi 2$) test to regulate the statistical changes among diverse groups by using a proposal statistical platform for social science (SPSS 2020). The possibility of ($P \leq 0.05$) was measured to be statistically significant. The examined parameters were offered in terms of means ± standard errors (S.E.), and variances between means of patients and controls were calculated by ANOVA test and the Least Significant Difference (LSD). The difference was measured significant when the possibility (P) value were ($\leq 0.05, \leq 0.01$).

Results & Discussion

1-Clinical sings

Clinical sings in COVID 19 patients were , fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia, some patients showed intermediate and mild clinical sings as shown in table (1).

Table (1) Clinical signs for COVID 19 patient .

NO.	Clinical signs	Number	Percentage%
1	acute	10	80%
2	Intermediate	10	80%
3	Mild	80	80%

2. IL-6 in COVID 19 patients

Serum of all patients with COVID 19 and those with acute or intermediate disease action contain higher level of IL-6 than healthy control group . IL-6 concentration was particularly increased in patients with intermediate disease and acute patients similarly than mild disease as corresponding with control groups.

Table(2) The Concentration of IL-6 in patients and controls

Group	NO.	Serum level of IL-6		
		Mean	Minimum	Maximum
Mild	80	500.00	180.00	440.00
Acute	10	900.00	300	900
Intermediate	10	1500.00	1300.00	1400.00
Control	10	70.00	50.00	80.00

Chemokines apply their biological activity through linking to certain cell surface receptors. An infrequent feature of greatest chemokine receptors is their great attraction for numerous ligands ([9]). inflammatory cytokines

such as IL-6, 10 &12 ,create irritable oxygen species. IL-8 excretion effects in an elevated employment of neutrophils into lung ([10],[11]). Lung I L -6 is noticed at less preservation grade at acute stage of COVID 19 impurity, while noticable rises in blood serum and liver grade can be detected patients with moderiate infection This rise in I L-6 associates completely with an elevated in T NF – α([12]).

3- level of gamma interferon

Current study showed that all patients with COVID- 19 cover higher level of gamma interferon than healthy control group , gamma interferon concentration was improved particularly with acute COVID patients, moderate patients and mild patients correspondingly . table(3)

Table(3) The Concentration of gamma interferon in patients and controls

Group	NO.	Serum level of gamma interferon		
		Mean	Minimum	Maximum
Acute	10	600.00	400.00	650.00
Intermediate	10	80.00	75.00	83.00
mild	80	20.00	17.00	21.00
Control	10	17.00	9.00	18.00

gamma interferon is a central cytokine to the of inflammatory pathogenesis routes. The T N F -α pro-inflammatory effect is facilitated via straight initiation of other pro- inflammatory cytokines([13])

The cytokine storm will activate a forceful round by the immune system to the body, cause ARDS and multiple organ failure, and lastly lead to death in severe cases of SARS-CoV- 2 infection([14])

4- Expression of CD54 and CD2 in COVID 19 patients

Results as in table (4) shown that there was highly significant differences in mean of CD 54 expression among COVID 19 patients and healthy control groups ($p<0.05$) ,the cell surface CD54 was over expressed in acute compared to intermediate COVID 19 patients, mild and healthy control groups individually

The results demonstrated in table (5) shows there was high statistically significant difference in mean of CD2 expression among COVID- 19 patients and healthy control groups ($p<0.05$)

To get rid of COVID 19 is related with vital multi-vague C D 4+ and C D 8+ T cell responses ,while persons that progress mild infection likely to have fragile, slimly dedicated responses [[15]]. CD8+ effector cells in the lung were initiate to have less serviceable proficiency, as proved by low IF N -y fabrication [[16]]. The determination of lung pathogens is frequently attended thru frail "CD8+ T cell response " antigens subsequent[[17]]. We exasperated to conclude the pathogenic status of C D74 over comparing of its expression during infection , our results make it clear that robust up-regulation of both C D2&C D54 manage a tough mark that lymphocytes in peripheral blood of COVID- 19 persons within formal of immune dysregulation. Acute determining infections are considered complete primary enlargement of "poly clonal C D 4+andCD8 + T –cell" residents that continued over allowance[[17],[18]]. On other hand, prolonged infections are related with temporary hindered responses that are frail and goal a slight array of MHC class I and II limited epitopes [[19],[20]]

Table(4) The expression of CD54 in patients

Group	NO.	Serum level of CD54		
		Mean	Minimum	Maximum
Intermediate	10	9.00	5.00	10.00
Acute	10	13.00	10.00	14.00
mild	80	6.00	4.00	7.00
Control	10	2.00	1.00	3.00

Table(5) The Concentration of CD2 in patients

Group	NO.	Serum level of CD2		
		Mean	Minimum	Maximum
Mild	12	10	7.00	12.00
Acute	4	35.00	20.00	40.00
Intermediate	7	14.00	11.00	15.00
Control	10	4.00	5.00	6.00

Ethical Clearance: Taken from department committee

Conflict of Interest Nil

Source of Funding: Self

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A Case Report on an Estimation of Financial Damage Occurring at A Rural University, South Africa

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Abstract

Background: Universities are power houses that generate new knowledge by carrying out research all over the world. Professors are promoted, appreciated and awarded as the academics who conduct research at universities. This did not happen in the case of Mr BM, who was humiliated and punished for his research activity.

Case History: Mr BM was excited by the prospect of carrying out research when he joined the university in 1996. He used his time and money in research and trying to find a gap in the lack of knowledge that he could close through his work. He was quite on his own in his department without even a secretary yet managed to find time for research either after hours or during holidays. Between 2001 and 2018, he published more than 100articles in around 25 different peer-reviewed journals. Mr BM boosted the good name of the university, but in exchange for it, he received three disciplinary enquiries, two suspensions and a forensic audit. All were intended to discredit him and to stop his research activity. The most painful was to cause him financial loss by decreasing his monthly salary. The history of damage and its results are discussed in this report.

Conclusion: Heavy financial loss was caused by the persistent disciplinary enquiries at the university. The extent could only be estimated by an actuarial consultant. The Ministry of Higher Education must take note of it.

Keywords*: research, promotion, financial loss, actuarial*

Introduction

The university's higher management constantly wants to know why this university ranks so low on the list of institutions producing research publications in South Africa. The usual answer they give is lack of funds. There is a vicious cycle relating to funding at the university. The more research one does, the more funds are available, and when more funds become available, there is a demand for more research projects to be carried out. Research publications bring money into university, which constitutes third-stream funding generates in universities in South Africa. There are universities in South Africa that generate a lot of funds through research publications. Publications are not only about money; it improves the good name of a university and improves its rating in the world.

The main problem in the faculty of rural University was that the position of faculty head was filled non-academic candidate. He was not a regular researcher yet have enormous power. This power was abused to humiliate and punish people in the faculty, as in the case of Mr BM. If the researcher did not include the name of a person in authority in publications, such people used their power to punish. Generally, it remained limited to small matters such as not being allowed to go on leave or not approving applications for promotion, etc. In the case of Mr BM, when they found that these small humiliations did not affect the work he was doing, so they designed some more rigorous methods. Most foreign staff accepts what those in powers tell them. These foreigners do not ask how and why things happen. This is the reason for the recruitment of foreigners in the faculty, as they do not ask any questions and are always ready to agree with those in authority. The culture of

employing foreigners has been also picked up by the recently appointed head of institution. He is comfortable with these foreigners, who have few choices, as they are not employable in other places, and they must survive. It is thus a win-win situation. The case of Mr BM is not the same. He did not wish to give them any co-authorship as a gift, and that led to numerous hurdles in his work. This is a culture at the university, if you work hard, you must face the problems. This kind of practice is common in some countries, historically English countries, where people have a concept of big and small men, and a small man does not ask any questions from a big man. Mr BM was humiliated long before his rigorous punishment in the form of disciplinary enquiries and suspension, but he simply accepted it. Mr BM never thought that a university where one works hard would dislike one.

Mr BM was determined that the truth must prevail. A research director recognised his work and encourages him to carry on at the same speed. Mr BM worked under three faculty head. These faculty head had one adviser. The first faculty head was very clever. He was appointed for three years but continued working as faculty head for almost 12 years by his convincing nature. The second faculty head took a power and had one adviser whom he later appointed as second in command expected a gift authorship, which was unethical. Secondly, they were confronted by the higher management when the issue of research was discussed. They could not explain why they could not produce research publications. This question led to trouble for Mr BM, and they wanted to eliminate him, so that this question would not be asked.

At the University this was practised in the 1990s. What happened at University was like what is currently happening at ESKOM in South Africa. ESKOM deals with power, and problems are visible, but universities' corruption can be hidden easily, as it is not an emergency. Mr BM was investigated forensically, but no report has submitted even after his retirement. The investigator took Mr BM's personal file from HR; that file was reported as lost by HR. Recently, the head of institution conducted an audit of the faculty, but the results were not made public.

Results

Mr BM received head of institution awards. In 2004, he was not allowed to apply for the reward. Then in 2006, the process started again, and BM was nominated to receive the award. On the day when the award was to be handed over to Mr BM, it was stopped, and a prepared cheque of R10 000 was cancelled; this was conceded by the head of institution himself in his reply to a dispute resolution letter in 2015. Mr BM received no cash payment in his personal research account after it had been prepared by the research office. The deputy head of institution research himself got the cash refund himself but did not pass this on to Mr BM. Personal research funds are supposed to be paid in cash on retirement and BM received an amount, but much less than what it should have been. His personal research account was tampered with by the research or finance department and fraudulently claimed. Mr BM wrote about six dozen letters to the research office, and head of institution, but not solution was reached. Mr. BM research account was also frozen and had not allowed me to utilize it. Prepared documents of conference attendance claims were rejected not paid after delays or even lost.

Discussion

When things deteriorated, the Council of Higher Education sent an assessor to investigate and submit report.[1] An assessor was appointed, and he submitted a report, but nobody implemented his recommendations. [1] He clearly mentioned that this university functioned like Afghanistan. There was failure of multiple organ systems. He paid attention to HR, but nothing changed. The independent assessor's effort went to waste; there should be no need to carry out such a time-consuming exercise if its recommendations are not to be implemented.

It is said that in Africa, people consider their stomachs first. They do not look at the country or their institutions. If things do not come into their account, they are not interested. This proved true in the case of Mr BM. In a ground-breaking judgment, the Constitutional Court pointed out that such an employee needs greater protection, as he is vulnerable to exploitation.[2] The Department of Education subsidises articles published in recognised journals through funds being paid into the accounts of universities. It is quite a significant amount and is an incentive to researchers to motivate them to carry out more research leading to publications. Of the amount received from the DoE, 60%goes to the researcher and

40% is divided among the research office (15%), head of institution office (10%) and the department (15%). This is according to the policy on research at the university. Mr BM brought millions of rand into the university funds between 2000 and 2018. Mr BM received hardly any funds from outside sources. The research office did not allow him to use the funds he had generated himself. Mr BM's research publications comprised almost 50% of the total number of publications of the faculty and about 25% of the whole university, according to the CHE report of 2011.[1]

There was an allegation of plagiarism against BM in 2008, and an enquiry was conducted by a top labour law practitioner. He found Mr BM not guilty. When Mr BM wrote a letter to the registrar of the university regarding revocation of the degree of the party involved, no action was taken. BM was not guilty, then the opposite party to be guilty, and his degree had to be revoked. There is a need of such blow to university so that they will not exploit like Mr BM. The federation congratulated its affiliate, NUMSA, on the successful conclusion of its long court battle against labour brokers.[3]

Similarly, in second DC, Mr BM, one of his articles was published by mistake in two different journals with a gap of two years. He was suspended and disciplinary enquiry started against him. The charges were less serious than first one, but the punishment was much severe than first one. When Mr BM has produced proof of two professors in his faculty who were also duplicated their article in two different journals, and they were also supposed to be suspended and DC must be carried out, but the faculty head is refused to do so. This kind of double standard showed that how malicious this faculty to Mr. BM.

Professional jealousy is understandable, but the level of punishment through a decrease in salary, multiple disciplinary enquires, and the intention to de-motivate Mr BM to the point where he would stop his research publications is not. However, MR BM persevered and kept working despite multiple problems created by the faculty and university with the help of union. Mr BM could have multiplied his publications, as he had enough experience, but was unfortunately limited by the DCs' and suspension. A meeting was held with the head of research in his office, and a full list of grievances was submitted, but nothing happened. Mr BM wrote several letters to head of institution but got not result. One email sent by head of institution in reply to Mr. BM as follows:

I request you to audit all the funds as long as you can go back. These funds are generated by me through research publications. These are two accounts. 1. Personal research publications account and 2. Department research publications account.

I am not getting any information, and nobody prepare to help me. It looks like that someone is dosing with bad intention. Attached herewith some examples or copy was issued by research office, but these are not only one. Every year (2001 to till date), I received money from DoE through research publications. Please take audit it from 2001 if possible.

I am concerned about the email below. Ms M reports to me and no staff member has the power to request her to institute audits. And in any event, this request is not reasonable.

Please refrain from this kind of thing in future.

Mr BM requested an audit of personal research funds because of the previous statement provided to him by the finance office was very problematic, as personal research money was not supposed to be used without his signature, and this statement showed that funds were being used by someone without his knowledge. This request to audit his funds was ignored by the head of institution. The process of getting refunds was very complicated that had already been spent in attending conferences. It became a project that could take from a month to a year to get a refund. It would start from a junior clerk in the research office, and then go to a senior clerk, before at last going to the director of research for a signature. The distance between two clerks is only a few metres but it would take a week to pass on the document. For Mr BM to write an article and publish it in a journal was much easier than to get a claim processed by the research directorate at the university.

It is difficult, but not impossible, to estimate the total loss in research funds owed to BM; only an actuarial assessment can determine it.

1. It involves a loss in cash through not getting research award money and cash payments from the

personal research account.

2. Mr BM was working on his own and should have been allowed to use the departmental research funds, but he was prevented from doing this and attempts were made to divert the funds.

3. Though Mr BM reported failure to pay and delays in payment, there was no change in the practice of payment.

4. Loss of productivity resulted from Mr BM not being allowed to enter the forensic pathology laboratory, and not being permitted to enter the university during suspension periods. Disciplinary enquiries are themselves problematic and take a lot of time (2008 to 2018) which Mr BM could have used in writing.

5. Articles that had been accepted were subsequently declined as a result of disciplinary enquiries.

Mr BM faced difficulty at all levels. It started with him not being allowed to work in the forensic pathology laboratory. If he published articles, then these were not submitted to the DoE for subsidy. The funds received from the DoE were also difficult to get. The OSD scale has given to all doctors who registered in health professions council and working in public sector .[4] This was not applicable to Mr. BM.

Conclusion

Direct financial loss could be calculated by an accountant, but the loss in the form of pain and suffering caused by persistent disciplinary enquiries could only be assessed and estimated with the help of an actuarial consultant. This concerns not only the issue of money, but also the culture of functioning of this university administration. The heads of institution of this university has displayed complete amnesia, tolerance and a blind eye to ongoing corruption. The assessor's report, issued in 2011, which made some recommendations, was put on the back burner. The heads of institution keeps changing like visitors, without showing any real interest in the improvement of the university.

Ethical Issue: Proper care was taken in this manuscript, as not to disclose directly to any institution or an individual, but if is knowingly or unknowingly someone find it out then it is not author intention. It is failure of system in rural university, and that led to victimization of Mr. BM. The author would like to high light this system failure in this report, not an individual or institution.

Funding: Self-funded

Conflict of Interest: None

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Estimation of Shortfall in Salary from 1996 to 2018 from Health and University, South Africa

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Abstract

Background: Professional jealousy is known to be rife among academics in various universities, but hardly reduces their salaries. The productive staff is generally protected from external as well as internal threats by the higher management of the university, but this did not happen in the case of Mr. BM.

Case history: Mr. BM was appointed as acting head of department, a medical officer against the post of professor and head of forensic medicine. He was not paid according to the University's conditions of service policy document. This was ignored by Human Resource department despite a resolution of the council. Mr. BM kept on receiving low salary to which he was entitled until his retirement after almost 20 years (1996-2018). This brought him close to financial ruin, as his pension was consequently also reduced by half. The estimated salary shortfall could be calculated, but the pain and suffering caused by three disciplinary enquiries and suspension are difficult to assess without the help of an actuarial consultant.

Conclusion: The shortfall in salary must be paid by the health, which is the major (80%) funder of his salary in a dual employment. This shortfall entailed differences in payment of basic salary as well as allowances between 1996 and 2018.

Keywords*: dual employment, abuse of power, financial ruin*

Introduction

Mr. BM was appointed as an acting head in January 1996, but a letter was only issued by the faculty head and confirmed by the Senate on 1996. He was a medical officer in the health. Mr. BM was working on his own like a full-time staff member of university, doing the work and assuming all the responsibilities of a professor and head. This was confirmed by the Registrar in 1997. He was also working as a full-time staff member in the health rendering all services, where he was working on his own. University interviewed him for a post of professor but later appointed him as a lecturer, which was like a discredit him. He was again interviewed for a post of professor but appointed as an associate against the post of professor. However, the salary of a professor was negotiated.

Mr. BM wrote to the University about his payment as per the policy document of university conditions of service, which was also later recognized by the council. The Council issued instructions to HR, which HR did not implement. Between 2005 and 2007, he started visiting the HR office, as someone had told him that it was always good to meet personally, as this was the culture at the university to solve problems, but in his case, it did not work out. In the end, Mr. BM was desperate for his outstanding payment, and wrote a letter stating his grievances to the head of the institution of the University, thinking that there had to be some light at the end of tunnel, but received no reply. Mr. BM was thinking that at least higher management would recognize his work, which had earned him five awards in research and had led to dozens of research publications between 2002 and 2007, which put 54 million rand in the pockets of ex-mineworkers.

When he was at the peak of research publications in 2008, disciplinary enquiries (DC) were instituted against

him, accusing him of plagiarizing somebody's work, although it was rather the reverse that was true. First DC, a Professor was a commissioner of enquiry from outside of university and gave a fair judgment. When Mr. BM then pointed out to the University that since he had not been found guilty, the person who used his work in his thesis had to be guilty, and his degree had to be revoked, it refused to carry out any enquiry against that person. This enquiry had just been concluded when another DC (2010) started, leading to suspension. This matter was supposed to be less punishable, as it was a self-plagiarism, but unfortunately the punishment was more severe. The charge was related to an article that had been published in two journals by mistake. It was a double jeopardy in terms of the law to have a similar enquiry in a short time. Mr. BM produced articles written by his 2 colleagues in the same faculty that were also self-plagiarized, but the head of faculty said that he wanted only to punish Mr. BM. It was surprising that Mr. BM was suspended and not allows entering in university, but his research work poster displayed in a conference organized by the faculty. An independent assessor inspected the University and mentioned it was like Afghanistan, with failure of multiple organs in 2011. He specified the HR department. One department published 25% of all research publications of the university, and about 50% of those in the Faculty. This was Mr. BM's department, where he was the only staff member.

The more one published, the more jealousy this generated among professionals in Faculty. The head claimed Mr. BM was working under him. This led to increased jealousy among his professional colleagues. The suspension lifted all of sudden as the faculty needed me conduct the examination of fourth year medical students, but DC was continued. Fortunately, the union refused to accept him after few meetings, as he refused to call the head of institution as a witness to appear in front of the enquiry committee. He said that he did not have the power to call the head of institution and when the union chairperson asked him for his letter of appointment; it transpired that he had only been appointed telephonically. Later, charges were withdrawn in the DC.

In a third DC, wanted to be sure of success by appointing a confirmed X man as a commissioner of enquiry. There were in fact no valid charges, as Mr. BM went on leave with permission from head, but the faculty head said that he had to get permission from him. The commissioner declared guilty abruptly and issued a letter immediately suspending one month's salary. The union made representations and the salary was paid after two weeks. Salary was stopped by instruction of faculty head by email. The faculty has also managed to be stopped from working in the laboratory although I was using it as a teaching platform for medical students. The University had been underpaying Mr. BM since 1996 and this continued till Mr. BM retirement in December 2018. In July 2009 the occupation-specific dispensation (OSD) scale was implemented for all health professionals registered with the Health Professions Council of South Africa and Mr. BM received a lump sum in December 2009.[2] The amount paid was less than it should have been, as Mr. BM was working on a higher level as a clinician. A HR staff member responsible for salaries increased his own salary, but constantly decreased the salary of Mr. BM. This was reported as prima facie evidence to the head of the institution, but no response was received. Subsequently, the same basic salary scale was maintained without any revision for almost five years. The University thus kept paying Mr.BM a lower basic salary, as well as overtime and rural allowance.

Methodology

The information on the shortfall in salary was collected from Mr. BM monthly pay slips from 1996 to 2018. Some gaps in determining the accurate salary of a month or year were dealt with by calculating the salary of the previous month and adding a 5% annual increase. The salary from 1996 to January 2001 was received directly from the health with fixed overtime. Mr. BM was a senior lecturer from 2001, getting 60% of salary from the health and 40% from the University, as per the agreement of the joint establishments. Overtime (53%) of basic salary, was also paid by health. In 2004, Mr. BM was appointed as associate professor, against the post of Professor receiving 60% of salary from the health through the University (dual employment). This post was against the post of professor, and Mr. BM was working in it till retirement in December 2018.

Results

The calculation to estimate the shortfall from 1996 to 2018 was conservative. The difference in salary between

a medical officer and a professor from 1996 to January 2001 was calculated based on a reference received from a professor appointed at the University. Estimates were calculated with approximately 5% annual increase or decrease in basic salary. From February 2001 to June 2009, the calculation was based on either pay slips or the appointment letter issued by the University. The gap in proof was covered by a 5% annual increase in salary that year.

Overtime calculation was carried out assuming that it was 53% of the basic salary, but for practical purposes it was accepted as 50% in calculations and decimal points was omitted. The salary paid to Mr. BM always followed a very irregular pattern and it is difficult to know how and why this happened. Most problems relating to the salary occurred between 2009 and 2018, which was also a troubled time of DCs. The scale of basic salary and over time changed at least three times in a year, despite BM performing the same duties for decades. Replies on dispute resolution from the head of institution were contradictory and yielded interesting findings. Less and less overtime was paid between 2010 and 2016; it has been calculated separately, as it is not covered by the difference in salary payment.

The OSD scale concerned national government funding and it is assumed that it was fully paid by the national government through provincial health to the University, but this needs to be confirmed.[1] In July 2009, the OSD scale was implemented by the national government and it was paid in that year, but after that it was never revised, and continued till 2013 and part of 2014.[2] (Figure 1). There was a slight change in the second half of 2014, and then from 2015 to 2018, but not based on the OSD scale. Figure 2 showing year wise the pattern of total claim. It is mostly between 2009 and 2018, when OSD scale was not revised in the salary of Mr. BM.[2]

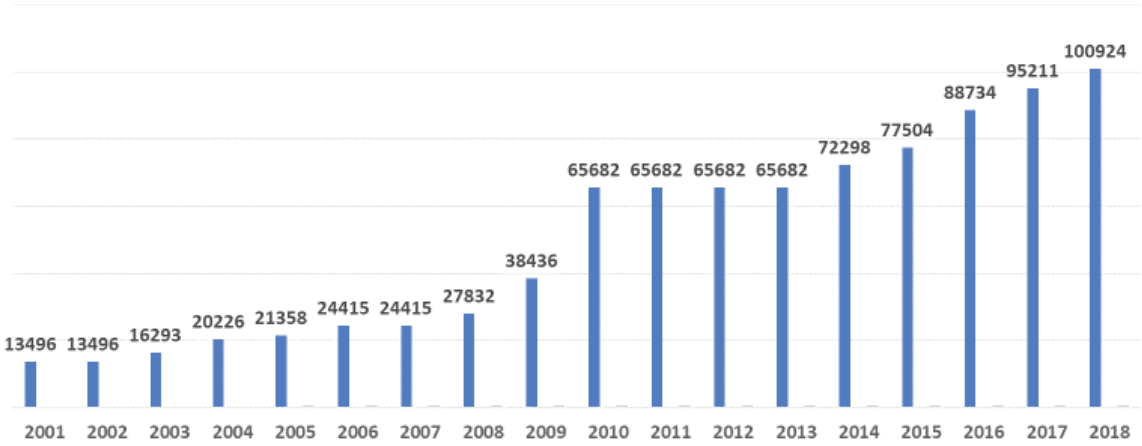


Figure 1. Year-wise pattern of salary paid from 2001 to 2018.

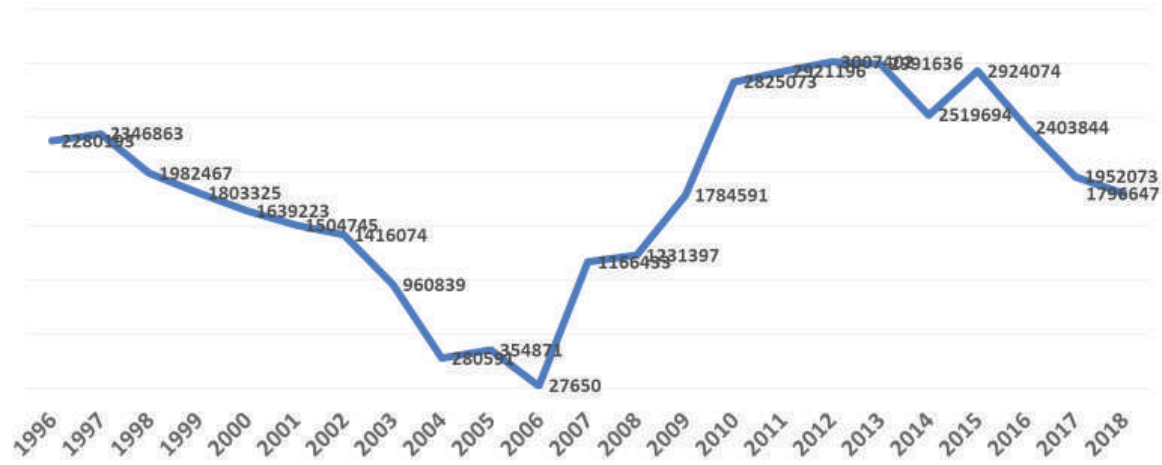


Figure 2. Year wise pattern of total claim from DOH between 1996 to 2018.

Since the basic salary paid was lower, the overtime and rural allowance were automatically also lower. The difference in each month's amount was calculated very conservatively, as the basic salary was supposed to be that of the post of chief specialist, but Mr. BM used only the top notch and grade of a principal specialist (level 12) for calculation. The amount of overtime payment was lower especially from 2010 onwards. However, the duty hours of work carried by Mr. BM was the same from 1996 to 2018 (proof attached). The estimate of overtime (50%) was calculated based on the basic salary by determining the difference in the payment to Mr.BM who was paid up to 22% less from 2010 to 2016 or sometimes not paid for overtime at all (Figure 2).

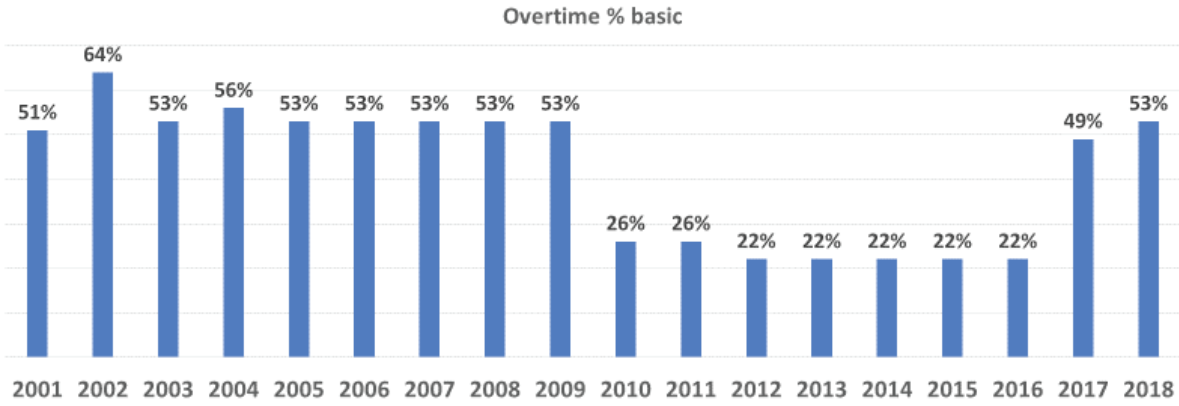


Figure 3. The pattern of Overtime paid in percentage of basic salary from 2001 to 2018.

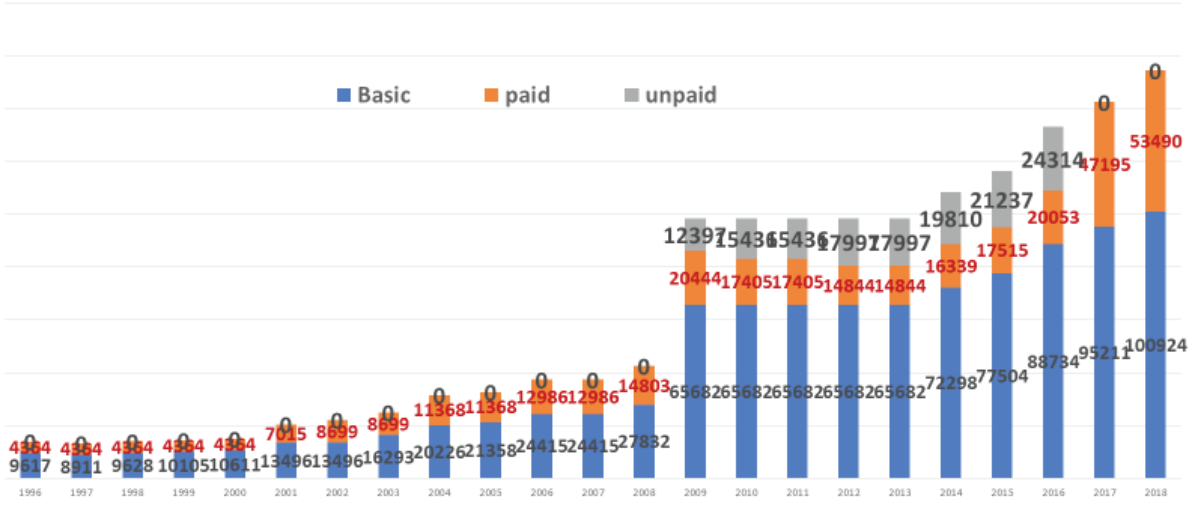


Figure 4. Deficit over time 1996 to 2018 which was not paid of basic salary especially from 2009 to 2016 by the University.

Similarly, the rural allowance was estimated by calculating a monthly and yearly discrepancy of 21% in the basic salary from 2001 to 2018 (Figure 3).

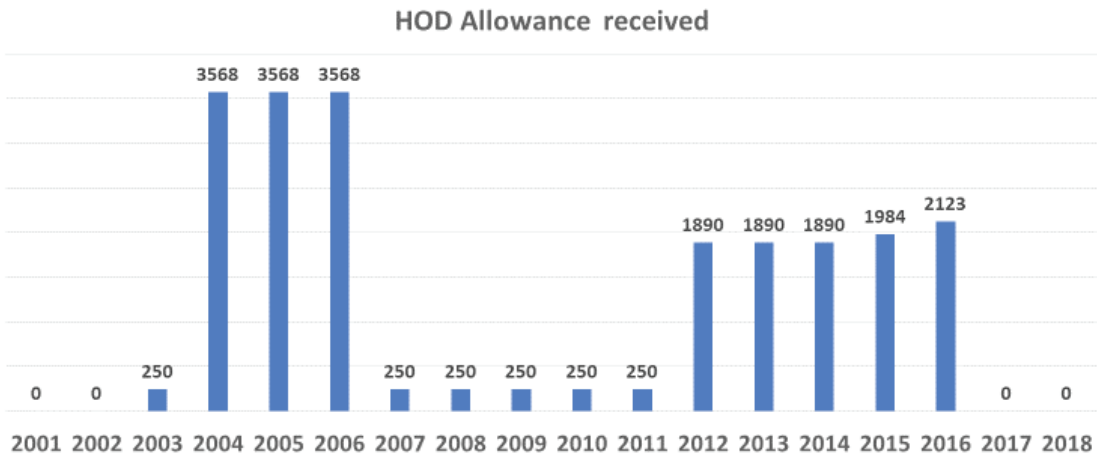


Figure 5. Pattern of Head of Department Allowance from 2001 to 2018.

Discussion

The health contributed the main portion of Mr. BM's salary directly (1996 to January 2001) and through the University (February, 2001-2018). Therefore, it must be held primarily responsible. As proven in a NUMSA case, in dual employment the employee is always sandwiched between two employers and is exploited by one.[3] In this case Mr. BM was exploited by both the health and the University. The procedure followed for the head of institution research award gives an idea of the humiliation suffered. Mr. BM received head of institution research award in 2002 and 2003. In 2004, they did not allow Mr. BM to participate. In 2006 Mr. BM, got the platinum award, but it has not been handed over to date despite a dispute being declared with the head of institution. Again, the 2007, 2008, and 2009

awards were announced, but not finalized. The matter was dealt with by the head of institution, and after that Mr. BM problems multiplied in the form of three DCs, two suspensions, a forensic audit and stoppage of his birthday bonus. There were staff who has not published even a single research paper, got promoted through inaugural professorial lecture within an hour bypassing all the hurdles of interview and short listing. Mr. BM's main concern is that non-payment means not being paid according to the university conditions of service applicable from 1996 to 2018. A letter from Mr. F, who was registrar finance, and an e-mail from him both reconfirm the claim for payment. Mr. BM submitted details of his grievances to the head of institution on 18 March 2008 but did not receive a reply. On 24 October 2011, the head of the Faculty himself wrote a letter to the Finance Officer regarding salary concerns from the joint establishment clinical staff, including Mr. BM. Subsequently the faculty head also wrote a memorandum to the head of institution, mentioning that since the implementation of the OSD in 2009, no annual adjustment had been implemented.[2] The faculty head wrote a similar letter on the same date about the non-payment of the HOD allowance, and Mr. BM's name was also included in the attached list of HODs. The faculty sent a list of joint staff members to HR for the back payment of 2010 and 2011, but Mr. BM's name was excluded from that list. This is evident from the letter received from HR Director. He mentioned that the faculty took this decision along with the finance officer. When asked who the specialist on salaries in the faculty was, he did not answer.

Mr. BM was acting HOD for almost two decades (1996-2015). He received a letter from the HR director: Mr. X renewal of contract. He was surprised to read it, as Mr. BM service had continued without any interruption since 1996. When the director was asked for a policy document, he did not reply. In 2015, the University prepared to merge four departments, and interviews were conducted. They displaced Mr. BM from the post of HOD at the University, but Mr. BM remained the HOD in the hospital, paying overtime by DOH and completed PMDS of his staff in hospital.

On 12 February 2015 all the clinical staff of the joint establishment wrote a letter to the Director HR about a salary adjustment, but nothing was done to resolve the problem. A letter was written to the HR Director regarding unfair labor practice, dated 26 February 2015, by an attorney, and later a CCMA hearing was held but not completed, as HR had lost Mr. BM's personal file. The CCMA was started but could not complete as HR supposed to bring that file to continue CCMA process. On 23 November 2015 the head of institution replied to a dispute referral to by the union lodged on 31 August 2015. He declined my claim without referring to the policy document. Mr. BM made great efforts in 2017 and 2018 as he was retiring, and the matter was going to affect his pension. A dispute resolution meeting was held with the head of institution. Mr. BM wrote many emails and letters, but he replied few of these. Mr. BM found it callous that a head of institution would not even answer questions on the shortfall of salary of a staff member. A dispute resolution meeting was held on 17 April 2017. The head of institution nominated himself as a facilitator. He also allowed a deputy director HR to write the minutes. Mr. BM was afraid and wanted to cut short the meeting as Mr. BM pension could be stopped. He could not solve the problem of the OSD scale, as he seemed to have made up his mind before the proceedings. The HR director mentioned that Mr. BM deserved to get his OSD scale and that the OSD scale money would come from the health.

Mr. BM contacted the national director of remuneration and he emailed that Mr. BM deserved to be paid on the OSD scale. It was wrong and against labor law practice to start paying this and then stopping it. In Mr. BM's case it was started and paid in 2009 and then not revised. Several letters written by the hospital management from the HOD, clinical managers, directors and CEOs of hospitals recommended that since Mr. BM was a HOD, he deserved remuneration according to the OSD scale. After a lot of difficulty Mr. BM succeeded in organizing a meeting between HR of the health. The meeting took place in on 12 September 2018, two days before Mr. BM was to receive his birthday bonus. It was unfortunate that this meeting led to the stoppage of Mr. BM birthday bonus on 15 September 2018. A colleague, a union member, accompanied to Mr. BM and has also received his salary less. He wrote a letter to the head of institution. HR emailed a letter agreed to pay to Mr. BM 15 October 2018. He was paid, but only half the amount. An independent assessor inspected the University and mentioned it was like Afghanistan, with failure of

multiple organs in 2011.[4]

It is interesting to know that one of Mr. BM's students was getting the OSD scale and earning almost double of Mr. BM. Both were working in the same hospital. She was a senior lecturer at the University, and then moved full-time to the hospital in 2011. Many other favored University employees were also assisted to join the hospital full-time in 2012 and left behind only a few staff members (foreigners). The arrangement was quite secret from the rural University. He also drafted a new agreement between the University and the health. In Mr. BM case the worst was that the post of chief specialist was abolished in the establishment, which closed the doors to being appointed on the full-time staff of the hospital. Many senior lecturers were getting higher salaries than Mr. BM on the university payroll, as he reported to HR. This was repeatedly reported to the head of institution but honorable chief has not applied his mind. BM. Salary is the pride of a human being, and it must receive urgent attention.

Declaration

The information is close to accuracy, as it was calculated at the lower side of estimation especially between 2001 and 2008. Any margin of error is minimal and if there is one, it is to the advantage of the health and the University. The calculation in estimating the total shortfall has been very conservative. All statements in this report are accompanied by hard documentary proof, which could be produced whenever necessary.

Conclusion

Gross underpayment occurred, and this needs to be rectified by the health as the custodian of major funding. It paid 60% of the salary plus 100% overtime; therefore, it contributed 80% of the salary. This resulted in exploitation of the individual as staff member and also in terms of his pension.

Acknowledgment : The author is thanks to all those who helped in getting information especial thanks to the Chartered Accountant who calculated the total amount.

Ethical Issues: Proper care was taken in this manuscript, as not to disclose directly to any institution or an individual, but if is knowingly or unknowingly someone find it out then it is not author intention. It is failure of system in rural university, and that led to victimization of Mr. BM. The author would like to high light this system failure in this report, not an individual or institution.

Conflict of Interest: The facts in this report could be submitted in demand. The references were not labeled but can be produced if necessary.

Source of Funding: Self-funded

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Mandibular Second Molar Protostylid: A Rare Case Report

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Abstract

Aim: The aim of this case report is to present a rare finding of protostylid on the mandibular second molar.

Background: Human teeth of both primary and permanent dentitions sometimes shows developmental malformation which results in alteration of their size, shape and structure and especially with the crown either in the form of anomalous cusps or an increase in the number of cusp. A protostylid is a supernumerary or accessory cusp found on the molars on the mesial half of the buccal surface.

Case discussion: This case report presents a rare finding of protostylid on the buccal surface of the permanent left second molar tooth in a 12 year old female patient. There was no similar finding seen on the other teeth and in other members of the family.

Conclusion: The dental polymorphisms described in this case report is some of the rarest and unique forms of the human dentition. Thus, the reporting and preservation of these structures can be important from the perspective of forensic odontology.

Clinical significance: Though this may not pose significant problem in most instances but its finding is of very much significant interest from the perspective of forensic dentistry.

Key Words: *Dental morphology, protostylid, mandibular molar, extra cusp*

Introduction

One of the most studied components of dental anthropology worldwide is dental morphology, as it seeks to understand the of frequency and variability of coronal and radicular morphology in human teeth.[1, 2] The morphological variations may be seen either involving the crown as anomalous cusps or involving the root in the form of an increase in the number of roots. One such morphological trait seen on the clinical crown is a protostylid, which is studied worldwide. It is a supernumerary cusp located on the mesial half of the buccal surface of molars. Protostylid was first described by Bolk in 1914 as, a "supernumerary tubercle or cusp". It results due to the fusion of taper supernumerary tooth with the adjacent normal tooth. Protostylid is usually located on the vestibular surface of the second and third lower molars and least seen in the first permanent molar. Dahlberg in 1950 identified it on the primary maxillary molars of an Eskimo skull as an accessory or supernumerary cusp. The "mesiobuccal edge prominence" were termed as protostylid by De Jonge- Cohen.[3] However, there is scarcity of literature on protostylids, primarily because of their low prevalence rate. This case report presents an incidental finding of a protostylid on the buccal surface of permanent mandibular second molar along with its clinical significance, especially in the field of forensic odontology.

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Case Presentation

A 12 year old female patient reported to the Department of Pediatric and Preventive Dentistry at Manipal College of Dental Sciences, Mangalore with chief complaint of presence of deposits in the teeth. The past medical and dental histories of the patient were not significant. On intraoral examination patient had permanent set of dentition and the lower left permanent second molar was structurally dissimilar to its antimere in that it had an extra cusp on the mesial half of the buccal surface. The anomaly was diagnosed to be a case of a unilateral protostylid of mandibular left second molar. However this interesting observation made was not relevant to the chief complaint of the patient and was only incidental. The protostylid was noted to be of triangular in shape, measuring about 3mm cervico-occlusally and 4 mm mesio- distally with base at around 1 mm above the gingival margin and its apex around 1mm below the occlusal level *(Fig 1*).

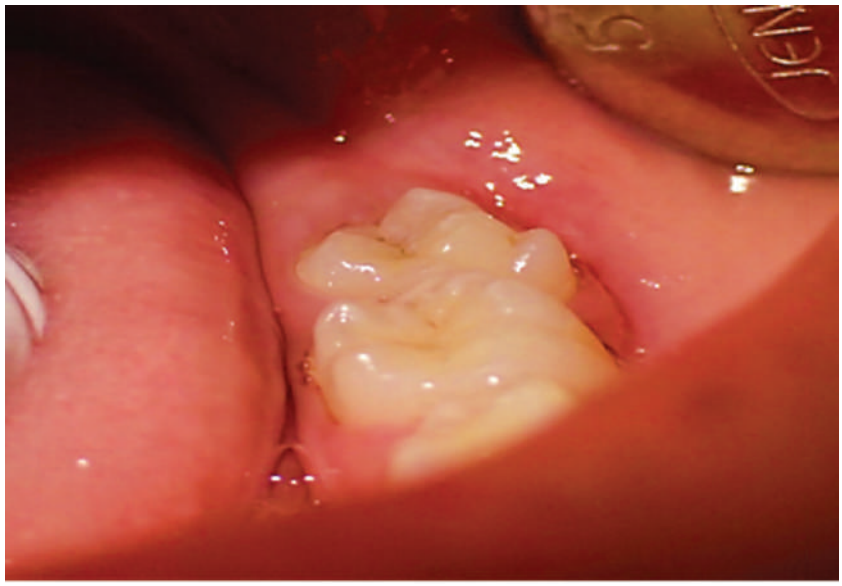


Figure 1: Intraoral image showing protostylid of the lower left second molar.

No similar structure was noticed on the other teeth neither in maxilla and mandible (*Figs 2 and 3).* The tooth was partially erupted. The protostylid was out of function since the tooth was partially erupted and not occluded with the opposing tooth therefore, no occlusal modification was done



Figure 2: Intraoral image of maxillary arch

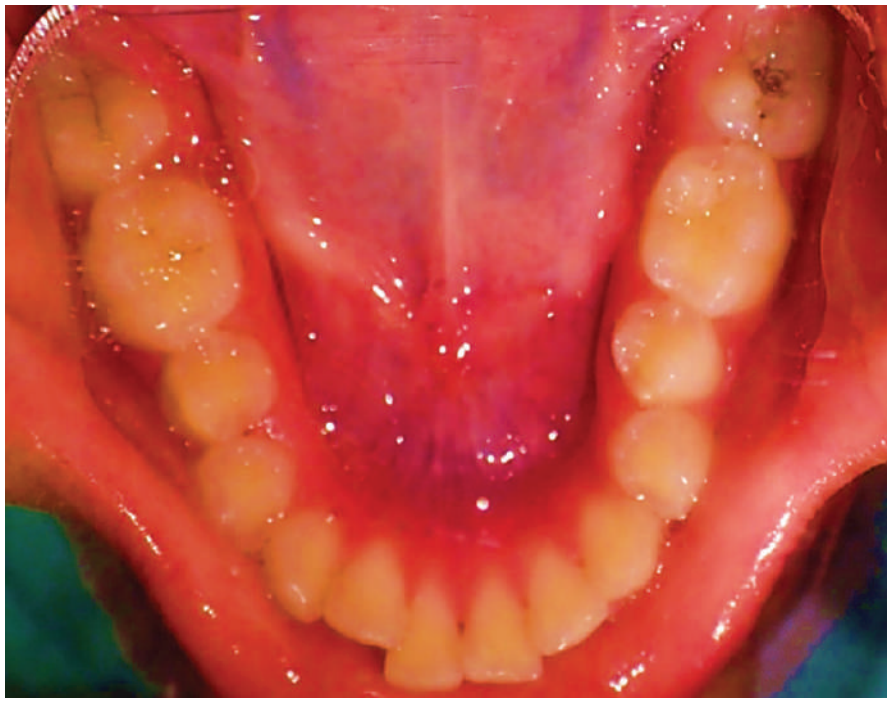


Figure 3: Intraoral image of mandibular arch

Discussion

Literature on dental anthropology has documented several kind of morphological variants, which include dental tubercle (on the lingual surface of the upper lateral incisors); disto- sagittal crest (on the buccal surface of the upper first premolar); carabelli's trait (on the lingual surface of the mesiolingual cusp of the upper molars); and protostylid (on the buccal surface of the upper and lower first molars). Earlier, the etiology of such variation was thought to be the over activity of the dental lamina leading to abnormally shaped cusp or extra cusp. At present it is believed that certain genes like PAX and MSX are responsible for this anomaly.[4, 5]

This case report presents a rare case of protostylid seen as an accessory cusp on mandibular second permanent molar in a child patient of 12 years. According to the literature its prevalence in Asian population is only 2 % [6] and existence of such an accessory cusp can thus provide important information on the ethnic or racial background of the individuals, with special significance in the field of forensic odontology. It has been reported that the presence of protostylid in primary molars is a predilection for its presence in permanent molars.[7] In our case, the parents were unaware of the presence of such an extra cusp in the primary molar and so we could only assume regarding its presence in the primary dentition. Upon asking the family history, parents revealed a negative history and even on clinical examination no such extra cusp was seen in parents and siblings.

The protostylid is a paramolar cusp (that does not make part of the functional occlusal table) that may vary in shape from a furrow to a free-apex cusp on the vestibular surface of the mesial vestibular cusp of the second lower deciduous molars and of the first and second lower permanent molars. It can also be expressed as a vestibular fossa or fovea on a furrow of vestibular development. Protostylid is categorized into eight categories by Arizona State University Dental Anthropology System (ASUDAS) [8] wherein zero and one is considered as absence of protostylid and two to seven is considered to be present. In the present case, protostylid belonged to the grade 7 as the presence of free vertex cusp was noted.

The presence of protostylid is of clinical significance, as it can lead to difficulty in stainless steel crown placement, failure of pulpectomy due to chances of missing a canal, interference with bracket cementation, banding and preventing appropriate alignment during orthodontic treatment, gingival and periodontal problems due to increased chances of plaque accumulations, increased caries incidence and even frictional keratosis when it is sharp.[9] Apart from the clinical complications,

its presence is of significance even from the point of view of forensic odontology. It may aid in identification of suspects through bite marks on living/ nonliving objects. Dental identification is primarily used to confirm the identities of unknown persons when identification by other means such as DNA or fingerprints is not possible in disaster situations that result in skeletonization, decomposition, severe burning or charring[10] of the individuals beyond recognition, especially in disasters that involve multitude of casualties.[11] Each individual have got different dental characteristics and features. This could help in identification of unknown individuals during mass catastrophes and more over being the strongest part of the body, teeth are durable and are capable of resisting damage unlike the other structures of the body.[11, 12] Thus, it is important that the dentist identifies such dental anomalies and maintains a record of the same.

In this case, intraoral periapical radiograph was not taken to avoid unnecessary radiation exposure to the patient, and also no occlusal modifications were carried out as the accessory cusp was not causing any functional complications. The patient was instructed to maintain a good oral hygiene, after making the parents and the patient aware of the condition and advised regular monitoring for early detection of dental caries.

Conclusion

Presence of additional tooth projections in children requires routine periodic dental evaluation. Early diagnosis and management are important if complications are to be avoided. The dental polymorphisms described in this case report is some of the rarest and unique forms of the human dentition. Thus, the reporting and preservation of these structures can be important from the perspective of forensic odontology.

Clinical Significance

Each individual have got different dental characteristics and features. From forensic odontology aspect presence of protostylid is of very much clinical significance. This could help in identification of unknown individuals during mass catastrophes and more over being the strongest part of the body, teeth are durable and are capable of resisting damage unlike the other structures of the body. Thus it is important that the dentist identifies such dental anomalies and maintains a record of the same.

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Protein Supplement Drinks, the Modern Killer that Induces Oxidative Stress in Mice Liver

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Abstract

Introduction: In past few years, the use of protein supplements is popular among body builders, as the strategy for weight control, and energy intake.

Aims: In the current study we aim to examine the effect of a protein supplement drink on oxidation-reduction systems in mice liver tissues.

Material and Methods: Twenty mice were randomly separated into two groups. The first experimental group; were feeding with a protein upplement and the other without protein supplement as a control groups over 12 weeks.

Results: The results highlighted that body weight was significantly lower (P =0.001) in the protein supplement group when compared with the second group. Additionally, we have observed significant differences in the level of malondialdehyde, total protein carbonyl, nitric oxide, and glutathione oxidized in liver tissue of the protein supplement group when compared with the control group.***Conclusion:*** Protein supplement drinks have a harmful toxic effect that can promote worse oxidative stress, especially on liver tissue, which could elevate the risk for the development of liver diseases in the long time.

Keywords*: protein supplement, oxidative stress, malondialdehyde, total carbonyl, nitric oxide, and glutathione*

Introduction

Protein molecules are performing a vast array of functions within the cells. Along with carbohydrates and fat, protein is one of the three macronutrients[1]. Constantly, Proteins are being degraded and synthesized in the body. Unlike carbohydrates and fats, amino acids are not stored in the body. Therefore, they should be gained from the diet, synthesized denovo, or produced by biodegradation of normal protein. The excess amino acids in the biosynthetic needs of cells are quickly degraded. As amino acids are oxidized, the nitrogen is converted to urea, uric acid, creatinine, and ammonia excreted by the kidneys [2]. The recommendation of protein intake for normal adults is 0.8 g/kg of body weight per day. Over the past few years, the consuming of protein supplement drinks (PSD) is gaining by body builders. Indeed, PSD is increasingly being recommended as one of weight control strategies. The protein supplement drinks appear to reduce body weight, appetite, energy intake, and improve plasma lipid profile. Despite the anti-obesity effects of PSD, the impact of such supplement on oxidative stress dose not examined [3,4].

During several metabolic reactions free radicals are generated, which contain unpaired electrons on outer orbital [5,6], and radicals can cause tissue damages as well as cell death because they are very reactive species [7].

When, the stringent balance between free radical generation and antioxidant defenses is disrupting the oxidative stress resulting, and that leads to undesirable damages for wide range of molecular species like lipids, proteins, and nucleic acids [8]. The cell-membrane polyunsaturated fatty acids are these targets of reactive oxygen species, in turn, lead to damage in the cell

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structure and function [9]. Also, lipid hydroperoxide decomposition leads to diversity of end products, such as malondialdehyde (MDA), which is now considered as a dependable marker of lipid peroxidation [10]. Additionally, total Protein carbonyl (TPC) are the products of the oxidative modification of proteins [11].

Also, Nitric oxide (NO) is a messenger molecule functioning immunity, in vascular regulation, and neurotransmission [12]. Previous researches have pointed the excess NO can play critical role in the altered pathophysiological mechanisms of diseases [13]. NO high concentrations are produced as a result of inducible nitric oxide synthase induction, and peroxynitrite formation [14].

While, anti- oxidant glutathione (GSH) has an important role in cascading of cellular defense versus oxidative injury. This molecule considered as the most available intracellular thiol-based antioxidant, that available in all living aerobic cells [15, 16].

Oxidative stress has been involved in the creation and complexity of diabetes mellitus, cancer as well as neurodegenerative diseases [17].

The aim of this study was to determine to what extent protein supplement drinks change the liver tissue content of total protein carbonyl, NO, MDA and GSH.

Materials and Methods

Animals and experimental design

A total of twenty albino male mice aged 2-3 months (22-30 g) were assigned into two groups (n=10), first ten fed with normal diet and the other fed with protein supplement drink (PSD) of whey protein. The cages were placed in a well-ventilated thermostatically regulated room (21±2ºC), relative humidity (40 to 60%), and (12:12) light-dark cycles. One week before to the experimental period, the mice were permitted for adaption to the experimental conditions. The experimental period lasted 4 weeks. The body weight of the animals was measured at the beginning of the experiment, and on the last day of the experiment. At the ending of the experimental interval, the mice were anesthetized with (ketamine-xylacine) and sacrificed. The internal organs (liver, spleen, and kidney) were extracted, washed in normal saline solution, then dried with a filter and weighed to calculate the relative organ weights (organ weight/body weight %). livers after weighing were immediately frozen in liquid N_2 and preserved at -80 ºC unto further analyses.

Experimental diet

Mice in the protein supplement drink (PSD) group were given protein supplement by oral feeding. The (Gold Standard 100% Whey) was purchased from a local market; PSD was weighted and fluxed in distilled water. The recommended consumption of Whey gold standard for humans is near (32g/ one intake). The mouse PSD dose (6.56 g/kg) used in this study was calculated according to Chen et al [18].

Chemical analyses

Liver homogenate samples for biochemical tests were collected and prepared according to the procedure provided by Elabscience colorimetric assay kits, and evaluated by the same assay kits for MDA, total protein carbonyl, NO and GSH.

Statistical Analysis

Statistical analysis: The data of the study were statistically analyzed using the statistical package for social science (SPSS) program (version 21, IBM Corporation, Somers, NY). The independent t-test analysis was carried out to compare between the mean values of the different parameters. Any two-tailed $P < 0.05$ was counted statistically significant result.

Results

The special effects of the protein supplement drink on the final body weight , relative weight of liver, kidney, and spleen of animals with PSD, and the control group, are shown in table 1.

The results highlight significant differences between the two groups on final body weight, relative weight of liver, kidney and the relative weight of spleen.

Table 1: Effects of the protein supplement drink on final body weight(g), the relative weight of liver, kidney, and spleen.

Parameter	Control group	PSD group	P value
Total Body weight (gm)	25.08 ± 1.84	21.9 ± 1.30	0.0003
Relative Liver weight (%)	5.15 ± 0. 1	5.24 ± 0. 2	0.7054
Relative Kidney weight (%)	1.31 ± 0. 26	0.64 ± 0.07	0.0231
Relative Spleen Weight (%)	0.71 ± 0. 18	1.39 ± 0.179	0.0150

Data are mean ± SEM, n = 10

Oxidative stress levels were determined through the assessment of oxidative end products of proteins (TPC), the oxidative end product lipids malondialdehyde (MDA), and nitric oxide (NO). All of the estimated oxidative damage products (TPC and MDA) were significantly higher ($P<0.0001$) in the PSD animals when compared to the control animals. While, there are a significant declination ($P<0.02$) in the concentration of glutathione reduced form in liver tissue of PSD animals when compared to the control animals, as in table 2.

Table 2: The levels of MDA, TPC, TNO, and GSH in liver mice tissues.

Parameter	Control group	PSD group	P value
MDA (ng/mg protein)	3.02 ± 0.41	5.98 ± 0.35	0.0001
TPC (µmol/mg protein)	29.06 ± 2.62	77.03 ± 2.09	0.0001
NO (µmol/g tissue)	0.106 ± 0.03	0.567 ± 0.01	0.0001
GSH (µg/mg protein)	9.82±2.15	3.57±1.99	0.0204

Results are expressed as mean ± SE. MDA, malondialdehyde. TPC, Total protein carbonyl. NO= (nitrite + nitrate), GSH, reduced glutathione.

Discussion

In the past few years, the use of protein supplements is popular among body builder, as a strategy for weight control, and energy intake [19]. Because the liver is particularly tending to oxidative damage and, due to the existence of many dialectical researches concerning its response to a high protein diet, we evaluated lipid and protein oxidation end products, and antioxidant status in liver tissues of mice fed a PSD. This is the first study that has compared oxidative stress induced by a chronic administration of PSD.

As a pointer of the toxicity of the PSD, we measured the changes in total body weight of mice, following 4 weeks. There were significant a difference in body weight loss when matched to the PSD group with their respective control group. In addition to that the relative organ weights were calculated. It is an important requirement to indicate the effect of xenobiotics present in PSD on certain organs between treated group and control group. So, the fresh weights of livers, kidneys, and spleen of mice were measured for recording of relative organ. There were no significant differences in the relative liver weights of mice fed with PSD compared to control group for 4 weeks. The differences were significant in

the relative weights kidneys and spleen of mice between PSD group and their respective control group after. Organs weight can be the most sensitive indicator of an experimental compound [20]. As a significant difference in organ weight between treated and control groups may occur. Actually, a compound that causes reduction in body weight and significant change in relative weights of most organs has a specific toxic effect on the organs. So, the results of the current study indicate that PSD has a harmful toxic effect.

Generally, the oxidative stress that caused by an imbalance between overproduction of reactive oxygen species and antioxidant defense [21,22]. In the current study, we have shown higher level of MDA, and TPC in liver tissue of PSD mice.

As proteins cannot be stored, high levels of protein intake lead to elevated amino acid oxidation to keep the homeostasis of amino acid in the body. The re-oxidation of reducing equivalents generated from amino acids oxidation can raise the free radical formation, via electron flow along the mitochondrial respiratory chain [23, 24].

The existence of free radicals has some harmful effects on several sensitive molecular and cellular components such as proteins, and membrane lipids [25]. As mentioned above the cell-membrane polyunsaturated fatty acids are the primary targets of reactive oxygen species, which, in turn,can lead to many damages in the cell structure and function [26].

The results of our study, suggest that PSD consumption used in the present study increases the levels of MDA, and TPC in liver tissue. The levels of MDA, and TPC in the liver are considered especially sensitive to oxidative stress results from PSD. The current study, also detected a significantly lower levels of GSH in the liver tissues of animals with PSD in respect to control group liver tissues. It is recognized that GSH is the most vital component of antioxidant tissue system defense and the only protective compound that can scavenges the hydroxyl radicals [27].

Previously it was confirmed that lowered levels of GSH lead to mitochondrial damage in the liver [28]. So, the reduced GSH levels that detected in this study can be a proof for the increasingly oxidative damage in liver despite the fact that enzymatic antioxidants were markedly increased.

In our research, we observe a significant change in the liver NO levels within the group feeding with PSD, possibly due to the difference between the liver cells metabolism and/or regulation of NO in them. A reduced release of NO is one of the classical markers of endothelial dysfunction. Also NO is decreasing in rats with chronic renal failure [29].

In conclusion, the data of this study suggests that the consumption of protein supplements may induce oxidative stress in the liver tissue of mice. So, further studies will be necessary to understand the mechanisms of PSD on other tissues.

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Medical Negligence Dispute Settlement in Indonesia

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Abstract

The doctor-patient relationship has a specificity that distinguishes it from the business actor relationship. Malpractice is a very general term. Medical malpractice, medical negligence, and medical risk are not regulated in Indonesia and requires medical knowledge to understand them. This is where the difficulty of proving negligence and medical risk in solving medical negligence disputes. Medical negligence disputes in Indonesia are settled by litigation (criminal and civil) and non-litigation. Non-litigation medical negligence dispute settlement is a breakthrough in resolving medical negligence disputes in Indonesia, namely mediation and the Indonesian Honorary Medical Disciplinary Council or *Majelis Kehormatan Disiplin Kedokteran Indonesia* (MKDKI). This study revealed that there are no clear rules regarding medical negligence, medical risk, and medical dispute resolution to produce identical rules based on structure, substance, and culture. The settlement of medical negligence disputes through mediation is an alternative in resolving medical negligence disputes in Indonesia to allow for legal certainty and legal protection for doctors and patients.

Keywords*: medical negligence, medical risk, medical disciplinary sanctions, litigation, and mediation.*

Introduction

A medical doctor is a noble profession because it saves lives and as such the relationship between a doctor and a patient is different from other relationships in any other profession. In the doctor-patient relationship, doctors are strongly influenced by the ethics of the medical profession. There are two types of relationship between doctor and patient in the effort to heal, namely relationship due to contract (therapeutic transaction) and relationship due to law. Both of these patient-doctor relationships bear legal and ethical responsibilities. According to the law, the doctor and patient relationship is an agreement whose object is in the form of medical services or healing efforts, known as a therapeutic transaction. The engagement that arises from the therapeutic transaction (healing) is called verbintenis inspanning, which is an engagement that must be done with care and effort (met zorg en inspanning). Because of his achievements in the form of effort, the results are uncertain. As a result, if the effort fails, in the sense that the patient does not recover or even die, this is a risk that must be borne by both parties.[1]

In Indonesia, no clear law regarding medical negligence has been put forward so far, so the definition of negligence or malpractice has not yet been formulated.[1] A person is said to be negligent when he/she acts indifferent, does not care, does not pay attention to the interests of others as is usual in social relations in the community. As long as the consequences of negligence do not bring harm or injury to others, or because it involves trivial matters, then there are no legal consequences.[2] The terminology of medical malpractice and medical negligence are two different things. Medical negligence has a broader understanding than

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negligence because, in addition to including the meaning of negligence, the term malpractice includes actions that are done intentionally (dolus, opzettelijk) and violate the law. In the sense of intentional implied there is a motive (mens rea, guilty mind) while the meaning of negligence is more intentional (culpa), less thorough, less careful, indifferent, indifferent, reckless. Ngesti Lestari and Soedjatmiko distinguish medical malpractice into two forms, namely ethical malpractice, and judicial malpractice, in terms of professional ethics and law.[3] The responsibility of doctors in criminal provisions is regulated in the Criminal Code,[4] Law of the Republic of Indonesia No. 29/2004 on Medical Practices, and Law of the Republic of Indonesia Number 36/2009 on Health. Civil liability is contained in the Civil Code regarding breach of contract and acts against the law.[5]

In Indonesia, medical negligence dispute settlement can be done by litigation and non-litigation. Cases of alleged malpractice are classified as offense complaints, so long as there is no complaint from the injured party or the patient, law enforcement will not act.[6] In criminal malpractice, the evidence is based on whether all criminal elements have been fulfilled, Article 184 of the Criminal Procedure Code states that evidence can be used to prove criminal acts, namely witness statements, instructions, and statements of the defendant.[2] Allegations of malpractice due to negligence must be proven that the doctor behaves inadvertently, what someone with a cautious attitude is doing properly, or otherwise doing what someone with a cautious attitude would not do in that situation. It can also be called negligence if the doctor's actions are performed below the standard of medical services. This is hard to prove by law enforcement officers because it requires adequate medical knowledge. Besides, regulations governing malpractice have not yet been established in Indonesia. Negligence is not a crime unless it harms or injury to others.[7]

In this regard, Adami Chazawi claims that not all medical malpractice is included in the realm of criminal law. Three conditions must be met, namely the doctor's inner attitude (in this case there is intentional/dolus or culpa); secondly, the requirements in medical treatment include medical treatment that deviates from the standards of the medical profession, standard operating procedures, or contains unlawful properties.[8]

Settlement of medical negligence disputes through criminal proceedings, the legal apparatus uses articles in the Criminal Code concerning negligence in particular Article 359 to ensnare doctors. This article raises problems because negligence referred to in this article is not the same as medical negligence. Negligence blamed on doctors is not necessarily negligence that could be a medical risk and vice versa. To prove negligence or medical risk law enforcement agencies have limitations. Existing regulations also do not formulate medical malpractice and medical risk. The weakness of this Criminal Code article raises problems in solving medical negligence disputes that can harm patients and doctors. In resolving disputes through the civil code also creates problems because of the difficulty of patients to conduct evidence and the tendency of expert testimony to defend doctors. The length of the judicial process and being defeated increasingly tiring the process of resolving medical disputes. Doctors are also psychologically burdened facing legal problems that will damage their good name and interfere with medical services.

In line with legal developments, dispute resolution outside the court offers new solutions for the resolution of medical negligence disputes through mediation conducted by MKDKI. Alternative medical dispute resolution is to overcome the increasing cases of litigation that have accumulated and can provide justice for both parties. Despite this alternative medical negligence dispute settlement still has shortcomings such as doctors can still be sued for legal proceedings, punishment is only disciplinary, and the compensation process is not regulated.

Several cases of doctors and patients in Indonesia show that there are legal problems in resolving medical disputes. The case that first appeared in Indonesia was the case of Dr. Setyaningrum where the doctor had been found guilty of negligence but ultimately in the Supreme Court was found not guilty. Other legal medical cases have become common knowledge where the patient does not find justice to the medical problems he/she faces, the patient does not know where to complain, and the resolution of protracted medical disputes. Besides that, many medical negligence disputes are lost in court which raises suspicion against doctors even though the lawsuit itself is vague because it is not supported by medical and legal logic.

Results and Discussion

Legal Certainty

One orientation of the legal goals according to Gustav Radbruch is legal certainty. According to Radbruch, legal certainty is the first guide to the law. The guideline is to make it positive, that is, to be sure. The law must be obeyed so that the law is truly positive.[9] Legal certainty is legal protection against arbitrary actions, which means that someone will be able to obtain something that is expected under certain circumstances. People expect legal certainty because with legal certainty the community will be more orderly. The law is in charge of creating legal certainty because it aims at public order.[10] Legal certainty also means legal security and protection for the parties against the arbitrariness of judges.[11]

Medical Malpractice and Risk

Medical malpractice is a term that always connotes bad connotation, stigmatization, blame. According to J. Guwandi medical malpractice can be divided into two groups:[12]

1. Deliberately (dolus, vorsatz, wilens en wetens handelen, intentional) are prohibited by statutory regulations.

2. Not deliberately (negligence, culpa) or due to negligence, for example neglecting the patient's treatment because of forgetfulness or carelessness so that the patient's disease gets worse and then dies (abandonment).

Medical risk, on the other hand, is an unwanted condition both by the patient and by the doctor, after the doctor tries his best to have met medical service standards and operational procedures standards, but accidents still occur. Thus this medical risk or accident contains elements that cannot be blamed (verwijtbaarheid), cannot be prevented (vermijtbaarheid), and the occurrence of unpredictability (verzienbaarheid).[14]

1. The results of a disease course or disease complication that has nothing to do with the medical actions taken by a doctor.

2. The results of a risk that can be avoided, namely;

a. The risk that cannot be known before (unforeseeable).

b. The risk that has been previously known (foreseeable) but is considered acceptable, and which the patient is aware of and has been given their consent/ approval to be carried out such as:

1. drug side effects, bleeding, and infections in surgery, etc.,

2. Risks of a high degree of probability and severity in certain circumstances, ie if these risky medical actions must be taken because they are the only way that must be taken (the only way), especially in emergencies.

In Indonesia, the notion of medical risk is not explicitly formulated in existing legislation. However, implicitly, medical risk is mentioned in several statements of informed consent, Article 45 of the Medical Practice Act, Ministry of Health Regulation No. 585 concerning the Approval of Medical Measures, and statements by the Executive Board of the Indonesian Medical Association regarding Informed Consent.

Settlement of Litigation Medical Negligence Disputes

Settlement of litigation medical negligence disputes through criminal and civil law. Medical disputes that often occur because of complaints or demands of patients that the doctor has or suspected wrongdoing and the most common is medical negligence. In the criminal law process, law enforcement then conducts an investigation and investigation into the alleged medical negligence case. Then the case was transferred to the attorney and finally the trial process. At trial, the prosecutor must be able to prove that the doctor has committed negligence and the defendant is also entitled to present witnesses or expert testimony to alleviate the defendant. Problems have arisen since the investigation process due to a lack of knowledge of law enforcement on medical issues, difficulty in proving, and neutral and credible expert witnesses. Law enforcers will use the article regarding negligence which results in death or disability of others. Negligence in offense is not the same as medical negligence, negligence about consequences. Negligence according to layman's understanding is often equated with medical risk. As a result of medical action, if it is

per applicable rules or procedures, it is not categorized as medical negligence, but it is called medical risk and the doctor cannot be blamed.

There is no formal law in Indonesia regulating medical malpractice and medical risk. This is a problem that always arises because often the medical risk is considered negligence by law enforcement.

The above problems cause the absence of legal certainty in medical disputes. The unclear mechanism for complaints of suspected medical negligence so that every alleged medical negligence was directly addressed by a doctor to the law enforcement officers even though the case was not necessarily medical negligence. Not all medical negligence must be resolved through criminal channels only on gross negligence that is recommended to be resolved criminally. In civil cases as well, patients must prove that the doctor has neglected and this is very difficult for patients and also judges who handle the case because of the difficulty of proof and find expert witnesses who are neutral and credible. The civil settlement will require time, money, and take up open ideas and justice. Litigation medical dispute settlement does not provide legal certainty and violates the principle of legal certainty. The absence of special rules governing medical malpractice, medical risk, and medical dispute resolution. Medical dispute resolution is in essence a specialist lex where the approach is to settle it specifically through professional organizations or mediation but the organization has not provided neutrality and professionalism in medical dispute resolution and needs to be regulated how the medical dispute resolution process involves the profession and law enforcement. A civil law legal system requires the upholding of the principle of legality but in the settlement of medical disputes through litigation, no clear rules are governing medical malpractice and medical dispute resolution.

Settlement of Non-Litigation Medical Negligence Disputes

Any person who knows or interests is harmed by the actions of a doctor or dentist in carrying out medical practice can complain in writing to the head of the Indonesian Honorary Medical Disciplinary Council (MKDKI). One non-litigation medical dispute resolution is through MKDKI located in Jakarta or provinces. MKDKI is under the Indonesian Medical Council. Its membership consists of doctors representing professional organizations and hospital associations and legal experts. This institution can punish doctors if they have been proven to have violated discipline. Penalties can be in the form of reprimands/written warnings, revocation of the registration certificate, and the obligation to attend education or training. If in the investigation found ethics violations, MKDKI forward complaints to professional organizations. Complaints to MKDKI do not deprive anyone of the right to report suspected criminal acts to the authorities and / or bring civil damages to court.

Until now the central MKDKI that has resolved complaints against doctors should have been formed at the regional or provincial level but is still constrained by various problems, especially funding. This is where the difficulty of patients to report doctors because of the distance MKDKI center and also their ignorance of this institution. At the same time, the patient may also file a civil suit or report to the police for criminal proceedings so that the doctor in this case is the party who is seriously harmed. Doctors are not necessarily guilty when complaints are still being processed by MKDKI.

MKDKI can be used as the initial step of the disputing parties before proceeding to the litigation process. MKDKI's decision must be confirmed whether it can be the initial evidence/clue that the doctor has made a mistake especially negligence. Vice versa if the doctor has been found not guilty then the court has the right to reject a lawsuit filed by the patient. For punishment, MKDKI may determine compensation for the disputing party. And MKDKI is also given the authority to facilitate the parties to the dispute to mediate. Because medical dispute resolution is a lex specialist, MKDKI has a place to make improvements to better provide legal certainty and justice for all parties. Law No. 36/2009 on Health provides a breakthrough regarding the resolution of medical negligence disputes, namely through mediation as a settlement of non-litigation medical negligence disputes. Mediation is also regulated in the resolution of disputes through civil lines. Mediation provides new hope for the resolution of medical disputes, especially doctor errors due to negligence. However, mediation needs to be regulated and combined with the role of MKDKI or professional organizations. The mediation

process must be more clearly regulated for the resolution of medical disputes so that the disputing parties feel certain and fair in their dispute resolution. Settlement of litigation medical negligence disputes which tend not to provide legal certainty can be thought to make non-litigation medical negligence dispute resolution a hope. The settlement of non-litigation medical negligence disputes is also a breakthrough.

Conclusion

Clearer regulation is needed regarding medical malpractice, especially medical negligence and medical risk. In the case of medicolegal especially regarding medical negligence, it can no longer be used as a general understanding of negligence in the Criminal Code, for this reason, it is necessary to study the existing rules to produce identical rules based on structure, substance, and culture. Institutions that determine who has the authority to investigate medical negligence must also be clear and provide comprehensive resolve medical disputes resolution mechanisms. The settlement of medical negligence disputes must be clear as to whether the litigation or non-litigation is resolved, to provide legal certainty and justice for the disputing parties. We need to consider resolving non-litigation medical negligence disputes into hopes and breakthroughs in resolving medical negligence disputes in Indonesia.

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Protective Strategies for the Ownership of Traditional Medicine Knowledge

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Abstract

This paper discusses the ownership of traditional medicine knowledge by drawing empirical data on the experience of the indigenous communities in Mentawai, Indonesia, and Sabah, Malaysia. For a long time, the acquisition of traditional medicine knowledge and the treatment of patients has been deemed the result of cultural heritage handed down from generation to generation. However, the complexity of the knowledge acquisition process and the skill displayed by the healer or kerei prove that traditional medicine knowledge qualifies as intellectual property. An appropriate protection strategy is identified based on the traditional knowledge ownership map in indigenous communities. This study reveals that not every traditional medicine knowledge and practices are "common properties". Therefore, the study suggests that traditional medicine knowledge, like any other intellectual properties, must be protected through intellectual property rights not only to protect the cultural and economic rights of indigenous people but also to protect the environment.

Keywords: *Traditional medicine knowledge, Indigenous people, Intellectual Property Rights, Sui generis, Mentawai and Sabah.*

Introduction

In general, the international community agrees that traditional knowledge plays a very important role within the community. This knowledge is important for both developed and developing countries where it is developed, preserved, and handed down from generation to generation. Although there is almost no disagreement on the need for the legal protection of such knowledge among law/policymakers, academics, NGOs, and indigenous people, there is no agreement on how it should be protected. One of the reasons is the difference in perception about the nature of ownership of traditional knowledge. The public considers traditional knowledge as a common heritage or property. The issue is whether traditional knowledge possessed by indigenous peoples is a public property that can freely be accessed to and obtained free of charge by anyone not a member of the community.

The academic debate regarding the ownership of traditional knowledge affects knowledge protection strategies. Without clear ownership of traditional knowledge, it will be very difficult to determine protective strategies. This is because the protection of traditional knowledge does not only sustain the knowledge but also how that knowledge can benefit its owners and society in general. Thus, the designed protection strategy is intended for fairness, expediency, and legal certainty.[1] As stated earlier, this paper discusses the ownership and protection of traditional medicine knowledge within indigenous communities in Mentawai (Indonesia) and Sabah (Malaysia).

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Owner of Medicine Knowledge

Traditional Medicine Knowledge owner is any person or group of persons within an indigenous community that develops medicinal knowledge and has the right and authority to maintain confidentiality and put the knowledge into practice. The research shows that, within indigenous communities in Siberut, Mentawai, particularly in the *Rereiket* area, three groups possess traditional medicine knowledge: the community, clans; and healers. While in Sabah, Malaysia, only two groups have such knowledge: the community and the healers.[2]

The healers are specialized in treatment/healing and have the authority to prescribe medicines because they are recognized and trusted by their community members. The Mentawai *kerei* are specialized in treatment and receive widespread recognition from the public. Within the indigenous community in Sabah, the healers consist of several groups, namely *bobolian and bomoh*, herbalist (*mongugusap*), and massage therapist. The existing knowledge of each of these healing groups differs from one another in the way to acquire knowledge, the rights and authority of the owner, and knowledge transfer methods. It can be inferred from the above grouping that medicinal knowledge is owned by:

a) Community;

b) Clan; and

c) Healers consist of Kerei, Bobolian, Bomoh, Herbalists; and Massage Therapists.

Procedures for Transfer of Medicinal Knowledge

Each group of owners knows the rules and procedures of traditional medicine transfer is based on tradition. Both in Mentawai and Sabah, in general, traditional medicine knowledge and patient treatment are acquired through the following processes: learning, discovering or developing, dreams, natural talent, and inspiration (divine gift). The learning process is a series of knowledge transfer activities and the value of living in a community, which is done consciously by teachers and pupils. In practice, the process of teaching the knowledge of medicine and treatment is done through oral teaching, direct observation; and apprenticeship. Teaching rules and procedures are various. Each healer has a unique method in knowledge transfer.

Indigenous people believe that the transfer of medicinal knowledge along with patient treatment is a very important occasion as they see it not just as a mere transfer between the giver and receiver of the knowledge (teachers and pupils) but also as the requirements to meet before the transfer occurs. These requirements include the rights and obligations of the parties involved in the transfer. Until these requirements are met, no transfer of medicinal knowledge can take place. These requirements, in general, can be grouped into two categories: primary requirements and secondary requirements. Primary requirements compulsory requirements. Prospective healers who fail to meet these requirements are denied the learning process. Secondary requirements are complementary requirements. Although these two categories of requirements are found in each group of healers, there are fundamental differences in what constitutes primary and secondary requirements between the healing groups in the Mentawai kerei healer and those in Sabah.

In the generally accepted rules within the healing groups in Sabah, primary requirements are immaterial and secondary requirements are material". A similar situation applies to the *kerei* tradition in Mentawai for potential healers "*mukerei*".[3] Only adult Males must comply with "immaterial" requirements to follow the *mukerei*. In addition to the immaterial requirements, a *kerei* candidate must also meet the "material" requirements which are usually negotiated and agreed to by the *kerei* candidate's parents or tribal leaders and a senior *kerei* who would be his teacher (*sipaumat*). This requirement is commonly referred to as *sakinia* (fee).

The type and the fee requested by the senior *kerei* vary. In terms of type, five are always requested, i.e., pigs (3-10), chicken (1-2), durian fruits, coconuts, and sago palms. These are the key conditions to comply with for the implementation of *mukerei.* The payment is made in several stages. First, during the mukerei, which is before the kerei inauguration party begins. It is known as *katotoili* payment (*saki katotoili*), and second, after the completion of the *kerei* inauguration party, which is done in the morning after an *alub* party. The third and last stage is the payment for special medicinal knowledge.

In addition to the mandatory material requirements above, senior kerei sometimes ask other requirements,

such as *rambutan* fruits ax, knife sharpener, mirror, and white clothes. Because these are complementary material requirements, they are not requested by every potential teacher.

From what precedes, it appears that each indigenous community has its procedure for the transfer of traditional knowledge. This is because every community has its tradition that spawns the principle and nature of medicinal knowledge ownership. Traditional medicine knowledge is handed down from one generation to another to preserve it. The tradition is the rule of normative life and an integral part of traditional medicine knowledge ~~existence~~ existing in an indigenous society.

In traditional societies both in Mentawai and Sabah, the transfer of medicinal knowledge and the treatment of patients is not just a mere transfer of information about medicine, but it is also a process to recognize the possession of medicinal knowledge. In the process of transfer, the donor and the recipient have rights and obligations depending on the nature of the ownership of the medicinal knowledge that is based on the prevailing tradition in a group of holders of indigenous knowledge. The following part discusses the nature of the ownership of indigenous medicinal knowledge.

Protecting Traditional Medicine Knowledge Through Intellectual Property Rights

Conceptually, there are two forms of protection of traditional knowledge: positive protection and defensive protection.[4] This distinction is based on the purpose or motivation behind the protection of traditional knowledge.[5] Positive protection under the law is the means provided to traditional knowledge owners to claim their rights. Meanwhile, defensive protection is the legal instrument and non-legal measure available to knowledge owners to prevent from them being taken advantage of by third parties.

The idea behind positive protection is to safeguard the rights of traditional knowledge holders or owners for commercial benefits.[4] In other words, this form of protection is used to drive economic benefits from the exploitation of traditional knowledge. Therefore, the recognition of legal rights over such traditional knowledge is required. These legal rights are in forms of intellectual property rights; and the legal establishment of Intellectual Property Rights (*sui generis*). The following is the elaboration of each legal means that can be used to protect traditional medicine knowledge.

Protection through Conventional Intellectual Property Rights

Intellectual property law is the method governing the creation, use, and exploitation of the results of the creative effort or mental (mental or creative labor). Intellectual property rights law provides private property rights to any work involving creative or mental activity.[6] The term "intellectual property" has been used since ages to refer to areas of the law relating to copyright, patents, designs, and trademarks as well as other rights associated with it.[5] This branch of the law is known as the law of conventional intellectual property rights.[7]

Some scholars criticize the protection of traditional knowledge through intellectual property rights. They claim that there is a clear line of demarcation between private rights and public rights. They argue that the ownership of traditional Knowledge is vague. Supporting this is Sardjono (2004) who argues that ~~t~~raditional medicine knowledge is a collective right and that individualistic patent may not be used to protect something that relies on communalistic value.[8] But it is worth noting that not every traditional knowledge is communal. The research conducted in Mentawai and Sabah reveals that not all ownership, especially ownership of traditional medicine knowledge is "common property". Concerning the ownership boundary lines introduced by Heller, much of the traditional medicine knowledge owned by indigenous peoples is private. A Kerei, who develops and discovers new and unprecedented medicinal, is recognized by their community and the society in general as the owner of that knowledge.

In the prevailing tradition of the Mentawai people, owners of medicinal knowledge have the right and authority to exploit their knowledge. They also have the right to sue and fines anyone unlawfully using their knowledge.

Furthermore, the right to possess and use medicinal knowledge is limited to those initiated by the knowledge owner.[8] The difference between medicinal knowledge possessed by an individual and that of the group lies in the nature of the access to such knowledge. Access

to medicinal knowledge possessed by an individual is open to the public (open access), whereas access to the knowledge owned by the healers very limited. This is consistent with Heller's basic concept of the boundary line of private property that claims that open access of others to a power source, the nature of private property ownership is shifted towards the 'commons'.

Every indigenous community has its own rules regarding the ownership of traditional medicine knowledge. Although this knowledge is viewed as common property, each indigenous community has its concept about it. Based on the tradition in other parts of Indonesia such as Java, Bali, and Lombok, access to traditional medicinal knowledge is open meaning that anyone, including foreigners, is allowed to learn it and use it any way that suits them.[7] Referring to Heller's theory, knowledge of traditional medicine in the community of Java, Bali, and Lombok can be considered as an open-access common property.[7]

The private aspect of the acquisition of traditional medicine knowledge can also be found in other Asian countries such as Thailand, India, and China. Whereas countries such as Ethiopia and Peru do not recognize private ownership over traditional knowledge. A system of joint ownership is applied in Peru instead, which means that indigenous peoples are entitled to the economic value of their knowledge despite ownership being open to the public.

It can be concluded, from what precedes, that not every traditional medicine knowledge - including knowledge of traditional medicine is common property. Thus, the idea that intellectual property rights are incompatible with the legal protection of traditional knowledge becomes irrelevant.

Another legal issue is the fulfillment of the requirements for obtaining substantial property rights in each relevant legal instrument, i.e., the requirement of novelty and inventive step to acquire a substantial patent. The legal protection of traditional knowledge involves patents, trademarks, and geographical indications. This paper is only concerned with protection through patents. Patent registration to fulfill substantive requirements is one of the major problems in the protection of traditional knowledge. Novelty and inventive step formalities are required to obtain a patent. The question is whether there is any legal concept related to patent that can best match/ accommodate the real condition of traditional knowledge owned by indigenous people. The requirement of the invention for patent varies from one country to another.[9] There are two legal concepts of novelty, i.e., *absolute novelty* and *relative newness*. Theoretically, every country is free to choose the type of novelty that can best accommodate the protection of traditional knowledge through the patent regime. In their patent law, both the United States and China chose the concept of "relative novelty" which allows them to provide patents on eligible traditional knowledge. An example of the protection of traditional knowledge through patent in the US is the formulation of 'azadirachtin' for storage stability.[10] Similarly, the concept of novelty also exists in European patent law on the impact of fungal oil 'neem' (fungicidal effect of neem oil).[11] This patent claims to control fungi on plants by using a stable extract from seeds of the neem tree. Both of these patents were challenged in court on behalf of traditional communities.

This has led to the cancelation of the patent by the Technical Board of Appeal of the European Patent Office on the ground that the invention was common knowledge of the indigenous people in India and that it did not meet the substantive requirements of EPO. The US Patent and Trade Mark Office (USPTO), on the other hand, ruled that the patent was valid as the invention meets the substantive requirement of novelty.[12]

Furthermore, the same also applies in determining the concept of inventive step. It is highly dependent on the patent laws of a country to formulate the inventive steps to ensure a qualitative difference. This requirement is a qualitative examination to ascertain whether the contribution of the invention is sufficiently creative.[13]

China uses prominent substantive features as an indicator to determine inventive steps in the formulation of its patent law. It is assumed that this delivers rapid progress compared to previous concepts.[14] This indicator is also used by the European Patent Office in assessing inventive steps. The above-mentioned indicators are generally accepted as measuring ropes for inventive steps in the field of traditional medicine in China.[15] In 2001 alone, China provided over 3000 patents on innovative developments in the field of traditional Chinese medicine.[16,17] Much of the medicinal knowledge

owned by the *kerei* in Mentawai and some herbalists in Sabah complies novelty and inventive steps. It is the knowledge of medicine they have always developed and opened to the public. The knowledge is transferred only on a limited basis according to the applicable methods. In India, for example, there are more than 10,000 patentable traditional inventions.[5] But the question as to how to determine novelty standards and inventive step within the country's patent law remains a challenge.

Establishment of Intellectual Property Law "*sui generis*"

Traditional Knowledge can also be protected by intellectual property laws (sui generis). If traditional knowledge is used for commercial reasons, then the economic aspect of its protection is only effective through international arrangements. Moreover, a regime of international law is required. Verma (2004) argues that the protection of traditional knowledge through the establishment of intellectual property law "sui generis" can be set at the national and international levels. He claims that the first thing to do at the international level is to stop taking unlawful actions against genetic resources along with traditional knowledge therein. The TRIPS Agreement could be an ideal tool compelling in this regard.[18] Article 29 of TRIPS requires member states to unlock the genetic origin and related knowledge and to provide evidence of prior informed consent (PIC) and benefit-sharing. Article 29 of the same Agreement requires World Trade Organization member countries to require patent applicants to disclose the invention and to allow it to be assessed by a relevant expert.

Furthermore, efforts to protect traditional knowledge at the international level can also be done through bilateral agreements between states. Through the principle of mutual recognition, two countries could agree on standards for the protection of traditional knowledge, particularly traditional medicine knowledge. The protection standards applied in one country could be recognized by another on the principle of reciprocity applied by WTO member countries and the copyrights of many countries in the 19th century. Countries could also agree to the establishment of a legal regime that protects traditional knowledge "*sui generis*" by learning from the experience of both the Berne Convention on the protection of Copyright and the Paris Convention on the Protection of Industrial Property. Countries involved in these two conventions have developed patterns allowing them to agree on protecting standards that have proved to be effective at the national level.

National Intellectual Property Rights "*sui generis*"

The protection of traditional knowledge protection intellectual property rights "sui generis" at the national level, in certain cases, seems to be more advantageous than at the international level. International tools that protect the economic aspect of traditional knowledge are not concerned with its development, and conservation. These processes can only be achieved through national law. The preservation of traditional knowledge is closely associated with the land and living environment of indigenous people. Protecting traditional knowledge does not just mean to recognize and provide exclusive rights over traditional knowledge. It must also encompass a legal mechanism that ensures the protection and preservation of the land, forest, and water of indigenous people. These must not only be protected and preserved, but they should not be taken away from indigenous people as they are the foundation of the perpetuation of the knowledge of traditional medicine. Verma (2004) argues that separating indigenous people from their surroundings is killing traditional knowledge instead of helping it to be developed and conserved.[18]

Another reason why protecting traditional knowledge at the national level is preferable is that not all of the traditional knowledge may be protected by conventional intellectual property laws.

It worth noting that traditional medicine knowledge is a knowledge that does not sometimes improve since it is owned collectively. Some community members simply feel entitled to use it without feeling obliged to develop it. This is partly because many indigenous people believe that it is a knowledge acquired through supernatural powers. Therefore, the protection of such knowledge is only possible under the law of intellectual property rights *sui generis*. Finally, it is important to bear in mind that the purpose of the protection of traditional knowledge should not only be for economic interests but its preservation.

Conclusion

The nature of the ownership of traditional medicine knowledge is largely determined by the way of acquiring such knowledge. Ownership over traditional medicine authorizes the owner (the healer) to treat, maintain confidentiality, and transfer the knowledge. Traditional medicine knowledge is privately owned both in Mentawai and Sabah. But it can also be owned collectively when it is under the control of the community at large. In both Mentawai and Sabah, knowledge of traditional medicine is accessible to people outside of the indigenous communities including foreigners based on the tradition and rules that apply.

Like any other intellectual property, traditional medicine knowledge can be and must be protected through Intellectual Property Rights law to obtain economic value either through legal property rights conventional such as patents, trade secrets, geographical indications, and the establishment of intellectual property law sui generis, both at national and international levels. To that end, it is important that parties i.e., indigenous people, companies, activists, and the government work together to bring about efficient and sustainable regulations and policies that guarantee the preservation of traditional heritage and the protection of both the environment and the rights of indigenous people.

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Effectiveness of Online Oral Health Education During the Covid-19 Pandemic

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Abstract

This study aims at analyzing the effectiveness of online public education about oral health during the COVID-19 pandemic. Studies show that humans touch their face 23 times in 1 hour and touch their mouth and its surroundings 4 times per hour with a duration of 1-12 seconds. This can cause the transmission of the virus through the oral, nasal, or conjunctival mucosa. How oral hygiene can contribute to reducing the risk of Corona Virus transmission between humans is the main focus of this study. Dental procedures carry high risk of exposure to Covid-19 through saliva, blood and other body fluids from patients, the use of high-speed instruments also increases the risk of infection in practice. The incubation period of 7-24 days exposes dentists and patients to cross-infection that can be transmitted through the oral cavity and respiratory tract. The study reveals that a great part of the population in Indonesia, especially in West is unaware of the transmission of the Corona Virus through dental procedures. Consequently, the study suggests that online oral education is suitable for raising the community's awareness of the Covid-19 pandemic.

Keywords*: Oral Health Education, the Covid-19 Pandemic, Corona Virus, and Dental Procedures*

Introduction

Education is the process of providing knowledge to improve individual abilities in order to form behavior change.[1]Oral Health Education is to provide information about the condition of the oral cavity using educational aids.[1,2] Oral health education is carried out by considering age, level of education as well as social and cultural conditions around the individual.[1,3,4,5]

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Health education can be done face-to-face or online with various methods such as lectures, written media, demonstrations, and game simulations.[1,2,6] Educational materials in the form of technological media are called e-learning (online). The online method changes the system directly which has been established for more than 25 years.[7] The educational method used in this study is an online demonstration method for respondents through podcasts, videos, posters containing educational material distributed on various social media platforms.[8]

The advantage of using online media is that it is easy to access, can be seen repeatedly, the ability to connect individuals from various regions.[9] This is in line with the education trend according to Han et. al (2019) namely by utilizing technology.[10] Technology facilitates education so that it can be accessed anytime and anywhere easily via cellphone or computer. Edu54cational material made

in various forms of multimedia is more interesting and interactive than written form so that it can increase motivation and concentration in viewing educational material.[11] However, behind the many advantages of online methods, there are several limitations, namely the number of people who have limited access to technology, the possibility of technical errors, and difficulty understanding the exact intent of the speaker. Online education is a solution during a pandemic.[12]

The Corona Virus Pandemic (COVID-19) is an infectious disease caused by the novel coronavirus.[13,14] This disease started in Wuhan, China since December 12, 2019 and on January 30, 2020 the World Health Organization (WHO) declared COVID-19 as an epidemic on an international scale.[13] This disease has several main symptoms such as fever, persistent dry cough, and myalgia. Accompanying symptoms such as sputum production, headache, diarrhea, and hemoptysis were also found.[13,15]

Covid-19 transmission can be through coughing, sneezing and inhalation of droplets containing the virus.[13,14] Oral, nasal, and mucous contact with the eye membranes can also be contagious. There are various clinical manifestations of this disease, such as manifestations on the skin and mouth of the patient.[13] WHO recommends maintaining hand hygiene by washing hands with soap in running water for 40 - 60 seconds, or the use of sanitizer containing alcohol for 20 seconds.[14]

The WHO declaration regarding the COVID-19 pandemic condition makes dental services limited to emergency cases. March 25, 2020, the Chief Dental Officer (CDO) in the UK recommends that all dental care be carried out by applying dental emergency triage and providing analgesics and antimicrobial care without involving contact between the dentist and the patient.[13] The dental emergencies mentioned, such as severe uncontrolled pain in the teeth, recurrent and widespread dental infections, permanent dental avulsions, severe trauma to the teeth dental procedures such as permanent cementation of the crown, and a biopsy.[13,5]

According to studies, humans touch the face 23 times in 1 hour which can cause transmission of the virus through the oral, nasal, or conjunctival mucosa.[14,16] Based on Kwok's study, humans touch the mouth area 4 times per hour, with a duration of 1 - 12 seconds.[15] Like hand hygiene, oral hygiene must also be done to reduce the risk of virus transmission. The act of maintaining oral hygiene can be carried out personally at home or in a professional manner.[14] Oral hygiene procedures can be performed by brushing your teeth with toothpaste.[14] During the COVID-19 pandemic, it is recommended to brush your teeth before and after being outside the house,[14] the duration of brushing is 2 minutes.[14,17]

The textures of toothbrushes vary, namely hard, medium, soft, and extra soft.[17,18] For COVID-19 positive patients, they must have personal oral hygiene products and not mix with other people, namely, toothbrush with soft bristles, toothpaste, and personal mouthwash and must be replaced after the patient's condition improves. Toothbrush disinfection can be done by soaking the toothbrush in chlorhexidine solution for 20 minutes before and after use.[19] Oral hygiene equipment for COVID-19 positive patients must be disinfected regularly after use.[14]

Dental procedures are a procedure that carries a high risk of exposure to Covid-19 through saliva, blood and other body fluids from patients, the use of high-speed instruments also increases the risk of infection in practice. The incubation period of 7-24 days exposes dentists and patients to cross-infection that can be transmitted through the oral cavity and respiratory tract.[13,15]

During the pandemic, dental procedures are limited to emergency measures. A strict patient screening procedure must be implemented as a first step namely by measuring body temperature and the patient must complete a questionnaire to identify whether the patient has symptoms of COVID-19 such as fever, persistent cough, and shortness of breath for 2 weeks before the visit. Patients should also report whether they have had contact with 2 or more individuals who have symptoms of fever or shortness of breath 2 weeks prior to the visit. If the screening results indicate the potential for COVID-19 infection in the patient, the visit must be postponed and the patient is expected to self-isolate and visit the nearest health facility.[13,15]

Standard procedure during the COVID-19 pandemic in all health facilities, namely social distancing, must be implemented in the waiting room, patients must wear a mask. Patient appointments should

be scheduled between patients to reduce contact in the waiting room, all magazines or toys should be removed from the patient waiting room. If this step cannot be implemented, then patients are required to wait in their respective vehicles.[15] Hand hygiene procedures must be applied by both dentist and patient when performing the procedure. Dentists should wash their hands before examining patients, after dental procedures, and after touching non-sterile surfaces and tools. Hands should be washed after direct contact with the oral mucosa, blood, body fluids and saliva.[13] Oral hygiene measures in practice must also be applied. Patients are asked to rinse their mouth before performing dental procedures with povidone iodine.[13,15]

During the procedure, the patient must wear a rubber dam to reduce the risk of aerosol contamination.[13,15] Dentists must wear personal protective equipment such as gloves, masks (N-95 or FFP2), face shields, and waterproof gowns or jumpsuits to protect the dentist from aerosol or blood splashes.[15] Waste generated from the treatment of COVID-19 positive patients must be disposed of in a separate place.[13]

Methods

This research is an effort of the Faculty of Dentistry, Andalas University to educate the public about dental care procedures during the COVID-19 pandemic. This research was conducted using a questionnaire for data collection which was carried out online and was carried out from 1 July - 30 July 2020, with 67 volunteers of Thematic field study students of the Faculty of Dentistry, Andalas University. The total respondents for this study were 3500 people. Respondent characteristic data taken is based on age, education level, and occupation of the respondent. The questionnaire consisted of 22 questions that were given online to respondents. The pre-test was carried out on all respondents, then given an intervention in the form of online education, namely by means of interactive video calls, videos, podcasts and educational posters that were distributed to various media platforms to the respondents. Post-test is given to see the degree of understanding of the respondent after the intervention. The data that has been obtained are then processed statistically.

Results

Characteristics of Respondents

The description of the characteristics of respondents based on gender, education level, and occupation. Based on the data, respondents with male gender is 1190 respondents (33.4%) while female respondents are 2393 respondents (66.6%). Respondents with the most recent education level, namely Senior High School is as many as 1916 respondents (53.4%).

Univariate Analysis

Based on the data, the question 11 in the *post-test* section received the most correct answers from as many as 3449 respondents (96.1%). Most errors in the *post-test* section is in question number 4 with 1114 respondents (31.0%). Question number 21 shows the *pre-test* results with the highest error, namely 2315 respondents (64.5%).

Bivariate Analysis

Table 1.1 Relationship between Gender and Pre-Test Results

	Gender				Total		p-value
Pre-test	**Male**		**Female**				
	f	%	f	%	f	%	
Insufficient	41	39,4	63	60,6	104	100	**0,006**
Sufficient	701	36,2	1238	63,8	1936	100	
Good	339	30,8	768	69,2	1106	100	
Total	1081	34,4	2069	65,5	3150	100	

Based on table 1.1, it can be seen that as many as 41 respondents (39.4%) were male and 63 respondents (60.6%) were female with Good category pre-test. It is known that the p-value is 0.006, so there is no relationship between gender and the pre-test results.

Table 1.2 Relationship between Gender and Post-Test Results

	Gender				**Total**		**p-value**
Pre-test	**Male**		**Female**				
	f	**%**	**f**	**%**	**f**	**%**	
Insufficient	3	100	0	60,6	3	100	**0,006**
Sufficient	94	39,0	150	61,0	244	100	
Good	985	33,9	1918	61,1	2903	100	
Total	1082	34,4	2069	65,5	3150	100	

Based on table 1.2 it can be seen that as many as 985 respondents (33.9%) were male and 1918 respondents (66.1%) were female with a Good category pre-test. It is known that the p-value is 0.016, so there is no relationship between gender and the results of the post test.

Table 1.3 Relationship between Education and Pre-Test Results

	Pre-test								**p-value**
Pre-test	**Insufficient**		**Sufficient**		**Good**		**Total**		
	f	%	f	%	f	%	f	%	
Kindergarten	4	3,8	15	0,8	6	0,5	25	0,8	
Primary School	6	5,8	130	6,7	43	3,9	179	5,7	
Junior High	20	19,2	192	9,9	85	7,7	297	9,4	0,000
Senior High	54	51,9	1039	53,6	605	55,0	1698	54	
University	20	19,2	563	29,0	368	32,9	951	30,1	
Total	104	100	1039	100	1107	100	3150	100	

Table 1.3 suggests that as many as 6 respondents (0.5%) have a kindergarten education, 43 respondents (3.9%) have elementary school, 85 respondents (7.7%) junior high school, 605 respondents (55.0%) high school, 368 respondents (32.9%) universities with Good category pre-test results.

Table 1.4 Relationship between Education and Post-Test Results

	Pre-test								p-value
Pre-test	**Insufficient**		**Sufficient**		**Good**		**Total**		
	f	%	f	%	f	%	f	%	
Kindergarten	0	0	2	0,8	23	0,8	25	0,8	
Primary School	2	66,7	17	7,1	160	5,5	179	5,7	
Junior High	0	0	37	15,4	261	9,0	298	9,5	0,000
Senior High	1	33,3	123	49,8	1579	54,4	1700	54,0	
University	0	0	65	27,0	880	30,3	945	30,0	
Total	3	100	244	100	2903	100	3150	100	

The above table shows that as many as 23 respondents (0.8%) had a kindergarten education, 160 respondents (5.5%) Elementary School, 261 respondents (9.0%) Junior High School, 1579 respondents (54.4%) Senior High School, 880 respondents (30.3%) universities with Good category post test results.

Table 1.5 Mean Score of Interventions

Variable	Pre-test		Post-test		Difference	p-value
	Mean	± Primary School	Mean	± Primary School		
Intervention	16,06	3,631	19,85	4,172	2,79	0,000

Table 1.5 suggests that the results of the statistical test showed that the average difference in knowledge before and after the intervention was obtained p-value of 0.000 ($p < 0.005$), it can be concluded that there was an influence on the intervention that was carried out.

Table 1.6 Interpretation of NGain

	Class	N	Mean	Standard Deviation	Standard Error Mean
NGain Percent	Intervention	3150	78,11	34,467	0,614

Based on table 1.6, it can be seen that the average (mean) value of NGain Percent for the intervention class is 78.11 or if rounded to 78%. Based on the category table of interpretation of the NGain value (%) above, it can be concluded that the method used is effective.

Discussion

The educational background of each respondent affects the level of knowledge. The results of the respondents' pre-test showed that high school and college education had good knowledge of oral health during the COVID-19 pandemic. This shows that the level of education plays a role in individual knowledge regarding oral health. This is consistent with the 2015 Gomes study and the 2020 Chen study, namely individuals with low educational degrees will have low health literacy,

poor diet, and poor oral health behaviors.[20,21] According to Passalacqua (2012), the level of education affects the level of knowledge about dental and oral health because the higher the level of individual education, the higher the possibility of the individual being exposed to dental and oral health promotion programs.[22]

Of all the pre and post test questions, question number 11 about masks is the result with the smallest percentage of errors. The educated community has understood the function of masks. The transmission of COVID-19 can be through droplets, direct contact and aerosols. Face-to-face conversation, breathing, coughing and sneezing can release droplets up to 1 meter away so that they can enter the mucosal surface. According to Wang (2020), the use of masks is the main effective preventive measure against the transmission of COVID-19 Virus infection.[23]

Question number 4 regarding the procedure for storing toothbrushes shows the highest error results. This illustrates that people who are educated do not understand that COVID-19 transmission is through toothbrushes. Toothbrush can be a medium for the development of microorganisms so it must be replaced periodically within 3 months.[13,17,18,19] Store the toothbrush in a dry place in an upright position until reuse.[18] Check the bristles regularly, if the bristles have been damaged before 3 months of use then the toothbrush must be replaced.[13,18] Bristles that are damaged too quickly (before 3 months) indicate that the individual is brushing their teeth too hard, while a toothbrush that does not break after more than 3 months of use indicates that the individual has not brushed his teeth properly and correctly.[13,17] Toothbrush replacement is also recommended if the individual has recovered from an infectious disease.[17]

Of all the pre-test questions, question number 21 regarding the maximum time to get oral therapy, the results of the pre-test answers have the highest error rate in the community. After education has been carried out, it can be seen that the increase in public knowledge about the timing of oral therapy during the COVID-19 pandemic. Oral therapy carries a risk of SARS-CoV-2 infection because of the procedure, which involves face-to-face communication with the patient and frequent exposure to saliva, blood and other body fluids, as well as the use of sharp tools. The corona virus can be transmitted within a distance of less than 2 meters and the duration of exposure is more than 15 minutes.[24] According to Bizocca (2020), the corona virus can survive in aerosol form and can survive on objects' surfaces. The corona virus becomes inactive indoors within 10-15 minutes.[25,26]

In the bivariate analysis of this study, gender did not affect knowledge about oral health. This is consistent with Azodo's 2015 study, namely the role of gender on perceptions of oral health, oral health problems, and oral health status.[27] However, the results of this study contradict the 2019 Etetafia study, which states that gender has an influence on knowledge of dental and oral health.[28] In this study, it was stated that women in Africa tended to have more knowledge about oral health than men.[28] This is due to the social role of women who are responsible for family health, so that women tend to be more interested in seeking health information.[28,29]

Education has an influence on respondents' knowledge. This is in accordance with the Halawany study in 2018 and Angelopolou in 2015 regarding dental education interventions in children.[30,31] Education about oral health aims to improve health literacy and provide changes in attitudes to individuals.[32,33,34,35] There are two aspects to the educational process, namely the perception and motivation of the individual.[33]

The method of providing education can be based on Dale's cone of experience.[2,36] Giving lectures, demonstrations, and simulations is an educational method that can be given the use of digital platforms such as social media as an educational medium can also be done to educate the public.[5,36] The percentage level of individual understanding will vary and depends on the teaching method used, individuals taught by the lecture method will remember 10% - 30% of the information provided, individuals taught by the demonstration method will remember 30% - 50% given, and individuals who taught by the simulation method will be able to remember 70% - 90% of the information given.[36] The success factors for a health education consist of the presentation of material based on the needs of the community as an educational target, techniques for providing materials tailored to the community's abilities such as effective planning of educational programs, the active role of the community during education, and a

comprehensive evaluation of the educational programs provided.[2,36,37,38]

Dental education should be provided in a way that is easy to understand and adapted to each level of the respondent's age group.[32,33] The application of an educational method must consider the values, culture, language, emotional and social needs of each respondent.[33] Each individual has different characteristics in processing the information received. This difference must be taken into consideration, so that education must be provided according to individual characteristics in order to be effective in achieving an understanding related to oral health.[20,35]

Education given by the lecture method must use simple and easy to understand sentences, avoid using health terms, and speak slowly. Education that is given personally can also use the personal experience of the target to facilitate understanding of the material.[39,40] Meanwhile, the education given on a demonstration is good to use simple pictures to make it easier for respondents to understand and to help focus on the material.[39,38]

Health education can also fail due to obstacles encountered during the process, such as the presenters do not have sufficient ability to educate respondents, the presenters do not consider patient characteristics in understanding information, language, culture, socioeconomic factors, and the education level of respondents also plays a role as a barrier to health education.[38,40,41]

Evaluation of oral health promotion based on Nutbeam consists of four levels, namely actions taken on oral health promotion such as education, outcomes - the consequences of education, namely health literacy, intermediate health outcomes - behaviors that are formed after health literacy, health and social outcomes- examination of health indicators such as plaque scores.[30,32] In this study, NGain's results were 78% so that online education methods were effective on respondents' knowledge. Increased knowledge of public oral health carried out online is suitable for the COVID-19 pandemic conditions. Oral health education is very important for the public to minimize the oral transmission of COVID-19.

Conclusion

Online education methods are effective on respondents' knowledge. Online oral education is suitable for the COVID-19 pandemic conditions. Oral education is very important for the community to break the chain of COVID-19. The results of this research show that there is public ignorance about toothbrushes as a medium for transmitting COVID-19. The study also reveals that there is public ignorance about the limitation of time for oral therapy during the COVID-19 pandemic. However, the study notes that the community's understanding of the function of masks to protect themselves from the transmission of COVID-19 was obtained.

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The Implementation of Honesty Principles in Therapeutic Agreements based on the Health Law Perspective in Indonesia

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Abstract

Covid-19 is a virus that has become an epidemic in almost all countries, including Indonesia. One of the reasons for the massive spread of Covid-19 is the patient's dishonesty in providing information related to their medical history and symptoms to doctors and health workers, causing patients not to be treated according to their complaints. The formulation of the problems in the research, namely: the legal basis for the application of The Honesty Principle in the therapeutic agreement and the legal consequences don't fulfill the honesty principle. The research method used is normative juridical research. The legal basis for applying The Honesty Principle refers to the general rules of contract law in the Civil Code and regulations in the field of health law in particular. The absence of The Honesty Principle in a therapeutic agreement includes an agreement that is defective and can be canceled and compensated for. Especially for an infectious disease outbreak such as Covid-19, a dishonest patient can be subject to a maximum imprisonment of 1 year and/or a maximum fine of Rp.1,000,000.

Keywords: *Covid-19, Honesty Principle, Therapeutic Agreement*

Introduction

Corona Virus Infection (Covid-19) is a virus that has now become an epidemic in almost all countries, including Indonesia. A total of 216 countries have confirmed 17,660,523 positive cases of Covid-19 and 680,894 people have died.[1] In Indonesia, 210,940 positive cases of Covid-19 have been confirmed and 8,544 people have died.[1] The factors that cause the Covid-19 virus to spread so rapidly are high community mobility, increased capacity for tests conducted on the community, and the application of health protocols that are not strict.[2] Besides, sometimes people do not provide true/honest information regarding their symptoms or history of illness to health workers. This is the case in several regions in Indonesia. There are lots of medical personnel at the Hospital who contracted from patients who seek treatment without wearing masks, are not honest about their travel history and symptoms of Covid-19 that have been felt. [3,4]

The above case, contradicts the patient's obligation to provide complete and honest information about his health problem.[5] The patient's dishonesty can lead to an incorrect diagnosis given by the doctor. In the end, the doctor's diagnosis will bring harm to the patient himself. Moreover, during the Covid-19 period, this patient's dishonesty could also harm doctors, as was the case above. In essence, the relationship between patient and doctor in health care is a partnership relationship that together establishes the right diagnosis. Therefore, this honesty principle does not only apply to doctors but also applies to patients to get the right diagnosis and protect medical personnel (in this paper it will be focused on a doctor) from disease transmission.

Based on the description above, this study will identify and analyze the application of the honesty principle in the Therapeutic Agreement based on health law in Indonesia with several problem formulations as follows especially about the regulation and legal consequence of the honesty principle in the therapeutic

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agreement. In connection with the idea of this study, it is known that previous studies discussed the following: analysis of legal protection for patients in therapeutic transactions[6]; the perspective of legal protection for patients, and liability for violations of therapeutic agreements based on civil law[7]; the legal relationship between doctors and patients as well as doctors' responsibilities in providing health services[8]; and legal protection for doctors in providing medical services[9].

Methods

The research method uses normative juridical research. Normative Juridical Research is research that examines legal products.[10] Legal materials include laws and regulations, books, journals, and other legal documents.[11] The approach method used includes: statute-approach, namely the approach method using analysis of statutory regulations.[12]; conceptual approach, which is an approach method by examining and understanding legal concepts related to the problem.[13]

Results and Discussion

Legal Basis for Implementation of Honesty Principles in Therapeutic Agreements Based on Health Law in Indonesia

Therapeutic agreements are agreements that arise because of an agreement in the field of health services between doctors and health workers and patients. Patient consent in administering medical action by a doctor can be done in writing or orally. Therapeutic Agreement is closely related to Informed Consent. Informed Consent is the consent given by the patient or the closest family after receiving a complete explanation of the medical or dental action to be performed on the patient.14 The first stage in the informed consent process is a discussion between the doctor and the patient to convey medical information regarding a medical procedure/research.

A therapeutic agreement is an agreement made between a patient and a doctor which creates an obligation for the doctor to try to make maximum efforts to cure the patient following the agreement made between the two and the obligation for the patient to pay for the cost of healing.15,16 Doctors will certainly pay attention to the health information submitted by the patient so that the diagnosis is correct. With honest communication between doctors and patients, it will lead to mutual trust. Therefore, in a therapeutic agreement, patients and doctors must be able to provide honest information to each other to create good cooperation and achieve the goals of health services. Juridically, the application of The Honesty Principle in an agreement is part of one's good faith in agreeing. In essence, good faith must cover all stages of the agreement, which begins with honesty when agreeing.17 This is as regulated in Article 1338(3) of the Civil Code which states that the agreement must be carried out in good faith.

Although the term therapeutic agreement is not known in the Civil Code, the Therapeutic Agreement cannot be separated from the provisions of the Civil Code (Agreement Law). Article 1319 of the Civil Code states that all agreements that have a special name or are not known by a certain name, then the agreement is subject to general regulations regarding the engagement in general. Besides, this therapeutic agreement was also born because of the principle of freedom of contract as stated in Article 1338 of the Civil Code. Therefore, the validity of the Therapeutic agreement also depends on the fulfillment of the conditions stated in Article 1320 of the Indonesian Criminal Code which requires 4 things, namely:1) Agreement;2) Proficiency;3) certain objects; and 4) Causa Halal. The Honesty Principleis part of the legality of an agreement as stated in Article 1321 of the Civil Code which states that a legal agreement must be free from error, coercion, or fraud. Besides that, Therapeutic Agreements which have a more specific nature (in the field of Health) of course have their own more specific and detailed rules in the realm of Health Law.

Some of the legal principles underlying the therapeutic agreement, namely: the principle of legality, the principle of balance, the principle of punctuality, the principle of good faith, the principle of honesty, the principle of prudence, and the principle of openness.18 Legal principles are meta-norms / rules which in essence are also rules of behavior. However, it is hoped that these principles can be implemented in the laws and regulations so that they can be directly applied by the community.

The honesty principle is the principle underlying the delivery of correct information, both by patients and doctors and health workers in communicating so that

it can help the patient's healing process. Obtaining the correctness of this information is a human right. The doctor and patient relationship in a therapeutic agreement is based on two kinds of human rights, namely the right to self-determination and the right to information.[19] Therefore, The Honesty Principle is very important to achieve a prosperous life for the parties, both patients and doctors. Especially during the Covid-19 period, everyone, both patients and doctors and health workers, both have the same human right to life as stated in Article 3 of the Declaration of Human Rights by the United Nations, namely "one has the right to life and security of person".[20]. Several rules that implement The Honesty Principle in the Therapeutic Agreement, both explicitly and implicitly, are:

1. Law Number 36 of 2009 concerning Health

This law shows that everyone has the right to balanced and responsible health information (Article 7). This information also means that everyone has the right to obtain information about their health data, including the actions and treatments they have or will receive (Article 8). Article 56 stipulates that every person has the right to accept or reject help actions after receiving information and understanding information about the action completely. What is of concern is Article 56 which allows people not to have the right to refuse medical assistance, that is, for people with diseases whose diseases are quickly transmitted to the wider community.

2. Law Number 29 of 2004 concerning Medical Practice

This law states that actions that doctors take against patients must obtain the consent of the patient (Article 45). This consent is given after the patient has received a complete explanation from the doctor. Of course, in this case, the doctor has the right to obtain complete and honest information from the patient or his family (Article 50(c)). This is balanced with the provision of Article 53(a) which stipulates that Patients also must provide complete and honest information about their health problems. Furthermore, patients receiving services in medical practice have the right to receive a complete explanation of medical procedures (Article 52(a)).

3. Regulation of the Minister of Health of the Republic of Indonesia Number 4 of 2018 concerning Hospital Obligations and Patient Obligations.

The Honesty Principlein the Terapeuitk agreement is also stated in Article 26 which states that patients are obliged to provide honest, complete and accurate information following their abilities and knowledge.

4. Regulation of the Minister of Health Number:290/ Menkes/Per/III/2008 concerning Approval of Medical Actions

This regulation is an implementation of Article 45 of the Medical Practice Law. However, in this regulation, there are no the honesty principle in the approval of medical action. However, all medical procedures must have the patient's consent after the patient is adequately informed.

5. Circular of the Director-General of Medical Services Number: YM.02.04.3.5.2504

This Circular Letter states that doctors have the right to complete and honest information from patients or their families. Vice versa, doctors also have the obligation to provide sufficient information about the need for medical action and the risks that may occur.

6. Law Number 4 of 1984 concerning Outbreaks of Infectious Diseases

This law discusses infectious disease outbreaks as is currently happening. The Covid-19 case is an outbreak of infectious disease as referred to in Article 1 letter a. This is because Covid-19 is a contagious disease where the number of sufferers has significantly increased beyond the usual conditions at certain times and regions. Ministerial Regulation Number 40 of 1991 is an implementing rule of this Law that regulates that everyone participates in the implementation of epidemic prevention efforts (Article 21). This participation can be carried out in one way, namely by providing information on the existence of sufferers or suspected sufferers of plague (Article 22) which of course this information must be conveyed honestly for the benefit of the wider community

Based on some of the rules and descriptions above, it is clear that the application of The Honesty Principle in the world of health is highly respected. Although there is

no clear provision that applies The Honesty Principle in a therapeutic agreement has been stipulated as the rights and obligations of each party when making a therapeutic agreement. The Honesty Principle is used as the initial foundation for enforcing the correct diagnosis so that the objectives of the Therapeutic agreement are achieved, namely maximum health services and patient healing and the safety of health workers in providing health services.

Legal Consequences of Fulfillment of Honesty Principles in Therapeutic Agreements

The Honesty Principleis the basis for conveying correct information from patients or doctors and health workers in communicating so that the expected recovery of patients can be realized. The Honesty Principlein the therapeutic agreement will be more inclined towards the subjective terms of the agreement stipulated in Article 1320(1,2) of the Civil Code, meaning that the agreement has a defect of will. This is because The Honesty Principlein a therapeutic agreement is information provided by patients to doctors or health workers, or vice versa. When from the beginning there has been no honesty conveyed by the parties in the therapeutic agreement, the subjective conditions have been violated and can cause the agreement to be canceled. Providing information that is not following the existing facts can be considered as fraud or an act of deception. Article 1328 of the Civil Code has stated that fraud is the reason for the cancellation of the agreement.

In the case of fraud in the making of an agreement, it means the party who states according to his will, but the will that is conveyed is something that is not honest and is deliberately directed to something contrary to the actual situation. The element of deception is not just a statement that is lying, but there is a series of lies, a series of untrue stories, and every act/attitude that is deceptive. The fraudulent activity is the name of the party listed in the therapeutic agreement.

The result of the cancellation of the agreement is the return to its original position as it was before the agreement.[21] In a therapeutic agreement, a cancellation can only be done before medical action is taken and the legal consequences that arise will be the responsibility of the party canceling. As with agreements in general, when there is a cancellation of the agreement, the party that has already received achievements from the other party is obliged to return it. The return can be in the form of compensation which is the right of the injured party. The compensation regulated in Article 1239 of the Civil Code consists of compensation for expenses, losses, and interest

The absence of The Honesty Principle in the conditions of the Covid-19 pandemic is included in actions that prevent the implementation of the outbreak response. When there is a patient who is dishonest in providing information to doctors and health workers regarding his travel history, he has fulfilled Article 14(1) of Law Number 4 of 1984 concerning Communicable Disease Outbreaks, for which he can be subject to a maximum imprisonment of 1 year and/or a maximum fine of IDR 1,000,000. Based on this explanation, it can be seen that the granting of approval for medical action or a therapeutic agreement does not eliminate legal liability if there is evidence of negligence in taking medical action which causes the patient to feel disadvantaged.

Conclusion

The legal basis for applying The Honesty Principle in therapeutic agreements generally refers to the general rules of contract law in the Civil Code and several further provisions which regulate in detail the matters that underlie the therapeutic agreement. The absence of The Honesty Principle in a therapeutic agreement is included in an agreement with a defect of will that can be civically canceled and compensation is requested. During an outbreak such as Covid-19, a dishonest patient can be subject to a maximum imprisonment of 1 year and/or a maximum fine of Rp.1,000,000, -.

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Study of Lipid Profiles levels in Iraqi children with GH Deficiency Before and after 6 Months GH Replacement Therapy

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Abstract

This present study aimed to quantify serum lipid levels [Total cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein –cholesterol (LDL-c), Very Low Density Lipoprotein –cholesterol(VLDL-c), High Density Lipoprotein–cholesterol (HDL–c)] quantified before and after 6 months of treatment in Iraqi children with HGH deficiency

This study was conducted at the National Center for Diabetes Treatment and Research of the Mustansiriya University in Baghdad and for the period from, (October 2016 to July 2017). The study included 200 samples divided into two groups. The patient group included 100 children with short stature including 52 males and 48 females with their age ranged from 5 to 12 years . For comparison, 100 samples (50 females and 50 males) of healthy children corresponding to patients with sex, age were selected as control group.

The results of the study showed Serum (TC), (TG), (LDL-C) and (VLDL) levels had a highly significantly increased in children GHD group when compared with controls group (P<0.001).

Regarding serum (HDL-C) levels there was a highly significantly decreased in children GHD group when compared with healthy children (P<0.001) before treatment, and after 6 months of treatment a decrease in fat levels was observed in patients [(TC), (TG) and (LDL-C), (VLDL), very significantly (p <0.001), while their HDL-C level was higher than before treatment .

To distinguish between GHD patients and the control group, the Receiver Operator Curve (ROC) analysis showed a descending order of significance for the indicators that showed significant differences before treatment. The serum level of LDL-C was 0.77, followed by (TG) (0.637) ,(VLDL-C) (0.637),(TC) (0.626), and (HDL-C) (0.614) respectively [this means that all variables managed to occupy a significant region of ROC

It was concluded from this study that there was a significant increase (P <0.001) in serum TG, TC, LDL-C and VLDL-C, while the level of HDL-C in the blood decreased significantly in GHD children compared with healthy children. After 6 months of treatment rhGH has improved the lipid profile of children with growth hormone deficiency.

Keywords: *Growth hormone deficiency , GH therapy, lipid profile.*

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Introduction

Human Growth hormone (hGH) A polypeptide protein hormone on a single base of protein containing two sulfuric bonds (disulfide bonds -S-S) the hormone is

produced and stored and then secreted by somatotropin cells in the frontal lobe of the pituitary gland [1] , GH necessary for normal growth during childhood and adolescence and influences bone mineralization and body composition in children and adults [2] . Growth hormone helps Consumption of stored fat in the body as a source of energy because The amount of carbohydrates stored in the body is small and insufficient to produce the necessary energy, so the hormone helps prevent the destruction of protein for use as an energy source[3, 4] . Cholesterol (TC), a waxy substance produced by the liver and found in certain foods, is needed to make vitamin D and some hormones, build cell walls, and create bile salts that help to digest fat[5] . Triglycerides (TG) are another class of fat or lipids, a mixture of fatty acids and glycerol circulated in the bloodstream. Triglycerides (TG) are necessary for life itself . they contain chains of high energy fatty acids providing much of the fuel needed for body cells to function . thus the cells remove TG from the VLDLs only when they need them as energy source , People with high triglycerides (TG) often have a high total cholesterol (TC) ,high low density lipoprotein (LDL-c) (bad) cholesterol and a low High Density Lipoprotein–cholesterol(HDL-c) (good) cholesterol level[6]. HDL is a class of lipoproteins produced by the liver and intestines , Sometimes it is referred to as 'good cholesterol' lipoprotein The High density lipoprotein level it normal or decrease in severe hypothyroidism because of a decrease in activity of cholesteryl-ester transfer protein (CETP) and hepatic lipase (HL),which are enzymes regulated by thyroid hormones[7].Very Low Density Lipoprotein –cholesterol (VLDL) particles have a diameter of 30-80 nm. VLDL transports endogenous products while chylomicrone transports exogenous (dietary) products. low density lipoprotein (LDL-C) is a type of lipoprotein that carries cholesterol from the liver to cells of the body. Sometimes it is referred to as bad cholesterol lipoprotein [8]

Materials and Methods

This study was conducted at the National Center for Diabetes Treatment and Research of the Mustansiriya University in Baghdad and for the period from October 2016 - July 2017)

The study included (200) samples divided into two groups. The patient group included 100 samples (52 males and 48 females) of children with growth hormone deficiency Informed consent was obtained from parents, 100 samples (50 females and 50 males) of healthy children corresponding to patients with sex, age, nutritional behavior and geographical area were selected as control group Informed consent was obtained from parents ,The study also included age groups for both sexes between (5-12) years and for both infected samples and control group. Serum (TC) , (TG) , (HDL-C), (LDL-C) and (VLDL-C) at baseline and after 6 months of treatment were measured.

Samples collection: Ten milliliters (10 ml) of peripheral venous blood was aspirated from each patient and control subject at time (8.30 – 10.30 a.m.) in the fasting state, and after an overnight's fast, venous blood samples was collected in plain tubes for serum collection.

Lipid Profile the [TC, TG, HDL-C, LDL-C and VLDL-C] were measured by enzymatic colorimetric method . (LDL-C), and (VLDL-C) were calculated according to the (Friedewald formula) [9].

Statistical Analysis

Statistical analysis done according to [10]

Results

METABOLISM OF LIPID

Table (1) summarizes the differences between healthy and HGH deficient children. The result indicates a significant increase ($p < 0.0001$) in serum(TG, TC, LDL-C and VLDL-C). (163.7± 15.2,90.9 ± 15.4,100.2 ± 7.8 and18.2 ± 3.1 mg/dl)respectively compared to healthy controls (156.8± 13.2,82.3 ± 16.9 ,92.4 ± 6 and16.5 ± 3.4 mg/dl)respectively compared to healthy controls (156.8± 13.2,82.3 ± 16.9 ,92.4 ± 6 and16.5 ± 3.4 mg/dl)respectively.

Regarding serum high density lipoprotein (HDL-C) levels there was a highly significantly decreased ($P<0.001$) in children GHD group (45.4 ± 5.3mg/dl) when compared with controls (48 ± 4.8 mg/dl) before treatment.

A 6 months of treatment with growth hormone has revealed a dramatic increase in levels of serum (HDL-C) (47.4 ± 4.4 mg/dl) in children with growth hormone deficiency. As presented in table (2), the effect of GHD

on increase the serum concentration of Serum HDL was also evaluated as a strong effect (Cohen's d = 1.33) , so the treatment with growth hormone has contributed to improved levels of serum HDL compared to what they were before treatment

To distinguish between GHD patients and the control group, the Receiver Operator Curve (ROC) analysis showed a descending order of significance for the indicators that showed significant differences before treatment. The serum level of LDL-C was 0.77, followed by (TG) (0.637) , (VLDL-C) (0.637),(TC) (0.626), and (HDL-C) (0.614) respectively [this means that all variables managed to occupy a significant region of ROC as shown in the table (3) and figure (1)below .

Table 1: parameters differences between growth hormone deficiency patients and Controls group.

HDL-high density lipoprotein, LDL-Low density lipoprotein, VLDL-Very Low density lipoprotein

Parameters	Area ROC	P value
Serum LDL (mg/dl)-before treatment	0.778	<0.001
Serum Triglycerides (mg/dl)-before treatment	0.637	<0.001
Serum VLDL (mg/dl)-before treatment	0.637	<0.001
Serum Total Cholesterol (mg/dl)-before treatment	0.626	0.002
Serum HDL (mg/dl)-before treatment	0.614	0.005

Table 2: parameters differences between growth hormone deficiency patients befor and after

Parameters	before treatment Mean ± SD	after treatment Mean ± SD	changes after 6 months of treatment Mean ± SD	Cohen's d	P Value
Serum total Cholesterol (mg/dl)	163.7 ± 15.2	160.1 ± 13.7	3.6 ± 1.5	-1.28	p<0.001
Serum Triglycerides (mg/dl)	90.9 ± 15.4	87.4 ± 14.9	3.5 ± 0.5	-1.94	p<0.001
Serum HDL (mg/dl)	45.4 ± 5.3	47.4 ± 4.4	-2 ±0.9	1.33	p<0.001
Serum LDL (mg/dl)	100.2 ± 7.8	95.2 ± 6.8	5 ± 1	-1.56	p<0.001
Serum VLDL (mg/dl)	18.2 ± 3.1	17.5 ± 3	0.7 ± 0.1	-1.75	p<0.001

Table(3): ROC area for selected measurements at baseline (before treatment) when used as test to diagnose GH deficiency differentiating it from healthy control. (Larger values of the measurement is associated with higher probability for having GH deficiency).

Parameters	Control group Mean ± SD	Patients group Mean ± SD	P Value
Serum total Cholesterol (mg/dl)	156.8± 13.2	163.7± 15.2	p<0.001
Serum Triglycerides (mg/dl)	82.3 ± 16.9	90.9 ± 15.4	p<0.001
Serum HDL (mg/dl)	48 ± 4.8	45.4 ± 5.3	p<0.001
Serum LDL (mg/dl)	92.4 ± 6	100.2 ± 7.8	p<0.001
Serum VLDL (mg/dl)	16.5 ± 3.4	18.2 ± 3.1	p<0.001

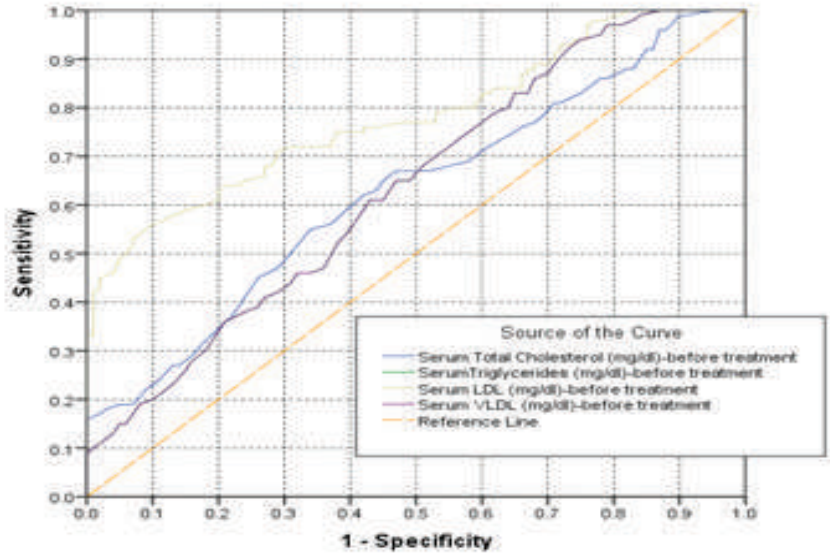


Figure 1. ROC Curve Showing the Trade-off between sensitivity and specificity for LDL, TG, VLDL, HDL and Cholesterol at baseline (before treatment) when used as test to diagnose GH deficiency differentiating it from healthy control

Discussion

Growth hormone deficiency (GHD) is a endocrine disease which could affected onto individual's life with childhood, adolescence while adulthood [11] . (GH) could boosts linear growth, quicken protein synthesis and catalyze bone growth; in addition to , it has determined effects onto the body formation, bone density, muscle strength and blood lipid level [12]. Growth hormone deficiency has a global incidence of 1: 4000 - 1: 10,000 worldwide [13]. HGH helps to consume fat stored in the body as an energy source. The amount of carbohydrates stored in the body is small and insufficient to produce the necessary energy, so this hormone helps prevent the destruction of protein to be used as an energy source[14] . Growth hormone deficiency (GHD) is a pathological condition it is caused by a lack of pituitary secretions where the body does not produce sufficient growth hormone. The most common cause of growth hormone

deficiency is pituitary tumors [15]. The origin of the disease may be fungal or acquired. For acquired causes: (15% unknown, 50% higher tumors of the pituitary gland, 5% of inflammatory wounds)[16] .

Previous studies suggest that the development of cardiovascular disease (CVD) and atherosclerosis begins in early childhood [17] . Children with growth hormone deficiency (GHD) patients are at risk of developing heart disease and atherosclerosis, including blood lipid disorder, impaired heart function and abnormalities in body composition due to their growth hormone deficiency [18] .

Dyslipidaemia during childhood with adolescence is an strong sign onto atherogenic risk to can contribute in evolution coronary heart disease (CHD) at adulthood[19]. Childhood atherosclerosis cannot be obvious, moreoer, atherosclerosis is a multifactorial disease with its roots during childhood[20] .

RhGH according to the reports in 1985 first prouction released , which able to support the final solution as treatment for all patients with GHD. Since while after that the rhGH has been approved in the rapid clinical application, the therapeutic effects of rhGH were demonstrated in wide way, as well as considered the predominant drug of the treatment GHD[21] , an effect rhGH therapy onto lipid metabolism became a centre from interest during these years [22] .

The results of Lipid profile study agreed with other studies The result indicate the presence of significant increase ($p < 0.001$) in serum TG, TC, LDL-C, and VLDL-C. Serum HDL-C levels were significantly decreased ($p < 0.001$) in GHD patients.

Patients with GHD frequently have an abnormal blood lipid profile which increase in(TC), (TG), (LDL–C) and (VLDL–C),while (HDL–C) showed decrease in patient with GHD compared to control.

where GHD children suffer from metabolic disorder for fats and RhGH therapy can improve blood lipid levels because GH promotes lipolysis regulates the rate of lipolysis in adipocytes by activating adrenergic receptors in adipocytes, thereby reducing the fat content in tissues [23]. GH had a significant effect in reducing fat by increasing blood lipid metabolism [24], and regulating the level of mRNA expression of hepatic LDL-C receptors, enhancing the liver's ability to absorb LDL-C and reducing the rate of LDL-C production [25].

moreover, some studies had observed that improvements at atherogenic danger factors are the poorerest following the withdrawal of GH therapy , which supports the beneficial effect of GH treatment onto cardiovascular danger. at 2015, an European Society of Paediatric Endocrinology emphasized that GH can improve a blood lipid levels with reduce a carotid intimal thickness, as supported by a results of our study . also , these results suggest that rhGH therapy has preventive monuments onto cardiovascular features at GHD children [26].

Our results agreed with a previous study conducted by [26] which noted this Children with untreated GHD carries a range of early cardiovascular risk factors, RhGH alternative therapy in GHD children can improve the image of blood lipids .

It was concluded from this study that there was a significant increase ($P < 0.001$) in serum TG, TC, LDL-C and VLDL-C, while the level of HDL-C in the blood decreased significantly in GHD children compared with healthy children. but After 6 months of treatment rhGH has improved the lipid profile of children with growth hormone deficiency.

In conclusion, GHD children develop lipid metabolic disorder, and rhGH therapy can improve the blood lipid levels.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq**.**

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The Association between The Thickness of the Endometrium at the Day of Human Chorionic Gonadotrophin Injection and the Outcome Intracytoplasmic Sperm Injection

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Abstract

Background: Clinically during ICSI, controlled ovarian stimulation (COS) is a famous way to increase the number of mature oocytes picked up. This method must be asseeed with a substantial danger of ovarian hyper stimulation syndrome (OHSS), which occurred if follicular induction is very strong. Along with multifollicular growth, COS is also resulting in increased serum concentration of estradiol (E2) that in turn may has effect on the receptivity of the endometrium .The high serum E2 concentration through COS may resulting either with an increased chance of pregnancy or an impaired reproductive score secondary to change in endometrial receptivity. **Aim of the study**: to assess the impact of thickness of the endometrium at the day of hCG administration on the winning of intracytoplasmic sperm injection (ICSI) cycles. **Patients and method**: A retrospective study in which 235 women who were undergo one time of ICSI cycle were evaluated in this retrospective study, All patients were treated with gonadotrophin agonist either short or long protocol. The patients were according to endometrial thickness they were categorized into three groups I (<7mm), II (7-14mm), and group III (>14mm). **Results**: the rate of clinical pregnancy was increased in-group II (7-14mm) than other two groups depending on endometrial thickness measurement.

Conclusion: endometrial thickness 7-14mm is associated with higher rate of clinical pregnancy.

Keyword: Endometrial thickness, *ICIS, controlled ovarian stimulation (COS), IVF*

Introduction

In vitro fertilization and embryo transfer (IVF-ET) is only way of assisted reproductive technique that make the couples to meet their chance of having a baby into a reality.(1)

IVF was firstly used to solve the problem of tubal blockage, however, is now widely used for treatment of other types of infertility including: unexplained infertility, endometriosis, male factor and ovarian dysfunctions (2,3)

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The implantation of an embryo found to be the most important way of the reproductive method. It includes a special biological phenomenon, by which the blastocyst closely attached to the maternal surface of endometrium to make the placenta that will form a connection between the developing baby and the maternal blood flow. (4) A good implantation needs a receptive endometrium, a good embryo at the stage of blastocyst and a rhythmic connection between maternal and fetal tissues. Implantation happened about 9 days after ovulation (6-12) days. (5)

Endometrial receptivity:

Endometrial receptivity defined as a special series of events that make the endometrium accepted to the receiving of an embryo. (6) The thickness of endometrium can be used as an indirect indicator for endometrial receptivity. (7) In sufficient uterine receptivity in IVF

is found to be account for about two third of failure of implantation, while the embryo itself is responsible one third of these failures. (8)

Factors that affect the receptivity of the endometrium:

1- Maternal age:

There is significant decline in human fecundity with increasing maternal age. (9)

2- Hormonal factors:

a- Estrogen and progesterone;

Serum levels of estradiol (E2) have a little value in assessing endometrial maturation, although there is association between endometrial thickness and estradiol levels in both natural and stimulated cycles. (10)

b- Gonadotropin hormones;

The conception rates is found to be increased in previously amenorrheic patient than in patient with normal cycle. This may be due to a beneficial effect of the higher gonadotropin levels on endometrial receptivity, in amenorrheic women (11)

c- GnRH agonist and GnRH antagonist;

The impact of the GnRh-agonist or GnRh-antagonist on corpus luteum or on endometrium and then on endometrial receptivity cannot be ignored because GnRh receptors have been recognized in both compartments. (12)

3-endometrial thickness and pattern:

Transvaginal ultrasound can be used as an alternative method in the assessment of endometrial receptivity. It has been found that the pattern and thickness of endometrium on the day before oocyte pickup may be a marker of the likelihood of having a pregnancy. (13)

4-endometrial volumetry:

A minimum volume of 2ml was needed for a receptive endometrium and no pregnancy occur when endometrial volume measured <1ml. (13)

5-Genetic factors:

There are many genetic factors are responsible for the success or failure of implantation. (14)

The aim of the study

This retrospective study was performed to assess the effect endometrial thickness at the time of hCG injection on ICSI patients who were undergone controlled ovarian stimulation (COS).

Patients and Method

A retrospective study was done in the fertility center of Al-Sader teaching hospital in Al-Najaf city. From February 2018 to February 2019, (235) couples underwent ICSI cycles because of tubal, ovarian, male and unexplained infertility. Exclusion criteria for the study including the presence of known intrauterine anomalies and ovarian stimulation other than GnRH agonist protocol (short & long).

In cycle day 2-3 hormonal assay in form of (FSH, LH, Prolactin,and E2) done along with vaginal ultrasound to detect the antral follicles count and to rule the presence of ovarian cysts or other pelvic pathology and seminal fluid analysis for the partner reviewed then according to above results, stimulation protocol was selected. Patients were screened and were negative for infection with HIV, hepatitis B and C.

Stimulation protocol:

Gonadotrpin releasing hormone (GnRH) agonist used for all women. Two main protocols used in this study;

1- long protocol: for suppression of the pituitary gland, GnRh agonist (decapeptly 3.7mg) was administered on the day of 21 of the menstrual cycle (mid luteal phase) vaginal ultrasonography was performed on day 2 or 3 of the next menstrual cycle to rule out the presence any functional ovarian cyst more than 10mm in size. Gonadotropin stimulation include 1-2 ampoules per day of recombinant FSH (Gonal-F^{R,} 75 UI). The starting dose was individualized according to patient's age, ovarian reserve test tailored according to her response after the first 4-5 days of stimulation.

2- short protocol or flare-up protocol: involves agonist treatment (decapeptyl 0.1mg S.C daily) for 10-14 days started one day before or concurrently with ovarian gonadotropines stimulation and the dose of gonadotropines adjusted as the long protocol(as described above).

For both protocols, follicular development was monitored by vaginal ultrasonography and serum E2 level starting from cycle day 8 or 9 and then every other day. At the day of human chorionic gonadotrophine (hCG) injection, the endometrial thickness was calculated by trans- vaginal ultrasound using mid-sagittal plane through the uterine body

All measurement were done by the same gynecologist.

Endometrial thickness was classified into three groups:-

I -(<7mm), II-(7-14mm), III-(>14mm).

Thirty- four to 36 hours following 10,000 IU of hCG administration, oocyte retrieval was performed under general anesthesia and by guided of transvaginal ultrasound. After aspiration, oocytes screened and injected (ICSI). The fertilization rate calculated as (number of embryos obtained per number of injected oocytes). After transfer embryo of, all women were receive luteal phase support using vaginal pessaries (progesterone 400mg twice a day (Cyclogest ^{R}) Started at day of ovum pick up for 16 days and continued if pregnancy occurred up to 10-12 weeks. Two weeks after embryo transfer, we check the level of βhCG and then after the clinical pregnancy was confirmed two weeks later by sonographic detection of the gestational sac and positive heartbeat. Clinical pregnancy rate calculated as the women with clinical pregnancy per number of women that did embryo transfer.

Embryos were classified according to the number of blastomeres, percentage of fragmentation & blastomere appearance as type 1, 2, 3 or 4.

Regarding the equality of embryos, good quality embryos are those of grade 1 and 2, while bad quality embryos are those of grade 3 and 4.

Statistical Analysis

After data entry the analysis were done by using SPSS (statistical package for social science) version 23. In which we use chi sequare(x^2) for categorical data and independent sample T-test for measurement data. P value <0.005 were considered significant.

Results

Table (1) shows clinical pregnancy rate was higher in group II 70/194(63.9%) than in group III 3/21(14.3%) and group I 1/20 (5%) it was statistically significant (p=0.003)

Table (1): Shows the comparison of the ICSI outcome according to endometrial thickness on the day of hCG injection.

		Endometrial thickness			P value
		<7 (N=20)	7-14 (N=194)	>14 (n=21)	
Clinical pregnancy rate	Negative	19 (95%)	124 (63.9%)	18 (85.7%)	0.003
	positive	1 (5%)	70 (36.1%)	3 (14.3%)	

Table (2) shows demographic characteristic of three groups according to endometrial thickness were no statistically different regarding age, BMI, hormonal assay (FSH,LH,E2 &Prolactin) and period of infertility.

Table (2): Demographic characteristic of women according to endometrial thickness at day of hCG administration.

Characteristic	Endometrial thickness			P value
	<7 Mean±SD	7-14 Mean±SD	>14 Mean±SD	
Age/ years	32±4.2	30±5.9	29.2±6.5	0.852
BMI(kg/m2)	25.5±4.9	27.3±3.1	28±2.4	0.638
FSH(IU/L)	2.5±3.4	4.7±2.5	3.2±2.3	0.2
LH(IU/L)	1.6±0.4	3.1±2.6	1±1.3	0.177
E2(pg/ml)	29±16.9	37.3±18.9	19.4±9	0.092
Prolactin(ng/ml)	21±2.8	25.5±18.5	23.4±8.5	0.911

Table (3): Shows that there is no significant differences found between types of the protocols and the type of infertility with endometrial thickness (P>0.05).

Table (3) the type of protocol and indication of ICSI according to endometrial thickness.

Variable		Endometrial thickness(mm)			P value
		<7	7-14	>14	
Protocol	Long	6	26	5	0.198
	Short	14	168	16	
Indication	Female	7	71	10	0.970
	Male	11	110	10	
	unexplained	2	13	1	

Discussion

Assessment of thickness of endometrium on the day of hCG injection is of great clinical importance. [15] In current study, we evaluate clinical pregnancy rate after classifying the endometrial thickness into three groups. We found that higher pregnancy rate reported in group 7-14mm (36.1%), while those level above 14mm the clinical pregnancy rate was (14.3%) and rapid decrease in clinical pregnancy rate in women with endometrial thickness <7mm (5%) but this difference is statistically significant (p=0.003).

An implantation failure occurs in thin endometrium. This finding is agree with Gonen Y, [13] this may be associated with oxygen tension. When the thickness of

endomertium measured by ultrasound and it is <7mm, the functional layer is thin or absent, and the implanting embryo would be much closer to the spiral arteries and the higher vascular and oxygen concentration. The high oxygen concentration near the basal layer could be detrimental compared with usual low oxygen tension of the surface endometrium. (13)

A limited number of studies have reported a detrimental impact of greatly increased endometrial thickness on pregnancy outcomes.

Weissman et al, found that an endometrial >14mm resulted in significant reduced implantation and pregnancy rate (16). This was supported by the finding of Rashidi et al. (17) Our results were with agreement to these both studies.

Higher pregnancy rate was found in group 7-14mm thickness and this agree with Kuc et al., 2011 stated that endometrial thickness of 12-13mm was associated with highest pregnancy rate (18). Okohue et al., reported the highest pregnancy rate with an endometrial thickness of 7-14mm (19).This also confirmed by Traub et al.,(20) and Chen et al,.(21)

However, the optimum endometrial thickness that predicts successful pregnancy is not definite yet.

Conclusion:

We can conclude that the endometrial thickness of 7-14 mm is associated with a higher pregnancy rate.

Recommendations

We advocate that in each ICSI center the effort should be made to institute protocols that will improve endometrial development as a mean of improving cycle outcome and avoid both thin and thick endometrium. Further larger randomized studies with more cases are needed and for longer duration of follow up to define appropriate protocols in clinical practice of ICSI cycle.

Conflicts of Interest: No

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Effects of Dance and Movement Therapy (DMT) versus Progressive Muscle Relaxation(PMR) on Depression among Elderly Residing in Selected Old Age Homes of Haryana: A Quasi Experimental Study

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Abstract

Depression is a universal health related problem which is increasing the life expectancy worldwide associated with morbidity and disability among elderly people. Elderly age group is a controversial issue as it is a natural process which presents a unique challenge for every individual in society. The objectives of the study were to assess and compare depression among elderly before and after administration of dance and movement therapy and progressive muscle relaxation, to determine the relationship between depression and to find out the association of depression with selected variables. Quantitative research approach, quasi experimental non equivalent control group pretest post test design was used to collect the data. Sixty participants (30 DMT group, 30 PMR group) were enrolled for the study those who fulfilled the inclusion criteria by using purposive sampling technique. Homogeneity was checked by enrolling the participants. Data was collected by using Geriatric Depression Scale (GDS) for assessing depression by interview technique. Analysis was done by applying non parametric test after K-S test to check normality of data. Thus findings of the study showed effectiveness of dance and movement therapy and progressive muscle relaxation on depression as the calculated Friedman test Chi square value (DMT χ^2 =51.44, p=0.00 and PMR χ^2 =52.91, p=0.00) was significant at 0.05 in both groups. There was no any significant difference between DMT and PMR groups in terms of depression. Depression among elderly was found to be statistically significant with duration of stay in old age homes and history of chronic illness i.e (Ψ=5.764, p=0.05) and (И=49.00, p=0.02) in DMT group, and depression among elderly was found to be statistically significant with history of chronic illness i.e (И=49.00, p=0.04) in PMR group. Conclusion: Dance and movement therapy and Progressive muscle relaxation was effective in reducing depression among elderly as there was significant difference within the group.

Keywords: *Depression, Dance and movement therapy, elderly, old age homes*

Introduction

Human beings have no exemption. The past era manifest substantial to increase in the population of elderly i.e a stratified set of medical, social, psychological, financial and ethical problems in elderly.[1] So there are predictable health problems among elderly which change or altered the elderly life and the most universal is depression.[2]

The world population is ageing and by 2025, the world's population is expected to include more than 830 million people at an age of 65 by 2030. With a relatively young population, India is still on the edge to become

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second leading number of elderly individuals in the world.[3, 4]

Depression is a state of low mood and disinclination or aversion to activity that can affect a individual's thoughts, behaviour, feelings, and sense of well-being. [5] The term "depression" can apply to a fleeting temper, or a constant change in temper/mood, a indication, or a disorder. Depression is categorized by constant low mood or sadness and is accompanied by both substantial and psychosomatic symptoms of at least two weeks period and with associated impact on social functioning.[6]

Dance therapy effects changes in feelings, cognition, physical functioning, and behavior.[7] Dance as psychotherapy has been used to recover from psychological and substantial well- being of a person.[8] Poco-Poco is the type of dance therapy which is used as the psychotherapy along with the music to relieve the mental illness.[9]

Progressive muscle relaxation is a psychotherapy that is used for relaxing mind and body by gradually tensing and soothing muscle of body.[10] Person will feels less pressure and have a good physical and emotional health. When the muscle is relaxed, the feelings of warmness and enormity are felt as a result.[11]

Elderly at the peak age feels impassiveness, worthlessness and insecurity, especially when living with these feelings in old age homes. It is very important to understand the factor which affects the quality of life of the elderly persons.[12,13] The quality of life also depends upon emotional interpretation that a person feels about life. Thus the quality of life is progressively more attributed as an estimation that is reliant on the person's prejudice.[14]

Methodology

The study was conducted during the period from October 2017- November 2018 in the state of Haryana, India. A sample of 60 elderly participated in this quasi experimental study with the prior permission from concerned authority of old age homes. Quantitative research approach was considered to be the most appreciated approach to assess the effectiveness of dance and movement therapy and progressive muscle relaxation on depression among elderly with non equivalent control group pre-test post-test design.

Inclusion Criteria: Elderly aged 60 years and above, residing in old age homes, willing to participate in the study, alert, oriented, and comprehend to respond, able to understand and speak Hindi and able to move without walking aids or any physical assistance.

Procedure

Screening was done on day 1with selected variables and by Geriatric depression scale for assessing depression with interview technique. Dance and movement therapy and progressive muscle relaxation was administered to elderly from 2nd day to 8th day as per intervention protocol. DMT contain 3 sessions i.e warm up session of 5 min duration followed by active session that involves all elderly to sit and do the chair step dance together for 20 min, further third session is for cooling or relaxing session for 5 min. Similarly Progressive muscle relaxation also include 3 sessions i.e warm up session that is done for 5 minutes followed by active sessions done for 20 minutes in this muscle are tensed for 5 seconds and relaxed for 20-30 seconds along with deep breathing and cooling session that is done for 5 minutes. Immediately after completion of 7 days intervention post test I was taken (day 8) and then after 1 week of post test I post test II was taken (day 16) for both DMT and PMR group.

Data Analysis

Descriptive statistics: Frequency, percentage distribution was used to describe selected variables, Chi-square was used to assess the homogeneity between two groups

Inferential statistics: Independent Mann-Whitney U test, Repeated measure Wilcoxon signed ranked test, Friedman test.

Results

Homogeneity was checked by χ^2 test was applied to compare the DMT and PMR group with respect to every selected variables. Hence both the group was homogenous except for gender (p=0.01).

Percentage distribution of DMT and PMR in terms of depression among elderly before administration of intervention is shown in Figure 1. In both group DMT

(80%) and PMR (67 %) majority of the elderly were having moderate level of depression.

In terms of Depression although the subjects in DMT group reported higher depression as compared PMR group, no significant difference was found between the group as calculated И value is (432) for depression among elderly and Z value 0.27 (p=0.78).

Similarly, И value in post test I and II was 434.5 and 352.5 respectively and Z value in post test I and II was -0.29 post test I (p=0.77) and -1.59 (p= 0.11) respectively.

Table 1: Mean rank, Sum of Rank, U and Z value of Depression after Administration of Dance and Movement Therapy and Progressive Muscle Relaxation among Elderly between DMT and PMR group

N=60

Observation	Group	Mean Rank	Sum of Rank	Mann-Whitney U Test (И)	Z value	p value
Depression (Pretest)	DMT group (n = 30)	31.10	933.00	432	0.27	0.78NS
	PMR group (n = 30)	29.90	897.00			
Depression (Post test I)	DMT group (n=30)	29.98	899.50	434.5	-0.29	0.77NS
	PMR group (n=30)	31.02	930.50			
Depression (Post test II)	DMT group (n=30)	33.77	1013.00	352.5	-1.59	0.11NS
	PMR group (n=30)	27.33	817.00			

NS -Not significant(p>0.05)

In DMT group the mean score of depression in pre test, post test I and post test II was 3.00, 1.38 and 1.62 respectively. Where the χ^2 (51.44) (p=0.00). Whereas in PMR group the mean score of depression in pre test, post test I and post test II scores was 3.00, 1.42 and 1.58 respectively. Where χ^2 (52.91) (p= 0.00).

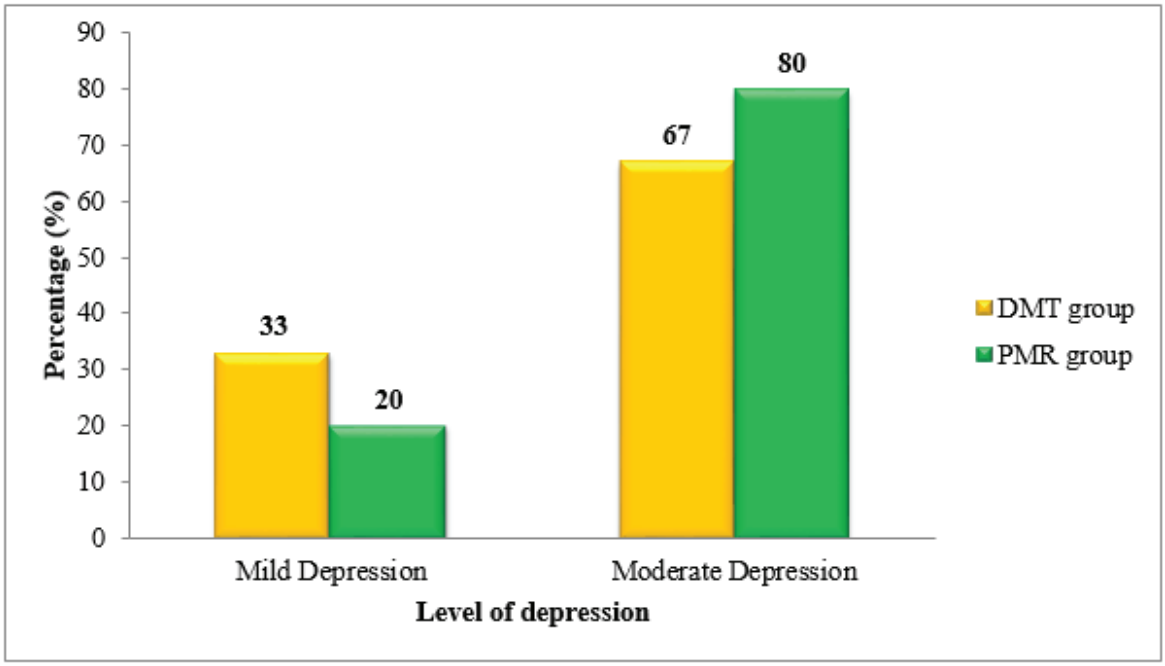


Figure 1 : Bar Diagram Showing Distribution of elderly in Terms of Level of Depression before Administration of Dance and Movement Therapy and Progressive Muscle Relaxation in DMT and PMR Group

Table 2: Friedman Test showing Mean Rank, Percentiles and Chi square value of Depression within the Group of DMT and PMR Groups

N= 60

Variable Depression	Test	Percentiles 25th	50th	75th	Mean Rank	Chi-Square	df	p value
DMT Group (n=30)	Pre test	8.00	10.00	10.25	3.00	51.44	2	0.00*
	Post test I	3.75	4.00	4.00	1.38			
	Post test II	4.00	4.00	4.00	1.62			
PMR Group (n=30)	Pre test	9.00	9.00	9.00	3.00	52.91	2	0.00*
	Post test I	4.00	4.00	4.00	1.42			
	Post test II	4.00	4.00	4.25	1.58			

χ^2 (2)=5.99 *- Significant ($p \leq 0.05$)

The results depicts the significant difference between pre test, post test I and Post test II i.e (p=0.00) in DMT group. This concludes that at the time of pre test participants had higher depression than post test I and post test II in DMT group and in post test I the participants had higher depression than post test II. Whereas in PMR group there was also significant difference between pre test, post test I and post test II i.e (p=0.00) respectively which shows that at time of pre test participants had higher depression than post test I and post test II.

Table 3: Wilcoxon Signed Ranked Test Showing Significant Mean Rank Difference within Groups in Term of depression in DMT and PMR group

N= 60

Group	Category	Z value	p value
DMT group (n=30)	Pre test Vs post test I	-4.81	0.00*
	Pre test Vs post test II	-4.81	0.00*
	Post test I Vs post test II	-2.00	0.04*
PMR group (n=30)	Pre test Vs post test I	-4.81	0.00*
	Pre test Vs post test II	-4.81	0.00*
	Post test I Vs post test II	-0.44	0.65NS

NS -Not significant(p>0.05) *- significant ($p \leq 0.05$)

Association of depression among elderly with selected variables was tested by Kruskal-Wallis (Ψ) and Mann- Whitney U test (И) among DMT and PMR group. Thus the findings revealed that in DMT group in post test I there was no significant association of depression among elderly with age, gender, religion, nature of previous habitat, educational status, marital status, source of income, duration of stay in old age homes, physical activities at present days, except duration of stay in old age homes and history of chronic illness i.e (Ψ =5.764, p=0.05) and (И=49.00, p=0.02) which was significantly associated with depression among elderly.

Further in post test II in PMR group, there was no significant association of depression among elderly with selected characteristic except history of chronic illness (И = 49.00, p=0.04).

Discussion

In the present study, two third of the elderly in DMT group were having moderate depression (67%) whereas in PMR group most of the elderly (80%) were having moderate depression before administration of intervention. The findings of the study is consistent with the study conducted by Deise AAP Oliveira, Lucy Gomes and Rodrigo F Oliveira et al. (2006) on prevalence of depression among elderly population at community centers in Taguatinga, where they found that half of the elderly (50%) were having moderate depression, least number of elderly were having mild depression (20%) and severe depression (14%) respectively.[15] Further these findings were contradictory to the study conducted by Ranjan et al (2013) where they found that 50 (70.42%) were having mild depression and 21(29.58%) had severe depression.[16]

In the present study Friedman test showed that there is a significant difference within the group in pre test, Post test I and Post test II in DMT group in terms of depression ($\chi^2 = 51.44$, p= 0.00). This infers that DMT was significantly effective in reducing depression. These study findings were similar to the study conducted by Vankova H. (2014) to evaluate the effects of dance and movement therapy on depressive symptoms where the result showed that there was significant difference within the group (p=0.00) in terms of depression in experimental group.[17]

In the presents study there was significant difference in terms of depression as calculated Friedman test for depression (F=52.91, p=0.00). It infers that progressive muscle relaxation was significantly effective in reducing depression. These findings are similar to the study conducted by Fong TCT, Cheung IKM and Yip PSF et al. (2016) on short term effects of dance and relaxation where they found that progressive Muscle relaxation was effective in reducing depression and improving quality of life as the calculated (p= < 0.05) was significant at 0.05 level of significance.[18]

In the present study there was a significant association of depression with selected variables after administration of DMT and PMR i.e duration of stay in old age home, and history of chronic illness as checked by using kruskal –wallis, Mann- Whitney U for depression which was significant at 0.05 level of significance. These findings are similar to the study conducted by Thilak SA, Sarada AK (2016) where they found that co-morbidity was associated with depression ($p \leq 0.05$) among elderly.[19]

Conclusion

Dance and movement therapy and Progressive muscle relaxation was effective in reducing level of depression among elderly

Conflict of Interest: NIL

Source of Funding: NIL

Ethical approval: Research ethics committee of Maharishi Markandeshwar (Deemed to be) University Mullana, Ambala (MMDU/IEC/973).

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Assess the Caregiver's Knowledge on the Adverse Effect of Antipsychotics in the Selected Psychiatric unit of South India

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Abstract

Background of the study: Antipsychotic drugs are chemicals that influence the brain and nervous system in various ways, and they are frequently used in the practice of psychiatry to treat a broad range of mental and emotional illness. The use of antipsychotics has reduced the number of patients hospitalized for mental illness up to 50 % in the United States. Researchers worldwide have compared various family interventions to enhance caregiver's knowledge of antipsychotics, its side effects and prevent relapse, which concluded that Psycho educational interventions are more effective in increasing knowledge and reducing stress among the caregivers. Psycho educational intervention is a systematic approach based on patients and family needs. The psycho education mainly emphasizes improving patient and family knowledge about the disease, coping with illness, and managing the adverse drug effects of psychotic drugs. The study aimed to assess the caregiver's level of knowledge on the adverse effects of antipsychotics and its association with the selected demographic variables

Methods: A descriptive survey design was used in this study with the one hundred study participants selected using a purposive sampling technique and visiting the tertiary hospital of Mangaluru, south India. A Self-structured knowledge questionnaire was administered to the study participants.

Results: The caregiver's knowledge of the adverse effects of antipsychotics were assessed using descriptive statistics. The results highlighted that more than half of the caregivers (67%) had inadequate knowledge of the adverse effects and management of antipsychotics, while 33% of them had adequate knowledge.

Keywords: *Schizophrenia, Caregivers, knowledge, antipsychotics, adverse effects.*

Introduction

In psychotic disorders, psychopharmacology is the "Gold Standard." The concept of psychopharmacology is widely used to treat specific psychotic disorders with significant effectiveness[1]. One among them is an antipsychotic drug that has chemicals that affect the brain and nervous system in various ways, and they are frequently used in the treatment of mental and emotional illness.[2] Around 500,000 patients were hospitalized in the United States for mental illness in the mid-1950s, but by 1973 that number had drastically fallen to 250,000, mainly due to psychoactive drugs.[3] Antipsychotics may be useful in relieving positive and negative symptoms. It is observed that antipsychotics improved the quality of life in most of the patients. But not effective in few other patients.[4] However, long term use of these drugs may lead to side effects such as extra pyramidal symptoms and metabolic syndrome.[5]These side effects and adverse reactions to drug therapy impact the lifestyle of the individual and caregivers. Therefore, mentally ill patients' caregivers need to have extensive knowledge of antipsychotics and its management of adverse effects. Existing research evidence suggests that imparting knowledge to the caregivers can reduce stress and

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improve the quality of care provided by them.[4] India, families are the primary caregivers to individuals with mental illness.[5]

However, due to a lack of awareness about treatment for mental illness and its care at home, they cannot support and realize the patient's psychological needs, which can lead to unsatisfactory progress of the illness and added burden to the caregivers.[6] Therefore all caregivers must understand the adverse effects of antipsychotics, precautions, and management while taking care of the mentally ill client.[7] It is the fact that caregivers get burned out, distressed, or burdened because of ignorance of the treatment and ways to manage their maladaptive behavior.[8] Hence imparting knowledge to the caregivers or family members on adverse effects of antipsychotics for mentally ill patients improves their favourable attitude towards psychiatric patients.[9]

In this background, we undertook this study to assess the caregiver's knowledge of the adverse effect of antipsychotics in the Psychiatric unit of 1200 bedded tertiary hospital, Mangaluru, in South India.

Materials and Methods

A descriptive survey design was adopted in this study as a research design. One hundred caregivers were recruited through a purposive sampling technique. However, the caregivers diagnosed with any co morbid disorders were not included in the study. Ethical clearance was obtained from the Institutional Ethics committee (Reference No-NUINS/CON/NU/IEC/2016-17). The necessary socio-demographic information was obtained from one hundred caregivers as per the inclusion criteria for the study. Fourteen item knowledge questionnaires were used to assess the caregiver's knowledge of antipsychotics treatment, management, adverse effects with the option of Yes / No/I do not know.

The knowledge questionnaire was validated by five experts from the field of psychiatry and pharmacist. The reliability of Chronbach's correlation and coefficient was 0.768(r= 0.768), which indicated that the tool is reliable. The level of knowledge was graded as inadequate knowledge (1-14) and adequate knowledge (15-28). The minimum score given is 1, and the maximum score is 28. The pilot study was conducted among ten caregivers of patients who have possessed similar characteristics of the main study subjects. The pilot study report showed that the research methodology, data collection instruments adopted in the study was feasible to conduct further research.

The primary study data were collected by obtaining written permission from the Medical Superintendent of the territory Hospital, Mangaluru, South India. The investigator-initiated the data collection by a self-introduction to the study participants. Study participants were explained about the objectives and purpose of the study, and informed consent was obtained. Confidentiality was assured before administrating the research tool. Data were analyzed using descriptive and inferential statistics. The collected information was summarized by using descriptive statistics.

Results

Section I: Socio-Demographic profile of the Caregivers

Table 1 -Distribution of demographic characteristics of the caregivers

n=100

Demographic characteristics of the caregiver		Frequency (F)
Age	18-30	7
	31-40	35
	41-50	58
Gender	Male	49
	Female	51

Cont... **Table 1 -Distribution of demographic characteristics of the caregivers**

n=100

Education	Primary	41
	Higher primary	21
	Graduate	24
	Postgraduate	7
	Professionals	4
	Others	3
Relationship with the patient	Father	10
	Mother	23
	Siblings	28
	Wife	10
	Husband	8
	Others	21
Number of years stayed with the patient	Less than 10	20
	11-20	21
	More than 20	59
Concern towards patient and illness	Concerned	94
	Not concerned	6
On regular treatment	Yes	90
	No	10
History of relapse	Yes	49
	No	69

Table1 shows the distribution of demographic characteristics of the caregivers. The majority were females and between the age group 41- 50 years (51% and 58 % respectively). Most of the caregivers had a primary education (41%). Among 100 caregivers, 33 were parents, 28 were siblings, 18 were spouse, and 21 were having other blood relationships with the patient. Most of the caregivers stayed for more than twenty years with the patient (59 %). The majority of caregivers showed concern towards the patient, i.e. 94 caregivers. The caregivers reported that 90% of the patients were on regular treatment, and 49 % had a relapse history.

Section II: Caregiver's knowledge of adverse effects of antipsychotics.

n=100

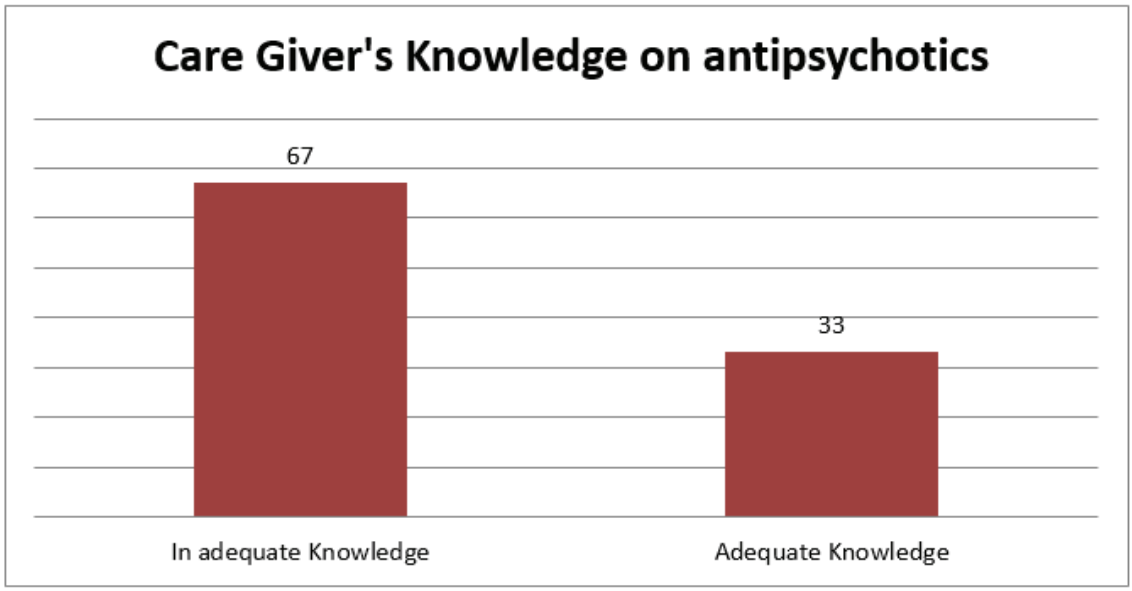


Figure 1- Care givers knowledge on adverse effects of antipsychotics

Figure 1.shows the level of knowledge on adverse effects of antipsychotic drugs among one hundred caregivers of the psychiatric patients who have visited the psychiatric unit of a tertiary hospital, Mangaluru, South India. Out of one hundred subjects, 67 of them had poor knowledge, and 33 of them had good knowledge of antipsychotics.

Discussion

The present study findings showed that the caregivers' age varies from 18 to 50 years, and most of them were above 31 years (93%). Moreover, 51% of subjects were Female. The majority of the study participants had a Family income of less than 5000 INR per month (46%). The present studies also highlighted that the majority of them were unemployed and had an education below high school, which was 58% and 62%, respectively.

The findings of the present study are in congruence with the research study conducted by. Manipal University on practice knowledge on atypical antipsychotic Drugs among Caregivers of Mentally ill Patients[9]. The study highlighted that the subjects' age was 21 to 50 years, and most of them were above 31 years of age, i.e., 76%. Male and female participants were equal in number, which was 50% in each. Also, the majority of the subjects had education below the secondary level. The majority of the finding is congruent to the present study because of the similar geographical area, which is in the Dakshina Kannada district. Both studies are done in a similar setting, the psychiatric unit with the same sample size and data collection method. However, this study findings were contrary to the subjects' occupation status, where most of the subjects were employed (83%)[9]. The occupational status is contrary to the present study finding since most of the participants were females from rural areas and homemakers. This given results t emphasized that most females from the rural population need empowerment with education and occupation.

The present study results show that most caregivers (67%) have inadequate knowledge of antipsychotics, and 33% have adequate knowledge. This finding contradicts the study conducted by waghela et.al [9] 45% of them had good knowledge, 45% had an average, and 6% had poor antipsychotics knowledge. However, the study's finding difference was noted since most of the study participants of the present study had primary education

and belonged to the rural community where exposure to psycho education is comparatively less because of stigma and ignorance regarding mental illness. There is a considerable gap between caregiver's knowledge of psychiatric treatment. Hence, immediate psychiatric treatment should modify their treatment protocol, which included an emphasis on the caregivers' physical and psychological well-being.

There was no significant association found between caregivers' knowledge and selected demographic variable in the present study. This study finding contradicts the study conducted by Waghela et.al[9] were the variables previous exposure to atypical antipsychotics, patient's diagnosis, age, education and occupation are associated with caregivers' knowledge at 0.05 levels of significance[7].However, the study's results difference probably because of the small sample size, and the majority of the participants are from the rural community. Also, there was no equal distribution of gender among the study participants. Furthermore, researchers can focus on the comparison of the knowledge level between different types of antipsychotics. It helps them to manage the symptoms.

Conclusion

Family/caregivers play a significant role in the care of the mentally ill individual. Understanding the patient's disease condition and the medication or treatment used to alleviate the symptoms reduces the caregivers' burden. It is essential to prevent relapse and manage the patient at home vigilantly and affectionately, reducing caregivers' burden. Imparting knowledge to the caregivers with the help of psycho education or any other medium can cause an increasing change in the attitude and quality of care among the caregivers. However, the rural population must be targeted to give health education on mental illness and reduce stigma.

Conflict of Interest: Nil

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The Analysis Effect Prenatal Yoga and the Expression gen MRHR-CRHR1 and mRNA FKBP5 with Real Time-PCR

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Abstract

Background: Stress triggers the release of corticotrophic release factor (CRF) from the hypothalamus which in turn stimulates the anterior pituitary to release adrenocorticotrophic hormone (ACTH).

Methods: The design of this study is a quasi-experimental approach / quasi experimental and one group design pre and post test design with group control. The sample in this study amounted to 24 respondents

Results: Based on the results of the study showed that the sample LA24 (7,935) was the highest sample fold fold change and the lowest in the sample LA15 (6,194). Whereas in the post test results it was found that the LB18 sample (10,580) was the highest sample in fold change and the lowest was in the LB15 sample (8,472).

Conclusion: There is a significant relationship / correlation between CRHR1 levels and FKBP5 levels ($p = 0.001 <\propto$). This is indicated by the high / strong correlation coefficient value which is 0.846. A positive value at 0.846 indicates that there is a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in CRHR1 levels and will further reduce the effect of FKBP5 levels in third trimester pregnant women.

Keywords: *prenatal yoga, CRHR1 gene, FKBP5 gene , real time-PCR*

Introduction

Pregnancy is a natural process, because it is natural for a woman to become pregnant and give birth, this will be a new experience for newly married women[1]. Pregnancy is a period when biomechanical and physiological changes occur quickly when the body adapts to support a developing fetus. Every woman experiences pregnancy with different experiences and with various physical and psychological symptoms that are interrelated[2].

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Mental disorders afflict about 450 million people worldwide Among mental disorders anxiety has as much as 31.2% in the general population: 36.4% for women and 25.4% for men. explained in his study that 54% of women reported anxiety in pregnancy. Anxiety in pregnancy not only affects the health of pregnant women but also affects the outcome of labor such as premature labor, prolonged labor, cesarean delivery, low birth weight, revealed the prevalence of pregnancy anxiety varies in different pregnancy trimesters with high levels in the first trimester and third trimester[3,4].

Stress is responded by activating the cardiorespiratory system, the locus ceruleus system (LC / norepinephrin (NE), the metabolic system and the HPA axis. hypothalamus-paryutary-adrenal Axis

(LHPA axis), then stimulates the hypothalamus and causes secretion of the hormone corticotrophin relesing hormone (CRH), stimulates the hypothalamus for ACTH secretion, increased ACTH secretion, causes increased secretion, cortisol. The hormone is released to maintain homeostasis in dealing with stress, both physical and psychological[5,6,7] Cortisol is assumed to be a stress biomarker, representing HPA system activity. Cortisol is a variable cause of stress conditions and will ultimately have a negative effect on immune function[8,9,10]..

Stress triggers the release of corticotrophic release factor (CRF) from the hypothalamus which in turn stimulates the anterior pituitary to release adrenocorticotrophic hormone (ACTH). ACTH then binds and stimulates the adrenal cortex to release glucocorticoids (cortisol in humans, corticosterone in animals)[11,12,13]. Glucocorticoids act on mineralocorticoid receptors (MR) and glucocorticoid receptors (GR) located in several brain regions such as the hypothalamus and hippocampus to initiate negative feedback which weakens further release. Glucocorticoids can also inhibit telomerase activity (TEL) which can cause a reduction in telomere length (TL)[14,15].

The benefits of yoga for pregnancy can be categorized into physical, mental and spiritual benefits, among others, the physical benefits of pregnant yoga through yoga postures (asanas): Practicing good posture, robust and strong throughout pregnancy, Smoothing blood flow, smoothing the supply of oxygen, nutrients and vitamins from food to the fetus, Strengthens the back muscles, makes it stronger to support the burden of pregnancy and the pelvic floor-perineum which functions as a birth muscle, so that it strengthens the burden of pregnancy and supports the bladder and large intestine[16,17].

In this study, researchers focused on the molecular regulatory genes for the GR function, namely the FKBP5 and CRHR1 genes. FKBP5 and CRHR1 are the main proteins that regulate stress responses. Where FK506 binding protein 5 encoded by genes (FKBP5) and G-protein coupled type-I CRH receptors encoded by genes (CRHR1), has been linked to several neuropsychiatric disorders (Mahon.PB, et al., 2013). CRHR1 was chosen in this study for several reasons, where CRH is one of the main stress factors in the CNS. CRHR1 encodes the G-protein coupled type I CRH receptor (CRHR1), a protein that is essential for activation of signal transduction pathways that activate mesolimbic and HPA axis responses to various types of stress. The CRHR1 receptor is very important for determining the initial HPA axis response to stressful events.

Materials and Methods

This study uses quantitative research with quasi experimental / quasi experimental approaches and one group design pre and post test design with group control. The sample in this study amounted to 24 respondents.

Criteria objektif:

a. Type I CRH receptor (CRHR1) gene expression is gene expression measured quantitatively using the real time PCR method which is assessed by the ratio of the CRHR1 gene mRNA to the mRNA of the control gene (housekeeping gene)

b. FK506-binding protein 51 (FKBP5) gene expression is gene expression that is measured quantitatively using real time PCR method which is assessed by the ratio of mRNA between FKBP5 gene and mRNA from control gene (housekeeping gene).

Examination ekspresi with real time-PCR

a. **Examination ekspresi mRNA CRHR1 with real-time PCR**

1) CRHR1 F: ACCTCATCACCGCCTTCATCC and

2) CRHR1R: AGCAGCCCTCGCCAAACAT (Gene bankXM_020113112.1)

3) House keeping gene:

(a) β-actin F: GGAAATCGTGCGTGACATTAAG

(b) R: CCTCTGGACAACGGAACCTCT (gene Bank:

(c) HQ386788.1)

QPCR conditions with a temperature of 95oC for 10 seconds and 60oC for 30 seconds as many as 40 cycles

b. **FKPBP5 mRNA expression check with realtime PCR**

1) FKPB5F: 5′-AAAAGGCCAAGGAGCACAAC-3′ and

2) FKPB5R: and 5′-TTGAGGAGGGGCCGAGTTC-3′

3) *House keeping gene:*

(a) B2 microglobulin

(b) F: 5′-CCAGCAGAGAATGGAAAGTC-3′

(c) R: 5′-GATGCTGCTTACATGTCTCG-3′

QPCR conditions with a temperature of 95oC for 10 seconds and 60oC for 30 seconds as many as 40 cycles

Data processing and analysis Shapiro-Wilk test, this test is subject to ratio scale data that is age, anxiety level, cortisol level, CRHR1 level, and FKBP5 level. Decision criteria by looking at the Sig or p-value, if the p-value> □ = 0

Result

Table 1 : FKBP5 level Control group of pregnant women without yoga

NO	Sample	Ekspresi (Fold change)	NO	Sample	Ekspresi (Fold change)
1	LA13	6.706	13	LB13	9.417
2	LA14	7.022	14	LB14	9.414
3	LA15	6.194	15	LB15	8.472
4	LA16	6.588	16	LB16	10.369
5	LA17	7.416	17	LB17	9.190
6	LA18	7.531	18	LB18	10.580
7	LA19	6.997	19	LB19	8.611
8	LA20	6.228	20	LB20	10.145
9	LA21	7.286	21	LB21	9.450
10	LA22	7.931	22	LB22	9.404
11	LA23	7.389	23	LB23	8.596
12	LA24	7.935	24	LB24	9.565

Based on the data in table 1 shows the sample LA24 (7,935) is the highest sample fold fold change and the lowest in the sample LA15 (6,194). While in the post test results it was found that the LB18 sample (10,580) was the highest sample in fold change and the lowest was in the LB15 sample (8,472).

Table 2: Comparison of CRHR1 levels Pre and post test between control and Treatment

Level CRHR1 (pre) (control)	Level CRHR1 (post) (control)	Level CRHR1 (pre) (Treatment)	Level CRHR1 (post) (Treatment)
5.333	8.835	6.854	5.204
6.183	8.235	5.613	5.141
5.401	7.560	6.408	5.324
5.409	9.457	5.515	5.434
5.530	9.020	5.854	5.599
5.658	8.178	6.774	5.485
6.351	9.212	6.351	5.648
5.826	8.439	6.300	5.697
6.633	7.800	6.165	5.784
6.565	9.321	6.617	6.230
5.173	8.327	5.467	6.349
5.399	8.603	6.949	6.583

Based on table 2 shows that CRHR1 levels in pre and post control have increased, as well as in CRHR1 levels both in pre and post test overall decreased means that there is a yoga effect on primagravidae mothers.

Table 3: Comparison of CRHR1 levels Pre and post test between control and Treatment

Level FKBP5 (pre) (control)	Level FKBP5 (post) (control)	Level FKBP5 (pre) (Treatment)	Level FKBP5 (post) (treatment)
6.706	9.417	6.367	6.105
7.022	9.414	7.743	6.173
6.194	8.472	7.898	6.576
6.588	10.369	6.877	6.399
7.416	9.190	7.323	6.484
7.531	10.580	7.438	7.188
6.997	8.611	7.782	6.622
6.228	10.145	7.400	7.277
7.286	9.450	7.502	7.331
7.931	9.404	7.903	7.454
7.389	8.596	7.916	7.406
7.935	9.565	8.105	7.564

Table 4. Correlation Test Results Between Variables In The GroupTreatment After Yoga Exercises

Correlation variable	n	coefisien Corelasi (r)	p-value
Anxiety level with FKBP5	12	0.819	0.001
Anxiety level with with kortisol	12	0.853	0.000
Anxiety level with dengan CRHR1	12	0.853	0.000
Cortisol with FKBP5	12	0.937	0.000
CRHR1 with KBP5	12	0.937	0.000
Cortisol with CRHR1	12	0.956	0.000
Cortisol with FKBP5	12	0.901	0.000
CRHR1with FKBP5	12	0.846	0.001

According In Table 4. shows that there is a significant relationship / correlation between anxiety levels with FKBP5 levels ($p = 0.001 < \propto$). This is indicated by the high / strong correlation coefficient value which is 0.819. A positive value at 0.819 indicates a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in anxiety levels and will further reduce the level of FKBP5 in third trimester pregnant women

Discussion

The results of the study were between cortisol levels and FKBP5 levels ($p = 0.000 < \propto$). This is indicated by the high correlation coefficient value which is 0.937. A positive value at 0.937 indicates that there is a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in cortisol levels and will further reduce the effect of FKBP5 levels in third trimester pregnant women. Furthermore, there was a significant relationship / correlation between CRHR1 levels and FKBP5 levels ($p = 0.000 < \propto$). This is indicated by the high correlation coefficient value which is 0.937. A positive value at 0.937 indicates that there is a positive relationship or a concomitant relationship, namely the influence of yoga exercises will result in a decrease in CRHR1 levels and subsequently will have an effect on reducing FKBP5 levels in third trimester pregnant women.

Stress induces activation of the HPA shaft, which is under the control of several neurotransmitter systems. corticotropin-releasing hormone (CRH) is released from the hypothalamus paraventricularis (PVN) nucleus into the portal venous system, where this hormone is transported to the anterior pituitary to stimulate the secretion of adrenocorticotropic hormone (ACTH) into the blood circulation. ACTH is transported to the adrenal gland cortex, where it activates the release of the primary stress hormone cortisol into the bloodstream[18]. Cortisol itself regulates the activation of the HPA axis through negative feedback by binding to glucocorticoid receptors (GR) in the anterior pituitary and in the hypothalamus. Chronic dysregulation of HPA axis activity in response to stress can be a risk factor for psychiatric illnesses such as depression, anxiety.[19]

In vitro experiments have shown that overexpression of human FKBP5 also reduces the hormone binding affinity and GR nuclear making it reasonable that changes in FKBP5 expression can also affect human GR function in vivo. FKBP5 expression is also induced by steroids, including glucocorticoids as part of an intracellular ultra-short negative feedback loop for GR activity. Increased transcription and translation of FKBP5 after activation of steroid receptors will then reduce the sensitivity of GR[,11,17,21].

Given the fact that polymorphism is associated with higher levels of FKBP5 that lead to GR resistance

and negative feedback disturbances, one can speculate that FKBP5 alleles are associated with slower returns to baseline levels of stress-induced cortisol as well as increasing the risk of psychiatric related disorders with stress[5,8.,20].

Peripheral FKBP5 blood mRNA expression was also reduced in patients with PTSD, consistent with the increased GR response observed in this disorder and FKBP5 expression predicted by cortisol in studies of victims of the 9/11 attacks in New York City. Several studies have investigated the relationship between FKBP5 polymorphisms and mood disorders. Major depression has been associated with GR supersensitivity in high-induction allele carriers FKBP5. In studies in mice, prenatal stress has been associated with decreased FKBP5 expression in the prefrontal cortex and this is restored by administering antidepressant drugs[21].

FKBP5 mRNA levels in the ventral hippocampus are significantly regulated by stress. Indeed, Fkbp5 expression increases significantly after CMS (chronic mild stress). Research evidence suggests that animals exposed to CMS (chronic mild stress) show changes in the FKBP5-GR system that are important for HPA axis function and GR activity[5,18]. Changes produced by CMS, which reproduce phenotypes such as depression, show decreased translocation and GR function in the ventral and dorsal hippocampus as well as in the prefrontal cortex, and we show that chronic treatment with duloxetine antidepressants is able to normalize some of these changes, especially in the prefrontal cortex[9,10,22.]

CRH dysregulation that is induced nerve activity in the CNS may also be highly involved in the pathology of depressive disorders, Neuroendocrine findings in posttraumatic stress disorder (PTSD) reveal that PTSD patients are characterized by low adrenocortical activity and high CRH levels in the CNS CRH also inhibits eating behavior even in experimental animals that lack food[7,11]. It is possible that one of the peptides associated with CRH, such as urocortin I or III, mediates this gastrointestinal action. CRH is also a major inhibitor of reproductive function in both sexes. For example, intense or prolonged stress has been shown to inhibit gonadotropin secretion. Additional central effects of CRF include weakening of sexual behavior[3,8].

Conclusion

There was a significant correlation / correlation between CRHR1 levels and FKBP5 levels ($p = 0.001 <\propto$). This is indicated by the high / strong correlation coefficient value which is 0.846. shows the influence of yoga exercises will result in a decrease in CRHR1 levels and FKBP5 levels in third trimester pregnant women.

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Risk Factors Related To Mastalgia

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Abstract

Background : Breast pain becomes more complicated in many humans. Various hospitals are planning for surgery in the initial stages. The main aim of this paper to find the various factors which cause mastalgia, this is mainly causing the abnormal conditions of the breast.

Methods: The experiments conducted on 400 patients and this is collected from various surveys, for patients older than the age of 40 the mammography and ultrasonography (US).

Results: The mean age was 44.23 ± 9.23 years. This group consists of 300 cases, the asymptomatic group consists of 100 persons. Due to the various reasons that are related to mastalgia ($p < 0.05$). These are based on their breastfeeding to the child. If it is more than 4 times or more were higher in the mastalgia group ($p < 0.04$). The breast imaging-reporting and data system (BI-RADS 2) mammography results were associated with mastalgia ($p < 0.05$). These two are more common in the mastalgia group ($p < 0.05$) that is Fibro cysts and fibro adenomas. Based on the previous history the malignant breast disease was simultaneously higher in the mastalgia group ($p < 0.05$).

Conclusions: Life stress, high caffeine consumption, smoking, lactation frequency, and benign disorders were factors identified to be related with mastalgia. Though a significant relation between mastalgia and malignant breast disease was detected in our study, more studies are still necessary to investigate this relationship.

Keywords: *Mastalgia · Surveys · Mammography. US findings. Pain · Surgery*

Introduction

The most common factor in women is Breast pain nothing but mastalgia which causes breast tenderness, hard burning sensation or tightness in the tissue, the pain may increase or maybe constant and this will occur some other times.[1] Due to the pain in the breast and tenderness, many women are fear with early symptoms of breast cancer, generally, these are not considered as disorders.[2-3]

Mastalgia is classified into two types such as cyclic breast pain (CBP) or non-cyclic breast pain (NCBP). CBC affects women at the age of 20 to 30 and it is up to 40 also. But still, it is menstruating. Mainly the pain occurs at the end of the menstrual cycle week or before the period occurs. At this time the breast is affected with the painful, tender, and swollen, based on the cycle the symptoms improve at another point. The pain occurs at the breast outer and upper parts of two breasts and this involve the underarm area as well. In the luteal period, the result of water increased in breast stroma produced by increasing hormone levels.[3-5] Menopause is the non-cyclic mastalgia. This may like sharpness, burning, or soreness in the breast.

Causative pathophysiology of pain stays hazy however is believed to be known with secretion factors (e.g., hormone-substitution treatment, oral contraceptives, pregnancy, menses, pubescence, and menopause) these variables will modify the steroid, Lipo-Lutin and prolactin levels.[6] Kind breast issue (e.g., fibrocystic changes), mind-expanding drugs, psychosocial factors and passionate stress square measure connected with breast torment.[2-7] Lactation problems (e.g., engorgement, mastitis, and breast ulcer), inflammation (Tietze disorder), Postthoracotomy disorder, Spinal and paraspinal issue and Referred

torment (e.g., pneumonic, heart, or vesica sickness) will likewise cause breast torment. Caffeine and vasoconstrictive utilization square measure likewise viewed as related with pain.[1] Cancer is a rare reason for breast torment.[4]

Breast pain associated with malignant growth is one-sided, non-cyclic, and extremely a lot of confined.[8]

The fundamental center is to research the components of inpatient history that influences the event of breast pain, and to decide the connection among mastalgia and considerate or malignant breast utilizing a huge arrangement of members.

Method

The research was completed in an exceedingly non-public Surgery facility in Iraq, between September 2018 and August 2019. This experiment was affirmed by the local human morals committee. The patients were relegated to at least one of 2 gatherings: the pain gathering, from women, admitted to the breast surgery outpatient center with mastalgia (n = 300); and therefore the symptomless gathering (the management gathering), including patients undergoing routine breast screening (n = 100). Patients UN agency was treated with oral contraceptives, psychoactive specialists, secretion medical care, or vas operators, even as patients below 18, or over 70 older were banished from this research.

A standard summary study for all patients within the groups was performed regarding their enthusiastic anxiety, utilization of caffein and phytotoxin. AN intensive physical assessment was performed for all patients. The gap between the areola and therefore the os indent was calculable, any length surpassed 33 cm was thought of as macromastia. Breast screening was done by means that of the diagnostic procedures for patients skilled than the age of 40; imaging (US) was done once necessary for any age. Breast thickness bit by bit will increase to type D, during this manner, drop-off the affectability of the diagnostic procedure. What is more, the imaging discoveries in the diagnostic procedure were ordered accordant to the Breast Imaging-Reporting and information system (BI-RADS)? The BI-RADS classes are characterized as BI-RADS zero, fragmented; BI-RADS one, typical; BI-RADS2, thoughtful finding(s); BI-RADS three, presumptively generous; BI-RADS four, suspicious abnormality; BI-RADS five, deeply resembling harm; and BI-RADS vi, presence of biopsy-demonstrated danger.

The NCSS 2007, and PASS 2008 Statistical Software (Utah, USA) programs were used for measurable examination to perform the mean, variance, return and proportion, the Mann-Whitney U take a look at was used to affirm a normal dispersion condition. Fisher's actual take a look at, Pearson's chi-squared take a look at, and Yates' progression rectification were used for the subjective evaluations of the data. Contrasts were thought of statistically vital once $p < 0.05$.

Results

Overall 400 females with a males period of 44.23±9.23years (approx 19-65) were enlisted. The mastalgia gathering, 300 patients, and, the well gathering, two hundred patients. Guys age was basically higher within the pain gathering (p = zero.001) (Table 1).The rate range of patients World Health Organization admitted extended gung ho feeling of tension was higher within the pain gathering (p < zero.005). alkaloid use and smoking were known with pain (p < zero.05). The lactation repeat rate disclosed associate degree on a awfully basic level higher association with bosom torment within the pain gathering (p < zero.05). The sort, shape, and laterality of bosom torment within the pain bundle area unit consolidated in Table a pair of. the speed range of patients of the pain bunch eaten soul, paracetamol, and non-steroid assuaging medicine were primarily higher (p < zero.05) (Table 3).

A total of 280 patients was given to mammographic screening, 209 patients from the pain gathering, and seventy one patients within the well gathering. The unendingly visit bosom thickness configuration was composed associate degree within the well gathering, and sort B saw as increasingly typical within the pain gathering (p < zero.01). In step with the BI-RADS categories, BI-RADS one was increasingly customary within the well occasion and BI-RADS a pair of within the commanding within the pain gathering (p < zero.05). The analysis of the U.S.A. photos disclosed the events of elementary bruises and fibro adenomas were higher within the pain gathering (p < zero.05). The assessment of the tomography discoveries between the 2 social occasions shows up in Table four.

Different sorts of six patients resolved to own BI-RADS four were within the pain gathering. Once the diagnostic assay, the psychoneurotic evaluations disclosed that 3 of those patients had a unsafe contamination, agitating cautious intervention.

Table 1. the comparison between the mastalgia and asymptomatic groups patients

		Total	Mastalgia	Asymptomatic	p
Age mean ± SD, years		44.23±9.23	45.25±9.50	42.85±10.20	0.001
Patients, n		400	300	100	
Stress, n (%)	none	85 (20.5)	52 (17.3)	33 (33.0)	0.001
	stressful	315 (79.5)	248 (82.7)	67 (67.0)	0.002
Caffeine consumption (coffee), n (%)	none	250 (62.5)	170 (56.7)	80 (80.0)	0.003
	1 cup/day	65 (16.2)	60 (20.0)	5 (5.0)	0.012
	≥ 2 cups/day	85 (21.3)	70 (23.3)	15 (15.0)	0.012
Smoking (cigarettes), n (%)	None	280 (70.0)	205 (68.3)	75 (75.0)	0.004
	5-10/day	71 (17.8)	55 (18.3)	16 (16.0)	0.125
	>10/day	49 (12.2)	40 (13.3)	9 (9.0)	0.007
Lactation (breast-fed infants), n (%)	0	30 (7.50)	10 (3.30)	20 (20.0)	0.001
	1	39 (9.80)	22 (7.30)	17 (17.0)	0.005
	2	97 (24.2)	65 (21.6)	32 (32.0)	0.154
	3	72 (18.0)	60 (20.0)	12 (12.0)	0.001
	≥ 4	162 (40.5)	143 (47.7)	19 (19.0)	0.035
BSO±TAH, n (%)	No	384 (96.3)	294 (98.0)	90 (90.0)	0.001
	Yes	16 (4.0)	6 (2.0)	10 (10.0)	0.005
Menopause, n (%)	premenopausal	312 (78.0)	252 (84.0)	60 (60.0)	0.001
	postmenopausal	88 (22.0)	48 (16.0)	40 (40.0)	0.002
Breast size, n (%)	Normal	368 (92.0)	280 (93.3)	88 (88.0)	0.001
	macromastia	32 (8.0)	20 (6.7)	12 (12.0)	0.002
Recent breast trauma, n (%)	No	391 (97.8)	292 (97.3)	99 (99.0)	0.055
	Yes	9 (2.2)	8 (2.7)	1 (1.0)	0.058
Breast cancer, n (%)	No	393 (98.2)	295 (98.3)	98 (98.0)	0.015
	Yes	7 (1.8)	5 (1.7)	2 (2.0)	0.005

SD, BSO±TAH: history of a prior bilateral salpingooophorectomy with or without total abdominal hysterectomy.

Table 2.Mastalgia patients' based on the pain the distribution is done

Breast Pain	Type	N	%
Radiation	Diffused	140	46.7
	Localized	160	53.3
Laterality	Bilateral	61	20.3
	Unilateral	239	79.7
Relation with menstruation	Cyclic	205	68.3
	Non-cyclic	95	31.7

Table 3. Analgesic consumption comparison between the mastalgia and asymptomatic groups

Analgesic consumption n (%)	Total	Mastalgia	Asymptomatic	P
None	350 (87.5)	255(85.0)	95(95.0)	0.001
Aspirin	9(2.2)	8(2.7)	1(1.0)	0.011
Paracetamol	17(4.3)	15(5.0)	2(2.0)	0.001
NSAIDs	24(6.0)	22(7.3)	2(2.0)	0.021

Table 4. Comparison of mammography and ultrasound findings between the mastalgia and asymptomatic groups

	Total, n (%)	Mastalgia, n (%)	Asymptomatic, n (%)	p
Mammographic breast density (pattern) (n=280)				
A	57 (20.4)	12 (5.7)	45 (63.4)	0.001
B	172 (61.4)	160 (76.6)	12 (16.9)	0.001
C	44 (14.7)	34 (16.3)	10 (14.1)	0.010
D	7 (2.5)	3 (1.4)	4 (5.6)	0.020
Mammographic category (n=280)				
BI-RADS 0	160 (57.1)	101 (52.3)	59 (67.8)	0.073
BI-RADS 1	30(10.7)	16 (8.30)	14 (16.1)	0.001
BI-RADS 2	70(25.0)	60 (31.1)	10 (11.5)	0.001
BI-RADS 3	10(3.57)	7 (3.63)	3 (3.45)	0.014
BI-RADS 4	6(2.14)	6 (3.11)	0 (0.0)	0.021
BI-RADS 5	4(1.4)	3 (1.55)	1 (1.15)	0.002
Ultrasound findings (n=285)				
Normal	35(12.3)	25 (13.5)	10 (10.0)	0.003
Simple cysts	20(7.02)	20 (10.8)	0 (0.0)	0.001
Fibroadenomas	45(15.8)	35 (18.9)	10 (10.0)	0.039
Fibrocystic disease	180(63.2)	100 (54.1)	80 (80.0)	0.015
Lymph nodes	3(0.78)	3 (1.62)	0 (0.0)	0.014
Complex cysts	2(0.70)	2 (1.08)	0 (0.0)	0.004
Fatty tissue necrosis	1(0.35)	1(0.54)	0 (0.0)	0.004

Discussion

Various factors are effecting mastalgia, The author [9] portrayed that the period of women grumbling of the pain in breast was in the range 35-55 years and that this side effect was once in a while experienced younger than 45. Present research, the male age of the all-out investigation group was 44.23±9.23years (territory 19-65 years), and the male age of the mastalgia group was higher lighting up that mastalgia happens in the perimenopausal period when the inconsistency of hormonal and menstrual beginnings. Numerous studies showed that there is a connection between breast torment and mental strain.[10–12] In this paper, the patients how had an unpleasant lifestyle were essentially higher in the mastalgia gathering. Numerous concentrates uncovered a connection between caffeine utilization and smoking with breast torment.[1-3-6] In our examination, there was a critical association between either standard caffeine utilization or substantial smoking (in excess of 10 cigarettes every day) and mastalgia.

Anomalies of the estrogen/progesterone proportion, which can happen on the off chance that one of these hormones increment, the other one will diminish, or expanded prolactin levels are identified with mastalgia.[13-14] The event of breast side effects, for example, expanding, irritation, and nodularity in the menopausal period, at that point the end of these manifestations in the postmenopausal period is potential because of the impacts of the estrogen hormone.[15-16]

In perspective on lactation, the level of ladies with a background marked by breast feeding4 or more newborn children were expanded in the mastalgia gathering. These outcomes shed light on the connection of breast torment with the expanded frequencies of lactation, which is identified with an expanded number of births, and therefore with an expanded introduction to significant levels of estrogen and prolactin. Additionally, at least 4 times of lactation may bring about anatomical changes in the breast tissue, particularly in the ductal framework, which could likewise expand the breast torment episodes.

An earlier reciprocal salpingo-oophorectomy with or without absolute stomach hysterectomy was identified with a fundamentally diminished event of mastalgia.

The mastalgia side effects which coordinate the announced investigations that found a huge connection between the asymptomatic gathering and postmenopausal state.[17-18]

Macromastia or breast hypertrophy is an uncommon ailment of the breast connective tissues wherein the breasts become exorbitantly enormous. In the event that the separation between the sternal score and the areola surpassed 33 cm this means that macromastia.[19] In our examination, the recurrence of macromastia was altogether lesser in the mastalgia gathering, recommending that macromastia probably won't be one of the components that reason mastalgia. Mastalgia may happen attributable to greasy tissue rot, or a strain in the Cooper tendons as aftereffects of either dull or entering injury to the breast. In our investigation, higher paces of injury history found in the mastalgia gathering.

Extreme mastalgia can happen in 10–22% of patients and requires medicinal medications. As an initial step approach, analgesics In our present examination, the mastalgia bunch utilized pain-relieving specialists more than the asymptomatic gathering, and the most picked pain relieving type was NSAIDs.

As breast thickness builds, the mastalgia does. In our examination, the sort B breast design was higher in the mastalgia gathering. Different designs demonstrated immaterial contrasts between the two gatherings. Mastalgia and generous breast issue is altogether related. Our examination indicated a higher recurrence of mastalgia in patients with discoveries of benevolent issue (BI-RADS 2). The connection between breast torment and kind issue distinguished utilizing the US is likewise easily proven wrong .The author announced that fibroadenomas and basic sores were the most widely recognized existing together benevolent sores.

Furthermore, the rate of fibrocystic malady recognized by the US was essentially higher in patients with mastalgia. Since the fibrocystic ailment is a substance demonstrating a summed up inclusion of the breast parenchyma, that it prompts diffuse breast torment isn't startling. Despite the high likelihood of the dwelling together of a dangerous breast tumor with non-cyclic breast torment, albeit uncommon, repetitive mastalgia can likewise be identified with harm.

Despite the fact that the aftereffects of our examination uncover a relationship among mastalgia and danger, this issue is as yet dubious, and there is as yet a requirement for enormous future investigations to uncover the relationship more precisely.

Conclusion

Symptoms like stress, coffee consumption, smoking, and other abnormal disorders are the key points found which is related to mastalgia. There is a relation between mastalgia and malignant breast disease is found in our research. In the future, more research is to be done.

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All writers announce that no issues are found in that and they have no direct or business monetary affectation related to distributing this article.

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Oral Health Care Guidelines for Patients with Diabetes Mellitus: A Review

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Abstract

Diabetes Mellitus [DM] is a metabolic disorder characterised by relative or obsolete insufficiency of insulin and resultant disturbances of carbohydrate metabolism. People with poorly controlled DM are at greater risk for dental problems. They are more likely to affect with oral infections. High and low blood sugar may also be able to cause many oral manifestations and some emergency situations in dental office. This article highlights on oral manifestations and dental considerations while treating these DM patients.

Keywords: *Diabetic, oral infection, hypoglycaemia, hyperglycemia*

Introduction

Diabetes Mellitus [DM] is one of the common medical problems affecting the general population. It is a metabolic disease characterized by dysregulation of carbohydrate, protein and lipid metabolism. There is no definitive cure for DM and it is most common endocrine disorder. The prevalence of DM is increasing steadily. It is one of the frequent pathologies that dentists encounter due to its high prevalence worldwide. [1] So Dentists play a major role in providing oral care to patients with DM.[2] They may detect undiagnosed cases of DM and refer these patients to physicians for further evaluation.

CLASSIFICATION AND PATHOGENESIS OF DM:

DM may be the result of multiple reasons. Genetic type of DM is the most common type. This type is also called as Primary, hereditary or essential DM.[3]Blood glucose level in normal person will be with in the range of 60 to 150 milligram per decilitre (mg/dL).in patients diagnosed as DM, these levels will be increased [**Table 1**].

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TABLE 1: Blood Glucose level in DM		
TEST	**DM**	**NORMAL VALUE**
Fasting	Equal or More than 126mg/dL	70 – 110 mg/dL
Post Prandial (2hrs after food)	More than 200mg/dL	140mg/dL
Random Blood Glucose	More than 200mg/dL	Below 200mg/dL

Insulin synthesized in beta cells of pancreas and secreted in to the blood in response to increased blood sugar level. Insulin plays an important role in the regulation of blood glucose. It promoting uptake of glucose from the blood in to cells and by its storage in the liver glycogen. It also helps in the uptake of fatty acids and amino acids as well as their conversion in to triglyceride protein stores. A lack of insulin or insulin resistance results in an inability of insulin dependant cells to use blood glucose as an energy source.[4]

GLYCOSYLATED HAEMOGLOGIN (HbA1c):

Longer-term diabetes control is carried out by monitoring the level of glycosylated haemoglobin. Haemoglobin is a protein attached to the red blood cells. Its main function is the transportation of oxygen around the body. When the haemoglobin molecule is surrounded by a high glucose level within the bloodstream then some of the glucose will become attached to the haemoglobin. This is called glycosylation. This haemoglobin will remain glycosylated until the red blood cell to which it is attached dies (approximately three months). The HbA1c is the ratio of glycosylated haemoglobin to unglycosylated haemoglobin given as a percentage.[5] This level approximates to the average blood sugar level within the last three months. The Glycosylated Haemoglobin values are 4% to 5.7% (Normal), 5.7 % to 6.4% (Prediabetic), Morethan 6.5% (Controlled DM) & Above 7.5% (Uncontrolled DM).

Based on the pathogenic process there are four types of DM are identified. [6]

- Type 1 DM →Insulin dependant DM, Which constitutes 5% to 10%
- Type 2 DM → Non Insulin Dependant DM, Which constitutes 90 % to 95%
- Type 3 or Secondary DM→ Due to other metabolic disorders/ Genetic defects of cell functioning of pancreas /Genetic defects of insulin action/ Any diseases of pancreas/ Endocrinal disorders/ Drugs/ Surgery
- Gestational DM → During Pregnancy

In Type 1DM, environmental factors such as viral infection and autoimmune reactions are the major causative factors. There is absolute insulin deficiency due to these factors which causes destruction or disappearance of insulin producing pancreatic beta cells.[7]This Type can occur at any age but more in childhood and adolescence. It leads to absolute insulin deficiency. Also these patients have high risks of severe complications.[4] Type 1 DM is also prone to autoimmune disorders such as Graves Disease, Hashimot's Thyroiditis and Addison's Disease. [4]

Type 2 DM is complicated because patient present with varying degree of insulin dependency and insulin resistance.[7]strong Genetic role is more in Type 2 DM.[8] This type of DM is results from impaired insulin function. The risk of this type DM is increases with Age, obesity and lack of physical activity.[9]

Type 3 or secondary DM are relatively uncommon. This type of DM is due to genetic defects of beta cell function or insulin action, diseases of pancrease, endocrinopathies, use of drugs, infections and certain genetic syndromes. Drugs like glucocorticoids, thiazides, dilantin and interferon alpha can also affect

insulin secretion. Certain viruses can also responsible for deta cell destruction [**Figure 1**].[4,9]

Gestational DM is a glucose intolerance with onset of first recognition during pregnancy. High incidences seen in pregnant women with overweight and older pregnant women. In Majority of cases (70%) glucose regulation will return to normal after delivery.[4] Around 30 % of women with history of Gestational DM will develop Type 2 DM with un 10 years after delivery.[10]

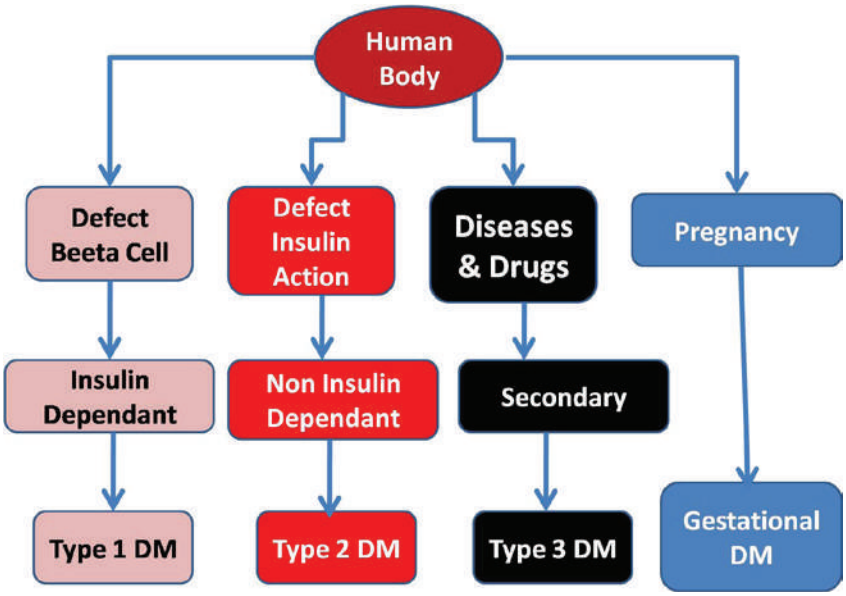


Figure 1: Types of DM

Clinical Signs & Symptoms:

In case of increased blood glucose level (Hyperglycemia), excessive urination occurs due to osmotic dieresis (Polyurea) and more glucose is excreted in the urine. Excessive loss of fluied leads to dehydration and excessive thirst (Polydipsia). It causes less glucose to body cells leads to increased hunger (Polyphagia). since the cells are unable to take up glucose, When this occurs, the body starts burning fat and muscle for energy, causing a reduction in overall body weight. These are signs and symptoms of DM. [11]

In DM patient body's natural buffering system is overwhelmed by the acidic ketones, this imbalance causes hyperventilation as the body attempts to regulate blood acid levels by getting rid of carbon dioxide in expired air.[12]People with uncontrolled DM have a higher chance of developing pancreatitis which causes swelling and inflammation of the pancreas, and may cause nausea, Vomiting and abdominal pain.[13]

Complications:[14,15]

Long term DM patients have an increased incidence of oral and general both Microvascular and Macrovascular complications [**Table 2**]. Microvascular complications are retinopathy causes loss of vision and nephropathy (Renal Failure).[16,17] Macrovascular complications are hypertension, hyperlipidemia, atherosclerotic cardiovascular disease and cerebrovascular disease. Other common symptoms are numbness and tingling sensation of extremities, oral parasthesia and burning sensation.These changes are result from microvascular injury involving small blood vessels that supply nerves (vasa nervorum).[18]

Impaired wound healing and susceptible to infections seen in poorly controlled DM.Diabetic wound healing is impaired because less oxygen can reach the wound As a result of narrowed blood vessels, and the tissues do not heal as quickly.[19]

TABLE 2: Complications of DM	
Oral Complications	• Gingival & periodontal disease • Salivary gland dysfunction • Oral Infections • Oral neuropathy
Long term complications	• Retinopathy • Nephropathy • Neuropathy • Macro vascular disease • Alteration in wound healing

ORAL MANIFESTATIONS OF DIABETES MELLITUS:

There are various oral manifestations reported in uncontrolled DM patients [**Table 3**]. These manifestations may relate to excessive loss of body fluids, altered response to infection, microvascular changes and increased glucose concentration in saliva.[8,20,21]

TABLE 3: Oral Manifestations of DM
• Gingivitis & Periodontal disease • Alveolar Bone Loss • Oral Candidiasis • Median Rhomboid Glossitis • Xerostomia • Dental caries • Acute oral Infection • Dry Socket • Halitosis • Tingling & Numbness • Taste disturbances • Burning Pain In The Oral Region • Lichenoid Reaction

GINGIVITIS & PERIODONTAL DISEASE: DM is a confirmed major risk factor for periodontitis. Risk increased in 3 times in patients with DM. the level of glycemic control is key importance in determining increased risk. Periodontitis is refered as sixth complication of DM and in 2003 ADA acknowledged this.Diabetes increases inflammation in the gingival and periodontal tissues leads to halitosis. Both type 1 and type 2 diabetes mellitus are associated with elevated levels of systemic markers of inflammation. The elevated inflammatory state in diabetes contributes to both microvascular and macrovascular complications, and hyperglycaemia can result in the activation of pathways that increase inflammation, oxidative stress and apoptosis.[22,23]

Elevated levels of CRP are also associated with insulin resistance, type 2 diabetes mellitus. The systemic inflammation that is associated with gingival and periodontal disease may therefore enhance the diabetic state. Adipokines may also contribute to susceptibility to both periodontitis and diabetes, and the proinflammatory properties of leptin may be particularly important in upregulating periodontal inflammation in people who are obese and have type 2 diabetes mellitus.[24] Diabetes also prolongs the inflammatory response to *Porphyromonas gingivalis,* a periodontal pathogen commonly found in the biofilm of patients with advanced gingivitis and periodontitis.[25]

ORAL CANDIDIASIS : Oral Thrush is a common problem and particularly for people with DM. Higher levels of glucose in the blood make candida all the more likely, so DM patients who have difficulty in controlling their blood sugar may find themselves particularly prone to fungal infections. Yeast feeds off of sugar. If DM isn›t well-controlled, blood sugar levels can increase to high levels. This increase in sugar can cause yeast to overgrow, particularly in the oral region. So body may develop a fungal infection in response.[26] Oral candidiasis

can be diagnosed by the differential patterns of mucosal changes like erythematous, pseudomembranous, and curd-like plaques.[27]

XEROSTOMIA : High blood sugar level cause dry mouth in people with both types of DM. The reason for these problems could be due to damage to the gland parenchyma, alterations in the microcirculation to the salivary glands, dehydration by hyperglycemia, disturbances in glycemic control, diabetic neuropathy, structural changes in the salivary glands and medications used to treat DM. The increased diuresis in people with DM leads to a significant decrease in extracellular fluids that directly affects salivary production. According to Moore, in periods of metabolic disorder, dehydration raises the osmotic gradient from the blood vessels in relation to the salivary glands, limiting saliva secretion and therefore exacerbating xerostomia symptoms. [28]

DENTAL CARIES: Due to the low sugar diet by most of DM patients , there is a reduced carious lesions, however reduced salivary flow rate caused by hyperglycemia causes glucose leakage into the oral cavity thereby facilitating the growth of aciduric and acidogenic bacteria and caries lesion development. If the patients also has oral fungal infections and under topical antifungal medications, these medications have high sugar content and also can promote caries.[29]

ACUTE ORAL INFECTION :Patients with diabetes are more susceptible to the development of various oral infections. As high blood sugar levels and the absence of its antimicrobial effects can weaken the patient's immune system defences can cause these infections. In addition, some diabetes-related health issues, such as nerve damage and reduced blood flow to the extremities, increase the body's vulnerability to infection [**Figure 2**]. [30]

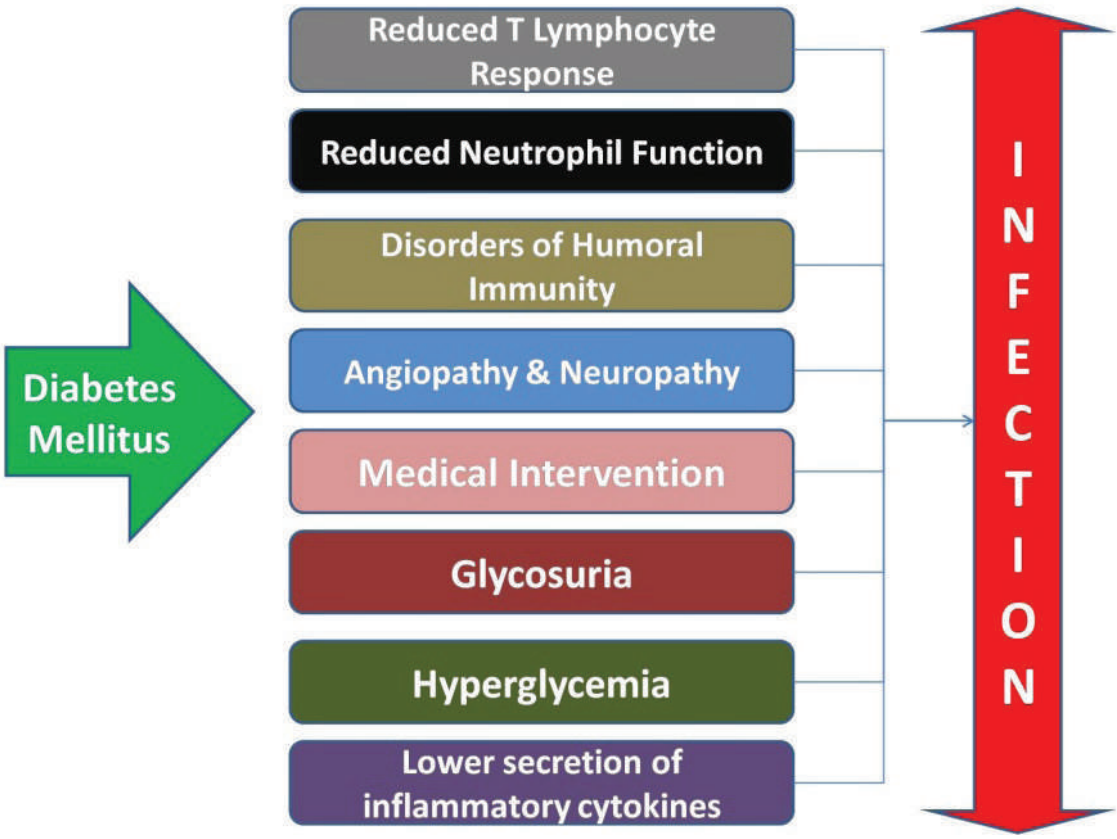


Figure 2: Pathophysiology of Infections Associated with DM

TINGLING & NUMBNESS :Uncontrolled high blood sugar damages nerves and interferes with their ability to send signals, leading to diabetic neuropathy. High blood sugar also weakens the walls of the small blood vessels (capillaries) that supply the nerves with oxygen and nutrients. Symptoms depend on the site of nerve damage and can include motor changes such as weakness; sensory symptoms such as numbness, tingling, pain or autonomic changes such as urinary symptoms. Tingling and numbness is usually peripheral neuropathy, or nerve damage in the arms, legs, hands, and feet. [31]

TASTE DISTURBANCES: Diabetes can sometimes cause a sweet taste in the mouth and is often accompanied by other symptoms. Additional symptoms include: reduced ability to taste the sweetness in foods. among diabetic or prediabetic patients, 5.7% had a sweet taste disorder and 8.6% had a salt taste disorder. Neuropathy also increases the threshold of taste. This sensory dysfunction can inhibit the ability to maintain a good diet and can lead to poor glucose regulation. [32]

BURNING PAIN IN THE ORAL REGION :Neuropathic pain in these patients can be manifested as burning pain or even as electric shock or stabbing sensation that these symptoms may be very debilitating. These pain sensations have a considerable effect on the physical and psychological functions. These Burning sensation or dysesthesia in the oral cavity of DM patients due to poor glycemic control, metabolic alterations in oral mucosa, angiopathy, candida infection, and neuropathy. [33]

LICHENOID REACTION :Little evidence supports a connection between diabetes mellitus and oral lichen planus. The oral lichenoid reaction in triad of oral lichen planus, diabetes mellitus, and hypertension (Grinspan syndrome) is probably an adverse effect of the drug therapy for diabetes mellitus and hypertension.[34]

EMERGENCY IN DENTAL CLINICS:

The emergency situation most likely encountered in the dental office is a patient with hypoglycemia or insulin shock. This condition is caused by an excessively high level of insulin due to the patient taking their daily dose of insulin with inadequate intake of carbohydrates. Symptoms of hypoglycaemia may range from mild, such as anxiety, sweating and tachycardia, to severe, such as mental status changes, seizure and coma. If dentists suspects that patient shows any symptoms of hypoglycaemia, they should immediately terminate dental treatment and administer immediately 15 g of a fast acting oral carbohydratesuch as glucose tablets, sugar , soft drinksor juice[**Table 4**].[35]

TABLE 4: Emergencies of DM Patients in Dental Office

Hypoglycaemia	INSULIN SHOCK	Acute emergency symptoms: • Confusion • Sweating • Tremors • Agitation • Anxiety • Dizziness • Tingling or numbness • Tachycardia • Seizures • Loss of consciousness.
Hyperglycemia	DIABETIC KETOACIDOSIS	No acute emergency symptoms

Hyperglycemia is Due to Low or absent plasma insulin levels for a long period of time. Because of the absence of insulin, glucose cannot enter cells, forcing the cells to metabolize fat and proteins to produce glucose. In the process ketones and other metabolic acids are produced leading to a condition known as diabetic ketoacidosis which, if not treated over a period of days, can lead to coma and death. The risk of hyperglycaemic crisis is lower than that of hypoglycaemia in dental clinic. Because it takes several days for ketoacidosis to occur, hyperglycemic patients do not exhibit acute emergency symptoms.[35]

DENTAL CONSIDERATIONS:

To provide competent care to DM patients, dental clinicians must understand the disease, its treatment, and its impact on the patients' ability to undergo and respond to dental care. To minimise the risk of an intraoperative emergency, clinicians need to consider some issues before initiating dental treatment. [36] We have to follow few general guidelines while treating DM patients. [**Table 5**]

TABLE 5: General Guidelines for the Treatment of of DM

A.	Take physicians consent
B.	Treatment in hospital setting
C.	Detailed Medical history
D.	Morning short appointments
E.	Check glucose level
F.	Continuous verbal contact with patients
G.	Monitor pulse, respiration & BP
H.	Defer any minor surgical procedures

It is always advisable to consult a patient's physician before any surgical procedure to consider adjustment of the patient's insulin and dosageand dietary needs during the postoperative period. In a poorly controlled diabetic, prophylactic antibiotic should be considered due to altered function of neutrophils. To minimize the risk of emergency dentists should consider few management steps before dental treatment [**Table 6**].

TABLE 6: Dental Considerations of DM PatientsDuring Treatment

TREATMENT	DENTAL CONSIDERATION
Orthodontic Treatment 37	• DM is not a contraindication for orthodontic treatment • Patients should have control of their glycaemic state • Under proper monitoring before starting active orthodontic treatment • Should apply light physiological force on teeth • Antibiotic prophylaxis for band and separator placement and orthodontic implant placement • For simple appliance, Antibiotic not required • Dentist should trained in management of hypoglycaemic condition
Oral Surgical Procedures 38	• Check blood glucose prior to procedure • Pre and post surgical antibiotics • Increase insulin dose in case of infection and delayed wound healing • Max permissible blood glucose level is fastin 180mg/dL & random 200mg/dL • In case of emergency procedures cut off point is max 234mg/dL • Dry socket is the complication • An early morning appointment will minimize the risk of stress-induced hypoglycemia
Periodontal Treatment 39	• Proper periodontal treatment is associated with reduction of HbA1c level • Blood glucose level should be fasting 160mg/dL • Routine periodontal assessment should be performed • Ask the patients about their level of glycaemic control • Patient education, oral hygiene instruction & monitoring treatment outcome • Conventional periodontal treatment is very effective in DM • Antibiotics as a part of periodontal therapy

Cont... **TABLE 6: Dental Considerations of DM PatientsDuring Treatment**

Endodontic Treatment 40	• Well controlled and free of complication patients are fit for treatment • Special consideration for acute infections • Acute infections managed with drainage, pulpectomy & antibiotics • Insulin doses should be increased if required • Early morning appointments • Pretreatment sedation, if required • Antibiotic prophylaxis not required for well controlled cases
Prosthodontic Treatment 41	• Stress reduction • Oral hygiene instructions • Mucostatic impressions • Uncontrolled cases, implants are contraindicated • Complications are –Abutment failure, tissue abrasions and fungal infections in denture patients, increased residual ridge resorption

It's very important for clinician to take detailed medical history of the DM patient. When reviewing medical histories, a clinician should be aware of the cardinal signs of DM. Should also ask about any hypoglycaemic episodes and recent test details and history of antidiabetic and other medications. There are variety of medications can alter blood glucose levels in the body. Few major surgical procedures may require adjustments in insulin or antidiabetic medication doses. Appointments should be scheduled at early morning since endogenous cortisol levels are generally higher at this time (cortisol increases blood sugar levels). For patients receiving insulin therapy, appointments should be scheduled so that they do not coincide with peaks of insulin activity, since this is the period of maximal risk of developing hypoglycaemia. Dentists should ensure that the DM patients has eaten normally and taken their medications. If patient skip the meal and takes the normal dose of insulin, then risk of hypoglycaemic episode is more. Patients with poorly controlled DM are at greater risk of developing post treatment infections and delayed wound healing. Therefore antibiotic coverage is necessary for such patients.[4]

Conclusion

Oral complications in patients with DM are considered major complications of the disease and can impress the patients' quality of life. Patients with poorly controlled DM must be considered at risk for oral complications and they should be informed of this risk. There is evidence that chronic and persistent oral complications in these patients adversely affect blood glucose control. Thus, prevention and management of oral complications due to diabetes are more important. Fully aware of managing medically compromised patients with different treatment modalities and cooperating with consulting patient's physicians is a key responsibility of a oral physician.

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The Effect of *Escherichia coli* on Pro-Inflammatory Mediators Level and Kidney and Liver Function of Sepsis in *Rattus novergicus*

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Abstract

Sepsis is a life-threatening state of organ dysfunction caused by a deregulation of the body's response towards an infection. The purpose of this study was to determine the pro-inflammatory mediators' level as well as kidney and liver organ function on the *Rattus novergicus* sepsis model injected intraperitoneally (i.p.) with *E. coli*. This study used 20 male rats (*Rattus novergicus*, Wistar strain) which were divided into 2 groups: rats without *E. coli* injection and rats were i.p. with 105 CFU of E. coli. After six hours, the level of pro-inflammatory mediators (TNFα, Hs-CRP, PCT, and MDA), kidney function (urea, BUN, and creatinine), and liver function (SGPT, SGOT, and the total of bilirubin) were examined. The data obtained were analyzed using T-Test. This study concluded that intraperitoneal injection of *E. coli* increased pro-inflammatory mediator levels which include TNFα, Hs-CRP, PCT, MDA level. It also increased kidney function. Interestingly, the injection only increased bilirubin total levels in the liver but did not show improvement in the SGPT and SGOT. In summary, there was a significant increase in TNF-α pro-inflammatory mediators, procalcitonin (PCT), malondialdehyde (MDA), urea kidney function parameters and BUN among treatment groups. The hs-CRP, creatinine, and total bilirubin experienced a significant increase.

Keywords: Sepsis, Escherichia coli, pro-inflammatory mediators, Rattus novergicus, kidney, liver

Introduction

Sepsis is a life-threatening state of organ dysfunction caused by a deregulation of the body's response towards an infection. Where there is excessive body response due to infections that can occur in blood, urine, lungs, skin and other tissues[1,2,3]. Septic shock is defined as a state of sepsis where blood circulation and metabolism are abnormal. This condition can lead to significant death[4]. The symptoms and signs of sepsis vary widely. They are very nonspecific and usually give a systemic sign of pain. Sepsis is characterized by fever, mental disorder, hypotension, decreased urinary excretion, and thrombocytopenia. If the patients do not receive adequate therapy, sepsis will develop and turn into respiratory failure, kidney failure, coagulation abnormalities and even death.

Until now, sepsis is still the leading cause of death in the intensive care unit[5]. Globally, there are 27-30 million cases of sepsis worldwide and every approximately 9 million die (1 person dies every 3.5

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seconds)[6]. In developing countries, such as Indonesia, the mortality rate due to sepsis is two to three times higher than in developed countries, such as the USA. At the Pendidikan Hospital in Yogyakarta, 631 sepsis cases were found in 2007 with a mortality rate of 48.96%[8]. Various epidemiological researches stated that the incidence and mortality of sepsis are higher in men than women, although the cause of these findings is still unexplainable. In 2012-2013 at the Dr. Saiful Anwar General Hospital in Malang, Indonesia, a total of 1026 patients were diagnosed with sepsis and 788 of them died (76.8%). Whereas 168 sepsis patients were sent to the Intensive Care Unit (ICU) and 78 of them died (46.4%)[9].

Sepsis can be caused by a variety of microorganisms including viruses, bacteria, fungi, and protozoa[9]. The main cause of sepsis is lipopolysaccharide (LPS) exposure. Gram-negative bacteria are involved in 60-70% of sepsis epidemic, with some regional variations. Gram-positive bacteria also play a role in 30-50% of sepsis cases[10,11]. In recent years, murine sepsis models have become quite interesting topics of debate, especially those related to human diseases and the development of new biological therapy. Some researchers have reported an association between murine and human response to sepsis at the genomic level[12]. Some others argued that there are biological similarities among them. For example, a comparison between the complex human condition and homogeneous endotoxemia model in a single genetic strain of *Rattus novergicus*[13]. Among animal sepsis models, the *Rattus novergicus* is commonly used. It is due to the ease of handling, the availability of its genetic manipulation, and the relatively low cost[14]. Therefore, this study used sepsis rat injected intraperitoneally with 10^5 CFU of *E. coli*.

Materials and Methods

This study was approved by the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia. This research uses an experimental post-test only control group design. The experimental animals used were male rats (*Rattus novergicus*), 7-9 weeks old, 150-170 grams body weight, had not undergone any treatment or had not received any chemicals, and were in a healthy condition. *Rattus novergicus* were divided into 2 groups: rats without E. coli injection and rats were i.p. with 10^5 CFU of *E. coli*. After six hours, the level of pro-inflammatory mediators (TNFα, Hs-CRP, PCT, and MDA), kidney function (urea, BUN, and creatinine), and liver function (SGPT, SGOT, and the total of bilirubin) were examined. The TNF-α, PCT, hs-CRP level was measured by using enzyme-linked immunosorbent assay (ELISA). Liver dysfunction was evaluated by measuring the total levels of bilirubin, aspartate aminotransferase (AST/SGOT) and alanine aminotransferase (ALT/SGPT). Renal dysfunction was analyzed by measuring the levels of urea, nitrogen, and creatinine. All examinations were analyzed by the auto analyzer. The experimental data obtained were analyzed with the T-test.

Results and Discussion

The data shows that *E. coli* infection caused a significant increase in TNFα, PCT, MDA, urea, BUN (p <0.05) as well as the hs-CRP, creatinine and total bilirubin (p <0.01). Whereas the SGOT and SGPT did not show significant improvement (p >0.05).

Table 1. The results of the level of pro-inflammatory mediators, liver, and kidney functions in rats.

Variables	Control (X±SD)	E. coli Infection (X±SD)
TNF-α	0.58±0.16a	0.84±0.02b*
PCT	0.76±0.21a	1.06±0.09b*
hs-CRP	0.72±0.24a	1.17±0.10b**
MDA	0.13±0.03a	0.53±0.23b*
Urea	15.90±3.50a	24.46±4.75b*
BUN	7.43±1.64a	11.44±2.22b*
Creatinine	0.30±0.07a	0.54±0.05b**
SGOT	66.40±18.04a	95.20±36.48a
SGPT	31.00±15.08a	42.40±15.34a
Total Bilirubin	0.24±0.05a	0.42±0.08b**

The increase in TNF-α and MDA level has been reported in previous research where CLP (caecal ligation puncture) sepsis rat model and *E. coli* i.p. injection sepsis rat model was able to significantly increase both levels[15]. The most commonly used parameter of sepsis in humans is hs-CRP which is an examination of acute-

phase proteins. This parameter is more sensitive than CRP and LED in showing acute inflammatory processes in humans and PCT to see the severity of sepsis. In this study, hs-CRP experienced a very significant increase in hs-CRP and a significant increase in PCT.

E. coli is generally the main causative agent of extraintestinal infections, such as neonatal meningitis, bacteremia, pyelonephritis, cystitis, prostatitis, and sepsis. Paradoxically, this microorganism is also a dominant facultative member of normal human gut microbiota. Adhesion of pathogenic bacteria to host cells is the first step in establishing infection. Further events include tissue colonization and, in certain cases, cellular invasion followed by intracellular multiplication or persistence. The adhesion process begins when the surface structure, known as an adhesin, binds to their specific ligands, receptor host cells or extracellular matrix proteins[16,17]. Many of the previous sepsis-*E. coli* study showed changes in pro-inflammatory cytokines. This study showed changes in kidney function, where there is a significant increase in urea, BUN and creatinine level along with changes in liver function that represented by a significant increase in total bilirubin. However, the SGPT and SGOT levels did not change. This was due to the fact that an increase in SGPT and SGOT enzymes usually occurred after hepatocyte cell damage. The increase in liver enzymes occurred when there was inflammation in the liver after more than 24 hours[18].

In the *E. coli* group and control group, both showed a significant increase in TNF-α pro-inflammatory mediators, PCT, MDA, urea, and BUN levels. There was also a very significant increase in hs-CRP level, creatinine level, and bilirubin total level. Nevertheless, neither SGPT nor SGOT showed any improvements. *E. coli* i.p. injection can be used as one of the options to create a sepsis rat model which was easier to do and did not hurt experimental animals compared to the CLP method.

Conclusion

In summary, there was a significant increase in TNF-α pro-inflammatory mediators, procalcitonin (PCT), malondialdehyde (MDA), urea kidney function parameters and BUN among treatment groups. The hs-CRP, creatinine, and total bilirubin experienced a significant increase.

Conflict of Interest : The author declare that they have no conflict of interest.

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Ethical Approval: This study was approved by the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

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Stress Related Temporomandibular Joint Disorders - A Comparative Study between Preclinical and Clinical Undergraduate Students of Dentistry

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Abstract

Objective: This cross-sectional study compared the proportion and severity of stress related TMD, gender predilection and influence of parafunctional habits in preclinical and clinical undergraduate students of dentistry.

Method: The data was collected using Fonseca's Anamnestic index and Zung's Self-rating Anxiety Scale. The results were analyzed as proportions and percentage. Chi square test was used. A 'p value' of less than 0.05 was considered as significant.

Results: Mild to moderate anxiety levels were seen in 6.4% females and 3.4% males .According to the FAI, 48.2% females and 67.8% males did not have TMD. Mild, moderate and severe TMD were seen in 41.1%, 9.2% and 1.4% of the females and 27.1%, 5.1% and 0% males respectively.

Conclusion: While the proportion of stress was higher in preclinical students, TMD was higher in clinical students. TMD observed in clinical students could be a manifestation of the stress they experienced in their former years.

Key Words – *Temporomandibular joint, Masticatory Muscles, Stress, Bruxism, Undergraduate, Dentistry, University, Students*

Introduction

Temporomandibular Joint Disorders (TMDs) are a comprehensive term, used to describe numerous related disorders involving the Temporomandibular Joints (TMJ), masticatory muscles, and occlusion. It is characterized by symptoms such as TMJ pain, restricted mouth opening, muscle tenderness and intermittent joint sounds. Secondary symptoms such as muscle fatigue, radiation of pain to the neck and temple, headaches and incoordination of jaw movement may also be present. [1-3]

The etiology of TMD is multifactorial, including biomechanical, neuromuscular and psychosocial factors such as traumatic injuries, occlusal interferences, postural changes, parafunctional habits such as bruxism, dysfunction of masticatory muscles, intrinsic and extrinsic changes of the structure of the TMJ, and emotional stress. [1,4]

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Emotional stress in individuals may lead to insidious habits such as bruxism. Bruxism is the involuntary grinding or clenching of teeth. It is usually an unconscious activity, whether the individual is awake or asleep; often associated with fatigue, anxiety, emotional stress, or fear, and frequently triggered by occlusal irregularities. Prolonged clenching results in excessive workload on the TMJ and the muscles of mastication, which leads to strain and fatigue of the muscles, causing TMD. [4,5]

Undergraduate students of dentistry are expected to excel in both theoretical and clinical knowledge and obtain a holistic approach to Dentistry. As an attempt to achieve this goal, dental institutions set up a heavy curriculum of lectures, with a composite union of both medical and dental subjects. Furthermore, the preclinical students (1st and 2nd year) experience a sudden change in curriculum, coupled with an unfamiliar living environment and increased academic load. On the contrary, the clinical students (3rd and 4th year) experience a sudden change from model work to clinical practice dentistry and have numerous clinical quotas to complete throughout the course.[6-8]

Innumerable published literature assessed the prevalence of TMD among university students. Most studies focused on the prevalence of signs and symptoms of TMD among students with variables such as age, gender or field of professional education, without marked emphasis on the causes of TMD, particularly, emotional stress.

This study was an effort to establish the relationship between emotional stress and TMD and to estimate and compare the proportion of stress related TMD in preclinical and clinical undergraduate students of dentistry, along with variables such as gender predilection and the influence of parafunctional habits. [9,10]

Materials and Methods

This cross-sectional study was conducted in Manipal College of Dental Sciences, Manipal Academy of Higher Education, Mangalore, Karnataka, India, among undergraduate students of Dentistry aged 17 to 24 from December 2018 to June 2019.

All full-time students willing to give written consent participated in the study. Students with a history of trauma to head and neck and those receiving orthodontic treatment were excluded.

This study was approved by the Institutional Ethics Committee of Manipal college of Dental Sciences, MAHE. An Informed Consent was taken from all students, and confidentiality of the study data was maintained. Out of 180 preclinical students and 200 clinical students 100 each of preclinical and clinical student agreed to take part in the study.

Sample Size:

With 95% confidence level and 92.1% power (d = 10%) with reference to p=50%, sample size comes to be 200, with 100 students each of preclinical and clinical students.

(n = 100×2 = 200)

$$n = [Z_\alpha^2\ p\ q] / d^2$$

Z_α = 1.96 at 50% confidence level

d = 10% relative precision

Sample selection:

The study questionnaires will be distributed among 200 students, 100 each of preclinical and clinical years. Among preclinical students, 50 students each will be selected at random from first and second year. Among clinical students, 50 students each will be selected at random from third and fourth year.

Sampling method: Stratified random sampling

The demographic details, the information sheets and study questionnaires (Fonseca's Anamnestic Index[11,12] and Zung's self-rating anxiety scale[13,14]), were distributed among 200 students, 100 each of preclinical and clinical years by Stratified Random Sampling. Among the preclinical students, 50 students each were selected at random from first and second year. Among the clinical students, 50 students each were selected at random from third and fourth year. The students were briefed about the questionnaires. After obtaining written informed consent, the subjects were asked to complete and submit the questionnaires.

Following the collection of the questionnaires, a short session was conducted by the chief investigators of the study to enlighten the students on the prevalence, causes, the signs and symptoms of TMD, with a special emphasis on the treatment options, and the methods to provide symptomatic relief for the same.

The data was collected and tabulated in Microsoft Excel and analyzed using the SPSS version 17.0. The results were analyzed as proportions and percentage. Chi square test was used to determine the strength of association between the variables. A 'p value' of less than 0.05 was considered as significant.

Ethics Approval number: 17134

Results

Of the 200 students (100 each of preclinical and clinical years) aged 18 to 23, females and males were 70.5% and 20.5% respectively. According to the FAI, 59% of the Preclinical Students and 49% of the Clinical Students did not have TMD. Mild, moderate and severe TMD was seen in 34%, 7% and 0% of the Preclinical Students and 40%, 9% and 2% of the Clinical Students respectively (Chi square test value=3.662, p=0.300). According to the FAI, 48.2% of the female students and 67.8% of the male students did not have TMD. Mild, moderate and severe TMD was seen in 41.1%, 9.2% and 1.4% of the female students and 27.1%, 5.1% and 0% male students respectively (Chi square test value=6.884, p=0.076). According to the Zung's SAS, 92% of the Preclinical Students and 97% of the Clinical Students had normal levels of anxiety. Mild-moderate anxiety levels were seen in 8% of the Preclinical Students and 3% of the Clinical Students. 'Marked to severe' and 'extreme' anxiety levels were not seen in the students (Chi square test value=2.405, p=0.121). According to the Zung's SAS, 93.6% of the female students and 96.6% of the male students had normal levels of anxiety. Mild-moderate anxiety levels were seen in 6.4% of the female students and 3.4% of the male students (Chi square test value=0.717, p=0.397).

Table 1: Group-wise and gender-wise score on Zung's Scale among the study participants

	NORMAL	MILD-MODERATE
Group		
Preclinical	8%	92%
Clinical	3%	97%
Gender		
Female	132 (93.6%)	9 (6.4%)
Male	57 (96.6%)	2 (3.4%)

[p value (Group-wise) = 0.121, p value (Gender-wise) = 0.397]

Discussion

This cross-sectional study investigated the association of emotional stress and TMD. It was observed that the proportion of TMD was higher in the clinical students as compared to the preclinical students. While 'severe TMD' (according to the FAI) was not seen in the Preclinical Students, 2% of clinical students experienced it. The female students had a higher proportion and severity of TMD as compared with male students. While 'severe TMD' was not seen in the male students, 1.4% of the female students experienced it. The proportion of stress was higher in preclinical students as compared with clinical students. Female students had a higher incidence of stress as compared with male students. Both 'marked to severe' and 'extreme' stress levels (According to the Zung's SAS) were not seen in the students.

Wahid et al. [9] performed a similar study on the prevalence and severity of TMD in undergraduate university students using the FAI, which was also used to obtain information on the presence of stress among students. It was a cross-sectional study conducted among four medical disciplines, namely, Dentistry,

Medicine, Pharmacy and Physical therapy. Out of 137 students who enrolled, 7.9% were observed with 'No TMD', 44.3% with 'Mild TMD', 44.3% with 'Moderate TMD' and 3.6% with 'Severe TMD'. It was observed that the MBBS students experienced severe TMD more commonly as compared to the other disciplines. The authors concluded that the mean level of stress and TMD is almost equivalent in the different medical disciplines, with a variation in only its level of severity. [10]

Augusto et al.[12] studied the prevalence of TMD and its association with perceived stress and Common Mental Disorders (CMD) in students. The FAI, perceived stress scale, and the self-reporting questionnaire (SRQ-20) was used for data collection respectively. The prevalence of TMD among the students was 71.9%, distributed as Light TMD dysfunction (50%), moderate TMD dysfunction (16.4%) and severe TMD dysfunction (5.5%), with a higher frequency seen among women (76.4%). It was thus concluded that there is a significant correlation between TMD and variables such as para-functional habits, perceived stress and CMD. [13]

Nomura et al.[10]assessed the prevalence of TMD among 218 dental students using the FAI. The group consisted of 96 men and 122 women, with an average age of 20 years. Of the students, 53.21% were observed with TMD, with 35.78% showing mild TMD, 11.93% moderate TMD and 5.5% severe TMD. While considering severe TMD alone, women were affected approximately 9 times more than men. Amongst the students with TMD, 76.72% students considered themselves tense people; 71.55% reported to clench or grind their teeth; 65.52% reported clicking of the TMJ; 64.66% reported frequent headache and 61.21% reported neck pain. [11]

Habib et al.[3] conducted a questionnaire study on the prevalence and severity of TMD among male university students using the FAI. In addition to this, the role of relevant medical and dental histories in the assessment of TMD was also addressed. It was reported that psychological stress (30.5%) and direct restoration (77%) were the most common entries in the dental and medical histories. [3]

Notable similarities exist between the study conducted and the ones mentioned above, namely, the common use of the FAI for assessing the presence of TMD with no supplemental clinical examination, and the analysis of emotional stress, para-functional habits, occlusal interferences and gender as influencing factors for the prevalence and severity of TMD.

A few distinct characteristics make this study unique. Of the ten questions in the FAI, only one gives information about the presence of emotional stress among the students. While most other studies relied on the FAI to evaluate stress, this study made use of the Zung's SAS for this data. Another salient component of this study was the educational session conducted by the chief investigators to brief the students on the prevalence, causes, signs and symptoms of TMD, with a special emphasis on the treatment options for the same.

Clinical examination to assess the signs of TMD was not performed in this study and may be considered for the next phases of the study. Due to feasibility issues and time constraint, it was not possible to conduct a cohort study, which would be the ideal method to establish causal association.

Conclusions

The proportion of stress was higher in preclinical students, whereas TMD was higher in clinical students. It can be inferred from these results that the increased stress levels in the preclinical students may be a consequence of a sudden change in curriculum, coupled with an unfamiliar living environment and increased academic load. The clinical students, on the other hand, may be more accustomed to the program. The symptoms of stress related TMD manifest over a period of time, as a result of the pathological changes in the structure of the TMJ and masticatory muscles. Thus, TMD experienced in the clinical students could be a manifestation of the increased stress levels that the students experienced in their former years.

Conflict of Interest : None declared

Source of Support : None

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Risk Factors Related to Mastalgia

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Abstract

Background : Breast pain becomes more complicated in many humans. Various hospitals are planning for surgery in the initial stages. The main aim of this paper to find the various factors which cause mastalgia, this is mainly causing the abnormal conditions of the breast.

Methods: The experiments conducted on 400 patients and this is collected from various surveys, for patients older than the age of 40 the mammography and ultrasonography (US).

Results: The mean age was 44.23 ± 9.23 years. This group consists of 300 cases, the asymptomatic group consists of 100 persons. Due to the various reasons that are related to mastalgia ($p < 0.05$). These are based on their breastfeeding to the child. If it is more than 4 times or more were higher in the mastalgia group ($p < 0.04$). The breast imaging-reporting and data system (BI-RADS 2) mammography results were associated with mastalgia ($p < 0.05$). These two are more common in the mastalgia group ($p < 0.05$) that is Fibro cysts and fibro adenomas. Based on the previous history the malignant breast disease was simultaneously higher in the mastalgia group ($p < 0.05$).

Conclusions: Life stress, high caffeine consumption, smoking, lactation frequency, and benign disorders were factors identified to be related with mastalgia. Though a significant relation between mastalgia and malignant breast disease was detected in our study, more studies are still necessary to investigate this relationship.

Keywords: *Mastalgia · Surveys · Mammography. US findings. Pain · Surgery*

Introduction

The most common factor in women is Breast pain nothing but mastalgia which causes breast tenderness, hard burning sensation or tightness in the tissue, the pain may increase or maybe constant and this will occur some other times.[1] Due to the pain in the breast and tenderness, many women are fear with early symptoms of breast cancer, generally, these are not considered as disorders.[2-3]

Mastalgia is classified into two types such as cyclic breast pain (CBP) or non-cyclic breast pain (NCBP). CBC affects women at the age of 20 to 30 and it is up to 40 also. But still, it is menstruating. Mainly the pain occurs at the end of the menstrual cycle week or before the period occurs. At this time the breast is affected with the painful, tender, and swollen, based on the cycle the symptoms improve at another point. The pain occurs at the breast outer and upper parts of two breasts and this involve the underarm area as well. In the luteal period, the result of water increased in breast stroma produced by increasing hormone levels.[3-5] Menopause is the non-cyclic mastalgia. This may like sharpness, burning, or soreness in the breast.

Causative pathophysiology of pain stays hazy however is believed to be known with secretion factors (e.g., hormone-substitution treatment, oral contraceptives, pregnancy, menses, pubescence, and menopause) these variables will modify the steroid, Lipo-Lutin and prolactin levels.[6] Kind breast issue (e.g., fibrocystic changes), mind-expanding drugs, psychosocial factors and passionate stress square measure connected with breast torment.[2-7] Lactation problems (e.g., engorgement, mastitis, and breast ulcer), inflammation (Tietze disorder), Postthoracotomy disorder, Spinal and paraspinal issue and Referred torment (e.g., pneumonic, heart, or vesica sickness) will likewise cause breast torment. Caffeine and vasoconstrictive utilization square measure likewise

viewed as related with pain.[1] Cancer is a rare reason for breast torment.[4]

Breast pain associated with malignant growth is one-sided, non-cyclic, and extremely a lot of confined.[8]

The fundamental center is to research the components of inpatient history that influences the event of breast pain, and to decide the connection among mastalgia and considerate or malignant breast utilizing a huge arrangement of members.

Method

The research was completed in an exceedingly non-public Surgery facility in Iraq, between September 2018 and August 2019. This experiment was affirmed by the local human morals committee. The patients were relegated to at least one of 2 gatherings: the pain gathering, from women, admitted to the breast surgery outpatient center with mastalgia (n = 300); and therefore the symptomless gathering (the management gathering), including patients undergoing routine breast screening (n = 100). Patients UN agency was treated with oral contraceptives, psychoactive specialists, secretion medical care, or vas operators, even as patients below 18, or over 70 older were banished from this research.

A standard summary study for all patients within the groups was performed regarding their enthusiastic anxiety, utilization of caffein and phytotoxin. AN intensive physical assessment was performed for all patients. The gap between the areola and therefore the os indent was calculable, any length surpassed 33 cm was thought of as macromastia. Breast screening was done by means that of the diagnostic procedures for patients skilled than the age of 40; imaging (US) was done once necessary for any age. Breast thickness bit by bit will increase to type D, during this manner, drop-off the affectability of the diagnostic procedure. What is more, the imaging discoveries in the diagnostic procedure were ordered accordant to the Breast Imaging-Reporting and information system (BI-RADS)? The BI-RADS classes are characterized as BI-RADS zero, fragmented; BI-RADS one, typical; BI-RADS2, thoughtful finding(s); BI-RADS three, presumptively generous; BI-RADS four, suspicious abnormality; BI-RADS five, deeply resembling harm; and BI-RADS vi, presence of biopsy-demonstrated danger.

The NCSS 2007, and PASS 2008 Statistical Software (Utah, USA) programs were used for measurable examination to perform the mean, variance, return and proportion, the Mann-Whitney U take a look at was used to affirm a normal dispersion condition. Fisher's actual take a look at, Pearson's chi-squared take a look at, and Yates' progression rectification were used for the subjective evaluations of the data. Contrasts were thought of statistically vital once $p < 0.05$.

Results

Overall 400 females with a males period of 44.23±9.23years (approx 19-65) were enlisted. The mastalgia gathering, 300 patients, and, the well gathering, two hundred patients. Guys age was basically higher within the pain gathering (p = zero.001) (Table 1).The rate range of patients World Health Organization admitted extended gung ho feeling of tension was higher within the pain gathering (p < zero.005). alkaloid use and smoking were known with pain (p < zero.05). The lactation repeat rate disclosed associate degree on a awfully basic level higher association with bosom torment within the pain gathering (p < zero.05). The sort, shape, and laterality of bosom torment within the pain bundle area unit consolidated in Table a pair of. the speed range of patients of the pain bunch eaten soul, paracetamol, and non-steroid assuaging medicine were primarily higher (p < zero.05) (Table 3).

A total of 280 patients was given to mammographic screening, 209 patients from the pain gathering, and seventy one patients within the well gathering. The unendingly visit bosom thickness configuration was composed associate degree within the well gathering, and sort B saw as increasingly typical within the pain gathering (p < zero.01). In step with the BI-RADS categories, BI-RADS one was increasingly customary within the well occasion and BI-RADS a pair of within the commanding within the pain gathering (p < zero.05). The analysis of the U.S.A. photos disclosed the events of elementary bruises and fibro adenomas were higher within the pain gathering (p < zero.05). The assessment of the tomography discoveries between the 2 social occasions shows up in Table four.

Different sorts of six patients resolved to own BI-RADS four were within the pain gathering. Once the diagnostic assay, the psychoneurotic evaluations disclosed that 3 of those patients had a unsafe contamination, agitating cautious intervention.

Table 1. the comparison between the mastalgia and asymptomatic groups patients

		Total	Mastalgia	Asymptomatic	p
Age mean ± SD, years		44.23±9.23	45.25±9.50	42.85±10.20	0.001
Patients, n		400	300	100	
Stress, n (%)	none	85 (20.5)	52 (17.3)	33 (33.0)	0.001
	stressful	315 (79.5)	248 (82.7)	67 (67.0)	0.002
Caffeine consumption (coffee), n (%)	none	250 (62.5)	170 (56.7)	80 (80.0)	0.003
	1 cup/day	65 (16.2)	60 (20.0)	5 (5.0)	0.012
	≥ 2 cups/day	85 (21.3)	70 (23.3)	15 (15.0)	0.012
Smoking (cigarettes), n (%)	None	280 (70.0)	205 (68.3)	75 (75.0)	0.004
	5-10/day	71 (17.8)	55 (18.3)	16 (16.0)	0.125
	>10/day	49 (12.2)	40 (13.3)	9 (9.0)	0.007
Lactation (breast-fed infants), n (%)	0	30 (7.50)	10 (3.30)	20 (20.0)	0.001
	1	39 (9.80)	22 (7.30)	17 (17.0)	0.005
	2	97 (24.2)	65 (21.6)	32 (32.0)	0.154
	3	72 (18.0)	60 (20.0)	12 (12.0)	0.001
	≥ 4	162 (40.5)	143 (47.7)	19 (19.0)	0.035
BSO±TAH, n (%)	No	384 (96.3)	294 (98.0)	90 (90.0)	0.001
	Yes	16 (4.0)	6 (2.0)	10 (10.0)	0.005
Menopause, n (%)	premenopausal	312 (78.0)	252 (84.0)	60 (60.0)	0.001
	postmenopausal	88 (22.0)	48 (16.0)	40 (40.0)	0.002
Breast size, n (%)	Normal	368 (92.0)	280 (93.3)	88 (88.0)	0.001
	macromastia	32 (8.0)	20 (6.7)	12 (12.0)	0.002
Recent breast trauma, n (%)	No	391 (97.8)	292 (97.3)	99 (99.0)	0.055
	Yes	9 (2.2)	8 (2.7)	1 (1.0)	0.058
Breast cancer, n (%)	No	393 (98.2)	295 (98.3)	98 (98.0)	0.015
	Yes	7 (1.8)	5 (1.7)	2 (2.0)	0.005

SD, BSO±TAH: history of a prior bilateral salpingooophorectomy with or without total abdominal hysterectomy.

Table 2.Mastalgia patients' based on the pain the distribution is done

Breast Pain	Type	N	%
Radiation	Diffused	140	46.7
	Localized	160	53.3
Laterality	Bilateral	61	20.3
	Unilateral	239	79.7
Relation with menstruation	Cyclic	205	68.3
	Non-cyclic	95	31.7

Table 3. Analgesic consumption comparison between the mastalgia and asymptomatic groups

Analgesic consumption n (%)	Total	Mastalgia	Asymptomatic	P
None	350 (87.5)	255(85.0)	95(95.0)	0.001
Aspirin	9(2.2)	8(2.7)	1(1.0)	0.011
Paracetamol	17(4.3)	15(5.0)	2(2.0)	0.001
NSAIDs	24(6.0)	22(7.3)	2(2.0)	0.021

Table 4. Comparison of mammography and ultrasound findings between the mastalgia and asymptomatic groups

	Total, n (%)	Mastalgia, n (%)	Asymptomatic, n (%)	p
Mammographic breast density (pattern) (n=280)				
A	57 (20.4)	12 (5.7)	45 (63.4)	0.001
B	172 (61.4)	160 (76.6)	12 (16.9)	0.001
C	44 (14.7)	34 (16.3)	10 (14.1)	0.010
D	7 (2.5)	3 (1.4)	4 (5.6)	0.020
Mammographic category (n=280)				
BI-RADS 0	160 (57.1)	101 (52.3)	59 (67.8)	0.073
BI-RADS 1	30(10.7)	16 (8.30)	14 (16.1)	0.001
BI-RADS 2	70(25.0)	60 (31.1)	10 (11.5)	0.001
BI-RADS 3	10(3.57)	7 (3.63)	3 (3.45)	0.014
BI-RADS 4	6(2.14)	6 (3.11)	0 (0.0)	0.021
BI-RADS 5	4(1.4)	3 (1.55)	1 (1.15)	0.002

Cont... **Table 4. Comparison of mammography and ultrasound findings between the mastalgia and asymptomatic groups**

Ultrasound findings (n=285)				
Normal	35(12.3)	25 (13.5)	10 (10.0)	0.003
Simple cysts	20(7.02)	20 (10.8)	0 (0.0)	0.001
Fibroadenomas	45(15.8)	35 (18.9)	10 (10.0)	0.039
Fibrocystic disease	180(63.2)	100 (54.1)	80 (80.0)	0.015
Lymph nodes	3(0.78)	3 (1.62)	0 (0.0)	0.014
Complex cysts	2(0.70)	2 (1.08)	0 (0.0)	0.004
Fatty tissue necrosis	1(0.35)	1(0.54)	0 (0.0)	0.004

Discussion

Various factors are effecting mastalgia, The author [9] portrayed that the period of women grumbling of the pain in breast was in the range 35-55 years and that this side effect was once in a while experienced younger than 45. Present research, the male age of the all-out investigation group was 44.23±9.23years (territory 19-65 years), and the male age of the mastalgia group was higher lighting up that mastalgia happens in the perimenopausal period when the inconsistency of hormonal and menstrual beginnings. Numerous studies showed that there is a connection between breast torment and mental strain.[10–12] In this paper, the patients how had an unpleasant lifestyle were essentially higher in the mastalgia gathering. Numerous concentrates uncovered a connection between caffeine utilization and smoking with breast torment.[1-3-6] In our examination, there was a critical association between either standard caffeine utilization or substantial smoking (in excess of 10 cigarettes every day) and mastalgia.

Anomalies of the estrogen/progesterone proportion, which can happen on the off chance that one of these hormones increment, the other one will diminish, or expanded prolactin levels are identified with mastalgia.[13-14] The event of breast side effects, for example, expanding, irritation, and nodularity in the menopausal period, at that point the end of these manifestations in the postmenopausal period is potential because of the impacts of the estrogen hormone.[15-16]

In perspective on lactation, the level of ladies with a background marked by breast feeding4 or more newborn children were expanded in the mastalgia gathering. These outcomes shed light on the connection of breast torment with the expanded frequencies of lactation, which is identified with an expanded number of births, and therefore with an expanded introduction to significant levels of estrogen and prolactin. Additionally, at least 4 times of lactation may bring about anatomical changes in the breast tissue, particularly in the ductal framework, which could likewise expand the breast torment episodes.

An earlier reciprocal salpingo-oophorectomy with or without absolute stomach hysterectomy was identified with a fundamentally diminished event of mastalgia.

The mastalgia side effects which coordinate the announced investigations that found a huge connection between the asymptomatic gathering and postmenopausal state.[17-18]

Macromastia or breast hypertrophy is an uncommon ailment of the breast connective tissues wherein the breasts become exorbitantly enormous. In the event that the separation between the sternal score and the areola surpassed 33 cm this means that macromastia.[19] In our examination, the recurrence of macromastia was altogether lesser in the mastalgia gathering, recommending that macromastia probably won't be one of the components that reason mastalgia. Mastalgia may happen attributable to greasy tissue rot, or a strain in the Cooper tendons as aftereffects of either dull or entering

injury to the breast. In our investigation, higher paces of injury history found in the mastalgia gathering.

Extreme mastalgia can happen in 10–22% of patients and requires medicinal medications. As an initial step approach, analgesics In our present examination, the mastalgia bunch utilized pain-relieving specialists more than the asymptomatic gathering, and the most picked pain relieving type was NSAIDs.

As breast thickness builds, the mastalgia does. In our examination, the sort B breast design was higher in the mastalgia gathering. Different designs demonstrated immaterial contrasts between the two gatherings. Mastalgia and generous breast issue is altogether related. Our examination indicated a higher recurrence of mastalgia in patients with discoveries of benevolent issue (BI-RADS 2). The connection between breast torment and kind issue distinguished utilizing the US is likewise easily proven wrong .The author announced that fibroadenomas and basic sores were the most widely recognized existing together benevolent sores.

Furthermore, the rate of fibrocystic malady recognized by the US was essentially higher in patients with mastalgia. Since the fibrocystic ailment is a substance demonstrating a summed up inclusion of the breast parenchyma, that it prompts diffuse breast torment isn't startling. Despite the high likelihood of the dwelling together of a dangerous breast tumor with non-cyclic breast torment, albeit uncommon, repetitive mastalgia can likewise be identified with harm.

Despite the fact that the aftereffects of our examination uncover a relationship among mastalgia and danger, this issue is as yet dubious, and there is as yet a requirement for enormous future investigations to uncover the relationship more precisely.

Conclusion

Symptoms like stress, coffee consumption, smoking, and other abnormal disorders are the key points found which is related to mastalgia. There is a relation between mastalgia and malignant breast disease is found in our research. In the future, more research is to be done.

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Verbal Aggressive Behaviours of Elementary School Age Children

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Abstract

Background: Verbal abuse has negative effects that will ultimately affect child development. Verbal abuse committed by parents may potentially leave a trauma in children's lives and affect their growth and development. Children's social environment where they carry out interactions may be the factor of verbal abuse of children.

Methods: The design of this study is cross-sectional, with total population of 328 children of school age. 259 children were selected with proportional random sampling technique. The independent variables were parental verbal abuse and psychosocial factors, while the dependent variable was children verbal aggressive behaviors. The instrument used was questionnaire sheets. The analysis performed was Logistic Regression Analysis.

Results: Verbal abuse behaviors were influenced by parental verbal abuse factor (p=0.003) and psychosocial factor (p=0.03).

Conclusions: Parenting style and children's social environment contribute to the probability of children verbal abuse. Family intervention is highly expected to prevent aggressive child behavior.

Keywords: *Verbal abuse, Verbal aggressive, School-age children*

Background

Children verbal aggressive behaviors are uncontrolled child behaviors that typically occur in home and school environment. These behaviors are the result of parental behaviors that unknowingly shape verbal aggressive behaviors.[1] The way parents communicate rules to their kids may have been done in an improper way. Sharp utterances that are deemed "okay" to be spoken such as calling children by names they do not like, underestimating children's abilities in front of their siblings, or yelling harsh words are forms of verbal abuse communications. That verbal abuse is common among Home Care aides and is strongly associated with physical abuse. We identified verbal abuse risk factors for which preventive interventions can be implemented. Child aggressive behaviors will certainly affect their psychological and psychosocial development in the subsequent stage of their growth.

Verbal abuse has negative effects that will ultimately affect child development. Children will constantly feel threatened and have difficulties to think ahead which in turn will cause them to act based on instinct without much consideration, replicating communications and utterances of their parents, resulting in verbal aggressive behaviors. Based on the record of The National Commission for Child Protection (*KPAI*), the number of violence against children shows an increase. In 2011 there were 261 cases of child abuse. In the past 4 years, cases of violence against children reached the highest rate in 2013 with a total of 1,615 cases. KPAI received 622 reports of cases of violence against children from January to April 2014 ranging from physical violence, psychological violence and sexual violence. There were also 12 cases of emotional violence. Of this amount, 51% of children experienced violence in the family, while 28.6% of children experienced violence in the school environment and 20.4% of children had experienced

violence in the community. As many as 60% are victims of mild violence in the form of verbal abuse or insults. Verbal communication abuse in children is mostly committed by parents.[2]

Verbal abuse is emotional abuse or treatment that hurts the child's feelings continuously so that it causes a bad and continuous influence on the child's emotional development, which includes the use of language which implies that the child is worthless or unloved, incompetent, and other utterances that describe parents' unmet expectations that are not appropriate to the child's age and child development, to the neglect and abandonment of the child's basic needs.[3] Verbal abuse against children will cause heartache and make children think that they are what their parents often say they are. If a parent says a child is stupid or ugly, then the child will think of himself that way.[4] Children will imitate the behavior of older people. If they are exposed to behavioral or verbal abuse then the child will do the same thing to others, and it will always be imprinted in their minds.[5]

These acts of violence can have physical or psychological effects. Physical effects may include actions that result in pain, bruising, broken bones to permanent disability.[6] Psychological effects may include fear, anger, sadness, guilt, confusion, loss of self-confidence and/or severe psychological suffering and even rebellion and avoidance of the environment. Efforts in preventing the occurrence of aggressive behavior in children is an effort that can be done in the family environment, school environment and community environment. The purpose of this study is to look closer at the effect of verbal abuse by parents and psychosocial conditions on aggressive verbal behavior in school-age children.[7]

Methods

This study used analytic research design with cross-sectional approach. The purpose of this study was to determine the factors influencing verbal aggressive behavior of elementary school-age children. The independent variable of this study was parental verbal abuse behavior and child psychosocial factors, while the dependent variable was child verbal aggressive behaviors. The population in this study were all students aged 11-12 years in Surabaya and Jombang Elementary Schools. The sample in this study were all sixth-grader students aged 11-12 years old at the Elementary Schools of Surabaya and Jombang with 259. The research sample was selected using a proportional random sampling technique. The instrument used in this study was a questionnaire. This study used the following instruments: Parental verbal abuse behavior questionnaire based on theories of parental verbal abuse forms. The questionnaire used the Guttman scale with fifteen (15) questions with "Yes" answer being worth one (1) and "No" answer being worth zero (0). The children's aggressive behavior questionnaire aimed to identify the forms of aggressive behavior that children did verbally which was measured based on five verbal aspects namely mocking, cursing, forcing, yelling, and scaring. Psychosocial instruments used instruments developed by the researchers, containing 8 questions, with answer "Yes" being worth (1) and "No" being worth (0). Data was collected at elementary schools in the Surabaya and Jombang areas. All instruments had been tested for validity and reliability. Data analysis was performed using logistic regression statistical tests.

Results and Discussion

1. Respondent Characteristics Based on Parents' Professions and Educations

Table 1. Respondent Characteristics Based on Parents' Professions and Educations

No.	Fathers' Professions	Total	(%)
1	Unemployed	15	5,7
2	PNS	65	25,1
3	Self-employed	107	41,3
4	Nomadic job	72	27,8
	Mothers' Professions		
5	Unemployed	74	28,6
6	PNS	19	7,3
7	Self-employed	95	37,2
8	Nomadic job	71	27,4
	Fathers' Education		
9	SD	22	84,9
10	SMP	89	34,3
11	SMA	105	40,5
12	Sarjana	43	16,6
	Mothers' Education		
13	SD	56	21,6
14	SMP	88	34
15	SMA	104	40,1
16	Sarjana	11	4,3

Based on the above table, it was known that there were 107 (41.3%) self-employed fathers and 95 (37.2%) self-employed women. While fathers with high school education totaled 105 (40.5%) people and mothers with high school education totaled 104 (40.1%) mothers.

2. Child verbal aggressive behavior based on parental verbal abuse and child psychosocial factors.

Independent Variables	Mild Child Aggression		Severe Child Aggression		F	%
	N	%	N	%	N	%
Mild Child Psychosocial	109	81,3	25	18,6	134	51,7
Severe Child Psychosocial	85	68	40	32	125	48,3
Mild Parental Verbal Abuse	164	79,6	42	20,4	206	79,5
Several Parental Verbal Abuse	30	56,6	23	43,4	53	20,5

Table 3. Independent Variable Significance

	Independent Variables	Significance Value
1.	Parental Verbal Abuse	0,003
2.	Child Psychosocial Factor	0,001
Logistic Regression Analysis, Nagelkerke R Square 0,080 squere, $\alpha = 0,05$		

Based on the above table, all independent variables significantly affected child aggressive behaviors, with significance value of parental verbal abuse of 0.003 and psychosocial factor of 0.001.

Discussion

Child psychosocial factors are one of the causes of aggressive behavior in addition to biological, familial, socio-cognitive, peer groups or groups, academic, community school factors.[8] Biological factors are related to genetic temperament, family factors are related to parenting, environmental factors are related to group pressure or rejection. Environmental factors also influence acts of violence on children. The environment can increase the burden of child care and cause sudden environmental problems which also play a role in verbal violence. Television as the most effective media in conveying various messages to the wider community has the highest potential to influence the violent behavior of parents in children.[9] Messages targeting the wider community have the potential to influence the violent behavior of parents in children. A study conducted by Fitriana et al. in 2015 showed that there was a relationship between the respondent's environment and the possibility of parents verbally abusing pre-school children in Pendowoharjo Village, Sewon District, Bantul Regency.[10] The environment has a big influence on the behavior of parents in verbal abuse against pre-school children. In accordance with the result of the study, it is known that parents who have a good environment have behaviors that tend not to verbally abuse their children. Conversely, parents who have a bad environment tend to verbally abuse their children.[11]

The results of the verbal abuse study of parents showed a high statistical value for the category of severe verbal abuse. Verbal abuse is one type of violence that often occurs in children. Verbal abuse is all forms of actions or utterances that have the nature of insulting, yelling, cursing, and frightening by uttering inappropriate words.[12] Parental verbal abuse can occur because of minimal parental knowledge, where parents do not understand the process of child development, parents force children to do something and become angry when they could not, yelling and berating them. In

addition, parent economic factor may also be the cause of verbal abuse. In addition, parent economic factor may also be the cause of verbal abuse. Poverty can cause violence in children because of the increasing crisis in life and parents do not have room to look for economic resources. Because of these economic pressures, parents experience prolonged stress, become sensitive and eventually become irritable. Childhood verbal abuse may also increase risk for the development of PDs by increasing the likelihood that youths will experience profoundly maladaptive thoughts and feelings, such as mistrust, shame, doubt, guilt, inferiority, and identity diffusion during their most critical years of psychosocial development. [13]

Child Verbal Aggressive Behavior

The result showed that some children at elementary school age exhibited mild verbal aggressive behavior and some others exhibited severe verbal aggressive behavior. Aggressive behavior that appears in individuals is closely related to anger that occurs in individuals. Aggressive behavior may be caused by the following action: There is an attack from another person, where the individual will reflexively give rise to an aggressive attitude towards someone who suddenly attacks or hurts whether with words (verbal) and with physical actions; the occurrence of frustration in someone.[14]

When individuals experience frustration, anger can arise which can arouse feelings of aggression; the expectation of retaliation or motivation for revenge, that is, when an angry individual is able to take revenge, the anger will be greater and the possibility of aggression will also increase. A study conducted by Sulastri in 2017 showed that most of these students made verbal remarks that mocked, cursed, forced, shouted, and frightened to hurt the feelings of friends or for their own pleasure. This proved that the children had received verbal attacks from other people.[15] Verbal aggressive behavior could arise due to situations such as provocation. Since the provocation is seen by the aggressor as a threat that must be dealt with by an aggressive response to negate the danger implied by the threat. In the face of threatening provocation, aggressors tend to believe that rather than being attacked it is better to attack first, or rather than being killed it is better to kill.[16]

Factors Influencing Child Verbal Aggressive Behavior

The result showed that there was an influence of verbal abuse of parents and child psychosocial factors on verbal aggressive behavior of school-age children in elementary schools. Factors that influenced parental verbal abuse were internal factors which included parental knowledge, in which many parents did not understand children's developmental needs and force them to do things that they were actually incapable of doing and are angry at, shout and insult them and parenting experience factor in which a child who was abused by their parents will become aggressive and do what was done to them to their children when they become a parent. In social theory, it is stated that children imitate aggressive behavior committed by their parents, or imitation. This happens because a child has a high tendency to imitate. Violence that is seen or experienced by children continuously will form a mindset in children that the environment in which they live is not a safe place for them, so they will tend to be suspicious and cause aggressive behavior. Children who experience constant violence also tend to have lower self-esteem. Low self-esteem leads to negative attitudes and reduces coping when frustrated, thus causing an aggressive behavior tendency in children.[18]

The dominant influence is the social change in people's lives characterized by events that often cause tension, such as economic competition, discrimination, mass media (e.g. pornography, porno-action), recreational facilities (such as PlayStation), and night clubs, such as discotheques. This condition is a driving factor for the emergence of destructive (negative) behavior in adolescents. This study also identifies the community factors that become one of the environments for adolescents, indicated by several respondents' answers that answer "often" and "sometimes" which indicated the dominant agreement regarding activities in the community.[19]

Verbal aggression is an aggression committed by utterance intended to hurt other people that may take forms as insults, cussing, coercing, yelling and scaring. Factors influencing school-age child aggressive behavior include biological factors that are typically comprehensive in nature, meaning that they can

affect all aspects of behaviors, from intelligence to immunity against stress. Biological factors that may influence one's aggressive behaviors include genetics, cerebral system and blood chemistry. The next factor is psychological factors, where the aggressive behaviors exhibited are closely associated with anger within oneself. Relationships with peers are a source of social influence that is closely associated with aggression. An adolescent peer group can refer to neighborhood people, sports teams, friend groups, and acquaintance. Peer influence or peer group depends on the specific setting and context.[17]

Aggressive behavior can arise due to several reasons, one of which is when there is an attack from another person, the individual will reflexively exhibit aggressive attitude towards someone and suddenly attack or hurt with words (verbal) or with physical actions. One influential psychosocial factor is childhood trauma (psychological) or in other words, experiences that destroy a sense of security, a sense of ability, and self-esteem that cause psychological injuries that are difficult to cure completely. Psychological trauma experienced in childhood tends to continue to be carried into adulthood, especially if the trauma is never realized by the child's social environment.[17-19]

Conclusions

There is a correlation between parental verbal abuse and child psychosocial factor with elementary school-age child aggressive behavior. Parents are advised to carefully word their utterances when conversing with their children.

Conflict of Interest: Nil

Ethical Clearance: This research has been proved by Health Research Ethics Committee, Faculty of Public Health universitas Airlangga

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The Relationship between Obesity and the Incidence of Hypertension in Young Adults (18-40 Years) in Tomohon City in 2019

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Abstract

Hypertension is an unfinished health problem until now. Hypertension is a disease that causes stroke and decreases the quality of life. One of the preventable causes of hypertension is obese. The purpose of this study is observing the relationship between nutritional status, in this case obesity, and the incidence of hypertension in young adults (18-40 years) in Tomohon City. By utilizing a cross sectional approach, this research was conducted in January-December 2019 on young adults aged 18-40 years who visited 7 community health centers in Tomohon. There were 4,040 samples who were willing to be involved in this study, who had never previously been diagnosed with hypertension by a doctor in any health facility. Based on the Pearson's chi-square test showed a p-value of age and the incidence of hypertension is 0.0001 < 0.05 with estimated risk of 2.206 (CI 95%: 1.917-2.593). P-value of gender and the incidence of hypertension is 0.0001 < 0.05 with estimated risk of 0.715 (CI 95%: 0.622-0.823). P-value of obesity and the incidence of hypertension is 0.0001 < 0.05 with estimated risk of 2.502 (CI 95%: 2.150-2.911). So, it can be concluded that there is a significant relationship between obesity and the incidence of hypertension

Keywords: *hypertension, obesity, gender, age, tomohon*

Introduction

Hypertension is a leading cause of cardiovascular disease and premature death worldwide, especially in low and middle income countries like Indonesia[1]. It is also a serious public health issue[2] . North Sulawesi Province, in which there is Tomohon city, is one of the contributors to high hypertension rates in Indonesia. According to Indonesia Basic Health Research (Indonesian: *Riset Kesehatan Dasar/*Riskesdas), Tomohon is the city with the highest prevalence of obesity in Indonesia from 2007 to 2018[3]

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Based on data from Health Office of Tomohon, hypertension has consistently been one of the main metabolic diseases since 2009. Stroke as a result of hypertension has become a non-communicable disease that is prevalent in the Community Health Center in Tomohon. It is supported by hospital records in Tomohon which state hypertension as a metabolic disease suffered by both inpatients and outpatients. Direct survey shows that hypertension is included in the top 5 metabolic diseases in this population[4]

Furthermore, according to Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC7), hypertension can be classified into Stage 1 (systolic 140-159 mm Hg and/ or diastolic 90-99 mm Hg) and Stage 2 (systolic >160 mmHg and/or diastolic >100 mmHg)[5]. Most young adults are in the Stage I hypertension category and are unaware of the condition. They then continue to be exposed to risk factors until the disease becomes more

severe[6] .

Risk factors for hypertension consist of: 1) non-modifiable risk factors such as race, age, sex, and heredity (family history of hypertension); and 2) modifiable risk factors, related to changeable behavior, such as obesity, stress, smoking habits, sedentary behavior, excessive alcohol consumption, excessive salt intake and hyperlipidemia[7]

Obesity, accompanied by changes in hormonal levels, inflammation and endothelium, triggers the stimulation of several other mechanisms contributing to hypertension and increases cardiovascular morbidity[8]. Obesity is classified according to body mass index (BMI): Low body weight <18.5, Normal body weight 18.5–23.9, Overweight 24.0–27.9, Obesity ≥ 28[9]. Obesity is a serious global health problem. This is evidenced by the increasing trend of the prevalence of overweight and obesity in the last 20 years.

In Indonesia, the prevalence increases from 10.5% in 2007 to 21.8% in 2018. In adult males, there is an increase from 13.9% in 2007 to 19.7% in 2013. Additionally, in adult women, there is a very extreme increase reaching 18.1%, from 14.8% in 2007 to 32.9% in 2013. North Sulawesi Province is always in the first rank with a prevalence of 24.1% in 2013 and 30.2% in 2018. This situation is influenced by the extreme value of Tomohon, which is 33% in 2013 and around 40% in 2018[3,10]

Moreover, several studies have shown that obesity has a significant relationship with the incidence of hypertension. A research conducted by Framingham concluded that being overweight contributes to hypertension cases[11,12]. It is in line with a research by Jullaman claiming that BMI classified as obese has 1.64 times the risk of suffering from Stage 1 hypertension than normal BMI[13].

Based on the aforementioned background, the researchers are interested in observing the relationship between nutritional status, in this case obesity, and the incidence of hypertension in young adults in Tomohon.

Material and Methods

By utilizing a cross sectional approach, this research was conducted in January-December 2019 on young adults aged 18-40 years who visited 7 community health centers in Tomohon. There were 4,040 samples who were willing to be involved in this study, who had never previously been diagnosed with hypertension by a doctor in any health facility. Measurements were carried out using standardized tools and procedures by competent nurses. Blood pressure measurements were carried out 3 times; the average of the three measurements was recorded as a result by ensuring that respondents were resting (inactive) and calm before being measured.

Findings

Table 1 : Distribution of Variables

Variable	Category	Total (n=4040)	%
Body Mass Index	Underweight	268	6.6
	Normal	1544	38.2
	Over Weight	1221	30.2
	Obese	1007	24.9
Obese Incident	No (< 28 Kg/m2)	3033	75.1
	Yes (> 28 Kg/m2)	1007	24.9

***Cont.*.Table 1 : Distribution of Variables**

Systolic Blood Pressure	Normal	1499	37.1
	Pre Hypertension	1807	44.7
	Stage 1 Hypertension	554	13.7
	Stage 2 Hypertension	180	4.5
Diastolic Blood Pressure	Normal	1563	38.7
	Pre Hypertension	1490	36.9
	Stage 1 Hypertension	731	18.1
	Stage 2 Hypertension	256	6.3
Hypertension Incidence	No (< 140/90 mmHg)	2936	72.7
	Yes (> 140/90 mmHg)	1104	27.3
Gender	Male	1599	39.6
	Female	2441	60.4
Age	18-25 years	1545	38.2
	26-30 years	807	20.0
	31-35 years	777	19.2
	36-40 years	911	22.5

Table 1 showed that the proportion of males was 39.6% and females was 60.4%. There were 6.6% of people with BMI in underweight category, 38.2% of people with BMI in overweight category, and 24.9% of people with BMI in obese category. Thus, it can be said that there were 1,007 or 24.9% obese people (> 28 Kg/m^2) and 3,033 people or 75.1% who were not obese (< 28 Kg/m^2). Besides, systolic blood pressure classified as normal was 37.1%, pre-hypertension was 44.7%, stage 1 hypertension was 13.7%, and stage 2 hypertension was 4.5%. Diastolic blood pressure classified as normal was 38.7%, pre-hypertension was 36.9%, stage 1 hypertension was 18.1% and stage 2 hypertension was 6.3%. Thus, there were 2,936 people or 72.7% not included in hypertension category (< 140/90 mmHg), and 1,104 people or 27.3% included in the hypertension category (> 140/90 mmHg).

Table 2 : The Relationship of Incidence of Hypertension Based on Age, Obesity, and Gender

Variable	Category	Hypertension			p-value
		No	Yes	Total	
Age	18-30 years	1865 (63.5%)	487 (44.1%)	2352 (58.2%)	0.0001
	31-40 years	1071(36.5%)	617 (55.9%)	1688 (41.8%)	

Cont... **Table 2 : The Relationship of Incidence of Hypertension Based on Age, Obesity, and Gender**

Obese	No	2352 (80.1%)	681(61.7%)	3033(75.1%)	0.0001
	Yes	584 (19.9%)	423 (38.3%)	1007 (24.9%)	
Gender	Male	1097 (37.4%)	502 (45.5%)	1599 (39.6%)	0.0001
	Female	1839 (62.6%)	602 (54.5%)	2441 (60.4%)	

Based on Table 2, results of Pearson's chi-square test showed a p value of 0.0001 <0.05. It indicates that there was a significant relationship between age and the incidence of hypertension with estimated risk of 2.206 (CI 95%: 1.917-2.593), meaning that people aged >30 years were estimated to have 2.2 times the risk than people aged <30.

The results of Pearson's chi-square test obtained p value of 0.0001 < 0.05, indicating that there was a significant relationship between obesity with estimated risk of 2.502 (CI 95%: 2.150-2.911). It can be interpreted that people with a BMI > 28 Kg/m^2 or classified as obese were estimated to have 2.5 times the risk of experiencing hypertension than those who were 2.5 times the risk of developing hypertension compared to people who were < 28 Kg/m^2.

The results of Pearson's chi-square test showed a p value of 0.0001 <0.05, which means that there is a significant relationship between gender differences and the incidence of hypertension with estimated risk of 0.715 (CI 95%: 0.622-0.823). It indicates that males had 0.715 times the risk of experiencing hypertension. Therefore, it can be said that females were more at risk of getting hypertension than males at the age of 18-40 years.

Discussion

The results showed that women are more at risk of getting hypertension than men at the age of 18-40 years. It proves a theory that as the age increases, the organ function decreases, and the prevalence of hypertension differs between males and females[14-18]. Besides nearly a quarter (24.9%) of research subjects of young adults aged 18-40 years were obese, still far below the results of the 2013 Indonesia Riskesdas which claimed the prevalence of obesity was 33% in 2013 and about 40% in 2018[3,19].

An interesting point about these results is that there were 30.2% of subjects who were in the overweight category. If it is combined with obesity, more than half of young adults in Tomohon had non ideal body weight or tended to be fatter than they should be. This is much higher than the average prevalence of obesity from Riskesdas results in Indonesia; from 10.5% in 2007 and 21.8% in 2018. In addition, North Sulawesi Province is always in the first rank with a prevalence of 24.1% in 2013 and 30.2% in 2018[3,10]. The prevalence close to results in this study can be seen in a research by Integrated Development Post for Non-Communicable Disease of Port Health Office (POSBINDU PTM KKP) Bandung in 2016 on 202 samples showing that the proportion of overweight and obesity was 54.9%[6]. This is also in accordance with research which found that 53.61% of respondents are obese[19].

Results found that there were 2,936 people or 72.7% not included in hypertension category (< 140/90 mmHg), and 1,104 people or 27.3% included in the hypertension category (> 140/90 mmHg). These are close to results of research conducted by Samakul et al., in Tomohon, finding that the proportion of hypertension sufferers was 27.1% and respondents who did not suffer from hypertension were 72.9%[20]. This result is far above the 2018 Riskesdas results which found that, based on a doctor's diagnosis in North Sulawesi, the proportion of hypertension was 13.2%. It was the highest result in Indonesia, considering that the national average percentage was 8.4%. However, it was below the results of the 2018 Riskesdas measurement, which was 33.12% for North Sulawesi[3]. The proportion of

this research is still far from the proportion obtained by Anggraini et al., 62.89% of the subjects had high blood pressure or hypertension and results of Posbindu PTM KKP Bandung in 2016 which found that the proportion of stage 1 hypertension was 41.7%[6,19]

The results of this study indicate a significant relationship between obesity and hypertension in Tomohon. This is in accordance with several previous studies conducted in various regions in Indonesia. One of them is a study conducted by Natalia in 2015 which found that obesity is the most dominant risk factor for influencing the occurrence of hypertension, which is 2.16 higher than normal respondents. This is reinforced by a research by Anggraini et al., which claimed that there is a relationship between obesity and hypertension ($p = 0.004$)[19]. The same thing was also conveyed by Rohkusmawara and Syarif which stated that respondents who were obese (BMI > 25 Kg/m^2) had a 1.681 times risk of suffering from stage 1 hypertension compared to those who were not obese[6].

Furthermore, previous research conducted by Anggara and Prayitno in showed a risk of 51.1 times. This may occur due to high fat intake[21]. It is supported by a research conducted by Kartika which stated that high fat intake has a 4.246 times risk of getting hypertension than those who consume low fat[13,21,22]. Sulastri et al., found that more than half of hypertensive patients were obese (56.6%) and there was a significant relationship between obesity and the incidence of hypertension ($p <0.05$; OR = 1.82) in Minangkabau ethnic community in Padang, this city is famous for its foods that contain lots of fat[23]. Results of research related to a high-fat diet carried out in young adults aged 18-40 years at Rurukan Health Center, Tomohon showed a significant relationship between unusual food consumption and the incidence of hypertension ($p = 0.032$)[24]. However, this study also shows different results from previous studies using 108 samples aged 45-60 years. It was only in one sub-district, Central Tomohon District, Tomohon in 2014 which showed no significant relationship between obesity and hypertension ($p = 0.639> 0.05$); although this study claimed that the prevalence of hypertension in the community was quite high (68.5%)[25].

Although the mechanism is still an area of this study research, the relationship between obesity and hypertension cannot be separated in both children and adults. Those that play a role in the pathogenesis of obesity-related hypertension that are considered important are activation of sympathetic nervous system, insulin resistance, and inflammation which can increase the profile of altered vascular function and consequently hypertension, and leptin and other neuropeptides that are thought to explain a possible link between obesity and development of hypertension[26].

Conclusions

Based on the results discussed, it can be concluded that there is a significant relationship between obesity and the incidence of hypertension. Additionally, there is a high proportion of people with hypertension and obesity in young adults in Tomohon City. Considering high prevalence of hypertension and obesity, it is necessary to optimize the prevention and control of non-communicable diseases.

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The Art and the Science of Manuscript Publication: Tips and Tricks for Health Science Students and Professionals

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Abstract

Health care professionals are bound by the duty of providing the best possible care to their clients. To abide by this, one needs to constantly learn and imbibe the advances in the field. Evidence-based practise should be built on the firm foundation of evidence synthesis. A professional may be a clinician, an academician, or a researcher per se, but contribution to the evolution of their profession is an indispensable responsibility. Conducting research is a scientific process which when combines with the art of writing the manuscript influences innumerable lives in more than one positive way. Publication of findings not only helps one in professional development but also helps others globally in upgrading their services to benefit humankind. The process of drafting and publishing a manuscript needs initial guidance that appears to be missing as a major part in the health science curriculum. In this article, we aim to outline the practical points to facilitate the process of writing a manuscript for publication. This article includes the information on types of accepted articles, the pattern of manuscripts, gold standard guidelines, tips to draft each section of the manuscript (including cover letter), and how to choose a journal for publication, which would help to reduce the time required to prepare a draft and to increase the probability of acceptance of the manuscript for publication.

Keywords: *manuscript, research, scientific paper, scholarly writing, journal publication*

Introduction

Researchers are known to be frontiers of knowledge. In the ever-evolving field of health science, updating knowledge and skills is utterly crucial. Publications constitute the basis of evidence-based practice, aimed at providing the most efficient patient care available and enhancing professional expertise (1). Although many professionals are keen about publishing their research, most find the process daunting as this skill set is underrated in the curriculum (2). Research plays a significant role in developing clinical practices and in implementing new health policies. A logical scientific approach is needed since it generates new claims (3). This review strives to throw light on the process of efficient drafting and publishing manuscript for novice writers.

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Types of accepted articles:

We witnessed that with the advancement in the quality of journals emerges the specificity of whether the manuscript complies with the journal's scope and aims. Furthermore, the qualitative and indexed journals are specific about considering the type of research that they would carry forward for a further editorial review. There are a few journals, which publish the original research exclusively, while some only publish case reports.

1. Systematic reviews and meta-analysis are the most gold standard articles in research, which fall into the first level of evidence in research.

2. Original research encompasses randomised trials, experimental studies, surveys etc. They form the most significant part of the level of evidence hierarchy in research. The original researches provide momentum

to a majority of reviews and case reports.

3. Non-research publications include review papers, book reviews, opinion papers, letters to the editor, case reports, case series, editorials, film reviews, 'unique' categories (e.g. clinical picture in the Lancet, ten-minute consultation in the BMJ), etc. They carry unprecedented importance in publications for providing an insight into the research ecosystem.

Guidelines/Checklist:

To upgrade the quality and accessibility of the research, the research scientists have developed gold standard guidelines to ease the cumbersome process of maintaining the quality of research. These guidelines assist the researchers in designing their research protocol and polishing it into a scientific manuscript. The EQUATOR (Enhancing the Quality and Transparency Of health Research) network is the most trusted database to explore the research guidelines, which provide explanations to assist researchers worldwide in framing their studies.

Majority of journals follow guidelines published by the International Committee of Medical Journal Editors, wherein the most common reporting guidelines (by EQUATOR Network and the NLM's Research Reporting Guidelines and Initiatives) include-

- Randomized controlled trials (RCTs) follow the Consolidated Standards of Reporting Trials (CONSORT) guideline,
- Systematic reviews and meta-analyses follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline,
- Studies of diagnostic accuracy follow the Standards for Reporting Diagnostic accuracy studies (STARD) guideline,
- Case reports follow the Consensus-based Clinical Case Reporting (CARE) guideline, etc.

In 2015, EQUATOR designed an easy flow chart to assist authors, peer reviewers and editors find the most relevant checklist and reporting guideline to carry out a study (Fig 1).

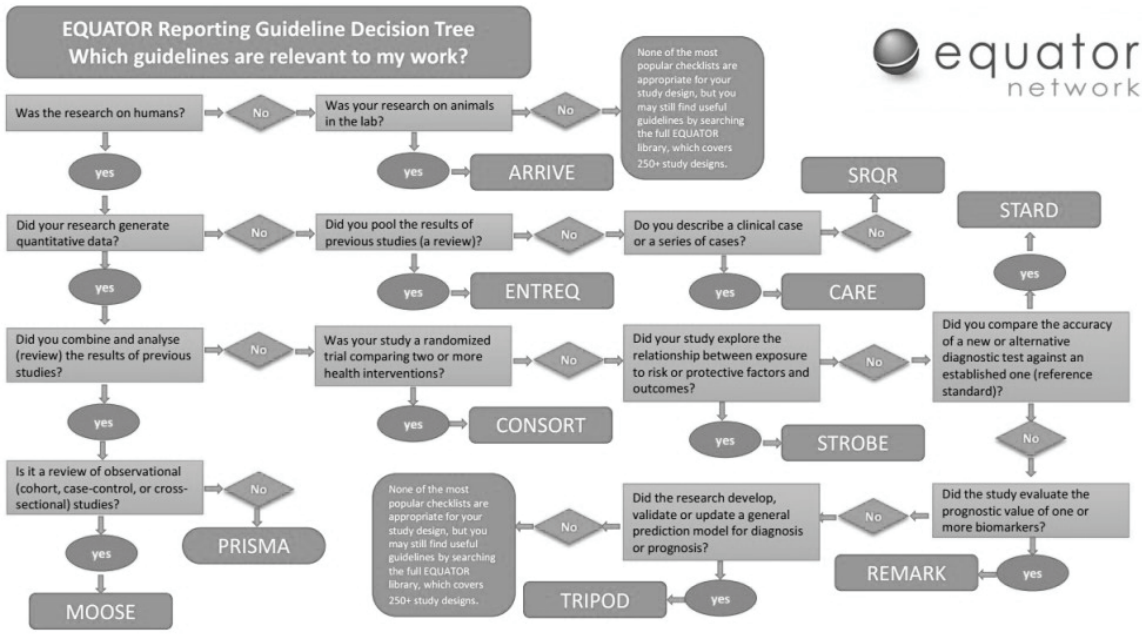


Figure 1. EQUATOR reporting Guideline Decision Tree.

Journal Indexing

Journal Indexing, also known as bibliographic indexes or bibliographic databases, refer to the collection of the scientific journals, which is arranged systematically according to streams, speciality, subjects, topics or area of interest (4). Some journals are indexed in multiple databases, whereas some adhere to a single indexing database. Often, the databases include journals after checking their brief criteria or quality. The researchers

extensively use the journals indexed in PubMed, Scopus, Web of Science, and Science direct databases to learn and cite the knowledge for their researches. The quote "Standing on the shoulder of the giants" perfectly fits this context. Furthermore, the journals are classified based on impact factor, quartiles (Q-ranking), h-index, cite index, and so forth. Nonetheless, there are some databases, which are commercially available and include journals without undergoing any qualitative check.

Globally, researchers recommend the journals indexed in Journal Citation Reports, which are acknowledged by the United States National Library of Medicine and National Institutes of Health shared website, PubMed (5). These journals comprehend the peer-review process, thereby ensuring the commencement of better studies for other researchers. Besides, if a medical, surgical, or scientific society supports a journal, or if it belongs to a well-known publisher, such as Springer, Elsevier, Wiley, and Taylor and Francis, it is suggestive of good quality (1). The ethical principle of most indexed journals implies, to prevent simultaneous submissions, a manuscript submitted to one journal should not be submitted to another simultaneously but can be submitted to the latter after rejection from the former.

MiSeRY

The prevalence of manuscript rejection syndrome (MiSeRY) is growing in the health care community. An increasing number of medical professionals attempting for manuscript publication and low acceptance rates by indexed journals are the key contributing factors to MiSeRY (6). The symptoms in this condition closely resemble those with the Kübler-Ross stages of grief but the time duration between the denial phase and the acceptance phase is markedly variable. It is more likely to be experienced by first authors and by authors who have high expectations of acceptance before the submission. Mastering the Coping and reLaxing Mechanisms (CaLM) and tempering the expectations taking in view the journal acceptance rates can help to alleviate MiSeRY. Senior authors, who have experienced MiSeRY in their developmental research years, develop strengthened Coping and reLaxing Mechanisms (CaLM), which protect against a probable state of enduring MiSery (7). However, some journals strive to minimize author expectations by publishing their low acceptance rates, and wise selection of manuscripts directed for external review enhances this effect (7).

Predatory journals

New researchers sometimes get confused in selecting the journal, understanding their format, open access policy, scope. Hastiness and eagerness to submit a manuscript can sometimes be indecisive, as according to the researchers, it can lead to manuscript submission in predatory journals, hijack journals, or cloned journals. Pseudo or predatory journals are those that accept and publish nearly all the submissions. They charge fees for the processing of the article (or publishing), inform about the unethical aspect after acceptance or do not notify at all, and falsely claim peer review. Authors must avoid submitting research manuscript in these journals and avoid citing articles from these journals. It is necessary to encourage researchers to crosscheck both the journal and the database to inspect a journal's current indexing.

Open-access journal

Publishing a manuscript in an open-access journal increases the size of the audience significantly since your article is freely accessible to the readers. However, researchers from low-income countries find it difficult to publish their work due to the high cost. Nonetheless, some qualitative journals allow free submission and publication of original researches. Besides, there are journals called hybrids in which some of the articles are open access.

Manuscript format

The basic formations or templates of the manuscript and word limits are available in the section 'instruction to authors' of each journal.

Most of the published articles follow 'IMRaD' pattern (7).

- **An introduction usually constitutes about 10 % of the manuscript. It must include a maximum of three paragraphs, which reveal what is known, what is unknown, and how does the study fill the gap or serve its purpose.**

TIP: The introduction section must not include 'Literature Review.' Furthermore, cite reviews to allow readers to find out more information.

- **Materials and Methods commonly include 6-9 paragraphs, constituting about 20 - 30 % of the manuscript depending upon the type and complexity of the study. This section should mention the steps**

followed in the study, which one can replicate later.

A trick for drafting a comprehensive section is incorporating 'STUD':

- **Subheadings to describe different sections**
- **Tests and statistical parameters**
- **Use of past tense to describe the procedure**
- **Description of methods in brief, and citation(s) to find more information.**

TIP: To present this section, researchers should go through 'Instructions for Authors' in the target journal to see the layout. Besides, go through previously published articles or sample reports on the journal website.

- **Results usually comprise of 6-9 paragraphs, constituting 20-30% of the manuscript. The findings obtained after putting the methodology in practice belongs to results. Use subheadings and logical order. Demonstrate the data, but do not discuss. Initial results characterize the environment or study representativeness before presenting the findings (e.g. table showing demographic data of the participants). The final data can be displayed in numbers, tables, graphics, and/or p values.**

TIP: Image can explain the findings better than the text as goes the quote "a picture is worth a thousand words."

Discussion and Conclusion

Discussion constitutes 20 – 30 % of the word count, and includes the description and interpretation of the findings. The importance of the study in changing the status quo is made comprehensive. The results of the study are compared with those of the previous studies (whether the study conforms or confronts or adds to what is already known?) and are seen in the view of related literature which also includes the limitations of the study (8) Besides, discussion recognizes clinical implications and future perspectives. The conclusion includes a paragraph about the takeaway message of the study. It may be included in the last paragraph of the discussion.

This standard structure:

- **Gives a logical flow to the content,**
- **Makes the manuscripts consistent and easy to read,**
- **Provides a "map" so that readers can quickly find the content of interest, and**
- **Reminds authors what content to include.**

Research Question

A good research question (RQ) provides detailed focus to the problem statement, details and refines the issue under study, directs data collection and analysis, and lays the context of research. Acronym 'FINERMAPS' represents a good research question (Figure 2) (3,9)

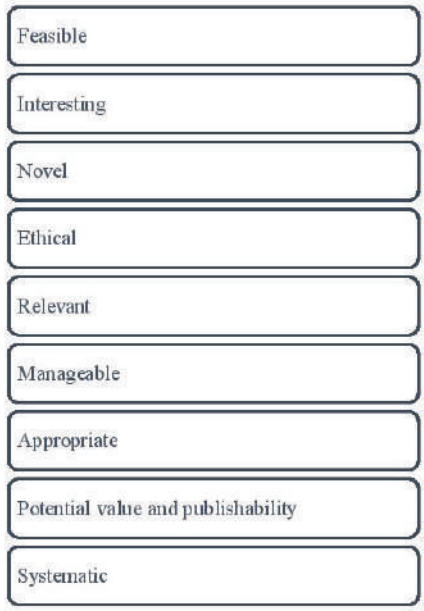


Figure 2 – FINERMAPS acronym.

Formulating the RQ involves:

1. Express the RQ in your own words.
2. Write down the RQ as thoroughly as possible.
3. Divide your question into concepts.
4. Specify the study population.
5. Refer to the intervention or exposure under investigation, if any.
6. Reflect the outcome of interest.

Writing sequence

The standard journal manuscript section usually follows the order 'Title, Abstract, Introduction, Materials

and Methods, Results, Discussion, and Conclusion,' this is not the finest order to compose the manuscript sections. Table 1 demonstrates a recommended strategy universally accepted to write a manuscript(10). This order, when followed, will assist in writing a logical and efficient manuscript.

Table 1: Strategy to write a manuscript (10)

1. Materials and Methods	**Start with writing these two first, while planning and execution of the experiment and while collecting the results.**
2. Results	
3. Introduction	Write these three sections next, after the analysis of results and after deciding which journal to submit for publication.
4. Discussion	
5. Conclusion	
6. Title	Write these at last since they depend upon other sections.
7. Abstract	

Title

The title is the first introduction of the work to the readers. Therefore, an effective title must be 'SECULAR':

- **Simple** to understand
- **Efficient** to provide a content overview
- **Concise** and in common terms
- **Unique** to grab attention and attract readers
- **Limiting word count** between 12 to 15
- **Avoiding jargon,** non-standard abbreviations and Roman numerals
- **Reader-friendly** and conforming to the journal requirement

The following parameters help to formulate a suitable title:

1. The aim of the research
2. The scope of the research
3. The narrative tone described by the type of research
4. The methodology and the results of the study

Three types of titles commonly used in journal articles are:

1. **Declarative**: It makes a declaration while summarizing the study and its results. This type provides the most insight into the contents of the paper.

2. **Descriptive**: It describes the study but falls short of revealing the results, and often includes details about the method.

3. **Interrogative**: It represents the research or restates the problem in the form of a question, commonly excluding the details of the study (11).

Tips: Some journals have specific guidelines for titles too!

Tricks:

- Form a sentence using the identified keywords liberally. Then, trim and reword the sentence.
- For longer titles, use a subtitle separated from the main title with a colon or a dash.
- A formula to structure:

[Result]: A **[method]** study of **[topic]** among **[sample]**

Example: Exercises enhance the quality of life: An

experimental study of tai chi in Osteoarthritis patients.

Abstract

An abstract should encompass '3S':

- Summary of the content
- Shortcut for saving time of busy researchers
- A stand-alone section that is complete in itself

Types of abstract-

Unstructured abstract: It is in paragraph form. The authors are free to present the summary within the prescribed word limit.

Structured abstract: It has predefined subheadings like IMRaD pattern. This makes it more informative and easier to read than the unstructured ones (12).

It is the most accessed part; it encourages researchers to read the entire paper further. However, most readers prefer to read only the abstract (11). An interesting and well-written abstract helps to accelerate the peer-review process. Usually, only the abstract of an article shows up in the indexing databases such as Web of Science or PubMed.

The abstract must answer the following questions about the manuscript:

- What was performed in the study?
- Why did you do it?
- What were the findings?
- What is the usefulness and importance of the findings?

TIP:

- For the abstracts, journals often lay a maximum word limit, which is roughly 250 to 300 words. It does not allow citations.
- These ensure the appearance of the entire abstract in the indexing services.
- Besides, the journals prescribe the type of abstract as per the article type to be submitted.

Keywords

Keywords are the tool, which assists the search engines to find relevant papers and provide more searchability of the research. Furthermore, the database search engines allow readers to find them. This enhances the number of people who will read and cite the article.

The 'ABC' rule to choose the keywords wisely:

- **A**ssure coverage of relevant terms
- **B**e specific to the field or sub-field of the research
- **C**onform to the manuscript content representation

A means to identify the keywords is to write a sentence describing the study and remove conjunctions and prepositions. This provides a list of terms, which one can use while searching the Medical Subject Headings (MeSH) terms in PubMed database (13) writing, and submitting a research manuscript to a peer-reviewed journal is described.\nSUMMARY: The research project and writing-related activities should be conducted concurrently along a clear timeline developed with input from all members of the writing team. Issues of conformance to standards of scholarly publishing (e.g., ordering of the author list, contributor acknowledgments, disclosure statements.

Tip: MeSH terms form effective keywords.

Tables and figures

The pictorial representations communicate with the findings clearly and quickly. This provides the work with a professional appearance and attracts readers.

A well-designed table has 'CDEF'.

- Clear and concise legend or caption to describe the table.
- Data divided into categories.
- Enough spacing between the columns and rows.
- Font legible and as per journal requirement.

Figures can be:

- images, which include scale bar and label important items,
- data plots, which label axes and specify units, or
- Schematics, which highlight only key elements.

TIPS:

- Figures with legends must supplement the reported data instead of simply repeating the already mentioned.
- A resolution of 72 PPI is usually sufficient for online publication, and 100 PPI in print. However, some journals recommend even higher resolution.
- Avoid manipulation of the images.
- While using any processing software, e.g. Inkscape, state its use.
- Keep copies of the original images if the reviewer requests for them during the review process.

References

The instruction for authors' column specifies the citation of the maximum number of references. The 'CLEAR' trick for reference citation includes:

- **Citation style** recommendation should be checked. The commonly used citation styles include the Vancouver style, American Psychological Association (APA) style, Harvard style, Chicago style, Modern Language Association (MLA) style, Oxford style, etc. However, most journals recommend Vancouver style (14).
- **Latest** references, not more than 10 years old, should be cited (exception is the first discovery or first mention of something in the literature).
- **Ease** the work using referencing software like Zotero, Mendeley etc.
- **Aim** to read the best evidence available.
- **Relevant** studies should be cited.

Formatting

Most journals recommend the following:

- Text is usually 12-point sized, double-spaced, left-aligned, and Times New Roman font (15).
- Use the same heading styles across the paper for up to three heading levels.
- Use three font sizes and three typefaces at the most.
- Mention the number the pages.
- Usually, the Figures and Tables altogether follow the references.

Quality of paper

The quality of the paper follows 'SUGAR':

- **Spellings:** They are as per the journal requirement for the type of English (American or British) and without errors.
- **Use of tense**: Follow the past tense for the methods and results, and present tense for general facts and conclusion. Most researchers recommend the use of an active voice.
- **Grammar**: Use determiners, articles, and verbs carefully.
- **Authors' ideas and presentation**: Maintain originality and correctness.
- **Reading fluidity**- Follow a logical flow throughout the article, like a story.

Plagiarism

Plagiarism refers to representing the work of another author or previous self-work without proper acknowledgement or citations. Plagiarism may contribute to a prompt rejection of the manuscript (16,17). There is ample software available for checking plagiarism, e.g. Turnitin and iThenticate, which are subscription-based. Before submitting the manuscript to the journal, it is preferable to keep a pre-check on manuscript plagiarism and keep it below 10 per cent.

Ethical clearance / Helsinki declaration

It is significant to take the Institutional Review Board (IRB) approval for the study before its implementation. Most peer-reviewed journals request a declaration that local IRB approved the study (14). Besides, the statement of IRB approval should include informed consent. While reporting *research involving human data*, the authors should indicate in the Methods section whether a legally qualified Ethics Review Committee (institutional or national) has evaluated and approved the followed procedures. Should there be no formal ethics committee available, then the study must follow the 2013 Helsinki Declaration.

Conflict of interest

Conflict of interest and bias is possible when a secondary interest (such as financial gain) affects a primary interest-related professional judgment (such as the validity of the research). Conflicts may emerge from financial relations, personal relations or rivalries, academic rivalry and intellectual convictions. Nonetheless, the readers must be able to judge whether the relationships and activities of an author are relevant to the content of a paper, which requires transparent disclosures. Complete disclosure by an author indicates a commitment to transparency and helps preserve trust in the scientific process. An intentional failure to disclose the relationships or activities set out in the journal's disclosure form is a form of misconduct. Many journals require that authors fill out a form for the disclosure of conflicting interests, apart from mentioning the manuscript.

Authors

The principal investigator is the first author who does the majority of work, and the sequence of co-authors follows the order of the magnitude of their contributions (13)writing, and submitting a research manuscript to a peer-reviewed journal is described.\ nSUMMARY: The research project and writing-related activities should be conducted concurrently along a clear timeline developed with input from all members of the writing team. Issues of conformance to standards of scholarly publishing (e.g., ordering of the author list, contributor acknowledgments, disclosure statements. The corresponding author is the one responsible for communicating with the journal during the publication process (14). International Committee of Journals Medical Editors (ICJME) has devised the authorship criteria in four sections, which encompass the substantial contribution, drafting of the content, approval of the manuscript, and the common agreement between the authors on carrying out of research and preserving the integrity of the entire study (18).

All those designated as authors must satisfy all four criteria. Besides, acknowledge those who do not meet these criteria. Non-author contributions include general supervision of a research group or general administrative support, writing assistance, technical editing, language editing, and proofreading. Author's contribution needs to be stated for many journals (13) writing, and submitting a research manuscript to a peer-reviewed journal is described.\nSUMMARY: The research project and writing-related activities should be conducted concurrently along a clear timeline developed with input from all members of the writing team. Issues of conformance to standards of scholarly publishing (e.g., ordering of the author list, contributor acknowledgments, disclosure statements.

Title page

The title page showcases the title of the article, details and affiliation of the authors with disclaimers, funding support, total word count, and sometimes the number of tables and figures.

Covering letter

This letter to the journal's editor represents an opportunity to sell the idea in the manuscript. It emphasizes on the submitted manuscript's relevance with the aims and scope of the journal and its efficacy to fill the gap in current knowledge. It is paramount to mention that the submitted manuscript neither has been published before nor is under consideration in any other journal.

Conclusion

It is paramount to comprehend the art of writing a manuscript while framing and writing one. The writing style of the manuscript must be simple yet powerful. To ensure the readers' engagement, the content of the manuscript should have fluidity. It is advisable to check the recommended templates on the journal website, meanwhile, maintain the manuscript quality. Nonetheless, ensure that the manuscript fulfils the aims and scope of the target journal. Beyond all considerations, one must check the plagiarism before submission to decrease the rate of manuscript rejection.

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Authors' contributions

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Data curation: WMN and AIS.

Formal analysis: AIS.

Funding acquisition:

Methodology: WMN CVG AIS.

Project administration: WMN.

Visualization: WMN.

Writing – original draft: WMN.

Writing – review & editing: WMN CVG AIS.

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Knowledge, Attitude and Practice of Undergraduate Students Regarding Toothbrush Contamination and Disinfection

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Abstract

Background: Toothbrushes play a significant role in disease transmission as they act as a reservoir for microorganisms and aid in increasing the risk of infection in any individual. In recent years, the necessity of toothbrush disinfection has gained importance. Toothbrush disinfection should be recommended as a routine practice for all individuals. This study aimed to assess the knowledge, attitude, and practice of undergraduate students regarding toothbrush contamination and disinfection.

Materials and methods: This study involved 446 undergraduate students between the age group of 18 to 23 years. A validated questionnaire was used to assess the knowledge, attitude, and practice of undergraduate students regarding toothbrush contamination and disinfection. The study showed that 24.89% of the students disinfected their toothbrushes and 75.11% did not use any disinfectant. 78.92% of the students feel that bacteria as the main mode of toothbrush contamination. 84.40% of the students feel that the disinfection of the toothbrush is necessary whereas 15.69% feel that toothbrush disinfection is not necessary. 61.29% of students did not know toothbrush disinfection.

Conclusion: Fewer students have sufficient knowledge about toothbrush contamination and practice the disinfection methods, whereas the majority of the students are unaware of the contamination of toothbrushes and the use of disinfectant methods.

Keywords *– Knowledge, Practice, Toothbrush, Disinfection, Contamination*

Introduction

Toothbrushes are essential for the removal of dental plaque and for maintaining oral hygiene, but the remnants on their bristles may encourage the growth of numerous microorganisms.[1] Toothbrush after the use may be contaminated with several bacteria and viruses. The toothbrush thus contaminated can play a vital role in the transmission of many oral diseases.[2] *Glass et. al.,* observed that the use of a contaminated toothbrush aggravates the injuries of oral tissues when compared with a germ-free toothbrush and may lead to septicemia due to microbial transmission.[3] Few studies also stated that toothbrushes of both healthy individuals and patients with illness contain both opportunistic and pathogenic microorganisms, in significant numbers which might induce cardiovascular, respiratory, and renal problems.[3]

Taji et.al., in his study reported that toothbrushes stored in bathrooms show higher contamination, levels, as the environment is contaminated highly by aerosol dispersion.[4] Many studies have recommended the need for disinfection of toothbrushes using different methods to prevent various diseases.[5,6] Disinfection of

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toothbrushes should be suggested as a regular practice to the patients.[5] Some of the recommended methods of disinfection methods are immersing in a disinfecting solution like Chlorhexidine gluconate, Listerine, Sodium hypochlorite 1%, spraying of antimicrobial solutions like Cetylpyridinium chloride on bristles, immersion in alcohol, and using microwave irradiation and ultraviolet light.[7] As contamination of toothbrushes occurs after repeated use, hence it is recommended to disinfect the toothbrush every time prior to brushing.[8] Thus the our study aimed to assess the knowledge, attitude and practices of undergraduate students regarding toothbrush contamination and disinfection.

Materials and Methods

A cross-sectional survey was conducted after obtaining ethical approval from the Institutional Ethical Committee. The participants were explained in detail regarding the importance of the survey and written informed consent was obtained from all the students who were willing to participate in the study. A total of 446 undergraduate students between the age group of 18 to 23 years, from Melaka Manipal Medical College, Manipal, participated in this study. The sample size was calculated based on the prevalence rate of a previous study.[9] A closed-ended questionnaire containing 15 items was modified from the previous literature[10] and distributed to the students. The questionnaire consisted of basic questions regarding the knowledge and practices regarding toothbrush contamination and disinfection. The responses from each student were collected and tabulated. The tabulated data were statistically analyzed using SPSS-15.0 with descriptive statistics and the Chi-square test. Differences with $P < 0.05$ were considered statistically significant.

Results

A total of 446 students, including 318 females (71.30%) and 127 males (28.48%) filled the questionnaire. Students ranged in age from 18 to 23 years (mean age ± standard deviation: 20.42 ± 1.75 years). Of the participants, 24.88% were dental students, 47.75% medical students, and 27.35% of students from the foundation course as seen in table 1.

A total of 24.89% of students disinfected their toothbrush and 75.11% of students did not disinfect their toothbrushes. A statistically significant difference between the two groups was seen (P-value <0.05*). A statistically significant (P<0.05) difference was also seen between the students who had and who did not have sufficient knowledge about toothbrush contamination and disinfection. Only 38.79% (n=173) of the students had prior knowledge regarding toothbrush contamination and disinfection. About 54.04% (n=241) of students did share their toothpaste with other individuals and 45.96 % (n=205) of students did not share their toothpaste with other individuals [Table 2]

The majority of the students (84.30%, n= 376) felt that the disinfection of toothbrush was necessary, and there was a statistically significant difference observed (P <0.05) between the students who felt the need for disinfecting the toothbrushes and who felt disinfection not necessary. Around 64.57% (n= 288) of students felt that every individual need to disinfect their toothbrushes and 23.99% (n=105) of the students felt that disinfection was required only for hospitalized and immunocompromised patients [Table 2].

About 86.55% (n=386) of the students believed that contact between toothbrushes was the main concern and 13.45% (n=60) of the students did not feel it important, and there was statistically significant difference observed (P <0.05). Most of the students reported that they stored their toothbrushes in open contact with the surroundings inside the bathroom (n = 311, 69.73%) and in a closed cabinet inside the bathroom (n =106, 23.77%), outside the bathroom in closed cabinet (n =14, 3.13%), outside the bathroom in open contact with the surroundings (n = 15, 3.36%). About 86.32% (n=385) of the students felt that sharing the toothbrushes was the normal mode of infection transmission from a toothbrush and 78.92% (n=352) felt that bacteria were capable of causing toothbrush contamination [Table 2].

The majority of the students (68.16% n=304) rated their oral hygiene to be good and 11.66% (n=52) of the students as excellent [Table 3]. The difference in self-rating of oral hygiene between the student was also statistically significant (P <0.05). The majority of the students (n=396, 88.79 %) brushed their teeth twice daily and 50.22 % (n=224) changed their toothbrushes once in three months [Table 4].

Table 1: Distribution of Study participants based on Age, Gender and Study Course

Age: Mean (SD)	**20.42(1.75)**	
Gender (n=446)	**n(%)**	**P value**
Male Female Transgender	127 (28.48) 318 (71.30) 1 (0.22)	<0.05*
Study participants (n=446)		
BDS MBBS FIS	111 (24.88) 213 (47.75) 122 (27.35)	0.0000000201 <0.05*

* Level of Significance <0.05*

Table 2: Knowledge regarding toothbrush contamination and disinfection among study participants

Questions	Yes n (%)	No n(%)	P value
Do you disinfect your toothbrush	111(24.89)	335 (75.11)	<0.05*
Do you have any knowledge about toothbrush cleaning and disinfection	173 (38.79)	273 (61.21)	0.00000213 <0.05*
Do you share your toothpaste with other individuals	241 (54.04)	205(45.96)	0.0975 ns
In your opinion is toothbrush disinfection necessary	376 (84.30)	70 (15.69)	<0.05*
If Yes, Everyone Special patient groups such as immunosuppressed individuals, hospitalized patients and children Not sure	288 (64.57) 107 (23.99) 51 (11.43)		<0.05*
In your opinion is the contact between toothbrushes an important issue	386(86.55)	60(13.45)	<0.05*
In your opinion which microorganism causes toothbrush contamination			
Bacteria Fungi Virus No Idea	352 (78.92) 43 (9.64) 3 (0.67) 48 (10.76)		<0.05*

Cont... **Table 2: Knowledge regarding toothbrush contamination and disinfection among study participants**

What is the common mode of transmission of infection from a toothbrush		
Sharing the same toothbrush holder Sharing the tooth paste Sharing the toothbrush Using frayed bristled toothbrush	25(5.61) 20 (4.48) 385(86.32) 16 (3.59)	<0.05*
Where do you store your toothbrush?		
In the bathroom - in a closed cabinet	106 (23.77)	<0.05*
In the bathroom - in open contact with the surrounding	311(69.73)	
Outside the bathroom - in a closed cabinet	14 (3.13)	
Outside the bathroom - in open contact with the surrounding	15 (3.36)	

* Level of Significance <0.05*, ns- Not significant

Table 3: Self –Rating of oral Hygiene by the study participants

How do you rate your oral hygiene	n(%)	P value
Excellent Good Fair Poor	52 (11.66) 304 (68.16) 88 (19.73 2 (0.45)	<0.05*

* Level of Significance <0.05*,

Table 4: Distribution of study participants according to on their practice regarding toothbrush care

How do you store your toothbrush	n(%)	P value
In a toothbrush holder shared with others Separately from the toothbrushes of others	177 (39.69) 269 (60.31)	0.0000164 (<0.05*)
How often do you brush your teeth		
Once a day Twice or more a day	50 (11.21) 396 (88.79)	<0.05*

Cont... **Table 4: Distribution of study participants according to on their practice regarding toothbrush care**

How often do you change your toothbrush		
Once every 3 months	224 (50.22)	
Once a month	72 (16.14)	0.934
Bimonthly	74 (16.59)	ns
After 3 months or more	76(17.04)	

* Level of Significance <0.05*, ns- Not significant

Discussion

The survey was done to assess the knowledge, attitude, and practices of undergraduate students about toothbrush contamination and disinfection. Approximately 61.21% of students had no knowledge about the disinfection of toothbrush and 75.11% of students did not disinfect their toothbrushes. However, 84.30% of students indicated that toothbrush disinfection was necessary. A statistically significant difference was found between students having knowledge about toothbrush disinfection and not having knowledge about disinfection.

Around 24.89% of the students disinfected their toothbrushes and statistically significant differences were found between the students who had knowledge of disinfecting the toothbrush, with a significantly higher number of students responding positively to whether toothbrush disinfection was needed. Many studies have assessed the oral health attitude and practices among undergraduates in different countries, found that most students brush their teeth at least twice a day.[11] In the present study, approximately 88.79% of the students brushed their teeth twice daily or more and 50.22% of the students felt that replacement of the toothbrushes should be done once in every 3 months. However, most of the previous studies are done only on the dental undergraduate, postgraduate students, and dentists.[9,10,13] In our study, medical undergraduate students are also included so the results cannot be directly compared.

Contamination of the toothbrushes occurs due to several factors like storage conditions, toothbrush location, sharing of toothpaste, microbes in the oral cavity, and cross-infection. Recommendations for storage of toothbrush include using cup hooks for hanging the brushes, using a brush box, using closed and vented containers.[12] In the present study 86.32% of the students felt that sharing the toothbrushes was the usual mode of infection transmission from a toothbrush and 78.92% felt that bacteria were capable of causing toothbrush contamination. The results of this study are similar to the previous studies, which also reported bacteria as the main causative agent for toothbrush contamination. [9, 10, 13] Earlier studies have observed a higher number of bacteria in toothbrushes stored in closed containers compared to those open environments.[10,12,14] Increased chances of bacterial survival on toothbrushes are observed with the use of a toothbrush cap. Generally, contamination may occur when toothpaste and brushes are shared, or when toothbrushes are stored with the toothbrushes of other individuals. Toothbrushes are made of nylon synthetic resin bristles, which reduces bacterial growth as they absorb less water and dries faster. However, if the bristles are not dried, water gets collected at the lowest portion of the bristles, and increases the humidity, thereby creating a conducive environment for bacterial growth. Storage of toothbrushes in a humid environment after use without disinfection can contaminate the toothbrush with microorganisms from the oral cavity or from the surrounding air.[13] In the present study most of the students reported that they stored their toothbrushes in open contact with the surroundings inside the bathroom and few students stored in a closed cabinet in the bathroom and outside the bathroom.

At present, no standard guidelines on toothbrush disinfection for healthy individuals are available even though contamination of toothbrushes with microorganisms may occur right after use, and increased contamination is seen with repeated use. Approximately 64.57% of the students in this study believed that the disinfection of toothbrushes should be done by every

individual. Only 23% of students thought that the disinfection of toothbrush was essential only for special patient groups.

Conclusion

The majority of the students surveyed had no knowledge about toothbrush contamination and never disinfected their toothbrushes. Some students had knowledge about disinfection but did not practice any disinfection methods. Fewer numbers of students practiced disinfection methods. Increased awareness has to be created about this topic for the adoption of toothbrush disinfection as a routine practice, among the public. The dentists need to spread awareness not only about maintaining oral hygiene but also on toothbrush disinfection.

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Clinical Evaluation of Local Drug Delivery of Chlorhexidine and Ornidazole in the Treatment of Gingivitis as an Adjunct to Scaling and Root Planing

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Abstract

Background: Local drug delivery to gingival sulcus avoids adverse effects seen with systemic therapy and there is very little information to substantiate the additive effect of the drugs, the current study makes an attempt in same regard.

Aim: The objective of this study was to compare the local application of ornidazole and chlorhexidine gel (Clorni^{TM} gel) as an adjunct to scaling and polishing, with scaling and polishing alone.

Materials and methods: 33 patients meeting inclusion criteria were randomly allocated to treatment groups by computerized randomly selection method. Indices were recorded at baseline at their first visit. Supragingival and subgingival scaling and polishing was performed using hand and ultrasonic instruments. The patients were evaluated clinically by using the gingival index, plaque index and modified sulcus bleeding index. On test side, Clorni^{TM} gel was applied subgingivally after nonsurgical periodontal therapy. On the control side, no intervention was done following subgingival scaling and polishing. Patients were recalled at 7th day for subsequent administration of gel on the same site and followed up at 21st day and 3 months to compare the gingival condition by using the indices.

Results: All subjects showed significant improvement over 3 months recall period, irrespective of test or controls. Statistical significance ($p< 0.05$) was observed at 7 days and 21 days for test group for the gingival index and at 21 days for the plaque index and modified sulcus bleeding index.

Conclusion: The topical gel when used as an adjunct to scaling and polishing had significant results.

Keywords: *inflammation, gingivitis, chlorhexidine, ornidazole*

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Introduction

Gingivitis has a widespread prevalence, and is seen more in the younger and older age groups and lesser in the middle age groups; with an average of 50-100% in dentate individuals.[1]

As shown by Carranza et al, it is characterized by color change, edema, bleeding and in severe cases, if left unchecked, may lead to progression to periodontal involvement. As a result of this inflammation, the microorganisms that enter the sulcus release their destructive enzymes and cause the dissolution of the cellular and intercellular elements. This leads to widening of intercellular spaces of the junctional epithelium and as consequently allows the endotoxins to penetrate deeper, leading to further destruction if left untreated.[2]

Local drug delivery is the local application of the drug to gingival sulcus, avoiding adverse effects with systemic therapy and achieving a much higher concentration locally.[3] Many drugs have been tried for the application at the site of inflammation in order to alleviate the signs and symptoms of gingivitis. Use of metronidazole,[4,] minocycline,[5] tetracycline,[6] chlorhexidine,[7] doxycycline[8] are some of the examples of local drug delivery. Studies have shown that, tetracycline, minocycline, chlorhexidineand metronidazole when used as a monotherapy[9-11] have shown clinically significant results as an adjunct to scaling and root planing (SRP). However, there are lack of sufficient clinical trials to substantiate the additive effect of the drugs.

Metronidazole, an antimicrobial agent belonging to the 5-nitroimidazole group, has been used successfully in treating chronic periodontitis, alone or in combination with amoxicillin. Ornidazole is another antibiotic that belongs to the 5-nitroimidazole group with comparable or better antibacterial properties.[4] Better efficacy and antibacterial properties in comparison with metronidazole have been reported in literature.[12] Similar results were demonstrated while comparing ornidazole to metronidazole.[13] Ornidazole has a lower minimum inhibitory concentration compared to metronidazole and hence, safer for the patient in terms of the development of resistance to the drug. Ornidazole is shown to have higher water solubility and inturn, the mean half-life of elimination from human plasma is almost double than that of metronidazole.[14] This gives ornidazole an edge over metronidazole, in that, the dosage can be reduced with less frequent administration.[15] Ornidazole has previously been used systemically, as an adjunct to non-surgical periodontal therapy.[4] However, it may induce unnecessary side effects or systemic actions which are not desired.

A new commercially available gel (Clorni^{TM} gel, Group Pharmaceuticals, Ltd, Mumbai, India) containing a combination of ornidazole (1% w/w) and chlorhexidine (0.25% w/w) was used in this study. Since both the drugs have their individual advantages, so may be when used in combination may exhibit additive/synergistic effect for the treatment of gingival disease. The gel contains Pluronic F127 poloxmer as the base, which serves as a potent vehicle for the drugs and allows a controlled release of the drug at the site of application.[16]

To the best of author's knowledge, there is no such study comparing the local application of ornidazole in the treatment of gingivitis. Hence, the aim of the present study is to compare the local application of ornidazole and chlorhexidine gel (Clorni^{TM} gel) as an adjunct to scaling and polishing, with scaling and polishing alone as control.

Methods

The present study was designed as a randomized, double-blind, controlled, split-mouth and parallel arm trial. The study was conducted in the Department of Periodontology, Krishnadevaraya College of Dental Sciences, Bangalore and the study protocol was presented to institutional ethical review committee before obtaining the ethical approval to conduct the study. 42 systemically healthy patients (18 males, 12 females), with a chief complaint of bleeding, swollen and/or red gums, were recruited from the pool of the patients referred to the out-patient department of Periodontology.

After obtaining a detailed medical, dental, periodontal and personal history, the suitable patients fulfilling the inclusion criteria were included in the study. The decided inclusion criteria were; 1) Age between 20-40 years, 2) Bilaterally involved teeth, with moderate to severe gingivitis, 3) No previous history of periodontal treatment. The patients were excluded from the study if; 1) Pregnant or lactating 2) Smokers, 3) Patients with systemic diseases, 4) Patients taking systemic antibiotics over the last 2 months, 5) Use of non-steroidal anti-inflammatory drugs, 6) Any reported or suspected drug allergies, 7) subgingival scaling or surgical periodontal therapy in the previous year.

Finally, 33 patients meeting the inclusion criteria were included in the study. All patients were informed about the study protocol, advantages and possible adverse effects of the drug to be administered, and a written informed consent was obtained from all the patients. The participants were randomly allocated to one of the two treatment groups by computerized randomly selection method. To avoid inter-examiner variation; a single examiner scored all the parameters. The patients were evaluated clinically by using the gingival index,[17] plaque index[17] and modified sulcus bleeding index. [18] The examiner was calibrated on 5 patients prior to the scoring of the patients in the study, and the intra-examiner agreement was 95%.

All the indices were recorded at baseline at the first visit of the study subjects. Following this, a thorough full mouth supragingival and subgingival scaling and polishing was performed using hand and ultrasonic instruments. Test and control side were selected by computerized random selection method. On the test side, the ornidazole-chlorhexidine gel was applied subgingivally after nonsurgical periodontal therapy, until it completely filled the sulcus, using a 23 gauge needle (Figure 1). Patients were asked to remain on the dental chair for 10 minutes without moth rinsing to avoid flushing of the applied gel. On the control side, no intervention was done following subgingival scaling and polishing. Finally, patients were advised with uniform oral hygiene instructions for plaque control before leaving the clinics.

All patients were recalled at 7th day for subsequent administration of gel on the same site. The gingival index, plaque index, and modified sulcus bleeding index were used to evaluate and compare the change in the gingival condition on both test and control sides. The patients were then recalled after 21st day and 3 months, to evaluate and compare the gingival condition by using above indices. Three patients were excluded from the study, because they failed to show up at follow-up visits. Finally, data from 30 patients were analyzed to formulate the results.

Results

All the patients were evaluated at baseline, 7 days, 21 days and 3 months. Gingival index, Plaque index and modified sulcus bleeding index were used to assess the healing in the gingival tissues in both the test and control sites.

The number of tests and controls selected were similar, showing that the split mouth cases showed similar severity in all the cases selected. As shown in Figures 2,3 and 4, all the subjects showed significant improvement over the 3 months recall period, irrespective of test or controls. The number of healthy sites increased over the 3rd month recall visit when compared to the baseline in both the test and control sites, with better results in the test group.

Statistical analysis was done using Student's t-test for intergroup comparison. Statistically significant results ($P< 0.05$) were observed at 7 days and 21 days for the test group (Figure 2) for the Gingival index and at 21 days (Figure 3 and 4) for the Plaque index and modified sulcus bleeding index (Table).

None of the patients showed adverse drug reactions during the course of treatment.

Table: Gingival index, Plaque index, Modified sulcus bleeding index assessment at various patient visits for test and control sites

Variable	Baseline-7days	Baseline-21days	Baseline-3months
1.	Gingival index		
Control	0.64±0.22	0.97±0.25	0.99±0.31
Test	1.12±0.35	1.42±0.36	1.29±0.32
P Value	0.0019*	0.0415*	0.1785

2.	Plaque index		
Control	0.76±0.18	0.95±0.26	1.05±0.28
Test	0.98±0.24	1.3±0.34	1.19±0.30
P Value	0.0952	0.0067*	0.6589
3.	Modified Sulcus bleeding index		
Control	0.62±0.18	0.86±0.29	1.05±0.25
Test	0.83±0.21	1.13±0.30	1.16±0.27
P Value	0.0658	0.0256*	0.3674

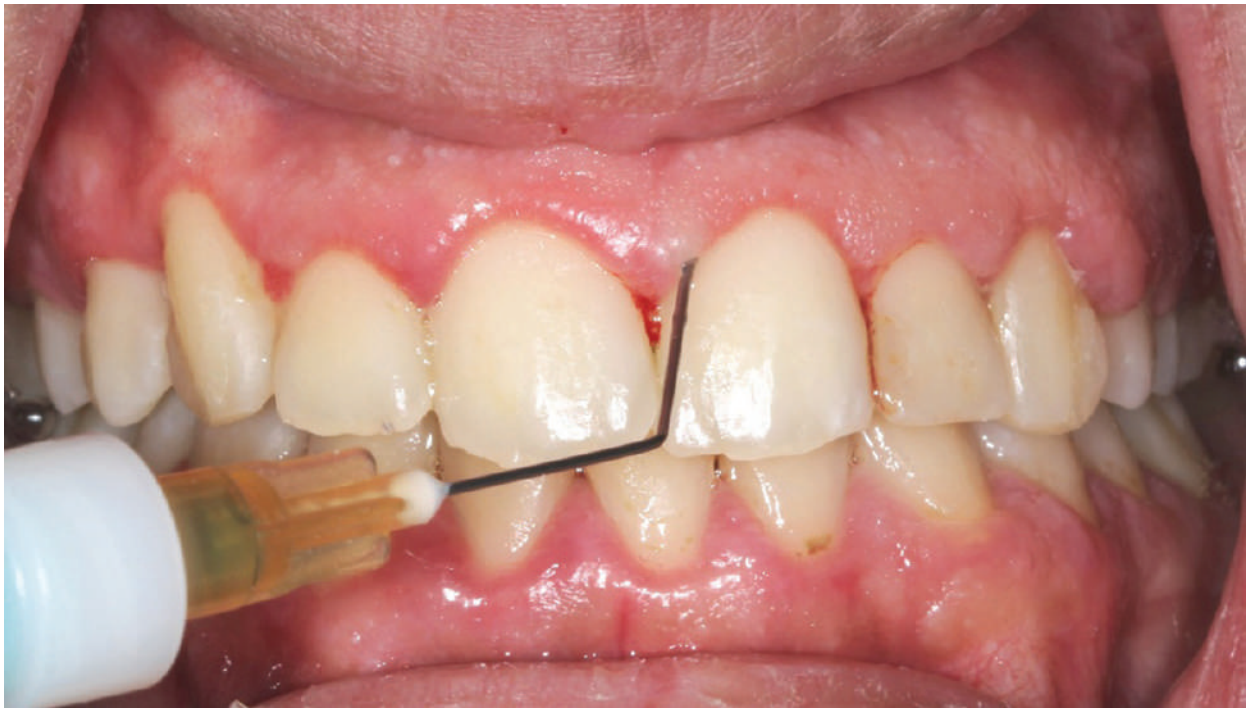
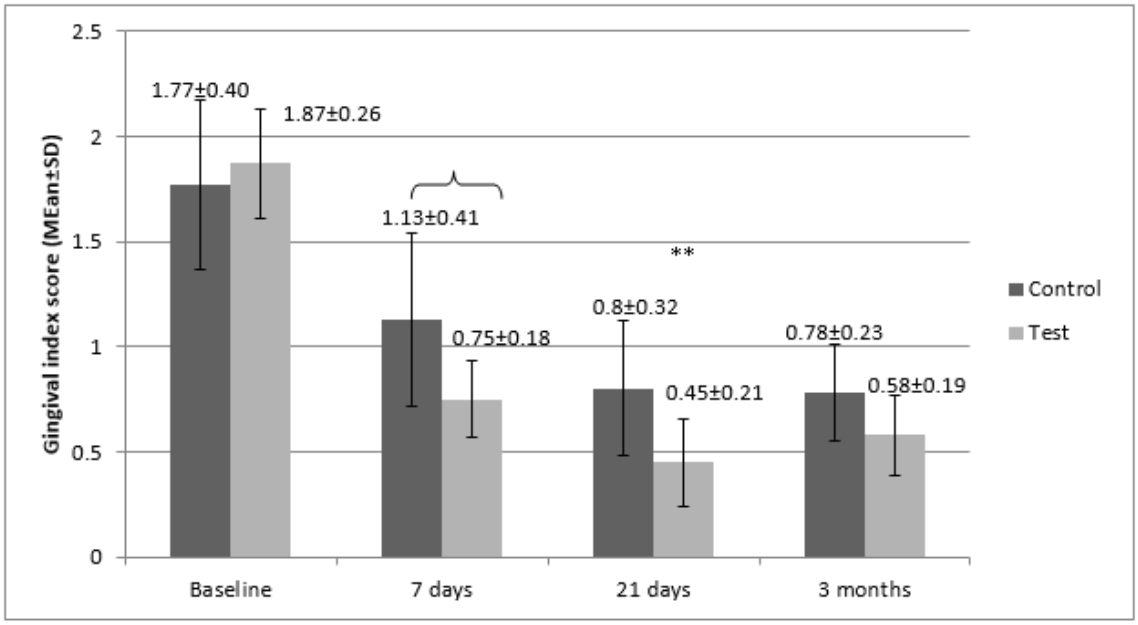


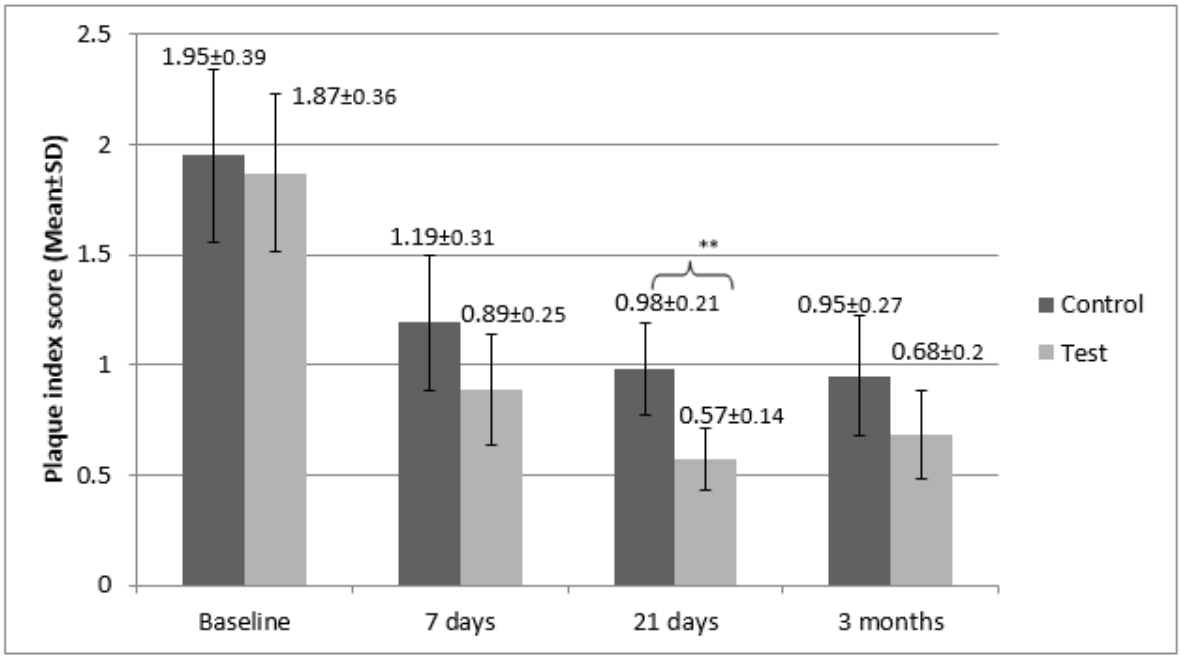
Figure 1: Placement of local drug delivery agent (Clorni gel)

****= Significance**



**= Significance

Figure 2: Gingival index assessed at various patient visits for test and control sites



**= Significance

Figure 3: Plaque index assessed at various patient visits for test and control sites

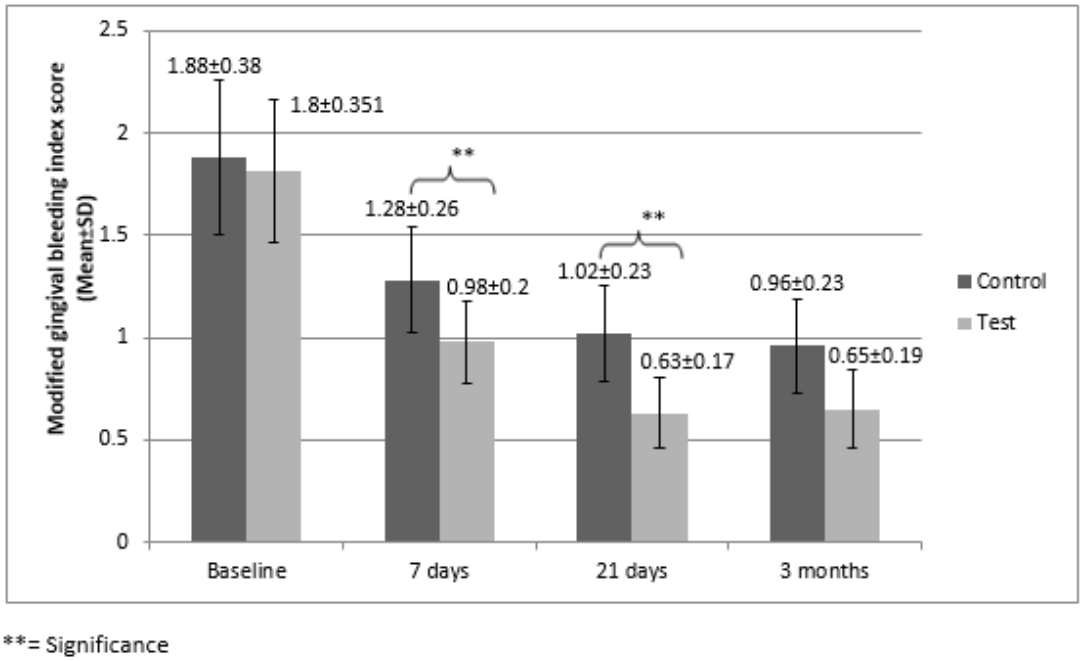


Figure 4: Modified sulcus bleeding index assessed at various patient visits for test and control sites

Discussion

This study shows the action of a newly commercially available Clorni^{TM} gel, containing a combination of Ornidazole and Chlorhexidine (CHX), when used as an adjunct to SRP to aid in faster healing of advanced gingivitis cases. This study, to the best of our knowledge is the first of its kind, using ornidazole as a local application formulation. Considering its systemic application and since ornidazole and metronidazole belong to the same group, the study was conducted on the basis of previous studies using local application of metronidazole, as shown by Slots and Rams.[4]

Chlorhexidine is an effective antimicrobial agent and shows action against a wide range of organisms.[19] Its mechanism of action relates to its inhibitory effect on the pellicle formation, alteration of bacterial adherence to teeth and due to its positive charge, it interacts with the negatively charged cell walls of the bacteriae and enters the cell, causing the lysis of the microbe. Chlorhexidine in a sustained release formulation, has been shown to be effective against subgingival plaque bacteria when used for 7-10 days. The antimicrobial effects were evident up to 6-9 months after treatment and clinical efficacy in terms of reduced bleeding on probing, reduced probing pocket depths and a gain in clinical attachment level were apparent.[20]

In our study the improvement in gingival status, plaque and bleeding were observed when gingival index, plaque index and modified sulcus bleeding index were used. An overall improvement was seen over 3 months in both test and control groups. However, significant improvement was seen in the test group at 7 and 21 days concerning gingival status and at 21 days when plaque index and modified sulcus bleeding index were considered.

The 20 ml/hour out flow of gingival crevicular fluid would be responsible for the 1 minute half-life of any gel within a periodontal pocket.[21] Furthermore, the lack of adherence of CHX to root surfaces[22] and its high affinity for blood and serum proteins[23]are also hypothesized to be among the reasons for its low subgingival substantivity.

Pluronic F127 is a member of the copolymer known as poloxomer, which is used as a vehicle for subgingival gel preparations. This component helps to provide a good bio-adhesive property to the preparation which helps in the permanence of the gel inside the sulcus, aiding in longer medicinal effects of the drug within the sulcus as suggested by Chávez et al, 2006.[16]

Also, the systemic administration of ornidazole has showed a significant reduction in the readings compared to the baseline readings as reported by studies by Kamma et al, 2000[24] and Pradeep et al, 2012.[15] When used in cases of chronic and aggressive periodontitis as an adjunct to scaling and root planning, Kamma et al (2000)[24] showed a probing depth reduction of 1.9 mm at the end of 6 months, when he used ornidazole with nonsurgical periodontal therapy to treat cases of aggressive periodontitis. Pradeep et al (2012) administered systemic ornidazole to 58 patients with chronic periodontitis, adjunctive to scaling and root planing and also showed significant results in treatment results.[15]

Systemic application has the possibility of systemic complications. As shown in our present study, no adverse drug reactions were noted with the local application of ornidazole. The mode of application and treatment method used in this study is simple, economical and effective.

However, further research needs to be done with larger sample size and longer follow up period, accompanied by microbiological tests, to validate the better results of Clorni^{TM} gel.

Conclusion

This study revealed that the topical gel as an adjunct to thorough scaling and polishing had significant results compared to baseline, and also statistically significant additional benefits when compared to scaling and polishing alone. Better clinical results have been seen when Clorni^{TM} gel has been used along with scaling and polishing. Therefore, this gel can be safely suggested as a good option in the treatment of severe gingivitis. Further studies are awaited, with a larger sample, to validate the treatment efficacy of this combination drug.

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Postmortem Cardiac Troponin-T levels in Heart Blood as a Marker to Diagnose Acute Myocardial Infarction

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Abstract

Sudden death accounts for approximately two-thirds of autopsies in Forensic Medicine. Natural death within 1 hour after the beginning of acute symptoms, is defined as Sudden Cardiac Death. Acute Myocardial infarction (AMI) is a serious and potentially lethal manifestation of coronary artery disease, affecting more than 7 million people worldwide each year and was proved to be a cause of sudden death. This study quantitatively analyzed the heart blood for Cardiac Troponin T (cTnT) levels within 48hours after death & correlated with the histological changes of myocardium.

Key words *: Acute Myocardial Infarction, Sudden Cardiac Death, Cardiac Troponin T,*

Introduction

The incidence of heart disease was less than 10% worldwide, at the starting of the 20th century. With the advent of 21st century, cardiac deaths accounted for 50% of all deaths in the developed world and one fourth in the developing world.[1]By 2020,heart disease will lead to 25 million deaths all over the world annually and coronary artery disease will take over infectious disease as the number one killer.[2]

One-6th of total populace lives in India and coronary disease represent 24% of all deaths. Among all the ethnic groups in the world, Indians have highest prevalence of coronary artery disease and the projected rise in the mortality rates is >100% in the next 25 years.[3]

In postmortem examination of dead bodies subjected to autopsy with probable sudden cardiac death, a diagnosis of myocardial infarction is usually made based on the finding of severe atherosclerotic occlusive coronary artery disease. The development of histological sequelae of the infarcted myocardium occurs only after significant time lag between onset of myocardial infarction and death.

Recently several studies have shown keen interest on the application of biomarkers to diagnose AMI. The inclusion of cardiac biomarkers like Troponin T enables to diagnose AMI in 1/3rd of patients who do not meet the criteria for diagnosis of myocardial infarction.

The prognostic value of Cardiac Troponin T is most important as it is not dependent on Age, Sex, ECG changes as well as levels of age old biochemical markers such as CKMB. Cardiac Troponin levels act as a specific and sensitive marker of cardiac cellular necrosis and myocardial infarction. The rise in Cardiac Troponin T levels occurs within 3-24 hours after AMI and persists up to 2 weeks in the living.[4]

In clinical practice, the cTnT assay has been approved for the diagnosis of AMI with high sensitivity and specificity. Few studies have been done to assess the use of serum cTnT as a biomarker in investigating coronary artery diseases.

The aim of this study is to estimate postmortem Cardiac Troponin T levels in blood to diagnose acute myocardial infarction in autopsy and to correlate Cardiac Troponin T levels with histopathological diagnosis of acute myocardial infarction in autopsy.

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Materials and Methods

This prospective study was conducted in the Mortuary of Chromepet Govt Hospital, Chennai and Department of Forensic Medicine, Sree Balaji Medical College, Chennai for a period of 10 months in 2019.

This study quantitatively analyzed the heart blood from the right and left ventricle and pericardial fluid for Cardiac Troponin T (cTnT) levels, in sudden deaths within 48hours after death, by a highly sensitive and rapid Cardiac Troponin T Electrochemiluminescence (ECL) immunoassay analyser and correlated with the histopathological findings of acute myocardial infarction. The study sample consisted of 12 hearts taken from cases of sudden deaths. A control sample (n = 6) consisting of deaths due to poisoning, and natural non cardiac causes like stroke, tuberculosis etc. were taken. Before getting into the study, Ethical clearance was obtained from the Institutional Ethical committee. (IEC)

In dead bodies with history of non traumatic sudden death subjected for autopsy, after making a longitudinal incision on the skin dissecting out the skin, subcutaneous tissue, fascia and muscle with a BP handle scalpel blade, reflecting out the skin together with the muscles, cutting the ribs at the costochondral junction along with the sternoclavicular joint disarticulating it and the sternum lifted up, dissected out exposing the thoracic cavity and the heart, blood was withdrawn from the pericardial cavity, right and left chamber of the heart with a 21G sterile syringe with needle and immediately transferred to a sterile sampling anticoagulant added test tube, corked with the stopper and sent for analysis at a private lab. Cardiac Troponin-T levels in heart blood serum was measured using Cobase411 immunoassay Analyser. T

The internal autopsy was followed by microscopic and toxicology examination. The cause of death was determined by correlating different histopathological features, with the levels of Cardiac Troponin T in heart blood.

Results

The study MI and control sample contained predominantly men (Table -1).While the average age of the control sample was 50(Table 2), the average age of the cardiac death sample was 52 (Table 2). In the control sample two patients died due to acute organophosphorus poisoning, three cases due to cerebrovascular accident/ metabolic encephalopathy, and one case due to pulmonary tuberculosis. Cardiac Troponin T level was markedly elevated **(>10ng/ml)** in all except one case of suspected MI in the study sample (Table -3) and also markedly elevated in the control sample except two cases (Table-4). Histopathological examination of the hematoxylin and eosin stained, heart slices, showed that four cases of the study group had evidence of early myocardial infarction with features of myonecrosis, waviness of fibers, nucleomegaly, pyknotic nuclei and polymorph infiltration (Table -5). Three cases of acute MI deaths demonstrated, myocardium with multiple foci of infarction showing hyalinised collagen fibers. In areas of old infarction, HPE revealed evidence of dense fibrous tissue replacing normal myocardial tissue. Four of the control cases showed normal histology of the myocardium, while two showed mild eosinophilia of the myocardial fibers with mild infiltration of polymorphs(Table -5).

Table: 1 Sex Distribution among the study sample and among the control group

	Study sample		Control sample	
Sex	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Male	11	91.7%	5	83.3%
Female	1	8.3%	1	16.7%
Total	12	100%	6	100%

Table: 2 Age group wise distribution of the study sample and control sample group

Study sample			Control sample	
Age distribution	**No. of Cases**	**Percentage**	**No. of Cases**	**Percentage**
20-40 years	3	25%	1	16.6%
41-60 years	6	50%	4	67%
61- 80 years	3	25%	1	16.6%

Cardiac Troponin T concentration levels in heart blood and pericardial fluid by highly sensitive Cardiac Troponin T quantitative assay

Table 3: Study group (n=12)

S.No.	Cardiac Troponin T Levels (ng/ml)(> 0.1ng/ml +ve result)			Postmortem Interval	Inference
	Left Ventricle	Right Ventricle	Pericardial Fluid		
1.	>10	>10	>10	8 hours	Markedly increased
2.	>10	>10	>10	10 hours	Markedly increased
3.	>10	>10	>10	19 hours	Markedly increased
4.	>10	>10	>10	16 hours	Markedly increased
5.	>10	>10	>10	16 hours	Markedly increased
6.	>10	>10	>10	12hours	Markedly increased
7.	>10	>10	>10	17hours	Markedly increased
8.	>10	>10	>10	5 hours	Markedly increased
9.	>10	>10	>10	12hours	Markedly increased
10.	0.063	0.051	0.093	6 hours	Normal
11.	>10	>10	>10	18hours	Markedly increased
12.	>10	>10	>10	24 hours	Markedly increased

Table 4: Control group(n=6)

S.No.	Cardiac Troponin T Levels (ng/ml) (> 0.1ng/ml +ve result)			Postmortem Interval	Inference
	Left Ventricle	Right Ventricle	Pericardial Fluid		
1.	>10	>10	>10	30 hours	Markedly increased
2.	>10	>10	>10	28 hours	Markedly increased
3.	>10	>10	>10	40 hours	Markedly increased
4.	0.05	0.06	0.03	6 hours	Normal
5.	0.05	0.08	0.06	8 hours	Normal
6.	>10	>10	>10	36 hours	Markedly increased

Table 5: Histopathological examination results of study group & control group

S.No.	Observations	Study group No. of Cases	Control group No. of Cases
1	Evidence of early myocardial infarction	4	nil
2	Evidence of early myocardial infarction and old infarction	3	---
3	Evidence of old infarction	2	2
4	Normal myocardial histology. No areas of infarction	3	4

$$\text{Sensitivity} = \frac{\text{Total MI cases with positive cTnT test/HPE results}}{\text{Total of all MI cases tested.}}$$

Sensitivity = $11 \div 12 \times 100 =$ **91.66%**

$$\text{Specificity} = \frac{\text{Total non MI/healthy cases with negative cTnT test/HPE results}}{\text{Total of all healthy cases tested.}}$$

Specificity= $4 \div 6 \times 100 =$ **66.66%**

Discussion

WHO defines sudden death as those that occurs within 24 hours of onset of terminal illness. Coronary atherosclerosis contribute about 80 % of sudden cardiac deaths. Establishment of cause of death in cases of sudden cardiac death is a challenging task to the Forensic Pathologist. Any apparent gross change of infarction takes 24- 48 hours following occlusion of major coronary, resulting in autopsy identification of early myocardial infarction difficult. Additional insight may be obtained from history, environment and circumstance of death. Even though minimal microscopic evidence are recognized as early as 6 hours, in the absence of gross changes the involved area may be missed when random blocks are taken for histopathological examination . Studies on human heart during autopsy for detection of early myocardial infarction are scarce.

The basis of using the measurement of a protein in blood to detect cell straightforward and requires consideration of a few major factors:

Criteria for a blood marker of cell death

I. Sensitivity : Abundance in cell, Location in cell

II. Sample timing : Mode of entry into blood, Half-life of elimination

III. Specificity : Distribution in different cells or organs

The myocyte is the major cell in the myocardium, and the heart's action is to pump blood. Because myocytes essentially cannot be regenerated, if heart cells die, then cardiac function has a high probability of being impaired. When the cell dies, the proteins inside the cell will be released, with proteins in the cytoplasm leaving the cell more rapidly than ones in the membranes or fixed cell elements. The most sensitive markers should be those in highest abundance in the cell, and because the major function of the heart is contraction, the proteins involved in contraction and producing the energy to support it should be good candidates for biomarkers of cardiac injury which could be detected in blood. Also, one has to consider the means by which the markers can reach the blood. Since occlusion of blood flow is the primary cause of myocardial infarction, most of the proteins reach the blood via the lymphatics where they are prone for degradation leading to delayed appearance in the blood. Finally, specificity can be achieved if the protein has a cardiac specific form. While utilizing abundant cardiac proteins involved in contraction or energy production seems obvious, it is not exactly the way the field evolved. Cell injury markers can be identified via via gene expression or proteomics. However, these are recent advances which required sequencing entire genomes and the development of technologies which were not available until recently. Table 6 Characteristics of various studies involving Cardiac troponin T as a biomarker to diagnose Acute Myocardial Infarction

Cont... **Table 6 Characteristics of various studies involving Cardiac troponin T as a biomarker to diagnose Acute Myocardial Infarction**

Author	Age group	Postmortem Interval (PMI) (hours)	Analysed sample	Cardiac Troponin T levels
Ellingsen et al.5	4–92	3–75	Serum	Elevated (1.95microg/l)
Zhu et al. 6	0–94	< 48	Serum, Heart blood, Pericardial fluid	Markedly elevated (Pericardial fluid < Heart blood<Serum)
Khalifa et al.7	—	6–20	Serum	Elevated
Remmer et al.8	25–54	8–141	Pericardial fluid and serum	Elevated
González-Herrera et al.9	27–95	5–34	Pericardial fluid and serum	Markedly elevated
Carvajal-Zarrabal et al. 10	24–74	<8	Serum	Markedly elevated ($p<0.05$)

Similar to the above mentioned studies (Table 6), our study also showed markedly elevated Cardiac Troponin T levels in heart blood of right ventricle, left ventricle and pericardial fluid (>10ng/l) and was statistically significant.

In our study, two cases of heart blood, cTnT level from the control sample was within normal range for living patients, while the remaining samples showed high levels, maybe due to autolysis that would be false positive of AMI in a living patient. , others have reported levels similar to living persons and have dismissed the role of hemolysis and autolysis[11].Some have propounded that Cardiac Troponin T levels may be elevated in many deaths because of nonspecific cardiac injury due to lack of oxygen during the agonal period.

The aims of this short study was to ascertain the relationship of deaths due to acute MI between Cardiac Troponin T levels and correlate them with the HPE findings. Limitations of his study include factors like cohort size, autolysis, variation in time since death, and cold storage duration. Inspite of these shortcomings, the data was analysed that may help our understanding of Cardiac Troponin T.

Conclusion

This study showed that there is a strong relationship between postmortem Cardiac Troponin T (cTnT) reactivity with death caused by myocardial infarction correlating significantly with histopathology findings.

One issue with this study and studies upto date, is that Cardiac Troponin T levels are tested in obvious patients with acute MI and compared with patients who died of non cardiac causes. Inevitably, all of these studies concluded that increased Cardiac Troponin T levels correlate with the cause of death and supported the gross and HPE findings.

Ethical Clearance: obtained from Institutional Ethical Committee

Source of Funding: Self

Conflict of Interest: Nil

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Biological Effects of Tumor Necrosis Factor Alpha (TNF-α) in Systemic Inflammation. Running title: TNF-α for systemic inflammation

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Abstract

Aim. The purpose of this article review is to investigate the biological effects of TNF-α in systemic inflammation at moderate levels. TNF-α is a product of macrophages, one of the body's defence systems that is active in the presence of a bacterial infection.

Background. TNF-α plays a role in host defence for bacterial, viral and parasitic infections. TNF-α is produced by macrophages and is activated by T cell lymphocytes, antigens, NK cells, and mast cells. TNF-α is usually not detected in healthy individuals but is often found in conditions of inflammation and infection in the serum. TNF-α works against leukocytes and endothelium, induces acute inflammation at low levels because TNF-α is a strong pyrogen. TNF-α plays a role in systemic inflammation at moderate levels. TNF-α causes pathological abnormalities in high levels of septic shock, because TNF-α is cytotoxic.

Riview Results. In the review of this article we get results about the biological effects of TNF-α on systemic inflammation at moderate levels and their role in the humoral and cellular immune systems.

Conclusion. TNF-α has a biological effect on systemic inflammation at moderate levels and has a strong role in the humoral and cellular immune systems.

***Keywords**: Tumor Necrosis Factor Alpha (TNF-α), Humoral, Cellular Immune, Systemic Inflammation*

Background

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine which plays a role in the inflammatory process, initiates polymorphonuclear (PMN) and activites it so that PMN can reach the site of infection. Tumor necrosis factor alpha (TNF-α) is the main cytokine in the acute inflammatory response to Gram negative bacteria and other microbes. [1]

Severe infections can trigger the production of large amounts of TNF-α which results in a systemic reaction. The main sources of TNF-α are mononuclear phagocytes and T cells that are activated by antigens, NK cells, and mast cells. Lipopolysaccharide is a patent stimulation of macrophages to secrete TNF-α. IFN-γ produced by T cells and NK cells also stimulates macrophages, including increasing synthesis of TNF-α.[2] TNF-α has several functions in the inflammatory process, which can increase the pro-thrombotic role and stimulate adhesion molecules from leukocyte cells and induce endothelial cells, play a role in regulating macrophage activity and immune responses in tissues by stimulating growth factors and other cytokines, functioning as regulators of hematopoietic and commitogen for T cells and B cells and neutrophil cell and macrophage activity. [3]

TNF-α also has beneficial additional functions including its role in the immune response to bacteria, viruses, fungi, and parasitic invasion. Almost all

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inflammatory processes result in activation of tissue macrophages and blood monocyte infiltration. This activation causes many changes in cells, including the production of TNF, IL-1, and IL-6, namely cytokines that cause multiple effects on the host. [4]

Among the many proinflammatory cytokines, tumor necrosis factor α (TNF-α) has an important role in the occurrence of systemic inflammation. Preclinical and clinical studies have reported that suppressing TNF-α expression can reduce the development of inflammation in many diseases, including typhoid fever caused by Salmonella typhi bacteria. Using anti-TNF antibodies can reduce systemic inflammation that occurs during the infection process. Besides the dissolved TNF-α receptor has the ability to reduce the induction of ischemia and the sequestration of neutrophils which play a role in injury to the infected intestine. [5]

Endogenous pyrogens, which can cause inflammation, apoptotic cell death, and mediate the release of various cytokines such as IL-6, IL-8 and IL-1 by stimulation of macrophages. Excessive production of TNF-α can cause various diseases in humans including atherosclerosis, cancer, and inflammatory bowel disease. [6] However, it is still unclear, and many studies are directly related to TNF-α. The molecular mechanism of TNF-α from clinical studies found that a decrease in levels of TNF-α in the blood level can control the inflammation that is currently occurring in the host in systemic inflammation. [7]

Riview Results

TNF-α plays a role in host defence for bacterial, viral and parasitic infections. TNF-α is produced by macrophages and is activated by T cell lymphocytes, antigens, NK cells, and mast cells. [8] TNF-α is usually not detected in healthy individuals but is often found in conditions of inflammation and infection in the serum. TNF-α works against leukocytes and endothelium, induces acute inflammation at low levels because TNF-α is a strong pyrogen. TNF-α plays a role in systemic inflammation at moderate levels. TNF-α causes pathological abnormalities in high levels of septic shock, because TNF-α is cytotoxic. The biological effects of TNF-α are as follows: [9]

1. Deployment of neutrophils and monocytes to the site of infection and activate these cells to get rid of microbes.

2. Encouraging the expression of molecular adhesion of vascular endothelial cells to leukocytes.

3. Stimulates macrophages to secrete chemokines and induces chemotaxis and leukocyte deposition.

4. Stimulate mononuclear phagocytes to secrete IL-1 with effects such as TNF-α.

5. Induces the same inflammatory cell apoptosis.

6. Stimulates the hypothalamus which induces heat, so-called endogenous pyrogens.

7. Production of large amounts of TNF-α can prevent myocardial contractility and vascular smooth muscle tone which lowers blood pressure or shock and weak cells that cause of kaheksia (severe metabolic disorders such as blood sugar drops to levels that are not possible to live).

8. Complications of septic shock syndrome caused by gram-negative or gram-positive bacteria characterized by vascular collapse. Some of the biological functions of TNF-α comprise cellular proliferation and differentiation, tumorigenesis, apoptosis or necrotic cells death, immunoregulators, lipid metabolism, coagulation and endothelial function.

TABLE 1 : Biological Effects of TNF-α [9]

Number	Location Target	Biological effect
1	Neutrophils	Deployment of neutrophils to the site of infection and activate these cells to get rid of microbes.
2	Monocytes	Deployment of monocytes to the site of infection and activate these cells to get rid of microbes.
3	Endothelial cells	Encouraging the expression of molecular adhesion of vascular endothelial cells to leukocytes.
4	Macrophage	Stimulates macrophages to secrete chemokines and induces chemotaxis and leukocyte deposition.
5	Mononuclear cells	Stimulate mononuclear phagocytes to secrete IL-1 with effects such as TNF-α.
6	Hypothalamus	Stimulates the hypothalamus which induces heat, so-called endogenous pyrogens.
7	Cardiac myocardial cells	Production of large amounts of TNF-α can prevent myocardial contractility and vascular smooth muscle tone
8	Vascular	Complications of septic shock syndrome caused by gram-negative or gram-positive bacteria characterized by vascular collapse
9	Inflamation Cell	Induces the same inflammatory cell apoptosis

Discussion

After invading mammalian tissue, bacteria activate complement and macrophage tissue. Activated macrophages secrete proinflammatory cytokines such as TNF-α, interleukin-1β (IL-1β) and IL-8 which

play a role in phagocytosis and enhance the T cell immune response. [10] In the blood, bacteria or bacterial products causal systemic inflammation characterized by activation of macrophages in the reticuloendothelial system, leukocytosis, release of cytokines and hypotension8. Some gram-positive organisms have a thick peptidoglycan layer that inhibits insertion of the C5b-9 membrane attack complex on bacterial cell membranes. Salmonella typhi can induce an inflammatory response in the respiratory tract through activation of TNFR. [11] The complex mechanism of host response to invasion by microbial pathogens includes the production and release of proinflammatory and immunomodulating cytokines, which are very necessary in stimulating leukocytes and other cells by pathogens. [12]

Cytokine synthesis is needed for host defence against infection. But an excessive inflammatory response will cause organ dysfunction and host death. Bacterial products in the form of peptidoglycan cell wall fragments and lipoteikoic acid have characteristics as immunostimulators, which can induce the release of TNF-α, IL-1β, IL-6, IL-8 and IL-10 from monocyte macrophage culture in mice. [13]

Salmonella typhi has several strategies to fight the killing of neutrophils, by releasing Chemotaxis Inhibitory Protein (CHIP), and Extracellular adherence protein and binding to endothelial ICAM-1 adhesion molecules. ICAM-1 inhibition prevents leukocyte adhesion, diapedesis and extravasation of blood flow to the infected part. After arriving at the site of infection, neutrophils release antimicrobial substances, including antimicrobial peptides, ROS, RNS, proteases and lysozyme. [14]

Defence against ROS from Salmonella typhi by releasing large amounts of antioxidant enzymes (eg catalase, pigments, superoxide dismutase) which neutralizes ROS and RNS. Severe bacterial infections usually cause host to improve the specific immunity response within 7 to 10 days to limit ongoing infections and reinfection. Immunity Response due to Salmonella infection in typhi. [15]

3. 1 The Role of TNF-α Humoral and Cellular Immunology

Two types of adaptive immunity, humoral immunity and cellular immunity, are mediated by different cells and molecules and each is designed to provide defence against extra and intra-cellular microbes. [16]

Humoral Immunity

Humoral immunity is mediated by proteins called antibodies, which are produced by cells called lymphocytes B. Antibodies enter the circulation and mucous fluid, then neutralize and eliminate microbes and microbial toxins that are outside the host cells, in the blood, extracellular fluid which originates from the plasma and inside the lumen of mucous organs, such as the gastrointestinal tract and the respiratory tract.

One of the most important functions of antibodies is to stop microbes which are on the mucosal surface and in the blood so that they do not gain access to host cells and do not form colonies in the cells and connective tissue of the host. In this way, antibodies prevent infection from developing. Antibodies cannot reach microbes that live and divide in infected cells. [17]

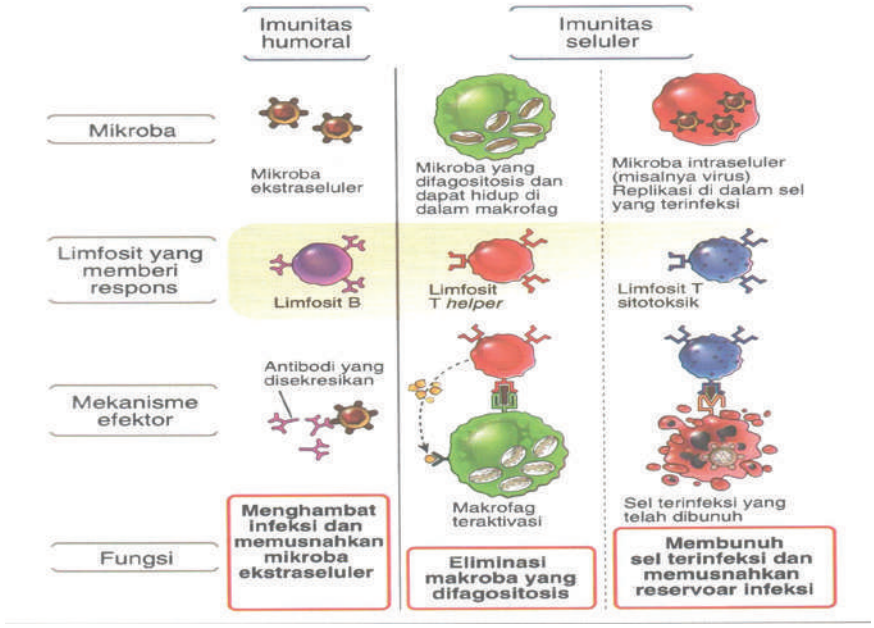


Figure 1

Humoral and cellular immunity [17]

One of the most important functions of antibodies is to stop microbes that are on the mucosal surface and in the blood so that they do not gain access to host cells and do not form colonies in the cells and host connective tissue (Fig. 1). In this way, antibodies prevent infection from developing. Antibodies cannot reach microbes that live and divide in infected cells. [18]

Once tied, B cells receive a signal to begin secreting this form of immunoglobulin, which is a process that initiates an optimal antibody response with the intention of eliminating antigens from the host. Antibodies are a heterogeneous mixture of serum globulins, which work together to demonstrate the ability to bind specific antigens. All serum globulins with antibody activity are called immunoglobulins. [19]

The bond between antigens and antibodies is not covalent, but depends on various bonds with weak forces, such as hydrogen bonds, van der Waals, hydrophobic bonds. Because of the weak nature of the bond, the success of the bond between the antigen and the antibody depends on the area that is very close and appropriate, which can be imagined as the contact between the lock and the lock (a lock and a key). Another important element in the humoral immune response is the complement system. [20]

The reaction between antigens and antibodies activates this complement system, which consists of a series of serum enzymes, and the end of the complement activation reaction is target cell lysis or increases the phagocytosis process by phagocytic cells. Complement activation also results in PMN (phagocytic polymorphonuclear) cell recruitment, which is part of the immune system's acquisition. This activity maximizes the effectiveness of the humoral immune response to invading agents. [21]

Humoral defence consists of complement, acute phase protein, mediator of phospholipid origin, cytokine IL-1, IL-6, TNF-α. Complement consists of a large number of proteins which, when activated, provide protection against infection and play a role in the inflammatory response. Complement acts as opsonin which increases phagocytosis, as a chemotactic factor and also causes bacterial and parasite lysis. [22]

Acute phase protein consists of CRP, lectin, and other acute phase proteins α1-antitrypsin, serum amyloid A, haptoglobin, C9, factor B and fibrinogen. The mediator from phospholipids is needed for the production of prostaglandins and leukotrienes. Both increase the inflammatory response through increased vascular permeability and vasodilation. [23]

The nonspecific immune system uses various soluble molecules. Other soluble factors are produced in a more distant place and are deployed to target tissues through circulation such as complement, acute phase proteins, mediators of the origin of phospholipids and cytokines such as IL-1, IL-6, and TNF-α. [24]

The main actors in the humoral specific immune system are B lymphocytes or B cells. B cells stimulated by foreign objects will proliferate, differentiate, and develop into plasma cells that produce antibodies. The main function of antibodies is defence against extracellular infections, viruses, and bacteria and neutralizes their toxins. [25]

Cellular Immunity

The defence against intracellular microbes is called cellular immunity because the process is mediated by cells called T lymphocytes. Some T lymphocytes activate phagocytes to destroy microbes that have been eaten by phagocytic cells into intracellular phagocytes. Other T lymphocytes kill various types of host cells infected with infectious microbes in their cytoplasm. In both cases, T cells recognize antigens that are displayed on the cell surface, which indicates the presence of microbes in the cell. [26]

There are various T cell subpopulations, each of which has the same specificity for an antigenic determinant (epitope), although the function is different. This is analogous to different classes of immunoglobulins, which have identical specificity but different biological functions. Existing functions originate from various T cell subsets, namely: [27]

1. Working with B cells, increasing antibody production. Such T cells are called helper T cells (TH) and the functions caused by the released cytokines provide various activation signals for B cells.

2. Inflammatory effects. When activating, certain subpopulations of T cells release cytokines, which induce migration and activation of monocytes and macrophages, which cause delayed-type hypersensitivity inflammatory reactions, and that subset of T cells is TDTH cells.

3. Cytotoxic effects. T cells in this subset become cytotoxic kiler cells which if contact with target cells will cause target cell death. These cells are called cytotoxic T cells (Tc).

4. Regulatory effects. Helper T cells can be divided into subsets of different functions determined by the cytokines they release, namely TH! And Mr. Both can regulate each other with negative effects.

5. Signal via cytokine. T cells and other cells involved in the immune system (eg macrophages) affect the effects of various lymphoid and non-lymphoid

cells, through the different cytokines that they release. So, directly or indirectly T cells communicate and collaborate with various cell types.

Over the years, researchers in the field of immunology have known that antigen-activated cells show a variety of effector phenomena. Only in the last century have they noticed the complexity of the events that exist with the activation of antigens and communication with other cells. [28]

Conclusions

The results can be concluded that there are TNF-α have the most significant biological effects on the systemic inflammatory process, namely the deposition of neutrophils and monocytes to the site of infection, stimulating the expression of molecular adhesion of vascular endothelial cells to leukocytes and stimulating chemokine secreting macrophages and induces chemotaxis and leukocyte deposition.

Ethical Approval: The study is literature riviews no ethical approval to support this study

Conflicts of Interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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Experiencing the Ancient Indian Healthcare Science of Ayurveda Through the Immersive Experiential Prism of a Scholar from the Global North West Visiting an Ayurveda Centre in Coastal Karnataka

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Abstract

Ayurveda is an ancient age-old practice that for many centuries has developed to become the principle teaching of how to live a healthy lifestyle: "Ayurveda, the ancient traditional medicine of India, defines health as a state of complete physical, mental and spiritual well-being. The focus of Ayurveda is on a predictive, preventive and personalized medicine. This is obtained through low-cost personalized counseling about lifestyle measures (diet, activities, etc.), trying to involve the patient directly in the process of healing, increasing his self-awareness and good relationships with other people and nature". Ayurveda approaches health from a holistic perspective. Its goal is not to reduce pain, as most modern medicine does, but to prevent it: "The person-centered point of view of Ayurveda mainly concerns the maintenance of an abiding health, preventing rather than treating diseases. If our health is compromised, the treatment resides in restoring the balance between the affected functions, the tissues, the environment and the whole organism. The nature of the individual is a parameter on which the whole system's scale is tuned."

Key words: *Ayurveda, public health, ancient India, healthcare, experience*

Introduction

Ayurveda is an ancient age-old practice that for many centuries has developed to become the principle teaching of how to live a healthy lifestyle: "Ayurveda, the ancient traditional medicine of India, defines health as a state of complete physical, mental and spiritual well-being. The focus of Ayurveda is on a predictive, preventive and personalized medicine. This is obtained through low-cost personalized counseling about lifestyle measures (diet, activities, etc.), trying to involve the patient directly in the process of healing, increasing his self-awareness and good relationships with other people and nature".[1] Ayurveda approaches health from a holistic perspective. Its goal is not to reduce pain, as most modern medicine does, but to prevent it: "The person-centered point of view of Ayurveda mainly concerns the maintenance of an abiding health, preventing rather than treating diseases. If our health is compromised, the treatment resides in restoring the balance between the affected functions, the tissues, the environment and the whole organism. The nature of the individual is a parameter on which the whole system's scale is tuned"[2]

Ayurveda answers one of the most fundamental aspects of public health within itself.

The study of prevalence and distribution of disease, in Public health, is a critical aspect in the foundational principles of Ayurveda. "CEA Winslow in 1920 defined

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Public Health as, "the science and art of preventing diseases, prolonging life, and promoting health and efficiency through organized community efforts"[3]

The definition emphasizes on three essential features; prevention of disease, prolongation of life, and promotion of health. These three essential features have also been noted by proponents of Ayurveda like great saint Charak and others. He mentions about the objectives of Ayurveda which indirectly defines public health and its objective."[4] Ayurveda is heavily influenced by the natural aspects of growth and development in life. In addition, Ayurveda embodies the elemental perspective of nature as well by looking at the human and nature's interactivity: "Individuals interact with the environment through the five senses. Perceptions are generated, processed and integrated into associative memories in a complex model of reality. This model enables the individual to adequately interact both with the received stimuli and with the environment"[5]

According to Ayurveda, these relationships determine the psychological structure, psycho-physical health and general balance of each organism. Health is a positive state, and it is the outcome of the adaptive feedback that each being establishes with the environment. In human terms, health is not merely a biological process; it is also a cultural one involving knowledge as well as the interaction with the situation in order to modify it."[6] Because of this, Ayurveda has developed to become the alternate form of medicine. Whereas modern 21st-century medicine relies on the latest medical technological advancements for answers, Ayurveda relies on the past. It, through centuries, has formed the dynamic, yet all-encompassing principles formed through the process of trial and error. Because of this, Ayurveda has been a viable source for human health development and disease prevention as its ancient, yet powerful remedies have shown time and time again to answer some of nature's hardest problems.

One of the most defining qualities of Ayurveda that makes the science so appealing to many is its use of natural elements to treat, cure, and prevent ailments. One example of this is Ayurveda stress and importance on the use of spices for treatments. This plays a tremendous factor in the public health network. As Dr. Manisha explained at the 3rd annual Sivananda Ayurveda Conference, "spices not only give food flavor, but they have tremendous medicinal power and can be very potent in their effects. In ancient times, spices were used to preserve as well as flavor foods, and during the middle ages, women often embroidered a spring of thyme into scarfs to protect their children against illness. Even today in India, garlands of cardamom are often worn as a preventative measure against disease"[7]

With such a long history and know for their positive effect on the human body spices that range from turmeric, coriander, cumin, ginger, garlic, and cinnamon have grown to be imperative to the process of treating patients. For example, spices can be used for patients who have diabetes, cancer, heart problems and much more: "Scientific studies have shown that turmeric (Curcuma longa Linn.) has anticancer properties—it protects DNA and stimulates detoxifying enzymes. It also promoted wound healing, protects against her disease by decreasing low-density lipoprotein (LDL) and triglyceride levels, is antithrombotic, fights against Alzheimer's disease"[8] Coriander, similar as Turmeric has therapeutic qualities as well: "Coriander (Coriandrum sativum Linn.) protects against heart disease: it decreases levels of lipid peroxide, increases activity of antioxidant enzymes,77 decreases total cholesterol, LDL and triglycerides, and increases high-density lipoprotein (HDL), has traditionally been used as a treatment for diabetes[8] Cumin is another example: "Cumin (Cuminum cyminum Linn.) is antidiabetic; it reduces blood sugar, glycosylated hemoglobin, plasma cholesterol, tissue cholesterol, phospholipids, free fatty acids, and triglycerides. Cumin was more effective in treating diabetes than the drug glibenclamide.[9]

These are just a few of the many spices used in Ayurvedic procedures and treatments. As described, spices play have a profound effect not only on food but on the body as well. Though just powder in most physical states, spices can be the answer to health problems not even drugs can cure, as noted by Sharma. With such benefits, Indian food itself becomes a healthy alternative way of eating, if cooked right. As many Indian dishes include a variety of the species mentioned above, the delivery of these ingredients not only makes it easy to consume for the patients but also ensures preventive measures against the development of future diseases as well. This is one of the first layers of prevention that

Ayurveda ensure through its ancient, yet effective form of the public health system.

In addition to spices, herbs are also part of this preventative and curative measure. For example, Ayurveda includes an extensive list of herbs that can be used for an assortment of treatments: "Ayurveda's materia medica is extensive, with more than 700 herbs described in detail in the ancient texts. The herbs and herbal mixtures utilized in Ayurveda are prepared by using the various parts of the plant".[10] These herbs, however, play a slightly different role than spices do in the sense that they are one, not often used in traditional Indian food and two, are used for a more toxic purpose: "Herbs are sometimes used singly but more often in combination to provide synergistic effects and mitigate toxic side-effects. Ayurveda does not recommend isolating the active ingredient because toxic side effects can occur and the synergistic benefits are lost".[10] Some of these include but are not limited to, Asparagus racemosus, Sida cordifolia, Abutilon indicium, and many more. As described by Sharma these herbs play a multipurpose role for the betterment of the human body: "The herb Asparagus racemosus Willd, promotes lactation in the postnatal period, Sida cordifolia Linn and Abutilon indicum encourage the growth of the baby.[9] These benefits provided by both herbs and spices are examples of how Ayurveda, from ancient times, has focused on promoting public health measures in society.

Though health promotion and preventative health have been a central pillar in the development of Ayurveda, what exactly does health mean to this form of medicine? According to Moradani: "Ayurveda, the traditional medicine of India and first of all anthropological/traditional medical systems, emphasizes the necessity of a more person-centered, personalized approach in medicine. The process of healing has to be based on people's whole bio-psycho-spiritual unity and equilibrium, including their relation to the environment and the way they perceive or "narrate" their own complex individual existence, both in sickness and health."[11] As described above, health is heavily influenced by how in-sync with the surrounding environment both physical and spiritual as well.

With having a proper relationship with these factors not only does a person live a healthier lifestyle, but in addition, they can easily heal from various diseases or injuries that they have contracted. Determinants in public health are extremely important when trying to analyze the incidence and prevalence of diseases in a community. For example, determinants such as sociodemographic status, genetics, behavior, environment, etc, play intertwining role in the status of health. Similar, in Ayurveda, there are determinants that are outlined that play an effective role in promoting and maintaining a proper health status in a community: "In Ayurveda the determinants of health are biological, ecological, medical, psychological, sociocultural, spiritual and metaphysical factors, all interdependent and wired together by the common concept of relationship. The harmonization and integration of these determinants in a complex system allow the emergence of what is identified as health."[11]

Through these determinants, Ayurveda helps create an outline for establishing its foundation of health that in turn provides a baseline for health promotion development and care. In Ayurveda, as noted before, there is a different perspective on care: "Health for Ayurveda is not a constant state of well being, it is rather an ideal space around an ideal condition in which the physiology can float around, the bigger the space, the healthier the individual. The crossing of the boundaries of this space towards disorder is what is called diseased state, but the strength of an organism lies in the ability to return back to his overall ideal condition. The role of Ayurveda is to keep this space as wide as possible and to facilitate the return in case of a disease."[12] Being based around this concept of idealism, Ayurveda approaches preventative health with a multidimensional, promotive, and curative approach: "But Ayurveda is an even more complex scientific and philosophical model of reality which accounts for its multidimensional preventive, health promotive and curative approach, and can give a wider spectrum of possible interventions and possibilities" This universality of Ayurveda allows it to encompass treatments for a variety of diseases and infections. In addition, this variance allows Ayurveda to approach preventative health in a multifactorial way.

One of the ways we can see the universality of health is through Ayurveda concept of prevention in Public Health. Prevention of disease as described by Dr. Halpern is based on four important concepts. The first is dependent upon the early detection promoted by the

mainstream medical community. The second involves preventing the effects of stress from harming us. The third method involved purifying our bodies as a way to keep environmental poisons (pesticides, heavy metals, industrial, chemicals, hormones, etc.) from harming us. Fourthly, prevention involves looking at our lifestyle and recognizing how our choices about how we live and act are producing stress which is slowly killing us. Throughout health care schemes you can see these concepts in play when trying to tackle the spread of disease. For example, the process of routine mammograms women likely to get breast cancer is a process of early detection. In regards to the second concept, the promotion of meditation, daily exercise, micronutrient support, and vitamins have all been sources to help reduce stress.

The growth of herbal alternative such as this, has led to the expansion of organic health food and water purification industries. "In addition, many new products are on the market to help us remove toxins from our bodies. Most of these are herbal alternatives (blood and liver purifiers) and purgatives or laxatives to cleanse the colon. Panchakarma, the Ayurvedic science of purification, also addresses these toxins, as well as toxins created internally through the poor digestion of food."[13] To address the fourth concept, reflection on one's habits and lifestyle can be a beneficial when trying to create positive environments. The idea of such a method is there not to necessarily reduce stress but finding ways to deal with it by learning the trigger factors in our daily lifestyle

Ayurveda's goal, unlike modern medicine, isn't necessarily to just treat but to also prevent it. This aligns with the ideals of public health which looks at the prevention and distribution of diseases within a community as whole: "In the area of disease prevention, Ayurveda teaches us that through a healthy lifestyle that is individually designed to be harmonious with our own unique nature (our constitution), and the practices of meditation and yoga, a person can reach their potential physically, emotionally and spiritually. In this state, the disease does not exist. It serves no purpose. For what is disease? It is our body communicating disharmony. Remove the disharmony, and you remove the disease". However what public health fails to address in preventative medicine Ayurveda answers. This is increasingly evident in the area of personalized medicine. Personalized medicine in Ayurveda is addressed in swasthavritta, a concept which looks at the maintenance of positive health: "It is defined as a state of resistance through the defense activities of the human body against the disease. And this can be attained by getting proper nutrition, minimal exposure to pollutants, reducing stress in one's life (Shweta et al.). This concept was addressed thousands of years ago, something which modern medicine is now finally addressing. Swasthavritta is multifaceted, in the sense that it is an incorporation of many preventative factors. For example, the contents of Swasthavritta include: 1. Daily regimen (Dincharya), 2. Seasonal Regimen (Ritucharya), 3. Occupational duties, 4. Precautionary Observances in sexual activity, 5 General rules of conduct for the well being in society, 6. Preventive measures against untimely old age, 7. Conduct and practices of realization (Shweta et al.). Through this process, Ayurveda highlights on three critical aspects of preventative health which includes personal hygiene, rejuvenating and invigorating agents, and practice of yoga.

For personal hygiene, Ayurveda talks about physical, physiological, and spiritual harmony. There are many ways that this can be achieved. For example, when looking at oral hygiene, Ayurveda stress heavily on the inculcation of natural sources: "According to Ayurvedic texts, it is recommended that Datuna (Chewing Sticks) in the morning as well after every meal prevents oral disease. The method of use is to crush one end, chew it and brush with it slowly. It is better to take this from a fresh plant. The stems used for Dante Pavan should be healthy, soft, without leave and knots. After cleaning the teeth with Dattuna, it is torn in two part and used to scrape the tongue coatings." When talking about rejuvenation, Ayurveda utilizes the concept of panchakarma. A detox process, different than what most modern medicine is aimed at: "Panchakarma is unlike any other detoxification program; it is fundamentally designed to remove a different form of toxin. While many toxins exist in our environment which accumulates and harm our bodies, Ayurvedic Panchakarma addresses a special toxin called ama which is formed within our own bodies. When ama is cleansed, the body can then begin the process of rejuvenation. Almost like pressing a restart button on a phone, the body can not jumpstart with a fresh state of mind and body: "Dr. David Frawley, renowned Vedic scholar, says in his book Ayurveda

and the Mind: "Panchakarma is the main Ayurvedic method for physical purification. It is useful for physical problems caused by excesses of the three doshas. Yet it can also be helpful for psychological problems caused by internal factors, emotions, and karma. In addition, the practice of yoga can as well lead to the alleviation of psychological problems: The primary means of yoga is to balance the mind by removing its tendency to enter states causing imbalance through the qualities of triguna. In Ayurveda, the primary emphasis is on promoting health and preventing pathology and restoring health when a person's bad habits and bad karma have compromised their state of health."

Though Ayurveda might be considered an ancient form of medicine for many, it nevertheless, exemplifies medicine to its real purpose: Preventing disease. As modern medicine looks towards current treatment, Ayurveda looks beyond that scope by asking how can we prevent this from happening again? Based on this fundamental concept, Ayurveda has formed its practices and remedies tailored to that prevention. Because of this, Ayurveda has been one of the first examples of preventative medicine and therefore a gateway to public health care. Having a history of 5,000 years under its belt, Ayurveda didn't merely come up with public health standards without any basis; instead, the concepts and standards outlined in Ayurveda have been a product of years and years of trial and error. Through this, the teachings in Ayurveda have been a guidebook and exemplification for modern medicine to look at and learn from.

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An Autopsy Study of Burn Injuries in Elderly Women

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Abstract

Fire is mischievously called "necessary evil". Burn injuries have long been described as among the most serious injuries that may affect human body. The issue of burns has always been a significant cause of morbidity and mortality in both developing and developed countries around the world and a major cause of global public health crises.[1,2] A 10 year retrospective study of death in elderly burn victims was under taken at St. Johns medical college hospital, Bangalore, India. It was observed that majority of the victims were woman, many of them sustained burns while performing "Puja" (ritual of worshipping God). All were dry burns. Face and upper part of chest was spared in 80% of cases. 80% victims sustained burns during morning hours. Total body surface area involved ranged from 50 to70**%.**

Keywords: *women, Burns Injury, Puja, Hospitalization.*

Introduction

Burns are injuries produced by application of dry heat such as flame, radiant heat or some heated solid substance like metal or glass to the body. Local injury to the body by heat may result from dry heat, application of hot bodies, licking by flames resulting in simple burns, moist heat leading to scalds, and corrosive poisons resulting in corrosive burns. Electric spark, discharges, flashes and lightening leads to electric burns.[3]

Of all the Medico-legal Autopsies, death due to burns is the second most common cause preceded by Road traffic accidents. In India, many of these cases are accidental, suicidal or homicidal. Many studies do exist as to the profile of death due to burns. However this study was undertaken to know the extent death due to burns in elderly, age, sex, mode of sustaining burns, total body surface of burns, duration of survival, cause of death and to suggest any remedial measures.

Burn-related injuries and deaths amongst women in India are likely to be caused by: kitchen accidents related to use of kerosene and flammability of garments; self-immolation or suicides; and homicides related to domestic violence. Evidence suggests that domestic violence (physical, sexual and emotional), which is widespread in India, could have an important role in these burn injuries.[4] Various studies have been undertaken and studied with regards to burns. But, nowhere a study due to burns sustained while performing Puja has been reported.

The purpose of this study was to highlight the mortality due to burns sustained by elderly women of a particular religion performing a ritual called Puja. This study was undertaken at SJMC over a period of 10 years

Puja is essentially a religious *ritual of Hindus*, or a form of communion with the Divine. *Puja* is a religious *ritual* which some *Hindus* perform every morning after bathing and dressing but prior to taking any food or drink. They offer prayers to the Deity of worship, lighting a lamp filled with oil or while pouring oil into it, which is usually kept at the ground level and also rotating around the burning lamp, elderly women

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have been victims of burns. They even offer fruits, flowers and various other offerings to the Deity and recite chants and hymns with eyes closed with devotion.

Materials and Methods:

A retrospective study was conducted at St. Johns Medical College, Bangalore. All medico legal autopsy records and emergency medicine records of elderly burn victims over a period of ten years (March 2001 to December 2010) were perused and descriptive statistics was collected in a systematic manner.

We compared the deaths sustained by elderly women over a period of 10 yrs due to burns sustained while performing Puja & burns sustained by women due to other domestic accidents and suicides. A Statistical analysis of the same was done later in order to understand the trends and patterns of burn deaths while performing Puja.

Results and Discussion

Burn is a problem prevalent worldwide, especially in developing countries.[5]

During the period of study, a total number of 120 victims of burns medico-legal autopsies were undertaken by our Department. In our study there was a preponderance to females compared to males similar to the study by Pateria[6].

Among the 120 cases majority were accidental deaths.Similar results were obtained by the study conducted by Bangal.[7] of these 20 cases were victims of accidental pooja burns. Puja burns constituted 16% of total burns cases.

All the victims who died belonged to predominantly female urban population. This may be because the majority of people admitted in our hospital are from Bangalore.

65% of victims died on auspicious days which were observed as Tuesday, Friday and Sunday. This is because more number of people perform puja during these days.

80% of the victims belonged to Hindu Brahmin Community. Also 80% of victims sustained burns while doing Puja in the morning hours. In hindu community Brahmins are known to perform pujas and morning hours are apt for performing majority of the pujas.

80% of the victims were aged >60 years and only 20% were <60 years. Clothings played a major role, Nylon saree being a risk factor, 80% of victims were found to be wearing Nylon Sarees.

They drape themselves with a saree, usually one end of the saree is left loose hanging around which is hanging at the back and at the level of knees and may come in contact with flame of lighted lamp and can catch fire while rotating around the lighted lamp. While being deeply engrossed in prayers, chances of catching fire are more. Also the poor reflexes in elderly women make them vulnerable to sustain burns. Unfortunately, while being engrossed deeply in prayers or meditating before the Deity of worship, lighting a lamp filled with oil or while pouring oil into it and also rotating around the burning lamp, elderly women have been victims of burns.

The leading cause of death being Septicaemia about 85%. Also 65% of victims died within a week of hospitalisation. All the cases in the present study were cases which were hospitalized at the burns ward of St.Johns Medical College Hospital, Bangalore who died in the hospital and subjected to Postmortem examination due to failure to respond to the treatment given and developing complications of burns such as primary shock, secondary shock, toxaemia, septicaemia, acute renal failure and others.

Leading Cause of Death was Septicarmia-85%

- Most of the cases were dry burns sustained due to flames.
- There was no smell of kerosene.
- There were no scalds seen.
- Majority of the deaths occurred within a week (69%)
- Most of the victims died due to Septicaemia (51%)

The total number of unnatural deaths in elderly women over a period of 10 years was analysed which was 50 cases. Out of which Pooja burns constituted 20 cases i.e, 40%; followed by Road Traffic Accidents 16

cases i.e.32%.

Table -1 - Distribution of elderly burns according to the day.

Days of week	No. of deaths (20)	Percentage
Monday	1	5%
Tuesday	5	25%
Wednesday	2	10%
Thursday	2	10%
Friday	4	20%
Saturday	1	5%
Sunday	5	25%

Table -2 - Age wise distribution of puja burns

Range	No. of deaths
30-40 years	1
41-50	1
51-60	1
61-70	10
71-80	7

Table-3 – Percentage and survival period of burn victims

Case No.	Percentage of burns	Days of survival
1.	80%	5
2.	45%	1
3.	35%	5
4.	30%	20
5.	35%	3
6.	50%	5
7.	50%	7
8.	60%	6
9.	50%	8
10.	90%	2
11.	40%	7
12.	70%	6
13.	80%	1
14.	70%	5
15.	75%	27
16.	40%	26

17.	35%	12
18.	40%	4
19.	35%	18
20.	55%	2

Table -4 - Percentage of burns in death due to puja burns

Range of percentage of Burns	No.	Percentage
0-25%	0	0%
26-50%	12	60%
51-75%	5	25%
76-100%	3	15%

Table -5 - Survival period in days of puja burns victims

Range	No. of cases	Percentage
≤ 7days	14	70%
7 – 14 days	2	10%
≥ 14 days	4	20%

Conclusion

The purpose of this study is to create awareness among elderly women while performing Puja and to take more precautions. As the elderly women suffer from poor reflexes, the family members can play an active role in helping the elderly and preventing casualties.

Further research on the social and economic resources available to the elderly burn population, particularly women, is warranted in order to provide cost effective quality care during acute hospitalization and upon discharge.

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Sudden Deaths in Mthatha Area of South Africa

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Abstract

Background: A sudden unexpected death in South Africa is considered an unnatural death, and requires an autopsy to determine the cause of death. These deaths were classified under a vague term, collapse, by investigating officers and relatives of the deceased.

Objectives: To assess the incidence of sudden deaths in Mthatha.

Method: This is a record review from 1993 to 2004 of the medico legal autopsies in Umtata General Hospital (UGH). All cases of unnatural deaths are brought by police to UGH mortuary**.**

Results: Four hundred and thirteen cases of sudden deaths were recorded over a period of 12 years (1993-2004) with an average of 11 deaths/100 000 population. The highest (22.7/100 000) was recorded in 1997, and the lowest (4.5/100 000) in 1996. Males outnumbered females 3.2:1. The commonest age group involved is between 31 and 40 years. The commonest underlying cause of death is pneumonia.

Conclusion: There is an increasing trend of sudden deaths in the population in Mthatha and it is most prevalent among males between 31 and 40 years.

Keywords: *sudden, death, natural*

Introduction

Sudden deaths are unexpected resulting from various causes, and in layman terms as called 'collapse.' Sudden death is defined as unexpected death occurring as a result of natural causes where loss of all functions occur instantaneously or within 6 hours of the onset of symptoms or collapse.[1] Sudden cardiac deaths cause between 300 000 and 400 000 deaths a year. It is the leading cause of death in men between 20 and 65 years of age.[2]

The HIV/AIDS epidemic in South Africa continues to grow at a rapid rate. Various projections of the demographic impact of HIV, based on the antenatal survey results, suggest that the disease will have a considerable impact on mortality in South Africa. While projections differ somewhat, they suggest that between 2000 and 2010, somewhere between 4 and 7 million South Africans will die from HIV/AIDS. Several studies showed that there is a role of HIV in sudden deaths directly or indirectly.[4,5] A number of studies comparing HIV infected and uninfected drug users have noted an increased sudden deaths from overdose for HIV infected. In Amsterdam the rate was 0.39/100 persons years for the uninfected but 1.42/100 person years for the HIV infected.[6]

The majority of sudden cardiac deaths in the young are due to inherited forms of heart muscle disorder and irregular heart function.[7] With an estimated US population of 296, 766, 821, this implies 163, 221 out-of-hospital sudden cardiac arrests occur annually in the US.[8] Hundreds of fit and healthy adults are dying annually in England for no apparent reason- and little concerted preventive action is being taken largely because there is no agreed term to describe the syndrome. [9] A French study on 1000 autopsies of those under 65 years showed a cardiac cause of death in 848 (85%). In 152 no cause was found.[10] Italian Law requires every athlete to have

an annual "Fitness Certificate" before they are permitted to participate in a sports club and schools have to take responsibility for their students' fitness to participate. [11]

The problem of death in individuals with HIV infection is of importance not only to individuals infected with HIV but also to health care professionals trying to provide accurate information concerning the natural history of HIV.[12] Sudden death in HIV-infected drug users have been reported. [4] There is an increased risk of sudden cardiac deaths if erythromycin and protease inhibitors are taken at the same time. [5] The purpose of this study is to estimate the cases of sudden deaths in this region.

Methods

This retrospective study covers the period January 1993 to December 2004, and was carried out in Umtata General Hospital (UGH) mortuary. This is located on the hospital premises, the teaching hospital of the University of Transkei in the Eastern Cape, South Africa. Now it is a level 1 hospital but remains a teaching institution. The mortuary provides services to Mthatha (Umtata) and Ngqeleni magisterial districts, which together have a population of approximately 400 000. Annually more than 1200 autopsies are carried out in this mortuary. All deaths from unnatural causes in the region are notifiable to the police, who then request medico legal autopsies. All cases of sudden deaths are brought to this mortuary and a medico legal autopsy is conducted.

Results

Four hundred and thirteen cases of sudden deaths were recorded over a period of 12 years (1993-2004) with an average of 11 deaths/100 000 population. The highest deaths 22.7/100 000, were recorded in 1997, and least 4.5/100 000, were in the year 1996. Males outnumbered females 3.2:1 (Table 1). The commonest age group involved is the one between 31 and 40 years with 24.4% of deaths, and least in the age group 1 to 5 years (5.2%) (Table 2). The systems involved in these sudden deaths were respiratory 19 (28.3%), cardiovascular and nervous 9 (13.4%) each (Table 3).

Table 1. Sudden deaths in Mthatha area of South Africa (1993 to 2004) (N=413).

Year	male	Female	Total	Sudden deaths/100 000 population per year
1993	27	6	33	8.2
1994	33	7	40	10
1995	38	5	43	10.7
1996	16	2	18	4.5
1997	71	20	91	22.7
1998	30	11	41	10.2
1999	30	4	34	8.5
2000	24	11	35	8.7
2001	22	5	27	6.8
2002	25	14	39	9.8

***Cont...*Table 1. Sudden deaths in Mthatha area of South Africa (1993 to 2004) (N=413).**

2003	56	9	65	16.3
2004	42	21	63	15.8
2005	54	13	67	16.7
Mean	40.2	10.5	34.4	13.9

Table 2. Age groups in sudden natural deaths in Mthatha area of South Africa 1996-2004 (N=365).

Age group	Male	Female	Total
1 to 10	11 (3%)	8 (2.2%)	19(5.2%)
11 to 20	17 (4.6%)	10 (2.7%)	27 (7.3%)
21 to 30	47 (12.9%0	13 (3.5%)	60 (16.4%)
31 to 40	69 (18.9%)	20 (5.5%)	89 (24.4%)
41 to 50	56 (15.3%)	14 (3.8%)	70 (19.1%)
51 to 60	42 (11.5%)	7 (1.9%)	49 (13.4%)
60+	35 (9.6%)	16 (4.4%)	51 (14%)
Total	277(75.9%)	88 (24.1%)	365 (100%)

Table 3. System related causes of death on autopsies in 2005 (N=67).

S.No	System	Number
1.	Respiratory system	19 (16 pneumonia, 2 PTB, 1 COPD)
2.	Cardiovascular system	9 (8 Cardiomyopathy, 1 CAD)
3.	Central nervous system	9 (4 meningitis,3 Subarachnoid Haemorrhage, 2 epilepsy)
4.	Gastrointestinal system	4 (1 enteritis, 1 infarction, 1 hepatic, 1 hernia)
5.	Reproductive system	1 (abruption placenta)
6.	Miscellaneous	8 (3 septicaemia, 1diabetes, 1 nephropathy,
7.	Undetermined	17

Discussion

This retrospective record review is only enquiry in this area related with sudden deaths. It provides important information that justifies expanded efforts to initiate and develop a programme for a well planned study. It is a trend in the mortuary to declare a person collapsed where there is no visible injury on body, and no circumstantial history is available at the time of death. This trend has been prevalent for years among investigating officers, mortuary staff, and medical officers who conduct autopsies. The problem of sudden death is not only important to the relatives of the deceased, but also to the health professionals trying to provide accurate information concerning the cause of death. This is more important in low resource areas such as Mthatha. Mthatha (Umtata) was the capital of former black homeland of Transkei. Facilities for health care services including medico legal investigations are at lower end of the spectrum in South Africa.

There is a lack of statistics on sudden deaths, and therefore it is difficult to compare with the findings of this study. Sudden cardiac deaths causes between 300 000 and 400 000 deaths a year in United States. [2] Four hundred and thirteen cases of sudden deaths were recorded over a period of 12 years (1993-2004) with an average of 13.5 deaths/100 000 population (Table 1). There is an increase in the number of sudden deaths from 1993 to 2004, but it is not a steady pattern. The peak was in 1997(22.7 per 100 000 deaths) (Table 1). There is no explanation for this sharp rise in 1997. However, the medico legal officers certifying deaths have changed. This is much higher than reported by a North American study, where the annual incidence of sudden cardiac arrests is 0.55 per 1, 000 population. With an estimated population of 296 million in the United States, this implies that about 163, 221 out-of-hospital sudden cardiac arrests occur. [6] There were only 9 deaths in 2005 as a result of cardiovascular disease. Of this only one had coronary artery disease, and 8 were due to cardiomyopathy. Ischemic heart disease is not common among African people. A study carried out by Seedat et al (1996) showed that coronary artery disease is still uncommon among the black population of South Africa. [13] Hypertrophic Cardio Myopathy is common. A study by Maron (1995) showed that 1 in 500 people have Hypertrophy Cardiomyopathy.[7] The ratio of males to females is 3.2:1. This is because males are much less bothered about their illnesses than females and they are detected only at autopsy.

It is surprising that sudden deaths in 2005 were due to pneumonia and pulmonary tuberculosis, 18 out of 19 involving the respiratory system, and meningitis 4 out of 9 involving the central nervous system (Table 3). Deaths from tuberculosis (TB) are increasing but TB-related sudden death (TBRSD) is rarely reported in the literature. The most common cause of TBRSD was tuberculous bronchopneumonia in 64%.[14] This infectious disease is opportunistic infections in immunocompromised such as HIV. Many HIV- seropositive patients who present with pneumonia have obvious clinical features of immunodeficiency. The possibility of mycobacterium tuberculosis infection should always be considered in immunocompromized patients. [15] A post mortem study carried out in Edinburgh showed that 9% of deaths are associated with AIDS. Significant encephalitis is also present in post-mortem diagnosis of AIDS.[12] The commonest age group involved in sudden deaths is 31 to 40 years, 24.4% **(**Table 2). It is the same age group who also has the highest rate of HIV infection. The prevalence of HIV was highest among women aged 25-34 years-more than 1 in 3 is estimated to be living with HIV.[16]

Conclusion

There is an increasing trend of sudden deaths and infectious diseases contribute to this trend. The need to investigate thoroughly the cause of sudden death is emphasized.

Ethical issue: Ethical permission was granted by the University of Transkei ethical committee (approved project No. 4114/1999). The names of all the cases were kept anonymous and not divulge to anyone.

Conflict of Interest: None

Funding: self-funded

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An Opinion based on the Autopsy Report on the Manner of Death by a Gunshot Injury: Concealed Murder or Accidental

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Background: Most of the forensic pathologist limited themselves to give a cause of death in a case of firearm death without taking any history. It neither help to the next of kin nor were the judiciary, as a result of it the chances of misdirected investigation are high, and therefore miscarried justice also high.

Objective: To high light the manner of death in a case of gunshot injury.

Method: A post mortem report and a brief history provided by a private agency to give an opinion on the manner of death.

Case history: Mr. A was died in a pistol gunshot injury by his colleague while on duty. The case history narrated that the gun was accidently fired during unloading by Mr. O. The intention of firing has to be determined. The cause of death was given by pathologist but no opinion on manner of death which was crucial to give in this case. The history, findings of postmortem report and manner of death has discussed in this report.

Conclusion: The manner of death is a concealed murder, warrants an investigation by a higher investigative agency such as Central Bauru of Investigation.

Keywords: *gunshot, accidental, intentional, death*

Introduction

Police officers, politicians and criminal nexus in India are known to everyone. Politician-police-criminal nexus flourishes because politicians decide on posting of officers at police stations.[1] (Opinion. Times of India 2020). There are a lot of reports where police officer were get killed by gangs, drug and sand mafia. Sometimes, honest police officer get killed because he is honest, and doing his duty honestly. [1] In 2016, 251,000 deaths resulted from firearms injuries worldwide without any war.[2] Firearm injuries account for the most homicides in the United States of America, with 46 deaths every day.[3] Some studies showed over 90 deaths every day caused by gunshot-related injuries.[4] Almost two-thirds of these deaths (59%) resulted from homicide, with just over a third (35%) as a result of suicide. [5]

After thorough search on literature the author could not find any literature where some has died as a result of fire during unloading the pistol. There are cases of homicide and suicide by gunshot injury but could not find an accidental fire during loading and unloading of pistol. The purpose of this case report is to high light the problem of fallacious misconception of accidental firing and death of another police officer during unloading of a gun.

Method

A post mortem report is submitted to give opinion on the manner of death. A brief history is also provided by a private agency. A team of police officer was chasing mafia who use to sell opium. These mafias are very hard core and also have some liaison with police officer who protects them. Mr. SI was a leader of team accompanied with three police man and a commando police (CP) officer who were chasing these criminals. While returning back, car tyre was punctured in a village where they stopped. The CCTV footage was provided where initial allegation of CP of killed himself was clearly denied. The SI fired from his pistol to CP, where

he is mentioned that the fire was an accidental during unloading of a pistol. CP was died on the way to Jodhpur city in India which was about 150 kms from the incident. The commando died on their way to hospital which took about 5 hours to reach in Jodhpur,

Discussion

It is unfortunate that such type of a death of a police officer is investigated by another police officer. It is very well known that there is always nexus between police and criminals. It is just like a team of soccer who is having their own player and also referees. This kind of team is certainly going to win. An appointed investigating officer must not be a member of this team, to protect the justice system, therefore this case must be handover to CBI. This is a perfect case to be investigated by a Central Bureau of investigation (CBI) to give a clear picture of death, so that an appropriate punishment to be given to the culprit. What a paradox in an Indian judicial system where a suicide by a film star is pushing towards murder and a clear cut murder is putting under accidental. Moreover a prime facie case of murder of a commando is not order for investigation by a police whereas a clear cut suicide is under investigation by a CBI. The Commando who was killed by a fellow police officer, suppose to investigate by a CBI.

A lousy postmortem was conducted in a hospital where they could not even solve the problem of deceased. What is the purpose of autopsy? It is not just dissection of a body and write a finding copied from a text book but one has to assertive in giving opinion related with each and every case. Here in this autopsy report, nowhere is try to answer the manner of death which was crucial to answer by forensic pathologist in this case. Unfortunate part in Indian legal system is that there is lack of accountability. Forensic pathologist has conducted an autopsy without any direction given by the investigating officer, and judicial officer accepted as such without any applying their mind. Here are few comments on the autopsy report

1. The author is having problem with this post mortem report as it is written by hand. At page 2. Entry wound the measurement is given in 3/4x ¼ x cavity deep and at the same time the examiner is writing it is circular in shape. How it can be? It is surrounded by circular ring of grease collar (inner), and abrasion, reddish (outer). How could he see the grease collar? No blackening, burning, tattooing present on body. Has he not examined the clothes? Have this examiner seen this grease collar or writing as some forensic book are traditionally writing in describing the wound.

2. In describing exit wound on the same page, $2x1^{1}/_{4}$ cm x cavity deep. Exit wound is present on posterior chest wall between both scapula, 19 cm below nape of neck, 11 cm from both scapulas. It means the exit wound is in middle of both scapulae. The examiner is showed in sketch diagram that the wound is more on the left side and also describe on left side wound of track. It is surprising that the examiner has written the protrusion of fat and muscle tissue is present from the exit wound which is not visible in photograph. Author would like to ask the examiner, from which book he is coping these findings?

3. On the same page, the entry wound is 22 cms from right shoulder and 15 cms above right nipple is very vague way of providing a location of a wound. It is rather useful to give from midline. A track was established between entry and exit wound on dissection. It passed from first inter costal space, and immediately reach to upper lobe of right lung (entry ¾ x1/4 cm), then passes right lung ($1x1^{1}/_{2}$ cm), then passes through right 4^{th} intercostals space to outside, left posteriorly. The measure the wound on lung is just impossible as it is get collapsed. How could he measure it?

4. Injured right clavicle, 2^{nd} rib, 4^{th} rib (posterior), and thoracic vertebrae T4/T5 are fractured. About 1200 ml clotted blood in right pleural cavity. All have written without any photographs.

The Gunshot entry wound on the right side upper part of the chest which is passing through intercostals muscle, and puncturing right upper lobe of right lung, and then bullet coming out from left of back of the chest. The direction of the wound is above downwardly, backwardly from right to left indicating that SI was standing in front of CP at a height than CP.

The author has gone through post mortem report and a brief history. Based on the facts submitted in post mortem report, it is a clear cut case of murder without any doubt. The probability of accidental firing has ruled out by the facts that

1. The entry gunshot wound is on the top of the trunk, which is most unlikely can happened in case of accidental unloading of a weapon.

2. The direction of the entry wound is from right to left, above downwardly and backwardly, again indicated that the accidental firing is not possible during unloading of a gun.

3. The position of assailant and victim. The victim is a little lower level than assailant as per looking the direction of the wound, which is very unlikely, that it could be happen during unloading of a gun.

4. It is discussed with several police officers the process of unloading and accidental firing and their comments were totally denying the fact of accidental firing. During training of a police officer, they use to teach how to load and unload a gun, and what are standard operating procedures?

5. The victim was collapse on the way to hospital, about 150 kms from the site of happening which is again suspecting some ulterior motive officer a police officer. He could have taken to nearby district hospital, and can get manage an emergency, but assailant did not seek that necessary.

Pistol has 12 cartridges in the magazine. During the loading of the Pistol, the magazine is taken out from the Pistol and cartridges are inserted inside the magazine and then again inserted inside the Pistol in the space provided in the back of the Pistol. The Pistol cannot fire during the loading as the firing with the Pistol requires pulling back the slide to cock the Pistol and some force is required to pull back the slide. If the magazine loaded with the cartridges is already in, the slide is pulled back to make it cock ready for fire. As for unloading, there is a button which is pressed to release the magazine and after that the magazine is pulled out. There is no chance of fire because trigger cannot be pushed in this process. The Pistol can fire only when deliberately the slide is pulled back for the purpose of fire otherwise there is no chance of fire during loading or unloading. If the person deliberately fires, it is murder if person intended is killed and if a person is killed by negligence, it is a separate offence under IPC. Homicide is another condition in which a person is killed when intended action was not to kill which is not applicable in this case. It is a pure case of murder. Where and with whom this has happened.

Conclusion

This is a case of a concealed murder. The victim was only taken to hospital after death. The evidence of the site and direction of firing are also very strong evidence in favour of murder, where to convert impossibility to possibility is not possible. It is fallacious assumptions that it is an accidental firing during unloading process. It deserves to get investigated by CBI so that this concealment of crime could not be successful.

Ethical issue: The names of all the cases were kept anonymous and not divulge to anyone.

Conflict of Interest: None

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Prevalence of Myths and False Beliefs Regarding HIV/AIDS among Xhosa Community in the Transkei Region of South Africa

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Abstract

HIV/AIDS is one of the main challenges facing South Africa today. It is estimated that of the 39.5 million people living with HIV worldwide in 2006, more than 63% were from sub-Saharan Africa. In 2005, about 5.54 million people were estimated to be living with HIV in South Africa, with 18.8% of the adult population (15-49 years) and about 12% of the general population affected.[1]

Prevention of HIV infection has to be tackled in various ways, not just to recommend condom use and circumcision. There are several cultural and social factors associated with spread of HIV infection. There is a general belief that illness (HIV/AIDS) and other bad occurrences are related to witchcraft. The illness is difficult for them to believe and to be explained. Infections like HIV cannot be explained to ordinary rural people and therefore there is a strong belief in witchcraft. People believe that HIV/AIDS is related to witchcraft, and could be managed by traditional healers. Poor people are less educated and therefore more ignorant about their illnesses. Most of young deaths where the deceased is sick from a long time were labeled as "*Isidliso*" otherwise called "black poison". Almost everybody who is suffering from HIV/AIDS has been taken to a traditional healer before been taken to medical practitioner in town. The widespread rape and forced sexual abuse of children is a serious social and health issue. There are several motives behind this unsocial and unhealthy epidemic, but the most dangerous is the strong belief in a myth of achieving a cure for a person's HIV/AIDS through sexual intercourse with a virgin.

Keywords: *HIV and AIDS, Myth and misbeliefs, virgin rape and witchcraft, traditional healers.*

Introduction

Tremendous challenges remain in the field of HIV education, prevention and care in South Africa. It is estimated that of the 39.5 million people living with HIV worldwide in 2006, more than 63% were from sub-Saharan Africa. In 2005, about 5.54 million people were estimated to be living with HIV in South Africa, with 18.8% of the adult population (15-49 years) and about 12% of the general population affected.[1] Nearly 60 children are raped every day in South Africa and while experts agree to disagree to the causes, or whether the pervasive belief in the so-called "Virgin Cure" prevents/cures HIV/AIDS is possibly responsible for this phenomenon**.** The culture-specific belief systems, which is not confined to South Africa, is found to exist in most other developing countries with large numbers of HIV/AIDS positive people in their populations.[2, 3] South Africa is strife-torn with major socio-economic problems as well as the myth that sex with a virgin can rid men HIV/AIDS and sexually transmitted diseases.[4] Thus, controlling HIV/AIDS in Africa needs a different approach. Poverty, social factors, and beliefs are the most potent co-factors for an HIV/AIDS diagnosis.

According to antenatal surveys in South Africa, in 1990 the HIV prevalence was 0.7%, and rose to 30.2% in 2005. This is an increase by 43 fold over a period of 16 years. There is no fall in HIV/AIDS cases in South Africa. Recent (2006) 'Think Tank' group meeting in Lesotho by SADC and UNAIDS, concluded that high levels of multiple partnerships by men and women

with insufficient condom use, combined with low level of circumcision are the key drivers of the epidemic in the sub-region.[5] In 2005, a randomized clinical trial of male circumcision for HIV prevention in Orange Farm, South Africa, found that the procedure reduced a man's risk of infection by at least 60%. This does not explain why HIV has spread so much in Southern Africa than in India, where circumcision is uncommon.[5] There is no strong evidence for this claim and so might be a wrong signal being sent to the society.

The resistance to change, the attitude of African people regarding their false beliefs and persistent myths about their sexual practices, is an obstacle to the prevention programs.[3] Africans are also unwilling to discuss their sexual practices. Their core beliefs are unquestionable. Africans are so difficult people in this respect that one cannot be resolved by reason or evidence. They have certain ideas, which are very dangerous such as rape of virgin is cure for HIV/AIDS. It is difficult for Africans to change their sexual practices through abstinence, monogamy and the use of condoms.[6]

The purpose of this report is to highlight the problem of myths or false beliefs, which is prevalent in this community, and there is hardly any way forward without addressing these obstacles through traditional leaders.

Methods

The third year medical students were asked to write myths prevalent in the community. Majority of the students were Xhosa speaking, born and bred in the local community in and around Mthatha. They were asked to write about myths and misbeliefs regarding HIV/AIDS in the community. Out of 98 students, 63 replied and wrote 42 myths prevalent in the community. These myths were analyzed, categorized, and tabulated.

Results

Table 1. Categories of Myths identified in Transkei region of South Africa.

Myths related with condoms	Myths related with sexual practice	Miscellaneous myths
No one eats a sweet from a paper (condom) or a banana with its peel on.	The cure of HIV is to have a sex with a toddler.	"Xhosa people believe that the witches can inject HIV into blood stream". If you chase HIV, it will run away.
Condoms are all rubbish. What if it stays in me? Scared to use it.	Have sex with a virgin to get HIV cure, and have a shower after having a sex to prevent from HIV transmission	HIV is a politically created horror. It does not exist.
Condoms do not give full satisfaction, as people believe that skin-to-skin contact is vital.	More sex partners you have, the more experience and knowledge you get and the less chances of getting HIV.	Traditional doctors say they can prepare some traditional medicine that can cure HIV.
Xhosa people do not like to wear swimming costume when diving.	If you keep the girl standing after sex without condom you can prevent HIV and pregnancy.	Rural people think that HIV is a myth; it is just a story to scare them from enjoying sex. Beautiful woman cannot be HIV positive.
Condom is meant for superman, not for real man.	Eating eggs before sex makes your chances of becoming HIV +ve, and impregnating a girl higher.	Nevirapine is given by women to their boyfriends to prevent HIV.

Cont... **Table 1. Categories of Myths identified in Transkei region of South Africa.**

Condoms have worms.	If man does not ejaculate inside, they will not get HIV/AIDS.	Having a diet of garlic and vegetables cure HIV/AIDS.
Condoms transmit HIV/ AIDS as it contains HIV infection	HIV/AIDS affects only uncircumcised boys, since they believe that foreskin is the carrier for HIV/AIDS..	HIV/AIDS is a curse put upon by the foreigners; it does not exist among us.
condoms are itching	You cannot enjoy sex without touching meat to meat.	eating pork is a cure for aids
Condoms can protect you from AIDS even from needle stick injuries.	You cannot get HIV infection during your first sexual encounter.	HIV is to punish by forces of the earth
A condom predisposes to fleas.	Trying to prevent HIV is a waste. You can get HIV from drinking water.	One gets HIV from monkeys, not from the people.
	If some one is HIV positive means he is caught by thorn prick	Muti can cure HIV/AIDS.
		HIV is a disease for human beings, not for dogs, and therefore, it should affect us.
		If you are trying to prevent death from HIV, surely you will from something else such as a car accident.
		HIV/AIDS is said to affect people who do wrong things in their lives.
		It is seen as a bad omen; HIV is high in those who drink VODKA; AIDS is common in Johannesburg not in the Eastern Cape; This infection is to reduce the population to conserve oxygen; People do not mind being HIV positive because they get a grant from government.

Discussion

South Africa is a very diverse and a complicated country inheriting enormous problems of the past such as economical inequity and racial discrimination. It has been liberated politically, but not economically and socially. Social fragmentation and moral values were distorted by apartheid and will take years to be healed. The rate of sexual abuse is increasing in the Mthatha area of South Africa. A recent (2008) study carried out by the author has showed that 2 378 victims of sexual assault were seen over a period of six years (2001- 2006). The average rate of sexual assault was 198 per 100 000 women a year. There was a tenfold increase of sexual assault from 39 per 100 000 women in 2001 to 417 per

100 000 women in 2006.[7] One-and-half-fold increase in HIV seropositivity has recorded over the period of 5 years (2000-2004). [8]

Prevention of HIV infection has to be tackled in various ways, not just to recommend condom use and circumcision. There are several cultural and social factors associated with spread of HIV infection (Table 1). The most popular and common belief among people in this area is called as '*inyama enymeni' or flesh*-to-flesh contact, denying use of condoms. There are several myths are associated with sexual practices (Table 1). The most dangerous and widely prevalent myth is of having a sex with virgin is cure of HIV.

A case study has carried out by the author has showed that a nine-year old female child who was brought to the Umtata General Hospital, a victim of the mistaken belief that sex with a virgin will cure an HIV-infected person or AIDS sufferer of his illness. The alleged rapist was an HIV-positive uncle of the child. The myth of the 'HIV/AIDS virgin cure' is prevalent in the community. [3]

The dictum of ABC is not functioning, and higher promiscuity and infidelity, and increasing number of rapes and children sexual abuse is fuelling the HIV prevalence. Abstinence is not possible in among young adults. In a highly promiscuous community, it is difficult to be faithful. Moreover, it is related with poverty. A hungry women need food for her child, is ready to do anything. Transkei is a poverty-stricken former black homeland, now a part of the Eastern Cape Province. Unemployment and the incidental violence are very high. Women are mainly responsible for bringing up their children. Condoms are widely distributed and freely available, but their use consistently is problematic. A study carried out in 2007 by the author showed that HIV prevalence is commoner among condom users.

There were 157 sexually active cases between 17 and 49 years of age in the study group. All had practiced some form of contraception. One had a tubal ligation, 65 (41.8%) had used condoms, 22 (13.9%) oral contraceptives (OC), 55 (35.4%) injectables, and 12 (7.6%) both barrier method and hormonal. The highest number (28 -17.7%) of HIV positive cases were in the age group of 17 to 26 years. A total of 42 (27%) were HIV positive of whom 26 (16.6%) had only used condoms, ten (6.4%) had used OCs and injectables, and six (3.8%) used both hormonal and barrier method. [9]

There is very few studies carried out on psychiatric disorders in this community. HIV infection and psychiatric disorders have a complex relationship. HIV infection could lead to psychiatric disorders, and psychiatric patients are more vulnerable to HIV infection. HIV is not only an illness, which is associated with stigmatization and discrimination, but also has several risks attached including physical and sexual abuse. There is a scarcity of literature on HIV, mental illness and sexual assault although they are a common trio.

There is a case report of an HIV-positive woman with a psychiatric disorder. She suffered from AIDS and periodic psychotic episodes. On a day when she had such an episode, she became a rape victim. [10] Conversely, mentally retarded children are more prone for sexual assault, and therefore more vulnerable for HIV infection. Protection of children has been identified as a priority in South Africa. Despite a commitment to uphold children's rights, much still needs to be done for the safety of mentally impaired children.

A recent (2009) published work of the author showed that over 2% of the sexual assault victims attending the Sinawe Centre were mentally impaired. Of these, 12.5% were HIV seropositive. [11] Mentally retarded perpetrators have infected a teenager seropositive. A case of a 13-year-old girl who was raped twice within three months and brought to the Sinawe Centre of the Nelson Mandela Academic Hospital. Failure to adhere to post exposure prophylaxis (PEP) has undermined the implementation of antiretroviral roll out programme by the government. She was HIV-seroconverted following sexual abuse.[12]

An earlier study (Meel, 2003) showed that more than 90% of victims of sexual assault in Transkei region, South Africa, were HIV-seronegative at the time of the incident. This was despite the fact that the community had a high prevalence of HIV. In sexual assault cases post-exposure prophylaxis (PEP) is recommended to prevent HIV transmission. Therefore, therapy with zidovudine (AZT) and lamivudine (3TC) is justified. There is a serious problem of not attending the patient for follow up.

There were 594 victims of sexual assault during the study period at Sinawe Centre from 2000-2003. Of these, 346 (58.2%) were children under the age of 15 years. Seventeen children (2.9%) were found to be HIV positive at the first test. Among the adults, 58(9.8%) tested HIV positive. Of the 225 who attended after PEP was introduced, only two were found to be HIV seropositive at the time of the incident. A second test was recommended after four weeks and a third after 12 weeks. The majority of the victims did not report for the second test, but all 35 who did come to be tested were seronegative. who did come to be tested were seronegative. Seventeen of those were between 11-15 years of age. Only seven victims came for the third test, and they, too, were negative. Nausea and vomiting were the commonest side effects of PEP treatment in four patients and one developed a generalized rash. Only one victim seroconverted. [13]

The stigma and discrimination attached to HIV/AIDS are hampering control of the disease. Family life has greatly been disrupted by the pandemic. AIDS causes illness, disability and death as well as severe economic and emotional disruptions to the families. The epidemic is well established in South Africa. The mortality will be doubled over the next five years. A broad range of coercive measures has been considered to be applied internationally in the interest of controlling the spread of HIV. Responsibility of the employers to their HIV/AIDS employees at workplace, choice of termination of pregnancy when a woman is HIV positive, attitude of health care provider to their HIV infected patients, informed consent for taking blood to protect from transmission of infection in a case of accidental prick, and forced resignation from employment. [14]

There is a general belief that illness (HIV/AIDS) and other bad occurrences are related to witchcraft. The illness is difficult for them to believe and to be explained. Infections like HIV cannot be explained to ordinary rural people and therefore there is a strong belief in witchcraft. Therefore, they should be targeted to control the HIV in the community. The strategic planners are optimistic to reduce HIV infection by 50% by 2011. Nevertheless, without reducing rapes by 50%, this would be very difficult. The ignorance and misconception in the minds of Africans about HIV is that it is not life threatening. Some like to remain HIV positive for financial gains as there is a government grant available to them.

Conclusion

Several widespread myths and misbeliefs are prevalent in the South African Community regarding its prevalence, transmission, and cure. Many of them are negating the efforts of control of HIV by the government and non-governmental organizations in the community.

Ethical Issue: This report approved by ethical committee of University of Transkei, South Africa for publication.

Conflict of Interest: None

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Metformin : as First-Line Treatment for Type 2 Diabetes Prevention

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Abstract

Objectives: To assess the effect of Metformin, this has multiple pharmacodynamic effects and prevention in type 2 diabetes (T2DM).

Design: Case - control study.

Setting: Outpatient clinics of Diabetic Endocrine Research Lab and patients reporting to medicine department of Sheth Vadilal Sarabhai General Hospital (Smt. NHL Municipal Medical College) , Ahmedabad, Gujarat .

Participants: A total of 240 patients, 100 control group healthy normal and nondiabetic 140 study group with Type 2 Diabetes treated with Metformin and not known with a contraindication for the use of Metformin were approached, given informed consent, and entered the study.

e .Intervention : Addition of Metformin to Insulin Therapy three times a day.

Primary Outcomes: Vitamin B12, Folic acid , Serum Homocysteine.

Results : Metformin Treatment compare with control, was associated with a mean difference in Vitamin B12 -340.26 and confidence 6.71 (95 % confidence interval 27.53 to 40.95: P= < 0.0001) , and in Folate concentration of -6.24 and confidence 0.18 (95% confidence interval 0.74 to 1.1:P=0.026) , and an increase in Homocysteine concentration of 18.97 and confidence 0.93 (95% confidence interval 3.83 to 5.69 : P= <0.0001) . In addition, decrease Vitamin B12 and Folate could be explained by the increase in Homocysteine. The average person with type 2 DM has 3 times the normal rate of gluconeogenesis, Metformin treatment reduces this by over one – third.

Conclusion : Metformin use for long term has been associated with increased homocysteine level and malabsorption of Vitamin B12 which is preventable and some researchers and our findings suggests , recommend screening or prevention strategies .Metformin's efficacy, security, profile, benefic Cardio Vascular & metabolic effects, and its capacity to be associated with other antidiabetic agents makes this drug the first glucose lowering agent of choice when treating patients with T2DM.

Key Words: *Diabetes mellitus type 2, Metformin, homocysteine, Vitamin B12 and folate.*

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Introduction

Metformin (sold as Glucophage) is an oral antidiabetic drug in the biguanide class and the first-line drug of choice for the treatment of type 2 diabetes, in particular, in overweight and obese people and those with kidney function[1, 2] its use in gestational diabetes

has been limited by safety concerns. The most common adverse effect of metformin is gastrointestinal irritation, including diarrhea, cramp, nausea, vomiting and increased flatulence[3].

Metformin have evidence of reduced vitamin B12 absorption due to calcium-dependent ileac membrane antagonism, an effect that can be reversed with supplemental calcium.Vitamin B12 that plays a very fundamental role in DNA synthesis and neurological function, hence an essential micronutrient required for Cardiovascular and Neuro-cognitive function[4,5]. Clinical Vitamin B12 deficiency and biochemical has been demonstrated to be highly prevalent among patients with Type 2 diabetesmellitus.

Type 2 Diabetes Mellitus is associated with a two to four fold increase in the risk of cardiovascular disease, which cannot be fully explained byimportant risk factor such as hyperglycemia, hypertension and dyslipidemia[6]. It also appears that Diabetes interact with risk factor to increase more risk of cardiovascular disease[7]. Hyperhomocysteinemia is a independent cardiovascular risk factor, specially in diabetic patient[8].For prevention of cardiovascular events in patients with Type 2 diabetes in addition for control of type 2 diabetes, identification and treatment of risk factor, such as hyperhomocysteinemia is critical[7].

It is well known that Metformin by decreasing plasma folate and Vitamin B12 levels can increase Serum homocysteine levels [7]. But data on this issues are sparse and conflicting [7,8,9,10].

In view of this consideration, the aim of the study is evaluating Metformin for the prevention in type 2 diabetes mellitus.

Materials & Methods

This study included total 240 patients,100 control group healthy patient and 140 study group with Type 2 diabetes Mellitus treated with Metformin , these patients have been referred to diabetic research laboratory and medicine department of Smt.N.H.L. Medical College , Ahmedabad , Gujarat . All patients gave informed voluntary consent to participate in the study. The study was approved by the ethic committee of Smt. N.H.L. Medical College.

We aimed to include the patient with Type 2 diabetes mellitus between 35 and 75 yrs of age during the study period from November 2012 to Dec 2016 and who had received a diagnosis of diabetes after 25 yrs of age, had never had an episode of keto-acidosis, and whose blood glucose lowering treatment had previously consisted of oral agents but now exclusive consist of insulin or insulin and Metformin. We excluded pregnant women and women trying to become pregnant and other serious medical or psychiatric disease. Patients were randomized into 2 groups trial and control, Trial group treated initially with 850 mg Metformin daily.

Doses of Metformin based on results of monthly Plasma glucose, as need be increased to 2000 mg daily .Whereas in control group 100 patientswere non diabetic without any disease and healthy normal .Exclusion criteria were intolerance of Metformin or drugs which effect plasma homocysteine levels in duration of study. Following Laboratory investigations were done in study and control group.Vitamin B12 by micro particle enzyme intrinsic factor assay, Folic acid by iron capture technology and glucose by Hexokinase, Serum homocysteine was determined by Fluorescence Polarization Immuno acid. All blood specimens were drawn at 0800 hrs after 12 hrs fast.

The normal level of Vitamin B12 are 208-963.5 pg/ml and folate is 7.2-15.4 ng/ml and below the normal range of this parameters is considered to be deficiency respectively [7,9]. The normal level of Serum Homocysteine are between 3.36–20.44 μmol/l. Serum homocysteine level of 20.44 μmol/l or more is considered to be hyperhomocysteinemia [6].

The data were analyzed using SPSS version 11 and P value less than 0.05 and 0.001 were considered to be statistically significant and highly significant respectively. Difference between mean Serum homocysteine levels in groups were assayed by student t- test and paired t- test. Pearson correlation test were applied to test correlation.

Result

In final analysis , there were 240 Indian adults , ≥ 35 to 75 years of age with Type 2 Diabetes using Metformin (male 92 and female 48) , 100 adults normal healthy in same age group (54 male and 46

female). During Metformin treatment homocysteine increased, mean serum homocysteine 29.79 µmol/l with standard deviation 5.06 was compared with 12.02µmol/l to 3.79 µmol/l in the control group. In the patient with Metformin in Type 2 Diabetes homocysteine level was compared in both male and female with control group. Serum homocysteine level was higher in male than female. That was corresponded by other studies (Alan et al., 2005:Passaro et al.2000)[09,10]. The geometric mean serum vitamin B12 concentration among those with Type 2 Diabetes taking Metformin was 132.92pg/ml with standard deviation 29.39. This was significantly lower than the geometric mean concentration in those without diabetes(Control Group) 467.2 pg/ml with standard deviation 207.81, P=0.0116 . The prevalence of Biochemical Vit B 12 deficiency was 6.4% among those taking Metformin ≥ 1-3 years , 4.2% among those taking Metformin > 3-5 years , and 8.2% among those taking ≥ 5 – 10 years (P=0.3319 for ≥ 1-3 years vs ≥ 5 – 10 years . Similarly in patient with Metformin related folate deficiency, mean serum folate concentration was 5.56ng/ml with standard deviation 1.71as compared with 11.06 ng/ml standard deviation 2.64 in the control group.

The demography and biological characteristics of the groups are shown in Table 1. Among Metformin users mean age was 62.22 ± 6.47 years, 66% were male and 34% were female.

Table:1 Socio demographics Profile / characteristics of study population analyzed .

	Metformin (n=140)	**Control (n=100)**
Demographics:		
Men:Women(n:n)	92:48	54:46
Age (years)	62.22(6.47)	50.75(11.46)
Duration of Diabetes Years	12.33(4.59)	NA
Insulin Treatment Years	8(9)	NA
Laboratory variables:		
Fasting plasma glucose(FBS)(mg/dl)	159.70(68.23)	90.81(13.01)
Post Prandial blood sugar(PP2BS) (mg/dl)	205.64(70.98)	100.33(12.54)
Vitamin B12 (pg/ml)	132.92(29.39)	467.16(207.81)
Folate (ng/ml)	5.56(1.71)	11.058(2.64)
Homocysteine (µmol/l)	29.79(5.06)	12.02(3.79)

*Values are in mean and standard deviation.

Compare with control, Metformin Treatment was associated with a mean difference in Vitamin B12 -334.24 and confidence 6.71 (95 % confidence interval 27.53 to 40.95 : P= < 0.0001) , and in Folate concentration of -5.50 and confidence 0.18 (95% confidence interval 0.74 to 1.1: P=0.026) , and an increase in Homocysteine concentration of 17.77 and confidence 0.93 (95% confidence interval 3.83 to 5.69 : P= <0.0001) .Comparing the patients and controls, the levels of Serum Homocysteine were highly significantly higher among patients than controls, while vitamin B12 and folic acid level was found significantly decreased in patients than controls P (<0.0001) for each parameter.

The effect of Metformin on the variables (serum homocysteine, folate and Vitamin B12) were re-analyzed with adjustment of age, previous Metformin treatment, duration of diabetes, gender , insulin dose and actual Metformin dose separately for each variable as well as all together, all of which did not materially change the result.

The our findings correlates and demonstrated as compare to published data (de Jager J 2010) that ,Metformin in Patients with Type 2 diabetes on long term (>5 yrs) lead to modest increase in Serum Homocysteine levels than patients exposed to short term (<5 yrs) and patients not exposed to Metformin: these changes were correlated . When we have calculated P value it was found to be statistically highly significant (p=<0.0001), and rate of significance is higher in study group than in control group.

The results and findings of our study correlates compared to published data (Wulffele at el, 2003) and which show that serum level of homocysteine in individuals with Type 2 Diabetes treated with It indicates that higher homocysteine level is observed in Type 2Diabetes treated with Metformin which is a well known risk factor for the disease of the cardiovascular system which seem to be the main cause of increased mortality in patients with Type 2 Diabetes. Metformin decreases hyperglycemia primarily by suppressing glucose production by the liver [3]. Works by suppressing glucose production by the liver.

Our study limitations are the smaller sample size over all and also we have a small number of female participants in comparison to males. This limitation may cause our inability to precisely evaluate completely the role of hyperhomocysteinemia in sex. Further study with more number of patients with more female participant is needed for more accuracy.

Conclusion

In the present study, we have observed and can evaluate and recommend on the use of metformin for the prevention of type 2 diabetes . After completing the studies, we have an idea about the levels of these investigations such asVitaminB12, folic acid Homocysteine, in patients with type 2 diabetes mellitus with Metformin, and our data provide a strong case for routine assessment of vitamin B-12 levels during treatment with Metformin.

Patients with T2DM have increased risks of various types of cancer. Tentative evidence shows metformin may decrease the risk of cancer. A direct action of metformin on cancer cells is suspected. Metformin exhibits a strong and consistent antiproliferative action on several cancer cell lines. Our findings are similar to those obtained by De Jager J et al , (2010) and Wulffele at el, (2003) that concluded in Patients with type 2 diabetes on long term (>5 yrs) Metformin lead to modest increase in Serum Homocysteine levels than patients exposed to short term to Metformin and patients not exposed to Metformin: these changes were correlated .This means that Metformin therapy carries a potential risk for development of vitamin B12 and folic acid deficiency but can be prevented by giving supplments.metformin modestly reduces LDL and triglyceride levels too.

The risk of developing Metformin associated vitamin B12 deficiency is greatly influenced by increasing age, Metformin dose and duration of use .Our data favours that homocysteine level is affected by both non-modifiable risk factor of atherosclerosis like age and sex and modifiable risk factors like vitamin B12 and folic acid. So, person with increasing age (age above 35 years) should comprise Vitamin B12 in their health check-up and if there is any abnormality treat the underlying cause which will help individual to stay away from CVD in upcoming days up to some extent which seem to be the main cause of increased mortality in patients treated with Metformin in Type2Diabetes.

The study highlights the necessity of checking

VitaminB12 in patients with type 2 diabetes mellitus during Metformin treatment in order to avoid this potential adverse drug reaction and preserve the beneficial effects of Metformin.

Finally, from our study we can conclude that as Metformin is generally can be recommended as a first line treatment for type 2 diabetes mellitus, as there is a good evidence that it decreases mortality[1] .It works by decreasing production of glucose by the liver[2]. Several other groups of drugs, mostly given by mouth, may also decreases blood sugar in T2DM[3].

Metformin is a cornerstone in the treatment of diabetes type 2, but make no recommendations on the detection and prevention of vitamin B12 deficiency during treatment.

Ethical Clearance- Took from NHLMMC IEC

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Conflict of Interest - Nil

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The Spiritual Care Competence Scale: A Confirmatory Study of the Malay Language Version

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Abstract

Background: Nursing competency is an integral part of providing patients with spiritual care. The aim of this study was to validate the translation of the spiritual care competence (SCC) scale to the Malay language version.

Methods: The cross-section study design applicable to the SCCS-M self-report questionnaire. Data were collected from staff nurses at the hospital Universiti Sains Malaysia. A total of 270 nurses participated in this study. Spiritual care competence was assessed with the 27-item SCC-M. Standard forward–backward translation was performed to translate the English version of the SCC into the Malay version (SCC-M). All the participants completed the SCC-M.

Results: The initial measurement models tested (6-factor models) did not result in a good fit to the data. Subsequent investigation of the confirmatory factor analysis (CFA) results recommended some modifications, including adding correlations between the item residuals within the same latent variable. These modifications resulted in acceptable fit indices for the 6-factor model: root mean square error of approximation (RMSEA) = .050, comparative fit index (CFI) = .900, Tucker–Lewis index (TLI) = .885, and standardized root mean square residual (SRMR) = .065. The final measurement models comprised all 27 SCC-M items, which had significant factor loadings of more than .40. The composite reliability was .696-.853 for 6-factors model.

Conclusions: These results suggest that the subscales in 6-factor SCC-M model are unique, the factors do not overlap much, and each factor explains different variance than the other factors. Therefore, the translated version of the SCC-M was valid and reliable for assessing the level of spiritual care competence among hospital nurses in Malaysia.

Keyword*: Spiritual; Validity and Reliability; Nurses; Translation; Competence.*

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Introduction

Spiritual Care Competence (SCC) is the knowledge, skills, and attitudes of nurses who provide patient's spiritual needs with implications and meaning as well as intent questions [1]. It refers to a range of skills used in the nursing process [2]. Such skills used in the professional field or nursing process include therapeutic relationships between nurse and patient, patient communication,

constructive listening, sensitivity, supplying patients with certain religious beliefs with religious services, supporting patients... etc. [3]. When nurses are conscious of their spiritual state, they may be conscious of their patients' spiritual situation [1]. Indeed, this awareness and spirituality in nurses is for creating obligation in the process of spiritual care. In nurses with higher spiritual well-being scores, age, occupation, and clinical career experience the psychological nursing performance was better. By comparison, when depression, anxiety, and stress levels were high, spirituality score was low [4].

The most often conducted spiritual nursing interventions by nurses were recorded as a result of analyzing the effects of local and international studies in the spiritual interventions. The influence of nursing interventions was substantial [5]. However, a nurse's spiritual nursing capability should be developed to recognize possible spiritual needs based on the awareness of the spirituality of the subject, and to conduct nursing interventions based on it [6].

Spiritual care competence scale (SCCS) focused on spiritual dimensions of assessing the competencies of nursing spiritual care. The tool was built on the basis of nursing competence profile [7], which relies on comprehensive literature conducted among nurses. It focused on the role of the nurse in providing spiritual care, spirituality as a side of the methods of nursing care and the involvement of the nurses in improving and developing the performance of nurses within the nursing institution.

The spiritual care competence scale (SCCS) consists of 6 domains with 27 items. The first domain is the 'assessment and implementation of spiritual care' contain six items. This domain refers to the ability to determine a patient's spiritual needs and problem, and to the planning of multidisciplinary spiritual care. This includes written intra- and inter-professional communication of spiritual needs and spiritual care. The second domain is the 'professionalization and improving the quality of spiritual care' contains six items. This domain contains the activities of the nurse aimed at quality assurance and policy development in the area of spiritual care. It refers to contributions to the institutional level that transcend the primary process of care, and with which the nurse also contributes to the promotion of professional practice. The third domain is 'personal support and patient counselling' contain six items. The dimension of this domain was the heart of spiritual care, with items operationalized in terms of interventions. They indicate the actual provision and evaluation of spiritual care with patients and their relatives. The fourth domain is the 'Referral to professionals' is contains three items. The dimension of this domain relating to cooperation with other disciplines in healthcare that take responsibility for spiritual care, for example, Imam. The fifth domain is the 'attitude towards patient spirituality' contain four items. This domain refers to personal factors relevant to providing spiritual care. The last domain is the 'communication' contain two items. This domain refers to the essential aspects of spiritual care between nurses and patients by contact and communication.

The author stated that the instrument could be useful for instructional and academic purposes to assist the capacities of the nurses in delivering spiritual care. Evaluating the nurses will help the researcher enhance the method and establish an effective educational program to enhance the competence of the nurses to provide the patients with spiritual care [6].

However, SCCS was used to assess the competence of nursing college students and staff nurses in hospitals with reliability and validity tested in several countries including the Netherlands, Korea, Brazil and Pakistan with various languages such as English, Korean, Brazilian Portuguese, Prison and the outcome of the reliability test was high from 0.61 to 0.80 [1].

Today, a limited study measure spiritual care in the nursing sector in Malaysia. Contributing to enhancing the evidence-based nursing practice by recognizing, applying nursing competency and checking the validity of the Malay version is required. This study therefore aims to measure the validity and reliability of the Malay version of SCCS among nurses in Malaysia.

Materials and Methods

Participants

The online survey included a total of 320 participants; the response rate was 84% with 270 questionnaires filled out and submitted to the researchers. All participants were staff nurses from various departments (Medical,

Surgical, Pediatric, Orthopedic, Emergency, Psychiatric, Operation rooms and Clinical Unit) of Hospital Universiti Sains Malaysia (HUSM) with at least one year of experience and diploma degree.

Measurement

SCCS self-assessment has 27 items, consisting of six areas: assessment and implementation of spiritual care (Item 1-6), professionalization and improving the quality of spiritual care (Item 7-12), personal support and patient counseling (Item 13-18), referral to professionals (Item 19-21), attitude towards patient spirituality (Item 22-25), and communication (Item 26 and 27) (1 pp. 138-140). The items used a 5-point Likert Scale (1= strongly disagree, 5= strongly agree). The questionnaire has a minimum of 27 scores, and a maximum of 135 scores so that below 64 is low spiritual competence, 64-98 suggests average spiritual care and above 98 demonstrates high spiritual competence. The questionnaire has simple instructions and can be done in 10-15 minutes. Previous studies indicated that the SCCS is a valid and reliable indicator of competence in spiritual care, and the Cronbach's alpha scores ranged from 0.56 to 0.82 [1, 8]. Determining the sample size depending on the number of items in the questionnaire. The sample to variable ratio, also referred to as the N: P ratios where (N) refers to the number of samples and (p) refers to the number of variables or items. This Study's N: P is 10:1. In Confirmatory Factor Analysis (CFA), the sample size needed is 270 participants [9].

Questionnaire Translation

Using forward and backward translation process, the bilingual (knowing both English and Malay) from the Center for Translation and Language in USM prepared the adapted translation of the English version of SCC scale into the Malay version. To finalize the Malay language version of the SCC scale, a consensus was reached on each translated item by sending the questionnaire to a lecturer who holds a PhD in English language translation and a lecturer with a PhD in the Nursing program.

Procedure

The cross-section study design applicable to the SCCS-M self-report questionnaire. Data collection by staff nurses at Hospital Universiti Sains Malaysia, Kelantan, Malaysia using a simple random sampling method. The researcher explained the study to the Head of Nursing in each department. The Head of Nurses shall explain the survey to the staff nurses and provide a link to all participants. The online survey consisted of the information sheet and the SCCS-M questionnaire.

Ethics approval

Ethical approval has been obtained from the Research Ethics Committee of the USM. A research information sheet was given to the participants prior to their inclusion in the study. Consent received when the staff nurses sent the completed questionnaire to the researchers. Participants were informed that their participation was anonymous and voluntary.

Statistical Analysis

Analysis of data using Mplus 8.0. Data screened for missing values prior to study. Out of the total of 270 questionnaires received, there were no responses with missing values. The CFA tested the correlation hypotheses between items depending on the fit indices, including the ratio of chi-square to the degree of freedom ($\chi 2/df$)< 5.0, root mean square approximation error (RMSEA) ≤0.08, comparative fit index (CFI) > 0.9, Tucker Lewis index (TLI) > 0.9, standardized root mean residual square (SRMR) ≤0.08 and p-value > 0.05 for the chi-square test [10-12]. Reasonable values of Cronbach alpha can be greater than 0.7 or 0.6 [13].

Results

Our sample consisted of 270 participants (male 7.4%, n = 20, female 92.6%, n = 251) from Hospital Universiti Sains Malaysia (HUSM). The average age was 35 years (SD = 8.4) and their ethnicity was Malay (96.7%), Chinese (2.6%) and Indian (0.7%). Their religion was Islam (96.7%), Buddha (2.2%), Christian (0.7%) and Hindu (0.4%).

Confirmatory factor analysis (CFA)

The result of the CFA in the initial model demonstrated poor data fit (see Table 1). However, all 27 items have factor loads greater than 0.40 with p values < .001. Further analysis was used to improve the model using the modification indices (MI) (Fig. 1). The findings

of the final model demonstrated an acceptable fit for the data (CFI.90; TLI.885, SRMR.065, and RMSE.050). Factor loads ranged from 0.497 to 0.870. The relative chi-square ($\chi 2$/df) was 1.66, which was less than 3 [14]. The final model was established without missing any of the items after the adjustment indices were applied. The average extracted variance (AVE) was 0.392–0.646 for the final 6-factor model (Table II). Some factors were lower than the recommended value of 0.50. However, the composite reliability (CR) was 0.696–0.853 for the final 6-factor model, which all the CR values were more than the recommended value of 0.60. According to Fornell and Larcker (15), even if AVE is less than 0.5, but composite reliability is higher than 0.6, the convergent validity of the construct is still adequate (see Table 2). Therefore, the model was considered to have adequate convergent validity. The SCCS-M had an appropriate model fit suggested above the recommended values ($\chi 2$/df, CFI, SRMR, and RMSEA) except for the TLI which was marginally below the recommended value. Overall, the SCCS-M was considered to have adequate convergent validity.

Table 1. Summary of the models' fit indices.

Model	CFI	TLI	SRMR	RMSEA (90%CI)	RMSEA p-value
First model SCCS	0.868	0.851	0.066	0.057 (0.050, 0.064)	0.059
*Final model SCCS	0.900	0.885	0.065	0.050 (0.042, 0.057)	0.508

*Measurement final model with correlated item residual (Q3 and Q2; Q6 and Q4; Q18 and Q13).
CFI= Comparative fit index
TLI= Tucker Lewis Index
SRMR= Standardized root mean square residual
RMSE= Root mean square error of approximation

Table 2. Factor loadings, average variance extracted and composite reliability for each factor in the Spiritual care competence (Bahasa Melayu version)

Factors and items	Factor loading	Average variance extracted (AVE)	Composite-reliability (CR)
Assessment and implementation of spiritual care		0.392	0.790
Q1	0.626		
Q2	0.568		
Q3	0.497		
Q4	0.742		
Q5	0.744		
Q6	0.537		
Professionalization and improving quality of spiritual care		0.459	0.833
Q7	0.741		
Q8	0.718		
Q9	0.541		
Q10	0.652		
Q11	0.712		

Cont... **Table 2. Factor loadings, average variance extracted and composite reliability for each factor in the Spiritual care competence (Bahasa Melayu version)**

Q12	0.683		
Personal support and counselling of patients		0.426	0.828
Q13	0.691		
Q14	0.647		
Q15	0.643		
Q16	0.639		
Q17	0.651		
Q18	0.645		
Referral to professionals		0.441	0.696
Q19	0.598		
Q20	0.656		
Q21	0.731		
Attitude towards the patient's spirituality		0.605	0.853
Q22	0.776		
Q23	0.870		
Q24	0.668		
Q25	0.783		
Communication		0.646	0.785
Q26	0.797		
Q27	0.811		

The standardized factor loadings for SCCS-M range from 0.497 to 0.870, P-Value < 0.001.

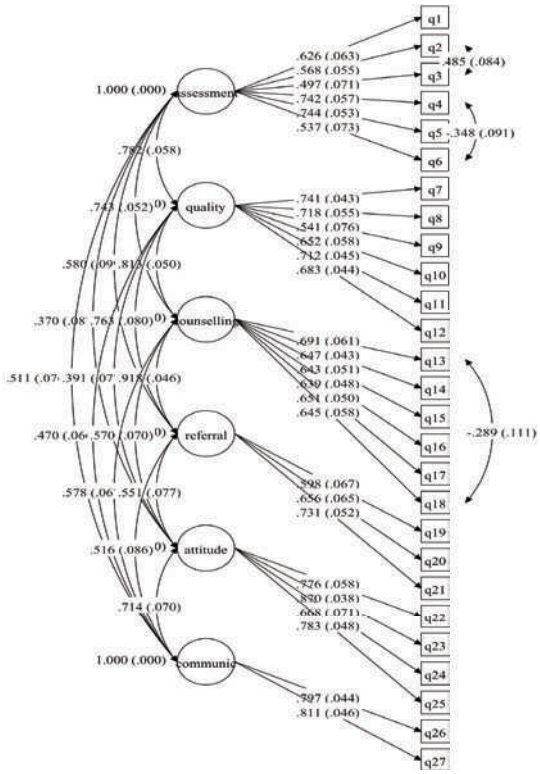


Figure. 1 Confirmatory factor analysis of SCCS-M

Internal Consistency

The Cronbach's alpha of the six factors SCCS-M was 0.787 for the assessment and implementation of spiritual care factor, 0.828 for the professionalization and enhancement of the quality of the spiritual care factor, 0.808 for the personal support and counseling factor, 0.685 for the patients referring to the professional factor, 0.851 for the patients' spirituality factor and 0.784 for the communication factor. Overall, the Cronbach alpha of the total six SCCS-M factors was 0.926, which demonstrated strong reliability (see Table 3).

Table 3. The consistency-reliability

Variables	Cronbach's alpha	Corrected items range
Assessment	0.787	0.726-0.787
Quality	0.828	0.473-0.665
Counselling	0.808	0.537-0.606
Referral	0.685	0.418-0.582
Attitude	0.851	0.619-0.783
Communication	0.784	0.646-0.646
SCCS-M	0.926	0.405-0.653

Discussion

In this study, the forward-backward method used to translate the SCCS English-language version into the Malay-language version is appropriate and understandable to Malaysians. Subsequently, the researcher verified the psychometric properties of the survey to provide an instrument for assessing the competence of the Malay nurses' community in the field of spiritual care. Perceived spiritual care is an essential aspect of the healthcare given to patients by nurses. As a result, the scale of the SCC should consider the concept of self-efficacy and be assessed with the population of Malay-nurses.

In this study, six factors and 27 items were produced without deleting any items similar to the standard scale of spiritual care competence [1]. Another study revealed a scale of competency in spiritual care with only three factors [16]. The CFA in this study showed that the first model did not fit the data well, with CFI (0.868) and TLI (0.851) values less than 0.90. However, all items had factor loads greater than 0.40; therefore, the MI checked for unnecessary items.

Based on the MI, three pairs of items had high MI values (Q3 and Q2; Q6 and Q4; Q18 and Q13). The model re - examined using CFA until the final model revealed that the accepted data fitted well (X2 / df = 1.66. CFI, 0.90; TLI, 0.885, SRMR, .065 and RMSE, .050). In the other hand, a study in Korea found that the CFI was 0.87, and the Normal Fit Index (NFI) was 0.85, which was somewhat incompatible with the fit criterion 0.90 [17]. A Turkish study showed that the Normal Fit Index (NFI) and the CFI were higher than 0.95, but the RMSEA was higher than 0.08. In fact, the model has an accepted fit model [16].

All the model correlations were smaller than 0.85, which demonstrated strong discriminatory validity [18]. Some of the AVE values were below 0.50. However, the composite reliability of the factors was (0.696–0.853) higher than the suggested value of 0.60 [19], which could also have adequate convergent validity.

The SCCS-M has been proven to have internal constancy with the sample studied. The total Cronbach alpha (0.926) was close to previous studies, such as the Brazilian Portuguese version recorded a total Cronbach alpha of 0.92 [20], and the Korean version with 0.95 [17].

The revised item-total correlation for all 27 items was also greater than 0.46. That proves that the scale of the SCCS-M has strong internal stability. The literature claimed that when the item-total correlations were lower than 0.3, it was not sufficient and the items did not help with the main factor check [21].

However, this tool can be utilized for practical and research purposes to evaluate student and staff nurse competencies in Malaysia towards spiritual care. These evaluations can provide insight for the nursing managers about the areas where nurses should receive training to become able to provide spiritual care to the patients. Also, it can be used in further research among nurses, before and after they have received educational programs or training in spiritual care, to evaluate whether they have improved their competencies in providing spiritual care.

Conclusion

The SCCS Malay version developed six factors and 27 items. The CFA reported that all items have loading factors greater than 0.40. The questionnaire also demonstrated high reliability. Overall, the SCCS Malay version has demonstrated an appropriate validity and reliable tool for assessing the competence of nurses in the field of spiritual care and can be used for research purposes. However, further study is necessary to consider the improvement of the tool.

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Conflict of Interest: The authors declare that there is no conflict of interest

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Ethical Statement: The study protocol has been reviewed and is hereby granted approval for implementation by the Human Research Ethics Committee of Universiti Sains Malaysia.

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Bovine Polyclonal Anti-eCG and eCG Combinated Vaginal Sponges Progesterone to Induce Estrus and Pregnancy of Etawa Crossbred Goats

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Abstract

Equine chorionic gonadotropin (eCG) like folligon is a gonadotropin hormon derived from blood sera in pregnant mare 30 till 140 days. The repeated use of eCG treatments for the induction of ovulation is generally followed can decrease in fertility. Bovine anti-eCG antibodies (Abs) as research result in Universitas Airlangga in 2019 can solve the problem of decrease in fertility in does. The objective of this study was to evaluate the reproductive performance of subjected to a short-term protocol for estrus induction using 50 mg of vaginal sponges' progesterone. The experimental animal used in this study comprised of 30 etawa Crossbred goats, with normal estrus cycle and 3 years old of age. The etawa crossbred goat are all healthy with a body score of at least three. 30 goats divided become 3 groups Group I as (T0) 10 goats are as controls Groups. (T0) was inserted sponge progesterone 50 mg of MPA and injected with PGF2α 7.5 mg in day 10 intramuscular (im) when T0 removal of the sponge were removed on day 11. Does on day 11 as T1 going on the removal of devices 24 h before removal inject 7.5 mg of PGF2α and 300 IU of eCG. T2 were injected in all the goats 7.5 mg of PGF2α, 300 IU of eCG at day 10 and 300 IU bovine anti eCG inject at day 11 when removal device on day 11. After 35 days, pregnancy was diagnosed by ultrasound scanning. Short-term protocols using either an intravaginal device was similarly effective to estrus induction 100% $p > 0.05$, and all goats from both groups had estrus manifestation within 30 h after device removal, duration of estrus 37 h and doing AI. In addition, no significant difference in pregnancy $p > 0.05$ on 35 days.

Keywords: *Animal breeding; etawa crossbred goats; progesterone; reproductive synchronization of estrus and pregnancy*

Introduction

The etawa goat could be a goat imported from India which is additionally called the Jamnapari goat. The tallness of male goat's ranges from 90 centimeters to 127 centimeters and the females as it were reach 92 centimeters. Male weights can reach 91 kilograms, whereas females as it were reach 63 kilograms. His ears were long and hang down. Arched brow and nose. Both male and female with brief horns. This sort of goat is able to create milk up to three liters per day. The crossover breed (half breed) of etawa goats with neighborhood goats is known as "Peranakan Etawa" or "PE" goats. PE goats are nearly the same estimate as etawa cross bred but are more versatile to Indonesia's nearby environment.

The synchronization dairy goats have been done in France that has been success in estrus and pregnancy rates greater than 65% then the condition is continued by artificial insemination with quality cement and experienced inseminator. Strategies which use progesterone or its analogs are based on their impacts within the luteal stage of the cycle reenacting the activity of characteristic progesterone delivered by the corpus luteum after ovulation[1,2,3,4,5]. Utilize of prostaglandins is an elective strategy for controlling generation by disposing of the corpus and actuating luteum an ensuing follicular stage with ovulation (the following, however, the study distinguishes the use of progesterone ear

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implants from commercial intravaginal progesterone devices as a material for estrus synchronization. It turned out that the results showed no difference between the use of progesterone devices and ear implants in demonstrating the success of estrus and pregnancy as equally great[6].

Cloprostenol in cycling goats using luteolysis may be induced by inject of PGF2α (2.5–5 mg, IM) as early as day 3. cloprostenol PGF2α (≥15 mg) In sheep is effective after day 5 of the cycle of estrus is day 0. Technical estrus synchronized by two doses of cloprostenol, 7–9 days apart in ewes 11–13 days apart in does. impregnated intravaginal sponges (medroxyprogesterone or fluorgestone) have been used resources for control of ovulation. Controlled internal drug release (CIDR) intravaginal plastic device impregnated with progesterone (300 mg) and vaginal sponge 50 mg MPA be synchronized in cyclic or anestrous does and ewes by administration of progestagens[7]. In small ruminants also be used CIDR to insert in the vagina for 7 days, and inject luteolytic as PGF2α 1 day before removal and estrus response is high within 72 hr[8]. Characteristics of goats when estrus is screaming while actively moving the base of the tail at the injection of 300 IU eCG combined with PGF 10 mg 24 hours before the removal of intra vaginal progesterone, at 43 to 45 hours after the removal of intra vaginal sponge devices these signs appear very lustrous dominant. If the granting of 300 IU eCG compared with 400 to 500 IU combined with PGF there is no real difference for the onset of estrus[9]. The use of eCG on an ongoing basis can cause anti-body in livestock which results in a decrease in the potential of eCG for its use. For this reason, it is necessary to neutralize the anti-body anti-eCG against eCG's ability to increase ovulation. If we inject eCG the same as we give antigens into the animal's body where the high molecular weight of about 60 kda can cause anti-body against eCG which if the next use occurs can affect the fertility of the livestock itself[10]. Polyclonal anti eCG bovine can be obtained when we inject superovulation doses with the help of active and active ingredients can be harvested polyclonal antibodies from bovine that are mass produced at the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, which first carried out the expansion of 1: 1 supreme charcoal and ethanol in 4°C ultra-centrifuge and filtered by CM Sephadex G-100 coloums chromatography[11].

Materials and Methods

The experimental animal use in the study is 30 Etawa crossbred goats. The goat has bred seen in its horns and teeth, with a body condition score of 3. The goat has been examined for its health in a healthy reproductive condition. The age of the experimental animal is around 3 years. 30 goats divided become 3 groups Group I as (T0) 10 goats are as controls Groups. (T0) was inserted sponge progesterone 50 mg of MPA and injected with PGF2α 7.5 mg in day 10 intramuscular (i.m.) when T0 removal of the sponge were removed on day 11. Does on day 11 as T1 going on the removal of devices 24 h before removal inject 7.5 mg of PGF2α and 300 IU of eCG. T2 were injected in all the goats 7.5 mg of PGF2α, 300 IU of eCG at day 10 and 300 IU bovine anti eCG inject at day 11 when removal device on day 11. After 35 days, pregnancy was diagnosed by ultrasound scanning. Dosage and administration to increase of fertility rate hormon form Intervet Holland named as Folligon 300 IU by i.m. route[12], bovine anti sera eCG 300 IU production from Universitas Airlangga research results and PGF2 7.5 mgSome types of data analysis that will be used are: Analysis of Variance Analysis (ANOVA) and Honest Difference Test (SPSS 22)[13] for duration of estrus and time of estrus data. The test result is significant if $p < 0.05$.

Results and Discussion

The key to this study is the role of Bovine polyclonal anti eCG obtained from the follicular eCG immunization in bulls which can be used to neutralize the negative effects of eCG. eCG can cause anti-body if injected into various animals, such as injected in goats can cause an antigen antibody reaction where a large molecular weight of about 60 kda can be considered as an antigen. Harvesting anti-body in goats with immunization model can be used to neutralize the anti-body in a humorous manner referred to as anti-body polyclonal abpo if in goats the name goat polyclonal anti-body[14]. Likewise, if eCG is injected at high doses or deserves to be called a superovulation dose, the commercialized adjuvant and body harvest can be done gradually once a week, repeated eCG treatments induce highly variable humoral immune responses among individual ewes; Significant associations were found between the anti-eCG response phenotype and some MHC class II alleles[15].

Treatment with P4 for 5 d was as effective as for 12 d to induce fertile estrus in FSH-treated anestrous

ewes[16], research on goats takes 10 days for removal of a progesterone 50 mg, the device as carried out in this study uses the same dose in Cross Bred Goats etawa[17]. The sialic acid content in eCG has a different effect than the anti-eCG Abs on eCG bioactivity can be explained by two hypotheses. The steric inhibition of the interaction of eCG with its receptors explains the inhibitory effect of some anti-eCG abs; the second way of working there is a conformational change in eCG by anti-eCG Abs bias through induction of inhibition or acceleration of the potentiation of the eCG bioactivity. It is important that this modulation of the eCG bioactivity by the anti-eCG Abs impacts primarily on the FSH bioactivity of eCG, which is very important and we know the effect of FSH to grow follicles on the surface of the ovaries[2]. eCG against anti body gonadotropins were created, it can moreover be normally emitted to humoral safe reaction to endogenous, exogenous gonadotropins. Developmentr of against gonadotropins movement, there's along absent until to go therapeutic anti body target can be reach the advertise[3]. The time of estrus When the device removed in general estrus response is high within 72 hr[4].

The effect of eCG treatment on pregnancy rates in synchronized heifers using norgestomet ear implants removed on day 10 and the sign of estrus will be shown at 56 h (18). As well as progestagen-[7] or prostaglandin-treated ewes[18]. In this way, the combination of PGF2 amid the early luteal stage with the male effect 'may be satisfactory elective for synchronizing oestrus earlier to artificial insemination within the nonappearance of past oestrus detection[10]. Using PGF the changeability within the timing of ovulation after treatment may be indeed more decreased by applying the 'male effect' coincidentally with the moment PGF2 treatment. The 'male effect', in spite of the fact that commonly utilized for actuating LH surge and ovulation amid regular anoestrus, moreover increments LH discharge, amid the breeding season, in cycling[11].

The result showed in the table are Estrus rate, 100% showed estrous response in all control group animals and this treatment showed a combination of vaginal sponge progesterone PGF2α, eCG and eCG antibody as well as the estrus deposition in goat time of estrus. When the device removed an average of 30 hours lasted and the duration of estrus was 37 hours. When we associate with sharing opinions on some of the results of the study are as follows: pGF2α with intra-vaginal progesterone sponge and its combination with eCG can sufficiently increase estrus synchronization and some reproductive and productive properties in Bark sheep by up to 95%. Real-time ultrasound scanning (US) via the transrectal route, pregnancy diagnosis, the present paper is to compare the accuracy of diagnosis of pregnancy using transrectal US Canary dairy goats were synchronized with an 11-day progesterone intravaginal) sponge followed by PGF2α and eCG 2 days before sponges' withdrawal. on Days 20, 22, 24, and 26 after mating.

Table 1. Bovine polyclonal anti eCG and eCG combinated vaginal sponges' progesterone to induce estrus and pregnancy of etawa crossbred goats.

Group of treatments	T0 (Deviceprog PGF2α)	T1 (Device prog PGF2α, eCG)	T2 (Device prog PGF2α, eCG anti eCG)
Number of Etawa Crossbred Goats	10	10	10
Duration of estrus (h) ±S.D.	37.00 ± 4.09	37.00±12.06	37.0 ± 11.3
Estrus rate %	100.00 (10/10)	100.00 (10/10)	100.00 (10/10)
Time of estrus When the device removed	30.8 ± 12.0	30.11 ± 13.8	30.03 ± 8.09
Pregnancy rate %	50.00 (5/10)	50.00 (5/10)	60.00 (6/10)

*The treatment has no effect on the measured variable (p >0.05).

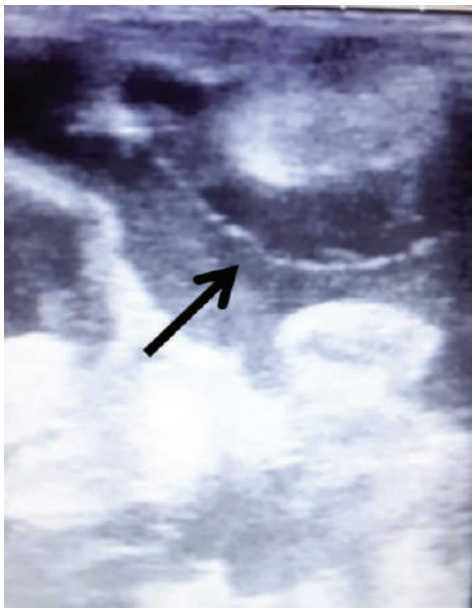


Figure 1. The result of pregnancy 35-day detection via ultrasonography showed.

Conclusion

In summary, based on the comes approximately of this ask, can be concluded that the eCG, bovine against sera eCG and PGF2α combination can increase the rate of estrus and pregnancy in etawa crossbred goats. Injected of eCG measurements of 300 IU and 300 IU eCG + sera eCG im has showed up no differences in p >0.05 ubsequently, utilizing of 300 IU eCG + bovine against sera eCG is surveyed to amplify the rate of estrus and pregnancy in etawa crossbred goats.

Conflict of Interest : The author declare that they have no conflict of interest.

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Compensation for Ex-Mineworkers in the Mthatha Region of South Africa: A Long Road to Travel

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Abstract

Background: Ex-mineworkers are sandwiched between scare resources and little hope of being re-employed. They do not have enough savings to support their families. Expenditure is at its highest when they return, as their children have grown and are in secondary or senior secondary school. Many are not re-employable because of poor health and some are disabled.

Objective: To highlight the compensation claims of ex-mineworkers in the Mthatha region of South Africa.

Method: Ex-mineworkers were examined and sent their reports to Medical Bureau of Occupational Diseases (MBOD) to claim compensation. The detailed histories of these ex-mineworkers were requested by a trained research assistant at the Benefit Examination Clinic (BEC).

Case Histories: Most mineworkers have indicated that the pride of being a male breadwinner is often lost, and their wives have to take over this role. Some take to drinking alcohol and run the risk of premature death. Everyone is healthy at the time of recruitment, but many return diseased. Pulmonary tuberculosis overshadows underlying silicosis among mineworkers. Oesophageal carcinoma is associated with silicosis and this could be the reason for its high prevalence in this region.

Conclusion: The MBOD recommended compensation in only 2.5% of cases during the last seven years in the Mthatha region. There is a long road ahead to get these claims paid out; meanwhile many of the claimants will die.

Keywords*: ex-mineworkers, psychosocial problems, compensation claims, pulmonary tuberculosis, silicosis.*

Introduction

The migrant labour system has had very extensive socio-economic effects in the Mthatha region of South Africa. At one stage it was estimated that 2 million of the 5 million black workers in South Africa were migrant labourers.[1] About 600 000 mineworkers were employed by the gold mining industry in South Africa alone.[2] Most of them were from the Transkei region. About 14% of the former mineworkers who visited the Umtata Benefit Examination Clinic (BEC) between April and August 2000 indicated that they were given no reasons for their retrenchment.[3] There is evidence of a huge accumulation of unrecognised, therefore uncompensated, cases of pneumoconiosis and/or tuberculosis among former mineworkers living in labour-sending regions such as Mthatha.[4] The purpose of this report is to highlight the problems of compensation in the Mthatha region of South Africa.

Patients and Methods

Ex-mineworkers were examined at the BEC, a clinic located in the chest section of Umtata (Mthatha) hospital, which previously (apartheid era) was known as the Henry Elliot hospital. The clinic was established by the author to provide voluntary services to ex-mineworkers every Wednesday from 10:00 to 13:00, with minimum resources. The establishment of this clinic was announced on community radio. It could be assumed fairly that ex-mineworkers presented themselves voluntarily. Those who attended could be considered a

cross-section of the population of ex-mineworkers. Full chest x-rays were obtained and physical examination of workers took place, while their mining history was recorded. Fingerprints were taken. The patient's name, age and occupational health registration number were also recorded. Patients were interviewed by a trained research assistant asking pre-designed questions about their psychosocial, familial and financial difficulties.

Case histories

The extremely high burden of lung disease in ex-mineworkers is an enormous challenge to the health services and compensation authorities. An X-ray-based study conducted by the author (2002) showed that 78.2% of ex-mineworkers had evidence of lung disease. Pulmonary tuberculosis (PTB) with or without silicosis was evident in 64.2% of the X-rays, silicosis with or without PTB in 34%, chronic obstructive pulmonary disease in 7%, and asbestosis in 1.5%.[5] The case below describes a wife taking over the role of her husband.

Case 1. This is an example of a family where the wife is the breadwinner and the husband is so ill that he cannot work. He is irritable and shouts at the children at the slightest provocation. This situation is compounded by his hearing impairment. When young, he was handsome and his wife was attracted to him and married him. Now she sells household items such as paraffin, candles, and soap. She is now the breadwinner, as shown in photograph 1.

Many are not re-employable because of their poor state of health and some being disabled. They get used to taking alcohol to drown their sorrows. The following is such an example.

BD, a-60-year-old ex-mineworker, died on 6 June 2000 at about 6.00. See photograph. His body was lying outside the gate of his house. He had come home late from a shebeen. He had the habit of jumping over the gate when it was locked. On the day of his death he was found on the other side. An off-duty policeman saw him, took him to his house, and informed his colleagues. The deceased had the habit of going to the shebeen in the evening and returning home the following morning and sleeping it off. At 60 years of age he was getting an old age grant and used that money for drinking. His wife left him as a result of the drinking problem and he was left with three children. His drinking started at the time he was retrenched. Two of his children were at school in standard 4 and standard 2 and the third was staying at home.

The discovery of gold provided the base from which South Africa was able to develop a substantial industrial capacity, but the mines demanded a reliable supply of labour, which could only be met by drawing in migrant workers from distant rural areas, both within and outside South Africa. The migrant labour system not only creates situations in which diseases such as tuberculosis and sexually transmitted infections flourish, but also serves to disseminate these diseases widely throughout the region.[6] Many of these migrants contracted fatal diseases in the mines. Historian Randall Packards[7] paints a fairly grim picture of this trend:

GK, a 49-year-old, was a retired mineworker from the goldmines. He earned a monthly wage of R1105, 66. He worked from 1977 to 1997, when he took a voluntary retirement package. He was awarded R14 031.48. He had five young children. In 1989 he was treated for PTB in the mine hospital and in 1998 for a relapse in the local hospital. At the same time cancer of the oesophagus was diagnosed. He had been a smoker and a consumer of alcohol. He died as a result of complications from the cancer.

Many mineworkers ended up disabled as a result of mining accidents. Many returned home without compensation and RM, the insurers, do not deal with old cases.

LT, a 40- year-old, started working underground in a mine in Elandsrand in 1984 and worked until September 1996. He died in 1999. When he left for the mines he was a healthy young man. He sustained a fracture while working underground and was discharged. After recovering he worked for a construction company for a meagre monthly salary of R260.

During an informal interview, a spouse described her ex-mineworker husband as "no longer the person I married." The man was impotent and could not meet her demands. The woman poisoned her husband. Her daughter revealed this before committing suicide.

ZZ, a 27-year-old female, hanged herself in Feb 2001. A suicide note revealed that she was taking her life because the mother was not close to her. The father had died of poisoning in 1998, but the police were not informed. He had worked in platinum mine in Rustenburg for 20 years. He was retrenched because of poor vision. His wife, the mother of the deceased, had poisoned the father as he used to sleep away from his wife. (The son-in-law of the elder brother of the father narrated this story.)

Little attention was paid to black mineworkers, even though they performed the most difficult jobs, such as drilling rocks at deep levels. The Occupational Disease in Mines and Works Act (ODMWA) used racial criteria in the past to determine the amount of compensation paid out to workers. White workers with PTB were paid 5.5 times more than black workers. White workers with silicosis were paid up to 13.6 times more than black workers.[8] Most of the ex-mineworkers are in their fifties (mean age 51 years), capable of re-employment if they are fit and healthy. However, many of them are ill and even if they are employed will get very low wages.

The family of an ex-mineworker was admitted to the local hospital with food poisoning. On inquiry it transpired that they had eaten the meat of a cow found dead. They decided to consume this meat as a result of poverty because they could not afford to buy meat from the butchery. Many ex-mineworkers live below the poverty line. Some get occasional work and earn a few rands, which is hardly enough to run a family.

Some ex-mineworkers suffer from mental illnesses. It is difficult to manage their illnesses, as health facilities in the rural areas of Transkei are inadequate.

On 11 May 2000, a 51-year-old ex-mineworker was knocked down by a car. It was a hit-and-run accident. He had a history of mental illness. He had been a mineworker in President Steyn Mines. After leaving the mine he was living without any money. He had been acting strangely, sometimes even running naked in the locality and collecting thrash. He had once been admitted to hospital in Queenstown in 1997.

A large number of ex-mineworkers have hearing impairments. Some of them are completely deaf. They find it difficult to apply for compensation, although their hearing loss is due to their mining job. If a miner applies for compensation, it has to be done within a year of retrenchment or retirement. In most cases this does not take place. As a result of this, most ex-mineworkers who end up with hearing loss get nothing in compensation.

A recent study conducted by the author showed that there is a high prevalence (54%) of hearing loss among ex-mineworkers. Of those affected, 33% were between 40 and 59 years of age. Twenty two percent who had worked in the mines for 10-20 years indicated loss of hearing.[9]

The returning miners affect their families and communities in two main ways. One is measurable and can be compensated. Disease and disability belong to this category. The other is issues such as their psychosocial impact. Diseases and disabilities could be assessed by age and the level of skills. The compensation commissioner's office makes use of such indices for payouts, either a lump sum or instalments. Only about 8-10% of mineworkers have been compensated. The liability for unpaid compensation to mineworkers for occupational lung diseases remains, and their families should be paid without any delay. There is a need for more clinics in rural Transkei so that mineworkers can enjoy easy access to medication for chronic diseases such as silicosis and chronic obstructive pulmonary disease from which they suffer. Doctors in public service are not fulfilling their legal obligation to submit claims for living and deceased ex-mine workers.[8] It is difficult to estimate the financial costs to the families because of ill ex-mineworkers.

The process of getting compensation is tremendously slow and inadequate, as shown in Table 1. Only 2.5% of ex-mineworkers were compensated between 1997 and 2000. In the meantime many ex-mineworkers have died. There is only one clinic in the area that serves the ex-mineworkers in the region.

Table 1: Statistics of ex-mineworkers from 1997 to August 2000.

Years	Examined	Re-examined	Compensated	RIP*
1997	601	------	9 (1.5%)	9
1998	1181	11	17 (1.4%)	18
1999	165	99	18 (11%)	20
2000(August)	80	62	7 (9%)	8
Total	2027	172	51 (2.5%)	55

*RIP= The rest in peace column indicates the subjects who were awarded compensation but died before receiving it.

Farming is the only productive work that most returning miners can perform. However, they lack land and farming skills . For as long as they worked in the mines the families were supported, but on their return they became dependent on others mainly because of poor and failing health. Most ended up destitute.

Conclusion

It is not only the ex-mineworkers who are often under psychosocial pressure, but also their families and the community as a whole. Extreme poverty is clearly evident in many families of ex-mineworkers and this need to be addressed by the government as a priority. These mineworkers must be adequately and promptly compensated before they die.

Ethical Issue: This report was approved by the ethical committee of the University of Transkei, South Africa for publication.

Conflict of Interest: None

Source of Funding: It is self-funded.

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Nurses – Midwifes Knowledge Concerning Polycystic Ovary Syndrome in Baghdad Hospitals

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Abstract

Background: Polycystic ovary syndrome is the most common ovary disorder characterize by complex of health problems that affects women of reproductive age due to hormonal imbalance and metabolic problems, so nurses have an important role in providing counseling and health education for women in reproductive age.

Objectives: To assess nurses knowledge about Polycystic ovary syndrome.

Methodology: A descriptive study was conducted on probability sample (systematic random) of (100) nurses and midwife who work in obstetric and gynecological departments from three hospitals at Baghdad City: AL-Karkh maternity Hospital, Al-Elwia Maternity Teaching Hospital and Baghdad Teaching Hospital. Study implemented for the period of February 24th 2015 to April 25th 2015. A questionnaire was used as a tool of data collection to fulfill with objective of the study and consisted of three parts, including demographic characteristics, personal experience with polycystic ovary syndrome, sources of knowledge regarding polycystic ovary syndrome and knowledge regarding polycystic ovary syndrome. A pilot study was carried out to test the reliability of the questionnaire and content validity was carried out through the 17 experts. Descriptive statistical analyses were used to analyze the data.

Results: In general the results of the study revealed that half of nurses were at age group (30-39) years , (35%) of study sample was Institute of medical technology/nursing, half of study sample work at obstetric and gynecological ward, (55%) of study sample were married and the highest percentage (93%) their sources of knowledge about Polycystic ovary syndrome from syllabus. Most of the study sample had high assessment level of knowledge about polycystic ovary syndrome.

Conclusions: Nurses had high level of knowledge regarding the polycystic ovary syndrome, the reason for this is due to nursing curriculum was effective in giving scientific knowledge about it.

Key Words: *Knowledge, Nurse, Polycystic ovary syndrome.*

Introduction

Polycystic ovary syndrome (PCOS) is common endocrine disorder for women in reproductive age and the prevalence of PCOS is rapidly increased worldwide , women with PCOs are at increased risk of endometrial cancer, infertility, late menopause and additionally metabolic abnormalities, including insulin resistance, type 2 diabetes mellitus, dyslipidemia and cardiovascular infection [1].

The effect of modernization and technological development reflects in our daily life and changed our lifestyle a lot. Food intake is focused progressively on sugar, fast food, and soft drinks. This unhealthy eating habits and lack of exercise leads to PCOS as well [2]. By royal college of obstetrician and gynecologists and the American college of obstetrician gynecologists the clinical feature of PCOS are irregular menstrual cycle or may be absent, hirsutism, loss of head hair, obesity, difficult to be pregnant or may be infertile, acne and oily skin [3, 4]. Mental health disorders that occur more frequently in women with PCOS including depression, anxiety, bipolar disorder and binge eating disorder [5].

A health risks or complications that women affects by PCOS not only on her reproductive health and system but women increase risk of serious health complication have lifelong consequence and it's type 2 diabetes, cardiovascular diseases and endometrial cancer [4] . By American Academy of Family Physicians Diagnosis of PCOS can usually be done with a careful history especially menstrual history cycle, physical examination, and specific laboratory testing and without or without ultrasonography or other imaging [6].

"A Nurse holds a critical role in health care that goes beyond the day to day duties" [7]. Nurses are in a position to provide comprehensive care to woman in reproductive age and who with the syndrome. basic elements of nursing practice should be included in the nursing education [7] .So scientific knowledge regarding PCOS to nurses will enhance the scientific background to play important role of nurses as health educator to enhance lifestyle and reduce the risk of PCOS among woman with syndrome.

Methods

Descriptive study was carried out upon nurses who work at Baghdad city hospitals in gynecological and obstetric departments. Study implemented for the period of February 24th 2015 to April 3rd 2015. Data collection will be gathered by questionnaire format, and Interview with nurses at Baghdad city hospitals. Probability sampling approach; (systemic random sampling) was utilized through following steps: 1. Researcher got a list of hospitals names that have maternity department 2. Researcher has a frame list of (232) nurses names who work in obstetric ward, delivery room and maternity theater room in three Baghdad city hospitals. 3. Research consisted of 100 nurses as size of study sample 4. Researcher calculated the sampling interval as standard distances between the elements chosen for the sampling. 5.The first element was selected randomly 6.The total sample was illustrated in table (1).The period of data collection for all hospitals was two months. The research study was conducted in three hospitals at Baghdad City: Al-Elwia Maternity Teaching Hospital, AL-Karkh maternity Hospital, and Baghdad Teaching Hospital. Nurses who work in obstetric ward, delivery room, obstetric theater room were selected as study sample. A questionnaire was used as a tool of data collection to fulfill with objective of the study and consisted of four parts, including demographic characteristics, personal experience with polycystic ovary syndrome, sources of knowledge regarding polycystic ovary syndrome and knowledge regarding polycystic ovary syndrome . Pilot study was carried out between the 2nd January to January 21 of 2015, on (10) nurse who work in maternity department to determine the reliability of questionnaire and content validity was carried out through the 17 experts.

Descriptive statistical analyses were used to analyze the data. Data were analyzed using the statistical package for social sciences (SPSS) version 16, and Excel. Through the application of descriptive statistical data analysis include (Frequencies, Percentage, Mean, Standard Deviation ,Mean score and Relative Sufficiency). All questions rated according to the following criteria: Yes= 2, No= 1.

So the cut-off-point = 1.5

Where relative sufficiency (RS %) are calculated by:

Reassessment scoring scales for binary dichotomous random variable, preceding scoring scales according following intervals: L (Low) 0.00 – 33.33; M (Moderate) 33.34 – 66.66; H (High) 66.67 – 100.

Table (1): Selection of study Sample According to Systematic Random Sampling from Six Hospitals in Baghdad City.

No.	Hospitals Name	Total Number	Sample Size
1.	AL-Karkh maternity Hospital	77	33
2.	Al-Elwia Maternity Teaching Hospital	106	46
3.	AL-Baghdad Teaching Hospital	50	21
Total		232	100

Results

Table (2): Distribution of Study Sample According to Demographic Characteristics

Demographic Characteristics	Nurses (n=100)	
Age/years	No.	%
20 - 29	34	34
30 - 39	51	51
40 - 49 15		15
± SD 32 ± 8		
Educational Level		
Secondary school nursing graduated	25	25
Secondary school midwifery graduated	22	22
Institute of medical technology/nursing	35	35
College of nursing	18	18
Job Title		
Skilled nurse	21	21
Professional nurse	35	35
Academic nurse	18	18
Skilled midwife	26	26
Location of Work		
Obstetric and gynecological ward	23	23
Delivery room	52	52
Theater room	25	25
Marital Status		
Single	45	45
Married	55	55

Table (2) Shows that the half of study sample (51%) was at age group (30-39) years while the lowest percentage (15%) of them group age between (40-49) ; with the mean age and SD was (32 ± 8) years.

Regarding educational level : The highest percentage (35%) of study sample was graduated from Institute of medical technology/nursing while the lowest percentage (18%) were from college of nursing .

Regarding job title : The highest percentage (35%) of study sample were Professional nurse while the lowest percentage (18%) were academic nurse.

***Regar*ding place of work:** The highest percentage (52%) of study sample work at obstetric and gynecologic ward ,while lower percentage were (23%) work at obstetric and gynecologic ward.

Regarding marital status: The highest percentage (55%) of study sample was married, while the lower percentage (45%) were single.

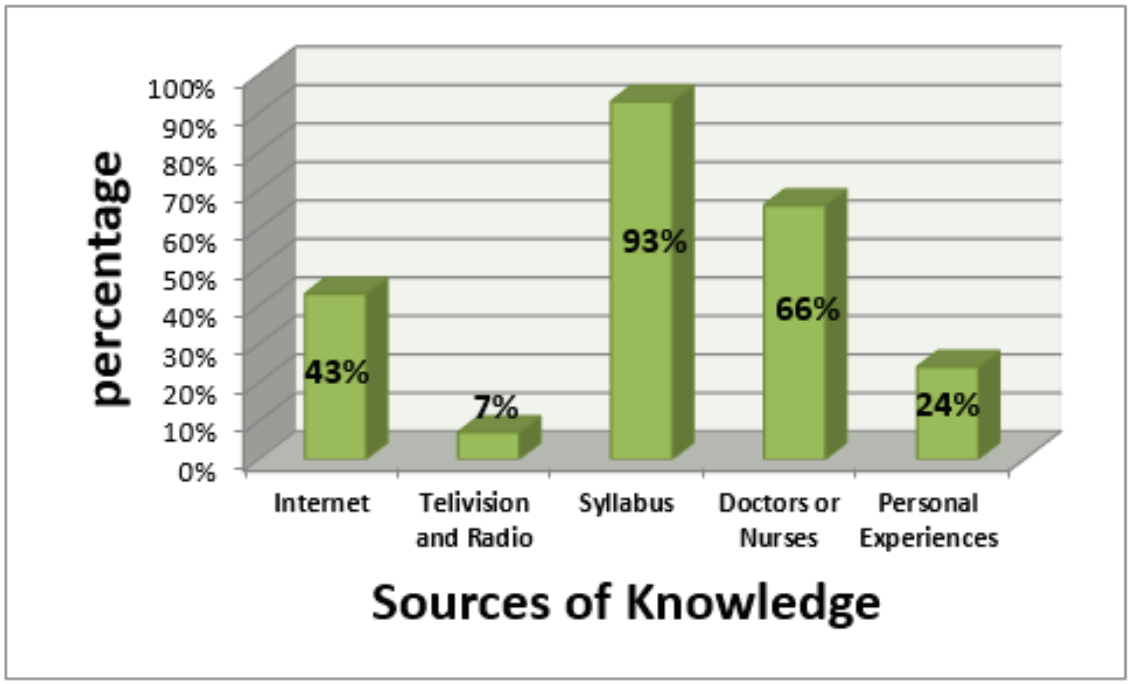


Figure (1): Sources of Knowledge about Polycystic ovary Syndrome in Study Sample

This figure shows that the highest percentage (93%) of studied sample reported they obtained knowledge regarding polycystic ovary syndrome from syllabus. Meanwhile, (7%) of the study sample obtained information from school.

Table(3) Distribution of (100) Nurses Knowledge Regarding Polycystic Ovary Syndrome

Item No.	Variables	Answer	F	%	MS	SD	RS	Ass.
1.	Definition							
1.1	Have you heard about the term called polycystic ovary syndrome	Yes	100	100%	2	0.00	100	H
		No	-	-				
1.2	polycystic ovary syndrome means patients have small multiple cysts in their ovaries	Yes	100	100%	2	0.00	100	H
		No	-	-				
1.3	Androgen is (male) hormone (e.g. testosterone))	Yes	100	100%	2	0.00	100	H
		No	-	-				
2.	**Causes of polycystic ovary syndrome**							
2.1	Obesity	Yes	37	37%	1.37	0.48	68.5	H
		No	63	63%				

Cont... **Table(3) Distribution of (100) Nurses Knowledge Regarding Polycystic Ovary Syndrome**

2.2	Family history	Yes	30	30%	1.3	0.47	50.15	M
		No	70	70%				
2.3	Decrease insulin action in body	Yes	97	97%	1.97	0.24	98.5	H
		No	3	3%				
3.	Signs and Symptoms polycystic ovary syndrome							
3.1	Absence of menstrual cycle or irregular menstrual cycle or	Yes	100	100%	2	0.00	100	H
		No	-	-				
3.2	Hair growth in Unusual amount in facial or body hair (hirsutism)	Yes	93	93%	1.93	0.30	96.5	H
		No	7	7%				
3.3	Acne problem (Severe) or oily skin	Yes	47	47%	1.47	0.50	73.5	H
		No	53	53%				
3.4	Hair loss from head more than normal	Yes	19	19%	1.19	0.49	59.5	M
		No	81	81%				
3.5	Increased level of testosterone	Yes	70	70%	1.7	0.48	85	H
		No	30	30%				
4.	Diagnosis (polycystic ovary syndrome can be diagnosis by)							
4.1	vaginal ultrasound	Yes	100	100%	2	0.00	100	H
		No	-	-				
4.2	Specific blood test	Yes	93	93%	1.93	0.25	96.5	H
		No	7	7%				
5.	Complication (polycystic ovary syndrome may leads to the following)							
5.1	Diabetes (sugar)	Yes	23	23%	1.23	0.55	61.5	M
		No	77	77%				
5.2	Heart diseases	Yes	23	23%	1.23	0.55	61.5	M
		No	77	77%				

Cont... Table

5.3	Reduced fertility(difficulty becoming pregnant)	Yes	93	93%	1.93	0.25	96.5	H
		No	7	7%				
5.4	Depression and psychological problems	Yes	58	58%	1.58	0.49	97	H
		No	42	42%				
5.4	Endometrial cancer	Yes	75	75%	1.75	0.46	87.5	H
		No	25	25%				
6.	Treatment of polycystic ovary syndrome							
6.1	Hormonal therapy	Yes	100	100%	2	0.00	100	H
		No	-	-				
6.2	Anti-diabetic medications (metformin) may be used to treat diabetes	Yes	28	28%	1.28	0.45	64	M
		No	72	72%				
6.3	Symptomatic treatment may be given to relief the symptoms of polycystic ovary syndrome	Yes	12	12%	1.12	0.42	56	M
		No	88	88%				
6.4	Surgery	Yes	100	100%	2	0.00	100	H
		No	-	-				

MS= Mean Score, RS Relative Sufficiency, Assessments Intervals Scoring Scales: [L: Low (0.00 – 33.33)]; [M: Moderate (33.34 – 66.66)]; [H: High (66.67 – 100)].

Table (3) shows that there were high mean score with high relative sufficiency (RS%) assess as high (H) in all items regarding to "definition of polycystic ovary syndrome".

Regarding to "Causes of polycystic ovary syndrome" were high mean score with high (RS%) assess as (H- High) in most items.

Regarding to "signs and Symptoms polycystic ovary syndrome" were high mean score with high (RS%) assess as (H- High) in most items.

Regarding to "Diagnosis polycystic ovary syndrome can be diagnosis by" were high mean score with high (RS%) assess as (H- High) in most items.

Regarding to "Complication of polycystic ovary syndrome" were high mean score with high (RS%) assess as (H- High) in most items.

Regarding to "Treatment of polycystic ovary syndrome" items were half of study sample were high mean score with high (RS%) assess as (H- High) and half of study sample were moderate mean score with high (RS%) assess as (M- moderate).

Discussion

The study conducted on 100 nurses who work in obstetric and gynecological department at three Baghdad city hospitals, the finding of this study reported that the highest percentage (93%) of studied sample were obtained knowledge regarding polycystic ovary syndrome from syllabus and due to this finding the nurses in this study were had high level assessment of

knowledge regarding polycystic ovary syndrome in most items as shows in table (3) so the nursing curriculum was effective in giving scientific knowledge polycystic ovary syndrome.

Conclusions

Based on study findings; The study showed that nurses had high level of knowledge regarding the polycystic ovary syndrome, the nursing syllabus was the prevalent sources. The study showed that (28%) of study sample suffering from polycystic ovary syndrome and most of them suffering from psychological problem.

Conflict of Interest: Nil

Sources of Funding: self

Ethical Clearance: obtained from the

- Ministry of Health/Al- Russafa Health Director
- Ministry of Health/Al- Karkh Health Director
- Ministry of Health/ Medicine City Director
- Ministry of Planning and Development Cooperation/Central Statistical Organization Technique and Information.
- Al-Elwia Maternity Teaching Hospital
- AL-Karkh maternity Hospital
- Baghdad Teaching Hospital
- All nurses- midwifes participant in the research have been approved before the questionnaire is started.

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The Effect of Repetition Simulation after Debrief Method on Learning Satisfaction and Learning Achievement in Midwifery Students at Universitas Airlangga, Indonesia

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Abstract

Background: Simulation and debrief are one kind of learning method used in midwifery education. Repetition of the simulation (redo) after debrief is expected to improve students' satisfaction and learning achievement. In Indonesia, the implementation of the simulation method has not been well structured and redo research is still limited.

Aims: This research examined the differences between students' satisfaction, learning achievement and implementation time when doing the first simulation and redo after debrief in the antenatal care subject.

Method: The study used a pre-experimental quantitative method that observes the level of satisfaction, learning achievement and time used when doing the first simulation and redo after the debriefing. The samples were 45 midwifery students using a total sampling technique. Data on students' satisfaction levels were obtained through the interview method with a questionnaire and the learning achievement and time were obtained through the observation method with a checklist graph. The data of the study were analyzed using the Wilcoxon signed ranks test.

Result: There were significant differences in the level of students' satisfaction and the learning achievement when doing the first simulation and redo after debrief, but there was no significant difference in the time allocated to finish the scenario when doing the first simulation and redo after debrief.

Conclusion: The Redo after debriefs simulation increases the level of students' satisfaction and learning achievement on midwifery students in the Faculty of Medicine, Universitas Airlangga, Indonesia.

Keywords: *Repetition of the simulation, Debrief, Satisfaction, Learning Achievement, Midwifery student*

Introduction

Midwifery is a profession that requires a lot of clinical expertise[1]. Health education requires a fundamental change in the method and process of learning so that students gain confidence and competence in playing their profession[2]. Self-confidence is one of the keys to clinical competence[3]. More than two decades of simulators and models have provided opportunities for students to learn both communication skills and procedure techniques and a safe environment, before being introduced to actual clinical learning[2]. Simulation activities can combine several types of elements in one activity and encourage students to reflect on their performance[2].

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Simulation and debrief are important learning methods in supporting the learning process, especially learning before students practice clinics directly to patients at the clinic so that they are expected to apply it when practicing clinics with the aim of improving patient safety[4]. The advantage of the simulation in the learning method is to increase knowledge and skills, besides that, simulation can also improve student performance, appearance and attitude because the learning process has been set to resemble the actual conditions of both playing roles and the environment. The simulation is carried out using a pre-prepared scenario[5].

Learning achievement is the main thing that must be determined in the scenario. Learning achievement is expected not only to focus on hard skills but also soft skills so that the student's attitude and performance after doing the simulation will be better[6]. Simulation-based education has increasingly become an important method in the clinical education and training of health students[7]. Learning satisfaction is the extent of satisfaction regarding the learning process and learning performance. Learning satisfaction as indicators of learning performance[8]. The previous research proves that there is a strong relationship between satisfaction with motivation and specialization in learning so that they will be more enthusiastic when studying, especially in terms of midwifery skills[1].

Repetition of the simulation (redo) after debrief is expected to improve students' satisfaction and learning achievement. The process of redo after debrief during the educational experience consists of more than reversing and avoiding making the same mistakes. Redo after the simulation gives students the opportunity to practice their skill in simulation much better. The lecturers that involved in the redo station can also focus more on students specifically to activate the simulation learning process[4,9]. This study aims to determine the effect of repetition simulation (redo) after debrief method on midwifery students' learning satisfaction and learning achievement in Universitas Airlangga.

Method

The study used a pre-experimental quantitative method that gives treatment in the form of debriefing to the research samples. The researchers analyzed the student's satisfaction level, learning achievement, and implementation time during the first simulation and redo after debrief. The sample size in this study was 45 midwifery students in the fourth semester using a sampling technique with total sampling.

Data on student satisfaction levels were obtained by interview method based on questionnaires while learning achievement and time implementation were obtained based on observations using the observation sheet or checklist. The level of student satisfaction was measured using a Linkert scale with five criteria namely not satisfied, less satisfied, partly satisfied, satisfied and very satisfied. Learning achievements are obtained from the results of observations based on learning adjectives that were predetermined in the simulation scenario. The execution time is obtained based on the results of observations using minute units. Data from the study were analyzed using the Wilcoxon signed ranks test. The ethical clearance of studies was taken from Faculty of Medicine, Universitas Airlangga No. 197/EC/KEPK/FKUA/2018.

Result

Level of Student Satisfaction

Based on table 1, the data shows that there is an increase in student satisfaction at a very satisfied level from the first simulation to the redo after debrief. The results of the analysis using the Wilcoxon Signed Ranks Test showed that there were significant differences in the level of student satisfaction in the first simulation with the redo after debrief with $p = 0.015$ at α: 5%

Table 1. Level of student satisfaction

Level of satisfaction	N (%)		X2
	First Simulation	Redo after debrief	
Not Satisfied	1 (2.22)	0 (0.00)	0.015*
Less Satisfied	0 (0.00)	0 (0.00)	
Partly Satisfied	10 (22.22)	4 (8.89)	
Satisfied	22 (48.89)	20 (44.44)	
Very satisfied	12 (26.67)	21 (46.67)	
Total	45 (100)	45 (100)	

Learning Achievement

Based on table 2, the data shows that the minimum learning achievement that can be achieved in the first simulation is less than at the time of redo. The results of the analysis using the Wilcoxon Signed Ranks Test showed that Learning achievement in the first simulation and redo after debrief showed significant differences with $p = 0.014$ at α: 5%.

Table 2. Learning achievement data during the first simulation with redo after the debrief

Description	First Simulation	Redo after debrief	X2
Mean	5.57	6.85	0.014*
Deviation standard	0.97	0.69	
Min Score	4	6	
Max Score	7	8	

Time Data

Based on table 3, the data shows that the time for implementing the redo after debrief is longer than the first simulation but the results of analysis using the Wilcoxon Signed Ranks Test show that there is no difference in the time needed for the first simulation and debrief simulation with p value 0.141 at α 5%.

Table 3. Time data during the first simulation with redo after the debrief (in minutes)

Description	First Simulation	Redo after debrief	X2
Mean	12.85	14.71	0.141*
Deviation standard	2.34	2.69	
Min Score	9	11	
Max Score	16	18	

Discussion

The simulation is an educational activity by utilizing simulation tools in order to create learning opportunities for participants[10]. Furthermore, debrief after simulation means to develop activities and processes that enable students to reflect on the experiences gained during learning. Simulations and debrief are important learning methods in supporting the learning process, especially learning before students practice clinics directly to patients at the clinic so that they are expected to apply it during clinical practice with the aim of improving patient safety[9,11]

Based on finding, there were significant differences in the level of student satisfaction when doing the first simulation and redo after debrief. Debrief help student to self-evaluation, critical thinking and to improve their knowledge and skill. The simulation repetition method after debrief (redo), proves that it will improve the ability of students to carry out the skills in accordance with the simulation scenario.

Redo after debrief gives students the opportunity to practice their skills in simulation. Redo help student to better doing so the student has experienced good better so the student did feel more satisfied when redo rather than in the first simulation because the student feels more confidence and good performance. The lecturers that involved in the redo station can also focus more on students specifically to activate the simulation learning process[4,12]. Learning satisfaction is the extent satisfaction regarding the learning process and learning performance. Learning satisfaction as indicators of learning performance[13].

Based on the study, there are significant differences in the achievement of learning outcomes after repetition. Students will do redo better and try to improve the simulation after debrief so that time doesn't make a difference[14]. Simulation helps students also get a gradual learning experience in which students learn from simple stages and then to more complex learning, namely clinical vehicles. Students can achieve better learning outcomes, as evidenced by increased attainment of minimum and maximum scores[15]. The advantage of simulation in learning methods is to improve knowledge and skills. In addition, simulations can also improve student performance/appearance and attitudes because the learning process has been arranged to resemble the actual conditions of both playing roles and the environment[3,16].

Debrief and feedback accounts for the reflective observation, abstract conceptualization and active experimentation components of the learning cycle by helping participants make sense of the simulation scenario and reflect on their practice to improve future performance[9,17]. Debrief helps students in evaluating during the first simulation so that when students do redo learning will get better and maximum learning achievement. The redo after debrief was perceived by students as a positive tool to enhance their own learning. The use of a staffed redo station may further enable student-focused learning. Future exploration of this learning and clinical reasoning in regards to deteriorating patients is warranted[4,18].

This study proved that by performing redo after debrief although there appears to be an increase in time to do redo after debrief there are no significant differences in the simulation implementation time in the first simulation with redo after debrief. Simulation is an educational activity by utilizing simulation tools in order to create learning opportunities for participants[1]. Debrief after simulation means to develop activities and processes that enable students to reflect on the experiences gained during learning a significant difference in the time because students doing redo better and try to improve simulation after debrief so time did not give a difference. During the debriefing, students discussed any emotions that they had about the simulation scenario as well as reflecting on and exploring their decision-making processes. Giving oral feedback to students enabled the faculty to be flexible with their questioning, allowed an immediate response from the student and permitted clarification of any misunderstandings[2,7].

The evaluation results when making a debrief will be a base for improvement when students were redone (repetition of the simulation) after debrief so that the time implementation for redo will be longer than during the first simulation but the students will feel more satisfied and better learning achievement so a redo becomes important to complete implementation for learning methods with simulation and debrief.

Conclusion

The Redo after debrief simulation increases the level of students' satisfaction and the learning achievement on midwifery student in Midwifery Study Program, Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia.

Conflict of Interest: None

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The Effectivesness Meaning in Life and Antiretroviral Therapy (Metart) Book as an to Promotion Adherence Antiretroviral Therapy among New People Living with HIV/AIDS Asesment Pre Intervention

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Abstract

Adherence to ART is the primary determinant of viral suppression. Adherence of at least 95% is needed for optimal suppression. One of relationship with HAART is meaning in life. **This study aimed** at assessment meaning in life and adherence antiretroviral therapy before intervention about METART book. **This method** use descriptive statistic analysis to explain social demographic, meaning in life, and adherence antiretroviral therapy. **The result** Characteristics gender people new living with HIV/AIDS this study is 75,6% is Male with 38,5% homosexual. The age range of respondents is in the adult (65,4%). Single merital status is 74.4%. The total respondent with ARV medication is 30.8% under one year. 74.4% has meaningful life but 65,4% respondent has lack adherence ARV. **Conclusion** this research get data pre intervention metart book about demographic, meaning in life, and adherence ARV.

Keywords*: Adherence ART, meaning in life, HIV/AIDS,*

Introduction

Adherence to ART is the primary determinant of viral suppression and the risk of transmission. it decreases the viral replication and viral load which in turn preserves the CD4 level, decreases the progress of AIDS, and reduces deaths[(1)]. Adherence of at least 95% is needed for optimal suppression[(2,3)]. Antiretroviral therapy has an impressive clinical effect in that WHO has a target that 90% of People Living with HIV/AIDS (PLWHA) already underwent ART by 2016, but the target realization is only 53%. From year to year, PLWHA who undergo antiretroviral therapy is increasing. In 2016, there are 19.5 million or about 53% of the total number of PLWHA, and in mid-June 2017 the number has progressed to 20.9 million or about 56.9% of the total number of PLWHA [(3,4)]. Although adherence is important to the outcomes of therapy(5).

Out of 231 respondents in Nepal , 87.4% (95% CI: 83.2–91.6%) of them had an optimal adherence level and 12.6% of the respondents had an adherence level less than the opti-mal within the last month. i.e. 87.4% of the respondents' attained 95% adherence to prescribed ART regimen[(3)]. Results of the evaluation of adherence to ART in Brazil using CEAT-VIH indicated that 50% (109/220) of the patients presented good adherence, 36% (79/220) presented strict adherence, and 14% (32/220) presented low adherence[(6)]. Based on the results research in Iran, 30.4% (65) of patients were non-adherent and 69.6% (149) showed good adherence to their medication protocols. The most important reason for discontinuation of medication was the patients' unknown personal reasons (n=60, 61.2%), from the non-adherence group[(7)]. Many determinant factors to present non-adherence; quality of life, distress psychology, meaning in life, self-efficacy, peer group, economy, health worker, stigma, motivation, gender [(3,7–11)].

Effect of non-adherence ARV increases mortality and morbidities. HIV/AIDS has effect to biological, psychological, social, and spiritual. Thus, prevention of co-morbidities, improving knowledge through health education. Psychoeducation should be conducted at the initial evaluation to reduce negative beliefs regarding antiretroviral therapy Assessment of anxiety and depression symptoms should be done throughout therapy as both psychological conditions are associated with patient adherence, success of treatment, and ultimately with patients' quality of life. This study researcher will be increasing of meaning in life to increasing adherence ARV people living with HIV/AIDS.

Material and Method

The study protocol and consent procedures were reviewed and approved by the Institutional Review Boards of Sekolah Tinggi Ilmu Kesehatan Ngudia Husada Madura with number of ethical clearance 709/ KEPK/STIKES-NHM-/EC/IX/2020. In accordance with the approved protocol, written informed consent was obtained from all study participants prior to data collection. This study is assessment pre intervention of meaning in life and antiretroviral therapy (METART) to promoting adherence antiretroviral therapy. Researcher use meaning in life questionnaire Indonesian version[12] and Morisky 8-scale[13] to assessment meaning in life and adherence antiretroviral therapy. Data analysis, descriptive statistic[14–16] were used to determine statistical differences in demographic variables (i.e., gender, education, marital status, CD4 status, Viral load status, Sexual orientation), meaning in life and adherence ART.

Result and Discussion

Demographic Characteristics

A total of 78 PLWHAs were invited to participate in the study. Demographic Characteristics

Are gender, sexual orientation, age, education level, merital status, ARV medication, CD4 status, and viral load status (Table 1).

Table 1.1: Demographic Characteristics people new living with HIV/AIDS n=78

Demographic data	Frequency (n)	Percent (%)
Gender		
Female	16	20.5
Male	59	75.6
Other	3	3.8
Total	78	100
Sexual-orientation		
Heterosexual	26	33.3
Homosexual	30	38.5
Bisexual	15	19.2
Other	7	9
Total	78	100
Age		
Adolescent	24	30.8
Adult	51	65.4
Elderly	3	3.8
Total	78	100
Education-level		
Middle-elementary school	7	9
High school	50	64.1
Undergraduate	20	25.6
Postgraduate	1	1.3
Total	78	100
Merital status		
Sigle	58	74.4
Meried	16	20.5
Window/windower/divorce	4	5.1
Total	78	100

Cont... **Table 1.1: Demographic Characteristics people new living with HIV/AIDS n=78**

ARV-Medication		
0-6 months	18	23.1
7-12 months	24	30.8
13-18 months	17	21.8
19-24 months	19	24.4
Total	78	100
Cd4-Status		
<200	13	16.7
200-300	13	16.7
300-400	20	25.6
400-500	14	17.9
>500	11	14.1
I don't know	7	9
Total	78	100
Viral load status		
Detections	16	20.5
Un-detections	22	28.2
I don't know	40	51.3
Total	78	100

Characteristics gender people new living with HIV/AIDS this study is 75,6% is Male with 38,5% homosexual. Homosexual is romantic sexual attraction, behavior with same sex [17][18][19]. The age range of respondents is in the adult (65,4%) age range which is above 25-45 where this age is a productive to developmental task of choosing a job and marriage. This time is also a time to build the peak structure of life [20]. Sigle merital status is 74.4%. the total respondent with ARV medication is 30.8% under one year. Almost respondent have CD4 under 500, a normal CD4 cell count is about 500 until 1.500 cells/mm^3. Clinicians use this test to monitor the destruction of CD4 cells, and it also monitors the effectiveness of the antiretroviral treatment (ART). For a physician, the CD4 cell count has become the best indicator of disease progression and is used to stage disease and guide medical therapy. Per the Center for Disease Control and Prevention (CDC), one of the indications for the diagnosis of AIDS is when CD4 cell count drops below 200 cells/mm^3. The decline of CD4 T cells can lead to opportunistic infections, and it increases mortality. The results of a viral load test are described as the number of copies HIV RNA in a milliliter of blood, when somebody has undetected of viral load is described if the copies of HIV RNA of blood in very low and its good for healthy [10,11].

Meaning in life

The result of meaning in life people with HIV/AIDS in this research is 74.4% has meaningfull life (Tabel 2).

Tabel 2: Meaning in life people living with HIV/AIDS n=78

Meaning in Life	Frequency (n)	Percent (%)
Meaningfull life	58	74.4
Low meaning in life	20	25.6
Total	78	100

Meaning in life is comprehended from every event that occurred and experienced both pleasant and distressing events. It will bring a person into a meaningful life so that the feeling of happiness will emerge, on contrary it can lead one to a meaningless empty life if they unsuccessful [23–25]. Meaning in life had a different function for each individual, but according to Mackenzie & Baumeister (2014), the function of meaning in life could be divided into three function themes[26]. The first function was to help someone in recognizing and discussing signs and patterns in the environment. The second function was for communication. The third function was for self-control. The meaning in life according to Starck (2014), was said to be the phase where a person reached his life goal[27]. The meaning in life have correlations with adherence ARV[11].

Adherence antiretroviral therapy

Table 3: Adherence antiretroviral therapy n=78

Adherence antiretroviral therapy	Frequency (n)	Percent (%)
High adherence	27	32.6
Middle adherence	26	33.3
Low adherence	25	32.1
Total	78	100

From the research results obtained that 59 respondents or 57.8% were adherence to antiretroviral therapy and the rest did not comply as much as 43 respondents or 42.2%. This level of adherence was seen from the accuracy of the dosage and the frequency of time-consuming ARV. The non-adherence of respondents based on the questionnaires due to forgetting to take the drugs by 26.5% and difficult to remember to consume the drugs by 28.4%.

ART treatment has the potential to reduce mortality and morbidity that associated with HIV infection, and improve the quality of life [28,29]. A person is said to be adherent in undergoing an optimal antiretroviral therapy if (> 95%) never forgets to take the drug, but it is recommended that the adherence is (100%). According to Bangsberg, Kroetz, & Deeks, (2007), ART adherence should be observed to discern the compliance level of the treatment, as some studies indicate that with treatment adherence of (95%) or more indicates the effectiveness of antiretroviral therapy[30], but on adherence (75%) shows a rise of viruses with retention against drugs[31]. Some patients fail to maintain ART adherence [32]. Optimal ART adherence was significantly associated with being virally suppressed[1,7,9,10].

Many factors to associate ART is motivation on the relationship between HIV stigma and ART adherence[10].

Conclusion

Characteristics gender people new living with HIV/AIDS this study is 75,6% is Male with 38,5% homosexual. The age range of respondents is in the adult (65,4%). Single merital status is 74.4%. The total respondent with ARV medication is 30.8% under one year. 74.4% has meaningful life but 65,4% respondent has lack adherence ARV.

Conflict of Interest: No conflict on interest

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Through the Life of their Spouses- Coping Strategies of Wives of Male Alcoholics

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Abstract

Background: Alcoholism is a major health issue and it affects the mental stability of the individual. It is also considered as a family disease and social problem because the alcoholism of one person in the family affects the entire family by mentally, economically and physically. Among family members, the wives of alcoholics are the most affected person by their emotional and physical attachment in the relationship. The wives of alcoholics often face domestic violence, marital conflicts, insecurity, social isolation, job losses or house lose due to the alcoholism of their partner and it leads them into psychological problems like stress, anger, shame, anxiety, depression, self- blame, hopelessness, low self- esteem and suicide tendency. Copings are the thoughts and actions used by an individual to deal with a threatening situation. Since wives of alcoholics are stressed due to the drinking habit of their spouse, they need to choose different coping strategies. Coping strategies are the intensive efforts used by the wives of alcoholics both behavioural as well as psychological to endure and decrease the stress associated with their husband's drinking habit.

Methods: In this study, researchers attempted to understand the coping strategies used by the wives of alcoholics. The study measured the level of Coping Strategies and significant factors associated with wives of alcoholics of Kottayam district of Kerala, India. Survey Design was used for the study. Census method used to collect the samples. **Conclusion:** Nearly half (42%) of the respondents have a medium level of coping strategies and 32% of the respondents have high level and 26% have low level of coping strategies. The study reveals that Coping Strategies has no significant difference between sociodemographic variables like age, religion, area of residence, type of family, education, occupation, family monthly income and duration of habit in the spouse. In this study, it is understood that the coping strategies of wives of alcoholics depend on the family atmosphere, behaviour of the husbands and genetic factor and personality of the wives.

Key words: *Alcohol dependents, Alcoholism, Coping Strategies, Wives of alcoholics,*

Introduction

Alcoholism is a condition in which an individual has a lack of control in drinking habit due to physical and emotional dependence to alcohol and it is a chronic disease which is characterised by uncontrolled drinking and addiction.

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Alcoholism affects the individual's physical and mental wellbeing hence some people think that it is an individual problem but actually it is also a family problem and social problem because an individual's excess drinking habit or alcohol dependency will adversely affect the symphony of family functions which will lead to domestic violence, marital dissatisfactions and quarrels between family members. As we know that families are the basic building block of every society and the unsatisfied and unstable families would lead to an unstable society. Hence the alcoholism needs to be considered as a family problem and social problem.

Alcoholism is one of the major health and social problems around the world, which results in 3.3

million deaths every year (WHO, 2018)[1]. India is one of the fastest-growing alcohol markets in the world. Urbanization, the large number of middle-class population and fast growing economy are the certain reasons behind an increase in the consumption of alcohol in India[2]. The per capita alcohol consumption of India is very low due to its large population however India is the largest consumer of alcohol in the world as compared to other countries.

Kerala has the highest per capita alcohol consumption of any state in India, at over 8 litres per person per year (NFHS-4 – 2015-16)[3]. This is well above the national annual average alcohol consumption, which is 5.7 litres per person per year. This increase of the alcohol consumption provides more than 40 percent of revenue to Kerala's annual budget however it is also adversely affecting the state, as per the Kerala Women's' Commission statement the 40 percent of road traffic accidents are directly linked to alcohol and 80 percent of divorces are also connected with alcohol[4].

Alcoholism is considered as a 'Family Disease' because if one member in any family is alcohol dependant the entire family will suffer either by physical or by mental harassment. The alcoholics experience the burden of the physical problems, but family members often share the emotional side effects of the person's addiction. Family members of alcoholics mostly experience anxiety, depression, financial crisis and social isolation.

The wives of alcoholics are one of the main victims of their husbands drinking habit due to the intimacy in their relationship. They mostly experience insecurity, sexual dissatisfaction, shame to face public and anxiety about their future as well as the future of their children. They also experience continuing financial problems, loss of job or home, physical injuries and mental illness which requires ongoing treatment, social isolation, and withdrawal from family and friends.

When there is a mental break down, the human being grasps different ways of coping with crisis. According to Lazarus (1984) "Coping is a process of managing taxing circumstances expending effort to solve personal and interpersonal problems and seeking to master, minimize or reduce stress or conflict"[5]. Coping strategies used by the wives of alcoholics are the concerted efforts both behavioural as well as psychological which are employed by them to tolerate and reduce the stress associated with their husband's drinking[6].

As per Folkman and Lazarus the Coping strategies can be classifieds in four groups: - Problem-Focused, Emotion-Focused, Support-Seeking and Meaning-Making. In this study, researchers focused on two major coping strategies: emotion-focused and problem-focused. The problem-focused coping involves some active action to alleviate stressful circumstances, and emotion-focused coping involves the efforts to regulate the emotional consequences of stressful events. Generally, women are using emotion-focused coping and the "tend and befriend" response to stress, but men using problem-focused coping and the "fight or flight" response[7].

Types of Coping Mechanism:

- Support- Discussing stressful situations with an intimate person is an effective method to reduce stress. Finding external support instead of self- isolating and internalizing in a stressful situation can help to reduce the adverse effects.

- Problem-solving- In this type of coping mechanism the individual first identifying the problem which causing stress and then creates the solution for effective managing of the problem.

- Relaxation- The relaxing activities like meditation, yoga, music listening or sitting in a calm place can help people to cope with stressful situations.

- Humour- It helps people to maintain the toughest situation to light and maintain perspective and prevent the situation to become overwhelming.

- Physical activity- Activities like running, swimming, walking, dancing, team sports will help the individual to forget or lighten the toughest situation of stress8.

The wives of alcoholics are trying different coping strategies at different stages of their marital life, in initial few years they trying tolerant styles with a hope to get things better and after few years if they are not getting any positive outcome the wives of alcoholics adopted withdrawal coping.

As we know alcoholism is a disease which can be treated with the support of family members especially with the help of wives of alcoholics. It is necessary to know the problems faced by the wives of alcoholics and

the level coping strategies adopted by them for effective family counselling and psychological support to them. Hence the researcher selected this topic for the study.

Materials and Methods

This paper is based on standardized scale and the data was collected with an interview schedule from wives of alcoholic attending family counselling and Al-anon meeting in a De-addiction centre of Kottayam district of Kerala. An interview schedule was used to collect data. The Coping Strategies Scale was developed by Folkman and Lazarus (1989) is used for the study. Survey Design was used for the study. Census method used to collect the samples. The researcher collected data's from all the respondents who were willing to participate in the study during the period of data collection (July 2019 – December 2019). The criterion for selection of the respondents was the wives living with the alcoholics who are under treatment at de-addiction centres in Kottayam District, Kerala, India. The universe consists of 200 spouses of alcoholics who were coming to the rehabilitation centre in ADARRT- IRCA (Research Rehabilitation & Treatment Centre), Pala, Kottayam-Kerala. (Established: 1984) for treatment of their husband and use of group counselling services. The spouses of alcoholics above 20 years of age are selected for the study.

Major findings:

The simple percentage of socio demographic data reveals the following information. It is observed that 30% of the respondents are in the 20-30 years age group. Nearly half of the respondents (49%) are Christian. Majority of the respondents (55%) are from the rural area. More than half of the respondents (56%) are from the nuclear family. It is seen that 29% of respondents are Graduates and are housewives respectively. Majority of the respondents (64%) have family monthly income above Rs.20000. Half of the respondents' husbands (50%) have above 10 years of a drinking habit.

Level of Coping Strategies

Nearly half (42%) of the respondents have a medium level of coping strategies and 32% have high and 26% have low level of coping strategies.

In this study, it is clear that the husbands drinking habits compelling the wives to adopt coping strategies to maintain marital life. In this study researcher identified that the respondents were using different types of coping techniques such as avoidance, competition, taking special actions, seeking support, adjusting their expectations, denial, self-blame. During the interaction with the respondents at the time of the interview, the researcher identified that the wives of male alcoholics adopting the coping strategies either to adjust with the drinking habit of their husbands or liberate their husband from alcoholism for the betterment of marital life and to secure their children's future. It is evident that the wives of alcoholics adopting the coping strategies for managing the marital life without breakups as the divorces will hamper the future of their children too. The respondents were bothered about the health and wealth of their husbands due to the alcoholism. Therefore they use the strategies as per their mental status, husband's character and family atmosphere. The coping used by the wives of alcoholics depends on whether their husband in a drunken state or not. Normally the wives of alcoholics are adopting rough and aggressive coping when the husbands are in a drunken state and they are converting their coping strategies into soft and kind when their husbands in a normal or non-drinking state.

Factors affecting the Coping Strategies

Table No. 1: ANOVA- Significance table for Coping Strategies and Socio demographic variables

Sl.No.	Variables	F -value	Table value	Significant/Not
1	Age	0.187	2.651	NS
2	Religion	2.607	3.042	NS
3	Area of residence	1.170	3.042	NS
4	Type of family	2.480	3.042	NS

5	Education	2.178	2.261	NS
6	Occupation	1.450	2.418	NS
7	Family monthly income	0.004	3.042	NS
8	Duration of habit in spouse	1.461	2.651	NS

NS-Not significant, * -Significant at 5% level, ** -Significant at 1% level

The above table shows that there is no significant difference between sociodemographic variables with coping strategies. In this study, it is evident that the age, religion, area of residence, type of family, education, occupation, family monthly income and duration of habit in spouse has no relation between coping strategies of the wives of alcoholics and coping strategies are connected with family circumstances of an individual or behaviour of wives of alcoholics and their husbands. The researcher identified that the coping mechanism differs from case to case even if sociodemographic variables are same in different individual due to the difference in their personality, husbands' personality or family atmosphere.

Discussion and Conclusion

Alcoholism is one of the major threats to the marital family life. In order to avoid this threat, it is to be treated timely by specialised programs like de-addiction therapy, alcohol anonymous, al-anon and family counselling. This specialised treatment helps the alcoholics to regain control over their life and maintain the life of abstinence. The success of these specialised programs is related to the co-operation of alcoholics and their wives. For this, the wives of alcoholics should know that alcoholism is a disease and can be cured with the help of all family members.

Chronic alcoholism of husbands leads the wives into the unstable mental conditions and which turns into family breakups, physical violence, divorce and suicide. In order to avoid these situations, the coping strategies of each individual need to be evaluated in detail and guided them for selecting appropriate coping strategies.

The wives of alcoholics required family therapy, couples' therapy, social skills training, parental training and psychiatric services which will help the wives of alcoholics to recognize the causes of their husbands' alcoholism and develop good solutions to resolve them.

It is identified that feelings of wives of alcoholics are often ignored when it comes to treatment. The alcoholic husbands may get professional treatment, residential care but the problems faced by the wives are left unnoticed. Mentioned below are some ways for wives of alcoholics to get much-needed support:

- A trusted friend or family to disclose their feelings.
- Join a support group for close relatives of alcoholics by online or offline groups.
- Visit mental health workers to evaluate mental health, to get counselling and other treatments if required.
- Learn the positive coping strategies and effective use of the same depending on the situation of family life.
- Practice of Yoga, Meditations, deep breathing exercises and prayers to get better relaxation of mind and soul.
- Spend more time with friends, family and peer groups for socialising.
- Take care of the children and help them to cope up with the alcoholic father.

In this study, it is revealed that the nature of coping strategies depends on the personality of the wives and their husbands. The coping strategies of wives of alcoholics have no relation between the sociodemographic variables such as age, religion, area of residence, type of family, education, occupation, family monthly income and duration of habit in the spouse.

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Effectiveness of Low Dose Over Standard dose CT for Detection of Urolithiasis: A Systematic Review

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Abstract

Computed Tomography (CT) of the Kidneys, ureter, bladder is prime investigation to study the pathologies of the Genitourinary system. Despite the advantages of CT, radiation exposure poses major risk. The objective of the review is to investigate the effectiveness of Low dose dose CT over standard dose CT for detection of Urolithiasis. The literature search was performed on PubMed, Scopus, CINHAL, Cochrane Library and Web of Science databases for original research articles published between 2000 and 2020. Research Articles that included the efficacy of low dose CT over standard dose CT in identification of Urinary stones were included. Total 4558 studies were detected from the literature search. After thorough screening 12 articles were included in the review. The Sensitivity of Low dose CT KUB was 80 to 99% and the Specificity ranged from 88 to 100%, the diagnostic accuracy of Low dose CT was 96.1%. From our study we conclude that Low dose CT KUB was effective in maintain the diagnostic confidence with optimal image quality and reduced radiation dose.

Keywords: *Low dose, CT KUB, Urolithiasis, Radiation dose*

Introduction

Urolithiasis is widely known problem across the globe. The incidence and prevalence rates of kidney stones may be affected by genetic, nutritional and environmental factors. The incidence of urolithiasis is 1 in 1,000 per year. The non-enhanced CT KUB was used for identifying urolithiasis in patients with acute flank pain and hematuria[1-3]. The radiation dose and risk associated with CT KUB is a major concern. Due to repeated CT KUB scans for assessment of urolithiasis has caused raise in radiation received by the patients. The National Dose Reference Level for CT KUB in UK for assessing urolithiasis is 6.44 mSv[4-5]. With respect to this issues of high radiation dose, low dose protocols for CT KUB has been used in recent years to reduce the radiation dose by maintaining optimum image quality[6-7]. The objective of the review is to investigate the effectiveness of Low dose CT over standard dose CT for identification of Urinary stones.

Design and Literature search strategy:

The systematic review was done according to Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA).

The literature search was performed on PubMed, Scopus, CINHAL, Cochrane Library and Web of Science databases for published original articles between 2000 and 2020. The keywords that were used in the literature search was: 'Urolithiasis', 'Renal Colic' 'CT KUB', Low dose CT'. Study retrieval methods from various data base are shown in Table 1.

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Table 1: Showing methods of study retrieval from database

Database	Year	No. of studies	Total Number
PubMed	2000–2020	935	4558
Scopus Direct	2000–2020	1100	
CINHAL	2000–2020	856	
Cochrane Library	2000–2020	664	
Web of Science	2000–2020	1003	

Inclusion criteria:

Original articles that were published in language English between 2000 and 2020

Studies reported CT KUB comparing low dose with standard dose were included

Exclusion criteria:

Articles with case studies, case reports, posters and narrative literature reviews were excluded as they did not fulfill the criteria.

Data extraction and analysis

Two independent reviewers extracted data individually from each article and any differences were solved following consensus between them.

Quality Assessment

The quality of articles was evaluated by two independent reviewers using QUADAS-2 assessment tool. Results were reported according to STARD guideline[8]

Results

A total of 4558 articles were detected by extensive literature search. 400 duplicates were excluded. 4136 studies were excluded after evaluation of article title and abstract content. Further 10 studies were excluded after examining the full text. Finally, twelve studies were included [Fig. 1].

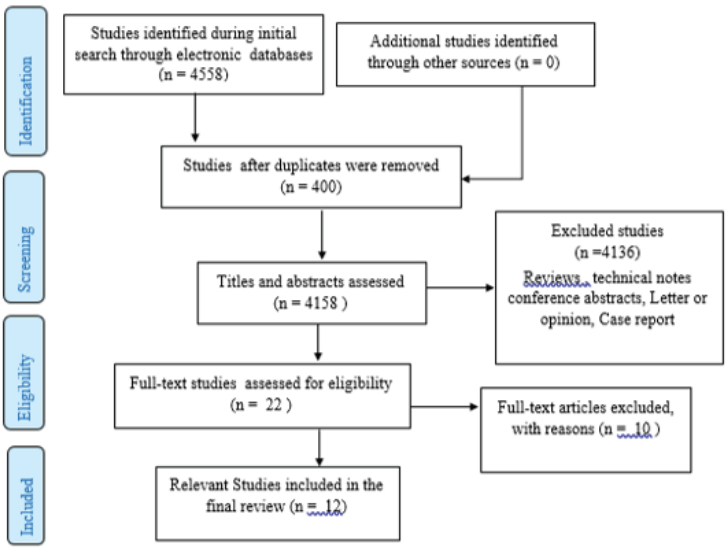


Fig. 1- Flow diagram of study selection process.

Characteristics of included studies:

After reviewing the inclusion and exclusion criteria, a total of 12 articles were included in the study. The study covers different regions of the world such as Germany, Belgium, South Korea, Switzerland, Belgium, America, France, UK, Kenya. A total of 1,439 sample size was included. All the included articles compared the low dose CT KUB with standard dose CT KUB. Table 2 shows the summary of sample characteristics of included articles.

Table 2: Shows the summary of characteristics of articles involved for the review

S.No.	Author (Year)	Country	Sample size	Dose (mSv)
1	Michael Hamm et al 9 (2002)	Germany	109 patients	1.1
2	DenisTack et al 10 (2003)	Belgium	106 patients	1.5
3	Bong Soo Kim et al 11 (2005)	South Korea	121 patients	1.7
4	Claudia Kluner et al 12 (2006)	Germany	142 patients	0.6
5	Pierre-Alexandre Poletti et al 13 (2007)	Switzerland	125 patients	1.7
6	Tom H Mulkens et al 14 (2007)	Belgium	300 patients	6
7	Mc Laughlin et al 15 (2014)	USA	33 patients	0.4
8	Mikael Fontarensky et al 16 (2015)	France	118 patients	1.4
9	T Meagher et al 17 (2001)	UK	69 patients	3.5
10	N Twahirwa et al 18 (2009)	Kenya	104 patients	-
11	John A Fracchia et al 19 (2012)	USA	101 patients	2.1
12	Christopher L Moore et al 20 (2015)	USA	201 patients	3.4

Discussion

Over the past years, there is enormous increase in use of CT KUB for diagnosis of urolithiasis. The CT KUB has been repeated as follow up for assessing the recurrent urolithiasis which lead to increase in radiation dose received. Urolithiasis often affect young patients who are relative risk of radiation induced carcinogenic effects. The sequential CT scans for evaluation of urolithiasis can result in carcinogenic effects, there subjects who are present in with recurrent urolthiasis [22,23,24].

Our review noticed the high sensitivity and sensitivity of low dose CT KUB. There was significant reduction in dose received by the patients with optimum diagnostic image quality. With the advent of automatic tube current and voltage modulation, iterative reconstruction techniques the dose levels in CT are almost equaling to that of X-ray [21].

Our review found the Sensitivity of Low dose CT KUB was 80 to 99% and the specificity ranged from 88 to 100%, the diagnostic accuracy of Low dose CT was 96.1%. The mean effective radiation dose of all the studies ranged from 0.48-3.5 mSv. All studies had reported higher sensitivity and specificity, one study reported very low sensitivity with reduced radiation dose[15].

Our study noted variation in dose reduction techniques used, methodology of determination of dose by using different conversion factors and also there is variation in classification of grouping based on BMI.

Recommendations

We recommend there is need for consensus on BMI based classifications, conversion factor which provides comfortable comparison of the studies and also recommend future studies to be based on advance noise reduction techniques such as iterative reconstruction techniques which provides optimum image quality at lower dose

Conclusion

Our review suggests that a low-dose CT protocol has highest sensitivity and specificity in identification of renal stones and could be used effectively for diagnosis of urolithiasis in renal colic patients. The low dose CT protocol would be really helpful in younger patients with recurrent urolithiasis as they are at the risk of effects of radiation dose.

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Perceived Social Support and Locus of Control among Children in Child Care Institutions

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Abstract

Introduction: Children in child care institutions are one of the most vulnerable groups in society. In many countries, they are living in orphanages or children's home for reasons, such as parent's death, abandonment, neglect, born with some medical conditions, poverty. Many nations are facing a struggle to care for these vulnerable group. Child care institutions provide children's home aid programmes, foster care, adoption and family reunification programmes. Research work related to children in child care institutions shed light into their wellbeing.

Aim: This study aimed to know the level of perceived social support and locus of control among children in child care institutions and relations of independent variables (age, gender etc.) on perceived social support and locus of control. This study was a survey design in nature. Census method was used for the study. 222 children between the age group of 10-18, from 5 children homes run by the Kerala government was selected for the study. Kerala. **Result:** This study reveals that there is no significant relationship between perceived social support and locus of control. Perceived social support is high among the children those who have an internal locus of control. It is seen that 53% of children have an internal locus of control and 47% of children have an external locus of control. **Conclusion**: It can be concluded that the children in child care institutions show average level of perceived social support and there is no relationship between locus of control. Internal locus of control in children is higher than external locus of control. The caretakers, parents and relatives need to support the children in a better way so that they feel cared and loved. It is important to train the children to have internal locus of control so that they take responsibility for the outcome of their lives.

***Key words**: Children, Child care institutions, Locus of control, Perceived social support.*

Introduction

Children are one of the most important part the world. They are inclusive of joy, energy and curiosity. They constantly explore all around them and in the course learn numerous things. The exploration and learning new things are very crucial to the development of the child. Individuals and peers, family, school. And community have a great influence on childhood development. Parents and society have to provide a healthy environment for the better growth and development of children. They are more vulnerable to exploitation and abuse than adults to the conditions.

Child care institution is the residency of children where they get institutional care in an organized manner. These care homes are keeping the best interests and protection of the child. And providing physical, psychological, emotional. moral, social, ethical and spiritual needs of children. Children ages between 10-18 are in the adolescence stage. It is a period of transitional when the individual changes physically and psychologically from a child to an adult. This transition involves psychological, emotional, social and intellectual changes. The period of transition leaves its mark on the individual's behaviour as they unsure of themselves and

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insecure in their status and hence naturally sometimes become aggressive self-conscious and withdrawn.

Social support has an important role in the development of children. It can help them to cope with stressful life events. Social support is the availability of people who are supportive, caring and loving. Perceived social support reflects an individual's perception of social support. Children from children's home are separated from the home environment which leads to loss of social support**.** Saron & Pierce[1] in their perceived social support refers to an individual's belief that social support is available, which is generally considered positive or negative and provides what is considered needed by that individual. Low perceived social support affects children's mental health.[1] Ebru Ikiza and Firdevs Savi Cakarb [2] Studies reveal that perceived social support and self-esteem are the major factors that correlate with various forms of regressive behaviour and adjustment of adolescents. Many works of literature say that low level of perceived social support causes emotional, behavioural problems and increased risk of mental health problems among children.[2] Allgower, Wardle & Steptoe[3] states that a lack of social support and lower perceived adequacy of social support have been linked to poorer mental and physical health**.**Thus, care providers have an important role to provide support to children, Social support influences adolescent's mental health, perceptions of stress and wellbeing. Better perceived social support enhances the mental health of children in child care institutions.

Locus of control is the personal belief system about the causes of his or her experiences. The concept of locus of control is proposed by Julian B. Rotter (1954).[4] It is the degree to which respondents believe that they have the control (Internal) over the outcome of events in their lives, as opposed to external forces beyond their control[4]. There are two types of locus of control, internal and external.[5] People with an internal locus of control believe that they are in control of their lives and people with an external locus of control believe that they are not in control of their lives[5]. Locus of control is often viewed as an inborn personality component. It is shaped by childhood experiences. James Sengendo and Janet Nambi [6] used the concept of the locus of control in their studies to show the relationship between the environment and individuals' assessment of their ability to deal with it and to adjust behaviour. Most orphans become vulnerable and predisposed to physical and psychological risks.[6]

Methodology

This research work aimed to study the perceived social support and locus of control among children in child care institutions. The **objectives** of the study were to assess the level of perceived social support of the respondents, to assess the type of locus of control of the respondents, to identify the relationship between selected variables in the socio-demographic profile (age, gender etc.) with perceived social support and locus of control of respondents and to find out the relationship between the key variables. The **research design** was survey design and an interview schedule were used to collect data from the respondents. **Piolet study** was done during July 2018.The researcher visited Government Children's home, Vellimaadukunnu, Calicut-Kerala and Government Children's Home, Trivandrum Kerala, for the study and found the possibilities of conducting the present study. Through interaction with inmates, the researcher understood that many of these children had to face many problems. **The pre-test** was conducted among 10 respondents of children's home inmates of Vellimadukunnu, Calicut, Kerala. It was done in September 2018. Through this study, the researcher moulded the questions to get responses correctly. The **universe** of this study consists of children staying in Government children's homes of Kerala State. The **sampling technique** used was the Census method. All 222 children between the age group of 10-18, from 5 children 's homes run by the Kerala government was taken for the study (Calicut, Trissur, Ernakulam and Trivandrum). The data was collected during the period between September 2019 to October 2019 as per the permission granted by the Government of Kerala.

Tools of data collection was an interview schedule which included the socio-demographic profile and 2 standardized scales. The Perceived social support scale developed by Simet and Farley (1998).[7] This is a 14 items scale. It is a 6 point scale and all these 14 statement are positive statements; the scoring is 1, 2, 3, 4, 5 and 6. The maximum possible score is 84 and the minimum possible score is 14.Higher the score, higher the perceived social support.14- 28= Poor perceived social support.29- 56= Average perceived social support 57-84= Good perceived social support .Locus of control scale was formulated by Rotter[8].He divided individuals in two personality classifications. Internal and external based on their perception of locus of control. J.B. Rotter 's Internal External Locus of Control Scale is a measure of personal belief .[8] It consists of 29 items. Six filler

items are:1,8.14,19,24 and 27. A high score (17-21) = External Locus of Control and a low score (6-16) = Internal Locus of Control.

Major Findings and Discussion

It is seen that 41% of the respondents are between the age group of 13-15 yrs. Majority (55%) of them are female and 69% of them are of Hindu religion It is seen that 20% of respondents are studying in eight standards. Nearly half (48%) of children are non-orphan and 42% of respondents birth order is that of the middle child. It is seen that 55% of respondents are from the rural area. It is found that 45% of respondents are staying in the institution between 1-3yrs. It is inferred that 47% of respondents came to the institution because of family problems.

It is inferred that 39% of the respondents have an average level of perceived social support. It is seen that 53% of respondents have an internal locus of control and 47% have an external locus of control. Higher Internal Locus of control (53%) means that the children believe that they are in control of their lives than the external factors. Socio-demographic variables such as class which respondents are studying and domiciliary have no significant difference with perceived social support. There is a significant difference between the year of staying in child care institutions and reason for institutionalisation with perceived social support. There is a significant association between domiciliary and years of staying in child care institutions with Locus of control. All the other socio-demographic variables have no significant association with Locus of control. There is no significant relationship between perceived social support and locus of control.

Richmond, Ross & Egeland [9] in their study states that Social support functions as a buffer to reduce distress and resilience for people in stressful events. Children who have lost their parents to other causes and other orphaned children reported similar levels of social support.[9] The present study shows that perceived social support is high among the children those who have internal locus control. This may be since children take responsibility for their lives and are happy with the social support given by caregivers.

Table No:1: Perceived social support and its influencing factors.

S.NO	Variables	't'/ANOVA	Significant or Not
1	Age	ANOVA	NS
2	Gender	"t"	**
3	Religion	ANOVA	NS
4	Class studying	ANOVA	NS
5	Parental status	ANOVA	NS
6	Domicillary	ANOVA	**
7	Order of birth	ANOVA	NS
8	Year of staying in institution	ANOVA	**
9	Reason for institutionalisation	ANOVA	*

NS=Not significant*=Significant at 5% level, **=Significant at 1% level

Table No:2: CHI square test results on Locus of control and its influencing factors.

S.NO	Variables	Significant or Not
1	Age	NS
2	Gender	**
3	Religion	NS
4	Class studying	NS
5	Parental status	NS
6	Domiciliary	*
7	Order of birth	NS
8	Year of staying in institution	**
9	Reason for institutionalisation	NS

NS=Not significant*=Significant at 5% level, **=Significant at 1% level

Table No:3: Correlation between perceived social support and locus of control

	Perceived Social Support	Locus of Control
Perceived Social Support	---	.025
Locus of Control	.	---

** - Significant at 1% level

Conclusion

The researcher conducted this study for understanding the perceived social support and locus of control of children in child care institutions. The result showed that the majority of respondents have an average level of perceived social support. There is a need for more social support should be provided to them. Internal locus of control in children is higher than the external locus of control. It can be concluded that there is no significant relationship between perceived social support and locus of control. Thus, it is inferred that the caretakers, parents and relatives need to support the children so that they feel cared and loved. There is a need to train the children to have an internal locus of control so that they take responsibility for the outcome of their lives. The awareness and insight about the problems of children in child care institutions can help the authorities to formulate innovative projects and schemes for the wellbeing of children.

Source of Funding-Self

Conflict of Interest-Nil

Ethical Clearance- Taken from Human Ethics Committee, Dept. of Social Work, Amrita Vishwa Vidyapeetham, Coimbatore, Tamil Nadu, India

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Effects of Smokers and Second Hand Smoke by Gender and Age on DALYs Stroke in Indonesia

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Abstract

Stroke is one of the causes of productive age losing time and work due to disability and death. This articles want to analyze the differences in the influence of smokers and second hand smoke by gender and age group to the value of DALYs stroke in Indonesia. This research is quantitative with cross-sectional design. It use BOD (Burden Of Disease) research data in 2018. The population is stroke sufferers. The sample is people who are exposed to cigarettes both as smokers and second hand smoke. Highest smokers DALYs value of stroke are found in ≥ 70 years old group. The value are 6041,33 for men and 810.82 for women. Lowest smokers DALYs value of stroke are found in <50 years old group. It value are 475.06 for men and 56,63 for women. Men DALYS value are 7,5 to 8,4 folds higher than women. Highest second hand smoke DALYs value of stroke are found in ≥ 70 years old group too. The value are 619.48 for men and 1322.03 for women. Lowest DALYs value of stroke are found in <50 years old group too. The value are 26.3 for men and 165.19 for women. Women DALYs value are 2,13 – 6,4 fold higher than men. Based on Kruskal-Wallis test result with CI level of 95% indicate significant difference for gender, age and individual exposed to smoke on DALYs stroke. There is a significant difference between sex, age, smoker and second hand smoke on DALYs stroke. Male smokers had higher DALYs than women. Female second hand smoke had higher DALYS than men.

Keywords: *Smoke, Smoker, DALYs, Stroke*

Introduction

Stroke is non infectious disease acutely attacking focal and global brain function with symptoms resulting in neurological deficit [1]. Recently it remains one of main risk factors for morbidity and mortality in the world and is predicted to cause 4,4% year of life lost due to smoking related-morbidity (DALYs) [2]. Smoking still becomes controversial. Beside it increases nation revenues and provides employment but there is also evidence that smoking is detrimental for health. Cigarette has harmful chemical components including alkaloid (nicotine), tar, carbon monoxide, nitrogen monoxide, nitrit compounds, chlorium, cadmium, sipermettrin, provenofos[3,4]. Smoker has 6 folds of risk to have stroke in comparison with non smoker[5].

Stroke is one of causing disease for disability and mortality [6] even it ranks third highest in the world only surpassed by heart disease and cancer [3]. Patient with stroke will loss significant part of his/her healthy or productive life. Public Health practitioners indicate stroke as global scale burdening disease which increase in last two decades. Stroke also burdens for each country and region[7].

Based on Tati Suryati[8], burden of disease can be measured by burden of disease method. The calculation uses Disability Adjusted Life Year (DALYs) method. Therefore, this research aimed to analyze difference between active smoker and second hand smoker (passive smoker) based on sex and age on stroke DALYs value in Indonesia in 2018.

Methode

It is quantitative inferential research with cross sectional design. The population is all stroke patients with sample those who are exposed to smoke both as active smoker or second hand smokers. The data is from Global Burden of Diseases (GBD) research in 2018. This

research supported by Ethical Clearance no: LB.02.01/2/KE.086/2018. The data result was managed by *Institute for Health Metrics and Evaluation in University of Washington in Seattle, Washington,* AS.

DALYs (*Disability Adjusted Life*) constitutes lost years rate for healthy life caused by early death (YLL) and disease or injury/disability (YLD). Years of life lost (YLL) is living year rate which is lost due to early death. Years lived with disability (YLD) is living year rate with non healthy condition (injury/disability)[8].

Value 1 of DALYs indicates the lost of 1 productive lived year. Higher DALYs value indicates higher disease burden in the region. In this article, stroke DALYs related to active smoker and second hand smoker (passive smoker). It was stroke DALYs in 34 provinces in Indonesia based on sex and age.

Age group based was divided into 3 groups. They are age < 50 years old, age between 50 – < 70 years old, and age ≥ 70 years old. Individuals in under 50 years old age group generally have good health. Individu between 50 - < 70 years old have more health problem, i.e : heart disease, stroke, prostate, gall stone. Individu at 70 years old and over have decreasing body function and other health problem. Individu in smoke exposed group were divided into two consisting active smoking and second hand smoke or passive smoker.

Data analysis used Kruskal Wallis test since the data was not normal distributed with Sig value or p = 0,000 <α=0,005. It also was not homogenous suitable Levene's test score with Sig value or p =0,000 < α=0,005, as following:

Results

Kruskal Wallis analysis result indicated that there is difference between sex, age, smoke exposed group (active smoker and second hand smoker or passive smoker) on stroke DALYs as following:

Tab1e 1. Kruskal-Wallis test Statistical Result on significance of gender, age, smoke exposed group difference on stroke DALYS in Indonesia in 2018

Grouping variabel	Analysis	DALYs Score(Convident interval level (CI= 95%) and α =5%	Conclusion
Gender	Chi-Square	7,801	
	Df	1	
	Asymp. Sig.	0,004	At level CI level of 95% significant difference exists (P= 0,004 < α (5%), between gender and stroke DALYs stroke
Age	Chi-Square	214,053	
	Df	2	
	Asymp. Sig.	,000	At CI level of 95%, significant difference exist (P= 0,000) < α (5%), between age group on stroke DALYs

Cont... **Tab1e 1. Kruskal-Wallis test Statistical Result on significance of gender, age, smoke exposed group difference on stroke DALYS in Indonesia in 2018**

Smoke exposed (active Smoker dan second hand smoker)	Chi-Square	28,877	
	Df	1	
	Asymp. Sig.	,000	At CI level of CI 95%,, significant difference exists (P=0,000) < α (5%), between active smoker and antara perokok dan second hand smoker on stroke DALYs with CI 95%

Kruskal-Wallis test result with CI level of 95% indicate significant difference exist for gender (p =0,004 < α =5%), age (p =0,000< α =5%) and individual exposed to smoke (active smoker and *second hand smoker*) p =0,000< α =5% on DALYs stroke .

Result of DALYs statistic descriptive of stroke based on group of individual exposed to smoke, age and gender in Indonesia in 2018 as indicated in Global Burden Disease (GBD) research in 2018 are following:

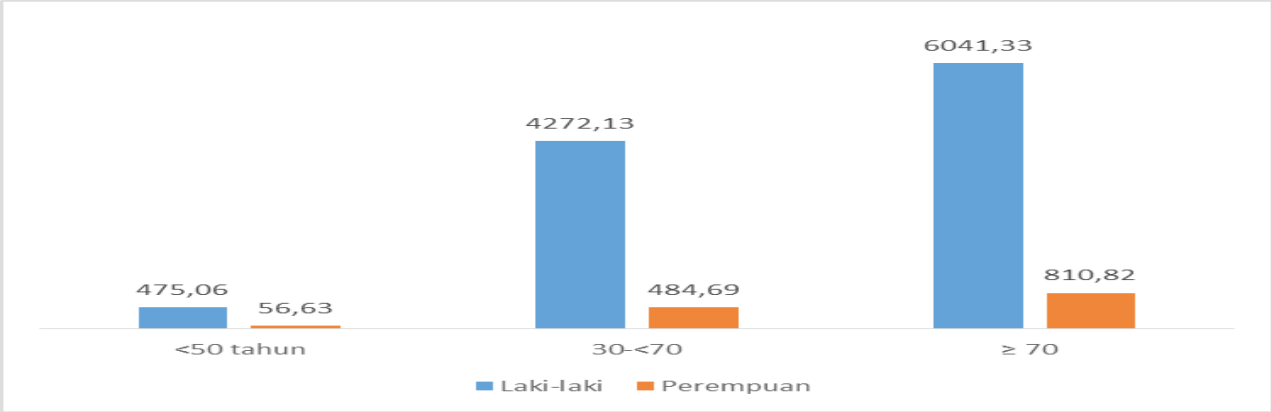
Table 2. DALYs value of stroke disease in active smoker and passive smoker based on Gender and Age groups in Indonesia year 2018

Type of smokers	Gender	Age (Year)	Mean DALYs stroke per 100.000 population	Std. Deviation
Active Smoker	Male	< 50	475.06	146.35
		50 - < 70	4272.13	1038.01
		≥ 70	6041.33	1155.92
	Female	< 50	56.63	50.34
		50 - < 70	484.69	305.07
		≥ 70	810.82	410.77
Passive Smoker (second hand smoker)	Male	< 50	26.31	8.96
		50 - < 70	298.73	76.17
		≥ 70	619.48	132.99
	Female	< 50	165.19	85.79
		50 - < 70	873.46	372.56
		≥ 70	1322.03	488.75

Smoker is not only found in men but also among women. The highest mean value of DALYs stroke among men smoker are found in ≥ 70 years old group. The mean value is 6041,33 per 100.000 population. The lowest mean value of DALYs stroke are found in < 50 years old group. The mean value is 475.06 per 100.000 population. The highest mean value of DALYs stroke among women smoker are also found in ≥ 70 years old group. The mean value is 810.82 per 100.000 population. The lowest mean value of DALYs stroke are also found in <50 years old group. The mean value is 56,63 per 100.000 population.

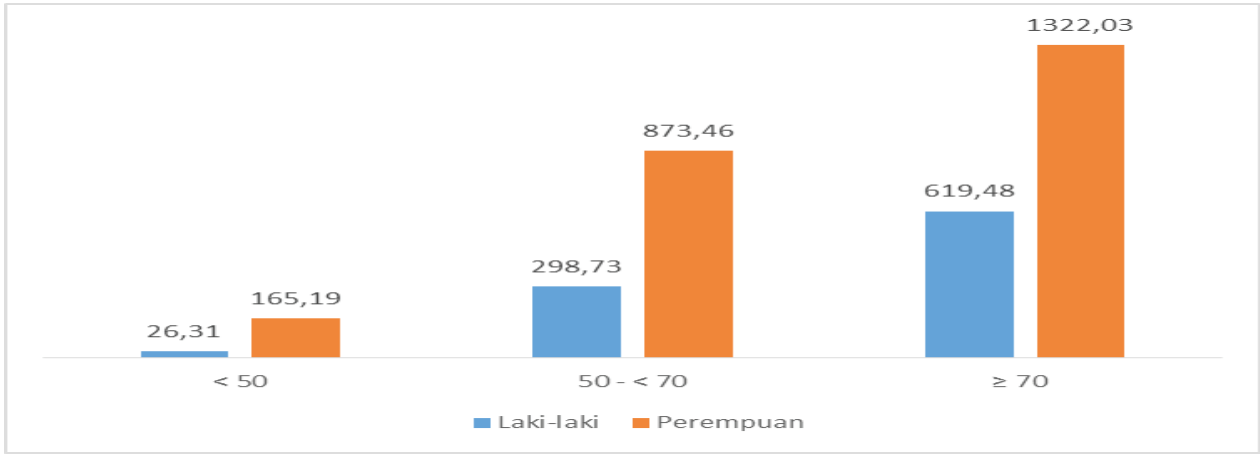
The highest mean value of DALYs stroke among men second hand smoke are found in ≥ 70 years old group. The mean value is 619.48 per 100.000 population. The lowest mean value of DALYs stroke are found in < 50 years old group. The mean value is 26.3 per 100.000 population. The highest mean value of DALYs stroke among women second hand smoke are found in ≥ 70 years old group. The mean value is 1322.03 per 100.000 population. The lowest mean value of DALYs stroke are also found in < 50 years old group. The mean value is 165.19 per 100.000 population.

Graphic 1 indicates comparison of DALYs value due to stroke as smoker at age ≥ 70 indicate that men have 7,5 folds higher than women. At <50 years old group indicates that men have 8,4 folds higher than women as shown in following table.



Graphic 1. Score of DALYs mean for Stroke disease in Smokers based on Gender and Age in Indonesia year 2017

The graphic 2 is shown the comparison of Stroke DALYs value of second hand smoke in ≥ 70 years old age group indicate that women have 2.13 folds higher than men. At <50 years old group indicates that women have 6,4 folds higher than men as shown in following table.



Graphic 2. DALYs mean score of Stroke on Second Hand Smoker based on gender and Age in Indonesia year 2018

Discussion

Cigarette problem along economy and health continuum has not been solved yet. Cigarette industry doesn't only support economy revenue but also create employment [3,4]

To sustain and increase the smoker number, industry promotes advertisement in printed and electronic media that depict a smoker man as luxurious, powerful, strong and rich. The advertisements were dominated by men, however smoking among women show increase. The ban of cigarette add that involves under 18 years old group has been passed however smoking among children aged 10 years old up in Indonesia keep increasing and according to Essential Health Research in 2018, there was 24,3%[9]. Meanwhile, to decrease smoker number, government educates public through add simultaneously with the cigarette adds where every commercial adds was concluded by health message. Health Ministry also promotes in every cigarette package about the danger of smoking for health both for men and women and also for the environment [10]

Result of GDR research proved that many people in Indonesia were still smoking both men and women. In South Sulwesi province, cigarette has become necessity for men hence the budget for smoking constitutes 3 biggest spending in household in addition to meal (rice and cassava) and protein (fish and egg). Each day they can smoke 10 to 20 cigarettes in average [11]. Indonesian smokers consume average 12,8 cigarettes. In global, smokers can consume 5 – 20 cigarettes a day (*cigarettes per day* atau CPD)[12.]

Globally smoking men consumes 49% or higher than women who consume 11% and the number increase annually(12). In Indonesia, men smokers were scored 47,5% or higher than women with only 1,1% and the result of Riskesdas (Essential Health Research) in 2018 stated that men smokers were scored 47,3% or higher than women with only 1.2%(9). Men smokers according to survey result were scored 73,7% as smoking fathers whereas smoking mothers were only 0,5%. Health consequence from smoking father in household constitutes significant influencing factor with p = 0,0001 on the low body weight in children (CM Best) and significantly correlates to stress at work (p = 0,004) and musculoskeletal problem (p=0,023).

Stroke case in Indonesia according to Riskesdas result in 2018 were 10,9% and 11% occurred in men and 10,9% in women. One of causing factor in stroke based on this research was due to smoking cigarette exposure(12). Stroke is non infectious disease which can result in disability and mortality or DALYs[6,13]. Globally there are 25,7 millions people with stroke, 6,5 millions death related to stroke and 113 millions people living with stroke related disability. Stroke is known as acute cerebrovascular incidence, it closely relates to brain tissue injury as main cause of mortality and disability in the world [14,15] .

Gender is important part in GDR research as basic data to calculate the burden of stroke due to smoking, since in addition to ethic group, gender is risk factor for stroke incidence (Martin, 2016). The result showed that there is significant difference (P =0,004 < α =5%) among gender on DALYs of smoking related stroke. Relative risk to have stroke in men and women who smoke 1 cigarette a day was men would have 1.25 to 1.38 fold to have stroke compared with non smokers. In Indonesia, DALYs of smoking related stroke in men were found highest in West Nusa Tenggara, Southeast Sulawesi, Papua and lowest scores were found in North Kalimantan, Jakarta, Gorontalo[12].

Smoking women have stroke risk 1,31 to 1,52 fold compared with non smoking women. In Indonesia, DALYs of smoking related stroke in women were found highest in Papua, West Kalimantan, Southeast Sulawesi, Jambi, and lowest score were found in North Kalimantan, East Java, Yogyakarta, North Sumatra, East Nusa Tenggara.Men and women who smoke 5 cigarette a day have relative risk (RR) to have stroke 1,30-1,42 fold (men) and women have 1,44-1,48 fold compared with non smokers. Men and women who smoke 20 cigarettes a day have relative risk to have stroke 1,64-1,82 fold and in men and women were 2,16 - 2,75 fold[12]. Based on gender, globally there is DALYs difference due to stroke where highest score found in Bulgaria, Macedonia, Georgia, China, and North Korea (11% –12%)[7].

Age becomes important part in GDR research to analyze burden of smoking related stroke since one of 10 factors to cause stroke in the world is age[6] . Data collection of respondent age is important to identify the age for first time smoking and the age of first time having

stroke. Youngest smoker age in Indonesia was 10 – 14 years old accounting for 0.7% and highest 45 – 49 years old or 29,6%. Analysis result in this research indicates significant difference (P =0,000< α =5%) among age group in stroke case. Result from other research also indicated the difference of age factor on stroke risk and mortality. Stroke case in Indonesia is found highest in 75 years old up group constituting 50,2% and globally age of 70 years old up causes increased stroke risk and mortality compared with younger age[16] .

Research in 32 countries indicated that there is more significant relationship (p<0□0001) between smoke exposed, heart disease, diabetes mellitus and stroke than hypertension[6]. Unhealthy life style like smoking, according to the result causes increased heart attack and stroke incidences[17]. Despite fact that stroke causes mortality and disability[15], smoking habit has not been decreasing in public even show an increase[12].

Smoking cigarette is influencing factor on stroke case that causes disability and mortality (DALYs) and exposed to smoking becomes mortality causes of 85-90% in chronic lung disease while seond hand smoking in women causes higher DALY of stroke than in men[18]. Non smokers who are frequently exposed to smoking in the room is called second hand smoker[18] and they are vulnerable group to have stroke[4,18].

Highest second hand smoke in men in Indonesia is found in East Kalimantan, North Sulawesi, Papua and the lowest is found in North Kalimantan, South Sulawesi, Gorontalo. Meanwhile in women total DALYs mean score for stroke per 100.000 population were found highest in North Maluku, Maluku, West Papua and lowest score were found in North Kalimantan, West Java and North Sumatra.. based on result of stroke DALYs calculation, it is stated that second hand smokers is one risk factor that can cause stroke DALYs to increase[19]. DALYs of stroke due to second hand smoker were found highest in women aged ≥ 70 years old of 1322.03 per 100.000 population. Research in New Zealand found that second hand smokers exposed to smoking in workplace caused 100 mortalities which could be avoided per year from lung cancer, coronary heart disease and stroke in collective[5]

Second hand smoker statistically showed significant correlation as risk factor to have stroke DALYs[19]. This research indicated significant difference on stroke DALYs score between active smoker group and second hand smoker group with P =0,000< α =5%. Smoking is main risk factor in survey on disease burden. It caused five millions of mortality (8,8%) in world population and it also caused 4,1% lived year to loss due to disability in the world [2]. Descriptive statistic analysis indicate that the highest DALYs stroke both as active smokers and second hand is found in ≥ 70 years old group.

DALYs score in men tend to be higher than in women since men who smoke cigarette were 47,5% more than women with only 1,1%[1]. Other research indicated that smoker men have 7.3 fold higher to have stroke risk than non smoking men. Women who expose to smoking cigarette have scored only 1.2 fold higher to have stroke risk than non smoking women[5]. Duration of smoking cigarette over 20 year will have higher risk to have cardiovascular problem than those who have been smoking in less 20 years duration [20].

Conclusion

The effect of cigarette exposure can cause an increase in stroke DALYs. Statistically, there are significant differences between sex, age groups and between smokers and second hand smokers. The highest DALYs stroke of smokers was found in men aged ≥ 70 years, with the ratio of DALYs strokes of men 7.5 times greater than the DALYS stroke value of women.

The highest DALYs stroke on second hand smoke was found in women aged ≥ 70 years, with a female ratio of 6.3 times greater DALYS stroke compared with men.

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Depression and Suicidal Ideation as a Consequence of Academic Stress among Adolescent Students

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Abstract

Many factors contribute to the stress being experienced by students but one common cause of academic stress in India is School. Such stress comes from too much homework, unsatisfactory academic performance, preparation for tests, lack of interest in a particular subject, and teacher's punishment. Stress from high expectations of teachers, parents, and self is usually an agony for students studying in schools[1].

Objectives: The study was conducted (i) to assess the level of academic stress and its consequences of academic stress among adolescent students (ii) to find the association between depression and demographic characteristics of adolescent students and (iii) to find out the correlation between academic stress and its consequence among adolescent students.

Material and Methods: 1204 adolescent students studying in schools of Belagavi city were selected by using stratified cluster sampling technique and were assessed for academic stress by using ESSA scale. The adolescents were assed for depression and suicidal ideation by using Kutchers depression scale appropriate statistical methods were used to interpret the data. **Results:** The results shown that the mean of the sample on the total academic stress score was 49.38 (SD=13.00) and 273 (i.e.22.67%) reported high level of academic stress. The depression assessment shown that 381 students (i.e. 31.64%), 625 (i.e. 51.91%) and 198 (i.e. 16.45%) have mild, moderate and severe levels of depression respectfully and the scores have shown that among N=1204 students 1021 students (i.e. 84.80%) have responded No and 183 students (i.e. 15.20%) have responded Yes for the suicidal ideation. **Conclusion:** The academic stress among students causes depression and suicidal ideation among adolescent and knowing and handling this stress may prevent adolescents from having difficult situations.

Key words: *Academic stress, Depression, Suicidal ideation, Adolescent students and consequences.*

Introduction

Academic stress is mental distress with respect to some anticipated frustration associated with academic failure or even unawareness to the possibility of such failure. Students have to face many academic demands for example - school examination, answering questions in the class, showing progress in school subjects, understanding what the teacher is teaching, competing with other class mates, fulfilling teachers and parents academic expectations. These demands may tax or exceed available resources of the students. As a consequence, they can be under stress and depression [2].

Academic concerns pervade the lives of students, interrupting aspects of their daily routine. Students are pressured to perform, pressured to fit in, pressured to commit, and pressured to live up to the expectations of others. They even set goals for themselves that are sometimes impossible to achieve [3].

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Most teenagers respond to stressful events in their lives by doing something relaxing, trying positive and self-reliant problem-solving or seeking friendship and support from others. But for others, the events pile up and the stressors are too great. In the Minnesota study teens who reported that they had made a suicide attempt had five additional "bad" events on their list: parents' divorce, loss of a close friend, and change to a new school, failing grades and personal illness or injury. It is significant that the young people who showed high degrees of depression and who had made suicide attempts reported over five of these "bad" events in the past six months, more than twice as many as the rest of the group.[4]

For ages, it was a popular notion that children do not suffer from depression. Teenagers with depression were more often than not labelled as being just "difficult" or "moody". It is only recently that the world is awakening to the fact that up to 20% of world's children and adolescents suffer from disabling mental illnesses including depression. WHO has included depression as one of the priority mental disorders in childhood and adolescents[5]. It is seen from Banerjee's report that every year about 25,000 students in the age group of 16 to 20 years commit suicide in the examination month (i.e. March to June). They are really worried about their future and desired results in the examinations[6].

Since the family, school and peer group form the major socializing influences on adolescents, the expectations or demands they make may convert into stressors. Hence, it is important to analyze the determinants that resulted in academic stress.

Thus identifying the determinants of academic stress and addressing the consequences like substance abuse, depression and suicidal ideation among school going adolescents in school setting may help students alleviate their experience of academic related stress and have less stressful and possibly more fulfilling school carrier.

Methodology

This cross sectional study involves the school going adolescent boys and girls of 13 to 19 years of age from rural and urban areas of Belagavi. The data was collected with the permission of district education office where the list of schools were obtained and then according to sampling criteria 12 schools were selected from north, south, east and west zones of Belagavi city and the head of the institutes of selected schools were approached for the permission to conduct the studies. The students were informed one day prior to the study and their ascent was obtained for the participation I the study.

The sample size was determined by considering all the variables like Academic stress, Depression, Substance abuse and suicidal ideation among adolescent students and among which the highest sample size was considered for the study the calculated sample size was 1160 and 1204 students were undertaken for the final study.

Sampling technique: Stratified Cluster Sampling

Schools of Belagavi city will be classified in to East, West, North and South zones, from each group cluster schools will be selected by using simple random method and the age group of 13 to 19 years adolescents will be selected from class VIII, IX, X, XI, and XII. The required numbers of Samples will be drawn using simple random with proportion to school strength. "Educational Stress Scale for Adolescents" was used to estimate the level of perceived academic stress. In present study as per the previous literatures reviewed depression and suicidal ideation were considered as consequences of academic stress and they were assessed by using The Kutcher Adolescent Depression Scale (KADS).

Statistical Analysis

To assess the relative influence of factors on educational stress, all variables were included in multiple logistic regression models to assess the relationship between the academic stress scores with its components and consequences i.e. substance use, depression and suicidal ideation. Stepwise linear regression was used to assess the influence of variables on academic stress levels of the students. Data were analysed by using the statistic software 20.00version. The statistical significance was set at 5%, level of significance ($p<0.05$).

Results

Table 1: Levels of academic stress of adolescents (n =1204)

Levels of academic stress	No of adolescents	No of adolescents
Low level	286	23.75
Average level	645	53.57
High level	273	22.67
Total	1204	100.00

One of the main objectives of the study was to assess the level of academic stress among adolescents of age group 13-19. The total no.of participants who reported the questionnaire i.e. Educational stress scale for adolescents (ESSA) where N=1204. The mean of the sample on the total academic stress score was 49.38 (SD=13.00).The table revealed that 286 (i.e. 23.75%), 645 (i.e. 53.57%) & 273 (i.e.22.67%) reported low level, average level & High level of academic stress respectively. [<Q1= Low, Q1-Q3 = Average and >Q3 = High where Q1 was considered as 44 & Q3 was 58].

Findings related consequences of academic stress among adolescent students

Table 2: Levels of depression among adolescent students (n =1204)

Levels of depression	No of adolescents	No of adolescents
Mild depression	381	31.64
Moderate depression	625	51.91
Severe depression	198	16.45
Total	1204	100.00

The depression scores have been assessed by using Kutcher's depression scale for adolescents. The results shown that 381 students (i.e. 31.64%), 625 (i.e. 51.91%) and 198 (i.e. 16.45%) have mild, moderate and severe levels of depression respectfully.

Table 3: Levels of suicidal ideation of adolescents

Levels of suicidal ideation	No of adolescents	No of adolescents
No	1021	84.80
Yes	183	15.20
Total	1204	100.00

Mean-0.25 & SD 0.67

One item was added along with depression questionnaires separately to assess the suicidal ideation among the adolescent students, the scores have shown that among N=1204 students 1021 students (i.e. 84.80%) have responded No and 183 students (i.e. 15.20%) have responded Yes for the suicidal ideation with Mean score of 0.25 & SD 0.67.

Findings related to correlation between academic stress scores and its consequences

Table 4: Correlation between academic stress and its components with depression scores by Karl Pearson's correlation method

Variables	Correlation between depression scores with			
	n	r-value	t-value	p-value
Total academic stress	1204	0.3514	13.0117	0.0001*
Pressure from study stress	1204	0.2824	10.2045	0.0001*
study work load stress	1204	0.1698	5.9728	0.0001*
Worry about grades stress	1204	0.2091	7.4138	0.0001*
Self exp stress	1204	0.3108	11.3379	0.0001*
Study despondency stress	1204	0.3333	12.2570	0.0001*
Suicidal ideation	1204	0.3929	14.8123	0.0001*

*p<0.05

The table 4 represents correlation between depression scores with academic stress and its components. The table clearly shows that a significant and positive correlation was observed between depression scores and total scores of academic stress of adolescents ($r= 0.3514$, $p<0.05$) at 5% level of significance. It means that depression scores increases (decreases) with increase (decrease) in academic stress scores. In other words the depression and academic stress scores are depending on each other.

A significant and positive correlation was observed between depression scores and Pressure from study a component of academic stress of adolescents ($r=0.2824$, $p<0.05$) at 5% level of significance. It means that depression scores increases (decreases) with increase (decrease) in Pressure from study. In other words the depression and Pressure from study a component of academic stress scores are depending on each other.

A significant and positive correlation was observed between depression scores and study work load stress a component of academic stress of adolescents ($r=0.1698$, $p<0.05$) at 5% level of significance. It means that depression scores increases (decreases) with increase (decrease) in study work load stress. In other words the depression and study work load stress a component of academic stress scores are depending on each other.

Similarly A significant and positive correlation was observed between depression scores other components of academic stress i.e. Worry about grades stress ($r=0.2091$, $p<0.05$), Self exp stress ($r=0.3108$, $p<0.05$), Study despondency stress ($r=0.3333$, $p<0.05$) and suicidal ideation scores ($r=0.3929$, $p<0.05$) of adolescents at 5% level of significance.

Interpretation and conclusion

This study was conducted to assess the academic stress and its consequences among adolescent students of 13 to 19 years of age group. Very few studies have been conducted on this area and in present study it was assessed that the depression and the suicidal ideation are the consequences of academic stress among the students. The findings of the study suggested that academic stress is directly proportional to depression and suicide among adolescent students hence the proper management of academic stress can prevent such consequences among adolescent students. The stress management programmes are very useful and also the findings can be utilized by policy makers to make changes in the educational system in India.

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Epidemiological Pattern of Head Injuries in Road Traffic Accident Victims Presenting to a Tertiary Care Hospital in South India

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Abstract

Introduction: In 2017, Karnataka registered 10,609 deaths due to Road Traffic Accidents (RTAs). Among all injuries, head and especially brain injuries were responsible for significant mortality and morbidity. RTAs account for a significant proportion of brain injuries. **Materials and Methods**: Of 1035 cases of head injuries visiting the trauma center of a tertiary care teaching hospital in south India, 650 head injuries were attributable to RTAs during the period between August 1, 2017 to July 31, 2018. Retrospective data was obtained from patient records taken from the medical records. After obtaining ethical approval, the records were analyzed in terms of age group variables, sex, time, day, season, accident event, means of transport, the practice of protective devices usage such as a helmet for 2 wheeler RTA victims and safety belt for 4 wheeler RTA victims, insurance status, type of head injury: external or internal and diagnosis**. Results**: The incidence of RTAs was higher in males (77.3%), younger age group: 20 to 30 years (30.15%), drivers (48.31%), rainy season (40%), afternoon hours (38%) and weekdays (69.23%). Usage of protective devices was low (13.54%).

Key words: *Epidemiological Pattern, Head Injuries, Road Traffic Accident, RTA, South India.*

Background

1,50,000 fatalities and 5,00,000 injuries were reported in 2016.[1,2,3] More than 87% of these deaths are in the working age group.[4] India sees 400 deaths due to RTAs every day. Uttar Pradesh accounts for the largest number of fatal accidents among the states. Karnataka ranked 4th among all the states of India for RTAs in 2017 with 10,609 deaths.[5] Among all types of injuries, especially the head and brain are chief cause of debility and loss of lives.[6] 50 percent of brain injuries are from RTAs. It is expected that traumatic brain injuries will account for the highest number of deaths and physical disabilities by 2020.[7,8,9,10,11]

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Materials and Methods

The study was designed to evaluate the epidemiological pattern of injuries of the head on patients traumatized by road accidents attending a hospital in a tertiary care teaching hospital in south India. The data collection period was from 10 October 2018 to 1 May 2019. Retrospective data was collected from all patient records for the one-year period, which includes all records of victims with serious injuries who visited the

hospital between 1 August 2017 and 31 July 2018.

Exclusion criteria were incomplete medical records, dead on arrival and discharge against medical advice patients. 650 patient records were analyzed for age, sex, time, day, mode of transport, usage of protective devices such as helmets for 2 wheeler users and seat belts in 4-wheelers. Statistical analysis was done with version 7.2 of Epi Info. The qualitative variables were expressed as a percentage. The quantitative variables were expressed in terms of average or median and standard deviation or categorized and expressed as a percentage. To test the difference between two media in the present study, Student's t-test was used. The significance level was set at 0.05.

Results

Out of over-all 1035 cases of head injuries, after applying the inclusion and exclusion criteria, 650 head injuries were analyzed. Of the 650 cases, women were 22.62% and men 77.38% with a ratio of 1: 3.48. This indicates that men are more vulnerable to RTA head injuries. Figure 1 shows that the average age of the subjects was 37.65 ± 16.30 years, with a maximum of 80 years and a minimum of 18 years.

69.23% of patients reported to the hospital on a weekday. The maximum number of head injury causing RTAs occurred in the rainy season (40%), followed by the winter season (35.85%) and the summer season (24.15%). 48.31% were drivers, 36% were passengers and 15.54% were pedestrians. 2 wheelers accounted for 65.38% followed by 4 wheelers 14.46%. 4.92% of subjects reported being under the influence of alcohol. Only 13.54% of subjects were using a helmet or seat belts at the time of the accident. 58.92% were minor head injuries, 19.85% were moderate head injuries and 21.23% were severe head injuries.

Laceration (60.46%), wounds to the head in 14.92% and abrasions in 10.15%. CT scan findings showed Sub Dural Hemorrhage in 16.46%, Sub Arachnoid Hemorrhage in 7.69%, bruising in 9.23%, 3.54% had widespread axonal damage and 9.08% had Epidural Hemorrhage. 73.08% were treated on an inpatient basis and 26.92 % were treated on an outpatient basis. 43.54% of patients visited the hospital directly while 56.46% were referrals from other hospitals. 90.15% of patients were discharged alive. 423 (65.08%) of the RTA patients had no insurance coverage and 227 (34.92%) had insurance coverage. The Median out of pocket expenditure (OOP) for those with insurance was 81,142.67 INR. Median OOP for those without insurance was 77,569.34 INR. P value is 0.5067: the difference is not statistically significant.

450 (69.23%) of the RTA patients arrived at the hospital on weekdays. 200 (30.77%) arrived on weekends. The season wise distribution of RTAs was 157 (24.15%) in the Summer season (Feb to May); 260 (40%) in the rainy season (June to September) and 233 (35.85%) in the Winter season (October to January). At the time of the RTA, 314 (48.31%) were drivers, 234 (36%) were passengers and 101 (15.54%) were pedestrians. 425 (65.38%) of the RTA victims were on 2 wheelers, 26 (4%) were on 3 wheelers, 94 (14.46%) were on 4 wheelers, 4 (0.62%) were on a cycle and 100 (15.38%) were pedestrians. 88 patients (13.54%) were using a protective device at the time of the RTA. 561 (86.31%) were not. 383 (58.92%) of the head injury cases were classified as mild, 129 (19.85%) were classified as moderate and 138 (21.23%) were classified as severe.

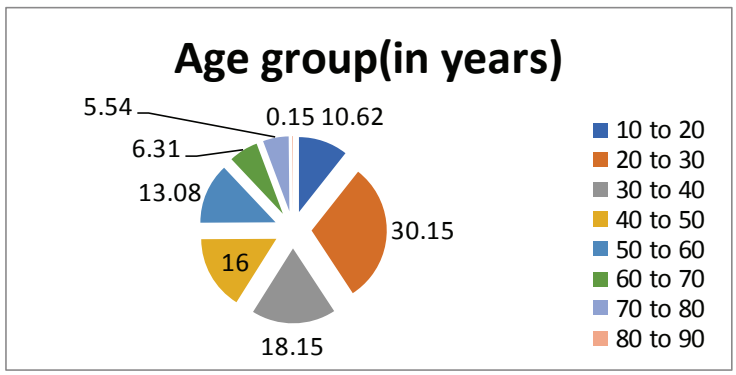


Figure 1. Age distribution of subjects

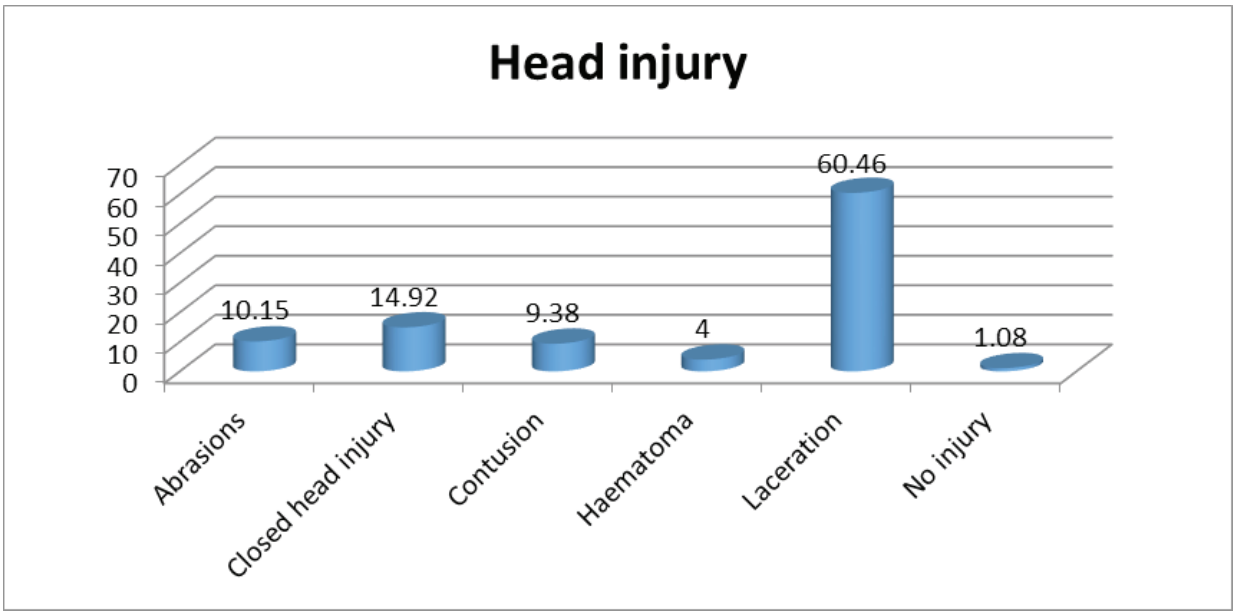


Figure 2. Distribution of types of external head injury

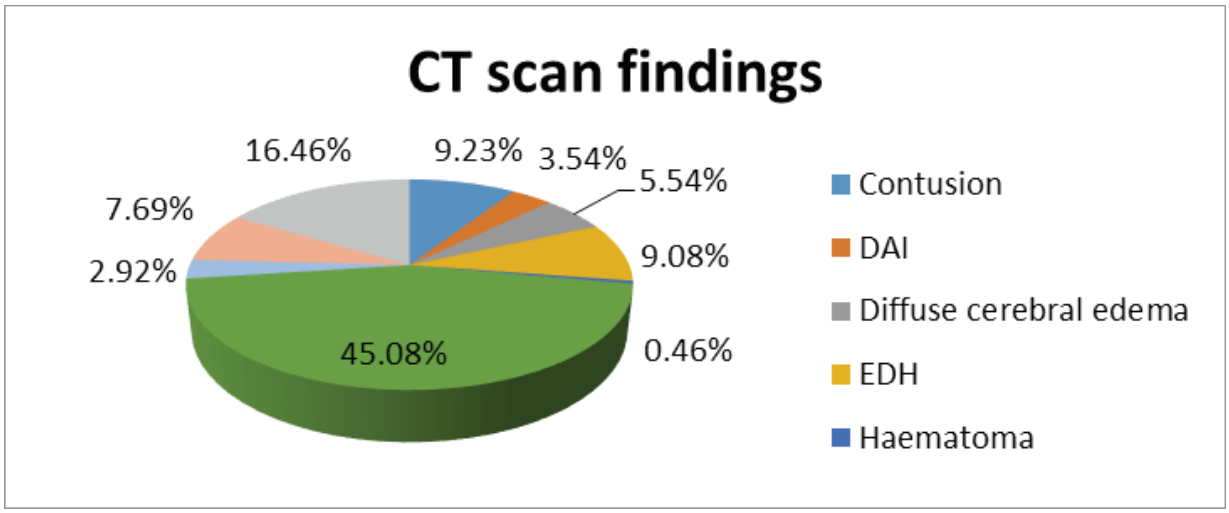


Figure 3. CT scan findings to assess the type of internal head injury

Table 1. Distribution of study subjects based on hospital arrival mode

Hospital arrival mode	Frequency	Percentage
Ambulance	26	4.00
Bystander	20	3.08
Police	2	0.31
Relative	602	92.62
Total	650	100

Table 2. Distribution of study subjects based on the time of arrival.

Time of arrival	Frequency	Percentage
Midnight 12.00 to 5.59 am	56	8.62
6 am to 11.59 am	158	24.31
12 noon to 5.59 pm	189	29.08
6 pm to 11.59 pm	247	38.00
Total	650	100.00

Table 3. Type of skull Fracture

Fracture	Frequency	Percent
Basilo Frontal	1	0.15%
Communited	5	0.77%
Depressed	10	1.54%
Frontal Bone	50	7.69%
Maxilla	3	0.46%
Nasal	4	0.62%
Normal	495	76.15%
Occipital	11	1.69%
Orbital	1	0.15%
Parietal	15	2.31%
Spinous	8	1.23%
Temporal Bone	35	5.38%
Zygomaticomaxillary complex	1	0.15%
Zygoma	11	1.69%
TOTAL	650	100.00%

Discussion

In this study, an overall of 650 retrospective data was analyzed based on the epidemiological model of road accident injuries. In the study of retrospective findings, there were 77.38% of men and 22.62% of women. A female: male ratio of 1: 3.42 observed in cases of maximum incidents. In this study, the maximum, ie 30.15% of the cases were in the age -group of 21-30 years, then by 18.15% of the age group of 31-40 years. The high sex ratio found can be documented to the fact that the percentage of men compared to women is higher in cases of injuries of the head, in road accidents. In India, men are bread generators for the family and, therefore, participate in outdoor activities exposing themselves to the risk of accidents. These results were similar to other studies.[12,13,14] The WHO World Report on Road Safety[15] showed that injuries due to road accidents were the major cause of lives lost amongst young aged 15 to 29 years. Jha S et al[16] noted that there was a maximum number of lives lost in the age group of 21-30 years with a ratio of women to men were 1:3.53. It was witnessed that the majority of cases of traffic accidents on the road, most of the patients were drivers (48.31%) followed by the passenger by category and about 15.54% were pedestrians. These results are like some other studies.[17,18]

This study discovered that only 4.9% of patients had history of alcohol consumption while driving. Drunken driving is a significant cause of RTAs.[19,20,21]. Government data puts the percentage RTAs and RTA deaths because of alcohol/drug use at 5.6 and 6.4% respectively. 48.31% of RTA patients were drivers (48.31%); 15.54% were pedestrians. 2 wheeler riders accounted for 65.38% of all RTAs, followed by 4 wheeler occupants (14.46%), which is similar to previous findings.[24,2526] The "WHO global report on road safety" (2009)[20] says pedestrians, cyclists, motorcyclists and their passengers accounted for 46% of life lost in world traffic. In the present study, 86.31% of RTA victims did not use protective devices (helmet for two wheelers and safety belts for 4 wheelers), similar to findings in other studies.[27,28,29] In the present study, 73.08% patients were treated on an inpatient basis and 26.92% of patients were treated on an outpatient basis. 56.46% of patients were referrals. 43.54% were direct admissions.

In this study, laceration (60.46%) was followed by closed head injuries (14.92%) (including internal fracture and internal bleeding), abrasions (10.15%), bruises (9.38%) and hematoma (4 %) respectively. Internal head injuries: SDH (16.46%), HSA (7.69%), bruises (9.23%), diffuse axonal lesion (3.54%), EDH (9.08%).

Frontal bone fracture (7.69%), temporal bone fracture (5.38%) and parietal bone fractures (2.31%). The results of detailed head injuries are present in very few studies, especially in living patients.[29,30] 58.92% were minor injuries, 21.23% were serious injuries and 19.85% were moderate injuries. 90.15% of patients survived. 9.85% of patients died.

40.62% paid between 10,000 to 50,000 INR; 17.85% paid between 0.00-5,000 INR, 16.46% paid between 50,000-1 lakh INR, 13.54% between 5,000-10,000 INR and 11.54% over 1 lakh INR. 34.92% were covered by insurance. 65.08% had no insurance coverage.

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Clinical, Laboratory and Radiologic Evaluations in Patients with Malignant Tuberculous Spondylitis

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Abstract

Background: Tuberculous spondylitis patient diagnosis still becomes a challenging task in orthopedics. Mycobacterium tuberculosis Beijing strain caused malignant tuberculous spondylitis in most patients (66.7%). Therefore, they proposed scoring system to estimate the prognosis of patients with malignant tuberculous spondylitis with some parameters, including abscess area, erythrocyte sedimentation rate (ESR), number of vertebral destruction, disseminated tuberculosis and infectious Mycobacterium tuberculosis stain.

Objectives: To perform clinical, laboratory and radiologic evaluations in patients with malignant tuberculous spondylitis using scoring system proposed by Yunus and Prijambodo

Methods: This retrospective cohort study was carried out from 2012 to 2015 in patients with malignant tuberculous spondylitis at Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, using Yunus and Prijambodo scoring. Subjects were evaluated in minimum 6 months after surgery. The evaluations included clinical (abscess recurrence), laboratory (ESR) and radiologic (bony fusion in spinal X-ray tomography).

Results: Of eight acute tuberculous patients, only 5 were evaluated. One had moderate prognosis and four had severe prognosis. No abscess recurrence found in patients after 20-month evaluation. ESR was below 20 mm/hour (12.8±7.8) and 80% of patients had bony fusion in spinal X-ray tomography.

Conclusion: Most patients with malignant tuberculous spondylitis with moderate and severe prognosis had better outcomes after 20-month evaluation. Further studies should consider other factors affecting tuberculous spondylitis patient's prognosis including host factor.

Keywords: *malignant tuberculous spondylitis, postoperative evaluation, Yunus and Prijambodo scoring*

Introduction

Mycobacterium tuberculosis commonly causes granulomatous spinal infections. Tuberculosis incidences in developed countries decreased over the past 30 years, with 10% bone and joint involvements and half of which were spinal tuberculosis. Tuberculous spondylitis incidences in Indonesia are still high. 98 tuberculous spondylitis patients were treated at Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, from 1 January 2008 to 31 December 2012 [1].

Tuberculous spondylitis diagnosis still becomes a challenging task in orthopedics. Previous studies reported that Ziehl-Neelsen stain had 87.5% sensitivity and 100% specificity in diagnosing tuberculous spondylitis, while acridine orange had 100% sensitivity [2]. Nevertheless, these methods require a relatively much bacteria (100,000 bacteria/mL). Diagnosis develops by using polymerase chain reaction (PCR) technique from fine needle aspiration with 83.3% sensitivity and 50% specificity [3]. A study found a correlation between malignant/virulence tuberculous spondylitis by measuring abscess and high tumor necrotizing factor alpha (TNF-α) level.

Nevertheless, there was a correlation between increased TNF-α and erythrocyte sedimentation rate

(ESR) that subsequently helped peripheral areas that could not examine TNF-α level. A study found 54.5% of virulent strain PCR positive gyrB gene with 18.2% polydrug-resistance and 9.1% multidrug-resistance, with PCR validation had 83% sensitivity and 80% specificity in diagnosing tuberculous spondylitis. In diagnosing tuberculosis, direct methods, including PCR, require specimens of infected area that are often difficult to obtain or too invasive. Some researchers have tried to detect mycobacterium tuberculosis' DNA in patient's peripheral blood. Studies on PCR with peripheral blood sample are beneficial for tuberculosis diagnosis [4].

Masuda et al., in their study in 104 pulmonary tuberculosis patients, found 35.4% of them were positive for acid bacillus testing, 62.5% were positive for culture testing and 14.5% were positive for PCR of peripheral blood. Mycobacterium tuberculosis bacterial virulence is obtained by evaluating its morbidity and mortality [5]. One of the causes of treatment failure in tuberculosis is the presence of Beijing strain that inhibits therapy effectivity. Beijing strain in 33% of Indonesian patients. In experimental animal model, Beijing strain shows high virulence level, severe tissue damage and lower survival rate. Tuberculous spondylitis is clinically divided into two: malignant/virulence tuberculous spondylitis and non-malignant/virulence tuberculous spondylitis. Malignant/virulence tuberculous spondylitis causes severe and multiple damages, skip lesion, higher ESR and abscess [1].

Malignant/virulence tuberculous spondylitis was caused by Mycobacterium tuberculosis Beijing strain as much as 66.7%. Tuberculous spondylitis with large abscess is dominated by Beijing strain as much as 80%, with abscess size 300-1000 mm. Tuberculous spondylitis with ESR 50-100 mm is found in Beijing strain as much as 71.4%. Tuberculous spondylitis with 2-level vertebral destruction is caused by Beijing strain as much as 75% and 60% with multilevel damage and all malignant/ virulence tuberculous spondylitis with disseminated tuberculosis caused by Mycobacterium tuberculosis bacteria [6].

Masuda *et.,al* (2016) in their research proposed a classification or scoring system in assessing tuberculous spondylitis with clinical virulence. Parameters measured in the study were: abscess size, vertebral destruction, disseminated tuberculosis/skip lesion that can be evaluated with MRI, high ESR and Mycobacterium tuberculosis strain that can be detected with PCR test [5]. We can estimate prognosis of each tuberculous spondylitis case from those parameters.

Method of Research

This retrospective-cohort study used malignant tuberculous spondylitis patients participated in the study conducted by Eviyanti *et.,al* (2018). All samples were followed up and evaluated for their clinical, laboratory and radiologic outcomes. New malignant tuberculous spondylitis patients diagnosed during the study period were evaluated for their scores and outcomes [1]. Malignant tuberculous spondylitis patients were treated at Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, from April 2013-June 2015. The inclusion criteria were male and female malignant tuberculous spondylitis patients with paravertebral abscess, multiple spinal collapse and/ or disseminated tuberculous spondylitis and ESR below 20 mm/hour.

We evaluated bony fusion in spinal X-ray tomography, ESR and abscess recurrence. Primary data were obtained from clinical, laboratory and radiologic evaluation results in tuberculous spondylitis patients with anti-tuberculosis drugs, former surgery procedures and classified based on scoring system (Table 1). Secondary data (patient identity, medical history and medication history) were obtained from medical records at Dr. Soetomo Teaching Hospital, Surabaya, Indonesia [7]. The data were categorized based on bony fusion in X-ray, ESR and abscess recurrence. The outcome results were compared with the predicted prognosis scores to test the scores' clinical reliability and usefulness. We processed the data using SPSS 20 SPSS, Inc., Chicago, IL.) [8].

Result

Of 8 samples, only 5 samples could be evaluated (Table 2). Three samples were excluded since one of them refused to participate in the study, while two others suffered from loss of control. Table 3 showed subjects' characteristics by sex, age, abscess size, ESR, vertebrae corpus destruction, skip lesion, Mycobacterium tuberculosis strain based on PCR test results, prognosis classification based on clinical, laboratory and radiologic

evaluations minimum 6 months after surgery. The subjects' average age was 25.8±8.32, average abscess size was 1704.40±1979.50 mL and average ESR was 81.00±19.46 mm/hour.

Male subjects were fewer than female subjects (40% vs 60%). Figure 1 showed 40% samples suffered from 2-level vertebrae corpus destruction and 60% had more than vertebrae corpus destruction level 2.

Figure 3 showed that Mycobacterium tuberculosis Beijing strain mostly infected tuberculous spondylitis subjects (60%). No subjects had good prognosis (0%), 20% had moderate prognosis and 80% had bad prognosis (Figure 4). No subjects had abscess recurrence (0%) after being clinically evaluated 20 months after surgery. The subjects' average ESR was 12.80±7.79 mm/hour. Most samples had bony fusion in spinal X-ray tomography (80%) after being radiologically evaluated 20 months after surgery (Figure 6).

Table 1. Yunus & Prijambodo classification in tuberculous spondylitis

Parameters	Value	Score	%	Beijing	%	Non- Beijing
Abscess	<300	1	22.2	1/6	16	1/3
	300-1000	2	55.6	4/6	66	1/3
	>1000	2	22.2	1/6	16	1/3
ESR	<50	0				
	50-100	1	77.78	5/6	84	2/3
	>100	2	22.2	1/6	16	1/3
Destruction	1 level	0				
	2 level	1	33.3	3/6	48	1/3
	>2 level	2	66.7	3/6	48	2/3
Skip lesion	Yes	2	11.1	1/6	16	0/3
	No	0	88.9	5/6	84	3/3
PCR	Beijing	4	66.7	6		6
	Non-Beijing	2	33.3	3		3

Annotation:

<4 : good prognosis

4-6 : moderate prognosis

>6 : bad prognosis

Table 2. Research data

No.	Name	Sex	Age	Abscess size	EMR	Corpus destruction	Skip Lesion	PCR	Yunus & Prijambodo scoring	Evaluation time (months)	Abscess recurrence	EMR evaluation	Bony fusion
1	Veranatul	F	19	253	94	2 vertebra	(-)	NB	5	24	(-)	19	(+)
2	Yurliani	F	22	2843	105	3 vertebra	(-)	NB	8	17	(-)	20	(-)
3	Sani	F	40	4665	69	2 vertebra	(-)	B	8	19	(-)	16	(+)
4	Sogol	M	22	375	81	5 vertebra	(-)	B	10	21	(-)	6	(+)
5	Rodandi	M	26	386	56	6 vertebra	(-)	B	9	19	(-)	3	(+)
6	Sukarni	F	48	816	121	2 vertebra	(-)	B	9	Drop out	Drop out	Drop out	Drop out
7	Sri Wahyuni	F	29	455	55	5 vertebra	(-)	B	9	Drop out	Drop out	Drop out	Drop out
8	Ummi	F	30	372	52	3 vertebra	(-)	NB	7	Drop out	Drop out	Drop out	Drop out

Table 3. Subjects' characteristics

Subjects' characteristics	
Sex	L = 40% P = 60%
Age (year)	25.8±8.32
Abscess size	1704.40±1979.50 mL
ESR	81.00±19.46 mm/hour
Corpus destruction	2 level = 40% > 2 level = 60%
Skip lesion	Yes = 20% No = 80%
PCR	B = 60% NB = 40%
Yunus & Prijambodo scoring	Good prognosis = 0% Moderate prognosis = 20%
Evaluation	Bad promotion = 80% 20.00±2.65 months
Abscess recurrence	Recurrence = 0% No recurrence = 100%
EMR evaluation	12.80±7.79 mm/hour
Bony fusion	Fusion = 80% No fusion = 20%

Discussion

Tuberculous spondylitis patients' morbidity and mortality could be estimated by determining patient's prognosis. Nevertheless, this diagnosis still becomes a challenging task in orthopedics.

Tabassum & Haider (2016) in their research argued that tuberculous spondylitis is clinically divided into two: malignant/virulence tuberculous spondylitis and non-malignant/virulence tuberculous spondylitis [9]. Mycobacterium tuberculosis Beijing strain mostly causes malignant/virulence tuberculous spondylitis (66.7%) that eventually results in severe and multiple damages, skip lesion, high ESR and abscess [10].

Most subjects in this study were females (60%), with age ranged from 19 to 40 (25.8±8.32). The subjects' abscess sizes were varied from 235 mL to 4,665 mL. This abscess is formed from the accumulation of reactive liquefaction and exudation products of leukocytes, caseous material, bone debris and bacillary tubercle. Some patient's symptoms cannot be seen until paravertebral abscess developed. In this stage, patient will have back pain complaint that may be visible and palpable in physical examination. Recurrent infections may wider abscess in the lumbar region as it expands to a new region with lowest pressure that subsequently forms a skin tract below inguinal ligament or gluteal region [11].

The subjects' average ESR were varied from 56 mm/hour to 105 mm/hour (81.00±19.46 mm/hour). ESR examination tends to be simpler and can be performed in the peripheral areas compared to TNF-α examination. Prasetyo and Prijambodo in their study found no correlation between abscess size and TNF-α. Their study also found a correlation between increased TNF-α level and ESR.

The subjects' corpus destructions were varied from 2 to 6 vertebrae corpus, and 60% of which suffered from more than 2 vertebrae corpus destruction. The higher the destruction level accompanied by larger kyphosis angulation, the longer the spondylitis disease experienced by the patient. Bone destruction occurs due to bone lysis that subsequently makes bone to be softer and flattened due to gravitation and thoracolumbar muscle pull. Bone destruction is exacerbated by secondary ischemia as results of thromboembolism, periarteritis and endarteritis [12]. Since gravitational transmission load in the thoracic vertebrae is more located in the anterior half of vertebral body, more compression lesions are found in anterior vertebral body that subsequently causes vertebral body to be flatter than its posterior [13].

In this study, Mycobacterium tuberculosis Beijing strain mostly infected tuberculous spondylitis patients (60%). This strain mostly caused malignant tuberculous spondylitis (66.7%). Beijing strain mostly dominated tuberculous spondylitis with large abscess (80%), with size ranged from 300 to 1,000 mL. This strain also caused tuberculous spondylitis with ESR 50-100 mm/hour (71.4%), 2-level vertebrae destruction (75%) and multilevel destructions (60%). In addition, this strain mostly caused malignant tuberculous spondylitis with disseminated tuberculosis.

Some parameters included in the study were abscess size, ESR, vertebral destruction, disseminated tuberculosis/skip lesion and infectious Mycobacterium tuberculosis. We found no good prognosis (0%), 20% moderate prognosis and 80% bad prognosis. All subjects had no abscess recurrence after being clinically evaluated 20 months after surgery. The subjects' average ESR was 12.80±7.79 mm/hour, and most subjects had bony fusion in spinal X-ray tomography (80%). The results of clinical, laboratory and radiologic evaluations showed most subjects with moderate or bad prognosis had better outcomes after being evaluated 20 months after surgery [14]. Therefore, further studies should consider other factors affecting tuberculous spondylitis patient's prognosis including host factor.

The host factor is patient's immunity to resist Mycobacterium tuberculosis infection. This depends on age, sex, nutrition status toxic factor and comorbidities. As infants and children, both males and females have weak immunity, and will only increase after puberty. Malnutrition (both in children and adults) will decrease resistance to disease [15]. Tobacco smokers and alcoholic are toxic factors that will lower immune system, as well as other corticosteroid or other immunosuppressant. Comorbidities like HIV, diabetes, leukemia also increase tuberculosis risk [16].

Conclusion

20-month clinical evaluation after surgery in acute tuberculous spondylitis patients showed no abscess recurrence and average ESR was 12.80±7.79 mm/hour (all subjects had ESR lower than 20 mm/hour). Most subjects had bone fusion in spinal X-ray tomography (80%).

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Ethical Clearance *:* This study obtained research ethical certificate from The Ethical Committee Dr. Soetomo Teaching Hospital Surabaya, Indonesia.

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International Human Rights Instruments and Indonesian Legal Protection For Persons With Disabilities

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Abstract

Persons with disabilities as Indonesian citizens, in fact have the same position, rights and obligations as other Indonesian citizens. This is guaranteed in the Indonesian Constitution. This study aims to discuss about how the legal protection for persons with disabilities in Indonesia. The juridical normative research method uses a statute approach and conceptual approach, and then the data is processed and analyzed qualitatively. Legal protection for persons with disabilities is the responsibility of the state. Indonesia has ratified the Convention on the Rights of Persons with Disabilities, which was followed up with the revocation of Law no. 4 of 1997 concerning Disabled Persons, then enactment of Law No. 8 of 2016 concerning Persons with Disabilities, which prioritizes the issue of human rights. Persons with disabilities are seen as subjects whose rights must be protected for the sake of their dignity. For the enforcement mechanism, the National Disability Commission was formed based on Presidential Regulation No. 68 of 2020, but the substance is not in accordance with the human rights and independent principles because it is attached to the Ministry of Social Affairs which should be the object of its supervision. Therefore the President must immediately revise it in accordance with the mandate of Law No. 8 of 2016.

Keywords: *Legal Protection, Persons with Disabilities, Indonesia*

Introduction

In Indonesia, the basic rights and freedoms of persons with disabilities are guaranteed by the constitution. Article 28H (2) of the 1945 Constitution states that "Every person has the right to receive facilities and special treatment to obtain equal opportunities and benefits in order to achieve equality and justice". In relation to 'special treatment', vulnerable groups of people are entitled to receive more treatment and protection with regard to their specificity (Article 5 (3) of the Human Rights Law) and every person with a disability has the right to obtain special facilities and treatment (Article 41 (2) of the Human Rights Law). Thus the state has an obligation to protect the rights and dignity of persons with disabilities, because the real responsibility of the state, especially the Government, is to protect, advance, uphold and fulfill human rights (Article 28 I (4) of the 1945 Constitution).

The general condition of persons with disabilities faced in the community is the low level of participation in various sectors, for example education, training, employment, and others. Persons with disabilities are also considered to be still excluded from the social environment, access to facilities, and limited public services[2,3]. Data on persons with disabilities from the 2018 National Socio-Economic Survey consisted of age groups of 2-6 years totaling 33,320,357 people (people with moderate disabilities: 1,150,173 people, people with severe disabilities: 309,784 people); The age group of 7-18 years was 55,708,205 people (people with moderate disabilities: 1,327,688 people, people with severe disabilities: 433,297 people); The age group between 19-59 years is 150,704,645 people (people with moderate disabilities: 15,834,339 people, people with severe disabilities: 2,627,531 people); The age group> 60 years is 24,493,684 people (people with

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moderate disabilities: 12,073,572 people, people with severe disabilities: 3,381,134 people)[4]. Hence, this study aims to discuss about how the legal protection for persons with disabilities in Indonesia.

Research method

The research method used is a normative juridical method[5], using a statute approach and conceptual approach[6]. The data used are secondary data obtained through library research. The data is processed and analyzed qualitatively which is then presented in the form of systematic writing[7].

International Human Rights Instruments of Persons with Disabilities

Universal Declaration of Human Rights (UDHR) is a common standard of the international community which must be maintained continuously through teaching and education to promote respect for the rights of freedom by taking progressive steps at both the national and international levels as universal recognition and effective adherence to people who are in the jurisdiction of a country[8,9]. Human rights conventions impose international obligations on States parties to incorporate international human rights standards into their national legal systems and provide enforcement mechanisms[10,11,12]. One of the new human rights conventions is the Convention on the Rights of Persons with Disabilities or CRPD. In accordance with modern human rights theory, the struggle for new rights is determined by several factors such as organized social movements that normatively convince the Government and the United Nations as the main guardians of human rights[13]. Previous models regarded disability as an individual medical problem so disability is usually described by way of condescending words such as 'dysfunction' or 'disability'. People with disabilities are considered 'objects' of charity, medical care, and dependent social protection[14]. On the contrary, the social model intends to replace this attitude by seeing people with disabilities as 'subjects' and active members of society, who are able to pursue their rights. In this model, responsibility for the difficulties experienced in everyday life by people with disabilities is transferred from the individual to the community and the way it is arranged. Social barriers such as discrimination, education, prejudice, etc. exclude people with disabilities from taking part in social life with the same rights and possibilities as others. The social model has also been criticized, because it is too focused on external obstacles and underestimates personal experiences[15]. Another model has been proposed is the affirmation model, but this is not considered as the opposite of the social model but as a model that develops and complements it[16].

Finally, the CRPD and its Optional Protocol were adopted during the UN General Assembly on December 13, 2006, open for signature on March 30, 2007 and took effect on May 3, 2008. Once international treaties came into force, the legal obligations of the international agreements became operative. In accordance with Article 38 (1) of the Statute of the International Court, treaty becomes a source of international law, especially for parties[10], and legally the parties must obey the treaty in good faith (pacta sunt servanda, Article 26 Vienna Convention on the Law of Treaties). Pacta sunt servanda is the basic principle of contract law that the agreement is binding on the parties and must be implemented in good faith. Good faith is a legal principle that comes from an integral part of pacta sunt servanda[8]. The principle of pacta sunt servanda and good faith are the principle of equal importance (equal importance). Every time a State violates the principle of pacta sunt servanda, it also violates the principle of good faith, for example if a country does not carry out its obligations originating from an agreement means it violates both principles[17].

The implementation of CRPD shows global concern to give serious attention to persons with disabilities. The CRPD aims to promote, protect and guarantee the overall and balanced fulfillment of all human rights and fundamental freedoms of all persons with disabilities and to increase respect for the dignity inherent in them. CRPD refers directly to the values promoted by the social model. The principle of fulfilling the rights of persons with disabilities is expressly stated in Article 3 of the CRPD, namely respect for inherent dignity, individual autonomy, including freedom to make choices, and individual independence; non-discrimination; full and effective participation and participation in society; respect for differences and acceptance of persons with disabilities as part of human diversity and humanity; equality of opportunity; accessibility; equality between men and women; respect for the evolving capacity of children with disabilities of children and respect for

the rights of children with disabilities to maintain their identity.

States parties must guarantee and advance the full realization of all human rights and fundamental freedoms for all persons with disabilities without discrimination in any form. The steps taken include: taking legislative and administrative steps in fulfilling the rights recognized by the CRPD; take policy to change or revoke the provisions of the law (laws), regulations (regulations), customs (customs), and applicable practices that contain elements of discrimination against persons with disabilities; adopt practical policies and programs in the context of protecting and promoting the rights of persons with disabilities; refrain from taking action and / or make discriminatory policies and so on (Article 4 CRPD). The CRPD emphasizes 15 groups of categories of rights for persons with disabilities. These rights are women's rights, children's rights, accessibility to adequate public facilities, right to life, legal rights, free from torture, free from exploitation, the right to citizenship, social rights and mobility, the right to information and expression, personal integrity and family, education and health, decent work and livelihoods, politics, and participation in culture.

National Legal Instruments to the Rights of Persons with Disabilities

Human rights as fundamental rights are universally recognized as rights inherent in humans because of their human nature and nature[18]. The inherent nature of human rights because it cannot be deprived or revoked[19]. Nowak said there are four human rights principles namely universality, indivisibility, interdependent, and interrelated[9]. Smith & van den Anker added equality and non-discrimination[20]. The indivisibility principle means that all human rights are equally important, therefore it is not allowed to exclude certain rights or certain categories of rights from their share[21]. According to Article 1 (1) of the Human Rights Law stated that human rights are a set of rights inherent in the nature and existence of humans as God's creatures and are His gifts that must be respected, upheld and protected by the state, law, government and everyone for the sake of honor and protection of human dignity and. Thus, humans should not be treated arbitrarily because humans have natural rights attached to him. Regarding the rights of persons with disabilities, Article 5 (3) of the Human Rights Law states that every person who belongs to a vulnerable group of people has the right to receive treatment and protection with regard to their specificity. Thus, every person with a disability has the right to obtain facilities and special treatment (Article 41 (2) of the Human Rights Law). This is reiterated in Article 42 of the Human Rights Law that every citizen with physical or mental disability has the right to receive special care, education, training and assistance at the expense of the state to guarantee a decent life in accordance with his human dignity.

In the framework of realizing equal rights and opportunities for persons with disabilities towards a prosperous, independent, and non-discriminatory life, legislation is needed that can guarantee their implementation. In 1997 Law No. 4 of 1997 concerning Disabled Persons, but is no longer in line with the paradigm of the needs of persons with disabilities. The law has been replaced by Law No. 8 of 2016 concerning Persons with Disabilities, where the State guarantees the survival of every citizen, including persons with disabilities who have a legal position and have the same human rights as Indonesian citizens and as an inseparable part of Indonesian citizens and citizens to live forward and develop fairly and dignified[22].

Legal Protection for Persons with Disabilities

Regarding legal protection for persons with disabilities, the Government of Indonesia signed the CRPD on March 30, 2007 in New York and has ratified it through Law No. 19 of 2011 concerning Ratification of the Convention on the Rights of Persons with Disabilities on November 10, 2011. This is a step forward of the Government of Indonesia in order to recognize the importance of protection and fulfillment and special attention for persons with disabilities. Legally according to the principle of pacta sunt servanda, Indonesia is obliged to implement it in good faith because international treaties that have been ratified must be implemented at the national level[23]. This is reinforced by Article 7 (2) of the Human Rights Law that provisions of international law that have been accepted by the Republic of Indonesia concerning human rights become national law.

The CRPD calls on the state parties to immediately accelerate in order to achieve equality between persons

with disabilities and non-disabilities. Equality requires policies that favor the realization of the rights of persons with disabilities, so affirmative action is needed from the government to remove barriers to social interaction so that persons with disabilities can access all public services independently. Law no. 4 of 1997 concerning Disabled Persons, is no longer in accordance with the paradigm of the needs of persons with disabilities so that it is revoked and replaced with Law No. 8 of 2016 concerning Persons with Disabilities, to realize the equal rights and opportunities for persons with disabilities towards a prosperous, independent, and non-discriminatory life[24,25]. In the framework of implementing the respect, protection and fulfillment of the rights of persons with disabilities, a National Disability Commission (KND) was established as an independent non-structural institution (Article 131). The KND has the task of carrying out monitoring, evaluation, and advocacy for the implementation of respect, protection and fulfillment of the rights of persons with disabilities, the results of which are reported to the President (Article 132). KND was formed based on Presidential Regulation No. 68 of 2020 concerning the National Disability Commission.

Conclusions

Legal protection and dignity of persons with disabilities are the responsibility of the state. Indonesia has ratified the Convention on the Rights of Persons with Disabilities, which was followed up with the revocation of Law no. 4 of 1997, then enactment of Law No. 8 of 2016 concerning Persons with Disabilities, which prioritizes the issue of human rights. Persons with disabilities are seen as subjects whose rights must be protected and respected for their dignity. For the enforcement mechanism, KND was formed based on Presidential Regulation No. 68 of 2020. However, the substance is not in accordance with the principle of human rights (independent principle) because it is attached to the Ministry of Social Affairs which should be the object of supervision. Therefore, the President must immediately revise it in accordance with the mandate of Law No. 8 of 2016 and CRPD.

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Goethite Nanofibers as Bio-Fillers in Dental Composite Resin

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Abstract

Composite resins represent an important member of biomaterials family that have been evolving due to the needs in biocompatibility and mechanical properties. Fillers in a variety of forms and types have been evaluated and the impact of fillers to improve the performance of the dental composites have been studied in vast. Various composite resin fillers tested had revealed its own strengths and weaknesses. Bio polymers when they are reinforced with biofillers can produce novel biocomposites. The biocomposites with the biofillers will replace and be a substitute for synthetic fibre-reinforced composites. In recent years, natural bio-fibres have gained attention as suitable reinforcements in polymeric matrices. These bio-fillers possess high specific strength and can be effectively used for dental composites resin. The most commonly occurring natural bio-fibres are the teeth of limpets. The teeth of limpets have a distinctive composite nanostructure which comprises of high-volume fractions of reinforcing goethite nanofibers to provide mechanical integrity. The limpet tooth is effective at resisting failure to abrasion. The goethite nanofibers are expected to be significant for novel dental biomaterials with extreme strength and hardness.

Keywords: *Bio-filler, Nanoparticles, Nano-filler, Dental Composite resin.*

Introduction

Composite resin are tooth-colored restorative materials with synthetic polymers, reinforcing fillers and silane coupling agents. The silane coupling agents helps to bond the reinforcing fillers to the polymer matrix. Each component of the dental composite resin is important for the success of the final restoration. The developments in commercial dental composites have been a focused on the modifications to the filler component[(1)].

Reinforcing fillers are particles added to any materials such as to plastics, concrete etc. They are added to bring out the better properties of the mixed material. Such materials can be in the form of solid, liquid or gas. Most of the materials used in dentistry contain fillers as a part of their composition. Fillers are aimed to improve the chemical and mechanical properties of the dental composite material[(1),(2)]. Combination of individually dispersed nano sized filler particles and nano clusters enhance the aesthetics[(3)].

TRADITIONAL FILLERS:

Initially, crystalline quartz and borosilicate or lithium aluminosilicate glasses were used as fillers. The quartz was ground or milled into particles of sizes ranging 0.1nm to 100nm. Crystalline quartz has an excellent optical match to the polymer resin. However, quartz can be very abrasive to enamel. The glass filler particles and the crystalline quartz filler particles are large in diameter and very hard to the surrounding polymer matrix. The hardness and the larger diameter ensured that as the surface of the composite was abraded, the polymer would wear at a faster rate than the fillers, leaving them raised and exposed from the surface. This makes the surface of the restoration rough and less natural like. Thus, polishability and esthetics were compromised[(1)(2)].

To improve the esthetics, the quartz filler particles where replaced with silica particles. These silicon dioxide particles have a diameter of approximately 0.04µm. The surface area of the silicon dioxide particles is more hence they require considerable amount of monomer to wet their surface. This characteristic of the silicon dioxide filler particle will limit the filler concentration to approximately 35 wt%, which in turn limits the mechanical properties of the material. The silica fillers though they have a good polishability but the

strength and stiffness values are considerably lower than other fillers incorporated composite resin.[1].

To improve the esthetic, optical and mechanical properties of composite resin, nano particles as filler has been added. Nano particles enhance wear resistance and reduce polymerization shrinkage. Fillers with nano sizes have an increased surface area which provides an increase in flexural strength. [2][4].

In spite of developing materials to lower stress and improving the strength in the materials, researchers have also focused on developing materials with bioactive characteristics. The ideal restorative material of the future will not only be able to withstand occlusal loads and develop low polymerization stress but also will have an antibacterial, and remineralizing, properties in addition to being biocompatible[5]

Although modifications have been tried with the resin monomer, most significant modifications in dental composite resin is been with the fillers. The modifications have been in the type, size, and distribution of the inorganic fillers. The effects of size, shape, and size distribution of different fillers. [6]

As many articles have covered the review of fillers in composite resin; this review focuses mainly on the availability and performance of bio-filler`s materials and focus on the goethite nanofibers as fillers.

BIO-FILLERS

A biomaterial by definition is a substance can be used to fix the defects, repair or replacement of defective organ in the body. These materials can be synthetic (synthetic - mineral or organic) or natural.[6]

The development of bio degradable and recyclable materials from renewable resources have drawn attention as they are found to be suitable replacements for synthetic materials. The plant fibers and cellulose based fibers are natural fibers and are the most commonly used bio-fibers. These bio fibers are used for reinforcing various types of polymer matrices[7][8].

Bio-fillers in dental composite resin act as a biomaterial and hence reduce the bacterial penetration into marginal gaps for composite restorations. With the advent of modern technology, various attempts are made to modify these fillers to make them more beneficial for a better material. Strong scientific and collaborative foundations presently exist for further development and improvement of the filler system, which will certainly continue to hold a pivotal role in the dentistry.[3]

The bio-fillers because of their properties such as biodegradability, and high specific strength, have received extensive attention as a replacement for traditional synthetic fillers. Hence, the bio-fillers are used in the fabrication of hybrid composites and bio-composites[8].

The mechanical and the physical properties of bio fillers filled composite resin varied considerably. The variations of the properties depends on the stiffness, physical and chemical composition of the material, type of particulates, and growth environment[9]

BIO-NANO-COMPOSITES

The inorganic fillers are added to the polymer matrix in dental composite resin is to improve the mechanical properties of the composite resin. With the aid of nanotechnology, the dental composite resin fillers have been manufactured in nano scale level. The intrinsic toughening of the composite resin with the bio nano filler prevents the micro crack formation and increases the material toughness.

The bio-fillers in nano scale has been introduced in the composite resin and these composites have been termed as bio-nano-composites. Interest in the use of bionanocomposites has been increased over the past 20 years. As a dental material, bionanocomposites has the ability in mimicking native tissue structure and properties. The bionanocomposites has the ability to endure high biting force and shows a good mechanical flexibility in harsh environment. Similar structure or properties to bionanocomposites are often found in dentin and enamel. Therefore, if the bionanocomposites replaces enamel or dentin, they are expected to act as single entity and stay for a longer period of time. [10]

GOETHITE NANOFIBRES AS BIO-FILLERS:

The inorganic phase of minerals in the biological system from highest animals to plants are made of bio minerals. Although bio minerals composed of calcium predominate, bio minerals of barium, strontium, iron,

and silica are also well known. Bio mineral generally occur as oxides, hydroxides, phosphates, and carbonates in the biological system [11].

This reinforcing bio minerals in many organism approaches nano metre in length, at the orientation is always in one-dimension. Which has been proposed as promoting flaw insensitivity and in turn increases the tensile strength of the bio mineral[11].

The aquatic snail (Limpet) teeth are an example of a biomineral produced biologically that is optimized for strength. Studies have shown that the teeth of limpets have the high aspect ratio mineral nanofibres of goethite with a reinforcing protein matrix[11].

LIMPET-THE AQUATIC SNAIL

The intertidal zones are mostly inhabited by primitive marine mollusks. These marine mollusks are Limpets (Mollusca, Gastropoda) and are characterized by their inverted cone-shaped shells. The limpet radula is a highly mineralized tooth structure. The radula contains more than 100rowsofteeth.The outermost rows of the teeth are actively used. During the growth and maturation of the radula, the radula advance in position to the scraping zone. The scraping zone of radula will be in contact with the substrate while feeding. Once the teeth start to function, they begin to wear down, at an average rate of one tooth row per day. At the same time, a new row of teeth emerges. The radular teeth has a membrane and it is manipulated by various muscles and cartilages which are used to graze or cut food. There exist seven basic types of radula morphology[12].

MINERALIZATION OF RADULA

According to the extent of mineralization the radulae of Limpets can be segmented into 4 stages. The First stage there is no mineralization formation. The organic framework of tooth from the odontoblast is initiated in this stage. In the second stage, mineralization formation occurs. The minerals are deposited at the cusp and the rows of teeth. In the third stage, the mineralization gets completed. The cusps of the radula are solid and firm with full mineral deposition. In the fourth and the final stage the fully grown mineralized teeth move and replace the worn-out teeth. The mineralization of tooth cusps begins in the posterior region of the radula. The limpet teeth are organic structures composed of chitin and proteins rich in tyrosine[13].

The mineralization of limpet teeth consists primarily of mineral nano fibres typically many micrometres in length but only a few tens of nanometers in diameter. These mineral nanofibers occupy a significant volume fraction of approximately 80% in mature teeth. The strength and hardness of the limpet tooth is critically dependent on the strength of the mineral nanofibres within the composite structure[11].

Mineralization of the limpet teeth begins with the formation of elongated crystals of goethite (-FeOOH) crystals. The goethite crystals are closely associated with the organic matrix. Hydrated amorphous silica phase fills the space between the goethite crystals. The pre-formed matrix of limpet teeth consists of organized arrays of densely packed chitin fibers, with no apparent gaps or holes for crystal growth, other than the few nanometers between adjacent fibers. The organization of the goethite crystals along with the amorphous silica plays an important role in determining the fracture and wear characteristics of the functional tooth. The orientations of the goethite crystals are controlled by the chitin fibers in the organic matrix. The orientation of the goethite crystals results in teeth which are self-sharpening and resistant to fracture.

As the radula develops in the radular sac, the iron is secreted into the radula from the superior epithelium. Copper in the limpet radula are involved in the enzymic cross linking of the organic component. Zinc in the radula plays a role in the control of iron oxide crystallization. Calcium in the radula is incorporated in the crystalline phosphatic mineral hydroxyapatite[14][15].

The goethite nanofibers with other minerals are expected to dictate the flaw tolerance. This might be due to their diameters are being below a critical threshold value of the order of tens of nanometres. The tensile strength of limpet teeth can reach values higher than spider silk, considered currently to be the strongest biological material, and only comparable to the strongest commercial carbon fibres.

The radula of Limpet structure highlights the efficiency of biological control in assembling a composite structure of nano fibrous goethite for optimal

strength and hardness. This corresponding structural design features are expected to be significant for novel biomaterials with extreme strength and hardness, such as next-generation dental restorations [11].

Discussion

It is apparent from the previous studies that the current composites have significantly improved clinical performance compared with their predecessors, especially in posterior teeth with respect to strength and hardness to resist crack or fracture. However, Considerable effort are expended in the laboratory to ensure that the new materials handle and perform at and above the level of existing materials in respect to strength for the posterior teeth and superior polishability in the anterior region. Laboratory composite resin will identify the weaknesses, and further refinements in formulations will then take place to maximize performance. There are, further changes awaited in dental composite formulation in the areas of new fillers which produce particles in high yields. These newly developed particles will ideally be very fracture-resistant and only minimally abrasive. In addition, the refractive index of the modified filler has to accurately match to existing as well as newly developed polymers. The continued development of fillers containing biocompatible material will have an enhanced therapeutic effect. [1]

Conclusion

The novel biomineral fillers such as goethite nanofiber present in the radula of the limpet with a nanofiber dimension with extreme strength and hardness present an alternative to the present fillers in the dental composite resin.

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Radiographic Methods as a Adjuvant Guide to Oral Pathologist in Diagnosis of Oral Cancer

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Abstract

The management of oral cancer is a multidisciplinary endeavour, as each patient presents the treating clinicians with a unique set of challenges the management of which impacts on both survival and quality of life. This article focuses on the different radiographic methods used in the diagnosis of oral cancer. Imaging techniques have become an important element in the field of oral oncology. Continued research has led to technical progress in the existing modalities and addition of newer, exiting and highly accurate techniques. Although this is a boon, it has created differences of opinion regarding the ideal choice of imaging techniques. Inappropriate choice of imaging modality can result in delay in diagnosis and treatment thereby inducing more suffering for the patients.

Keywords*: Oral cancer, Radiography, CT scans.*

Introduction

Oral cavity squamous cell cancers form a significant percentage of the cancers seen in India. While clinical examination allows direct visualization, it cannot evaluate deep extension of disease. Cross-sectional imaging has become the cornerstone in the pretreatment evaluation of these cancers and provides accurate information about the extent and depth of disease that can help decide the appropriate management strategy and indicate prognosis. Early cancers are treated with a single modality, either surgery or radiotherapy while advanced cancers are offered a combination of surgery, radiotherapy and chemotherapy. Imaging can decide resectability, help plan the precise extent of resection, and indicate whether organ conservation therapy should be offered. Quality of life issues necessitate preservation of form and function and pretreatment imaging helps plan appropriate reconstruction and counsel patients regarding lifestyle changes.

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WHY DO WE NEED TO RADIOGRAPHS EVEN AFTER HISTOPATHOLOGICAL CONFIRMATION OF ORAL CANCER:

Despite continued research and advances the survival rates have not improved significantly . This is because of the two main reasons. Firstly, the oral cancer lesions are detected only at an advanced stage when they have invaded the deeper structures and metastasized to another location, most likely the lymph nodes of the neck[1]. Secondly, there could be an inappropriate assessment of the lesion in terms of depth and extent, bone invasion, status of lymph node involvement and distant metastasis, thereby leading to inappropriate treatment planning and therapy failure[2,3]. Hence it is important not only to detect the tumors at the earliest but also make an adequate assessment which is critical for appropriate planning of surgical, radiation and chemotherapy treatment[4].

Histopathological examination by biopsy is a useful method to confirm diagnosis and to estimate the malignant potential of tumor. However digital examination needs mastery of skills for consistent accuracy and objectivity,

more so in inaccessible intra oral sites. Accurate lymph node assessment and appropriate staging are also difficult with clinical examination alone[5,6].

APPLICATIONS OF IMAGING IN ORAL CANCERS :

ADVANCES IN THE FIELD OF IMAGING HAVE PROVED TO BE A BOON IN:

i) In finding information regarding location, extent, depth, proximity to anatomical structures and lymph node assessment.

ii) Helps in grading the stages of cancer and determining the treatment modality.

iii) It guides during cancer treatments by focusing treatments on the tumors and thereby minimizing the damage to the adjacent normal tissues.

iv) And post-operatively to determine the treatment response

v) It plays a major role in monitoring cancer recurrence in patients previously treated for cancer[7].

IMAGING MODALITIES ARE AVAILABLE:

Ø RVG (Intraoral radiography)

Ø Conventional OPG (Panoramic radiography)

Ø Nuclear medicine Scintigraphy

Ø Ultrasonograhy (US)

Ø Computed Tomography (CT)

Ø Magnetic Resonance Imaging (MRI)

Ø Positron Emission Tomography (PET)

Ø Positron Emission Tomography/ Computed Tomography (PET/CT)

Ø Single Positron Emission Computed Tomography (SPECT)

IOPA/RVG (Intraoral radiography)

On regular basis of screening the patient, sometimes the complaint of patient can be common pain, swelling or mobility in tooth , in such cases basic IOPA/ RVG can reveal abnormal intraosseous changes which can be alarming. These Imaging can be useful in determining any suspicious abnormalities that might be cancerous.

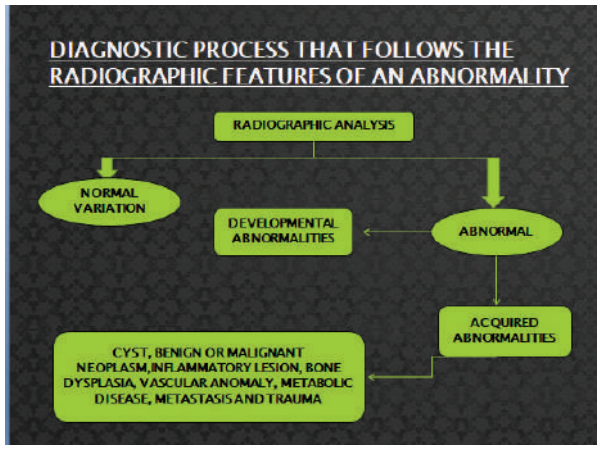


Figure 1 -Diagnostic process that follows the radiographic features of an abnormality

CONVENTIONAL OPG (PANORAMIC RADIOGRAPHY)

Conventional imaging techniques with x-rays are perhaps the most familiar type of imaging. Orthopantomography (OPG) provides a good overall view of the jaws and the dentition[7]. A combination of OPG and intraoral radiography play an important role in the detection of bone invasion especially in determining the supero-inferior extent in carcinoma of the mandibular gingiva. This assessment of the superoinferior extent is one of the most important factors influencing surgeons in choosing between a rim or segmental resection of the mandible. A study comparing OPG combined with intraoral radiography and CT in detecting the supero-inferior extent of tumor invasion of mandible, concluded that there was no difference in the diagnostic accuracy between the two techniques[8]. The sensitivity of OPG was lower than CT, MRI. Hence, it was suggested that decision to resect the mandible as a part of the management of oral cancer should be taken on the evidence of clinical examination, periosteal stripping and at least two imaging techniques that compliment each other in terms of specificity and sensitivity. A combination of OPG and Scintigraphy has high specificity and sensitivity thereby giving accurate results[9].

CT- COMPUTED TOMOGRAPHY

Numerous prospective and retrospective studies

have investigated various imaging methods such as orthopantomogram (OPG), CT scan, Denta scan, MRI, Bone scan and SPECT for assessing mandibular invasion in oral squamous cancers[10,11]. Comparison has also been made with clinical examination and periosteal stripping. Although initially imaging was pronounced inaccurate as compared to clinical examination, subsequent studies conclusively proved the role of imaging in assessing mandibular invasion. CT was found to have the highest specificity (87%) while SPECT and MRI had the highest sensitivity (96-97%)[12]

CT scans of the head/neck/chest are routinely employed in assessment of oral cancer and are excellent in highlight cortical destruction, potential cervical node metastases and pulmonary metastases.

disadvantages with the use of CT. Presence of amalgam fillings and metallic prosthesis in the oral cavity can result in artifacts and obscures the anatomy and pathology in the sections where devices are included. A CT scan is not as useful in cases where soft tissue malignancies are required to be studied[13,14]. Hence a CT is not recommended in cases of carcinomas of tongue and gingiva, instead MRI is preferred.

CT scan is also currently used for radiation therapy planning, with a specific positioning and immobilizing device. It will play an essential role, along with MRI, in the development of conformal radiation therapy[15].

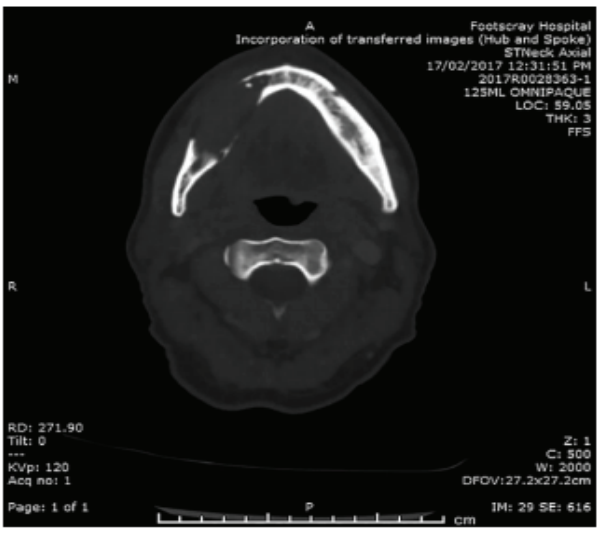


Figure 2-Image of Axial of CT scan-Head

MRI- MAGNETIC RESONANCE IMAGING

MRI of the neck is excellent at evaluating the soft tissue extent of the tumour, extent of marrow infiltration of the mandible or maxilla and assessment of intra or perineural involvement. MRI can assess the presence of marrow edema and hence bone infiltration which cannot be appreciated with CT. MRI can better assess the occult metastasis as the lymph nodes give the same signal intensity as the primary tumor[16]. In cases of salivary gland tumors, MRI can better assess the fine details of the gland. MRI can also accurately depict the T stage, another factor with bearing on prognosis and treatment. Extension to the extrinsic muscles (upstaging disease to T4a), encasement of neurovascular bundle, invasion and base tongue are well seen . These features influence the choice of therapy (single or multiple modality) and the extent of surgical resection that can vary from wide excision to partial glossectomy to total glossectomy (for tumors involving bilateral neurovascular bundles)[17,18].

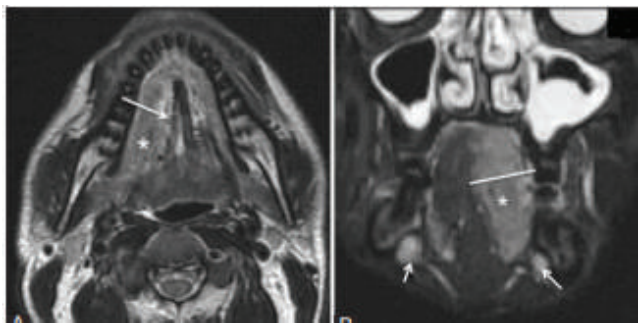


Figure 3-Image of Magnetic resonance imaging of head

ULTRASONOGRAPHY:

Ultrasound uses sound waves with frequencies above those humans can hear. A transducer sends sound waves traveling into the body which are reflected back from organs and tissues, allowing a picture to be made of the internal organs. Ultrasound, used either intraorally for accessible tumours, or more commonly for assessment of cervical nodes can be combined with a fine-needle aspirate for cytologic assessment of suspicious cervical nodes. In head and neck malignancies it is mainly limited to the tumors in salivary glands and detection of lymph nodes metastasis. For lesions in the parotid, submandibular and sublingual glands, ultrasound is an ideal tool for initial assessment[19]. These are relatively superficial structures accessible by high resolution ultrasound, which provides excellent resolution and tissue characterization without a radiation hazard. The main advantages are low cost and easily accessible

technique and can easily guide a fine-needle aspiration[20].

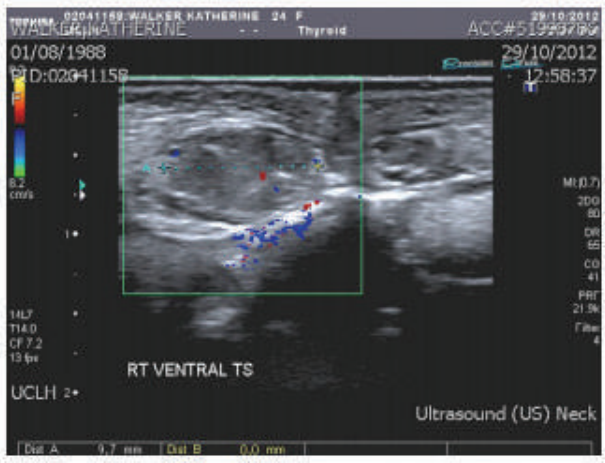


Figure 4 - Image of Ultrasonography of the tongue measuring the dimensions of the tongue tumour

POSITRON EMISSION TOMOGRAPHY/ COMPUTED TOMOGRAPHY (PET/CT):

PET/CT is a physiological imaging for assessment of metabolism within the tissues. It is the fusion of the two techniques of PET and CT. CT helps in localization of the tumor anatomically and PET helps in characterization, thereby providing information physiologically[21]. The main application is in the assessment of patients with cancer using the **glucose analogue 2-[18] fluoro-2-deoxy-Dglucose (FDG).** Cancer cells have increased glucose utilization and FDG PET/CT is used to investigate the increased FDG metabolism of malignant cells compared with non-malignant cells. PET is often combined with an anatomical imaging modality (CT or MR), and is a functional scan, where a radiotracer is administered intravenously to the patient; the tracer is preferentially taken up by cells with a high metabolic rate (a characteristic of many oral cancers). Infection and inflammation however can also provide similar radiologic appearances. It is most often used in advanced (stage 3 or 4 disease, or in salvage/recurrent cases) and the assessment of metastatic disease.

FDG PET/CT plays an increasing central role in the detection and management of head and neck cancers because most of the cancers in this region are squamous cell carcinomas. This technique is particularly useful in assessment of occult primary tumors and lymph nodes which are missed in other radiographic techniques. It helps in accurate staging of the carcinomas also assess the post treatment results and responses. The residual tumor and recurrences are also better assessed by this technique. PET/CT better assess the operability upto 95%. This technique is highly sensitive. The specificity is compromised and there are false positive results when there are other infections present. For example in case of post chemotherapy, the patients immunity is low and prone for infections. This can result in false uptake of the radiotracer and false positive results. It cannot be used in cases of uncontrolled diabetes where the blood sugar levels are higher than 150mg/dl[22,23].

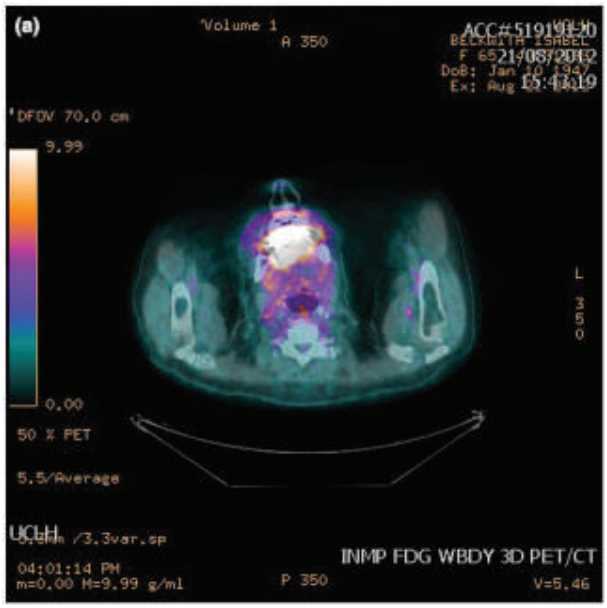


Figure 5a - Image of assessment of metastasis using 3D PET/CT



Figure 5b - Image of PET-CT showing extensive uptake in the floor of mouth and bilateral cervical nodes

BONE SCINTIGRAPHY:

Bone imaging with radionuclides is a non-invasive technique for demonstrating the osteoblastic lesion of the skeletal system. It is based on the uptake of a radiotracer that occurs where bone is being formed or repaired. It can be particularly helpful in tumors of head and neck which invade the adjacent bones. A bone loss upto 5 percent can also be detected with accuracy with a bone scan. It is also of particular importance in distant metastasis in the bones.

SINGLE POSITRON EMISSION COMPUTED TOMOGRAPHY - SPECT/CT:

SPECT/CT is a imaging technique that combines the functional information from Scintigraphy with the anatomical information from Computed Tomography into one set of images. It measures the concentration of chemicals injected into the body, and provides images of the chemical function of body parts of interest. In this technique the image obtained by a gamma camera is a 2-D view of 3-D distribution of a radionuclide[24,25].

Conclusion

Oral cavity cancer is a challenging disease with high mortality rates; dentists and dental specialists play a critical role at all stages in the management of patients. Choice among further sophisticated imaging modalities depend on number of factors like availability, affordability and clinical situation. If clinical findings are suggestive of bone involvement, the preferred imaging method is CT as it assesses bone architecture better. If the clinical findings are suggestive more of soft tissue involvement and nodal metastasis, MRI is the preferred imaging modality. In addition to these, several new techniques that aid in diagnosis and staging of oral carcinomas like SPECT and PET/CT are also available. PET/CT is superior and is of particular value in investigation of occult primary tumors, staging primary disease, detection of residual and recurrent lesions.

Prevention through education about smoking cessation and safe alcohol consumption is critical, detection and early referral of premalignant lesions and oral cancers and ongoing surveillance, follow up and preservation of oral health are just a few of the many roles of the dental practitioner in the management of oral cancer.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Biodentine as a Perforative Repair Material- A Review

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Abstract

Perforations are anomalous communications between the root canal system and the external dental surface that connects the pulp cavity to the periodontal tissue. Current advances in biomaterials has made the recovery of tooth structure and function possible even in the most complicated cases. Ideally, perforations should be immediately repaired with a biocompatible material to seal the communication between the perforation site and the gingival sulcus in order to achieve a more favorable prognosis. This review article is focussed on Biodentine as perforative repair material, and has found to provide good biocompatibility, bioactivity, high compressive strength and a short setting time.

Keywords : *Biodentin, perforation, repair, biocompatible*

Introduction

Perforation can be defined as mechanical or pathological communication between the tooth surface and root canal systems.[1] They may be iatrogenic in cause or pathologic communications between the root canal system and the attachment apparatus. Perforation of the pulpal floor of the molar is one of the most difficult situation to handle in clinical situations. If a perforation occurs, the clinician is faced with the dilemma of repair, surgery or extraction. Prevalence of accidental root perforations ranges 2-12%.[2]As the perforation creates a portal of exit in the root canal system it has to be sealed as quickly as possible, since periodontal involvement arising from the perforation can become irreversible with time.[3] Many techniques have been described for the repair of the furcation perforations; they can be broadly classified into surgical and non surgical methods. The most preferable method is the non surgical method since, surgical method can lead to pathological pocket formation.[4]

The non surgical method involves the placement of the sealing material in to perforation through the access cavity and produces a good seal to avoid fluid movement, which will lead to failure of the treatment. Many materials have been used to seal the furcation perforation from zinc oxide based materials, zinc oxide eugenol cement, super ethoxy benzoicacid , calcium phosphate, glassionomer, Resin modified glass ionomers, Composites, Mineral Trioxide Aggregate.[5-7]

Biodentine is a calcium-silicate bio active material. It has superior mechanical, physical and handling properties comparable to most commonly used restorative materials. It is used as a temporary enamel restoration and permanent dentine restoration. It is used during root perforations, apexification, resorptions, retrograde fillings, pulp capping procedures, and dentine replacement. It is a powder liquid system, powder composed of Tri-calcium silicate, Di-calcium silicate, Calcium carbonate and oxide, Iron oxide, Zirconium oxide. Liquid consist of Calcium chloride, Hydro soluble polymer.[8]

CLINICAL APPLICATIONS OF BIODENTINE:

Biodentine has a wide range of applications including endodontic repair (root perforations, apexification, resorptive lesions, and retrograde filling material in endodontic surgery) and pulp capping and can be used as a dentine replacement material in restorative dentistry. The material is actually formulated using the MTA-

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based cement technology and the improvement of some properties of these types of cements, such as physical qualities and handling makes up Biodentine. [9]

It is easy to handle owing to its ease of manipulation and a short setting time approximately 12 minutes, has high alkaline pH and is a biocompatible material makes it a favourable material for perforation repair. In a study by Guneser et al., Biodentine showed considerable performance as a perforation repair material even after being exposed to various endodontic irrigants as compared to MTA. Biodentine was compared with white MTA (ProRoot) and glass ionomer cement (FujiIX) using human fibroblasts, both white MTA and Biodentine were found to be less toxic compared to glass ionomer during the 1- and 7-day observation period.[10] [11]

PHYSIO-CHEMICAL PROPERTIES OF BIODENTINE :

Working time of Biodentine is up to 6 minutes with a final set at around 10-12 minutes. The density of Biodentine is 2.26g/cm and porosity of 6.8%. There is an increase of the electrical resistance along with the porosity reduction. Therefore, even after the initial setting of Biodentine, the material continues to improve in terms of internal structure towards a more dense material, with a decrease in porosity. The setting of Biodentine is illustrated by a sharp increase in the compressive strength reaching more than 100 MPa in the first hour. The mechanical strength continues to improve to reach more than 200 MPa at 24h. After 2 hours, the hardness of Biodentine was 51 HVN and reached 69 HVN after 1 month. The reported micro hardness values for natural dentine are in the range of 60-90 HVN. Biodentine has surface hardness in the same range as natural dentine. Biodentine contains zirconium oxide allowing identification on radiographs. According to the ISO standard 6876, Biodentine displays a radiopacity equivalent to 3.5 mm of aluminum. This value is over the minimum requirement of the ISO standard (3 mm aluminum). This makes Biodentine particularly suitable in the endodontic indications of canal repair. [11][12]

BIOCOMPATIBILITY OF BIODENTINE :

Biocompatibility of a dental material is a major factor that should be taken into consideration specifically when it is used in pulp capping, perforation repair or as a retrograde filling. During the aforementioned procedures, the material is in direct contact with the connective tissue and has the potential to affect the viability of periradicular and pulpal cells. Therefore, it is essential that toxic materials are avoided and materials promoting repair or that are biologically neutral are preferred during procedures in which the material is directly in contact with the surrounding tissue. Dentine element uptake is prominent for Biodentine. There is presence of high release of calcium for Biodentine. [13] [14]

Biodentine consistency is better suited to the clinical use than MTA's. Biodentine presentation ensures a better handling and safety than MTA. Biodentine does not require a two step obturation as in the case of MTA. As the setting is faster, there is a lower risk of bacterial contamination than with MTA. Adding to its ability to be used as dentine substitute, Biodentine could safely be used for each indication where dentine is damaged. Therefore, it is an advantage for the clinician and the patient. [15]

Fig 2: Occlusal view

BIODENTINE AS PERFORATIVE REPAIR MATERIAL :

Biodentine is fast setting, so if perforations are communicated to the oral cavity, use is recommended. Another factor to consider the Biodentine is their coloration, similar to the dental tissues and produces no staining of these. The Biodentine cement is selected as perforation filing material due to its satisfactory bond strength to dentin compared with the MTA, as in repair furcation perforations situations. It is easy to handle owing to its ease of manipulation and a short setting time approximately 12 minutes, has high alkaline pH and is a biocompatible material makes it a favourable material for perforation repair.[16,17] In a study by Guneser et al., Biodentine showed considerable performance as a perforation repair material even after being exposed to various endodontic irrigants as compared to MTA. [18]The satisfactory tissue response and cytotoxicity compared with other cements based on calcium silicate were decisive factors for the choice of Biodentine cement as filling material for root perforation.[19]

Biodentine was found to significantly increase TGF-B1 secretion from pulp cells.[20]TGF is a growth factor whose role in angiogenesis, recruitment of progenitor cells, cell differentiation, and mineralization has been highlighted in recent studies.Biodentine can induce the synthesis of a dentin- like matrix by human odontoblast-like cells in the form of mineralization nodules that have the molecular characteristics of dentin. Additionally, the FTIR analysis has previously shown that this mineralized material was a specific deposition, which had the same mineral and organic composition of dentin.[21]This can also stimulate cell growth and induce Hydroxyapatite (HA) formation on the surface of the material when exposed to the simulated body fluid. HA have been shown to induce bone formation, growth and maintenance at the bone-material interface in vivo and this can be reproduced and demonstrated in vitro by soaking HA in simulated body fluids .This is of prime importance during the process of healing as Silica can induce the mineralisation function of cells by affecting cell proliferation and gene expression.

Zanini et al. [22] also evaluated the biological effect of Biodentine on murine pulp cells by analysing the expression of several biomolecular markers after culturing OD-21 cells with or without Biodentine. Their results, consistent with other studies, were in favor of Biodentine, which was found to be bioactive due to its ability to increase OD-21 cell proliferation and biomineralization.

Laurent et al. [19] indicated that though the interactions between pulp capping materials and the injured pulp tissue are yet unclear, there is growing evidence on the role of growth factors, with TGF-□1 being the most important one. These factors' main role is the signalling of reparative dentinogenesis. In a recently published article, they assessed the reparative dentin synthesis capacity of Biodentine as well as the ability to modulate TGF-□1 secretion by pulp cells which has previously shown to be released from dentine by calcium hydroxide.[23,24] Using an entire human tooth culture model, they showed that, upon application on the exposed pulp, Biodentine had the potential to significantly increase TGF-□1 secretion from pulp cells and induce an early form of reparative dentin synthesis.

Biodentine has bioactive properties, encourages hard tissue regeneration, and provoke no signs of moderate or severe pulp inflammation response. The material has the ability to maintain a successful marginal integrity due to the formation of hydroxyapatite crystals at the surface which enhances the sealing ability. Due to its superior sealing potential, there is no risk of microleakage which may cause the pulp to become infected or necrotic and jeopardize the success of vital treatment procedures. The hard tissue formation due to calcium hydroxide is a defense response of the pulp against the irritant nature of the material whereas calcium silicate based materials are compatible with the cell recruitment.[25]

Conclusion

A perforation is an artificial communication between the root canal system and the supporting tissues of the teeth. When not properly managed, root perforation complicates the treatment and deprives the prognosis. A wide range of materials have been proposed in literature to seal the perforations.Biodentine has a wide range of applications in endodontics and has shown remarkable results as a perforation repair material. It is a calcium silicate-based bioactive material.In our review we have found that biodentinehas superior mechanical, physical and handling properties comparable to most commonly used restorative materials and can because of its ease of manipulation, short setting time, high alkaline pH and biocompatibility. Hence it is a favourable material for perforation repair.

Ethical Clearance – Not required since it is a review article

Source of Funding – Nil

Conflict of Interest – Nil

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Esthetic Replacement of Missing Antetior Tooth with All Ceramic Restoration (IPS E.max Cad) – A Case Report

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Abstract

In Dentistry, ceramic restoration has become more popular among other dental restorative material, because of its superior esthetics, good biocompatibility as well as excellent mechanical and physical properties. Among dental ceramics -Lithium Disilicate (LD) glass ceramics and Polycrystalline Zirconium Dioxide Ceramics are used as a dental biomaterial and also the material of choice for esthetic replacement of missing anterior teeth.These materials exhibit better esthetics, color stability, biocompatibility as well as better strength. Theyact as a most promising restorative materials because it has favourable mechanical and physical properties compared to other restorative materials.

Keywords*; all ceramic, lithium disilicate, zirconia ceramic. esthetic, biocompatibility*

Introduction

Fixed denture prostheses are the treatment option, used to replace damaged or missing teeth or teeth that have been lost. They are attached to prepared abutment teeth using restorative cement material so that they cannot be removed. In 1960s, metal ceramic has been considered as the gold standard of crown and bridge because of its satisfactory mechanical and physical properties as well as its esthetics results and marginal adaptations which is clinically acceptable[1].

In recent decades, increasing demand from patients for natural-appearing and bio-safety reasons, metal free restoration hasled to the development of all-ceramic materials with improved mechanical characteristics that ensure suitable longevity. These restorative materials are now replacing traditional metal-ceramic restorations. Among dental ceramics - Lithium disilicate (LD) glass ceramics and Zirconia ceramic are the material of choice for esthetic replacement of missing anterior teeth.

Lithium disilicate is a glass ceramic with a unique structure and can be produced by means of both pressable and Computer Aided Design-Computer Aided Manufacturing (CAD-CAM) processing. It shows good mechanical properties (flexural strength 350 - 450 MPa), has excellent translucency and is more suitable than zirconia ceramic especially in esthetic areas[2].

A Case Report

A 24 years, male patient came to the Department of Prosthodontics, Sree Balaji Dental College & Hospital with a chief complaint of missing tooth in upper front jaw region for past 1 year. The patient presented no significant medical history.

Clinical examination revealed that maxillary left central incisor was missing (fig.1and 2) and the tooth was extracted due to trauma before 1 year. On radiographic assessmentrevealed that presence of adequate bone support from abutment teethand sufficient amount of space available for either fixed or removable prosthesis.

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Possible treatment options are fixed or removable prosthesis. Patient wants fixed restoration, so removable treatment plan was eliminated. Patient also need not wants any invasive treatment procedure (Dental implant) hence various fixed treatment options were discussed with the patient and replacement of the missing tooth was planned with conventional fixed partial denture. The patient signed the informed consent, and accepted the treatment plan for fixed partial denture.

Procedure

The primary impression was made with irreversible hydrocolloid (fig.3) impression material (Zhermack-tropicalgin, BadiaPolesine[Rovigo] Italy). The cast was poured in type-3 dental stone (DPI, Mumbai, Maharashtra, India). After the primary casts obtained, it was mounted in a articulator and wax mock-up was done. Then the putty index was made.

A conventional tooth preparation was done in maxillary 11 and 22 incisor region (fig.4) Shoulder type finish line was given in 11 and 22 regions for all-ceramic restoration. For all – ceramic restoration, the tooth preparation in 11 and 22 regions need1.5 to 2mm labiolingual reduction and 2mm incisal reduction. Flat-end tapered diamond was used for shoulder type of finish line. Before tooth preparation shade selection should be done.

Once the tooth preparation has been done, retraction cord was placed for retraction of gingival tissue. Then the master impression was made. The material of choice used for impression procedure were Zhermack elite HD+ soft putty and Zhermack elite HD+ normal set light body (fig.5), using the double mix technique. For better esthetics and phonetics, tooth colored provisional restoration(fig.6) was given in maxillary anterior region. The impressions were poured using type IV dental stone. The restorative crowns were designed and fabricated using CAD – CAM technology. After fabrication of permanent prosthesis, it should be checked in patient mouth for better adaptation and shade matching, then the all ceramic restoration was cemented(fig.7) using 3M ESPE RelyX^{TM} U200 resin cement.



Fig 1: Intra Oral Examination



Fig 2: Occlusal view

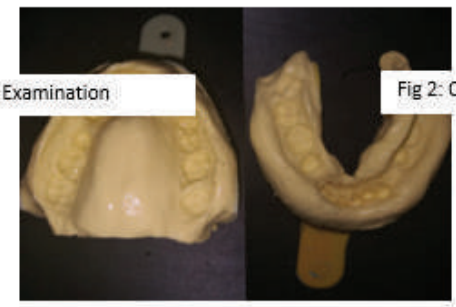


Fig 3: Diagnostic impression



Fig 4: Tooth preparation



Fig 5: Master impression



Fig 6: Temporary restoration



Fig 7: Permanent restoration



Fig 8: Post-

Discussion

The replication of natural teeth in anterior region for patients having high esthetic demands has become more challenge for dentists. The reproduction of the color in the cervical portion is more challenging because a thinner layer of material must be used to prevent harm to the pulpal tissue and also most critical area for selecting the color shade in fixed prosthesis. Shade selection is an important factor because the color of a restoration should be indistinguishable from that of the adjacent teeth. The color matching of a restoration is determined by the core materials, porcelain thickness, fabrication process, and by surface properties such as texture[3].

In recent decades, the demand for better estheticsby the patient had increased over the year. The tooth color restorations like all ceramics and composite material play a major role in fulfilling the esthetics demand of the patient. The use of all ceramic restorations has increased

due to its superior esthetics, color stability, and its biocompatibility[4].

Lithium disilicate is a glass ceramic with a unique structure. This material has glass ceramic phase in which crystalline fillers are added during manufacturing to improve the strength, thermal expansion and contraction behaviour of ceramics. The crystalline phase of $Li_2Si_2O_5$ makes for up to 70% of the material, grows by nucleation and forms many randomly oriented interlocking crystals, contributing to the superior strength (flexural strength of 350 – 450 MPa) than the alumina or leucite ceramics. The crystalline phase orientation could also cause crack deflection and blunting, accounting for the improved strength.

It has excellent translucency due to good compatibility between the glass ceramic phase and the crystalline phase which keeps internal light scattering within the material to a minimum. Highly esthetic results can be achieved with this material.

It is commercially available as ingots that can be pressed to obtain full-contour forms or sub-structures, as milling blocks that can be milled by CAD-CAM technology, and also act as a veneering material that can be hot pressed onto all-ceramic frameworks[5].

Harda et al has reported that lithium disilicate is more translucent than zirconia, which means the superior esthetic property of monolithic lithium disilicate[6].

Conclusion

From this clinical report it was concluded that replacement of missing anterior teeth is more challenging one for the dentist. All ceramic restoration - Lithium disilicate (LD) glass ceramics and Zirconia ceramic are the material of choice for esthetic replacement of missing anterior teeth. Lithium disilicate is a glass ceramic with a uniquestructure. It shows good mechanical properties (flexural strength 350 - 450 MPa), has excellent translucency and is more suitable than zirconia ceramic especially in esthetic areas

Ethical Clearance – Not required since it is a case report

Source of Funding – nil

Conflict of Interest – nil

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Role of Platform Switching in Success of Dental Implants- A Meta-Analysis

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Abstract

Aim: To analyze through systematic review and meta-analysis of implant failure rates, marginal bone loss (MBL) in patients who received platform-switched implants and platform-matched implants and to determine literature based evidence.

Materials and Methods :

Data: Search terms used in combination are dental implant, platform switch, platform mismatch, switched platform, implant – abutment design.

Sources : An electronic search engine without time/ language restrictions was taken from Google Scholar, Pubmed.

Clinical Significance: Platform switching for maintaining peri-implant bone levels has gained popularity over last few years. As philosophies of treatment alter over , a periodic review of different concepts are necessary to refine techniques thereby forming a basis for optimum treatment.

Keywords: *Dental implants, Meta- analysis, Marginal bone*

Introduction

Dental implants have become a standardized and predictable treatment modality for the rehabilitation of partially and completely edentulous arches, resulting in restoring the normal function, esthetics, speech, and comfort in harmony with stomatognathic system[1].

Various studies have been conducted on the effectiveness and predictability of dental implant therapy on which Dr. Branemark in 1968, first proposed the concept of osseointegration.

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Wide-diameter dental implants introduced in the late 1980s, created a situation where mismatched standard-diameter abutments were used simply because of the lack of commercial availability of components to match the wide-diameter implants. It was found that these implants exhibited less-than-expected initial crestal bone loss. This remodeling is termed as saucerization[2].

The ability to reduce this crestal bone loss may have several advantages such as improved esthetics, higher bone to implant contact and better primary stability. To minimize this crestal bone resorption, several techniques and procedures such as non-submerged technique, utilizing micro-roughness on implant neck surface and platform switching have recently been developed [3].

A better understanding of the biologic principles of bone healing around dental implants is needed and studies have demonstrated that bone resorption at the implant-abutment junction (IAJ) may be caused by an inflammatory cell infiltrate, that forms a zone around

the IAJ. Though not fully understood, the current theory of platform switching is related to the physical repositioning of the IAJ away from the outer surface of the implant and the surrounding bone, thereby avoiding the inflammatory infiltrate away from the width of the platform switched dental implant.

Considering the key importance of crestal bone loss to implant success, several preclinical and clinical studies have investigated a variety of confounding biologic and biomechanical factors contributing to CBL. The micro gap at the junction of an implant platform and the abutment has been suggested as a major contributor. Thepresent meta-analysis was conducted to investigate the impact of platform switching on marginal bone levels around platform switched and platform matched dental implants

Aim & Objectives

To analyze through systematic review and meta-analysis of implant failure rates, marginal bone loss (MBL) in patients who received platform-switched implants and platform-matched implants.

· To Collect Reliable Journal Articles Reporting The Marginal Bone Loss Among Platform Switched Dental Implants And Platform Matched Dental Implants.

· To Compare The Parameters Across The Different Articles.

· To Address The Limitations In The Collected Data.

Methodology

- A structured electronic systematic search without time or language restrictions was undertaken from March 2009-2019 in the following databases.

- PubMed library and Google scholar was searched for articles using MeSH terms.

- The following question in the PICO format (Patient, Intervention, Comparison and Outcome) was developed in patients treated with dental implants, are there any differences among platform- switched restorations compared to platform matched ones in terms of peri-implant marginal bone loss and implant survival.

- In the electronic search, 475 articles were identified , 372 articles were obtained after removal of duplicates and 36 full text articles were assessed for eligibility based on inclusion criteria. 29 articles were included in qualitative analysis and 7 articles were included in the quantitative analysis

Search Terms :

• The following combinations of keywords were used :

• Dental implant , Platform shifting, Platform switch, Platform switching, Mismatched abutment, non-platform switched implants, platform design, switched abutment, crestal bone remodeling, marginal tissue, Implant- abutment junction, Crestal bone loss, Marginal bone preservation, Marginal bone loss, Marginal bone changes, dental implants, and implant design.

Inclusion Criteria :

• All clinical trials,

• Randomized Control Trials

• Prospective studies,

• Case-control studies with a minimum of 10 implants/ subjects.

Exclusion Criteria :

• Animal studies,

• Biomechanical studies,

• Finite element analysis,

• Case reports,

• Studies in the absence of a comparison group.

STUDY SELECTION AND DATA COLLECTION :

• During the first step of selection, studies were retained based on the screening of the title and abstracts, later the final stage of screening involved full- text reading using a predetermined data extraction form to confirm the eligibility of each study based on inclusion and exclusion criteria.

• In addition bibliography of these selected articles were also scanned to obtain the maximum number of results possible. A total of 475 articles were obtained from Pubmed And Google Scholar , after removal of duplicates and 36 full text articles were assessed for eligibility based on inclusion criteria. 29 articles were included in qualitative analysis and 7 articles were included in the quantitative analysis.

Study Design :

· A meta-analysis on the platform switching in dental implants

PRISMA FLOW CHART

Results

Characteristics Of The Studies Included And Comparison Of Mean marginal Bone loss considering RCT'S with an observation period (Table 1)

Sl. No:	Study name	Platform switched			Platform matched		
		Mean	SD	95% CI	Mean	SD	95% CI
1.	Enkling (2011) -1 year -25 implants	0.19	0.47	-0.37,0	-0.34	0.35	-0.5,0
2.	Enkling et al(2013) -1 year -25 implants	-0.34	±0.35	0.50, 0.00	-0.19	±0.47	-0.37, 0.00
3.	Silvio et al(2014) -1 year -36 implants	0.84	±0.23	0.73,0.95	0.93	±0.26	0.81, 1.05
4.	Silvio et al (2016) -3 year -36 implants	1.06	±0.24	0.93, 1.15	1.09	±0.31	0.85, 1.13
5.	S.Rocha et al (2017) -5 years -59 implants	0.27	±0.44	0.26,0.76	0.09	±0.85	0.07, 0.64
5.	S.Rocha et al (2017) -5 years -59 implants	0.27	±0.44	0.26,0.76	0.09	±0.85	0.07, 0.64
6.	**Laura Lago et al (2018) -1 to 5 years -100 implants**	**-0.17**	**±0.67**	**0.71**	**0.34**	**±0.54**	**0.33**
7.	**Ana Messias et al (2019) - 5 years -121 implants**	**0.19**	**±0.53**	**0.33,0.43**	**0.04**	**±0.58**	**0.03, 0.43**

META- ANALYSIS :

· Mean difference of the mean bone implant contact was calculated for platform switched dental implant and platform matched dental implant and compared using student T test at confidence interval of 95%

· Graphically the data was represented as forest plot.

· Meta-analysis was done using MEDCALC software version 19.1

· In the electronic search, 475 articles were identified. Using the key words "platform switched and platform matched implants "372 articles were obtained after removal of duplicates and 36 full text articles were assessed for eligibility based on inclusion criteria. 29 articles were included in qualitative analysis and 7 articles were included in the quantitative analysis.

· Pooled estimates from the 7 studies were analysed using a continuous random effects model meta-analysis, the variable analyzed was crestal bone loss of implants placed in platform switched and platform matched dental implants.

META-ANALYSIS: CONTINUOUS MEASURE: TABLE – 2

Variable for studies	study_name
1. Intervention groups	
Variable for number of cases	N
Variable for mean	Mean
Variable for SD	Sd
2. Control groups	
Variable for number of cases	N
Variable for mean	mean_1
Variable for SD	sd1

Study	N1	N2	Total	SMD	SE	95% CI	t	P	Weight (%)	
									Fixed	Random
Enkling (2011)	25	25	50	1.259	0.306	0.645 to 1.873			5.46	12.80
Enkling et al(2013)	25	25	50	-0.356	0.281	-0.921 to 0.208			6.47	13.25
Silvio et al(2014)	36	36	72	-0.363	0.235	-0.832 to 0.106			9.22	14.05

Silvio et al (2016)	36	36	72	-0.107	0.233	-0.572 to 0.358			9.36	14.08
S.Rocha et al (2017)	59	59	118	0.264	0.184	-0.0997 to 0.628			15.10	14.86
Laura lago et al (2018)	100	100	200	-0.835	0.147	-1.125 to -0.545			23.61	15.37
Ana messias et al (2019)	121	121	242	0.269	0.129	0.0155 to 0.523			30.76	15.59
Total (fixed effects)	402	402	804	-0.0722	0.0714	-0.212 to 0.0679	-1.012	0.312	100.00	100.00
Total (random effects)	402	402	804	0.000827	0.234	-0.459 to 0.461	0.00353	0.997	100.00	100.00

TEST OF HETEROGENEITY :

Q	58.8846
DF	6
Significance level	P < 0.0001
I2 (inconsistency)	89.81%
95% CI for I2	81.54 to 94.37

FOREST PLOT :

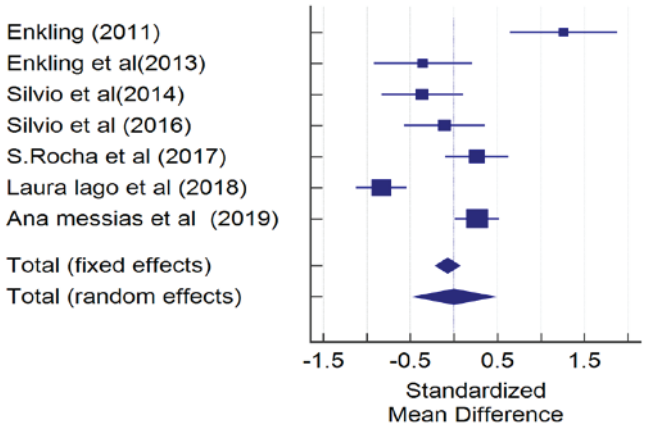


FIGURE 1, Forest Plot Depicts the Mean Differences of Marginal Bone Loss between Platform switched And Platform matched Implants.

FUNNEL PLOT:

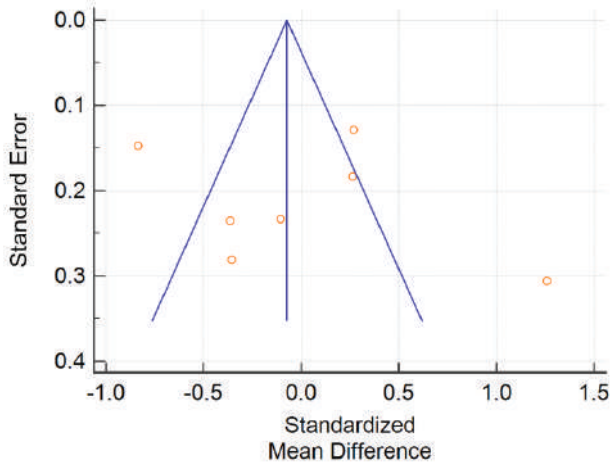


FIGURE 2, Funnel Plot For Detection Of Publication Bias

META-ANALYSIS INTERPRETATION :

FOREST PLOT :

· The main outcome of the meta-analysis is a forest plot, a graphical display as in figure 1, the X-axis (horizontal axis) forms the effect size scale (odds Ratio or Relative Risk), plotted on the bottom of the plot. Each row except bottom two, represents a study's effect size estimate in the form of a square (black box) the bigger the box, the more contribution to the study and a (95%) confidence interval and a vertical line which is known as the line of no effect / null effect.

· The diamond at the bottom of the forest plot shows the results when all the individual studies are combined and averaged. The horizontal points of the diamond are the limits of the 95% confidence interval and are subject to the same interpretation as any of the other individual studies on the plot.

· In this analysis, diamond shape 'Pooled Data ' found on the line of no effect which means there is less statistical significance or no statistical significance with the use of platform switched dental implants compared with platform matched dental implants.

FUNNEL PLOT :

Funnel plot has the following characteristics,

· The plot ideally resembles a pyramid or inverted funnel, with scatter due to sampling variation. The shape is expected because the studies have a wide range of

standard errors. If the same size, the studies would fall on the horizontal line.

In this analysis, Few studies (3 out of 7) were scattered out of the funnel plot it shows that there was a possible error in the studies, which may due to poor methodological design, inadequate analysis, heterogeneity (size of the effect differs according to study size) and sampling variation.

Discussion

Platform switching for maintaining of peri-implant bone levels has gained popularity over last few years. As philosophies of treatment alter over time, a periodic review of different concepts is necessary to refine techniques thereby forming a basis for optimum treatment. Crestal bone loss is considered an important criteria for the evaluation of the implant outcome and evidence for the presence or absence of peri-implant tissue health. Therefore, efforts were made to preserve the peri-implant marginal bone levels stable throughout and following the prosthetic loading phase.

Platform switching technique is one among the various approaches to achieve marginal or crestal bone stability. With the platform switching technique, there is a close adaptation of soft tissues, increased bone-implant contact area, good per mucosal seal, and hence better emergence profile is obtained thereby improving the esthetics.

Recently, published systematic reviews and meta-analyses supported this assumption by confirming the effectiveness of the platform switching technique[4] significantly limiting marginal bone resorption around dental implants, while the cumulative estimated implant success rate was statistically less significant between both intervention groups of platform switched and platform matched dental implants.

All the studies included in the meta-analysis were RCT's, the analysis of the methodological quality of some of them revealed some potential criticisms i.e bias for reporting incomplete outcome data, due to dropouts or exclusion after randomization, some authors did not report the periodontal health status of the patients included in the study.

The platform switching technique and effects of platform switching were found via electronic database, of which 475 records were found. 36 articles were fully assessed for eligibility , 29 met the inclusion criteria for the qualitative analysis, and 7 were found eligible for the quantitative, to be included in meta-analysis. Considering the results of RCT's included in this meta-analysis, three studies stated that the difference of marginal bone levels were remarkably in favor of the platform switched group. However a statistical analysis has to be done to calculate the level of significance, and four studies indicated the difference of marginal bone levels was less significant in the platform switched group compared to those utilizing platform matched implant-abutment connections.

The possibility cannot be ignored, but it is not known whether the loss of marginal bone is a long term process. Only drawback is, only few studies are available with long term follow up. Moreover, it is debatable whether such mean difference may have clinical significance. Several hypotheses trying to explain this phenomenon have been raised in the literature. Some studies have shown that bone resorption around the implant neck does not begin until the implant is uncovered and exposed to the oral cavity, which invariably leads to bacterial contamination of the gap between implant and abutment. The bacterial reservoir in the microgap may continuously invade the bone through micro-spaces. Toxins produced by the bacteria create a zone of toxicity at the level of microcap, resulting in peri-implant inflammation and typical bone loss upto 1st thread.

Especially periodontally compromised smoking patients with treated periodontitis revealed significantly more peri-implant marginal bone loss compared to periodontally healthy smokers , therefore smoking and the history of periodontitis should be considered risk factors for marginal bone loss around dental implants.

However, studies done by Enkling[5] et al in 2011, Enkling[6] et al in 2013, Silvio[7] et al 2014, Silvio[8] et al 2016, S.Rocha[9] et al in 2017, Laura lago et al in 2018, Ana messias[10] et al in 2019 cautiously summarized their findings, especially on emphasizing the bone-preserving effects on platform switched implant and platform matched implant. Nevertheless, within the limitations of the recently published RCTs included for meta-analysis presented here, the results of former systematic

reviews confirmed, a less significant difference between the mean MBL change at dental implants with a PS implant – abutment compared to a PM implant-abutment configuration.

Atieh[11] et al, in their systematic review and meta-analysis involving a total of 10 RCTs, reported significantly lower bone loss in implants restored with platform switching vs platform matching.

Annibali[12] et al in their systematic review with a follow-up of 36 months reported that there is no significant difference in implant success rate between platform matching and platform switching. Implants restored by platform switching showed a lower degree of marginal bone loss over time of 0 to 0.99 mm and - 0 to 2.02 mm for platform matching dental implants. In fact, there seems to be a relationship between a higher degree of discrepancy and a smaller amount of bone loss.

Herekar[13] et al, in their systematic review of 10 RCTs and 5 controlled clinical trials, showed that marginal bone loss around implants restored by platform switching was statistically less significant than that of implants restored by platform matching −0.34 mm. Strietzel et al, in their systematic review and meta-analysis, reported that most studies showed a favorable trend toward platform switching.

Santiago[14] et al analyzed a total of 17 RCTs and 8 prospective studies. This meta-analysis revealed a significant reduction in marginal bone loss for implants restored with platform switching compared with implants restored with platform matching. Another highlight, regardless of the P value, is the clinical relevance these findings may offer, since the difference between the two techniques is seldom greater than 0.5 mm. If possible error in the measuring method is taken into account, the comparative advantage of bone-level implants restored by platform switching over the tissue-level implants restored by platform matching may be limited by this value.

The last, as reported by Hsu, Lin, and Wang [15] (2017), seems to be a crucial factor for the preservation of marginal bone levels, contributing to the success of the rehabilitations. Notwithstanding that and taking into consideration the validity of these results, we believe that any patient receiving implants in any healed bounded or free end edentulous areas would benefit from the use of PS prosthetic components from the early stages of the rehabilitation (i.e. from surgery onwards), as long as strict hygiene and motivation is practised with follow-up programme is done to ensure patient compliance.

Summarizing the findings of the included publications, especially among the RCTs remarkably more studies indicated results favoring less significance for the use of PS technique to prevent MBL changes. Therefore, heterogeneity among the study conditions is supposed to have a crucial influence on study outcomes.

The results of the study in the forest plot suggested that the study done by Enkling et al in 2013, Silvio et al in 2014, had a significant impact on the platform switching technique. Whereas study done by Enkling in 2011 , S Rocha et al in 2017, Ana messias et al in 2019 had a less significant impact in this technique. The pooled estimate of the study (diamond shape) lied in the center of line of no effect stating that ,there is less significant difference with use of platform switched when compared with platform matched dental implants.

Meta-analysis of 7 RCTs revealed a significantly less mean MBL change at PS implants compared with PM implants, thus confirming the supposed bone level stabilizing effect of PS implant-abutment configurations at least when considering short-term observations. The longest follow-up period within the RCTs was reported up to 60 months i.e.5 years by S.Rocha et al. (2017) and Laura lago et al (2018).

Limitations

· First, few clinical studies with less numbers of patients were available for data extraction. Second, there was not enough evidence from RCT in the selected literature.

The impact of positioning the micro-gap on soft tissue margin changes around PS implants lacks documentation and may not allow for any conclusions.

Platform Switching may have a positive effect on bone preservation in the first year, but after five years, the marginal bone change is insignificant, as compared to that at one year, around both PS and PM implants.

More homogeneous studies with a longer follow-up

are needed to confirm platform switching phenomenon to reduce crestal bone resorption, and health of peri-implant soft tissue and hard tissue .

Summary and Conclusion

· The meta-analysis of 7 RCT'S revealed a significantly less mean MBL change at implants with a PS – implant –abutment configuration compared with PM-implant-abutment design. However, within the limitations of the recently available publications of the RCT'S and PCCS, the tendency revealing from the studies results favors less significance of the PS technique to prevent or minimize peri-implant marginal bone loss, compared to implants with PM abutments.

· The results of the present study suggest that there is a significantly less MBL at implants with platform-switching than on implants with platform matching. Moreover, precisely designed and appropriate clinical trials with larger samples and longer follow-up periods are required for literature based evidence.

Ethical Clearance – Institutional Ethics Committee, Sree Balaji Dental College and Hospital, Committee registered with DCGI. (Registration no: SBDCH/IEC/04/2019/22)

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Review Article

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Emerging and Re-Emerging Virus

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Abstract

Infectious diseases remain a major cause of human and animal morbidity and mortality leading to significant healthcare expenditure. However, enormous successes have been obtained against the control of major epidemic diseases, such as malaria, plague, leprosy and cholera, in the past. The vast terrains and extreme geo-climatic differences and uneven population distribution present unique patterns of distribution of viral diseases. Dynamic interplay of biological, socio-cultural and ecological factors, together with novel aspects of human-animal interphase, pose additional challenges with respect to the emergence of infectious diseases. The important challenges faced in the control and prevention of emerging and re-emerging infectious diseases range from understanding the impact of factors that are necessary for the emergence, to development of strengthened surveillance systems that can mitigate human suffering and death. Viral pathogens are known to cause outbreaks that have epidemic and pandemic potential which would result in severe range of mortality and Health care expenditure on a scale depending on the pathogenicity of the virus.

Keywords*: Avian influenza - CCHF - emerging infections - Nipah virus - COVID-19- Respiratory viral infections - re-emerging virus - rotavirus - viral diseases*

Introduction

The emergence of new human pathogens and the re-emergence of several diseases in the current decade are of particular concern.[1] Emerging pathogens may be described at a basic level as those diseases whose occurrence has been shown to have risen in recent decades or that have threatened to increase in the future. Several factors underlie the emergence of such diseases, including population growth, poverty and malnutrition, increased domestic and global connectivity, economic factors leading to population migration, the prevalence of immuno-suppressive diseases in social practices, unplanned urbanization, deforestation and changes in agricultural practices such as mixed agriculture.[2] Genetic variations in infections were also responsible, to some degree, for these outbreaks. It is estimated that about 60% of infectious diseases and 70% of emerging human infections are of zoonotic origin, two-thirds of which arises from wildlife, like COVID-19.[3] Habitat destruction due to unplanned urbanization has placed human at contact with animals and arthropod vectors of viral infection.[4] Such interactions have been one of the major causes for increased human susceptibility to infections by novel pathogens, in the absence of specific immunity in these population.[5]

Respiratory viral infections, arboviral infections and bat-borne viral infections represent three major categories of emerging viral infections.[6] Infection aerosols of the tracheo bronchial tree represent efficient means for spread of viral pathogens affecting the respiratory tract. Pandemic influenza H1N1, highly pathogenic avian influenza (A1) injection (H5NI) and the Middle East respiratory Syndrome coronavirus (MERS-CoV) also

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the COVID-19 pathogen that pose severe threat in this category.[7] Arthropod -borne viruses have constantly been the reason for emerging and re-emerging disease including Crimean - Congo hemorrhagic fever (CCHF), dengu , chikungunya, encephalitis. Severe and major arboviral pathogens of human belong to the three genera of Flavivirus, Alphavirus and Nairovirus.[8]

Several bat-borne viruses have also come into prominent notice, best exemplified by Nipha virus disease, COVID- 19 severe fever with thrombocytopenia virus (SFTV), as well as Ebola virus disease.[9] Theme of re-emergence of the virus is often seen in a more virulent forms following their initial discovery.[10] There have also been cases of discovery of novel pathogens in the country, the example include CHPV, CCHP and KFD virus in the 1950s-196 but their pathogenic and public health significance Remained unexplored for a long time.[9,11] Several of these infections to take a heavy toll on the animal and agricultural industry. [12]The economic costs associated with such infections can be heavy as can be inferred from the high cost of medical and intensive care, days of productive work lost, impact on travel and tourism ban on export of agricultural produce and products from affected regions. The major emerging and re-emerging viral infections of public health importance have been reviewed that have already been included in the Integrated Disease Surveillance Programme so that control and prevention of emerging and re-emerging infectious diseases could be handled through understanding the impact of factors that are necessary for the emergence and to develop and strengthen surveillance systems that can mitigate human suffering and death.

Material and Methods

Article collection for this systematic review was collected and analysed from the PMC database, Mediline embrace Cochrane. Necessary articles selected based on this importance to this review article on emerging and re-emerging viral threat. From which 30 articles were reviewed and selected using scholarly search engines of PubMED & Google,Inc.

Emerging Viral infections identified as public health threats

Viral pathogens are known to cause outbreaks that have epidemic and pandemic potential. [13,14]Integrated Disease Surveillance Programme (IDSP) is a laboratory-based, IT enabled system in the country for Surveillance of epidemic prone disease. During 2017, the IDSP showed a total of 1683 outbreaks of such disease of which 71 % of those outbreaks were caused by viral pathogens while the rest 29 % were non-viral. Subclinical and sporadic infection as well as those not identified by the health facility are often missed by the surveillance system.[4,13]

Impact of Mass gatherings and emerging viral infections

Mass gathering opportunities create situations for human proximity within very close distances and the challenges they present to the maintenance of sanitation, a considerable public health concern. Transmission of respiratory and gastrointestinal infection remains a major concern during several large-scale assemblies, for example outbreak of cholera at Kumbh Mela festival in 1817. [15]Large scale gatherings provide platforms for exchange of genomic material and thereby evolution of pathogens, including viruses.[1617]

Nosocomial Transmission and Emerging infection

Institutional care of vulnerable people with compromised immune systems may present opportunities for transmission of viral infection.[16,18] Appreciable risks also exist at dental clinics, haemodialysis units etc. where sterilization/disinfection practices for patient care instruments are not followed stringently. Hospital-associated transmission of infection was a prominent finding during the outbreak of Nipah infection in Bengal and Kerala, where several health care staff fell victims to the infections.[19]

Laboratory Accidents/Lapses in Biosafety practices

There is a current investigation going on at WHO regarding the outbreak of COVID-19 at Wuhan, China. Several experts believe that the initial outbreak of COVID-19 is from the Wuhan Institute of Virology, a microbiology laboratory under the Chinese Academy Science and investigations are ongoing.[20,21] Neglect of laboratory biosafety requirements as well as laboratory accidents may also lead to the occurrences of emerging/ re-emerging infections. Recently there was a report on the development of buffalopox (BPX) lesions on the

palm of a biomedical researcher following a shrapnel injury, warranting surgical treatment and leading to delayed healing.[22] Such reports, through infrequent emphasize the need for stringent adherence to biosafety guidelines to be observed in research involving viral agents with human pathogenic potential.[23]

Current Scenario of Emerging Viral Infections

Despite an elaborate armamentarium to tackle microbes, emerging infectious diseases remain a crucial global challenge. Emerging infections can have a significant impact on human well being and can cause gigantic monetary misfortunes. Expanded checking and reconnaissance by associations, for example, the U.S. Centers for Disease Control and Prevention(CDC)and the World Health Organization (WHO), and a few other distributed reports of cases, outbreaks and pandemics have identified numerous unrecognized viral pathogens developing globally with increasing frequency. Historically, Asia is believed to be the epicentre of several emerging viral diseases, (2 influenza pandemics in the previous millennium, SARS in China, avian influenza in Hong Kong etc.) that are of significant global public health importance. Acute respiratory disease claims over four million deaths every year and causes millions of hospitalization in developing countries every year. Over 200 viral pathogens, belonging to the families Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Coronaviridae, Adenoviridae and Herpesviridae cause respiratory infections in humans. Influenza, parainfluenza, respiratory syncytial virus (RSV) and adenoviruses remain important respiratory pathogens. Human meta pneumovirus has been recognised worldwide as a pathogen of significance.[24]

COVID-19:

Coronavirus disease 2019 (COVID-19) is characterized as illness caused by a novel coronavirus now called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; once in the past called 2019-nCoV), which was first distinguished amid an outbreak of respiratory illness cases in Wuhan City, Hubei Province, China. It was initially reported to the WHO on December 31, 2019. On January 30, 2020, the WHO declared the COVID-19 outbreak a global health crisis. On March 11, 2020, the WHO declared COVID-19 a global pandemic, its first such designation since declaring H1N1 influenza a pandemic in 2009. [25] [26]

Illness caused by SARS-CoV-2 was named COVID-19 by the WHO, the acronym got from "coronavirus disease 2019. " The name was picked to avoid stigmatizing the infection's causes as far as populations, geography, or animal associations. [20]

Influenza:

Influenza infections having a place with the orthomyxoviridae family are the incessant causes of epidemics and pandemics affecting humans. Influenza pandemics have happened earlier in 1918 swine influenza 1957 Asian flu 1968 Hong Kong flu 1977 Russian flu and the recent pandemic of 2009 pandemic influenza a H1N1 influenza infection type an is exceptionally variable shows consistent antigenic variation and is a major cause of pestilences and pandemics. The surface antigenic glycoproteins undergo two major kinds of antigenic variation viz. Antigenic shift and antigenic drift. Antigenic shift is the consequence of major changes in one or both the surface antigens [haemagglutinin ha and neuraminidase na] causes occasional pandemics. Three mechanisms may be operative in the antigenic shift leading to development of pandemic influenza strains viz. Genetic reassortment direct transfer from avian/mammalian host to humans and virus recycling. Antigenic drift results because of minor changes in HA or NA and causes frequent epidemics. influenza infections are constantly developing and show universal dispersion in nature animals and humans.[27]

Severe acute respiratory syndrome-associated coronavirus (SARS-CoV):

SARS was first reported in the Guangdong territory of China in February 2003 demonstrating human-to-human transmission. The disease caused an estimated 8000 cases and in excess of 750 deaths in more than 12 countries. The WHO gave a global alert about the disease on March 13, 2003. Although the cases generally remained restricted to China, a couple of cases were accounted for from North and South America, Europe and Asia. No case, be that as it may, has been accounted for from India.[28]

MERS-CoV:

MERS-CoV is a zoonotic viral illness causing respiratory contamination which was first revealed in Saudi Arabia in 2012 and has since spread to 26 unique nations. More than 2207 laboratory-affirmed cases and 787 deaths have happened because of MERS-CoV disease globally, since 2012. The clinical range of illness associated with MERS-CoV ranges from asymptomatic contaminations to acute respiratory distress syndrome, coming about in multi-organ failure and death. The case-fatality rates (CFRs) have remained high at 3-4 for every 10 cases. Limited information as of now exists about the transmission dynamics of this infection, and complete treatment and a prophylactic vaccination remain unavailable till date. Proof for secondary, tertiary and quaternary cases of MERS following from a solitary contaminated patient also exists, even in the absence of mutations conferring hyper-virulence. No case of contamination with this infection has been identified in India up until now. Bats are believed to be the natural supply of this infection, and many patients built up the illness after contact with camels. India is home to a great decent variety of bat species and has a substantial camel population. The nation also reports heavy passenger traffic from the Center East, as part of pilgrimage, work, the travel industry and trade. These facts call for preparedness and surveillance against this infection in the country.[29]

RSV:

RSV is an important pathogen causing acute lower respiratory tract contamination (ALRTI) in small kids. It can also affect more older adults and immuno-compromised individuals. Estimates indicate an annual frequency of approximately 34 million scenes of ALRTI associated with RSV disease in kids aged five years or less.[26] RSV contaminations also lead to about 3,000,000 cases of hospitalization and about 66,000-199,000 deaths, with more than 99 percent of the deaths detailed from growing nation. Considering the general health significance, the WHO has started a pilot venture for RSV surveillance in its six locales, using the entrenched platform of Global Influenza Surveillance and Reaction System. The exact weight and impact of RSV diseases in the nation should be concentrated in depth. [30,31]

Nipah virus: Nipah virus (NiV) and Hendra virus (HeV) are closely related members of the family Paramyxoviridae and are included in a new genus, Henipavirus. Nipah virus, a zoonotic paramyxovirus, is an emerging virus endemic in Southeast Asia. Beginning in September 1998 a major outbreak of disease in pigs and human beings appeared in the northern part of peninsular Malaysia, resulting in 265 infected individuals and 105 deaths, most of whom were farmers or abattoir workers who had come in close contact with infected pigs. Nipah virus associated encephalitis outbreaks with a high mortality occurred in Bangladesh between 2001 and 2007. A similar outbreak which was retrospectively identified to be due to Nipah virus was reported in 2001 from Siliguri, West Bengal, not far from Bangladesh. It has a unique genetic signature and may have co-evolved within local natural reservoirs. Patients usually present with fever, malaise, headache, myalgia, sore throat, nausea and vomiting, sometimes accompanied by vertigo and disorientation. Severe cases progress to encephalitis, which may be complicated by seizures and coma. Procedures for the laboratory diagnosis of Nipah virus include serology, histopathology, PCR and virus isolation.

Hantavirus:

The occurrence of 'hantavirus pulmonary syndrome' in 1993 in the Southwestern United States due to the emergence of a disease caused by viruses known for decades, was attributed to the relatively abrupt and short-lived weather changes due to the El Nino effect. The El Nino effect provoked heavy rainfall across the southern states in the US, leading to proliferation of vegetation and consequently the rodent population, the natural hantavirus hosts which are asymptomatic carriers of the virus. As the population of rodents, especially deer mice (Peromyscus maniculatus) exploded and they sought food and shelter in human dwellings, people were exposed to the aerosolized animal droppings, urine and saliva, which lead to the infection. The outbreak has continued since and by December 1, 2009, a total of 534 cases of hantavirus pulmonary syndrome with 36% mortality have been reported in the United States. Hantaviruses are the most widely distributed zoonotic rodent borne viruses known to cause two significant clinical syndromes-haemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome

(HPS), also referred to as Hantavirus cardiopulmonary syndrome (HCPS). Hantaviruses belonging to the family Bunyaviridae, genus Hantavirus are enveloped RNA viruses and currently up to 22 species and more than 30 genotypes have been Described. There are no specific antiviral drugs for treatment of all hantavirus infections. Ribavirin used in clinical trials in China for HFRS has been reported to cause significant reduction in fatality. Prevention of exposure to rodent excreta is the best preventive strategy

Ebola:

Ebola hemorrhagic fever is a viral disease caused by Ebola virus (a member of the Filoviridae family or filoviruses) that results in nonspecific symptoms (see symptom section of this article) early in the disease and often causes internal and external hemorrhage (bleeding) as the disease progresses. Ebola hemorrhagic fever is one of the most life-threatening viral infections; the mortality rate (death rate) may be very high during outbreaks (reports of outbreaks range from about 25%-100% of people infected, depending on the Ebola strain). Because most outbreaks occur in areas where high-level intensive care supportive public health services are not available, survival rates are difficult to translate to potential outbreaks in Ebola-affected areas with more resources. Ebola viruses are highly contagious once early symptoms such as fever develop. The infected patient sheds infectious viruses in all body secretions (bodily fluids); direct contact with any of these secretions may cause the virus transmission to uninfected individuals. Definitive pathogenesis of EVD is still unknown due to the lethal nature of the virus and rarity of human studies. Concurrent transmission of EVD occurs through two main routes; animal-to-human and human-to-human contacts. The early clinical diagnosis of EVD is challenging due to the vague presentation that usually overlaps with multiple differential diagnoses, including malaria, dengue fever, typhoid fever, meningococcemia, and other bacterial infections.[32]

Management of viral infection

Many viral infections resolve on their own without treatment. Other times, treatment of viral infections focuses on symptom relief, not fighting the virus. For example, cold medicine helps alleviate the pain and congestion associated with the cold, but it doesn't act directly on the cold virus.

There are some medications that work directly on viruses. These are called antiviral medications. They work by inhibiting the production of virus particles. Some interfere with the production of viral DNA. Others prevent viruses from entering host cells. There are other ways in which these medications work. In general, antiviral medications are most effective when they are taken early on in the course of an initial viral infection or a recurrent outbreak. Different kinds of antiviral medications may be used to treat chickenpox, shingles, herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), HIV, hepatitis B, Hepatitis C and Influenza.[33]

Vaccines can reduce the risk of acquiring some viral illnesses. Vaccines are available to help protect against the flu, hepatitis A, hepatitis B, chickenpox, herpes zoster (shingles), cancer-causing strains of human papillomavirus (HPV), measles/mumps/rubella (MMR), polio, rabies, rotavirus, and other viruses. Vaccines vary in effectiveness and in the number of doses required to confer protection. Some vaccines require booster shots to maintain immunity.

Conclusion

The earth of extreme geo-climatic diversity, faces a constant threat of emerging and re- emerging viral pathogens of public health importance. There is a need for strengthening disease surveillance in the world focusing on the epidemiology and disease burden. There is also a pressing need to gain detailed insight into disease biomics, including vector biology and environmental factors influencing the diseases. It is also important to strengthen the emergency preparedness for these diseases and response by focusing on 'one health' approach.

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Matrix Vesicle Mediated Mineralization - A Review

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Abstract

Teeth are composed of the mineralized tissues dentine and cementum, and a mineralized structure, the dental enamel. The pulp may also mineralize in response to aging processes and/or pathologic aggressions. The process of mineralization is specific for each dental tissue. Matrix vesicles have been implicated in the mineralisation of calcified cartilage, bone and dentin. It is an orchestrated sequence of ultrastructural and biochemical events that lead to crystal nucleation and growth. Matrix vesicles appear to mineralize by concentrating calcium and phosphate at a protected site close to the inner leaflet of the vesicle membrane. Calcium may be attracted by its affinity for acidic phospholipids of the vesicle membrane, and phosphate may be concentrated by the action of transmembrane phosphatases of the matrix vesicle membrane. Evidence is accumulating to suggest that alkaline phosphatase of the matrix vesicle membrane functions as a phosphotransferase or phosphate vector, transporting PO_4 across the vesicle membrane. The influx of phosphate ions into the matrix vesicle is mediated by several proteins such as TNAP, ENPP1, Pit1, annexin and so forth. The catalytic activity of ENPP1 generates pyrophosphate (PPi) using extracellular ATPs as a substrate, and the resultant PPi prevents crystal overgrowth. However, TNAP hydrolyzes PPi into phosphate ion monomers, which are then transported into the matrix vesicle through Pit1. Accumulation of Ca^{2+} and PO_4^{3-} inside matrix vesicles then induces crystalline nucleation, with calcium phosphate crystals budding off radially, puncturing the matrix vesicle's membrane and finally growing out of it to form mineralized nodules. Their exact role, if any, in the nucleation of hydroxyapatite mineral, and its association with collagen fibres The organic Matrix has been debated and is controversial. Several hypotheses have been recently introduced to explain in greater detail how Matrix vesicles function in biomineralization. This review will summarise recent advances, and ultra-structural and biochemical aspects on Matrix vesicles - mediated mineralization.

Keywords: *Matrix vesicle, mineralisation, TNAP, ENPP1, PHOSPHO1, mineralised nodule.*

Introduction

Matrix vesicles refer to small spherical bodies, around 20 to 200 nm observed in pre-mineralized Matrix of dentin, cartilage and bone. They are bounded by lipid bilayer, and are often associated with small crystals of calcium phosphate mineral [1] . Mineralised bone matrix consists of abundant calcium phosphate, type I collagen and non - collagenous organic materials[2]. When the least matrix vesicle contains abundant protein and lipids that are known to chelate inorganic phosphatase (Pi) and Ca^{2+} [3]. These vesicles are often reported to be found in osteoid, mental dentin and calcifying tendons [4]. The density of these particles appear to decrease with the increasing compactness of collagen fibrils in mature bone [5]. Multiple rational theories which describe mineral crystallisation exist. One of the most discussed theories is the new creation of apatite through collagen polypeptide stereochemistry with Ca^{2+} and inorganic phosphate (Pi), where apatite crystals precipitate and propagate from an amorphous phase, in the Gap zone of collagen fibres [6]

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Bone mineralisation is divided into two ultra-structural phases. They are primary and secondary mineralisation. Primary mineralisation is biologically orchestrated by osteoblast and osteoclast during bone modelling and remodelling. It is achieved by fine – tuning synthesis of organic materials and subsequent apposition of calcium phosphates. Secondary mineralisation is a phenomenon by which bone mineral density elevates it after primary mineralisation. This is achieved physicochemically, through crystal maturation and by osteocyte network. The primary mineralisation has two distinct phases: matrix vesicle mediated mineralization and collagen mineralisation [2].

Previously, our team had conducted numerous original studies [7-13] and surveys [14-21] over the past 5 years. Now we are focussing on applying this knowledge to write the review on new recent advancements in various fields. In this review, the ultra structural and biochemical evidence of matrix vesicle mediated mineralization is introduced.

Ultrastructural Evidences of Matrix Vesicle - Mediated Mineralization In Bone

In matrix vesicle mediated mineralisation, osteoblast secrete matrix vesicles on which the membrane transporters and enzymes involved in mineralisation are equipped [22]. Before the introduction of matrix vesicle mediated mineralisation theory, alkaline phosphatase theory was followed. According to alkaline phosphatase theory, alkaline phosphatase supplies more phosphate ion by hydrolyzing phosphate substrates. Then accelerate to form crystalline calcium phosphate [23].

Bone mineralization initiates inside the matrix vesicle. Matrix vesicles are small extracellular vesicles enveloped by a plasma membrane secreted by osteoblast [24]. Chondrocytes and pre-osteoblast differentiate into hypertrophic chondrocytes and osteoblast respectively [25]. These two cell types are mineralisation – competent cells that regulate Matrix calcification by modification of extracellular matrix composition and releasing matrix vesicles [26].

The initial step of extracellular matrix calcification by promoting the deposition of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$ in their lumen [27]. Ion channels and transporters present in matrix vesicle membranes act for Ca^{2+} [28] and inorganic phosphate uptakes into these organelles [29]. By accumulating Ca^{2+} and inorganic phosphate, matrix vesicles create an optimal environment to induce the formation of hydroxyapatite [30]. Then, the breakdown of matrix vesicle membrane releases hydroxyapatite crystals into the extracellular where the mineralization is propagated [3]. These crystals serve as a template for the formation of crystalline arrays, leading to tissue calcification [30]. The extracellular matrix contains sufficiently high concentration of Ca^{2+} And inorganic phosphate concentration to propagate the mineralisation. Negatively charged proteins of the matrix interact with minerals and thus, control their growth, orientation and size [31].

Ultrastructural and Biochemical Function Of Enzymes And Membrane Transporters For Matrix Vesicle - Mediated Mineralization

Abundant Ca^{2+} was evenly distributed in the peripheral region of the matrix vesicle, while PO_4^{3-} was predominantly associated with organic material such as collagen fibrils [32]. Inside the matrix vesicle, "needle-shaped" crystalline calcium phosphates form a stellate assembly, grow in all direction, and then, come out of the vesicle penetrating the plasma membrane to form mineralised nodules, also referred to as calcifying globules [33]. Several enzymes and membrane transporters found in the Matrixx by cycle are involved in metallisation. Among these enzymes and transporters, TNAP, ENPP1, ANK, PHOSPHO1, PIT1, appear to play pivotal roles in phosphate transport on matrix vesicle mediated mineralisation [2].

Tissue Nonspecific Alkaline Phosphatase (Tnap)

One of the most important enzymes that initiate mineralisation inborn must be TNAP, a glycosylphosphatidylinositol anchor enzyme associated with cell membrane. TNAP is responsible for the production of inorganic phosphate. Hence it is called a potent inducer of mineralisation [34]. The distribution of TNAP on cell membranes is not uniform in osteoblast, which are polar cells with distinct basolateral and secretary domains [35].

The ratio of inorganic pyrophosphate (PPi) to inorganic phosphate (Pi) is important in the promotion or restriction of minerals in physiological tissue. TNAP

is one of the regulators of the extracellular PPi/Pi ratio. TNAP, encoded by Alpl, is abundant on the surface of matrix vesicles derived from osteoblast, hypertrophic chondrocytes and odontoblast [36]. TNAP produces an environment surrounding matrix vesicles conducive to mineralization not only through regulation of PPi/Pi Ratio but also through modulating the phosphorylation status of osteopontin. Osteopontin inhibits nucleation and growth of hydroxyapatite [37]. Hypophosphatasia, is a clinically heterogeneous heritable disorder characterised by defective bone mineralization which is caused due to impaired TNAP activity due to mutation of TNAP gene [38]. The phosphatase associated with the osteoidal aspect of TNSALP, which is restricted to the basolateral domains of osteoblasts [39].

Ecto-nucleotide Pyrophosphatase/ Phosphodiesterase (ENNP1)

ENPP1 he is a member of the family ENPP family of proteins. It is composed of 2 N-terminal somatomedin B (SMB) - like domain, a catalytic domain and nuclease like domain. The catalytic and nuclease like domains for bone mineralisation and SMB like domains for insulin signalling respectively [40]. ENPP1 is one of the regulators of the extracellular PPi/Pi ratio [41]. ENNP1, that's not have a major PPi generating role, but rather can act as a 'backup' phosphatase in the absence of TNAP. This role as a 'Plan B' phosphatase is proposed as the reason why the PHOSPHO1 , mineralisation of the axial skeleton can occur occasionally [42].

An Emerging consensus now emphasises the central role of the matrix vesicle enzymes TNAP and NPP1, in conjunction with the cell associated and ankylosis protein (ANK), in regulating the onset of calcification [43]. NPP1 along with ANK function to suppress mineralization by increasing the extracellular concentration of the calcification inhibitor, PPi (pyrophosphate) [44]. In human infants, severe ENPP1 deficient state's were recently linked to a syndrome of spontaneous infantile arterial and periarticular mineralization [45].

Ankylosis (Ank)

Ankylosis is an non-enzymatic plasma membrane pyrophosphate (PPi) channel. It is encoded by mouse progressive ankylosis (Ank) gene [46]. Ank Gene appears to regulate both intra and extracellular levels of an important inhibitor of hydroxyapatite crystal formation [47]. The infant carrying Ank mutation gene caused a 3-to-5 fold decrease in extracellular level of inorganic pyrophosphate in contrast to stimulatory effect on intracellular inorganic pyrophosphate. With the loss of ANK activity, the extracellular inorganic pyrophosphate levels attenuate, the intracellular inorganic pyrophosphate levels rise, and unregulated mineralisation begins in joints and tissues [2]. Extracellular inorganic pyrophosphate is formed from extracellular nucleoside triphosphate (NTP) by NPP1 and exported from cells is through the action of ANK. It is hydrolysed to inorganic phosphate by TNAP [1].

PHOSPHO 1

PHOSPHO 1 is Enzyme highly expressed in minimising cells like in bone and cartilage, with systematic name phosphoethanolamine phosphohydrolase [48]. PHOSPHO 1 is a Phosphate and a member of the halo acid dehalogenase superfamily. It is essential for the initiation of skeletal mineralisation [41]. PHOSPHO1 appears to serve for initial mineralisation inside matrix vesicles [49]. PHOSPHO1 appears to serve for initial mineralization inside matrix vesicle [2].

FORMATION AND DEVELOPMENT OF MINERALIZED NODULES

Mineralised nodules is a globular assembly of numerous needle shaped mineral crystals that has been exposed to an extracellular environment for matrix vesicles [2]. Osteopontin is suited to the task of regulating mineralisation because it effectively inhibits apatite formation and growth [50]. Osteocalcin may play an important role in the globular assembly of needle shaped mineral crystals, probably binding together the organic component of the crystal [51]. Zinc (Zn^{2+}) has long been known to play important roles in mineralization and ossification of skeletal tissues, but the mechanisms of Zn^{2+} action are not well understood. the effects of Zn^{2+} on mineralization in a cell culture system in which terminal differentiation and mineralization of hypertrophic growth plate chondrocytes was induced by retinoic acid (RA) treatment. Addition of Zn^{2+} to RA□treated cultures decreased mineralization in a dose□dependent manner without affecting alkaline phosphatase (APase) activity. Characterization of matrix vesicles, particles that initiate the mineralization process, revealed that vesicles

isolated from RA-treated and RA/Zn^{2+}-treated cultures showed similar APase activity, but vesicles from RA/Zn^{2+}-treated cultures contained significantly less Ca^{2+} and Pi. MVs isolated from RA-treated cultures were able to take up Ca^{2+} and mineralize in vitro, whereas vesicles isolated from RA/Zn^{2+}-treated cultures were not able to do so [52].

ULTRASTRUCTURE OF COLLAGEN MINERALIZATION

After the beginning of matrix vesicle mediated mineralization, mineralized nodules would contact the surrounding collagen fibrils. There are two theories explaining collagen mineralisation: Hole zone theory and one supporting that mineralization. Initial mineralization begins in the collagen fibrils, 'holds'. While decorin/biglycan-double knockout mice revealed osteopenia as a result of impaired GAG - linking to decorin and biglycan cone protein, mineralization was not stimulated [53].

Conclusion

Our current knowledge of mineral vesicles is undoubtedly building towards the foundation in understanding the complex mechanism of the development of matrix mineralisation. It causes a series of orchestrated ultrastructural and biochemical events in bone to achieve proper mineralisation, a variety of membrane transporters and enzymes are put to work.

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Brain Distinguish Speech From Music - A Review

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Abstract

Human potential is available both to speak and to listen to music. These are the important features in a person's life. Music aims at the language system's own efficacy that relies on the disposition of speech. Both music and speech share a tone of voice. The Relationship between music and speech has been a topic of much debate. In general, the idea that we have a rational left brain and a creative right brain is a myth, but how the brain circuitry for processing music and language works is still not clear.The topic is important because it speaks directly to the nature of evolved human cognition, these features overlap at times which leads to neuro comparative study of this process. This review was done based on articles obtained from various platforms like PubMed, PubMed central and Google scholar. They were collected with a restriction in time basis from 1970-2020. The inclusion were original research papers, in vitro studied among various conditions and articles that contain pros and cons. Exclusion criteria came into account for review articles, retracted articles and articles of other languages. All the articles were selected based on the brain distinguishing between song and speech.The information about the brain separating speech from song was searched in relevant search engines and relevant researches. The Knowledge and current point of analysis. Then Consensus we're established. This study explains that the right and left hemisphere of the brain distinguish between the song and speech and also this study gives clear information about the following.

Keywords: *Music, Speech, Hemispheres, Brain, Cognition*

Introduction

Human potential is available both to speak and to listen to music. These are the important features in a person's life. Music aims at the language system's own efficacy that relies on disposition of speech. Both music and speech share a tone of voice. Speech and music are human universals, and people around the world often blend them into vocal songs. This entwinement of the speech and music cognitive domains is a challenge for the auditory cognitive system. Speech and musical sound are thoughts that differ in details of their acoustic structure and thus activate different receptive preferences of the left and right auditory cortices of the brain. There are doubts about degradation in each acoustic dimension that would affect the brain activity. They found that degradation of the spectral dimensions only influenced activity in the right auditory cortex, and only during melody perception.in contrast, degradation of the temporal dimensions affected only the auditory cortex, and just during speech perception. This shows the differential response. Each hemisphere relies upon the kind of acoustical data in the stimulus. Music and speech exploit different ends of the spectro temporal continuum and that hemispheric specialisation may be the nervous system's way of optimising the processing of these two communication methods.

It has been known for decades that the two hemispheres respond to speech and music differently,

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but the physiological basis for this difference remains a mystery[1]. Brain regions are similarly and differently involved in listening and converting the production of singing related to speech. Use of auditory motor self monitoring consonance in the singing, brain regions involved processes and prediction to be differentially active for singing more than for speech[2]. Neural connections help process music and speech coactivation of brain regions, neuroimaging studies neural circurities for language recycled during evolution for musicality served as a springboard for language emergence[3]. Brain regions frontal, temporal lobes, activates speech and song and its acoustic features[4]. Language focuses on Broca's area, prefrontal cortex, Wernicke's area, superior temporal gyrus. lexicon accessed,articulate fasciculus,Left and right brain, per motor,somato-sensory and inferior temporal gyrus,left plenum temporal which has greater memory[5]. Previously our team has conducted numerous clinical trials,studies and reviews about the upcoming topics [6–12]. The main aim of this study is to understand the difference and responses that separates the song from speech.

MATERIALS AND METHODS

This review was done based on articles obtained from various platforms like PubMed, PubMed central and Google scholar. They were collected with a restriction in time basis from 1970-2020. The inclusion were original research papers, in vitro studied among various conditions and articles that contain pros and cons. Exclusion criteria came into account for review articles, retracted articles and articles of other languages. All the articles were selected based on the brain distinguishing between song and speech.

They are determined by article title, abstract and complete article. When article holder websites were analysed on the topic of brain distinguishing between speech and song, more than 2000 articles and based articles were found, when it was shortlisted based on inclusion and exclusion criteria, the number of articles were lowered to 100 articles. When timeline and other factors were quoted only 25 articles came into play. This article is reviewed from 25 articles collected. The data was collected and quality analysis of the collected data was done using Health Evidence's Quality Assessment Tool (*Health Evidence^{TM} Quality Assessment Tool*, no date) and the data was shown in a tabular column. (Table 1). The knowledge at current point of time analysed and thus the consensus was established.

Table 1: QUALITY ANALYSIS OF THE STUDIES REFERRED :

AUTHOR	YEAR	QUALITY ANALYSIS
Daniel Samler	2020	Strong(9)
Callan DE	2006	Moderate(6)
Jackendoff.R	2009	Moderate(6)
Belin.D	2000	Moderate(6)
Koelsch.S	2002	Moderate(7)
Jancke	2020	Moderate(7)
Peretz	2015	Moderate(6)
Evelina	2012	Strong(8)
Corinne	2013	Moderate(7)
Aniruddh	2013	Strong(8)
Samuel	2015	Moderate(7)
Baheerati	2018	Moderate(5)
Fathima	2016	Moderate(6)
Rj I	2016	Moderate(6)
Priya J	2015	Moderate(7)

Table 1: QUALITY ANALYSIS OF THE STUDIES REFERRED :

Dave PH	2016	Moderate(5)
Koelsch S	2002	Moderate(6)
David	2019	Moderate(6)
Shruthi	2018	Moderate(5)
Choudhari	2016	Moderate(7)
Iyer PK	2019	Moderate(6)
Gayatri	2018	Moderate(7)
Swathy	2015	Moderate(6)
Renuka	2015	Moderate(7)
Timothy CN	2019	Moderate(7)

Discussion

Two of the most uniquely human uses of sound is enabled by specialised neural systems in different brain hemispheres adapted to respond differently to specific features in the acoustic structure of the song.music and speech are often inextricably entwined and ability of human to recognise and separate words from melodies in a single continuous sound wave represent a significant cognitive challenge.Brain decode sound using two separate measures.one assesses how quickly sound fluctuates over time.The other detects the frequencies in a sound stroke.In left hemisphere you are much more likely to have a language impairment than if your stroke is in the right hemisphere.When it was scanned through fMRI it resulted that people decoded sounds same as the songbirds do by separating sounds time.Related elements from the frequencies it contains and processing the information using two different groups of specialised brain cells.Hence when song is heard it engages both the hemispheres. Degradation of the temporal information impaired speech recognition but not melody recognition. On the other hand,the perception of the melody decreased only with spectral degradation of the song. There are certain researches that conclude that speech is separated in the left hemisphere of the brain and melody in the right hemisphere of the brain[13]. A particular article explained neural overlap occurs in music and speech[14]. Music has a high impact on people[15]. Several recent studies suggest that speech and music perception recruit shared computational systems,and a common substrate in the Broca's area for hierarchical processing has recently been processed[16]. Growing body suggest musical training which has an impact in the processing of speech[17]. Previously our team has also conducted studies and done reviews most importantly based with lab animals [18–22] and few were in vitro[23,2425].

Limitations Of Study

- Intensive study can't be done.
- Study could have been done by analysing or testing human beings.
- Basic Analysis gives inaccurate results.

Future Scope

- Speech and song separation is only discussed within the right and left hemisphere.
- Physiological connections have been a mystery for which more research in the future could give answers.
- Future analysis done regarding this topic could bring clarity to the present researchers.

Conclusion

Brain is one of the important organs which controls the whole body. It has many functions by its neuronal connection. This review may help the people to understand better how the brain separates speech and song by the right and left hemisphere. This helps to interpret the neuronal connections and the control of

speech and song.

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Conflict of Interest: All the authors declare no conflict of interest in the study

Ethical Clearance: As it is a review article so it is not required.

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Animal Model Used in Dentistry-A Review

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Abstract

The main aim of the study was to know about the various dental studies carried out on animal models. Animals have been targeted for various medical investigations for human observations. From the early nineteenth century, there was a significant increase in the use of animals for scientific experiments . Compared to small animal models like rodents, large animal models are superior in many aspects for the study of human diseases and pre-clinical therapies.This is significant to develop new medicines for newer diseases emerging day by day in the current world. Animals are also used for the safety test to know the potential of medicines before they are being clinically introduced in suffering patients to make sure it doesn't show adverse effects and to check the safety of other chemicals as well. A review is done on different dental studies carried out in animal models which were collected from search engines like pubmed , google scholar, google chrome and from various research articles.Quality analysis was done using the ideal method. The review helps in attaining complete knowledge about dental related studies on animal models. Studies like these can prove to be helpful in further development concerned with humans. This review gives a clear view about the different dental studies carried out in animal models and it's observation and outcomes.

Keywords: *Animal models, dental study, development, procedures*

Introduction

Animals are used in scientific research to help us understand our own bodies in a better way and how they work. This is significant to develop new medicines for newer diseases emerging day by day. Animals are also used to safety test potential medicines before they are tested in people to make sure it doesn't show adverse effects and to check the safety of other chemicals as well. Animals , right from the fruit fly to the mouse, are widely used in scientific research occurring in numerous platforms[1]. They are very crucial for allowing scientists and researchers to learn more about human biology and health, and for multiple developments.The use of animals in scientific research has always been the subject of heated debate for the longest time. On the one hand it is considered morally wrong to use animals in this way solely for human benefit which is painful. But, removing animals completely from the lab would impede the understanding of health and diseases, and consequently affect the development of new and vital treatments. Although sometimes these studies can reduce the quality of life of these animals , thorough regulations are in place appropriately to ensure that they are carried out in a proper way. To help minimise the harm animals usually experience while being studied in the laboratory, researchers are required to follow a set of these protocols which include the 'three Rs'.

These are

Replace: Replacing, where and whenever possible, experiments using animals with alternative techniques such as cell culture in microbiology labs, computer modelling or human volunteers instead of animals, everytime[2].

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Reduce: Reducing the number of animals being used and by improving experimental techniques and sharing information with other researchers so that the same experiments aren't being done by the other fellow researchers.

Refine: Refining the way the animals are being taken care of. To help minimise any kind of stress or pain, by using less invasive techniques wherever possible and improving medical care and living conditions for such animal models [3].Animals have been targets for investigations and observations for mankind.Animal research has been important in the development of many major medical advances. Studies that use animals have played a role in the prevention or treatment of conditions as diverse as tuberculosis, diabetes, polio, parkinson's disease, muscular dystrophy and high blood pressure[4]. From the early nineteenth century , there has been a significant increase in the use of animal scientific experiments[5]. Studies carried out in ex-vivo I.e procedures done in animals for research are mainly done before performing them in-vivo. The most preferred animals used for medical and dental studies are rats, mice, guinea pigs, rabbits, beagle dogs, goats, and nonhuman primates[6]. The selection of an experimental model is determined according to research objectives, as well as constraints such as housing of large or nonstandard animals[7,8].Small animal models generate substantial and relevant data on the interactions between the soft and hard tissue, especially during inflammation [9].

On the other hand, the major drawback of using small animals is that they show very limited similarity in dentins of humans when dental experiments are concerned[10]. Compared to small animal models like rodents, large animal models are superior in many aspects for the study of human diseases and pre-clinical therapies but their usage is difficult[11]. The dental anatomy of larger species of animals resembles human dentoalveolar architecture more closely than that of smaller species[12]. They allow a more direct interpretation of the data and translation of the knowledge to clinical settings. Several large-animal species, such as nonhuman primates, dogs, sheep and miniature pigs, have been traditionally used in periodontal research. Nonhuman primates have oral structures and teeth similar to those of humans and have naturally occurring dental plaque, calculus, oral microbial pathogens and periodontal disease[13]. Mice are the smallest species used extensively in dental studies. There are several advantages of a mouse as a model. They are extremely cost-effective when compared to larger animals.Rabbits are also preferably good small animal models for studying the impact of inflammation on periodontal wound healing and regeneration. However, with the new advancement of ex vivo animal models, it has become easy to investigate disease pathogenesis and to test the efficacy of newer therapeutic modalities with the reduced usage of animal models[14].

The appropriate guidelines to use animals in researches is as follows :

- Depending only on the need,one should allow and provide facilities to use animal models for all kinds of research purposes.
- It is recommended for all the researchers that one should not use animals unless it is an unavoidable situation and circumstance.[15]
- It should be ensured that unnecessary pain or injury to the animals should be evaded.
- It should provide adequate care, housing, and make sure that the animal models used for research purposes are physically comfortable and in good state of health.
- Sources of experimental animals for research should be from recognized animal facilities where there will be availability of genetic, health, and nutritional status of each animal model.[16]
- It should provide training facilities to the scientific researcher and the supporting staff those who take care of the animals during experiment.[17]
- Alternative animal models should be used to replace experimental animals wherever possible and least usage should be kept in mind.[18]
- Procedures which are painful should ensure appropriate use of anesthesia.
- Forming ethical committees will ensure the minimal usage of animal models.[19]

Different animal Profiles

It is extremely important to know about animal

profiles prior their use in any field of research. Animal profile includes physiological norms of commonly used laboratory models like mouse, rat, hamster, guinea pig, rabbit, cat, dog, and monkey etc. Maturity of age , adult weight, respiratory rate, rectal temperature, pulse rate per minute, and life span are some of the physiologic norms of the healthy animal models to follow during selection of research.[2021]

Table 1.1 Different animal profiles used in various fields of research

Animal	Age at maturity:	adult weight:	respiratory rate:	rectal temperature:	pulse rate per min:	life span:
Mouse	6–8 weeks	25–30 g	90–180 per min	37.4°C	600 on average	1.5–2 years
Rat	10–12 weeks	200–300 g	80–150 per min	37.5°C	300 on average	2.5–3 years
Hamster	6–8 weeks	80–100 g	40–120 per min	37.6°C	450 on average	1.5–2 years
Guinea pig22	16–20 weeks	400–500 g	60–110 per min	38.6°C	150 on average	4–5 years
Rabbit	24–32 weeks	2–2.5 kg	35–56 per min	38.7°C	133 on average	4–5 years
Dog 23	1–1.2 years	12–15 kg	14–28 per min	38.6°C	95 on average	10–15 years
Monkey 24	4–5 years	10–12 kg	30–54 per min	38.4°C	200 on average	15–20 years
cat	30–35 weeks	3–5 kg	20–30 per min	39.5°C	110 on average	8–12 years

The current study discusses the various researches in the field of dentistry and related areas of interest. This study would enable one to know about procedures carried out in animal models and its outcome as well(figure 1).

Materials And Methods

A review literature was done on different dental studies carried out in animal models. The system and data based searches for the relevant articles were done and collected from search engines like pubmed , google scholar, google chrome and from various research articles. The articles were primarily dated back till 2000 but few references were dated even earlier. About 50 articles were collected , analysed and reviewed. Quality analysis was done using the ideal method using the health evidence (quality analysis tool) and it was scored as weak , moderate and strong and also by grading them on various levels from level 1 to 5.

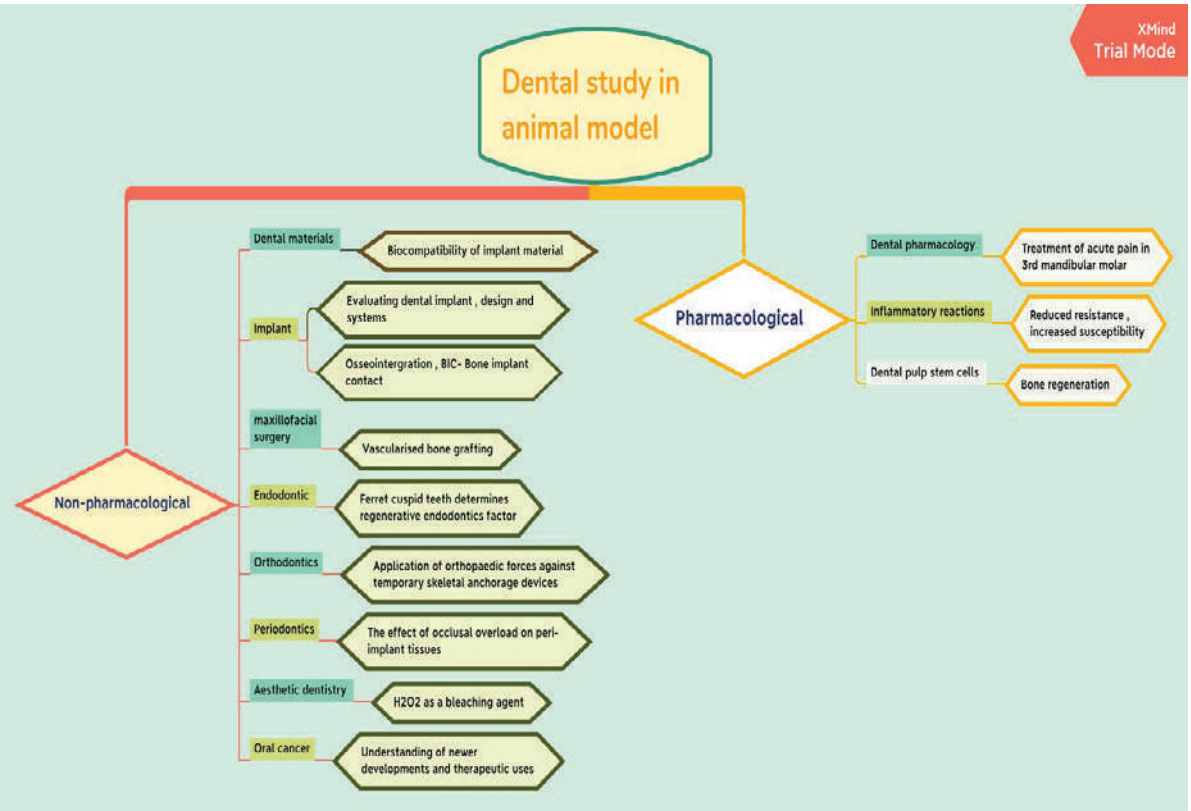


Figure 1: Flow chart describing the branches of studies involved.

Discussion

Dental pharmacology and animal model

Treatment of acute pain after the removal of mandibular 3rd molars can be used to study in a dental pain model and compared to the analgesic efficacy of a new, orally administered NSAID, lornoxicam, with another potent NSAID in a double-blind. Placebo-controlled randomized was used in the previous literature.[25]

Oral and maxillofacial surgery

The oral maxillofacial region of miniature pigs is similar to the ones seen in humans. The similarities include anatomy, physiological development , pathophysiology and the disease occurrence as well[2627]. Reaction of bone tissue was determined after placement of two different types of in vascularised bone grafting , the results revealed that a hydroxyapatite-coated implant as well as a titanium screw-type implant might be applied with a vascularized bone graft for jaw reconstruction.

Relationship between dental erosion and GERD

GERD-Gastro esophageal reflux disorder is a condition where the mucosal layer gets eroded due to stomach acid reflux into the esophagus . The study reveals the fact that there is an existence of a relationship between the dental erosion and GERD. Dental erosion was only detected in the reflux rats first .Relationship between dental erosion and GERD was observed First step of dental erosion caused by GERD is the loss of surface enamel induced by regurgitation of an acidic liquid and acidic gas[28].

Biocompatibility between dental materials

The impact of bone compression on bone to implant contact of an osseointegration is called bone impact contact The biocompatibility of two implant materials, vitallium and poly-methyl methacrylate (PMMA), was evaluated in an animal model in which bone formation and resorption are independent of each other. Neither material accelerated resorption, but formation was inhibited 12% by vitallium and 38% by PMMA[29].

Endosteal dental implants

One-hundred and twenty endosteal dental implants were inserted bilaterally in the mandibles of 30 adult mongrel dogs after bilateral extraction of all premolars. describes an animal model that is useful in evaluating dental implant designs and compares the results with those from humans[30].

Regenerative endodontics

Study was done to investigate ferret cuspid teeth as a model to investigate the factors involved in regenerative endodontics.Each animal was anesthetized and perfused with 10% of buffered formalin[31]. Block sections including the mandibular and maxillary canine teeth and their surrounding periapical tissues were obtained then radiographed, decalcified, sectioned, and stained with the H and E (hematoxylin-eosin) to determine and study the various stages of apical closure in these teeth.Thus the study concludes that ferret cuspid teeth can be used to investigate various factors involved in regenerative endodontics that cannot be tested in human subjects easily [32].

Speed surgical orthodontics(SSO)

Speedy surgical orthodontics (SSO), which is an innovative orthodontic treatment, involves the application of orthopedic forces against temporary skeletal anchorage devices following the suprasegmental corticotomy to induce movement of specific dental segments[33]. For the study 5 beagle dogs were divided into 2 groups and their 6 maxillary incisors were retracted en masse by applying 500 g orthopedic force against a single palatal mini-plate.All animals were killed on the 70th day, and their periodontal structures were processed for histologic analyses and scanning electronic microscopy (SEM)[3435].

Effect of occlusal load

Evaluates the effect of occlusal overload (OV) on peri-implant tissue health in animal studies.Data on OV on stable implants are limited and conflicting. OV may lead to bone loss in the presence of dental plaque and to an increase in BD in areas where plaque control is performed[36].

The complex microenvironment of any periodontal wound creates many challenges linked with multi tissue regeneration of periodontal lesions. Recent characterization of mesenchymal stem cells-like populations residing in periodontal ligament tissues has shown that these cells exhibit features of postnatal stem cells. The aim of this study was to assess the regenerative potential of (PDLSCs) also called allogeneic periodontal ligament stem cells in a rat periodontal fenestration defect mode and to identify an optimal end time-point suitable for quantitative assessment of tissue regeneration[37].

Radiology

Hepatic cryoablation was done in rabbits with VX2 tumors and in two healthy rabbits. Nonenhanced and dynamic contrast material–enhanced computed tomography (CT) and magnetic resonance (MR) imaging and power and color Doppler flow ultrasonography (US) were performed 7–8 days after cryoablation.In this animal model, recurrent tumor typically appeared as focal nodules at the cryolesion periphery. The rim and central foci of enhancement, doppler flow, and increased signal intensity on T2-weighted MR images can be normal findings after hepatic cryoablation[38].

Implantology

Development of optimal interface between bone orthopaedics and dental implants has been taking place for many years and animal models are often an essential step for testing of orthopaedic dental implants prior their use clinically in humans[39]. Some of the more commonly available and frequently used models are of the dog, sheep , goat, pig , rabbit for evaluation of bone-implant interactions[40,41].Insertion Torque Resonance Frequency , Analysis of dental Implant systems in animal Model Loaded with Implants[42].Thirty-two implants were placed in 16 beagle dogs maximum insertion torque values were recorded. After a healing period of 8 weeks the implants were loaded for 3 months , the animals were then sacrificed[43]. Studies from animal subjects indicate that irradiated bone has a greater risk of implant failure than non-irradiated bone[44]. Animal clinical studies differ on the importance of dose effect on implant location regarding implant survival[4546]. Influence of multiple variables with different animal models monkeys, dogs, pigs, rats, rabbits, sheep leading to results that might not be comparable reliably on clinical implications on

performance in humans , estimate percentage of patients who would experience different amounts apical root shortening[47]. Diamond coated titanium dental implant used in nanodentistry: Nano-crystalline diamond-coated titanium dental implants – A histomorphometric study in adult domestic pigs[48].Twenty four MWP-CVD diamond-coated and twenty four un-coated dental titanium-alloy implants were placed in the frontal skull of eight adult domestic pigs. To evaluate the effects of the nano-structured surfaces on bone formation, a histomorphometric analysis was performed after 2 and 5 months of implant healing.[49]

Aesthetic dentistry

Hydrogen peroxidase, commonly known as H2O2 is a bleaching agent used in aesthetic dentistry. It possesses bleaching efficacy due to its ability of penetrating the tooth structures and oxidized organic molecules. The inflammatory tissue response in rats teeth after dental bleaching is performed and seems to be greater in diabetic rats. Reproducible Experimental model that directly relates Kronick biphosphate administration development, osteonecrosis with and without the extraction with no drug involved. New animal models of BRONJ after zoledronic acid administration, dental extractions achieving bone changes are similar or superior[50].

Inflammatory reaction infection

Diabetes mellitus is a complex multisystemic disorder with elevated prevalence. Patients' diabetes with reduced resistance to bacterial infection and decreased tissue repair ability will eventually contribute to an increased susceptibility to inflammatory reactions infections[51].

DPSCs- Dental pulp stem cells:

Evaluating frequency of dental pulp stem cells for bone regeneration. Stem cells are the ones with self regenerating ability[52]. They are capable of differentiating into multiple cell lineages as a promising cell-based tissue. Significantly no healing period is generally accepted as a prerequisite for osseointegration of dental implantology[5354].

A histological assessment of intruded teeth:

The study evaluated the histological examination of surgical repositioning of the intruded dog hit upon the pulpal and surrounding tissue. 30 teeth in 10 adult dogs, between the age group of 2 to 4 were used. The observation was done at the seventh, 15th and 30th postoperative days; it was later concluded that the pulpal necrosis, external root resorption and losses were common sequelae to serve traumatic intrusions[55].

Canine periodontics

Periodontal disease refers to a group of inflammatory diseases caused by bacterial plaque in the periodontium and are ranged in various stages. Periodontal disease had an enormous impact on humans and veterinary medicines due to its high prevalence. The commonest animal as a periodontal disease model is the dog and they are used for having useful approaches for understanding human periodontal diseases in a new therapeutic and preventive measure[56].

Maxillary sinus floor elevation

The study uses 12 fresh heads of 4 merino sheep , 4 Marciano granadina goats , 4 large white pigs. Two schools from each species were used and several 3-D pictures of them were taken. The study concludes that the approach of maxillary sinus in sheep, goat and pigs from the buckle vestibule required a previous surgical enlargement of the vestibule[57].

Animal model in oral cancer research

Oral cancer is the world's 11th most common human new place yeah. No animal model, of course, is perfectly applicable to any kind of human cancer. Even so, it is generally agreed that animal model is a virtually inevitable preclude to understanding how new development takes place and helps in assessing efficiency of new therapeutic approaches for such cases[58].

Conclusion

In conclusion, the review shows that there have been multiple experimental studies that have been carried out in animals and are being done currently as well the main purpose is to make the best out of these places ex-vivo studies. This will make future research easier and simpler by knowing appropriate usage of animal models and different procedures carried out in them.

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Lasers in Endodontics- A Review of Literature

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Abstract

Lasers are commonly used to reshape our gums during root canal procedures and to kill bacteria. Application of lasers in dentistry has been increased due to its safety and effectiveness in dental treatments. Lasers have been used as coadjuvant treatment in endodontic therapy with respect to both low-intensity laser therapy (LILT) and high-intensity laser treatment (HILT) to improve clinical procedures' success rate. The major procedures done with lasers in endodontics are dentin hypersensitivity, removal of carious tissues, tooth preparations, pulp capping or pulpotomy and also in root canal treatment.Lasers are often used to remove mouth lesions and to soothe discomfort. A thorough literature search was performed using the database like Pubmed, google scholar, BioRxiv, MESH database using the keywords 'light amplification device' and 'Endodontics' and also their types with no date and year restrictions.12 articles with similar data have been found which were analysed and have been included in this study. Talulation were done using google documents. The recent articles discussed in this study help us in gaining further knowledge about lasers and their usage in endodontics. The various methods of using lasers discussed in this article has been widely used by dental practitioners with 100% success rate. So it has been concluded that lasers are well established instruments. Upcoming research based on lasers are showing more benefits and ability to perform less invasive procedures with greater comfort of patients has led to the development of lasers in modern dentistry especially in endodontics.

Keywords: *Endodontics; Light amplification devices; Soft tissue and hard tissue lasers; Diode lasers ablation; Analgesic effects.*

Introduction

Lasers are generally used to reshape our gums during root canal procedures and to kill bacteria. Lasers can also be used for excising a small piece of tissue to examine cancer cells known as biopsy or removal of lesions. Lasers are also used to remove mouth lesions and soothe pain[1].

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Application of lasers in dentistry has been increased due to its safety and effectiveness in dental treatments. The major procedures done with lasers in endodontics are dentin hypersensitivity, removal of carious tissues, tooth preparations, pulp capping or pulpotomy, enamel erosion , and also in root canal treatment. Lasers are also used for tooth bleaching and diagnosis of dental pulp vitality. Root canal preparation can also be done during Rotary instruments but dealing with lasers is comparatively easier[1,2].

The term LASER is an abbreviation for 'Light Amplification by The stimulated Emission of radiation'. There are various types of application of lasers in endodontics that are mainly classified as hard tissue lasers and soft tissue lasers. Paste containing Bioactive Glass and Topical cream containing Casein Phosphopeptide has the ability to remineralize the tooth

structures[3]. The various hard tissue applications are to prevention of caries, bleaching, restorative removal and curing, cavity preparation, dentinal hypersensitivity and for various diagnostic purposes. Whereas incase of soft tissue application it includes wound healing, removal of hyperplastic tissue, Photodynamic therapy and also photostimulation of few lesions. Usage of lasers has been proved as the most effective treatment as it provides a painless, specific, efficient, cost efficient, scoreless and comfortable dental treatment[4]. When compared to previous studies post operative pain is felt after using endodontic needle and endoactivator which is now replaced by painless laser therapies. Carbon Dioxide, Neodymium Yttrium aluminium garnet and Er.YAG used in case of both hard and soft tissue application but this is quite an expensive treatment so not recommended most of the time and it may also cause pulpal injury. Usage of low cost devices is preferred widely known as Low level laser therapy or biostimulation[3,5].

Lasers after approaching an appropriate wavelength for melting the hard tissue, This is said to be the main application of laser technology In surgical endodontics[6]. Chlorhexidine with sodium hypochlorite, neem and tulsi can be used for irrigation during endodontic surgeries[7,8]. Lasers used for Dentinal hypersensitivity can be classified into low output power lasers and Middle output power lasers. Initially a low out power laser has been used to support wound healing and also healing of fractures[9]. They also have an anti-inflammatory effect which helps in stimulation of nerve cells[10].

Microwave Amplification by stimulated emission radiation was developed later. Lasers are used in determination of pulp vitality and also in Apicoectomy, Retrograde and endodontics apical cavity preparation and periapical curettage[10].

There are lasers Generate visible light, ultraviolet rays, Infrared rays, Invisible rays. Generally, electrons jump from higher energy levels to lower energy levels to emit light photons**.** Lasers are also used to diagnose Dental pulp vitality**.** The main aim of our study is to provide knowledge about usage and purpose of lasers in Endodontics[11].

Materials and Methods

A thorough literature search was performed using the database like Pubmed,google scholar, BioRxiv, MESH database using the keywords 'light amplification device' and 'Endodontics' and also their types with no date and year restrictions. The language is restricted to English. 12 articles with similar data have been found which were analysed and have been included in this study. Talulation were done using google documents.

Result and Discussion

Applications of lasers in endodontics

The main usage of lasers in endodontics will be discussed in this chapter. The first and foremost application as a diagnostic tool for endodontics is the analgesic effects of laser, Dentin hypersensitivity, Treatment of vital pulpal tissue by pulpectomy and direct pulp capping[12].

Root canal disinfection and irrigation, Access cavity preparation and root canal orifice enlargement[13], Root canal wall preparation, sweeping of the root canal and irrigation, sterilisation or disinfection of the root canals[14], obturation with gutta percha or resin, removal of the temporary cavity filling materials- Root canal sealing materials and also fractured instruments inside the root canal[15].

Lasers are also used incase of vertical root fracture diagnosis and treatment[16], laser assisted obturation removal and gutta percha obturation material[17].

Usage of lasers in endodontic surgeries include: Flap preparation, cutting bond to prepare window access to the apex of the roots[18], Apicoectomy, Root end preparation for retro fill amalgam or composite and also for removal of pathological and hyperplastic tissue[19] .

Variation in between hard and soft tissue lasers and their application in endodontics

Hard tissue application is for prevention of caries, bleaching, restorative removal and curing, cavity preparation and also for diagnostic purposes but soft tissue laser application is for wound healing, removal of hyperplastic tissues to uncover the impacted tooth[20].

Mechanism of lasers

The first and foremost mechanism of lasers is the direct application of laser irradiation on the electric

activity of nerve fibres which happens within the dental pulp[21].

The second mechanism of lasers modification of the tubular structure of dentin by melting or fusing of hard tissue layer or smear layer and then subsequently dentinal tubules are sealed[22].

Lasers use Photo-Thermal and photomechanical effects which is resulting from the interaction of different wavelengths and various other parameters on the target tissue. Especially dentine, Smear layer, debris, residual pulp and bacteria in their aggregate forms[23].

This can also be told as a light beam from the optical fibre entering the tissue has been Absorbed in the blood cells and fibre tips and collects the scattered light which inturn provides information about the pulp vitality. This provides a painless, invasive and scarless treatment but takes longer than other vitality determination techniques**.**

Lasers for diagnosing pulp vitality

LASER Doppler flowmetry is a minimally - intrusive assessment technique and measures the flow of pulp tissue through blood. Benefits include; The technique is more objective and more reliable in evaluating the Pulp Tissue Safety. These have benefits in data storage, for Measurements which are to be compared later. Includes drawbacks, Its technique is sensitive and requires putty splint preparation to hold the probes, and it's costly[24].

Dentinal hypersensitivity

Lasers used in treatment are divided into two different groups: 1. Lasers with low power output (He-Ne and Gallium / Aluminum/ Lasers with arsenide [Ga / Al / As]), 2. Lasers with low power output (Nd : YAG and CO2 Lasers) Lasers. Clinically and under SEM, Kumar and Mehta assessed the Nd : YAG Laser Irradiation Effectiveness alone and in combination 5% Sodium fluoride varnish used in dentine care feeling hypersensitive. They discovered Nd : YAG combination Laser and 5 percent varnish of sodium fluoride appeared to be impressive effectiveness as compared with a single therapy, Aranha et al. evaluated dentine hypersensitivity in the treatment of Impact of the lasers Nd : YAG and Er: YAG on dentine reduction permeability through sealing of open tubules.

Tests showed that the Er : YAG laser was 60 mJ, 2 Hz and the Nd : YAG laser is useful for decreasing at 1.5 W, 15 Hz permeability to the dentin. Laser efficiency for treating DH varies from 5% to 100%, depending on the laser type and parameters to diagnosis. Studies suggested the Nd: YAG Laser, Er : YAG laser and Ga-Al-A reduced laser low-level hypersensitivity to dentine[25].

Root canal shaping

The shaping of root canals aims at removing organic tissues and facilitates irrigation and the obturation of canals. Laser spotlights can be delivered via an optical fiber that enables better delivery the root canals are open. The technique requires more extension of the root canal before the laser probes by conventional methods can be put into a canal[26].

Use of lasers in periradicular surgery

Apicectomy is an surgical procedure in which the root apex is used removes; removes adjacent periapical tissues and curetted simultaneously. LASER which is used for surgery in a bloodless Surgical environment by vaporization and coagulation of tissue and the sealing of little blood vessels. Where the cut surface irradiates, the surface is sealed and sterilised. Er : YAG laser could even cut hard dental tissue without substantial use damage to thermal or to structure. Miserendino which uses CO2 lasers. The diagnosis of secondary apical abscess in an apicoectomy was capable of sealing the dentinal tubules in the apical portion of the rooting and sterilization of the Gouw-Soares region under investigation use of the Er: YAG, Nd: YAG and Ga-Al-As periapical lasers clinically performed surgery[27].

Photodynamic therapy for malignancies

This therapy has been employed Incase of oral mucosa malignancies particularly multi focal squamous cell carcinoma. Here there will be a generation of reactive oxygen species which inturn damages the cells associated with the blood vascular network directly and triggers necrosis and apoptosis. This activates the host immune response and promotes the anti tumour immunity by activating macrophages and T lymphocytes[28]. This treatment is very successful and approximately has a success rate of 90%.

Analgesic effects of the laser

Nd-YAG is widely used as an analgesia in endodontics. Wavelength of Nd-YAG interferes with the Sodium pump mechanism which inturn change the cell membrane permeability and also alter the endings of sensory neurons temporarily, Which leads to block depolarisation of C and A fibres of the nerves I.e fast and slow conduction fibres. Evaluation of pulp vitality can be done using an electric pulp tester[28,29]. This application in the oral cavity reduces the firing frequency of the nociceptors (Pain receptors). Local carbon dioxide laser irradiation will reduce the pain associated with orthodontic force application without involvement of the tooth[30].

Indications and contraindications of laser in endodontics:

Indication of lasers in endodontics are teeth with lateral canals which leads to periodontal involvement, Pulp necrosis and purulent pulpitis, gangrenous changes, periapical lesions upto 5mm or more[31].

Laser treatments cannot be done in person who have advanced periodontitis caries, deep crown and root fracture and obliterated root canals in endodontic treated teeth[32].

Application of carbon dioxide lasers in endodontics:

Co2 lasers have been available in the medical and dental field for the past 25 years. They possess 10,600 nm of infrared wavelength that is highly absorbed by water. They are continuous wave lasers that can be operated in gated wave modes including super pulsed modes. Co2 lasers have a bactericidal action but there are no reports shouting nerve analgesic Irradiation effects of Co2 lasers in endodontics[33]. This laser can be used for performing root canal procedures.

Application of nd yag lasers in endodontics:

ND YAG lasers have a wavelength of 1064 nm and can be used to extract bacteria from contaminated teeth[34]. This is the first true pulsed laser introduced in dentistry. ND YAG lasers are used for cleaning and shaping in endodontics and they also play a role of analgesia. They are very effective in prevention of dental decay[35].

Xenon fluoride lasers

Xenon fluoride lasers are used to seal the exposed dentinal tubules. They are also used for pulp capping,checking the pulp vitality and for root canal treatments In endodontics. They have a visible light of 400nm -750nm and this is commonly used in endodontic treatments. Chlorhexidine helps in local intensity enhancement of lasers[36].

Table: 1: Lasers in Endodontics:

Lasers in endodontics	wavelength	uses in endodontics
CO2	Beam of Infrared light with the principle wavelength bands cantering on 9.4-10.6 micrometers	Dentinal hypersensitivity, pulp capping and pulpotomy, modifications of root canal walls, sterilisation of root canal system
Argon	488nm(Blue)- peak and 514nm(green)- Relatively short	caries removal, Root canal and tooth preparation
Nd:YAG	infrared-1064nm	used for root canal wall preparation, smear layer can be eradicated completely with sealing of dentinal tubules.
Er:YAG	infrared light-2940 nm	access cavity preparation, root canal shaping and cleaning

Laser safety

Even though lasers are very easy to use there are few precautions that have to be taken care of while using lasers. First precaution is usage of protective eye wear with a specific wavelength. Warning sign supposed to be posted outside the nominal hazard zone[37]. Every office should be appointed with an laser safety officer to proper usage of lasers, to coordinate the staff in training and to check the use of protective eyewear and also to prevent laser injury and to provide treatment on inflammation caused due to lasers[38].

Commercially available lasers

1)Dentsply sirona:

These majorly manufacture diode lasers, they are soft tissue lasers used in wound healing and periodontal treatments[39].

2)Biolase:

Biolase epic pro soft tissue lasers, This Laser has greater penetration, control and consistency than Co2 Laser[40].

3)Picasso:

Picasso lite is a laser used in soft tissues and is used for surgical procedures in soft tissue. This Laser has a multi tip handpiece.[41]

4)Fotona:

Fotona sky plus is an Er.YAG Laser with a wavelength at 2940nm, it is used in both hard tissue and soft tissue lasers. This is a highly customisable Laser[42].

Conclusion

So it has been concluded that lasers are well established instruments. Upcoming research based on lasers are showing more benefits and ability to perform less invasive procedures with greater comfort of patients has led to the development of lasers in modern dentistry especially in endodontics.

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Role of Dermal Fillers in Aesthetic Dentistry

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Abstract

Dermal fillers are gel like substances which are injected beneath the skin.It is also used to soften creases and enhance facial contour. The potential promise of the role of dermal fillers in Aesthetic dentistry involves important clinic uses. Dermal fillers are an important tool in the armamentarium of an aesthetic surgeon in the management of ageing skin. A surge within the use of fillers has been witnessed thanks to increasing awareness among people, easy availability of fillers and increased enthusiasm amongst the dermatologists and plastic surgeons to use this modality. In this era of evidence-based medicine and litigations against doctors, Oral Surgeons should be vigilant about different acts of omission and commission in the use of fillers.Dermal fillers have revolutionized the sector of cosmetic dermatology, as evidenced by the presence of an outsized number of products within the market. Even though fillers have been classified as a cosmetic device and have been FDA approved only for wrinkle management, they have been used for several other aesthetic and non-aesthetic indications too. This article briefly discusses the dos and don'ts with reference to dermal fillers.The review is done to fulfill the need to evaluate prevailing awareness regarding the application of dermal fillers in dentistry. Further, the review aims to establish the differences between filler injections in a dental procedure. The review also aims to consolidate recent advances in dermal fillers and its subsequent role in cosmetic dentistry.Search engines like NCBI- pubmed,mesh, google scholar were used to review articles since the year 2000 which were consolidated and analysed. The review provided an extensive outlook regarding the popularity ,extent of use ,demand and its advanced administrative techniques in aesthetic dentistry .

Keywords: *Dermal Fillers, Aesthetic Dentistry ,Fillers ,Facial Enhancement.*

Introduction

Dermal fillers are agents which can be injected beneath the skin inorder to restore or enhance facial aesthetics[1]. According to a study by Al Hamdan[2]. it is proved to be an effective non surgical solution when compared to invasive cosmetic surgical procedures[3]. Common comparison between filling agents like hyaluronic acid versus mechanism of action of botulism toxin is always a debate in terms of efficiency [4]. Botulism toxin is a purified protein which is harmless to facial muscles thereby preventing wrinkling of skin[5]. However, there are significant differences between botox and dermal fillers in terms of durability, composition and mode of application[6].

Considered to be one of the popular facial rejuvenation treatments, which can be a cost-effective way to look younger without surgery or downtime, there are multiple options of FDA approved filler products recommended for surgical use[7]. Hyaluronic acid fillers are the common temporary fillers used , hence are mostly recommended for first-time filler patients[8]. These are

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believed to typically last for 6 to 18 months[9].Injections to the lips are expected to wear out a little faster than those present in the nasolabial folds[10]. Certain hyaluronic acid fillers, like VOLUMA, are deciphered to last longer, but are limited to certain areas, such as the buccal region[11].

Synthetic fillers tend to last much longer, since they are not readily absorbed by the body. They can be a great alternative for an indicative patient. Fat injections are expected to last indefinitely when compared to the synthetic fillers however, some of the injected fat is expected to not survive[12]. Initially, one could attempt to overfill the treatment area, resulting in a fuller look that will gradually settle into a more natural appearance later[13] . Alternatives to dermal fillers include topical lotions, microdermabrasion and chemical peels.This review aims to differentiate the role of dermal fillers and its varied applications and limitations in modern dentistry[14].

TYPES OF DERMAL FILLERS IN AESTHETIC DENTISTRY

Biodegradable fillers

Biodegradable fillers are impermanent agents that can last for a limited time of volume augmentation, from months up to 12 months, but will eventually be metabolized by the body. Some of the quantity effect is thanks to a transient inflammatory response to skin fillers with associated edema. However, these volume effects will diminish soon after injection.[3] Subsequent fibroblast activation and neocollagenesis are often another two factors for volume augmentation, but they only end in partial filler engraftment into the encompassing tissue. Current biodegradable fillers stimulate neocollagenesis for more sustained aesthetic improvements and carry a coffee risk of adverse events or serious complications. Although permanent agents offer significant clinical benefits, short-term of volume effect, simple correction and sometimes reversible within the event of adverse effects make biodegradable fillers attractive to patients and plastic surgeons worldwide.

Generally, biodegradable filler spread on the market currently includes: mucopolysaccharide (HA), bovine collagen, calcium hydroxylapatite (CaHA) and injectable poly-L-lactic acid (PLLA).

Platelet rich plasma:

Platelet-rich fibrin matrices derived through the collection and centrifugation of blood, is approved by the FDA as a medical device designed for the safe and rapid preparation of autologous platelet-rich plasma (PRP) for use in orthopedic surgery. For cosmetic applications, PRP is injected into the face to stimulate cell proliferation via the discharge of growth-promoting proteins[3].Histological examination shows activated fibroblasts and new collagen deposition at the site of injection[5]. Injection is an office-based procedure used to fill scars and rhytides with only minor transient ecchymosis and edema.Additional studies are required to evaluate the efficacy and safety of platelet-rich fibrin matrices for soft-tissue augmentation[15].

Injectable poly-l-lactic acid:

Injectable PLLA is biocompatible, biodegradable, biostimulatory, synthetic filler that has got to be injected into the reticular dermis or subcutaneous fat. Animal studies have revealed that PLLA are ready to stimulate the proliferation of dermal fibroblasts with subsequent endogenous production of collagen. Histological studies in humans have shown gradual dissolution of the injected PLLA and dermal in-growth of type I collagen over 8 to 30 months after injection. PLLA is gradually degraded by nonenzymatic hydrolysis into water and CO2 over approximately 9 to 24 months.[16]

Short-term adverse events, including swelling, bruising, erythema, pain, inflammation, and pruritus, are frequently, but they usually disappear in a few days. The rate of granuloma formation has been reported as high as 44%.The formation of granuloma greatly influences patient's appearance. Treatment of granulomas includes surgical excision and intralesional injection of corticosteroids. Surgical excision isn't recommended except as a final resort. The corticosteroids used to treat granulomas need to be injected repeatedly. There are also severe systemic adverse effects, which is very rare, with only one case reported as an anaphylactic reaction necessitating treatment interruption.[17]

Calcium hydroxylapatite (CaHA):

CaHA may be a biocompatible, biodegradable, resorbable and biostimulatory filler that contains

microspheres which may stimulate the endogenous production of collagen. The product features a texture resembling native soft tissue and migration is minimal. Histopathologically, microspheres of CaHA stimulate almost no foreign body reaction and that they appear bluish in color and round or oval in shape. It is suggested that the microspheres of this implant are degraded by enzymatic breakdown instead of phagocytosis. Injectable microspheres of CaHA are successfully used for correction of lipoatrophy of HIV patients receiving antiprotease treatment and for smoothing moderate wrinkles[3]. When this agent is injected within the lips, it tends to induce a high incidence of nodules. Migration to a distant location from the injection site, a foreign body granulomatous reaction, seen as blue-gray microspheres in the extracellular matrix or within multinucleated giant cells has also been reported[6].

Polylactone based dermal filler:

A promising new biodegradable collagen stimulatory filler, composed of 70% aqueous carboxymethylcellulose (CMC) gel carrier and 30% synthetic polycaprolactone (PCL) microspheres has recently been introduced to the market. Its unique tuneable longevity gives the dermal filler variable durations for up to 4 years and is therefore ideal for those seeking long-lasting results.[18]

The PCL-based dermal filler is proved to be safe and sturdy in use in facial treatment and in hand rejuvenation during a clinical test .However, further clinically study and safety study should be introduced before it might be finally used as a bio stimulory filler in human.[18]

Cross linked cmc:

Five-eight chemically cross-linked CMC is now available as skin filler for the correction of facial defects and imperfections. It was first used in the pharmaceutical industry since the 1960s as an excipient and for drug delivery. A commercially available product supported cross-linked CMC is Erelle™ (Total Action, Bioitech Italy Ltd, Rome, Italy). It consists of a non-particulate, viscoelastic, monophasic gel supported cross-linked CMC in isotonic saline . One study of CMC injection in 350 patients with 3-year follow-up revealed that CMC could effectively and durably correct nasolabial wrinkles for 9-12 months[4].

Autologous fat:

Fat grafting is revolutionizing cosmetic surgery by providing methodologies to less invasively transfer adipose tissue . The initial attempts at soft-tissue augmentation revolved round the surgical use of autologous fat to reconstruct facial scars in 1893.It then were largely used for nasolabial folds injection, forehead augmentation, temporal augmentation, mid face lift. PRP and cell-assisted lipotransfer using adipose-derived stem cells have recent been developed to enhance the survival rate of fat grafting[19] . Adverse effects include prolonged edema and ecchymosis which can fade several days after injection. There is also a risk of necrosis and infection[20].

Permanent fillers

Permanent fillers include polymethyl-methacrylate (PMMA), silicone, polyacrylamide hydrogel, polyvinylpyrrolidone-silicone suspension, polyalkylimide gel, polyvinylhdydroxide microspheres suspended in polyacrylamide gel .Permanent fillers are non-resorbable and will provide long-lasting revolumization results. They could also induce fibrogenesis and collagen production, but complications like granulomas are more frequent in injection with such filler[21].

Paraffins:

Paraffins were initially used for aesthetic procedures to revive facial volume and contours, but complications like granulomas and paraffinomas years after treatment have restricted their use for aesthetic treatment.[22,23]

Silicones:

No silicone product for soft tissue augmentation has been approved by the FDA. The major indication for FDA-approved products is detachment of the retina with removal of the fabric after reattachment. In soft tissue augmentation, removal of silicone isn't performed. The use of liquid silicon is off label. Adverse effects have also been noted after use for tissue augmentation. After illegal silicone injection, the silicone embolism syndrome has been observed with potential fatal outcome in 24% of patients. Symptoms and signs of the "silicone syndrome" include dyspnea, fever, cough, hemoptysis, pain , hypoxia, alveolar hemorrhage, and altered consciousness.[24,25]they need almost been

abandoned nowadays.

Poly methyl – methacrylate:

PMMA is rigid, transparent and colorless, thermoplastic permanent skin filler with low cost, easy accessibility, and potential to realize lasting results. PMMA injections are related to several side effects; especially they'll cause some undesirable effects within the eyelids and periocular region.

First-generation polymerized PMMA microspheres are purified with diameter greater than 20 μm, which may produce foreign body granulomas. Complications of PMMA injection were classified as nodular masses, inflammation, allergies and skin hypopigmentation. The most affected sides were the lips (46%), followed by periocular, nasolabial folds, forehead, and cheeks. PMMA injection to the periocular region may lead to erythema, hardening of the local tissues, edema, and formation of nodules and eyelid malposition, which are associated with fibrotic nodules, giant cell inflammation[26].

Polyacrylamide gel (aquamid):

Aquamid has been used extensively for soft tissue augmentation and body contouring for two decades. Aquamid may be a biocompatible and nonabsorbable hydrogel consisting of 97.5% water and a couple of 5% cross-linked polyacrylamide (PAAG). The gel is manufactured through polymerization of the acrylamide monomers and N, N'-methylenbisacrylamide. Aquamid is currently approved in several countries in Europe, European Conformity marked in Europe in 2001 for facial augmentation and minor body contouring,

After injection, the implant is encapsulated and surrounded by fibroblasts and microphages, theoretically preventing migration. Many studies have supported the usage of Aquamid for the treatment of varied rhytides, facial contouring, and correction of HIV lipoatrophy.

For the past decade, Aquamid has gained popularity as injectable filler. Similar to other facial fillers, there are reported cases of inflammation, nodule and granuloma formation, and delayed hypersensitivity reactions. [27]

Polyvinylpyrrolidone - silicone suspension :

This is a permanent filler comprised of particles of polymerized silicone elastomer, 100-600 μm in size, dispersed during a carrier of polyvinylpyrrolidone .The suspension has been mostly used for lip augmentation and therefore the correction of facial rhytids. It should be injected in the subcutaneous tissue[21].

Polyvinylhydroxide microspheres suspended in polyacrylamide gel:

This is a permanent filler composed of co of a suspension of 6 polyvinyl hydroxide microspheres suspended in 2.5% polyacrylamide gel and has been used mostly for lip augmentation .[28]

APPLICATION OF DERMAL FILLERS IN AESTHETIC DENTISTRY

Enhancement of micro aesthetics

Therapeutically dermal fillers could be administered during the treatment of angular cheilitis [29]It can be further used during volumerisation of interdental papilla loss (black triangles)and others[30] Dermal fillers is known for its long history of use in facial aesthetics [30] At present, temporary options vary from hyaluronic acid,calcium hydroxyapatite ,polylactic acid and PMMA [31]

Hyaluronic acid is considered to be one of the extensively used dermal fillers. It's primary application involves subperiosteal augmentation . It is done to aesthetically enhance esthetics.[1]It involves specific triangular spaces below the tooth contact [2]According to the study done by Abdul Jabbar MH et al, 2011[1]. Hyaluronic acid is considered to be the chief requirement in the overlay technique procedures. [3]

Enhancement of macro esthetic profile

Enhancement of macro esthetic profile is highly influenced by the type of dermal fillers used. Dermal fillers can be primarily employed for facial soft tissue augmentation as mentioned earlier. [4]This can be correlated to the role of implants in micro aesthetics. [5]The procedures can be coupled with intra oral procedures to provide especially during the fabrication of prosthesis.[6]result in outstanding patient outcomes. [31]

Dermal fillers can be used to support the maxillary corners to partially edentulous patients especially in the cases of missing canines[14]. Lip augmentation involves support provision to lips ,shortening of lip length ,repose

and appearance of fuller maxillary lip[32]. It can also be incorporated in typical dental procedures in edentulous patients which can be extended to dentate as well[19].

RECENT ADVANCES IN DERMAL FILLERS IN AESTHETIC DENTISTRY

Ideal advance training methods along with experience and maintenance of the same proves extraordinary clinical results in clinical dentistry [33]Further ,safety during the use of dermal fillers can be constantly monitored by maintenance of long track record which will result in outstanding patient outcomes [31]

As mentioned earlier, **Hyaluronic acid** is a naturally occurring substance which is a predominant component of human skin.[12] It helps keep skin plump and hydrated. HA fillers are typically soft and gel-like. The results are temporary, lasting 6 to 12 months or longer before the body gradually and naturally absorbs the particles. Most HA fillers are infused with lidocaine to help minimize discomfort during and after treatment.[34] **FDA approved HA fillers include:**

- Juvéderm products: Juvéderm XC, VOLUMA, VOLBELLA, VOLLURE
- **Restylane products: Restylane, Restylane Silk, Restylane Lyft, Restylane Refyne, and Restylane Defyne**
- **Belotero Balance**[35]

Calcium hydroxylapatite is a naturally occurring substance, which is a primary component of our bones.[36]When used in a filler, the calcium particles are nearly microscopic and suspended in a smooth gel. [36]The consistency of a CaHA filler is typically thicker than that of a hyaluronic acid filler and typically last longer as well, about 12 months for most patients.[37] Calcium hydroxylapatite is also reported to help stimulate natural collagen production, and it is typically used for deeper lines and wrinkles. **FDA approved CaHA fillers include Radiesse**.[38]

Poly-L-lactic acid is a biocompatible , biodegradable synthetic substance. It has been used for many years in medical devices, such as dissolvable stitches. [39]Poly-L-lactic acid products are technically classified as "collagen stimulators,"[40] as their main mechanism to smooth fine lines is by helping your skin rebuild natural collagen—the filler gel itself dissipates a few days after treatment. Poly-L-lactic acid is typically used to treat deep facial wrinkles, and results can last more than 2 years.[41] [42] **FDA approved Poly-L-lactic acid fillers include Sculptra Aesthetic.**[38]

Polymethylmethacrylate (PMMA) is a synthetic, biocompatible substance that has been used in medicine for much of the last century.[43] In dermal fillers, PMMA takes the form of a "microsphere" or tiny ball, that remains beneath the skin indefinitely to provide continued support.[44]PMMA fillers will also contain collagen, a naturally occurring substance in the skin that provides structure and firmness.[45] **FDA approved PMMA fillers include Bellafill (formerly known as Artefill).**[46]

COMPLICATIONS AND CONTRAINDICATIONS

Various patient related, skin related and systematic factors create a major impact during the administration of dermal fillers[38]. Hence contraindications should be known and instructions of use for the chosen filler is an essential criteria which is to be strictly adhered to. [47] [48] Patients with multiple allergies and a history of anaphylaxis are advised not to be treated without prior care.[49]Moreover, prospective patients having abnormally thin skin, skin atrophy due to administration of corticosteroids or also due to long term topical/ peroral steroid use are commonly advised not to undergo such treatments.[50]

Conditions like anetoderma, vermiculate atrophoderma, rheumatoid arthritis associated with skin thinning in the dorsum of the hand are contraindicated for superficial or medium depth placement fillers. One of the previous studies[51], suggests that candidates with very eyelids,cheeks and many fine wrinkles are also contraindicated for fillers.[52]

STRATEGIES TO DEAL WITH COMPLICATIONS

Numerous complications can be prevented during intraoral treatment by exacerbating the infections adjacent to the region to be treated.[53] Otherwise, which might result in complications due to the

presence of pathogenic substance/ organism at the site of administration. [51]If a patient is suffering from an ongoing skin infection located close to site of filler administered, then it is required not to treat the patient till the subsidence of the ongoing infection.[7] Various such conditions include viral infections like HSV, perioral human papillomavirus, mollusca contagens, bacterial infections- streptococci[28], yeast infections - extensive pityrosporum folliculitis. Physicians are suggested to make a convincing judgement based on the proximity to the treatment area. [48]

Conclusion

Consolidated concepts from major aspects of dermal fillers and its role in aesthetic dentistry reveals efficient outcomes at dental practice. However, it is essential to administer the same with required care and precautions.

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Nano Glass Ionomers - A Review

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Abstract

Restorative dentistry deals with materials that are used to fill cavities and prevent the infection of caries from recurrence. We have many restorative materials like amalgam, zinc phosphate, zinc oxide eugenol, GIC. Glass Ionomer Cement (GIC) has been a part of therapeutic dentistry for over three decades. It is one of the dental restorative materials used in dentistry for filling cavities and used as luting cement for the attachment of orthodontic brackets. It is a solid material with satisfactory appearance. It discharges fluoride which has a remineralising effect. It also additionally empowers high quality and rebuilding tendency in restorative dentistry. It is more susceptible to fracture and is more susceptible to fracture as it cannot bear heavy masticatory load. GIC generally lasts for five years and is quite affordable(price) also. Nanotechnology in dentistry has various implications and it tends to improve the existing properties of the materials in numerous ways. When nanoparticles are introduced into GIC, they can help in better adhesive mechanism to teeth so that they can reduce the fracture occurrence as well as the leakages. It also improves the longevity of the restoration, improves the aesthetic appearance and overcomes the surface roughness seen in conventional GIC. Nano particles are small and occupy the space in the matrix which can improve the strength of the material. Nanoparticles improve sustained fluoride release, increase the strength, improve the antibacterial activity, and can be used clinically as an efficient material. The drawbacks of the conventional materials are overcome by the nanotechnology.They play a very important role in dentistry to improve the efficiency of many materials involved in dentistry.This investigation is mainly focussing on the use of nanotechnology in glass ionomer cements dealing with its properties, points of interest and hindrances.

Keywords: *Glass ionomer cement, fluoride release, physical properties, antimicrobial effect, aesthetic appearance, properties of cement, nanotechnology.*

Introduction

Glass ionomer cement is utilised for a wide range of uses in dentistry [1]. They are tooth coloured restorative material which bond chemically to tooth and release fluoride for a longer period. They are also material of choice for restoration in the primary teeth. Their bonding mechanism can seal and protect the pulp and prevent leakage at the margins which decreases the possibility of failure of restoration. The coefficient of thermal expansion of GIC is similar to dentin in humans so that the marginal leakage possibility is less. When GIC is placed in cervical cavities it is durable also. GIC is available in different shades but still surface finish,variations related to finishing and polishing, surface staining still remain a concern. GIC has changed the restorative approaches, particularly in minimal invasive dentistry. GIC synthetically holds fast to tooth structure through trade of particles which forestall microleakage,secondary caries and pulp contamination [2.] It is ideally utilised since they bond chemically to both enamel and dentin. It is exceptionally biocompatible

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and goes about as a store of fluoride by taking fluoride ions from dentifrices and discharging it after some time [3]. The early conventional glass ionomer materials were technique sensitive, slow setting, opaque when set and can cause premature surface deterioration [4]. Most of these problems are overcome in newer generations of glass ionomer cement [5]. Bioactivity implies cellular growth, proliferation and antibacterial effect. Glass ionomer cement contains bioactive properties due to presence of fluoride and silicate. Each modification in GIC has a significant outcome when considered in relation to effect on final properties [6]. Glass ionomer cement have other uses such as luting agent in crowns and other indirect restorations [7].High fluoride content gives anti caries effect [8,9]. Tooth coloured restorative material helps in better aesthetic appearance though it has drawbacks viz. being brittle which means it is prone to fracture,poor wear and tear resistance and sensitivity in oral cavity[10]. Nano particles are of the size ranging from 10-20 micrometer. They have better bonding properties because of their occupancy of the space in the material. Their bonding mechanism is micro mechanical interlocking which is because of chemical nature and surface texture. Nano light curing type of GIC is also available for use. They are clinically used for Class 1 temporary restoration and sandwich restoration in type 3 and type 5 along with core build ups. Incorporation of nanoparticles have improved mechanical properties of GIC with enhanced features like elastic modulus and increase in compressive strength. Similar to crystals in enamel, some crystals like nano hydroxyapatite introduced in GIC can favour the remineralisation of enamel [11]. Conventional glass ionomer cement had low flexural and tensile strength and high modulus of elasticity so they were much more prone to fracture. Addition of nano crystal has shown positive impact on compressive, tensile and flexural strength of GIC. It has also improved the chemical stability and water solubility. Glass ionomer cement is considered to be good material for root caries and for sealants. GIC becomes stronger with the progression of reactions more resistant to moisture [12]. When nanoparticles were introduced there was a decrease in working and setting time of the cements. They have better survival rates when compared to conventional GIC [13]. Some particles like nano TiO2 particles are introduced to show enhanced antibacterial effect [14]. Some particles like zinc oxide when introduced in GIC, the GIC shows increase in anti bacterial activity without modifying mechanical properties and are found to be better alternatives to conventional GIC. Fluoride release from the matrix of GIC helps in antibacterial effect. When compared with conventional GIC , nano modified GIC releases less amount of fluoride from the matrix [15,16]. Even though glass ionomer cements have less chair side time and ease of use, they are also prone to maximum leakage [17]. Restoration of noncarious cervical lesions often poses a challenge to the clinician.Various restorative materials are available and modifications of GIC enhances their properties [18]. Conventional glass ionomers were difficult to manipulate as they are sensitive to moisture imbibition during the early setting reaction. Incorporation of nano technology in dentistry has so many advantages. They are antibacterial in effect and lead to ultimate reduction of periodontal disease. Nowadays even nano technology is introduced in mouthwash and oral hygiene products so that they can reduce caries , dentin permeability [19,20]. Nano hydroxyapatite particles when introduced can also lead to enamel remineralisation effect. Nano glass ionomer cement is said to have many clinical applications and helps in conservation of tooth structure by their adhesive mechanisms [21,22].

Materials and Methods

The review of literature was done by collecting the articles related to the article. 20 articles were collected from google scholar and pubmed. The articles were searched for using keywords like nano glass ionomers , modifications of GIC, bond strength , polish properties. They were collected from the period of 2000-2020. the inclusion criteria were aesthetic outcome, polish properties and wear resistance. Exclusion criteria includes stress bearing properties.

NANOPARTICLES INCORPORATED IN GIC:

The incorporation of nanoparticles in conventional GIC has led to improved properties. When nano hydroxyapatite particles were introduced they improved the fluoride release, mechanical properties as well as the reduction in bacterial invasion. When titanium oxide was added they improved the mean compressive fracture strength of the material[23]. When silica is introduced it improves the shear bond strength, compressive and flexural strength, they also improve the microhardness

property [24]. When zirconia particles are added, they strengthen the material with reduction in brittleness [25]. Biocompatibility and bioactivity are also improved when niobium pentoxide is added[26]. Barium sulfate was seen to influence the setting and working time of GIC. When titanium oxide was added it improved physical properties.

FLOURIDE RELEASE

Nano technology increases the longevity of GIC, as the nano glass filler particles are incorporated into them. Nanotechnology increases the mechanical properties and aesthetic outcome of nano glass ionomers to facilitate strong ionic bonding to the tooth. They have improved the stability and sustained fluoride release. Carious lesions are one of the most common problems faced by children. Secondary caries are the recurrence of caries after removal of the existing caries. When there is fluoride release from the restorative material it prevents the emergence of secondary caries. GIC contains fluoride which is clinically cariostatic and acts as a fluoride reservoir. They also undergo remineralisation which is a unique property [18,27.] The fluoride release can remineralise the enamel and can counteract the appearance of secondary caries. Incorporation of nanoparticles have led to sustained release of fluoride [28.] According to another view, fluoride release is initially high but later eventually decreases [29.] When nano GIC was light cured or chemical cured it affected the fluoride release. Addition of CaF particles in nano modified GIC affect the fluoride releasing property [14]. Another study showed that nano hydroxyapatite dentifrice produced better effects along with remineralisation potential [30]. Within the limitations of an in vitro study it was concluded that cumulative release of fluoride was higher in nano glass ionomers, resin modified GIC when compared to resin composite and compomer [31]. Another in vitro study also shows that remineralising effect was additive due to incorporation of zinc reinforced synthetic nano hydroxyapatite on caries like lesion in human teeth [32]. Enamel erosion when prevented we can avoid the occurrence of secondary caries [21,33–35.]

STRENGTH PROPERTIES

High mechanical strength is seen in GIC. It is more stable chemically. When more small sized particles are added it makes the material stronger and tougher. Addition of nFAp particles help in the positive impact of compressive, tensile, and flexural strength to GIC. When MgO nanoparticles were added it showed that they were proved to be a clinically more effective restorative material.Setting time of GIC decreased when macro particles were replaced by nanoparticles. Increase in compressive strength but the effect is being more pronounced when it contains nano glass particles [36]. According to another study cumulativeF release decreases when macro particles are replaced by micro particles. Bonding was greatest in nano modified GIC [37.] Mechanical strength was more in nano GIC compared to unmodified GIC. According to another study, the property of improved surface hardness was found when nanoparticles were incorporated into GIC [38.] However, decrease in compressive strength was observed in yet another study after thermal cycling [39.]

BIOMECHANICAL DEGRADATION

Biodegradation occurs due to many factors like humidity, biofilm, type of material and abrasion. Also undesirable roughening of surface was also observed [40]. There have also been studies which produce contradictory result viz. low roughness values. It has also been reported that nano fillers did not show any influence on roughness and hardness concerning the degradation resistance [41]

ANTIMICROBIAL ACTIVITY

Anti microbial means the reduction in occurrence of bacteria. The presence of bacteria can lead to caries and destruction of teeth.They determine the stability of teeth. Prevalence of micro leakage is more if they are present [42,43]. It is a very important property when the cement is being used as a luting cement, restorative material because bacteria may still be present in the walls of preparation and can cause infection if microleakage is present after their usage. There have also been contradictory results like more chemical bond strength is seen so there is reduction in leakage. Nano silver incorporation has depicted inhibition halos in an agar plate. According to another study they show that enhanced bactericidal effect . Teratogenicity of nano silver particles is unknown [44,45.]

PIT AND FISSURE SEALANTS

Dental caries are still a concern. The modern restorative dentistry focuses on natural tooth conservation and minimal invasive dentistry. Atraumatic Restorative Treatment (ART) is one such mechanism in minimal invasive dentistry. In ART,they remove the caries and demineralised tissue with the help of hand instruments. We use GIC as a pit and fissure sealant so that they can seal the fissures at risk for manifestation of caries. Another study has shown lower viscosity and formed a resin tag of sufficient length and the sealant is showing better results when compared to conventional sealant [46,47.] Glass ionomer cement had superior seal property when compared to other restorative materials [48].Pit and fissure sealants are mostly used for preventing caries formation [49,50]

AESTHETIC OUTCOME

Glass ionomer cement is a tooth coloured restorative material which can be used anteriorly for aesthetic purposes. They are available in different shades. They do not stain easily and are also affordable to people. They are also durable and can last for about 5 years. They are similar to human dentin so very aesthetic in purpose. They are not much sensitive to water and have better adhesion to the tooth. They can also undergo finishing and polishing almost immediately. When ytterbium ions along with aluminium fluoride ions are introduced in the material they show similar chemical and physical properties and produce the uniform surface and improve the aesthetics along with better finishing and polishing property[51].

Limitations

Nano glass ionomers are technique sensitive. Conventional GIC are very brittle and fracture prone and not appropriate for their usage in posterior stress bearing areas like molar teeth [52.] Nano GIC have high creep which causes reduction in marginal integrity. Due to lack of studies nano glass ionomers are in the initial stage of use in clinical dentistry. We have to control certain aspects of nanoparticles in GIC, such as correct indication, good isolation, and choice of right material for each situation to be of use without any doubt or apprehension.

Future Scope

Nanoparticles can be incorporated to improve the strength of the GIC so that they can be used in posterior stress bearing areas. They can be modified to decrease leakage and improve the marginal integrity. Further research is needed in biochemical stability of glass ionomer cements, supported by both the dentists as well as the industry which is a need of the hour.

Conclusion

Glass ionomer cement is a tooth coloured restorative material. Introduction of nanotechnology in it will improve the properties. Nano technology is also technique sensitive which is a concern but the pros of introducing the nanoparticles in GIC outweighs the cons of it. Still research is yet to be conducted in nano GIC so that it can overcome the drawbacks.

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Shade Matching in Fixed Partial Denture Patients

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Abstract

Shade matching is a significant process in fixed dental prosthesis where the selection needs special attention and systemic approach in covering all aspects. It is a fact that this approach is mainly used for achieving the goals related to the morphological and optical acceptance within the community settings. A total of 960 patients were taken and analysed for shade matching in fixed partial denture. The data collection is done from the dental archives of the patient management software system. The study is done under university setting. The results were analysed using SPSS software version 20 by IBM.T he result observed is that there is a highly achieved shade match with positive correlation and significant values ($p<0.05$) in the fixed partial denture patients. From the overall study it was found that quality of shade match in fixed partial dentures is clinically good. Special attention and effort to be performed during shade selection to get accurate shade match in fixed dental prosthesis.

Keywords *:Aesthetics; Shade match;Shade selection.*

Introduction

Colour matching in aesthetic restorative material with the natural teeth is the common approach in shade matching.[1,2].The aesthetic success is dependent upon the close shade matching between the dental restoration and the adjacent teeth[3,4].The shade matching is done to adapt restoration with the surrounding tissues[5,6,7,8]. The demanding aspect within the aesthetic dentistry is regarding the duplication and characterisation of colour[5,9,10].The shade perception is psychophysiological response it is regulated by the human eyes and sensory structures of brain towards the reflection of light spectrum from any object[2,11,12].Some elements give direct demand and impact on the colour of a restoration[13,14].The common elements that gives impact includes lightning conditions, thickness of porcelain, soft tissue colour, core material, soft tissue shade selection system, firing process and fabrication[15,16,17]. The judgement made on shade selection has some factors that include ambient light, surrounding colour, clothes, makeup of the patient and chromatic perception of the dentist[18,19,20].These factors results in frequent errors while performing shade selection[21,22]There are various approaches in shade selection method which is used for aesthetic success in shade match[23,24].The study was to determine shade matching in fixed partial denture patients [25,26]

Materials and Methods

The study is done in a university setting. This study was approved by the institutional ethics board. Two reviewers are involved in the study. These samples were taken from patients who checked in the clinic from June 2019 to April 2020.Total number of sample data included 960 patients who were subjected to fixed partial denture treatments. The case sheets were verified with the help of photographic examination and interim procedure notes. To minimize the sampling bias we included all the data available and there was no sorting of data done. Internal validity of the study included all those undergoing fixed partial denture treatment. The external validity of the study is to find the replication of results in different time periods. Data collection was done from the dental archives of the patient management software system patented by Saveetha Dental College. The data obtained from the category fixed dental prosthesis, shade match criteria and the data is tabulated. The data was reviewed by one external reviewer. The data was imported to SPSS software and the variables were

defines. Chi square test was done on the data obtained using SPSS software version 20 by IBM. The age and gender were independent variables and shade match was dependant variable. Correlation and association type of analysis was done.

Results and Discussion

The best shade selection increases the natural appearance of a shade and the acceptance of the prosthesis. The data collected from the patient management software were tabulated in SPSS and the descriptive method statistics were obtained. Out of 960 patients,39 were in age group 18-20,241 were in age group 20-30 years,317 were 30-40 years,171 were 40-50 years,129 were 50-60 years,48 were 60-70 years,15 were 70-85 years. The frequencies calculated are tabulated and depicted in (Table 1 and Table 2) .Chi square test was done between age group and colour match. Results showed statistically significant differences in shade matching among the groups,(P<.0.001).with very good and good shade matching being the most predominant ones (Figure 1).The results obtained states that the shade match is highly achieved and it consensus with the study by [16,26,7] which states that the shade match in the age group has high satisfactory population with very low crevice in shade match of the fixed partial dentures.

According to the study of [27,28]the clinical steps in managing the predictable colour management in the aesthetic restorative dentistry depicts the results where the shade selection was accurate in the limited cast group selected and natural appearance was obtained. An understanding of the science of colour, shade perception and their modification is important if success must be attained in expanding field of aesthetic dentistry[29,30.] The shade match is highly achieved in sextant two as the anterior perception of shade match plays a very important role in the aesthetic appearance of the patient [31,32].The shade matching is performed visually using dental shade guide. The guide used in study is VITA which is the most popular guide and it gives best results which is accurate.[33]

From the present study it was observed that shade match is highly achieved in all age groups in fixed partial denture patients.

Table 1 : Table Depicts the Total Number of Patients According to Each Age Group.

Age Group	Total
18-20	39
20-30	241
30-40	317
40-50	171
50-60	129
60-70	48
70-85	15
	960

Table 2 : Table Depicts the Total Number of Patients Showing the Frequencies of the Shade Match

Age Group	Very Good Shade Match	Good shade match	Slight mismatch	Obvious mismatch	Gross mismatch
18-20	18	18	3	0	0
20-30	94	134	13	0	0
30-40	130	156	25	5	1
40-50	73	91	7	0	0
50-60	50	54	24	1	0
60-70	21	25	1	0	1
70-85	2	12	1	0	0

Shade matching in Fixed Partial Dentures across age groups

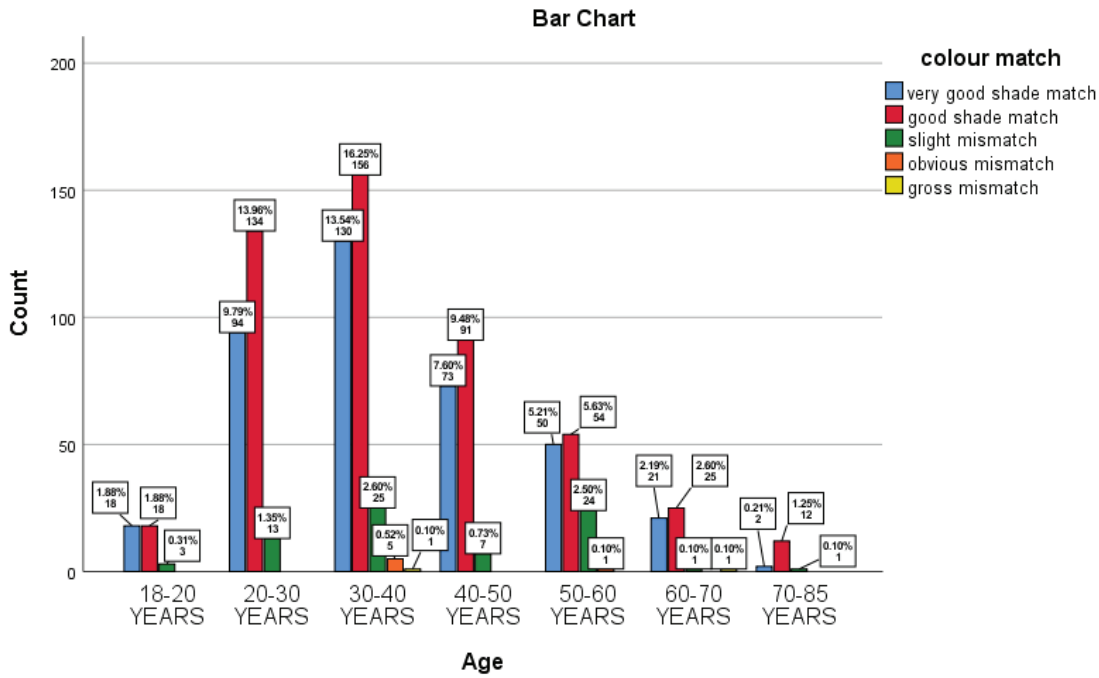


Figure 1 : Bar graph showing the frequencies of the shade match depending upon the age groups of the patients.the Frequencies of shade match are plotted in Y axis and the number count of patients Depending on the age group is plotted in X Axis. the Shade Match frequencies are represented as very good shade match denoted by colour Blue, good shade match is denoted by colour Red, slight mismatch is denoted by colour Green, obvious mismatch is denoted by colour Orange and gross mismatch is denoted by colour Yellow. There is statistically significant differences in shade matching among the groups, with very good and good shade matching being the most predominant ones.(Pearson Chi Square Test ;P<.0.001).

Conclusion

From the present study it was observed that shade match is highly achieved in all age groups in fixed partial denture patients.Shade matching done for fixed partial dentures in patients who responded in this study was very satisfactory.Shade matching is important stage of prosthetic work and in fixed dental prosthesis. The traditional methods and the new methods offered by the technical advancements should be factored in

for appropriate shade selection in fixed partial dentures. The layers,degree of translucency,opacity and the way colours are reflected will make the process of shade selection and manufacturing of prosthesis more skill demanding to achieve the aesthetically attractive results. Special attention and effort to be performed in shade selection to get accurate shade match in the fixed dental prosthesis.

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Prevalence of Fluorosis among Patients Visiting a Private College and Hospital in Chennai, India

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Abstract

Dental fluorosis is a tooth malformation caused by the intake of excess fluoride from various sources, such as water, food, and air, during tooth development and mineralization. The regional concentration of fluoride and extensive application of fluoride to prevent dental caries have resulted in the high incidence of this malformation. Though many institutional studies on fluoride concentration and its adverse effects have been conducted , the result is highly scattered and needs to be systematically summarized for better utilization. Hence the aim of this study was to assess the prevalence of fluorosis and its severity among the patients visiting a private teaching hospital in a non fluorosis endemic area in Chennai. It is a retrospective cross sectional study was carried out and the case records of patients having fluorosis was collected by reviewing patient records and analyzing the data of 86000 patients from June 2019- April 2020. Patients screened for Dean's Fluorosis index were selected. The data was collected and subjected to statistical analysis and correlation using the SPSS software. The results showed that fluorosis was found to be more prevalent in males (71.5%) than females (28.5%) , it was also found that 37.5 % of the patients had mild fluorosis. It was observed that patients presented with mild fluorosis especially in the upper arch in our hospital.

Keywords: *Correlation; Fluorosis; Gender; Severity; Teeth*

Introduction

Dental fluorosis is a fluoride-induced condition, which leads to specific interruption in tooth formation, mineralisation and an aesthetic condition, in which enamel formation is altered and hypo mineralized [1]. The regional concentration of fluoride and extensive application of fluoride to prevent dental caries have resulted in the high incidence of this malformation. Calcium, phosphate, and fluoride ions play an important role in the battle between demineralization and remineralization processes and accordingly modify the susceptibility of tooth to caries progression [2]. Carbonate apatite is also the material found in calcifications in the vascular walls [3]. The clinical appearance of dental fluorosis is distinguished by lustreless opaque white patches within the enamel which can become striated, mottled and/or pitted [4]. The diagnosis of these lesions is based on clinical history[5]. The incidence of dental fluorosis reaches 80–90% in some high-fluoride areas [6]. The Bureau of Indian Standards (BIS) has set the standards for fluoride levels in drinking water with maximum desirable limit as 1.0 mg/L and permissible limit in the absence of alternate source as 1.5 mg/L [7]. Fluorosis has its impact on 21 states of India [8]. India is situated within the geographical fluoride belt and in locations where fluoride content is high in rocks or soil, leaching of fluoride occurs, causing excess fluoride level in groundwater. However, the extent of fluoride in water

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also depends on the natural solubility, presence of other minerals, the acidity of the soil and amount of water present, which explains high fluoride content in the groundwater [9]. In a study done in the Madurai district of Tamil Nadu, India showed that due to high concentration of fluoride in groundwater , dental fluorosis was a major problem among people residing in that area [10]. About 20 out of 35 states and union territories of India were identified as endemic for fluorosis and about 66 million people in these regions are at risk of fluoride contamination [11].

It is known that fluoride content in drinking water is the primary cause of fluorosis [12]. Clinically ,it is very important to understand varying factors which may alter the course of any condition [13]. It is suggested that other factors like altitude of residence, climate, dietary habits, tea consumption, nutritional status of the child, duration of breastfeeding, infant formulae and use of fluoridated toothpaste have an influence on the prevalence and severity of dental fluorosis [14,15]. A study done in the central plateau district of Nigeria showed that the prevalence of fluorosis was significantly higher in the high altitude parts of the district than the low altitude parts [16]. Yet another study indicated that toothpaste , if swallowed by young children , is a risk factor but this risk is under the parent's control: less toothpaste can be used and low fluoride toothpaste can be introduced [17]. Whole saliva is mainly composed of fluid produced by major and minor salivary glands[18]. A previous study suggests that a combination of fluoride excess and calcium deficiency may lead to the development of a mixed picture of osteosclerosis and osteopenia [17,19]. Despite diagnostic and therapeutic advances over the decades, the disease remains a challenge for medical and dental professionals [20].

It is important to educate the community on dangers of using water with excess fluoride and other associated risk factors of fluorosis to ensure good health [21]. Patients from different backgrounds and localities come to dental clinics seeking for treatments but they might have other underlying conditions like fluorosis which show evident changes in the tooth structure. Thus the treatment plan should change accordingly in order to treat the main complaint along with other conditions like fluorosis.

Hence the aim of this study was to assess the prevalence of fluorosis and its severity among the patients visiting a private teaching hospital in a non fluorosis endemic area in Chennai.

Materialsand Methods

Study Setting

This is a retrospective study regarding patients with fluorosis who have visited Saveetha Dental College and Hospitals in between June 2019- April 2020. The approval for this study setting was obtained from the Institution Ethics Board. The sample size of n= 488 patients in which sampling bias was minimized with the verification of photographs. The main purpose of photography in dentistry is the registration of clinical information in the oral cavity [22] The study was reviewed by two reviewers and it was cross verified. All patients data diagnosed with fluorosis were included for the data analysis. Cases with Incomplete record entry in the system were excluded from the study.

Data Collection

The case records of patients having fluorosis was collected by reviewing patient records and analyzing the data of 86000 patients. The data of these patients was collected and tabulated. It included parameters – Patients ID, Age , Gender, Teeth and severity of fluorosis. Age was categorized into 14-18 years, 36-55 years and patients more than 55 years. Severity of fluorosis was grouped into questionable, very mild, mild, moderate and severe fluorosis.

Statistical Analysis

All the parameters were tabulated and assessed for statistical significance using the SPSS software [23]. Percentage , mean, standard deviation, frequency of parameters were employed in the analysis. Chi square test was used to detect the significance between age, gender, severity of fluorosis and teeth involving fluorosi. P value less than 0.05 was considered to be statistically significant.

Results and Discussion

The results of this study show that among the total participants, 71.5% of them were males whereas 28.5% of them females [Figure 1]. It was observed that 63% of patients presented with fluorosis involving upper arch,

9% of the patients had fluorosis involving lower arch, and about 28% of the participants had generalized fluorosis [Figure 2]. 37.5% of the patients presented with mild fluorosis, 30.5% of the patients showed moderate fluorosis, 21% of the patients had very mild fluorosis, 9% had severe fluorosis and 2% of the patients had questionable fluorosis [Figure 3]. Among the participants , 82% of the patients were in the age group 19-35 years, 13% of the patients were between 36-55 years, 4% of the patients were 14-18 years and 1% of the patients were more than 55 years old and above age groups [Figure 4]. Correlation between the teeth involved in fluorosis and severity of fluorosis was observed [Figure 5] but p value >0.05 was found to be statistically not significant.

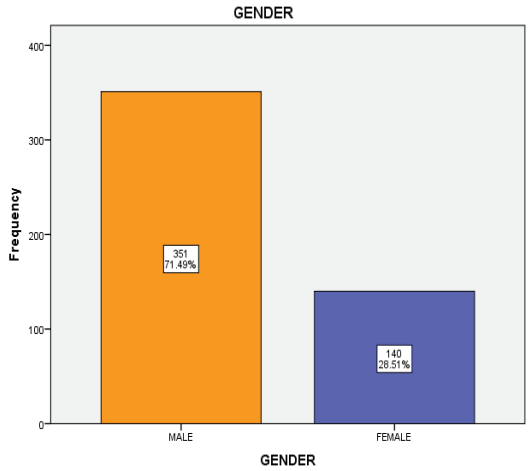


Figure 1: Bar graph depicting the prevalence of fluorosis between different gender of patients visiting the outpatient department at a private college hospital. X axis represents the Gender of the patients and Y axis represents the frequency of patients having fluorosis. The colour Orange denotes the number of male patients and the colour Blue denotes female patients. The graph shows that among the patients having fluorosis, 71.4% of them were males and 28.5% were females indicating that fluorosis was more commonly seen in males among the patients visiting the institution.

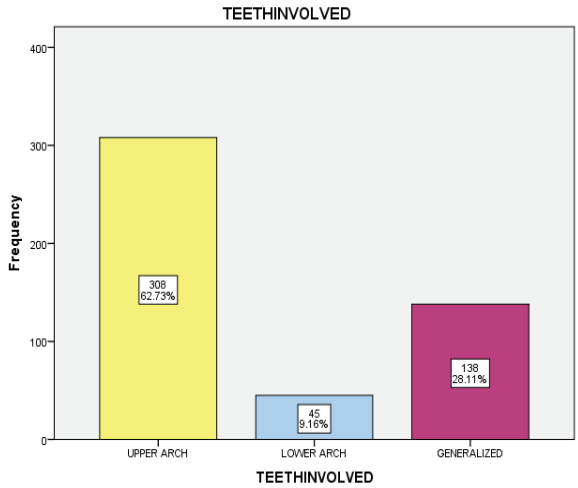


Figure 2: Bar graph depicting the teeth involved in fluorosis among patients visiting the outpatient department at a private dental hospital. X axis represents the teeth involved in fluorosis and Y axis represents the frequency of patients having fluorosis. The colour Yellow denotes upper arch, Blue denotes lower arch and the colour Magenta denotes generalized fluorosis. The graph shows that among the patients having fluorosis, 62.7% of them had fluorosis in the upper arch, 28% had generalized fluorosis and 9.2% of them had fluorosis involving the lower arch indicating that fluorosis was most commonly seen in the upper arch among the patients visiting the institution.

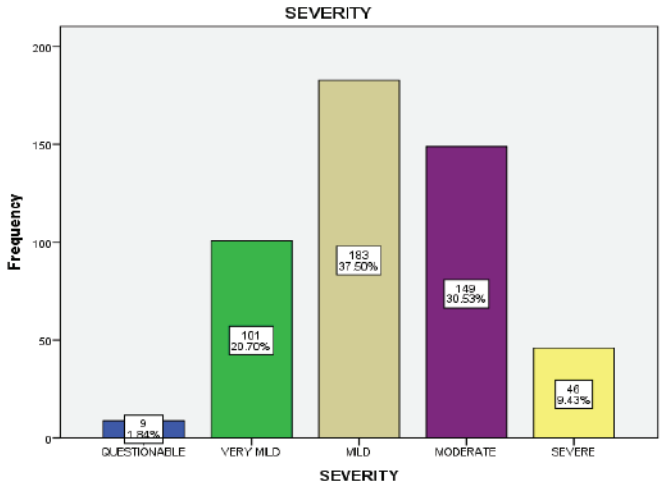


Figure 3: Bar graph depicting the severity of fluorosis among patients visiting the outpatient department at a private dental hospital. X axis represents the severity of fluorosis and Y axis represents the frequency of patients having fluorosis. Blue colour denotes questionable fluorosis, green denotes very mild fluorosis, beige denotes mild fluorosis, purple denotes moderate fluorosis and yellow denotes severe fluorosis. The graph shows that among the patients having fluorosis, 37.5% of them had mild fluorosis, 30.5% had moderate fluorosis, 20.7% had very mild fluorosis, 9.4% had severe fluorosis and 1.8% of them had questionable fluorosis indicating that mild fluorosis was most commonly seen among the patients visiting the institution.

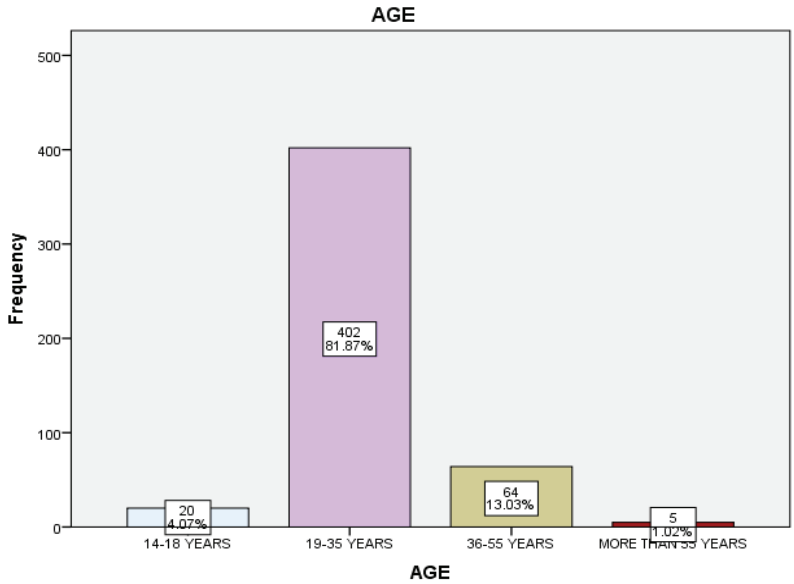


Figure 4: Bar graph depicting the prevalence of fluorosis between different age groups of patients visiting the outpatient department at a private dental hospital. X axis represents the Age of the patients and Y axis represents the frequency of patients having fluorosis. The colour blue denotes 14-18 years, purple denotes 19-35 years, brown denotes 36-55 years and red denotes more than 55 years. The graph shows that among the patients having fluorosis, 81.8% of them were between 19-35 years, 13% belonged to the age group 36-55 years, 4% were between 14-18 years and 1% of them were more than 55 years old indicating that fluorosis most commonly occurred in the age group 19-35 years among the patients visiting the private institution.

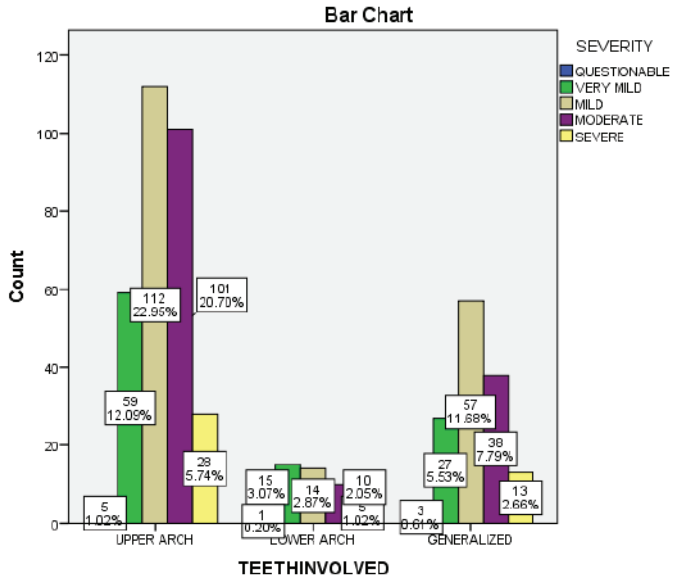


Figure 5: Bar graph depicting the correlation and association between severity of fluorosis and teeth involved in fluorosis among outpatients visiting a private dental hospital. X axis represents the teeth involved in fluorosis and Y axis represents the severity of the fluorosis. The above graph shows that 63% of the patients presented with fluorosis of upper arch out of which a maximum of 23% of them had mild fluorosis. However the severity of fluorosis and teeth involved was not statistically significant. Pearson Chi-Square analysis done p-0.502 and hence, it is not significant.

Dental fluorosis is a condition of enamel hypo-mineralization caused due to the effects of excessive fluoride on ameloblasts during enamel formation [24,25] . Dental fluorosis in teeth exhibits the first sign of fluoride toxicity in the form of "mottled enamel" [26]. The main cause is the water containing excess fluoride content especially ground water of particular communities [27]. Dental fluorosis has several stages. Initially, the teeth become chalky and opaque as an outcome of subsurface hypomineralization. As dental fluorosis progresses, the teeth lose enamel and increasingly develop pits and grooves [28]. If the condition is mild the enamel has opaque white areas covering 50% of the tooth surface [29]. There is a propensity for affecting the entire body leading to skeletal fluorosis and may cause debilitating disabilities compromising the quality of life [30]. Dental fluorosis is more common in permanent dentition than in deciduous dentition [31]. The excessive fluoride intake must occur during the period of tooth formation, since the fluoride appears to affect the activity of ameloblasts [32]. In our study, severity of fluorosis was calculated using Dean's fluorosis index. The success of any treatment for fluorosed teeth depends on the severity of the fluorosis [33,34]. According to Dean's index, Questionable fluorosis includes occasional white fleckings and sightings of enamel, Mild fluorosis includes white opaque areas involving more of the tooth surface, Moderate and severe fluorosis includes pitting and brownish staining of tooth surface [35]. In Spite of these obstacles, several groups have successfully gleaned important insights from the focused comparison of disparate microarray results [36] . Many laboratory, clinical and dental public health researchers after more than 70 years of research have concluded that fluoride is a double–edged weapon, where its deficiency increases the risk for dental caries and excess consumption increases the risk for dental fluorosis [37]. Despite diagnostic and therapeutic advances over the decades, this condition remains a challenge for medical and dental professionals [38] . It is known that a concentration of 1 parts per million (ppm) of fluoride in water offered significant protection against dental caries [39]. The decline in dental caries prevalence and incidence in developed countries over the last two decades is considered to be largely due to the widespread use of fluoride [40]. The fluoride produces a dose-dependent

effect on the dentition [41].

In this present study, it is seen that more males presented with fluorosis with a percentage of 71.5% whereas females were only 28.5%. This is in concordance with a study done among the school children of Nalgonda district in Andhra Pradesh [42]. Previous studies done by Molina *et al* and Sabokseir *et al* showed similar results stating that around 53% of the participants who had fluorosis were males and the remaining were females [43,44]. Firempong *et al* in their study stated that 54.5% of the people diagnosed with fluorosis were males and only 45.5% were females [45]. However Idon *et al* and Molina *et al* in their study reported a contradictory result showing that the prevalence of fluorosis is more common among the females [46,47]. The probable reasons for prevalence in males could be that their daily work schedule ,access and lifestyle which may prevent them from utilising the different preventive strategies enforced in their localities .A study done in the Prakasam district of Tamil Nadu shows that the prevalence of dental fluorosis was 82.04% and compared to urban areas, the prevalence of dental fluorosis was higher in rural areas [48]. Another study done in the Cuddalore District of Tamil Nadu revealed that only one-third of children had experienced dental fluorosis of 31.4% [49]. This might be because in an endemic fluorosis area, a great amount of fluoride is incorporated into food materials and ingested into the body.

Our study also revealed that most of the participants presented with mild fluorosis (37.5%). This is in concordance with previous studies performed by Damelash *et al* and Khan *et al* which revealed that there was a high prevalence of mild fluorosis [50,51] . Another study showed similar results stating that the majority of the participants presented with mild fluorosis [52].This solely depends on the concentration of fluoride in the ground water of the locality and in the diet consumed by the participants. The severity of fluorosis could depend on the difference in exposure to fluoride or on the individual characteristics of the participants.

In our study, a major proportion (63%) of the participants presented with fluorosis in the upper arch. This is in concordance with a similar study done in North-Western Villages of Makoo which reveals that maxillary teeth were more affected by fluorosis [53]. Other studies done by Mehta D et al and Lasrsen et al show similar results stating that there is more than 50% risk for maxillary teeth especially central incisors and first premolars to get affected by fluorosis [54,55]. In another study by Larsen et al, it is stated that the prevalence of fluorosis is least in lower incisors and increases in the upper molars, incisors and canines [56]. This is because fluorosis affects teeth like maxillary incisors, canines and first molars which mineralize last. Hence maxillary teeth are more affected than mandibular teeth. Although Chi square test did not reveal any significance between the teeth involved and severity of fluorosis (p value 0.5>0.05).

In our present study, about 82% of the sample size presenting with fluorosis were in the 19- 35 years age group. This is in concordance with a study done by Akinkugbe *et al* that shows similar results stating that the majority of the patients having fluorosis were between 18-45 years [57]. However Szpunar et al in his study shows contradictory results stating that prevalence of fluorosis increased in the age group 8-10 years. [58]. This is because, at early ages there is little control of expectoration of the residues of the toothpaste during brushing and this leads to the ingestion of toothpaste in children. Hence children are more commonly affected by fluorosis.

Thus fluorosis can be prevented by having an adequate knowledge of the fluoride sources, knowing how to manage this issue and therefore, avoid overexposure [59]. Providing decreased fluoride content water to the community can be useful in the management of this condition as a whole [60].

The study was geographically limited and predominantly consisted of the South Indian population. Data which were unclear were excluded thereby reducing the sample size. Within the limit of the study , it was found that 488 cases of fluorosis reported to the private dental college and hospital and showed a male predilection majorly affecting age groups of 19 to 35 years. To ascertain the results of this study and to increase the level of significance, the sample size and the geographic area of coverage should be extended to at least most parts of South India. Thus the study helps in gaining knowledge about the prevalence of fluorosis which will help in taking up further interventions like

setting up of community defluoridation units and improving the awareness about the current scenario.

Conclusion

Although dental fluorosis is an irreversible condition of the enamel of teeth, it can be prevented if the level of fluoride in water is optimum. Regular water testing, routine medical check-up camps, and continued health awareness program would definitely benefit the community residing in fluoride endemic areas.

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Conflict of Interest: None to declare

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Correlation of Clinically Desquamative Gingivitis and Histopathology at a Private College Hospital in Chennai, India - A Retrospective Study

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Abstract

Desquamative gingivitis is an elucidating term used to demonstrate epithelial desquamation erythema, erosions and vesiculobullous lesions. It is a clinical manifestation that can be caused by several systemic disorders. Detection and differentiation between conditions that manifest desquamative gingivitis has been a continuing problem. Accurate clinical, histologic and serologic investigations are often required to differentiate among desquamative gingivitis disorder. This study focuses on understanding the correlation between clinically desquamative gingivitis and the histopathological reports. A retrospective cross-sectional study was conducted using the patient records from the department of periodontics and oral pathology, Saveetha Dental College from June 2019- April 2020 and patients who underwent treatment for desquamative gingivitis were selected by nonprobability sampling Microsoft Excel® 2016 (Microsoft office 10) data spreadsheet was used to collect data and later exported to SPSS® statistical package for social sciences for Windows (version 20.0, SPSS Inc, Chicago IU, USA). From the data analyzed it is observed that the overall incidence of desquamative gingivitis was higher in females (60%) predominantly in the age group of (51-60) years (50%). The most predominant histopathology report diagnosed Oral lichen Planus (70%). The correlation between clinical diagnosis and histopathology reports was statistically significant with $p<0.05$. Within the limits of the study, a significant number of patients with desquamative gingivitis had a female predilection predominantly in the age group of 50-60 years. The most common histopathology report of clinically desquamative gingivitis was Oral Lichen Planus. As clinically suggestive desquamative gingivitis may be an initial symptom of a systemic disease, the need for biopsy as a diagnostic tool should be made mandatory.

Keywords: *Oral Lichen Planus, Pemphigus Vulgaris, Mucous Membrane Pemphigoid, Linear IgA disease, Desquamative Gingivit*is

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Introduction

Desquamative Gingivitis is a descriptive clinical term for desquamation, erosion, vesicles, and bullae that involve the free and attached gingiva. [1] The term "desquamative gingivitis" was first described by Tomes and Tomes in 1894, but it was Prinz in 1932, who established [2] that it was a descriptive term used to define the presence of the erythema, desquamation, erosions and blistering of the marginal and attached gingiva.[3] In 1960, Mc Kathy and Co were the first to suggest that

desquamative gingivitis is not an entity but a gingiva response to a variety of systemic disturbances of varied etiologies. [4] Furthermore, in 1964, Glickman and Jerome demonstrated that this alteration of the gingiva was secondary to other systemic conditions.[5] It is usually associated with autoimmune blistering disorders like pemphigus Vulgaris, pemphigoid, and oral lichen planus and other systemic disorders. [4,6]

The pathogenic mechanism of desquamative gingivitis involves the separation of epithelium from the underlying connective tissue by a disease that can cause either a subepithelial or an intraepithelial vesicles [6–8]. Desquamation can also be triggered by intentional local factors (Nikolsky sign) or intentional factors like masticatory forces, poor restoration, and calculus[9,10] and habits like smoking and tobacco chewing. [11]Cicatricial pemphigoid (CP) is an autoimmune vesiculobullous disease which targets various components of the basements membrane such as BP 180 and laminin 332 resulting in the formation of subepithelial vesicles.[12,13]

Pemphigus Vulgaris is an autoimmune disease which shows its initial symptoms of desquamative gingivitis.[14] In this disease, antibodies are directed against desmosomal proteins (desmoglein 1 and 3) of the cells in a spinous layer which leads to the formation of intraepithelial blisters.[15,16] Lichen planus is an autoimmune disease that manifests desquamative gingivitis. In lichen planus, subepithelial separation is induced due to the destruction of the basal cell layer of the epithelium resulting in loss of adhesion in connective tissue.[17] lichen planus is associated with oxidative stress in saliva as the levels of various biomarkers were seen to be altered in the disease [18,19]. However, these markers are of no help in the Oral Tongue Squamous Cell Carcinoma.[20] high degree of Reactive Oxygen Species (ROS) release leads to heightened oxidative damage to gingival tissue, periodontal ligament, and alveolar bone. [21] Oral leukoplakia which is a potentially malignant disorder and oral lichen planus have different etiopathology but their clinical representation can be very similar. [22–24] Ankyloglossia otherwise called tongue-tie restricts the cleansing activity due to limited movement of the tongue leading to gingivitis and other varied periodontal diseases. [25]

Establishing the correct diagnosis of the underlying disease is of paramount importance in order to provide proper treatment.[26] The appearance and location of lesions must alert the clinician and proper photographs must be taken for future references.[27,28]. Hence, the aim of the study is to find the correlation between clinically diagnosed desquamative gingivitis and histopathology diagnosis.

Materials and Methods

Study Design And Setting

This pilot retrospective study examined the records of 86,000 patients from June 2019- April 2020 undergoing treatment at Saveetha Dental College and Hospital, Chennai in the department of periodontics. Ethical approval was obtained from the institutional ethics committee. The study population included patients who underwent treatment for desquamative gingivitis at the Outpatient Department of Saveetha Dental College.

Data were collected from the hospital's patient database records which were used to identify n= 86,000 patients from which n=10 patients who were diagnosed for desquamative gingivitis and underwent treatment for the condition.

Relevant data like patient's age, gender was recorded. Patient:s age groups were divided as (20-30) years,(30-40) years, (40-50) years, (51-60) years, and (61-70) years. Repeated patient records and incomplete data were excluded. Data were reviewed by an external reviewer. Data were recorded in Microsoft Excel® 2016 (Microsoft office 10) and later exported to SPSS® statistical package for social sciences for windows versions, 20.0, SPSS Inc, Chicago IU, USA), and subjected to statistical analysis. A Chi-square test was done and $p < 0.05$ was considered significant.

Results and Discussion

The final dataset consisted of n= 10 patients of Indian origin who underwent treatment for desquamative gingivitis.

The prevalence of desquamative gingivitis based on age was found to be higher in the (51-60 years) age group (50%) followed by (31-40 years) age group (20%), following which (20-30 years), (40-50 years) and (61-70

years) showed (10%) each. (figure 1) and was found to be higher in females (60%) than males (40%) (figure 2). The prevalence of desquamative gingivitis as a clinical diagnosis was about (50%) while oral lichen planus was about (40%) and vesiculobullous lesions were about (10%) (figure 3) The most prevalent histopathology report of the clinically suggestive desquamative gingivitis was Oral Lichen Planus (70%) followed by 'Not Available' (20%) and Vesiculo Bullous Lesion (10%) (figure 4). Biopsy was subjected for (30%) of the patients and the rest of the (70%) were ill-advised. (figure 5) About 30% of the biopsies were done after initiating treatment for the differential diagnosis. (figure 6) The correlation of clinical diagnosis of desquamative gingivitis and histopathology reports was found to be significant with $p< 0.05$ (figure 7).

The data for this retrospective study was based on the residents of Chennai seeking treatment at Saveetha Dental College and Hospitals.

Desquamative gingivitis is a group of non-plaque induced gingival disorders according to the current classification of periodontal diseases and conditions. It appears very early during its clinical course. Most cases of oral lichen planus, Mucous Membrane Pemphigoid and Pemphigus Vulgaris initially present as gingival lesions.[29,30]

In the current study, the prevalence of desquamative gingivitis on the basis of age was observed to be higher (50%) in the 51-60 years age group when compared with other age groups. Few studies stated that Desquamative gingivitis generally has a peak during the fourth and sixth decade of life. They had also reported that Desquamative Gingivitis cases were rarely associated with children and adolescents.[31–34]. In a similar study by Suresh L et al, the median age of the patients was 58 years. [35] A study by Leao et al, stated that the reason behind this could be related to hormonal changes that occur during menopause, which occurs generally in the 5th decade of life.[7] Another study by Yih et al stated that it occurs due to the lack of oestrogen and progesterone which occurs at menopause. [36] All the previously literature is in concordance with our results.

The current study showed that females(60%) had a higher predilection with Desquamative Gingivitis than males. There is a female predilection for all the diseases which clinically represents Desquamative Gingivitis except for pemphigus and linear IgA disease. [35,37] In a study by Suresh L et al, there was a female predilection to diseases like Oral Lichen Planus and Mucous Membrane Pemphigoid which are associated with Desquamative Gingivitis.[35] These findings could be because of hormonal changes that occur during menopause in women. [7]

A few studies state that desquamative gingivitis is a clinical symptom that may represent an underlying disease. [37,38]. In the current study, 50% of the diagnosis which was made based on the clinical appearance was Desquamative gingivitis. When the disease is confined to the gingiva, the diagnosis becomes difficult. Clinical presentation of the generalized symptoms makes the definitive diagnosis of Desquamative gingivitis more difficult. The clinical symptoms of Desquamative Gingivitis is polymorphous and the presence of plaque and calculus complicates the diagnosis. Many diseases like Oral Lichen Planus [17,39] and other vesiculobullous diseases such as Mucous Membrane Pemphigoid [40,41], Pemphigus Vulgaris[30,42], etc. present as desquamative gingivitis so diagnosis from the clinical symptom becomes difficult.

This current study found a higher prevalence (70%) of Oral lichen planus based on the histopathology reports of the clinically suggestive desquamative gingivitis. A few similar studies were done by Suresh L et al and Lo Russo et al stated that the clinical diagnosis of Oral Lichen Planus was the most common disease presenting with Desquamative Gingivitis. [35,38]. The frequency of desquamative gingivitis in patients with lichen planus is lower than in cicatricial pemphigoid and pemphigus Vulgaris. However, due to the significantly higher prevalence of lichen planus compared to cicatricial pemphigoid and pemphigus Vulgaris, desquamative gingivitis is most commonly attributed to lichen planus. [38,43] According to a few different studies, Mucous membrane pemphigoid was responsible for (35%-48%) of the cases of desquamative gingivitis whereas Oral Lichen Planus and Pemphigus Vulgaris accounted for about (24%-45%) and (3%-5%) respectively. [30,41] In the current study, the possible reason for the higher prevalence of oral lichen Planus may be because of its epidemiology, which occurs in 1 to 2% of the population. [38]

In the current study, there was an unusual contradiction in histopathology in which a particular case was diagnosed and treated for both Oral Lichen Planus and Pemphigus Vulgaris. This may be due to the nonavailability of Immunofluorescence in which case the reporting pathologist for the second time could have probably considered bullous lichen planus in lieu of the previous report and clinical presentation. In a study by Bernt A et al, 14 patients with desquamative gingivitis were first subjected to histopathological and immunofluorescence studies before treatment with tetracycline was started.[44] In a study by Roger et al, 41 patients with desquamative gingivitis were treated. Biopsies were done initially and the tissue was subjected to light and direct immunofluorescence microscopy. Based on the histopathological findings the present disease was established and treatment started thereafter with topical corticosteroids and systemic dapsone. [29] In all previous clinical studies, treatment was started after conducting histopathological and immunofluorescence studies.

In our study,70% of patients were not advised for biopsy probably due to comorbid factors such as Uncontrolled Diabetes, Anticoagulant Therapy, Compromised general health, Chronic Kidney Disease, Hypertension, and various other ailments which could have probably delayed the clinician to advice incisional biopsy. Literature suggests that patients should be carefully evaluated prior to biopsy if they have comorbid conditions to avoid unnecessary complications.[45]In order to relieve the symptoms the treating physician could have empirically started the patient on medication.

Desquamative gingivitis can mimic plaque related gingival inflammation, as painful symptoms impair maintenance of oral hygiene and cause accumulation of dental plaque.[46–48] this can cause a delay in diagnosis as clinicians might confuse with plaque-induced gingivitis and which potentially increases the risk of morbidity and the risk of life-threatening complications.[49,50] However, typical and distinctive oral and skin lesions presenting in a specific location can present as a valuable aid in the differential diagnosis.[38] It is known that desquamative gingivitis is a clinically descriptive term and not an actual disease and also knowing the fact that it may be a manifestation of severe systemic disease and that only histopathology will help us in finding the definitive diagnosis. This shows an increasing need to mandate biopsy as the disease goes undiagnosed or misdiagnosed without subjecting the patient for biopsy and also without the histopathology reports. Making a definitive diagnosis by differentiating these disorders from one another is important because the treatment, management, and prognosis differ among the disorders.

Upon conducting histopathological studies characteristic features are seen in different pathological states. Acantholytic keratinocytes (Tzanck cells) are seen in Pemphigus Vulgaris, subepithelial or intraepithelial vesiculation and necrotic keratinocytes in Erythema Multiforme, basal cells liquefaction degeneration and lymphocytic band-like sub basilar infiltrate in Oral Lichen Planus and Keratinocyte vacuolization with lamina propria edema in Lupus Erythematosus. [51] These findings direct the diagnosis and treatment planning of the affected individual in an efficient manner.

The limitation of the study includes geographic limitations as the study was conducted only among the South Indian population predominantly. The study included small sample size and hence the results obtained cannot be generalized.

The future scope of the study will be better and will yield accurate results if different ethnic populations are considered along with a larger study population.

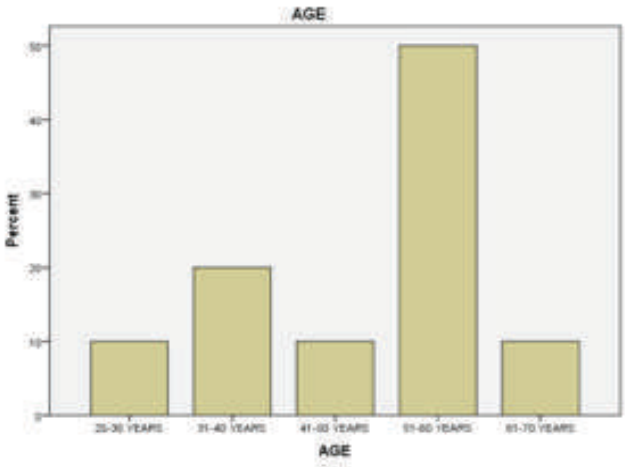


Figure 1: This bar graph represents the percentage of desquamative gingivitis based on age. Age is distributed as 20-30 years, 31-40 years, 41-50 years, 51-60 years on the x-axis. From the graph, it is evident that desquamative gingivitis is more prevalent in the 51-60 years age group.

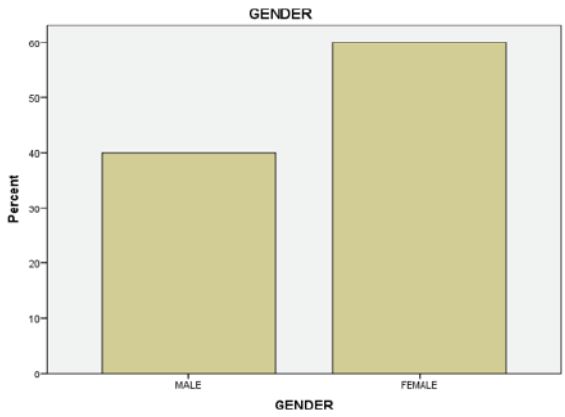


Figure 2: This bar graph represents the percentage of desquamative gingivitis based on gender. Parameters were Males and females and were represented on the x-axis. From the graph, it is evident that desquamative gingivitis is more prevalent in females than males.

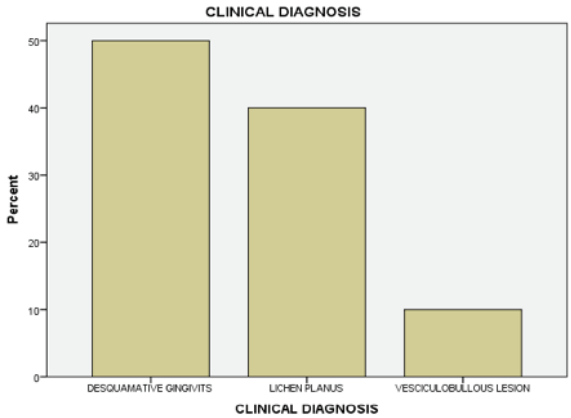


Figure 3: This bar graph represents the percentage of clinical diagnosis of desquamative gingivitis where the parameters like Desquamative gingivitis, lichen planus and Vesiculobullous lesion are represented on the x-axis. From the graph it is evident that desquamative gingivitis was the most prevalent clinical diagnosis. Wow

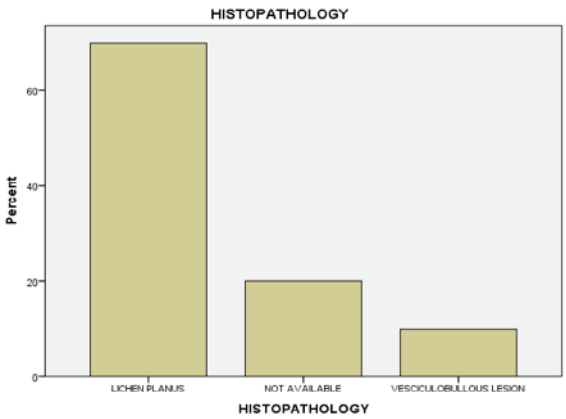


Figure 4: This bar graph represents percentage of Histopathology diagnosis of Desquamative gingivitis where Parameters like lichen planus, Vesiculobullous lesion, and not available are represented on the x-axis, From the graph, it is evident that lichen planus is the most prevalent histopathology diagnosis of desquamative gingivitis.

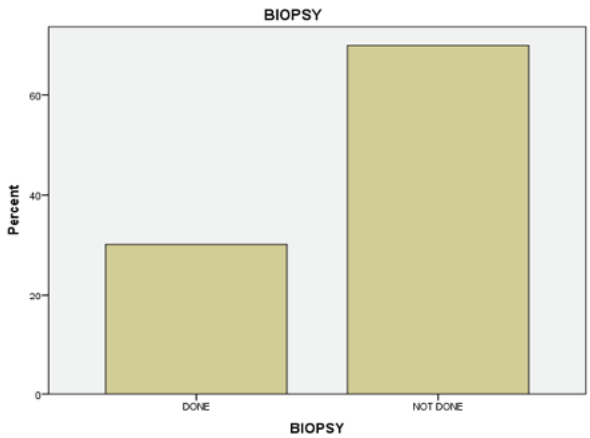


Figure 5: This bar graph represents the percentage of Biopsy status in Desquamative gingivitis where Parameters were 'done' or 'not done' were represented on the x-axis. From the graph it is evident that not done was the most prevalent status of biopsy.

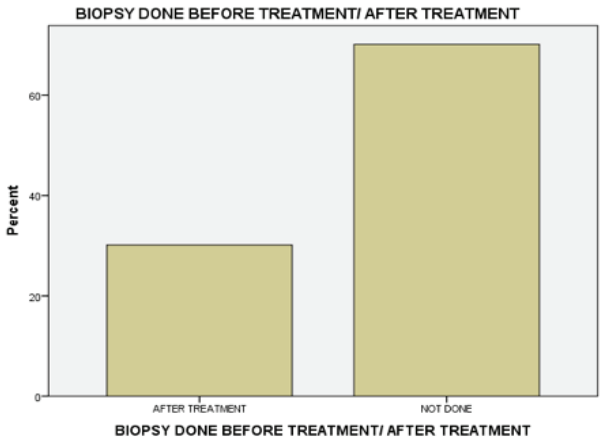


Figure 6: This bar graph represents the percentage of Biopsy status whether it was done before or after desquamative gingivitis where the parameters included 'after treatment' and 'not done' on the x-axis. From the graph, it is evident that most of the biopsies were not done.

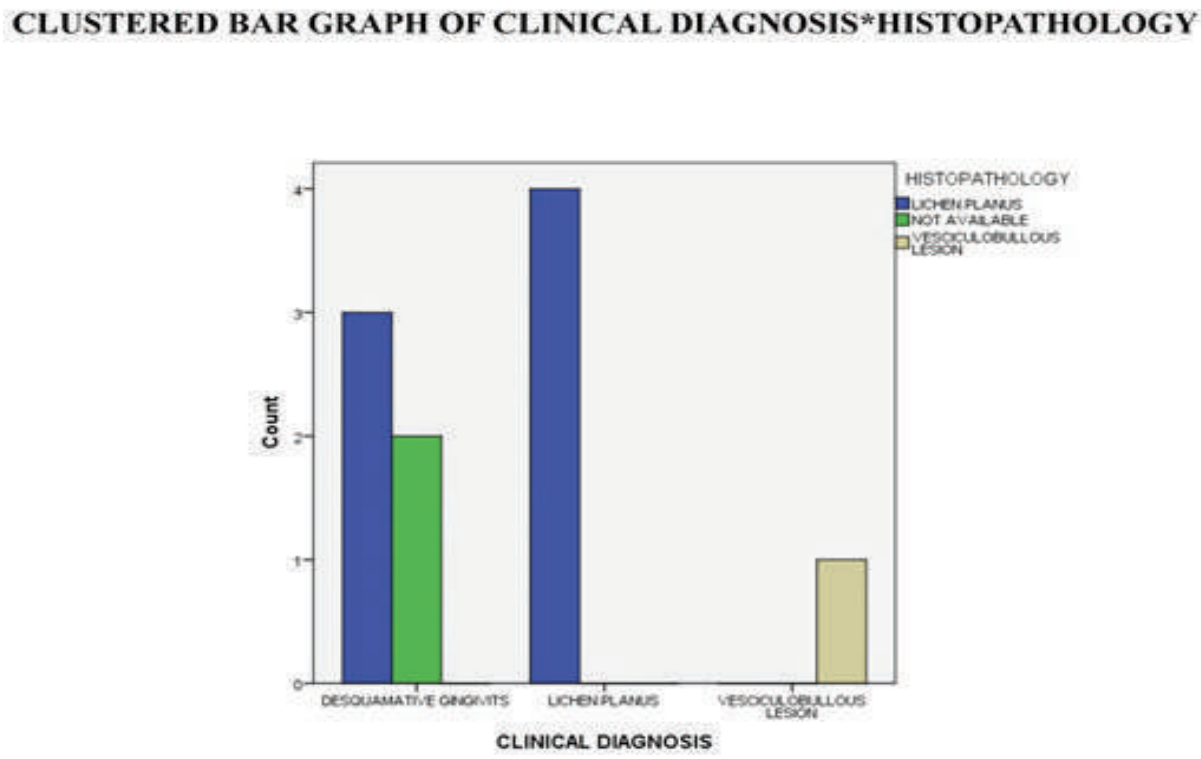


Figure 7: This bar graph depicts the association between the clinical diagnosis and histopathology reports of the study population. Different clinical diagnosis are represented in various colours on the X axis, Blue represents Lichen Planus, green represents data not available and brown represents Vesiculobullous Lesions. Y axis represents the histopathology reports of the study population on a scale from 0 to 4. There is a significant association between the clinical diagnosis and histopathology reports. (Pearson chi square test; p value =0.015, statistically significant)

Conclusion

Within the limits of the present study, it is observed that desquamative gingivitis was more prevalent in females and predominantly in the age group of 51-60 years. The most prevalent histopathology diagnosis was oral lichen planus. Also, there was a positive correlation between clinical diagnosis and histopathology reports with $p<0.05$. This study helps us to understand the importance of biopsy as a diagnostic tool and histopathology results in diagnosing clinically significant desquamative gingivitis. It is known that desquamative gingivitis is a clinically descriptive term and not an actual disease and also knowing the fact that it may be a manifestation of severe systemic disease and that only histopathology will help us in finding the definitive diagnosis hence, a biopsy must be made mandatory. Also, patient education and motivation in biopsy must be considered a high priority whilst determining the treatment plan for Clinically suggestive Desquamative Gingivitis.

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Conflict of Interest: None declared.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Research Article

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Prevalence of Odontogenic Tumours in a Dental Hospital in Southern India

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Abstract

Odontogenic Tumours (OTs) pose as a heterogenous group of lesions exhibiting diverse clinical behaviour and histopathological types which can range from hamartomatous lesions to malignancies. Although a large number of studies have been done to record the epidemiology of odontogenic tumours, there is a paucity of information where its prevalence is concerned in South Indian cities. Thus, the aim of our study is to highlight the prevalence of various types of odontogenic tumours in a predominantly South Indian hospital set up so as to improve clinical understanding and handling of patients with said tumours. A retrospective cross-sectional study was conducted after reviewing and analysing the data from patient records over the last five years. Patients with histopathologically established odontogenic tumours were selected without any age restriction. The data was collected and descriptive statistics were performed. The findings of our study hinted at a benign odontogenic prevalence with a male predilection (68.6%). The posterior region of the mandible was the most commonly involved site (60%) and ameloblastomas followed by odontomas and adenomatoid odontogenic tumours were the most prevalent odontogenic tumours in our hospital set up. An asymptomatic trend with multilocular radiolucencies was evidently observed. Through the results of our study, we hope to elucidate the prevalence of odontogenic tumours in the South Indian population. In essence our findings suggest a male predilection in the posterior region of the mandible and that Ameloblastomas and Odontomas are the most common odontogenic tumours. Due to the large number of asymptomatic presentations, proper diagnosis and management is key. Thus, further studies need to be done to overcome the limitations of our study and to confirm our findings.

Keywords: *Odontogenic Tumours; Follicular Ameloblastoma; Unicystic Ameloblastoma; South India.*

Introduction

Odontogenic Tumours are heterogeneous lesions that are derived from epithelial or ectomesenchymal tissues or sometimes, both. They can range from hamartomatous lesions to malignancies and exhibit a plethora of clinical behaviours and histopathological types [1–3]. They can either be found within the jaw bones (central type) or in the mucosal tissue that overlies the tooth bearing areas (peripheral types). Although the etiology is unknown OTs can be primarily classified into malignant and benign tumours. The majority of benign tumours seem to arise de novo, whereas in malignant tumours they may arise de novo but they more commonly arise from their benign precursor. Essentially, the classification of odontogenic tumours is based on its interactions between the epithelium and the odontogenic ectomesenchyme. But this classification is constantly under debate so it is frequently met with new additions and the removal of existing entities.[4,5] Ameloblastomas

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in general are common (among OTs), benign tumours of the odontogenic epithelium that more often occur in the mandible than the maxilla. Although they are rarely metastatic and progress very slowly, the lesions cause severe disfigurement of the face and the jaw. [6–15] Odontomas are benign tumours associated with tooth development [16–24]. It is a dental hamartoma indicating that it is composed of normal dental tissue but has grown in an abnormal manner. It is of type types – compound and complex. [25–29] Adenomatoid Odontogenic Tumours (AOTs) arise from the enamel organ or the dental lamina.[30] Nearly two by third of its cases are associated with an impacted canine.[31,32] It is fairly uncommon and is seen mostly in younger people with a female predilection.[33] Odontogenic Myxomas are uncommon benign tumours arising from embryonic connective tissues associated with tooth formation. It mainly consists of spindle shaped cells and scattered collagen fibres, distributed in a loose mucoid material. As the tumour expands it has been known to infiltrate adjacent structures like sinuses in the case of maxillary tumours and the ramus in the case of mandibular tumours.[34–37] Odontogenic Fibromas are slow growing, uncommon gingival masses. It can sometimes cause tooth displacement and has no particular gender predilection.[38–41] Ameloblastic carcinoma is a rare tumour that develops in the jaw bones from enamel forming epithelial cells.[42,43] It can cause painful swellings that only progresses, with no response to chemotherapy. Although some patients may even be asymptomatic, some rare symptoms include trismus, dysphonia and voice loss.[44–46] As tumours always pose a threat to a person's wellbeing, our study aims to enumerate its prevalence and to analyse any gender, site, radiographic or clinical presentation predilection.

There is a marked difference in the patterns of incidence as reported by several individuals attempting to study the epidemiology of odontogenic tumours. Thus, our study is engineered to notice any difference in prevalence trends in recent years as compared to older documentations and to elucidate the presence of variations amongst different geographical locations and ethnicities in regard to the prevalence of different types of odontogenic tumours.

Materials and Methods

Study Design and Setting

This retrospective study reviewed the records of patients who visited the hospital over the last five years to undergo various treatments at Saveetha Dental College, Chennai. Ethical approval was obtained from the Institutional Ethics Committee. The study population included patients with histopathologically established odontogenic tumours with no age restrictions. They were separated according to their sex, type of tumour, site, recurrence, clinical and radiographic presentations. Mentally or physically disabled individuals were excluded from the study due to their potential difficulties faced during participation.

Data Collection

The records of patients who visited Saveetha Dental College in the last five years were analysed and were used to identify 35 patients in the hospital database undergoing various treatments, but with histopathologically established odontogenic tumours. Relevant data such as patient age, sex, site, type of tumour, history of recurrence, clinical and radiographic presentation were obtained from their general examination, clinical examination, personal details and histopathological reports and was recorded. Repeated patient records and incomplete entries were excluded. The data obtained was then verified by an external reviewer.

Statistical Analysis

Data was recorded in Microsoft Excel 2016 (Microsoft Office 10) and was later exported to the Statistical Package for the Social Sciences for Windows. (Version 20.0, SPSS, Inc., Chicago, USA) and was subjected to descriptive statistical analysis.

Results and Discussion

The final dataset consisted of 35 patients of predominantly South Indian origin undergoing various treatments but with histopathologically proven odontogenic tumours among the total 1550 reported biopsy cases. The sites were grouped as the mandibular posterior region, mandibular anterior region, maxillary anterior region and 'mandible' for tumours that occurred in the premolar region or throughout the entire lower jaw. Out of these sites, the one with the highest prevalence of odontogenic tumours was the mandibular posterior region (60%) followed by the mandible (25.7%), the mandibular anterior region (11.4%) and the maxillary

anterior region with the least (2.9%) as inferred from Figure 1. Overall, the mandible was more susceptible to the prevalence of odontogenic tumours when compared to the maxilla. The mean age of the study sample was 33.09 years. According to Figure 2, the age group with the most number of established odontogenic tumours was found to be 'above the age of 40 years'. There was a clear male predilection when its incidence was considered with 68.6% as opposed to 31.4% of females. (Figure 3)

Based on the type of tumour, follicular ameloblastoma was the most common (34.3%), followed by unicystic ameloblastoma (including all types - 17.1%), plexiform ameloblastoma (14.3%), granular cell ameloblastoma (8.6%), 5.7% for both types of odontomas and adenomatoid odontogenic tumours and 2.9% being the least for proliferative ameloblastoma, odontogenic myxoma, calcifying cystic odontogenic tumour, peripheral odontogenic fibroma and ameloblastic carcinoma. (Table 1)

Recurrence was observed in 14.3% of the cases as suggested from our data in Table 2 and Figure 4. The clinical presentation was recorded under two groups – asymptomatic swellings (60%) and symptomatic swellings (17.1%) with the rest of the data not available because of the absence of its mention in the case reports. (Table 3) The radiographic presentations was recorded under four groups – multilocular radiolucency (40%), unilocular radiolucency (28.6%), unilocular with an impacted tooth (11.4%) and 2.9% of radio-opaque masses with the rest unavailable as inferred from Table 4 and Figure 5.

Thus, the most common trends observed was a male predilection; involvement of the posterior mandible; rarely recurring asymptomatic swellings radiographically presenting with multilocular radiolucencies and a tendency to occur more commonly above the age of 40 years. Ameloblastomas (follicular type) followed by odontomas and adenomatoid odontogenic tumours were the most frequently prevalent OTs.

The data for this retrospective study was based on residents of South Indian cities seeking treatment at Saveetha Dental College, Chennai. According to our results, 24 males out of 35 patients (68.6%) and 11 females out of 35 patients (31.4%) were affected showing a predilection in favour of males in regard to the prevalence of odontogenic tumours. This is in accordance with most studies - In a study conducted by Gupta B *et al.* in 2010 sampling a similar south Indian population in the state of Tamil Nadu, it is suggested that the mandible especially the posterior part was the most commonly involved site and that ameloblastomas were the most prevalent type of odontogenic tumour (67.69%) followed by AOTs (9%) and odontomas (7.77%). Most cases were benign with only a few incidences of malignancies. This is in line with our findings, except for the fact that odontomas and AOTs were equally prevalent in our study. This could be an attribute to our difference in sample size of 35 patients as opposed to 489 cases.[47] In another study by Ezekiel *et al*. in 2002, they concluded that ameloblastomas were the most common odontogenic tumour and that tumours occurred more often in the mandible than the maxilla. There was also a gender predilection to males (57%) as opposed to females (43%). Some of the most common clinical findings were pain, swellings and loosening of teeth. There were many records of asymptomatic cases with most being benign.[48] These suggestions are in line with our findings. This could possibly be due to our comparable population sizes of 35 and 78.

In a study by Varun A.I *et al.* in 2016, they reported that the prevalence of odontogenic myxomas was around (0.04% – 3.7%) [49] which is in line with our results of 2.9% for the same. In another study by Siar C.H *et al.* in 2000 they report that peripheral odontogenic fibromas account for (1.2% - 4.7%) [50] of all odontogenic tumours which is in accordance to our finding of 2.9%. In a study of 12 cases of ameloblastic carcinomas by Li J *et al.* in 2014 they concluded that ameloblastic carcinoma is a rare type of tumour, occurring only in 2.23% [51] of patients which is comparable to our findings of 2.9% for the same. Since several studies over the last decade exhibit a similar pattern of incidence, there is no conclusive evidence of a change in the trends surrounding the prevalence of OTs.

Although retrospective, our study does present with certain limitations such as geographical barriers and diversity of opinions during clinical examination and radiographic interpretation. Thus, further multicentre studies must be performed to ascertain the true prevalence of the south Indian population.

Prevalence of Types of Odontogenic Tumours		
Type of Tumour	**Frequency**	**Percentage**
Follicular Ameloblastoma	12	34.3
Plexiform Ameloblastoma	5	14.3
Proliferative Ameloblastoma	1	2.9
Granular Cell Ameloblastoma	3	8.6
Unicystic Ameloblastoma Type I, Type II and Type III	6	17.1
Odontogenic Myxoma	1	2.9
Odontoma Type I and Type II	2	5.7
Calcifying Cystic Odontogenic Tumor	1	2.9
Peripheral Odontogenic Fibroma	1	2.9
Adenomatoid Odontogenic Tumor	2	5.7
Ameloblastic Carcinoma	1	2.9
Total	35	100.0

Table 1 - Table representing the frequency and percentage of prevalence of OTs based on its type with follicular ameloblastoma (34.3%) being the most common followed by unicystic ameloblastoma (17.1%). Hence ameloblastomas are the most prevalent odontogenic tumours.

Recurrence		
	Frequency	**Percentage**
Yes	5	14.3
No	30	85.7
Total	35	100.0

Table 2 - Table representing the frequency and percentage of prevalence of recurrence among the OTs with a statistically significant negative predilection towards recurrence (30 out of 35 patients). Hence recurrence is less common for odontogenic tumours.

Clinical Presentation		
	Frequency	**Percentage**
Asymptomatic Swelling	21	60.0
Symptomatic Swelling	6	17.1
NA*	8	22.9
Total	35	100.0

Table 3 - Table representing the frequency and percentage of various clinical presentations of OTs where asymptomatic presentations (60.0%) are more common when opposed to symptomatic presentations (17.1%). Hence it is more common for odontogenic tumours to go unnoticed for a long time. *Not Available

Radiographic Presentation	**Frequency**	**Percentage**
Unilocular Radiolucency	10	28.6
Unilocular with an Impacted Tooth	4	11.4
Multilocular Radiolucency	14	40.0
Radiopaque Masses	1	2.9
NA*	6	17.1
Total	35	100.0

Table 4 - Table representing the frequency and percentage of various radiographic presentations of OTs where multilocular radiolucencies (40.0%) followed by unilocular radiolucencies (28.6%) are the most frequent presentations. Hence tumours which present with multilocular radiolucencies radiographically are more likely to be commonly prevalent compared to other tumours. *Not Available

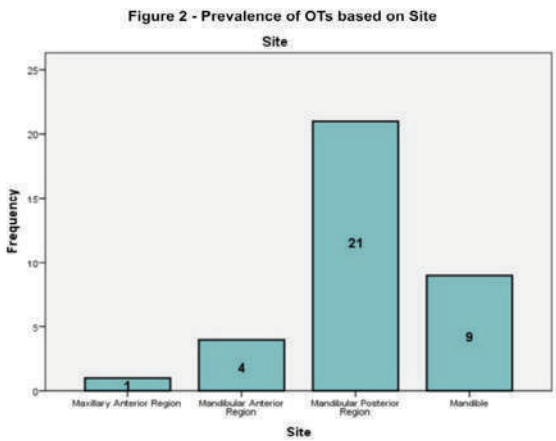


Figure 1 - Bar chart representing the prevalence of OTs based on site with a predilection towards the posterior region of the mandible with 21 out of 35 patients across the scale of study population frequency in the 'y' axis and site in the 'x' axis. Hence the most common site for odontogenic tumours is the mandibular posterior region.

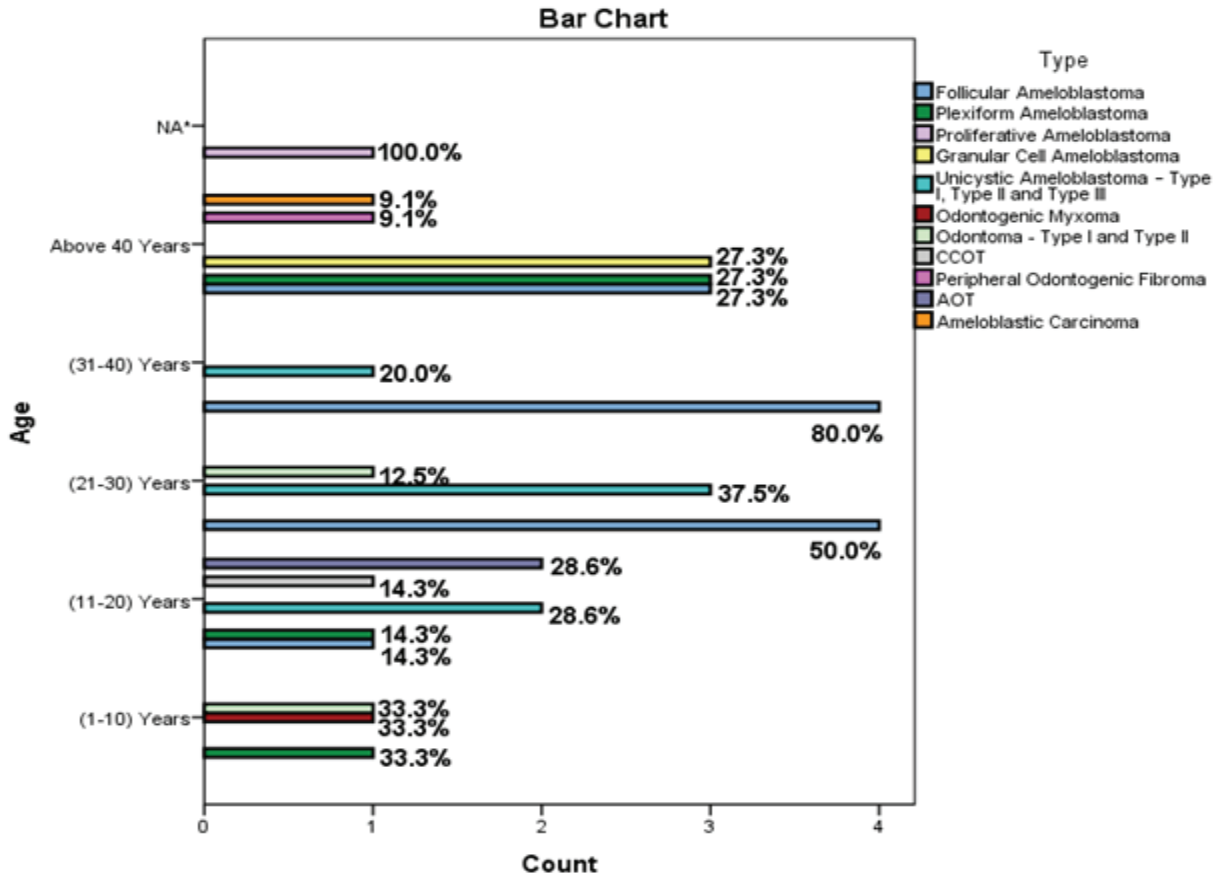


Figure 2 - Bar chart representing the association between the various types of OTs and age, showing a higher incidence of various types of OTs above the age of 40 years across the scale of study population count in the 'x' axis and the age groups of (1-10) years, (11-20) years, (21-30) years, (31-40) years and above 40 years in the 'y' axis. Here, the colour blue represents follicular ameloblastoma, dark green represents plexiform ameloblastoma, lilac represents proliferative ameloblastoma, yellow represents granular cell ameloblastoma, turquoise represents unicystic ameloblastoma (inclusive of all types), maroon represents odontogenic myxoma, pale green represents odontoma (inclusive of all types), grey represents calcifying cystic odontogenic tumors, pink represents peripheral odontogenic fibroma, violet represents adenomatoid odontogenic tumours and orange represents ameloblastic carcinoma. People above the age of 40 years are more likely to develop odontogenic tumours and this was found to be statistically significant. Chi square value, p = 0.001 (p< 0.05) *NA-Not Available

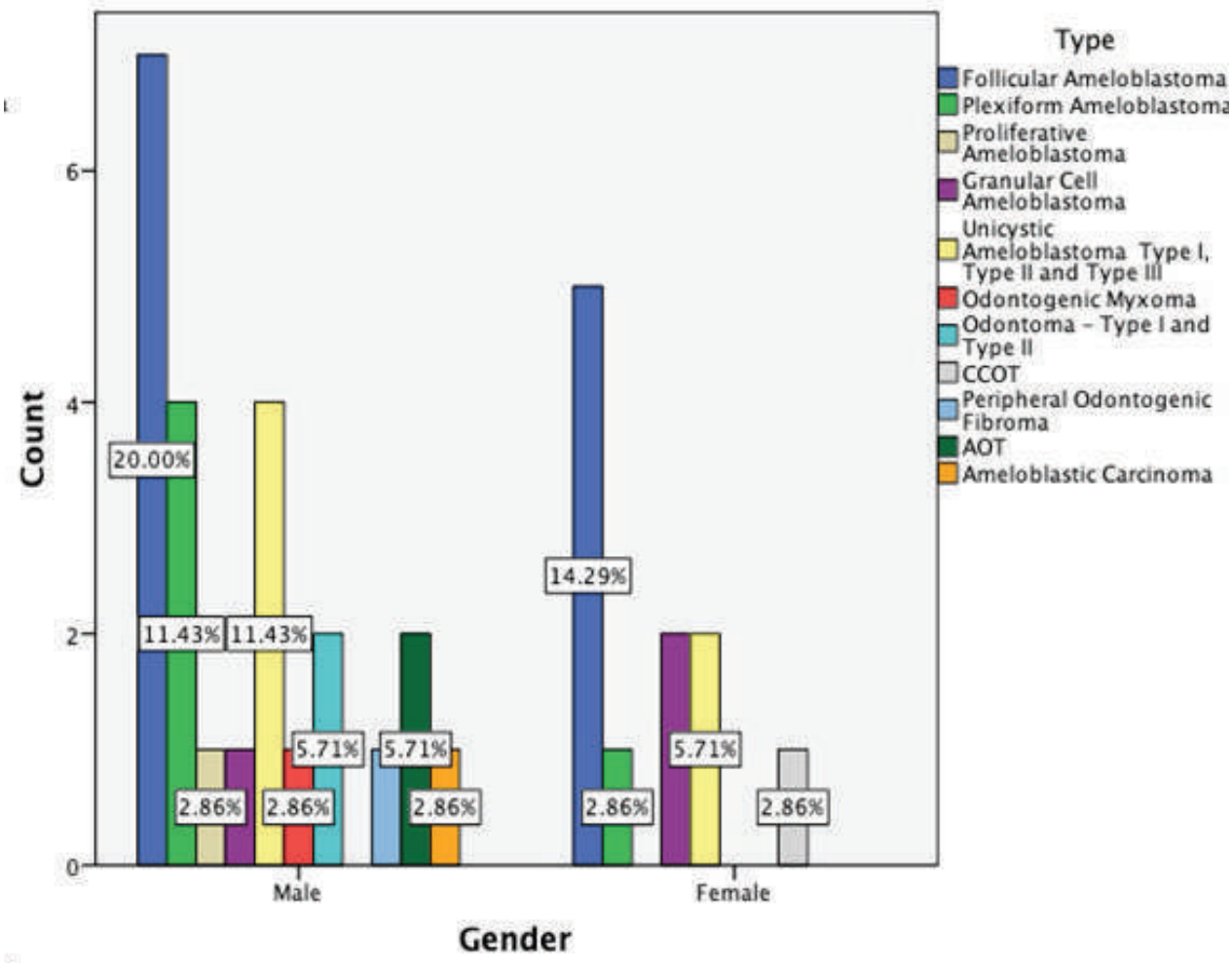


Figure 3 - Bar chart representing the association between the various types of OTs and gender where there is an existing predilection towards males across a scale of gender in the 'x' axis and study population count in the 'y' axis. Here, the colour dark blue represents follicular ameloblastoma, light green represents plexiform ameloblastoma, mustard represents proliferative ameloblastoma, purple represents granular cell ameloblastoma, yellow represents unicystic ameloblastoma (inclusive of all types), red represents odontogenic myxoma, turquoise represents odontoma (inclusive of all types), grey represents calcifying cystic odontogenic tumors, blue represents peripheral odontogenic fibroma, dark green represents adenomatoid odontogenic tumours and orange represents ameloblastic carcinoma. Males are more likely to develop odontogenic tumours than females, although this was not found to be statistically significant. Chi square value, p = 0.583 (p>0.05)

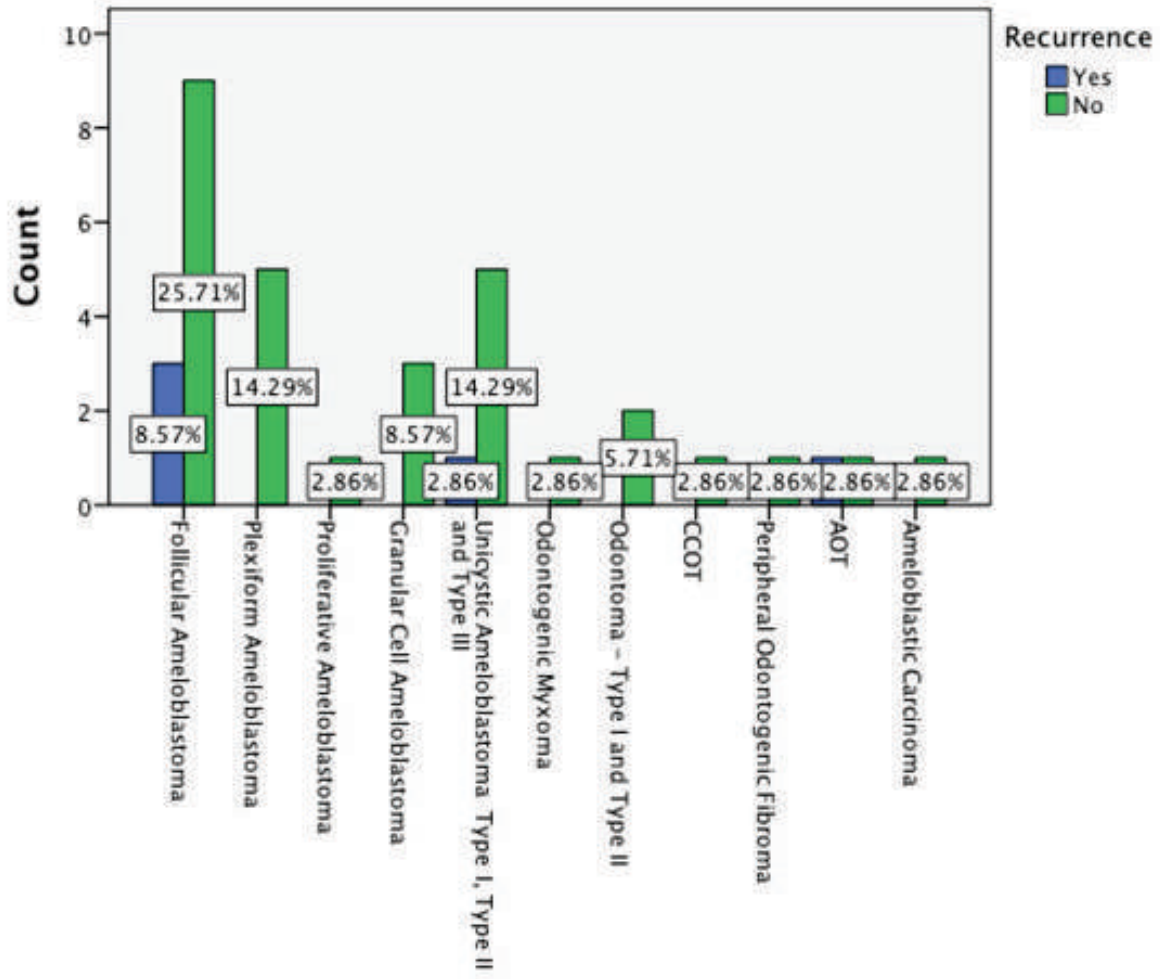


Figure 4 - Bar chart representing the association between the various types of OTs in percentage based on recurrence where there is an existing negative predilection for recurrence across a scale of type of OT in the 'x' axis and study population count in the 'y' axis. Here, the colour blue represents the presence of recurrence and green represents the absence of recurrence. Out of 11 types of odontogenic tumours, only 3 types (follicular ameloblastoma, unicystic ameloblastoma and AOT) have exhibited a history of recurrence.Follicular ameloblastoma was the most common OT to recur (8.57%), although this was not found to be statistically significant. Chi square value, $p = 0.837$ ($p>0.05$)

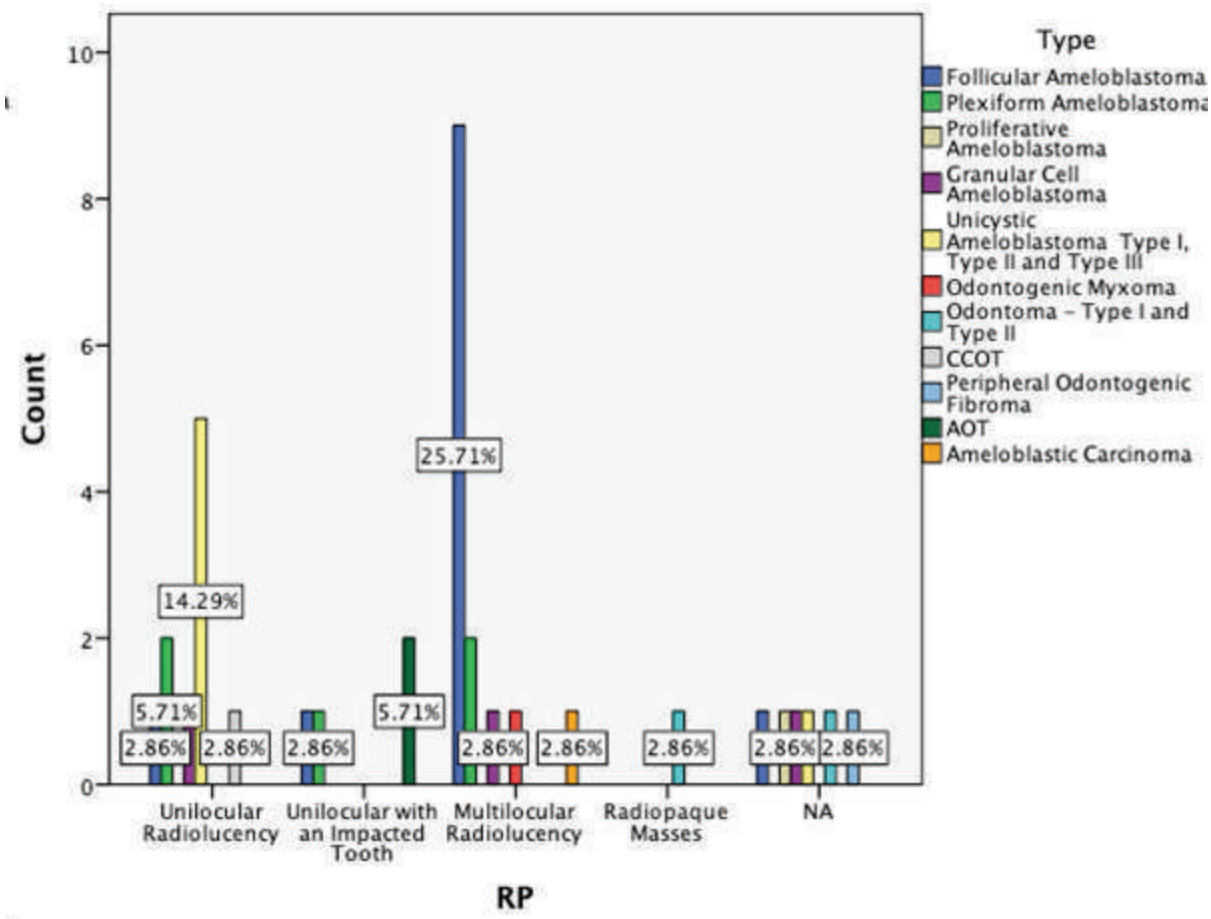


Figure 5 - Bar chart representing the association between various types of OTs and their radiographic presentation. Radiographic representations of OTs are in the 'x' axis and the study population count in the 'y' axis. Here, the colour dark blue represents follicular ameloblastoma, light green represents plexiform ameloblastoma, mustard represents proliferative ameloblastoma, purple represents granular cell ameloblastoma, yellow represents unicystic ameloblastoma (inclusive of all types), red represents odontogenic myxoma, turquoise represents odontoma (inclusive of all types), grey represents calcifying cystic odontogenic tumors, blue represents peripheral odontogenic fibroma, dark green represents adenomatoid odontogenic tumours and orange represents ameloblastic carcinoma. Follicular ameloblastoma most commonly presents as a multilocular radiolucency (25.71%) and this was found to be statistically significant. Chi square value, p = 0.04 (p< 0.05)

Conclusion

Within the limits of this study, there is an existing male predilection in the mandibular posterior region. Most cases are benign, occur after the age of 40 years and are asymptomatic with multilocular radiolucencies. On considering a time span of the last five years, we have 1550 established biopsy cases out of which 35 were odontogenic tumours. Thus, the prevalence rate of odontogenic tumours is approximately 2.26%. The most prevalent type of odontogenic tumour found was ameloblastoma followed by odontomas and adenomatoid odontogenic tumours. Overall, there was no noteworthy change in trend with regard to the pattern of prevalence of OTs. But due to our aforementioned shortcomings and controversy of literature, further research has to be done to confirm our existing findings. As they are predominantly asymptomatic, proper diagnosis and timely management is required.

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Conflict of Interest: None Declared

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Determination of Sex Using Mastoid Process among South Indian Population - A Review

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Abstract

Identification of an individual is of utmost importance in conditions of natural calamities or in case of man made disaster. Determination of sex becomes the primary priority in the process of identification of a person by a forensic investigator during the time of crime investigations, natural disasters and the ethnic studies. Determination of sex is one of the vital for the identification of an individual. Often the fragmentary remains are available for forensic identification of an individual making sex determination difficult. When the pelvic bone is unavailable, the skull is also widely considered the best indicator of sex. Skull is important in this regard as it resists adverse environmental conditions over time. The first indicator for the sex determination is the pelvic bones and pelvis which is more accurate than the skull bones. The skull is the most dimorphic and easily sexed portion of the skeleton after pelvis, providing up to 92% reliability. The mastoid process can thus be used as a best indicator of sex as well as ancestry of populations and individuals. Mastoid process is a dimorphic bone situated at the basolateral region of the skull. This mastoid process is placed in the posterior aspect of the temporal bone. Mastoid process is one of the two projections situated behind the ear. The mastoid process provides an attachment for certain muscles of the neck. This mastoid process is a fragmentary piece of skull, is ideal for sexual dimorphism as it is resistant to damage due to its anatomical position at the base of the skull. The mastoid process of the skull plays a vital role in determination of the sex as it is the most di-morphic bone present at the baso-lateral region of the skull. Detailed morphometric analysis of mastoid process, mastoid triangle will help in the planning of surgical intervention involving the skull base. This review will be useful for the anatomist, neurosurgeon, radiologist and morphologists clinical anatomists to carry out further research work. The aim of the present review is the determination of sex using mastoid process and mastoid triangle among south Indian population

Keywords: *Morphometry; sex determination; mastoid process; mastoid triangle; forensic investigation.*

Introduction

Historically, identification of individuals is one of the most challenging and difficult subjects that man has confronted. The concept of identity, with few significant variations, is the same as the assertion of that identity is a set of physical characteristics, functional or psychic, normal or pathological, that define an individual [1–3]. Nowadays, human identification is a universal process based on scientific principles, mainly involving fingerprinting, the objective of which is to identify and register individuals for both civil and criminal identification purposes [4–6] the application of the knowledge of physical anthropology for the purpose of forensic medicine constitutes forensic anthropology [7–9]. Anthropology is the study of measurements or proportions of human body such as bones, muscle and adipose tissue according to sex and age for identification purposes. Forensic anthropology is the application of the

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science of physical anthropology and human osteology in other words the study of human skeleton Achievement of sex determination depends upon the completeness of the skeleton.

Sex classification is more precise in pelvic remains than the skull but whole and complete pelvic bone is not always available for analysis[10–12]. Skull is probably the second best region of the skeleton to determine sex often fragmentary remains are available, instead of complete skeletons for forensic evaluation. Furthermore, the petrous part of temporal bone is resistant to destruction and damage such as burning [13–15]. The mastoid region is favorable for sex determination as it is one of the most protected region and resistant to damage[16,17]. The mastoid process is a pyramidal bony projection from the posterior section of the temporal bone. It is located just behind the external acoustic meatus and lateral to the styloid process in the skull. The mastoid process characteristics are of great utility in the determination of sex. The mastoid Triangle (MT) is a triangle constructed between the landmark pterion, mastoidale and asterion. Parameters like mastoid process length can be used as predictors to determine gender [18,19]

Materials and Methods

Articles were collected from PMC database, Medline database. Using keywords, mastoid triangle, mastoid process, sex determination using bones, morphometry of the skull. The total number of articles collected were 34. All the collected articles were systematically analysed for review of its content based on determination of sex using mastoid process among south Indian population .

Results and Discussion

Morphology and Morphometry of Skull

Skull is the most dimorphic and easily sexed portion of the skeleton after the pelvic bone, providing upto 92% reliability. Determination of sex from skeletons is a vital work for a forensic anthropological analysis [20–22] skull is the most ordinarily used skeleton part in forensic anthropological analysis. Studies indicate that pelvis is that the most reliable indicator of sex assessment, and skull is that the other sexual dimorphism is extremely important in studies of morphologically, skeletal biology and human evolution. Skull is important in the determination of sex as it resists adverse environmental conditions over time. Dimorphic characteristics of sex are often studied metrically and morphologically, although morphologic studies pose several problems like difficulties with quantification and interobserver error[23]. Investigation into the usability of geometric morphometric analysis in assessment of sexual dimorphism [24,25]. The posterior part of the inferior surface of the skull is formed by the occipital bone.

Prominent features of posterior part of inferior surface are the foramen magnum and associated occipital condyles, jugular foramen, mastoid notch and the squamous part of the occipital bone up to the external occipital protuberance and the superior nuchal lines, hypoglossal canals (anterior condylar canals) and condylar canals (posterior condylar canals) [26]. It may be a vital landmark for anthropology, anatomy, forensic pathology, surgeons and other medical fields for the skull base [24].

Determination of Sex Using Bones

The microscopic age changes that occur in human cortical bone were measured by counting the amount of osteons, osteon fragments and non-Haversian canals. The microscopic determination of age in human bone [27]. Sex determination is one of the first and basic steps of assessing the biological profile. Although the analysis of DNA is the most reliable method for sex determinationit is also the most expensive and time consuming method, which can also be hindered by local conditions [28,29].

During the reconstruction process, numerous issues may arise, including bone fragmentation and poor preservation of skeletal remains, coupled with the complexity of human skeleton [30]. Osteometric studies using individual bones exhibiting sexual dimorphism have been reported among different population

Mastoid Process

In the skull, the mastoid bone is robust and tough making it resistant to physical damage. The mastoid region is favourable for sex determination for two reasons it's compact structure of the petrous portion and it's protected position at the base of the skull. Hence, it is commonly found intact in skeletons of very old age. Even though the skull is fragmented, the mastoid stays intact. The mastoid process characteristics are of great

utility in determination of sex. Studies based on human skeletal remains for determination of sex have been the topic of interest among the researchers.

The mastoid region is considered as one of the slowest and later growing regions of the cranial cavity and this mastoid region shows a higher degree of sexual dimorphism in adulthood. The mastoid process is a conical prominence which projects from the undersurface of mastoid portion of temporal bone situated behind the and lateral to the tactic and external acoustic meatus and lateral to the styloid process. The mastoid process is larger in males than in females. Difference between the size of mastoid process in males and females could be due to the variation in growth of mastoid process in both males and females along with response of stronger muscle actions of the muscles attached to it. The important role of this bone is that, it is a point for attachment of several muscle the of the neck such as splenius capitis, longissimus capitis, posterior belly of digastric and sternocleidomastoid, because of these muscle attachments mastoid tend to be larger in males and smaller in females. Determination of sex through skeletal remains is an age old and time tested method.

In the understanding of humans the sex determination from skeletal remains has began ever since Hippocrates period, but thorough knowledge was acquired in post renaissance period. Such knowledge is applied in time of war, crimes, genocides, or mass disaster [11]. In the skull, the mastoid bone is robust and tough making it resistant to physical damage. The mastoid region is favorable for sex determination for two reasons – the compact structure of the petrous portion and its protected position at the base of the skull [31]. The mastoid process can be used as a marker of determination of sex in unidentified human skeletal remains. The articles reviewed for the determination of sex using mastoid process by morphometric parameters were subjected for quality analysis and the confidence level scoring was given as level 1, level 2, level 3 and graded accordingly as low, moderate and high (Table 1). The articles were subjected for scoring with a scale ranging from 1 to 10, represented in Table 2.

Mastoid Triangle

The mastoid triangle varies in different age groups. The perimeter, area, dimensions and the angles of the mastoid triangle were analysed for their utility in determination of sex [32]. The mastoid triangle is one of the poor indicator of sex . The sex difference has been observed to be statistically significant for all measurements except for the angles of the opisthion-Bimastoid triangle the highest value for determination of sex for Asterion- Mastoidale length of right side and next is the Bimastoid breadth. This is useful for determination of sex of unknown bones [33,34]. Heron's formula was used for calculating the area of the mastoid triangle in mm . It needs to be highlighted here that the mastoid triangle does not constitute a flat surface but a slightly curved one, and hence, the area calculated in the study cannot be considered as a true area but area for a two dimensional planar view.

Limitation of the Study

Less articles were reviewed and specific parameters were discussed and considered.

Future Scope

With this we can conclude that the results obtained from the present review may be of use to the forensic experts, neurosurgeons, anthropologists, morphologists clinical anatomists carrying out further research work

Conclusion

This review concluded that the morphometric values of the mastoid process are variable. The mastoid process is a good indicator of sex when all the parameters are considered length is the best parameter when individual parameters are considered. So the knowledge of these variations may guide neurosurgeons to approach in the management of neoplasms and other pathology related to this region. The area of mastoid triangle is considered as a poor indicator of sex. The mastoid triangle plays an important role as a landmark. It is of particular interest in the field of forensic medicine to identify fire victims and also used for intracranial surgical approaches. The result of this study offers a good opportunity to identify sex using the mastoid process apart from pelvimetric and craniometric analysis of sex determination.

Table 1: Quality Analysis showing the articles reviewed for the determination of sex using mastoid process by morphometric parameters were subjected for quality analysis and the confidence level scoring was given as level 1, level 2, level 3 and graded low, moderate and high.

S.No	Author Name	Year	Level	Quality
1	Luiz Airton Saavedra de Paiva	2003	Level 1	High
2	Johnson	2020	Level 1	High
3	Sekar	2019	Level 1	High
4	Alves ES	1965	Level 2	Moderate
5	Seppan	2018	Level 1	High
6	Krishna	2916	Level 1	High
7	Arbenz GO	1988	Level 1	High
8	Nandini	2018	Level 1	High
9	Subashri	2016	Level 1	High
10	Jigyasa Passey	2015	Level 2	Moderate
11	Thejeswar	2015	Level 1	High
12	Sriram	2015	Level 1	High
13	Bass WM	2004	Level 3	Low
14	Kalmey JK	1996	Level 1	High
15	Keerthana	2016	Level 1	High
16	Li Luo	2013	Level 1	High
17	Pratha	2016	Level 2	Moderate
18	Pretorius E	2006	Level 3	Low
19	Menon A	2016	Level 1	High
20	Ropper	1993	Level 1	High
21	Samuel	2015	Level 1	High
22	Nauma	2016	Level 1	High
23	Archana Singh	2019	Level 1	High
24	Željana Bašić	2013	Level 2	Moderate
25	Thenmozhi	2016	Level 1	High
26	Kerley	1965	Level 1	High
27	Gibbon V	2013	Level 1	High
28	Bidmos MA	2010	Level 3	Low
29	Bruzek J	2006	Level 2	Moderate
30	Rajni	2017	Level 1	High
31	TanujKanchan	2013	Level 1	High
32	Sinhorini	2019	Level 1	High
33	Deepali Jain	2013	Level 2	Moderate
34	Choudhari	2016	Level 1	High

Table 2: Showing scoring of the articles for quality analysis with a scale range of 1 to 10 based on the article content, parameters analysed, sample size, statistical analysis and discussion.

S.No	1	2	3	4	5	6	7	8	9	10	Yes	No
1	☑	☑	☑	✖	☑	☑	☑	☑	☑	✖	8	2
2	☑	☑	✖	☑	☑	☑	☑	☑	☑	☑	9	1
3	☑	✖	☑	✖	☑	☑	☑	☑	☑	☑	8	2
4	☑	✖	☑	☑	☑	✖	☑	✖	✖	☑	6	4
5	☑	☑	☑	☑	☑	☑	☑	✖	☑	☑	9	1
6	☑	✖	☑	☑	☑	☑	☑	☑	☑	☑	8	2
7	☑	☑	☑	✖	☑	☑	☑	☑	☑	✖	8	2
8	☑	☑	☑	☑	☑	☑	✖	☑	✖	☑	8	2
9	☑	☑	☑	☑	☑	✖	☑	☑	☑	☑	9	1
10	☑	✖	☑	✖	☑	✖	☑	☑	☑	✖	6	4
11	☑	✖	☑	☑	☑	☑	✖	☑	☑	☑	8	2
12	☑	☑	☑	✖	☑	☑	☑	☑	☑	☑	9	1
13	☑	☑	✖	✖	☑	✖	✖	☑	✖	✖	4	6
14	☑	☑	☑	✖	☑	☑	☑	☑	☑	✖	8	2
15	☑	☑	☑	☑	☑	☑	✖	☑	☑	☑	9	1
16	☑	☑	✖	✖	☑	☑	☑	☑	☑	☑	8	2
17	☑	☑	✖	☑	☑	☑	✖	✖	☑	☑	7	3
18	✖	✖	☑	✖	☑	✖	✖	☑	✖	☑	4	6
19	☑	☑	☑	☑	☑	☑	☑	☑	✖	☑	9	1
20	☑	☑	✖	☑	☑	✖	☑	☑	☑	☑	8	2
21	☑	✖	☑	☑	☑	☑	☑	☑	☑	☑	9	1
22	☑	☑	☑	✖	☑	☑	☑	☑	✖	☑	8	2
23	☑	☑	☑	✖	☑	☑	☑	☑	☑	✖	8	2
24	☑	☑	✖	☑	☑	✖	☑	☑	✖	☑	7	3
25	☑	☑	✖	☑	☑	☑	☑	☑	☑	☑	9	1
26	☑	✖	☑	☑	☑	☑	✖	☑	☑	☑	8	2
27	☑	✖	☑	✖	☑	☑	☑	☑	☑	☑	8	2
28	✖	✖	✖	✖	☑	✖	✖	✖	✖	☑	2	8
29	✖	✖	☑	☑	☑	☑	☑	✖	✖	✖	5	5
30	☑	✖	☑	☑	☑	☑	☑	☑	☑	✖	8	2
31	✖	☑	☑	☑	☑	☑	☑	☑	✖	☑	8	2
32	☑	☑	☑	☑	☑	☑	☑	✖	☑	☑	9	1
33	✖	☑	☑	☑	☑	☑	✖	✖	☑	✖	6	4
34	☑	☑	☑	✖	☑	☑	☑	☑	☑	✖	8	2

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Ethical Clearance: As it is a review article so it is not required.

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DNA Profiling in Forensic Dentistry

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Abstract

The recent advances in molecular biology have revolutionized all aspects of dentistry. Deoxyribose Nucleic Acid (DNA), the language of life yields information beyond our imagination with respect to our body health conditions. DNA is present in chromosomes and is responsible for storing all the genetic material and they are not the same for all individuals. Forensic dental comparison has been used for identification of humans in cases where body tissues are damaged or prolonged exposure to the environment has made the identification impractical. The use of DNA profile tests in forensic dentistry provides a new perspective in human identification. This article summarizes the recent literature on use of this technique in identification of unidentified human remains, highlighting the importance of DNA in the cases of forensic investigation.

Key Words: *DNA profiling; forensic dentistry; human identification; molecular biology.*

Introduction

The discipline of Forensic Dentistry was introduced to the curriculum of the Brazilian Dental Schools after issuing of Decree no. 19852, amended in 1931. Since then, this specialty has continued to evolve, showing remarkable scientific and professional maturity lately[1]. The role of dental restorations, and radiological identification among forensic odontology has declined, whereas the molecular biology and laboratory procedures are increasing at a fast rate in efficiency and availability[2]. The Deoxyribose Nucleic Acid (DNA) extracted from the teeth of an unknown individual is matched with DNA isolated from known ante mortem samples such as stored blood, tooth brush, hairbrush, cervical smear, biopsy, to a parent or sibling. This is the usual procedure in DNA analysis[3].

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Several biological materials are used for isolation of DNA and accomplish the laboratory tests for human identification, including bone tissue, hair bulb, biopsy sample, saliva, blood, etc.It is possible to obtain DNA from all human body tissues but only with variations in the quality and quantity of the DNA extracted from each tissue. Teeth play an important role as the subject of DNA studies as the dental hard tissue physically encloses the pulp and offers an anatomical configuration of great durability[4]. When morphologically evaluated, even a single tooth provides valuable information regarding the individual to whom the tooth belongs [5,6,7]. The importance of Forensic Dentistry in the field of human identification, mainly when there is little remaining material to perform these identifications has brought dentists to work with forensic investigation and make them more familiar with the new molecular biology technologies. Previously our team had conducted numerous original studies [8-14] and surveys [15-22] over the past 5 years. Now we are focusing on applying this knowledge to write the review on new advancements in various fields. This article summarizes the recent literature on use of this technique in identification of unidentified human remains, highlighting the importance of DNA in the cases of forensic investigation.

BASIS OF DNA FINGERPRINTING

Each individual has a unique DNA sequence. The DNA is the same for every cell in a person. Every person has a different DNA fingerprint which will identify only that individual. The knowledge of DNA science is the only way to identify a particular individual. DNA fingerprinting or DNA profile are the only codes that reflect a person's DNA makeup, which can also be used to identify individuals. The repetitive sequence is highly polymorphic and unique to each individual. It is generally seen as long tandem repeats (midi satellites), short tandem repeats (STR; mini satellites) and distributes repetitive sequences. A gene particularly codes a separate protein which is found in a segment of DNA. The function of the remaining 95% or more of the DNA is not known and is called non-coding DNA or junk DNA[23]. Variations in DNA sequence called polymorphisms can be used both to differentiate and to correlate individuals[24]. A variable number tandem repeat is a location in a genome where an organized tandem repeat is from a short nucleotide sequence. These are commonly found on many chromosomes, and often show variations in length (number of repeats) among persons. Each variant acts as an allele which is inherited, allowing them to be used for identification of a person or a parent. Their analysis is useful in genetics, forensics, and DNA fingerprinting.

DNA AND FORENSIC DENTISTRY

The main factors which are exogenous may limit the retrieval of information from body remnants and stops the processes of human identification are the elements present or associated with heat and its impacts[25]. In this sense, the teeth play an important role in identification and criminology, due to the high uniqueness of dental characteristics in addition to the relatively high degree of physical and chemical resistance of the dental structure[26]. Due to the resistant nature of dental tissues to environmental assaults, such as incineration, immersion, trauma, mutilation, decomposition and microbial action, teeth represent an excellent source of DNA material. In a tooth, dentin and pulp are rich sources of DNA which can be successfully extracted where pulp is vascular and dentin is avascular[27]. Total production of genomic DNA obtained from dental samples may range from 6 μg to 50 μg DNA[28]. A study stated that the polymerase chain reaction method enables the differentiation of an individual from another, with a high level of reliability and with about 1 ng of the target DNA. Thus, abundance of quality DNA can be extracted from a tooth which is an important advantage in DNA analysis[29]. DNA is preserved in the teeth and bones for a very long period and thus is a valuable source of information. Ancient DNA (aDNA) analysis can be carried out in samples that are hundreds to tens of thousands of years old[30]. Even though 99% of the bodies were identified using dental records or fingerprints and only 1% of forensic identification was made by DNA profiling[31].

SOURCES OF DNA IN TOOTH

The teeth differ in shape and size (dimensions) but have similar histological structure .The dentin is a connective tissue that forms the major structural axis of the tooth. The dentin on the crown of the tooth is covered by enamel . The tooth is attached to the bone through periodontal ligament . The enamel origin is ectoderm and is a highly mineralized tissue. Furthermore, it is an acellular and avascular structure without nerves. Soft tissue within the coronal and radicular pulp chamber consists of odontoblasts, fibroblasts, endothelial cells, peripheral nerve, undifferentiated mesenchymal cells and nucleated components of blood which are rich sources of DNA. Odontoblastic processes that extend into dentinal tubules, soft tissue within accessory canals, cellular cementum, adherent bone and periodontal ligament fibres are less frequently used in anatomical locations of DNA. Pulp tissue is most commonly used because it is usually abundant and has blood supply and has least chance of contamination by nonhuman DNA. Sampling of the pulp tissue is done in three ways: crushing, vertical or horizontal splitting, and by endodontic access.

DNA EXTRACTION

DNA extraction process is composed of 3 different stages: cell rupture or lysis (which allows use of several techniques for effective rupture of the cell membranes), protein denaturation and inactivation (by chelating agents and proteinases in order to inactive elements, such as proteins), and finally DNA extraction itself[32]. The techniques of DNA extraction most often employed in Forensic Dentistry are the live and natural method (composed of phenol-chloroform and used for high molecular weight DNA, laborious, time consuming, with

a higher likelihood of errors, given the use of multiple tubes and can only be done if abundance of sample is available); Chelex 100 (the fastest with the lowest risk of contamination, yet very expensive); FTA Paper (composed of absorbent cellulose paper with chemical substances, which speed up its use); and isopropyl alcohol (containing ammonium and isopropanol, which is less expensive and also an alternative to the organic method)[33].

TYPES OF DNA

Genomic and mitochondrial are two types of DNA which are used in forensic sciences. The genomic DNA is found in the nucleus of each and every cell in the human body and represents a DNA source for most forensic applications. The teeth are an excellent source of genomic DNA. Mitochondrial DNA (mtDNA) is another type of material that can be used when the extracted DNA samples are too small or degraded, such as those obtained from skeletonized tissues, the likelihood of obtaining a DNA profile from mtDNA is higher than that with any marker found in genomic DNA[34]. Various biological samples such as hair and teeth that lack nucleated cellular material can be analyzed with mtDNA.

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

RFLP is used for analyzing the variable lengths of DNA fragments that result from digesting a DNA sample with "restriction endonuclease" which is a special kind of restriction enzyme. The separate sections of DNA at a specific sequence pattern is known as a "restriction endonuclease recognition site". RFLP requires relatively large amounts of DNA. Hence, cannot be performed with the samples degraded by environmental factors and also takes longer time to get the results[35].

PCR

Polymerase Chain Reaction (PCR) is used to amplify the amount of DNA material available, so DNA analysis is carried out by sufficient quantity. To proceed the special enzymes and DNA primers are required. These primers resemble the probes with known constant sections of DNA but they are unlabeled. They are designed to know constant sections of DNA at the ends of variable regions to be amplified. The principle of PCR is that the DNA has a special feature that it can duplicate itself. This is usually done by unwinding the DNA strands and each strand helps in synthesis of new strands. By PCR technique, amplification of specific DNA segments dependent on the employed primer is done. The standard PCR reaction runs through 30 cycles in a couple of hours which results in amplification of original DNA by over 109 times[36].

The DNA found can be of two types, they are genomic DNA and mtDNA. The teeth are excellent in PCR analyses and allows comparing the collected postmortem samples to known antemortem samples or parental DNA. Main advantage of mtDNA is the high number of copies per cell (from hundreds to thousands of organelles). PCR was performed in a thermal cycler. More recently, application has also been found in molecular biology, where different methods are used in optimizing PCR.

STRs TYPING

In forensic samples taken from humans, the study of DNA is usually performed by Short Tandom Repeat (STR) analysis, which can be defined as hypervariable regions of DNA that present consecutive repetitions of fragments that have 2-7 bp[37]. The Federal Bureau of Investigation has chosen 13 specific STR loci to serve as the standard for the Combined DNA index system[38]. STR was used on 45 DNA samples from teeth obtained from unidentified bodies buried in 1995 and exhumed in 2000, and pulp showed the strongest PCR amplification signals[39]. STR testing is being used for forensic identifications, making a revolution on human identification and paternity tests.

mtDNA ANALYSIS

mtDNA is the strongest tool in forensic identification since it possesses high copy number, maternal inheritance, and high degree of sequence variability. Each offspring has the same mtDNA as their mothers since the mitochondrion of each new embryo comes from the mother's egg cell and the nuclear DNA is contributed by the father's sperm. It is used in finding missing persons, comparing the mtDNA profile of unidentified remains with the profile of a potential maternal relative can be an important technique[35]. However, mtDNA analysis is a

very costly technique and is less informative. Thus, this analysis is not used in all forensic laboratories directed at resolution of crimes.

Y - CHROMOSOME ANALYSIS

DNA-polymorphisms on the human Y chromosome are valuable tools for understanding human evolution, migration and for tracing relationships among males[40]. Most of the length of the Y chromosome is inherited as a single block in linkage from father to male offspring as a haploid body. Hence Y chromosomal DNA variation has been mainly used for investigations on human evolution and for forensic purposes or paternity analysis[41].

X - CHROMOSOME STR

Chromosome X specific STR is used in the identification and the genomic studies of various ethnic groups in the World[42]. Since the size of X-chromosome STR alleles is small, generally including 100-350 nucleotides, it is relatively easy to be amplified and detected with high sensitivity[43]. X-chromosome STR (X-STR) markers are a powerful complimentary system especially in deficiency paternity testing.

SNPs

Single Nucleotide Polymorphism (SNP) detection technologies are used in scanning the new polymorphisms and helps in determination of the allele(s) of a known polymorphism in target sequences[44]. SNP detection technologies have evolved from labor intensive, time consuming, and expensive processes to some of the most highly automated, efficient, and some inexpensive methods. Local, target, SNP discovery relies mostly on direct DNA sequencing or on denaturing high performance liquid chromatography[45].

AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (Amp FLP)

AmpFLP,amplified fragment length polymorphism was also put into practice during the early 1990s[46]. This technique is faster than RFLP analysis and used in PCR to amplify the DNA samples. AmpFLP analysis can be highly automated, and allows in creating easy phylogenetic trees based on comparing individual samples of DNA. Owing to its relatively low cost and ease of set-up and operation, AmpFLP remains popular in lower income countries[47].

Conclusion

The finding of DNA fingerprinting has revolutionised the concept of identification. It is sensible to anticipate that future advances in DNA technology will reduce the time and cost factor for identification of unknown deceased. It has also revolutionised the forensic identification procedures. Therefore, dental professionals who are working on the field of Forensic Dentistry should include these new technologies in their work, as several methods are available for DNA extraction from biological materials, yet similarity of the protocols adopted for such purpose has not been reached so far. The field is developing to find out many riddles in the human genome.

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Effects of Temperature on Mechanical Properties of Dental Restoration - A Review

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Abstract

The aim of the review was to determine the effect of temperature on mechanical properties of dental restoration in the oral environment . Dental restorative materials are exposed to temperature ranging from ten degree to five degree celsius, since the properties of dental material are sensitive to temperature of this magnitude it is important to define the effect of services temperature on the mechanical properties of dental composites. Increase in the mechanical properties of compressive strength, diametral tensile strength, compressive elastic modulus and hardness for all investigated composites with increase of temperature. The mechanical properties of nanohybrid composites were less sensitive to temperature change. Ultimate strength decreases linearly increasing temperature strength is higher for the lower volume fraction material and is decreased by the presence of a small percentage of very large particles. Elastic modulus and yield strength decrease sigmoidally with increasing temperature and depends only on particle volume fraction. In the clinically significant temperature range ultimately decreases to 14%,the decrease in elastic modulus is either 6 or 11% and yield strength decreases to 45%. Dental restoration like gold, silver amalgam are often unaffected even after prolonged exposure to fire. Dental tissues and restoration can undergo a series of changes which correlate with temperature to which they are exposed. The temperature condition of the oral environment can significantly affect the mechanical properties of dental restorative materials.

Keywords: *Dental restoration;; effective temperature; gold amalgam; mechanical property; resin composites.*

Introduction

A dental restoration or dental filling is a treatment to restore the function integrity,and morphology of missing tooth structure resulting from caries or external trauma as well as to the replacement of such structure supported by dental implants . The variation in the oral environment can affect the mechanical properties of dental restorative materials. Eating hot or cold foods and drinking beverages are the causes of the most extreme temperature variation in the oral cavity. In addition to the temperature variations in oral condition,water or other liquids such as saliva,beverages or components may influence their mechanical properties. [1 2 3].The mechanical instrumentation may not be sufficient to remove bacteria from root canals owing to complex anatomy. The mechanical properties such as modulus of elasticity, stress, strength, toughness, brittleness, fatigue. The mechanical properties of the dental composites temperature from 5 to 50 degree celsius. dental restorative materials undergo a series of changes which are corrected well with a varying temperature to which they were exposed.[4 5]. Heat induced alteration to dental and restorative materials can be of great interest to forensic dentistry. Knowing the specific optical behaviour of dental materials can be of high importance

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as recognition of changes induced by high temperature can lead to the determination of material which was used in dental restoration. Dental avulsion is a type of traumatic tooth injury because it causes damage to several structures and results in the complete displacement of the tooth from its socket in the alveolar bone.[6 7] The aim of the study was to determine the effect of temperature on mechanical properties of dental restoration.

Materials and Methods

The literature review was carried out from Scopus and pubmed databases from 2000 till date. Five step process involved in selection of articles. They are identification of relevant articles, section of articles, data extraction and charting and final analysis and report. Recent similar, relevant publications are taken into account for the collection of knowledge and to get clear about it. Articles collected through Medline-embrace, cochrane library. Number.of. Articles-39.

MECHANICAL PROPERTIES

Mechanical properties of importance to dentistry include brittleness compressive strength, ,ductility , elastic modulus, fatigue limit , flexural modulus, flexural strength, fracture toughness, hardness, impact strength, malleability, percent elongation,poisonous ratio , proportional limit , shear modulus, shear strength, tensile strength, torsional strength,yield strength and young's modulus. Dental caries is the most common cause for the loss of enamel in a clinical situation and it is easily detectable. [8 9]. Mechanical properties are defined by law of mechanics that is the physical science dealing with forces that act on bodies and the resultant motion, deformation or stress that those bodies experience when force acts on the body tending to produce deformation. Their resistance developed within the body to the external force.the internal resistance the body to the external force is called stress. Mechanical properties and parameters that are measures of the elastic strain or plastic strain behaviour of dental material include elastic modulus also called young's modulus. Pulp canal obliteration is characterized by deposition of hard tissue within root canal space and yellow discoloration due to change mechanical properties.[10 11]. Strain is expressed as change in length per unit length of the body. When a stress is applied ductility is the ability of material to withstand permanent deformation under a tensile load without rupture.young's modulus is the ratio of stress to strain proportional limit Is greatest stress that may be produce in a material such that stress is directly proportional to strain. [12 13]

MECHANICAL PROPERTIES OF GOLD

The available restorative material, direct filling gold is the gold filling material that is still used in restorative dentistry while most of the metals can be welded and alloyed at a temperature above the temperature [14 15] .The pure gold can be cold welded. Malleability exceeds all other metals it may be reduced by beating to sheets of 1/250,000 of an inch in thickness.ductility Is most ductile of all metals.the importance of minimum requirement for the mechanical properties of dental gold alloy arises from the large stress. Advantages are strength, maintaining high polish,perfect adaptation to cavity walls because of ductility insoluble in oral fluids. Disadvantages are inharmonious color can lead to sensitivity to hot and cold. It is not indicated on the subject of stress of mastication.[16 17 18]

MECHANICAL PROPERTIES OF AMALGAM

Amalgam is strongest in compression and weaker in tension and shear. The compressive strength of a satisfactory amalgam restoration should be at least 310 MPa. Creep is a slow change in shape caused by compression due to dynamic intraoral stress. The rate at which amalgam develops strength is an important characteristic. Amalgam is much weaker in tension. Tensile strengths of amalgam are only a fraction of their compressive strength cavity design that should be constructed to reduce tensile stress resulting from biting forces. High early tensile strength is important to resist fracture by biting forces.[19 20]. The effects of porosity facilitate stress concentration. Propagation of crack corrosion and fatigue failure of amalgam restoration. Modulus of elasticity in high copper alloys tends to be stiffer than low copper alloys, when rate of loading increases, values of approximately 62 GPa have been obtained. 380 MPa for low copper amalgam, 414 MPa for high copper amalgam. Dentin has a relatively low elastic modulus and as much tooth structure as possible should be preserved to prevent the dentin from bending which allows amalgam to bend and for tensile stresses to develop. Amalgam cannot withstand high tensile strength. There is danger that will be flexed with tension.

The effect of the mercury content upon the compressive strength of amalgam. Dental amalgam is an alloy made by mixing mercury with a silver tin alloy. Silver tin alloy to which varying amounts of copper and zinc has been added.[21 22 23] Advantages are easy to manipulate,wear resistant,well condensed and amalgam has good comprehensive strength. Disadvantages are unnatural appearance ,tarnish and corrosion, lack of mechanical adhesion to the tooth structure [24].

MECHANICAL PROPERTIES OF RESIN COMPOSITE

Resin composites are the material of first choice for anterior and posterior dental restoration due to their excellent optical properties associated with adequate mechanical properties. Strongly depends on some factors, such as filter content, integrity of the organic to filters, adhesion. Porosity percentage of the cured material and on the oral environment. Strength of composite depends on the ability of the coupling agent to transfer stresses. The crack propagates to a bonded filter, the crack must pass around the particle since it is stronger than the matrix and the interfacial bond. [25 26]. The final stiffness or rigidity of a resin composite may play a compensating role in coping with polymerization stress. Thick resin layer stacking of this layer provides sufficient elasticity to relieve stress of resin composite. There is no correlation between fracture toughness and filter content by volume of flowable composite but hardness and fracture toughness tend to be linearly proportional to filter of fracture toughness values.Advantages are mechanical properties than traditional and microfilled composites, wear resistance, high compressive strength, high modulus of elasticity. Disadvantages are heavy metal glass filters are softer and prone to hydrolysis so long term durability of these resins lowered.[27 28]

MECHANICAL PROPERTIES OF GLASS IONOMER CEMENT

Strength has both tensile and compressive strength is greater than conventional glass ionomer cement. Compressive strength is fairly high up to 200 MPa. Flexural strength is fairly low 5 to 40 MPa and shear strength is fairly low, 3 to 5 MPa. Coefficient of thermal expansion similar to tooth structure. Dimensional changes shrink on setting, expand with water absorption. Abrasion resistance is greater than conventional glass ionomer cement due to silver particles incorporation. Modulus of elasticity tends to be relatively lower than conventional glass ionomer cement.[29 30]. Advantages are inherent adhesion to the tooth surface, coefficient of thermal expansion similar to tooth structure and low thermal conductivity. Disadvantages are opacity in glass ionomers cement is higher than resin and less polishability than resin. It also has poor wear resistance and poor tensile strength.[31]

EFFECTS OF TEMPERATURE

Dental restoration the amalgama at 200 degree Celsius and 400 degree Celsius. Lost its shine due to mercury operation and showed an opaque black colour from 600 degree celsius[32 33].At higher temperature silver metallic shine on the roots probably due to mercury decomposition, the pink pigments root and crown between 1000 degree celsius and 1100 degree Celsius. [34] The composite Restoration turned to a bright brown colour with white veins at 400 degree Celsius and increased its shine. [35 36]. Resin composite is used for class 2 fracture. At 600 degree Celsius resin change to grayish black colour. At 200 degree Celsius the amalgam and composite filling should be a marginal contraction probably due to the evaporation of the mercury loss of the organic matrix.[37 38 39]

Conclusion

In the oral environment, dental restorative materials are usually exposed to temperatures ranging from 10” to 50°C. High temperatures reduce the stiffness. Low temperature increases the stiffness. Higher annealing temperatures cause mechanical properties of amalgam to deteriorate. With the help of the review, we are able to conclude that temperature can significantly affect the mechanical properties of dental restoration.

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Therapeutic Potential of Stem Cells From Human Exfoliated Deciduous Teeth(Shed)- A Review

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Abstract

Stem cells are groups of undifferentiated cells that have the ability to become any cell. Recent studies have shown that the stem cells present in the pulp of human teeth can also be used for the same purpose. Stem cell therapy is a safe procedure. The physician must follow proper cell administration techniques. Patients must also be screened for treatment candidacy as all people may not be a candidate for stem cells. Dental pulp stem cells are stem cells present in the dental pulp, which is the soft living tissue within teeth. They are pluripotent, as they can form embryoid body-like structures in vitro and teratoma-like structures that contained tissues derived from all three embryonic germ layers when injected in nude mice. Recent advancements prove that stem cells from exfoliated deciduous teeth prove more effective than the permanent ones. In a clinical trial stem cells extracted from children's baby teeth were used to regrow the living tissue in teeth damaged by injury. In the future they may be used to replace cells and tissues that have been damaged or lost due to disease. The review has covered various aspects to find if SHED is a potent product. Its viability and sustention as discussed by various authors has been discussed. Role of SHED in pulp tissue engineering, neural tissue engineering, their differentiation towards hormone secreting cells etc has been discussed. Hence the stem cells from human exfoliated deciduous teeth also present another opportunity to dentistry to contribute to the development of tissue engineering.

Keywords: *stem cells, deciduous teeth, exfoliated, SHED, Therapeutic potential*

Introduction

Stem cells are specialised human cells that have the ability to develop into any cells. They can repair damaged tissues.Stem cells are of various origins,they are derived from bone marrow, blood, umbilical cord. Dental stem cells derived from wisdom teeth and milk teeth have the ability to regenerate a whole tooth when they are obtained from pulp. Dental pulp is a highly vascularized connective tissue encapsulated in mineralized structure formed by enamel, dentin, and cementum. The dental pulp is a source of different populations of stem cells, such as dental pulp stem cells (DPSC) in permanent teeth and stem cells from human exfoliated deciduous teeth (SHED) [1]. A naturally occurring exfoliated deciduous tooth is similar in some ways to an umbilical cord, containing stem cells that may offer a unique stem-cell resource for potential clinical applications [2]. Possibility of cell-based therapies to treat disease, which is often referred to as regenerative or reparative medicine is becoming a promising field of research on stem cells[3]. SHEDs have high proliferation potency and are multipotent mesenchymal stem cells. Taking these advantageous properties together, SHEDs are one of the candidate cell types for tissue regeneration study [4]. Tooth loss is a common condition that has been encountered in clinics, it affects mastication and also a series of physiological and psychological problems. The

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tooth root is complex , it consists of hard and soft tissues including the cementum, dentine and periodontium [5]. The potency and quality of adult stem cells in the body is inversely proportional to the age. The perspective of obtaining SHED from deciduous teeth is quite practical because everyone sheds deciduous teeth[6] . Previously our team had conducted numerous original studies [7-13] and surveys [14-21] over the past 5 years. Now we are focusing on applying the knowledge to write the review on new recent advancements in various fields. This review focuses on the therapeutic potential of SHED and its various applications in different tissues.

BASIC CHARACTERISTICS OF STEM CELLS

In recent advancements, stem cell therapy has become a very promising and advanced scientific research topic. The development of treatment methods has evoked great expectations. Stem cells have great potential to become one of the most important aspects of medicine. In addition to the fact that they play a large role in developing restorative medicine, their study reveals much information about the complex events that happen during human development[22]. SHED are heterogeneous populations of cells that have been isolated from exfoliated deciduous teeth [4].

STEM CELLS IN DENTISTRY

Stem cells have the ability to repair and regenerate dental tissues like dentine, teeth, bone, cartilages, skin, adipose tissues, and glands. The research of stem cells in scientific and therapeutic potential in scientific and therapeutic potential in oro-facial diseases is yet not reached its pinnacle but future is flamboyant in regenerative dentistry. Scientists have reported for the first time that baby /milk teeth, the temporary teeth that children begin losing around their sixth birthday, contain a rich supply of stem cells in their dental pulp. The scientists say this unexpected discovery could have important implications because the stem cells remain alive inside the tooth for a short time after it falls out of a child's mouth, suggesting the cells could be readily harvested for research [3].

4. VIABILITY OF STEM CELLS:

The blood supply to the pulpal tissues enters through the apical area of the tooth. Hence, when a tooth is extracted, the pulp should appear red in color, indicating that the pulp received blood flow up until the time of removal, which is indicative of cell viability.During an extraction, if the pulp is gray in color, it is likely that blood flow to the pulp has been compromised, and thus, the stem cells are likely necrotic and are no longer viable for recovery. Tooth that are very loose, either through trauma or disease (e.g. Class III or IV mobility), often have a severed blood supply, and are not candidates for stem cell recovery. That is why the recovery of stem cells from deciduous teeth is preferred after an extraction versus the tooth that is "hanging on by a thread" with mobility. Pulpal stem cells should not be harvested from teeth with apical abscesses, tumors or cysts [23] .

SUSTENTION OF STEM CELLS

After an extraction of either a temporary, permanent tooth or another appropriate surgical procedure, the dentist places the tooth/ tissue sample in a screw-top vial containing a hypotonic phosphate buffered saline solution, which provides nutrients and helps to prevent the tissue from drying out during transport. Keeping a tooth into this vial at room temperature induces hypothermia. The vial has to be carefully sealed and placed into the thermette, a temperature phase change carrier, after which the carrier is then placed into an insulated metal transport vessel. The thermette along with the insulated transport vessel maintains the sample in a hypothermic state during transportation. The viability of the stem cells depends on both time and temperature, and careful attention is required to ensure that the sample will remain viable. Time from harvesting to arrival at the processing storage facility should not exceed 40 hour [23,24] .

CHARACTERISTICS OF SHED

SHEDs are a different population of cells that are isolated from dental pulp tissues which remain in exfoliated deciduous teeth. Similar to the mesenchymal stem cells (MSCs), SHEDs exhibit fibroblast□like appearance, attaches on plastic tissue culture surface, express mesenchymal stem cell surface marker and have multipotential differentiation ability (4). SHEDs have higher multiplication rate when compared to dental pulp stem cells (DPSCs) and bone marrow derived mesenchymal stem cells (BMMSCs)[2,25] . This

could be due to the high expression of genes related to cell proliferation and extracellular matrix in SHEDs comparing with DPSCs [25]. MSCs can be isolated from many tissue types. Though, there is no specific marker to clearly identify these cells. According to the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy, the minimum criteria to identify MSCs are as follows [26]. First, the isolated MSCs should attach to plastic tissue culture plates [9]. Second, expression of MSCs to several specific surface markers, namely CD105, CD73 and CD90[9] . They should not express CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR[9]. Finally, MSC should be able to differentiate into osteoblasts, adipocytes and chondroblasts in vitro[26]. The following section describes general SHEDs' characteristics and addresses MSCs' characteristics of SHEDs according to these criteria.

ROLE OF SHED IN DENTAL PULP TISSUE ENGINEERING

Despite the introduction of new materials, medicines, and tools for the clinical management of dental pulp diseases, the principles of root canal treatment have not evolved significantly from the disinfection and obturation paradigm. This well-established approach presents high rates of success in the daily clinics, but it is very rarely based on the substitution of organic tissues with synthetic and, in many cases, inactive materials [27]. This often restricts the completion of root development in immature teeth [28] . Hence, the development of clinically approachable techniques that allow the regener- ation of a functional dental pulp that is capable of depositing organized and mineralized matrix is of great interest [27,29]. The shift towards regenerative endodontics leads to the rescue of tooth viability and further development of the root structure.The capacity of SHED to specialise into fully functional odontoblasts are capable of depositing a mineralized structure comparable to dentin invivo was observed later. Similar to the previous study, SHED were seeded within a scaffold cast in a tooth slice and implanted subcutaneously into the dorsum of mice. 32 days after implantation, a dental pulp- like tissue was centripetally formed in the pulp chamber of the tooth slice. The tissue formed with SHED had a positive expression for the markers of odontoblastic differentiation such as dentin sialophosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1). During the experiment, mice had been injected with the periodical injections of tetracycline hydrochloride to reveal the deposition of mineralized matrix. Remarkably, well-defined fluorescent lines originated from the chelation of calcium ions in the newly deposited dentin offered the evidence that SHED can differentiate into fully functional odontoblasts invivo [30].

SHED FOR NEURAL TISSUE ENGINEERING

Neurodegenerative disorders are characterized by the loss of neurons, leading to functional disabilities. Although some of them, such as Parkinson and Alzheimer's disease, mainly affect the older people, they are not part of the natural aging process. Parkinson disease affects 9.7 to 13.8 per 100,000 population, while Alzheimer's disease has become a major public health concern as the world's population ages [30,31]. In addition to these diseases, there are approximately five million people living with traumatic brain disability in the United States alone [32]. Other injuries such as stroke, peripheral nerve injury, and spinal cord injury also is a huge burden to society. Due to the limited regenerative capacity of the nervous system, stem cell-based therapies have emerged as treatment options. The neural crest-cell origin of the dental pulp makes SHED an interesting cell model for neuron tissue regeneration research [33]. The neural developmental potential of SHED invivo was demonstrated by Miura and colleagues by injecting SHED into the dentate gyrus of the hippocampus of mice. The cells survived in the environment which was provided and it continued to express neural markers such as NFM for more than ten days [2]. The potential of SHED to undergo neurogenic differentiation invivo opened avenues for the use of these cells as an alternative model to treat different neuron-related conditions like focal cerebral ischemia, spinal cord injuries, Alzheimer's disease, and others. The enhanced regeneration observed with SHED not only applies to lesions in the central nervous system, but also was observed in peripheral nerve injuries. It was possible to promote the regeneration of sciatic nerve defects in rats by treating the lesions with SHED conditioned media. The axon density and number of regenerated myelinated fibers observed in the group treated with SHED conditioned media were similar to the autograft used as a control [25] .These promising results pertaining to the neuron differentiation potential of SHED in several in vitro settings and animal models

increase the interest of using these cells as an alternative to treat different neuronal diseases and injuries [1] .

SHED DIFFERENTIATION TOWARDS HORMONE SECRETORY CELLS

In addition to their therapeutic use in dental pulp and neuroregeneration, SHED also has the potential to be used in the treatment of liver diseases and diabetes. Organ transplantation could be the choice of treatment for patients suffering from fatal liver conditions such as cirrhosis and hepatocellular carcinoma. However, the lack of donors encourages the development of therapeutic alternatives [1] . Under proper stimulation, SHED expresses a set of hepatic markers such as hepatic nuclear factor-4 alpha, alpha-fetoprotein, and insulin-like growth factor-1. Remarkably, 90% of the hepatocytes that were obtained were positive for the expression of albumin. In addition, there were significant increases in the concentration of urea in the median amount of cytoplasmic glycogen storage within the cells after differentiation [34] .

SHED IN ADIPOGENIC DIFFERENTIATION

A total of 1×10 5 DFCs and SHEDs were seeded into each well of a 6-well plate. When the cells reached 70% convergence, the culture medium was replaced with a complete α-MEM which was supplemented with 10% FBS, 2 mm insulin (Sigma, USA), 0.5 mm isobutylmethyl- xanthine (IBMX; Sigma, USA), and 10 nM dexamethasone (Sigma, USA)(35). α-MEM supplemented with 10% FBS served as the negative control. The medium was refreshed every 2 days. After 15 days of culture, the cells were washed three times with PBS after fixation with 4% paraformaldehyde for 15 min and then incubated with a 0.3% Oil Red O (Sigma, USA) solution for 15 min. After washing thrice with PBS, cells were periodically observed and were photographed under a phase-contrast inverted microscope (Olympus, Japan). The expression of adipogenic genes PPARγ2, LPL and adiponectin was analyzed using real-time PCR[6].

SHED IN OSTEOGENIC DIFFERENTIATION

A total of 1×10 5 DFCs and SHEDs were seeded into each well of separate 6-well plates. Upon reaching a level of 70% convergence, cells were cultured in osteogenic medium containing 10% FBS, 10 mM L-glycerophosphate (Sigma, USA), 100 μM dexamethasone (Sigma, USA), and 50 μg/ml ascorbic acid (Sigma, USA) for 21 days. α-MEM which was supplemented with 10% FBS served as the negative control. The medium was refreshed every 2 days. After 3 weeks, cells were washed twice with PBS after fixation with 4% paraformaldehyde for 15 min and then incubated with a 0.1% alizarin red solution (Sigma, USA) in Tris-HCl (pH 8.3) at 37°C for 30 min. After washing twice with PBS, cells were observed and photographed under a phase-contrast inverted microscope (Olympus, Japan). The expression of the osteogenic genes BSP, ALP and OCN was analyzed using real-time PCR. Relative expression levels were calculated using the $2-\Delta\Delta CT$ method and normalized to the reference GAPDH gene [6].

FUTURE SCOPE

Teeth derived from autologous cells, such as dental pulp derived stem cells and SHED, which can be deposited in cell banks, can be produced remains to be established. Immune responses to the cells used are unknown but these have to be understood before any clinical trials can be performed. With more extensive research it may be possible, in future, to regenerate the structures of the teeth .

Conclusion

As the stem cells from human exfoliated deciduous teeth can be obtained from naturally "disposable" tissues without significant morbidity to host and with limited ethical concern, they present another opportunity for dentistry to contribute to the development of tissue engineering. Several studies offer evidence that SHED can differentiate into odontoblasts, neurons, hepatocytes, endothelial cells, alpha cells, and others. The wide variety of cell types creates a plethora of opportunities for the use of SHED in tissue regeneration processes. There is still the need to deepen the understanding of the mechanisms underlying the differenti- ation processes before SHED-based therapies can become a clinical reality .

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Curcumin A Better Medicine to Treat Coronavirus- A Review

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Abstract

Curcumin, a yellow pigment, which is a bioactive compound in Turmeric, is a polyphenol derivative used as an herbal medicine to treat remedies. Coronavirus disease-2019 (COVID-19) is caused due to coronavirus which is an infectious and dangerous virus. Coronavirus causes severe infections in humans and also in different species of animals. Curcumin has multi properties in which one of them is anti-viral, which may help in the prevention and treatment of COVID-19. Curcumin can be taken by the means of food and studies show that there are no side effects, caused due to its consumption. Turmeric is the main source of Curcumin; RDA of Curcumin is 3mg/kg/day. Some of its properties include: anti-viral, anti-inflammatory, anti-bacterial, anti-parasitic, anti-tumor, anti-microbial activities. It also has the ability to gene transcription the viral strains like coronavirus, and maybe a possible treatment in treating COVID-19. Curcumin as exhibits anti-viral properties and acts on viral strains for gene transcription, can be a possible treatment in prevention and treating COVID-19.

Keywords: *Curcumin; Turmeric; COVID-19; antiviral activity; symptoms; treatment.*

Introduction

Curcumin is a yellow pigment which is a bio active compound present in Turmeric. Curcumin is mainly used as a therapeutic agent, it is so because of its multi-properties which include: antiviral activity, antifungal and anti-bacterial activities, antioxidant activity, and acts also as an anti-inflammatory agent and also has anticancer effects. Main aim of this study is to review whether curcumin can act as a better medicine than the drug treatment [1] in treating COVID 19. Coronavirus are viruses which are infectious and can be transmitted from person to person and this diseased state is called COVID-19. There are etiologic agents [infectious substances, materials reasonably expected to contain a pathogen] responsible for causing severe infections, they are enveloped RNA viruses [non-segmented, positive sense, short noncoding molecules that consist of 21–25 nucleotides in length [2]] which are mainly found in humans and mammals [3]. Curcumin is a polyphenol derivative which is mainly used as an herbal remedy and dietary spices, present in Turmeric [4]. Curcuminoids refers to a family of active compounds which includes curcumin. Curcuminoids play a significant role in fundamental disease control at a cellular level, possessing anti-bacterial, anti-microbial, anti-viral, anti-tumor and anti-immunomodulatory properties [5]. It is mainly known for its anti-inflammatory and anti-cancer properties [6 7]. Turmeric is the main source of Curcumin which is used for many remedies [8]. Curcumin also has the ability to affect gene transcription [9], as it down regulates specific gene transcription in tumor cells according to this study [10]. It also possesses skin protection capacity and wound dressing properties [11]. It also performs COVID-19 viral gene transcription. According to this study [12] Curcumin has the ability to inhibit herpes simplex virus.

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Some of the previous studies were compared, and Curcumin is a spice, which is effectively used as a drug to treat various viral and bactericidal infections [13] [14]. In India it is being used as a primary agent to treat arthritis and muscular disorders, and in China it is used as a topic analgesic and flatulence, to treat chest pain, hepatitis and colic conditions [15]. Relief in symptoms like excretion of sputum, cough is normalized and no or less dyspneic conditions [16] observed due to Curcumin administration [17] [18] [19] [20] [21] [22] [23] [24] [25].

The curcumin can be modified in addition with silver nanoparticles which are used in treating respiratory syncytial virus infections [26]. Curcumin shows a bunch of treatmentive aspects which include, anti-inflammatory activity [27] [effectivity in rats- pathological changes observed in albino rats [28]] , antioxidant activity [29], antiprotozoal activity [15], Nematocidal activity [30] [31], antibacterial activity [32], anti-tumor activity [33] [pseudotumors treatment [34]], and anti-venom activity [35]. Curcumin due to its anti-inflammatory and antioxidant capacity [36] which exhibits negative influence towards obesity [which may be linked to thyroid functioning] [37] is a treatmentive option. Curcumin can also be used as therapeutic treatment for eye disease [38] like amblyopia [39], dry eye disease, conjunctivitis, corneal wound healing [38]. Curcumin also acts as a potential inhibitor of COVID-19 main protease (M^{pro}), reported by the study [40] but still no proper and specific treatment and therapies has been found for the new strain of CoV [41]. The key concept of this review is to find whether the use of Curcumin will do any better in treating a viral infection such as COVID 19? Main focus on the antiviral activities of the Curcumin.

The aim of the study is to find whether Curcumin can treat COVID 19 better than any medical drug treatments available in the market, possible therapies to treat COVID 19 and to find whether Curcumin can act as a potential drug against coronavirus helping in treatment of COVID-19.

CURCUMIN AND COVID 19

COVID-19

Coronaviruses (CoV), they are the main causative and etiological agents which causes severe infections both in humans and animals mainly mammals, this virus has been associated with the respiratory tracts in numerous cases and it also causes disorders in the digestive tract systematically [40]. Coronaviruses infect various species of animals, including avian species and reptiles and also mammals. Curcumin can be used as a possible treatment option for the COVID-10 pandemic, it can also be used as a preventive factor for coronaviruses according to this study [42]. Coronaviruses were first discovered in the 1930's in domestic chickens who were infected with acute respiratory infection caused by IBV (infectious bronchitis virus) [43].

Epidemiology

COVID-19 epidemic has spread very quickly, as it approximately took 30 days for its spread [44]. Findings suggest that the transmission has taken place either through airdrop lets or possibly from an infected person to another from an unknown source [45]. Evidence found has reported the main cause due to huge clusters of infected family members and medical workers, so it was confirmed that COVID 19 can be transmitted from human to human interaction [46] [47] [48] .

Symptoms

Coronavirus type NL63 will cause lower respiratory tract syndrome symptoms which is acquired in early life [49]. Symptoms of respiratory tract illness were primarily found in very young, elderly and immunocompromised individuals [50]. Human Coronavirus is found in small children as a minor proportion of infections [51]. Coronavirus had some symptoms of acute respiratory virus infection like fever, Myalgia and sore throat, which were found common in SARS-COV too [52].

Controlling measures

Further control and prevention of the spreading of the disease globally, but no specific therapies and treatments have been found to cure COVID-19 [41]. Investigation to find possible treatment for COVID-19 are still lacking [53]. Nutrition supplement, symptomatic treatment and anti-viral treatments are needed as active intervention for the severe as well as mild patients affected with COVID-19 [54]. The transmission roots intermediate host with intense surveillance has to be mentioned [54].

Therapeutic treatment

Therapeutic Treatment of Curcumin induces treatment of arthritis and muscular disorder and is used as topical analgesic and flatulence, used to treat hepatitis and also cancer [15]. Curcumin in turmeric can possibly act as a treatment option for curing 2019 new Coronavirus it is also used as food preservative [55], and possesses the following therapeutic properties.

1) Anti-inflammatory [56]

2) Anti-bacterial [57]

3) Antiviral [58]

4) Anti-fungal [59]

5) Anti-tumor [60]

6) Antispasmodic [61]

7) Hepatoprotective [62]

8) Nephroprotective treatment of dementia [63]

9) Effectiveness on AIDS (Auto immune deficiency syndrome) [64]

Curcuma Longa

Turmeric is a spice which has its root called curcuma longa. They exhibit multi-therapeutic activities like anti-inflammatory, anti-human immunodeficiency virus, anti-bacteria, antioxidant effects and nematicidal activities [65]. Curcuma longa belongs to the family Zingiberaceae which is native to India, commonly known as the term turmeric. In vitro it exhibits activities like anti-parasitic, anti-spasmodic and gastrointestinal effects it also inhibits carcinogenesis and cancer growth (anti-tumor activity).

Some medicinal properties exhibited from different roots are effective and they can be administered topically, orally and also by inhalation [66].

Beneficial effects of Curcumin

Curcumin exhibits a bunch of therapeutics by important activities are, they exert anti-inflammatory activity, anti-oxidant effect, antiviral and antifungal actions [66]. Previous studies have shown that curcumin is non-toxic to humans [67]. Curcumin is the bioactive compound, which is powerful and has antioxidant action like vitamin C, vitamin E and beta-carotene of Turmeric which plays a major part in cancer prevention, liver protection and premature aging, and also treats respiratory problems [68].

Antiviral properties of Curcumin

Curcumin exhibits good microbial activity, it helps in development of vaginal microbial gel which will act as a defense system against viral infections [69]. Curcumin when compared with systemically acting drugs, the drugs were more potent in binding with the COVID-19 M^{pro} [70]. Curcumin- copper acts as a broad spectrum antiviral agent against vesicular stomatitis virus, coxsackie virus, respiratory syncytial viruses [71]. Curcumin acts as an agent to inhibit cancer growth, and sensitizes human colorectal cancer. Curcumin clearly has anti allergic properties and its related compounds possess the same activity [72]. Curcumin can hopefully act as a treatment of coronavirus infection [73], it can also be possibly used as a preventive treatment for COVID-19 [74].

Curcumin is a bioactive agent exclusively present in turmeric, it's a polyphenol derivative, used as an herbal remedy and also as a dietary spice almost globally [4]. Turmeric being its main source, curcumin is having special therapeutic effects like anti-inflammatory and anti-cancer properties thus used to treat numerous conditions [66], it is also used extravagantly as a skin protector and also for wound healing and dressing purposes [11]. It has the capacity for viral gene transcription in this case on COVID-19 disease viral strains [43] and miRNA has been tested as a novel molecular pathway/ target in therapies of various heart diseases [75 76 77].

It's an important dietary source for Indian meals, present in turmeric and its bioavailability increases when it's combined with piperine (black pepper extract) which tends to boost immunity, and also taken with lipids. Curcumin daily intake or RDA is about 3 mg/ kg/day, when it's combined with essential oils it can be taken as 500mg twice a day which is 1000mg/day as the recommended amount [5].

It can hopefully be used as an antiviral drug for the treatment of coronavirus infection, it's also a possible preventive and treatmentive agent to COVID-19. So, curcumin against inflammatory response in our body could act as inhibitors which has phagocytic capacity

which may prevent from viral and bacterial infections, its effective against viral disease and infections [78 79 74].

It's important to consume curcumin in a routine because it's a multi-faced compound which has its therapeutics properties like: anti-oxidant, anti-parasitic, antitumor and anti-viral and more. It's an adjuvant in anti-HCV therapy, acts like an antioxidant, it exhibits chemopreventive and chemotherapeutic activities [56], it has antiviral activity against human immunodeficiency virus (HIV) in AIDS, it also acts as an inhibitor in the exhibition of breast cancer cell line and acts as an anti-cancer chemical in high doses.

Its main biological and pharmacological actions resemble its anti-viral properties. Curcumin effectively acts against dengue virus, influenza virus, zika virus and also chikungunya virus [5].

Previous studies include: to reduce the inflammatory response of the temporal lobe in Alzheimer's disease [80]. Studies include its action against human papillomavirus infection and cervical cancer treatments [81]. And was found when curcumin is combined with sodium valproate has shown response to treat bipolar disorder [82]. And its invasive is resistant to melanomas as it inhibits proliferation and invasion of the significant pathway [83 84].

Conclusion

From this meticulous review it was observed that curcumin is a best and better medicine to treat against various diseases. Though it has various properties against various diseases, it has very good antiviral properties. Thus the present study suggested that curcumin may be the promising medicine to mitigate COVID-19 infection.

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Ethical Clearance As it is a review article so it is not required.

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Different Histological Stains Used for Assessing the Morphology of Cementum - A Review

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Abstract

Cementum is a unique least mineralised tissue with only little information known on cementogenesis. Hence various stains have been identified to view the morphology of cementum. Cementum is critical for attachment of the tooth to the surrounding periodontal ligament (PDL). This study aims to review various stains used for assessing the cementum morphology.The study setting is a review where the information about the topic is obtained from various search engines like pubmed and google scholar by searching the keywords and the study was made. The cementum is a distinct and unique mineralized hard tissue which shares many similar properties with the dentin and bone. There are various methods of histotechniques for studying morphology and development of cementum. Upon overview of various histological approaches for studying and staining of cementum through light microscopy are H&E, Toluidine Blue, Alcian blue, Picrosirius red, etc. Bone sialoprotein, osteopontin, DMP1, etc., are the staining methods using immunohistochemistry. Cementum annulations are used in forensic analysis to determine the age of the body. Hence, studying about these stains might help to understand the morphology of cementum deeply and thus give accurate details.

Keywords: *Stains; tissue fixing; cementum; microscope.*

Introduction

Cementum is a thin, mineralised tissue covering the root dentin surface and is one of the least mineralised tissues to be understood with little known information on cementogenesis[1],[2].Histological stains used for aiding the microscopic study of tissues. They consist of dyes that are capable of binding with these tissues and stain them based on their composition,affinity, interaction between stain and the dye used. Various stains when combined with other stains have been found to be more effective. The most commonly used stain is H and E.Modified Gallego's stain was originally derived from Lille's stain that is used in differential staining of hard tissue of teeth[3,4].The best stain that is used to identify stained decalcification and ground tissue of the tooth is the cresyl violet. Other stains like Toluidine blue, PAS under conventional light and fluorescent microscopy can be used [5]. Additional imaging methods like scanning and transmission electron microscope and micro CT etc can also be used for histological staining.[6]

The stains are used to provide contrast to colourless cells and tissues for rapid ease and evaluation of the morphology. It also enhances fibrillar collagen to detect cementum defects. The advantages of these stains are that they can be used to view the ground section of the tooth, considered as an alternative to immunohistochemistry by pathologists as it gives proper insight to character and mineralised deposits. But it has certain limitations that though H and E and TB stains offer results, it is not optimal for cementum visualisation. No single stain

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describes the nature of hard tissue. [6,7]

Gallego in 1954 did a study to identify the mineralised components using gallego stain in decalcified and ground tissue. Robert J. Levey in 1956 did a study on differential staining of cementum using modified gallego's iron fuchsin stain and found that it stained red whereas dentin and bone stained green. Sandhya et al in 2015 conducted differential staining of hard tissue of tooth, bone and pathological lesions using the stains to identify the nature of calcifications and found that enamel stains were pink and immature hard tissue deposition stained shades of red and green.[7]

Previously, our team had conducted numerous organisational studies [8-14] and surveys [15-22] for the past 5 years. Now we are focusing on applying this knowledge to write a review on new advancements in various fields. The research was done as it provided faster and better diagnosis to trace pathological lesions. Hence the study aims to review all the stains used to assess cementum

Materials and Methods

The study setting of this research is a review The information about the topic is obtained by searching for keywords and the data was collected through search engines like pubmed and google scholar. The articles collected were related to data that included histological stains, IHC stains, promising new stains and microscopes for visualising cementum. It excluded articles related to other categories of Hard tissues.

ANIMAL AND HUMAN SAMPLES

The most commonly used models are rodents especially transgenic mice to study the critical regulators of cementum formation but it is technically challenging because the tissues are small and stages of tooth development occur rapidly compared to humans [23]. Hanford miniature pigs were used to harvest tissues of mandibular first molars at 13 - 16yrs of age at University of Washington and human samples from de identified teeth and discarded dental tissues were obtained for conducting research activities. All these procedures were approved by their respective institutional committees [6,24]

TISSUE FIXATION, DECALCIFICATION, PROCESSING AND EMBEDDING

It was found that pig and mouse tissue was fixed in Bouin's solution for about 18-24hrs and by storing it in 70% ethyl alcohol at 4 degree Celsius and human tissues were fixed in 10% neutral buffered formalin. Mouse samples which are of 8yrs age or more were decalcified in acid flagellates solution (AFS) for 2 to 3 weeks at 4 degree Celsius. Molar Pig tissue was decalcified

in 8% EDTA and 25% formic acid . The human molar teeth were decalcified in 20% of EDTA at 4degree Celsius for about 5weeks and these decalcified tissues were processed by automatic tissue processors embedded in paraffin in base molds at 5micron in thickness for mouse and human and 7microns for pig. Bouin's solution is used as it provides high quality morphology along with in situ hybridisation detection of mRNA but might also cause demineralisation of bone and teeth thus making it a poor choice. Hence EDTA and AFS considered a better choice than it [6]

HISTOLOGICAL STAINING

Hematoxylin and Eosin Stain

It is the most common differential stain used. It consists of 2 components haematoxylin and eosin which are differentiated in acid alcohol. Hematoxylin has properties similar to that of basic dye staining nuclei of the cell intense blue and other tissue components in varying shades of blue whereas Eosin Y is an acidic dye that counterstains cytoplasm and extracellular fibres pink and red blood cells intensely red. It showed that cementum was found as basophilic spheroidal lobule staining while dentin was found with eosinophilic staining with pale pink colour due to the presence of extracellular matrix. CEJ and incremental lines are prominent under this stain. AEFC layer of cervical root shown as a basophilic layer formed on the root dentin surface. It is widely used in pathological biopsies for providing contrast to colourless cells and tissues to study their morphology apart from histological staining. It also gives a good contrast between dentin and AEFC and acellular cementum and mantle dentin. [27,25]

Toluidine Blue

Basic dye that is used without any counterstain. Contains deparaffinized sections that are stained and dehydrated in alcohol,after which they are cleared

in xylene. It showed deep blue cementum and pale blue dentin and allowed actual visualisation of CEJ. AEFC stained dark blue.It also stains acidic mucopolysaccharides.The small integrin binding ligands N- linked glycoproteins family members BSP and OPN fall under mucopolysaccharides [It enabled better differentiation under light microscope and produced maximum contrast between cementum and cementoid, bone and osteoid thereby exhibiting metachromasia property. Apart from this, it has been used to visualise mouse CIFC in transgenic mouse models and CDJ in human teeth. It is found markedly improved when compared to H and E.Some studies have talked about the staining of acellular cementum due to the presence of rich ECM content [25,26,27]

Alcian Blue With Nuclear Red Fast Staining

It is counterstain that contains deparaffinized sections in 1% alcian and 3% acetic acid sol. cleared in xylene and stain acidic mucopolysaccharides. It showed pink areas of cementum and blue patchy areas of dentin. The cervical and apical regions of dentin were pale pink and the contrast was found to be more appreciable. It provided excellent contrast for the acellular cementum layer present in mice by showing intense blue colour and PDL in bright pink colour and also provided fine details about dentinal tubules. It is superior to H and E and TB stains but rarely used [25,28,29]

Picrosirius Red (PR) Staining

Strong acidic dye that was used to stain collagen by reacting with basic amino groups. It contains deparaffinized sections in 0.2% of aq sol. of phosphomolybdic acid hydrate rinsed in deionised water and placed in 0.4% direct red and 1.3% of 2,4,6-trinitrophenol and 0.4%of HCL and left for dehydration. When combined with polarised light microscopy, it enhances birefringence of collagen fibre varying from maximum intensity to lesser intensity depending on the organisation and angle of collagen and PDL fibres compared to angle of polariser and makes visible the sharpey's fibres inserting through the acellular cementum along with tissue intrinsic fibres.It was also observed that different types of collagen display different interference colors under this staining in polarised light. Collagen1 forms bright yellow or red colour and its fibres are thick and tightly packed whereas collagen3 displays green colour forming thin fibres and loosely packed. But Yellow fibres are found predominant than green fibers wh lesser amounts of types4,5,6. [30,31,32,33]

IMMUNOHISTOCHEMISTRY STAINS

Bone Sialoprotein

It shows very strong localisation in thin acellular extrinsic fibre cementum layer and strong staining in bone with a little staining in soft tissues. It is also present in PBL layer and diffuse localisation to pick CIFC layer. It is important in cementogenesis [2,34,35]

Osteopontin

It is present widespread in PDL, highlighted in AEFC layers as strong as BSP. It is also present in CIFC thereby providing a good contrast from underlying dentin and bone in definite patterns indicating its presence in cemental lines. It provides cell Matrix interactions during bone remodelling and is used in investigating developmental biology of the cementum and thereby used often as great markers.[2,6,36,37]

Dentin matrix acidic phosphoprotein1

It is found in higher concentrations around osteocytes and then Dentinal tubules. It was found in cellular cementum's Matrix mainly around the embedded Cementocytes parallel to osteocytes[38].It is said to localise around a cellular cementum and study on transgenic mice revealed the potential role of DMP1 at cementum Dentin interface[6]. It is often weak when compared to BSP and OPN. Hence not recommended as a primary marker for that tissue[39]

MICROSCOPE

Light Microscope

It is used mainly to estimate the age to the nearest accuracy for all age groups . It has lesser r value and its reliability is 74 percent but better than stereo microscope. When the relationship between chronological and estimated age was found using light microscope, it showed stronger r value [40,41]

Phase Contrast Microscope

Cementum annulations are more clearly visible in this microscope and hence found reliable for counting

the incremental lines and also used for age estimation. It shows stronger r value, [41–43,40]

Polarised Microscope

When correlation between chronological age and number of cementum annulations were found in both males and females using polarised microscopes, it showed a higher r value than all other microscopes.Better among other microscopes used, for assessing cementum annulations.But this statement was contradicted in a study conducted by Kaur et al where they found that the cementum annulations were clearly visible under the phase contrast microscope It showed better discernibility and annulation, hence preferred over light microscope.It shows less standard error of estimate (SEE) [41,40-44]

Stereo Microscope

When a stereo microscope was used to find the relationship between the estimated and chronological age, it showed a weaker r value correlation and the p values produced by it are not found significant among all age groups when compared to all other microscopes,hence not considered that much. A significant coefficient of correlation of about 0.000 was found with all microscopes except stereomicroscope [40]

PROMISING STAINS

Gallego's Iron Fuchsin Stain

Human teeth use this stain.Apart from this, calcified and noncalcified tissues can also be used.

It showed that cementum was stained in contrast to dentin and PDL. Bone reversal lines was found to be positively stained [40,45]

Sudan Black B

Species used here are rats and human fetal tissues. For this stain, pyridine treatment is necessary before processing of tissue. Not used to study cementum explicitly but clear clear pattern of staining can be found for bone reversal lines and mineralisation front seen in dentin was also found in cementum as well[46]

Procion Dyes

The species used here are mice, rats, rabbits, cats, monkeys and even dogs. 12 structurally related dyes were evaluated to see their ability to serve as vital dyes for mineralisation structures. Sme were found to be capable to mineralise even in decalcified tissue by light microscopy[47]

Metal Staining, Rio Hortega, Polak Mitochondria And Achucarrro - Hortega Tannic - Silver Methods

The species used here is hypercementosis human teeth . It makes an emphasis on cementum shape, sharpey's fibre insertion and cementocyte imaging [48]

Fluorescent Labeling With Vital Dyes (Tetracycline, Calcein, Alizarin)

The species used here are monkeys. This is used to track cementum regeneration through vital dye dynamic histomorphometry[49]

Cresyl Violet And Periodic Acid Schiff Staining Method

The species used here is human teeth. It is used to mark cementum incremental lines[50]

The limitation of the study was that more number of articles are needed to analyse the application of alcian blue and PR staining as these are being rarely used by researchers even though they have a lot of advantages. Cementum annulations are usually used to estimate the age of the dead body. Hence, the future aim is directed towards approaches of newer stains and establishing a better understanding the morphology of cementum and its use in forensic department

Conclusion

Cementum is a unique and distinct mineralised tissue. Hence, different approaches for studying cementum is very essential to develop therapeutic strategies in regeneration of cementum.

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Rejuvenation of Old People with Stem Technology

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Abstract

Rejuvenation is a process of reverse practice of the process of aging. This can be done by stem cells stimulated by the induced growth factors in an active cell division. Senescent cells can be removed by the immune system, this is destroyed by gene therapy. There are some preformulated drugs available which are the precursor thought of this article.In the last few decades, there has been a substantial increase in the population of people over 60 years of age. Most of them maintain a good general health and physical activity and fitness. For these individuals there is a good number of dermatologic procedures, medications, and cosmetics that can be prescribed to improve the aspect of skin aging, providing an improvement in their self-esteem and quality of life as a result of their better look. The aim of the research is to study the rejuvenation of old people with stem cell technology

Keywords: *Gene therapy, stem cells, senescence, grafting, growth factors.*

Introduction

Rejuvenation is the reverse practice of the aging process by the repair of damaged cells and replacement of the damaged cells by a new tissue. The cell loss can be reversed by suitable exercise of muscles or by replacement of the muscle using tissue growth mechanism. This can be done by stem cells stimulated by the induced growth factors in an active cell division. Senescent cells can be removed by the immune system, this is destroyed by gene therapy. The gene therapy kills or removes the suicidal genes. And this process kills only the senescent cells. Some rejuvenation drugs which are responsible in the market just reverses the protein crosslinking. This is developed by some enzymatic methods[1]. Some vaccination which helps in rejuvenation of old cells is by removing the extracellular garbage. This provides the arrival of immune cells. The intracellular substance which brings the process of scenesense can be removed by the infusion of many new enzymes that degrade the junk that even our own natural enzymes cannot upgrade[2]. Cancer cells use gene therapy which might require deletion or usage of other genes to add or cleave on others. Telomerase is used to determine the activity of other enzymes.

The normal cell considers the cancer cells as immortal, and this can be compensated by telomerases. Old human cells can be rejuvenated by stem cell technology. They become more youthful by having a coaxing property that expresses proteins[3]. The pluripotent cells are the implications for ageing research. They have high popularity among the scientists and researchers too. Stem cell regenerative capacity persists throughout life and hence they are used to produce organs and other things like in the field of medicine. They are used in the synthesis of insulin. The productive regenerative responses, abandonment of the tissue maintenance, repairs in the old. Progeneric changes and other biochemical signals show the sign of young cells behaving like old in early days of the synthesis[4]. This can be due to the accumulation of the metabolic stress or environmental damage or prolonged entropy which

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could accumulate [4,5].

Induced pluripotent stem cells combine by the rational means,there is no 100% possibility that the induced pluripotent stem cells can get attached to the part in the process of grafting.They obtain the histo compatible tissues .They are highly patient specific.They help in the treatment of diseases like osteoarthritis,muscle wasting [4–6].This keep us away from aging and aging related diseases.Older people might control their emotions and focus more to make the most of life,Hence increased stress leads to disappearance of hair. The aim of the research is to study the rejuvenation of old people with stem cell technology.

Hematopoietic Stem Cells

The hematopoietic stem cells increases with the age rather than others ,The proportion of CD341, Lin2, CD341, CD382CD901, CD45RA2 protein increases in eldery bone marrow in case of humans and mice ,There is a general preference over the myeloid over lymphoid differentiation[7,8]. First possibility is on the basis of clonal alteration method ,obtained by the homogenous population of hematopoietic stem cells[9]. They are generally the myeloid cells[1,10]. Accumulation of mitochondrial mutations in the hematopoietic stem cells observed in people have an affected functionality of hematopoietic stem cells[11].

Tissue -Specific Stem Cells

Mesenchymal stem cells are the cells which form the tissue component. They include the bone marrow and other tissues capable of mobilizing, proliferating terminally like osteoblasts and adipocytes[12]. The mesenchymal stem cells are capable of differentiating into neurons, glial cells and hepatocytes[13]. The mesenchymal stem cells can accumulate DNA changes in an in vitro culture. This causes exchange in differentiation capacity[14]. Exciting possibility is reprogramming the somatic cells back to pluripotent stem cells.

Stem Cell Characteristics Changed By Microenvironment

The stem cells are the special type of the cells which can repair and regenerate themselves. They can be obtained from the bone marrow, skeletal muscles, pulp etc[15],[16]. they are subjected to a characteristic change by the microenvironment, this causes the mutated forms of the stem cells. The stem cells are mutated by selecting a reasonable gene, and that gene selected for the mutation are the satellite cells.

Satellite Cells

Satellite cells are the part of the tissue which acts as the path of mutation of the cells. Sarcopenia is the part from compromised satellite cell function. Studies of C1C12 cells say that the Dna methylation occurs due to C1C12 cells and their cause muscle regeneration[17].The satellite cells form the bridge between the normal cells and the other mutated cells or the mutation, they play a major role in the rejuvenation of the cells.

Cancer Cells

They are highly proliferating cells which causes an increased amount of mutation[18]. This is due to provokement of the DNA amplification, deletion or addition. The main cause may be due to the deficiency of the tumor suppressor gene and the DNA repair mechanism[10,19]. The Intracellular enzymes are highly degradable. The enzymes cannot be degraded by the normal enzymes present in the body. Cancer cells use gene therapy to delete the genes for the telomerase and to eliminate telomerase. These are independent mechanisms[20]. The normal cell changes into immortal cancer cells. The compensation for telomerases are given by the stem cells.

A World Where None Ages

A world where none ages is a good one than mass disasters. These people who tend to live wanted a better and quality life in their future. Even if its out of ethics, the external life and everlasting youths gives strength to the ideas that cures for human aging and this is eventually developed

Longevity Regulators And Anti Ageing Drugs

Longevity regulators include the commercially available anti aging drugs in the market. These can extend the life span by 50%. The humans of longevity pathways have short lived model systems. Life extension process curing a chronic or malignant form of a disease[21]. They develop interventions and they cure helpless disease like cancer[22]. Cancer is treated by chemotherapy first and

they are removed by manual or laser method. They are named according to the site of cancer[23].

Road Map To Life Extension

Theories involved in gene therapy, stem cells and synthetic biology leading the pact by disruptive medical technologies[23]. They have the greatest possibility or the life extension. SENS, the strategies for engineered negligible senescence is the largely based medicine for reversal of aging

Re-Engineering Humans For Longer Life

By the process of gene therapy and stem cell technology, we can make even a new person. Hence called as re-engineered humans. Advances in genome editing by the zinc finger TAL(transcription activator like)[24]. Nucleases alter the human genetic code, causing regeneration. The research practices includes the forced expression in mice by a single transcription factor that regenerates and fully involved thymus is the best example for the pluripotency, a single factor that reverses the effect of aging. Some types of stem cells are responsible for that[25]. The intrinsic and extrinsic effect of the mutation of the cells and muscles have a possible regeneration and causes a high blood level of chemokines, which negatively regulates neurogenesis.

Discussion

Haematopoietic stem cells are the main reason for partial regeneration[26]. The pooling of blood can show the person young, because of this the gene therapy is done in bone marrow. The stem cells are the undifferentiated cells which can be transformed into any kind of cell,[27]. These kinds of cells can be obtained from adipocytes, pulp chambers, etc[28]. they can be taken to modify the cell according to the patient and can be placed again to the patient. The pluripotent cells are the implications for ageing research. Stem cell regenerative capacity persists throughout life and hence they are used to produce organs and other things like in the field of medicine. They are used in the synthesis of insulin[23]. The productive regenerative responses, abandonment of the tissue maintenance, repairs the old cells. Progeneric changes and other biochemical signals show the sign of young cells behaving like old in early days of the synthesis Stem cells can be modified according to the environment. They are also called as the compensatory cells. Wherever there is a loss of cell. The stem cells transform to form that kind of cell. They are the cells which are responsible for the mutation[29],[30]. These cells specify the loci of the differentiable or mutable cells[31]. Hence they play a major role in the regenerations of the cell. Reengineering of the cells by the process of stem cell technology and tissue engineering leads to regeneration of the cell. This can be caused by a single or multiple factor.

Conclusion

This review is necessary as far as now, because the lifespan of the homosapiens are getting less. There is an increased mortality rate than the birth and people of very less age are prone to death now-a-days .The only way to control the mortality is by the process of rejuvenation. Aging is the single greatest risk factor for developing Alzheimer's disease, Parkinson's disease, and other brain diseases. It slows down your body's ability to repair itself. But if we could rejuvenate tissues in aging bodies we could prevent brain diseases by slowing down aging.

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Recent Advances in HIV Diagnosis (Orasure and Oraquick)

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Abstract

Acquired Immunodeficiency syndrome(AIDS) is an infection caused by the Human Immunodeficiency Virus(HIV). The infection straight forwardly influences the cells of the insusceptible framework, especially the T lymphocytes, bringing about various sharp contaminations prompting the terminal condition AIDS. Since no antibodies are accessible for this shocking illness, HIV disease is a danger and can without much of a stretch be named as a revile upon mankind. The Centers for Disease Control and Prevention (CDC) and numerous general wellbeing boards of trustees advocate for the fast HIV test that is accessible. Tests that can distinguish HIV contamination biomarkers inside 30days of disease, when beginning safe reactions are mounted. The OraQuick ADVANCE Rapid HIV - 1/2 Antibody test is sensitive– utilizing subjective immunoassay to distinguish the antibodies of HIV-1 and HIV-2 out of an oral liquid. Orasure Technologies is enabling the worldwide network to improve wellbeing and health by giving access to precise fundamental data. OraQuick and Orasure determination are rapid, flexible, accurate, simple and patient preferred to perceive antibodies to all HIV-1 subtypes, especially in making nations.

Keywords *: HIV, AIDS, Orasure, OraQuick, Rapid diagnostic kits*

Introduction

Acquired immunodeficiency syndrome(AIDS) is an infection caused by the human immunodeficiency infection(HIV). HIV contamination is an amazingly present danger and can without a very remarkable stretch be named as a berate upon mankind. Set of scientists initially observed and saw the closeness of AIDS as a real disease following an extension in the event of remarkable enterprising contamination and malignancies among regardless among in any case among otherwise healthy homosexual men[1]. The Centers for Disease Control and Prevention (CDC) and numerous general wellbeing council keep on pushing for the most delicate quick HIV test that is accessible, both to recognize positive patients when they are at their generally irresistible, and to guarantee that negative patients are really negative when beginning a pre-exposure prophylaxis routine[2]. Tests that can identify HIV disease biomarkers within 30 days of disease, when initial immune responses are mounted[3]. The infected patient's IgM reaction is regularly used to identify intense disease inside a 20–25 days of window period after contamination [4].

Quick HIV immunizer tests have altered HIV analysis by encouraging the testing of a large number of individuals around the world[5]. The accessibility of reasonable, purpose of administration HIV testing is particularly significant in low-pay, high HIV trouble nations which do not have the money related and innovative assets to perform progressively refined research center based assays[6]. OraSure Technologies is enabling the worldwide network to improve wellbeing and health by giving access to precise fundamental data. OraSure is a pioneer in the turn of events, assembling and dispersion of purpose of care demonstrative tests, sub-atomic assortment devices and different innovations intended to recognize or analyze basic ailments. Its first-

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to-showcase, imaginative items incorporate fast tests for the recognition of antibodies to HIV[7].

HIV Transmission

HIV might be transmitted through certain body liquids that are fit for containing high concentrations of HIV. These liquids incorporate blood, semen, vaginal and rectal emissions, and breast milk. Notwithstanding the various courses by which HIV-1 can be transmitted beginning with one individual then onto the following, there is usually a sorted out and reproducible appearance in the blood of viral and host markers of sickness following a clinically productive transmission event[8,9]. Transmit HIV through various specific activities[10]. Most regularly, individuals get or transmit HIV through sexual practices and needle or syringe use[11]. Just certain body liquids, blood serum, rectal liquids and breast milk from an individual who HIV can transmit [12]. These liquids must interact with mucous layer or harmed tissue or straightforwardly infused into the circulatory system.

Epidemiology of HIV

The AIDS case announcing arrangement of the Centers of Disease Control and Prevention (CDC) is the main complete national population based information accessible to screen the epidemic[13]. Information is valuable in assessing ailment predominance and frequency, revealed AIDS cases are just clinical tips of something larger impacts created by HIV contaminations[14]. HIV seroprevalence reviews are data for their description of the size of the epidemic, however they speak to individuals whose date of contamination is unknown[15]. HIV rate information is for more data for observing the current course of the epidemic[16]. Since HIV contamination isn't reportable in all states and on the grounds that most investigations of HIV have excluded agent tests[17]. HIV observation gives data that is of restricted incentive in forecasting the fate of the epidemic[18]. Better developed social epidemiologic information known risk practices could give information about possible transmission and future spread[19,20].

Immune markers of HIV

When HIV has entered the body, the insusceptible framework starts hostile to HIV counter acting agent and binds to dendritic cells which convey the infection to CD4+ T cells in lymphoid tissue establishing the disease[21,22]. Virus replication quickens delivering massive viremia and wide dispersal of virus all through the body's lymphoid tissues[23]. An immune reaction against the infection causes some protection yet a constant relentless contamination is set up. The creation of cytokines and cell divisions that direct the resistant reaction for protection likewise cause HIV Virus replication quickens conveying massive viremia and wide dispersal of infection all through the body's lymphoid tissues[24]. There is a rapid turnover of CD4+ T cells that ultimately leads to their destruction and to a change in lymphoid tissues that prevent immune reactions. Anyway it can take one to a half year for an individual exposed to HIV to produce measurable quantities of antibody[25].

The immune reaction is weakened as memory t cells (CD4+CCR5+) are Destroyed. During development, HIV proteases divide the poly-proteins[26,27]. Into individual functional HIV proteins[28]. The mature various are able to cause another host cell. Maturation happens either in the forming buds or subsequent to maturing from the host cell. Tests to distinguish HIV with the nearness of antibodies to HIV, not simply the infection. Although a few tests are sensitive, there is a "window period". This is the period between the beginning of disease with HIV and the presence of distinguishable antibodies to the infection. On account of the most sensitive anti- HIV tests as of now suggested, the window period is around three weeks. This period might be longer if less sensitive tests are utilized[29].

Recent advances in technology have led to various simple rapid tests being developed. Most of these tests come in a kit and require no reagent, equipment, training, or specified temperature controls, and tests can be performed at any time[30]. OraQuick and Orasure diagnosis are rapid, flexible, accurate, simple and patient preferred to recognize antibodies to all HIV-1 subtypes, particularly in creating countries.

ORAQUICK AND ORASURE

The OraQuick® ADVANCE Rapid HIV-1/2 Antibody Test is a solitary use, subjective immunoassay to recognize antibodies of HIV-1 and HIV-2 in oral fluid, fingerstick whole blood, venipuncture whole blood and plasma specimens [31,32]. The OraQuick® ADVANCE

Rapid HIV-1/2 Antibody Test is normal for use as a condition-of-care test to help in the finish of tainting with HIV-1 and HIV-2. This test is proper for use in multi-test estimations proposed for quantifiable endorsement of quick HIV test results[33]. Clinical assessment of the OraQuick ADVANCE Rapid HIV-1/2 immunizer test has uncovered high affectability and explicitness. The test has numerous significant applications, expanding the open doors for willful directing and testing, and as a device for the scale-up of antiretroviral treatment in asset constrained settings[34]. Rapid, Affordable, quick, minimal effort, delicate, and explicit tests are expected to recognize antibodies to all HIV-1 subtypes, particularly in creating nations[35]. In addition in provincial regions assets are restricted, research center technicians much of the time inaccessible, phlebotomy is troublesome and socially less favored HIV test results can end up being extremely testing, Although oral liquid – based quick testing has a few alluring highlights and give helpful and feasible choice to blood – based testing[36].

History of Orasure and Oraquick

OraQuick is a HIV test made by Orasure Technologies and affirmed by the United States Food and Drug Administration in 2012. Orasure Technologies was chosen as one of the region's top places to work[37]. Perceived as a 2016 Top workplace, Orasure was only one of 15 organizations who were chosen among employers[38]. In 2011, Orasure gained DNA Genotek, a main supplier of oral liquid example collection, adjustment and planning products for molecular analytic applications. Furthermore, in 2012, Orasure got FDA endorsement for the Oraquick in-home HIV test, the first and only oral liquid fast OTC HIV test affirmed in the US and in 2019, Orasure gains CoreBiome, a microbiome administrations supplier, a urine sample collection device developer [39].

Orasure and OraQuick recent diagnosis,which is very much grown these days. The definite determination of HIV contamination at any age requires finding testing that confirms the presence of HIV[40]. HIV disease definitely in children aged less than 18 months, measures are required to recognize the infection or its parts i.e Virological test[41,42]. Maternal HIV immune response is moved to the infant latently during pregnancy and afterward decays with a half – life 28-30 days in non breastfed infants[43,44].

OraQuick ADVANCE Rapid HIV-1/2 Antibody Test

The OraQuick ADVANCE Test is FDA endorsed to recognize antibodies to HIV-1 and HIV-2 in entire blood, plasma, or oral fluid samples. The assay strip in the device consists of a membrane of nitrocellulose stripped with control and test lines[45]. The test line is striped with both HIV-1 and HIV-2 synthetic peptides and adjusted streptavidin (a biotin-binding protein). HIV- specific antibodies are presented with the sample, and, if present, will interact with biotinylated HIV-1 and HIV-2 peptides and colloidal gold marked with Protein A, which are impregnated in various segments of the device[46]. This sample reagent mixture then flows up the nitrocellulose, with complexed gold particles binding to the test line segments in different "sandwich" setups. The presence of HIV-1 as well as HIV-2 antibodies in the tested specimen is shown by the presence of a reddish purple coloured band at the test line area of the device within 20–40 minutes[47].

Conclusion

The review briefly outlines that OraQuick ADVANCE and Orasure HIV -1/2 Antibody Test detects antibodies to HIV-1 and HIV-2 in 20mintues and this diagnosis is rapid, flexible, accurate, simple and patient preferred. This rapid test showed good sensitivity in detecting antibodies against HIV from oral samples making diagnosis easy. These tests detect IgM antibodies of acute infection within windows period based on capacity, and detect seroconversion approximately 20-25 days after infection..

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Conflict of Interest : NIL

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Identification of Latent Lip Prints in Forensics - Review

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Abstract

Identification plays a major role in crime investigation. One of the most effective methods of identification are fingerprints. Like fingerprints, lip prints are also unique for individuals and remain the same throughout life. They are not influenced by environmental changes, trauma and diseases. The uniqueness of lip has been proven by researchers by using shape analysis and colour information. Lip prints can also provide some important information in crime investigations and have the ability to distinguish individuals and are used in human identification purposes. Human lips recognition is one of the most emerging methods of human identification that originates from forensic and criminal practice. Lip prints are captured by police technicians and further analysis provides valuable evidence. The use of lip prints first started in 1950 and researches were carried out in 1970. The wrinkles pattern on the lips has individual characteristics like fingerprints. Cheiloscopy is a forensic investigation method that deals with the study of elevations and depressions which form a characteristic pattern on the external surface of the lips. This is unique for individuals. Lip print recording is used in forensic investigation that deals with identification of humans, based on lip traces. Lip prints can be a basis for conclusion such as sexes, habits and character of that event. At a crime site lip prints can either be visible or latent. Print produced by a traditional lipstick is easily identifiable. Latent print investigation is important in resolving any criminal act. Latent lip prints are invisible prints and can be identified by using fingerprint powder. Long lasting lipsticks produce prints that are invisible and require reagents to develop the prints. The porous and multicoloured surfaces are the surfaces in which a print is difficult to identify and cannot be easily seen. Cups, glasses, tissue papers and napkins may have imprints of the suspect's lips.

Keywords : *Lip print , cheiloscopy , Forensic identification , latent lip prints.*

Introduction

Identification is important in any crime investigation. Cheiloscopy is a forensic investigation method that deals with recognition of humans based on lips traces. The wrinkles pattern on the lips has individual characteristics.[1,2] The wrinkles and grooves on labial mucosa, called as sulci labiorum forms a characteristic pattern known as lip prints and the study of this is cheiloscopy.[3] It can be defined "as a method of identification of a person based on characteristic arrangements of lines appearing on the red part of lips or as a science dealing with lines appearing on red part of the lips".[1,2] Like fingerprints, this is unique for individuals.[4] Fischer in 1902 was the first anthropologist to analyse the furrows on the red part of the human lips.[5] However, it was only in 1932 that Edmond Locard, one of France's greatest criminologists, recommended to use lip prints in personal identification and criminalization. LeMoyne Snyder in his book Homicide Investigation, written as early as 1950, mentions the use of lip prints in the identification of individuals. The lip prints are uniform for the whole of life and characteristics of a person can be used to verify the presence or absence

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of a person from the crime, provided there has been consumption of beverages, drinks, usage of cloth, tissues or napkin etc., at the crime scene.[6] Dr. Martins Santos in 1960 proposed that these lip characteristics could be used in personal identification and proposed a simple system for classifying lip prints.[1,2] In 1967, Suzuki made investigation of the measurement of lips, the use and the color of rouge and method of its extraction to obtain data for forensic application.[4] Later in 1971 Suzuki and Tsuchihashi, conducted a study and used their own classification.[1] Analysing in depth and establishing further facts and truth in lip prints will certainly help as useful evidence in forensic dentistry.[2] Previously our team had conducted numerous original studies [7-13] and surveys [14-21] over the past 5 years. Now we are focussing on using this knowledge in writing reviews on recent advancements in various fields. The idea for this review stemmed from the current interest in Forensic Identification. The aim of the study is to assess the identification of latent lip prints in forensics.

Lip Prints in Forensics

In the past there were different modalities used for identification like fingerprints, MN blood group system, and DNA fingerprinting: of these, finger printing is most widely used. As knowledge of using fingerprints for identification is increasing in the general population, offenders are taking care not to leave behind fingerprints at a crime scene. So cheiloscopy can also be used as an additional tool for crime investigation. Cheiloscopy is an upcoming tool in crime investigation. Though fingerprints and DNA comparison are most commonly used, additional tools like cheiloscopy and palatoscopy can be used for identification.[22] External surface of the lip has many elevations and depressions forming a characteristic pattern called lip prints, examination of which is referred to as cheiloscopy.

Like the fingerprints, this is unique for individuals. A lip print is a surface with visible elements of lines representing the furrows. This characteristic pattern helps to identify the individuals since it is unique for individuals. If the lines are not clear, individual identification of human beings based on this trace is extremely difficult, unless the trace contains more individual characteristics like scars, clefts etc, and often identification ends with group identification.[23] Lip print patterns appear to be genotypically determined, unchanged from birth.[24] The lips can be horizontal, elevated, or depressed, and according to their thickness, it is possible to identify the following four groups:

- Thin lips (common in the European Caucasian),
- Medium lips (from 8 to 10 mm is the most common type),
- Thick or very thick lip (usually having an inversion of the lip cord and is usually seen in negroes),
- Mix lips (usually seen in Orientals). [25,26]

The importance of cheiloscopy is linked to the fact that the lip prints are unique to one person, except in monozygotic twins.[27] Like fingerprints and palatal rugae, the lip grooves are permanent throughout life. It is possible to identify lip patterns as early as the 6th week of uterine life.[28]

The oily and moist secretions from sebaceous and salivary glands placed at the vermillion border and subsequent moisturization from the tongue enables the formation of a latent lip print whenever there is contact and is likely to be encountered and should be suspected to be present on the scene of the crime.[29,30]

Photographs, cigarette butts, drinking glasses, cups, letters, and windowpanes are among the items that can bear lip prints and should be examined and photographed before any other examination is made. Latent or invisible prints left at the crime scene have been a source of interest for some time. The development or extraction of lip prints is a matter of providing color contrast between the print and its background, so that it can be photographed or otherwise preserved for later comparison. Latent prints are invisible prints and are developed by powders, chemical means, or laser-induced luminescence. The powders most commonly used are aluminum powder, cobalt oxide, and magnetic powder. It may be applied with an atomizer, or by brushing, being the generally accepted method. The material is spread with a brush of soft and extremely fine hair. This brush is dipped sparingly in the powder and a few light taps are given to shake off the excess material. Then the brush is drawn very lightly across the latent print. When sufficient material has adhered to the print, residue is brushed away and the print photographed. If

the latent print cannot be satisfactorily photographed owing to its location, it can be "lifted" using a variety of pliable, adhesive materials. These can be pressed against the latent impression being dusted with powder and then covered with some transparent substance. Alternatively, transparent lifters similar to "scotch tape" may also be used.[26]

CLASSIFICATION OF LIP PRINTS

In 1967 Santos was the first person to describe lip grooves. He divided them in to four types namely

1. Straight line, 2. Curved line, 3. Angled line, 4. Sine-shaped line

Martin Santos Classification (1966)

I. Simple Wrinkles

a. Straight lines

b. Curved lines

c. Angled lines

d. Sine shaped curve

II. Compound Wrinkles

a. Bifurcated

b. Trifurcated

c. Anomalous

Suzuki And Tsuchihashi's Classification

Suzuki and Tsuchihashi (1970) have put forward a classification of lip prints also known as Tsuchihashi classification, these are the most widely used classification in literature.[31–33]

Type I: clear cut grooves running vertically across the lip

Type ll: Straight grooves which disappear half way instead of covering the entire. breadth of the lip

Type lll: Fork grooves in their course

Type lV: Intersecting grooves

Type V: Reticulate grooves

Type VI: Undetermined

Renaud's Classification

A. Complete vertical

B. Incomplete vertical

C. Complete Bifurcated

D. Incomplete Bifurcated

E. Complete Branched

F. Incomplete Branched

G. Reticular Pattern

H. X or Coma form

I. Horizontal

J. Other forms (Ellipse, Triangle)

Afchar -bayat Classification (1979)

A1 :Vertical and Straight grooves, covering the whole lip

A2 :Vertical and Straight grooves, but not covering the whole lip

B1 :Straight branched grooves

B2 :Angulated branched grooves

C :Converging grooves

D :Reticular pattern grooves

E :other grooves.[31]

RECORDING VISIBLE LIP PRINTS

Photographs

When the lips are photographed, proper lighting should be focused on the lips at an angle that accentuates the contrast between the white and dark areas. The resulting Lip Print photographs should be of correct natural size. The photographic method involves photographing the Lip Print (either direct or latent and subsequently developed), and comparing it with photographs of the lips of the suspects or photographs of the Lip Print of the suspects. Photographing of the lips can often be very tricky and subject to errors as

the central area of the lips and the angles of the lips are never in the same plane and leads to focusing errors that result in unsharp or blurred or partial images of the lips. This always calls for recording the Lip Print and then photographing them and then comparing the two photographs. With the advent of digital photography, the trend is to record direct digital imaging using digital cameras. Several courts, particularly those in the U.K. reject the evidence if the very first photograph is a direct digital image. On the grounds that there is no proof that the digital image has not been previously manipulated using any of the available digital imaging software including Adobe Photoshop. The court in the U.K. insists on having the primary records based on conventional photographs and a willingness to accept digital analysis of such photographs.[1]

Traditional Lipstick

In addition to fingerprints, lipstick prints are of forensic interest. Lipstick smears show indirect proof of a relationship or contact between a victim and a suspect or a suspect and a crime scene. Extracting DNA from saliva on a lipstick print is valuable in an investigation. Traditional lipstick produces a print that is easily identifiable.[24] However, the cosmetics industry has developed long-lasting lipsticks that often do not leave visible prints. Different lipsticks have different compositions. During the manufacture of long-lasting lipstick, the oil content is reduced to minimum. It is necessary to find other development methods that are more sensitive to oils and more easily applied.[34]

RECORDING LATENT LIP PRINT

Fingerprint Powder

This technique is one of the oldest and most common methods of latent print detection. It involves the application of finely divided particles that physically adhere to the aqueous and oily components in latent print residue on nonporous surfaces. Early practitioners used a variety of locally available ingredients to make their own dusting powders including charcoal, lead powder, cigar ashes[35] powdered "washing blue," powdered iron, and talc. Commercial powder manufacturers tend to label powders by color, such as black, white, silver, gray, and so forth, rather than labelling the ingredients. Particles that serve as good fingerprint powders include carbon black, lamp black, talc, kaolin, aluminium, metal flake, and dolomite.[36]

Other effective and widely used latent print powders are flake metal powders made from aluminium, zinc, copper, brass, stainless steel, iron, cobalt, and nickel. Particle size, shape, relative surface area and charge appear to play roles in it.[37–39]

In recent years, lipsticks have been advanced that do not leave any visible trace after contact with surfaces such as clothing, tissue paper, ceramic, and glass. These are the latent lip prints which can be developed with fingerprint powders such as black powder, gray powder. Fingerprint powder development is a convenient and practical way of collecting latent prints but the quality of latent print is the deciding factor and also the surface and sensitivity of the technique. It is necessary to find other development methods that are more sensitive to oils and more easily applied.[40]

Magnetic Powder

Another kind of powder, called magnetic or magna powder, allows for application with a magnetized rod that has no bristles. This sort of powder can be light, dark, or fluorescent and utilizes the ferromagnetic properties of iron powder mixed with pigment powders. The magnetized applicator (magna brush) is immersed into the powder, picking up a ball of the iron and particle mixture, essentially forming its own brush. This ball serves as an effective carrier for pigment particles and is passed back and forth over the substrate to develop latent impressions. There are two ways to preserve a powdered impression. The most common and simplest method is lifting. The second method is photographic representation of the latent prints.[37]

Lysochrome Dyes

Lip prints are genotypically unique and stable. At the site of crime, lip prints can be either visible or invisible. To develop lip prints various chemicals such as lysochrome dyes, fluorescent dyes (Nile blue, Nile red), etc. are available which are very expensive. Vermilion and indigo dye are readily available, naturally derived, and cost-effective reagents available in India.[34] Lysochrome is a generic term for compounds that have the ability to dye fatty acids. Their molecule contains

a portion that dissolves in contact with fat and another that is responsible for colour. Lysochromes are effective when used on long-lasting lipstick prints on porous surfaces, such as paper or fabric, where detection is usually difficult. Thus, lysochromes are a useful group of compounds for locating and developing recent as well as older latent lip prints. Lysochromes seems to be effective in both powder and solution application forms. Sudan black, has been used as a solvent in ethanol and water to develop latent fingerprints on surfaces contaminated with foodstuffs, oils and other fatty substances. All lip prints contain lipids thereby of the three reagents used, the best results were with Sudan black, followed by Oil Red O and Sudan III. Recently lysochrome dyes are being used for colorless lip prints. Dyes like Nile red are also used to obtain lip imprints. These dyes are visualized under ultraviolet or blue light.[41]

FACTORS (TRANSFER CONDITIONS)

Pre-transfer Conditions

These are affected by age, gender, occupation, disease, and the application of lipstick or any other material applied on lips.

Transfer Conditions

These are the conditions of the surface being touched, including texture, surface area, surface curvature or shape, surface temperature, condensation, contaminants, and surface residues. The pressure applied during contact, including lateral force, also contributes to transfer conditions.

Post-transfer Conditions

Environmental factors are forces that affect the quality of latent prints after deposition. Examples of these factors are physical contact from another surface, water, humidity, temperature and time factor.[37,42]

FACTORS (SURFACES)

The type of surface expected to bear a latent print is an important step towards successful development. Three types are

Porous Surfaces

Porous substrates are normally absorbent and contain materials like paper, cardboard, wood, and other forms of cellulose. Porous surfaces (tissue paper and cloth fabric).

Nonporous Surfaces

Nonporous surfaces do not absorb. These surfaces repel moisture and often appear polished. They include glass, metal, plastics, lacquered or painted wood, and rubber. Latent prints on these substrates are more susceptible to damage because the fingerprint residue resides on the outermost surface. Dye stains, powders, and vacuum metal deposition are the best choices to use on these surfaces. Nonporous ones (ceramic tile).[43]

Semi Porous Surface

Type of substrate that does not easily fit into the first two categories but should be mentioned is considered semi porous. Semi Porous surfaces are characterized by their nature to both resist and absorb fingerprint residue. These surfaces contain glossy cardboard, glossy magazine covers, some finished wood, and some cellophane. Semi Porous surfaces should be treated with processes intended for both nonporous and porous surfaces.[37]

The limitation is limited no of articles are available and more molecular level studies are needed.

The future scope is quick identification of the suspects in crime investigation and thereby saving the time in the crime investigation.

Conclusion

Lip prints thus hold potential promise as a supplementary tool along with other modes in forensics. In latent lip prints, use of lysochrome dyes and fluorescent dyes are very effective. Nile red is a very effective reagent for the development of latent lip prints on difficult surfaces like porous and multicoloured surfaces. Cheiloscopy can also serve as a very important tool in identification of a person. The uniqueness of lip print needed to be confirmed and accepted. A standard and uniform procedure has to be developed for the collection, development and recording of lip prints and the ensuring comparison.

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Bio Terrorism and Future - A Review

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Abstract

Biological warfare or biowarfare is the use of biological toxins or infectious agents such as bacteria, viruses, and fungi with the intent to kill or incapacitate humans as an act of war. It is a criminal act against unsuspecting civilians using pathogenic biological agents. Bioterror and Biological Warfare agents are most often colorless, by-and-large odorless microorganisms (bacteria, viruses, fungi) or toxins (usually protein toxins) derived from microorganisms that can be spread in air as aerosols or in food or drink to infect as many people as possible. They are easily concealed, and thus difficult to detect before an attack. Their main advantages to terrorists are allowing easy escape and causing panic and chaos within a civilian population. Their aim is to overwhelm emergency medical departments at local hospitals and clinics. However, there are ways to help protect yourself against bioterror agents and by extrapolating biological warfare agents and to help identify an attack when it occurs.

Keywords: *bioterrorism, Biological Warfare, combat bioterrorism, impact, biothreat organisms.*

Introduction

Bioterrorism, bioweapons, or biowarfare is a term used to portray the purposeful or intentional use, spread/ discharge, scattering or creation of living life forms, poisons, and synthetic concoctions of creature or plant inception to deliver such ailments that can mischief or cause passing of people, creatures, plants, and even the general environment[1]. The psychological militant can spread it through air, water, soil, and food, and they can take scarcely any hours to a few days to cause the impact. More up to date pathogens are developing step by step which lose a significant danger in rewarding hospitalized patients. It is an expansive subject that incorporates different scope of living beings and compound poisons which are utilized to spread fear, peril, hazard, and threat[2].This can be performed by an individual having personal stakes or for counter, however it can likewise be supported by some legislature as a major aspect of a political agenda[3] . In prior days, Bacillus anthracis and little pox infections were utilized as a wellspring of bioterrorism in the West and even as of late, Bacillus anthracis is a normally utilized operator for bioterrorism[4]. This type of psychological oppression is the least demanding on the grounds that the specialists are accessible effectively, don't cause sound or impact, can cause hurt across the outskirts, and are far reaching for the most part as it can clear out the whole populace[5]. There are three general classifications which indicate the term bioterrorism The classes depend on the danger running from most noteworthy to direct and in the long run to gradual[6]. Bioterrorism is genuine, clear, and steady in the cutting edge time. Thusly reinforcing of commonplace Environmental Protection Agencies (EPA), usage of self-observing projects by the

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enterprises, and enactments, for example, the "polluter pays'' standard can assist with securing our natural resources[7] .Research is the foundation of arrangements. Accordingly, the private party ought to be urged to put resources into investigating this field[8].

A bioterrorist assault might be hard to recognize from a normally happening infection [9] sickness flare-up. Agents should initially look at the etiology and the study of disease transmission of an episode to distinguish its source, method of transmission, and people in danger. Certain intimations may show whether a flare-up is the consequence of deliberate arrival of microorganisms. Normally happening ailments are endemic to specific zones and include conventional patterns of transmission; a few infections happen occasionally, and sentinel cases are not uncommon[10]. Conversely, an ailment flare-up because of bioterrorism could happen in an endemic-illness region, whenever of year, all of a sudden, and relying upon the etiologic operator and method of transmission, in enormous numbers—a huge number of cases may happen unexpectedly[11]. Public wellbeing authorities must be suitably sharpened to the chance of bioterrorism when examining sickness flare-ups.

Bioterrorism

Bioterrorism includes the deliberate discharge or scattering of organic agents[12]. These specialists are microscopic organisms, infections, creepy crawlies, parasites, or poisons, and might be in a normally happening or a human-changed structure, similarly in natural fighting. Further, present day agribusiness is defenseless against hostile to farming assaults by fear based oppressors, and such assaults can genuinely harm economy just as buyer confidence[13].The later damaging movement is called agrobioterrorism and is subtype of agro-terrorism.Bioterrorism is the purposeful arrival of infections, microscopic organisms, poisons or other hurtful operators to cause ailment or demise in individuals, animals, or plants[14]. These specialists are normally found in nature, however could be transformed or changed to expand their capacity to cause malady, make them impervious to ebb and flow drugs, or to build their capacity to be spread into the environment[15] .Biological operators can be spread through the air, water, or in food. Organic specialists are alluring to fear mongers since they are amazingly hard to distinguish and don't make disease for a few hours a few days. Some bioterrorism operators, similar to the smallpox infection, can be spread from individual to individual and a few, similar to Bacillus anthracis, cannot[16]. Hepatitis B is an irresistible malady brought about by hepatitis B infection which influences the liver.It can be transmitted through a few different ways through immediate or aberrant contact with blood , oral liquid , mist concentrates and so forth

Bioterrorism might be supported in light of the fact that organic specialists are moderately simple and reasonable to get, can be handily spread, and can cause across the board dread and frenzy past the real physical damage[17].Military pioneers, in any case, have discovered that, as a military resource, bioterrorism has some significant impediments; it is hard to utilize a bioweapon such that lone influences the foe and not well disposed forces [18].A natural weapon is helpful to psychological oppressors primarily as a technique for making mass frenzy and disturbance to a state or a country[19].

History

Contagious diseases and other biological weapons were recognized for their potential impact on armies or people as early as the 14th century BC . The Hittites might have produced the first documented example of BW by sending diseased rams (possibly infected with tularaemia) to their enemies to weaken them [20]. In the fourth century BC, the Greek historian Herodotus relates that Scythian archers used to infect their arrows by dipping them in a mixture of decomposing cadavers of adders and human blood. According to our modern interpretation, this mixture might have contained Clostridium perfringens and Clostridium tetani, as well as the snakes' venom [21] In the third century BC, the military commander Hannibal of Cartagena set fire to the enemy's fleet (belonging to King Eumenes II of Pergamon) with pots full of venomous snakes. Similar examples are reported by historians or, for cases closer to our epoch, by anthropologists of the use of arrows or other vessels infected with different products extracted from animal parts or plants in order to attack the human enemy [22]. Similarly, the use of arrows for the transmission of plague is suggested by some allegorical documents, such as the drawing painted in 1437 by an anonymous artist on a wood cover used by the

government of Siena to protect official documents (Fig. 1). In the Middle Ages, a famous although controversial example is offered by the siege of Caffa (now Feodosia in Ukraine/Crimea), a Genovese outpost on the Black Sea coast, by the Mongols. In 1346, the attacking army experienced an epidemic of bubonic plague. The Italian chronicler Gabriele de' Mussi, in his Istoria de Morbo sive Mortalitate quae fuit Anno Domini 1348, describes quite plausibly how the plague was transmitted by the Mongols by throwing diseased cadavers with catapults into the besieged city, and how ships transporting Genovese soldiers, fleas and rats fleeing from there brought it to the Mediterranean ports[23] Given the highly complex epidemiology of plague, this interpretation of the Black Death (which might have killed >25 million people in the following years throughout Europe) as stemming from a specific and localized origin of the Black Death remains controversial. Similarly, it remains doubtful whether the effect of throwing infected cadavers could have been the sole cause of the outburst of an epidemic in the besieged city. However, this episode of the use of cadavers in order to infect a population remains a landmark in the history of BW [24]. Similar examples of the use of the technique of catapulting infected cadavers can be found throughout the modern period, from the siege of the Bohemian city of Carolstein by Lithuanian troops in 1422 to the siege of the Swedish army in Reval (Estonia) in 1710 by the Russians

Laboratory detection and surveillance for bioterrorism:

Globally, there are laboratories established to address bioterrorism. According to the Centers for Disease Control and Prevention (CDC), there should be a laboratory response network which should include clinical microbiology laboratories that are responsible for identifying, detecting, and reporting bioterrorism[25]. In addition, they must suggest the measures that can be adopted to remedy the effects .Development of resistance in pathogens to antimicrobials is threatening mankind. Intrinsic and mechanisms act together to make the pathogen more and more potent against most of the available therapeutic drugs**.**The laboratory response network can be grouped category wise, dealing with the issue, like a category A laboratory can be a primary laboratory that can help detect such agents which can likely cause bioterrorism effects and so they should be equipped to conduct primary analysis.[26]The B category laboratories can help to isolate and identify the exact agent responsible for bioterrorism and work for its remedial measures and design such strategies. Similarly, the category C laboratories should work to identify those agents that can cause potential damage as a bioterrorism agent in future; they all must act as public health laboratories [27]Mostly, microbiologists are capable enough to work out the effects and remedies for bioterrorism and the population suffering or at risk to suffer from its impacts.[28] However, toxicologists also have a significant role to offer in this regard, as they are the ones who can identify the toxins from food, air, water, and even soil sources that can have an impact on the entire population and pose a risk to the overall public health.

Impacts

Bioterrorism and its potential for mass destruction have been subjects of increasing international concern. Approximately 17 countries (including five implicated as sponsors of international terrorism) may have active research and development programs for biologic weapons [29]. Moreover, groups and individuals with grievances against the government or society have been known to use or plan to use biological weapons to further personal causes.[30] Only modest microbiologic skills are needed to produce and effectively use biologic weapons. The greatest, but not insurmountable, hurdle in such an endeavor may be gaining access to a virulent strain of the desired agent[31]. Production costs are low, and aerosol dispersal equipment from commercial sources can be adapted for biologic weapon dissemination. Bioterrorists operating in a civilian environment have relative freedom of movement, which could allow them to use freshly grown microbial suspensions (storage reduces viability and virulence.[32] Moreover, bioterrorists may not be constrained by the need for precise targeting or predictable results.

The impact of a bioterrorist attack depends on the specific agent or toxin used, the method and efficiency of dispersal, the population exposed, the level of immunity in the population, the availability of effective postexposure and/or therapeutic regimens, and the potential for secondary transmission[33]. Understanding and quantifying the impact of a bioterrorist attack are

essential to developing an effective response.

Biothreat organisms

The biothreat organisms are classified into 3 categories. They are Category A that can be can be easily disseminated or transmitted from person to person;result in high mortality rates and have the potential for major public health impact;might cause public panic and social disruption; and require special action for public health preparedness.[34] The organisms are Anthrax (Bacillus anthracis)Botulism (Clostridium botulinum toxin)Plague (Yersinia pestis)Smallpox (variola major) Tularemia (Francisella tularensis) Viral hemorrhagic fevers, includingFiloviruses (Ebola, Marburg)Arenaviruses (Lassa, Machupo).[35] Category B that are are moderately easy to disseminate;result in moderate morbidity rates and low mortality rates; andrequire specific enhancements of CDC's diagnostic capacity and enhanced disease surveillance[36]. Among the bacteria that colonises the mouth, treponemes dominate because they proliferate rapidly in devitalised tissue with the availability of a simple source of nutrient The organisms are Brucellosis (Brucella species) Epsilon toxin of Clostridium perfringens. Food safety threats (Salmonella species, Escherichia coli O157:H7, Shigella)Glanders (Burkholderia mallei)Melioidosis (Burkholderia pseudomallei)Psittacosis (Chlamydia psittaci)Q fever (Coxiella burnetii)[37].Category C that are available ;ease of production and dissemination; and potential for high morbidity and mortality rates and major health impact. The organisms are Nipah virus and hantavirus and now coronavirus too.[38]

1.6 Preventive measures

The prevention of use of biological weapons should endure to be a top priority for the nation. In the production of bioweapons, which deliver a highly infectious, contagious and lethal pathogen, relatively smaller investment of money, time and effort are required. Recent advances in biotechnology, bioinformatics and molecular genetics has great benefits for human health, but state proliferators and sophisticated terrorists misuse the results of such research to develop more effective biological weapons[39] .The knowledge, materials, and technologies needed to make and use a biological weapon are readily accessible around the world. Pathogens are pervasive in nature and can be found everywhere. The possibility of synthesizing pathogens particularly viruses, using technologies are inexpensive and globally available. Public health, medical, military, and law enforcement experts have met in a number of settings in efforts to identify the most threatening of the biological weapons, specifically those weapons that merit priority concern in the development of public health and medical preparedness measures.

During an event of biological warfare, it is not the muscle power or the powerful ammunition that will come to the rescue, it is the efficiency of an applied science methodology from microbiological scientists that will protect the soldiers and citizens. During an emergency, an immediate intervention will be the need of the hour to reduce the mortality and morbidity rate. Antibiotics, active and passive immunizations, and antiviral agents can be used for this purpose. Since diseases resulting from biological warfare agents and disasters have an incubation period of days, there is a short time period in which proper post exposure prophylaxis may be given.

Conclusion

Bioterrorism is real and evident and persistent in the modern era. During an emergency, an immediate intervention will be the need of the hour to reduce the mortality and morbidity rate. Antibiotics, active and passive immunizations, and antiviral agents can be used for this purpose. Since diseases resulting from biological warfare agents have an incubation period of days, there is a short time period in which proper post exposure prophylaxis may be given. Detection of the pathogenic agent and intervention with appropriate drugs with proper knowledge and awareness of the public is essential to control the spread of the disease.

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Conflict of InteresT: Nil

Ethical Clearance: As it is a review article so it is not required.

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Colour Stability of Composite Resins - A Review

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Abstract

Composite resins are the most preferred dental materials for anterior and posterior teeth. They have an important role in aesthetic and restorative dentistry. Paramount importance of composite resins are they have an Optical appearance, glossy surface and colour stability. There are three types of composite resins Conventional(Packable), Flowable(Bulkfill),Microfill. Hybrid,Fluoride releasing composites. Color change of composite restoration in different color media during the time is a common problem in esthetic dentistry, causing the need to replace restoration and spending a great deal of cost and time to patients. The aim of this study is to present a short review of recent literature available on the association of colour stability of composite resins. The article was done after reviewing several articles on the colour stability of composite resins. The effect on colour stability of composite resins depends on type of solution exposure time and composition of material. In clinical practice, patients should be aware of the staining effects, as staining in anterior teeth may lead to unaesthetic appearance.

Keywords: *Colour stability , Esthetic Restorative materials, Discolouration , Surface roughness.*

Introduction

Colour stability of composite resin is a crucial property influencing clinical longevity , which continues as a challenge inherent to material [1] .Colour changes can occur because of staining in a superficial layer of resin composite , water absorption ,surface roughness ,smoking and diet [2]. Intrinsic discoloration can occur as a result of physio mechanical reaction within the fabric material example the filler and resins matrix properties [3]. It is documented that the original colour of composite resins are often changed within a particular period of time[4] . Long term colour changes in composite restorations can occur due to surface and marginal staining, microleakage, wear dependent surface changes and internal material deterioration ,which may compromise the visual acceptability of those restorations and end in additional expenses for replacement [5,6]. Secondary caries were the most main reason for failure of resin composites , followed by discoloration. This process concerns patients and dentists and consumes time and money. Not all alterations might be related with surface degradation [7]

Discoloration of composite resins are often caused by internal or external factors . Internally induced discoloration are permanent and are relatable polymer quality , filler type and amount ,as well because the synergist is added to the photoinitiator system [8]. The resin's affinity for extrinsic stains is modulated by its conversion rate and physico chemical characteristics with water sorption rate being of particular importance [9] . Within the mouth due to superficial degradation or a small penetration and adsorption of staining agents at the superficial layer of composite resins discoloration of surface subsurface of resin restoration may result. Moreover externally induced discoloration is often

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associated with surface roughness, surface integrity and therefore the polishing technique [10,11] .

Generally it's recommended that resins should be placed in 2mm increments to urge sufficient light transmittance and complete curing of composite resins [12] .The application of composite resins in an incremental technique and light weight curing each increment individually may be a time-consuming procedure[13] .

In addition to colour alteration a number of these products also can cause superficial degradation on the composite surface by reducing its microhardness which could possibly favour more superficial staining[14]. Corroborating this theory Okte Et Al showed decreased microhardness valves of microhybrid and nano resin composite after immersion in both coffee and wine solutions [15] .

Materials and Method

Study setting was made by sampling reviews. Number of articles taken 25. Search engines used are "Google Scholar and PUBMED". Search terms are used in relation with colour stability of composite resins. There are five steps selected on the article.They are Identification of clear subjects , Identification of relevant articles, Selection, Data Extraction and Charting , Analysis and report. The articles selected based on recent similar and relevant publications.Article selection include articles with recent advancement in colour stability of composite resins. Scoring was done for all 25 articles.

Composite resins

Composite resins represent one of successes of biomaterial research ,since they replaced biological tissue in both appearance and performance. A minimum of half posterior direct restoration placements now believe composite materials [16]. Unfortunately demands on these restoration with regard to mechanical properties, placement and need for in-situ curing leave significant room for advancements, particularly the mechanical properties of polymerization shrinkage and polymerization induced stress ,thermal expansion mismatch, Fracture abrasions and wear resistance marginal leakage and toxicity [17] . Ultimately, these shortcomings reduce a restoration's lifetime and represent the drive factor for improvement in dental composites clinical evaluations and laboratory based studies focused on composite durability also still highlight this need for brand fresh materials[18]. Review discussed development of polymeric composite resins focussing on methods for reducing polymerization shrinkage and achieving improvements in biocompatibility and wear resistance [19,20] .

Photo initiation

Though non-photoinitiated polymerization are still performed and studied, photoinitiated polymerization have enormous value in controlling polymerization temporally,allowing adequate time for placement and manipulation of restorative before curing [21,22] . Clinically desirable visible light has reduced energy per photon relative to UV light ,which limits the selection of suitable inhibitors and sometimes necessitates multicomponent initiators. Additionally there remains concerns over the toxicity of amine co initiators that are used with camphorquinone. Ultimately,these needs drive the investigation of a replacement photoinitiator system [23].

Colour testing

A colorimetric evaluation to the CIELab system was performed by a blind trained operator at five experiments periods immediately after light polymerization and at 7 ,14 ,21, 28 days of staining process.colour of the specimens was measured with spectrophotometer against a background in order to stimulate absence of light in mouth against a white background [24] . FilTek supreme has almost the same matrix formulation as FilTek Z250 & P60 with exception of containing small amounts of TEGDMA which might be responsible for the high water absorption and discolouration rates. Chlorhexidine application, either in an aqueous solution or associated with the acid conditioner, preserves durability of the resin-dentin interface. Further *in vivo* studies are needed to clarify whether the use of a 2% CHX-containing acid preserves resin-dentin interface after long-term function [25]. According Umand Ruyte Et Al Cola had lowest pH and it might damage surface integrity of composite resin materials.It did not produce any as discoloration as coffee and tea possibly due to its lack of yellow colourant.Both coffee and tea contained yellow colourant which had different polarities,It is

noteworthy that materials which contained TEGDMA showed higher discoloration values meaning TEGDMA was responsible for discoloration due to hydrophilic character [26] .In clinical practices patients should be aware of staining effects of drinks tested in this study, while practitioners should take into consideration the staining susceptibility of composite resins [27] .

Effects of foods And beverages on composite resins

According to Bagheri Et Al [28] , composite resins are able to absorb water,then it is capable of absorbing other fluids resulting in its discoloration and in the reduction of mechanical properties due to polymer matrix degradation [29] . Fluid absorption occurs mainly as direct absorption in resin matrix.Glass filler particles will not absorb water onto the surface. The ability of composite to absorb fluids is strongly related to the nature of material, where hydrophobic material with absence of hydroxyl group in their composition showed low water sorption but were stained by hydrophobic solutions,whereas hydrophilic materials with high degree of water sorption were stained by hydrophilic substances in aqueous solutions the other substances that also had an impact on composite colour were ketchup another condiment is very popular among populations all over the world and its red colour is attributed to a substance called lycopene is also insoluble in water [30] .

The colour of grapes comes from anthocyanins, which are water soluble vacuolar pigments that may appear red, purple or blue according to pH [31] . Differently from the pigments found in mustard and in ketchup anthocyanins are hydrophilic and so is the caramel colouring,pigment present in soy sauce. In order to prevent the mechanical retention of these pigments on the composite surface,all specimen were washed and brushed before each evaluation [32] Immersion of specimens in deionized water did not alter colour of composite to a considerable extent,Although a perceptible colour alteration could be noticed after 7 days in this group. Fontes Et Al believe that low pH of fruit juice affects the resin surface increasing pigment absorption. According to Mundim Et al [33] , patients should last and whether their eating habits may influence quality and longevity of restoration . Knowledge of effects of staining potential on the surface of composite resin could guide clinicians as regards instruction, give their patients after operator procedure, to assure better colour [34] stability and long term maintenance of restoration.

Tooth brushing simulation on composite resins

Degradation of composite material can occur due to mechanical and chemical factors from the oral environment which can cause changes in surface roughness, loss of surface gloss and increased discolouration of material affecting esthetic quality of restoration [35] . In non stress bearing areas main causal factors of texture changes are the relationship between biodegradation and oral hygiene procedures [36] . Abrasions of composite resins were affected more by the toothpaste's abrasiveness than by toothbrush filament diameter. It is important to notice that the pattern of toothbrushing wear on restorative materials was the result of interaction of several factors and studies have reported the effects on GIC, pit and fissure sealants. [37]According to Gurgan et al mouthrinses can affect the hardness of restorative materials, alcohol content is not only a factor that has softening effect on materials for example showed higher roughness resulting saliva immersion compared with a mouthrinse [38] .

Conclusion

Composites have undeniably obtained a prominent place among the filling materials utilized in direct methods. Their significant stylish prospects offer ascent to an assortment of restorative signs, which keep on developing because of the extraordinary flexibility of the introductions offered; likewise, these materials preserve the tooth structure better since they are held by cement techniques as opposed to relying upon cavity plan. With this help of a review article we can conclude that Color stability is the ability of materials to retain their original color. Daily intake of food with staining ability such as tea, coffee, and cola can compromise esthetics of restorative materials. Understanding the property of color stability and the comparative analysis of various restorative materials will help a clinician to choose the materials as per the diet habits of the patients and ensure predictability of success. It will also enable the clinician to educate and counsel the patient about the effects of specific chromogenic ingredients in the diet such as tea, coffee, and wine on the color stability of the restorative material used.

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Prevalence of Nance Space Maintainer as a Treatment Modality among Children between the Age Group of 6-10 Years

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Abstract

The early loss of deciduous teeth may lead to a decrease in arch length and result in crowding, rotation, and impaction of the succedaneous teeth. Space management plays a pivotal role in preventing these malocclusions. This study aims to assess the prevalence of Nance space maintainers among children between the age group of 6-10 years in Chennai. A retrospective cross-sectional study was conducted using the patient records from June 2019 to April 2020, and children who underwent space maintainer therapy were selected by non-probability sampling. Data was collected and then subjected to statistical analysis using Statistical Package for Social Science for Windows (version 20.0, SPSS Inc., Chicago Ill., USA). The prevalence of nance space maintainer among 6-10 year old children was found to be 76.5%, with most of them being delivered to 8 year olds. More males than females underwent nance space maintainer therapy. 53.85% of the 6-10 year olds receiving nance space maintenance therapy were found to be treated by the post graduate students (p>0.05, statistically not significant). The prevalence of nance space maintainer as a treatment option among the mixed dentition phase in this study seems to be high when compared to other studies. Considering the limitations of the nance appliance, extensive research needs to be done to find alternatives to nance space maintainer therapy and to spread awareness among dentists on the necessity of such further advancements.

Keywords: *Preventive orthodontics, Space maintainer, Nance appliance, Mixed dentition, Space management*

Introduction

The guidance of eruption and proper development of the primary, mixed, and permanent dentitions is a fundamental element of comprehensive oral health care for all pediatric patients[1]. It must contribute to the development of a permanent dentition that is stable, functional, and esthetically acceptable and subsequent normal dentofacial development[2]. Timely diagnosis and prompt treatment of developing malocclusions can have both short-term and long-term advantages while attaining the orthodontic goals of functional efficacy, structural balance and esthetic harmony[3].

Preventive orthodontics is defined as "*the action taken to preserve the integrity of what appears to be a normal occlusion at a specific time*". Early recognition of a future problem forms the basis of preventive orthodontics. One of the major challenges in Paediatric dentistry is the management of space loss due to the premature loss of primary teeth[4]. Any interruption in the sequence of transition from deciduous to permanent dentition leads to an occlusion that is not functionally stable and esthetically pleasing. Hence, there exists

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a need to prevent such malocclusions at the primary stages in order to benefit the patient's functional as well as psychological development[5]. Space maintenance is concerned with the maintenance of space that is lost by the early loss of a primary tooth by means of a passive appliance or by means of gaining the space that is lost. Numerous appliances can be used for space maintenance based on the patient's age, growth and development of dental arches and patient compliance[6]. Space maintainers may potentially eliminate the ill consequences of early loss of primary teeth and the need for complex orthodontic treatment at a later stage[7].

Maintenance of the primary second molar space is key for guiding the eruption of the first permanent molar, else it may lead to mesial migration, space loss and reduction in arch length. It is particularly imperative when the leeway space is paramount to treat any crowding or in order to preserve the midline[8]. Conventionally, the treatment of choice for maxillary loss is the Nance appliance. The nance appliance (NA) is a modification of the maxillary lingual arch and was described by Nance in 1947. It has ever since been the conventional treatment choice for premature loss of deciduous maxillary posteriors. The NA does not contact the anterior teeth, but approximates the anterior palate. The palatal portion has an acrylic button incorporated in it that provides resistance to anterior movement of the posterior teeth. The molar bands are connected to the acrylic button using a 0.9 mm round stainless-steel wire [9-10].

Though the nance appliance is widely used, it has many limitations including soft tissue irritation, which is of major concern. Bacterial and food debris accumulation often leads to palatal inflammation and pain. The acrylic button may get embedded in the soft tissue in case of palatal tissue hypertrophy from poor oral hygiene or distortion of the appliance[11]. This study sheds light on the current scenario of the nance appliance as a treatment modality in the early mixed dentition phase by assessing the prevalence of Nance space maintainer as a treatment modality among children between the age group of 6-10 years in Chennai.

Materials and Methods

Study Design and Setting

This pilot retrospective study examined the records of 100 patients from June 2019-April 2020. Ethical approval was obtained from the Institutional Ethics Committee. The study population included children who underwent nance space maintainer treatment by means of non-probability sampling. Children with mental or physical disability, unable to maintain oral hygiene and children with active lesions were excluded from the study.

Data Collection

Out of the patient records, 13 patients were identified to be undergoing nance appliance therapy. Relevant data such as patient age, sex and operator qualification was recorded. Repeated patient records and incomplete records were excluded. Data was verified by an external reviewer.

Statistical Analysis

Data was recorded in Microsoft Excel/2016 (Microsoft office 10) and later exported to the Statistical Package for Social Science for Windows (version 20.0, SPSS Inc., Chicago Ill., USA) and subjected to statistical analysis. Chi square test was employed with a level of significance set at $p<0.05$.

Results and Discussion

The final dataset consisted of 13 patients of Indian origin undergoing nance appliance therapy. The mean age for NA treatment was 6.35 years (standard deviation = 1.579 years). The age group associated with the greatest prevalence of NA as a treatment option was 8 years, followed by 6 and 7 years (Table 1, Figure 1). The prevalence of NA among 6-10 year olds as a treatment modality was found to be 76.5%.

61.5% of the children receiving NA therapy were males showing a male dominance (Figure 2).

Among the NA delivered to the 6-10 year old age group, 53.8% were delivered by PG students and 46.2% of them were delivered by UG students (Figure 3).

There was no statistically significant difference between the operator's qualification and the age groups receiving NA therapy ($p=0.557$). Most of the NA was delivered by both PG students and UG students equally. 23.08% of NA was delivered to 6 years old by PG

students and 23.08% delivered by UG students to 8 years old. The least number of NA delivered was by UG students to 6 years old children. (Figure 4).

Table 1: Patients undergoing NA treatment between age group of 6-10 years old

		No. of patients	Percentage (%)
Age (years)	6	4	30.8
	7	4	30.8
	8	5	38.4
	9	0	0
	10	0	0
Total		13	100

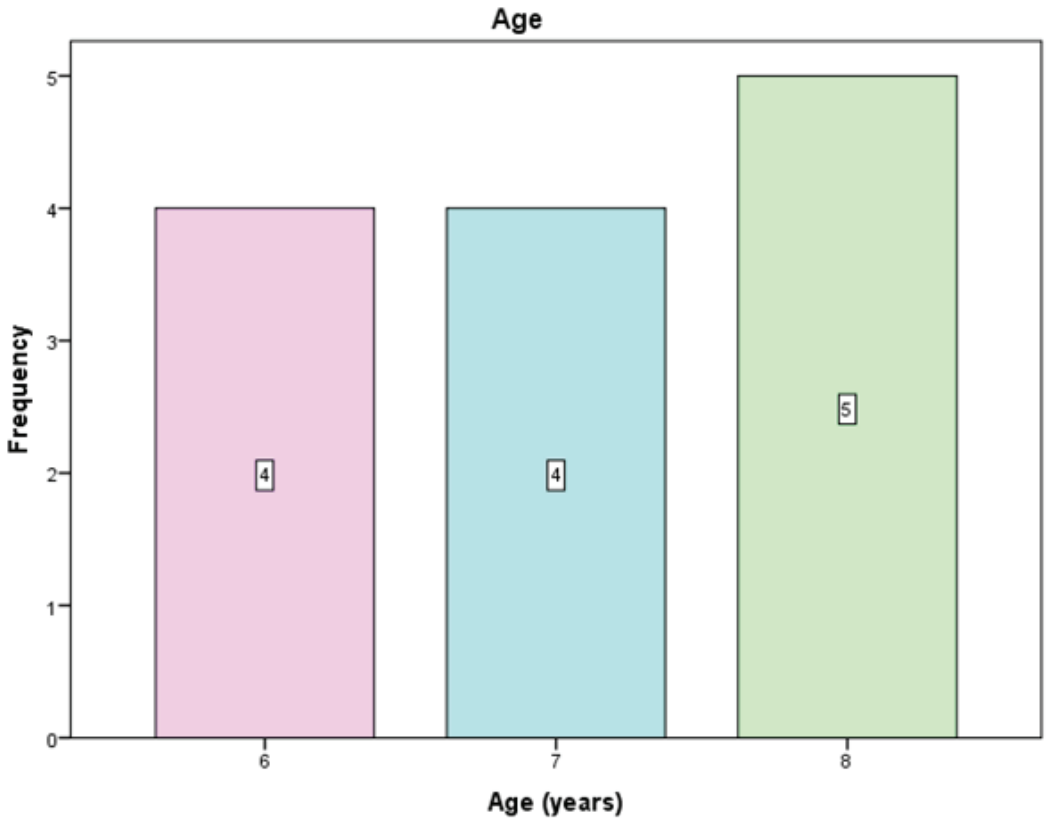


Figure 1: Bar graph shows the age wise distribution of NA treatment. X axis depicts the different age groups and Y axis depicts the number of NA delivered. Pink color denotes 6 years, blue color denotes 7 years and green color denotes 8 years. 38.4% of NA were delivered to children of 8 years of age, followed by 30.8% to children of both 6 years and 7 years of age.

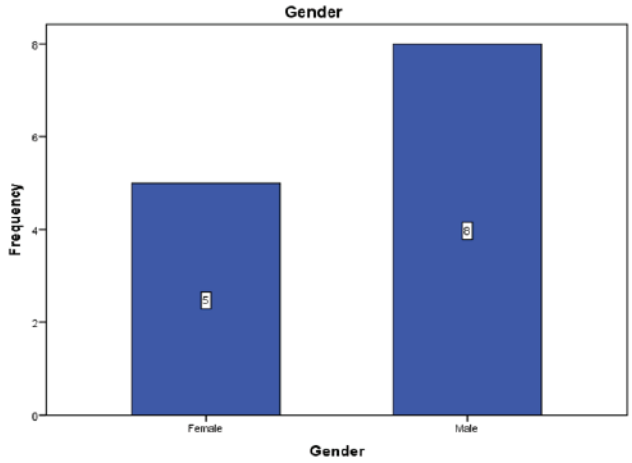


Figure 2: Bar graph depicts the gender distribution of children receiving NA treatment. X axis represents the gender and Y axis depicts the number of NA delivered. 61.5% were males and 38.5% were females.

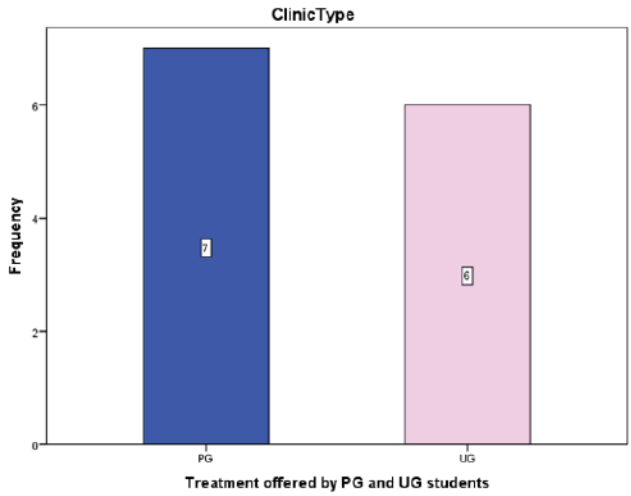


Figure 3: Bar graph depicts the operator qualification of those who delivered NA. X axis represents the operator qualification and Y axis depicts the number of NA delivered. Blue color denotes the PG students and pink color denotes the UG students. 53.8% of NA was delivered by PG students, 46.2% of NA was delivered by UG students.

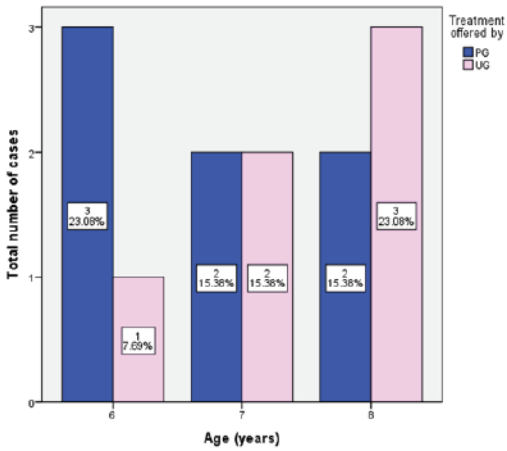


Figure 4: Bar chart depicts the association between operator qualification and the age groups treated with NA. X axis depicts the age groups and Y axis depicts the number of NA delivered. The blue color indicates

the NA delivered by PG students and the pink color indicates the ones delivered by UG students. 23.08% of NA was delivered to 6 years old by PG students and 23.08% delivered by UG students to 8 years old. The least number of NA delivered was by UG students to 6 years old children. However, there was no statistically significant difference between operator qualification and the age groups to which NA was delivered. (Chi square test, p=0.557- not statistically significant)

The data for this retrospective study was based on residents of Chennai seeking treatment at Saveetha Dental College. Currently, there are no existing studies investigating the prevalence of nance space maintainer as a treatment modality among the children with mixed dentition phase in Chennai. Since all the data available was included without a sorting process, no bias was expected in the selection of patients. The current study aims to shed light on the current scenario of nance appliance as a treatment modality in the early mixed dentition phase, which is the golden time for prevention of malocclusions as described in literature[12].

In a study by Kumar et al,[13] it has been established that children belonging to the age group of 5-12 years in Chennai are more prone to dental caries. In another study by Jayashri Prabakar et al,[14] it has been proven that primary dentition is more prone to dental caries and there exists a need for treatment at its earliest possible stage [15–26]. This explains the higher prevalence of NA treatment among 6-10 year olds in this study, particularly the 8-year olds. The parent's encouragement and active participation in the child's oral health care are crucial in influencing the dental health of the child[27].

There exists a wide variation in the prevalence of malocclusion in Indian population, ranging from 20-43%, which may be due to disparities in ethnicity and nutritional status[28]. A study by Rajendra Reddy et al [29] showed 52% of the 6-10 year olds in Nalgonda had malocclusions. The most common reason for these malocclusions being premature loss of deciduous teeth due to dental caries[14]. The study by Danalakshmi Jayachandar et al [30] showed that 34.46% of 5-10 year old children in Chennai had early loss of primary teeth with boys showing an increased incidence of 54.64%. The most common missing tooth in the study carried out by Danalakshmi Jayachandar et al was found to be the first molar (43.8%) followed by second molars (33.2%). The study also showed a greater prevalence for early loss of primary teeth was among the age group of 8–9 years, which is similar to the results of the study. The probable reason for this similarity is that both the studies have been carried out in the same geographical location.

There exists a male predilection for NA therapy in the current study. This may be due to the fact that males are more prone to early exfoliation of primary teeth as established by previous literature. It has been established that males are more prone to traumatic injuries as well as dental caries[31]. This may be attributed to a variety of reasons. One of the reasons being an increased feeding in male children by mothers due to their preference of a male child than a female child, especially in India, leading to an increased susceptibility to dental caries[32]. An increased male predominance may also be due to differences in diet, geographical location, cultural differences, especially in a country like India that believes in an ancient belief of male priority [27,33-34].

In the current study, the prevalence of NA among 6-10 year olds was found to be 76.5%, with most of them being delivered by postgraduate students,[35] probably because of their higher clinical experience aiding them in treatment planning, course requirements and superior clinical skills. The prevalence of NA therapy in this study seems to be higher when compared to previous literature[6]. The study by Qudeimat MA et al [36] at a pediatric dental clinic in the UK showed a 11% prevalence of NA. Another study by Baroni et al [37] also showed a low prevalence of 31.15% for NA therapy. The current study is also not coherent with the low prevalence NA in the study by H. Shamsaddin et al [38] but is coherent in terms of the male predominance.

Despite the popularity of the nance appliance, it has many drawbacks including soft tissue irritation, which is of foremost concern. Bacterial and food debris accumulation often leads to palatal inflammation and pain. Poor oral hygiene or distortion of the appliance may result in the embedding of the acrylic button in the soft tissue because of palatal tissue hypertrophy. Hence, other appliances like the Transpalatal arch

came into use[11]. The current study shows that despite its drawbacks, NA is continuing to be used as a popular treatment modality for mixed dentition patients in Chennai in the prevention of malocclusions. This suggests the need to spread awareness amongst dentists in India on the disadvantages of the NA and encourage the use of alternative treatment modalities.

Conclusion

Within the limitations of this study, it was found that the prevalence of nance space maintainer as a treatment modality among the children between 6-10 years in Chennai seems to be higher when compared to literature from other countries. Considering the deleterious side effects of using the NA, it can be concluded that there exists a paramount need for more extensive research to be done in this area to find alternatives for NA therapy and to spread awareness among dentists in India on the dire necessity of such further advancements.

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Conflicts of Interest: Nil

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Factors That Determine Areas Into Containment Zones of A Disease- A Review

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Abstract

Containment zones, or areas with strictly enforced restrictions regarding social interactions and self-protective behaviour, are basically demarcated to ensure that in the early stages or at the least, as soon as possible, a disease is confined to particular areas and not allowed to spread to unaffected areas. Influenced by ecological, environmental, pathogenic and demographic factors, which still are just a few of the many formative factors, implementing such demarcations have helped prevent worsening situations in many cases. Building containment zones require analysing experiences from previous attacks of a disease, mathematical modelling and social spread pattern identification, as well as understanding the progression of the disease from rural areas to urban metro cities. Moreover, on account of the diverse characterisation of a geographical area and its population, all the parts of the area will not be uniformly affected. Thus, developing containment zones and implementing them require strategic planning. Further, if effectively dealt with and reviewed, containment zones even transform back into safe zones. This review will assess the necessity of a containment zone, its emergence and factors influencing it, its relation with hotspots and finally, how it could be transformed into safe zones, free of disease constraints.

Keywords: *Containment zone, hotspot, disease spread, demographic, environmental factors, Buffer zone, Safe zone.*

Introduction

Generally, a given geographic area with positive cases of infectious diseases, and where it is found to be tough to restrict the rates of cross-infection, is considered as a containment zone. Further spread is prevented by imposing behavioural and social restrictions. Hotspots, on the other hand, are areas with a concentrated disease occurrence. Even if it's just a small area, if those affected are confined to the same area, the entire area becomes a hotspot. Containment zones and hotspots are demarcated by assessing risk factors, high probability of spread, and attitude of the people in the area [1]. A containment zone may or may not become a hotspot, but a hotspot can become a containment zone. A containment zone will have harsher restrictions to check the further progression of a disease to such an extent that even essential services can be curbed [2].

Based on the extent of cases or contact mapped by local response teams [3], containment zones are categorized. In the case of an immediate need where mapping might cause a delay, an approximate radius of 3-5 km about an area is designated as a containment zone, that will be updated as and when mapping results are obtained. A buffer zone i.e. a zone of larger radius, than the containment zone, encircling it, will also be declared [4].

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Building containment zones include analysing experience from previous attacks of a disease, mathematical modelling and social spread pattern identification, understanding progression from rural areas to urban metro cities [5]. Besides, on account of the diverse characterisation of a geographical area and its population, it is only unlikely that all the parts of the area will be uniformly affected. Thus, developing containment zones and implementing them require strategic planning.

Large scale developments in the fields of agriculture, hydro-industry and extensive population movements like immigration & emigration, have inflicted an invasion of sorts into the natural environment [6]. Thus, it becomes necessary to understand parasitic and zoonotic diseases. Urbanization has brought rural diseases into urban areas where the course of the infection is apparated by greater populations of vectors and hosts [7]. It is to be noted that so far there has been only little work done in linking biomedical aspects of a disease with the socio-economic effects caused due to them. Or the latter must be just too extensive to comprehend adequately [8].

Recognition of the potential for a widespread infliction due to a disease and mobilisation, that too, timely, of required public health operations and mathematical modelling to predict any further cause of a disease is necessary in the initial stages itself [9]. These might also be accompanied by prophylactic medication, movement restrictions and other non-pharmaceutical interventions [10]. The rapid detection, investigation, and reporting of human cases enable mathematical modelling and analysing what possible actions at the moment would be required. In a study [11] it has been cited that timely deployment of adequate drugs to 80% of the population of a containment zone can ensure the disease does not progress beyond the initial stages.

Materials and Methods

Around 30-40 articles were selected from portals like Google Scholar, PubMed, and Semantic scholar. They were analysed and reviewed based on their relevance to the study, after which their content was interpreted and incorporated into the present study. Uncontrolled variables like time frame of disease, pathogenesis etc. were excluded while control variables including demographics, human-caused natural habitat destruction etc. were included in the study.

Disease emergence

A disease can be a familiar one resurfacing, or a completely unfamiliar newly surfacing one. However, this does not differentiate between some common driving factors in disease emergence, in both cases, like ecological and environmental changes including natural disasters, deforestation, droughts, agricultural interventions, demographic and behavioural aspects like population migration, urbanization, international trade and travel, technology, microbial adaptation [12] and public health measures [13].

Certain situations of conflict characterised by a civil strife are again more prone to disease emergence with frequent periods of violence, insecurities, economic downfall, poverty and after effects still ongoing after a war or disturbance, severe enough to sustain that long [14].

An infection might be introduced into the community through travel, commerce, or war, especially with the current widespread globalisation of the service sector. Certain studies cite examples of pathogens invading raw materials in cargo ships, and ending up in the larger batch of the manufactured products [15]. Inadequate surveillance, response teams, destroyed infrastructure and health systems, and poor disease control programs and infection control practices, accompanied by poor coordination among humanitarian organisations contribute to disease development [16].

Detecting and controlling emerging diseases already is a challenge as such, due to multiple uncontrolled factors like pathogenic mutations and suitable environments or niche [17]. Civil conflicts only add on to these hassles, leading to further population displacement, overcrowding, inadequate water and sanitation deficiency while in vector-borne diseases, enhanced transmission by vectors during the initial stages itself. Vulnerability to contracting infectious diseases in an area is very much linked to long term lack of investment in health, education, public works, malnutrition, low prophylaxis coverage, and chronic stress [18].

Disease amplification

In the past (2000), Uganda saw an outbreak of

Ebola [19] which was attributed to poor infection control practices in health care facilities itself . In poor resource settings, sub-standard supply and maintenance of personal protective equipment, insufficiently trained staff, unhygienic practices [20]and inadequate sterilization protocol amplify disease transmission [21].

Delays in detection and reporting due to weak surveillance systems [22], limited laboratory infrastructure and expertise, and delayed specimen collection may impede the confirmation of the pathogen [23]. Poorly trained health staff and unorganized logistics are added factors that prevent the reach of drugs and medication to the wider public at the time of need [24].

Transmission heterogeneity [25] is a phenomenon observed in different pathogens where, within the same species, there tend to be differences in the mode of transmission making it unpredictable to outline a definitive pattern. Variability in host behaviour, host & pathogen genetics, co-infection and cross-infection patterns, and environmental exposure [26] are some variables leading to transmission heterogeneity. Variable population densities, environmental characteristics and some spatial characteristics which if understood can enable determining patterns of transmission. Pathogenic resistance to drugs is another factor contributing to disease amplification with resistance leading to confusing diagnoses and inappropriate drug regimens or outdated drugs [16,27].

Poor treatment compliance and insufficient procuring of drugs or interrupted treatment with sudden displacement or irregular health access [28] are some factors of amplification of diseases. International disease spread may occur through movements of refugees, relief workers, private sector employees, animals and products, especially those directly from factories and industries where a large number of people remain confined to the workplace [29].

Demarcating containment zones

A particular zone demarcated from the rest of the zone and feasibly maintained, large enough to include all infected persons in the vicinity and those in contact with them, is included as a containment zone [30]. Meanwhile, the surrounding 'buffer zone' of larger radius is to conduct surveillance for new cases and to see if the containment operations are effective. This enables modifying, continuing or terminating the containment operations [31].

The theoretically most inclusive form of a containment zone is the circular form with a defined radius, however, in reality, containment zones will be geographically determined. Movements of people, case contacts, administrative boundaries and natural boundaries, infrastructure and essentials like power, sanitation, food supply, contamination etc. affect/ influence containment and buffer zones [32]. If human-to-human transmission is still detected outside the containment and buffer zones, the WHO aids government authorities to constantly assess the situation and expand containment operations further [33].

Pathogenic factors

Several pathogens exist which infect across various species where contact rates and transmissibility vary [34]. It becomes necessary to also identify hosts that amplify pathogen transmissions and super spreaders to manage multi-host diseases. These hosts increase susceptibility of differing species to the disease on exposure, and ironically, tend to have a greater social interaction and reside in populated zones. They elevate overall disease prevalence in multi species communities [35].

The distribution of pathogens within an environment influences burdens on the host. For example, even in situations with a lesser pathogenic population, if a host comes across a concentrated pathogenic region, he will still be infected only, even from that less populated zone [36].

Environmental factors

As discussed previously, spatial heterogeneity also plays an important role in determining pathogenic prevalence. Hotspot or transmission rates may act as source areas for pathogens to traverse across landscapes to affect less infected areas [37]. Even a specific environment may facilitate elevated survival rates of pathogen or amplification hosts which are equally dangerous. In a study by Spira et al [38], the bacteria Vibrio cholerae was observed to thrive well in water hyacinth colonies causing areas with such colonies to become hotspots of the disease cholera.

Demographic factors

Alteration in community structure, contact rates and host susceptibility in humans, plants and all the occupants of an area are associated with anthropogenic changes such as genetic drift, which alter population and habitat size, changing reduced population diversity in host population, reducing host immunity, causing spatial variability [39]. Nutrient enrichment also elevated disease risk through pathogen abundance virulence even though this is probably being utilised by the human population [40]. A study by Lol et al [41] showed how nutrients enhanced in an agricultural land also caused a greater Anophele vestiti pennis (vector) population.

Further, day care facilities, hospitals, farms, markets and regional differences in socialising also enable hotspot development. The main areas of disease transmission include schools where multiple children aggregate wood places, construction sites where labourers work together, etc. A study by Chong et al [42] showed how the Nipah virus was contracted by Malaysian families from Bangladeshi nurses. Other 'social animals' like rodents and their 'lek' behaviour especially in feeding sites, watering holes etc, cause greater transmission of pathogens.

Cluster size, time lapse, and mitigation protocol since early cases determine the furthering of conditions in a containment zone. Geographical characteristics coupled with the interactions of social structures based on the area are determinants of the ease of pathogenic transmission. The feasibility of initiating and maintaining containment operations for the better, require consultation with the WHO and must aim to assess the general security of the situation, international support, financial and technical resources, food, water and sanitation, and the national government's willingness to address the situation [43].

Table 1: Factors that determine disease emergence in population

Factors that determine disease emergence in population	
Pathogenic factors	Vectors Amplification hosts Multi-drug resistance Multifactorial-health conditions
Environmental factors	Human animal interactions Climatic conditions Spatial heterogeneity
Demographic factors	Age Multi-factorial health conditions Human animal interactions Population distribution Urbanisation Social behaviour/interaction Population migration

Discussion

Thus, the review has highlighted what hotspots and containment zones are, what do they root from and how they enable controlling of diseases. Mostly, socioeconomic, environmental and ecological factors are attributed to the emergence of containment zones. One aspect to be noted is that containment zones are born out of necessity rather than of environmental or other extrinsic causative pathogenic factors.

A common finding is the emphasis on vectors, amplification hosts, population distribution, urbanisation, and social behaviour/interaction [44], as risk factors for the development of containment zones. Another study, [45] however argues that delayed detection could be due to unfamiliarity with disease and cannot be addressed in the same way as lack of infrastructure, which might be one of the reasons for the development of containment zones.

Age, population density, population movements, multi-factorial health conditions and human animal interactions can also be other factors that determine containment zones (Table 1). Again, it is reiterated that social contact occurs more within homes and workplaces, than in the course of travelling, so population migration can only be conditionally considered as a risk factor [46].

Containment zone formation is tedious enough but the recovery time surpasses this in terms of challenges faced for which rapid action is required. Antiviral medication [47], non-pharmaceutical [48] interventions, dispatching health care workers, consistent surveillance all form a part of outreach protocols and response team motives, to deal with situations alike.

Communication plays an important role [49] in dispatching information and implementing better preventive methods. Local, regional, national and even international public communication must form a support system for the affected and must guide and organise ways of information dispatching to those in the vicinity of containment and buffer zones. This also is a key to international coordination which might bring about a holistic betterment of the affected zones. With greater awareness, the public would also follow stringent practices for protecting oneself including disinfecting household items, using sanitizer, consuming immunity booster and stocking on protective masks and gloves [50,51]. The emphasis of careful determination of the affected zones is exhibited in one such case, in the city of Mumbai, India, where instead of selecting entire districts as containment zones in the face of the extensive spread of the Covid-19 disease, two regions, the Dilshad Gardens and Nizamuddin, are selected as hotspots, making population directed treatment, feasible [52].

Frequent physical reminders on the status of the situation are also necessary incentives to the population. Establishing clear cut exit and entry points, screening procedures, watch on the transit through sea, air, or land, and administering chemoprophylactic doses are necessary aspects of containment operations.

The review is however limited only to the discussion of controlled aspects of a setting, which are at least quantifiable and measurable. It does not highlight abstract factors like the genetic disposition of an individual or the pathogenesis of the disease [53].

Conclusion

The review has assessed possible risk factors, demographic patterns, measures taken in the development of containment zones and has highlighted barriers and deficiencies in a social setting that might necessitate the formation of such zones. Future considerations might as well involve necessary measures to improve outreach across communities and countries, standardize quantification and assessment of disease progression and preparatory efforts to equip nations with resources to handle unfamiliar disease conditions.

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Occlusal Adjustments in Complete Denture- A Review

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Abstract

A complete denture is a removable appliance used when all the teeth have been lost. It needs to be prosthetically replaced. Occlusal adjustment procedure or bite adjustment is performed to remove tiny interferences that keep teeth from coming together properly. The maxilla and mandible relationships must be established during the fabrication of complete dentures. Adjustment of occlusion is necessary to account for inherent errors. This research is seen as scoping literature review. In seeking to identify the relevant literature from the past twenty years, we used common databases such as the Pubmed and Google scholar online websites. 37 articles are found relevant to the topic. 18 articles are reviewed for this study. The obtained articles were later read thoroughly and understood. From this study, it is clear that when placing complete dentures, the occlusion should be corrected, as it is important for speaking, mastication. This occlusal adjustment is necessary to eliminate errors apparent at the try- in stage, correction of occlusal disharmony. This provides a balanced contact between the teeth in the jaw. By comparing with other research and reviews, this review gives a detailed explanation about occlusal adjustments. Further studies have to be done on malocclusions.

Keywords: *Complete denture, edentulous, occlusal adjustments, features, adjustments on proximal side.*

Introduction

Complete denture is a removable appliance, used when all teeth have been lost.[1] It is a tissue supported prosthesis [2]. Occlusal contact means that the cusps present on the proximal side of maxilla will contact with the groove of the mandible [3]. Occlusal adjustment is necessary to account for inherent errors caused by processing changes. Importance in this complete denture is the tooth extraction which may be due to dental caries, periodontal diseases and trauma [4]. This complete denture is mostly used by the old aged people. Also this denture is used for the esthetic appearance. It provides comfort to the patient. It helps in the proper functioning of the oral region.[5] It provides esthetics to the people [6]. The main difficulty in placing the complete denture is, the patient with natural opposing dentition and maxillary retention [7].

Compared to previous research, they have evaluated the occlusal contacts in a completed denture using pressure sensitive sheets.[8] They concluded that, pressure sensitive sheet system is able to pressure the occlusal contact at every contacting point. This method was considered as a useful method for the occlusal analysis [9]. Another study was carried out to investigate the occlusal pressure pattern of complete denture wearers. In this study, they used a computer- based device to measure the occlusal pattern. [10]They concluded that peak ratio is very important for the estimation of occlusal adjustments [11].

The main uses of this complete denture is, it replaces all the missing teeth[12]. As it is a removable appliance, it can be easily cleaned and helps to maintain good oral

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hygiene. Also, it provides esthetics to the patients and helps in the mastication process and phonetics. The main aim of this study is to explore detailed information about the complete denture and occlusal adjustments. This review is expected to help people, who want to know about the complete denture and occlusal contact adjustments.

Materials and Method

This research is seen as a scoping literature review. We did not follow a systematic review or meta analysis. In seeking to identify relevant literature from the past 20 years, articles are collected from the Pubmed and Google scholar online websites. [13] [14] [1]. Total number of articles found related to my topic is 37. From this,18 articles are reviewed in this study. [15] [16] [17].

Articles collected are related to the complete dentures, occlusal adjustments, evaluation of occlusal patterns, removable appliances. Articles related to other categories are excluded for this study. The obtained articles were later thoroughly read and understood.

PROCEDURES DONE PREVIOUS TO INSERTION OF COMPLETE DENTURE

Before placing or inserting the complete denture, roentgen cephalometric recordings should be done.[18] The cephalometric analysis was based on electronic measurements of linear and angular morphological variables. Next is the alveolar ridge reduction, which is more rapid during the first 3 months of denture wear and particularly during the post extraction period [19]. The effects on speech sounds caused by alterations of the oral cavity dimensions with complete denture of different morphology were analysed [20]. The effects of these changes on the relative duration of separate sounds in a word and the patient adaptation period were also analysed [21].[22].

CAUSATIVE FACTORS FOR ERRORS IN COMPLETE DENTURE

The problems may be transient and may be essentially disregarded by the patient. [23]Factors which cause problems are 1. Adverse intra- oral anatomical factors 2. Clinical factors 3. Technical factors 4. Patient adaptational factors. Of these four factors the major is patient adaptational factors [24]. Quality of residual edentulous ridges and quality of new complete dentures predict patients to the use of new complete dentures [25]. Complete denture fractures are more common. These features may be due to material factors and clinical or technical factors [26]

IDENTIFICATION OF ERRORS IN COMPLETE DENTURE

Problems in complete denture can be easily identified by incorporating microchips, a radio based tagging transponder into a complete denture [27]. Inter condylar width and inter- dental width serves as a guide for setting up complete denture. With the help of these widths, problems of complete denture can be identified. [28]. In the majority of the patients, the most complaint in wearing complete dentures is because of technical errors in the denture construction [29].

CORRECTION OF ERRORS IN COMPLETE DENTURE

The combination of laboratory and clinical remount procedures with occlusal corrections enhances the patient's comfort [30]. The errors present in the patient are inevitable [31]. Hence for the comfort of patients, laboratory remounting is an important procedure that needs to be followed as a regular step after processing of each and every denture [32]. Nowadays there is an increase in the interest in computer engineered complete dentures [CECDs]. The advantages of this are reduced number of visits, improved fit & retention of electronic archiving [33]. This reference shows that complete dentures fabricated with a normal and convenient method had significantly large occlusal contact areas compared with the other method [34] [35].

Results and Discussion

From the articles reviewed, study has been done on the efficacy of the occlusal analysis system and peak ratio is useful for the evaluation of the occlusal adjustments. Discussion has been done on the occlusal adjustments and the importance of prosthodontic treatments. But the current review involves only the occlusal adjustments. Parameters have been done for the patients with the disorders. Occlusal pressure patterns are observed using pressure sensitive sheets. Other treatments like the facebow transfer fabrication for occlusal splints

of complete denture have been done. This is done on the basis of biomedical rationale. Analysis of occlusal contacts with the accuracy and reliability has been done. Evaluation of occlusal adjustments for the masticatory process. The treatment involving occlusal adjustments would be the denture restoration. Discussion on the discomfort associated with denture wearers, loosening of dentures.

This current review has explored the effect of dentures and the adjustments made for complete dentures. Literature review has been done for the factors related to chewing efficiency in edentulous patients. And problems with the age related adaptations.

TABLE :1: DESCRIPTION OF INCLUDED STUDIES

S.no	AUTHOR	YEAR	KEY FINDINGS	QUALITY
1	Suzuki Tetsuzya, Kumangi, Hiroshi	1997	Efficacy of new occlusal analysis system	Moderate
2	Kenji Okuma, Shiezgo, Hirani	2004	Peak ratio is used for the evaluation of occlusal adjustments.	Moderate
3	J F McCord, A A Grant	2000	Discomfort associated with the dentures, loosening of dentures and the problems of adaptation.	Strong
4	Alex Koper D D S	1997	Discussion on occlusal patterns and the method of fabrication.	Strong
5	Anthony Au, Iven Klienberg	2016	Evaluation of occlusal adjustments by occlusal pressure pattern in complete denture wearers	Strong
6	Iwayo Hayakawa	2004	About the facebow transfer, fabrication of occlusal splints in complete denture.	Moderate
7	Farah Razakhan, Rabia Ali, Aiman Sheikh	2018	Occlusal considerations in the implant therapy.It is a biomedical rationale.	Moderate
8	Yuriko Komagamine, Manabu Kanazawa	2016	Analysis of occlusal contacts in complete denture with more accuracy and reliability .	Strong
9	Wen Quiao Zhou, Die Liu, Tao Chen	2016	Impressions of complete denture fabrication for the masticatory performance.	Moderate
10	Sandile K Mpoungose, Greta Aimee	2016	Complete denture restoration, a temporary treatment in edentulous patients.	Strong

Conclusion

From this review, it was concluded that all the information reviewed would be expected to help clinicians to know about occlusal adjustments in complete denture. All the discomforts associated with the complete dentures are assessed. Future studies has to be done for the malocclusions related to complete denture.

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Ethical Clearance: As it is a review article so it is not required.

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Discovery of Life in Solid Rocks, on Seabed Inspires New Search for Martian Life - A Review

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Abstract

Life on Mars has been considered a possibility only in the wildest of wild dreams and that too only among scientists. It was considered a far from happening possibility as it wasn't based on the environment of Earth but of a different planet as a whole. Newly found microorganisms could help in finding the proof of life on the planet Mars. If successful, it could help in various ways for our planet, Earth. Researchers have found billions of bacteria live in tiny cracks in volcanic rocks below the ocean floor, more than nine miles below the ocean surface and a further 300 feet below the ocean floor. And they think similar tiny, clay-filled cracks in rocks on or below Mars' surface could be a life containing hub. To find a correlation between the environment on the surface of Mars and that of which is beneath the seabed. A thorough literature search was performed using the database like PubMed, Google scholar, BioRxiv, MESH, Google Cochrane database using the keywords 'Martian surface' and 'rocks beneath seabed' with no date and year restrictions. The language is restricted to English. 16 articles with similar data have been found which were analyzed and have been included in this study. The recent articles discussed in this study help us in gaining further knowledge about the 'relation between Mars and Earth' and how it affects life on earth. We could come to a result that there are slight correlations between the environments of both the planets. This review would also help us be enlightened about how far we humans have come to realize the correlation between life on earth and that of the possibility of Mars.

Keywords : *Microorganisms, seabed, volcanic rocks, environment*

Introduction

Life on Mars has been considered a possibility only in the wildest of wild dreams and that too only among scientists. It was considered a far from happening possibility as it wasn't based on the environment of Earth but of a different planet as a whole.[1] This had brought doubts on the possibility of the research being successful. This review would help us be enlightened about how far we humans have come to realize the correlation between life on earth and that of the possibility in Mars.[2] Researchers have found billions of bacteria live in tiny cracks in volcanic rocks below the ocean floor, more than nine miles below the ocean surface and a further 300 feet below the ocean floor. And they think similar tiny, clay-filled cracks in rocks on or below Mars' surface could be a life containing hub. For around 3.8 billion years, the upper oceanic crust, known as the ocean floor, was continuously formed on Earth. Underwater volcanoes release 2,200 degrees Fahrenheit lava which solidifies into basaltic rock as the hot rock reacts to the depths of the cold oceans. It has been established that hydrothermal vents along the ocean floor support bacteria and other life that turn minerals into energy rather than light. Like Earth, Mars also has a basaltic crust, which was formed four billion years ago. And sub-surface water and methane were found on the

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Red Planet in the last few years.

When the lava from the underwater volcanoes cool, the cracks form creating narrow spaces of less than one millimeter across. They 're packed with mineral-infused clay from millions of years of sediment and accumulation. Bacteria then find a nice home inside them and settle in. These 'clay-filled-crack' solid rocks are considered to be a million years old hence these newly discovered single celled creatures beneath the seafloor are clues of life on Mars.[3] They are such clues which would provide enough information on the existence and continuity of life on Mars[4] and that very existence of life helping the life on earth and also its resources.[5] The bacteria discovered in these tiny cracks of the former volcanic rocks are living proof of survival in the most extreme of extreme environments.[6] The cracks present in the rocks are the homes of a large community of bacteria. Such a large amount of bacteria is present in the community as much as 10 billion bacterial cells per cubic centimeter.[7] Researchers estimate that since these rocks are the home to such a large number of bacteria, it is as dense as the human gut[8] which is approximately 10 billion bacteria per 0.06 cubic inches. Such a large number of microorganisms in such a small space with such extreme conditions is quite baffling.[9] Hence as the possibility and exceptional facts are way beyond what was considered possible, research is very important and required to hook up for life on mars to that on earth.[10] Because all of the above had seemed to be only a mere possibility earlier, and now as it seems possible, search for life on Mars has become one of the top priorities of the current leading nations. It has been entered on the list of important and prioritised research in most developing countries[11] because in case it turns out to be successful, then the resources from Mars can be traded in for that on earth which can hence help in rejuvenation of the resources of earth.[12] Hence it's very much required to draw a connection between the planet Mars and Earth.[13]

Single-celled microorganisms

These newly discovered microorganisms are those single celled creatures which live deep beneath the seafloor where the environment is at such extreme conditions[14] where life wouldn't have been considered possible. The microorganisms seem to have great tolerance to extreme levels hence the microorganisms which live deep beneath the seabed help provide clues about life on Mars.[15,16] To find the correlation between the life on Mars samples from both of the areas are required. To obtain the colonies of bacteria so that it's characteristics can be studied under the microscope, research had to perfect a new method of cutting the rocks into ultra thin slices.[17,18]

Techniques to find correlation

Firstly certain techniques had to be developed to find a correlation between the life on earth and that of Mars. For such interplanet connections, the availability of resources to create such a connection and thus finding proof was difficult.[19] The entire procedure of establishing the correlation seemed quite as close to impossible. Even if proof from the Martian surface had been obtained, the correlation had to be created and hence proof from earth also was required.[20] To help with findings from earth, scientists had to find proof of life which would support the atmosphere of Mars.[21,22] Certain microorganisms, to be more precise certain bacteria were discovered to be living within clay filled solid rocks.[23] To be within such rocks is practically considered a wonder and awe since such rocks have been formed from mostly the lava from volcanoes.[24] These microorganisms should have extremely good resistance to the odds of the exigency of bad environment and temperature. If it is able to withstand such extremities,[25] it also might have the possibility of being able to withstand the temperature of that of Mars.[26]

Proof from both planets

Cutting the rocks into ultra thin slices is very much required to obtain better results and cutting of rocks is very difficult when compared to other specimens.[27] When studied under the microscope, it showed that these microorganisms were very much alive in the tiny cracks preset inside the volcanic rocks.[28] Once these rocks were cut open, to be analyzed under the microscope, it was found that these cracks had aerobic bacteria present within them which underwent processes similar to that of human cells.[29] The microorganisms had processes which were similar to the energy making processes of human beings. This shows that these microorganisms also relied on oxygen and other organic nutrients for making energy to sustain life and the future generations.[30]

Rocks which were assumed to have had microorganisms buried within it were chosen from the seabed and even rocks were collected[31] from the Martian surface using space rovers. These rocks were examined, analysed and also checked for any link between each other so that the study could be confirmed to be worthy to continue. When a connection was tried to be drawn out between the samples (of Mars and Earth's seabed), the condition of the rocks beneath the seabed were found to be similar to that of the Martian rock.

Once the interlink was formed between the rocks on Mars and that beneath the seabed on earth,[32] it had been confirmed that this research is meant to continue as it has great potential and also life on Mars could be checked. After the connection had been formed in this research, the need for its continuation was considered very crucial and important as it could be a breakthrough in science.[33] Hence all of the powerful nations have considered it as their top priority to do further research on. The check for similarity between the rocks would have a great possibility in how the research would help in future rejuvenation of the[34,35] resources of earth.

Requirement of efficient research

Like mentioned earlier hardcore research is required to come up with solid proof of a link between the pattern of life within the two planets.[36,37] If the hypothesis of correlation between the Martian rocks and those of earth prove to be a success, it could help bring earth back to its former glory.[38–40] There can be obstruction to the study such as lack of proper evidence from the surface of Mars, limited access to the depths of the seabeds, vitality of microorganisms.[41] But there is a scope in future for the relation between life on Earth and Mars.[42,43]

Conclusion

Life on a planet other than Earth was believed to be practically impossible earlier. But slowly the level of confidence on the fact of occurrence of any correlation between the planets (Mars and Earth) increased[44] which sparked up the interest of many more institutions and countries to come together and work. In case the hypothesis put forward proves to be right, the phase of life on earth would change. There are remarkable similarities and major variations between the two planets. We might characterize Mars as having a poor relationship with the Earth. We just scratched a couple of sites on its surface though. The man remains unaware of most of its secrets.

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Conflict of Interest: Nil

Ethical Clearance: As it is a review article so it is not required.

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Type of Study: ***Retrospective study***

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A Comparative Retrospective Analysis on Choice of Taper and Preparation Sizes among the Postgraduate Students in Mandibular Molars with and without Periapical Pathosis

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Abstract

Preparation of the root canal system is recognised as being one of the most important stages in root canal treatment. Cleaning and shaping is the crucial phase that eliminates infection. It involves removal of vital and necrotic tissues from the root canal system along with root dentin. The aim of the present study is retrospective analysis on choice of taper and proportion sizes among the postgraduate students in mandibular molar with and without periapical pathosis. It is a single centred retrospective study, data was collected after reviewing 86000 records of the patients between June 2019 and March 2020.Tapers used in the preparation of the root canal system for mandibular molars have been assessed. In this study we observed that significant association between taper of preparation and periapical pathosis ($p<0.05$). Within the limitations of the study, 6% taper has been used for the teeth with periapical pathosis. 4% and 6% taper has been used in teeth without periapical pathosis.

Keywords : *Endodontic Therapy, Mandibular Molars, Periapical pathosis, Root canal treatment, Tapers.*

Introduction

Preparation of the root canal system is recognised as being one of the most important stages in root canal treatment.[1–3] It includes the removal of vital and necrotic tissues from the root canal system, along with infected root dentin and , in cases of retreatment, the removal of metallic and nonmetallic obstacles. It aims to prepare the canal space to facilitate disinfection with the help of irrigants and medicaments.[4,5] Thus, canal preparation is the essential phase that eliminates. Prevention of reinfection is then achieved through the provision of a fluid tight root canal filling and a coronal restoration. Although mechanical preparation and chemical disinfection cannot be considered separately and are commonly referred to as chemomechanical preparation or biomechanical preparation.[6–8]

However, studies have observed that the current instrumentation and irrigation techniques are not completely effective in the elimination of debris and bacteria from the apical third.[9,10] The difficulty in the removal of bacterial debris from the apical third has been attributed to the narrow canal space, the complex canal morphology, inadequate flushing of irrigants and variations in diameter of the root canal.[11–13] The enlargement of the apical area has been advocated to ensure an adequate depth of penetration of the irrigant for better cleansing.[1,4,14] However, the extent of apical enlargement required is a matter of debate. Preparation to larger apical sizes has been suggested by its protagonists to be the most efficacious way of cleaning and disinfecting the canals. Larger apical preparations allow better removal of infected dentin[15–18], enhance the flushing action of irrigants in the apical region and significantly reduce the bacterial load in the root canal

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system.[19–24]

The drawbacks of larger apical preparations include undesirable deviation from the original shape of the canal, weakening of the root and procedural complications like ledge formation, transportation and perforation.[25–27] The aim of the present study is to retrospectively analyse on the choice of taper and proportion size among the postgraduates students in mandibular molars with and without periapical pathosis.

Materials and Methods

The following single centred retrospective study was performed, data was collected from reviewing patients records and analysed the data of 86000 patients between June 2019 and March 2020. After reviewing all the records,1326 patients records were selected who underwent root canal treatment in mandibular molars. All the records were cross checked with the help of the radiographs by other reviewers to eliminate the sampling bias. Individuals excluded from this study were, fractured instruments, 2% taper, anteriors, premolars, upper molars. Inclusion criteria was 4% taper, 6% taper, lower molars, multi visit RCT, single visit RCT.

Considering single centred retrospective study, multiple operators are involved in treatments to reduce selection bias. The parameters assessed in the study are age group, gender, taper, multi visit and single visit. The collected data was entered in the excel sheet. After grouping, the parameters data was copied into the SPSS software and statistical analysis was done.

Statistical Analysis

Statistical analysis was done using SPSS (SPSS inc., version 23, chicago,IL, USA Norman H. Nie, Dale H. Bent, C. Hadlai Hull). To describe the significance between two groups, Chi square test was used where p-value < 0.05 was considered statistically significant.

Results and Discussion

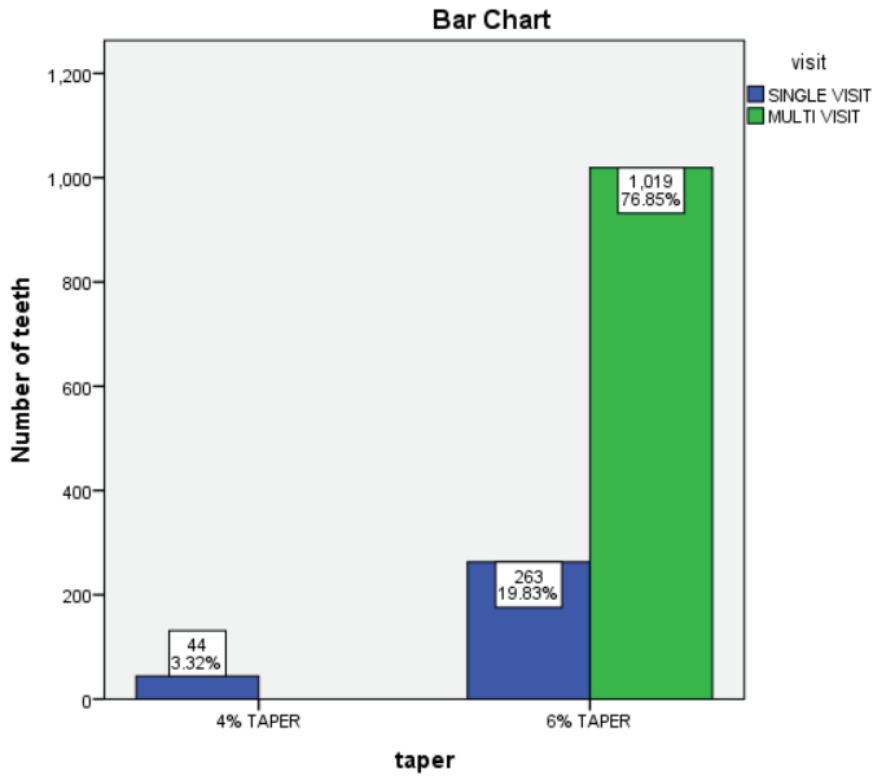
Frequency tables were used to describe the variables included in the study. Data on age included for the present study is represented in (Table 1), among the included age groups most of them were old aged (51-60) ranging around 44.7%. The association between 4% taper and 6% taper with single visit, multi visit is shown in (Table 2 and chart 1). The relation between tooth and taper is shown in (Table 3 and chart 2) where the mandibular 2nd molar was prepared only with 6% and the 1st molar was prepared with 6% and 4%, but the maximum was with 6% taper. In this study, we contemplate that there is significant association between taper of preparation and periapical pathosis (p value <0.05).

Table 1 : Frequency of age distribution of patients undergoing root canal treatment, which shows that 51-60 years have undergone more number of root canal treatments(44.7%) followed by 41-50 years (23.7%).

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18-30	192	14.5	14.5	14.5
	31-40	227	17.1	17.1	31.6
	41-50	314	23.7	23.7	55.3
	51-60	593	44.7	44.7	100.0
	Total	1326	100.0	100.0	

Taper	Visit		Chi square value	P value
	Single visit	Multi visit		
4% Taper	44	1019	151.058	.000
6% Taper	NIL	263		

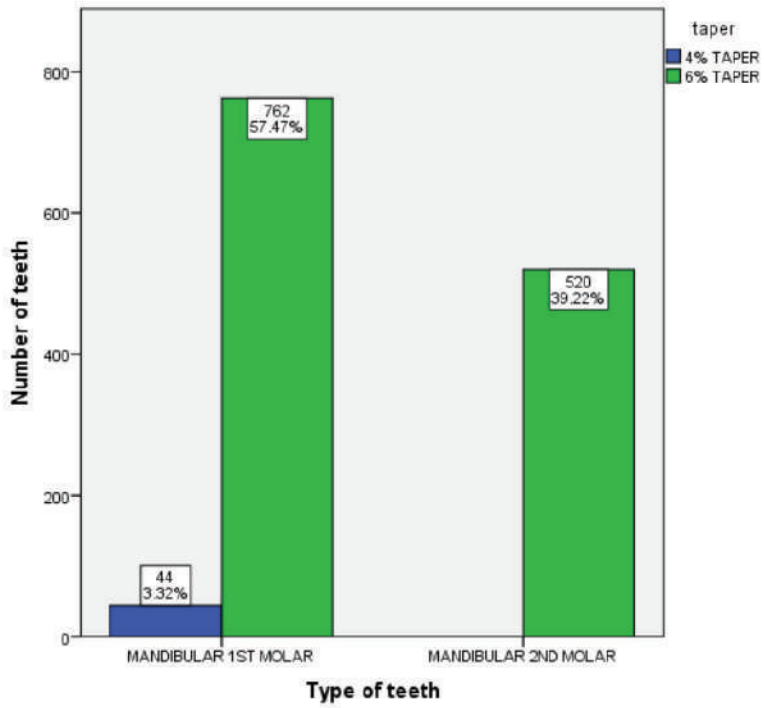
Table 2: This table represents association between 4% taper and 6% taper and vist where single visit is teeth without periapical pathosis and multi visit is teeth without periapical pathosis. However Chi square test, p = 0.000, significant difference seen (p<0.005)



Bar chart 1 - This bar chart represents the association between taper used in multi visit(teeth with periapical pathosis) and single visit (teeth without periapical pathosis) , X axis represents the taper used and Y axis represents the number of teeth ; blue color depicts single visit (without periapical pathosis) and green color depicts multi visit (with periapical pathosis). The chart shows 4% taper was used in single visit that is teeth without periapical pathosis (3.32%); 6% taper was used in multi visit that is teeth without periapical pathosis (76.85%) and in single visit (19.83%). However Chi square test, p = 0.000, significant difference seen (p<0.05)

Taper	Teeth		Chi square value	P value
	Mandibular 1st molar	**Mandibular 2nd molar**		
4% Taper	44	NIL	29.361	.000
6% Taper	762	520		

Table 3 : This table represents association between mandibular 1st molar, mandibular 2nd molar with 4% taper and 6% taper. However Chi square test, p=0.000, significant difference seen(p<0.05)



Bar chart 2 - This bar chart represents the association between teeth and taper, X axis represents type of teeth that are mandibular 1st molar, mandibular 2nd molar and Y axis represents the number of teeth; blue color depicts 4% taper and green color depicts 6% taper. The chart shows in mandibular 1st molar 57.47% used 6% taper , 3.32% used 4% taper and in mandibular 2nd molar 39.22% used 6% taper.However Chi square test, p=0.000, significant difference seen(p<0.05)

The present study was mainly targeted in evaluating the choice of taper preferred by endodontic postgraduates for the endodontic therapy in mandibular first and second molars. The reason for choosing only the mandibular molar is because the chances of possible iatrogenic events are more [28],the procedure and protocol for treating mandibular molars is complex as compared to maxillary molars. In regards to the endodontic literature the shape and apical preparation sizes chosen for a case based scenario is different. According to the present literature evidence, increased tapers and preparation sizes have proven to induce more healing than compared to the counterpart [29] the reality is shape should be optimal to clean more, given that no optimal large size has ever been defined.[30] But, the present trend with introduction of sophisticated technology, the enhanced healing rates were observed in cases with minimally compromised dentin.

The results of the present showed that overall choice of taper in both the visits was 6%. However,Endodontists also choose 4% taper in single visit cases mainly aimed to conserve the root dentin and none of the operators choose 4% taper for multi visit cases. So, statistically results showed the favourance of operators to 6% taper ($p < 0.05$) as compared to 4% tapered preparations in both the protocols. So, our study was primarily aimed at evaluating the choice of taper for single or multi visit and to assess any choice of taper and its significance in periapical pathosis. The choice of taper in a clinical scenario is multifactorial and declared to be choice based rather than evidence based. One cannot solely rely completely on taper for enhanced healing. Many in vitro literature has shown that an increase in taper often leads to increased failure of endodontically restored teeth[29,31]. Ideally a single visit root canal treatment should concentrate on the pulpal debridement and multi visit protocol has to concentrate on the removal of maximal amount of infected dentin with aiming in bacterial biofilm reduction. Literature has shown its favourance towards the increased tapers as compared to reduced tapers[31]. So, the views of the operators of the present study were in concordance with the available literature evidence.

Primarily when evaluated, there is still an ambiguity in preferable taper and apical preparation size advisable for specific cases although one cannot generalise a standardised common taper and preparation size for all cases. Especially, a clinical decision on specified taper and preparation sizes for a specific tooth undergoing endodontic therapy varies from a clinical condition, canal curvature and intricate root canal anatomy and ultimately based on the operators decision. But, compared to the operators choice and experience the decision should be taken based on the literature evidence it is stated that, increased apical preparation sizes showed improved healing outcomes on clinical radiographic evaluation.[31–35]

Within the limited information currently available, the 6% taper was used in all the cases with periapical pathosis. It is unknown if in root canal systems with previous vital pulps versus necrotic pulps with no noticeable periapical pathosis. The enlargement of the apical size would result in better healing outcome. However, vital pulps or necrotic pulps with absence of periapical pathosis have better prognosis.[12,36–38] Data from systematic reviews state that

Our results contrary to the findings of previous studies that evaluated the effect of the apical size of canal preparation on treatment outcome to date.[39–41] Hence the use of common taper in all teeth cannot be justified with a small sample. Present study was limited in evaluating the choice of taper among the cluster of endodontists in a university set-up. Although preliminary data was obtained on the choice of taper in different visits, Future studies should better analyse the effect of taper used on the healing outcome with longer follow ups.

Limitations

The limitations of this study was, it has small sample size, cannot be generalized to all teeth using this data. The period of evaluation was also very less.

Conclusion

Within the limitations of the study 6% taper has been used for the teeth with periapical pathosis 4% and 6% taper has been used in teeth without periapical pathosis. 6% taper was the preferred choice among endodontists, reason for choosing this taper was improve the flow of irrigant especially at the apical third.

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Conflict of Interest: Nil

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Atraumatic Restorative Treatment for Managing Dental Caries - A Review

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Abstract

The study aims to create awareness about the newly developing atraumatic treatment and its benefits. Atraumatic restorative treatment is an alternative treatment that is used for the removal of caries using hand instruments only, so therefore no electricity or anaesthesia is required and the pain experienced is minimum. This technique is very useful for children, elderly, special needs patients and patients who feel fear and anxiety about dental treatment as in atraumatic restorative treatment that is more conservation of tooth structure, minimise in trauma and so there is reduced amount of pain due to small cavity preparations. Atraumatic restorative treatment is less painful and is more patient-friendly than that of conventional caries treatment. The fundamental instruments for ART are mouth mirror, explorer, pair of tweezers, dental hatchet, small and medium-size excavator, spatula, amalgam carrier/applier. ART uses only hand instruments for opening/enlarging the cavity and for removing carious tissue. The amount of carious tissue that should be removed depends mainly on the cavity depth. In cavities of shallow and medium depth, carious tissue is removed up to firm dentine. In deep/very deep cavities, in which there is no sign of pulp exposure, pulp inflammation and/or history of spontaneous pain, some soft dentine can be left in the pulpal floor/wall to avoid pulp exposure. This method of treatment is more useful for people in underexposed areas where they are also able to maintain good oral health.

Keywords: *Atraumatic restorative treatment, instruments and material consideration, conventional treatment, the survival of ART, advantages of ART.*

Introduction

Atraumatic restorative treatment is an alternative treatment that is used for the removal of caries using hand instruments only[1]so, therefore no electricity or anaesthesia is required and the pain experienced is minimum[2]. Originally atraumatic restorative treatment was created for the use in developing since it doesn't require electricity or sedation[3]. All the more as of late, Atraumatic restorative treatment has gotten progressively acknowledged in evolved nations due to its atraumatic approach compared to the pressure and torment experienced by patients [4,5]. This technique is very useful for children, elderly, special needs patients and patients who feel fear and anxiety about dental treatment as in atraumatic restorative treatment that is more conservation of tooth structure, minimise in trauma and so there is reduced amount of pain due to small cavity preparations.[6]

Atraumatic restorative treatment is less painful and is more patient-friendly than that of conventional Caries treatment.[7] Many researchers have shown that the ART approach is more effective in managing single surface cavities in both the deciduous and permanent teeth. There is no difference in survival rates between single surface

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atraumatic Restorative treatment restorations when compared to amalgam restorations in the permanent dentition[8]. The surface wear of ART reclamations utilising high consistency glass Ionomer following two years is slow. Workmanship sealants utilising High consistency glass Ionomer are held longer than ART sealants utilising low thickness glass ionomer following three years. ART consists of removing the decayed tissue using hand instruments and then restoring with a good adhesive material usually high viscosity glass ionomer cement[9]. Pain is absent so local anaesthesia is hardly needed and use of rotary instruments has minimised patients anxiety and discomfort. Studies have proved that ART is a more friendly approach when compared to other restorative treatments. The ART restoration took less time to be done than that of conventional treatment.[10]

Glass Ionomer cement, they are a constant trend in the market and they fulfil many functions and also in aesthetic requirements. They are inexpensive when compared to resin composite[11]. In Atraumatic restoration by powder to the liquid ratio we can get high viscous or condensable GIC, that has high mechanical properties as compared to traditional GIC that was developed for Atraumatic restorative treatment.[12] GICs the material of decision for ART because of its physical and synthetic properties. Such properties incorporate it's grip to dental structures, biocompatibility, substance set response and fluoride discharge-take up which adds to GICs preventive character. One of the significant downsides of GIC is the moderately low crack quality and higher occlusal wear rate in contrast with amalgam and present composite material[13]. Recent clinical trials have proven that single surface ART restoration using high-velocity GIC in both primary and permanent dentition has a higher survival rate than that of medium viscosity GIC Casein phosphopeptides (CPP) stabilize amorphous calcium phosphate (ACP), localize ACP in dental plaque[14]. Calcium phosphopeptides help to remineralise surface lesions in enamel[15]. Dental erosion is caused by acid attacks, either from extrinsic sources such as consumption of acidic beverages, occupational acid exposure such as wine tasters, and workers from battery industries or intrinsic sources of reflux of gastric acid into the oral cavity in conditions such as gastroesophageal reflux disease, anorexia nervosa, and bulimia.[16]

The standards of the ART strategy are not new. One of the foundations of the customs administration of a patient with various caries sores is to balance out the Caries procedure by unearthing all the sores and putting zinc oxide-based dressing[17]. The ART method depends on logical discoveries and supporters that this training is done appropriately; therefore the time engaged with doing a solitary rebuilding might take up to 20 minutes. The teeth are then re-established using a cutting edge, fluoride filling material. Most adults have higher dental anxiety during their childhood[18], so those people have not fully benefited from good dental care and they have all undergone more of its extractions rather than restorations. ART technique can be useful to this group of people. Patients with medical or physical disability undergo treatment with local anaesthesia[19]. The ART technique could be suitable for providing care for these patients. The procedures can be carried out in patients' homes, hospital or dental chairs. Use of these techniques in patients' homes will require minor adaptations like provision of the suitable light source. Some of the children may prefer anaesthesia for all dental treatment this may be due to the anxiety of fear or previous bad experience. Utilising of ART procedures for the treatment of youngsters may assist with accomplishing helpful, preventive and mental consideration as the kid to figures out how to conquer his or her feeling of trepidation of customary dental treatment[20]. ART appropriately utilised would give a substantially more worthy prolong to dental consideration than the ordinary infuse, drill and fill philosophy. Thus ART has a definite place in modern clinical practice as most of the practitioners of following this technique whenever there is a temporary restoration if needed[21]. However the success of the treatment requires additional steps like adequate caries removal, and training in the use of the technique is essential to avoid claims of supervised neglect by not carrying out the procedure thoroughly[22]. Recalling a review of the patient has to be undergone. Nervous patients who benefit from this technique gradually introduced to conventional treatment techniques.[23]

Materials and Methods

A systematic review of the scientific literature was done in preparation of the manuscript. The systems and databases were searched for relevant articles from Pubmed and Google scholar from the year 2000.

Databases of intended journals were searched for keywords such as Atraumatic restorative treatment, instrument and material consideration, the survival of ART, advantages of ART etc. Exclusion criteria were case reports, review and studies in other languages.

Discussion

Dental caries is a sugar dependent ailment that harms tooth structure and because of loss of mineral segments, may, in the end, lead to cavitation. Dental caries is the most predominant sickness worldwide and is viewed as the most significant weight of oral well-being. Ordinary treatment (drill and fill) include the utilisation of rotary burrs under sedation[24]. The requirement of electricity, costly handpieces and profoundly prepared dental well-being workforce may restrict access to dental treatment, particularly in immature areas.[25] To beat the confinement of ordinary treatments was grown for the most part for treating Caries in kids living in underserved territories where assets and officers, power and prepared labour are constrained[26]. ART is a minimally invasive approach that involves the expulsion of tissues using hand instruments, usually without the use of anaesthesia and electricity-driven equipment, and restoration of the dental cavity using an adhesive material. ART is perfectly aligned with modern concepts in healthcare, with adequate maximal effort regarding preventive approach and minimally invasive procedure. Also, ART has the other following advantages, the use of easily available and inexpensive hand instruments rather than more expensive electrically driven dental equipment, conservation of sound of tooth tissue through chemical adhesion of glass ionomers; limitations of pain, minimising the use of local anaesthesia, and low-cost.[23] As a result; many people living in less-developed areas can receive oral care using ART. Moreover, ART is also comfortable for patients with physical disabilities. Although initially developed to provide restorative dental treatment in areas of difficult access, ART or modified ART techniques are being increasingly introduced into dental clinics in industrialised countries.[20] ART may reduce pain experienced when compared with conventional treatment[27].ART is used to remove caries from the hard dentin caused by Streptococcus Mutans and lactobacilli. Chlorhexidine-gluconate is used to reduce the S. Mutans and lactobacilli [28,29].

The fundamental instruments for ART are mouth mirror, explorer, pair of tweezers, dental hatchet, small and medium-size excavator, spatula, Amalgam carrier/applier. To improve working permeability, an exceptional light source is fixed to a pair of spectacle frames that is powered by a rechargeable battery source. This unit likewise allows amplifying the glass to be connected. The essential materials are gloves, cotton rolls and pellets, glass Ionomer material, Vaseline, wedges, plastic strips and mortar.[30] Like other treatments ART also requires a proper patient to an operator position. Several devices have been developed and one among them is the light, weight cushion headrest that is attached to a short end of the table combined with the foldable cushion for the comfort of the patient receiving the treatment. The tooth or the teeth to be treated is isolated with a cotton wool roll. Enamel hatchet replaces the bur it is used to widen the entrance of the lesion by placing at the entrance and rotating backwards and forwards[31]. By rotating the instrument the unsupported enamel will be removed by creating an opening for the small excavator to enter depending upon the size of the cavity. A small or medium-sized excavator is used to remove caries. To remove calcified canal small hand files such as a size 6, 8 or 10 K-file are used to negotiate further into a system when coronal preparation has taken place[32]. Alternating between rotary and hand instrumentation while constantly bathing the pulp chamber of the tooth can expedite negotiation to the apex.[33] Glass ionomer cement is mixed and filled into the cavity; the mixed material is inserted using the flat end of the applier and plugged into the corners of the cavity either side of the excavator or with a ball burnisher.[34] Avoid air bubbles and the material is also placed in the pit and fissures. Vaseline is coated over the glove finger to prevent the glass ionomer from sticking to the glove. Place the finger over the mixture, apply pressure for a few seconds and remove the finger. [35] Treating and restoring anterior teeth is of great challenges nowadays for dentists because of aesthetics and now it is made easier and treated successfully with porcelain laminate veneers[36]. In ART procedure if there is any case of dental avulsion the doctors will not have any other way of storing the teeth rather than natural products like milk, coconut water green tea extract. [37]Intracanal medicaments are used for the essential step in destroying the bacteria in root canals; however, in modern endodontics, shaping

and cleaning may be assuming greater importance than intracanal medicaments as a means of disinfecting root canals. Until recently, formocresol and its relatives were frequently used as intracanal medicaments.[38]

Dental caries has been considered the most common global disease. Conventional methods involve the use of electric drills to clear away decayed areas of the tooth before filling. Local anaesthesia is normally injected to prevent pain during the procedure. Conventional treatments require highly trained dental health personnel, access to electricity, appropriate tools are more expensive[39]. All these factors may limit access, especially in underdeveloped regions. ART is used for managing dental decay, that involves the expulsion of decay tissues using hand instruments, without the use of sedation and electrical equipment and rebuilding of the dental cavity with an adhesive material, glass ionomer cement, composite resin, resin-modified glass ionomer cement. [40,41]Glass ionomer cement is the dominantly used restorative material used for ART. GIC restorative materials have advantages such as the ability to bond chemically to enamel and dentin, biocompatibility with pulpal tissues less potential to induce recurrent the least, inhibition of enamel demineralisation good cavity seal, ease of use and low-cost[42]. ART utilizes just hand instruments for opening/amplifying the pit and for expelling carious tissue. The measure of carious tissue that ought to be evacuated relies fundamentally upon the cavity depth. In cavities of shallow depth, the expulsion of carious tissue is up to firm dentine. In deep cavities, we're there is no sign of pulp exposure, pulp inflammation or any history of spontaneous pain, some amount soft dentine is left back in the pulpal floor/wall to avoid pulp exposure.[43,44]

The advantage of Atraumatic of ART is the easy availability and relatively inexpensive and instrument rather than expensive electricity is driven by dental equipment[17]. It is a biologically friendly approach involving the removal of only decalcified tooth structures, which result in relatively small cavities and conserve sound tooth tissue, there is a limitation of pain thereby minimising the need for local anaesthesia[45]. It is a straightforward and simple infection control practice without the need to use sequentially autoclave handpieces. The chemical adhesion of the glass ionomer that reduces the cut sound tooth tissues for retention of restoration material; the leaching of fluoride from the glass ionomer prevents secondary caries development. and probably [46]remineralise carious dentin. The ART technique is a non-threatening oral procedure. This characteristic has a great advantage of making oral care more popular among the population, in particular, the young. Fear inducing situations caused by dental equipment are not involved, and there is no sound of a drill or from suction equipment. The maximum number of instruments in the mouth at any one time is similar to that used for an oral examination, the mouth mirror in one hand and a working instrument in the other, thus ART is patient-friendly.[47,48].

Conclusion

The greater part of the world's population has no access to restorative dental care. One of the main obstacles is the easy traditional manner of treating caries, which relies on electricity-driven equipment. The basic concept of the ART technique is the removal of the calcified dental tissues using only readily available hand instruments, following the modern concept of cavity preparation, and the use of a high technology adhesive restorative material. ART is a technique that has the potential to make oral care more available to a larger part of the world population than before.

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Correlation of Dental Fluorosis with Dental Caries among Adult Population Attending A Private Dental College in Chennai

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Abstract

Fluoride is known as a double edged sword, for both its anticariogenic activity and dental fluorosis in excessive exposure. Results on whether dental caries and dental fluorosis are positively correlated or negatively correlated vary among different researches, thus making it an unsolved area. Hence this study aims to correlate dental fluorosis to dental caries among the adult population attending a private dental college in Chennai.The study includes 258 participants aged 18 years and above, with dental fluorosis. Recorded data was retrieved from patient records in Saveetha Dental College. Record details related to the number of carious lesions present was analysed and Dean's Fluorosis Index (1942) was used to assess the severity of fluorosis among the study population. Descriptive statistics and Pearson correlation were used to analyze the data. Among the participants, 36.4% had carious lesions ranging between 2-3, followed by 27% having more than 4 carious lesions. Mild form of fluorosis was seen among 34.8% of the study population, 9.3% experienced a severe form of fluorosis. Weak correlation between dental caries and dental fluorosis was found at r = 0.03. For the study population, weak correlation between dental fluorosis and dental caries were found, though prevalence of fluorosis was high among the participants. Given the prevalence of fluorosis, defluoridation of water should be considered as a priority.

Keywords - *endemic fluorosis, fluoridation, dental caries, adult, tamil nadu*

Introduction

Dental fluorosis also called mottling of enamel is a developmental disturbance of enamel caused by excessive ingestion of fluoride during tooth development. For fluorosis to develop, the excessive exposure to fluoride must occur during the period of tooth formation, as it will interfere with the ameloblast activity producing a defective matrix causing deposition of calcium fluorapatite crystals. One of the principal reasons for dental fluorosis is the result of drinking water drawn from ground water sources containing a high fluoride content [1] usually more than 2.0 ppm [2,3]. Dental fluorosis manifests as white opaque striations running across the teeth in milder forms whereas advanced stages appear as stained pitted, porous enamel prone to wear and fracture. The reason fluorosis is known as a double edged sword is because there is a strong link established between carious lesions and fluoridation where fluoride gels, fluoride based sealants [4-6], pastes, varnished were proven to prevent dental caries from 23-36% as observed in populations compared to placebo [7], but in excessive quantities causes dental fluorosis and in extreme cases, skeletal fluorosis.

Dental caries is an irreversible [8-10], multifactorial disease of the oral cavity [11] caused mainly by microbial plaque adhesion [12] and local factors like deleterious

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habits [13], poor oral hygiene, salivary gland dysfunction [14] raising ultimately causes problems in mastication leading to nutritional problems [15,16], speech, daily activities [17] and presents as a global burden of oral disease. Evidence suggests fluoride based sealants and such products have been preferred to prevent dental caries [18].Many reports suggest caries prevalence tends to decrease with increasing fluoride level, thus indicating a negative association between fluoride and caries [19] , however a few studies report a positive correlation between fluoride and dental caries as well, especially in high fluoride areas [20]. Due to scarcity in studies relating to these parameters and the varying results, the relationship between them is still undecided.

Fluorosis is worldwide in distribution and endemic in at least 25 countries. As of the data in the year 2014, the population at risk as per population in habitation with high fluoride was 11.7 million. States like Rajasthan, Gujarat, Andhra Pradesh are the worst affected states, while Tamil Nadu, West Bengal, Uttar Pradesh, Bihar, Assam are mildly affected states [21] . Even though prevalence studies related to dental caries and fluorosis are plenty especially among young population, there is a scarcity of research done to correlate dental caries to dental fluorosis and since parts of Tamil Nadu are fluoride endemic areas, this study was designed and conducted to assess the correlation of dental caries to dental fluorosis among adult population, attending a private dental college in Chennai. The objectives of the study are to determine the caries prevalence, to determine the severity of dental fluorosis, to correlate dental fluorosis with dental caries among study participants.

Materials and Method

A retrospective study was done by evaluating and analysing 258 patient case records visiting a dental hospital from August (2019) - January (2020) with dental fluorosis. Prior to the start of the study, ethical approval number (SDC/SIHEC/2020/DIASDATA/0619-0320) was obtained from Scientific Review Board, Saveetha Dental College, SIMATS. Data such as age, gender, number of carious lesions, severity of fluorosis were documented. The validated, reliable tool, Dean's Fluorosis Index 1942 was used for scoring. The scoring method used is, 0 - Normal, 0.5 - Questionable, 1 - Very mild, 2 - Mild, 3 - Moderate, 4 - Severe. Patient records with age 18 and above with fluorosis were included in the study. Patients under special care and incomplete case records were excluded from the study. The 258 patient case records were reviewed and cross verification was done through intraoral photographs by the examiners. The caries status was recorded as the number of carious lesions present. The data thus collected, was entered in excel sheet and imported to IBM SPSS Version 23.0 for statistical analysis. The data was analyzed using descriptive statistics and Pearson's correlation. Statistical significance was set at $p < 0.05$

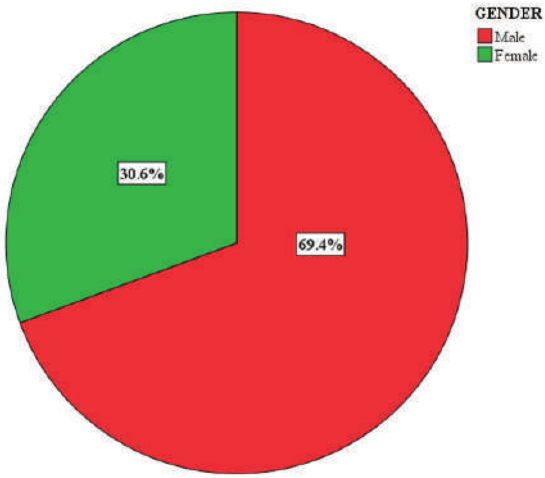


Figure 1: The pie chart represents the distribution of study participants based on gender, where 69.38% were males and 30.62% were females, thus showing a male predominance in the current study population.

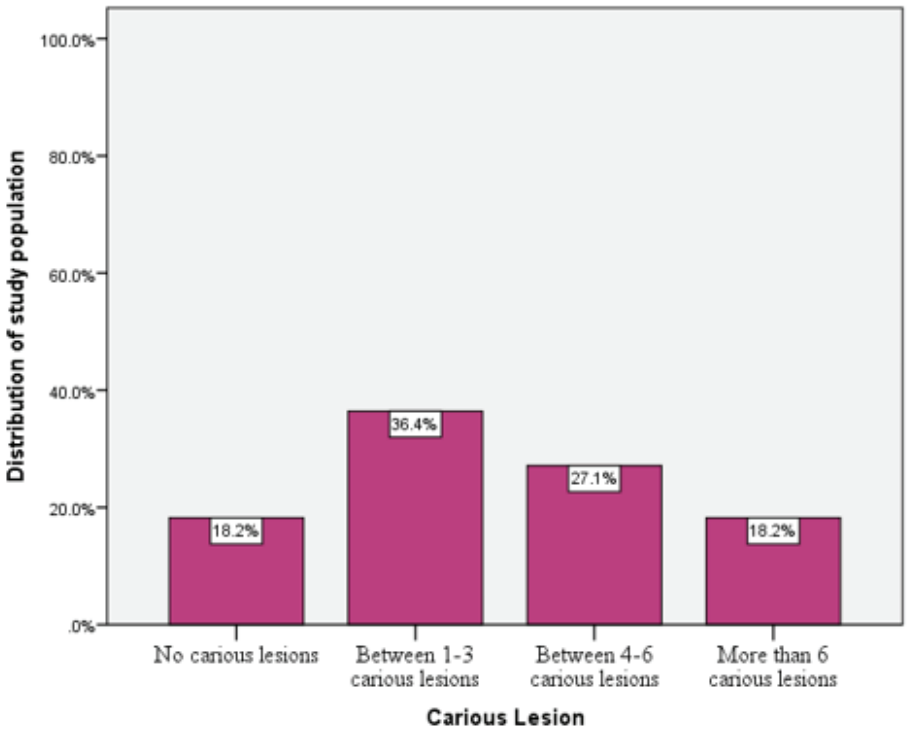


Figure 2: The Bar Graph represents the distribution of study participants based on the number of carious lesions. X axis represents the number of carious lesions and Y axis represents the percentage distribution of participants based on number of carious lesions. Among the study population, the majority of the population (36.4%) had 1-3 carious lesions.

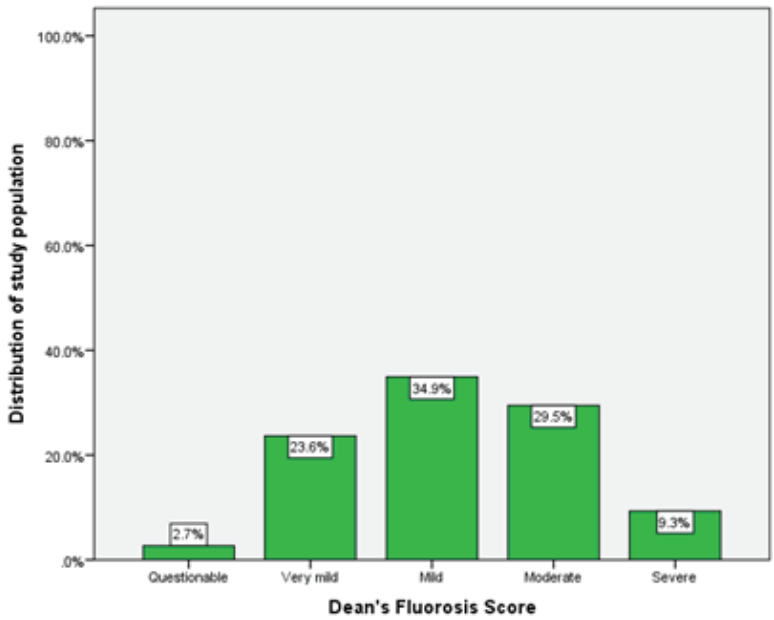


Figure 3: The bar graph represents the distribution of participants based on the Dean's Fluorosis Index. X axis represents the severity of fluorosis based on Dean's Fluorosis Index criteria. Y axis represents the percentage distribution of participants based on severity of fluorosis. Among the study population, the majority of the participants (34.9%) had mild fluorosis. Hence, a mild form of fluorosis was prevalent among the study population.

Table 1: The table represents the correlation between Dean's Fluorosis score and carious lesions. Pearson correlation test was used; *correlation coefficient (r) - .035; p value - 0.5; hence statistically not significant.

Variables	Pearson correlation value (r)	p- value
Dean's Fluorosis score and Carious lesions.	.035*	0.5

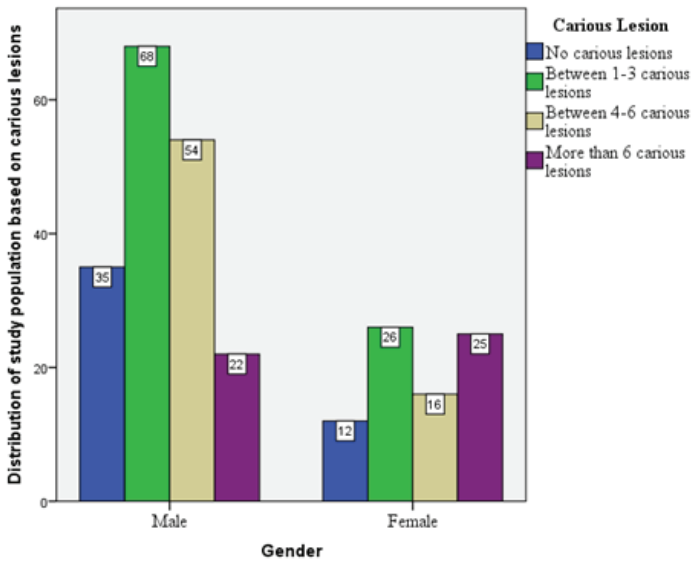


Figure 4: The bar graph represents the association between gender and carious lesion. X axis represents the genders, male and female. Y axis represents the distribution of study population based on carious lesions. Prevalence of dental caries in total was more in males (55.8%) as compared to females (25.8%). Chi square test was used to determine the association and it was found to be statistically significant. Pearson Chi square value = 14.218, df = 3, p value = 0.003, hence statistically significant.

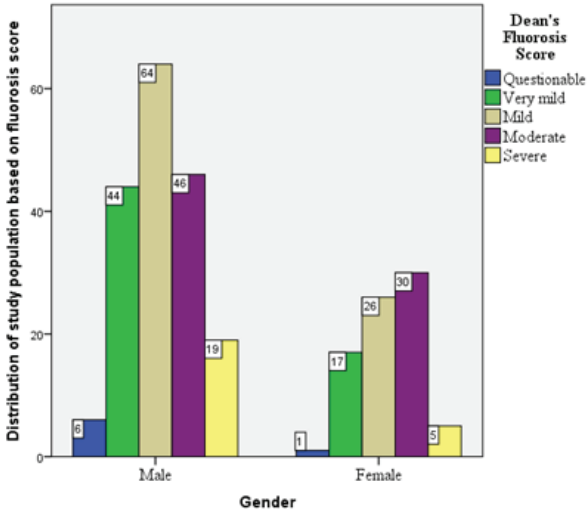


Figure 5: The bar chart represents the association between gender and fluorosis. X axis represents the genders, male and female and Y axis represents the distribution of study population based on fluorosis score. Chi square test was done and severe form of fluorosis was seen more in males (7.3%) as compared to females (1.9%), there was no significant association observed. Pearson Chi square value = 5.110, df = 4, p value = 0.2, hence not statistically significant.

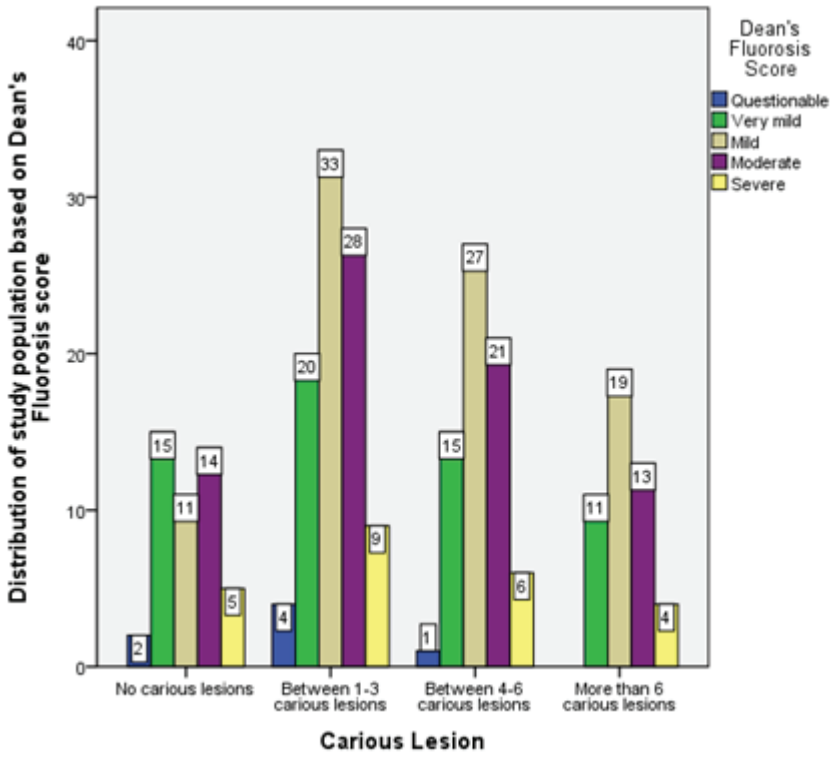


Figure 6: The bar chart represents the association between dental fluorosis and carious lesion. X axis represents the carious lesion and Y axis represents the distribution of study population based on Dean's fluorosis score. Chi square test was done, over all mild forms of fluorosis was more prevalent (70.6%) with regard to carious lesions but no statistical significance was observed. Pearson chi square value = 7.363, df = 12, p value = 0.8, hence not statistically significant.

Results and Discussion

The study comprises 258 participants, out of which 69.4% were males and 30.6% were females, as seen in Figure 1. The mean age of the participants were 28.0±8.8. The distribution of participants according to number of carious lesions shows 36.4% had between 1 - 3 number of carious lesions, 27.1% had between 4-6 number of carious lesions, 18.2% of the study participants had more than 6 numbers of carious lesions and similarly 18.2% had no present carious lesions (Figure 2). Figure 3 depicts the distribution of participants according to Dean's fluorosis score, which reveals among the study population, 34.9% had mild degrees of fluorosis, followed by 29.5% having moderate fluorosis, very mild fluorosis was seen among 23.6% of the participants, 9.3% had severe degrees of fluorosis and about 2.7% had questionable degrees of fluorosis. A statistically significant association (p = 0.003) was found between gender and carious lesions (Figure 4). Among the study population, those who had questionable fluorosis, only 4 of them had carious lesions between 1-3, among the 61 participants who had very mild fluorosis, around 20 of them had carious lesion between 1-3, followed by 15 of them having no carious lesion and 15 having between 4-6 carious lesions, and 11 of the participants had more than 6 carious lesions. About 90 participants had mild fluorosis, out of which 11 had no carious lesions, 33 had carious lesions between 1-3, 27 participants had carious lesions between 4-6 and more than 6 carious lesions were seen in 19 participants. In the current study, around 76 participants had moderate fluorosis, out of which 14 experienced no carious lesions, 28 of them had carious lesions between 1-3, 21 participants had carious lesions between 4-6, more than 6 carious lesions were seen among 13 participants. Among the 24 participants who had severe forms of fluorosis, 5 had no carious lesions, 1-3 carious lesions were seen among 9 of them, 6 participants had between 4-6 carious lesions

and 4 participants had more than 6 carious lesions. The association between dental fluorosis and carious lesion was found to be statistically insignificant (Figure 6). Pearson correlation revealed weak non significant correlation between carious lesion and fluorosis score (r = 0.03) as shown in Table 1.

Fluoride continues to be the cornerstone of caries prevention programs, that's why dental caries and fluorosis have been studied together in many researches aiming to seek associations between these two. There still exists a debate whether dental fluorosis increases, decreases or has no effect on risk of caries [22,23]. This study was conducted among the adult population attending a private dental college in Chennai, seeking treatment. The caries prevalence was high in this current study at 81.6%, similar studies reported high prevalence of caries among adults, similar to Patro BK et al 's study [24] which reported caries prevalence of 82.4% among adults, also the caries prevalence in Tamil Nadu is high in even infants and children as reported in a study [25] . The findings in the present study were found to be lower than that reported in WHO Oral Health Country Profile which was 94% [26]. However, various studies have reported lower prevalence of caries among different populations [27]. The variance in the study results could be attributed to factors like geographical location, age difference, cultural and dietary differences.

In the current study, 34.9% of the study participants had mild fluorosis, a similar finding was reported by Idon PI et al [28] where 32.1% participants reported mild fluorosis. A contrasting finding of 18.5% mild fluorosis was found in a study done by Sebastian ST et al [29], which coincides with the findings by Chaudhry M et al [30]. Dental fluorosis is mainly because of the concentration of fluoride in drinking water which varies with geography, hence the reason for contrasting results.Tamil Nadu is one of the 19 states with high fluoride contamination in drinking water, varying from 1.0 - 48 mg per litre [31]. A weak correlation between dental fluorosis and dental caries (r = .035) was reported in our study, which was contradictory to the findings by Grobleri SR et al [20] , Woondwossen F et al [32] , whereas similar findings to our studies have been reported as well by other studies [33]. In the current study, a statistically significant association was observed between gender and carious lesions, similar to other studies [34,35]. However, no there was significant association between gender and fluorosis, contrary to other studies [36] which could be attributed to the varying levels of fluoride in water and is a geographical factor. The retrospective nature of the study is a limitation, also since it was done in a hospital setting, the result might not be truly representative of the general population. Dentists are distributed throughout a community and they can be a part of an effective surveillance network [37, 38] to conduct more studies on this topic, so as to gather more evidence to solve this uncertainty regarding the correlation between dental caries and dental fluorosis.

Conclusion

Within the limits of the study, the result revealed a weak correlation between dental caries and dental fluorosis. Further studies should be conducted among larger populations to make the evidence stronger.

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Conflicts of Interest - Nil

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Preference of Self Etch Vs Total Etch among Dental Practitioners in India- A Survey

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Abstract

Buonocore introduced Adhesion into dentistry 1955. According to the preference of the practitioners, each generation of adhesive was replaced by the next generation which overcame the disadvantages of the previous generation. Now, the adhesive which is in use are Self Etch, Total etch Adhesives and Resin modified approach as it has unique properties of self adherence to the tooth. The concept of total etching or etch and rinse technique was given way back in the early 1980s by Fusayama. With this background, the aim of the present study is to survey the preference of Self etch VS Total etch among dental practitioners. A Cross-sectional survey was conducted among the dental practitioners about their preference for self-etch and total-etch adhesive. The data was collected and statistically analysed using the SPSS software version 23. Descriptive statistical analysis was carried out and the chi-square test was used and the p-value was calculated, with a p-value less than 0.05 to be statistically significant. The present survey included 209 dental practitioners of different categories with a clinical experience of at least 3 months, 32.69 % of the practitioners had clinical experience of 10 years and more than that. 71.15 % of the practitioners preferred the usage of Total Etch and remaining 28.85 % preferred the usage of self etch. According to the Practitioners, 68.75 % considered the total etching technique as the best technique and 31.25 % considered self etch as the beat technique. From this survey, it is seen that the majority of the practitioners preferred using total-etch compared to self etch. Despite having its disadvantage it is preferred by the practitioners.

Keywords: *Self etch, Total etch, Smear layer, Adhesive Systems, Dentin bonding Agent*

Introduction

About 50 years ago, adhesives evolved into dentistry. but the challenging point in adhesives is the effective bonding to the hard tissues. Bonding the adhesive to the enamel is easy and durable whereas bonding to dentin is a bit difficult and complicated procedure, a time-consuming procedure. The basic requirements for adhesives such as user-friendly, increased bond strength, less technique sensitivity etc., introduced different generations of adhesives as per requirement. Now the adhesives which are currently being used are Self etch and Total Etch adhesives [1]. The usage of the adhesives is according to the preference of the practitioner and clinical necessity [2]. The evolution adhesives into dentistry is illustrated as in (Figure 1).

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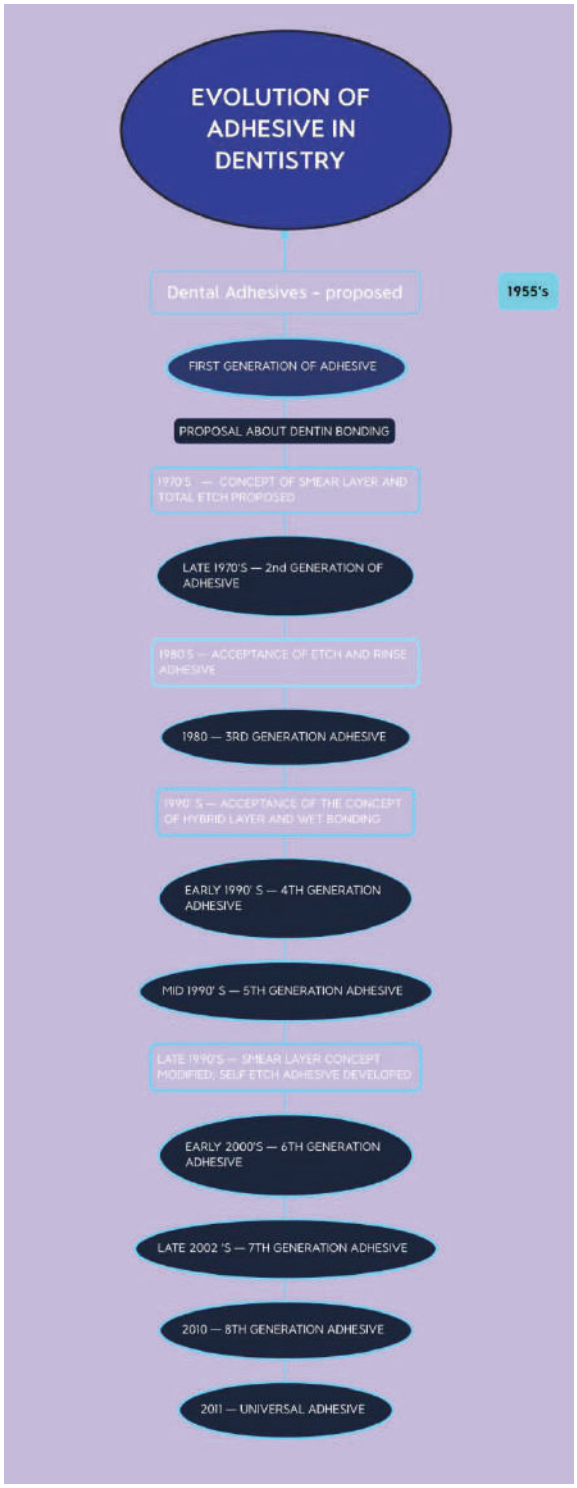


Figure 1 Elucidates the Evolution of adhesives into Dentistry

Total Etching Technique :

The concept of total etching or etch and rinse technique was given way back in the early 1980s by Fusayama. The turning point in the evolution of adhesives used in dentistry was the ability to remove the smear layer completely which was achieved in the 4th generation adhesives Total etch adhesive can be classified based on the number of clinical steps involved as 3 steps technique and 2 steps technique [3]. 4th generation adhesives come under 3 step technique, Etchant, Primer and Bonding agent are used in 3 different clinical steps. The characteristic feature of 4th generation is the ability to etch both enamel and dentin simultaneously, which is achieved using acid (Phosphoric acid) for 15 – 20 sec. The concept of wet bonding is used here to prevent the disintegration of collagen. The drawback is increased time consumption due to the increased number of clinical steps, this led to the need for newer adhesives evolution [4,5].

The 5th generation of Adhesives was introduced in the mid-1990s. This generation involves two clinical steps as Acid etching followed by a combination of primer and bonding agent. The advantages of this generation of adhesives are decreased time consumption and high bond strength. The resin – dentin interface is more prone to degradation, which increases postoperative sensitivity is the main disadvantage. Bonding to dentin is less reliable and difficult to bond compared to enamel; this is due to organic constituents present in the dentin [6,7].

Mechanism Of Bonding:

When the surface is etched with acid it exposes the collagen fibres that are devoid of hydroxyapatite crystals. The bonding will occur by the diffusion or infiltration of the resin within the collagen which forms the hybrid layer. After the polymerisation, the hybrid layer provides micromechanical retention for the restoration [8]. The hydrophilic and ionic monomers combine which results in the bonded interface. The solvents present in the adhesive are difficult to evaporate and get entrapped within the adhesive layer after polymerisation [9,10] .

Total etch technique is a gold standard technique and also the oldest technique of adhesion, used even till date, but they are incapable of preventing Nano leakage despite its clinical durability and performance [11,12].

Self Etch Technique

The self-etching technique was introduced into dentistry in the early 2000s. The self etch are classified as two-step and one-step adhesive based on the number of

clinical steps involved. Two-step adhesive includes 6th generation of adhesive, the steps involved are etchant and primer together and bonding agent separately or etchant separately and primer and bonding agent together [13]. The one-step adhesive includes 7th generation adhesives, Etchant, primer and bonding agent are used together in a single step. They can be used to etch the etched or even unetched enamel or dentin surface. They are also called as universal or Multimode adhesive [14]. The self etch adhesives contain water as the ionising medium which enables better adhesion but there are exceptional conditions like Acetone based adhesive [15,16].

These adhesives can demineralise and infiltrate the surface of the tooth, the adhesive can also penetrate for the same time and same depth. When the monomers in the adhesive and the tooth surface interacts there will be a formation of Adhesive – Tooth interface [17,18]. Based on this interaction, adhesive are classified as (i) Ultra Mild – interaction depth is few hundred nanometres (pH > 2.5) (ii) Mild – Interaction depth around 1 µm (pH approximately 2) (iii) Intermediately strong – interaction depth around 1-2 µm (pH 1- 2) (iv) Strong – interaction depth several µm (pH <1). When strong adhesives are used there will be the formation of typical resin tags but when mild or ultra-mild adhesives are used the resin tags are formed rarely, in case even if they are formed resin will get infiltrated because of the resin tag demineralisation [19].

Mechanism Of Bonding

Adhesives bond to the tooth surface by either micromechanical interlocking or by chemical bonding. Micromechanical bonding provides strength to bear the stress wherein chemical bonding increases the longevity of the restoration by reducing hydrolytic degradation [20]. The acid monomers will react with the hydroxyapatite crystals which are composed of specific carboxylic or phosphate group [4 –Methacryloxyethyl trimellitic acid (4 – MET), Phenyl P, 10- Methacryloxy decyl dihydrogen phosphate (10 – MDP)]. When 4 – MET acts as an adhesion-promoting monomer when carboxylic acid gets attached to the aromatic group. The aromatic group has hydrophobic properties which reduce the acidity of the carboxyl groups, 4-MET forms a compound by interacting with Calcium in Hydroxyapatite crystals and forms Ca-MET salt by ionic bond formation [21].

With this background, the present study aims to analyse about the preference of the dental practitioner for Total etch and Self etch adhesive and also study about reasons for their preference of adhesive among the dental practitioners.

Materials and Methods

A questionnaire was prepared which consisted of questions related to self etch and total-etch adhesive system. This questionnaire was circulated among dental practitioners of 21 years and above including the UG Students, PG Students, UG practitioners and PG practitioners were included in this study. Since this is randomly sampled study it was circulated randomly to all the dental practitioners through an online survey portal. The framed questions were uploaded in an online platform i.e., Google Forms and circulated to all the dental practitioners and were asked to fill the survey. The data was collected and analysed statistically using SPSS software 2.0. Chi-square test and Pearson correlation analysis were used for the data analysis, with a p-value less than 0.05 to be statistically significant.

Inclusion Criteria: The dental practitioners of all categories and clinical experience of at least 3 months were included for this study.

Exclusion Criteria: The UG dental students in 1st and 2nd year of study, persons with no clinical experience and knowledge were excluded from this study.

Results and Discussion

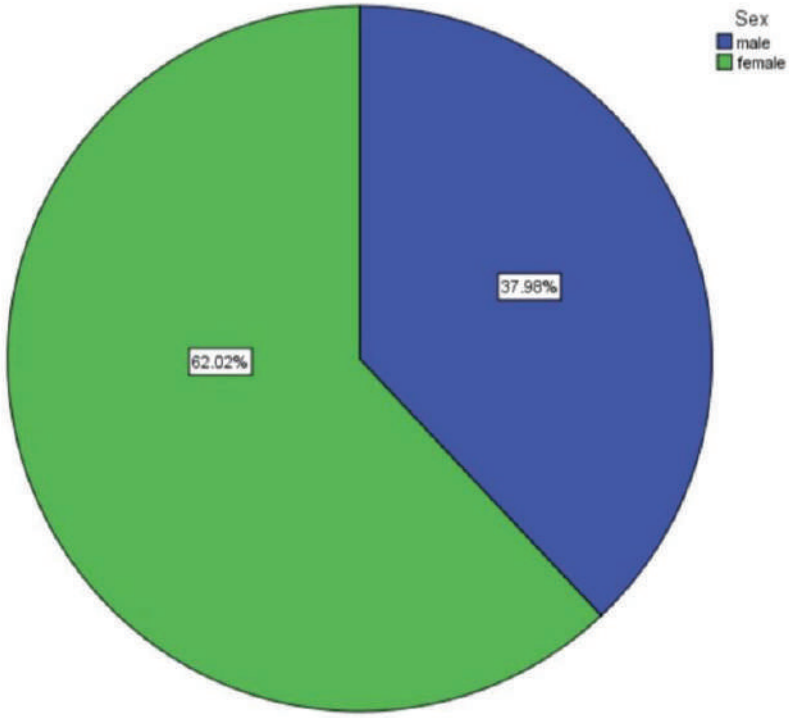


Figure 2, depicts the Gender of the practitioner who participated in the survey. The male practitioners are represented in Blue colour and female practitioners are represented by Green colour. 62.02% were female practitioners and 37.98% were male practitioners. This was a random sampling and there was no intention on emphasizing female dental practitioners to participate in the survey.

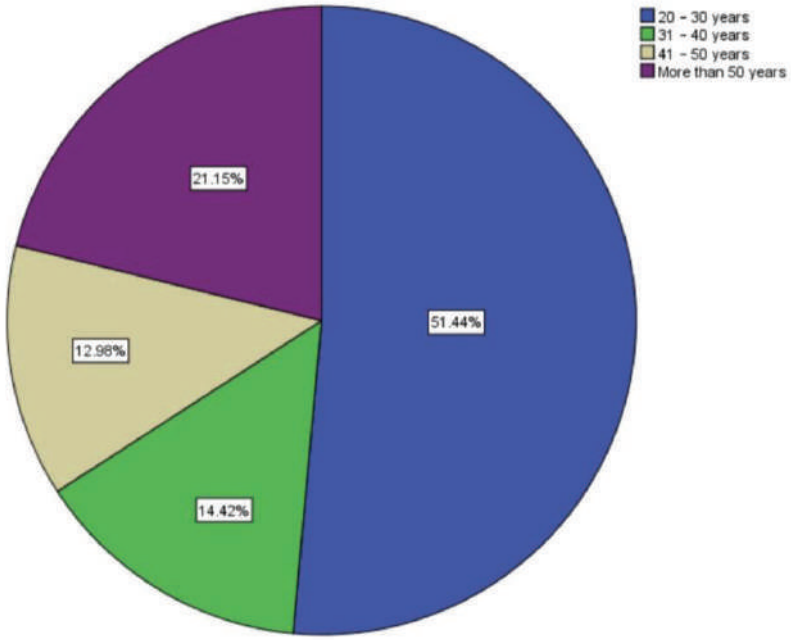


Figure 3 depicts the Age group of the practitioner, 51.44% were in the age group of 20 - 30 years (Blue), 14.42% in the group of 31 - 40 years (Green), 12.98% in the age group of 41 - 50 years(Brown), 21.15% in the age group of more than 50 years (Violet). Since there are no previous studies regarding the preference among adhesive, the data cannot be compared [22].

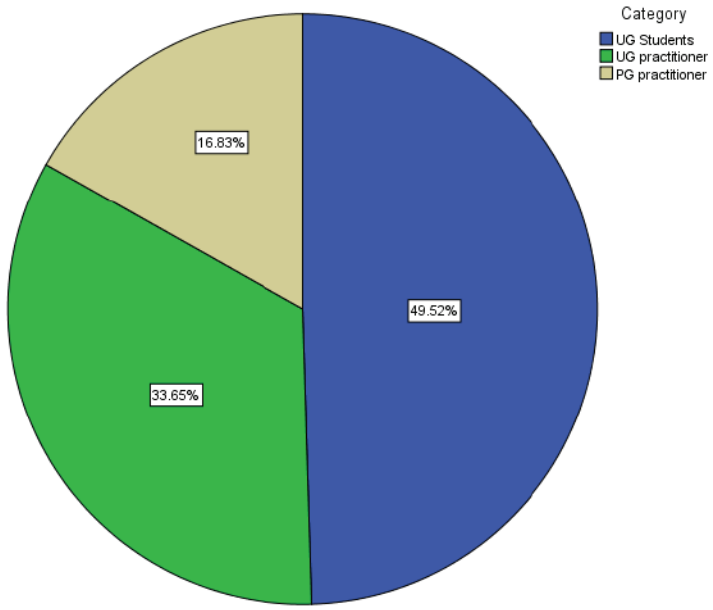


Figure 4 depicts the category to which the practitioner participated in the survey belongs, it was reported that 49.52 % were UG Students (Blue), 33.65 % were UG practitioners (Green), 16.83% were PG practitioners (Brown).

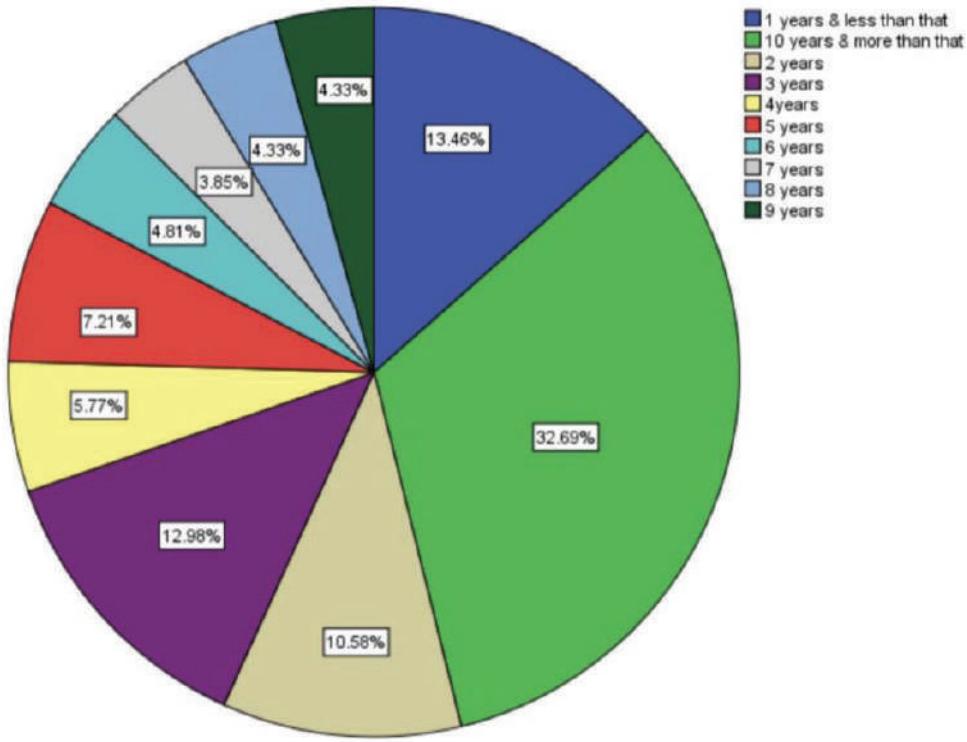


Figure 5 portrays the Clinical experience of the practitioner and it is reported that 13.46% had an experience of one year and less than that (Dark Blue), 32.69% had an experience of 10 years and more than (Light Green), 10.58 % had an experience of 2 years (Brown), 12.98 % had the experience of about 3 years (Violet), 5.77 % had an experience of 4 years (Yellow), 7.21 % experience of 5 years (Red), 4.81% experience of 6 years (Light Blue), 3.85 % experience of 7 years (Grey), 4.33 % experience of 8 years (Indigo) and practitioners with 9 years of clinical practice were 4.33 % (Dark Green).

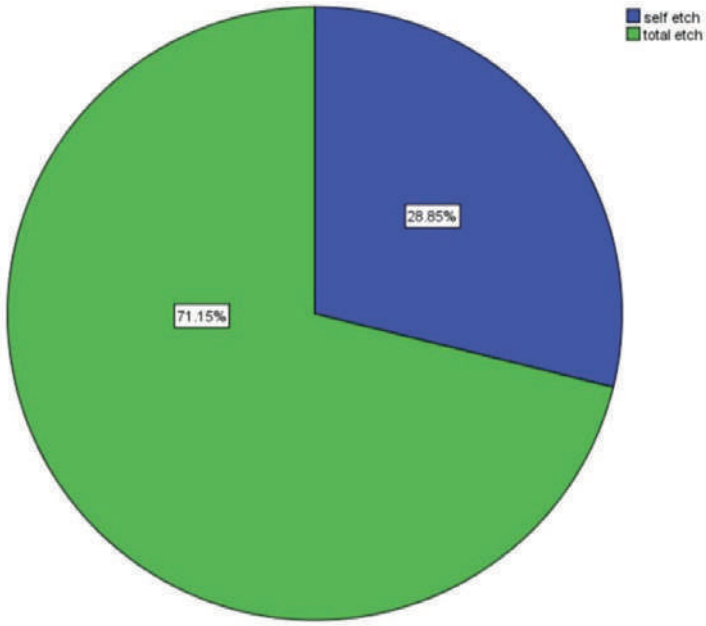


Figure 6, depicts the preference of adhesive by the Practitioner during their clinical practice, from the survey it is seen that that majority of practitioners 71.15 % preferred using Total etch (Green) and 28.85 % preferred using Self etch Adhesive (Blue).

The total-etch technique preference might be due to some lectures and practitioners who guided them must have strongly preferred towards total etching technique. But the self-etching technique also has its unique features, indications and contraindications [23]. So we cannot conclude that neither total-etch nor self etch preference alone[24,25]. The preference should be also based on the clinical preference, for example, cavities with exposed dentin, there will be a preference of self etch but due to Esthetic preference [26–28], it is less indicated or not even indicated since the cavity walls are located in the enamel [29,30].

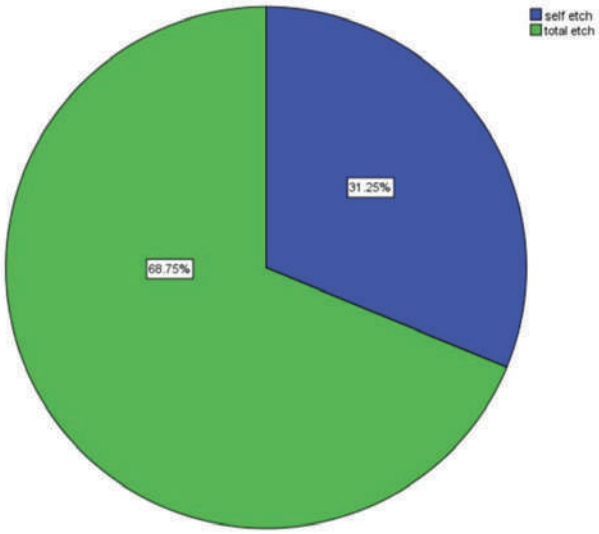


Figure 7 denotes the Best adhesive according to the Practitioner despite their usage in their clinical practice, 68.75% practitioners preferred usage of total-etch (Green) and 31.25% practitioners preferred self etch (Blue) as the best etching technique.

Despite using a particular adhesive in clinical practice, there would be an adhesive which the practitioner prefers as the best technique, it will differ among the practitioners. There are equal advantages and disadvantages in both the techniques and reason for selecting that particular adhesive is also controversial [31,32]. In a study conducted by

Mithra Hedge et al., it was concluded that self etch adhesives are the best and they are also preferred widely, this is in contrast to the present study wherein total-etch is preferred widely [33].

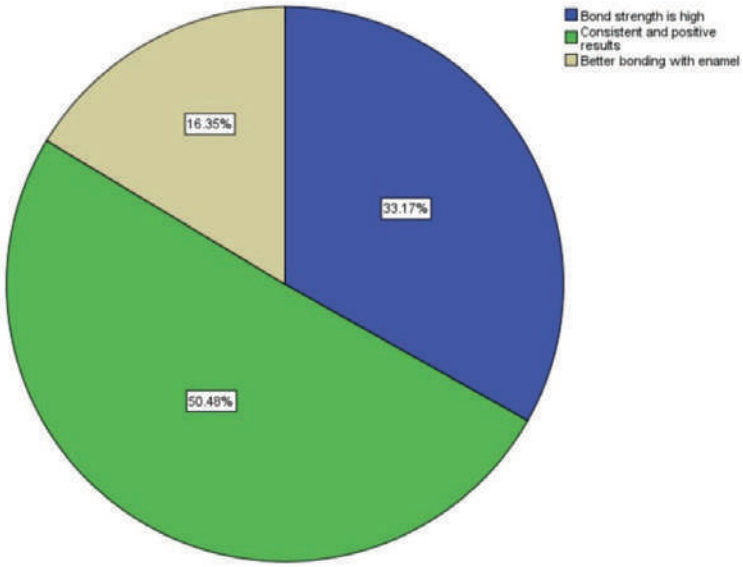


Figure 8 elucidates the reason for preferring Total etch by the practitioners, and it was reported that 33.17 % for high bond strength (Blue), 50.48 % preferred it for the positive results which were seen in clinical practice (Green) and 16.35 % for the better bonding with enamel nature (Brown).

When total-etch is widely preferred among the practitioners there should be a reason for that preference. The high bond strength nature is one of the reasons for its preference [34,35]. A study conducted by Michael schauseil et al., Evaluating the shear bond strength between self etch and total-etch adhesive concluded that there is no difference in the shear bond strength between both the adhesives [36].

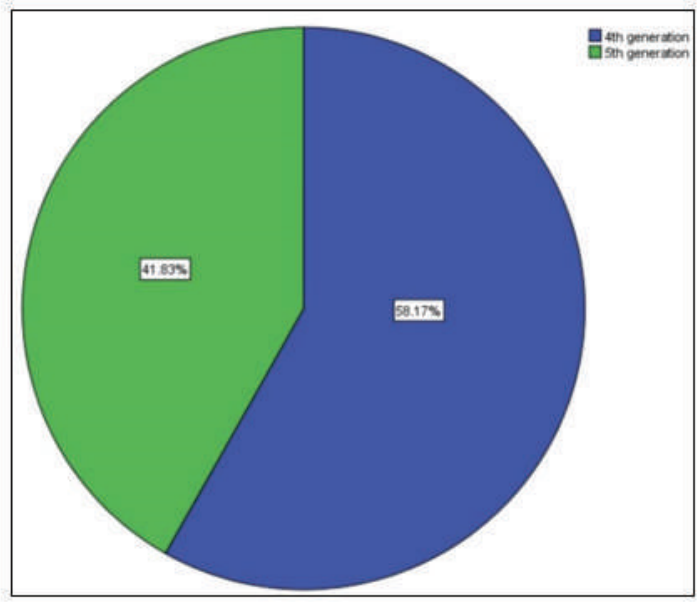


Figure 9 elucidates the preference of generation in the total-etch adhesive among the practitioners where it is seen that 58.17% preferred using 4th generation (Blue) and 41.83 % preferred using 5th generation adhesive (Green).

4th generation involves 3 steps Acid etching, Primer and Bonding agent are used in three different steps, which leads to the formation of the hybrid layer and increases bond strength. In 5th generation, adhesives involve two

steps Acid etchant followed by a combination of primer and bonding agent [37,38]. These adhesives decreased the time consumption and also improved the prevention of collagen collapsing in demineralised dentin but they will be more prone to water degradation [39].

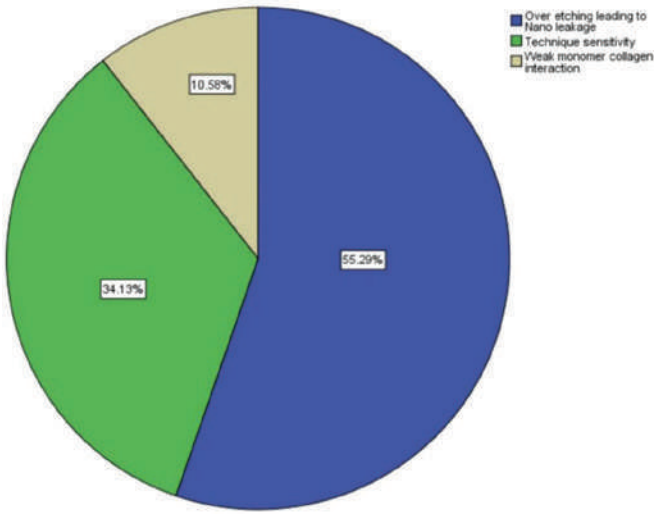


Figure 10 represents the disadvantages of total-etch adhesive according to the practitioners where it is documented that 55.29% did not prefer the usage of total-etch because over-etching leads to nano leakage (Blue), 34.13 % did not prefer because of Technique sensitivity (Green) and 10.58% for weak collagen - monomer interaction (Brown).

The technique sensitivity is attributed in dried dentin as there are more chances of collapsing the demineralizing dentin and low monomer diffusion which interrupts the hybrid layer formation, it not only contributes technique sensitivity it also plays an antagonistic role of water in bonding [40,41]. In which over setting leads to the separation of the component in the adhesive I.e., hydrophobic and hydrophilic which leads to the formation of voids in the resin - dentin interface [42,43].

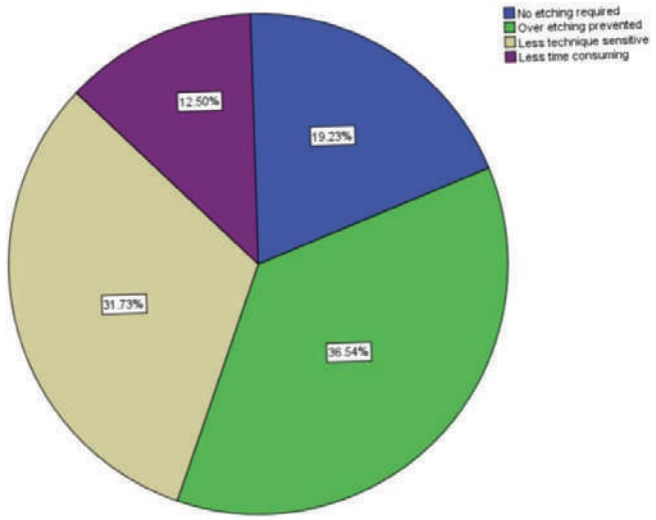


Figure 11 portrays the advantages of self etch according to the practitioner where it was recorded that, 19.23% for the property of no etching requirement (Blue), 36.54% preferred since over-etching is prevented (Green), 31.73% preferred for less technique sensitivity (Brown), 12.50% preferred for decreased time consumption (Violet).

Self etch adhesives reduce post-operative sensitivity since the residual smear plugs are left behind which leads to lesser exposure of dentinal tubules compared to total-etch [44,45]

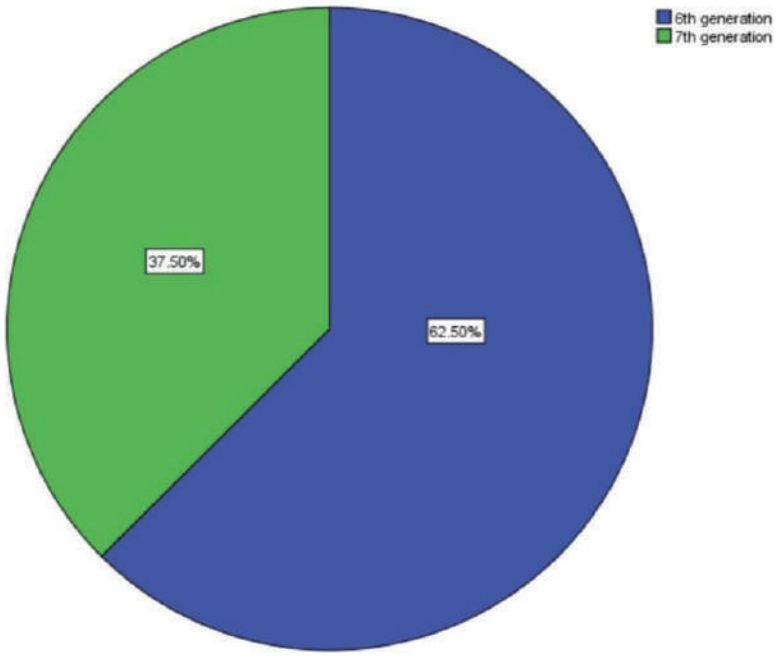


Figure 12 represents the preference among the generation of Self-etch among the practitioners, where 62.5% preferred 6th generation of adhesive (Blue) and 37.5 % preferred 7th generation of adhesive (Green).

6th generation adhesives have better adherence to the dentin in comparison to the total-etch adhesive. 7th generation adhesives are all in one adhesive [46]. They have lower initial and long term bond strength of any adhesive is a major disadvantage [47].

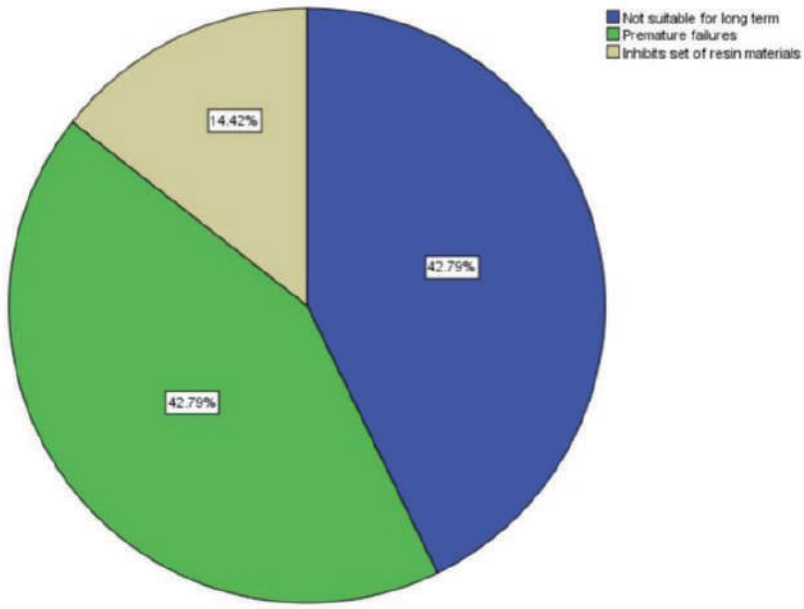


Figure 13 denotes the disadvantages of self etch adhesive among the practitioners, an equal number of practitioners reported that 42.79 % it is not preferred because they are not suitable for the long term (Blue) and due to premature failure (Green), 14.42% did not prefer due to inhibition of setting of resin materials (Brown).

When a thin layer of adhesive is applied it inhibits the polymerisation due to decreased Oxygen content [48,49]. In contrast to it when the adhesive is applied in multiple layers it increases the bond strength and premature failures are also prevented [50].

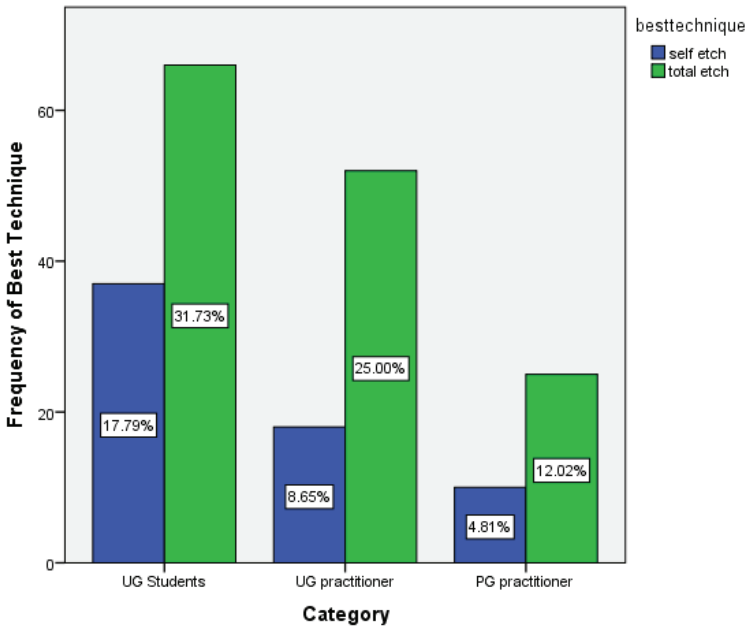


Figure 14 represents the association between Category of the Practitioner and Preference of Best adhesive according to the practitioner. The X-axis represents the Category of the Practitioner and Y-axis represents their Preference for adhesive. Blue colour represents Self etch and Green colour represents Total Etch technique was preferred mostly in all categories of the practitioners, 31.73% (n = 66) of the UG students, 25% (n = 52) of the UG practitioners and 12.02% (n =25). It is also seen that the UG students mostly preferred the usage of Total Etch compared to the other categories of practitioners. However this is statistically not significant with chi-square value - 2.162 and p-value = 0.3 (p-value > 0.05) hence insignificant.

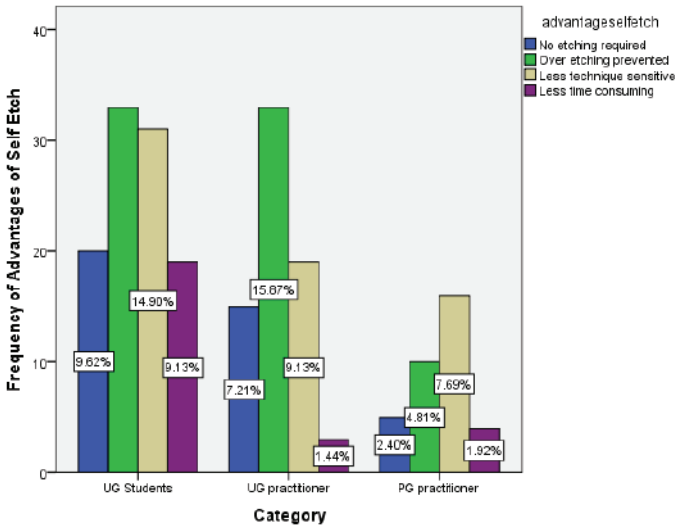


Figure 15 denotes the Chi-square analysis between Category of the Practitioner and Advantages of self etch according to the practitioner. The X-axis represents the Category of the Practitioner and Y-axis represents Advantages of Self etch according to the practitioner. Blue colour represents No etching required, Green colour represents Over etching is prevented, Brown colour represents Less technique sensitivity, Violet colour represents Less time-consuming.There are different reasons for preferring Self etch among the practitioners; Among the UG students 9.62% (n = 20) prefer for No requirement of etching, 14.90% (n = 33) since the over-etching is prevented, 14.85% (n = 31) for the less technique sensitive nature and 9.13% (n = 19) for less time consuming nature. Both UG students and UG practitioners mainly preferred Self etch because the Over etching is prevented than the PG practitioners. There is also a significant difference between the Category of the practitioner and the advantages of Self Etch with Chi-square value - 13.388 and p-value - 0.03 (p-value < 0.05) hence significant.

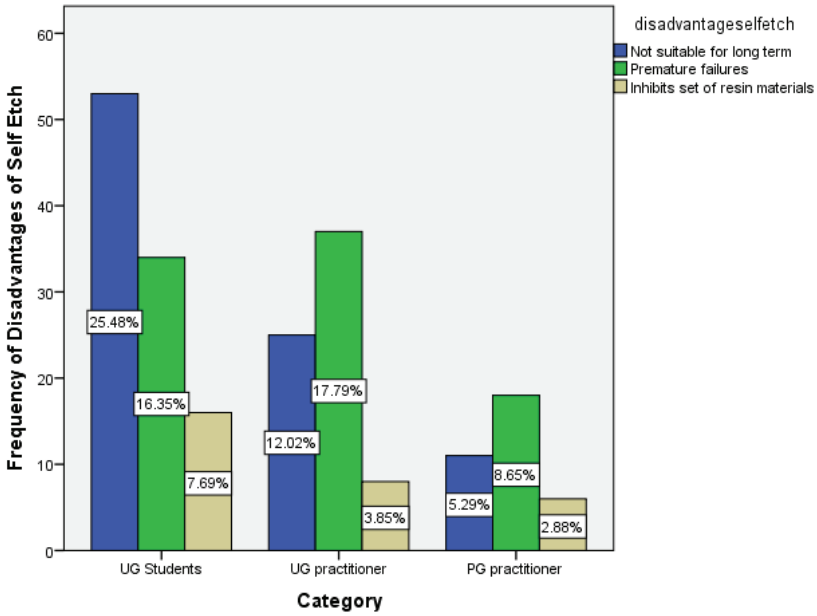


Figure 16 portraying the Chi-square analysis between Category of the Practitioner and Disadvantages of self etch according to the practitioner. The X-axis represents the Category of the Practitioner and Y-axis represents the Disadvantages of self etch according to the practitioner. Blue colour represents Not suitable for the long term, Green colour represents Premature failures and light brown colour represents inhibits set of resin materials. Majority of the UG students, 25.48% (n = 53) reported that self etch is not suitable for the long term, among the UG practitioners 17.79% (n = 37) considered Premature failures as a disadvantage, among the PG practitioner 8.65 (n = 18) also reported Premature failures as a disadvantage of Self etch. UG students found more disadvantages in self etch more than the UG and PG practitioners. This is proven statistically significant with a Chi-square value - 8.957 and p-value - 0.04 (p-value < 0.05), hence significant.

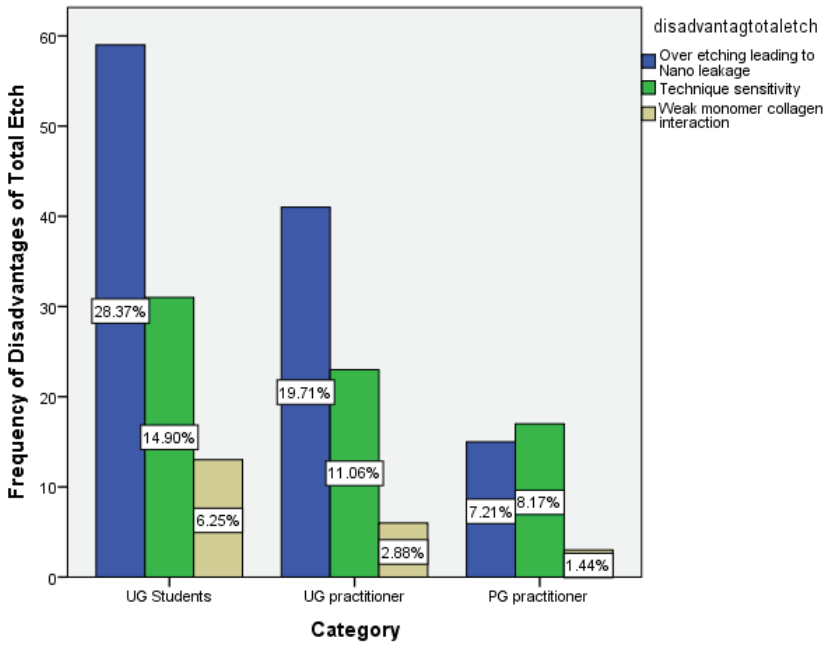


Figure 17 elucidates the Chi-square analysis between Category of the Practitioner and Disadvantages of Total etch according to the practitioner. The X-axis represents the Category of the Practitioner and Y-axis represents Disadvantages of Total etch according to the practitioner. Blue colour represents Over etching leading to nano leakage, Green colour represents Technique sensitivity and light brown colour represents weak monomer collagen interaction.

Among the UG students it is seen that 28.37% (n = 59) considered Over etching leading to nano leakage as a disadvantage of total-etch, 19.71% (n = 41) also opted Over etching leading to nano leakage as a disadvantage of total-etch, but among the PG practitioners Technique sensitivity was considered as the disadvantage by 8.17% (n = 17). There is no significant difference between the UG students, UG and PG practitioners with Chi-square value - 4.567 and p-value - 0.3 (p-value > 0.05) hence statistically not significant.

A comparative study between the Category of the Practitioners and different parameters like the best adhesive according to the practitioner (Figure 14), Advantages of Self Etch technique (Figure 15), Reason for preferring Total etch Adhesive (Figure 16) and Disadvantages of Total etch Adhesive were done using Chi-Square analysis in SPSS software version 23. It is also seen that the UG students mostly preferred the usage of Total Etch compared to the other categories of practitioners. However, this is statistically not significant (p-value > 0.05). Both UG students and UG practitioners mainly preferred Self etch because the Over etching is prevented than the PG practitioners. There is also a significant difference between the Category of the practitioner and the advantages of Self Etch with (p-value < 0.05). UG students found more disadvantages in self etch more than the UG and PG practitioners. This is proven statistically significant (p-value < 0.05). Chi-square analysis between Category of the Practitioner and Disadvantages of Total etch according to the practitioner. There is no significant difference between the UG students, UG and PG practitioners (p-value > 0.05) statistically.

Limitations

It is a cross-sectional study conducted online with a small sample size. With an increase in sample size, preference of adhesive among a wide group of practitioners can be obtained.

Future Scope

Based on the preference among the practitioner's adhesive type can be improved with the required criteria by the practitioners. The disadvantages of adhesive can be fulfilled so that there will be better clinical usage of adhesive.

Conclusion

From the present study, it can be concluded that the total-etch adhesive system was more likely preferred compared to self etch adhesive among the practitioners in their clinical practice. Despite the disadvantages of total-etch adhesive. However further studies can be conducted to overcome the disadvantages of total-etch adhesives to provide better clinical results.

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Conflict of Interest: The authors declare no Conflict of interest.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Association of Systemic Diseases with Periodontitis among Patients Attending A Private Dental College in Chennai - A Record based Study

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Abstract

Oral cavity works as a continuous space of infectious agents and its condition reflects in progression of systemic diseases. Recent studies state that periodontal disease may be a potential marker to systemic diseases. Aim of the study was to determine the association of periodontitis and systemic disease among patients attending a private dental college. Russell's periodontal index was used to measure the periodontal disease.This study included all periodontal index patient records from 18- 80 years old who visited the dental college in the month of January 2020 and a total of 136 records were reviewed. Descriptive statistics and chi square tests were used for statistical analysis. In the sample population (N= 136), 64.71% males and 35.29 % females were present. Age distribution of the records showed that 16.91 % of the study population were 18-35 years old; 36.03% were 36-50 years and 47.06% were 51-80 years. It was found that 0.74% of the study population had normal gingiva; 8.09% had simple gingivitis; 23.53 % had beginning destructive periodontitis; 53.68% had established destructive periodontitis and 13.97 % had terminal periodontal disease. Results revealed that 35.56 % of the study population reported to have systemic disease. Significant association was found between age and periodontal disease; age and systemic disease ($p<0.05$). Results of the present study showed that there was no significant association between periodontal status and systemic disease condition of the study population ($X^2 = 5.826^{a;}$; $p>0.05$)

Keywords: *Periodontitis, systemic disease, diabetes, hypertension , Russel's periodontal index*

Introduction

Periodontitis is defined as an inflammatory disease of supporting tissues of teeth caused by specific microorganisms resulting in progressive destruction of periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession or both[1]. The diversity of bacterial species in the periodontal flora , the variation in composition of floras from individual to individual and variation in host response to bacterial species are some of the major reasons that the specific etiology of periodontal diseases has not been clearly established.[2,3]

Some of the most common organisms associated with periodontal diseases are Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Campylobacter rectus and Actinobacillus actinomycetemcomitans, as well as the treponemes[4]. Periodontitis and periodontal diseases are true infections of the oral cavity. There is an equilibrium that exists between microbial challenge and host's immune response; any alteration to that with addition of other modifying factors is responsible for clinical manifestation of periodontal disease. The oral cavity works as a continuous source of infectious agents, and its condition often reflects progression of systemic

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pathologies.[5] Pathogens of the subgingival microbiota can interact with host tissues even without direct tissue penetration, and the subgingival microbiota accumulate on the oral cavity to form an adherent layer of plaque with the characteristics of a biofilm.[1]

Many studies have proven the role of oral microflora in oral diseases. [6,7] and their importance with aging and nutrition.It was shown to increase with the increasing age and malnutrition.[8,9]. In our country, dental caries is given much importance and people are having better knowledge about dental caries than periodontal diseases. Many studies have proved the importance of age with respect to dental caries and people's attitude and practice about dental caries.[10–13].There are even ways to prevent dental caries from occurring . studies have proven use of fluorides to prevent dental caries and use of antimicrobial agents.[14–20]

Recent evidence suggests that periodontal infection may significantly enhance the risk for certain systemic diseases or alter the natural course of systemic conditions; and conditions in which influences of periodontal infection are documented include coronary heart diseases (CHD) and CHD-related events such as angina and infarction, atherosclerosis, stroke, diabetes mellitus; preterm labor, low-birth-weight delivery; and respiratory conditions such as chronic obstructive pulmonary diseases.[21]

In a study conducted by SS oberai et al in 2016, the systemic disease patients had high severity of periodontal disease [22]. similarly Qi- wei Yao et al found periodontal disease has increased susceptibility to oral cancer[23]. Kodovanzenti G et al has established an association between periodontitis and acute myocardial infarction in 2014[24]. The high prevalence (42.3%) of periodontitis among adults makes it an important area of research for early diagnosis. It has been established as a potential marker for systemic disease[25]. But these studies lacked to establish a causal relationship and use of radiographs . Since periodontal diseases are a marker of systemic disease , it is important to do further research about it and have knowledge about prevention , intervention of the periodontal disease and to identify the highly susceptible ones.

Aim of the study was to determine the association of periodontitis and systemic disease among patients attending a private dental college

Materials and Methods

Study Setting

This was an institution based study which was conducted in Saveetha dental college.

Study population

Study population included all the Russel;s periodontal index patients who have attended college in the month of January 2020. The patients from the age 18-80 years were included and they should have been interpreted by Russels periodontal index

Sampling

All the Russels periodontal index patients who have been interpreted were included in the study within the age group of 18-80 years old and their case sheets were reviewed (136) . Cross verification was done by photographic verification of the patient. To avoid sampling bias, everyone who has undergone Russels periodontal index within the age group in that time period was included. Internal validity of the study was established by the use of gold standard periodontal index, Russels periodontal index (1956).

Index used

The Periodontal Index (PI) was developed by Russel A.L. in 1956. All the teeth are examined and scoring values are 0,1 ,2,4,6,and 8. A PI score of 0 indicates no overt inflammation in the supporting tissues or loss of function owing to destruction of supporting tissues. A PI score of 1 corresponds to mild gingivitis (overt inflammation in the free gingiva, but not circumscribing the tooth), whereas a PI score of 2 indicates gingivitis circumscribing a tooth. A PI score of 4 can be assigned only if dental radiographs are available. A PI score of 6 indicates gingivitis with a pocket formation. A PI score of 8 indicates advanced destruction of supporting tissues with loss of masticatory function, although this was not observed in any of the patients in the present study. There are no scores of 3, 5 or 7 in the Russell PI. It has a rule that "when in doubt assign the lesser score" . The instruments used in this index are mouth mirror and non calibrated probe.

Data collection

The patient details were taken from the patient records and about 136 records which recorded Russels periodontal index were included in the study. The data was collected and reviewed by 2 examiners and entered in the MS excel sheet. The variables of the results were explained using tables and graphs directly from SPSS software

Statistical Analysis

Data was imported in IBM SPSS software version 20.0. The independent variables were age, sex and systemic disease condition, while the dependent variables were the interpretation of Russel's periodontal index. Descriptive statistics and chi square tests were done to identify the distribution and association between systemic disease and systemic disease.

Results and Discussion

In the present study, after excluding the incomplete data, the total sample size of the study was 136 in which 64.71% were males and 35.29% were females (figure1). Results showed that 16.91% of the study sample were in the age group of 18-35 years old; 36.03% were present in the age group of 36-50 years and 47.06% were present in the age group of 51-80 years. (figure 2). Results of the study revealed that 0.74% of the study population had normal gingiva; 8.09% had simple gingivitis; 23.53 % had beginning destructive periodontitis; 53.68% had established destructive periodontitis and 13.97 % had terminal disease.(figure 3). Among the study population,, 35.56 % had systemic disease and 84.44% of the study population were free of systemic diseases (figure 4)

The distribution of periodontal diseases among age groups were as follows: in the age group of 18-35 years, 0% had normal gingiva, 21.73% had simple gingivitis, 26.08% had beginning destructive periodontitis, 47.82% had established destructive periodontitis and 4.34% had terminal disease. In the age group of 36-50 years, 0% had normal gingiva, 4.08% had simple gingivitis, 32.65% had beginning destructive periodontitis, 55.1% had established destructive periodontitis and 8.16% had terminal disease. In the age group of 51-80 years, 1.56% had normal gingiva, 6.25% had simple gingivitis, 15.62% had beginning destructive periodontitis, 54.68% had established destructive periodontitis and 21.87% had terminal disease. This showed that as the age increases the severity of periodontitis increases. There was a statistically significant association found between age and periodontitis with a chi square value of 16.908^{a} ($p=0.031$)(figure 5).

Results of the distribution of systemic diseases among age groups showed that in the age group of 18-35 years, there was 0% prevalence of systemic diseases ; in the age group of 36-50 years , 18.36% had systemic diseases and 81.63 % were free of systemic diseases. In the age group of 51-80 years , 61.9% had systemic diseases while 38.09% did not have any history of systemic diseases. This clearly stated that age was directly proportional to prevalence of systemic diseases in the present study. There was a statistically significant association found between age and systemic diseases with a chi square value of 38.096^{a} ($p=0.000$)(figure 6). There was no significant association found between periodontal status and systemic disease ($X^2 = 5.826^{a;}$; $p>0.05$) (figure 7)

Although the direct cause for periodontitis is oral bacterial infection, its progression and severity depend on a number of genetic and environmental factors Several epidemiological studies in different populations demonstrate a relationship between smoking and periodontal disease.[26] Cigarette smoking is arguably the strongest behavioral risk factor for the incidence and progression of periodontitis. It is also important to note that although nonsmokers universally respond better to periodontal treatment than do smokers, there is nevertheless substantial evidence of clinical improvement in smokers after treatment, indicating that smoking as a risk factor will compromise rather than prevent tissue healing.[27]As healthcare professionals, we have a role in preventing disease occurrence and progression on all the occasions. [28,29] So it's our duty to inform the patients about the predilection of periodontal disease, identify high risk individuals and create awareness [30]

In the present study, more male population (64.71%) when compared to females. In a similar study done by Balaji et al in 2018, 59.4% males participated in their study [31] but in a contrastive study done by TS Sekhon et al more female participants (52.14%) been reported.[32]

In the present study, 16.91% belonged to 18-35 years, 36.03% were from 36-50 years and 47.06% were

from 51-80 years old. Similar findings were found in a study done by SS Oberai et al in 2016 [22]. But in a contrastive study done by TS Sekhon et al in 2015, only 18% were above 60 years old[32]

In the present study , 35.56% had prevalence of systemic diseases. In a study by Cottone HA , every 2/3rd of Indian had medical history[33]. Similar findings were found (33.49%) in a study conducted in 2005;[34] but in a contrastive study conducted in central India, only 4.05% had systemic diseases.[35]. In the present study, prevalence of periodontitis was 67.65% among the study population. Similar findings were found in a study by Balaji et al in 2018 in which 54.2% had periodontal diseases.[31] But in a contrastive study conducted by Rajesh Shankaranpillai in 2010,low prevalence of periodontitis was found[36]

In the present study, no significant association was established between periodontal status and systemic disease. Similar findings were found in a studies in which period

But the major cited articles state that Diabetes mellitus, CVD, and respiratory disease are associated with a higher severity of periodontal disease. [22,39–41]

There were certain limitations to the study. One of the limitations was the record based study design. Lack of external validity and small sample size are other limitations. Another limitation was the individual systemic diseases were not assessed in the study

Further research should be focused on individual systemic disease association with periodontitis in a prospective design including more samples.

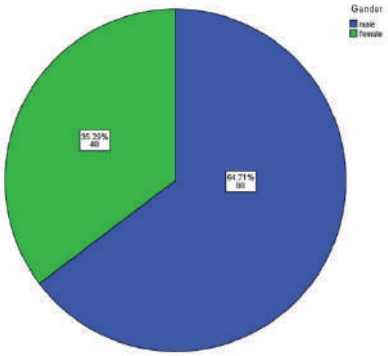


Figure 1 shows the gender distribution in the study population representing male predilection (males (64.71%) and females (35.29%))

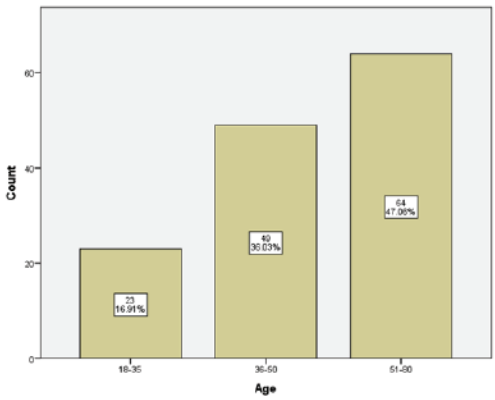


Figure 2 shows the distribution of age in the study sample. X axis represents the age group of the study participants and Y axis represents the number of people present in each group. More people were distributed in the 51-80 age group(47.06%)

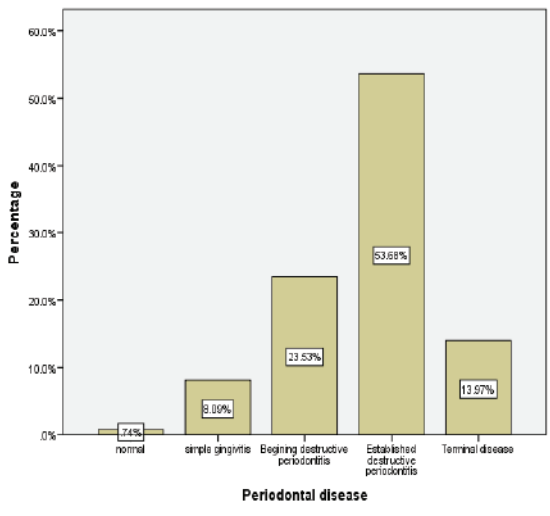


Figure 3 shows the distribution of periodontal disease in the study sample. X axis represents the periodontal disease and Y axis represents the percentage of study samples present in each score group. Established periodontal disease was the most prevalent among the study population (53.68%)

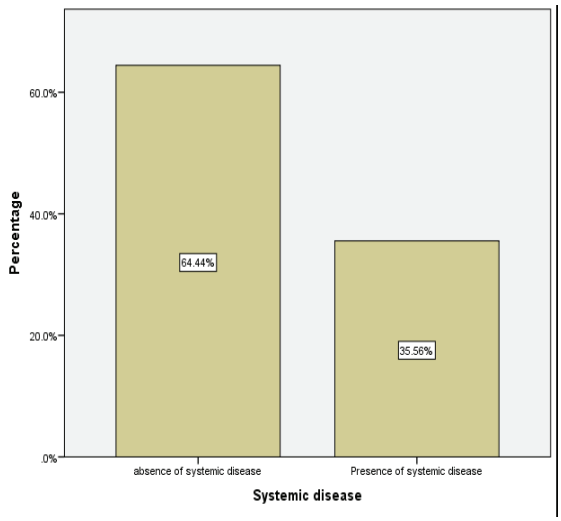


Figure 4 shows the distribution of systemic diseases in the study population.X axis represents the presence and absence of systemic disease and Y axis represents the percentage of samples present in each group. Prevalence of systemic diseases in the sample is 35.56%

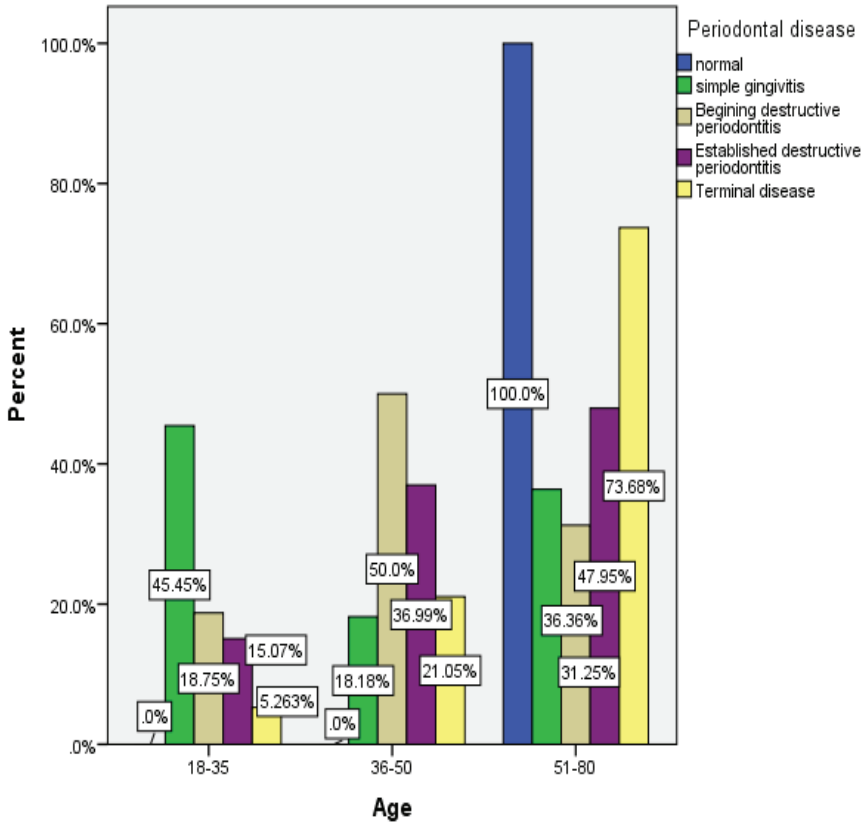


Figure 5 represents the association between age and periodontal disease. X axis represents age groups and Y axis represents periodontal disease scores. Established destructive periodontitis and terminal periodontal disease scores were more among 51-80 years old study participants than participants in 18-35 and 36-50 yrs, suggesting that advanced periodontitis was more among older people and this association was found to be statistically significant (p value = 0.031, Chi square value = 16.908[a])

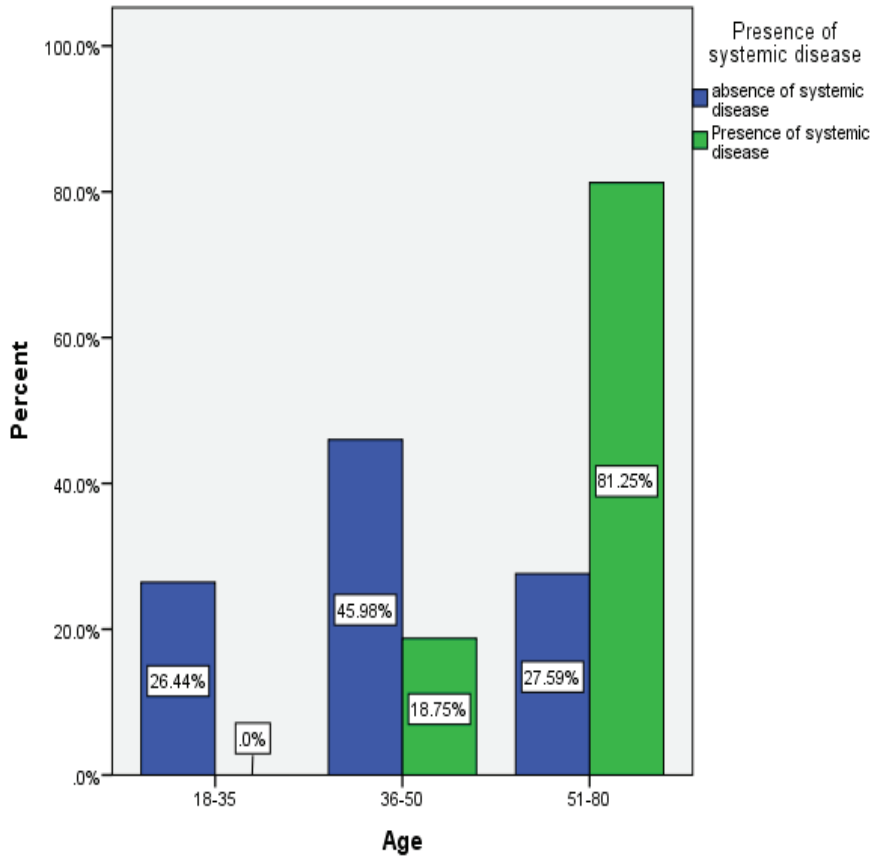


Figure 6 representing the association between age and systemic disease. X axis represents age groups and Y axis represents systemic disease. Systemic disease was more prevalent in the 51- 80 years old group than the other two age groups, suggesting that as age increases the prevalence of systemic diseases increases and this association was found to be a statistically significant association (p value = 0.000. Chi square value = 38.096[a])

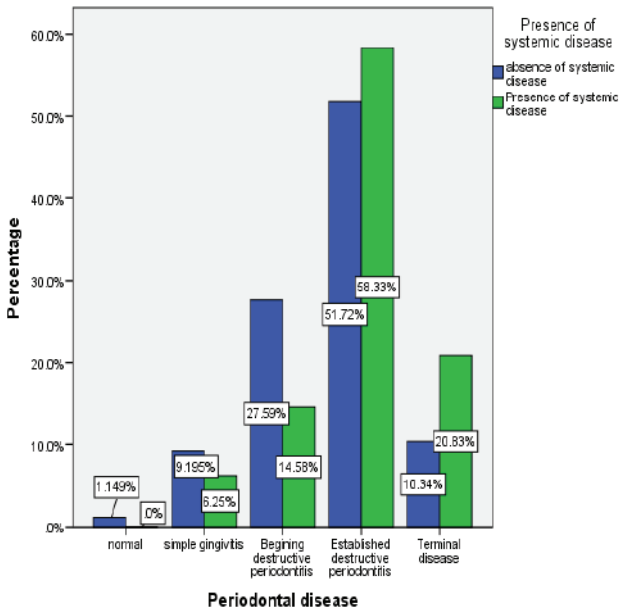


Figure 7 representing the association between periodontal disease and systemic disease. X axis represents periodontal disease and Y axis represents percentage of participants with or without systemic disease. Established destructive periodontitis and terminal disease was more prevalent in people with systemic disease but this association

was found to be not statistically significant (p value = 0.212). Chi square value = 5.826[a]

Conclusion

Within the limitations of the study, the results of the current study showed that there was no statistically significant association between periodontitis and systemic diseases.

Acknowledgments: We would like to acknowledge Information Technology Department of the institution for their help and assistance

Conflict of Interest - Nil

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Comparative Analysis of Different Sizes of Prefabricated Metal Posts Placed in Relation to Premolars- A Retrospective Analysis

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Abstract

Endodontically treated teeth which have extensive structural defects due to caries, access cavity preparation require post and core management for retention purpose.The clinical decision as to whether an endodontically treated tooth requires a post and a crown poses a challenge to dental practitioners.This study aims at comparing different sizes of prefabricated metal posts in relation to endodontically treated premolars.This retrospective clinical study evaluated the patients who received prefabricated metal posts in endodontically treated premolars in Saveetha dental college, Chennai from June 2019 to March 2020. Over 373 patients records of prefabricated metal posts, 157 cases were selected based on the inclusion and exclusion criteria.The collected data was imported to excel sheet , analysed using SPSS software.Results showed that the Group B (post size-2)(diameter of 0.85mm) reported greater preferences than other groups in relation to prefabricated metal posts in endodontically treated premolars.The comparative analysis in relation to endodontically treated premolars with prefabricated metal posts showed post size of 2(diameter of 0.85mm) showed greater preferences than other groups.

Keywords: *Metal posts, Post width, Post size, Premolars, Prefabricated posts.*

Introduction

Intraradicular posts are commonly used to restore endodontically treated teeth when their remaining coronal structure cannot provide adequate support and retention for the restorative material[1]. Cast post and core systems were the standard techniques for many years. However, demands for simpler procedures led to the development of prefabricated posts, initially made from metal and recently from non metallic as the glass fibre posts[2].

A key element in the post selection is the amount of remaining coronal tooth structure and incorporation of ferrule[3].Endodontically treated teeth with moderate to severe coronal tooth loss have demonstrated a success rate of 90.6% after 5 years of service when restored with cast post and cores[4]. Previous invitro and invivo studies showed that non metallic posts should be used when broad coronal dentin is remaining and the crown is well supported by remaining tooth structure; otherwise cast post and core may be used when there is moderate to severe loss of tooth structure[5].

A systematic review showed that the presence of ferrules with 1.5-2mm has a positive effect to ensure proper resistance form to the tooth, prevent fracture from the forces, and dislodgement of the post[6]. Oliveria

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showed that the fracture resistance of the teeth with 0,1,2mm /3mm of remaining coronal tooth structure reinforced with prefabricated non metallic posts did not differ among them and to the group with no remaining coronal tooth structure restored with cast post and cores[7].

Tik et al studied the root widths to determine the best size of the posts, they reported that 1.10 mm size of post is the most appropriate one for the upper central incisors, being responsible by proportion of one third of the root[2,8]. In other studies, best results were obtained with less conservative post diameters. Kivance et al found that posts with increased diameter (roots with 1 & 1.5 mm of remaining coronal structure) presented significant higher fracture resistance than the posts with less diameter[8,9]. Also a finite element analysis demonstrated that when the diameter of the post was 50% of the root, the stress distribution of the post and dentin were most favourable.

Post heads are either flat,spherical, serrated and the most commonly used core materials are composite, glass ionomer cement, resin based luting cement[10–12]. The advantages of glass ionomer cement as core material includes bonding to tooth structure and fluoride release, remineralization in case of carious lesions.

The aim of this study was to compare and analyse the different sizes of prefabricated metal posts in relation to endodontically treated premolars.

Materials and Methods

As it is a retrospective study, several factors such as sample size, sample selection, data collection and analysis influence the validity of this study. This retrospective clinical study evaluated the patients who received prefabricated metal posts (MANI,EG brand) in endodontically treated premolars, Saveetha dental college, Chennai.

DATA EXTRACTION- Over 373 records of prefabricated metal posts cases, 157 cases were selected for this study based on inclusion and exclusion criteria from June 2019 to March 2020.

Inclusion criteria- Patients aged 18- 60 years , endodontically treated premolars treated with prefabricated metal posts(MANI,EG brand).

Exclusion criteria- Teeth other than molars which received posts, teeth which received other post and core systems such as FRC post, acst post were excluded from this study.

The collected data was entered in Microsoft excel sheet and grouping of parameters done in excel sheet. The four parameters in these studies were age of the patient, gender of the patient, teeth number,post size used.

After grouping of parameters , data copied to SPSS software. The statistical analysis between different groups was carried out in SPSS software. Chi square test was done to compare the remaining coronal tooth structure to other three parameters – age, gender. teeth number.

TABLE 1:Based on Age of the patient, grouping done as follows

AGE	GROUP
18-30	1
30-40	2
40-50	3
50-60	4

TABLE 2:Based on Gender of the patient, grouping done as follows

GENDER	GROUP
Female	1
Male	2

TABLE 3: Based on Teeth number of the patient, grouping done as follows

Teeth number	Group
First quadrant	1
Second quadrant	2
Third quadrant	3
Fourth quadrant	4

TABLE 4: Based on Post size used, grouping done as follows

Post size used	Group
Size 1(Diameter-0.65mm)	A
Size 2(Diameter-0.85mm)	B
Size 3(Diameter-1.05mm)	C

Chi square test was done to assess these parameters. The outcome data was represented in the form of tables and graphs. The four tables represent the prefabricated metal posts in premolars done based on the age, gender, teeth number of the patient. The graphs represent the correlation between these parameters- Correlation of age and post size used in endodontically treated premolars, gender and post size used in endodontically treated premolars, teeth number and post size used in endodontically treated premolars.

The bar charts are represented by percentage- for example, X axis represents the age of the patient, Y axis represents the percentage of post size used in endodontically treated premolars.

These type of correlation were represented for all these parameters such as prefabricated post size used based on age, gender, teeth number parameters

Results and Discussion

In this study, analysing and comparing different prefabricated post sizes used in endodontically treated premolars, Group B (post size-2 having diameter of 0.85mm) reported greater preferences than Group A(post size-1 having diameter of 0.65mm), Group C(post size-3 having diameter of 1.05mm).

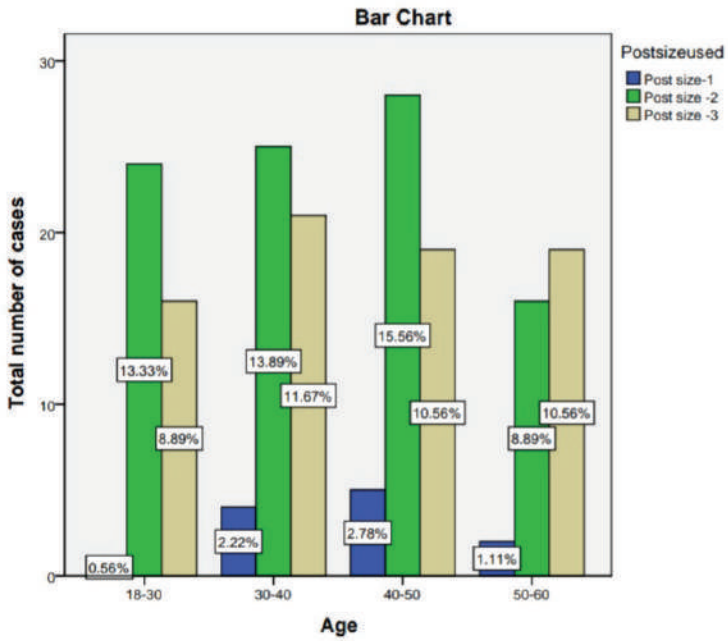


Figure 1- Bar graph showing correlation between age and post size. In the graph, X axis represents the age of the patient and Y axis represents the absolute count. Blue colour represents post size 1, green colour represents post size 2 and grey colour represents post size 3.In this graph, depending on the frequency of post size used based on age group, maximum cases in the age group of 18-60 years accounted for post size of

2 having diameter of 0.85mm. Group B(post size-2 having diameter of 0.85mm) showing greater preferences than other groups.There is a significant difference among groups(p value is 0.02, p<0.05, Chi Square test).

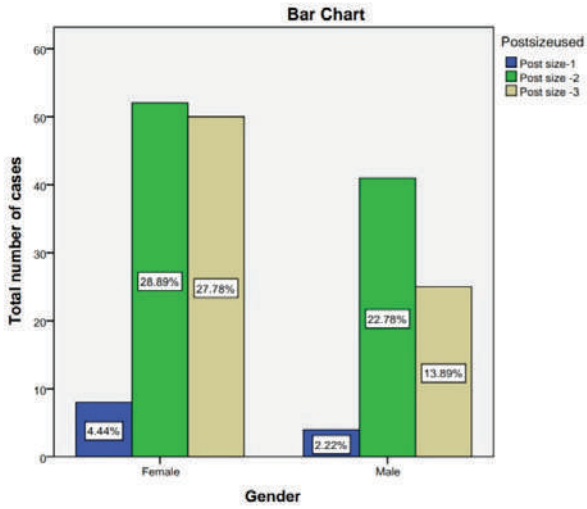


Figure 2-Bar graph showing correlation between gender and post size. In the graph, X axis represents the gender of the patient and Y axis represents the absolute count..Blue colour represents post size 1, green colour represents post size 2 and grey colour represents post size 3. In this graph, depending on the frequency of post size used based on gender, maximum cases in the males and females of 18-60 years accounted for post size 2 having a diameter of 0.85mm.Group B(post size-2 having diameter of 0.85mm) showing greater preferences than other groups.There is a significant difference among groups(p value is 0.03<0.05, Chi Square test).

Figure 3:

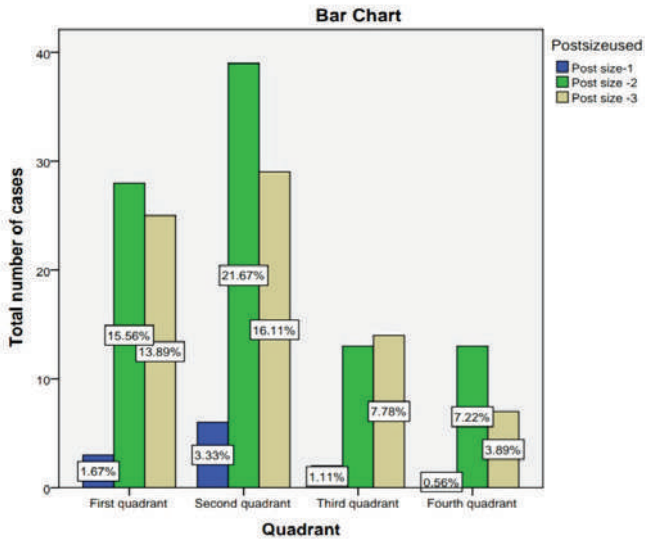


Figure 3-Bar graph showing correlation between teeth number and post size. In the graph, X axis represents the teeth number of the patient and Y axis represents post size used. In this graph, depending on the frequency of post size used based on teeth group, maximum cases in all quadrants accounted for post diameter 2 having a diameter of 0.85mm except in the third quadrant in which post diameter 3 having a diameter of 1.05mm is preferred.Group B(post size-2 having diameter of 0.85mm) showing greater preferences than other groups.There is a significant difference among groups(p value is 0.02<0.05, Chi Square test).

Achieving a post diameter that is strong to support the occlusal loads and does not interfere with the resistance of the remaining tooth structure is one of the major aims in rehabilitation. The comparison of different post thickness (1 and 1.5mm) showed no statistically significant differences in both materials[13]. This results suggests that when restoring anterior endodontically treated teeth, thin post can be indicated. Thus, with a minimum preparation of root canals, the capacity of reinforcement and the resistance to fracture is maintained[14].

Studies showed that a smaller post diameter might be used to avoid excessive wear of inner root dentin during the post size preparation once the amount of remaining dentin wall around the post is directly related to fracture resistance of tooth[15].However some studies indicated that posts with increased diameter were more resistant and provided more resistance to restored teeth and led to less stress distribution of remaining dentin. Another study indicates a more conservative root canal preparation being a 1mm size (one third of the root) able to perform proper resistance[16].

Understanding the biomechanical factors that affect the ability of post to retain a restoration and to protect the remaining tooth structure is essential for the design of post core systems[17]. Parameters such as length,diameter,configuration,surface roughness, material used in the post affects its retention and strength[18].

Parallel sided posts have more retention than tapered posts and longer, wider the posts, greater is its strength and retention. Yet,over enlargement of the channel for insertion of large diameter post may weaken the root because of loss of dentin and results in root fracture under functional load[19].

Stress also increases as the post diameter increases. Therefore the need to preserve and protect the remaining dentin for maximum root strength is equally as important for retention[20].Hock stated that strength of root is directly related to bulk of dentin. Trabert found that teeth with 1.25mm diameter steel dowels were less prone to fracture than either untreated control teeth.

Colmon suggested a maximum diameter of 1.1mm and recommended that dowel diameter be one third the diameter of the root. Standlee proposed that at least 1mm of sound dentin be maintained around the entire circumference of channel.In addition, a sufficient buccal dentin wall must be conserved in maxillary anterior teeth because of its function as fulcrum towards horizontally directed force[21].

Dowel channels with 1 mm buccal dentin walls were more prone to fracture. Two patterns of root fracture were identified[22] such as Oblique fracture, Horizontal fracture at post's end.

Dowel channels with 3mm buccal dentin walls generally failed due to cement failure rather than root fracture[23].If remaining buccal wall at the entrance of dowel channel is 1mm/less, a parallel sided dowel form should be avoided in tapered root to prevent perforation/ undermining of dentin wall at dowel's end. It may also produce root fracture at the region of dowel's end under horizontal impact[24].

The influence of remaining coronal structure on the survival of root canal filled teeth has been noted to be at greater extent.Finally among the multiple factors that have influenced the prognosis of restorations with posts, excessive removal of root dentin when preparation decreases resistance to fracture and it should be avoided.It is similar to many factors that influence the endodontic therapy such as proper disinfection, shaping, placement of intracanal medicament[25–30].Similarly, many factors influence the vital status of pulp in case of inflammation, traumatic injury,calcified canal[31,32].The pulpal diagnosis should be made properly with advanced armamentarium[33,34].

Many luting cements used for post and core cementation have many advantages such as aesthetic property[35,36], proper adaptation of the post to the walls.

To avoid a wedge like action from a tapered dowel, the dowel and core should have a positive stop/seat. This can be obtained from core design well adapted undersurface, perpendicular to the root axis, to prevent the dowel from entering the canal beyond the predetermined limit[37].

Limitations

The limitations of this study were confined to a smaller number of populations.For comparing between

maxillary, mandibular premolars, criteria should have been different as the canal anatomy differs.

Future scope

The future studies can focus on the sizes of prefabricated metal posts in relation to molars with a larger population included in comparison.

Conclusion

Within limitations of this study, it can be concluded that the prefabricated posts of size 2 having a diameter of 0.85mm were highly preferred in relation to endodontically treated premolars with prefabricated metal posts.

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Role of MMPS in Periodontal Diseases

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Abstract

Matrix metalloproteinases (MMPs) belong to a family of structurally related zinc-dependent proteolytic enzymes that are known to play a key role in the catabolic turnover of extracellular matrix (ECM) components. Research studies to date have indicated that MMPs regulate the activity of several non-ECM bioactive substrates, including growth factor, cytokines, chemokines and cell receptors which determine the tissue microenvironment. Disruption of the balance between the concentration of active metalloproteinases and their inhibitors (TIMPS) may lead to pathological changes associated with uncontrolled ECM turnover, tissue remodeling, inflammatory response, cell growth and migration. This brief review presents some information on MMPs role in inflammatory, metabolic and cancer abnormalities related to the salivary glands, as well as MMP- related aspects that lead to the formation of human dentinal caries lesions. In oral diseases, the most relevant biological fluid commonly used for diagnosing periodontal diseases in saliva. Conventional treatment successfully reduces the levels of MMPs inhibits the progressive breakdown of gingival and periodontal ligament collagens.

Keywords: *Matrix Metalloproteinases; Periodontal Diseases; Tissue Inhibitors.*

Introduction

Periodontal disease is a common, complex, inflammatory disease characterised by the destruction of tooth supporting soft and hard tissues of periodontium, including alveolar bone and periodontal ligament[1]. Although the inflammation is initiated by bacteria, the tissues breakdown events that lead to the clinical signs if disease result from the host inflammatory responses that develop to combat the challenge presented by the subgingival biofilm.

Extracellular matrix (ECM) macromolecules play a key role in development and morphogenesis. Matrix metalloproteinases are also called matrixins, and are an important family of metal-dependent endopeptidases responsible for the degradation of ECM components. Expression of the 28 matrixins genes in humans is transcriptionally controlled by inflammatory cytokines, growth factors, hormones and cell-cell and cell-matrix interactions. Matrixins activities are also regulated by the activation of the precursor zymogens and inhibition by endogenous inhibitors, tissues inhibitors of metalloproteinases(TIMPs). Thus, the balance between MMPs and TIMPs are critical for the eventual ECM remodeling. Previously our team had conducted numerous original studies [2-9] and surveys [10-16], over the past 5 years. Now we are focusing on applying this knowledge to write the review on new advancements in the field.

CLASSIFICATION OF MMPS

Based on the substrate specificity MMPs are classified into the following types [10,17] They are Collagenases-MMP-1, the substrates present in it are col-1,2,3,6,8,10 and gelatin, its specific role is over expression in the liver effectively attenuates fibrosis and causes hepatocyte proliferation. MMP-8, the col-1,2,3,7,8,10 and aggrecan, its specific role is promoted leukocyte infiltration in TNF- induced acute hepatitis. MMP-13, the substrate used are col-1,2,3,4,9,10 and gelatin, its specific role in liver diseases are acute liver injury and accelerates liver fibrosis. MMP-2, the substrates are

gelatin, col-1,2,3,4,7,10, its specific role is expressed during liver ischemia. MMP-9 , the substrates used were col-4,5 and gelatin, their role is expressed during Ischemia. Stromelysins are MMP-3, the substrate used are col-2,4,9,10 and gelatin, its specific role is expressed in hepatocellular carcinoma. MMP-10, the substrates are col-4,laminin, fibronectin and elastin, its specific role are hepatocellular carcinoma, MMP-11, the substrates are col-4, fibronectin, laminin, its specific functions are expressed in normothermic ischemia injury. MMP-19 the specific role of it is promoting TGF-p signalling in development of liver fibrosis. Matrilysins contains MMP-26 and MMP-7, its substrates are fibronectin, laminin, Col-4, gelatin and its function is expressed in biliary atresia fibrosis. Membrane type MMPs contain MMP-14, which has Gelatin and fibronectin as substrates and its role is shown in highly invasive hepatocellular carcinoma and MMP-15 and MMP-16 also contain the same substrates as MMP-14 but their role is expressed only on partial hepatectomy, MMP-17, MMP-24, MMP -25 are some of the membrane type MMPs. Other MMPs are MMP-12, MMP-20, MMP-22 and MMP 28.

STRUCTURE OF MMPS

MMPs are structurally similar but differ in substrate specifically. The following features are seen in the structure[18], they are single peptide, propeptide, Furin-cleavage site insert, catalytic domain, Fibronectin like repeats, hinge region, hemopexin domain and membrane insertion extension. While primary structures of these metalloproteinases domains have little homology among families, the overall protein folds are similar. A typical MMP consists of a propeptide of about 80 amino acids, a catalytic metalloproteinase domain of about 170 amino acids, a linker peptide of variable lengths (also called the 'hinge region') and a hemopexin (Hpx) domain of about 200 amino acids. Exceptions to this are MMP-7 (matrilysin 1), MMP-26 (matrilysin 2) and MMP-23; they lack the linker peptide and the Hpx domain and MMP-23 has a unique cysteine-rich domain and an immunoglobulin-like domain after the metalloproteinase domain. While the primary structures of these metalloproteinase domains have little homology among the families, the overall protein folds are similar. In 1994 3D structures of the catalytic domain of collagenases (MMP-1 and MMP-8) were determined by X-ray crystallography by several groups and subsequently crystal structures of proMMP-3 lacking the hemopexin domain and the active full-length MMP-1 in 1995. Since then, a large number of 3D structures of MMPs have been determined both by X-ray crystallography and by NMR spectroscopy including full-length proMMP-1, proMMP-2 and the proMMP-2–TIMP-2 complex.

MODE OF ACTION

Mode of action of MMPs are as follows [18,19]

- MMPs may affect cell migration by changing the cells from an adhesive to non-adhesive phenotype and by degrading the ECM.

- MMPs may enter and alter the ECM microenvironment leading to cell proliferation, apoptosis or morphogenesis.

- MMPs may modulate the activity of biologically active molecules such as growth factor receptors by cleaving them or releasing them from the ECM [20.]

- MMPs may alter the balance of protease activity by cleaving the enzymes or their inhibitors.

REGULAGION OF MMP ACTIVITY

The activity of MMP against extracellular matrix substrates is regulated at 4 gates [21]

1) Transcriptional regulation of MMP genes
2) Precursor activation
3) Differences in substrate specificity
4) MMP inhibitors

Alpha-2-macroglobulins, they capture the active MMP by a unique venus-fly trap mechanism activated by cleavage of a bond in the "bait" region. This cleavage leads to hydrolysis of a liable internal thio-ester bond and covalent cross-linking of a nascent glutamyl residue to lysyl side chains exposed on the surface of the attacking proteinase [22]

The first TIMP was described in 1975 as a protein, in the culture medium of human fibroblasts and in human serum, which was able to inhibit collagenase activity. The molecular weight of this protein was later shown to be 28.5kDa. Since then, three new TIMPs have been

discovered in different species, and have been designated TIMP-2,-3,-4, respectively

MATRIX METALLOPROTEINASES AND BONE RESORPTION:

The role of MMPs during bone resorption is not totally clear but new insights suggest that MMPs also play a key role during this event. Osteoclasts are unable to attach to the bone surface if the mineralised bone matrix is covered by an osteoid layer. Osteoid is composed of type 1 collagen, proteoglycans, glycoproteins and native types 4 and 9 collagens.

Osteoblast-derived collagenase (nMP-13) seems to be mainly responsible for degradation of non mineralised osteoid layers covering bone surfaces. MMP-13 is expressed in human breast carcinomas[23], articular cartilage from arthritic patients. Osteoblasts, periosteal cells and fibroblasts during human foetal bone development [24,25] and postnatally in bone remodelling[26]. MMP-13 is as efficient as MMP-1 and MMP-8 in digestion of type 1 collagenase [27,28]. Cleavage of collagen 1 by MMP-13 seems to be the initial step of the entire bone resorption process. Subsequent, denatured collagen fragments are also degraded by gelatinases MMP-2 and MMP-9, it cleaves acid insoluble type 1 collagen at 37 degree celsius and presents strong proteolytic activity against denatured type 1 collagen and type 4 collagen[27]. Other direct evidence of the inhibition of MMPs by chemically modified tetracyclines can prevent bone loss [29,30].

MMP IN PERIODONTAL DISEASES:

In periodontal disease, MMPs play a key role in degradation of ECM, basement membrane and protective serpins as well as in the modification of cytokine action and activation of attachments apparatus is the hallmark of periodontal diseases [31,32]. Organisms like porphyromonas gingivalis and aggregatibacter actinomycetemcomitans do produce collagenases for the breakdown of ECM but they do not help much in periodontal collagen degradation and resident gingival and periodontal ligament fibroblasts produce collagenases that are thought to be involved in normal tissue turnover. The inflammatory reaction associated with periodontitis may damage the surrounding cells and connective tissue structures, including alveolar bone, causing tooth loss[32,33]. Inflammatory cells such as neutrophils and macrophages produce MMPs, with neutrophils being the major source of collagenase and gelatinase in inflammatory diseases such as periodontitis[31]. MMP-3 is effective at degrading proteoglycans and fibronectin. Epithelial cells can also produce elevated levels of these enzymes, which may facilitate the apical migration and lateral extension of the junctional epithelium and the subsequent loss of connective tissues attachment. Inflammatory cells, particularly neutrophils, are thought to play a particularly important role in MMP- mediated PDL destruction [34,35]. Epithelial cells can also produce elevated levels of these enzymes, which may facilitate the apical and lateral extension of junctional epithelium[36].

ESTIMATION OF MMP IN PERIODONTAL DISEASES:

Matrix metalloproteinases can also process various bioactive non matrix substrates, such as cytokines, chemokines, growth factors and immune mediation, thereby mediating both anti and pro-inflammatory processes. Therefore, the levels of matrix metalloproteinases should not be interpreted solely as surrogate markers of time destruction but also as part of physiological or anti-inflammatory defence[37]. Based on these biochemical or immunological findings, the oral fluids have been a target for extensive research on diagnostic utilisation of MMPs and their regulators as potential candidates for PDL diseases. Various proteomic techniques are being used to estimate the levels of these MMPs.

ROLE OF MMP INHIBITORS IN PERIODONTAL DISEASES:

Inhibiting MMPs can be an effective adjunctive treatment in the management of periodontitis as they are important mediators in the connective tissue breakdown in periodontitis. Inhibitors fall into three categories:

1) Collagen peptidomimetics and nonpeptidomentics- a. Peptidomimetic MMP inhibitors, b. Batimastat, C .Marimastat, d. Non peptidic MMP inhibitors. E. BAY 12-9566, f. AG3340, g. BMS-27529, h.CGS-27023A.

2) Tetracycline derivatives - a. Doxycycline, b. Col-3(metastat)

3) Bisphosphonate.

A disturbed balance between MMPs and TIMPs might contribute to the disease process in degenerative diseases. In some cases, the occurrence of MMPs and TIMPs in body fluids such as saliva, gingival cervical fluid(GCF) or serum provides additional information about the progression of the disease. In health periodontal tissue, TIMP levels are generally higher than in inflamed periodontal tissues, in which MMP levels exceed TIMP levels. The more severe the information the higher the concentration of MMP. The inhibition of MMP expression or activity, or increased TIMP expression might reduce tissue destruction in periodontitis.

Different inhibitors include

a. Alpha -2- macroglobulins

b. Tissue inhibitors of metalloproteinases

c. Inhibiting antibodies

d. Synthetic inhibitors

During inflammation, however, the later high molecular weight protein may escape the vasculature and also function in the extracellular matrix. Multiple synthetic peptides have been formulated in an attempt to synthesise more specific chelators including hydroxyzine acid derivatives.

Conclusion

MMPs are important components in many biological pathological processes because of their ability to degrade ECM compounds. The use of a host modulatory agent such as MMP inhibitor can assist with conventional treatment for periodontitis and, when used adjunctively, can enhance and make clinical therapeutic responses more predictable in more susceptible patients.

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Prevalence of Midline Fracture in Mandibular Complete Denture

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Abstract

Patients wearing complete dentures whether both arches or a single mandibular denture pose a great problem of midline fracture. They are more evident in patients who have retained their natural maxillary teeth. Few reports are available on the incidence of mandibular complete denture and its management. This study aims to evaluate the prevalence of midline fractures in mandibular complete denture .This study was designed as a retrospective clinical study. All the patients with Complete dentures who have been treated at the department of Prosthodontics from June 2019-March 2020 were included in the study. A total of 915 patients wearing complete dentures in the age group 40-80 years were included in the study .Various parameters namely midline fracture , denture age and the arch were evaluated.The data was analysed and results were recorded. In the present study , out of 915 only 11 patients reported with complete denture fractures predominantly within 5 years of denture use. Midline fracture was found to be more prevalent in mandibular complete denture than maxillary complete denture.Patients wearing dentures for less than or equal to 5 years had a higher frequency of denture fracture in both maxilla(0.22%) and mandible(0.66%).The prevalence of midline fracture in mandibular complete denture was found to be 17.2%.

Keyword: *Complete denture, Fracture , Midline , Mandible*

Introduction

Complete or partial edentulism is a result of poor oral hygiene [1] , periodontal diseases [2] and dental caries[3] eventually leading to loss of teeth.[4].Most of the individuals wearing complete dentures are elderly patients and a few who have less remaining natural teeth. Complete dentures serve as an artificial replacement of edntulous patients[5]. It not only provides esthetic function but also phonetics as well as masticatory functions in the complete denture wearers.[6] It has been considered a one of the frequent prostheses fabricated for patients with high levels of satisfaction.[3,7]

Various materials have been used for the fabrication of complete dentures. Wood , ivory, bone and alloys have been used in the ancient times[8]. More recently acrylic dentures are the most widely used prosthesis due to their aesthetic value and ease of manipulation.More advanced technology such as CAD-CAM [9]have been developed over the past few years, where the manufacturing process reduces the chair time. [10]Commonly complete denture final insertion takes at least 5 appointments but computerized complete dentures need two clinical appointments ,clinical data collection and second appointment involved placement of computer-engineered complete denture [6,11,12]. A third day at the clinic could be possible for a trial placement. Acrylic material or

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polymethacrylate is the most common denture material used for the fabrication of partial or complete denture [13,14], due to good esthetic characteristics[13], low water absorption [15]and,adequate strength [16]easy repair and simple processing technique. Disadvantages include porosity,presence of residual monomer ,increased finishing time, brittle and unevenness.[17]

Over the years of denture usage , they become more resistant to fractures , especially if not maintained properly [16,18]. Some denture wearers do not clean their dentures, use them even at night making it more feeble[19]. Many causes of denture fractures have been identified [20], they can be extraoral like accidents from falling or trauma or accidental dropping.[17,21] Or they can be subjected to areas of stress maximally intraorally, improper occlusion, placement of artificial teeth in the buccal slope of,the ridge or against the palate, pressure from opposing natural teeth, poor retention and stability, and resorption of residual ridge, presence of high,frenal attachments, prominent mid palatine suture, palatal or lingual torus, hard or soft tissue,undercut.[22]

The proposed classification system is as follows.[23,24]

- Class I: The fracture line passes through the midline between the central incisors extending to the posterior extension.
- Class II: The fracture line passes through other than midline in a diagonal direction extending to the posterior extension.
- Class III: The fracture line is moon shape passing through the labial or buccal flange.
- Class IV: The fracture line passes through dentoalveolar structure of the denture, involving two or more teeth.
- Class V: The fracture of a part of artificial tooth or separation of a single tooth from denture

The midline fracture is often a result of flexural fatigue. [25]Other causes that could potentially cause a fracture are defects in the denture that can be due to malaligned laboratory procedure.[26] It can be thin denture base, porosity, air bubbles, deep scratches etc. There proposed classifications for denture fracture are as follows.[27] However we are going to base this study on midline fractures that are class 1. The current study was done with the objective to find the prevalence of midline fracture in mandibular complete denture, so that preventive measures can be undertaken to avoid new incidence and improvement in the prosthesis can be made.

Materials and Methods

This is a retrospective study that aims in determining the prevalence of midline fracture in mandibular complete dentures. All the patients wearing complete dentures who were treated at the Department of Prosthodontics, Saveetha Dental College and Hospital ,Chennai between June 2019 and March 2020 were selected for the study. A total of 915 patients were identified.. The approval for this university setting was obtained from the institution's aesthetics board.Case Sheets that were duplicated, non specific samples and filled with incomplete information were eliminated with the verification of photographs. The study was reviewed by 2 reviewers and was cross verified. Data obtained were chronologically recorded and tabulated in the excel sheet. The dependent variables were age, gender, ethnicity and independent variables were presence of midline fractures in mandibular and maxillary complete denture as well as the denture age. The data obtained were further analyzed using Statistical Package for Social Sciences version 22 (SPSS).Percentile, frequency and correlation and association type of analysis were employed in this study.The results were tabulated.Chi square test was used to detect the significance $P<0.005$ was considered statistically significant.

Results and Discussion

Total number of patients wearing Complete dentures were 915 out of which 11 reported with the complaint of fractured denture. The prevalence of midline fracture was found to be 17.2% and mandibular midline fracture was more common than maxillary fractures.

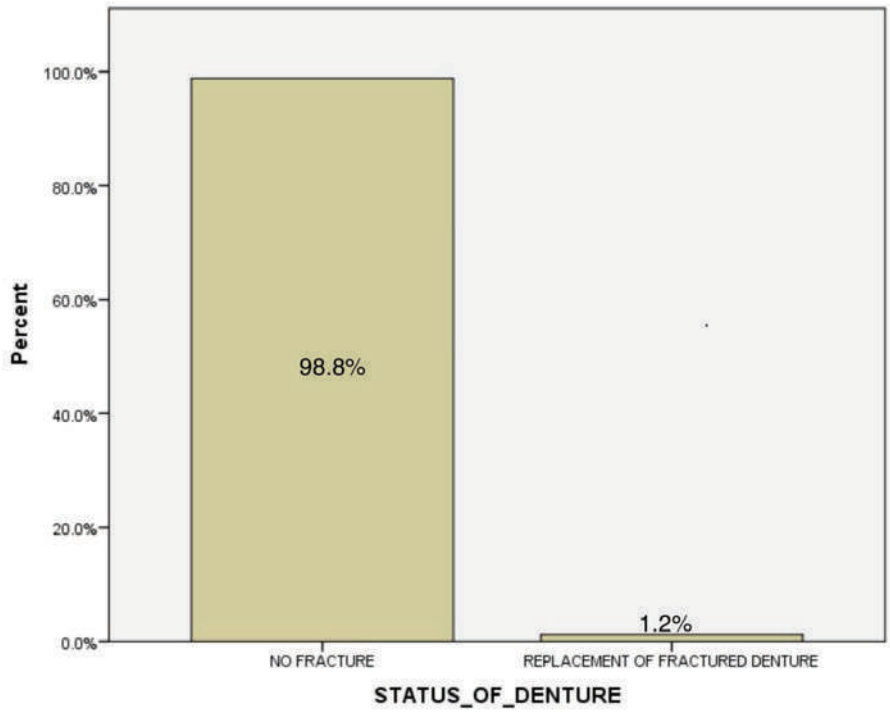


Figure 1 : Represents the status of the complete denture among the edentulous population visiting the hospital. X axis denotes the status of denture as no fracture and replacement of fractures denture and Y axis denotes the percentage .Majority of the patients(98.8%) did not have any complaints with the denture and only a few required replacement of fractured denture(1.2%).

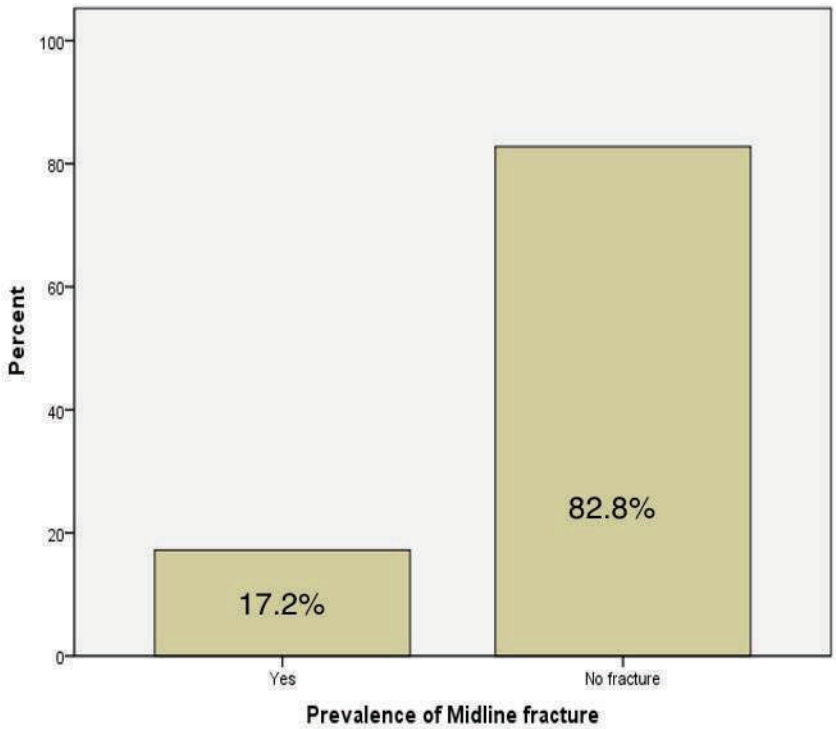


Figure 2 : Shows the prevalence of midline fractures among complete denture wearers. X axis denotes the prevalence of midline fracture and Y axis denotes the percentage . Midline fracture was present in 17.2 % of the population that reported to the hospital .

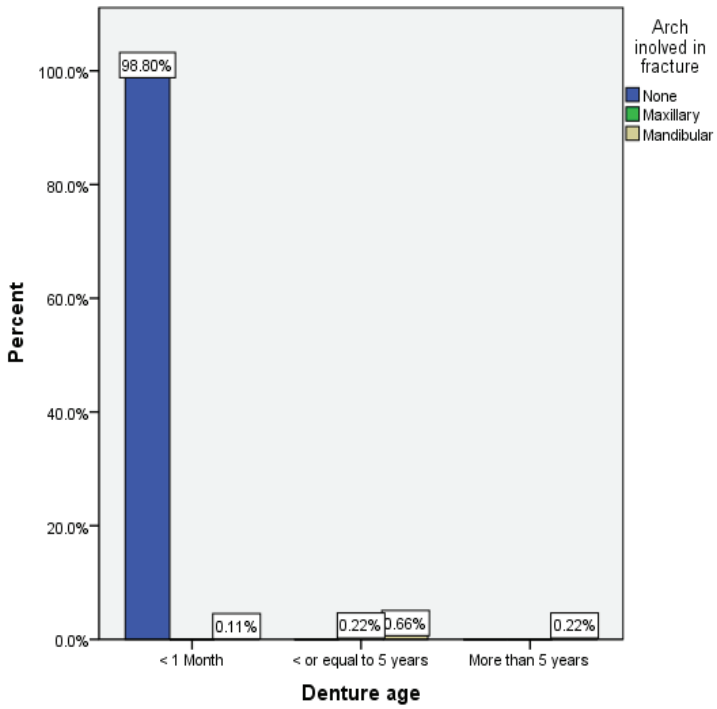


Figure 3: Bar graph represents the association between the Denture age and occurence of midline fracture in maxillary and mandibular denture. X axis represents the Arch of the dentures and Y represents the percentage of denture age worn by the edentulous patients. Blue color indicates that none of the arches were having midline fracture , green and yellow bars represent the maxillary and mandibular arch involving midline fractures respectively. Patients wearing dentures for less than or equal to 5 years had a higher frequency of denture fracture in both maxilla(0.22%) and mandible(0.66%) than those who wore dentures for more than 5 years(mandible, 0.22%) . Chi square test was done and association was found to be statistically significant.P value = 0.001 (<0.05).Mandibular complete denture was found to be more prevalent in the incidence of midline fracture in all the denture age groups.

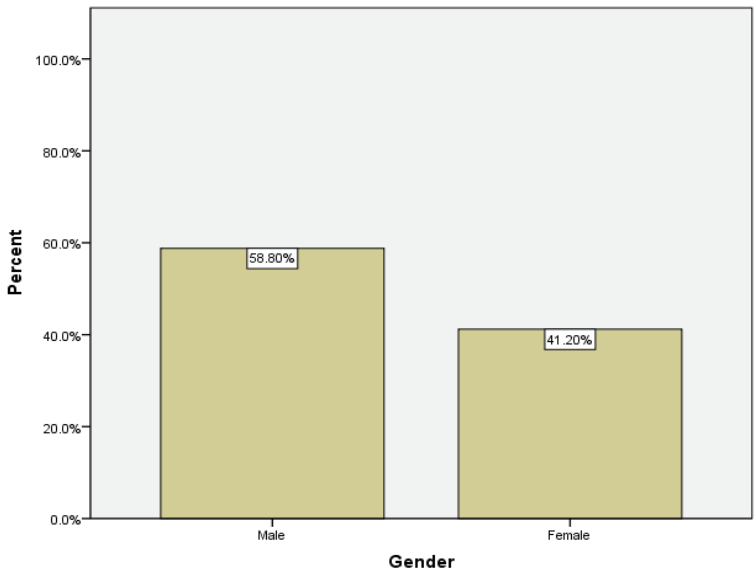


Figure 4: Represents the gender distribution of complete denture wearers. X axis denotes the Gender and Y axis the percentage. Men were more prevalent with 58.8% than women who were 41.2%.

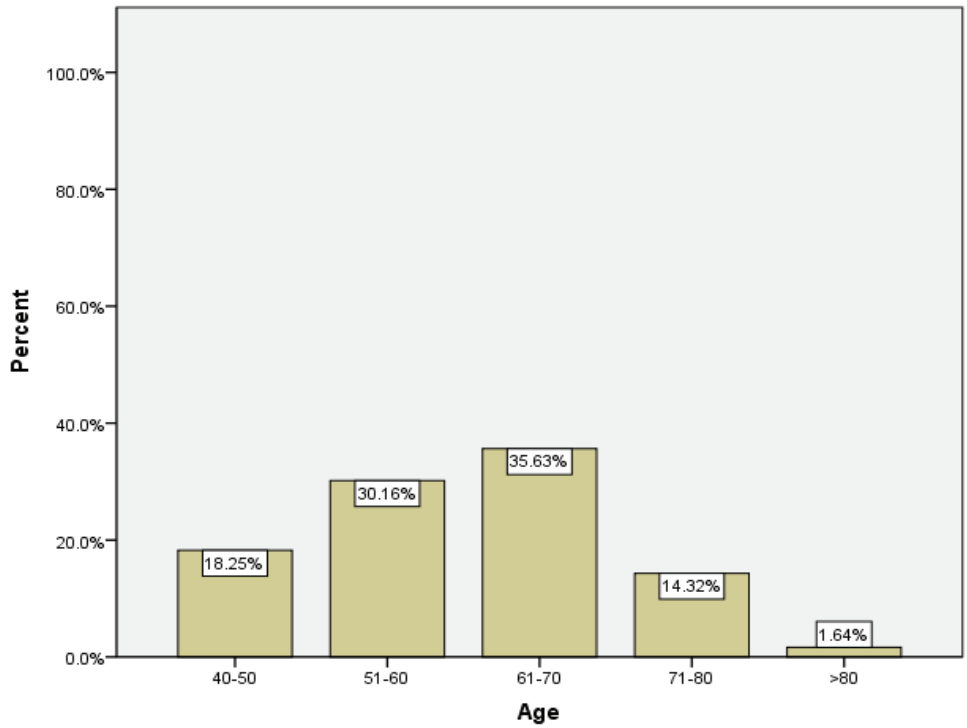


Figure 5: Represents the age distribution of complete denture wearers with X axis representing the age group and Y axis representing the percentage. Most common age groups wearing complete dentures were 61-70 yr olds (35.63%) followed by 51-60(30.16%), 40-50(18.25%), 71-80 (14.32%) and > 80 (1.64%).

Even though many developments have been made in the dental materials, techniques, and fabrication of dentures, fracture of the prosthesis and its incidence still remains as a major problem.Patients who wear complete mandibular denture against maxillary natural teeth or with maxillary complete denture often face the problem of midline fracture in their dentures.[28]

In the present study it reveals that the age group 61-70 years showed the majority of the patients wearing complete dentures reported with midline fractures. However age of the individual does not relate to the occurrence of fractures. Moreover it could be the prevalence of complete denture wearers in that age. According to Raina et al [29]where the demographics of the patients wearing complete denture showed participants > 60 years of age.

Denture age could be an important factor in considering the cause of fractures in complete dentures. In this study , patients wearing complete dentures for less than 5 years were found to be more susceptible to midline fractures. Similar results were obtained from a survey conducted by Ali et al[30] . However ,Shakir et al stated that the majority of the denture fractures occur between 6 to 12 months.[30,311]. Dimensional failures in the laboratory during fabrication of the denture or a weak denture material could be the reason for failure of dentures . It could also be the result of breakdown of the material and fatigue phenomenon.[32]

There was slight male predilection in our study. This result was also similar to the study of Jalil et al , where there was a significant relationship between type of denture fractures with gender P=0.001. The incidence of midline fractures were observed in all of the reported fractures in the mandibular complete dentures and none among the patients wearing maxillary complete dentures. Choudhary et al revealed similar results in their study where class 1(midline fracture)was the most common denture fracture[24].

In the current study, in comparison with maxillary denture, the midline fracture was more prevalent in mandibular complete dentures . The lesser surface area and thickness of the mandibular denture in the

central part could be the reason for the fracture[24,33]. The instability according to Dan et al was the most common cause of lower complete denture midline fractures.[34] On the contrary , Smith has reported maxillary Complete denture midline fracture is usually after 3 years in clinical use. The most commonly affected site could be the incisal notch. It appears that the incisal notch has a significant role in fracture of upper complete denture. Fracture near the midline in the maxillary denture could also contribute to cyclic deformation during masticatory function[33].

Sherry said most of the fractures may be due to accidental dropping the denture. One limitation of this study is the inability to identify the cause of fracture. Poor occlusion , heavy occlusal contact and faulty teeth setting outside the ridge are some of the other causes of denture fracture[35].

Auto Polymerized, heat , light and microwave resins are some of the repairing techniques for fracture of a denture. Auto polymerised resin is considered the most popular due to its easy handling and quick repair due to low cost. Further studies should be conducted to identify the cause of fracture in a larger sample size[36].

Conclusion

This study showed maximum prevalence of midline fracture in mandibular complete denture among the age group 61-70 years. Therefore proper patient education on the handling of newly fabricated dentures should be conducted to reduce accidental mishaps along with definite prosthodontic guidelines and principles should be followed in the construction of the prosthesis to avoid new incidences of midline fractures.

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Conflict of Interest : There is no conflict of interest

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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A Retrospective Analysis of the Most Frequently Infected Maxillary Primary Tooth in Pediatric Patients Visiting A University Hospital in Chennai

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Abstract

An abscess is defined as a collection of pus that has built up within the tissue of the body. Space infection can be defined as a rapidly spreading, bilateral, indurated cellulitis which usually occur in the suprahyoid soft tissues, the floor of the mouth, and both sublingual and submaxillary spaces without abscess formation.This research comprehensively studies and assesses the most commonly associated primary tooth in the maxillary arch of a patient which gets commonly infected by an abscess or space infection.Since abscess and space infection can affect the daily routine of an individual especially in the Paediatric population which shows more effects. Understanding, identification and early diagnosis helps in preventive care for patients before complications.This is a retrospective descriptive study in Chennai population. Our study is an institution based retrospective descriptive study carried out in between the month of June2019-March2020. Data of patients aged below 13 years who came with chief complaints of pain,infection and who had undergone Pulpectomy and extraction procedures were included in the study sample. SPSS software is used for statistical analysis. Based on statistical analysis results were tabulated. Most prevalent Primary tooth in Upper Arch to be infected with Abscess or Space infection is found to be 54. Around 347 out of 1100. Primary maxillary right first molar is the most prevalent tooth to be affected with abscess/space infection. Within the limitations of the study , the most frequently associated primary tooth in maxillary arch with space infection or abscess is maxillary right first primary molar [54] which shows direct correlation with the age group of 7-10 years.

Keywords: *Primary Tooth, Abscess, Space infection, Children, Extraction, Pulpectomy*

Introduction

An abscess is defined as a collection of pus that has built up within the tissue of the body[1]. Space infection is defined as rapidly spreading, bilateral, indurated cellulitis which usually occur in the suprahyoid soft tissues, the floor of the mouth, and both sublingual and submaxillary spaces without abscess formation. Although not a true abscess, it resembles one clinically and is treated similarly. Abscess and space infection were usually caused by a bacterial infection. Orofacial infections are one of the most common health care concerns in children and are a frequent cause for dental consultation worldwide [2,3]. Microorganism related to oral cavity are the most important causative agents in osteomyelitis, aspiration pneumonia, bacterial endocarditis, halitosis, periodontal disease, abscesses, pulpitis, dental caries, space infection in children, cerebral infarction, coronary heart disease, and preterm low birth weight [4–6]. Fluoride application reduces occurrence of dental caries and caries related infections [7,8]. In order to prevent

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systemic involvement Early diagnosis, management and recognition of orofacial infections in children play an important role. [9,10]

Most orofacial infections are found to be odontogenic in origin, but others are self-limiting in nature [11,12]. Odontogenic infections have polymicrobial sources with mixed aerobic bacteria and anaerobic bacteria [13]. Paediatric orofacial infections, disease has more ability to progress in a rapid manner, by producing significant systemic symptoms including fever, dehydration, and airway compromise[14]. Because of the possibility of progression to systemic disease, early management and recognition of orofacial infections in children is necessary [15,16]

In children, unlike adults, the location of various anatomic infections is thought to be helpful to guide for proper diagnosis and management and treatment [17,18,19]. In case of early diagnosis of abscess and space infection can be treated with endodontic procedure-pulpectomy. Success of pulpectomy procedure depends on instrumentation[20] and behaviour management. Usage of rotary instruments in pulpectomy procedure consumes less time [21,22,23,24,25]. In severe cases tooth has to be surgically extracted [26,27]. Since abscess and space infection can affect the daily routine of an individual especially in the paediatric population which shows more effects. Understanding, identification and early diagnosis helps in preventive care for patients before complications.This retrospective descriptive study limited to the Chennai population. This research comprehensively assesses the most commonly associated primary tooth in the maxillary arch of children which gets commonly infected resulting in an abscess or space infection.

Materials and Methods

Our study is a retrospective descriptive study. For this study we got approval from the Institutional Ethical Review Board (SDC//SIHEC/2020/DIASDATA/0619-0320) and our study was carried in a university hospital setting in a major city of Tamil Nadu, South India. After getting approval Case sheets of 5516 paediatric dental OP patients aged below 13 years who visited the University Hospital in between the month of June 2019 and March 2020 were considered. In that 5516 around 2527 case sheets of patients with chief complaints of pain, irritation in the oral cavity underwent pulpectomy and extraction procedure were taken into study. 1100 case sheets were obtained by filtering those patients who aged below 13 years who visited the University Hospital with chief complaints of pain, irritation in oral cavity underwent pulpectomy and extraction procedure especially in maxillary arch were analysed. The collected data were filtered and a case sheet of patients below the age of 13years was considered.

These 1100 data were analysed , even photographs, radiographs in the image gallery of the patient online case sheet records were verified. In order to reduce, minimise and avoid the occurrence errors, verified case sheets were once again cross verified by another examiner. Verified case sheet details were tabulated in excel under columns of age, gender, patient with abscess/ space infection , maxillary arch tooth number in FDI notation ,treatment done by dentist to patient. Excel tabulated data is transferred to SPSS software for statistical analysis. Descriptive statistics and chi square tests were conducted to evaluate differences between groups with significance level at 95% confidence interval ($P<0.05$).

Result and Discussion

Based on analysis results were obtained and tabulated. The frequency distribution of age of patient where around 315 patients fall between the age group of 7-10 years , 296 patients between 11-13 years, 255 patients between 0-3 years and 234 patients between 4-6 years (Figure 1). Considering the percentage of Age distribution of patients in our study 28.6%(315nos) lie between 7-10 years and 26.9%(296nos) lie between 11-13 years. Which goes in relation with study done by Ingle NA, et al, where 6 to 10 years - 9.46%[28] there are several reasons like dental caries, infection,trauma for high prevalence in this age group.

The frequency distribution of gender of patient where around 589 patients were male and 511 patients were female (Figure 2). On seeing the percentage of gender distribution in our study around 53.5%(589nos) were males and the remaining 46.5%were females=46.5% who had infection. This is in relation with a previous study done by Manal Al-Malik et al where 64.8% males and 35.2% females had infections[5]. The reason stated was boys are hyperactive, prone to infection, etc.

The frequency distribution of individual tooth considered in our study (Figure 3) where 357 nos -54, 247 nos -51, 207 nos- 52,84 nos-62 , 63 nos- 62, 52 nos -53, 48nos- 55, 31 nos – 64 , 11nos- 65 and 6 nos -63 is involved. The frequency distribution of type of infection associated with tooth in the study (Figure 4) where around 994 teeth are not associated with any infection, 136 teeth had abscess and 20 teeth had space infection. On seeing the percentage of Infection distribution around 85.8% cases had no infection, 12.4% had Abscess and 1.8% had Space infection. This is in relation with previous studies where the authors say orofacial infections constitute approximately 20% of total maxillofacial and oral surgery admissions [5,29].

The frequency distribution of the type of treatment done by dentists to patients in the study (Figure 5)where 628 patient teeth were extracted, 466 patients were treated with pulpectomy and 6 patients didn't go for any treatment. The gender wise categorisation of frequency distribution of type of infection (Figure 6) where 516 male had no infection, 63 male had abscess and 10 had space infection, 428 female had no infection, 73 female had abscess and 10 had space infection. The Age wise categorisation of frequency distribution of type of infection (Figure 7) where about 119 patients were present with abscess and 14 patients present with space infection between the age group of 7-10years.

The individual tooth wise categorisation of frequency distribution of infection in patients (Figure 8) where infection is more prevalent in 54. Most prevalent primary tooth in the upper arch to be infected with abscess or space infection is found to be 54 (Figure 8).

Primary maxillary right first molar is the most prevalent tooth to be affected with abscess/space infection. On seeing percentage of most frequently associated primary tooth in maxillary arch with space infection or abscess is maxillary right primary molar =32.5%, maxillary right primary central incisor=22.5% maxillary right primary lateral incisor= 18.3% This is in relation with previous study where the author says in almost 84% cases primary posterior teeth were considered to be a major source of infection.

The most commonly affected teeth were the primary first molars[5]. This can be due to mastication where molars play an important role, habits like bruxism, difference in chronological age like 1st primary molar erupt before 2nd primary molar so higher plaque accumulation and lack of prophylactic method and improper brushing in posterior region can be taken as several cause for space infection. Chi square tests were carried for frequency distribution of individual teeth which got infected, age by individual tooth in the study, age by infection, gender by individual tooth association, gender by individual tooth in the Study shows p value statistically significant.

Abscess and space infection in children aged below 13 years can significantly affect the quality of life of children. It also leads to missing school hours and overall well being of the child. Considering the impact caused by abscess and space infection in children early diagnosis and treatment planning plays an important role. Understanding the most commonly affected tooth with infection can help in implementing better preventive strategies, evidence from the present study can be utilized for these purposes. Our study had few limitations such as its a single centered study, subjects were not available for direct examination and we relied on photographs and case sheets of patients.

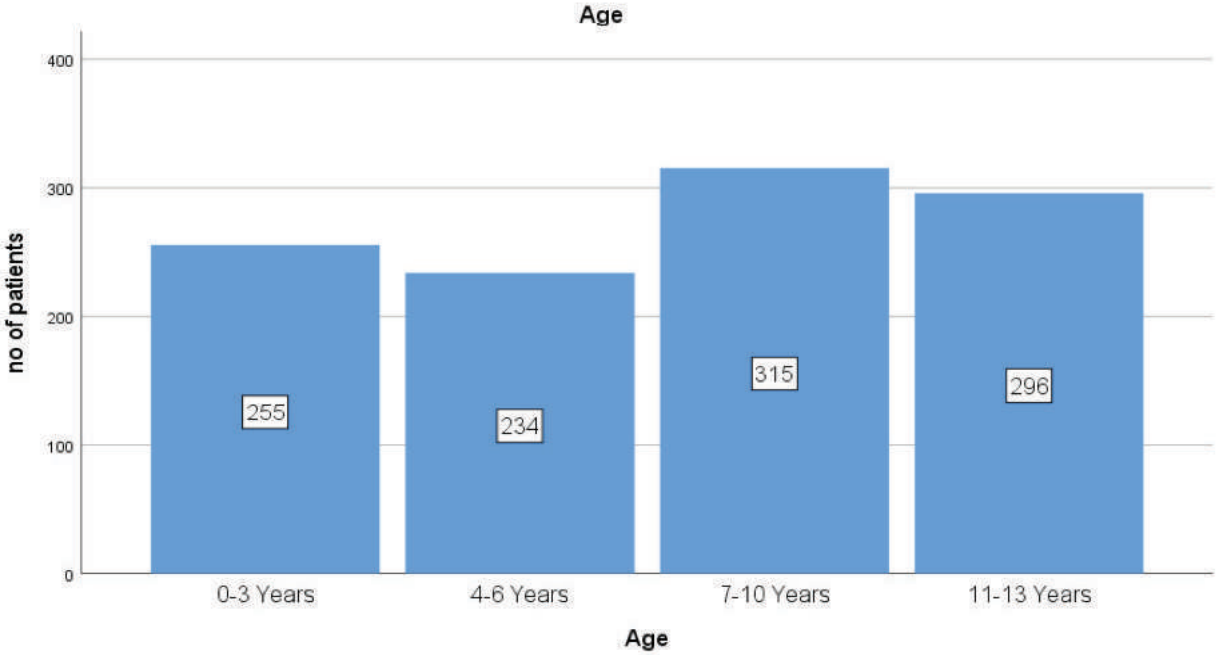


Figure 1: Bar graph representing frequency distribution of age group of children in the sample population. X-axis represents age groups and Y-axis represents the number of children in each age group. Most of the patients who underwent extraction/ pulpectomy procedures were between the age group of 7-10 years (315).

Figure 2: Bar graph representing frequency distribution of gender of children in the study population. X-axis represents gender and Y-axis represents the number of children. Most of the children who underwent extraction/ pulpectomy procedure were males (589) compared to females (511).

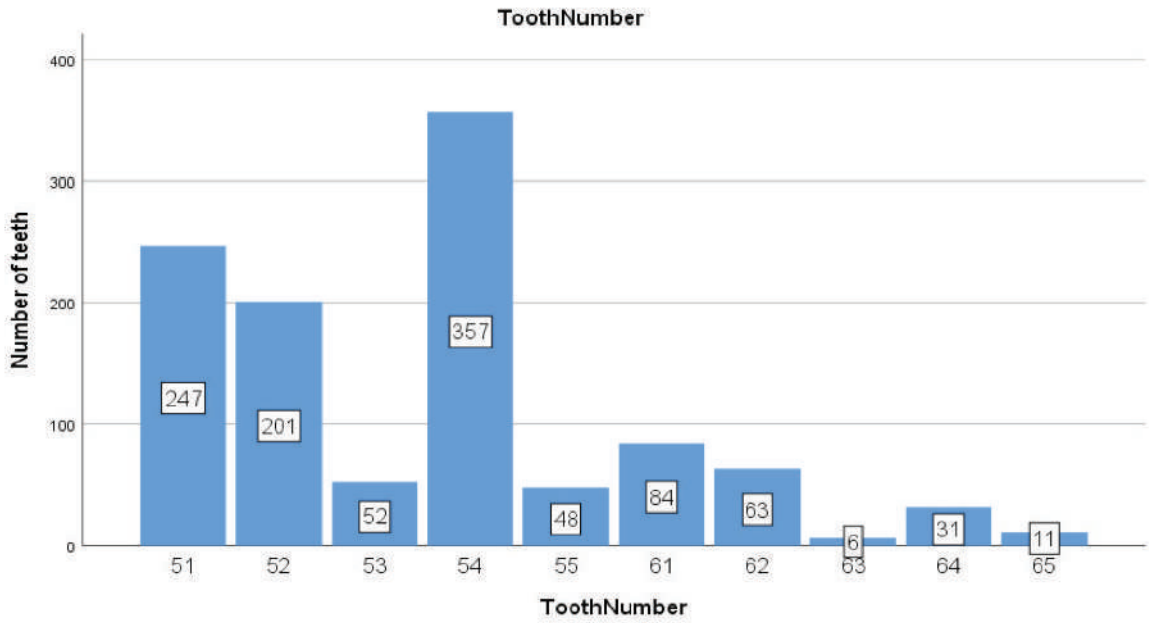


Figure 3: Bar graph representing frequency distribution of individual tooth in FDI notation. X-axis represents tooth number in FDI notation and Y-axis represents the number of times the tooth was involved. Graph shows that most of the children's individual tooth which underwent extraction/ pulpectomy procedures was Maxillary right first primary molar (357).

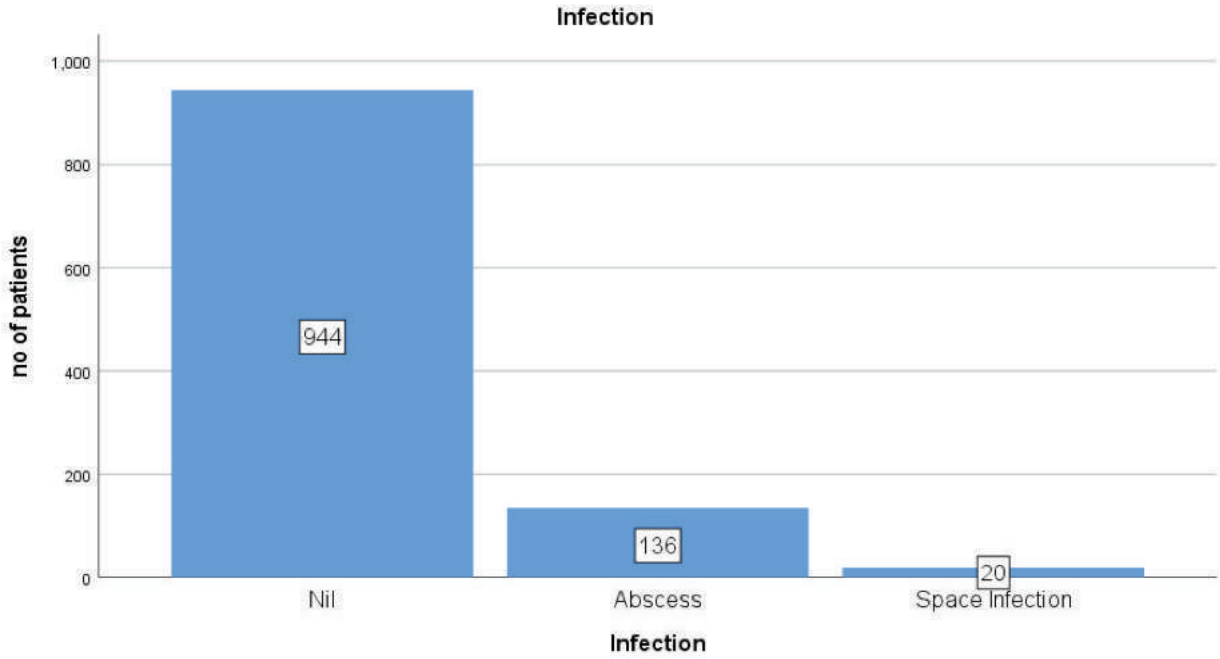


Figure 4 : Bar graph representing frequency distribution of type of infection. X-axis represents the type of infection and Y-axis represents the number of patients. Most of the patients had no infection (944), abscess was seen in 136 children and space infection in 20 children.

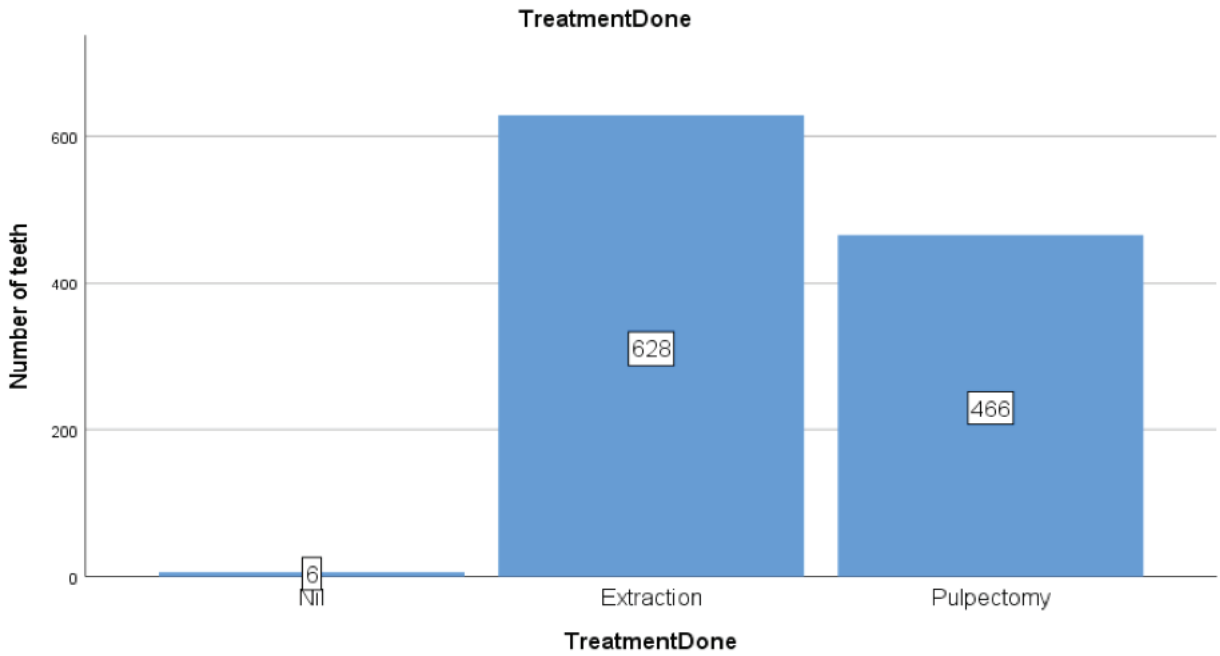


Figure 5: Bar graph representing frequency distribution of type of treatment provided by dentists for children . X-axis represents the type of treatment provided by the dentist and Y-axis represents the number of teeth treated. Most of the children in the sample population underwent extraction (628) followed by pulpectomy (466). For 6 children no treatment was done.

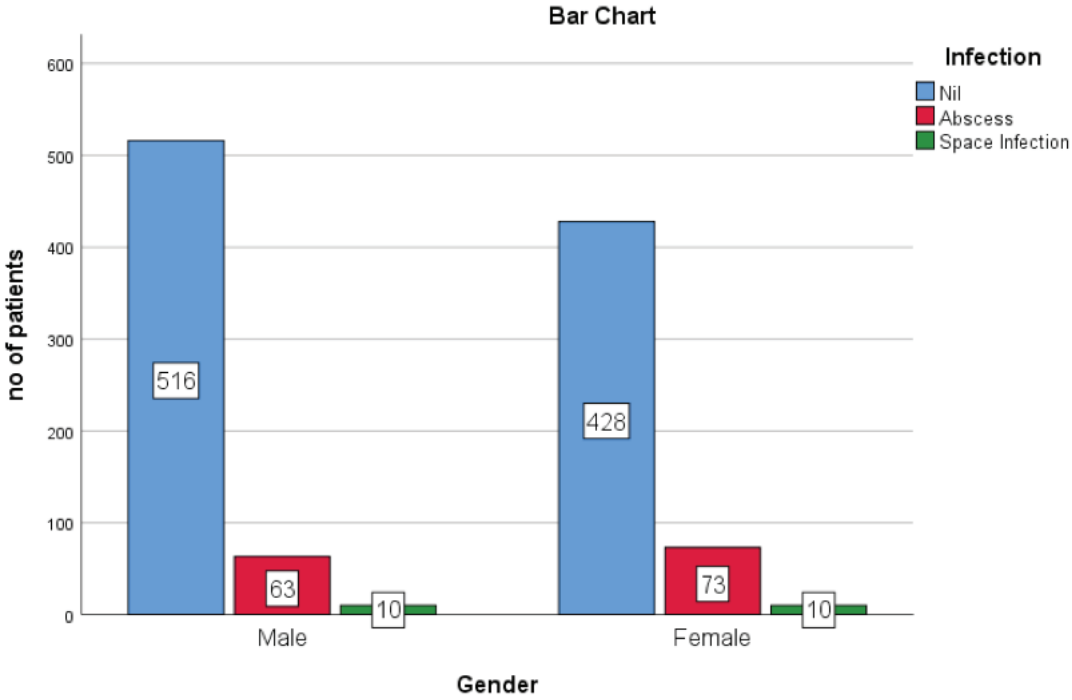


Figure 6: Bar graph representing association between gender and frequency distribution of type of infection . X-axis: gender wise categorisation of frequency distribution of type of infection (blue colour= no infection ; red colour= abscess; green space infection). Y-axis: No.of Patients.There was no difference between boys and girls in type of infection.Chi square test was done and the association was found to be not significant.(p value was =0.180 p>0.05 statistically not significant).

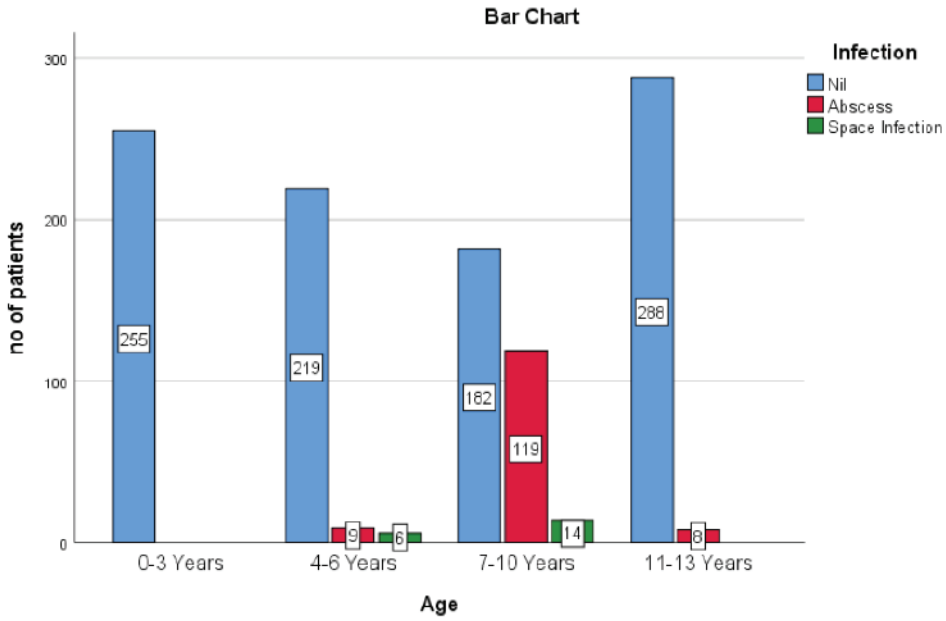


Figure 7: Bar graph representing association between age and type of infection . X-axis represents age groups and x clusters represent types of infection (blue colour= no infection ; red colour= abscess; green space infection). Y-axis: Number of children with different types of infection.There was a difference in occurrence of infection between age groups with higher occurrence among the age group of 7 to 10 years. Chi square test was done and the association was found to be significant.(p value was =0.000 p<0.05 statistically significant).

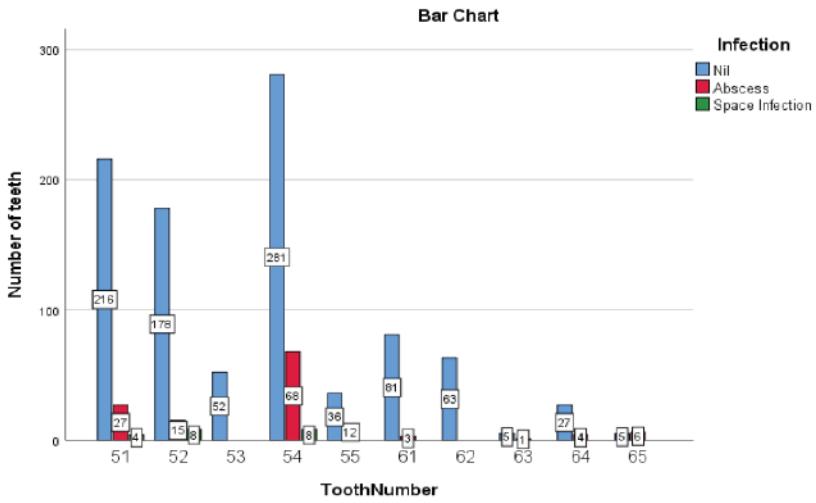


Figure 8: Bar graph representing association between individual tooth and type of infection . X-axis represents tooth number in the FDI system of notation and x cluster shows types of infection(blue colour= no infection ; red colour= abscess; green= space infection). Y-axis represents the number of teeth. Higher occurrence of infection was noticed in maxillary right first primary molar compared to other teeth. Chi square test was done and the association was found to be significant. (p value = 0.000 <0.05 statistically significant).

Conclusion

Within the limitation of the study , Most frequently associated primary tooth in maxillary arch with space infection or abscess: is Maxillary Right First Primary Molar [54] which shows direct correlation with the age group of 7-10 years. 11.5 % of children who underwent extraction or pulpectomy presented with abscess or space infection.

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Conflict of Interest: Nil

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Most Commonly Associated Primary Tooth in Mandibular Arch with Space Infection or Abscess - A Retrospective Study

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Abstract

Oral cavity is considered to be an ideal niche for the growth of microorganisms. Despite the advances in paediatric dentistry infections in the oral cavity remains to be a major problem in children. Infections in the jaws of a child have the ability to spread rapidly due wide marrow space. In children, unlike adults, the location of anatomic infection is a helpful guide for diagnosis and management which is essential to prevent progression.The objective of this study is to determine the most frequently associated primary mandibular tooth with space infection or abscess.The present study was a retrospective observational study conducted in a university hospital located in South India. The sample included Patients below 13 years who underwent extraction or pulpectomy of mandibular primary teeth. Patient records between June 2019-March 2020 were reviewed.This was followed by Excel tabulation of data . Data analysis was done using SPSS software. Descriptive statistics such as frequencies and percentages were elicited and chi square test was done to check association between different variables. The most common infection was found to be abscess 6.7%. The most frequently associated primary tooth in the mandibular arch with space infection or abscess was found to be the primary right mandibular second molar 22% Within the limits of the study, the most frequently associated tooth with infection in the mandibular arch is the right second primary molar and shows correlation with the age group 7-10 years.Based on study results, posterior teeth were more susceptible to infection than anterior teeth.Space Infection and abscess had a male predilection.

Keywords: *Space infection, Abscess, Odontogenic origin,Pediatric patients, Primary mandibular teeth.*

Introduction

Oral cavity is considered to be an ideal niche for the growth of microorganisms. Despite the great advances in paediatric dentistry infections in the oral cavity remains to be a major problem in children[1]. Majority of the orofacial infections are odontogenic in origin, whereas the others are self-limiting in nature. An odontogenic infection may arise from a tooth or its supporting structures are secondary to dental caries, periodontal disease, or pericoronitis .They can range from localised abscesses to deep space head and neck infection[2].

Orofacial space infections of odontogenic origin which are quite common in children originate from partially erupted teeth, traumatized teeth or necrotic pulp [3 4]. However, Seow has stated that developmental abnormalities like dentin dysplasia, dentinogenesis imperfecta and dens evaginatus, may also lead to space infections[5]. The study by Sawle RF et al 1988 reveals that acquired conditions like pre eruptive intracoronal resorption of dentin can result in space infection [6]. Based on previous studies it is found that most of the orofacial

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infections arise as result of neglected dental caries [7,8] . Neglected dental caries in turn lead to exposure of the pulp causing severe pain especially in children[9,10] . Controlling pain and prevention of further caries can be managed by the use of rotary instruments [11 12 13]. Therefore controlling further spread of dental caries is indeed an important measure to prevent the occurrence of oro facial infection [14 15].

Infections in the jaws of a child have the ability to spread rapidly due wide marrow space and also because of the less density of the bones in children [16]. Rapid progression of Infection produces significant systemic symptoms. Moreover, abscess formation and cellulitis are quite exaggerated in children and failure to render correct treatment may result in severe complications like blindness,cavernous sinus thrombosis, septicemia,brain abscess and airway obstruction[17].

Previous studies reveal that oro facial infection is most commonly associated with primary posterior teeth[18 19].Further it also states that submandibular space is frequently susceptible to orofacial infection. However, the majority of studies on space infection and abscess was conducted in relation to maxillary teeth and only a few studies were conducted in relation to the mandibular arch. In children , unlike adults, the location of anatomic infection is a helpful guide for diagnosis and management which is essential to prevent progression[20] . It also helps to provide the necessary preventive care before any complication. The aim of this study is to determine the most frequently associated primary mandibular tooth with space infection or abscess in children of Chennai.

Materials and Methods

Study setting:

This study is a university hospital based retrospective cross sectional study conducted among patients who visited the hospital. Large sample size contributes as a major advantage for this study. Data collected was reliable and with photographic and radiographic evidence. The present study was approved by the Institutional Ethical Review Board[SDC/SIHEC/2020/DIASDATA/0619-0320].

Sampling:

The study population included 5516 patients below 13 years who had visited the university hospital from June 2019- March 2020. Among these patients around 2527 patients had undergone extraction and pulpectomy procedures. By simple random sampling a sample size of 1000 was selected, which included patients below 13 years in which 734 patients had undergone extraction and the remaining 266 had undergone pulpectomy treatment in the mandibular arch. Cases were cross verified with the help of clinical photographs. Incomplete records were excluded from the study.

Data Analysis

This was followed by Excel tabulation of parameters. and then the data was exported to SPSS version 20.0 . Descriptive statistics and relation between variables were determined using chi square test with significance level set at $p < 0.05$.

Results and Discussion

The present study was conducted on patients below 13 years. 49.4% cases lie in the age group between 7-10 years, which is the most common age group. 36.6% cases were recorded in children between 4-6 years .10.6% cases were recorded in children between 11-13 years (Figure 1).The results are in consensus with study of Manal Al Malik et al . He has stated that the 6-10 age group is the most susceptible age group for oro facial infection[21]. The reason stated for this finding is due to the mixed dentition and partially erupted teeth. Space infection and abscess were predominantly seen in boys(54.6%). (Figure 2) The results are in consensus with the study of Faban Carl et al. According to him, orofacial infection distribution had a male predilection [22] (62.4%). Ozsurekci Y et al also highlighted that a male predominance is associated with orofacial infection with a male female ratio of 1:1.4[23] . The reason behind this finding may be due to the hyper active nature of boys and also diet of male children can result in increased DMFT score. Frequent susceptibility to trauma due to their aggressive nature also supports this finding.

Abscess was the most commonly encountered infection 6.7% followed by space infection. However, infection was not associated with 91.9% of the cases (Figure 3) . This finding goes in hand with the previous study done by Nagaveni et al . Her study stated that abscess was the most common infection (12%) [24].

Ozsurekci Y et al also reveals that abscess was the most common infection 20% followed by space infection 12%. This is mainly because abscesses are frequently associated with systemic disease. Frequent trauma and Vitamin D deficiency also contribute towards formation of abscess. Vitamin D deficiency may lead to bacterial infection which in turn leads to abscess formation.

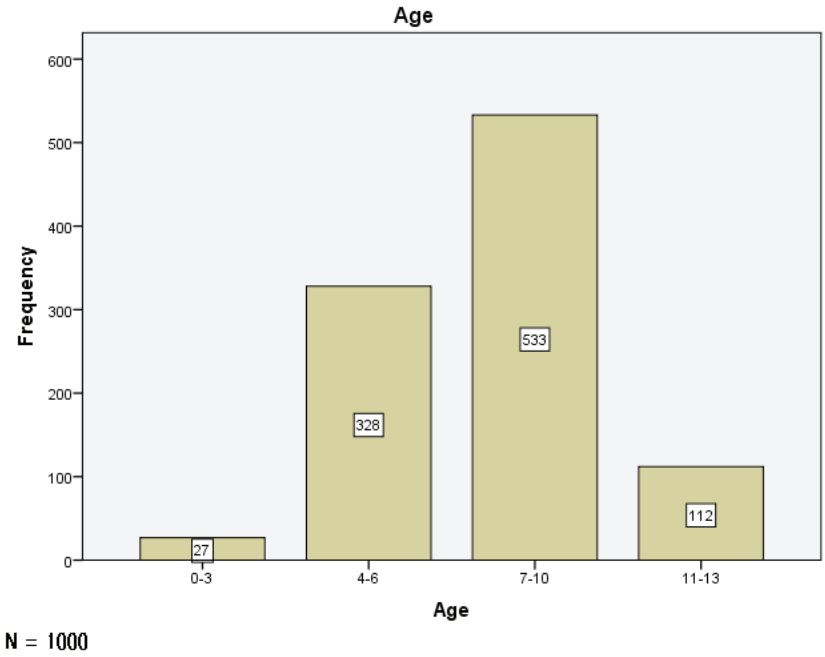


Figure 1 : Bar graph represents the frequency of age distribution among patients.X axis denotes different age groups of patients . Y axis denotes the number of patients.Majority of the cases lie in the age group between 7-10 years (533); followed by 4-6 years (328);followed by 11-13 years (112);and 0-3 years (27)

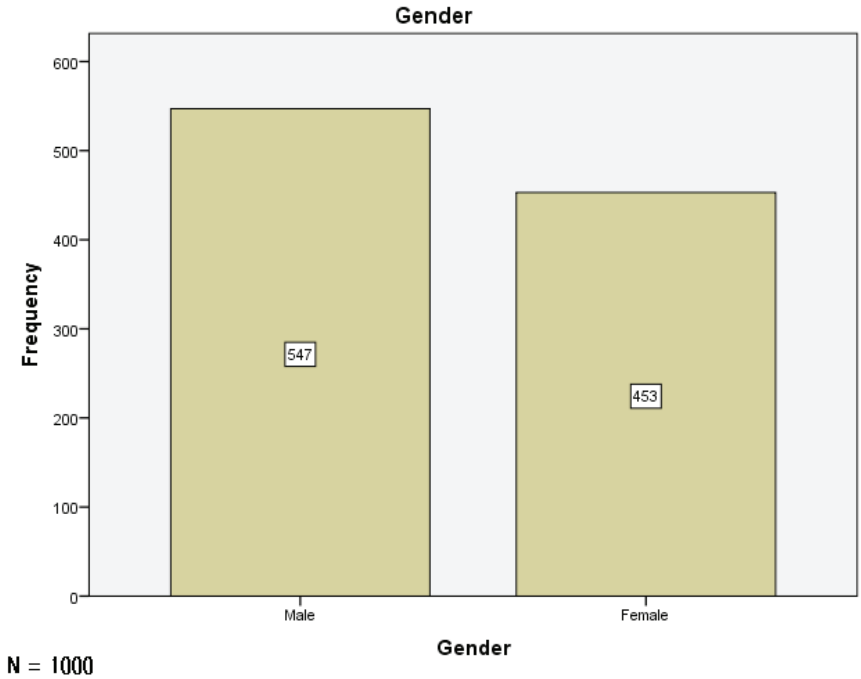


Figure 2: Ba r graph represents the frequency of gender distribution among patients. X axis denotes gender of patients. Y axis denotes number of patients.Majority of the the patients were male children (547)

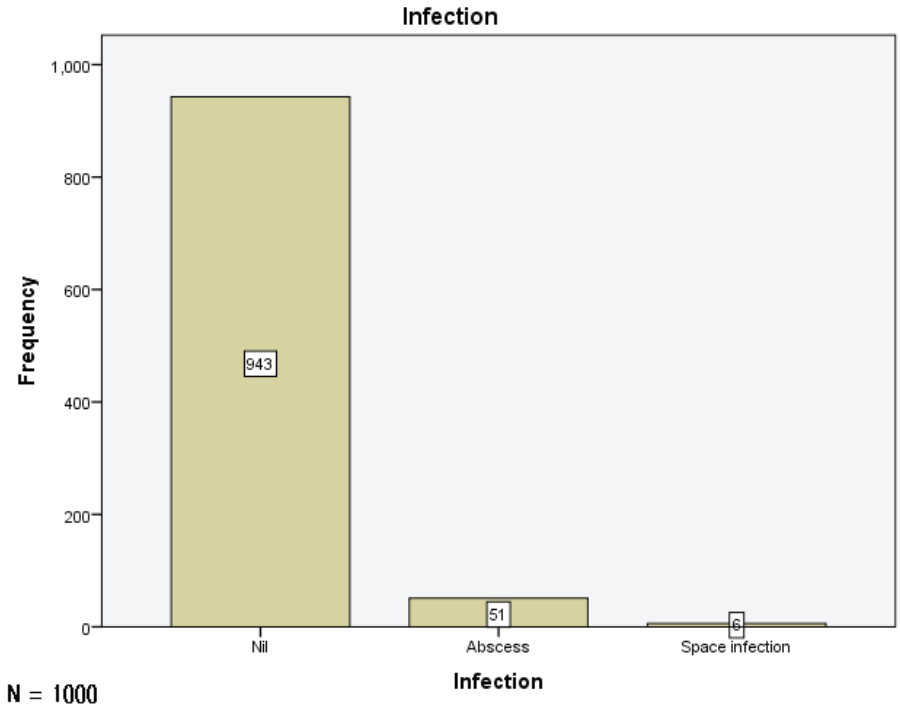


Figure 3 This bar chart represents the prevalence of Infection among study samples .X axis denotes the type of infection. Y axis denotes the number of Children.(O represents no infection). Abscess was the most common infection (51); followed by space infection (6).

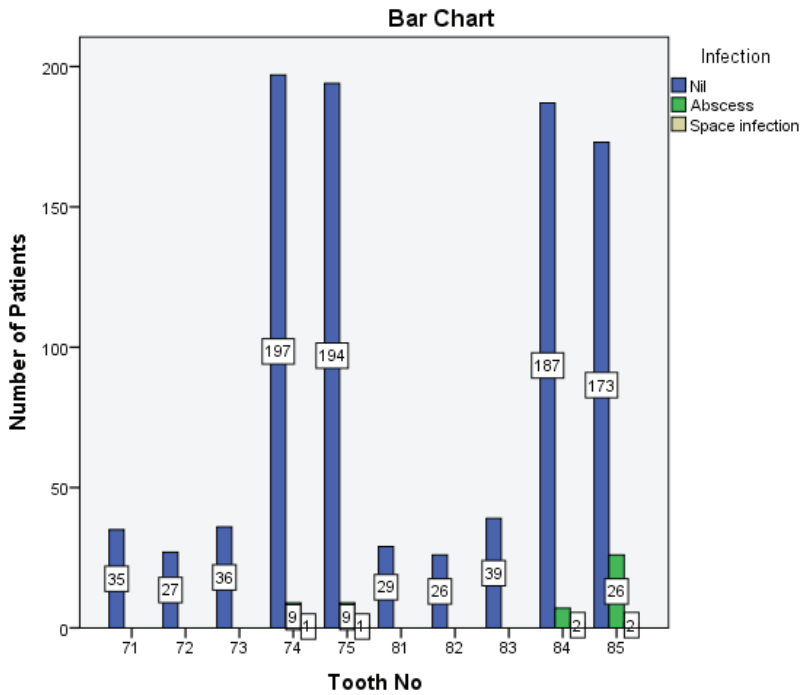


Figure 4 represents the association between individual teeth in mandibular arch and type of infection. X axis represents Tooth number in the FDI system of nomenclature and x cluster denotes tooth with or without infection. Y axis represents the number of teeth. Blue colour denotes no infection, green denotes abscess and beige denotes space infection.Chi square test was done and the association was found to be not significant. (p value= .164 ;p value>0.05 Statistically not significant)

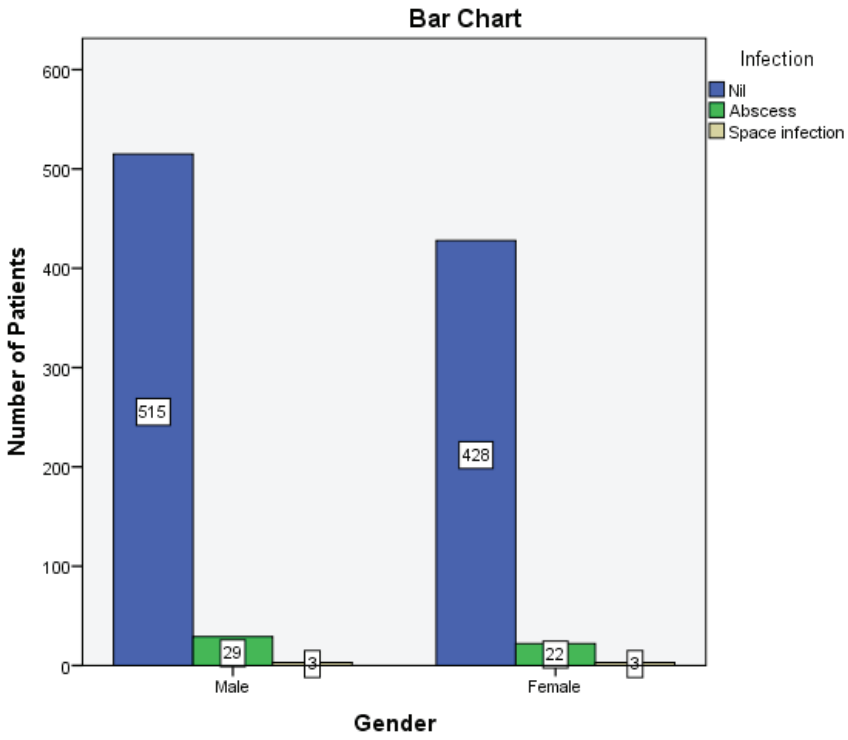


Figure 5: Bar graph represents the association of gender with infection. X axis denotes the gender of children and x cluster represents the children with or without infection. and Y axis denotes the number of children. Blue colour denotes no infection, green denotes abscess and beige denotes space infection. There was no difference in occurrence of infection between boys and girls. Chi square test was done and the association was found to be not significant. (p value = 0.321 >0.05 statistically not significant).

The primary mandibular right second molar is the most frequent tooth associated with space infection or abscess 22% followed by primary mandibular left first molar which is 21% and primary mandibular right first molar 9.9% (Figure 4).This results are in consensus with the study of Manal Al Maliket al. He stated that primary posterior teeth are more susceptible to orofacial infection. This is mainly due to the accumulation of plaque and calculus in the posterior region due to the improper brushing.Children tend to pay more attention to the anterior region. However, Bruno et al stated 84 as the most frequent tooth associated with orofacial infection[25]. This may be due to the different geographic location and larger sample size. There was no statistically significant difference in occurrence of infection between boys and girls based on chi square test; (p value= .321 (p value>0.05 not significant) (Figure. 5).

Patient management may vary with the severity of odontogenic infection,restorability of the tooth concerned, ability to achieve sufficient anaesthesia. Febrile patients with acute signs of spreading infection such as odynophagia and stridor are managed by carrying out extraction . Extraction not only allows complete drainage of infection but it also reduces the likelihood of a recurrent episode in which more morbidities and life-threatening complications could entail. Pulpectomy is another common method of management. Young uncooperative patients who are not willing for pulpectomy can be managed by pulp opening [26 27] .Following pulpal opening, the tooth was always dressed with an intracanal medicament and access cavity closed

Infections in the jaws of a child have the ability to spread rapidly due wide marrow space and also because of the less density of the bones in children .It may result in complete destruction of the permanent tooth germs and if undiagnosed it can reach the critical growth centers of jaws like the condyle. The condylar region

of the mandible is the most sensitive area which can be grossly disfigured in case of growth disturbances . This study focuses on the most frequently associated mandibular tooth with infection which is nothing but the location of anatomic infection which acts as a helpful guide in understanding the prevalence of various teeth affected and which inturn will help in initiating various preventive measures.

The limitation of this study was that it cannot be generalised to another population since our study was a unicentered study conducted in the South Indian population.In future this study can be done on a larger population with a multicentered approach .

Conclusion

Within the limitations of the study it can be concluded that primary posterior teeth were more susceptible to space infection or abscess rather than anterior teeth in the mandibular arch.The most frequently associated tooth in the mandibular arch was the right second primary molar mainly in the age group of 7-10 years.

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Conflict of Interest: Nil

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Most Frequently Extracted Primary Tooth in Mandibular Arch of Children - A Retrospective Study

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Abstract

The goal of Pediatric dentistry is to preserve the primary teeth. Extraction of primary teeth is one of the most frequent procedures which has to be performed in pediatric clinics. The common cause of extraction includes dental caries followed by dental trauma. Other causes include the diseases involving the periapical tissues. Knowing the prevalence or most commonly extracted tooth can help in establishing essential preventive strategies. Hence, the aim of the present study was to analyse the frequently extracted primary tooth in the mandibular arch of children. The study was a retrospective study done under a University setting.The data collected by reviewing and analysing 7415 patients records who visited the university hospital during the time period of June 2019 to March 2020. Inclusion criteria was children who underwent single and multiple extractions of mandibular primary teeth. A total of 1169 case records were collected, tabulated and entered in excel. Using SPSS software the data was subjected to descriptive statistics and Chi-square tests. Prevalence of boys who underwent extraction was 54%. Extraction was more prevalent in the age group of 7-12 years.The most frequently extracted primary tooth in the mandibular arch of children based on the study was the mandibular left first primary molar which accounts for 19.9%. From the data obtained , the study concludes that the most frequently extracted primary tooth in children is the left first molar and found to be predominantly in boys.

Keywords: *Extraction - Primary Tooth - Mandibular Arch - Children- Dental Caries.*

Introduction

Pediatric dentistry is better termed as age defined speciality that includes all the dental procedures. Pediatric dentistry deals with children from birth till adolescence period includes treatment for differently abled children and focuses on providing preventive and preservational care.[1]

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The primary teeth play an important role in the dentition of the child. Deciduous teeth are unsuitably referred to as "Temporary Teeth". Primary tooth acts as a guide to permanent dentition in their optimal position,occlusion and is responsible for esthetics, functions of mastication, phonetics. [2] Primary teeth play an imperative role in the self-esteem of the preschool children. [3] Children's lives are greatly influenced by their parents. Parent's knowledge and belief play an important role in the child's oral health and maintenance .Teaching and guiding the child about the oral habits in their early childhood will prevent complications in future.

The oral health of the primary dentition should be taken care of with utmost attention by the parents and children. Dental neglect is defined as parents' persistent failure to take precautions and provide necessary dental treatment to maintain the child's oral health and to

ensure their freedom from pain and infection. [4] As the children are more suspected of dental problems, if left untreated and will affect the future permanent dentition. For example premature loss of the teeth affects the child's nutrition, self-confidence, growth ,sleep and leads to malocclusion of permanent dentition. Can be due to reduced arch length required for succeeding teeth which predisposes to crowding, rotation, midline shift, impaction of permanent teeth. [5]

Conservative and endodontic treatments are advised when the pulp has the ability to recover when the cause for irritation is removed. [6–10] When endodontic procedures are no longer applicable for treatment, extraction of teeth is done to avoid further complications. And space maintainers are given to prevent malocclusion problems further in permanent dentition. So the extraction is one of the frequently done procedures in dental Pediatric clinics.

There are various causes that lead to the extraction of the primary tooth . Out of which the extraction done due to dental caries accounts for the predominant cause.[11] As dental caries is considered a complex process of demineralization and dissolution of the substance of the teeth leading to cavitation. The etiology is multifactorial and involves initiation and progression of caries. Increased risk of caries can occur due to decreased fluoridation. [12]

Early childhood caries is a common problem encountered in children. One of the chronic diseases which is prevalent and affects life in different aspects. [13]The caries are virulent and are seen to begin in early life soon after dental eruption. The caries develop on smooth surfaces and progresses rapidly. There is high probability of subsequent caries to occur in permanent dentition.[14 15] Thick labial frenum contributes to plaque accumulation which turns to caries this occurs due to difficulty in cleansing the area.[16]

Premature eruption of the permanent tooth without the shedding of the primary tooth is also a major cause. That signifies the reason that extraction is mainly carried out in the mixed dentition period for most of the children. Extraction is also done as an interceptive treatment in children to prevent malocclusion as part of orthodontic treatment.[17] Other causes for extraction include trauma, failure of the pulp, Periapical problems, Congenital disorders, ectopic eruption of permanent tooth specifically the permanent first molars. [18 19]

The aim of the study was to analyse the frequently extracted primary tooth in the Mandibular arch of children.

Material and Methods

Study Setting:

The present study is a retrospective cross sectional study which includes patients who visited an University hospital in Chennai, Tamil nadu. The present study was ethically approved by the Institutional Ethical Committee. (SDC/SIHEC/2020/DIASDATA/0619-0320). The study was carried out on children who visited the Department of Pediatric and Preventive Dentistry during the time period of June 2019 and March 2020 and had undergone the treatment of extraction in the lower arch. The pros of the study accounts for a large sample size and availability of reliable data which can be cross verified from patient radiographs and clinical pictures.

Sampling:

The study is a retrospective study with adequate internal and external validity. The data was acquired by reviewing and analysing 7415 patient records who underwent treatment in the Department of Pediatric and Preventive Dentistry during the time period of June 2019 to March 2020. The sample included patients under the age group of 0 to 15 years. Sample size was 6344 children. Inclusion criteria was children who underwent single and multiple extractions of primary teeth in the mandibular arch and also case sheets which were completely filled. Differently abled children and children with other systemic conditions were excluded from the study. Also case sheets which were incomplete and partially filled were excluded. Sampling bias was avoided by including all cases with a history of extraction meeting the inclusion criteria. The final sample size of 1169 patients. Any gross data which had the possibility of bias and could affect the studies was not included. All the data collected was cross verified by photographic and radiographic examination

Analysis :

The data was entered and tabulated in excel. The

data tabulation in excel was according to S.no, PID, Age, Gender, Extracted tooth. The data was then transferred to Statistical Package for Social Sciences (SPSS version 20) software and analysed.The data obtained was subjected to descriptive statistics and chi square tests. Frequency tables, cross tabulation tables were derived. The data was represented by the means of bar graphs. Results were correlated and associated. p value of <0.05 was considered to be significant.

Results and Discussion

The study evaluated the patients who had undergone treatment for extraction of primary teeth in mandibular arch. The total number of patients included in the study were 1169 patients. All the patients had visited the Department of Pediatric and Preventive Dentistry. The patients underwent extraction treatment for various causes like pain, caries, trauma, orthodontic treatment,periapical problems.

Among the study sample, the number of boy children were 629 out of the total 1169 patients and girl children were 540 out of 1169 patients. Boys (53.8%) underwent more extraction procedures compared to girls (46.2%) (table 1) (figure 1).

The age range of the study was patients under 15 years. Based on the data collected the age group was categorised as age group 0-3 which includes 11 patients , age group 4-6 which includes 193 patients ,age group 7-12 which includes 906 patients, age group 13-15 years which includes 59 patients. The common age group for extraction was found to be 7-12 years. The mean age group was found to be 8 years.The prevalence of extraction in the 0-3 age group was 0.94%, 4-6 years was 16.5%, 7-12 years was 77.5% and 13-15 years was 5.05%.(table 2).

The primary teeth in the lower arch was notated in FDI system.The frequently extracted tooth in the lower arch is the primary molars (77.5%) out of which (19.93%) are the left first molars which are 233 in number followed by 230 right first molar (19.6%), 225 left second molar (19.2%) and 218 right second molar (18.6%) (table 4) (figure 2).

The study reveals that extraction carried among the boys was higher compared to that of girl children. Increased tendencies of dental trauma are seen among males in the society [20]. Occurence of trauma in the primary dentition is common in the age group of 2-4 years. Preschool going children are indulged in various physical activities which induce traumatic injury because in that particular age there is lack of motor coordination.[21] Development of ranula occurs due to trauma or obstruction of salivary gland.[22]

In comparison with previous literature, (Mohammed A et al) states a similar evidence that extraction is more common in male who were presented with poor oral hygiene which denotes their carelessness and ignorance in maintaining good oral hygiene.[23] Whereas

(Chukwuneke F et al) states a different evidence that extraction is more common in females as they are more conscious about their health related issues than males.[24]

Parent's observance and care of dental health of children is very essential. Proper periodic tooth brushing and supervision of a child's healthy diet and nutrition plays an important role in maintaining oral hygiene. [25] The prevalence of extraction in the 0-3 age group was 0.94%, 4-6 years was 16.5%, 7-12 years was 77.5% and 13-15 years was 5.05%.Children belonging to age group 7-12 years were more in a number a total of 906 patients (77.5%) compared to other age groups.The study reveals that extraction is common among the age group of 7-12 years. That is due to the fact the mixed dentition period occurs during this age group. [26 27]

In comparison with previous literature, (Mohammed A et al) states a similar evidence as the percentage of extraction was greater in the age group between 6-8 years and 9-11 years as the shedding time of primary teeth is between 7-12 years. [23]. (Demiriz et al) provides similar evidence that extraction is common in 10 years of age as the children are in the mixed dentition period which makes it difficult to provide good oral hygiene.[28]

In the left arch of the mandible 36 central incisors (3.08%), 36 lateral incisors (3.08%), 56 canine (4.79%), 233 first molars (19.93%), 225 second molars (19.2%) were extracted.In the right arch of the mandible, 40 central incisors (3.42%), 40 lateral incisors (3.42%), 55 canine (4.7%), 230 first molars (19.67%), 218 second molars (18.65%) were extracted.The study reveals that lower primary molars are the frequently extracted tooth

which is followed by secondary molars.(table 3)

Poor dexterity and difficulty in accessing the tooth brush in the posterior region accounts for food accumulation and plaque accumulation in this region. This paves way for the streptococcus mutans and colonizes the occlusal fissure areas and develops to caries. When the caries are left untreated the pulp of the tooth becomes necrotic. Incase of pulpectomy failure, extraction procedure is carried out.[29 30]

In comparison with previous literature, (Samuel S et al) (Azodo CC et al) states a similar evidence that primary molars are the frequently extracted tooth due to the fact that the last primary teeth to be shed is second deciduous molar and earliest permanent teeth to erupt is the first permanent molar hence subjected to dental caries.[31 32] . (Mukhopadhyay et al) states a similar evidence that primary first molars are the frequently extracted tooth as the success rate pulp therapy treatment done in first primary molars is low.[33]

Extraction is a common procedure performed in the Pediatric Dentistry. Early diagnosis and treatment helps to prevent extraction procedures and improve the quality of life of the patient. The study helped in identifying that the posterior teeth were frequently extracted and a healthy diet ,nutrition and proper brushing techniques is to be followed to maintain a good oral hygiene which prevents complications. The limitation of the study includes that it is a single centered study that does not represent ethnic groups**.**

Table 1: Depicts the frequency distribution of the gender predilection of the children who underwent extraction procedure. Boys (53.8%) underwent more extraction procedures compared to girls (46.2%).

Gender	Frequency	Percent
Boys	629	53.8
Girls	540	46.2
Total	1169	100

Table 2: Depicts the frequency distribution of the age of the patients who have undergone extraction.Higher incidence of extraction was noticed in age group of 7 to 12 (77.5%)

Age Group	Frequency	Percent
0-3 age group	11	0.9
4-6 age group	193	16.5
7-12 age group	906	77.5
13-15 age group	59	5
Total	1169	100

Table 3: Depicts the frequency distribution of the frequently extracted primary tooth in the mandibular arch of children. Higher incidence of extraction was found in Mandibular left primary first molar with 19.9%.

Extracted lower primary tooth	Frequency	Percentage
Extraction of 71	36	3.1
Extraction of 72	36	3.1
Extraction of 73	56	4.8
Extraction of 74	233	19.9
Extraction of 75	225	19.2
Extraction of 81	40	3.4
Extraction of 82	40	3.4
Extraction of 83	55	4.7
Extraction of 84	230	19.7
Extraction of 85	218	18.6
Total	1169	100

Table 4: Shows the association between the type of primary teeth extracted in mandibular arch(shown in FDI system of notation) and different age groups. Higher number of extractions was seen in the age group of 7-12 years.Chi square test was done and p value was found to be 0.00 (p value <0.05,statistically significant).

AGE	EXTRACTION OF PRIMARY TOOTH										TOTAL	P VALUE
	71	72	73	74	75	81	82	83	84	85		
0-3 age group	3	1	0	2	0	3	2	0	0	0	11	0.000
4-6 age group	8	9	3	43	29	11	7	1	48	34	193	
7-12 age group	24	25	49	184	179	26	30	50	177	162	906	
13-15 age group	1	1	4	4	17	0	1	4	5	22	59	
TOTAL	36	36	56	233	225	40	40	55	230	218	1169	

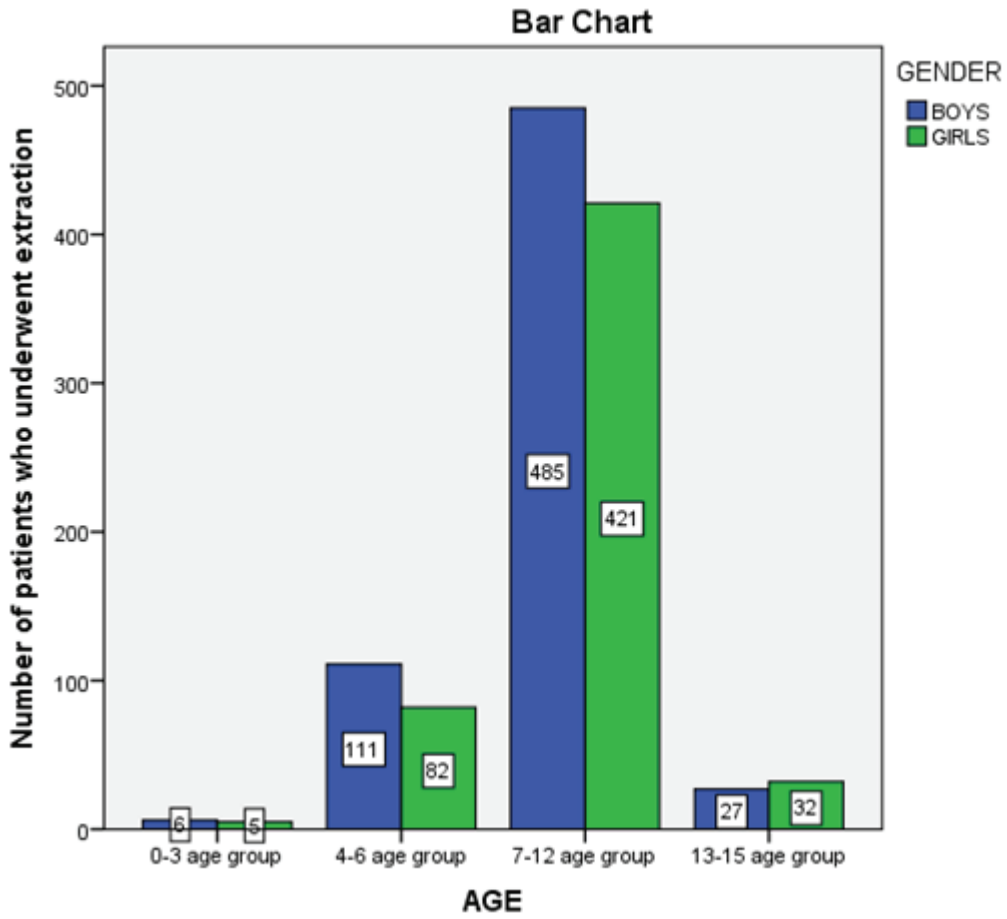


Figure 1:Depicts the association between age group and gender in children undergoing extraction. X-axis represents the age group of patients based on gender as boys (blue), girls (green). Y-axis represents the number of patients who underwent extraction. There was no difference between the number of teeth extracted between boys and girls at different age groups. Chi-square test was done and the p value was found to be 0.452 (p value > 0.05, statistically not significant).

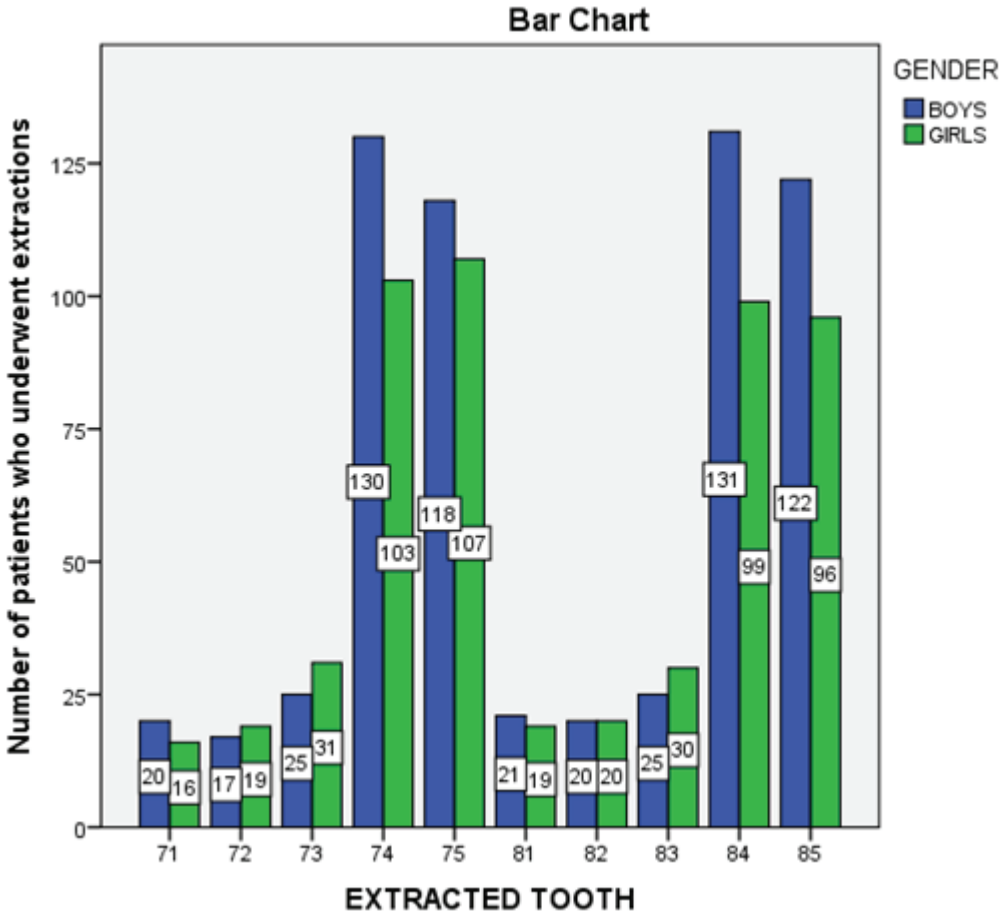


Figure 2: Depicts the association between type of tooth extracted and gender. X-axis represents the frequently extracted tooth in the FDI system of tooth notation) based on gender as boys (blue), girls (green). Y-axis represents the number of patients who underwent extraction. There was no difference between boys and girls and the type of teeth extracted.Chi-square test was done and the p value was found to be 0.716 (p value > 0.05,statistically not significant).

Conclusion

Within the limits of the study we conclude that the most frequently extracted primary tooth in the mandibular arch of children is the primary left first molar and is carried out predominantly in boys under the age group of 7-12 years.

Acknowledgement: The study was supported by the Research Department of Saveetha Dental College. We extend our gratitude to our colleagues who provided insight and expertise that greatly assisted in the research.

Conflict of Interest: There is no conflict of interest.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Type of Study *– Retrospective study* *DOI Number:10.37506/ijfmt.v14i4.12391*

Assessment of Working Length of Root Canal in Mandibular First Primary Molar in Children Aged between 2 to 6 Years

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Abstract

In the endodontic glossary the working length is defined as the distance from a coronal reference point to the point at which canal preparation and obturation should terminate. Pulpectomy is a process of complete removal of the necrotic pulp from the root canals of primary teeth and filling them with an inert resolvable material so as to maintain the tooth in the dental arch. A University based study was conducted on mandibular primary first molar. Various pathologies such as pain, swelling, abscess and resorption were considered. Data collection was done by reviewing the patient records and analysing the data of 501 patients from June 2019 to March 2020, then excel tabulation, Statistical analysis was done using SPSS and Pearson chi-square test was performed. The total number of patients seen were 501, age ranged from 2 to 6 years. Out of the total population 42.91% (215) were female and 57.09% (286) were males. The mean length of mesio buccal canal was 10.76 mm, mesio distal canal – 10.88 mm, and the distal canal was 11.2 mm in the mandibular first primary molar. Pulpal pathology with mean working length of the root canals were as follows, In pain the mesio buccal root had 10.76 mm, mesio lingual root had 10.81 mm and the distal root had 12.01 mm. Abscess the mesio buccal root had 10.75 mm, mesio lingual root 10.79 mm and the distal root 12.03 mm. Swelling the mesio buccal root had 10.75 mm, mesio lingual root had 10.80 mm, and the distal root had12 mm, Resorption mesio buccal root had 10.77 mm, mesio lingual root had 10.78 mm, and the distal root had 11.8 mm. In regard to various pathogens such as pain,swelling, abscess and resorption the working length was determined as 10.76 mm for mesio buccal root, 10.88 mm for mesio lingual root and 11.2 mm for distal root of the mandibular first primary molar.

Keywords*: Primary mandibular first molar, determination of working length*

Introduction

In the endodontic glossary the working length is defined as the distance from a coronal reference point to the point at which canal preparation and obturation should terminate [1]. Pulpectomy is a process of complete removal of the necrotic pulp from the root canals of primary teeth and filling them with an inert resolvable material so as to maintain the tooth in the dental arch [2]. The main objective of pulpectomy in the primary teeth is to retain the primary tooth as a fully functional component in the dental arch. The advantage of this process is that it provides integrity to the damaged tooth which provides spacing required for a new tooth to grow, and plays a role in self-esteem of the preschool children and also in speech development, function and esthetics [3,4].

Bacteria plays an important role in pulpal and periapical diseases. Dental caries is the most infectious disease in children. It is a complex process of demineralization and dissolution of the substance of the teeth leading to cavitation. Increase in malondialdehyde concentration in saliva can cause dental caries [5]. Tooth brushing is the primary method of maintaining good oral hygiene [6]. Fluoride when present in minimal quantity can prevent dental caries [7,8]. Dental neglect can lead to poor oral health, which increases the risk of dental caries in neglected children [9]. Frenal attachment can lead to accumulation of milk which can lead to dental caries [10].

The primary objective of cleaning and shaping is to remove the organic debris from the pulpal canal [11]. The conventional method of cleaning and shaping is done using hand files, which is a time consuming procedure which may cause a negative effect in the behaviour of the child, whereas with the use of rotary instruments the procedure time is reduced which increases the patient cooperation [12,13]. The different techniques that have been proposed for canal preparation include corono-apical and apico-coronal [14]. The clinical use of paediatric rotary Kedo-S files was effective during root canal preparation of primary teeth with reduction in instrumentation time [15]. These instruments debride the uneven walls and improve the quality of obturation [16,17]. Previously our team had conducted Systematic review [18] over the past 5 years. Now we are focusing on epidemiological surveys. The idea of this survey stemmed from the current interest in our community.

The process of determining accurate root canal length is a vital part for a successful root canal treatment in primary teeth in order to minimise the periapical injury and possible damage to erupting permanent teeth [19]. The accurate root canal length of primary teeth is difficult to predict because of the pattern of root resorption, physiological process or a pathological process due to periapical pathology. In the primary teeth the size, shape, and position of the root apices alter continuously, because of this it becomes difficult to accurately determine the root canal length of primary teeth [20,21]. Therefore, one of the main important things in the root canal treatment of primary teeth is to determine how far instruments should be advanced within the root canal and at what point the preparation and restoration should terminate [22,23].

There are various methods of determining the root canal length of a primary tooth are tactile sensation method (TSM), conventional radiography (X-ray), radiovisiography (RVG), and the electronic apex locator (EAL) method. Electronic apex locator methods give accurate root canal length when compared to other methods (24). Working length determination is an important factor for the success of root canal treatment, the canal should be prepared till the apical constriction or apical foramen [25]. The use of arbitrary Working length in anxious children is to reduce the duration of treatment for better cooperation from the child and to improve the quality of the treatment. Therefore the main objective is to determine the working length for Dravidian children aged 2 to 6 years.

The challenges faced are the negative attitude of the patients and parents due to fear of the treatment, difficulty in cleaning, shaping and obturation due to the tortuous canal anatomy of the teeth with resorbing and open apices [26,27].

Comparison of different methods of working length determination like digital radiography, conventional radiography and by using Electronic apex locator concluded that the accuracy in measuring working length in primary teeth was achieved by using electronic apex locator. With continuous advancements in the technology of EALs, the correct use of apex locators has a definitive place in clinical Pedodontics and their day to day use in clinics can reduce chairside time, limit radiation and achieve more cooperation from the children [28].

This research is very important for the success of pulpectomy and to avoid complications like perforation of the canal, overfilling or retained pulp tissue which may lead to failure of the treatment and cause reinfection .The aim of this study is to determine the working length of lower first primary molar in children aged between 2 to 6 years.

Materials and Methods

A University based study was conducted among 501 patients in mandibular first primary molar. Various pathologies such as pain, swelling, abscess and resorption were considered. Mandibular first primary molars that were indicated for pulpectomy were taken into consideration. The study was conducted with the approval of the Institutional Ethics Committee [SDC/SIHEC/2020/DIASDATA/0619-0320]. Two researchers namely DG and MR are involved in this study. Cross verification was done by two reviewers and the measures taken to minimize the sampling bias are by including all the data available with no sorting process.Inclusion criteria included the mandibular first primary molar in children aged between 2 to 6 years, the teeth that had demonstrated extensive caries, and teeth that showed the presence of two thirds of the root length radiographically. Bias was avoided by including all mandibular molar

teeth with pulpal pathologies. Stringent protocol in doing the working length determination using the conventional and digital radiography method was followed.

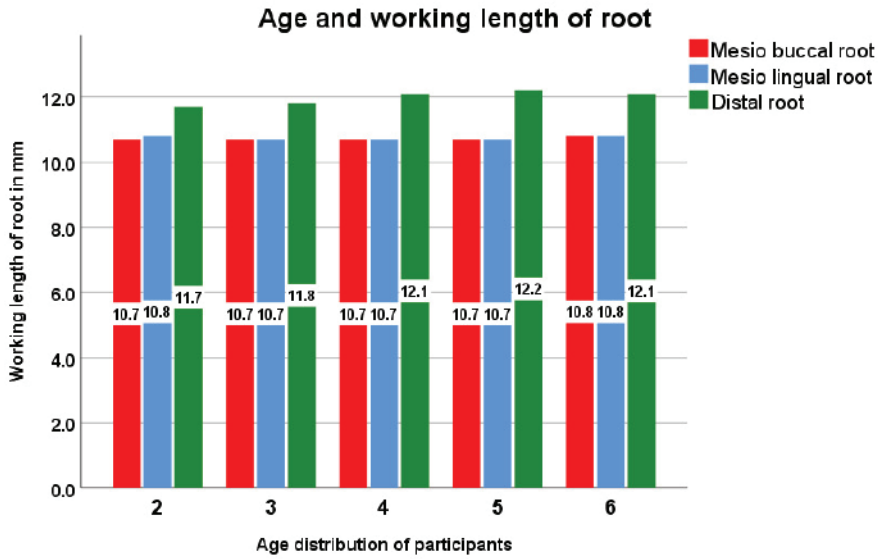
Data collection was done by reviewing the patient records and analysing the data of 501 patients from June 2019 to March 2020, excel tabulation of the data, statistical analysis was done using spss and pearson chi-square test was performed in the mandibular first primary molar in children aged between 2 to 6 years was done.

Incomplete or censored data was excluded from data. Statistical analysis was done by exporting the data to SPSS and Descriptive distribution of working length of mandibular first primary teeth was obtained.

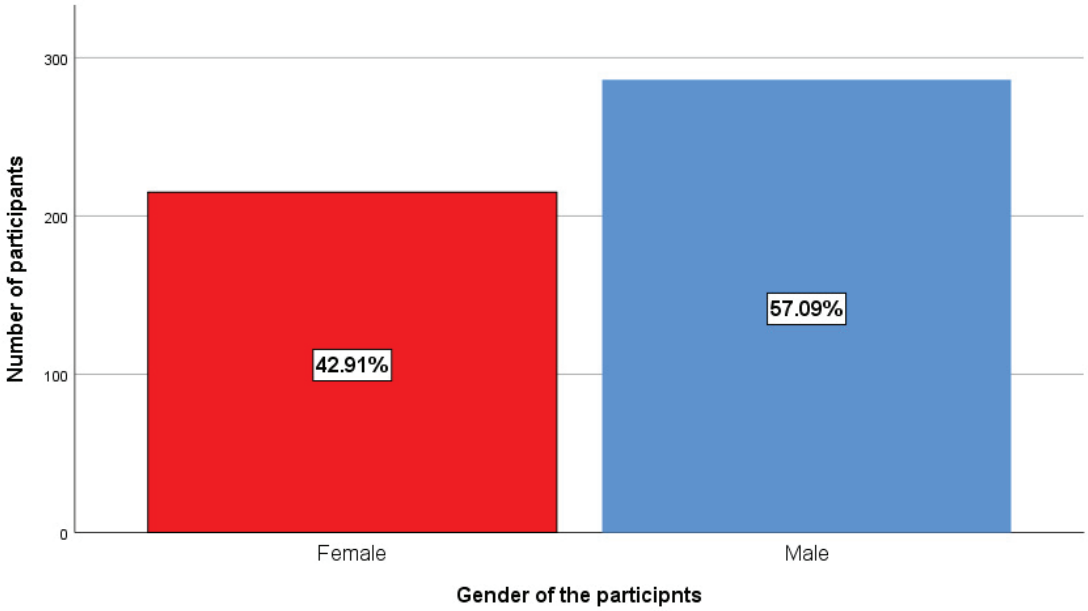
Results and Discussion

The total number of patients seen were 501, whose age ranged from 2 to 6 years (graph 1) with 4.46 years being the mean age. Out of the total sample 42.91 % (215) were female and 57.09% (286) were males (graph 2), there was no significant difference in the working length of mesio buccal, mesio lingual and distal roots between males and females as seen in graph 3.

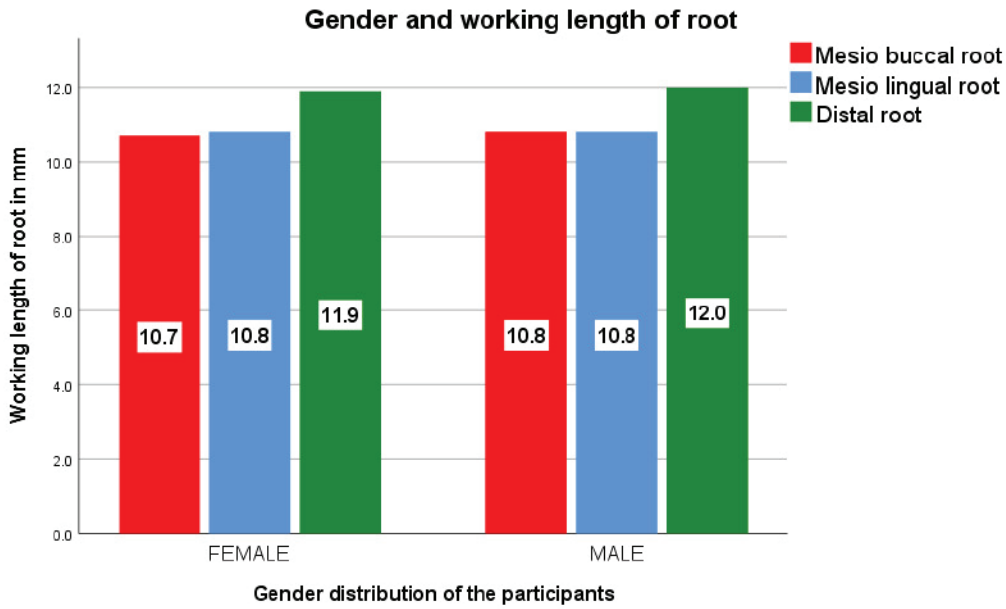
The four pulpal pathologies that were mainly treated in the mandibular first primary molar were pain, abscess, swelling, and resorption. Pulpal pathology with mean working length of the root canals were as follows, In pain the mesio buccal root had 10.76 mm, mesio lingual root had 10.81 mm and the distal root had 12.01 mm. Abscess the mesio buccal root had 10.75 mm, mesio lingual root 10.79 mm and the distal root 12.03 mm. Swelling the mesio buccal root had 10.75 mm, mesio lingual root had 10.80 mm, and the distal root had12 mm, Resorption mesio buccal root had 10.77 mm, mesio lingual root had 10.78 mm, and the distal root had 11.8 mm. Association of Mesio buccal canal with pulp pathology was done as shown in graph 4, Mesio lingual canal with pulp pathology was seen in Graph 5 and distal canal with pulpal pathology was seen in Graph 6.



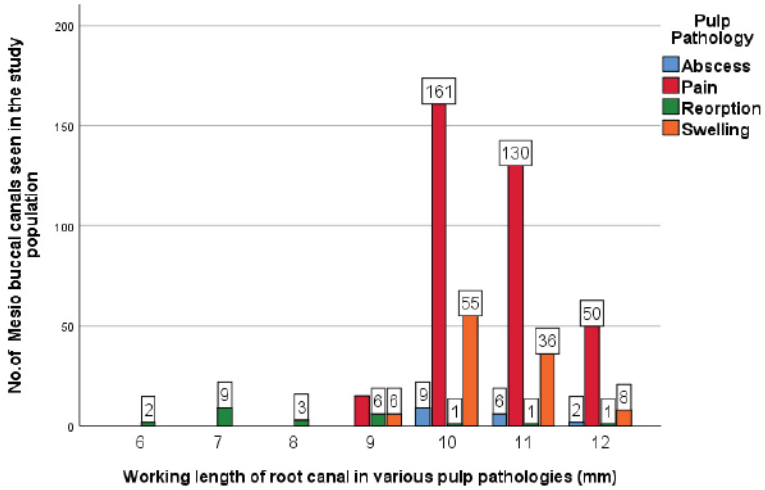
Graph 1 - This graph shows association between the age of participants and working length of root in mandibular first primary molar in children aged 2 to 6 years where red colour represents mesio buccal root with 10.8 mm in the age group 6, blue colour represents mesio lingual root with 10.8 mm in the age group 2 and 6 and green colour represents distal root with 12.2 mm in the age group 5. There was no mean difference between the working lengths in the age group of 2-6 years.The X axis represents the age of the participants and the Y axis represents the Working length of root in mm (mesio buccal, mesio lingual and distal root). Chi square test was done P = 0.998 for mesio buccal root (>0.05 - indicating statistically not significant), P = 0.847 for mesio lingual root (>0.05 - indicating statistically not significant) and P = 0.518 for distal root (>0.05 - indicating statistically not significant).



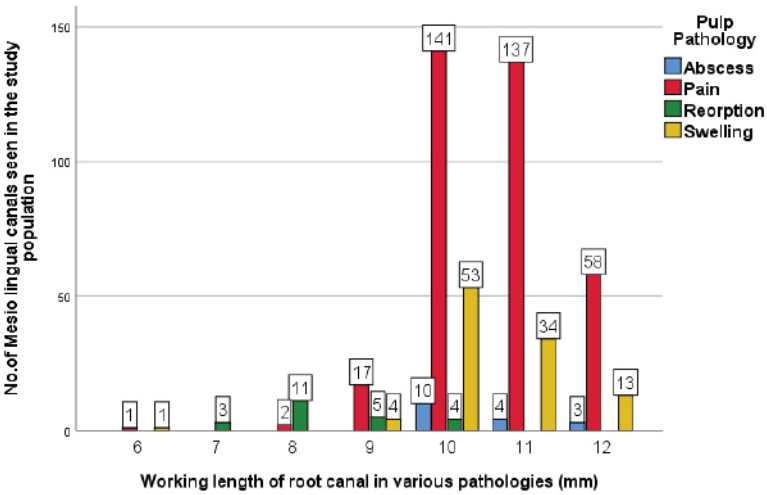
Graph 2 - Bar graph showing the descriptive distribution of gender of participants in mandibular first primary molar in children aged 2 to 6 years where red colour represents females with 42.91% and blue colour represents males with 57.09% The X axis represents the Gender of the participants and the Y axis represents the number of patients.



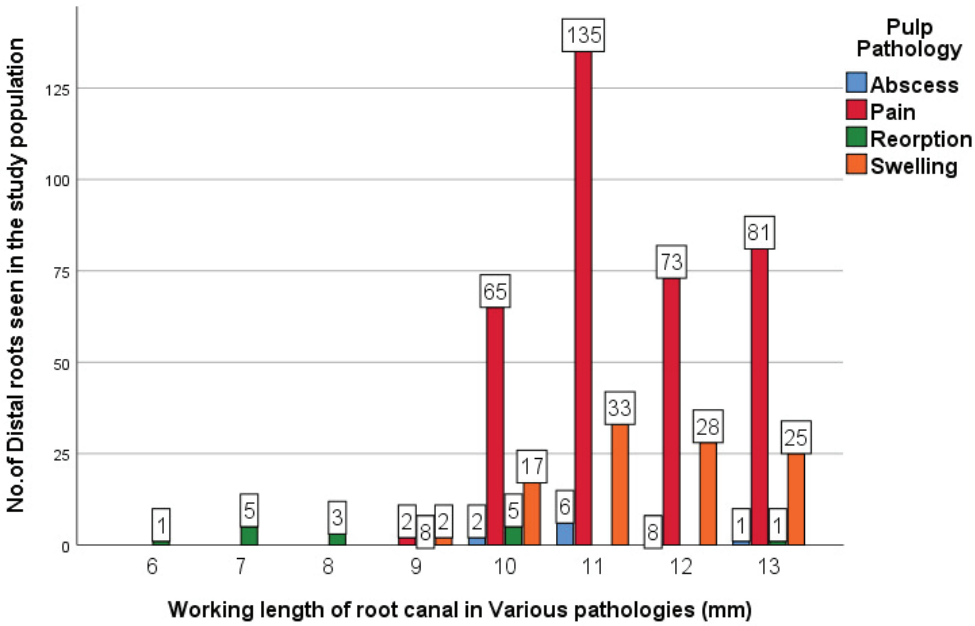
Graph 3 - This graph shows association between gender of participants and working length of root in mandibular first primary molar in children aged 2 to 6 years where red colour represents mesio buccal root, blue colour represents mesio lingual root and green colour represents distal root. There was no difference in the working length of mesio buccal, mesio lingual and distal roots between males and females. The X axis represents the gender distribution (Male and female) of the participants and the Y axis represents the working length of the root in mm (mesio buccal, mesio lingual and distal root). Pearson Chi square test was done, P = 0.00 for mesio buccal root (<0.05 - indicating statistically significant), P = 0.01 for mesio lingual root (<0.05 - indicating statistically significant) and P = 0.000 for distal root (<0.05 - indicating statistically significant).



Graph 4 - This graph shows association between pulp pathology (Abscess, Pain, Resorption and Swelling) of teeth considered for pulpectomy and working length of mesio buccal canal in mandibular first primary molar in children aged 2 to 6 years where blue colour represents Abscess, red colour represents pain, green colour represents resorption and yellow colour represents swelling. The X axis represents the working length of root canals in mm and the Y axis represents the number of Mesio buccal canals on a scale of 0-200 (count). The mean working length of root canal in mesio buccal canal is more when the patient has only abscess (10.97 mm) when compared with other pathologies such as Pain (10.87 mm), Swelling (10.75 mm) and Resorption (8.36 mm). Pearson chi-test was done P = 0.000 for mesio buccal root (<0.05 - indicating statistically significant).



Graph 5- This graph shows association between pulp pathology (Abscess, Pain, Resorption and Swelling) of teeth considered for pulpectomy and working length of mesio lingual canal in mandibular first primary molar in children aged 2 to 6 years where blue colour represents Abscess, red colour represents pain, green colour represents resorption and yellow colour represents swelling. The X axis represents the working length of root canals in mm and the Y axis represents the number of Mesio lingual canals on a scale of 0-200 (count). The mean working length of root canal in mesio buccal canal is more when the patient has Pain (10.94 mm) when compared with other pathologies such as Abscess (10.93 mm), Swelling (10.83 mm) and Resorption (8.53 mm). Pearson chi square test was done P = 0.000 for mesio lingual root (<0.05 - indicating statistically significant).P = 0.000 for distal root (<0.05 - indicating statistically significant).



Graph 6 - This graph shows association between pulp pathology (Abscess, Pain, Resorption and Swelling) of teeth considered for pulpectomy and working length of distal canal in mandibular first primary molar in children aged 2 to 6 years where blue colour represents Abscess, red colour represents pain, green colour represents resorption and yellow colour represents swelling. The X axis represents the working length of root canals in mm and the Y axis represents the number of Distal canals on a scale of 0-200 (count). The mean working length of root canal in mesio buccal canal is more when the patient has only Pain (12.275 mm) when compared with other pathologies such as Abscess (11.80 mm), Swelling (11.79 mm) and Resorption (8.79 mm). Pearson chi square test was done P = 0.000 for distal root (<0.05 - indicating statistically significant).

One of the most important goals in pediatric dentistry is to preserve the primary teeth until eruption of their succedaneous teeth in an attempt to prevent primary tooth loss sequels such as disturbance in mastication, speech, esthetics and correct tooth spacing .

According to this study the most common age group undergoing pulpectomy was observed to be 4 years and the age ranged from 2 to 6 years. Similarly in a study conducted by Yin-Lin, et al. he compared the working length of mandibular molars in the age group of 2-8 years,where the highest number of pulpectomies were done in the age group of 4 [29].

The mean working length was determined as 11.79 mm with apex locator and with digital radiography the length was observed as 11.98 mm, in this study it was observed that there was a decrease in the mean working length of resorption (8.56 mm), swelling (11.13 mm), and abscess (11.24 mm) and to avoid the radiographic error electronic apex locator should be used to determine the working length by Neena, I. E., et al. [30]. Literature is sparse in determining the working length of primary teeth. This study has an advantage of large sample size and of the same ethnicity, which will help in determination of working length .

Male patients had undergone more pulpectomy than female children. However there were no significant differences in the working length of the primary mandibular first molar based on gender [31].

In this study the mean working length of the distal root was longer than the mesial roots. The distal root showed the longest measurement (11.99 mm) followed by mesio lingual root (10.8 mm) and mesio buccal root (10.73 mm), which was in accordance with Reddy, N. Venugopal, et al [32]. where distal root showed maximum working length with a mean of 8.95 mm, whereas mesio

buccal root showed minimum length with a mean of 7.85 mm, but in contrast to the findings of Zoremchhingi et al [33] . This difference might be attributed to the different populations examined. Better knowledge of root length may be useful for determining the working length which prevents over or under instrumentation wherein debris removal might be hampered.

The pulp pathology was compared with the working length of the root in the mandibular first primary molar. Out of 501 children, 359 had pain, 105 had swelling, 23 had resorption, and 17 patients had abscess. The most commonly diagnosed pulp pathology in children was observed to be pain. During pain distal root was observed to have maximum working length of 12.28 mm, whereas mesio buccal root was found to have minimum working length of 10.87 mm.

The Limitations of the study were the radiographic shortcomings like angulation, distortion of image which were not included, Comparison should be done with other groups which included apex locators, CT and CBCT to measure the working length in mandibular first primary molar. The future scope is that all the parameters of root canal morphology and root angulations should be considered for the success of treatment in children and for a better scope of the study.

Conclusion

Within the limits of this study it is concluded that the mean working length was observed to be 10.76 mm for mesio buccal root, 10.88 mm for mesio lingual root, 11.2 mm for distal root. Pulpal pathology with mean working length of the root canals were as follows, teeth in which pain were present the mesio buccal root was 10.76 mm, mesio lingual root had 10.81 mm and the distal root had 12.01 mm in length respectively. In teeth with abscess the length of mesio buccal root was 10.75 mm, mesio lingual root was 10.79 mm and the distal root 12.03 mm. In case of swelling the mesio buccal root was 10.75 mm, mesio lingual root was 10.80 mm, and the distal root 12 mm.In case of resorption mesio buccal root was 10.77 mm, mesio lingual root was 10.78 mm, and the distal root was 11.8 mm.

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Prevalence of Hypertension in Completely Edentulous Patients

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Abstract

Edentulism is the state of having lost all of one natural teeth. Cardiovascular disease is one of the leading causes of premature death among Non communicable disease. Hypertension increases the risk of Cardiovascular events. Tooth loss is defined as separation of tooth from its supporting structure and this can be caused by tooth caries , failure of endodontic treatment, periodontal disease, fractured tooth. The aim of the study is to investigate the prevalence of hypertension in completely edentulous patients.This retrospective study was conducted among patients aged 25-85 years reporting to Saveetha Dental college and hospitals . For data collection, we reviewed patients records and analysed the data of 86,000 patients between june 2019 and March 2020. Collected data are entered in the excel sheet. Data analysis and results done through SPSS. Prevalence of completely edentulous patients is more common among 56-65 years of age . Male are more commonly affected. Prevalence of hypertension is more in males compared to females.Within the limits of the study , prevalence of completely edentulous patients is more common in 56-65 years of age and males are more common compared to females.

Keywords: *Completely edentulous , Hypertension, Male, prevalence, age.*

Introduction

Edentulism is the state of having lost all of one's natural teeth . [1] Monitoring the occurrence of an oral end state such as edentulism is important because it is an indicator of both population health , the functioning and adequacy of a country's oral health care system . [2]Tooth loss is defined as separation of tooth from its supporting structure and this can be caused by tooth caries , failure of endodontic treatment, periodontal disease, fractured tooth.[3]

Hypertension is defined as an abnormal elevation of systolic and diastolic pressure above normal range.[4] The normal range of systolic and diastolic pressure will be 100 - 140 mmHg and 60 - 100 mmHg respectively. [5] It can affect the physical and social lifestyle of a patient that may have a role in increased risk of hypertension.[6] Complete edentulism has been shown to be associated with the development of hypertension. The comfort, function, and esthetics must be restored altogether while treating a completely edentulous patient. One of the main objectives in selecting and arranging artificial teeth is to produce a prosthesis that defies detection.[78]

A previous study showed that the means of blood pressure were higher in patients with complete denture. [9]Tooth loss has been reported to be associated with an increase in cardiovascular diseases.[10] Hypertension increases the risk of cardiovascular events , well known risk factors for hypertension like lack of physical activity, obesity etc . Apart from complete denture other options are available for replacing missing natural teeth such as fixed prosthesis, implant retained over dentures [11], RPDs ,still play a major role in prosthetics rehabilitation owing to financial issues, patients compliance and residual height of edentulous ridges.[12] [13] Some authors showed

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that the prevalence of hypertension in Malaysia was 66.8 . [14] The prevalence of edentulism in the United States is around 30-35% among older adults and while Malaysia is around 30-40% [1516].

In addition to well known risk factors for hypertension, obesity , lack of physical activity, smoking , low income, education level and several studies have shown an independent association between tooth loss and blood pressure . [17] However the relevant literature is not conclusive about the association. Tooth loss and periodontal diseases are considered to be the markers of oral health and however complete tooth loss is a total failure of oral health .[18,19] Volzke et al., study showed an inverse association between hypertension and complete edentulous patients among men but not in women. [20,21] Aloe Vera has its uses in various systemic diseases like diabetes, hyperlipidemic patients etc . It has beneficial effects to prevent microbial infections such as caries etc., because caries , periodontal diseases are one of the causes of tooth loss.[2223]

Compromised oral hygiene resulting in the accumulation of plaque and calculus induces inflammatory changes in the adjoining areas which leads to subsequent pathogenic microbial colonization.[24] The bacteria most commonly affect tooth loss is streptococci. [25] A multidisciplinary population based study reported that prevalence of systolic blood pressure is high in Brazilian population. A study in the South African population reported that complete edentulous is a risk indicator for hypertension . [26]Establishing a healthy oral environment and maintaining proper oral hygiene are the primary objectives .[27] Increased life expectancy and increase in population, creates high demand in dental care especially in the field of prosthodontics. [28]Furthermore, there is a need for further studies on diverse populations to strengthen the prevalence of hypertension in completely edentulous patients. Hence the aim of our study is to investigate the prevalence of hypertension in completely edentulous patients.

Materials and Method

A retrospective study was conducted among patients aged 25-85 years reporting to Private dental institutions, Chennai. Ethical approval was obtained from the Institutional Scientific Review board . Number of people involved in this study were two researchers and one guide.

Sample methods :

Non probability screening of the specified population, All case sheets reviewed from June 2019 to March 2020. The inclusion criteria will be completely edentulous patients and hypertension. Internal validity will be non probability inclusion and external validity will be homogenisation, replication and cross comparison.

Data collection :

For data collection, we reviewed patients records and analysed the data of 86,000 patients between june 2019 and March 2020. We recorded all the patient details and systemic diseases like hypertension and tabulated. The sample size considered was 915.Tabulation of patient details will be name of the patient ,age , sex and presence or absence of hypertension. Imported to SPSS and variables were defined.

Data analysis:

We considered age, gender and prevalence of hypertension in completely edentulous patients as presence and absence of hypertension. We categorised age as 6 groups [25-35 , 36-45 , 46-55 , 56-65 , 66-75 , 76-85] and then based on age group, we scored the data as 25-35 as 1, 36-45 as 2 so on… Then we categorised gender and scored as Male [1] , female [2]. Finally we categorised presence or absence of hypertension and scored the data like presence of hypertension as [1], absence of hypertension as [2].

After checking and coding the responses , we used SPSS for data entry and data analysis. Descriptive statistics like frequency and percentage were calculated for categorised variables. We performed Chi square test and cross tabulation between presence or absence of hypertension, age,gender of the patients. Independent variables will be age, gender and Dependent variables will be hypertension. Cross tabulation and chi square test was done for age and presence or absence of hypertension, gender and presence or absence of hypertension. Statistical significance was determined using P value less than 0.05.

Results and Discussion

This retrospective study included 915 patients

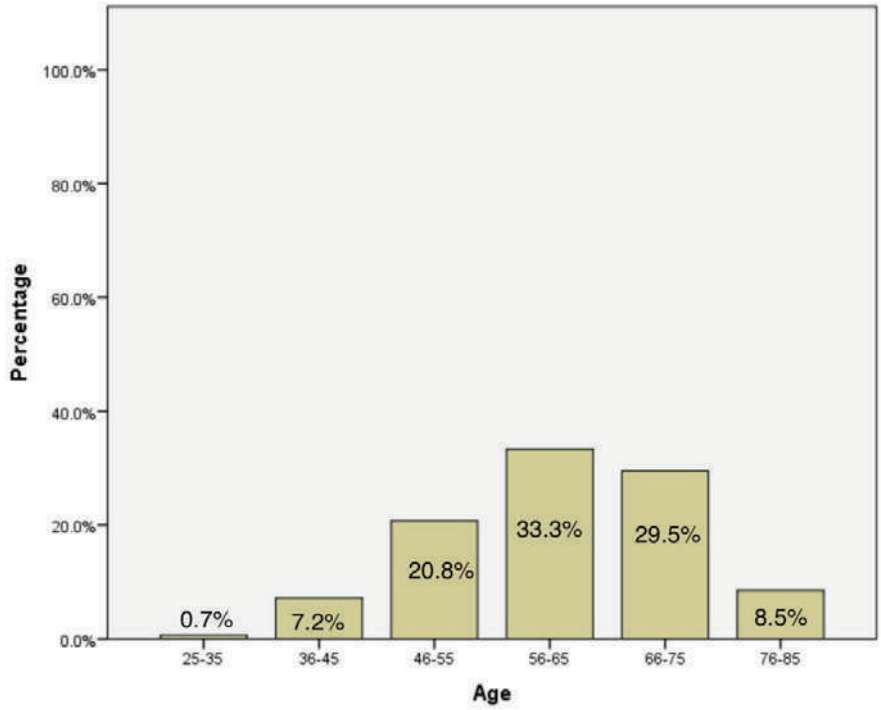
reported to Private dental institutions. Bar graph shows the distribution of the study population based on age groups. prevalence of completely edentulous patients is more common in 56-65 years of age groups (Graph 1) Bar graph shows the distribution of the study population based on gender . Among 915 patients , 58.8% were males and 41.2% were females. Prevalence is more common in Male compared to females.(Graph 2) Bar graphs show the distribution of the study population based on presence or absence of hypertension. It is evident that the presence of hypertension will be 20.3% and absence of hypertension will be 79.7%. (Graph 3) Bar chart depicting association between gender and presence or absence of hypertension in completely edentulous patients. Association between gender and presence or absence of hypertension in completely edentulous patients was done using chi square test (p= 0.952) and was found statistically non-significant (p>0.05)(Graph 4). Bar chart depicting association between age groups and presence or absence of hypertension in completely edentulous patients. Association between age groups and presence or absence of hypertension in completely edentulous patients was done using chi square test (p= 0.007) and was found statistically significant (p<0.05) (Graph 5)

Oral health is fundamental for general health, functioning and well-being. The Global Burden of Disease 2010 Study estimated that oral conditions (untreated caries, severe periodontitis and severe tooth loss) accounted for almost 3 % of all years lived with disability. Edentulism is a final marker of disease burden for oral health common among older adults populations. Various research aimed at improving oral health should take into account social as well as biological determinants. Prevalence of hypertension was significantly different in China and South Africa ($p < 0.001$) compared to other countries.

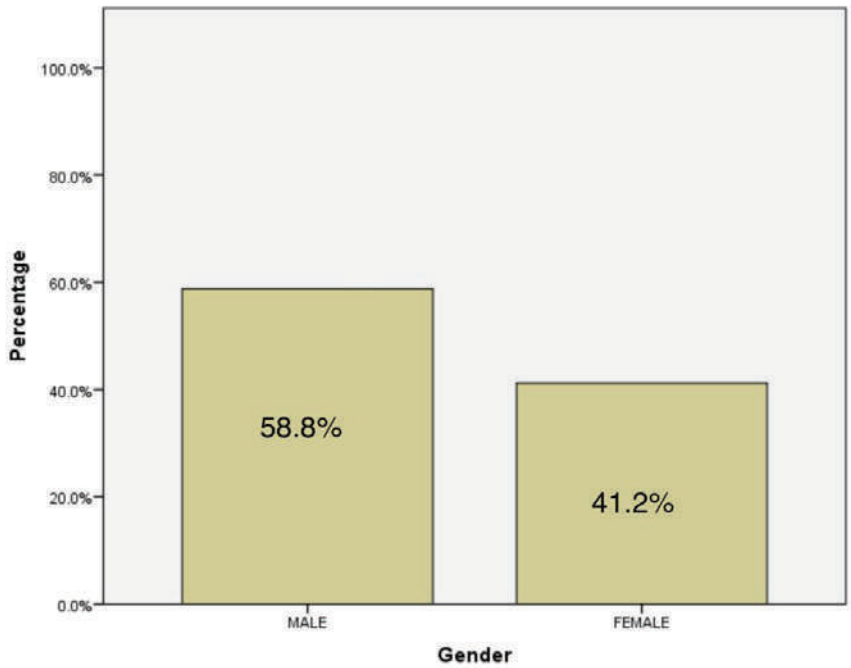
This retrospective study was done to determine the prevalence of hypertension in completely edentulous patients. In this study , prevalence of completely edentulous patients were more common in (56-65) years of age . It is more common in Male compared to female . Prevalence of hypertension was common around (56-65) years of age and with Male predominance . P value less than 0.05 will be statistically non significant. Laxman Singh Kaira et al reported similar evidence that prevalence was common in (51-65) years of age . [29]

However, our study followed a similar protocol to the above mentioned study but our results differed from their study i.e Enami et al., reported a different evidence that prevalence of completely edentulous patients were more common above 70 years of age. [30] Nonetheless, several studies have reported an association between tooth loss and hypertension. [31] In cross sectional study Mustafa Al.Ahmad et al., found that tooth loss is significantly associated with hypertension in post menopausal women. [32]Prevalence of completely edentulous patients more in Male compared to females . Laxman et al., reported similar evidence that the prevalence is more common in Male but Mitsuhiro Sanada et al., reported different evidence that post menopausal women had more prevalence compared to male.

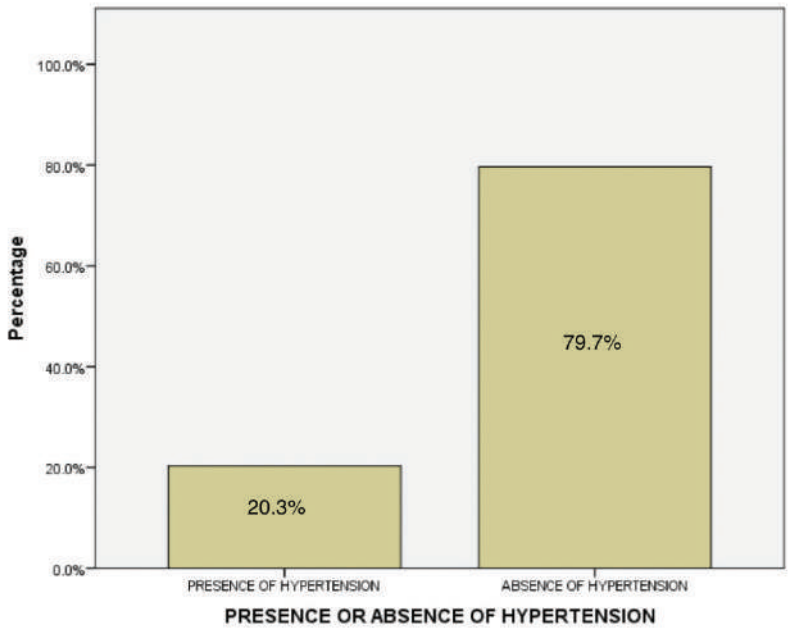
Prevalence of hypertension were more common in (56-65) years of age in completely edentulous patients with male predominance. Akira Taguchi et al., reported that post menopausal women had more prevalence compared to male. Ayo - Yusuf et al., reported similar evidence (50-59) years of age had more prevalence of hypertension. [33] Correlations between age and presence or absence of hypertension were analysed and found to be significant ,whereas correlations between gender and presence or absence of hypertension were analysed and found to be statistically non significant.Limitation of the study was relatively small sample size , single centred study and non representation of all ethnic groups or populations.



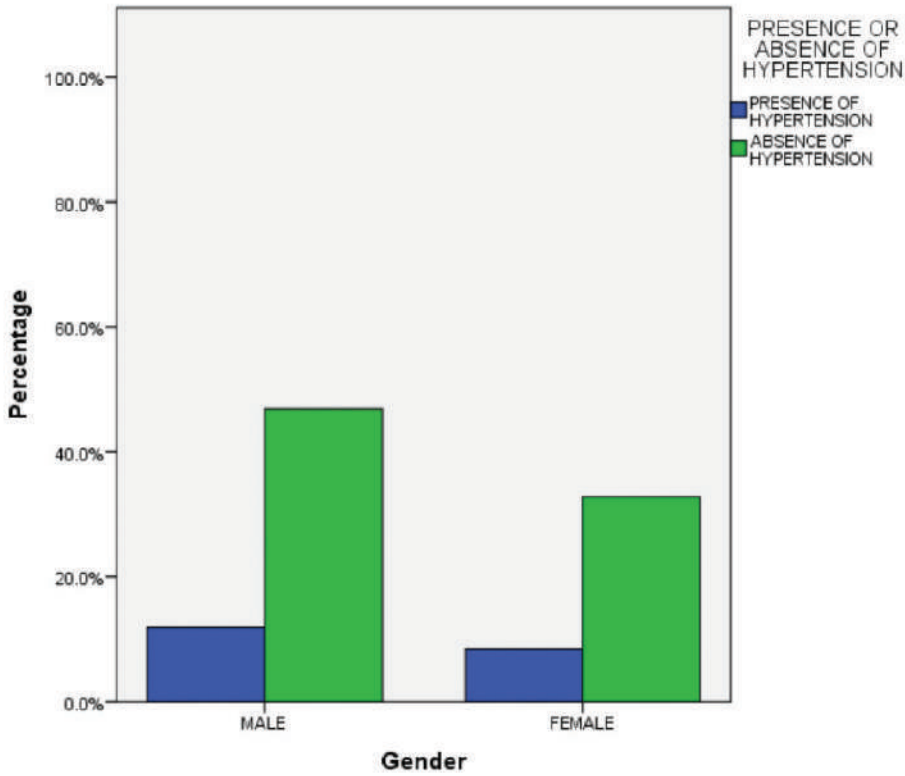
Graph 1: This graph represents percentage distribution of study population based on age groups . Bar graph shows the distribution of the study population based on age groups. The X axis represents the age group and the Y axis represents the numbers of patients. It is evident that prevalence of completely edentulous patients is more common in 56-65 years of age groups



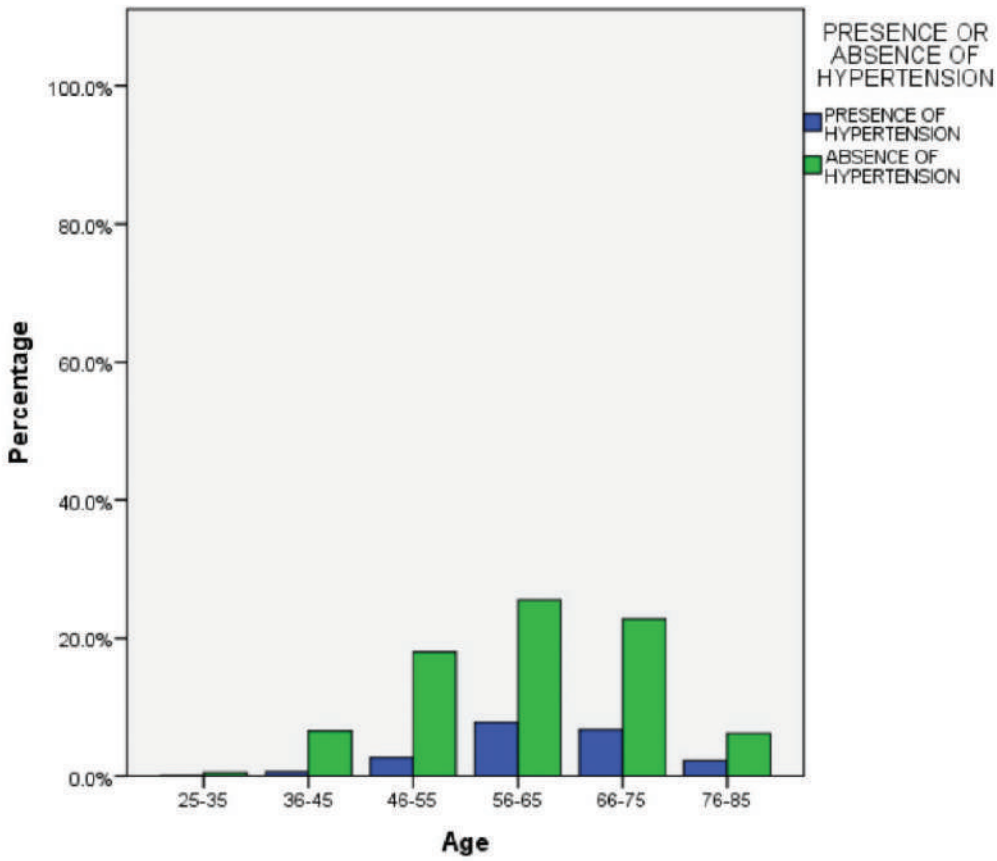
Graph 2: This graph represents percentage distribution of study population based on gender . Bar graph shows the distribution of the study population based on gender . The X axis represents gender and the Y axis represents the numbers of patients. Prevalence is more common in males compared to females.It is evident that 58.8% were males and 41.2% were females.



Graph 3: This graph represents percentage distribution of study population based on presence or absence of hypertension. Bar graph shows the distribution of the study population based on presence or absence of hypertension.The X axis represents presence or absence of hypertension and the Y axis represents the numbers of patients. It is evident that the presence of hypertension will be 20.3% and absence of hypertension will be 79.7%.



Graph 4: Bar chart depicting association between gender and presence or absence of hypertension in completely edentulous patients. X axis represents gender and Y axis represents numbers of patients where blue colour denotes presence of hypertension and green colour denotes absence of hypertension .Association between gender and presence of hypertension in completely edentulous patients was done using chi square test and was found statistically non significant ((P= 0.952,P>0.05)



Graph 5: Bar chart depicting association between age groups and presence or absence of hypertension in completely edentulous patients. X axis represents age groups and Y axis represents numbers of patients where blue colour denotes presence of hypertension and green colour denotes absence of hypertension . Association between age groups and presence or absence of hypertension in completely edentulous patients was done using chi square test and was found statistically significant (P=0.007,P<0.05).Hypertension was more prevalent in the age groups 56-65 and 66-75 years when compared to other age groups.

Conclusion

Within the limits of the study , prevalence of completely edentulous patients is more common (56-65) years of age and Male are more commonly affected. Hypertension prevention was more common in Male compared to females. While studies are yet exploring the prevalence of hypertension , it is essential that these findings need to be discussed with primary care providers.Further studies on inter professional practice among dentists and physicians in management of hypertension.

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Conflict of Interest : Nil

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Temporomandibular Joint Disorder among Patients Attending Private Dental College, Chennai

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Abstract

Temporomandibular joint and muscle disorder commonly called "TMJ ‘’ disorder, are a group of conditions that cause pain and dysfunction in the jaw joint and the muscle that control the jaw movement. The general and clinical consequences of discomfort, pain and disturbance of mastication are similar in most patients. TMD can be caused by injury to the jaw, TMJ or muscles on head and neck, such as from a heavy blow. Other causes includes: Grinding or clenching the teeth (puts a lot of pressure on TMJ), dislocation of the soft cushion or disc between the ball and socket, presence of osteoarthritis or rheumatoid arthritis in the TMJ, stress which can cause a person to tighten muscles in the face and jaw as to clench the teeth. Some symptoms of TMD are like - Pain and tenderness, clicking, popping or limited jaw opening or there will be swelling on the side of the face.Therefore, the aim of this study is to determine the prevalence of TMJ disorder attending private dental colleges in Chennai. Case sheet of all the patients reporting to the OPD of Saveetha Dental College with TMJ problems.The study was conducted between June 2019-March 2020. This university setting study a sample of 61pateints.Data tabulated with parameters of age, gender and TMD types Microsoft excel 2016 (microsoft office 10) was used to collect data and later was imported to spss software for statistical analysis. Descriptive statistics having frequency, percentage and chi-square test done for association.The prevalence of TMD Temporomandibular joint disorder was found to be more among males than females. Among the age groups of 11-25 years, 26-40 years and above 40 years, TMJ disorder was more prevalent between 26-40 years age group (64.41%). Prevalence of TMD Degenerative disorder, Disc condyle disorder and MPDS among them Disc condyle is more common(61.02%) as compared to others. The association between age and TMD was found to be statistically highly significant using chi-square test ($p<0.001$).Within the limit of this study,male were more affected and the disc condyle disorder was more common among study participants in the age group between 26-40 year.

Keywords – *TMJ, TMD, Clenching, Heavy bow, stress, trauma,*

Introduction

The temporomandibular articulation is composed of bilateral, diarthrodial temporomandibular joints (TMJ)[1].Each joint is formed by a mandibular condyle and its corresponding temporal cavity(glenoid fossa and articular eminence).The TMJ and its associated structure play an essential role in guiding mandibular motion and distributing stress produced by every day task such as chewing, swallowing and speaking.Some dental pain is caused cavities or stress and if you are stressed to the point of clenching your jaw and grinding your teeth,which develop pain in TMJ[2].

TMJ disorders (TMD) are a class of degenerative neuro-skeletal conditions associated with morphological and functional deformities.Invasion of bacteria into the

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joint space cause intracapsular and pericapsular infection of the TMJ is rare[34]. TMD include abnormalities of the intra articular disc position and/or structure as well as dysfunction of the associated musculature.Self reported system of temporomandibular disorder include report the problem related to masticatory system such as pain while chewing food sometimes children cannot explain property whether it is caused by caries or any other reason[5].TMD is characterized by the clinical sign of pain and malfunction occurring jointly and separately

1. Pain in the temporomandibular joint (TMJ).

2. Articular sound.

3. Pain in the muscle of mastication.

4. Stiffness of jaws.

5. Limited mouth opening.

6. Signs and symptoms that may be associated with orofacial pain and/or cervicoscapular problems.

A malfunction is an expression of disturbance of functionality. TMJ disorders are common and often self limited in adults. However, only few with TMJ symptoms require treatment and even fewer develop chronic or debilitating symptoms.Fluoride are present in the environment.Excessive systemic exposure of fluorides can lead to disturbance of bone homeostasis[67]. Sometimes TMJ disorder caused due to trauma or accident,acute trauma to the jaw and/or muscles of the jaw is a leading cause of TMJ injury and can often be attributed to car accidents, falls, etc[8]. Causes of TMJ disorder include parafunctional habit, emotional stress anxiety occlusal interferences, malpositioning or loss of teeth , postural changes, dysfunction of the masticatory musculature and adjacent structure, intrinsic and extrinsic changes in TMJ structure and/or a combination of such factor[9].

TMJ disorder diagnosed there is no widely accepted standard test now available to correctly diagnose TMJ disorders. Because the exact causes and symptoms are unknown, identifying disorders can be difficult and confusing[10]. Currently, health care providers note the patient's description of symptoms, take a detailed history and examine problem areas including head, neck, face and jaw. Facial pain can be a symptom of many conditions, such as sinus or ear infection, various types of headaches, and facial neuralgia[11]. Ruling out these problems will help in identifying TMJ disorder.

TMJ disorder can be treated but the more Effective treatment requires more knowledge about the cause, signs & symptoms[12]. Treatment can be like conservative treatment which is considered as the simple treatment in which we have to do all the things which help in relieving discomfort.

Self-care practices like eating soft foods, applying ice packs, avoiding extreme jaw movement and learning techniques for releasing and reducing stress and sometimes by opting for preventive treatment like pit and fissure sealant[13,14]for prevention of dental caries which ultimately reduces the risk of caries development and tooth loss. For many people with TMJ disorder, short term use of over the counter pain medicines [NSAID].Natural products offer a novel method for preventing and treating inflammatory diseases and are potential tools for TMJ arthritis management [15,16]. Dentists may recommend an oral appliance also called a stabilization splint or bite guard, which is a plastic guard that fits over the upper or lower teeth.Nutrition in jaw fractures treated with intermaxillary fixation is more compromised. Healing is impaired in malnourished, critically ill, elderly, and patients with prolonged stay in hospital and hence nutrition in the form of enteral, parenteral, and oral sip feeding plays a major role in providing nutritional care[17]. Stabilization splints are the most widely used treatments for TMJ disorder. Other types of treatment, such as surgical procedure, invade the tissue[18]. Surgical treatments are controversial, often irreversible and should be avoided as much as possible. There have been no long-term clinical trials to study the Safety and effectiveness of surgical treatment of TMJ disorders. Failure to respond to conservative treatment, for example,does not automatically mean that surgery is necessary. If surgery is recommended, be sure to have the doctor explain it to you, in words you can understand, the reason for the treatment, the aisle involved, the other type of treatment that may be available.

Materials and Methods

This record based, descriptive study was conducted among patients reporting to the OP of Saveetha Dental College. Case sheets of all the Patients of OP Department

of Saveetha dental college were reviewed for a period of ten months [JUNE 2019 and MARCH 2020]. Sample of 61 patients regarding Temporomandibular joint disorder were taken.

Prior to the start of the study,ethical approval was obtained from Scientific Review Board,Saveetha Dental College,SIMATS University.Data consisting of age,gender and TMJ disorder types was retrieved from the records of patients who visited dental college from october 2019-march 2020..

The age was grouped into (11-25 years),(26-40 years) and above 40 years.The gender include male and female.TMJ disorders types-Disc condyle disorder,Degenerative disorder and MPDS.Data was recorded and tabulated in ms excel.Then the tabulated data expressed by means of frequency and percentage. Chi-square test was employed to find the association between age and TMJ disorder, Gender and TMJ disorder with level of Statistical significance at $p<0.05$.

Results and Discussion

TMD is similar to musculoskeletal disorders in other parts of the body,and similar treatment approach can generally used.It is important for dentists to rule out disorders that mimic same as temporomandibular disorder, and to identify non TMJ disorder that may have negatively impact the patients TMD symptoms and offer the patient to take treatment.In this study we take a various age groups among them the age group which had higher prevalence for temporomandibular joint disorder was (26-40 years age group (64.41%) followed by 11-25 year age group as shown in figure 1.The most common age group was found to be (26-40 years).Supported studies done by (Muthukrishnan et al.,and Yang et al.) state in their studies that the young adult age group between(26-36 years) were maximum affected by TMJ disorder[19].

Some literature also state that there is no proper studies done on the prevalence of the middle age group patient. Some studies oppose this result done by (Yadav et al.,and Mathew et al.), in their studies they say that the old age group is more prone to TMJ disorder as their bone becomes weak they also say there is no any exact reason found behind TMJ disorder.[20,21]

Out of 61 patients, 32 were males and 27 females patients in figure 2.In a study done by (H et al., and Charizopoulos et al,) state that the male are more prevalence to TMD disorder which is similar to this study[22,23],some studies done by (Bagis et al and Wizel et al) stated that females are more prevalent than the male due to nutrition differences and many other factors [24,25]. This hold true as male are more prone to TMD disorder as they have habit and more prone to trauma.There are different types of TMD disorder like degenerative disorder, disc condyle disorder and MPDS among them the more prevalence we observe the disc condyle disorder types(61.02%) followed by MPDS (35.59%) as shown in figure 3. Supportive study done by (Turp et al). state that disc condyle is more frequently seen as compared to other types of TMJ disorder[26].

It is apparent from Figure 4 which shows a statistically significant association between Age and TMJ disorder using Chi-square test. The frequency distribution of Disc condyle disorder (n=14) was found to be more in the age group of 11-25 years. Similarly, Disc condyle disorder (n=22) was found to be more in the age group of 26-40 years than MPDS. A total of 5 study participants in the age group more than 40 years reported with MPDS..Above age 40 year we can only see MPDS types of TMD disorder.Young adults have habits of betel nut chewing and they are also more prone to road accidents[29].In male disc, condyle disorder is more prominent as compared to female shown .

Figure 5 depicts the association between Gender and TMJ disorder where disc condyle disorder was more prevalent in Male (n=21) study participants than females(n=15). But on the other hand, MPDS was found to be more prevalent in female (n=11) study participants than males(n=10) and was found to be statistically insignificant using chi-square test. Supportive studies (Koca et al)[27] state that male are more to frequently seen with TMJ disorder, some study disagree with that(Pontes et al) [28] they say that females are more prone to disc condyle disorder.As a patient ages the attrition rate increases which changes the maxillary mandibular relationship which poses a risk of TMD disorder.

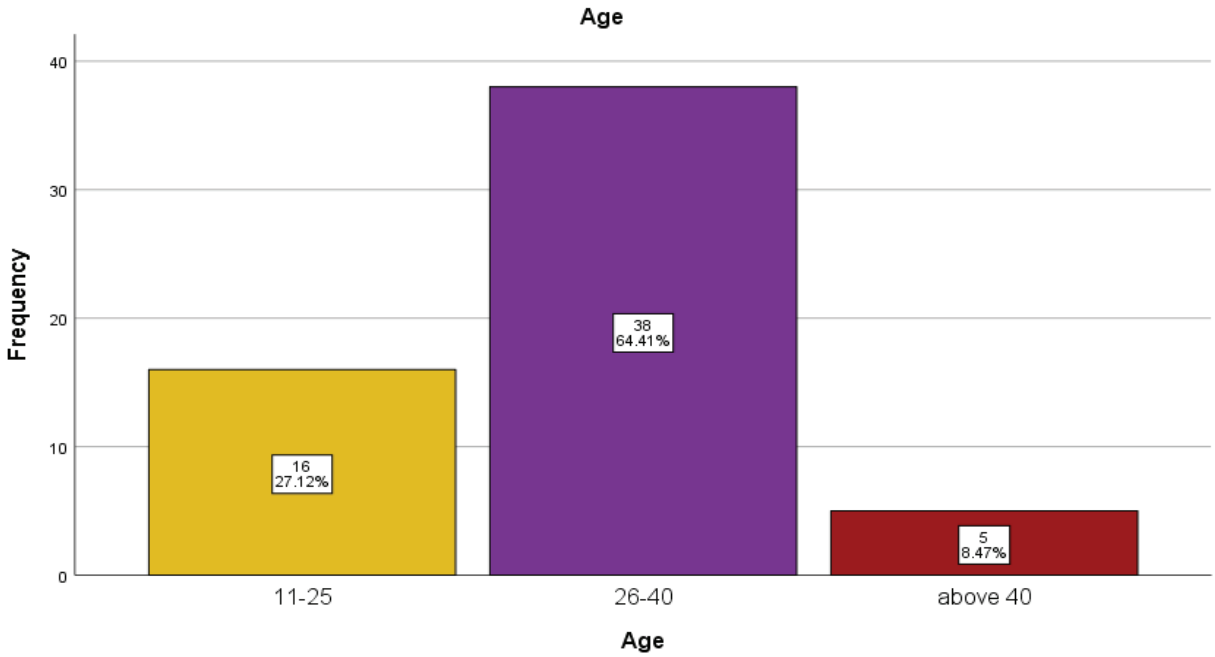


Figure 1- This graph represents the distribution of study subjects based on age group.X axis represents the age range (11-25 year),(26-40 year) and above 40 years, y axis represents the frequency of participants. Age range from 26-40 years were found be more (64.41%) followed by age range 11-25 years.(27.12%)

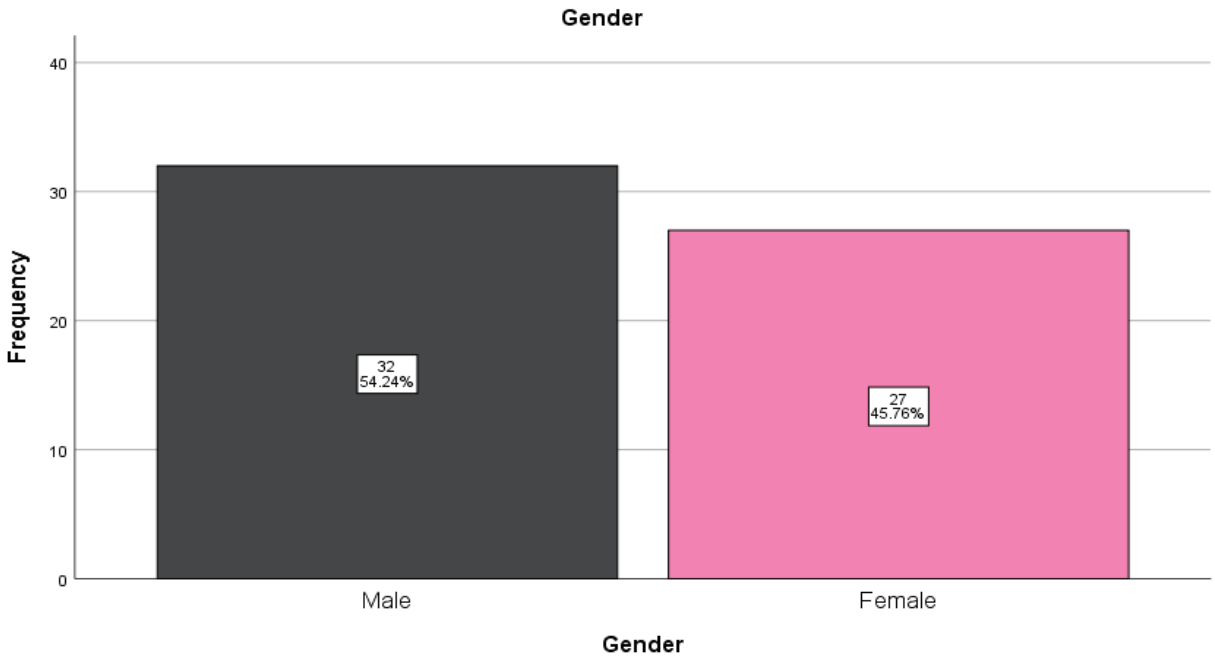


Figure 2:The graph represents the distribution of study subjects based on gender. X axis represents the gender y axis represents the frequency of study subjects. Out of 61 study subjects, 32 were male 32(54.24%) and 27(45.76%) were female

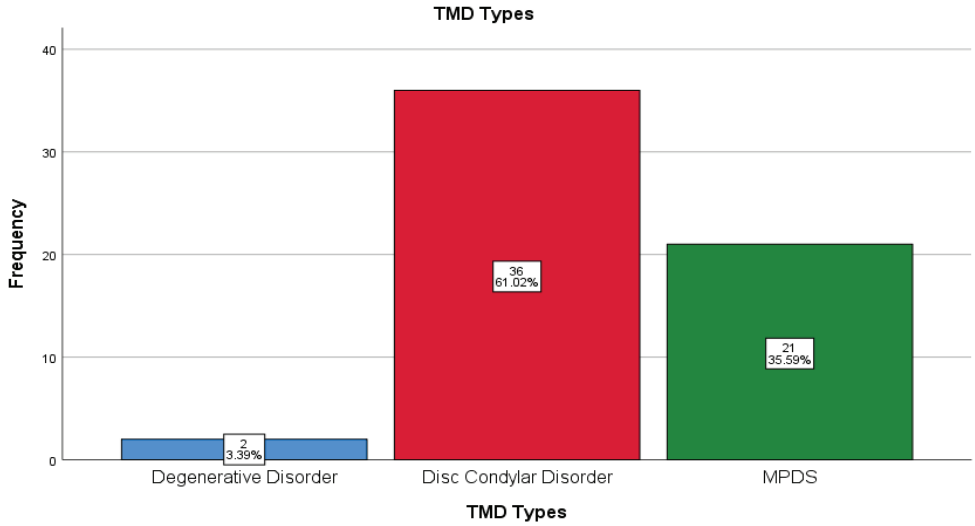


Figure 3:The graph represents the prevalence of Temporomandibular disorder types. X axis represents the TMD types like Degenerative disorder, Disc condyle disorder and MPDS y axis represent the frequency of participants. Where disc condyle disorder is more in number(61.02%) followed by MPDS (35.59%).

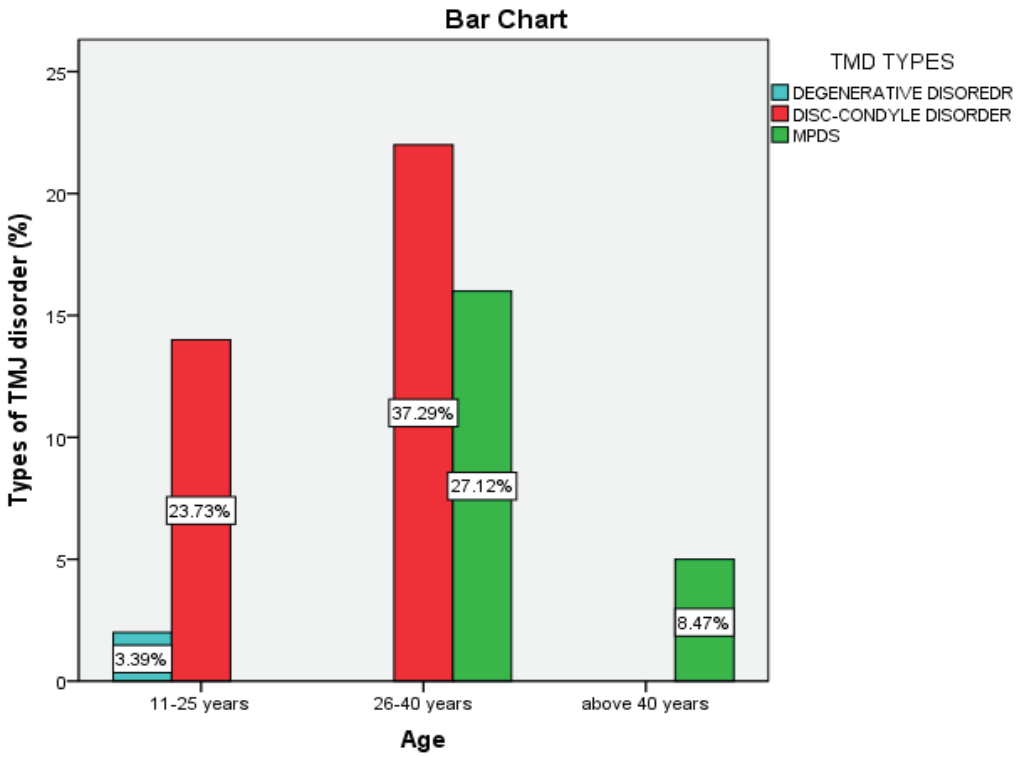


Figure 4 Bar graph represents the association between the age and Temporomandibular joint disorder types. X axis represents the age range 11-25 years,26-40 years and above 40 years and Y axis represents the TMD types, where blue colour denotes the degenerative disorder, red colour denotes disc condyle disorder and green colour denotes the MPDS. The association between age and Temporomandibular joint disorder types was analyzed using Chi square test and was found to be statistically significant [Chi- square value= 22.301; (p value<0.001)]. Hence proving disc condyle disorder and MPDS were more prevalent in the age group between 26-40 years.

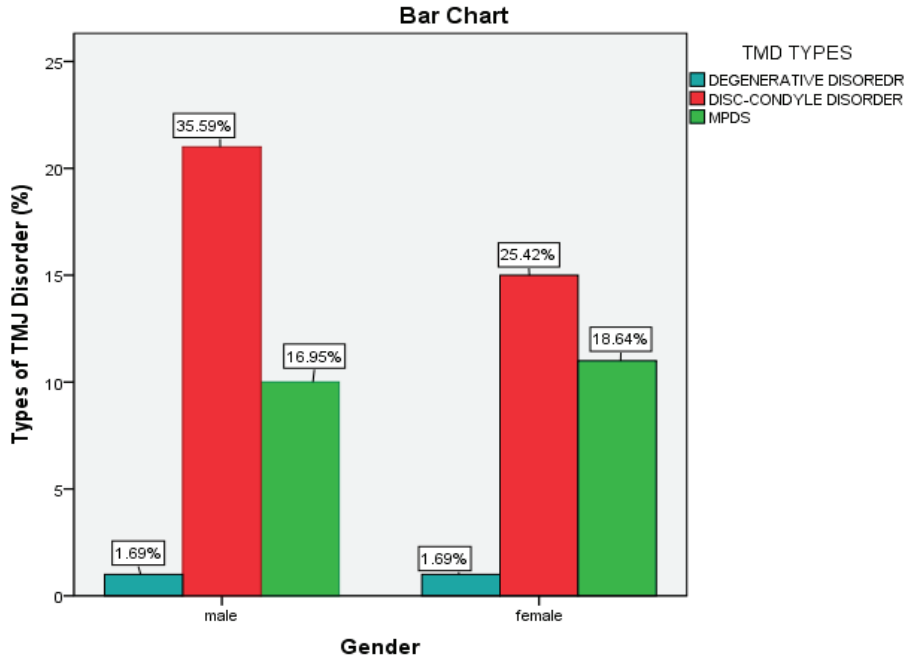


Figure 5 Bar graph represents the association between the gender and Temporomandibular joint disorder types. X axis represents the gender and Y axis represents the TMD types-degenerative disorder, disc condyle disorder and MPDS, where blue colour denotes the degenerative disorder, red colour denotes disc condyle disorder and green colour denotes the MPDS. Chi square test was done and association was found statistically not significant [Chi- square value=0.628; p>0.05].Hence proving that disc condyle disorder was more prevalent in males where females were more affected with MPDS.

Conclusion

Within the limit of this study the age group of 26-40 years had higher prevalence of TMD disorder (Disc Condyle disorder). With respect to gender wise distribution, Disc Condyle disorder was found to be more prevalent among males than females and MPDS was more common in females than males.

Contribution- All authors are equally contributed for the study design, methodology and data collection.

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Conflicts of Interest: None to declare.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Antimalarial Drugs Used in the Management of Viral Infections-A Perspective

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Abstract

Antimalarial drugs are antiparasitic agents which are naturally derived from plants used in the treatment of malaria.There are various classes of antimalarial drugs seen.They are Artemisinin derivatives, aryl-amino alcohols, aminoquinolines and antimicrobial drugs.The mechanism of action of antimalarial drugs is to prevent the polymerisation of heme which results in the death of the parasite.Antimalarials are also used in the treatment and prophylaxis of viral infections which is being discussed in this review. Around 40 articles from Pubmed, Google scholar which are relevant to the topic are taken and discussed.The articles which don't fall into the criteria are excluded.The points from the article are taken and discussed.The review methods,the different classes of drugs like artemisinin derivatives, aryl-amino alcohols, aminoquinolines and antimicrobial drugs are also discussed. The antimalarial drugs are widely used in the prophylaxis and treatment of various existing and emerging viral infections.Newer viral infections have no resistance against antimalarial drugs which proves to be very useful.

Keywords: *Antimalarials, parasites, viral infections ,resistance*

Introduction

Antimalarial drugs are anti-parasitic agents which are naturally derived from plants used in the treatment of malaria.There are various class of antimalarial drugs seen.They are Artemisinin derivatives, aryl-amino alcohols, aminoquinolines and antimicrobial drugs.The mechanism of action is to prevent the polymerisation of heme which results in the death of the parasite. Artemisinin derivatives are the most effective of antimalarial drugs. Artemisinin derivatives used in vitro are proven to be effective against human cytomegalovirus.[1] Artemisinin and Artesunate have been proven to be used for the treatment of Hepatitis B and C infections. Dihydroartemisinin is effective against bovine viral diarrhoea virus.[2] Quinine sulphate tested invitro is proven to have been effective against dengue virus.

Mefloquine is also proven to be effective against Zika virus infections.Quinine which is derived from barks of Cinchona trees are proven to be effective against Swine flu. Previous research articles are taken into account.One of the studies about artemisinin used invitro against hepatitis B production is taken.The result of this study shows that the combination of artesunate and lamivudine shows synergistic effects against the infection.[3] Another study about the usage of chloroquine against influenza prevention in a randomised placebo trial is taken.The result however shows that chloroquine is not effective and alternate drugs are required for the treatment of influenza.[4]

Recently, hydroxychloroquine is being recommended for the prophylaxis and treatment of COVID-19 or coronavirus infection which is a global epidemic in recent days. SARS coronavirus which causes

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acute respiratory obstruction in the past have been treated with chloroquine with combinations of antiviral drugs.[5] The aim of this review is to study the characteristics of antimalarial drugs used in the management of various viral infections.

Materials and Methods

Review Methods

About 40 articles are chosen for this review. The articles which are taken are chosen based on the relevancy to the topic.The articles which are in other languages are excluded and the articles which are not related to the topic are excluded.The data for the review was extracted based on the characteristics of the article.[6]

Artemisinin Derivatives

Artemisinin derivatives are drugs which are derived from a plant called

Artemisia annua,a herb.These drugs are safe,well tolerated with lower potency in lower or normal dosage but high potency in larger dosages.The main derivatives discussed here are artemisinin and artesunate.[7] Artemisinin is a semisynthetic derivative which is poorly soluble. Artemisinin is proven to be effective against hepatitis B and C infections in vitro.Artemisinin is activated by iron molecules which dissolves to free radical formation.[8]

Artesunate which is another derivative has the highest activity and is effective against herpes simplex viruses.It is taken by intravenous route for the protocol treatment of malaria. Artesunate is also involved in the inhibition of human immunodeficiency virus replication and hence seen in antiretroviral therapy in the treatment of Acquired Immune Deficiency Syndrome.[9] Artesunate has a synergistic action with antiviral drugs like maribavir, lamivudine, ganciclovir, foscarnet, cidofovir, letermovir etc.[10,11]Artesunate is effective against Epstein-Barr virus infection in low micromolar ranges and acts on epithelial cells and lymphocytes.[12]

Aryl- Amino Alcohols

The important drugs in this classification are quinine sulphate and mefloquine. Quinine sulphate is the oldest drug in existence used in treating malaria.[13] Quinine sulphate in micromolar range and not in toxic doses have reduced the number of plagues formed and also in Herpes Simplex Virus-1 in vitro.[14] Quinine sulphate is also said to be effective in the treatment of dengue virus infections.

Mefloquine combined with mirtazapine acts on the 5-HT2A serotonin receptor. It inhibits the entry of JCPyV into glial cells preventing the diffusion and the infection of oligodendrocytes.[15–18] Mefloquine is widely used in the prophylaxis of mild to moderate malaria.[19] Lumefantrine with combination with artemether is called CoArtem commercially. It is used in treatment of non severe malaria.

Aminoquinolines

The important drugs in this classification are chloroquine and hydroxychloroquine. Chloroquine has low toxicity and high tolerance and is used as an antiviral drug. Chloroquine is used in combination with antiviral drugs to produce synergistic effects.[20] Chloroquine is also used in the treatment of amoebiasis, systemic and discoid lupus erythematosus, sarcoidosis,porphyria cutanea tarda and in decreasing the symptoms for rheumatoid arthritis.[21] Hydroxychloroquine is used in the treatment of RNA virus infections.It is also used in the treatment of lupus erythematosus and rheumatoid arthritis.[22] Hydroxychloroquine goes by the commercial name Planequil.

Anti- Microbial Drugs

The drugs seen here are atovaquone, doxycycline and sulfonamides. Atovaquone is an antimalarial drug used in pregnant women.Atovaquone is an analogue of ubiquinone and is used to treat a very serious lung infection called Pneumocystis pneumonia.[23] Doxycycline is a semisynthetic tetracycline used in the combination treatment of malaria along with quinine.[24] Sulfonamides are a group of drugs that are prescribed to treat urinary tract infections,bacterial meningitis,bronchitis, eye and ear infections etc.[25,26] Recent studies have demonstrated the activity of sulfonamides as they act on latent herpes viruses, Epstein-Barr virus and Kaposi sarcoma virus.[27,28]

Discussion

In this review, we discussed the previous articles related to antimalarial drugs used in the management

of viral infections. We have evaluated the drug actions of artemisinin derivatives, aryl- amino alcohols, aminoquinolines and other antimicrobial drugs.

Artemisinin derivatives such as artemisinin and artesunate are taken into account.They produce synergistic actions when combined with other antiviral drugs and are effective against hepatitis B and C and also inhibit the replication of human immunodeficiency virus.Aryl- amino alcohols such as quinine sulphate and mefloquine are earlier derived drugs which are effective in treatment of Herpes simplex virus-1 and Zika virus infections.[29,30] Quinine sulphate might cause a condition that affects the heart rhythm by QT prolongation in the electrocardiogram readings.[31] Mefloquine should not be taken with hydroxychloroquine as it increases the risk for occurence of seizures.[32–34]

Aminoquinolines such as chloroquine and hydroxychloroquine has low toxicity, high tolerance and hence used in the anti- retroviral regimen therapy. Hydroxychloroquine and diabetes drugs taken together will cause hypoglycemia.[35] Hydroxychloroquine should not be taken with other cardiac drugs as it causes dangerous cardiac arrhythmias.[36] Other antimicrobial drugs like atovaquone, doxycycline and sulfonamides are used in the treatment of Epstein-Barr virus and Kaposi sarcoma virus infections.[37] The complications like hepatic inflammation and fibrosis are caused due to chronic viral deposition.[38] Sulfonamides antimicrobials can be combined with trimethoprim to make them bactericidal.[39,40] Other natural agents like Acacia species have less toxicity and good efficacy.[41,42] The main drawback of antimalarial drugs is their efficacy.The efficacy of these drugs are declined due to the emergence of drug resistant organisms.It can be corrected by usage of nanoparticles for targeted drug delivery and thus rendering excellent efficacy.[43]

The limiting factors of usage of antimalarials are the usage of older drugs.Different dosages given for each infection, ongoing trials on the usage of drugs are also considered to be a drawback. The uncertainty of the adverse effects of the drugs on healthy individuals should be considered.Recently, hydroxychloroquine is recommended in the prophylaxis and treatment of COVID-19 or coronavirus infections. SARS coronavirus in the early 2000's was treated back then with a combination of chloroquine with antiviral drugs.[44]

Conclusion

The antimalarial drugs are widely used in the prophylaxis and treatment of various existing and emerging viral infections.Newer viral infections have almost no resistance against antimalarial drugs which proves to be very useful.Newer antimalarial drugs with a wide spectrum against viral infections might bring favourable results.

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Ethical Clearance:- Not Required

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Ayurvedic Science-An Undergraduate Perspective

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Abstract

Ayurvedic medicine is considered as the world's oldest medical system. Ayurveda originated in India over 5000 years ago in the magnificent Himalayas.Ayurveda is a pseudoscientific system of medicine with historical roots in the indian subcontinent. Ancient Ayurvedic texts also taught surgical techniques,including rhinoplasty,kidney stone extractions,sutures, and the extraction of foreign objects. It is still practiced in the South Indian population. There are studies showing data that about 80% of Indian patients use ayurvedic therapy instead of allopathy. This is a cross sectional study conducted among 100 dental students through a questionnaire with the help of an online survey link. The responses show 79% of the participants agree that knowledge about Ayurveda is important to dental students. 80% of the participants agree that Ayurveda should be integrated with modern medicine. Dental students have a good knowledge about Ayurvedic medicine and medicinal herbs that are used in the process.

Key words: *Ayurvedic medicine, Modern medicine , Medicinal herbs.*

Introduction

Ayurveda is a conjugation of two Sanskrit words Ayus means life and Veda means science thus Ayurveda means science of life.[1] Ayurveda originated in India more than 5000 years ago and is often called as (Mother of all healing)[2]. The purpose of Ayurvedic medicines was to synchronise and maintain the body, mind and spirit. This balance is believed to lead happiness , health and prevent illness [3]. Ayurveda believes in 5 basic elements(space, fire,water ,earth and air) which manifested in the human body as three basic humours known as tridosas (vata,pitta and kapha). These three tridosas govern creation, maintenance and destruction of bodily tissues as well as the assimilation and elimination. Ayurveda basically comprises fruits,vegetables,spices and natural herb essence which helps in treating the diseases without causing any side effects on the patient. Meanwhile , allopathy cures the illness in no time, but leaves behind some side effects like allergies,pain etc..[4]. Ayurveda also helps dental professionals in enhancing oral health and promoting periodontal health status.[5]

According to the shalyatantra and shalakya tantra (one of the branches of Ayurveda) 65 varieties of oral diseases can arise in 7 body locations - 8 on the lips, 15 on the alveolar margin, 8-in teeth, 5 on the tongue, 9 on the palate, 17 in the oropharynx and 3 in a generalised form. For these types of diseases Ayurveda advocates procedures such as dant Dhanvanti (Brushing), jivha Lekhana (tongue scraping) and gandoosha (gargling) or oil pulling and tissue regeneration therapies for maintaining proper oral health [5,6]. Chewing sticks in the morning as well as after every meal is also recommended

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in Ayurveda to prevent oral diseases. The most famous herbal chewing stick is the neem [7]. Herbs with medicinal properties are useful and effective sources for treatment of various disease processes[8]

The main aim of this study is to assess the knowledge of dental students about Ayurvedic science and different medicinal herbs used in the procedure.

Materials and Methods

Study Design

A cross sectional survey was conducted among first year dental students to evaluate their knowledge and awareness level on ayurvedic science and medicine. The study population was 100 participants. The participants did the survey voluntarily and know incentives were given to them. The survey was conducted in the month of May , 2020. Ethical approval and informed consent from the participants were obtained.

Survey Methodology

The questions were prepared after extensive literature. The questionnaire was reviewed and amendments were made to improve clarity of questions and eliminate ambiguous responses. The survey methodology was a structured questionnaire with both open and close ended questions [9]. It consists of a brief introduction regarding research objectives. 12 questions were circulated to the participants through Google Forms.

Data Analysis

Only completed filed online forms were included in this study . The filled responses were verified by the 2 viewers and collected on the same day. The entered data was analyzed using SPSS Software.Descriptive analysis was performed to calculate frequencies of categorical variables [10,11 ,12 ,13 ,14] .

Results and Discussion

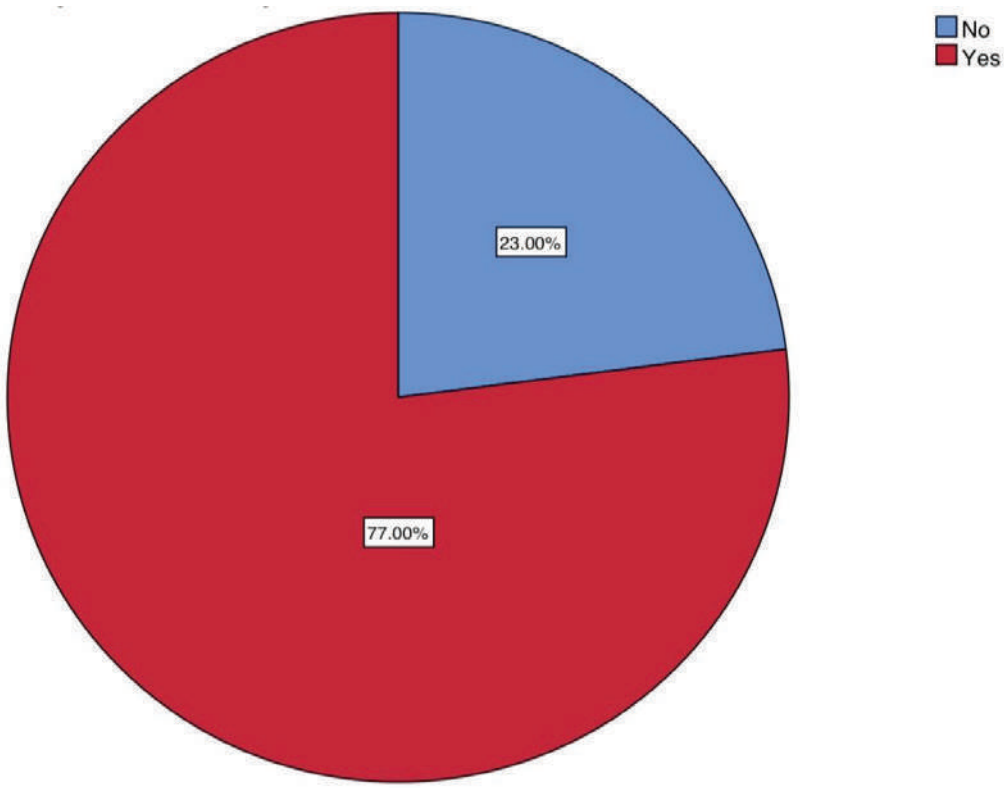


Figure 1:The pie chart shows responses to the question "Ayurveda is considered the world's oldest medical system". The Majority of the participants are aware(77%,red) whereas the remaining 23% are unaware about this fact(blue).

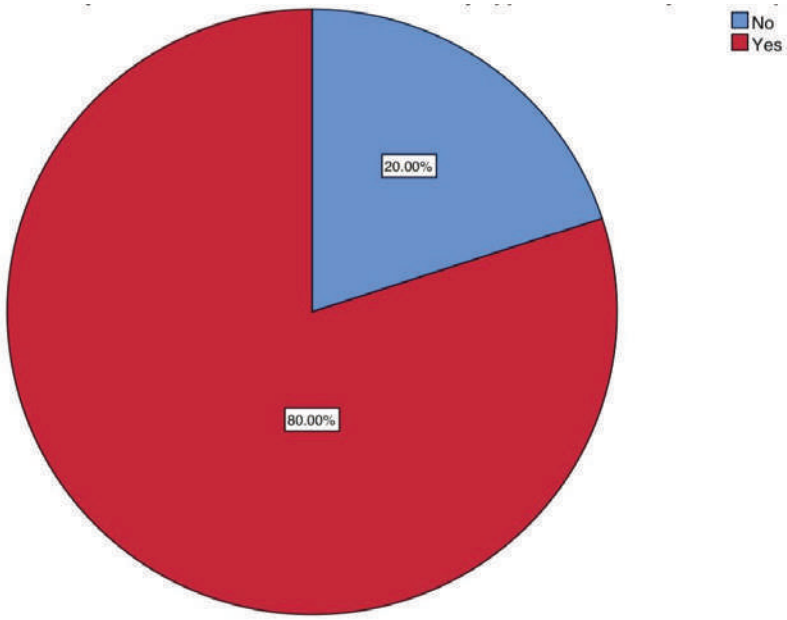


Figure 2:The pie chart shows responses to the question "Ayurveda successfully applied in dentistry". The majority of the population are aware that ayurvedic medicine can be successfully applied in dentistry as antiseptics and antioxidants(80%,red) whereas the remaining 20% are unaware about this fact(blue).

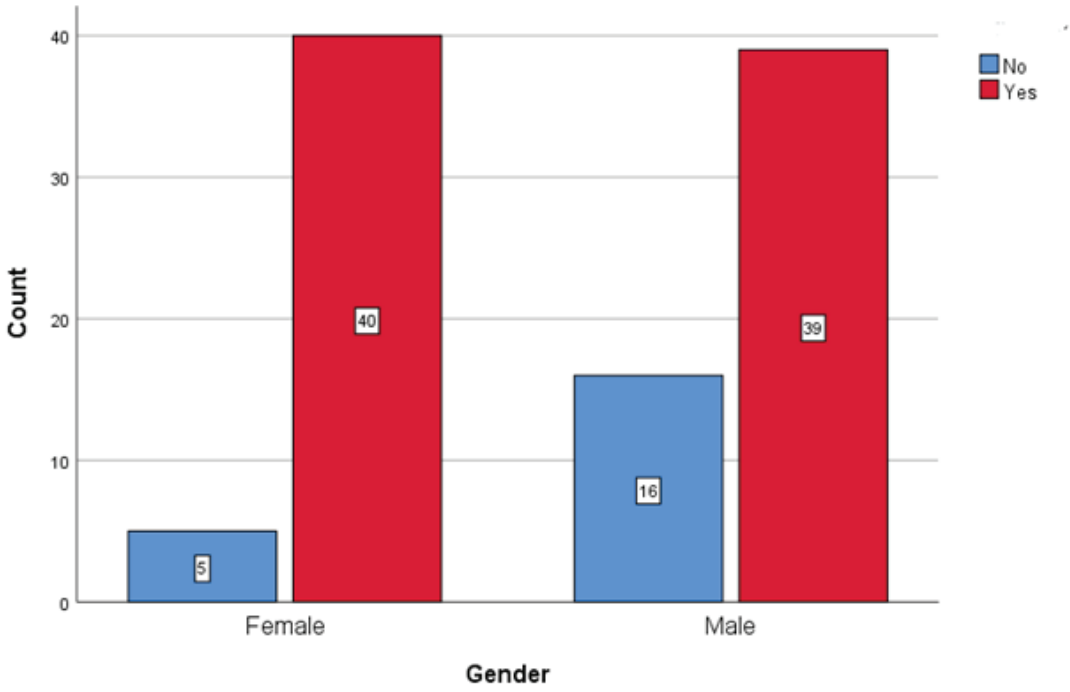


Figure 3: Bar chart representing the association between gender and knowledge of ayurveda important to dental students. X axis represents gender and Y axis represents knowledge of ayurveda important to dental students.Out of 79% of the public who are aware(red), 40% constitute female and the remaining 39% are male. Hence more women think that knowledge of ayurveda is important to dental students.Chi square test was done and the association was found to be statistically significant(Person's Chi square value:4.823,df:1,P value:0.0289(<0,05)).

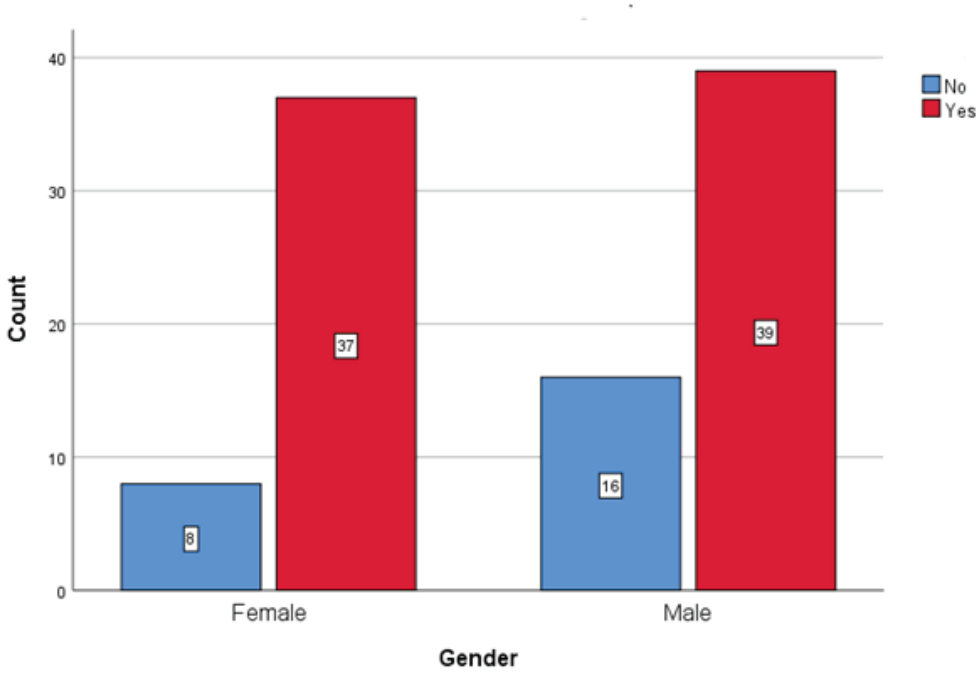


Figure 4: Bar chart representing the association between gender and usage of ayurvedic medicine . X axis represents gender and Y axis represents usage of ayurvedic medicine.Out of 76% of the public who are aware(red), 37% constitute female and the remaining 39% are male. Hence more men use ayurvedic medicine and it was also proved to be effective.Chi square test was done and the association was found to be statistically non significant(Person's Chi square value:1.737,df:1,P value:0.188(>0,05).

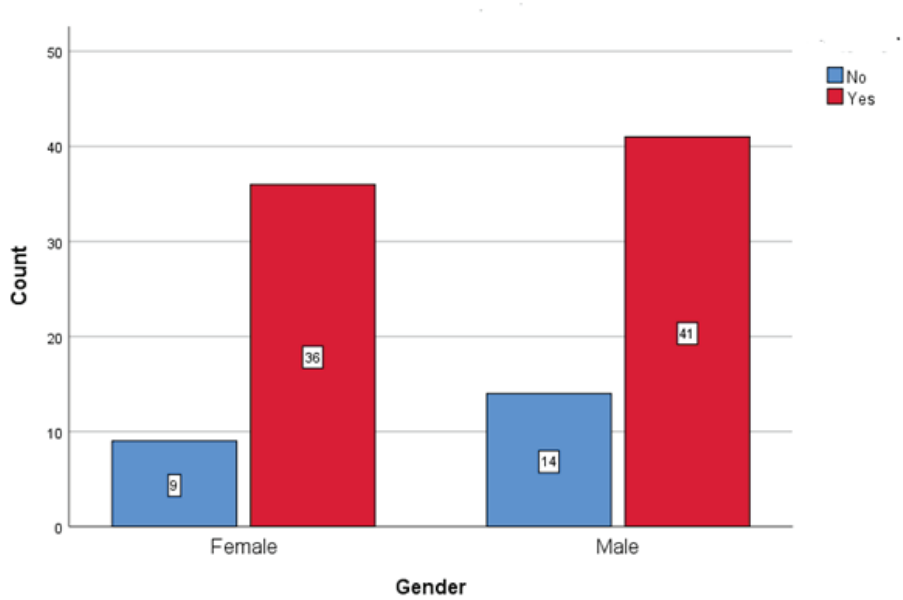


Figure 5: Bar chart representing the association between gender and ayurvedic medicine considered the world's oldest medical system. X axis represents gender and Y axis represents ayurvedic medicine considered as a world's oldest medical system. Out of 77% of the public who are aware(red), 36% constitute female and the remaining 41% are male. Hence majority of the men are aware that ayurvedic medicine is considered the world's oldest medical system. Chi square test was done and the association was found to be statistically non-significant(Person's Chi square value:0.416,df:1,P value:0.519(>0,05)).

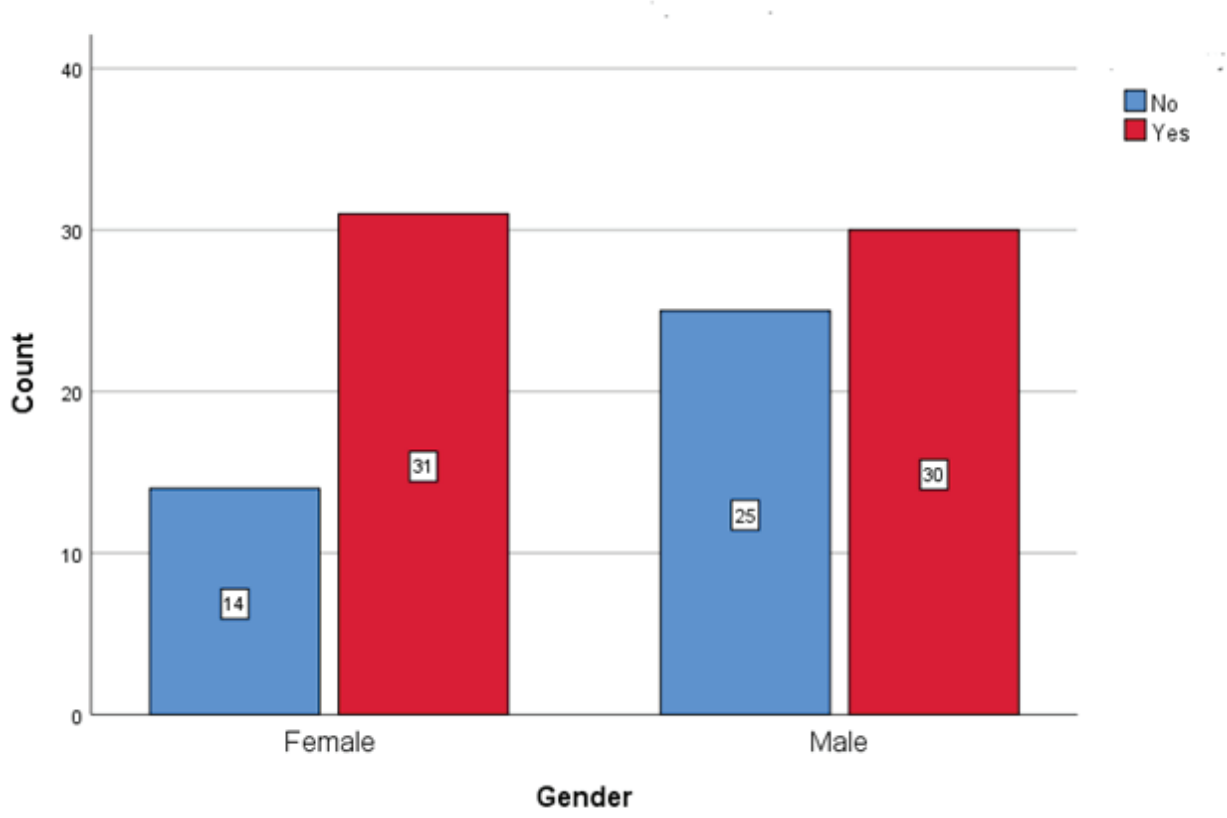


Figure 6: Bar chart representing the association between gender and real meaning of ayurveda. X axis represents gender and Y axis represents real meaning of ayurveda. Out of 61% of the public who are aware(red), 31% constitute female and the remaining 30% are male. Hence majority of the women are aware that the real meaning of ayurveda is science of life. Chi square test was done and the association was found to be statistically non-significant(Person's Chi square value:2.140,df:1,P value:0.143(>0,05)).

Survey population was sufficient enough to reach a conclusion regarding the knowledge of dental students about Ayurvedic science. This survey was conducted among college students who are within the 18 year age group. Out of 100 responses given 55% were male and 45% were female, where most of the students have used Ayurvedic medicines. 77% of the dental students know that Ayurvedic medicine is considered to be the world's oldest medical system [figure 1]. 80% of the dental students know that Ayurvedic medicine can be successfully applied in dentistry as antiseptics and antioxidants[figure 2]. The bar graph representing Chi square analysis between gender and importance of ayurveda. X axis represents gender and Y axis represents importance of ayurveda towards dental students. Out of 79% of the public who are aware(red), 40% constitute female and the remaining 39% are male. Hence more women think that knowledge of ayurveda is important to dental students.The result was found to be statistically significant(Person's Chi square value:4.823,df:1,P value:0.0289(<0,05))[figure 3]. The bar graph representing Chi square analysis between gender and usage of ayurvedic medicine. X axis represents gender and Y axis represents usage of ayurvedic medicine. Out of 76% of the public who are aware(red), 37% constitute female and the remaining 39% are male. Hence more men use ayurvedic medicine and it was also proved to be effective.Chi square test was done and the association was found to be statistically non significant(Person's Chi square value:1.737,df:1,P value:0.188(>0,05)) [figure 4]. The bar graph representing Chi square analysis between gender and ayurvedic medicine is considered the world's oldest medical system. X axis represents gender and Y axis represents ayurvedic medicine considered the world's oldest medical system. Out of

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From the responses it was evident that the majority of the respondents are aware of the origins of Ayurveda which was around 5000 years ago in the magnificent Himalayas, and the meaning of Ayurveda is science of life. There are also responses obtained for the most common element used in Ayurvedic medicine is oil, the most commonly used herbal extract is Salvadora Persica and Acacia gum, about Shiro Abhyanga massage, integration of the Ayurveda with modern medicine. The present research has origins from the team of investigators where previous studies were done based on clinical reports,interventional studies like [15,16,17],in vitro studies like, [10,11,12,14]and systematic reviews [15,18,19, 20, 21 ,22] .In a study done by Mankar NN, 93.40% of the participants think that knowledge of Ayurveda is important to dental students /medical students.56% 0f participants stated that they have used Ayurvedic medicine and it was proved to be effective. 78% of the participants think that Ayurveda should be integrated with modern medicine[23] . In a study done by Suchita R Gawde ,20% of the participants prefer using Ayurvedic medicine. 60% of the participants agreed that cooperation of Ayurveda therapies into practice would result in increased patient satisfaction and attract more people [24] . In a study done by H.Katole,27.11% consumers prefer using Ayurvedic medicine [25]. Even though many strict protocols were followed during this study there are certain limitations as this was a cross sectional study done only among 100 dental students. Dental students are not much aware about the various procedures that are practiced in Ayurveda like Shiro Abhyanga massage. The future goal of Ayurveda is to promote good health among individuals and cure deadly diseases like diabetes, cancer, arthritis and HIV with natural ingredients .

Conclusion

Ayurvedic medicine is considered as one of the best medical systems in the world because each and every ingredient that is used for producing a product is natural and does not result in any side effects.

According to our survey it is observed that dental students have a very good knowledge about Ayurvedic science and the medical herbs used in it.

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Ethical Clearance: Not Required

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Novel Mutations Associated with Amelogenesis Imperfecta- A Review

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Abstract

Amelogenesis imperfecta [AI] is a congenital disorder that presents with a rare abnormal formation of the enamel, external layer of the crown of teeth, unrelated to any systemic or generalized Conditions. AI causes teeth to be usually small, discolored, pitted or grooved and prone to rapid wear and breakage. These defects which vary among affected individuals can affect both primary teeth and secondary teeth. About 14 forms of amelogenesis imperfecta have been described so far. They are distinguished by their specific dental abnormalities and by their pattern of inheritance. Additionally, amelogenesis imperfecta can occur alone without any other signs and symptoms or as a part of a syndrome that affects multiple parts of the body.

Keywords*: Amelogenesis imperfecta; gene mutation; enamel; dental.*

Introduction

Amelogenesis imperfecta [AI] is a congenital disorder that presents with a rare abnormal formation of the enamel, external layer of the crown of teeth, unrelated to any systemic or generalized conditions.[1,2] Tooth enamel is the most highly mineralized structure in the human body, with 85.1. of its volume involved by hydroxyapatite crystals[3]. The physical properties and physiological function of enamel are directly related to the orientation, disposition Composition, and morphology of the mineral components within the tissue[4]. During organogenesis, the enamel transitions from a pliable tissue, which is almost entirely devoid of proteins.[5] During genesis deflection of the unique molecular and cellular activities might be responsible for the patterns observed in amelogenesis imperfecta.[6]

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The exact incidence of amelogenesis imperfecta is uncertain. Estimates vary wildly, from 1 in 700 people in northern Sweden to 1 in 14,000 people in the United states[7], among which hypoplastic AI represents 60 - 73% of all cases, hypomaturation AI represents 20-40%, and hypocalcification AI represents 7%[8]. A complex interplay of genes results in the formation of enamel, growth and enamel mineralization. The most severe type is mostly caused by truncating mutations in the *FAN83H* gene[9,10].

Classification of Amelogenesis Imperfecta

Amelogenesis imperfecta can be classified based on the phenotype[11], based on the clinical, microradiography and histopathological findings, and on the phenotype and mode of inheritance[12,13]. Most commonly accepted classification is the one being proposed by Witkop in the year 1988, which classified AI into mainly 4 types *viz.,* hypoplastic AI, hypomaturation AI and hypocalcified type based on developmental stages of enamel and hypoplastic-hypomaturation with taurodontism with several other subtypes under each type[14].

Table 1: Different types of amelogenesis imperfecta

Type 1 - Hypoplastic	IA hypoplastic, pitted autosomal dominant IB hypoplastic, local autosomal dominant IC hypoplastic, local autosomal recessive ID hypoplastic, smooth autosomal dominant IE hypoplastic, smooth X-linked dominant IF hypoplastic, rough autosomal dominant IG enamel agenesis, autosomal recessive
Type 2 - Hypomaturation	IIA hypomaturation pigmented autosomal recessive IIB hypomaturation IIC snow capped teeth, X-linked IID autosomal dominant
Type 3 - Hypocalcified	IIA autosomal dominant IIB autosomal recessive
Type 4 - Hypomature hypoplastic enamel with taurodontism	IVA hypomaturation - hypoplastic with taurodontism, autosomal dominant IVB hypoplastic - hypomaturation with taurodontism, autosomal dominant

1. Type 1 - Hypoplastic

The enamel of abnormal thickness due to malfunction in an enamel matrix formation. Enamel is extremely thin but hard & translucent and should have random pits & grooves. Condition is of an autosomal dominant, autosomal recessive, or x-linked pattern. Enamel differs in appearance from dentine radiographically as normal functional enamel.[15,16]

2. Type 2 - Hypomaturation

Enamel has a sound thickness, with a pitted appearance. It is less hard compared to normal enamel, and is prone to rapid wear, although not as intense as Type 3 AI.[17] Condition is of an autosomal dominant, autosomal recessive, or x-linked pattern. Enamel appears to be comparable to dentine in its radiodensity on radiographs.[18]

3. Type 3 - Hypocalcified

Enamel defect because of malfunction of enamel calcification, therefore enamel is of normal thickness but is extremely brittle, with an opaque/chalky presentation. Teeth are susceptible to staining and rapid wear, exposing dentin. Condition is of an autosomal dominant and autosomal recessive pattern. Enamel appears less radiopaque compared to dentine on radiographs.[20]

4. Type 4 - Hypomature hypoplastic enamel and taurodontism

Enamel has a variation in appearance, with mixed features from Type 1 and Type 2 AL. All Type 4 AI has taurodontism in common. Condition is of the autosomal dominant pattern. Other common features may include an anterior open bite,[19] taurodontism, the sensitivity of teeth. Differential diagnosis would include

dental fluorosis, molar-incisor hypomineralization, chronological disorders of tooth development.[20]

INHERITANCE PATTERNS OF AI

AI may be inherited in three manners which are X-linked form, autosomal dominant, or autosomal recessive trait. Apparently molecular genetic tools will allow more precise diagnosis. [21,22]Different genes are related to different forms of inheritance, located at different genomic sites. *AMELX* is associated with X-linked form which displays disorganized hypoplastic enamel.[23] Another gene shows both autosomal dominant and autosomal recessive inheritance patterns within the 4q13.3. has been identified as being allocated with on autosomal recessive inheritance.[24]

1. X-linked forms of amelogenesis imperfecta

X-linked amelogenesis imperfecta (XAI) shows the typical pattern of X-linked inheritance. Heterozygous females can transfer the mutant gene to children of either sex with the danger of this being 50% The condition affects males and females in strikingly different ways. Males show the trait fully [25]. They may have teeth that have only a thin layer of enamel of normal color and translucency, or the enamel may be of normal thickness but poorly mineralized with loss of translucency and/or a yellow-brown discoloration[26]

2. Autosomal dominant amelogenesis imperfecta

Autosomal dominant AI (ADAI) typically affects one or more individuals in each generation of a family.[27] There may be consistency in the clinical manifestations in every affected individual or there may be the variable expression, resulting in substantial or subtle differences between different affected individuals in the same family[28]. The phenotype in ADAI could also be predominantly or exclusively hypoplastic, manifested by thin enamel and spacing between the teeth, or in some pedigrees by rough, irregular, or randomly pitted enamel.[29,30] If the prime defect is in the amount of enamel matrix produced, the enamel will be hard, normally translucent, and not subject to significant attrition.[31]

3. Autosomal recessive amelogenesis imperfecta

Autosomal recessive AI (ARAI) should be considered if there is known consanguinity in a family with an affected individual. This may be more often encountered in certain ethnic and cultural groups where intermarriage within the family may be more common (for example, AI in association with cone rod dystrophy, a syndromic condition[32,33]. ARAI will also be more prevalent where there is a high frequency of the mutant gene in a population, such as in some Polynesian communities [34]

NOVEL MUTATION ASSOCIATED WITH AI

The first AI causing mutation was identified in the gene encoding the enamel matrix protein known to make up the bulk of the secreted enamel organic matrix [32][TABLE 2]. The EMP gene evolved from a standard ancestral gene and formed a part of the secretory calcium-binding phosphate gene cluster. The enamel matrix protein include amelogenin [*AMELX*] which makes up around 90%, of the EMD, [33] secrete *AMELX* last with remaining ameloblastin (AMBN) and *ENAM* [34]

GENE	Mode of inheritance
FAM83H	Autosomal dominant
FAM20A	Autosomal recessive
AMELX	X-linked form
ENAM	Autosomal dominant
MMP20	Autosomal recessive
KLK-4	Autosomal recessive
WDR72	Autosomal recessive
SLC4A4	Autosomal recessive
LAMB3	Autosomal dominant
ITGB6	Autosomal recessive

Table 2: Different gene mutation reported in AI

Enamel is a highly mineralized structure in the body with 85% of its volume occupied by hydroxyapatite crystals.[35] During the organogenesis, the enamel metamorphoses to form soft, pliable time to its final form which is almost devoid of protein.The final composition of enamel is a reflection of its unique molecular and cellular activity that happens during its genesis[36] deviation from this pattern may lead to amelogenesis imperfecta (AI)[37]. With an underlying genetic cause, the etiology of AI includes febrile illness or vitamin deficiency, local infection or trauma, fluoride ingestion congenital syphilis, birth defects or idiopathic factors[38]. *ENAM* and *AMELX* encode extracellular matrix proteins of the developing enamel and *KLK-4* and *MMP20* encode proteases that help corrupt organic matter from the enamel matrix during the development phase of amelogenesis[39]. *SLC24A4* encodes a calcium transporter that intervenes calcium transport to develop enamel during tooth development. Less is thought about the function of other genes implicated in amelogenesis imperfecta.[40]

MANAGEMENT OF AI

The management of individuals affected by AI has been described in three phases viz., temporary phase, transcriptional phase and permanent phase. The first phase of management is directed during primary and mixed dentition.[35] The transcriptional phase occurs during the period of tooth eruption and continues till adulthood and the final stage or permanent phase occurs in adulthood. In primary dentition, the dental treatment of affected children aims to ensure favorable conditions for the eruption of the permanent teeth as well as for the normal growth of the facial bones and TMJ.[36] In the mixed dentition, the treatment goals are to preserve tooth structure, maintain Tooth Vitality, decrease tooth sensitivity, vertical dimension, and Improve esthetics. when permanent first molars and anterior teeth erupt orthodontic and prosthetic assessment is essential.[37] However, rehabilitation in the mixed dentition is complex, since teeth have different eruption sequence, and definitive treatment cannot be rendered until the complete eruption of permanent dentition.[38] In the permanent dentition, the final treatment objective is to diminish tooth sensitivity and to restore the vertical dimension of occlusion, the function of well as esthetic is the final treatment often starts is soon in the clinical height of the crown and gingival tissue have been stabilizes and the pulp time has reduced.[39]

Conclusion

Amelogenesis imperfecta is a hereditary disorder affecting enamel often leading to physiological stress in patients resulting in poor aesthetics. One of the greatest challenges faced by the clinician in the total rehabilitation.[40–42] The treatment plan with respect to Al patients may be influenced by factors such as age, socioeconomic status and other comorbid conditions. Early plans for treatment, precise surgical procedure in order to meet the aesthetic and functional demands of patients with modern technology is warranted in the present scenario.[43,44]

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Methods of Online Teaching and Its Efficiency- A Review

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Abstract

Online teaching has become a more common way to learn in college settings for the past 10 years therefore, curriculum designers must evaluate the best ways in which to deliver information and assure student knowledge in an online form. Various methods of teaching are known- analytical, episodic, narrative, which provide different advantages and disadvantages. They differ on the basis of discussions and include factors such as discussion initiation, maintenance and assessment. Chickering's seven principles of good practice are used to design courses and the usefulness of each principle and suggests ways in which the principle can be implemented effectively. In an online forum, other efficiency determining factors have been discussed, which include communication, flexibility, feedback, quality of course content among adapting to student needs.

Keywords- *Online teaching; Narrative method; Episodic method; Efficiency of online teaching; Chickering's principle*

Introduction

The world of education is currently undergoing a huge transformation as a results of the digital revolution [1]. All around us people are learning with the help of the latest technologies: children are playing complex video games, students are taking courses at online institutions, and adults are consulting Wikipedia. New technologies create learning opportunities which challenge traditional schools and colleges and enable people of all ages to pursue learning on their own terms. People round the world are taking their education out of faculty into homes, libraries, Internet cafes, and workplaces, where they will decide what they need to find out , when they want to find out , and the way they need to find out.

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The important work of researchers within the 1980s and 1990s helped define teaching effectiveness within the traditional classroom setting and provided support for the usefulness and validity of student ratings in evaluating teaching. In addition, the research in online teaching indicates that the web environment is analogous to the normal environment in some ways , yet shows important differences like the changing roles of students and instructors and the importance of careful planning. Bruner's classification which mentioned two modes of analytical and narrative thought was used as a theoretical framework. Accordingly, two teaching methods, narrative and episodic, were identified [2] and have been discussed in detail further in the article. Distance education has grown to be a fast growing delivery method for imparting knowledge. It provides improved student access and higher degree completion rates [3] There is an array of technologies available to provide web based discussions and instructions [4], some of which are discussed in the article. These courses however, primarily, are text driven [5], where the instructor has a significant impact on the efficiency [6] and the quality of content of the web based course [7]. It enquires about student perception and faculty feedback which assists campus leaders and/or school administration to change

policies necessary to improve the teaching and learning conditions [8].

Certain previous studies were used to examine the perspective of students. Findings from previous studies indicated that narrative teaching with higher interactivity level, was a preferred method [9]. The interactivity among online instructors and the perceived success of courses were moderately correlated. Participants who possessed strong computer skills preferred teaching methods which involved lower interactivity. Some participants preferred combining both, the narrative and episodic methods. Such findings inform educators about possible changes to improve the quality of online teaching[10]. Communication, feedback, flexibility, student and instructor roles, and the quality of course materials have been the focus of some studies of online teaching and learning. In one study of 104 online instructors, instructors reported that students were required to take on different roles in their learning and that students needed to be more actively involved while instructors should take on more of a facilitative role [11].

Online teaching differs from traditional courses in a number of ways , including instructor and student roles, communication, interaction, and adaptability. To find out about the online teaching methods and their efficiency, a literature review was conducted to look at empirical studies, including quantitative, qualitative, and mixed methods, also as literature reviews related to online teaching methods which were published in peer-reviewed journals since 1985. Online teaching methods, online discussion, online instruction, and online instructor role were some of the keywords used.

The aim of this study was twofold. First was to identify the recommended online teaching methods and second, in order to explore learners' perceptions about the identified teaching methods. Second was to correlate the learners' perceptions with learners' characteristics, which involve experiences, computer skills, and activity ratios in online discussions.

Materials and Methods

This review analyzed 68 articles taken from the year 2000- 2020 (till date)[12]. Cross references were also included. The sampling and data collection was done by search engines like pubmed, google scholar, and from various other journals. There was a clear five step process in selection of these articles. Identification of clear objectives, identification of relevant articles, selection, data extraction and analysis and report.

Results and Discussion

Previously our team had conducted numerous clinical trials [13–22] and lab animal studies [23–26] and in-vitro students [27–31] over the past 5 years. Now we are focussing on reviews. The idea for this review stemmed from the current interest in our dynamics of the education community, owing to the lockdown period.

Online education has become a permanent component of higher education. However, there are still major issues that need to be addressed to improve online courses. Particularly, online learning systems provide such a different platform for learning that a redefinition of the roles of instructors in online learning systems is needed.

The study showed that the programming abilities of the participants had a strong negative association with their favored methods of teaching. Participants with higher computer skills felt more confident and independent in online courses, and thus preferred less support and involvement from their instructors.

Distance education has become a fast-growing delivery method in higher studies. It is important to inquire about impressions of campus environments by the students and faculty. Reasons for offering online courses include- improved student access- higher degree completion rates- appeal of online courses to nontraditional students.

The essence of narrative teaching is its approach of employing interactive dialogue to construct knowledge. It helps learners build the knowledge by engaging with the content and teacher in interactive dialogue. Knowledge is transmitted to students in online discussions and authority and control is imposed by applying rules such as setting required numbers and lengths of postings in discussion.

Teaching aids will help students in gaining interest in the subject, consequently showing good performance in their academics[32]. It helps in discussing many problems faced by the students in routine activities in a college

and suggesting ideas to improve the student's academic performance [33] Since iPads are compact, they can be used as high-end devices for learning needs of students digitally [34]. Formal clinical teaching for senior medical students needs to be used more regularly to improve standards [35] E-learning provides a more efficient, cheaper, and potentially better alternative. E-learning needs to be introduced and made readily accessible to everyone [36]. Modern technology helps in improving the classroom management practices since students prefer activity-based learning. They find modern technology more helpful for them in understanding than the conventional method [37]. For subject specific studies, certain articles were reviewed. Proper utilization of newer technologies along with the traditional teaching methods will certainly lead to a better understanding of gross anatomy and will eventually improve students' performance [38]. In a study conducted among 100 students of Saveetha Dental College, Chennai, it was found that about 75% of students feel that it is useful to learn through video tutorials for preclinical prosthodontics [39]. Flipped classrooms showed promising results among final year graduate students as compared to conventional classroom groups in the subject of conservative dentistry and endodontics. Designing the curriculum for flipped classroom lectures takes some additional time than conventional classes. The number of distinctions obtained in flipped classroom groups was higher than conventional classroom groups[40]. Technology has been very efficient in bringing new electronic gadgets. Audio visual aids deemed more effective [41]. Now with the new technologies, many colleges and schools have started using smart boards instead of blackboard [42].

Creating videos, animations, and web sites can be helpful. Memorizing information becomes less important with the web available. Sharing customized apps, providing peer feedback, composing design proposals, keeping design journals can improve efficiency of digital methods.

Instructors also say that online classes can provide valuable resources for special needs students. For instance , teachers have indicated that students with learning disabilities contribute more to the course than they would in a typical classroom. This may be because of the increased anonymity in online courses.

In addition, shy students who would not ask questions in class appear to be more likely to participate and ask questions online due to the increased anonymity. Online learning can thus be particularly helpful in increasing the involvement of shy students or students with learning disabilities.

Narrative Teaching

There were primarily two methods of teaching-narrative and episodic, which were identified for fulfilling pedagogical roles based on previous literature.

Narrative and episodic are two contrasting modes of thoughts which can be used as two distinctive teaching and learning methods in education [43] and two distinctive teaching methods in online discussions [44].

Narrative has a Latin root that suggests close connection with knowledge or skillful practice [45]. The essence of narrative is its approach of building knowledge through interactive dialogue. This method helps the learners construct their knowledge via engaging them in interactive dialogue with the content as well as the teacher. A number of studies [46,47] developed interactive activities for learners, allowed them to learn the subject through dialogue, and encouraged them to construct meaning through reflecting on and revising their understanding of the subject. Robertson suggested that narrative teachers engaged in dialog with learners provide a more effective method of teaching than non-narrative or episodic teachers with a low degree of involvement in dialogue with the learners. Narrative teachers participate explicitly in online dialogue and build a common framework with students in which all further interactions grow. Knowledge is built in the narrative method by dialog in online discussions and authority and influence is established by asking questions and proposing ideas throughout the dialogue.

There is widespread interest in studying and using the narrative method as a way of knowing, learning and teaching in a variety of disciplines such as literacy criticism, philosophy [2,48] ,cognitive psychology, anthropology, research methodology [45,49] postmodernism teaching, learning, and curriculum [43], and interactive media in education [46,50,51] and online courses [52,53]. Despite such interests, as Hazel suggested, the definition and characteristics of the narrative teaching method are

vague, specifically in online education.

Discussion Initiation - The main functions of discussion initiation are proposing goals, forming prompts for discussion, making group discussions, and setting norms and agenda (25, 26). Narrative is a transformative teaching mode which constructs knowledge while episodic is a mode of transmission in which knowledge is received.

Narrative instructors encourage learners to learn by creating and exploring the meaning on the basis of their own experiences, whereas episodic instructors allow students to receive prepared and lectured subjects. Narrative instructors provide a flexible syllabus and try to modify it based on student preferences during the course, while episodic instructors use a preplanned and fixed syllabus during the course.

Discussion Maintenance- The main functions of the maintenance of discussions include controlling and monitoring the discussion [54] and guiding, coordinating, energizing, and perpetuating discussion by seeking opinion and information. In addition, facilitating debate, commenting on posts, controlling engagement and changing the conversation speed are defined as management of the debate.

Discussion Assessment- The duties of the instructors include roles to improve the level of conversation by providing input and creating a critical cycle between learners and instructors.

The aim of narrative mode assessment is to help learners achieve a higher learning order while in episodic mode it is to calculate how often learners obtain a particular body of knowledge. Narrative instructors provide formative feedback during the course of the discussion that encourage students to improve their understanding while episodic instructors provide summative feedback at the end of the course.

Episodic Teaching

In the episodic method, knowledge is transmitted to students in online discussions and authority and control is imposed by applying rules such as setting required numbers and lengths of postings in discussion. Episodic instructors provide more space for students to collaborate and rely on each other to develop their understanding of the topic at hand [55].

The key difference between the two teaching methods is that analytical teaching is explanatory while narrative is interpretative. The analytical method suggested by Doll is very similar to the episodic method suggested by Robertson. In both methods, teachers are lecturers who are outside the learning process and explain the subjects so the learners receive the material. In episodic teaching, context is separated, knowledge is transmitted and received as well. The control is external [56]. Episodic instructions require summative feedback at the end of the course.

Chickering's Seven Principles

Principle 1: Encourage Contact Between Students and Faculty

Blignaut and Trollip [57] and Young [58] indicate that most online students desire routine interaction with instructors and that they perceive themselves as learning more as a result of increased interaction. Students have also reported feeling less isolated when they have more interaction with the instructor [59]. The faculty and administrators also indicate that the presence of teachers in the course is one of the most important success factors. Finally, increased interaction between the instructor and the student was not only considered important by students and faculty, but the increased interaction resulted in better academic performance.

Principle 2: Develop a Reciprocity and Cooperation Among Students

Many studies indicate that students are more satisfied with online courses when they have a high level of interaction with other students [60]. Studies also indicate that students perceive themselves as having learned more when they had high levels of interaction with other students and they felt more prepared to complete their assignments as a result of their interaction with other students [61].

Principle 3: Encourage Active Learning

They indicate that students need to relate the material to their own lives. This requires that students talk about what they are learning and write about it, not just memorize the material for tests. This type of pedagogy

is within the constructivist realm. It is learner-centered rather than teacher driven. Niederhauser, Bigley, Hale, & Harper [62] noted that their online students had improved in their ability to be self-directed and do independent research.

Principle 4: Give Prompt Feedback

Feedback can be an essential tool in order to focus on future learning efforts. Chickering and Gamson [63] state that students should also be given frequent suggestions for improvement. Research suggests many online faculty agree on the importance of transparent and prompt grading among online instructors as a highly valued competency. Young also found that students support the principle of prompt feedback. In regard to giving feedback, Wang and Newlin [64] found that they could use feedback after the first week of class in order to encourage non-participating students to become more involved in the course.

Principle 5: Emphasize Time on Tasks

Many research studies suggest that more time is spent on learning tasks through internet courses. Bachman and Panzarine compared a traditional class to a class that had traditional and web components. The students with the components on the web spent more time interacting with other students and doing the course research. Other studies have found that students in online courses went beyond the assignment requirements, spending extra time on the course [65].

Principle 6: Communicate High Expectations

Suzanne Young found that online students thought teachers were more effective when they motivated students to perform at their best. Therefore, many students appear to want to be challenged to work hard. In addition, Niederhuaser found that their online students felt challenged and empowered to learn within the online forum. Finally, it has been shown that the constructivist approach employed in many online learning classes imposes high standards on learners [66] and has led to positive outcomes.

Principle 7: Respect Diverse Talents and Ways of Learning

Research indicates that most instructors agree that a range of learning opportunities should be offered, but that most instructors still do not incorporate a variety of learning tasks [67]. To allow them to integrate a range of learning activities, Smith and Valentine recommend further instruction for both conventional and online teachers. One way to make the courses more successful is by discussing a range of learning styles. For example, Suen [68] found that her students taking an online epidemiology class were demonstrating a variety of learning style preferences. Therefore, she effectively addressed these preferences using various options for learning such as small group discussions, telephone contact with other students, and email. Other teachers advocate using case studies, powerpoint presentations, and video conferencing.

EFFICIENCY AND EFFECTIVENESS

Learning is a social activity that is strengthened when instruction is carefully facilitated by an instructor. The instructor's role and responsibilities in an online course involve carefully designed, primarily written communication with the learners. An effective instructor can provide corrective feedback and encouragement, motivating the students to stay on task and to achieve the learning goals. Online learning should not be an isolated, independent activity but rather one in which students and instructors are partners in learning. Effective communication is one of the most important elements of a successful online course. The demands of communicating in an online course can be overwhelming; the volume of e-mail messages alone can quickly become a huge burden. Hara and Kling [69] advise students and instructors to be realistic regarding their expectations and to learn to manage their involvement in the course so that it does not become problematic.

Flexibility is one of the most often cited advantages of online courses, according to both students and instructors. Students in Northrup's [70] study reported that flexibility was one of the most important factors in choosing to learn online, even though most of the students reported that they could have taken a campus-based course.

Providing meaningful examples for students helps them to make important connections with the course content. In a traditional classroom, students can easily ask an instructor to clarify fuzzy concepts. In an online

classroom, answers to those important questions are delayed, sometimes causing frustration and reducing motivation to learn.

Students in the current study reported that the best courses are the ones in which instructors demand high-quality work from the students. Marsh [71] addressed one of the myths related to effective teaching: Teachers who give students less work, fewer challenges, a slower pace, and higher grades are rewarded with higher evaluations for their teaching by the students. In fact, Marsh found that when teachers give more work that demands high quality, those teachers in turn are seen as more effective by students.

An online instructor must design the course in advance, preparing materials, schedules, assessments, and even discussion topics. Once the course begins, an effective teacher must give considerable attention to facilitating the course. The instructor is fully absorbed with communication, including e-mail, threaded discussions, and chats, and must work hard to meet the varied needs and demands of the students. This includes providing a structured yet comfortable classroom environment and communicating with students in a consistent, thoughtful, and personal way.

DIGITAL TEACHING METHODS

The future of higher education is likely to be driven by the willingness to adapt and grow with the use of technologies in teaching, learning, and research. Google Apps for Education (GAFE) is a powerful cloud-computing solution that works for students regardless of their location, time, or the type of device being used. GAFE is used by thousands of schools and universities worldwide to make effective use of collaboration tools for students and faculties, with the primary objective of enhancing teaching and learning [72]. In particular, GAFE tools enable users to work together virtually on documents, presentations, and projects in the cloud. GAFE is used to develop course websites, as a complement to traditional classroom instruction, with the aim of delivering coursework to students. For this research study, a group of computer science students from the University of Ghana were surveyed to understand the impact of GAFE use on their performance and satisfaction. The study was conducted after in-class deployment of GAFE during the fall 2013 semester. When asked if using the GAFE-based course website improved their performance, over 84% answered "yes." Additionally, about 91% of the participants indicated that they were more satisfied with the courses using GAFE than those using traditional methods of instruction with no or moderate use of technology, where the least proportion of content is delivered online; the remaining 9% noted that they were moderately satisfied. Overall, the respondents were satisfied to some degree.

Other methods include creating videos, animations and other websites. Memorising information becomes less important with the web available [73]. This improves quality learning instead of just memorising. Sharing customised apps, providing feedback, proposing design proposals, keeping online design journals can also be useful [74]. Hybrid courses that are a combination of online and face to face meetings is gaining popularity and are more readily available in higher education. Some frequently used online applications include Google Classrooms, Zoom Cloud, Google Hangouts, Webinars. However, the allover problem in digital methods of education is the low persistence rate of students in online classes.

LIMITATIONS

There is a low persistence rate of online students in research methods, classes which raises the question whether online teaching should be equally effective in a different course. There are limited resources, which include time and expertise, which may limit the opportunities for interaction. Some educational programs do not fit into an online setting. For such courses online teaching may not suffice.

FUTURE SCOPE

Instructions can leverage online interactions with peer experts, course content and assessment. Enquiring students and faculty will give better insights. This can be done by conducting surveys which have a considerable large sample size in order to have a significant result. It will help in assisting campus leaders in changing policies that will lead to improvement of teaching and learning conditions if necessary. Advancement of technology might be helpful in development of a user friendly successful app which could bring a revolutionary change in online teaching.

Conclusion

Online teaching varies in a variety of ways from conventional courses including instructor and student positions, connectivity, interaction and versatility. However, little work has been carried out to explore student opinions on the features of successful online teachers.

It seems that, for expanding E-learning, a combination of online and face to face guidance can possibly have an acceptable effectiveness. Meanwhile, for archiving such combined methods, the appropriate ground, containing related hardware and software must be provided in universities.

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Inhibition of Root Canal Medicaments by Dentin and Its Implication

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Abstract

Disinfection of root canal using root canal medicaments is considered to be an essential step for a successful endodontic treatment. Ideal requisite of intracanal madicaments is the disinfection of the root canal and rendering it free of bacteria without causing harm to the tooth and oral tissues. Most commonly used intracanal medicaments are calcium hydroxide, chlorhexidine and iodine potassium iodide. Sodium chloride is not preferred as a medicament due to its property of irritating the surrounding tissues. Dentin has an inherent inhibiting effect on intracanal medicaments as it has the buffering effect which tends to neutralise the pH of the medicaments rendering them with decreased antibacterial property. The antibacterial effect of a medicament indicates its level of action. More the antibacterial action, less the dentinal inhibition. A review of scientific literature was done using databases such as pubmed, google scholar, MESH and so on. The time frame of the articles taken is between 2000 to 2020. The results are based on previous studies done on this topic. Newer methods such as bioactive glass, photon induced acoustic streaming and gentlewave system were introduced to overcome this discrepancy in disinfecting the root canal. Even though the success rates of endodontic treatments are high, it can be improved by researching and using newer and better materials for elimination of bacteria. Thus, in this review we have analysed the relation between root canal medicaments and their inhibition by dentin.

Keywords: *Root Canal Medicaments; Chlorhexidine; Calcium Hydroxide; Iodine Potassium Iodide; Inhibition; Enterococcus Faecalis; Antibacterial Property.*

Introduction

Success or failure of an endodontic treatment depends on many factors. One of the most important prerequisites is the disinfection of the root canal and rendering it free of bacteria. One of the common etiologies of periapical diseases is the invasion of the infected root canal by bacteria which can be the result of periapical inflammation which is also caused by bacterial infection[1]. Disinfection of the root canal using medicaments is considered to be an essential step for a successful endodontic treatment. In the modern days however, shaping and cleaning is preferred more as a means of disinfection[2]. But, cleaning and shaping, use of rotary and hand instrumentation, alone is not enough in cases of severe infections and in root canals with complex anatomies[3]. Instrumentation of canals with such anatomy, such as, oval shaped canals, can lead to buccal and lingual recesses or extensions which serves as a collection point for necrotic debris and biofilms[4–7]. This can lead to the failure of the treatment and recurrent infections[8,9]. Further disinfection after instrumentation is required in order to increase the success of the endodontic treatment. Usage of phenols and its derivatives and compounds of formaldehyde and formocresol, previously preferred, are now not used due to their action as irritants[10]. Formocresol is also said to lead to brain injury by anaphylactic shock. Biocompatibility and stability are properties important for root canal medicaments. The long acting activity and stability of calcium hydroxide has made it one of the most widely used medicaments. For a root canal treatment, a single visit treatment is considered advantageous over multiple visits[11]. In case of multiple visit treatment, calcium hydroxide is the most preferred medicament. Root canal medicaments act by disinfecting the root canal and rendering it clean and free of bacteria and infected

tissues[12,13]. The antibacterial effects of medicaments can be analysed by their action against microorganisms such as Enterococcus faecalis and Candida albicans. The more the antibacterial action, the lesser is the dentinal inhibition. Some of the most commonly used root canal medicaments are calcium hydroxide, chlorhexidine and iodine potassium iodide[14].

Chlorhexidine is widely used as an irrigant and disinfectant and is mainly available in form of salts[15]. The original salts were CHX acetate and CHX hydrochloride. Both were replaced with CHX digluconate due to their insufficient solubility in water. CHX is a cationic molecule with a pH of 5.5 - 7[16]. It has a central hexamethylene chain which connects two biguanide groups and has two symmetric 4-chlorophenyl rings in the sides. It acts better against gram positive bacteria than gram negative bacteria[17]. The bacterial cell wall is destroyed by the attachment of CHX to the surface of the bacteria rendering it permeable. The subsequent entering of the medicament into the bacteria causes the precipitation of the cytoplasm damaging the self repair mechanism. The function is usually bacteriostatic. Chlorhexidine was found to be inhibited by dentine powder Invitro. However, at higher concentrations, the effect of inhibition was not observed[18].

Calcium hydroxide is the most commonly used root canal medicament. It has a high ph, 12.5 to 12.8, and has less solubility in water compared to tissue fluids[19]. It is not soluble in alcohol. When in the oral cavity, calcium hydroxide gets soluble in the tissue fluids on direct contact with the tissues. Calcium hydroxide exerts antimicrobial action by direct contact and high pH. It creates an alkaline environment which is not not suitable for microorganisms by release and diffusion of hydroxyl ions[20]. However, the rate of diffusion is slow due to the inhibitive and buffering action of the dentin. Some microorganisms are not susceptible to the action of calcium hydroxide. These include Enterococcus faecalis and Candida albicans[21]. CHX is usually used in combination with calcium hydroxide to improve its antimicrobial property[22]. Calcium hydroxide is inhibited in the dentin by the buffering action of dentin which neutralises the pH and reduces the antibacterial property[23].

Iodine potassium iodide (IKI) when used as a 2% solution, acts as a root canal disinfectant. When compared to sodium hydroxide, which is not used as a disinfectant much these days die to their bad odour, taste, cytotoxicity, damaging effect on dentin, harmful effects on the mechanical properties of nickel titanium instruments and its negative effect on the bond strength of bonding system, IKI has a pleasant odour, taste and lower toxicity[24]. IKI can penetrate deep until 1000 μm when irrigated in dentin for 5 minutes. It has rapid microbial activity against Enterococcus faecalis, Streptococcus sanguis, Fusobacterium, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, Staphylococcus aureus and Candida albicans[25]. It is generally used in concentrations ranging from 1% to 5% with the minimum active concentration of IKI being 1%. IKI also shows less toxicity and tissue irritation than chlorhexidine, camphorated monoparachlorhenol, formocresol and sodium hypochlorite. Combination of IKI with calcium hypochlorite tends to increase its antimicrobial properties[26]. The mechanism of action of IKI has not been clearly understood. The level of inhibition of dentin is dependent on the concentration of the medicament. However, IKI showed high antimicrobial properties in both high and low concentrations[27]. But, at diluted concentrations of 0.2% - 0.4%, the dentin exhibited strong inhibitory effect and if IKI is preincubated in dentin for 1 hour, the bactericidal effect was completely abolished. It is a possibility that dentin has limited capacity to inactivate IKI and a solution of stronger concentration remained viable and killed the bacteria in 5 minutes. In previous studies, the rapid loss of antibacterial activity has also been noted[28].

Finally, this review is aimed at assessing the inactivation of root canal medicaments by dentin and its implications.

Materials and Methods

A review of scientific literature was done in preparation of the manuscript. The relevant articles were collected from databases such as pubmed, google scholar, MESH etc. The Time frame of the articles are between the year 2000 to 2020. Around 25 articles were collected, analysed and reviewed. The articles were collected on the basis of containing keywords such as

root canal medicaments, inactivation, antimicrobial properties, dentin powder etc. All topics irrelevant to the subject are excluded. The results of this review are based on previous studies done by other esteemed authors.

INHIBITIVE AND BUFFERING ACTION OF DENTIN:

Calcium hydroxide is one of the main medicaments which displays its antimicrobial activity due to its high pH. A few earlier studies indicate that Enterococcus faecalis may survive in the root canal disinfected by calcium hydroxide, due to the buffering mechanism of dentin[29]. This action of dentin is against the pH rise by alkaline medicaments. The mechanism of action of calcium hydroxide medicaments is the ability of the medicament to dissociate and release calcium and hydroxyl ions which leads to increase in pH and ultimately results in bacterial cell toxicity[30]. However the dentinal structure has a buffering effect which causes pH variations and reduces the antimicrobial action of calcium hydroxide in the root canal. Such interaction between alkaline medicaments and dentine leads to negative impact on their performance and impaired disinfection of the canal[31]. In a previous study, it was observed that invitro inhibition of root canal medicaments such as calcium hydroxide was observed when the medicament was incubated with dentin taken from the canal walls as it reduced the pH significantly. On the other hand, dentin taken from the pulp chamber floor did not play such an active role of inhibiting the alkaline medicaments[32]. A 1.8% dentine powder prevented the eradication of Enterococcus faecalis by saturated calcium hydroxide solution.

TIME DEPENDENT INHIBITION OF MEDICAMENTS BY DENTIN:

It is a generalised opinion that medicaments tend to get inhibited and use their activity in the root canal environment. The inhibitory effect of dentin on calcium hydroxide due to its buffering action is seen to decrease with time[33]. In previous studies it was seen that the pH of the dentin located in the root canal was seen to increase from 6.2 to 7.4 in about 1 hour of placement and increased upto 10.8 within 24 hours of placement of the medicament[34]. The antibacterial property of calcium hydroxide is mainly mediated by the release of hydroxide ions into the surrounding aqueous environment and the rate of such ionic dissociation tends to increased time[35–38]. The synergistic effect of the hydroxide ions and iodoform aids in overcoming the buffering activity of the dentin leading to the rise in pH[39].

INHIBITION OF IODINE POTASSIUM IODINE AND CHLORHEXIDINE DIGLUCONATE BY DENTIN:

The most effective inhibitors of chlorhexidine digluconate were seen to be dentin matrix and heat killed microbial cells[40]. Dentin which is pretreated with citric acid or EDTA tended to display only a slight inhibition of the medicament[41]. Similar to calcium hydroxide, while dentin displays inhibition of the medicament within 1 hour of administration, the level of inhibition decreases with time and no inhibition is seen at 24 hours of application of the disinfectant. IKI can also be inhibited by dentin, dentinal matrix and heat killed microbial cells and application of EDTA to dentin lead to little or no inhibitory effect[42]. IKI is a strong medicament and does not get inhibited at high and medium concentrations[43,44]. Different components of the dentin are responsible for inhibition in divergent patterns of the antibacterial activity of chlorhexidine digluconate and potassium iodide. Inorder to lessen the inhibitory effect, it is better to chemically heat dentin before application of medicaments[45].

ASSESSING THE ERADICATION OF *Enterococcus faecalis* BY MEDICAMENTS IN PRESENCE OF DENTIN:

In most medicaments, the inhibitory effect of dentin would not be observable at a higher concentration[46]. Calcium hydroxide tends to completely lose activity against Enterococcus faecalis in presence of dentin[47]. Presence of dentin induces a strong inhibitory effect on diluted iodine potassium iodide whereas a stronger solution of IKI displayed reduced activity against Enterococcus faecalis[48]. While chlorhexidine digluconate showed reduced activity against dentin initially, extended incubation, upto 24 hours, leads to loss of 99% viability of bacteria. Sodium hypochlorite also displays inhibition by dentine and is also not much preferred as a disinfecting agent[11].

NEWER METHODS OF ROOT CANAL DISINFECTION:

Newer and better methods of root canal disinfection are developed in order to get better results[49]. Bioactive glass can be used as an alternative to calcium hydroxide[50]. Bioactive glass which contains silicon dioxide, sodium oxide, calcium oxide and phosphorus pentoxide has a superior disinfecting effect. It does not irritate or affect dentin and can induce dentin mineralisation[51]. It is also used as an intracanal dressing. The antibacterial effect of bioglass increases when mixed with dentin unlike other medicaments which get inhibited[52,53]. Chitosan nanoparticles act synergistically with chlorhexidine inorder to eliminate qmore number of colony forming units[54]. Photon induced acoustic streaming of antibacterial fluid within the canal by creating successive shockwaves[55]. Another system known as Gentlewave system aids in cleaning the root canal by generation of various broad spectrum sound waves through different physical, chemical and biological mechanisms[56].

Limitations:

Even though studies have been made to analyse the inhibition of medicaments by dentine, no adequate quantitative data is available. Since disinfection is a technique which has been used for a longer time, dental practitioners are skeptical to try newer products. The mechanism of action and inhibition of some medicaments are not yet available and have to be researched upon.

FUTURE SCOPE:

Further research can aid in discovery of newer products with better properties and functions. Usage of newer techniques can create better treatment plans and treatment success rates. Since disinfectants are commonly used materials for endodontic procedures, ways to overcome inhibition can go a long way for success of the treatment in the long run. Awareness must be created among dentists about the availability of newer products that can help expand their horizons and aid in doing better clinical treatments.

Conclusion

The success rates of endodontic treatments are high and can be improved by using newer and better materials for elimination of bacteria. Consideration of the medicament's therapeutic action and level of inhibition based on the type of treatment is crucial. Newer materials can be researched upon as many of the commercial preparations fail to fulfil the requirements for an ideal root canal medicament. Thus, we have analysed the relation between root canal medicaments and their inhibition by dentin.

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Novel Trends in Drug Delivery and Application of Curcumin in Dentistry

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Abstract

The frequent use and misuse of the currently used therapeutic agents have led to the evolution of resistant strains of common pathogens as well as increased incidence of adverse effects associated with their usage. Hence, the search for phytochemicals isolated from plants is considered as a good alternative source. The minimum number of plant species worldwide has been phytochemically investigated until date, there is a great potential for discovering novel bioactive compounds and drugs. The design and development of herbal nanoparticles have become frontier research in the nanoformulation arena. Curcumin, a hydrophobic polyphenol (diferuloylmethane) is a potent Phyto molecule obtained from turmeric (*Curcuma longa*, Family-Zingiberaceae) has a wide range of biological activities in chronic diseases and a large number of in-vitro and invivo studies in both humans and animals reported that curcumin has provoked properties like anti-inflammatory, antioxidant, antimicrobial, antiseptic, antimutagenic, hepatoprotective and immunostimulant. Due to these properties, it is quite useful in dentistry. It has a role in the treatment of periodontal diseases and oral cancers. But the clinical application of curcumin was limited due to its poor oral bioavailability, which may result from its poor water solubility, its poor pharmacokinetic profile, rapid metabolism, and rapid elimination which ultimately results in poor bioavailability upon oral administration. Therefore the introduction of novel drug delivery technologies gained the importance to achieve modified delivery of herbal drugs by increasing the therapeutic value and provides a solution towards increased bioavailability of curcumin. However, there is a scarcity of research and information in this field and much work is needed to further investigate the pharmacokinetics, enhance the delivery at the target tissue, the bioavailability, and the medicinal value of curcumin. In this review, various nanoparticles, micellar formulations, cyclodextrin inclusion compounds, and liposomes have been reported in order to improve the bioavailability, solubility, and efficacy of curcumin.

Keywords:*bioavailability, Curcumin, drug delivery system, nanoparticles.*

Introduction

Medicinal plants are used as a traditional treatment agent for numerous human diseases. Plants are the main source of different types of phytochemicals with numerous biomedical applications and can be used for the management of many diseases like diabetes, cancer, hepatotoxicity and microbial infection [1,2,3,4,5, 6,7]. Nanotechnology is a rapidly growing field and plays an important role in most of the advanced sciences, medicines, and technology areas. It led to the development of novel strategies to manipulate minute particles resulting in the production of nanoparticles .Nanoparticles prepared from plant materials such as selenium,zinc oxide ,silver are extensively explored for their therapeutic potential in many diseases [8,9,10,11,12,13,14,15]. Curcumin is a yellow-colored phenolic compound extracted from the spice herb Curcuma longa. It has attracted particular

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attention in dentistry due to its broad spectrum of biological and pharmacological activities, such as anti-inflammatory, anti-oxidant, anti-microbial,anti-tumor, anti-coagulant, anti-virus properties. Curcumin is a safe, non-toxic, and effective alternative for many new drugs[16]. Commercially available curcumin contains a mixture of 75 % curcumin,15%demethoxycurcumin, 5 %bisdemethoxycurcumin, and volatile oils. Nevertheless, the majority of the orally administered curcumin is detected in urine and feces, traces are detected in blood plasma[17]. Although 10 or 12 g/mL of orally administered curcumin in humans leads to serum curcumin level of 50 ng/ mL, it is lower than a value to achieve curcumin therapeutic effects such as controlling inflammation, cell growth, apoptosis, etc [18]. The low bioavailability of curcumin is due to its low water solubility, instability in low pH values results in difficult absorption, and limited clinical use . At the same time, after oral curcumin dosing, it is rapidly metabolized in the intestine [19].In order to overcome these limitations of curcumin by novel drug delivery systems and to improve the Curcumin bioavailability.

NOVEL DRUG DELIVERY SYSTEM OF CURCUMIN:

Solubility enhancement by manipulation of crystalline forms:

Manipulations of the crystalline state are one of the classical methods to increase solubility or the dissolution rate of insoluble curcumin [20].

Amorphous form of curcumin :

Amorphous forms non-crystalline materials and their structure similar to that of a frozen liquid. They are thermodynamically unstable and most energetic forms, which may result in higher solubility or a higher dissolution rate . Oral absorption of amorphous curcumin was studied in rats. The study reported that the absorption of the amorphous material was rather rapid, the oral bioavailability (AUC) was significantly improved.

Solid dispersions:

Solid dispersion technology transforms crystalline materials into amorphous materials. Here, an active substance (crystalline)embedded in a carrier is transformed into an amorphous form in the dispersion. This method is used to improve the dissolution and bioavailability of Curcumin[21]. A few curcumin solid dispersions were recently studied in rats. These results suggest that manipulation of the curcumin crystal itself has limited potential for absorption enhancement, while amorphous curcumin in solid dispersions led to significant increases in bioavailability [22].

Complex formation:

Cyclodextrins (CDs) are cyclic oligosaccharides that can sequester insoluble compounds within their hydrophobic cavity, resulting in improved solubility and enhanced chemical or enzymatic stability. A hydroxypropyl-β-CD inclusion compound with curcumin showed enhanced oral absorption in a rat study[23].In a rat study, cyclodextrin led to 10-20-times greater amount of total curcumin in the plasma than pure curcumin powder after oral administration**.** Bioavailability was about 0.2%, which is four times that of standard curcumin.

Phosphatidylcholine complexes :

A molecular complex of curcumin with phosphatidylcholine can be formed by refluxing in organic solvents. These carriers can increase permeability by interacting with membrane components[24]. Meriva is a product composed of complexes of curcuminoids (curcumin I, II, and III) with lecithin (mainly phosphatidylcholine). Plasma concentrations of the three components were assayed after oral administration to healthy volunteers. Of note, complex formulation increased the absorption of curcumin II much more than that of curcumin I [25]. Thus, the major plasma curcuminoid after administration of the complex was not curcumin I but curcumin II .

Nanoparticles:

Nanoparticles can provide greater penetration of membrane barriers because of their small size. Besides their size, their potential for targeting specific organs through modification makes them excellent drug carriers . The other advantages of this technology include its simplicity, ease of scale-up, and narrow particle size distribution.

Nanocrystal solid dispersion of curcumin:

It was prepared using the NanoMill-01 wet-

milling system, in which polystyrene beads micronized curcumin crystals [26]. The resulting nanosuspension was composed of curcumin, hydroxypropyl cellulose SL, sodium dodecyl sulfate (SDS), and water .

Polymeric nanoparticles:

Due to the small size and excellent biocompatibility, polymeric nanoparticles can circulate in the bloodstream for a longer time. The widely researched synthetic polymers include chitosan poly(D, L-lactide co-glycolide) (PLGA)and PEG for the curcumin nanoparticle formation [27]. These polymers can be combined to form copolymers, which could be a promising drug carrier for the site targeting and sustained action.

Solid lipid nanoparticles:

SLNs are made up of natural or synthetic lipids or lipids, such as lecithin and triglycerides, which are solid at human physiological temperature [28]. It has unique properties such as smaller size, larger surface area, the interaction of phases at the interfaces, and these are attractive for their ability to improve the performance of pharmaceuticals and other materials. Kakkar et al,2011 prepared curcumin-loaded solid lipid nanoparticles (C-SLNs) for the improvement of its oral bioavailability[29].

Liquid formulations:

Liposomes are closed spherical vesicles consisting of the lipid bilayer that encapsulate an aqueous phase in which drugs can be entrapped [30]. With the advantages of high biocompatibility, easy preparation, and chemical versatility used to improve the therapeutic activity and safety of drugs for many years [31]. So, liposomes have found wide application in enhancing curcumin's bioavailability and efficacy. All curcumin-containing formulations were effective in inhibiting cell proliferation in vitro cell culture.

Self-emulsifying drug delivery system:

It is used to solve the low bioavailability problems of poorly soluble drugs. When such a liquid system is released in the lumen of the GI tract, it disperses to form a fine (micron/nano) emulsion with the aid of GI fluid. This leads to the in situ solubilization of the drug that can subsequently be absorbed [32]. The self-micro emulsifying delivery system can significantly increase curcumin dissolution in vitro and bioavailability in vivo studies

Micelles:

The self-association of amphiphile into small aggregates (diameter less than 100 nm) is called micelles. In aqueous solution, the aggregates have a hydrophobic core surrounded by a hydrophilic layer. The hydrophobic drugs are then dissolved in the core, thus forming an aqueous solution of the drugs usually for parenteral dosage[33]. Wu et al,2011 designed a class of water-dispersible hybrid nanogels for intracellular delivery of hydrophobic curcumin[34].

DENTAL APPLICATIONS OF TURMERIC :

Periodontal problems:

Topical application: It provides relief from periodontitis and gingivitis. It is recommended to rub the gums and teeth with this paste twice daily.

Mouthwash: The chlorhexidine gluconate as well as turmeric mouthwash can be effectively used as an adjunct to mechanical plaque control methods in the prevention of gingivitis and plaque[35].

Subgingival irrigant :

A 1% curcumin solution can cause a better resolution of inflammatory signs than chlorhexidine and saline irrigation as a subgingival irrigant [36].

Pit and fissure sealant:

Pit and fissure sealant reduces the incidence of dental caries on the tooth surfaces. Acrylic monomer and one colorant selected from the group consisting of annatto extract, turmeric extract, and β-Apo-8'-Carotenal help to produce the pit and fissure sealant. [37],[38].

Anticancer properties:

Curcumin has an anti-cancer property due to its effect on biological activities of carcinogenesis including oncogene expression, cell cycle regulation, apoptosis, tumorigenesis, and metastasis [39]. It can be used in the treatment of oral cancer. It potentiates the effect of chemotherapy and enhances the effect of radiotherapy.

Precancerous lesions :

Its role in the treatment of various precancerous conditions like oral submucous fibrosis, leukoplakia, and lichen planus has also been studied . The local symptoms of burning sensation and pain were reduced and partial reversal of opening of the mouth was also observed [40].

Conclusion

Low oral bioavailability is one of the major reasons for curcumin unsuccessful in achieving therapeutic outcomes in spite of its pharmacological properties. In order to increase its absorption through the intestinal membrane, a higher concentration at the membrane surface is most important. Curcumin delivery systems with increased solubility and stability, or accessibility, in or to the GI tract, were introduced. In addition to the advances in delivery technology, simple and reproductive analytical methods should be developed. In the near future, with advances in science and technology, the therapeutic or preventive benefits of curcumin are expected to surface.

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Prevalence of Reticular Oral Lichen Planus: A Retrospective Study

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Abstract

Oral lichen planus(OLP) is a common chronic mucocutaneous disorder with an immune mediated pathogenesis. Etiology of OLP is unknown, but it is thought to be the result of an autoimmune process with an unknown predisposing factor. The clinical presentation of OLP ranges from mild painless white keratotic lesions to painful erosions and ulcerations. It has various oral manifestations with the reticular type being the most common. The erosive and atrophic variants are less common but most likely to cause symptoms. Topical corticosteroids constitute the mainstay for symptomatic lesions of OLP. The aim of this study is to investigate the prevalence of reticular variant of oral lichen planus.Materials and Methods: In the present study a total of 68 patients were included. The demographic, clinical and treatment details were recorded. All the collected data was analyzed by SPSS IBM software Results: Among 63 patients with OLP 60.3% were females showing a bilateral involvement in the buccal mucosa (92.1%) with $P<0.05$. The reticular variant constituted (37.1 %) of all the total cases and erosive variant was the most common (57.1%).Corticosteroids were the most common drug. Conclusion:Within the limitations of the study it is evident that OLP has more female prevalence, erosive form was more frequent followed by the reticular form of Lichen planus. Buccal mucosa was the most commonly affected site and majority of the patients were treated using Corticosteroids.

Keywords: *oral lichen planus, reticular lichen planus, erosive lichen planus, epidemiology*

Introduction

The term Lichen planus (LP) is derived from the Greek word "leichen" meaning tree moss and the Latin word "planus" meaning flat[1]. Erasmus Wilson first described the condition LP in 1869, as a chronic disease affecting the skin, scalp, nails and mucosa, with possible rare malignant transformation.[2]

Oral Lichen Planus (OLP) is a chronic inflammatory disease, it is the mucosal counterpart of cutaneous lichen planus. The disease affects 0.5-2% of the general population. This disease most commonly involves middle aged patients of 30-60 yrs age group and females are more prone with the ratio of 1.4:1[3]. Children are rarely affected. The clinical history confirms the relationship between OLP and oral cancer. Therefore it should be considered a precancerous lesion, however the malignant transformation rate is around 6% only and is considered as an oral potentially malignant disorder(OPMD).[1]

The pathogenesis remains unknown,although recent data have shown that immunological mechanisms may play an important role as a causative or contributing factor[4]. It is a T-cell mediated autoimmune disease in which the auto-cytotoxic CD8+T cells trigger apoptosis of the basal cells of the oral epithelium[5]. Oral Lichen Planus can be associated with other autoimmune disorders such as myasthenia gravis, alopecia areata, vitiligo & ulcerative colitis. Some patients with OLP

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have a higher concentration of serum antibodies against desmoglein compared to healthy controls, which may be another indication of involvement of autoimmunity in the pathogenesis of OLP[6].

In 1968, Anderson divided OLP into 6 clinical forms: reticular, plaque-like, papular, atrophic, erosive and bullous[7]. It is not uncommon for the same patient to present with multiple forms of OLP. Among these types reticular and erosive types are the main types The reticular lesions, the most recognized form of OLP, are often asymptomatic and appear as multiple papules with a network of small, raised, whitish-gray, lacy lesions referred to as Wickham's striae[8]. Wickham's striae are usually bilateral and seen on buccal mucosa, mucobuccal fold, gingiva and rarely on palate, tongue and lips[9]. OLP involving the gingiva is termed as "desquamative gingivitis" which [10]clinically manifest as a fiery red erythema of attached gingiva. OLP lesions which are associated with patchy brown melanin deposits are termed as inflammatory melanosis[11].

Erosive OLP presents as presents as a mix of erythematous and ulcerated areas surrounded by finely radiating keratotic striae. The lesions of erosive OLP migrate overtime and tend to be multifocal. Patients with this form of OLP can often present with symptoms ranging from episodic pain to severe discomfort that can interfere with normal masticatory function[12]. OLP lesion, especially the erosive variant is associated with poor oral hygiene. It remains to be known if the lesion secondarily impacts the ability of the patient to maintain a good hygiene or the poor oral hygiene is a contributor to the lesion.[13,14] The prognosis of this lesion in good with repeated bouts of relapses and remissions and the erosive variant alone has a malignant transformation potential.[15] The variants OLP which are likely to undergo malignant transformation are also capable of showing changes in the salivary m-RNA.[16]. Interestingly none of the OLP lesions are associated with the usage of tobacco habits.[17,18]The plaque form of OLP mimics leukoplakia in that appears a white homogenous, slightly elevated, multifocal smooth lesion, affecting the tongue & buccal mucosa[19]. Bullous and papular forms are rare in the oral mucosa.This retrospective study is done to assess the prevalence of reticular lichen planus among oral lichen planus patients.

Materials and Methods

In this retrospective study the archived patient records of the department of Oral Medicine and Radiology, Saveetha Dental College were collected and the data was assessed from the time period of March 2019 - April 2020. From the records of 86000 patients all known cases of OLP were picked up. Ethical clearance was obtained from the Institutional Ethics Committee of Saveetha Dental College (ethical approval number : SDC/SIHEC/2020/DIASDATA/0619-0320). The main advantages of this study were that the data was all prevalidated and the main disadvantages were that it was an unicentric study and only a single ethnicity of the population was studied.

The patient records were reviewed and analyzed, variables recorded were the age, gender, variant of OLP, the site of the lesion and the therapy used. The assessment was done by 2 observers. The data was randomly cross verified by either recalling the patients or having telephonic conversations with the patient. The internal validity of the study was established as the data was collected from a verifiable and standardised database. The external validity is established as the data is from a clinical setup which is duplicatable.

The collected data was entered into Microsoft Excel and exported to SPSS IBM software. chi-square test was done which was used to study the association between site and occurrence of the lesion ; and therapy and the variant of the lesion by chi-square analysis.

Results and Discussion

A total of 63 cases of oral lichen planus were taken during the study period. Among them 39.7% were male and 60.3% were female[Table-1],aged (26-73) years with a mean of 38.5 years. The prevalence of OLP according to the affected site when analysed shows the most common site of involvement to be the buccal mucosa[Fig-1] which is statistically significant. The study also showed the distribution of various forms of lichen planus erosive pattern was more in number followed by reticular pattern of lichen planus[Fig-2].According to the therapy used in treatment of oral lichen planus when analysed reveals 81% were treated using corticosteroids and 19% were treated using a combination of corticosteroids and antihistamines, among which Corticosteroids were the

most used line of treatment in OLP patients[Fig-3], which is statistically significant.

Table-1 : Gender distribution of the cases.

GENDER	PERCENTAGE
Male	39.7%
Female	60.3%

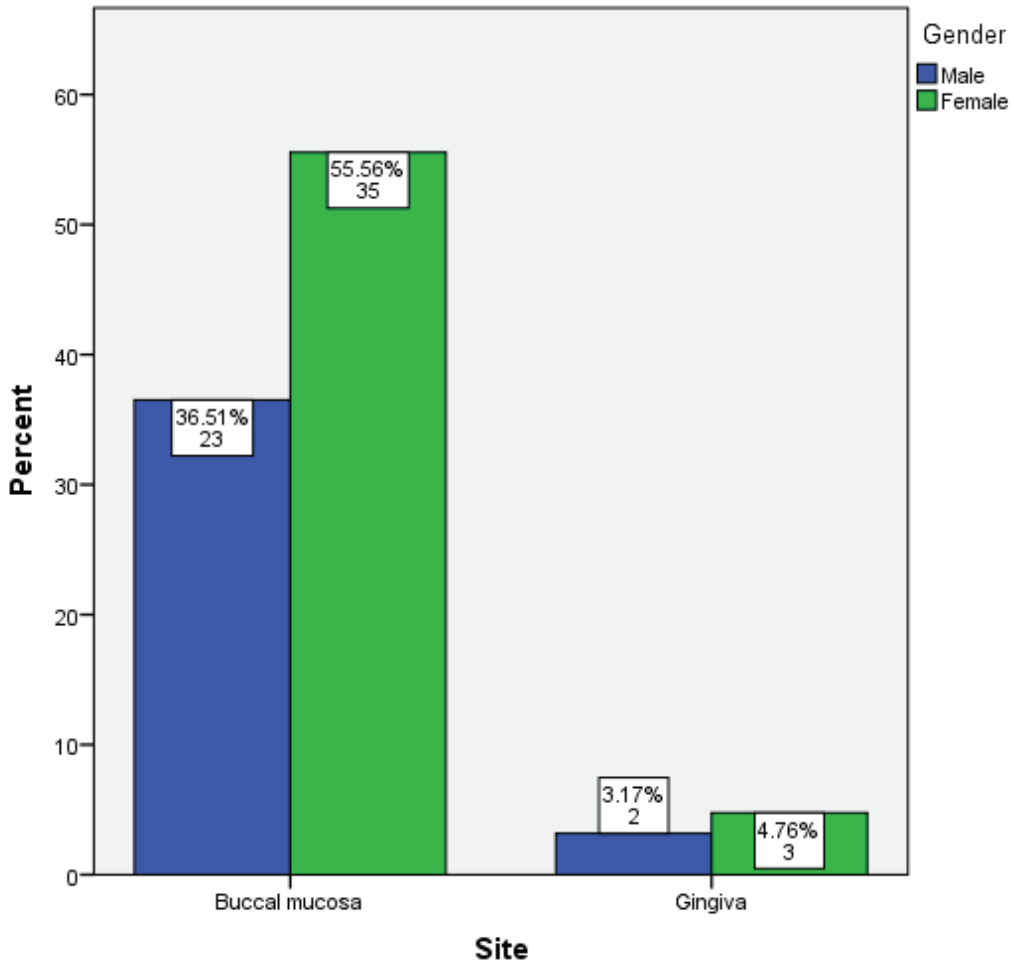


Figure 1: This is a clustered bar graph representing the association of the site of occurrence of the lesion with the total number of patients on a gender basis. The X axis represents the site of occurrence of the lesion and Y axis represents the total number of patients. The blue colour represents the male gender and green colour represents the female gender. A total of 58 patients presented with lesions in the buccal mucosa out of which 23 (36.51%) were males and 35(55.61%) were females and 5 patients in the gingiva of which 2(3.17%) were males and 3(4.76%) were females. A chi square analysis was done to compare the gender and site of occurrence which gave a P value - 0.043 (P<0.05) which is statistically significant indicating buccal mucosa is the most predominant site for the occurrence of the lesion.

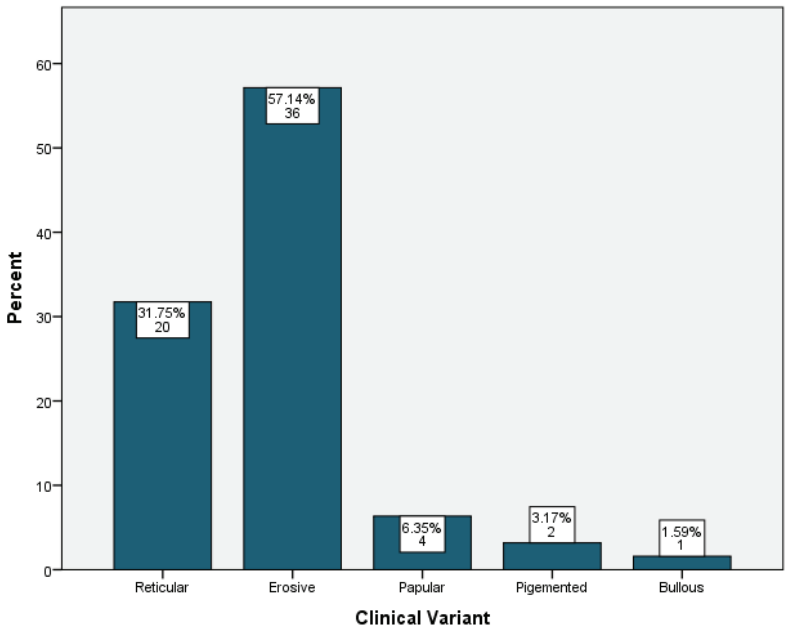


Fig-2: This is a bar graph showing the relative frequency of variants of Oral Lichen planus with the total number of patients.The X-axis represents the variants and Y-axis represents the total number of patients. The blue colour represents the various clinical variants. The most commonly seen in the erosive type 36(51.75%)of Oral Lichen planus was the most prevalent type followed by reticular variant 20(31.75%). The other types namely papular had 4(6.35%) of cases , pigmented 2(3.17%) and bullous 1(1.59%)

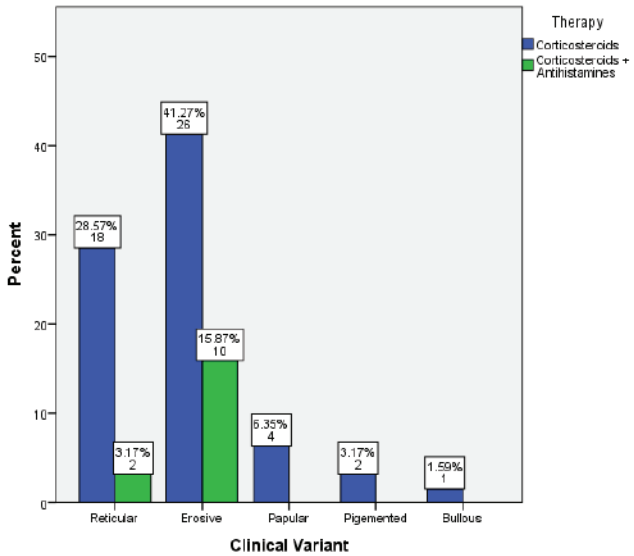


Fig 3: This is a clustered bar graph showing the association between therapy used for the different variants of oral lichen planus with the total number of patients. X-axis gives the clinical variants and y axis gives the total number of patients.The blue colour represents corticosteroids and green colour represents corticosteroids& antihistamines. In the reticular variant 18(25.87%) of patients were prescribed corticosteroids and 2(3.17%) of patients prescribed both drugs. In the erosive variant 26(41.27%) of patients were prescribed corticosteroids and 10(15.87%) of patients prescribed both drugs.In the papular variant 4(6.35%) patients were prescribed corticosteroids. In the pigmented and bullous 2(3.17%) and 1(1.59%) of patients were prescribed only steroids. A.Chi-square analysis was done to compare the clinical variants with the therapy used for treatment which gave a P value-0.049(P<0.05) which is statistically significant implying steroids as the most common drug.

On analysis of the data, it was found corticosteroids were used predominantly in the management of reticular OLP while the combination of antihistamine and steroid was used in the management of erosive OLP.

According to the World Health Organisation OLP is categorized into a group of potentially malignant disorders[20], while its most severe complication is the progression into oral squamous cell carcinoma. It accounts for 90% of malignant tumors in the head and neck region. It is commonly diagnosed in middle aged individuals. It is a multifactorial disease. The malignant process is based on increased proliferation of basal layer cells under the influence of mediators from inflammatory infiltrate that activate different pathways leading to tumor formation[21].

Reticular lesions that are asymptomatic[22] generally require no therapy but only watchful observation. In general management should be aimed at treating atrophic and erosive/ulcerative lesions, alleviating accompanying symptoms and reducing the potential risk of malignant transformation[23]. Topical corticosteroids are available adhesive vehicles or can be used as mouth rinses[24]. Empirical evidence suggests that mouth rinses are of value to patients with wide spread OLP. Systemic steroid therapy is reserved for patients in whom lesions are recalcitrant to topical steroids.Steroids are generally used in the management of several immune mediated disorders.[25]

The histological findings of OLP are a number of epithelial changes, the amount and extensions of which vary. They include epithelial orthokeratotic hyperkeratosis, hyperkeratosis, parakeratosis, epithelial atrophy or hyperplasia, acanthosis,saw-tooth rete pegs, liquefaction and degeneration, single cell necrosis called colloid bodies[25,26]. The superficial dermis shows a dense, well-defined, band-like inflammatory infiltrate composed of lymphocytes and macrophages[27]. Histological evaluation is also important as solely clinical criteria may result in false-positive findings for lesions similar to OLP.

In this retrospective study we assessed the demographic and clinical features of oral lichen planus patients along with the therapy used for treatment. In this study we observed that out of 63 patients, there was more of female prevalence than males, females were 60.3% which was in accordance with Mostafa Betal, who found 68.7% females were affected by OLP[28]. This was also seen in MCarbone et al; study where more than 60% of the patients affected by OLP were females[29].On the contrary Anita D Munde et al in their study showed that 61.7% males were affected whereas our study showed only 39.7% of male prevalence[9]. Others like Rohan et al also showed 75.4% male prevalence in oral lichen planus[30].

Bilateral involvement of buccal mucosa was seen in 92.1% of the patients followed by gingiva in our study. Soma Susan et al also showed similar results in their study where 77% of the patients showed presence of OLP in the buccal mucosa. Munde et al also showed that buccal mucosa was the most affected site.

Our study showed that the erosive form of Lichen Planus was the most prevalent clinical type which was not in accordance with Rohan Sachdev et al, their study showed 67.6% of reticular variant of oral lichen planus[31]. Carbone et al's study revealed that 42% of the patients were using topical corticosteroids, whereas our study showed that 81% of the patients were using corticosteroids. Along with steroids nutritional supplements also can be prescribed for the patients.[32,33]

The data for this study had been obtained from our internal archives and similar studies that have been done in the past had matching results with the existing literature evidence.[33] There have also been observational studies done from the archived database which has yielded positive results. [10,34]

The limitations of this study was the smaller sample size and the follow up with the recurrence potential of the lesion is not recorded. A future study incorporating a larger sample size might be required.

Conclusion

Our study shows that the erosive variant of OLP which is the symptomatic variant while the reticular variant which is asymptomatic is the second most commonest variant. Patients with OLP should be counselled as the nature of this chronic condition and different approaches of treatment. Patients should be informed that they may experience altering periods of symptomatic remission and exacerbation. Clinicians

should maintain a high index of suspicion for all intraoral areas that appear unusual. This vigilance is especially important in patients with Oral Lichen planus as it has the potency to transform into a carcinoma.

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Mentor Mentee Strategy in Academic Success -A Review

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Abstract

Mentor Mentee is a symbiotic relationship which is aimed at advancing academic excellence and career satisfaction for both mentor mentee equally. Mentors play a vital role in providing emotional and career support thereby prominently helpful in the decision making process. They are capable of making diverse conditions for mentees which promotes the level of confidence and leadership equalities in them. Effective mentorship is one of the determinants for academic success.The mentors must be able to create a network of support for the mentees, and maintain balance to manage the stress through effective communication which will help to facilitate desirable attributes and practices. Mentors must be able to plan, organize, manage meetings and create a schedule for maximizing academic success . The significant mentor mentee relationship is often challenged by increased academic work , managing stress and multiple work at a time. The mentors act as a catalyst for advancing academic ranks, career selection, leadership roles, income and overall job satisfaction. There must be a need to cultivate an efficient mentoring relationship , chosen from the mentees perspective and propose a strategy of managing up to guide the mentees action. Positive mentor mentee relationships are pivotal for advancing academic success and career satisfaction.

Keywords : *Mentor-Mentee; steadfast relationship; collaborative alliance; effective interaction.*

Introduction

Mentoring has been defined as a part of the nurturing process in which a skilled and/or an experienced person, serves as a role model, teaches , encourages and counsels a less skilled and/or less experienced person for the purpose of promoting the latters professional and/or personal development [1]. It is mostly considered as a lifelong process particularly important for the career development in academic medicine and plays a vital role for the mentee to develop confidence in his/her own work [2]. They facilitate various fields of interest such as career selection , career advancement, publication productivity and achievement of grant funding [3].

Mentor Mentee is a symbiotic relationship which is aimed at advancing career and career satisfaction for both the mentor mentees equally[4]. Ideally, it is classified as a dynamic collaborative, reciprocal relationship focused on a mentees personal and professional development . Mentees benefit from various mentors in order to gain exposure to a wide variety of style, opinion and experiences [5] .

Effective mentorship is one of the determinants for academic success [6] . To enhance the development of mentorship within academic institutions and prevent deterioration of these vital relationships, it is necessary to understand the effect of mentorship on the lives of the mentor and the mentee as well [7]. However, the significant mentor mentee relationship is challenged by increased academic work, managing stress and multiple work at a time. The variables associated with this symbiotic

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relationship involves the impact on intervention of productivity and on career satisfaction [8].

Mentors play a crucial role in facilitating the establishing the priorities of the mentees and trajectories [9]. They act as a catalyst for obtaining advancing academic ranks, career selection, leadership roles, income and overall job satisfaction. Mentors need to be approachable, accessible,altruistic,understanding , patient, honest and dedicated.

The mentees tend to develop a positive relationship with their mentors by asking support, developing resources that will refine the mentors skills. They need to be open minded and must gain the courage to ask questions openly along with the ability to manage multiple responsibilities at a time [10]. The aim of this review is to explore the phenomenon of an efficient mentor mentee relationship. Moreover to evaluate the prevalence of the mentorship and its effect on career development.

Characteristics of Efficient Mentorship Relationships

Mentor Mentee relationship is built on the basis of mutual respect and open communication between the mentor mentee in order to express a clear expectation of view thereby advancing academic success [11].

In particular, both the mentor mentee must develop passion to succeed in his/her own career. Moreover commitment, hard work and dedication plays a significant role in empowering career success and facilitating successful relationship [12]. Regular contact must be maintained between the mentor mentee inorder to develop trust between the mentor and mentee and evaluate their progress thereby making their mentees accountable for their actions towards their respective goals [13]. Good and efficient mentorship is vital for career success,developing leadership and communication skills.

Roles of A Mentor

Mentors play a key role in establishing connections with potential research collaborators, preparing manuscripts, presentations, academic reports, providing networking opportunities, advising on career progress and providing navigation on management on career progress and providing navigation on management of various responsibilities at a time [14]. Efficient mentors tend to check the strength and weakness of the mentees along with the working on behavior,management , personal communication, professional ethics , collaborative alliance[15]. They facilitate guidance in decision making,thereby making a distinction between telling the mentees on what to do and help the mentee to make a decision [16]. Mentors balance power and develop a positive relationship between the mentees. They help the mentees to gain confidence in all the work they do [17].

Impact on Personal Development and Career Guidance

Mentorship plays a vital impact on enhancing career guidance and management of personality characteristics. Mentors are capable of making diverse contributions for mentees which can help open doors to opportunity . These mentees can help open doors to opportunities. These must be a need to cultivate efficient mentoring relationships, chosen from the mentees, prospective and propose strategy of managing up to guide mentees actions [18]. They play a vital role in enhancing careers particularly for the medical students, fellow technicians, physicians in various other disciplines. According to the studies conducted by Wingard, it was noted that in a structured mentoring program for the junior faculty at the University of California San Diago, the program was multifaceted and included many professional development workshops, career planning, counseling sessions, formal mentoring sessions and community network building. The programs significantly increased self assessed confidence in the mentees academic roles and skills in various fields of interest such as professional development, education and administration with increase in self efficiency screen of 52%, 33% and 76% respectively [19].

Effective Communication

Open communication is one of the most vital aspects in enhancing interpersonal and professional trust with the mentees [20] . The modes of communication include verbal and non verbal cues, digital/handwritten materials of encouragement. Efficient communication facilitates supportiveness,personal engagement, well chosen words, confidence, integrity, credibility which acts as an integral part of the communication with the respective mentee [21]. The mentors must develop a habit

of efficiently listening to their mentees at all times as staying focused and being a keen listener is a key to being a successful mentor [22]. They need to develop the willingness to have the openness to investigate, consider multiple views of their mentees.This will help to reduce conflicts and attain a sustainable relationship [23].

The mentees must develop the habit of sharing their views openly even if it differs from the mentors point of view. This will help to improve efficient communication skill development and problem solving skills [24]. The mentees must get acquainted with their mentors and focus on techniques towards academic success.

Collaborative Problem Solving

Stress is a main barrier that comes across the mentor mentee relationship[25]. Therefore,it's necessary for the mentors to assist their mentees in identifying the root cause of the problem thereby helping the peers to advance problem solving skills [26]. A study reported that mentors must focus on helping their mentees to obtain the resources , provide opportunities, protect and advise them from time to time [27]. Some studies identified the possible failures in mentoring leading to disastrous situations between the intellectual property of the mentor and the mentee [28].

BARRIERS TO MENTORSHIP

Time plays a crucial role in the lives of both the mentor and the mentee .The lack of recognition is considered as a significant barrier for the mentors. Many mentors fail to take up mentoring as a profession and neglect the view of their mentees. Some reports have focused on the lack of financial incentives for mentorship has failed to bring about the outcome [29].

Mentees fail to establish a significant relationship with their mentors as a result they fail to express their views and don't improve their communication skills [30]. Lack of available mentors is recognized as a crucial issue [31] .

ROLE OF MENTOR IN RESEARCH GUIDANCE

The mentors equip their mentees in providing adequate guidance for various comprehensive research studies. According to the various reviews done in the field of pharmacology, it can be comprehended that certain novel drug options can be used to develop antihyperglycemic effects from the ethanolic leaf extract of *Caralluma fimbriata* [32]. The characteristic feature of cytotoxic effects of the ethanolic extract of the *Caralluma fimbriata* helps to reduce cell proliferation [33]. Several reviews have proven the anti inflammatory, antimalarial and anti viral properties of the neem leaf, *Azadirachta indica* that is effective against various deadly disorders like hepatitis, malaria, cancer and other viral infections [34]. The mentors assist their mentees in the development of nanotechnology thereby reducing the cytotoxicity and increasing the biocompatibility of the anti cancer drugs [35]. A similar study was conducted to evaluate the cytotoxic activity of the *Acacia catechu* bark against the human squamous cell carcinoma-25 to confirm the induction of apoptosis [36]. To enhance ease of learning, mentees of the Saveetha Dental college have reported various other studies to detect the induction of the apoptosis thereby inhibiting the cancer cell proliferation in vitro and in silico using the styrene substituted biscoumarin [37]. The mentors play a vital role in making the mentees aware about the various novel interventions using oligonucleotide based therapies in the treatment of asthma and Chronic Obstructive Pulmonary Disease (COPD) [38]. In addition to it, they help to reinforce and increase scientific knowledge on the cytotoxic effect of the SCC-25 cells using *Acacia catechu* along with imbibed traits such as hepatoprotective, antipyretic, antidiarrheal, hypoglycemic, anti-inflammatory etc [39]. Positive mentorship helps in undertaking various strategies to understand the role of oxidative stress for the pathogenesis of the chronic liver diseases [40]. The related advancements for the treatment of the chronic liver diseases is due to the cavitation of the hepatic stellate cells [41]. Mentors in certain studies helped their mentees to evaluate the cytotoxic effect of syringic acid against human hepatoma HepG2 cell lines causing wide range of relief among the patients [42]. In addition to that,it was observed that the selenium nanoparticles are better as anticancer, non- toxic, and biocompatible operators than selenite and selenate compounds [43].

The mentors help in promoting sustainable approaches to the mentees for the biosynthesis of zinc oxide nanoparticles thereby producing anticancer and antioxidant agents [44]. Similarly the silver nanoparticles (AgNPs) were produced using *Garcinia mangostana* bark for showing potential larvicidal effect [45]. Various other

plants for green synthesis of silver nanoparticles include herbal plant Cassia alata and their antidermatophytic potential ranges are observed [46,47]. Moreover the Brassica oleracea extract also facilitates the synthesis of the zinc oxide nanoparticles thereby promoting anti bacterial property [48].

Conclusion

Mentor-Mentee is a symbiotic relationship which is aimed at advancing academic excellence and career satisfaction for both the mentor- mentees equally. Positive mentors–mentee relationships are pivotal for advancing academic success. It facilitates partnership between the mentees for developing personal and professional growth. Efficient mentoring enhances trust and respect among the mentees which will help the mentees to share their information , resource by being enthusiastic, supportive along with developing collaborative problem solving skills. Mentors play a crucial role in producing network opportunities, advising on career progress and providing navigation for the mentees on management of various responsibilities at a time. Efficient mentors tend to check the strength , weakness of their mentees along with the behavior management, personal communication,collaborative alliance. They balance power and develop a positive, steadfast relationship with their mentees. Mentees must be expressive and share their point of views openly thereby develop their communication skills and manage stressful situations with confidence. Open communication is one of the most vital aspects in enhancing interpersonal and professional trust with mentees. Certain barriers to mentorship includes time, management of stress and multiple work at a time, lack of financial incentives and availability of recognized mentors. Therefore mentoring is an evolving relationship which requires time and attention to develop academic excellence. Hence sufficient awareness must be given to implement mentoring techniques for academic success and career guidance.

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A Current Review on Gut Microbiota For Healthy Life

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Abstract

The human gastrointestinal tract is highly populated by a vast and diverse community of different microbes. Every individual is provided with a unique gut microbiota which varies according to their food habits and dietary intakes. This human gastrointestinal tract gut microbiota has various functions by participating in host mechanisms or metabolisms acts as or gives protection against the pathogens invading microbes into the gut,and also play an important role in facilitating the immune system development and function. An slight alteration even in this natural flora of gut in the intestine can cause infectious disease and disorders to the human. Various infections due to antibiotics and gut bacterial communities,bacterial interactions,mood disorders,metabolic disorders,diabetes,allergic disease,inflammatory bowel disease,hormonal and many neurological processes can happen. Hence, it is very important in maintaining the natural flora of the gut as it maintains a healthy gut microbiota , structural integrity and protection. Recently ayurvedic medicines are used over allopathy for its less sides on the body and for maintaining a healthy gut microbiome. This review aims on the current status of the gut microbiota for healthy life under key problems identification and maintaining the current healthy gut microbiota in the human gastrointestinal tract.

Keywords*: Gut ; Microbiota ; natural flora ; disease ; function ; healthy ; gastrointestinal tract ; infections.*

Introduction

The human gastrointestinal tract gut microbiota have received increasing attention over the last years. Every individual is provided with a unique gut microbiota profile. [1] Intestinal microbiota is a natural flora of the human gastrointestinal tract which is a diverse community of various different types of microbes. Natural flora of the human intestinal gut have different compositions and are linked with various health and disease. [2] Microbiota has different taxonomic factors ,although these gut microbiota have been explored from several decades, investigations of the various role of microorganisms residing in the human gut have been attracted much attention beyond classical infectious diseases.[3] Different types of microbes may include firmicutes, Bacteroidetes, actinobacteria and proteobacteria are some microbes in the gut microbiota. These microbes maintain the natural flora microbiota.[4] The human gastrointestinal tract is populated by a wide range of diverse communities of microbes.The natural flora gut microbiota maintains and plays a major role in various host functions and different diversity of the microbiota.[5] Human gut microbiota is the natural flora so even a small alternation or disturbance in this natural flora will lead to many infectious disease and gut interactions and many disorders.[6] This natural flora is based on the individuals food habits, their dietary intake for maintaining a normal healthy gut. [7]

Recent articles show that common medications including all antibiotics and laxatives,can alter gut microbiome. These medications will increase the risk

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of intestinal infections, gut disorders,skin rashes,colon cancer ,obesity,mood disorders, diabetes,lung cancer[8]. These allopathy medicines have a lot of side effects and therefore ayurvedic medicine is used more recently. Hence, many herbs are explored for their pharmacological profile and can be used for management of diseases such as cancer [9,10] diabetes, oxidative stress induced liver injury,[11] [12]inflammation[13,14,15,16,17,18]As nanomedicine is ruling the industry now for its excellent properties people are using various plants and plant products for preparing eco friendly nanoparticles [19] to cure various disease [20,21,22]As increased antibiotics cause various damages, ayurvedic and nanomedicine is playing a vital role which may be opted for better protection of gut microbiota.

GUT - NATURAL FLORA

Human intestinal microbiota is the natural flora of the human gastrointestinal tract which is of a diverse community of a wide range of differing types of microbes. This natural gut flora aims at maintaining various host metabolisms, functions and diversity of the microbiota. Human microbiomes have differences even in healthy individuals with occupying different habitats such as the gut, skin, and vagina of every individual . This diversity remains unexplained although diet , host genetics , environmental factors and the early microbial exposure has also been implicated.[23] As numerous studies have already reported changes in the natural gut microbiota is not only during obesity, diabetes and liver disease but also cancer and even neurodegenerative disease. So this human gut microbiota is viewed as a potential source of novel therapeutics.

As every individual is provided with a very unique gut microbiota profile ,the natural flora of the human intestinal gut has different compositions and are linked with various health and disease .The microbiota has different taxonomic factors. They maintain the functional characterization of a healthy gut microbiota and plays a major role in maintaining the structural integrity of the gut mucosal barrier , as a specific role in host nutrient metabolism, immuno modulation and also acts as a protection against the pathogens or against invading microbes in the gut microbiota.

Health and Immune Response

As gut microbiota is an important component for the development of gut immune system and the gut immunological homeostasis which is influenced by the host - microbe interactions . As an underdeveloped immune system and immune functions,which would be restored by the colonisation of certain bacteria such as segmented filamentous bacteria. Other hand ,symbiotic microbes maintain the immune balance through both direct and indirect pathways. A good immune system and gut interactions is always necessary for maintaining the normal gut flora free from any disease and disorders. Healthy intestinal microbiota that contributes to host resistance to infection through their involvement in the development of various host immune systems and complex interactions.

GUT MICROBIOTA DISORDERS AND DISEASES

Gut microbiota have various relations in mood disorders of each individual. Approximately 10% of the world's total population is mood disorders due to socio economic burdens of significant individuals. Among the general population, people with mood disorders tend to have a Higher rate of mortality and a very decreased life expectancy.But however the underlying mechanism of gut interactions and alterations of mood disorders are not sufficiently characterised . The human body can be considered as a meta organism made up of its own eukaryotic cells and trillions of microbes that colonise on the superficial body sites , such as the skin , airways and gastrointestinal tract. The co-evolution of hosts and microbes brought about a variety of molecular organisms. Genetic and environmental factors contribute to the major cause of mood disorders such as genetic vulnerability and susceptibility , chronic non infectious inflammation, oxidative stress, neurotransmitter imbalance , insufficient signalling by many factors and abnormalities.

As the natural flora of the human gastrointestinal gut relates to various types of disease such as inflammatory bowel disease , type -I diabetes , mood disorders, hormonal disorders neurotransmitter modulation and cancers[24] As said in previous articles the oral microbiota to an extent influence on the oral squamous cell carcinoma.

Inflammatory Bowel Disease

The intestinal microbiota has communities colonising the small and large intestine, bowel movements play an important role in a multitude of host functions. Alterations in intestinal gut microbiota, of intestinal bacteria functions in the energy extraction from food ,production of key metabolites for the host physiology and also in the protection and response to gastrointestinal infectious disease.[19]Normally in a human gut upto 100 trillions of bacteria colonise as estimated 200 to 1000 distinct bacterial species. And the intestinal epithelium provides an essential barrier which ensures a space between the microbes and the host in the gut microbiota .

Type I Diabetes

Gut microbiota always has a correlation between diabetes mellitus and obesity as reviewed in many articles .[25] Changes in the gut microbiota have been implicated in the emergence of both diabetes and obesity in humans .These changes appear to be attenuated by dietary interventions, as a fibre enriched diet may improve the insulin resistance. Type I diabetes is a debilitating autoimmune disease that results from the T cells mediated destruction of insulin producing Beta cells.[26] Type I diabetes incidence was increasing during the past several decades in all the developed countries around the world, suggesting that these changes in the environment may influence disease pathogenesis due to the alterations in the natural flora of gut microbiota.[27]

Neurotransmitter Modulation

Gut microbiota is not only essential in maintaining structural integrity of host, maintaining immune system and metabolic health but also seems to have influence in the development of central nervous systems, neurodegenerative disease and neuro immune mediated disorders . Gut microbiota consumes a wide range of neurotransmitters such GABA,[28] acetylcholine, serotonin [29] , dopamine and histamine. As known , more than 90% of the neurotransmitter, serotonin, in the body of humans are produced in the gut, which can affect emotion regulation when transmitted to the CNS. As the natural flora of the human gastrointestinal gut relates to hormonal disorders and neurological processes also. So, it is mandatory to maintain a healthy and normal gut metabolisms. As the human gastrointestinal gut plays a wide range of functions and helps in maintaining a good health, understanding the disturbances and alterations in the natural flora of gut is essential.[30]

Conclusion

This review article highlights the importance of a healthy microbiota. Every individual is provided with a very unique gut microbiota, having various functions, that plays a major role in maintaining the structural integrity of the gut mucosal barrier ,specific role in host nutrient metabolism, protecting against the invading microbes in the gut. The normal flora of gut microbiota depends on dietary and food habits of the individual and also age. Any small disturbance in the gut microbiome can lead to various diseases . Healthy gut can be maintained by increasing the intake of fibre rich food, fruits with citric acid and vegetables. Avoiding unnecessary antibiotics can also maintain a normal and healthy gut microbiota.

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Oncogenes Associated with Oral Cancer - An Update

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Abstract

Oral cancer and oropharyngeal cancer represent a large group disorder that falls into the head and neck category and is considered as the most leading cause of death in developing countries. The prime reason for the high mortality rate of patients suffering from oral cancer is the delay in the diagnosis of the type and grade of oral cancer and also in the offering of prompt treatment. A report from India demonstrated that 35% of oral squamous carcinoma contains H-ras gene mutations which are an oncogene. Advances in the field of oncogenes have created an instrument to examine the various phases of carcinogenesis, including progress from premalignant to metastatic stages. These systems have direct importance to oral malignancy where the precancerous stage is very much characterized. Primer investigations into the articulation and capacity of oncogenes recommend anomalies including the individual proliferation of at least three kinds of these oncogenes. Some of the oncogenes associated with oral cancer are studied in this article and the factors contributing to its molecular basis like chromosome instability and telomerase activity are also discussed briefly. There are many risk factors and other predisposing factors that may affect the diagnosis, prognosis, and progression of oral cancer and are also discussed in this article.

Keywords: *Proto-oncogenes; Oncogenes; Oral cancer.*

Introduction

Oral cancer is considered to be the most common form of cancer affecting males with a greater prevalence in the Asian-population. Around 90% of head and neck cancer are typed as oral squamous cell carcinoma (OSCC)[1]. OSCC is the 6th most common type of cancer with poor clinical indications which needs to be surpassed with advancements in its molecular basis. Oncogenes are capable of inducing cell transformation and is hence implicated in the molecular basis for cancer[2, 3]. The gain of function mutations in oncogenes are considered to be the prime etiological factors influencing the transformation of a proto-oncogene into an oncogene[4]. Prevailing changes most often happening in proto-oncogenes in addition to certain tumor silencing genes (TSGs) bring about an increase in the magnitude of the disease. Environmental exposure to pro-carcinogens, along with habits such as pan, gutka, and tobacco usage also increases the transformation and progression of the disease.

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Oncogenes

Proto-oncogenes assume a significant role in controlling cell division and apoptosis during development and improvement. In such a situation when a proto-oncogene becomes mutated, or the cell makes additional duplicates of the proto-oncogene, the gene becomes hyperactive and leads to uncontrolled cell division, eventually leading to the advancement of a malignant growth cell from an ordinary cell. When a proto-oncogene is actuated by a transformation, we at that point allude to it as an oncogene. So it's the

initiation of oncogenes that is one of the processes that add to the formation of a tumor. Proto-oncogenes transform to oncogenes which are an essential part of cancer progression[5]. Point mutations in proto-oncogene results in a constitutively active protein[6] which is the result of localized gene amplification of a DNA strand containing proto-oncogenes.[7] There are many types of oncogenes involved in the progression of oral cancer some of which are discussed.

RISK FACTORS ASSOCIATED WITH ORAL CANCER

The use of smoking and smokeless tobacco such as pan, gutka, etc., seems to be the principal risk factor associated with oral cancer in Asian subcontinent[8]. Other habits, occupations, environmental exposure, sharp tooth, etc may be responsible for the increased vulnerability of the patient towards oval cancer. Some or all of these factors may contribute to the prognosis of the patient and if identified as a precancerous or cancer-prone condition may even to its early diagnosis. There is a low prevalence of screening and poor awareness of oral cancer and precursor lesions[9]. The risk of oral cancer in smokers is more than the non-smokers' population. Even passion smoking is associated with some risk. The danger of creating oral malignant growth relies upon the term and recurrence of tobacco use. Smoking may prompt malignant growth in the mouth or throat, and oral tobacco items are related to disease in the cheeks, gums, and internal surface of the lips.

Virus

Viruses may also be responsible for the development of cancer[10] some of the strongly associated viruses with oral cancer are Epstein Barr virus (EBV), human papillomavirus (HPV 16 and HPV 18)[11] and herpes simplex virus[12]. Other lifestyle choices may also have an impact on the procurement of oral cancer and its progression like occupation, frequency of exposure and so on. Be that as it may, no persuading proof exists that they are a hazardous factor in OSCC. In this manner more investigations are required so as to explain the various parts of infection contribution. So preventive measures against viral agents like vaccinations play an essential role[13] acquisition of a virus that may also serve as a prelude to cancer.

MOLECULAR BASIS

Carcinogenesis is a complex, multistep process with altered signal transduction pathways in case of oral cancer[14]. Dysplasia may be due to premalignant lesions such as leukoplakia, erythroplakia, lichen planus and so on[15]. The state of dysplasia when discovered early could aid in identifying the specific treatment modality.[16] One of the crucial segments of oral cancer is the transformation of proto-oncogenes into oncogenes. Chromosomal instability is a common feature of solid tumours[17], which is responsible for cancer development and progression. Malignant cells consistently contain unusual quantities of chromosomes, called the aneuploid state, which prompts modified measurements and articulation of the entirety of the functions carried on a given chromosome. As malignant cells quickly develop and isolate, they don't adapt well to additional chromosomes, which prompts anomalous chromosome isolation and extra changes in chromosome number as the cells keep on separating. This property of cancer cells is named chromosomal instability.

Telomerase activity was detected in 89% of malignant and 5% of normal oral mucosal tissue[18] which indicates that activation of telomerase is frequent in oral squamous cell carcinoma. This is also clinically useful marker for prognosis of oral cancer. Cell cycle regulation is important in the progression of oral cancer. The overexpression of cycle regulators promote oral cancer progression by enhancing cell proliferation with prevention of GI phase arrest[19] There is a strong relationship between oral squamous cell carcinoma and DNA repair genes[20]. The x-ray repair cross complementing group seems to be a risk factor for predisposition of oral squamous cell carcinoma. Understanding the molecular basis is important because it helps in the development of gene therapies[21] that target specific genes.

Type of Oncogenes

Nuclear Transcription Regulators

They are present in the nucleus and give rise to encoded proteins that are transcription factors. Though they contain many subdivisions, the most involved genes with oral cancer are jun and fos[22]. The most commonly found complex in cancer tissues was the c-fos-Jun D

complex. The current discoveries demonstrate that c-Jun; the significant part of the AP-1 transcription factor is modified in dysplastic epithelium and oral SCC. The overexpression of c-Jun reveals its job in early carcinogenesis. Henceforth, c-Jun may act in various instrumental ways and pathways that lead to a harmful change in oral cells.

Intracellular Signal Transducers

Ras and raf are the most involved genes with oral cancer. The Ras gene gives rise to protein serine kinase and the raf gene generates GTP/GDP binding protein[23]. The H-Ras protein was fundamentally overexpressed in oral carcinoma[24,25]. The vast majority of the OSCC cases indicated positive recoloring with moderate articulation. Most of H-Ras positive cases were found in people with numerous hazard propensities including tobacco usage in some form. H-Ras expression expanded in cases influenced with buccal mucosa site and higher evaluation of carcinoma. Relative mRNA level of H-Ras was essentially raised in oral carcinoma. Protein and mRNA levels of H-Ras were ineffective in relation.

Apoptosis Inhibitors

Numerous genes viz., Bcl-2, Bcl-x, Bcl-xL, Bcl-2-related protein A1, BAG-1, and survivin have been found to be associated with oral cancer. The process of mitochondrial apoptosis is regulated by pro and anti-apoptotic proteins that belong to the BCL-2 family. A balance between the pro and anti-apoptotic protein ensures proper regulation of the programmed cell death which influences the development of an organism. Any dysregulation in the BCL-2 pathway can prevent the process of apoptosis eventually leading to the uncontrolled proliferation of cells facilitating tumor development, progression, and resistance to cancer therapy[26].

Cell Growth Proteins

In case of cancer, growth factors are deregulated. There is over-expression of transforming growth factor x by autocrine mechanism. Thus there is alteration of mutation of the all surface receptor genes there by producing a ligand-independent mitogenic signal[27]. The G-protein coupled receptors are the surface receptors most associated with turnover growth and metastasis and are involved in signal transmission.

DIAGNOSTIC PROPERTY

Oncogenes serve as poor prognostic markers[28] as their presence suggests the initiation or progression of cancer. Oncogenes can serve as diagnostic markers and help in the early diagnosis for example to detect precancerous lesions and mutated oncogenes are distinguished from proto-oncogenes[29]. Confined, community oriented systematic research will help the study of oncogenes in oral cancer reach new heights like advancements in the diagnostic methods and targeted therapy. Some of the microbial infections may also predispose to many future complications in chronic presentations and drug resistance may also play a role [30, 31, 32, 33] which also include cancer and other iatrogenic diseases as well. So knowledge of the properties of potential natural medicaments may prove useful to prevent the chronic persistence of these infections [34, 35] without any drugs but just by incorporating them into our lifestyle. Some of the microbes for example like *Acinetobacter baumannii* is a recently identified orodental pathogen and gene studies [36,37,38,39] pertaining to this may prove imperial in understanding its pathogenesis and so on. Many contemporary medications[40] against these microbes as well as others and preventive measures[41,42] are also being studied through further research.

Conclusion

Cancer is a complex genetic disorder which involves abnormalities in multiple genes which are implicated in one or more biological processes including cell cycle regulation, DNA repair, replication, regulation of gene expression etc. The review highlights the common genes involved in the development and progression of oral cancer. Epigenetic mechanisms also work simultaneously to modify the gene expression of proto-oncogenes. Further research on these epigenetic controllers would aid in deriving strongest association between these oncogenes and oral cancer.

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Safety of Dental Restorative Materials – A Survey among Undergraduate Dental Students

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Abstract

Usage of proper dental restorative materials is very important in dentistry. One who is doing the course of dentistry should have sound knowledge on these restorative materials. Furthermore, it is important to know the view of budding young dentists about safety of dental restorative materials. The aim of present study was to assess the awareness and perception of undergraduate dental students towards safety of dental restorative materials. A study was carried out by using multiple-choice structured questionnaire among 262 students, the questionnaire was prepared in such a way that we can assess the attitude, their perception and awareness towards safety of dental restorative materials. The data was collected and statistically analysed. Although, most of the participants were having moderate level of awareness towards safety of dental restorative materials. The level of perception of some respondents towards amalgam related and other restorative materials related issues was found to be improved, participants with a better perception and attitude are having more knowledge and awareness towards safety of dental restorative materials. Within the limitations of the study, usage of alternative materials to amalgam should be improved.

Keywords: *Dental restorative materials, Dental students, Awareness, Safety*

Introduction

Two types of restorative materials are commonly used in dentistry, they are designated depending on whether they can be applied directly to the tooth or require fabrication of the restoration in the dental laboratory. Restorative materials are used to restore tooth damage that occurs as a result of dental caries. When a tooth is badly decayed or seriously infected, root canal treatment should be done.[1] Dental materials are used for direct restoration of a tooth in order to save its function while indirect materials include preformed metal crowns, dental porcelain and cast restorations. There are two main types of dental tools used for placing fillings: plastic instruments and condenser.[2] The principal material types for direct restorations are: Direct amalgam (silver-tin-copper alloy and approximately 50% mercury), Resin-based composite materials, Modifications of RBCs (poly-acid modified composites); compomers and giomers (glass filler modified composites). Glass-ionomer cements/water-based cements: Self-setting ("pure" glass ionomers) or more usually, light cured (resin modified glass-ionomers). Long-term temporary materials e.g. reinforced zinc oxide-eugenol cements. Chlorhexidine-releasing methacrylate dental composite materials have antimicrobial activity.[3]

The indications for use of restorative materials span from small cavities to extensive loss of tooth substance.[4,5] Materials are employed for cavities in primary teeth; for cavities in permanent teeth, ranging from "minimal interventions" to the need for extensive replacements and/or build-procedures; replacement or repair of failed or less satisfactory restorations, or materials are used in

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people with compromised health and having dental caries on certain locations, e.g. root caries.[6] The development of 'smart composites', Amorphous Calcium Phosphate Composites that respond to oral microflora by releasing chemotherapeutics or antimicrobials such as calcium and fluoride, may circumvent some of the shortcomings of composite restorations. Research into a material that is based on the technology of glass ionomers, low shrinking resins and high strength filters with simple handling and acceptable longevity is in progress. Glass ionomer cements exhibit a number of advantages over other restorative materials, by bonding a restorative material to tooth structure, the cavity is theoretically sealed, protecting the pulp, eliminating secondary caries and preventing leakage at the margins.[7]

The most common different types of restorative materials are: Amalgam, composites, glass ionomer, resin ionomers.[8] Amalgam are commonly used in dental fillings which are heavily loaded mostly in posterior restorations, composite are mostly used in aesthetic dental fillings and veneers. Glass ionomers are commonly used for small non load fillings, cavity liners, cements for crowns and bridges and resin ionomers are used for small non load fillings, cavity liners and cement for crowns and bridges.[9]

Dental materials which have widespread applications in dentistry, inspite of their good physical, mechanical properties and excellent esthetic characteristics, may inturn cause some side effects. Chlorhexidine has powerful antibacterial properties and can be especially helpful in maintaining a healthy mouth after procedures such as scaling and root planing.[10] The side effects of some restorative materials may lead to severe lesions in the oral cavity or far from the application place of the materials.[11,12] Dental materials maybe categorized into temporary or permanent materials and the related side effects can be studied based on clinical view, region and the type of material.

All artificial materials release substances into the oral environment and imply some risk of side effects and adverse reactions. Amalgam has been associated with general health concerns, while local oral effects from different restorative materials are reported.[13,14] The majority of the cases of side effects of dental filling materials are linked with dental amalgam. However, the reporting of composites and cements after the 2008 amalgam ban, decrease in harmful reactions has been observed.[15,16] The aim of present study was to assess the awareness and perception of undergraduate dental students towards safety of dental restorative materials.

Materials and Methods

The present study was the survey, conducted online through docs.google.com. The study was conducted among undergraduate dental students, from various places of India, and email addresses of the respondents were collected for study purposes, a list of 300 emails were collected and questionnaires were sent to them.

A self designed, close ended questionnaire consisting 10 questions was prepared. It was prepared in such a way that we can easily know the perception, attitude and awareness about safety of dental restorative materials among undergraduate dental students. The questionnaire was uploaded in Google docs. The web link was sent to 300 members in total, out of which 262 have responded. The responses were collected, compiled, and tabulated in MS Excel sheet, the data was then subjected to statistical analysis using SPSS-16, the statistical tests done are chi-square tests.

Results and Discussion

TABLE 1: Percentage distribution of responses to questions on awareness and perception level about restorative materials.

Question	Responses - Yes	Responses - No
Are you aware of amalgam controversy?	54.5%	45.5%
Would you recommend an alternative to amalgam?	81.8%	18.2%

Cont ... **TABLE 1: Percentage distribution of responses to questions on awareness and perception level about**

Are you bothered about the environmental issues of mercury in dental offices?	54.5%	45.5%
Is your patient worried about the colour of the restoration?	72.7%	27.3%
Do you prefer composite over amalgam?	81.8%	18.2%

TABLE 2: Percentage distribution of responses to questions on knowledge level about amalgam safety.

QUESTION	RESPONSES - YES	RESPONSES - NO	RESPONSES - MAYBE
Is amalgam safe to use?	27.3%	36.4%	36.4%
Do you think composite restorations are better than amalgam restorations?	54.5%	18.2%	27.3%
Do you think using amalgam causes many side effects?	45.5%	36.4%	18.2%

TABLE 3: Percentage distribution of responses to questions on knowledge level about material allergy.

QUESTION	RESPONSES - YES	RESPONSES - NO	RESPONSES - NOT AWARE
Are you aware that the methacrylate monomers are allergens in resin based materials like composites, resin modified GIC?	18.2%	54.5%	27.3%
Is there any relationship between mercury released from dental amalgam and neurological diseases?	54.5%	27.3%	18.2%

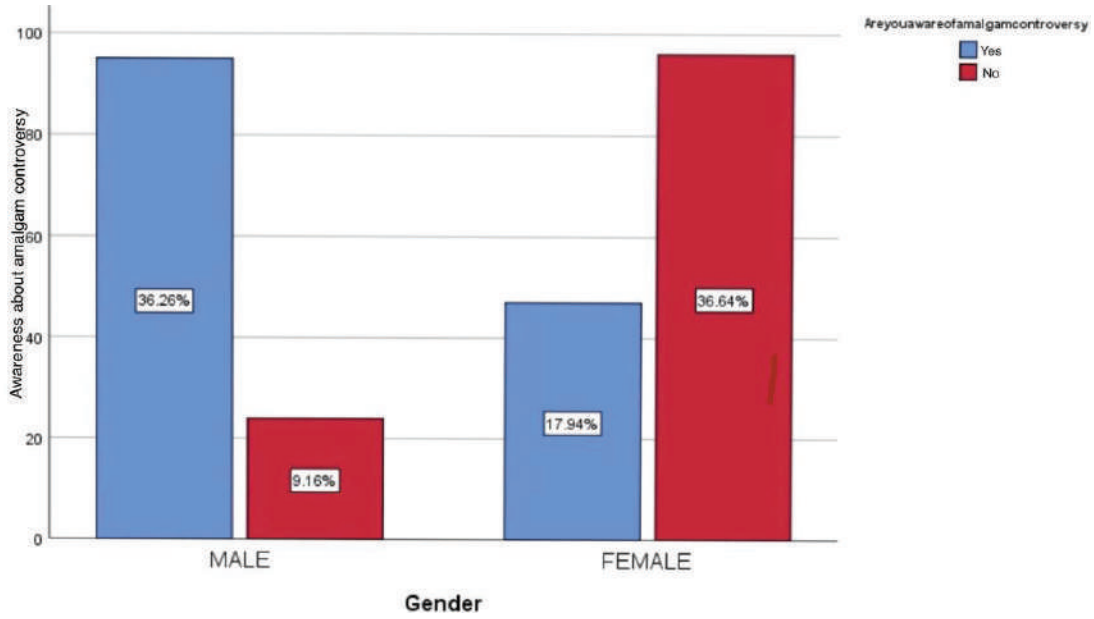


Figure 1: Bar chart showing association between gender of students and responses to the question, Are you aware of amalgam controversy. X axis represents the gender and the Y axis represents the number of responses. Blue represents yes and red represents no. 36.26% of males reported yes and 9.16% of males reported no. 17.94% of females reported yes and 36.64% of females reported no. Male participants are more aware regarding the controversy of amalgam compared to females. Chi square test; p value=0.00 (<0.05) indicating statistically significant.

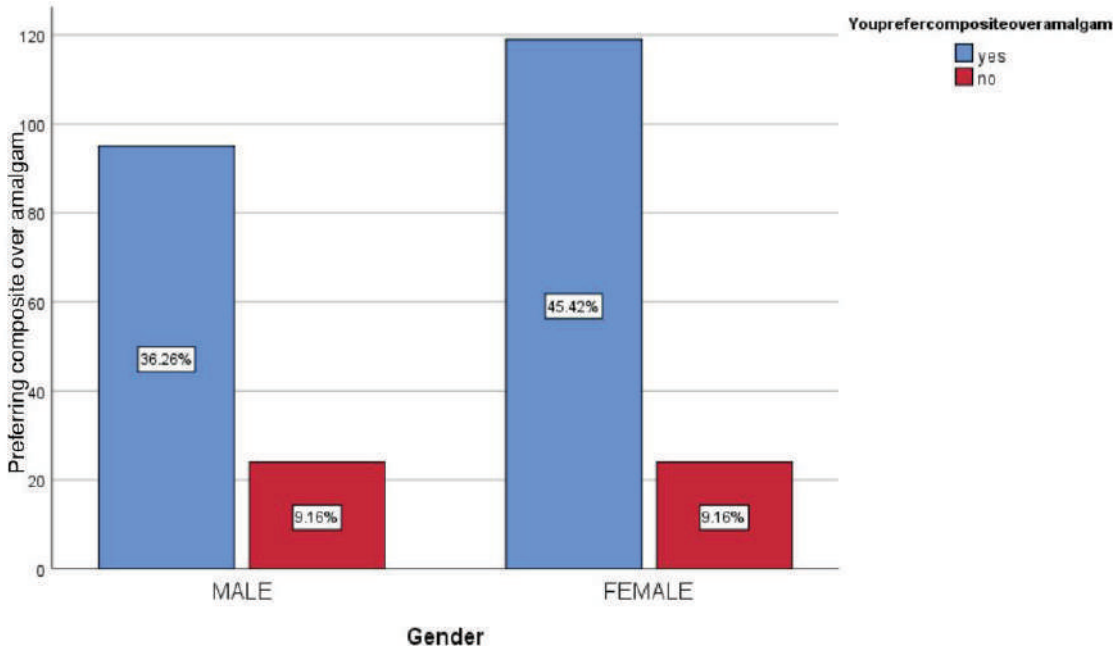


Figure 2: Bar chart showing association between gender of students and responses to the question, Would you recommend composite over amalgam. X axis represents the gender and the Y axis represents the number of responses. Blue represents yes and red represents no. 36.26% of males reported yes and 9.16% of males reported no. 45.42% of females reported yes and 9.16% of females reported no. Female participants prefer composite over amalgam compared to males. Chi square test; p value=0.481 (>0.05), indicating statistically not significant.

Out of 300 students approached, 262 have responded and the responses are statistically analysed. In this study, 54.5% of the respondents are aware of amalgam controversy, and the rest 45.5% are not aware (Table-1), more percentage of the respondents are aware about the amalgam controversy when compared to the study done by JE Dodes in 2001 and Simon JF in 2011.[17,18] 36.4% of students think that amalgam is unsafe, 36.4% students are uncertain whether amalgam is safe or unsafe, 27.3% of students think that amalgam is safe (Table-2). Dental amalgam contains elemental mercury. It releases low levels of mercury in the form of a vapor that can be inhaled and absorbed by the lungs. High levels of mercury vapor exposure are associated with adverse effects in the brain and the kidneys.[19,20] 81.8% of students recommended an alternative to amalgam and 18.2% don't recommend an alternative to amalgam (Table-1). When compared to the study done by Balevi B in 2014, more percentage of the respondents recommended an alternative to amalgam.[21,22] 54.5%of the students are bothered about environmental issues of mercury in dental offices and the 45.5% are not aware **(**Table-1**)**, when compared to the study done by keenan jr, more percentage of students are bothered about environmental issues of mercury in dental offices.

54.5% of the respondents think that composite restorations are better than amalgam restorations 18.2% of students think that it's not. 27.3% students opted for maybe (Table-2**)**. Composite filling is the better choice because it supports the natural tooth structure and in the process makes it stronger.[23] 45% of students think that using amalgam causes many side effects. 36.4% don't think that and the rest 18.2% opted the option maybe (Table-2), compared to previous study done by Hariyani M, more percentage of the respondents think that amalgam causes side effects.[24]

72.7% of respondents agreed that their patients are worried about the colour of restoration and the rest 27.3% did not agree, amalgam restorations look odd on the tooth, it is not an aesthetic material (Table-1). 54.5% of students don't think that methacrylate monomers are allergens in resin based on materials like composite and resin modified GIC, 27.3% are not aware of and the rest 18.2% agreed that methacrylate monomers are allergens (Table-3). Awareness about methacrylate monomers need to be improved. 81.8% of students prefer composite over amalgam and the rest 18.2% don't prefer (Table-1), compared to previous studies, more percentage of respondents prefer composite over amalgam.[25] 54.5% students think that, there is the relation between mercury released from dental amalgam and neurological diseases, 27.3% don't think that and the rest 18.2% are not aware (Table-3). Currently, there is no evidence that dental amalgam causes any severe neurological diseases. [26]

The response rate of the study is pretty much satisfactory, the study may be limited because only a selected population of Indian undergraduate students were studied. Consequently the data may not be a true representation of all undergraduate students. In the current study the number of undergraduate dental students who agreed on the safety of amalgam was slightly lower than that reported by kharirulden and others.[27] However, in the current study, a higher number of students agreed that amalgam is unsafe. Fewer patients believe that amalgam is unsafe compared with previous studies. [28]

The association between gender of students and responses to the question, Are you aware of amalgam controversy was done. 36.26% of males reported yes and 9.16% of males reported no. 17.94% of females reported yes and 36.64% of females reported no. Male participants are more aware regarding the controversy of amalgam compared to females. Chi square test; p value=0.00 (<0.05). Hence, it is statistically significant (Figure 1). The association between gender of students and responses to the question, Would you recommend composite over amalgam was done. 36.26% of males reported yes and 9.16% of males reported no. 45.42% of females reported yes and 9.16% of females reported no. Female participants prefer composite over amalgam compared to males. Chi square test; p value=0.481 (>0.05). Hence, it is statistically not significant (Figure 2). Compared to previous reports[29], the current study reveals that fewer undergraduate dental students would oblige, requesting patients to get their amalgam removed. This matter may also be dentist dependent. The rate of agreeing that their patients are worried about the colour of restoration is higher than the previous studies.[30] In present study, 54.5% of the students were bothered about mercury issues in dental office, this is also higher than the previous study done by mantyla et al in 1976.[31,32]

Conclusion

There is a need for improvement of usage of alternatives to amalgam and awareness about restorative materials and safety also should be improved. Within the limitations of the study, a moderate level of awareness about restorative materials was observed among the undergraduate dental students.

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Coronavirus Disease 2019: Corona Viruses and Blood Safety - A Review

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Abstract

The main aim of the study is to understand the current information of corona transmission and blood safety measures taken during handling blood safety products of corona infected patients.Coronaviruses are in the subfamily Ortho Coronaviridae of the Coronaviridae family.It is a type of viral virus that causes inflammation in your nose, sinus, or upper throat.Most coronaviruses are not harmful.The virus may result from pneumonia, respiratory failure, septic shock, and death. Many complications of Coronavirus disease 2019 (COVID 19) may result from a condition known as cytokine release syndrome or a cytokine storm. This study mainly aims at the awareness and perception of rural and urban people about covid-19 virus. .Finding advance in blood safety to avoid blood contamination. It will help to provide a proper diagnosis of the disease and understand current information on coronavirus transmission and blood safety measures during the course of the infected patient's blood safety procedures.To assess public health risks and the risk of occupationally acquired disease which may be associated with the management of infectious waste, understanding these factors is required.The storage, processing and examination of blood specimens from Covid-19 patients must be carried out by qualified staff in suitably equipped laboratories, previously educated on the technical and safety procedures.A blood-borne illness is a disease which can spread by blood and other body fluids contamination. Blood can contain various types of pathogens, mainly microorganisms like bacteria and parasites, and non-living infectious agents such as viruses. Through taking preventive steps in the handling of blood products and storage of blood products for infection, contamination will be reduced and the public will not be affected.

Keywords: *Blood safety, Coronavirus, Disposal, Handling, Spread, Blood borne.*

Introduction

Coronaviruses make up the Ortho Corona Virinae sub - family and are enveloped viruses with a single-stranded RNA genetic code as well as a nucleocapsid helical uniformity in the family Coronaviridae, order Nidovirales and realm Ribovirus.[1] The genome size of coronaviruses varies between around 26 and 32 kilobases[2], one of the largest among RNA viruses.They have distinctive club-shaped spikes that extend from their surface , producing a picture reminiscent of the solar corona from which their name derives in electron micrographs [3].

A coronavirus is a type of viral virus that causes inflammation in your nose, sinus, or upper throat.Most coronaviruses are not harmful[4].The World Health Organization described SARS-CoV-2 as a new form of coronavirus in early 2020, following an outbreak in China in December 2019. Coronavirus disease 2019 (COVID-19) is a disease caused by SARS-CoV-2 that

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can cause inflammation in the respiratory tract. It can affect the upper respiratory tract (sinuses, nose and throat) or lower respiratory tract (windpipe and lungs)[5]. It spreads the same way that other coronaviruses do, primarily through contact between persons.The other coronaviruses cause most of the colds that affect us during the year but do not pose a significant threat to otherwise healthy people. The 2019 novel coronavirus (COVID-19) is an infectious infection that can transmit directly or indirectly from one person to another, causing respiratory diseases ranging from common cold to acute respiratory syndrome[6]. This virus was first discovered in Wuhan , China. COVID-19, according to the World Health Organization, is a major health problem and has a higher risk of extreme disease, which is spreading rapidly around the world. A total of 87,137 confirmed cases were reported worldwide until March 3, 2020. Of these, 2977 (3.42 percent) were fatal.

BLOOD SAFETY IN CORONA INFECTION

Spread of Coronavirus:

Respiratory infectious diseases can be transported by droplets of various sizes: if the droplet particles are > 5-10 μm in diameter, they are called respiratory droplets, and if they are < 5 μm in diameter, they were also made reference to it as aerosol droplets.According to available data, COVID-19 virus is transmitted mainly among people through respiratory secretions and communication paths. Airborne communication wasn't really noted in an assessment of 75,465 COVID-19 cases in China.Transmission of droplets occurs when a person is in close contact (within 1 m) with someone with respiratory symptoms (e.g., coughing or sneezing) and is therefore at risk of exposure to potentially infectious respiratory droplets through his / her mucosa (mouth and nose) or conjunctiva (eyes)[7,8]. In the immediate environment around the infected person transmission may also occur via fomites. Consequently, COVID-19 virus transmission can occur through direct contact with infected people and indirect contact with surfaces in the immediate environment or with items used on the infected person (e.g., stethoscope or thermometer)[9].Airborne transmission is distinct from droplet transmission because it refers to the presence of microbes within droplet nuclei, which are usually referred to as particles < 5 μm in diameter, can remain in the air for long periods of time, and can be transmitted to others over distances greater than 1 m.i.l.e,Endotracheal intubation, bronchoscopy, open suction, nebulizer therapy, manual ventilation before intubation, disconnection of the patient from the ventilator, non-invasive positive pressure ventilation, tracheostomy and cardiopulmonary rehabilitation[10]. There is some evidence that infection with COVID-19 can lead to bowel infection and can be found in faeces. To date, however, only one study has developed the COVID-19 virus from a single stool specimen. To date, no cases of faecal−oral COVID-19 virus transmission have been reported[11].

Blood Borne Disease and Coronavirus:

Bloodborne pathogens are microorganisms that are carried in the blood, such as viruses or bacteria, and can cause disease in humans.There are many different bloodborne pathogens including malaria, syphilis, and brucellosis, most commonly hepatitis B (HBV), hepatitis C (HCV), and the HIV virus.Bloodborne pathogens such as HBV and HIV can be transmitted by contact with contaminated human blood and other body fluids such as.A blood-borne illness is a disease which can spread by blood and other body fluids contamination[12]. Blood can contain various types of pathogens, mainly microorganisms like bacteria and parasites, and non-living infectious agents such as viruses. CDC-NIOSH cites, in particular , three bloodborne pathogens, all viruses, as being of primary concern to health workers: HIV, hepatitis B (HVB), & hepatitis C (HVC)[13].

It is more useful to classify diseases that are not usually transmitted directly by blood contact, but rather by insects or other vectors as vector-borne diseases, even though the causative agent may be found in the blood. Because it is difficult to determine what pathogens any given blood sample contains, and certain blood-borne diseases are lethal, all blood (and any body fluid) is regarded as potentially infectious by common practice. Precautions for blood and body fluid[14] are a kind of infection prevention procedure that aims to prevent transmission of this form of disease.

Needle Stick Injury:

Many healthcare workers are at increased risk of unintended needle stick injuries because of the environment in which they work. Injury from infected

sharp instruments and needles poses a significant occupational threat for healthcare employees[15]. The prevalence of such viruses is unknown in the hospital patient population and in healthcare workers There is a chance of occupational transmission of bloodborne pathogens such as Aids, hepatitis B and C and other diseases[16]. The overall risk of HIV transmission to a health care worker following percutaneous exposure to HIV contaminated blood was estimated at 3 in 1000. According to a WHO study, the estimated annual proportion of health care workers (HCW) exposed to blood-borne pathogens worldwide was 2.6 per cent for HCV, 5.9 per cent for HBV, and 0.5 per cent for HIV, corresponding to around 16,000 HCV infections and 66,000 HBV infections worldwide. Injuries recorded by standard occupational reporting systems can underestimate the true rate of injury as much as 10 times[17]. Needle stick injuries have major indirect effects in the delivery of healthcare, especially in developing countries, where the skilled workforce in the population is already reduced in terms of the disease burden. Such accidents not only potentiate health effects, but also cause emotional distress in health care personnel, leading to missing workdays and directly affects the health care services and resources.

Handling of Blood Safety in Clinical Laboratory:

The storage, processing and examination of blood specimens from Covid-19 patients must be carried out by qualified staff in suitably equipped laboratories, previously educated on the technical and safety procedures[18]. In all cases, national laboratory biosafety standards should be followed, and general information is also available at the World Health Organization (WHO) laboratory. .In Modena, blood specimens from Covid-19 patients are treated in a Biosafety Level (BLS)-2 laboratory equipped with Biological Safety Cabinets (BSC) of Class II. Both cabinets are fitted everyday with an internal waste (containing 0.5% bleach) where any polluted biological material is discarded[19]. Both laboratory staff need to wear personal protective equipment. In detail, personnel must wear disposable gloves, laboratory coats, and surgical masks when working in the laboratory area, required to prevent the spread of unwanted droplets[20]. Such measure is also necessary to avoid the spread of the infection in the event of an asymptomatic infection by a researcher. Laboratory clothing is kept in the laboratory, and should never be used outside. For all ongoing tests doors of the laboratory are kept locked[21].

The storage, processing and examination of blood specimens from Covid-19 patients must be carried out by qualified staff in suitably equipped laboratories, previously educated on the technical and safety procedures[22]. Local laboratory biosafety standards should be followed under all cases, and general guidance is also available in the World Health Organization (WHO) Laboratory Biosafety Manual. Blood specimens from patients with Covid-19 are treated in the Biosafety Level (BLS)-2 laboratory equipped with Class II Biosafety Cabinets (BSC) in Modena. All cabinets are equipped with one every day internal waste (containing 0.5% bleach) where any possible contaminated biological material is discarded[23].

Inside the laboratory there is a distance of at least 1 m between people, and where possible the presence in each room should be limited to just one person. If this is not possible, it is important not to have two operators sitting too close to the same cytometer or computer using the same instrument (e.g., like a 180 cm cabinet) or two researchers[24].

Disposal of Corona Infected Waste Product :

To assess public health risks and the risk of occupationally acquired disease which may be associated with the management of infectious waste, an understanding of these factors is required[25].Infectious waste means that active pathogenic microorganisms are present at a sufficient concentration to infect a susceptible human host[26]. If the level of pathogenic organisms is below that which can enter and reproduce inside the host, there is no risk of illness.A patient in a healthcare facility, an experimental animal in an infectious disease vivarium, or the culture medium used to propagate an infectious agent may be the source of the infectious wastes.The principal responsibility for the safe handling and disposal of infectious waste rests with the waste generator. That duty continues to the final disposal point and though other parties are involved in the processing of the waste[27]. The generator will carry out inspections or take other steps to ensure that the waste is properly treated and disposed of, even though the handling of infectious waste is also a problem for waste hauliers and

operators of treatment facilities. Innovations in waste management like in the educational system are also upcoming in this pandemic era. Additionally, federal, state , or local regulations may have to be observed to control medical waste disposal and record keeping. Wearing protective gloves and frequent handwashing are the most critical practices for all workers handling infectious waste. Handling of infected objects gloves and a laboratory coat are recommended. When soiled, or damaged, gloves and clothing should be changed[28].

The type of laboratory activity will determine whether further protective measures are necessary. Laboratory operations with a high risk of contamination caused by infectious fluid spills or droplet production on plastic-backed absorbent bench paper should be carried out[29]. Workers who handle infectious waste in an autoclave should wear a rubber apron, sturdy shoes, asbestos-free heat-resistant gloves, and face shield to protect against injuries that may occur when the autoclave is loaded or unloaded.The method of choice for treating large quantities of hazardous waste , animal carcasses and toxic bedding products is incineration. Because incinerators are usually located some distance from the laboratory, additional precautions are needed to handle and package infectious waste.[30]

Conclusion

While coronaviruses mainly cause mild to serious respiratory infections, it is worth considering the potential for transfusion transmission. Coronavirus infection can result in a spectrum of diseases, from mild respiratory symptoms to severe, life-threatening pneumonia. Therefore, it is critical to consider the potential for transmission of this infection by blood transfusionBy taking preventive measures in the handling of blood products and in the disposal of infectious blood products, contamination will be minimized and there will be no public effect.Those patients recovering from COVID-19 should avoid donating blood for at least 28 days after symptom resolution and completion of therapy.This might avoid spread of infection to others.

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Matrix Metalloproteinases (MMPS) and its Role in Cancers - A Review

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Abstract

The main aim of this study is to analyze the role of Matrix metalloproteinases genes in cancer progression and metastasis. Matrix metalloproteinase genes were identified in humans, and many are involved in cancer. Extracellular matrix (ECM) degradation by matrix metalloproteinase not only enhances tumour invasion but also affects tumour cell behaviour and leads to cancer progression. This review highlights recent developments in the cellular and molecular mechanisms of matrix metalloproteinase that influence tumour cell growth, invasion, and metastasis. A review with recent information about matrix metalloproteinases and its role in cancer collected from search engines from the articles dated from 2000-2020. The recent articles discussed in this review help to gain knowledge and understanding of Matrix metalloproteinases in cancer invasion and progression, metastasis, angiogenesis, and aid in the analysis of the mutations caused which aid the medical field to prevent tumour progression.

Keywords: *Matrix metalloproteinases, tumour growth, metastasis, angiogenesis, tumour invasion, matrix metalloproteinase inhibitors*

Introduction

Cancer is one of the world's leading causes of illness and death. As a result, the past two decades of biomedical research have yielded vast quantities of knowledge about the molecular events that occur during carcinogenesis and the signalling pathways that participate in the development of cancer. A key role in this process is the molecular mechanisms of the complex interplay between the tumour cells and the tumour microenvironment[1].

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Matrix metalloproteinases consist of a multi-gene family of zinc-dependent extracellular matrix (ECM) remodelling endopeptidases involved in pathological processes such as carcinogenesis, physiological processes like wound healing, uterine involution, organogenesis, and pathological conditions such as inflammatory, vascular, and autoimmune disorders. Here, their activity plays a pivotal role in tumour growth and multi-stage invasion and metastasis processes, including proteolytic ECM degradation, cell-cell, and cell-ECM interactions, migration, and angiogenesis[2]. Matrix metalloproteinases are considered a possible biomarker of diagnosis and prognosis in many cancer forms and stages. An increasing number of matrix metalloproteinase inhibitors (MMPs) were subsequently developed and evaluated in various clinical trials[3].

While several proteases are involved in ECM degradation, it is known that a group of metalloproteinases called matrix metalloproteinases or matrixes play a major role. Diseases like arthritis, atherosclerosis, fibrosis, and cancer include ECM turnover associated with uncontrolled matrix activities.

Particular attention has been drawn to involving matrix metalloproteinase in cancer metastasis, as it increases the prospect of developing matrix metalloproteinase inhibitors as a new type of cancer treatment. Recent findings suggest that matrix metalloproteinase are also involved in vascularization and initial growth of tumours, in addition to involvement in metastasis. In this review, we discuss the current understanding of the role of matrix metalloproteinase in cancer metastasis and tumour progression[4]. The number of articles used in the present study was the articles that were collected from various search engines. The articles relevant to matrix metalloproteinase and its role in cancers were collected from the period of 2000 to 2020 (to date). It is a five-step process in the selection of articles – identification of clear objectives, identification of relevant articles, selection of data, data extraction, analysis and report.

MATRIX METALLOPROTEINASES

Types of matrix metalloproteinase :

They are classified into collagenases, gelatinases, stromelysins, and matrilysins based on the specificity of matrix metalloproteinase for ECM components. Of the eight distinct matrix metalloproteinase structural classes: five are secreted, and three are matrix metalloproteinase (MT-Matrix metalloproteinase) of the membrane type[5]. MT-Matrix metalloproteinases are bound to the cell membrane by covalent bonds, the most obvious way to tether matrix metalloproteinase activity to the cell membrane. Another way of locating the cell surface is by binding the integrins in the form of secreted matrix metalloproteinase or CD44 or by interacting with cell-surface associated heparan sulfate proteoglycans, collagen type IV or extracellular metalloproteinase inducer (Matrix metalloproteinaseRIN)[6]

Role of Matrix metalloproteinases:

Matrix metalloproteinase-1 (Interstitial collagenase):

Matrix Metalloproteinase 1 (MMP-1) degrades collagen type I, a major constituent of bone ECM Matrix metalloproteinase-1 was significantly down-regulated while TIMP-1 levels were increased in a smooth muscle cell (SMC) mechanical strain model, in a time- and pressure-dependent manner. The matrix metalloproteinase-1 expresses fibroblasts, keratinocytes, endothelial cells, monocytes, and macrophages. In addition, a bet-expression screen set up to identify the molecules needed for motoneuron development also led to matrix metalloproteinase1 being isolated. In primary culture, matrix metalloproteinase-1 encoding mRNA was expressed at significantly higher levels in Human OS cells than normal human bone cells.

Matrix metalloproteinase-2 (Gelatinase-A, 72 kDa gelatinase):

Whole-mount RNA hybridization in situ characterized matrix metalloproteinase2 ‘s pattern of expression. Matrix metalloproteinase2 expression takes place widely in the embryonic CNS which contrasts with matrix metalloproteinase 1 Matrix metalloproteinase-2 expression and beta-catenin loss have a part to play in ESC pathogenesis and progression. It has recently been shown that the expression of the enzymes in vitro and the size of the C6-glioma in vivo in the animal model was reduced by DNAzyme produced against matrix metalloproteinase-2 mRNA. Decreased E-cadherin plays a significant role in the development of both the ESC and EEC[7]

Matrix metalloproteinase-3 (Stromelysin 1) :

The size of IGFBP-3 degradation products produced by matrix metalloproteinase-3 is identical to that produced by serum during pregnancy. Subgroup E stromelysin contains stromelysin-1 (MMP-3). In recent research on atherosclerotic plaque stability, a series of apoE / matrix metalloproteinase double knockout mice were used to show that matrix metalloproteinase-3 restricts plaque growth and improves plaque stability, thus playing a protective role[8].

Matrix metalloproteinase-7 (Matrilysin, PUMP 1):

Matrix metalloproteinase-7 (MMP-7), the matrix-degrading enzyme, plays a major role in cancer invasion and metastasis. Studies have shown that antisense oligonucleotides to matrix metalloproteinase-7 inhibit a higher rate of spread of H-infected gastric gland cells. Cultures PyloriMatrix metalloproteinase-7 mRNA was expressed in 53 percent of primary stomach cancers but not in the normal stomach mucosa, fibroblasts, or mesothelial cells. Induction of matrix metalloproteinase-7

occurs during epithelial cell response to bacterial infection[9]

Matrix metalloproteinase-8 (Neutrophil collagenase):

In most mammals, the connective tissue contains neutrophil collagenase, a collagen cleaving enzyme[10]. It is often referred to as MMP-8 or MNL-CL[11]. This has an exclusive pattern of expression in inflammatory conditions and is thus unique in the metalloproteinase matrix family MMPMatrix metalloproteinase-8 mRNA and protein have been expressed in all 3 forms of human in situ atheroma cells[12].

Matrix metalloproteinase-9 (Gelatinase -B, 92 kDa gelatinase):

It is also known as collagenase IV type 92 kDa, gelatinase B (GELB) 92 kDa matrix metalloproteinase-9 releases skit and this allows BM repopulating cells to be translocated to a permissive vascular niche that facilitates the differentiation and reconstitution of the stem/progenitor cell stream[13].

Stromelysin 2 (MMP10):

For humans, the matrix metalloproteinase10 gene encodes the Stromelysin-2 enzyme, also known as the matrix metalloproteinase-10 or transin-2 matrix[14].

Matrix metalloproteinase-10 (Stromelysin 2):

For humans, the matrix metalloproteinase10 gene encodes the Stromelysin-2 enzyme, also known as the matrix metalloproteinase-10 or transin-2 matrix[15].

Stromelysin-3 (MMP11):

The gene matrix metalloproteinase11 encodes Stromelysin-3 (SL-3) or (MMP-11) in humans[16]. The role of Matrix metalloproteinase-11 in neointima formation was tested in wild-type (MMP-11+/+) and matrix metalloproteinase-11–deficient (MMP-11−/−) mice using a vascular injury model matrix metalloproteinase-11 overexpression has possibly been linked with ovarian carcinoma aggressiveness[17].

Matrix metalloproteinase-11 (Stromelysin-3) :

The gene matrix metalloproteinase11 encodes Stromelysin-3 (SL-3) or (MMP-11) in humans. The role of Matrix metalloproteinase-11 in neointima formation was tested in wild-type (MMP-11+/+) and matrix metalloproteinase-11–deficient (MMP-11−/−) mice using a vascular injury modelMatrix metalloproteinase-11 overexpression has possibly been linked with ovarian carcinoma aggressiveness[18].

Matrix metalloproteinase-12 (Macrophage metalloelastase):

Animal models conducted research into the role of matrix metalloproteinase-12 in the development of COPD in humans smokers and suggested a predominant role for matrix metalloproteinase-9 and matrix metalloproteinase-12 in pulmonary inflammation pathogenesis[19].

Matrix metalloproteinase-13 (Collagenase 3):

The human gene matrix metalloproteinase13 encodes the enzyme Collagenase 3. Expressed into human OA and RA by chondrocytes and synovial cells, matrix metalloproteinase-13 is thought to play a crucial role in cartilage degradation. It has been documented that the degradation of the connective tissue growth factor in wound tissue was prevented transiently in matrix metalloproteinase-13 KO miceMatrix metalloproteinase-13 remains the primary matrix metalloproteinase released by chondrocytes, when stimulated with retinoic acid, to degrade their matrix[20].

Matrix metalloproteinase-14 (MT1-matrix metalloproteinase) :

Membrane type 1-matrix metalloproteinase (MT1-matrix metalloproteinase or matrix metalloproteinase-14) is a major pro-matrix metalloproteinase-2 activator and is essential for the development of the skeleton. Cleavage of membrane-bound native MT1-matrix metalloproteinase with several recombinant matrix metalloproteinases including both active MT1-matrix metalloproteinase and matrix metalloproteinase-2 is generated in vitro[21].

Matrix metalloproteinase-15 (MT2-matrix metalloproteinase) :

Ueno et al. identified a correlation between the positive nodal status and the 15 mRNA expression. On E10 hypocellular ECs. Mice with targeted snail

knockdown were exhibited and associated with decreased expression of mesenchyme cell markers and downregulation of the family member of the matrix metalloproteinase MMP, matrix metalloproteinase15[22].

Matrix metalloproteinase-16 (MT3-matrix metalloproteinase):

This is the new name for MT3-matrix metalloproteinase [Membrane-type matrix metalloproteinase-3], according to a numerical nomenclature for the matrix metalloproteinases. MT3-matrix metalloproteinase expression in human cartilage is elevated in end-stage osteoarthritis. Under pathological conditions, PDGF and fibronectin can upregulate the expression of matrix metalloproteinase-16 through cultured vascular smooth muscle cells[23].

Matrix metalloproteinase-17 (MT4-matrix metalloproteinase) :

The matrix metalloproteinase-17 (MT4-matrix metalloproteinase) is a member of the subfamily MT-matrix metalloproteinase. Eyes are anchored to the plasma membrane via a glycosyl-phosphatidylinositol (GPI) anchor that provides these enzymes with a specific collection of regulatory and functional mechanisms separating them from the rest of the matrix metalloproteinase family[24].

Matrix metalloproteinase-18 (Collagenase 4, xcol4, Xenopus),:

Matrix metalloproteinase-18 is expressed in migratory macrophages, and bands for matrix metalloproteinase-18 corresponding to mRNA are found in CB tissue[25].

Matrix metalloproteinase-19 (RASI-1, occasionally referred to as stromelysin-4) :

Matrix metalloproteinase-19 was revealed in laser capture microscopy as a novel mediator followed by microarray analysis in the hyperplastic epithelial cells adjacent to biotic regions. This has functions in cell proliferation, migration, angiogenesis, and adhesion, expressed in human epidermis and endothelial cells. For this gene, Yu et al. , 2012, identified several transcript variants that encode different isoforms.[26]

Enamelysin (MMP20):

An amelogenesis imperfecta was associated with a matrix metalloproteinase-20 mutation which alters the normal splice pattern and results in premature ending of the encoded protein.[27]

Matrix metalloproteinase-21 (X-matrix metalloproteinase):

Matrix metalloproteinase-21 increases the ability of certain solid tumours to enter and metastasize tumour matrix metalloproteinase-21 expression was examined on immunohistochemistry assay in 296 cases of gastric cancer.[28]

Matrix metalloproteinase-23A (MMP-CA):

Matrix metalloproteinase 23a does not have the signal chain, as opposed to other matrix metalloproteinases. It is suggested it could function intracellularlyMatrix metalloproteinase-23 has a short prodomain and contains a single cysteine residue that may be part of the cysteine-switch process working to preserve the latency of the enzymes.[29]

Matrix metalloproteinase-24 (MT5-matrix metalloproteinase):

TIMPs inhibit all matrix metalloproteinase, except matrix metalloproteinase-24, matrix metalloproteinase25 (MT6-matrix metalloproteinase). Membrane-type matrix metalloproteinase (MMP -25, also known as MT1-, MT2-, MT3-, MT4-, MT5-, and MT6-matrix metalloproteinase) are structurally similar to other matrix metalloproteinase types but are anchored to the cell membrane ‘s exterior. It is highly expressed in leukocytes and some tissues of cancer.[30]

Matrix metalloproteinase-26 (Matrilysin-2, endometase):

Matrix metalloproteinase-26 has 998 mRNA nucleotides and no type of transcript. RT-PCR, the study of immunofluorescence, and cytometry determined the matrix metalloproteinase-26 mRNA and protein expression by. This is the minor part of the metalloproteinase matrix. E encoded protein degrades type IV collagen, fibronectin, fibrinogen, casein, vitronectin, alpha-1-antitrypsin, alpha-2-macroglobulin, and insulin-like growth factor-binding protein 1, and activates matrix metalloproteinase9 through cleavage [31].

Matrix metalloproteinase-27 (MMP-22, C):

The matrix metalloproteinase-27 mRNAs are usually expressed at a lower level[32].

Matrix metalloproteinase-28 (Epilysin):

Matrix metalloproteinase-28 (MMP-28, epilysine) is highly expressed in the skin by keratinocytes, the nervous system developing and regenerating, and a variety of other normal human tissues. Matrix metalloproteinase-28 expression is associated with cell proliferation during epithelial repair and is spatially and temporarily tightly regulated during wound repair matrix metalloproteinase-28 expression is upregulated in primary keratinocytes by TNF-alpha therapy [33].

Role of matrix metalloproteinase in Cancer Progression :

Tumour cells participate in many tumour microenvironmental interactions involving extracellular matrix (ECM), ECM-associated growth factors, and cytokines, as well as surrounding cells (endothelial cells, fibroblasts, macrophages, mast cells, neutrophils, pericytes, and adipocytes) during carcinogenesis formation. The local microenvironment relies on four cancer hallmarks that include migration, invasion, metastasis, and angiogenesis [34].

Matrix metalloproteinase is an important molecule in these processes as they degrade specific molecules of cell adhesion and thus modulate interactions between cells and cells and ECM. The development of cancer includes multiple steps, including tumour growth and multi-step invasion, metastasis, and angiogenesis processes, all of which can be modulated by a matrix metalloproteinase. The tumour microenvironment expression of matrix metalloproteinase depends not only on the cancer cells but also on the neighbouring stromal cells [35]Matrix metalloproteinase exercise their proteolytic activity and break down the physical barriers, promoting angiogenesis, invasion of tumour cells, and metastasis. Tumour growth and angiogenesis often rely on the increased availability of signalling molecules, such as growth factors and cytokines, by making those factors more available to cancer cells and the tumour microenvironment. This occurs either by extracting them from the ECM (IGF, bFGF, and VEGF) or by shedding them out of the cell surface (EGF, TGF-α, HB-EGF) [36].

Angiogenesis is also strongly modulated by the release of angiogenesis negative regulators, such as angiostatin, tumstatin, endostatin, and endorepellinMatrix metalloproteinase also modulate cell-cell and cell – ECM interactions by manipulating E-cadherin and integrins, influencing both cell phenotype (EMT) and cell migration, respectively. The over-expression of matrix metalloproteinase in the tumour microenvironment depends not only on the cancer cells but also on the neighbouring stromal cells that are induced in a paracrine manner by the cancer cells [37]. Cancer cells stimulate host cells such as fibroblasts to form an essential source of matrix metalloproteinase through interleukin secretion and growth factors, and direct signalling by extracellular matrix metalloproteinase inducers. Therefore the cellular source of matrix metalloproteinase may have important effects on its role and operation. In this regard, for example, neutrophils express matrix metalloproteinase-9 free of TIMP-1, which leads to more readily activating the proteinase.[38]

Specifically, they may promote or inhibit the development of cancer depending on the tumour stage, tumour site (primary, metastasis), enzyme localization (tumour cells, stroma), and substrate profile among other factors. For example, matrix metalloproteinase-8 provides a protective effect in the metastatic cycle, decreasing the metastatic potential of breast cancer cells when overexpressed. Similarly, the expression of matrix metalloproteinase-8 in tongue squamous cell carcinoma is associated with improved patient survival, and it is proposed that this protective action is possibly associated with the role of estrogen in tongue sq growth.[39]On the other hand, in some particular circumstances, matrix metalloproteinase-9 may act as a tumour promoter in the carcinogenesis cycle, as well as an anticancer enzyme at later stages of the disease. This dual function is based on the results in animal models, where matrix metalloproteinase-9 knockdown mouse models showed a reduced incidence of carcinogenesis whereas tumours developed in matrix metalloproteinase-9 mice were substantially more aggressive.

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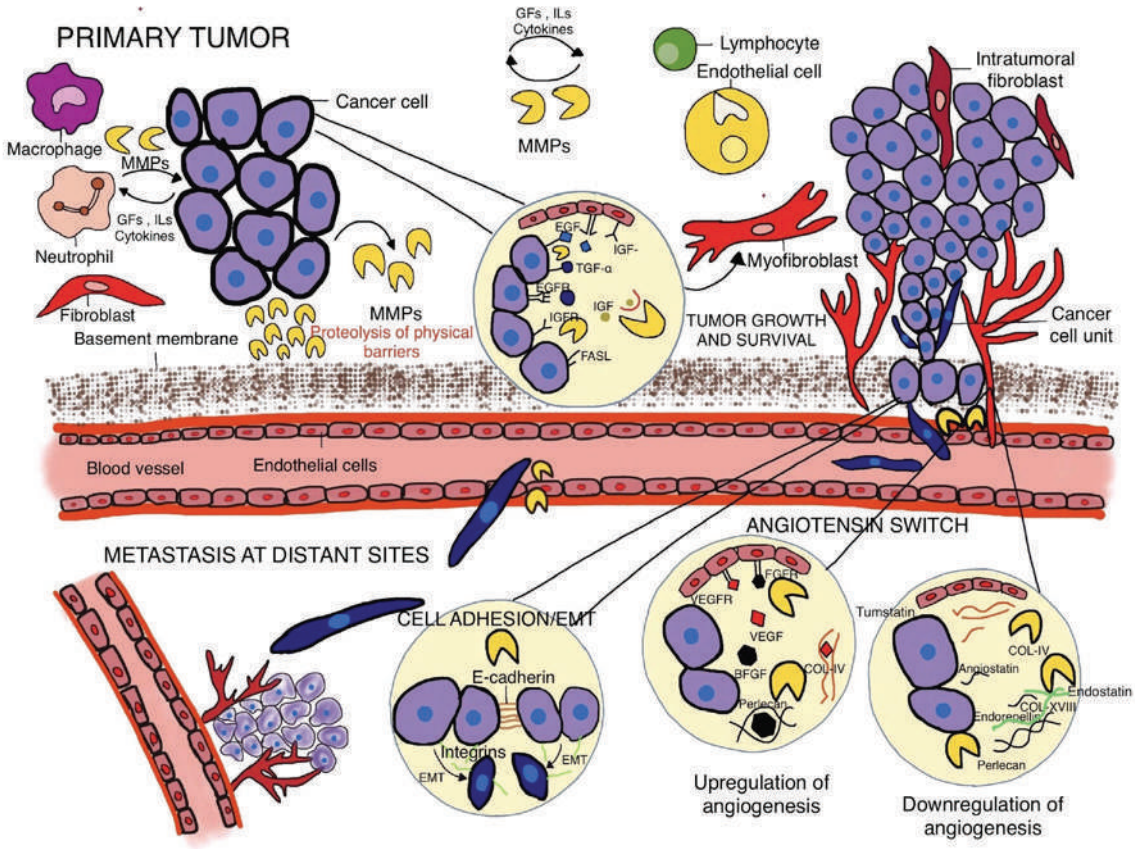


FIGURE 1: Role of matrix metalloproteinase in cancer progression.

Matrix metalloproteinases and Cancer Invasion :

The ECM is a dynamic structure that orchestrates the cells' actions through interaction with them. A cancer cell requires the proteolytic activity of matrix metalloproteinase to degrade physical barriers during local expansion and intravasation in nearby blood vessels, extravasation, and remote invasion. Localization of matrix metalloproteinase to specific cell surface structures, called invadopodia, is necessary during invasion for their ability to promote invasion. Such structures reflect the place where there is a significant degradation of the ECM. Invadopodia uses proteinases related to transmembrane invadopodia, like matrix metalloproteinase-14 (figure 2) [41].

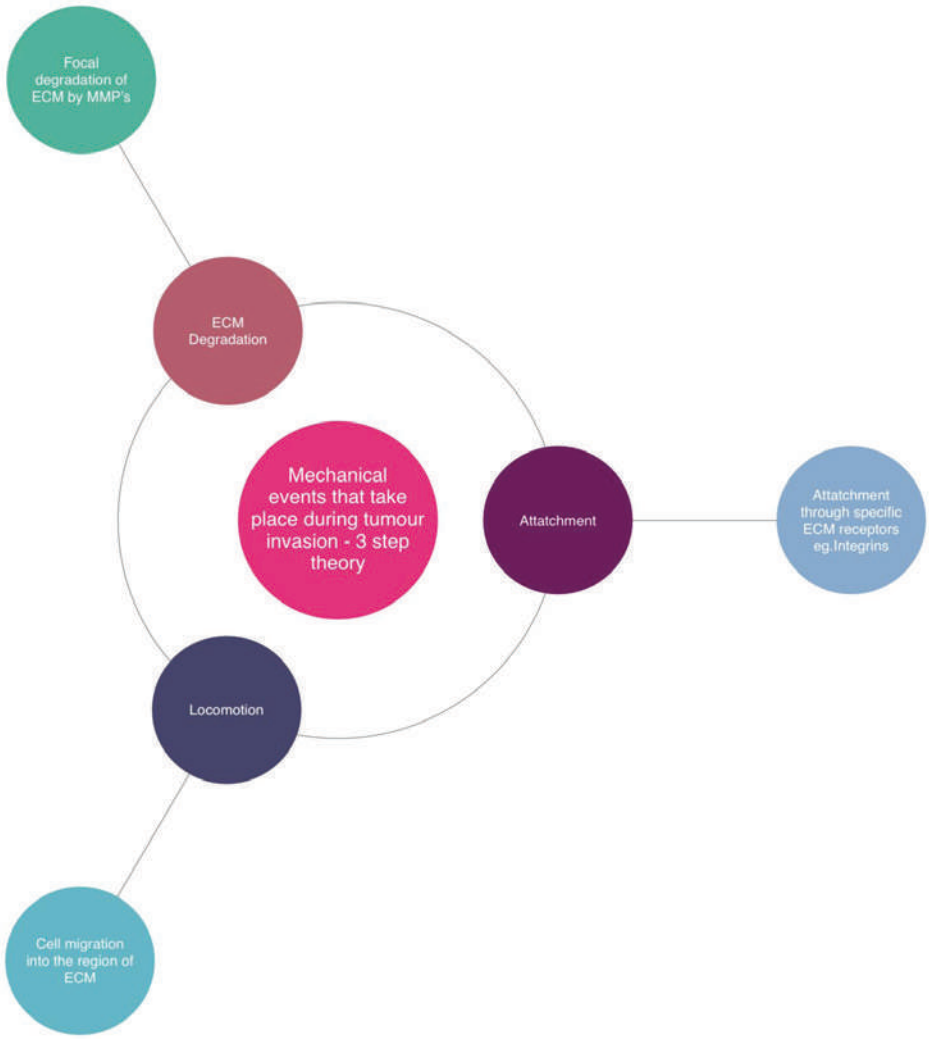


FIGURE 2: Role of matrix metalloproteinase and ECM in tumour invasion.

Matrix metalloproteinases and Tumour Angiogenesis :

Matrix metalloproteinase has a dual role in tumour vasculature as they can function as both positive and negative angiogenesis regulators depending on the time point of expression during tumour angiogenesis and vasculogenesis as well as the availability of substrates. The matrix metalloproteinase family's main players involved in tumour angiogenesis are mainly matrix metalloproteinase-2, -9, and matrix metalloproteinase-14 and, to a lesser degree, matrix metalloproteinase-1 and -7. For cancer cells to keep growing and begin migrating, new blood vessels need to be created [42]. The first step in this cycle is to remove the physical obstacles by degrading the ECM and produce pro-angiogenic factors thereafter. Indeed, matrix metalloproteinase-9 participates in the angiogenic transition as it increases the bioavailability of essential factors in this cycle, such as the most effective tumour vascular endothelial growth factor (VEGF) and the specific fibroblast growth factor (bFGF), by degrading extracellular components such as collagen type IV, XVIII and perlecan, respectively [43].

matrix metalloproteinase tightly controls the angiogenic balance, but they can also down-regulate the formation of blood vessels by producing degradation fragments that inhibit angiogenesis. These molecules include tumstatin, endostatin, angiostatin, and endorepellin, which are produced by type IV, XVII collagen, plasminogen, a serine proteinase plasmin inactive precursor, and perlecan cleavage (figure 3) [44].

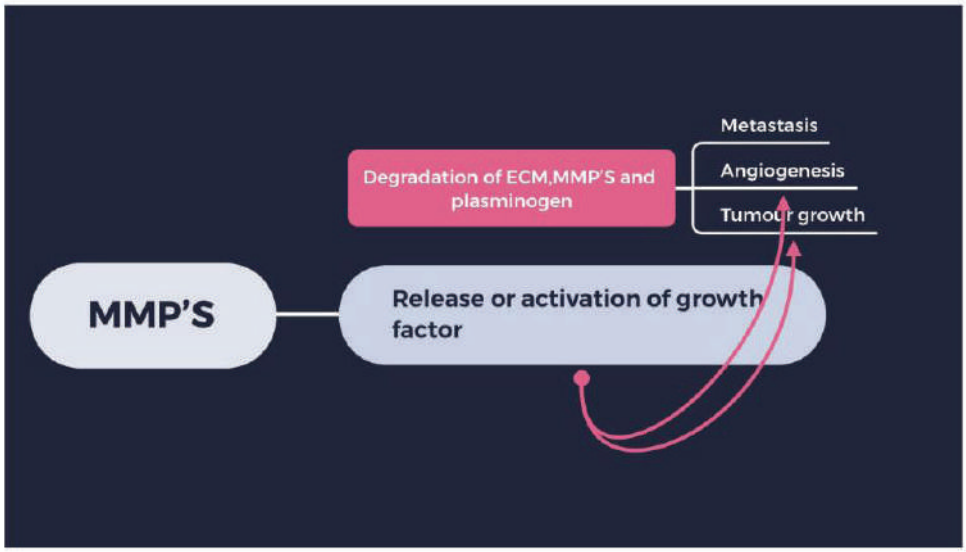


FIGURE 3: Role of matrix metalloproteinase In tumour angiogenesis.

Matrix metalloproteinase and Cancer Proliferation:

matrix metalloproteinase contributes to tumour cell proliferation by many pathways. In particular, they can modulate growth factor bioavailability and cell-surface receptor function. Even the ADAM family is interested in the above operation. Members of the matrix metalloproteinase and ADAM families that release cell-membrane-precursors of multiple growth factors such as insulin-like growth factors (IGFs) and epidermal growth factor receptor (EGFR) ligands promoting proliferation; Several matrix metalloproteinases (MMP-1,-2,-3,-7,-9,-11 and-19) and ADAM12 break down IGF-binding proteins which regulate the growth factor 's bioavailability [45]. Cell proliferation mediator EGFR is implicated in cancer development as it is over-expressed in over one-third of all solid tumours. With the action of matrix metalloproteinase-3, -7, ADAM17 or ADAM10, increased shedding of EGFR membrane-anchored ligands, including heparin-binding EGF (HB-EGF), transforming growth factor (TGF)-α, and amphiregulin was observed during cancer progression[46]Matrix metalloproteinase and ADAM also regulates proliferation signals through integrins since the shedding of E-cadherin results in translocation of β-catenin to the nucleus, leading to cell proliferation. It is worth noting that the inactive proform of TGF-β, an important molecule in cancer, is proteolytically activated by matrix metalloproteinase-9, -2, -14 in a similar way [47].

One of the main findings that emerged from several studies is the crucial role of glycosaminoglycan (GAG)-matrix metalloproteinase-GF interactions, leading to the activation of pro matrix metalloproteinase and their subsequent proliferative effects. Notably, GAGs chains may recruit matrix metalloproteinase to release growth factors from the cell surface and, as a result, induce cancer cell proliferation matrix metalloproteinase-7, for example, exerts a high affinity for sulfate chains in heparan. Based on this notion, heparan sulfate chains on cell surface receptors, such as some CD44 variant isoforms, anchor the proteolytically active matrix metalloproteinase-7, resulting in the HB-EGF cleavage[48].

Matrix metalloproteinase and Cancer Cell Apoptosis:

Degrading matrix enzymes impart both apoptotic and antiapoptotic action matrix metalloproteinase and ADAMs, especially matrix metalloproteinase-7 and ADAM10, relay anti-apoptotic signals from the cell surface to cancer cells by cleaving Fas ligand, a death receptor Fas transmembrane stimulator. This proteolytic activity inactivates the Fas receptor and causes apoptosis resistance and chemical resistance to cancer cells or encourages apoptosis of neighbouring cells depending on the method. In addition, proteolytic shedding of tumour-

associated major histocompatibility proteins with ADAM17-related complex class-I proteins will inhibit natural killer (NK) cytotoxicity to cancer cells[49]Matrix metalloproteinase can, in particular, contribute to the anti-apoptotic effect by indirectly activating serine/ threonine kinase Akt / protein kinase B via the EGFR and IGFR signalling cascadesMatrix metalloproteinase also facilitates apoptosis, most likely indirectly by altering the composition of the ECM; for example, by splitting laminin, which affects the signalling of the integrin [50].

Matrix metalloproteinase and Cell Adhesion, Migration, Epithelial to Mesenchymal Transition :

Cell movement is strongly correlated with matrix metalloproteinase and ADAMs proteolytic activity, regulating the complex ECM – cell and cell-cell interactions during migration. Initially, mysterious peptide production through the degradation of ECM molecules such as collagen type IV and laminin-5 promotes cancer cell migration. In controlling cell migration, some integrins play an important role as they can serve as substrates for matrix metalloproteinase [51].

Several matrix metalloproteinases (MMP-2,-3,-9,-13,-14) has been over-expressed in association with epithelial-mesenchymal transition (EMT), a highly conserved and fundamental morphological transition phase. In particular, epithelial cells are actively down-regulating cell-cell adhesion systems during this event, losing their polarity and acquiring a mesenchymal phenotype with reduced intercellular interactions and increased migratory ability. Contact between the cells is hindered by ADAM10 shedding of E-cadherin, resulting in disrupted cell adhesion and EMT induction, accompanied by increased cell migration. By cleaving E-cadherin, matrix metalloproteinase-1 and -7 also appear to contribute to this morphological transition[52]. Recent studies indicate the implication of matrix metalloproteinase-28 in the proteolytic activation of TGF-β, a strong inducer of EMT, leading to EMT.

It should be remembered that the interaction between hyaluronan and its main cell surface receptor, CD44, contributes to the activation of signalling molecules such as Ras, Rho, PI-3 kinases and AKT, thereby facilitating the progression of cancer[53,54]. A recent study indicated that hyaluronan promotes cancer cell migration and increased metalloproteinase matrix secretion through Rho kinase-mediated signalling, specifically the increased active form of matrix metalloproteinase-2 [55].

Matrix metalloproteinase and Immune Surveillance :

By recruiting tumour-specific T-lymphocytes, NK cells, neutrophils, and macrophages, the host immune system is able to recognize and attack cancer cells. Cancer cells, by contrast, evolve mechanisms of escape using matrix metalloproteinase to gain immunity [56]. Matrix metalloproteinase shed T-lymphocyte interleukin-2 receptors-α to inhibit their proliferation. In addition, TGF-β is released as a result of matrix metalloproteinase activity, a major suppressor of T-lymphocyte reaction against cancer cells. Likewise, matrix metalloproteinase reduces the vulnerability of cancer cells to NK cells by producing a bioactive fragment from an inhibitor of 1-proteinase[57]. A number of studies have also demonstrated the ability of matrix metalloproteinase to effectively cleave or control the mobilization of many members of the CC (β-chemokine) and CXC (α-chemokine) chemokine subfamilies, affecting leukocyte infiltration and migration [58]

Pharmacological Targeting of matrix metalloproteinase :

Hence, matrix metalloproteinase is suitable for therapeutic intervention by synthetic and natural inhibitors, giving prospects for future studies. Multiple therapeutic agents, called inhibitors of matrix metalloproteinase (MMPs), were developed to target matrix metalloproteinase in an attempt to control their enzymatic activity. Even though clinical trials with these compounds in most cases do not produce the anticipated effects, the field of matrix metalloproteinases is still in development.

TABLE 1: Potential matrix metalloproteinase inhibitors

Matrix metalloproteinases	Type of Drug/source	Enzymes Inhibited
Synthetic inhibitors		
Batimastat	Peptidomimetic	matrix metalloproteinase-1,-2,-3,-7,-9
Marimastat	Peptidomimetic	Broad Spectrum
Tanomastat(BAY12-9666)	Non Peptidomimetic	matrix metalloproteinase-2,-3,-9
Prinomastat(AG3340)	Non Peptidomimetic	matrix metalloproteinase-2,-3,-7,-9,-13
BMS-275291	Non Peptidomimetic	matrix metalloproteinase-2,-9
CGS27023A	Non Peptidomimetic	matrix metalloproteinase-1,-2,-3
Minocycline	Chemically modified tetracycline	matrix metalloproteinase-1,-2,-3
Metastat(COL-3)	Chemically modified tetracycline	matrix metalloproteinase-1,-2,-8,-9,-13
SB-3CT	Reform proenzyme structure	matrix metalloproteinase-2,-9
INCB7839	Small molecule sheddase inhibitor	ADAM-10,-17
Off Target inhibitors		
Bisphosphonates	Analogue of PPi	matrix metalloproteinase-1,-2,-7,9,MT1,MT2matrix metalloproteinase
Letrozole	Nonsteroidal inhibitors of aromatase	matrix metalloproteinase-2,-9
Neovastat(AE-941)	Extract from shark cartilage	matrix metalloproteinase-1,-2,-7,-9,-13
Genistein	Soy isoflavone	matrix metalloproteinase-2,-9,MT1-,MT2-,MT3-matrix metalloproteinase

Matrix metalloproteinases Inhibition and Anticancer Therapy :

Matrix metalloproteinase synthesis is inhibited by multiple agents that prevent them from interacting with molecules that direct their action to the cell surface or inhibit their enzymatic activity [59].

Inhibition of matrix metalloproteinases synthesis :

Matrix metalloproteinase synthesis is inhibited directly by transfecting mRNA or oligonucleotide antisense cells or by attacking mRNA with RIBOZYMES. This means it has been used in mouse models to downregulate matrix metalloproteinase7 or 9 to reduce the stress or metastasis of tumours. Indirect strategies for reducing matrix metalloproteinase expression are signal transduction pathways inhibition that induces matrix metalloproteinase transcription. Several medicines in clinical trials inhibit signals of tyrosine kinase receptors and levels of matrix metalloproteinase. Halofuginone, a COCCIDIOSTAT used in poultry, is a drug that controls the expression of the matrix metalloproteinase genes and the experimental metastasis of cancer cells. Coccidiostat is a drug used to treat coccidiosis, which is a protozoan-induced intestinal disease[60].

Inhibiting interactions between matrix metalloproteinase and other proteins:

Matrix metalloproteinase-2 is prevented from binding to αvβ3 integrin so as to inhibit matrix metalloproteinase interaction with other proteins. This form of strategy may be evaluated in clinical practice by directly targeting the cancer-promoting feature, and the compound shows promising results in animal experiments [61]

. Exploiting matrix metalloproteinase activity:

This has developed many cytotoxic agents that are activated by a matrix metalloproteinase. It is effective for treating tumours. Cytotoxic agents, such as recombinant proteins containing ANTHRAX TOXIN fused to a matrix metalloproteinase cleavage site, are activated by cell surface matrix metalloproteinase cleavage and internalized by the cell, followed by cell death[62]

Blocking of matrix metalloproteinase :

Common endogenous inhibitors (TIMPs), comprising a family of four protease inhibitors, inhibit the matrix metalloproteinase:

- TIMP-1
- TIMP-2
- TIMP-3 and
- TIMP-4TIMPs

might have matrix metalloproteinase-independent cancer-promoting activities [63]

Future Perspective :

Perspective research into neoepitopes will provide important and novel means for cancer diagnosis, prognosis, and increased efficacy of treatment. However, in order to take full advantage of neoepitopes as highly useful biomarkers of cancer, understanding the physiological processes and signalling pathways that control their generation is very critical. And the ultimate aim of modern diagnostic studies would be to use highly accurate biomarkers based on non-invasive mechanisms. Currently, receptors, cell adhesion molecules, growth factors, and enzymes with their associated protein substrates (e.g., matrix metalloproteinase and extracellular matrix components) are all hot areas of study in cancer drug production and diagnostic assays [64].

Conclusion

Thus, this review gives a clear knowledge about different types of matrix metalloproteinases and their role in cancer progression and metastasis to analyze the gene mutations in cancer cells in order to prevent and reduce cancer progression in the future.

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Role of Lasers in Composite Restorations

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Abstract

The term LASER is an acronym for 'Light Amplification by the Stimulated Emission of Radiation'. The first laser was introduced in the 1960's by Miaman. Laser is used to treat a number of various dental conditions like composite restorations etc.. It is most commonly used in restorations for treating hypersensitivity, tooth decay, gum diseases and whitening teeth. Various kinds of lasers such as ND:YAG, Er:YAG, Er:Cr:YSGG are used in various dental procedures. Laser seems to be made for effective removal of dentine and enamel with only minor side effects such as thermal damage. The capability of Er:YAG lasers for the removal of hard tissues in dentistry was exhibited as of now in 1989, Er:YAG (erbium-doped yttrium aluminum garnet) lasers have been progressively utilized in dental practices and are turning out to be increasingly more an agreeable technique for caries evacuation for patients, as conventional cavity drilling may cause noise and pain. Laser received FDA approval for caries removal and cavity preparations. The use of laser therapy also seems to be promising from many recent researchers. Laser therapy induces surface roughness or even improves bond strength or decreases marginal leakage. There seems to be a general consensus on the fact that Er:YAG is one of the best suited types of lasers because it's efficiency in dentine is very good without any damage of pulp. Er:YAG lasers are a lot utilized in small scale blasts that may bring visible irregularities. In addition, pain reduction in comparison to bur assisted preparation of cavities has been clearly demonstrated.

Keywords*: Lasers, composite restorations, Er:YAG lasers, compositional changes, Dentistry.*

Introduction

The term LASER is an acronym for 'Light Amplification by the Stimulated Emission of Radiation'. The first laser was introduced in the 1960's[1,2] by Theodore Miaman. Maiman, at the Hughes Research Laboratories in Malibu, CA, built the first functioning laser by using a mixture of helium and neon. In 1961, a laser generated from crystals of yttrium-aluminum-garnet treated with 1-3% neodymium (Nd:YAG) was developed. It is used for nonsurgical sulcular debridement in periodontal disease control and the Laser Assisted New Attachment Procedure (LANAP). In 1962, the argon laser was developed, whereas, the ruby laser became the first medical laser to coagulate retinal lesions, when it was used in 1963. In 1964, Patel at Bell Laboratories developed the CO_2 laser. It has a very high affinity for water, resulting in rapid soft tissue removal and hemostasis with a very shallow depth of penetration. Lasers have been used in dentistry since 1994 to treat a number of dental problems. Yet, despite FDA approval, no laser system has received the American Dental Association's (ADA) Seal of Acceptance as an alternative to more traditional treatment. The use of lasers in dentistry has increased over the past few years. Because of their many advantages, lasers are indicated for a wide variety of procedures [3,4]. It is most commonly used in restorations for treating hypersensitivity

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and tooth decay. Nowadays, diode lasers are being extensively used in the field of dentistry. Diode lasers are used in some kind of procedures like aesthetic gingival recontouring, soft tissue crown lengthening, exposure of soft tissue impacted teeth, removal of inflamed and hypertrophic tissue, frenectomies, and photostimulation of the aphthous and herpetic lesions. Lasers seem to have been made for effective removal of dentine and enamel with only minor side effects such as thermal damage. The potential of Er:YAG (erbium-doped yttrium aluminum garnet) lasers for ablation of hard tissues in dentistry was demonstrated already, Er:YAG lasers have been increasingly used in dental practices and are becoming more and more a comfortable method for caries removal for patients, as conventional cavity drilling may cause noise and pain. The repair and the extension of composite restorations is an everyday task for dentists. However, the existing composite restoration may have been in the oral environment for a long, and often unknown, period of time, and in many cases the dental clinician is unaware of which product has been used to create the restorations [5,6,7]. During repair of a composite restoration, the prepared cavity usually exposes enamel and dentine [8,9,10]. For that reason, the usage of lasers is required to obtain adequate bond strength to the tooth structure. Many studies have been carried out to investigate the usage of lasers bonding between composites/ enamel and composite /dentine and it gives acceptable tensile strength and shear bond strength (SBS) between the composite and the tooth [11,12]. Thus the main aim of this study is to know about the role of lasers and various laser types in composite restoration procedures.

LASERS IN COMPOSITE RESTORATIONS:

At present days, there are numerous lasers which can be used efficiently in restorative dentistry for various procedures as the results yielded by employing lasers are promising. Various kinds of lasers can be employed in composite resin restorations such as Er:YAG, Nd:YAG (neodymium-doped yttrium aluminum garnet) , Diode lasers, CO_2 lasers, Er:Cr:YSGG (erbium/chromium-doped yttrium scandium gallium garnet)[13,14] etc..

Er:YAG Lasers:

Er: YAG lasers are a lot utilized in small scale blasts that may bring about tiny and plainly visible irregularities. Safe utilization of lasers likewise [15,16] be the fundamental objective of proposed or future laser treatment. Laser beam cutting with Er:YAG lasers are suitable for short pulse duration and have low mean beam power, high laser beam intensity. Er:YAG lasers have a tissue applicability used on hard and soft tissues[17]. Hard tissue lasers which were developed during the 1990s have the capability to prepare enamel for restoration and reduces, eliminates the audibility of drills and reduces the micro fractures [18,19]. Low power Er:YAG lasers which have 100 milliwatts could operate the middle opened infrared region with the cape of hard and soft tissues [20–22].

Nd:YAG Laser:

These lasers have a wide range of utilizations in the procedures such as laser spectroscopy and composite restorations. Nd:YAG laser is a four-level laser framework, which implies that the four vitality levels are associated with lasers of composite resin restoration activity. These lasers work in both beat and ceaseless mode. Nd:YAG laser produces laser light usually in the close infrared locale of the range at 1064 nanometers (nm). It likewise emanates laser light at a few unique frequencies including 1440 nm, 1320 nm, 1120 nm, and 940 nm. Nd:YAG laser fluorescence which helps in the detection of dental decay could demonstrate the presence of lesions and calculus [23,24].

Diode Lasers:

The dynamic mode of the diode laser is a strong state semiconductor made of aluminum, gallium, arsenide, and infrequently indium, which produces laser frequencies, running from roughly 810 nm to 980 nm. All diode frequencies are ingested fundamentally by tissue shade (melanin) and hemoglobin. They are inadequately consumed by the hydroxyapatite and water present in the finish. Explicit strategies incorporate tasteful gingival reshaping, delicate tissue crown, frenectomies, and photostimulation of the aphthous and herpetic sores.

Argon Lasers:

Argon lasers are a unique laser used specifically in composite restorations. It was invented by William Bridges at the Hughes Aircraft Labs in 1964. The argon laser doesn't employ the use of filters. Rather, it

produces one frequency of blue light (i.e., the light is monochromatic) having a band width of just 40 to 45 nm. Furthermore, the brilliance of the light can be set to the producer's details for ideal effectiveness, one of a kind for each brand of composite pitch. Thoroughness and the depth in composite restorations are increased using the argon lasers. Argon lasers in composites are used to improve shear bond strength in both enamel and dentine.

MORPHOLOGICAL CHANGES ON TOOTH UPON LASERS:

Er:YAG laser irradiation may cause morphological changes in the enamel causing the formation of rough surfaces, cracks, prone to bacterial accumulation[25]. Laser action with temperature rise causing morphological alterations that are directly related to frequency increment, causing demineralization of sound dentine[26]. Er:YAG lasers have potential treatment of dental decay cause alteration to the conventional drill may reduce mechanical damage and can remove the hard dental tissues. Thus, Er:YAG lasers cause extensive cracking lines in dentine surfaces with a smear layer with sound dentine[27]. It reduces the mechanical damage and removes the hard tissue. Cavity walls and borders disclose typical morphological aspects after ablation Er:YAG laser treatment. The smear layer is efficiently removed. Neither knoop hardness nor Ca/P ratio evaluation on the cavity floor revealed any significant difference between laser and bur treatment. If any, only minimal thermally induced changes of dental hard tissue composition is produced by Er:YAG and only minimal local thermal damage follows Er:YAG irradiations. The surface morphology and the chemistry of dentine may influence the bond strength to dental restoration materials. Enamel acid etching after laser treatment increases the etching depth if evaluated with the help of X-ray tomography.

COMPOSITIONAL CHANGES:

The degree of compositional changes helps in the retention of tissues to diminish or expand its dissolvability lighted to finish or dentine (CO_2, Nd:YAG lasers). Laser illumination can build the CA or P structure which may result from the evaporation of organic compounds. This causes the adjustment in warm reactions Er:Cr:YSGG laser light with CA:P proportion stays unaltered [28,29]. Corrosive carving causes substance changes and expands the dentine penetrability, dentin, wetness and furthermore builds the potential for mash crabbiness [30,31]. Laser application may cause comparative corrosive carving that causes the high unpleasantness without any dental damage in deep underlying tissues.

BOND STRENGTH:

Mean modified shear bond strength for light and laser groups ranges from 10.80 MPa to 13.31 MPa which are recommended for orthodontic treatment and also for both curing modalities[32]. Laser irradiation causes surface modification by increasing its bond strength in resin cements. Er:YAG laser irradiation causes adhesion of resin to dentine that improves bond strength of acid etching and increases tensile strength highly for acid etched human dentine[33]. Thus, irradiation by the Er:YAG laser at 450 mJ has bond strength ($p<0.05$) level. Many authors found similar tensile bonds to acid etching samples. If Er:YAG treatment was combined with acid etching, higher bond strength was found than with laser treatment alone. If the tensile bond strength on dentine was not adversely affected by different water flow cooling rates, it was of importance to optimise the water flow on enamel to prevent the formation of non appetite CaP phases on the enamel surface, which may compromise adhesion. For composites repair, Er:YAG lasers as the conditioning method showed a significant improvement in tensile bond strength in comparison to classical methods such as air abrasion, salinization, hydrofluoric acid and their combination, reaching mean values of 22.92MPa [34].

ENERGY SOURCES:

Energy sources for the lasers for etching the tooth surfaces derived from blue coloured halogen curing lamps. Infrared CO_2 lasers, blue coloured plasma arc lamps, cool blue argon lasers used and 980nm GAA1As (Gallium-Aluminum-Arsenide) lasers are used which are capable with different energy sources [35,36]. Enamel and dentine surfaces can be etched utilizing Er:YAG lasers with 2.94 miniaturized scale frequency for 250 small scale beat terms which can be achieved with 300 mJ for enamel and 250 mJ for root surfaces[37,38]. Camphoroquinione initiation with blue light of frequency running of 400 to 500 na have an expansive pinnacle of 480 nm. Argon lasers improve the tear bond quality for the lacquer and dentine [39,40,41].

ADVANTAGES:

Dental lasers which emit energy convey end cutting by methods altered for specialists' clinical technique. Erbium lasers can expel unhealthy tooth structure,carious sores with higher water content cause solid tissues may cause an end of microfracture. It brings down pulpal temperature and causes quicker recovery.

CONTRAINDICATIONS:

Lasers can't be used for teeth already filled and also for performing dental procedures involving removal of old fillings. In tooth preparations, the Erbium family of lasers are unable to remove gold and vitreous porcelain and have no interaction with the restorative material and also in most composite restoration.

Conclusion

There seems to be a general consensus on the fact that Er:YAG is one of the best suited laser types for cavity preparation because it's efficiency, especially in dentine, is very good without any danger of pulpal damage if working under sufficient water cooling. In addition, pain reduction in comparison to bur assisted preparation has clearly been demonstrated making it possible to work without local anaesthesia in most instances. Er:YAG is also efficient in removing composite restoration, however, little is known on its ability to ablate dental ceramics, GIC and gold. Ablation of amalgam should be avoided because it is not efficient and because it leads to mercury evaporation. Thus, the role of lasers in composite restorations adds to major use in dental procedures.

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Use of Urinary Reagent Strips in Testing Cerebrospinal Fluid For Meningitis - A Review

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Abstract

Analysis of cerebrospinal fluid (CSF) is needed to make a meningitis diagnosis to assist in the proper management of patients. There is a good correlation between the results of reagent strip test and laboratory methods of CSF protein, sugar, and leukocyte estimation. CSF supplies nutrients to tissue in the nervous system. CSF eliminates waste products from cerebral metabolism. Cerebrospinal fluid has three main functions: CSF protects against trauma on the brain and spinal cord. Meningitis is a rare infection that affects the delicate membranes called meninges, which cover the brain and spinal cord. Index test was done for testing meningitis using urinary reagent strips. An intrusive outpatient procedure used to remove a sample of cerebrospinal fluid (CSF) from the spine's subarachnoid area is a lumbar puncture (LP), also called a spinal tap. A urinary test strip or dipstick is a simple medical tool used in normal urinalysis to assess abnormal differences in a patient's urine. There is a good correlation between the results of reagent strip test and laboratory methods of CSF protein, sugar, and leukocyte estimation. The review gives a clear view and knowledge about the usage of urinary reagent strips in measuring CSF for meningitis.

Keywords: *CSF; meningitis; urinary reagent strips; index test; definitive test; leukocyte.*

Introduction

Analysis of cerebrospinal fluid (CSF) is needed to render a diagnosis of meningitis to aid in the proper treatment of patients. There is a strong connection between the results of reagent strip test and laboratory methods of CSF protein, sugar and leukocyte estimation[1]. The estimation of CSF cell count is required while the estimation of sugar and protein levels requires appropriate laboratory help.Such services are often not accessible online[2].

Resource-limited environments, and the turnaround times are long even in environments where available. There are currently no prompt point-of -care tests available for meningitis diagnosis[3]. Using urinary reagent strips can be calculated the presence of glucose, fat, leukocytes, erythrocytes and pH in a body fluid[4]. In the past, CSF cellularity and chemistry were calculated using reagent strips, but the results were variable and the process did not gain popularity. Such strips can be an ideal test if proved effective to help clinicians make a simple bedside diagnosis of meningitis and start treatment[5]. This will greatly help health care workers who work in resource-limited environments[6].

We planned this analysis to evaluate whether urinary reagent strips are used to make a semi-quantitative assessment of Fat, glucose, and leukocyte esterase activity (as contrasted with CSF glucose, fat, and cell count) are sufficient to differentiate between normal and abnormal CSF samples[7]. This review has been done by

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a thorough literature search. Diagrams were made using brushes redux application.

URINARY MULTI REAGENT STRIPS IN CEREBROSPINAL FLUID ANALYSIS

Automatic urine test strip analysis using automated urine test strip analyzers is a well-established procedure in urinalysis of modern times[8]. Calcium, plasma, glucose, bilirubin, urobilinogen, ketones, leukocytes, creatinine, microalbumin, pH, ascorbic acid, and protein can be assessed[9].

Simply put one drop of your urine sample on a single chemical dipstick pad and wait for the time needed to measure the sample against the chart[10]. So proceed to the next strip check, and so forth[11]. Only be sure that the eyedropper, and any container used for urine collection, is sterile[12].

Using a collection jar to collect urine mid-stream, Then dip the strip into the sample for no more than 2 seconds and extract any excess by wiping the bottle side test strip and later write the results after 60 seconds (write after 90-120 seconds for checking for Leukocytes)[13]. Urine Reagent Strips (Figure 1) are strong plastic strips onto which are attached several different reagent area[14]. The test is designed to detect one or more of the following urine analytes: Leukocytes, Glucose, Ketone (Acetoacetic acid), Bilirubin, Blood, Specific Gravity, Protein, Urobilinogen, Nitrite, Ascorbic Acid, and Multiple Reactive Strips[15].

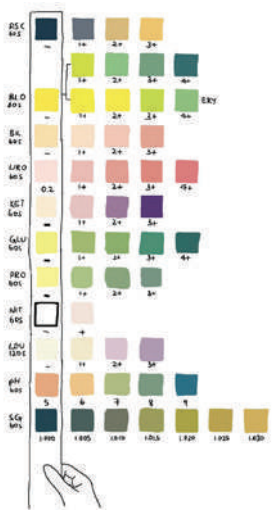


Figure 1: A model diagram of Urinary Reagent Strip. The diagram was made using brushes redux application.

Uses Of Urinary Reagent Strips:

Diagnostic reagent strips are widely used in clinical urine and blood analysis, particularly for monitoring the concentration of glucose[16]. Tests are collected instrumentally or visually as quantitative thresholds and outputs. Dry reagents are used in a number of ways when producing strips[17]. A reagent strip is a thin sheet of paper that is impregnated with a reagent(= a material that induces a chemical reaction) of a particular substance contained in a body of fluid of test for that substance. Through urine, reagent strips react with protein and show its presence and level[18]. Doctors are calling for a urine test to help diagnose and treat a variety of disorders including kidney , liver, diabetes, and infections[19]. Urine may be tested for specific proteins, sugars, hormones, or other chemicals, some bacteria, and their acidity or alkalinity[20]. If the urine is alkaline, 1 ml of dilute acetic acid will help to dissolve phosphates that may obscure the elements that have formed. The two medical providers able to detect sexually transmitted diseases (STDs) using a urine test are chlamydia and gonorrhea. Most STDs or sexually transmitted infections (STIs) don't cause overt physical signs or symptoms, as healthcare providers often call them[21].

CerebroSpinal Fluid (CSF):

Cerebrospinal fluid has three main functions: CSF protects against trauma on the brain and spinal cord[22]. CSF supplies nutrients to tissue in the nervous system.CSF eliminates waste products from cerebral metabolism[23]. This is a continually generated and consumed watery fluid that flows throughout the brain and across the surface of the brain and spinal cord in the ventricles. The abbreviation is CSF[24]. The CSF obtained after a lumbar puncture is tested to diagnose disease. A combined cycle of diffusion, pinocytosis and active transfer produces the cerebrospinal fluid (CSF) from arterial blood into the choroid plexuses of the lateral and fourth ventricles. Ependymal cells produce a small amount too[25]. The ventricle volume is approximately 25 ml.

The concentration of proteins is lower in both cisternal and ventricular CSF.Normal CSF is composed of 0-5 mononuclear cells[26]. Measured at lumbar puncture (LP), the CSF pressure is 100-180 mm H2O (8-15 mm Hg) with the patient lying on the side and 200-300 mm

with the patient sitting up[27]. Cerebrospinal fluid has three main functions: CSF protects against trauma on the brain and spinal cord.CSF supplies nutrients to tissue in the nervous system. CSF prevents brain absorption of waste materials[28]. Although CSF's primary purpose is to protect the brain inside the skull and act as a shock absorber for the central nervous system, CSF also circulates blood-filtered nutrients and chemicals, and eliminates waste products from the brain.CSF is formed in the brain via the choroid plexus and then reabsorbed into your bloodstream[29]. A CSF sample is typically obtained by the operation of a lumbar puncture often known as a spinal tap[30]. A sample analysis involves calculating and testing for: the fluid strain. This test is performed for calculating pressure inside the CSF and for collecting a fluid sample for further research[31]. Testing of CSF may be used to identify some neurological conditions. This may involve infections (such as meningitis) and damage to the brain or spinal cord[32].

Meningitis:

Meningitis is a rare infection that affects the delicate membranes — called meninges— which cover the brain and spinal cord[33]. The first symptoms are usually fever, vomiting, headache and feeling unwell.Limb discomfort, pale skin, and cold hands and feet frequently manifest earlier than rash, neck stiffness, aversion to bright lights and uncertainty. Septicaemia can occur with meningitis or without it[34].

Actually there are five types of meningitis- bacterial, viral, parasitic, fungal and noninfectious— each categorized by disease origin[33]. Bacterial meningitis is serious, and can be fatal. Death can happen in as few hours[35]. Most people are recuperating from meningitis. However, the infection can cause permanent disabilities (such as brain injury, hearing loss, and learning disabilities [3,36].

Bacterial meningitis is serious, and can be fatal. Death can happen in as few hours.Most people are recuperating from meningitis[37]. However, the infection can cause permanent disabilities (such as brain injury, hearing loss, and learning disabilities)[38].

Index Test and definite tests:

Index research was performed by one of the investigators using urinary reagent strips that can detect ten parameters, including protein, glucose, and esterase leukocytes[39]. With the help of a pipette, 2–3 drops of undiluted CSF were applied to protein, glucose, and leukocyte esterase patches and the color change was reported and interpreted using the color grading provided by the manufacturer[40]. Leukocytes in cerebrospinal fluid:A standard CSF WBC count is < 5 cells / mm3, and the reagent strip requires at least 10 cells for detection and has an upper limit of 500 cells / mm3 for detection. The reaction does detect the presence of esterase on granulocytes, however.Using the color grading given by the manufacturer (less than 10 granulocytes / mm3, no colour; 10-75 granulocytes / mm3, 1 +; 75-500 granulocytes / mm3, 2+;>500 granulocytes / mm3, 3 +), we used undiluted CSF and interpreted the test result[41]. CSF glucose: A typical level of CSF glucose is 2/3 of the plasma glucose.CSF glucose levels dropped in bacterial meningitis and fungal meningitis. We calculated if CSF glucose was below or above 50 mg / dL, using the reagent strip. The interpretation of the findings on the reagent strip was no color change as less than 50 mg / dL and any color change as greater than 50 mg / dL[42]. CSF protein:Normal CSF proteins vary from 15 to 45 mg / dL; the strip protein detection range is between less than 30 mg / dL and 500 mg / dL.We decided if the CSF protein was < 30 mg / dL (normal), 30 to 100 mg / dL, or > 100 mg / dL.Total color definition on reagent stripes was as follows: less than 30 mg / dL, no color change; between 30 and 100 mg / dL, 1 +; between 100 and 500 mg / dL, 2 +; and more than 500 mg / dL, 3 +[43]. A definite test should be an objective blind investigator who has done conclusive research. The tests performed were cell count by Neubauer 's chamber followed by a differential count on two centrifuged smears: one stained with hematoxylin and eosin stain, and the other stained with Leishman stain along with an automated analyzer's protein and sugar estimate[44].

Urinary Reagent Strips In Measuring CSF:

It is commonly known as an index test. With the help of a pipette, 2–3 drops of undiluted CSF were applied to protein, glucose, and leukocyte esterase patches, and the color change was reported and interpreted using the color grading given by the manufacturer[45]. The reagent stripe is designed to detect the range of 15 to 500 cells / mm 3 leukocytes. A spinal tap is necessary to obtain

cerebrospinal fluid (CSF) for a definitive meningitis diagnosis. The CSF also indicates reduced sugar (glucose) levels in people with meningitis, along with an elevated white blood cell count and decreased protein[46]. CSF check up treats bacterial meningitis[47]. Typical findings include: increased opening pressure, high protein, and hypoglycorrhachia.The appearance of the fluid is sometimes blurry or turbid.bThe concentration of CSF leukocytes is normally elevated with pleocytosis of a neutrophilic nature.A red color occurs with fresh blood or brownish with old blood. The protein elevation is believed to be due to blood-brain barrier disruption and serum protein leakage into the CSF. In bacterial meningitis, CSF proteins are typically the largest, and can exceed 1,000 mg / dL.If the CSF appears blurry it may indicate that white blood cells or proteins are tainted or accumulation. If the CSF appears bloody or red it may be a sign of bleeding or inflammation of the spinal cord. Increased CSF pressure can be due to increased intracranial pressure (skull pressure)[48]. The concentration of proteins is lower in both cisternal and ventricular CSF.Normal CSF is composed of 0-5 mononuclear cells[49]. Measured at lumbar puncture (LP), the CSF pressure is 100-180 mm H2O (8-15 mm Hg) with the patient lying on the side and 200-300 mm with the patient sitting up. This test is identical to a blood test where a needle is inserted into the blood test artery[50].

Procedure For Testing CSF:

An intrusive outpatient procedure used to remove a sample of cerebrospinal fluid (CSF) from the spine's subarachnoid area is a lumbar puncture (LP), also called a spinal tap. Many techniques can also be used to extract CSF, but they are used mainly in people who have spine deformities or are unable to undergo a normal lumbar puncture.A needle can be inserted at the base of the brain under the occipital bone[51]. You can drill a hole directly into the skull too[52]. If appropriate CSF is obtained it will be sent to a laboratory[35].The laboratory technicians must position the CSF in medium cultured dishes. The plates are supervised for the development of infectious organisms.If no growth occurs, the test is considered average, or negative.The test is considered positive if the CSF detects bacteria , viruses or fungi[53]. CSF culture tests will help the health care provider decide what causes the symptoms.You may need more checking, such as: Blood crops, Total Blood Count (CBC), CT scanning or magnetic resonance imaging (MRI); Your health care provider will support you in drawing up a recovery plan to best address and relieve the symptoms you encounter[54]. Within the CSF a population of CSF is used to detect infectious species.The CNS is vulnerable to bacterial, viral and fungal infections. A culture of CSF can help to diagnose a variety of disorders including: Bacterial Meningitis or Infection, Mummy diseases, Bleeding in the brain[55](hemorrhage of the subarachnoid), Epilepsia, Multiple sclerosis, Lyme Borrelia, Syndrome of Guillain-Barré. CSF pressure can also be calculated concurrently with the execution of a CSF culture[56].

Advantages and Disadvantages of measuring CSF for diagnosing meningitis:

Although CSF's primary purpose is to protect the brain inside the skull and act as a shock absorber for the central nervous system, CSF also circulates blood-filtered nutrients and chemicals, and eliminates waste products from the brain[57]. Urinary strips in testing of CSF for meningitis helps in biochemical analysis of CSF, whether the levels of glucose and protein are altered or maintained. This can be an ancillary method to confirm the biochemical parameters. However it shows the biochemical status of the CSF, the reagent strips will give only semi quantitative levels of the glucose and protein value. The cell count is not possible with the strips even though the leukocytes count is in urine. The CSF culture is not harmful while there are risks in the selection of CSF.Risks of puncture to the lumbar include: Inconvenience or pain during treatment,Bleeding of the spinal cord, particularly in people with low platelet count or blood thinners (thrombocytopenia), Headache[58] following leakage of CSF, Infection of Virus, Shock to the nerves. Lumbar punctures usually should not be performed on someone with a brain tumor or cyst.The operation can cause brain damage in these cases, and even death[59]. By avoiding strenuous activities on the day of the operation and remaining well hydrated you will reduce the risk of headache. A non-prescription medication, like acetaminophen (Tylenol), can help relieve back pain or headache[60].

Conclusion

The analysis offers strong insight and information on the use of urinary reagent strips in the calculation of CSF for meningitis. The principle of the chemical reactions

and colour reactions will be maintained both in the urine sample and cerebrospinal fluid sample. These concepts kindled the researchers in the field of neuropathology and made them to analyse the use of reagent strips in CSF analysis. Many successful results were obtained with a few limitations which were mentioned in this article.

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Role of Iron Deficiency in Chronic Hair Loss

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Abstract

Anemia is a condition in which you lack enough healthy red blood cells to carry sufficient oxygen into the tissues of your body. Anemia can make you feel weak and tired. Iron deficiency anemia is the most common form of anemia, which occurs when there is not enough mineral iron in your body. To generate hemoglobin your body needs iron. If your blood supply doesn't have enough iron, the rest of the body can't get the amount of oxygen it needs.Hair is a fast-proliferating organ with a high blood supply requirement. The length and size of the hair depend on the hair cycle anagen, growth stage. At the center of these systems, however, lies the blood supply in the scalp, the oxygen delivery system, and nutrients that are provided to the subcutaneous blood flowing hair follicles. It is not yet clear what role iron plays in hair loss. This research had the purpose of determining the relationship between iron and hair loss. Iron hair loss deficiency may look like traditional hair loss patterned by males and females. A study published in the Journal of Korean Medical Science found that iron may not only play a role in hair loss, but may also cause hair to fall out in a manner similar to genetic baldness of male and female patterns. The aim of the study is to analyse the role of iron deficiency in chronic hair loss. Most widely cited nutritional causes of hair loss include iron, one of the key micronutrients in metabolism of our body. This review gives a clear knowledge about the different roles played by iron deficiency in the body, specifically its role in chronic hair loss.

Keywords: *Anemia, iron deficiency, nutritional, hair loss, alopecia*

Introduction

Iron is a trace factor essential for various metabolic functions in cells. Because iron is toxic in excess, tight control is required to prevent iron deficiency (ID) or iron overload [1]. The adult body has an iron content of 3–4 g[2]. The standard western diet contains approximately 7 mg iron per 1000 kcal; however, each day it is usually only absorbed in 1–2 mg[3]. The usual Western diet contains about 7 mg of iron per 1000 kcal, but normally only 1–2 mg is absorbed every day[4]. There are two forms of iron in the human diet: heme iron and nonheme iron. Heme iron is meat-derived, and is well absorbed.Anemia is defined by the World Health Organization as blood hemoglobin values below 7.7 mmol / l (13 g / dl) in men and 7.4 mmol / l (12 g / dl) in women[5]. Usually a full blood cell count, peripheral smear, reticulocyte count, and serum iron indices are used in the anemia cause assessment. The severity of anemia depends on the amount of hemoglobin / hematocrit in the patient[6]. Microcytic, hypochromic erythrocytes and weak iron stores define iron deficiency anaemia[7]. The mean corpuscular volume is the measure of the total volume of red blood cells, and mean concentration of corpuscular hemoglobin is the measure of hemoglobin concentration in a given volume of packed red blood cells[8]. Standard reference ranges for mean corpuscular volume are 80–100 fL, and mean concentration of corpuscular hemoglobin is 320–360 g / l [9]. Poor iron bioavailability of the diet is the primary cause of iron deficiency anemia in developing countries [10,11]; however, reduced iron absorption and blood loss

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account for the more likely iron deficiency etiologies in developed countries [8,12]. Reduced absorption of iron may also be the result of atrophic gastritis or malabsorption syndromes especially celiac disease[13]. Postoperative gastrectomy (partial or total) and intestinal resection or bypass can also lead to secondary iron deficiency anemia through reduced iron absorption[14]. Chronic blood loss from genitourinary, gynecological, or gastrointestinal tracts accounts for the bulk of iron deficiency anaemia causes[15]. Excessive menstruation is the most common etiology of iron deficiency anaemia in premenopausal women[16]. Hair is a fast-proliferating organ with a high blood supply requirement[17]. The length and size of the hair depend on the hair cycle anagen, growth stage. At the center of these systems, however, lies the blood supply in the scalp, the oxygen delivery system, and nutrients that are provided to the subcutaneous blood flowing hair follicles[18]. It is not yet clear what role iron plays in hair loss[19]. This research had the purpose of determining the relationship between iron and hair loss. Iron hair loss deficiency may look like traditional hair loss patterned by males and females. A study published in the Journal of Korean Medical Science found that iron may not only play a role in hair loss, but may also cause hair to fall out in a manner similar to genetic baldness of male and female patterns.

CONSEQUENCES OF IRON DEFICIENCY ANEMIA IN HAIR GROWTH

Hair Loss

Hair loss is one of the most common aesthetic problems among the world's population, with 50% of men and almost 50% of women over the age of 50 suffering from this blemish [20]. Despite being part of the natural aging process, which also affects the scalp, it can negatively affect the quality of life of each individual, with situations that can lead to a state of psychological distress and demoralization. It is important to understand whether hair loss comes within the context of Normal physiological process, or otherwise, if an abnormal process is Complication caused by problems relating, for example, to Scalp or bulb to the scalp. The definition of all hair loss types, baldness and alopecia is often complex and reductive, since they are always the result of several causes that lead to the emergence of these pathologies[21].

Androgenetic alopecia is one of the most common causes of men's hair loss, which can be traced back to androgenic hormone activity and Dysfunction of 5-alpha-reductase enzyme leading to increased hair weakness. In women androgenetic alopecia is one of the main causes, but due to the reduced presence of androgens, the incidence is extremely minor[22]. Negative chemical and physical treatments applied to women's scalp are responsible for hair weakening which often leads to hair loss. Conversely, in spring and autumn, the common causes which can be associated simultaneously in men and women thinning hair are different, such as the so-called seasonal hair loss.

Even this phenomenon should be kept under control, albeit temporary, so as not to compromise any already critical situations.Other common causes which undermine normal hair function are

Also related to insufficient and unbalanced dietary intake, deficits in critical trace elements, genetic factors, hormonal imbalances or adverse activities that may change the hair growth cycle equilibrium [23,24]. Modern nutrients and some food supplements that are advertised will help the patient mitigate and reduce hair loss, compensating for future changes and nutritional shortages that may accentuate or worsen hair loss in the long run. Food supplements are often used to enhance hair growth although scientific studies do not always support the correlation between the use of specific components and the prevention of hair loss [25]. In addition, the use of different vitamins and minerals for hair loss treatment is often based on traditional practices or commercial grounds, and several Nutrients are inserted into the composition of dietary supplements to comply with the rule "one size fits all". In fact, some elements are traditionally known to play a significant role in hair physiology, while others are often present in the composition of certain food supplements without sufficient scientific evidence to support their use. Non-pathological hair loss diagnosis includes a detailed review of each individual's nutritional deficiencies to restore the hair's biological functions and achieve normal physiological conditions.

Iron Deficiency

Iron deficiency anemia arises when the iron intake balance, iron stores, and iron loss from the body is

insufficient to fully support erythrocyte production[26]. Iron deficiency anemia seldom causes death although there is a major effect on human health. The condition is quickly diagnosed and managed in the developed world but is still ignored by the doctors[27]. In comparison, in underdeveloped nations it is a health issue that affects significant parts of the population. Overall, worldwide, particularly among underprivileged women and children, the prevention and effective treatment for iron deficiency anemia remains dreadfully inadequate. Iron-related erythropoietic demands are produced by three variables: tissue oxygenation, erythrocyte turnover and hemorrhage loss of erythrocytes[28]. In the absence of hemorrhage, illness, or altered physical activity, the tissue oxygenation requirements and erythrocyte development usually remain constant throughout adulthood. Around 20 mL of senescent erythrocytes are cleared daily, and the 20 mg of iron in those cells is recycled for new erythrocyte production. Due to a shorter half-life of iron deficiency anemia circulating erythrocytes, iron is recovered in these patients earlier but the amount of iron in each microcytic erythrocyte is reduced[29]. Iron deficiency anaemia is usually associated with low iron saturation of the transferrin available. Iron is loaded from three sources onto diferric transferrin: gut (diet), macrophages (recycled iron), and liver (stored ferritin iron)[30] Overall, before the host develops anemia, iron stores are reduced or lost. The demands for erythrocytes must therefore be met by dietary and erythrocyte-recycled iron. If iron losses occur, the newly developed erythrocytes may decrease hemoglobin, resulting in a reduction in the amount of iron supplied by the same number of senescent erythrocytes. In children and other classes, 30 percent – 50 percent of anemia is caused by iron deficiency[31].

As 1.6 billion people are anemic [32], several hundred million demonstrate anemia for iron deficiency. As such, the iron deficiency is the world's most severe cause of anemia.Iron deficiency anemia affects a subset of the two billion nutritionally iron deficient people worldwide [33]. Hence the iron deficiency health burden can be extrapolated from the global incidence of anemia. Anemia is not distributed evenly across the globe, as underdeveloped geographies are fivefold increasing. The prevalence of anemia among young children in some global regions is > 50 percent , and even approaches 100 percent in some places [34]. In the same regions, with the exception of nonelderly men, 40 percent -50 percent of the population remains anemic at all ages. By comparison, in developed nations the burden of the disease is far less.In the United States, the incidence of anemia caused by iron deficiency in all age groups and gender groups is reduced [35]. About 10 million people in the United States, however, are iron deficient, including 5 million people with iron deficiency anaemia. The clinical spectrum of signs and symptoms for this disorder is largely dependent on the severity of the anemia. The hemoglobin values used to define anemia vary by age, sex, race and whether the blood was sampled from the capillary (finger stick) or venous (phlebotomy) source [36]. In the absence of automated testing, it has been shown that portable devices or hemoglobin color visual matching are 95 percent accurate to classify the hemoglobin level within 1–2 g / dL of reference values [37,38]. Patients can be complaining about poor mental performance or cold intolerance [39]. Fatigue and associated exercise dyspnea are recorded regularly. Though rare at presentation, glossitis or dysphagia may be identified [40,41]. Recognition of such characteristics can trigger appropriate laboratory tests and therapy.[42]

Relationship Between Iron Deficiency And Hair Loss

Loss of scalp hair is not a life-threatening condition which can cause psychiatric discomfort and adversely affect the quality of life. Hair is a rapidly proliferating organ with a lot of blood supply requirements. The association between micronutrients and hair loss has thus been tested in many studies since the 1960s. The most widely cited hair loss nutrition causes include iron, which is one of our body 's key micronutrients in metabolism. It is well known from its diverse functions that iron deficiency is linked to many pathological conditions. The role in hair loss however is not yet well known. Reviewing articles documenting the relationship between ID and hair loss including female hair loss pattern (FPHL), telogen effluvium, alopecia areata, alopecia universalis or totalis, some [43] advocated the association between ID and hair loss and others opposed it. Serum ferritin level may be used as a very early marker to detect the ID. It is a major iron-binding protein in non erythroid cells that reflects storehouses of total body iron. It declines as iron reserves decline from the

very early stage of ID [44]. Since only ID can induce quite low serum ferritin (FC) concentration, an ID specific FC is very common. However, it can play a role as a reactive protein in inflammation, patients with active infection and/or inflammation should not be included in the analysis when we consider that FC is used to detect IDs.

Hormonal status can affect hair loss, and should also be taken into account when interpreting the clinical condition.In previous research, the mean level of ferritin and transferrin concentration in patients with diffuse telogen hair loss was statistically slightly smaller than in non-hair loss subjects (16.3+/-12.6 vs. 60.3+/-50.1, ng / mL; P<0.0001 and 20.3+/-9.7 vs. 28.3+/-11.8 percent; P=0.006, respectively).Total iron binding potential in patients was also slightly higher than in control group (367.8+/-58.2 vs. 319.2+/-60.1 microg / dL; P=0.004). For nine iron deficiency anemia patients (Hb < 12 g / dL), eight patients reported telogen hair loss (odds ratio: 10.5, 95 percent CI: 1.2-90.7; P=0.013); At serum ferritin levels, the odds ratio (95 percent confidence interval) for diffuse telogen hair loss was 21.0 (4.2-105.0).Women with an iron deficient status are at risk for loss of telogen hair. More evident is the essential role of serum ferritin in hair loss. Serum ferritin levels below or equivalent to 30 ng / mL are closely associated with telogen hair loss in women with no systemic inflammation or any other underlying disorders[45]. This research had the purpose of determining the relationship between iron and hair loss.

Conclusion

From the above review we can analyse that iron deficiency plays an important role in the body. Iron deficiency anemia arises when the iron intake balance, iron stores, and iron loss from the body is insufficient. Though loss of scalp hair is not a life-threatening condition it can still cause psychiatric discomfort and adversely affect the quality of life. From the above study we can conclude that iron plays an important role in the body, specifically in chronic hair loss.

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Rast and Eucast in Blood Test- A Review

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Abstract

An allergic response is the hypersensitive immune response to a substance which is normally harmless or would not cause an immune response in everyone. An allergic response can cause several symptoms such as itching, inflammation and tissue injury. The manifestation of allergies is recognized in the form of urticaria, swelling, burning, pain, and dryness of the mucosa, stomatitis. radioallergosorbent test (RAST) which is the radioallergosorbent test used for the confirmation of allergic reaction in the body. This test specifically deals with the IgE antibodies which are responsible for the allergic reactions on the body. It is very essential to cross-check the exact history related to allergy. Individual sensitivity and population sensitivity, both are equally responsible for skin allergy are the factors which are to be considered. Skin tests become negative under conditions of severe allergic reactions which is determined by graded therapy by freedom from contact and using epinephrine administration. RAST test is a two-stage procedure. RAST- test is performed according to the guidelines of European Committee on Antimicrobial Susceptibility (EUCAST). The reliability of the test is high compared to other skin tests. Specificity based on two radioallergosorbent tests is high as 90% for inhaled allergens. It shows excellent reproducibility across a full measuring range with the help of the calibration curve. The RAST test is considered to be extremely sensitive. It shows high specificity which binds to allergen-specific IgE. This article mainly deals with the RAST procedure, advantages and disadvantages of the test.

Keywords: *RAST test, EUCAST protocols, skin allergy, contamination.*

Introduction

The RAST test is defined as a radioallergosorbent test. EUCAST is a committee that stands for "European Committee on Antimicrobial Susceptibility Testing". The first clinical case on allergy was reported by Dr. John Bostock in 1819. RAST test is used for the detection of specific IgE antibodies which are responsible for allergic reactions on the body. IgE which is an immunoglobulin is produced in two forms in our body. One is secreted as an alternative splicing form and the other form is the membrane-bound form [1]. Individual sensitivity and population sensitivity, both are equally responsible for the causation of skin allergy are the factors which are to be considered. It is very essential to cross-check the exact history related to allergy. Various clinical examinations should be performed via specific confirmatory tests. The difference in structure present between IgE constant domains along with all other immunoglobulin classes is mainly concerned. Physical effects of allergic reactions in certain tissues can trigger the mutagenic triggers before transformation of malignant cells occur. Radiation therapy is given in order to avoid the cancer cell growth and division, thus killing the cancer cells. This therapy can lead to destruction of a normal blood cell due to exposure of ionising radiation [2]. Buccal smear is useful for diagnosing Malignancy, dermatoses.

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Liquid paraffin as a rehydrant for air dried buccal smear [3]. The amount of hemoglobin is determined spectrophotometrically. These RBC cells are developed from hematopoietic stem cells which shows alteration with respect to age, sex, and dental diseases [4,5]. For the diagnosis of malignancy such as breast cancer, ovarian cancer blood test is taken as initial step[67] .Expert rules of the EUCAST committee were designed to assist the clinical microbiologists and to describe specific actions that are to be taken in response to antimicrobial susceptibility tests which in turn result in a more specific outcome[8]. These include the recommendations on topics such as reporting, which include responding to inferring susceptibility against agents from results with one, result suppression which might be inappropriate, and editing of results from intermediate to resistant or from susceptible to intermediate or resistant based on inferred resistance mechanism. It is based on current microbiological and/or clinical evidence. EUCAST expert rules also include the exceptional resistance phenotypes and intrinsic resistance phenotypes. These phenotypes have not yet been reported and it's rare [9]. The execution of the EUCAST expert rules is mostly dependent on MIC breakpoints which are used to define rules. Setting up of pertinent clinical breakpoints, which is based on patient's treatment and particularly not on the mechanisms of resistance detection. This may cause specific modification of some expert rules in the future and leads to success. The knowledge on diagnosis of the disease prognosis is very important. Several methods are used to obtain proper knowledge of specimen slides such as game based learning about the morphological features rather than using conventional slide methods. Even popplet notes are used widely comparing popplet notes while learning about the prognosis of disease and Rast test changes [10,11]. There is a requirement of personal protective measures such as helmet, face mask, head cap, safety shoes, gloves in order to avoid contamination while collecting the blood sample[12]. This review article deals with the importance of the RAST test in case of allergy and differentiation of this test with a normal skin test to analyze the merits and demerits of EUCAST and RAST in a blood test. Literature searches were limited to studies about RAST sample collection, maintenance, advantages, and disadvantages.

RAST AND EUCAST

Allergic Response:

Skin testing and allergy blood testing are used in confirming allergen sensitization[13]. Indoor allergens are damp old mattresses, unwashed pillows, and duvet, apple cider vinegar, close contact with pets, cigarette smoke, gas-fired cooking stoves, boilers [14,15]. Outdoor allergens are density, grass or tree pollen, exposure to roadways, or power stations [16]. Occupational allergens are contacted with isocyanate, flour, laboratory animals, resin-wood dust, biological enzymes, latex usage, etc [17]. skin testing with penicillin skin test reagent was proven to be very reliable in predicting the risk of anaphylactic reaction with penicillin. Other types of allergic responses can also be noticed under hysterectomy conditions mainly in women[18] and even after the application of particular vaccines. Prausnitz and Kushner were first to find the transferrable tissue sensitizing factor in serum responsible for allergic reactions. IgE in the body is produced in two forms such as an alternative splicing form which is secreted and the other is the membrane-bound form[19]. Ingredients in dental amalgam (including mercury), bonding, or porcelain can result in redness, swelling, and irritation of the gums [20,21]. In sensitized atopic individuals it can be noticed that allergic response induces cross-linking of high affinity[22]. The manifestation of allergies is recognized in the form of urticaria, swelling, burning, pain, and dryness of the mucosa, stomatitis. It may cause life-threatening conditions such as laryngeal edema, anaphylaxis, and cardiac arrhythmias, hypertension. Individual sensitivity and population sensitivity, both are equally responsible for skin allergy are the factors which are to be considered. It is confirmed that there is no use of probiotics and vaccines for the control of allergic reaction[23]. It is essential to obtain proper history, related to allergy and clinical examination should be done using various confirmatory tests [24]. Skin biopsy is done to understand the histopathologic changes that occur in Hansen's disease[25].

Rast Test Vs Other Skin Tests:

The two main allergy tests are skin tests and blood tests to detect an allergic response in the body. A scratch test is the most common allergy test against a tiny bit of an allergen on the skin by pricking the outer layer of skin and making a small scratch on the skin[26]. Percutaneous

skin testing is the most common- screening method used at present[27]. Intradermal testing (ID) is done for aeroallergens which show negative Production screen test. Skin test requires the discontinuation of antihistamines which have antihistaminic effect at regular intervals ranging from days to weeks before testing [28]. RAST may be preferred over skin tests [29,30]. Special test for food allergy which was introduced by Kolmannskog and Haneberg in 1986 [31]. Skin tests become negative under conditions of severe allergic reactions which is determined by graded therapy by freedom from contact and using epinephrine administration[32]. A skin prick test is the puncture test while the scratch test is used to check immediate allergic reactions of 50 different substances at once[33]. Skin prick test is usually done to identify allergies such as pollen, mold, pet dander, dust mites, and foods. In adults, this test is usually done on the forearm[34] .

Rast Procedure:

RAST test is a two-stage procedure. The first stage is the collection of a patient's serum as a source from which the IgE antibody is recognized. It is reacted with an allergen which has been covalently coupled to a solid-phase support system, most commonly a filter paper disk or microcrystalline cellulose particles, Sephadex particles, or agarose[35]. Once the incubation period is over, washing of the solid-phase allergen is done to remove the nonspecific serum components. Then the IgE antibody which is attached to solid-phase is reacted with a radiolabeled anti-IgE antibody in the second stage; after another overnight incubation, the complex is washed again and counted in a gamma scintillation counter[36]. The number of radioactive counts present is directly proportional to the amount of IgE antibody which was present to the particular solid-phase allergen. Thus, the reaction depends on two critical reagents, the insolubilized antigen and the radiolabeled anti-IgE [37]. The most common method used to prepare the solid-phase allergen is to react to the allergen solution with cyanogen bromide-activated polysaccharides. The procedure we use with the filter paper disks is a little different and is essentially the method that is used by workers at Beecham Laboratories in England.

Eucast Guidelines: EUCAST is the European Committee on Antimicrobial Susceptibility Testing. The most objectives of EUCAST are to harmonize antimicrobial breakpoints in Europe [38] and to act because the breakpoint committee for the EU Medicines Agency (EMEA) during the registration of latest antimicrobial agents, as defined in an exceedingly Standard procedure agreed between the EMEA, EUCAST and also the pharmaceutical industry in 2005 [39]. The responsibility for the development of harmonized breakpoints rests with the Steering Committee along with consultation regarding the national breakpoint committees, the EUCAST General Committee, the susceptibility testing devices manufacturers, and the pharmaceutical industry [40]. The International Standards Organization (ISO) is currently developing a worldwide standard to see MICs by microdilution[41]. EUCAST breakpoints are going to be implemented within the susceptibility testing systems of the national breakpoint committees in Europe. Disk-diffusion susceptibility testing methods that implement EUCAST breakpoints include the BSAC [42], CA-SFM, and SRGA systems. Antimicrobial resistance surveillance systems in which MIC values are collected can start using EUCAST epidemiological cut-off values and clinical breakpoints as they become available. The consequences of adjusting breakpoints may be assessed by retrospective comparison of knowledge interpreted by previous breakpoints and by EUCAST breakpoints

Uses in Various Disease:

There is vast usage of the RAST test in the detection of allergens nowadays. Allergic disorders could protect the body against cancer development via promoting immune surveillance[43]. IgE could stimulate the production of reactive oxygen metabolites and nitric oxide, which are important in tumor eradication[44]. Some of the diseases and allergies in which RAST test is used are tested to detect the presence of new aeroallergens—e.g. Parietaria Judaica - southern England, allergic rhinitis which is one of the lower respiratory tract[45], the conjunctival allergic responses[46], several skin allergies, stye[47], gastrointestinal tract allergies, which are frequently affected by the allergic disease [48]. It is also useful in the improvement of the treatment outcome in several allergic diseases such as asthma, a common respiratory illness [49]. It is also used in the detection of the COVID-19 disease which is an infectious disease that spreads through air droplets and causes respiratory illness affecting mostly the respiratory organ. Immediate

hypersensitivity to insulin is a rare condition among patients suffering from type 2 diabetes mellitus, particularly after the development of recombinant human insulins, which are less immunogenic than former animal preparations[50]. Atopic dermatitis in which rashes are developed that appears to be a severe exacerbation of eczema after application of vaccinations can be tested through the RAST test. Even in disease conditions like Alibert-Bazin syndrome or granuloma fungoides, is the most common form of cutaneous T-cell lymphoma, which generally affects the skin, but may progress internally over time it is used. Nocturia is the condition of frequent urination during night and can be noted during diabetes mellitus[51]. Noctiva is used to treat nocturia. Noctiva caused several side effects such as nasal congestion, sneezing, bronchitis, dizziness[52]. RAST test is used in the diagnosis of the allergic reaction caused by given noctiva.

Advantage:

The reliability of the RAST test is high [53]. It is not influenced by any concurrent drug treatment. It is performed when there is a widespread skin disease that is more prevalent. It is one the completely safest test to undergo for the allergic response [54]. Specificity based on two radioallergosorbent tests is high as 90% for inhaled allergens. It shows excellent reproducibility across a full measuring range with the help of the calibration curve. It shows high specificity which binds to allergen-specific IgE [55]. The RAST test is considered to be extremely sensitive. Another type of RAST test is CAP-RAST with allergens which are covalently coupled using a cellulose carrier over a large surface area. The patient's serum containing IgE is added to specific IgE reacts with the bound allergen. Non-specific IgE is washed away by enzyme-labeled antibodies against human IgE which is added with a bound complex that is incubated by fluorescence substrate [56]. This test is perceived as a "Gold standard" for in vitro IgE testing.

Disadvantage of RAST test :

The RAST test takes longer to perform. It is also less cost-effective. It is less sensitive than skin testing for detection for clinically relevant allergies. Sometimes it shows false-positive results that are obtained due to cross-reactivity between homologous proteins which are cross-reactive with carbohydrate determinants[57]. The predictive values associated with the RAST test shows clinical evidence of allergy using ImmunoCAP which cannot be applied to other test methods [58]. The awareness of perception and knowledge on hematological report plays a major role in determination of allergic response and diagnosis of the disease.

Conclusion

The above article determines the advantage and disadvantages of the RAST test and the exact view on how the test is performed. This test is widely used for confirming allergen sensitization. The specificity of the test is based on two radioallergosorbent tests as high as 90% for inhaled allergens. It is essential to obtain proper history, related to allergy and clinical examination should be done using various confirmatory tests. The test ensures that the RAST test is done under the EUCAST protocols and the safest test for allergic reactions.

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Can Achyranthes Aspera Be Used In Dentistry?

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Abstract

Plant kingdom serves the best source of remedy for all sorts of problems of the human body. Microbial species that are found in almost every habitat present in nature accounts for about 60% of the Earth's total biomass. The World Health Organisation (WHO) has stated that more than 80% of the people in the world rely upon traditional medicines for their primary treatment. On that note *Achyranthes aspera* commonly called as chaff flower has got immense therapeutic values. Each and every part of the plant, most importantly it's seeds, roots and shoots possess great medicinal values. *Achyranthes aspera* belongs to the family *Amaranthaceae*. The Plant is a stiff erect annual herb. *Achyranthes aspera* has been used as a folk medicine in Australia, India and Kenya It is useful in treating cough, renal dropsy, fistula, scrofula, skin rash, nasal infection, chronic malaria, impotence, fever, asthma, piles and snake bites. Their stem parts can be used as an emmenagogue, anti arthritic, laxative, ecbolic, abentifacien, anti helminthic, antiviral, anti plasmodic, anti hypertensive, anti coagulant, diuretic and anti tumor agent. The ash from the burnt plant can be mixed with mustard oil and a pinch of salt and the tooth powder obtained is useful in cleaning teeth, treating pyorrhea and tooth ache. Their roots can also be powdered and used as a tooth powder and the roots itself were used as brushes. In India and Terai of Nepal, the juice of the plant is applied to relieve toothache. The stem of the plant was also used as a toothbrush after removing the thorns. The infusion of the twig is also used as a wash for tooth pain. Their usage in dentistry is remarkable as it has got good anti-cariogenic properties and anti inflammatory properties which has been discussed in detail in the current review article.

Keywords: *Achyranthes aspera; Streptococcus mutans; Anti-cariogenic activity; Anti-inflammatory activity*

Introduction

Microbial species that are found in almost every habitat present in nature, accounts for about 60% of the Earth's biomass [1]. Herbal medicines have been used since time immemorial. Plants have got an extraordinary ability to synthesize aromatic substances which are the useful phenols or their oxygen substituted seriatives [2]. The maintenance of good health of the people in most of the developing countries forms the normative basis of the usage of traditional medicines [3]. The World Health Organisation, known for the importance of these valuable medicinal plants, has made an attempt to list all the medicinal plants present globally and has listed more than 20,000 species [4]. It also states that more than 80% of the population worldwide rely upon traditional herbal medicine for their primary health care [5] . The presence of numerous bioactive constituents of immense therapeutic value with no or less side effects make them an exemplary source of alternative medicines [6]. There exists a plethora of knowledge, information and benefits of these plants in our ancient literature. There are about more than 4,00,000 plant species present all over Earth [7]. But only 6% have been studied for their biological properties and only 15% have been phytochemically investigated [8]. This shows that research on plant

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species and their extracts must be greatly increased [9] . On that note, the current study deals with one of such plant species of immense therapeutic values which is *Achyranthes aspera*. Each and every part of the plant, most importantly it's seeds, roots and shoots possess great medicinal values[10]. *Achyranthes aspera* belongs to the family *Amaranthaceae* and is a stiff erect annual herb [11]. *Achyranthes aspera* has been used as folk medicine in Australia, India and Kenya [12]. It is useful in treating cough, renal dropsy, fistula, scrofula, skin rash, nasal infection, chronic malaria, impotence, fever, asthma, piles and snake bites. This review highlights the usage of the traditional herb, *Achyranthes aspera* in dentistry and its anti-cariogenic and anti-inflammatory properties.

Taxonomic Classification [13]:

Kingdom: Plantar

Sub Kingdom: Tracheobinota

Super Division: Spermatophyta

Division: Mangoliophyta

Class: Mangoliophsida

Sub class: Caryophyllidae

Order: Caryophylla

Family: Amaranthaceae

Genus: Achyranthes

Species: aspera

Vernacular Names [14]

Latin: *Achyranthes aspera*

English: Prickly Chaff flower, Rough Chaff tree, Red chaff tree

Sanskrit: Aghata

Hindi: Latjira, Chirchira

Gujarati: Safad, Aghedo

Tamil: Nayuruvi

Telugu: Uttaraene

Malayalam: Kadalad

Punjabi: Kutri

Unani: Chirchitaa

Ayurvedic: Apaamaarga, Chirchitaa, Shikhari, Shaikharika

Geographical Distribution:

Achyranthes aspera is found all over Balochistan, Sri lanka, Tropical Asia, Africa, Australia and America [15]. In India, it is easily found everywhere on roadsides and field edges as a weed throughout up to an altitude of about 2100m [16]. It is also found in plenty in the South Andaman Islands.

Morphological Features [17]

Root: Cylindrical root, 0.1 - 1.0 cm thick, slightly ribbed, gradually tapering, yellowish brown in colour, has got secondary and tertiary roots.

Stem: Yellowish brown, square, branched, hairy, cylindrical, solid, hollow when dry.

Leaf: Simple, subsessile, slightly acuminate, estipulate, obovate, petiolate, opposite, decussate and Pubescent.

Flower: Arranged in long spikes form in inflorescence. Bisexual, Greenish white, numerous, sessile, bracteate with two bracteolates, actinomorphic, hypogynous and syncarpous.

Phytochemical constituents:The major chemical constituents are carbohydrates, protein, glycosides, alkaloids, tannins, saponins, flavoides, lignin.

6. *Achyranthes Aspera* In Dentistry:

Since ancient times, sticks of stems and roots of plants have been widely used by people for brushing. The chewing sticks of the traditional plants possessed anti microbial properties and also cleansed the teeth mechanically [18]. *Achyranthes Aspera Linn* is the scientific name of the Indian prickly chaff flower that was widely seen at road sides and farms throughout tropical and subtropical regions [19]. This is also known as Apamarga. Its Tamil name is Nayuruvi which was described by siddhas as the powerful herbal. All parts of the plants including root, seed, lead and flowers are used as the medicine to cure even dangerous diseases. Their

roots had been powdered and used as a tooth powder and the roots itself were used as brushes [15]. In India and Terai of Nepal, the juice of the plant is applied to relieve toothache[16]. The stem of the plant was also used as a toothbrush after removing the thorns. The infusion of the twig is also used as a wash for tooth pain [17].

It makes teeth brighter, odour free and ensures the maintenance of strong and healthy gums [18]. Only few researchers across the world have tested the anti-cariogenic properties of *Achyranthes aspera* but their other pharmacological properties were widely tested across the globe by many researchers.

Anticariogenic Properties Of *Achyranthes Aspera*:

A study tested the anticariogenic activity of *Achyranthes aspera* against *Streptococcus mutans* [19] The lowest concentration of the extract that had inhibited the growth of the tested microorganisms for both the stem and root extracts is found to be about 2.5% [19]. While for the Gold standard chlorhexidine, the zone of inhibition obtained was 19 mm at 10 µl volume at 2% concentration[19]. *A. aspera* extract has shown statistically significant zones of inhibition. *A. aspera* has shown marked antibacterial activity against *S. mutans*[19] . Another study shows that the leaf extracts of the plant had more significant effects against the salivary microflora than the stem and root extracts of the same plant [20] A study by Murugan K et al., 2013 has recorded the biofilm inhibition percentage against *S.mutans* obtained for methanol, benzene, petroleum ether and aqueous extracts (125 µg/mL) of the plant to be ≤94%, ≤74%, ≤62% ≤42%, respectively. This again proves that *A.aspera* has got great anti cariogenic properties [21].

A study has shown that petroleum ether extract of leaves of *Achyranthes aspera* had shown broad spectrum antibacterial potential predominantly against Gram negative bacteria than Gram positive bacteria[22]. Similarly, Benzene extract of the stem of the plant had also shown good antibacterial activity against gram negative bacteria than gram positive bacteria [23].

The ethanolic extracts of leaves, stem and root parts of *Achyranthes aspera Linn* had been evaluated for antimicrobial activity against salivary microflora by a study [24]. The salivary samples were collected from children who had mixed dentition age group with moderate caries activity. Antibacterial assay was done by agar diffusion method [24]. The results were then evaluated with 0.2% Chlorhexidine which had been used as a standard. The seasonal and less palpated plant, *Achyranthes aspera* showed significant medicinal properties [24]. The crude ethanol leaf extract that was obtained from *Achyranthes aspera* plant was found to be an effective antimicrobial agent against the salivary micro flora having a comparable activity with that of chlorhexidine mouthwash [24]. The study also confirmed the antimicrobial potentials of the plant in its stem and root extract. Thus the study supports the folklore application of the plant as a preventive remedy for various microbial diseases of hard and soft tissues in the oral cavity[24].

A study by Jebashree et al., 2011 had evaluated the anticariogenic activity of ethyl acetate extracts *A. aspera.* The study had shown higher antibacterial activity against *S. mutans* than any other solvent extracts[25]. From these we can infer that *Achyranthes aspera* has got great anti-cariogenic properties. A study by Prabhat et al., 2005, had reported that methanolic extracts of *A.aspera* possessed antimicrobial activity while the study by Khan et al., 2010, had demonstrated that the ethanol and chloroform extracts of the seeds of *A. aspera* had shown mild to moderate antibiotic activity against *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa* [26 ,27]. Studies by Alam et al., 2009 [28]; Londonkar R et al., 2011 [29]; Naidu et al., 2006 [30]; Elumalai et al., 2009 [31] had demonstrated the potential Antimicrobial activities of *A.aspera.* Further studies on the anti cariogenic properties of the plant can substantiate their potential against the oral pathogens.

***Achyranthes Aspera* Gel In The Management Of Chronic Periodontitis:**

A study by Ramanarayana Boyapati et al., 2017 had conducted an experiment evaluating the usage of *Achyranthes aspera* in the management of chronic periodontitis[32]. The study has concluded that *A. aspera* gel when delivered locally along with scaling and root planing had shown a beneficial effect. *A. aspera* gel as a non-surgical local drug delivery system proved to be without any side effects in the management of periodontitis[32]. *A. aspera* gel has got strong anti-

inflammatory effects in addition to its antioxidant activity which has been discussed in the next paragraph[32].

Anti Inflammatory Properties Of *Achyranthes Aspera*:

There are many studies that show *Achyranthes aspera* has got a good anti inflammatory property An alcohol extract of *A.aspera,* 375 and 500 mg/kg was tested in carrageenan induced hind paw oedema and cotton pellet granuloma models in male albino rats. The alcoholic extract showed a maximum inhibition of rat paw edema of 65.38% and 72.37% after 3 hours [33].

In a chronic test the extract has inhibited 40.03% and 45.32% reduction of the granuloma weight in the sub acute cotton pellet granuloma model[34] Another similar study has also shown that alcohol extract has suppressed granuloma formation in both acute and chronic inflammation [35]. The orally administered ethanolic seed extracts of 100 and 200mg/kg and silver nanoparticles of *Achyranthes aspera* in carrageenan induced paw edema in rats has shown significant inhibition of edema and inflammation [36].

Future Scope:

The review article has explained the anti cariogenic activity of the plant extract. In recent times, the world has shifted towards the herbal cures because of the pronounced cumulative and irreversible reactions of modern therapeutic drugs. The field of nano technology is booming. The *Achyranthes aspera* mediated green synthesis of nanoparticles can enhance the antimicrobial effect of the plant. Especially the green synthesis of silver nanoparticles can be carried out which has got an excellent antimicrobial property[37]. Their quantum effects attribute to their unique mechanical and physicochemical properties[38]. Their increased surface area permits the coordination of various ligands [39]. For these reasons, they are greatly known for their applications in diagnosis, drug delivery and treatment of various diseases[40]. Applications of silver nanoparticles in dentistry is a growing field that has enhanced and stabilized various biomaterials and devices that are already in use in dentistry [38]. However, they cannot be used as such because of it's toxic nature [41]. But green synthesis of nanosilver makes it harmless and also enhances its pharmacological properties [42]. So studies owing to nanotechnology have got a great scope in future.

Conclusion

The article has reviewed the anti cariogenic properties of the plant *A.aspera* in detail. From our article, we can conclude that *A.aspera* has got a lot of uses in dentistry and has got a good anti cariogenic and anti inflammatory property.

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Most Frequently Extracted Permanent Tooth in Patients Below 18 Years of Age Visiting a University Hospital in Chennai –A Retrospective Study

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Abstract

The extraction of permanent teeth should not be an insignificant act, specially in children, because of the terrible repercussions on eruption phenomena, the harmony of the arches, the primary capabilities of chewing, swallowing, breathing and phonation. Loss of permanent teeth has negative consequences functionally, psychologically and socially, especially for children and adolescents. The aim of this study is to analyse the most frequently extracted permanent tooth and to evaluate the reasons for extractions in patients below 18 years of age visiting a university hospital in Chennai in a period of 10 months from June 2019 March 2020. The data of 7415 patients was reviewed and patients from 6-18 years of age undergoing permanent teeth extraction were selected for this study. Chi-square test was used to detect the significance between age, gender, reasons, and extracted tooth number. P value less than 0.05 was considered statistically significant. In this study we observed that the most frequently extracted permanent teeth in patients from 6-18 years of age are upper premolars, followed by lower first molars and the main reason for extractions were therapeutic & dental caries. Within the limits of this study, the most frequently extracted permanent teeth in patients from 0-18 years of age is upper premolars, followed by lower first molars and the main reason for extractions were therapeutic & dental caries.

Keywords: *extraction, prevalence, dental caries, permanent tooth, children.*

Introduction

Preservation of primary and permanent teeth from getting prematurely lost is the most essential goal directed towards the treatment provided for a pediatric dental patient [1,2], as any teeth will participate in the stimulation and development of the dental arches, aid in normal occlusal relationship, maintain esthetics, assist in speech development and functional mastication as well as enhance the improvement of quality of life. [3]

The extraction of permanent teeth should not be an insignificant act, specially in children, because of the terrible repercussions on eruption phenomena, the harmony of the arches, the primary capabilities of chewing, swallowing, breathing and phonation.[4] The first tooth to appear into oral cavity is the first permanent molar at the age of 6-years, hence called the 6-year molar. The first permanent molar is the strongest and the largest among all the teeth.. It is the keystone of the occlusion; it determines the shape of the lower part of the face and conditions the position and health of the other permanent teeth. [5]

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Tooth loss has diverse harmful effects on an individual e.g. impairment of masticatory function, unpleasant aesthetics, impaired phonetics, temporomandibular dysfunctions, psychological issues, social withdrawal and decrease in confidence level [6]. Common indications for extraction of permanent teeth includes dental caries and its sequelae (e.g. pulpitis and periapical infections), periodontal diseases, orthodontic treatment, malpositioned or impacted teeth, tooth fracture, retained deciduous teeth,supernumerary teeth, prosthetic considerations, and preparation for radiotherapy [7–12]

One of the most effective ways in declining the prevalence of dental caries and its sequelae is by using fluoride.[13–15] If dental caries is not treated on time, it might lead to progression of caries into the pulpal region which might require invasive treatment using rotary instruments.[16–21] If the caries is still not arrested and treated, it might lead to further complications which might indicate extraction of the teeth.

The decision to extract permanent teeth must be reasoned and incorporated into a global treatment plan that often requires collaboration with various other dental specialties.The analysis of the causes of permanent tooth loss is of interest to practitioners and decision-makers in order to develop control techniques to be integrated into overall dental public health programs.[22,23] It is from this perspective that an indirect approach based on the search for reasons for these permanent tooth losses by extraction has been developed and used in many countries. [24]

In the past decade a limited number of epidemiologic investigations have been conducted to determine the reasons given by dentists for extractions of permanent teeth and the majority of these studies have been carried out in European countries, such as Finland, France, Scotland, England and Wales, Norway, and Germany

To organize and implement adequate strategies for the prevention and the treatment of oral diseases more information is required about the reasons for extraction of permanent teeth. Tooth mortality provides some of the necessary information on the prevalence of dental diseases, on the availability of dental treatments, on the attitude towards dental extractions, and it is crucial for the planning of dental health services.

This study was carried out with the aim to determine the most frequently extracted permanent teeth in patients ranging from 6-18 years of age and also to evaluate the various reasons and pattern of tooth loss to assess any changing trends that will help in improving the level of oral hygiene and dental awareness among patients , thereby reducing their early extractions and consequent adverse sequelae.

Materials and Methods

Study Design and Setting:

This retrospective study examined the records of patients from June 2019-March 2020 undergoing treatment at a University Dental College in Chennai.

Data Collection:

Out of the patient records of 7415 children and adolescents who visited the hospital between June 2019 to March 2020, 528 patient's records undergoing permanent teeth extraction between the age group of 6-18 years were selected for this study.

The approval for this university setting study was obtained from the Institution Ethics Boards[SDC/SIHEC/2020/DIASDATA/0619-0320]. Sampling bias was minimised with the verification of photographs. Relevant data such as patient's age, gender, extracted teeth number, and reason for extraction were taken into consideration . Repeated and incomplete patient records were excluded from the study to prevent possibility of bias.

Statistical Analysis

Data was recorded in Microsoft Excel 2016 (Microsoft Office 10) and later exported to the Statistical Package for Social Science for Windows (Version 20.0, SPSS Inc., Chicago, Illinois, USA) and subjected to statistical analysis. Percentages, frequency of parameters were employed in the analysis. Chi-square test was used to detect the significance between age, gender, reason , and extracted teeth number.

Results and Discussion

In the present study, a total of 528 patients were selected as the final sample population after screening of 7415 patient records. In the study sample the percentages

of patients between 6-12 years and 12-18 years are 17.8% and 82.2% respectively. (Graph 1)

Out of the total patients screened, male patients were 47.7% and female patients were 52.3%. (Graph 2) .In the present study, the most frequently extracted permanent teeth were upper premolars, followed by lower premolars, and lower first molars (Graph 3)

Among both the age groups and both genders, upper premolars were the most frequently extracted permanent teeth followed by lower premolars, and lower first molars.(Graph 4 & 6).The most common reason for extraction among both the age groups and both genders, were therapeutic extractions, decayed teeth/dental caries and root stumps. (Graph 5 & 7)

Increasing the knowledge about the pattern and reasons for teeth extractions are often useful to dental practitioners so as to provide better information about dental disease prevalence, dental care availability, and attitudes towards teeth mortality [25,26]

This study was done to determine the most frequently extracted permanent teeth in patients below 18 years of age . The age of the patients selected was 6 – 18 years of age. Majority of the patients (82.2%) were within the age group of 12-18 years . These findings were similar with previous studies [24]. Children in the 12-15 age group acquired more extractions than others. This indicates that the number of permanent teeth extracted increases with age. Pain & decayed teeth were the main reason for consultation and concerned the majority of patients. Studies by several authors [27,28] have produced similar results. This is linked to an economic scenario and/or a lifestyle that is not compatible with a "preventive-conscious" approach. Difficulties in access to dental care, lack of dental facilities, lack of financial resources or lack of information and education on oral health may additionally constitute a barrier to systematic dental visits or consultations as soon as the first signs of dental complication appears.

The difference in the number of permanent teeth extraction between males (47.7%) and females (52.3%) was not significant .Though in several studies it was revealed that females have a higher proportion for extraction than males.[4] The reason for this might be the low self-care and especially dental care in our female population. It could also be due to lack of awareness, dependency and difficult approach to dental facilities. Several studies revealed that the presentation of males who were seeking treatment by extraction was larger than females [29] which may indicate carelessness and the ignorance of boys to maintain good oral hygiene.

The results of this study indicates that therapeutic extractions for orthodontic treatment and extractions due to dental caries are the main causes of tooth extractions with upper and lower first premolars being the most extracted teeth followed by lower first molars respectively. These findings are in correspondence with several studies. Orthodontic treatment is more often considered a priority when standard levels of prosperity are high. The findings of Agerholm et al 1986 revealed lower proportions of extractions for young people being essential due to caries and higher proportions of orthodontic extractions in S.England compared to Wales and N. England. [30]

Stephens et al reported that, in patients under 20 years of age, extraction for orthodontic purposes accounted for 33% of extractions in a certain Canadian population.[31] Murray et al, in a survey of Ontario general dental practitioners, reported that orthodontic considerations were the main reasons for permanent tooth loss in childhood. [32]

Extractions for orthodontic reasons were more common in mandibular and maxillary premolars [22,33]

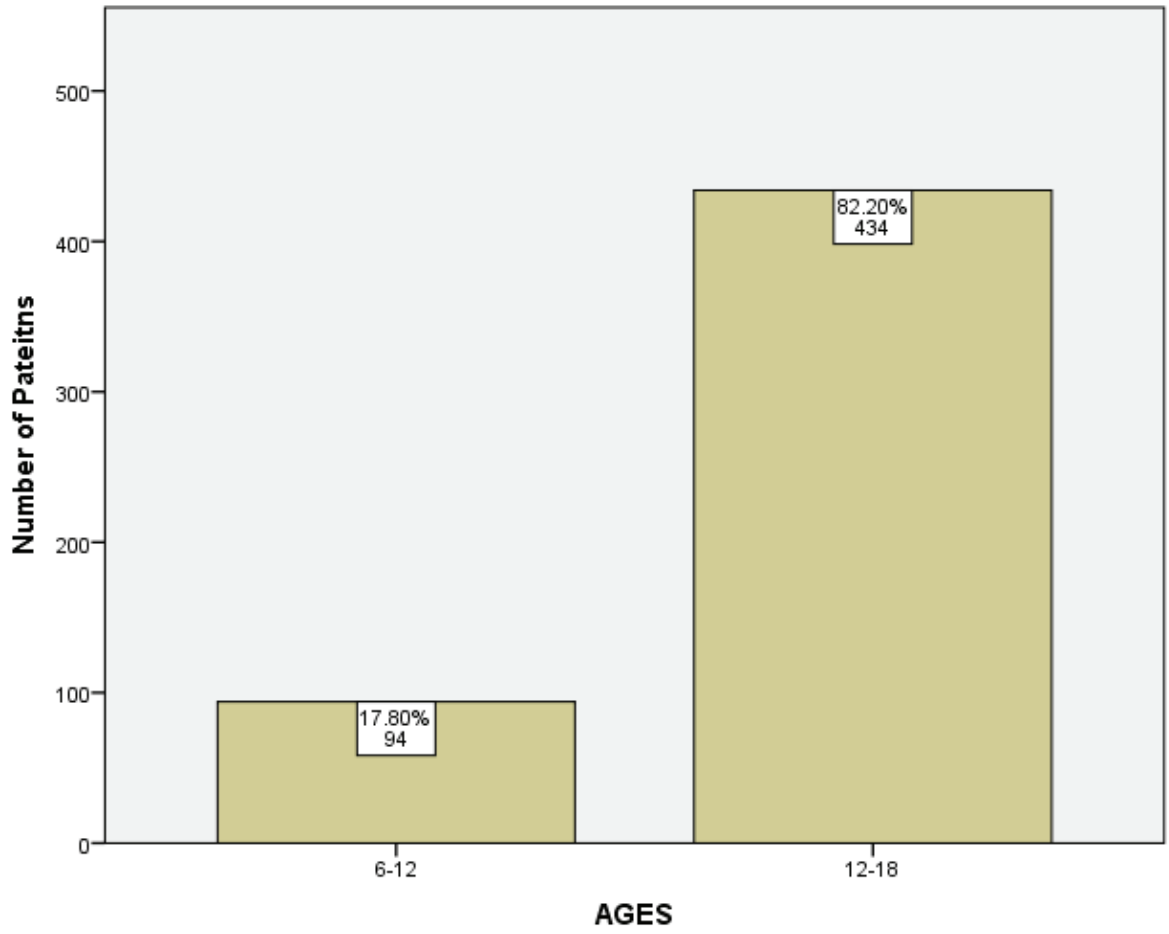
Concerning dental caries, the first permanent molar was the foremost affected tooth as being the earliest permanent tooth to erupt, in addition to its morphological features facilitate the accumulation of food and plaque, which can favor the bacterial invasion that ends to development of caries.[29]

The single predominant reason for tooth loss however appears to vary in several parts of the world, being primarily a function of socio-economic status, dietary and oral hygiene practices among other factors.[34]

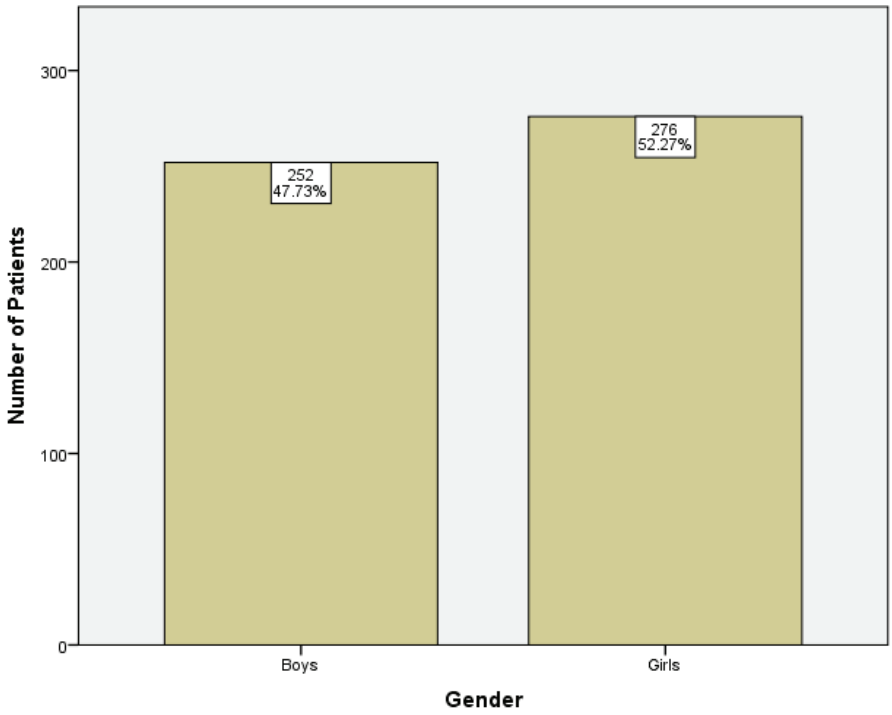
Although the study has some limitations like population bias, as the samples are of one particular population and does not represent all the ethnic groups or populations from around the world. A new study with a population group which is larger and more ethnically

diverse than the current one. Additional information regarding the results can be obtained which will hold more validity.

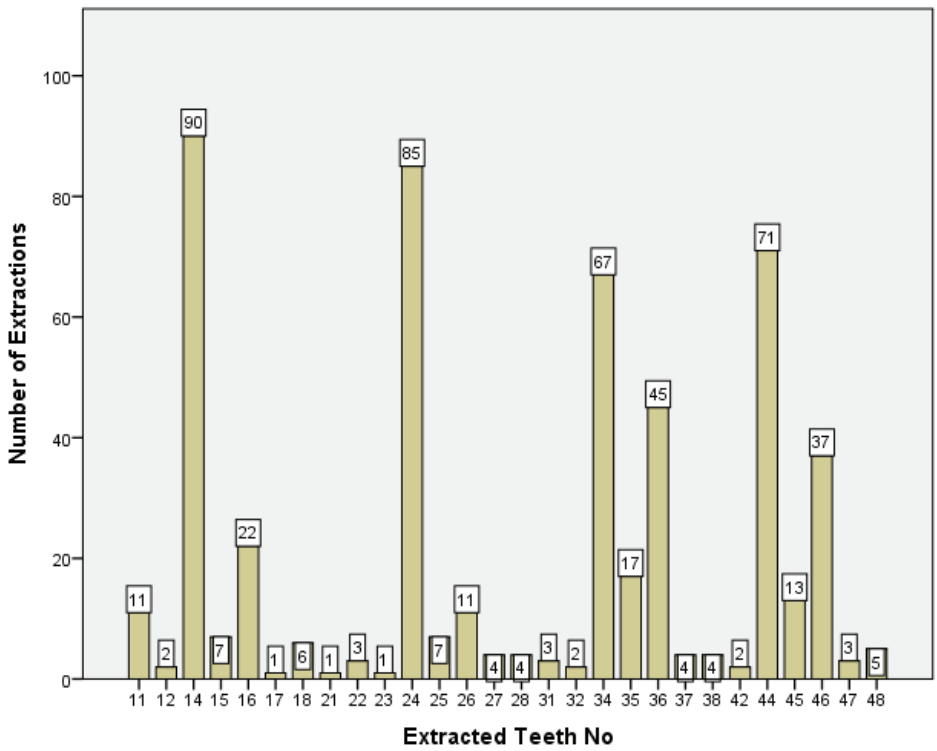
It is also recommended that community awareness programs should be initiated and people must be emphasized for vigorous oral hygiene maintenance. Government authorities must facilitate the dental organizations for community awareness campaigns. A proper health system including efficient dental care programs focusing on prevention and treatment of these diseases should be created and developed on a larger scale.



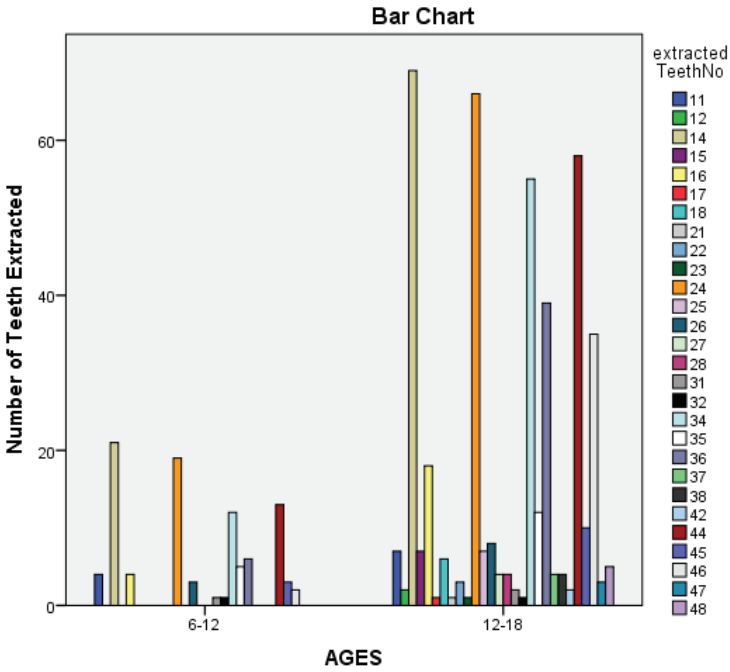
GRAPH 1: Age distribution of children in the study sample. X-axis denotes the age groups and Y-axis denotes the number of patients. The percentages of patients between 6-12 years and 12-18 years are 17.8% and 82.2% respectively.



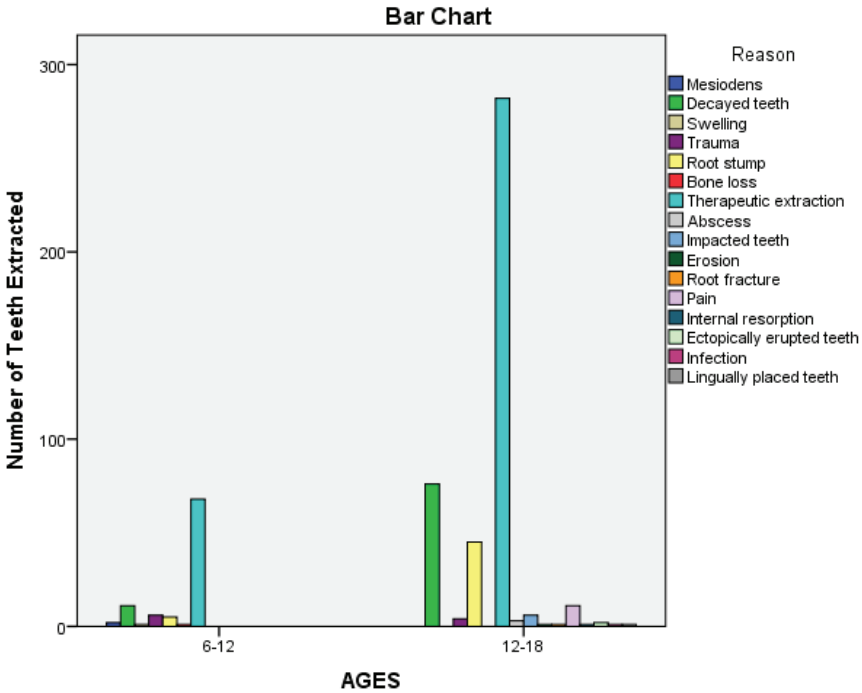
GRAPH 2: Gender distribution of children in study sample. The X-axis denotes the gender and Y-axis denotes the number of patients. The percentages of male patients were 47.73% and female patients were 52.27%.



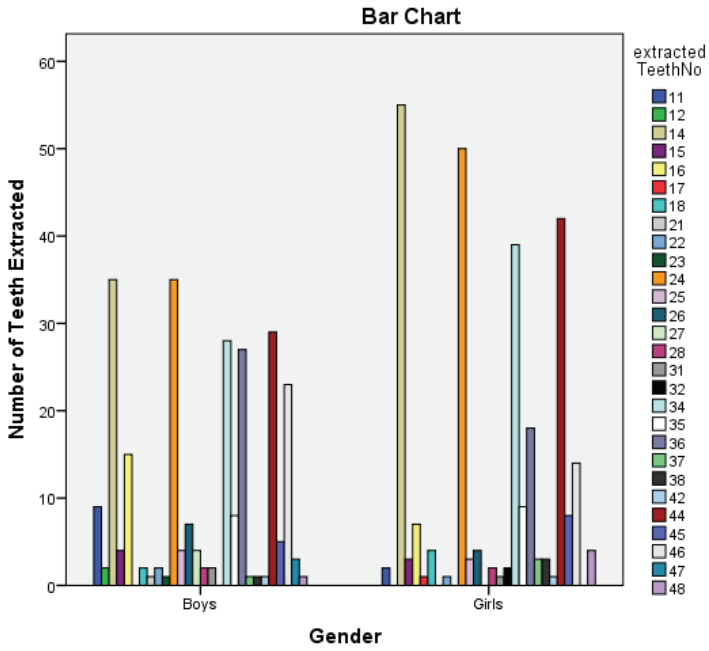
GRAPH 3: The graph denotes the total number of extractions done for each permanent tooth. X-axis denotes the teeth extracted in the FDI notation system and Y-axis denotes the number of extractions. The most frequently extracted permanent teeth were upper premolars, followed by lower premolars, and lower first molars



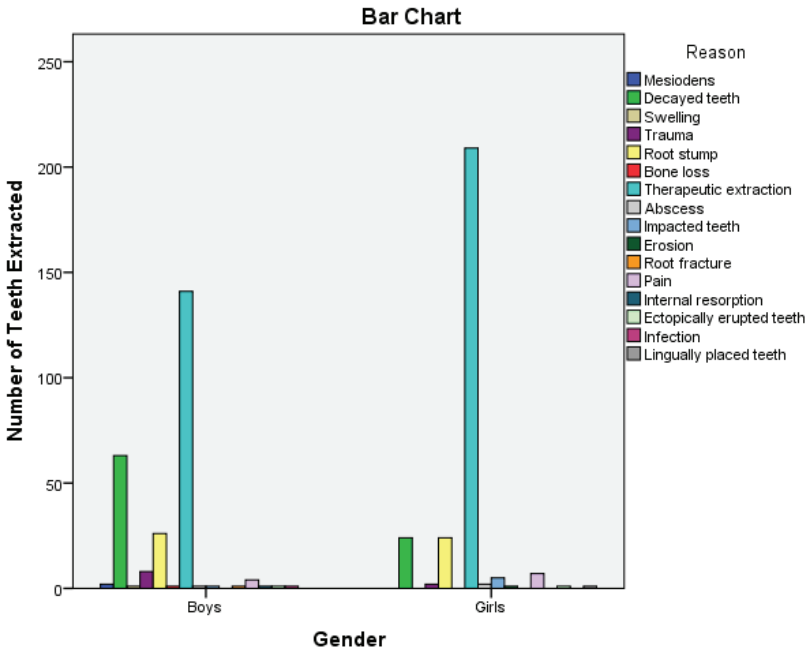
GRAPH 4: The graph represents the association between individual permanent teeth extracted at different age groups. X-axis denotes the age groups and Y-axis denotes the number of individual teeth extractions. There was no difference in individual teeth extracted at different age groups.Chi square test was done and the association was found to be not significant.(p value was =0.053 p>0.05 statistically not significant).



GRAPH5: The bar chart compares the various reasons for extraction of teeth at different age groups. X-axis denotes the age groups and Y-axis denotes the number of teeth extractions due to various reasons. Therapeutic extraction was the most common reason for extraction at different age groups. Chi square test was done and the association was found to be significant. (p value = 0.001 <0.05 statistically significant).



GRAPH 6: The graph compares the number of extraction of individual teeth(represented in the FDI system) with gender. X-axis denotes the gender and Y-axis denotes the number of individual teeth extractions. Females underwent higher number of extractions especially of upper first premolars compared to males. Chi square test was done and the association was found to be significant. (p value = 0.029 <0.05 statistically significant).



GRAPH 7: The graph represents various reasons for extraction between different genders. X-axis denotes the gender and Y-axis denotes the number of teeth extractions due to various reasons. Therapeutic extractions was the common reason for extraction among both the genders.Chi square test was done and the association was found to be significant. (p value = 0.001 <0.05 statistically significant).

Conclusion

Within the limits of the study, the most frequently extracted permanent teeth in patients from 6-12 years of age is upper first premolars, followed by lower first premolars, and lower first molars. The main reason for extractions were therapeutic & dental caries. This study will further pave the way for better diagnosis and treatment planning, and will also help in creating awareness.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Assessment of Partial Edentulism Based on Kennedy's Class IV Classification- Retrospective Study

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Abstract

Kennedy's classification provides immediate visualization, recognition of prosthesis support and assessment of design features of removable partial denture. Missing Teeth in patients with the long span Kennedy class IV situation demands biomechanical balance and aesthetic improvement. The long-span condition complicates the problem because of the unavailability of sufficient number of abutments to support the prosthesis. Conventional removable prosthesis and fixed partial denture are not advised for the same reason. The system of classifying the partially edentulous arch would be an enormous aid in providing a precise word picture of a particular dental arch that is to be discussed. Aim was to determine the prevalence of Kennedy's class IV classification among the partial edentulous patients residing in chennai who had undergone treatment for the replacement of missing tooth in the department of Prosthodontics,saveetha dental college,chennai,India. Study samples of 1341 cases were obtained from the data of 86000 patients between June 2019 and March 2020. Statistical software used for analysis was the SPSS(statistical package for the social sciences) which is designed by IBM and the statistical tests used were frequency tables along with bar graphs to analyse and compare the obtained results. The results showed that the patients with Kennedy's class IV were found to be the most prevalent in the male population. The findings of the present study showed that Prevalence of partial edentulism based on Kennedy's class IV was commonly occuring in the male population and also reveals that lower arch has a higher incidence of Kennedy's class IV.

Keywords: *Kennedy's class IV ,arch ,gender ,assess, edentulism*

Introduction

Tooth loss has an impact on an individual's oral health quality of life at biologic,psychological and social levels. The prevalence and extent of tooth loss have decreased significantly in many countries during recent decades[1,2,3]. Prosthetic rehabilitation is done to regain function, speech and esthetics[4]. The purpose of partial edentulous arches provides communication between dental college students, technicians about the case for planning good treatment and to design the partial denture. It also predicts the difficulties commonly occurring with particular removable partial denture design[5,6,7,8,9,10,11,12]. The prevalence of edentulousness has been used as an indicator to evaluate the efficiency of oral health services as well as to show the oral health of a population. It has been monitored in several countries for decades. The rate of edentulousness has declined particularly in the western countries, which is at least partly attributed to improved oral health services. However, its prevalence is still high in some other countries. The Stability of the removable Prosthesis is maintained by counter resisting the occlusal forces exerted on the restored arch-curve by use of a posteriorly directed path of placement for the prosthesis.[13,14,15,16]

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The variation in number and location of the edentulous space and its relationship to the remaining natural teeth necessitates to classify the patient edentulous arches[17].An RPD rehabilitating Kennedy class IV edentulous span can be considered successful if it does not cause gingival irritation and subsequent periodontal damage, the clasp arms are inconspicuous, and it is retentive.Kennedy class IV edentulous space may be short-span, where only incisors are missing or long-span, where anterior teeth and some premolars are missing. Later, even if the abutments are periodontally sound, a fixed prosthesis would result in an unacceptable cantilever effect. With the recent trends in dental health care that favour natural dentition preservation a disease in edentulous patients number is predicted[18,19,20,21]

Various prosthetic treatment options are available for partial edentulism, including fixed partial prostheses (FPP), implants, or removable partial dentures (RPDs). However, RPDs may have limited retention and stability due to the double-support system. In case of an RPD with a free end (e.g. distal extension RPDs), these aspects could worsen because of the propensity of the prosthesis to rotate during masticatory function. The denture-users of Kennedy's class II and IV categories might experience problems such as pain, occlusal disharmony, or soft tissue injury under the denture base or connector due to displacement of the distal extension of the prosthesis. The RPDs are most commonly used in clinical practice because they are economical, and facilitate easy care of the remaining teeth.

There are more than 65000 potential combination of partial edentulism pattern in maxillary and mandibular arches,hence it is logical to classify partially edentulous arches that have common characteristics and to facilitate communication among different dental professionals[22,23,24] .These disparities may be attributed partly to the increased availability and accessibility to oral disease prevention and control programs, as well as to increase in the awareness of importance in oral health. The study of trends in tooth loss,comparing rates of occurrence between different populations,may provide important information about risk factors for tooth loss,potential changes in oral health status,and possible causes of these changes[25]. The aim of this study was to analyse Kennedy's class IV among the patients who visited Saveetha Dental College.

Materials and Methods

Study Setting:

This study was carried out in a university setting which consists of subjects predominantly South Indian population. Advantages of the study includes available data,similar ethnicity.Disadvantages of this study is the fact that it is unicentred study and the geographic locations ,trends are not assessed. Approval of the study is by the ethical board of Saveetha university.Number of people involves 3 reviewers. A Guide, Researcher and a reviewing expert.

Sampling:

This is a retrospective study in which the samples were considered from the time period of september 2019 to march 2020. Case sheets reviewed for the research include patients with partial edentulous condition and cross verification of the required samples done by reviewing experts. Measures were taken to minimize the sampling bias.

Data Collection/tabulation

The Study samples of 1341 cases were obtained from the patient records and analysed the total data of 86000 patients between June 2019 and March 2020. The required data i.e,patients with removable partial Denture were collected and entered in a methodical manner in an excel sheet for the tabulation of data and further statistical analysis data was validated by 1-2 external reviewers and all the non specific, unclear or incomplete data were excluded from the study.

Analytics

Statistical software used for analysis is the SPSS(statistical package for the social sciences) which is designed by IBM and the statistical test used were frequency tables along with bar graphs to analyse and compare the obtained results. Independent variables include ethnicity,age and gender. Dependent variables includes, missing teeth and arch involved

Results and Discussion

Out of the total sample size 1341 cases,Result values obtained were, (Table 1)males showed 58.8% and females were 41.1%Also observed that the

increased age of male population was higher. And also this study reports that the occurrence of Kennedy's class IV is predominantly higher in the lower arch (Table 2 and Figure 1). According to Niarchou et al., noted that Kennedy's class were more frequent among males than females thus is in concordance with similar studies and also with literature[26]. Spakota B et al., observed that females are more edentulous compared to males but at the same time they are more concerned about replacing the missing teeth. This may be due to the dependency upon the males for this dental treatment to save the teeth. However, they are more conscious about their appearance which explains their preference for replacement of missing teeth[27]. Several studies have observed that prevalence of partial edentulism is more common in mandibular arch than maxillary arch as follows.

There was a significant association between male patients with the missing teeth in the lower arch of Kennedy's class IV classification(Pearson Chi-Square Value-0.918; $p>0.05$) Hence it was not significant (Figure 3). Prabhu et al., noted that partial edentulism was more common in the mandibular arch compared to maxillary arch. This is due to the fact the mandibular teeth erupt earlier in the oral cavity which is more for higher caries rate and higher chance of the tooth to get extracted[28]. Gad et al., Basutkar et al.,and Charyeva et al., noted that frequency of partial edentulism was higher in the mandibular arch compared to maxillary arch[29,30,31]. However there were few limitations encountered in this study .

The study contained some data that were unclear of certain reporting parameters such data were not considered. Another limitation was the geographic limitation i.e, assessment of predominantly South Indian population. Further this study is an unicentered study. Future research should focus on panel data to better understand the relationship between gender,arch involvement and the development of Kennedy's class IV. The scope of this study is that the prevalence and association of Kennedy's class IV with various parameters is essential to record.

Table 1: Table showing the frequency distribution of gender among patients with Kennedy's class IV partial edentulism.Male patients with Kennedy's class IV classification were 41.1% and females were 58.6%.

GENDER	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
MALE	789	41.1	41.1	41.1
FEMALE	550	58.6	58.6	58.6
TRANS GENDER	2	0.3	0.3	0.3
TOTAL	**1341**	**100.0**	**100.0**	

Table 2: Table showing the frequency distribution of missing teeth/arch among patients with Kennedy's class IV partial edentulism. The distribution of upper arch is 48.2%,lower arch is 49.0% and both arch is 2.8%.

MISSING TOOTH / ARCH	FREQUENCY	PERCENT	VALID PERCENT	CUMULATIVE PERCENT
UPPER ARCH	647	48.2	48.2	48.2
LOWER ARCH	657	49.0	49.0	49.0
BOTH ARCH	37	2.8	2.8	2.8
TOTAL	**1341**	**100.0**	**100.0**	

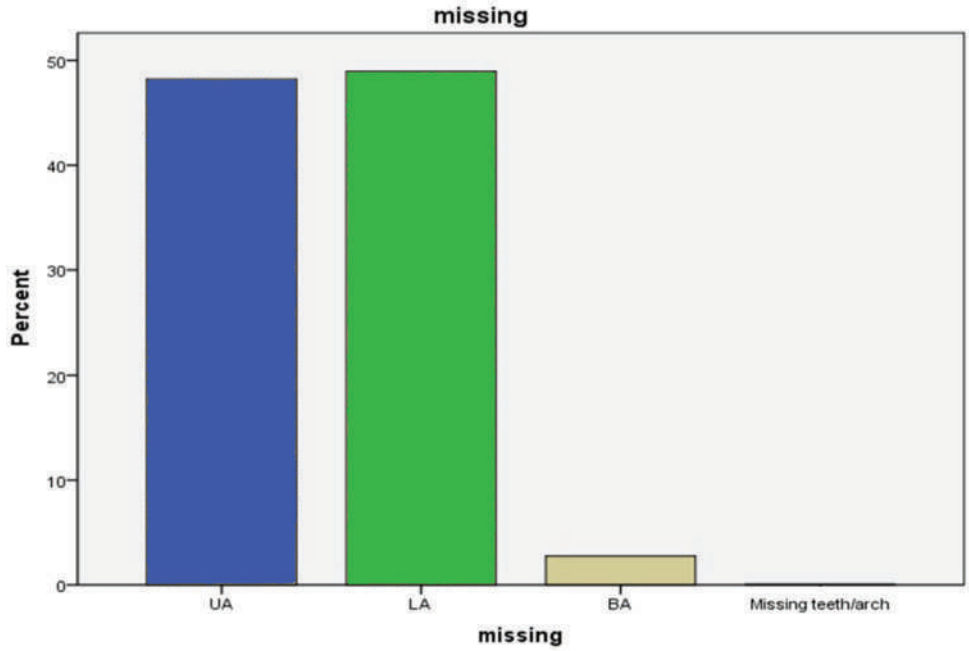


Figure 1: Bar graph showing the distribution of missing teeth in different arches, upper arch (Blue),lower arch Green) and both arch (Yellow) across a scale of 1-100. x axis represents the arch containing the missing tooth based on kenney's Class IV and y axis represents the number of patients under each category. There is a slight increase in the number of missing teeth in lower arch than upper arch.

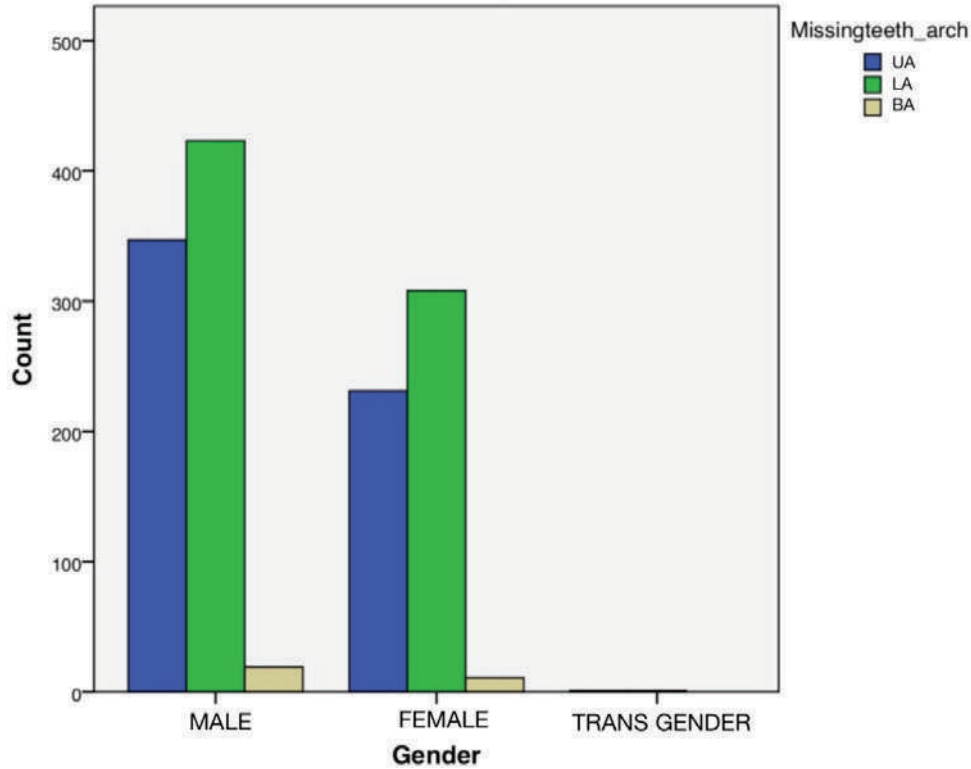


Figure 2: Bar graph depicting the association between the Gender and the missing teeth based on Kennedy's class IV Classification in different arches. X axis represents gender and Y axis represents the frequency of missing teeth in different arches. Blue colour denotes upper arch, Green colour denotes Lower arch, and Yellow colour denotes Both arch. Missing teeth were more in the lower arch among males than females. However, chi square tests revealed Pearson's chi square value : 0.948 df: 4 p value=0.918 (>0.05), statistically not significant.

Conclusion

Further evaluation based on bigger sample size, multi location studies with details on the oral hygiene,status of loyalty could be helpful. The findings of the study showed that Prevalence of partial edentulism based on Kennedy's class IV was commonly occuring in the male population and lower arch has a higher incidence of Kennedy's class IV.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Comparison of the Prevalence of Space Maintainers among Different Age Groups and Genders in Children

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Abstract

The space maintainers are mainly the devices that have been specially designed to maintain a space or to create an additional space that was lost due to the premature loss of the primitive teeth. They are mainly available in the various types with different types of indications. The main reasons for the premature loss of the teeth has been indicated for the extraction due to the dental caries or trauma or any type of genetic disorders.The main aim of this study is to estimate the most common type of the space maintainer that has been used among the different age and gender groups. All the patients are being considered in the study with the age group of 3-12 years, they must mainly have clinically missing teeth. The results are obtained and tabulated for the statistical analysis, both descriptive and inferential test which is done by using SPSS version 23. From the above study the results are obtained as, the age distribution of this particular study is mainly about the age groups 1-5 years(48%) and 6-12 years (52%),the gender distribution of this study is males(62%) and females (38%). The most common type of the space maintainer used is the Band and loop space maintainer. Within the limits of this particular study the most common type of the space maintainer indicated is the band and loop space maintainer for the age group of 6-12 years and has the higher gender predilection among the male children.

Keywords : *Band and loop space maintainer, Dental caries,Malocclusion, Space management.*

Introduction

The term Space maintainer was first coined by JC Brauer in 1941 and it is defined as a device used for maintaining a space in a given arch which has been previously occupied by a tooth or a group of teeth[1]. These space maintainers do not play an important role in the development of new teeth. The major function of the space maintainers is to guide the eruption of the upcoming permanent teeth in its appropriate space in order to prevent from the impaction, crowding or the supraeruption of the teeth[2]. There are many types and the designs that have been developed for the available space maintainer after the loss of the primary dentition[3]. An ideal space maintainer should maintain the entire space, which is mesiodistally and labio lingually that has been created after the loss of the primitive teeth[4]. They should be simple in the construction of the adequate strength to withstand the functional forces that are developed from the mastication and to restore the function as much as possible . They have been broadly classified into based on their fixation pattern ; fixed, semi-fixed or removable ; based on their type of the construction with or without the loops, wires and the bands ; based on their laterality as the unilateral or bilateral and based on their site as the maxillary or mandibular arches. There are mainly

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various considerations taken for the placement of the space maintainer. In case of the failure of placement of space maintainers in patients mainly lead towards the malocclusion[5].

The main indication for the use of the space maintainer is in the case of the premature loss of the primary dentition when the concern exists as the regards of the proper development and alignment of the newly growing or erupting dentition. Initially the teeth with dental caries are subjected to endodontic treatment [6 7 8] and when they have poor prognosis they have been subjected to the extraction of the teeth, which later on lead to the placement of the space maintainer. The various types of the space maintainers used are band and loop, lingual arch, Nance appliance, transpalatal arch, groper's appliance etc. Each type of the space maintainer has the specific indications with various advantages and disadvantages

The main reason for the premature loss of the teeth is mainly due to the indication for the extraction due to the dental caries which occurs mainly due to the consumption of a high level of sweet content diet[9], improper brushing habits[10], trauma[11] and even certain types of genetic disorders like the down syndrome etc. The education of the proper oral hygiene maintenance has to be given to the children this can be done by showing them a proper brushing technique, fluoride application twice in a year[12], regular dental visits[13]. The success rate of any particular treatment mainly depends on the quality of that particular treatment in case on a endodontic treatment the success rate depends over the obturation quality [14,15] and the patient must not report back with any lesions or secondary caries [16], likewise in case of the space maintainer it must helpful in guiding the eruption of the permanent teeth in the available space. The various compilations of the space maintainer include the increased risk of the infections in some cases, plaque accumulation, local pain and the discomfort[17], oral ranula[18], improper frenal attachment[19], interference with normal development and the alignment of the erupting succeeding teeth and the complications associated with appliances like breakage, loss or the displacement. There have been numerous pilot studies done to know about the different types of space maintainers. This research is mainly done to identify the prevalence of the space maintainer and its indication among the young patients, so it will fulfill the needs for this procedure and the awareness among the patients[20]. The main aim of this study is to estimate the most common type of the space maintainer that has been used maong the different age and gender groups.

Materials and Methods

This retrospective study setting was mainly a university setting and a single centred study. The various advantages they had are large available data and similar ethnicity and the disadvantages of this particular study was mainly the geographical limitations and the isolated populations.

The data collection has been done from 89000 cases sheets that were reviewed and analyzed individually. The non-probability convenience sampling method has been used. The sample data were collected from June 2019 – March 2020. Ethical approval was obtained from institutional ethical committee (Ethical approval number: SDC/SIHEC/2020DIASDATA/0619-0320) The inclusion criteria for the study were mainly the patients undergoing extraction due to caries, patients between the age group of 3-12 years with no other lesions, medical history and systemic complications, children who received any type of space maintainer and complete available data were considered. The exclusion criteria for this study were patients above the age group of 12 and patients with medical complications, incomplete and censored data. The case sheet verification was done using the photographic method. To minimize sampling bias simple random sampling was done.

The collected data were subjected to statistical analysis using the SPSS software by IBM of version 23 in which both the descriptive and the inferential test has been done which was the Chi-square test.

Results & Discussion

From the above done study, the results were obtained and tabulated, among which the age distribution of children for the space maintainer treatment was 2-5 years (48%) and 6-12 years (52%) [Figure 1] ,the gender distribution of this study shows that from the involved children are males (62%) and the females (38%) [Figure 2] and the most used space maintainer for the children was mainly the band and loop space maintainer [Figure 3] which has been used for about 50% of the patients.

The correlation between the age group and the type of the space maintainer mainly shows that the age group of 6-12 years were more prevalent for the space maintainer placement with band and loop space maintainer as the most commonly used type. This was mainly statistically significant [p=<0.05][Figure 4]. The correlation between the gender and the type of the space maintainer shows that the males were more prevalent for the placement of space maintainers with band and loop as the most commonly used type. They were statistically non-significant [p=>0.05][Figure 5].

Early losses of the deciduous teeth have often been studied because of their relevance and association with the occlusal abnormality[21], this mainly occurs due to the improper oral hygiene among the children, so that in the mixed phase space maintainers are used to maintain the space corresponding to the permanent successor teeth. The loss of the arch length resulting from this process can lead to the development of the severe malocclusions in the permanent dentition.

The age and gender distribution of the study of which age were mainly been grouped into two groups like 1-5 years and 6-12 years as they were the stages of the primary dentition and the mixed dentition respectively, so by which the study shows that the patients of the age group 6-12 years were more prevalent for the space maintainer placement this was similar to the findings seen in the previous studies [22]. The gender predilection shows that the males were more prevalent than the females for the space maintainer placement this was mainly due to the habit of frequent snacking which increases the risk of the increased dental caries among the male children and so they are highly indicated for the extraction which was a type of premature loss of the teeth and this was similar to the results of the previous literature [23].

The most commonly used type of the space maintainer was the band and loop space maintainer this was similar to the previous literature [24] in which band and loop space maintainer was the most commonly indicated for the premature loss of the teeth, and this was mainly due to the various reasons like patients have mainly unilateral loss of the teeth [25] which has been more common and it mainly has the advantage of easy fabrication [26].

The correlation between the age and the type of the space maintainer shows that patients of the age group 6-12 years were more prevalent for the space maintainer placement with a positive correlation and this finding was mainly similar to various studies [27,28]. The correlation between the gender and the type of the space maintainer shows that the males had the higher prevalence for the space maintainer placement and this finding was not statistically significant.

The possible limitations of this study was mainly a lesser sample size. The future scope is that they can be subjected to a multicentric study and mainly can be involved for the study of longevity of the space maintainer and to determine the proper indication for the space maintainer placement and if they are provided with a higher sample size and the various other ethnicities can provide a better results.

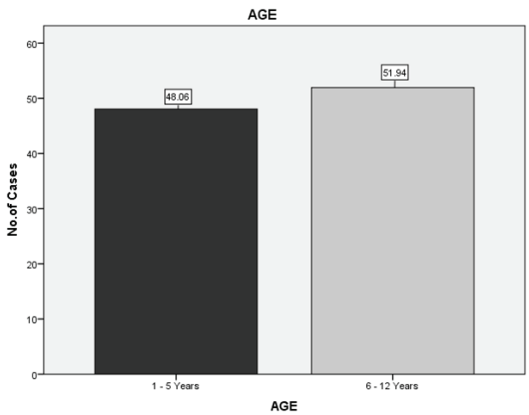


Figure 1 : Bar graph showing the age wise distribution of the children with the space maintainer treatment, in which 52% belongs to the 6-12 years age group and 48% belong to the 1-5 years age group.

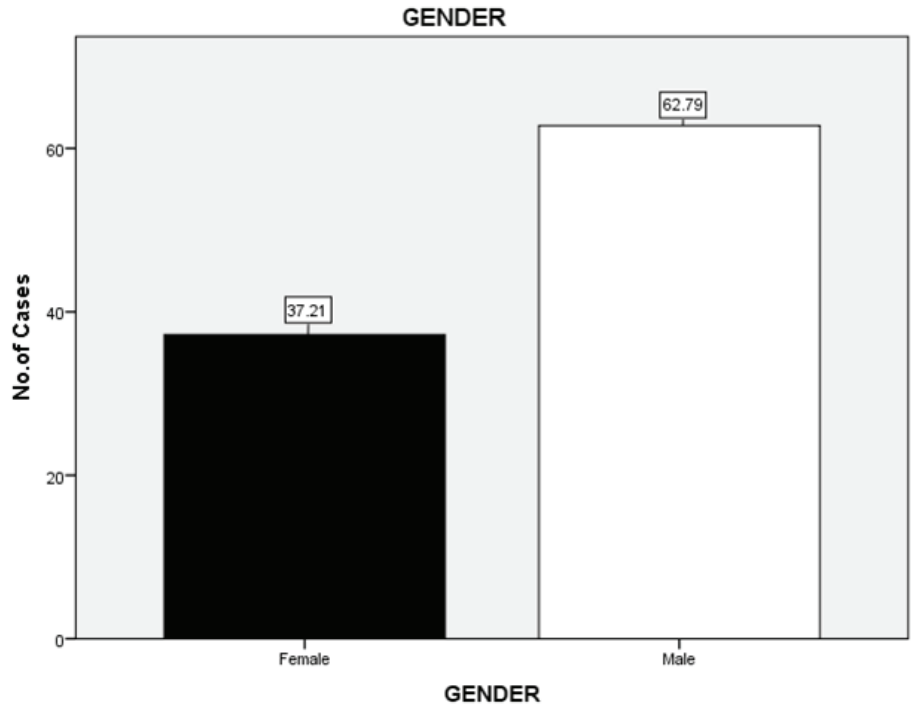


Figure 2 : Bar graph showing the gender distribution of children with space maintainers, 62% belongs to males and 38% were females.

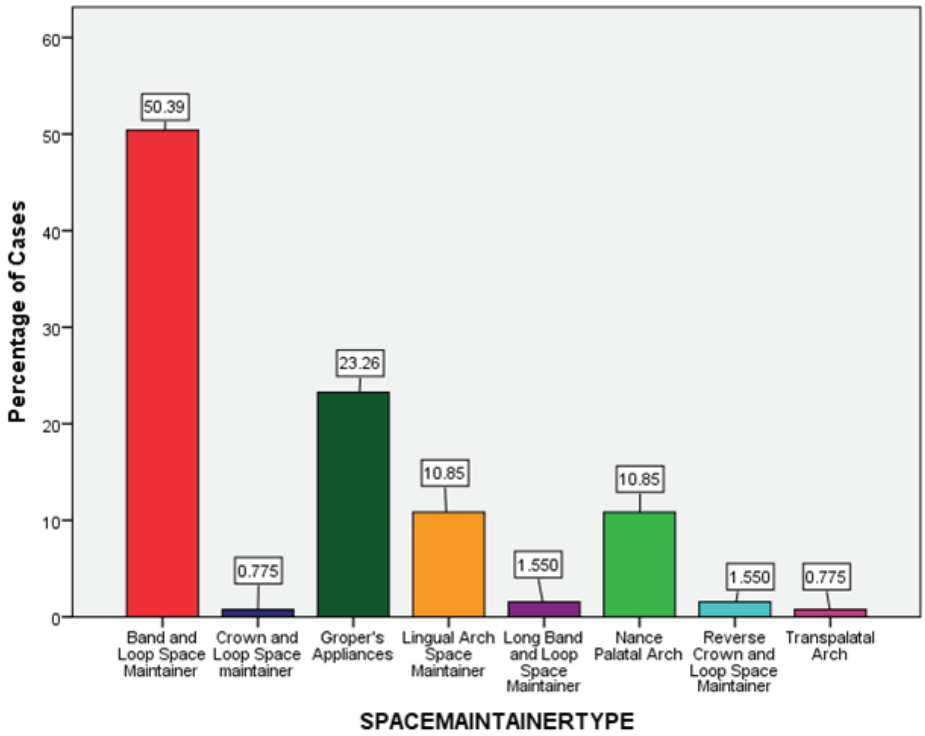


Figure 3: Bar graph showing the frequency distribution of different types of the space maintainers delivered to the children. Red colour represents band and loop space maintainer, Dark blue denotes crown and loop space maintainer, Dark green denotes Groper's appliance, yellow denotes the lingual arch, purple denotes the long band and loop, light green denotes the nance palatal arch, light blue denotes reverse crown and loop space maintainer, pink colour denotes transpalatal arch. Band and loop space maintainer was most commonly used among children(50.39%).

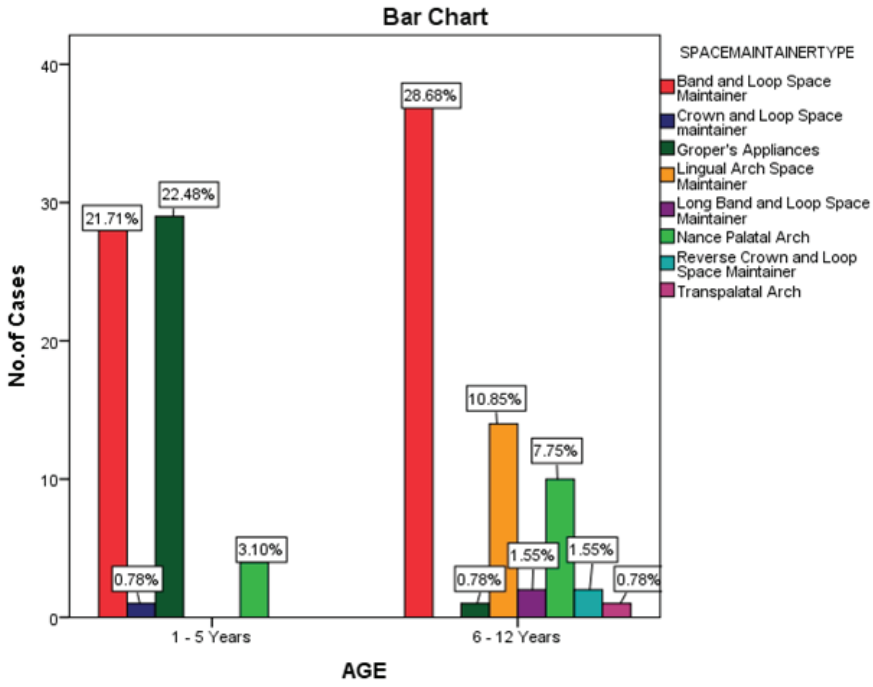


Figure 4 : Bar Graph depicts the association between the age of the child and the type of space maintainer placement. X axis represents the different age group of the children and Y axis represents the number of cases. Red colour represents band and loop space maintainer, Dark blue denotes crown and loop space maintainer, Dark green denotes Groper's appliance, yellow denotes the lingual arch, purple denotes the long band and loop, light green denotes the nance palatal arch, light blue denotes reverse crown and loop space maintainer, pink colour denotes transpalatal arch. Band and loop space maintainer was the most commonly used space maintainer in both the age groups, However groper's appliance was more commonly used in the 1-5 years age group the other space maintainers were more pertained to mixed dentition. This difference was statistically significant (Chi-Square test; p-value = 0.000 -significant)

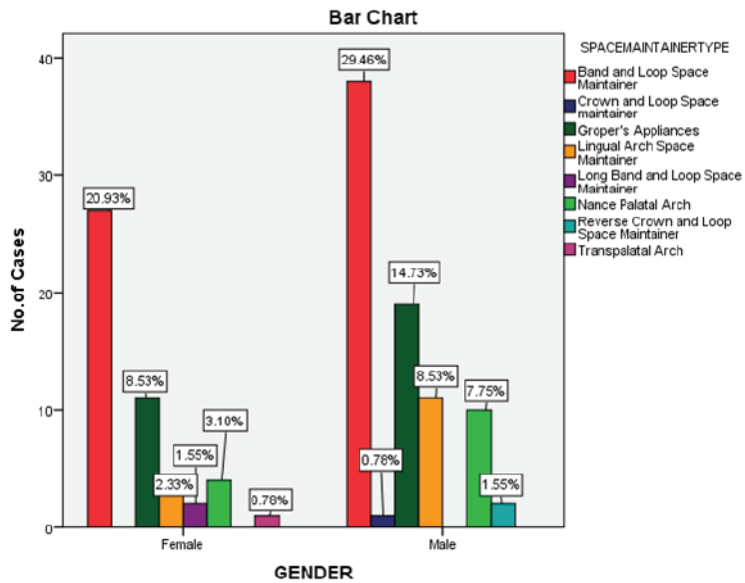


Figure 5 : Bar Graph depicts the association between the gender and the type of space maintainer placement. X axis represents the patients of different genders and Y axis represents the number of patients with space maintainers.

Red colour represents band and loop space maintainer, Dark blue denotes crown and loop space maintainer, Dark green denotes Groper's appliance, yellow denotes the lingual arch, purple denotes the long band and loop, light green denotes the nance palatal arch, light blue denotes reverse crown and loop space maintainer, pink colour denotes transpalatal arch. Children who were male had higher chances of receiving space maintainers when compared to females which was not statistically significant, among which band and loop space maintainer were as most commonly used in males. (Chi-Square test; p-value=0.125-not significant)

Conclusion

Within the limits of the present retrospective study, the most commonly indicated space maintainer was the band and loop space maintainer among the patients of age group of 6-12 years with a higher gender predilection among the male children.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Type of Study: Original Research *DOI Number: 10.37506/ijfmt.v14i4.12416*

An Estimation of Blood Glucose Level in Children with Early Childhood Caries- A Retrospective Study

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Abstract

ECC is one of the most common oral conditions seen in children. It is primarily seen on the central incisors in the form of a white spot, which later spreads to the posterior teeth as well. This is a serious concern for the biological and the social life of a child. Treatment of this condition is also expensive making it difficult for the economically backward to avail treatment. It has a lot of etiological factors including diet, socio economic status and poor oral hygiene. It is a condition seen in children below the age of 6. The aim of this study is to find the level of blood glucose in children with early childhood caries and to evaluate whether there are any abnormalities in blood glucose level compared to normal reference level. A retrospective descriptive study was conducted using patient records of a university dental hospital in Chennai from June 2019-March 2020 among patients diagnosed with ECC who had their blood sample taken and their blood glucose levels recorded. Descriptive statistical tests were employed.Point biserial correlation analysis was done to find out the relationship between blood glucose levels in boys and girls with ECC. ECC was most prevalent in the age group of 3 and the mean blood glucose of the participants was found to be 88.22 mg/dl. There was no significant correlation or difference in the mean blood glucose level of males and females with ECC (Point biserial correlation coefficient=0.07). Based on the results, there were no abnormalities in blood glucose values of children with ECC.

Keywords*: Early childhood caries, Children, Caries, Blood glucose, Relationship.*

Introduction

The primary aim of pediatric dentistry is to preserve the primary teeth since it acts as a natural space maintainer to guide the eruption of permanent teeth [1,2]. This becomes challenging due to the high prevalence of trauma between the age of 2 and 4 [3] ,presence of other biological conditions [4] and due to diseases like Early Childhood caries(ECC), which is one of the most common diseases of the oral cavity in children. It is also a major oral health condition, especially in socially backward populations. [5] Its widespread nature and expensive cure makes it a difficult problem to deal with. The condition is also known as nursing bottle caries, it is the presence of one or more decayed, missing or filled tooth surfaces in any primary tooth in preschool children between birth and 71 months of age. It usually begins as a white spot lesion in the upper primary incisors and then spreads as the caries starts to set in. Information relating to the non cavitated carious lesions in young children is very important as it provides additional components of understanding problems of caries in toddlers. [6]

In the early phase, ECC is recognized as a dull, demineralised enamel which advances rapidly to full

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blown decay along the gingival margin. [7] It can be seen either on the labial side, the lingual side or both(depending upon the factors which lead to the evolution of caries). It is primarily seen in school children between the age of 2-5, sometimes extending till 8 years. Various studies conducted all across the globe show the highest prevalence in Africa and SouthEast Asia. [8] In India, the state with the highest prevalence was found to be Andhra Pradesh at 64%. [9] and the lowest prevalence was found in Sikkim at 41.92%

The etiology of ECC is varied and is well established. Some of the factors include, poor oral hygiene,[10] carbohydrate rich diet, [11] microflora, incorrect feeding habits[hence known as nursing bottle caries] and biological anomalies(eg. High frenal attachment [12]). When microflora is concerned streptococcus mutans and streptococcus sobrinus were found in high association with ECC [13] .But, very few studies were found which tested the relation of glucose level in children and ECC. All the articles found regarding this topic were from the early 20th century [14]. Studies showed a positive relation between carbohydrates and a fat rich diet and the incidence of Early Childhood Caries. [15] Fluoride levels in the oral cavity also dictate the presence of carious lesions in children. [16,17]

A cause of the initiation of ECC could be lipid peroxidation, which has been known to cause alterations in the structure and function of the host cells by producing malondialdehyde(MDA) as the byproduct. This along with cell damage in the host tissue affects the immune mechanism in saliva, leading to initiation and progression of dental caries. [18] A study by Subramanyam et al showed an increase in the MDA levels of the patients with ECC in children below the age of 6 in Chennai. [19]

Since increased carbohydrate consumption is the main etiology of ECC, a question arises whether there can be any increase in the average blood glucose level of children with ECC. The increased glucose in the blood may lead to more potential cariogenic property and it may lead to the teeth providing more substrate for the microbes to feast on, leading to their uncontrolled multiplication and formation of caries via demineralisation of the enamel. The aim of this study is to find the level of blood glucose in children with early childhood caries and to evaluate whether there are any abnormalities in blood glucose level compared to normal reference level.

Materials and Method

Study Design and Setting

This descriptive study examined the records of patients fromJune 2019-March 2020 undergoing treatment at a University Dental College in Chennai. The records were collected from the hospital's record database. Ethical approval was obtained from the Institutional Ethical Review Board(Approval number 0619-0320). The study population included children under the age of 6 who visited the Department of Pediatric Dentistry, underwent blood test and treatment under general anesthesia by means of simple random sampling. Children with any systemic conditions were excluded from the study.

Data Collection

Out of the patient records of the 1557 0-6 year old patients who visited the hospital between June 2019 to March 2020, 122 patient's records undergoing treatment for ECC were analysed . Relevant data such as patient age, gender, RBS recorded at the time of visit were recorded. Repeated and incomplete patient records were excluded from the study to prevent possibility of bias. Data was verified by an external reviewer.

Statistical Analysis

Data was recorded in Microsoft Excel 2016(Microsoft Office 10) and later exported to the Statistical Package for Social Science for Windows (Version 20.0, SPSS Inc., Chicago, Illinois, USA) and subjected to statistical analysis. Descriptive statistical tests were employed. Point biserial correlation analysis was done to find out the relationship between blood glucose levels in boys and girls with ECC.

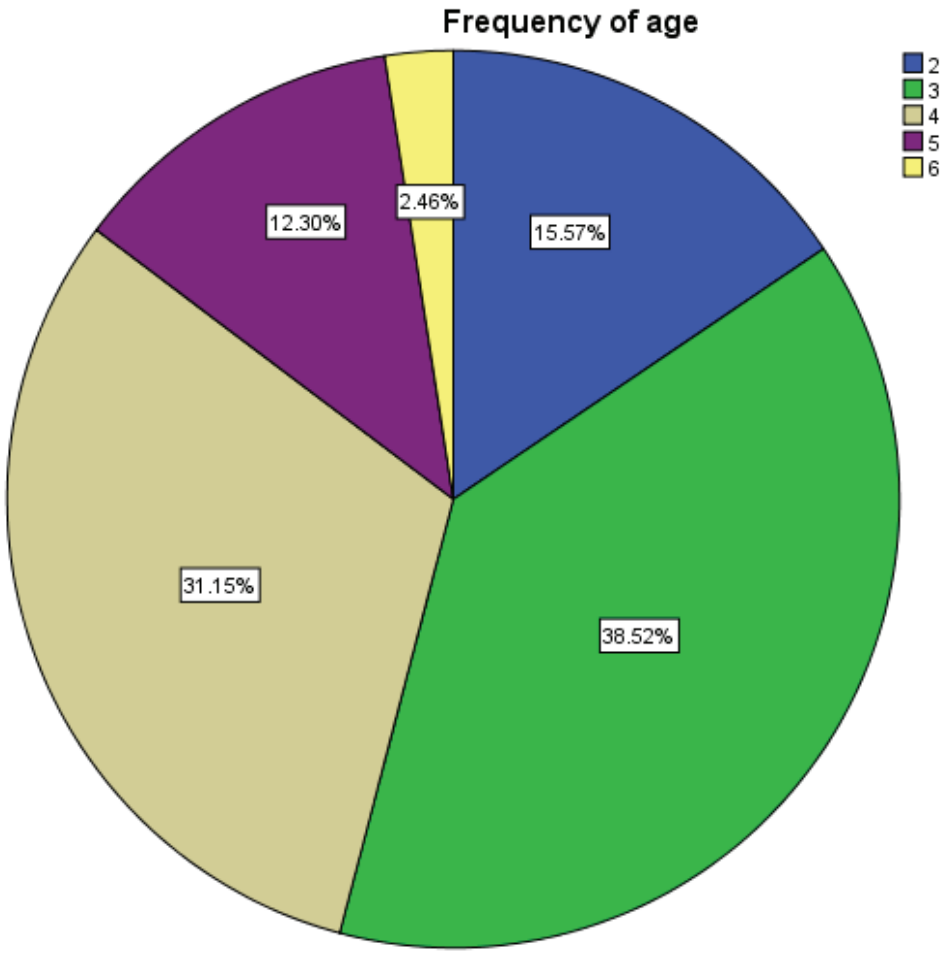
Results and Discussion

The final data set consisted of 122 patients of Indian origin who underwent treatment under general anesthesia. The mean age for children with ECC treatment was 3.48 years ± 0.981 years. The age group associated with the greatest prevalence of ECC was 3 years[38.52%], followed by 4 years[31.5%]. The prevalence of ECC among 2 year old children was found

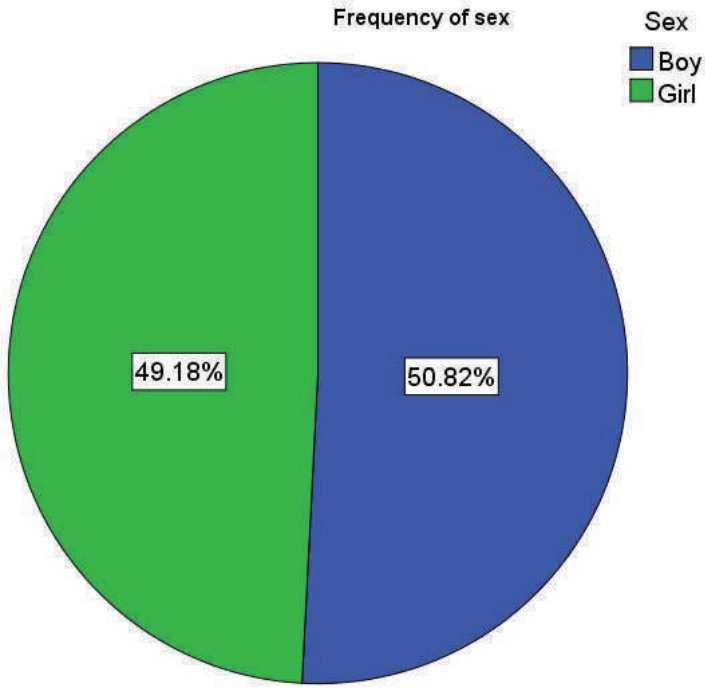
to be 15.1%. [Graph 1]

50.8% of the participants were male and 49.2% were female.[Graph 2] 54.8% of the participants in the 0-3 age groups were male showing a male dominance, although the differences between the gender were not statistically significant. The mean blood glucose level of the male participants was 87.6 mg/dl [Graph 3] and the mean blood glucose level of the female participants was 88.87 mg/dl [Graph 4].

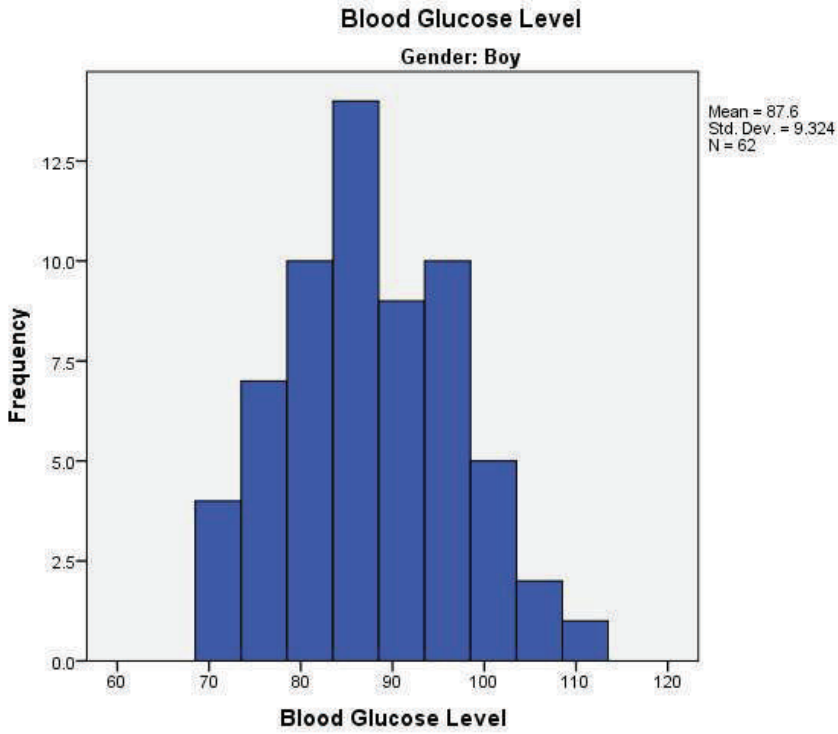
The mean blood glucose level was found to be 88.22 mg/dl in the total population. It was found that 10% of the study population had a blood glucose above 100g/dl. [Graph 5]. Point biserial correlation coefficient was elicited and value was found to be .07 (close to zero) suggesting no relationship/correlation between blood glucose levels of boys and girls with ECC.[Graph 6]



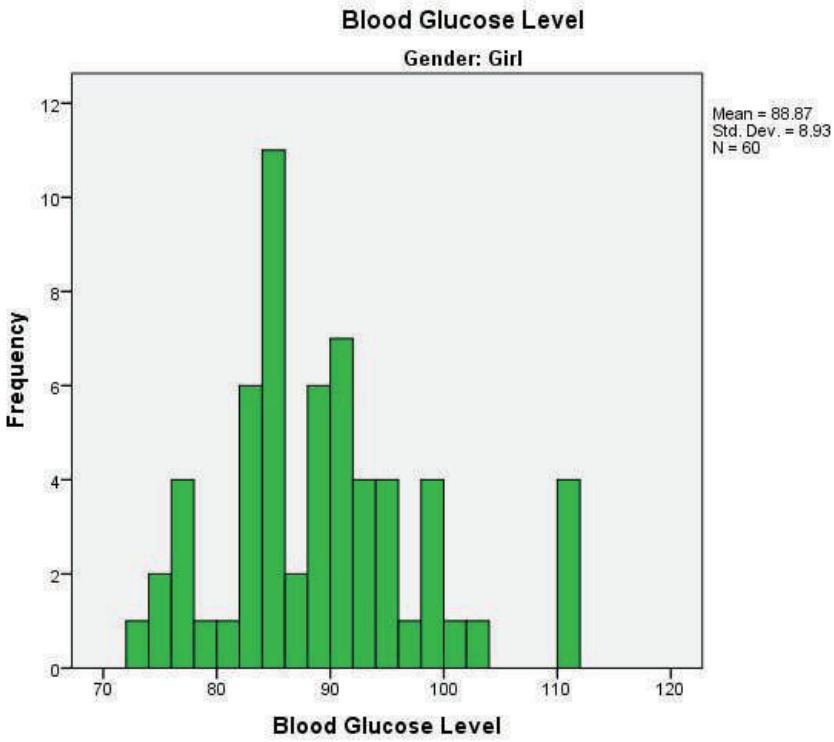
Graph 1: Pie chart representing frequency distribution of age of study population. Blue represents the frequency of 2 year olds, green represents frequency of 3 years olds, beige represents the frequency of 4 year olds, purple represents the frequency of 5 year olds and yellow represents the frequency of 6 year olds. 38.52% of the participants were 3 years old, 31.15% were 4 years old, 15.17 were 2 years old, 12.30 were 5 years old and 2.46% were 6 years old.



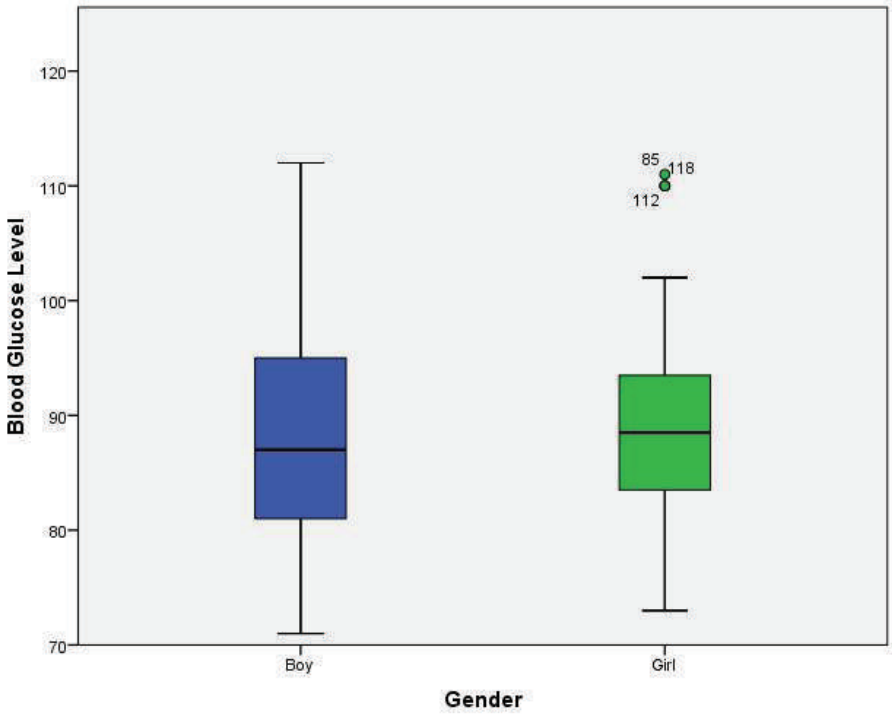
Graph 2: Pie chart representing frequency of gender of population who underwent treatment under general anesthesia. Blue represents the participants who were boys and green represents the participants who were girls. 50.82% of the participants were boys and 49.18 were girls.



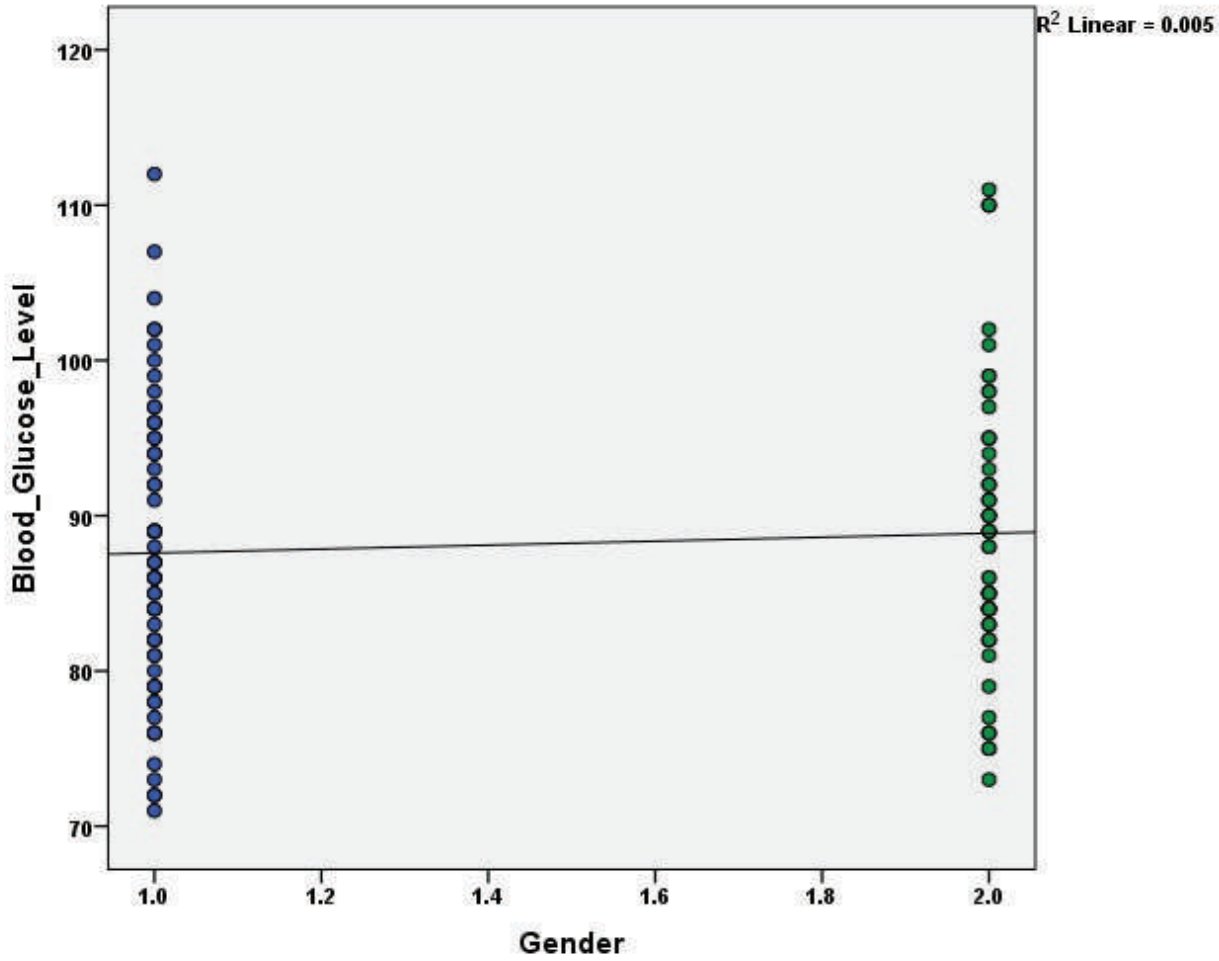
Graph 3: Histogram depicting Frequency of different blood glucose levels in boys. The mean blood glucose level was found to be 87.6±9.324 mg/dl.



Graph 4: Histogram depicting Frequency different blood glucose level in girls. The mean blood glucose level was found to be 88.87±8.93 mg/dl.



Graph 5: Box plot depicting the Correlation between blood glucose levels and gender. X axis represents the different genders of the participants of the study and the Y axis represents the blood glucose level of participants. There was no difference in blood glucose levels between boys and girls with ECC. Point biserial correlation coefficient was elicited and value was found to be .07 (close to zero) suggesting no relationship/ correlation between blood glucose levels of boys and girls with ECC.



Graph 6: Scatter plot depicting the Correlation between blood glucose levels and gender. X axis represents the different genders of the participants of the study and the Y axis represents the blood glucose level of participants. There was no difference in blood glucose levels between boys and girls with ECC. Point biserial correlation coefficient was elicited and value was found to be .07 (close to zero) suggesting no relationship/ correlation between blood glucose levels of boys and girls with ECC.

A study done by Rudesil et al showed the normal value of blood glucose level in children to be between 60 to 100 mg/dl [14].The data for this retrospective study was based on residents of Chennai seeking treatment at a university hospital in Chennai. Currently, there are no existing studies investigating the relation of blood glucose level to incidence of ECC. Since all data was included without a sorting process, no bias was expected in selection of patients. The current study aimed to assess whether any variation in the blood glucose levels existed in ECC patients.

A study done by Bansal et al showed a strong association of anemia with ECC due to iron deficiency [20] Ling et al also showed a similar result in one of his studies [21] where he showed a correlation between ECC and atherosclerosis. These associations show that there can be systemic manifestations as a result of ECC.

Diet has been shown as one of the most consistent etiological factors in ECC. A study done by Hu et al showed a positive correlation between dietary patterns and ECC showing that diet plays an important factor in determination of the spread of ECC. [22]. Due to the major role played by carbohydrate rich diet in etiology, the estimation of blood glucose levels and any variations can be suspected, hence this study was conducted.

Usually, pulpectomy is done for the treatment of caries which involve the pulp. [23,24]. A survey showed 50% of its participants to be using rotary instruments while doing a pulpectomy. [25] A study showed ProTaper rotary files had significantly less instrumentation time when compared to other instrumentation methods. [26] However a study by Lakshmanan et al showed that the Kedo-S rotary system provided the best quality of root canal filling in the shortest instrumentation time.[27]

The extent of ECC in children can also be due to increased neglect of dental care. A study done by Gurunathan et al, showed that there was a higher dental neglect score in people with secondary education. [28] This could lead to decreased awareness on the importance of oral health, especially in childhood. Proper care should be taken during the development of dentition to prevent oral hygiene issues. This includes brushing regularly, rinsing the mouth after every meal and having regular dental checkups. A study by Govindaraju et al showed chewable toothbrushes to be a good alternative for pediatric patients. [29]. Studies like these show different methods to appeal to the psychology of a child to satisfy him while making sure that his oral hygiene is maintained.

Since the consumption of glucose in excess leads to increased glucose in the blood over a short period of time , it may be possible to see a link between blood glucose level and ECC. But it will not be possible in this study as there was no normal control group to compare it with i.e the blood of children who do not have ECC was not taken due to ethical reasons. Studies by Watson et al showed the mean glucose level of children before they underwent surgery to be 100 mg/dl. Since articles comparing blood glucose levels of diseased children are very few and are old, no conclusion can be drawn based only on data on one group. However, our study showed that more than 10% of the children had blood glucose levels above 100mg/dl. This may be a reason for concern and also a predictor for future type 2 Diabetes in children when they grow into adults. Unless and until longitudinal studies which follow up children till adulthood and probing their diabetic status are done we won't be getting conclusive evidence.

Furthermore, the limited inclusivity of the population of this study considering its focus only on residents of Chennai could have played a part in the results acquired. A future study showing the correlation of blood glucose levels of children with ECC and a control group with normal children without ECC might yield more prominent results.

Conclusion

Within the limitations of this study, it was found the patients with ECC had blood glucose levels within the normal limits($p>0.05$) with a mean value of 88 mg/dl, however 10% of the study population had an RBS value 100mg/dl which can be a risk factor for future diabetes. [Figure 6] A clear association if present can only be established with further longitudinal studies.

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Source of funding : Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Caries of First Primary Molars Requiring Glass Ionomer Cement Versus Composites as Restorative Materials

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Abstract

In the present study comparison of the clinical preference of glass ionomer versus resin composite in primary molars by dentists was performed. This was done to evaluate which material will provide better clinical performance for pediatric patients based on esthetic , functional and biological properties.Data collected from a patient management software. A total of 1609 children were included in the study who required class 1 restorations . The data was tabulated on an excel sheet and transferred to SPSS software where statistical analysis was further performed. 232 children were indicated for composite restoration and 1377 children were indicated for glass ionomer cement for class 1 restorations. There was no statistical significant difference between both the restorative material groups (p>0.05).We observed that dentists preferred glass ionomer cement over composite to restore class 1 cavity in primary molars.

Keywords*: Glass ionomer cement, Composite, Class 1 restoration, Primary molars.*

Introduction

The field of dentistry has witnessed a vast range of evolution from diagnosing a variety of oral diseases to treating these conditions.[1,2,3] ,[4] Barring this dental caries still remains the most common disease found in children.[5,7,8] Class 1 restorations are caries affecting pits and fissures on occlusal, buccal and lingual surfaces of the permanent as well as primary teeth.

For several years amalgam was the preferred material for restoration of decay in primary molars which provided satisfactory results. Due to concerns related to aesthetics patients tend to not prefer amalgam as a restorative material. Dentists also do not prefer amalgam due to concerns related to toxicity.[9] Many alternatives to amalgam have been frequently used to restore cavities. Materials with increased aesthetics and strength are preferred.

Behaviour management is the most commonly faced challenge by dentists.[10–13] If children are uncooperative many problems are faced by dentists while restoring the cavity , the most common being moisture control.[14] Studies involving identifying a suitable restorative material for a particular age group is limited.

Resin composites have advantages like aesthetics, minimal cavity preparation and also has a certain amount of clinical reliability[15]. Glass ionomer cement on the other hand has certain advantages like anti-cariogenicity due to its fluoride release.[16] It has a similar coefficient of thermal expansion to dentin. It is not as technique sensitive as composite resin.

In the view of this available data this study was conducted to evaluate these two restorative materials on the basis of preference by dentists to restore first primary molars.

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Materials and Methods

The study was based on a university setting . The ethical approval was given by the Institutional Scientific Review Board , Saveetha Dental College and Hospitals . There were three reviewers involved in the study which comprised of one external reviewer. The data was collected from a patient management software where the input was inserted as “class 1 restoration” under the heading “treatment done” in primary molars.

The data was then tabulated on an excel datasheet and sorted according to requirements.

A total sample of 1609 children was obtained. The type of material used had to be sorted from the data sheet. The data after being verified by an external reviewer was imported to SPSS software and statistical analysis was performed.

Statistical analysis

Descriptive analysis was used to assess the prevalence rate of both the restorations used.

Chi square test was performed to compare the prevalence rate between both the groups. All analysis was performed using statistical software (SPSS inc version 2.0, Chicago IL USA) and p value less than 0.05 was considered statistically significant.

Results and Discussion

Out of a sample of 1609 class 1 restorations, 1377 were restored with glass ionomer cement and 232 were restored with composite resin. Out of all the primary molars 657 were maxillary primary first molars and 952 were mandibular primary first molars.

There was no statistically significant difference between both the type of restorative material and primary first molar with class I cavity (p value is 0.273) (figure 1 and table 1)

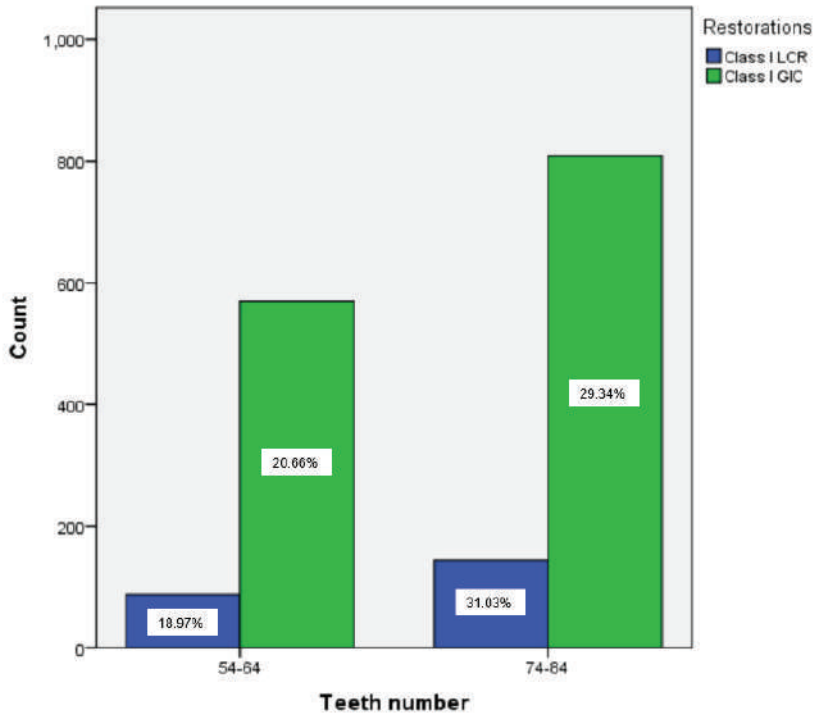


Figure 1: Bar graph representing the association between the type of restorative material and first primary molars with class I cavity, where blue represents composite restoration and green represents glass ionomer cement restoration. X axis represents the arch in which the teeth were restored and Y axis represents the number of teeth included in the study. There is no significant difference between the type of restorative materials and the first primary molars with class1 cavity. Chi square test value is 1.2012 and the p value is 0.273. ($p>0.05$). Hence, it is not significant.

Table 1 : Chi square test to represent comparison between both the type of restorative material and first primary molar with class I cavity.

	value	df	P value
Chi square	1.2012	1	0.273
Likelihood ratio	1.21	1	0.271

Dental caries is an oral health condition affecting most of the children.[17] It is essential to treat caries in the primary dentition in order to inhibit the spread of microorganisms and to maintain space for the permanent tooth to erupt correctly into position. Carious teeth when not treated for a long time might lead to pain and affect their quality of life too. It is very essential to prevent caries at the earliest stage for this reason. Techniques to restore carious primary teeth have changed over the past few years as new materials have been developed which are favourable to the dentist as well as the child.. The most meaningful way of assessing the efficacy of any restorative material is by clinical trials. It is very essential to identify , assess and evaluate materials for restoring primary teeth. Managing an uncooperative child is the biggest challenge faced by pediatric patients. This makes it essential for usage of a material that is less technique sensitive to assist pediatric dentists while restoring primary teeth. Type of restoration to be used always depends on the location and the extent of the caries.

Composite is considered esthetic but is a highly technique sensitive material. It is essential to maintain moisture control while restoring teeth hence a material should be selected based on this criteria.[18]Glass ionomers on the other hand have potential qualities over few other restorative materials. These include fluoride release and chemical bonding.In a systematic review done by Nedelijkovic it was observed that composite resin was associated with secondary caries.[19]

Restorations ensuring good clinical outcome is challenging while restoring primary teeth.

Restoration of primary teeth is different from that of adults due to factors like behaviour of the child and handling properties.[20]Primary molars are considered to be the best space maintainers and early loss of these teeth may lead to the permanent molar encroaching into the space and subsequent malocclusion.Space In the present study it was noted that out of 1611 class 1 restorations , maintenance of dentists preferred to restore 1379 teeth with glass ionomer cement. The reason for this might be because of the numerous properties of glass ionomer cement being favourable for primary teeth.Fluoride releasing properties allows glass ionomer cement to prevent demineralization and promote remineralisation. The adhesive nature of glass ionomer cement allows better retention and minimises microleakage.Its ease of manipulation is also one of the factors that might lead to its preference in restoring primary teeth. The manipulation of glass ionomer cement is extremely flexible and it allows dentists to manipulate or alter its physical properties for a particular dental application.All the advantages of glass ionomer cement makes it one of the most preferred materials for restoring primary teeth despite composite resin being highly esthetic.

Multiple operators were involved in deciding the treatment plan for the entire sample size making it a limitation of the study. Another limitation for the present study was that reasons for the preferred material for the tooth to be restored by the dentist was not assessed.

Further studies can be performed with a larger sample size. More parameters can be assessed and different materials can be used and compared for restoration. This will help us in selecting an ideal material to restore primary teeth.

Conclusion

In the above study , we observed dentists preferred glass ionomer cement over composite for class 1 restorations in primary molars. It is essential to perform further studies related to this topic in the future in order to select an ideal material for restoration of primary teeth.

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Source of Funding : Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Knowledge and Awareness on Infant Oral Health among Parents

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Abstract

One of the most common problems that children and infants face, which is due to lack of awareness and knowledge on the same, is Early Childhood Caries. If left untreated, this could complicate various diseases and this signifies the importance of a dental examination in infants. The main aim of this study is to analyse the knowledge and awareness on infant oral health among parents. A self administered questionnaire was circulated via an online google forms link among 150 parents and the statistical software used was SPSS version 22. The statistical test used was the descriptive statistics and Chi square test. The p value less than 0.05 was considered statistically significant. The confidence level of the study was set to 95%. In our study, 40.67% felt that the infant's oral cavity must be cleaned with wet cotton. 76.67% felt that infant's tooth development affects general health and 44.67% that parafunctional habits affect tooth development. Among the different educational groups of parents who had answered, the PG level educated parents had the highest knowledge among infant oral health. The PG level parents had the highest knowledge and awareness on infant oral health while secondary school level had the least knowledge. In general, the South Indian parents had a moderate level of awareness but, however still presented a need to increase this knowledge and awareness levels.

Key words: *Awareness, oral health, infant, parent, Early Childhood caries, level of education.*

Introduction

The practice of infant oral health refers to the task of keeping one's mouth clean and free from diseases caused by bacteria. Typically, children belonging to the age group 1 year and below fall under the category of infants.[1] Dental assessments for infants, even before the eruption of the primary teeth is extremely essential as it helps in the detection of Early Childhood Caries which if left untreated can complicate to various dental diseases. Any infant oral care program must include the potential risk assessment on risk factors associated with ECC, preventive treatments, education of parents on the importance of oral health and establishment of a positive impression about the dental clinic towards the child.[2] Across the nation, due to lack of proper awareness and lack of early assessment, 54.3% of infants suffer from ECC which not only affects the oral health but also the general health of the individual. To enhance this field, 10% of funds from the World Health Organisation is donated to infant oral health.[3] For both infants and adults, oral health implies the cleaning or maintaining the cleanliness of both the lips and mucosa. In infants, special care has to be taken to ensure that no food debris is left behind after a meal. The maintenance of hygiene helps in the proper development of the primary teeth and better oral health with suppressing the possibility of acquiring a heart disease as the infant develops in age.[4]

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Scientific researches suggested that poor knowledge and attitude among parents are the major reasons for bad oral health among infants. The reason behind this lack of awareness arises from the difference in socio-economic backgrounds and the poor literacy rates among parents.[5] The majority of parents show or possess a wrong attitude and mindset towards dental health care and in turn become the cause for their child's poor oral health. On conducting surveys to test the existing knowledge it was found that only 68% of the parents were aware of the methods used to clean the infant's oral cavity and the time from which this practice should be initiated.[6] Among other studies conducted, medical students had an opinion that tooth decay was one of the leading causes for ECC and the inability to express this led to the lack of dental treatment.[7] In most cases, family physicians and pediatricians often see the child upto 6 times before the child turns 2 years of age. These appointments are crucial to increase the awareness and opportunities of infant oral health and a need to spread awareness on the same.[8] Most parents however, prefer the choice of their pediatricians for a dental examination which usually occurs before 1 year of age. Pediatricians are also considered as oral health service providers.[9] The aggregation of bacteria can be reduced and a foetus' oral health can be improved by reducing the pathogenic bacteria. Lifestyle modification in association with this and therapeutic intervention must be practiced.[10]

Despite the previous researches conducted, they focused only on clinical health care and there were no standard measures for a defined and proper oral health care condition. The present study was conducted to eliminate these limitations and provide a proper standard for infant oral health. Proper awareness of UIP reduces millions of child deaths every year. Previously our team has conducted numerous original studies [11 – 17] and surveys [18 – 25] over the past 5 years.The main aim of this research is to analyse the knowledge and awareness on infant oral health among parents in the South Indian population and the need to spread awareness on the same.

Materials and Methods

The present study population consisted of parents in the South Indian population through an online setting. The study design was a questionnaire based cross sectional study, conducted in 2020 and approval was obtained from the Institutional Review Board. The total number of people involved in the study was 150 participants. The sampling method used was a simple random sampling. The following questionnaire was circulated via an online google forms link:

The questionnaire consisted of close ended questions and the validity checking was conducted by the faculty members of the institution. Google forms were used for the collection of data and the statistical software used was the SPSS software version 22. The statistical analysis was descriptive statistics to summarize demographic data and chi square analysis to analyse survey data. The p value less than 0.05 was considered as statistically significant and the confidence level taken was 95%.

Results and Discussion

Table 1: Table showing Frequency of responses

S. No	Question	Options	Response Percentage
1.	When should an infant's first dental visit be?	Immediately after birth	22%
		Within 6 months of eruption of first tooth	22.67%
		Whenever necessary	51.33%

Cont... **Table 1: Table showing Frequency of responses**

2.	How would you clean an infant's oral cavity?	Wipe with dry cloth	22%
		With wet cotton	40.67%
		With cotton and toothpaste	14%
		Soft toothbrush and toothpaste	15.33%
		Don't know	8%
3.	Do you think infant's tooth development affects general health?	Yes	76.67%
		No	23.33%
4.	Is it necessary for a parent to know the development rates of an infant's tooth?	Yes	78%
		No	22%
5.	Which of the following do you think can lead to irregular infant teeth?	Thumb sucking/tongue thrusting	36%
		Mouth breathing	19.33%
		Both	44.67%
6.	Does genetics affect the development of primary teeth?	Yes	79.33%
		No	20.67%
7.	Can prolonged breastfeeding practice have a potential increase on the risk of dental caries?	Yes	60%
		No	40%
8.	Do you think early loss of milk teeth can lead to malocclusion of permanent teeth?	Yes	66.67%
		No	33.33%
9.	Which of the following methods are useful in preventing ECC?	Maintaining good oral habits in infants	61.33%
		Anti caries vaccine	20.67%
		Both	18%

Cont... **Table 1: Table showing Frequency of responses**

10.	Do you think primary teeth are as important as permanent teeth?	Primary teeth are not important at all	18.49%
		Primary teeth are slightly important	19.18%
		Both are equally important	38.36%
		Yes	10.27%
		No	6.85%
		Don't know	6.85%
11.	When does the kid's first teeth begin to emerge?	Before 1 year	54.36%
		1st year	28.85%
		2nd year	2.68%
		3rd year	7.38%
		Don't know	8.72%
12.	When should a mother start cleaning her child's teeth?	When the first milk tooth erupts	55.33%
		When all the milk teeth have erupted	25.33%
		When the first permanent tooth erupts	15.33%
		Don't know	4%
13.	What size of toothpaste should be placed on a child's toothbrush?	Pea size	54.67%
		Bean size	24%
		Half the length of the toothbrush	17.33%
		Don't know	4%
14.	At what age should a child start brushing unassisted?	1-3 years	31.33%
		4-6 years	38%
		7-9 years	24.67%
		>15 years	3.33%
		Don't know	4%

Cont... **Table 1: Table showing Frequency of responses**

15.	Food plays an important role in oral health?	Yes No Don't know	74.67% 20.67% 4.67%
16.	Does sugar consumption play a major role in oral hygiene?	Yes No Don't know	66.67% 26.67% 6.67%
17.	Fruit juices are more carcinogenic than whole fruits?	Yes No Don't know	68.67% 22.67% 8.67%
18.	Frequency of sugar consumption plays a greater role in producing caries than does the total amounts of sugar consumption?	Yes No Don't know	68.67% 22.67% 6.67%
19.	Retentiveness of food in the oral cavity is linked to dental caries?	Yes No Don't know	66.67% 26% 7.33%
20.	Does fluoride prevent dental caries?	Yes No Don't know	67.33% 22.67% 10%
21.	Do you think there is a need to improve parent knowledge on infant oral health?	Yes No	88.67% 11.33%

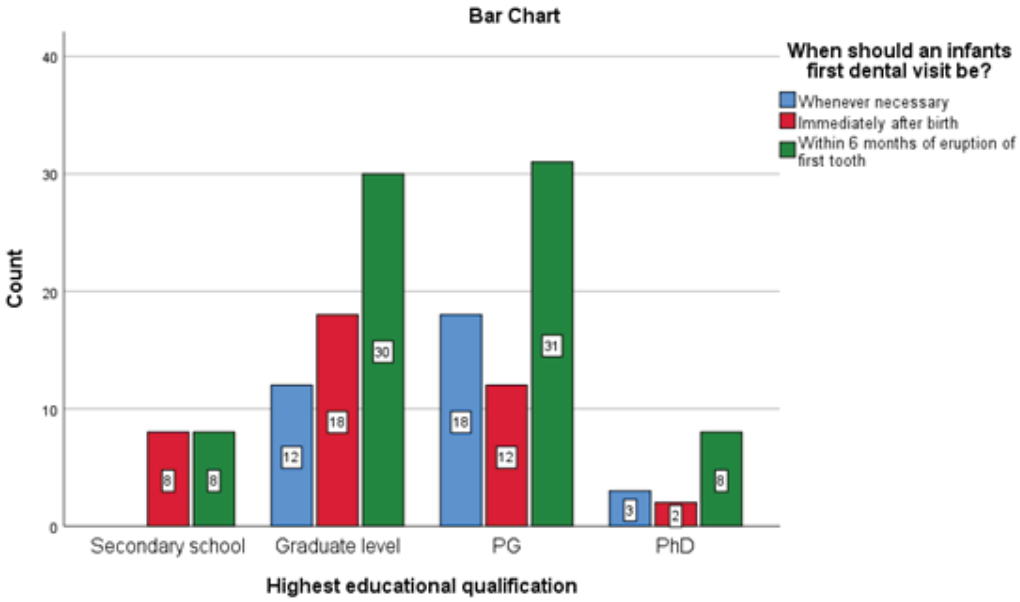


Figure.1- Bar graph showing comparison of responses between highest educational qualification and infant's first dental visit. X-axis represents the highest educational qualification of parents and y-axis represents the number of parents responded. Blue colour represents whenever necessary, red colour represents immediately after birth and green colour represents within 6 months of eruption of the first tooth. PG level of educated parents showed more awareness (31) on an infant's first dental visit, but it was not statistically significant (Chi square test p=0.096 - Indicating statistically not significant).

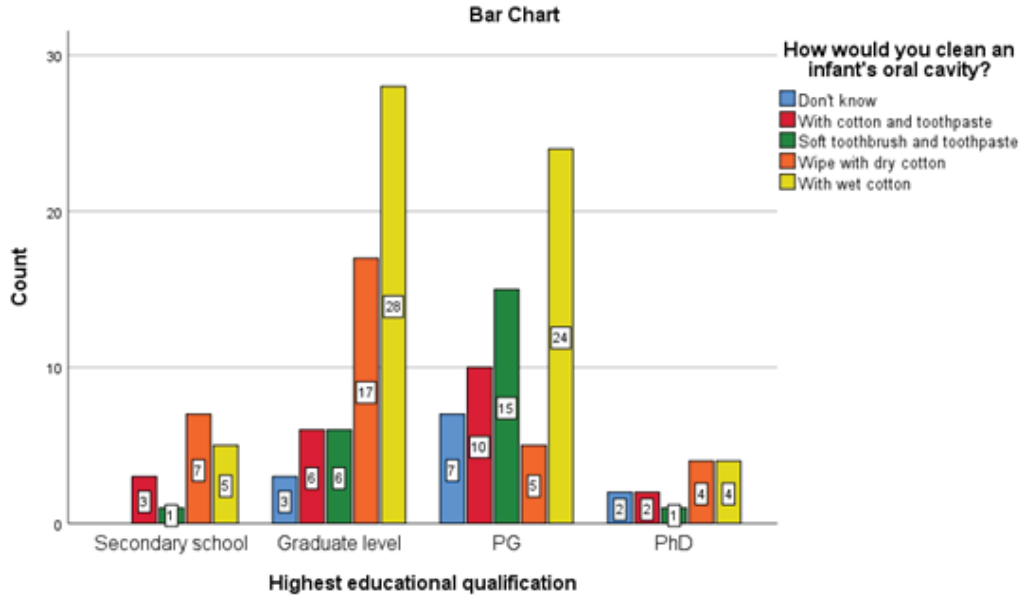


Figure.2- Bar graph showing comparison of responses between highest educational qualification and cleaning of an infant's oral cavity. X-axis represents the highest educational qualification of parent and y-axis represents the number of parents who responded. Blue colour represents don't know, red colour represents cotton and toothpaste, green colour represents soft toothbrush and toothpaste, orange colour represents wipe with dry cotton and yellow colour represents wet cotton. Graduate level educated parents showed more awareness (28) on cleaning an infant's oral cavity, but it was statistically significant (Chi square test p=0.034 - Indicating statistically significant).

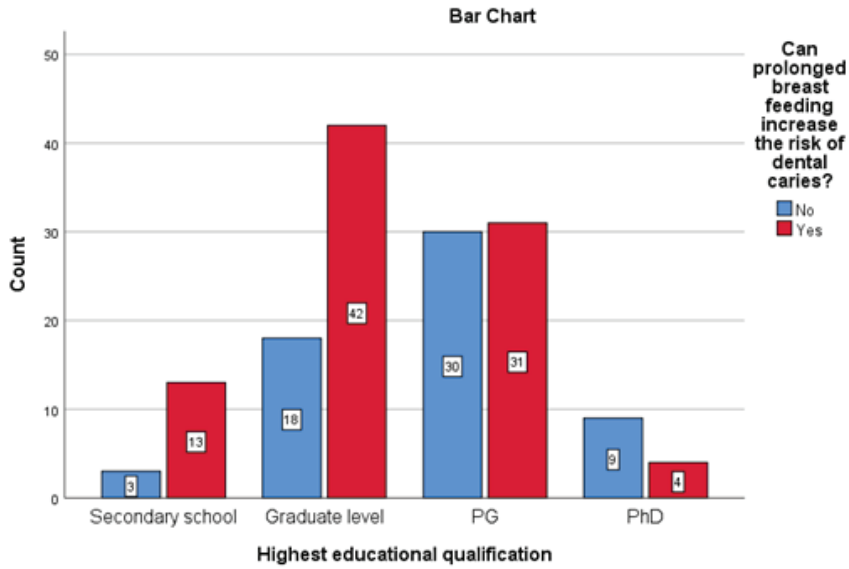


Figure.3- Bar graph showing comparison of responses between highest educational qualification and relation between breastfeeding and caries. X-axis represents the highest educational qualification of parent and y-axis represents the number of parents who responded. Blue colour represents no and red colour yes. Graduate level educated parents showed more awareness (42) on the relation between breastfeeding and caries, comparison was statistically significant (Chi square test p=0.006 - Indicating statistically significant).

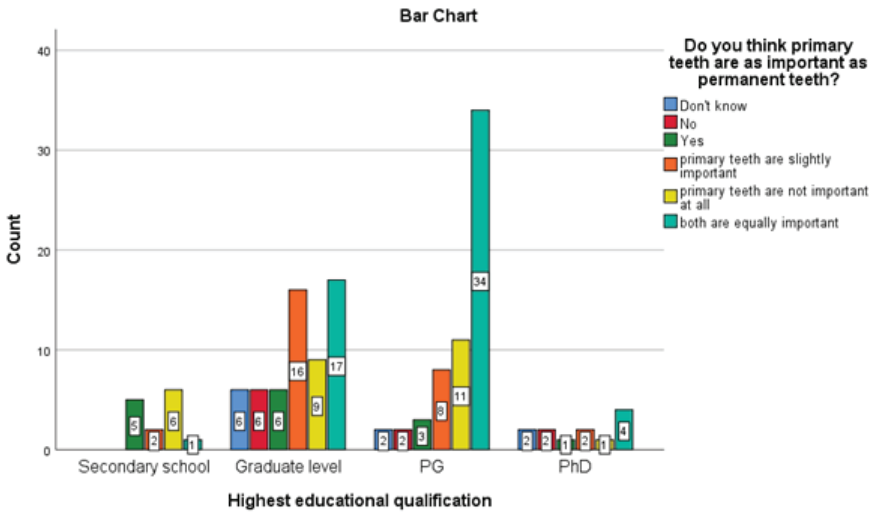


Figure.4- Bar graph showing comparison of responses between highest educational qualification and importance of primary and permanent teeth. X-axis represents the highest educational qualification of parent and y-axis represents the number of parents responded. Blue colour represents Don't know, red colour represents No, green colour represents Yes, orange colour represents primary teeth are slightly important, yellow colour represents primary teeth are not important at all and teal colour represents both are equally important. PG level educated parents showed more awareness (34) on the importance of primary and permanent teeth, and comparison was statistically significant (Chi square test p=0.001 - Indicating statistically significant).

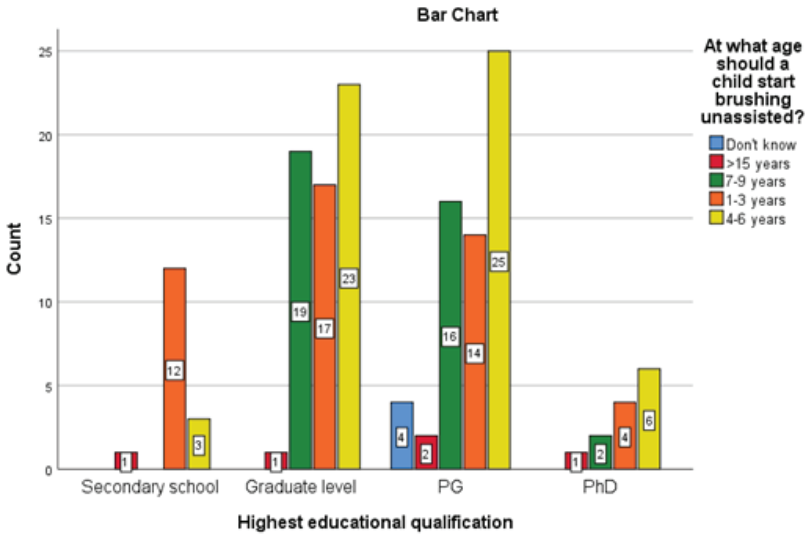


Figure.5- Bar graph showing comparison of responses between highest educational qualification and association between age and unassisted brushing. X-axis represents the highest educational qualification of parent and y-axis represents the number of parents who responded. Blue colour represents Don't know, red colour represents >15 years, green colour represents 7-9 years, orange colour represents 1-3 years and yellow colour represents 4-6 years. PG level educated parents showed more awareness (25) on association between age and unassisted brushing, but it was not statistically significant (Chi square test p=0.060 - Indicating statistically not significant).

On collecting and analysing the results obtained, it was found that 25.2% of the population belonged to the age group 30-39 years and 41.1% of the population had a highest educational qualification of PG level. 21.33% of the parents belonged to the income group ranging from 75000-100000 Rs

51.3% of the parents felt that an infant's first dental visit should be within 6 months of the eruption of the primary tooth. 40.67% of parents had chosen to clean an infant's oral cavity with wet cotton and 76.67% that the infant's tooth development affects the general health. 22% of the parents felt that knowledge on the teeth development rates were unnecessary and 44.67% that various parafunctional habits can lead to irregular infant teeth. 20.67% of parents felt that genetics and development of primary teeth were unlinked. 60% were aware that prolonged breast feeding can lead to dental caries and 66.67% that malocclusion can be caused by the early loss of milk teeth. 61.33% of parents opted that good oral hygiene can prevent ECC and 38.36% that both primary and permanent teeth are equally important . 54.36% that an infant's tooth develops before 1 year and 55.33% choose that cleaning should occur when all the first milk teeth have erupted. 54.67% chose that pea size amount of toothpaste should be used to clean the infant's tooth . 38% of parents chose that a child should start brushing unassisted between 4-6 years of age and 74.67% that food was related to oral health. 66.675 of individuals felt that sugar consumption was related to oral hygiene and 68.67% that frequency of sugar consumption had a greater impact than amount of sugar consumed. 66.67% voted that retentiveness of food in the oral cavity was linked to caries. 67.33% were aware that fluoride could prevent caries and 88.67% that parent knowledge had to be improved. (Table 1)

On comparing the levels of awareness based on the highest educational qualifications among 150 South Indian parents, the following results were obtained. 31 Post-graduate level educated students had chosen that an infant's first dental visit should be within 6 months of the eruption of the first tooth (Figure.1) and 10 post graduate level parents felt that an infant's oral cavity must be cleaned with wet cotton and toothpaste (Figure.2) 50 postgraduate level parents voted that infant's teeth development affects general health. 48 post graduate level parents chose that parent knowledge

on development rates were essential and 28 postgraduate level parents that parafunctional habits can cause irregular teeth. 49 graduate level educated parents agreed that genetics affects tooth development and 42 graduate students that prolonged breast feeding can lead to an increased risk of dental caries (Figure.3). 41 graduate level educated level parents voted that early loss of milk teeth can cause potential malocclusion. 16 postgraduate level of education parents opted that anti-caries vaccine can prevent ECC and 34 of them voted that both primary and permanent teeth were essential (Figure.4).

35 post graduate level educated parents believed that the kid's first teeth begin to erupt before 1 year of age and 35 of them responded that cleaning of infant's teeth should begin when the 1st milk tooth erupts and 35 of the postgraduate level parents that a pea size amount of toothpaste should be used for cleaning. 25 post graduate level educated parents believed that 4-6 years was the ideal age for a child to start brushing unassisted (Figure.5). 46 graduate level educated parents felt that food was related to oral health while 41 of the postgraduate level educated parents felt that sugar consumption affects oral hygiene. 42 post graduate level educated parents were aware that retaining of food in the oral cavity was associated with caries and 42 post graduate educated parents knew that fluoride helps in preventing dental caries.

51.33% of the parents responded that an infant's first dental visit should be within 6 months of the eruption of the first tooth, 26.67% for immediately after birth and 22% for whenever necessary. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (20.66%) had chosen the correct answer i.e. within 6 months of eruption of first teeth. (Figure.1) The p value was found to be 0.096 which was statistically not significant.

40.67% of the parents chose to use wet cotton for cleaning the infant's oral cavity, 22% for dry cotton, 15.33% for soft toothbrush and toothpaste and 14% for cotton and toothpaste. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (6.67%) had chosen the correct method of cleaning i.e. with cotton and toothpaste. (Figure.2) The p value was found to be 0.034 which was statistically not significant.

76.6% of the parents were aware that an infant's tooth development affects general health and 23.3% that it doesn't. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (33.33%) had opted for the correct answer that infant's tooth development affects general health. The p value was found to be 0.104 which was statistically not significant. Similar results were obtained by Adimoulame, et al with 70% of the population who agreed for the same [26]. Overall, it can be inferred that most of the parents were aware that infant's tooth development affects general health.

78% of the parents felt that development rates of infant's teeth was essential and 22% that it was unnecessary . On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (32%) had opted for the correct answer that development rates of an infant's teeth were essential. The p value was found to be 0.405 which was statistically not significant.

19.3% of parents felt that mouth breathing can lead to irregular teeth, 36% for thumb sucking and tongue thrusting and 44.67% for both. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (19.33%) choose the correct option i.e. all of the above. The p value obtained was 0.137 which was statistically not significant. Similar results were obtained by P.M. Castelo, et al with 34.3% on parafunctional habits [27]. In general, it can be inferred that the majority of the parents were fully aware that parafunctional habits such as the above could cause irregular teeth.

79.33% of parents were aware that genetics affects primary teeth development and 28.67% were unaware. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (32.67%) had chosen the correct answer that development rates of the teeth are affected by genetics. The p value obtained was 0.331 which was statistically not significant.

60% of the parents knew that prolonged breast feeding practice can increase the risk of caries and 40% were unaware of this fact. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (28%) had correctly opted that prolonged breast feeding practices could lead to dental caries. (Figure.3) The p value was found to be 0.006 which was statistically significant.

66.67% of the parents were aware that early loss of milk teeth can lead to malocclusion of permanent teeth and 33.33% were unaware. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (27.33%) correctly chose that malocclusion could potentially be due to the early loss of milk teeth. The p value was found to be 0.428 which was statistically not significant. Similar results were obtained by A. Sanguida, et al with 77%. The above results lead us to the inference that most of the parents were aware that malocclusion has a direct relation with the early loss of milk teeth.

20.67% of the parents felt that anti-caries vaccines can prevent ECC, 61.33% for good oral habits and 18% for both . On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (27.33%) had correctly opted for both anti caries vaccine and good oral habits as methods of prevention of ECC. The p value was found to be 0.121 which was statistically not significant. Similar results were obtained by S.A. Mani with 53% for good oral hygiene [28]. Overall, it can be inferred that good oral hygiene can prevent early childhood caries which most of the parents were aware of.

19.18% of parents felt that primary teeth are slightly important, 18.49% that primary teeth are not important. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (22.67%) had chosen the correct answer i.e. that primary and permanent teeth are equally important. (Figure.4) The p value obtained was 0.001 which was statistically significant. Similar results were obtained by A. Alsheri with 62.63% agreeing that both primary and permanent teeth are essential. From the above discussion, it can be inferred that the awareness level of importance of primary and permanent teeth among parents were high.

28.65% of the parents chose that the kid's first teeth begin at 1 year, 2.68% of the parents for second year, 7.38% for third year. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (23.33%) had chosen the correct answer i.e. less than a year for emergence of the child's first tooth. The p value obtained was 0.098 which was statistically not significant.

15.33% of parents begin to clean a child's teeth when the first permanent teeth erupt, 25.33% when all the milk teeth have erupted, 55.33% when the first milk tooth erupts. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (25.33%) had chosen the correct answer i.e. cleaning of oral cavity should commence when the infant's first milk tooth erupts. The p value was 0.0483 which was statistically not significant. Similar results were obtained by A. Alsheri, et al with 63.34% for when milk teeth start to erupt. Overall, the inference obtained can be stated that the awareness of cleaning an infant's oral cavity was quite low.

17.33% of parents chose to use toothpaste for half the length of the toothbrush, 24% for bean sized and 54.67% for pea size. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (23.33%) had chosen the correct answer i.e. pea sized amounts of toothpaste for cleaning. The p value obtained was 0.171 which was statistically not significant. Similar results were obtained by A. Sanguida, et al with 45% for pea size. It can be inferred that most of the parents were aware of the amount of toothpaste to be used while cleaning an infant's oral cavity.

3.33% of parents choose above 15 years for a child to brush unassisted, 24.67% for 7-9 years, 31.33% for 1-3 years and 38% for 4-6 years. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (16.67%) had chosen the correct answer i.e. 4-6 years was the ideal age for unassisted

brushing. (Figure.5) The p value obtained was 0.060 and was statistically not significant. Similar results were obtained by Adimoulame Sanguida, et al with 46.8% for 4-8 years. Overall, it can be inferred that the majority of the parents were aware of the ideal age for unassisted brushing in children.

74.76% of the parents felt that food affects oral health and 20.67% that food doesn't affect oral health. Chi square analysis proved that On comparing parents of different educational levels, through chi square test, it was found that the maximum number of graduate educated parents (30.66%) had chosen the correct answer i.e. food affects oral health. The p value was found to be 0.641 which was statistically not significant. Similar results were obtained by A. Alsheri, et al with 56.2%. The above discussion obtains an inference that most of the parents were aware of the relation between food and oral health.

68.67% of parents were aware that sugar consumption affects oral hygiene and 22.67% that it doesn't. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (27.33%) had chosen the correct answer i.e. sugar consumption was related to oral health. The p value was found to be 0.954 and was statistically not significant. Similar results were obtained by A. Alsheri, et al with 95.4%. The inference obtained shows that a majority of the parents were aware of the ill effects of sugar consumption on oral hygiene.

68.67% of the parents felt that frequency of sugar consumption affected caries and 22.67% that it doesn't. On comparing parents of different educational levels, through chi square test, it was found that equal number of graduate and postgraduate educated parents (27.33%) had chosen the correct answer i.e. frequency of sugar consumption affected caries. The p value was found to be 0.492 which was statistically not significant. Similar results were obtained by A. Sanguida, et al with 92.4%. Overall, it can be inferred that most of the parents were aware that sugar consumption is linked to dental caries.

66.67% of parents felt that food in the oral cavity was linked to caries and 26% disagreed with this statement . On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (42.6%) had chosen the correct answer i.e. food retained in the oral cavity is linked to dental caries. The p value was found to be 0.775 which was statistically not significant. Similar findings were obtained by A. Alsheri with 92.2%. The inference obtained shows that a majority of the parents were aware that food retained in the oral cavity can lead to caries.

67.33% of parents felt that fluoride can prevent dental caries and 22.67% that fluoride does not prevent dental caries . On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate educated parents (28%) had chosen the correct answer i.e. fluoride can prevent dental caries. The p value was found to be 0.068% which was statistically not significant. Similar results were obtained by A. Sanguida, et a lwith 25.7%. Overall, most of the parents were aware that fluoride plays a major role in prevention of dental caries.

88.67% of parents felt that there was a need to improve parent knowledge on infant oral and 11.33% that there wasn't a need. On comparing parents of different educational levels, through chi square test, it was found that the maximum number of post graduate eșducated parents (36.67%) had chosen the correct answer i.e. there was a need to improve parent knowledge on infant oral health care. The p value was found to be 0.945 which was statistically not significant.

The present study however suffered a limitation due to less number of sample size and a homogenous population. The study provides a scope to improve the knowledge among parents particularly the secondary school level educated parents.

Conclusion

The present study concluded that the post graduate parents had the highest awareness level among infant oral health and the secondary school level educated parents had the least knowledge on the same. Among the South Indian population, there exists a moderately high level of awareness on the infant oral health practices and early childhood caries. Dental assessments for infants, even before the eruption of the primary teeth is extremely essential as it helps in the detection of Early Childhood Caries which if left untreated can complicate to various dental diseases.

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Conflict of Interest: The author declares that there was no conflict of interest in the present study.

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Analysis of Pulp Capping Procedures Done in Permanent Molars in A University Setting - A Retrospective Study

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Abstract

Direct and indirect pulp capping are the treatment choices to maintain integrity and health of dental pulp. Direct pulp capping is used when pulp is visibly exposed due to caries, trauma or iatrogenic causes. Indirect pulp capping generally performed on teeth with deep cavity preparations with or without caries remaining. Many authors have reported regarding pulp capping materials. The aim of this retrospective study was to analyse the practice of pulp capping procedure based on age, gender, tooth number and pulp capping materials. Data regarding pulp capping procedures that were recorded between June 2019 to March 2020 were retrieved from the patients management software of Saveetha Dental College, Chennai. Data that was retrieved then evaluated by 2 viewers. Cases with incomplete documentation or double entries were excluded from this study. Data from the Excel sheet was imported to SPSS for statistical analysis. Chi-square test was done to assess the association between age, gender, tooth types, type of pulp capping and pulp capping material. This study showed a statistically significant difference between age and tooth number; tooth number and pulp capping materials ($p<0.05$). It was seen that lower molars underwent significantly more pulp capping procedure in the age group of 18-30 years. In lower molars, calcium hydroxide was the widely used pulp capping material.

Keywords: *Pulp capping; retrospective; vital pulp; permanent molars*

Introduction

The primary objective for pulp treatment is to preserve integrity and heath of dental pulp. Even though a tooth can survive without a vital pulp, it is important to maintain vitality of the pulp. Therefore the objective of direct and indirect pulp capping is to treat reversible pulp injuries [1]. Soon after the eruption, permanent first molars may show extensive caries or are associated with hypoplasia [2] . If it is not treated properly eventually it will lead to the infections of the pulp and surrounding periapical tissues [3]. For clinicians, one of the most challenging clinical scenarios is the management of pulpally involved immature permanent molars. Root canal treatment would stop opposition of dentin along the canal walls and pulp chamber leaving the tooth with thin dental walls and make it prone for fracture almost in all teeth [4].

Indirect pulp capping treatment is preferred for teeth with deep carious lesions approximating the pulp but without signs or symptoms of pulp degeneration. The deepest layer of remaining carious dentin will be covered with a biocompatible material to avoid pulp exposure and additional trauma to the tooh. The most commonly used pulp capping materials are calcium hydroxide, zinc oxide eugenol paste (ZnO) and glass ionomer cements. The rationale for indirect pulp capping is that few viable bacteria will remain in deeper dentine layers and will be inactivated once the cavity

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has been sealed properly. The main objective for this procedure is to preserve pulp vitality, by arresting the carious process, promoting dentin sclerosis, stimulating formation or tertiary dentin and remineralize carious dentin. Few studies stated that dentin bonding agents can be used in indirect pulp capping [5]. It was seen that materials such as zinc phosphate cement, amalgam, zinc oxide eugenol cements, polycarbonate cements, glass ionomer cements, resin adhesives and cryanolates does not give predictable success for the procedures. Use of calcium hydroxide was superior but unpredictable [6].

More recently, better results for pulp capping procedures was achieved by using materials like mineral trioxide aggregate (MTA) and it was statistically significant when compared with formocresol. Removal of the microorganisms from the root canal system is necessary for the successful outcome of any root canal treatment [7]. The activation of irrigants using Endo activator can reduce postoperative effectiveness rather than using endo needles [8]. Remaining dentin thickness is an important parameter for the success rate of root canal treatment [9]. Irrigants play an important role in debridement and disinfection of the root canal space[10] . Dental avulsion can be considered as one of the most severe types of traumatic tooth injuries [11] . Dental traumatic can also lead to pulp canal obliteration [12]. The various reasons for dental trauma are falls, traffic accidents, fights and sports injuries [13]. Dental erosion can be referred to as loss of tooth structure due to chemical processes and not due to bacteria [14]. Dental caries are easily detectable and reversible at an early stage [15]. Non-carious loss of tooth structure in the cervical region is a very common clinical condition in elder patients [16]. A veneer can be referred to as a thin sheet of material which is placed in the front surface of the tooth for aesthetic purposes [17]. Clorhexidine is an antibacterial used as an antiseptic[18]. Matrix metalloproteinases (MMPs) act as an important role in the efficient tissue turnover and remodeling [19]. Effective root canal disinfection can be achieved by using sodium hypochlorite [20]. Diagnostic aids play a crucial role for a successful treatment plan [21]. The aim of this study was to analyse the practice of pulp capping based on age, gender, tooth number and pulp capping materials.

Materials and Methods

A university set up was selected for this study which provided easy accessibility for data and provided a population of similar ethnicity for this study. There were two reviewers for analysis of data for this study. The variables that were considered were tooth type, type of pulp capping, pulp capping materials used, age and gender of patients that underwent pulp capping. We reviewed patient records and analysed the data of 86000 between June 2019 to March 2020. The data with complete case history and documentations were included in this study. Incomplete cases or double entries cases were excluded from this study. Final sample size for statistical analysis was 183 patients. Data was then tabulated in Microsoft Excel Sheet and then transferred to SPSS software system. After entering data in SPSS software, variables were verified and association (Chi-square test) was evaluated between age and tooth number; age and types of pulp capping; tooth number and types of pulp capping; tooth number and pulp capping materials.

Results and Discussion

In the study, we have observed that there was a statistically significant difference between age and tooth number; tooth number and pulp capping materials with p value ($p<0.05$) (Table 1 & 6). Pulp capping procedures were most predominantly done in patients aged 18 to 30 years old. It was significant in lower molars than upper molars [Bar chart 1]. Patients aged 18 to 30 years old mostly underwent pulp capping compared to the population aged more than 50 years. Based on the association of age with type of pulp capping, it was seen that the population age of 18 to 30 years was the commonest age group that underwent indirect pulp capping followed by population aged 31-40 years, population aged 41-50 years and population aged 50 years and above [Bar chart 2].

Based on the distribution of gender against tooth number, it was observed that male and female populations mostly undergo pulp capping procedures mostly in mandibular molars than in maxillary molars [Bar chart 3]. In the current study, it was observed that in both genders, indirect pulp capping was done more compared to direct pulp capping but was not statistically significant ($p>0.05$) [Bar chart 4].

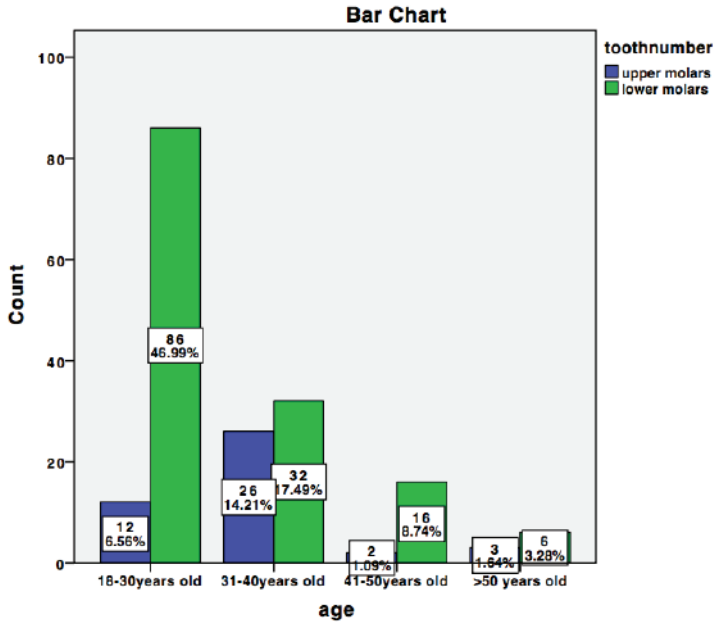
Based on the distribution of tooth number against types of pulp capping, it was revealed that indirect pulp capping was done more frequently in both upper and lower molars compared to the direct pulp capping but without statistical significance. [Bar chart 5]. Considering the choice of pulp capping material, it was observed that calcium hydroxide was used with a statistically significant difference compared to MTA in both upper and lower molars [Bar chart 6]. Overall, calcium hydroxide was the most predominant pulp capping material for both direct and indirect pulp capping. [Bar chart 7].

The current study analysed a total of 429 case history for pulp capping procedures and it was found that a total of 183 cases had undergone pulp capping procedures in molars in which 128 of them were male and 55 were female. Pulp capping was done mostly in patients aged 18 to 30 years old with patients over 50 years who minimally undergo pulp capping procedures. Studies have shown that in older patients , the typically more fibrous dental pulp has a reduced ability to overcome insult [22]. Pulp capping in molars with deep cavities were highly prevalent in mandibular jaw compared to maxillary jaw. This finding is similar to study from Luen et al [23] who evaluated ten-year incidence of dental caries in adult and elderly Chinese patients and observed mandibular molars were apparently most susceptible to caries. In addition, Manji and Fejer Skov [24] stated that lower molars were the most severely affected in the entire dentition.

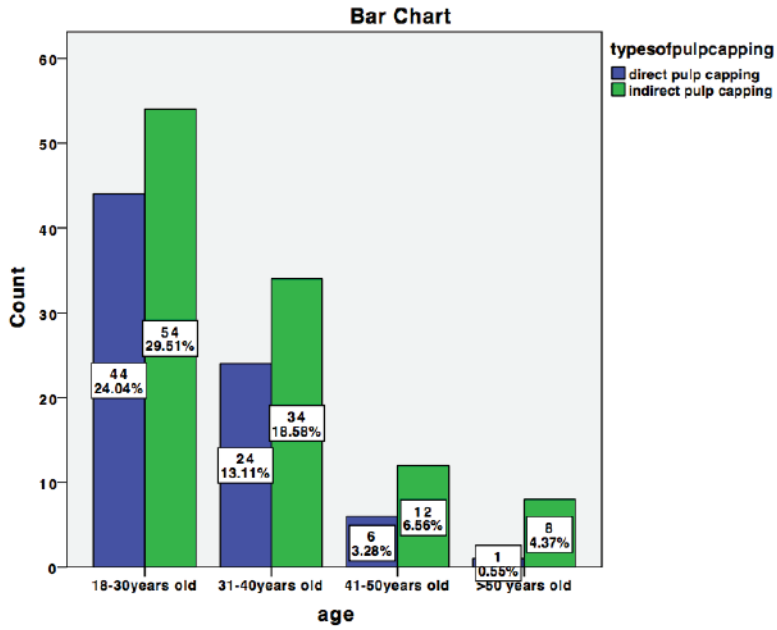
In the present study, calcium hydroxide was highly preferred for pulp capping material either in maxillary or mandibular teeth compared to MTA and Ca(OH)2+RMGIC. This can be explained by study from Baume et al [6] in which even though MTA is viable and perhaps better option than Ca(OH)2 and Ca(OH)2+RMGIC for pulp capping procedures, traditional powder or liquid formulations of MTA have significant drawbacks such as not user friendly, require very precise mixing, can lead to tooth discolouration, manipulation and placement can be challenging. Furthermore, traditional MTA formulations need long setting time, usually two to four hours, with one study that simulated intraoral condition finding that the MTA formulation tested (Pro root MTA) was still not set even after six hours [25].

It was observed that male population highly undergone pulp capping procedures compared to females. It also meant that the prevalence of dental caries in male much higher than females. This is in contrast with study from John et al [26] which explained that female populations usually had a higher prevalence for dental caries than male. Higher caries prevalence among females were often explained by factors such as earlier eruption of teeth among girls, therefore longer exposure of girls teeth to cariogenic and environment, easier access to food supplies by women and frequent snacking and pregnancy.

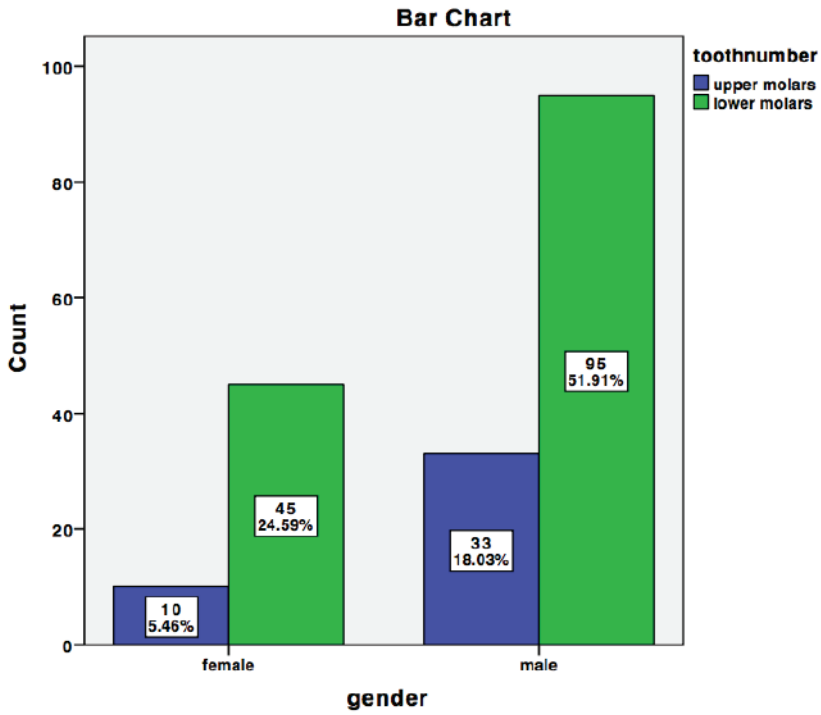
The limitations of this study is that the sample size is small and the retrospective design. Therefore, it does not provide results for the entire population. Further studies to be performed with large sample size. Prospective study designs with longer follow up to be done to evaluate the clinical performance of calcium hydroxide and MTA as pulp capping materials



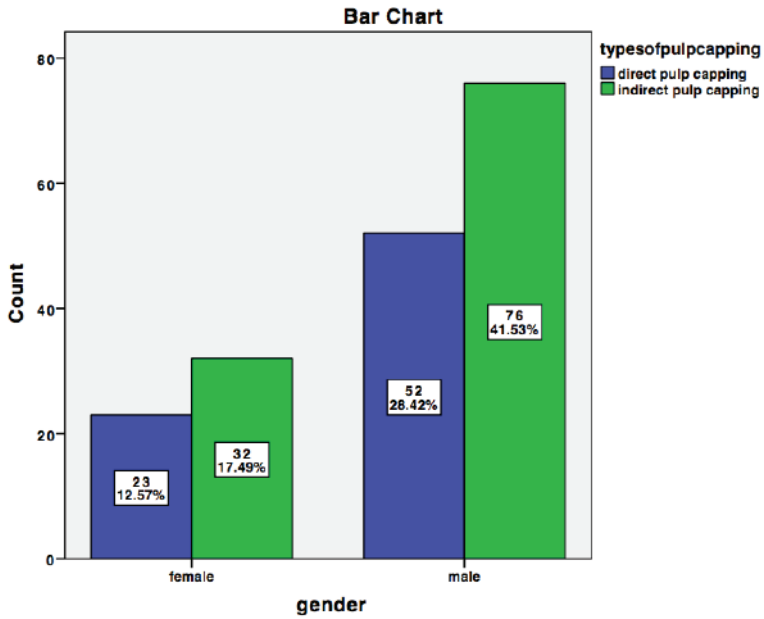
Bar chart 1: represents association between age and tooth number where blue colour denotes upper molars and green denotes lower molars. X axis represents the age and Y axis represents the number of pulp capping procedures. Chi square test was done and the association was found to be statistically significant. P value was 0.000 (<0.05). Pulp capping was done highest in lower molars (77%) in all age groups.



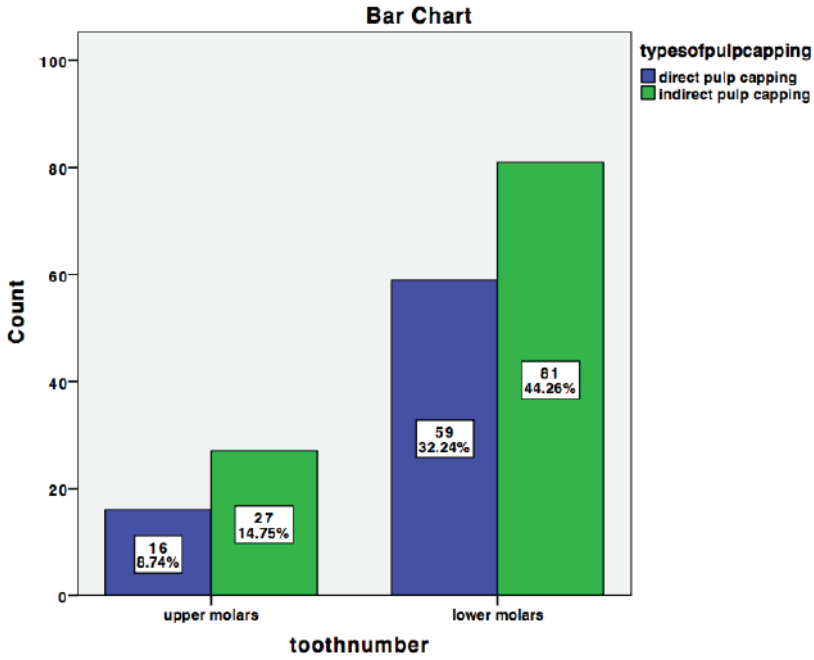
Bar chart 2: Bar graph representing association between age and types of pulp capping where blue colour denotes direct pulp capping and green colour denotes indirect pulp capping. X axis represents the age of the population and Y axis represents the number of pulp capping procedures. Chi square test was done and the association was found to be statistically not significant. P value was 0.223 (>0.05). However, indirect pulp capping was more commonly done in all age groups.



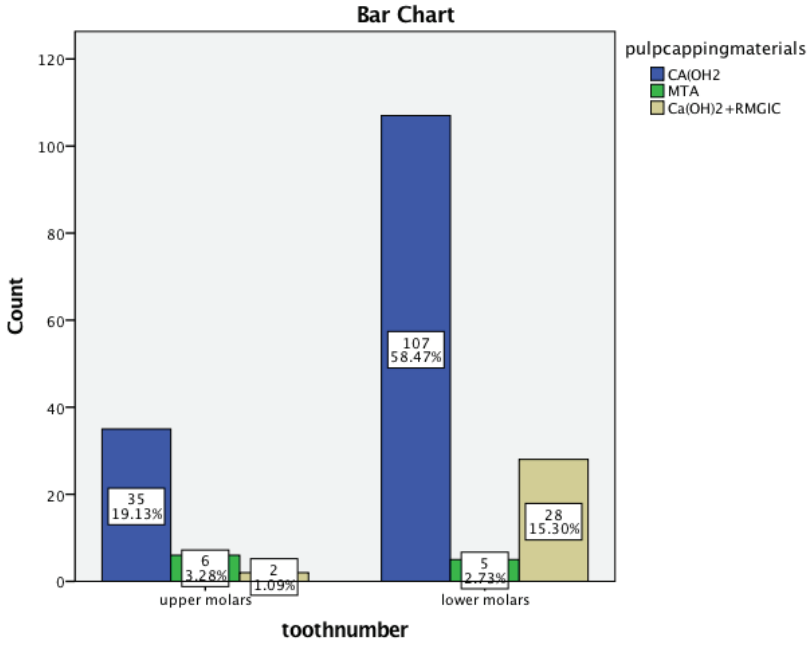
Bar chart 3: Bar graph representing association between gender and tooth number where blue colour denotes upper molars and green colour denotes lower molars. X axis represents gender of the population and Y axis represents the number of pulp capping procedures. Chi square test was done and the association was found to be statistically not significant. P value was 0.266 (>0.05). However, pulp capping procedure was done more in lower molars in both the gender



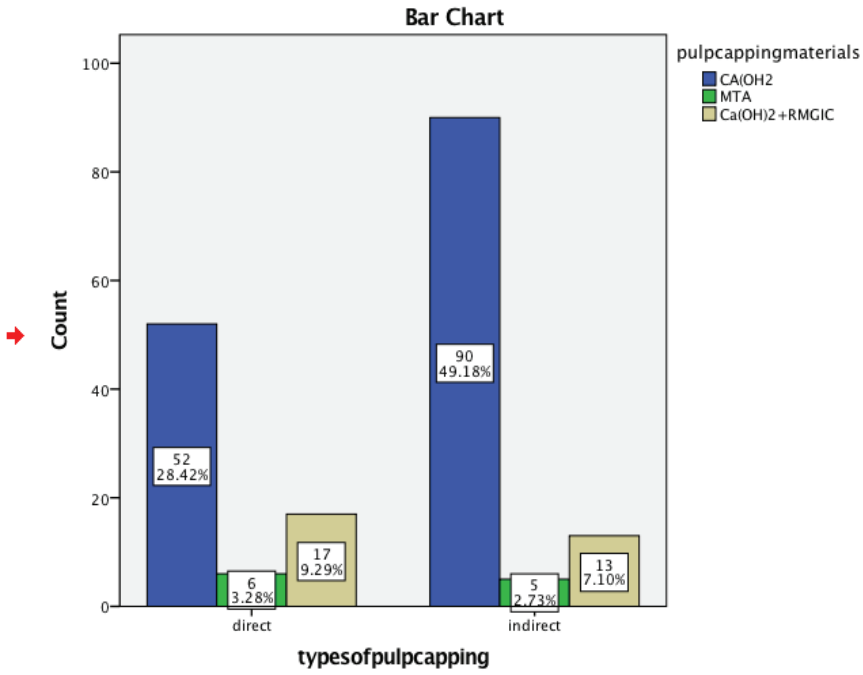
Bar chart 4: Bar graph representing association between gender and types of pulp capping where blue colour denotes direct pulp capping and green denotes indirect pulp capping. X axis represents gender of the population and Y axis represents the number of pulp capping procedures. Chi square test was done and the association was found to be statistically not significant. P value was 0.880 (>0.05). However, indirect pulp capping procedure was more commonly followed in both the gender



Bar chart 5: Bar graph representing association between tooth number and types of pulp capping where blue colour denotes direct pulp capping and green colour denotes indirect pulp capping. X axis represents tooth number and Y axis represents the number of pulp capping procedures. Chi square test was done and the association was found to be statistically not significant. P value was 0.5650 (>0.05). However, indirect pulp capping procedure was followed more than direct pulp capping in both upper and lower molars.



Bar chart 6: Bar graph representing association between tooth number and types of pulp capping materials where blue colour denotes Ca(OH)2, green denotes MTA and brown colour denotes CaOH)2+RMGIC. X axis represents tooth number and Y axis represents number of pulp capping procedures. Chi square test was done and the association was found to be statistically significant. P value was 0.05 (=0.05). Ca(OH)2 was the most commonly used pulp capping agent (78%) in both upper and lower molars.



Bar chart 7: representing association between types of pulp capping and pulp capping materials where blue colour represents Ca(OH)2, green denotes MTA and brown colour denotes Ca(OH)2+RMGIC. X axis represents types of pulp capping and Y axis represents number of pulp capping procedures. Chi square test was done and the association was found statistically not significant. P value was 0.082 (>0.05). However Ca(OH)2 was the most common pulp capping material used.

Conclusion

Within the limitations of the study, it can be concluded that lower molars underwent significantly more pulp capping procedure in the age group of 18-30 years. In lower molars, calcium hydroxide was the widely used pulp capping material.

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Conflict of Interest : Nil

Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320

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Awareness on the Methods to Overcome Depression among Undergraduate Students in An University Hospital Setting

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Abstract

Depression is a mental disorder characterized by persistent depressed mood , loss of interest in activities, significant impairment in daily life .It is a mood disorder of feelings of sadness,loss of anger and interfere in people's life activitiesThis is a prospective observational study .A well structured questionnaire is administered through online google forms link.This study is approved by the scientific review board of Saveetha Dental College. From the data statistics using SPSS, 67% were aware of methods to overcome depression, 34% felt behavioural changes is a symptom of depression, 27% agreed eating disorder is a symptom for depression and 8% agree that insomnia was a symptom for depression. The association between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant. We have observed that association between gender and responses to awareness on methods to overcome depression was statistically significant ($p = 0.043$). From this study we may conclude that many students are aware about depression and the methods to overcome depression.

Keywords: *Depression, stress, online survey, stress management techniques*

Introduction

Depression is a mental disorder characterized by persistent depressed mood , loss of interest in significant impairment in daily life .It is a mood disorder of feelings and sadness ,loss of anger,interfere in people's daily life activities.The people with clinical depression or other problems of depression can get old traditional treatments.In first stage of treatment they don't get expert help .In this stage they use self help materials like books,tapes called bibliotherapy[1]. About most of the population in the United States believe the Chronic Obstructive Pulmonary Disease is due to depression and anxiety[2] .Mobile applications are used as an excellent means to reduce depression.It is used to get rid of mental health problems[3]. The main symptoms of depression is anhedonia that is inability to experience pleasure from activities which are enjoyable.It is found that patients with heart disease ,liver disease,blocking of arteries have high depression compared to normal people.[2]

These days in the United States, an antidepressant Sertraline (Zoloft) is used to correct the imbalance of chemicals which reduces the depression.It gives good results in reducing depression[4].Music is known for its mending properties,it is used in relieving stress and depression.,music gives pleasant state and decreases the depression and decreases depression and stress .Ian Hackie etal felt that the burden of depression is due to economic and social cost[5].Paul Farrand felt that cognitive behaviour therapy can be used to reduce depression it helps to improve the emotional regulation for the Armed Forces.In one such study uses rats for diabetic activity[6] .Serotonin transporter inhibitor is used to treat depression by increasing the 5 hydroxytryptamine in the brain[7,8].

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ME Thase feels that psychotherapy can be used to treat patients who do not give response to antidepressant medicines[8]. For children the treatment suggested for depressive disorder is mostly play therapy[9]. Ladson Hinton feels that family members have key roles in the life of old depressed men as the expectation of old men would be care from their loved little ones[10]. Some feel depression can be reduced by dance movements as when we dance out,all the depression and stress vanishes away[11]. Some studies show even functional impairment causes depression;these impairment are the cause of depression[12]

Previous studies on nanoparticles[13,14], natural products[15–23,24], enzymatic studies[25] enriched my research knowledge and improved my passion for research.Nowadays the most common problem is depression.This research focuses on how to overcome depression and the methods to overcome depression .

Materials and Methods

This was a prospective observational study .The advantages of the study were economical,easy to create,wide reach,gathers large data,quick interpretation. The questions were circulated to the age group of 18 to 24 years and circulated among 100 undergraduate college students.The sampling method used in our study was simple random sampling .

The questionnaire was a self structured questionnaire administered through google forms to the participants. Each output variable was collected as ordinal data and the collected data were represented as pie charts.A statistical test was done using a software SPSS. Statistical test used descriptive analysis and frequency percentage. The list of output variables were demographic information,eating disorder,sleep patterns,stress management techniques. The method of representative of each output variable pie chart. The association between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION:

From the data statistics using SPSS; 67% of participants are aware of methods to overcome depression. 34% feel behavioural changes were a symptom for depression;27% felt eating disorder is a symptom for depression;8% agreed that insomnia was a symptom of depression. 33% agreed that being with friends was a method to overcome depression .19% agreed that social media as a method to overcome depression ;22% agreed that sleeping as a mode overcome depression. 70% agreed that depression is a mental health disorder characterized by depressed mood. 52% agreed that drugs reduce depression ;48% feel drugs do not reduce depression. 71% felt that depression leads to suicide.

We have seen the association between gender (X axis) and responses to awareness on the methods to overcome depression (Fig 1), responses to awareness on symptoms for depression (Fig 2), responses to awareness on methods to overcome depression (Fig 3), responses to awareness on the definition of depression (Fig 4),responses to awareness on antidepressant drugs (Fig 5),responses to awareness on suicidal tendency due to depression (Fig 6).

We have observed that association between gender and responses to awareness on methods to overcome depression was statistically significant ($p = 0.043$) and association between gender and responses to awareness on anti - depressive drugs was statistically significant ($p = 0.025$). The statistical associations between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant.

According to previous studies, 43.6% were aware about depression[26]. 83.4% felt behavioural changes are the major symptom of depression;95% felt depressive disorder is a disability[27] 64% agreed that depression affect memory [28]. In the study by Shukri it was important to know about childhood obesity as it is also a cause for depression [29] .71% feel depression leads to suicide 9.5% students attempted suicide in the previous study.This may be caused due to depression.

GRAPHS:

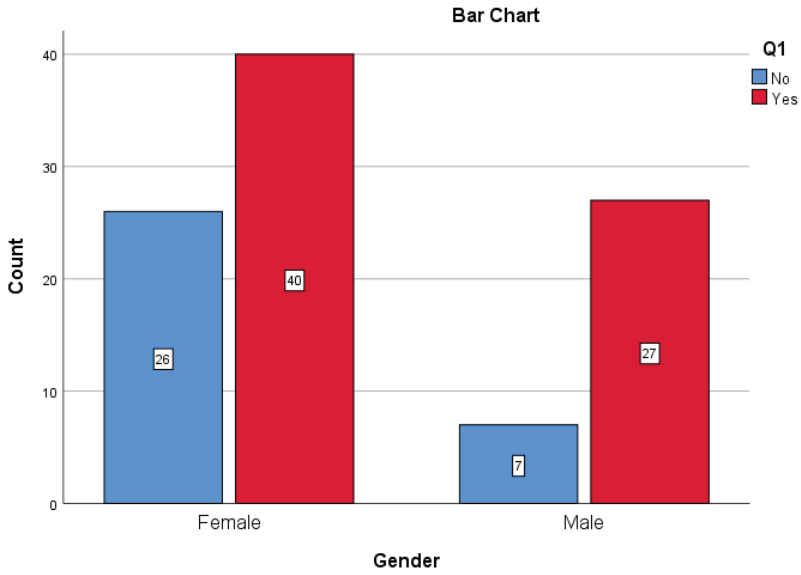


Figure 1: Bar chart depicts the gender wise responses regarding awareness on the methods to overcome depression. X axis depicts the gender and Y axis depicts the frequency of responses. Red colour bar denotes a positive response and blue colour denotes the vice versa.Majority, 40% of women reported they were aware, while only 27% reported they were aware on methods. Chi square test was done and it was found to be insignificant statistically (Pearson Chi square value = 3.589), P=0.058

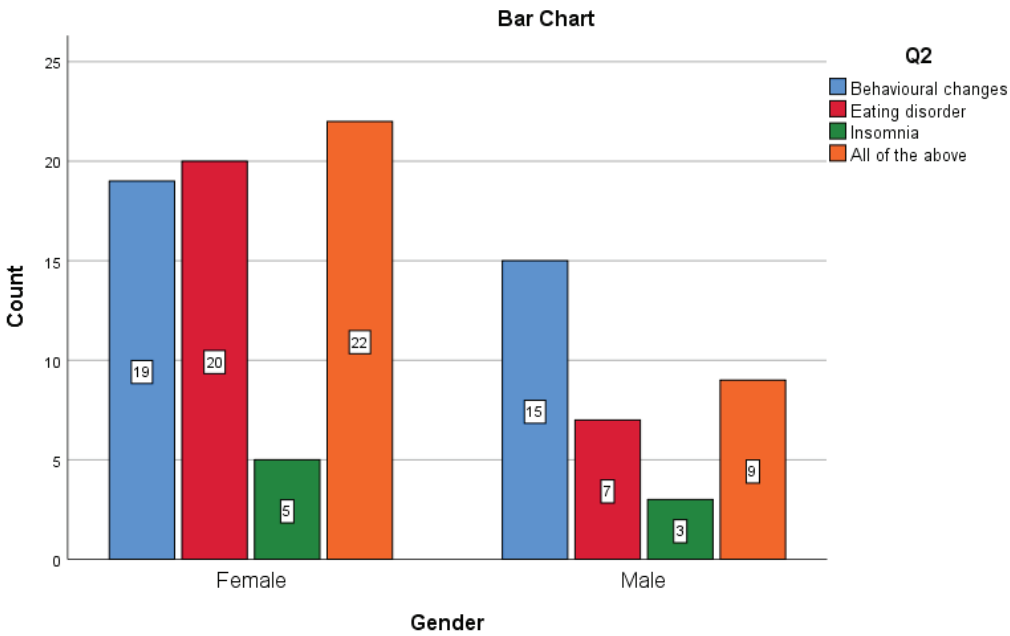


Figure 2:Bar chart depicts the association between gender(X axis) and responses to awareness on symptoms for depression (Y axis). 22% female reported behavioural changes,eating disorder,insomnia are the symptoms of depression and 16% male reported behavioural changes are the symptoms of depression.Blue denotes behavioural changes ,red denotes eating disorder,green denotes insomnia and orange denotes all of the above.Female were more aware of the symptoms of depression . Chi square test was done and was found not to be significant statistically (Pearson Chi - Square value = 2.720, p =0.437).

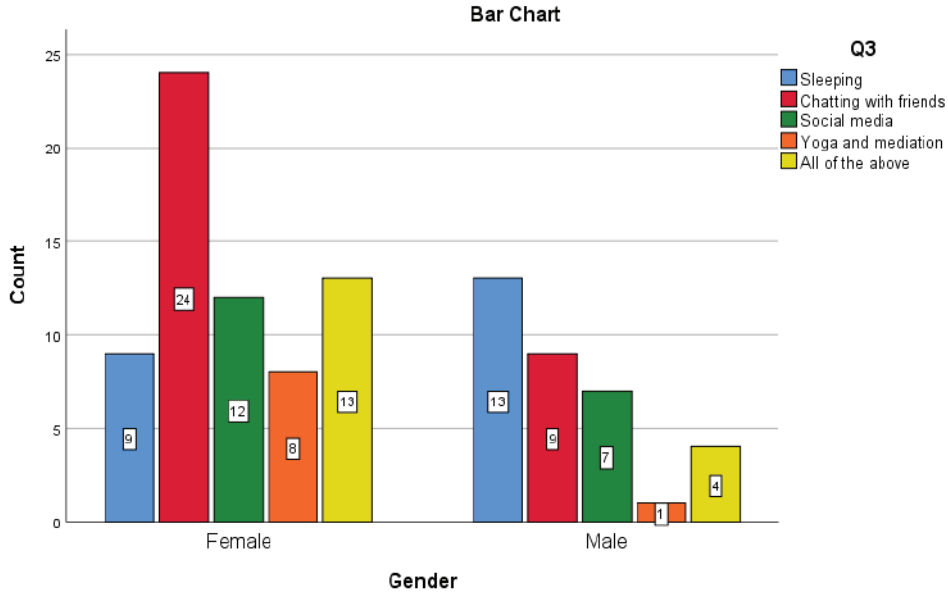


Figure 3:Bar chart showing the association between gender (X axis) and responses to awareness on preferred methods to overcome depression (Y axis). 24% females reported chatting with friends as a means to reduce depression and 13% male reported sleeping to reduce depression.Blue denotes sleeping, red denotes chatting with friends, green denotes social media, orange denotes yoga and meditation and yellow denotes all the above options. It was found that females were more aware than males. Chi square test was done and it was found to be statistically significant (Pearson Chi square value = 9.838, P=0.043).

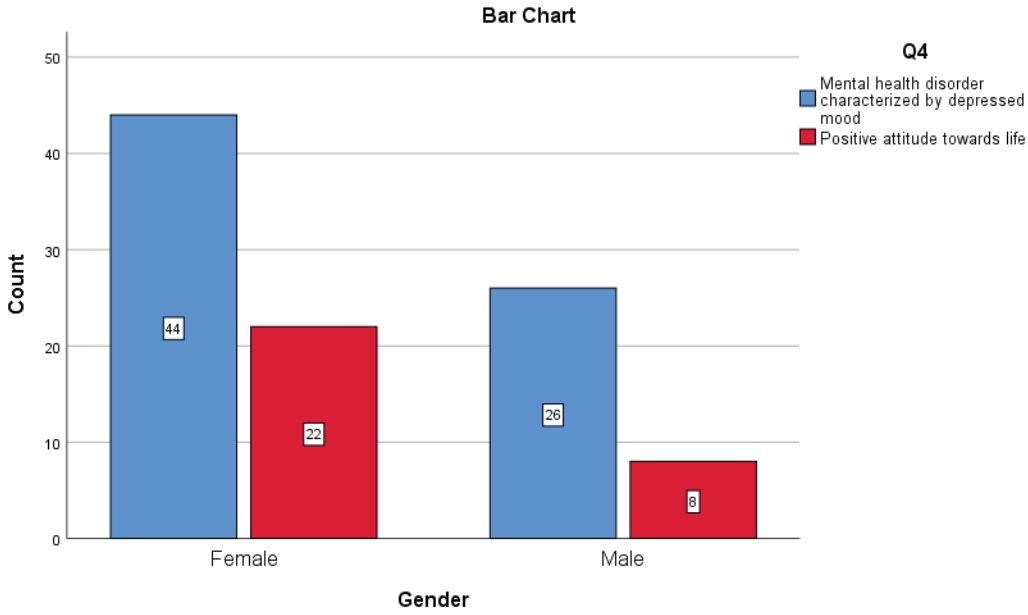


Figure 4: Bar chart showing the association between gender(X axis) and responses to awareness on the definition of depression (Y axis). 44% female reported depression is defined as a mental health disorder characterized by depressed mood and 26% male reported depression is defined as mental health disorder characterized by depressed mood.Blue denotes mental health disorder characterized by depressed mood and red denotes positive attitude towards life.Females were more aware than males. Chi square test was done and it was found not to be statistically significant. (Pearson Chi square value = 1.027,P=0.31).

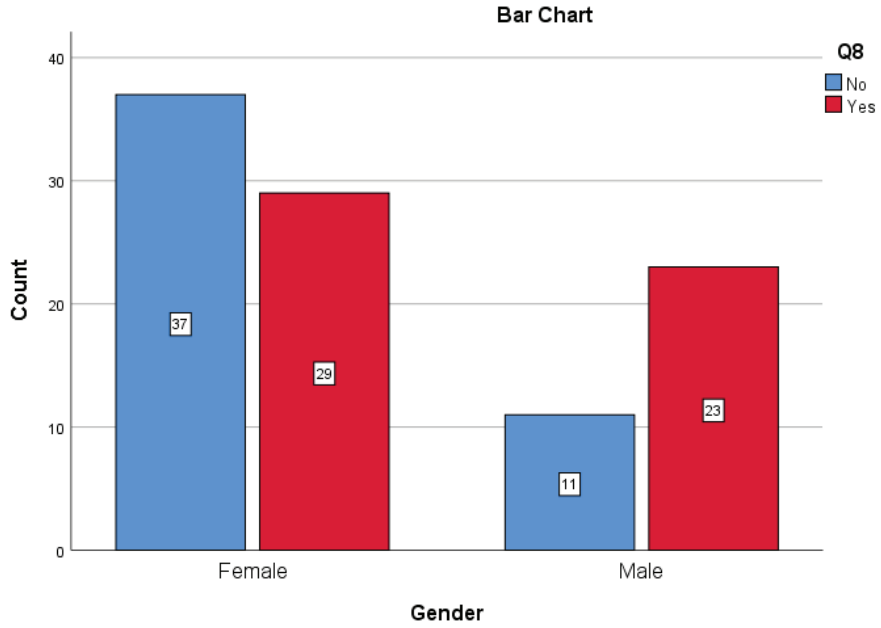


Figure 5:Bar chart showing the association between gender (X axis) and responses to awareness on anti depressive drugs (Y axis). 37% females and 23% of males reported drugs don't reduce depression. Blue bar denotes no and red bar denotes yes, implying females were more aware than males. Chi square test was done and it was found to be statistically significant (Pearson Chi square value = 5.053, P=0.025)

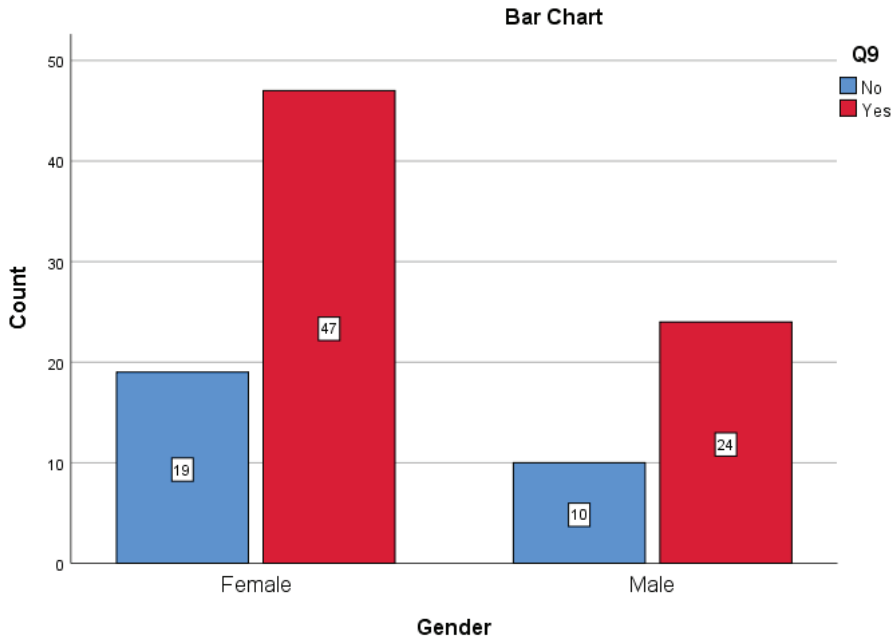


Figure 6: Bar chart showing the association between gender (X axis) and responses to awareness on suicidal tendency due to depression (Y axis). 47% females and 24% females reported depression leads to suicide. Blue denotes a negative response and red denotes positive response.Female were more aware than males.Chi square test was done and it was not statistically significant. (Pearson Chi square value = 0.004, P=0.948)

Conclusion

Depression is a group of conditions associated with the elevation or lowering of a person's mood. The causes of depression are death or loss of a person,serious illness, personal problem like social isolation,personal conflicts.The symptoms of depression are fatigue,insomnia,overeating ,restlessness,aches ,pains,suicidal thoughts,From this survey we can conclude that many students were aware of depression and the methods to overcome it.

Acknowledgment: We thank Saveetha Dental College for providing us the support to conduct the study.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Correlation between Hard and Soft Tissue Lower Anterior Facial Height of Different Skeletal Pattern on South Indian Population

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Abstract

The soft tissue is in close contact to the underlying skeletal hard tissue and takes up whatever form is dictated by the hard tissue. Yet, there exists some variations in cases of which, the soft tissue thickness varies in certain places to camouflage the skeletal deficiency. Hence, the purpose of this study was to evaluate the correlation of hard and soft tissue lower anterior facial height (LAFH) of different skeletal patterns on the south indian population. The study consisted of 30 lateral cephalograms, which were divided into three groups based on the skeletal relationship: Group A - Class I skeletal pattern, Group B - Class II skeletal pattern and Group C - Class III skeletal pattern. All the groups contained 10 radiographs each of that particular skeletal pattern. FACAD software was used to do the digital tracing and analysis. The obtained results were tabulated and were subjected to statistical analysis using SPSS software version 23. The results of Pearson's correlation test revealed that Group A and Group B were positively correlated with statistical significance, whereas Group C was statistically insignificant. Hence, soft tissue LAFH can be used as a valuable indicator and an adjunct in diagnosis and treatment planning of orthodontic cases.

Keywords: *Hard tissue; Lower anterior facial height, Lateral cephalogram, Soft tissue.*

Introduction

Lateral cephalometric readings of the hard tissue structures are often used as the diagnostic criteria in classifying the type of malocclusion, based on which treatment plans are formulated. The soft tissue however, is not given much consideration as it is not very apparent in lateral cephalograms. They also cannot predict the outcome and final soft tissue position, as the soft tissue doesn't accurately mimic the hard tissue changes to maximum extent.[1] This makes it essential for the establishment of a standard, for soft tissue structures pertaining to the different types of skeletal relationship.

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Vertical discrepancies are one of the most commonly encountered types of skeletal malocclusion in everyday life.[2] Vertical discrepancies cause an increase or a decrease in the overall length of the face. They are usually termed as either a high angle case, a low angle case or a normal angle case. This can be due to a variety of reasons, like the rotation of mandible, rotation of occlusal plane, inclination of palatal plane, development of dentoalveolar segment etc.,[3]

The soft tissues are closely adherent to the hard tissue. They reflect the hard tissue configuration underneath. In certain cases, the soft tissues also effectively mask the skeletal defects, thereby effectively camouflaging the condition. The extent upto which the soft tissue thickness covers different regions of the face varies from person to person. Gender also plays a crucial role in variation of soft tissue thickness of the face due to a variety of factors such as difference in growth spurts, hormonal changes, etc,. This is commonly referred to as sexual dimorphism, that establishes the need for different standards of soft

tissue structures in males and females.[4,5]

Lower anterior facial height is a direct reflection of the soft tissue morphology.[6] Increase or decrease in the lower anterior facial height can be a direct reflection of the type of growth pattern that influences the patient's facial form.[4] Saxby and Freer in their study have evaluated the correlation between lower anterior facial height and soft tissue form in vertical and horizontal planes.[7] Soft tissues more often than not, tend to exaggerate the jaw discrepancies.

Ethnicity and race have also been found to be an important factor influencing the soft tissue configuration. The relationship between hard and soft tissue profiles are variable as they are closely re;ated to the hard tissue as shown in studies done by Kasai.[8]

The purpose of this retrospective study was to evaluate the correlation between hard and soft tissue lower anterior facial height (LAFH) on different skeletal patterns of the south indian population.

Materials and Methods

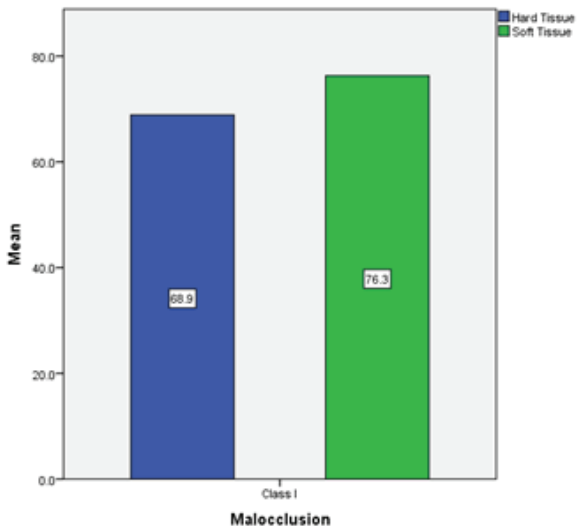
The retrospective study consisted of a total of 30 lateral cephalograms. The lateral cephalograms were divided into 3 groups, based on the skeletal pattern as :

- Group A - Class I skeletal pattern
- Group B - Class II skeletal pattern
- Group C - Class III skeletal pattern

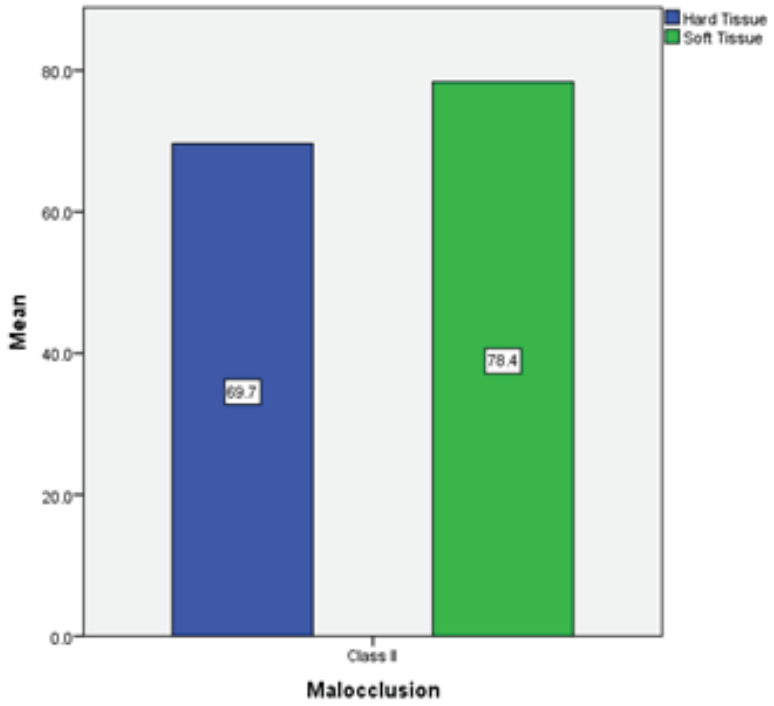
Each group contained 10 radiographs each, pertaining to the specific skeletal relationship. The data for the study was randomly collected from the Saveetha Institute of Medical and Technical Sciences (SIMATS) university database. Radiographs taken in proper natural head position with proper exposure were chosen for this study. Lateral cephalograms of syndromic patients and those with missing teeth were excluded from this study. Ethical approval was obtained from the institutional review board. FACAD software was used for digital cephalometric tracing and analysis. Points were plotted for hard tissue lower anterior facial height (LAFH) from anterior nasal spine to menton (ANS-Mn) and for soft tissue LAFH from Subnasale to Soft tissue menton (Sn-Mn'). The linear measurements were obtained and were subjected to statistical analysis using SPSS software version 23 for windows. Pearson's correlation test was performed to determine the correlation between the hard and soft tissue LAFH in different skeletal patterns.

Results and Discussion

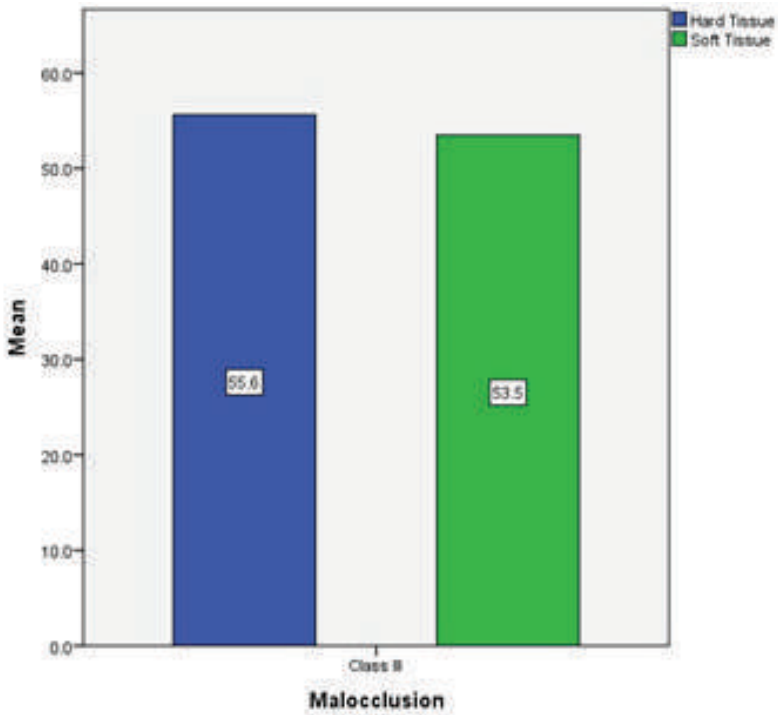
Descriptive statistics showing the mean and standard deviation of Class I, Class II and Class III skeletal patterns are shown in (Graph 1, 2, 3).



Graph 1: Descriptive statistics showing mean of hard and soft tissue LAFH in Class I. Bar chart showing mean value of hard and soft tissue lower anterior facial height (LAFH) in Class I skeletal relationship. The mean hard tissue LAFH is 68.9 whereas the mean soft tissue LAFH is 76.3 (x axis - Skeletal relationship, y axis - soft tissue thickness; blue colour - hard tissue LAFH green colour - soft tissue LAFH)



Graph 2: Descriptive statistics showing mean of hard and soft tissue LAFH in Class Bar chart showing mean value of hard and soft tissue lower anterior facial height (LAFH) in Class II skeletal relationship. The mean hard tissue LAFH is 69.7 whereas the mean soft tissue LAFH is 78.4 (x axis - skeletal relationship, y axis - soft tissue thickness; blue colour - hard tissue LAFH, green colour - soft tissue LAFH)



Graph 3: Descriptive statistics showing mean of hard and soft tissue LAFH in Class III ar chart showing mean value of hard and soft tissue lower anterior facial height (LAFH) in Class III skeletal relationship. The mean hard tissue LAFH is 55.6 whereas the mean soft tissue LAFH is 53.5 (x axis - Skeletal relationship, y axis - soft tissue thickness; blue colour - hard tissue LAFH, green colour - soft tissue LAFH)

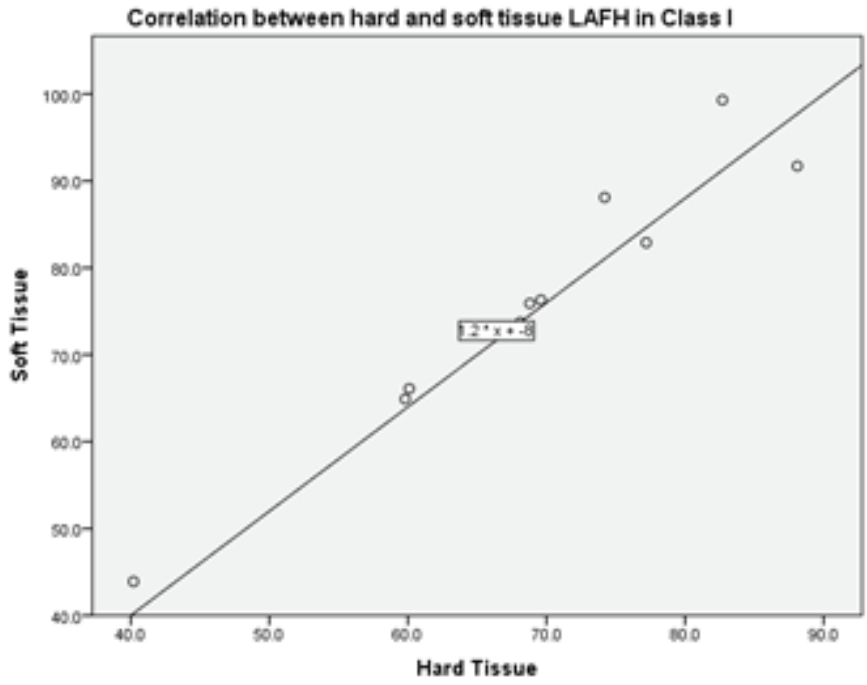
The results of the Pearson's correlation test for Class I skeletal pattern is shown in (Table 1 and Graph 4). There is a positive correlation between the hard and soft tissue LAFH in Class I skeletal pattern which is statistically significant as the P value is <0.05.

Table 1: Pearson's correlation test between hard and soft tissue LAFH of Class I

Correlations

		Hard Tissue	Soft Tissue
Hard Tissue	Pearson Correlation	1	.968**
	Sig. (2-tailed)		.000
	N	10	10
Soft Tissue	Pearson Correlation	.968**	1
	Sig. (2-tailed)	.000	
	N	10	10

**Correlation is significant at the 0.01 level (2-tailed).



Graph 4: Scatter dot graph showing correlation between hard and soft tissue LAFH of Class I. Scatter dot chart showing correlation between hard and soft tissue lower anterior facial height (LAFH) in Class I skeletal relationship shows that there is a positive correlation between hard and soft tissue LAFH as it falls within the straight line and is also of statistical significance as p value is <0.05 (x axis - hard tissue LAFH, y axis - soft tissue thickness)

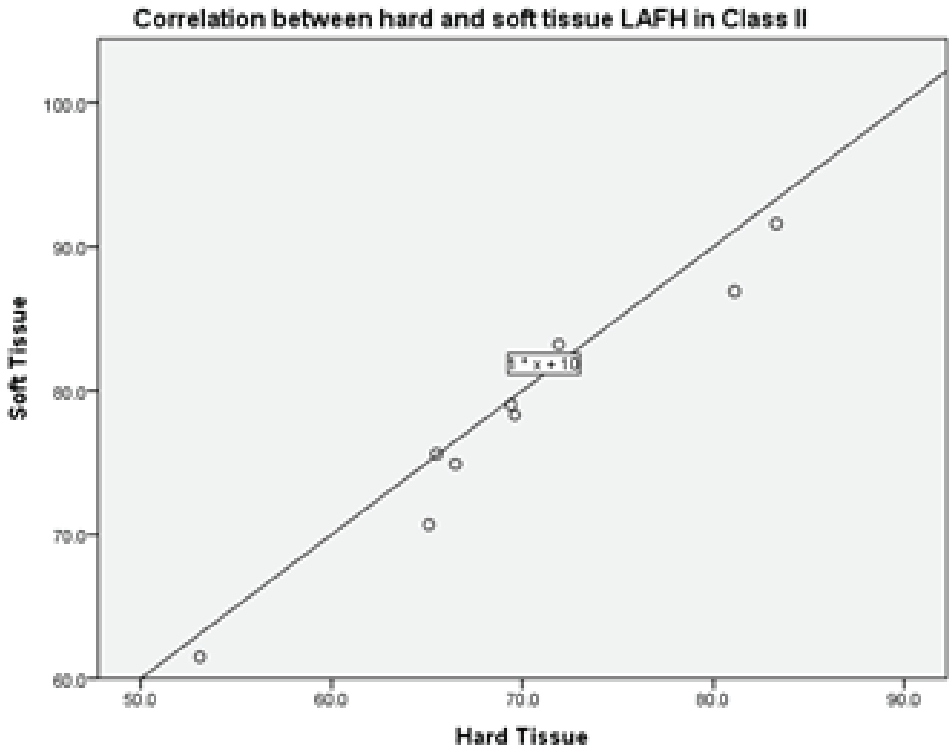
Results for Class II skeletal pattern are depicted in (Table 2 and Graph 5), which also shows a positive correlation between the hard and soft tissue LAFH. The results are also statistically significant with the P value of < 0.05.

Table 2: Pearson's correlation test between hard and soft tissue LAFH of Class II

Correlations

		Hard Tissue	Soft Tissue
Hard Tissue	Pearson Correlation	1	.975**
	Sig. (2-tailed)		.000
	N	10	10
Soft Tissue	Pearson Correlation	.975**	1
	Sig. (2-tailed)	.000	
	N	10	10

** Correlation is significant at the 0.01 level (2-tailed).



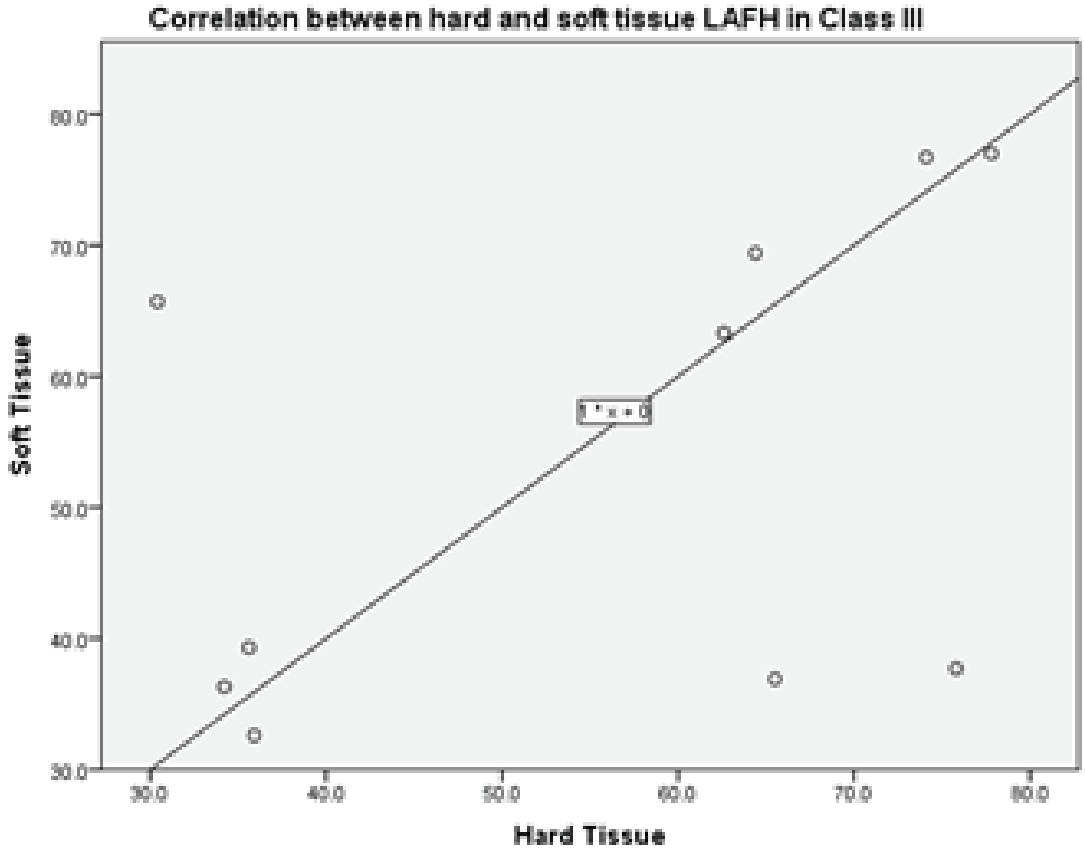
Graph 5: Scatter dot graph showing correlation between hard and soft tissue LAFH of Class II. Scatter dot chart showing correlation between hard and soft tissue lower anterior facial height (LAFH) in Class II skeletal relationship shows that there is a positive correlation between hard and soft tissue LAFH as it falls within the straight line and is also of statistical significance as p value is <0.05. (x axis - hard tissue LAFH, y axis - soft tissue thickness)

The results for Class III skeletal pattern however, is statistically no significance shown in (Table 3 and Graph 6).

Correlations

Table 3: Pearson's correlation test between hard and soft tissue LAFH of Class III

		Hard Tissue	Soft Tissue
Hard Tissue	Pearson Correlation	1	.448
	Sig. (2-tailed)		.194
	N	10	10
Soft Tissue	Pearson Correlation	.448	1
	Sig. (2-tailed)	.194	
	N	10	10



Graph 6: Scatter dot graph showing correlation between hard and soft tissue LAFH of Class III. Scatter dot chart showing correlation between hard and soft tissue lower anterior facial height (LAFH) in Class III skeletal relationship shows that there is a positive correlation between hard and soft tissue LAFH as it falls within the straight line, but there is no statistical significance as p value is >0.05. (x axis - hard tissue LAFH, y axis - soft tissue thickness)

Previously, our team had conducted numerous clinical trials [9], in vitro studies [4,10–14], Finite element studies [1516,17]

and a couple of prospective studies [18–22] over the past 5 years. Now we are focusing on this retrospective study, done with the data obtained from our vast database. The idea for this study stemmed from the current interest in our community on the soft tissue paradigm shift.

This study was conducted to assess the correlation between hard and soft tissue LAFH of different malocclusions. The age of the subjects were maintained over 18 years to address the aspect of growth. Only the average growth pattern in all malocclusions with a mean FMA angle of 25-35^{o} was chosen to maintain the uniformity of the chosen samples.

The positive correlation in Class I and Class II skeletal patterns show that the hard and soft tissue LAFH are within their appropriate proportions and are also statistically significant. However, the insignificant results of Class III skeletal pattern could be due to the variations in the soft tissue stretching due to the prognathic mandible. As a result of which, the soft tissue chin thickness is commonly reduced in Class III patients.[23]

Our team has conducted numerous clinical trials over the past 5 years on various topics pertaining to the hard and soft tissue characteristics of the face and its implications on treatment planning and its outcome. This current study aims at addressing the contribution of soft tissue in the increase of lower anterior facial height.

Increase in the lower anterior facial height is more commonly seen in patients with vertical growth pattern. They often exhibit a leptoprosopic facial form that is usually termed as long face syndrome. On radiographic evaluation, they have several diagnostic indicators such as the gonial angle, basal plane angle, mandibular plane angle, etc., that further confirm the diagnosis.[4]

Lateral cephalograms, ever since its discovery by broadbent in the year 1931 has been a boon for orthodontists.[24] They are considered as supplemental diagnostic tools that aid during treatment planning and also planning of surgical cases. If taken with proper exposure, even the soft tissue structures can be visualised with ease and can be of much relevance in treatment planning. Soft tissue cephalometric analysis makes use of lateral cephalograms to analyse and interpret soft tissue changes in facial profile of a patient.[25,26] Besides, they are much more feasible as they are less expensive and pose much lesser threat of exposure to radiation, when compared to CBCT and CTs. Hence, they were opted in this study for analysis of soft tissue and hard tissue LAFH.

Vertical dimension control has always been one of the most difficult malocclusions to treat.[27] They can be corrected by virtues of intrusion, extrusion of teeth or by redirection of the mandibular growth direction. Jain RK et al has conducted a study in which he has discussed and compared the usage of mini implants, j-hook headgear and intrusion arches for the purpose of intrusion. Similarly, Felicita AS et al has reported the effects of mini implants on retraction and intrusion of anterior teeth. Sivamurthy et al in his study has reported the use of mini implants and its stress distribution during intrusion as well as retraction in a finite element method study. Of all the parameters that contribute to LAFH, mandibular growth rotation is one of the most significant causes. Infact, AFH has been confirmed to have a strong influence on vertical disproportions.[28]

In today's time where the paradigm has shifted towards a soft tissue dictated treatment approach, it is of absolute importance to establish norms for soft tissue structures as well, similar to their hard tissue counterparts. This stands true in case of lower anterior facial heights as well, since ultimately we treat the patient for their face and not for their radiographs.

Conclusion

Soft tissue LAFH are predictable diagnostic indicators in determining the skeletal hard tissue discrepancies.

Authors Contribution: All authors have equal contribution in bringing out this research work.

Conflict of Interest: None.

Source of Funding : Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Dry Mouth for Older Patients- Problems and Treatments - A Survey among Dental Undergraduates

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Abstract

Dry mouth is a common complication faced by elderly people. It causes weakness and discomfort. Dry mouth is responsible for significant oropharyngeal disorder and pain which in turn leads to impaired quantity of lifestyle. Hyposalivation may cause dry mouth. The reasons for dry mouth may vary. Survey was conducted online. Questionnaire which consisted of 10 questions related to the present survey was prepared and distributed through google forms. Study population consists of 100 dental undergraduates. Data were collected and statistically analysed. This created an awareness on knowledge about dry mouth, its cause, its problems and treatments for older patients and knowledge about this topic helps the dentist to plan treatments for such patients. This study shows that there is a moderate to high level of knowledge on this topic among dental undergraduates. It is important for dental students to be aware about this topic, so that better diagnosis plans and relevant treatments can be done. Majority of the population are aware that dry mouth is a common condition among older patients and may cause weakness and discomfort.

Keywords: *Dry mouth, problems and treatments for dry mouth, causes of dry mouth, disorders of dry mouth, elderly patients*

Introduction

Saliva is an opalescent slimy fluid containing 99% water and 1% dissolved solids. The significant organic solutes in the saliva are mucin, a glycoprotein which serves as a lubricant and the enzyme ptyalin (salivary α−amylase) that initiates the digestion of starch. Saliva maintains oral homeostasis [1]. Hyposalivation leads to dry mouth. Dry mouth is a common complication faced by elderly people. Dry mouth is responsible for significant oropharyngeal disorders and pain which in turn leads to impaired quantity of lifestyle. The reasons for dry mouth may vary [2]. Reduced saliva flow or change in biochemical composition of saliva are the common reasons for dry mouth[3]. The other reasons are local salivary disorder, plethora of medication and medical condition [4]. Treatments for dry mouth have to be designed according to the cause. Patients with dry mouth suffer from problems like difficulty in chewing, swallowing and speaking. Older people are more prone to dry mouth because they suffer from other ailments and take more medications which may lead to dry mouth [5]. Patients who are suffering from dry mouth and also wearing complete dentures usually have many complications like pain and discomfort, difficulties in normal functions, cracking at corners of mouth, burning sensation of tongue, alteration in taste, failure of denture retention.

Persistent dry mouth will lead to significant and permanent oral and pharyngeal disorders [6]. Dry mouth

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may also occur due to stroke, diabetes, thrush in mouth and autoimmune diseases. Snoring and breathing while keeping the mouth open also results in dry mouth. Smith et al., have examined the difference in decreased saliva flow rate among 3 different age groups[7]. Lin et al., have done an epidemiologic study and concluded that the female patients have higher prevalence for dry mouth than males [8]. Ship et al., in their study observed that prevalence of xerostomia increases with age and estimated that about 30% of the patients are in the 65 years or older age group [9]. Fox et al., in their study, pronounced that sjogren syndrome also leads to dry mouth in elderly[10]. Van der reijden et al., in their study, list out the treatment methodologies for oral dryness and related complaints in sjogren syndrome [11]. Furness et al., observe that there is no strong evidence for effectiveness of topical therapies in dry mouth[12]. Jacqueline et al., stipulate that initial evaluation of patients with dry mouth is essential for providing better treatment and also in preventing oral diseases [13]. Not drinking enough fluids, sleeping with your mouth open, dry hot weather, eating dry food etc are some of the reasons for dry mouth [14]. These lead to many periodontal problems like dental caries leading to discomfort difficulties in normal functions of oral cavity.

Dry mouth is a common condition among older people which leads to weakness and discomfort. It is important for dental students to be aware of this affliction so that better diagnosis and plan relevant treatment can be given [15]. Previous research articles predominantly focus only on medications as treatment for dry mouth[16] and not on the alternative therapies. But home treatment and minor lifestyle alterations may also help to relieve symptoms of dry mouth. Some are frequent water intake, using ice chips while feeling dry in the mouth and restriction to sugar free items. Aim of this survey is to analyse the knowledge among dental undergraduates on dry mouth in older patients, its problem and treatments.

Materials and Methods

Study design,area and study population:

A survey was conducted among dental undergraduates. The sampling from this survey is about 100. Participants aged 17-25 are considered. Participants in this survey are volunteers and no incentives were provided to the participants. The survey was conducted in the month of april, 2020.

Survey instruments:

A questionnaire was prepared after extensive review and amendments were made to improve clarity of the questions and eliminate ambiguous responses. The survey instrument was a structured questionnaire with both open and close ended questions. 10 questions were prepared related to the present survey and were circulated to the participants via google forms.

Data Analysis

Only completely filled online forms were included in the study, the filled responses were verified by two reviewers and the collected data was entered on the same day. The entered data were analysed using SPSS statistics 19. Descriptive analysis was performed to calculate frequencies of categorical variables. [17–21]

Results and Discussions

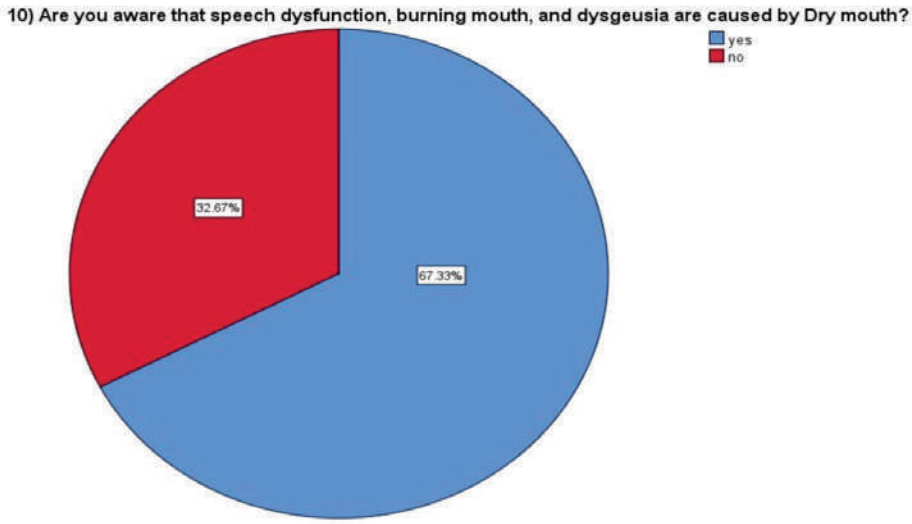


Figure 10: The pie chart depicts awareness on the symptoms of dry mouth

Majority (67.33%,blue) were aware and few (32.67%,red) weren't aware

Figure 1: The pie chart depicts awareness on the symptoms of dry mouth. Majority, 67.33% (blue) were aware and around 32.67% (red) weren't aware.

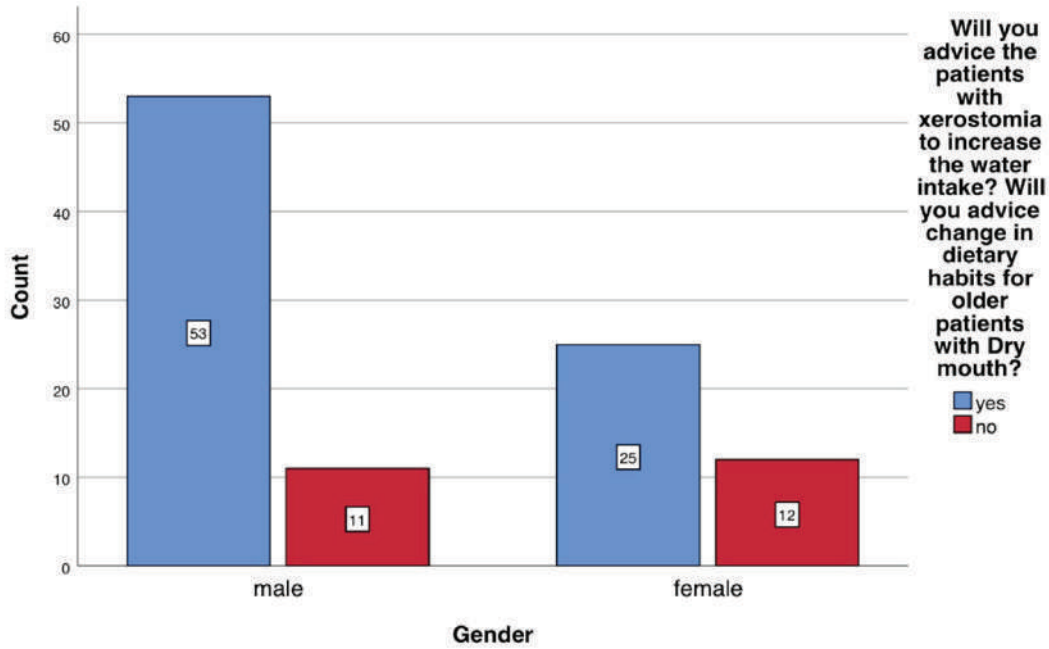


Figure 2: Bar graph representing association between gender and opinion of dental students on advising to change dietary habits in xerostomia patients. X axis represents gender and Y axis represents the number of participants' opinion on advising to change dietary habits in xerostomia patients. 78 participants felt that it is necessary to change dietary habits for xerostomia patients. Out of 78 participants, 53 participants were male and 25 participants were female. There is no significant difference in opinion between male and females. Chi square value- 3.098, df- 1, p value= 0.078 (>0.05). Hence statistically not significant.

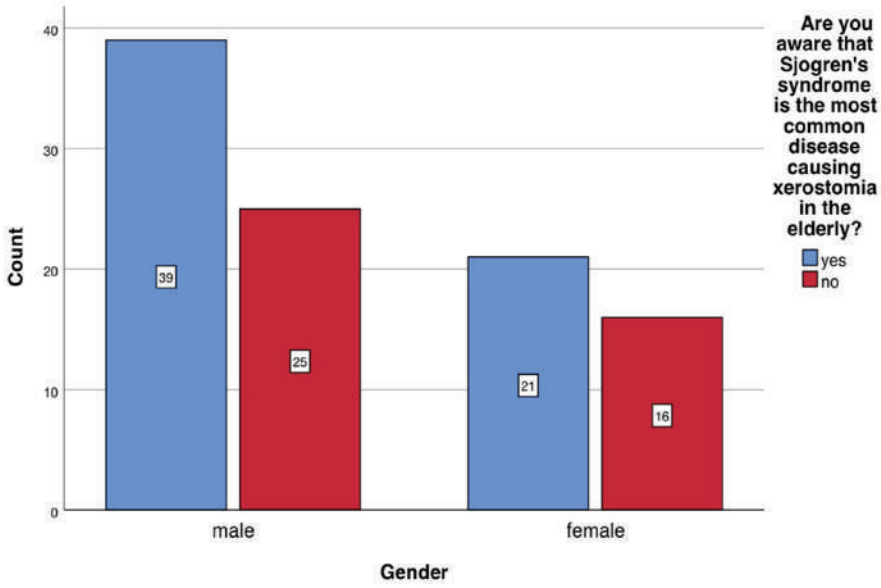


Figure 3: Bar graph representing association between gender and awareness of sjogren's syndrome as a causative agent of xerostomia in elderly. The X-axis represents gender and the Y axis represents the number of participants aware of sjogren's syndrome as a causative agent of xerostomia in elderly. Out of 60 participants who were aware that sjogren syndrome is the most common disease which causes xerostomia in elderly, 39 participants were male and 21 participants were female. There is no significant difference in awareness between male and females. Chi square value- 0.170, df-1, p value=0.680 (>0.05). Hence statistically not significant.

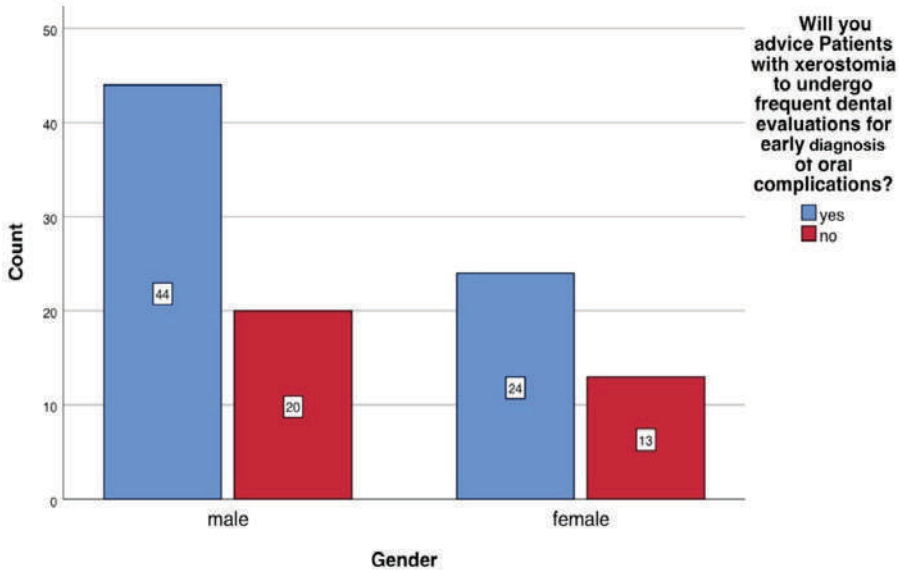


Figure 4: Bar graph representing association between gender and opinion of dental undergraduates on advising frequent dental evaluation for xerostomia patients. X axis represents gender and Y axis represents number of participants' opinion on advising frequent dental evaluation for xerostomia patients. 64 participants felt that it is necessary to advise xerostomia patients to undergo frequent dental evaluation for early diagnosis of oral complications, in which 44 participants were male and 24 participants were female. There is no significant difference in opinion between male and female. Chi square value-0.161,df-1, p value= 0.688 (>0.05). Hence statistically not significant.

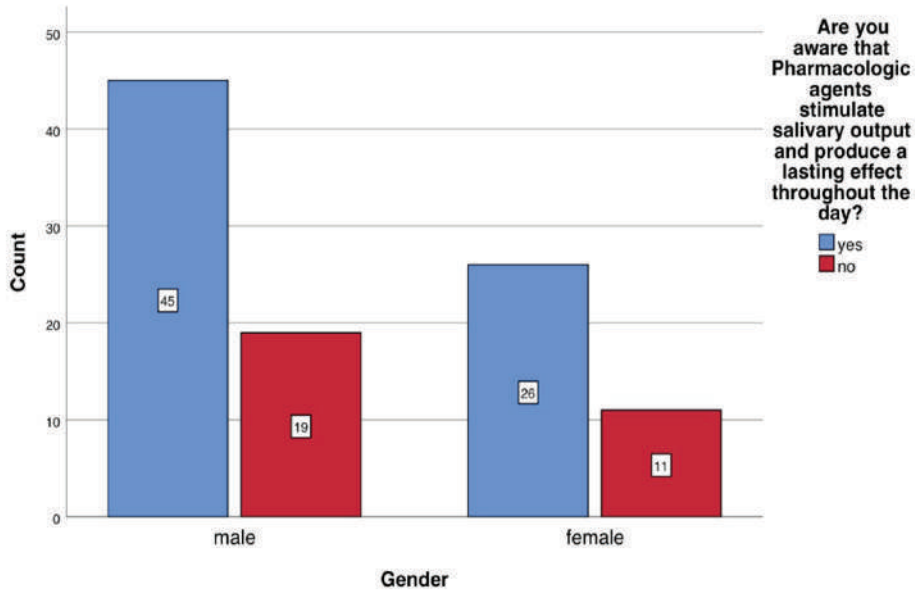


Figure 5: Bar graph representing association between gender and effect of pharmacologic agents in stimulating salivary output. X axis represents gender and Y axis represents the number of participants aware of the effect of pharmacologic agents in stimulating salivary output. Out of 71 participants who were aware that pharmacological agents stimulate salivary output, 45 participants were male and 26 participants were female. There is no significant difference in awareness between male and female. Chi square value- 0.903,df- 1, p value= 0.966 (>0.05). Hence statistically not significant.

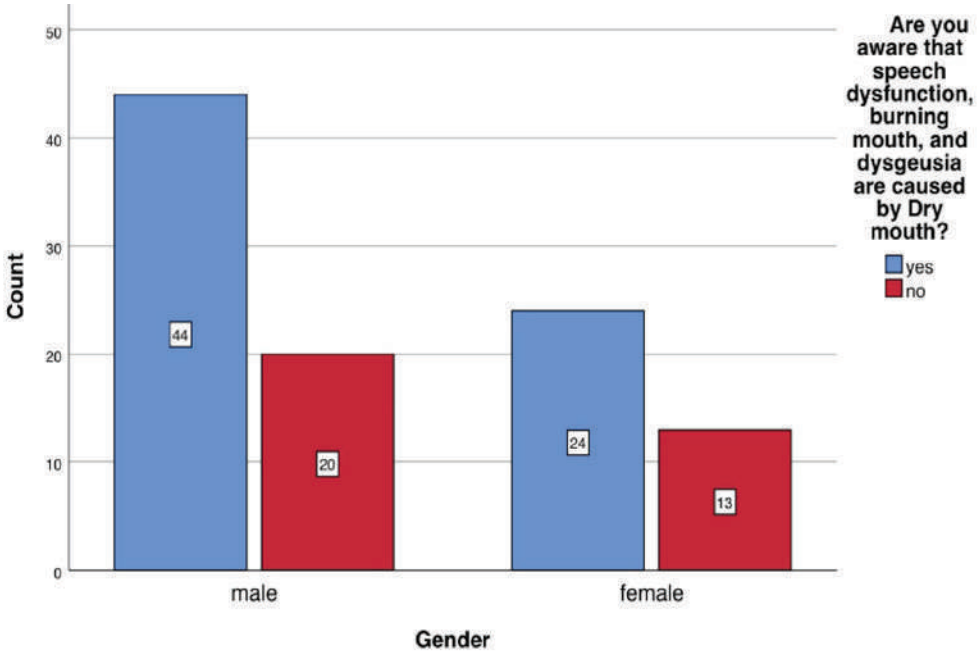


Figure 6: Bar graph representing association between gender and awareness on the symptoms of dry mouth. X axis represents gender and Y axis represents the number of participants aware of the symptoms of dry mouth. Out of 68 participants who were aware that dry mouth causes speech dysfunction, burning mouth, and dysgeusia, 44 participants were male and 24 participants were female. There is no significant difference in awareness between male and female. Chi square value- 0.161, df- 2, p value= 0.688 (>0.05). Hence statistically not significant.

Table 1: Questionnaire regarding the etiology and awareness of oral effects of xerostomia

S.No	Questions	Results in percentage	
		Yes	No
1.	saliva helps maintain a neutral oral pH and provides a reservoir of calcium and phosphate ions to remineralise teeth	72.28%	27.72%
2.	medicine intake by older patients may cause dry mouth	70.30%	29.70%
3.	Candidiasis and mouth sores in older patients may be due to dry mouth	61.39%	38.61%
4.	sjogren syndrome is the common disease causing xerostomia	59.41%	40.59%
5.	awareness about discomfort caused due to dry mouth in elderly patients	86.14%	13.86%
6.	change dietary habits in xerostomia patients	77.23%	22.77
7.	xerostomia patients to undergo frequent dental evaluation for early diagnosis of oral complications	67.33%	32.67%
8.	pharmacologic agents stimulate salivary output & produce a lasting effect throughout the day	70.30%	29.70%
9.	dental instability in CD patients may be due to dry mouth	61.39%	38.61%
10.	speech dysfunction, burning mouth and dysgeusia are caused by dry mouth	67.33%	67.33%

Table 1 shows that, 72.28% of the population were aware that saliva helps maintain a neutral oral pH and provides a reservoir of calcium and phosphate ions to remineralise teeth whereas 27.72% weren't aware**.** When asked about whether medicine intake by older patients may cause dry mouth, 70.30% said yes and 29.70% said no. 61.39% said yes that oral infections like candidiasis and mouth sores in older patients may be due to dry mouth but 38.61% didn't agree with this. 59.41% of the population were aware that sjogren syndrome is the common disease causing xerostomia in the elderly whereas 40.59% aren't. On the question of awareness about discomfort caused due to dry mouth in elderly patients, the majority of the

population(86.14%) said yes, they were aware & few (13.86%) said no, they weren't. When asked about the opinion of dental students on advising to change dietary habits in xerostomia patients. Majority (77.23%) said yes and a minor population (22.77) said no**.** 67.33% felt that it is necessary for xerostomia patients to undergo frequent dental evaluation for early diagnosis of oral complications but 32.67% of the population didn't feel so. 70.30% were aware that pharmacologic agents stimulate salivary output & produce a lasting effect throughout the day and 29.70% weren't aware. On the question of awareness about whether dental revention and dental instability in CD patients may be due to dry mouth. 61.39% said yes and 38.61% said no. 67.33% of the population is aware that speech dysfunction, burning mouth and dysgeusia are caused by dry mouth. Whereas 32.67% of the population are not aware about this fact. Figure 1 depicts awareness on the symptoms of dry mouth. Majority, 67.33% (blue) were aware and around 32.67% (red) weren't aware. When gender was compared with the opinion of dental students on advising to change dietary habits in xerostomia patients, p value obtained was 0.078 and it was not statistically significant [Figure 2]. When gender was compared with awareness of sjogren's syndrome as a causative agent of xerostomia in elderly, p value obtained was 0.680 and it was not statistically significant [Figure 3]. When gender was compared with the opinion of dental undergraduates on advising frequent dental evaluation for xerostomia patients, p value obtained was 0.688 and it was not statistically significant [Figure 4]. When gender was compared with the effect of pharmacologic agents in stimulating salivary output, p value obtained was 0.966 and it was not statistically significant [Figure 5]. When gender was compared with awareness on the symptoms of dry mouth, p value obtained was 0.688 and it was not statistically significant [Figure 6].

The present research has origins from the team of investigators where previous studies were done based on clinical reports,interventional studies like[22–24], in vitro studies like[17–19,21], systematic reviews[22,25–31]. In one of the questions in the survey, the respondents were asked if they were aware of the fact that dry mouth causes extreme discomfort. For this 86.14% of the participants responded in affirmative, that is they are aware. The balance 13.86% were not aware of this fact. Similar query was included in a previous survey conducted by raside et al.,[32] in which majority responded that they were aware. On whether they were aware that in older patients, medicine intake may lead to xerostomia, 70.3% answered that they were aware and 29.7% replied in negative. In an allied study conducted previously on the risk factors of hypo-salivation and the impacts on xerostomia by Nikeandees et al., 2017 70.5% of the population responded that they are aware of the impact of medicines in causing xerostomia in older patients [33]. On the awareness that dry mouth can lead to oral infections like mouth sore, candidiasis in older patients, 61.39% of the participants consented and 38.61% replied that they were not aware. Interestingly, during an analogous survey conducted by Jannan et al., 2016 it was found that only 45.2% knew this fact [34]. In the survey, the participants were also asked if they were aware that sjogren syndrome is the common ailment that causes xerostomia, 76% replied that they were aware. This awareness figure is considerably higher than a survey conducted by Abdelghany et al., 2011 in which only about 59.41% acknowledged this[35].

A few limitations that can be seen in the research is that the sample size (100 participate) is minimum, so the results may vary with other research. The study may not be too accurate and also this research is just a sample view of reality. It is not very deep.

This study gives different information about dry mouth, its case, its problems and treatment for olders patients. Future, this survey could be extended among different types of population with large sample sizes.

Conclusion

Dry mouth is a common condition that can be seen among older patients. It causes weakness and discomfort. It is important for dental students to be aware so that better diagnosis plans and relevant treatments can be done. Majority of the population are aware about dry mouth, its problems and treatments among older patients.

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Conflict of Interest : The author declares that there was no conflict of interest in the present study.

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Ethical Clearance: Not required

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Role of Bruxism in Prosthetic Treatments- A Survey

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Abstract

Bruxism is a parafunctional habit of grinding of teeth and it results in an overload of occlusal loading that puts on more pressure on the teeth and the subsequent prosthetics causing them to fail, it is a common phenomenon affecting nearly 30% of the general population but is not looked into with deep detail. A survey of 16 questions was sent to 114 respondents. The questionnaire was made on Google Forms, it had several options for each question to get a response as accurate as possible. The incomplete responses were rejected and the data was collected and compiled using SPSS software. The compiled data was compared with the results of other studies and the important questions were discussed. This study conclusively found that the awareness of Bruxism and its effect on prosthetic treatments is high and that the data can be used in future treatments resulting in reduced failure of prosthetics due to habits such as

Keywords: *Bruxism, prosthetics, survey, effect on treatment*

Introduction

Bruxism is an action that involves the individuals grinding or clenching their teeth.[1] This habit increases the amount of occlusal loading. The additional pressure makes the prosthetic lose its integrity, which makes it unusable. Considering that dentures are used primarily by patients who are edentulous, to replace the functions of the teeth lost, their unhealthy habit means that they have an unsatisfactory final product that will with use, get severely damaged. [2,3]

Bruxism is surprisingly common and with the research conducted so far does not seem to have had serious side effects.[4] While not being a major cause of harm, it does cause its fair share of discomfort during prosthetic treatments.and in the long severe damage to dentures and in cases of implant, even implant failure and results in complications late on, especially after treatment finishes.[5], [6,7] While there are factors like misplacement of the Temporomandibular joint or in some cases an uncorrected habit from childhood, stress also plays a role in Bruxism. Stress comes in various types such as pathophysiological, psychosocial or even hormone related as there is a link between urinary catecholamine and the levels of salivary cortisol, and bruxism , or it is simply because of psychological stress experienced by the person.

The topic of bruxism has long been characterized as an unacceptable or an disgusting habit but the true cause of this parafunctional habit is because of one of three reasons which are anxiety , hormonal imbalance and even misalignment of jaws and it is surprisingly common with 3% of the general population having symptoms of the same and the fact that it's effect on a variety is unknown and for this reason further research is needed. This will only help to increase the awareness among patients and Doctors through which it will find better means of treatment.

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Materials and Methods

Study Design

The survey has a large variety of answers to choose from for the answers of the respondent to be more specific. The survey was conducted in the month of April 2020 and the respondents were aged between 17-23, all were dental undergraduates.

Survey Instrument

The questionnaire was prepared after an extensive review with amendments further made to improve clarity of the questions. The survey was structured and had close ended questions. 16 questions were prepared and were sent to the participants via Google Forms.

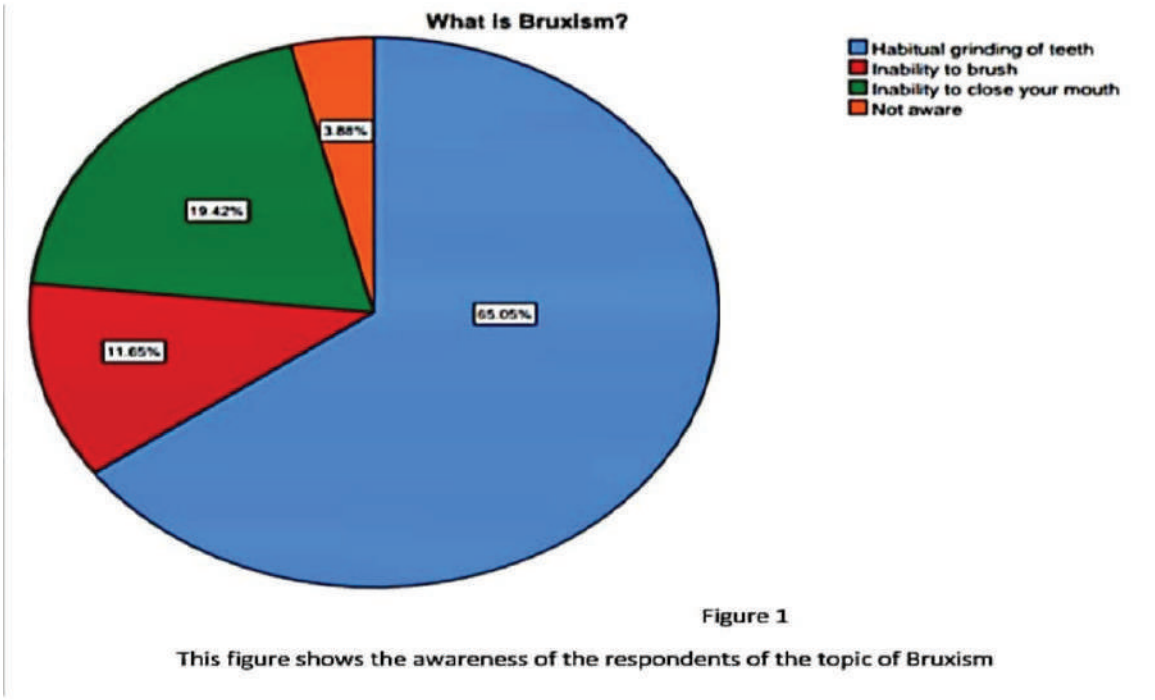
Analytics

The consisted of incomplete responses which were rejected and was reviewed if the data collected was entered the same day. The collected data was analysed using SPSS statistics 19 Descriptive analysis was performed to calculate frequencies of suitable variables and to create pie charts. [89101112]

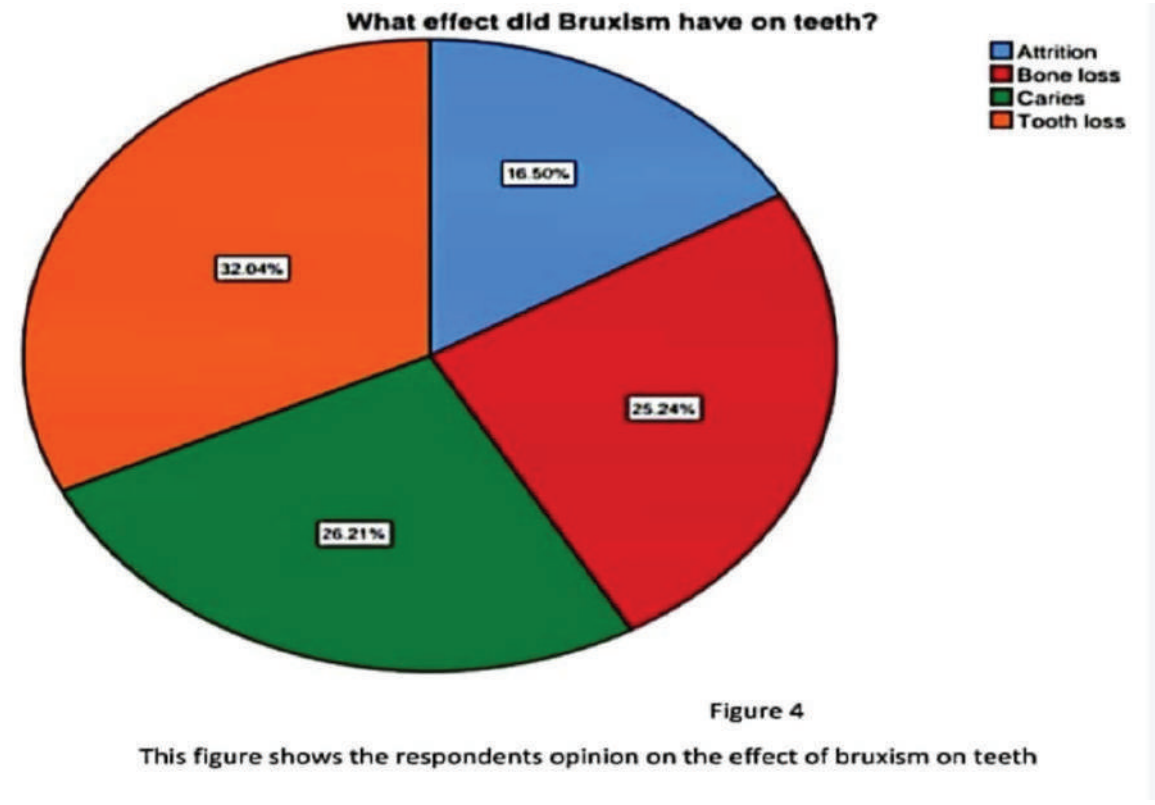
The individual graphs made for each question help to determine the percentage of the responded for its further analysis. Some important questions are discussed below

Results and Discussions

To know the significance of this study, we had to know the familiarity of the respondent's with the topic of Bruxism. 65.05% were aware that it is the grinding of teeth (Graph 1). (Graph 2) shows the respondents opinion on the effect of Bruxism on teeth. 32.64% chose tooth tooth loss, represented by orange colour, 26.21% chose caries that are represented by green, 25.24% chose bone loss as a possible effect of bruxism which is represented by red and 16.50% chose attrition which is represented by blue. To counter Bruxism as a phenomena, 35.92% felt it would be more effective to place the implant in a more medial position to the actual position of the tooth[13] . To counter the effects of Bruxism, 57.28% would prefer to use mouth guards but this was not similar to the findings of Michelle Omerbornn et al 2011, who said only 8% preferred mouth guards [14] and the 26.21% preferring medication is similar to Shilpa Shetty et al 2010 who found 20%.[2]. (Graph 3). (Graph 4) depicts the association between gender and the awareness about the familiar features of Bruxism X-axis represents Gender Y-axis represents number of respondents awareness on familiar features of Bruxism. Habitual grinding of teeth was the most selected option across both the genders showing no statistical significance. Pearson's Chi square value= 0.551, p value=0.907, (>0.05) hence statically non significant. (Graph 5) depicts the association between gender and the awareness of the possible causes of Bruxism. The X-axis represents Gender and the Y-axis represents the number of respondents aware of the possible causes of Bruxism. Even though most of the males have opted childhood habits but the options seem to be equally distributed among women for the possible cause of bruxism but on analysis there seems to be no statistical significance. Pearson's Chi square value= 5.914, p value= 0.116 (>0.05) hence statically not significant. (Graph 6) depicts the association between gender and the awareness of the effect of Bruxism on teeth. The X-axis represents Gender and Y-axis represents the number of respondents familiarity with the effect of Bruxism on teeth. Most of the males opted carries while the females opted tooth loss but there seems to be no statistical significance among genders on various effects of Bruxism on teeth. Pearson's Chi square value= 5.007, p value= 0.171, (>0.05) hence statically non significant.



Graph 1: This figure shows the awareness of the respondents on the topic of Bruxism. 65.05% were aware that bruxism meant the habitual grinding of teeth (Blue).



Graph 2: This graph shows the respondents opinion on the effect of Bruxism on teeth. 32.64% chose tooth tooth loss, represented by orange colour, 26.21% chose caries that are represented by green, 25.24% chose bone loss as a possible effect of bruxism which is represented by red and 16.50% chose attrition which is represented by blue.

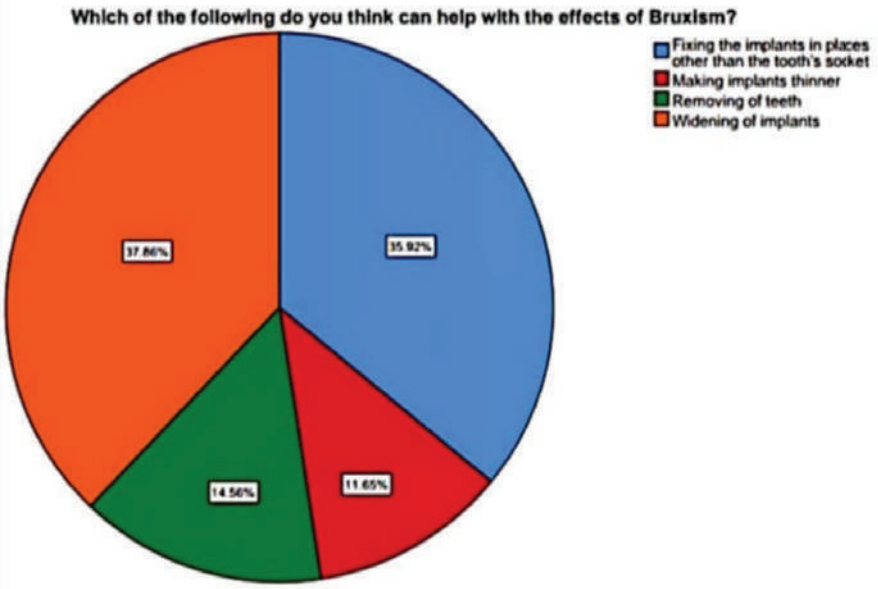
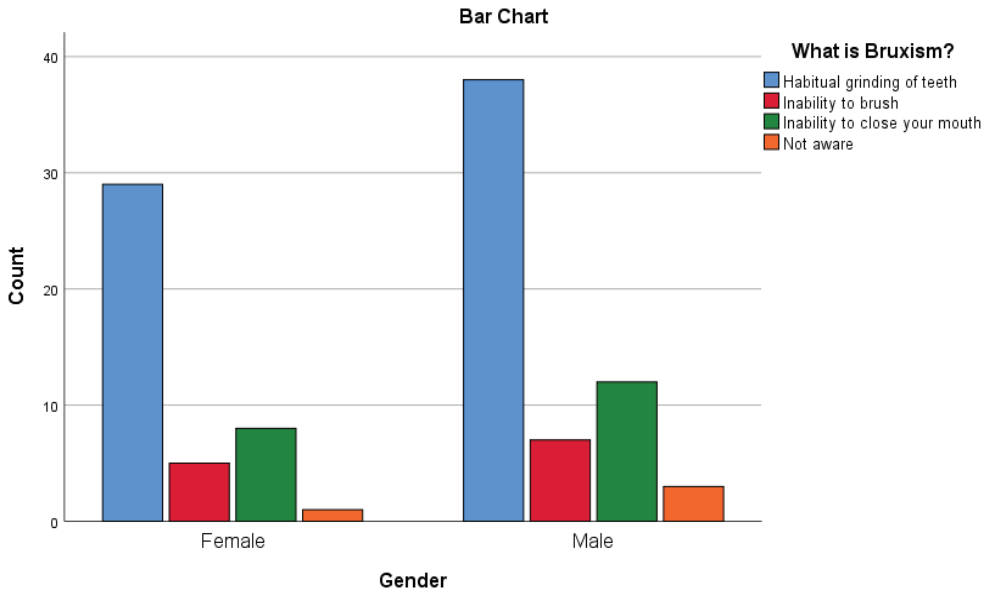


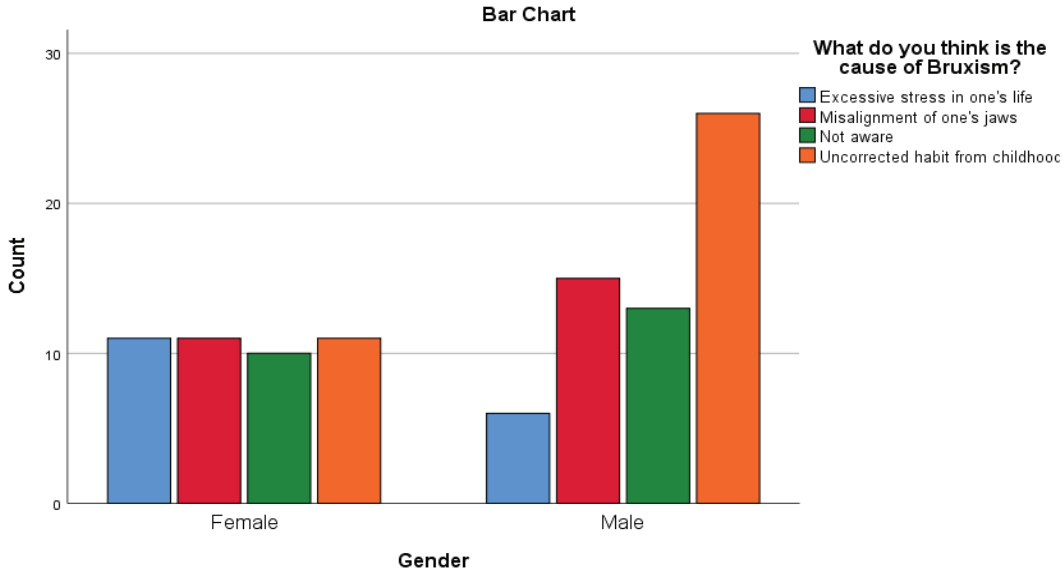
Figure 11

This graph shows the result to which the respondents thought would help the most with effects of bruxism

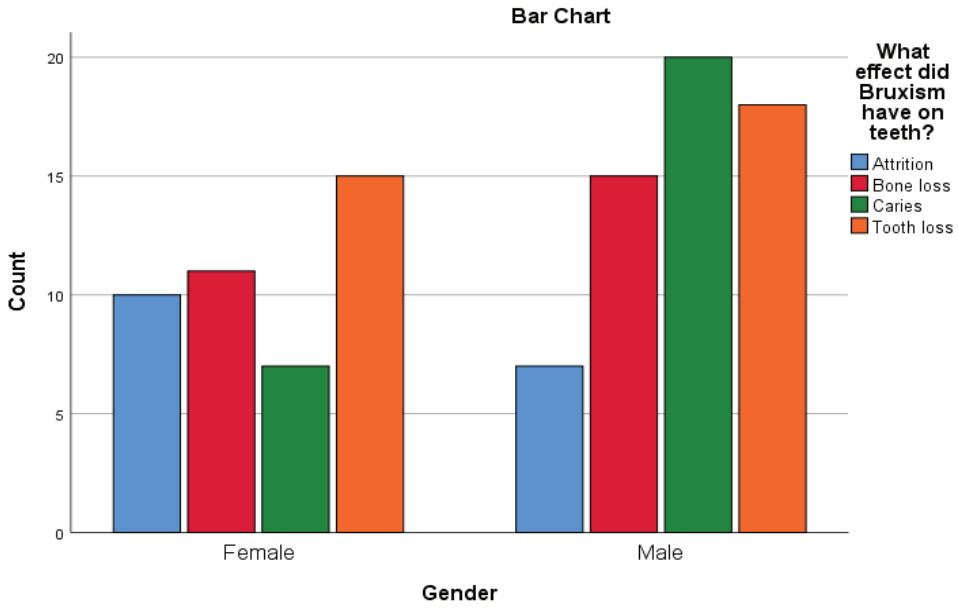
Graph 3: This graph shows respondents' opinion as to what would help with the effects of Bruxism. 35.92% chose fixing the implants mesial to their actual position, represented by blue, 14.56% chose the removal of teeth and it is represented by green. 37.86% felt widening of the occlusal surface of the implant would help, represented by orange and 11.65% felt making the implant thinner would help and it is represented by red.



Graph 4: Bar graph depicts the association between gender and the awareness about the familiar features of Bruxism X-axis represents Gender Y-axis represents number of respondents awareness on familiar features of Bruxism. Habitual grinding of teeth was the most selected option across both the genders showing no statistical significance. Pearson's Chi square value= 0.551, p value=0.907, (>0.05) hence statically non significant.



Graph 5: Bar graph depicts the association between gender and the awareness of the possible causes of Bruxism. The X-axis represents Gender and the Y-axis represents the number of respondents aware of the possible causes of Bruxism. Even though most of the males have opted childhood habits but the options seem to be equally distributed among women for the possible cause of bruxism but on analysis there seems to be no statistical significance. Pearson's Chi square value= 5.914, p value= 0.116 (>0.05) hence statically not significant.



Graph 6: Bar graph depicts the association between gender and the awareness of the effect of Bruxism on teeth. The X-axis represents Gender and Y-axis represents the number of respondents familiarity with the effect of Bruxism on teeth. Most of the males opted carries while the females opted tooth loss but there seems to be no statistical significance among genders on various effects of Bruxism on teeth. Pearson's Chi square value= 5.007, p value= 0.171, (>0.05) hence statically non significant.

Table 1: Questionnaire regarding the awareness of Bruxism and the responses in %

S.No	Questions	Results in percentage	
		Yes	No
1.	Awareness of the respondents on the topic of Bruxism, that bruxism meant the habitual grinding of teeth	65.00%	35%
2.	Bruxism could be fatal ?	59.22%	40.78%
3.	Do any slight modifications to the denture could help with Bruxism	69.90%	30.10%
4.	Bruxism had a major role in tooth wear and loss.	64.08%	35.92%
5.	Do enzyme levels cause Bruxism .	30.10%	69.90%
6.	Is Bruxism self curable?	51 .4%	48.6%

Table 1 shows the Awareness of the respondents on the topic of Bruxism and the response, that bruxism meant the habitual grinding of teeth , Will the habit Bruxism could be fatal. 59.22% said yes and 40.78% chose no. Slight modifications to the denture could help with Bruxism, respondents' opinion on 69.90% chose yes and 30.10% chose no. Respondent's opinion as to if Bruxism had a major role in tooth wear and loss, 64.08% said yes and 35.92% said no. any change in enzyme levels cause Bruxism, responses were 30.10% Yes and 69.90% No. Is Bruxism self curable? 51 .4% responses were Yes and 48.6% No. The present research has origins from the team of investigators where previous studies were done based on clinical reports, interventional studies [12,16,17], in vitro studies [9,18] and systemic reviews [15, 19, 20,21, 22, 23] .

The limitations of this study is we used a small sample size, upon using a larger sample size the results can turn out to be very different.

In the future, this study can be used for increasing awareness and finding new methods for treatments of patients who are suffering from Bruxism.

Conclusion

This study shows that the awareness of bruxism and its effect on prosthetic treatments is high. This data can be used in future treatments, resulting in reduced failure of prosthetics due to habits such as Bruxism.

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Ebola Virus and Its Immunopathogenesis - A Review

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Abstract

Ebola virus, a highly pathogenic DNA virus, is associated with severe systemic complications in both man and animals. Ebola virus is transmitted through direct and indirect contact. The first Ebola virus outbreak was due to the zoonotic transmission followed by human to human. These viruses encompass serious pathophysiological events like systemic viral replication, immune suppression, abnormal inflammatory responses, major fluid and electrolyte losses and high mortality. Diagnosis and laboratory tests, typically real time reverse transcription PCR is used to detect viral genome. The 2013 - 2016 outbreak was classified by WHO as a public health emergency of international concern, which drew attention to the challenges of diseases caused by infection with Ebola virus and societal preparation to handle future epidemics. This review throws insight on the immune-pathogenesis of ebola viruses from the articles retrieved from various search engines like Pubmed and Google scholar.

Keywords*: Ebola virus, Immuno-pathogenesis, Viral marker, Genome detection, Markers*

Introduction

Ebola virus disease was formerly known as ebola hemorrhagic disease. In Guinea during December 2013 the first case of ebola virus was reported. Ebola virus was found to be one among Filoviruses' family. Ebola virus disease is caused by many clades viz., BDBV, EBOV, SUDV or TAFV[1]. Until 2013, most Ebola virus outbreaks originated from middle Africa [2]. Ebola virus is a dangerous form of virus which has no treatment. Although the first case of human infection was probably acquired from an animal, all subsequent cases are likely to have arisen from human-to- human transmission [3]. The duration of epidemic growth in each region of each country, and the number of people at risk, were determined in part by the timing and magnitude of the interventions against Ebola. After the epidemic peak in each country, the case incidence declined most quickly in Liberia and more slowly in Sierra Leone and Guinea [4].

None of the health care workers reported to be infected globally and had died from ebola virus disease. So, it appears that aggressive supportive care can reduce mortality. However, there is no definite treatment for this virus, so it will lead to mortality [90%] [5]. Ebola virus is one of the most deadly pathogens known to infect humans and the origin of infection was a carrier who survived EVD in November 2014 [2,7]. In this outbreak, which generated 13 confirmed and probable cases, infection spread from Nzerekore to Macenta prefecture in Guinea and into Mon- rovia [8]. The persistent risk of infection from survivors is another reason (in addition to locally high rates of transmission) why we can expect any large Ebola epidemic to have a protracted end, demanding heightened surveillance, with routine testing of live and dead persons in whom EVD is suspected, for months after an outbreak ended [9]. This review thus highlights an overview on ebola virus and its immuno-pathogenesis.

Ebola Virus: An Overview:

The literature of the review was retrieved from search engines such as Pubmed, MESH, Google scholar and from other online sources**.** All the articles relevant to the topic of the review were included. The articles with general information were excluded from the study. The review was purely based on the previous reports published by other esteemed authors.

EBOLA VIRUS STRUCTURE AND GENOME:

Ebola virus is a RNA virus with the length of the viral particle around 300~1500 nm. It occurs primarily on the African continent. Strains from Zaire, Sudan, Tai Forest, and Bundibugyo with Zaire Ebola virus are considered as the lethal strain [10,11]. Researchers have found a fifth strain termed Reston in the Philippines. The strain infects primates, pigs, and humans and causes few if any symptoms and no deaths in humans. Ebola virus consists of 5 viral species within the genus ebolavirus [12]. It was found that people with Ebola virus had sudden onset of fever and malaise, accompanied by other nonspecific signs such as myalgia, headache, vomiting and diarrhea [10]. Among EVD patients 30% - 50% experienced hemorrhagic symptoms [13]. The outbreak was more during 1967, that time people were affected with EVD massively. 20 EVD outbreaks across central africa, species like Zaire ebolavirus, historically proven high case fatality upto 90% [14].

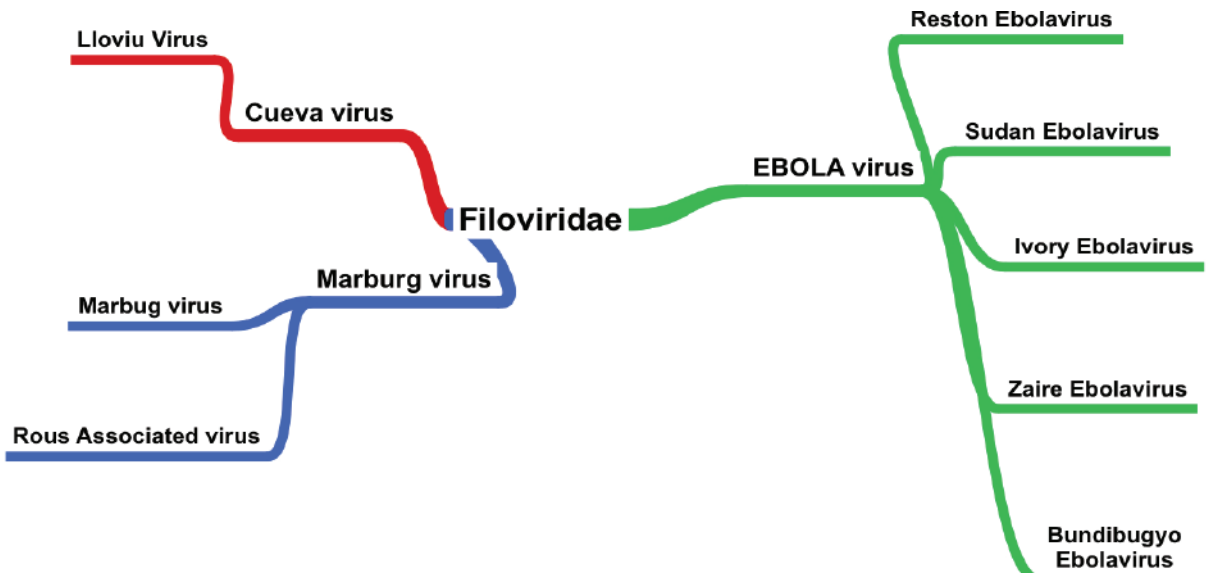


Figure 1: Taxonomy and clades of EBOLA virus

CLINICAL MANIFESTATIONS:

Ebola virus is most commonly transmitted when secretion from an infected patient comes in contact with mucosa or conjunctiva. Ebola virus replication requires attachment to a cell's membrane, binding to specific cell receptors and fusion with the cell's membrane. The virions glycoprotein [15,16], the outer capsule, is responsible for the attachments of the virus to the cell [17,18]. Ebola virus does not fuse to the cell membrane[19]. Death occurs as a result of circulatory shock or more commonly, multisystem organ dysfunction [20]. Nucleosome remodelers have been identified which causes repression of viral infection during the early stages [21]. Viral hemorrhagic disease, caused by members of the flaviviridae, Bunyavidae, viral families are characterised by fever, bleeding diathesis followed by circulatory collapse and death [22]. Filaviviridae family viruses are responsible for yellow fever and dengue fever [23].

IMMUNE RESPONSES

The patients who have poor immune response and high inflammatory response are mostly affected with Ebola virus disease. Host innate immune responses are vital in restricting the spread of viral infections including that of Ebola virus [24]. Antibodies could also function by recruiting cells and proteins of the immune system to destroy and clear the pathogen and its infected cells [25]. In most of the cases, both humoral and cellular immune response plays a vital role [26]. Cellular immune response involves the role of the natural killer cells and other immune cells[27]. The proinflammatory cytokines do play their role in viral evasion and protection. Both neutralizing and cross-reacting antibodies help in viral

clearance in the infected individuals [24].

DIAGNOSTIC METHODS TO DETECT THE ANTIGENS

Laboratory diagnosis of Ebola is achieved in two ways: detection of infectious particles (or particle components) in affected individuals and measurement of specific immune responses to Ebola virus**.** The incubation period of ebola virus disease is from 2 to 21 days, with shorter incubation periods correlating with exposure to a larger viral load. Viremia correlates with the abrupt onset of symptoms and signs of the disease [28]. According to WHO's protocol, it is like the sudden onset of high fever and headache, vomiting, diarrhoea, stomach pain, dyspnea, hiccuping [29,27]. Conventional diagnostic methods were replaced with RT-PCR tests for Ebola virus, developed by the CDC, and was first evaluated on serum samples collected from acutely ill patients [30]. Serological diagnostic methods to detect both IgG and IgM antibodies are also employed in certain conditions[31].

TREATMENT STRATEGIES

Although vaccination probably helped to reduce the rate of transmission after April 2015, the dominant interventions used have been the classic methods of Ebola control. Finding symptomatic cases and tracing potentially infected contacts, isolating cases, admitting patients to specially designed Ebola treatment centers, and providing supportive clinical care; and ensuring safe and dignified burial are also in the guidelines in the ebola viral disease prevention [32]. With no proper treatment strategies until now, increase in mortality rate is by immuno-pathogenic mechanisms that leads to develop shock and DIC [33]. It is capable of causing death in humans and non humans within a few days of exposure [34]. The epidemics that have occurred during the past 4 decades have been in low income countries with limited healthcare resources [35]. Most countries do not have laboratory tests, like CBC, TBC and cardiac out measurement are rare [36]. In addition, tests must be performed in a biosafety level - 4 laboratory.

If the patient cannot maintain fluid balance because of gastrointestinal illness, IV crystalloid fluids should be administered [37]. Hypoxia is reported to occur with Ebola virus disease. Many people have survived Ebola virus disease [38]. Their convalescent serum has been administered to others who were actually affected with Ebola virus disease with anecdotal success [39]. Early vaccines like AVI - 7537 & AVI - 7288 were used for the treatment of Ebola virus and found to be less effective for about 84% of the population [40]. A non - neutralizing antibody that cross reacts with glycoprotein effective neutralization of virus [41].

PREVENTION STRATEGIES

While licensed vaccines against Ebola virus infection are still against Ebola virus infection are still not available. A number of vaccines against Ebola virus have been developed and showed good effects among animal models. This development needs a focus and a proper, adaptable, non - harmful / Hazards vaccine can be invented [42,43]. Coordinated medical services, careful handling of infected cases are the main preventive measures of ebola virus [44]. Preventive measures can be followed by distancing from the infected people, their body fluids, and the bodies of anyone who has died from the disease. Preventive strategies must also be taken to avoid contact contact with wild reservoir animals, like bats and monkeys, and their meat.

Conclusion

Conclusively, this review had highlighted an overview on the EBOLA virus and its immunopathogenesis. With no treatment strategies discovered yet, preventive measures are considered as the best method from acquiring the disease. Conventional, serological and molecular methods are available to detect and screen the viral markers with limitations. This review also emphasizes on the need for vaccine and drug discovery against EBOLA virus.

AUTHOR CONTRIBUTIONS :

S. UMAYAL

1. Execution of the work.
2. Data collection.
3. Drafting of Manuscript.

SMILINE GIRIJA . A.S

1.Concept and design of the study.

2. Validation of the data collections.

3. Revision and proofreading of the review

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Prevalence of Anterior Crossbite in Children Visiting A University Hospital in Chennai - A Retrospective Study

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Abstract

Anterior crossbite is a common malocclusion in early mixed dentition.Teeth with proper occlusion plays an important role in speech, mastication, deglutition. Determining its prevalence is important for early correction. The objective of the study was to assess prevalence of anterior crossbite in children visiting university hospital Chennai. A sample of 4355 patients of age 6-13 years visiting the hospital were taken. We reviewed patients records, analysed data of 7415 patients between June 2019 to March 2020 and clinical findings are recorded.The data is then tabulated in microsoft excel. Chi square test is used for comparison of groups. The data is analysed with the help of SPSS software.In this study we observed that 3.7% of the total patients showed incidence of anterior crossbite. Prevalence of anterior crossbite is observed to be more in age groups between 10-13 years followed by 6-9 years. Within the limitations of the study, 3% of the children visiting university hospital have anterior crossbite.

Key words: *Anterior crossbite , class I, Class II, Class III, Malocclusion, Children*

Introduction

Anterior crossbite is a typical malocclusion and it is generally evident in early mixed dentition. Salzman defined anterior cross bite as the lingual placement of maxillary incisors in relation to the opposing mandibular teeth when both arches are in centric occlusion [1]. Clinically anterior crossbite presents with reverse overjet, premature bite leading to displacement of anterior cross bite, gingival recession along with mobility of anterior tooth associated with crossbite [2].

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A crossbite can be of dental or skeletal origin or a combination of both [3]. Crossbite of all incisors is rare except the patients with class3 jaw relation [4]. Factors like lingual eruption of incisors, retained deciduous incisors ,crowding, trauma are reported as causes of anterior crossbite [5]. Previous studies show that prevalence of anterior crossbite is 7-10% in children with mixed dentition [6]. Survey in Iraq reported that prevalence of anterior crossbite is about 5.5% [7]. A recent study reported the prevalence of anterior cross bite is 7.09% in age group between 3-5 years.

Treating anterior crossbite is essential in primary and early mixed dentition,irrespective of skeletal or dentoalveolar origin [8]. Correcting anterior crossbite in the earliest will eliminate displacement of mandible ,further complications and centric relationship is obtained [9]. If anterior crossbite is neglected it leads to abrasion of labial surfaces of upper anteriors, causing damage to overall periodontal health [5,9].Therefore,early rectification of anterior crossbite will eliminate complications.

Anterior crossbite is the major esthetic concern which results in various skeletal and dental deformities, if left untreated. As a child grows their crossbite has an impact on social and emotional well-being. Children with anterior crossbite are prone to caries due to improper occlusion there is accumulation of food and bacteria, to minimise optimum concentration of fluoride is necessary to prevent caries, where as imbalance in the concentration of fluoride causes increased caries incidence and skeletal problems [10,11]. Chewable toothbrush can be used as an alternative to manual toothbrush to remove plaque especially in children [12]. Evaluation of malondialdehyde (MDA) level in saliva helps in detection of caries, it is known that children with caries have a high level of MDA in saliva [13] .

Anterior crossbite if left untreated it leads to abrasion of teeth which in turn leads to sensitivity and ultimately pulp exposure of teeth. Restorative treatment for caries should be advised before proceeding to orthodontic treatment. Pulpectomy is the commonly performed treatment to save the primary tooth in the cases of deep caries and pulp exposure [14]. Rotary instrumentation in primary teeth results in marked decrease in instrumentation time and improves obturation quality[15]. kedo-S paediatric rotary file is used for pulpectomy procedures in primary tooth due it's uniform and predictable quality of obturation [16,17,18].Obturation is more convenient with rotary than manual obturation [17,19]. There is a need to attain extensive knowledge on the use of rotary file to perform pulpectomy procedures in primary teeth [20].

Many studies were done on dental malocclusion in various communities. Ethnic,behavioural, and nutritional variations have likewise been assessed.There is extensive variation in figures acquired by researchers as different researches have followed different criteria for surveying the occlusal characteristics [5,9,21]. This laid emphasis on the need to standardize criteria for surveying malocclusion.The current study spread awareness and helped in Understanding prevalence of anterior crossbite in chennai population.

Management of dental traumatic injuries of primary tooth carry major importance in terms of preventing malocclusion in early stages [22]. Thorough examination of soft and hard tissues of oral cavity plays a vital role in treating malocclusion [23,24]. Dental negligence in children is the failure of a parent or guardian to meet the child's principal oral health care needs [25].Information on prevalence if anterior crossbite is necessary for early intervention and prevention of further skeletal discrepancies and the above stated dental diseases from occurring. The current study aims to assess the Prevalence of anterior crossbite in children visiting a university hospital in Chennai.

Materials and Methods

This retrospective study examined the records of patients from 01 june 2019 to 31st march 2020 who visited saveetha dental college and hospital. Ethical approval was taken from the institutional review board/ SDC/SIHEC/DIASDATA/0619-0320. The study population included patients with age ranging between 6-13 years. The study sample included both male and female gender, predominantly south indians.

The study population was 7415 pediatric patients who visited university hospital. Sample size was 4355 pediatric patients in which 187 patients in the hospital database were diagnosed with crossbite. The necessary data such as age, gender, type of cross bite, molar relation was recorded. Incomplete patient records were excluded.Data was recorded in Microsoft Excel and exported to the statistical package of social science for windows (SPSS) and subjected to statistical analysis. Chi square tests are used for comparison of groups.

Results and Discussion

The study sample consisted of 4355 patients, about 2070(47.5%) of children belonged to the age group between 6-9 years and 2285 (52.5%) of children belonged to the age group between 10-13 years (Graph -1). Among 4355, 2389(54.9%) of the children were boys and 1966(45.1%)of the children were girls (Graph -2).

Among patients 4355 patients one hundred twenty nine (2.96%) had anterior crossbite, 58 (1.3%) were anterior crossbite with posterior crossbite and 4168 (95.7%) with No crossbite (Graph -3).

Children in group 6-9 years showed incidence of anterior crossbite(1.17%), 0.39% were anterior and posterior crossbite and 45.97% no crossbite. In the

age group between 10-13 years incidence of anterior crossbite is 1.79% followed by anterior and posterior crossbite (0.94%) and 49.74% of children had no crossbite (Graph-4).

Anterior crossbite In relation to gender when analysed showed girls with incidence of 1.58% of anterior crossbite, 0.48% showed anterior crossbite along with posterior crossbite and 43.8% accounts for No crossbite. In males, 1.38% showed incidence of anterior crossbite, 0.85% showed both anterior and posterior crossbite, 52.63% with no crossbite (Graph -5).

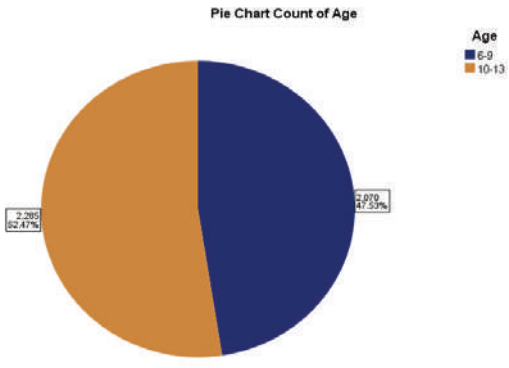
This study found that of 4355 patients attending the university hospital ,3.7% had anterior crossbite.The results of anterior crossbite are similar to the results of other studies conducted in Asian region[26],[27]among the anterior crossbite patients The percentage of males is 2.5% and females is 3.5% This findings were similar to that reported among school children in Brazil[28]. This shows contrast result with the study performed children in kerala[29]

Among the recorded crossbites,58(1.3%) were anterior cross with posterior crossbite and with a finding of 95.7% no crossbite cases. According to Dacosta et al.reported that 14.3% had coexisting of posterior crossbite which is not similar to the study it may be due to the small sample size of 85 and the patients with no crossbite is not included in the study[30]. The majority of the patients presenting with anterior crossbite have class 1 molar relation. This finding is in agreement with that of other studies [31] [32]

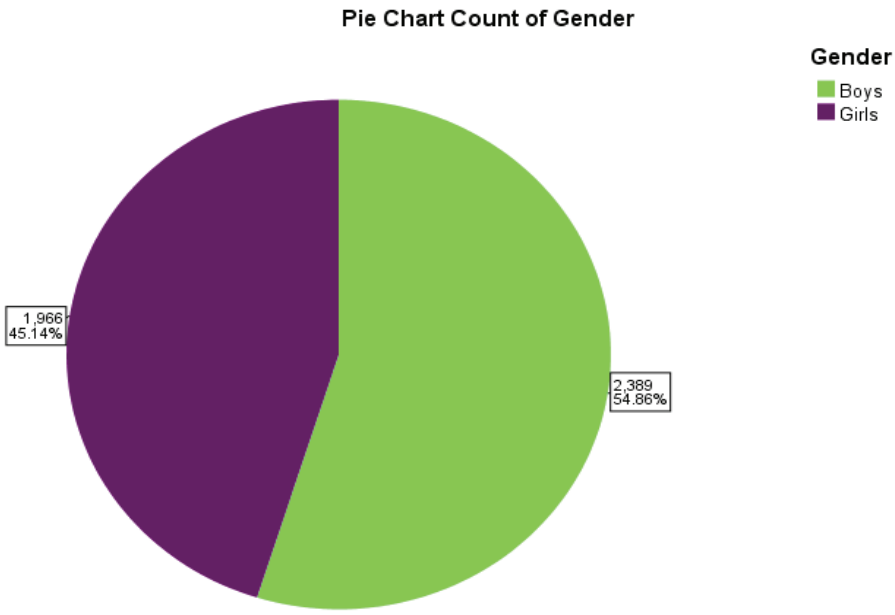
The present study showed that anterior crossbites were significantly more frequently occurring than posterior crossbites.These findings concur with findings of dacosta[30] Adegbite and Nnachetta[33,34] in prevalence studies on Nigerian school children.According to results of this study incidence of crossbite is prevalent.This study serves as a step for planning necessary preventive, interceptive measures and helps in early correction of the malocclusion. This study provides baseline data enabling the dental practitioners to plan their treatment to avoid compromising the prognosis for later orthodontic treatment and even encourages development of normal occlusion.

This study is limited as it has only recorded malocclusion in the age group of 6-13 years which cannot be generalized to the entire population. Besides, orthodontically treated cases were not included which can underestimate the commonness of occlusal characteristics. Subjects are not available for direct examination.The examination is fairly based on photographs and case sheets available in the DIAS.

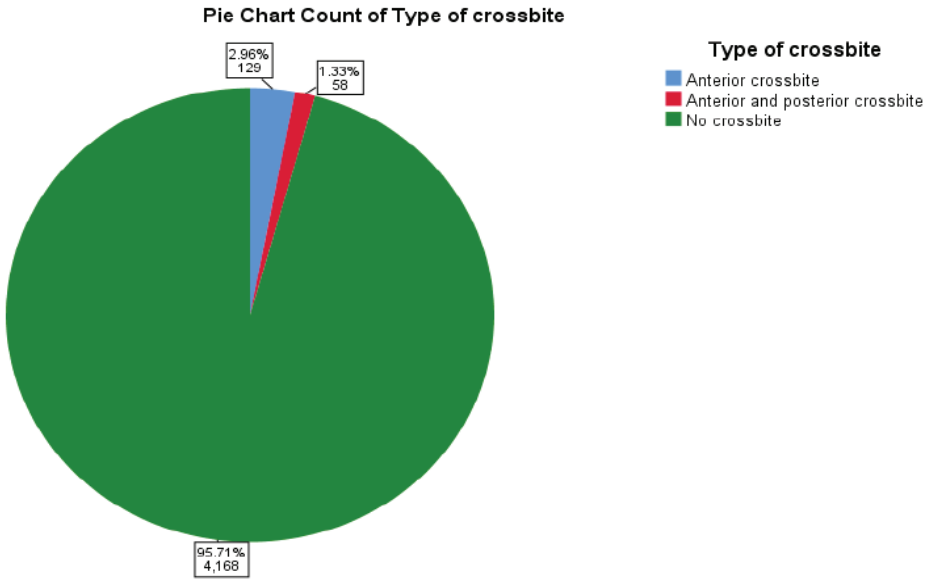
Further research is needed as anterior crossbite is a major esthetic and functional concern. Study can be extended for further diagnosis and treatment planning. Proper counselling on awareness of early correction is required.Thus the study serves as an evidence and adds to the consensus that can be utilised for further studies at the larger population and clinical studies.



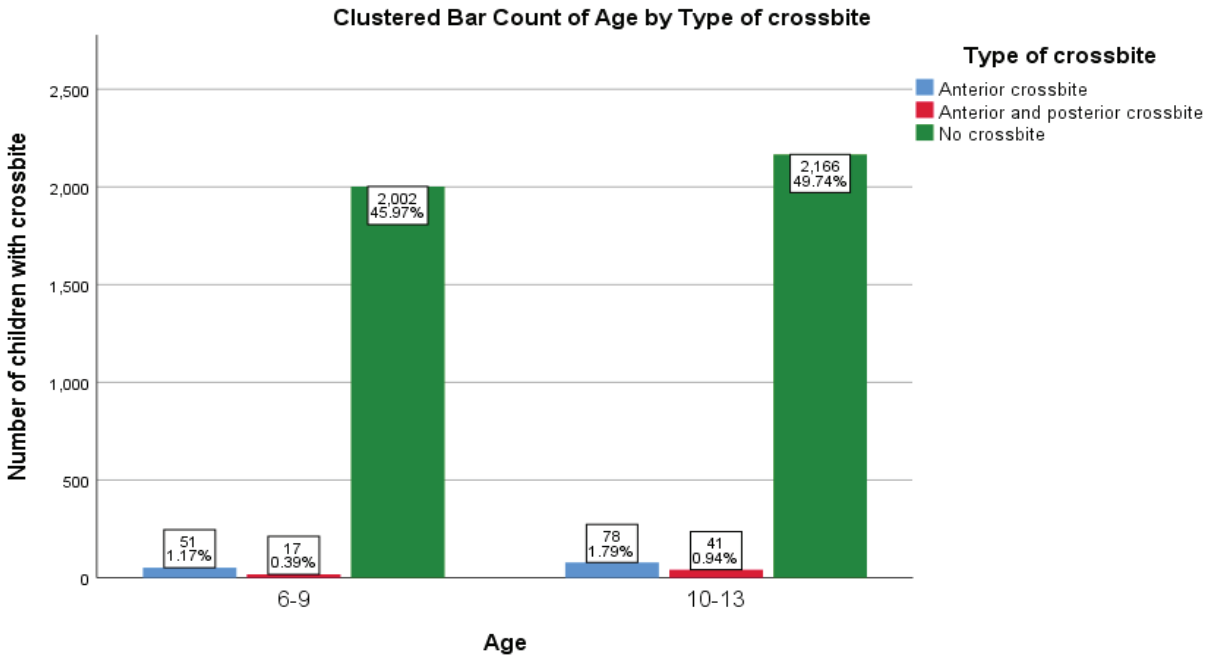
Graph 1 : Pie chart showing age distribution of children in sample population. Dark blue colour denotes children of age group between 6 to 9 years and brown colour denotes children of age group between 10 to 13 years. 52.47% of the children belonged to the age group of 10 to 13 years and 47.53% of the children belonged to age group between 6 to 9 years.



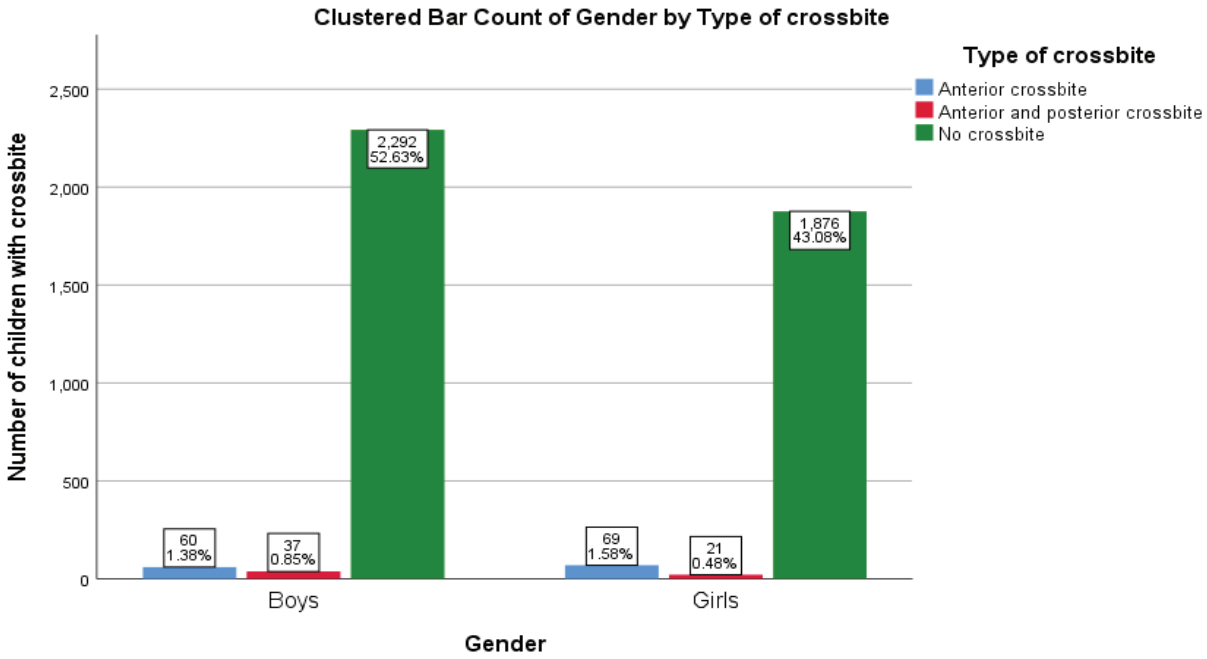
Graph 2 : Pie chart showing gender distribution of children in sample population. Light green colour denotes boys and purple colour denotes girls. 54.86% of the children were boys and 45.14% of the children were girls.



Graph -3 : Pie Chart showing prevalence of type of crossbite in study population where blue colour denotes anterior crossbite, red colour denotes anterior and posterior crossbite and green colour denotes no crossbite. Prevalence of anterior crossbite was 2.96%.



Graph 4: Barchart shows the association between various age groups and types of crossbite.X axis represents age group and Y axis represents frequency distribution of type of crossbite. Blue colour denotes anterior crossbite, red colour denotes anterior and posterior crossbite and green colour denotes no crossbite. The occurrence of anterior crossbite in children was more in age group between 10 to 13 years. Chi square test was done and the association was found to be significant. (p value = 0.003 <0.05 statistically significant).



Graph -5: Barchart showing association of types of crossbite with gender. X axis represents gender and Y axis represents frequency of type of crossbite. Blue colour denotes anterior crossbite, Red colour denotes anterior and posterior crossbite and green colour denotes no crossbite. There was no difference in the type of crossbite between boys and girls. Chi square test was done and the association was found to be not significant.(p value was =0.063 p>0.05 statistically not significant).

Conclusion

Within the limits of this study, we observed that 2.96% of the total patients showed prevalence of anterior crossbite. Anterior crossbite is observed to be more incident in the age group 10-13 years followed by 6-9 years. It is considerably significant to the dental specialist to examine the early signs of malocclusion particularly during developmental stage because of consistent occlusal changes that occur during the early growth of children.

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Conflicts of Interest : None declared

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Eclia Test- Review

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Abstract

Chemiluminescent immunoassay is a modification of standard enzyme immunoassay which is a biochemical technique that has evolved to be used in recent times. Immunoassay is a diagnostic biochemical test used to quantify an analyte or a specific substance from blood or other body fluids. Electrochemiluminescence immunoassay (ECLIA) is a new developing method to determine antibodies in human being's blood sample.this has evolved from various other immunoassay methods that were previously used for detection of enzyme/antibody etc. The test takes place following three principles. Electrochemiluminescence immunoassay testing has been used to detect many markers such as typhoid, cardiac, tumor markers and various infectious diseases in in-vitro analysis. It is also frequently used in the detection of recombinant specific treponemal antigens. Research articles were searched from search engines such as MeSH, pubMed, Core, Google Scholar, Cochrane etc. the time period considere was from 2000 - 2020 which is 20 years. Articles were studied and data were collected under different headings. In Spite of many advantages of immunoassays, they have some limitations. Immunoassays rely primarily on activity between analyte and the natural antibody, they may have more intrinsic imprecision than other methods used in medicine analysis. Biological markers might produce false positive results and usage of viral markers is still not found for the process of electrochemiluminescence immunoassay test. Late night salivary cortisol concentration, an indicator of cushing's syndrome, is detected using this test. This research provides in-depth knowledge and awareness of electrochemiluminescence immunoassay tests and paves the way for better diagnostic methods for the future.

Keywords: *Electrochemiluminescence, Antigen/ antibody detection, Light night salivary cortisol, Diagnostic test.*

Introduction

Chemiluminescent immunoassay is a modification of standard enzyme immunoassay which is a biochemical technique that has evolved to be used in recent times. It is often used as a diagnostic tool in medicine[1].It is expressed by a luminescence molecule which happens by the emission of visible or near to visible radiation that occurs due to transmission of electrons from excited state to ground state. The potential energy that is an indication of detection of an indicator[2].

Immunoassay is a diagnostic biochemical test used to quantify an analyte or a specific substance from blood or other body fluids. The molecule observed by the immunoassay is frequently referred[3] to as the analyte and in some instances proteins, although it may exist different sorts of atoms, of different types and sizes, as long as the specific antibody has sufficient attributes for the assay produced. Analyses in natural liquids e.g. serum or excrement are often assessed using immunoassays for medical and investigation purposes[4].

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Electro chemiluminescence immuno assay is a new developing method to determine antibodies in human being's blood sample.this has evolved from various other immunoassay methods that were previously used for detection of enzyme/antibody etc[5] Determination of antibody is done by chemical reactivity with materials like ruthenium, osmium etc., that are coated on monoclonal antibodies and on reaction produce luminescent molecules hence the name. This production of luminescent molecules makes determination uncomplicated[6].

The test takes place following three principles: Competitive principles for example small analytes, Sandwich principle for large analytes, Bridging principle for dilution of antibodies in the sample. All these three principles put together makes this test sensitive and less time consuming [7]. Enhances like phenolic and its derivatives are used to ameliorate the sensitivity of the test to detect antibodies quicker. Every test follows a sequential procedure to express the result/analysis. Like wise electro chemiluminescence immuno assay also follows a fully automated procedure that reduces time and any errors possible [8].

The various advantages of electrochemiluminescence immunoassay test is that it is an extremely sensitive and time thrifty method to determine the antibody in the blood, which makes it a very potential test for many disease diagnosis. It is also automated so there are minimal or no errors possible while doing this test[9]. The chances of false positive and false negative results are obvious. Just like all any expediency having a negative aspect, viral markers[10] and biological markers are still not usable in this method[11]. Enhancers that are used to improve the chemi-luminescent reaction are enzyme protectors which increase the time of exposure of light without much reduction in the intensity[12].

Electrochemiluminescence immunoassay testing has been used to detect many markers such as typhoid, cardiac, tumor markers and various infectious diseases in in-vitro analysis. It is also frequently used in the detection of recombinant specific treponemal and some bacterial[13,14] antigens. It is also helpful in detecting the prevalence of congenital hyperthyroidism.In addition the working dynamic range is wider compared to previous immuno assay methods. The relationship between the intensity of luminescence produced and the concentration of substance measured is one-dimensional[15].

The importance and availability of such accurate results producing tests have to be known world wide which is why research on this test providing in-depth knowledge and awareness of the same paves path for better diagnostic methods for future.Research articles were searched from search engines such as MeSH, pubMed, Core, Google Scholar, Cochrane etc. the time period considere was from 2000 - 2020 which is 20 years. Inclusion criteria for articles were electrochemiluminescence immunoassay test application, electrochemiluminescence immunoassay test for detection of various pathogenesis proteins and comparative studies between different immuno assay. Exclusion criterion was presence of other systemic diseases that causes complications in testing procedures. Articles were studied with standard quality assessment tool[16] and data were collected under different headings such as electrochemiluminescence immunoassay sampling and its collection, Processing of sample, Analytics from Saliva, Advantages and Disadvantages. All the data were analysed thoroughly to conclude with the processing and analysis of electrochemiluminescence immunoassay test to provide a proper article on its working principle and give more evidence.

ELECTROCHEMILUMINESCENCE IMMUNOASSAY - ECLIA

Electrochemiluminescence Immunoassay Sampling And Its Collection

Electrochemiluminescence detection is a combination of sensitivity, dynamic range, and convenience that is not matched by any other diagnostic method[17]. It is a biological assay involving multi array technology and electrochemiluminescence which increases the speed and density of information of biological assays. The procedure requires less time and effort[18]

Electrochemiluminescence immunoassay test is done on dried blood spots to detect the antibodies. Blood samples are collected in conventional methods and stored in plain vacutainers[19]. Serum and plasma are separated by centrifuging the blood for 20 minutes and then transferred to analyse Plasma collected with

anticoagulants and are centrifuged for 15 minutes and transferred to analyse. Anticoagulants used are sodium citrate, ethylenediaminetetraacetic acid (EDTA), heparin which are some commonly used blood thinning agents during sample collection. Sodium citrate chelates the free Calcium ions (Ca^{2+}), and inhibits the coagulation of blood. Though it has the ability to do so they have weak anticoagulative effects and poor sensitivity[20]. Ethylenediaminetetraacetic acid (EDTA) also chelates the free Calcium ions (Ca^{2+}), and inhibits coagulation. It doesn't affect red blood cells but affects platelet aggregation. Cell preservation is optimum in Electrochemiluminescence immunoassay. High concentrations of ethylenediaminetetraacetic acid (EDTA) are hypertonic in comparison to red blood cells therefore high caution is required during collection of small amounts of blood samples as the red blood cells would shrink Heparin so far has been the best anticoagulant used for blood sample collection and storage. Heparin inhibits antithrombin III. It is a strong anti coagulant[21]. It is stable in high temperatures also. Heparin is hard to hemolyse and causes leukocytes aggregation. After blood samples are collected they are analysed for antibodies/ antigens[22].

Processing of Samples

After collection of samples and separating them, the sample is firstly incubated with specific anti T3 antibody labelled with ruthenium complex which is also known as biotinylated T3, along with Streptavidin coated paramagnetic microparticle[23] Free binding sites of the labelled antibody are occupied by the formation of antigen - hapten complex and this whole complex is bound by micro-particles of biotin and streptavidin[24]. After the second incubation, the immune complexes are transferred to a measuring cell where the complexes are magnetically trapped on a working electrode[25]. The unbound reagent and sample are washed away with a buffer. In the reaction the chemiluminescent reaction is electrically estimated to produce light. The amount of light provided by the reaction is indirectly proportional to the concentration of antigen/ antibody present in the sample tested[26].

Electrochemiluminescence immunoassay is Roche's technology for detection by immunoassay[27]. Based on the combination of this technology and well designed specific and sensitive immunoassay electrochemiluminescence immunoassay test device provides reliable results. The use of ruthenium - complex and tripropylamine has led to the development of Electrochemiluminescence immunoassay. The reaction for detection of the reaction complex is stimulated by voltage applied to the sample solution which also facilitates for the precise control over the reaction. Electrochemiluminescence immunoassay technology follows many immunoassay principles while providing superior performance and better sensitive results[28].

Analytics from Saliva

Saliva test or Salivaomics is a diagnostic procedure that involves biochemical laboratory analysis of saliva which is an important biological fluid that can be used for steroid assay and identification of various hormones and markers of endocrine, immunologic, inflammatory, infectious and other types of conditions. It is very useful for assaying steroid hormones like cortisol, genetic material like ribonucleic acid (RNA), proteins such as enzymes and antibiotics and a variety of other substances including natural metabolitesTesting of saliva is used for the screening of various conditions and diseases such as Cushing's syndrome, anovulation, human immunodeficiency virus (HIV), cancer, etc[29] it is one of the least invasive tests. They give the most reliable results. It is the best method for immediate detection of any condition as the oral fluid is well connected to the bloodstream. The proponents of saliva testing are that there is an ease in collection of sample, safety, non-invasiveness, affordability, accuracy and capacity to circumvent venipuncture. Collection of multiple samples [30]can also be readily obtained. It is also useful for performing chronobiological assessment for long duration. Collection of passive drool facilitates large sample collection. It has the ability to freeze and the left over specimen can be used for future purposes.

Late night salivary cortisol concentration is detected using this test. Late night salivary cortisol is an indicator of cushing's syndrome. Therefore electrochemiluminescence immunoassay test is preferred for bio-screening. Late night salivary cortisol has an effect on endogenous hypercortisolism which in turn leads to adrenal incidentalomas[31] When the salivary cortisol during the screening is very low, synthetic

glucocorticoids are added which cross reacts to give results for immune assay.When cortisone - cortisol ratio remains constant it is indicative of contamination by topical hydrocortisone. Late night salivary cortisol measurements every 6 months are essential for absence or recurrence of symptoms. Electrochemiluminescence immunoassay test is a wide spread, cost effective screening test for Cushing's syndrome. It is also more advantageous being fully automated and less time consuming[32].

Advantages

Electrochemiluminescence immunoassay test is highly specific in recognition of antigens and antibodies and is used for detection of aflatoxin. Inexpensive practical screening is possible. It is a highly sensitive portable device. As there is absence of background optical signal there is an ease in control and the changing of electrode potential can be done manually to detect even ultra trace levels of antibodies. It has narrow emission bands which makes it highly sensitive. The band range of absorption is extremely wide. It has high resistance to photo bleaching. Nano crystals are added as semiconductors that increase sensitivity and rapid analysis of samples is possible.Semiconductors that are added are nanomaterials containing Cadmium sulphide (CdS), Cadmium selenide (CdSe), Cadmium telluride (CdTe) etc. addition of these semiconductors facilitate label free biosensing by low density lipoproteins. Magnetic nanoparticles[33] increase signal and sensitivity of immune devices. The simple precept for Electrochemiluminescence is that the luminescence substrate and the factor in the response lose electrons on the floor of an electrode, so as to be oxidized[34]. The electron donor loses a hydrogen ion (H^+) to turn out to be a sturdy lowering agent, which reduces the luminescence substrate to the excited state, and then the luminescence substrate releases photons to return to the floor state[34,35]. This system is carried out persistently on the floor of the electrode, and photons are continuously launched to hold the substrate awareness constant. The detection scope of electrochemiluminescence immunoassay of this method is wider than that of Enzyme linked immunosorbent assay (ELISA).

Electrochemiluminescence or electrogenerated chemiluminescence is a kind of luminescence produced during electrochemical reactions between analytes and antigen/ antibody complexes in the reacting solutions. Intermediates that are produced undergo exthothermic reaction to produce an excited state that luminates upon relaxation to a lower - level state. This luminance of the emitted photon correlates to the energy gap between the excited and relaxed state of the molecules[36]. The advantages of immunoassays are their ability to concurrently check for multiple antigen/antibodies, give speedy results and take proper usage of confirmatory testing. However, immunoassays may cross-react with different antigen/antibodies and change in ability and specificity. Therefore, unexpected immunoassay effects should be translated with caution and verified by confirmatory tests[37].

Disadvantages

Though this test is extremely sensitive and helpful there are few disadvantages as well. Peroxydisulfate solution with dissolved oxygen makes it difficult for antibodies to bind to ruthenium etc. and in relatively scarce quantities makes it difficult to separate[38]. Convenient method is by immobilizing antibody by single electrode to greatest extent by its difficulty to graft as it deactivates/degenerates the antibody. High concentration of substance reduces the signaling capability as less enzymes are labelled with antigen and remaining settle in the bottom of the microtiter plate. When there is no catalysis of substrate there is no signal produced. High levels of ruthenium and other substances in multiple sites tend to cause loss of biological activity of molecules[39].

In Spite of many advantages of immunoassays, they have some limitations. Immunoassays rely primarily on activity between analyte and the natural antibody, they may have more intrinsic imprecision than other methods used in medicine analysis[40]. The specificity of immunoassays is based on the protein directed to the analyte, yet some are not extremely specific/selective. Due to the ruthenium complex coating done for electrochemiluminescence immunoassay it is extremely selective and gives better and accurate results. This shows a good innovative [41]development in immunoassay generations.

Jie, Guifen, et al 2009 has stated that immuno sensor is better with biotinylated monoclonal

antibody labelled with ruthenium complex than plain antibodies[42]. Yao, Wu, et al 2008 has mentioned that electrochemiluminescence immunoassay tests are used for various diagnostic purposes such as monitoring the function of hormones, occurrence of any tumor markers, prevalence of infectious diseases by certain pathogens etc.[43] Gagnon, N, et al 2018 has tested various principles that are followed by the test.[44] Raff, H 2013 found that the particles like ruthenium, osmium etc are attached to agglutination, and the reporter molecules conjugate with target molecules. Non target molecules bind to antibodies.[45]

CONCLUSION

Electrochemiluminescence immunoassay is a widely used diagnostic method to find pathogens from body fluid with the help of antigen - antibody complex and production of luminescence based on the reaction. It is also cost effective and consumes very less time. Simple errors are avoided as it is an automated process. It has high specificity compared to other immunoassays. It is helpful in detection of various markers like tumor marker, infectious pathogens etc. Semiconductors increase the sensitivity of the device. It has a wider detection scope compared to Enzyme linked immunosorbent assay (ELISA). This study has reviewed various uses and applications and unique features of electrochemiluminescence immunoassay test and provides in-depth knowledge in understanding the method of electrochemiluminescence immunoassay test and given better knowledge and open up any advancements and new ideas for further improvement in the diagnostic method.

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Amount of Tooth Reduction in Fixed Partial Denture

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Abstract

Fixed Partial Denture is a restoration that is used or otherwise securely retained to natural teeth, tooth roots and dental implants abutments to furnish the primary support for the prosthesis. Tooth preparation for fixed dental prosthesis is a common procedure in clinical practice among general dentists should perform correctly. The aim of this present study was to evaluate the trends in the amount of tooth structure reduction in fixed partial denture done by dental students.Three thirteen patients were examined for the study. Each patient's prepared teeth were noted under certain parameters. Data collection was taken from DIAS, a dental archive. Three thirteen patients' details of fixed partial denture were taken and the collected datas statistically analysed by a software SPSS. The Parameters observed were over reduction,under reduction, rough surfaces ,pulpal damage and finish lines..Over reduction during tooth preparation was the most common error followed by pulpal damage ,rough surfaces and under reduction.However there was no statistically significant differences between undergraduate and postgraduate students with respect to all the above mentioned parameters.

Keywords: *Fixed partial denture, Tooth preparation, Pulpal damage, Undercuts, Finish line.*

Introduction

Tooth preparation for fixed partial prosthesis is a common procedure in clinical practice, which all general dentists should perform correctly [1]. However, it could be difficult to always obtain a predictable result, especially for dental students or young doctors, they could make mistakes in their learning curve leading to inadequate results [2,3]. Unlike other human substances, dental tissue doesn't have regenerative capacity [4]. Therefore the removal of dental biological material should be planned and executed with maximum attention [5].

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The purpose of a fixed prosthodontic therapy may vary from the restoration of a single tooth to the rehabilitation of the complete occlusion [6]. A single tooth can be fully restored both functionally and aesthetically [7]. A missing tooth can be replaced by a fixed prosthesis, increasing patient masticatory competence and maintaining or improving dental arches function, often elevating a patient's self-image [8,9]. Tooth preparation should have specific geometrical characteristics to provide necessary retention and resistance to the vertical and lateral forces acting on the restoration [10]. The most important element of retention is the presence of two opposing vertical surfaces [11]. The axial walls of the preparation should taper slightly to allow the cementation of the artificial crown. The more parallel are the axial walls the greater is the retention [12]. However, it is impossible to obtain parallel surfaces without producing undercuts.The longer is the preparation the greater is the retention [13]. Teeth with larger diameter need a greater length to prevent dislodgement [14]. Proper occlusal and axial reductions are essentials to provide enough space, allowing a good functional morphology and structural durability [15]. Moreover, no more than

necessary dental tissues should be removed in order not to jeopardize tooth structure and retention of the restoration [16]. However, it could be difficult to obtain always predictable results, especially for dental students or young doctors, they could make mistakes in their learning curve leading to inadequate results. The various factors to be considered while tooth preparation depends on the type of restoration, type of luting cement used, optimal aesthetic requirement, retention and resistance form, position of the margins, and tooth alignment [17]. In-depth knowledge and an understanding of the various criteria is a prerequisite to the development of optimum tooth preparation. This enables a clinician to find a best combination of compromises among the applicable biological, mechanical, and esthetic considerations.

Tooth preparation produces depth-orientation grooves on the vestibular and incisal surfaces, with a round-end tapered diamond as reference for removing tooth structure [18].The occlusal reduction is performed by removing the tooth portions between the orientation groves with the same bur. After the functional cusp bevel is made, a no. 17L bur is used to smooth the planes of the occlusal reduction [19]. Three vertical groves are performed in the vestibular surface with a flat-end tapered diamond. All tissues between the depth-orientation channels are removed. The proximal reduction is performed with a needle narrow diamond, avoiding damage to the adjacent teeth. The lingual and proximal surfaces are then cut with a torpedo diamond. Different finishing lines may be created. A chamfer is considered the preferred choice for veneer metal restoration. Several Authors have shown how this finishing line exhibits the least stress, with the lowest failure rates. A shoulder finishing line is used for all-ceramic crowns, minimizing stresses with its width and preventing the possibility of the porcelain fracture [20]. However, it is the more destructive finishing line for the tooth tissues. On the other hand, the knife-edge is the more conservative finishing line, but it may result in an over-contoured restoration [21,22]. The most demanding challenges for prosthodontists are the control of depth and direction of tissue removal. Hence, the aim of this present study was to evaluate the trends in the amount of tooth structure reduction in fixed partial dentures done by dental students.

Materials and Methods

This study is a retrospective cross sectional study. This study was conducted among patients who have undergone fixed partial denture procedure in Saveetha Dental hospital, chennai. It included demographic data of the patients of each individual.

Data Collection - The data collection was done by reviewing the patients record for tooth preparations done for fixed partial dentures and analysed the data of 86000 patients between June 2019 and March 2020. The fixed partial denture data collected was classified according to age, gender, pulpal damage, rough surface, over reduction ,under reduction and year of graduation .

A customised examination was used to collect data and a specific table for collected data records was prepared. All the general examination and the clinical examination of patients are correlated and the data relevant to the topic and collected and tabulated. The data was then analysed using spss software, inference of the study is given below.

Results and Discussion

Pulpal damage during tooth preparation

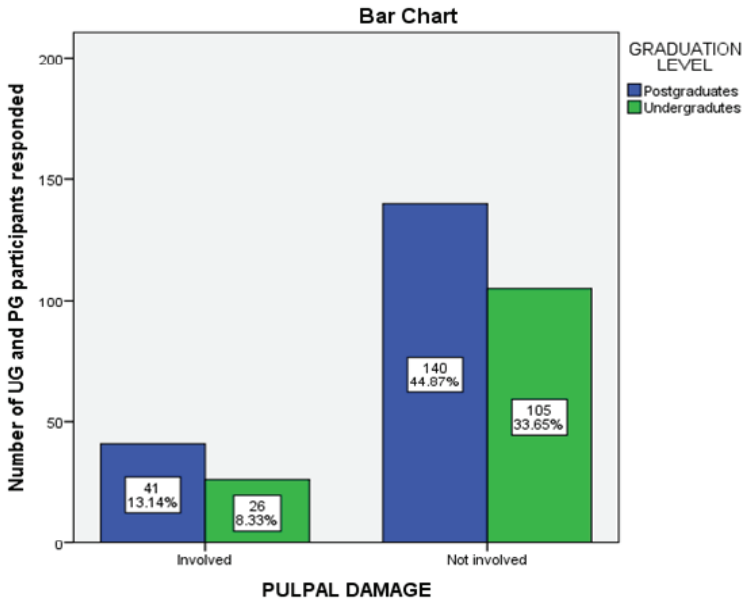


Figure 1 - The bar graph shows the association of UG and PG students with respect to pulpal damage to the tooth and X axis represents frequency of pulpal damage and Y axis represents the number of UG and PG participants responded (Blue - Postgraduates) and (Green - Undergraduates). 140 postgraduates and 106 undergraduates perceived to have no involvement of pulp during tooth preparation. Chi square test was done P value was found to be 0.552 (p value > 0.05, statistically not significant).

Rough surfaces during tooth preparation

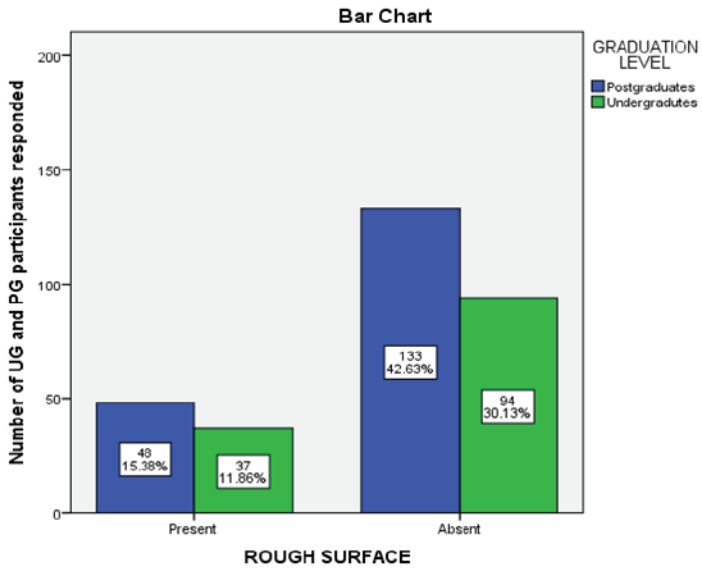


Figure 2 - The bar graph shows the association of UG and PG students with respect to the rough and irregular surfaced tooth preparation. X axis represents frequency of rough and irregular surfaced tooth preparations and Y axis represents the number of UG and PG participants responded (Blue - Postgraduates) and (Green - Undergraduates). Preparations done by postgraduates appear to have less irregular and rough surfaces than the undergraduates,however statistically significant differences were not observed.(Pearson Chi square test;P=0.736,P>0.05).

Over reduction during tooth preparation

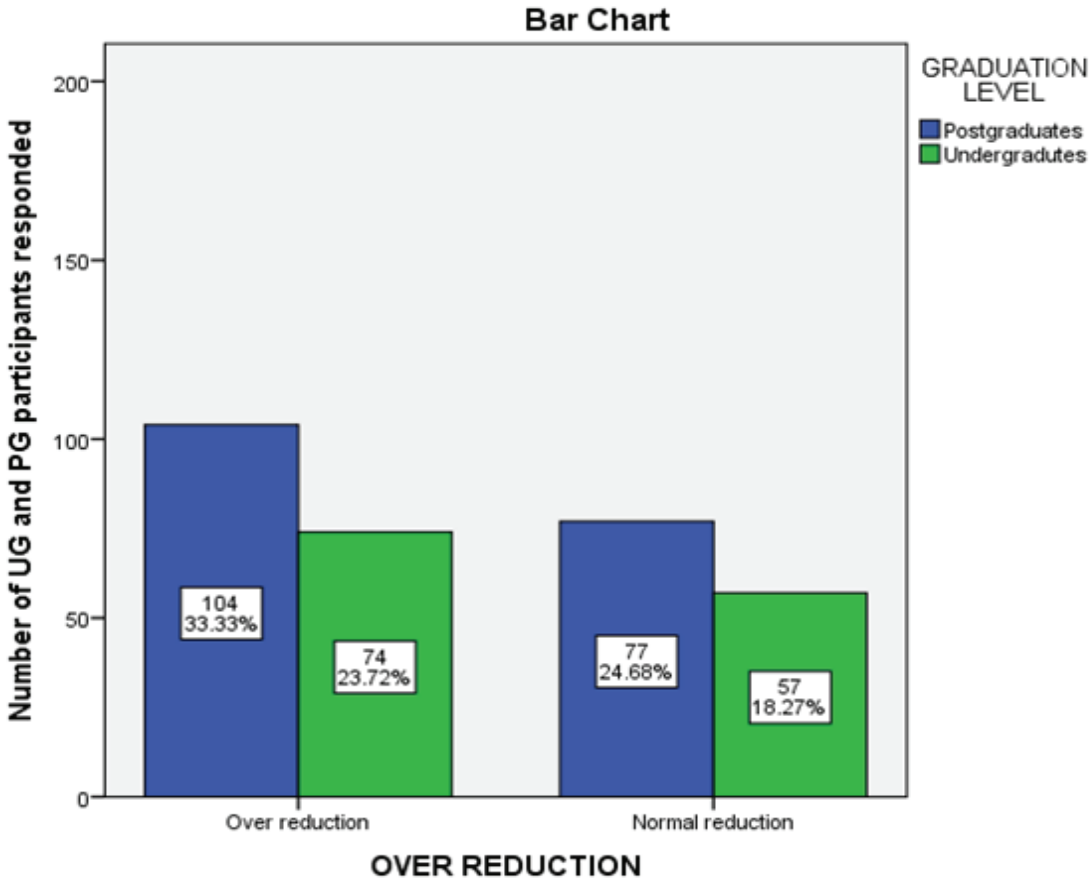


Figure 3 - The bar graph shows association of UG and PG students with respect to the over reduction during tooth preparation. X axis represents frequency of over reduced and normal reduced tooth preparations and Y axis represents the number of UG and PG participants responded (Blue - Postgraduates) and (Green - Undergraduates). 104 postgraduates and 74 undergraduates are perceived to have over reduction than normal reduction. Chi square analysis was done and P value was found to be 0.864 (p value > 0.05, statistically not significant).

From the current study postgraduates have participated more than undergraduates. According to the study [23], done by Karlsen et al, it was found that the margin of restoration should be located near the gingiva, which doesn't correlate with the current study. Most restorations have margins in the gingival crevice, and permanent tissue damage is common. The relationship between periodontal health and the restoration of teeth is intimate and inseparable. Although it is widely accepted that the best restorative margin is one that is placed coronal to marginal tissue.

An adequate reduction of teeth will provide proper retention and resistance for the bridge to adapate [24]. First phase in reduction technique includes : Axial and Occlusal reduction depth which is achieved by placing N°1 bur along the vestibular and oral gingival margin of the tooth. The round and flat stopping surface of the bur doesn't allow it to go beyond a depth of 1 mm.

The second phase is Actual reduction. Actual reduction of anterior teeth is done between the grooves with the N° 3 bur. The third phase is finishing line preparation. The N° 4 bur is utilized to plan the wrapping up line. The smooth proximal portion of the bur permits to utilize the axial surfaces of the cast to direct the dental

practitioner within the arrangement and the fourth phase is Tooth surface refining. The N° 5 bur refines the tooth surface. It is used to round the line angle of the stump and eliminates any undercut.

It is also proven that, non involvement of the pulp are the majority in the studies and it is good for better retention [25]. Involvement of pulp during tooth preparation may lead to several complications during and after treatment, whereas anticipated exposures of abutment teeth pulp during tooth preparation is included in a patient's treatment strategy regardless of whether or not teeth present with pulpal pathology, unanticipated exposure may create delays in treatment and necessitate reassessment of the treatment plan by the dentist and the patient.

According to the studies [18,26], it says that tooth occlusal convergence is an essential criteria to be considered in tooth preparation. It shows the benefits of the control of depth and direction of tooth tissue removal as well as better definition of tooth finishing line.According to the studies [19,20], Renggli H and Valderhaung they suggested that pocket depth and loss of attachment are one of the main reasons for the failure of fixed partial dentures. Several studies indicated that poor marginal adaptation, subgingival margin placement, and over-contoured crowns can contribute to localized periodontal inflammation [27].

According to the studies [28], it was proven that presence of rough surface in the prepared teeth may provide better resistance and retention for the teeth, which is contradictory to the current study [29]. Failure of tooth preparation leads to damage or rough surface of the teeth. Rough margins reduce adaptation.

According to the study done by Syu JZ et al [30], it was seen that fit of the patients depends upon the finish line that is given to the prepared teeth. In our current study, the majority of the students have obtained shoulder finish line, which helps in stress concentration lesser than classical shoulder [31]. Shoulder finish line provides less distortion of crown margins, adequate bulk, good crown contours, and can attain good esthetics. Shoulder with bevel is given to get proper marginal adaptation and the bulk of the material border.

Conclusion

Within the limits of present study, over reduction during tooth preparation was the most common error followed by pulpal damage ,rough surfaces and under reduction. However there were no statistically significant differences between undergraduate and postgraduate students with respect to all the above mentioned parameters.

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Prevalence of Periodontal Abscess among Patients Visiting A Private Dental College and Hospital in Chennai, India

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Abstract

Periodontal abscess is a frequently occurring condition in the oral cavity in which periodontal tissue such as bone, cementum, periodontal ligament and gingival are destroyed. It is more often than not associated with periodontal pockets and periodontitis and is a marker of active bone destruction in the periodontal region. It is the third most frequent dental emergency and its treatment has been a challenge for many years. The aim of this study is to assess the prevalence of periodontal abscess among patients reporting to a Dental University Hospital, Chennai. A retrospective cross-sectional study was conducted after reviewing patient records and analysing the data of 86000 patients between June 2019 and March 2020. Microsoft Excel® Data spreadsheet was used to tabulate the data. The variables involved were gender, age group, site and chief complaint. Data was then exported to the Statistical Package for Social Sciences (SPSS) for Windows (Version 19, 2010) for further analysis. The age groups were 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years, 61-70 years and 71-80 years. From the data analysed through the Statistical Package for Social Sciences (SPSS) for Windows (Version 19, 2010), it was observed that the overall incidence of periodontal abscess was higher in males (68.2%) than females (31.8%). 31-40 year olds had the highest incidence at 39.4%. The most common site was sextant 4 (22.7%). Pain was the most observed complaint (45.5%). Most common site of occurrence was sextant 1 in males and sextant 4 in females. (Chi square = 0.832 < expected value of 2.55, data is statistically significant) Within the limitations of the current study, periodontal abscess has a male predilection between age group 31-40 years with most common site being sextant 4. Pain was the most common symptom reported.

Keywords*: Periodontal abscess, pain, bacterial infection, lower anterior teeth etc.*

Introduction

Periodontal abscess is a localised acute bacterial infection which is confined to the tissues of the periodontium.[1] It is a distinctive process occurring in the periodontium and not the tooth pulp, resulting in localised collection of pus and communicating with the oral cavity through the gingival sulcus. Based on the course of the disease it can have both acute and chronic origin.[2] Release of reactive oxygen species leads to heightened oxidative damage to gingival tissue, alveolar bone and periodontal ligament.[3] Cytomegalovirus and EBV have often been found in periodontal lesions.[4,5]

It is a moderately painful clinical condition which demands emergency treatment in over 60% patients. The abscess teeth with severe destruction, associated with deep periodontal probing depths, mobility, bleeding and suppuration.[6]

The prevalence of periodontal abscess is relatively high in the general population and it can affect the prognosis of the infected tooth.[7,8] Among all emergency dental conditions periodontal abscess represents approximately 8% of all in the world.[9] It is also the third most common dental emergency preceded by pulpal infection and pericoronitis.[10] *Porphyromonas gingivalis*, *Prevotella intermedia*, and other *Prevotella* spp. are the predominant bacteria of human periodontal abscesses.

They are generally present in the oral cavity at various sites such as mucosa, saliva, pockets etc. [11,12]

The most commonly involved teeth in the periodontal abscess have been reported as lower anterior teeth, upper anterior teeth and lower molars.[7] Periodontal abscess in impacted third molars can have multiple complications and have poor prognosis.[13] Ankyloglossia restricts the cleansing activity of saliva leading to gingivitis and periodontal abnormalities.[14] The prevalence of periodontal abscess increases with age and it has a higher frequency in males than in females.[15]

Periodontal abscess if not treated in time can also lead to loss of a tooth. In such cases more furcated teeth are lost than non furcated teeth. Although periodontal abscesses are more common in anterior teeth the prevalence is found to be higher in posterior teeth due to furcation involvement.[16]

Members of the MMP family are known to cause local tissue breakdown and play an important role in periodontal abscess formation.[17] Often, due to close proximity, squamous cell carcinoma [18,19], proliferative leukoplakia[20], inflammatory myofibroblastic tumour[21], tongue carcinoma[22] mimic benign inflammatory conditions such as dentoalveolar abscess, periodontal abscess etc. leading to misdiagnosis. Other differential diagnosis for periodontal abscess include pyogenic granuloma, peripheral giant cell granuloma etc. [23]

The aim of the current study is to find the prevalence of periodontal abscess in the patients reporting to the outpatient department of Saveetha Dental College and Hospitals, Chennai.

Materials and Methods

Study Design And Setting

This is a retrospective cross-sectional study in which patient records from a Dental University Hospital were obtained. Data was collected for patients reporting to the Department of Periodontology from June 2019- April 2020 who were diagnosed with periodontal abscess.

Data Collection

A total of 66 cases of periodontal abscess (n=66) were identified after reviewing and analysing the data of 86000 patients. The inclusion criteria was patients of all age groups and genders with periodontal abscess. Other relevant data such as gender, age, chief complaint, patient ID. were also recorded. Clinical photos and radiographs were used to verify the site of abscess. Data was also verified by an external reviewer.

Statistical Analysis

Data was recorded in Microsoft Excel® and later exported to the Statistical Package for Social Sciences after proper coding of the variables involved. Parameters included were gender, age, site and chief complaint including pain, swelling, pain with pus discharge, mobile teeth, pain with mobile teeth, pain with swelling and pus discharge, carious tooth, pain with swelling, food lodgement, missing teeth, pain with swelling and bleeding gums, pain with burning sensation, and pus discharge respectively.

Thereafter, the data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) for Windows (Version 19, 2010). Chi square test was employed with the level of significance set at $p<0.05$.

Results and Discussion

The sample size was n=66. The age distribution was 11-20 years (3%), 21-30 yrs (12.1%), 31-40 yrs (39.4%), 41-50 yrs (22.7%), 51-60 yrs (15.2%), 61-70 yrs (6.1%) and 71-80 yrs (1.5%). (Table 1) The gender distribution was Males (68.2%) and Females (31.8%). (Table 2) Incidence of periodontal abscess at various sites was sextant 1 (21.2%), sextant 2 (13.6%), sextant 3 (12.1%), sextant 4 (22.7%), sextant 5 (12.1%) and sextant 6 (18.2%). (Table 3) The chief complaint of reporting patients were distributed as Pain (45.5%), swelling (9.1%), pain with pus discharge (1.5%), mobile teeth (7.6%), pain with mobile teeth (1.5%), pain with swelling and pus discharge (1.5%), decayed tooth (4.5%), pain with swelling (18.2%), food lodgement (3%), missing teeth (1.5%), pain with swelling and bleeding gums (3%), pain with burning sensation (1.5%) and pus discharge (1.5%). (Table 4) The most common site was upper right posterior teeth in males and lower left posterior teeth in females. (Chi square = 0.832 < expected value of 2.55) (Graph 1)

The site of occurrence was found to be most common in age group 11-20 years was sextant 2 and 5,

in 21-30 years was sextant 4 and 6, in 31-40 years was sextant 1 and 4, in 41-50 years was sextant 4, in 51-60 years was sextant 1, in 61-70 years was sextant 3, 4, 5, 6 and in 71-80 years was sextant 3. (p=0.500, p>0.05data is statistically not significant) (Graph 2)

Among all age groups the most common chief complaint was reported pain except for the group of 71-80 year olds where the most common complaint was pain with swelling. (p=0.996, p>0.05, data is statistically not significant) (Graph 4)

At each site of periodontal abscess the most common chief complaint associated with it was pain except for sextant 4 where the most common complaint was pain with swelling. (p=0.802, p>0.05, data is statistically not significant) (Graph 3)

Table 1: Table showing frequency and percentage distribution of age in patients with periodontal abscess among patients reporting to the outpatient department of a Dental University Hospital, Chennai, India. 11-20 years (3%), 21-30 yrs (12.1%), 31-40 yrs (39.4%), 41-50 yrs (22.7%), 51-60 yrs (15.2%), 61-70 yrs (6.1%) and 71-80 yrs (1.5%)

Age groups	Frequency	Percent
11-20 years	2	3.0
21-30 years	8	12.1
31-40 years	26	39.4
41-50 years	15	22.7
51-60 years	10	15.2
61-70 years	4	6.1
71-80 years	1	1.5
Total	66	100.0

Table 2: Table showing frequency and percentage distribution of gender among patients with periodontal abscess reporting to the outpatient department of a Dental University Hospital, Chennai, India. Females 31.8%, Males 68.2%

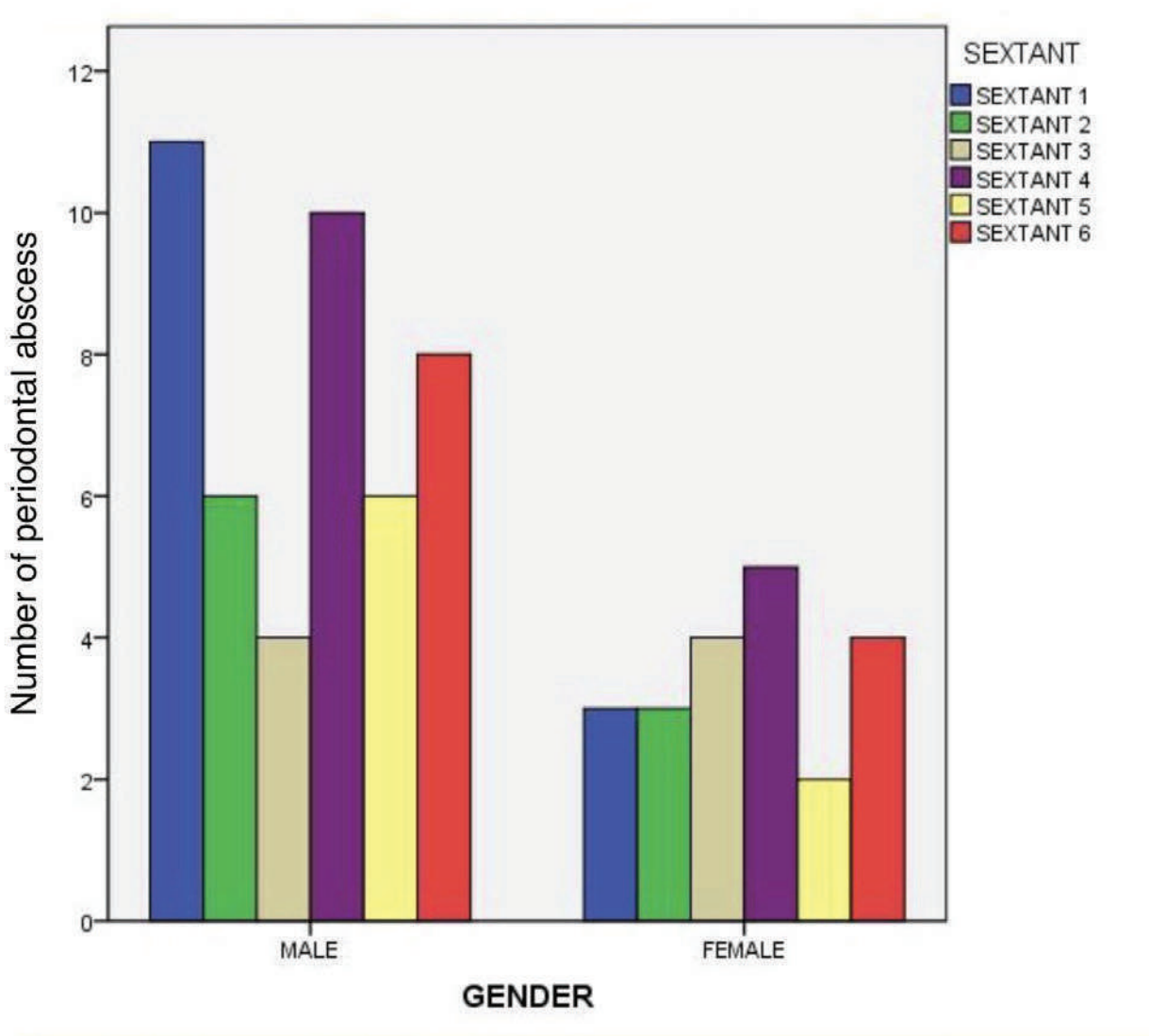
Gender	Frequency	Percent
Male	45	68.2
Female	21	31.8
Total	66	100.0

Table 3: Table showing frequency and percentage of distribution of site of occurrence of periodontal abscess patients reporting to the outpatient department of a Dental University Hospital, Chennai, India. sextant 1 (21.2%), sextant 2 (13.6%), sextant 3 (12.1%), sextant 4 (22.7%), sextant 5 (12.1%) and sextant 6 (18.2%)

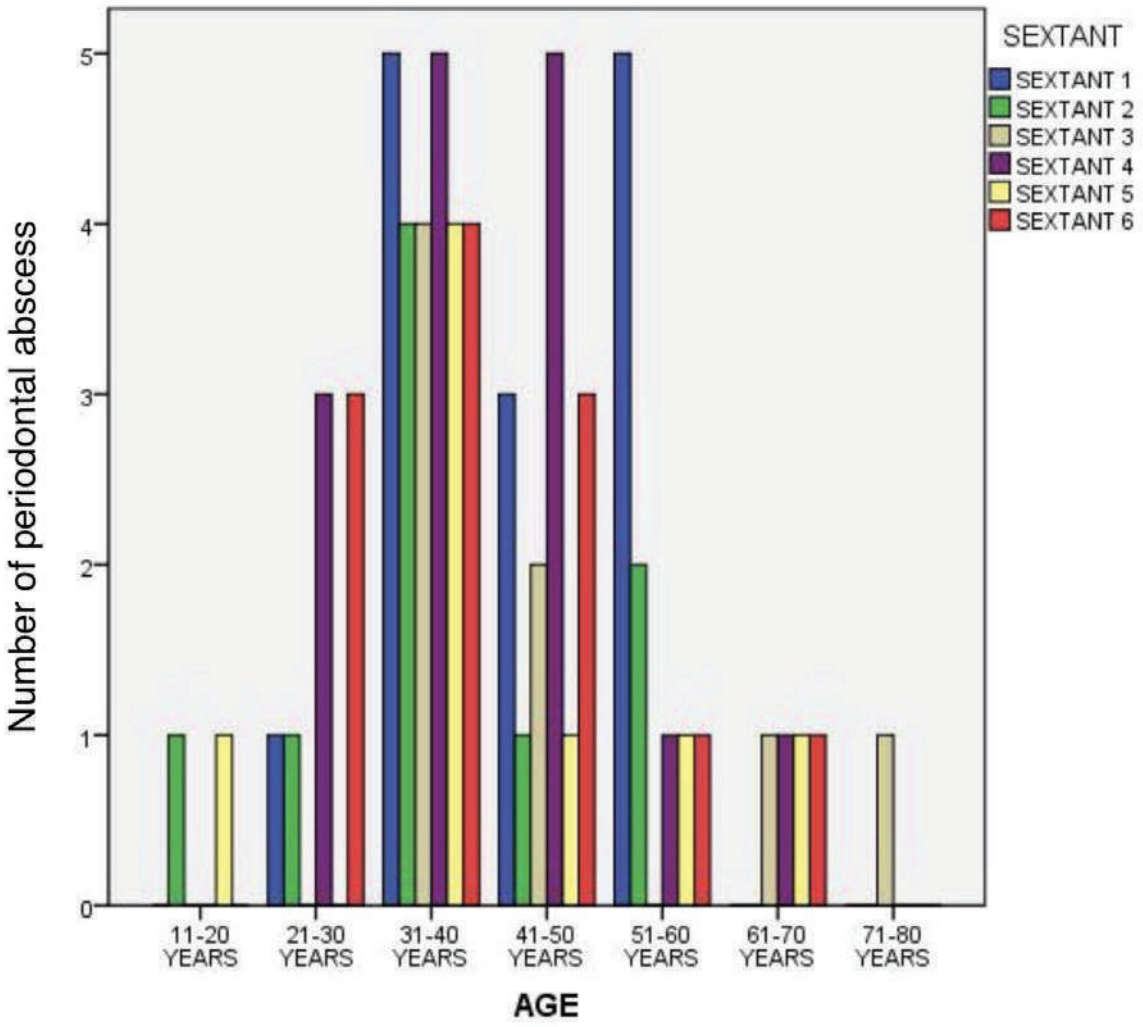
Site	Frequency	Percent
Sextant 1	14	21.2
Sextant 2	9	13.6
Sextant 3	8	12.1
Sextant 4	15	22.7
Sextant 5	8	12.1
Sextant 6	12	18.2
Total	66	100.0

Table 4: Table showing frequency and percentage of distribution of chief complaint in patients with periodontal abscess reporting to a Dental University Hospital, Chennai, India. Pain (45.5%), swelling (9.1%), pain with pus discharge (1.5%), mobile teeth (7.6%), pain with mobile teeth (1.5%), pain with swelling and pus discharge (1.5%), decayed tooth (4.5%), pain with swelling (18.2%), food lodgement (3%), missing teeth (1.5%), pain with swelling and bleeding gums (3%), pain with burning sensation (1.5%) and pus discharge (1.5%)

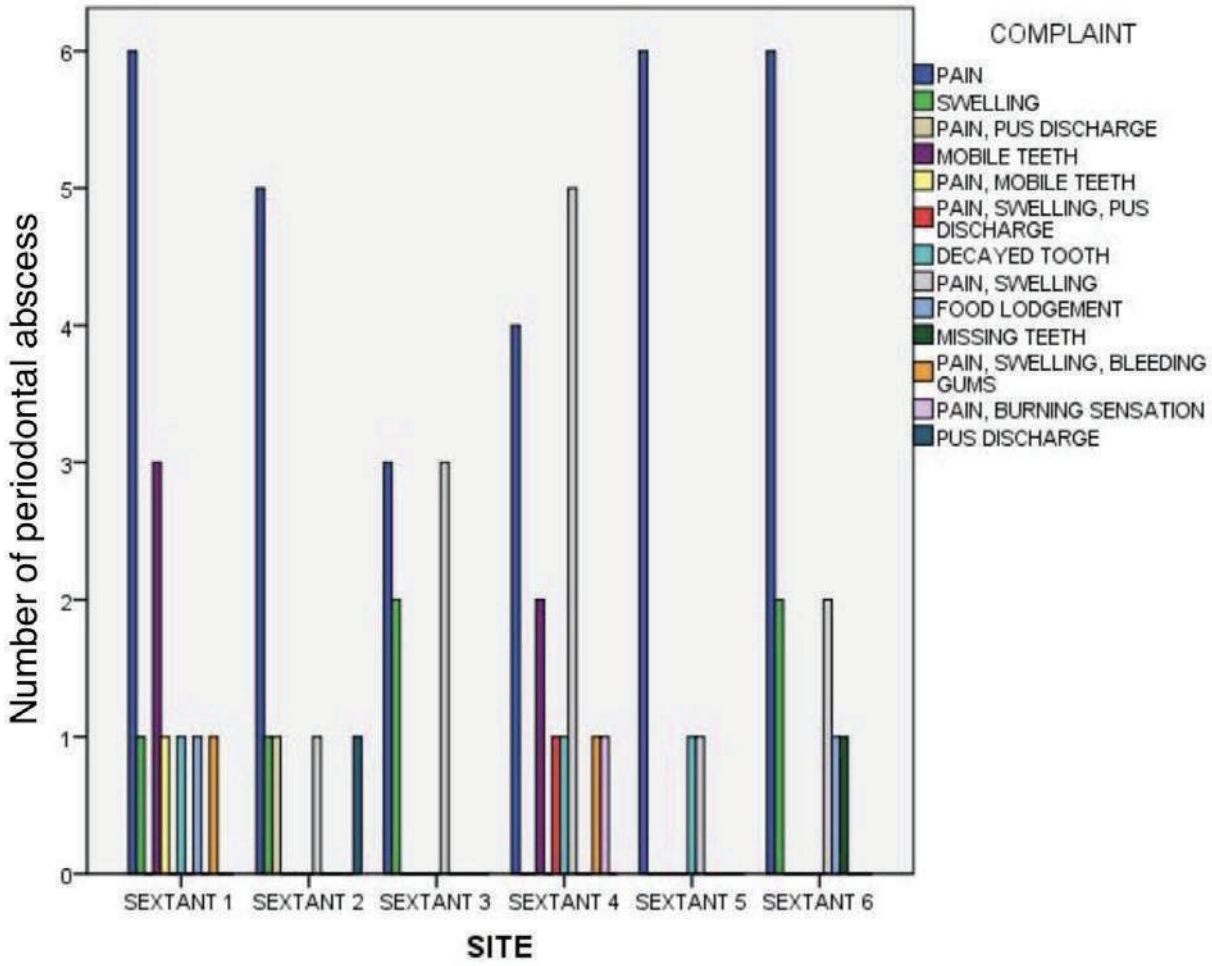
Chief complaint	Frequency	Percent
Pain	30	45.5
Swelling	6	9.1
Pain and pus discharge	1	1.5
Mobile teeth	5	7.6
Pain and mobile teeth	1	1.5
Pain, swelling and pus discharge	1	1.5
Decayed tooth	3	4.5
Pain and swelling	12	18.2
Food lodgement	2	3.0
Missing teeth	1	1.5
Pain, swelling and bleeding gums	2	3.0
Pain and burning sensation	1	1.5
Pus discharge	1	1.5
Total	66	100.0



Graph 1: Bar graph showing frequency of distribution of site of periodontal abscess in males and females. X axis denotes gender and Y axis denotes number of cases of periodontal abscess. This graph shows the number of cases of periodontal abscess in each sextant among males and females in patients reporting to a Dental University Hospital, Chennai, India. Blue colour represents sextant 1, green represents sextant 2, white represents sextant 3, purple represents sextant 4, yellow represents sextant 5 and red represents sextant 6. At all sites periodontal abscess occurs more commonly in males except at sextant 3 where there is equal predilection. However this data is statistically not significant. (Chi-square test; p-value=0.832; p>0.05; statistically not significant)

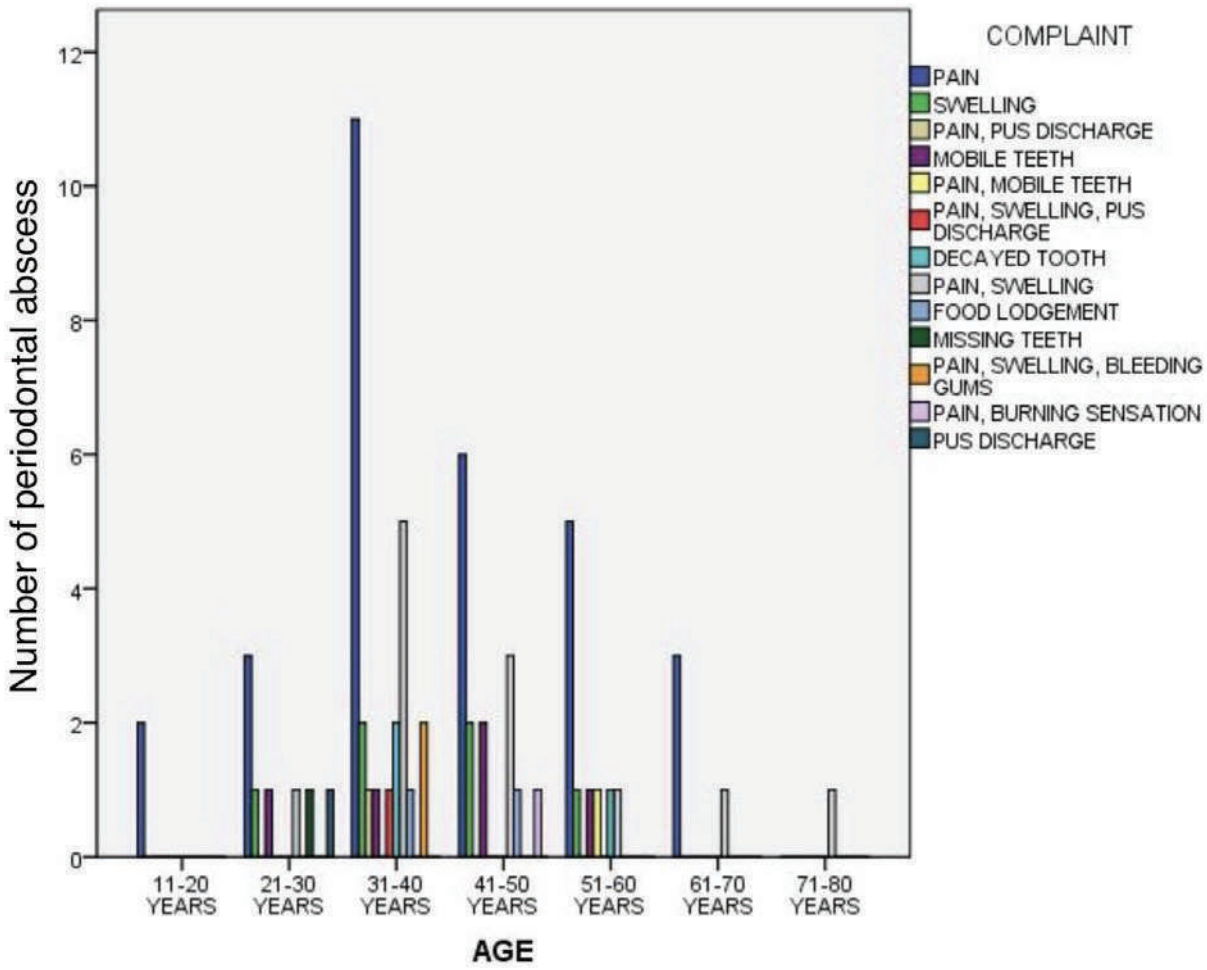


Graph 2: Bar graph showing frequency of distribution of site of periodontal abscess in different age groups. X axis denotes age group and Y axis denotes number of cases of periodontal abscess. This graph shows the number of cases of periodontal abscess in each sextant in different age groups among patients reporting to a Dental University Hospital, Chennai, India. Blue colour represents sextant 1, green represents sextant 2, white represents sextant 3, purple represents sextant 4, yellow represents sextant 5 and red represents sextant 6. Most common site of periodontal abscess in age group 11-20 years was sextant 2 and 5, in 21-30 years was sextant 4 and 6, in 31-40 years was sextant 1 and 4, in 41-50 years was sextant 4, in 51-60 years was sextant 1, in 61-70 years was sextant 3, 4, 5, 6 and in 71-80 years was sextant 3. However this data is statistically not significant. (Chi-square test; p-value=0.500; p>0.05; statistically not significant)



Graph 3: Bar graph showing frequency of distribution of chief complaints at each site.

X axis denotes site of periodontal abscess and Y axis denotes number of cases of periodontal abscess. Dark blue represents pain, light green represents swelling, light brown represents pain with pus discharge, purple represents mobile teeth, yellow represents pain with mobile teeth, red represents pain with swelling and pus discharge, cyan represents decayed tooth, grey represents pain with swelling, indigo represents food lodgement, dark green represents missing teeth, orange represents pain with swelling and bleeding gums, lilac represents pain with burning sensation and light blue represents pus discharge. Pain is the most common symptom at all sites. However this data is statistically not significant. (Chi-square test; p-value=0.996; $p>0.05$; statistically not significant)



Graph 4: Bar graph showing frequency of distribution of chief complaints within each age group.

X axis denotes age group and Y axis denotes number of cases of periodontal abscess. Dark blue represents pain, light green represents swelling, light brown represents pain with pus discharge, purple represents mobile teeth, yellow represents pain with mobile teeth, red represents pain with swelling and pus discharge, cyan represents decayed tooth, grey represents pain with swelling, indigo represents food lodgement, dark green represents missing teeth, orange represents pain with swelling and bleeding gums, lilac represents pain with burning sensation and light blue represents pus discharge. Pain is the most common symptom in all age groups except in 71-80 year olds where pain with swelling was seen more commonly. However this data is statistically not significant. (Chi-square test; p-value=0.802; p>0.05; statistically not significant)

In the present study the prevalence of periodontal abscess based on gender was observed to have a higher male predilection. There was a higher incidence in males at 68.2% while females were at 31.8%. A study done by Al. Zaidi et. al. stated similar findings with a higher percentage of involvement in males than females.[15] Such a finding has been related to the higher prevalence of oral habits such as smoking among males when compared to females. Although the relation between smoking and periodontal abscess also exists among the female population.[24]

The most common complaint of the reporting patients was observed to be pain (45.5%), followed by pain with swelling (18.2%) and swelling only at 9.1%. Pain with and without other symptoms were seen in overall 72.7% cases. Azodo et. al. stated that most patients with periodontal abscess reported severe,

spontaneous, throbbing, radiating or non-radiating types of pain which was relieved temporarily on usage of analgesics.[25] In another study by Herrera D et. al. 90% of patients with periodontal abscess reported with pain.[26] Study conducted by Ibrahem 2008, 90% of the patients reported pain and more than 75% of the abscesses had moderate to severe scores related to swelling, edema and redness. Bleeding occurred in all abscesses, while suppuration was detected in 70% and 85% of teeth.[27] Pain, swelling and erythema were noted to be the most common symptoms by Shweta et al. 2013. [28]

The most common site affected by periodontal abscess were found to be sextants 1, 3, 4, 6 – 74.2% all of which include the premolars and molar. Studies done by Azodo et. al. and Pop MM et. al. all state that the most common site of periodontal abscess is posterior teeth.[25,6] 69% of periodontal abscesses were associated with a molar tooth in a study by Herrera et al.[26] According to Lee 2011 more posterior teeth involved with periodontal abscess (64%) as compared to anterior teeth (36%).[29] However, there are other studies such as one by Arowojolu MO et. al which states that the most common site in decreasing order of incidence is – lower incisors, upper incisors, upper first molars, upper second molars, lower molars and premolars and finally canines.[30] Jaramillo A et. al. also stated that the most commonly affected teeth were lower anterior teeth, upper anterior teeth and lower molars.[7] These studies are contradictory to our findings. A possible reason for such an opposing finding could be that in old age the immune responses of a person fades which leads to infection at unfavourable sites as well.[31] The higher prevalence of posterior teeth could be due to the furcation involvement.[6] Another study by Gary JL et. al. stated that maxillary incisors and premolars have the lowest rate of involvement.[32] According to Chace and Low in 1993 the majority of teeth with periodontal abscesses extracted due to poor prognosis were maxillary second molars (38.2%), followed by maxillary first molars (25.5%), and mandibular second molars (16.4%). [33]

In the current study periodontal abscess is most commonly seen in the middle age group (31-40 years) at approximately 40%. Studies by Tadjoedin FM et. al. has shown that periodontal diseases increase in incidence as the person ages.[34] It has also been noted that chronic hyperglycemia such as in case of diabetes mellitus may trigger deficiencies in the immune system, increasing the risk of infections, including in the oral cavity. [35]

Periodontal abscess is highly prevalent in the population and should be managed at the earliest as its most common presenting feature is pain and swelling which causes extreme discomfort to the patient. If not treated at an early stage it can cause various complications such as septicemia, cavernous sinus thrombosis, brain abscess, shock, mandibular fracture and in some cases death. Periodontal abscess and its complications thus become a substantial burden on individuals, communities, and the health-care system.[28] A clinician should take these factors into consideration while treating a patient.

Limitations

This study is of shorter duration and limited population as it was conducted only among the South Indian population. The small size was small and thus the results obtained cannot be generalized. So, to ascertain the findings of our study we have to do further studies in the future with large sample size and longer duration.

Future Scope

The future scope of the study will be better and will yield accurate results if different ethnic groups are considered along with a larger sample size.

Conclusion

Within the limits of the study, it can be concluded that among the patients of Saveetha Dental College, the prevalence of periodontal abscess is higher in males of middle age group. The most common symptom is pain and the most common site of occurrence is upper right posterior teeth among males and lower left posterior teeth among females.

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Role of Vitamin C in Immune Function of Human Body

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Abstract

Vitamin C is an important micronutrient for humans with pleiotropic function associated with its ability to donate electrons. It is a potent antioxidant and a cofactor for a family of biosynthetic and gene regulatory enzymes. Vitamin C contributes to immune system defense by supporting various cellular functions of both the innate and adaptive immune system. Vitamin C supports epithelial barrier function against environmental oxidative stress. Vitamin C accumulates in phagocytic cells like neutrophils, and may enhance chemotaxis, phagocytosis, generation of reactive oxygen species and ultimately microbial killing. It is also needed for apoptosis and clearance of the spent neutrophils from sites of infection by macrophages. Vitamin C deficiency leads to impaired immunity and better susceptibility to infections. In turn infections significantly impact on Vitamin C levels thanks to enhanced inflammation and metabolic requirements. In contrast treatment of established infections requires a significantly higher dose of vitamin to catch up on the increased inflammatory response and metabolic demand.one of the most reasons people take vitamin c supplements is to spice up their immunity as vitamin C is involved in many parts of the immune system. First, vitamin c helps encourage the assembly of white blood cells known as lymphocytes and phagocytes which helps to protect the body against various infections. Vitamin C is a strong antioxidant that can boost blood antioxidant levels and vitamin c rich foods and supplements have been linked to reduce blood uric acid levels. Many studies show vitamin c intake lower risk of several cancers and also reduced risks of eye diseases . Vitamin Cis an essential nutrient for healthy immune function. The main aim of the paper is to know the role of Vitamin C in immune function of the human body.

Key Words: *Immune system, Vitamin C, ascorbic acid, Lymphocytes.*

Introduction

The immune system may be a multifaceted and complicated network of specialised organs, tissues, cells, proteins and chemicals, which has evolved so as to guard the host from a variety of pathogens such as bacteria, viruses, fungi and parasites as well as cancer cells[1,2]. It can be divided into epithelial barriers and cellular and humoral constituents if either innate and acquired immunity[3]. These constituents are intact in multiple and highly complex ways[4]. More than half a century of research has shown Vitamin C to be a crucial player in various aspects of the immune system, particularly immune cell function. Vitamin C is an essential nutrient which cannot be synthesized by humans due to loss of a key enzyme in the biosynthetic pathway[5]. Severe Vitamin C deficiency leads to the doubtless fatal disease scurvy. Scurvy is characterized by weakening of collagenous structure resulting in poor wound healing and impaired immunity. Individuals with scurvy are highly susceptible to potentially fatal disease such as pneumonia In turn infections can significantly impact on Vitamin C levels due to enhanced inflammation and metabolic requirements[6]. Early on it was noted that scurvy often

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followed infectious epidemics in population epidemics in population and cases of scurvy have been reported following respiratory infection[7–9]. This is particularly for individuals who are already malnutritioned.Although the amount of Vitamin C required to prevent scurvy is relatively low(i.e 10mg/day)[10]. The recommended dietary intake for Vitamin C is up to one hundred fold higher than other vitamins [11]. A diet that supplies 100-200mg/day of Vitamin C provides adequate intake is required to preve hypervitaminosis . Vitamin C has a number of activities that could conceivably contribute to the immune modulating effects. It is a highly effective antioxidant, thanks to its ability to readily donate electrons, thus protecting important biomolecules [proteins, lipids, carbohydrates and nucleic acids] from damage by oxidants generated during normal cell metabolism and through exposure to toxins and population. Vitamin C is also a cofactor for a family biosynthetic and the gene regulatory monooxygenase and dioxygenase enzymes. The vitamin has long been known as a cofactor for the lysyl and prolyl hydroxylase required for stabilization of the tertiary structure of collagen and is a cofactor for two hydrolysis involved in carnitine biosynthesis, a molecule required for transport of fatty acid into mitochondria for generation if metabolic energy. Vitamin C in the regulations of gene transcription [12] . And cell signaling pathway through regulation of transcription factor activity and epigenetic marks. Our review explores various roles of Vitamin C in the immune system including barrier integrity and wound healing and leucocyte function and modulating effects of Vitamin C in the context of infection conditions leading to Vitamin C insufficiency[13].

A Review type of study is to understand the significant role of Vitamin C in immune function of the human body by retrieving related articles. The sampling data collection search engines are pub med, google scholar,research gateway and so on. Inclusion Criterias included are articles related to vitamins, Vitamin C, human body, immune function and exclusion criteria are articles not related to human body and immune functions.

VITAMIN C IN HUMAN IMMUNE FUNCTIONS

Functions of Immune System

The function of the precise immune response is aimed towards a lively removal of foreign antigen. In the case of cellular immunity, T.helper effector cells function in the active requirement of cells, such as macrophages which phagocytize and remove foreign substances. Cytotoxic cells bind foreign antigen bearing cells and kill them[14]. This is especially an advantage in the case of replicating agents such as viruses because it can limit the spread of the virus. The function of B cells is to produce antibodies which binds antigen and facilitates its removal from the body[15]. An antibody / antigen complex can initiate a spread of various processes aimed toward antigen elimination including facilitative, phagocytosis complement activation, antibody dependent cellular cytotoxicity and mastocyte degranulation. The entire process is finely turned. There are a variety of regulatory mechanisms involved in its control of immune responses including T suppressor cells, anti-idiotypic regulation and antigen elimination[16]. This regulatory control is critical in avoiding lymphoproliferative disease and immunopathologic response. When immune responses result in disease they are formed hypersensitivities . The ultimate function of the response is to provide protection from foreign substances. Since there are a variety of important steps and regulations in the immune system[17].

Table 1: Role of Vitamin C in Immune mechanisms.

Immune system	Function of Vitamin C
Epithelial barriers	Enhances collagen synthesis and stabilization Protect against ROS induced damage Shortens time to wound healing in patients
Phagocytes	Act as an antioxidant Enhance motility/chemotaxis Enhance phagocytosis Facilitate Apoptosis Decrease Necrosis
B and T lymphocyte	Enhance differentiation and proliferation Enhance antibody levels
Inflammatory mediators	Modulate cytokine production Decrease histamine levels

Barrier Integrity and Wound Healing

The skin has numerous essential functions, the primary of which is to act as a barrier against external insults including pathogens [18]. Skin contains kill molar concentrations of Vitamin C with higher levels found in the epidermis than the dermis(Table 1) [19]. Vitamin C is actively accumulated into the epidermal and dermal cells[20,21] via the two sodium dependent Vitamin C transporter[22]. Clues to the role of Vitamin C in skin come from the symptoms of Vitamin C deficiency scurvy which is characterized by bleeding gums, brushing and impaired wound healing [23]. These symptoms are thought to be a result of the role of vitamin C as a cofactor for the prolyl and lysyl hydroxylase enzymes that stabilize tertiary structure of collagen[24]. Cell culture and preclinical studies have indicated that Vitamin C enhances epithelial barrier function via number of different mechanisms [2526].

Vitamin C Insufficiency Conditions

Numerous environmental healthy conditions can have an impact on Vitamin C status. Tobacco smoke is an underestimated pollutant in many parts of the world. Both smokers and passive smokers have lower plasma and leukocyte Vitamin C levels than non –smokers. The antioxidant property of vitamin c enables it to protect lung cells exposed to oxidants and oxidant mediated damage caused by various pollutants, heavy metals, pesticides and xenobiotics [27].Individuals with diabetes are of greater risk of common infections . A decrease in plasma vitamin C reading has been observed in studies of type 2 diabetes and a major cause for increased Vitamin C in type 2 diabetes is thought to be the high level of oxidation stress caused by hyperglycemia[28]. Elderly people are particularly susceptible to infections due to immunosenescence and decreased immune cell function[29].

Vitamin C and Leukocyte Function

Leukocytes like neutrophils and monocytes actively accumulate vitamin C against a degree gradient, leading to values that are 50-100 fold above plasma concentration[30]. These cells accumulate Vitamin C concentration at a dietary intake of 0-100mg/day]. DHA is then rapidly reduced to ascorbate intra cellularly to give levels of about 10mm. It is believed that the accumulation of such high Vitamin C concentrations

indicates function within these cells[31]. Thus Vitamin C could modulate redox-sensitive cell signaling pathways or by directly protecting important cell structural components[3233].

Vitamin C and Infection

A major symptom of the Vitamin C deficiency disease scurvy is the marked susceptibility to infections, particularly of the respiratory tract with pneumonia of the respiratory tract with pneumonia being one of the most frequent complications of scurvy and a major cause of death [3435].Cases of acute lung infection have shown rapid clearance of chest x-rays following administration of intravenous Vitamin C[36]. Beneficial effects of Vitamin C on recovery are noted in pneumonia. An elderly person hospitalized because of pneumonia, who was determined to have very low Vitamin C levels[37]. Administration of Vitamin C reduced the respiratory symptom score in the mare severe patients. It is likely that the low Vitamin C levels observed during respiratory infections are both a cause and consequence of the diseases[38].

Some others Benefits of Vitamin C

Cardiovascular health: Vitamin C may widen the blood vessels and this could help protect against heart disease and hypertension or high blood pressure. Cholesterol levels: There are found to be lower in individuals with adequate levels of Vitamin C[39]. Cataract: Vitamin C may help lower the risk of cataracts as well as age related macular degeneration . Anemia: Vitamin C enhances the absorption of ironLead levels may be reduced if there is an adequate intake of Vitamin C [40]. In dental disorders the role of Vitamin C in stabilizing the collagen and prevents gum bleeding[41]. As an antioxidant Vitamin C acts as an anticancer agent also[42].

Vitamin C is a key for a strong and well-functioning immune system Vitamin C supports the production of interferons. Interferons are produced when the presence of pathogens is detected. They facilitate the ability of cells to initiate protection of cellular defenses[4344]. Vitamin C enhances the function of phagocytes. Phagocytes are a kind of white cells that surround pathogens and other dangerous particles; once the intruders are captured they are digested and neutralized enzymatically[45]. Some of the primary cells of the immune system have levels of Vitamin C upto 80 times higher than levels found in plasma. Vitamin C inhibits apoptosis of various forms of T lymphocytes. Vitamin C increases the assembly of 13 lymphocytes. Vitamin C increases the assembly and activity of antibodies. Good antibody function is vital for a healthy immune system [46]. Vitamin C supports the activity of natural killer cells. Natural killer cells are small lymphocytes which will attack directly cells such as cancer cells and kill them[47]. Vitamin C can make bacterial membranes more permeable to some antibiotics Vitamin C is a fanatical supporter of healthy immune function[46,47].

Conclusion

Overall Vitamin C appears to exert a mass of beneficial effects on cellular functions of both the innate and adaptive immune system. Although Vitamin C may be a patent antioxidant protecting the body against endogenous exogenous oxidative challenges. It's likely that its action as a cofactor for varied biosynthetic and gene regulatory enzymes plays a key role in immune-modulating effects. It's apparent that Vitamin C is important for the immune system to mount and sustain an adequate response against pathogens, while avoiding excessive damage to the host. Epidemiological studies indicate that hypovitaminosis C remains relatively common worldwide and Vitamin C deficiency is the fourth leading deficiency. Ensuring adequate intake of vitamin through the diet or via supplements especially in groups such as the elderly or in individuals exposed to risk factors for Vitamin C insufficiency is required for proper immune function and resistance to infections.

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A Survey on Knowledge about Oxidative Stress and Inflammatory Disease among Dental Students

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Abstract

Oxidative stress is caused by an imbalance between production and accumulation of oxygen reactive species in cells and tissues which can lead to various neuronal degenerative diseases and also oral diseases such as periodontitis and dental caries. Long term inflammation can lead to an inflammatory disease which is also due to impairment of the immune system in healthy tissue. In oral cavity it may cause conditions such as periodontitis, gingivitis.The aim of the study was to assess the knowledge and awareness regarding oxidative stress and inflammatory disease among dental students . A survey was conducted among 100 dental students in a private dental college. The questionnaire was circulated through google forms. Ten questions related to oxidative stress and inflammatory disease were included in the study and the responses were collected and analysed using SPSS.Chi-Square test was carried out and $p < 0.05$ was considered as significant. It was observed that 80% of students were aware about increase in oxidative stress and 60% of students were aware of oral disease such as periodontitis which helps in treatment plan of the patients .85% of students thinks that stress was also one of the reasons for increasing oxidative stress.80% of students were with the opinion that increased oxidative stress increase the chance of getting various inflammatory disease.50% of students were aware that using antioxidant supplements increase the survival rate of patient and showed better therapeutic response.It was observed that students were aware of clinical symptoms and the effects of oxidative stress in oral health and various reasons for causing inflammatory disease. However, still more knowledge regarding this will help in early diagnosing of disease caused by oxidative stress and inflammatory disease.

Keywords: *Oxidative stress; inflammatory disease ; oral disease ; anti oxidants ; free radicals.*

Introduction

Oxidative stress is imbalance between oxidants and antioxidants in the body. It is the result of overproduction of free radicals including reactive oxygen species. Oxidative stress may be systemic as well as localised [1] ; which can lead to cell damage . Oxidative stress can be responsible for the induction of several diseases both chronic and degenerative and also speeds up the ageing process and causes acute pathologies[2] . Oxidative stress plays an important role in the pathogenesis of various chronic liver diseases (CLD) and increasing evidence has confirmed the contributory role of oxidative stress in the pathogenesis of drugs and chemical-induced CLD[3]. Oxidative stress can even cause cancer as it damages DNA. It causes various other diseases such as cardiovascular disease, neuroscience disease, respiratory disease and kidney disease [4]. Oxidative stress affects different tissues and systems and it also plays a significant role in delayed sexual maturation both in male and female[5]. Besides the detrimental effects on human health, it plays a significant role in radiotherapy by

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using prooxidant agents [6]. Oligonucleotide therapy can also be used for treating various COPD and respiratory diseases[7].

Radiation therapy plays a key role in cancer treatment, where prooxidants are used which is chemicals that induce oxidative stress. But recent studies showed that redox dysregulation originating from metabolic alteration represents the specific vulnerability of malignant cells so it is used in radiation therapy [8]. Since oxidative stress plays an important role in the pathogenesis of metabolic disease mainly diabetes mellitus.*Caralluma fimbriata* extract may help in diabetes by inhibition of alpha-amylase and alpha-glucosidase[9].Many articles documented that DNA damage caused by the increased oxidative stress can result in colon cancer due to the eventual progression of adenoma to carcinoma. The cytotoxic effect of *Caralluma fimbriata* showed a good effect on colon cancer cell line [10]**.** Increased oxidative stress causes various cancers in which effective nano-carrier-loaded therapeutic drug delivery methods have shown promising potential in treating lung cancer as its target is to control the growth of tumour cell [11]. Coumarin is explored due to their diverse biological activity [12]. The effect of *A.catechu* ethanolic bark extract on human squamous carcinoma SCC-25 cells showed in apoptosis induction possibly by molecular mechanisms and has good result in treating human oral carcinoma [13].SeNps can open ways to new regular strategies for treating illnesses like malignancy, various cancer radiation therapy[14]. Biosynthesized zinc nanoparticles can be used as a good antioxidant[15]. Hence, various research shows plants are good candidates for managing different diseases including oxidative stress and inflammatory diseases.

Inflammatory disease is when the immune system attacks the body tissues, resulting in inflammation. Inflammatory bowel inflammatory disease is one of the most prevalent diseases[16]. Various other inflammatory diseases such as rheumatoid arthritis, vasculitis, myositis, are also present. This is due to inflammation that can be sometimes misdirected and it attacks its immune system which leads to various other diseases. The inflammatory process significantly affects the periodontium and causes the periodontitis. Endotoxins, cytokines and protein toxins released as a result of inflammation can penetrate the gingival epithelium which ultimately results in gingivitis[17]. Phenotypic activation of hepatic stem cells plays a central role in the progression of hepatic fibrosis which is caused majorly due to chronic inflammation [18]. There is no curing of the condition, the only treatment for the inflammatory disease is available such as medication, rest, surgical means and exercise. Many articles suggested that periodontitis can be treated with plant extract *Azadirachta Indica* (neem) which has excellent activity against periodontal pathogens[19]. *Acacia catechu* Willd (*Fabaceae*), commonly known as catechu, has good antiinflammatory properties which prevent many inflammatory diseases[20]. Syringic acid (SA) has been studied for its hepatoprotective, anti-inflammatory, immunomodulatory, free radical scavenging, and antioxidant activity[21]. The antibacterial activity of zinc oxide nanoparticles was determined by agar well diffusion method against Gram-positive and Gram-negative bacteria.

Many studies documented the relationship between oxidative stress and inflammatory disease[22]. Oxidative stress is the main pathogenic role in chronic inflammation which leads to inflammatory disease such as rheumatoid arthritis[23,24]. Recent studies proved that damage caused by oxidative stress results in various inflammation, even neuroinflammation and cell death which ultimately leads to memory loss[25]. Aim of the study is to analyse the knowledge and awareness among oxidative stress and inflammatory disease and the relationship between oxidative stress and inflammation. Many articles convey either oxidative stress or inflammatory disease. But this article emphasizes the importance of knowledge about oxidative stress and inflammatory disease.

Materials and Methods

In this study, a survey was conducted among 100 dental students of a private dental college by circulating questionnaire . Google forms and links were shared in various social media. 10 questions consisting related to oxidative stress, inflammatory disease and relationship between oxidative stress and inflammation were included in the study. Undergraduates, postgraduate students of the private dental colleges were only included in the study.The data was collected and analysed using SPSS software version 23 and the statistical significance was checked using Chi-Square analysis . ($p < 0.05$ was considered as statistically significant)

Result and Discussion

The responses received were analysed using SPSS software and were used for the discussion. Figure-1a depicts that 92.4% of students were aware of the increase in oxidative stress. This awareness helps in early diagnosis of disease caused by oxidative stress and helps in quick recovery of patients. Increase in oxidative stress may be due to increased production of free radicals and these excess free radicals will damage various cells and cause disease. So, awareness of the increase in oxidative stress is more important. 83.8% of students were aware and knew that oxidative stress is a diagnostic marker in diseases such as cardiovascular disease,(fig-1b). This knowledge helps students to diagnose that rapid increase in oxidative stress may lead to various cardiovascular disease, neurogenic disease and even cancer. So, understanding the importance of oxidative stress and diagnostic disease. In the case of cardiovascular disease, there is a significant increase in oxidative stress which can be diagnosed by chemiluminescence assay, direct assay etc

81.1% of students were aware that due to oxidative stress increase oral disease such as periodontitis occurs (fig-1c).Since periodontitis is one of the most common diseases, students must be aware that oxidative stress can also cause periodontitis which can even cause various other diseases. Diagnosing and treating oxidative stress can prevent patients from various diseases by oxidative stress. Bacteria such as *Streptococcus mutants* are the main cariogenic bacteria causing dental caries. Zinc is used as an antimicrobial, it has been added to mouthrinses and toothpaste to control dental plaque, inhibit calculus formation, and reduce halitosis.[26]

86.7% of students felt that stress can also be one of the reasons for developing the inflammatory disease (fig-1d). But studies on this reveal that inflammation will damage joints and bones which leads to abnormality. So chemicals are released as a result of stress which increases the inflammation in the body which leads to chronic inflammation causing inflammatory disease. So, awareness about this can prevent inflammatory disease.

Fig-1e depicts that 65.7% of students felt that using an antioxidant supplement during radiation therapy is useful whereas 27.6% of students feel that supplemental antioxidants during chemotherapy may cause side effects. But recent studies on radiation therapy with antioxidant supplements reveal that antioxidants don't interfere with therapy, increasing patient survival and therapeutic responses[27]. So, using antioxidants will increase the survival rate of patients. 78.1% of students were aware about prevalence among inflammatory diseases bowel inflammatory disease is more prevalent (fig-2a). The immune system may be attacked by the virus, bacteria attack their immune system and also a virus, bacteria, which leads to inflammation of the bowel. If this inflammation is chronic and continuous for a long time it causes bowel inflammatory disease. Since there is more probability to enter bacteria, virus to get is more prevalent of bowel inflammatory disease. Extraintestinal complications may occur in the oral cavity which causes aphthae, uncommon lesions etc which are symptoms of bowel inflammatory disease. An article suggested that biosynthesized silver nanoparticles have the potential in treating the inflammatory bowel disease[28].

Figure-2b depicts that 82.9% of students know that due to oxidative stress, the inflammatory disease occurs. But even the fact is many studies reveal that oxidative stress is also one of the causative agents in developing inflammation. This is due to increased oxidative stress causing various damage to tissues and cells that leads to inflammation which causes inflammatory disease.From the figure-2c, 66.7% of students are aware that x-ray reveals oxidative stress causing inflammatory disease. The major diagnosing tool to diagnose inflammatory disease is x-ray. The wide availability, low cost, limited time helps in easy accessing of joint space and diagnosing of inflammatory disease. So, students must know the uses of x-ray in diagnosing inflammatory disease. 66.7% of students were aware that joint stiffness and loss of function in the inflamed area is also a clinical symptom of inflammatory disease(fig-2d). Since these are major clinical symptoms associated with inflammatory disease, it is mandatory to know how to diagnose inflammatory disease and treat it. From figure-2e, it is clear that 84.8% of students know that rheumatoid arthritis is also an inflammatory disease. Rheumatoid arthritis occurs due to autoimmune response of the body causing joint inflammation, pain and stiffness in joints. There is also treatment available such as administration of NSAIDs, steroids can be helpful but this condition cannot be cured. An article suggested that consuming *Brassica oleracea* which includes broccoli, cabbage which is rich

in antioxidants and plays an important role in decreasing oxidative stress and particular tissue level which tends to decrease condition[29].

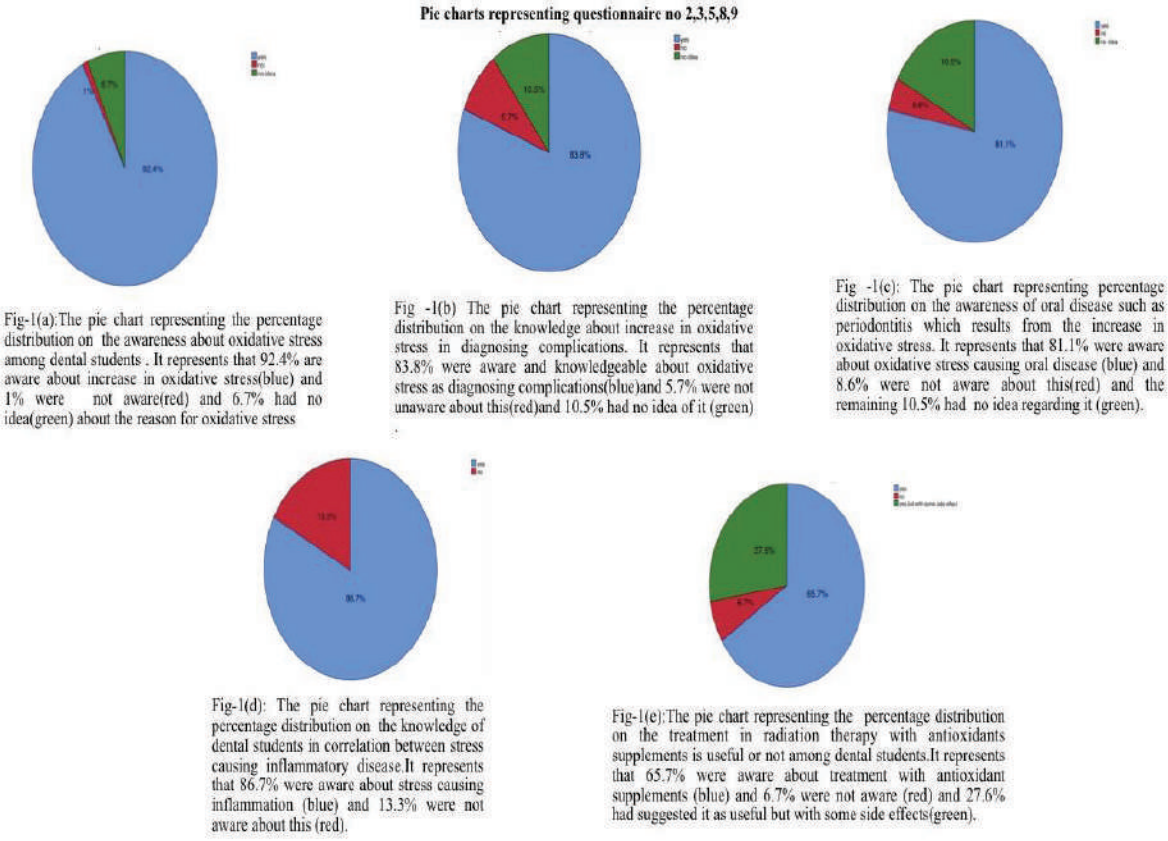


Fig-1(a):The pie chart representing the percentage distribution on the awareness about oxidative stress among dental students . It represents that 92.4% are aware about increase in oxidative stress(blue) and 1% were not aware(red) and 6.7% had no idea(green) about the reason for oxidative stress

Fig -1(b) The pie chart representing the percentage distribution on the knowledge about increase in oxidative stress in diagnosing complications. It represents that 83.8% were aware and knowledgeable about oxidative stress as diagnosing complications(blue)and 5.7% were not unaware about this(red)and 10.5% had no idea of it (green)

Fig -1(c): The pie chart representing percentage distribution on the awareness of oral disease such as periodontitis which results from the increase in oxidative stress. It represents that 81.1% were aware about oxidative stress causing oral disease (blue) and 8.6% were not aware about this(red) and the remaining 10.5% had no idea regarding it (green).

Fig-1(d): The pie chart representing the percentage distribution on the knowledge of dental students in correlation between stress causing inflammatory disease.It represents that 86.7% were aware about stress causing inflammation (blue) and 13.3% were not aware about this (red).

Fig-1(e):The pie chart representing the percentage distribution on the treatment in radiation therapy with antioxidants supplements is useful or not among dental students.It represents that 65.7% were aware about treatment with antioxidant supplements (blue) and 6.7% were not aware (red) and 27.6% had suggested it as useful but with some side effects(green).

Figure 1: Pie Chart representing questions 2,3,5,8 and 9

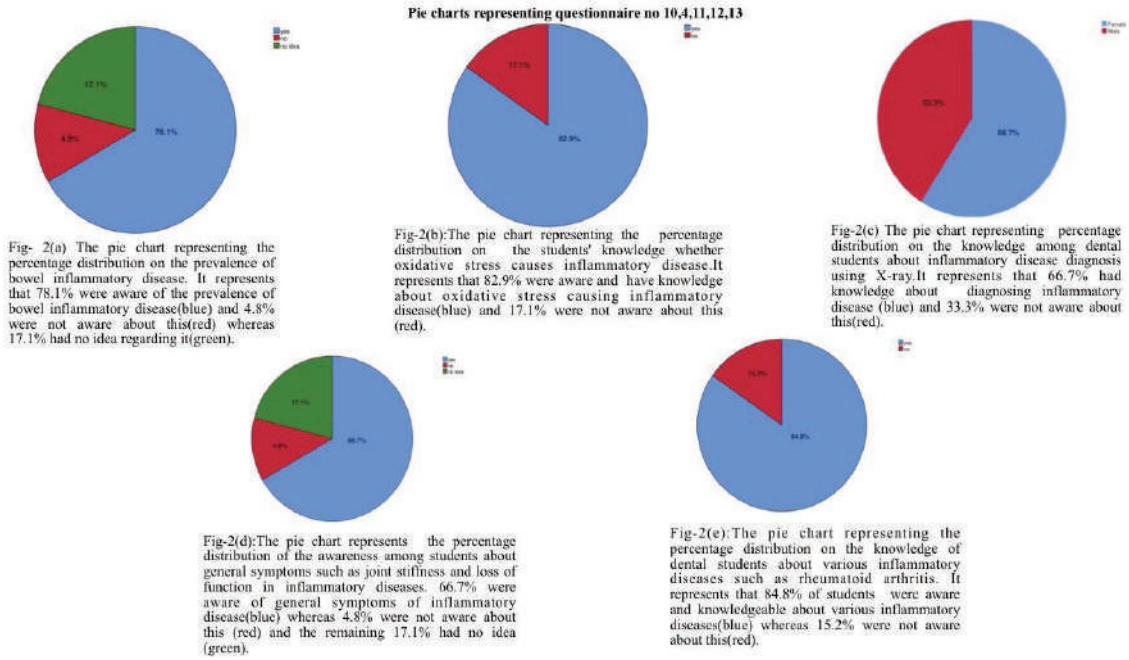


Fig- 2(a) The pie chart representing the percentage distribution on the prevalence of bowel inflammatory disease. It represents that 78.1% were aware of the prevalence of bowel inflammatory disease(blue) and 4.8% were not aware about this(red) whereas 17.1% had no idea regarding it(green).

Fig-2(b):The pie chart representing the percentage distribution on the students' knowledge whether oxidative stress causes inflammatory disease.It represents that 82.9% were aware and have knowledge about oxidative stress causing inflammatory disease(blue) and 17.1% were not aware about this (red).

Fig-2(c) The pie chart representing percentage distribution on the knowledge among dental students about inflammatory disease diagnosis using X-ray.It represents that 66.7% had knowledge about diagnosing inflammatory disease (blue) and 33.3% were not aware about this(red).

Fig-2(d):The pie chart represents the percentage distribution of the awareness among students about general symptoms such as joint stiffness and loss of function in inflammatory diseases. 66.7% were aware of general symptoms of inflammatory disease(blue) whereas 4.8% were not aware about this (red) and the remaining 17.1% had no idea (green).

Fig-2(e):The pie chart representing the percentage distribution on the knowledge of dental students about various inflammatory diseases such as rheumatoid arthritis. It represents that 84.8% of students were aware and knowledgeable about various inflammatory diseases(blue) whereas 15.2% were not aware about this(red).

Figure 2: Pie Chart representing questions 4,10,11,12 and 13

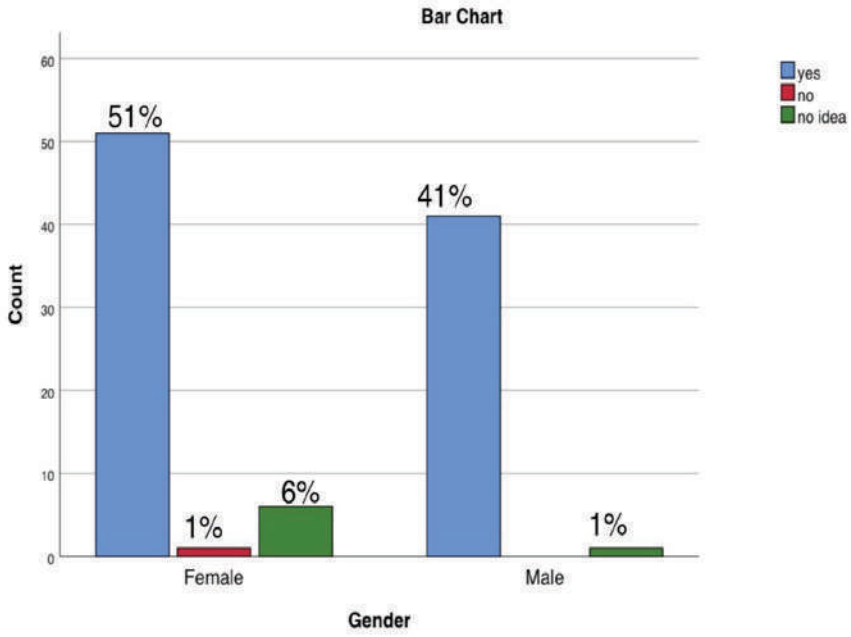


Figure 3 : The bar chart representing the association between gender and awareness about increase in oxidative stress. (X- axis represents Gender and Y- axis represents no.of. responses). Blue colour represents the awareness about increase in oxidative stress and red colour represents that they were not aware about oxidative stress and green colour represents that they have not any idea regarding it. 51% of the female dentists and 41% male dentists were aware of oxidative stress .Chi -Square test was carried out for statistical analysis ; p value = 0.204 , which was not statistically significant.

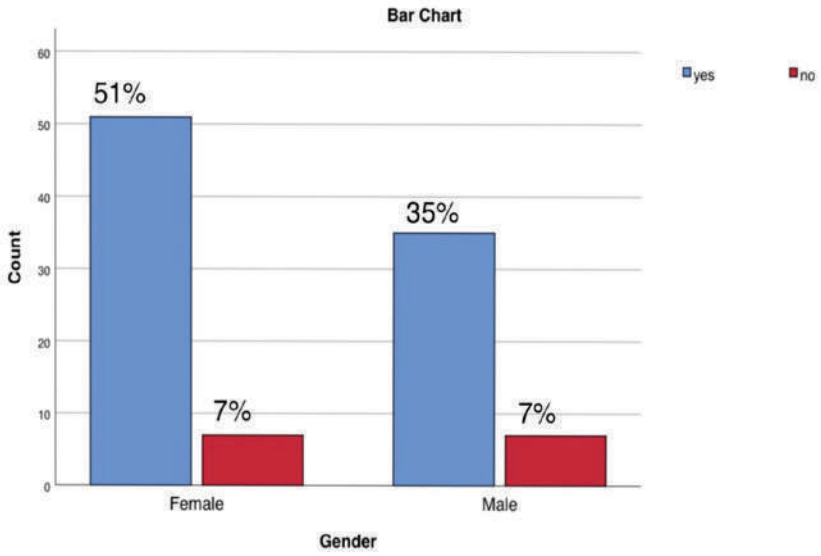


Figure 4: The bar chart represents the association between gender and awareness about oxidative stress causing inflammatory disease. (X- axis represents gender and Y - axis represents no.of. responses). Blue colour represents the awareness and red colour represents lack of awareness .51% female dentists and 35% male dentists were aware that oxidative stress can lead to inflammatory disease. Statistical analysis was done with Chi -Square test ; p value = 0.513 , which was not statistically significant.

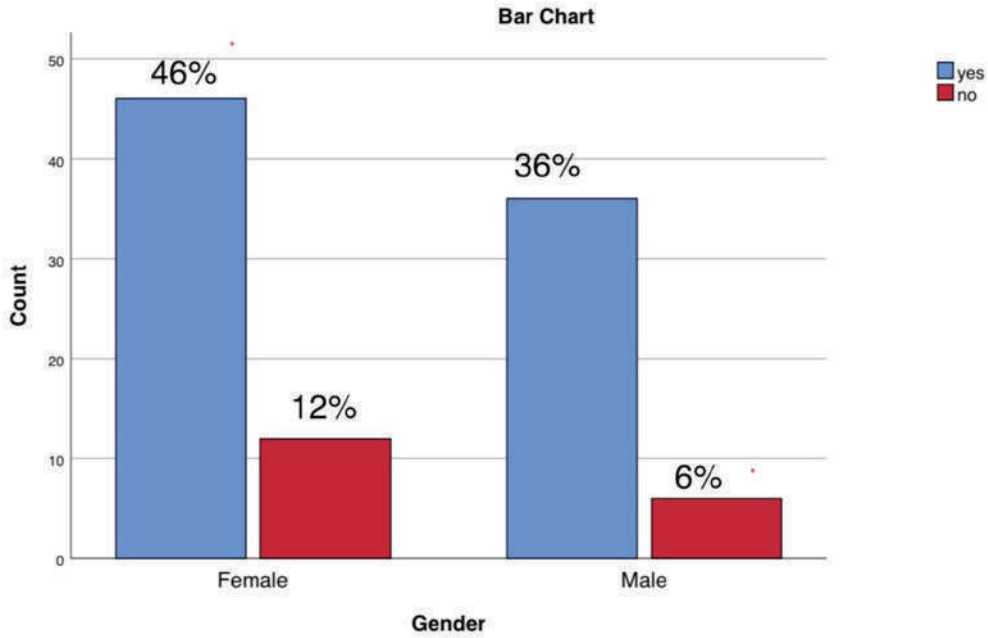


Figure 5: The bar graph represents the correlation between age and correlation between stress causing inflammatory disease. (X axis represents Gender and Y axis represents no.of. responses).Blue colour represents the awareness and red colour represents lack of awareness. 46% of dentists among the female population and 36% male population were aware that stress can lead to inflammatory disease Statistical analysis was carried out with Chi -Square test ; p value = 0.411, which was not statistically significant.

From the charts, it is clear that an increase in oxidative stress leads to various other health issues and also various other oral health problems which ultimately leads to loss of teeth. Tracing the reasons for increased oxidative stress can help in protecting from various diseases. Since increased oxidative stress has an important role in the pathological process of inflammatory disease, awareness of oxidative stress will help in preventing disease caused by inflammation. Since there is no medication and treatment of condition caused by inflammation, only treating the condition helps in temporary pain relief of the patients. So wide knowledge and awareness of the interconnection between oxidative stress and inflammation help in the prevention of various diseases.

Conclusion

Lifestyle and dietary measures can help in reducing oxidative stress. Oxidative stress reduction helps in preventing inflammatory disease. Eating balanced food, healthy food, limiting the intake of processed foods, exercising regularly, quitting smoking, reducing stress, reducing exposure to pollution can decrease oxidative stress. Inflammation and oxidative stress are closely related and linked with pathophysiological processes and both of them take part in chronic inflammatory disease. Although identification and treatment of primary abnormality are of great clinical importance, there must be more awareness among the practitioners which help in easy and quick understanding . Hence, students must be aware of all the reasons for oxidative stress and inflammatory disease. Awareness programmes and workshops may be conducted to improve the knowledge on oxidative stress and inflammatory diseases.

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Awareness on Application of Antacids among Undergraduate Students

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Abstract

Antacids are usually a class of medication that neutralize stomach acidity and makes its pH more neutral. Commonly used self prescribed medications. Antacids usually consist of magnesium, calcium carbonate and aluminium salts in various compounds or in combination. Gastric acid related disorder could be treated using antacids. Ingestion of antacids must be followed only after ingestion of Meals. The main aim of the survey was to create awareness on application of antacids among undergraduate students. A questionnaire was prepared and administered to 100 participants through an online Google forms link. Study participants included undergraduate students of 18- 26 age group. Method of representation of each output variable was in pie chart form. The result was then statistically analyzed using SPSS software. The result was analyzed as 64% were aware on the application of antacids. 55% think antacids are only used to neutralize acidity but other research shows it can reduce activity of pepsin. 45% feel common side effects of overusing antacids are constipation, Diarrhea, Kidney stones. It shows that severe side effects are rare, although they can occur in high-risk patients. But minor problems like change in bowel habits can occur frequently. This study concludes that antacids have a high neutralizing capacity and give relief and comfort. Also inactivate Pepsin to relieve symptoms and provide temporary relief and comfort. Natural foods like mint, milk, Banana, ginger can be taken instead of antacids.

Keywords: *Antacids, acidity, pH, comfort, Awareness.*

Introduction

Antacids are usually a class medicine that neutralize stomach acidity and makes its pH more neutral. Commonly used self-prescribed medications are Antacids [1]. Antacids usually consist of magnesium, calcium carbonate and aluminium salts in various compounds or in combination. Gastric acid related disorder could be treated using antacids [2]. Ingestion of antacids must be followed only after ingestion of Meals. By consuming antacids, severe side effects are very rare, but may occur in High-risk patients. However, minor problems like change in bowel habits can occur Frequently [3]. The more common side effects of consuming antacids are diarrhea, Constipation and so on, which can be managed by alternating the agents or by using one of the various antacids mixtures [4]. Normal antacid dose taken for treating ulcer is 120 mmol / day to 1008 mmol / day. Low dose antacids may increase mucosal resistance [5].

It is revealed that net effect of antacids is reduction of gastric acidity and aluminium containing antacids exhibit the cytoprotective activity or enhancement of

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natural mucosal defense mechanism [6]. The antacid - induced resistance to acidification largely corresponds to the therapeutic antacid activity [7]. Pregnancy causes various hormonal changes in the body that can actually increase the risk of developing gum diseases [8]. Antacids may help in preventing gum disease [9]. Antacids with low dosage can be as effective as H2 blockers both in terms of healing capacity and in relieving ulcer symptoms [10]. To reduce the concentration and total load of acids in gastric juice with a pH ranging from 1.3 pH to 5.0 pH is the ultimate goal of antacids therapy [11]. In the prevention of relapse, consumption of one tablet of antacids, four times a day was found to be effective [12].

There are no proper research or survey done on application of antacids among post

Graduate students. The main aim of the survey was to create awareness on application of antacids among undergraduate students.

Materials and Methodology

Self-administrated questionnaire was designed based on awareness. The questionnaire was distributed through an online Google forms link. The study Population included undergraduate students belonging to the 18- 26 age group. The Participants were explained about the purpose of study in detail. The questions were carefully studied and the participants marked the corresponding answers.

The measures that were taken to minimize the sampling bias were that the Validity was checked both internally and externally. Sample method carried out was simple Random sampling. Method of representation of each output Variable was in pie chart form where descriptive statistics were used to create pie charts.

The result was then statistically analyzed using SPSS statistic 19 software [13,14,15,16,17]. The survey has been completed in the month of may 2020.

Result and Discussion

This survey was done among undergraduate students in order to analyze awareness on application of Antacids among them. This included 100 participants where the result was statistically analyzed as 45% were female and 45% were male [Figure 1]. 64% were aware of the application of antacids [Figure 2]. 20% feels Heartburn is the symptoms for which antacids are commonly used for, 21% feels Abdominal pain, 13% feels nausea and 46% feels all of these symptoms [Figure 3]. 30% feels constipation is the common side effect of overusing antacids, 12% feels Diarrhea, 13% feels Kidney stones, and 45 % feel all of these [Figure 4]. 35% feel a single dose of any antacids taken in an empty stomach acts for 10- 30 minutes, 37 % feel 30-60 minutes and 28 % feel more than 1 hour. [Figure 5]. 42% think antacids can be taken on an empty stomach [Figure 6]. 19% think mint is the natural food that can be taken instead of antacids, 11% think milk, 13% think Banana, 11% think ginger and 46% think all of these natural foods [Figure 7]. Chi square analysis between gender and opinion on antacids are only used to neutralise acidity in which 32% female and 23% male responded yes. The P value was 0.042 and it is Statistically significant [Figure 8]. Chi square analysis between gender and opinion on consumption of antacids in which 15% female and 32% male responded to all of the above (feeling of restlessness, nausea, indigestion). The P value was 0.001 and it is Statistically significant [Figure 9]. Chi square analysis between gender and opinion on antacids are mainly used to reduce damage and relieve pain in which 26% female and 29% male responded yes. The P value was 0.702 and it is Statistically non significant [Figure 10]. Chi square analysis between gender and opinion on gelusil or digene is one of the best antacids used in which 31% female and 20% male responded yes. The P value was 0.016 and it is Statistically significant [Figure 11]. Chi square analysis between gender and opinion on treatment with antacid alone is asymptomatic in which 35% female and 29% male responded yes. The P value was 0.129 and it is Statistically non significant [Figure 12].

The present research has origins from the team of investigators where the previous studies were done based on clinical reports, interventional studies like [18,19,20], in vitro studies like [13,14,15,17], and systematic review like [18,21,22,23,24,25]. From this survey, it is analyzed that 42% think antacids can be taken on an empty stomach. Antacids suspension should be given at least often as one hour after each meal and at bedtime. The dose should be planned in terms of milli equivalents of acid neutralizing capacity and should be adjusted according to the type of disease under treatment [26]. 58% feel antacids are only used to neutralize acidity. It is revealed that antacids also

reduce the activity of pepsin and have the capacity to bind bile acids apart from neutralizing acidity [27]. 45% feel common side effects of overusing antacids are constipation, Diarrhea, Kidney stones. It shows that severe side effects are rare, although they can occur in high-risk patients. But minor problems like change in bowel habits can occur frequently [28]. 55% feel antacids are mainly used to reduce damage and relieve pain. 71.1% feels antacids are helpful mainly in ulcer healing where they were treated with a low dose antacid regimen which is 1 tablet, 120mmol / day [29]. 51% feel gelusil or digene is one of the best antacids used whereas it is revealed that antacid which is one tablet four times daily, cimetidine which is 400 mg at night or a combination of the two to be approximately equally effective and it's better antacid [30].

There is no exact solution found to eradicate problems like gastric ulcers and so on with the help of antacids as it provides only temporary relief and comfort. This survey can also be carried out on postgraduate students to analyze their level of awareness on application of antacids.

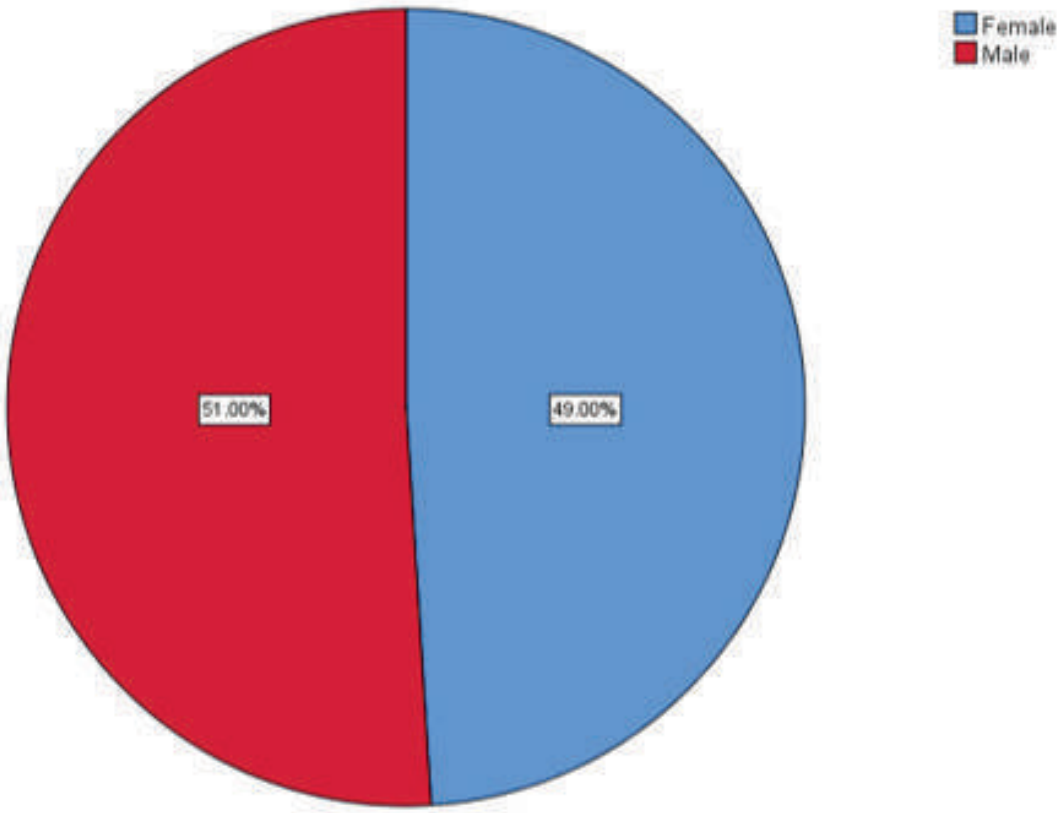


Figure 1: Pie chart represents gender of participants in the study which shows response for the question on gender. 51% (Red) of the participants were male and 49% (Blue) participants were female.

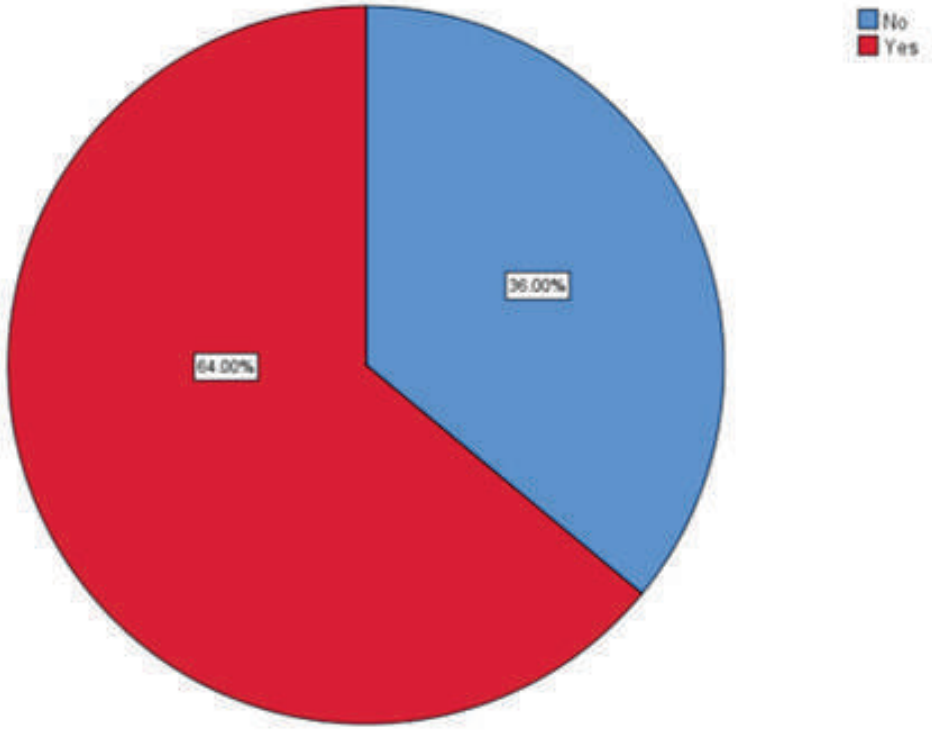


Figure 2: Pie chart represents awareness on the application of antacids which shows response for the question on awareness on the application of antacids. 64% (Red) were aware and 36% (Blue) were not aware.

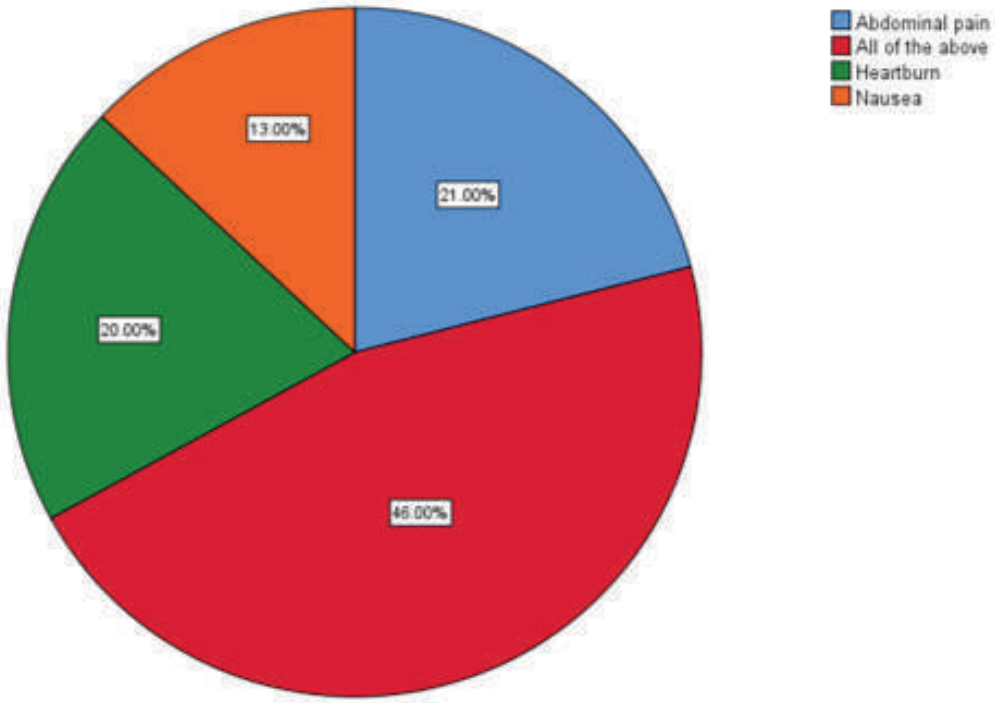


Figure 3: Pie chart representing the opinion on symptoms for which antacids are being used which shows response for the question on symptoms for which antacids are being used. 21% (Blue) responded to abdominal pain, 20% (Green) responded to heartburn, 13% (Orange) responded to nausea and 21% (Red)

responded to all of the above.

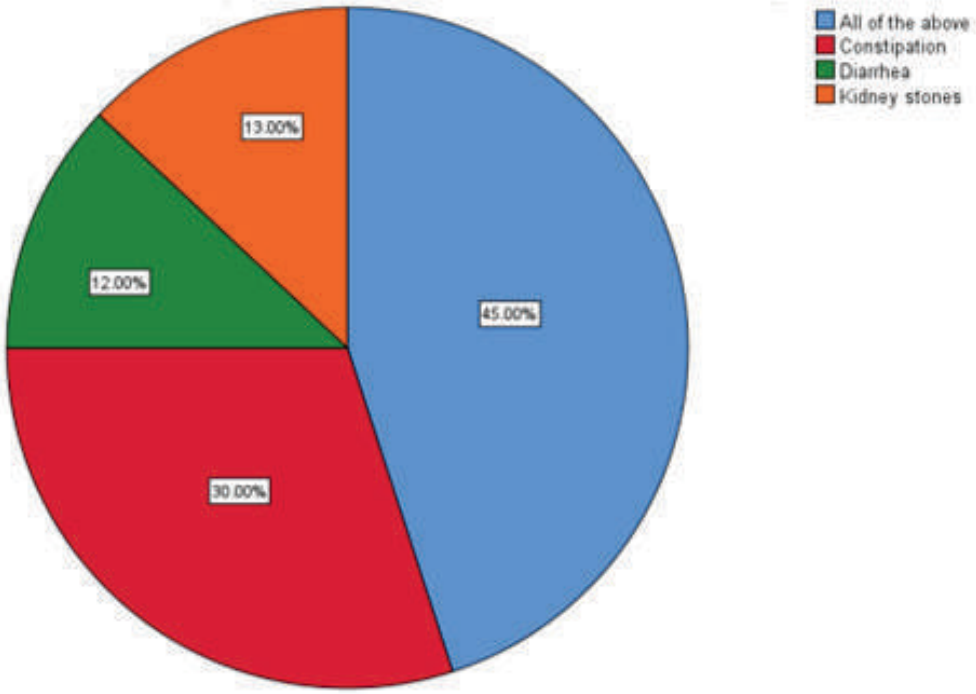


Figure 4: Pie chart representing the opinion on common side effects of overusing antacids which shows response for the question on common side effects of overusing antacids. 30% (Red) responded to constipation, 13% (Orange) responded to kidney stones, 12% (Green) responded to diarrhea and 45% (Blue) responded to all of the above.

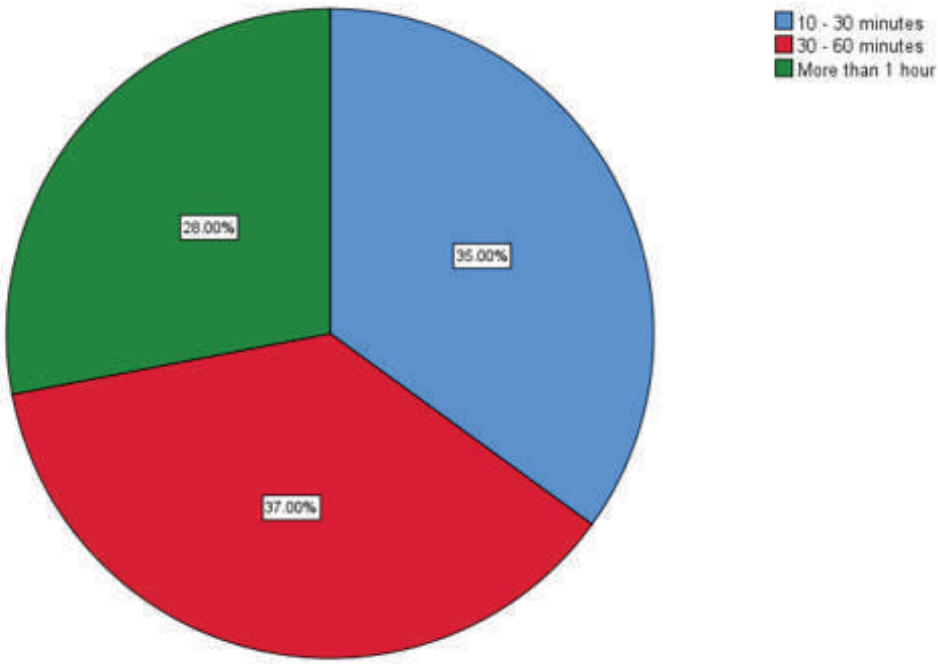


Figure 5: Pie chart representing the opinion on duration of effectiveness of antacids taken in an empty stomach which shows response for the question on duration of effectiveness of antacids taken in an empty stomach. 35% (Blue) responded to 10-30 mins, 37% (Red) responded to 30-60 mins and 28% (Green)

responded to more than 1 hr.

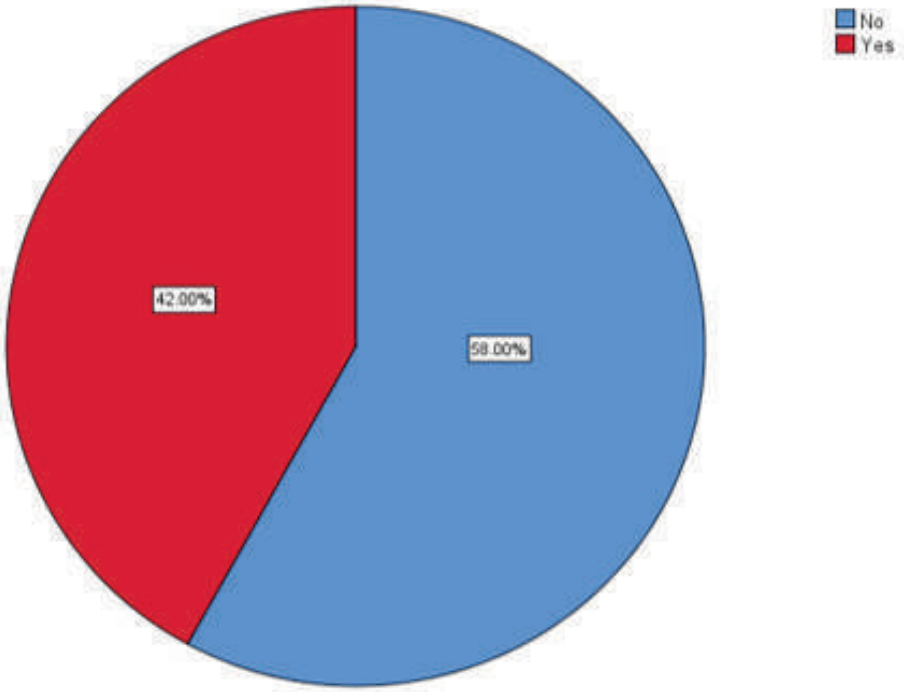


Figure 6: Pie chart representing the opinion on antacids taken on an empty stomach which shows response for the question on whether antacids can be taken on an empty stomach. 42% (Red) responded to yes and 58% (Blue) responded to no.

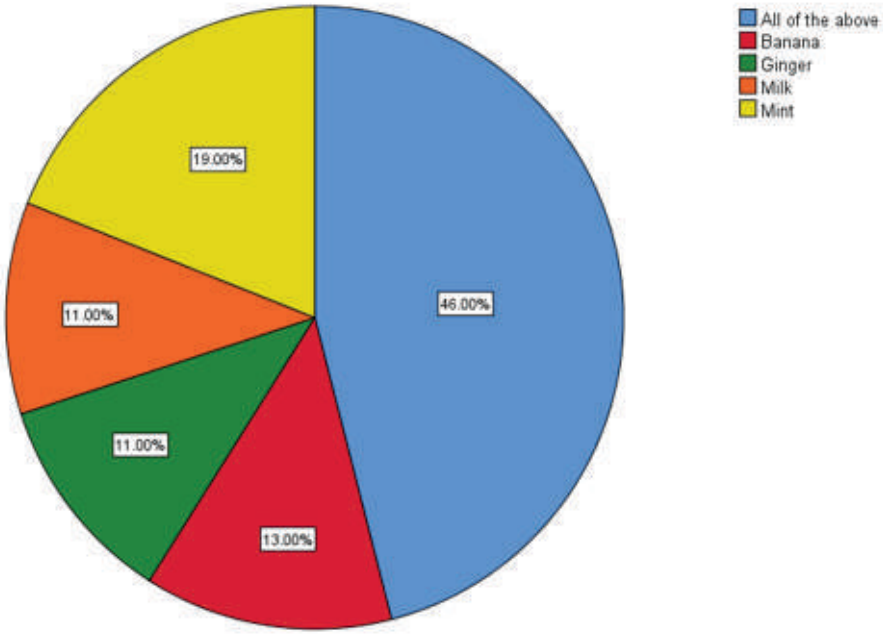


Figure 7: Pie chart representing the opinion on natural food that can be consumed instead of antacids which shows response for the question on natural food that can be consumed instead of antacids. 13% (Red) responded to bananas, 11% (Green) responded to ginger, 11% (Orange) responded to milk, 19% (Yellow) responded to mint and 46% (Blue) responded to all of the above.

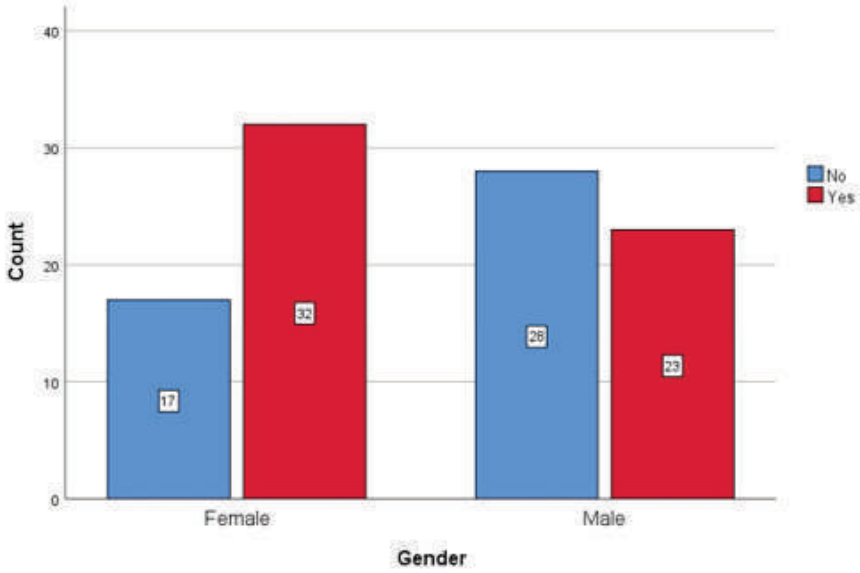


Figure 8: Bar chart representing association between gender and opinion on antacids are only used to neutralise acidity where red denotes yes and blue denotes no. 32% female and 23% male responded yes. X axis represents gender and Y axis represents the number of participants responded regarding antacids are only used to neutralise acidity. Out of 55% of the participants who responded yes, 32% constitute female and 23% constitute male. Hence females have a stronger opinion that antacids are only used to neutralise acidity. Chi square test was done and the association was found to be statistically significant (Pearson's chi square value = 4.123, df = 1, P value = 0.042(<0.05)). Hence statistically significant.

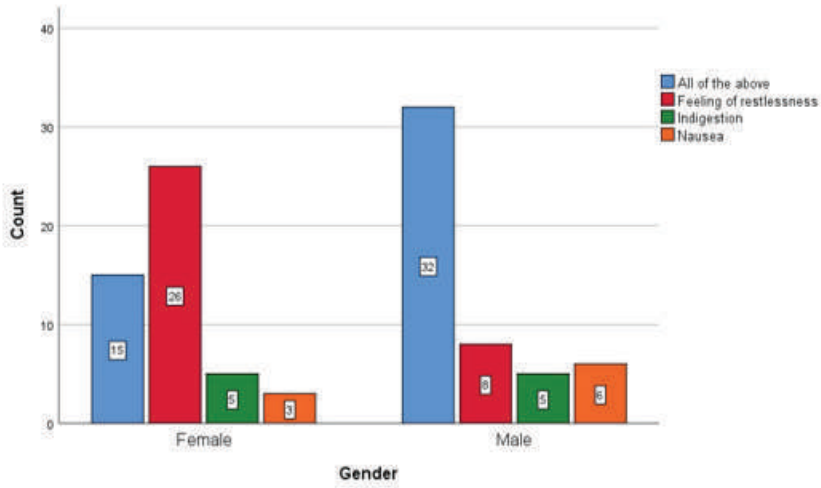


Figure 9: Bar chart representing association between gender and opinion on consumption of antacids where red denotes yes and blue denotes no. 15% female and 32% male responded to all of the above. X axis represents gender and Y axis represents the number of participants responded regarding consumption of antacids. Out of 47% of the participants who responded all of the above, 15% constitute female and 32% constitute male. Hence males have a stronger opinion than females on consumption of antacids incase of indigestion, nausea and feeling of restlessness. Chi square test was done and the association was found to be statistically significant (Pearson's chi square value = 16.645, df = 3, P value = 0.001(<0.05)). Hence statistically significant.

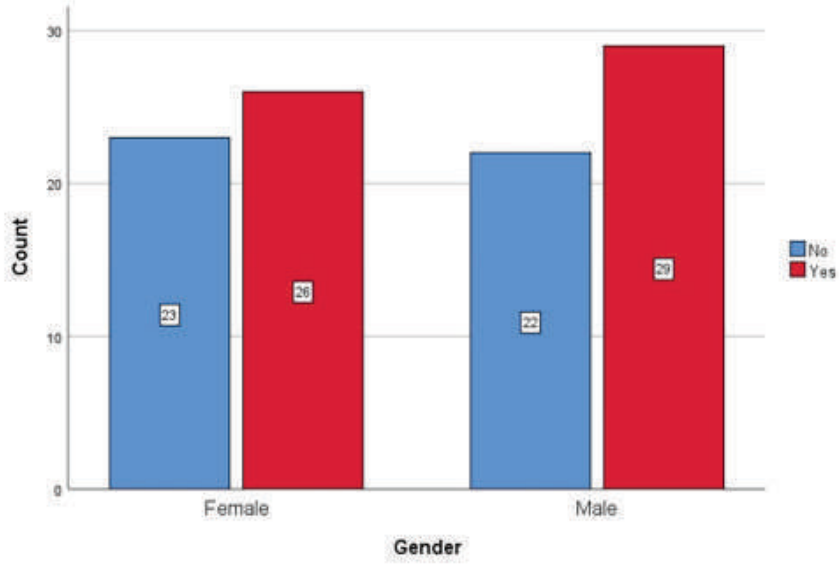


Figure 10: Bar chart representing association between gender and opinion on antacids are mainly used to reduce damage and relieve pain where red denotes yes and blue denotes no. 26% female and 29% male responded yes. X axis represents gender and Y axis represents the number of participants responded regarding antacids are mainly used to reduce damage and relieve pain. Out of 55% of the participants who responded yes, 26% constitute female and 29% constitute male. Hence males have a stronger opinion on antacids are mainly used to reduce damage and relieve pain than females. Chi square test was done and the association was found to be statistically non-significant (Pearson's chi square value = 0.146, df = 1, P value = 0.702(>0.05)). Hence statistically non-significant.

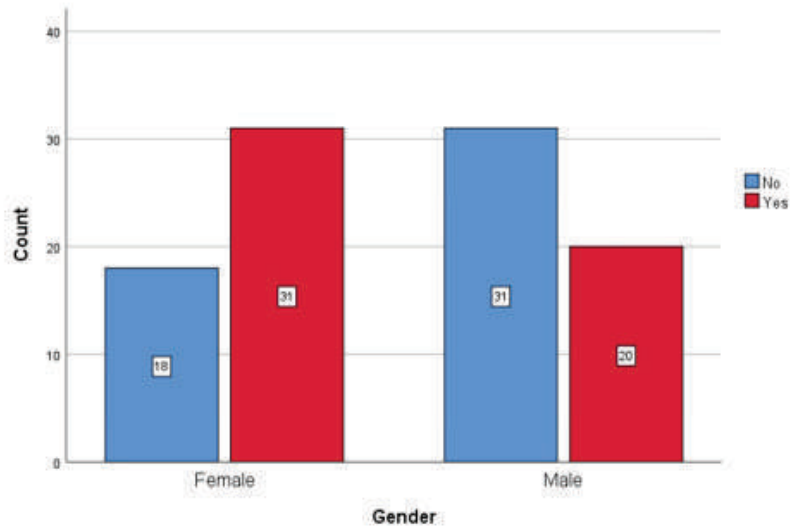


Figure 11: Bar chart representing association between gender and opinion on gelusil or digene is one of the best antacids used where red denotes yes and blue denotes no. 31% female and 20% male responded yes. X axis represents gender and Y axis represents the number of participants responded regarding gelusil or digene is one of the best antacids used. Out of 51% of the participants who responded yes, 31% constitute female and 20% constitute male. Hence females have a stronger opinion on gelusil or digene is one of the best antacids used than male. Chi square test was done and the association was found to be statistically significant (Pearson's chi square value = 5.784, df = 1, P value = 0.016(<0.05)). Hence statistically significant.

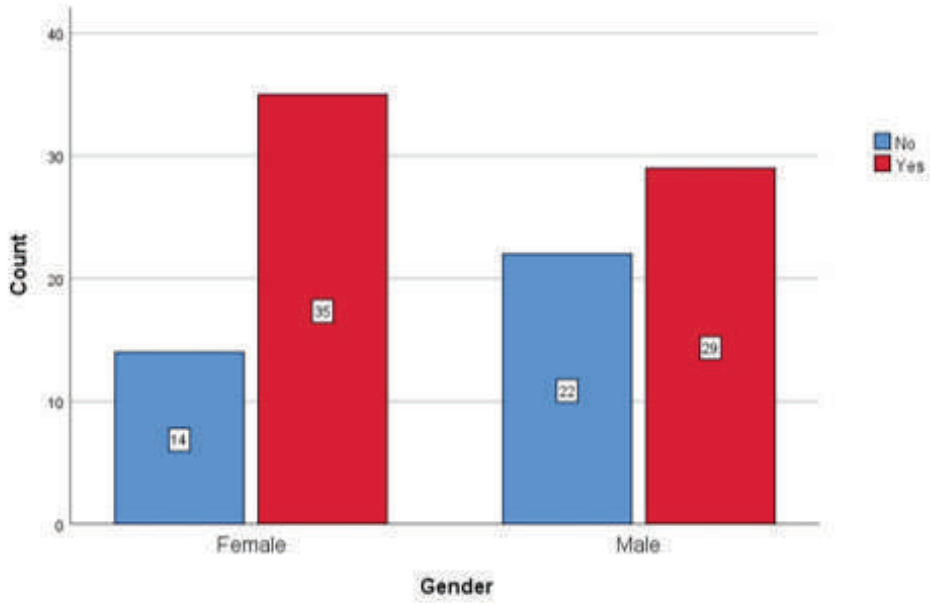


Figure 12: Bar chart representing association between gender and opinion on treatment with antacid alone is asymptomatic where red denotes yes and blue denotes no. 35% female and 29% male responded yes. X axis represents gender and Y axis represents the number of participants responded regarding treatment with antacid alone is asymptomatic. Out of 64% of the participants who responded yes, 35% constitute female and 29% constitute male. Hence females have a stronger opinion on treatment with antacid alone is asymptomatic than male. Chi square test was done and the association was found to be statistically non-significant (Pearson's chi square value = 2.301, df = 1, P value = 0.129(>0.05)). Hence statistically non-significant.

Conclusion

This study concludes that there is a moderate level of knowledge about antacids among the undergraduate students. As antacids are mainly used for acidity, which is a common problem of present generation due to different lifestyles and food habits, more research has to be carried out for better understanding on the application of antacids.

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Conflict of Interest : The author declares that there was no conflict of interest in the present study.

Source of Funding : Self

Ethical Clearance: Not Required

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Osteosynthetic Materials Used in Open Reduction and Internal Fixation of Facial Fractures in University Hospital Setting- A Retrospective Study

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Abstract

Facial fractures and management form a significant quantum of work for maxillofacial surgeons. Understanding the appropriate surgical techniques and materials can avoid complications like infections, implant loosening, improper healing and resurgeries. The aim of the study is to compare the two most commonly used osteosynthetic materials for open reduction and internal fixation - stainless steel and titanium. A retrospective data collection was done from March 2019 to June 2020 for a period of 10 months. Case records of patients who reported with facial trauma and underwent open reduction internal fixation(ORIF) were reviewed. There were 105 patients who reported with facial trauma, among which 47 patients needed ORIF. Parameters analysed were age, gender, site of fracture and choice of plate material. Among these, 44 were males and 3 were females. 35 patients were below 35 years and 12 patients were above 35 years. 65% were treated using Titanium plate & 35 % with stainless steel plate. Chi square test was performed to study the statistical significance and p value was found to be (0.030)<0.05, statistically significant proving titanium is more used in patients below 35 years of age.This study showed that titanium plates were most commonly preferred over stainless steel plates. This is in consensus with existing literature that titanium plates are widely used for ORIF in facial fractures. However, though 35% of our study group still preferred to be treated with stainless steel plates. Whether age or socioeconomic reasons played a role in the choice of treatment needs to be further evaluated with larger sample size and multicentre studies among our population.

Keywords: *Open reduction internal fixation;Facial fracture; Stainless steel;Titanium; Trauma*

Introduction

The surgical treatment of craniomaxillofacial region involves restoration of ideal form and function[1]. Complete healing of facial fracture was highly reported after taking into principles of orthopaedics like closed reduction, open reduction and internal fixation. Especially when the fractures cannot heal on their own, have been treated by ORIF by maxillofacial surgeons globally[2]. There are constant advancements in use of many materials from rigid metals to alloys to bioresorbable plates, In the middle of the 1980s Champy popularized the use of stainless steel for ORIF[3]. Stainless steel was thought to be toughest material to withstand stress and was comparatively cheaper,but it had the disadvantage of high surface damage. Also it was difficult for adaptation.

To overcome this there were researches done and many concluded titanium as a superior option. Titanium was reported for its unique property called osseointegration. It had shown better adaptation, low surface damage, higher stress. Hence they can be preferred for most maxillofacial procedures[4], though cannot be generalized for all procedures like mandibulectomy [5,6].

Also the release torque of titanium is higher than its insertion torque which accounted for its overall less loosening during stress application. But the cost of hardware and hypersensitivity reactions were reported as disadvantages[7]. Researchers stated that there were more radiographic scatter in stainless steel than compared to others like vitallium and titanium. Francel et al stated

that there would be a need for removal of titanium plates as the biohazards were higher which would significantly increase procedure expenses of patients[8].

Common sites of fracture in maxillofacial region are the nasal bones, mandible, zygomatico maxillary bone and orbit. Angle fractures are quite common considering that the angle of the mandible forms an area of lower resistance which contains a thicker upper border, a thin basilar bone, and the presence of an impacted mandibular third molar [9,10].

Plate removal can be a stressful procedure for the patients as it would require a second surgical procedure under general anesthesia[11]. Though the procedure of plate removal may be minor, the anaesthetic procedures, anxiety, stress and minimal hospital stay can be burdening to the patients [12,13–15]. Various schools of thought debating the need to remove plates electively vs not are found in literature [16,17]. So the choice of an ideal material is a debate globally with varied conclusions based on studies and case reports.

The main aim of this study is to compare the osteosynthetic materials used or preferred based on their properties,location of usage,complications,post operative results.

Materials and Methods

Study Type & Setting:

This is a retrospective study and all data were collected by reviewing and analysing 105 trauma case sheets of Saveetha Dental college and Hospitals over a period of 10 months from June 2019-March 2020. Ethical clearance was obtained for the study from the Institutional Ethical Committee

Participants:

Among the 105 cases sheets reviewed, 58 patients were treated with closed reduction and were excluded from the study. Patients who were treated by Open Reduction and Internal Fixation alone were included and it accounted for around 47 patients where 44 were males and 3 were female patients. All data were tabulated and cross verified by 2 reviewers.

Data Analysis

Data retrieved were compiled in an excel sheet and imported to IBM SPSS Statistics Version 20. Statistical analysis was performed using chi square statistical test for significance. The independent variable is the plate material and dependent variables were age, gender and site of fracture.

Results and Discussions

All cases selected were operated in the same institution with the same team of surgeons. Surgical protocols were standardised and post operative medications both antibiotics and analgesics were common for both the plates [18,19].

It was seen that 55% of males (24/44) and 66% of females (2/1) preferred titanium over stainless steel (Fig-1). However the overall number of males who were treated were more than females treated[20]. Among males an almost equal number of patients (20/44) preferred to be treated with stainless steel.

65% of patients treated with ORIF were of age less than 35years and preferred titanium(22/35) over stainless steel. 70% of patients above 35 years of age preferred stainless steel (9/15) over titanium (Fig-2). Chee Wei Lee et al stated that most fractures were observed during the early 30s and hence we established a relation between age over the material preference[21].

55% of mandibular fractures were treated with stainless steel whereas titanium was preferred over 50% of maxillary fractures and was the choice of preference overall for all fractures(26/47). Chi square test was performed. The p value was found to be 0.148, implying that there is no significance between location of fracture and the material used (Fig-3). All fractures involving orbit were only treated with titanium mesh or plates. This is possibly to avoid chances of infection and eventful needs of plate removal at later stages which can be very difficult.

Researchers stated that most reported cases of ORIF were in the mandible in Indian population in his earlier studies [22]. Dube et al also stated that the second most common region of facial fracture is seen in the middle third of face especially in the zygomatic region and in nasomaxillary region[23]. Thus a study between location of

fracture and the preference of material was established.

Kaura et al stated that 61% of facial fractures were reported to hospitals due to road traffic accidents followed by trauma due to assaults (23%)[24,25]. Such cases are prone to infections and necessitate the choice of the most inert biocompatible material. Based on this hypothesis, titanium is most preferred in the panfacial trauma or fractures involving the middle third of the facial skeleton. But there have been certain documentations stating the effects of titanium on bones causing hypersensitivity and preferred stainless steel.

Sensitivity to titanium is characterized by the local presence of abundant macrophages and T lymphocytes and the absence of B lymphocytes, indicating Type 4 hypersensitivity. Researches are going on to develop a technique using flow cytometry, for the purpose of detecting the activation of lymphocytes stimulated by a metal, and measuring different mediators (cytokines, inflammatory mediators) released in response to the metal. Sahoo et al in his experiments found that there was osteoid tissue deposition around titanium which was a unique property lacking in stainless steel[26].

Veneable et al stated the presence of osteolysis around the fixation site when using stainless steel[27]. Branemark et al in his studies proved that there was microscopic evidence of osseointegration seen between bone and titanium[28]. Titanium plates possess little biohazards and the second surgery to be performed for their removal has been minimal.

Hence, they remain the choice of preference for their strength and osseointegrative property[29]. Also full or complete recovery from fractures can be obtained faster in using titanium over stainless steel due its various properties like osseointegration, high release torque,strength[30].

Thus incase of complicated fractures or multiple fractures or to enhance faster recovery titanium plates can be preferred. There has been global consensus with the result and this can be taken as evidence to practice.

Certain limitations in this study were less sample size and unequal gender distribution. The presence of other confounding factors like literacy and socio economic status were not well documented and hence could not be analysed.

Future prospects of this study can be established in larger sample size and also to find microscopic tissue level changes involved in both materials used for ORIF and so there can be a fair and conclusive evidence for their predominant preference and usage.

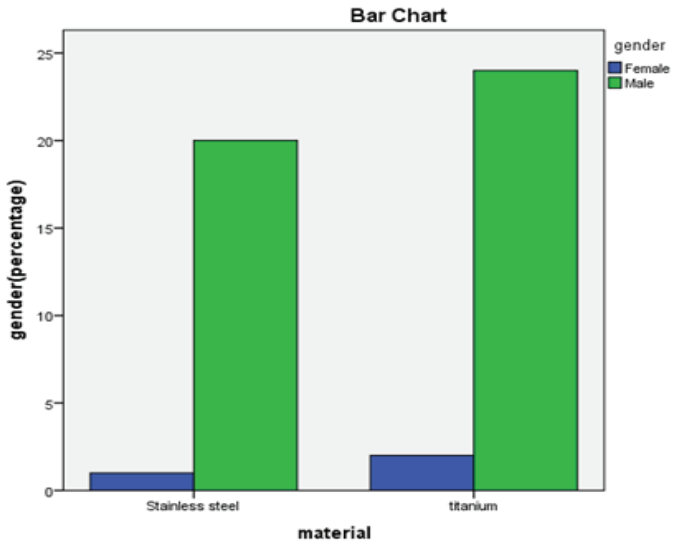


Fig-1: Bar graph representing the association between gender and the material used.X-Axis: Material Used (Titanium And Stainless Steel); Y Axis-Number Of Patients In Both Gender (Male-Green;Female-Blue). Chi square test was performed and the association was not significant. P value = 0.682 > 0.05, hence not significant proving osteosynthetic materials can be preferred irrespective of gender.

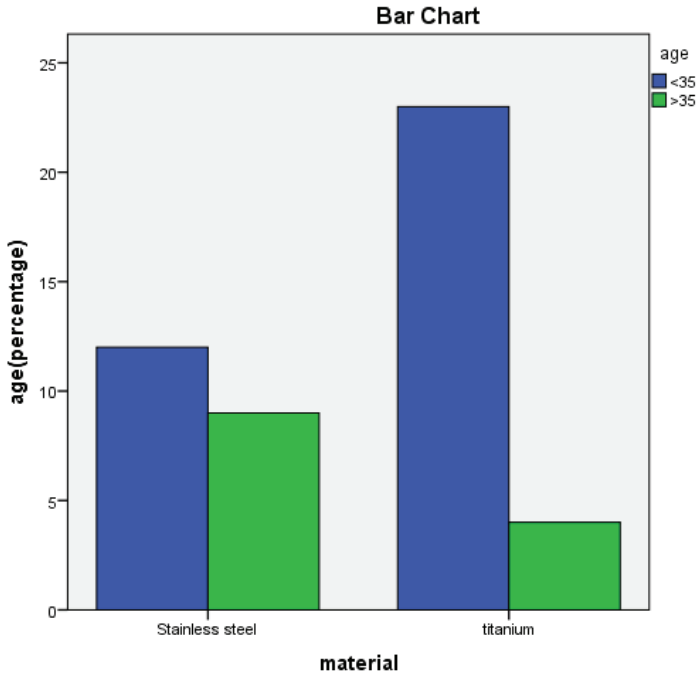


Fig-2: Bar graph representing the association between age and the material used.X-Axis: Material Used (Titanium And Stainless Steel); Y Axis-Number Of Patients In Both Age category(>35-Green;<35-Blue).Chi square test was performed and the association was significant. P value = 0.03 < 0.05, statistically significant proving titanium is the preferred material of choice by patients below 35 years of age .

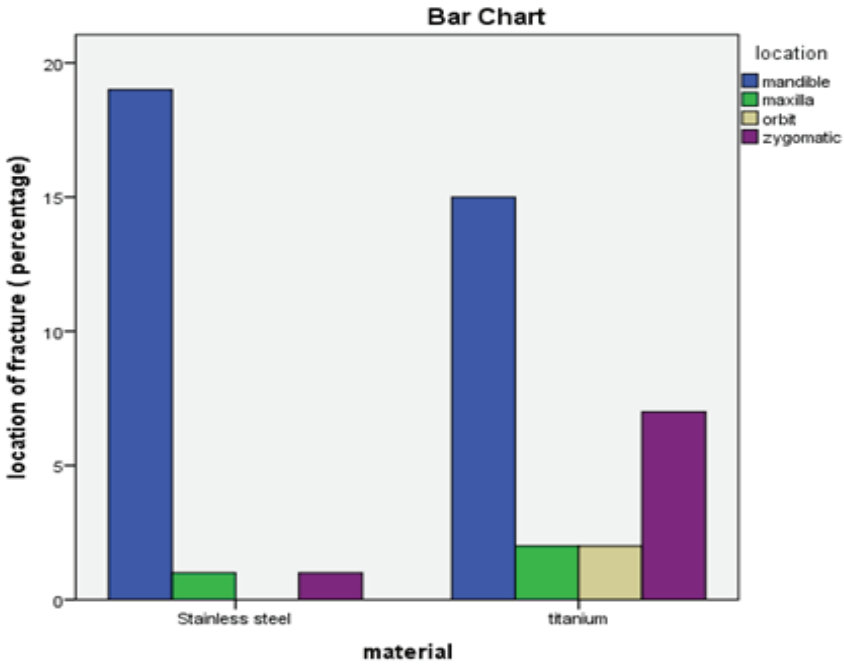


Fig-3: Bar graph representing the association between location of fracture and the material used.X-Axis: Material Used (Titanium And Stainless Steel); Y Axis-Location of fractures (maxilla-Green;mandible-Blue;orbit-Grey; zygomatic-Purple).Chi square test was performed and the association was not significant. P value = 0.148 (> 0.05), hence not statistically significant proving titanium is more used irrespective of location of fracture.

Conclusion

Conventionally two biomaterials namely titanium and stainless steel are been used for Open reduction and Internal Fixation.There has always been a comparison between the preference of both the materials at different setup.Within the limitations of the study it can be concluded that titanium was considered over stainless steel for ORIF of facial fractures irrespective of age,gender and location because of their strength and properties like less surface damage,less scatter in radiographs and osseointegration.

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IgG4 Related Lung Disorder -A Review

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Abstract

IgG4 disorder is an autoimmune disorder. Its manifestation in the lung causes fibroinflammatory disorder. It is one of the chronic inflammatory disorders. IgG4 is the subclass of IgG. This review is analysed based on the IgG4 related disorder. Nearly 50 articles were reviewed and this study was conducted for a period of nearly 15 years.The IgG4 related disorder is a very rare disorder in the world. Moreover, It is an autoimmune disorder like Systemic lupus erythematosus. It is related to systemic disorder and chronic inflammatory disorder IgG4 varies high degree of fibrosis and it is prompted by the response of steroid therapy.It is the most common form of immunoglobulin. Ig G also known as secretory immunoglobulin.Ig G4 is a subclass of IgG comprising only 4% in gamma immunoglobulins . IgG4 does not bind to polysaccharides. Increased levels of IgG4 in serum usually found in patients suffering sclerosing pancreatitis, cholangitis and interstitial pneumonia caused by the infiltration of IgG4 positive plasma cells. IgG4 has a molecular weight of 159. IgG4 related disorder recognised as one of the rare autoimmune disorders which can affect multiple organs and cause organ failure sometimes. It involves the complement system and interacts with the antibody. Generally IgG4 related disorder is recognised as one of the most rare autoimmune disorders which can affect multiple organ failure sometimes. It involves complement system and interact with antibody. Generally, IgG4 related disorder most commonly present in in beekeper, animal laboratory workers and individuals who have undergone allergen immunotherapy possess high serum level of IgG4.it is one of the acquired Type II hypersensitivity disorder. This review mainly aims to know the reason behind the clinical lung disorder.

Keywords*: IgG4, Complement system, autoimmune disorder, treatment, clinical findings*

Introduction

Ig G4 is a subclass of IgG comprising only 4% in gamma immunoglobulins . IgG4 does not bind to polysaccharides. Increased levels of IgG4 in serum usually found in patients suffering sclerosing pancreatitis, cholangitis and interstitial pneumonia caused by the infiltration of IgG4 positive plasma cells. IgG4 has a molecular weight of 159. 12 amino acids are found in the hinge region, two inter H chain disulfide bonds are found. Normal serum level of IgG4 is 0.56 g/l. IgG4 related disorder is a novel recognised fibroinflammatory disorder and it is usually manifest as generalised swelling in various organs with pathologic findings showing the IgG4 rich plasma cell infiltration, chronic inflammation and fibrosis. It may involve any organ showing the elevated serum IgG4 concentration[1]. The initial differential diagnosis for IgG4 related disorder includes panbronchiolitis, an inflammation found in bronchioles which causes severe cough,large amount of sputum and exertional breathlessness,sarcoidosis and the hypersensitive pneumonitis.In chest computed tomography, it reveals the bilateral diffuse centrilobular

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nodules, tee in bud pattern, bronchial wall thickening and multifocal ground glass opacities. It shows some mediastinal lymph node enlargement and also shows multiple mosaic attenuation.[2]. It may involve multiple organs including lungs, mediastinum , liver and pancreas. It is characterized by tumefactive lesions with lymphoplasmacytic infiltrates with abundant IgG4 positive plasma cells, storiform fibrosis and usually elevated serum IgG4 concentration[3]. This disease is also related to numerous conditions like autoimmune pancreatitis, Riedel thyroiditis, tubulointerstitial nephritis[4]. The clinical treatment for IgG4 can be found by reviewing the articles.There are certain drugs administered in this disorder like Glucocorticoid, azathioprine, 6 mercaptopurine, cyclophosphamide, rituximab are given. In this article, morphological views can be found.Glucocorticoid is the first line drug used in treatment of IgG4 related disorders we can find remission induction in all patient is active, conditions which mimics IgG4 related disease are inflammatory bowel disease, Rhinosinusitis, sjogren syndrome, sarcoidosis, xanthogranuloma,splenic sclerosis[5]. The Etiological factor of IgG4 Related disorder could be congenital or acquired [6] the lymphocytic infiltration is seen in respiratory disorder.[7]Usually the stye is seen in this disorder observed in the monkey[8]. The stye is an infection of sebaceous gland [9] This study mainly focuses to know the reason behind the every clinical symptoms produced in IgG4 related lung disorder.The article can be reviewed with the help with the help of search engine. Nearly 36 articles are reviewed with the help of search engines. The articles were collected based on their pathogenesis , clinical manifestation, diagnosis, treatment of IgG4 related disorder. The period of study from 2005 -2020. The article which is not related with this review other than IgG4 related disorder, IgG4 related cardiovascular system, IgG4 related kidney are not considered.

LUNG DISORDERS RELATED TO IgG4

IgG4 Related to Interstitial Lung Disorder

IgG4 disorder is related with IgG4 positive plasma cell suggestive of grade 1 lymphomatoid granulomatosis. presence of IgG4 positive plasma cell with lack of atypical cell favours IgG4 related disorder[10]. IgG4 related interstitial lung disease have shown without any systemic manifestation have been reported.The pathological findings are examined reveals that the thickening of bronchovascular bundle and alveolar septa by infiltration of plasma cell , lymphocyte and few eosinophils.[11]There is an increased attenuation to IgG4 related lung disorder is seen and it is characterized by elevated serum IgG4 level and IgG4 positive plasma cell infiltration into multiple target organs includes lung.[12]IgG4 related disease is characterized by by high serum IgG4 concentration,sclerosing inflammation with numerous IgG4 positive plasma cell and steroid sensitivity irrespective of any organs[13] Immunostaining shows the infiltrations present in interstitium of CD3 positive T cell and CD 20 positive B cell as well as numerous CD38 positive, IgG4 positive plasma cells.[14] The X ray finding tells that there is ground glass appearance and honey comb associated with interstitial pneumonia[15] Respiratory disorder is the most common disorder in the world and its prevelence is seen among old aged patient[16]The count of white blood is decreased generally in the autoimmune disorder like IgG4 disorder, sjogren syndrome,multiple sclerosis,etc.[17]The Infiltration of IgG4 in the Alveolar septum is clearly visible in immunostaining. Interstitial pneumonia is strongly associated with autoimmune pancreatitis. There is an infiltration of IgG4 positive plasma cells were also observed.[18]

IgG4 Related Lung Disorder Diagnostic Feature:

Elevated IgG4 serum level is the key to confirm the IgG4 related disorder. It shows the presence of hypergammaglobulinemia, hypocomplementemia, tissue eosinophilia, and the lymphoplasmacytic infiltration.[19] The gold standard diagnosis of IgG4 Related disorder , identification of typical histopathologic feature (i.e., rich lymphoplasmacytic infiltration, storiform infiltrate, obliterative phlebitis) in context of significant IgG4 positive plasma infiltrate.[1]. Elevations in serum IgG4 concentration is considered as one of the most important diagnostic features in IgG4 related disorder. Most of the research articles shows that the IgG4 Related Disorder shows the various organ manifestation particularly in organs like pancreas, kidney, biliary tree appearance, salivary gland.[20] The cardinal feature of IgG4 related disorder or multiple organ swelling that are often considered as increased concern for malignancy[21]there is the relative predominance of lymphoplasmacytic

and sclerotic components like pseudolymphomatous, mixed and sclerosing.[22] The histopathologic diagnosis of the IgG4 related lung disorder is always the best in identification of IgG4 related lung disorder. It is often related to the abundent infiltration of IgG4 positive plasma cells. The patients with this disorder were shown to have immunocompromised problems[23]. So the histopathological picture of disorder is a very important aspect.[24] Usually for the histopathological study, the microscopic slide paraffin should be added for clear visibility.[25]

Pathogenesis of IgG4 Related Disorder:

IgG4 related lung has an analogue to sarcoidosis, another systemic disease in which the diverse organs manifestation linked by the unique histological appearance.Disparate disorder such as Mikulicz syndrome,kutter tumour, multifocal fibrosclerosis and eosinophilic angiocentric fibrosis [22]. The two main features that links disparate manifestation of IgG4 related disorder are characteristic of histological appearance and elevated IgG4 positive plasma cell within the tissues.[1] The serum IgG4 concentration upto 40% of patients have shown biopsy proven IgG4 related disorder.[26] There are some studies have shown the correlation between the Helicobacter pylori and constituent of pancreatic epithelial cell suggest gastric Helicobacter pylori infections triggers the autoimmune pancreatitis in genetically predisposed individuals through antigen antibody cross reaction. It involves activation of many complementary systems.[27] [28]The significant role of Th2 cytokines with the contribution of innate immunity factors such as toll like receptors, macrophages and basophils.The alveolar macrophages are activated by interleukins which causes inflammation are overexpressed by the B-cell activating factor and contributes to the chronic inflammation which causes the development of fibrosis.[29] Wheezing is commonly seen in patients with respiratory problems and old aged patients. The only way to alleviate wheezing is breathing exercise.[30]

Treatment of IgG4 Disorder with Drugs

IgG4 related lung disorders treated with rituximab have shown a prompt decrease in IgG4 serum level. The level of liver enzymes gradually decreased and continued to decrease further after 6 months administration of rituximab.[31] . Although rituximab shows the adverse effect like B cell depletion it is effectively used in treatment of IgG4 related disorder.[32] Rituximab is a chimeric monoclonal antibody which is directed against the B lymphocytes which has a specific antigen CD 20. CD 20 is first expressed in bone marrow.[33]Glucocorticoids and B cell depletion strategies appear to be effective[34] Corticosteroid treatment for this disease follow-up for two months have shown the complete resolution of the disease.[35] Dupilumab is a monoclonal antibody directed against the interleukins and also inhibits the signalling of cytokines. It is potentially used in treatment of allergies and atopic diseases.Subcutaneous administration of dupilumab have shown poor control on atopic manifestation. It is a steroid sparing drug.[36]Dupilumab is a drug which serves as an effective in treatment of the IgG4 related disorder and it is also considered as safe drug though has adverse effects.[37]Usually the stem cells also been used as one of the treatment procedure for treating the IgG4 related disorder[38] The radiotherapy is also in treatment but it can impose various damage to the cells[39]the red blood cell count is relatively decreased[40].In order to avoid that fruit consumption should be there.[41]

Clinical Features of IgG4 Related Disorder

IgG4 Related disorder involves multiple organs which causes faIlure. The affected organs show the orbital pseudotumor [A renal mass Decembers renal cell carcinoma] and nodular lesion in lungs. Lymphadenopathy is the common clinical feature in IgG4 or related disorder.Asthma and allergies present nearly in 40% of the patients. There is a presence of solid nodular , thickening of bronchovascular and interlobular septa, alveolar interstitial resembling the honeycomb , bronchiectasis and diffuse ground glass opacity.[42]Physical examination of IgG4 related disorder patients has shown reduced breathing sound at the bottom of lung and blood gas analysis reveals moderate hypoxemia. Blood test revealed that there is mild anaemia, increased inflammatory marker (erythrocytes sediment rate, and C reactive protein) and increased plasma level IgG4 (230mg/dL).CT scan of lung reveals the partially organized left pleural effusion, small area of atelectasis in lingula and anterior basal segment of left lower lobe and increased volume of lymph node seen in paratracheal and subcarinal station. IgG4 related disorder affects any organ with exception of synovium which is

usually present in joints.Haematological findings have revealed lymphadenopathy, eosinophilia and polyclonal hypergammaglobulinemia.[43].

Lung manifestation of IgG4 related disorder revealed to be diverse. They include disease tracks along the bronchi and blood vessels. Images have revealed pulmonary nodules, ground glass opacities, pleural thickening or interstitial lung disease[44]. The clinical symptoms include cold, cough, haemoptysis, dyspnea, pleural effusion and chest pain are present. Many patients with IgG4 related disease have shown allergic features like atopy is seen, eczema, chronic sinusitis and asthma are seen in this disorder. It causes irreversible tissue damage to any organs.[45,46] Some patients have presented the infection of pneumonias, deep vein thrombosis, gastrointestinal bleeding during the treatment of steroid. It has been reported that one patient have died due to presence of IgG4 related disease with respiratory failure due to pneumonia for eight months after the glucocorticoid therapy.[4748] Histopathological features are lymphoplasmacellular infiltration with IgG4 positive plasma cell Storiform fibrosis and obliterative phlebitis. This leads to functional destruction. There is a presence of a mixture of Th1 and Th2 cytokines immune response whereas the role of pathogenic IgG4 antibodies are still unclear. Occurrence of this disease in organs due to dysregulation of the immune system.[49]

Conclusion

To conclude, IgG4 is a rare autoimmune disorder manifested due to lymphoplasmacytic infiltration and elevated serum IgG4 positive cells. It can also manifest multiple organ failure. Glucocorticoids being the first line drug for IgG4 related disorder, other drugs like rituximab, dupilumab and other steroidal therapies were also recommended to treat the IgG4 related lung disorders. This review had thus provided an overview of the IgG4 associated lung disorders and its associated therapeutic measures with its limitations and challenges.

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Artificial Intelligence in Dentistry - A Survey

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Abstract

Today's artificial intelligence is invading our everyday lives such as digital assistants like ALEXA, SIRI. While we may think about sci-fi imagination when heard about artificial intelligence, the future of Artificial intelligence in dentistry is very real. With the enormous increase in the documented information and patient data, intelligence software and computation of this data is a necessity. While advances in artificial intelligence, neural networking, image recognition, speech recognition, etc have transformed the field of medicine and dentistry in many ways and yet a number of drawbacks, challenges have to be overcome. A self-designed survey study was conducted among 100 individuals of the general population. The questionnaire was designed in the manner to assess their knowledge on the treatment and vaccines for COVID-19 infections. The questionnaire contains a set of 10 questions. These questions were distributed through an online survey link. The study population was well explained about the purpose of this study. The questions were carefully studied and the responses of each individual has been recorded. The results were statistically analysed through an online platform named google forms. The AI has the potential that can be applied in various fields of science. Thus most of participants were aware about the usage of AI in dentistry.

Keywords: *Artificial Intelligence, digital assistance, software, science fiction.*

Introduction

Today's artificial intelligence is invading our everyday lives through digital assistants like ALEXA and SIRI. While we may think about sci-fi imaginations when heard about AI, the future of AI in dentistry is very real . With the enormous increase in the documented information like patient's data, computation of this data through intelligent software is a necessity [1] . From data processing, finding relevant information using neural networks, diagnosis of a particular disease, introduction to augmented reality and virtual reality in dental education. AI has a number of applications in the field of medicine and dentistry, Introduction of robotics in the field of surgery has increased precision and the surgical procedure is predictable. Improvement in the AI, digitization of the new era in the field of dentistry, the future aspects are extremely promising. Augmented reality and virtual reality are used widely in the field of dental education for creating situations that could stimulate clinical work on patients and eliminates all the risks.

Previous researches say that As virtual assistance they perform a number of simple tasks in a dental clinic with a greater precision and less manpower is said by Dr. Sunali S. Khanna [2] , in the year 2017. Some tasks include booking in coordination appointments, alerting

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the patient and dentist about check ups, assisting in clinical diagnosis and treatment. AI is now available in orthodontic diagnosis, treatment planning and treatment monitoring, says Sonali Vijay Deshmukh [3] in the year 2018. In every field like endodontics and implantology, AI has a major role to play in making precise surgical guides. Thus, application of artificial intelligence based on dentistry is not a myth but turning into a reality. AI is used increasingly in the aid of diagnosis in the field of medicine, says PM Speight [4] , et al, in 1995. The value of neural networks in identification of individuals with high risk of oral cancer in clinical examination or health education.

While advances in AI like neural networking, image recognition, speech recognition have transformed the field of medicine and dentistry in many ways, yet a number of drawbacks, challenges to be overcome. The future of AI needs to solve the challenges created by AI. The aim of this study is to analyse the need for AI in the field of dentistry.

Materials and Method

Study Design:

This study setting includes an online survey involving undergraduates of age between 18 - 23 from different locations. Ethical approval and informed consent from the participants is required. The number of people involved are the study participant and analyser, sampling - the sample size of the previous study was 196, a study on patient's awareness towards the role of AI in dentistry, Akanksha Singh [5] , et al, 2019. Power calculation using G power software. The sampling method is stratified random sampling. The measure taken to minimize the sampling bias is stratification and matching independent variables in a selected sample. The internal validity is the usage of a pre tested questionnaire. The external validity is homogenization and replication of the experiment.

Survey Instrumental:

Data was collected. A questionnaire contains a set of 10 questions with a validity checking. Data collection software was used. Data manipulation/ clean up in excel spreadsheet. The list of output variables that are to be assessed are the effect of knowledge, age on the use of AI in dentistry. The method of representation of each output variable is pie chart.

Analytics:

The statistical test used here is the chi square test. The statistical software used is a SPSS [6 7 8 9 10] . The list of independent variables are the knowledge and age. The list of dependent variables is the AI. The type of analysis used is the correlation and association in chi square. The steps followed in software analysis.

Result And Discussion

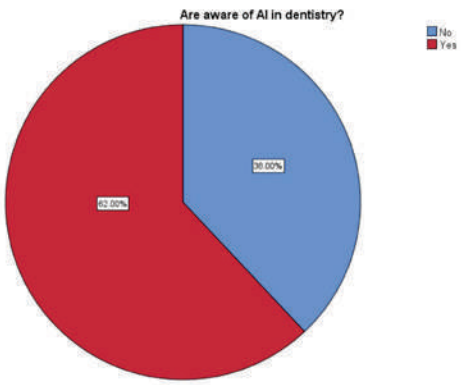


FIGURE 3

Figure 1: Pie chart showing the responses to questions about AI in dentistry. Majority of respondents have replied no (62%). Blue represents no and red represents yes.

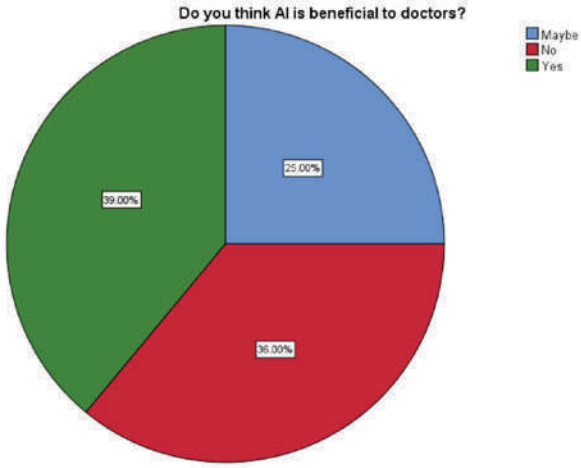


FIGURE 4

Figure 2: Pie chart showing the responses to questions about the benefits of AI to doctors. Majority of the respondents have replied yes (39%)Green represents yes, red represents no and blue represents maybe.

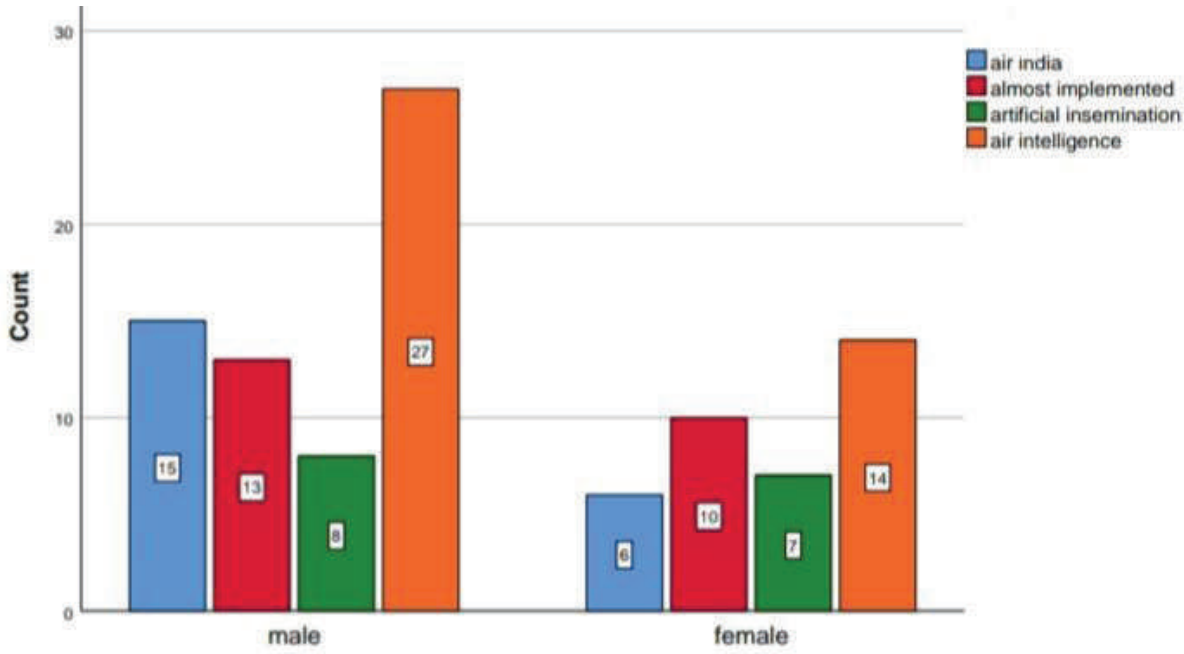


Figure 3: Bar graph showing the comparison of responses based on gender to the questions on abbreviation of AI, where blue represents air india, red represents almost implemented, green represents artificial insemination and orange represents artificial intelligence. Majority awareness responses(27%) were given by males. X-axis represents gender of the participants and Y-axis represents the frequency. There is no significant difference in responses between gender.Pearson Chi square test - 1.799, P value - 0.615(p>0.05- Statistically not significant).

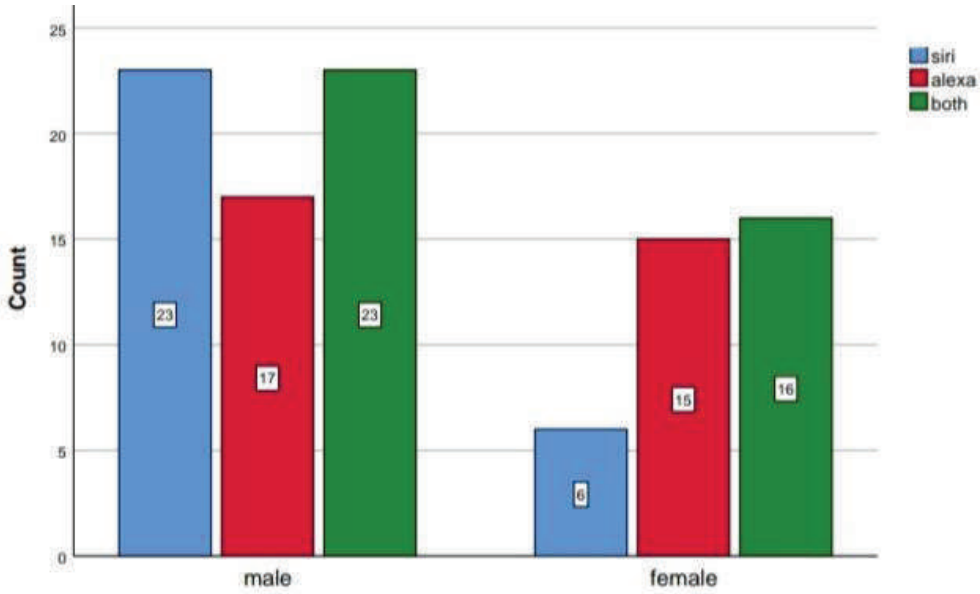


Figure 4: Bar graph showing the comparison of responses based on gender to the questions on the technology of AI like alexa, siri, etc, where blue represents siri, red represents alexa and green represents both alexa and siri. Majority of responses (23%) were given by males. X-axis represents gender of the participants and Y-axis represents the frequency. There are no significant differences in response between gender. Pearson Chi square test - 4.919, P value - 0.085. (p>0.05- Statistically not significant).

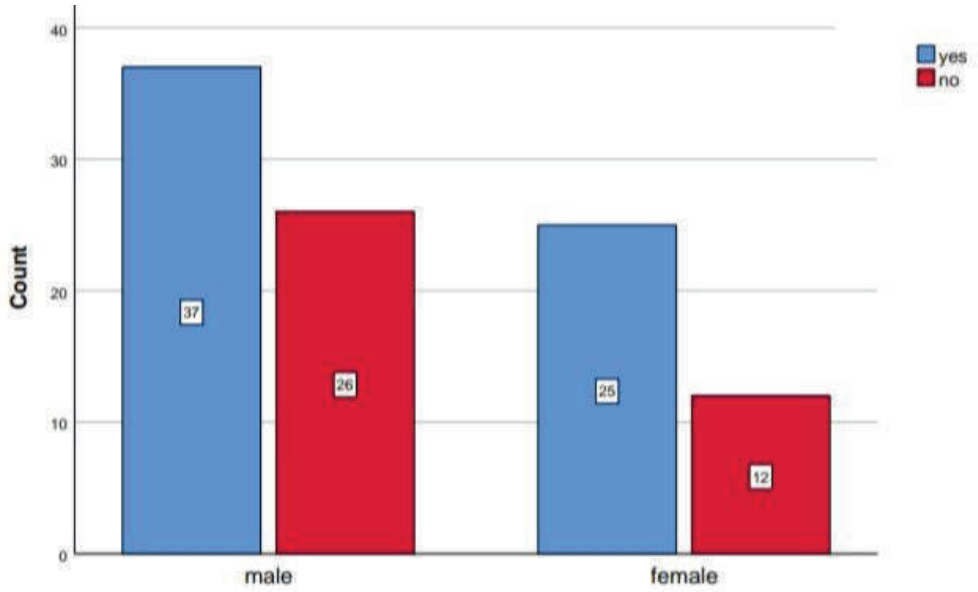


Figure 5: Bar graph showing the comparison of responses based on gender to the questions on the awareness of AI in dentistry where blue represents yes and red represents no. Majority of the responses (37%) were given by males. X-axis represents gender of the participants and Y-axis represents the frequency. There are no significant differences in response between gender. Pearson Chi square value - 0.773, P value - 0.379. (p>0.05- Statistically not significant).

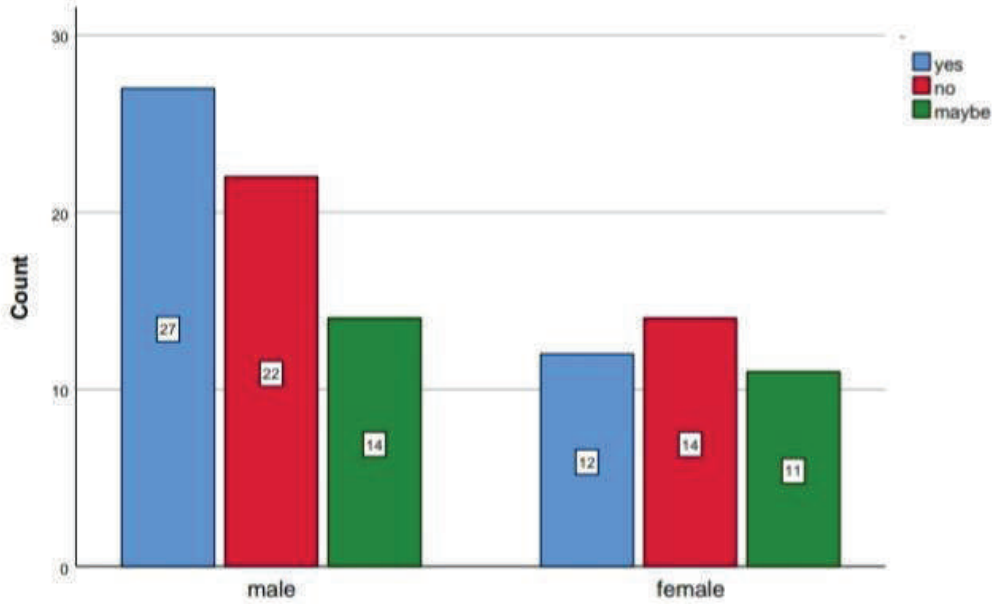


Figure 6: Bar graph showing the comparison of responses based on gender to the questions on the benefits of AI to the doctors, where blue represents yes, red represents no and green represents maybe. Majority of the responses (27%) were given by males. X-axis represents the gender of the participants and Y-axis represents the frequency. There is no significant difference in responses between gender. Pearson Chi square value - 1.230, P value - 0.541. (p>0.05- Statistically not significant).

The questionnaire is distributed and various responses have been collected for each and every question. The results were statistically studied and were analysed.

In figure 1, it shows about the awareness of AI in dentistry, Blue represents 62% student says that they are aware and Red represents 38% student says that they are not aware. In figure 2, it shows the beneficial of AI towards doctors, Blue represents 39% student says yes, Red represents 36% student says no and Orange represents 25% student says maybe. In figure 5, it shows about AI, as a confidentiality and data security, a major threat, blue represents 44% student says yes, Red represents 38% student says no and Orange represents 18% student says maybe. In figure 3, Chi square test was done to analyse association between gender and knowledge of full form of AI, in which 27% of males and 14% of females were aware(p value=0.615). It was found statistically not significant. In figure 4, Chi square test was done to analyse association between gender and the technology of AI, in which 23% of males and 16% of females were aware (p value = 0.085). It was found statistically not significant. In figure 5, Chi square test was done to analyse the association between gender and awareness of AI in dentistry, in which 37% of males and 25% of females were aware (p value= 0.379). It was found statistically not significant. In figure 6, Chi square test was done to analyse association between gender and knowledge of AI beneficial to doctors, in which 27% of male and 12% of females were aware(p value=0.541). It was found statistically not significant.

The present research interest was initiated from the team of investigators where previous studies were done based on clinical reports, interventional studies like [11 12 13], in vitro studies like, [6 7 8 10] and systematic reviews [11 14 15 16 16,17 18]. Compared to the previous studies. When said about the results of AI are accurate, Mcgrath SP [19], et al, in the year 2001 said that machines as a classification of dental images and visual search, 57% accuracy is observed when humans classify images. But 84% accuracy is observed when the machine classifies the image.

When said about AI that is helpful in diagnosis, Alexander L. Fogel [20], et al, in the year 2018, says that AI is described in prevention, detection, diagnosis and treatment of diseases, 57% says that it is helpful for AI diagnosis and is revolutionised in an article titled as Artificial Intelligence as power and digital science - A

review. Even Lin K [20 21] , et al, in the year 2020, said that AI improves outcomes, detection and diagnosis of oral cancer by 53%, was said in an article titled as Improving oral cancer and outcomes and imaging through AI - A review.

When said about AI as a threat, Andreas Holzinger [22] , et al, in the year 2019, says that AI enhance trust in medical professionals as future systems in our body and 49% feel that AI technology as a threat, in an articles titled as Causability and Explainability of AI in medicinal field - a review. Thus, in the above articles, most of the results from the previous literature are more or less similar to the results of this survey .

The limitations of this study is that the questionnaire may contain error captions. Due to the small scale of sample size, results may vary. The future scope of this study is that the present data has 100 responses. The sample size is small, among the general population. In future, more numbers of sample size can be used to study different types of population and for better results [23] .

Conclusion

AI has the potential that can be applied in various fields of science. There is a growing demand in the field of medicine, for AI to approach as transparent, trustworthy, etc for human experts in medicine. Thus, the participants of this study were aware about the usage of AI in the field of Dentistry.

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Conflict of Interest: The Author declares that there was no conflict of interest in the present study.

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Ethical Clearance: Not Required

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Knowledge and Awareness about Importance of Aesthetics in Dentistry

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Abstract

Esthetic dentistry can be defined as the art and science of dentistry applied to create or enhance beauty of an individual within functional and physiological limits. Esthetics is the branch of philosophy dealing with beauty. In dentistry, The theory and philosophy that deal with beauty[1] and the beautiful, especially with respect to the appearance of a dental restoration, as achieved through its form of colour. The aim of this study is to assess the dental esthetics awareness among dental students. Survey was conducted online. Questionnaire which consists of 10 questions was prepared and distributed through google forms. Study population consists of 100 dental students. This creates awareness about aesthetics and helps to understand the importance of aesthetics and also provide required technology. This studies shows a high level of self consciousness and the findings of the studies prove that even slight variations have greater impact on physiological functional and physical aspects.

Keywords: *Aesthetic dentistry, Beauty, appearance of teeth.*

Introduction

Esthetics is the branch of philosophy dealing with beauty. In dentistry, The theory and philosophy that deal with beauty[1] and the beautiful, especially with respect to the appearance of a dental restoration, as achieved through its form of colour. Esthetic dentistry can be defined as the art and science of dentistry applied to create or enhance beauty of an individual within functional and physiological limits. The naturals of the system is emphasized by showing by examples from nature and how artists and designers use it [2] when overall dental appearance is considered several factors of significance, including tooth colour, shape, and position and general arrangement of teeth [3]

Previous research states that advancement in dental care means that today's adults expect not only to keep most of their teeth for most of their lives, but that they should look good as well. [4]

And however even as dental professional we may not fully realize what a life changing event it is have an undesirable smile [5]

Compared to previous studies the level of aesthetic requirement in clinical practise has increased over the past decade, and this made it necessary for dentist to explore this field in order to satisfy the existing demand ton this field [6] and also aesthetic dentistry provides a new dimension of treatments that is rewarding and gives the dentist another means of artistic expression [7] and hence aesthetic dentistry is applied to create or enhance beauty of an individual within functional and physiological limit. The aim of this study is to assess the dental esthetics awareness among dental students.

Materials and Method

Study design, Area and study population:

A survey was conducted among dental students about knowledge and awareness about aesthetics in dentistry. The sample size of this survey is a total of 100 people. Participants who belong to dental precipitation in this study were voluntary and no incentives were provided to the participants. The survey was conducted in the month of April 2020.

Study Instruments:

A questionnaire was prepared after extensive review of the existing literature. The questionnaire was reviewed and amendments were made to improve clarity of pertinent questions and eliminate ambiguous responses. The survey instrument was a structured questionnaire with both open and close ended questions. It consists of a brief introduction regarding the purpose of the study, questions pertaining to demographic data and question regarding research objective 10 questions were circulated to the participants in a google.[8]

Data Analysis

Only completely filled online forms were included in the study. The full response was verified by two reviewers and the controlled data was entered on the same day. The entered data were analysed using SPSS. Descriptive analysis was performed to calculate frequencies of categorical variables.[9,10,11,12,13]

Results and Discussion

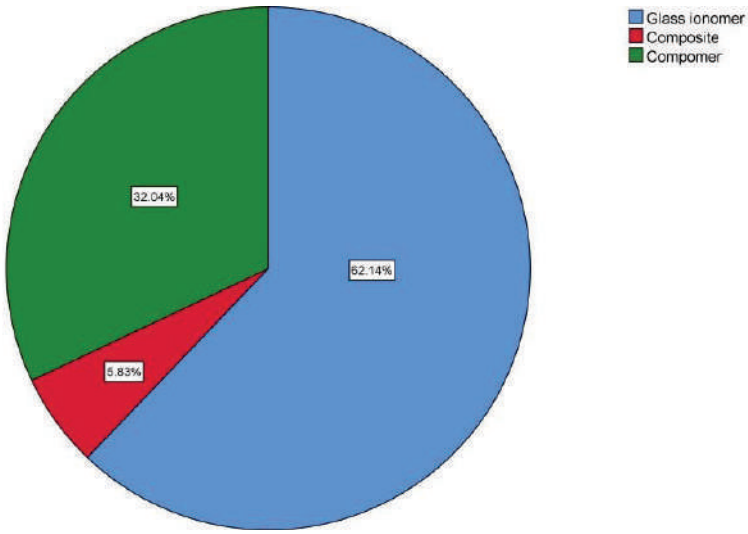


FIGURE 01: Pie chart depicts that knowledge and awareness among study participants regarding restoring class V cavity. 62.14% responded for glass ionomer (blue), 5.83% responded for composite (red) , 32.04% responded for compomer (green)

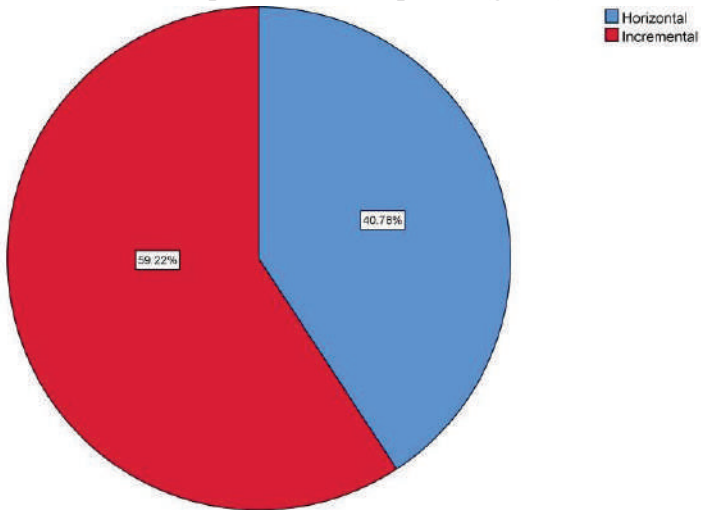


FIGURE 02: Pie chart depicts that Knowledge and awareness among study participants regarding technique used for placing composite resin restoration. 40.78% responded for horizontal technique(blue), 59.22% responded for incremental technique (red)

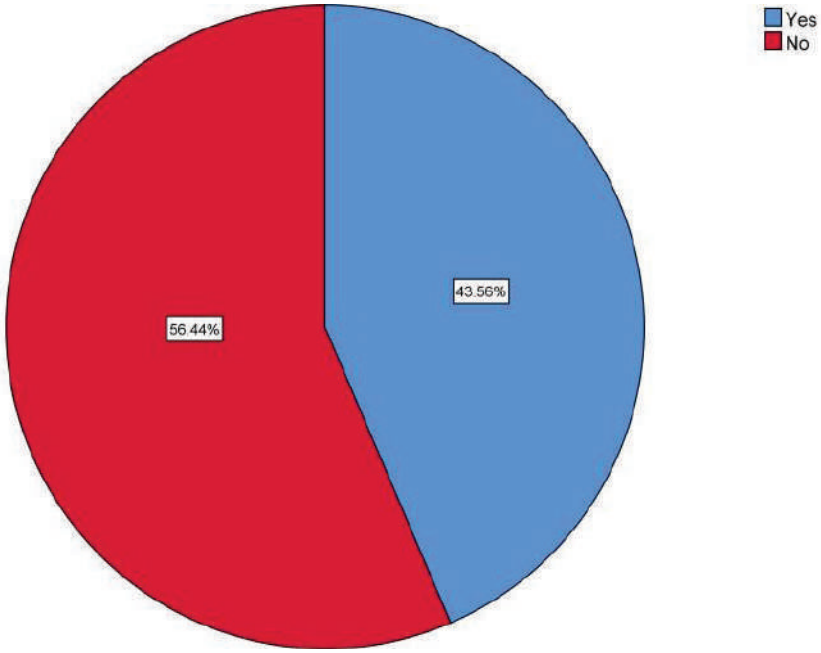


FIGURE 03:Pie chart depicts that Knowledge and awareness among study participants regarding importance of retainer. 43.56% responded for yes (blue), 58.44% responded for no (red)

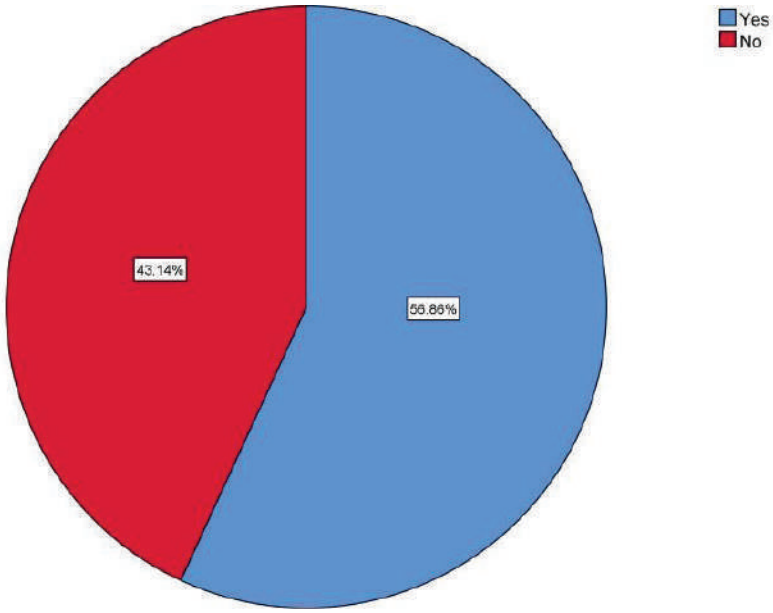


FIGURE 04: Pie chart depicts that Knowledge and awareness among study participants regarding importance of retainer. 56.86% responded for yes (blue), 43.14% responded for no (red)

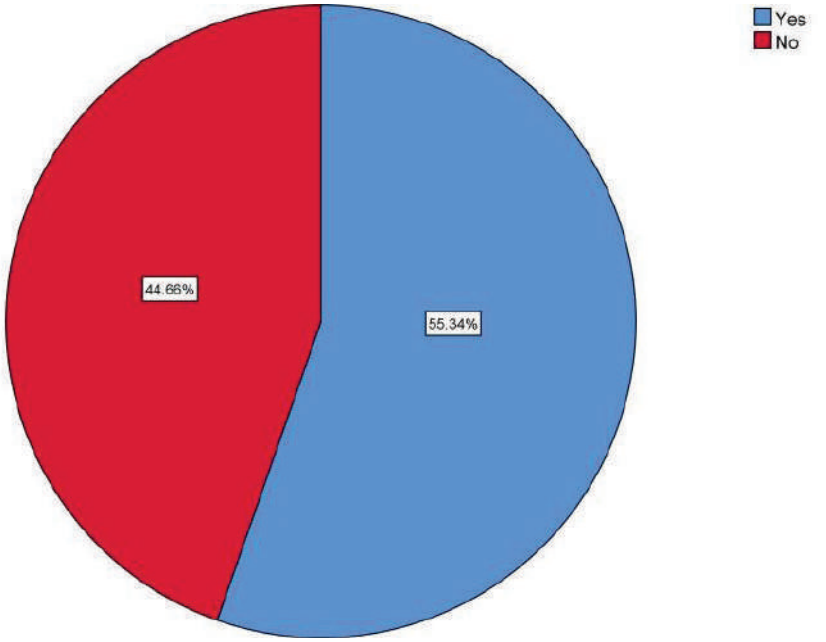


FIGURE 05: Pie chart depicts that knowledge and awareness among study participants regarding porcelain bonding veneer. 55.34% responded for yes (blue), 44.66% responded for no (red)

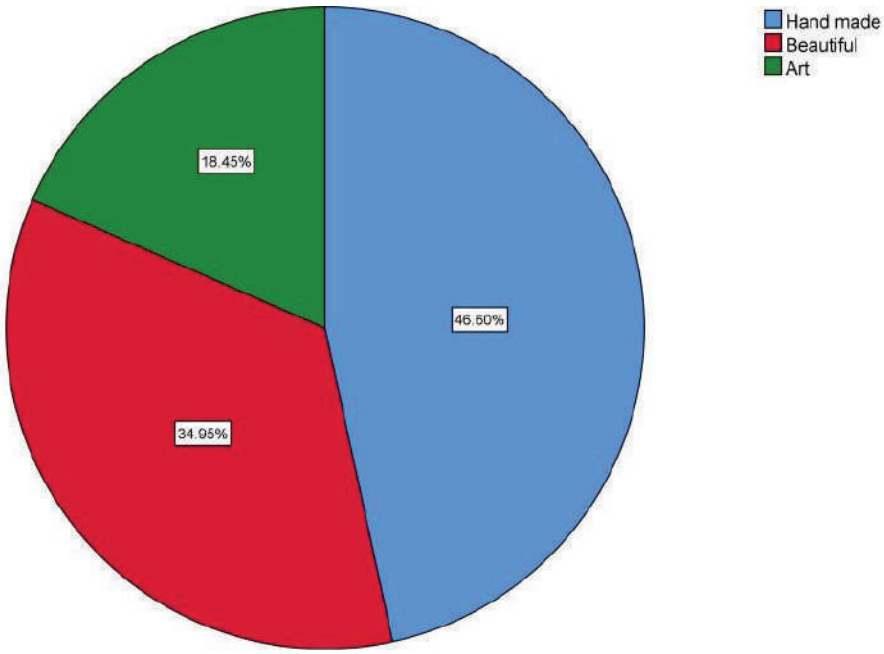


FIGURE 06: Pie chart depicts that awareness among study participants regarding aesthetics. 46.50% responded for hand made (blue), 34.56% responded for beautiful (red),18.45% responded for art

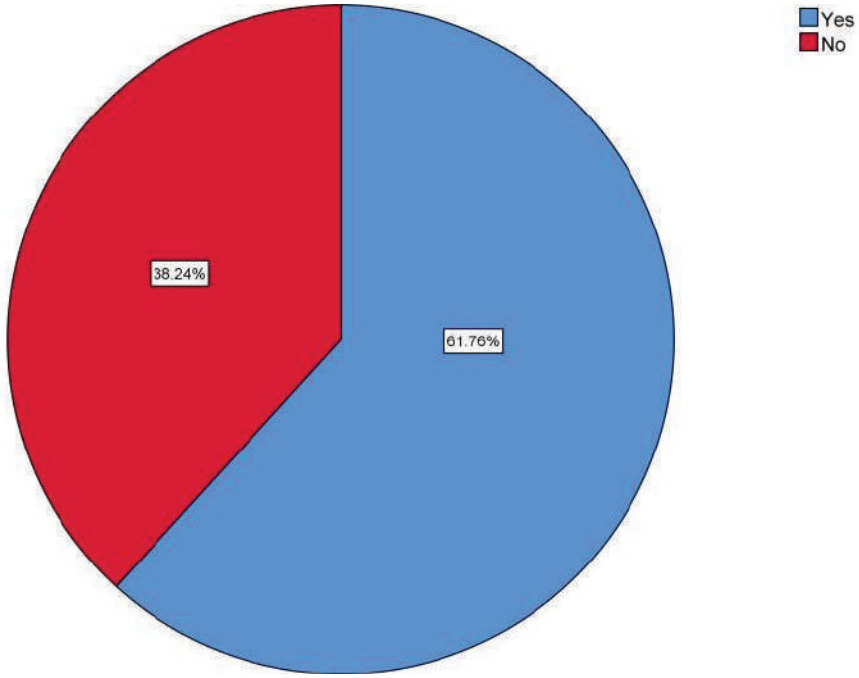


FIGURE 07: Pie chart depicts that awareness among study participants regarding snap on smile and evidence based practice. 61.75% responded for yes (blue), 38.24% responded for no (red)

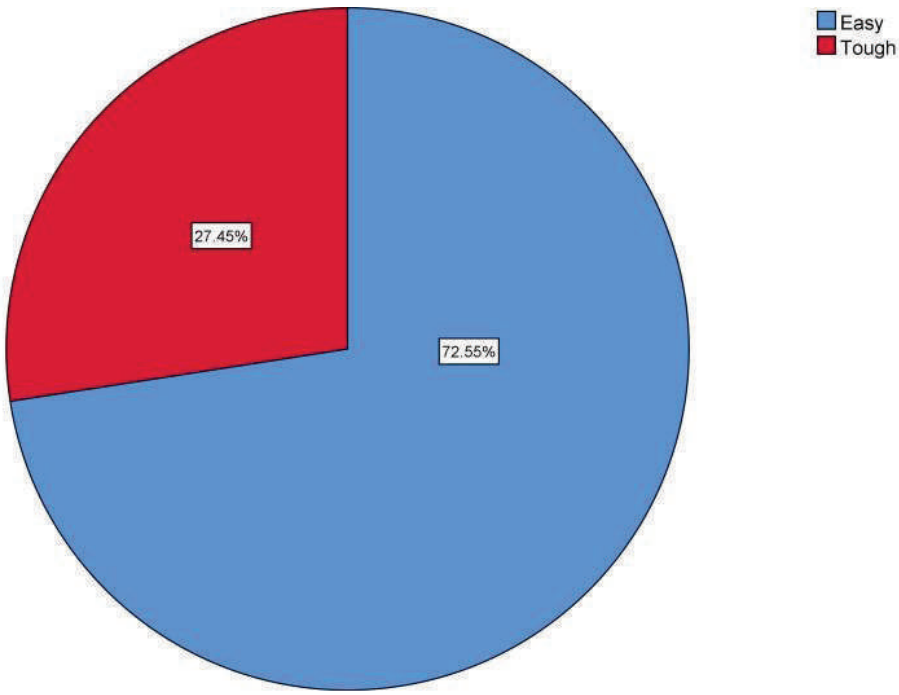


FIGURE 08: Pie chart depicts that awareness among study participants regarding importance of shade matching in prosthodontics. 72.55% responded for easy (blue), 27.45% responded for tough (red)

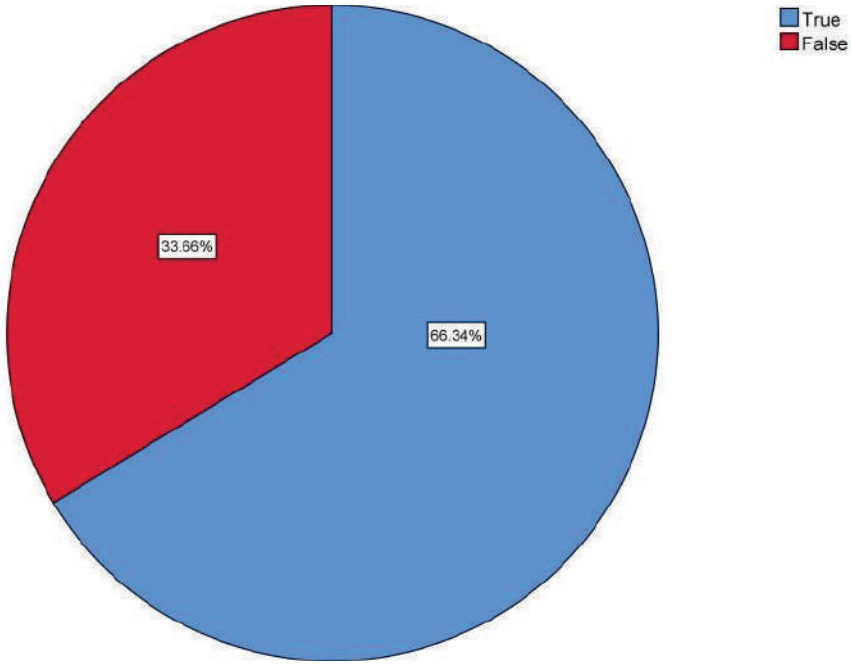


FIGURE 09: Pie chart depicts that awareness among study participants regarding importance of shade matching in prosthodontics. 72.55% responded for easy (blue), 27.45% responded for tough (red)

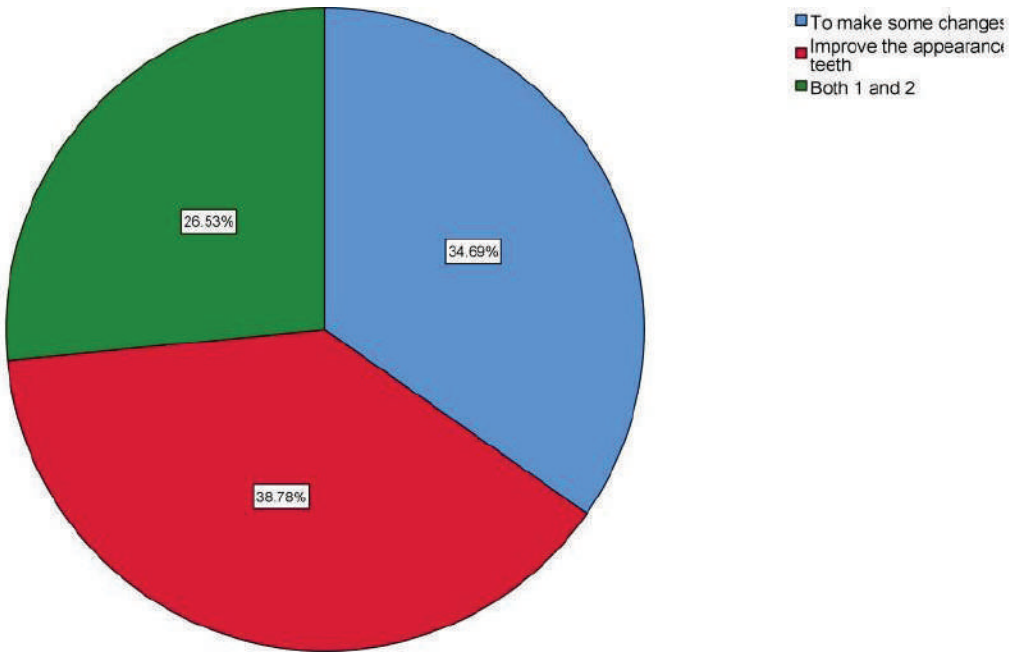


FIGURE 10: Pie chart depicts that awareness among study participants regarding importance of shade matching in prosthodontics. 34.60% responded for making some change (blue), 38.78% improve the appearance (red), 26.53% responded for both

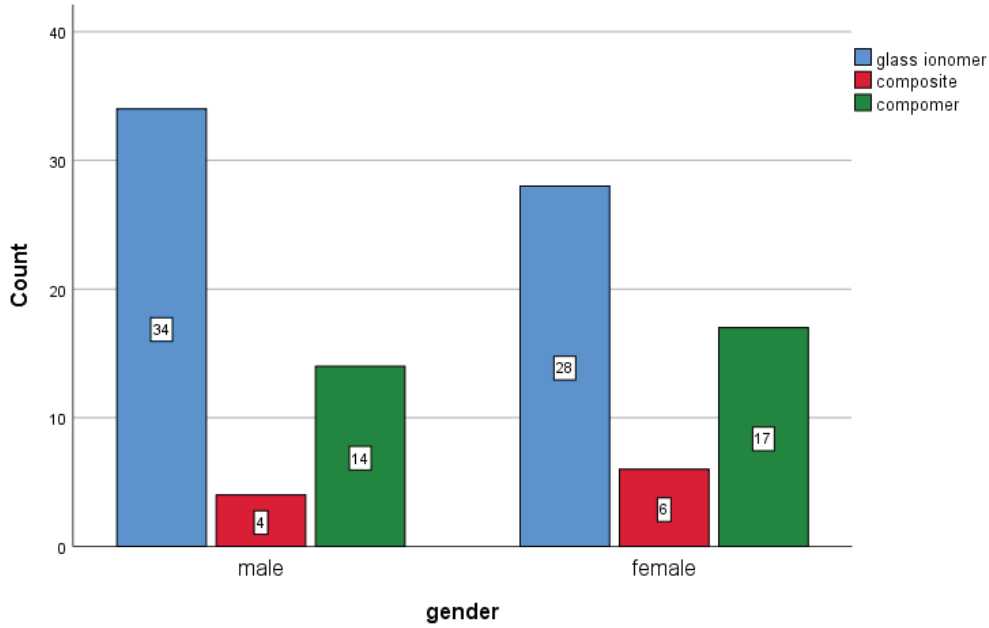


Figure 11: Bar chart represents the association between gender and awareness in restoring class V cavity. X axis represents the gender and Y axis represents the number of participants responding regarding awareness in restoring class V cavity by glass ionomer (blue), composite (red) and compomer (green). Males(34%) are more aware of restoring class V cavities by glass ionomer than females (28%). Chi Square test was done and the association was found to be statistically not significant. Pearson's Chi Square value:1.261, P value:0.532 (>0.05), Statistically not significant.

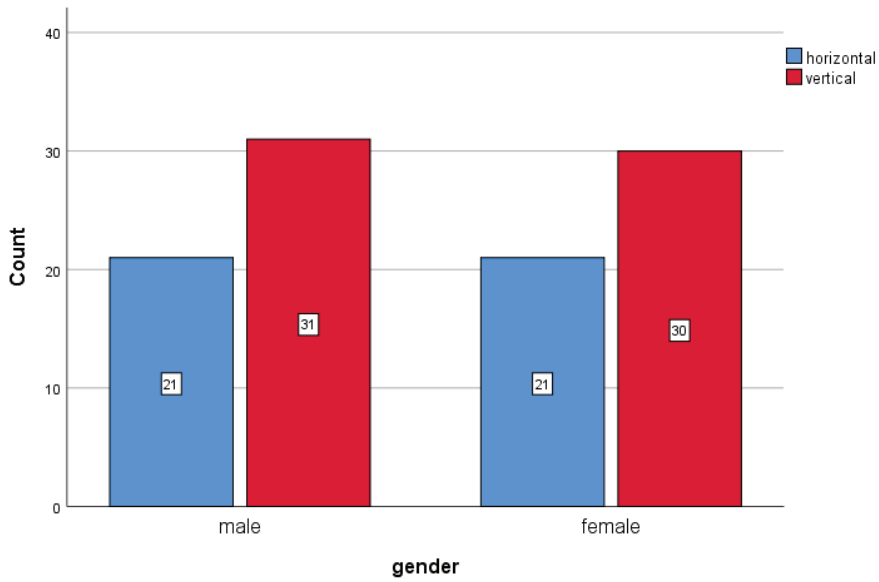


Figure 12: Bar chart represents the association between gender and technique used for placing composite resin restoration. X axis represents the gender and Y axis represents the number of participants regarding technique used for placing composite resin restoration by horizontal (blue) and incremental/vertical (red). Males (31%) are more aware of the technique used for placing composite restoration by vertical technique than females (30%). Chi Square test was done and the association was found to be statistically not significant. Pearson's Chi Square value:0.007 , P value:0.0935(>0.05), Statistically not significant.

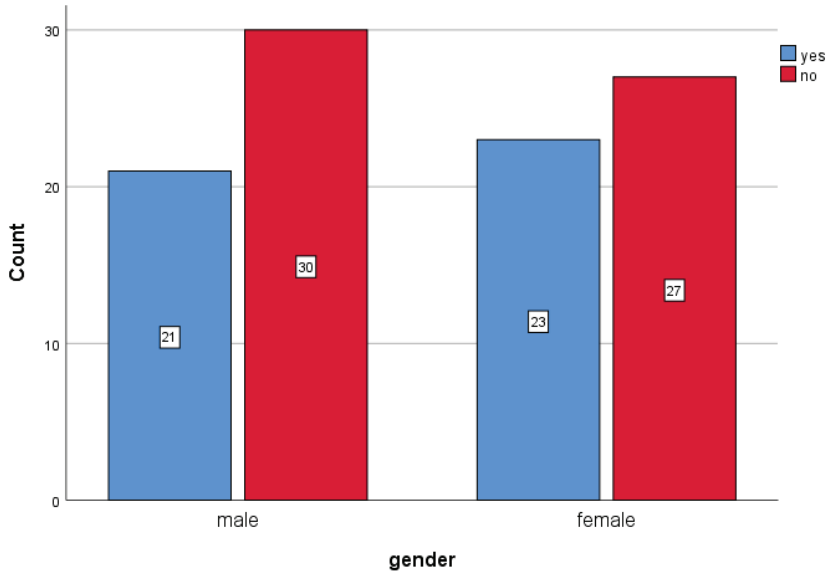


Figure 13: Bar chart represents the association between gender and Knowledge and awareness among study participants regarding the importance of retainers. X axis represents the gender and Y axis represents the number of participants responding regarding the importance of retainer. Females(23%) are more aware about the importance of retainers than males (21%). Chi Square test was done and the association was found to be statistically not significant. Pearson's Chi Square value:0.239, P value:0.625 (>0.05), Statistically not significant.

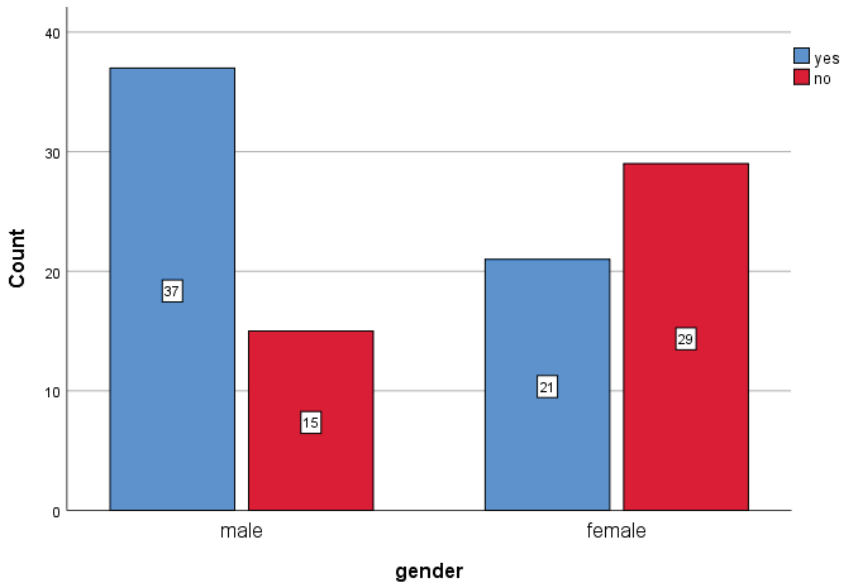


Figure 14: Bar chart represents the association between gender and Knowledge and awareness regarding study results showing the dimension change after composite build up . X axis represents the gender and Y axis represents the number of participants responding regarding study results showing the dimension change after composite build up. Males (37%) are more aware about study results showing the dimension change after composite build up than female (21%).Chi Square test was done and the association was found to be statistically not significant. Pearson's Chi Square value:8.833, P value:0.003 (<0.05), Statistically significant.

A total of 100 students participated in the survey of these participants 62.4% of them preferred to use glass ionomers for restoring class v cavity, 32.04% of them preferred to use composite and 5.83% of them preferred to use compomer (figure 1). 59.22% of them preferred to use horizontal techniques for placing composite resin restoration, 40.78% of them preferred to use incremental technique (figure 2). 42.72% will tell their patients to wear retainers, 55.34% of them will not tell their patients to wear retainers (figure 3). 56.31% of them answered yes for the study results showing the dimension change composite build up 42.72% answered no (figure 4). 55.34% of them said that porcelain veneer bonding is as strong as enamel bonding veneer, 44.66% of them answered no (figure 5). 34.95% of them said the meaning of aesthetics is beautiful, 46.60% of them answered handmade and 18.45% of them answered that it belongs to art (figure 6). 61.17% of them said that a snap on smile is an evidence based practice and 37.86% of them answered no (figure 7). 71.84% of the students answered that shade matching in prosthodontic is easy and 27.18% of the students answered that shade matching in prosthodontics is tough (figure 8). 65.05% of has accepted aesthetic dentistry is devoted to improving the appearance of teeth , 33.01% of them have not accepted (figure 9). 36.89% of them said that the importance of aesthetics is to improve the appearance of teeth,33.01% of them answered that the importance of aesthetics is to make some changes, 25.24% of them answered both (figure 10).

The present research has origins from the team of investigators where previous studies were done based on clinical reports, interventional studies like [14], [15],[16], in vitro studies like,[10],[11],[12,9] and systematic reviews [14] ,[17],[18] ,[19], [20],[21] .Chi square test was done to analyse association between gender and knowledge and awareness in restoring class v cavity in which 34% of the male and 28% of the female answered glass ionomer and 4% of the male and 6% of the female answered composite (P value:0.532). It was found statistically non significant (figure 11).Chi square test was done to analyse association between gender and technique used for placing composite resin restoration in which 21% of the male and 21% of the female answered horizontal technique and 31% of the male and 30% of the female answered incremental technique (P value:0.0935). It was found statistically not significant (figure 12). Chi square test was done to analyse association between gender and awareness regarding importance of retainer in which 21% of the male and 23% of the female answered yes (P value:0.625), It was found statistically not significant (figure 13). Chi square test was done to analyse association between gender and awareness regarding study results showing the dimension change after composite build up in which 37% of the male and 21% of the female answered yes(P value:0.003). It was found statistically significant (figure 14)

Compared to previous studies, 92.3% of them are satisfied with this smile [22] but in this study only 61.17% of them are satisfied with their smile. The result is different from the previous study and as the population involved is limited in this study[23] This survey shows, more that 85% of the females are satisfied with function of speech and 14.9% of the females are not satisfied with the function of speech whereas in this study 36.89 of them are satisfied in improving the appearance of teeth and 33.01% of them are satisfied in making some changes teeth.[24] This study conclude that 26.8% of the people are satisfied with shade matching whereas 70.5% of the people are not satisfied with shade matching but in this study 71.84% of them are satisfied with shade matching and 27.18% of them are not satisfied in shade matching. The results of both studies are almost the same.

Limitations of this study include the sample size, results may vary as it has less population, this study creates awareness about aesthetics and helps us to understand the importance of aesthetics and also provide required technology to improve the educational learning among students.

Conclusion

This study shows a high level of self consciousness and the findings of the studies prove that even slight variation have greater impact on psychological, functional and physical aspects

Acknowledgement: We thank Saveetha Dental College for providing us the support to conduct the study

Conflict of Interest: Nil

Source of Funding : Self

Ethical Clearance: Not Required

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Type of Research: Review *DOI Number: 10.37506/ijfmt.v14i4.12436*

Properties of Denture Liners - A Review

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Abstract

Denture liners are the materials applied to the tissue side of a denture coming in contact with the soft tissue. It cushions the contact of the denture with the tissues. The usage of the denture liners is to alleviate the trauma associated with complete dentures. The failure in adhesion, changes in rough surfaces and hardness are the favourable factors for microbial accumulation and compromise the liner's durability and causes the oral health conditions like denture stomatitis. The adhesion can be improved with the usage of different substances and surface treatments. The changes in the rough surface and hardness can compromise the material's lifespan. This review deals about the properties of adhesion, roughness and hardness of the denture liners.

Keywords: *Denture liners, denture liner adhesion mechanism, surface roughness, sealants application, hardness.*

Introduction

The denture liners have been widely used in the dentistry to shape prostheses surface in contact with the soft tissues in the oral cavity. The failure in adhesion, changes in rough surfaces and hardness are the favourable factors for microbial accumulation and compromise the liner's durability and causes the oral health conditions like denture stomatitis, implant loss, peri-implantitis and osseointegration delay as well as respiratory problems that can interfere with the rehabilitation treatment success and the quality of the life [1]. The silicone based soft dentures doesn't require an external plasticizer to be soft over a long period of time [2]. The silicone based soft dentures are similar to silicone impression materials. It can manage bruxism, chronic soreness etc., The silicone based soft dentures withstands extrusion forces, energy, viscosity, tensile strength and tear strength and room temperatures. The usage of the denture liners are used to alleviate the trauma associated with complete dentures [3]. The long term soft denture lining materials, despite their established clinical efficacy, the use of Long Term Soft Denture Lining materials (LTSDL) has been limited due to the unfavourable effects of the oral environment on some of their mechanical and performance characteristics [4]. The silicone based denture liners are the most commonly used denture liners due to its more stable hardness, absorption and adsorption and solubility than acrylic based denture liners [5]. The silicone based soft dentures are the relining materials that have no odour or no taste and offer a stronger adhesion and exceptional durability than the other denture liners [6].

Most importantly, all of the lining materials exhibit an aging solution which has specific tendency towards discoloration and the available cleansers are not fully effective and can alter the mechanical properties of the denture liners [7]. The denture liners are processed in laboratories (heat polymerized) and dentist offices (self-polymerized) because of their easy and quick application [8]. The term "soft denture liners" refers to a class of resilient materials used to reline denture base surface

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in contact with the occlusal stress bearing oral mucosa. The denture liners are noninvasive and relatively more economical if compared to make a new denture. Patients prefer resilient liners over the hard ones, because they improve comfort [9]. The denture liners can be either that are usually made of polymethacrylate or resilient, in which plasticizers are added to the resin and silicone elastomers. The resilient materials are also classified as short term and long term products [10]. The long term resilient denture liners maintain their resilience for more than 30 days upto 1 years whereas short term liners can be used upto 30 days. The resilient liners are intended to be elastic, absorb energy and act on the cushion effect [11].

There are some disadvantages like presence of surface defects and porosity, residual taste after use, tendency to pick up odours, water uptake, poor adhesion to acrylic resin, proneness to change of color, difficulty to clean and premature hardening due to plasticizers solubilization [12]. The successful relining depends on the bond strength between the liners and the resin base [13]. This lack of bonding leads to debonding, diminishing the procedures longevity and may occur due to an inefficient bond to the denture or low cohesive strength.

The sealants application, surface treatments and physical mechanical changes resulting from disinfection among others, improve adhesiveness, rough surface and maintain the liners initial hardness [14].

DENTURE LINER ADHESION MECHANISM

The aging alters the adhesive properties of the denture base polymers and liners, leading to flaws on the materials. The failure in adhesion causes the rough surfaces, change in hardness and microbial accumulation that compromise the liner's durability [15]. The important factors for the considerations relative to denture liners adhesion are surface roughness, hardness, antimicrobial agents etc., [16]. The bond between the prosthesis and the denture liner begins with the dissolution of resin by solvent, swelling of surface layers and the evaporation of the solvent. The liner monomers diffuse, penetrate the resin pores and form an interpenetrating polymeric network [17]. The larger the surface swelling, the deeper the porous layer and as a consequence, the better the adhesion between the liner and the denture base [18].

The bond strength between the liner and denture base was assessed through primer application, where the layer of the GC resin primer was applied on the polyamide surface, through an adhesive such as bonding agent that is a reline material partner [19]. According to Ohkubo et al, dentures used for an extended period of time are difficult to reline because microorganisms produce methyl mercaptan, which causes liner detachment even after the primer dissolution [20].

Silicone liners are mechanically superior and more durable than resin liners, however they lack Chemical adhesion and adhesive flaws can be associated with the bonding agent. The adhesive failures between liners and prosthesis are increased after 30 days of storage in water, suggesting that their bonding gradually weakens over time [21]. The air abrasion with silica and silanization had failed to improve bond strength of silicone resilient lining to the prosthesis and defects produced by the 30 μm particles were not sufficient for the liner material penetration [22]. Some organic solvents such as the usage of poly methacrylate and ethyl acetate improve silicone liners adhesion to polymethylmethacrylate because they lead to softening and porosities that enhance adhesive penetration. The adhesion can be enhanced by ethyl acetate as a bonding agent [19].

TREATMENTS TO IMPROVE DENTURE LINER'S ADHESION TO THE PROSTHESIS

The treatments to improve denture liner's adhesion to the prosthesis includes the treatment with acetic acid, tribochemical silica coating, polymethylmethacrylate (PMMA) with methyl formate - methyl acetate (MF-MA) and application of laser [23]. A study showed an increase in silicone based liners bond strength to urethane dimethacrylate base following laser application. The oxygen plasma treatment would improve the tensile bond strength between silicone based soft liner and thermocycler denture base [24]. The plasma is a partially or wholly ionized gas that contains highly reactive particles including electronically excited electrons, sonic and free radical species and photons in the short wave UV range. The plasma treatment is a gentle method to change the characteristics of the topmost layer of the polymer [25]. Mechanical surface preparation of denture base with lasers or alumina abrading had no significant effects on the bond strength compared to untreated

acrylic interfaces [26]. Considering experimental urethane acrylate oligomers-based photopolymerized soft liners, no significant difference in adhesion was observed after 1 day or 12 months of storage in water at 37℃ [27].

LINER'S ADHESION TO DIFFERENT TYPES OF PROSTHESIS

To improve the bonding between polyamide prosthesis and self polymerizable resin liner is by treatment with tribochemical silica and 4-methacryloxyethyl trimellitate anhydride in methylmethacrylate initiated by tri-n-butyl borane (4-META / MMA-TBB) resin [28]. Polyamides can be chemical resistant materials due to their high degree of crystallinity. A study showed that the flaws in the liners adhesion to UDMA prosthesis due to its highly reticular nature that hinders the monomer penetration [15].

The adhesion of hardliners to thermoplastic acrylic resin is similar to that of conventional thermal polymerized acrylic resin. But its results were different from polyamide, since they are chemically resistant [29]. The weak adhesion between the resilient resin base liners and prosthesis is explained by the absence of monomers associated with the non reticulated amorphous polymers. The glass fibers reinforced PMMA showed increased adhesion to the liners since the fibers were previously filled with non reticulated polymers containing PMMA in micrometric scale [19].

SURFACE ROUGHNESS

There are several methods to remove contaminants from the liners. But it is important to assess their effects on the surface since the cleaning solutions penetrate the resin which can change the morphology and the immersion time and concentration can alter the polymer structure [30]. The self polymerizable hard liner's roughness can be increased by the immersion of hard liners in sodium perborate and exposure of radiation with microwaves due to the immersion temperature and oxygen released by the perborate from the sodium perborate [31]. Bubbling from the oxygen release is a mechanical cleaning mechanism [32]. A study found that decrease in roughness is related with the brushing and disinfection with sodium perborate and chlorhexidine gluconate and related it to crosslinked agents that decrease the acrylic resin solubility in the organic solvents [30].

The results of one heat polymerized denture based acrylic resin and another auto polymerized reline resin with cleaning agent resulted in no changes in roughness which was associated with short immersion time [33]. There is an increase in roughness of hard liners because of the porosities that are formed due to the release of residual monomers and plasticizers and from the increase in the temperature during disinfection with microwaves [34]. There is an increase in the roughness when organic solvents such as MMA were applied on PMMA as an attempt to improve the adhesiveness to the silicone based liners because these solvents degrade the surface of the silicone based liners and alter its morphology.

SEALANTS APPLICATION FOR SURFACE ROUGHNESS

The surface sealant application reduces the roughness produced by brushing in silicone and resin based liners, with a more definite effect for siloxane-based material. But it showed no reduction in the roughness even after a sealant was applied to silicone based and methacrylate based liners [14]. There are several situations that increase a liner's roughness, which is a favouring factor for bacteria accumulation. The surface sealants protect the liners against water absorption and damage from substances like chemicals, saliva, food and brushing and coating defects and also reduces porosities and fissures [35]. But there is still no consensus on whether roughness is reduced or not when a surface sealant is applied to the liner.

HARDNESS

According to the specific ISO standards, liners can be classified into type A (soft) and type B (extra soft) from the measurement taken 24 hours after preparation of specimens [36]. A study says that resilient liners compromise changes in hardness over time [37]. Generally, hardness can be defined as the penetration resistance and it is increased in resin liners that are subjected to warm water bathe following polymerization and it is associated with reduction in residual monomers. The hardness of experimental photopolymerizable soft liners based on urethane acrylate oligomers was almost similar to silicone or acrylic resistant liners [27]. Cazacu et al, found that higher hardness values for a

thermostable silicone tested as liner, equivalent to that of addition silicone changes in hardness can also be caused by temperature fluctuation in the oral cavity and due to changes in pH [38]. Immersion in different solutions increased the liner's hardness. But Rezende-Pinto et al found that the reduction in the self-polymerizable hard liner's hardness regardless of the chemical solution or with water immersion [9].

The change in the hardness of the acrylic resilient liners occurred after 1 month of use by patients and smoking patients resulted in higher hardness value, which is probably due to the heat exposure [39]. The frequent use of cleaners kept the liners soft and also delayed their hardening process. the increase in acidity of the saliva was associated with an increased hardness, but this association cannot be generalized [1]. Thus the maintenance of materials hardness is critical for their longevity. It should be noted that the hardness has a direct relation with the viscoelastic properties which are responsible for distributing and tension generated during its clinical function [40]. Sealant application on resilient methacrylate can be effective in preserving hardness [41]. The higher the hardness value, lower the materials ability to absorb the impact of mastication. Decrease in hardness values may sometimes lead to superficial changes and retention of the oral pathogens.

GENERAL CONSIDERATIONS

The failure of adhesion between the prosthesis and liner will compromise the procedure durability and favours microbial colonization and stomatitis [42]. The adhesive failure is associated with the bonding agent. The use of solvents on silicone based liners improve adhesion on the PMMA base [43]. Surface treatments like with acetic acid or with tribochemical silica are required to adhere liners to polyamide denture bases [44]. It is important to preserve the hardness value of the liners so that the liners can maintain its elasticity for a long period of time. The selection of liner should be based on the procedure's objective, considering the serviceability and expected results from the considerations [45].

Conclusion

The diversity of methods presented the properties in a diverse manner, showing that subsequent studies are necessary to meet better utilization and indication of liners regarding hardness, roughness, and adhesion. Based on the present results, further in vivo investigations with randomized controlled trials are necessary to compare the performance and properties of these denture liners' modifications in clinical use. The adhesion to the prosthesis base is improved with treatments like those with acetic acid etc.,. The use of solvents seems to improve the adhesion of the reliners to the PMMA base. Most of the cleansing agents compromise the hardness and elastic modulus. Changes in roughness can lead to the formation of microbial colonization, increase the risk of oral and systemic infections and decrease the quality and life of the dentures. Among the various disinfection methods, minor changes in the hardness and roughness properties of the denture liners were observed when incorporating antimicrobial agents into the liners. Unlike acrylic based long term soft denture liners (ALTSDL) , the viscoelastic properties of silicone based long term soft denture liners materials (SLTSDL) are more appropriate and stable over time. Contrary to ALTSDL, the hardness of SLTSDL is stable over time. The sorption and solubility of SLTSDL is significantly lower than ALTSDL.

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Type of Study: *Retrospective Study*

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Comparison of Open Vs Closed Reduction of Mandibular Angle Fractures-A Retrospective Institutional based Study

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Abstract

Fractures of the mandible, particularly in the angle region have been found to have a high incidence. Clinical and radiographic examination along with the basis of displacement help to decide the treatment modality for these fractures. Open reduction is generally performed when there is significant deviation and closed reduction for simple fractures. The most common modality being Open Reduction with Internal Fixation(ORIF). The aim of the study is to compare Open Vs closed reduction in patients with fracture in the angle region of the mandible. This retrospective study involved a sample size of 18 patients with mandible fracture in the angle region. Case records were collected, tabulated and imported to SPSS for statistical analysis. Descriptive statistics and chi-square tests were adopted for association of various parameters. Angle fractures were most commonly seen in males(89%) than females. RTA and assault were the most common reason for trauma caused - 39% & 33% respectively. 89% of the treatment done was through open reduction with majority being treated by internal fixation(94%). Angle fracture management depends upon the severity of the displacement to decide the method of reduction and type of stabilisation. Open reduction with internal fixation was the most common treatment adopted in this study .

Keywords: *Open reduction, Closed reduction, Internal fixation, Angle fracture*

Introduction

There has been an increased trend of fractures of mandibles in the recent past. Among these, the most common site affected is the angle region of the mandible.[1] Mandible fractures occupy the second most frequent incidence in facial traumas with an incidence of 38%. [2]. Another study showed the incidence of 63.8% of the maxillofacial traumas affecting the mandible.[3]

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The etiology of fracture is very much related to the site of the mandible. The angle region of the mandible is a thinner cross sectional area bearing third molars and is considered to be the 'lever' area.[4,5] The reason behind these fractures is most frequently due to Road Traffic Accidents (RTA)[6], assault or interpersonal violence. Increased incidence of this happening in the urban region suggests that people do not tend to follow safety rules and regulations for their protection. This negligence and carefree attitude leads to accidents. The most prone are the youngsters who are often under the influence of alcoholism. [7]

Diagnosing the types of mandibular fractures in the correct site is very essential. Detailed history of the trauma occurrence, clinical examination,[8] radiographic examination guide for most effective treatment planning. The most useful for radiographic examination are the panoramic radiographs and tomography sometimes.[9]

The management of these fractures are often influenced by several factors. They are associated with occlusal disharmony, pain, edema, laceration of soft tissue, hematoma, deviation of jaw, pain on mastication and while opening and closing of the mouth. [10,11]

Fracture management classified on the basis of method of reduction is dealt as open or closed reduction. Open reduction can further be subclassified as rigid or non-rigid fixation. Angle fractures are generally treated by an open reduction method. Open reduction is performed when there is significant deviation of the jaw, communitive fracture and instability. Closed reduction is preferred when it's a simple fracture with not much deviation and less severe [12]. Displacement of the jaw can influence the dimension of the airway at all levels[13]. Champy et al, while performing several investigations suggested that the most effective management of angle fractures is that plate location should be along the superior border of the mandible [14,15,16]

The most common modality used is the internal fixation. Open reduction and rigid internal fixation bring back the normal function, show easier maintenance and functional/ anatomical reduction with immobilization of the fracture.[17] However there arises complications which range from 7.5%-29% and is due to the use of compression and two points of fixation. Infection and malocclusion have shown to have high in the list of complications.[18] These can be reduced only when there are certain protocols developed. Extensive blood loss is also a complication in various surgical procedures which can be reduced with the help of pharmacological agents.[19] Post operative pain is reduced by prescribing NSAIDs like Ketorolac or Paracetamol which have been proven to be very effective.[20] Post operative care is also of importance for effective wound healing. BTX injections have been proved to assist wound healing of the facial laceration and also obtain good immobilization.[21] It has become difficult to determine the actual rate of complications of angle fractures because there are very few studies to this focus. Quality and quantity of post surgical care has to be similar in all regions of the world. [22] Previously, our team had conducted numerous clinical trials[23], in vitro & lab animal studies and reviews[24,25] over the past 5 years and now we are focussing on epidemiological surveys. The idea for this survey stemmed from the current interest in our community.

The aim of the study was to compare open and closed reduction in patients treated for mandibular fractures in the angle region. Method of fixation was also noted in order to acquire knowledge on the most effective one.

Materials and Methodology

Study Setting

This is a retrospective study of patients with Angular Mandible Fractures. It revolves around a university setting study having patients visiting Saveetha Dental College and Hospitals. The approval for this study was given by the Institutional Ethical Committee (Ethical Approval Number - SDC/SIHEC/2020/DIASDATA/0619-0320). The sample size for this study was 18 patients having mandible fracture pertaining to angle region. Sampling bias was minimized by verifying the photographs.

Data Collection and Tabulation

Retrospective data of 86,000 patients between June 2019 and March 2020 were retrieved and the patient records were reviewed and analysed for overall patients reported with maxillofacial trauma . The datas pertinent to the present topic were extracted from the overall data collected and tabulated. Tabulation included information/ parameters like Name of the patient, Age, Gender, Etiology of the fracture, method of reduction- open/closed reduction, type of fixation- internal fixation/ IMF.

Statistical Analysis

After further verification of data by an external reviewer, it was imported to the SPSS software by IBM for statistical analysis. Percentages, mean, frequency of certain parameters were employed in the analysis. Chi-square test was used to detect the significance between open/closed reduction and type of fixation. p value <0.05 was considered to be statistically significant.

Results and Discussion

The following are the results obtained from the analysis – It was seen that there were 16 male and 2 females affected.(Table 2) The cause of the fracture was mainly due to Assault and RTA with 38.9% and 33.3 respectively. Other reasons include work injury and trauma(Table 1). The major finding of this study was to compare open/closed reduction and it is shown that

incidence of open reduction(88.9%) was extremely high when compared to closed reduction(11.1%)(Table 3). When focussing on the type of fixation, it was seen that 94.4% was by internal fixation and only 5.6% accounting for IMF (Table 4). Crosstabulation as shown in Table 5 to find the association between method of reduction and type of fixation using Chi-square tests revealed a significant result (p value < 0.05). This explains that open reduction with internal fixation was found to be high and very few were with IMF. The descriptive statistics and association is represented in the form of tabulations and bar graphs below-

Table 1- This table shows the number and percentage distribution of etiology of mandible fracture. The two most common reason for mandible angle fracture is Assault(38.9%), RTA (33.3%) and the least common is work injury(5.6%)

Etiology	Number of Patients	Percentage (%)
RTA	6	33.3
Assault	7	38.9
Trauma	4	22.2
Work Injury	1	5.6
TOTAL	18	100.0

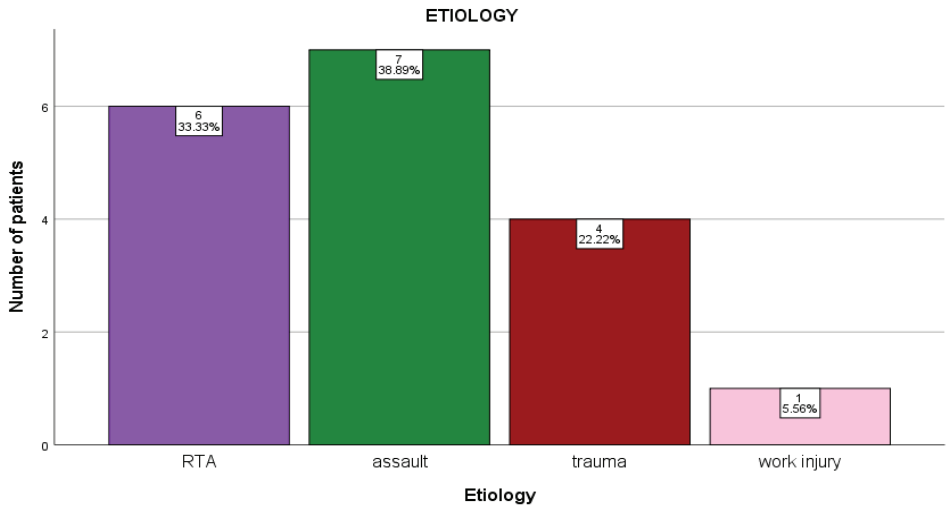


Figure 1- This graph shows distribution of various causes leading to mandible fracture in the angle region where X axis denotes Etiology and Y-axis denotes the number of patients. Violet colour denotes RTA, green denotes assault, brown denotes trauma and pink denotes work injury.. It shows that the two most common reasons for mandible fracture in the angle region are assault(38.9%)and RTA(33.3%).

Table 2- This table shows the number and percentage distribution of gender variation of patients with mandible fracture. It shows that males are more affected(88.9%) than females(11.1%).

	Number of Patients	Percentage (%)
Male	16	88.9
Female	2	11.1
TOTAL	18	100.0

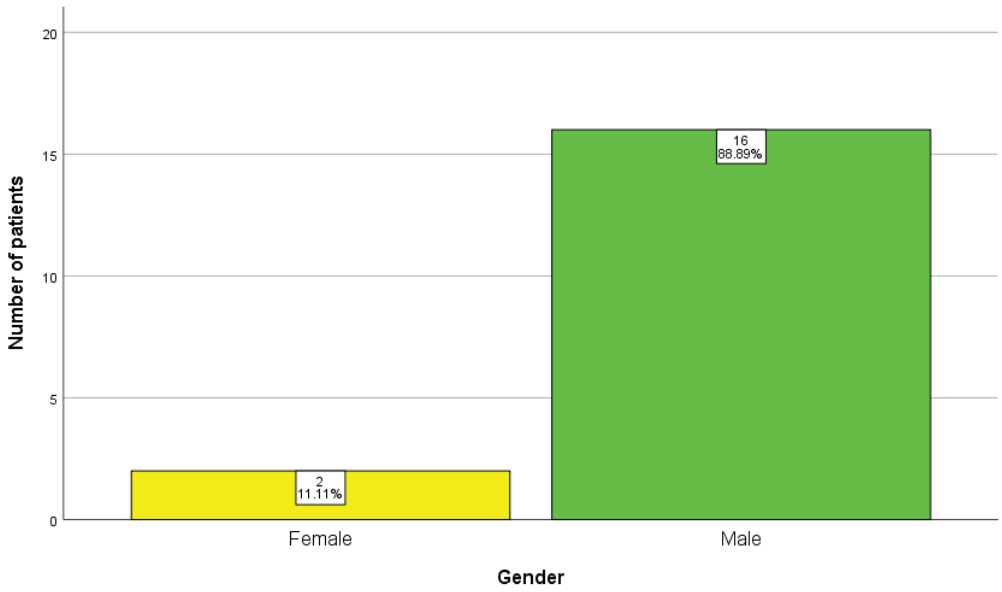


Figure 2 - This graph shows the number distribution of gender variation of patients with mandible fracture in the angle region where the X axis denotes gender and Y-axis denotes the number of patients. Yellow colour denotes female patients, green denotes male patients. It shows that males are more affected(88.9%) than females(11.1%).

Table 3 - This table shows the number and percentage distribution of the method of reduction adopted for treating the fracture. It shows that open reduction is more commonly adopted(88.9%) than closed reduction (11.1%).

	Number of Patients	**Percentage (%)**
Closed Reduction	2	11.1
Open Reduction	16	88.9
TOTAL	18	100.0

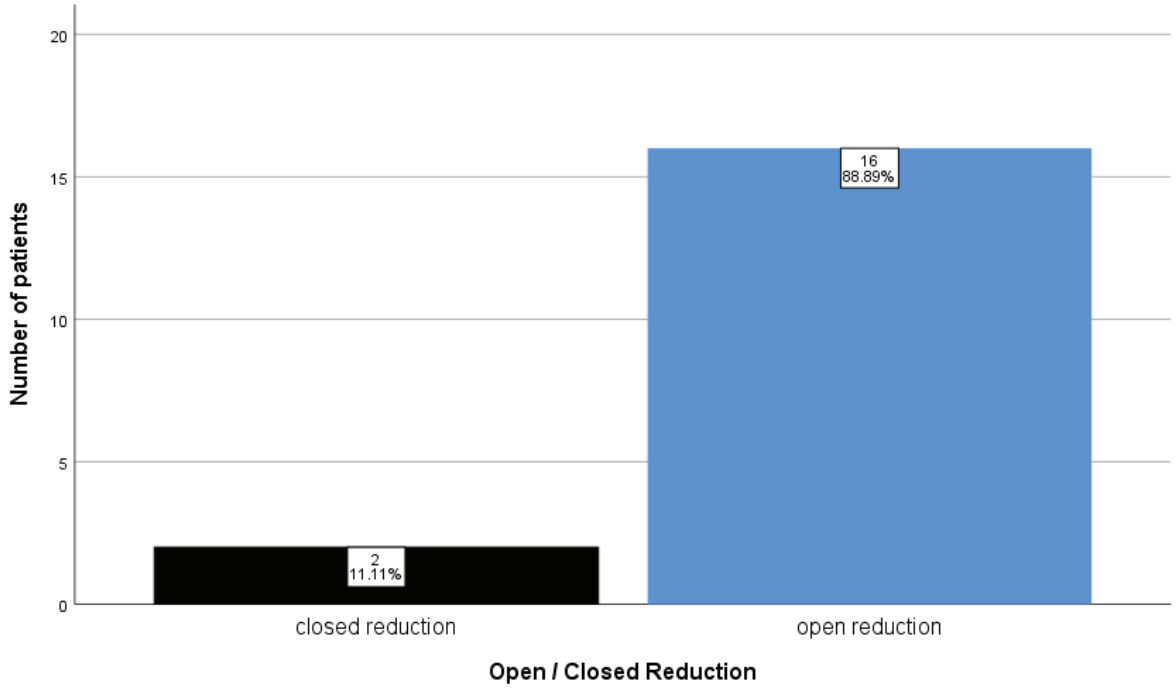


Figure 3 - This graph represents the number distribution of the method of reduction adopted for treating the fracture where X axis denotes type of reduction and Y-axis denotes the number of patients. Black colour denotes closed reduction and blue denotes open reduction. The graph shows that open reduction is more commonly adopted(88.9%) than closed reduction (11.1%).

Table 4- This table shows the number and percentage distribution of the type of fixation adopted for treating the fracture. The most commonly adopted type of fixation is internal type of fixation(94.4%) whereas IMF accounting for 5.6% only.

	Number of Patients	Percentage
Internal Fixation	17	94.4
IMF	1	5.6
TOTAL	18	100.0

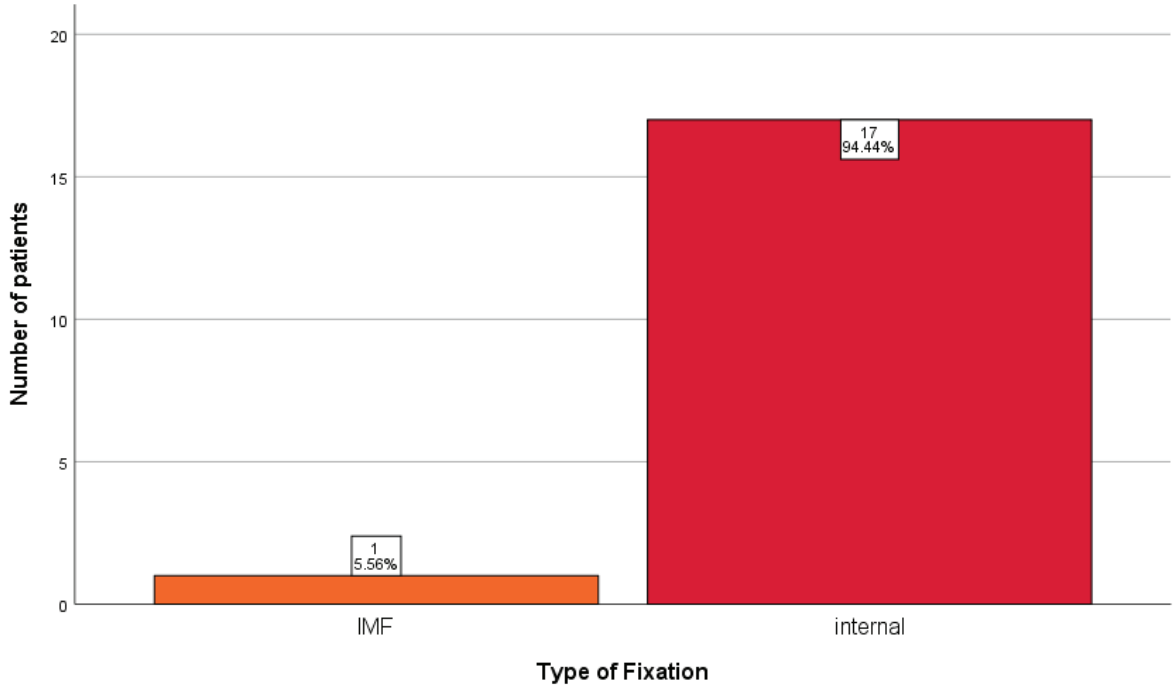


Figure 4- This graph represents the number distribution of the type of fixation adopted for treating the fracture where X axis denotes type of fixation and Y-axis denotes the number of patients. Orange colour denotes Intermaxillary Fixation (IMF) and red denotes Internal fixation. The graph shows that the most commonly adopted type of fixation is internal type of fixation(94.4%) whereas IMF accounting for 5.6%.

Table 5 - This table shows the association of method of reduction and type of fixation. Chi-square-statistically significant - p value - 0.004 (< 0.05).

	Value	df	Asymptotic Significance(2-sided)	Exact Significance (2-sided)
Pearson Chi-square	8.471	1	0.004	
Continuity Correction	1.621	1	0.203	
Likelihood Ratio	4.952	1	0.026	
Fisher's Exact test				0.111
N of Valid Cases	18			

The mandible,a single bone connecting to skull by temporomandibular joint is responsible for functions like mastication, phonation, swallowing and occlusal harmony[26] Since the mandible arch is open, located in the lower portion of face, it is more prone to road traffic accidents and interpersonal violence[27]. Studies have shown that assault and Road Traffic Accidents are considered to be the primary causes of mandible fractures. High incidence of Road Traffic Accidents could be attributed to poor road conditions, speed limit violation, drunk and drive and no proper safety measures being taken while driving[3].

Our study shows that the main etiology behind fractures was primarily due to assault(38.9%) and RTA (33.3%) as shown in Figure 1. Few others were due to trauma, interpersonal violence or a work injury. The proportion of males being affected was extremely higher than females. As seen in Figure 2, males are about 8 times more affected than females which is in agreement with a study where there was male predominance as well [28,29]. Male predominance is due to the fact that they are more easily prone to road traffic accidents, interpersonal violence often influenced with alcoholism [3,30].

The various sites of mandibular fractures include angle, condyle, symphysis region etc. wherein angle region being the most common region. Complete clinical history is very essential for the diagnosis of the fracture. Radiological evidence like orthopantogram and CT scans favour in diagnosing and give us a clear view of the extent of fracture[31].

Incidence of open or closed reduction as a treatment modality has been determined in this study. Figure 3 shows that the percentage of open reduction of mandibular angle fracture is 89%. Other literature studies also are in support of increased open reduction as the adopted treatment modality[9]. They have different approaches to treat and are often associated with complications such as sepsis or infection commonly seen in angle fractures of the mandible. Rate of infection ranges from 5% - 32% with angle fractures and can sometimes be associated with grossly carious and periodontally involved teeth which have to be extracted[12].

The main aim of closed or open reduction treatment in mandibular angular fracture is to establish functional harmony [2]. Open reduction Internal Fixation(ORIF) in mandibular angle fracture shows greater prevailing treatment modality accounting 94% in our study. Association of method of fixation and reduction revealed that open reduction is generally adopted with internal fixation.[3]

Closed reduction or Inter Maxillary Fixation(IMF) is least adopted (Figure 5) among prevailing modality of fixation in mandibular angle fracture. The ORIF is more advantageous over IMF,as ORIF brings back the mandibles anatomy and functions effectively with early postoperative recovery,which is in concordance with other studies. [3,17] It necessitates for even more development in this modality otherwise lack of skills and knowledge leads to non adherence of these procedures[32].

The limitations of the study is that it includes a very small sample size, single centered and completely relies on inpatient records for the analysis. The results are compromised in a retrospective manner and providing treatment recommendations based on such a small sample size is challenging. Therefore, a well designed, extensive study should be done in the near future to implicate the result of those studies in clinical practise.

Conclusion

Within the limits of the present study, open reduction with internal fixation(ORIF) was found to be adopted as the common treatment modality in patients with mandible fractures in the angle region. The fracture showed more prevalence in males and the most common etiology was found to be assault and road traffic accidents(RTA).With this small sampled retrospective study it is difficult to implicate the result of this study into maxillofacial operating criterias. .Hence a large sized multicentered randomized control trial should be done to to generalise the outcome of this study

Conflict of Interest: The authors declare no conflict of interest.

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A Retrospective Analysis of the Most Frequently Root Canal Treated Tooth in Pediatric Patients Visiting a University Hospital In Chennai

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Abstract

Aim: The aim of this study was to determine the most common permanent tooth which requires RCT in patients below 18 years of age.

Materials and methods: A retrospective cross sectional study of 581 subjects to determine the most common permanent tooth which requires RCT in patients below 18 years of age was carried out. The investigation was done to relate the permanent tooth which requires an RCT to age and gender. Chi - square test was the statistical test used for correlation in this study.

Results: Among the 581 subjects, 238 were males (41%) and 343 were females (59%); Age 6-10 years (18.42%), Age 11-14 years (69.71%), Age 15-17 years (11.88%); Mandibular right first molar (25%); 11-14 years most common age group (p=0.063) that underwent RCT; Higher number girls underwent RCT (p=0.006).

Conclusion: Thus based on analysis it was found that permanent mandibular right first molar is the most common permanent tooth which requires RCT in patients below 18 years of age and there is a female predominance seen. Mandibular first molar was the most common permanent tooth that underwent RCT in the study due to its high susceptibility to caries.

Clinical significance: This study serves as a beneficial tool for the identification of common permanent teeth undergoing an RCT which in turn can lead to implementation of correct preventive strategies and awareness among dentists as well as the general population.

Keywords: *Young permanent tooth, Pulp necrosis, Root Canal Treatment, Children, Gender.*

Introduction

Functional dentition is an important factor in the process of growth and development of the child patient, therefore preservation of the primary, permanent teeth in a functional state is a major task in achieving good oral health in children. [1,2]

Young permanent teeth are more often sensitive to dental caries which affect the pulpal health of teeth leading to pulp necrosis. [3,4] Several risk factors may affect the dental pulp: one of the most injurious agents of dental pulp is caries disease, caused by oral microorganisms. Of these factors dental caries, periodontal disease and dental trauma have been generally reported to be the major and the commonest causes of pulp necrosis and periapical periodontitis. [5–8]

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Hence the need for RCT arrives. Tooth preservation being the ultimate goal of modern dental care, RCT is an available, efficacious therapeutic strategy to retain the teeth. Root canal treatment is one of the most technically demanding procedures and a very specialised aspect in restorative dentistry requiring high level of technical skills. The main objective of the root canal treatment is to make the affected tooth/root of a tooth, a proper functional unit which is free from symptoms and pathogenesis and is biologically compatible. [9,10] On the other hand pulpectomy is a dental procedure in which all of the material in the pulp chamber and root canal of a tooth is removed. This procedure is recommended when the pulp has an infection that cannot be resolved. Previously our team had conducted numerous clinical trials focusing on reducing post operative pain, efficient instrumentation techniques for primary tooth. [11–18] Information on reasons for and pattern of a treatment are necessary for understanding the disease, performance of previous treatment, determination of cost effectiveness and devising future facilities based on patient need. [19,20]

It is also important to notice that the RCT treatment has invariably led to a decline in the tendency amongst dentists to extract diseased teeth, since this treatment aims at the prevention of progression of infection, preservation of normal periradicular tissues and the restoration of the treated to its proper form and function in the dental arch. [21] Teeth loss / absence due to periodontal disease, dental caries , trauma / some genetic disorders not only affect basic oral functions but also the aesthetic appearance and quality of life. [8] The other fact which is evident from various previous studies is that the dental awareness in the population is much less than desired. Patients usually present when they have pain or advanced stage of dental disease i.e. complicated dental diseases and periodontal diseases. The late presentation and symptomatic visits attitude has made endodontic treatment inevitable among us. Demanding treatment at late stages may relate to several psychological factors such as dental anxiety, financial costs, perceptions of need and lack of access. [5,22] Thus there is a need to educate children and their parents on the importance of early presentation of dental clinics and the possible sequel of late presentation in terms of morbidity, cost and time. [23] With this background in mind this study also creates awareness for the dentists and the general population.

Since there were no previous studies to encounter the commonest permanent teeth which require RCT in children in the South Indian population, this study was designed to fulfill this lacunae and leads to implementation of correct preventive strategies. Previously our team had also conducted numerous clinical trials which has led to the implementation of correct preventive strategies.[24–29] This research also aims for the achievement of the same.

Materials and Methods

A retrospective cross-sectional study in the hospital setting of University Hospital was conducted. All the case records of patients below 18 years of age who had undergone RCT in permanent teeth in the University Dental Hospital between June 2019 - March 2020 were included for this study and all the records of patients with any major systemic illness or special health care conditions were excluded from the study. After obtaining the ethical clearance from the institutional ethical committee board of University Dental Hospital (Ethical Approval number: SDC/SIHEC/2020/DIASDATA/0619-0320), the list of 581 the subjects who were below 18 years of age and had undergone an RCT treatment during the study period were retrieved by reviewing 7415 case sheets. The demographic and clinical data especially age, sex and teeth involved were retrieved from the Dental records. Cross verification of data was done by using the photographs available in the image gallery to avoid errors available in the case sheet. Cross verification was done by another examiner who reviewed the photographs, radiographs to reduce the data errors available in the case sheet. The main advantage of such data collection was that data was readily available and it included larger sample size and larger distribution of data. Data collected were entered into a spreadsheet and analysed subsequently using the Statistical software SPSS (Version 20). Frequency and percentage were calculated from the study variable. Descriptive statistics was used to summarise the variable in the data set. Chi square test was employed to test the association involving discrete data with the level of significance set at $p<0.05$.

Results

A total of 581 children aged between 6-17 years of age were treated at the University Dental hospital between study periods as mentioned above. Among

these two hundred thirty eight were males (41%) and three hundred and forty three were females (59%).

With regards to teeth undergone an RCT, Table 1 indicates that the permanent mandibular right first molar is the most common permanent tooth (25%) to get treated followed by the permanent mandibular left first molar (20.7%). It was found that 186 children had undergone RCT in anterior teeth 399 in the posterior teeth region.

It was noticed from the results that a greater proportion of children who belonged to the age group of 11-14 years had undergone root canal treatment (69.7%), followed by the children in the age group of 6-10 years (18,4%) and the age group of 15-17 years (11.9%) (Figure 1) but there is no statistically significant association between age and the individual teeth that underwent RCT in this study (p=0.063) ; however mandibular right first molar is the most common tooth that has undergone an RCT in almost all age groups (Figure 3).

Another finding of this study was that the girls (59%) underwent RCT more than the boys (41%) (Figure 2) and that there is a statistically significant association observed between the gender and teeth (p= 0.006); it can be found that the mandibular right first molar is the most common tooth that underwent an RCT in both boys and girls (Figure 4).

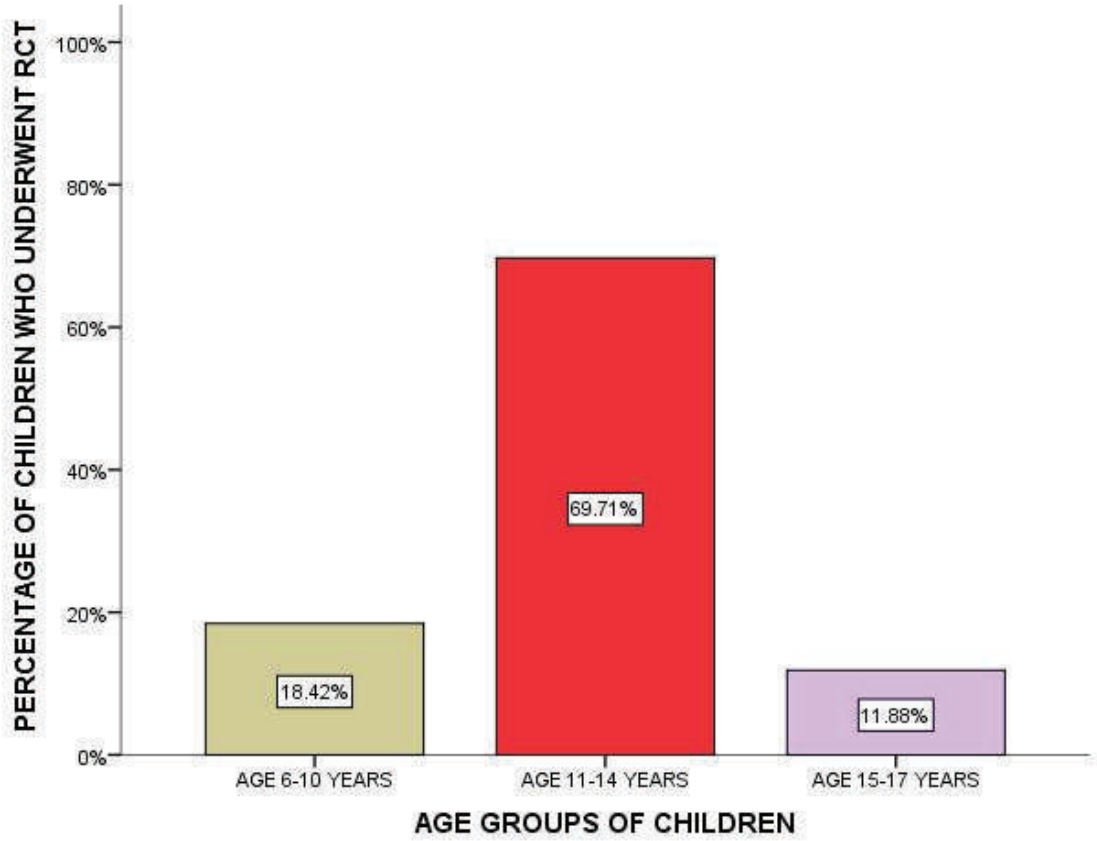


Figure 1 Image representing the frequency distribution of the different age groups that underwent RCT in the permanent teeth. X-axis shows the age groups while Y-axis shows the percentage of children who underwent RCT. Higher number of children in the age group of 11-14 years (69.71%) underwent RCT.

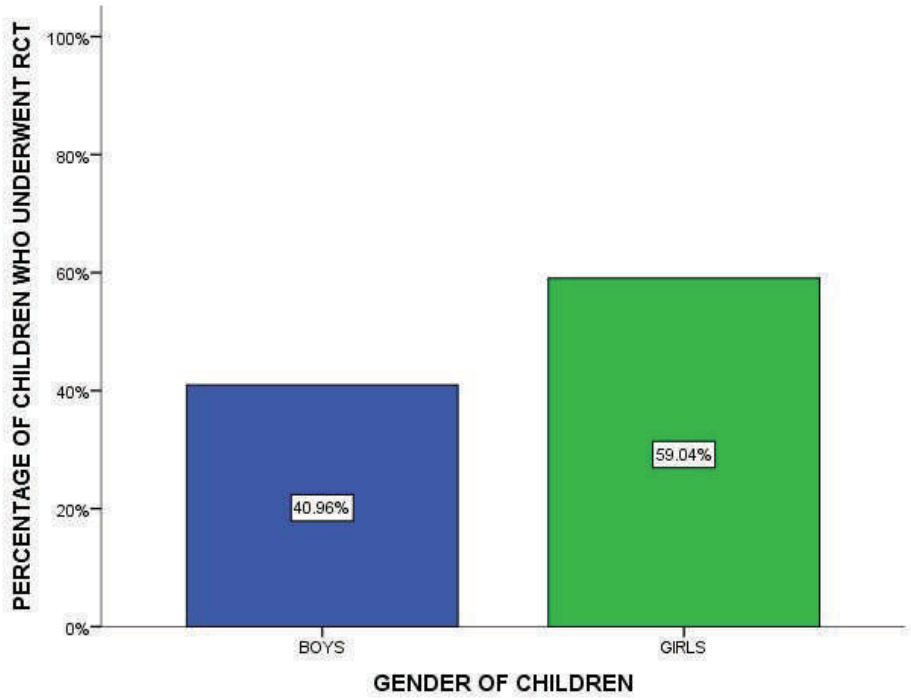


Figure 2 *Image representing the frequency distribution of girls and boys who underwent RCT treatment. X-axis shows the gender while Y-axis shows the percentage of children who underwent RCT. Higher number of girls (59.04%) underwent RCT than boys.*

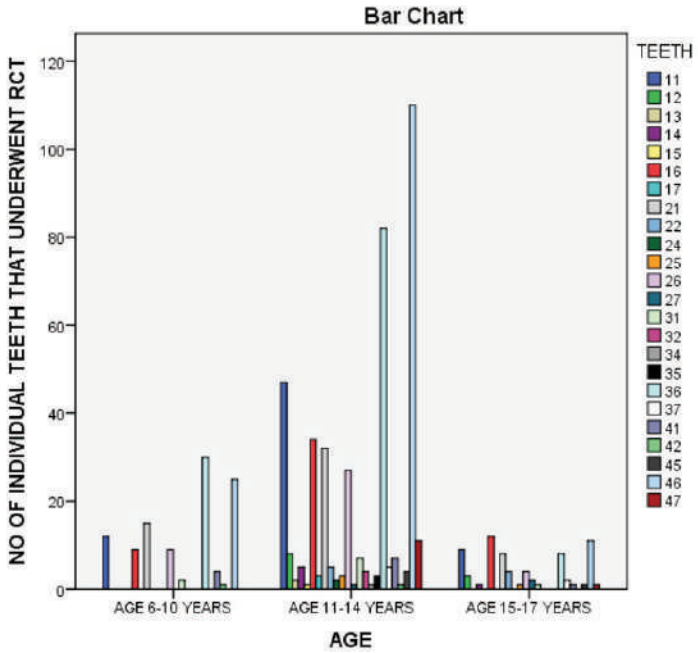


Figure 3 *Image representing the association between different age groups and the RCT treated teeth. X- axis represents the age groups with tooth number in the X cluster in the FDI system of notation and Y- axis represents the number of individual teeth that underwent an RCT treatment. Chi square test was done and the association was found out to be not statistically significant. Pearson Chi-square value: 61.483 , DF: 46 , p value : 0.063 (>0.05) hence not statistically significant, however 11-14 years is the most common age group in which a higher number of children underwent RCT treatment in permanent teeth.*

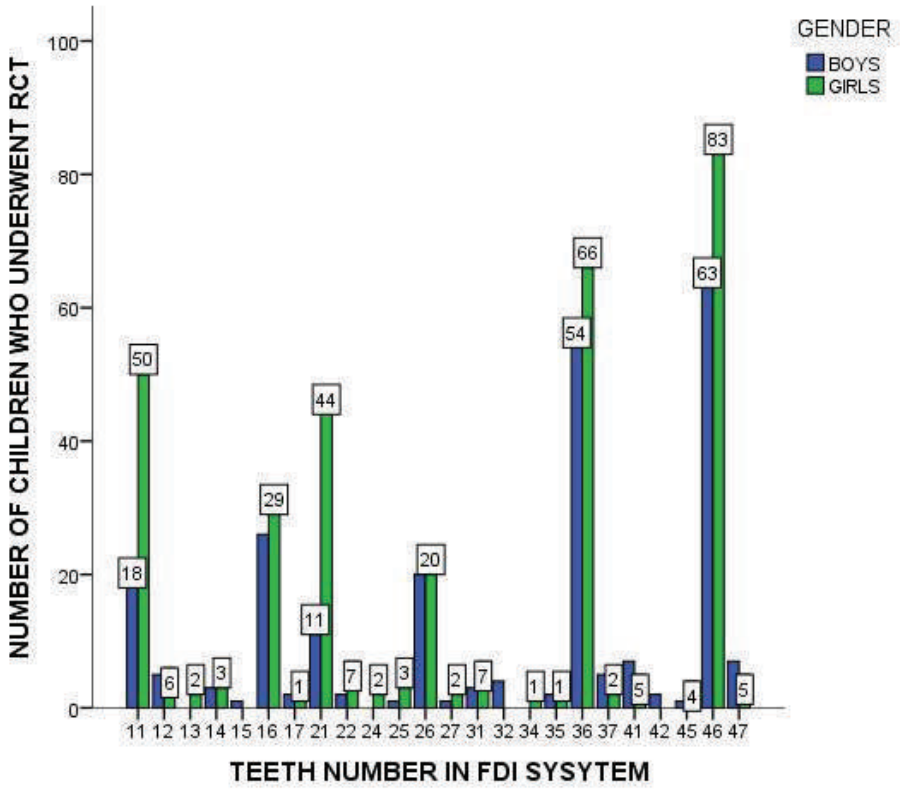


Figure 4 Image representing the association between gender and the RCT treated teeth. X- axis represents the individual tooth in the FDI system of notation and Y axis represents the number of children who underwent RCT. Blue colour represents boys and green colour represents girls. Chi square test was done and the association was found out to be statistically significant. Pearson chi-square value :43.632, DF:23, p value :0.006 (<0.05) hence statistically significant, proving that the need for root canal treatment was greater in girls compared to boys and the most frequently affected teeth were mandibular right first molar, followed by mandibular left first molar, maxillary right central incisor and maxillary left central incisor.

Table 1 Comparison of percentage distribution of different permanent teeth that underwent RCT. It can be inferred from this table that the permanent mandibular right first molar (46) is the most common tooth that underwent an RCT in children below 18 years of age (n=146, 25 %).

TEETH NO. IN FDI SYSTEM	FREQUENCY	PERCENTAGE
11/12/13	68/11/2	11.7/1.9/0.3
14/15	6/1	1/0.2
16/17	55/3	9.5/0.5
21/22	55/9	9.5/1.5
24/25	2/4	0.3/0.7
26/27	40/3	6.9/0.5

***Cont*... Table 1 Comparison of percentage distribution of different permanent teeth that underwent RCT. It can be inferred from this table that the permanent mandibular right first molar (46) is the most common tooth that underwent an RCT in children below 18 years of age (n=146, 25 %).**

31/32	10/4	1.7/0.7
34/35	1/3	0.2/0.5
36/37	120/7	20.7/1.2
41/42	12/2	2.1/0.3
45	5	0.9
46/47	146/12	25/2.1
TOTAL	581	100.0

Discussion

Data on the frequency and distribution of root canal treated teeth may reflect the attitude towards such treatment as well as the need and demand for it. In this study it was noted that the root canal treatment was needed mostly in the mandibular right first molar, followed by mandibular left first molars. This agrees with the findings of the study by Ridell et al. 2003. [30] The most probable reason for this finding was that the mandibular first molar is the first permanent tooth to erupt in the oral cavity hence it was more prone to caries. In addition the mandibular molars are more susceptible to food stagnation than the maxillary molars. Also it can be inferred that many parents may fail to recognise them as permanent teeth and thus neglect them through bad oral hygiene practices such as ineffective oral hygiene measures. Another reason for their susceptibility may be related to their period of eruption prolongation which makes oral hygiene measures ineffective. This also indicated the fact that preventive measurements for lower molars are extremely necessary. [5,31–36] However Augusto et al., reported an occurrence of 68% of maxillary posterior treated teeth and 32% of mandibular posterior treated teeth in a study conducted in Sweden. [37] This could be related to the reasons such as the findings may be attributed to a particular population. However the overall consensus agrees with the findings of our current study.

Since the prominent factors necessitating RCT are caries related , it is therefore not surprising to observe the pattern of prevalence of RCT with respect to the teeth involved tends to follow the reported order of susceptibility of teeth to caries : Mandibular 1st molars; maxillary 1st molars; maxillary central incisors; maxillary lateral incisors; maxillary first and second molars; maxillary second molars; mandibular 2nd molars; maxillary and mandibular canines with mandibular central and lateral incisors being the least susceptible teeth. Similar findings were seen in the study conducted by many researchers. [4,7,38]

About 59% of the sample population who underwent root canal treatment were females in the present study. The reason was that females had been reported to be more concerned about their oral health; hence they appeared to be better motivated to demand for oral health care and were found to attend dental services more often for various treatments than males who visit their dental professionals only when it is absolutely necessary. Another study by Ajayi et al., reported that the reason for female predominance to dental caries may be the facts of earlier tooth eruption in girls (and therefore increased time of exposure to cariogenic processes). Females may be more prone to dental caries due to excessive snacking of cariogenic food, hormonal and/or physiological differences. These may be the reasons why a larger proportion of the children who underwent root

canal treatment were females in the present study . This was in agreement with various previously conducted studies. [19,22,39,40] . Although studies by Ahmed MF et al in the year 2000 and Dadpe et al 2018 reported a higher requirement of root canal treatment in males [10,21] .The reason suggested by them was males were physically active hence, have an increased susceptibility to falls and other forms of injuries, however Dadpe et al reported an over representation of males in their study populations. Overall consensus from various other studies also reported a higher need for endodontic treatment in females compared to males.

Another finding of this study was that, the greatest percentage of patients who received root canal treatment belonged to the age group of 11-14 years (69.7%) and was in agreement with the previous study as caries prevalence increases as age increases.[19,41,42]

This study also found that the posterior teeth required a higher number of root canal treatments than the anterior teeth which is in agreement with the studies previously conducted. [30,37,39] The higher incidence of maxillary central incisors (20%) being the second most RCT treated tooth in our study, can be attributed to factors such as trauma which is commoner in children. The maxillary incisors due to their location are more susceptible to injury as compared to the rest of the teeth. In addition to this, the person's aesthetic appeal could be the most attractive factor for the higher incidence of root canal therapy in maxillary incisors in this study. Similar findings are seen in the study conducted by Al - Negrish et al.,2019 . Who proposed that the upper teeth appear more prominent than the lower teeth during smile making the patient more interested to preserve the upper teeth. [43]

The strength of this study was that the records of patients seen during the period of study were used; therefore there was no issue regarding declined participation from patients and in addition there was no issue regarding improper patient selection. The weakness of the study being a retrospective study, there was no possibility for direct interactions and examinations with the patients and the study relied only on the radiographic and clinical photographs also the sample size was limited and confined to only the South Indian population. However this study will shed light for future studies that study an even larger population. Continued advancements in the field of research can lead to the implementation of correct preventive strategies. More prospective studies can be conducted to clarify the reason for commonly undergoing RCT treatment.

Conclusion

Thus in this study it was determined that the permanent mandibular right first molar is the most common tooth which requires a root canal treatment in a patient below 18 years of age and there is statistically significant female predominance of about 59% (p=0.006) and the most common age group that underwent an RCT treatment was 11-14 years.

Clinical Significance

This study served as a beneficial tool for the identification of common permanent teeth undergoing an RCT. The data derived from the study is of high clinical value for evidence based practice and can be used for implementation of correct preventive strategies and imparting awareness among dentists as well as the general population.

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Conflict of Interest : Nil

Ethical Approval: Ethical clearance from the institutional ethical committee board of University Dental Hospital was obtained prior to the study (Ethical Approval number: SDC/SIHEC/2020/DIASDATA/0619-0320).

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Survey on Recent Advances in Root Canal Disinfection

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Abstract

Root canal treatment is a procedure done to remove the infected pulp of the tooth to prevent infection. The most important step in root canal treatment is cleaning and shaping which is done to disinfect the root canal. Persistent microorganisms after root canal treatment, can lead to failure of the endodontic treatment. Survey questionnaires were prepared, using an online survey portal it was distributed randomly to 100 participants via social media application. Responses were tabulated and graphs were plotted. From the survey results it was evident that knowledge regarding the recent advancements of root canal disinfection is notable. There was no significant association between the field of practice (specialists / general practitioners) and their response to the questions. Overall, the survey showed a good knowledge and awareness among the responders about the recent advancements in irrigants, used in the root canal treatment.

Keywords: *Root canal disinfection; Biofilm; Sodium Hypochlorite; Chlorhexidine; EDTA*

Introduction

Root canal harbors a variety of microorganisms, which possess a great challenge to dentists in achieving complete disinfection of the root canal system. Antimicrobial strategies for the removal of biofilm is of utmost importance [1,2]. The most frequent microorganisms in root canal treatment are gram negative anaerobic rods, gram positive aerobic and facultative rods, lactobacillus species, gram positive facultative streptococcus species. Obligate anaerobes are easily removed during root canal treatment, streptococcus, enterococcus and lactobacillus once established, are likely to be persistent if effective treatment and irrigation are not done properly thereby leading to failures[3]. Challenges in effective biofilm removal during root canal treatment arise due to anatomical complexities and dentin composition[4].

Conventional antimicrobials such as sodium hypochlorite (NaOCl) are most commonly used in biofilm removal but does not provide adequate disinfection when dealt with tooth having root anatomical complexities [5]. A proper endodontic therapy requires the use of a proper irrigant and intracanal medicament to ensure success in the treatment and prevent infection [6]. Sodium hypochlorite has been used as a potential root canal irrigant as early as the 1920s [3]. Antibacterial property, its tissue dissolving capacity and its toxicity are all dependent on the concentration used for its action. Use of 'full strength' 5.25% leads to adverse reactions and decrease in strength of dentin. Demineralisation agents such as EDTA were bought to use due to its high biocompatibility and great efficacy in smear layer removal [7,8]. EDTA was slightly better than NaOCl in removal of the smear layer and opening of the dentinal tubules [4,9]. Chlorhexidine, is another widely used irrigant due to its high antimicrobial activity, but it lacks tissue dissolving capacity [4].

The available irrigants have some limitations related to it and hence, newer irrigants were developed.

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Some recent advances in root canal disinfection include MTAD, tetraclean, electrochemically activated solution, ozonated water, photon-induced disinfection and herbal extracts. MTAD is a mixture of tetracycline, acetic acid and detergent for usage as final rinse before obturation[10]. Tetraclean similar to MTAD, it is a mixture of antibiotic(doxycycline), detergent and acid; but with varying concentrations[11]. Tetraclean causes high biofilm degradation when compared to MTAD [11]. Ozonated water is a powerful antimicrobial agent against viruses, bacteria, fungi, protozoa and hence useful in root canal irrigation [12,13]. The killing ability of ozonated water was comparable with 2.5% of NaOCl when irrigated with sonication [12,13]. Methylene Blue has been used in photon-induced disinfection targeting gram positive and gram negative oral bacteria [14,15]. Use of methylene blue and red light (665 nm) showed 97% reduced bacterial viability [16–18]. Herbal alternatives [29] show good antibacterial properties. Triphala, Licorice, cranberry extract, green tea, neem, tulsi, conifer, poplars, generic clusia flowers are some of the herbal extracts used for root canal disinfection[19] .

In photodynamic therapy use of photosensitizer followed by light illumination of sensitized tissue are used for killing microorganisms [20,21]. Photon induced photoacoustic streaming is based on radial firing tips with laser impulses of energies at 15Hz for average 0.3W for root canal disinfection. Gentle wave irrigation aims to clean the root canal through generation of different physicochemical mechanisms including a broad spectrum of sound waves[22].

Conventionally used irrigants such as chlorhexidine [23,24], sodium hypochlorite [25] requires proper shaping of the canal for better penetration of the irrigant [26,27]. Use of herbal alternatives as root canal irrigants, has been increasing over the years with usage of licorice, cranberry extract and passion fruit juice as they have good advantages in bactericidal activity [28,30]. Various surveys [31], in vitro studies [25,32,33] and molecular studies[34] have been conducted to analyse the endodontic therapy which can be employed to improve the outcome of treatment procedures [35] .

The purpose of this survey was to analyse the knowledge and awareness regarding the usage of newer root canal irrigants in clinical practice and their advantage over conventional irrigants.

MATERIALS AND METHODS

A cross-sectional study was conducted across various specialists and general practitioners in Chennai. Convenient sampling was done and questionnaires were randomly distributed to 100 participants, comprising 45 specialists(endodontists) and 55 general practitioners respectively. A self administered questionnaire was created using Google Forms in English and was distributed. It took about 5-10 minutes to complete the survey. The questions were directed towards analysing the awareness in recent advances in root canal disinfection. With the help of the survey portal, the results were obtained and graphs were plotted accordingly. The results obtained were then compared with the responses derived from specialists and general practitioners for a comparative analysis. The questionnaires mentioned in the survey mentioned in the table below.

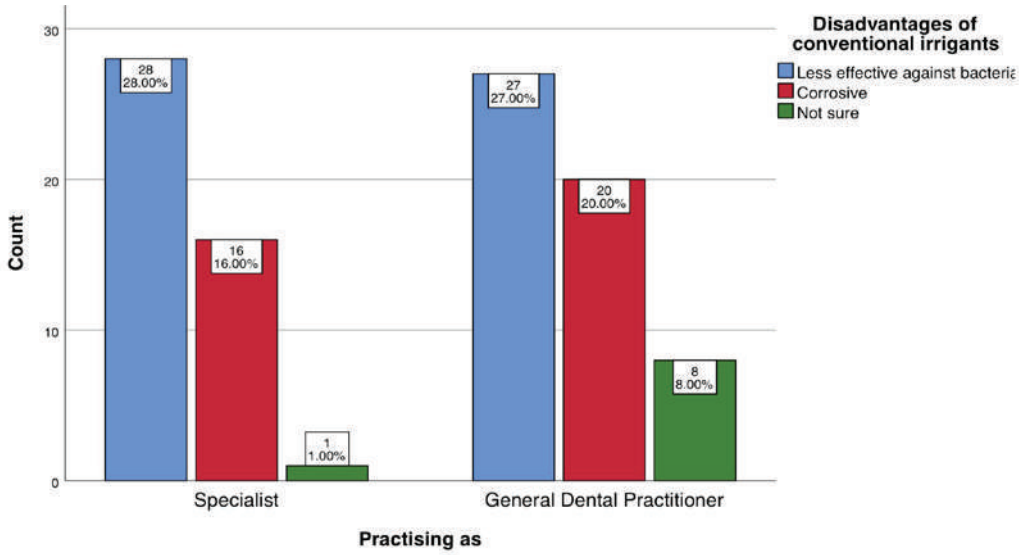
QUESTION	OPTIONS
What are some of the disadvantages of conventional irrigants?	• Less effective against bacteria • Corrosive • Not sure
What happens if bacteria is not completely removed from root canals?	• Failure of endodontic treatment • Re-infections • Not aware
Are you aware that the newly developed irrigants have higher potential for root canal disinfection and has advantages when compared with conventional irrigants?	• Yes • No

Cont... **Table 1: Questionnaires included in survey**

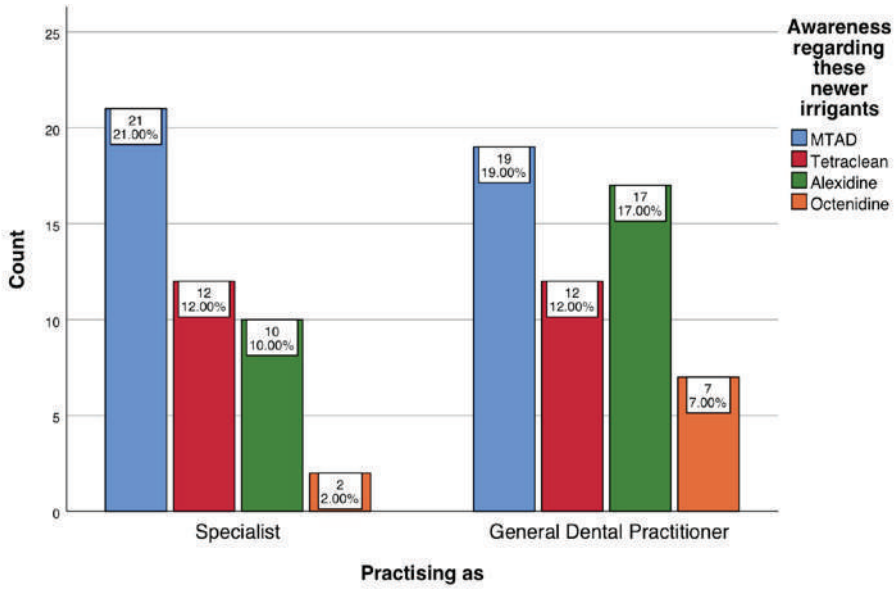
Are you aware of some of these root canal disinfectants mentioned below?	• MTAD • Tetraclean • Alexidine • Octenidine
How efficient do you think is the use of herbal extracts in root canal disinfection?	• Highly efficient • Not so efficient • Not aware/ not sure
Are you aware of the use of antibacterial nanoparticles for root canal disinfection?	• Yes • No
Are you aware that MTAD does not erode dentin whereas other conventional irrigants do?	• Yes • No
What do you think are some advantages of MTAD?	• Root Surface conditioning • Smear layer removal • Both of the above • None of the above
Do you know that Tetraclean can disaggregate biofilm better when used with NaOCl?	• Yes • No
Are you aware that ozonated water could neutralise LPS(Lipopolysaccharide) within the root canal?	• Yes • No
Do you know that antimicrobial photodynamic therapy can be used with conventional root canal treatment?	• Yes • No
What do you think are some advantages of photon-induced photoacoustic streaming?	• Doesn't require use of extensive instrumentation • Effective debridement, no thermal effect on dentinal walls • Effective removal of calcium hydroxide from root canal
Are you aware that lasers are highly effective in root canal disinfection?	• Yes • No
What do you think are some advantages of recent advances in disinfectants?	• Broad spectrum of antimicrobial effect • Effective removal of vital and non-vital tissue • Improve the fluid dynamics • Not sure

Results and Discussion

From the survey, the following results were observed and demonstrated below (graphs 1-6)

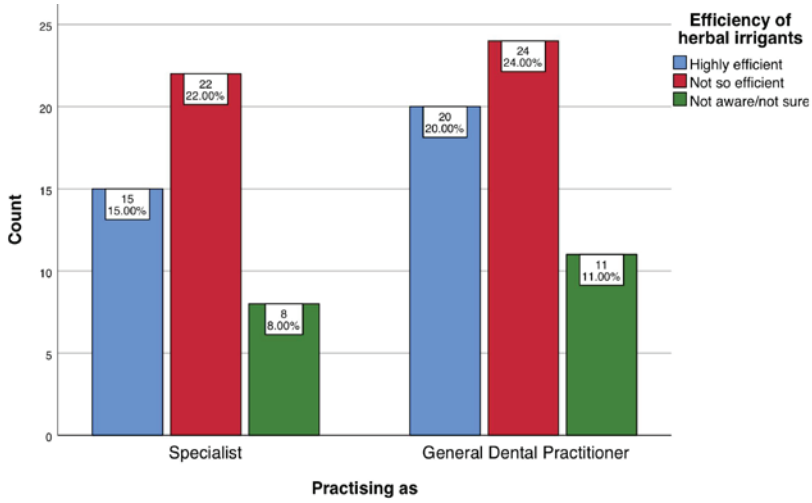


Graph 1: Bar graph represents the association between the field of practice (specialists / general practitioners) and their opinion regarding the disadvantage of conventional irrigants, X-axis represents the field of practice and Y-axis represents the number of participants. The blue bar represents that conventional irrigants were less effective against bacteria, red bar represents irrigants were corrosive in nature and the green bar represents indecisiveness. Majority of the respondents (28% of specialists and 27% general practitioners) opted conventional irrigants were less effective against bacteria. Chi square test was done and association was not significant (p value- 0.084; p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) of the dentists and their response to the question.

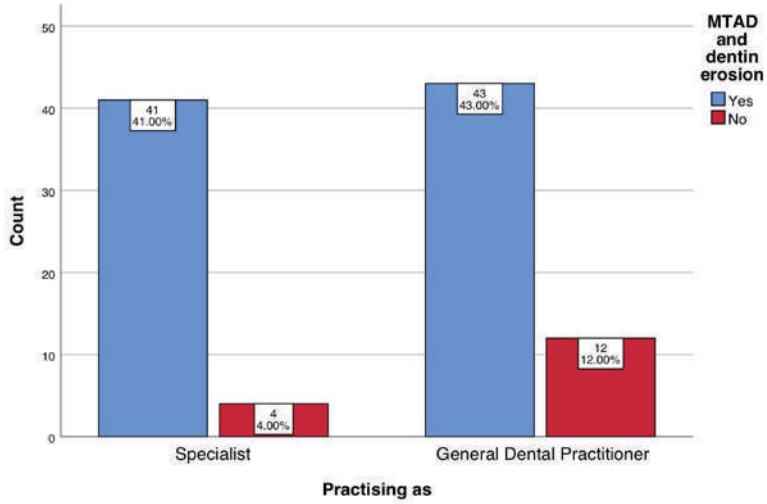


Graph 2: Bar graph represents the association between the field of practice (specialists / general practitioners) and their awareness of recently developed irrigants, X-axis represents the field of practice (specialists / general practitioners) and Y-axis represents the number of participants. The blue bar represents MTAD, the red bar represents Tetraclean, green bar represents Alexidine and the orange bar represents Octenidine. Majority of the respondents (21% of the specialists and 19% general practitioners) were aware of the MTAD which is a mixture of doxycycline, citric acid and a detergent. Chi square test was

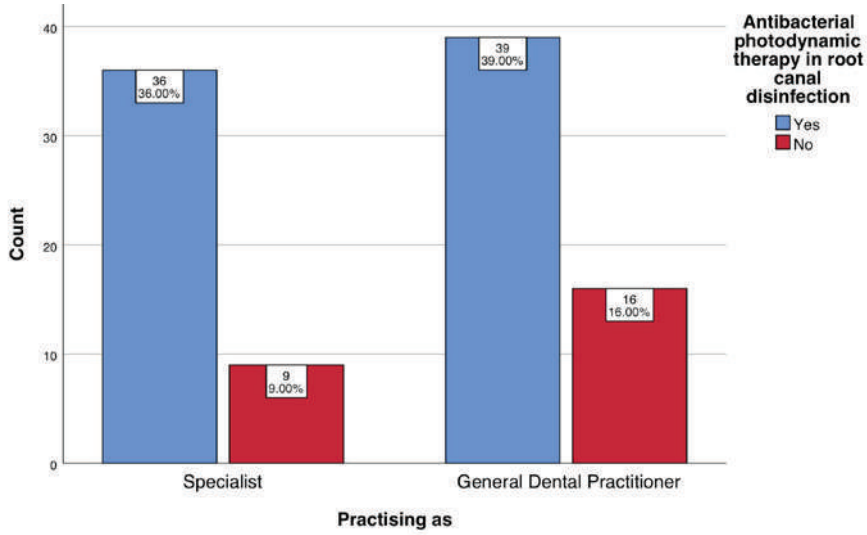
done and association was not significant (p value- 0.292; p>0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



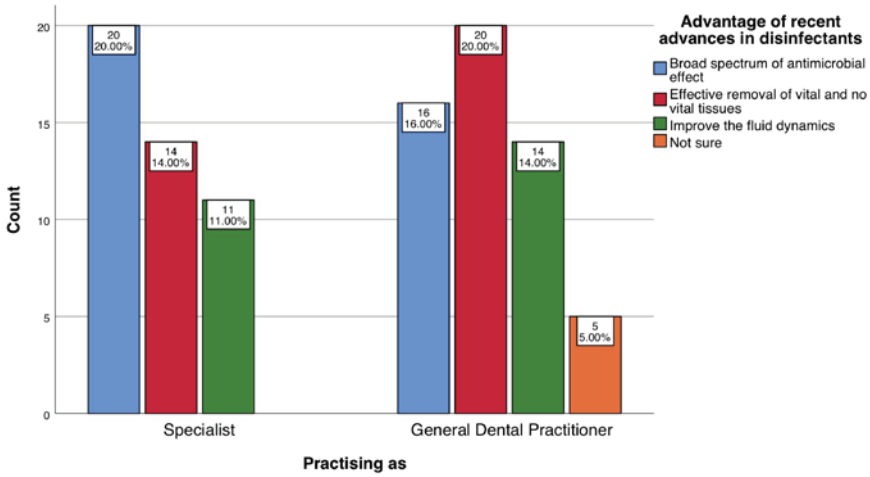
Graph 3: Bar graph represents the association between the field of practice (specialists / general practitioners) and the efficiency of herbal disinfectants, X-axis represents the field of practice (specialists / general practitioners) and Y-axis represents the number of participants. The blue bar represents herbal irrigants were highly efficient, red bar represents not so efficient and the green bar represents unfamiliarity or indecisiveness. Majority of the respondents (22% of specialists and 24% of general practitioners) responded that herbal disinfectants were not so efficient in root canal disinfection. Chi square test was done and association was not significant (p value- 0.870; p>0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



Graph 4: Bar graph represents the association between the field of practice (specialists / general practitioners) and awareness of dentin erosion that occurs during the use of MTAD. X-axis represents the field of practice (specialists / general practitioners) and Y-axis represents the number of participants. The blue bar represents yes- as an agreement to the statement(or question) and the red bar represents no- as a disagreement to the statement(or question).Majority of the respondents (41% specialists and 43% of general practitioners) were aware that MTAD can erode the dentin. Chi square test was done and association was not significant (p value- 0.079; p>0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



Graph 5: Bar graph represents the association between the field of practice (specialists / general practitioners) and the knowledge regarding the usage of antibacterial photodynamic therapy in conventional root canal treatment, X-axis represents the field of practice (specialists / general practitioners) and Y-axis represents the number of participants. The blue bar represents yes- as an agreement to the statement(or question) and the red bar represents no- as a disagreement to the statement(or question). Majority of the respondents (36% specialists and 39% of general practitioners) were aware that the antimicrobial photodynamic therapy could be used in conventional root canal treatment. Chi square test was done and association was not significant (p value- 0.296; p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



Graph 6: Bar graph represents the association between the field of practice (specialists / general practitioners) and the advantage of newly developed irrigants, X-axis represents the field of practice (specialists / general practitioners) and Y-axis represents the number of participants. The blue bar represents a broad spectrum of antimicrobial activity, the red bar represents effectiveness to remove vital and non vital tissues, the green bar represents improvement of fluid dynamics of irrigants used and the orange bar represents indecisiveness. Majority of the respondents (20% specialist and 16% general practitioners) concluded that the advantage of newly developed irrigants is the broad spectrum antimicrobial activity. Chi square test was done and association was not significant (p value- 0.115; p>0.05) proving there was no significant association between the field of practice (specialist / general practitioner) and their response to the question.

Presence of microorganisms within the root canal threaten to cause re-infections leading to the failure of the endodontic treatment [2].Hence, complete root canal debridement is very important [6]. Conventional irrigants such as NaOCl and chlorhexidine, have been employed for disinfection from as early as the 1920s [3,6]. Through various researches, it has been found that the conventional disinfectants have some disadvantages that tend to hamper a complete disinfection of the canal[36]. This has given rise to the development of newer irrigants such as MTAD, Tetraclean and Octenidine to challenge the disadvantages of the conventional irrigants, and also have improved properties in canal disinfection [37]. Various researches related to endodontics have been published that suggest that proper treatment is necessary for successful endodontic therapy [38,39].

The survey sought to analyse the knowledge and awareness of the recent advancements in the dental irrigants used during root canal disinfection. From the varied responses of the individuals, it was clear that a clear knowledge regarding the recent advances in root canal disinfection was present. Conventional irrigants are widely used for root canal disinfection during root canal treatment and they have been employed most commonly. Conventional root canal irrigants revealed that it had some disadvantages such as corrosive to the dentinal layer and reduced effectiveness against certain bacterial species such as enterococcus and lactobacillus. Development of newer variety of irrigants thus began for effective disinfection and also possess additional advantages such as improvement of fluid dynamics and effective bacterial removal. Participants in the survey are well aware of the recent advances in root canal irrigation and were ready to imply them in their clinical practice. There was no significant difference in the responses of the specialists as well as general practitioners, which means that the majority of the general practitioners were also aware of all the recent advances in root canal irrigation. However, there were some limitations in terms of small sample size and that many of the responders might have had an unwillingness to answer resulting in false responses being generated in the survey result. The scope in this study is that it can be used to create knowledge among individuals regarding recent advances in root canal disinfection.

Conclusion

Within the limitations of the study, the knowledge, attitude and practice regarding recent advances in root canal disinfection were analysed. It showed that the majority of the dentists were aware of newer advancements and the advantages of the same over conventional irrigants and were ready to imply them in their daily practice. There was no significant association between the field of practice (specialists / general practitioners) and their response to the questions. Overall, the survey showed good knowledge in the awareness of recently developed disinfectants among the dentists.

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Survey on Management of White Spot Lesions

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Abstract

White spot lesion occurs due to the demineralization of enamel.The white spot lesion is also caused by hypomineralization, fluorosis etc. It can be identified by visual as well as radiographic methods. The white spot lesion can mainly be prevented by maintaining proper oral hygiene. Other methods of management of white spot lesions include fluoride therapy, calcium phosphate, resin infiltration and CPP-ACP. The usage of remineralizing agents also helps in preventing and treating white spot lesions. This survey was conducted with a questionnaire about the management of white spot lesion and was circulated among 100 specialists and general practitioners. Data collection was done and analysed. The graphs were plotted according to the responses obtained from the survey. The results of the survey showed that the participants prefered the proper maintenance of the oral hygiene to prevent the white spot lesion. In case of no proper oral hygiene the participants preferred the usage of different techniques available to revert demineralisation. There was no significant association between the field of practice (specialist /general practitioners) and their response to the question. The participants had a good knowledge regarding the management of the white spot lesion and most of them were aware regarding the different modalities of treatment available and implied them in their clinical practice.

Keywords: *White opacity; hypomineralization; fluoride; CPP - ACP; Resin infiltration*

Introduction

White spot lesion is the demineralization or decalcification of enamel [1]. The initial lesion formation in caries is called as white spot lesion [2]. It can also occur due to hypomineralization, hypoplasia and fluorosis . The risk of white spot lesion is due to improper oral hygiene maintenance, acid producing bacteria, low salivary volume flow rate and fermented carbohydrates [3]. It is also called as white opacity because of its white opaque appearance during the scattering of light and appears as decalcified structures where the calcified structures are demineralized [4 5]. The common site for white spot lesion is the labiogingival surface of the lateral incisors and the least common site of white spot lesion is maxillary posterior segments [6] The development of the white spot lesion requires the coexistence of four factors namely fermentable carbohydrates, bacterial plaque, susceptible tooth surface and period for the development of white spot lesion [7].

Diagnosis of white spot lesion is done using visual inspection and radiography, transillumination methods, ultrasonic methods, electrical conductivity, polarization-sensitive test, optical coherence tomography including new technological methods for diagnosis of white spot lesion [8]. The most commonly used method in the diagnosis of white spot lesion is visual identification and radiography method. The differential diagnosis factor included is hypomineralization that is caused by fluorosis [1], molar-incisor hypomineralization [9 10] and traumatic hypomineralization.[11]

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The management of white spot lesion is by remineralization of demineralized enamel [12].Fluoride application in the form of toothpaste, mouthwash, varnishes, gels and foam can promote remineralisation. The fluoro apatite formed is seen to be more durable than the hydroxyapatite [13] [14]. Other management techniques include the usage of calcium phosphate, sealants, vital tooth bleaching, microabrasion and resin infiltration [11]. Chlorexidine also reduces the white spot lesion [15] [16] [17]. Tooth bleaching agents are minimally invasive and provide a uniform appearance [18]. Administration of phosphoric acid reduces the white spot lesion. Microabrasion treatment can improve the enamel surface texture and remineralize enamel. In resin infiltration method the diffusion pathway of dissolved minerals and acids causes obstruction of pores. The resin infiltration produces good strength and they prevent the cavitation of the tooth. The masking also showed good colour stability [19]. The usage of natural products like milk help the recovery of white spot lesions. Bioactive composites are also used in the treatment of white spot lesions [20] [21] [21,22]. White spot lesions can also be managed using the laminates and veneers [23] [24] Grape seed has remineralization capacity hence it can be used in the treatment of white spot lesion [25] [26].

The previous studies conducted by our team in the field of conservative dentistry and endodontics [27–34] paved the way to carry on with this research.This survey was performed to analyse the knowledge, attitude and practice regarding the management of white spot lesions among the general practitioners.

MATERIALS AND METHODS

A survey questionnaire was prepared and circulated among the specialists and general practitioners to analyze and evaluate the techniques used in the management of the white spot lesion. This survey was conducted through an online portal, questionnaires (Table:1) were prepared using google forms and circulated among 100 participants comprising of 63 specialists and 37 general dental practitioners randomly using social media; it took about 5-10 minutes to complete the survey. Responses obtained from the survey were tabulated in an excel sheet and analysed using SPSS software. The charts were plotted and the results were compared with responses obtained from the participated specialists and general dental practitioners.

The table below shows the questionnaire prepared and circulated among the respondents,

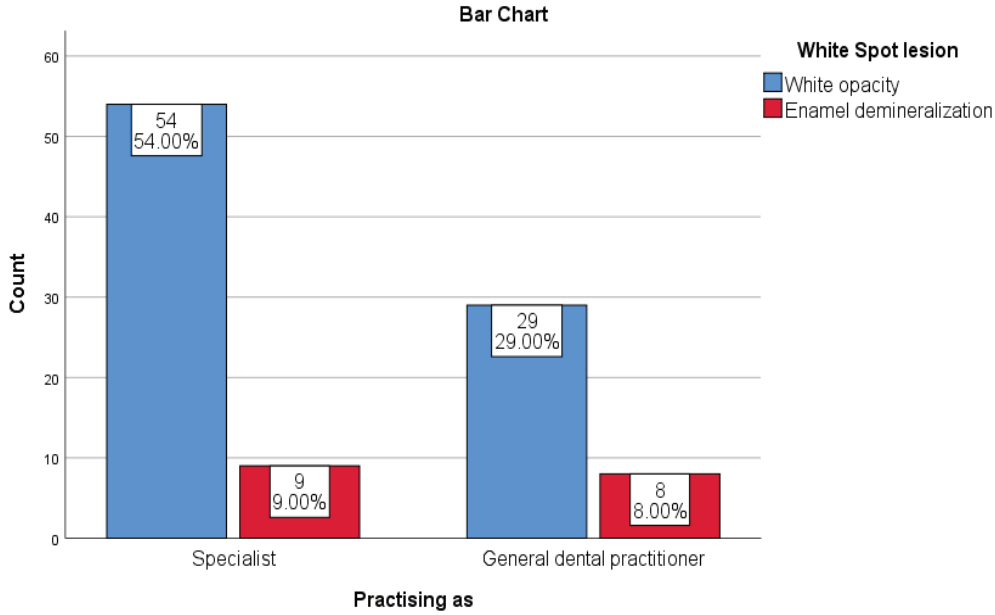
Questions	Options
What is white spot lesion?	● White opacity ● Enamel demineralization ● Decalcification ● Others
How to identify white spot lesions?	● Visual observation ● Radiography ● Others
How many cases of white spot lesions will you encounter in your practice per month?	(Numbers to be mentioned)
Can we get rid of white spot lesions?	● Yes ● No
Is white spot lesion reversible?	● Yes ● No
Can white spot lesion be managed by using 35-37% of phosphoric acid gel?	● Yes ● No ● Not sure

Cont... **Table 1 Questionnaires included in survey**

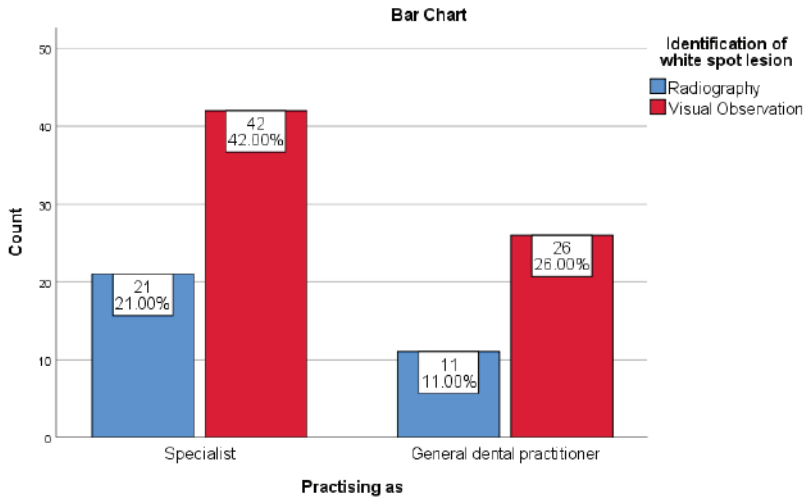
Can white spot lesion be treated by using fluoride?	• Yes • No • Sometimes
Is enamel microabrasion useful in the removal of white spot lesion?	• Yes • No
Which one do you prefer in the treatment of white spot lesions?	• 35-37% phosphoric acid • Fluoride • Remineralization with fluoride, calcium and phosphate. • All the above
Can white spot lesion be treated at home?	• Yes • No
If so how can it be treated at home?	• Oral hygiene • Fluoride mouthrinse • Fluoride based toothpaste • Remineralising paste (CPP-ACP) • Any other
What can we do to prevent the white spot lesion?	• Oral hygiene maintenance • Agents with fluorides • Antimicrobial agents • Other
Among the classes of white spot lesion which is easily curable?	• Class 1 • Class 2 • Class 3 • Class 4
Does white spot lesion form cavities?	• Yes • No • May be

Results and Discussion

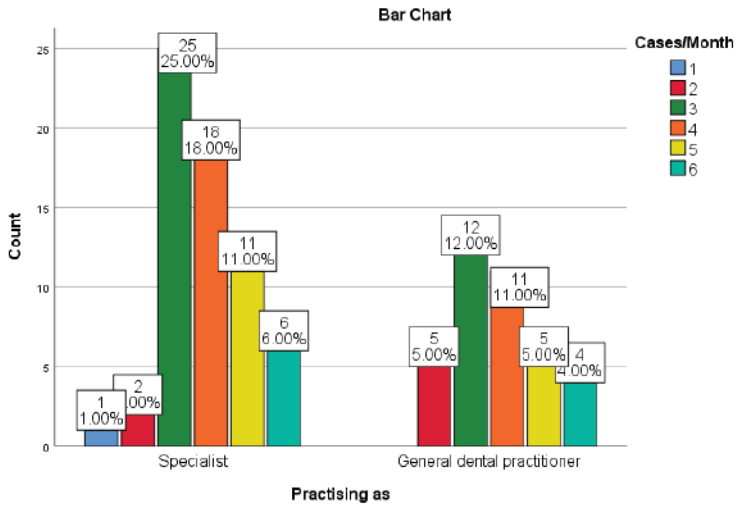
From the survey, the following results were observed and demonstrated below (graphs 1 - 6)



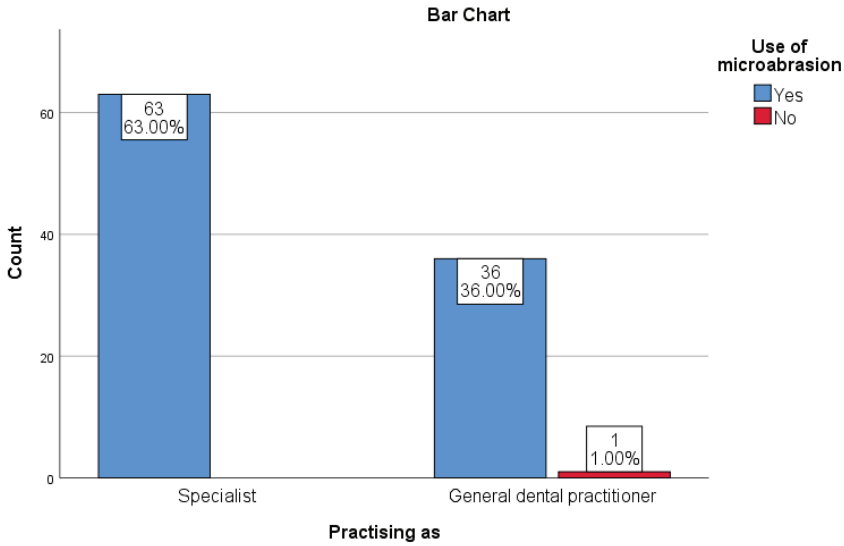
Graph 1 - Bar graph showing the association between field of practice (specialists / general practitioners) and white spot lesions. X axis represents the field of practice, Y axis represents the number of participants. Blue bar represents the dentist who turned up with answer white opacity, red represents the dentist who turned up with the answer enamel demineralization. Majority of the respondents (54% of the specialists and 29% of general practitioners) opted for white opacity. Chi square test was done and association was not significant (p value - 0.346;p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



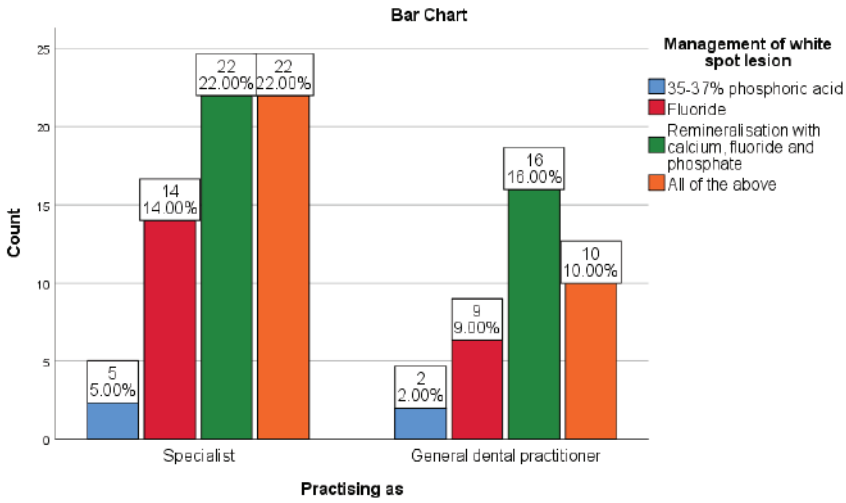
Graph 2 - Bar graph showing the association between field of practice (specialist / general practitioner) and identification of white spot lesion. X axis represents the field of practice, Y axis represents the number of participants. Blue bar represents the dentist who turned up with answer radiography, red represents the dentist who turned up with the answer visual observation. Majority of the respondents (42% of the specialists and 26% of general practitioners) opted for visual observation. Chi square test was done and association was not significant (p value - 0.709;p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



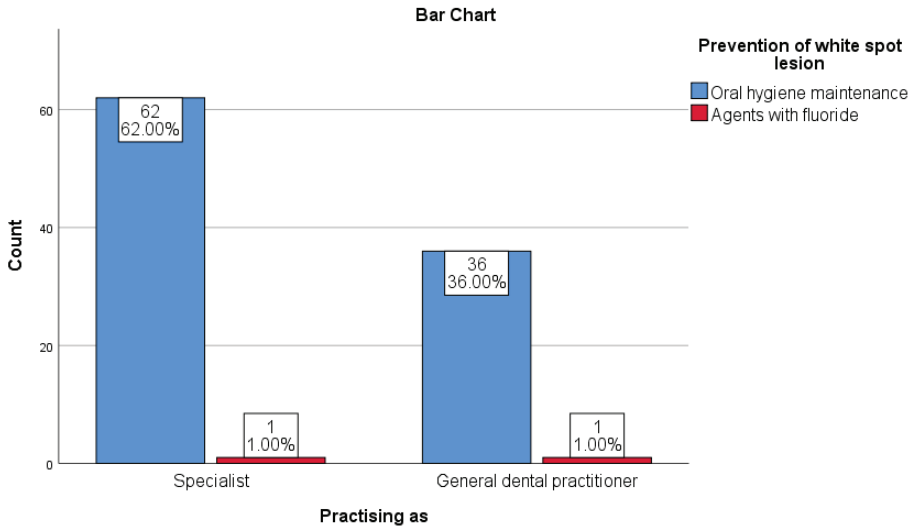
Graph 3 - Bar graph showing the association between field of practice (specialist / general practitioner) and identification of white spot lesion. X axis represents the field of practice, Y axis represents the number of participants. Blue bar represents the dentist who turned up with answer 1 patient per month, red represents the dentist who turned up with the answer 2 patient per month, green represents the dentist who turned up with the answer 3 patients per month, orange represents the dentist who turned up with the answer 4 patients per month, yellow represents the dentist who turned up with answer 5 patients per month, light blue represents the dentist who turned up with answer 6 patients per month. Majority of the respondents (25% of the specialists and 12% of general practitioners) opted for 3patients/month. Chi square test was done and association was not significant (p value - 0.447;p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



Graph 4 - Bar graph showing the association between field of practice (specialist / general practitioner) and identification of white spot lesion. X axis represents the field of practice, Y axis represents the number of participants. Blue bar represents the dentist who turned up with yes, red represents the dentist who turned up with the answer no. Majority of the respondents (63% of the specialists and 36% of general practitioners) opted for yes. Chi square test was done and association was not significant (p value - 0.190;p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



Graph 5 - Bar graph showing the association between field of practice (specialist / general practitioner) and identification of white spot lesion. X axis represents the field of practice, Y axis represents the number of participants. Blue bar represents the dentist who turned up with answer 35-37% phosphoric acid, red represents the dentist who turned up with the answer fluoride, green represents the dentist who turned up with the answer remineralisation using fluoride, calcium and phosphate, orange represents the dentist who turned up with answer all of the above. Majority of the respondents (22% of the specialists and 16% of general practitioners) opted for remineralisation using calcium, fluoride and phosphate. Chi square test was done and association was not significant (p value - 0.768;p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.



Graph 6 - Bar graph showing the association between field of practice (specialist / general practitioner) and identification of white spot lesion. X axis represents the field of practice, Y axis represents the number of participants. Blue bar represents the dentist who turned up with answer oral hygiene maintenance, red represents the dentist who turned up with the answer agents with fluoride. Majority of the respondents (62% of the specialists and 36% of general practitioners) opted for oral hygiene maintenance. Chi square test was done and association was not significant (p value - 0.700;p >0.05) proving there was no statistical significant association between the field of practice (specialists / general practitioners) and their response to the question.

The initial caries lesions are seen as a white opaque lesions on the enamel surface [12]. The main and the common way of identifying a white spot lesion is by visual observation by the dentist [35]. It is clear that the topical application of fluoride and remineralising cream on the white spot lesion helps in treating the white spot lesion. The recent studies also show that the white spot lesions can be treated by primary and secondary prevention [36]. The use of fluoride toothpaste on a daily basis helps in reducing the white spot lesion[4]. Hence, as the white spot lesion causes cavities, operative procedures to be involved after cavitation. So as to prevent the tooth surface the white spot lesions should be prevented in early stages. The survey results helped in analysing the knowledge, awareness and practice on the management of white spot lesion, from the responses collected participants had a clear knowledge regarding the management of white spot lesion. They were aware of different treatment modalities available regarding the management of white spot lesions.The prevention of white spot lesion before the demineralisation and treatment after demineralisation are very important in managing such situations. Majority of the participants in the study were aware of the techniques used in the management of white spot lesions and implied them in their clinical practice to reverse the condition. There was no significant association found between specialists and the general practitioners in the responses obtained, which means both the general practitioners and the specialists are aware of the strategies available for the management of white spot lesions.The limitation of the study included the smaller population that was involved, some had false responses and irrelevant answers. The scope of the study was to find the knowledge about the management of the white spot lesion among the dentist.

Conclusion

Within the limitation of the study, the knowledge, attitude and practice regarding the management of white spot lesions were analysed. It showed that the majority of participated dentists were aware of different treatment strategies available for the management of white spot lesions and most of them were implying them on daily practice for the prevention and treatment of white spot lesions. There was no significant association between the field of practice (specialist /general practitioners) and their response to the question. Overall, the survey showed good knowledge on the management of white spot lesions.

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Ethical Clearance: Not Required

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Recent Advances in Apex Locators- A Review

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Abstract

Apex locator is an electronic device used in endodontics that determines the position of the apical construction and it determines the root canal space length. Usually the correct working length determination is identified by using radiograph ,but electronic apex locators are being used increasingly nowadays. These electronic apex locators reduce the number of radiographs required and assist where radiographic methods create difficulties. Detection of root canal perforation is also considered as one of the roles of an apex locator. The benefit of apex locators include that these devices are much less affected by fluid conductive media in the canal. Some experience in the use of the apex locator will allow the clinician to recognize the differences immediately. It is used to find the working length determination, cementing the file in place, extracting the tooth and locating the file under magnification in the root canal . This review assesses the advantages and advances that would enable the use in dentistry and determination of working length.

Keywords: *Apex locators; Root canal; Radiograph; Apical foramen.*

Introduction

Apex locator is an electronic device used to measure the length of the root canal space. Removal of pulp tissue, necrotic material and microorganisms from the root canal is essential for the endodontic therapy. The development of the electronic apex locator has helped to make their assessment of the working length more accurate and predictable. The use of any electronic apex locator in combination with other radiographs is of greater precision in the determination of length of the root canal [1]. First generation apex locators use the resistance method for determining working length. This instrument measured the opposition of flow of current and was named as resistance-based apex locators. There are some modifications made under new machines that use the less than 5 microA were introduced. New machine is an endodontic meter S II [2]. Some of the previous researches have evaluated intracanal medications used, instrumentation methods on postoperative pain [3,4] Second generation apex locators use the impedance method for working length determination. These instruments measure an opposition of flow of alternating current and are named as impedance based apex locators. This utilises the current of a single sequence. Few examples are Formatron IV, Sono explorer and Endocater[5]. The determination of endo motor integrated apex locators is used [6] .Third generation apex locators use frequencies instead of a single one to measure the impedance in order to determine the working length. This is called comparative impedance type apex locator and frequency-based apex locators. Examples are Endex and Root ZX apex locators[7].

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Root ZX can be used to safely and conveniently locate the apical foramen and this will reduce the number of radiographs taken. There will be a reduction or decrease in the impedance when the instrument reaches the apical constriction[8]. Fourth generation apex locators use multiple frequencies to measure the impedance to find the working length. Multi frequency measurement system is used to calculate the distance from tip of the file to foramen by measuring the changes in the impedance between the electrodes. Examples are canal pro apex locators [9]. Although certain development of fifth and sixth generation apex locators are coming up.Negotiation of canals is mostly reported in case of endodontic therapy [10,11]. Veneers and laminates help in enhancing the shape of the teeth [12,13]

GENERATIONS	EXAMPLES	OPERATIVE BASE
FIRST GENERATION	Root canal meter, Endodontic meter, Dentometer, Endo radar.	Measurements of electrical resistance.
SECOND GENERATION	Sono explorer, Endocater, Digipex, Formatron IV, Endodontic meter S II.	Measurements of electrical impedance.
THIRD GENERATION	Endex or Apit, Root ZX, Dentaport ZX, Endod, Mini apex locators, Endy, Apex finder.	Using two different frequencies at the same time in order to measure difference or ratio between two currents.
FOURTH GENERATION	Bingo 1020, Raypex 4, Propex apex locators, Novapex, Ipex, Apex DSP.	Using two or more non-simultaneous continuous frequencies in order to measure the difference or ratio between two currents.
FIFTH GENERATION	Propex II, Apex locator joypex 5, I-Root, Raypex 5.	Measures the capacitance and resistance of the circuit separately.

Materials and Methods

A systematic review was done in manuscript preparation. The systems and databases search from relevant articles from Pubmed and Google scholar. Databases of intended journals were searched for keywords in this review.

Discussion

BINGO 1020 APEX LOCATORS

Bingo 1020 claims to be the fourth generation apex locating device and its unit uses two separate frequencies of 400 Hz and 8 kHz similar to the current third generation units [14]. The study found that bingo 1020 is found to be as reliable as the Root ZX and also user-friendly. It is also accurate as Root ZX , that is easier for a beginner to use briefly in the canals. Bingo and apex finder model instruments or more accurately without flaring [15,16].

PROPEX APEX LOCATORS

Propex is a multi frequency-based apex locator that is based on the same principle of other such modern devices that use multiple frequencies to determine the root canal length. One of the important characteristics of a propex is that the calculation is based on the energy of the signal, whereas other apex locators use the amplitude of the signal [17]. Propex is the most clinically suitable apex locator. In the majority of the cases, propex determined the apical constriction with high accuracy. After extirpation of the pulp, the smallest distance to the apex locator is obtained, determining that it is more accurate [18].

IPEX APEX LOCATORS

The ipex apex locators claimed to be a fourth generation apex locator, but it can measure the capacitance and resistance simultaneously to determine the location of their file tip of the canal [19]. Ipex apex locators were able to determine the accuracy of the working length in multi rooted primary teeth[20].

PROPEX II APEX LOCATORS

Propex II apex locators are the multi frequency based apex locator that is based on the fifth generation apex locator that uses multiple frequencies to determine the length of the root canal. It measures the energy of the signal with multi signal frequencies [21,22]. Propex II was more accurate than the radiographic method in determining the working length of the root canal [23]. In certain studies, it was concluded that propex II and Root ZX apex locators were able to determine the position of the apical foramen accurately [24].

RAYPEX 5 APEX LOCATORS

Raypex 5 apex locators are the fourth-generation device that uses two units that are 400 Hz and 8 kHz . It is performed equally well irrespective of the irrigant used. Irrigant solutions did not affect the accuracy of the working length[25] . Previous study based on the comparison of accuracy of Raypex 5 and Root ZX determined . There observed a slight decrease in both apex locators in the presence of blood in the root canal. It is considered that clinicians consider the possibility of a decrease. Therefore Raypex 5 and a Root ZX electronic apex locators are not influenced by the presence of blood in the root canal space [26]

SIXTH GENERATION APEX LOCATORS

Sixth generation apex locators served as the efficient diagnostic tool for detecting root perforations as they are based on impedance principle rather than the conventional gradient method [27]. The method of measuring the working length of the canal depending on the canal's moisture, implemented in the first Bulgarian apex locator . It is so called a sixth generation that is an adaptive type. Measurement with an adaptive apex locator provides for eliminating the necessity of drying or moistening the canal .This adaptive apex locator overcomes the disadvantage of popular fourth generation lower accuracy on working with canals and also devices of fifth generation difficulty in working dry canals. They continuously define humidity of the canal and immediately adapt to the dry or wet canal [28].

WORKING LENGTH DETERMINATION

Working length is the distance from the coronal reference point to the point in which the canal preparation terminates. Working length for instrumentation should be established at the level of apical constriction[29]. Accurate determination of working length is one of the key factors in endodontic therapy. Electronic apex locators reduce

the number of radiographs required and also helps in assisting the radiographic methods of working length determination [30,31]. One of the previous studies compared the accuracy of Dentaport ZX, Raypex 5 and elements of diagnostic units in establishing the working length. It is concluded that none of the tested devices or electronic apex locators show the full accuracy on working length determination [32]. The anatomical variations in apical constriction location, size, type of tooth and age makes the working length determination unreliable [33,34]. One of the reasons about the radiographic rate determination of the working length is, it lacks accuracy based on radiographic apex rather than the terminus. Working length is obtained with the radiograph by positioning the tip of the file from the radiographic apex . It is found that working length is based on the location of the minor foramen rather than the apex[35].

CLINICAL FEATURES AND PATIENTS USE

Root perforation is a serious dental complication that occurs in 3 to 10 percent of the root canal treatments. Perforations at the periodontal ligament are diagnosed and detected earlier and immediate treatment is required for those conditions. New generation electronic apex locators may be considered as the reliable aid in detecting the root perforations [36,37]. Patients with resin modified glass ionomer restoration have no sensitivity. Endoactivator influences postoperative pain than the endodontic needle.[38,39] Calcifications and dental shavings interfere with the performance of the apex locator and it is shown that packing of the debris in the apical third of the canal will affect the accuracy of these electronic apex locating devices. It is considered that pre-flaring of the can also improve the efficacy of the Apex locators[40]. Protaper as rotary instruments are used in CBCT root canal preparation Apex locators can reduce the patient's radiation exposure and better determine the working length.[41] Electromagnetic interference from dental instruments including electronic apex locators has the potential to interfere with the cardiac pacemakers [42,43].

Table : 2 : Comparison of apex locators and technique of working length determination based on the previous studies

STUDY	TYPE OF EALs STUDIED	AIM OF THAT STUDY	RESULTS
Saatchi M et al.,2016 25	Raypex 5 apex locators	Comparison of Raypex 5 with radiography in determination of working length.	No significant difference seen.
Weiger R et al., 1999 23	Propex II Apex locators	Comparison of Propex II and radiography in working length determination.	Propex II is more accurate than the radiography.
Tinaz AC et al.,2002 17	Propex	Comparison of propex and radiography in the working length determination.	propex is more accurate than the radiography.
Cianconi L et al.,2010 29	Root ZX and Propex apex locators	comparison of EALs and radiography in working length determination.	It is found that Root ZX and propex is more accurate than radiography.
Stober EL et al.,2011 20	Root ZX	Comparison of Root ZX and radiography in working length determination.	No significant difference seen.
Plotino G et al.,2006 18	Root ZX, Elements diagnostic, precision AL and Raypex 5.	Comparison of EALs and other methods of working length determination.	EALs are more accurate than others.

Conclusion

From this type of review study, some advances, clinical efficacy and generations of apex locators are included. Proper data for this study is not fully available. Future research should elaborate on the advanced techniques in using apex locators clinically and use of the patient is mostly required. Apex locators generation should be elaborately required.

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Immune Boosting Drugs -A Myth or Reality - A Review

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Abstract

The concept of boosting immunity was a popular one. Although the lay public is exposed to information on how to boost immunity and the only evidence-based approach to this is vaccination. The aim of this review was to analyze such immune boosters as a myth or reality and the information about immune-boosting drugs available on the Internet. Of the 30 approaches to boost immunity recorded, the top ones were diet (76%), fruit (70%), vitamins (65%), antioxidants (54%), probiotics (52%), minerals (49%), and vitamin C (56%). Interestingly, vaccines ranked 27th, with only 12% mentioning them. Commercial websites are an important component of the information available to the public on the topic and thus contribute to providing information that is biased. Vaccines have developed society by saving lives and dramatically reducing the burden of infectious disease. Most successful vaccines have been developed empirically, but recent advances in immunology are beginning to shed new light on the mechanisms of vaccine-mediated protection and the development of long-term immunity. The role of vaccination is to generate long term protection against diseases. Prime-boost vaccine strategies could improve cellular and also humoral immunity in several animal models, among various vaccine modalities. Although natural infection will often provide lifelong immunity, current vaccinations need a booster dose to achieve durable protective humoral immune responses, regardless of whether the vaccine is based on infection with replicating live-attenuated vaccine strains of the specific pathogen or whether they are derived from immunization with inactivated, non-replicating vaccines or subunit vaccines. This review discusses the recent advances in immunization and the immune boosters and decides whether these are a myth or reality.

Keywords *immune boosters; vaccine; health; immunological memory; protection.*

Introduction

Immunity is the major mechanism of the host to defend against the eradicating disease which is caused by infectious agents. Successful vaccinations could help in boosting immunity. Substances that help in the stimulation of the immune system by inducing activation or increasing activity of any of its components are known as immunostimulants or immunostimulators. They are available as drugs and nutrients.[1] Immune boosters include vitamins, minerals, antioxidants, probiotics, other supplements, and the dietary complementary and approach chiropractic or yoga and the vaccination.[2] A booster dose or booster vaccination is a re-exposure of immunizing antigen which is exposed after the initial immunization. [3] It is intended to increase the immunity back to protective levels against that antigen after memory has deteriorated overtime against that antigen. Arthus reaction, patients receive a booster dose but already have a high level of antibody in the body may develop, an inflammation that is caused by a localized form of Type III hypersensitivity induced by high levels of IgG antibodies.[4]

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Ginseng is a characteristic rejuvenator and furthermore it is a safe and non-harmful substance to the body and goes about as an immune booster, vitality, and health and helps the body to adapt to different forms of stress. Besides ginseng, regular uptake of vitamin-D also helps in preventing yourself from frequent diseases.[5] Immunity can be improved by dietary supplements and plant products. Plants products are extensively evaluated for their acting various use in different disease conditions like cancer, diabetes, hepatotoxicity. [6 7 8] Plants such as *Caralluma fimbriata,Azadirachta indica,Acacia catechu ,Syzygium cumini, Garcinia mangostana,Brassica oleracea* are evaluated for their various pharmacological activities.[9 10 11 12 13 14 15 16]Amla fruits and aloe vera have been gaining wide popularity in recent years in India as well as in other parts of the world, but the mechanism of action of most of these fruits and plants has not been subjected to thorough scientific investigations. Hence much research is carried out in this field. Plant based nanoparticles are extensively used for the preparation of various formulations and treatment of many diseases. [17 18 19 20 21 22 23 24]Indeed, findings suggested that these plants and their bioactive metabolites are effective in balancing and proper functionality of the immune system through various modules of immune modification like stimulation and suppression.

IMMUNE BOOSTING DRUGS

One of the most common misconceptions in vaccinology is that inactivated vaccines and subunit vaccines are weaker immunogens and will require booster vaccination whereas live, attenuated vaccines are expected to elicit the same degree of durable protection as that achieved by natural infection.[25] This does not appear to be the case since, with the possible exception of rubella, essentially all common childhood vaccines require booster vaccination regardless of whether they are non-replicating vaccines or live, attenuated vaccines. Measles and mumps, for example, are often described as "childhood infections" because a person is typically infected once and then maintains lifelong immunity thereafter.[26] However, unlike wild-type strains of measles and mumps, infection with the attenuated vaccine strains of these viruses is insufficient to provide long-term protective immunity after a single dose and a 2-dose regimen is required in order to achieve sustained protection and broad herd immunity.

Supplement through Diet

Low fat and plant related help in boosting the immune system. The immune system relies on white blood cells that produce antibodies to combat bacteria, viruses, and other invaders. Vegetarians have been shown to have more effective white blood cells when compared to nonvegetarians, due to a high intake of vitamins and low intake of fat.[27] Eating a low-fat diet may also be protective. Studies have shown that limiting dietary fat helps strengthen immune defenses. Research also shows that oil may impair white blood cell function and that high-fat diets may alter the gut microbiota that aid in immunity.[28]

Maintaining a healthy weight can also benefit the immune system. Obesity has been linked to increased risk for influenza and other infections such as pneumonia. Plant-based diets are effective for weight because they are rich in fiber, which helps fill you up, without adding extra calories. Fiber can also lower BMI, which is linked to improved immunity. A plant-based diet has also been shown to reduce inflammatory biomarkers.[29].

Vitamins

Vitamins are the vital substances that our body needs daily to grow. Vitamins play an important role in various immunity processes which include lymphocyte activation and proliferation, T-helper-cell differentiation, the production of specific antibody isotypes and regulation of the immune response, etc. Vitamin C is the biggest immune system boosters that is required daily because the body doesn't produce or store it. Vitamin E is a powerful antioxidant that is present in nuts, spinach, etc. Vitamin A helps to strengthen the immune system against infections. It also acts as a regulator in tissue and cell growth. Many products claim to support or.boost immunity. The capability of the immune system reduces as the age increases and the body becomes prone to more infectious diseases.

The immune system is a network of specialized organs, tissues, cells, proteins, and chemicals, which has evolved in order to protect the host from a range of pathogens, such as bacteria, viruses, fungi, and parasites, as well as cancer cells. Early on, it was noted that scurvy often Nutrients 2 of 25 followed infectious epidemics in populations, and cases of scurvy have

been reported following a respiratory infection. This is particularly apparent for individuals who are already malnourished.[30]. To prevent Hypervitaminosis C, a regular and adequate intake of vitamins was required to the body because the body storage capacity for water soluble vitamins was very low. Epidemiological studies have shown that hypovitaminosis C which is plasma vitamin C < 23 μmol/L was relatively common in Western countries whereas vitamin C deficiency that is <11 μmol/L was the fourth leading nutrient deficiency in the United States. [31]There are several reasons why vitamin C dietary recommendations are not met, even in countries where food availability and supply would be expected to be sufficient. These include poor dietary habits, lifestyles either limiting intakes or increasing micronutrient requirements such as smoking and alcohol or drug abuse, various diseases, exposure to pollutants and active and passive smoking and poor socioeconomic status and limited access to nutritious food. Even in industrialized countries can be at risk due to lifestyle-related factors, such as those on a diet or eating an unbalanced diet, and people facing periods of excessive physical or psychological stress.

VACCINATION

A vaccine is a biological preparation that provides a specific infectious disease with successfully acquired immunity. A vaccine usually contains an agent that resembles a microorganism that causes disease and is mostly produced from weakened or killed types of the microbe, its toxins or one of its surface proteins. The agent activates and kills the body's immune system to identify the agent as a threat and to further identify and kill all of the microorganisms that could be associated with that agent in the future. Vaccines can be prophylactic (to prevent or mitigate the effects of a future infection being investigated by a natural or "wild" pathogen), or therapeutic, such as cancer vaccines.[32,33]

Some vaccine safety issues relate to how vaccines interact with the immune system (for example, too many vaccines) or just how the immune system operates in various circumstances (for example, natural infection versus immunization). Although these issues should be treated equally, it is important to understand them in the sense of how the immune system functions. Vaccine immunization, like normal infections, causes long-lasting immunity[34] Yet unlike normal infection, immunization does not gain such high immunity rates.[35]

ROLE OF IMMUNE BOOSTERS IN HEALTH

Vaccines have given numerous examples of overcoming adversity and even a prerequisite for a solitary sponsor inoculation is a little cost to pay for solid invulnerability without requiring serious or possibly hazardous infectious conditions rather than the confirmed ones. In any case, a few immunizations are just somewhat viable much after different vaccinations(e.g., acellular pertussis) and the advancement of better immunization methodologies that require fewer promoters is a significant objective, particularly in creating nations in which routine inoculation can be entangled by calculated difficulties for finishing essential inoculation regimens. One approach to improve immunizations is to analyze effective antibodies and recognize potential factors that might be engaged with deciding long term immunization adequacy. In expansive terms, antibodies or vaccines can be sorted into three general classes: multivalent non-protein antigen, monovalent protein antigen, and multivalent protein antigen. As indicated by the engraved life expectancy model for acceptance of enduring plasma cells incitement of T cell-free counteracting agent reactions will be generally momentary without T cell help. Albeit monovalent protein antigens will obtain better, increasingly tough immune response reactions (because of obtaining of T cell help), the most enduring counteracting agent reactions are anticipated to happen when a multivalent antigen triggers solid B cell initiation just as viable T cell help.[36]

Polysaccharide antibodies, for example, Pneumovax-23 can evoke B cell initiation through the grouping of the B cell receptor (BCR) yet without a related protein in the immunization, this happens without related T cell help by CD4+ T follicular partner cells (TFH).[37] This T-independent immune response reaction gives just a restricted term of assurance in senile subjects and antibody reactions decrease to gauge levels inside 3–5 years after inoculation. Additionally, T-free counteracting agent reactions neglect to build up memory, and re-immunization with refined polysaccharides have been found to bring down serum neutralizer reactions, and reduction in the recurrence of antigen-specific memory B cells. To conquer these confinements, more

current polysaccharide immunizations, for example, Prevnar-13 are conjugated to a carrier protein (e.g., CRM197, a detoxified freak of diphtheria poison) and this, not just outcomes in counter acting agent reaction of higher ardentness, yet immunological memory is all the more seemingly perpetual.[38]

IMMUNE DISORDERS

The immune system begins to develop in the embryo and is localized. In several parts of the body. Immune cells develop in the primary organs like bone marrow and thymus. Immune responses occur in secondary organs like spleen etc. Immune disorders have been known to occur where the body produces antibodies that attack its own tissues, leading to deterioration and, in some cases, tissue destruction. [39]

Immune disorders are Respiratory, Inflammatory, Cancer, AIDS. The dysfunction of the immune system is an immune disorder. According to the International Union of Immunological Societies, more than 150 primary immunodeficiency diseases (PIDs) have been characterized.[40]However, the number of acquired immunodeficiencies exceeds the number of PIDs. Those caused by inherited genetic mutations are the primary immune deficiency diseases. Secondary or acquired immune defects, such as a virus or immune suppressing medications, are caused by something outside the body.[41]

RESPIRATORY DISORDERS

Viral rhinorrhea

Viral rhinorrhea is an acute, inflammatory state of infectious origin in the upper airways. Side effects of nasal congestion, discharge, and sneezing plus impaired alertness and fever atypically high sublingual temperature relative to clock-hour normative level intensify at night and are typically rated worse at morning awakening, but at the highest midday cough frequency.

Allergic rhinorrhea

Allergic rhinorrhea- immune system-mediated upper airways hypersensitivity to the environment and other antigens is expressed as inflammation of the tissue of the respiratory tract, activation of the mucus gland, and dilation of the blood vessels. The main signs of nasal congestion, discharge, and sneezing; swollen, itchy, watery eyes; and itchy mouth, nose, and throat slowly worsen late at night and overnight; Sleep and overall quality of life was commonly compromised. However, recalling and prospective daytime self-assessment studies typically rate symptoms worse when morning sleep awakens.[42]

Inflammation and cancer

Inflammation is often linked to cancer development and progression. Genetically stable are the cells responsible for cancer-associated inflammation and thus do not experience the rapid development of drug resistance; thus inflammation targeting provides an effective strategy for both cancer prevention and cancer therapy. Several factors induce tumor-extrinsic inflammation, including infections with bacteria and viruses, autoimmune disorders, obesity, cigarette smoking, exposure to asbestos, and excessive alcohol intake, both of which raise cancer risk and promote malignant progression. In comparison, cancer-intrinsic or cancer-induced inflammation can be caused by mutations that cause cancer and can lead to malignant development by recruiting and stimulating inflammatory cells. Both extrinsic and intrinsic inflammations can contribute to immunosuppression, providing a favored backdrop for the development of tumors.[43]

Symptomatic treatment

Although the principle of treatment of autoimmune movement disorders should focus on treating the underlying neuroinflammatory process it is often necessary to treat symptoms. A pragmatic summary of some commonly used symptomatic medications used in these disorders. It should be noted that these disorders can be dynamic and changing, and drug withdrawal or escalation may be required depending on disease evolution. It is also commonly observed that there is a higher risk of side effects with some of these agents, such as dystonia or neuroleptic malignant syndrome in anti-NMDAR encephalitis patients given neuroleptics. Optimizing the sleep wake cycle is often a priority to allow the child and family to rest. Important underlying principles with symptomatic management were to try and use medications that may improve multiple symptoms and to reduce the severity to enable comfort with unnecessarily using high doses that may lead to higher risk in dose related adverse effects.[44,45]

Conclusion

Immune boosting drugs were the most essential therapy. Immunotherapy was only a single therapy that is not very effective. combination of the conventional method was most appropriate. Eating more fruits and vegetables can keep the immune system strong. Vitamins and supplements can help ward off disease and make people feel better faster. A positive mindset can boost the immune system.

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Awareness of Hand Hygiene among Dentists- A Questionnaire Survey

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Abstract

Dentistry is a service sector where the doctors are in close contact with the patients. Hand hygiene is very important in the dental practice as it can prevent infection to a great extent. The aim of the survey was to assess the importance of hand hygiene among dentists. A Questionnaire of 15 questions was prepared and circulated in the google forms, the responses were collected, compiled, and updated on the SPSS software for data analysis. Pie charts and bar graphs were used to evaluate and interpret the results. Chi-Square test was carried out to check statistical significance and p value <0.05 was considered as significant. The results obtained from the dentists who participated in the survey provide that the dentists were aware of hand hygiene protocol and the survey showed that hand hygiene is important to every dentist in their dental practice . 98.1% of the population agreed that maintenance of hand hygiene by the dentists prevents the spread of infections; 81.1% of the dentists participated were aware that 7 steps involved in the hand hygiene protocol ; 85% of the dentists were aware that, a minimum of 20 seconds are required for proper handwashing to maintain the hand hygiene, All the dentists participated in this survey agreed that hand hygiene has to be maintained definitely before the patient contact; 97.2% of dentists also suggested that hand hygiene maintenance is more necessary among the health care workers and 74.8% of the dentists agreed as hand hygiene is the most important criterion among their dental clinics. Thus, This survey could make awareness among the dentist regarding hand hygiene protocol.

Keywords *Hand hygiene; Dentist ;awareness ;Infection spread; Steps.*

Introduction

Hand washing is a technique of hand hygiene where different formulations are used to remove or reduce the no. of microorganisms. The Hand Sanitizer is such a formulation used for hand hygiene that reduces the pathogens and maintains the hand hygiene for the users.

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The World Health Organisation suggests the techniques for hand hygiene that includes seven steps procedure or protocol [1]. Thus, this hand hygiene protects the clinicians, practitioners, and common people to prevent the spread of diseases due to contact and to control its transmission [2]. It is beneficial to make hand sanitizer using natural products other than chemicals. The hallmark of this study in the field of medicine is that hand hygiene is the major criterion in controlling the infectious spread, especially in the medical and dental procedures. The hand hygiene provides health care against the infections and acts as a protective measure against the disease caused due to the contact [3]. During dental procedures, the contact with the dentist from the infected patients, especially with their saliva, can cause infections to other patients and the dentist. Hence, it is mandatory to follow hand hygiene protocols to avoid community spread infections. Hand

sanitizers can also be considered as drugs since they prevent infections. The different formulations [4] used for hand hygiene include lotions, surgical hand sanitizers, alcohol-based scrubs, etc. All these can reduce infection [5]. Hand hygiene is the main practice provided to reduce the risk of the spread of infections from human - to - human[6].

Naik S in his study stated that only a small portion of dentists was aware of CDC rules and guidelines, hence rules and significance of CDC are to be improved among the dentists [7]. Darawad M.W in his study proposed that only half of the dentist population is aware of the effects of hand hygiene provided by hand sanitizer. Thus he stated that a survey on hand hygiene has to be enhanced to create awareness among people.[8].

The lacunae of this study include the sample size which was taken within a limited geographical area. The other factors also include that there is no restriction of age mentioned and the dentist was alone concentrated to fill this survey, where other medical and non-medical professionals could have also been included to create awareness among people [9]. The challenges faced also include the unaffordability of different classes of people to buy sanitizers and other formulations. In some cases, the hand sanitizer and other chemical formulations can also cause adverse effects such as itching, irritation, inflammation, etc.The study aim of the study was to assess the awareness on hand hygiene and to highlight the important effects of hand hygiene among dental professionals.

Materials and Method

A total number of 106 dentists participated in this study. The sampling method used in this study was random survey sampling. To minimize sampling bias certain measures were taken which includes framing straight forward questions which sounds simple and the questions were kept short and clear. A self-structured survey questionnaire was prepared which consists of 15 Questions. These questions were self framed and the validity checking of these questions was made by three internal experts in the university. The questionnaire included the steps involved and the importance given by the dentist on hand hygiene protocol. The dependent variables included the Awareness, hand hygiene, and the independent variables include the wash techniques, steps, and formulations.Google forms were used to circulate the questions and the responses were collected, the data analysis was carried out using SPSS software. Chi square test was used for statistical analysis and p value < than 0.05 was considered as significant.

Results and Discussion

A total of 106 dentists of different age groups have participated in this survey out of which 50 were male and 56 were female. (Figure 1a) represents the response to the question regarding the hand hygiene program wherein 57.55% attended the training program and 42.5% of the dentists did not attend the training. The World Health Organisation suggests that hand hygiene is the most powerful preventive measure over infections. Also, the study suggests that people who have attended the hand hygiene programs have created a greater impact on hand hygiene over them [10]. The hand hygiene programs were not developed and organized in rural areas which restricts the people and dentists to educate themselves with the effect of hand hygiene [11]. Thus it is important to attend the hand hygiene program. (Figure 1b) depicts the prevention in the spread of infection by hand hygiene for which “yes it is prevented’’ is suggested by 99.06% of dentists and “no it doesn’t” is suggested by 0.94% of dentists. Infections are prevented by following the proper protocol of hand hygiene steps, especially by the health care workers, as their hands are the vehicle of transmission [12].

Some formulations of hand sanitizer also produce adverse effects to skin making worse, thus the effectiveness has to be checked before usage [13]. (Figure 1c) indicates the opinion of dentists on Hand nails hinder the hand hygiene protocol -Yes it hinders is suggested by 69.81% of dentists; no it doesn’t is suggested by 27.36% and maybe by 2.83% of dentists. The nails serve as a bacterial burden where the microorganisms commonly reside, thus the nails make it difficult to clean and to maintain proper hand hygiene.[14]. Nails can be cleaned using alcohol-based rubs but long nails hinder the hand hygiene protocol [15]. (Figure 1d) represents the steps involved in wash techniques as per WHO includes the result as, 3.77% of the dentist suggested as 5 step technique and 6 steps are given by 7.55% of dentists and 7 step technique were given by 81.13% and finally 7.55% of dentists suggested

as 8 step technique. The World Health Organisation suggested the hand hygiene protocol of 7 steps were the most effective steps, conventionally involved to prevent the transmission [16]. Interventions in the steps of the hand hygiene were made for better protection but the effectiveness is involved in 7 step management [17]. (Figure 1e) portrays the awareness of the necessity of hand wash technique among the health care workers, "yes" necessary was suggested by 97.17% of dentists and ``no, not necessary'' is given by 2.83% dentists. Interventions are proved to improve the hand hygiene practices among the practicing nurses and healthcare providers who are more in need and are in direct contact with patients[18]. Thus another study provides information that only a few had a clear knowledge of hand hygiene protocol and established the techniques regularly. Hence to avoid cross-infection, education, and information to them are required [19]. (Figure 2a) provides information on hand hygiene products that are detrimental to the skin in which 42.45% of dentists suggest as "yes" it is detrimental and 48.11%. dentists suggest "no" not detrimental and 9.43% as "maybe".

The hygiene products also include the sanitizers that in some cases suggest having adverse effects like allergies due to the formulations [13]. In most cases, the alcohol-based sanitizer provides utmost reduction from microorganisms and efficacy against pandemic disease as COVID-19 [20] . (Figure 2b) represents the duration of handwashing with soap and water which was suggested as 10 seconds by 0.94% of dentists; 20 seconds by 84.91% of dentists and 30 seconds by 14.15% of dentists. The efficacy is extended and appropriate, accurate washing for at least 15-20 seconds is recommended for the reduction of contamination [21]. But 15 seconds were also proved as non-inferior with faster to kill microorganism effectiveness [22]. (Figure 2c) depicts the necessity of hand hygiene before patient contact -"yes" is suggested by the whole 100% of dentists. In a study, hand hygiene has been improved among the Healthcare workers before patient contact which leads to a result of the reduction in the transmission of infection and diseases provided to be effective against Covid-19 [23]. Hand hygiene health is the main route in the transmission of diseases to patients. Thus hand hygiene before patient contact has to be maintained to prevent contamination [24] .

(Figure 2d) depicts the importance of hand hygiene at the dental office as 74.5% of dentists have answered as "most important", 24.5% of dentists have answered as "moderately important" and 1% have answered to be "less important". Hand hygiene maintenance enhances and provides a better environment in the dental clinics which are included under infection control management. The disposal of the PPE and hand hygiene plays an important role in this [25] . (Figure 2e) depicts the response to the question, CDC hand hygiene guidelines, adversely affecting the skin of hands for which "yes" is suggested by 31.13% of dentists and "No" it does not affect is suggested by 63.21% and maybe is given by 5.66% of dentists. The result of this survey shows that dental professionals were moderately aware of the Hand hygiene protocol. In which most of them were well educated on the importance of dental hand hygiene and implemented at their clinics.

When comparing the awareness of hand hygiene to prevent the spread of infection with gender, it was shown that females had a higher level of awareness about the hand hygiene when compared to males (p=0.342), which was statistically not significant (figure 3). When comparing the knowledge about the steps involved in the hand hygiene protocol with gender , it was shown that females had a better knowledge about the steps involved in the hand hygiene protocol compared to the males (p = 0.119), which was statistically not significant (figure 4). When comparing the awareness of the necessity of hand hygiene among dental assistants with gender , it was shown that females had a higher level of awareness that hand hygiene was necessary for dental assistants compared to the males involved in this survey (p=0.493), which was statistically not significant (figure 5). When comparing the knowledge about the duration taken for hand wash technique with gender, it was shown that females had a higher level of knowledge when compared to the males involved in this survey (p=0.635) which is statistically not significant (figure 6)

Scientists are exploring plants for screening active phytochemicals and many plants like *Caralluma fimbriata*[26,27], *Acacia catech*u.[28–30] were evaluated and reported with antidiabetic, anticancer, and antimicrobial properties [31,26,27]. Many invivo studies have been conducted for hepatoprotective effects [32,33]. Green synthesis of nanoparticles such as silver [34,35], zinc[37], and

selenium[36] was reported to have fewer side effects compared to other methods of nanoparticle synthesis. These drugs have fewer side effects and help in better treatment for many diseases [38,39,40]. It provides many properties for betterment. These phytochemicals, extracts, and nanoparticles may be incorporated for the preparation of eco-friendly hand sanitizers for better maintenance of hand hygiene among dentists.

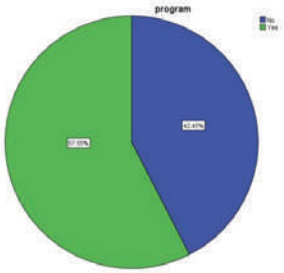


Figure 1(a) : Pie chart representing the percentage distribution of dentists attended the hand hygiene program before. 57.55% responded as 'yes' (green) attended and 42.45% responded 'No'(blue).

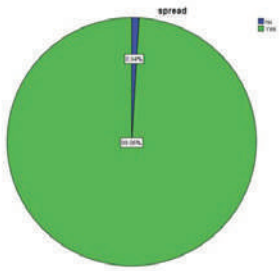


Figure 1(b) : Pie chart representing the prevention in the spread of infection by hand hygiene, 99.06% responded as 'yes' (green) of the study population and 0.94% responded 'No' (blue).

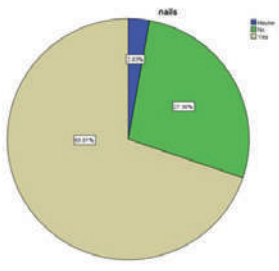


Figure 1(c): Pie chart represents the hindrance of nails in hand hygiene protocol. 69.81% of the dentists responded as 'yes' (buff), 27.36% responded as 'no' (green) and maybe (blue) by 2.83% of dentists.

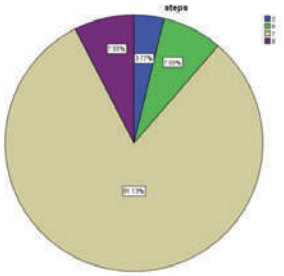


Figure 1(d) : Pie chart represents the steps involved in the hand hygiene protocol, 81.13% responded as 7 steps(buff), 7.55% as 6 steps (green), 7.55% of 8 steps (purple) and 3.77% responded as 5 steps (blue).

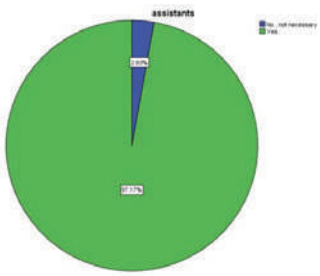


Figure 1(e): Pie chart represents the necessity of hand hygiene among health care workers, 97.17% of the dentists participating responded as 'Yes, it's necessary' (green) and 2.83% responded as 'No' (blue).

Figure 1

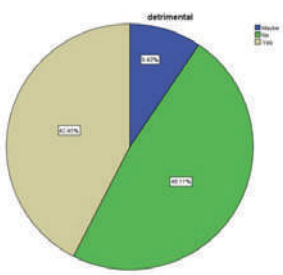


Figure 2(a) : Pie chart represents that hand hygiene products are detrimental to the skin, 42.45% responded as 'Yes' (buff), 48.11% responded as 'No' (green) and 9.43% responded as 'maybe' (blue).

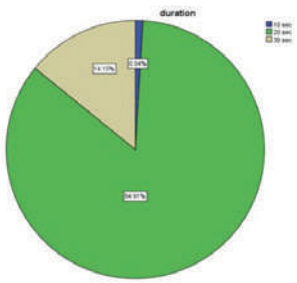


Figure 2(b) : Pie chart represents the duration for hand washing, 0.94% responded as 10 seconds (blue), 84.91% responded as 20 seconds (green) and 14.15% of dentists responded as 30 seconds (buff).

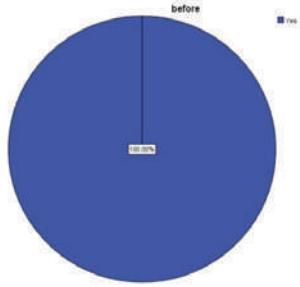


Figure 2(c) : Pie chart represents the necessity of maintenance of hand hygiene before patients contact, all the participants responded as 'yes' 100% (blue).

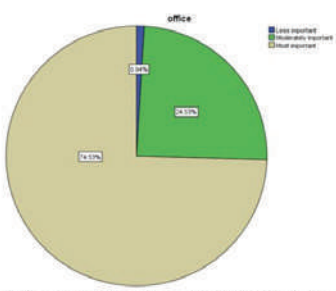


Figure 2(d) : Pie chart represents the maintenance of hand hygiene at dental offices, 74.8% responded as most important (buff) , 24.3% answered as moderately important (green) and 1% of dentists responded as less important (blue).

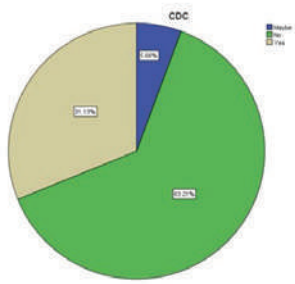


Figure 2(e) : Pie chart represents the CDC guidelines adversely affect the skin, 31.13% responded as 'yes' (buff), 63.21% responded as 'no' (green) and 5.66% of dentists responded as 'maybe'(blue).

Figure 2

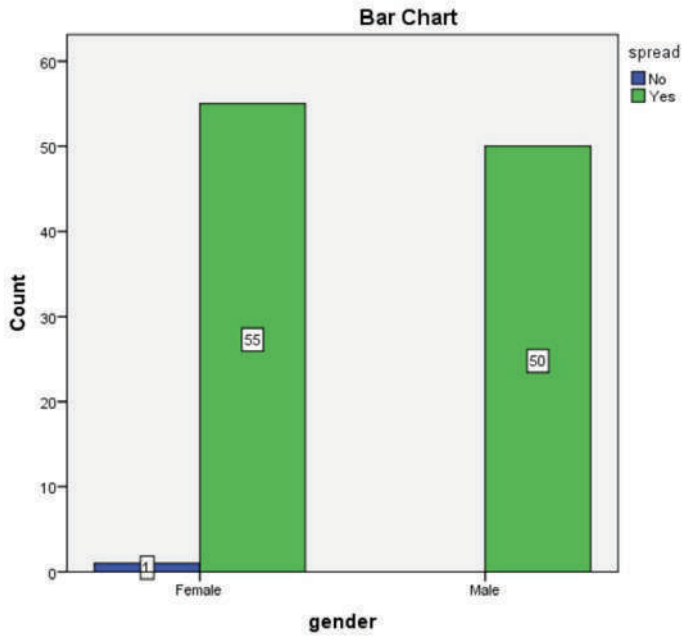


Figure 3: Bar graph depicting the association between gender and the awareness of hand hygiene to prevent the spread of infection; where blue denotes No and green denotes yes. X-axis represents the gender and Y-axis represents the number of respondents.Chi square test showing p = 0.342 (p > 0.05 indicating statistically not significant). So there is no significant association between gender and awareness on hand hygiene prevents the spread of infection.

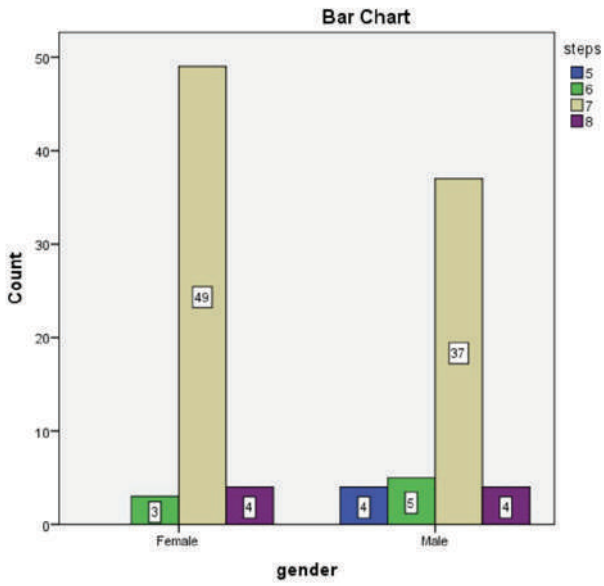


Figure 4: Bar chart depicting the association between gender and the steps involved in the hand hygiene protocol where blue denotes 5 steps , green denotes 6 steps , yellow denotes 7 steps and purple denotes 8 steps. X-axis represents the gender and Y-axis represents the number of respondents. Chi square test showed p = 0.119 (p >0.05 indicating statistically not significant). So there is no significant association between gender and the steps involved in the hand hygiene protocol.

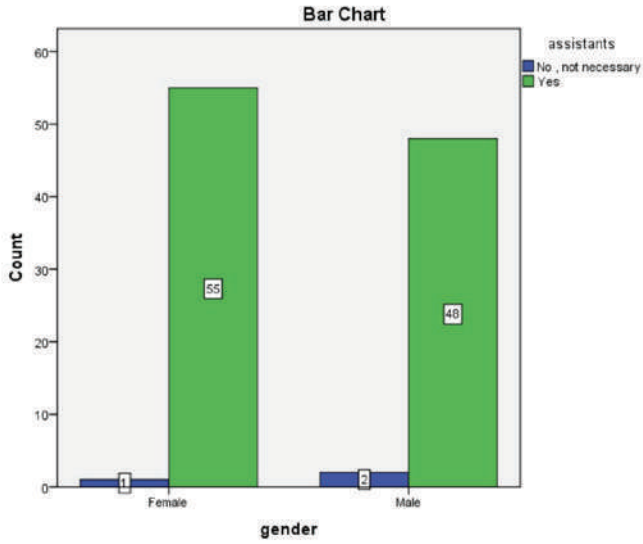


Figure 5: Bar chart depicting the association between gender and the awareness on necessity of hand hygiene among dental assistants where blue denotes No, not necessary and green denotes Yes necessary. X-axis represents the gender and Y-axis represents the number of respondents. Chi square test showing p = 0.493 , so (p > 0.05 indicating statistically not significant). So there is no significant association between gender and the awareness of the necessity of hand hygiene among dental assistants

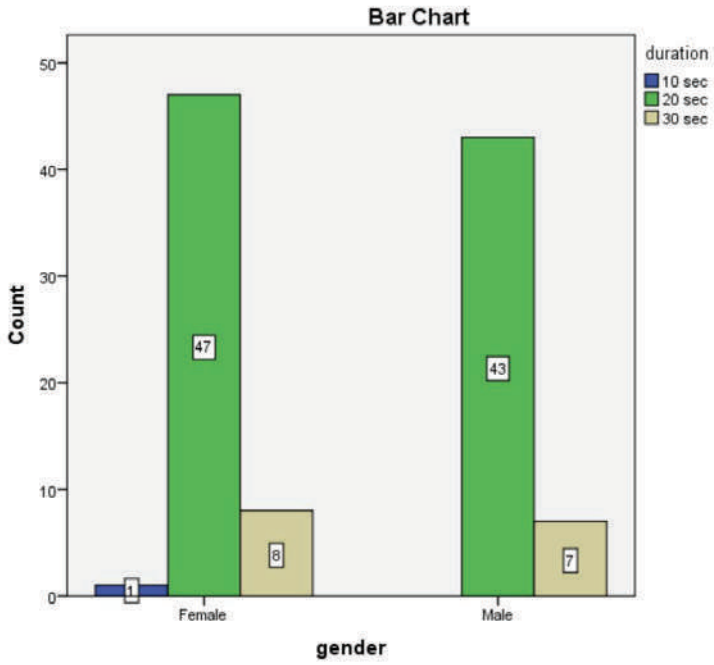


Figure 6: Bar chart depicting the association between gender and the awareness of duration taken for hand wash technique where blue denotes 10 seconds, green denotes 20 seconds, yellow denotes 30 seconds. X-axis represents the gender and Y-axis represents the number of respondents. Chi square test showing p = 0.635 (p >0.05 indicating statistically not significant). So there is no significant association between gender and the awareness of duration taken for hand wash technique

Annexure -I

S.no	Questions	Options
1.	Age	above 25
2.	Gender	Male/Female
3.	Have you attended a hand hygiene program before?	yes/ no
4.	Is contact the main route of transmission of potentially harmful germs to patients?	yes/no
5.	Do you think hand hygiene prevents the spread of infections to patients?	yes/No
6.	Do you believe that there is an association between hand hygiene and infection control?	yes/No
7.	Do hand nails hinder hand hygiene protocol?	yes/no/maybe
8.	How many steps of hand wash techniques are involved as per WHO	5/6/7/8
9.	Is it necessary to teach dental assistants on the Hand Wash technique?	yes/no
10.	Do you think hand hygiene products are detrimental to the skin?	yes/ no/ Maybe
11.	What is the duration of handwashing with soap and water?	20 secs/30 sec/10sec
12.	If you follow CDC hand hygiene guidelines, does it affect our skin?	yes/no/maybe
13.	Does hand rubbing & washing are performed in sequence?	yes/No
14.	Is it necessary to maintain hand hygiene before patient contact?	yes/ No/ Maybe
15.	How important is hand hygiene at your dental office?	Most important/ moderately important/ less important / not important at all

Conclusion

Hand hygiene is mandatory to prevent communicable diseases, especially in dental practice as the dentists are in close contact with patients. This survey could bring awareness about the need for hand hygiene to the readers as well as to dentists.

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Source of Funding: Self

Ethical Clearance: Not Required

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Knowledge and Awareness about Craniofacial Surgery among Dental Students - A Survey

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Abstract

Craniofacial surgery is a subspecialty in dentistry that deals with congenital and acquired deformities of head, neck, skull, face and jaw and associated structures. Craniosynostosis, craniofacial clefts, cleft lip and palate , teachers collins syndrome are a few typical defects treated by craniofacial surgeons.Craniofacial surgery reconstructs damaged bone and tissue and improves the appearance of disfigured areas of the face and head. For children with craniofacial anomalies, early surgery can minimize the impact of these conditions on growth, development, and function. Craniofacial surgery fellowship. Such fellowships are accessible for members with residency in oral and maxillofacial surgery , plastic and constructive surgeries. Those surgeons may be awarded as single degree or dual-degree surgeons, in US, cleft and craniofacial centers are available in special centres like academic institutions.The questions were prepared and distributed through an online survey planet link the participants well explained about the study and the results were collected and statistically analysed. From the analysed results we can see that the majority know about craniofacial surgery is but not in depth . The awareness must be made on craniofacial surgery among undergraduate dental students. From the survey research done To evaluate the undergraduate dental students are aware and have knowledge on craniofacial surgeries and techniques but not in depth.

Keywords: *craniofacial surgery,craniofacial anomalies , knowledge , dental undergraduates*

Introduction

Craniofacial surgery is a surgical subspecialty that deals with congenital and acquired deformities of the head[1]. Although craniofacial treatment involves manipulation of the bones ,craniofacial surgery is not tissue based/specific; craniofacial surgery deals with bone,skin,nerve,muscle ,teeth and other related structures/anatomy [2 3]. There a more than a number of different surgeries in Craniofacial surgery .Facial anomalies typically treated by craniofacial surgeons includes craniosynostosis, rare craniofacial clefts, cleft lip and palate,micrognathia, teachers Collins syndrome, Crouzon's syndrome and many other conditions [2,4] . Computer assisted surgery has been more and widely used in craniofacial surgery in recent years.[5] The various advantages of the techniques are emphasized from a surgical and form a teaching standpoint . In recent decades the evolution of procedures to treat craniosynostosis has resulted in improvement in perioperative morbidity including decreased blood loss and transfusion ,shorter operation,and shorter hospital stays [67]. The main goals of craniofacial surgery is to maintain hemodynamic stability and oxygen carrying capacity and to prevent and treat hyper fibrosis and delusional coagulopathy during the surgery [8]. Over transfusion and transfusion related side effects should be minimised .The recent explosion in our understanding of development biology and genetics has enhanced our understanding of craniofacial biology [8,9]. While studies have reported [9] that it is not possible to summarise each and every new craniofacial findings and development[10]. Ongoing research in bone biology has brought new cutting-edge technologies into everyday use in craniofacial surgery. Nonetheless, when osseous defects the craniomaxillofacial skeleton are encountered, autogenous bone grafting remains the criteria standard for reconstruction . [11].The novel and emerging field of bone-tissue engineering holds great

promise as a limitless source of autogenous bone studies have reported [12,13]. According to the core principles of bone crafting in craniofacial surgery physiology is of great permanent importance . Bone grafting in craniofacial survey ;donor site morbidity and operative risk among the limitations of the autogenous bone grafting harvest[14].

In this survey research was done to assess the awareness and knowledge of undergraduate dental students on craniofacial surgeries through an online survey.

Materials and Method

Study Design:

This was an experimental study based on a questionnaire. The sample size of this study is 128. There was voluntary involvement by the participants and no incentives were provided for the participants. The sampling method done in this survey is simple random sampling and the measures taken to minimise biasing is by using software and avoiding leading questions

Survey Instrument:

Questionnaire was formatted after reviewing the existing literature, the questions were distributed through an online survey platform. The participants were well aware of the survey , the participants were explained about the purpose of this study in detail. The response was, therefore, estimated by personally collecting the completely filled questionnaire from the samples.**.** The answer was marked carefully by the participant. The questions consisted of 10 prepared questions.

Analysis

The results were collected and analysed carefully. Using software SPSS [15–19] and the type of analysis done is descriptive analysis. The independent variable age, sex, gender. The dependent variable of this survey is dental students.

Result and Discussion

The data was collected and analysed This study is to estimate that the majority of the undergraduate students are aware of Craniofacial surgery and have knowledge in craniofacial surgery but not in depth. This is the result analysed from the survey done above.

As the survey was done on a small scale basis the results were taken and analysed carefully and statistically. In (figure 1) Chi square test was done to analyse the association between gender and awareness on craniofacial surgeries in which 30% of female and 27% of the male answered they are aware and 24% of the female and 19% of the male answered they are not aware (P value:0.752). It was found statistically not significant. In (figure 2) Chi square test was done to analyse the association between gender and awareness on procedures under craniofacial surgeries. Out of 17% participants who opted craniofacial reconstruction, 7% constituted female and 10% constituted male .Out of 20% participants who opted craniosynostosis, 10% constituted female amd 10% constituted male .Out of 27% participants who opted cleft lip and palate,10% constituted male and 17% constituted female. Out of 36% participants who opted all the above, 20% constituted female and 16% constitutes male.(P value:0.539). It was found statistically not significant. In (figure 3) Chi square test was done to analyse the association between gender and awareness on craniofacial surgeries including brain and eye. In which 21% of females and 27% of the male answered they are aware and 33% of the female and 19% of the male answered they are not aware (P value:0.048). It was found statistically not significant. In (figure 4) Chi square test was done to analyse the association between gender and awareness on craniofacial doctors treated the same as doctors, In which 29% of female and 28% of the male answered they are aware and 25% of the female and 18% of the male answered they are not aware (P value:0.471). It was found statistically not significant. In (figure 5) Chi square test was done to analyse the association between gender and awareness on craniofacial surgeries are tissue specific, In which 32% of female and 25% of the male answered they are aware and 22% of the female and 21% of the male answered they are not aware (P value:0.621). It was found statistically not significant. In (figure 6) when asked the participants if they knew about craniofacial anomalies 51.11% of the participants knew about craniofacial anomalies (represented in red) and the rest of the participants that is 48.89% said that they are not aware of craniofacial anomalies (represented in blue). The present research work was elicited from works done by investigators, where previous studies

were done based on clinical reports, interventional studies like [20,21] ,in vitro studies like [15,17–19,22–25] , and systematic reviews,[14,26,27,28].According to previous Similar study all predoctoral dental undergraduate students showed learning diagnosis, treatment,awareness on craniofacial surgery and similar to our study. In (figure.10) When asked the participants if they are aware of craniofacial surgeons are being treated the same as MBBS doctors 50.37%(represented in red) said no that they are not being treated in the same way and the rest of the participants that is 49.63% (represented in blue)said that they are being treated in the same way.

According to previous similar study undergraduates should have minimum knowledge awareness on craniofacial surgery. There are no opposing findings to this novel article. Limitations of this study results are that of small population, low sample size and homogeneous population and the answers are biased and could also include more questions and more type of population these are the limitations of this very study. From this study the future scope is that the study must be done in depth and finalized and the population/sample size of the research must be more detailed analysed on this research topic.

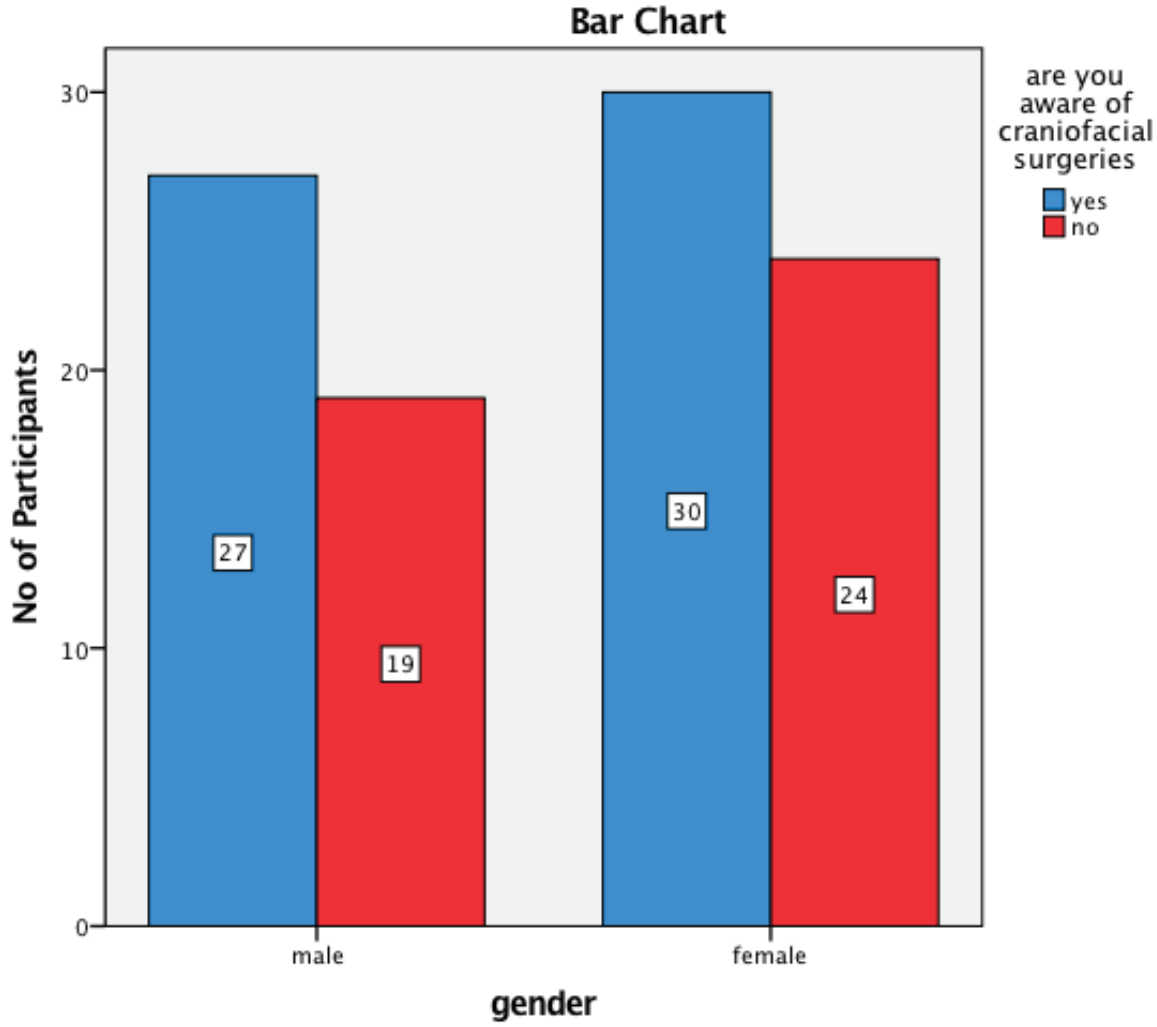


Fig.1 : Bar chart represents the association between gender and awareness on craniofacial surgeries. X-axis represents the Gender and Y-axis represents the number of participants who were aware (blue) and not aware (red). Out of 57% of the participants who were aware, 30% constituted female and 27% constituted male. Females are more aware than males about craniofacial surgeries. Chi square test was done. Pearson's Chi square value: 0.100, DF - 1, P value: 0.752(>0.05) hence it is statistically not significant.

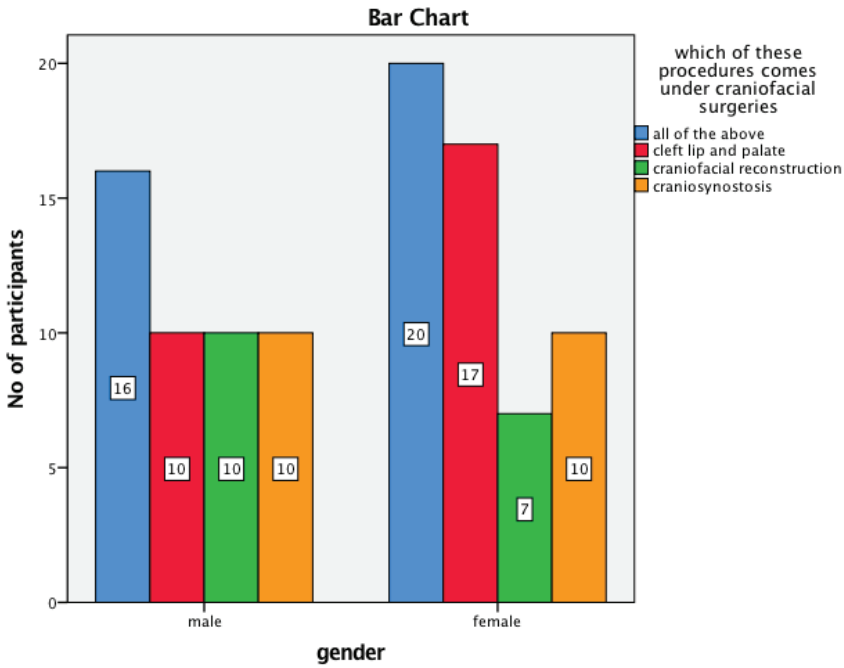


Fig.2: Bar chart represents the association between gender and awareness on procedures under craniofacial surgeries. X-axis represents the Gender and Y-axis represents the number of participants responding awareness on procedures under craniofacial surgeries. Out of 36% participants who opted all the above, 20% constitutes female and 16% constitutes male. Females were more aware about the procedures under craniofacial surgeries than females. Chi square test was done and the association was found statistically not significant. Pearson's Chi square value: 2.163, DF - 3, P value: 0.539(>0.05) hence not significant.

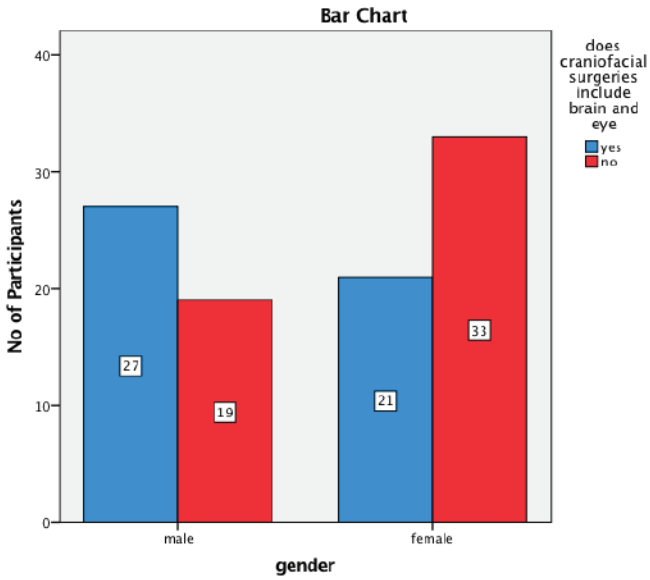


Fig.3 : Bar chart represents the association between gender and awareness on craniofacial surgeries including brain and eye. X-axis represents the Gender and Y-axis represents the number of participants who said yes (blue) and no (red). Out of 48% participants who were aware, 21% constitute female and 27% constitute male. When compared to male, females were slightly more aware Chi square test was done and the association was found statistically not significant. Pearson's Chi square value: 3.904, DF - 1, P value: 0.048(>0.05) hence not significant.

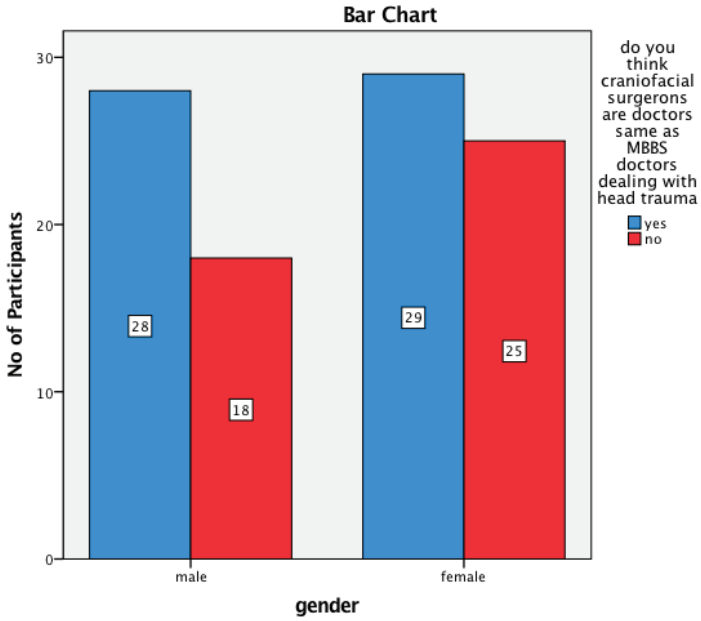


Fig.4 : Bar chart represents the association between gender and awareness on craniofacial doctors treated the same as doctors . X-axis represents the Gender and Y-axis represents the number of participants said yes (blue) and no (red). Out of 57% participants who were aware, 29% constitute female and 28% constitute male.When compared there is no significant difference in responses between male and females. Chi square test was done and the association was found statistically not significant. Pearson's Chi square value: 0.520, DF - 1, P value: 0.471(>0.05) hence not significant.

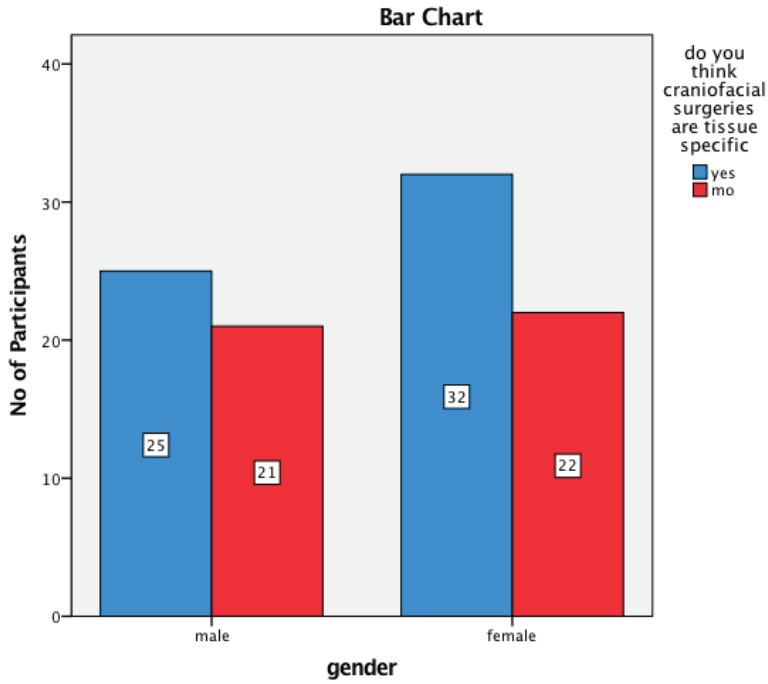


Fig.5 : Bar chart represents the association between gender and awareness on craniofacial surgeries are tissue specific. X-axis represents the Gender and Y-axis represents the number of participants said yes (blue) no (red).Out of 57% of the participants who were aware, 32% constitute female and 25% constitute male. When compared to male, females were slightly more aware. Chi square test was done and the association was found not to be significant statistically. Pearson's Chi square value: 0.244, DF - 1, P value: 0.621(>0.05) hence not significant.

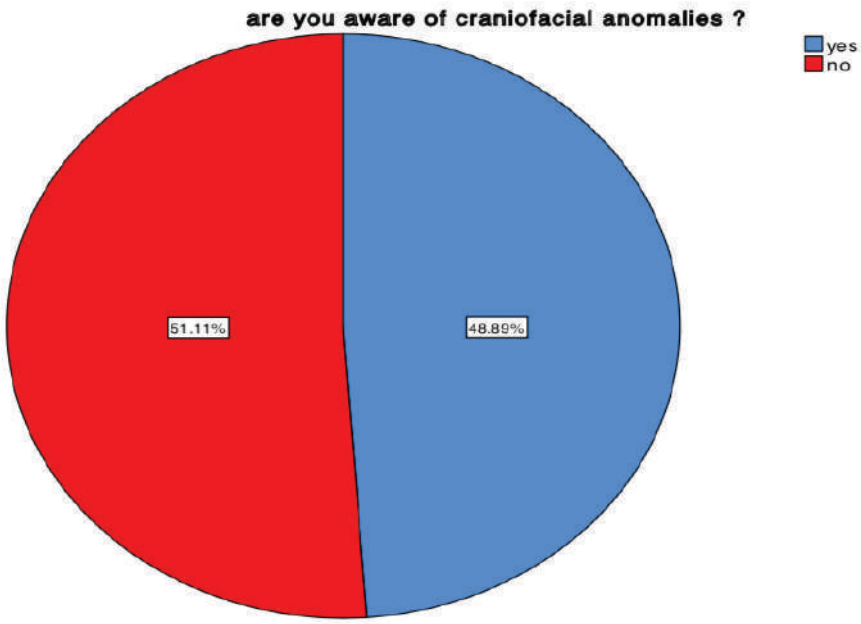


Fig.6: Pie chart depicts knowledge among study participants regarding awareness of craniofacial anomalies. 51.11% responded no (red) and 49.89% responded yes (blue)

Conclusion

Within the limitations of the study following conclusions can be drawn,

The undergraduate students are moderately aware of craniofacial surgery and what it deals with but in-depth knowledge about craniofacial surgery can be gained with further academic progression.

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Conflicts of Interest : The author declares that there was no conflict of interest in the present study.

Source of Funding : Self.

Ethical Clearance: Not Required

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Role of Microorganism as Bioremediation

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Abstract

Through agriculture, industry, and everyday life, harmful chemicals have been discharged into the world's air, soil, and water. Depending on their fixations, these substances can have ruinous outcomes on environments, just as cause serious harm to people and different living beings close by. This chemical has been removed by the process of bioremediation,it is a biological mechanism of recycling wastes into another form that can be used and reused by other organisms.Nowadays, the world is confronting the issue of various natural contamination. Bioremediation has been viewed as a domain of well disposed, modest and effective methods for natural reclamation. Since microorganisms comprise a key factor of this innovation, information on the nature and molecular components of their resistance to expanded overwhelming metal fixations is essential. Microorganisms are basic for a key alternative for conquer challenges.Microorganisms survive in all places in the biosphere because their metabolic activity is astonishing; then come into existence in all over a range of environmental conditions.The nutritional capacity of microorganisms is totally changed, so it is utilized as bioremediation of natural pollutants.Bioremediation is profoundly associated with corruption, eradication, immobilization, or detoxification of chemical waste and physical hazardous materials from the including through the exhaustive and movement of microorganisms. Microorganisms are the significant players in checking such burdens and reusing components by mineralizing or parceling poisons, even in situations poor in supplements or lacking accessibility of significant electron contributors and acceptor.The aim of this review article to discuss the importance of microorganisms in bioremediation.

Keywords: *Detoxification;chemical;microorganisms;ecosystem;environment*

Introduction

Microorganisms in nature have consistently separated the waste, and people have consistently (intentionally or unconsciously) utilized them in farming, household, and mechanical activities[1] and it also plays a vital role in bioremediation.Bioremediation refer to the process of the utilization of microorganisms to corrupt contaminants that present natural and human dangers.

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Bioremediation forms normally include the activities of a wide range of organisms acting in equal or succession to complete the degradation process.Both in situ (in place) and ex situ (evacuation and treatment in other place) remediation approaches are used. The adaptability of organisms to degrade a huge amount of contaminants that makes bioremediation an innovation that can be applied in various soil conditions [2]. The in situ approaches can reduce disruptive engineering practices, bioremediation is still not a common practice[3]. From an ecological perspective, bioremediation relies upon the different associations between three factors:substrate (toxin), life forms, and condition.The usage of bioremediation as a biotechnological procedure involve the microorganisms for illuminating and expelling threats of numerous contaminations through the biodegradation from the environment[4].Microorganisms act as a most important pollutant to excavate the tool in soil, water, and sediments;

mostly due to their advantage over other remediation procedural protocols. Microorganisms restore the natural surroundings and prevent further pollution. Bacteria are broadly microbial creatures, and in this manner it makes biodegradation and bioremediation as important one. There are barely few toxic microorganisms, so there is likely a life form ready to separate any given substrate, when furnished with the correct conditions (anaerobic versus aerobic environment, sufficient electron donors or acceptors, etc.)[5]. Bacteria like streptococcus aureus, Acinetobacter baumannii, Enterococcus faecalis,can help management of bioremediation [6,7,8,9]viruses cannot act as bioremediation[10].

Few Previous articles were referred to,in that role of bioremediation is to enhance the microorganisms with appropriate nutrients and other chemical compounds that will empower them to destroy the contaminant .In order to work the bioremediation system with microorganism it requires the the optimum levels of nutrients and other chemicals essential for their metabolism [11],some bacteria acts as a bioremediation that remove the pollutants,contaminant from the environment. Bacteria that can oxidize aromatics including benzoate, chlorobenzoate, and toluene, coupling the reaction with the decrease of oxygen, chlorate, or nitrate. Due to the high concentration of benzene contamination, especially in ground and surface water, D. aromatic is especially useful for in situ bioremediation of this substance[12]. The new version of bioremediation applications primarily use bacteria, with comparatively few attempts to use fungi. Fungi have important roles ,its participation in the cycling of elements through decomposition and transformation of organic and inorganic materials. These qualities can be converted into applications for bioremediation that separate natural mixes and decrease the dangers of metals. It is able to oxidize a diverse amount of chemicals and survive in harsh environmental conditions such as low moisture and high concentrations of pollutants. Therefore, fungi are potentially an extremely powerful tool in soil bioremediation [13,14,15]. The first Fungi which helps to degradation of organic pollutants is P. chrysosporium . Various research has shown that it has strong potential for bioremediation in pesticides, PAHs, dioxins, carbon tetrachloride, and many other pollutants. Among fungal systems, the P. chrysosporium has selected to demonstrate as bioremediation.Other notable species of white rot fungi include Pleurotus ostreatus and Trametes versicolor.[16]

The main differences between the previous article and this article is that they discussed only about the role of microorganisms in bioremediation but this review article concentrates on techniques followed during the bioremediation that helps to remove the pollutants from the environment. Aim of this review article is to emphasize all about current knowledge on the role of microorganisms in bioremediation and the techniques in bioremediation.

Materials and Method

Review of scientific literature was done in preparation of manuscript. This system and data base searched for relevant articles from PUBMED and GOOGLE SCHOLAR. About 50 articles were collected and analysed and reviewed .Databases of the journal were searching for articles based on the keyword bioremediation microorganism techniques ,cross references were also included .

Biological treatment of man-made waste has successfully been implemented for decades. Municipal and waste water and agriculture and agricultural waste are treatment in bioreactor as activated sludge in water ponds or in fluidized bed reactor containing an active biofilm of microorganism[17].Bioremediation use the characteristic feature of microorganism for transformation,mineralisation or complexation by coordinating those capacities towards natural and inorganic ecological contamination.

Discussion

Microorganism in bioremediation

Microorganisms act against the pollutants only when they have access to a variety of material compounds to help them generate and nutrient to build more cells [18].Besides bacteria they are also efficient in heavy metal bioremediation. Microorganisms have developed the capabilities to protect themselves from heavy metal toxicity by various mechanisms such as adsorption,uptake,oxidation. Some bacteria like Alcaligenes faecalis ,Bacillus pumilus,Brevibacterium iodonium,Staphylococcus ,Acinetobacter baumannii are gaseous methyl mercury [19,20,21,22]. As a bioremediator, there are biological agents which are used to clean the

contaminated site in the area. Bacteria, archaea and fungi are some of typical prime bioremediator[23]. Some of microorganisms can be biomediator which can be used as mouthwash[24,25].Bioremediator also consist of pesticides that kill the unwanted soil microorganism that causes toxicity to environment[26]. The bacteria which performs more antibacterial activity in orange peel has bioremediation [27,28,29] . Finally it is ecocompatibile as well as economically feasible [30].Figure 1 depicts the process of bioremediation which involves microorganisms and other substrates.

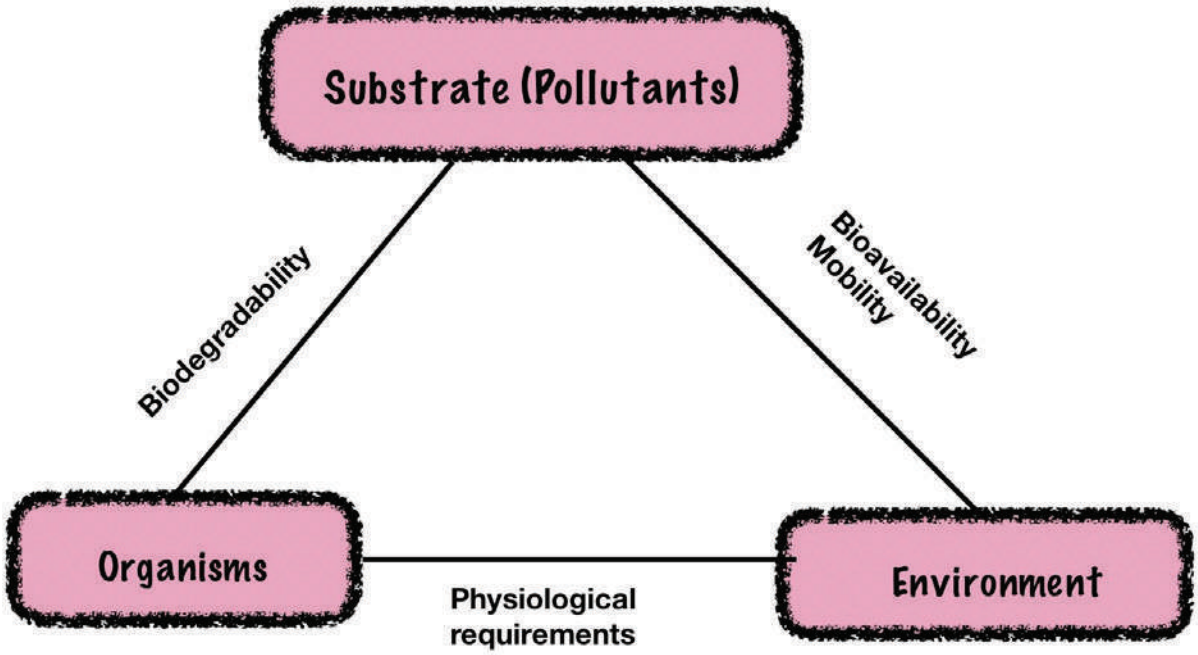


Figure 1 Bioremediation process

Factor affecting microbial bioremediation

Microbial and pollutants are not uniformly spread in the environment.The controlling and optimising of bioremediation process is complex system due to many factor.These factor included capable of degrading the pollutants,availability contaminants to microbial population and environment factor (type soil,temperature,PH,the pressure of oxygen and nutrient.

Biological factor-Major biological factor included is mutation,horizontal gene transfer,enzyme activity,interaction,its own growth until,critical biomass is reached,population size and composition[31],n6-adenosine methylation bacteria is also one of biological factor that affect the bioremediation[32]

Environment factor- Physicochemical bioavailability of pollutants contaminants,concentration, solubility, chemical structure and toxicity[33]. The environment factor microorganisms is a metabolic is a metabolic characteristic of microorganism.This mechanism only succeeds when the microorganism grows in the optimum temperature and PH.some of leaves like laures nobies can be used as bioremediation[34]

Availability of nutrition-The appropriate quality of nutrient is a favourable strategy for increasing the metabolic activity of microorganism and thus biodegradation rate in cold environment [35]. Biodegradation in aquatic environments is limited by the availability[36]. The artificially well-developed mixture of bacterial strain along with inorganic nutrients such as

phosphorus and nitrogen is pumped into the ground or applied to oil spill areas as required for treatment. This increases the rate of bioremediation at the target site.

Principle of bioremediation

The aim of bioremediation is enhanced by the giving the optimum level of nutrient and other essential chemicals that is required to degrade.It is effective,when it has suitable environment that allow the growth to the microorganism and is allow them to perform the microbial activity.There are several parameter that require for the growth of microorganism [37]. The principle of bioremediation is based on the process of degradation.Degradation is the process of removal of unwanted organic toxicity refers to harmless or naturally occurring compounds like carbon dioxide,water,inorganic compounds which are safe for human,animal plant and aquatic life. Many mechanism and pathway have been executed for the biodegradation of wide variety of organic compound in absences or in presences of oxygen

Biostimulation

Biostimulation is widely used to stimulate the nature occurring microbial communities providing them with nutrients and other needs to break down contaminants. Biostimulation can be achieved through change in PH,moisture,aeration or addition of electron acceptors,donors such as phosphorus,nitrogen,oxygen carbon.It is potentially useful for the treatment of less frequently encountered contaminants spill such as pesticides,particularly herbicides[38]. Biostimulation is dependent on the organisms and which requires the environment for their comfort which makes them easier to alter in a way that will have the desired bioremediation effect.

Bioattenuation

It is also called as naturally attenuation is the eradication of pollutants concentration from the surrounding. It is carried out in biological processes; it may include physical phenomenon and chemical reaction. Terms such as biotransformation are included within the more general attenuation[39]. The processes involved are largely due to biodegradation by soil microorganisms and to some extent by interaction with soil matrices. This procedure is frequently considered as a"do nothing" arrangement, yet it requires consistent checking of the contaminant in soil. The time required for natural attention relies upon site conditions and the particular contaminant.

Due to persistent nature and lack of appropriate degrading microorganisms, natural attenuation of a pesticide in some soils takes a long duration.

Bioaugmentation

Another bioremediation approach is termed bioaugmentation,where organism selected for high degradation abilities are used to inoculate the contaminated situ[40].It is also used to ensure that insitu microorganism can totally remove and alter these contaminants to ethylene and chloride which are non toxic[41]. Because of having diverse metabolic profiles to change into less complex and harmless end product[42].The potential of the microorganism and important features is to enable the cells to be functionally active and persistent under the desired environmental conditions. Based on the prior knowledge of microorganism communities inhabiting the target site ,competent microbes should be selected.The stresses that hamper microbial development may remember variances or limits for temperature, water content, pH, exhaustion of supplements, and furthermore possibly poisonous toxin levels in the debased found that organisms having the capacity to corrupt the natural contaminations in societies neglected to do likewise in regular frameworks. The recommended potential explanations behind bioaugmentation disappointment were: issues concerning the adjustment of the vaccinated microorganisms; insufficiency of substrate; rivalry among presented and indigenous biomass; utilization of other natural substrates in inclination to the toxic; and predation.

Genetically Engineered Microorganisms

Genetically engineered microorganism whose genetic material has been already changed by applying genetic engineering technique inspired by natural otherwise artificial genetic exchange microorganism. It has improved the utilization and elimination of hazardous unwanted waste under laboratory condition creating genetically modified organism[43]. Genetically Engineered microorganisms have potential for

bioremediation application in soil ground water etc .

Advantage of bioremediation

Bioremediation is eco friendly and sustainable[44] and it is relatively ease in implementation[45]. Effective way of remediating natural ecosystems from the number of contaminants and acts as an environment friendly option.Bioremediation is a natural process and accepted by the public as a waste treatment process for contaminated material such as soil. There is an increase in number and release of harmless products due to degradation of microbes. The residues for the treatment are usually harmless products such as carbon dioxide, water, and cell biomass.Bioremediation is useful for the complete destruction of a wide variety of contaminants. Many hazardous compounds can be transformed into harmless products. This reduces the chance of future liability associated with the treatment and disposal of contaminated material.

Disadvantage of bioremediation

It is difficult to extrapolate from bench and pilot scale studies and the biological process is often specific -important site factors required for success include the pressure of metabolically capable microbial population and appropriate level of nutrients and contaminants. Research is expected to create and build bioremediation advancements that are reasonable for destinations with complex blends of contaminants that are not equally dispersed in the earth. It might be available as solids, fluids, and gases. Organic procedures are exceptionally explicit. Significant site factors required for progress incorporate the nearness of metabolically able microbial populaces, reasonable natural development conditions, and suitable degrees of supplements and contaminants.

Conclusion

Through agriculture, industry, and daily life, harmful chemicals have been released into the earth's air, soil, and water. Depending on their concentrations, these substances can have destructive consequences on ecosystems, as well as cause severe damage to humans and other organisms nearby. Soil pollution is of special importance because of its impact on surface, groundwater and air contamination and can easily spread and be consumed by humans.Biodegradation is a very fruitful and attractive option to remediating,cleaning,managing and recovering techniques for solving pollutants' environment through microbial activity .The advantage is the use of technology and its increasing popularity through time.It relies on stimulating the growth of certain microbes that use contaminants like oil, solvents, and pesticides as a source of food and energy. These microbes consume the contaminants, converting them into small amounts of water and harmless gases like carbon dioxide

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Recent Advances in Nutraceutical and Functional Food

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Abstract

Food is just fortified into nutraceuticals and functional food. Nutraceuticals or bioceutical is a pharmaceutical alternative which claims nutritional sources. Functional foods are modified food which improve health by providing benefits beyond those provided by traditional nutrients. Eating habits and trends in food production and consumption have healthy environmental and social impacts.Blood pressure effects from different nutraceutical products have been studied with the specific food rich in minerals, lipids, whole protein and amino acid. Nutraceuticals range from isolated nutrients, specific diets to genetically engineered designer food herbal products and processed food such as cereals, soup and beverages. Recent experiment has shown that it may also inhibit plaque formation by bacteria in the mouth by the basic mechanism of inhibiting biofilm formation. Plaque is the primary cause of gingivitis.When there is an increased plaque accumulation, there is also an elevation of microbial count in the saliva and biofilm. The plaque deposition around the gingival margin constituting bacteria that are anaerobic and aerobic is capable of initiating periodontal disease and destruction. Fruits and vegetables contain thousands of biologically active phytochemicals some of which likely interact in a number of ways to prevent disease and promote health. Rapid changes in diet and lifestyle may enhance the expression of the harmful genes, which manifests in a sequence. There is an emergence of chronic disease in developing countries. Both pharmaceuticals and nutraceuticals compounds might be used to cure or prevent diseases. Therapeutic injections like lipotropic injection are also shown to improve the result of diet and exercise.Compounds not suspected of having any antibacterial properties such as pesticide were shown to increase the minimum inhibitory concentrations of antibiotics against a strain of staphylococcus aureus both alone and in conjunction with antibiotics where Carbapenem are often considered to have a broad spectrum of activity .

Keywords: *Blood pressure;Disease;Food supplement;Functional food;Nutraceutical factor.*

Introduction

Food is just fortified into nutraceuticals and functional food. Nutraceuticals or bioceuticals are pharmaceutical alternatives to nutritional sources. Functional foods are modified foods which improve health by providing benefits beyond those provided by traditional nutrients. It also includes items such as cereals, breads and beverages. But the commonest thing of both will be an enhanced physiological benefit. Excluding water, all food is a mix of several macro, micro and phytonutrients and are therefore complex systems [1]. Eating habits and trends in food production and consumption have healthy environmental and social impacts. Diet has implications on gut health that improve complexities such as ulcerative colitis, Crohn's disease , irritable bowel syndrome which result from excessive growth of intestinal microbial flora [2]. The interest in nutraceutical and functional foods stem from research efforts to recognize properties and possible application of the nutraceutical substance ,coupled with public

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attraction and consumer demand [3]. Blood Pressure effects from different nutraceutical products have been studied with the specific food rich in minerals, lipids, whole protein and amino acid [4]. Common genetic variants of Fat mass Obesity also known as alpha ketoglutarate dependent dioxygenase have been positively related with blood pressure in patients with hypertension [5]. Several special nutraceuticals are ginseng, echinacea, omega -3 and cod-liver oil .In current times, nutraceuticals have collected sustainable interest due to possible nutritional safety and medicinal effect [6,7].

Nutraceuticals range from isolated nutrients, dietary supplements and specific diets to genetically engineered designer food herbal products and processed food such as cereals, soup and beverages [8]. Cranberry juice cocktail is now marketed as a functional food with a structure /function claim regarding support of urinary tract health .Recent experiment has shown that it may also prevent plaque formation by bacteria in the mouth by the basic mechanism of inhibiting biofilm formation [9,10,11]. Plaque is the primary cause of gingivitis [12,13]. When there is an increased plaque accumulation, there is also an elevation of microbial count in the saliva and the biofilm. The plaque deposition around the gingival margin constituting bacteria that are anaerobic and aerobic is capable of initiating periodontal disease and destruction[14]. Medicaments [15,16] like kin perio gel can be used to treat gingivitis. There is evidence that certain beverages and food can protect against gingivitis[17].

Fruits and vegetables contain thousands of biologically active phytochemicals some of which likely interact in a number of ways to prevent disease and promote health [18]. They also act as an antimicrobial agent [19,20]. In a study, overweight participants found the consumption of whole pea flour and fractionated yellow pea flour at doses equivalent to half a cup that is used to reduce insulin resistance ,while wholePea flour reduced adiposity in women. Rapid changes in diet and lifestyle may enhance the expression of the harmful gene ,which manifests in a sequence. There is an emergence of chronic diseases in developing countries [21]. Both pharmaceutical and nutraceutical compounds might be used to cure or prevent disease but only pharmaceutical compounds have government sanction with no evident protective effect [22]. Therapeutic Injection [23] like lipotropic injections are also shown to improve the result of diet and exercise. Compounds not suspected of having any antibacterial properties such as pesticide were shown to increase the minimum inhibitory concentrations of antibiotics against a strain of staphylococcus aureus both alone and in conjunction with antibiotics [24] where Carbapenem [25] are often considered to have a broad spectrum of activity .

Nutraceutical Factor:

The proper organizational scheme for nutraceuticals can vary. Conditions like hypertension, hypercholesterolemia, clotting inadequacies related to diabetic conditions etc, are areas these superfoods work on. N-3 fatty acids, phytosterols, quercetin and grape flavonoids are all nutraceuticals. Meanwhile, oncologists may be more interested in those substances that target anticariogenic activities. However, the anticariogenic triterpene limonin being soluble in lipids and bitter to taste, is not used as a functional food ingredient [26]. Legislation therapeutic product or more beneficial to patients mainly protecting public health and assuring is effective [27].

Traditional and Nontraditional Nutraceuticals:

The natural, whole foods we consume are traditional nutraceuticals. With updated information on their nutrient status, we are just inclined to consume them better. The natural nutrients present in the common grains, fruits, vegetables, poultry and dairy products that we consume are beneficial in a holistic manner, providing essentials like Omega-3-fatty acids (as in cod) and lycopene (from tomatoes). Chocolates and teas have been noted in some studies to contain health-benefiting attributes [28]. Foods obtained through agricultural breeding or fortification with nutrients so as to increase their nutritive values, are non-traditional nutraceuticals [29].

Global Demand of Nutraceutical:

In some nutraceutical industries, the segment of dietary supplement of herbal and natural products is not available because of global demand it is commonly regulated[30]. Nutraceuticals are generally reported to have a good safety profile with few unwanted side effects and high bioavailability [31].

Allergy and Nutraceuticals:

Allergy is a hypersensitivity disorder of the immune

system.An allergic reaction usually occurs when a person's immune system reacts to normally harmless substances [32]. An increased activation of white blood cells and basophils by the antibody immunoglobulin E, inflammatory responses are generally mediated, making these reactions due to consuming certain foods unique [33]. Quercetin protects low-density Lipoprotein from becoming damaged especially to blood vessels . Diabetic patients are at higher risk of blood vessels damaged from oxidative stress, quercetin is beneficial in these patients [34].

Food Supplements:

In industrialized countries, there is virtually no restriction on the strength of the nutritional supplement that can be purchased. The significant limitation on the sale of supplements is that the disease and cure related monetary claims cannot be made [35]. Basically, the vitamins, fats, proteins and carbohydrates required in a healthy diet in adequate quantities, are provided by functional foods [36].

Food and Health:

Food habits developed in early infancy , development of food habits is a complex process that may be influenced by factors such as region ,family structure, habit, income and technological advancement [37,38]. Several epidemiologic studies over the last 50 years have clearly shown that diet dominated by fruits, vegetables and some dietary fibres prevent and reduce the risk of chronic disease and promote human health [39] .Dietary intake of fruit , vegetable and whole grains strongly associated with a reduced risk of developing chronic disease such as cardiovascular cancer [40].

Probiotic, Prebiotic and Synbiotic:

Probiotics are living microorganisms which upon ingestion in a certain number exert health benefits beyond inherant basic nutrition [41]. Prebiotic is a specifically fermented ingridient, or a fibre that permits explicit changes, both in the synthesis and additionally movement of gastrointestinal flora and resultantly presenting benefits on the well-being of the host [42]. Synbiotics are the synergistic combination of probiotics and prebiotics [43].

Biotechnology for Functional Food:

Food sources with expanded dietary benefits include stable starchy crops such as yams that have been altered to the substance of starch which has been related to Type II diabetes [44]. Different breeding varieties sometimes lead to malnutrition [45]. Fungi and algae [46] can also be used as functional food as they are rich in marine minerals like iodine, magnesium, potassium and calcium.

Conclusion

Lifestyle of modern age people have faced many challenges, especially health issues such as obesity, osteoporosis, cancer, diabetes and several chronic problems. Nutraceuticals and functional food play an important role in controlling such disease with the health benefit to people .It helps in nutritional, immunology and physiological function with the prevention or treatment of disease.

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Will Poly Ether Ether Ketone Outshine the Existing Dental Materials? - An Overview

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Abstract

PEEK stands for Poly Ether Ether Ketone.. It is a semi crystalline polycyclic aromatic linear polymer. It is a white and a radiolucent material that is rigid in nature. PEEK is the latest invention in the field of dentistry. It is known that this material can prove as a substitute for the existing dental materials. It is claimed that this has better properties than other materials. PEEK has been the center of attention for a lot of scientists looking for better alternatives to the existing materials. Various researches and reviews have been carried out in detail to study this material. The role of Poly Ether Ether Ketone in dentistry by itself has been a majorly studied topic. A literature review was carried out using a systematic search strategy and articles were found using keywords. Literature was taken from databases like PubMed and Google Scholar. Articles that discussed the properties of PEEK and its applications were included. Other articles which had data regarding the structure and its advantages were also included. This review summarized the properties of Poly Ether Ether Ketone. Its structure, and other applications in different dental materials were also discussed in detail.

Keywords: *Dentistry; Dental Materials; Implant Materials;Modifications of PEEK; PEEK;Properties*

Introduction

PEEK stands for Poly Ether Ether Ketone. It is a semi crystalline polycyclic aromatic linear polymer. It gained attention because of its great thermal stability. It is a white and a radiolucent material that is rigid in nature [1]. PEEK is the latest invention in the field of dentistry. It is known that this material can prove as a substitute for the existing dental materials. It is claimed that this has better properties than other materials [2]. It portrays many qualities that make it better than other materials. It is a strong material and has been proposed to be used in the framework for RPDs in dentistry [3]. RPDs and FPDs are the primary treatment choice for a partially edentulous patient. They should be able to provide comfort and aesthetic restoration to the patient [4]. The replacement of teeth by a denture can be called successful depending on a variety of factors [5].

Poly Ether Ether Ketone is being researched widely because it possesses many qualities that are not present in the existing conventional materials. It is known to be non-allergic and brings about lesser or no hypersensitivity or allergic reactions. It has a lower affinity to plaque; hence it will not facilitate lodging of infection causing bacteria and will protect the tooth from caries. To achieve higher aesthetic stability, polishing of a dental material is very essential. PEEK can be highly polished and hence will provide an aesthetic result. It has a good wear resistance. It will not wear out easily and add to the life of the dental appliance/material [6]. As discussed earlier, it has high temperature stability and is also more aesthetic [7]. This is important because when a patient is being given a denture, providing aesthetics and restoring normal function is very essential[8] [9]. Another advantage is that it has poor electrical and thermal

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conductivity and low frictional properties[10 11].

PEEK has been the center of attention for a lot of scientists looking for better alternatives to the existing materials. Various researches and reviews have been carried out in detail to study this material. The role of Poly Ether Ether Ketone in dentistry by itself has been a majorly studied topic. The applications of PEEK have been studied in the field of Implantology as well as Prosthodontics [12]. It has been researched to see if it can be used as a material for forming the framework of removable dental prostheses[13]. It has widely been studied to check if it can be the constituent of dental implants. Dental implants require stress bearing materials that do not give way easily and that also provide osseointegration without causing allergies. Various researches have been carried out to study PEEK as a dental implant material.

As every concept has certain downfalls, even PEEK possesses some disadvantages. Firstly, it is bio inert. It doesn't react much with the surrounding tissues, which makes it difficult to apply in the field of Implantology[14]. It may cause resorption of bone due to various causes. All of this will eventually lead to failure of the dental prostheses. It is hydrophobic in nature, and will not interact with bone; hence, more research is required to substitute it completely as an implant material [15]. It may also bring about allergic reactions in certain cases which cannot be predicted beforehand. It is not yet developed to its full potential and extensive research has to be carried out to understand its properties in detail.

This review on PEEK has been carried out because it has a tremendous potential that is yet to be discovered fully. It has excellent properties that are much better in various aspects when compared to the existing dental materials. It can be used in different areas like implants and RPD frameworks as well. It is a strong, durable and highly aesthetic material which can also be polished well. It can be introduced as an alternative to many existing materials. The aim of this research is to understand the emerging importance of PEEK as a material in dentistry.

What is PEEK?

PEEK stands for Poly Ether Ether Ketone. It is a polycyclic, aromatic, semi-crystalline structure that is best known for its array of properties. These properties are best suited for certain dental products hence PEEK has been introduced into the field of dentistry. It is basically a white/colourless thermoplastic polymer. It portrays high strength and good wear resistance amongst its various other plus points. It also has a high temperature resistance when compared to other dental materials. It has already been widely under use in the field of Implantology and prosthodontics [16].

Properties of PEEK:

PEEK has numerous properties that all contribute to its widely gained attention and property. It has a very low elasticity modulus, which is similar to bone[17]. It is known to be an insoluble material. The insoluble nature is attributed to its high temperature stability and low density. It is a white and rigid material that is also radiolucent. The radiolucency of this material proves very advantageous for its application in dentistry. PEEK has a low plaque affinity hence it would be helpful in preventing plaque induced dental diseases. Low plaque affinity ensures lesser periodontal and other dental problems. This is because Dental plaque is known to cause periodontal irritation.[18].Some studies showed that Aloe Vera also ensures lesser plaque affinity[19]. It has a great thermal stability thus, does not distort easily even under high temperatures. PEEK can be produced using CAD/CAM techniques which have gained importance due to the large number of benefits[20].

Exclusive features:

Due to the endless number of features that PEEK presents, it has gained popularity across various fields. The properties are being studied extensively to suit the particular requirements of the field. PEEK is a high performance polymer that is employed in medical as well as engineering fields. This wide range of application across various fields is due to its extremely favourable and distinct properties. It exhibits a good level of biocompatibility in most cases. As discussed earlier, it has excellent mechanical properties. It can be called an all in one material that can be employed in the dental field and yield better results[21 22]. PEEK is an inert material and does not exhibit any toxicity effects as far as facts are known. Because it will not cause any allergy/ toxicity, it can be substituted in patients that are allergic to titanium metal [23 24]. The modulus of elasticity of PEEK is in sync with that of bone, which adds on to the plus points[25]. Because of these numerous extravagant

properties, PEEK can be set to replace the conventional PMMA material in the upcoming years [26]. It is proven as the result of studies that PEEK does not cause cell damage in any way[27].

Applications of PEEK in Implantology:

PEEK is widely used as an implant material. When being employed as an implant material, it is necessary to maintain integrity of the implant. The stress to the bone has to be reduced[28]. While placing an implant, there are various factors to be taken into consideration. The fatigue caused due to masticatory forces should also be considered[29]. The material of the implant is extremely important [30]. It is said that PEEK can replace Titanium as an implant material in the future.

PEEK as temporary crowns:

PEEK is also used to make crowns that are produced through the process of Computer Aided Design and Computer Aided Milling. Ceramics are also used to replace missing teeth. But recently, PEEK proves to have more advantages over conventional materials [31]. Its advantage is that it is highly resistant to fracture. Such crowns are advantageous because they do not bring about attrition of the opposite tooth.

Role of PEEK in Orthodontics:

Another application of PEEK is the reinforcement of NiTi wires with this material. It may add to the flexibility of the material that is widely used in the field of Orthodontics. The most frequent application of PEEK is in the construction of Denture bases used in Dentistry. Prosthetic Rehabilitation is of utmost importance in patients that are missing teeth or have any bony defects[32].

PEEK in Maxillofacial Reconstruction:

PEEK can be directly bound to composites through polymerisation by light. This adds to the aesthetic component. Another interesting application of PEEK is seen in Maxillofacial Reconstruction. Conventionally, materials like PMMA, Copolymers, Silicone were used. Nowadays, even PEEK is being used to make prosthesis to reconstruct facial features [33 34].

Added Benefits:

As discussed in the above sections, PEEK can successfully replace titanium metal in the dental field. This can be done to provide optimum results to those that have allergic reactions to the titanium metal. It is a known fact that it has high strength, and it is also studied in vivo that PEEK has shown resistance to degradation. These properties compiled together would make the material- PEEK an ideal material for an implant[35]. PEEK should be used in making denture base materials whenever it can be used as it can preserve the supporting structures of the prosthesis[36]. It has lesser plaque affinity than conventional PMMA[37].

Modifications:

To overcome a few of the downfalls, PEEK has been modified by incorporating other materials like nanoparticles or reinforcement by fibre, carbon, etc. These modifications enhance the cell adhesion properties and thus, enhance the biocompatibility as well. When modified, it can also reduce peri-implant inflammation. To increase its elastic modulus and gain an altered resultant, PEEK has also been reinforced with Fibre, Carbon and Glass. The surface modifications lead to an alteration in the surface characteristics of the material. It improves the mechanical properties and hence improves biocompatibility as well[38]. This material was also modified and then used to make dental prostheses. These resultant prostheses made from modified PEEK, were lighter in weight, provided a better fitting and also was more functional [39]. Because PEEK is an inert material, various modifications have been tried and analyzed to make it more interactive and this also indirectly leads to a rise in tensile strength[40]. In recent times, microbes have started gaining resistance and easily bring about infection. Most infections are treated using oral antimicrobials. But to prevent this, incorporation of PEEK with Nanoparticles that have an anti-microbial activity may prove to be useful [4142]. Recently, more efforts are being taken to increase the bioactivity of PEEK as an implant. This is done by introducing bioactive materials [43].Lately a research was conducted to modify PEEK and use it as a substitute for fabrication of a resin bound fixed dental prostheses[44].

Carbon Fibre Reinforced and Glass Fibre Reinforced PEEK:

As discussed above, PEEK has been incorporated along with other materials to provide better properties.

Carbon-fiber reinforced PEEK showed greater compressive strength when compared to conventional. [45] Carbon-fiber reinforced PEEK has a modulus similar to that of bone and it can withstand fatigue strain [46] .In comparison to unfilled PEEK, glass fibre reinforced PEEK is proven to have increased mechanical strength and increased rigidity. [47] The tensile strength and flexural strength of GF/PEEK is higher than those of pure PEEK.[48]

BioHPP from PEEK family:

BioHPP is a High Performance Polymer belonging to the PEEK family. It is used in fabrication of prosthetic restorations. It is known to be the most stable non-metal material used in the field of dentistry [49] . It is a material with a wide range of advantages. It can resist fracture, it can be easily trimmed and is shock absorbing (ideal for implants). This material is non toxic and extremely biocompatible. As per clinical reports, it is suggested that BioHPP can be used to construct complete arch frameworks. [50] This material has been used as an alternative to make prosthetics over dental implants.[51] BioHPP has been in use in the field of medicine for more than 20 years due to its characteristic properties and low cost. [52]

Conclusion

This review was conducted to understand the basics of Poly Ether Ether Ketone (PEEK). It is a heavy polymer that belongs to the family of Poly Aryl Ether Ketone (PAEK). It is a semi crystalline polymer that is white and rigid in nature. It has great properties like strength, thermal stability and wear resistance amongst others. It is known that PEEK is used for dental procedures, implants and denture bases. A variety of properties were analysed in this review. There are multiple advantages as well as disadvantages for this material. The full potential of this material is not yet discovered. It may develop in the coming years and expand its range of applications.

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Prevalence of Root Canal Treated Anteriors in Patients Seeking Orthodontic Treatment

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Abstract

A definitive relationship exists between a root canal treated teeth and orthodontic tooth movement. Aim of this study was to assess the prevalence of root canal treated anteriors in the patients reporting for orthodontic treatment. Orthopantomogram of 550 patients were assessed for presence of any endodontically treated upper and lower anterior and was re-verified with the clinical data. Descriptive Statistics was used to to represent the data.Chi- Square test was done to evaluate the association of RC Treated anteriors between the genders.Prevalence of endodontically treated anterior teeth was found 0.4% was found in the current study. Highest prevalence was of maxillary central incisors ,secondary to trauma , in 96% cases. Maxillary Central Incisors are expected to be involved in trauma more frequently and therefore have higher incidence of root canal treatment. Multi-centre approach is needed before generalising the results to the entire population.

Key words:*Prevalence; Anterior Teeth; Endodontically treated teeth; Orthodontic Patients.*

Introduction

Root Canal Treatment and Orthodontics have been linked since years.There has been constant debates and dimorphism in opinion about success of Root Canal Treatment.Literature suggests there may be increased risk of root resorption when orthodontic force is applied to the endodontically treated tooth or teeth. [1–4]There is no solid literature to report as how orthodontic factors influence root resorption in Endodontically Treated Teeth. Orthodontically Induced External Root Resorption occurs in two forms: a) On the surface by loss of cementum. b) If this surface is apical end of root ,it is manifested as shortening of tooth or blunting of root.[5,6]Various studies have reported various degrees of EARR in orthodontic patients ranging from 48-66%.[5–7]

Root Resorption can be: A) Mild or Clinically Insignificant (less than 2mm) ; B)Severe or Clinically significant with more than 4mm of Root Resorption or more than one-third of root length resorbed. The laters occurs frequently during orthodontic treatment and has been reported to occur in 14.5 % incisors.[8–10]Etiology of External Apical Root Resorption is not fully understood .[11–13]Multiple factors like- individual's susceptibility, genetic predisposition [14], anatomical features , orthodontic treatment mechanics are few which can be held responsible for EARR [9,15]. The extent to which these factors affect Root Resorption is unpredictable and controversial.Orthodontic treatment, Orthodontic Tooth Movement and Root Canal Treatment makes an essential triangle of concern . Reports have shown that endodontically treated tooth / teeth respond similarly to vital teeth to orthodontic force [6,16–18]. However, there exists another view that endodontically treated teeth are more susceptible to EARR.

The purpose of this study is to know the prevalence of endodontically filled anteriors in patients reporting for orthodontic treatment and to retrospectively assess cause of same, and ultimately update our data about present prevalence rate of RC-filled teeth and enhance our knowledge about employing necessary measures in our practice to minimize the effect of orthodontic treatment on EARR of such endodontically treated teeth.

Material and Method

This retrospective study was conducted in the Department of Orthodontics , Saveetha Dental College and Hospital.

Patient Selection:

The pretreatment orthopantomogramme of all patients who had reported to the department since June, 2019 till March,20202 for orthodontic correction were eligible to be included in the study.The sample size of 550 was calculated using G*power software,based on calculation from previous study . In each sample 12 anteriors were assessed which equals to 6000 anterior teeth in the entire sample population.The teeth assessed were: Permanent Maxillary and Mandibular : Central Incisors, Lateral Incisors and Canines i.e 12 teeth per individual were assessed .

Inclusion Criteria:

Patients seeking orthodontic treatment.

Age: Patients above 12 years of age (marking the eruption of all permanent anteriors in upper and lower arch)

Non-syndromic/Syndromic Patients : because the aim was to assess overall prevalence.

Exclusion Criteria:

Patients younger than 12 years.

Patients in which canines were yet to erupt/or impacted.

Data Collection:

The data collection from the electronic patient database.This database stores every information pertaining to history , general examination , required treatment , patient consent , treatment details and all radiographic records of nearly 86000 patients. This database was reviewed based on inclusion criteria and dental history and clinical status of each eligible patient was obtained and tabulated.OPGs were individually downloaded from each patient's radiographic record.

Data Assessment:

Once the data collection was completed, each detail of patient was tabulated, OPGs were assessed and if any endodontically treated was present, a cross evaluation was done by checking through clinical data.The reason for Root Canal Treatment was evaluated from Patients Dental History Record.All the data was tabulated.Once data collection was done, the results were obtained.Chi-Square test was done to see associations between RC treated anteriors and Gender.

Results and Discussion

Prevalence of endodontically treated is found to be 0.4 % .In total six - thousand anteriors analysed, 26 anteriors were RC treated (FIGURE:1) .In 26 RC treated anteriors , 18 were of Male and 6 belonged to female population.(GRAPH 1)

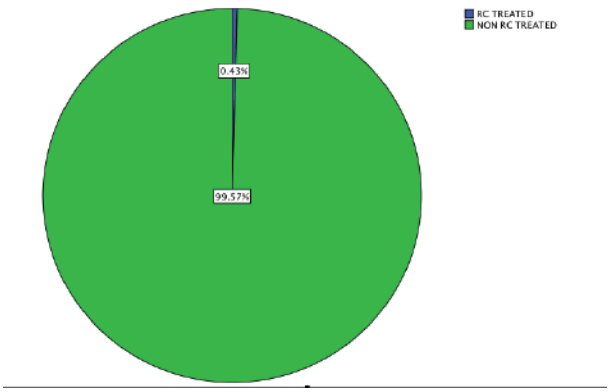


FIGURE 1: The Pie Chart showing frequency of RC treated anteriors in orthodontic population.Blue colour denotes patient who had Root Canal Treated Anterior teeth and Green color denotes the population without any Root Canal treated anteriors. A total of 0.43% (26-blue) Anterior Teeth Were Found To Be Rc Treated Of 6000 Anteriors Assessed.

Individual prevalence of Maxillary Central Incisor being endodontically treated was highest with 73% .15% of Mandibular central incisor accounts of total RC treated anteriors. Maxillary laterals and canines have an individual prevalence of 4% each of total RC treated anteriors. Least was Maxillary Canines with none being endodontically treated in all 550 OPGs assessed(FIGURE-2). Chi -Square test revealed that there was a statistically significant association of RC treated anteriors between males and females (p-0.011, p<0.05) (FIGURE -3)

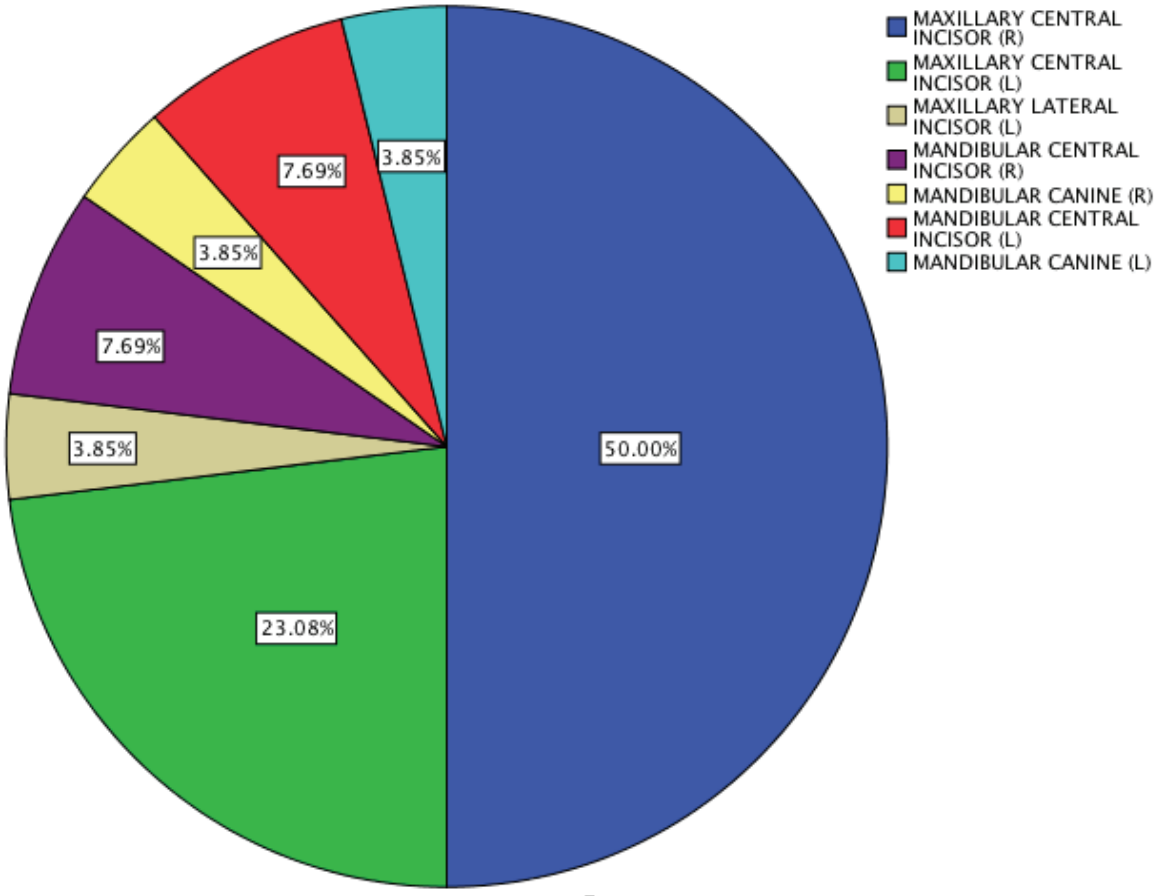


FIGURE 2:This Pie Chart represents the individual percentage of Endodontically treated Anteriors .Of all RC treated anteriors 50% were Right Maxillary Central incisors (Blue) and 23.1 % were Left Maxillary Central incisors(Green). Overall, 73.1% of RC treated anteriors were Maxillary Central Incisors. Mandibular Right Central incisor(Purple) and Mandibular Left Central incisor(Red) were endodontically treated 7.7% orthodontic patients respectively.Maxillary Lateral Incisor (Beige), Right Mandibular Canine (yellow)and left mandibular canine (blue) each contributed 3.8% of total Root Canal Treated Anteriors.

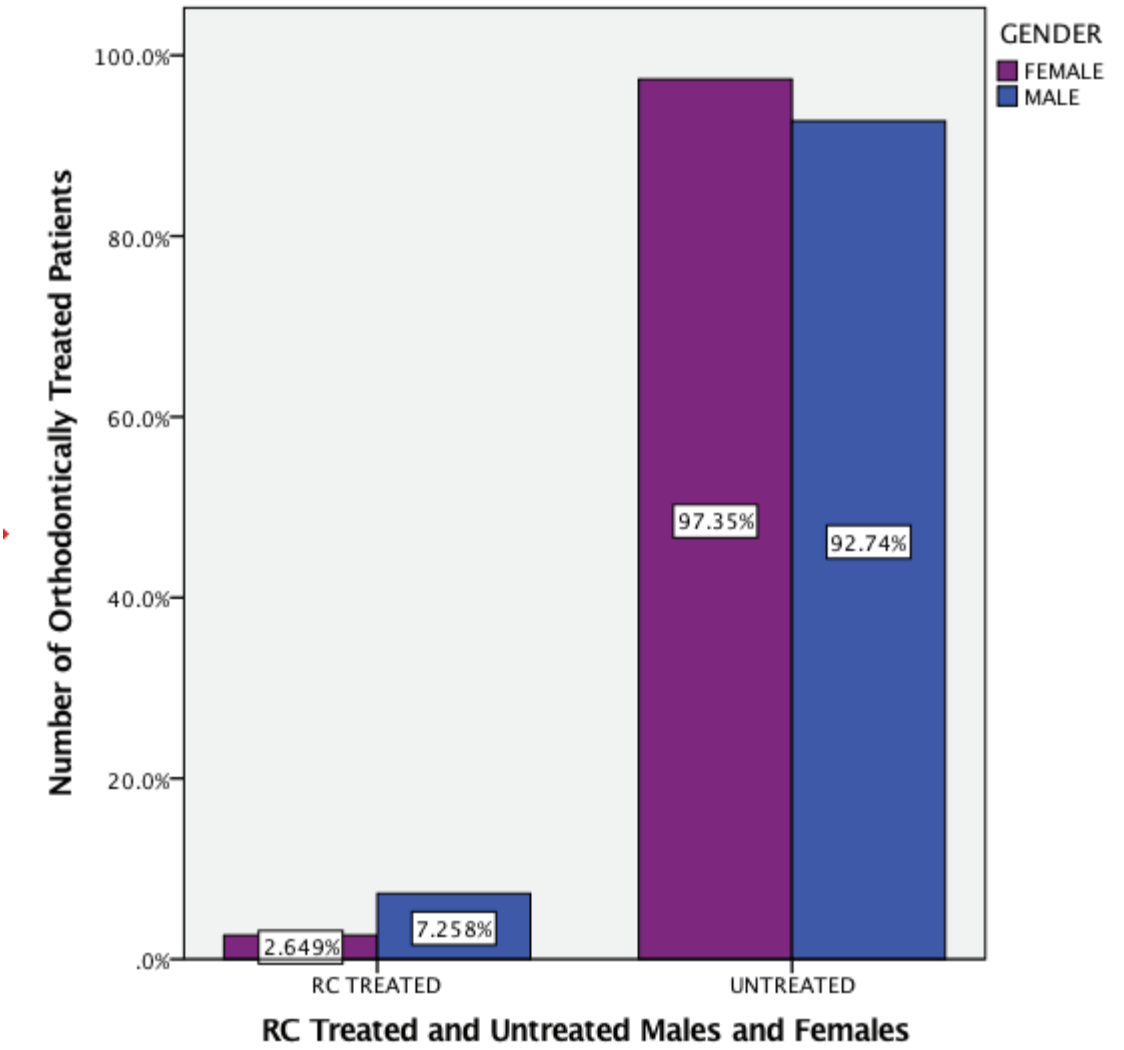


FIGURE 3: Bar Chart represents the association between RC treated anteriors amongst male and female orthodontic patients.X-Axis represents Root Canal Treated and Untreated Anteriors in Males and Females ,where as Y-axis represents the number of patients treated orthodontically. Purple colour denotes the females and blue represents the male orthodontic patients. Male orthodontic patients (7.25%-blue) have higher number of Root Canal Treated Anterior.This is statistically significant (Chi-Square Test;p-value- 0.01<0.05 statistically Significant)

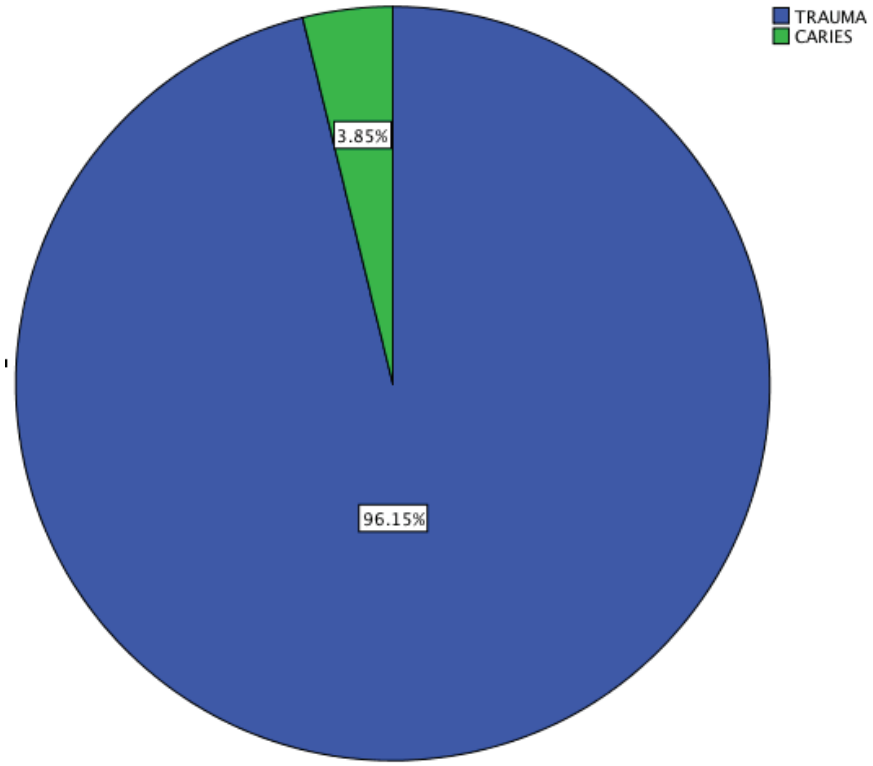


FIGURE 4: The Pie Chart Representing the distribution of the Cause Of Endodontic Treatment In Anteriors from the past Dental History Of Patient .Blue denotes teeth that have been treated endodontically secondary to trauma.Green represent anterior teeth which were endodontically treated due to caries. Majority of anteriors i.e 96.15% (blue) had experienced trauma and the remaining 3.85% underwent root canal treatment due to carious involvement.

In 96.2 % cases, the cause for undergoing root canal treatment was trauma to anterior tooth/teeth, remaining 3.8% were endodontically treated secondary to caries. (FIGURE- 4)

Data obtained from the current study suggests that a lesser percentage of anteriors are endodontically treated .However, even less percentage of anteriors being involved the risk of these endodontically treated anteriors getting affected by orthodontic tooth movement does not reduce.Maxillary Central Incisors accounts for more than two-third of total endodontically treated anteriors. The similar data has been suggested by previous studies that maxillary central incisors are more prone to trauma owing to proclination of these anteriors[19–21] .An angulation of more than average associated with variety of malocclusion is a common finding .A pool orthodontic patients reports with chief complaint of forwardly placed anteriors and in few instances also report history of trauma manifesting from a Elli's Class 1 fracture to avulsion .Thus it may be common to find maxillary central incisors being endodontically treated.

Mandibular central and lateral incisors are less likely to get trauma however they might be prone to caries due to poor oral hygiene and be involved pulpally .Maxillary and Mandibular Canines were rarely affected, except only with one patient where a proximal caries involved the mandibular canines , deep enough to undergo endodontic treatment.A previous epidemiological study by Cleen et al, carried out on Dutch population including 183 subjects reported a prevalence rate of 2.3%.With the increased sample size of the current study a prevalence of 0.4% can be considered significant.

Various orthodontic movements, exerts a variable degree of force on the tooth/teeth leading to variable degree of root resorption[22,23] .Orthodontic factors such as: (i)Type of tooth movement , (ii) Rate of tooth movement , (iii) extent of tooth movement,(iv) Anchorage demand on the teeth, (v)Type of appliance

used and (vi)Phase of orthodontic tooth movement may increase the risk root resorption, even more if the teeth are endodontically treated.[24–26]

Tooth Movement like Intrusion possesses an increased risk of apical root resorption , the force being concentrated at the root apex . Faster and farther the tooth is moved , risk for resorption tends to increase. Ameteur orthodontic mechanics may increase risk of root resorption .A larger percentage of orthodontic patients reports with a chief complaint of proclined anteriors and there exists previous literature supporting that trauma to anteriors is not very uncommon .Precaution to treat these patients orthodontically must be taken.We as an orthodontist must ensure a treatment protocol with minimum disharmony to the teeth [27]. Orthodontic tooth movement further must not degrade the condition of the endodontically treated teeth and therefore a more physiologic treatment mechanics must be involved in treating a patient with RC treated anteriors,as in most cases, the anteriors are the ones to be retracted for a longer distance.A sequential treatment protocol must be followed to ensure appropriate remodelling of the bone and the teeth. [28]

Previously our team had conducted numerous clinical trials [29–35] and lab animal studies [36–40] and in-vitro[41–43] studies over the past 5 years.Now we are focusing on epidemiological studies .The idea for this study stemmed from the current interest in our community. Patients, since the beginning must be informed about the status of the endodontically treated teeth and be updated about the same during the course of treatment.A patient consent pertaining to the status of endodontically treated teeth must be obtained before initiating the treatment.

Conclusion

Prevalence of endodontically treated teeth was found to be 0.4% , with maxillary central incisors accounting for major percentage amongst all anteriors.As an orthodontist a thorough evaluation of such teeth must be done before commencing the treatment. Knowledge about potential complications and treatment alternatives in such cases must be enhanced.

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Source of Funding : Self

Ethical Clearance: Not Required

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Importance of Zero Patient in Pandemics

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Abstract

The patient zero is the first reported patient in a pandemic within a population, or the first documented patient incorporated in a pandemic study. Some scientists claimed that it's important to examine primary cases . The term patient zero refers to the person identified as the first carrier of a communicable disease in an outbreak of related cases. They act as a symbol for initial actions that have major downstream consequences.The primary recorded victim of the Ebola infection was a 44 year old teacher named Mabola Lokela,who passed away on 8thSeptember 1970, 614 days after the clinical manifestation started .An infant in the Lewis House at 40 broad Road is viewed as the patient zero in the 1854 cholera outbreak in the Soho neighborhood of London .Pandemic is a disease outbreak that spreads across countries or continents and it affects more people and takes more lives than epidemic.It is an epidemic which takes place on a scale that crosses universal limits, generally influencing individuals on an overall scale. Detection of patient zero gives new insight to epidemiologists about the nature of first transmissions into a population.it's important to find the first person infected with the pathogen.By knowing person's history of patient zero we can determine the outbreak of the disease and the route of transmission .Discovering them might be significant in certain occasions, to know whether they are still alive and transmitting the infection. Identifying a "patient zero" is a rife with potential to provoke blame and scapegoating. Patient zero triggers a "desperate hunt" and is continuously giving expression to communal fears about dangerously reckless behaviour,Researchers have found people harbour negative attitudes towards Patient zero.The patient zero issue comprises of finding the source of a pandemic outbreak given,perceptions at a later time.Contemplating the patient zero issue we additionally tackle the issue of finishing and rectifying the observation to people and false test results

Keyword *:patient 0,pandemic ,first carrier ,epidemic ,index case*

Introduction

The patient zero is first reported patient in a pandemic within a population are the first documented patient incorporated in a pandemic study .It refers to the primary case of a condition, syndrome or a contagious disease [1,2] .The patient zero is significant as we can characterise the extent of the disease, estimate the hazard and comprehend what it will take to control the outbreak [3]. The primary recorded victim of the Ebola infection was a 44 year old teacher named Mabola Lokela,who passed away on 8thSeptember 1970 614 days after the clinical manifestation started[4]One year old Emilie Ouamouno was found to be the patient zero of the Ebola in 2014 [5].Mallon travelled from Ireland to the US as a young person after which she looked for some kind of employment as a cook. Unfortunately she got infected with typhoid fever, a potentially harmful disease.In 1907 Mallon was investigated and identified as a patient zero. Sspecialist isolated and imprisoned her on an island [6].Gaeton Dugas a Quebecois Canadian Airlines steward and was the first HIV patient who is known as "Patient zero".Dugas passed away on Quebec city on March 1984

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because of renal failure cost by AIDS [7,8].HIV-infected patients oppose an elevating risk of cardiovascular disease .N6-adenosine methylation is a promising recent molecular target in hypertension and cardiovascular diseases [9].Some scientists claimed that it's mandatory to examine the individual or animal first brings a bacterium or virus into a populace [10,11].The patient zero issue comprises of finding the source of a pandemic outbreak given,perceptions at a later time.Contemplating the patient zero issue we additionally tackle the issue of finishing and rectifying the observation to people and false test results [12] .

Discussion

Pandemic:

Pandemic is a disease outbreak that spreads across countries or continents and It affects more people and takes more lives than an epidemic [13,14].It is an epidemic which takes place on a scale that crosses universal limits, generally influencing individuals on an overall scale [15].A disease or condition is not always a pandemic merely because it is widespread or kills many people [16].Seven cholera pandemics have taken place in the last 200 years, with the first pandemic beginning in India in 1817 [17].Insufficient treatment of human waste and insufficient treatment of drinking water extraordinarily encourage its spread [18]

Patient zero:

Patient zero also known as index case is the first reported patient in a disease epidemic within up They may or may not specify the source of infection ,the possible transmission but may bring awareness of an emerging outbreak [1].Patient zero is a popular medical term which refers to the first person infected in an epidemic/pandemic [11,19].It refers to the person identified as the first carrier of a communicable disease in and out related cases.They act as a symbol for initial action that have major downstream consequences [20].Combination of ancient and hereditary research discloses the mistake and hype that led to the term patient zero and accusing of one man for the spread of HIV across North America [21,22].Patient zero was affected by a hepatitis A virus with a dissimilar genetic fingerprint not directly linked with the outbreak cases.This disease has repeatedly found to be in low levels in the community [23].

Significance of Patient Zero:

Detection of patient zero gives new insight to epidemiologists about the nature of first transmissions into a population [24].It's important to find the first person infected with the pathogen [25].By knowing person's history of patient zero we can determine the outbreak of the disease and the route of transmission [25,26].In silico examination, the harmful genes can be utilized as a possibility to build new medicinal target antagonists to pathogens [27].In silico examination, the harmful genes can be utilized as a possibility to build new medicinal target antagonists to pathogens [28].Information obtained from Patient zero are useful to scientists .Once a patient zero identified we can understand more about disease and can find the cure. Discovering patient zero might be significant in certain occasions, however just in the case where they are still alive and transmitting the infection [29]. Finding the patient zero will permit doctors to figure out where it originated from. This in turn gives the doctors an approach to stop, control and to study more about the disease [30].The phrase patient zero is currently utilised in the media to cite the primary cause for infectious outbreaks as the source of action has more far-reaching consequences [9,31]

Patient zero -toxic term:

Identifying a "patient zero" is a rife with potential to provoke blame and scapegoating. Patient zero trigger a "desperate hunt" and are continuously giving expression to communal fears about dangerously reckless behaviour. Researchers have found people harbour negative attitudes towards Patient zero [32].Patient zero identification is done for a good cause but the term reaches the public in a negative way.People believe them hostile,immoral against humanity.Thus this creates depression in patient zero and makes them an outcast [33,34].

Patient zero of various diseases:

Mary Mallon also called Typhoid Mary worked as a cook in New york city for seven families. Within two weeks of her employment the employers developed typhoid fever. Mary Mallon was not suspected as she was in good health. She was quarantined twice and died after 3 decades of isolation. She is said to have infected 51 people, 3 of them died [8,35].Gaetan Dugas, a flight attendant, was suspected to be the patient zero for HIV

AIDS in America. Dugas was charming, handsome and had many sex partners per year. Dugas had a sociopathic behaviour i.e., intentionally infecting others with the virus.Articles in newspapers published him as "MAN WHO GAVE US AIDS '' [36,37].64-year-old Liu Jianlun, a Guangdong specialist, transmitted SARS universally by contaminating other super-spreaders during a stay in the Hong Kong Metropole Inn in 2003 [38].A infant in the Lewis House at 40 broad Road is viewed as the patient zero in the 1854 cholera flare-up in the Soho neighborhood of London[39]. Edgar Enrique is a patient zero for flu pandemics, he recovered and a bronze sculpture was raised in this respect [40].

COVID -19 Patient zero:

Coronavirus disease 2019 (COVID-19) is a contagious disease originated from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).It was first reported in December 2019 in Wuhan, China, and has brought about a progressing pandemic [41].Normal manifestations include fever, hack, exhaustion, dyspnea ,anosmia ageusia . While most cases bring about mild symptoms , some advance to wet lung (ARDS) ,multi-organ failure , septic shock, thrombus and embolus [42].Wei Guixian, was selling shrimps at the Huanan Seafood Market. She developed symptoms on December 10. Wei went to a local clinic for treatment. There was no improvement and she went to Eleventh hospital and finally landed in Wuhan Union hospital.She was finally quarantined and her strange illness was finally diagnosed as having Coronavirus [43].Early examinations have indicated that about 25% of individuals who went to the medical clinic with extreme COVID-19 contaminations had diabetes.Diabetes mellitus is a set of metabolic disorders where there are high glucose levels over a extended period.It is a significant medical issue in India[44 45]

Case study of kerala :

Three students returning from wuhan were asked to report to their nearby hospitals for screenings and the students were immediately quarantined out of worry that someone could bring the virus .They were then given treatment for 28 days [46,47] .With no vaccine in sight the state has relied on the information obtained from patient zero and aggressive tried and tested system of early testing contact tracing and isolation ,social distancing promotions plus longer quarantine period ,Kerala has stem the spread of the virus [48].

Conclusion

Identifying Patient zero helps us to understand pathogenesis of the disease .It is useful to think historically about the divergent interests of two different groups keenly following spread of infection during an epidemic members in public , public health workers.If patient zero are not found we won't have a clear idea about disease transmission and etiology.patient o is determined for diseases like HIV,ebola and corona

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Mitochondrial DNA and Ageing

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Abstract

Mitochondrial DNA is a separate genome located in the cytoplasm of nearly all eukaryotic cells.Its importance in developmental outcome has often been neglected. However, its transmission and replication are strictly regulated during early development, as they are integral to the viability and health of the offspring.The accumulation of mitochondrial DNA mutations has been proposed as a potential mechanism in the physiological process of ageing and age-related disease. Although mitochondria have long been anticipated as a perpetrator of ageing, there was little experimental evidence to link these changes directly with the cellular pathology of ageing. Recently, considerable progress in understanding basic mitochondrial genetics and in identifying acquired mtDNA mutations in ageing has been made. Furthermore, the creation of mtDNA-mutator mice has provided the first direct evidence that accelerating the mtDNA mutation can result in premature ageing, consistent with the view that loss of mitochondrial function is a major causal factor in ageing. This review will, therefore, focus on recent developments in ageing research related to the role played by mitochondrial DNA.

Keywords: *Mitochondria, DNA, mitochondrial diseases, ageing, inflammation, mutations, deletions, ROS production.*

Introduction

Mitochondria plays a central role in ageing [1]. The initial free radical theory suggested that reactive oxygen species (ROS), which are constantly generated through normal metabolism, are causing ageing, as well as associated degenerative diseases, by introducing irreversible damage to membranes, proteins and DNA [2]. Early studies on isolated mitochondria, where many of the redox elements were held in an artificial state, have suggested that 1–4% of the total oxygen consumed was used to generate ROS [3]. However, subsequent studies, under more physiological conditions, have estimated this basal value to ~0.2% [4]. Even with this lower estimated value of ROS generation, the mitochondrial electron transport chain (ETC) still remains the major site of ROS production in a cell. This fact has led to the suggestion that mitochondria are also prime targets for oxidative damage and that accumulation of defective mitochondria is a major contributor to ageing[5] . Indeed, an increased number of dysfunctional mitochondria are found in a number of animal models and human tissues with increasing age [6]. Besides, mitochondria are the only organelles in the animal cell that possess their own DNA, mitochondrial DNA (mtDNA), with the limited arsenal of DNA repair processes, further supporting the idea that mitochondria might be particularly sensitive to accumulation of damage[7]. The mitochondrial theory of ageing, a correlate of the free radical theory, is also based around the idea of a vicious cycle , in which somatic mutations of mitochondrial DNA provoke respiratory chain dysfunction leading to enhanced ROS production and in turn to the accumulation of further

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mtDNA mutations[8].Harman's conjecture was that the generation of reactive oxygen species (ROS), which he viewed as likely coming from the mitochondria, gave rise to the subsequent accumulation of damaged proteins, lipids, and DNA, thereby fueling aging and age-related diseases in an inevitable but stochastic process[9] . While the notion that increased mitochondrial ROS directly causes aging has fallen into disfavor , in its wake, other aspects of mitochondrial biology have grown increasingly more appealing[10]. Some links between aspects of mitochondrial biology and aging have been the subject of many excellent recent reviews [11]. Here, we focus on those aspects of mitochondrial physiology that have perhaps the strongest connection to human aging. These processes include the emerging role that mitochondria play in inflammation[12]; how dysregulation of mitochondrial quality control and age-related mitochondrial dysfunction contributes to aging, age-related mitochondrial dysfunction, and the notion of retrograde signaling; and why a little mitochondrial stress might ultimately be a good thing[13].

mt DNA:

Mitochondria are structures within cells that convert the energy from food into a form that cells can use[14]. Each cell contains hundreds to thousands of mitochondria, which are located in the fluid that surrounds the nucleus (the cytoplasm). Although most DNA is packaged in chromosomes within the nucleus, mitochondria also have a small amount of their own DNA. This genetic material is known as mitochondrial DNA or mtDNA[15]. In humans, mitochondrial DNA spans about 16,500 DNA building blocks (base pairs), representing a small fraction of the total DNA in cells[16]. Mitochondrial DNA contains 37 genes, all of which are essential for normal mitochondrial function. Thirteen of these genes provide instructions for making enzymes involved in oxidative phosphorylation[17]. Oxidative phosphorylation is a process that uses oxygen and simple sugars to create adenosine triphosphate (ATP), the cell's main energy source. The remaining genes provide instructions for making molecules called transfer RNA (tRNA) and ribosomal RNA (rRNA), which are chemical cousins of DNA. These types of RNA help assemble protein building blocks into functioning proteins[18]. mtDNA is a separate genome located in the cytoplasm of nearly all eukaryotic cells . Its importance in developmental outcome has often been neglected. However, its transmission and replication are strictly regulated during early development, as they are integral to the viability and health of the offspring[19] . Failure to regulate these processes during oogenesis , in the embryo, and prior to gastrulation can have deleterious consequences for the fetus and the offspring. Human mitochondrial DNA was the first significant part of the human genome to be sequenced.This sequencing revealed that the human mtDNA includes 16,569 base pairs and encodes 13 proteins. Since animal mtDNA evolves faster than nuclear genetic markers it represents a mainstay of phylogenetics and evolution . It also permits an examination of the relatedness of populations, and so has become important in anthropology and biogeography.

Mitochondrial DNA and disease:

Most mtDNA alterations are neutral polymorphisms, which have proved useful in tracking human migrations [20]. The first pathogenic mtDNA mutations were identified in 1988 [21]. Since then, over 250 pathogenic mtDNA mutations (point mutations and rearrangements) have been characterised , shown to cause a wide variety of diseases with a heterogeneity of phenotypes and a variable age of onset [22]. The exact prevalence of mtDNA disease is notoriously difficult to ascertain due to clinical heterogeneity of mitochondrial diseases and the plethora of known causative mutations. The onset of clinical symptoms, phenotypic variability, and variable penetrance of mitochondrial diseases are governed by a number of factors, including the threshold effect, mitotic segregation, clonal expansion, and a genetic bottleneck.Leigh syndrome is a progressive neurodegenerative condition, which particularly affects the brain, diencephalon, and basal ganglia. There are characteristic neuropathological features, but newer neuroimaging techniques can now easily detect these lesions in life.Leigh syndrome is due to severe failure of oxidative metabolism and can be due to a variety of different genetic defects affecting the mitochondrial genome. The clinical features associated with depletion syndromes depend upon the organ(s) which have t DNA depletion[7]. On the whole, these are severe disorders and present in childhood with severe muscle weakness, progressive encephalopathy, or liver failure. Pearson syndrome is a rare disorder of infancy characterised by sideroblastic anaemia with pancytopenia and exocrine

pancreatic failure[23]. The clinical course in these children can be severe leading to early death. In those that survive the blood disorder improves but they later develop the clinical features of KSS. In these children, there is a very high level of large-scale single mtDNA deletion present in all tissues [24].One of the most common presentations of mtDNA disease in adults is CPEO. CPEO is characterised by a progressive paralysis of the eye muscles leading to impaired eye movement and ptosis.

Mitochondria and the inflammation of ageing:

Aging is associated with evidence of an activation of the innate immune system that leads to a condition known as "inflammaging" [25]. This is particularly true of elderly individuals who age less successfully than their peers, as both the frail and the pre-frail exhibit higher circulating levels of inflammatory mediators such as C-reactive protein, IL-6, and fibrinogen[26] . These relationships exist even in the absence of associated age-related diseases (e.g., cancer, cardiovascular disease, etc.), suggesting a potential causative relationship between activation of the immune system and accelerated aging . Perhaps because of its stability and ease of measurement, the data are most compelling with respect to circulating IL-6, as elevated IL-6 serum levels are predictive of incident disability, frailty, walking speed, and overall mortality . Analysis of T cells from elderly individuals suggests that although basal levels of cytokine production are elevated, invoked responses to a cytokine challenge are reduced [27].Besides evidence for augmented basal cytokine signaling, chronic activation of the inflammasome with elevated IL-1β levels appears to identify a subset of elderly individuals with higher rates of essential hypertension, arterial stiffness, and all-cause mortality . Consistent with this, evidence for activation of the NLRP3 inflammasome is seen in a wide range of age-related conditions, including atherosclerosis , Alzheimer's disease and metabolic syndrome. Interestingly, a number of preclinical observations suggest that the activation of the inflammasome may be maladaptive[28]. There is increasing evidence that mitochondria might play an important role in the inflammaging phenotype [29]. The immune system is capable of sensing and responding to tissue damage and views the release of intracellular molecules in an analogous fashion to dangerous pathogens. This "danger theory" argues that certain molecules released from senescent or dying cells might constitute signals that trigger an immune response , often termed damage-associated molecular patterns (DAMPs). In that context, the mitochondria, derived several billions of years ago from the endosymbiosis of α-proteobacteria into a eukaryotic cell, represent a smorgasbord of potential immune-stimulating DAMPs. Putative mitochondrial DAMPs include the release of mitochondrial DNA (mtDNA), *N*-formyl peptides generated by translation of mitochondrial-encoded protein, and unique lipid species such as cardiolipin, a unique phospholipid enriched on the inner mitochondrial membrane . In the case of circulating mtDNA, immune activation occurs, in part, by activation of Toll-like receptor 9 (TLR9) signaling, while for mitochondrial proteins, activation occurs through binding to formyl peptide receptor-1. This was first demonstrated in the case of trauma, where the release of mtDNA and formyl peptides activated circulating neutrophils to mediate tissue injury.

Functional significance of mtDNA:

Mitochondrial DNA has strands. These strands are present in the ratio of 1 mitochondrian : 2- 10 Mitochondrial DNA(mtDNA). Humans inherit them maternally. The involved mechanisms include dilution of sperm mtDNA within the fertilized egg. The uniparental mtDNA pattern is found in most animals and plants. The strands are susceptible to oxygen - reactive species and contain proteins with significant DNA repair capacity. They are susceptible to oxidative damage. The mtDNA mutations cause inherited diseases and aid the process of ageing. There are anything between 100 and 10,000 copies of mtDNA present per human cell. This genetic material is rearranged via recombination and remains unchanged, when passed on from parent to offspring. The mtDNA helps track ancestry back to hundreds of generations. It is compared in forensic science, to identify corpses and skeletal remains that are unidentified. The strands are used in combination with anthropological and missing persons. mtDNA facilitates the assessment of genetic relationships between individuals and quantifying evolutionary relationships. Dedicated mtDNA data helps biologists to construct a relationship network among various sequences. The sequences become larger, with evidence of a distant or non - existent relationship between species. It is highly susceptible to somatic or non - inherited mutation[30].These occur within the DNA of some cells and are not passed

down generations. Classic somatic mutations in mtDNA are seen in cancer of the colon, breast , liver and stomach and even leukemia and lymphoma. Mitochondrial DNA is not able to completely repair itself and hence , this leads to additional somatic mutations. When building blocks of nucleotides are altered, the energy producing ability of mitochondria is reduced and this leaves the immune system fighting infection with little or no help from these strands [31].The DNA strand is very important to biologists and foreign experts when attempting to establish vital relationships between people. It is being explored to answer the common questions that have plagued man for centuries on the possible evolutionary routes and possible age - reversing.Within the human body , these essential building blocks back up and strengthen the immune system to keep a number of ailments at bay[32].

Mitochondrial DNA keeps us young:

The extended inflammatory infiltrates suggest that mitochondria could produce ROS can act as signaling molecules for inflammasome activation[33]; unfortunately,there are no report measurements of oxidative stress,but cells depleted of mtDNA are usually characterized by diminished oxygen consumption and ROS production[34],suggesting that oxidative stress should not mediate the ageing phenotype observed here. However, the following two major consequences were observed in a cell model of mtDNA depletion using the same strategy as that used in the depleter mouse ; a significant rearrangement of histone acetylation due to indirect alterations in the citrate levels and a reduction in cell proliferation due to a reduction in the membrane potential and destabilization of Hif1alpha .While the type of epigenetic rearrangement that occurs during ageing is unclear .Hif1a depletion has been shown to lead to an accelerated aged skin phenotype in mice.Additionally, skin mitochondria with depleted DNA display a loss of mitochondrial cristae presumably due to the loss of F1/FO ATP synthase dimers .Indeed ,the rearrangement of ATP synthase dimers could predispose cells to death by opening the permeability transition pore channel [35].There is no report of measure of cell death (that was observed in the mutator mouse);nonetheless, since multiple mitochondrial molecules can act as damage-associated molecular patterns (DAMPs),an increase in PTPC -mediated cell death could cause the extensive release of mito-DAMP and ,ultimately,the observed increase in inflammation .The recovery of the skin phenotype is more intriguing .The mutator mouse model provided important insight into how mitochondria can induce an ageing phenotype by affecting haematopoietic and neural stem cell self-renewal capacities [36].

Conclusion

There is a growing body of evidence that mitochondria play a central role in the ageing process. Mitochondrial dysfunction and mtDNA mutations have been shown to amplify during the course of ageing. Results obtained from mtDNA mutator mice have further strengthened the role of mitochondria in organismal ageing. However, lack of increased oxidative stress in the mtDNA mutator mice has raised reasonable doubts in the direct connection of mtDNA mutations with increased ROS production. However, this does not imply that there is no age dependent accumulation of oxidative stress and increased ROS production, neither is it a final proof that oxidative stress does not play an important role in the generation of mtDNA mutations. Indeed, there is extensive literature demonstrating amplified oxidative damage to the different cell compartments with increasing age, not least in mitochondria and mtDNA. However, reviewing this literature is beyond the scope of this article that was intended to focus primarily on the role of mtDNA mutations in ageing. The role of mitochondrial dysfunction in human ageing must now be revisited and additional studies of respiratory chain capacity and mtDNA mutation load in different organs from humans of different ages must be performed to further validate this hypothesis. It may also be possible to perform additional genetic experiments in the mouse to test this hypothesis, for example creating mice with enhanced mtDNA polymerase proofreading capacity or enhanced mtDNA repair capacity. Such mouse mutants should have a longer than normal life span if somatic mtDNA mutations have a causative role in ageing. Mitochondria are now at the center stage in human disease and ageing and we should look forward to exciting developments in this field during the coming years.

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Relationship of Tooth Mobility with Systemic Diseases among Patients Reporting Private Dental College in Chennai

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Abstract

Periodontitis begins with bacterial infection of the oral structures and if not properly treated eventually leads to tooth loss. Over the past decade, a growing body of scientific evidence suggests an exquisite association between oral infection and systemic diseases. The purpose of our study was to compare the tooth mobility which is the main indicator of periodontal diseases with systemic diseases. An institutional study was conducted using case records of patients attending private dental college from July 2019- March 2020 . A total of 71 case sheets of patients who had recorded tooth mobility index were retrieved and data was collected. Data analysis was done using SPSS software version 23.0. Chi-square association was done to explore the relationship between tooth mobility and systemic diseases. From the study results, 50.70% of participants belong to the age group of 36-55 yrs, 40.85% were females and 59.15% were males, thus showing male predominance in the current study population. 66.197% of study participants have absence of systemic diseases and 33.803% have systemic diseases. 4.2% of participants with systemic diseases and 2.12% of participants without systemic diseases have mobile teeth between 17-24 (GROUP IV). No statistically significant association was found between tooth mobility and systemic diseases.(P = 0.858). The results of the present study indicate that prevalence of increased number of mobile teeth was found among participants with systemic diseases. But the association was not statistically significant. Knowledge of disease interrelationships and their associations may prove to reduce patient risks and prevent systemic disease outcomes and hence further studies to be carried out to assess the interrelationships.

Keywords*: Tooth mobility; systemic disease; tooth loss; Periodontal disease.*

Introduction

Gingival and periodontal diseases in their various forms, have afflicted mankind since the dawn of history. Diseases of the periodontium occur in childhood, adolescence and early adulthood but the prevalence of periodontal disease, tissue destruction and tooth loss increases with age [1]. Periodontal diseases are oral disorders characterized by inflammation of the supporting tissues of the teeth. Usually, periodontitis is a progressively destructive loss of bone and periodontal ligament (loss of the attachment apparatus of the teeth). Periodontitis has documented risk factors, including but not limited to specific plaque bacteria, smoking, and diabetes mellitus. The oral cavity contains almost half of the commensal bacteria in the human body, approximately 6 billion microbes representing 300 to 500 species reside in the oral cavity. The oral microbial ecosystem is remarkably dynamic [2]. As a consequence, there has been a resurgence of interest in oral microbial

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ecology, mucosal immunity and associations with periodontal disease which leads to mobility of teeth [3].

Teeth play a vital role in the oral cavity. They contribute in the process of mastication, speech and maintaining the facial aesthetic. Edentulous can result in disturbance in daily-living activities such as chewing adequately as well as causing emotional problems [4,5]. Loss of self confidence and concern about appearance and self image are the emotional impacts of tooth loss[6]. Early tooth loss or premature exfoliation of teeth is defined as loss of tooth in the oral cavity prior to the normal expected period of time. Since there are no references to address the exact natural expected age for loss of permanent dentition early loss is a relative term. There are both local and systemic factors that could result in such a phenomenon. In developing countries like India , oral cavity is prone for many diseases like dental caries, periodontal disease, oral cancer, malocclusion, fluorosis etc. These diseases affect different age groups in various aspects. Various treatment options are emerging for treating the oral diseases thus leading them a quality life [7– 16].

Poor oral hygiene could cause caries and periodontal disease leading to loss of teeth [17,18]. Additionally, there are a number of systemic diseases such as diabetes, leukemia, hyperthyroidism etc. whose effect on the oral cavity could make the teeth susceptible to exfoliation. They could increase the risk of caries as a result of interrepling the normal function of salivary glands, or affect the periodontal tissues, which support the teeth [19]. In addition, periodontal disease is a prevalent disorder with risk parameters that contribute to mobility in terms of decreasing oral function and increasing tooth loss. Risk factors have been studied extensively in dentistry and include specific bacteria. Smoking and systemic disease like diabetes mellitus.

Initially link between systemic disease and tooth mobility in unidirectional. Currently, there is increasing evidence that the relationship may be bidirectional [20,21]. This knowledge of disease intra-relationship may prove vital in intervention strategies to reduce patient risks and prevent systemic disease outcomes. In this study, we aim to focus on the systemic disease that can lead to tooth mobility or early tooth loss and to determine the relationship between tooth mobility and systemic diseases.

Materials And Methods:

Study setting

The present study was conducted by reviewing patient records from July 2019-March 2020 visiting Saveetha Dental College and Hospitals. Among them 71 case records of patients aged 18-80 years to whom tooth mobility index recorded were retrieved .

Ethical approval:

Ethical approval was obtained from the Institutional Review Board (IRB) of the University to use the data from case records (SDC/SIHEC/2020/DIASDATA/0619-0320). Informed consent was obtained from the patient at the time of screening procedure. Case sheets with informed consent were included in the study.

Sampling

All the patients for whom tooth mobility index have been recorded were included in the study and their case sheets were reviewed. Internally validity of the study was established by using universally accepted tooth mobility Index.

Index used and scoring criteria

Tooth Mobility Index [(22)] was used in the present study. Study population was categorized into five groups based on the number of mobile teeth in their oral cavity. When the patients didn't have any mobile tooth, they were categorized as Group I. Participants with less than 8 teeth were categorised as Group II. If the number of mobile teeth was between 9-16, the participants were grouped as group III. Participants having mobile teeth between 17-24 were grouped as Group IV. When the number of mobile teeth falls between 25-32, they are categorized as group V. Among the patients to whom tooth mobility index recorded, data related to their systemic diseases were collected and reviewed.

Statistical Analysis

The collected data was entered in MS Excel and imported in IBM SPSS software version 23.0. The independent variable were age and sex and systemic

disease while the dependent variables was tooth mobility. Frequency distribution of age, gender, systemic diseases was done using descriptive statistics. Chi-square test was done to determine the association between systemic diseases and tooth mobility.

Results and Discussion

Measuring the degree of tooth mobility is an important part of any thorough periodontal examination. Tooth mobility is the measurement of horizontal and vertical tooth displacement created by examiner's force. Even a tooth with significant alveolar bone support reveals proper tooth mobility [23]. Very often, the course of periodontal disease is modified by the systemic disorder of patients. Distribution of study subjects based on age showed that 19.71% participants were between 18-35 years, 50.704% were between 36-55 years and 29.77% were above 55 years(Figure 1). Figure 2 represents the distribution of study subjects based on gender. Among 71 participants, 40.85% were females and 59.15% were males. The systemic disorder exerted the effect in a generalized manner and so affects the occurrence and management of the periodontal conditions [24]. Figure 3 represents the distribution of presence or absence of systemic diseases among the study subjects. 66.197% didn't have systemic diseases and 33.803% reported to have systemic diseases. Periodontal diseases are prevalent disorders with risk parameters that contribute to morbidity in terms of decreasing oral function and increasing tooth loss [23]. From the results of the present study , systemic diseases like diabetes Mellitus , female sexual hormones condition hypopituitarism, hyperthyroidism , primary hyperparathyroidism ,osteoporosis hypophosphatasia, hypophosphatemia, acatalasia etc., could result in tooth loss and tooth mobility was determined [4]. Number of participants with systemic disease was 33.83%.This was almost similar to the study conducted by Mealey et al [25]. In contrast to these findings, a study conducted by researchers [26] ,60% of periodontitis patients with tooth mobility suffer from the same type of systemic diseases. According to a study, it was reported that 85.96% of patients suffering from periodontitis had at least one type of systemic disease. The findings of the present study were less than the studies by Georgiou et al [27]. This could be because of the differences in patient data recording systems. Also other studies included systemic diseases such as osteoporosis, rheumatoid arthritis, sinusitis , tumours etc whereas in our study we had included only diabetes and hypertension.

Periodontitis is due to the injury to the periodontal membrane and many risk factors contribute to periodontal infections [28]. Evidence exists which suggests a relationship between periodontal status and nutrition, alcohol consumption, socioeconomic status and stress levels [29-31].Smoking is the best established of the modifiable risk factors for developing periodontal disease[32]. Diet we consume also plays a major role in periodontal diseases[33]. Nutrition can influence the growth, development and metabolic activities of the periodontium; the high rate of cell turnover in the periodontal tissues requires that essential nutrients are readily available. The health care professionals are expected to provide treatment with all the knowledge and skill and as a public health dentist we should give proper awareness and proper healthcare to the patients even in the crisis period [34,35].

In this study hypertension and diabetes mellitus were the most frequent conditions detected and were significantly higher in periodontitis patients with tooth mobility. This is in agreement with previous studies [36]. From our study results, we found that there was no statistically significant relationship between systemic diseases and tooth mobility. Figure 4 cluster bar depicts the association between tooth mobility and systemic diseases. 4.2% of participants with systemic diseases and 2.12% of participants without systemic diseases have mobile teeth between 17-24(Group IV). Majority of the participants with systemic disease(70.21%) and without systemic disease(75%) belong to Group II . Previous studies show that systemic diseases are among elderly aged [37]. Such a finding implies that public healthcare dentists should need to actively seek changes and address more for elderly people.

In the present study, we found that there was a trend for mild mobility to severe mobility of teeth. Majority of patients with systemic diseases were in mild mobility of teeth and number decreased towards severe mobility of teeth. Tooth mobility score, however, was not having statistically significant association with systemic diseases.This is in accordance with the previous study results [37,38]. They stated that none of the systemic disease

in their study was significantly associated with tooth mobility, periodontal disease severity. Further implications to be done to elucidate whether periodontal infections could be a true risk factor for some important systemic conditions and provide more insight to the association of periodontitis and systemic disease.The present study has certain limitations attributed to study design which is a retrospective study. Further longitudinal prospective studies with standardized measuring instruments are needed to prove the hypothesis.

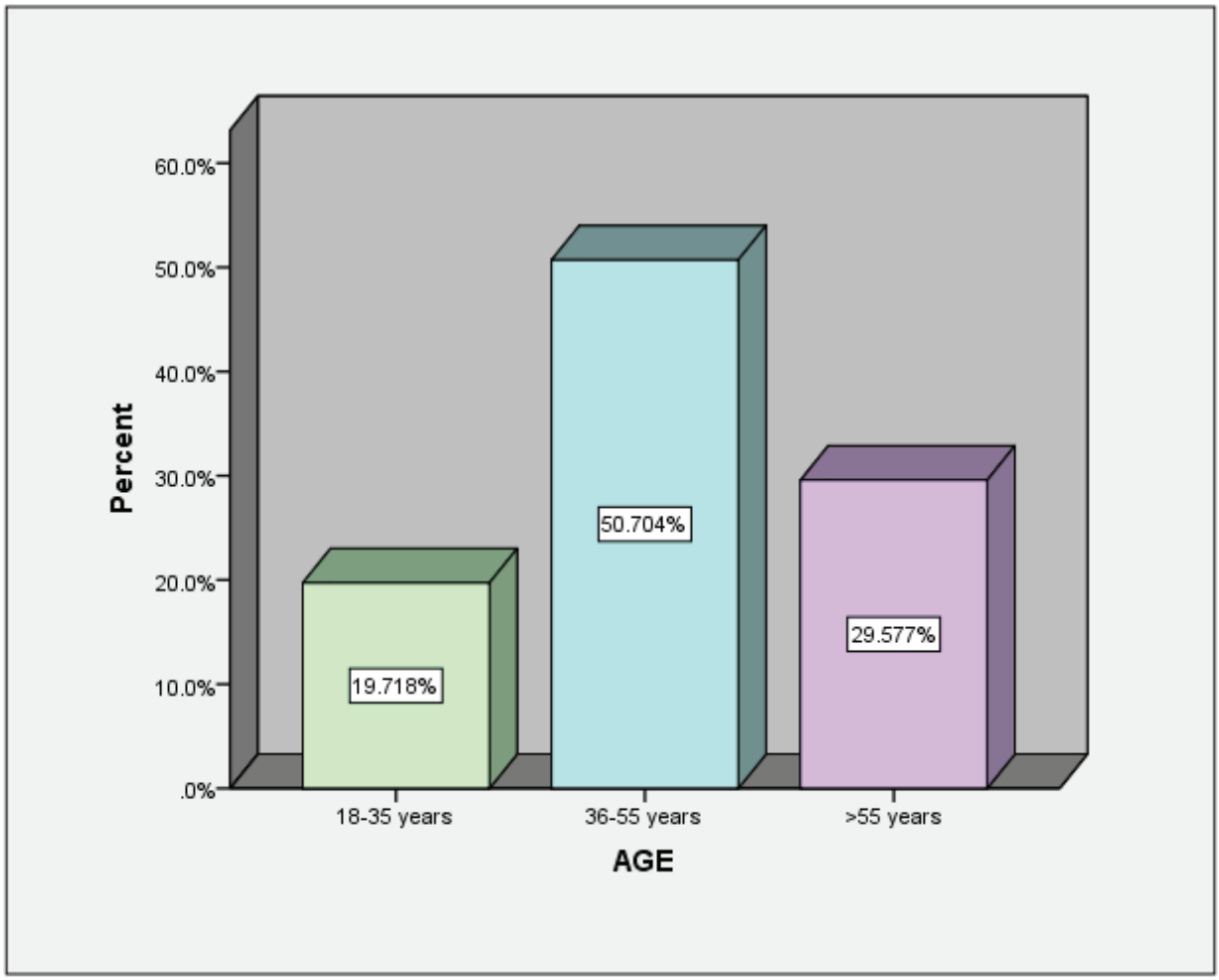


FIGURE 1: Bar diagram showing the percentage distribution of study subjects based on age. X axis represents the different age groups of the study participants and Y axis represents the percentage of the participants' age groups. 19.71% participants were between 18-35 years, 50.704% were between 36-55 years and 29.77% were above 55 years. Almost half of the study population belongs to the age group of 36-55 years.

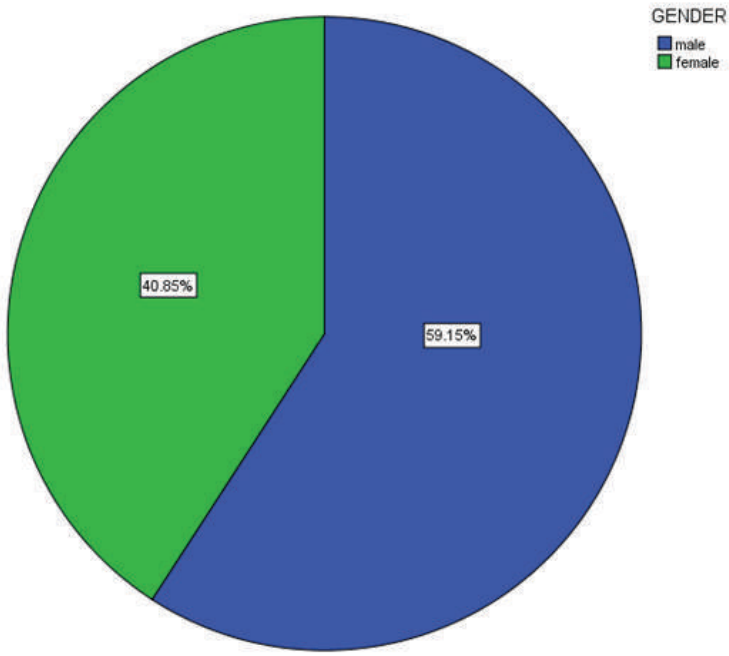


FIGURE 2: Pie chart represents the percentage distribution of study subjects based on gender. Among 71 participants, 40.85% were females and 59.15% were males, thus showing male predominance in the current study population.

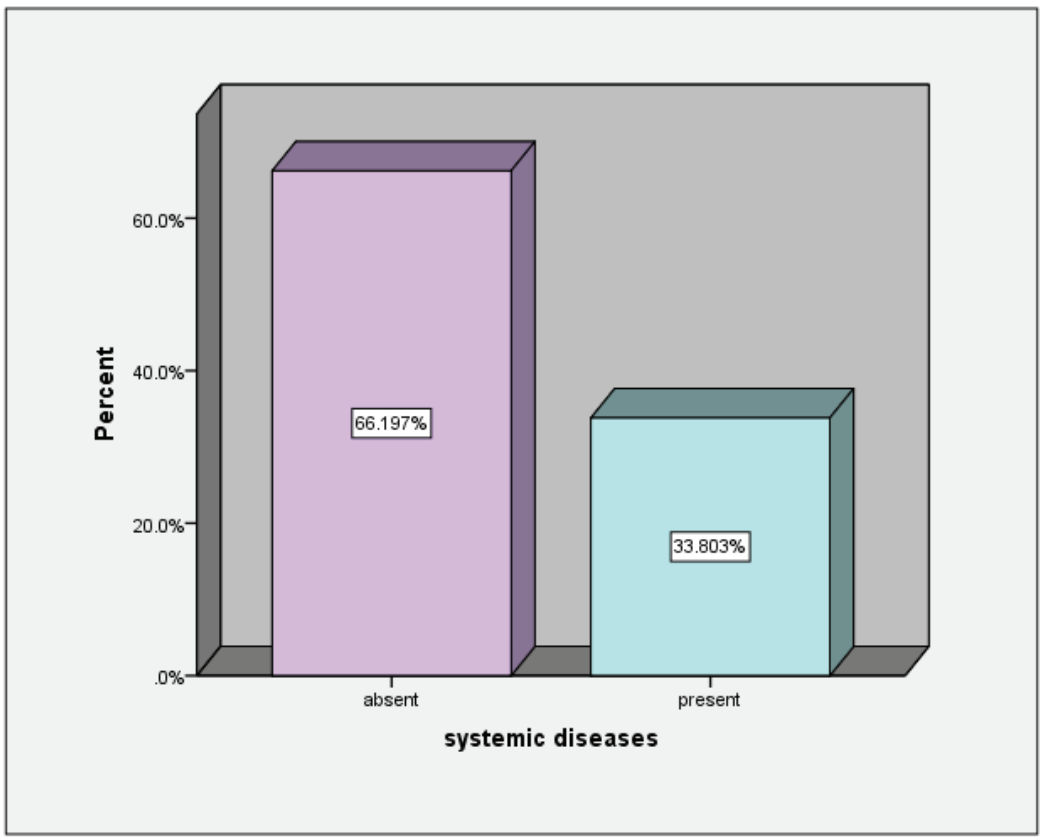


FIGURE 3: Bar graph depicting the percentage distribution of presence or absence of systemic diseases among the study subjects. X axis represents presence or absence of systemic diseases. Y axis represents the percentage of the study population with and without systemic diseases. 66.197% of the study population didn't have systemic diseases and 33.803% have systemic diseases.

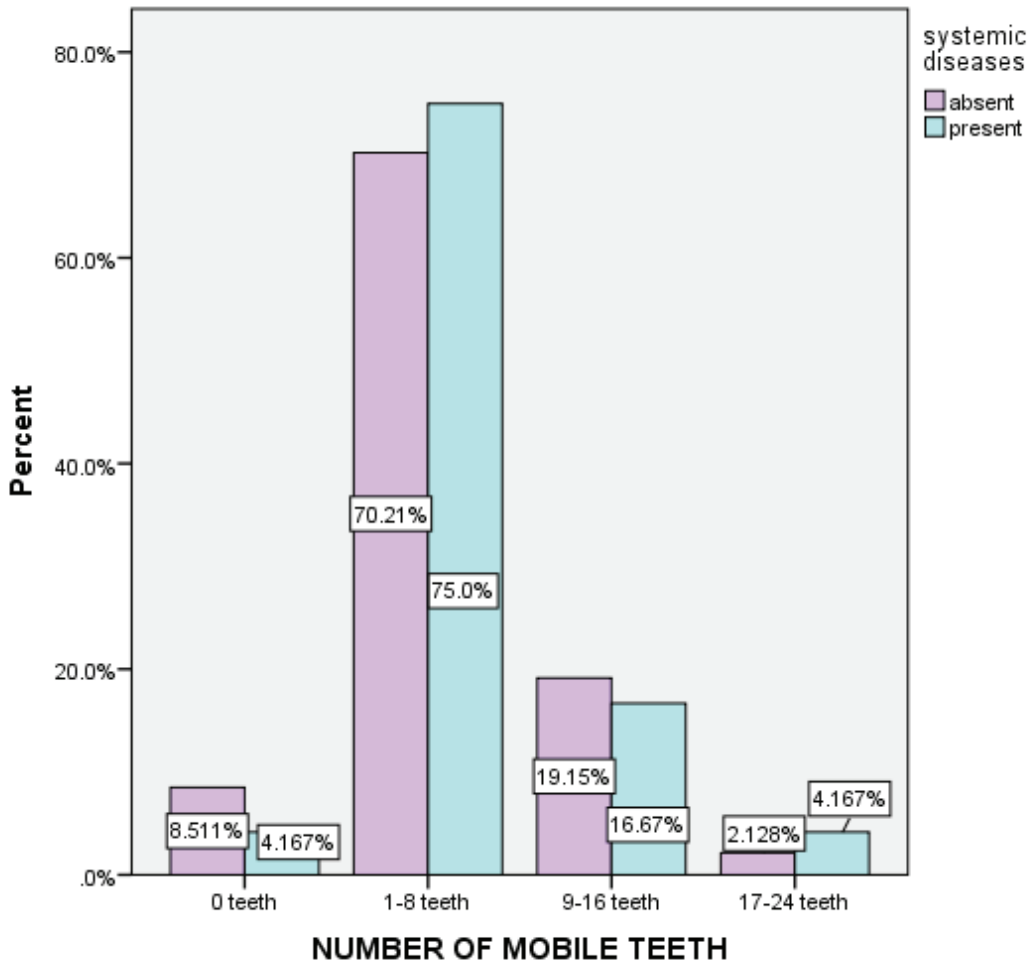


FIGURE 4: Clustered bar graph depicting association between number of mobile teeth and systemic diseases. X axis represents the number of mobile teeth and Y axis represents the percentage of study population with and without systemic diseases. Most of the participants without systemic diseases (70.21%) and with systemic diseases(75%) belong to Group II (1-8 mobile teeth). Highest number of mobile teeth(17-24) was found among 4.16% of the study participants with systemic diseases and 2.12% participants without systemic diseases. Chi-square test revealed no statistically significant association between tooth mobility and systemic diseases using (Chi-square value - 0.794 ; p value - 0.858).

Conclusion

Within the limits of the study, it was found that participants with systemic diseases comparatively had an increased prevalence of more mobile teeth than participants without systemic diseases. But this difference was not statistically significant. Further studies should be conducted with a larger sample population to assess better inter-relationships between tooth mobility and systemic diseases.

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Conflicts of Interest: Nil

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Evaluation of Clinical Marginal Adaptations of the Copings Fabricated in Cad Cam - Retrospective Study

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Abstract

CAD CAM Technology is a computer aided design and manufacturing system for the production of crowns. A variety of metal and all ceramic materials is available for the crown fabrication. The accuracy and the fit of the prosthesis depends on the CAD CAM system and each component individually like the milling and the scanner units. The aim of the present study was to determine the marginal adaptations of copings fabricated with CAD CAM and to analyse which technique has been used commonly to check the marginal fit of the copings. The study was carried out on a hospital setting with ethical approval. The records of the patients were collected and Chi square test was done to evaluate the results. From the overall study, it was found that there were different methods for the evaluation of fit of the copings. Most of the FPD patients were in the age group of 26 to 40 years. Most common method for checking marginal fit adaptation was RVG/IOPA. (p value =0.37) which is not statistically significant, followed by the catching on probing and the fit checker.

Keywords: *Copings, CAD CAM, Marginal fit adaptations, IOPA/RVG, Fit checker, Catching on probing.*

Introduction

The demand for better esthetics by the patients has increased over the years [1]. The use of all ceramic has increased due to its esthetics, colour stability and biocompatibility, but it is not indicated for long span bridges until the introduction of Zirconia [2]. According to the Millennium research group, it has been estimated the use of all ceramic crowns by 2015 from 40% to almost 70% [3]. The weakest link in the FPD treatment is the tooth restorative marginal interface. The marginal fit is an important factor [4]. For the long term success of the prosthesis. Poor form may cause dissolution of cement, percolation of the fluid, further leading to secondary caries [5]. McLean has given a clinically acceptable, marginal fit to be within 120 micrometer [6]. Sailer et al.m in 2006 stated all ceramic restorations failed 10.9% to 21.7% cut to secondary caries [7].

The advent of CAD CAM systems using optical scanning and computerised processing produced excellent fit and adaptation. Systems milling partially sintered zirconia could produce marginal values of about 34 to 78 micrometers [8, 9].

Digitalisation in prosthodontics have paved the way for new generation ceramics with unprecedented strength and performance [10]. Different methods have been used to assess marginal and internal adaptations of indirect restorations. Clinically, marginal fit can be analysed using a probe, dental mirror and adequate illumination and magnification [11, 12]. Marginal fit can also be indirectly evaluated radiographically and through epoxy resin replicas by light and electron scanning microscopy [13]. It is also possible to cross section the prepared tooth or master due the restoration for direct [14].

Assessment of the space correspondent to the cement gap- a surrogate measure of the marginal and internal adaptation under a microscope [15]. A popular

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non destructive method used to assess the marginal and internal fit in viva and in vitro is the silicone replica technique. A light body PVS material is used to record the space between the preparation and the internal surface of the restoration [16]. Recently, micro computed tomography (micro CT) imaging has also been used as non destructive methods to evaluate the Archibald and internal adaptations of indirect restorations [17]. There is consensus on which is the best non destructive method in evaluating the marginal and the internal adaptations of indirect restorations [18, 19]. All the previous studies mentioned in the review have compared the silicone replica technique with the cross sectional technique. Based on Beer-Lambert law proportionally between a known thickness and the light transmitted through the material can be assumed [20, 21]. Therefore the objective of this study was to determine the most commonly used technique or the evaluation of marginal fit adaptation in copings fabricated in CAD CAM.

Materials and Methods

The present study is a retrospective study carried out in a hospital setting under a specific population predominantly South Indian population. It is a single centred study with a small sample size. It was carried out under Institutional Review board approval. In this study, data of the patients were collected by complete analysing of data of 86000 patients between June 2019 and March 2020. Data including patient name, age, gender, radiographic verification of the copings and the marginal fit adaptations were collected. And for further analysis the collected data was cross verified by another examiner. The collected data was tabulated using Excel Spreadsheets and the data was analysed using SPSS software version 19. The statistical test used in this study was Chi square test with p value<0.05 and confidence interval of 95%.

Results and Discussion

From the present study, it was found the different methods were used for the evaluation of marginal fit of the copings. Out of which the most common was the radiographic evaluation. (IOPA/RVG) (p value=0.37) which is statistically not significant. RVG/IOPA was the most commonly used technique which was followed by the fit checker used to analyse the marginal fit in the posterior region in most of the cases (Fig 1). This study analysed the commonly used method for the evaluation of marginal fit in copings fabricated in CAD CAM. According to the study, RVG/IOPA was the most commonly used method to evaluate the marginal fit. And the second common was the fit checker, mostly used in the posterior regions. The most common age groups of FPD patients were seen in the age groups of 26 to 40 years. Age group 18 to 25 years (20.3%) most commonly involved sextant 1, age group 25 to 40 years (52.8%) involved sextant. Age group 41 to 60 years (43.0%) mostly involved sextant 3 and the age group above 60 years(9.8%) involved sextant 4. In the correlation between the age groups involved, 26 to 40 years of age was the common age group with FPD patients. The use of IOPA/RVG was common in all the six sextant. Particularly in sextant 5 and sextant 2 (44%) and (39.2%) catching on probing was used to evaluate the marginal fit adaptation. In sextant 4 (26.6%) primarily the fit checker was used (p=0.037) statistically not significant. Comparing the gender in the present study, the males had a higher predilection of FPD when compared to the female population (Fig 2). In the correlation between the sextant involved and the types of coping evaluation: RVG/IOPA was commonly used in all the six sextants. Followed by the catch on probing technique and the fit checker.

Marginal and internal adaptations is one of the most important factors for the success and clinical longevity of the crowns. Marginal misfit may lead to hypersensitivity, secondary caries and periodontal problems. It may also result in thick cement film, exposing the luting material and promoting its dissolution over a period of time [22]. Poor internal adaptations can lead to lack of restoration retention and poor resistance form from the tooth restoration complex. According to previous studies, it was reported that microscopes can result in inaccurate measurement, projection errors and rounding of the margins [23]. The cross sectioning technique may result in distortion of the restoration during sectioning. The silicone replica technique is considered the reliable method to evaluate the marginal fit, on the other hand it may result in tearing of the silicone film, sectioning plane error. Veneers fabricated using leucite reinforced lithium disilicate exhibited the least marginal discrepancy followed by lithium disilicate ceramic [24]. The micro CT imaging technique allows a two or three dimensional assessment of the marginal fit adaptations

of the restorations, non destructively.

Marginal discrepancy severely affects the long term success of All ceramic complete veneer crowns. Masticatory forces cause fatigue to the dental luting agents. It was reported that there was an effect on the periodontium and the tooth most commonly in the lower sextants due to poor oral hygiene [25]. Beuer et al., suggested there was higher prevalence in the posterior teeth following extractions [26]. Nawafleh et al. According to the study concluded, 85.5% of caries and missing teeth were seen among the age groups between 20 to 34 years [27]. Seo et al, said that the prevalence of decay and missing teeth was about (94.3%) among the age groups 35 to 45 years [28]. The success of all ceramic crowns included factors namely, esthetic value, resistance to fracture and third being marginal fit [29]. RVG provided best results with superior image recording capabilities [30]. Fit checker brought about newer advancements in checking the marginal fit accuracy of the prosthetics [31]. Therefore, the marginal fit and internal adaptation of indirect restorations is critically important in restorative dentistry. The limitations of the present study was single centred and short study samples.

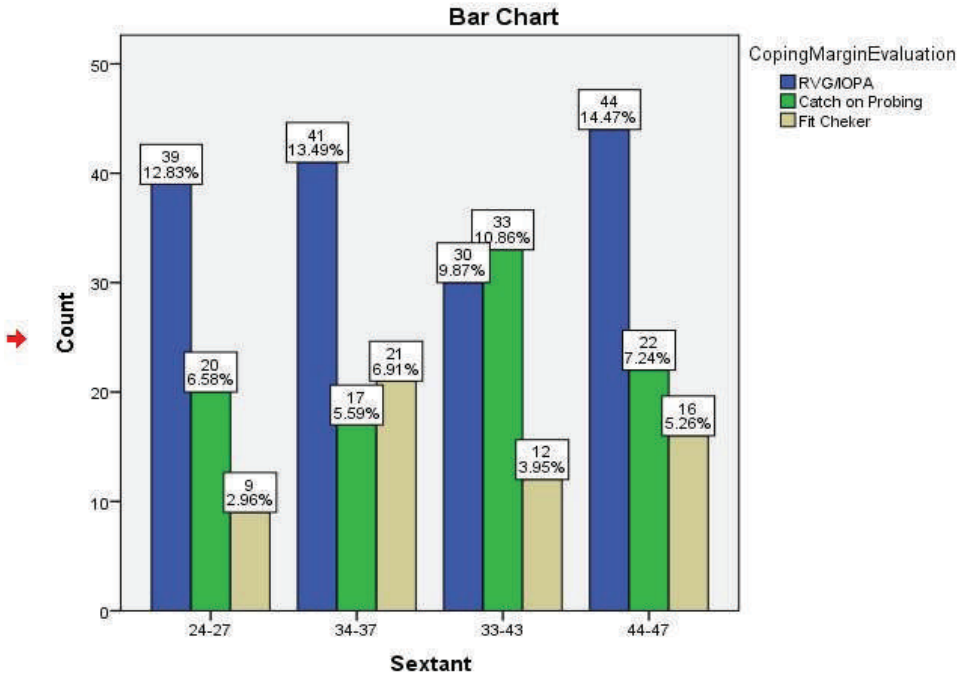


Figure 1: Bar chart depicting the association between the sextants involved and the methods of coping evaluation being used. The X-axis denotes the sextants and Y-axis denotes the percentage of the method of coping evaluations. Blue denotes RVG/IOPA, green denotes catching on probing and yellow denotes fit checker. IOPA/RVG is the most common method used for marginal evaluation of coping than catching on probing and the fit checker, however it is statistically not significant. Pearson's Chi square test value :13.390; df : 6; p value = 0.37 (> 0.05)

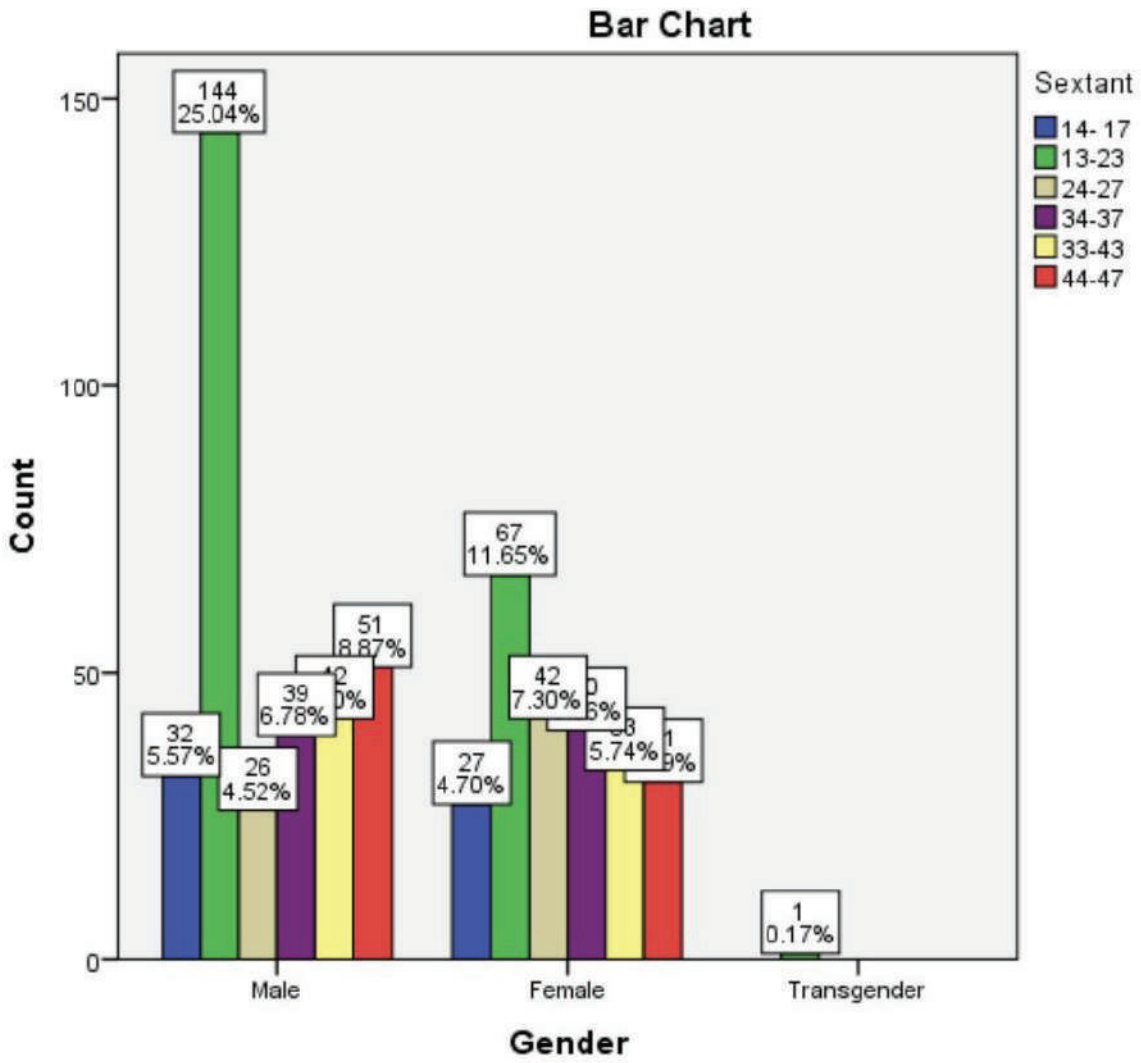


Figure 2: Bar chart depicting the association between the gender and number of FPD done in each sextant. X axis denotes the gender and Y axis denotes the number of FPD in each sextant. Blue denotes sextant 1, green denotes sextant 2, beige denotes sextant 3, purple denotes sextant 4, yellow denotes sextant 5 and red denotes sextant 6. Chi square test was done and the association was found to be significant. Pearsons's chi square value: 25.210 ; df : 10; p value : 0.005 (<0.05) hence statistically significant, proving that males have undergone more FPD treatment than females.

Conclusion

Radiograph has been the gold standard for many years for evaluation of fit of fixed dental prosthesis. According to the limitations of the study IOPA/RVG was found to be a commonly used method to evaluate the marginal fit of the crown fabricated in CAD CAM followed by catching on probing and the fit checker. Males have undergone FPD more than females mostly in the second sextant.

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Type of Research : **Retrospective Study.** *DOI Number: 10.37506/ijfmt.v14i4.12453*

Prevalence of Ridge Defects According To Sieberts Classification in Patients Undergoing Fixed Partial Denture - A Retrospective Analysis

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Abstract

In prosthetic dentistry, dentists may face challenges in treating patients with alveolar ridge defects in edentulous areas. Alveolar ridge defect may occur due to injury, trauma, denture wear and periodontitis. Based on Sieberts classification, it can be classified into three classes. class I- buccolingual loss of tissue , class II - apicoronal loss of tissue and class III - both loss of tissue. It is important to close the ridge defect by replacing the tooth loss and achieve good esthetic, phonetic and mastication . According to classification, a proper treatment plan and alternative can be determined for successful outcomes.The aim of this study is to assess the association of sieberts classification for edentulous ridges in selecting the pontic design in FPD.

This study was conducted in saveetha dental college. A total number 778 patients with alveolar ridge defects was selected. The data was collected using patient treatment details used in SIMATS and data was analysed using SPSS software. Based on results, class I ridge defect was more common among all age groups and also in all 6 sextrants . Class III ridge defect was more common in males compared to females. The $p<0.005$ which is statistically significant . As age increases the ridge defects vary. It was concluded that the prevalence of Sieberts classification helps in suggesting various management techniques or treatment planning to ensure that the prognosis and treatment outcomes can be better and successful.

Keywords: *Alveolar ridge defect; Pontic design; ridge augmentation; Siberts classification.*

Introduction

An alveolar ridge deficiency or ridge collapse may occur , when tooth is extracted, or due to injury , trauma , denture wear. Ridge deformities can create esthetic and functional dilemmas for the patient and restorative dentist[1]. Recently fixed prosthesis is more popular than removable prosthesis due to comfort, function and aesthetics[2]. Ridge deformities have both soft tissue (papilla and attached gingiva) and bony alveolus components. Soft tissue deformities can occur when surgical incision is made in delicate areas. MRSA (Methicillin-Resistant Staphylococcus aureus) in particular, has become the leading cause of skin and soft tissue infections[3]. Deformities of bone can occur following extractions of a tooth that has a thin dentoalveolar previous endodontic surgery, endodontic failure, and iatrogenic bone removal, intentional bone removal to gain a purchase, root fracture or periodontal bone loss[4]. With the increasing incidence of periodontal diseases and development of antibiotic resistance, there is a global need for alternative treatment modalities that is safe, effective and economical[5]. Blood investigations do not appear to be clinically useful for diagnosis[6]. Proper dental care during pregnancy is very important, since pregnancy causes various hormonal changes in the body that can actually increase the risk of developing dental diseases[7].

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Selection of teeth has been a major concern during replacement of teeth for completely edentulous conditions[8]. It is recommended to keep the dentures well relieved from the gingival margin wherever possible[9]. The mean microgap at the implant-abutment interface at the platform level at the external, middle, and internal points for both original abutments and non original abutments was found to be within clinically acceptable limits[10]. Marginal fit can be influenced by several factors like the type of finish lines, thickness of the die spacers, preference of restorative materials, processing techniques for fabrication and the choice of luting agents[11]. Veneers fabricated using leucite reinforced lithium disilicate exhibited the least marginal discrepancy followed by lithium disilicate ceramic, aluminous porcelain, and zirconia-based ceramics[12]. Furthermore it is also essential for the selection of pontic for the patient and indication for the patient to undergo surgical interventions to relapse the ridges[13]. The internal surface of the implant undergoes a series of changes with fabrication of restoration[14]. Masticatory forces cause fatigue to the dental luting agents, adversely affecting the retention of these cement-retained crowns[15].

Siebert has classified residual ridges into three categories based on the amount of ridge destruction. Class I - buccolingual loss of alveolar tissue with normal apicoronal height. Class II- apicoronal loss of alveolar tissue with normal buccolingual width. Class III- both apicoronal height and buccolingual width loss of tissue. Prosthetic rehabilitation is done to regain function, speech and esthetics[16]. The height and width of the residual ridges at the edentulous site decides the type of pontic that will be seated on the ridge and in all probability should maintain and mimic the appearance of its neighbouring teeth to the possible extent[17]. For teeth requiring crowns or replacement in the form of fixed dental prosthesis, gingival retraction becomes a mandatory procedure which aids in recording the prepared and unprepared surfaces of the abutment tooth[18]. The magnetically retained maxillofacial prosthesis provides an esthetically acceptable facial contour, functionally prevents food spill over from the oral cavity and boosts a patient's psychology to present themselves socially[19]. Interproximal embrasures should be left open to permit easy cleaning.

The aim of this study is to assess the association of sieberts classification for edentulous ridges in selecting the pontic design in FPD in Private dental college, Chennai.

Materials and Method

It is a single centered retrospective study conducted at Saveetha dental college and hospitals, Chennai. A total 778 patients, predominantly South Indians were included in the study. Ethical clearance was obtained from the International review board. The study was conducted from june 2019 to march 2020. Validation to the study was done by undergraduate, postgraduates and all faculty members of Saveetha dental college.

Data collection was done by using patient management software which has all patients records . It is a recording system of all patients of all data related to the medical and dental history of patients and treatment done in Saveetha dental college. The collected data was tabulated under following parameters - name, age, gender, region, Sieberts classification and pontic design . The main variables include Ridge classification and pontic design.

The data analysis was performed using SPSS software (version 19). The chi square test and pearson correlation was done. The chi square test was used to compare the data and checked for the distributions at 0.05 level of significance for effect of statistical significance.

Result and Discussion

A total of 778 patients with alveolar ridge defects were selected in this study.Out of 3 classes, in this study it was found that class I ridge defect (51.8%) was more common among all age groups irrespective of gender. And it was also observed the class IV type was more common in males compared to females.

Based on the results, the prevalence of ridge defects were more in males than females. Seibert class III defect was most common in males (38%) and class II defect was most common in females (31%). (Graph 1) The correlation between gender and type of sieberts classification showed the chi square value 0.000 which is statistically significant. According to the study by shahroom et al., also, male gender has high prevalence in having alveolar ridge deformities with 54.5%[1].

Class I ridge defect was seen in all 6 sextant. Class II ridge defect (23.5%) was seen in sextant 5 that is from 33-43. Class III was mostly seen in sextant II that is from 13-23 (34.2%).7.9% of class IV defect was observed in sextant 6 that is from 44-47. (Graph 2) The correlation between sextant and Sieberts classification was 0.067 which is not significant.

Class I defect was most common in patients above 60 years (58.1%). 21.1% of patients of age 18- 25 had class II ridge defect in common . Class III defect (31.2%) was most common in the 18 -25 age group. Around 14.3% class IV defect was seen in patients above 60 years.(graph 3) The correlation between age and sieberts classification, the chi square was 0.008 which is statistically not significant. In the study by shahroom et al., patients in the age within 40–49 years old have a high incidence of alveolar ridge defect with 50.9%[1].

Besides, the study of prevalence in Sieberts classification was intended to give a clear image on treatment choices and alternatives. The primary goal of closing the defect and replacing the tooth are to restore the loss of function, esthetic and natural appearance, but the goal can only be achieved if the final prosthesis is modified according to the prevalent situation[20].

In a study done by Tanka et al., a segmental osteotomy procedure with an interpositional graft is done on a patient with class II alveolar ridge defect[21]. Most of the studies have shown that this technique is practical and a predictable procedure with low incidence of complications and high probability of successful treatment outcomes [22].

According to sieberts classification, the quantification of the magnitude of the ridge deficiencies was not included by Siebert. The integrity of soft tissue also depends on the type of prosthesis given[22]. Therefore modification of siberts classification was introduced by allen in 1985 [1].

The need for the study is to determine the type of design fabrication based on the prevalence of ridge defect and also for knowledge and practise of pontic design selection by general practitioners and to evaluate the different pontic design and their complication among people.

The limitations of the current study are, it is a single centered study with small population size and does not include any ethic groups or races.

This study can be used for further study for a larger population and to create awareness in selecting the pontic design for FPD based on ridge defect and for better and successful treatment outcomes.

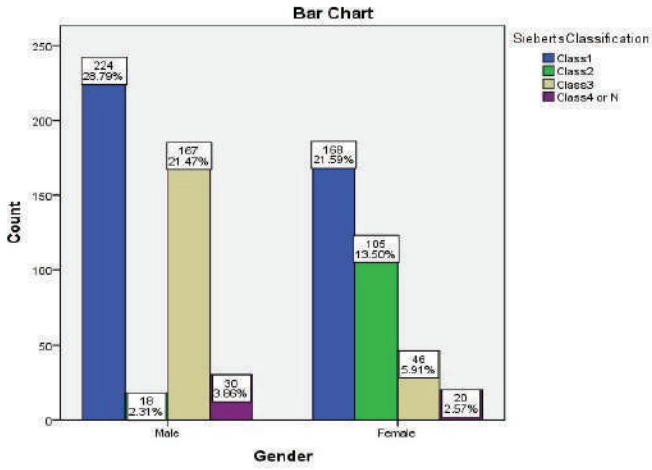


Fig 1 - Bar graph depicting the association between gender and sieberts classification. X-axis denotes gender and y-axis denotes number of patients. Blue colour denotes Class 1, green denotes class 2, yellow denotes class 3 and purple denotes class 4 or N. Chi square test was done and the association was found to be statistically significant. Pearson chi square value:129.561; df : 3; p value : 0.00 (<0.05) hence statistically significant, proving males have more ridge defects than females.

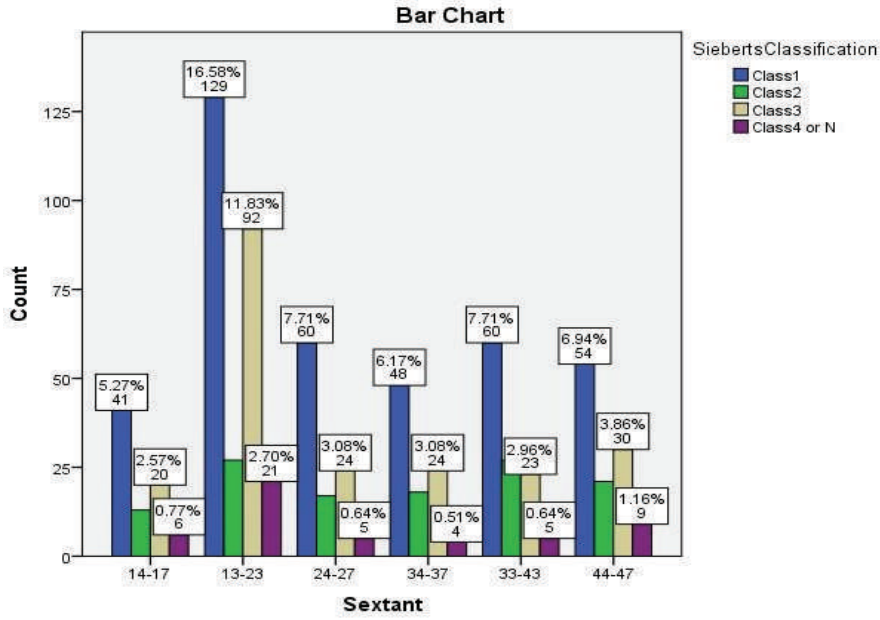


Fig 2 - Bar graph depicting the association between sextant and siebert classification. The x-axis denotes sextant and y- axis denotes the number of types of ridge defects.The colours of Graph 2 represent Sieberts classification where blue represents Class 1, green represents class 2, yellow represents class 3 and purple represents class 4 or N. Class I ridge defect was more common in all sextants, however Chi square test was done and association was not statistically significant. Pearson chi square value : 23.869; df : 15; p value : 0.67 (> 0.05).

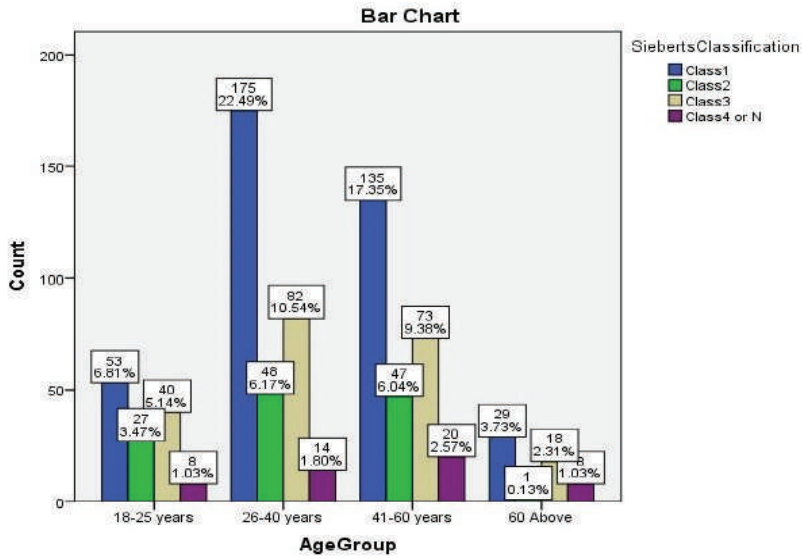


Fig 3 - Graph depicting the association between age and sieberts classification. The x-axis denotes the age group and y-axis denotes the number of patients with ridge defects. The colours of Graph 3 represent Sieberts classification where blue represents Class 1, green represents class 2, yellow represents class 3 and purple represents class 4 or N. The Chi square test was performed and association was found to be statistically significant. Pearson chi square value : 22.375; df : 9; p value : 0.008 (< 0.05) which is statistically significant proving that class I, II, III, IV ridge defect is more common in the age group of 26-40 years.

Conclusion

The prevalence of Seibert's classification helps to provide various management techniques and plans for better treatment outcomes for the patient. Within the limitation of the study, it was found that males have more alveolar ridge defects compared to females. In association with sextant; class I ridge defect was more common in all the sextant. However within the age group between 26-40 years had class I,II,III,IV ridge defects in common.

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Conflict of Interest: The authors declare no conflict of interest.

Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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A Smile Analysis in Completely Edentulous Patients Treated by Graduate Students

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Abstract

To evaluate smile analysis in completely edentulous patients. Smile is a main component in presentations of a human being favoring social acceptance. A non harmonic smile decreases the beauty of the face causing discomfort in the social activity Edentulous in a condition of being toothless. Details were collected from patients visiting dental college and reviewed patient records who underwent denture treatment between june 2019 and march 2020. In the present study we analyzed visibility, fullness, teeth size, buccal corridor space and smile line.. The study reviewed the patients who had undergone treatment between June 2019 – April 2020.Data was entered in a methodological manner and was verified manually. The available data was subjected to statistical tests using - Chi square test statistical software- SPSS software by IBM.The present study indicates the dentures constructed by training students are good at fullness, visibility and teeth selection criteria but there is lack of adequate buccal corridor space and smile curves being incorporated into dentures.

Key words*: smile line; visibility; complete denture; fullness; buccal corridor space*

Introduction

Increased life expectancy and increase in population, creates high demand in dental care especially in the field of prosthodontics.[1] Prosthetic rehabilitation in patients with bony defects is critical.[1,2] Good appearance is not considered a variety sign but literally a need and dentistry has a fundamental role in obtaining it. Since the face is the exposed area of the body and constitutes an important component of the mouth a prominent line.[3] The smile constitutes an important component in the presentation of a human being favouring social acceptance.[4] A non harmonic smile is one of the most important facial expressions that demonstrate friendship, pleasant sensation and appreciation.[5] Smile according to webster a change of facial expression involving aa brightening of the eyes, an upward curving of the corners of the mouth with no sound and less muscular distortion of the features laugh that may express amusement, pleasure, tender, affection, approval, restrained with irony decision or any other emotions.[6] Frush and Fisher and Jamerson discussed the concept of smile line and defined it has the harmony between the curvature of the incisal edges of the maxillary anterior teeth and the upper border of the lower lip.[6–8] According to sarbi smile line is the amount of vertical tooth exposure. On smiling the height of the upper lip relative to the maxillary central incisors and crown height.[9]

Different socio demographic variables such as age,gender, and systemic disease May affect satisfaction with complete dentures.[10] The comfort, function, and esthetics must be restored altogether while treating a completely edentulous patient.[11] One of the main objectives in selecting and arranging artificial teeth is to produce a prosthesis that defies detection.[12]

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Complete dentures consist of two main parts, namely the artificial teeth and the denture base.[13] The artificial tooth is used to restore the appearance of the natural tooth, its occlusion oral function and to assist in word pronunciation. [13,14] Patients requiring complete dentures usually expect comfort first, followed by harmonious appearance and efficiency.[15] Besides, on receiving their first dentures, patients often expect to appear similar to when they had their natural teeth.[16] Therefore, correct selection of artificial teeth is essential to achieve a pleasant esthetic outcome.The anterior teeth are the ones primarily selected to satisfy esthetics[17]. The selection requires scientific knowledge as well as artistic skill of the dentist.[18] Teeth that are of a proper size are essential in achieving a natural looking denture.[19]

With increasing dental awareness, the scope of implant therapy has increased manifold.[20] This most advocated therapy for replacement of teeth, however, holds many prosthetic complications such as crown loosening because of short abutments, esthetic failures, ceramic fracture, and inappropriate proximal contacts leading to food accumulation, and associated peri-implant diseases.[21] The prosthetic component failures of the dental implant have also been frequently associated with screw loosening or fracture hence many people opt for complete denture.[22]

The denture base is the foundation of the artificial tooth and can be used to restore the appearance of the natural tooth. It is recommended to keep the dentures well relieved from the gingival margin wherever possible as in recent decades, microbial resistance has reached an incredibly alarming level. [23-,24] It is important to restore the esthetics of a complete denture patient to re-store their appearance. Restoring the function as well as aesthetics is equally important.[25] It is also important to give proper oral hygiene instructions to the patient as acute spreading bacterial infection affects the quality of the denture.[26] Proper dental care during denture wearing is very important.[27]

Materials and Methods

The present study was done in Saveetha dental college Chennai with required approval from the Ethical board. .Retrospective study data analyzed from june 2019- feb20. Case sheet verification was done by 2 expert reviewers .To minimize sampling error, inclusion of all available data was done and verified by the experts. The screening led to a pool of 4048 patients reporting to clinics and the search narrowed 355 patient details based on the inclusion and exclusion criteria on complete denture treatments. Inclusion criteria include both arch complete denture treatments,photographs with good frontal views,patients with no facial abnormalities or asymmetries. Exclusion criteria include single complete denture, any maxillofacial defect associated with complete dentures. Evaluation was done using photographs taken at the time of treatments. The evidence was looking at various parameters such as lip fullness, teeth visibility, buccal corridor space and smile line. These details were scored as adequate or inadequate to look for the parameter differences. These parameters define the denture esthetics and a skill which requires to be developed by the dental operator. Data was entered in a methodological manner and was verified manually. The available data was subjected to statistical tests using - Chi square test statistical software- SPSS software by IBM.

Results and Discussion

The retrospective analysis of 355 complete dentures patients details revealed that more than half of the subjects were above 50 years. Males were 205 (57.2%) and females were 150 (42.8%)(figure1)The denture visibility was good in 73.17%% of the cases, and there was adequate lip fullness in about 75.20% of the cases. With regard to buccal corridor space there was a high number of inadequacy in 51.53% cases and smile lines were not following lower lip curvatures in 58.36% of cases. (figure 2-6)

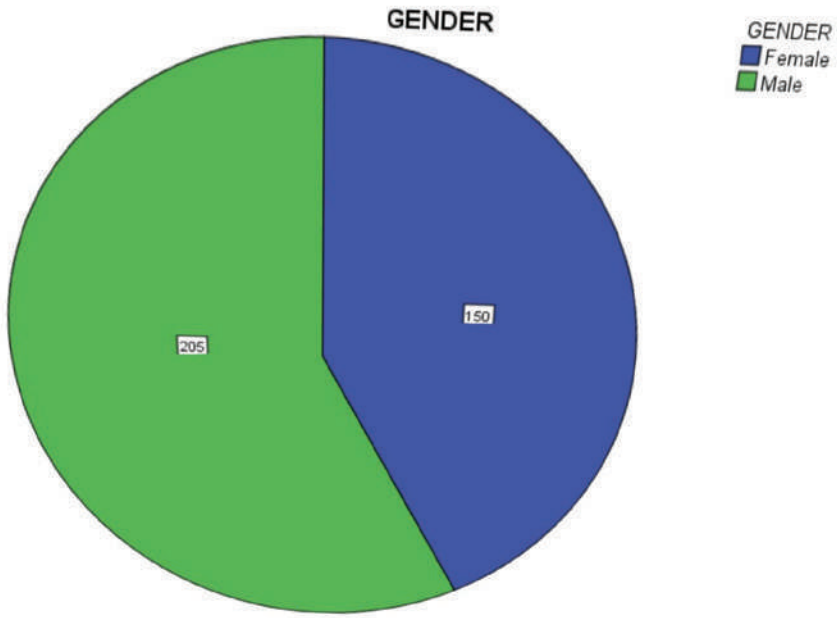


Figure 1: The pie chart represents the distribution of study participants based on gender. The graph shows a higher percentage of male participants with 57.2%(205) and females with 42.8% 150. Blue represents female distribution and Green represents male distribution.

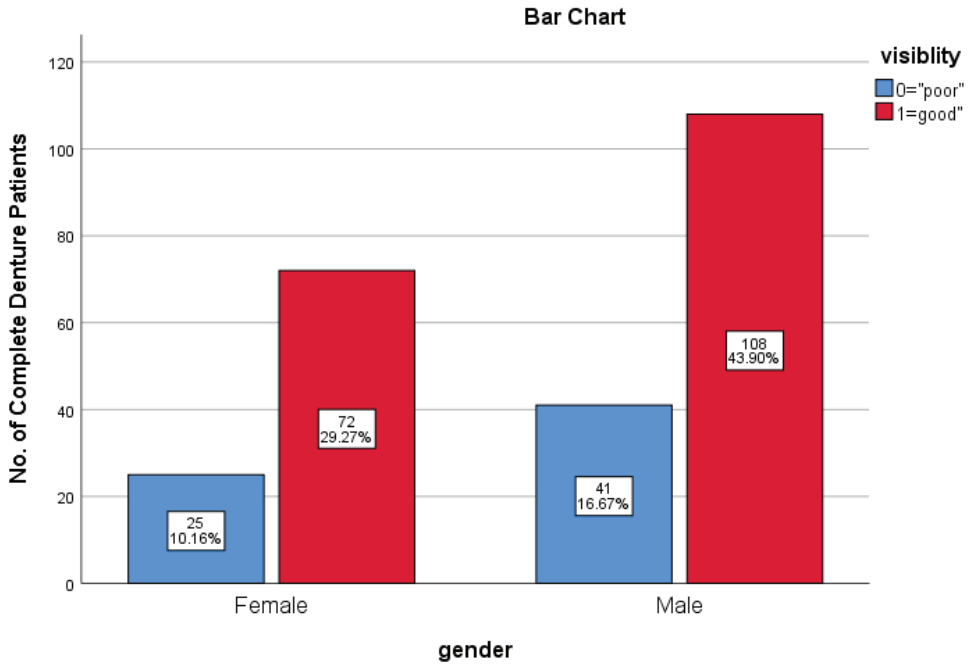


Figure 2 - Bar chart showing the association between Gender and visibility of anterior teeth X axis represents the gender and Y axis represents the percentage of visibility .Chi square test was done and association was found to be statistically not significant .Pearson chi square value 0.877 ,DF:2, P value - 0.445 (> 0.05) statistically not significant.Proving that in the dentures delivered by graduate students anterior teeth visibility was the same in both males and females.

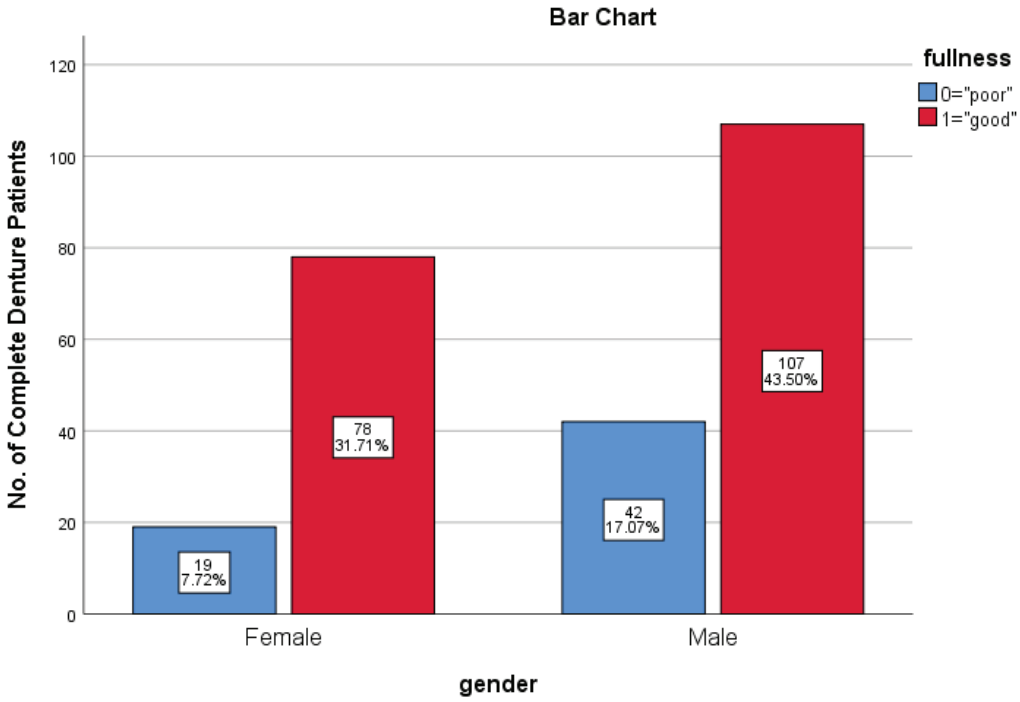


Figure 3 - Bar chart showing the association between Gender and fullness of anterior teeth X axis represents the gender and Y axis represents the percentage of fullness.Chi square test was done and association was found to be statistically not significant .Pearson chi square value 0.971 ,DF:2, P value - 1.000 (> 0.05) statistically not significant. Proving that lip fullness was the same in both genders.

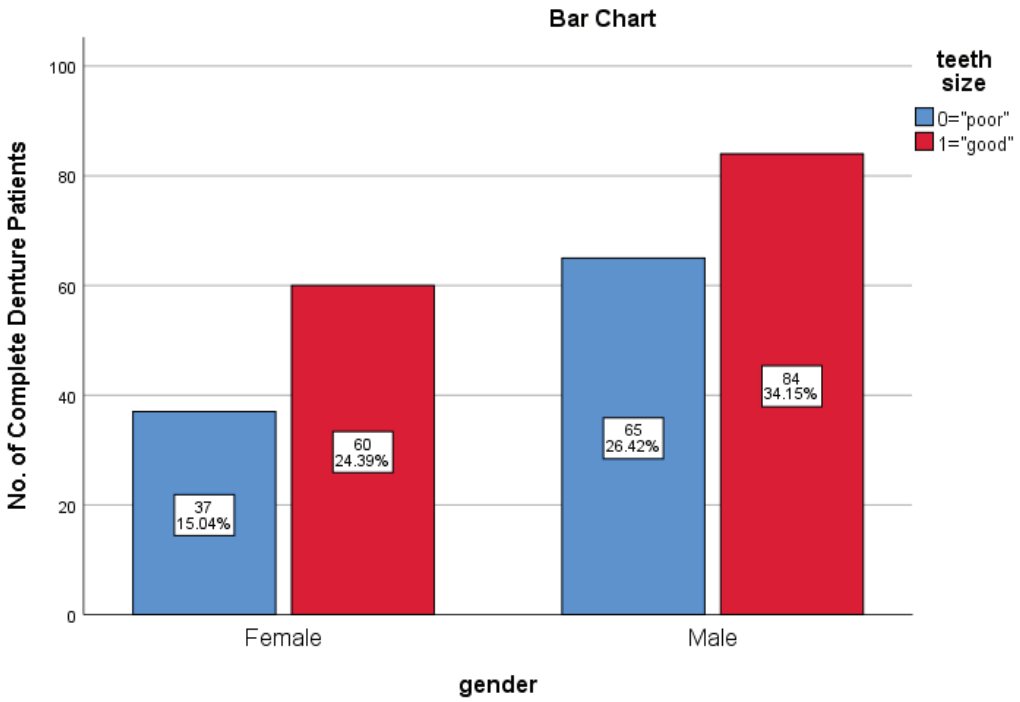


Figure 4 - Bar chart showing the association between Gender and teeth size of anterior teeth X axis represents the gender and Y axis represents the percentage of teeth size.Chi square test was done and association was found to be statistically not significant .Pearson chi square value 0.940 ,DF:2, P value - 0.853 (> 0.05) statistically not significant.Proving that teeth sizes were not different among the genders.

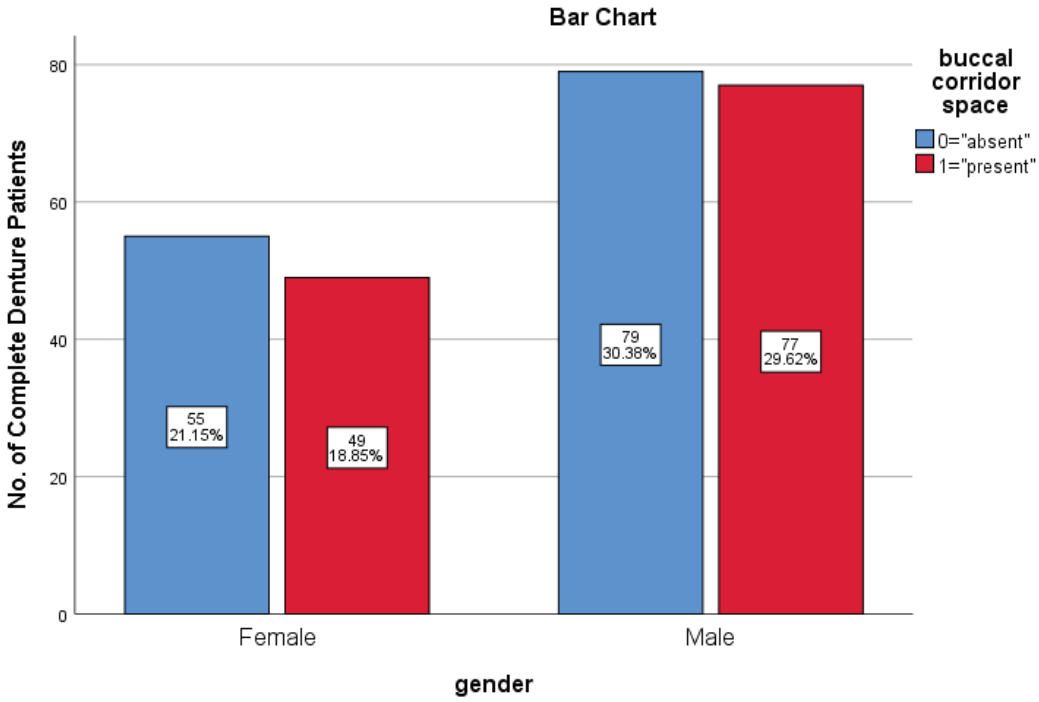


Figure 5- Bar chart showing the association between Gender and buccal corridor space X axis represents the gender and Y axis represents the percentage of buccal corridor space.Chi square test was done and association was found to be statistically not significant .Pearson chi square value 0.759 ,DF:2, P value - 0.677 (> 0.05) statistically not significant. Buccal corridor space was not visible both in males and females.

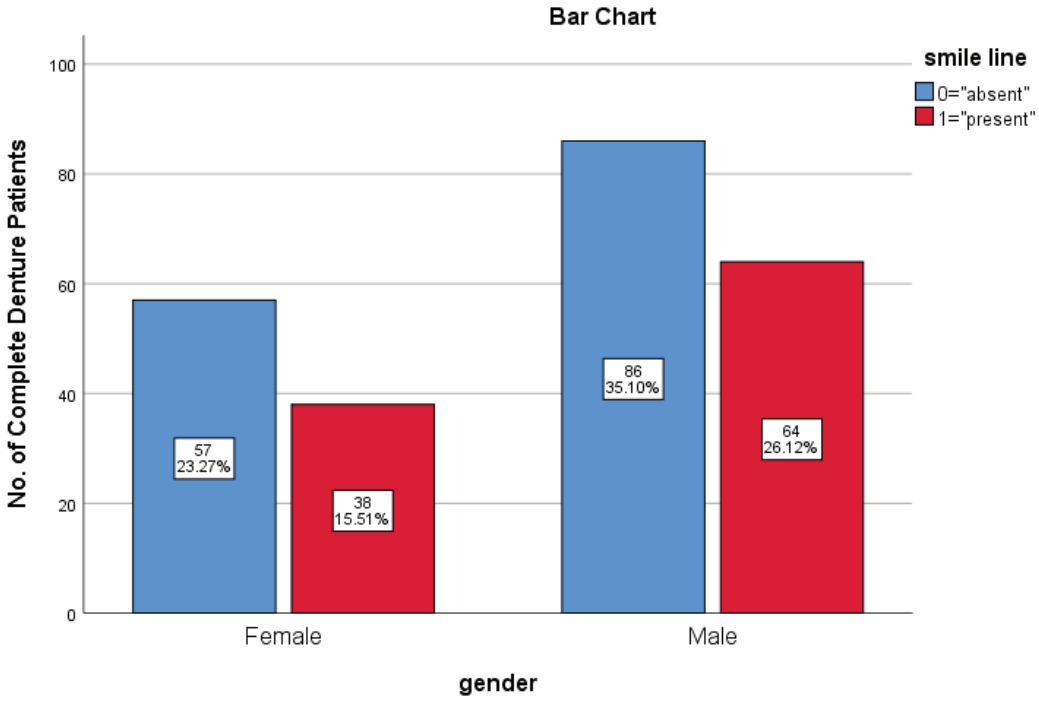


Figure 6 - Bar chart showing the association between Gender and smile line. X axis represents the gender and Y axis represents the whether smile line were seen or not .Chi square test was done and association was found to be statistically not significant .Pearson chi square value 0.759 ,DF:2, P value - 0.6777 (> 0.05) statistically not significant. Proving that smile lines are not different in males and females.

In the present study it was observed that the smile line curve was inadequate in both genders but predominantly more seen in females. Al.juboori MS et al. too reported similar findings with females as they have a 2.5 times more prevalent to high smile curve whereas males have more of low smile curve.[28] The Buccal corridor – defined by Frush and Fisher as the space created between buccal surfaces of position teeth and commissure of the lips when the patient smiles.[29] The teeth should fill partially and a visible black back drop add to the esthetics. This feature was again inadequate in both gender with more females not having adequate buccal corridor space in the present study with statistically non significance p> 0.05- chi square test. A study by Trite Trikku et al differed in our opinion as they suggest attractive smiles had least buccal corridor display and maximum inter canal and inter molar width.[30] The visibility, fullness parameters associated with the complete dentures were adequate and were statistically insignificant.

The smile line curve to follow the lower lip line is important and requires more visualization and patient cooperation during denture trial. The most common issues during denture trial being lack of retention also plays a role in misleading the denture trial step. There are studies reporting on issues relating to poor denture retention and esthetic error during denture trial.[31] Currently there are reports on computer based images or smile designing softwares available, may be use of the same can reduce such errors in denture construction.[32] The buccal corridor space is a area of differences among different clinicians. As many orthodontists look at buccal corridor space presence as not very esthetic, but others differ with this opinion.[33] The presence of buccal corridor space adds to the value of smile by acting as a backdrop and making smile pleasant.[34] There are differences in the point of skeletal changes or increased buccal pad thickness, which sometimes dictate the adequate visibility of the buccal corridor space.[35,36]The present study can be extended as a multi center study with adding or including other speciality in having an opinion on buccal corridor space and esthetic smile.[37]. Also digital smile designing or planning of complete denture at the diagnostic stages and executing the same can be an alternate solution.[38]

Conclusion

The present study indicates the complete dentures constructed by undergraduate students are good at anterior lip fullness, teeth visibility criteria, but there is lack of adequate buccal corridor space and smile line curves being incorporated into dentures which make up to the complete esthetic impact.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Hemoglobin Levels and the Associated Factors in the Patients Visiting A University Hospital in Chennai, India

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Abstract

Hemoglobin is a protein molecule present in the red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from the body tissues to the lungs. The normal Hemoglobin level for males is 13.5 - 17.5 -grams per deciliter and for females is 12.0 - 15.5 - grams per deciliter. However, Adult men and women have different haemoglobin levels, which are independent of iron status. Estimation of Hemoglobin levels is essential due to several reasons and this research is unique in the aspect of its importance in dentistry. This study aims at the assessment of Hemoglobin levels of patients, based on their gender visiting Saveetha dental college outpatient department. This is a comparative and descriptive study for which the data was obtained from reviewing the patient records. The data collection was from the Undergraduate,postgraduate clinics, Saveetha dental college, SIMATS. The data was collected and compiled followed by its statistical analysis by using the SPSS software by IBM. Out of the total sample size (1825 cases), various hemoglobin result values obtained were, 5-8 g/dl - 5.2%; 8-12 g/dl - 38.0%; 12-16 g/dl -52.3%; and 16-20 g/dl - 4.5%. Where 12-16 g/dl was considered as normal level Hemoglobin levels among males showed 65.0% under normal range; 27.12% anaemic ; 7.88% above normal level and Hemoglobin levels among females showed 35.54 % under normal range ; 64.33 % anaemic ; 0.13% above normal level. Chi square test between gender and Hemoglobin result values resulted in a P value < 0.05 statistically significant. Hemoglobin levels among the age group 1-31 years were 38.4%; 32-55 years were 45.2%; and above 55 years were 16.4%; Chi square tests between various age groups and Hemoglobin result values resulted in a P value < 0.05 statistically significant. Our study assessed the association of Hemoglobin levels of patients with various parameters such as age, gender and result values. The overall results show a Male predilection in hemoglobin levels and females having lesser hemoglobin levels than their normal range. The study also reveals that Anemia is strongly associated with females when compared to males and both males and females attain the peak in hemoglobin levels during their young and Middle Age and gradually come down during old age.

Key words: *Hemoglobin, anemia, gender, age, association, comparison.*

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Introduction

Hemoglobin is a protein molecule present in the red blood cells that carries oxygen from the lungs to the body's tissues and returns carbon dioxide from tissues to the lungs.[1] A Hemoglobin molecule consists of four globin chains, two alpha-globin chains and two beta-globin chains. Each globulin chain contains an important iron-containing porphyrin compound termed

heme. Hemoglobin is a metalloprotein.Embedded within the heme compound is an iron atom that is vital in transporting oxygen and carbon dioxide in our blood. The iron contained in hemoglobin is also responsible for the red color of blood.[2]

Hemoglobin also plays an important role in maintaining the shape of the red blood cells.[3]. In their natural shape,[4] red blood cells are round with narrow centers resembling a donut without a hole in the middle. Abnormal hemoglobin structure can, therefore, disrupt the shape of red blood cells and impede their function and flow through blood vessels. [5]

The normal range of Hemoglobin in males is 13.5 - 17.5 -grams per deciliter and females is 12.0 - 15.5 - grams per deciliter.[6] Fluctuation in hemoglobin levels, known as hemoglobin variability, is a well-documented phenomenon.[7] Low levels of hemoglobin and serum iron are suggestive of iron deficiency anemia. Anemia is a condition where the body suffers with lack of red blood cells or hemoglobin. Anemia affects about one-third of the global population. In India, the prevalence of anemia among adolescent girls was 56% and this amounts to an average 64 million girls Several factors have been found to affect hemoglobin variability, including drug-related issues, such as pharmacokinetic parameters, patient-related differences in demographic characteristics, and factors affecting clinical status, as well as clinical practice guidelines and treatment protocols [8].However, early detection and prompt diagnosis can lead to better prognosis and help in the implementation of successful clinical treatment.[9] A study by William G Murphy in 2015 revealed that Men and women have different mean haemoglobin levels in health in venous blood — women have mean levels approximately 12% lower than men.[10]. Adamu AL et al in 2017, in his study, revealed that Among women, the odds of anemia were higher among urban residents and those with higher socioeconomic status. Increasing age was associated with higher anemia prevalence in men. However, on gender comparison, a larger number of women were anemic when compared to men [11]. Research by Gautam S et al in 2019 also revealed an Overall percentage of about 41% of women aged 15–49 years were anemic. [12]

Anaemic disorders are often associated with orofacial signs and symptoms and have several oral manifestations[13] .The manifestations include conjunctiva and facial pallor, atrophic glossitis, angular stomatitis, dysphagia, magenta tongue, midfacial overgrowth, osteosclerosis, osteomyelitis and paraesthesia/anaesthesia of the mental nerve. [14].Orofacial petechiae, conjunctival haemorrhage, nose-bleeding, spontaneous and post-traumatic gingival haemorrhage and prolonged post-extraction bleeding are common orofacial manifestations[15] Dental management of patients of anaemia requires interdisciplinary care with the consultation of the treating dentist with haematologist.[16]Patients with abnormal hemoglobin conditions tend to experience orthopnea in dental chair along with dizziness, headache, red facial colouring and dyspnea; further, abnormal hemoglobin levels also influences wound healing to a great extent. [17]. Recording the required data serves as a precaution is in the interest of the patient and also a record for future uses..[18,19]. Hemoglobin levels are thus examined prior to procedures such as extraction or any suspicion of anemia in the patient. Estimation of Hemoglobin levels is thus an essential examination, as a dental practitioner, as Hemoglobin level has its own importance in dentistry.[20]

This study aims in assessment of Hemoglobin levels of patients along with various parameters such as age, gender and result values obtained and throws some limelight on the Hemoglobin levels analysis , as its knowledge is of importance for a dental surgeon.

Materials and Methods

Study Design and Setting

This Study was carried out in a university setting which consists of subjects of predominantly South Indian population. Approval for the study is by the ethical board of Saveetha University (applied). Number of people involved include 3 reviewers - A Guide, researcher and a reviewing expert.

Data Collection

This is a retrospective study in which the samples were considered from the time period of June 2019 to March 2020. Case sheets reviewed for the research includes All patients applicable for study and cross verification of the required samples were performed by a reviewing expert. Measures were taken to minimise the sampling bias. These are inclusion of only clear

and readily available data Followed by simple random sampling. Both Internal and external validation was also obtained to carry out the study. The required data for the study was obtained from reviewing about 86000 patient records to. The required data- i.e, the Hemoglobin levels of males and females who visited the out patient department were collected and entered in a methodical manner in an excel sheet for the tabulation of data and further statistical analysis. Data was validated by 1-2 external reviewers and all the non specific , unclear or incomplete data were excluded from the study.

Statistical Analysis

Statistical software used for analysis is the SPSS (statistical package for the social sciences) by IBM and the statistical tests used were Pearson's chi square tests[21], frequency tables along with bar graphs to analyse and compare the obtained results. The type of analysis performed was correlation and association. Independent variables include ethnicity, reasons for hemoglobin examination , age, gender and the dependent variables include Hemoglobin levels of males and females.

Results and Discussion

Results Summary

Out of the total sample size (1825 cases), distribution of males and females for hemoglobin examination revealed 57.0% males and 43.0% females **(Figure 1).** various hemoglobin result values obtained were, 5-8 g/dl - 5.2%; 8-12 g/dl - 38.0%; 12-16 g/dl -52.3%; and 16-20 g/dl - 4.5% (**Figure 2)**, Where 12-16 g/dl was considered as normal level Hemoglobin levels .Hemoglobin levels among the age group 1-31 years were 38.4%; 32-55 years were 45.2% and above 55 years were 16.4% **(Figure 3).** males showed 65.0% under normal range; 27.12% anaemic ; 7.88% above normal level and Hemoglobin levels among females showed 35.54 % under normal range ; 64.33 % anaemic ; 0.13% above normal level; Chi square test between gender and Hemoglobin result values resulted in P value < 0.05 statistically significant.**(Figure 4).**

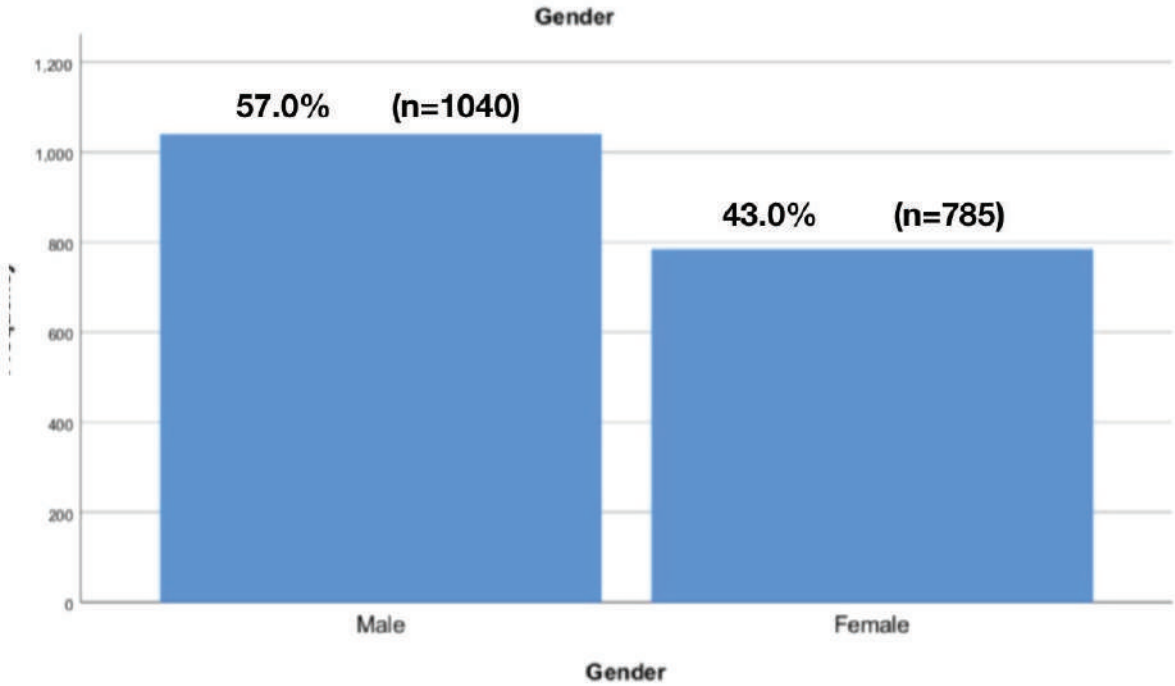


Figure 1 - Bar graph showing the distribution of males and females for hemoglobin examination. x axis represents gender and y axis represents the number of patients under each gender for hemoglobin examination on a scale of 1-100%. Males were found to be significant over the females.

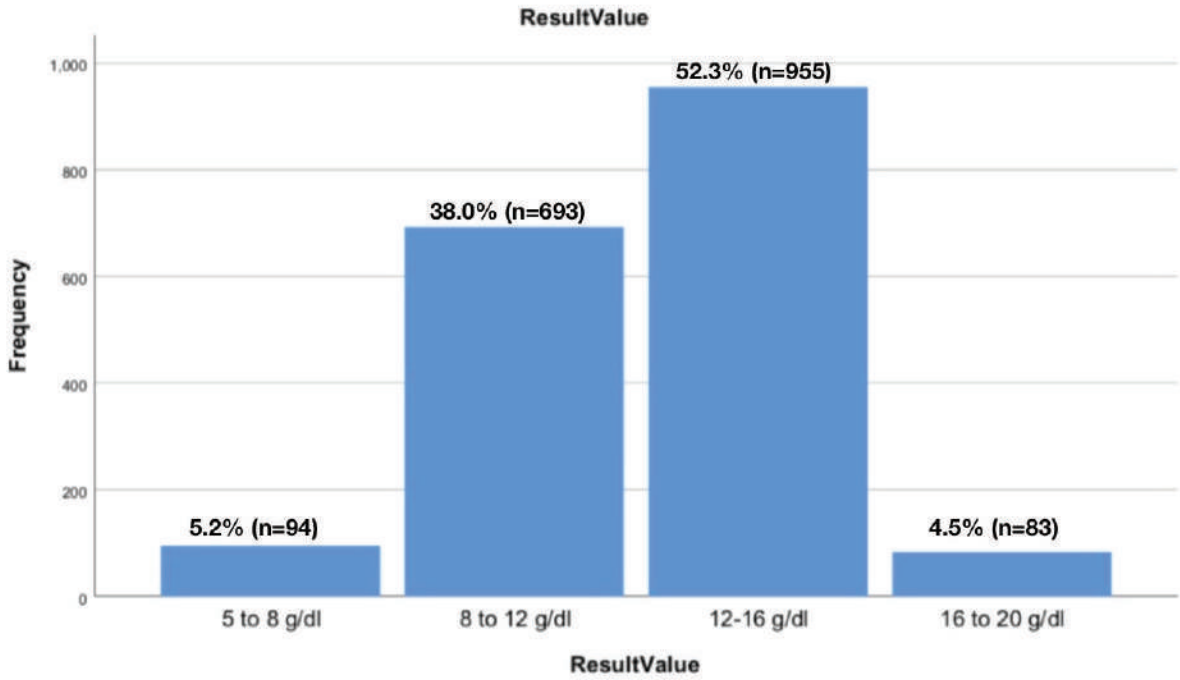


Figure 2- Bar graph showing the distribution of various result values for hemoglobin examination. x axis represents result values and y axis represents the number of patients under each category of result values for hemoglobin examination on a scale of 1-100%. 12-16g/dl range was found to be significant over others followed by 8-12g/dl, 5-8g/dl that and 16-20 g/dl which was the least prevalent.

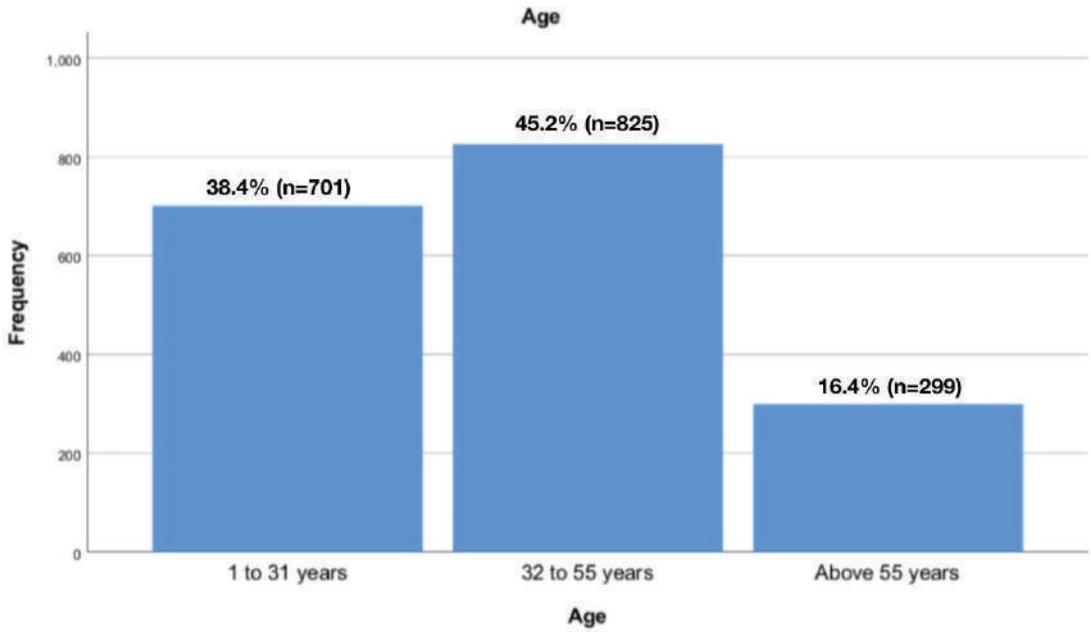


Figure 3- Bar graph showing the distribution of various age groups for hemoglobin examination. x axis represents age groups and y axis represents the number of patients under each age group for hemoglobin examination on a scale of 1-100%. 32-55 years age group was found to be significant over others followed by 1-31 years age group and above 55 years age group which was the least prevalent.

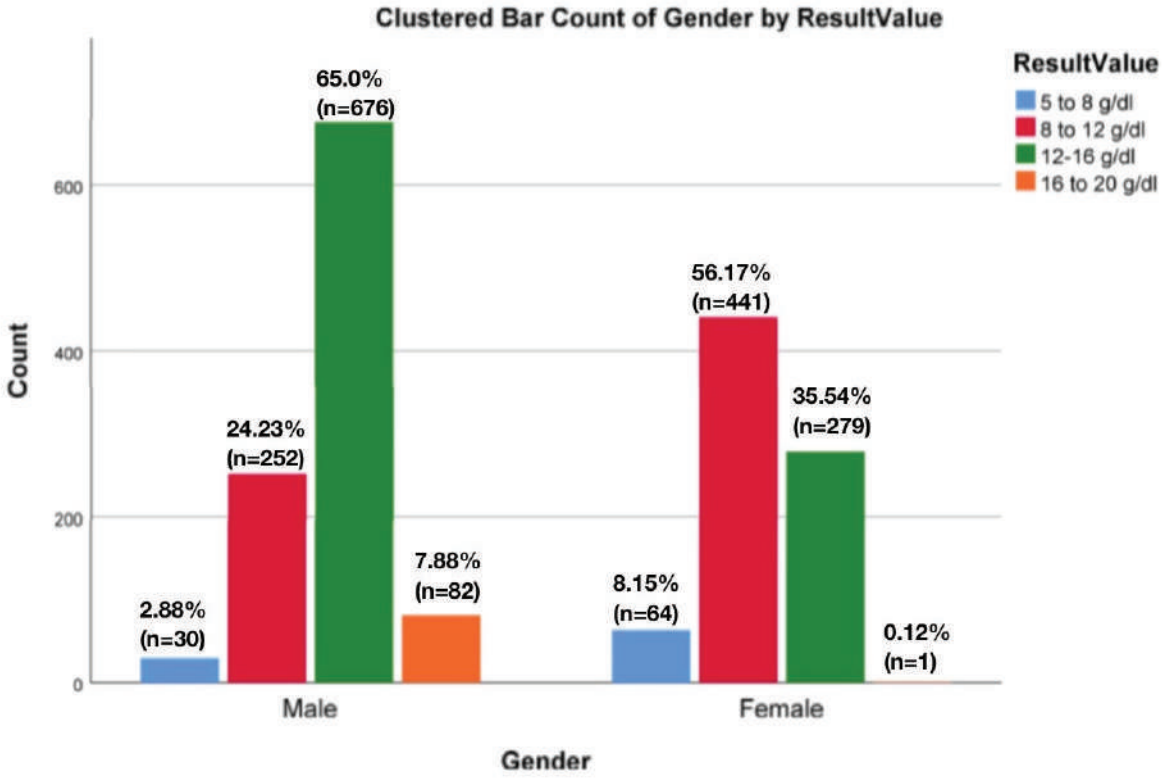


Figure 4- Clustered bar graph depicting the association of gender and hemoglobin result values on a scale of 1-100%. Blue colour depicts 5-8g/dl, red colour depicts 8-12 g/dl, green colour depicts 12-16g/dl and orange colour depicts 16-20 g/dl. The graph reveals that most of the males have their hemoglobin levels in the 12-16 g/dl range, whereas most of the females have their hemoglobin levels in the 8-12 g/dl range. Chi square test between gender and Hb result values reveals p value<0.05 (chi square value=277.719, df=3, p value=0.011) statistically significant. Thus, gender can be considered as a determinant parameter to correlate with the Hemoglobin levels.

Discussion

The need to transport oxygen and remove carbon dioxide from animal tissue is a fundamental requirement of life, independent of age or sex. The role of iron in humans and many other mammals is central to this process. Haemoglobin concentration and red blood cell count are important diagnostic indicators for anaemia in humans.[22] Not only anemia, they can also play a diagnostic tool in detection of certain severe disease conditions such as hodgkin's lymphoma, aplastic , iron deficiency anemia,Hypothyroidism or even oral squamous cell carcinoma [23],[18],[24] apart from other diagnostic tools such as saliva.[25]

Association of hemoglobin result values and gender revealed males- 65.0% under normal range; females - 35.54 % under normal range Chi square test - P VALUE < 0.05 statistically significant and the normal range was observed significantly among the males. A study by WT Kimberly et al in 2013, stated that Females have lesser Hemoglobin levels than their normal range when compared to males. The reason being the hormonal environment that is a possible cause for the observed sex difference in red blood cell count and haemoglobin and serum ferritin concentrations in human[26].Adding to this result, the study by William G Murphy et al in 2014 also stated that Hemoglobin levels has a Male predilection[10]. And this is prevalent in the modern women who have a different reproductive history from those in the past. They reach sexual maturity at an earlier age, have fewer pregnancies, and breast feed for shorter periods; as such they menstruate for more years than women in the past. Menstruation is the principal cause of iron loss in women [10] which is similar to our study finding and is in

concordance with the literature that also is compounded by the fact that females are the vulnerable gender for iron deficiency anemia owing to excessive blood loss during menstruation.

Association of hemoglobin result values and Age revealed 1-31 years - 38.4%; 32-55 years - 45.2%; above 55 years- 16.4%. Chi square test- P VALUE < 0.05 statistically significant. Maximum Hemoglobin levels were attained by the middle aged population. W.W Hawkins et al in 2001 revealed that Females, in their period of adolescence and middle age attain maximum Hemoglobin levels; whereas males, in their middle age and old age, attain The Maximum Hemoglobin levels. The reason for reduced hemoglobin levels in older age groups is because with increasing age , people tend to develop more systemic diseases and health problems and most of them affect the hemoglobin levels.[27] Another study by N Hanafusa et al in 2014 also supported our results by stating that Hemoglobin levels decreases with age, and that lesser Hemoglobin levels is prone among the elderly population, which is caused by a few diseases and conditions that cause your body to produce fewer red blood cells than normal [28] again similar to our study findings as well as the literature that states that hemoglobin count can be associated with a disease or condition that causes body to have lesser red blood cells,that can occurs if the body produces fewer red blood cells than usual, destroys red blood cells faster than they can be produced or have excessive blood loss.

Analysis of hemoglobin result values revealed 5-8 g/dl among 5.2%; 8-12 g/dl among 38.0%; 12-16 g/dl among 52.3%; 16-20 g/dl among 4.5%- predominant populations were within normal Hemoglobin levels. BJ Lee et al in 2016, in his study, revealed that anemic conditions are strongly associated with females, especially above 60 years of age or during the menopause period in females, the reason being iron deficiency anemia [29] . Study by Devi K et al revealed that adolescents are not much more prone to anemia as they have lesser disease conditions, healthier lifestyle with adequate nutritional status when compared to the aged population.[30] Our study findings, thus is in concordance with similar studies and also with the literature.

The overall implications and highlight of this study is the knowledge of hemoglobin levels and their importance in dentistry. The patients visiting dental clinics may have several disease or medical conditions and maybe on regular medications or without medications. Many of these systemic conditions have manifestations in the oral cavity, among which the altered hemoglobin levels is the common condition affecting various age groups, irrespective of males and females. The alterations in hemoglobin values, is thus significant in any dental practice and there is lesser knowledge regarding the relative frequencies of these alterations and its correlation with parameters such as age, gender and dental diseases associated among the south Indian population, which forms the major strength of this study.

However, there were a few Limitations encountered in this study. This study contained some data that were unclear of certain reporting parameters , such data were not considered.

Another limitation was the Geographic limitations i.e, assessment of predominantly South Indian population. Further, this study is Unicentered and the sample size collected for males and females were unequal.

The future prospects of this study is that the Knowledge and association of Hemoglobin levels with various parameters is essential for a dentist. The alterations in hemoglobin value is considered to be significant in any dental practice, and there is little information regarding the relative frequency of these alterations and its correlation with age, gender and the related dental manifestations. Further, The higher prevalence of anemia in women when compared to men suggests the need for substantial improvement in the nutritional status of women[31] The increased disease burden compared with the past studies highlights the needs to reconsider the existing nutritional policies.

Conclusion

Our study assessed the association of Hemoglobin levels of patients with various parameters such as age, gender and result values. The overall results show a male gender predominance in the and females having lesser hemoglobin levels than their normal range. The study also reveals that Anemia is strongly associated with females when compared to males and both males and females attain the peak in hemoglobin levels during

their young and Middle Age and gradually comes down during old age.

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Conflict of Interest: None declared.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Complications after Gingival Recession Coverage Procedure - A Retrospective Cohort Study

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Abstract

Gingival Recession is one of the common esthetic problems reported by patients for which recession coverage procedures like either flap or gingival grafting techniques are routinely practiced with high predictability and outcome. However these procedures also endup in morbidities like bleeding, necrosis etc. Hence the aim of the present study is to assess the complications after the recession coverage procedure. A retrospective evaluation was conducted to find out subjects who had undergone the gingival recession coverage procedure between June 2019- March 2020 at the University Dental Hospital. For the included subject, data regarding complications if present, demographic factors, other clinical factors associated with the complication were extracted. Out of 23 subjects, the prevalence of complications after gingival recession coverage procedure is 60.8%. Out of this post operative bleeding was the highly prevalent complication (43.5%), followed by tissue necrosis (17.3%) associated with subepithelial connective tissue graft. There were no reports of postoperative infection. A significant association was found with class 3 Miller's recession defect and tissue necrosis.(P=0.05, chi square analysis). However considering the retrospective nature of the study, future literatures must be emphasised to analyse better patient outcomes and minimise the complications.

Keywords: *Gingival recession coverage,tissue necrosis, postoperative bleeding, infection*

Introduction

Gingival recession has become one of the common dental complaints reported by patients nowadays. It is the apical shift of marginal gingiva from its normal position on the crown of the tooth[1]. The etiology of gingival recession is found to be multifactorial such as traumatic toothbrushing, lack of oral hygiene leading to plaque accumulation, abnormal oral habits and frenal pull which may lead to tooth sensitivity, unaesthetic appearance and caries [2]. Surgical treatments like free graft and pedicle flap are indicated when the gingival recession causes functional or esthetic problems [3]. The pedicle flap technique includes coronally advanced flap, laterally displaced flap, rotational flaps, double papilla technique in which adjacent gingival tissue is displaced to the defect site. In graft techniques like free gingival graft & connective tissue graft, it requires a second surgical site to procure the connective tissue either from palate or tuberosity to be placed over the defect site[4]. Of this subepithelial connective tissue graft (SCTG) is considered the gold standard technique till now, for its higher predictability and esthetic outcomes [5–13].However in spite of this good clinical outcome, it always requires a second surgical site for the graft procurement which predisposes the patient to operative & post operative complications [14,15]. During operative procedure especially while procuring graft from palate, nicking of greater palatine blood vessels can lead to profuse bleeding [15,16]. Also postoperatively this technique especially free gingival graft (FGG) leaves an open surgical wound that may start bleeding even on slight provocation during

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regular oral activities, and also contribute to pain, infection, swelling[17]. Sometimes the graft placed over the defect site if not of adequate thickness or handled improperly may undergo necrosis [18,19]. Necrosis can also develop due to inadequate revascularisation that results from improper stabilisation of the graft etc [20–25]. All these together predisposes to greater morbidity and to less acceptance of these treatment procedures by the patients [26,27]. A thorough knowledge on the appropriate selection of suitable technique for a specified gingival recession defect and identification of the risk factors associated with the complication can greatly prevent this occurrence. Hence the aim of this study is to assess the prevalence of complications after gingival recession coverage procedures and identify the risk factors associated with it.

Materials and Methods

This is a retrospective clinical study that is performed to evaluate the complications of recession coverage procedure in University dental hospital. After obtaining the ethical clearance form the Institutional Review Board of University Dental Hospital, the list of all the subjects treated with gingival recession coverage were retrieved by reviewing the 86000 patient's record who have visited the hospital during the study period from June 2019 to March 2020 based on the following criteria.

Inclusion criteria:

- Subjects above 18 years of age
- Subjects who underwent gingival recession coverage procedure between June 2019 and march 2020
- Subjects who have finished at least 2 consecutive postoperative followup.
- Subjects with gingival recessions associated with either anterior or posterior teeth or both
- Records with complete data and photographs during followup visits

Exclusion criteria:

- Smokers
- Records with incomplete data of clinical examination

The search resulted in a total of 23 patients who underwent one of the following recession coverage procedures like Subepithelial connective tissue graft, free gingival graft, and coronally advanced flap with 13, 4 and 6 subjects respectively. All the procedures in this study were performed by the post graduate students specialising in periodontics. Before the start of surgery, all the included subjects underwent a scaling and root planing. After the appropriate recession coverage surgical procedure, hemostasis was achieved in all the cases before disposal. Standard oral hygiene and post operative instructions with medication as required were prescribed for the patient. For suture removal and post operative review, the patient was recalled after 14 days during which the healing was documented and photographed. The age range of patients included for this study was 19-47 years.

From the preoperative and postoperative clinical clinical finding documented and photographs available for the included subject the demographic data like age, gender, site of gingival recession, clinical data like gingival recession defect characteristics, type of recession coverage attempted, operative and post operative clinical observations and findings were retrieve and summarised in Table 1. The collected data was tabulated in excel sheet. The data is imported and transcribed in Statistical Package for the Social Sciences, version 17(SPSS, IBM corporation). Descriptive analysis was based on quantitative variables and frequencies for categorical variables. Pearson chi square test was employed. P less than or equal to 0.05 was considered statistically significant with a confidence interval of 95%.

Results and Discussion

Table 1: Shows the Descriptive Statistics on frequency and distribution of all the study variables like Age, Gender, Characteristics of Recession Defects, Type of Recession Coverage Procedure and the Site Performed.

VARIABLE	FREQUENCY	PERCENTAGE (%)
Number of included subject	23 (1 defect in each subject)	-
Gender		
Male	13	58.8
Female	10	41.2
Age (Mean - 34.06)	Range (19-47)	-
Recession defect Characteristics (Based on Millers Classification)		
Class I	9	34.7
Class II	5	19.2
Class III	12	46.1
Recession coverage technique used		
Recession coverage with flap	6	26.1
Recession coverage with graft	17	73.9
Site of recession coverage procedure		
Incisor	18	78.3
Canine	4	17.4
Premolar	1	4.3

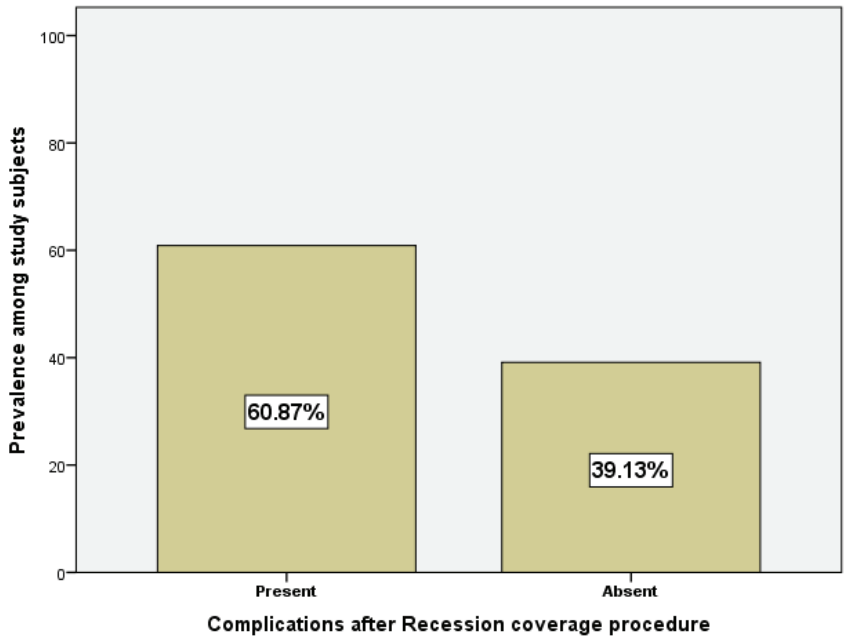


Figure 1: Bar graph shows the prevalence of complications after recession coverage procedure. X axis represents the complications after recession coverage procedure and Y axis represents the percentage of participants. Out of 23 subjects, 60.8%(14) showed postoperative complications whereas 39.1%(9) does not show any complications.

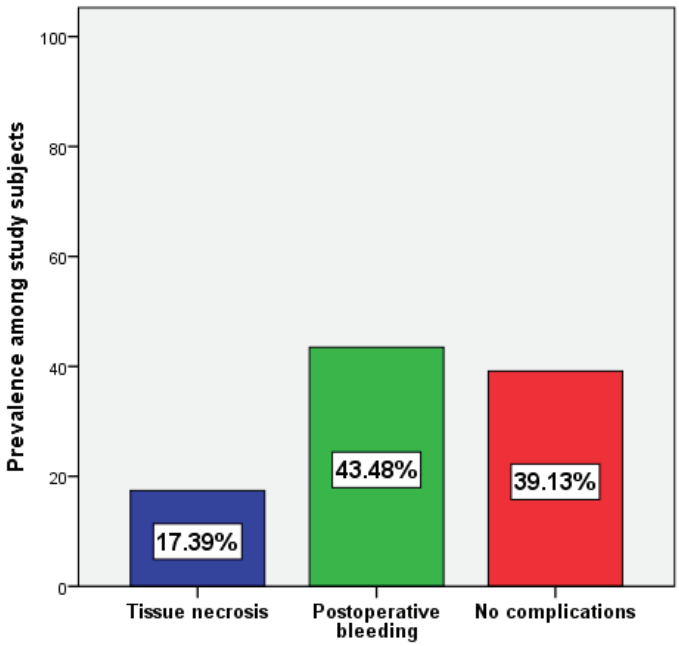


Figure 2: Bar graph shows the prevalence of each postoperative complications after recession coverage procedure. X axis represents the different postoperative complications and Y axis represents their respective prevalence (blue- tissue necrosis, green- postoperative infection, red- no complications). From the graph, the prevalence of tissue necrosis and postoperative bleeding was found to be 17.3% and 43.4% whereas no postoperative infection was reported. Only 39.1% does not show any complications.

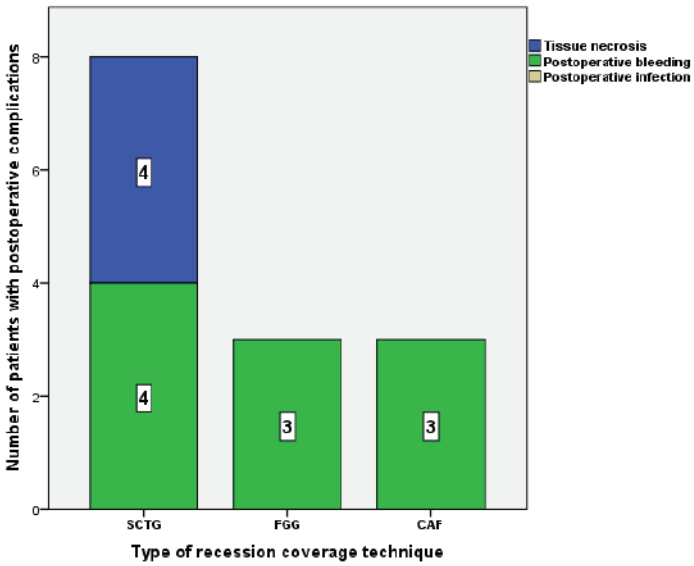


Figure 3: Bar graph shows the association of postoperative complications and the type of recession coverage technique used. X axis represents the type of recession coverage technique and Y axis represents the frequency of postoperative complications (Blue-tissue necrosis, green-postoperative bleeding, green-infection). Chi square test was done and showed statistically significant association between postoperative complication and SCTG technique. Chi-square test value:2.240 ,df:2, P=0.05 p<0.05 significant.Hence, the postoperative complications after recession coverage procedure are significantly higher among SCTG technique compared to FGG and CAF. (SCTG - Subepithelial Connective Tissue Graft, FGG - Free Gingival Grafts, CAF - Coronally Advanced Flap).

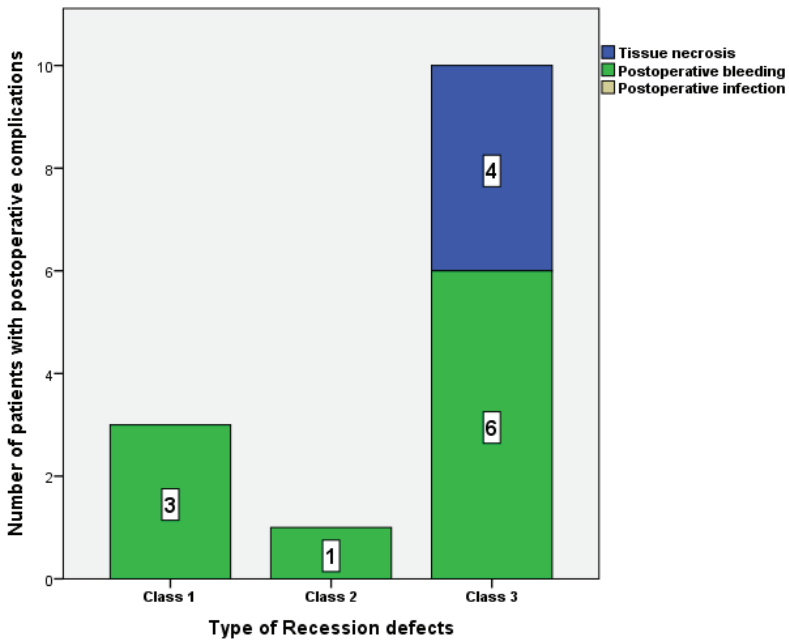


Figure 4: Bar graph shows the association of postoperative complications and type of recession defects. X axis represents the type of recession defects and Y axis represents the frequency of patients with postoperative complications (Blue - tissue necrosis, green - postoperative bleeding). Chi square test was done and was found to be statistically significant. Chi-square value -4.439, df:2, P=0.05, significant. Hence the total number of complications were higher among class 3 recession defects when compared to class 1 and 2 defects.

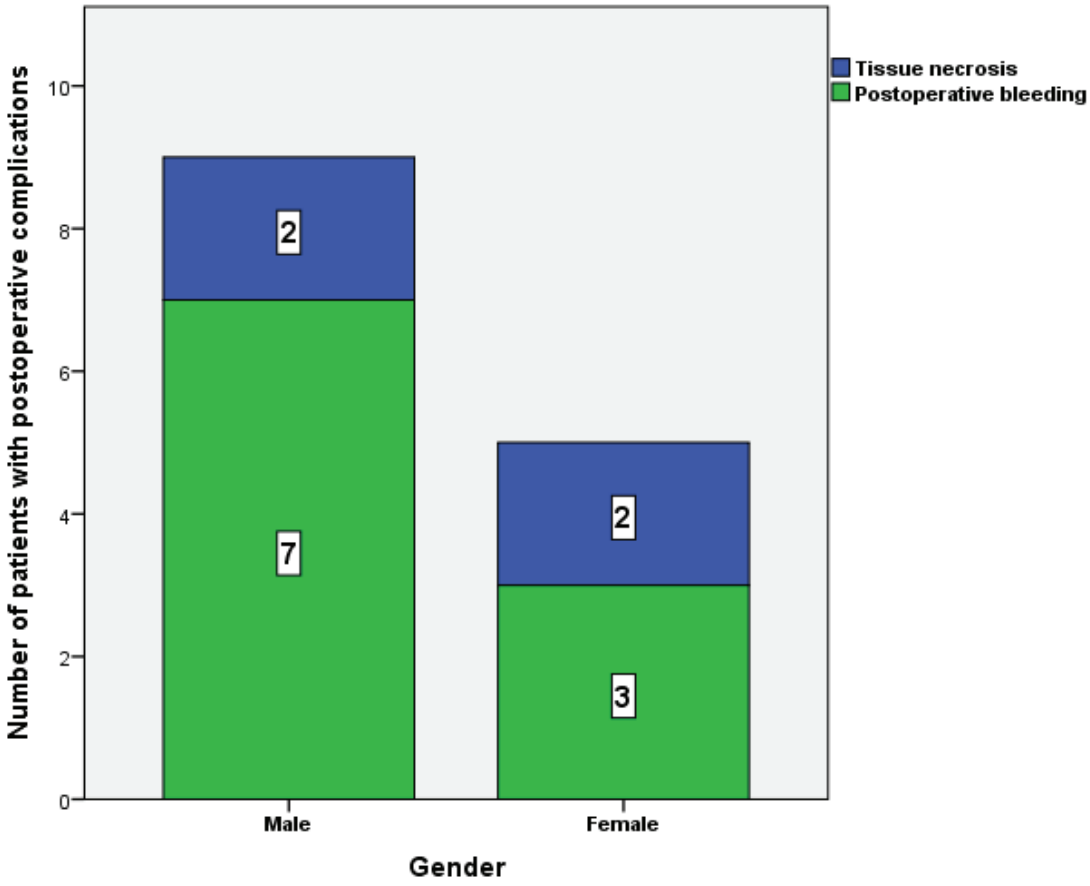


Figure 5: Bar graph shows the association of gender and postoperative complications of gingival recession coverage procedure. X axis represents the gender of the participants and Y axis represents the frequency of patients with postoperative complications. (Blue - tissue necrosis, green - postoperative bleeding). Though males reported more postoperative complications than females, there was no statistically significant association found between the prevalence of complications and the gender (Chi-square value:0.750,df:1,P=0.38). Hence postoperative complication after recession coverage procedures are equally prevalent among both genders.

In the present study, the results show there is a higher prevalence/ incidence (60.8%) of postoperative complications with gingival recession coverage procedures at the university dental hospital. Post operative bleeding was the most commonly reported (43.5%) complication among the study subjects. The results are concurrent with earlier reports by Del Pizzo et al, where 33% of the cases reported postoperative bleeding with respect to FGG[28] . This is evident in our study, where subjects who underwent graft technique for recession coverage contributed to higher incidence of postoperative bleeding than the pedicle flap techniques. The major causes for postoperative bleeding after graft techniques from the donor could be the mechanical trauma from oral activities that could distort the blood clot that was achieved during operative procedure. However the trauma can be prevented by use of protective acrylic stents that should be worn until initial healing of the open wound site is observed[29]. The chances of bleeding complications are even higher when the graft donor site lies in the course of the greater palatine vessels. This can be mostly prevented by adequate knowledge about the course of the blood vessels, but cannot be ruled out in all cases due to anatomic variation in the course[30].

Next to postoperative bleeding, tissue necrosis was reported in four patients (17.3%) who underwent SCTG technique. Similar findings are seen in the study conducted by Zorzano et al who reported a 25% prevalence rate of tissue necrosis[18] . Another study conducted by

Gobbato et al reported a 17% prevalence rate of tissue necrosis in SCTG which is slightly lower compared to the present study[20]. Necrosis usually happens when the tissue vascularity is compromised especially in case of gingival grafts transplanted from palate to recipient site, unless the graft is properly stabilized over a vascular bed, the revascularization of the graft is questionable and can highly result in necrosis. It also depends on the presence of adequate thickness of graft for initial survival and to resist surgical trauma[31]. No necrosis was seen at defects treated with pedicle flaps which might be due to adequate vascularity of flaps which are still connected to the donor sites. In contrast, Harry et al reported no graft necrosis in relation to SCTG [27].

A significant association was found between class III recession defect (40%) and tissue necrosis but not with class I and class II. According to Machtei et al., in their study analysed the root coverage among patients with class III millers recession reported that initiation of graft necrosis is one of the factors for partial root coverage [26, 32, 33].

The Current study reported no postoperative infection at the surgical sites. Similar to this Zorsano et al, also reported no incidence of infection with CTG[18] . The absence of postoperative infection in our study might be due to the strict aseptic surgical protocol carried out, with good oral hygiene maintenance and prescription of systemic antibiotics and mouthwashes.

The limitations of the study are the retrospective study design and smaller sample size. Hence further research with prospective longitudinal study design and larger samples are necessary to come up with stronger evidence.

Conclusion

Within the limits of the study, recession coverage procedure results in higher prevalence of complications especially postoperative bleeding and tissue necrosis and Class III recession defects increase the risk of the complications.

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Liquid Biopsies in Cancer Diagnosis

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Abstract

Over many years, the concept of precision medicine has dramatically renewed the sphere of clinical oncology; the advent of patient-tailor-made therapies has appreciably stepped forward all measurable outcomes. Liquid biopsy is a modern method that commences unexpected perspectives. It includes the detection and isolation of circulating tumor cells, circulating tumor DNA and exosomes, as a supply of genomic and proteomic records in patients with most cancers. Many technical hurdles had been resolved to newly advanced techniques and subsequent-generation sequencing analyses, permitting a wide software of liquid biopsy in a huge variety of settings. Initially correlated to analysis, liquid biopsy facts at the moment are being studied for cancer analysis, with a bit of luck consisting of screenings, and most significantly for the prediction of reaction or resistance to given remedies. In specific, the identification of particular mutations in genes can be useful resources in therapeutic choices, both in the appropriateness of remedy and within the superior identity of secondary resistance, aiming to early diagnose disease progression. Still, the utility is far from fact however ongoing research is leading the manner to a new era in oncology. The main aim of this study is to assess the importance of liquid biopsy in cancer diagnosis.

Keywords*: Liquid biopsy, cancer, exosomes, circulating tumor cells, circulating tumor DNA, oncology.*

Introduction

Liquid biopsy is the sampling and analysis of nonsolid biological tissue, primarily blood[1]. It is a test done on a sample of blood to look for cancer cells form a tumor that might be circulating in blood or pieces of DNA from tumor cells that are in the blood. It is used to assist cancers at an early stage [2]. It will also be used to assist plan treatment or discover how the treatment is running or if cancer has come back [1]. Being able to take more than one sample of blood over time, may additionally assist medical doctors to apprehend what kind of molecular changes are taking place in a tumor [3].

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Liquid biopsy is an easy and non-invasive opportunity for surgical biopsies which allows doctors to find information about tumors via a simple blood pattern[4]. Traces of cancer's DNA inside the blood can deliver clues about which remedies are most possible to work for affected people[2]. A liquid biopsy takes a look that can locate epidermal increase component receptor gene mutations, which occur in 10-35 % sufferers with non-small lung cancers, will assist to pick out the proper treatment for the affected person at the right time[5]. Much of early research on liquid biopsies has been in lung, breast, and prostate cancers however this generation is predicted to affect all forms of cancer[3]. Oxidative stress plays an important role in the pathogenesis of various diseases especially in chronic liver disease[6]. Hepatic fibrosis is considered to be the major issue in liver diseases[7]. The non-invasive nature of liquid biopsies, which require 5 milliliters of blood, means they're a lot easier to tolerate and the method is quicker than a surgical biopsy [8].

Tolerability and comfort are the main raise for sufferers[3]. The biggest advantage lies inside the ability of liquid biopsies to locate sickness development or remedy resistance long earlier than it would cause clinical signs and symptoms or seen on imaging scans [4,9]. Most cancers have a couple of genetic mutations and they'll now not have the equal ones in all parts of the most cancers[1]. The tissue samples eliminated for biopsy won't show all mutations whereas liquid biopsies offer a stepped forward danger of detecting those genetic modifications[5]"Liquid biopsies might be a -changer in cancer diagnosis," stated Miro Venturi."In terms of affected person acceptability and sickness control, the blessings of non-invasive, quick, and without difficulty repeatable exams are clear[10]. And inside the long term, liquid biopsies might also in the long run be used to capture symptoms of most cancers early, earlier than symptoms arise[11]. This could make a big distinction in the way we recognize and deal with cancer[12].There is increased mortality with cancer because of the toxic side effects of cancer chemotherapy and radiation therapy[13]. So medicinal plants are used as a substitute for conventional drugs because of their increasing demand for plant-based natural products. *Caralluma fimbriata* which is found in dry regions of Tamil Nadu has been known for its anticancer activities with no adverse effects [13,14]. It is also used against human colon cancer cells[13]. *Azadirachta indica* also has various medicinal properties[15]. Recent studies use herbal medicine as potent anticancer drug candidates[16]. Coumarin plays an important role in the drug discovery process and is also used to treat cancer[17]. *Acacia catechu* is known for its hypoglycemic, free radical scavenging and antioxidant properties. In this study, it has been used against SCC-25 human oral squamous carcinoma cell line[18].

Liquid Biopsy- Investigating Tumor

Cancer is one of the main causes of death, with 9.6 million cancer deaths in the world[3]. Hepatocellular carcinoma is the second most common cancer and is associated with increased mortality in the last two decades[19]. The improvement of technology has led to the sphere of precision oncology, which accommodates treatment regimens to the molecular traits of every patient's tumor [20]. The modern widespread genetic profiling of tumors commonly involves the usage of tissue biopsies. Despite their invasive nature, tissue biopsies are associated with many barriers, inclusive of patient chance, sample coaching, sensitivity and accuracy, procedural expenses, and invasiveness[21]. This makes the manner incompatible for clinical longitudinal tracking [22]. Furthermore, a significant dilemma of tissue biopsies is that they fail to seize intratumoral and anti-metastatic genetic heterogeneity, impacting the accuracy[23].

Liquid biopsies have an excellent capacity to triumph over these existing sampling barriers. Such biopsies comprise the sampling and analysis of liquid organic sources, usually blood, for cancer analysis, screening, and prognosis[12]. The 'tumor circle', described because the subset of circulating additives is derived from cancer tissue and may be immediately or circuitously used as a source of most cancers biomarkers in liquid biopsies[24] . These components encompass circulating tumor proteins, circulating tumor nucleic acids (ctDNA and ctRNA), CTCs, EVs, and TEPs [23]. Liquid biopsies present numerous advantages over conventional tissue biopsies), and technological improvements in sample isolation[25]. The first critical milestone in this subject was reached in 2016 with the FDA approval of the first companion diagnostic test for lung cancer primarily based on the ctDNA content of a liquid biopsy.

OTHER METHODS OF CANCER DIAGNOSIS

There are various methods of a cancer diagnosis. Cancer detection often involves radiological imaging. Imaging is used to check the spread of cancer and prognosis of treatment, and to monitor cancer oncological imaging is more varied and accurate[26]. Different imaging techniques aim to find the most suitable treatment option for each patient. Imaging techniques are often used in combination to obtain sufficient information[27]. These include computed tomography, Magnetic Resonance Imaging, Ultrasonic endoscopic examination, mammography, and isotopic diagnosis[28].

Laboratory tests are carried out when it is suspected that a patient has cancer. Normally blood samples are taken for monitoring blood counting[26]. Genetic testing can be useful in connection with certain cases of cancer [12]. A final diagnosis of cancer is based on an examination of tissues or cells under microscopic by a pathologist[12].

CIRCULATING TUMOR DNA

CtDNA comprises the fraction of circulating cellular- DNA originating from cancer cells. These consist of brief nucleosome-related fragments and longer fragments encapsulated inside EVs [3]. The mechanism of ctDNA encompasses apoptosis, necrosis, lysis of CTCs, and lively secretion from the tumor[29]. The proof of the suitability of ctDNA as a cancer biomarker came with the identity of KRAS gene mutations in ctDNA from the blood of patients with pancreatic cancer[1].

Both qualitative and quantitative data may be acquired from ctDNA evaluation. Quantitative information may be received from the measurement of the mutant allele fraction and is a reflection of the tumor[30]. It finds application inside the detection of minimal residual ailment (MRD) and occult metastases and within the monitoring of treatment response and therapeutic effectiveness [2]), The detection of ctDNA after treatment is an excessive-sensitivity and excessive-specificity predictor of relapse. Qualitative records can be sourced via the profiling of mutations, amplifications, deletions, and translocations in ctDNA allowing the identification of genetic alterations associated with the reaction, as a result assisting selection-making for customized control [30,31]. Other qualitative data obtained through ctDNA evaluation includes an assessment of methylation popularity[29].

CIRCULATING TUMOR CELLS

CTCs are tumor cells that are indifferent from one tumor and can be found in the peripheral blood of sufferers[32]. Their presence is thought to be fundamental to the development of metastasis. CTCs present systemically via energetic intravasation, with the epithelial-to-mesenchymal transition as a fundamental step or through passive shedding from the primary tumor[33]. This latter mechanism is supported through the presence of CTC aggregates or circulating tumor microemboli inside the blood[32].

The records acquired from CTCs are quantitative as well as phenotypic through single-cell genomics or proteomic profiling[34].CTCs have superpotential as tools for the analysis, tracking, prognosis, and prediction of reaction to remedy, and also for the discovery of novel drug objectives. The best records available from CTCs are their variety, which is a prognostic predictor for many cancers, which includes metastatic breast, colon, and prostate cancer[35]. The power of CTC counts as a criterion for the choice of first-line treatment in metastatic breast cancers is currently being investigated[36].

Despite numerous analytical systems and technology to be had for CTC analysis, their translation into medical practice is restricted by means of their isolation from the blood[37]. Challenges consist of their extreme rarity, fragility, and physical and phenotypic heterogeneity[30]. Each of these options has advantages and drawbacks, and best their mixture can support a comprehensive characterization[38].

EXTRACELLULAR VESICLES

Extracellular vesicles are membranous particles released from all cellular types underneath physiological and pathological conditions, in addition to following distinct forms of stimuli, inclusive of proteases, ADP, thrombin, inflammatory cytokines, increase factors, biomechanical shear and pressure inducers, and apoptotic indicators [3,5]. They can be observed in almost every physical fluid, especially blood[3]. EVs had been diagnosed as essential mediators of intercellular communication, regulating and collaborating in a plethora of physiological and pathological procedures, such as cancer. Based on their biogenesis, content, and secretory pathways, EVs may be divided into two large classes: exosomes and microvesicles [5].

The suitability of EVs as cancer biomarkers lies within the reality that the molecular cargoes they create can be considered a cell of origin[39]. The advantages of EVs are many [22]. EVs are usually produced and launched in abundant portions and in greater amounts compared with CTCs[40]. Similar to ctDNA and CTCs, EVs can be a supply of quantitative and qualitative information. Quantitative facts comprising EV can detect the presence of malignant sickness and tumor burden[41]. For instance, circulating exosome tiers are elevated in breast and pancreatic cancer and the number of circulating microparticles is higher in patients with a couple of myelomas as compared with healthful people[42].

SEQUENCING TECHNIQUES

Next-Generation Sequencing

There are various sequencing techniques in liquid biopsy. NGS has emerged beyond the decade as a primary method for sequencing DNA and acquiring genetic statistics[25]. NGS is primarily based on the analysis of several hundreds of thousands of DNA sequences in parallel observed by either series alignment to a reference genome. Despite its excessive sensitivity and specificity, NGS indicates a random error charge between 0.1% and 1% depending on the carried out platform making the detection of ctDNA through uncommon mutations in the general ctDNA[43,44]. According to this remark, many protocols have been changed to enhance and extend the detection of uncommon mutations[25].

PCR based methods

PCR represents a rapid and cheap approach for the amplification of nucleic acid. Polymerase Chain Reaction is used to make billions of copies of specific DNA samples.PCR can determine gene duplication or deletion[25]. Furthermore, melting curve analysis immediately after PCR can identify small mutations, down to single changes. These techniques are becoming easier and faster and can be multiplexed[45]. Real-time PCR methods are favorable options for the analysis of cancer markers[43]. PCR looks for changes in genes or chromosomes, which may help find and diagnose a genetic condition or disease such as cancer [43]. The AS-PCR is commonly used in medical settings to stumble on single nucleotide variation (SNV) or small insertion/deletion in formalin-constant, paraffin-embedded tumor tissues. Overall, PCR based assays is a promising device for detecting mutations as a low-cost powerful may be feasible in ordinary medical exercise[46].

Methylation Sequencing

DNA methylation plays an important role in regulating normal development and carcinogenesis. The current understanding of biological roles of DNA methylation is limited to its role in the regulation of gene transcription, genomic imprinting, X chromosome inactivation[44]. In the past 2 decades, a large number of changes have been identified[47]. These alterations fall into two main categories, namely hypermethylation of tumor suppressor genes and hypomethylation of oncogenes respectively [44]. The development of DNA methylation markers for cancer detection holds the promise of being accurate, sensitive, cost-effective for risk assessment, early diagnosis, and prognosis.[43]

ADVANTAGES

Liquid biopsy has numerous advantages. It is substantially less invasive while compared to tissue biopsy. Test results are generally available a great deal earlier than standard tissue biopsy [48]. Test results are generally available a great deal earlier than standard tissue biopsy[21] . Using a liquid biopsy, it is feasible to make an early diagnosis. Liquid biopsy can be used to estimate a threat for metastatic relapse progression. Liquid biopsy may additionally permit for stratification and real-time monitoring of treatment options[28]. It can be able to higher perceive therapeutic goals. The ease and frequency of a liquid biopsy test give bones over tissue biopsy [4]. The test may be easily repeated and used often as essential to monitor a patient ‘s development. Liquid biopsy is commonly much less expensive to carry out than tissue biopsy [3].

CLINICAL APPLICATIONS

Initial Diagnosis

Liquid biopsy at initial diagnosis may be beneficial for prognostications[12]. Another capacity benefit of liquid biopsy over strong tumor biopsy is that, is unresectable cancers [21,49]. There can be inadequate tissue available form this aspirate of DNA sequencing and liquid biopsy has a non-invasive supply of DNA available. [28]Regardless it might be imprudent to use mutation detected in liquid biopsies for choosing the first-line remedy unless the frequency of a mutant allele is excessive enough to warrant the entire self-being that mutation is likely to be derived from the tumor itself[21].

After Surgery

Liquid biopsy taken after surgical treatment is promising in this context [1]. There is already research displaying that sufferers who have circulating tumor DNA or CTCs following surgery are likely to relapse. In the present state, the sensitivity for detecting disease is far from 100%. A negative test should therefore be considered as another feature [50].

After additional therapies

Liquid biopsy is able to detect easily recurrence prior to tumors becoming radiographically or clinically

apparent, potentially giving clinicians a larger window of opportunity during which treatment regimen could be altered [28]. Once a patient relapses, a liquid biopsy may reveal new mutations not present in the primary tumor that could guide choice for second-line therapy[51]

Screening the cancer

Using liquid biopsies before cancer is clinically detected is discussed last because it is the most difficult application, but also has the greatest potential to reduce morbidity and mortality from cancer[52]. Cost and specificity are less of an issue, sensitivity is more important, as patients untreated on the basis of false-negative tests are likely to die[8]. Much controversy around screening tests is based on relatively low positive predictive values [2]. A related problem is an overdiagnosis, detection of cancers that are indolent and never would cause morbidity or mortality if they remain undetected. [1]

LIMITATION

Liquid biopsy is not considered a popular procedure although there is increasing use of these assays, tissue biopsy remains widespread for affirmation and analysis of sickness, such as numerous cancers and for detection of characteristics of sickness.[32] At present liquid, the biopsy is not used as a substitute for tissue biopsy.[53] More assistance for scientific utility is needed. There is presently, no longer a widespread usage of liquid biopsy taking a loop in clinical networks. More variation in scientific trials is required on the cost of liquid biopsies in medical Settings to support the clinical utility of tests [22]. The studies are needed to assess the test's accuracy, and its ability to identify various tumor types. Test sensitivity challenges still exist[52]. It is also given that circulating tumor cells or DNA are relatively rare compared to the number of hematological molecules found in blood samples, there are challenges to test 's detection ability.[44]

THE FUTURE SCOPE

During the past decade, liquid biopsy has received tremendous attention[28]. But yet the technique is not a standard tool in the clinical oncologist arsenal [54]. One often misunderstood, but critical point is that screening tests do not detect cancers that are very early[8]. All they do is detect cancer earlier than they could be detected. Therapeutics require biomarkers, to stratify responders from non-responders, thereby treating patients most likely to benefit and preventing unnecessary harm to unnecessary patients[3]. Liquid biopsy is likely to provide such markers for any type of cancer[1]. The method of balancing current, intolerable under diagnosis, with potential overdiagnosis is a challenge that further research will hopefully solve. However, a solution to this problem will be possible if reliable early diagnostic tests for major cancer are developed and used[55]. Nanotechnology has also emerged as a promising tool in the treatment and management of cancers[56]. Nanotechnology is also known for its role in target-specific delivery of drugs[57]. Selenium nanoparticles have a vital prospect in the field of medicine and are used as anti-cancer agents [58].Zinc oxide nanoparticles synthesized from *Mangifera indica* has been known for its antioxidant activity and cytotoxic effects on lung cancer[59]. Antibacterial properties of nanoparticles were evaluated[60]. Green synthetic methods of nanoparticles are simple, non-invasive, and eco-friendly [61]. Further studies can be done to evaluate the importance of nanotechnology in the diagnosis and treatment of cancer. With rapidly advancing technology and with accelerating interest in liquid biopsy from both academia and industry, looking forward to the day when liquid biopsy that detects cancer becomes a routine part of preventive medicine [54].

Conclusion

Liquid biopsy has a powerful role in helping patients to get the right treatment. Molecular analysis of cancer is required to optimize patient treatment. New methods such as next-generation show immense promise for the future. Liquid biopsy is coming of age and will change practice, it will enable oncologists to use drugs intelligently to combat the change in individual cancer as they happen the elegant and powerful technique, fulfill the promise of becoming rapid, reliable, and non-invasive decision-making tools.

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Therapeutic Approach on Oral Mucositis

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Abstract

Oral mucositis is a erythematous ulcerated lesion that causes soreness and tissue damage. The lesion leads to severe pain. It occurs on the comparative of nutrition diet, poor oral hygiene and local and systemic infections. The pathophysiology of the oral mucositis consists of five phases where initiation phase leads to injury to the cell. Which leads to activation of transcripts factors which leads to upregulation of inflammatory cytokines and tissue damage as results in subsequent bacterial colonization and cytokines mediated damages . The final phase involves healing through signalling and epithelium proliferation. Maintaining good oral hygiene reduces the severity of the effect and decreases bacteremia . It can be diagnosed by LLLT low level laser therapy. Also can be treated by growth factors, locally applicable gels immune modulators and hematopoietic agents Based on the development of clinical and scientific knowledge standard treatment plan and further research and analysis is needed for management of mucositis. The severity of oral mucositis also may be controlled by advanced plant based or nano based phytochemicals.

Keywords : *ulcerative lesion, local and systemic infections, mucositis.*

Introduction

Advances in the understanding, prevention and treatment of oral diseases during the past 50 years have led to a significant increase. As the goal of modern dental care is oral health, this cannot always be achieved or maintained. Often, only an arrest of disease progression can be attained, and chronic asymptomatic infections may persist. In addition, chronic or malignant diseases of the mucosa are increasingly common. The mouth has thus become a significant potential source of both infection and inflammation that contributes to the total burden of disease and to overall health and well-being.[1]

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The oral microbiota is both rich and unique. A similar microbiota does not exist elsewhere. In healthy individuals, viridans group streptococci constitute the majority of the indigenous oral flora. However, the most common bacteria seen are facultative anaerobic streptococci and anaerobic Gram-negative bacilli like Prevotellae and *Fusobacterium* spp., etc . Currently, a significant proportion of Prevotellae isolates worldwide are β-lactamase producers, and β-lactam antibiotics alone are thus ineffective against these organisms. For this reason, metronidazole has been used in combination with penicillin in order to achieve improved efficacy against anaerobes.[2]

Many antibiotics and synthetic oral hygiene products have diminished clinical efficacy, due to the resistance developed to antibiotics and synthetic chemicals, especially in persons with HIV/AIDS. Synthetic substances also have several adverse effects such as vomiting, diarrhoea and teeth staining . In order to overcome these shortcomings, plants and other natural antibacterial substances are now attracting attention as useful and alternative antimicrobials to be incorporated

into mouth rinses and toothpastes. Since plants are the sources of more than 25% of prescription and over-the-counter drugs, conventional medicine is increasingly becoming receptive to the use of antimicrobials derived from plants and other natural products. The rise in the use of herbal medicines has renewed interest in the effects of plant extracts to control plaque and other oral diseases . Screening for antimicrobial agents isolated from plants is a feasible approach to the identification of natural compounds with antimicrobial properties against dental pathogens .In fact, compounds that possess antimicrobial activities against oral pathogens are now being isolated from plants traditionally used as oral remedies .[3]

Oral mucositis is an erythematous ulcerative lesion caused by radiotherapy or chemotherapy . It is characterised by oral erythema , severe pain and can predispose to septicaemia in neutropenic patients.[4]. It affects the quality of the life and nutrition state of the patient who may require hospitalisation, thus increasing health care cost. [5]. Oral complications that arise with chemotherapy or radiation therapy include mucositis , xerostomia , bacterial , fungal or viral infection, osteoradionecrosis , dental caries , loss of taste and other oral complications in oral cavity. Oral mucositis also represents a major nonhematologic complication of cytotoxic chemotherapy and radiotherapy associated with significant morbidity pain , dysgeusia , odynodysphagia , subsequent dehydration and malnutrition.[6] . Several oral toxicities can also compromise the delivery of optimal cancer therapy protocols , like dose reduction or treatment schedule modification may be necessary to allow for resolution of oral lesion [7]. The risk of developing mucosal injury increases with the number of chemotherapy cycles and previous episodes of chemotherapy induced mucositis. Drugs affecting DNA synthesis exhibit more pronounced mycotoxin effects [8,9] . It has been estimated that there is an increased risk of mucositis development with blooms and continuous infusions compared to prolonged and repetitive administration of lower dose of cytotoxic agents [10,11]. It may also occur in bone marrow transplantation and receive high dose chemotherapy also for patients receiving radiation therapy for cancer of head and neck region may develop oral mucositis [12]. The degree and duration of mucositis in patients treated with radiation therapy are related to radiation source , cumulative dose ,dose intensity, volume of radiated mucosa , smoking ,alcohol consumption,oral hygiene [13–15].

The recent findings suggest that natural products are with less side effects and can be used for the management of different diseases such as diabetes, cancer, hepatotoxicity, and microbial infections [16,17,18]. Various plant species have been used but, the mode of action of each varies and it has not been subjected to through scientific investigations. So scientific exploration to screen drugs for different diseases for the treatment in a natural way will be important [19]. Plant based products hold a good therapeutic potential to prevent the abnormalities with additional benefit of low cost. Findings suggest that these plants and their bioactive metabolites are effective in proper functioning like stimulation and suppression based on the abnormalities.[19,20,21,20,22]. Many nanoparticles such as silver, selenium, and zinc oxide play a major role in preventing many diseases including oral mucositis [23,24,25,26]

PATHOPHYSIOLOGY OF ORAL MUCOSITIS:

A biological model for chemotherapy and radiotherapy induced oral mucositis proposed by various authors appeared to be more generally applicable to alimentary mucositis [27] .The fundamental mechanism for radiation induced and chemotherapy induced mucositis is believed to be similar [28].The pathogenesis of mucositis undergoes five stages which is of

1. Initiation of tissue injury : where chemotherapy/ radiation induced cellular damage resulting in death of basal epithelial cells. The generation of reactive oxygen species by radiation or chemotherapy will lead to initiation of mucosal injury [29].

2. Upregulation of inflammation through signal : The free radicals activate second messengers that transmits signals from receptor on the cellular surface to the inside of the cell. Which leads to tissue injury and cell death. [30]

3. Signalling and amplification : Up regulation of pro inflammatory cytokines such as tumour necrosis factor- alpha, produced by macrophage,causes injury to mucosal cells and also activates molecular pathways that amplify mucosal injury [31],[27].

4. Healing: This phase is characterised by epithelial proliferation as well as cellular and tissue differentiation,restoring the integrity of epithelium.[32]**.** The degree and extent of oral mucositis that develops in any particular patient and site appears to depend on factors such as age, gender,systemic diseases as well as tissue specific factors.[33]**.**In some cases it is indicated that pathways associated with pro-inflammatory molecules including cyclooxygenase-2,nuclear factor kappa and IL-6 are upregulated in oral mucositis.[34]**.**

CLINICAL MANAGEMENT OF ORAL MUCOSITIS:

The management of oral mucositis has been largely palliative although targeted therapeutic management has been developed. There are several guidelines for management of oral mucositis **.**[8]

Pain control :

The primary symptoms of oral mucositis is pain,which significantly affects the food intake,mouth care and quality of life.Using saline mouth rinse;ice chips and mouth rinses containing 2% of anaesthetic lidocaine.[35]. Some other topical mucosal biocompatible agents are also available but these are not anesthetic but are postulated to reduce pain by forming a protective coating over ulcerated mucosa..[36]**.**

Nutritional support :

Uptake of nutrition is compromised by pain associated with severe oral mucositis.In addition taste change can also occur secondary to chemotherapy and or of due to to radiation therapy.[37].Soft diet and liquid diet is recommended when oral mucositis is present .In case of patients undergoing Hematopoietic cell transplantation total parenteral nutrition is given through indwelling catheter,like Hickman line. [37]**.**

Palliation of mouth :

Patients undergoing cancer therapy often develop transient or permanent Xerostomia and hyposalivation[38]. chewing of sugarless gum to stimulate salivary flow .Sip water as needed to alleviate mouth dryness,using of cholinergic agents as necessary.[9]

Management of bleeding:

Patients with thrombocytopenia, as a result of high-dose chemotherapy (eg, hematopoietic cell transplant recipients), bleeding may occur from the ulcerations of oral mucositis. [16,38] Local intraoral bleeding can usually be controlled with topical hemostatic agents such as fibrin glue or gelatin sponge [39,40]. Patients platelet counts below 20,000/ml may receive platelet transfusion because of the risk for spontaneous internal bleeding, which may have grave consequences especially in the central nervous system.[39]**.**

MANAGEMENT OF ORAL MUCOSITIS :

Several agents have been tested to reduce the severity of mucositis or its prevention.

Cryotherapy:

It has been hypothesised that topical administration of ice chips to the oral cavity during administration of chemotherapy results in decreased delivery of chemotherapeutic agents to the oral mucosa [17].It is mediated through local vasoconstriction and reduced blood flow [41]**.**The cryotherapy reduces the severity of oral mucositis.Ice chips are placed in the mouth beginning 5 minutes before administration of chemotherapy and replenished as needed for up to 30 minutes[42,25]

Growth factors :

The pathogenesis of oral mucositis mainly involves proliferation of epithelial cells.Thus Various growth factors can increase epithelial cell proliferation [43].Growth factors like recombinant human Keratinocyte growth factor-1 significantly reduces oral mucositis in patients with haematologic malignancies.[44,38]**.** Intravenous human fibroblast growth factor -20 is used currently in reduction of mucositis secondary to high dose of chemotherapy. [45,46,47]

Low level laser therapy (LLLT):

Low level laser therapy can reduce the severity of chemotherapy and radiation induced oral mucositis[26]. As LLLT reduces the levels of reactive oxygen species and pro inflammatory cytokines Which involves in pathogenesis of mucositis [18].The usage of LLLT requires adequate training and technology.[8]**.**

Antioxidants :

Amifostine is thought to act as a scavenger for harmful reactive oxygen species that are known to potentiate mucositis [19,23,24].Also the use of amifostine was recommended for the prevention of esophagitis in patients receiving chemo radiation.[20,48] **.** RK 2020(Rx-kinetix) consists of antioxidants N-acetylcysteine in a proprietary matrix for topical application in the oral cavity . [7,49]**.**

Anti inflammatory agents :

Benzydamine hydrochloride is a non steroidal anti inflammatory drug used to inhibit proinflammatory cytokines including TNF - alpha[18] . This can be applicable for patients receiving moderate dose radiation therapy [50]. Glutamine may reduce mucosal injury by reducing the production of proinflammatory cytokines and cytokines related apoptosis [51,38]

ECONOMIC IMPACTS :

Chemotherapy patients with significant oral mucositis require supportive care measures such as use of total parenteral nutrition, fluid replacement and prophylaxis against infection [21,52]**.** Radiation induced oral mucositis also has a significant economic impact because of the cost associated with pain management,[51]liquid diet ,management of secondary infections and hospitalisation. [53]**.**

CLINICAL CONSIDERATIONS OF ORAL MUCOSITIS :

Chemotherapy induced oral mucositis ,lesions are usually limited to non keratinised surfaces like soft palate , buccal mucosa ,lateral and ventral tongue . The severity of oral mucositis is directly proportional to the dose of the radiation administered . One who receives radiation more than 5000 mc to the oral mucosa will develop severe ulcerative oral mucositis. Rebamipide is a relatively new mucoprotective agent which enhances preservation of existing epithelial cells and replacement of lost tissue through a multitude of actions. Recent studies have shown varied success of Rebamipide in the management of chemoradiotherapy induced mucositis.[54,55]. Topically applied melatonin in the evacuated sockets following tooth removal reduced oxidative stress and inflammation and accelerated the healing process. Thus, by being directly deposited in the oral cavity, it has the capacity to treat oral disorders and pathologies by reducing inflammatory responses in the gingiva and periodontium.[56]. Phytochemicals, such as Curcumin, turmeric extract, has attracted great attention for its therapeutic benefits in clinical oncology due to its chemopreventive, antitumoral, chemo sensibilizing and radio sensibilizing activities against various types of oral complications.[57]

Conclusion

This is a chronic ulcerative lesion that affects the oral health . Based on the development of clinical and scientific knowledge standard treatment plan and further research and analysis is needed for management of mucositis. The severity of oral mucositis also may be controlled by advanced plant based or nano based phytochemicals.

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Association between Tooth and Type of Instrument Fractured During Root Canal Treatment

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Abstract

Instrument fracture is a common unfortunate event that takes place during the root canal treatment. A good knowledge about the different instruments that can fracture, causes of fracture, predilection of teeth towards fracture of instruments, etc will help the clinician to be more alert. It will also help clinicians gain confidence towards methods of retrieval of these instruments. The aim of this study was to analyse the association between the tooth and the type of instrument separated during root canal treatment. The study was based on an online setting. Data was collected retrospectively. Patient reports of 86000 patients that reported to the institution between June 2019 and March 2020 were reviewed. Data of instrument retrievals done from December 2019 to March 2020 was collected. Excel sheet tabulations were made involving tooth number, type of instrument fractured and method of retrieval. Descriptive statistics was done on SPSS software. Association between the tooth and type of instrument was analyzed with Chi Square test. No statistical significant difference was seen between the tooth and type of instrument fractured. ($p>0.05$). The tooth type does not influence the type of instrument fractured significantly.

Keywords: *Instrument separation ; instrument fracture ; tooth type ; instrument retrieval ;retrieval technique ; root canal treatment.*

Introduction

Root canal preparation is a procedure in endodontics during which microbial pathogens present in the root canal system are eliminated. It is probably the most important stage of non surgical endodontic treatment. Because canal disinfection is achieved by mechanical debridement and use of irrigants and medicaments, enlargement of the root canal space is essential to facilitate the flow of the irrigating agent[1] as well as for the placement of filling material later on. Today, the concept of cleaning and shaping is drifting towards use of irrigants for cleaning and disinfecting canals more than mechanical shaping of the canals[2]. Endodontic instruments for cleaning and shaping have come a long way since their first invention. With the developing technology various inventions have been made even in this field, beginning with innovative designs for diagnostic instruments like pulp vitality testers[3], constantly changing the designs of these instruments. The trend is slowly changing from using various sizes of stainless steel files to using NiTi files[4], to using a single file for the entire preparation[5]. There have been suggestions for non instrumentation techniques to clean and obturate the root canal[6,7], however this technique is still experimental. There have been various advances even in materials and techniques used for irrigation and intracanal medication, with new materials being introduced into the market constantly.

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A well informed choice must be made as to which material to use in different clinical scenarios. A survey was done to evaluate the use of intracanal medications by dental practitioners[8]. The detailed mechanism of action, interactions, toxicity, etc of each material must be analyzed before using it clinically[9]. Interaction of different irrigants may lead to formation of precipitates which may cause toxic and carcinogenic effects on tissues [10]. Infact, research today is oriented towards nipping the problem at the bud. Remineralizing pastes, mouth rinses, etc are being introduced that prevent dental caries and cavitation thus reducing the need for endodontic treatment [11,12]. New restorative materials, involving resin composites with exceptional properties are being manufactured to improve the long term success of restorations [13]. Advances in laminates and veneering techniques and materials have made it possible to treat discoloured vital teeth more conservatively without the need for intentional endodontic treatment [14].

In spite of technology being so advanced, improving the efficiency of cleaning and shaping instruments, instrument fracture or separation is a common and unfortunate mishap that takes place. No material is infallible, hence an instrument will fracture if its ultimate strength is exceeded. The most commonly fractured instruments are files, reamers, broaches, peeso reamers, GG drills, paste fillers, spreaders, etc. Stainless steel files or reamers are typically operated manually, and fracture is often a result of overuse and is associated with pre-existing distortion of the instrument which acts as warning sign [15]. On the other hand, rotary files may fracture without any warning signs. Incidence rates of intracanal instrument fracture have been reported between 0.25% and 6% for hand instruments while it is seven times higher for rotary instruments [16]. Reasons for instrument fractures apart from manufacturer related factors are flexural fatigue or torsional loading [17,18].

Incase of an experienced clinician, fracture of an instrument does not adversely affect the outcome of an endodontic treatment. Successful treatment depends on proper debridement and disinfection of the root canal system and preventing reinfection by placing a good quality coronal restoration [19]. The tooth and type of instrument fractured also play an important role in the treatment outcome.

It is well documented that tooth influences the probability of instrument fracture, but there is no evidence that there is an association between the tooth and the type of instrument fractured. This knowledge will alert the clinician in case he encounters such situations. Hence this study aims at analyzing the association between the tooth and type of instrument fractured. It further analyzes the association between the instrument fractured and the method used for its retrieval.

Materials and Methods

DATA COLLECTION AND ANALYSIS

This study was conducted in an institutional online setting. One researcher and one guide were involved. Approval was obtained from the institutional review board. Data was collected retrospectively. Patient records of 86000 patients that reported to the institution from June 2019 to March 2020 were reviewed. Data of all instruments separated and retrieved from December 2019 to March 2020 was collected. Excel sheet tabulations were constructed for tooth number, instrument type and technique of retrieval. Data was imported into SPSS software (Version 23.0).

Statistical Analysis

Descriptive statistics was done to evaluate the frequency and distribution of instrument separation in the institution. Chi square test was used to determine the association between tooth and instrument type as well as instrument type and technique of retrieval.

Results and Discussion

A total of 18 instrument separations and retrievals took place in the institution from December 2019 to March 2020. Maximum number of instruments separated in maxillary anteriors (33.3%), followed by maxillary molars and mandibular anteriors (22.2% each), followed by mandibular molars (16.7%) and least by maxillary premolars (5.6%) [Figure 1].

50% of the instruments fractured were rotary NiTi files; 22.2% were H files; 11.1%were K files and 16.7% were others (including reamers, broaches, etc) [Figure 2].

The most common technique of retrieval of separated instruments in this study was by ultrasonics

(44.4%), followed by mechanical technique(33.3%), and least by manual technique (22.2%) [Figure 3].

There was no significant association seen between the tooth and type of instrument fractured ($p<0.05$). All K files fractured in maxillary anteriors. Most rotary files fractured in mandibular anteriors and all molars. [Figure 4].

There was no significant association observed between the type of instrument fractured and technique of retrieval ($p<0.05$). All H files were retrieved ultrasonically. Most rotary files were retrieved with ultrasonics or mechanically [Figure 5].

The intracanal breakage of root canal instruments causes endodontists considerable anxiety [20]. This is because this unfortunate event is associated with many complications ranging from post operative pain to large periapical inflammation, infections and swelling. Inflammatory markers can be used to assess the periapical inflammation present. Recently, MMP-3 gene expression was shown to have higher levels in inflammatory conditions [21]. Post operative pain may also arise due to various other factors including method of instrumentation used, irrigants, irrigation techniques[22], obturation techniques, sealer used, etc. In case of retaining the fractured fragment, the treatment outcome is not always predictable even after sufficient disinfection of root canals. Hence the best outcome is achieved on removal of the separated fragment [23].

In this study, a total of 18 instruments were separated from which 33.3% were separated in maxillary anteriors, 22.2% in maxillary molars and mandibular anteriors, 16.7% in mandibular molars and 5.6% in maxillary premolars. This is in contradiction to the previous studies which stated that molars have higher incidence of instrument separation [24,25]. In a study done by Ungerechts et al, a higher frequency of instrument fracture was found in molars (1.8%) compared with premolars (0.7%) and incisors (0.3%) [24]. A higher incidence in these teeth may be due to the fact that molars usually show the presence of curved canals or calcifications. This may be a predisposing factor for excess fatigue on the instrument causing it to fracture. Various techniques have been described in literature to negotiate calcified canals that may help reduce the incidence of instrument separation [26]. The reason for a contradictory finding in our study could be the small sample size which has skewed the data towards anteriors. Maxillary and mandibular anteriors are most prone to dental trauma which may require endodontic treatment. Even severely traumatized teeth including avulsion can be saved by reimplantation if extraoral dry time is minimal. For this, various storage media are recommended [27]. In cases of mild trauma involving Ellis Class 1 and 2 fractures, endodontic treatment may not be necessary. A survey was done to assess the modalities of treatment for such teeth by dental practitioners [28].

When the type of instrument fractured was analyzed, 50% of the fractures occurred in rotary instruments while the other half was stainless steel. Out of these, 11.1% were K files, 22.2% were H files and 16.7% were others including reamers, broaches, etc. This is in accordance with a study by Iqbal et al in 2002, which stated that 85% of files fractured were NiTi rotary files while 15% were stainless steel [16]. It is in contradiction with a study by Ungerechts et al which stated that most of the fractured instruments were K reamers, K files, K flex (71%) and NiTi instruments (18.4%) [24]. This may be due to the fact that almost 70% of the RCTs done in the institution are done by postgraduates who use rotary files and hence the recorded instrument separation is more for rotary files.

Ultrasonics were used for instrument retrieval 44.4% of the time. 33.3% used mechanical technique while 22.2% used manual technique. This is in accordance with an article by Nagai et al and Nimet et al which stated that ultrasonics are one of the best techniques of instrument retrieval endodontics. [29,30] The reason for this may be that most post-graduate students trained for instrument retrieval have easy access to ultrasonics and endodontic microscopes in the institution. The manual technique involves the use of hand files (H- files), spoon excavators or various gripping devices like fine hemostats, pin pliers or Stiglitz forceps. Except for H-files that may be inserted into the root canals, all of the others are applicable only for fragments that extend into the pulp chamber[31]. Mechanical techniques include the use of extraction tubes, Messaran technique, canal finder system, etc.

No significant association was observed between the tooth and the type of instrument fractured. Previous studies have shown that cyclic fatigue increases with

increase in curvature of canals. This increases the tendency of rotary files to fracture in curved canals such as mesial canals of lower molars, disto buccal canals of upper molars, premolars, etc. [32]

No significant association was observed between the type of instrument fractured and method of retrieval, although a slight predilection of using ultrasonics for retrieval of stainless steel files was observed.

The limitation of this study is that it is restricted to a single small population in a confined geographical area. The sample size is too small to make any strong conclusions. The data was retrospectively collected in a small time frame of four months. This may have increased the bias of the study. Hence more long term research needs to be done in this subject on a larger population for the conclusions to be generalized.

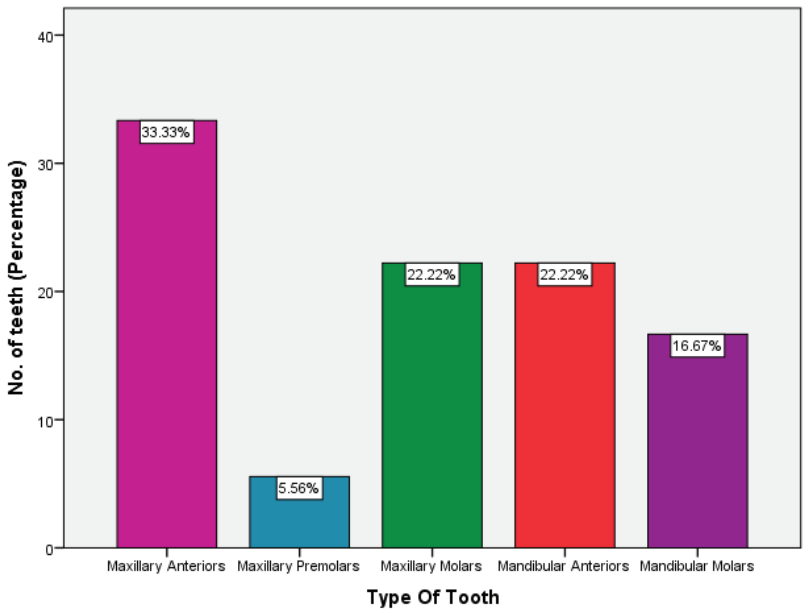


Figure 1: Bar graph representing the percentage distribution of teeth in which instrument separation had occurred. Pink bar represents maxillary anteriors, blue bar represents maxillary premolars, green bar represents maxillary molars, red bar represents mandibular anteriors and purple bar represents mandibular molars. Maximum separation occurred in maxillary anterior teeth (33.3%), followed by maxillary molars and mandibular anteriors (22.22% each), mandibular molars (16.7%) and maxillary premolars (5.6%).

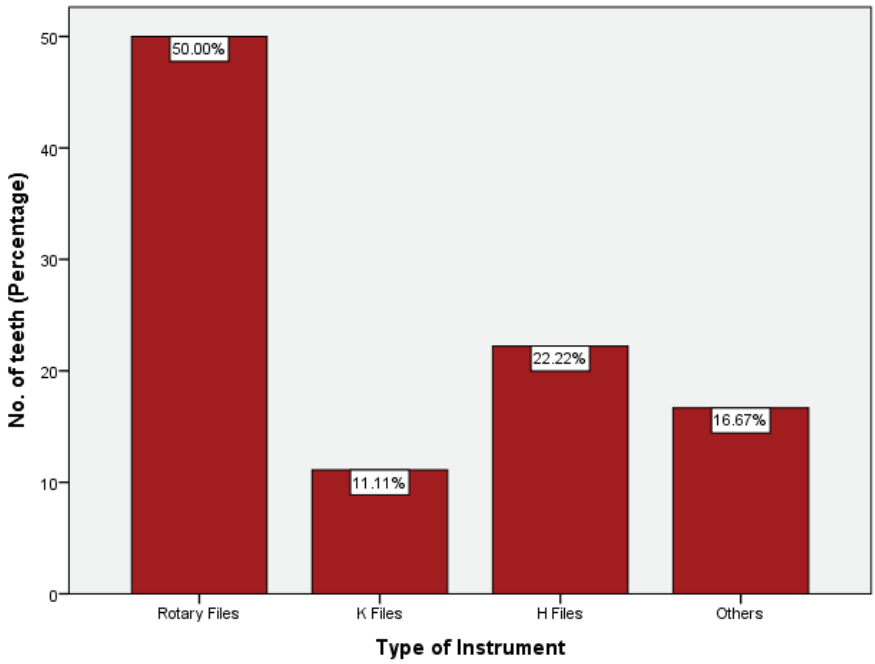


Figure 2: Bar graph representing the percentage distribution of type of instrument separated and retrieved. Maximum separations of rotary files were seen (50%), followed by H files (22.22%). 11.11% separations were of K files and 16.67% were other instruments including reamers, broaches, etc.

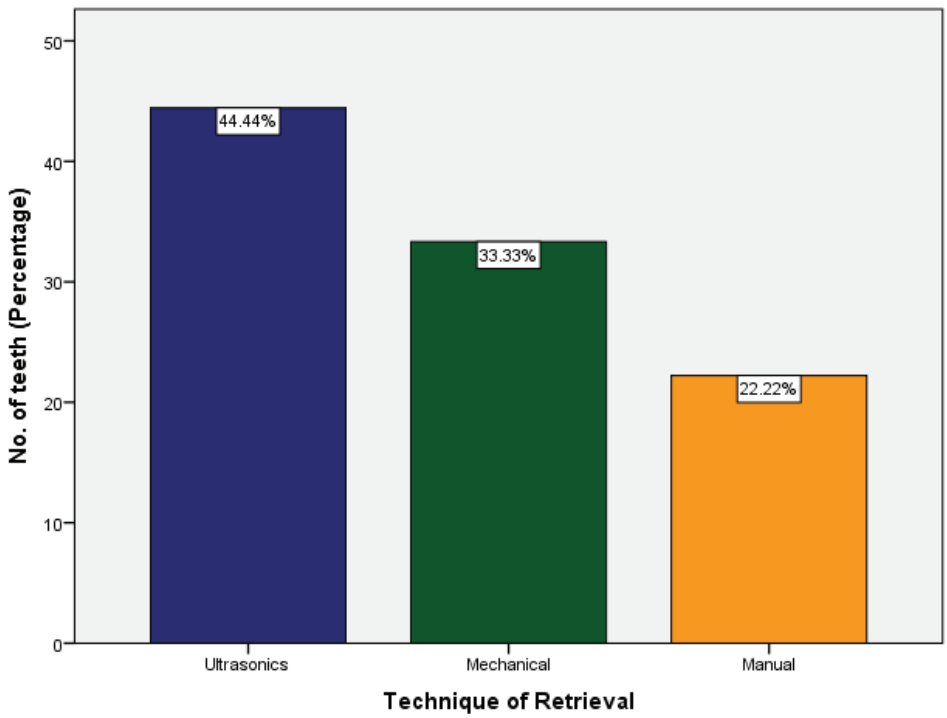


Figure 3: Bar graph representing the percentage distribution of techniques used for retrieval of separated instruments. Blue bar represents ultrasonics, green bar represents mechanical techniques and orange bar represents manual techniques. Maximum instrument retrievals were done by ultrasonics (44.4%), followed by mechanical techniques (33.3%) and manual technique (22.2%).

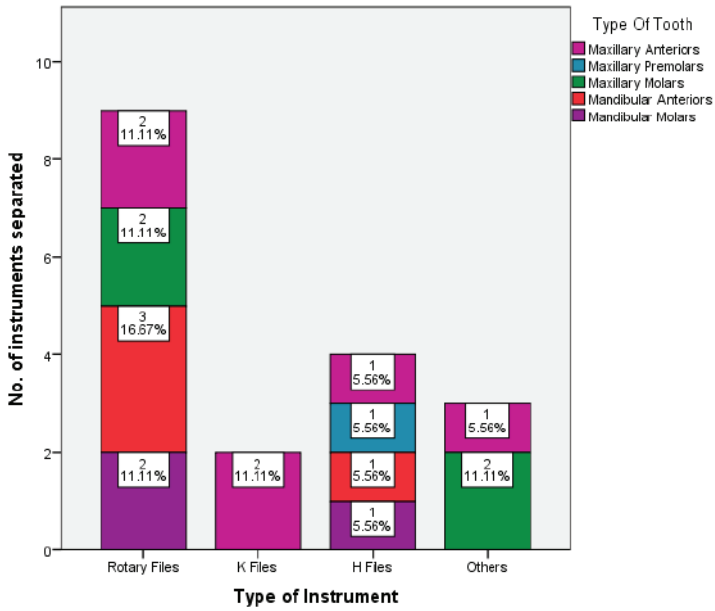


Figure 4: Bar graph representing association between tooth and type of instrument separated. Type of instrument separated was represented on the X axis and the number of instruments separated in each tooth was represented on the Y axis. Pink bar represents maxillary anteriors, blue bar represents maxillary premolars, green bar represents maxillary molars, red bar represents mandibular anteriors and purple bar represents mandibular molars. Association was statistically insignificant (p=0.342), although maximum rotary file separated in mandibular anteriors and all K files fractured in Maxillary anteriors. Chi square test was used to analyze the association.

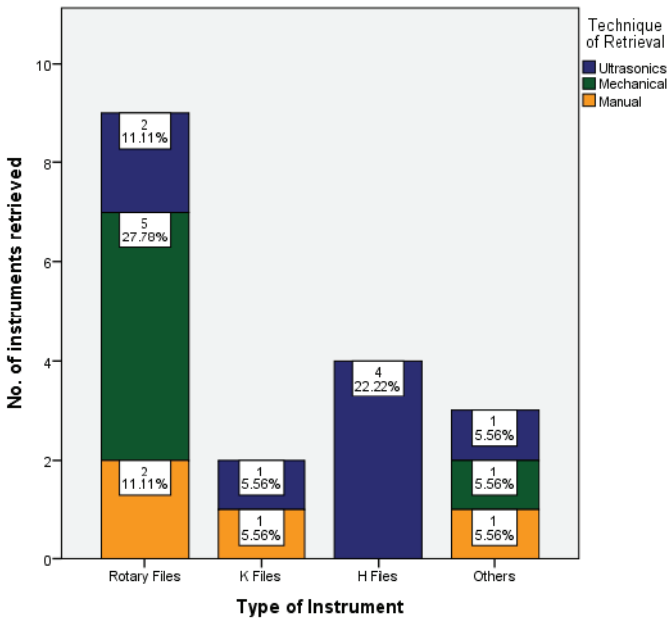


Figure 5: Bar graph representing the association between type of instrument separated and technique of retrieval. Type of instrument was represented on the X axis and the number of instruments retrieved for each technique of retrieval was represented on Y axis. Blue bar represents ultrasonics, green bar represents mechanical techniques and orange bar represents manual techniques. Association was statistically insignificant (p=0.176), although H files were retrieved by ultrasonics and the mechanical techniques were used more commonly for retrieval of rotary files. Chi square test was used to analyze the association.

Conclusion

Within the limitations of this study no significant association was observed between the tooth and type of instrument separated during the root canal treatment. Additionally, no significant association was observed between the instrument fractured and the method of retrieval.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence and Incidence of Desquamative Gingivitis among Patients Visiting a Private Teaching Hospital in Chennai, India

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Abstract

Desquamative gingivitis (DG) is a term indicating epithelial desquamation, erythema, erosion and/or vesiculobullous lesion of the attached and marginal gingiva. The lesions of DG mainly involve the buccal aspect of the gingiva. The term desquamative gingivitis is not a specific diagnosis , but describe several mucocutaneous disorders major of which are oral lichen planus (OLP), mucous membrane pemphigoid (MMP), pemphigus vulgaris (PV). The aim of the study was to assess the prevalence and incidence of desquamative gingivitis among the patients visiting the outpatient department of periodontics at a private teaching hospital , Chennai, India. This is a descriptive study which was performed under a university setting in which the data of patients who were diagnosed with desquamative gingivitis in the department of Periodontics at a private teaching hospital, Chennai from June 2019 to March 2020 was collected by reviewing patients records and the analysis of data of 86000 patients was done. The collected data was compiled , reviewed, tabulated and imported to SPSS software (version 22.0) for statistical analysis. The prevalence of desquamative gingivitis was found to be less 0.1% and had a female predilection (67%) and patients between 50 to 60 years of age were more commonly affected by DG. Most cases of DG presented with burning sensation in the mandibular gingiva (50%) . The most common treatment of DG was found to be pharmacotherapy mainly corticosteroids (50%) . Thus the knowledge of prevalence of desquamative gingivitis and correlation with various parameters is essential in dental practice for clinical implementation.

Keywords: *Desquamative gingivitis; systemic conditions; erythema ;corticosteroids*

Introduction

The current classification system for periodontal diseases and conditions includes, ‘‘gingival manifestations of systemic conditions’’ among the non plaque–induced gingival disorders [1,2].

Desquamative gingivitis (DG) is a term indicating epithelial desquamation, erythema, erosion, and/or vesiculobullous lesions of the attached and the marginal gingiva[3]. The Lesions mainly involve the buccal aspect of the gingiva of the anterior teeth in a diffusive pattern , but can also occur at any site in the gingiva with variable extent [4,5]. The term ‘‘desquamative gingivitis’’ is not a specific diagnosis, but describes several mucocutaneous disorders and systemic conditions [6]. It has been reported that there is an association of DG with oral lichen planus (OLP) , oral lichenoid lesions (OLL), pemphigus vulgaris (PV), mucous membrane pemphigoid (MMP), paraneoplastic pemphigus (PNP), erythema multiforme (EM), graft versus host disease (GVHD), lupus erythematosus (LE), epidermolysis bullosa acquisita

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(EBA), linear immunoglobulin A (IgA) disease (LAD), foreign body gingivitis, plasma cell gingivitis, chronic ulcerative stomatitis, dermatitis herpetiformis, and psoriasis[7] . Besides their heterogeneous nature, all of these disorders share two features in common : an immune mediated pathogenesis and possibly a common clinical appearance, that is the so-called ‘‘desquamative gingivitis’’. DG is very often associated with mucous membrane pemphigoid (MMP)[8–10] , followed by oral lichen planus (OLP)[11,12] and PV[13–15]. Overall, MMP, OLP, and PV are the most common causes of DG, with the first two accounting for 80% of the cases [16–18].

DG may represent an early sign of these disorders, which is important for the early detection and management, as many of these diseases are systemic in nature and are associated with a high morbidity and poor prognosis[19] . However , there is no evidence that DG per se can cause loss of attachment and alveolar bone destruction [20–22]. DG can present as a wide range of oral and gingival symptoms that can significantly compromise a patient’s oral hygiene and represents a potential risk factor for long-term periodontal health . The gingival features vary from erythema to erosive and/ or ulcerated areas. Intact vesicles or bullae can also occur but usually rupture quickly in the mouth [23,24]. When lesions of DG are recognized, the first step is to record a proper medical history. The onset and progression of the gingival lesions should be investigated carefully because most disorders associated with DG have a subacute onset, and the patient is generally unaware of it especially in cases of OLP and MMP while patients with PV will have a period in which symptoms and lesions arise [14,25].

Careful clinical examination of the oral mucosa is extremely important in assessing DG-associated disorders [26–29]. It is important to look for oral lesions with a specific appearance that will help in clinical diagnosis of different DG associated disorders[30] particularly for OLP lesions as they may generally involve multiple oral sites and show characteristic features [31–33]. Each DG associated disorder is characterized by different oral and systemic implications [34–36]. The treatment for DG is aimed at controlling the lesions and their symptoms thus preventing disease progression [37–39]. Supportive care is always important and includes hydration [40,41], nutritional support and elimination of precipitating factors [6,42–44] .

The purpose of the study was to associate age and gender of the patients who have desquamative gingivitis with chief complaint, symptoms, site affected, habits of the patient, past medical history , drug history, clinical appearance of different parts of the oral cavity which will aid the clinician to be prudent for overall evaluation of the patient and then execute treatment planning.

Materials and Method

This was a descriptive study which was performed in a university setting where the required data of patients who were diagnosed with desquamative gingivitis in the department of Periodontics at a private teaching hospital, Chennai from June 2019 to March 2020, was collected by reviewing patients records and the analysis of data of 86000 patients. The collected data was cross verified using photographs, reviewed by a reviewing expert and tabulated in Microsoft excel . The Sample size of the total number of patients diagnosed with gingivitis was n = 34859 of which patients diagnosed with desquamative gingivitis was hound to be n= 6 . The ethical approval of the current study was obtained from the institutional ethical board (Ethical approval number : SDC/SIHEC/2020/DIASDATA/0619-0320) . The tabulated data was imported to SPSS software (statistical package for social studies) version 22.0 (IBM corporation) for statistical analysis . To minimize sampling bias, collection of data was done by simple random sampling methods within the university. There is high internal validity and low external validity . The study included patients with healthy gingiva, gingivitis and lesions associated with desquamative gingivitis. Incomplete , censored and repeated data were excluded from the study.

Results and Discussion

The current study shows that gingivitis (71%) is the most common finding and prevalence of desquamative gingivitis (DG) is less than 0.1% [Figure 1] . There is female predilection (67%) indicating that females are most commonly affected with DG [Figure 2] . Figure 3 shows that patients of 50 to 60 years of age are commonly affected with DG. The most common chief complaint associated with patients with DG is the burning sensation of the gingiva (50%) followed by pain (33%) and bleeding (17%) . In most of the patients the mandibular gingiva was the common site to be affected (50%) while

33% of the patients presented with lesion in both maxilla and mandible. The maxilla was the least common site to be affected (17%) . The duration of symptoms associated with DG ranged from several days to years. Most patients showed symptoms for a duration of 1 year (50%) while few had duration of about 1 to 3 months (33%) and 1 to 10 days (17%) . All the patients had lip biting and mouth breathing habits. 33% of the patients had past medical history and drug treatment history for diabetes and hypertension . Most patients showed clinically healthy alveolar mucosa (50%) while an equal number of patients showed red and white lesions (17%) . 17% of the patients showed clinically healthy gingiva while 83% of the patients showed red , inflamed and soft gingiva with the absence of stippling . 50% of the patients showed clinically healthy mucosa , while the mucosa of 50% of the patients showed frictional keratosis , white striations with red pigmentation or erythematous area . The clinical examination of the tongue , floor of mouth, palate and other sites were normal. The most common treatment for DG is pharmacotherapy (50%) followed by scaling and curettage (33%) and proper diet intake (17%) . Corticosteroids was the treatment of choice for 50% of the patients . 33% of the patients required no treatment while 17% of the patients were advised to take proper diet [Figure 4]. Figure 5 shows the association of age, gender and chief complaint of the patients with desquamative gingivitis. Burning sensation of the gingiva was more common among males (66.6%) than in females (33.3%) and bleeding and pain of gingiva were only experienced by females (100%) and not males [Figure 5].

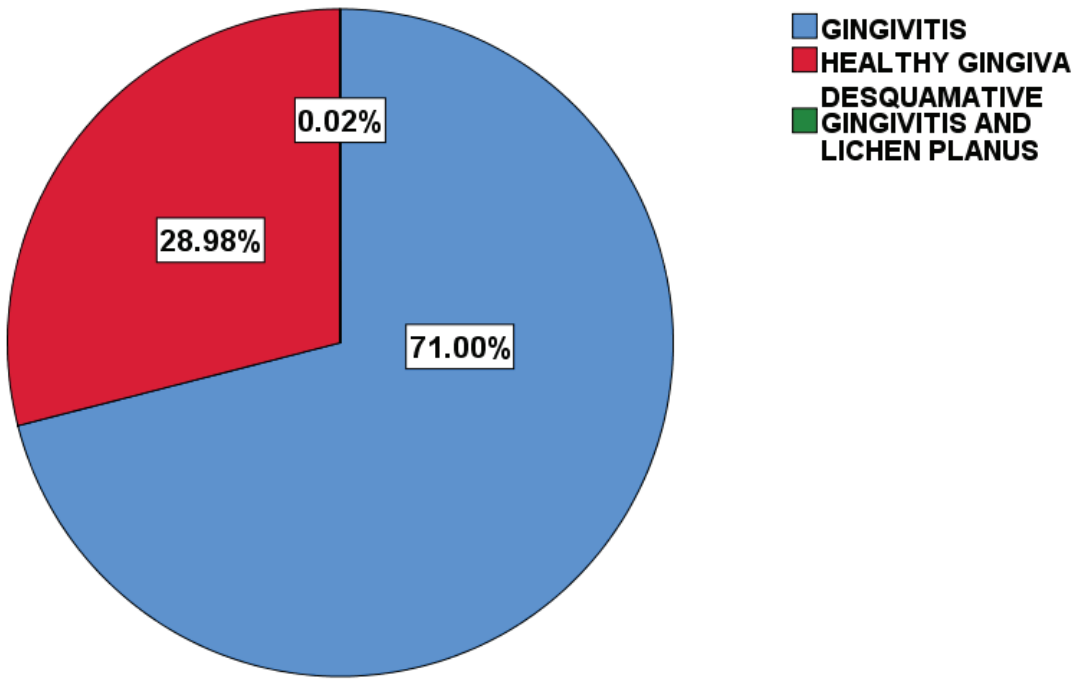


Figure 1 : The pie chart depicts the prevalence of desquamative gingivitis among patients visiting the out patient department of periodontics at a private teaching hospital in chennai. Blue denotes gingivitis , red denotes healthy gingiva and green denotes desquamative gingivitis and lichen planus. The prevalence of desquamative gingivitis is found to be less than 0.1%.

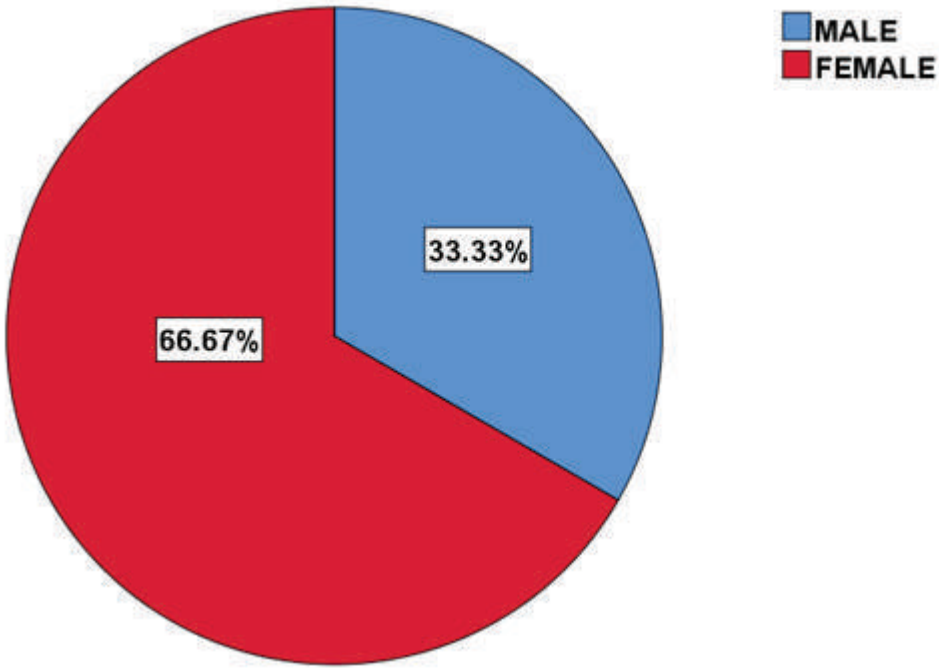


Figure 2 : The pie chart depicts the prevalence of desquamative gingivitis between different gender of patients visiting the out patient department of periodontics at a private teaching hospital in chennai. Blue denotes males and red denotes females. There is a high prevalence of desquamative gingivitis among the females . However, this is not statistically significant (Chi square test; p =0.414 ; p>0.005; Hence not significant)

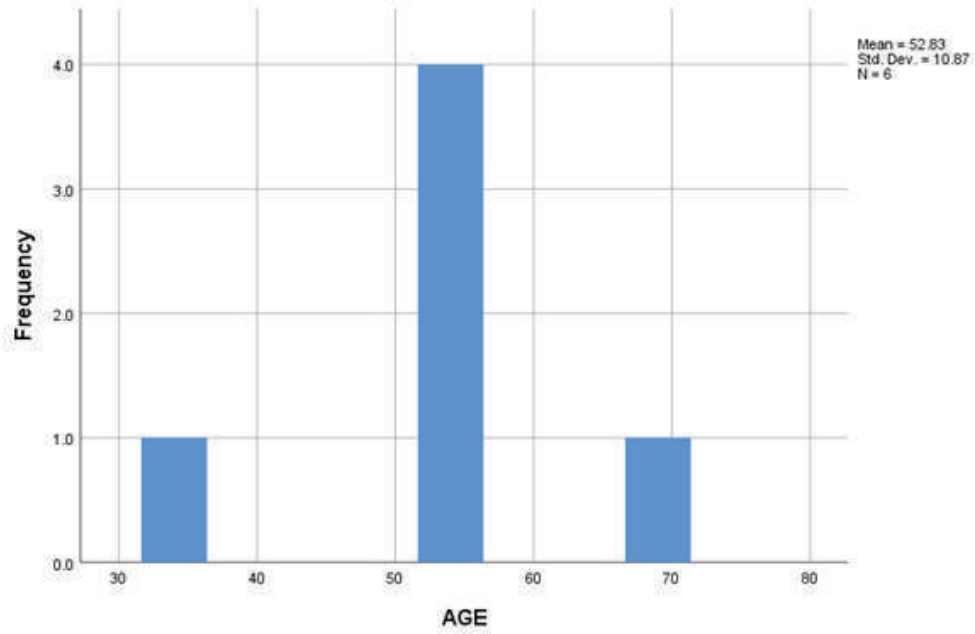


Figure 3 : The bar graph depicts the prevalence of desquamative gingivitis among different age groups of patients visiting the out patient department of periodontics at a private teaching hospital in chennai. The frequency of the number of patients is plotted on the Y axis on a scale of 0 to 4 and the age of the patients is plotted along the X axis on a scale of 30 to 80. Desquamative gingivitis was found to be more prevalent among individuals of age between 50 to 60 years. However, this is not statistically significant (Chi square test ; p =0.955 ; p>0.005; Hence not significant)

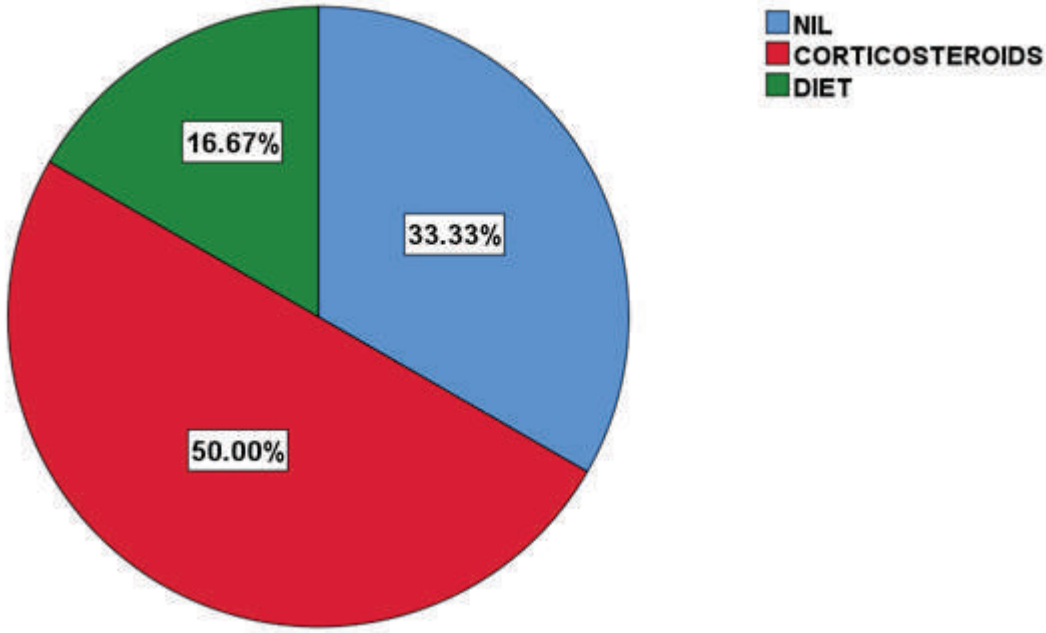


Figure 4 :The pie chart depicts the pharmacotherapy for treating desquamative gingivitis at a private teaching hospital in chennai. Blue denotes no medication, red denotes corticosteroids and green denotes diet. Corticosteroids was found to be the treatment of choice for desquamative gingivitis .

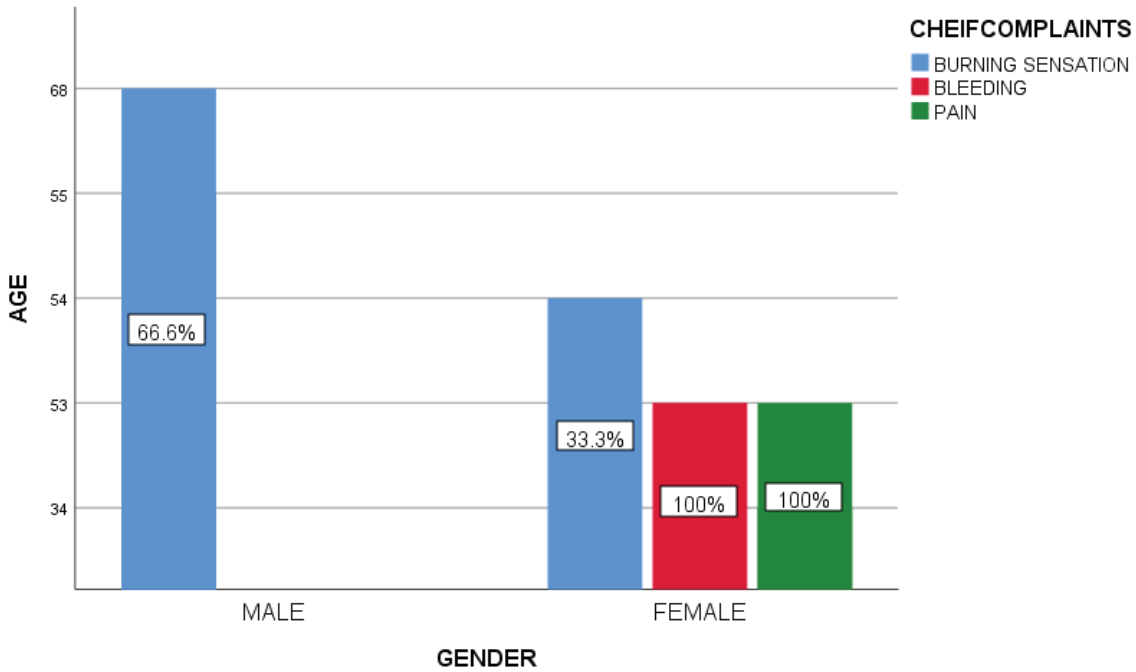


Figure 5: The bar graph depicts the percentage association of age , gender and chief complaints of the patients with desquamative gingivitis. The gender of the patient was plotted along the X axis and age of the patients was plotted along the Y axis. Blue denotes burning sensation of the gingiva, red denotes bleeding of the gingiva and green denotes pain. It is evident that burning sensation of the gingiva was more common among the males than in females and bleeding and pain of gingiva were only experienced by females and not males. Pearson's chi square value p=0.199; statistically not significant (p>0.05).

	GENDER	AGE	CHIEF COMPLAINTS	SITE	DURATION
Chi-Square	.667a	.667b	1.000c	1.000c	1.000c
df	1	4	2	2	2
Asymp. Sig.	.414	.955	.607	.607	.607
a. 2 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 3.0.					
b. 5 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 1.2.					
c. 3 cells (100.0%) have expected frequencies less than 5. The minimum expected cell frequency is 2.0.					

Table 1 :The table shows the chi square test for gender , age , chief complaint of patients with desquamative gingivitis , site affected and duration of desquamative gingivitis at private College Hospital in Chennai. It is evident that they are statistically not significant (p>0.005).

Desquamative gingivitis is a clinical finding with several etiologies. Among the etiology, the most common etiologies are oral lichen planus, cicatricial pemphigoid, and pemphigus vulgaris, although various other differential diagnoses exist. The presence of desquamative gingivitis often results in poor oral hygiene whose consequences include periodontitis and tooth loss. Though certain mucosal findings may directly suggest the presence of a particular diagnosis, a thorough history, physical examination, and appropriate dermato pathological and immunopathologic assessment is necessary for narrowing this broad differential diagnosis.

In our current study shows desquamative gingivitis was less than 0.1 % [Figure 1] . This is in concordance the study conducted by La Russo L et al., who stated that only 125 patients reported with clinical features of DG from the year 2004 to 2007 [7]**.** The decreased prevalence of lesions associated with desquamative gingivitis may be due to the improvement in the tools and techniques for immunologic analysis. Advancements in the knowledge and clinical practice, with a better understanding of the pathogenesis of the disorders associated with desquamative gingivitis and improved immunologic evaluation may have contributed to a decrease in the number of cases of desquamative gingivitis [45].

Our study showed female predilection which indicates that desquamative gingivitis was more common in females (67%) than in males (33.3%) [Figure 2]. The chi square test values of the prevalence of desquamative gingivitis among different genders was found to be statistically not significant (p>0.005) [Table 1]. This is in agreement with the studies conducted by Carbone M et al., and Skalvounou A et al.,[46,47] **.** Studies show that prevalence of disimmune disorders were more common in females[47]. The increased prevalence of desquamative gingivitis among females may be attributed to the hormones such as oestrogen and progesterone which changes in its levels during puberty, pregnancy, menstruation and menopause in females[48]. Other reasons may be due to intake or oral contraceptives, genetics and stress. Literature states that stress causes the decrease in saliva secretion thereby aids in the formation of dental plaque[49].

Desquamative gingivitis was found to be more common in patients of 50 to 60 years of age . The chi square test value of the prevalence of desquamative gingivitis among different age groups was statistically not significant (p>0.005) [Table 1]. This is in accordance with the study conducted by Sklavounou A et al., who stated that desquamative gingivitis is more common in

middle age and elderly females [47]**.** Carbone M et al., and seen in ages between 60-80 years[46]**.** Age is an important factor not only for diagnosis but also for formulating the treatment. It is also a differential parameter, considering the fact that some diseases have their onset in a specific age group. The conditions associated with DG usually have a peak of incidence between the fourth and sixth decade of life. Reports show that cases of children and adolescents are very uncommon [50–52]. The incidence of desquamative gingivitis in elderly people may be attributed to the poor diet intake, changes in salivary gland and salivary secretion and changes in the oral mucosal membrane.

The most common chief complaints of patients with DG in our data analysis was found to be the burning sensation of gingiva (50%) followed by pain (33.3%) and bleeding of the gums (16.7%) being the least common .The chi square test value for the chief complaint of patients diagnosed with desquamative gingivitis were statistically not significant (p>0.005) [Table 1]. Burning sensation was more commonly seen among the male patients (66.6%) while bleeding and pain was experienced by only female patients and not male patients .Dommy Aet al.,in his study reported that the most common chief complaint was burning sensation which is aggravated on consumption of spicy food[53] .Non plaque induced gingival erythema, gingival desquamation and intra oral lesions are major diagnostic criteria for DG which may be the cause of the burning sensation, pain and bleeding associated with DG. According to the study conducted by Leao J.C et al., Gingival soreness was the chief complaint of 92% of the patients while few patients complained of ulceration of oral mucosa (5%) [54]**.**

Our study shows that the lesions associated with DG were most commonly found in mandibular gingiva (50%) and least common in maxillary gingiva (16.7%) . 33% of the lesions involved both maxilla and mandible .The chi square test value for the site of occurence of desquamative gingivitis was statistically not significant (p>0.005) [Table 1]. Literature cited immune mediated disorders , most commonly affecting the buccal mucosa, tongue, gingiva, and labial mucosa of the mandible while the involvement of the palate, floor of the mouth, and upper lip are uncommon [55]. The study conducted by Carbone M et al., shows contradictory results stating that the maxilla is more commonly affected[46]. Ziskin D.E et al., and Dommy A et al., stated that the lesions may be present throughout the entire gingiva in the form vesicles or bullae [53,56]. The contradictory results may be due to the smaller sample size and difference in geographic area and ethnicity in our study.

The duration of symptoms of desquamative gingivitis was 1 to 10 days in most of the patients (50%) in our study. The chi square test value of the duration of symptoms of desquamative was statistically not significant (p>0.005) [Table 1]. The duration varied between studies conducted by different authors [53,54]. This may be due to the smaller sample size , usage of medication by the patient ,presence of systemic diseases and the difference in the duration of symptoms of various lesions associated with desquamative gingivitis.

All the patients reported with DG were found to have the habit of lip biting and mouth breathing and 67% of the patient presented with past medical history and drug history associated with diabetes and hypertension . Mouth breathing causes constant drying of the oral cavity, increased viscosity of saliva and loss of cleansing action which results in plaque deposits causing gingival inflammation which can lead to the manifestation of the underlying systemic mucocutaneous disorders . Studies have to be done to see for any direct correlation of these habits and Underlying pathology which results in the manifestation of the systemic condition on the gingiva. The first diagnostic step in identification of lesions associated with DG consists of taking careful medical history. The need for a detailed history is to help in the correct diagnosis of lesions associated with desquamative gingivitis as the diagnosis may be difficult and complicated. Determining etiologic factors that cause the lesions and arriving at the diagnosis of the underlying systemic disease can take a long time. The onset and progression of gingival lesions should be carefully investigated as most disorders associated with DG have a subacute onset, and the patient is generally unaware of it (especially OLP and MMP) [57–59]. The drug history of the patient plays an important role as local hypersensitive reactions may be caused due to several drugs , mouthwashes and even tooth pastes, which may play a causative role in few patients. Drugs such as aspirin, alpha blockers, chemotherapy medications, phenytoin and sulpha drugs cause mouth sores which

may exacerbate the lesions. Medications which can induce antimetabolic injury of oral mucosal epithelium include chemotherapeutic agents and few medications are found to cause stomatitis and oral ulcers.

Proper intraoral examination is required for the correct identification of various lesions associated with desquamative gingivitis[60]. The study showed that 66% of the patients were found to have clinically healthy alveolar mucosa while an equal number of patients were found to have red and white lesions .The alveolar mucosa is a thin, non keratinized mucosal layer covering the alveolar process of the maxilla and the mandible and is loosely attached to underlying bone and is continuous with the mucosa of the cheek, lips, tongue, and palate. Most red lesions of the oral cavity may be inflammatory in nature while some may be caused due to trauma, nutritional deficiencies and genetics. Red and white lesions are caused due to the necrosis of the oral epithelium. Ziskin D.E et al., and Dommy A et al., stated the presence of large vesicles and multiple bullae [53,56].

In the current study, 83% of the patients presented with red, inflamed and soft gingiva with absence of stippling which is a common indication of periodontal disease. Leao J et al., and Robinson N.A et al., reported that most patients presented with gingival erythema and areas of gingival desquamation [54,61]. Lo Russo L et al., reported 1 case of DG with no oral symptoms [7,62]. Most of these conditions associated with DG presents with a similar clinical appearance of the gingiva and can be only distinguished from each other by histologic presentation. This appearance of the gingiva in desquamative gingivitis may be due to the release of proinflammatory cytokines such as the tumour necrosis factor alfa, interleukin-1 and recruitment of inflammatory cells which causes the inflammation of the gingiva . These cytokines may also upregulate matrix metalloproteinases, which break down collagen and lead to the loss of periodontal attachment and bone destruction[63]. Reduced oral hygiene caused by gingival discomfort and bleeding on flossing which leads to dental plaque accumulation aggravates the clinical severity of inflammation[64].

The clinical presentation of desquamative gingivitis involves the gingival alteration which is not associated with biofilm formation and is framed within the gingival manifestations of systemic conditions. According to the clinical affectation, desquamative gingivitis can be distinguished into two types; one which are Mild forms, that are acute in nature and are characterized by the presence of erythema and mild desquamation without ulceration. On the other hand severe forms have erythema, desquamation, and painful ulcerations that affect the free and adhered gingiva[65] . The clinical characteristics of the disease varies according to the type and severity of the lesions.

Equal number of patients presented with normal and abnormal mucosa . Abnormal mucosal lesions may present as erythematous plaques in different areas of the oral mucosa. They may be due to infectious agents, metabolic disorders, endocrinopathies, neoplasms, developmental abnormalities, genetics, immunological disturbances and even injuries. Dommy A et al., and Maderal et al., in their study, stated that the mucosa is usually presented with erythema which is most frequently presented in sites such as the buccal mucosa [53,66]. Abnormal mucosal lesions may mostly occur due to lesions associated with erythema multiforme [67,68]. Lesions begin as areas of erythema with edema and progresses to erythematous plaques and bullous and erosive lesions with pseudomembrane formation.

Pharmacotherapy was the common treatment in most of the patients (50%) followed by scaling and root planing (33.3%) . This is in accordance with the study conducted by Nisengard R.J et al., who stated that pharmacotherapy is the most common and effective treatment followed by scaling and root planing and atraumatic oral hygiene [69–71]. The reason for the use of pharmacotherapy is that the systemic and topical pharmacological management of the underlying condition may contribute to improve the oral hygiene thereby reducing the overall inflammation. Other oral hygiene procedures must be atraumatic, as any mechanical and traumatic procedures may induce the exacerbation of lesions [11,72].

The use of corticosteroids was the most common medication (50%) while 16.7% of the patients were prescribed a proper diet [Figure 4]. Nisengard R.J et al., had stated that corticosteroids is the treatment of choice for desquamative gingivitis [69,73,74] .Topical corticosteroids such as hydrocortisone hemisuccinate and triamcinolone acetonide are the mainstay of therapy desquamative gingivitis and lesions associated with

it as these gingival lesions are often recalcitrant to therapy. The reason for the use of corticosteroids is that they are involved in a wide range of physiologic processes including stress response, immune response and regulation of inflammation . Topical corticosteroids must be considered a treatment of choice unless the disease is very extensive. Systemic therapy is considered for those with severe refractory disease [75].

Overall, desquamative gingivitis is a rare condition affecting majorly the female population in their fifth and sixth decade of life due to several reasons such as lack of nutrition, systemic illness and ill fitting dentures . Most patients complain of the burning sensation of the gingiva and pain which may be due to the desquamative nature of the lesions. The lesions were more commonly present in the mandibular gingiva and presented with symptoms from 1 to 10 days. The duration of symptoms may largely depend on the sample size of population , the past medical history of the patients and intake of medications by them. Lip biting and mouth breathing habits were associated with all the patients with DG and are found to cause the dryness of the mouth, decreased viscosity of saliva and loss of self cleansing ability. The intra oral examination revealed that the majority of the patients showed clinically healthy alveolar mucosa while an equal number of patients presented with red and white lesions which may be due to the atrophy of the oral mucous membrane . Majority of the patients showed red, inflamed and soft gingiva with the stippling indicating the presence of disease. Pharmacotherapy was the most common treatment in most of the patients as it helps in the management of the underlying disease, followed by scaling and root planing. The use of corticosteroids was the most common and effective medication used for DG as they are involved in a wide range of physiologic processes including stress response, immune response and regulation of inflammation .

The study was geographically limited and predominantly consisted of the South Indian population. Data which were unclear were excluded thereby reducing the sample size. Within the limit of the study , it was found that desquamative gingivitis presented in less than 0.1% of the patients, majorly affecting females of age 50 to 60 years . To ascertain the results of this study and to increase the level of significance, the sample size and the geographic area of coverage should be extended to at least most parts of South India. Conducting a multicentered study with extended geographic area and wide range of population in future we can obtain better results. Establishing the proper diagnosis is an absolute prerequisite for the effective treatment of the underlying disease in DG patients. Thus this knowledge of prevalence of DG and correlation with various parameters is essential in a dental practice for clinical implementation.

Conclusion

Desquamative gingivitis may be the clinical manifestation of a mucocutaneous disease process that recapitulates its pathogenesis in the mucosa of the oral cavity. Recognition of DG as a clinical sign of a mucocutaneous disease provides the alert clinician with a valuable diagnostic asset Thus the regular and thorough examination of the oral cavity in such patients is of paramount importance. Finally, establishing the proper diagnosis is an absolute prerequisite for effective treatment of the underlying disease in DG patients. In addition to topical or steroid treatment, elimination of DG requires excellent oral hygiene and replacement of any intraoral ill-fitting prosthesis.

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Assessment of Alveolar Ridge Defects based on Sibert's Classification - A Retrospective Study

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Abstract

Alveolar ridge defect may occur due to injury,trauma,normal healing after extraction, denture wear and periodontitis. Based on sibert's classification, it can be classified into three classes: class I (buccolingual loss of tissue), class II (apico coronal loss of tissue) and class III (both loss of tissue). It is important to close the ridge defect by replacing the tooth loss and to achieve good esthetic, phonetic and mastication. The aim of this study was to assess the prevalence of alveolar ridge defects using seibert's classification among partially edentulous patients.This study was conducted in a university setting among the outpatient population of a dental college.We reviewed patients records and analysed the data of 86000 patients between June 2019 and March 2020. 32,837 patients data were included from 86000 patients and were assessed and the data was tabulated in excel and later was imported to SPSS software by IBM and descriptive statistics were done and results were analysed graphically. The statistical analysis were performed using chi-square test.Based on the results, 50.5% males,51.4% females and 40% transgender have class I alveolar ridge defects; 0.7% male,0.8% females and 0% transgender have class II alveolar ridge defects;1.3 % males,1.4% females and 0% transgenders have class III alveolar ridge defects; 47.5% males, 46.4% females and 60% transgender have normal edentulous ridge.To conclude, the Sibert's classification helps in suggesting a proper treatment plan for the patient to ensure that the treatment outcomes turn out to be successful.

Keywords- *edentulous, sibert's classification,alveolar ridge,esthetics*

Introduction

Prosthodontists may face challenges in treating patients with alveolar ridge defect in partially edentulous patients [1].Local alveolar ridge defect can be seen as a deficient volume of limited extent of soft tissue and bone within the alveolar process. The edentulous space may be due to loss of tooth, due to trauma during extraction or congenital defects which eventually leads to alveolar bone loss. This loss in the alveolar bone causes the intrusion of the overlying soft tissue during healing which ultimately creates contours [2] .These contours possess difficulty in fabrication of prosthesis from an aesthetics point of view. [3] It also leads to food impaction and difficulty in speech due to percolation of saliva.[4] As a prosthodontist, it is required to replace the missing tooth and close the ridge defect for patients, for achieving aesthetics, kinetics, phonetics and mastication [5]. A variety of ridge deficiency classification have been described pertaining to both hard and soft tissue defects.

Siberts (1983) presented a classification of ridge defects to assess deficiencies in form, function and aesthetics(6). This classification takes into account both hard and soft tissues [7,8]. Siberts class I defects describe

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ridges deficient in the horizontal dimension. Siberts class II defects describe ridges deficient in the vertical dimension [9]. Sibert's class III defects include ridges deficient in both the horizontal and vertical dimensions [10]. Few studies have been done on the prevalence of alveolar ridge defect using seibert's classification.

Treatment of alveolar ridge defects includes ridge augmentation proposed by Langer, Kaldhal et al., and Calanga.[11]It can be classified into soft tissue augmentation and hard tissue augmentation procedure[6]. Soft tissue augmentation procedure is the roll technique for Class I alveolar ridge defects, interproximal graft technique and free gingival graft for Class II alveolar ridge defects and class III alveolar ridge defects.[6,12] Ridge augmentation is mostly done for Class I alveolar ridge defects.For ClassII and ClassIII alveolar ridge defect, bone augmentation technique by either autogenous grafts, allografts, or xenografts is done[13]. Other treatment modalities include removable partial denture, fixed partial dentures and Andrew's bridge. [13] Few studies have been done on the prevalence of alveolar ridge defect using Siebert's classification on fixed partial denture patients. Many studies have presented case reports on various treatment options for alveolar ridge defects.Therefore, the purpose of the study is to assess the prevalence of Siberts classification among partially edentulous patients to achieve good treatment outcomes for the different ridge defects.[10,14,15]

Materials and Methods

Sample Collection:

This study is in a university setting, it is a cross sectional retrospective study. the different advantages for taking up a study in university setting easy retrieval of records, the available data is from the same ethnicity. The disadvantages being the study is located in one geographical location, Limited population and same ethnicity. We reviewed patients records and analysed the data of 86000 patients between June 2019 and March 2020.32,837 patients data were included out of 86000 patients the study was approved by the university ethical committee. Patients who were completely edentulous were excluded from this study. Meanwhile, single partially edentulous site, multiple partially edentulous site, excessive ridge defect, and anterior or posterior ridge defect were included in this study. The alveolar of the patient is classified based on the siberts classification.

Sample Size:

Total of 32,832 patients is included in this study

Statistical Analysis

The data collected was tabulated in excel and was then imported to SPSS software by IBM. then, descriptive statistics was computed. Chi-square test was done. The chi-square test was used to compare the data and check for the distribution at 0.05 level of significance for effect of statistical significance. Results were analysed graphically, for both frequency distribution and statistical significance.

Results And Discussion

In this study, 43.97% males and 34.78% females have class I defects. 0.49% male and 0.49% females have class II defects. 1.3 % males,1.4% females and 0% transgenders have class III defect; 0.90% males and 0.74% females have normal edentulous ridge.(Figure 3)

Normal ridge is seen more in younger age and class I ridge more after 40 years whereas, in transgender below 35 years, more number of class I defect is seen and above 35 years, more number of normal ridge is prevalent.(Figure 2)Males in the age group of 1 to 15 years mostly have normal edentulous ridge, 15 to 30 years both class I defect as well as normal ridge is equally prevalent. After 30 years class I defect is more prevalent than other defects. Females of all age groups have equal prevalence of normal, class I ridge[16]. This study shows the prevalence of alveolar ridge defect according to Seibert's Classification. Siebert, classified the alveolar ridge defect according to the presence of deficiencies in form, function, and esthetics. [17,18]In these studies, normal ridge had the most number of incidence, followed by Class I and Class II with least prevalence was class III. According to these studies also, male gender has high prevalence in having alveolar ridge deformities with 54.5% and also patients in the age within 40–49 years old have a high incidence of alveolar ridge defect with 50.9%. However, no study has been done on the correlation of alveolar ridge defect with age and gender. Modification of Siebert's classification was introduced by Allen et al. [19] in the year 1985 which included the magnitude of the ridge: This classification was meant to

aid in the treatment planning and prognosis of the patient with alveolar ridge defect. The main problem of alveolar ridge defect is anterior tooth loss ,which is difficult to treat due to esthetic factors [12,19].

Besides, other problems might also be encountered such as lack of emergence profile, lack of root eminence, lack of marginal gingiva, and presence of black triangles in interdental papillae area which is a hindrance esthetically [20]. Black triangles can be described as dark appearance of alveolar tissue above the pontic [21].The main reason for alveolar ridge deformities is due to trauma to the alveolar process during extraction. After extraction, the process of healing of the bone and soft tissue took place. However, due to the trauma, the soft tissue will collapse into the bone defects which create contours which make esthetic functional prosthesis would be difficult [22]. Therefore, the management of alveolar ridge defect can be classified into hard tissue augmentation and soft tissue augmentation. [6] There are various treatment options to treat alveolar ridge defect such as the roll technique for Class I defect and interproximal graft technique for Class II and Class III defect, free gingival graft, bone grafting using both inlay and onlay grafting technique either autogenous grafts, allografts, or xenografts, ridge augmentation using bone graft followed by implant placement, removal partial denture, fixed partial denture and Andrew's bridge. [12]

To achieve an esthetically successful pontic, all criteria including replication of the form, contours, incisal edge, gingival embrasures, and color of adjacent teeth should be met. [21] Besides, the study of prevalence in Siebert's classification was intended to give a clear image on the treatment choices and alternatives to achieve successful outcomes. In a study done by Abrams et al.,[23] they reported that the prevalence of anterior ridge deformities of partially edentulous patients was 91% similar to the current study which is 91.6%. Class III defects were the highest with 55.8% followed by Class I defects with 32.8% and Class II defects with 2.9%. In a study done by John et al.,[24] bone defects in the posterior mandibular tooth region show a maximum defect with 33.8% followed by maxillary posterior with 19.9%. Since the prevalence of Class III defect is the highest compared to Class I and Class II, many articles have described the treatment outcome for Class III defects patients in their case report article.[25,26,12]In Class III defect, Andrew's bridge is the best option due to the challenging situation with esthetics and severe alveolar ridge defect. Andrew's bridge was introduced by Dr. James Andrew of Amite Louisiana in the year 1975.[27] It is a combination of a fixed dental prosthesis and a removable dental prosthesis and commonly used for anterior edentulous area. It replaced the teeth within the bar area which were incorporated with the fixed dental prosthesis. The removable dental prosthesis received retention from the vertical wall of the bar.

The advantages of Andrew's bridge system are the advantages of fixed and removable partial dentures with better esthetics, hygiene along with better adaptability, and phonetics [12]. Besides, it is economical and comfortable for the patient. Other advantages of this technique process are the flexibility and stabilizing quantities of the prosthesis. In a study done by Snehal and Amberkar[12,18]the clinical case report suggests that soft tissue augmentation with subepithelial connective tissue graft is a promising treatment in a condition with Class I defect.

The study was done by harvesting the connective tissue graft from the palate along with metal ceramic restoration. The advantages of this technique are maintenance of adequate blood supply, and the use of stents or hemostatic agent can be avoided and healing by first intention which provides greatest comfort to the patient [18] postoperatively.[18] Apart from that, the disadvantages of this technique are the limited volume of graft which depends on the size of the graft and increases prone to necrosis in case of large grafts.[28] In a study done by Tanaka et al.,[29]a segmental osteotomy procedure with an interpositional graft is done on a patient with Class II alveolar ridge defect. Most of the studies showed that this technique is practical and a predictable procedure with low incidence of complications and a high probability of successful treatment outcomes.[30]

Lack of alveolar bone height (apico coronal) condition can be overcome using various vertical guide bone regeneration procedures, alveolar distraction osteogenesis, titanium mesh, or only bone graft. According to this, other literatures Nor Syakirah Binti Shahroom, et. al.[31] In this study class III ridge defects had the most number of incidence-40 % class I- 33.3%, classII-18.3 %, males have high prevalence of 54.5

% and patients within the age group of 40 to 49 years have higher incidence of alveolar ridge defects 50.9 %. However, no study was done on the correlation of alveolar ridge defect with age and gender [16,32]. Class I ridge defect and normal ridge were equally prevalent in both genders. The results of this present study are morally relevant due to such a huge sample size of 32,837 patients.The limitations of this study were the same ethnicity and the same geographical location. Further cross sectional study with more sample size from different ethnicity, different geographic locations.

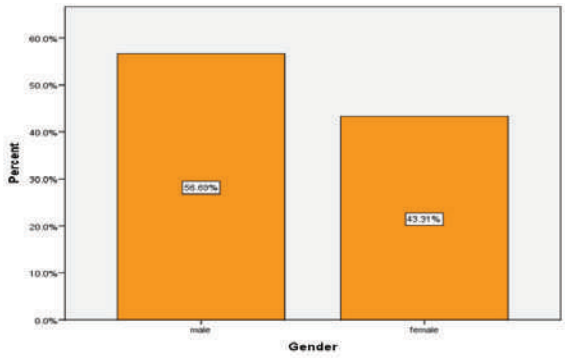


Figure 1: This graph represents the distribution of study subjects based on gender.X-axis represent gender and Y-axis represents the frequency distribution of the individual gender.Out of 32,832 patients,56.69% were males and 43.31% were females.

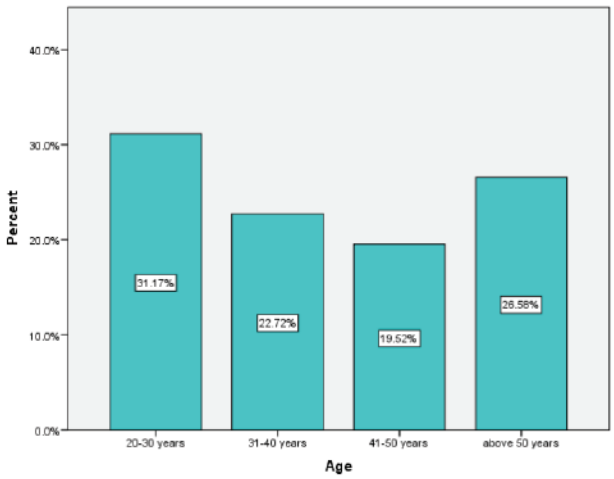


Figure 2: This graph represents the distribution of study subjects based on age group.X-axis represents age group and Y-axis represents the frequency distribution of the different age groups. Out of 32,832 patients, 31.7% patients belong to the age Group of 20-30 years, 22.72% patients belong to the age group of 31-40 years,19.52% patients belong to the age group of 41-50 years and 26.58% patients were above 50 years of age.

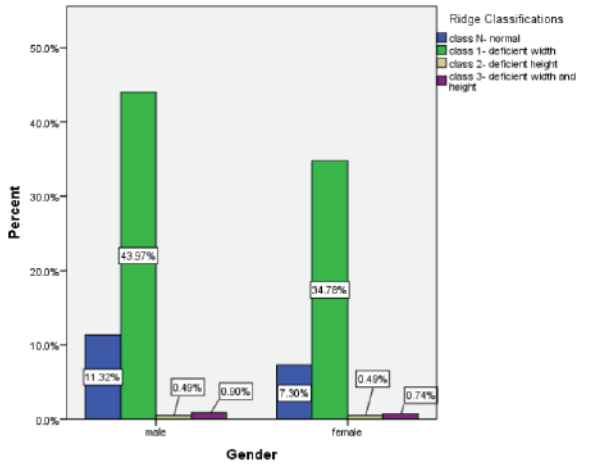


Figure 3: Bar graph represents the association between gender and alveolar ridge class according to seibert's classification.X-axis represents the gender and Y-axis represents the ridge classification where blue colour denotes normal ridge,green colour denotes class I ridge, yellow colour denotes class II ridge and violet colour denotes class III ridge.Chi-square test was done and association was found to be statistically insignificant [Pearson's Chi Square value:5.910, DF:6, p value:0.433(>0.05)]. Even Though it is statistically insignificant, majority of the male participants (43.97%) had Class I ridge pattern (deficient width) than female participants.

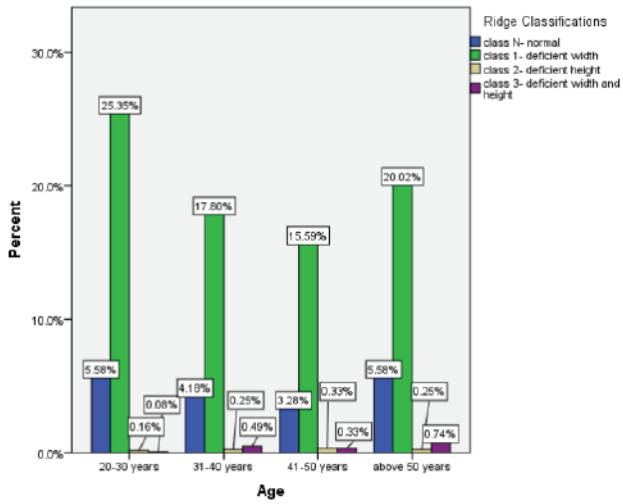


Figure 4: Bar graph represents the association between age groups of the patients and alveolar ridge defect according to seibert's classification.X-axis represents the age groups of the patients and

Y-axis represents the ridge classification where blue colour denotes normal ridge,green colour denotes class I ridge, yellow colour denotes class II ridge and violet colour denotes class III ridge. Chi-square test was done and association was found to be statistically significant. [Pearson's Chi Square value:426.482, DF:9,p value:0.00(<0.05)]. Majority of the participants distributed in the age group between 20-30 years (25.35%) had Class I ridge pattern (deficient width) than the other age groups.

Conclusion

jIt is important to assess the alveolar ridge deficiencies among patients with edentulism. According to the amount of destruction, it can be classified based on siberts classification. through this various treatment modalities can be suggested to the patient to ensure that the prognosis and treatment outcome turn out to be successful. Within the limit of this study, it is seen that class I ridge defect and normal ridge is most prevalent in all the three genders with slight deviation with age.

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Impact of Positive Thoughts on Immunity

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Abstract

Immunity plays a major role, by protecting us from various foreign antigens.It is broadly classified into two major groups, active immunity and passive immunity. Foods such as citrus fruits, broccoli, garlic, ginger, yogurt, and almonds are immune boosting foods.The positive thoughts also have a good impact over diseases like diabetes and cancer and it can be overcome by the positive thoughts. Positive thoughts are directly proportional to the positive effects on immunity.The positive thoughts are directly proportional to the positive effects on immunity by controlling or decreasing stress level. The decrease in the positive thoughts have direct impact on health, thus it paves a way for occurrence of disease. The aim of the present study is to assess the knowledge of positive thoughts on immunity among the general population. An online based survey was conducted among 100 people on the impact of positive thoughts on immunity. The results were analyzed and represented as graphs. The present study thus concluded that based on the survey people had less knowledge about the impact of positive thoughts on immunity.

Keyword: Immunity; positive thoughts; impact

Introduction

Immunity is an ability of an organism to resist a particular infection or toxin.It is broadly classified into two major groups, active immunity and passive immunity [1] .The adaptive component of immunity has T cells which activates the antibodies in the body to act against the antigen [2]. The active immunity results from production of antibodies by an individual's own immune system while passive immunity is a short term immunity from an external source [3].

Foods such as citrus fruits, broccoli, garlic, ginger, yogurt, and almonds are immune boosting foods. Eating a healthy diet, exercising regularly, getting adequate sleep for eight hours are some of the factors to boost our immune system [4]. The positive thoughts also have a good impact over diseases like diabetes and cancer and it can be overcome by the positive thoughts [5]. The decrease in immunity results in many health disorders mainly impaired vision, etc. The decrease in immunity results in many health disorders mainly impaired vision etc. [6].

The brain of the current generation has been exposed to radiations of electronic gadgets due to their addiction, which leads to suppression of their brain cells and inturn decreases their immunity [7]. There is a relationship between obesity and the immune system, therefore it is necessary to keep an eye on immunity and lead a healthy life [8].

The positive thoughts is a mental attitude expecting favourable results in all situations. It has a big impact on one's physical and mental health [9]. It motivates us to develop positive vibrations and attain peace of mind [10]. Focusing on good things, practicing positive self-talks, spending time with positive people, and

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practicing gratitude are some of the methods to increase an individual positive thoughts by eliminating negative thoughts [11]. Many patients have overcome major diseases with their positive mindset, thus a positive mindset helps us to lead a healthier life [12]. Electronic learning like Ipad learning is believed to provide a more positive mindset towards the world compared to textbook learning systems among students [13].

Positive thoughts are directly proportional to the positive effects on immunity. Positive thinking protects health during high stress levels [14]. The positive thoughts are directly proportional to the positive effects on immunity by controlling or decreasing stress level. The decreases in the positive thoughts have direct impact on health, thus it paves a way for occurrence of diseases [15]. Owing to the multifold increase in exposure to positive thoughts, this helps the person to cure the disease and also helps immunity.

The aim of the study is to assess and improve the knowledge about positive thoughts and to understand the importance of positive thoughts on immunity.

Methods and Materials

The present study has conducted questionnaire based online surveys on immunity and positive thoughts, a total of 100 participants participated in this survey. The obtained results were analysed by applying Pearson chi square test through SPSS. The results were represented as pie charts and graph bars.

Results and Discussion

A total number of 100 participants participated in the study. Results were collected and data has been analysed. Results were analysed in the form of bar graphs

The responses of the survey were collected and analysed and represented as bar graphs. The following table shows the results for the survey conducted (Table 1).

Table 1:

S. No	Questionnaire	Responses	
1.	Are you aware that nutritional food can boost our immunity?	73% of the people aware that the nutritional food can boost our immunity	27% unaware
2.	Do you think that our lifestyle has an impact on our immunity?	70% of the people accepted that lifestyle has an impact on our immunity	30% unaware
3.	Are you aware that we can fight diseases by keeping our immunity high?	70% of the respondents have believed	30% unaware
4.	Why is a positive mindset so important?	75% believed that positive thinking prevents of release of stress hormone, damage in mental health and to distress the mind	3.9% believe to prevent releasing of stress hormones, 14% believe to prevent damage in mental health, 6% believe to distress the mind
5.	Have you taken any measures so far to convert your negative thought into positive thought?	73% of the people have taken steps to convert their negative thoughts into positive ones	27% have not taken any steps so far

***Cont.*. Table 1**

6.	Have you experienced a situation where your positive thoughts helped you to recover in hard times?	67% of the respondents experienced a situation where their positive thoughts helped them to recover in hard times	33% have not dealt with such situations
7.	Are you aware of the term immunity?	83% of the people were aware of the term immunity	17% were not aware
8.	Are you aware of positive thoughts on positive action?	67% of the people believed that the positive thoughts influencing a positive actions	33% not accepted
9.	Are you aware that an affirmation of positive thoughts to one helps us to increase our confidence?	81 % of the people believed that affirmation of positive thoughts to oneself helps increase our confidence	19% not accepted

Awareness of the impact of lifestyles on immunity was associated with gender was statistically not significant, *p* value is 0.446 (>0.05), even though statistically not significant 45 % of the female participants are aware of the impact of lifestyle on immunity [Figure 1] and the association between gender and awareness of fighting disease found to be not significant, *p* value is 0.841 (*p* value > 0.05). Even though statistically not significant 45 % of females are aware of fighting disease by keeping immunity high [Figure 2]. The association between gender and knowledge on the importance of positive mindset found to be statistically not significant, *p* value is 0.428 (*p* value > 0.05). Even though 50 % of females are aware of knowledge on the importance of positive mindset [Figure 3].

Figure 4 represents the association between knowledge on converting negative thoughts to positive thoughts and genders. The association between gender and knowledge on converting negative thoughts to positive thoughts found to be statistically not significant, *p* value is 0.115 (*p* value > 0.05). But 43 % female and 31 % male are aware . Similarly, the association between gender and knowledge on positive thoughts resulting in positive action found to be statistically not significant, *p* value is 0.115 (*p* value > 0.05. But when compared to male (24%), females (44 %) are more aware [Figure 5].

In the present study, 72% of the people believe that nutritional food helps to increase their immunity. Similarly, it was emphasized that nutritional deficiency must be treated in order to reduce the risks of infections and diseases.[15] In the current study, 70.3% of the people believe that lifestyle has an impact on immunity . Similarly a study reported that the lifestyle has a great impact on immunity, it is due to the change in lifestyle in recent years, many new diseases are being developed [16]. In the present study, 69.6% of the people have believed that one can fight diseases by keeping good immunity by intake of immunity boosting food on a regular basis [17]. Only 74.5% of the people were aware of the advantages of positive mindset, which prevents stress hormones releasing. A study says that the positive mindset is very important in today's world. Through having positive mindset one can achieve good body and mind [18]

For infants mothers milk is necessary to keep the immunity good. The present study has demonstrated that 72.5 % of the people were aware of the importance of mother's milk on immunity. The mother's milk contains abundant antibodies and thereby increases immunity and also provides ideal nutrition [19] In addition, the positive thought tends to improve memory as well and keeping good thoughts may calm and relax the mind, which leads to maintaining good memory of an individual [20].

For increasing or to maintain immunity, it is important to keep the mind positive. So that, to keep a positive environment, yoga and meditations play a major role in keeping the mind healthy and removing the negative thoughts [21-22]. The various ailments, disorders, syndromes have a direct impact on mind, which in turn causes negative thought leads to weak immune systems. One must always be aware of the present situations and act upon it in a positive mindsets [23-30]. The limitation of the present study is minimal sample size and survey based analyses. But the present results may be useful in future studies.

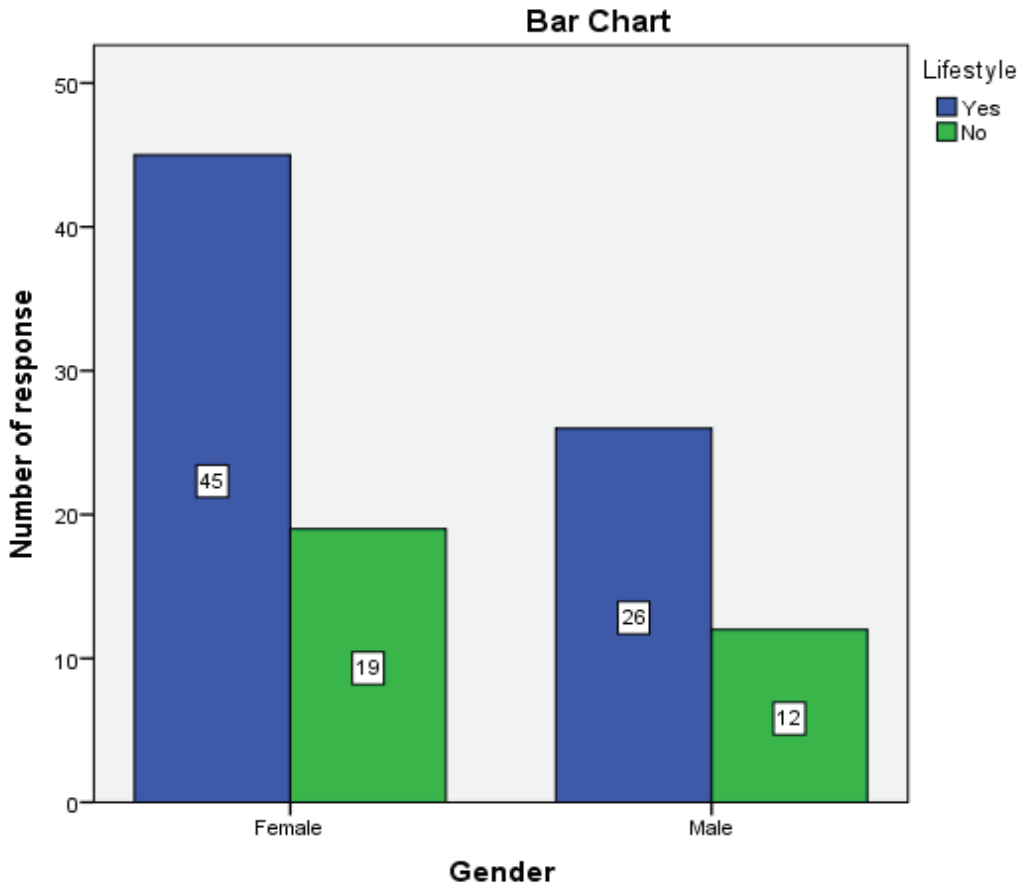


Figure 1: The bar graph represents the association between awareness of impact of lifestyles on immunity and gender. X axis represents the gender and Y axis represents the number of responses.Blue colour denotes awareness and green colour unawareness.Pearson chi-square test shows p value is 0.446 (>0.05). The association between gender and awareness of impact of lifestyle found to be statistically not significant. Even though statistically not significant 45 % of the female participants are aware of the impact of lifestyle on immunity.

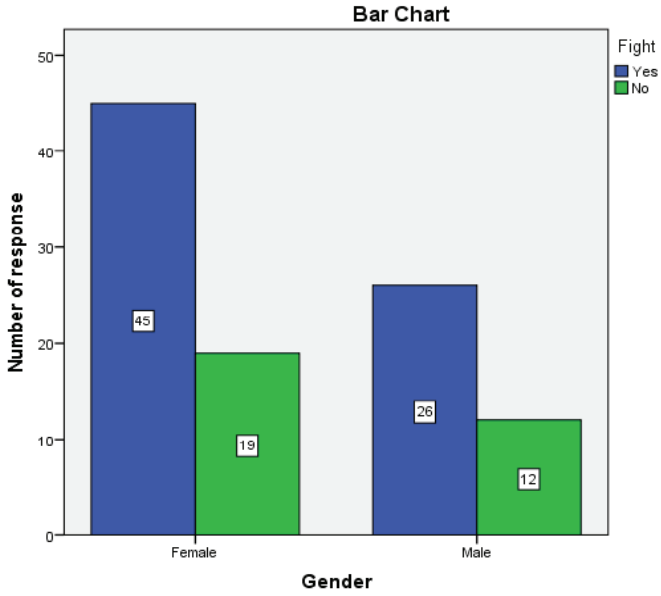


FIGURE 2: The bar graph represents the association between awareness of fighting disease by keeping our immunity high and gender. X axis represents gender and Y axis represents the number of responses. Blue colour denotes the awareness and green colour denotes unawareness. Pearson's chi Square test shows *p* value is 0.841 (*p* value > 0.05). The association between gender and awareness of fighting disease found to be not significant. Even though statistically not significant 45 % of females are aware of fighting disease by keeping immunity high.

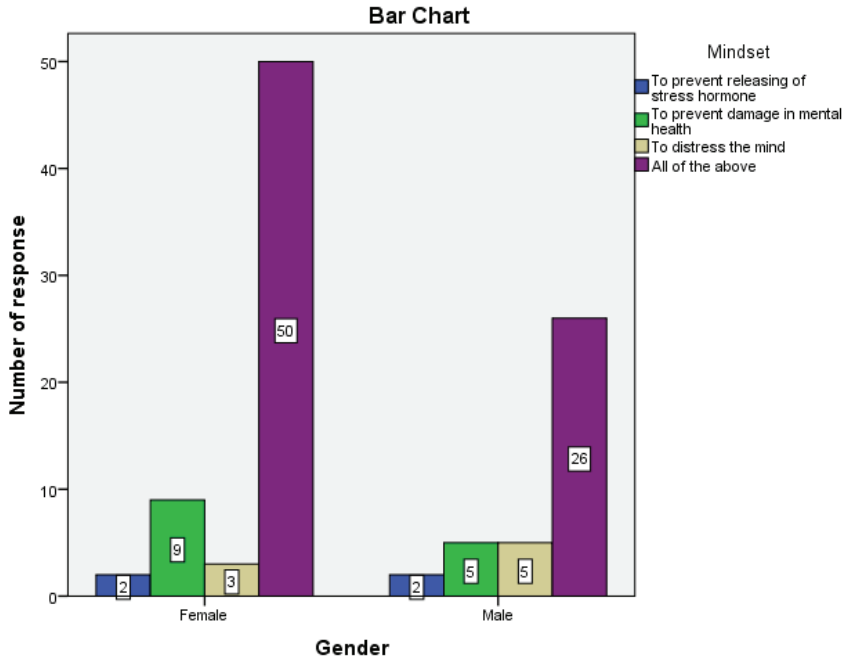
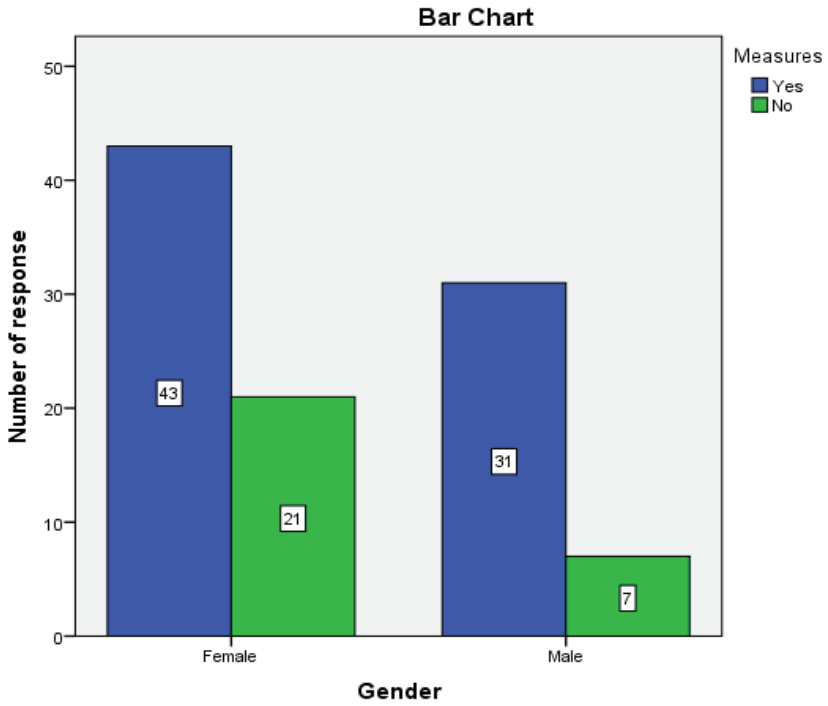


Figure 3: The graph represents the association between the knowledge on importance of positive mindset and gender. The X axis represents gender and Y axis represents the number of responses. Pearson chi square test, *p* value is 0.428 (*p* value > 0.05). The association between gender and knowledge on the importance of positive mindset found to be statistically not significant. Even though 50 % of females are aware of knowledge on the importance of positive mindset.



Figures 4: The bar graph represents the association between knowledge on converting negative thoughts to positive thoughts and genders. The X axis represents gender and Y axis represents the number of responses. Pearson chi square test, *p* value is 0.115 (*p* value > 0.05). The association between gender and knowledge on converting negative thoughts to positive thoughts found to be statistically not significant. But 43 % female and 31 % male are aware .

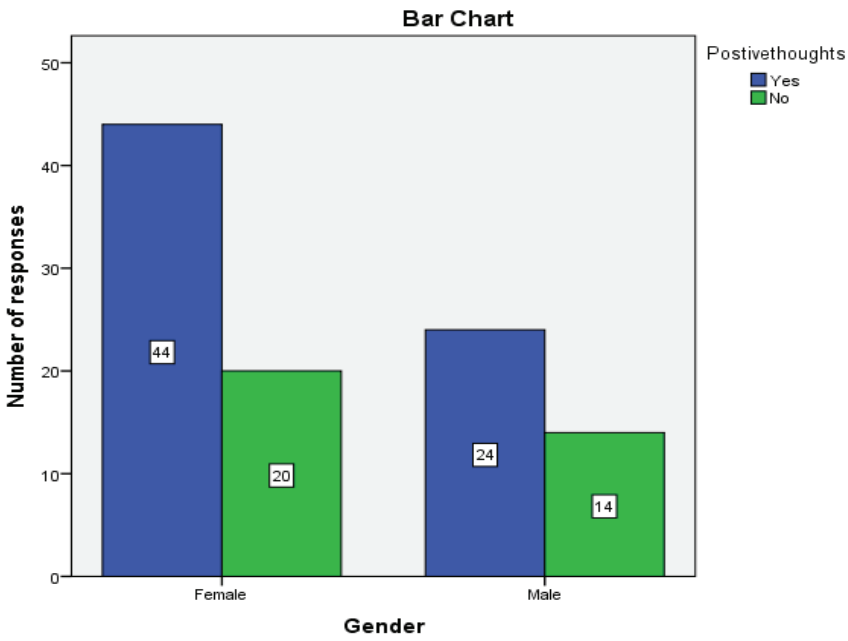


Figure 5: The bar graph represents the association between knowledge on positive thoughts resulting in positive action and gender. The X axis represents the gender and Y axis represents the response. Pearson chi square test, *p* value is 0.115 (*p* value > 0.05). The association between gender and knowledge on positive thoughts resulting in positive action found to be statistically not significant. But when compared to male (24%), females (44 %) are more aware.

Conclusion

The present study thus concluded that the positive thoughts have greater impact on maintaining a good immune system and lead a healthy life. From this study, the majority of the participants have got awareness about positive thoughts on the immune system.

Conflict of Interest: The authors have none to declare.

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Quantitative and Qualitative Analysis of Dental Caries among Different Age Groups of Patients in a Private Dental College Hospital, Chennai, India

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Abstract

Dental caries is the major health disorder caused by the interaction of oral microbes on the dental hard tissues. Dental caries are multifactorial in nature of etiology and pathogenesis. According to WHO almost 36% of the population have dental caries. Thus the aim of the current study is to quantitatively and qualitatively analyse the prevalence of dental caries among different age groups of patients visiting Saveetha dental college and hospital outpatient department services. All the cases reported between the month of June 2019 to March 2020 for dental caries of different classes were chosen for this study. Data of patients were retrieved from dental records. Data of patients with class I to class VI dental caries and root caries were included and age, gender, tooth affected were analyzed, tabulated and was subjected to statistical analysis. From the data analysis through SPSS it is observed that, the overall incidence of dental caries were higher among male patients (54.7%), between the age group of 15 to 30 years (45.4%) and was commonly seen in primary and permanent posterior tooth.(72.6%). Within the limitations of the present study, dental caries had male predilection, predominantly between the age group of 15 to 30 years,Class 1 caries primarily reported in the molars.

Keywords: *Dental caries, Multifactorial, Caries susceptibility, Caries index*

Introduction

Dental caries is a multifactorial infectious disease caused by the interaction of bacteria on enamel.[1-2] Dental caries is defined as an irreversible and a microbial disease of the tissues of the teeth, which is marked by demineralization of the inorganic portion of the tooth and destruction of the organic substance of the tooth, which often leads to cavitations.Of late, caries is on the increasing trend to become a major public health problems worldwide, with nearly 60–90% of young adults and about 100% of adults have dental cavities, often resulting in pain and discomfort.[3] According to the World Health Organization (WHO) report, approximately 2.43 billion people (36% of the population) have an active tooth Caries. Additionally, nearly all adults have dental caries at some point in their lifetime. Caries is prevalent in children, as well.[4] WHO has reported that 9% of youngsters (620 millions) have tooth caries.[4] There are several indices to ascertain dental caries; decayed, missed, and filled teeth (dmft) index and prevalence of untreated dental caries are of foremost significance.[5] With regard to the Histopathology of caries lesions, both enamel and dentine have different zones including the dark zone, transitional zone, body and surface zone.[6]

There are numerous theories explaining the pathogenesis of dental caries, and it is one established as a multifactorial disease with many risk factors.They have revealed several etiological factors for caries like oral cavity bacterial components; anti- oxidant activity [7-8],overall oral health status, including the frequency and efficacy of plaque removal; the quantity and frequency of sugar consumption; and components of saliva.[9-10] Additionally, there are several studies that have reported

the connection between oral health and a few demographic and socioeconomic factors like the age, sex, race, case history.[9,10] Oral microorganisms that ferment sugars[11] , host susceptibility, Dietary habits, have to coexist for caries to initiate and develop.[12-13] Also,the effect of nocturnal feeding is an important factor for increased prevalence of untreated caries lesions among children with Early childhood caries.[14] To add on, patients with OSMF often present with compromised oral hygiene, thus are more susceptible to dental caries.[15-17] Besides there are studies that states that, children with cleft lip/ palate face predisposing factors to acquire dental caries, and patients with cleft palate conditions generally shows high susceptibility to caries and poor oral hygiene, because of the difficulty in achieving adequate plaque control associated with dental anomalies and defects.[18] There is also a significant association of enamel defects with dental caries. The defect identified to be more frequently associated with dental caries was a variant showing both opacity and enamel hypoplasia.[19]

Even though the general prevalence of dental caries decrease in developed countries, caries continue to be an important oral health defect in most of the developing countries.[20] A study conducted in Lithuania showed that the general prevalence of cavities was 78.3% .[21] A study in 2013 in Brazil showed that 75% of the participants had enamel defects.[22] The survey on oral health (2016) indicated that the incidence of caries among adolescents in urban and rural areas of Tamil Nadu was 61.4%, with a mean DMFT score of 2.03.[23] In the 5- to 7-year age bracket, 34.9% children were found to be caries-free, while 65.1% showed one or more carious lesions. Within the 8 to 10 years age limit , the prevalence of dental caries was found to be 56.7%. 45.4% of individuals between the 11 to 13 year age bracket had caries involved teeth. The caries prevalence among males was found to be 59.3%. At an equivalent time, 51.7% of females were found to be suffering from caries.[24] In a study conducted in China in 2013 on caries prevalence it was concluded that the prevalence of tooth decay was 67.5% in elderly population in northeast China, and therefore the DFT index was 2.68±3.40.[25] Also, with regard to metabolome of caries, metabolic derivatives related to oxidation and chemical degradation are found at higher levels in archaeological dental calculus than fresh samples.[26]

At different ages, caries intensity may vary. The carious process often develops during the primary months after the tooth eruption but much less in adulthood and adulthood. These processes are mainly related to the mechanisms of ionic exchange between the mouth and hard dental tissues. Age is one of the most important factors influencing the structure ,and chemical composition of dental hair tissues. Not only the structure of enamel, but also the metabolic process in it alters with air leading to difference in caries incidence among different age groups.[27] While discussing the consistent trend of caries rates being higher in females than males, all associated factors must be considered. Women's roles in their community alongside other social factors, like differing salivary flow rates and compositions, dietary habits, hormonal changes during pregnancy, and particular variants of the AMELX gene must all be included within the assessment of woman's caries risk assessment.[28]

Thus there are so many studies done previously on the incidence of dental caries among different age groups, genders and also on assessment of different etiological factors of dental caries. This prompted us to conduct a study in a private dental college and hospital in chennai, India, so as to chart out the magnitude of the dental caries among different age groups within the population. Thus, the aim of the current study was to quantitatively and qualitatively analyse the prevalence of dental caries among different age groups of patients Saveetha dental college and hospital outpatient department which is a private college hospital in chennai, India.

Materials and Methods

This was a retrospective study conducted under a university setting. Ethical approval for the current study was obtained from the institutional ethical board (Ethical approval number: SDC/SIHEC/2020/ DIASDATA/0619-0320). This study had advantages of easy access to the software, large data availability yet also had disadvantages of smaller sample size and geographic limitation. In the current study the data of patients who visited Saveetha dental college from June 2019 to April 2020 were retrieved from the dental records.The Sample size for the study was n = 30232. Patients of all age groups (from 2 to 86 years) and genders (both male and females) with dental caries of all classes along with root

caries were included in the current study. Other patients without dental caries were excluded from the study.

All the case sheets included in the study were approved and verified by an external reviewer to avoid errors while recording. Also Cross- verification of data was done with photographs[29] and direct communication with dentists. Data for the study was retrieved and the collected data were tabulated in the excel sheet. Parameters such as age, gender, the tooth affected and the class of dental caries were included, correlated and analysed. This data was then imported to SPSS by IBM after coding. Frequency of all the parameters considered were drafted. Parametric and non- parametric correlations were done. Following which graphs were made. Non parametric tests were generated by clicking onto legacy dialogue, chi square test was run and P value was determined to verify the significance of each of the variables considered and the results were interpreted and analysed statistically.

Results and Discussion

Out of the total patients who visited Saveetha dental college between June 2019 to April 2020, 30232 patients were found to have dental caries of various class types. Of the total patients, male patients were observed to have a higher incidence of dental caries (54.7%) in comparison to females (45,3%), p value is less than 0.05 and shows statistical significance (graph1). It was also observed that the Caries generality between 1 to 15 years were 11.7% , between 15 to 30 years were 45.4%, among people of 30 to 50 years were 33.7%, and was 9% in the age group between 50 to 80, and 4% above the age of 80 years with p value less than 0.05 (graph 2). Occurance of dental caries were reported to be higher in primary and permanent molars (72.6%)with $p<0.05$. With regard to the class of caries, dental caries class 1 (according to GV BLACK' S classification) was higher (74.7%) followed by class 3 dental caries with $p<0.05$ (Graph3).

Dental caries is a chronic, non-communicable disease requiring attention for prevention and treatment. In the current study the Prevalence of dental caries based on the gender was observed to have a higher male predilection**.** Dental caries are in higher incidence in male (54.7%) than females (45.3%). The overall prevalence of dental caries in the study conducted by Jayashri prabakar et al in 2016, showed that the total caries prevalence was 47.3% which is less than the reported caries prevalence of India (i.e. 53.8%) in National Oral Health Survey [30] and proved that males (47.5%) were slightly more affected by caries than females (46.9%). This was similar to the study done by Goenka P, 2018 who stated that incidence of caries was 59.3% in males and, 51.7% in females[31], yet there were other studies contradicting this results such as the one conducted by Marina Ferraro et al, 2010 proved that Dental caries had female predilection and the reasons owing to differing salivary flow rates, compositions, dietary habits, hormonal changes during pregnancy, and particular variants of the AMELX gene in women.[32] Other study done by John R Lukacs et al, 2006, shows that Higher caries prevalence was among females due to earlier eruption of teeth and pregnancy pregnancy.[33] Also according to Mustafa Demirci et al, 2010 Females (59.1%) showed a higher incidence of caries than males (40.9%).[34] This lack of Similarity in the results could be attributed to unbiased data, social factors, area of study (South Indian population) considered.

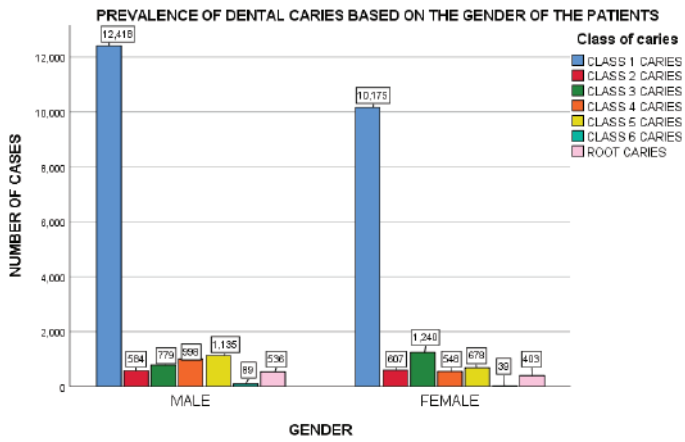
The Maximum occurance of dental caries was between 15 to 30 years (45.4%), followed by 30 to 50 years (33.7%) and least among 50 to 90 years(9.4%) of age, this proves that prevalence is higher in adolescents and young adults. This was in concordance with the study done by Mustafa Demirci et al, 2010 who stated that prevalence of Caries was most common among individuals aged between 17 to 25 years.(34) And according to Reifur KD et al, The prevalence of dental caries were higher among adolescents that ranged from 59% to 90.4%.[35] This was in concordance with the results of Subha poorani et al, 2015, who concluded that the incidence of dental caries in the age-group of 36-45 years to be 63.4%.[36] The reasons for higher prevalence of dental caries among adolescents were attributed to increased consumption of sugary beverages and foods, low home water fluoridation, lower parental education. It is also narrated that pulp stone occurring in adolescents is significantly associated with carious teeth, which suggests a causative relationship of chronic pulp irritation leading to the formation of pulp stones.[37] Thus with this study, it can also be concluded that higher emphasis had to be made regarding the purpose of fluoridation and consumption of carbohydrates among children and young adults.

On commenting about the frequency of dental caries in different tooth and tooth surfaces it could be analysed that the occurrence of dental Caries was higher in primary and permanent posterior teeth (72.6%). This result is in concordance with the study conducted by Mustafa Demirci, 2010, who stated that molars were the most significantly affected by dental caries at 45%.[34] Generally human teeth present anatomical details related to their function during mastication with elevations, depressions, convexities and concavities. Grooves and depressions in the teeth are always the most significant anatomical landmarks of concern for dental caries, along with occlusal contact points. The sulci of the human teeth are V- shaped, thus impaction of food and debris are not uncommon, contributing to microbial colonisation. Especially in molars the anatomical landmarks like the mesial and distal surfaces below the contact points, grooves, fissures, sulci, cervical thirds, triangular and central fossa are the major areas for dental caries.[38-39] Thus, regular dental check up and better oral hygiene practices should be emphasised by the dentists.

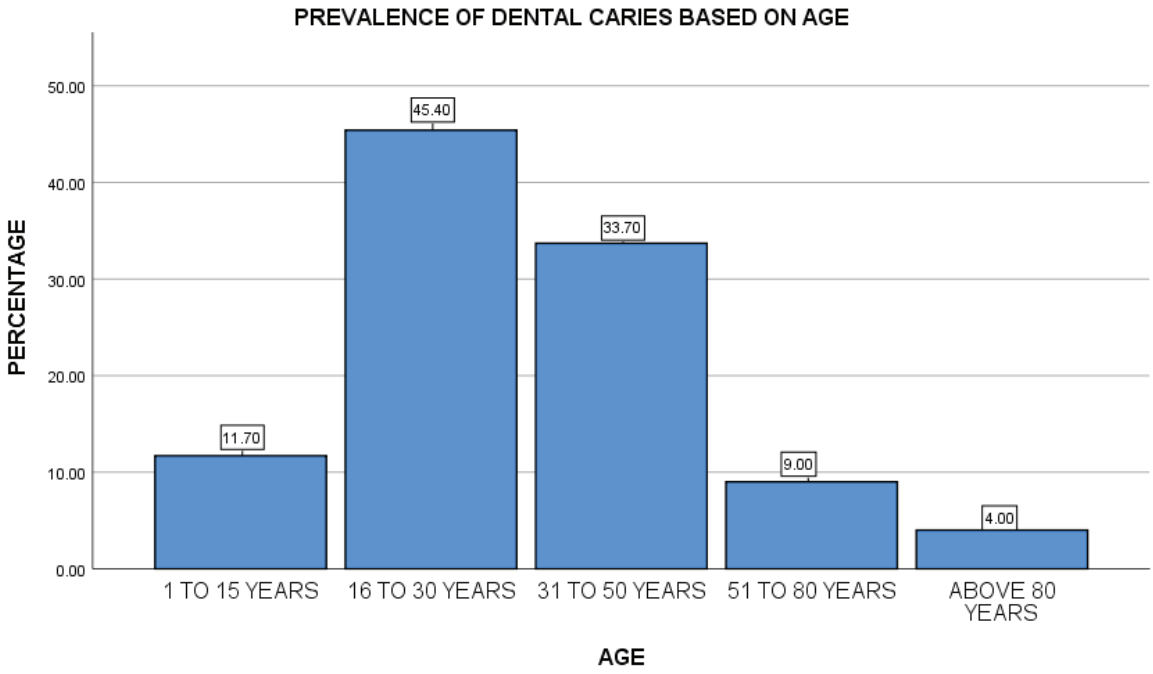
Establishing the relationship between different class of queries dental caries of class one type is higher (74.7%) followed by class three caries. And class six type of care is being the least prevalent. This result is in concordance with the one conducted by Mustafa Demirci 2010, Who stated that occlusal fissures contribute significantly to carries involvement with the frequency of 52.7% to 66.3%.[34] Also Talabani et al, 2015 concluded that Higher caries class is of class 1 Type involving occlusal surface (61.3%).[40] The reason can be attributed to easy food impaction on fissures and anatomical landmarks that results in higher class 1 type of caries and class 6 is low as debris impaction will not be higher on the Cuspal planes and in lines of the tooth. Thus, Awareness on food impaction and brushing techniques can be brought by the dentist to improve oral health and to avoid formation of periapical granulomas with further caries progression.[41] This was also quoted by Gheena sugumaran et al, in 2015 who stated that Cumulative analysis exposed a lacunae in the awareness of oral hygiene and knowledge regarding oral health thus, implying an urgent need for awareness initiative for oral health at the grassroots level in primary educational institutions.[42]

Limitations of this study include Geographic limitation as predominantly South Indian population were only considered, and was a Unicentric study with few Incomplete and unclear data. The Future scope of this study will yield a better and more accurate result when Different ethnic populations are considered.

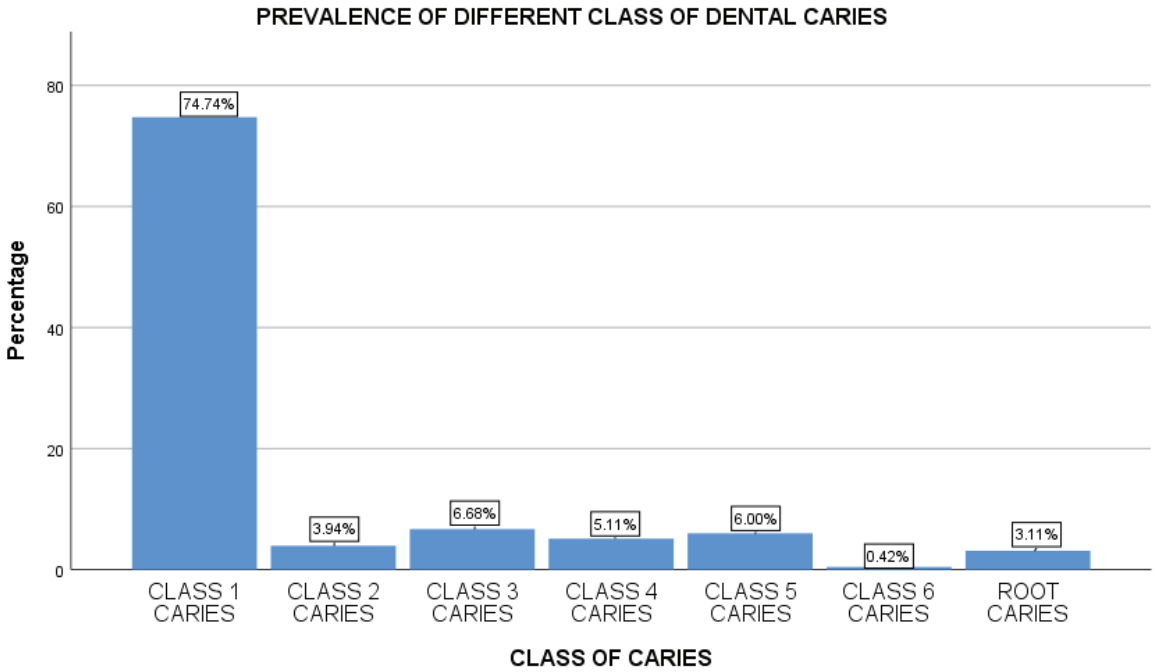
With the current study as a platform, a newer classification of dental caries according to its incidence based on age, gender, class type can be formulated to enable dentists gain a thorough knowledge on susceptibility groups and will also aid in improving general public oral health.



Graph 1 : Bar graph showing the correlation between the gender of patients and different class of dental caries. Class 1 caries are represented in blue, class 2 caries in red, class 3 caries in green, class 4 caries in orange, class 5 caries in yellow, class 6 caries in turquoise and root caries in pink. The X axis represents the frequency of dental caries among male and female patients and Y axis scale shows the total count of cases from 0 to 12,000. From the graph it is evident that all the class of dental caries except class III were highly prevalent among male patients and class III dental caries had slight female predilection. This finding is statistically significant. (Pearson Chi-SquareValue:347.5 ;p=0.000- statistically significant).



Graph 2: Bar graph showing the Prevalence of dental caries based on age. Dental caries are represented in blue. The X axis shows caries prevalence among different age groups and Y axis scale shows the percentage of dental caries from 0 to 100. From the graph it is evident that dental caries were prevalent highly among the patients of age group 15 to 30 years. This finding is statistically significant. (Pearson Chi-SquareValue:12704.2 ;p=0.000- statistically significant).



Graph 3 : Bar graph showing the Prevalence of different classes of dental caries. Dental caries are represented in blue. The X axis shows the prevalence of different classes of caries and the Y axis scale shows the total percentage of dental caries from 0 to 100. It is evident that dental caries of class 1 type are higher than other class of caries followed by class III dental caries while class VI has had the least occurrence.

Conclusion

Within the limitation of the present study, caries had male predilection, predominantly between the age group of 15 to 30 years, primarily reported in molars and in majority was class 1 type. Thus, as a clinician, it is important to evaluate every surface of the tooth while examining the oral cavity and better brushing techniques and awareness on oral health should be brought by dentists to maintain public oral health.

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Conflict of Interest: Nil.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Assessment of Partial Edentulism Based on Kennedy's Class I - A Retrospective Study

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Abstract

Tooth loss is mainly due to the incidence of carious lesions or teeth, periodontal diseases. The aim of the study was to evaluate the edentulous status and assessment of Kennedy's class I in patients visited to saveetha dental college for the last 3 months. Records of 86000 patients who visited saveetha dental college between June 2019 and March 2020 were reviewed and the data for the present study was segregated from Nov 2020- Jan 20120 with the input data being the presence of kennedy's class 1 findings. Data collected was calculated, tabulated, analysed and compiled using the SPSS statistical software. The prevalence of partial edentulism based on kennedy's class I findings was 14.3%, was more in old aged individuals above 60 years with a frequency of 40.5% and between age 51-60 at a frequency of 35.7%. Association was done between gender, age and partial edentulism with Kennedy's class I findings in maxillary and mandibular arch.The association between age and arch partial edentulism showed there was no statistical significance since p=0.051. The association between gender and arch partial edentulism was statistically significant since p=0.021. Within the limitations of this study, the prevalence of partial edentulism based on Kennedy's class I was more in males than females and the maxillary arch was commonly affected than the mandibular arch.

Keywords: *Age, Gender, Kennedys class I, Maxilla, Mandible, Prosthodontic rehabilitation, Tooth loss*

Introduction

Tooth loss is a general phenomenon that occurs due to physical, physiological, social causes [1]. The general causes for tooth loss are mainly caries and periodontal disease and other causes like trauma, etc [2]. Partial edentulism is one or more teeth missing in the oral cavity. It can be pertaining to an arch or a quadrant. Kennedys class I partial edentulism is seen in a patient who has bilateral free-end saddles, i.e. they have missing teeth in posterior areas bilaterally. There are no further posterior teeth to the edentulous area. It is defined as a bilateral edentulous area posterior to the remaining natural teeth [3].

The major drawbacks of tooth loss to a particular individual are drifting and tilting of adjacent teeth, supra eruption of opposite teeth, altered speech, changes in facial appearance and psychological dissatisfaction [4]. Lack of confidence, weight loss and restricted dietary and social activities are some of the major impacts adversely affecting the quality of life. The various causes and patterns of tooth loss in the population aid in indicating the levels of oral hygiene, dental health awareness and the management of exodontias [5]. The level of oral health status and hygiene, indicated the treatment regimen for the patient.

Various patterns of tooth loss vary in the prevalence between various countries and geographic areas with countries and depends on demography [6]. Tooth Loss has its prevalence in the elder age groups due to various

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reasons, and has been present evidently in patients of older age groups [7]. The studies also indicate that the incidence of partial edentulism is different between males and females [8]. Males have higher incidence, due to increased exposure to different environment like working environment, social and personal habit with increased incidence to smoking habit, alcohol abuse, etc [9,10,11].

Prevalence of class I kennedy's classification was seen with lesser incidence with compared to kennedy's class II and kennedy's class III incidence[12]. This was observed both in maxillary and mandibular arches with the gender prevalence of males over females[13]. Males showed increased prevalence of partial edentulism in both arches[14].

Treatment planning for Kennedy Class I cases provides a challenge to the restoring dentist. After carefully listening to the patient's desires and fully analyzing the patient's study models, radiographs, periodontal charting, dexterity, and medical history, a successful prosthesis can be fabricated that will meet and exceed the patient's desires and will play a significant role in preserving the patient's remaining dentition[15]. The treatment modalities for kennedys class I indicated partial edentulism are replacement with removable partial dentures, precision attached removable partial dentures, implant supported rehabilitation [16].

The maintenance of bilateral arches in Kennedy's class I condition presents a challenge to the dentist treating the patients. In implant supported over dentures, to prevent displacement of the denture, precision attachments or conventional clasps have been widely used [17]. The dentures should support from the teeth, the mucosa and the underlying residual alveolar ridges. In removable partial dentures the rotational effect plays a major effect in the displacement of the denture, this cannot be completely eliminated but can be minimised by using single implants placed bilaterally in the distal part of the denture bearing area is a remedy for this problem [18]. For stabilising displacement of dentures in vertical direction, single implant placement chiefly in the first molar region can be done to stabilize the denture [19]. The cost of implants and its placement is costly and individual not able to afford treatment with implant placement and removable partial dentures, alternative treatment involves temporary partial dentures [20]. This dentures are less supporting and is a challenge for stabilization of the denture in kennedy's class I cases. For better stabilization, the prosthesis requires to be a fixed prosthesis with the presence of implants to remove all the vertical, horizontal and torsional forces exerted on the edentulous ridges [21].

This study was conducted to check the prevalence of partial edentulism based on kennedy's class with respect to age of the person and the gender of the individual [22].

Materials and Methods

The study setting consists of patients visiting Saveetha dental college for the last 3 months with Kennedy's class I findings. Also, it is a randomised study. Approval of the data was done by the institutional ethics committee, saveetha university. From the total data of 86000 patients registered from june 2019-march 2020, the sample size for the present study was analysed and retrieved from the student quota report with people undergone RPD from November 2019 to January 2020 and out of which 42 patients had positive kennedys class I partial edentulism findings. These patients were segregated and cross verified through photographs. This data was tabulated and analysed with the SPSSsoftware importing. Variable definition was done with the insertion of tables and graphic importing and illustration. Patients with incomplete data were excluded.

Analytical and statistical testing of the data was done with statistical software SPSS IBM version 20.0. The dependent variable for the subjected study is RPD (removable partial denture) and the independent variable for the study includes age and gender. This study contains a descriptive analysis method. Following software analysis, data transfer is done with SPSS version 20.0.

Results and Discussion

The prevalence of partial edentulism based on Kennedy's class I findings among partially edentulous patients was 14.3%. The prevalence of partial edentulism based on Kennedy's class I was present in most patients above 60 years with a frequency of 40.5% and between age 51-60 at a frequency of 35.7%. (Figure 3- frequency of kennedy's class I incidence based on age). The prevalence of partial edentulism based on Kennedy's class I was seen higher in the maxillary

arch with a frequency of 59.5% and a lower frequency of 40.5% in the mandibular arch. (Figure 5- frequency of kennedy's class I incidence based on maxillary and mandibular arches) The mean age with the evidence of partial edentulism in patients was 62.4 years. In males, prevalence is more in patients who are above 60 years of age. In females, evidence is higher in the 51-60 age group and the above 60 years age group and is more prevalent in the mandibular arch. (Figure 4- association between age and kennedy's class I findings, Figure 2- association between gender and kennedy's class I findings)

The study indicated that the findings were with respect and increased prevalence in the male gender [23]. Kennedys class I finding has a prevalence of male predilection with a frequency of 56% [24]. It was mainly due to the exposure of male population to different environmental consensus which leads to the incidence for caries and other causes due to demographic concerns [25]. The connection between oral health and tooth maintainability is complex [26]. There are various other reasons like economic status of the patient, etc where the patient is not in a position to afford the restorative and preventive treatment which are the causes for the extraction of the respective tooth causing partial edentulism [27,28]. The overall consensus agrees with the respective study [22]. Thus all the responses are implicated in clinical practice.

The study shows more prevalence of kennedy's class I in the maxillary arch and in the older individuals [29]. The incidence of partial edentulism was more in the maxillary arch because of the sequence of tooth eruption and there was increased incidence or carious lesions in the maxillary arch of frequency 62% [30]. Thus , this increases the chances of extraction finding in the particular tooth in the oral cavity respectively [31]. There are chances of caries incidence in the mandibular arch of 53% which is caused as a result of poor oral hygiene [32]. This causes tooth loss in the respective site of incidence of caries [33,34,35]. Thus, overall consensus agrees with the results of the study and evidence adds to the consensus to be recorded in the clinical practice.

This study is a unicentered study and the data is unclear in certain parameters. The particular data is considered among the population. The ethnicity of the population is an important factor of the patient [22]. Thus, in these cases a different set of population is considered.

The studies conducted are related to partial edentulousness patterns of tooth loss. The study helps to create an awareness of the importance of oral health and expresses the importance of oral health and the education of the maintenance of oral health. The measures to reduce tooth loss is to be introduced to cause reduced incidence of tooth loss and increased effective introduction of prosthodontic rehabilitation.

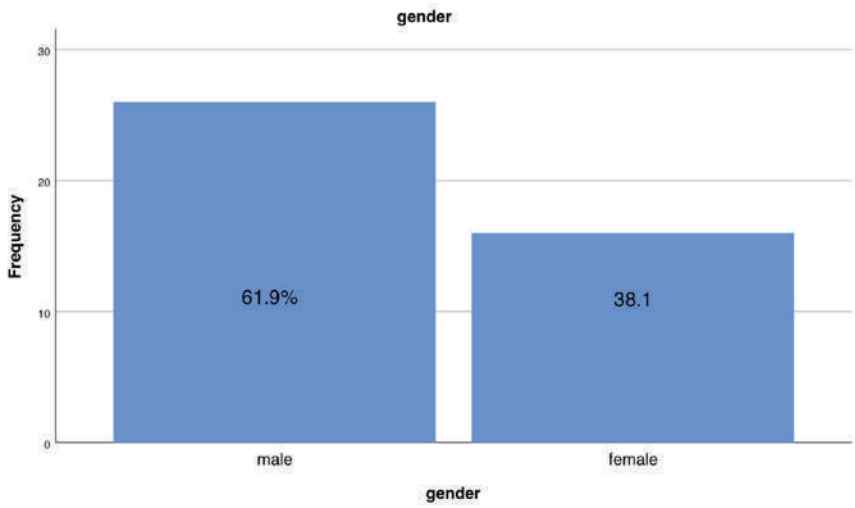


Figure 1- describes the frequency of male and female edentulism. X axis- describes the frequency of gender towards kennedys class I edentulism. Y axis- the frequency of data with respect to gender. Males showed 61.9% incidence where females showed 38.1% incidence.

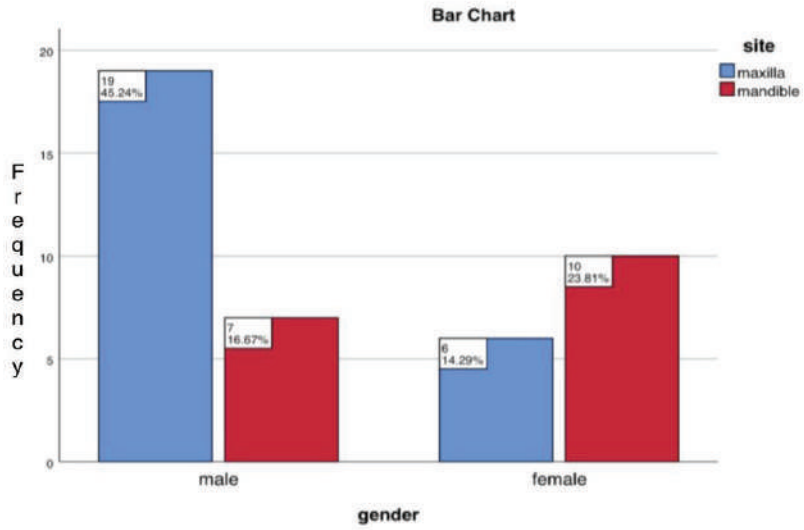


Figure 2: Describes the association between gender and partial edentulism with Kennedy's class I findings in maxillary and mandibular arch. X axis- Describes gender. Y axis- Describes the frequency of partial edentulism based on kennedy's class I in maxillary and mandibular arch. Blue colour denotes maxillary arch and red colour denotes mandibular arch. There was 45.24% incidence of kennedy's class I in maxillary arch and 16.67% incidence of kennedy's class I in the mandibular arch among male individuals and 14.29% incidence of kennedy's class I in the maxillary arch and 23.81% incidence of kennedy's class I in the mandibular arch among the female individuals. Chi-Square test was done and association was found to be statistically significant p=0.021, males had more maxillary arch partial edentulism than mandibular arch partial edentulism among kennedy's class I.

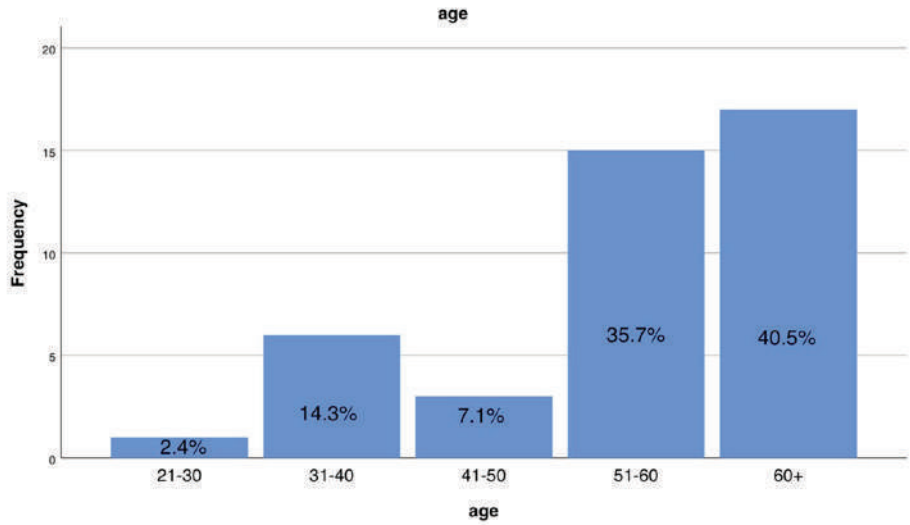


Figure 3: Describes the frequency of partial edentulism with kennedy's class I findings with respect to age. X axis - describes the frequency of age towards kennedy's class I partial edentulism. Y axis - describes the frequency of the respective data. There was an incidence of 2.4% in patients between 21-30 years, 14.3% in patients between 31-40, 7.1% in patients between 41-50 years, 35.7% in patients if age 51-60 years and 40.5% incidence in patients above 60 years of age.

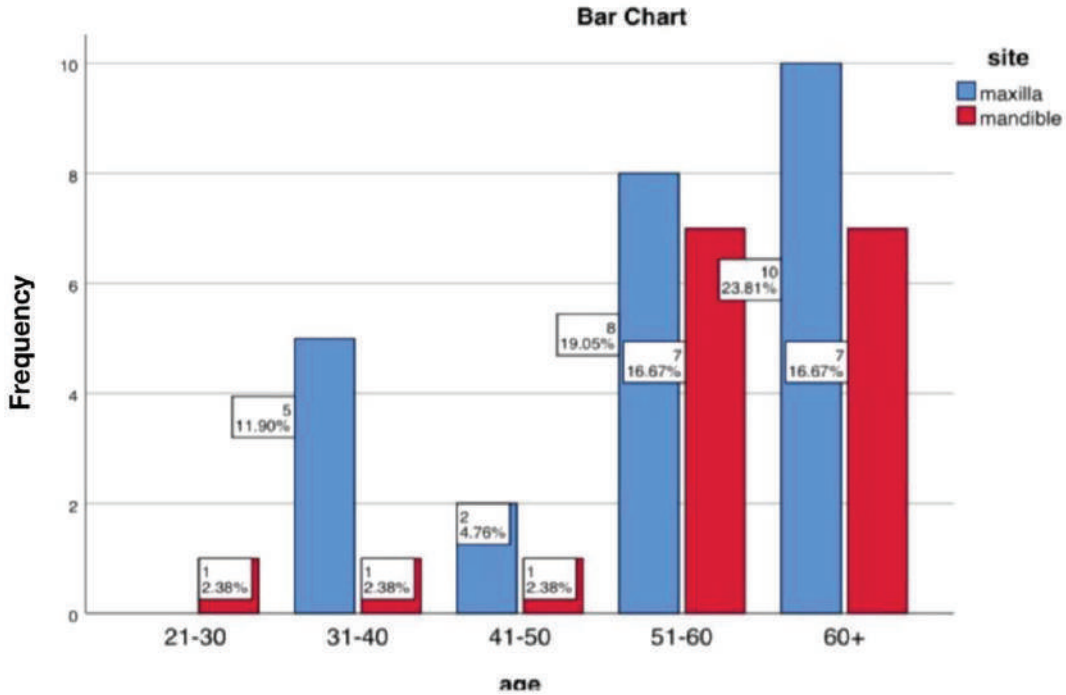


Figure 4: Describes the association between age and partial edentulism based on kennedy's class I findings in the maxillary and mandibular arch. X axis- describes the age groups. 21-30years, 31-40 years, 41-50 years, 51-60 years and 60+ years. Y axis- describes the frequency of partial edentulism based on kennedy's class I in the maxillary and mandibular arch. Blue colour denotes maxillary arch partial edentulism and red colour denotes mandibular arch partial edentulism. Chi square test was done and association was found to be statistically not significant p=0.051. Even though it was not significant, majority of the patients distributed in the age group above 60 years showed higher prevalence of partial edentulism based on kennedy's class I findings in the maxillary arch.

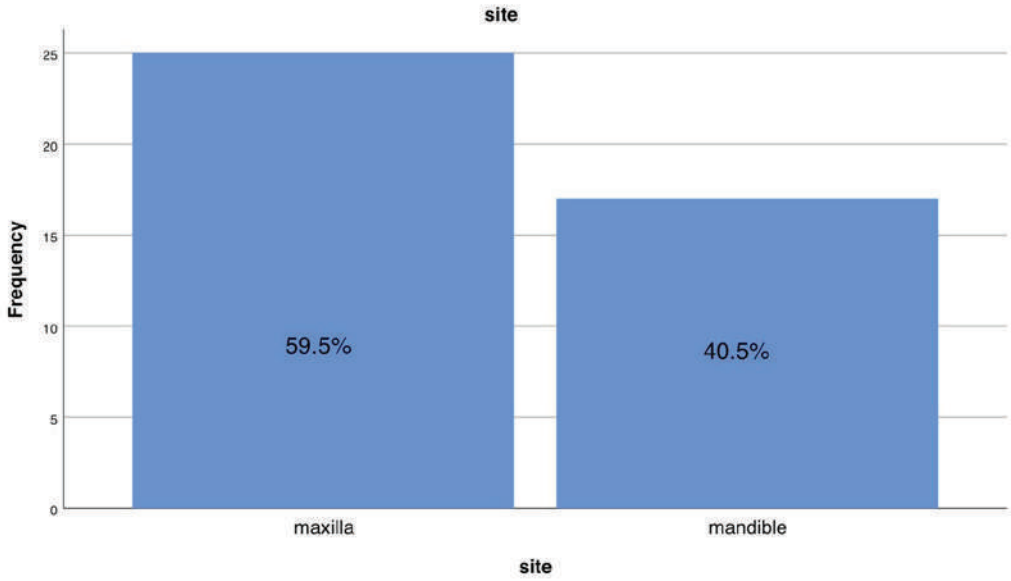


Figure 5: The frequency of partial edentulism with kennedys class I findings with respect to maxillary and mandibular arches. X axis- shows the frequency of kennedy's class I finding in relation to the maxillary and mandibular arches. Y axis- shows the frequency for kennedy's class I findings. There was an incidence of 59.5% in the maxillary arch and 40.5% incidence in the mandibular arch.

Conclusion

Within the limits of the study, male population had a high incidence of partial edentulism with kennedys class I classification and maxillary arch partial edentulism was found to be more common to mandibular arch partial edentulism.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Evaluation of Site Predilection of Alveoloplasty in Complete Denture - A Retrospective Study

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Abstract

Alveoplasty is the common pre prosthetic procedure. It is done to provide a better anatomic environment and create proper supporting structures for denture construction. The aim of the pre prosthetic surgery is to improve the better retention and stability of complete denture. Alveoloplasty is done to improve the quality and condition of the hard and soft oral aiding structures, so that they can provide retentive, supportful and stable dentures.The aim of the study is to evaluate the site predilection of alveoplasty procedure for complete denture.The Retrospective study was conducted in the University setting. Data were collected from the patients who visited Dental college. We Reviewed patient records and analysed the data of 86000 patients between June 2019 and March 2020, 120 patients data were included out of 86000 patients. The reports included Age , Gender , anatomical site of alveoloplasty done. Records on each case were examined and tabulation of the data was done in excel sheets followed by statistical analysis using SPSS software with Chi - Square test.The results were obtained as graphs and charts. The study had a total sample of 120 subjects out of which 52.5% were male and 46.7% were female. Carried out in a age group of 33 - 83 years. 35% of participants in the age group of 55 - 65 years had undergone Alveoloplasty more when compared to other age groups. 62% of Males underwent Alveoloplasty more when compared to females (38%). Alveoloplasty was performed more in lower arch (32.9%) when compared to other sites. Within the limitation of the study ,Males underwent Alveoloplasty more when compared to females. Alveoloplasty was performed predominantly more in lower arch when compared to other sites.

Keywords: *Edentulism;Preprosthetic Surgery;Residual ridge;Lower arch*

Introduction

Edentulism is a debilitating and irreversible condition and is described as the "final marker of disease burden for oral health". Complete loss of all teeth results in complete edentulism [1,2]. Smile is the key component of the self esteem of an individual [3,4]. Bone loss is an ongoing process following tooth loss, affecting the mandible four times more than the maxilla [5] Edentulism was found to have a significant effect on residual ridge resorption , which leads to a reduction in the alveolar bone and the size of the denture bearing area. This reduction affects face height and facial appearance.[6] Edentulism patients are at greater risk of cardiovascular disease [7] Edentulism may induce oral dyskinesia, defined as abnormal involuntary, patterned or stereotyped and purposeless orofacial movements [1]

Alveoloplasty is the preprosthetic surgery done to improve the quality and condition of the hard and soft oral aiding structures, so that they can provide retentive, supportful and stable dentures[8,9].It is carried out by recontouring and smoothening the alveolar arches followed by covering them with healthy soft tissue which helps in providing a stable and retentive oral prosthesis

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[10] .Alveoloplasty is the term used to describe the trimming and removal of the labiobuccal alveolar bone along with some interdental and interradicular bone and is carried out at the time of extraction of teeth and after extraction of teeth. The main objective is a) Hyperplastic replacement of resorbed ridges b) Unfavourable located frenular attachments c) Bony prominences , undercuts [11,12].

The pattern of bone loss differs in maxilla from mandible. In maxilla, the usual resorption is on the buccal and inferior portion of the alveolar ridge[13]. The pattern of edentulous bone loss results in upward and inward loss of structures [14,15]. In the anterior maxilla, there is less horizontal bone loss and posterior drift of anterior rest is seen more than in edentulous mandible[16,17]. In the posterior maxilla, there is an invented drift of posterior rest. The width of maxilla is reduced[11].The mandible is subjected to the effect of early atrophy to a much greater extent than the maxilla, and many reasons for this are postulated. Most are based on the vascular phenomena related to the changing blood supply to the mandible that occurs with increasing age, with a consequence that periosteal compression by a prosthesis results in ischemia which hastens the rate of bone loss.[18]

The purpose of this procedure can be two-fold:

1. When performed before (partial or complete) denture construction, it's used to optimize the shape of the patient's jawbone (ridge) so to avoid complications with appliance insertion, comfort stability, and/or retention

2. When performed in association with tooth extractions, it also establishes a jawbone shape that helps to facilitate the healing process that follows

3. An alveoplasty also aims to facilitate the healing procedure as well as the successful placement of a future prosthetic restoration.[19]

Some patients require minor oral surgical procedures before receiving a partial or complete denture to ensure the maximum level of comfort [20,21]. A denture sits on the bone ridge, so it is very important that the bone is the proper shape and size [22,23]. One of several procedures that might be needed to be performed to prepare the mouth for a denture include bone smoothing and reshaping, removal of excess bone, and/or removal of excess gum tissue [24,25].

However, the aim of the is to determine the evaluation of site predilection of alveoloplasty in complete denture by retrospective analysis.

Materials And Methods

The retrospective study was conducted in a hospital setting. The available data with similar ethnicity was collected from the particular geographic location. The trends in other locations were not assessed in this study setting. Ethical approval was given by the institutional ethical committee.We Reviewed patient records and analysed the data of 86000 patients between June 2019 and March 2020. About 120 patients data were included out of 86000 patients. Case sheets were reviewed and cross verification done by another examiner to avoid errors. To minimise the sampling bias all available data was included and no sorting process was done. The parameters include age, gender , the site of alveoloplasty done followed by complete denture.

These data were entered in an excel sheet and tabulated and SPSS importing was done including chi - square test. Site of alveoloplasty, complete denture, residual ridge were considered as dependent variables, Age and gender were considered as independent variables.The data was correlated with site of alveoloplasty followed by complete denture with respect to age and gender were analysed.

Results and Discussion

During the period of June 2019 - March 2020, a total of 120 subjects underwent Alveoloplasty. Out of which 52.2% of Male and 46.7% of female carried out in a age group of 33 - 83 years. The anatomical site of Alveoloplasty was included as upperI arch, lower arch and both arch. The highest percentage of participants who had undergone Alveoloplasty were the age group of 55 - 65 years (42participants, 35%). While the lowest percentage of participants aged between 77 - 83 years (4 participants, 3.3%). The other age group had similar rates like 14.2% were in the age group of 33 - 43 years ; 29.2% were in the age group of 44 - 54 years; 17.5% were in the age group of 66 - 76 years (Figure 1). The frequency distribution of gender in which 52.5% (63 participants) of Male and 47.5% (57 participants) of

female had undergone Alveoloplasty (Figure 2). The frequency distribution of anatomical site of alveoloplasty in which the highest percentage of participants had undergone Alveoloplasty in lower arch (47 participants, 39.2%); 35.8% of participants had undergone in upper arch ; 25% of participants had undergone Alveoloplasty in relation to both arch (Figure 3).

Kushal D. Gangwani et al…[26] studied with a sample of 35 subjects in which 25 were men and 10 were women. Studied with the age group of 38 - 83 years. 60 - 65 years of participants underwent Alveoloplasty more. 62% of Male had undergone Alveoloplasty predominantly more when compared to females (38%). He concluded that the goal of Alveoloplasty is to gain favourable tissue support for the designed prosthesis while conserving as much soft tissue and hard tissue as possible.Previous literature was consensus with the present study.

Nandhana S et al.. conducted a retrospective study with a sample size of 30 subjects in which 18 were male and 12 were female. Male had undergone alveoloplasty more when compared to females (56% : 46%). He concluded that ill fitting dentures and unstable prosthesis can be the outcome of not performing the Alveoloplasty immediately after post extraction. Previous literature consensus with the present study.

Medha Vivek Bhuskute… conducted a retrospective study with a sample size of 64 subjects in which 34 were Male and 32 were female. Studied with a age group of 40 - 86 years in which 58 - 65 years of participants underwent Alveoloplasty more.53% of Male had undergone Alveoloplasty in relation to lower arch. Males had undergone Alveoloplasty predominantly more when compared to females.Previous literature consensus with the present study.

Veeramalai Nadu Devaki et al….[11,27] conducted a retrospective study with a sample size of 42 subjects in which 31 participants were female and 11 participants were Male. Studied with the age group of 46 - 72 years in which 65 years of aged participants underwent Alveoloplasty more.73% of females had undergone Alveoloplasty more when compared to females.Previous literature was not in consensus with the present study.

Teeth have a major role in facial appearance, speech, and eating. There is much evidence on oral health [28]. Edentulism has a negative influence in oral health function, social behaviour and day to day activities [29]. Compromised oral function leads to self esteem and a fall in psychosocial well being [30].Edentulous people may restrict themselves from participating in social activities as they feel embarrassed to speak, smile or eat in front of others, which eventually keep them in an isolated state. In these individuals wearing dentures could improve their facial and oral appearance. It can improve social interactions which may increase their self esteem and can lead to psychological well being [31]

This study was undergone with a small sample size, hence it should be generated for larger population study. This altered response was obtained because of the absence of patterns own perception which was affected by the time of calling, social factors.In future long term research will be done with larger populations.

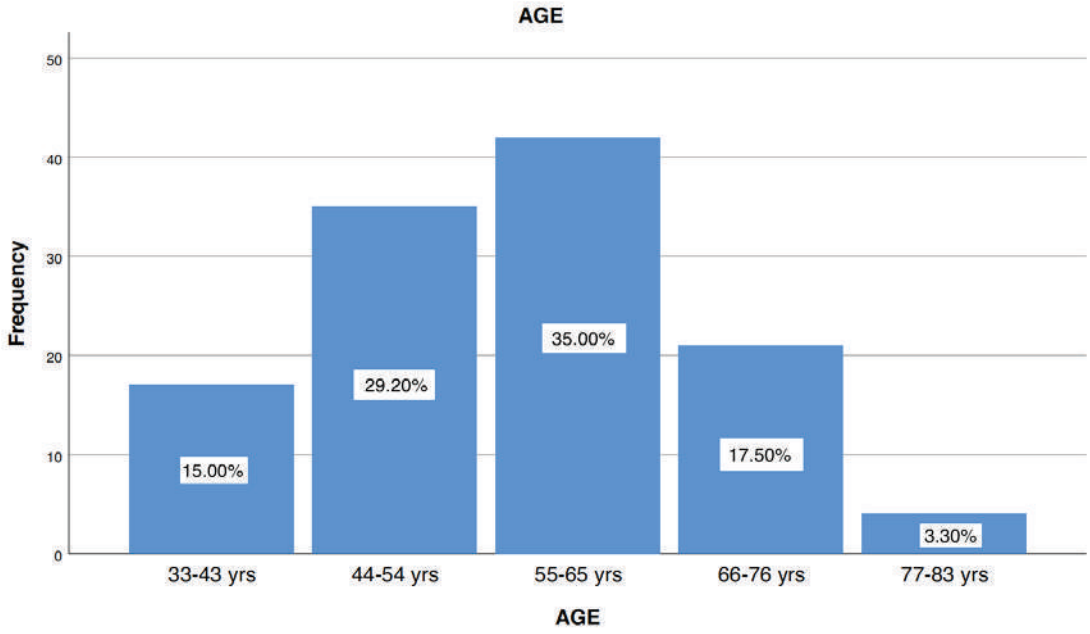
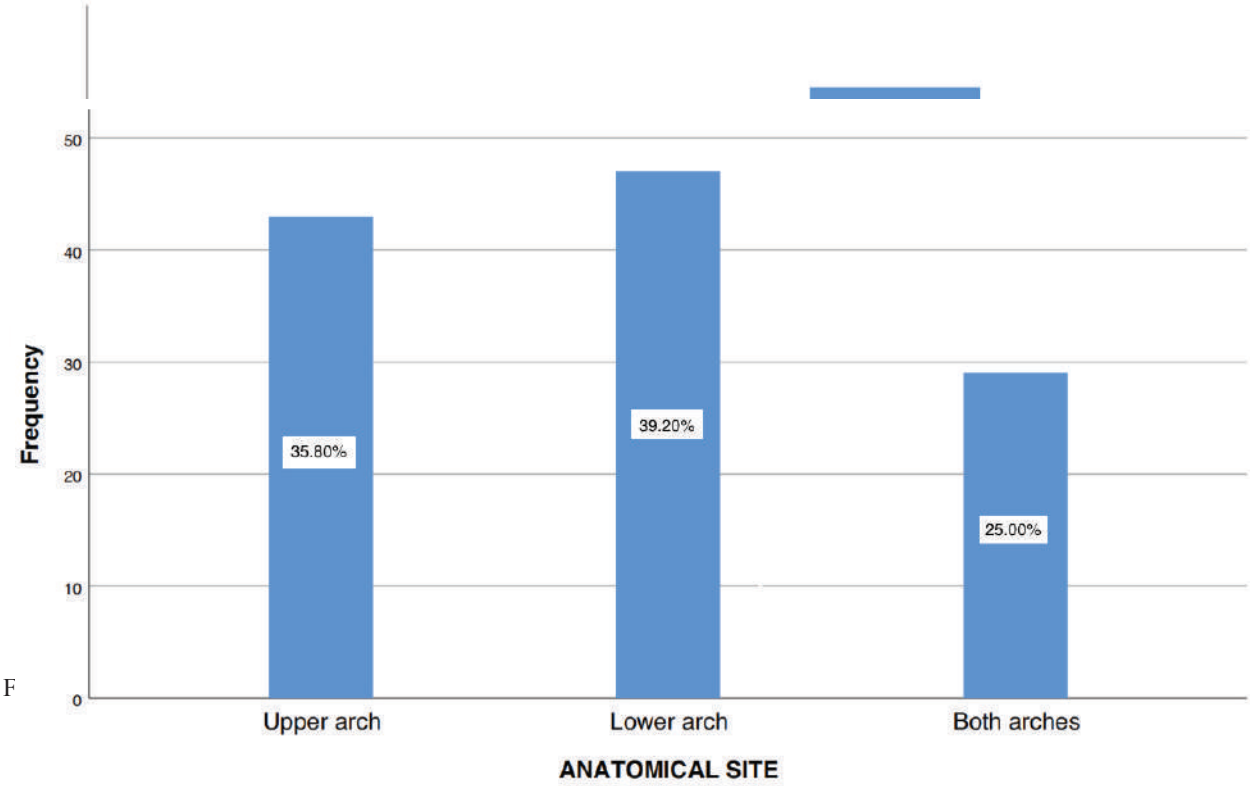


Figure 1: Bar graph represents the age group of people who had undergone Alveoloplasty. X axis represents the age group and Y axis represents the frequency of patients who had undergone Alveoloplasty. It shows that the age group of 55 - 65 years (35%) was seen predominantly more when compared to other age groups.



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Figure 3: Bar chart represents the frequency of anatomical site. X axis represents the anatomical site and Y axis represents the frequency of patients who had undergone Alveoloplasty. It shows that Alveoloplasty was performed predominantly more in lower arch (39.20%) when compared to other sites.

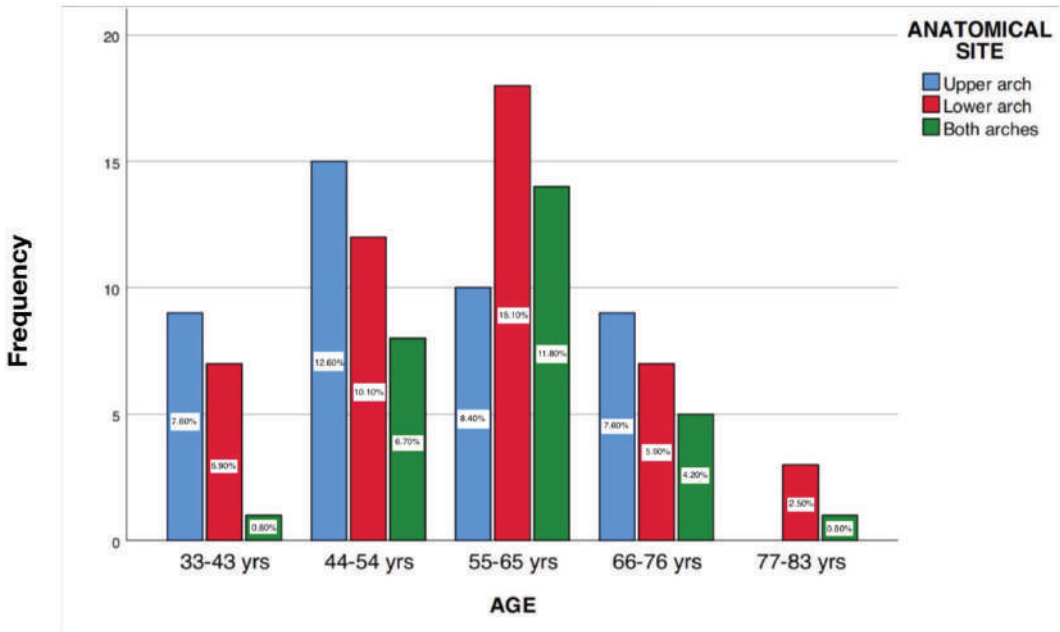


Figure 4: Bar chart represents the association between age group and the anatomical site of alveoloplasty. X axis represents the age group and Y axis represents the frequency of patients who had undergone Alveoloplasty. It shows that the age group of 55 - 65 years had undergone Alveoloplasty predominantly more with respect to lower arch (Red) when compared to other age groups, upper arch (Blue) and Both arch (Green). The association was found to be statistically not significant using chi square test (Pearson's Chi square : 10.897, DF: 8, p value: 0.208 (p>0.005)). Even though it is not significant Alveoloplasty was done predominantly more in the age group of 55 - 65 years.

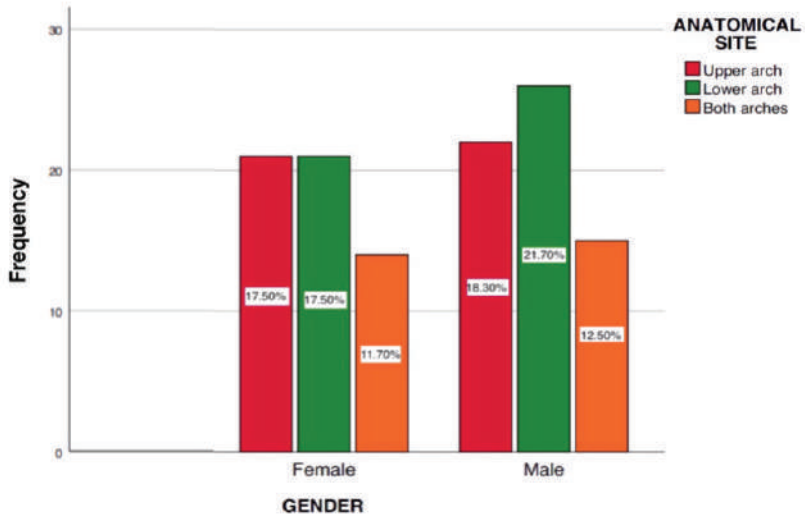


Figure 5: Bar chart represents the association between gender and the anatomical site of alveoloplasty. X axis represents the gender and Y axis represents the frequency of patients who had undergone Alveoloplasty. It shows the Males had undergone Alveoloplasty predominantly more in relation to the lower arch (21.70% - Green), upper arch (18.30% - Red) and both arch (12.50% - orange) when compared to females. The association was found to be statistically not significant using chi square test (Pearson's chi square: 12.180, DF: 6, p value: 0.621 (p>0.005)).

Conclusion

Alveoloplasty was performed more in lower arch (32.9%) when compared to other sites. Within the limitation of the study, Males underwent Alveoloplasty more when compared to females. As far as the arch was concerned alveoloplasty was performed predominantly more in the lower arch when compared to the upper arch.

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Assessment of Periodontal Status in Patients with Oral Lesions - A Retrospective Study in South Indian Population

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Abstract

Oral potentially malignant disorders (OPMD) are defined as the risk of malignancy being present in a lesion or condition either at the time of initial diagnosis or at a future date. They have the potential to undergo malignant transformation.Some of the potentially malignant disorders that are related to oral squamous cell carcinoma are Oral submucous fibrosis (OSMF), Leukoplakia, Erythroplakia, & Lichen Planus. There is a dual relationship between Oral carcinoma and periodontitis.Oral lesions may have an indirect effect on initiating periodontitis. Periodontitis can be considered as one of the risk factors for the development of oral cancer. The aim of the study is to assess the periodontal status of patients who are diagnosed with oral lesions in Saveetha Institute of Medical & Technical Sciences (SIMATS). This is a retrospective study, carried out by analysis of the patient records from June 2019 to March 2020 were assessed. Parameters assessed were - Age, Sex, Systemic status, Periodontal parameters (Periodontal probing depth - PPD, Loss of Attachment - LOA, Bleeding on Probing - BOP), periodontal status (Healthy gingiva, Gingivits, Periodontitis) & Severity of periodontitis (Mild, moderate & severe). Data was analysed using SPSS version 23.0. Chi square test was done to compare the differences in proportion. A total of 192 subjects were included in the study. 93 patients (48.4%) were diagnosed with OSMF, whereas 99 patients (51.6%) were diagnosed with Leukoplakia. Males constituted 93.75% of the study population and females constituted 6.25% of the study population. Age group of the study population ranged from 20 years to 80 years. Mean age of the study population is 42.25 years. 51.04% of the patients were diagnosed with gingivitis, 43.23% of the patients were diagnosed with periodontitis & 5.73% of the patients had healthy gingiva. Chi square test results showed statistically significant association between oral lesions and habits, with a p value of 0.000. Leukoplakia was found to be more prevalent among smokers & OSMF was found to be more prevalent among patients who had the habit of chewing Areca nut, Hans, Mawa and Pan. However, there was no statistically significant difference between oral lesions based on periodontal status & severity of periodontitis.Oral lesions can be considered as an attributing factor for development of periodontitis.

Keywords *- Leukoplakia; Oral lesions; Oral submucous fibrosis; Periodontitis; Premalignant lesion.*

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Introduction

Oral carcinoma is a public health problem in many countries [1]. About 3,00,000 new cases of oral cancer are reported every year worldwide and the majority of the cases are detected in developing countries. Around one third of the cases are seen in India. The number of cases has been increasing over the few years in developed countries [2]. About 90% of the oral cancers are reported as squamous cell carcinoma. Oral potentially malignant disorders (OPMD) can develop into oral squamous cell

carcinomas (OSCC). The term OMPD includes around 20 oral lesions and conditions that have the potential to undergo malignant transformation [3]. Some of the potentially malignant disorders that are related to oral squamous cell carcinoma are Oral submucous fibrosis (OSMF), Leukoplakia, Erythroplakia, & Lichen Planus [4]. OSMF is most prevalent in the Southeast Asian countries, ranging from 3.2 – 17.6% [5]. Leukoplakia, Erythroplakia & OSMF have potential risk factors including arecanut, slaked lime, tobacco and alcohol [6]. These habits are also risk factors for development of oral squamous cell carcinoma. Several other factors that are associated with oral cancer include Human Papillomavirus infection, stress and low socioeconomic status [7–9]. Poor oral hygiene, Periodontitis and chronic irritation caused by dentures or other appliances are considered to be risk factors for OSCC [110].

Periodontitis is a chronic inflammatory oral disease caused by gram negative bacteria residing in the tooth supporting structures [11–14]. It is a multifactorial disease, and is influenced by several risk factors [15]. The disease progresses as a result of host immune response to bacteria, producing breakdown of connective tissue and alveolar bone [16–18]. The endotoxins released by the subgingival flora acts as a prerequisite for periodontal destruction. They stimulate the host immune cells like the polymorphonuclear neutrophils (PMNs), macrophages, B & T lymphocytes to produce cytokines, proteolytic enzymes, and acute phase proteins that mediate tissue destruction [19–21]. Consequences of periodontitis include loss of tooth, loss of function and unpleasant esthetics [22,23]. Over the past few decades, several advancements in clinical and radiographic diagnosis are in practice [24]. Early diagnosis and treatment planning of periodontitis is of primary importance, as it eventually leads to tooth loss [25].

Research has proved the association between periodontitis and oral carcinoma, signifying an immune – inflammatory pathway [26,27]. Specific transcription factors act as a link between inflammation and cancer. These factors, on stimulation, have the ability to increase the expression of genes that are involved in production of inflammatory mediators and proliferation of cancer cells. Chronic inflammation activates certain cellular pathways that cause initial cell transformation and cancer progression. On persistent accumulation of cytokines, chemokines, and reactive oxygen species, the inflammatory mediators activate oncogenes and inactivate tumor suppressor genes that lead to uncontrolled cellular proliferation. This may produce genetic alterations that increase the risk of cancer development [28,29]. Tezal et al found that potentially malignant oral disorders are involved in immuno inflammatory pathogenesis that could possibly be associated with periodontitis [30]. Another study done in Pomerania showed that leukoplakia is associated with gingival inflammation [31].Considering the above reasons the present study was conducted to examine the periodontal status of the patients with OSMF & Leukoplakia in the South Indian population.

Materials & Method

This is a retrospective study, carried out by analysis of the patient records from June 2019 to March 2020 were assessed. The study design was reviewed and approved by the Ethical Committee of Saveetha Institute of Medical and Technical Sciences (SIMATS).Data from 200 patients who were diagnosed with OSMF and Leukoplakia at SIMATS were reviewed in the study. Case sheets with incomplete data were excluded from the study. A total of 192 patients were included in the study.

Parameters assessed -

Demographic details including Patient name,Patient identity number (PID No.), Age, Sex, Habits, systemic status were recorded. Periodontal parameters including Bleeding on probing (BOP), Periodontal probing depth (PPD), Loss of Attachment (LOA), and Periodontal status (Healthy gingiva, Gingivitis, Periodontitis), Severity of periodontitis (Mild, moderate, severe periodontitis) were taken into account in this study.

Group I (n = 93) included patients who were diagnosed with OSMF, Group II (n = 99) included patients who were diagnosed with Leukoplakia. Habits were categorized into use of Areca nut, Ghutka, Hans, Mawa, Pan & Tobacco smoking. Systemic status were categorized into systemically healthy patients, and systemically compromised patients (Diabetes & Hypertension). Periodontal status were categorized into healthy gingiva, gingivitis and periodontitis.

According to Centers for Disease Control & Prevention - American Academy of Periodontology (CDC-AAP) case definition,

a) Mild periodontitis - ≥2 interproximal sites with CAL ≥3 mm, and ≥2 interproximal sites with pocket depth ≥ 4 mm (not on same tooth) or one site with pocket depth ≥5 mm

b) Moderate ≥ 2 interproximal sites with CAL ≥ 4mm (not on the same tooth) or ≥ 2 interproximal sites with pocket depth ≥ 5mm (not on the same tooth).

c) Severe ≥ 2 interproximal sites with CAL ≥ 6mm (not on same tooth) and ≥ 1 interproximal site with pocket depth ≥ 5mm [32 33].

Statistical Analysis

The data obtained analysed using statistical software SPSS version 23.0 (Statistical Package For The Social Sciences). Chi squared test was performed to assess the relationship of oral lesions with gender, habits,periodontal status and severity of periodontitis. A difference was considered to be of statistical significance if $p < 0.05$.

Result & Discussion

A total of 192 subjects were included in the study. 93 patients (48.4%) were diagnosed with OSMF, whereas 99 patients (51.6%) were diagnosed with Leukoplakia (Figure 1). Males constituted 93.75% of the study population and females constituted 6.25% of the study population (Figure 2). Age group of the study population ranged from 20 years to 80 years (Figure 3). Mean age of the study population is 42.25 years. 51.04% of the patients were diagnosed with gingivitis, 43.23% of the patients were diagnosed with periodontitis & 5.73% of the patients had healthy gingiva (Figure 4).

Chi squared test was performed to assess the relationship of oral lesions with gender, habits,periodontal status and severity of periodontitis. Association between oral lesions and habits was found to be statistically significant with a p value of 0.000. Hence,from figure 5 it is evident that Leukoplakia was found to be more prevalent among smokers & OSMF was found to be more prevalent among patients who had the habit of chewing Areca nut, Hans, Mawa and Pan. Figure 6 shows that association between oral lesions and periodontal status was found to be statistically insignificant. Chi square test results showed no statistically significant difference between oral lesions based on severity of periodontitis (Figure 7).

Periodontitis is a common oral disease caused by bacterial infection, leading to irreversible attachment loss, alveolar bone loss and eventually to tooth loss. It is a multi-factorial disease influenced by several risk factors like smoking, diabetes, socioeconomic status, oral hygiene, and genetic susceptibility [34]. The extent, severity and course of the periodontal disease is dependent on these risk factors. Periodontitis is related to various oral pathological conditions including pre-malignant and malignant lesions.Smoking is the most important risk factor for oral leukoplakia and for periodontitis [35]. Periodontal sites are commonly involved in proliferative types of leukoplakia [36]

Recent research has focussed on the molecular and cellular pathways that act as a link between inflammation and cancer [37]. Two pathways link inflammation and cancer. In the intrinsic pathway, oncogenes that enhance the expression of inflammatory mediators and produce an inflammatory environment are activated. Whereas the extrinsic pathway drives the inflammatory conditions which predispose to cancer. Transcription factors like nuclear factor kappa Beta (NF-kB), and cytokines like Tumor necrosis factor and other chemokines act as important orchestrators for both intrinsic and extrinsic pathways [38]. Thus, inflammation is a key component of the tumour microenvironment.

Mean age of the study population is 42.25 years. This finding is similar to that of the study by N Prakash Rai et al, where the mean age was 47.7 years [39]. This is not in accordance with the study by Rathod S et al, where the mean age was 35 years [40]. This result was opposing to the findings of the studies by Shenoy & Hedge, Bansal et al, Sinor et al & Gaszynska et al, where the mean age was 71.2 years, 70.49 years, 71.2 years, 75.5 years respectively. All these studies focussed on geriatric population [41–44].

In the present study, Males constituted 93.75% of the study population and females constituted 6.25% of the study population.This finding is opposing to the study by Sajankumar RP et al, where 57.03% of the study

population were males and 42.97% of the population were females.[32] It was opposing to the study by Shenoy RP et al, where 54.9% & 45.1% of the participants were males and females respectively[41]

In the current study, the percentage of OSMF was 48.4% & leukoplakia was 51.6%. However, studies conducted by Tang et al & Yang et al, showed that OSMF was present in 5.49% & 23.2 % of the study population respectively [45,46]. This lower prevalence is because smaller communities were studied (Aborigines of Taiwan & Elderly Venezuelan population).

In this study, 4.17% of the study population, belonging to the OSMF group had healthy gingiva & 1.56% of the study population, belonging to Leukoplakia group had healthy gingiva (Figure 6). These findings are opposing to the study by Sajankumar RP, where no patient had a healthy gingiva [32]. Whereas in Shaheen et al, Agrawal et al & Mary et al studies, 0.91 % of the patients had healthy gingiva. [47–49].

Results from the present study shows that 40% of the patients with OSMF and 51% of the patients with Leukoplakia were diagnosed with periodontitis. There are several reasons for the association between malignant lesions of oral cavity and periodontal disease. Periodontal pathogens have the ability to convert nitrate into nitrite or acetaldehyde [50]. Evidence suggests that in patients with periodontal infection, there is production of carcinogenic metabolites. Increased level of mediators and inflammatory markers are considered to be associated with development of tumors. Compromised epithelial barrier in periodontitis allows the carcinogens produced by alcohol and tobacco consumption to penetrate the underlying tissues [27]. According to Nourelahi M et al, the rate of occurrence of oral malignancy is significantly related to tooth loss and other periodontal parameters such as clinical attachment loss and alveolar bone loss. Hence, periodontal disease can be considered as an independent risk factor for oral cancer [51] .

Limitations and future scope -

Further longitudinal studies are needed to investigate the periodontal status in patients with oral lesions with larger sample size. Dose dependent relationships between the habits, oral lesions & periodontitis should be studied. Effect of treatment of oral lesions on periodontal status should be assessed in future studies.

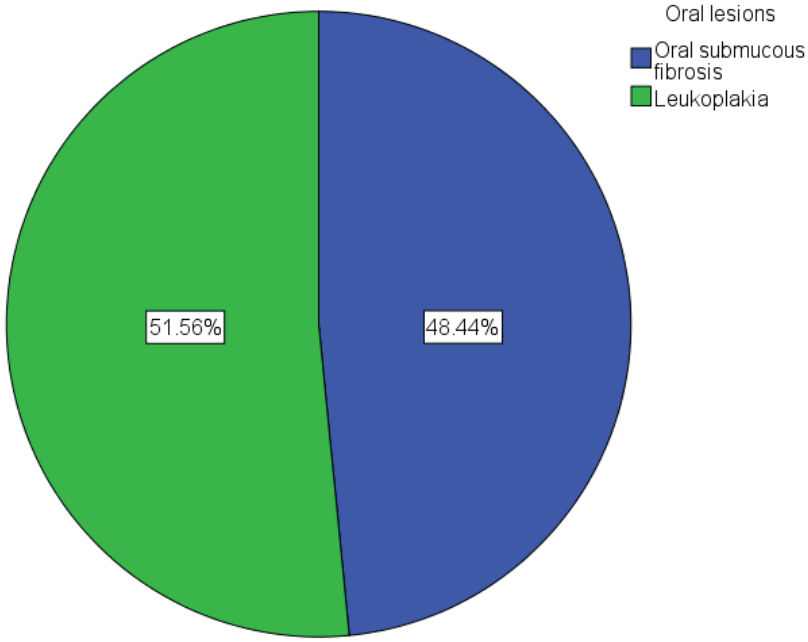


Figure 1 : Pie chart representing distribution of the oral lesions in the study population. Blue denotes Oral submucous fibrosis (OSMF) and green denotes Leukoplakia. 51.56% of the study population were diagnosed with leukoplakia and 48.44% of the patients were diagnosed with OSMF.

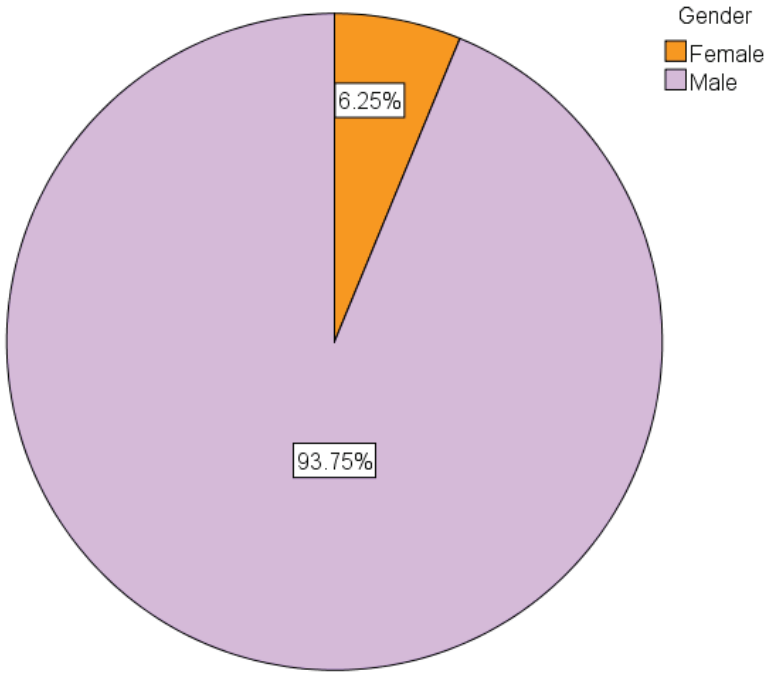


Figure 2 : Pie chart representing distribution of the gender in the study population. Purple denotes males and orange denotes females. 93.75% of the study population were males and 6.25% of the participants were females.

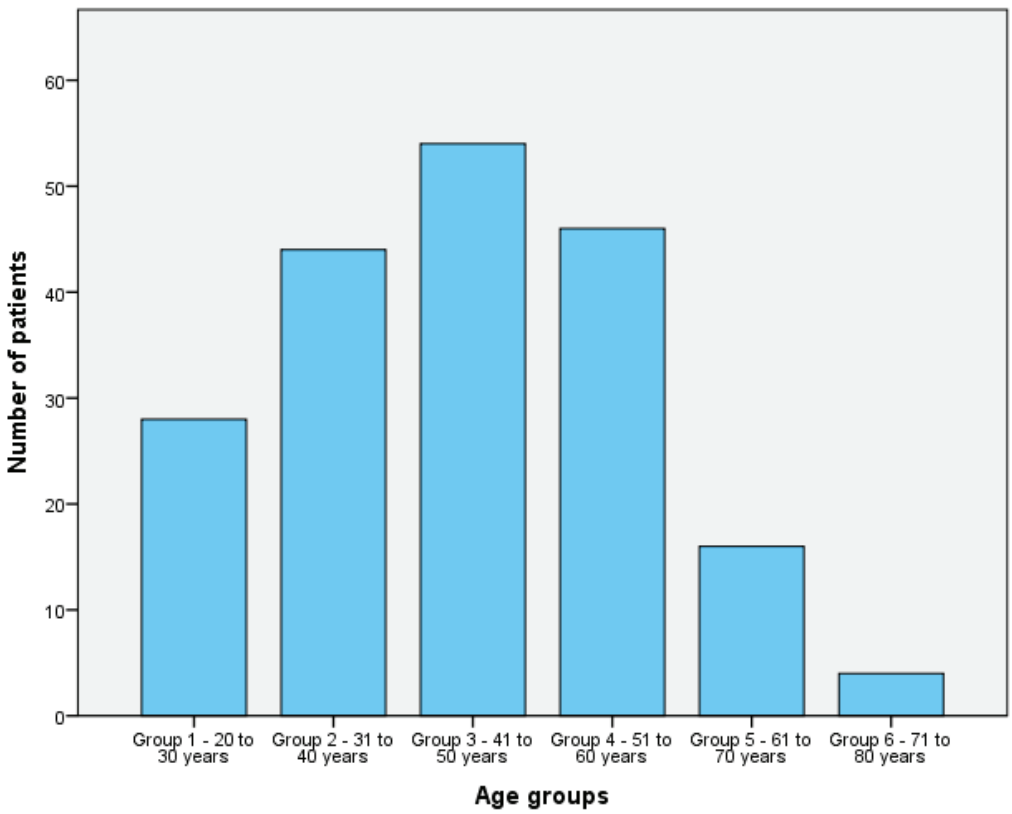


Figure 3 : Bar graph showing age groups of the study population. X axis represents the different age groups (20 - 30 years, 31 - 40 years, 41 - 50 years, 51 - 60 years, 61 - 70 years & 71 - 80 years) & Y axis represents the number of patients. Maximum patients of the study population belonged to the age group of 41 to 50 years.

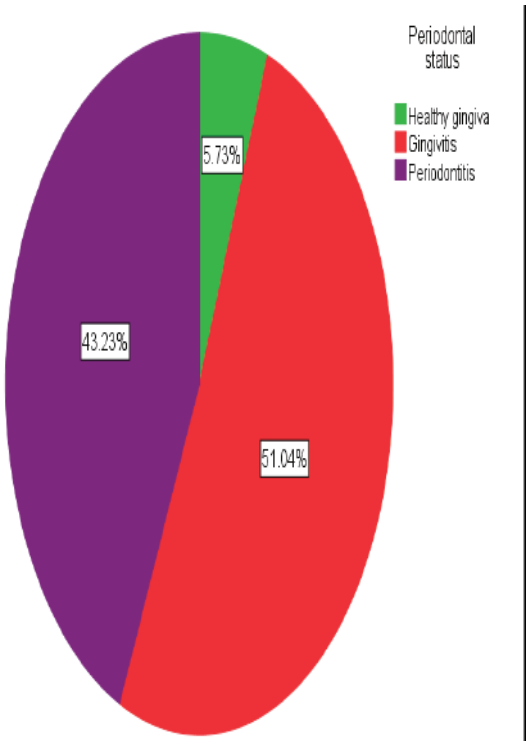


Figure 4: Pie chart representing distribution of periodontal status of the study population. Green denotes healthy gingiva, red denotes gingivitis and purple denotes periodontitis. 51.04% of the patients were diagnosed with gingivitis, followed by periodontitis (43.23%) and healthy gingiva (5.73%).

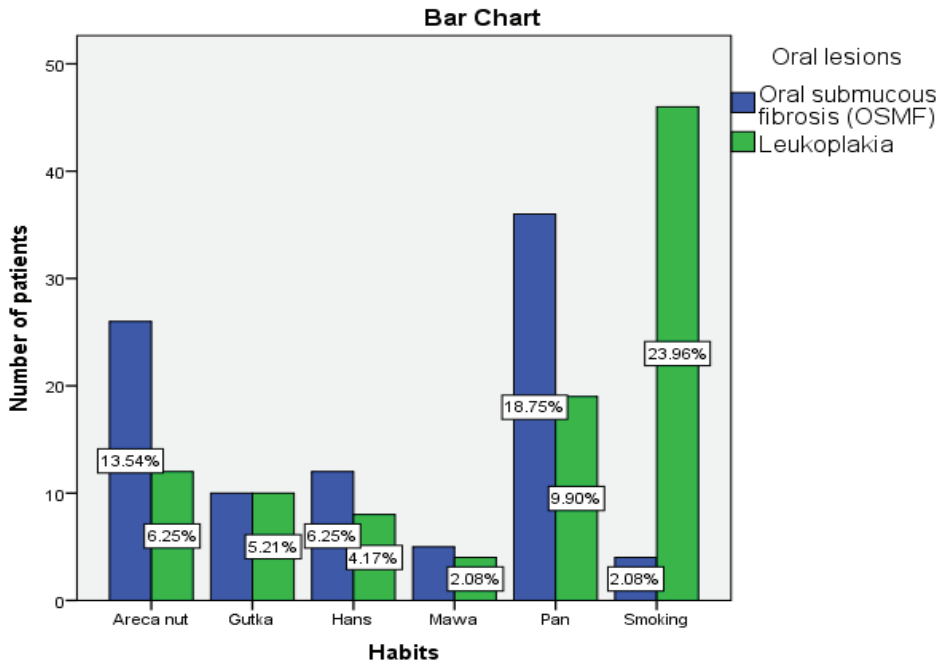


Figure 5: Bar graph depicting the relationship between the Oral lesions and habits of the patients. X axis represents the habits of the patients and Y axis represents the number of patients. Blue denotes Oral submucous fibrosis (OSMF) and green denotes Leukoplakia. Chi square test was done and association between oral lesions and habits was found to be statistically significant. (Pearson's Chi square value : 46.46, df - 5, p value - 0.000 - significant). Therefore, Leukoplakia was found to be more prevalent among smokers & OSMF was found to be more prevalent among patients who had the habit of chewing Areca nut, Hans, Mawa and Pan.

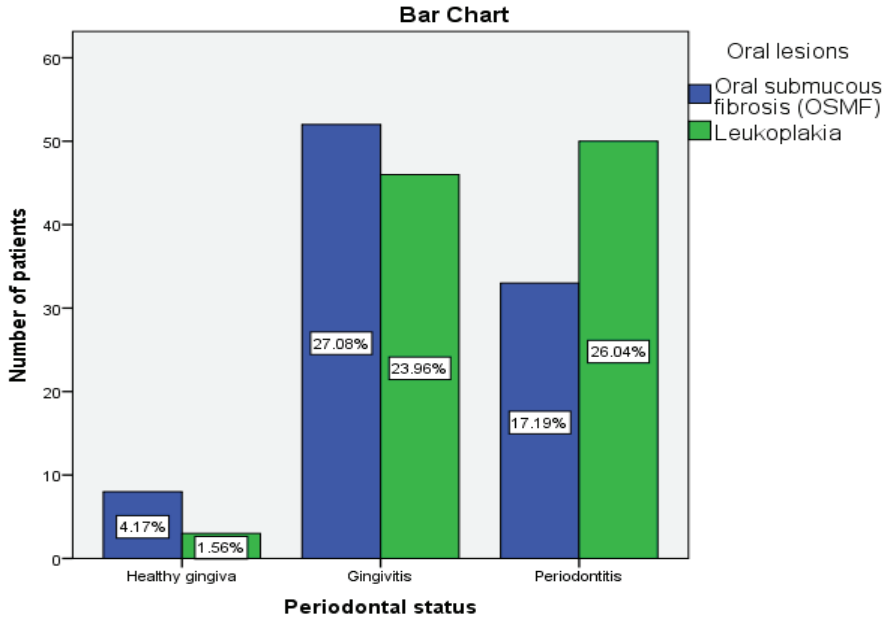


Figure 6: Bar graph depicting the relationship between the Oral lesions and periodontal status of the patients. X axis represents the periodontal status of the patients and Y axis represents the number of patients. Blue denotes Oral submucous fibrosis (OSMF) and green denotes Leukoplakia. Chi square test was done and association between oral lesions and periodontal status was found to be statistically insignificant. (Pearson's Chi square value : 5.7, df - 2, p value - 0.051 - insignificant). However there was no statistically significant difference between oral lesions based on periodontal status, the oral lesions were more prevalent in the gingivitis and periodontitis group when compared with patients who had healthy gingiva.

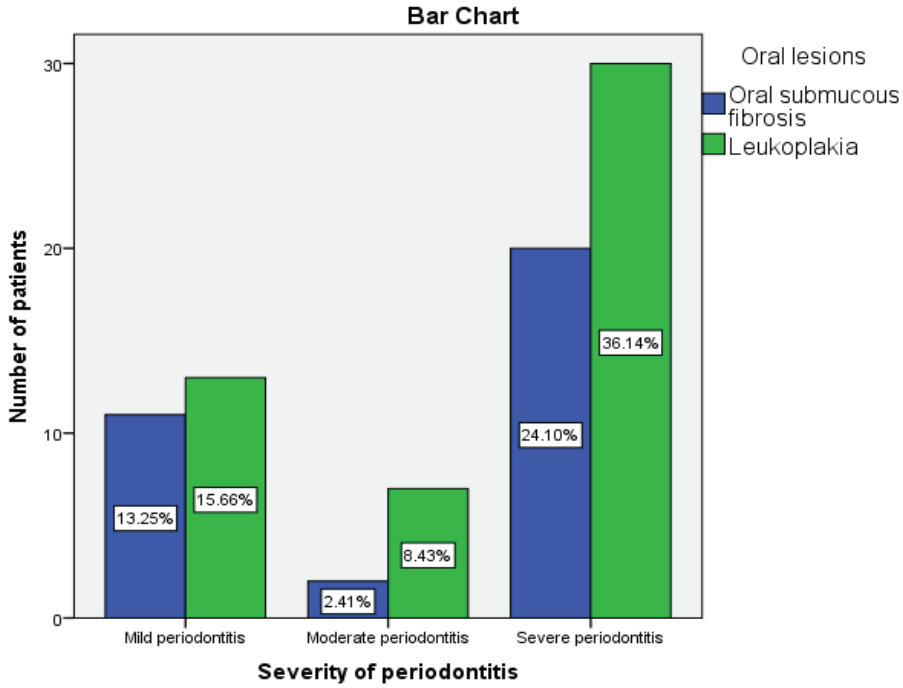


Figure 7: Bar graph depicting the relationship between the Oral lesions and severity of periodontitis. X axis represents the mild, moderate and severe periodontitis and Y axis represents the number of patients. Blue denotes Oral submucous fibrosis (OSMF) and green denotes Leukoplakia. Chi square test was done and association between oral lesions and severity of periodontitis was found to be statistically insignificant. (Pearson's Chi square value : 1.52, df - 2, p value - 0.466- not significant). However there was no statistically significant difference between oral lesions based on severity of periodontitis, the severe periodontitis group had more number of oral lesions than patients who had mild periodontitis.

Conclusion

Based on this evidence, dentists should consider periodontal disease as an attributing factor for development of oral carcinoma. It is also advisable to provide periodontal therapy for patients with oral premalignant disorders to achieve ideal therapeutic results.

Acknowledgment - Nil

Conflict of Interest - Nil

Source of Funding - Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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The Effectiveness of Splints in Temporomandibular Joint Disorders

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Abstract

TMD is defined as a muscular and articular disorder. The primary goal in the treatment of temporomandibular joint disorder is to ease pain or mandibular dysfunction with Conservative treatments such as splint therapy, and manipulation. occlusal splint insertion and physical treatment are the most commonly used conservative alternatives through which approximately 90% of all TMD cases can be successfully treated. The aim of the study was to assess the effectiveness of splints in temporomandibular joint disorders. Sample was collected from June 2019 to March 2020 from Saveetha Dental College and Hospitals, Chennai. Data collected is tabulated in Excel and statistical analysis using SPSS is done to obtain results. Out of 61 patients, 59% were male and 45.9% were female. The mean age of the participants is 33.21 years. Within the limitations of the study, it was concluded that splint therapy was effective for 59.02% patients with temporomandibular joint disorder patients. Also, splint therapy was more effective in males (32.79%) as compared to females (26.23%).

Keywords*: TMD; Splint therapy; Myofascial pain; TMJ; Internal derangement*

Introduction

Temporomandibular joint disorder is defined as a muscular and articular disorder[1]. Temporomandibular joint is formed by condylar process of mandible and mandibular fossa in the base of the skull.This term includes anatomical, histological, and functional anomalies in the functioning of the muscular and/or articular components of the system that are accompanied by highly varied clinical signs and symptoms. Most common symptoms include discomfort, pain, and disturbances of mastication .TMD is a prevalent disorder most commonly observed in individuals between the ages of 20 and 40 years[2]. Therefore this is termed As non-specific term, temporomandibular joint disorder. .Accordingly, TMD is characterized by clinical signs of pain or malfunction occurring jointly or separately. Dental anxiety is a common problem which results in the avoidance of dental care, and is one of the factors to increase pain as patients do not seek help immediately [3,4]. Therefore immersive virtual reality is an effective distraction tool to alleviate the anxiety of the patient[5]. The characteristic signs are pain in the TMJ joint, articular sounds,pain in the muscles of mastication and anomalies in mandibular movements [6].

Oral health care environment has become a helpful setting for early detection of most of the lesions etc in patients [7,8]. Sometimes TMD can also occur due to certain pre malignant conditions such as oral submucous fibrosis that causes limited mouth opening and severe pain [9,10]. It has been observed that BTX injections can be the least invasive method, which can provide

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relief of intractable symptoms in patients who have failed to show any improvement with the conventional modalities of TMD treatment . Injecting measured doses of BTX into specific sites in the major muscles of mastication can achieve sufficient masticatory muscle relaxation, decreased pain, and improved function and mouth opening[11]. The primary goal in the treatment of temporomandibular joint disorder (TMD) is to ease pain or mandibular dysfunction [12]. It is very important for the patient to be aware of the various basic procedures and treatments carried out in order to cooperate with the dentist[13]. Most management strategies aim to eliminate symptoms.TMD treatment can be divided into two categories: conservative method and surgical method.

Conservative treatments include medication, habit modification, counseling, physical therapy, splint therapy, and manipulation[14]. NSAID Medications such as ketorolac and paracetamol are taken by patients for temporary relief due to its effectiveness [15,16]. Surgical treatments include arthrocentesis, arthroscope, arthroplasty, discectomy, and temporomandibular joint (TMJ) reconstruction [17].Orthognathic surgery involves movement of jaws in all three planes, patients having TMJ dysfunction undergoing orthognathic surgery are likely to have significantly improved signs and symptoms post surgery[18]. It is observed that sometimes temporomandibular joint disorders can occur due to maxillofacial trauma, or road traffic accidents that affect the TMJ directly or indirectly as well [19,20]. Oral splints (OS), or occlusal splints, are removable occlusal devices used for the diagnosis of TMDs as well as therapy for the dysfunction of the relationship between the mandible and the maxilla[21]. A conservative, reversible method is widely accepted to be the first choice for TMJ treatment. As one of the conservative treatments, oral splint has been frequently used for internal derangement and myofascial pain treatment [22]. As for the mechanisms of occlusal splint therapy, wearing an occlusal splint is considered to cause alterations in mechanical sensitive input arising from periodontal tissue and spindle afferents in the jaw closing muscle and decrease in intra-articular pressure in TMJ [22]. Occlusal splint is often successful, but the length of time required to reach a pain-free normal range of motion is suboptimal [23] . Although oral splints are sometimes designed to stabilize the occlusion, or prevent wear of the dentition, a common application in the management of TMDs aims to protect the TMJ discs from dysfunctional forces that may lead to disc perforations or displacements [24].

Previous studies have been done that stress on the importance of conservative splint therapy to ease pain caused due to TMD. However a number of challenges are faced while the study is conducted. According to Lee, Hye-Sung, et al, conservative splint therapy is highly effective, but these do not achieve a satisfactory outcome in some cases. A surgical method should be considered in such cases[25]. According to Adibi SS, et al [26], Gray R, et al [27], it explains about the importance and need of splint therapy however it requires a longer duration of action to accomplish a pain free state and patient cooperation is a limitation in the study. Splint therapy can only be successful if minor cases and cannot treat complicated TMD conditions.

This study aims to improve quality and to enable Increase maximum mouth opening , to limit parafunctional habits that act as a predisposing factor for TMD. The main goal Is to decrease the pain and discomfort for the patients. Appliances are often used in conjunction with other forms of treatment such as physiotherapy or medication. The aim of this study was to assess the effectiveness of splint therapy in patients with temporomandibular joint disorder based on age and gender of the patient.

Materials and Methods

Study setting

The setting to conduct the study is a university setting. This enables easy retrieval of data over a specific period of time. The study was conducted with the approval of the Institutional Ethics Committee [SDC/SIHEC/2020/DIASDATA/0619-0320]. One guide and one researcher were involved in the study.

Sampling

Data is collected from June 2019 to March 2020 and cross verification is done with photographs. Sampling bias is minimised by the inclusion of all the data available and exclusion of in appropriate data. Internal validity consists of samples from University which has proper records and gives proper recall and review results and external validity is that the results can be generalised as samples of One ethnic group is chosen.

Data collection and Statistical analysis

Data to conduct the study is obtained by reviewing patients records and analysing the data of 100 patients reporting to the institute. Excel tabulation based on age, gender, TMD and splint therapy entered. Results are generated using the statistical analysing told SPSS. Incomplete/censored data is managed by excluding it from the data. Analyses used is a descriptive analysis – mean, range with tables and charts done using statistical software SPSS. Independent variables are age, sex and dependent variables a splint therapy and effectiveness the type of analysis done is correlation an association.

Results And Discussions

A total of 61 patients were assessed. The age range was from 15 to 56 year. The details on age distribution is given in (Figure 1). The mean age of the participants was 33.21. Out of the 61 patients, 33 (54.10%) were males and 28 (45.90%) were females, as shown in (Figure 2). Moderate-quality evidence suggests that splint therapy reduced pain in the temporomandibular joint (TMJ) area . It was observed that 36 patients (59.01%) found the splint therapy to be effective while 25 patients (40.98%) did not find splint therapy completely effective, as shown in (Figure 3). Association between effectiveness of splint therapy due to TMD with age and gender of the patient was done, as shown in (Figure 4 and 5).

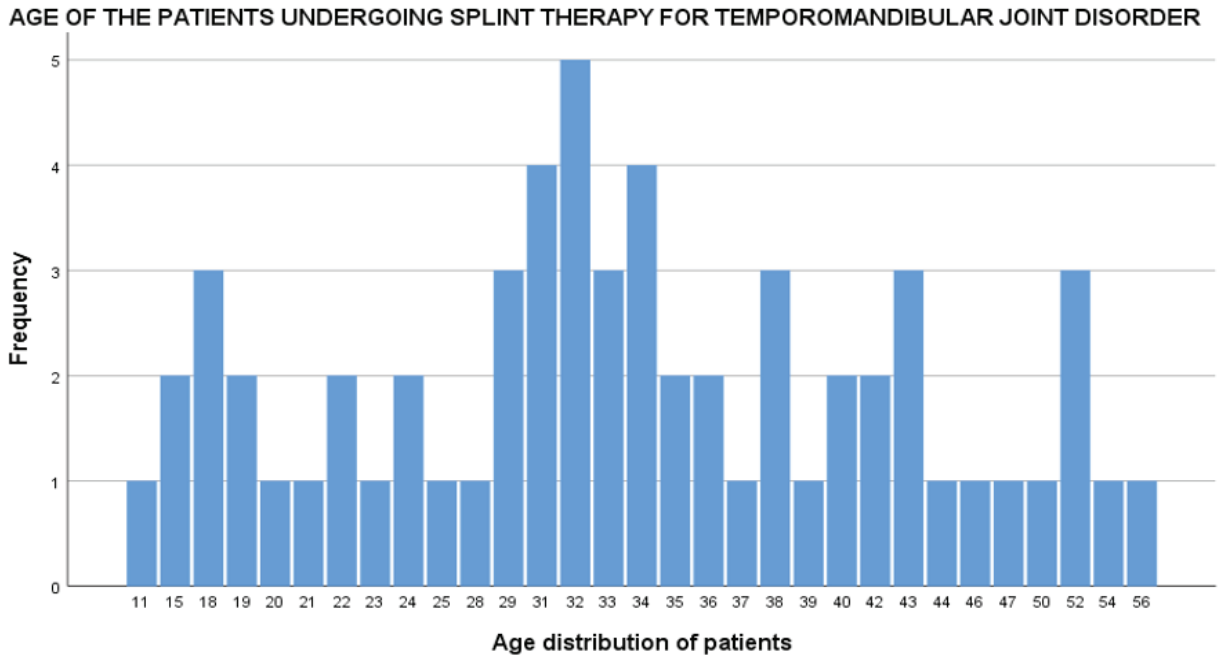


Figure 1: Bar graph showing distribution of age of the patients undergoing splint therapy due to temporomandibular joint disorder. The mean age of the participants were 33.21 years. X axis represents the age distribution of the patients and Y axis represents the number of patients in each age group.

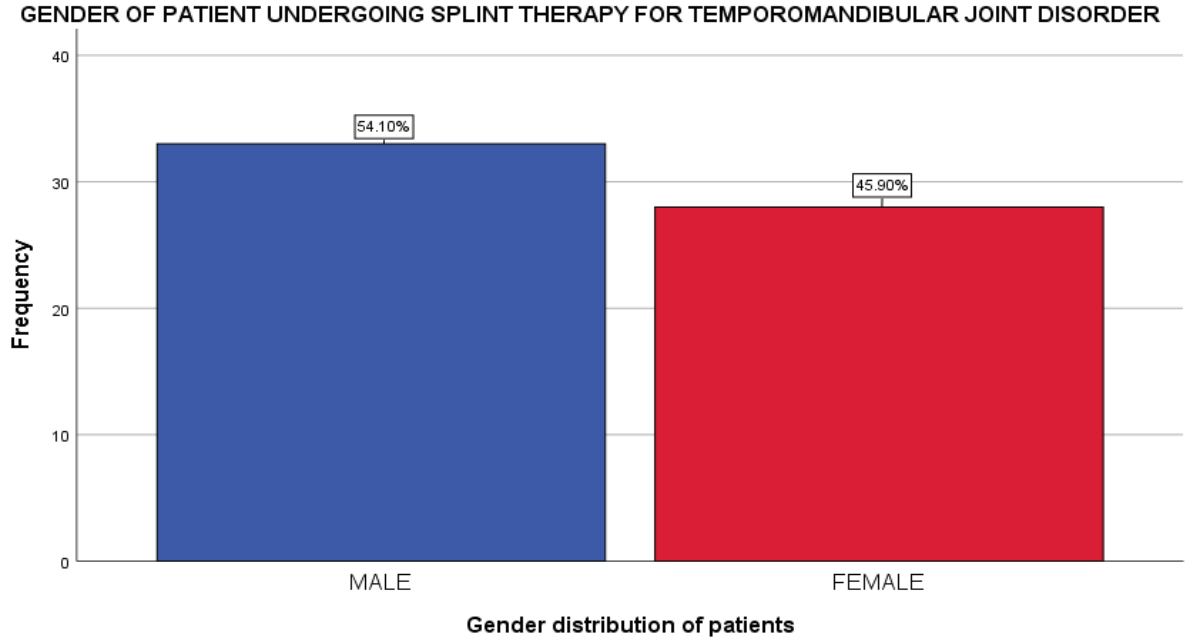


Figure 2: Bar chart showing distribution of gender of the patients undergoing splint therapy due to temporomandibular joint disorder. Blue denotes male participants with 54.1% and red denotes female participants with 45.90%. X axis represents the gender distribution of patients and Y axis represents the number of patients in each gender.

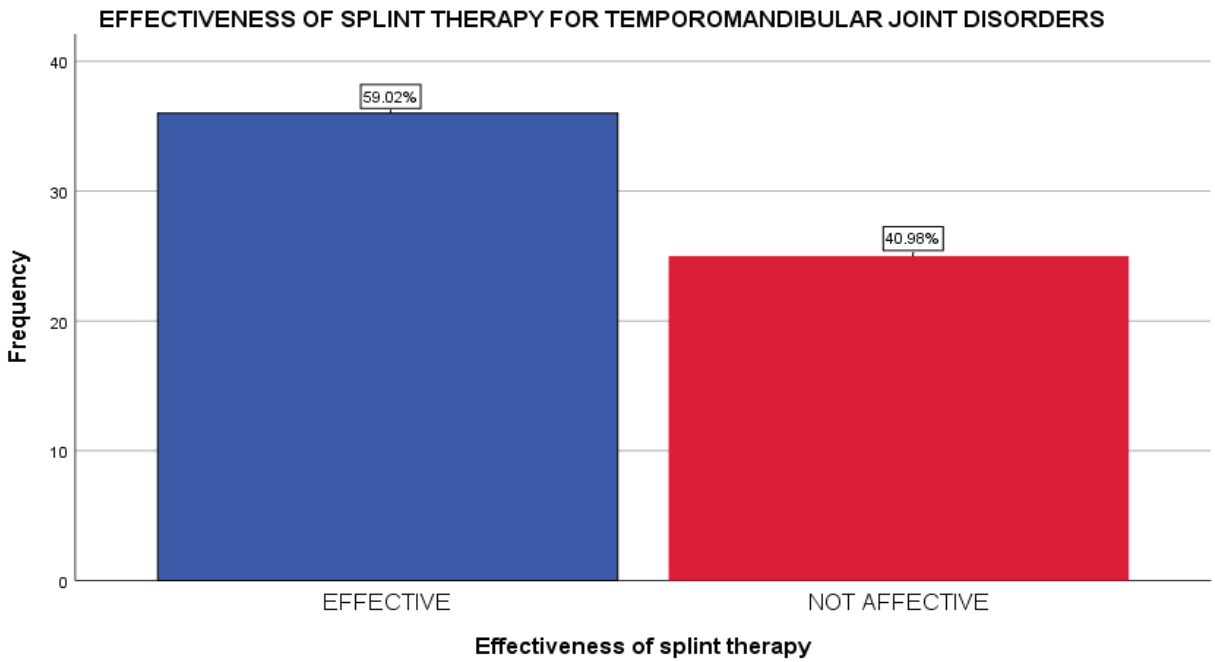


Figure 3: Bar chart showing distribution of effectiveness of splint therapy among the patients with temporomandibular joint disorder. Blue denotes effectiveness of splint therapy due to TMD with 59.02% and red denotes non- effectiveness of splints therapy due to TMD with 40.98%. X axis represents the effectiveness of splints therapy in patients and Y axis represents the number of patients.

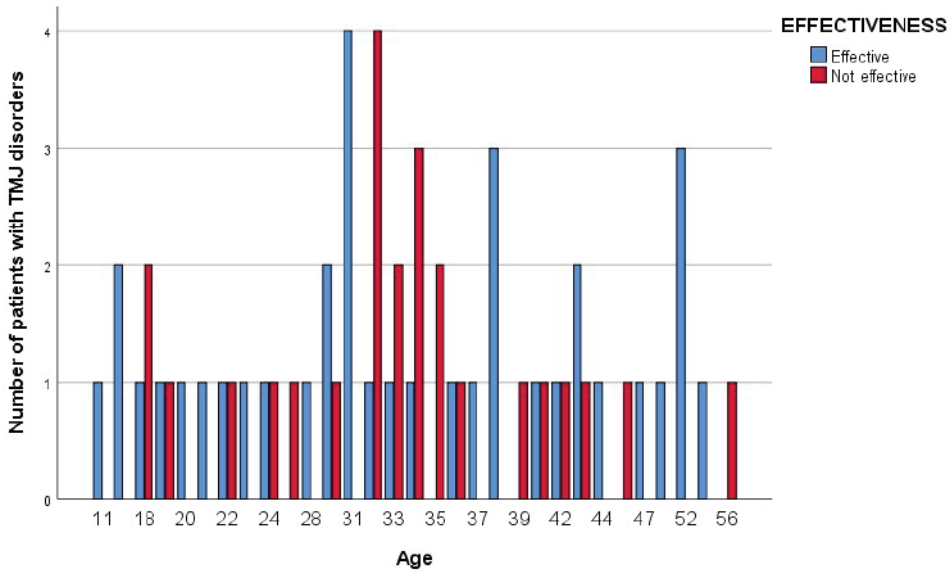


Figure 4: Bar chart depicting the association between age and effectiveness of splint therapy in TMD patients. X axis represents the age of the patients and Y axis represents the number of patients with TMD. Blue bars represent effectiveness of splint therapy due TMD and red bars represent non effectiveness of splint therapy due to TMD. The splint therapy was highly effective in the age group of 31 years with 6.56% followed by 38 and 52 years with 4.92%. Splint therapy was not effective in the age group 32 years with 6.56% followed by 34 years and 35 years with 4.92% and 3.28% respectively. Chi square analysis was done (p = 0.78; p >0.05 which was statistically not significant)

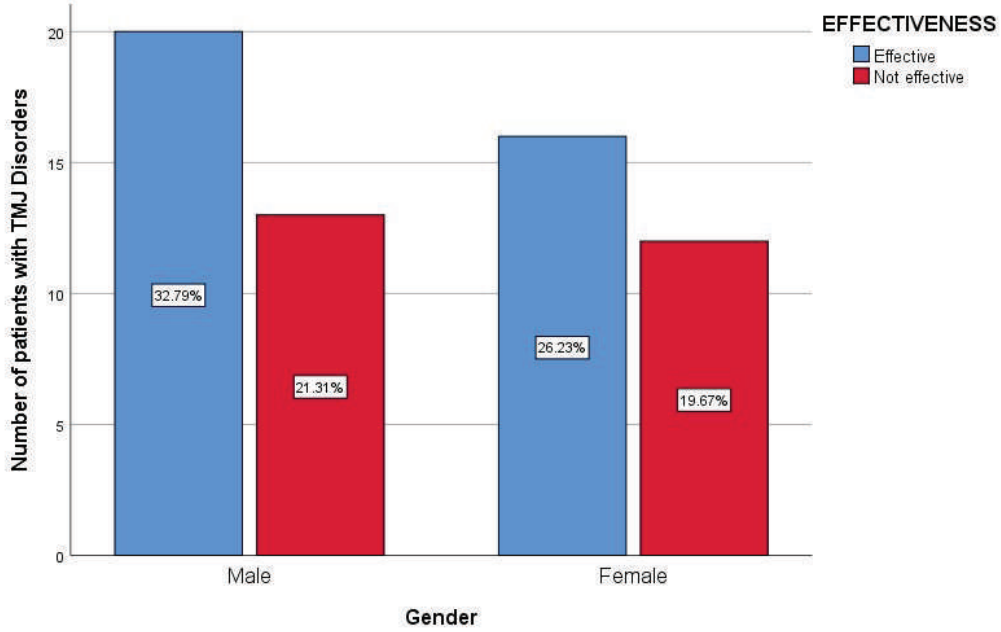


Figure 5: Bar chart depicting the association between gender and effectiveness of splint therapy in TMD patients. X axis represents the gender of the patients and Y axis represents the number of patients with TMD. Blue bars represent effectiveness of splint therapy due TMD and red bars represent non effectiveness of splint therapy due to TMD. The splint therapy was highly effective in males with 32.79% followed by 26.23% in females. Splint therapy was not effective for some male patients with 21.31% followed by 19.67% in females. Chi square analysis was done (p = 0.41; p >0.05 which was statistically not significant)

According to this study, The mean age of participants is 33.21 years. Similarly in a study by Wassell, R. W. Et.al, The patients reporting of the temporomandibular joint disorder were aged 18 years and above with the mean age of 30.2 years. In another study by Lee .HS. et al, the mean age of participants reporting with TMD and for a conservative management was 22.59 years [25]. Therefore these studies support the results of our study that the age group encountered for TMD are aged between 18 to 65 years.

In a study by Jokestad et al,out of 76 patients, 45 were males and 31 were females. In a study Contradicting to our finding by Lee HS., et al, out of 43 patients , 3 were males and 40 were females [25]. This difference in the ratio of male and female is due to a limited sample size and limited population in each study.

According to this study , splint therapy was effective and reduced pain and discomfort caused by TMD in 36 patients (59.01%) however almost 25 patients (40.98%) did not find treatment with splint therapy alone to be very effective in reducing the discomfort due to TMD. In a similar study by Ebrahim, et al , more than 50% of the patients were found to have complete relief of pain post splint therapy [28]. Carraro and Caffesse, et al claimed that the adoption of occlusal splint reduced TMJ pain by approximately 70% and muscle pain related to increased muscle activity by around 85.2% [29]. Similarly, Wassell R.W. et al, stated that 17 out of 36 patients had less than a 50% reduction in pain at six weeks.The pain for a few participants did not reduce immediately and took up to 6 to 12 months for complete ease of pain [30]. Similarly in a study by Madani AS.et al, Out of 20 patients in their study , The numbers of pain-free patients due to splint therapy were 12 in group 1 , 5 patients were pain-free post physical therapy in group 2 and 9 patients were pain-free post physical therapy in addition to splint therapy group 3. It was concluded that splint therapy appeared to be the best treatment method for reduction of pain and discomfort with TMD compared to the other two methods studied as 12 patients out of 20 were pain-free [31].Limitations of a study is a limited population covered and a small sample size. Larger samples could be taken and assessed over a longer period of time [32]. Future scope of the study is that other parts of the population should be studied.

Conclusion

Within the limitations of the study, it was concluded that splint therapy was effective for 59.02% patients with temporomandibular joint disorder patients. Also, splint therapy was more effective in males (32.79%) as compared to females (26.23%).

Conflict of Interest : There were no conflicts of interest as declared by the authors.

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Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Occlusal Morphological Patterns of Permanent Mandibular First Molar

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Abstract

Teeth are informative indicators for the study of human populations, serving as markers and the

bases for comparisons of genetic origin. Mandibular first molar is represented as 36 or 46 in the FDI system. The occlusal surfaces of posterior teeth occlude with the opposing occlusal surfaces of posterior teeth. The occlusal morphology differs in each posterior tooth. The occlusal aspect of posterior teeth is developed into cusps (5 or 4 or 6) and grooves (Y or + or X pattern). These cuspal patterns and groove patterns help in forensic identification in cases like gender determination and also to identify the ethnic background of an individual. Variation in degree of expression and frequency of teeth in dentitions of different populations is different which may be helpful for forensic identification. The aim of the study was to determine the prevalence of different occlusal morphological patterns of permanent mandibular first molars. The study included a predominantly South Indian population. Approval was received from the Institutional Review Board. Study was conducted with 100 participants who were randomly selected. The occlusal morphology patterns (number of cusps and groove pattern) of left and right permanent mandibular first molar were assessed from the photographs of the oral cavity and the date was recorded. Results showed that the most prevalent number of cusps in permanent mandibular first molar was 5 cusps (71%) and Most prevalent groove pattern in permanent mandibular first molar was Y pattern. It was also observed that the combination of the 5Y pattern was more prominent in females than in males.

Key Words: *groove pattern, cuspal pattern, permanent mandibular first molar, prevalence.*

Introduction

A dental trait in humans can be a valuable diagnostic tool in anthropological studies for classifying and characterizing different ethnic groups. Many studies have attempted to relate the prevalence of dental morphologies with different factors. Several variations, such as variations in the size, number of cusps, and groove pattern, have been observed in the mandibular molars of diverse populations. [1] The study of dental morphological characteristics is important in anthropological research as it can provide information on the phylogenetic relationship between species, as well as variations and diversities within a population.[2,]

Although tooth morphology may be an indicator of genetic distances between populations it should be viewed with caution. Almost all dental anatomy textbooks describe permanent mandibular first molars as 'Y' groove patterns with 5 cusp numbers. However, there may be variations such as the mandibular first molar having 4 cusps and also might have '+' pattern. [3]

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Gregory and Hellman and Hellman described variations in occlusal surfaces of the mandibular molars and developed morphological categories as "5 Y," "5+," "4Y" and "4+." And Takeshi Matsuda has used "6Y," "5Y," "4Y," "6+," "5+," "4+," "6X," "5X," "4X" pattern for mandibular

molars.[4]. The cusps, ridges, and grooves that decorate the crown surface also vary within different species of primates, together with the number and form of tooth roots.[5]

Dental morphological details are valuable for understanding variations among species and add information for phylogenetic and genetic studies. It is useful for collating and distinguishing populations as there are varying degrees of expression of dental features. Analysis of racial differences in dental morphology has led to the opinion that the common origins of people are reflected to a certain degree in their similar phenotypic patterns. [6]

Since only limited data are available on the prevalence of occlusal morphology patterns of mandibular first molars in the south indian population, there seems to be a need to investigate traits in this population. Previously our team had conducted numerous original studies [7-13] and surveys [14-20] over the past five years. Now we are focussing on using this knowledge and exploring the prevalence studies. The idea for this study stemmed from the current interest in forensic identification. The aim of the study was to determine the prevalence of occlusal morphological patterns of permanent mandibular first molar among south indian population.

Materials and Methods

A total of 100 participants were selected randomly. The study included a setting of a varied population predominantly South Indian population. The study was conducted in 2020 and approval was taken from the Institutional Review Board. Participants' name, gender and age were recorded. Total number of cusps and different groove patterns of right and left permanent mandibular first molars were examined from the oral cavity photographs taken.

Inclusion criteria

1. Permanent mandibular first molars free from occlusal caries.

2. Presence of bilaterally completely erupted permanent mandibular first molars.

3. Permanent mandibular first molars showing clear occlusal outline with all cusps and groove pattern.

Exclusion criteria

1. Participants with restorations and prosthesis in the permanent mandibular first molar.

2. Presence of unilateral erupted permanent mandibular first molar.

3. Participants with caries in permanent mandibular first molars.

The collected photographs were separated based on gender. The number of cusps and groove pattern of mandibular first molar was analysed from each photograph. We managed the incomplete or censored data by telephone communications. Statistical test used was descriptive statistics and chi square test. Statistical software used was SPSS software. P value less than 0.05 was considered as statistically significant and the confidence level is 95%.

Results and Discussion

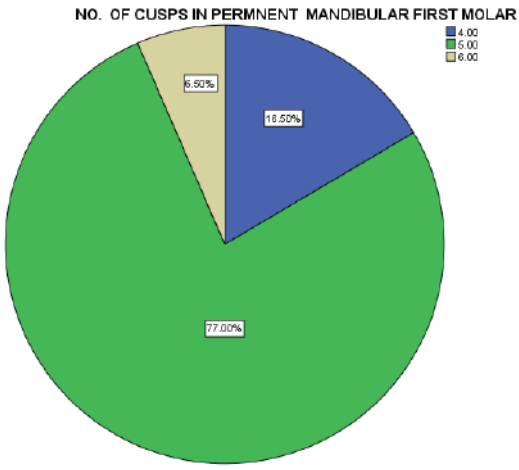


Figure 1 : Pie chart showing distribution of cuspal patterns in permanent mandibular first molar. The most predominant pattern was 5 cusps 77% (green colour), followed by 16.5% 4 cusps (blue colour) and 6.5% 6 cusps (khaki colour)

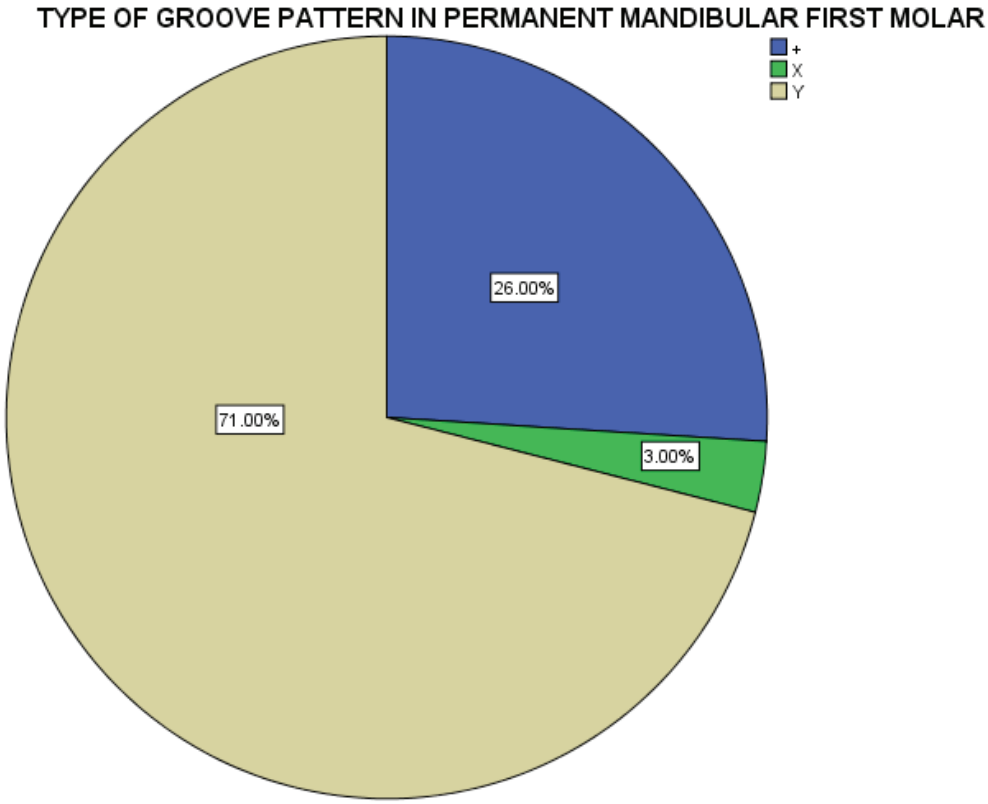


Figure 2 : The pie chart shows the distribution of groove patterns in permanent mandibular first molar. The most predominant pattern was Y pattern 71% (khaki colour), followed by 26% + pattern (blue colour) and 3% X pattern.(green colour)

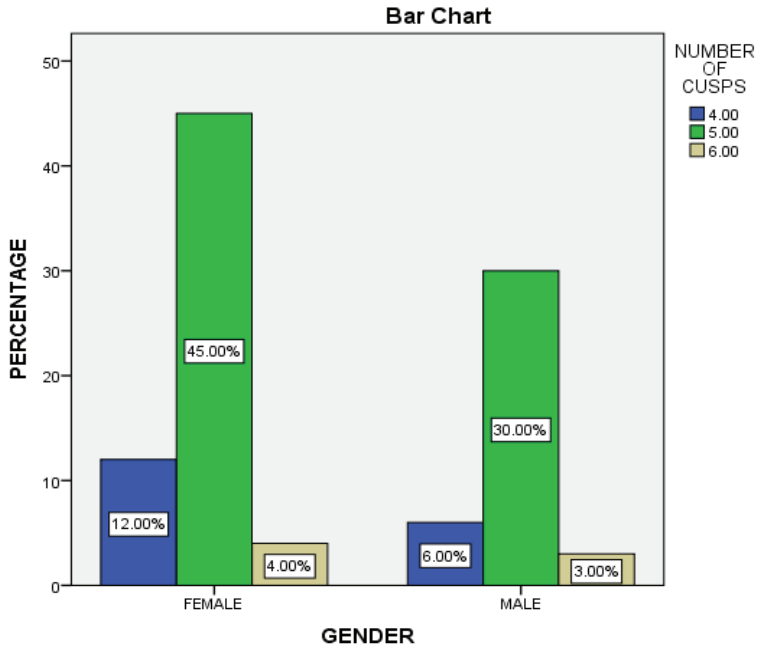


Figure 3 : Bar graph showing association between gender and number of cusps in permanent mandibular first molar. X-axis shows gender and Y-axis shows the percentage of participants. Green denotes 5 cusps, blue denotes 4 cusps and khaki denotes 6 cusps. The most predominant cuspal pattern in both genders was found to be 5 cusp patterns, with females showing slightly higher, but was statistically not significant. Chi square analysis, p=0.853 (p>0.05 indicating statistically not significant).

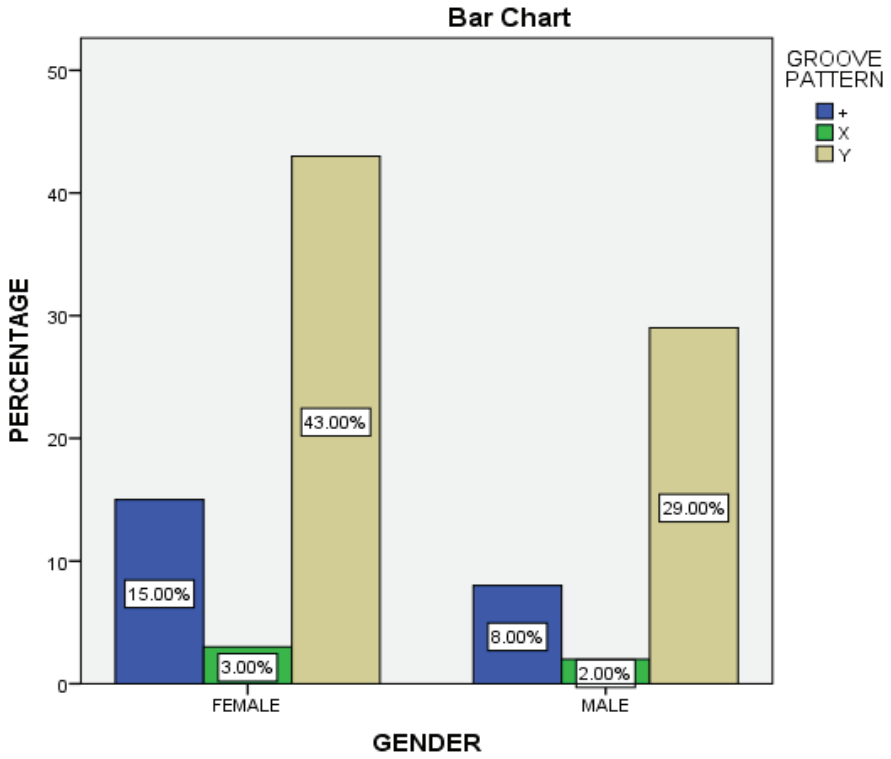


Figure 4 : Bar graph showing association between gender and groove pattern in permanent mandibular first molar. X-axis shows gender and Y-axis shows the percentage of participants. Khaki colour denotes Y pattern, blue denotes +, and green denotes X pattern. The most predominant groove pattern in both genders was found to be Y pattern with females showing slightly higher, but was statistically not significant. Chi square analysis, p=0.894 (p>0.05 indicating statistically not significant)

Table1 : Comparison of our study with the previous literature :

	Most prevalent cusp pattern	Most prevalent groove pattern	Most Prevalent no. of cusps in 36	Most Prevalent no. of cusps in 46	Most Prevalent groove pattern in 36	Most Prevalent groove pattern in 46	Most Prevalent no. of cusps in males	Most Prevalent no. of cusps in females	Most Prevalent groove pattern in males	Most Prevalent groove pattern in females
Present study	5	Y	5	5	Y	Y	5	5	Y	Y
Sneha et al	5	+	-	-	-	-	5	5	Y	+
Dholia Et al	5	Y	5	5	Y	Y	5	5	Y	Y
Guo Et al	5	Y	-	-	-	-	-	-	-	-
Hasund Et al	5	Y	-	-	-	-	-	-	-	-

The most prevalent number of cusps in the present study was 5 cusps (77%) (Figure 1). Our study was in accordance to the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent number of cusps in the mandibular first molar was 5 cusps.(Table 1) This indicates that the most prevalent cuspal pattern in different populations was the 5 cusp pattern.

The most prevalent groove pattern in the present study was Y pattern (71%) (Figure 2). Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent groove pattern in the mandibular first molar was Y pattern .(Table 1). But the study by Sneha et al showed that the most prevalent groove pattern was + pattern. (62%), which was not in accordance with our study. This indicates that the groove pattern could be different in different populations

The most prevalent number of cusps in 36 in our study was 5 cusps(74%) . Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent number of cusps in 36 was 5 cusps (Table 1) . This indicates that the most prevalent number of cusps in 36 among different populations was the 5 cusp pattern.

The most prevalent number of cusps in 46 in our study is 5 cusps (78%). Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent number of cusps in 46 was 5 cusps (Table 1). This indicates that the most prevalent number of cusps in 46 among different populations was 5 cusps.

The Most prevalent groove pattern in 36 in our study is Y pattern (73%). Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent groove pattern in 36 is Y pattern(Table 1). This indicates that the most prevalent groove pattern in 36 among different populations was Y pattern.

The Most prevalent groove pattern in 46 in our study is Y pattern (71%). Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent groove pattern in 46 was Y pattern(Table 1). This indicates that the most prevalent groove pattern in 46 among different populations was Y pattern.

Most prevalent number of cusps in males in our study is 5 cusps (71%). Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent number of cusps in males was 5 cusps(Table 1). This indicates that the most prevalent number of cusps in males among different populations was 5 cusps.

Most prevalent number of cusps in females in our study is 5 cusps (79.5%). Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent number of cusps in females was 5 cusps(Table 1). This indicates that the most prevalent number of cusps in females among different populations was 5 cusps.

Most prevalent groove pattern in males in our study is the Y pattern (66.7%) Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent groove pattern in males was Y pattern(Table 1). This indicates that the most prevalent groove pattern in males among different populations was Y pattern.

Most prevalent groove pattern in females in our study is Y pattern (73%). Our study was in accordance with the previous studies like Sneha et al, Dholia et al, Hasund et al and Guo et al [21 - 23] which showed that the most prevalent groove pattern in females is Y pattern(Table 1). But the study by Sneha et al disagree showing that the most prevalent groove pattern in females is + pattern (48%). This indicates that the most prevalent groove pattern in females could be different among different populations .

Comparison between gender in our study, showed that both genders showed the most prevalent cusp as 5 cusps and most prevalent groove pattern to be Y pattern (figure 3, 4). This showed that there were no gender differences on cuspal and groove patterns in our study.

The limitations of the study include less sample size

and homogenous population. This study expects a larger population in further studies for better understanding of the prevalence pattern of mandibular first molars, as it can be a source for forensic identification.

Conclusion

From the study, it was observed that the most common occlusal morphology in permanent mandibular first molar is 5 cusps and Y groove pattern. It was also observed that the combination of the "5Y" pattern was slightly more in females than in males. Thus it is concluded that more studies on different populations is needed to see variation in degree of expression pattern,which may be helpful for forensic identification.

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Conflict of Interest

The author declares that there were no conflict of interest in the present study

Source of Funding: Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Dental Caries in Children with and without Anterior Crossbite : A Case - Control Study

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Abstract

Dental caries and malocclusion are one the most common dental health problems affecting individuals worldwide.There are conflicting opinions about the contribution of malocclusions to the development of dental caries and periodontal disease. The aim of the study was to determine the dental caries experience in children with and without anterior crossbite. Case sheets were reviewed from the dental treatment records for dental caries in children with anterior crossbite over 9 months. Inclusion criteria for case sample required children to be above the age of 10 and below the age of 18 and with presence of anterior crossbite. Final sample size taken for the study was 12 children. Data was analysed using SPSS software and Mann-Whitney was done. The results showed that the mean DMFT score for children with anterior crossbite is 0.75 and for the control group is 2.67. Within the limitations of the present study, children with anterior crossbite (case) showed lower incidence of dental caries than the control group.

Keywords : *Dental caries, anterior crossbite, incidence, dental health*

Introduction

Malocclusion is a problem affecting the tooth where the teeth are misaligned as compared to a standard normal. Some of the common non-complex malocclusion is crowding, overbite, underbite, open bite and crossbite. These are deviations from the ideal occlusion as proposed in many literature. Malocclusion is usually an inherited condition but on occasions, can also be due to oral habits like thumb sucking and mouth breathing which causes imbalance in the forces acting on the teeth leading to this condition. In a study conducted in Rajasthan, India reported a prevalence rate of 36.42 percent for malocclusion and another study conducted in South India reported that the prevalence rate for malocclusion is 19.9 percent and 15 percent in Karnataka and Tamil Nadu, respectively.[1,2] In this present study, crossbite in particular is the malocclusion of concern. Anterior crossbite is the term used to define a malocclusion due to palatal positioning of the upper (maxillary) anterior teeth in relation to the lower (mandibular) anterior teeth. Anterior crossbite can be categorized into skeletal and dental, where in dental, the etiological factors result from a lingual path of eruption for maxillary incisors, trauma to the anterior teeth, supernumerary like mesiodens, crowding and few others.[3]

In a South Indian population study consisting of 1554 subject samples, 7.3 percent of the subjects had anterior crossbite.[4] Several complications may arise due to this condition, and dental caries is of the utmost concern. Dental caries is the destruction of dental hard tissue made up of inorganic material caused by the acid by-products released from the bacterial breakdown of carbohydrates mainly, which is the food particles that are lodged on the surfaces of the teeth.[5] Dental caries

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if left untreated, would progress down the layers of the teeth, causing more severe dental problems like pulpitis and abscess.[6,7] Dental caries is the most common dental health problem, followed by periodontal disease and malocclusion. The prevalence rate of dental caries varies from 49 percent to 83 percent across different countries.[8] In a recent study by Anand et al.[9], he reported that the incidence rate of dental caries experience among primary school children across India to be 78.9 percent. Previous studies on dental caries documented that the incidence rate was reported higher in individuals with malocclusion compared to individuals without any form of malocclusion.[10] According to Ralph et al.[11], anterior and posterior crossbite patients had higher DMFT score compared to patients without malocclusion. This study is aimed at determining the prevalence rate of dental caries in children with and without anterior crossbite and to investigate if they could be correlated. This knowledge will enable health professionals to educate the common people about the seriousness of malocclusion and the need to seek treatment for it, besides encouraging dental practitioners to consider dental caries in the treatment planning stage which eliminates the need for invasive methods.[12]

Materials and Methods

This retrospective study was conducted under a university setting. Ethical approval for this study was granted by the institute's ethical committee (ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320). Consent to use treatment records for research purposes were obtained from parents/guardians at the time of patient entry into the university for dental needs. The retrospective data were collected by obtaining and analysing the 89000 dental case records of the university from June 2019 to March 2020. The inclusion criteria for the current study were children between the age of 11-17 years, presence of anterior crossbite involving any anterior teeth, complete photographic and written records regarding the crossbite and DMFT/dmft scores. Age and gender matched controls i.e. children without anterior crossbite, were taken according to the relevant cases obtained from the inclusion criteria. The exclusion criteria were patients above 18 years of age and below 11 year of age, incomplete and censored dental records and absence of photographic evidence of anterior crossbite. The selected case and control group were examined by three people; one reviewer, one guide and one researcher. The patients' case sheets were reviewed thoroughly. Digital entry of clinical examinations and intra oral photographs of selected subjects were assessed and this included the assessment of every patients' dental caries experience by calculating their individual DMFT index score. A pretested format was used to record the caries score based on the DMFT Index. The examiner was trained to add data on the DMFT score to calculate caries experience for both case and control group by tabulation using excel software. Data analysis was done using SPSS PC Version 23.0 (IBM;2016) software for statistics. The dental caries score for case and control group was compared by Mann-Whitney U-Test.

Results and Discussion

The study population consisted of 24 children, with 12 children in the case group (children with anterior crossbite) and 12 children in the control group (without anterior crossbite)(Figure 1). All the children were aged 11-17 years old with mean age of 13 years old for both case and control sample. In the case and the control group, 6 children (50%) were males and 6 children (50%) were females respectively. (Figure 2) In the case group, 6 out of 12 children had a DMFT score of 0. But in the control group 2 out of 12 children had a DMFT score of 0. The mean DMFT score for children with anterior crossbite was higher (0.75) than children without anterior crossbite (2.67), but this difference was not statistically significant (Figure 3). (Mann-Whitney U-Test; $p \geq 0.05$).

It is important to identify these dental caries in children as they do not only impact aesthetics, but also function if left untreated, leading to debilitating conditions and instill psychological trauma in a child.[13,14] This is in particular to children with malocclusion such as anterior crossbite because of higher retention of food substance in areas of malocclusion as given in a study.[10] There were no significant differences in gender between both groups, as there was no gender predilection of significant value as reported in a study by Srikanth et al.[15]. The present study data as analysed does not support the theory that dental caries experience is higher in anterior crossbite patients than compared to control group. The sum of mean rank for case group was 416 and 522 for control group.

Higher DMFT mean score was recorded in the control group compared to case group, contradictory to the findings in the study that reported anterior crossbite patients had a higher mean DMFT score than the control group. The reason as to why the incidence is higher has yet to be investigated and information on it is scarce, thus more studies are required to find the etiological factor as well as the predilection. A plausible explanation could be the orientation of teeth to one another that makes it an area prone to food entrapment.[16] Dental caries could lead to space loss which is also a precursor for malocclusion development.[17–19] As other literature supports evidence of higher DMFT score in malocclusion patients, this study shows that DMFT score is lower for case group than compared to control group. The reason as to why the results of this study contradicts other studies could not be identified. In fact, it maybe is not a single definitive factor, but rather a collection of confounding factors that has not been taken into considerations in this study that has cumulatively contributed to the outcome of this study.

The limitations of the study were that this was a unicentric study with geographic limitations, limited sample size and has lower external validity. The oral hygiene habits were not considered, as dental caries incidence is reduced in patients with good oral hygiene than compared to one with poor oral hygiene practice. Patients with good oral hygiene negates the conducive environment required for the growth of microorganism that is responsible for demineralization of tooth hard tissue. Besides that, the patients' diet history was also not taken into account as an individual's dietary practices has been proved to affect the individuals dental caries experience. On top of that, history of past dental visits of both groups were also not assessed in particular as they may have revealed factors such as prior treatments that may have led for the development of caries such as orthodontic appliances which makes it harder for the patient to maintain their oral hygiene as it is less accessible to clean areas between the appliances and the teeth.

Socioeconomic factors which play an important role in the development of dental caries were also not assessed. Low socioeconomic status, low monthly household income and low educational level are generally analogous with less opportunity to dental services and proper oral health care products, poorer awareness on oral health and oral hygiene which led to a greater incidence and severity of dental caries.[20–25]

The advantages of the present study were that this was a case-control study with age and gender matched controls to provide better results and high internal validity. The future scope of this study would be to increase the sample size by making it multicentric which could yield better results and higher correlation with varied interpretations.

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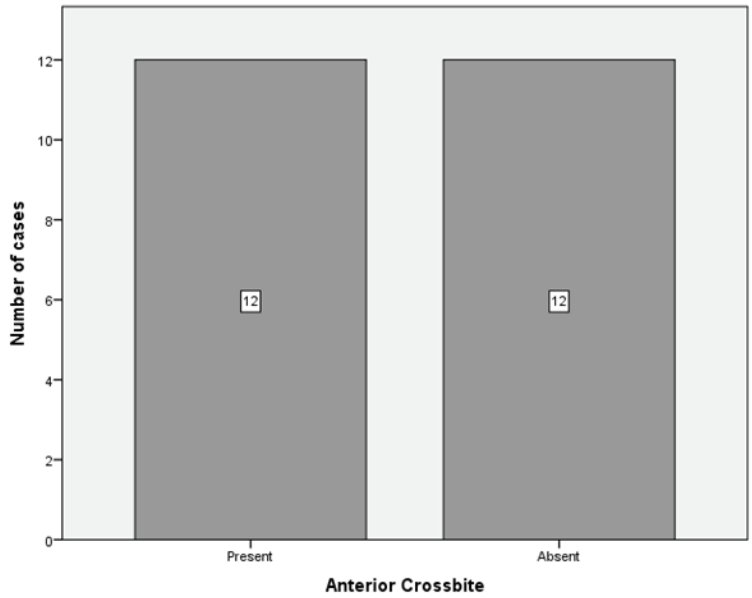


Figure 1- Bar graph showing number of cases in case (with anterior crossbite) and control (without anterior crossbite) group in the total sample population. The X-Axis represents incidence of anterior crossbite and the Y-Axis represents the number of cases. Notice the equal number of children in the case and control group.

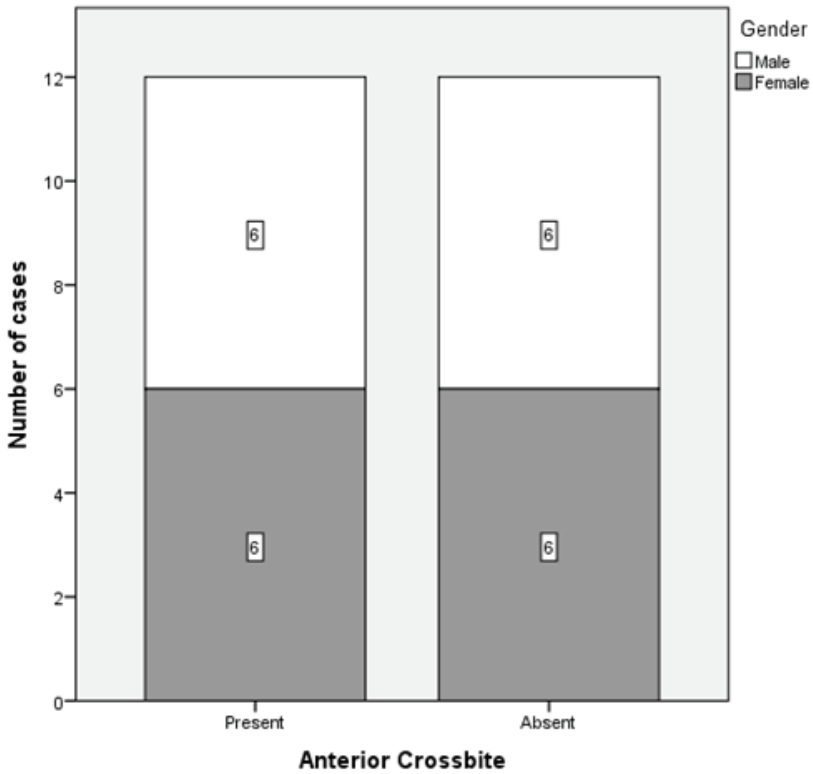


Figure 2 - Bar graph showing the gender distribution in case group (with anterior crossbite) and control sample (without anterior crossbite). The X- axis represents the presence and absence of anterior crossbite and the Y-Axis represents the number of cases, white represents children who were males and grey represents children who were females. Note the equal distribution of gender.

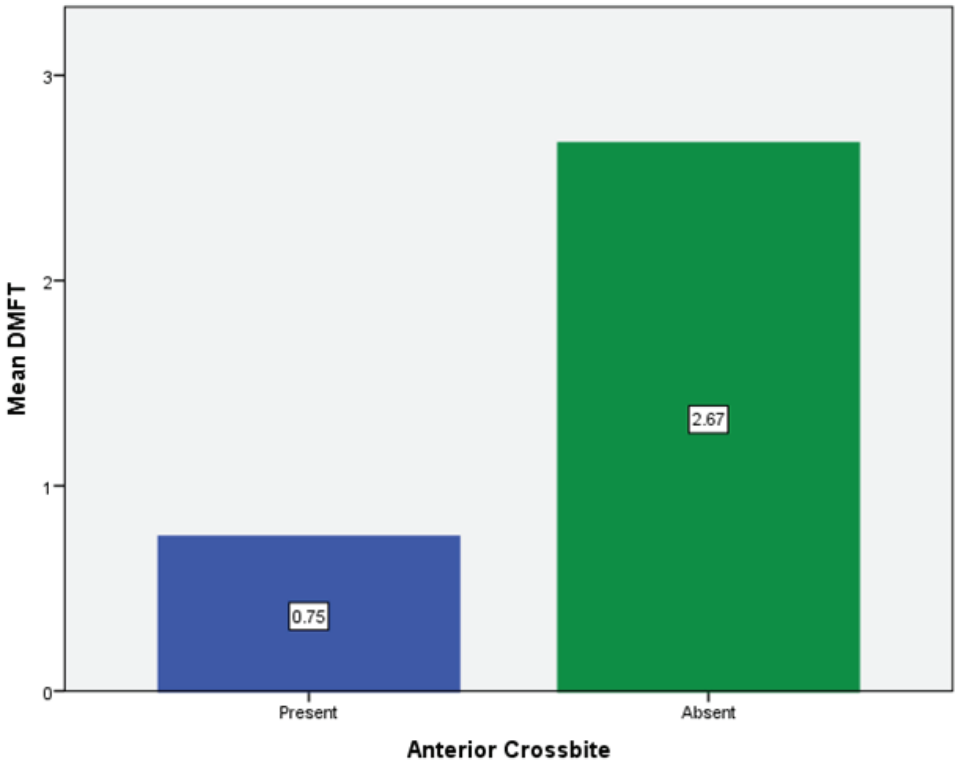


Figure 3 - Bar graph shows the mean DMFT score for both case (with anterior crossbite) and control (without anterior crossbite). The X-Axis represents the presence and absence of anterior crossbite and the Y-Axis represents the mean dmft score and blue represents children with anterior crossbite and green represents children without anterior crossbite. Children with anterior crossbite had lower mean DMFT/deft score (0.75) when compared to children without anterior crossbite (2.67) However this difference was not statistically significant (Mann-Whitney U-Test; p-value = 0.189 - statistically not significant)

Conclusion

Within the limitations of the present study, the mean DMFT score of children with anterior crossbite was higher than children without anterior crossbite. Higher number of children with anterior crossbite had no dental caries when compared to children without anterior crossbite.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Knowledge and Awareness of Primary Teeth and Their Importance among Parents

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Abstract

The primary dentition for children is, perhaps, the most essential and yet the most neglected concern by a parent. Often, these parents who are responsible for the health of the primary dentition of their children feel that primary teeth eventually shed, and hence do not deserve heed to providing adequate oral health. The aim of this study was to evaluate the parental knowledge, attitude, and practices on the importance of primary dentition of their children. A well structured, self-prepared questionnaire was circulated among parents of the dental students within the university. The results obtained were collected and analysed to find out the awareness levels among the parents and to find out which group had the highest knowledge on primary teeth. The statistical tests used were descriptive analysis and Chi square tests. The confidence level was 95% and the data was considered statistically significant if P value < 0.05. In our study, it was found that among the parents, graduated individuals had the highest knowledge on primary dentition, especially the Post graduate level parents

Key Words: *Dentition; primary teeth; children; awareness; parents.*

Introduction

Primary teeth are valuable assets of a child. The primary dentition involves 20 teeth. Regularly these teeth are alluded to as deciduous teeth. These teeth will be shed (lost) as the permanent teeth erupt. They play a vital role in eating, phonetics, aesthetics. Parents are considered as the primary Knowledge Source of maintaining a child's oral health. Child rearing and health habits for children undoubtedly lag a long term influence determining a child's oral health.[1] Indian Parents "have very low knowledge and Awareness on primary teeth and their Importance. Parents who believed that baby teeth were important had children with significantly lower Caries.

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The establishment for sound perpetual teeth in kids and young people is laid during the first year of life. A few examinations have demonstrated that inappropriate recurrence of diet, undesirable dietary patterns, and lacking oral cleanliness measures during the initial 3 years of life are identified with tooth rot in children. A dismissed carious essential teeth further builds the danger of creating caries in changeless teeth and in this manner influencing personal satisfaction. Although permanent teeth are already partly formed in children aged 0–3 years, an eruption occurs from about 6 years on. As the first molar erupts, the oral cavity constitutes of mixed dentition, thus heightening the risk of caries.

In certain cultures, little value is given to primary teeth, which resulted in Caries and early loss of the primary dentition. Previously our team had conducted numerous original studies [3-9] and surveys [10-17] over the past 5 years. Now we are focussing on epidemiological surveys. The idea for this survey stemmed from the current interest in our community. The aim of this study was to evaluate the parental knowledge, attitude, and practices on the importance of primary dentition of their

children.

Materials And Methods

This cross sectional questionnaire study was conducted in 2020 and approval was received by the institutional review board. The Sample Size was 140 parents. Random Sampling Method was followed. Questions were in English. The nature and purpose of the study was explained and strict Confidentiality was assured. The Questionnaire was self structured with 15 questions in total.

Data Collection software used was Google Forms. Analytics software used was SPSS, IBM. Pie Charts and bar diagrams are used as methods of representation of each output variable. Descriptive Statistics and Chi square test was used[18]. P value less than 0.05 was considered as statically insignificant and confidence level set as 90%.

Results and Discussion

In the present study, 70% agreed that frequency of sugar consumption affects consumption compared to amount of sugar consumed, 64.29% agreed the maintenance of good dental health can prevent dental Caries, The correct option was mainly coming from the graduate level educated parents.when asked about milk teeth, 37% of graduated parents answered as 'teeth which erupt first', When asked about the first dental visit, 31% of parents answered it as 6 months after eruption of first tooth. 35% of the parents said that all primary teeth will Shed, 35% of graduated Parents answered that there are 20 milk teeth. (Table 1)

Table 1: Table showing percentage of responses to the questionnaire

1. What are Milk teeth?	A. A.teeth which erupt first	55.71%
	B. B.teeth which are permanent	34.29%
	C. C.dont know	10%
2. How many milk teeth are there?	A. A.20	47.86%
	B. B.32	41.43%
	C. C. Dont know	10.71%
3. Will all primary teeth shed?	A. A. Yes	55.71%
	B. B. No	41.29%
4. At what age should a child start brushing unassisted?	A. A. 1–3 years	37.68%
	B. B. 4–6 years	44.83%
	C. C. 7–9 years	17.89 %
5. When do milk teeth start to erupt?	A. 6 months	14%
	B. 10 months	52.86%
	C. 1 year	35%

Cont... **Table 1: Table showing percentage of responses to the questionnaire**

6. When should an infant's first dental visit be?	A. Immediately after birth	28.57%
	B. Within 6 months of eruption of first tooth	52.86%
	C. Whenever necessary	18.57%
7. Do you think early loss of milk teeth can lead to malocclusion of permanent teeth?	A. A. Yes	68.57%
	B. B. No	31.43%
8. Do you think primary teeth are as important as permanent teeth?	A. Yes	63.57%
	B. No	36.43%
9. When should a mother start cleaning her child's teeth?	A. When the first milk tooth erupts	43.28%
	B. When all milk teeth have erupted	41.79 %
	C. When the first permanent tooth erupts	14.93%
10. Food plays an important role in the oral health	A. Yes	77.14 %
	B. No	22.86%
	C. Dont know	0%
11. Frequency of sugar consumption plays a greater role in producing caries than does the total amount of sugar consumed	A. Yes	70%
	B. No	30%
	C. Dont know	0%
12. Retentiveness of a food in the oral cavity is linked to dental caries	A. Yes	64.29 %
	B. No	35.71 %
	C. Dont know	0%
13) Does fluoride prevent dental caries ?	A. Yes	43.57%
	B. No	35.71%
	C. Maybe	20.71%
14. Is it ok for a child to sleep with a milk bottle in its mouth ?	A. Yes	61.43%
	B. No	38.57%

Cont... **Table 1: Table showing percentage of responses to the questionnaire**

15. How often do you take your child to visit the dentist ?	A. Once in 3 -6 months	46.43%
	B. Once in an year	47.16%
	C. Not at all	6.4%
16.How often do you change your child's toothbrush?	A. Once in 3 -6 months	55.71%
	B. Once in an year	34.29%
	C. less than 3 months	10%

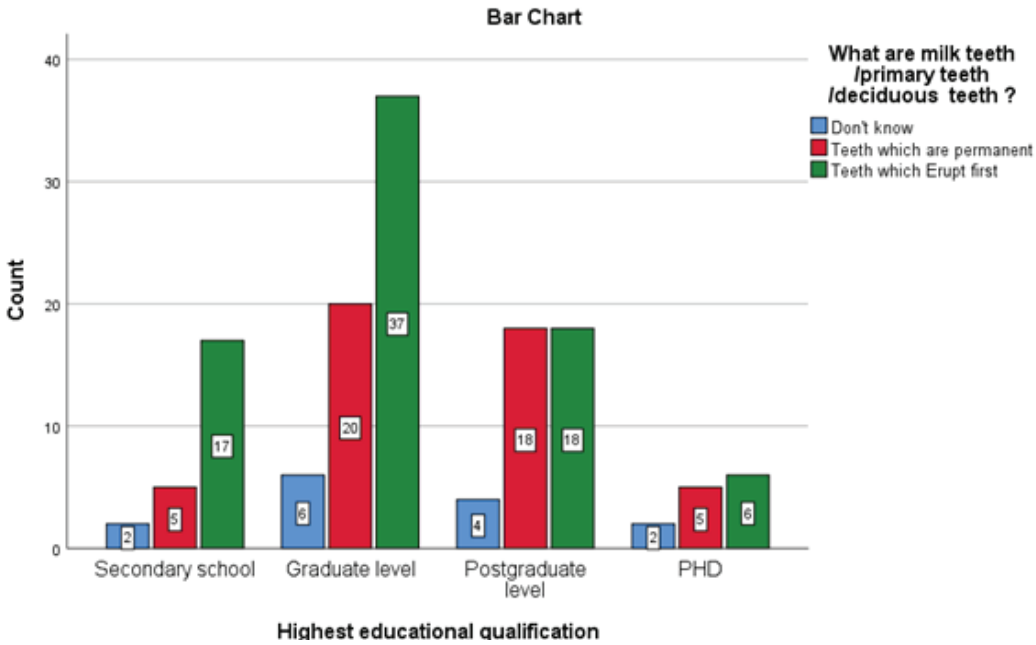


Figure 1: The bar graph showing the chi square analysis of association between different education levels of parents on knowledge of milk teeth. X-axis represents the highest educational qualification; Y-axis represents the count of parents; Green colour represents the teeth which erupt first, Red colour represents the teeth which are permanent, Blue colour represents don't know. Graduate level educated parents showed more awareness(37),But it was not statistically significant [chi Square test, p-value=0.497(p>0.05,indicates statistically not significant)

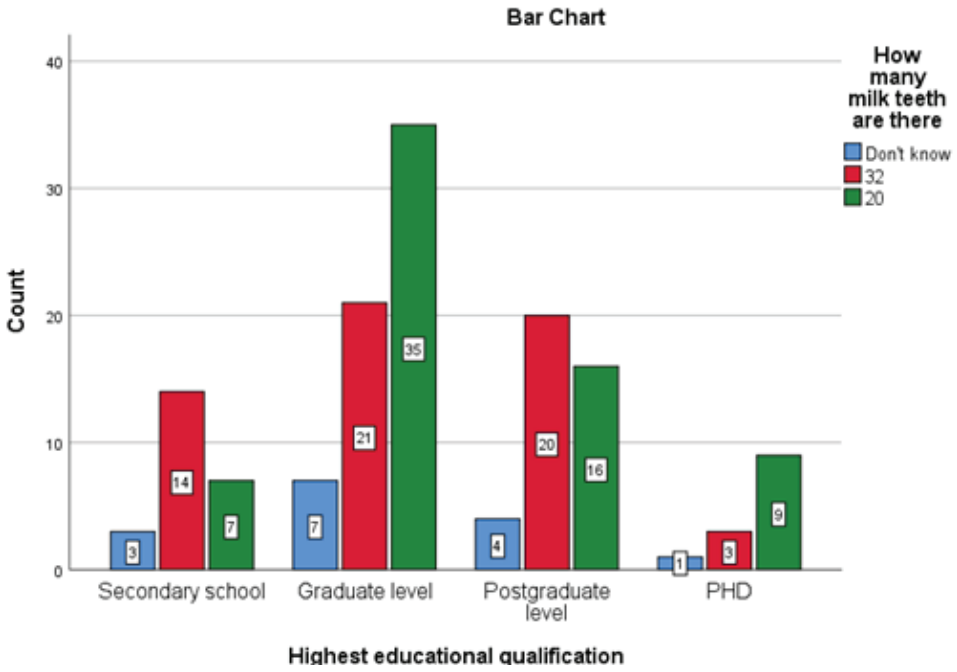


Figure 2:The bar graph showing the chi square analysis of association between different education levels of parents' knowledge on the number of milk teeth. X-axis represents the highest educational qualification; Y-axis represents the count of parents. Green colour represents 20 teeth, Red colour represents 32 teeth, Blue colour represents don't know.Graduate level educated parents showed more awareness(35) on correct number of milk teeth, But it was not statistically significant(Chi square test p=0.178, p>0.05- indicating Statistically not significant)

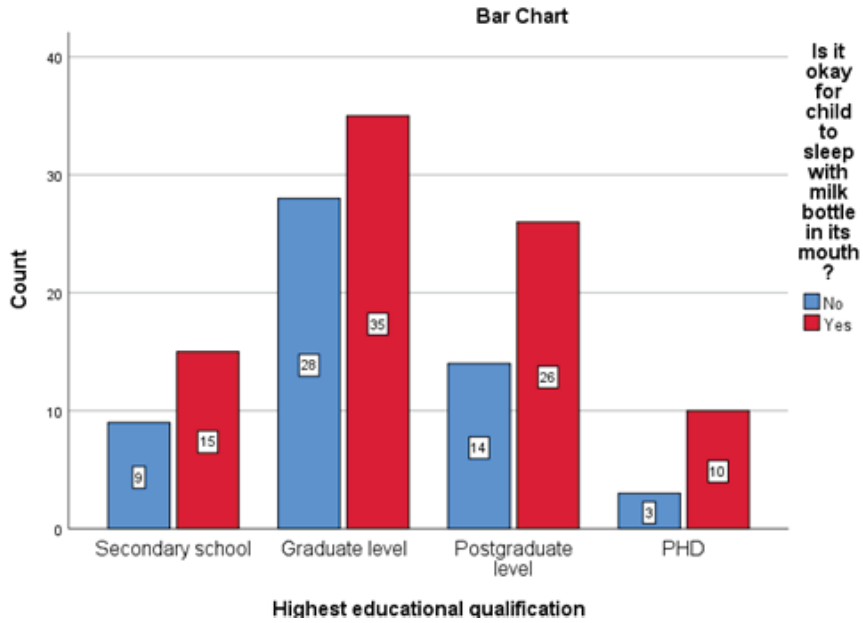


Figure 3: The bar graph showing the chi square analysis of association between different education levels of parents' knowledge on whether it is okay for a child to sleep with a milk bottle in his mouth or not. X-axis represents the highest educational qualification; Y-axis represents the count of parents; Red colour represents Yes, blue colour represents No. Majority of the parents of all education levels did not have awareness on whether it is okay for a child to sleep with a milk bottle in his mouth or not. Comparison between different educated level parents was not statistically significant (Chi square test p=0.432, p>0.05- indicating Statistically not significant)

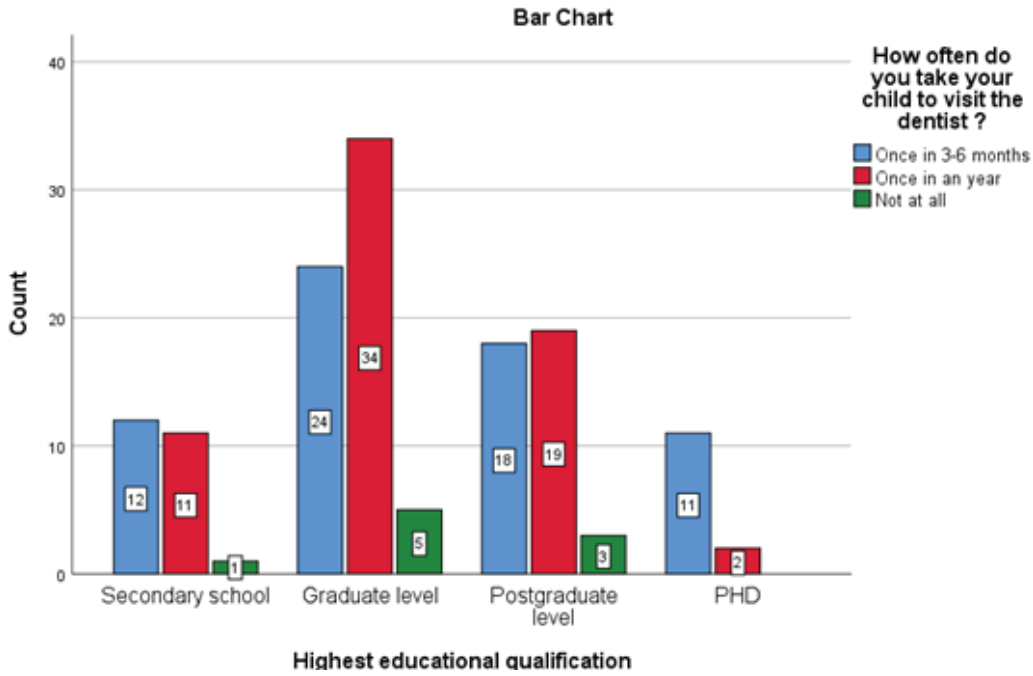


Figure 4: The bar graph showing the chi square analysis of association between different education levels of parents' knowledge on taking their children to visit the dentist. X-axis represents the highest educational qualification; Y-axis represents the count of parents; Red colour represents once in a year,blue colour represents once in 3-6 months, green colour represents not at all.Graduate level educated parents showed more awareness (34), But it was not statistically significant (Chi square test p=0.131, p>0.05- indicating Statistically not significant)

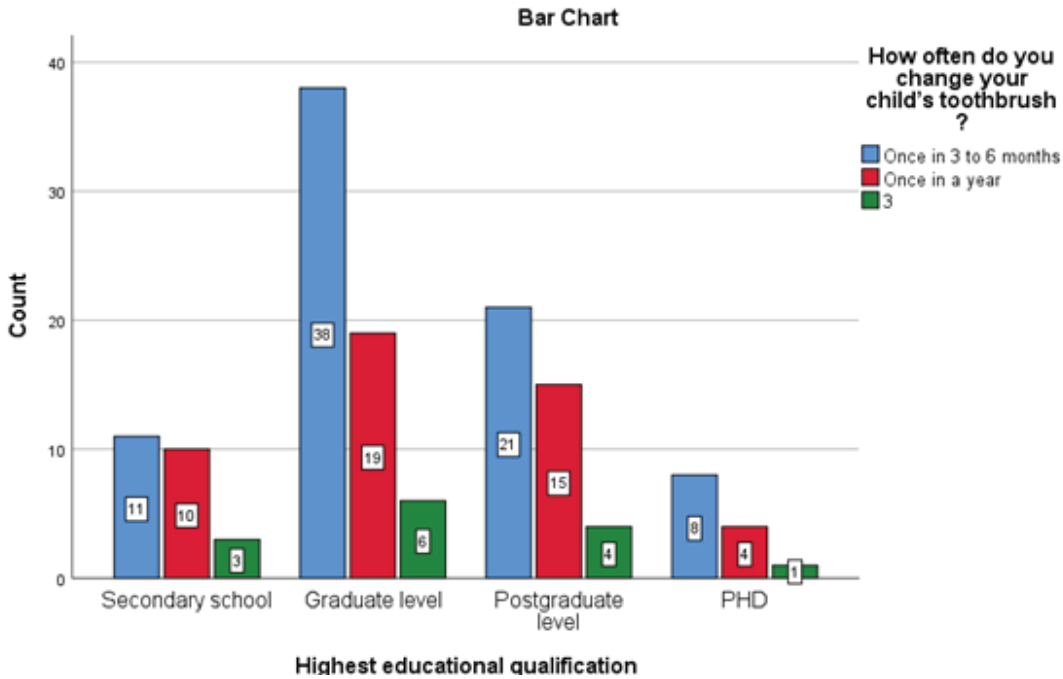


Figure 5:The bar graph showing the chi square analysis of association between different education levels of parents' knowledge on changing their children's toothbrush. X-axis represents the highest educational qualification; Y-axis represents the count of parents; Red colour represents once in a year,blue colour represents once in 3 -6 months, green colour represents once in 3 months. Graduate level educated parents showed more awareness(36), But it was not statistically significant. (Chi square test p=0.92, p>0.05-

indicating Statistically not significant)

What are milk teeth ?

55.71% people Responded that they are the tooth which erupts-first, 34.29% responded that they are teeth which are Permanent : 10 % didn't know . 37% of the Graduate level educated parents answered as teeth which erupt first (Figure.1), showed (p)= 0.497 (P>0.05)- (Statistically not significant). Similar findings were seen in studies like Vennila chandran- Et al[20] and Jyotsna Vittoba Shetty et.al [21] . Milk teeth are the onc which erupt firsts and the awareness about it was average among parents

How many Milk teeth are there ?

47.86% of the participants said that there are 20 milk teeth,41.43% - Said that 32 milk teeth are there, 10.71% - said that they don't know. 35% of the Graduate level educated parents said it's 20 milk teeth(Figure.2). (p)= 0.178 ,(P>0.05)(Statistically not significant) .The research done by Jyotsna Vittoba Shetty[21] and Vennila chandran were similar to our findings. The awareness on the number of milk teeth was found to be average.

Will all primary teeth Shed ?

55.71% of the participants responded that they will shed, 41.29% of the participants said they won't shed. 35 % of the Graduate level educated parents said that all milk teeth will shed.(p) = (0.138) (Statistically not significant) Similar Findings were seen in research done by Jyotsna Vittoba Shetty Et. Al[21] , Vennila chandran Et. Al[20]- and Sarnat H. Et Al.[1] All milk teeth in Infants sheds at different times and awareness on it is average among parents

At What Age should a child start Brushing Unassisted ?

44.83% of the population said that it's between 4-6years, 37.68% of the population said that it's between 1-3 years, 17.89 % of the population said that it's between 7- 9 years. 31.71 % of Graduate level educated parents responded as a 4-6 year age group. (p) = 0.687 (Statistically not significant) . The research done by Pinkham Et al[22] is similar to this study. Unassisted brushing Can be from 4 - 6 years.

When do milk teeth start to erupt?.

14% of the respondents said it's 6 months, 52.86% of the respondents said that they will erupt from 10 months,whilst 35% of them said it's from 1st year. 35% of Graduate level educated parents said it's 10 months. (p) = 0.770 : (Statistically not significant). The research done by Abdulrahman alshehri [23] is similar to this study. Milk teeth start to erupt before 1 year of age.

When should an Infant's First Dental Visit be ?

52.86% of people said that the first visit should be within 6months of first teeth , whilst 28.57% of them said that it should be Immediately after birth,and 18.57% said whenever necessary . 36% of the Graduate level educated parents said that its ": within 6 months of eruptions first teeth. (p)= 0.750 ; (p>0.05), (Statistically not significant) . Similar finding was found in the research done by Adimouleme Sanguida[17], Opposite Finding to this study was found in the research done by Amid 1. Ismail, et al., in which participants responded first to a dental visit 2-3 years after teeth erupts. This disagrees with present study,which is due to differences in the study population. Infants first dental visit, should be after 6 months of eruption the first teeth and awareness to this was found to be average.

Do you think early loss of Milk teeth can lead to Malocclusion of permanent teeth?

68.57% of the participants said that loss of milk teeth can lead to malocclusion of permanent teeth(24) (p) = 0.746 (Statistically not significant), 31.43% of the Graduate level educated parents responded that early loss of milk teeth don't lead to malocclusion of permanent teeth . 46% of the Graduate level educated parents said yes, Similar findings were found in the research done Adimoulane Sandigua[17]- Early loss of teeth causes malocclusion. And awareness of this was present among parents.

Do you think primary teeth are as Important as permanent teeth ?

63.57% of the people responded as Yes, 36.43% of the people opted for the option -'No' . 41% of the Graduate level educated parents said it's important. (p)=0.925, (Statistically not significant) . Similar research is done by Abdulrahman Alshahri[23]. Both teeth

are very important and have their own significance.

When Should a mother start cleaning her child's teeth ?

14.93% of the participants opted for the option- when first permanent teeth erupt, 41.79 % of them opted for the option 'when all milk teeth start to erupt', whilst 43.28% of the participants opted for the option- 'when the first milk teeth erupt'. In the graduated Parents 28% of them opted for the option ,when 1st milk tooth erupts. (p)=0.939 (Statistically not significant) .Similar findings were found in the research done by Abdulrahman Alshahri[23], Cleaning a child's teeth should be done after the eruption of the first milk tooth.

Food plays an Important role in oral health

77.14 % of the population said yes whilst 22.86% said No. 52.6% of the Graduate level educated parents said yes. (p) = 0.558 (Statistically not significant). Similar findings were found in the research done by Abdulrahman Alshehri[23]. Food plays an Important role in oral health

Frequency of sugar consumption plays a greater role in producing caries than the total amount of Sugar consumed? 70% of the people said yes whilst 30% said no. 49% of the Graduate level educated parents said yes. (p) = 0.317 (Statistically not significant). The research done by Adimoulame Sanguida[17] is similar to this study. The Frequency of Sugar affects caries more than the amount of sugar and awareness was present among parents.

Retentiveness of a food in the Oral cavity is linked to Dental caries ?

35.71 % of the people said No , 64.29 % of the people said ,Yes. 44% of the postGraduate level educated parents said Yes. The research done by Abdulrahman Alshehri[23] was similar to our study. Dental caries are caused due to the retention of food in the oral cavity[25].

Does fluoride prevent Dental Caries ?

20.71% of the people said maybe, 43.57% of the people Said Yes, whilst 35.71% said No . 24% of the Graduate level educated parents said Yes. (p) = 0.230 (Statistically not significant). Similar Findings were found in the research done by, Adimoulane.Etal [17], gruyten. J. Et. Al[26] , Schroth Et. Al[27] and Clarke Mist. Al[28] . Fluoride prevents Dental Caries, but the awareness about it was average among parents.

Is it okay for a child to sleep with a milk bottle in its mouth ?

61.43% of the people said yes, 38.57% of the people said no. 35 % of Graduate level educated parents agreed (Figure.3). The research done by Jyotsna vittoba et Al[21] was similar to this study. It's not okay to sleep with a bottle in your mouth, but many of the parents were not aware of it. This is one of the reasons for development of rampant caries.

How often do you take your child to visit the Dentist?

47.16% of the population said once in a year, 46.43% of the participants said it's once in 3-6 months, whilst 6.4% of the people said not at all. 34% of the Graduate level educated parents said it's once in 6 months. (Figure.4) (p)=0.131(Statistically not significant). The research done by Jyotsna vittoba Et al[21] was similar to this study . Taking the child to the dentist once in 6 months is advisable

How often do you change your child's toothbrush ?

Once in 3-6 months is the option selected by 55.71%, less than 3months is the option selected by 10 % whilst Once in a year option is selected by 34.29 % of the population. (Figure.5) (p)=0.928 (Statistically not significant) . The research done by Jyotsna vittoba Shetty Et. Al [21] similar to this study. Toothbrushes should be changed once in 3-6 months[29,30]

The limitations of our study include limited sample size, homogenous population and regionalism, overcoming which we can get more accurate results. The study shows that Secondary school level parents had less knowledge. Need awareness programmes on infant oral health to boost the knowledge on this topic.

Conclusion

Knowledge and awareness on Primary teeth among the parents was found to be moderate. The Graduate level educated parents have a higher knowledge on Primary teeth and its importance, Secondary school level educated parents have lesser awareness on primary teeth. Post graduate level parents have the highest

awareness on infant oral health.We can conclude that there is definitely a need to create more awareness on primary dentition and its importance .

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Conflict of Interest: The author declares that there were no conflicts of interests in the present study.

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Ethical Clearance: Not required

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Artificial Intelligence in Dentistry - A Review

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Abstract

Artificial Intelligence (AI) is a breakthrough in the field of technology which is rapidly progressing and has captivated the minds of researchers across the globe. Ever since its discovery, dentistry has witnessed some of the exceptional achievements. Hence, this technology as the future of dentistry is abutting the implementation of its application. While, in no ways, AI can replace the role of the dentist, it is of prime importance to be aware of the possibilities to integrate this technology in the future of a gratifying and successful practice. The aim of this review is to analyse the role and applications of artificial intelligence in dentistry. The field of artificial intelligence has transformed medicine and dentistry in several ways. AI can only assist the clinician in performing the tasks efficiently, but in no way, the intellect of the human knowledge skill and treatment planning.

Keywords *: Artificial Intelligence, dentistry*

Introduction

Various technological advancements had occurred in the field of dentistry in the previous decade. In newer times, technologies are consistently developed and support the principles that attempt to mimic the human brain functioning to develop solutions. AI is defined as the study of intelligent agents, any device that perceives its environment and takes action that maximizes its chance of successfully achieving its goals. The term AI was coined within the 1950s and refers to the thought of building machines that are capable of performing tasks that are normally performed by humans. Computer based diagnosis is gaining momentum thanks to its ability to detect and diagnose lesions which can go unnoticed to the human eye, thereby paving way for holistic practice.[1]

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Artificial Intelligence :

Artificial Intelligence is usually called machine intelligence and is demonstrated by machine in contrast to the natural intelligence displayed by humans and other animals.[1] AI alternatively could also be stated as a topic handling computational models which will think and act rationally. AI research are often defined because the study of intelligent agents, any device that perceives its environment and takes action that maximize its chance of successfully achieving its goal.[2]

History dates back to as early as 400BC when Plato envisaged a basic model of brain function. Since then, the sector of science has witnessed various inventions with the arrival of technology for creating a model which will stimulate the functioning of the human brain[3]. The process of the constant search has given rise to what's referred to as Artificial Intelligence (AI), which may be a highly evolved system capable of mimicking functioning of the human brain.[4] This unexampled nature of the human brain has always made researchers and scientists inquisitive from time immemorial.

Artificial intelligence is defined as the field of science and engineering concerned with the computational understanding of what's commonly called intelligent

behaviour and with the creation of artifacts that exhibit such behaviours. Computer base diagnosis is gaining momentum thanks to its ability to detect and diagnose lesions which can go unnoticed to the human eye, thereby paving way for a holistic practice. The varied techniques of AI which are being applied in industry include Artificial Neural Networks (ANN) , which are being applied in dentistry include Genetic Algorithms (GA) and symbolic logic. Following its interception in 1959 when the primary computational trainable neural networks were developed, the sector of drugs and dentistry has witnessed innumerable research using AI.[5]

A machine learning prediction technique is an algorithm that estimates an unknown dependency between a group of given input variables and its output variables. When such dependency is observed, it can predict the longer term output by identifying the target function that best describes the behaviour governing the input-output patterns.[6] AI is capable of communicating with a video camera and can automatically detect the root canal orifices in all the teeth in the oral cavity. The Applications of Artificial Intelligence are abundant and more research has been directed towards maximizing benefits.

Applications of AI In Dentistry :

Caries Detection

Dental caries is one of the most common problem affecting people of all ages, it can be of nursing bottle or rampant caries affecting childrens, pit and fissure caries, smooth surface caries, root caries mostly seen in gediatric patients.[7,8] It can be defined as microbial disease of the calcified tissues of tooth, characterized by demineralization of the inorganic portions and destruction of organic structures. Acids produced by fermentation of carbohydrates such as sucrose, glucose, fructose, by the plaque bacteria such as streptococcus mutans leads to subsurface demineralization of the tooth enamel. The periods of disease activity vary greatly and it is influenced by various risk factors. Subsequent damage to dentin-pulp complex leads to painful conditions like acute/chronic pulpitis.[9,10]

Diagnostic aids refers to tools, procedures or technologies that are used in the determination of diagnosis.[11, 12,13,14] Dental caries is diagnosed by clinical methods and radiographic examination of teeth is usually by using intraoral periapical radiographs, bitewing radiographs, orthopantomography. Fermentable dietary carbohydrates are the key players in causation of caries. After intake of these sugars, the ph of dental plaque falls to 4.5 to 5 within 1 to 3 minutes and it takes another 10 to 30 minutes to return back to neutral ph. The critical ph of plaque is 5.5 and tooth demineralization starts if ph drops below this level. Caries producing bacteria readily use up the sugars and produce sticky polysaccharides called dextrans which helps in adhering the plaque firm to tooth surfaces and causes further acid production and tooth decay.[15,16,17,18] By using smart microchips, we can assess the patients food intake, diet activity and oral ph levels irrespective of whether it is a pediatric, adult or gediatic case. Thereby it helps in assessing the caries activity, helps in diagnosing initial or incipient caries cases, and accordingly we can manage the cases by alteration in diet patterns or preventing the tooth decay.[19,20,21]

Orthodontics :

AI helps in orthodontic diagnosis and treatment planning[22]. The data is fed into the system the set algorithms and AI software helps in predicting tooth movements and final outcomes of the treatment.[23]

Oral Medicine and Radiology:

Artificial Intelligence can be used as a useful modality in diagnosis and treatment of lesions of oral cavity and can be employed in screening and classifying suspicious altered mucosa undergoing premalignant and malignant changes.[24] Artificial Intelligence might accurately predict a genetic predisposition for oral cancer for a large population. The use of ANNs in the diagnosis subtypes of temporomandibular disorders has been studied by Bas Betal[5]

Correct diagnosis is the key to a successful clinical practice. In this regard, adequately trained neural networks can be a boon to diagnosticians, especially in conditions having multifactorial etiology. In head and neck imaging modalities, it can be integrated with imaging and cone-beam computed tomography to identify minute deviations from normalcy that could have gone unnoticed by the human eye. ANN is found to act as a second opinion to locate the minor apical foramen ,

thereby enhancing the accuracy location on radiographs, working length determination by radiographs [25]. It is also found that sufficient sensitivity, specificity and accuracy to be a model for vertical root fracture detection in digital photography and in diagnosing proximal dental caries[26].

Prosthodontics :

AI combined with designing softwares can help the dentist to design the best possible and aesthetic prosthesis considering a number of factors like facial measurements , anthropological calculation, ethnicity, and patient desire[27]. Another breakthrough in this field is the use of CAD CAM technology[28] which creates 2 and 3D models. Virtual Reality Stimulation (VRS) can be used to simulate facial profile during treatment.

Oral And Maxillofacial Surgery :

The tremendous utilization of artificial intelligence in oral and maxillofacial surgery is alongside the evolution of robotic surgery where the human body motion and human intelligence is stimulated. AI software programs have assisted the surgeon in planning surgeries with reduced operation time thereby preserving prior to the actual surgery with higher intra operative accuracy[29].

Forensic Odontology :

Dental age estimated is based on the sequence of tooth formation and eruption time.Stages of tooth formation can be observed radiographically. Radiographic methods of age estimation are commonly used due to availability of the pre and post treatment records and also the ease of availability of the data can be retrieved any time[30].

Periodontics:

Deep learning analysis using radiographs can assist in diagnosing and treatment planning of periodontal diseases by enabling the early detection of periodontal changes, bone loss, and changes in bone density.[31]

Pediatric Dentistry:

In pediatric dentistry, Artificial Intelligence have many potential applications which would change the behavioural pediatric practice in future. Also, with the help of larger datasets, machine learning algorithms will only become more sophisticated over time. AI is also gaining pace in the early orthodontic tooth movement with customized AI driven appliances which would have better acceptability by the new generation. AI enabled restorative dentistry with design computer-aided manufacturing technology is already established in adult dental practice and would emerge a boon to restorations and aesthetics. Pain control with AI enabled devices is the new, smarter way towards injection-free pedodontic practice The various 4D goggles, movies, animations and virtual reality-based games can be used as a behaviour modification aid effectively for pediatric patients.[32]

Dental Implants:

The dental technicians position the abutments already assembled on a 3D printed model, with the implant analogues inserted, and scan them with a desktop scanner. This shows obtaining the anatomy and relative position of the abutments, including the margin line. Although this is often possible, it's an additional step that forces the technician to print a model, with related costs and problems , but in particular to model the definitive zirconia restorations.

These additional steps can now be avoided by using AI. Artificial Intelligence is defined as the ability of a system to get information from external data and learn from them, which use those learnings to realize the objectives and goals through flexible adaptation. Machine learning, as a subset and foundation of AI, is the ability of computer systems to perform specific tasks to approximate human cognition, without using explicit instructions, counting on patterns and mathematical models. CAD software is often 'instructed' to save lots of the stereolithographic file of the individual abutment, modelled by the technician, during a specific folder, then to retrieve it automatically when needed. The dentist can capture a replacement of an intraoral impression of the abutment with the correct position, which are generally subgingival. The portion which is relative to the abutment, that can be recognised and eliminated.

Future Of Artificial Intelligence :

As technology continues to invariably the dental community stands on the precipice of an AI revolution that will forever change the way dentistry performed from both a clinical and practice management perspective. Already, several practice management products have

been introduced which incorporate AI, including a digital assistant that uses natural - languages processing to replace the traditional point and click interfaces and a learning based schedule optimizer.

Moreover, deep learning AI techniques will begin to increasingly impact clinical dentistry. These tools are able to spot abnormalities in images that even clinicians might overlook, while executing its findings in realtime to seamlessly integrate into any practice workflow. As deep learning systems continue to grow in intelligence, the tools will augment diagnostic accuracy. On the contrary, this rise of the machines will enable to perform at a higher level while essentially making the job much easier.

Challenges Of AI :

We see three reasons why dentistry has not fully adapted to AI technologies. Proper analysis of these reasons will help to make dental AI technologies better and facilitate their uptake in clinical care. First , medical and dental data are not as available and accessible as other data, due to data protection concerts and organisational hurdles. Data is often locked within segregated, individualized and limited interoperable systems. Datasets lack structure and are often relatively small, at least when compared with other datasets.[33] Second, processing data and measuring and validating results is often insufficiently replicable and robust in dental AI research. It remains unclear oftentimes used for both training and testing leading to data snooping bias. Third, the outcomes of AI in dentistry are often not readily applicable. The single information produced by most dental AI applications will only partially inform the required and complex decision- making clinical care[34].

Conclusion

The field of artificial intelligence has transformed medicine and dentistry in several ways. Though Artificial Intelligence systems are a greater asset in dentistry and dental education, the human biological system is complex and it is to be noted that these technological advancements are still the beginning of innovations and discoveries of mankind. Furthermore, AI can only assist the clinician in performing the tasks efficiently, but does not replace the intellect of the human knowledge, skill and treatment planning.

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Awareness about Face Bow and its Types among Dental Professionals- A Questionnaire based Survey

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Abstract

A face bow is a caliper like instrument used to record spatial relation from maxillary arch to the anatomical points and transfer the jaw relation to an articulator. It orients the dental cast in the same relationship to the opening axis of the articulator. There are mainly two types of face bows - Kinematic and Arbitrary face bows. Arbitrary face bows inturn have many subtypes based on anterior and posterior reference point indicators and the mechanism of inter condylar distance equilibrium.The questionnaire containing questions about face bows and its types was circulated to about 100 participants through google docs - online survey. The study was approved ethically by IERB.The responses generated from the survey were converted into pie charts and recorded. The answers obtained are discussed by comparing with the previous articles.The survey study shows how much Dental professionals know about face bows and its types and implementing them in their practice.

Keywords: *Dentists, Hinge axis, Face Bows, Kinematics and Arbitrary Face bows.*

Introduction

A face bow is a caliper like instrument used to record spatial relation from maxillary arch to the anatomical points and transfer the jaw relation to an articulator. It orients the dental cast in the same relationship to the opening axis of the articulator. There are mainly two types of Face bows - Kinematic and Arbitrary face bows.[1] Kinematic face bows contain adjustable caliper ends and locates the exact axis of rotation and hinge axis. Arbitrary Face bows relates maxilla to the exact position of condylar axis and transfer relation. Arbitrary face bows are classified based on posterior, anterior reference point indicators and mechanisms of inter - condylar distance equilibrium.[2] Based on posterior reference indicators, they are infra orbital and nasion pointers. Based on the mechanism of inter condylar distance equilibrium arbitrary face bows are classified to spring bows, calibrated condylar rods and slidematic face bows. Face bow follows oriental jaw relations.[3] Face bows are most widely used in prosthodontic rehabilitations. They are used in the complete denture fabrication. Face bow records are transferred to articulators to prevent any occlusal errors since the distance from condyle to teeth is crucial. In the prosthodontic procedures, failure to use the face bow often leads to error in occlusal records.[4] Hinge axis is a major component in the masticatory movement of the mandible and hence it cannot be disregarded. Hinge axis accurately captured and transferred to the articulator represents the occlusal aspect of a patient. Hinge axis is determined by the type of face bow used.[5]

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Previous studies that are based on the effects of direct and indirect face bow transfer on the horizontal condylar guidance values in a semi adjustable articulator. The results from the study shows a statistically significant difference in the HCG values in direct and indirect face bow transfer.[6]

Dental professionals need to be aware of the types of Face bows as they help to find accurate Hinge axis of patient and ensure proper occlusion and good treatment. Face bows are widely used for good complete denture fabrication and reduce error in occlusion.[7] The main aim of the study is to create awareness among dental professionals about the use of Face bows and its types through a survey.

Materials and Methods

The Study setting is an online survey conducted using a self prepared questionnaire to 100 participants in the south indian population. The previous studies done based on Face bows had a population of 30 participants and 10 participants.[8,9] The internal validity in the study is the pre-tested questionnaire and the external validity is the homogenisation and replication of experiment and cross verification with the existing studies.

The questionnaire is circulated online among dental professionals and the data collection software used is Google Forms. The responses obtained are converted to pie charts for representation

The statistical test used here is the chi-square test and the statistical software is SPSS software. The independent variable here is age,gender and qualifications and the dependent variables are the facebow usage,applications and any complications of facebow if any. The statistical analysis here is done by chi-square test and correlation analysis test. In this, the type of analysis used for correction and association is the chi-square test.

Results and Discussion

In this survey study,we have discussed about the awareness of facebow usage among dental professionals from the responses gathered from the online survey.This survey contains questions based on their knowledge about the types of facebow,the individual uses of each facebow,correct handling of facebow and the reference points of facebow.The results are converted to pie charts for easy handling.

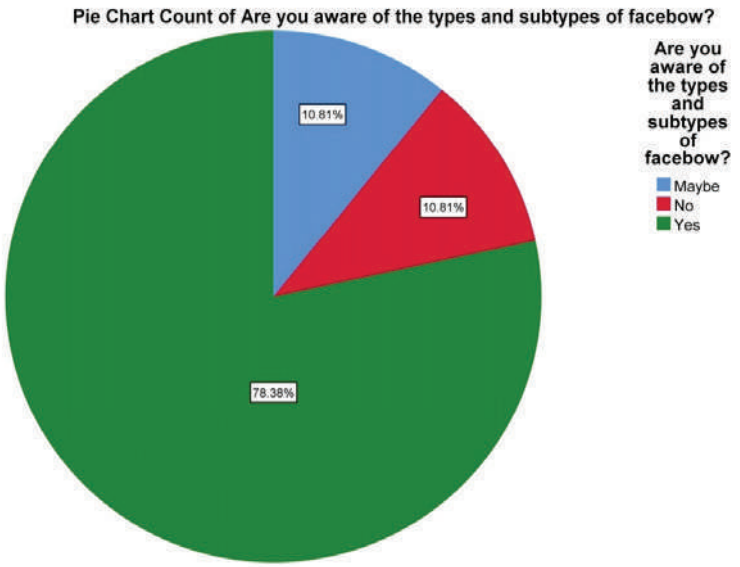


Figure 1: Pie chart representing frequency distribution of the response showing the awareness on the types of face bow. 78.38% answered yes(green), 10.81% answered no(red) and 10.81% answered maybe(blue)

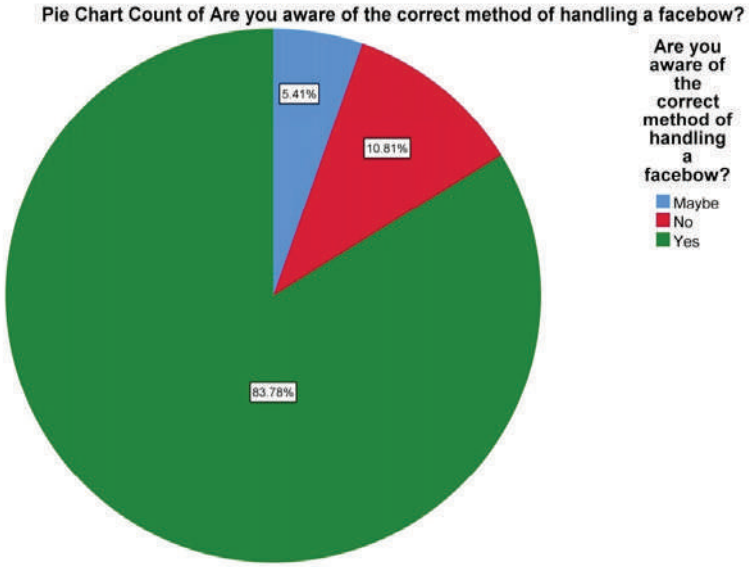


Figure 2:Pie chart representing percentage response on knowledge about the method of handling a facebow. 83.78% answered yes(green), 10.81% answered no(red) and 5.41% answered maybe(blue).

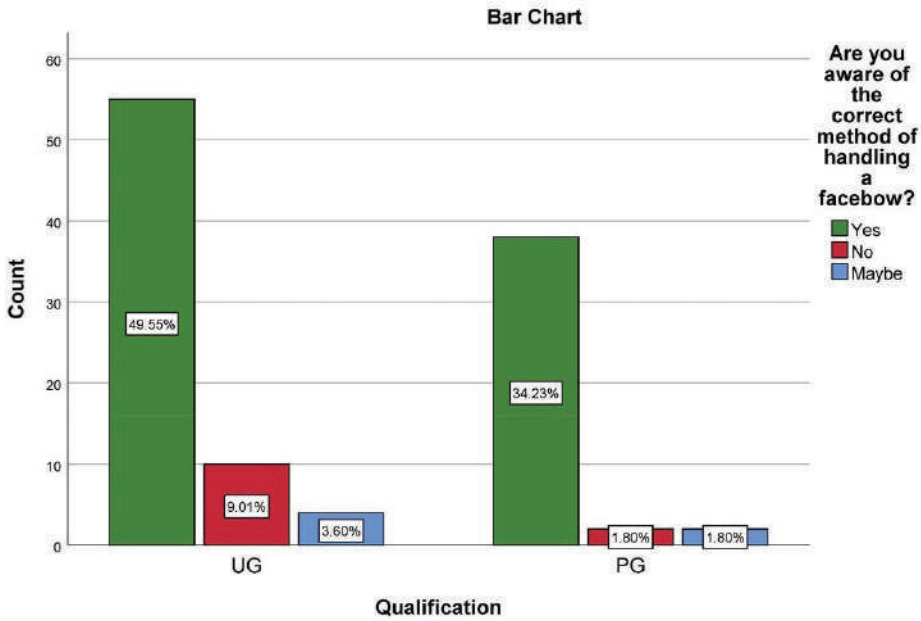


Figure 2.1: Bar graph representing the percentage response on knowledge about the method of handling a face bow. X-axis represents the qualification whether the professional is an undergraduate or postgraduate.Y-axis represents the percentage of awareness of handling of face bows. Green colour represents yes, red colour represents no and blue colour represents may be.Higher number of undergraduate professionals answered yes and there is a difference in responses between undergraduate and postgraduate professionals. Pearson Chi square test: 2.700a ; df: 2; p value: 0.259(>0.05), hence it is statistically not significant. This proves there is a similar percentage of awareness about the method of handling face bows among both UG and PG professionals.

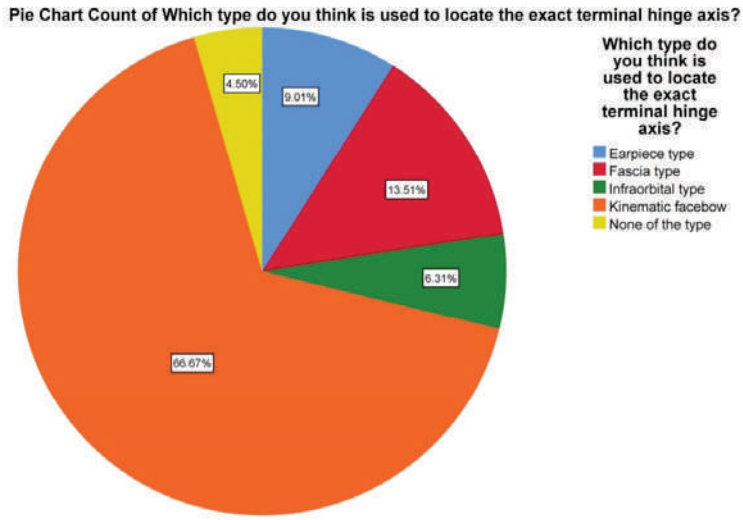


Figure 3: Pie chart representing frequency distribution of percentage response for the type of facebow used in locating the exact terminal hinge axis.9.01% answered earpiece type(blue), 13.51% answered fascia type(red), 6.31% answered infraorbital type(green), 66.67% answered kinematic face bow(orange) and 4.5% answered none of the above(yellow).

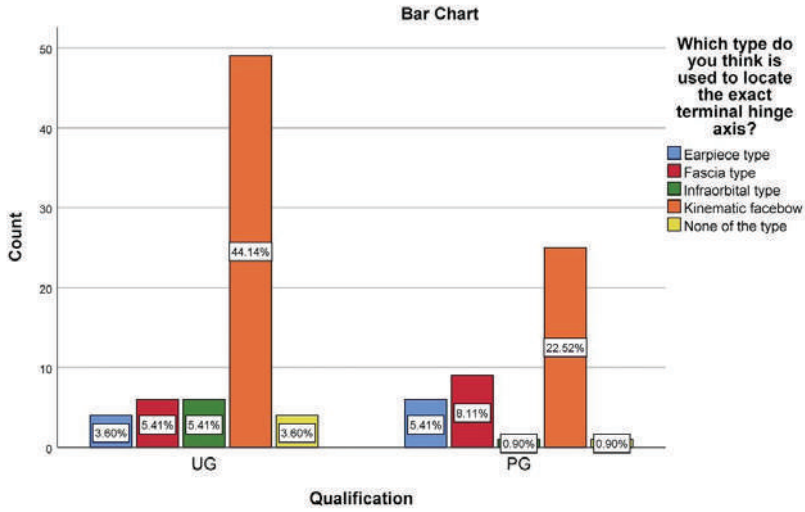


Figure 3.1: Bar graph representing the percentage response on the type of face bow used in locating the exact terminal hinge axis.X-axis represents the qualification whether the professional is an undergraduate or postgraduate.Y-axis represents the percentage of knowledge on the type of facebow used to locate hinge axis. Blue colour represents earpiece type, red colour represents fascia type, green colour represents infraorbital type, orange colour represents kinematic face bow and yellow colour represents none of the above. Higher number of undergraduate professionals answered kinematic face bow and there is a difference in responses between undergraduate and postgraduate professionals. Pearson Chi square test: 8.065a ; df- 4; p value: 0.089(>0.05), hence it is statistically not significant. This shows that there is a similar level of awareness about the type of face bow used in locating the exact hinge axis among both UG and PG professionals.

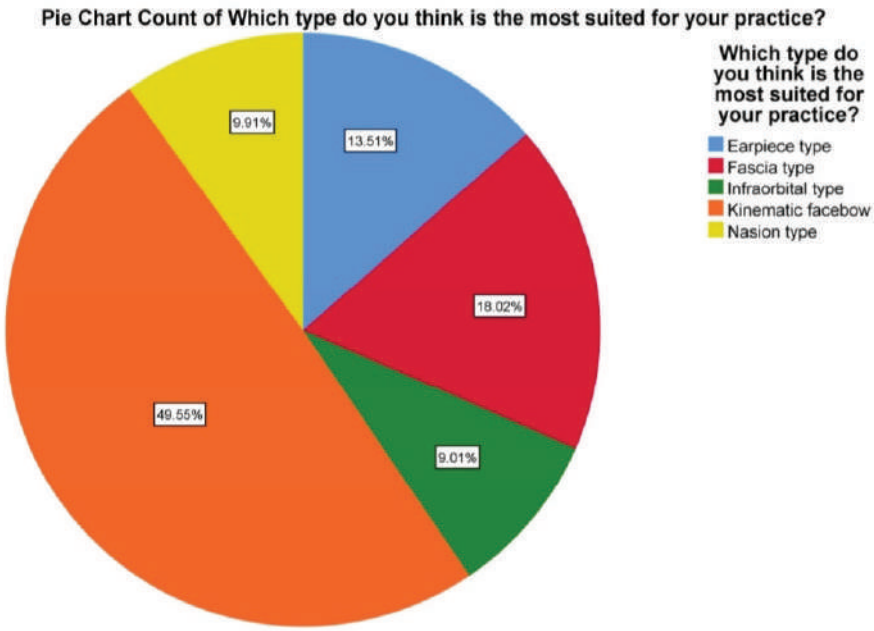


Figure 4:Pie chart representing the frequency distribution of the percentage response for the type of face bow used in dental practice.13.51% answered earpiece type(blue), 18.02% answered Fascia Type (Red), 49.55% Kinematic Face Bow (Orange), 9.01% Infra Orbital type (green), 9.91% (Yellow)

The figure 1 is about the participant's awareness of types of facebow. 78.38% responded positively, 10.31% responded negatively and 10.31% responded probably. Facebow usage is mandatory for remounting of maxillary cast in articulator having a fixed axis of opening.Failure in usage of a facebow leads to an error in occlusion.[10]

The figure 2 is about the method of handling facebows by the participants. 83.78% answered positively, 10.81% responded negatively and 5.41% answered probably. Facebow handling should be done properly to prevent errors in occlusion.[11] Figure 2.1 shows the percentage response on knowledge about the method of handling a face bow. X-axis represents the qualification whether the professional is an undergraduate or postgraduate.Y-axis represents the percentage of awareness of handling of face bows. Green colour represents yes, red colour represents no and blue colour represents may be.Higher number of undergraduate professionals answered yes and there is a difference in responses between undergraduate and postgraduate professionals. Pearson Chi square test: 2.700a ; df: 2; p value: 0.259(>0.05), hence it is statistically not significant. This proves there is a similar percentage of awareness about the method of handling face bows among both UG and PG professionals.

The figure 3 is about the knowledge on facebow used in finding the exact terminal hinge axis. The answers are 9% answered earpiece types, 13.5% answered fascia type , 6.3% from infraorbital type,66.6% answered kinematic face bow and 4.5% none of the above. Usually, Oriental Jaw relations are used in facebow to record occlusion. The position of the facebow and its relation to the articulator is important.[12–18], The kinematic Face bow connects the caliper ends to the exact hinge axis location.[19] Figure 3.1, Bar graph representing the percentage response on the type of face bow used in locating the exact terminal hinge axis.X-axis represents the qualification whether the professional is an undergraduate or postgraduate.Y-axis represents the percentage of knowledge on the type of facebow used to locate hinge axis.Blue colour represents earpiece type, red colour represents fascia type, green colour represents infraorbital type, orange colour represents kinematic face bow and yellow colour represents none of the above. Higher number of undergraduate professionals answered kinematic face

bow and there is a difference in responses between undergraduate and postgraduate professionals. Pearson Chi square test: 8.065a ; df- 4; p value: 0.089(>0.05), hence it is statistically not significant. This shows that there is a similar level of awareness about the type of face bow used in locating the exact hinge axis among both UG and PG professionals. professionals should know the reference points both anterior and posterior reference points. The posterior reference points are Baron point, Gysi, Snow and Denar point. The pointers used for finding anterior reference points are infraorbital and Nasion type facebows.[20] The pointers used for posterior reference points are Facia and earpiece type facebow.[21] The Face bow that uses external auditory meatus as a reference point is a kinematic face bow.[22] The figure 4 is about the participants choice about the type of Face bow used in Dental practice. The options are earpiece type, fascia type , infra orbital type , Kinematic Face bows and Nasion type. The responses are 13.5%, 18%, 9%, 49.5% and 9.9%. The figure 12 is about the awareness about the complications of Face bow usage. 68.5% responded positively, 15.3% responded negatively and 16.2% responded probably. The common complication that arises during Face bow usage are restriction of movement, different ear types in different individuals and alter arch for each individual.

The limitations of the study is that there are a minimum number of articles included. The other complications are the limited study population where the possible outcomes may vary. The results may vary with a bigger population.[23]

In the future awareness of Face bow types reduces errors in occlusion and proper usage ensures better treatment.[24–27]Virtual face bow transfer is a virtual procedure in which a digital maxillary cast is transferred to a virtual articulator using reverse engineering devices which is a futuristic approach.[28] Facebow transfers are used in prosthetic dentistry to record the anteroposterior and mediolateral spatial positions of maxillary cusps in relation to transverse opening and closing of mandible of the patient.[29–31]

Conclusion

Dental professionals must be aware of the types of Face bows as it helps in accurate detection of Hinge axis and proper occlusion. Incorrect records cause discomfort to patients during complete denture treatment. The above results conclude that the dental professionals require more information on types of Face bows to reduce errors in the future.

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Prevalence and Assessment of Periapical Lesions in Subjects Reporting to a Private Teaching Hospital, Chennai, India : A Retrospective Study

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Abstract

Apical or periapical lesions are inflammatory processes which are generally caused by microorganisms or their by-products and manifest due to defense response of the host to the stimulus in the root canal system. The aim of this study was to assess prevalence of periapical lesions among the south indian population. This was a descriptive study, where the data of the patients who reported to the dental clinics in saveetha dental college, SIMATS, Chennai, India, was obtained from the department of conservative dentistry and endodontics. Data was further analyzed, recorded in microsoft excel software and was subjected to statistical analysis. n = 15 for periapical lesions. From the data analysed through SPSS, it is observed that the prevalence of periapical lesions among non - vital teeth among the outpatients in the department of oral pathology is 1.1%. The age group of 60 - 70 years was the most commonly observed (60%). Male predilection was noted (67%). Radiographic examination was performed for 40% and histopathological examination was performed for 6.7% of the teeth with periapical lesions. 100% of the teeth observed in the current study were non - vital. Periapical abscess was the most common finding (53.3%). Occurring mostly in anterior maxillary, anterior mandibular and posterior mandibular regions (26.7%) each. The most commonly observed treatment modality was extraction (73.7%). Within the limitations of the current study , there exists increased prevalence of periapical pathology above 60 years, predominantly in the age group of 60 - 70 years, primarily reported in the undergraduate clinics. Routine dental checkup with both radiographic and histopathological examination of the periapical lesions is essential for the survival of the tooth and is beneficial for the community.

Keywords: *periapical lesions, histopathology, radiograph, extraction, dental caries, periapical abscess, periapical cyst.*

Introduction

Periapical or apical lesions are a set of chronic inflammatory processes where the causative factor is the presence of microorganisms or their by- products which are present in the periapical tissue .[1,2] The reason for the formation of the lesion is due to the reaction of the host tissue as a defense mechanism against noxious stimulus .[3,4] The formation of the periapical lesions occur frequently with dental caries as a predecessor .[5,6] Preceding the formation of periapical lesions, there commonly occurs the inflammation of the pulp which is known as pulpitis .[7] This pulpitis is of 2 types namely, acute and chronic pulpitis .[8] The common sites at which lesions occur are the apex and surrounding the apex .[9] For radiographic examination, a physician usually

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observes the size of the lesion, location of the lesion, periodontal ligament space widening, lamina dura and bone loss .[10] For the histopathological examination, the most commonly observed features included the cell predominance , type of epithelium present, presence of wall, cavity and blood vessels .[11–13]

Previous literature showed that periapical lesions were the most common inflammatory processes that are chronic in nature which are present in the jaws .[14] It was also found that this process most predominantly affects people in the third to fourth decade of life .[15] In a study conducted by N.A. Luna et al, 2009, it was observed that 79% of the root canal treated teeth presented with a lesion of some kind .[16] Due to lack of proper diagnosis and a wrong treatment decision, the end result is an unresolved condition that persists even after treatment .[17,18] There is presence of a widely held view that most of the periapical lesions, to be exact, almost half of all periapical lesions were thought to be cysts.in a study conducted by Ramachandran. N et al ,1996, found that out of the entire study population, cysts were found to be the least , making up merely 15% of all periapical lesions with radicular cysts being the lowest incidence among cysts thereby proving the previous thought invalid .[19] The major difficulty that was faced by researchers in previous studies was the presence of undefined radiographic characteristics which further affected the clinical validity of the diagnosis .[20]

The requirement of the current study was that there was a presence of a major inconsistency between the clinical findings, radiographic findings and histopathological findings of periapical lesions .[21,22] There was also the absence of certain clinical markers which affected the final diagnosis and treatment adversely .[23–25] This showed that clinical and radiographic findings alone were insufficient for diagnosis and that histopathological examination was of absolute necessity .[26,27] The type of radiograph will also determine the diagnosis. Newer imaging modalities such as cone beam computed tomography (CBCT) and orthopantomogram (OPG) aid in better analysis of the lesion to provide a precise diagnosis .[28] A prompt follow up is also required in order to study the disease progression post treatment .[29] The study will aid dental professionals in understanding the need for histopathology as an essential tool in achieving precise diagnosis which will cause the condition to resolve .[30,31] Thus the aim of the current study was to assess prevalence of periapical lesions among elderly subjects reporting to a private dental college and hospital.

Materials and Methods

The study was designed as a comparative and a descriptive study .[32] where the data of all the patients reporting to saveetha dental college , SIMATS, chennai , India with periapical lesions were obtained from the department of conservative dentistry and endodontics where 86000 patient records were reviewed and analysed between june 2019 and march 2020. This was a university setting and the study was conducted in the dental clinics of saveetha dental college. This setting came with various pros and cons. The pros included the presence of a versatile population and an abundant availability of data.Some of the cons included the study taking place in an unicentred setting and possessing a very limited demographic. The dependent variables in this study included the radiographic markers and histological markers. The independent variables include the age of subject, gender of the subject and the frequency. This was a correlation and association type of analysis. The selection of the study population was performed at random . This population was selected from the patients who visited the undergraduate and postgraduate dental clinics in saveetha dental college. The approval to undertake this research study had been approved by the ethical board of saveetha university (applied). n = 15 cases were reviewed and cross verification was performed by an additional reviewer. The minimisation of sample bias was performed by an additional reviewer, acquiring all the data from within the university and as an additional measure, simple random sampling was performed .[33] There was a presence of high internal and external validity. The data was then arranged in a methodical manner using microsoft excel software and was tabulated on the basis of 8 parameters namely, age , gender, radiograph, histopathology, tooth vitality, clinical diagnosis, site and treatment performed. Data pertaining to presence of non - vital teeth among patients were also assessed to determine the incidence of periapical lesions among these non vital teeth. For the non - vital teeth the age groups of the patients and the site of occurrence were noted. The data was validated by an additional reviewer. Any incomplete or censored

data that was present in the collected data was excluded from the study. Statistical analysis of the compiled data was performed using IBM SPSS statistical analyzer. Chi square test was done for statistical analysis .[10,34,35] The inclusion criteria for this study was outpatients with presence of periapical lesions irrespective of their age or gender. The exclusion criteria included outpatients who did not have the presence of periapical lesions.

Results and Discussion

The total sample size was n = 15 for patients who had periapical lesions. For patients who had non - vital teeth, n = 1310. The prevalence of periapical lesions in the dental clinics was found to be 1.1 %, meaning out of 1310 non vital teeth only 1.1 % of them had the presence of periapical lesions. The mean age group of the study for periapical lesions was found to be 70.5 years with the age group of 60 - 70 years being the most prevalent. Among non vital teeth, the most commonly observed age group was 28 to 48 years.male predilection (67 %) was observed in the case of periapical lesions. Radiographic examination was performed for 40 % and histopathological examination was performed for 6.7 % of the study population. As mentioned previously 100 % of the teeth showing periapical lesions were observed to be non vital. Periapical abscess was the most commonly observed (53.3 %). Occurring most commonly in anterior maxillary, anterior mandibular and posterior mandibular regions with (26.7%) of the population each. Most common site for non vital teeth was found to be the posterior mandible (44.2 %). The most commonly observed treatment modality was extraction which was done for 73.3 % of the cases with periapical lesions whereas in the case of non - vital teeth 100% of the affected teeth underwent root canal treatment.

The treatment choice for periapical lesions may be influenced by a variety of reasons such as the size of lesion, extent of lesion, association to surrounding structures and patient cooperation. There is no set protocol for management of periapical lesions and there is still ongoing debate regarding an ideal protocol which also addresses conservative management of the lesion .[36] Periapical changes occur in concordance to the changes that occur in the pulp over a period of time almost in every case. This does not mean that periapical pathology cannot occur without pulpal pathology, so assessment of the disease process is of absolute necessity .[37] If the disease process is found to be of origin from the pulpal tissues, the microbial infection and cell death could occur not only at the apex but also periapically. This is what dental professionals observe as a radiolucency in the periapical region .[38] Assessment of the patient's history is also essential to rule out the presence of congenital or hereditary factors that may be pertaining to the formation of periapical lesions .[39]

Among all the non - vital teeth the most predominant age group was 28 to 48 years (44.6 %) followed by 8 to 28 years (38.8 %), 48 to 68 years (15.6 %) and 68 to 88 years (0.8 %) . in a recent study by G. Kandemir et al, 2019 it was seen that similarly a younger age group was seen with presence of non vital teeth, with the majority of the vitality loss occurring in the age group of 18 to 40 years .[30] The primary reason for the loss of tooth vitality at a young age is dental caries which could be influenced by a variety of factors such as diet, poor oral hygiene, lack of professional dental treatment .[31,32] When we observed periapical lesions, contrasting results in relation to non vital teeth were observed as the most common age group with presence of periapical lesions was 60 - 70 years (60 %) followed by 70 - 80 years (27 %) and 80 - 90 years (13 %). Periapical were seen in an older study population unlike non vital teeth. In a previous study conducted by Akinyamoju AO et al, 2014, contradicting results were observed, the peak age of incidence was found to be 20 - 30 years .[18] Also in the study conducted by Gbadebo SO et al, 2014 , the peak age of incidence was found to be 32 years .[21] The primary reason for these literature findings could be due to the primary cause of periapical lesions being an endodontic pathology. immune response is increased in a younger age which could induce proinflammatory cytokines which is followed by periapical pathology .[33] The reason for the findings of our study is again dental caries but due to the reduced healing capacity of the body as it ages and its inability to fight infection could be the cause of periapical pathology in older individuals .[34] Thereby the findings of our study were not in concordance with that of literature. -

Among periapical lesions a male predilection was observed constituting 67 % and females constituting 33%. In a study conducted by Enriquez FJ et al, 2015, a female predilection was observed with females

constituting 65 % and males constituting 35 % .[35] Similarly the study conducted by Akinyamoju AO et al, 2014,observed a female predilection with females making up with 51.9 % .[18] Another study by Silva BSF et al, 2017, showed the presence of a male predilection similar to the one observed in the current study .[7] The study conducted by Gbadebo SO et al, 2014 also showed a male predilection with 53 % .[21] The results obtained from literature were varied and this could be due to the difference in study setting, population, clinical condition. Male patients were also found to a greater incidence of periapical abscesses.

Radiographic analysis was performed for 40 % of the study population and was not done for the remaining 60%. Likewise histopathological examination was performed for a mere 6.7 % of the population which further increases the need for the current study to increase awareness regarding the use of histopathology for a spot on diagnosis. In a study performed by Kruse C et al, 2017, radiographic diagnosis was performed for 100 % of the study population and histopathological examination was done for 42% of the population. Reasons for this could be improved methods and techniques for diagnosis, age of the patient, existing condition of the tooth, awareness of the dental professional and patient compliance some of which may have been lacking in the setting of the current study .[48,49]

As mentioned previously all the teeth in the current study were non - vital (100 %). In a study conducted by Kuc et al, 2000, it was found that 93.8 % of the teeth were non vital which is in concordance with the results of the current study .[50] From this we can infer that the vitality of the tooth plays a role in the majority of cases for the formation of periapical lesions .[51]

The most commonly observed lesions were periapical abscesses with 53.3 % followed by periapical cyst with 33.3 % and finally radicular cyst with 13.3 %. Chi square test revealed the distribution to be insignificant. In the studies such as the ones conducted by Akinyamoju AO et al, 2014 and Gbadebo SO et al, 2014 .[18, 21] where the most commonly observed lesions were periapical granulomas and periapical cysts respectively. In another study conducted by Çalışkan MK et al, 2016, the most commonly observed lesions were periradicular granulomas constituting 72 % of the study population .[39] Reason for this inconcordance may have been due to variation in the population and setting but further research is required to correlate race with the type of periapical lesions. More probable reasons may include lack of awareness of the lesion and misdiagnosis.

We also observed the common sites of incidence of both non vital teeth and periapical lesions. In the case of non vital teeth the most common site was the posterior mandible with 44.2 % followed by anterior maxilla (26.8 %), posterior maxilla (22.3 %) and anterior mandible (6.4 %). The reason for this could be due to the increased incidence of dental caries in the posterior region such as that observed in the study conducted by AH Wyne et al, 2008 .[53] Similarly in periapical lesions most common sites were anterior maxillary, anterior mandibular and posterior mandibular regions (26.7%) each the reasons for which could be similar to that of non vital teeth.

The most common treatment modality chosen for periapical lesions in the current study were extraction being done for 73.3 % of the population followed by no treatment for 20 % of the lesions and finally periradicular surgery for 7 % of the population. In a study conducted by Gbadebo SO et al, 2014 [21], it was found that the entire study population had undergone periradicular surgery. The reason for this inconcordance could be due to availability of infrastructure, patient compliance, experience of the dental professional and awareness regarding the treatment of the lesion.

In this study geographic limitation was present where the population was predominantly south indian. Some of the collected data was unclear, incomplete or the reporting parameters were not considered. Another limitation was that the study was a unicentred study taking place only within saveetha dental college, SIMATS, Chennai, India .[54,55] The study could pave the way for new research which will focus on the prevention of periapical lesions and also provide methods for the improved assessment of lesions which will lead to better treatment prognosis. A greater sample size combined with a variety in ethnicity which will yield a better result.

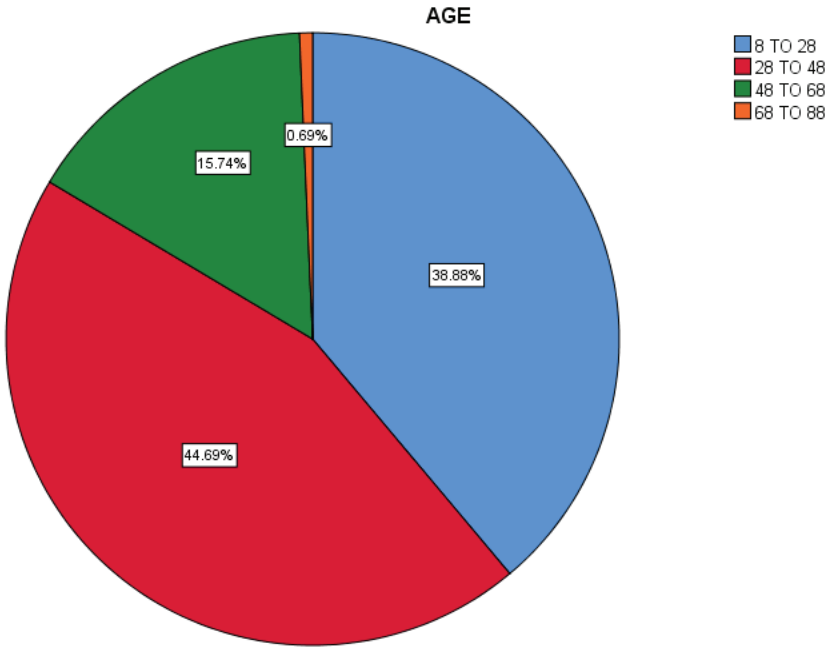


Figure 1 : This pie chart represents the distribution of non-vital teeth among different age groups on a scale of 1 – 100 % where blue colour denotes age group of 8 to 28 years, pink colour denotes age group of 28 to 48 years, green colour denotes age group of 48 to 68 years and orange colour denotes age group of 68 to 88. Most commonly observed age group was 28 to 48 years followed by 8 to 28 years, 48 to 68 years and finally the least commonly observed age group was 68 to 88 years .

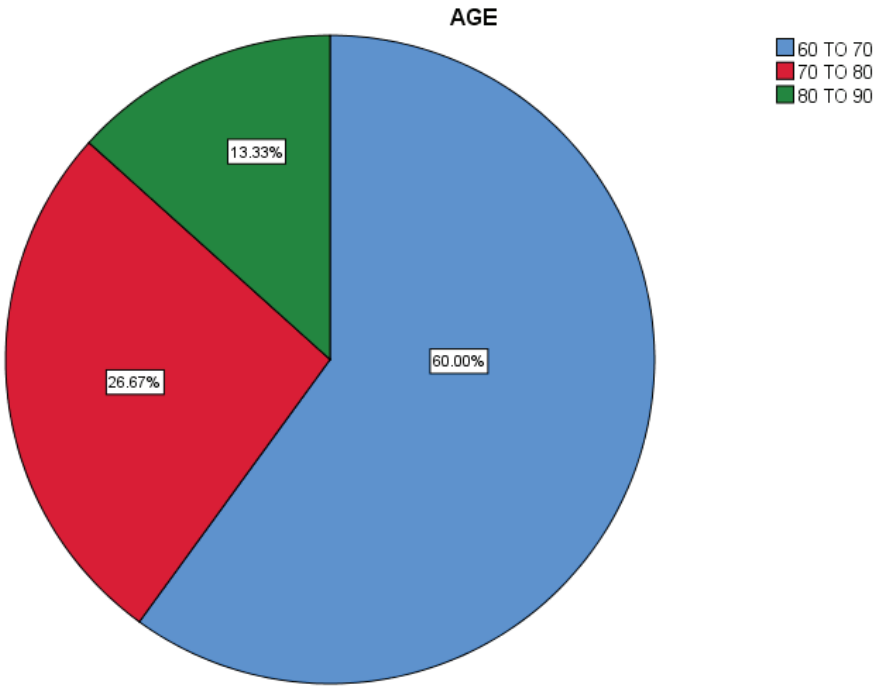


Figure 2 : This pie chart represents the distribution of periapical lesions among different age groups on a scale of 1 – 100 % where blue colour denotes age group of 60 to 70 years, pink colour denotes 70 to 80 years and green colour denotes 80 to 90 years. Most commonly observed age group was 60 to 70 years followed by 70 to 80 years and finally the least commonly observed age group was 80 to 90 years .

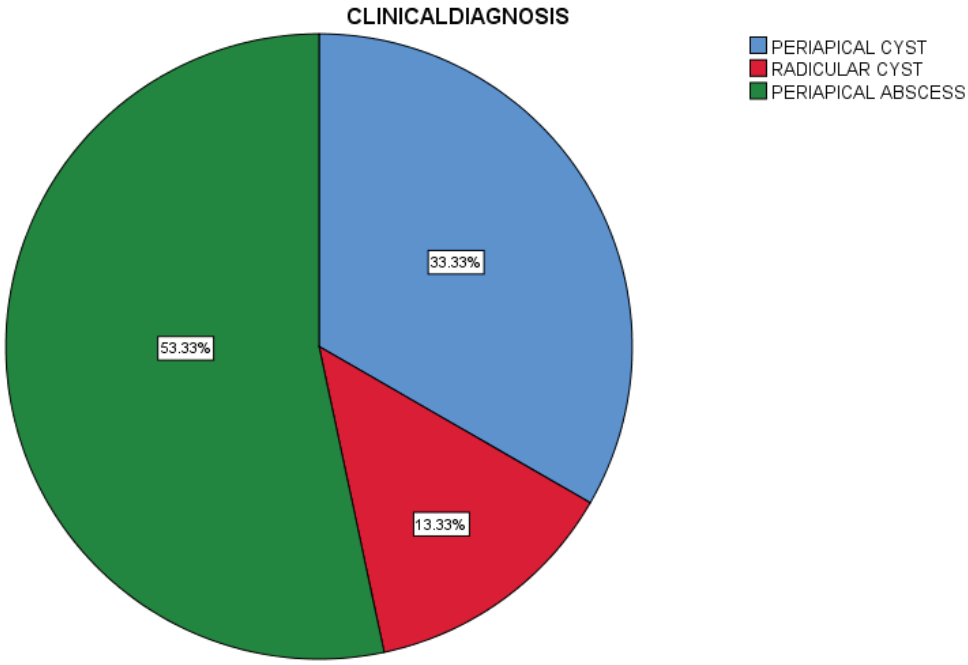


Figure 3 : This pie chart represents the distribution of clinical diagnosis of periapical lesions on a scale of 1 – 100 % where blue colour denotes periapical cyst, red colour denotes radicular cyst and green colour denotes periapical abscess. Most of the lesions were found to be periapical abscesses which is the most common followed by periapical cyst and radicular cyst.

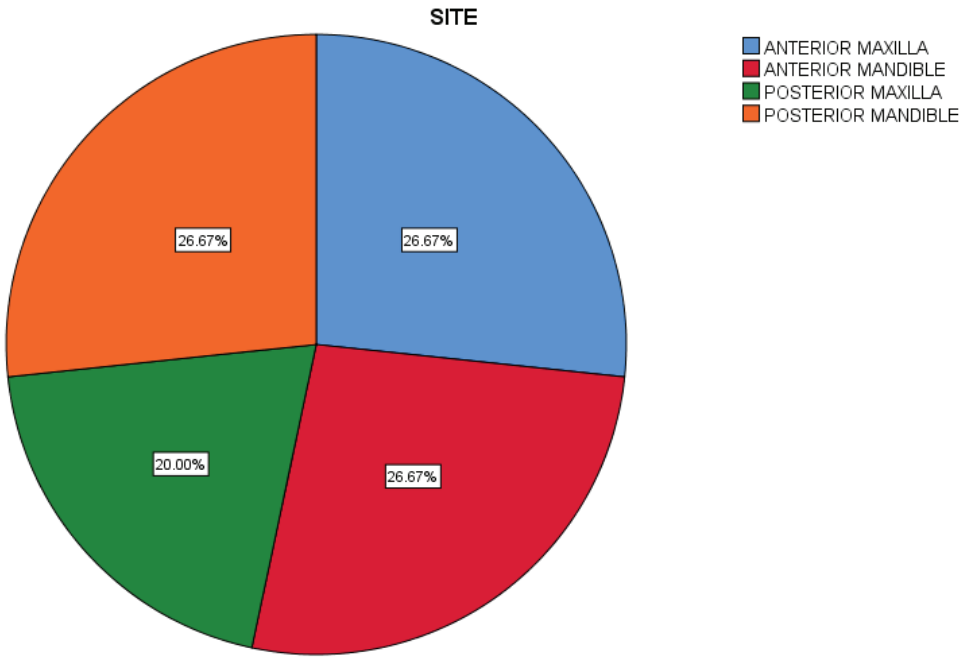


Figure 4 : This pie chart represents the distribution of sites associated with periapical lesions on a scale of 1 – 100 % where blue colour denotes anterior maxilla, red colour denotes anterior mandible, green colour denotes posterior maxilla and orange colour denotes posterior mandible. The most common sites where periapical lesions were observed were the anterior maxilla, anterior mandible and posterior mandible followed by posterior maxilla.

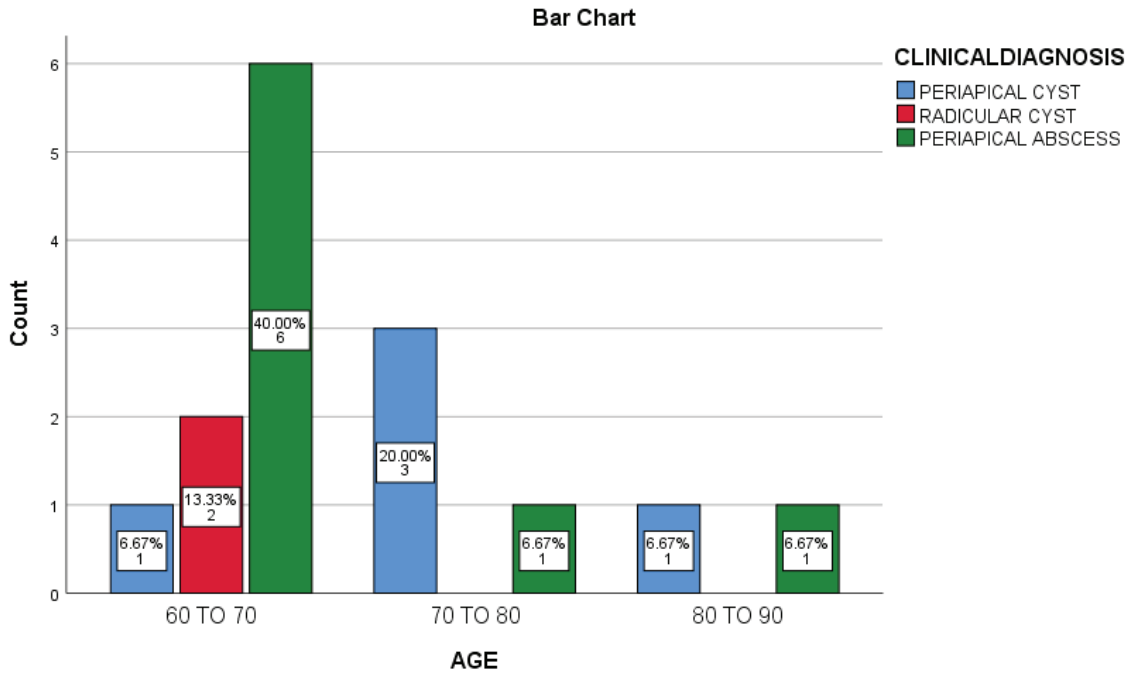


Figure 5.1 : Bar graph showing association of age of the population with the clinical diagnosis of the periapical lesions. X axis represents the age of the population and y axis represents the count and percentage of clinical diagnosis. Periapical abscess was common among 60 to 70 yr old subjects. Chi square test was done and the association was found to be statistically not significant. Pearson's Chi square value: 5.823, DF: 4, p value: 0.213 (>0.05) statistically not significant. Hence there is no age predilection for any particular type of periapical lesion.

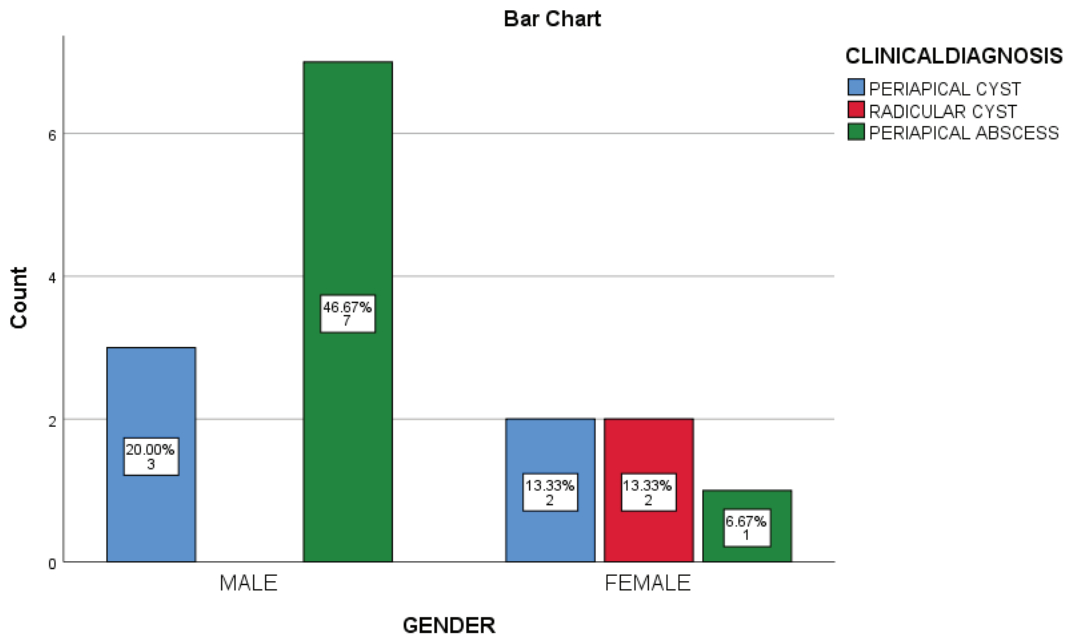


Figure 5.2 : Bar graph showing association of the gender of the population with the clinical diagnosis of the periapical lesions. X axis represents the gender of the population and y axis represents the count and percentage of clinical diagnosis. Chi square test was done and the association was found to be statistically significant. Pearson's Chi square value: 5.663, DF: 2, p value: 0.05 (=0.05). Hence statistically significant showing males have a higher incidence of periapical abscess compared to females.

Conclusion

Within the limitations of the current study, there exists increased prevalence of periapical lesions in the age group of 60 - 70 years and males had a higher prevalence of periapical abscess compared to females. Routine dental checkup with both radiographic and histopathological examination of the periapical lesions is essential for the survival of the tooth and is beneficial for the community.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Assessment and Study of Types of Finish Lines in Tooth Preparation - An Observational Study

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Abstract

The fixed and removable prosthodontics aims at the maintenance and preservation of the tooth structure . Restoration of teeth is possible only if sufficient space is created for the application of thickness of material required . The types of finish lines given for all ceramic , metal ceramic and all metallic tooth preparations are Chamfer , Shoulder finish line, etc , The aim of this observational study is to assess the different types of finish lines given in the tooth preparation . The study is focused to assess the different types of finish lines in tooth preparation and to assess the most preferred type of finish line as the tooth margin . Around 1000 different tooth preparations were observed to assess the types of finish lines . The data was obtained from 86,000 archived patient records . All the collected data were reviewed and statistically analysed . In the present study , most of the patients were 31 to 45 yrs old (42 %) . Among the patients , 56% were Male . Majority of the tooth preparations were done in Sextant 2 (35%) . The most preferred type of finish line was the Shoulder finish line (77 %).The study has observed that the shoulder finish line was the most preferred type of finish line .

Keywords : *Tooth preparation ; Types of finish lines ; Choice of finish lines*

Introduction

The fixed and removable prosthodontics aims at the maintenance and preservation of the tooth structure . Restoration of teeth is possible only if sufficient space is created for the application of thickness of material required . It is given for better marginal adoption and fitting of the restoration [1 , 2 , 3] . The types of finish line given for all ceramic , metal ceramic and all metallic tooth preparations are Chamfer , Shoulder , Knife edge finish lines [4] . They are given on the basis of the requirement and tooth structure . Ideally , all finish lines should be placed supragingivally [5 , 6] . Due to esthetic and carious considerations [7 , 8 , 9] , however subgingival placement of the finish line is preferred [10 , 11 , 12] .

No one type of finish line can be used for all crown preparations . In lower anterior teeth or periodontally treated teeth [13] , the knife edge finish line appears to be the treatment of choice [14] . The long term goal desired by a patient and the doctor is durability and longevity [15] . This can be achieved initially by tooth preparations that are clinically sound [16] . But another important factor that contributes to this is the selection of the finish line . Preference for a particular type of finish line preparation allows ample room for various criteria like the position of the teeth contributing to esthetics [Anterior or Posterior] [17] , Restoration material [Metal or Ceramic] [18] and the bulk of the restorative material [19 , 20] . The indications and the contraindications for each type of finish line will be reviewed . The ultimate goal in fixed & removable prosthodontics is the maintenance & preservation of the remaining dentition [21] . In short teeth and preparation for Porcelain and Porcelain - Gold crowns , the full shoulder bevelled preparation is the treatment of choice

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. In full Gold and Acrylic veneered Gold crowns , as in endodontically treated , the Chamfer preparation is the treatment of choice [22] . The various types of finish lines are Chamfer , Heavy chamfer , Shoulder , Round/Radial shoulder , Slope shoulder , Shoulder with bevel , Knife edge , Chisel edge & Butt joint finish lines .The present study was conducted to assess and study the various different types of finish lines in tooth preparation and to assess the most preferred type of finish line as the tooth margin .

Materials and Methods

The observational study was done in a university setting . Samples of 10 months from June 2019 - March 2020 was reviewed . A collected data of 868 patients were examined by 2 reviewers , Finish lines and tooth preparation were the inclusion of the data . All available data were included without any sorting process to minimise sampling bias . Non probability inclusion was done to ensure internal validity and Homogenization & replication of experiment was done for external validity .

The data from 86,000 patients visited Saveetha dental college during the time period of June 2019 to March 2020 were reviewed and the data of all tooth preparations were collected . Non specific samples were excluded from study . The collected data was entered in an excel sheet and the data verification was done by an external reviewer . Then the data were imported to statistical analysis . The variables were defined and SPSS by IBM was the statistical software used for analysis . Chi - square test was done by keeping age & gender as independent variable and tooth preparation & type of finish line as dependent variable . Correlation analysis and association analysis were done.

Results and Discussion

Among the sample size of 867 tooth preparations , 42 % of the patients were 31 to 45 yrs old , 32 % were 18 to 30 yrs old , 21 % were 46 to 60 yrs old and 5 % of the patients were above 60 yrs . In the total study population (Figure 1), 56 % were Male and 44 % were Female (Figure 2). 35 % of the total tooth preparation was done in Sextant 2 , 14 % in Sextant 3 , 14 % in Sextant 6 , 13 % in Sextant 4 , 13 % in Sextant 5 and 11 % of the preparations in Sextant 1 . There was a statistically significant difference between the sextants in receiving fixed partial denture treatment with Sextant 2 being the predominant one receiving treatment (Chi Square test ; $P < 0.05$)(Figure 3).

The most preferred type of finish line was Shoulder finish line - 77 % . Chamfer finish line was preferred in 13 % , Radial shoulder in 3 %, Heavy chamfer finish line in 3 % , Feather edge & Knife edge finish lines in less than 1 % of tooth preparations . There was a statistically significant difference between the finish lines placed in fixed partial denture treatment,with shoulder finish line being the predominant one receiving treatment (Chi Square test ; $P < 0.05$).

(Figure 4).There was a statistically significant difference between the sextants in receiving fixed partial denture treatment.with Sextant 2 being the predominant one receiving treatment (Chi Square test ; $P < 0.05$) (Figure 5).

Age of the patients in the study

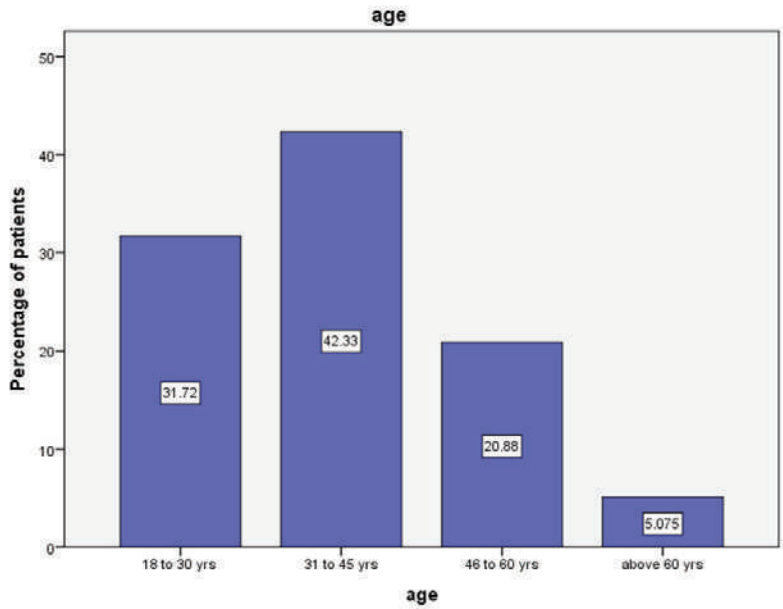


Figure 1 : The bar graph represents the age of the Patients in the study . The X - axis represents the Age groups and Y - axis represents the percentage of the patients . Among the total sample size of 867 patients , 31.7 % (275) were 18 to 30 yrs old , 42.3 % (367) were 31 to 45 yrs old , 20.9 % (181) were 46 to 60 yrs old and 5.1 % (44) were above 60 yrs old . Tooth preparations were predominantly done in patients of 31 to 60 yrs of Age (63.2 %) . N = 867 .

Gender of the patients in the study

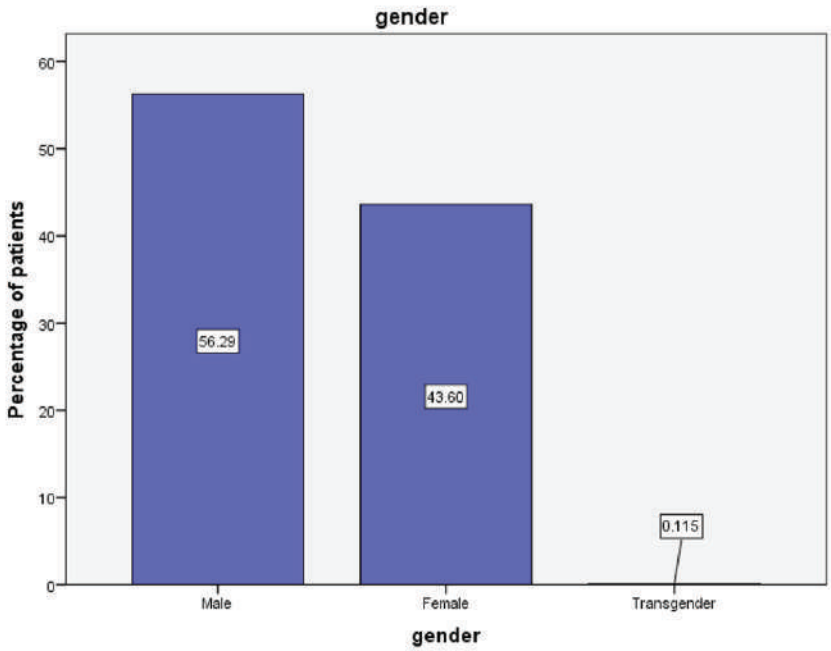


Figure 2 : The bar graph represents the gender of the Patients in the study . The X - axis represents the Gender and the Y - axis represents the percentage of the patients . Among the total sample size of 867 patients , 56.3 % (488) were Male , 43.6 % (378) were Female and 0.1 % (1) were Transgender . Thus Males were more prevalent to Fixed partial denture treatment than Females (56.3 %) . N = 867 .

Teeth sextants involved in the study

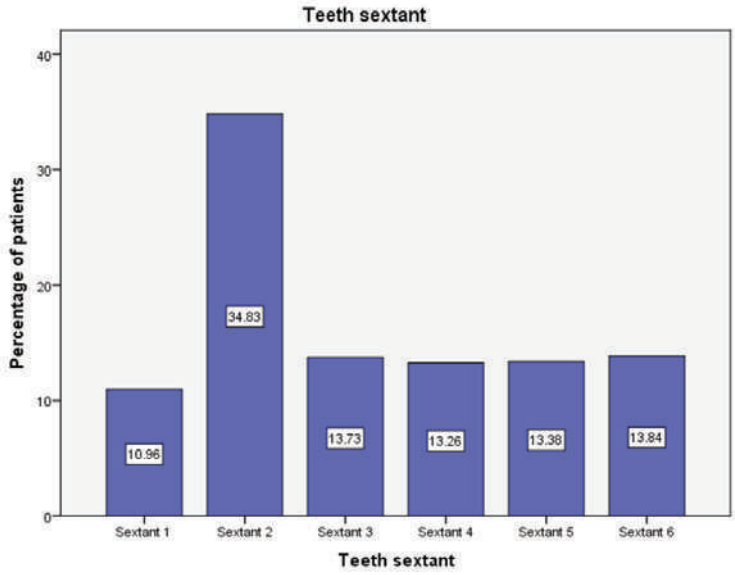


Figure 3 : The bar graph represents the teeth sextant of tooth preparation .The X - axis represents the Teeth sextant and the Y - axis represents the percentage of the patients . Among the total sample size of 867 patients , 11 % (95) of tooth preparations were done in Sextant 1 , 34.8 % (302) were in Sextant 2 , 13.7 % (119) were in Sextant 3 , 13.3 % (115) were in Sextant 4 , 13.4 % (116) were in Sextant 5 and 13.8 % (120) were done in Sextant 6. There was a statistically significant difference between the sextants in receiving fixed partial denture treatment with Sextant 2 being the predominant one receiving treatment (34.8 %) . N = 867 .

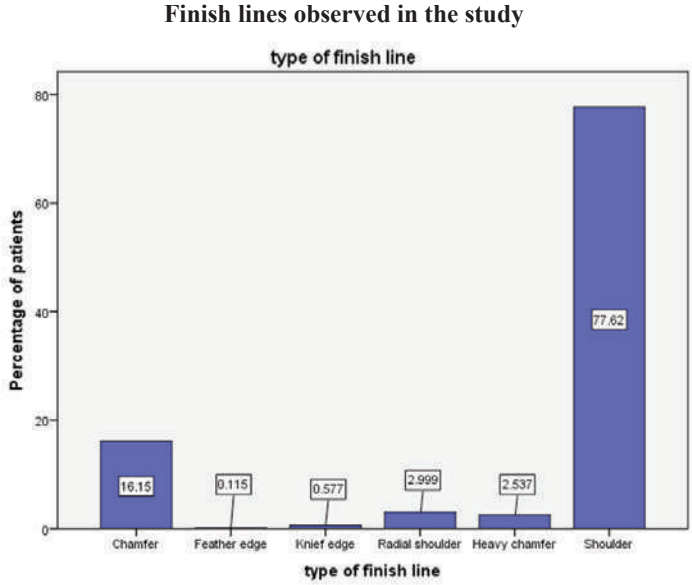


Figure 4 : The bar graph represents types of finish lines in tooth preparation . The X - axis represents the type of finish line and the Y - axis represents the percentage of the patients . Among the total sample size of 867 patients, 16.1 % (140) of the finish lines were Chamfer finish line , 0.1 % (1) were Feather edge finish line , 0.6 % (5) were Knife edge finish line , 3 % (26) were Radial shoulder finish line , 2.5 % (22) were heavy chamfer finish line and 77.6 % (673) were Shoulder finish line .There was a statistically significant difference between the finish lines placed in fixed partial denture treatment, with shoulder finish line being the predominant one receiving treatment (77.6 %) . N = 867

Finish lines in Teeth sextants in the study

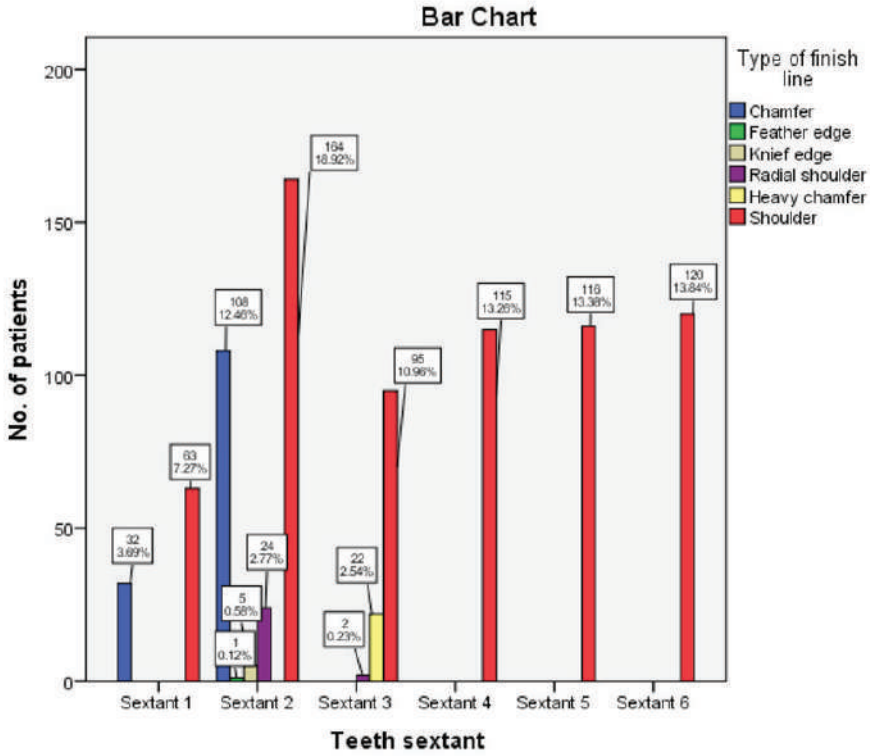


Figure 5 : The bar graph represents the association of Teeth sextant and the type of finish lines . The X - axis represents the Teeth sextant and the Y - axis represents the frequency of type of finish line such as Chamfer (Blue) finish line , Feather edge (Green) finish line , Knife edge (Tan yellow) finish line , Radial shoulder (Violet) finish line , Heavy chamfer (Yellow) finish line and Shoulder (Orange) finish line .There was a statistically significant difference between the finish lines in various Sextants and type of finish line placed in fixed partial denture treatment, with Sextant 2 being the predominant one receiving treatment and Shoulder finish line being the predominantly preferred one as the tooth margin . This was found to be statistically significant ; p = 0.01 (Pearson Chi Square test ; P < 0.05) . N = 867 .

The ideal finish line should have the following characteristics including , Shallow levels nearly parallel to cavosurface should be avoided because the restoration will be too thin at this area and may chip easily . The discrepancy decreases with the increase in angulation of the bevel and the bevel should not produce a very acute margin , which can lead to fracture of the wax pattern during removal [23] . Shoulder finish line has a gingival finish wall perpendicular to the axial surfaces of the teeth . If the marginal wall was at 120 degree to the axial wall , then it is termed a sloping shoulder. A shoulder finish line was preferred for all ceramic restorations [24] , where sufficient thickness of the margin was required for structural durability [16] . All anterior restorations were fabricated with a shoulder margin where aesthetics was the primary concern . The placement of finish lines influences the fabrication of the restoration and the final outcome of the treatment [25] .

In the present study , we concluded that among the total sample patients , Male gender was predominant. On the other hand , the study by Geiballa GH , et , al ,. [26] had opposing findings of female predominance. Also the study by Shillingburg HT , et , al ,.[16] had opposing findings of Female predominance . This opposing finding may be due to personal hygiene of the individual . In the present study , we concluded that the majority of the tooth preparations were done in Sextant 2 . The study by Rosenstiel SF , et , al ,. [22] had similar findings . In the present study , we concluded that the Shoulder finish line was the most preferred type of finish line done in tooth preparations. The study by Shillingburg HT , et , al

,. and Cho L , et , al ,. [27 , 16] had similar findings . On the other hand , the study by Tjan AHL , et , al ,. , Grajower R , et , al ,. and Noonan JE , et , al ,. [28–30] had differential opposing findings .

This study was single centered and large data for various different finish lines needs to be analysed further.. This study also doesn't represent all ethnic groups and populations .Future scopes were observation of more population , Assessment of the expectations of the patients , Assessment of adaptation of the prosthesis and other choices of finish lines .

Conclusion

This study observed that there was a statistically significant difference between the finish lines in various Sextants placed in fixed partial denture treatment,with shoulder finish line being the predominant one as the tooth margin . Predominantly tooth preparations were done in Sextant 2 , the Anterior region .

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Trends in Prosthodontics - A Review

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Abstract

Prosthodontics is concerned with the impact of tooth or tissue damage and partial or complete loss of teeth on oral function in its broadest sense. It deals with this largely through prosthetic replacement. The discipline occupies a major portion of a dental school curriculum, and dental practitioners usually devote much of their practice to prosthodontic services. It is continuously evolving consequent to the rapid advancements in dental biomaterials science, clinical and laboratory techniques and technologies, education, research, therapeutics, literature, and interdisciplinary developments. While the basic process of making dentures has changed little over the past several decades, new materials and techniques can help laboratories and clinicians provide functional, esthetic restorations that offer exceptional value to patients. Implant treatment is a tremendous adjunct to removable prosthodontics in the treatment of edentulous patients, but it is not within the financial reach of all dental patients.In future, prosthodontics has a very good scope and it is going to be beneficial for the patients who are edentulous.Technology developments are very much needed in future for its very well establishments .This review is aimed to discover some fascinating facts about the trends that are prevailing in prosthodontics and its future scope.

Keywords *: prosthodontics;trends;developments;future education; research.*

Introduction

Prosthodontics is a well defined and broad dental specialty catering to a wide range of oral rehabilitative treatment needs of the community. Prosthodontics has continuously evolved as a result of progress in biomaterial science,laboratory technology, multidisciplinary advancements and clinical techniques [1]. In the past several years, prosthodontics has begun to establish itself as a "knowledge-based" speciality, and not just a "skill-based" specialty. This change will be the key foundation in our ability to prosper. Emerging concepts, materials, and technologies impact the education, research, and practice of prosthodontics [2]. Many paradigm shifts and watershed events have signaled the end of historical boundaries in the dental profession such as osseointegration, internet, and tissue-engineering [3]. The future prospects of our discipline can be examined and understood only in the context of current trends and prospects [4].

Several other authors like Carlson , et al ,2006 , [5] have described in his study that the main focus in prosthodontics is that it has shifted from removable prosthesis to fixed dentures . implant-supported restorations have attracted intense interest in the dental community.Even authors like Kumar CP , et al , 2016 [6] have described in his overview about the enormous advancements that took place in various disciplines that's related to Prosthodontics .

True need can only be identified in a dialogue between the professional and the patient.Dental status and income , but not age, place of residence, nor concern for dental appearance, influence desire for implant

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treatment at the end of the studied 10-year period. There is no objective need in prosthodontic treatment. Manifests need and demand change over time, and are influenced by the patients' attitude and situation, and by the dentist's practice profile [7].

The aim of this review is to explore some detailed information regarding the Trends in prosthodontics .

Materials and Method

This research was conceived as a scoping literature review. First we sought existing reviews in the last decade that had assessed the all the possibilities of trends in prosthodontics.We did not follow a systematic review or meta – analysis .In seeking to identify relevant literature from the last 20 years , we accessed databases that are commonly used to index general dental health including pubmed , google scholar [8–13] , Searches of the reference list from the relevant review article were also employed to identify further relevant studies .Search terms included 'prosthodontics ' ; ' trends ' ; 'development ' ; ' future education ' ; ' research ' . Considered research was limited to titles that's related to prosthodontics , computer – aided manufacturing in prosthodontics , trends in removable prosthodontics , future value of prosthodontics and all other articles that were related to any other categories were excluded . The level of evidence of the reviewed articles were categorized as per the criteria of Centre for Evidence - Based Medicine , Oxford ,UK [14]

Number of articles selected : 33

Number of articles with similarity : 27

Number of articles with known concept : 7

Number of articles with recent advances : 8

RECENT HISTORY AND CURRENT TRENDS

Any kind of prognostication needs to be based on cur-rent realities. As far as they are known, these are complex. Prosthodontics has evolved from many years of clinical experience, into what may be considered to be an expense-related hierarchy of prosthodontic treatment options .

Threats to the integrity of the dentition have been through the application of prosthodontic solutions in the belief that a loss of arch intactness would result in a reduction in oral health and function. Such a therapeutic rationale gave rise to the '28-tooth syndrome', which dictated the need for complete dental arches, i.e. 28 teeth, 14 in each arch [15] . Regardless of earlier attempts at implants, [16] it was the principle of osseointegration that launched the intensive development of implants in dentistry in 1965, which has since revolutionized the treatment of edentulous patients [17 18] .

Management Trends

A central goal of prosthodontics is to stabilize the occlusion and restore oral function, an approach that has enjoyed success due to the wide range of treatment possibilities available for oral rehabilitation [5] ,so it has to be in control. Cost as a constraint for treatment is evident from a global perspective of poverty, but a reality also in the wealthiest countries, where many people cannot afford high-cost dental treatment [19] .A modified application of digital dentistry is the quantification of the effect of the proposed treatment prior to the active treatment phase. This takes advantage of the software precision in measurements and quantification. On the 3D models, volumes and distances can be precisely measured , and in the dental practice, analysis of tooth preparation can occur prior to prosthesis fabrication [20].

EVIDENCE OF THE VALUE OF PROSTHODONTICS

Prosthetic rehabilitation is done to regain function , esthetics and speech [21]. Evidence based clinical care has recently been widely discussed in the context of both medicine and dentistry .Evidence based care is defined as the provision of treatment based on a combination of the highest quality relevant research findings , the clinicians skills , and the patients particular needs . The quality and quantity of relevant research are not always are not always sufficient, however [4]. Modern prosthodontics can affect the most wonderful solutions through oral rehabilitation, but prosthodontists are in danger of letting their advanced technology block out their vision of humanistic priorities. The international prosthodontic community should provide guidance into ways and means of helping the disadvantaged achieve an improved quality of life [6].

CLINICAL IMPLICATION

The clinical skills required to deliver excellent complete denture care are also paramount to successful implant prosthodontics (fixed and removable) and esthetic dentistry. The most common gums disease are gingivitis and periodontitis , [22] . Acrylic resin denture can affect the periodontal structures of a tooth [23]. Marginal discrepancy severely affects the long term success of All ceramic complete veneer crowns [24] and ceramic restorations [25] .In cement retained restorations , there are numerous advantages for cement-retained prosthesis over screw-retained prosthesis such as passive casting, axial loading, accessibility, progressive loading, etc [26]. Even so, the opportunities to develop these skills and the interest appear to be decreasing at the same time that the need is projected to increase. In service to our patients, the profession must examine this trend closely [27]. The use of silicone maxillofacial elastomers are advantageous because it provides a wide range of customization, light weight, life-like appearance, ease of intrinsic and extrinsic coloring, non-allergenic, tissue compatibility, ease of construction and dimensionally stability [28]. The basic techniques used in all ceramic fabrications include powdered liquid glass base system, pressable glass base system, and computer-aided design/ computer-aided manufacturing (CAD/CAM) system [29].

PROSTHODONTICS : A PAST WITH FUTURE

As one of the oldest dental specialties, prosthodontics has a long history of innovation and adaptability. This overview of the field presents landmarks in the development of prosthodontics from mediaeval times to the present and speculates on some future trends [1] . Currently, dentists have a reasonable way to deliver an important health service to the population at large. However, only with a rapid advancement in the concept of delivery of total prosthodontic care can the best interests of the public be maintained [30] . Projections of future prosthodontics needs [31] show that the number of bridges needed by the elderly will increase 2.0 times and the number of dentures needed by 1.5 times over the next 20 years , then reach a plateau in the subsequent 10 years .

Results and Discussion

S.No	Author	Year	Type Of Study	Key Points	Quality of Study
1.	Hobkirk JA	2005	Review	Prosthodontics has a long history of innovation and adaptability	Strong
2.	Yumpu.com	2020	Review	Reframing the Future of Prosthodontics Growing Prosthodontics	Moderate
3.	Sadowsky , et al	2005	Review	Historical changes have taken place in dentistry	Strong
4.	Akagawa	2006	Review	Achieve greater recognition for prosthodontic	Strong
5.	Carlsson , et al	2006	Review	Prosthodontic treatment of depleted, damaged dentitions varies widely, but the replacement of missing teeth	Moderate
6.	Pradeep kumar , et al	2016	Review	Prosthodontics is a well defined and broad dental specialty catering to a wide range of oral rehabilitative treatment needs of the community.	Moderate

7.	Narby	2011	Review	An emancipatory perspective with the patient-dentist dialogue was regarded as central for an optimal treatment result in the prosthetic treatment decision-making process.	Strong
8.	Levin	1994	Review	Threats to the integrity of the dentition have been through the application of prosthodontic	Strong
9.	Branemark , et al	1997	Review	Implants are well developed .Used for prosthodontic treatments .	Moderate
10.	Ekeland , et al	2003	Review	Implants are the principles of osseointegration.	Strong
11.	Owen , et al	2004	Review	Prosthodontics are high cost efficient treatment	Strong
12.	Abduo , et al	2004	Review	Prosthodontics is defined as the dental specialty pertaining to the diagnosis, treatment planning, rehabilitation	Strong
13.	Burton Melton , et al	2000	Review	The clinical skills required to deliver excellent complete denture care are also paramount to successful implant prosthodontic	Moderate
14.	Love WB	1976	Review	The geographic areas served by licensed dental mechanics will increase	Moderate
15.	Kanatani , et al	2001	Review	Future in prosthodontics , bridges are needed.	Moderate
16.	Sukotjo , et al	2008	Review	preclinical prosthodontics clock hours are on average shorter than other schools	Strong
17.	Ong , et al	1999	Review	The undergraduate dental curriculum is undergoing rapid change and revision in dental schools	Strong
18.	Cooper , et al	2009	Review	Prosthodontics is the specialty responsible for restoration of individual teeth and replacement of missing teeth and supporting structures	Weak

19.	Ariga P , et al	2018	Review	Various anthropometric measurements have been used to determine the size of maxillary anterior teeth.	Strong
20.	Jothi S	2017	Research	In total 22 patients were wearing partial dentures.	Strong
21.	Duraisamy R , et al	2019	Research	The mean microgap at the implant-abutment interface at the external, middle, and internal points was 1.597, 1.399, and 1.831 μm	Strong
22.	Subhashree , et al	2016	Review	Microbial resistance has reached a high level in recent times .	Strong
23.	Ganapathy D , et al	2016	Research	ANOVA inferred a statistically significant difference between the four test specimens with regards to vertical and horizontal marginal discrepancy after cementation ($F=9.092$, $p<0.001$), ($F=10.97$, $p<0.001$).	Strong
24.	Subashree S , et al	2016	Review	Aloe vera is used for various purposes and also for the curing of the skin infections.	Strong
25.	Ranganathan , et al	2017	Research	The cervical and incisal marginal discrepancy scored $F = 243.408$, $P < 0.001$ and $F = 180.844$, $P < 0.001$, respectively.	Strong
26.	Vijayalakshmi , et al	2016	Review	Cellulites are the powerful skin bacterial infection .	Strong
27.	Ganapathy , et al	2017	Review	The abutment screw loosening has been a common clinical mishap affecting the success of the implant in the long run.	Strong
28.	Suvitha, et al	2016	Cross sectional study	Awareness towards patients that missing teeth has to be restored with ceramics.	Strong
29.	Ashok , et al	2014	Research	Two piece magnet retained hollow lip bumper prosthesis was fabricated to reduce the weight of the denture and to attain esthetics.	Strong
30.	Venugopalan , et al	2014	Review	Patients with orocutaneous fistulas suffer from discomfort in terms of facial esthetics	Strong
31.	Kanna Abinaya , et al	2018	Review	For teeth replacements , that is in case of crown placement , gingival retraction should be done	Strong
32.	Basha Farhat , et al	2018	Review	Proper dental care during pregnancy is very important.	Strong
33.	Ajay R , et al	2017	Original study	In relation to the subgroups, the bond strength of $Zn_2(PO_4)_3$ was higher in Group I than in Group II. The bond strength in subgroup IV was superior in both Group I and Group II ($547.170 N \pm 5.752$ and $531.975 N \pm 6.221$ respectively.	Strong

Prosthodontics curriculum clock hours, prosthodontics teaching participation, and plans for specialization were also analyzed , hypothesized that reduced hours and perceived stress in the prosthodontics curriculum might impact students' choice of specialty at HSDM. The shortened preclinical didactic and laboratory exercises in prosthodontics at HSDM affect student anxiety, but not their didactic and clinical performances or their decisions in choosing their graduate program. Problem-based learning (PBL) tutorials help the students to integrate preclinical and clinical knowledge and skills in prosthodontics [32].

The undergraduate dental curriculum is undergoing rapid change and revision in dental schools worldwide. Decreasing edentulism, the advent of the clinical dental technician, and advances in technology (such as dental implantology) have led to changes in patient management and treatment options for general dental practitioners in New Zealand [33] . Prosthodontics is the specialty responsible for restoration of individual teeth and replacement of missing teeth and supporting structures, where education and experience have been focused on esthetics, comfort, and function. The American College of Prosthodontists (ACP) has made commitments to strengthen the quality and scope of both undergraduate and postgraduate educational programs and to support prosthodontics educators [34].

CONCLUSION

Prosthodontics was second only to oral surgery in its appearance as a dental specialty. It has proven to be a remarkably acceptable and innovative branch of the subject as it has evolved and responded to expanding and sophisticated patient needs and technologies. Assessment of its current trends, innovative thoughts generated, emerging technologies etc, as well as how these are contributing to overall shaping the future of prosthodontics.

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Review on Caries Preventive Effect of Fluoride Toothpaste

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Abstract

Dental caries is defined as localized destruction of tooth tissue, mainly due to bacterial action causing demineralized foci on peripheral surfaces. Normally, children have the habit of brushing their teeth once a day using fluoridated toothpaste. Due to this,there is less decay of tooth in them, as fluoride being a mineral prevents tooth decay. There is a relationship between fluoride toothpaste and dental caries, if a toothpaste having 1000ppm of fluoride content, is associated with less decay of tooth, so the fluoride has caries preventive effect. The aim of the review is to find out the role of fluoride in caries preventive effect, to know fluoride toothpastes correct concentration to avoid fluorosis and other fluoride related ailments and finally to understand the advantages of fluoride toothpaste as preventive effect of dental caries. This research was conceived as scoping literature review. This review has accessed existing reviews and researches in the last decade mostly, through PMC database, MeSH, Google Scholar, Pubmed, Medline, CrossRef and the search terms included were 'dental caries', 'fluoride toothpaste', 'caries preventive effect', 'fluoride'. Considered research was limited to manuscripts related to English, dental caries, fluoride toothpaste (as caries preventive effect) and this review did not include or consider other oral problems, other preventive methods of dental caries, natural components that prevent caries attack. The description of included studies for the review is tabulated. The role of fluoride in dental caries, its correct concentration in fluoride toothpaste is known and the preventive effect of fluoride toothpaste on dental caries is well understood through this review. With these general guidelines, there is more possibility of preventing dental caries and excessive fluoride will not lead to fluorosis due to awareness of correct concentration of fluoride in fluoride toothpaste.

Keywords: *Dental caries; fluoride toothpaste; caries preventive effect; fluorosis.*

Introduction

Dental caries is defined as localized destruction of tooth tissue, mainly due to bacterial action causing demineralized foci on peripheral surfaces [1]. Normally, children have the habit of brushing their teeth once a day using fluoridated toothpaste. Due to this,there is less decay of tooth in them,as fluoride being a mineral prevents tooth decay [2]. There is a relationship between fluoride toothpaste and dental caries, if a toothpaste having 1000ppm of fluoride content, is associated with less decay of tooth, so the fluoride has caries preventive effect [3]. Methods of fluoride use may be different like fluoridated water, milk, varnish,etc and there are many methods of use but for classification, should be community based, individual based, professional based. By knowing this, we can give absolute fluoridated treatment for caries prevention. There was wrong classification as systemic and topical use which is now not considered, because the new classification gave better scope of treating /preventing caries [4,5] .

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The previous research by Gail Topping and Andrea Assaf,2005 [6], has concluded that daily use of fluoridated toothpaste in young permanent dentition should be more than 1500ppm. The study observed that fluoride toothpaste more than 1500ppm have superior caries preventive effect, and has also observed that caries preventive effect is higher in supervised brushing. Another research by Valeria CC Marinho,2004 [7], has concluded that topical fluorides in addition with fluoride toothpaste has modest reduction in dental caries, so both fluoride applications have better enhancement than using only fluoride toothpaste.

The limitation of most previous researches on this topic of review is that they have given effect of fluoride toothpaste mostly in children. The fluoride toothpaste effect is not discussed /studied in other age groups, like in adolescents. The dental caries preventive effect is not studied in most caries susceptible populations, even not in different regions, locality, different temperature, altitudes is not provided. Dental caries is once a problem mainly, as of now it is a rising problem above all age groups. So, there is a need to know the prevention method. The most used preventive measure is fluoride toothpaste which is of low cost remedy to prevent dental caries. The aim of the review is to find out the role of fluoride in caries preventive effect, to know fluoride toothpastes correct concentration to avoid fluorosis and other fluoride related ailments and finally to understand the advantages of fluoride toothpaste as preventive effect of dental caries.

Methodology

This research was conceived as scoping literature review. This review has accessed existing reviews and researches in the last decade mostly, through PMC database, MeSH, Google Scholar, Pubmed, Medline, CrossRef [8–13] and the search terms included were 'dental caries', 'fluoride toothpaste', 'caries preventive effect', 'fluoride'. Considered research was limited to manuscripts related to English, dental caries, fluoride toothpaste (as caries preventive effect) and this review did not include or consider other oral problems, other preventive methods of dental caries, natural components that prevent caries attack. The period of duration considered is 1965 to 2020. The total number of articles found on typing the topic is 62,200 and the number of articles actually relevant to the topic is 84 found by searching using keywords. The number of research articles that are used in writing the review is 45. Quality of articles used was assessed using Quality assessment tools and graded as strong, moderate and weak. The level of evidence of the reviewed articles were categorised according to the criteria of Centre for Evidence-Based Medicine, Oxford, UK [14].

DENTAL CARIES

Introduction of Dental Caries

Dental caries is defined as localized destruction of tooth tissue by bacterial action causing demineralized foci on the peripheral surface of enamel [1]. It can also be defined as localized demineralization of hard tissues of teeth by action of acid derived from food debris or sugar [15]. This is due to dissolution of hydroxyapatite crystals which results in loss of organic matrix of both enamel and dentin [16].

Causes of Dental Caries

Streptococcus mutans is the main cause for dental caries which leads to formation of plaque biofilm. This plaque biofilm ensures caries development in any individual [17]. Actually, dental caries are due to the presence of four variables such as cariogenic bacteria (caries producing bacteria, like S.mutans), sugar(carbohydrate fermentation also causes dental caries), susceptible tooth surface and time. The variables are most important to know for the cause of caries attack [18]. Dental caries is due to localised destruction of bacterial fermentation of dietary carbohydrates. It has factors also like ecological imbalance, physiological equilibrium, as sugar is also a main variable considered here [19].

Prevention of Dental Caries

Preventive factors such as salivary calcium, phosphate and proteins present in salivary flow helps in prevention of dental caries. Presence of a small amount of fluoride in saliva acts as antibacterial agents [20]. New possible advanced preventive method is by CO_2 laser. Irradiation of enamel by CO_2 alters the hydroxyapatite crystals and reduces activity. The CO_2, laser treatment along with fluoride treatment is found to be more beneficiary [21]. Fluoride is the most effective material in

control of dental caries. The other methods of fluoride use can be fluoride toothpaste, fluoride mouth rinse which can be easy to use for any individual [22]. Recent study for caries prevention is by grape extract. The grape extract has bacteriostatic activity and also has collagen crosslinking properties which will help in tooth remineralisation [23]. Other than CO_2 laser treatment, whitening LED is used. It reduces the growth of Streptococcus mutans which ultimately prevents dental caries. This treatment will allow full arch irradiation [24].

FLUORIDES PREVENTIVE EFFECT

Fluoride's Role in Dental Caries

Fluorine combines with free hydroxyapatite crystals in enamel. It then forms fluorohydroxyapatite which gets redeposited in enamel. This will form protective covering over the enamel. This leads to prevention of dental caries [22].

Previous Research on Preventive Effect

Children normally brush their teeth once a day using toothpaste with fluoride. It showed less decay effect which was studied by Valeria CC Marinho, etal,2003 [2]. Another study by Tanya Walsh, etal,2010 [3] has concluded that toothpaste with 1000ppm of fluoride is associated with less tooth decay. The study was effective to know the correct concentration of fluoride content in toothpaste. Supporting the above studies stated, study by Gail Topping and Andrea Assaf, 2005 [6] has concluded that daily use of fluoride toothpaste in young permanent dentition with more than 1500ppm has superior preventive effect on caries. All of the previous studies considered a range of 1000ppm-1500ppm would be the correct concentration of fluoride content in fluoride toothpaste for the best caries preventive effect.

Fluoride Toothpaste

The topical fluorides in addition with fluoride toothpaste will give modest reduction of caries. So, it is advised that not only fluoride toothpaste will help you out of caries, it needs some additional application to have long lasting, superior preventive effect [7]. To know the relationship between fluoride toothpaste and caries, is that if lower fluoride formulations will have lower risk of fluorosis which is balanced, but it will have higher risk of caries. So, more fluoride formulation means lower risk of caries attack [25]. Fluoride toothpaste, lactate ion, polyvinylmethylethermaleic anhydride, all of these help in enamel remineralisation [26].

OTHER MEANS OF FLUORIDE USE

Other Methods

Methods of fluoride use are classified as systemic,topical,community based,individual and professional based. Different methods will be water fluoridation, fluoride mouth rinse,salt fluoridation,milk fluoridation. There are many methods of fluoride use, which will help us to prevent caries [27]. These methods might prevent further periodontal diseases [28].

Fluoride Varnish

Fluoride varnish is more frequently used nowadays. It may not provide additional caries protection but has relatively low caries activity [29]. Fluoride varnish prevents occlusal, proximal, smooth surface caries. So fluoride varnish can also be used as an application but does not have a superior preventive effect for caries [30].

Results and Discussion

Table 1: Description and quality of included studies

S.NO	Author	Year	Quality of study	Key points	Level of evidence
1	Burnett G.W, etal	1968	Moderate	Dental caries is defined as localized destruction of tooth tissue, mainly due to bacterial action causing demineralized foci on peripheral surfaces.	Level 3
2	Silk H	2014	Moderate	Dental caries is defined as localized demineralization of hard tissues of teeth by action of acid derived from food debris or sugar.	Level 3
3	RJ Gibbons	1975	Strong	Dissolution of hydroxyapatite crystals which results in loss of organic matrix of both enamel and dentin.	Level 1
4	Qiuxiang Zhang, etal	2019	Strong	Streptococcus mutans is the main cause for dental caries which leads to formation of plaque biofilm.	Level 1
5	BA Kassim, etal	2006	Strong	Dental caries are due to the presence of four variables such as cariogenic bacteria, sugar,, susceptible tooth surface and time.	Level 2
6	Robert H Selwitz Dr, etal	2007	Weak	Dental caries is due to localised destruction of bacterial fermentation of dietary carbohydrates. It has factors like ecological imbalance, physiological equilibrium.	Level 5
7	John DB	2000	Moderate	Preventive factors such as salivary calcium, phosphate and proteins present in salivary flow helps in prevention of dental caries.	Level 3
8	Lidiany Karla Azevedo Rodrigues, etal	2004	Moderate	Irradiation of enamel by CO2 alters the hydroxyapatite crystals and reduces activity.	Level 3
9	Haiyang Sun, etal	2020	Strong	The other methods of fluoride use can be fluoride toothpaste, fluoride mouth rinse which can be easy to use for any individual.	Level 2
10	Delimont, etal	2019	Strong	The grape extract has bacteriostatic activity and also has collagen crosslinking properties which will help in tooth remineralisation.	Level 2

***Cont...* Table 1: Description and quality of included studies**

11	Feng Luo, etal	2020	Strong	Fluorine combines with free hydroxyapatite crystals in enamel. It then forms fluorohydroxyapatite which gets redeposited in enamel. This will form protective covering over the enamel.	Level 2
12	Valeria CC Marinho, etal	2003	Strong	Children normally brush their teeth once a day using toothpaste with fluoride. It showed less decay effect.	Level 2
13	Tanya Walsh, etal	2010	Strong	Toothpaste with 1000ppm of fluoride is associated with less tooth decay.	Level 1
14	Gail Topping, Andrea Assaf	2005	Strong	Daily use of fluoride toothpaste in young permanent dentition with more than 1500ppm has superior preventive effect on caries.	Level 1
15	Holt RD, Murray JJ	1997	Moderate	If lower fluoride formulations will have lower risk of fluorosis which is balanced, but it will have higher risk of caries. So, more fluoride formulation means lower risk of caries attack.	Level 3
16	Liisa Seppa	1990	Moderate	Fluoride varnish is more frequently used nowadays. It may not provide additional caries protection but has relatively low caries activity.	Level 3
17	Jonathan E Creeth, etal	2020	Strong	Fluoride toothpaste, lactate ion, polyvinylmethylethermaleic anhydride, all of these help in enamel remineralisation.	Level 2
18	Ratilal Lalloo, etal	2020	Moderate	Fluoride varnish prevents occlusal, proximal, smooth surface caries.	Level 3
19	Valeria CC Marinho	2004	Strong	The topical fluorides in addition with fluoride toothpaste will give modest reduction of caries.	Level 2
20	Ellwood RP, etal	2008	Moderate	Different methods will be water fluoridation, fluoride mouth rinse,salt fluoridation,milk fluoridation.	Level 3
21	Choa Park, etal	2020	Strong	Whitening LED reduces the growth of Streptococcus mutans which ultimately prevents dental caries. This treatment will allow full arch irradiation.	Level 1

Dental caries is the localized destruction of bacterial action on the peripheral surface of enamel [1] and it is also localized demineralization by acid from food debris or sugar [15]. Main cause of dental caries is Streptococcus mutans which leads to formation of plaque biofilm. The L.Plantarum FBT9 has a stronger inhibitory effect on cariogenic bacteria [17]. The hot steeping extract of tea contains catechins which have a higher inhibitory effect on biofilms [31]. Lactobacillus fermentum Tc UESC01 is an antimicrobial agent against S.mutans. It is anti-adherent, and has bactericidal activity against planktonic cells of S.mutans [32]. Fluoride toothpaste should be of 1000ppm, has association with less decay [3] and more than 1500ppm, has superior preventive effect on caries [6]. Daily use of 0.2% sodium fluoride (NaF) mouth rinse is the most effective method. 0.05% NaF mouth rinse along with 1100 to 1500ppm in fluoride toothpaste [33] and 1100ppm of fluoride toothpaste for daily use is recommended [34]. So, normal range would be 1000ppm to 1500ppm in fluoride toothpaste to have better caries preventive effect. The abutments, implants, veneers are independent of dental caries [35 36 37,38], facial prosthesis and lip bumper prosthesis [39] does not give caries preventive effects [40] and also only restorations does not provide a remedy for caries [41]. Oral hygiene is very important during pregnancy, as it also affects the developing foetus and health of it [42].

The limitations of the present review is only recent studies have been included,the older studies were not included. The study populations of previous researches are different, as many factors are responsible for inducing dental caries. The review should consider a wide range of studies, considerations of other factors of dental caries, caries preventive effect of fluoride toothpaste with inclusion of population of systemic disease, non oral habit population and the study setting should be in particular region.

Future scope of the present review article is that many toothpastes (especially whitening toothpaste) have more fluoride content, which leads to toxicity. The normal range should be between 1000ppm and 1500ppm. This review will create an awareness of selection of toothpaste with correct fluoride concentration which is helpful in prevention of dental caries.

Conclusion

The role of fluoride in dental caries, its correct concentration in fluoride toothpaste was made clear and the preventive effect of fluoride toothpaste on dental caries is well understood through this review. With these general guidelines, there is more possibility of preventing dental caries and excessive fluoride will not lead to fluorosis due to awareness of correct concentration of fluoride in fluoride toothpaste.

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Orthodontics and Smile Correction in Teenagers-A Survey

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Abstract

Orthodontics is a branch of dentistry that deals with diagnosis, prevention and correction of malpositioned teeth and jaws. Correction of malpositioned teeth is done by moving teeth and adjusting the underlying bone with the help of certain devices. Ideal age for undergoing orthodontic treatment is between 8 to 14 years. Orthodontics treatment is required for malocclusion of teeth to establish proper bite and to improve aesthetics. Malocclusion affects the individual self satisfaction, self confidence and causes emotional and mental disturbances. The aim of the study is to evaluate the awareness on orthodontics and smile correction in teenagers. A cross sectional survey was conducted among teenagers to evaluate the awareness about orthodontics and smile correction in teenagers.. The study population in the study are the teenagers with a sample size of 100. The questionnaire consisted of 20 questions and was shared to teenagers using online survey platform. 80% of the participants were aware about orthodontics and 20% were not aware. 41% of the respondents have worn braces before and 59% have not. 27% of the participants felt that their teeth alignment is perfect, 29% did not feel that way and 34% said almost perfect. 75%of the participants feel that orthodontic treatment is necessary for malocclusion of teeth, 7%did not feel as necessary and 18% maybe felt as necessary. From the results obtained, we can include that the moderate level of awareness is seen in teenagers about orthodontic treatment. More awareness is required as treatments are more effective when done at an early age.

Keywords :*Orthodontics; smile correction; awareness; teenagers; orthodontic treatment; malocclusion*

Introduction

Orthodontics is a branch of dentistry that deals with diagnosis, prevention and correction of malpositioned teeth and jaws. Correction of malpositioned teeth is done by moving teeth and adjusting the underlying bone with the help of certain devices. Ideal age for undergoing orthodontic treatment is between 8 to 14 years. The appliance used to move the teeth could be removable appliances or fixed appliances. Malocclusion of teeth is not a disease but it is an abnormal arrangement of the teeth and the way the upper and lower teeth fit together [1]. The common etiology of malocclusion are genetic, environmental, and ethnic factors [2]. Orthodontics treatment is required for malocclusion of teeth to establish proper bite, to correct the sequence of malocclusion, to improve aesthetics, to reduce susceptibility to accidental injury of anterior teeth with severe proclination, to correct abnormal habits and muscle activity, to enhance better cleansing and reduce the susceptibility to caries and periodontal disease. The common diseases of the gums are the gingivitis and periodontitis [3]. The periodontium is assessed by methods such as plaque score, bleeding on probing and probing pocket depth [4].

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Malocclusion affects the individual self satisfaction, self confidence and causes emotional and mental disturbances [5].Apart from the serenity of malocclusion, the rate of starting orthodontic treatment depends on other background factors such as age, sex and socio economic status [6]. The children's perception about the need of orthodontic treatment and satisfaction with their alignment of teeth was associated with their mother's perception [7]. Hence parental influence has a more motivating effect than the malocclusion severity on children . Awareness regarding orthodontic procedures are significantly higher in girls than boys. Among girls, awareness was significantly higher in urban areas than girls in rural areas [8].

Awareness regarding orthodontic treatment in teenagers is important for approaching dental treatment at correct age. Orthodontic treatment undergone at early age is more effective as the bones are more soft and pliable than late teens and adults. It is important to educate the teenagers about the benefits about orthodontics. The benefits also include improving the quality of life, improving one's self confidence and psychological and social changes [9]. The aim of the study is to evaluate the awareness on orthodontics and smile correction in teenagers.

Materials and Methods

Study Design

A cross sectional survey was conducted among teenagers to evaluate the awareness of orthodontics and smile correction in teenagers. The sampling method is simple random sampling method. The sample size of this study is 100. The participants did the survey voluntarily and no incentives were given to them. The study was conducted in the month of may,2020. Ethical approach and informed consent from the participants were obtained.

Survey Instrument

The survey instrument which was a questionnaire was prepared after extensive review of the existing literature. The questionnaire was reviewed and amendments were made to improve clarity of the questions to eliminate ambiguous responses. The questionnaire consisted of 20 questions with both open and closed ended questions. The questionnaire was shared to teenagers using online survey platform.

Data Analysis

Only completed surveys were taken for analysis and the incompleted surveys were eliminated. The statistical test used is descriptive statistics . All the responses obtained were tabulated and reliability of the data was checked. Frequency table was prepared for each question and analysed using spss data analysis software [10–14].

Results and Discussion

Out of a total of 100 teenagers participated in the survey, 16% belonged to the group of 12 to 14, 36% belonged to the age group of 15 to 16 and 48% which is the majority belonged to the age group of 17 to 19, 59% were females and 41% males. 80% of the participants were aware about orthodontics and 20% were not aware (figure 1). When asked about the conditions that require braces treatment, 41.8% said irregularly arranged teeth, 16.2% siad smile problem, 32.5 % said space between teeth and 9.3% were not aware (figure 2). The majority 67% of the participants said that a perfect smile is important for self confidence, 7% said no and 26% chose maybe (figure 3). In the chi square analysis between gender and awareness about orthodontics, the p value obtained was 0.263 and it was statistically not significant (figure 4). In the chi square analysis between gender and awareness about the right age for wearing braces, the p value obtained was 0.634 and it was statistically not significant (figure 5). In the chi square analysis between gender and participant opinion on whether orthodontic treatment is necessary, the p value obtained was 0.664 and it was statistically not significant (figure 6).

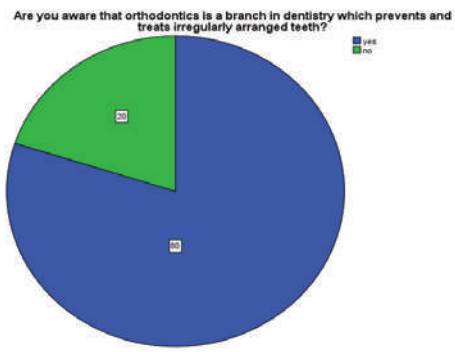


Figure 1: Pie chart showing the awareness of orthodontics among teenagers. 80% of the participants were aware (blue) and 20% were not

aware (green). Majority of teenagers are aware about orthodontics while a small group of participants are not aware.

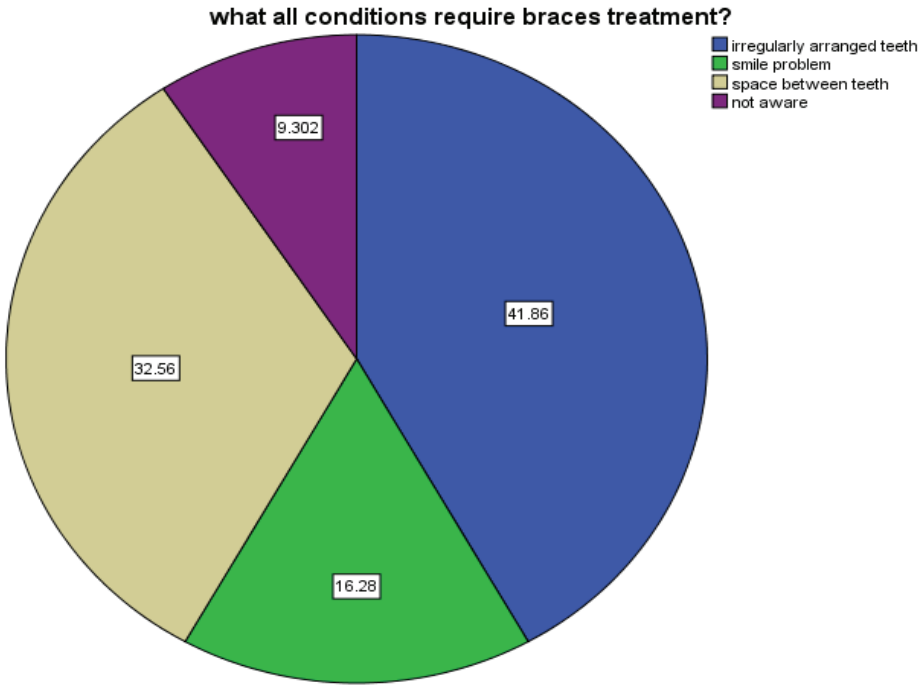


Figure 2: Pie chart showing the response for the conditions that require braces treatment. 41.9% said irregularly arranged teeth (blue), 16.2% said smile problem (green), 32.5% said space between teeth (cream) and 9.3% said that they are not aware (violet).

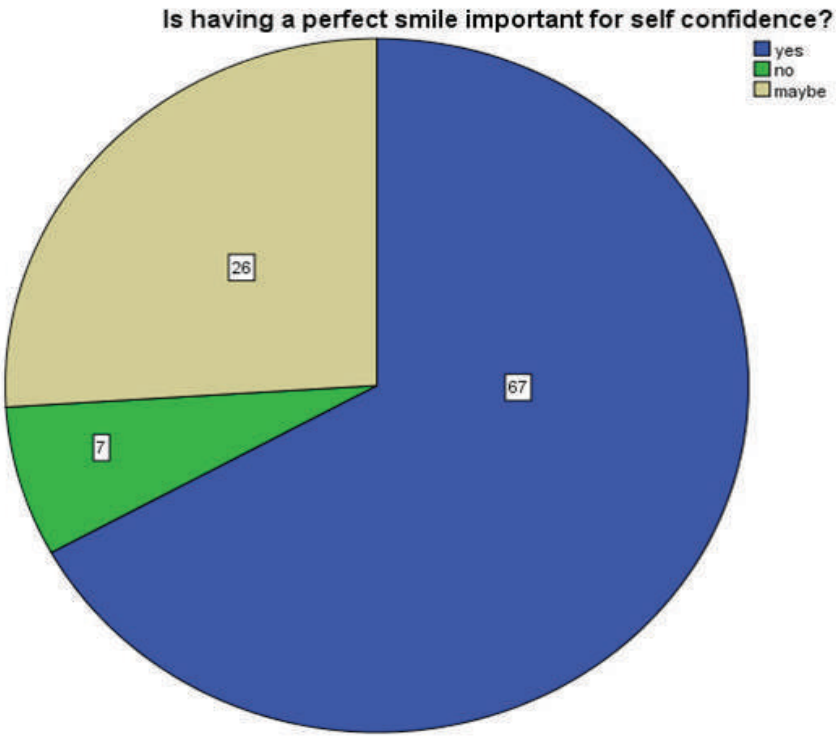


Figure 3: Pie chart showing the responses about whether a perfect smile is important for self confidence. 67% said yes (blue), 7% said no (green) and 26% said maybe (cream).

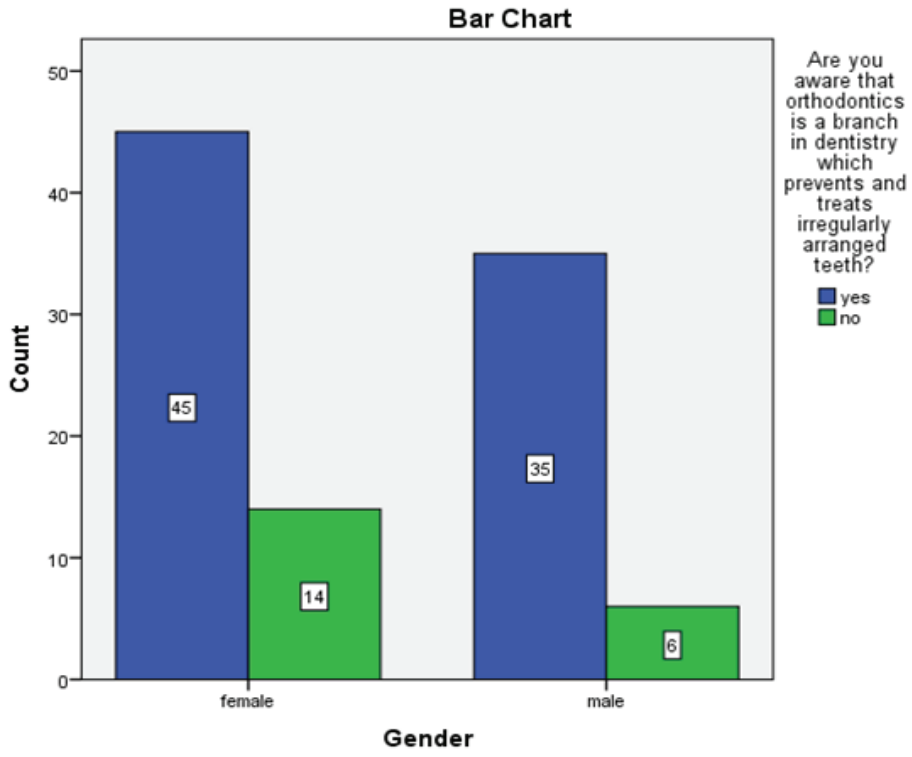


Figure 4: Bar graph represents the association between gender and awareness about orthodontics. X axis represents the gender and Y axis represents awareness about orthodontics. There was no statistically significant difference in the awareness between males and females. Pearson's Chi square value: 1.251, p value = 0.263(>0.05).

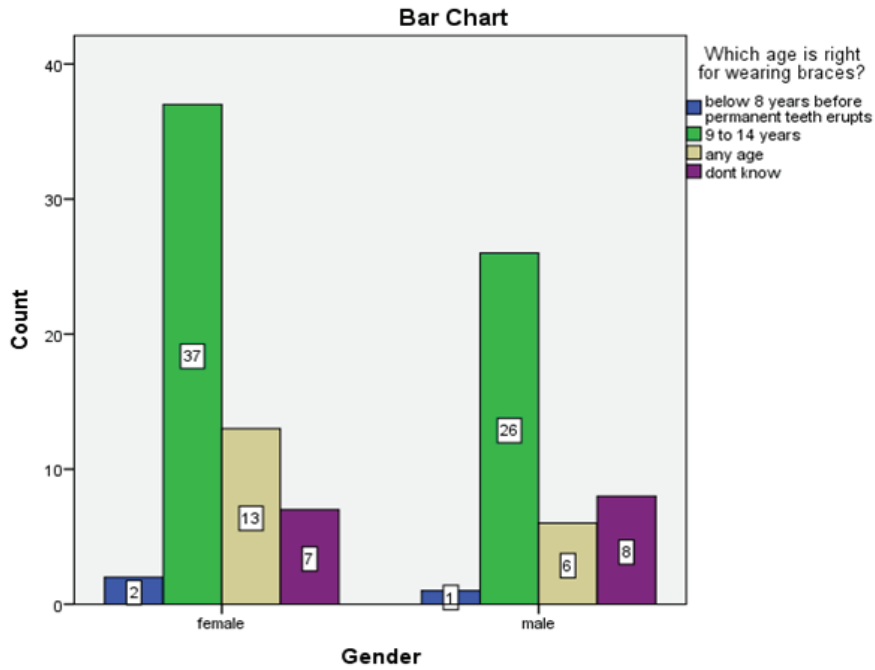


Figure 5: Bar graph represents the association between gender and awareness about the right age for wearing braces. X axis represents the gender and Y axis represents the awareness about the right age for wearing braces. Though males were more aware than females the difference was statistically not significant. Pearson's chi square value: 1.715, p value: 0.634(>0.05).

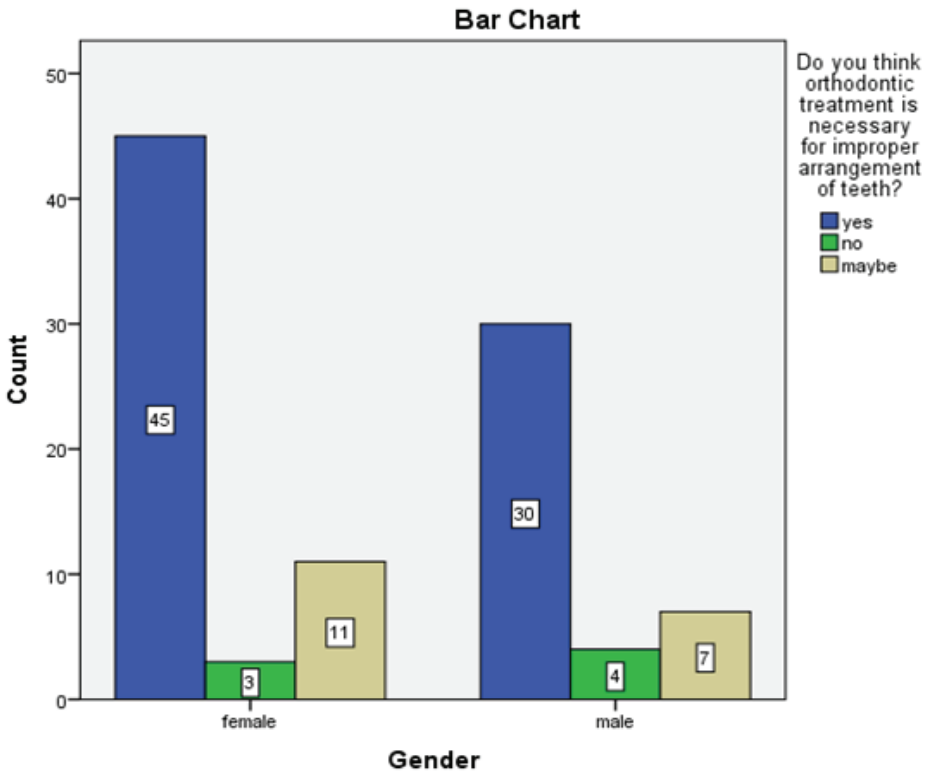


Figure 6: Bar graph represents the association between gender and participant opinion on whether orthodontic treatment is necessary. X axis represents the gender and Y axis represents participant opinion on whether orthodontic treatment is necessary. Females were more aware than males. There was no statistically significant difference in the awareness between males and females. Pearson's chi square value: 0.818, p value: 0.664(>0.05).

Table 1: Questionnaire regarding the awareness of Orthodontics and the responses in %

S.No	Questions	Results in percentage	
		Aware	Not Aware
1.	Awareness on orthodontics	80%	20%
2.	Right age for Braces?	63%	15%
3.	Do you experienced or aware about changes after orthodontic treatment	53%	47%
4.	orthodontic treatment is necessary for malocclusion of teeth	75%	25%
5.	Is orthodontic treatment painful?	82%	18%
6.	The duration of the orthodontic treatment	68%	32%
7.	Reasons for irregularly arranged teeth	90.4%	9.6%
8.	Improper alignment of teeth increases risk of caries,	70%	23%
9.	The cost of orthodontic treatment is high	68%	22%

Table 1 shows the responses for awareness on orthodontics, 80% of the participants were aware about orthodontics and 20% were not aware. When asked about the right age for braces, 2% said that the right age for braces are below 8 years before the eruption of tooth eruption, majority of then, 63% choose 9 to 14 years as the perfect age for braces,15% admitted that they are not aware about it. Hence 63% of the participants were aware about the right age for wearing braces. 53% of participants found significant differences after orthodontic treatment, and 34% not aware. 75%of the participants feel that orthodontic treatment is necessary for malocclusion of teeth, 7% did not feel as necessary and 18% may be felt as necessary. Most of the participants viewed orthodontic treatment as necessary. 75% were aware that tooth extraction is required mostly for braces treatment, 25% were not aware. 82% of the participants said that they will undergo orthodontic treatment if required knowing that it's painful, 18% said no. 68% of the participants are aware about the duration of the orthodontic treatment and 32% were not aware. When asked about the reasons for irregularly arranged teeth, 34.6% said genetics, 31.4% said oral habits, 24.3% said other reasons and 9.6% are not aware, this response shows that the majority of 90% are aware of the reasons for misaligned teeth and 9.6% are unaware. 70% of the participants were aware that improper alignment of teeth increases risk of caries, 7% said no and 23% were not aware. 68% of the teenagers participated said that the cost of orthodontic treatment is high, 10% said no which 22% were not aware about the cost.

The present research has origins from the team of investigators where previous studies were done on clinical reports, interventional studies like [15–17], in vitro studies like [14], and systemic reviews [18–22]. In a survey conducted among parents in Kanchipuram district, 76.5% of the parents of children of age 5-17 years considered orthodontic treatment essential and 55.9% of the parents were anxious for seeking treatment. In our study, 75% of the participants considered orthodontic treatment as necessary. The results are very much similar to our results [23].

In another survey among preadolescents, 45.1% were aware about orthodontic treatment whereas in our study, 80% were aware. Our survey, the awareness level is significantly higher probably due to the age difference. Previous study was among preadolescents while our study included teenagers as teenagers tend to be more conscious of their looks then preadolescents [8].In a study conducted in Berlin city among school children, 76.9% of the subjects were satisfied with their dental aesthetics. Our study only 37% of the participants felt that their teeth alignment was perfect and 34% felt that it was almost perfect [24].

In a previous study, a survey was conducted among students in which only 2 people had previously received orthodontic treatment and 72.9% had friends or relatives who had received orthodontic treatment. In our study 41% of the participants had previously received orthodontic treatment which is significantly much higher than the previous study. 89% of the participants in our study have a relative or a friend who were braces which is also significantly higher in our study [25]. In a previous study, 94.8 % of the participants felt that aligned teeth are important for facial esthetics. In our study, 69% of participants said that proper alignment of teeth is necessary for a perfect smile, which is significantly less than the previous study [26]. Some of the limitation in this study include, the smaller samples size of 100. Larger sample size will give more accurate data. Wide variety of population can be included, our study only included teengers.

Conclusion

From the results obtained, we can conclude that the awareness about orthodontics and smile correction in teenagers is convincing. Most of the teenagers are aware about orthodontic treatment but there is still a group of teenagers who are not aware. More awareness should be created as treatments are more effective when done at an early age. Awareness about orthodontic treatment and procedures will also make the teenagers more cooperating for the dental treatment.

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Conflict of Interest: The author declares that there was no conflict of interest in the present study.

Source of Funding: Self.

Ethical Clearance: Not Required

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Awareness of Oral Hygiene among Dental Students-A Survey

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Abstract

Attitudes of dental students toward their own oral health affect their oral health habits and also have a possible influence on the improvement of the oral health of their patients and society.The aim of this survey is to create awareness among dental students on oral hygiene.A survey with 17 questions was prepared and sent via google forms to 100 dental students.The responses were collected and analysed.Most of the dentists are aware that oral health determines the overall health of an individual.Many among the population use mouthwash,rinsing with water etc to avoid bad odour. Aspects of a dental student's oral health attitude and behavior improved but this improvement was limited.Thus, the students should be motivated to become an example of oral health for the society, for which few steps to motivate them toward better oral health are proposed.This approach would also be helpful in disseminating positive oral health concepts to the general public.

Keywords:*oral health; population; students; dental; hygiene*

Introduction

The prevention of oral health disease is the most acknowledged and efficient method of ensuring oral health.Oral health is now renowned to be equally important in relation to general health.Dental student so oral health attitude reflects their understanding of the importance of the disease prevention and their commitment to improve their patient's oral health.7[1]People's oral health knowledge,behaviour and status are influenced by many factors including culture,environment and social customs.[2]In future,today's students of dentistry will provide dental services and will be responsible for public oral health education,thus it is important to investigate their knowledge,attitude and behaviour regarding oral health.[3]Numerous studies have found oral behaviour to vary from among people of different countries and communities.Oral health attitude also differs among gender.[4]

To improve oral health of the population,WHO has set the promotion of self care of as one of the goals for the year 2020.Recommended oral self care (ROSC) includes tooth brushing more than once in a day,lesser consumption of sugar containing snacks daily once or rarely or regular use of fluoride containing toothpaste. Since dentists are expected to be role models to their patients,evaluation of ROSC of dentists the self will provide an estimate of the extent to which that follow ROSC.[5]In previous studies many industrialised countries have experienced a dramatic decline in dental caries prevalence among students.The reason for the improved oral health are complete but may involve a more sensible approach to sugar consumption,improved oral hygiene practice, topical Fluoride application,effective use of oral health services and preventive programmes.[6] To keep the oral health hygienic aloe vera can also be used.It has also been used in dentistry for its beneficial properties

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in various conditions[7]. There is a need to provide oral health education for pregnant women since it is during the time of pregnancy that chances of infection increases and it may also affect the developing foetus.[8]

Compared to other studies ,this survey covers only limited population.The dental institutions are an integral part of the oral health care system.They help train the future professionals and expand scientific knowledge through research.The oral hygiene attitude and behaviour of dental health professionals affect their oral self care habits along with their ability to motivate patients to undertake preventive oral health measures.[9]This study was conducted to access the oral health attitudes and behaviours among dental students.The study also creates awareness in having a good oral health.

Materials and Method

Study design,Area and study population:

A survey was conducted among various dental college students to assess the knowledge and awareness of good oral habits.The sample size of this survey is 100 dental students.Participants who were more than 16 years of age and below 29 years of age were included in the study.Participation in the study was voluntary and no incentives were provided to the participants.The study was conducted at the period of April 2020.

Survey in instrument:

The study instrument which was a questionnaire was prepared after extensive review of the existing literature.17 questions were prepared which was circulated among the 100 dental students via online-Google forms.The survey instrument was a structured questionnaire with both open and close ended questions. It consists of brief introduction regarding the purpose of the study, questions pertaining to demographic data and questions regarding the research objective-knowledge,awareness etc.

Data Analysis

Only completely filled online forms were included in the study.The filled responses were verified and entered.The entered data was analysed using SPSS.[101112,1314]Descriptive analysis was performed to calculate frequencies of categorical variables.

Results and Discussion

A total of 100 students participated in the survey. Of these participants 60.4% of them were females and 39.6% were male, the age group was between 16- 29 years.

73.3% were aware of what bad oral hygiene can cause.(pie chart 1) In the Chi square analysis between gender and the frequency of brushing each day ,the p value was 0.416 which is >0.05 and therefore it is not statistically significant(figure 2).In the Chi square analysis between gender and smoking it was analysed that it was statistically significant as the p value was 0.012 which is < 0.05(figure 3).In the chi square analysis between gender and the frequency of visiting the dentist ,the p value was found to be 0.262 due to which it is not statistically significant(figure 4). In Chi square analysis between gender and those who are aware that oral determines body health, the p value was found to be 0.320 and not statistically significant(figure 5). In the Chi square analysis between gender and the time taken to brush their teeth,the p value was found to be 0.513 and not statistically significant(figure 6).

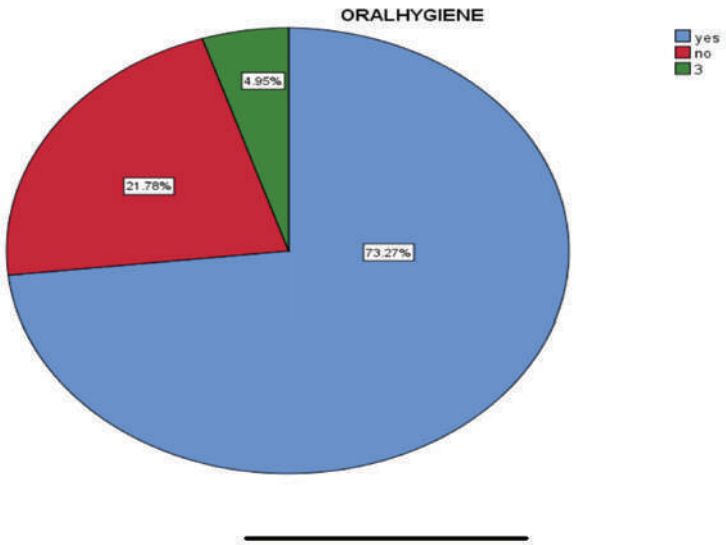


Figure 1: The pie chart showing response to the question- "Are you aware about the diseases caused if oral hygiene is not maintained? Majority of the respondents said yes(73.27%). Blue represents yes and red represents no and Green represents not sure.

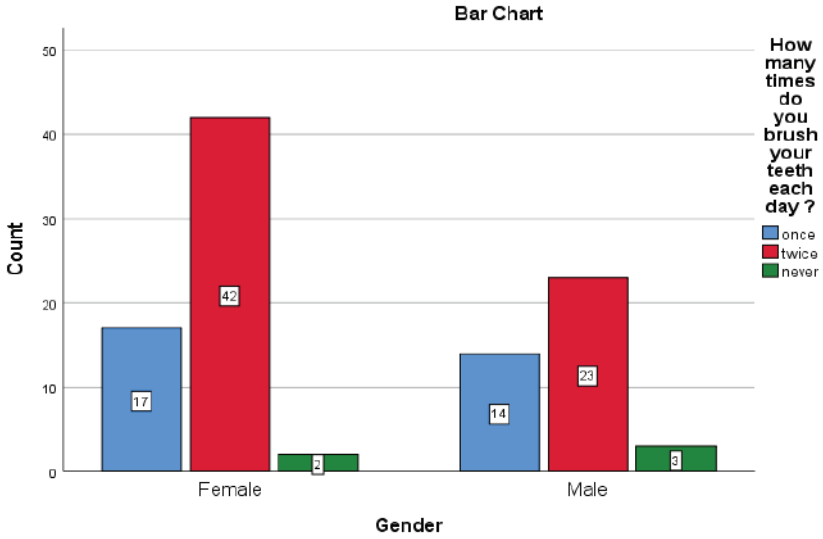


Figure 2**:**The bar graph represents the association between gender of the population and the frequency of brushing teeth every day.The X axis represents the gender of population and Y axis represents the number of participants responded .Blue represents once,red represents twice and green represents never. More number of females brushed twice a day compared to males, although this was found to be statistically not significant. Pearson Chi square test, p value = 0.416(>0.05) .

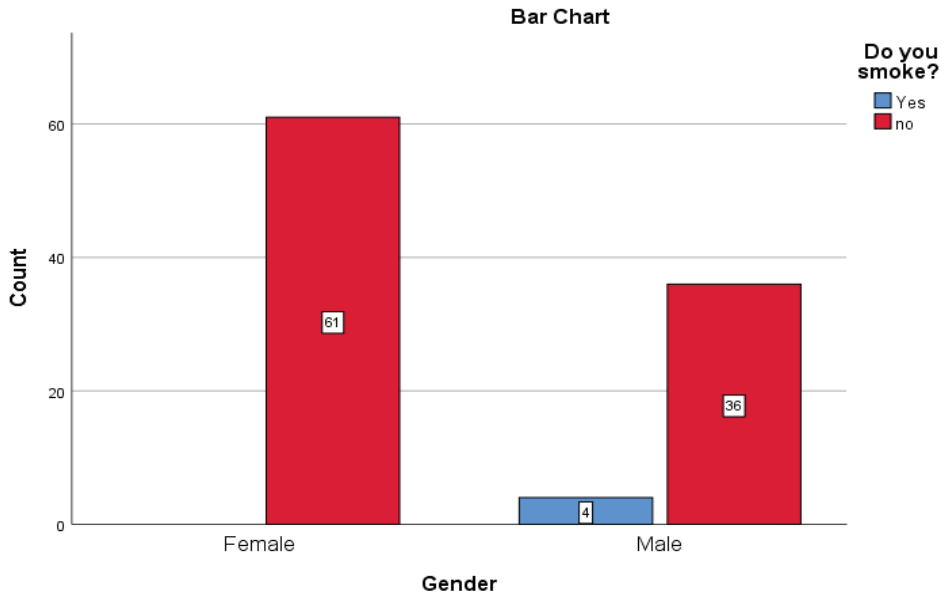


Figure 3:The bar graph represents the association between gender of the population and those who smoke. The X axis represents the gender of the population and Y axis represents the number of participants responded.Blue represents yes and red represents no.Females do not have the habit of smoking compared to males and this was found to be statistically significant.The Pearson Chi square, p value = 0.012(<0.05) .

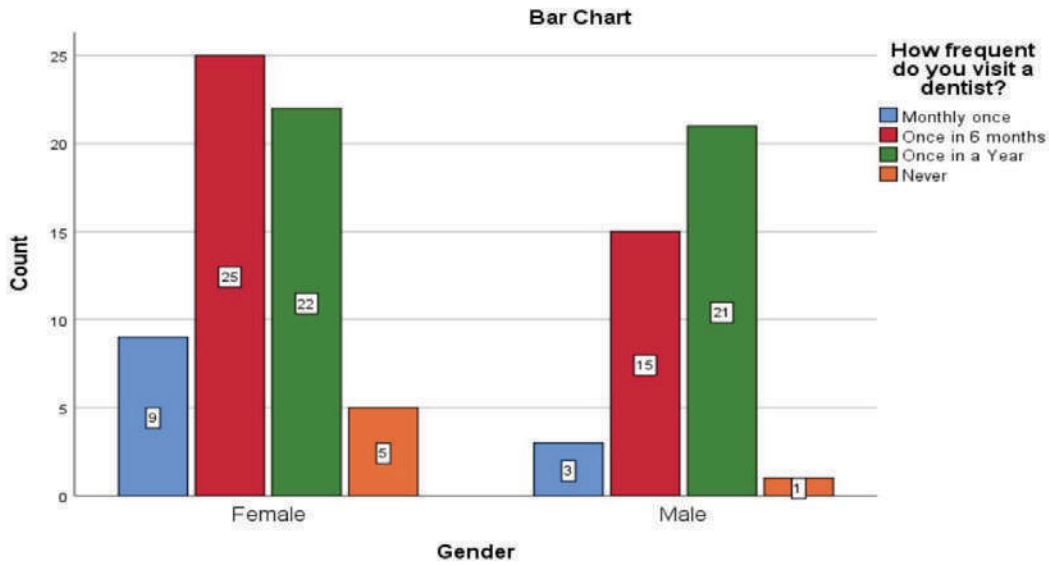


Figure 4:The bar graph represents the association between gender of the respondents and the frequency in visiting the dentist.The X axis represents the gender and Y axis represents the number of responses. Blue represents monthly once,red represents once in 6 months,green represents once in a year and orange represents never. Most of the females visited dentists once in 6 months whereas most of the males visited the dentist once in a year. There was no statistically significant difference in the frequency of dental visits between males and females. Pearson Chi square value was 3.996,df was 3, the p value was 0.262(>0.05).

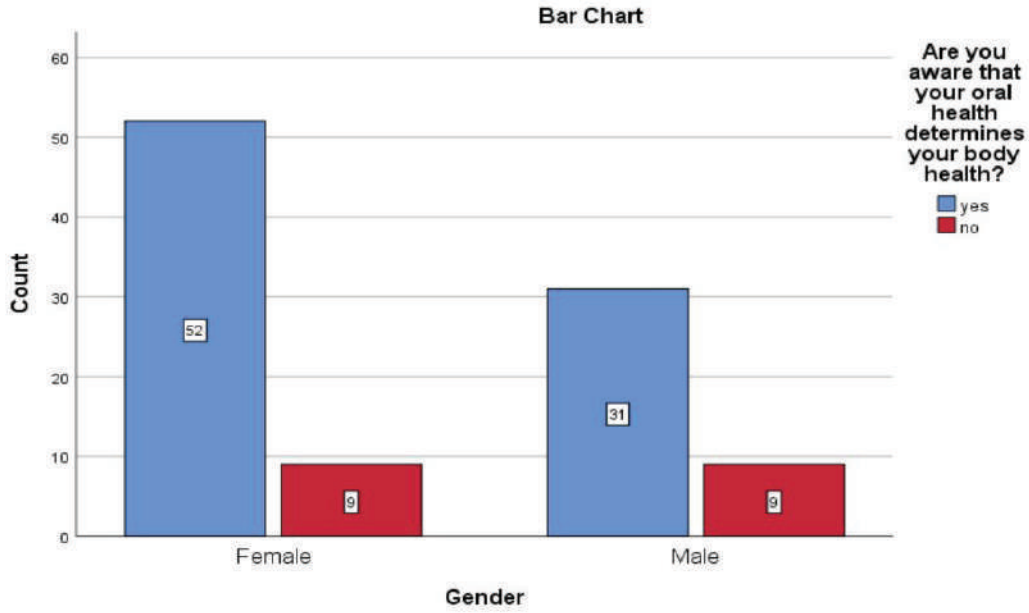


Figure 5:The bar graph represents the association between gender of the respondents and those who are aware that oral health determines body health.The X axis represents the gender and Y axis represents the number of participants responded. Blue represents yes and red represents no.Majority of the females are aware that oral health determines body health. There was no statistically significant difference between the genders on their awareness that oral health determines body health. Pearson Chi square test, p value =0.320(>0.05).

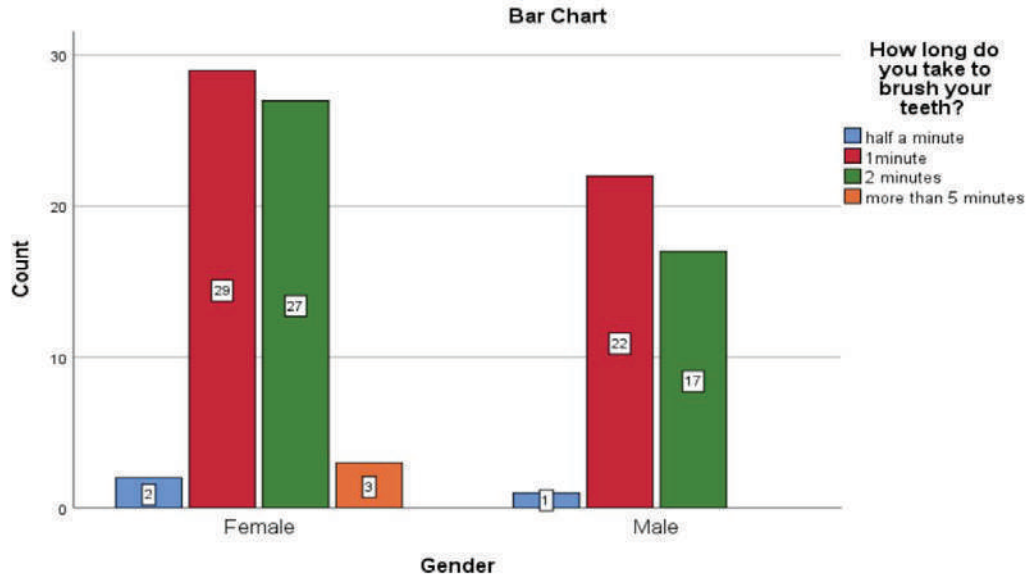


Figure 6:The bar graph represents the association between gender of the respondents and the time taken to brush their teeth.The X axis represents the gender and Y axis represents the number of participants responded. Blue represents half a minute,red represents 1 minute,green represents 2 minutes and orange represents more than 5 minutes. Most of them brush their teeth between 1-2 minutes. Majority females brush their teeth for 1-2 minutes compared to males. There was no statistically significant difference in the brushing time between males and females. Pearson Chi square, p value = 0.513(>0.05)

Table 1: Questionnaire regarding the awareness of Oral Hygiene habits and the responses in %

S.No	Questions	Results in percentage	
		Aware	Not Aware
1.	Brushing twice per day is good for health	64%	36%
2.	It is advised to brush at least two minutes to brush your teeth	50%	50%
3.	Habit of using mouthwash is good for oral health	53.47%	46.53%
4.	Habit of using Dental Floss for maintaining good oral hygiene	26.73%	73.27%
5.	Habit of using toothpicks is not good for oral health	21.86%	78.24%
6.	Bad mouth odour is a sign of bad oral health	46%	54%
7.	Are you aware that oral health determines body health	82%	18%
8.	Frequently have bleeding gums?	17%	83.%
9.	If you smoke, causes dental problems	96.04%	3.8%
10.	Do you have frequent deposits on your tongue	64%	36%
11.	Are you aware about the diseases caused if oral hygiene is not maintained?	73.27%	26.83%

Table 1 shows the responses in percentage about the awareness of oral hygiene habits among the participants. When asked for brushing habits to the respondents, the majority of the responses were brushing twice a day 64% and 36% once a day, this shows most of the people are aware that brushing twice is good for health, among them 50% of the participants brushes at least 2mins. 53.47 % has a habit of using mouthwash to maintain good oral health, but most of the respondents 78.24% do not use dental floss. When asked for Habit of using Dental Floss for maintaining good oral hygiene, 26.73% are aware of using dental floss and 73.27% are not aware of using dental floss. Responses for the question "Do you know the adverse effects of using toothpicks frequently".Majority of the respondents said no (78.24%). responses of the question- "Are you aware that bad breath is a sign of bad oral health?".Majority said no.(54%). The response to the question- " Are you aware that oral health determines body health." Majority of the respondents said yes(82%). Deposits on your tongue, can lead to bad odour, majority of the responses were yes (64%), smoking leads to dental problems, majority 96.04% were aware. 83% don't have bleeding gums and 17% have bleeding gums during brushing. The response to the question- "Are you aware about the diseases caused if oral hygiene is not maintained? Majority of the respondents said yes(73.27%).

The present research has origins from the team of investigators where previous studies were done based on clinical reports,interventional studies like[151617],in vitro studies like[141011],and systematic reviews[1819202122].

Compared to previous studies,a total of 84% dental students and 72% medical students visited the dentists at least once in their lifetime [23] .Whereas in this study 94.1% of dental students visited dentists at least once in their lifetime.This study's results of the previous results,as dentists are more aware of the causes and consequences of bad oral health.

In the previous study, only 31% revealed that they use dental floss on a daily basis.[24]whereas in this study 26% of them use dental floss on a daily basis which is similar to the previous study.Only limited population use dental floss as dental students are aware of the consequences.

34.9% of the students follow oral self health habits strictly.[25]whereas in this study 80% follow oral self health habits which is in contrast to the results of the previous study. This difference in result may be due to the population of the study.The sample size of the study is minimum and the homogenous group of study population are the limitations of the study.

Conclusion

This study provides an understanding of oral health knowledge, behaviour and status among dental students, which may help to promote and reform oral health education and establish a model for clinicians and dentists to work together for improving oral health, and also creates awareness among dental students.Therefore the awareness about good oral health has been created among undergraduate dental students.

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Conflict of Interest: The author declares that there was no conflict of interest in the present study.

Source of Funding: Self

Ethical Clearance: Not Required

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Awareness on Alzheimer's Diseases among College Students

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Abstract

Alzheimer's disease is the common form of dementia and possibly contributes about 60-70%. of Cases .There is no cure for Alzheimer's disease and drug therapy for disease is still in its infancy. Approved medication for the treatment of probable Alzheimer disease helps control the Symptoms of Alzheimer's disease **.** This study involves college students in the age of 17 to 20 years. A well Structured questionnaire comprising questions covering socio-demographic information , knowledge, altitude, perception was framed and adminstrested to these participants through an Online survey. The results were collected and were represented in pie charts. The association between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant. More awareness may be created on the risk factor and complications of Alzheimer's diseases among the younger generation.

Keywords *:Awareness; Alzheimer's disease; dementia; online survey*

Introduction

Alzheimer's disease is a common form of dementia that possibly contributes about 60-70% of Cases. Other types of vascular dementia , dementia with Lewy bodies in which a group of diseases that contribute to frontotemporal dementia. The boundaries between subtypes are in distinct and mixed forms mostly co-exist [1]. There is no cure for Alzheimer's disease and drug therapy for the disease is still in its infancy . Approved medication for the treatment Alzheimer's disease, helps control Symptoms of Alzheimer's disease but does not slow down the process progression or reverse [2]. Alzheimer's disease is characterized with mild cognitive impairment and dementia· Pathological changes that underlie Alzheimer's disease begin to accumulate for years or even decades, before emotional, physical or cognitive Symptoms occurs, at which the onset of a gradual and progressive decline in cognition occurs [3] Many physicians do not screen for cognitive problems in their practices unless they receive complaints from either the patient of their family [4] previous studies has demonstrated that quality care of the person with dementia is feasible in acute care setting. When attention to the special needs of patients with dementia is given [5]Currently, only symptomatic treatment can be given to patients with Alzheimer's diseases, and concentrate on potential prophylactic strategies[6]. Recent studies explain that dementia,mainly in men, may be declining in western countries. it is unclear which is the reason behind the dementia that are declining and this could be underpinned by better management of vascular risk [7]

Alzheimer's disease is substantially increased among aged 15 years and more with a progressive decline in memory, thinking , language and learning capacity etc. Alzheimer's diseases should be different compared to normal age - related decline in cognitive function, Which is more graduate and associated

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with less disability. Disease often begins with mild symptoms and ends with severe brain damage. People with dementia lose their ability at various rates [1] . The German psychiatrist Du. Alois Alzheimer was credited for the first time a dementing condition which later becomes known as Alzheimer's disease In landmark 1906 conference lecture and subsequent 1907 article put alzheimer's described the case of Auguste D, a 51-year old women with a peculiar disease of cerebral corter who had presented with progressive and language impairment, disorientation , behavioural symptoms like hallucinations, delusions, parabola and psychosocial impairment [2]. Alzheimer's disease (AD), a very common dementia in elders is a chronic neurodegenerative disease affecting approximately 30 million people worldwide in 2015 [8] Previous studies state that someone in the country will develop Alzheimer's disease every 66 sec . By the year 2050, every case of Alzheimer's is expected to develop within 33 seconds, concluding nearly 1 million new cases per year. In the year 2013, official death certificates recorded 84,767 death cases of Alzheimer's disease, making it the sixth largest cause of death in the United States .The fifth largest cause of death in Americans who age more than 65 year [9] The treatment is currently targeted towards symptomatic therapy although trials are underway Alzheimer's diseases [10]. Researchers indicates the that people are very concerned about the status of their cognitive health and development of AD [11]

In the past, Alzheimer's disease dementia was difficult to differentiate from other dementia causing pathogens, however with the advent of advanced medical imagéng techniques, including molecular imaging have given clinicians and researchers much greater insight into the neuropathological process of their patients aiding in diagnosis. Regardless of these advances, the recognitication of Alzheimer's disease remains one primarily formed on the clinical history and presentation of the patients as examined by the physician , According to the most recent diagnostic Criteria , the clinical onset of Alzheimer's disease dementia one can be divided into 3 broad periods : preclinical, MCI and Alzheimer's disease can be divided dementia and the lengths of time elapsed during these different periods of disease is on the order of decades [12]. Several of these pathologic changes may occur decades before symptom onset, leaving ample time for implementing prevention strategies that target the earliest stages of the disease. [13] .The current population has seen the rise of unaging and other biomarkers to characterize preclinical disease before the development of significant cognitive decline . At last, we suggest future directions and predictions for dementia - related research and potential therapeutic interventions. One of the biggest challenges faced by neuropsychologists over the past oo years is to understand the cognitive and behavioral manifestation of dementia and at their relationship between brain pathology [14]

Patients who have subjective cognitive decline have been found out as a useful population in whom we need to look for preclinical Alzheimer's disease [15] Recent research on childhood obesity [16],natural products in health and diseases [17,18,19,20,21] ,nanoparticles [22,23,24,25], studies on cancer cells [26,27,28,29,30] improved my passion for research and chose this topic to create awareness on Alzheimer's diseases in the society.The aim of this study is to create awareness and knowledge and to determine the attitude and practice towards Alzheimer's disease among College Students

Materials and Methods

This study involved both male and female in the age group of 19-23 years among College students. A well-structured questionnaire comprises 15 questions. This was conducted through an online survey. The online survey Software used was Google forms software. This is a cross sectional descriptive survey that is conducted among 100 students College Students . The results were collected and Tabulated. The descriptive statistics were done using the SPSS software. The results were analysed using statistical analysis.

Results and Discussion

In the survey conducted among 100 college Students 66.3% were female and 33 .7% were males. 81.2% were aware of Alzheimer's disease whereas 18.8% were not aware of Alzheimer's disease. 45.5% of the participants think Alzheimer's disease is curable and about 54.5% say it not curable. 76.2% think memory loss is the symptom for Alzheimer's disease. 43.6% think Alzheimer's disease and dementia are the same and about 56.4% think it is not the same. 47.5% thinks there is no cure for Alzheimer's disease.

37.6% agreed that they have disorientated time and place, often forgetting days of a week. About 50.5% agreed that they have memory loss and 49.5% agreed that they don't have memory loss. 36.6% agreed that memory loss get's worse day by day and 63.4% agreed that it doesn't get worse day by day. 79.2% agreed that they gain information from this survey.

We have seen the association between gender and awareness on symptoms of Alzheimer's disease [Fig 1], awareness on differentiation between Alzheimer's disease and dementia [Fig 2], awareness on treatment for Alzheimer's disease [Fig 3], awareness on Alzheimer's disease [Fig 4], awareness on memory loss getting worse everyday [Fig 5], awareness on gaining information about Alzheimer's diseases after doing this survey [Fig 6].

In the Study done by Wendy Smyth et al 2012 regarding the opinion of the participant thinking if Alzheimer's diseases and dementia are the same 62% agreed that yes and 38% agreed that No [5]. In the Study done by Miri Kim et al, 2020 majority of the participants agreed that younger people are affected more [8] And in Study done by Maria .S. Sabbagh et al, 2011 , 45.7% agreed that younger people are affected more[4].

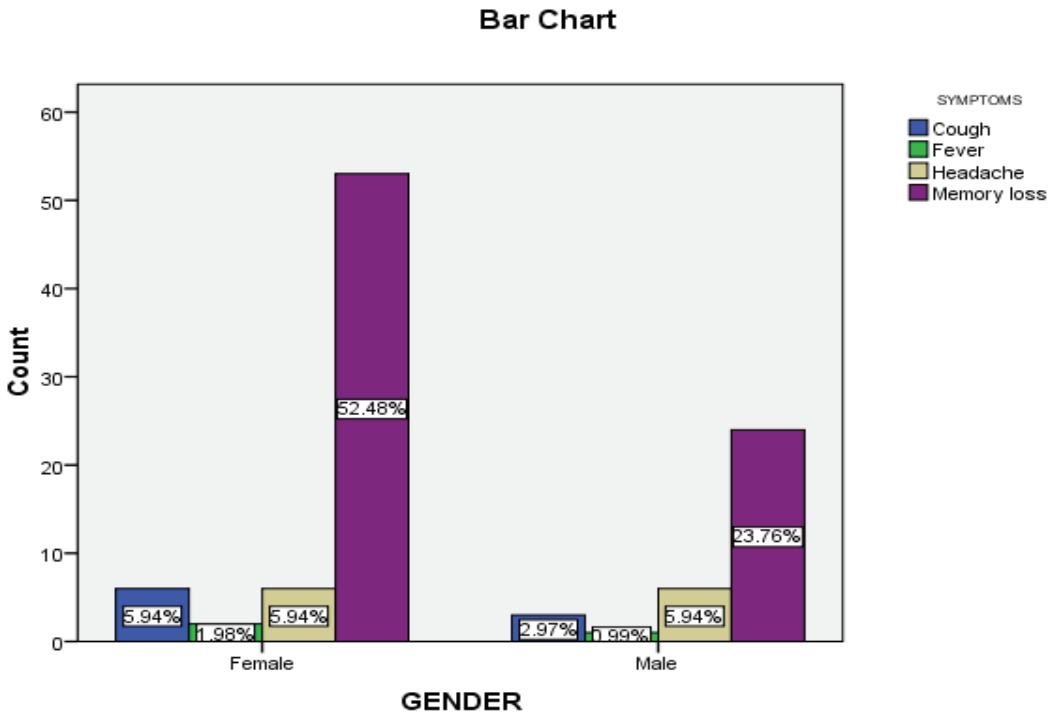


Figure 1:Bar Chart depicts the association between gender (X axis) and responses of awareness on symptoms of Alzheimer's disease (Y axis). 52.48% of female and 23.78 % of male agree that memory loss is a symptom . Blue colour denotes Female and green colour denotes Male .The majority of the female population (52.48%) agree that memory loss is a symptom of Alzheimer's disease . The association was found by chi square test (Chi square value= 1.649) which shows (p value=0.648) no statistical significance.

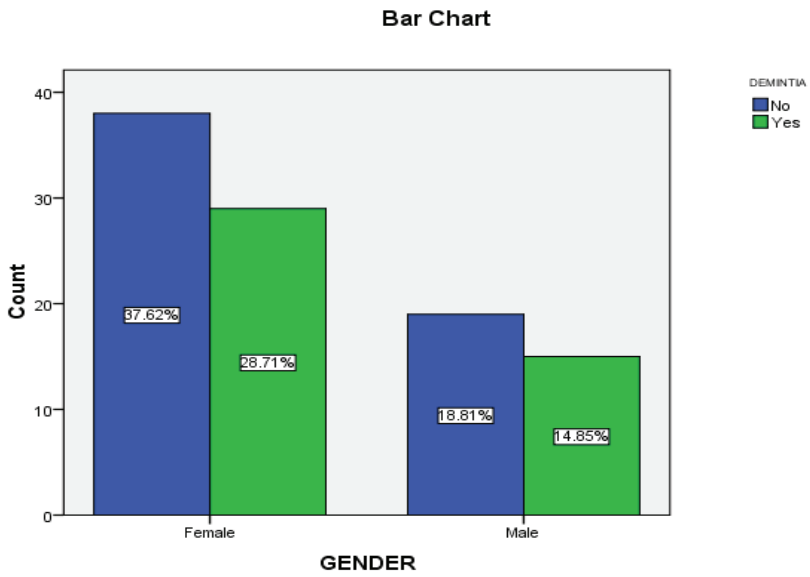


Figure 2:Bar Chart depicts the association between gender (X axis) and response of awareness on differentiation between Alzheimer's disease and dementia (Y axis). 28.71% of female and 14.85% of male accept dementia and Alzhemier's disease are the similarity .Blue colour denotes Female and green colour denotes Male. The majority of the female population (37.62%) agree that dementia and Alzheimer's disease are not similar. The association was found by chi square test (Chi square value= 0.004) which shows (p value = 0.936) no statistical significance.

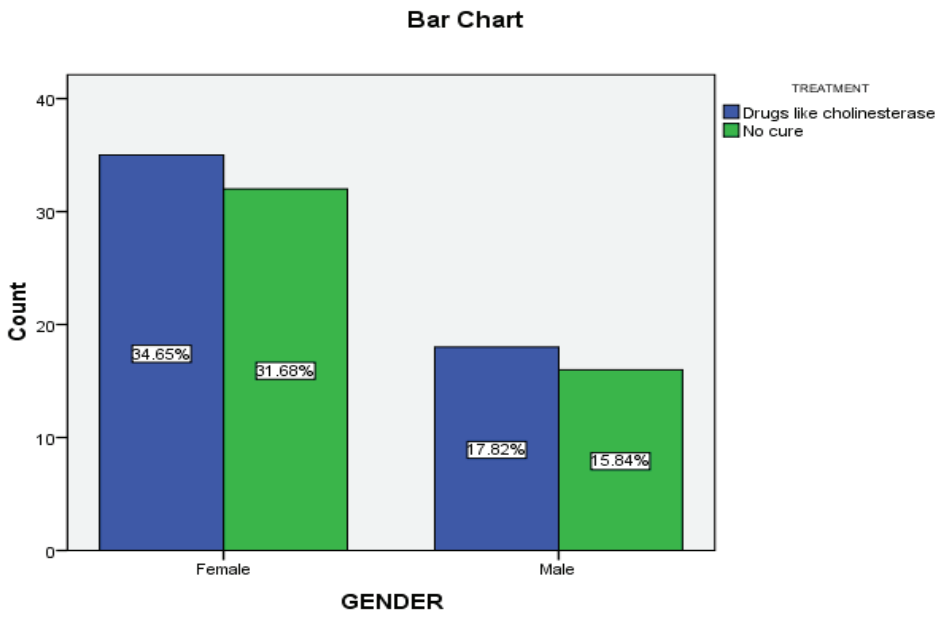


Figure 3 : Bar Chart depicts the association between gender (X axis) and responses of awareness on treatment for Alzheimer's disease (Y axis).34.65% of female and 17.82% of male admit cholinesterase as one of the best treatments for Alzheimer's disease .Blue colour denotes Female and green colour denotes Male. The majority of the female population (34.65%) responses that drug like cholinesterase is the best treatment for Alzheimer's diseases .The association was found by chi square test (Chi square value= 0.004) which shows (p value = 0.947) no statistical significance

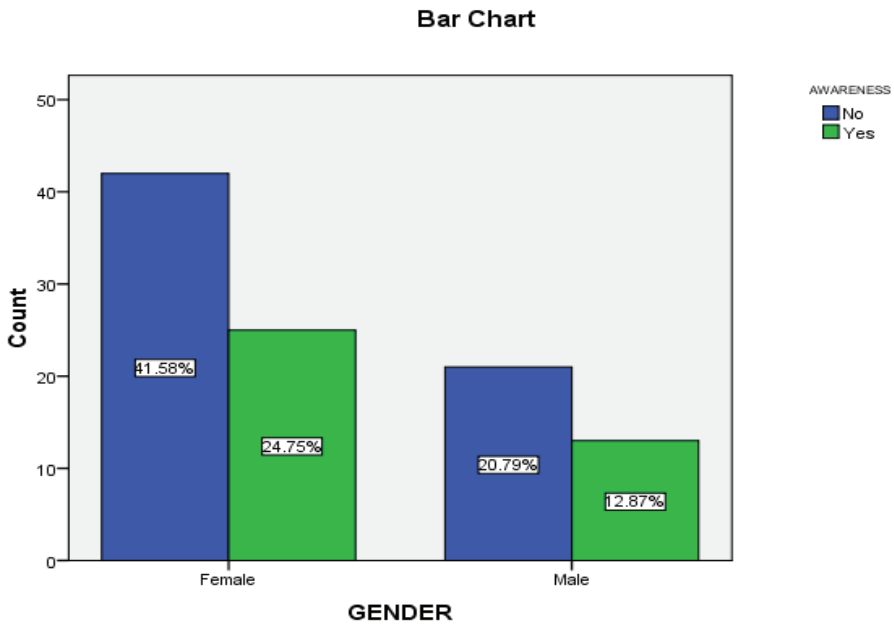


Figure 4 :Bar Chart depicts the association between gender (X axis) and responses of awareness on Alzheimer's disease (Y axis). 24.75% of female and 12.87% of male have enough awareness about Alzheimer's disease.Blue colour denotes Female and green colour denotes Male .The majority of the female population (34.65%) agrees that there is no enough awareness on Alzheimer's diseases . The association was found by chi square test (Chi square value= 0.008) which shows (p value = 0.928) no statistical significance.

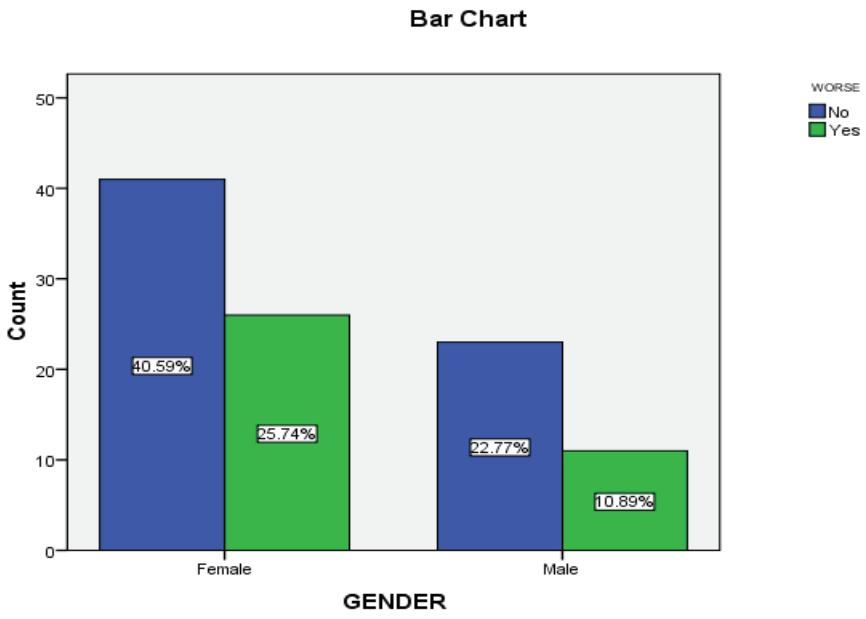


Figure 5:Bar Chart depicts the association between gender (X axis) and response of awareness on memory loss getting worse everyday (Y axis). 25.74% of female and 10.89% of male population says memory loss gets worse day by day. Blue colour denotes Female and green colour denotes Male.The majority of the female population (34.65%) responded that the memory loss doesn't get worse . The association was found by chi square test (Chi square value= 0.242) which shows (p value = 0.525) no statistical significance.

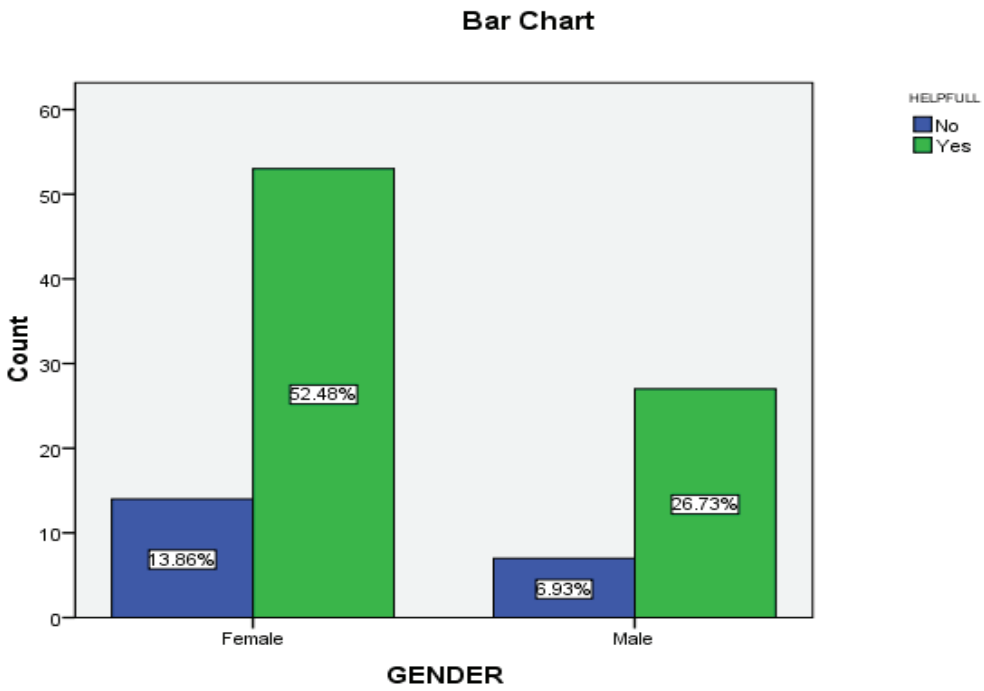


Figure 6 :Bar Chart depicts the association between gender (X axis) and response of awareness on gaining information about Alzheimer's diseases after doing this survey (Y axis).52.48% of female and 26.73% of male agree that this survey was helpful.Blue colour denotes Female and green colour denotes Male .The majority of the female population (52.48%) respond that this survey was helpful to learn more about Alzheimer Diseases . The association was found by chi square test (Chi square value= 0.405) which shows (p value = 0.971) no statistical significance.

Conclusion

From our study we can conclude that students are having good knowledge and awareness of Alzheimer's disease and its symptoms, treatment etc, but still few participants are not fully aware of Alzheimer's disease. Seminars in educational institutions, active lifestyle modifications and memory workshops may be required to bring awareness on Azheimer's disease among the community.

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Conflict of interest - All the authors declare no conflict of interest in the study.

Ethical Clearance: Not Required

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Knowledge on Emergency Management of Avulsed Tooth among Dental Students

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Abstract

Tooth avulsion is the most common dental trauma.The prevailing society considers the undergraduate preclinical students as dentists and so it becomes essential to possess knowledge and to be aware of the first aid and emergency management of dental trauma. The aim of the study is to assess and compare the knowledge of emergency management of tooth avulsion among dental students of different years. A descriptive cross sectional study with 160 students as sample size was conducted. The participants were grouped into three as Group A (Preclinical BDS students), Group B (Clinical BDS students) and Group C (Interns). A self structured questionnaire was distributed among the population and the data were collected and analysed with SPSS software. Comparison between the groups was done by Chi square test with the significance $p<0.05$. The results were mostly statistically significant. The preclinical students showed active participation and had quite knowledge (63.7%) on emergency management of tooth avulsion. Knowledge and awareness on emergency management of tooth avulsion was average among preclinical students, whereas good in clinical students and better among interns.

Keywords: *Dental trauma, tooth avulsion, emergency management, Dental students, awareness and knowledge.*

Introduction

Tooth avulsion is one of the critical forms of dental trauma. Tooth avulsion is the absolute displacement of a tooth from its socket due to accidental and non accidental injury [1] .These types of dental injuries occur chiefly between the ages 8 and 11 years when the child is exposed in a school environment and thus falling accidents leading to dental trauma is a natural happening [2]. The Injury of tooth avulsion when occurring on permanent dentition, it mainly involves the upper front teeth [3]. The growth of teeth is much influenced by genetics, race and ethnicity [4]. The clinical condition of such dental trauma is found to be that the socket is found to be empty and filled with coagulum and it can be extended to further complications involving pulp necrosis and periapical inflammation [5]. Being one of the serious dental trauma, emergency management becomes an essential criteria. The management of avulsed teeth depends on whether it is primary or permanent dentition. The management of avulsed teeth in terms of primary dentition, only clinical and radiographical following is necessary as far as the permanent successor is erupted and replant is not advisable[6]. In case of permanent dentition, the tooth is picked from the crown and cleaned under cold running water prior to repositioning and even the individual can bite on a handkerchief to clasp it to the socket. The management of tooth avulsion grants a paramount importance to the storage medium used. It should be a physiological media such as milk solution or saline and should not be water [7]. Also saliva is considered as the good storage medium beside milk and saline as it is the

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product of oral mucosa and salivary glands[8].This form of dental injury frequently found in individuals having active participation in sports [9].

In the prior studies, with a similar population of students from nine universities in Khartoum state, accommodated nearly 1575 participants. That study concluded that most of the second year students do not have sufficient knowledge on emergency management of avulsed teeth [10] . Another survey study on knowledge of emergency management of tooth avulsion with the population of 102 general dental practitioners in Kathmandu district, concluded that the observed population does not have sufficient knowledge on management of avulsed teeth[11] . In addition, a study undertaken with the population of 182 general dental practitioners attending the annual Malaysian Dental Association conference, reported that the knowledge of the population on managing avulsed teeth needs to be improved [12]. These studies have been limited within the factors that the response rate is low; the framed questionnaire was not circulated among the common category and also due to lack of guidelines.

General dental practitioners have the responsibility of diagnosing and treating dental trauma and thus they should be well educated and aware of emergency management [13]. The new and freshly undergraduate dental students are generally considered as dentists in the community and they might be called for any dental trauma emergency for which the students should process the knowledge of management. Thus all dental professionals including students must possess the knowledge of first aid in case of dental injuries [14–16]

This awareness benefits the patient with good prognosis and better treatment according to the legal ethics followed by dentists [17] . Better treatment also depends on empathy which is a healthcare provider-patient relationships[18]. The present study involves only undergraduate students which is exclusive and lacking in previous studies. Previously our team had conducted a similar survey [19] and now we are focusing on epidemiological surveys. The idea for this survey stemmed from the current interest in our community. The aim of the study is to assess the knowledge on emergency management of avulsed teeth among dental students.

Materials and Methods

The present study was conducted among dental students in 2020. This questionnaire based cross sectional study which was approved by the institutional review board which has undertaken many successful studies [20]. Number of participants took part in the survey was 160 which were distributed among three groups as group A consists of Pre clinical BDS students (1st and 2nd year), group B consists of clinical BDS students (3rd and 4th year) and group C comprises interns. The current study was undertaken with a convenient sampling method.

The questionnaire was self structured and modified from the previous similar study questionnaire[10–12] with demographic information and nine knowledge and awareness based close ended questions. The questionnaire validity checking was done by the faculty members of the institution. The questionnaire was circulated and data were collected using data collection software, Google forms. From the data obtained it was analysed and cleaned up to excel sheet. Then the data for each question is represented in the form of pie charts and bar charts.

Analysis of data was carried out by SPSS by IBM, statistical software. Descriptive statistics was done to summarise demographic data and chi square test to analyse the survey data. The p value less than 0.05 was considered as statistically significant and the confidence level is 95% .

Results and Discussion

The data was analysed and it was obtained that 45% of the population are male and 55% were female. 63.7% were pre clinical students, 20.6% were clinical students, 15.6% were interns. 83.13% were aware of dental trauma and 16.8% were unaware of it. When experience on avulsed teeth was questioned, 51.8% had experience on avulsed teeth and 48% had not experienced it. 68.13% of the observed population stated avulsion occurs mostly in primary dentition while 31.88% stated as permanent dentition. 56.25% were aware of emergency management whereas 43.75% were unaware. About 48.75% were unaware of proper medium of storage of avulsed teeth while 15.63% answered milk. 80.6% of the population considered time as an important factor while 61.8% were unaware of it. 59.38% of the considered

population lack knowledge on carrying out RCT in replanted avulsed permanent tooth. Similarly 57.5% of the respondents were unaware of commencement of RCT after replantation while 10.6% answered as 6 months after replantation. (Table 1)

Table 1: Table showing percentage of responses:

1. Are you aware of dental trauma and it's complications	• Yes	83.13%
	• No	16.87%
2. Do you have any experience of avulsed tooth	• Yes	51.88%
	• No	48.13%
3. Avulsion occurs mostly in	• Primary dentition	68.13%
	• Permanent dentition	31.88%
4. Are you aware of emergency management of tooth avulsion	• Yes	56.25%
	• No	43.75%
5. What is the proper medium for storage	• Milk solution	15.63%
	• Saline	4.38%
	• Hold the tooth in mouth itself/saline	6.88%
	• Any of the above	8.75%
	• Water	5%
	• Pack the tooth in ice	6.25%
	• Seal the tooth in plastic wrap	4.38%
	• Don't know	48.75%
6. If an avulsed tooth is covered with dirt and you would like to replant it, how would you prepare the tooth?	• Wash tooth in water	21.25%
	• Scrub the tooth gently with a toothbrush	12.50%
	• Rinse tooth in saline	10%
	• Put tooth straight back into socket, with no Pre treatment	6.88%
	• Don't know	49.38%

Cont.. **Table 1: Table showing percentage of responses:**

7. Do you think that time is an important factor for management of avulsed tooth	• Yes	30.63%
	• No	7.5%
	• Don't know	61.88%
8. Would you carry out RCT (Root canal treatment) after replantation of the avulsed permanent tooth?	• Yes, if tooth was immature with an open apex	13.75%
	• Yes, if tooth was fully formed with closed apex	6.25%
	• Yes, only after the patient develops signs and symptoms	10%
	• No	10.63%
	• Don't know	59.38%
9. If you were to carry out RCT on replanted tooth, when would you start the treatment?	• Immediately after replanting the tooth	18.75%
	• 7-10 days after replantation and before splint removal	13.13%
	• 6 months after replantation	10.83%
	• Don't know	57.50

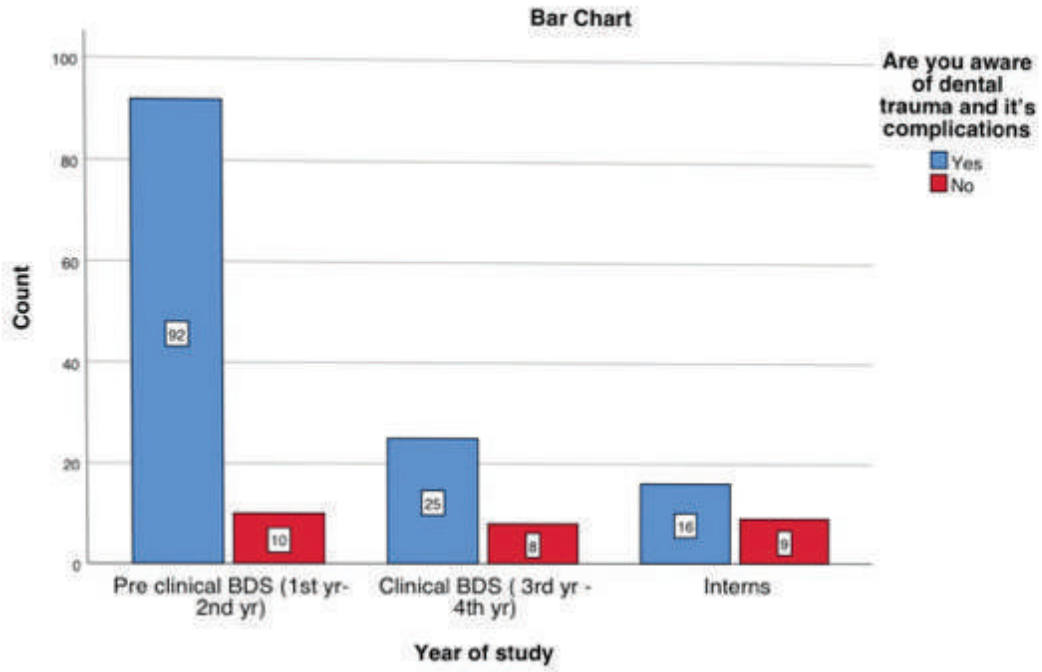


Figure 1: Bar graph showing association between different years of study and awareness on dental trauma and its complication. X axis represents different years of study and the Y axis represents the number of students. Blue denotes yes and red denotes no. Pre clinical BDS (1st year- 2nd year) students (92) show more awareness on dental trauma than students of other year and was statistically significant. (Chi square test showing, p= 0.03 - Indicating statistically significant).

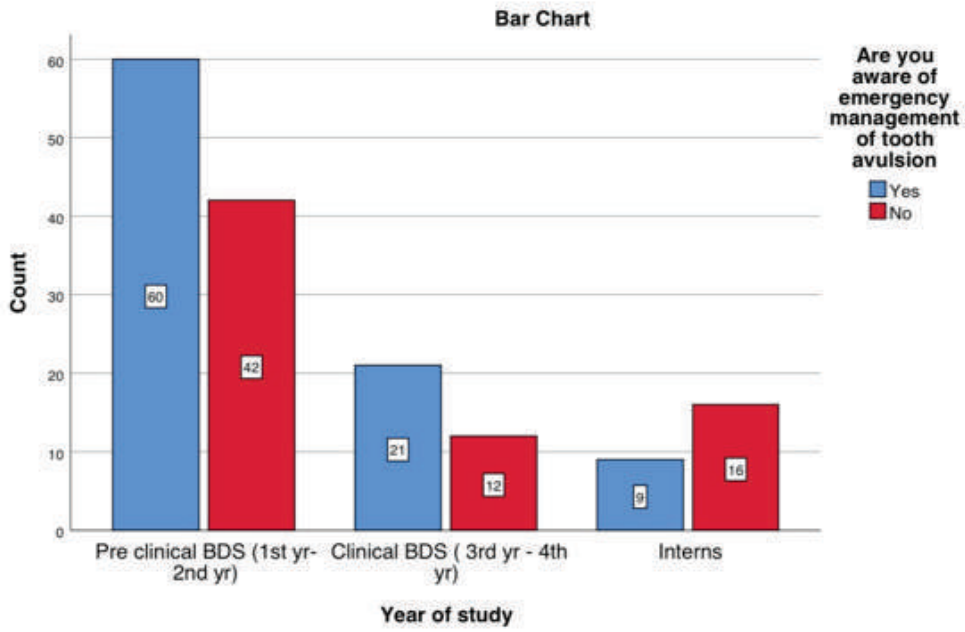


Figure 2: Bar graph showing association between different years of study and awareness on emergency management of tooth avulsion.X axis represents different years of study and the Y axis represents the number of students. Blue denotes yes and red denotes no. Pre clinical BDS (1st year- 2nd year) students (60) show more awareness on emergency management but it was statistically not significant. (Chi square test showing,p= 0.07- Indicating statistically not significant).

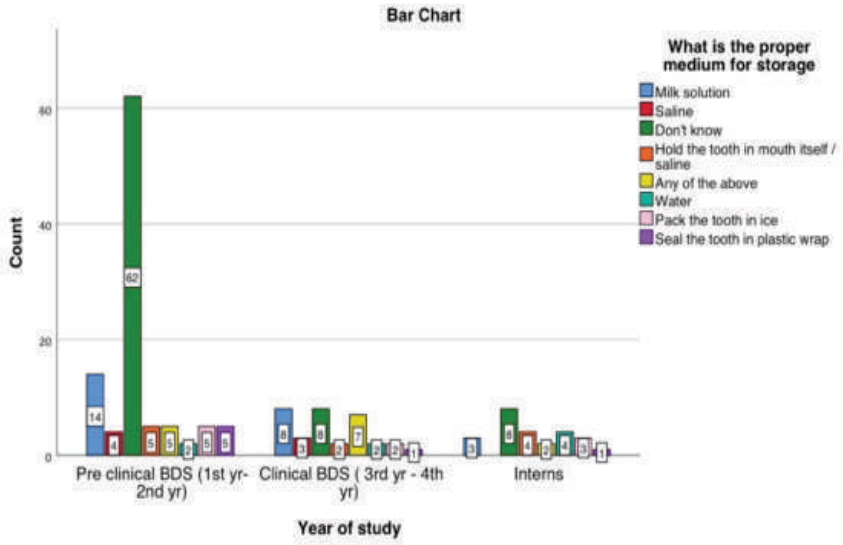


Figure 3: Bar graph showing association between different years of study and knowledge on proper storage medium of avulsed teeth.X axis represents different years of study and the Y axis represents the number of students. Blue denotes milk solution, red denotes saline, dark green denotes don't know, orange denotes hold the teeth in mouth itself/saline, yellow denotes any of the above, light green denotes water, pink denotes pack the tooth in ice and violet denotes seal the tooth in plastic wrap. Pre clinical BDS (1st year- 2nd year) students (62) showed more response as don't know on the proper storage medium and was statistically significant. (Chi square test showing,p=0.002- Indicating statistically significant).

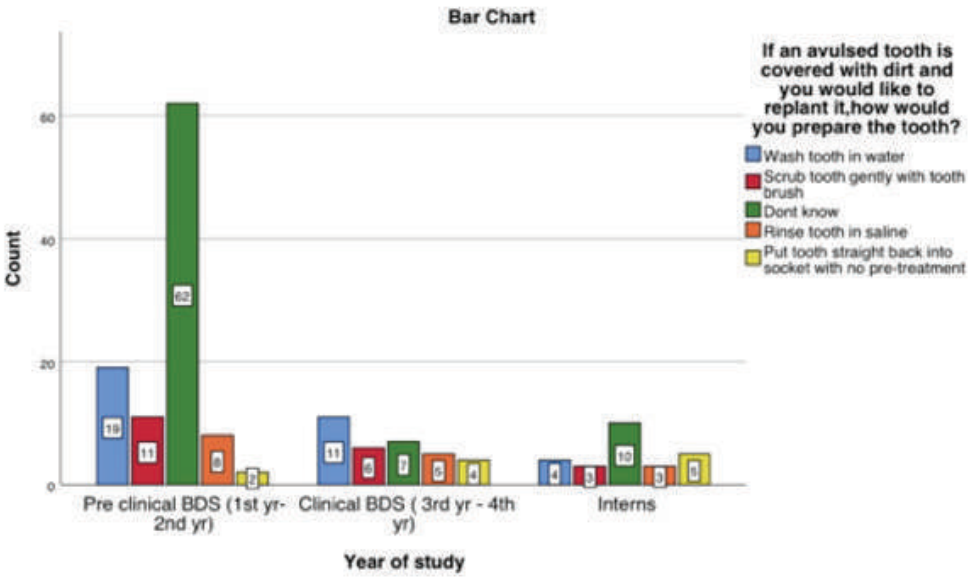


Figure 4: Bar graph showing association between different years of study and methods of cleaning an avulsed teeth before replanting. X axis represents different years of study and the Y axis represents the number of students. Blue denotes washing teeth in water, red denotes scrub the teeth gently with toothbrush, green denotes don't know, orange denotes rinse teeth in saline, yellow denotes put the teeth into the socket without any pretreatment. Pre clinical BDS (1st year- 2nd year) students (62) showed more response on don't know option for different methods of cleaning an avulsed teeth before replanting and was statistically significant. (Chi square test showing, p= 0.002 - Indicating statistically significant).

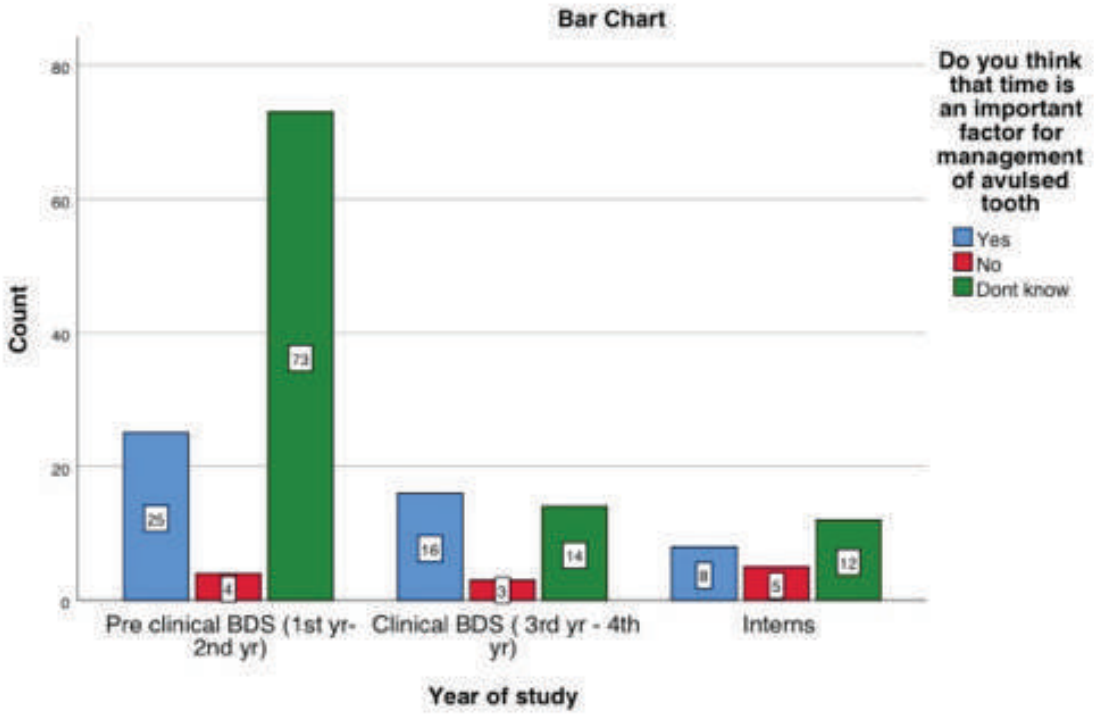


Figure 5: Bar graph showing association between different years of study and knowledge on time is an important factor for management of tooth avulsion.X axis represents different years of study and the Y axis represents the number of students. Blue denotes yes and red denotes no and green represents don't know. Pre clinical BDS (1st year- 2nd year) students (73) showed more response to the don't know option for time being an important factor in emergency management and was statistically significant. (Chi square test showing,p= 0.003- Indicating statistically significant).

In our study, the participants were questioned whether they were aware of any dental trauma and its complications 83.13% of the population gave a positive answer of yes. 92 out of 102 pre clinical students, 25 out of 33 clinical students and 16 out of 25 interns gave positive response [Fig.1]. A comparison between different years of study showed (p)= 0.03; p<0.05 and thus it is statistically significant. There exists an opposite study by Fujita.Y et.al., in which the interns gave a more positive response of 91.2% [1] . This difference is because the pre clinical students belonging to our population have awareness on dental trauma which is necessary.

Tooth avulsion was experienced by 51.88% of our population in which 55 out of 102 pre clinical students, 18 out of 33 clinical students and 10 out of 25 interns established positive response. The comparison between years of study about their experience of tooth avulsion showed (p)=0.43;p <0.05 which is statistically significant. In the similar study by Fernandes A.V et.al., with a similar population, the response showed that 81.4% of them had not witnessed tooth avulsion [21]. This opposite finding is due to the exposure of students to clinics which creates more awareness.

The participants were enquired about the type of dentition in which avulsion is mostly seen, 68.1% of population gave a positive response of primary dentition. 82 of 102 pre clinical students, 19 of 33 clinical students and 8 of 25 interns responded positively. This when compared between years of study showed (p)=0; p<0.05 and thus it is statistically significant. An opposite finding by Fernandes A.V et.al., in which 60.4% of the population stated response as permanent dentition [21]. This contrast result is due to the lack of knowledge on avulsion in the observed population the opposite study.

The awareness on emergency management of avulsion were questioned for which 56.25% responded positively. 60 out of 102 pre clinical students, 21 out of 33 clinical students and 9 out of 25 interns gave yes as an answer [Fig.2]. Comparison between different years of study showed (p)=0.07; p> 0.05 and thus statistically insignificant. In a previous study by Azmi M A et.al., 89.8% of interns were aware and not preclinical students [10]. This difference between the studies adds an evidence that the preclinical students of our population possess quite knowledge on avulsion.

The population was investigated about the proper medium of storage and only 16.5% of our population gave positive responses as milk. 14 out of 102 preclinical students, 8 out of 33 clinical students and 3 out of 25 interns reacted positively as milk which is an accurate storage medium [Fig.3]. The comparison between years of study showed (p)= 0.002; p<0.05 which is statistically significant. There exists opposite findings by Azmi MA et.al., in which 64.3% gave positive response and in another study by Fujita Y et.al., in which 57.4% responded positively. These positive responses were given by interns [1,10]. This again indicates that preclinical students of our population were aware of important guidelines.

When the population were questioned about the cleaning of dirt and tooth preparation, 21.25% of them responded that it should be cleaned under water which is a positive response.19 out of 102 preclinical students,11 out of 33 clinical students and 4 out of 25 interns answered positively [Fig.4]. Comparison between years of study showed (p)= 0.002; p<0.05 which is statistically significant. In the study by Fujita Y et.al., 64.7% and in another study by Fernandes AV et.al., 67.8% of similar populations have the same positive response [1,21]. This shows that preclinical students of our population learnt the necessary guidelines for a tooth to be replanted.

The participants were asked about their awareness on time as a factor for treatment of avulsion and 30.6% of responses were positive as yes in which 25 out of 102 preclinical students, 16 out of 33 clinical students and 8 of 25 interns gave an affirmative answer [Fig.5]. Comparison between the year of study showed (p)= 0.003; p<0.05 which is statistically significant. A supportive study by Fernandes A V et.al., establishes 88.5% positive response. Yet another supportive study by Azmi M A et.al., in which 38.4% of preclinical students showed positive response [10,21] . Thus the population was aware of one of the necessary guidelines for replantation.

The participants were enquired about the possibility of carrying out RCT after replantation in an avulsed tooth. Only 10% of respondents gave an affirmative response that RCT is advisable only if there are any signs and symptoms developed. 8 of 102 preclinical students, 5 of 33 clinical students and 3 of 25 interns stated positive responses. This result is supported by the study by Dalia Abdullah et.al., in which 53% of the population were aware that RCT can be done only after the development of signs and symptoms [12]. Comparison between the year of study showed (p)=0.05; p<0.05 which is statistically significant.

The participants were asked about the significant period to commence RCT on a replanted tooth for which 13.3% of respondents answered as 6 months after replantation and is a positive response. 8 of 102 preclinical students, 5 of 33 clinical students and 4 of 25 interns reacted positively.Comparison between years of study showed (p)= 0.06; p>0.05:and thus statistically significant. The similar response of 54.5% in a similar population was observed in the previous study by Dalia Abdullah et.al., [12].

Limitation And Future Scope:

Limitations of the current study include less sample size, homogeneous population i.e., consideration of only dental students and restriction of sample to specific local regions. Everyone cares about their systemic health more than oral health, so it is our duty to make them aware of dental injuries also [22,23]. The knowledge and awareness of emergency management of any dental trauma should be conducted as classes or any activities to improve the prognosis and better treatment. During treatment, it is better to carry out evidence based dentistry like photographing the before and after treatment conditions,etc., which would also enhance the awareness of patients and knowledge of the dentists [24,25] . Also dentists should be careful about molar incisor hypomineralisation, ethical issues and other factors during replant [26,27]. However, this treatment of dental trauma involves RCT and replantation and not any

complications like biopsy implant [28]. But the curing process involves the nutrition they intake from dietary sources [29]. Since a tooth is a weapon secretly besides masticatory work, it becomes of paramount importance to protect it [30] .

Conclusion

Knowledge and awareness on emergency management of tooth avulsion was average among preclinical students, whereas good in clinical students and better among interns. Being a dental professional, knowledge of first-aid and emergency management of dental injuries is an essential and thus awareness programs should be conducted.

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Ethical Clearance- Not required

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Retrospective Study on Patient Compliance Following Tooth Extraction

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Abstract

The aim of this study is to investigate the follow up and compliance of patients who sternly follow the post operative instructions after non surgical permanent tooth extraction. Data collection were taken from patients who reported to the college from a particular time period which is from June 2019 to March 2020. The number of extractions and reviewers were obtained from dental archiving software which is offered by the university. The statistical test that was run was the chi square test using statistical software SPSS by IBM. The results were represented in the form of graphs and pie charts. In this study , the most common age group were 41-50 years of age(20.38%) and >60 years of age for extractions (25.75%) and reviews respectively and the most common gender predilection was found to be male subjects more than female subjects (53.55%). The number of patients who returned for reviews were very less (3459 out of 21648) compared to the total number of extractions done. Hence, awareness programs must be initiated and people must be emphasised for vigorous oral hygiene maintenance.

Keywords: *Extraction,Compliance,Post operative instructions, Post operative complications, Dry socket*

Introduction

In dentistry, various treatment procedures are done to treat dental problems. [1] Extractions are the most common surgical procedure in Oral Surgery and it appears well documented in literature. Dental extraction is the most common procedure carried out by dentists such as extraction of mandibular third molar before an orthognathic surgery [2] and it is a common model for evaluating the efficacy of analgesics for acute dental pain relief. It is often associated with swelling, pain, and trismus. The pain of tooth extraction is likely to be one of the most severe pain that an individual experiences during his or her life. [3] A similar study done in Saveetha dental College, Dept of Oral and Maxillofacial Surgery, was to compare the effect of application of 0.2% chlorhexidine gel, a eugenol based paste, together with a control group on the postoperative incidence of alveolar osteitis in patients having third molars extracted. The chlorhexidine group showed less incidence of alveolar osteitis than other reported studies and also less pain, inflammation, infection, and better wound healing than the control group. [4]

Several authors have described that there are many factors that influence in easing the extraction procedure as well as the impact of this procedure on the quality of life of patients during the postoperative care [5]. Study done in Saveetha Dental College showed that tranexamic acid was the best protocol in the management of blood loss, minimizing the operating time, and providing the best surgical field. Maxillary impacted third molar is

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removed before or during maxillary osteotomies to prevent postoperative infection [6]. However, the post extraction period is also impacted by the understanding of the patient and the subsequent implementation of the guidelines presented by the professional in order to reduce the morbidity, complications and to improve the quality of lifestyle of the patient. [7]

Postoperatively, it is very important to control the pain and surgical site should be well-managed to improve the treatment outcomes [8]. The postoperative period is influenced by the understanding of the patient and the successive achievement of the instructions presented by the professional in order to minimize morbidity, complications, and improve the quality of life of the patient. [9] Oral squamous cell carcinoma is one of the most commonly occurring oral lesions, in which extraction of hopeless teeth is done before radiotherapy. In some salivary gland pathology, like ranula, mucocele occurs due to sharp teeth that can be either conservatively managed or extracted. [10]

In cases of Oral submucous fibrosis, restricted mouth opening which can cause difficulty in extractions and may lead to complication like oroantral communication.It showed that buccal fat pad graft proved to give better results as the interposition material for closure of oroantral communication as it has good patient acceptance, rapid epithelization, minimal donor site morbidity and minimal intra and postoperative complications [11].In some cases of inferior alveolar nerve block for removal of mandibular molar, facial paralysis can occur. The ability to use Botox as an adjuvant and primary mode of treatment for various maxillofacial disorders offers exciting treatment options for dentists and patients in the future [12]. One of the most common causes of maxillofacial trauma is road traffic accidents, in which extraction of teeth in the fracture line is removed.

Administration of local anesthesia is one of the procedures which results in increased anxiety level among patients. [13]. Pre-operative dental anxiety is a major predictor of pain experienced by patients during dental extractions. Hence, it is important to reduce anxiety before treatment to reduce pain during the treatment. Pharmacologic modalities like sedation can be used for reducing anxiety and pain related to the treatment in indicated patients. [14] Adequate and effective communication between doctor and patient increases the level of understanding and therefore reduces postoperative complications.. In order to achieve therapeutic achievements, patients must obey and be cooperative with the clinical prescriptions. Interestingly, there is a large possibility that stress may vary in terms of gender and hence result in a difference in healing of the socket .Follow-up is essential to support the patient in recovery. According to Monaco, Staffolani, Gatto and Checchi, age and gender along with smoking and alcohol consumption may cause variations in postoperative complications[9].

Any study related to patient compliance and follow up rate has its own challenges. This could be due to the travel time for the patient, lack of understanding of the post-operative instructions and such other reasons. The aim of this study is "to investigate the follow-up rate and compliance of patients who sternly follow the post-operative instructions after non-surgical permanent tooth extraction through health status of the socket"

Materials and Methods

This study was done in a university setting. The data collected was obtained from the institution. Ethical approval was obtained from the Institutional scientific review board, Saveetha Dental College and Hospitals. There are three reviewers for this data namely the guide, the researcher and the reviewer. The data was from a time period of one year which was from June 2019 to March 2020. Cross verification of the data was done by one additional reviewer. Inclusion of all available data was done to minimize the sampling bias.

Data was reviewed from the case record of 21,648 patients and then the data was extracted and filtered using the exclusion and inclusion criterias. The exclusion criteria includes primary teeth extraction and incomplete database and inclusion criteria includes permanent teeth and complete database. The collected data was entered and sorted in excel sheets and then was exported to SPSS software. The incomplete or censored data were excluded from the study.

The statistical analysis used was chi square test and the statistical analysis used was SPSS by IBM. The list of independent variables includes tooth number and the dependent variable includes age and gender. The type of

analysis done was correlation and association.

Results and Discussion

A total number of patients included in the study was 21,648. Out of which 21,648 (85.9%) patients underwent extractions and the number of patients who reported for follow up were 3549 (14.1%). Incidence of Male subjects were higher for extractions (53.5%) compared to female subjects (11594 out 21,648)(Fig 1). The most common gender among those who reported for reviews were male subjects (1895 out of 3549) (53.3%) (Fig 2)The most common age group found among extractions were 41-50 years of age (20.38%) (Fig 3) and for those who reported for review was age greater than 60 years (27.75%) (Fig 4). Correlation between the 7 different age groups of patients tand the gender of the patients that underwent extractions. (Fig5) Correlation between the 7 different age groups of patients tand the gender of the patients that reported for reviews. (Fig 6)

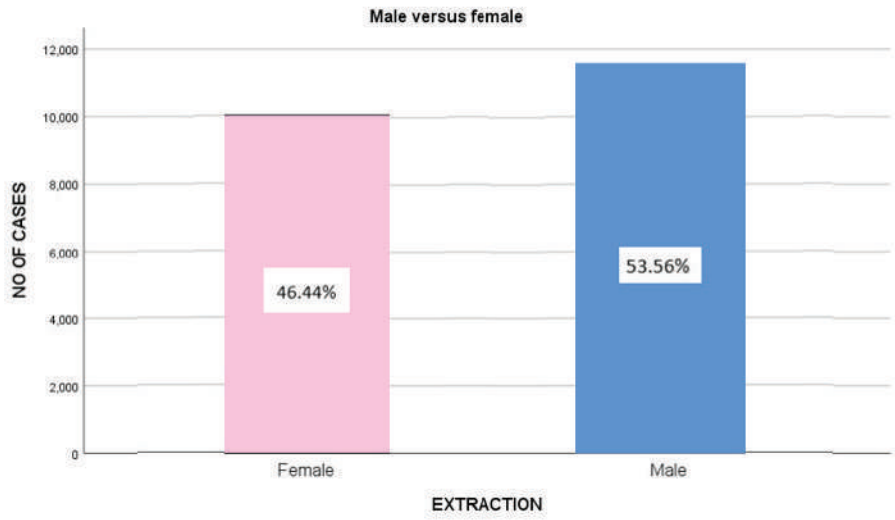


Fig 1: Graph 1 represents the number of extractions done based on gender of the patient. The x axis represents the gender of the patients and the Y axis represents the number of patients studied. Male 11,594 shown as blue in the graph and female subjects 10,054 shown as pink colour in the graph.

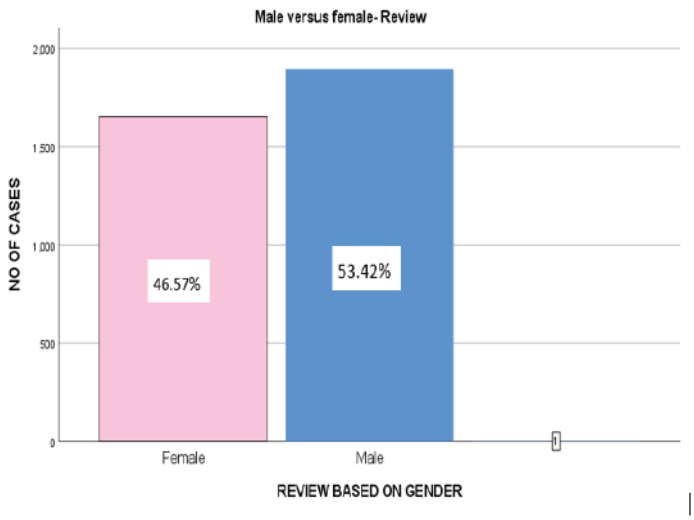


Fig 2: Graph 2 represents the number of patients that reported for reviews based on gender. The x axis represents the gender of the patients and the Y axis represents the number of patients studied. Male subjects shown in blue colour in the graph again show a higher incidence compared to female subjects depicted in pink colour, showing a 1895 out of total patients of 3549. (53.3%)

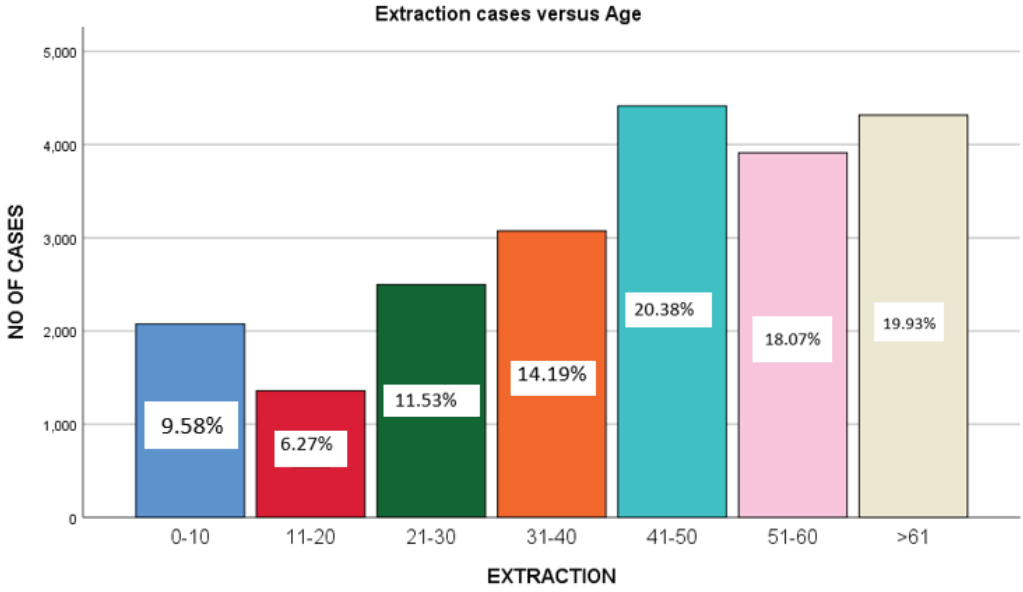


Fig 3: Graph represents the number of extractions done based on age. The x axis represents the age of the patients which was divided into 7 different categories and the Y axis represents the number of patients studied. The 7 categories were namely 0-10= 2076, 11-20= 1358 , 21-30= 2498, 31-40= 3073, 41-50= 4414, 51-60= 3913 and >60 years= 4316. 20.38

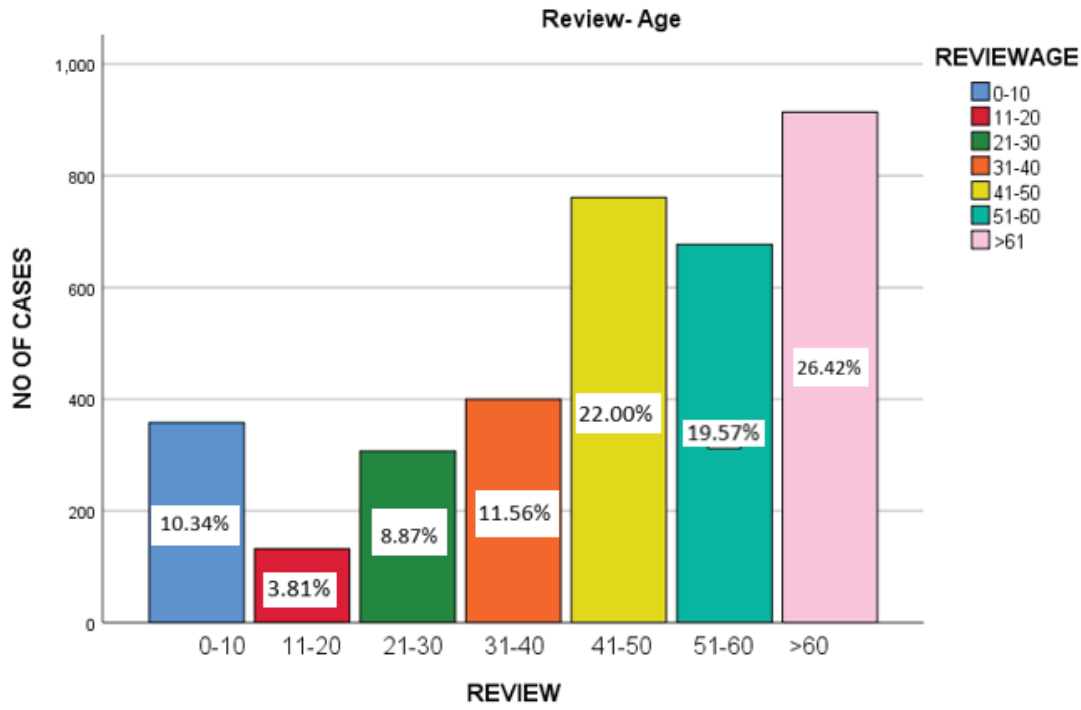


Fig 4**:** Graph represents number of patients who reported for reviews based on age of the patient. The x axis represents the age of the patients which was divided into 7 different categories and the Y axis represents the number of patients studied. The 7 categories were namely 0-10= 358, 11-20= 132, 21-30= 307, 31-40= 400, 41-50= 761, 51-60= 677 and >60 years= 914. The total number of subjects that appeared for reviews were 3549 out of 21,648.

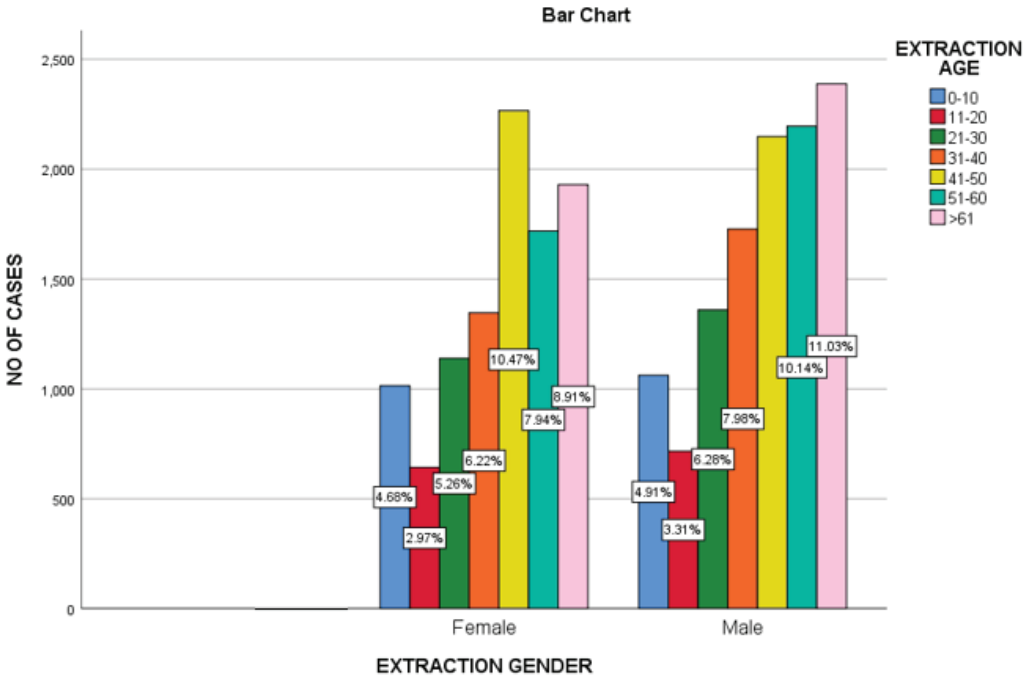


Fig 5: Bar graph shows the correlation between the 7 different age groups of patients tand the gender of the patients that underwent extractions. X axis represents the gender of the patients in 7 categories and Y axis represents the number of patients who underwent extraction. Chi square test was done and association was found to be statistically significant. Pearson's chi squared test value 76.553 Df: 12, p value:0.000 <0.5 Hence statistically significant. The graph infers that male subjects underwent more number of extractions than female subjects.

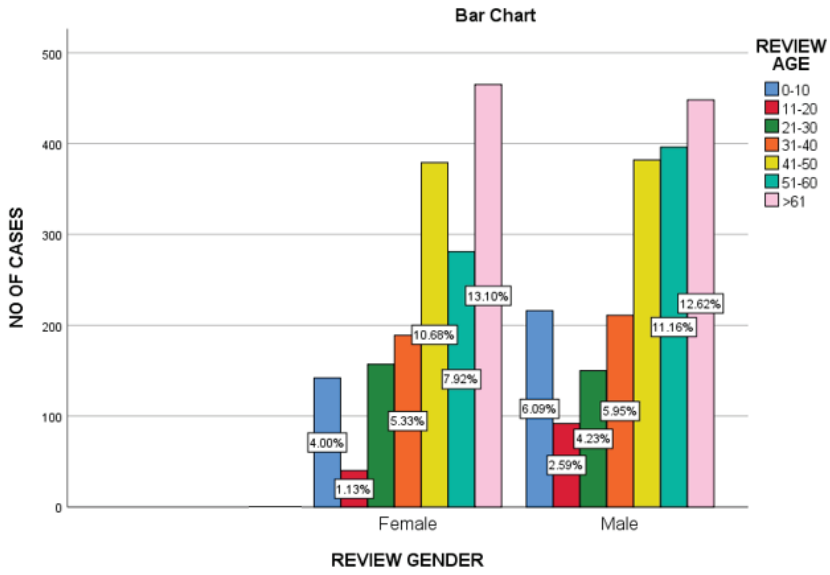


Fig 6: Bar graph shows the correlation between the 7 different age groups of patients tand the gender of the patients that reported for reviews. X axis represents the gender of the patients in 7 categories and Y axis represents the number of patients who reported for review. Chi square test was done and association was found to be statistically significant. Pearson's chi squared test value 43.584 Df: 12, p value:0.000 <0.5 hence statistically significant. The graph inferes that female subjects reported for reviews more compared to male subjects.

From this study we observe that the number of patients who are diagnosed are more than the number of patients who underwent the treatment. The most common gender predilection who underwent the treatment and those who reported for the reviews were males. The most common age group were found to be 41-50 years for extraction and >60 years for review.

AGE GROUP- EXTRACTION

The most common age group for extraction seen was 41-50 years. The highest incidence of extraction was observed at the age of 41- 50 years. The results are almost similar to the studies conducted in other regions. This could be due to poor oral hygiene maintenance, age related periodontal problems, bone atrophy, tooth wear, smoking and increased risks of co-morbidities in Adults. Although this is not in consistent with the study at Department of Khyber College of Dentistry, Peshawar in which higher incidence of proportion of patients had undergone extraction at a younger age i.e. 26 – 35 years[15]

AGE GROUP- REVIEW

The most common age group for review was around >60 years. Farheem et al 2014(P-value (p=0.489)) shows that there was no statistically significant association between age and socket status. [9]

GENDER- EXTRACTION AND REVIEW

The most common gender predilection in those who had an extraction and those who reported for reviews were males. These results are consistent with the study conducted in Lahore in which a large proportion of male population has undergone extraction as compared to females. [15 16 17]

However, this was inconsistent with a study conducted in University of Sulaimani, which showed females were in higher proportion for extraction as compared to males a clear predominance of male patients was observed in this study, correlating with the reports published earlier. [16]. Females are more commonly affected than males with a ratio of 1:1.4 [17] The reason for this might be the low self-care and especially dental care in our female population. It could also be due to lack of awareness, dependency and difficult approach to dental facilities. The knowledge acquired must be transferred into daily practice. This can be achieved by a change in their attitude towards oral hygiene maintenance. Inclusion of oral health-oriented education programs in their curriculum would improve their knowledge and behaviour and they would be a good model to the community.[18] The importance of training regarding Biomedical waste management also must be emphasized as the lack of proper and complete knowledge about biomedical waste management impacts practices of appropriate waste disposal. [19] Irrespective of the year of study, the majority of the students showed a negative attitude towards HIV/AIDS patients and only a few among the interns showed a positive approach towards treating HIV patients. [20 21]

Conclusions

The limitations of study could be short duration and cross sectional type of study design. But similar types of studies have been conducted in other parts of the world like one in Afghanistan where sample size was 184 and study duration was three months. It is recommended that this study should be conducted in broad horizon and should be designed longitudinally so that the results should be more representative of the population. It is also recommended that community awareness programs must be initiated and people must be emphasized for vigorous oral hygiene maintenance. Government authorities should facilitate the dental organizations for community awareness campaigns. A proper health system including efficient dental care programs focusing on prevention and treatment of these diseases should be created and developed on a larger scale.

Conflict of Interest:

The authors would like to declare that there is no conflict of interest among the authors.

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Awareness on Oral Hygiene and Practices among General Population

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Abstract

India is the sixth biggest country by area and is the second-most populous country. Factors contributing to the steady rise in prevalence of oral disease include poor oral health awareness and practice among fellow people. Oral health knowledge is considered to be an essential prerequisite for health-related behavior, although only a weak association exists between knowledge and behavior. Keeping a healthy oral profile requires joint efforts from the dentist as well as the patient himself. The study aims to analyze knowledge and awareness of oral hygiene and practice among the general population A descriptive cross sectional survey was conducted among 100 patients, visiting a private dental institution, Chennai, through a self administered questionnaire. The responses were collected, tabulated in excel sheet and analysed using SPSS software. 97% of the participants were using brush with hard bristles. 81% changed their toothbrush. 40% of the subjects visited a dentist when they were in pain. This shows very less care about oral hygiene which leads to the future undesirable outcome. This study concludes that there is an urgent need for comprehensive educational programs to promote good oral hygiene and impart education about oral hygiene practices.

Keywords: *Oral hygiene; oral health; awareness; oral profile;oral hygiene, Knowledge*

Introduction

Oral disease is a major public health concern owing to their high prevalence and their effects on the individual's quality of life [(1)]. The possible etiological factors leading to these oral diseases, developmental problems, poor oral hygiene, and traumatic incidents. [(2),(3)] Oral hygiene behavior and oral health care regimens should be positively reinforced. Lack of knowledge is one of the reasons for the lacunae in following oral hygiene practices. Keeping a healthy oral profile requires joint efforts from the dentist as well as the patient himself. One of the most important factors that decide the dental health of a population is the outlook of its people toward their dentition

We keep reading about studies done to judge the oral health and oral hygiene practices at so many places scattered all across the globe to know about how much the people are aware of the knowledge and awareness of oral hygiene and how they are practicing oral hygiene. Furthermore, even the people living in cities, despite having easy access to dental care, fall prey to dental diseases due to their negligence in dietary habits and an unhealthy lifestyle [(4).] Globally, with increasing age there is also an associated increase in the calculus among children and adolescents. Oral hygiene maintenance may be a totally ignored practice in people of the low socioeconomic class of developing countries. Good oral hygiene is the foundation of a healthy mouth and prevents 80% of all dental problems. Primary prevention through tooth brushing has paramount importance in the prevention of dental health problems.

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One of the most important factors that decide the dental health of a population is the outlook of its people toward their dentition [5], Keeping a healthy oral profile requires joint efforts from the dentist as well as the patient himself which works in joint venture of both dentists. Previously our team had conducted numerous original studies [6–13] and surveys [14–20] over the past 5 years. Now we are focussing on epidemiological surveys. The idea for this survey stemmed from the current interest in our community. Therefore the study aims to analyze knowledge and awareness of oral hygiene and practice among the general population.

Materials and Methods

Study Design:

A cross sectional study was conducted among 100 participants. A simple random sampling was used to select the study participants.

Ethical Considerations:

Returning the filled questionnaire was considered as implicit consent with no need for signing a written consent. Ethical approval for the study is obtained from the Institutional Review Board (IRB) .

Study Method:

Self administered questionnaire of 13 close ended questions was prepared and it was distributed among undergraduate dental college students of private dental institutions through online survey forms "google forms". The collected data were checked regularly for clarity, competence, consistency, accuracy and validity. Demographic details were also included in the questionnaire.

Statistical Analysis

Data was analysed with SPSS. Descriptive statistics as number and percent were calculated to summarise qualitative data. Chi square test was used to analyze and compare the level of knowledge on oral hygiene and practises among the general population. The confidence level was 95% and of statistical significance $P < 0.05$. Finally, the result was presented by using bar charts and frequency tables.

Results and Discussion

The survey included 100 patients of which 97% were male and 3% were female(Table 1).

27% people brush their teeth occasionally, 24% of them were brushing once a day, 22% were brushing twice a day, and none of them brushed more than 2 times a day which is very less as compared with 75% of the elderly Chinese adults in urban areas in a study by Zhu et al(21).

Around 52% of the subjects brush their teeth in vertical direction, which is the most dangerous method of brushing but the results are in consensus with the study done by Zhu et al (21). where 60% of the sample did the same.Approximately 97% of the participants were using brush with hard bristles and only 3% of the sample used a soft toothbrush, which is less than that observed among Zhu et al [21] study where 27% of the sample uses the soft bristles. Chi square test was used to compare gender and the type of brush used, where P value= 0.604(statistically not significant)(figure 1). 81% changed their toothbrush and the remaining 19 % answered that they don't change their toothbrush regularly. 45% change their toothbrush once in 3 months, and surprisingly reamning 32% change their brush only when it is useless. 88% of the participants were aware of interdental aids. 57 % of the participants washed their mouths after eating . Chi square test was used to compare gender and mouth washing after eating, where P value= 0.272(statistically not significant)(figure 2). 93% of the participants used dental floss and remaining 7% did not use flossing. The results show lacunae in the knowledge on interdental aids.

Only 66% of the studied population said that they clean their tongue and the remaining 34% don't clean their tongue . Chi square test was used to compare responses based on gender to the question on tongue cleaning, where P value= 0.319 (Figure 3) (statistically not significant). The results from the present study clearly indicate missing lack of awareness of oral hygiene among study participants. 58 % of the participants used a mouthwash for cleaning their mouth after eating. Chi square test was used to compare gender and awareness on using mouthwash, where P value= 0.119(statistically not significant) (Figure 4). Furthermore, 88% reported halitosis. This study is in contrast with that of an

epidemiologic survey by Miyazaki H, et al [22] in Japan 24% of the individuals complained about bad breath. Our study showed that 40% of the subjects visited a dentist when they had a dental pain, which is similar to the study done by Nabil Al-Beiruti (69.5%) [23].

Knowledge and awareness of oral health is very poor in India, due to poor socio – economic conditions. In addition to this, two thirds of people have never seen a dentist. Lack of awareness about the role of regular dental checkups in detecting and preventing dental diseases necessitates the need for public education. As dentists, it is our responsibility to educate and motivate people to visit a dentist.

Table 1: Depicts percentage of responses on Knowledge and awareness on Oral Hygiene and practices among General Population.

S.no	Question	Choices	Percentage
1.	Gender	● Male ● Female	● 97% ● 3%
2.	Frequency of cleaning the teeth	● Once daily ● Twice daily ● More than twice ● Occasionally	● 24% ● 22% ● 18.3% ● 27%
3.	Technique used for brushing	● Horizontal ● Vertical ● Circular ● combined	● 24% ● 52% ● 18% ● 6%
4.	Type of toothbrush used	● Hard ● Soft	● 97 % ● 3%
5.	Knowledge On changing the tooth brush	● Yes ● No	● 81% ● 19%
6.	Frequency of changing the toothbrush	● When Useless ● Once in 3 months ● Every 6 months	● 32% ● 45% ● 23%
7.	Knowledge on use of interdental aids	● Yes ● No	● 88% ● 12%
8.	Knowledge on rinsing the mouth after eating	● Yes ● No	● 57% ● 43%
9.	Type of interdental aid used	● Floss ● Interdental Brush	● 93% ● 7%
10.	Awareness of cleaning the tongue	● Yes ● No	● 66% ● 34%
11.	Awareness on using mouthwash	● Yes ● No	● 56% ● 44%
12.	Odour from mouth after eating	● Yes ● No	● 88% ● 12%
13.	Frequency of visiting dentists	● Never ● Only in problem ● Once in 3 months ● Once in 6 months ● Any other	● 11% ● 40% ● 11% ● 36% ● 2%

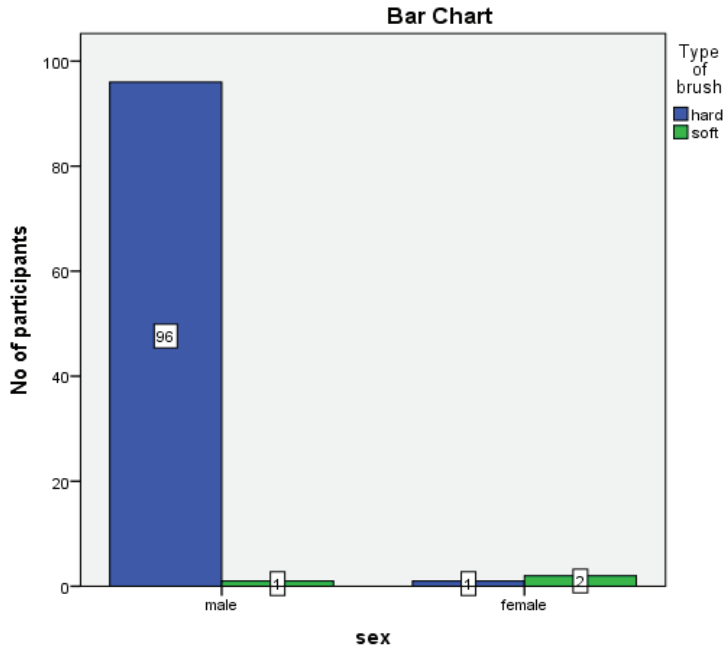


Figure 1:Bar graph showing association between gender and type of brush used, where (blue) hard, (green) soft. X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response (96-blue) was among males. Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.604 (p>0.05)hence not statistically significant.

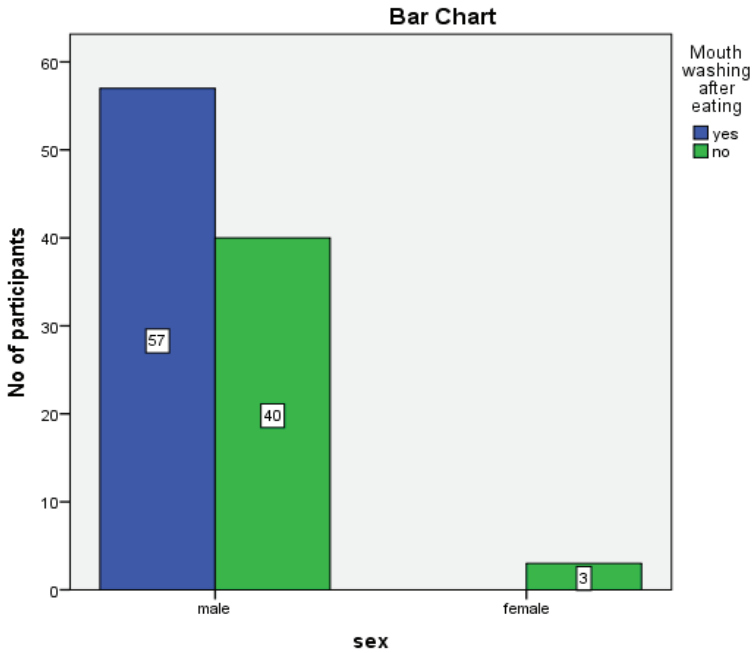


Figure 2: Bar graph showing association between gender and habit of mouth washing after eating, where yes(blue),No(green) X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response was among males(53). Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.272 (p>0.05) hence statistically not significant.

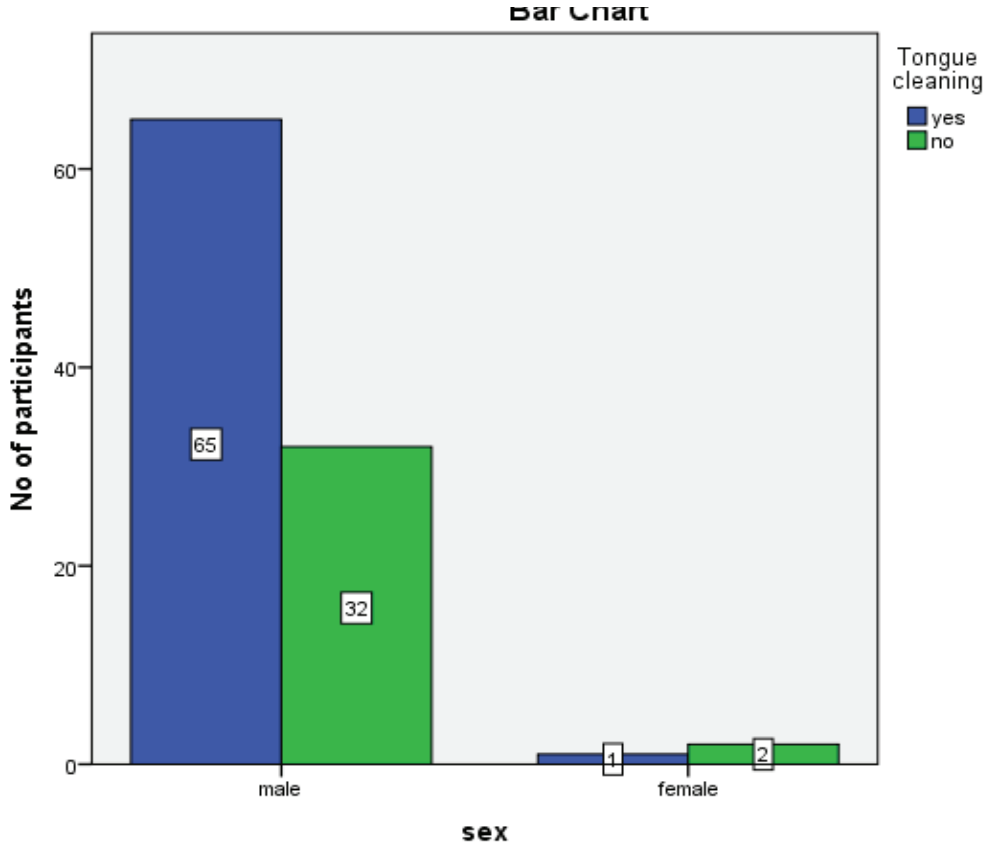


Figure 3: Bar graph showing association between gender and tongue cleaning, yes(blue),No(green). X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response (65) was among males. Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.319 (p>0.05) Hence not statistically significant

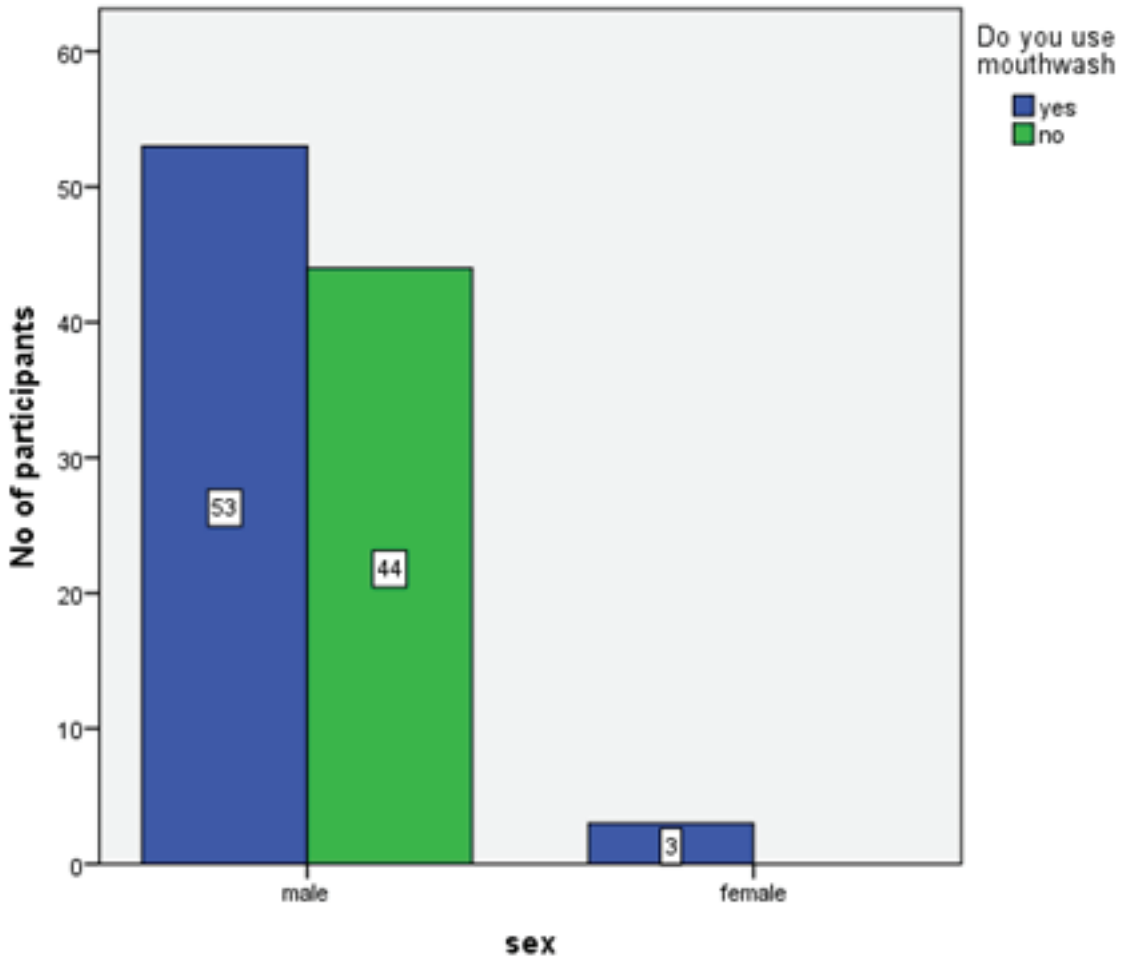


Figure 4:Bar graph showing association between gender and use of mouthwash, where denotes yes(blue), no(green). X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response was among males(53). Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.319 (p>0.05) Hence not statistically significant

Conclusion

The results of the present study indicates that the study participants have good knowledge on oral hygiene and practices.From the present study it can be inferred that among the general population both males and females had adequate knowledge on oral hygiene and practices.

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Prevalence of Space Maintainers as A Treatment Modality in Maxillary Arch among Children between the Age Group of 6 to 12 Years- A Retrospective Study

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Abstract

Early loss of primary teeth can affect the arch length and thereby lead to malocclusion. Space maintainers were introduced to effectively maintain the space after a premature tooth loss. This study aims to assess the prevalence of various space maintainers provided in the maxillary arch among children aged 6 to 12 years and describes the trends in the use of space maintainers to bring about better understanding about these appliances. The patient records from June 2019-April 2020 of children who underwent space maintainer therapy in maxillary arch were selected by non- probability sampling. Data was collected and exported to the Statistical package for social science for windows (SPSS version 20.0,IL,Chicago,USA) for analysis. The results of the study showed that among the space maintainers provided, Band and loops (51.6%) were commonly used followed by Groper's appliance (27.4%). The rarely provided space maintainers were Transpalatal arch (1.6%). The use of space maintainers in the maxillary arch had a male predominance (62.9%). The age of the children for whom space maintainers were commonly provided were 8 year olds (53.2%). Age and type of the space maintainer given was found to have no strong association ($p=0.264$, $p>0.005$).

The study draws attention to the fact that space maintainers are widely used in Paediatric dentistry as a part of preventive orthodontics. In this study, Band and loop space maintainers were prevalent. Male dominance was observed and use of space maintainers especially in the age of 8 years was common. There was no association between age and the type of space maintainers used. Extensive research based on space maintainers and the dentist's preferences and skill regarding the same has to be done. Alternatives for the current space maintainers have to be found to overcome their drawbacks .

Keywords *: Band and loop; Caries; Gropers appliance; Premature loss; Space maintainers*

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Introduction

Child's growth and development is influenced by primary dentition. It affects appearance, speech, mastication and also helps to guide the eruption of permanent teeth into the occlusal plane[1–4]. Exfoliation of primary teeth and emergence of permanent teeth is considered to be a normal physiological process [5–7]. Several factors like extensive caries, trauma etc, which

causes loss of primary teeth can lead to mesial migration of teeth and can overall manifest as malocclusion in the form of crowding, impaction, supraeruption etc [8–11]. The apt way to eliminate these problems is by preserving the deciduous tooth in the arch till it's normal exfoliation time is attained[12,13]. Hence, it is usually said that deciduous teeth act as best space maintainers for permanent dentition[14–17].

Space maintainers are usually indicated to preserve the space created by premature loss of primary teeth in either of the arches. There are various fixed space maintainers among which Band and loop space maintainers are one of the most frequently used [18]. They are used because of their high success rates [19] . But there are also various disadvantages associated with them like cement disintegration, caries forming along the margins of the band and prolonged construction time [20]. This had led to numerous pilot studies that suggests the use of newer adhesives like Glass fibre reinforced composite resins (GFRCR), Ribbond and Everstick as an alternative to fixed space maintainers [21,22]. For maxillary arch with bilateral loss of teeth, there are two options of fixed space maintainers, namely Nance palatal arch and Transpalatal arch space maintainers [23]. Transpalatal arch has lesser food impaction and improved compatibility with soft tissues when compared with Nance palatal arch space maintainers [24].

Deciduous maxillary anterior teeth are often lost due to early childhood caries, systemic conditions or syndromes and trauma [25,26]. This has known to affect the child's speech development as well, especially labiolingual sounds . Inappropriate speech compensations can develop if these teeth are missing [27] , and it can also affect the esthetics and thereby lower the self esteem of the growing child . This led to the introduction of Gropers appliance [28]. Although various appliances are available for different clinical situations, they usually have their very own adverse effects. This study sheds light on etiological factors that makes the children prone to early loss of tooth and also gives importance to why the particular appliances were preferred. It focuses on understanding the current scenario in use of space maintainers in maxillary arch for children aged 6 to 12 years.

Materials And Methods

A retrospective study of space maintainers used in the maxillary arch was carried out by examining the records of 100 patients from June 2019 to March 2020. Institutional ethical committee clearance was obtained for data retrieval and usage as needed for the study (SDC/SIHEC/2020/DIASDATA/0619-0320). Patients with incomplete records and double entries were excluded from the study. Type of space maintainer given was confirmed using photographic evaluation. For each child, details on type of space maintainer, age and gender were collected.

Statistical Analysis

Following the collection of data of children who underwent space maintainer therapy in maxillary arch by non- probability sampling. Data was subjected to statistical analysis.

Microsoft Excel 2016 data spreadsheet was used to collect data and later exported to the Statistical package for social science for windows (SPSS version 20.0,IL,Chicago,USA). The distribution percentages were obtained following which correlations were assessed using Chi square tests.

Results and Discussion

The final data set consisted of 62 children within the range of 6-12 years who were given space maintainers in the maxillary arch .

Age group associated with the highest use of space maintainers was found to be 8 years (53.2%) and the least was observed in children aged 9 and 11 years (3.2% each).[Table 1] Gender distribution among the children who had space maintainers in the maxillary arch depicts male predominance (62.9%).[Figure 1]

Band and loop space maintainers were most commonly provided (51.6%), followed by Groper's appliance (27.4%), and the least provided were Transpalatal arch (1.6%).[Table 2]

The type of space maintainer provided and the age of the child showed no significant association (p=0.264). [Figure 2]

The data for this study was based on residents of

Chennai seeking treatment at Saveetha dental college, Chennai. There is dearth in literature about studies assessing the prevalence of types of space maintainers among children in their mixed dentition phase in the given region. There was no sorting process and all of the data available was included to avoid sampling bias. This study sheds light on use of different space maintainers and their prevalence as a treatment modality to prevent malocclusion or other consequences of early tooth loss in a growing child.

In a study conducted by N. Venugopal Reddy et al, it was established that the maximum percentage of premature loss of deciduous teeth occurred in the age of 8 years, followed by 7 and 9 years making them in need of space maintenance [29]. These findings are similar to the present study which suggests that space maintainers were provided frequently at higher percentages to 8 year old (53.2%) children and the most uncommon age group were 9 and 11 years (3.2%) [Table 1]. Also in another study by S.Syed Shaheed Ahamed et al, there was significant early loss of primary teeth in 8 year old children[30] . The reason can be that the early loss of primary teeth is associated with oral hygiene, consumption of soft drinks, and previous experience of dental caries.

In the present study [Figure 1], there was a male predominance (62.9%) in use of space maintainers. This can be explained as an increase in premature loss of primary teeth in male children than females which was established in a study by Dhanalakshmi Jayachander in Chennai [31]. In another study by Saravanan et al, it was concluded that male children were subjected to longer feeding periods when compared to the female children irrespective of their economic status which led to higher DFT scores among them [32].

Among the type of space maintainers used [Table 2], Band and loop space maintainers were most often provided than the other space maintainers in the given study population . This is coherent with the results of the study conducted by Muawia et al, establishing that band and loop space maintainers were often given to children[33] . This can be because the most commonly affected tooth by early loss was found to be primary molars in a study by Leite-Cavalcanti et al [34]. This in turn means that since band and loops were given following loss of primary molars, they were provided more. Another reason can be that deciduous molars are critical for streptococcus mutans colonization as they erupt into the oral cavity between 16 to 29 months of age and have deep fissured occlusal surfaces that act as windows of infectivity[35].

Groper's appliances were the second most prevalent space maintainers in the present study (27.4%) [Table 2]. The reason can be that maxillary anterior teeth were affected by trauma more often compared to the mandibular teeth, and central incisors were the most affected teeth [36] . This will lead to increased requirements for Gropers appliance. The least preferred appliances were Transpalatal arch space maintainers (1.6%) which was contradictory to the study by Ari Kupietzsky, which claims that Transpalatal arch have more benefits than Nance palatal arch, like better maintenance of arch width, tissue compatibility and addition of bite planes[37]. This result can change with increased sample size and can vary according to study populations.

In the present study [Figure 2], Groper's appliances were frequently provided for children aged 6 years (17%). However, there was no statistically significant association between the type of space maintainer given and the age of the patient. This can be due to loss of tooth due to extensive caries irrespective of age and due to other reasons like trauma and syndrome causing aggressive periodontitis which invariably leads to early loss of tooth and requirement for space maintenance.

8.TABLES AND GRAPHS

Age of the children	Frequency	Percentage
6	8	12.9
7	12	19.4
8	33	53.2
9	2	3.2
10	5	8.1
11	2	3.2
Total	62	100

Table 1: Age distribution of children with space maintainers in maxillary arch.

Table shows the age distribution of the study population. Highest preference for space maintenance was observed among children aged 8 years (53.2%) and the least in children among the age groups of 9 and 11 years (3.2% each)

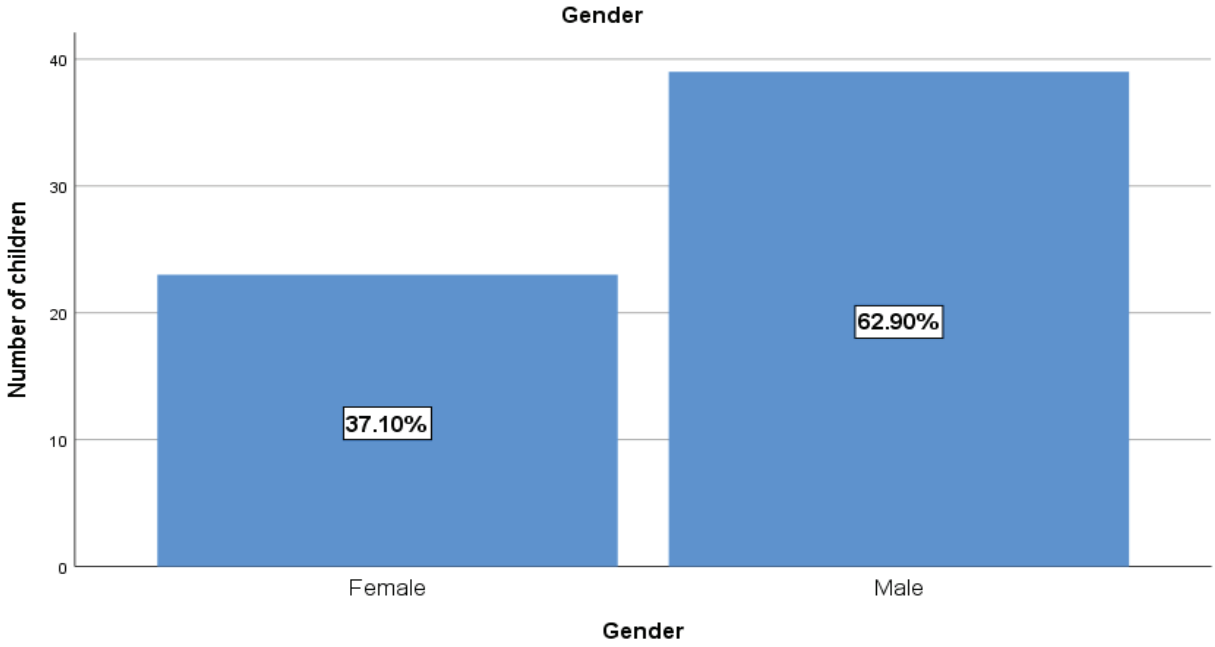


Figure 1: Gender distribution of space maintainers.

Graph shows the gender distribution of the study population (N=62). X axis represents the gender and Y axis represents the number of children. 62.9% were males, while 37.1% were females.

Type of space maintainer	Frequency	Percentage
Band and Loop	32	51.6
Groper's appliance	17	27.4
Nance palatal arch	12	19.4
Transpalatal arch	1	1.6
Total	62	100

Table 2: Distribution of space maintainers in maxillary arch among children.

Table shows the prevalence in the use of space maintainers in the maxillary arch among children. 51.6% were Band and loop space maintainers, followed by Groper's appliances (27.4%), and the least provided were Transpalatal arch (1.6%).

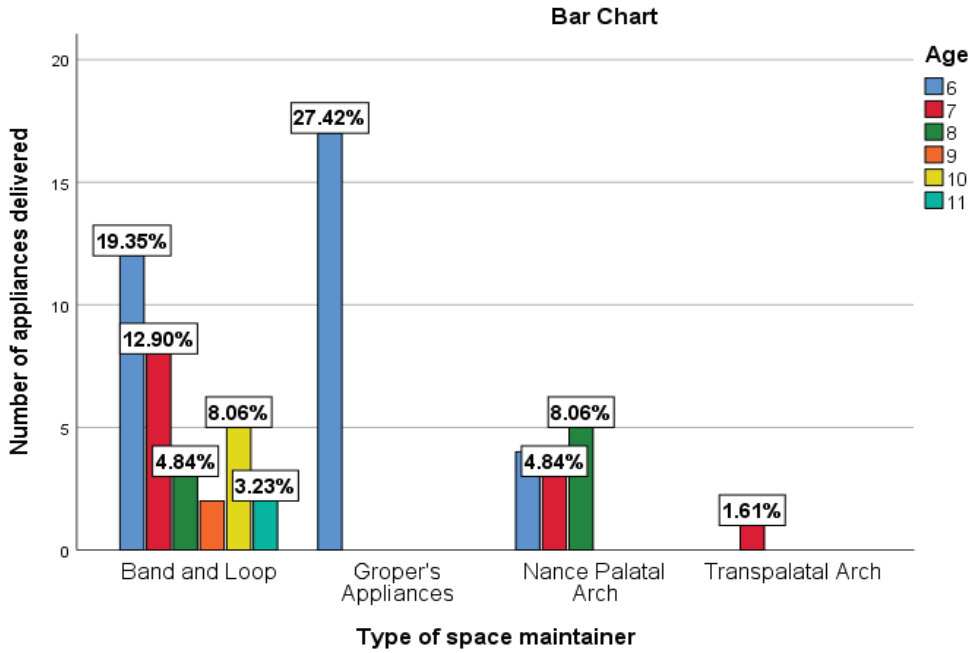


Figure 2: Bar graph depicting the association between type of space maintainer provided and the age of the patient. X-axis represents the types of space maintainer and y-axis represents the number of appliances delivered. Blue depicts 6 years, red depicts 7 years, green depicts 8 years, orange depicts 9 years, yellow depicts 10 years and cyan depicts 11 years. Groper's appliances were mostly provided for children aged 6 years (17%). There was no statistically significant difference between the type of space maintainer provided and the age of the child (Pearson Chi-Square test, p value- 0.264, p>0.05).

Conclusion

Within the limitations of the present study, it was found that Band and loop space maintainers were predominant. Prevalence was higher in male children than females. It was also observed that the most common age associated with use of space maintainers in the given study population were children aged 8 years .

Conflict of Interests : No conflict of interests

Acknowledgements: The authors of this study would like to express their gratitude towards everyone who facilitated and enabled us to carry out this study successfully.

Source of Funding : Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Incidence of Non Habit Associated Oral Squamous Cell Carcinoma among Patients in a Private College Hospital - A Retrospective Study

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Abstract

Oral squamous cell carcinoma is considered as one of the major prevalent oral cancers in the world. The major risk factors considered are tobacco and alcohol. However, in the present there is an increased incidence of non habit associated squamous cell carcinoma. This study aims to evaluate the incidence of non habit associated squamous cell carcinoma visiting our college hospital. A total of 50(n=50) patients with squamous cell carcinoma visited Saveetha Dental College were included in this study. Datas were collected by reviewing the patients records and analysed the data of 86000 patients visited between June 2019 and March 2020. All the datas were recorded and analysed statistically using SPSS software. From the analysis through SPSS, it was found that the overall incidence of non habit associated squamous cell carcinoma was 56% with male predilection and it was found to be statistically significant($p<0.05$). The results of our study shows that there is a significant number of patients without habits presented with oral squamous cell carcinoma with a cause unknown in our hospital.

Key words: *Oral squamous cell carcinoma, non habits,systemic disease, smoking, alcohol, hereditary.*

Introduction

Oral squamous cell carcinoma (OSCC) is the most common form of oral cancer and the sixth common malignancy in the world [1,2]. India is considered a high risk region for oral cancer due to increased prevalence of smoking, alcohol drinking and tobacco use [3–5]. However, a recent trend in India shows that there is an exaggerated incidence of oral squamous cell carcinoma in females without habit history [6]. The most common site affected is the buccal mucosa[7]. The factors influencing the oral squamous cell carcinoma are smoking, alcohol, diet and nutrition, virus, radiation, facial and genetic predisposition, oral thrush, immunosuppression, use of mouthwash, syphilis, dental factors and occupational risks [8–10]. The other factors which play a major role in oral squamous cell carcinoma are impaired ability to repair DNA damaged by mutagens, an impaired ability to metabolize carcinogens, deficiencies of vitamin A, E, C or trace elements and immune defects. The premalignant disorders that can progress to oral squamous cell carcinoma are erythroplakia, leukoplakias, actinic cheilitis, lichen planus, sideropenic dysphagia, submucous fibrosis, dyskeratosis congenita and discoid lupus erythematosus [11].

The most common age group of patients who were prevalent to oral squamous cell carcinoma are over 50 years of age [12]. However, in the last few years there

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has been an increased prevalence of squamous cell carcinoma in patients who are younger than 45 [13]. It is known that the major risk factors associated with oral squamous cell carcinoma are smoking and alcohol [14]. The combination of smoking and alcohol seems to increase the carcinogenic effect [15].

The etiological factors associated with non habit associated squamous cell carcinoma are viral infections like HPV, EBV, immunosuppression, familial factors, genetic predisposition, chronic mechanical irritation, dietary factors and hormonal factors [16–18]. Although, The mechanism of how it causes squamous cell carcinoma is not clear. It has been suggested that non smoking individuals have a greater probability of developing tumors related to HPV than individuals who smoke [14]. This study aims to evaluate the incidence of patients with non habit associated squamous cell carcinoma visited Saveetha Dental college.

Materials and Methods

In the present retrospective study, a total of 50 patients(n=50) visited Saveetha Dental College were included. Data were collected by reviewing records of 86000 patients. Sample collected was from June 2019 to March 2020. All the case sheets included in this study were approved and reviewed. Also, cross verification of data was done by photographs.

Parameters such as age, gender, presence or absence of habits like smoking, pan chewing and presence or absence of any systemic illness were collected. Approval from the ethical committee was taken before the start of the study. All these dates were entered in Microsoft excel sheet and imported to SPSS by IBM and the statistical analysis was done by Chi Square test. The results were interrupted in graphs.

Results and Discussion

Out of these 50(n=50) patients, 76% were male and 24% were female(Figure 1). From the analysis, 56% of patients with oral squamous cell carcinoma were associated with no habits and 44% of patients with squamous cell carcinoma presented with habits(Figure 2). 9% of patients had a history of smoking and 91% were found to be non smokers(Figure 3). 38% were associated with paan chewing habit(Figure 4). 57% were presented with no systemic illness and 43% presented with systemic diseases like diabetes, hypertension and cardiovascular disorders(Figure 5).

Oral squamous cell carcinoma is the most prevalent cancer in the world [19–24]. The most common etiologies are smoking and tobacco. Tobacco constitutes about 50 substances with carcinogenic potential like nitrosamines and aromatic hydrocarbons. These metabolises covalently bonds with DNA of keratinocytes stem cells forming DNA adducts. These are responsible for critical mutations involved in DNA replication. The metabolism of these carcinogens involves oxygenation in cytochrome by p450 enzyme and conjunction by glutathione- S- transferase [25–28]. Apart from tobacco and alcohol, there are various factors that influence the cause of oral squamous cell carcinoma.

Girod et al. 2009 [29] stated that squamous cell carcinoma was more favorable in male population. Our study findings stated that there was an increased number of Male patients with squamous cell carcinoma. Saxena et al. 2019 [17] stated that oral squamous cell carcinoma was more prevalent in patients without exposure to potentially harmful irritants. They also suggested that human papillomavirus, genetic counselling, hormonal and dietary factors are to be considered to correlate the cause and effect of non habit associated oral squamous cell carcinoma. Our study findings stated that squamous cell carcinoma was more prevalent in patients who are not exposed to any habits. Our study finding was in concordance with the literature. Few studies [30,31] stated that there was a relative increase in patients without habits associated with oral cell carcinoma and it was more prevalent in females. Our study findings(increased prevalence of non habit associated squamous cell carcinoma) was similar with the results of the literature.

Vargas-Ferreira et al. 2012 [14] stated that about 80% of patients with smoking habits reported with squamous cell carcinoma. Our study findings was that only 9% of patients with squamous cell carcinoma reported smoking habits. Our study findings are not in concordance with the literature. The reasons that affect our study results are patient declaration, raising trends and in our study we analysed the existing data. Wu et al. 2010 [32] reported that the diabetes mellitus could be related to oral squamous cell carcinoma. Our study reported only 43% of patients with systemic illness were associated with squamous

cell carcinoma, but we have seen a small but concerning percentage of patients with systemic illness who were associated with squamous cell carcinoma.

The limitations of this study was the incidence of patients with non habit associated squamous cell carcinoma was observed with the fact that the subjects examined represent a selected population. The exact cause of patients reported with non habit associated squamous cell carcinoma is unknown. Further studies can be done with larger sample size.

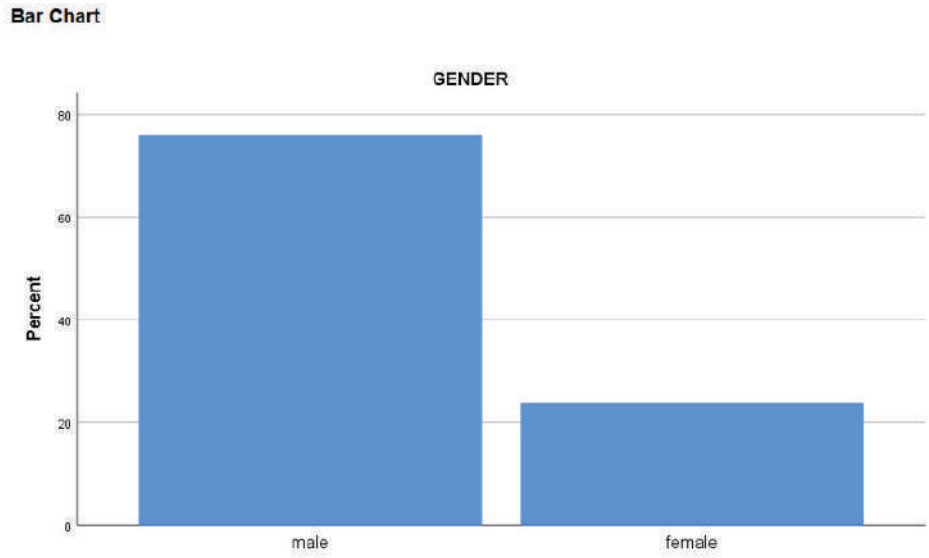


Figure 1: Bar graph showing the gender distribution among squamous cell carcinoma. X axis showing the gender and Y axis showing the percentage of patients with squamous cell carcinoma. From the graph, it was evident that squamous cell carcinoma has Male predilection.

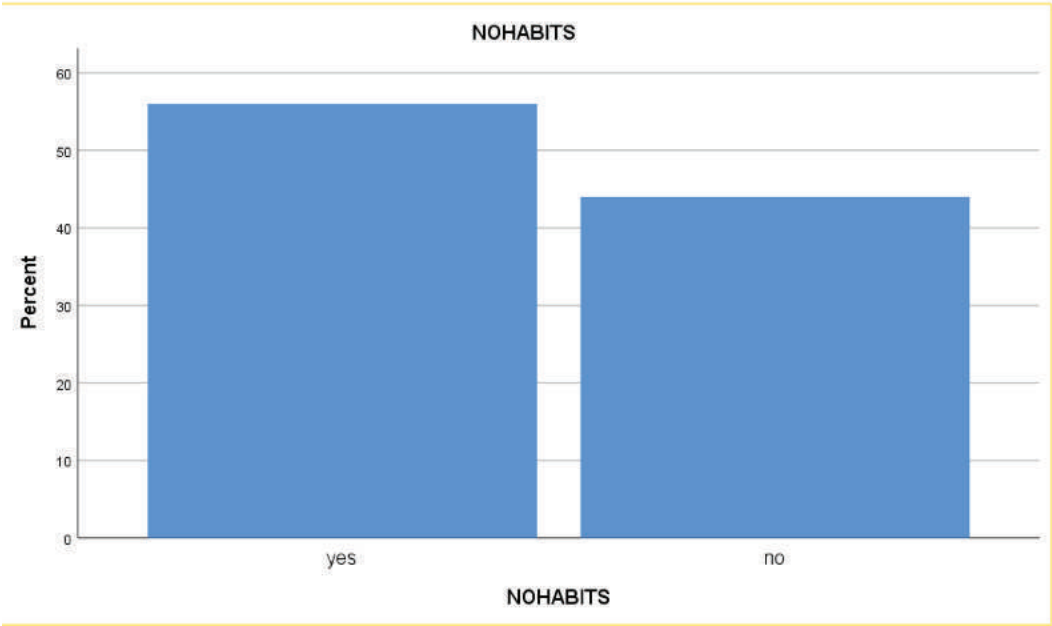


Figure 2: Bar graph showing prevalence of non habit associated squamous cell carcinoma. X axis showing the presence or absence of habit and Y axis showing the percentage of patients with squamous cell carcinoma. It was evident that there is an increased number of patients with non habit associated squamous cell carcinoma.

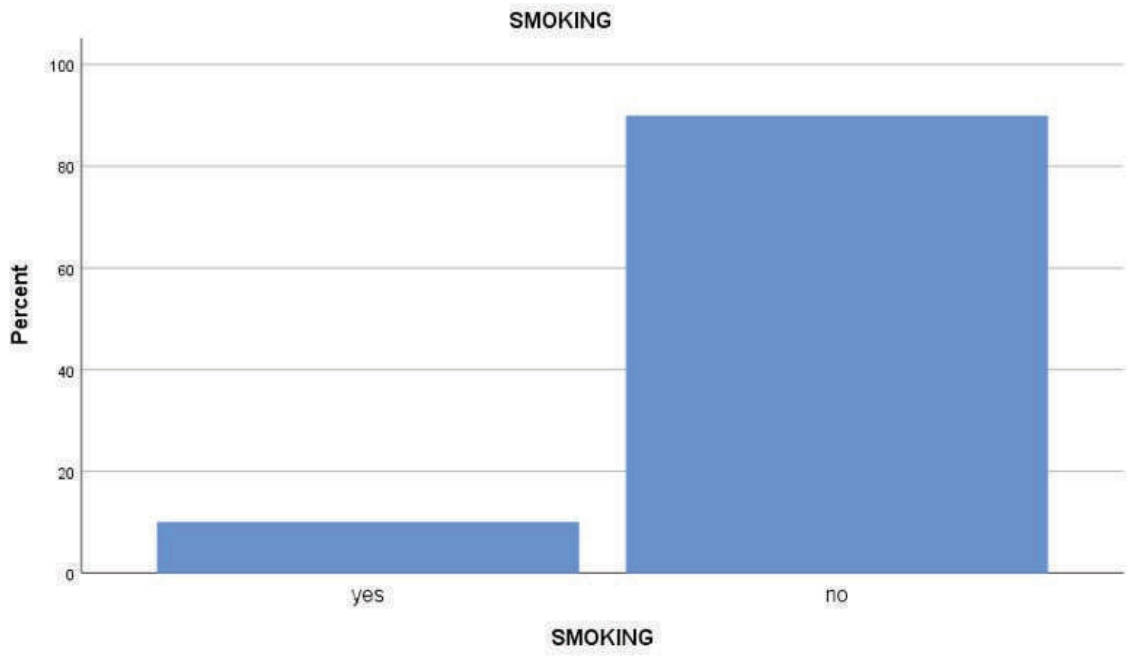


Figure 3: Bar graph showing the association of smoking and oral squamous cell carcinoma. X axis showing the presence or absence of smoking habit and Y axis showing the percentage. From the graph, it was found that most of the patients presented with squamous cell carcinoma were non smokers.

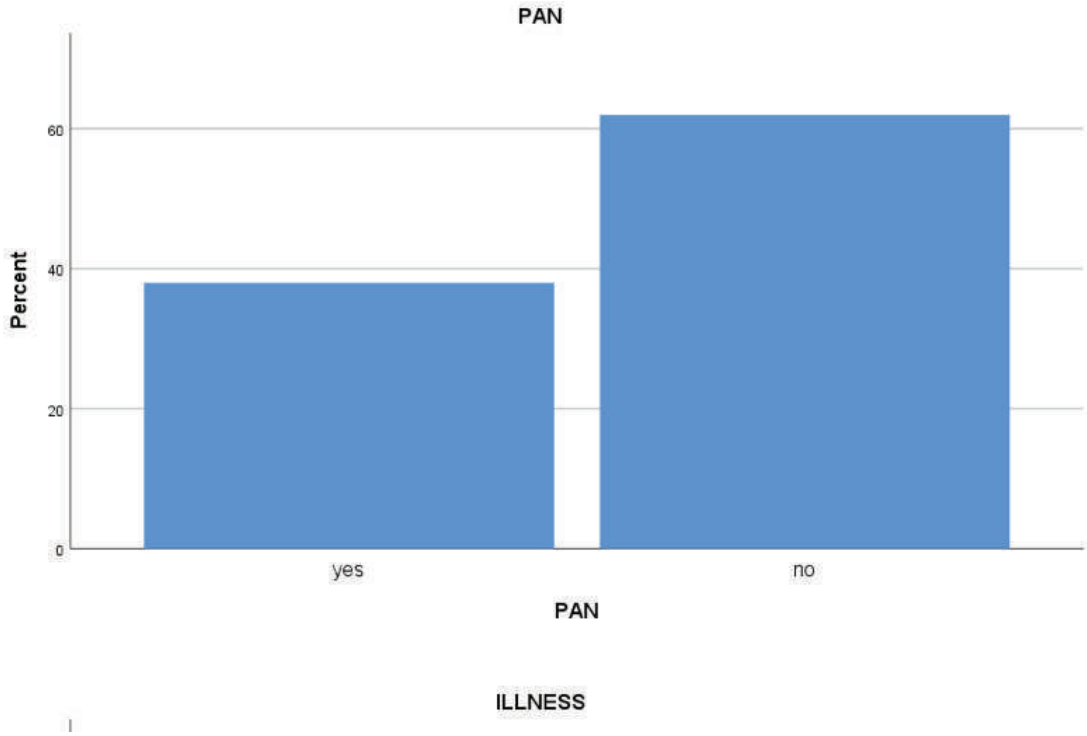


Figure 4: Bar graph showing the association of paan chewing and oral squamous cell carcinoma. X axis showing the presence or absence of paan chewing habit and Y axis showing the percentage. From the graph, it was found that most of the patients presented with squamous cell carcinoma were non paan chewers.

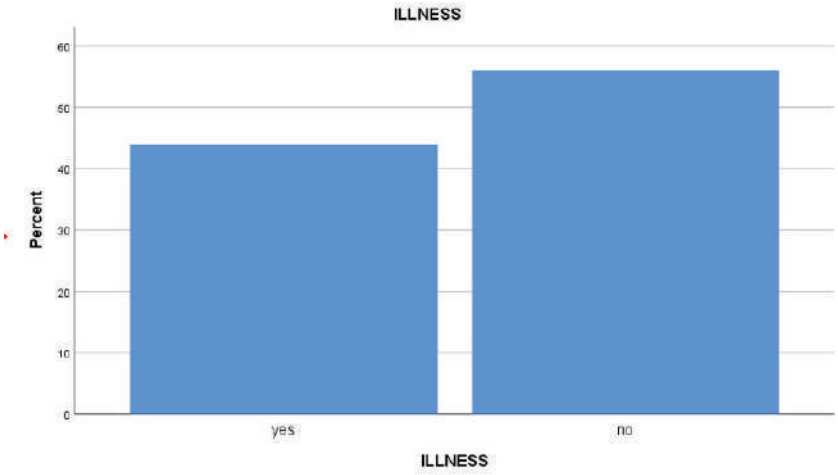


Figure 5: Bar graph showing the association of systemic disease and oral squamous cell carcinoma. X axis showing the presence or absence of systemic diseases and Y axis showing the percentage. From the graph, it was found that highest number of patients presented with squamous cell carcinoma were associated with no systemic disease.

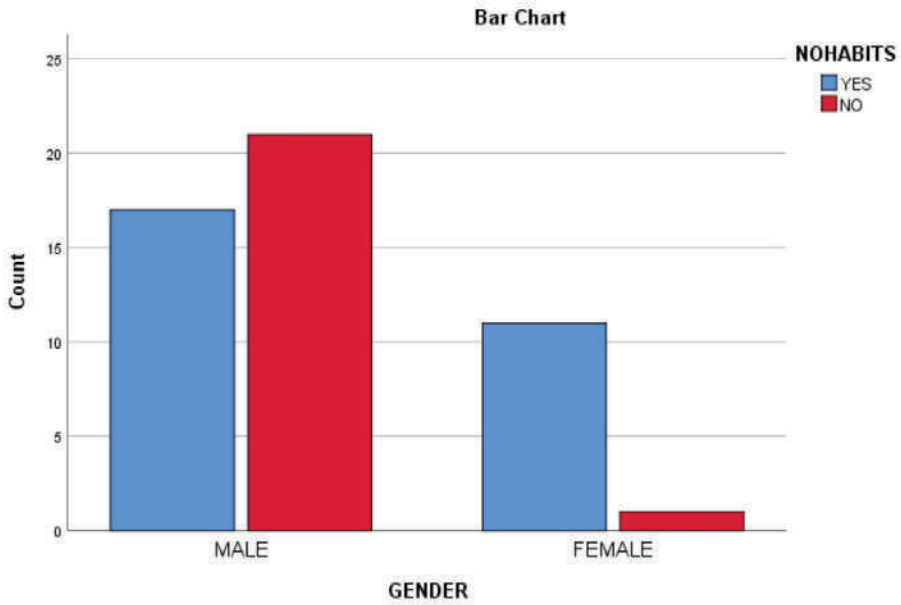


Figure 6: Bar graph showing the association of squamous cell carcinoma and gender. X axis represents the gender and Y axis represents the number of patients with squamous cell carcinoma. Here, blue colour denotes the presence of squamous cell carcinoma associated with no habits and red colour denotes the squamous cell carcinoma associated with habits. There is a significant increase in the male patients with non habit associated squamous cell carcinoma (Chi Square test; p value 0.004- statistically significant).

Conclusion

The result of this study shows that there is a significant number of patients without habits like smoking, pan chewing presented with oral squamous cell carcinoma with the cause unknown in our hospital. So, it is important to investigate possible factors associated with the occurrence of oral squamous cell carcinoma. A detailed investigation should be done for a better prognosis.

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Prevalence of Dental Plaque among Adults Attending Private Dental College in Chennai City- A Descriptive Study

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Abstract

Dental plaque is the community of micro organism found on a tooth surface as a biofilm, embedded in a matrix of polymers of host and bacterial origins of clinical relevance is that fact that biofilms are less susceptible to antimicrobial agents, while microbial communities can display enhanced pathogenicity. There are certain plaque induced oral diseases like chronic gingivitis, gingival enlargement, recurrent gingivitis,periodontitis. The Dental Plaque index was introduced to determine better oral hygiene by grading the dental plaque accordingly the present study was designed to assess prevalence of dental plaque among adults visiting saveetha dental college.Case sheets of all the patients of OP Department of Saveetha Dental College for the period of two months(Dec 2019- Jan 2020) were reviewed from patient record. Among their reviewed data, 388 appropriate study samples were selected and tabulated in MS excel.Then the tabulated data were transferred to statistical software SPSS Version 20.O. Descriptive statistics were expressed by means of frequency and percentage. Independent t test was used to compare the Mean Plaque index based on Gender and One way ANOVA was used to compare the Mean Plaque index based on age groups. A statistically significant difference was observed between the selected age groups which shows that the mean Plaque Index score was found to be high among participants aged more than 45 years. Even Though there was no significant difference on comparison of mean Plaque Index score based on Gender, the Plaque was found to be more prevalent among female participants than males.Within the limits of study, the prevalence of dental plaque was high among participants aged more than 45 years and females had a higher Mean Plaque Index score than males.

Keywords- *Dental plaque;plaque index; micro organisms; poor oral hygiene.*

Introduction

Dental plaque is the community of microorganisms found on a tooth surface as a biofilm, embedded in a matrix of polymers of host and bacterial origin [1,2] of clinical relevance is that biofilms are less susceptible to antimicrobial agents,while microbial communities can display enhanced pathogenicity[3]. The structure of the plaque biofilm might restrict the penetration of antimicrobial agents, while bacteria growing on a surface grow slowly and display a novel phenotype , one consequence of which is a reduced sensitivity to inhibitors[4].

Plaque is natural and contributes like the resident microflora of all other sites of the body to the normal development of the physiology and defenses of the host[5,6]. Irritant extraneous substances that are sick to the tooth

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surface include bacterial plaques, dental calculi,residual proteins from food and cloured substances , among which dental plaques and dental calculi are the most important localized causes of periodontal disease [7,8].

Accumulation of plaque can cause the rapid onset of gingivitis and long term exposure to plaque can lead to the loss of periodontal attachment and bone support.Long term exposure to plaque also can lead to the demineralization and destruction of the teeth through caries [9–11]. Removal of plaque is an effective way to help prevent periodontal disease and caries,but if deposits remain on the teeth across time, destruction periodontal disease,caries or both may occur [12].Main factor of both the disease is deposits and bacterial dental plaque accumulation on the tooth surface [13,14]. Thus, regular removal of dental plaque from the tooth surface by both personal oral hygiene practices such as tooth brushing and professional periodontal treatment are the most important steps of oral care [15-16,17]. Periodontal therapy starts by training the patient on oral hygiene and mechanical removal of deposits from the tooth surface [18]. Following the mechanical periodontal treatment it is very important to sustain individual oral hygiene practice in terms of maintenance of dental and periodontal health [19,20]. Thus, it is obligatory to raise consciousness about appropriate plaque control by teaching the patients oral hygiene practice in terms of either success of treatment, maintenance of oral health [21].

Additional research has elaborated on the influences of other factors, including diet,local oxygen tension,salivary factors, tooth position and disaster [22], all of which influences dental plaque accumulation [23–26] Other variables, influencing plaque accumulation include caries, which is also one of the important dental diseases causing tooth loss in adult humans. Practices of preventive dentistry making dental plaque control a habit, is enabled through patients motivation [27]. Despite motivation and training, the properties of patients regarding dental plaque control might get back to pretreatment level in because of time and may not apply what had been taught until the patient goes for a session [28].

Even though the patient has very good motivation in the beginning , the patient's efforts concerning daily oral care might decrease in time. Thus it is important for continuation of oral health to determine motivation level of patients at intervals during active periodontal treatment and periodical session, and continue motivation programs by taking time into consideration to reduce the plaque control and reduce occurrence of periodontal disease [29,30]. Aim of the study is to assess the prevalence of dental plaque among adults attending dental college of chennai city.

Materials and Methods

The present record based, descriptive study was conducted among patients reporting to the OP of Saveetha Dental College with dental plaque. Case sheets of all the Patients of OP Department of Saveetha dental college were reviewed for a period of two months [JUNE 2019 and MARCH 2020]. Simple Random Sampling was carried out to select a total of 388 patients. The descriptive study was done by two examiners and the approval for this study was obtained from the Institutional ethics committee,Saveetha University.

Assessment of dental plaque was carried out by plaque index(PI) by Silness P and Loe H(1964). A score of 0 was assigned when no plaque is present, a score of 1 was assigned when a thin film of plaque adhering to the free gingival margin and adjacent area of the tooth, a score of 2 was assigned when moderate accumulation of plaque within the gingival pocket and adjacent tooth surfaces, a score of 3 was assigned when there is a abundance of soft matter within the gingival pocket.

The data assessed and tabulated were age,gender,plaque index score.Later obtained data were tabulated and entered in the MS excel sheet. The data was imported to SPSS version 20.O and the variable definition process was done by using tables and graphical illustration.The results were expressed by means,standard deviation & percentage Descriptive statistics were expressed by means of frequency and percentage. Independent t test was used to compare the Mean Plaque index based on Gender and One way ANOVA was used to compare the Mean Plaque index based on age groups.. The level of statistical significance was set at a value $P<0.05$

Results And Discussion

The study sample consisted of 388 cases of which 21.9% of the subjects are 18-25 years old, 37.7% of the

subjects were 26-35 years old, 18.9% of the subjects are 36-45 years old and 21.4% of the subjects are > 45 years old and (refer Fig 1).Out of of 388 study participants, 58.9% were females and 41.3% were males (refer Figure 2).

Comparing the Mean Plaque index and Age of the study subjects . The mean Plaque index score for the subjects between 18 to 25 yrs, 26 - 35 yrs, 36-45 yrs and above 45 years age group were 0.96 ± 0.89, 0.97± 0.46, 1.02± 0.51 and 1.28± 0.62 respectively. One way ANOVA was used to find the difference between the different age groups with respect to Mean Plaque index and was found to be statistically significant (F value- 5.14; $p<0.05$) which signifies that study subjects in the age group above 45 years had a higher Mean Plaque index score when compared to other groups (refer Figure 3).

Comparison between Mean Plaque index and Gender Of the study subjects . The mean Plaque index score score for male and female study subjects were 0.96 ± 0.89, 0.98± 0.46 and 1.09± 0.73 respectively. Independent t test was used to find the difference between the male and female subjects with respect to Mean Plaque index and was found to be statistically insignificant (t value- 1.65; $p>0.05$). Even though the test was found to be statistically insignificant, female study subjects had a higher mean Plaque index score compared to male study subjects(refer Figure 4).

According to this study, there were a total of 388 patients who reported to the OP during december 2109-january 2020 of saveetha dental college.Out of which 21% of the patients were in the age group of 18-25 years, 37% in 26-35 years, 18% in 36-45 years and <45 years is about 21%. Distribution of patients based on gender and recovered with plaque index were 60% of males and 40% females were recorded.

According to Rizwan M sanodi et al [31]There was much awareness about oral health and hygiene however awareness about oral health and hygiene however makes it more prevalent to dental plaque in this study it is proven females are also prone to dental plaque, most likely age group above 50 years seems to have poor oral hygiene.

According to Sreenivasan et al [32]., dental plaque is significantly high in women,who maintain poor oral hygiene and have severe periodontal disease. According to Schaeken MJ et al [33].,In this study it is said that an increase in salivary secretion causes dental plaque formation which eventually helps bacteria to grow. According to Antina Schulze and martin busse [34].,systemic disease also causes significant increase of dental plaque in women, according to their study type 2 diabetic patients who are women are more prone to periodontal infection and disease which shows significant dental plaque in women.

In a study done by AL Ansari [35]et al., the prevalence of plaque induced gingivitis shows the result that males are more prevalent to gingivitis as well as the central plaque.Opposing studies may also be present accordingly to know better significance and results.According to Addy M et al [36].,The prevalence of dental plaque is significant in posterior teeths than the anterior due to food accumulation and poor brushing technique and the study also proves plaque index helps in appropriate scoring of dental plaque. According to Marakami et al [37]., the prevalence of dental plaque induced gingival conditions are common in females with post menstrual problem or any other hormone related problems,which can alter the severity of the plaque induced inflammation.Which may lead to attachment loss of alveolar bone and which significant loss of teeth.Moreover, most of the studies compared above,proves that prevalence of dental plaque in female population, there are also opposing studies to prove makes are more prone to dental plaque .But still in this study the p value is significant to prove that females are more prone to dental plaque, though current study possess few limitations such as very small sample size.

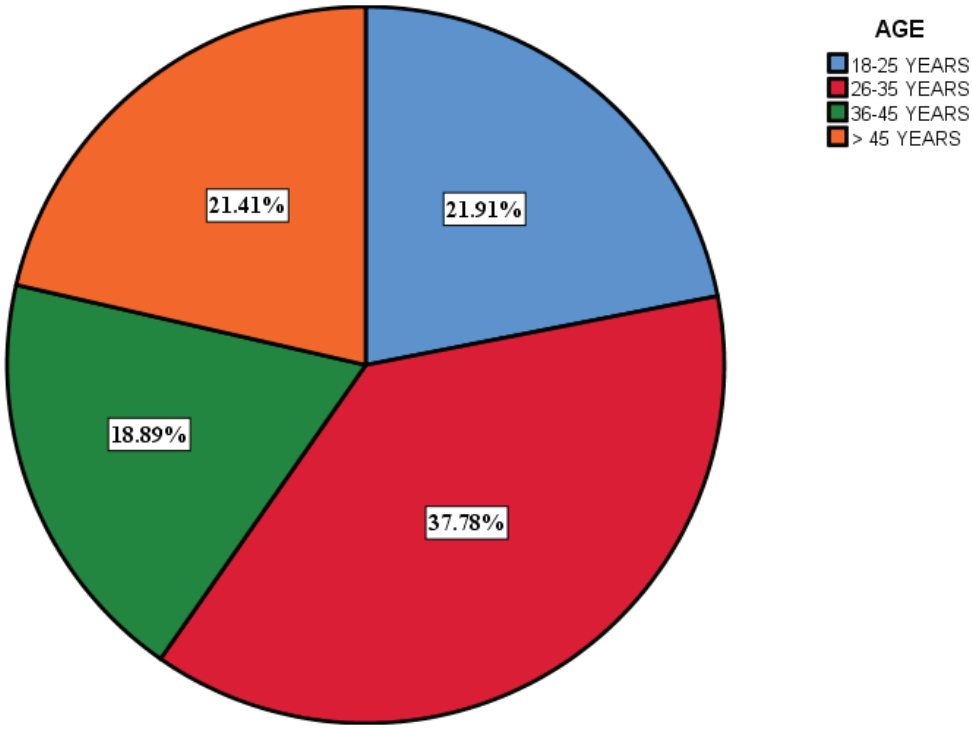


Figure 1- The pie chart represents the distribution of study subjects based on age. 21.9% of the study subjects were distributed in the age group between 18-25 years which is denoted by blue colour, 37.7% of the study subjects belongs to 26-35years of age group denoted by red colour , 21.9% constitute to age group between 36-45years denoted by green colour and 21.4% constitute study subjects above 45 years old denoted by orange.

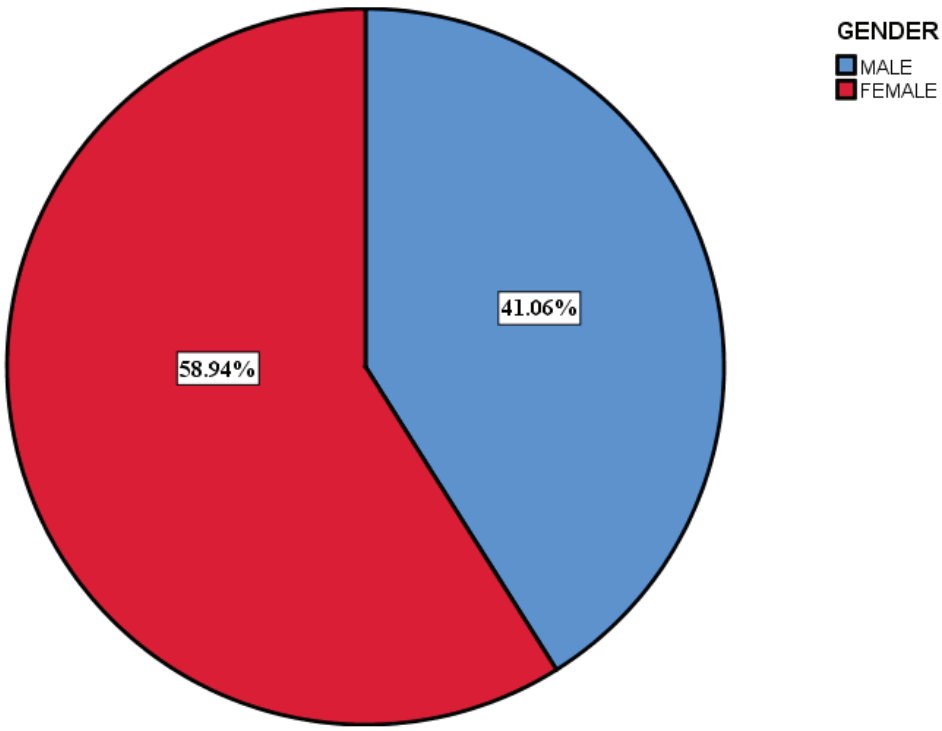


Figure 2- Pie chart represents distribution of study subjects based on Gender, where blue colour denotes male which is about 41.06% and red colour denotes females which is about 58.94%.

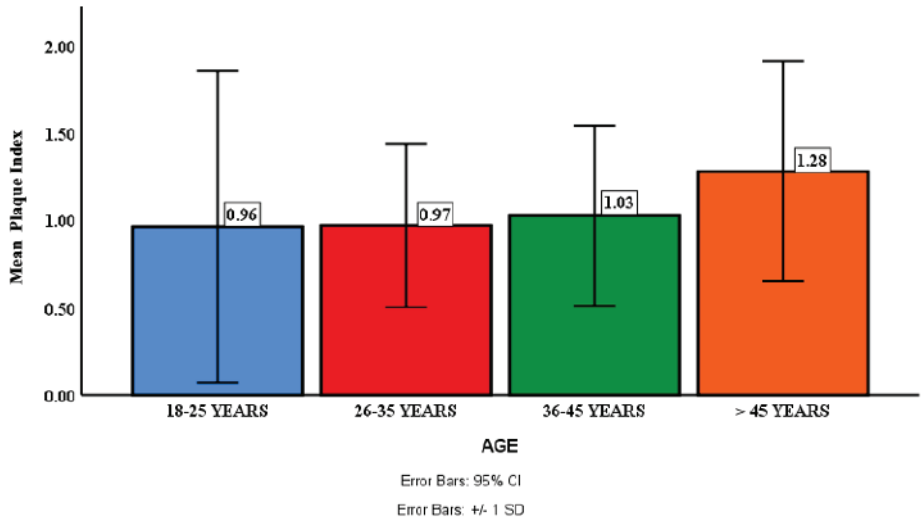


FIGURE 3 : The bar graph represents the Comparison between Mean Plaque index and Age of the study subjects . The mean Plaque index score for the subjects between 18 to 25 yrs, 26 - 35 yrs, 36-45 yrs and above 45 years age group were 0.96 ± 0.89, 0.97± 0.46, 1.02± 0.51 and 1.28± 0.62 respectively. One way ANOVA was used to find the difference between the different age groups with respect to Mean Plaque index and was found to be statistically significant (F value- 5.14; p<0.05) which signifies that study subjects in the age group above 45 years had a higher Mean Plaque index score when compared to other groups.

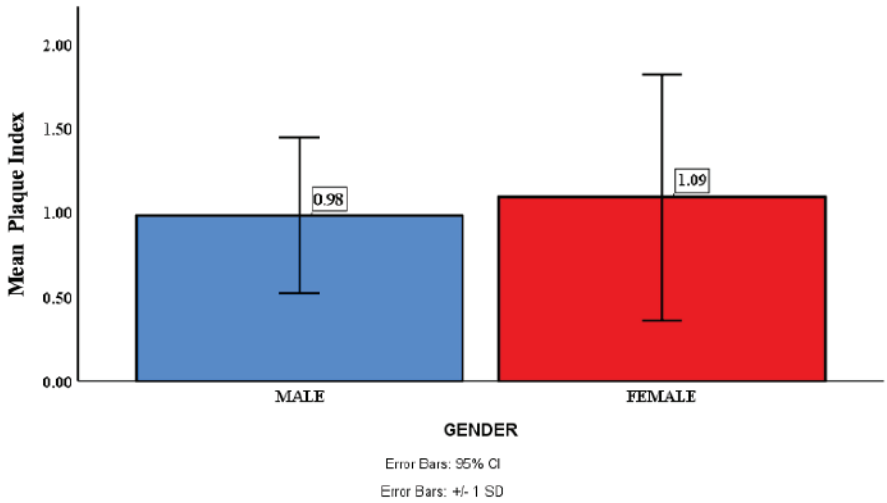


FIGURE 4 : The bar graph represents the Comparison between Mean Plaque index and Gender of the study subjects . The mean Plaque index score score for male and female study subjects were 0.96 ± 0.89, 0.98± 0.46 and 1.09± 0.73 respectively. Independent t test was used to find the difference between the male and female subjects with respect to Mean Plaque index and was found to be statistically insignificant (t value- 1.65; p>0.05). Even though the test was found to be statistically insignificant, female study subjects had a higher mean Plaque index score compared to male study subjects.

Conclusion

The prevalence of dental plaque was high among participants aged more than 45 years and females had a higher Mean Plaque Index score than males.Poor oral hygiene and systemic disease and also few other food habits causes females prone to plaque.Further measurements can be taken to control dental plaque by maintaining good oral health and brushing habits.

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Conflicts of Interest: Nil

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence and Distribution of Unerupted/Impacted Teeth Among Individuals 18-25 Years of Age Visiting Outpatient Department of Private Dental College and Hospital in Chennai, India

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Abstract

The term unerupted / impacted teeth defines a failure of the tooth to erupt within the expected time into the dental arch. Impaction is a common dental anomaly where the tooth has not erupted beyond the eruption period. The incidence of unerupted / impacted teeth differs among different populations and in different ethnic groups. The aim of this study was to assess the prevalence of unerupted/ impacted teeth among patients of 18-25 years of age visiting Private Dental college and hospital, Chennai. This is a descriptive study which is performed under a university setting where all the patients reported to Private Dental college and hospital, chennai. The data was collected by reviewing patients records and analysed the data of 86000 patients who underwent extraction between June 2019 to March 2020. The population size of the study was found to be n=296 .Data was collected , tabulated ,statistical analysis was done by SPSS – IBM.(Statistical Package for Social Studies)**.** From the statistical analysis, it is observed that the total number of extraction done between 18-25 years was found to be n=7339 (96%) out of which only n= 295 (4%) was found with an incidence of impaction. The overall prevalence of unerupted/ impacted teeth were higher in males(55.74%) predominantly between the age groups of 24 years (18.8%) and was commonly reported to third molars (41.9%)**.** Within the limitation of the current study, it is found that unerupted / impacted teeth are more common among 24 years of age with male predilection with higher incidence of mandibular molars.

Keywords : *Unerupted tooth , extraction , Type of impaction, frequency of impaction, site of impaction.*

Introduction

The word impaction originated from the Latin word “impact” meaning organ or structure, William stated that impacted tooth is the one which is completely or partially unerupted beyond the eruption period[1]. The term unerupted tooth defines a failure of the tooth to erupt within the expected time into the dental arch and positioned against another tooth, bone, or soft tissue so that its further eruption is disrupted beyond the time usually expected eruption time [2] . The failure of eruptions may be due to systemic and local factors ,which includes cleidocranial dysplasia, Down’s syndrome, and also deficiency of arch length[3,4]. The incidence of unerupted / impacted teeth differs across different populations and different ethnic groups [5,6]. The causes of impaction may be multifactorial usually due to adjacent teeth, dense overlying bone or soft tissue, size of the mandible or maxilla , lack of space in the jaw and the path of eruption, abnormal positioning of tooth bud, or due to any pathological lesions [7,8] . There is a correlation

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between the development of cystic lesions, pericoronitis ,and dental caries with unerupted /impacted teeth [9] .

Some dental surgeons believe that unerupted/ impacted teeth should be removed except, in certain cases like canines [10]. Usually canines may just remain and do not cause any further problems[11]. Thus surgical intervention is not required [12,13]. However, removal of asymptomatic, pathology-free, unerupted / impacted teeth isn't a medical consensus [14,15.] The classifications of impaction enables the oral surgeon to determine the difficulty in removal of the unerupted / impacted teeth. The primary factor in determining the difficulty is accessibility that is determined by adjacent teeth or other structures which paves a way for the teeth to erupt[16]. The majority of classification systems are based on analysis on a radiograph [17,18]. The most frequently considered factors are site of impaction ,type of impaction and severity of impaction .[19]

In most of the studies the impaction rate is higher for third molars when compared with other teeth [20]. The cause is due to inadequate space between the distal surface of the second mandibular molar and anterior border of the ascending ramus of the mandible [21]. However, recently there is no literature regarding incidence and prevalence of unerupted/ impacted teeth [18,22]. The purpose of this study can help to achieve more relevant information of many factors such as gender, age , systemic disease , site of impaction , angulation , severity, type and prevalence of impaction. Thus , The aim of this study was to assess the prevalence of unerupted/ impacted teeth among 18-25 years of age visiting Private dental colleges and hospitals, Chennai, India, by assessing demographic and morphologic factors that can lead to the condition .

Materials and Methods

This was a descriptive study which was performed under a University setting where all the patients between 18-25 years of age reported to Private Dental College and hospitals, Chennai , India. The data was collected by reviewing the patients records and analysed the data of 86000 patients who underwent extraction between June 2019 to March 2020. The ethical approval was obtained from the Institutional Ethical Committee(ethical approval number : SDC/SIHEC/2020/DIASDATA/0619-0320). The population size of the study who underwent extraction due to unerupted/impacted teeth was found to be n=296. The data was cross verified with photographs and was compiled for statistical analysis on SPSS(version 22.0) software. The minimising sampling bias was done by collecting data within the University and by using the simple random sampling method . There was a high internal validity and low external validity in our study. The patients between 18-25 years of age and the patients who underwent extraction due to impacted /unerupted teeth were included in the study. Improper and incomplete data ,repeated data ,extraction done for other reasons were excluded. Chi square test was used to compare the groups ($p<0.05$) was considered significant and the results were interpreted .

Results and Discussion

In the current study , The total number of extraction done among 18-25 years of age was found to be n=7339 (96%). Out of which only n= 296 (4%) was found with an incidence of an unerupted / Impacted teeth. [Figure 1] represents the distribution of study subjects with unerupted / Impacted teeth. [Figure 2] denoted the prevalence and distribution of unerupted / impacted teeth among different age groups. The result of our study showed unerupted / impacted teeth were more prevalent among 24 years of age (18.8%). [Figure 3] represented the prevalence of gender in relation to unerupted / impacted teeth. The total subjects of unerupted / impacted teeth examined were n=296(4%) of which males constituted (55.74%) and females (44.26%). In our study the most commonly associated chief complaint was found to be the presence of pain (84.98%) followed by absence of pain (14.68%) and presence of discharge (0.31%). Nearly (11.82%) of the patients presented with past medical history. Nearly (1.69%) of patients had swelling at the site of impaction .[Figure 4]denoted the prevalence and distribution of unerupted / impacted teeth in individuals of different tooth numbers,of which mandibular third molars showed higher prevalence (41.9%)than maxillary third molars. The total number of impaction was found to be more prevalent in the third quadrant with a common type being mesioangular (38.31%) [Figure 5]. By correlating age, gender, and tooth number males showed more prevalent on unerupted / impacted teeth than females but both males and females showed equal predilection for mandibular third molars with a mean age of 24 years [Figure 6]. Chi square tests for age, gender, tooth number was found to

be statistically significant (p=0.00).

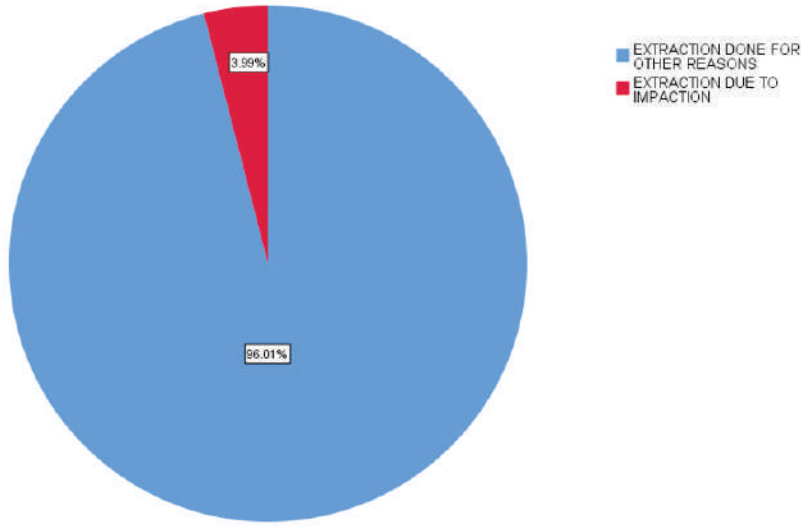


Figure 1 : Pie chart depicting the Prevalence and Distribution of Unerupted / Impacted Teeth Among 18-25 Years of Age Visiting Private Dental College and Hospital in Chennai . Blue colour denotes extraction done for other reasons. Red colour denotes extraction done for impaction. Extraction done for other reasons among 18-25 years of age was found to be(96%) and the extraction done due to impaction was found to be (4%) . (Chi Square test ; p value =0.00 ; p<0.05; hence significant).

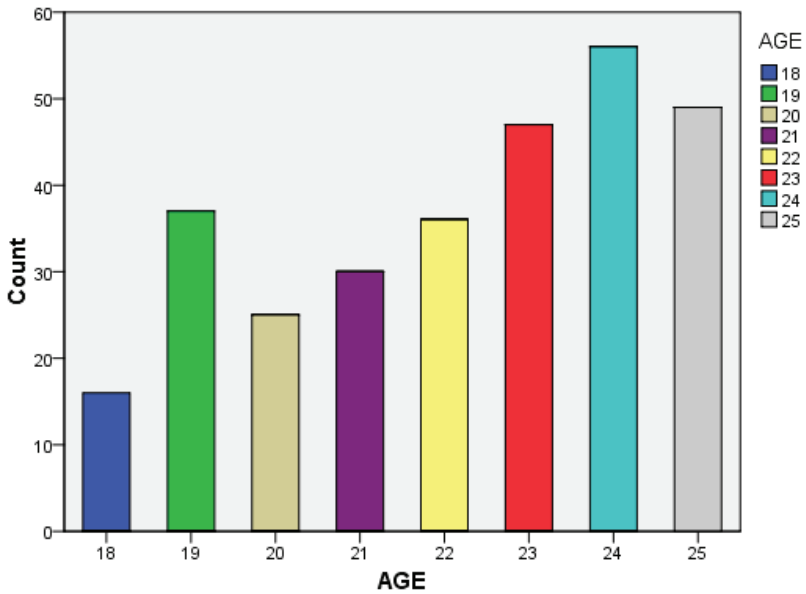


Figure 2 : Bar graph depicting the Prevalence and Distribution of Unerupted / Impacted Teeth Among Different Age Groups Visiting Private Dental College and Hospital in Chennai The graph has been plotted with the frequency taken on the scale from 0-60 on y -axis representing the unerupted / impacted teeth .The age of patients who underwent extraction for unerupted / impacted teeth is plotted on the scale 0-25 along x-axis .Blue colour denotes 18 years of age . Green colour denotes 19 years of age . Brown colour denotes 20 years of age .violet colour denotes 21 years of age . Yellow colour denotes 22 years of age . Red colour denotes 23 years of age .light blue colour denotes 24 years of age . Grey colour denotes 25 years of age . It is evident that the prevalence of Impacted / Unerupted teeth was more common in patients of 24 years of age. (Chi Square test ; p value =0.00 ; p<0.05; hence significant).

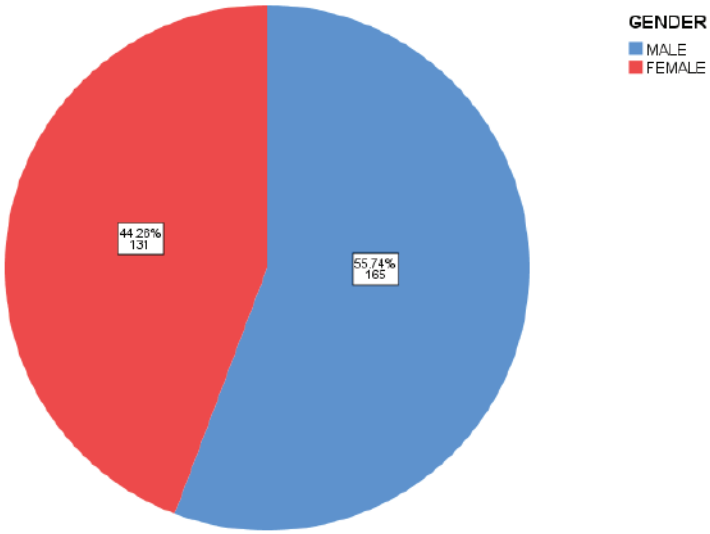


Figure 3 : Pie chart depicting Prevalence and Distribution of Unerupted / Impacted Teeth Among Different Genders Visiting Private Dental College and Hospital in Chennai . Blue colour denotes males. Red colour denotes females. Pie chart shows that Unerupted /Impacted teeth were higher in males . (Chi Square test ; p value =0.04 ; p <0.05; hence significant).

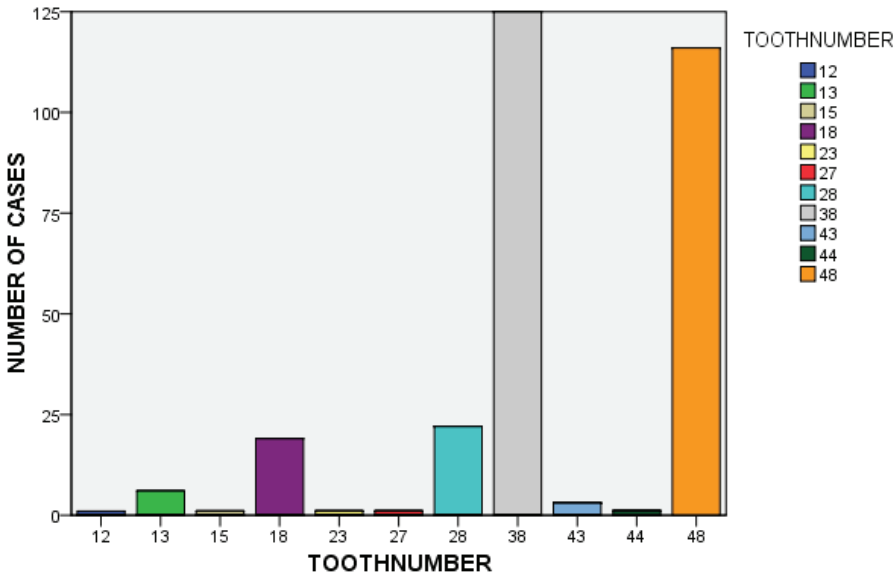


Figure 4: Bar graph depicting the Prevalence and Distribution of Unerupted / Impacted Teeth in Different Tooth Numbers in Individuals Among 18-25 Years of Age Visiting Private Dental College and Hospitals in Chennai. The frequency of unerupted /impacted teeth is plotted on scale 0-125 along y axis. The frequency of tooth number is plotted on the x-axis. Blue colour denotes the tooth number 12. Green colour denotes the tooth number 13. Brown colour denotes the tooth number 15. Violet colour denotes the tooth number 18. Yellow colour denotes the tooth number 23. Red colour denotes the tooth number 27. Light blue colour denotes the tooth number 28. Grey colour denotes the tooth number 38. Purple colour denotes the tooth number 43. Dark green colour denotes the tooth number 44. Orange colour denotes the tooth number 48. The prevalence of unerupted / impacted teeth was most frequently found in the lower left 3rd molar. (Chi Square test ; p value =0.00 ; p<0.05; hence significant).

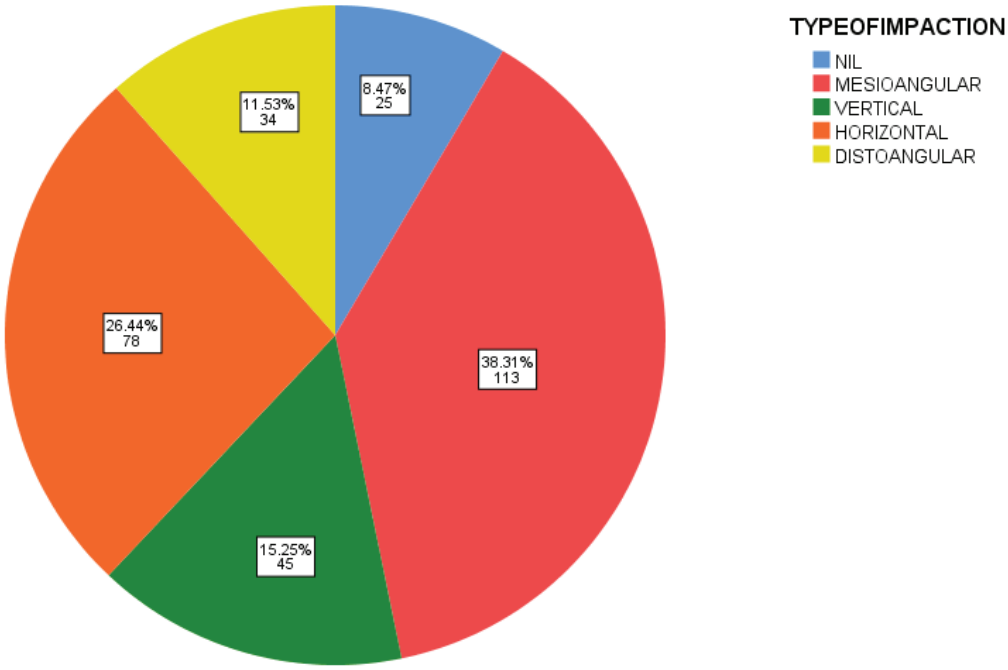


Figure 5: Pie chart depicting the assessment of type of impaction among patients who underwent extraction for unerupted / impacted teeth in Private Dental Colleges and Hospitals in Chennai. Red colour denotes Mesioangular type of impaction. Yellow colour denotes a distoangular type of impaction. Green colour denotes vertical type of impaction. Orange colour denotes horizontal type of impaction. Blue colour denotes absence of any type. Mesioangular was the most commonly found type of impaction among the patients with impacted /unerupted teeth . (Chi Square test ; p value =0.00 ; p<0.05; hence significant).

Figure 6 : Bar graph depicting the Correlation of Age,gender and Tooth Number of Patients Who Underwent Extraction for an Unerupted / Impacted Teeth in Private Dental Colleges and Hospitals in Chennai. The graph has been plotted with the frequency of mean age taken on the scale from 0-25 on y -axis and tooth number on x-axis. Blue colour denotes males. Red colour denotes females. It is evident that males showed more prevalent on unerupted / impacted teeth than females but both males and females showed equal predilection for mandibular third molars with a mean age of 24 years.(Chi Square test ; p value =0.00 ; p<0.05; hence significant).

A tooth which was unable to erupt physiologically into its functional , anatomic position with time is said to be unerupted / impacted teeth [23,24]. The current study showed that the prevalence of unerupted /impacted teeth among 18-25 years of age was found to be (4%)

[Figure 1]. The main reason for the impaction was due to lack of space of the path of eruption or may be due to abnormal positioning of tooth bud [25] . The present study indicated that there are predisposing variables which were associated with impaction [26]. Age was a predisposing factor associated with impaction [20]. A tooth that appeared impacted at the age of 18 years may have as many as chances as 30-50% of erupting fully , except horizontally impacted molars [27] . Impaction was more prevalent in individuals among 24 years of age (18.8%) [Figure 2]. The results of this study are in agreement with the study conducted by Seidu A Bello et al 2011 , suggesting that impacted/unerupted teeth are more prevalent in patients with an age range of 19-26 years. [28]

Gender is another predisposing variable which can be associated with impaction [29]. The results of the present study showed that males had undergone more number of extraction due to impaction (55.74%) compared to females (44.26%) [Figure 3]. The findings of the study were similar to study conducted by Enabulele et al 2017 , stating that males (80%) had higher impaction than females (20%) [30]. This supports the assertion that

there are sex- specific mandibular traits with the sexual dimorphism , clearly observed with bigger jaw bone in males [31]. Similarly , the study conducted by Nagaraj T et al 2016, reported that males had higher impaction than females[32] . But contradictory result was found in the study conducted by Korener et al 2015 , stated that the possibility of dietary influence on the physique , role of genetic factors or a combination of genetic and dietary factors have been proposed to have effect on low prevalence of unerupted / impacted third molar this may be the reason for fewer number of males presented compared to females [33, 34] .

.In our study pain was the most commonly associated chief complaint (84.98%). The pain may be due to the type of impaction, or any pathological growth or condition[1,35] . Similar results showed in the study conducted by Seidu A Bello et al 2011 , and Nagaraj T et al 2016 [36,32]. In our study nearly 11.82% of the population had a history of Diabetes mellitus and hypertension etc . Kemp et al 1980 , in his study mentioned that there was no history with signs of associated pathology and also Junko Mat Suyama et al 2015 , clearly mentioned that the medical history was unremarkable[37]. Only (1.69%) of the patients presented with swelling . The study done by Nagaraj T et al 2016, presented that swelling was associated in most of the cases and the root cause for wisdom teeth swelling is a natural response by the body ,when injured or any infection , so the body wants to bring oxygen rich blood , red blood cells and necessary nutrients to the affected area [32] .

Mandibular third molar was found to be more prevalent than maxillary molar (41.9%) [Figure 4]. Two reasons could be thought , one is due to inadequate space between the distal surface of the second mandibular molar and anterior border of the ascending ramus of the mandible and improper oral hygiene [38] . This study was in concordance with a study conducted by P.Santhosh et al 2015, Nagaraj et al 2016 , revealed that mandibular third molars had higher prevalence of impaction than other teeth [32]. The total number of impaction was more prevalent in the third quadrant with mesioangular being the common type (38.31%)[Figure 5]. As the mandibular growth stops , leading to shortage of retromolar space which is a major etiological factor of mandibular third molar impaction [39]. Osunde et al 2014 , obtained the similar result (ie) mesioangular being the most common type due to their late development and maturation, path of eruption and lack of space in mandible at later age [40].

By correlating age ,gender and tooth number males had higher impaction than females [Figure 6]. But both males and females showed equal gender predilection on mandibular third molar with a mean age of 24 years. The chi square test was compared among age, and tooth number was found to be (p= 0.00), statistically significant. It was observed that the total number of extraction done between 18-25 years was found to be n= 7339 (96%) out of which only n=295 (4%) was found with an incidence of impaction .

The overall inference of our study among prevalence of unerupted / impacted teeth were higher in males ,(55.74%) predominantly between the age groups of 24 years (18.8%) due to sex- specific mandibular traits with the sexual dimorphism clearly observed with bigger jaw bone in males with higher incidence of Mandibular third molars [41] . This might be due to inadequate space between the distal surface of the second mandibular molar and anterior border of the ascending ramus of the mandible and improper oral hygiene [42].

The limitation of our study is unicentered with a limited demographic area of smaller sample size . By investigating the prevalence and pattern of impaction and its association with pathological conditions and also this might help broaden existing knowledge about epidemiology of diseases associated with teeth impaction and improve our clinical management to minimize false interpretations .

Conclusion

Within the limitations of the current study , it is found that unerupted / impacted teeth are more common among 24 years of age with male predilection with higher incidence of mandibular molars. This was the most recent and perhaps first ever studies to evaluate the prevalence of unerupted /impacted teeth among 18-25 years of age visiting outpatient Department of Private Dental college and hospitals .In our study only 4% of population had unerupted / impacted teeth when compared to other studies from different countries and regions. Importance of regular dental checkups which could lead to an early detection of unerupted / impacted teeth and institution of appropriate measures before

complications is encountered .

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Angular Bone Defects in Chronic Periodontitis Patients with and without Systemic Diseases

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Abstract

The aim of the current study was to assess the distribution of angular defects in both maxillary and mandibular arches in chronic periodontitis patients with and without systemic diseases. This retrospective study was conducted among 200 patients who reported to Saveetha Dental College and Hospitals, Chennai from June 2019 to March 2020. A total of 200 chronic periodontitis patients were enrolled and assessed for number of angular bone defects using panoramic radiographs. The study participants were divided into two groups. Group 1 (n=100, 50 males and 50 females): chronic periodontitis patients with systemic diseases; Group 2 (n=100, 50 males and 50 females): chronic periodontitis patients without systemic diseases. Data regarding the number of angular defects of the study population were collected and analysed. Among 100 systemically healthy patients, 102 sites presented with angular defects. Among 100 systemically diseased patients, 320 sites presented with angular defects. In gender wise comparison, the number of angular defects were higher in females as compared to males. When the distribution of angular bone defects were compared between maxillary and mandibular arches, angular bone defects were predominantly observed in mandibular arch. Therefore, this study showed higher prevalence of angular bone defects in chronic periodontitis patients with systemic diseases (320 sites) as compared to the ones without systemic diseases (102 sites).

Keywords: *Periodontitis; Bone loss; Angular defects; Alveolar bone, Bone resorption*

Introduction

Chronic periodontitis is the most common inflammatory periodontal disease which leads to changes in normal architecture of the alveolar processes. Alveolar bone destruction is primarily caused by bacterial plaque that leads to inflammation of periodontal tissue. Bacterial plaque induces an increase of osteoclast formation and activity through direct or indirect mechanisms. Equilibrium between bone formation and bone resorption is thus shifted to favor the latter and results in the destruction of alveolar bone.[1–3] The possible factors in the pathogenesis of the bone defects are tooth anatomy and position, the relationship of adjacent marginal ridges and cementoenamel junctions and open contact points with resultant food impaction as well as traumatic lesions affecting the attachment apparatus.

Periodontal bone loss from periodontitis may be either horizontal or vertical, leading to the formation of defects contained within the bone. An intrabony defect may be detected by radiographic means, bone sounding, or by visual examination of the bone defects during periodontal surgery.[4–8] Angular bone defects have

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been associated with trauma from occlusion [9,10] and this association is not exclusive since angular bone loss defects are associated with teeth with normal function and in occlusion.[11–13] Most of the angular defects are due to the apical propagation of the subgingival plaque.[14–16]

Osseous defects occur either single or in different combination forms. The identification of osseous defects is clinically challenging as the osseous surgeries are based on diagnosis. The complexity of the disease grants importance to the use of imaging methods in the detection of such alterations. Radiography plays an important role in periodontal diagnosis mainly because radiographs can reveal the amount and type of damage caused to the alveolar bone.[17]

In this context, the present study was undertaken to assess the distribution of angular defects in both maxillary and mandibular arches in chronic periodontitis patients with and without systemic diseases.

Materialsand Methods

This retrospective study was conducted among 200 chronic periodontitis patients who reported to Saveetha Dental College and Hospitals, Chennai from June 2019 to March 2020. Data regarding the number of angular defects of the study population were collected using panoramic radiographs and analysed. A total of 200 chronic periodontitis patients were enrolled and assessed for number of angular bone defects using panoramic radiographs. The study participants were divided into two groups. Group 1 (n=100, 50 males and 50 females): chronic periodontitis patients with systemic diseases; Group 2 (n=100, 50 males and 50 females): chronic periodontitis patients without systemic diseases. Differential and inferential statistics were done for data summarization and presentation. The study protocol was approved by the Institutional Ethical and Review Board, Saveetha Dental College and Hospitals, Chennai.

Results and Discussion

A total of 200 chronic periodontitis patients were enrolled and assessed for number of angular bone defects using panoramic radiographs.

Among 100 systemically healthy patients, 102 sites presented with angular defects. Among 100 systemically compromised patients, 320 sites presented with angular defects. (Figure 1) In gender wise comparison, among 50 males without systemic diseases, the number of angular defects were 42. Among 50 males with systemic diseases, the number of angular defects were 120. Among 50 females without systemic diseases, the number of angular defects were 60. Among 50 females with systemic diseases, the number of angular defects observed were 200. (Figure 2) When the distribution of angular bone defects were compared between maxillary and mandibular arches, 298 sites with angular bone defects were observed in mandibular arch and 124 sites with angular bone defects were observed in maxillary arch. (Figure 3). There is a significant difference between the angular defects in systemically healthy patients and systemically compromised patients.Chi square test p value is 0.01(<0.05) which was clinically and statistically significant (Figure 4) .

The common radiographic representation of chronic periodontitis is infrabony defects which is commonly referred to as vertical or angular defects. A vertical defect is a pathologic phenomenon related to the process of irregular bone resorption seen in advanced forms of periodontal disease. Although vertical defects are considered a sign of progressive or advanced disease, their occurrence in the population has been but little investigated.[18–22]

The present retrospective study assessed the number of angular defects in chronic periodontitis patients with and without any systemic diseases. In the present study, it was observed that the number of angular defects were higher in chronic periodontitis patients with systemic diseases when compared to chronic periodontitis patients without systemic diseases.

Chapple ILC et al[23] in the consensus report of the joint EFP/AAP workshop on periodontitis and systemic diseases suggested a strong association between periodontitis and various systemic diseases and conditions. Kuo LC et al[24] conducted a systematic review to assess the inter-relationships and interactions between periodontal diseases and systemic diseases and concluded that most of the literature evaluated in the systematic review supported a modest association between periodontitis and systemic diseases.

Singh PK et al[25] demonstrated that the distribution of angular bone defects were associated with chronic

periodontitis. Albander JM et al[26] in a systematic review suggested that the systemic diseases and conditions can affect the periodontal attachment apparatus and cause loss of periodontal supporting tissues resulting in bone loss. Our finding is in accordance with the previous studies as the majority of angular bone defects were observed in chronic periodontitis patients with systemic diseases compared to the one without systemic diseases.

In the present study when gender wise comparison was done, females presented with more angular defects when compared to males. Zhao H et al[27] investigated the alveolar bone status chronic periodontitis patients using CBCT and found out that females had higher degree of bone loss when compared to males. Similarly, Kasaj A et al[28] assessed the alveolar bone loss and angular defects in panoramic radiograph and found that the majority of the bone defects were detected among females when compared to males. Our finding is in agreement with the previous study.

In the present study, when the distribution of angular bone defects were compared between maxillary and mandibular arches, the majority of the angular bone defects were observed in mandibular arch when compared to maxillary arch. In a study by Prichard J et al[29] mandibular arch showed higher number of angular defects than maxillary arch. Our finding is in line with the previous study.

The limitations of the present study is minimal sample size and the confounding factors like age, smoking, genetic factors, oral hygiene, stress were not considered. Therefore, extensive research needs to be done by considering the influence of various risk factors that aggravates periodontal destruction.

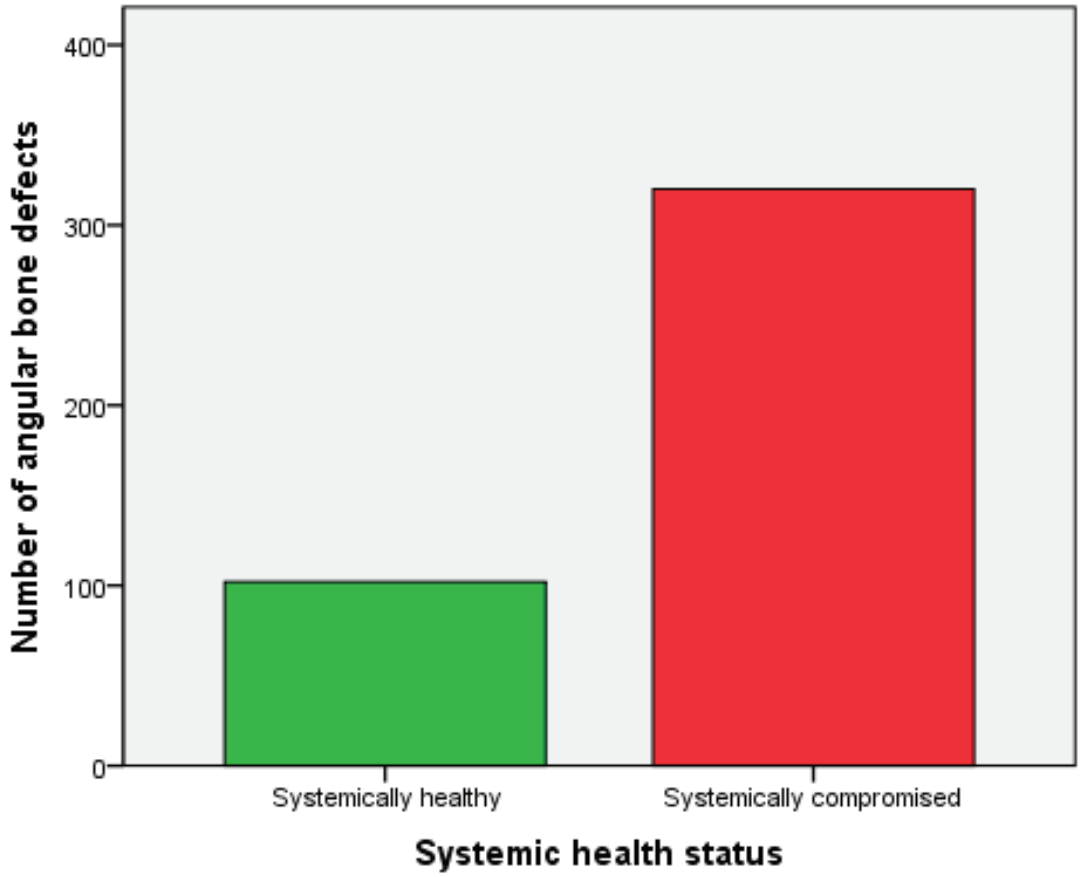


Figure 1: Bar graph representing the distribution of number of angular bone defects based on systemic health status. The X axis represents the systemic health status of the patients and the Y axis represents the number of angular defects. It was seen that the number of angular bone defects found in systemically healthy patients and in systemically compromised patients were 102 and 320 respectively.

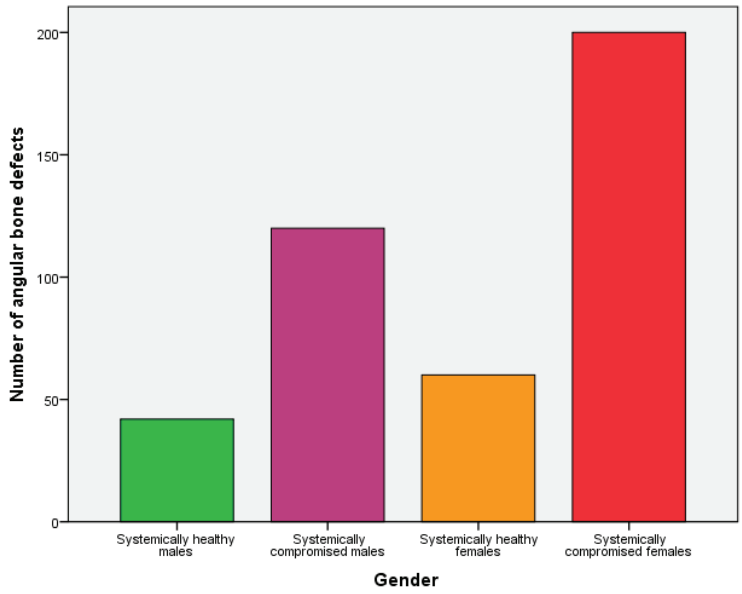


Figure 2: Bar graph representing gender wise distribution of the number of angular bone defects. The X axis represents the gender and the Y axis represents the number of angular defects. It was seen that the systemically healthy males presented 42 angular defects (green colour) and systemically healthy females presented with 60 angular defects (orange colour) and systemically compromised males presented with 120 angular defects (purple colour) and systemically compromised females presented with 200 angular bone defects (red colour). It was seen that systemically compromised females had more angular bone defects and systemically healthy males had least number of angular bone defects.

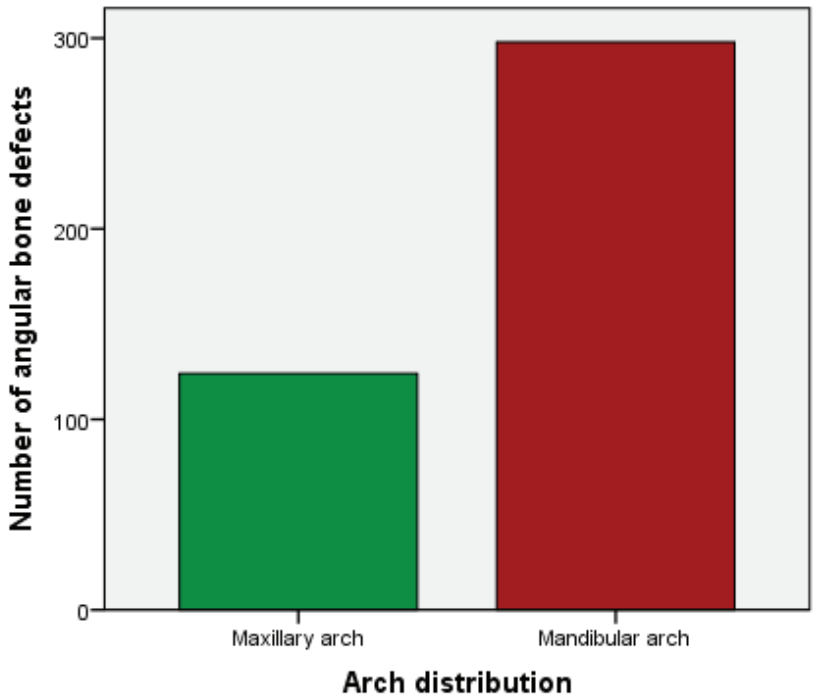


Figure 3: Bar graph representing archwise distribution of the number of angular bone defects. The X axis represents the archwise distribution and the Y axis represents the number of angular bone defects. It was seen that the maxillary arch exhibited 124 angular bone defects (dark green colour) and the mandibular arch showed 298 angular bone defects (dark red colour).Mandibular arch presented with more number of angular defects (298 sites) than the maxillary arch (124 sites).

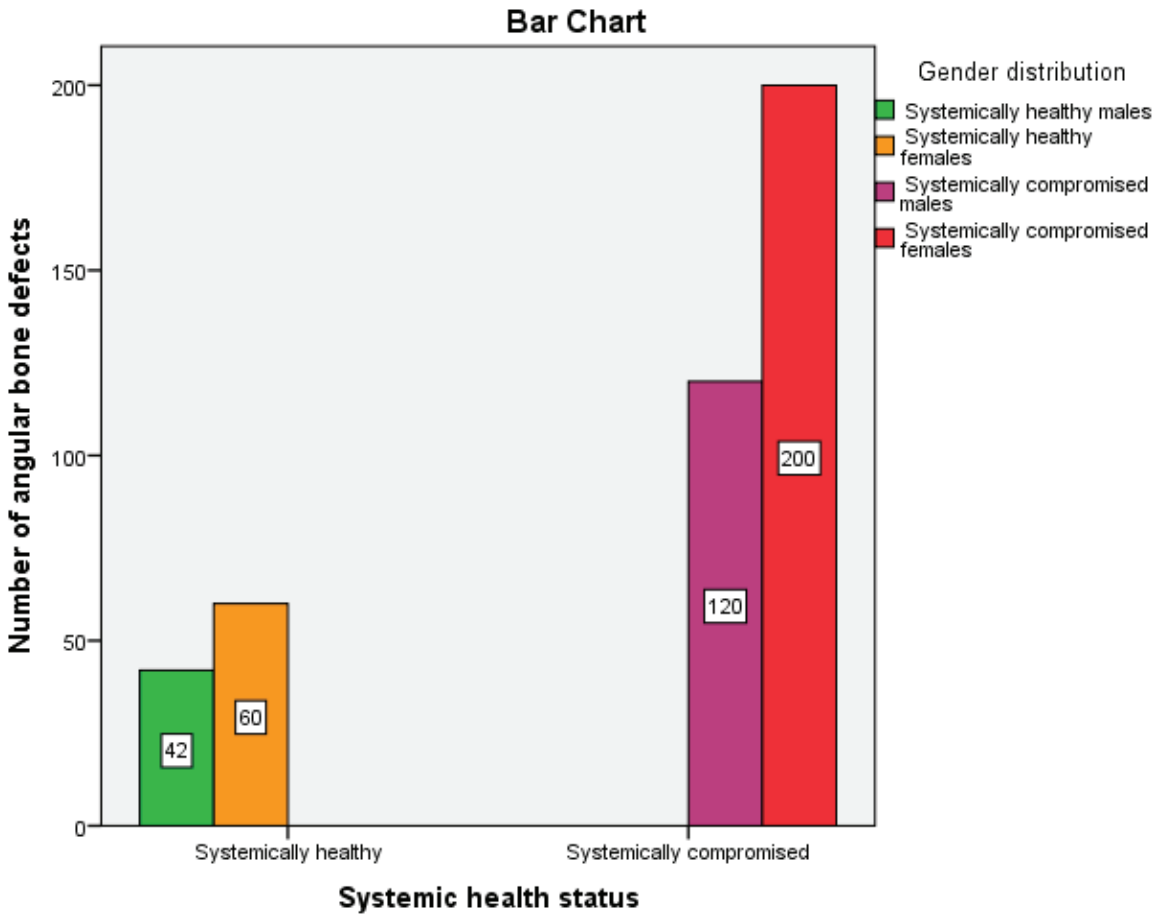


Figure 4: The bar graph showing association between systemic health status of the patients and the number of angular bone defects. The X axis represents the systemic health status of the patients and the Y axis represents the number of angular bone defects. The systemically healthy male patients presented with 42 angular bone defects (green colour). The systemically healthy female patients presented with 60 angular bone defects (orange colour). The systemically compromised male patients presented with 120 angular bone defects (purple colour) and the systemically compromised female patients presented with 200 angular bone defects (red colour).The systemically compromised patients presented more angular defects than the systemically healthy patients.There was a significant association between systemic health status of the patients and the number of angular bone defects. Chi square test was done; p=0.01; statistically significant (p<0.05) .

Conclusion

Within the limitations of the study, it was concluded that there was higher prevalence of angular bone defects in chronic periodontitis patients with systemic diseases (320 sites) as compared to the ones without systemic diseases (102 sites).

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Conflict of Interest: None declared.

Source of Funding: Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Assessment of Postoperative Obturation Quality of Retreatment Cases - A Retrospective Study

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Abstract

Conventional endodontic therapy emphasizes many factors which mainly emphasize root canal disinfection. However there is a probability of endodontic therapy to give an unfavourable outcome such as persistent clinical symptoms and unfavourable periapical healing. The frequency of failure is reported to be around 20% and various factors contribute to it which mostly relies on the treatment procedure followed by the previous operator. It is of paramount importance that postgraduate doctors should learn to treat these case scenarios with high effectiveness. This study aims to assess the postoperative obturation quality of retreatment cases done by graduate doctors. The study was done by using data collected from electronic medical records of Saveetha Dental College, Chennai, India. The time frame of data collection was seen from June 2019 to March 2020. A total sample size of 230 was assessed radiographically and the quality of obturation was analyzed using 2 grading criteria in compliance with 2 operators. From the cases assessed, about 80% had achieved correct radiographic length of obturation and about 70% of obturation was not of optimal quality. Quality of obturation was not adequate. The length of root canal filling was desirable whereas the quality of condensation was not adequate about 60% of the assessed cases

***Keywords:** Retreatment, Dental graduate, Electronic medical records, retrospective studies, Root canal obturation*

Introduction

The application of orthograde endodontic retreatment is fairly common clinical practice especially for an endodontic specialist [1]. Various epidemiological studies have been done showing a need for endodontic treatment due to the increased number of poor root canal therapy and persistent clinical symptoms for the clinician [2,3].

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It is well known that endodontic treatment has a viable outcome and is dependent on various factors such as operator factors, healing outcomes, tooth complexity and patient related factors [4]. All these factors have an invariably high influential role influential factor to the treatment outcome which draws the necessity for an endodontic retreatment for better favourable outcomes [5]. The reasons for endodontic failures are due to various factors, which is more commonly due to previous treatment procedures [6,7].

It is already known that there is a correlation which takes place between periapical destruction and improper obturation [8]. It is seen that the voids and unfilled spaces of the root canal play an influential role for infections, especially microorganisms, to easily penetrate and affect the periapical tissues [9]. This could again cause more harm to the patient than benefit them in the long run.

One of the theories that can be used by dental practitioners which have already been proven is Praxic Concept theory (PC), by Kvist et al [10]. This theory suggests that operators in order to assess the periapical health continuity on a scale of "no lesion" and "big lesion" in either extremities can help the operator to assess the periapical health. This helps the operator to grade the disease and the gravity of the lesion suggesting the need for Endodontic retreatment in clinical scenarios [11].

Different authors have suggested two modalities of endodontic retreatment. One an orthograde approach and next a retrograde approach (surgically). According to various authors it was seen that an orthograde approach had a much better successful outcome with a success rate seen to be around 80% [12,13].

The decision making process at a postgraduate endodontic program may be different to other employed in a private practice setting due to their different concepts and philosophies, patient and educational requirements and has an influential role in the need for Endodontic Retreatment [14,15].

Our study aims to assess the post obturation quality of Endodontic retreatment by postgraduate students.

Material and Methods

All the data was obtained via Electronic Medical records of Saveetha Dental College, Chennai, India. Demographic data such as patients age, gender, tooth number was obtained. Assessment data was obtained through the database and was manually analyzed by 2 calibrated operators who assessed the radiographs based on 2 criterias. In case of disagreement a third operator was asked to reach a conclusion for the same.

The criterias assessed were the following

A) Length of the root canal filling

B) Quality of the root canal filling

The assessment was done and the scoring criteria was done by criteria given by Hommez et al [16]. The length of root filling scoring criteria (A) was assessed from score 1-3

1) 1- Root filling terminating 0-2mm short of radiographic apex (acceptable)

2) 2- Root filling terminating >2mm from the radiographic apex (unacceptable)

3) 3- Root filling extending beyond the radiographic apex (unacceptable)

Quality of the root filling (B) was divided into score 1-2

1) 1- Homogenous root filling, good condensation, no voids, visible(acceptable)

2) 2- Inhomogenous root filling, poor condensation, voids visible

A total sample of 280 was recorded from the time frame June 2019 to March 2020. From all the duplicate entries, incomplete entries and samples subjected to inclusion criteria, a total sample size of 227 was achieved

The inclusion criteria being -

1) Patients aged 18-70 years

2) Tooth subjected to only endodontic retreatment

The exclusion criteria being -

Patients below age 18 and above age 70 years

Incomplete data

Patients with some records missing were excluded

Results and Discussion

The statistical analysis was carried out using SPSS 21.0 (IBM Corp, USA). Demographic data such as patient gender, tooth number, age was recorded and the results showed length of root canal filling showing 60% being acceptable and 70% the quality of obturation was seen to be unacceptable. A chi square test was done to assess the potential outcome predictors with level of significance set at 0.05 and ($p<0.005$), a statistically significant difference was seen

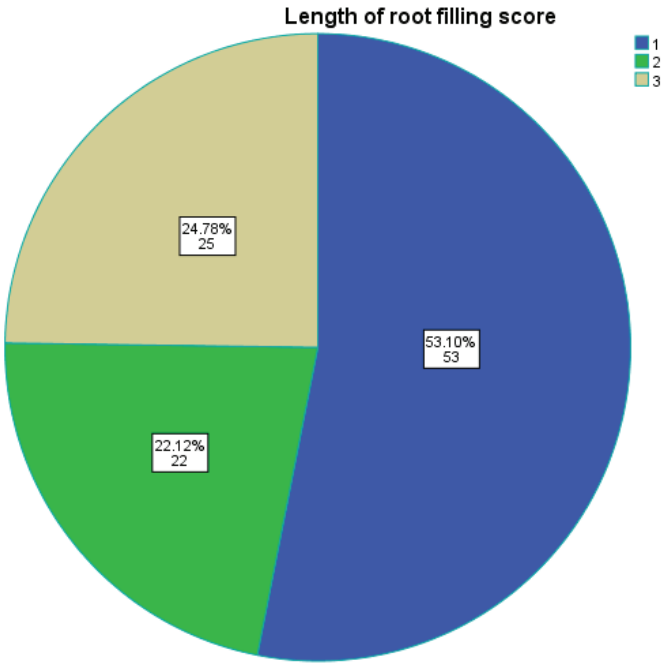


Figure 1: Pie chart denotes the frequency of distribution based on the length of root canal filling in Endodontic Retreatment cases. Blue color denotes Score 1- Root filling terminating 0-2mm short of radiographic apex (acceptable), Green color denotes Score 2- Root filling terminating >2mm from the radiographic apex (unacceptable), Yellow color denotes Score 3- Root filling extending beyond the radiographic apex (unacceptable). The frequency distribution of the length of root filling was seen to be highest for Score 1 - 53 (53.10%), followed by Score 2 - 22 (22.12%) and Score 3 - 25 (24.78%).

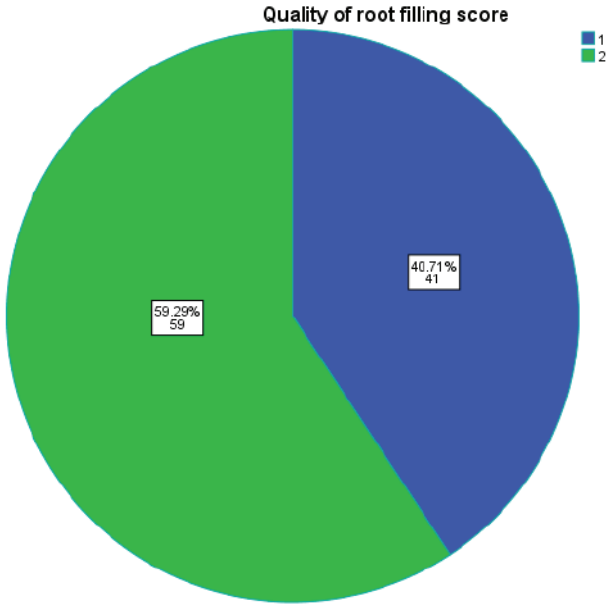


Figure 2: Pie chart denotes the assessment of quality of root canal filling in Endodontic Retreatment cases. Green color denotes Score 1- Homogenous root filling, good condensation, no voids, visible(acceptable). Blue color denotes Score 2- non homogenous root filling, poor condensation, voids visible. The frequency distribution of the quality of root filling was seen to be highest for Score 2 - 59 (59.29%) followed by Score 1 - 41 (40.71%)

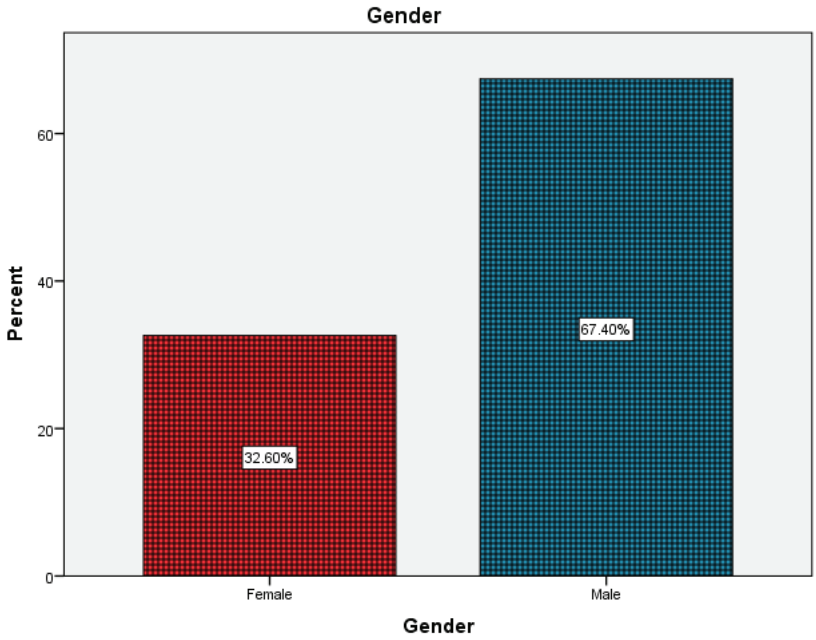


Figure 3: Bar chart denotes the Gender distribution for Endodontic Retreatment with X axis denoting the gender and Y axis denoting the percentage. From the data assessed it was seen that the male population was seen to be 153 (Blue) (67.4%) and female population being 74 (32.6%)(Maroon)

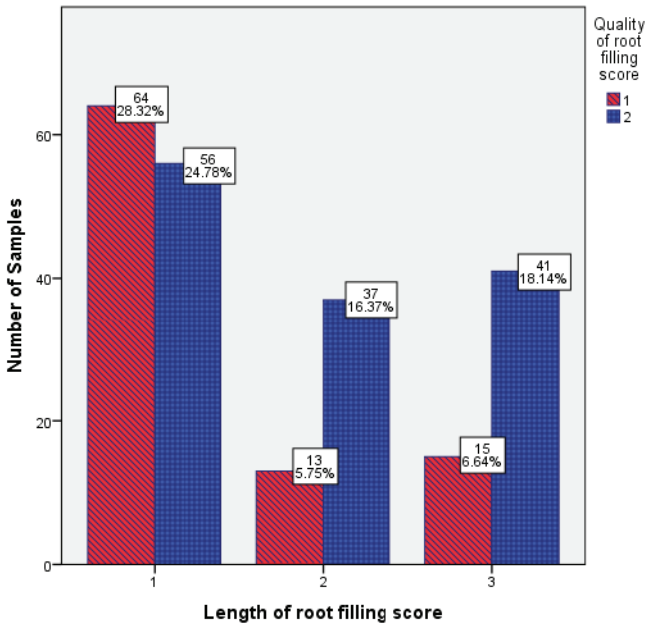


Figure 4: Bar chart denotes the association between the length and quality of the root filling. X axis denotes the length of the root filling score and Y axis denotes the frequency distribution of samples. The bar diagram denotes the frequency and its associated percentages Red color denotes Score 1 and Blue Color denotes Score 2 for quality of root filling score. It is seen that higher frequency of distribution is seen in Score 1(length)/Score 1(quality)- 64 (28.32%) denoting adequate quality. Score 1(length)/Score 2(quality) was seen to be next highest 56 (24.78%) denoting the length of root filling was adequate but the quality of root filling was not adequate. Score 2(length)/Score 1(quality) was seen at 13 (5.75%), Score 2(length)/Score 2(quality) at 37 (16.37%), Score 3(length)/Score 1(quality) at 15 (6.64%) and Score 3 (length)/Score 2(quality) at 41 (18.14%). Chi square test was done to analyze the correlation between the length of root filling and quality of root filing and was found be statistically significant (p value =0.000)

Endodontic retreatment consists of various factors such as cleaning and shaping of the previously obturated canals and three dimensional filling of their cleaned space [17,18]. This treatment depends on various factors such as feasibility of the access to the root canal system and the restorability of the teeth in the long run [19].

To successfully accomplish this procedure all the obstructions preventing direct access to the root canal are removed such as dentinal overhangs and the intracoronal restorations and other obstructions within the canal are removed such as root canal filling materials and other foreign objects [20–22]. Followed by this various obturating materials such as semisolid, solid materials are removed such as pastes and cements [23]. Patency filing is achieved till the minor constriction followed by adequate cleaning and shaping is done to reduce the microbial load. For this to be achieved simultaneous usage of both instrumentation and irrigants should be used judiciously in this case scenario [4,24–26].

The use of different GP solvents are advised to be used but only in minute quantities. The commonly used being chloroform, xylene, halothane and shown to have some amount of cytotoxic activity when extruded periapically [27]. Different methods are now currently being followed for gutta percha removal such as retreatment instrumentation, hand/rotary instruments or the use of ultrasonics which have shown better results compared to previous known methods [28].

In our results it was seen more endodontic retreatment samples were done on Male and commonly were done on anterior teeth. More of the retreatment was done in the age group above 45 years old. All these have an influential role was given by Imura et al [29] who stated that the outcome of success of endodontic treatment varied considerably with age factors playing a crucial role in periapical healing, also other factors they had noticed was the presence of flare-ups in most infected teeth [30,31]. It was also seen that age had a role in which the more the age increased, the lesser the healing of periapical lesions due to lesser mechanism of body's resistance to pathogens as described by Estefan et al [32].

The increased usage of Intracanal medication is crucial for Endodontic retreatment case scenarios such that it was seen to use full strength NaOCl irrigation in infected canals that fail to completely disinfect the infected root canal dentin [33–36]. Sjogren et al [37,38] has suggested the usage of (Ca(OH2)) paste as a 7-day dressing has shown to eliminate bacteria which survived even after the mechanical preparation [39]. Due to this it is always advised to do a two visit treatment as suggested by Yoldas et al [40] who have shown to have lesser postoperative complications in their multivisit therapy.

In our study we had done a association analysis based on the analysis done it was seen a statistically significant difference was seen between the length of filling and quality of filling score and hence proves that even if the length of root canal filling was achieved the quality of root canal filling also plays a crucial role in the prognosis of endodontic treatment in long run. This was supported by Alves et al [41] in their prospective study which showed higher incidence of endodontic flare ups in obturations with voids. This all findings were in agreement with common findings of all previous studies to be done in a postgraduate assessment

Study Limitations

1) More assessment criteria can be done for better quantitative analysis

2) Skill of an operator had an influential outcome in our study

Future Scope

1) More grading sample required

2) A prospective based sample would give better results of the long term prognosis which is crucial for the validation of treatment

Conclusion

From the results achieved, it is seen that the quality of the obturation was seen to be far from optimal despite the desired root length filling length being achieved by postgraduate students. This could be due to various endodontic complications which could have hindered the outcome and influence the obturation quality. More emphasis should be done in the postgraduate program to handle this case scenario since it is one of the most difficult treatments carried out by the specialist. This can be achieved by the mastery of various techniques and more emphasis on the programs which could prove beneficial in the long run and also help them treat any refractory endodontic disease.

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conflict of interest

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Ethical Clearance

It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Type of research *: Retrospective study.*

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Prevalence of Kennedy Classification in Partially Edentulous Patients - A Retrospective Study

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Abstract

The aim of the study is to evaluate the prevalence of partial edentulism according to Kennedy's classification in the South Indian population. A retrospective study was done in which 1419 removable partial dentures were fabricated from 1 June 2019 till 1 March 2020 were included. Data was reviewed from the patients records and analysed the data of 86,000 patients between June 2019 and March 2020 that were documented in a private institution. The data was analyzed using descriptive statistics and Chi square test and pearson correlation was done for checking correlation between age, gender and type of classification. Out of 1419 removable partial dentures, 58.4% were fabricated for males. Class III had the highest prevalence overall and in males and females. There was no significant relation between the type of Kennedy classification of partially edentulous patients with age and gender. Prevalence of Kennedy's Class III was the most common in the South Indian population. The prevalence was more in patients above 50 years of age. Male had more prevalence than females. Gender and age did not affect the prevalence of types of Kennedy's classes.

Keyword: *Partial edentulous, Missing teeth, Classification, Kennedy's classification.*

Introduction

Partial edentulism is when one or more teeth are missing. There are various causes for partial edentulism like caries, truma, periodontal problems, congenitally missing, internal resorptions, etc [1–3]. According to many studies, caries is the main cause of tooth extraction [4–7]. Bruce et al [8], also found that incidence of tooth loss due to caries is about 83% and due to periodontal problems is 17%. Caries mainly occur when the existing prosthesis has open margins which can lead to destruction of the tooth [9,10]. Partial edentulism causes problems for patients and the dentist. Patient related problems are decreased self esteem, lifestyle compromises, inability to eat, esthetic compromises, etc [11,12]. Clinical complications include drifting of teeth, supraeruption of opposing dentition, reduced space due to tipping of the abutments into the edentulous space, loss of occlusion, spacing between the teeth, alteration in speech, temporomandibular disorders, loss and degradation of alveolar bone of edentualous space and adjacent teeth, etc [1,13–18]. Treatment for partial edentulism can be removable or fixed depending on patients choice. Fixed options include Fixed partial dentures and implants [19,20].

Partially edentuous patient have different number and location of the missing teeth and their relation to the natural teeth, there are possibly more than 65000 combinations of partial edentulism in both arches, hence its important to classify them for easy communication between dentists, students and between dentist and laboratory technician [7,14,21–24]. Various classifications are available for partially edentulous patients like Beckett, Godfrey, Swenson, Avant, Cummer, Kennedy, Applegates, Friedman, Neurohr, Bailyn, Wild, Skinner [6,25,26]. Kennedy classification is the most widely accepted classification as it has visual representation of all the classes, easy to communicate, classifies both tooth and tissue born edentulism [25,27–31].

Kennedy's classified partial edentulism into: Class I: Bilateral edentulous space presents posterior to the remaining natural teeth, Class II: Unilateral edentulous space present posterior to the remaining natural teeth, Class III: Unilateral edentulous space with natural teeth both anterior and posterior to it, Class IV: Single edentulous area present in anterior to remaining natural teeth crossing the midline [32,33]. Kennedy classification was done according to order of the incidence of the type of partially edentulism present at that time. Hence, this

study was done to evaluate the prevalence of partial edentulism according to Kennedy's classification in the South Indian population.

Materials and Methods

A retrospective study was done in a private institution. Ethical clearance number was SDC/SIHEC/2020/DIASDATA/0619-0320. The clinical portion of this retrospective study was conducted over a 9 month period i.e from 1 June 2019 to 1 March 2020 and included patients who had undergone replacement of missing teeth with removable partial denture. A total of 1419 removable partial dentures were evaluated for the prevalence of partial edentulism according to Kennedy's classification

2.1.Inclusion Criteria : Age of 20–60 years irrespective of gender, race, community and socioeconomic status having partially edentulism in either maxilla, mandible or both the arches.

2.2.Exclusion Criteria: Patients having complete edentulism and missing third molars.

The data of 86,000 patients documented between June 2019 and March 2020 were reviewed and analysed. The data collected of partial edentulism of each patient was recorded and tabulated according to Kennedy's based on the groups - gender and age. To evaluate the prevalence of partial edentulism in the South Indian population according to Kennedy's classification based on age and gender. Statistical analysis was done using SPSS Statistics Software for windows, version 20.0. The data was analyzed using descriptive statistics and Chi square test and pearson correlation was done for checking correlation between age, gender and type of classification

Results and Discussion

Out of 1419 removable partial dentures, 32.3% were fabricated for patients in the age group of above 55 years. 58.4% of the removable partial dentures were fabricated for Males. Out of the total population 23.2% of the population had Kennedy Class III edentulous space, which had the highest prevalence (Graph 1).

Prevalence of Kennedy classification in partially edentulous patients according to gender showed that Class III had the highest prevalence in males (23.6%) and females (22.7%) (Table 1, Graph 2). Prevalence of Kennedy classification in partially edentulous patients according to age showed the highest prevalence of Class III in 36-45 years of patients (Table 2). Chi square test showed no significant difference in type of kennedy classification according to age and gender.

Partially edentulous cases can be easily classified for easy description and communication [34]. In our study, Kennedy classification was used because it is simple, easy for communication, picturaistic, logical, known worldwide, most common classification used. The present study was done to assess the prevalence of a type of Kennedy classification occurring in partially edentulous patients attending a private institution.

In our study the prevalence of partial edentulism was more in patients above 50 years of age. These results were similar to results obtained by Gad et al and Gupta et al [29,35,36]. There was no significant age difference in type of Kennedy classification for partially edentulous patients. But there are studies in literature which had correlation seen between age and type of Kennedy classification for partially edentulous patients. The frequency of males who underwent treatment for partial edentulism was more than that of females. Similar results were obtained by Owall et al [37]. Study done by Rana et al and Sapkota et al [36,38] showed more frequency in females compared to males. There was no significant gender difference in partially edentulous patients. Similar results were seen by Marcus et al [39]. Study done by Arandi et al showed dissimilar results [40].

Prevalence of Kennedy Class III (23.2%) was highest followed by Kennedy Class II (21.1%). The results for the highest prevalence of Kennedy Class III is similar in many studies done previously [21,40–42]. But a study done by Farahioon et al, showed that the most common prevalence of partial edentulism according to Kennedy's classification was Kennedy Class II. And Gad et al [35], showed that Class I had the highest prevalence. The second most common type varied from one study to another. Least common type of Kennedy class was class IV. This result was similar to study done by Arandi et al [40].

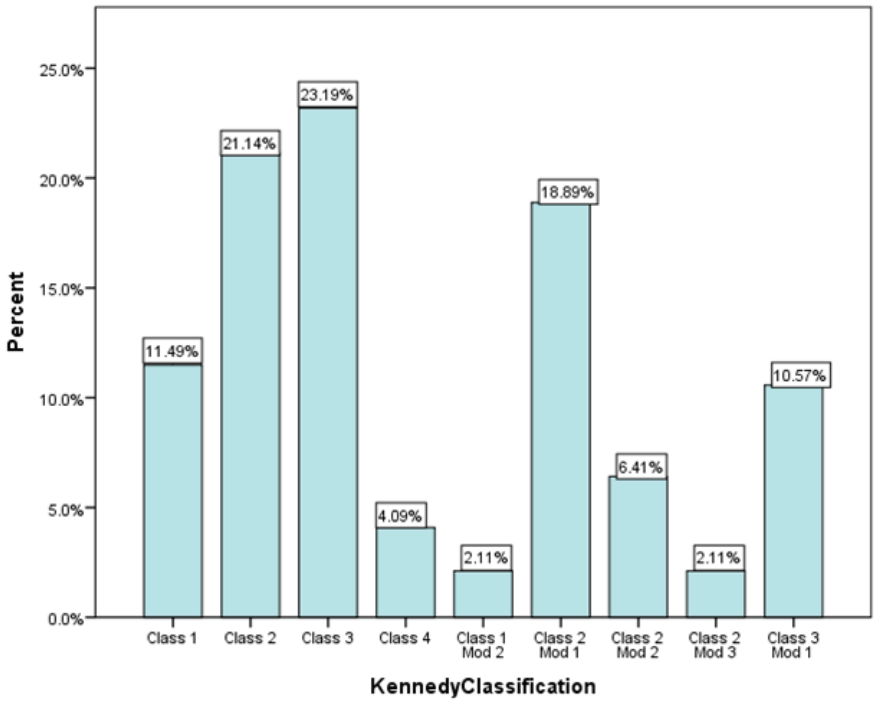
Limitation of our study is that it is done in an institutional setting, hence there are limited samples. Prevalence in maxilla and mandible was not considered in our study.

9.1 Table 1: Association between gender and Kennedy's classification. Chi-square test was done, p value was 0.064 showing that it is statistically not significant, proving that there is no association between gender and type of Kenndy's classification.

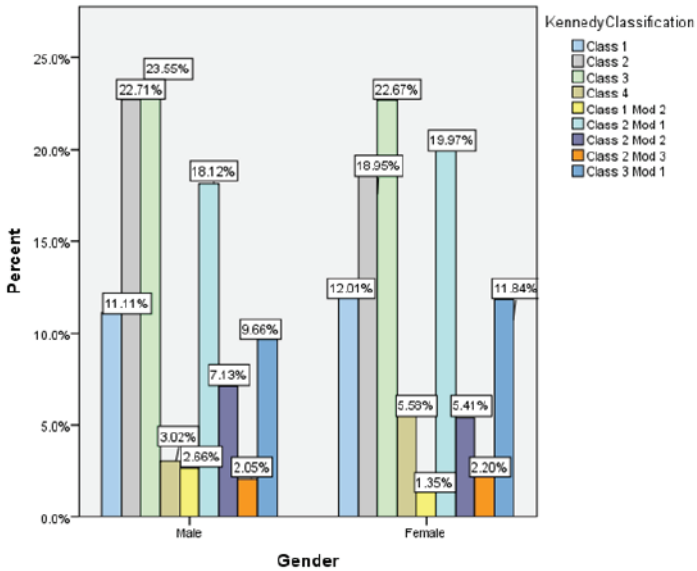
Kennedy's classification	Gender		Chi-square value	p value
	Male	Female		
Class 1	11.1%	12.0%	14.766	0.064
Class 2	22.7%	19.0%		
Class 3	23.6%	22.7%		
Class 4	3.0%	5.6%		
Class 1 Mod 2	2.7%	1.4%		
Class 2 Mod 1	18.1%	20.0%		
Class 2 Mod 2	7.1%	5.4%		
Class 2 Mod 3	2.1%	2.2%		
Class 3 Mod 1	9.7%	11.8%		

9.2 Table 2: Association between age and Kennedy's classification. Chi-square test was done, p value was 1.00 showing that it is statistically not significant, proving that there is no association between age and type of Kennedy's classification.

Kennedy's Classification	Age					Chi-square value	P value
	18-25 yrs	26-35 yrs	36-45 yrs	46-55 yrs	Above 55 yrs		
Class 1	11.1%	10.9%	10.8%	13.0%	10.7%	3.390	1.00
Class 2	22.2%	20.4%	21.2%	20.8%	21.6%		
Class 3	22.2%	23.1%	24.2%	22.5%	23.3%		
Class 4	3.2%	4.1%	4.0%	4.4%	3.9%		
Class 1 Mod 2	1.6%	2.0%	2.4%	2.0%	2.2%		
Class 2 Mod 1	22.2%	19.0%	18.5%	18.8%	18.7%		
Class 2 Mod 2	6.3%	6.8%	6.1%	6.4%	6.5%		
Class 2 Mod 3	1.6%	2.7%	2.0%	2.0%	2.2%		
Class 3 Mod 1	9.5%	10.9%	10.8%	10.2%	10.9%		



9.3. Figure 1: Bar graph showing percentage distribution of type of Kennedy's Classification. X-axis represents the type of Kennedy's Classification and Y-axis represents percentage distribution. The 23.91% distribution was for Kennedy Class III, which was highest.



9.4. Figure 2: Bar graph showing association between gender and Kennedy's classification. X-axis represents gender and Y-axis represents percentage distribution of type of Kennedy's classification according to gender. Sky blue colour represents Kennedy Class 1, Grey colour represents Kennedy Class 2, Pale green colour represents Kennedy Class 3, Golden yellow colour represents Kennedy Class 4, Yellow represents Kennedy Class 1 Modification 2, Electric blue colour represents Kennedy Class 2 Modification 1, Purple colour represents Kennedy Class 2 Modification 2, Orange colour represents Kennedy Class 2 Modification 3, Blue colour represents Kennedy Class 3 Modification 1. Chi-square test was done. Pearson's Chi-square value: 14.766, p value: 0.064 (>0.05), hence, statistically not significant, proving that there is no association between gender and Kennedy's classification.

Conclusion

Prevalence of Kennedy's Class III was the most common in the South Indian population. The prevalence was more in patients above 50 years of age. Male had more prevalence than females. Gender and age did not affect the prevalence of types of Kennedy's classes.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Vitality in Fixed Partial Denture Done by the Undergraduates - A Retrospective Study

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Abstract

Fixed partial denture is any dental prosthesis that is luted, screwed, or mechanically attached or otherwise securely retained to natural teeth, tooth roots, and/or dental implants/abutments that furnish the primary support for the dental prosthesis and restoring teeth in a partially edentulous arch; it cannot be removed by the patient. This study is aimed to evaluate prevalence of vitality of pulp before and after tooth preparation done by undergraduates for fixed partial denture. A retrospective study was done in which 955 teeth were prepared for fixed partial denture from 1 June 2019 till 1 March 2020 were included. Data was reviewed from the patients records of 86,000 patients documented in a private institution. Statistical analysis was performed to assess the vitality of pulp before and after tooth preparation done by undergraduates for fixed partial denture. There was a statistically significant difference when postoperative vitality was compared in different age groups ($p<0.05$). Out of 200 abutments that were non vital postoperatively only 48% of the abutment had indication for endodontic therapy. Lack of experience of dental students can be the reason for pulpal exposure during preparation. It might be difficult for students to get the parallelism of the abutments mainly in the multiunit fixed dental prosthesis. Treatment planning should be done carefully along with the students and a beforehand mock preparation should be done on plaster models instead of direct preparation of the vital abutments, this can also help students to perform different types of treatments adequately.

Keywords: *Vitality, Abutment, Fixed Partial Denture, Pulpal Exposure.*

Introduction

Fixed partial denture is any dental prosthesis that is luted, screwed, or mechanically attached or otherwise securely retained to natural teeth, tooth roots, and/or dental implants/abutments that furnish the primary support for the dental prosthesis and restoring teeth in a partially edentulous arch; it cannot be removed by the patient [1]. They are used mainly for replacing missing teeth or teeth for which fixed partial dentures have had insults from caries, trauma, any periodontal disease [2]. Dental pulp health can be affected by dental disease, trauma and restoration and further treatment of these problems can lead to various pulpal tissue degenerative problems [3]. Failures of Fixed Dental Prosthesis include biologic failures which include endodontic problems of the abutment, caries or any periodontal problems [3,4,5,6.] Mechanical failures of Fixed Dental Prosthesis include loss of retention, failure of porcelain, open margins of the framework, wear of ceramic, poor contours, poor margins, fracture of the abutment, etc. These failures can have a detrimental effect on the health of the pulp [7, 8].

There are various studies which have shown pulp necrosis after fabrication of Fixed Dental Prosthesis [9–11]. A study done by Bergenholtz & Nyman [9], had observation period of 8.7 years in which patients treated for advanced periodontal disease were reviewed and out it was found that 15% of the abutment teeth had pulpal necrosis, compared with only 3% in teeth which were not used as abutment.In a study done by Cheung *et al*, 4% of vital teeth developed pulpal necrosis after placement of single crowns [12, 13]. Other studies reported that 5.7% of teeth had received root canal treatment after 1–6 years of cementation of fixed dental prosthesis [11, 14] .

Fixed Dental Prosthesis preparation can have a detrimental effect on pulpal integrity, even though

preparation is kept as conservative as possible [15,16]. Detrimental effects are mainly due to the preparation process as it involves cutting of enamel and dentin, which leads to destruction of the odontoblastic process [17, 18]. This effect is further increased due to impression techniques used, which require drying the cut surface of the dentin [19, 20]. Bacteria are present in the form of saliva and carry almost continuously in the entire process from preparation till the final cementation and even after cementation if there are open margins present [7, 21, 22]. The literature shows that each step in the fabrication of a fixed prosthesis can potentially insult the pulp [11, 5]. This study was done for evaluation of prevalence of vitality of pulp before and after tooth preparation done by undergraduates for fixed partial denture.

Material and Methods

A retrospective study was done in a private institution. Ethical clearance number was SDC/SIHEC/2020/DIASDATA/0619-0320. The clinical portion of this retrospective study was conducted over a 9 month period i.e from 1 June 2019 to 1 March 2020 and included patients who had undergone Fixed Partial Dentures treatment. A total of 955 Fixed Partial Dentures were fabricated.

Inclusion Criteria : Patient undergoing FPD, age within 25 to 50 years, both male and female.

Exclusion Criteria: Single crowns, Implant placement patient, FPD done by post graduates.

The data of 86,000 patients documented between June 2019 and March 2020 were reviewed and analysed. The data collected was entered, tabulated and analysed for seeing the prevalence of vitality of pulp before and after tooth preparation done by undergraduates for fixed partial denture. Statistical analysis was done using SPSS Statistics Software for windows, version 20.0. Pearson correlation was done to determine that is there any statistical significant difference between vitality of pulp before and after tooth preparation done by undergraduates for fixed partial denture.

Results and Discussion

Out of 955 patients, 28% Fixed Dental Prosthesis were done in the age group of 26-35 years. 59.6% of the patients were Males. Out of the total population 79% abutments were vital at the end of the treatment.

Association between gender and preoperative condition of abutment showed no significant difference with Chi-square value of 3.076. There was no significant association between gender and postoperative vitality of abutment used for fixed partial denture (p value.0.05) (Table 1).

Similarly association between postoperative vitality with its preoperative condition according to gender had no significant association (Table 2). Association between age and preoperative condition of abutment had no significant relation ($p>0.05$). But association between age and post operative vitality of the abutment teeth was statistically significant ($p<0.05$) (Table 3, Figure 1). Association between postoperative vitality of abutment teeth with its preoperative condition according to age had statistically significant association (Table 4). Association between preoperative condition of abutment teeth and postoperative vitality of abutment showed no significant relation (Table 5, Figure 2).

This study aimed to evaluate the number of abutments going for endodontic therapy during Fixed Denture Prosthesis treatment done by undergraduates. There are many prospective studies that are done on endodontic treatment requirements for prosthetic treatments, but very few studies examine the vitality of the pulp preoperative and postoperative and pulpal exposure during tooth preparation [23, 24].

When the preoperative condition of teeth was not approaching pulp in the age group of 18-25 years many pulpal exposure were seen during preparation, this can be due to high level of pulp chamber in young adults. In a study done by Uzgur et al [25] showed that the incidence of vital abutment teeth that required endodontic therapy during the tooth preparation stage was about 0.7%. These results were similar to other studies done [0.6%] [26, 27].

Tooth preparation according to its principle is difficult as it involves making the wall of the abutment parallel to each other [28]. It is difficult when teeth are not aligned properly. Primary failures in Fixed partial denture fabricated by dental students occur mainly due to lack of experience [29]. Earlier studies were done by dentists rather than dental students.

According to the available literature 3-38% of the teeth that undergo fixed partial denture treatment have undergone pulp necrosis. However, the only clinical study that has checked the loss of vitality of the abutment during the time of preparation was done by Napankangas et al [30] and one retrospective study done by Raustia et al [29].

In our study, there was a significant difference seen when the postoperative vitality was compared according to age. In young patients the pulp chamber is higher than older patients, hence chances of exposure are more and therefore tooth preparation should be kept minimal mainly in the anterior region [31, 32]. As if the pulp is exposed it needs to undergo endodontic treatment which is assumed to weaken the abutment teeth. In a previous study it has been noted that on long term follow up, if the abutment is endodontically treated before or during tooth preparation it has to undergo extraction [33]. Hence, maintaining the vitality of pulp is an important factor that should be considered during tooth preparation. There are many clinical variables that can affect the exposure of abutment, but it is difficult to develop a causal relationship, hence its difficult to determine the factors that cause pulpal exposure during tooth preparation and factors that can prevent exposure [29]. Lack of experience of dental students can be the reason for pulpal exposure during preparation. It might be difficult for students to get the parallelism of the abutments mainly in the multiunit fixed dental prosthesis. Treatment planning should be done carefully along with the students and a beforehand mock preparation should be done on plaster models instead of direct preparation of the vital abutments, this can also help students to perform different types of treatments adequately.

Limitation of our study is that it is done in an institutional setting, hence there are limited samples. As it is an institutional study there can be operator bias, protocol bias seen. The clinical scenarios during fixed partial denture tooth preparation in our study were not the same as there were different operators and different clinical conditions.

Table 1: Association between gender and preoperative condition of teeth, gender and postoperative vitality of teeth. Chi-square test was done, p value was 0.063 showing that it is statistically not significant, proving that there is no association between gender and preoperative condition, gender and postoperative vitality of teeth.

		Gender		Chi-square value	P value
		MALE	FEMALE		
Preoperative Condition of teeth	Approaching Pulp	49.4%	44.1%	3.076	0.215
	Not Approaching Pulp	50.6%	55.9%		
Postoperative Vitality	Vital	74.9%	67.1%	5.534	0.063
	Non Vital	25.1%	32.9%		

Table 2: Association between preoperative condition of teeth and postoperative vitality of teeth after fixed partial denture was done according to gender. Chi-square test was done, p value was 0.277 and 0.08 showing that it is statistically not significant, proving that there is no association between preoperative condition of teeth and postoperative vitality of teeth after fixed partial denture was done according to gender.

			Gender		Chi-square value	P value
			MALE	FEMALE		
Preoperative Condition of teeth	Approaching Pulp	Vital	74.2%	66.7%	2.566	0.277
		Non Vital	25.8%	33.3%		
	Not Approaching Pulp	Vital	75.7%	67.5%	3.067	0.080
		Non Vital	24.3%	32.5%		

Table 3: Association between age and preoperative condition of teeth, age and postoperative vitality of teeth. Chi-square test was done, p value was 0.000 showing that it is statistically significant, proving that the 26-35 year age group had more vital teeth after preparation was done for fixed partial denture. Association between preoperative condition of teeth with age had p value of 0.47 showing that it is statistically not significant.

		Age (%)					Chi-square value	P value
		18-25	26-35	36-45	46-55	Above 55		
Pre operative Condition of teeth	Approaching Pulp	45.7%	51.3%	48.2%	40.4%	47.6%	3.548	0.47
	Not Approaching Pulp	54.3%	48.7%	51.8%	59.6%	52.4%		
Postoperative Vitality	Vital	59.1%	82.4%	70.7%	77.1%	61.9%	26.94	0.00*
	Non Vital	40.9%	17.6%	29.3%	22.9%	38.1%		

*The Chi-square is statistically significant at the 0.05 level.

Table 4: Association between preoperative condition of teeth and postoperative vitality of teeth after fixed partial denture was done according to age. Chi-square test was done, p value was 0.036 showing that it is statistically significant, proving that in the age group of 46-55 years though the preoperative condition of teeth was approaching pulp, the postoperative vitality was maintained after preparation for fixed partial denture. Association between teeth with ideal tooth structure and postoperative vitality had p value of 0.001 showing that the association is statistically significant, proving that in the age group of 18-25 years there were more pulpal exposure seen during tooth preparation.

			Age					Chi-square value	P value
			18-25	26-35	36-45	46-55	Above 55		
Preoperative Condition	Approaching Pulp	Vital	62.1%	79.4%	67.4%	81.8%	62.5%	10.30	0.036*
		Non Vital	37.9%	20.6%	32.6%	18.2%	37.5%		
	Not Approaching Pulp	Vital	56.5%	85.6%	73.7%	73.8%	61.4%	19.86	0.001*
		Non Vital	43.5%	14.4%	26.3%	26.2%	38.6%		

*The Chi-square is statistically significant at the 0.05 level.

Table 5: Association between preoperative condition of teeth and postoperative vitality of teeth used as abutment for fixed partial denture. Chi-square test was done, p value was 0.821 showing that it is statistically not significant, proving that there is no association between preoperative condition of teeth and postoperative vitality of teeth used as abutment for fixed partial denture.

		Preoperative Condition of teeth		Chi-square value	P value
		Approaching Pulp	Not Approaching Pulp		
Postoperative Vitality	Vital	71.4%	72.2%	0.051	0.821
	Non Vital	28.6%	27.8%		

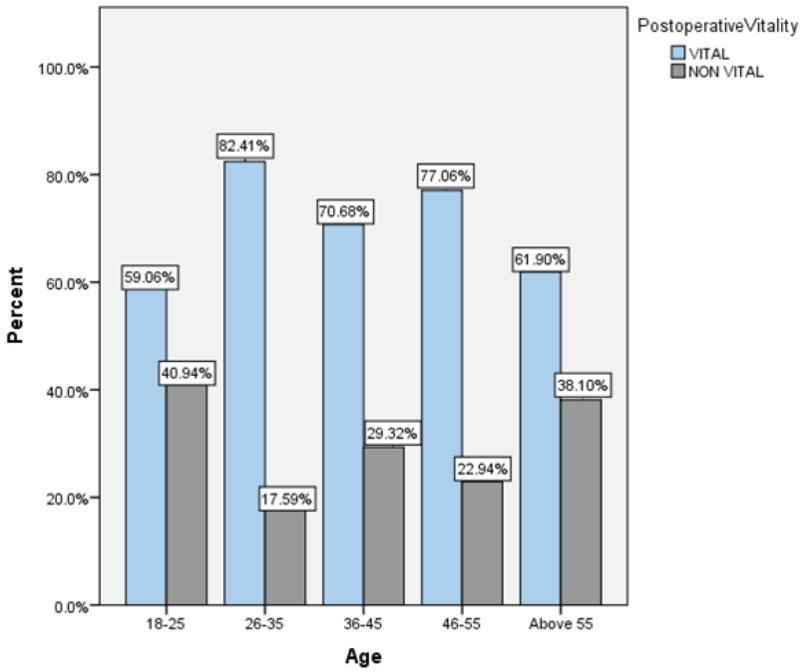


Figure 1: Bar graph showing association between age and postoperative vitality of teeth. X-axis represents different age groups involved in this study and Y-axis represents percentage distribution of postoperative vitality of teeth. Blue colour represents vital teeth postoperatively and Grey colour represents non vital teeth postoperatively. Chi-square test was done and association was found to be statistically significant. Pearson's Chi-square value: 26.946, p value: 0.000 (<0.05) hence statistically significant, proving that postoperative vitality varies in different age groups with highest frequency in the age group of 26-35 years.

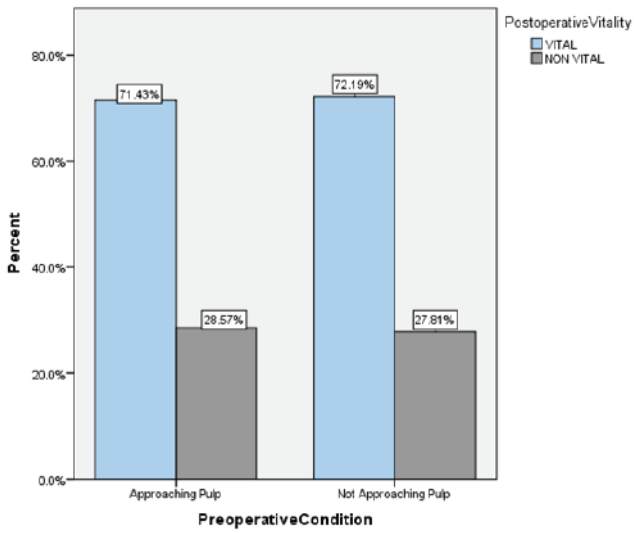


Figure 2: Bar graph showing association between preoperative condition of teeth and postoperative vitality of teeth for abutments used for fixed partial denture. X-axis represents the preoperative condition of teeth and Y-axis represents the percentage of postoperative vitality of teeth. Blue colour represents vital teeth postoperatively and grey colour represents non vital teeth postoperatively. Chi-square test was done. 72.19% of teeth which were not approaching pulp were vital after fixed partial denture treatment was done. Pearson's Chi-square value: 0.51, p value: 0.821 (>0.05) hence is statistically not significant, proving that there is no association between preoperative condition of teeth and postoperative vitality of teeth for abutments used for fixed partial denture.

Conclusion

Vitality of the abutment is the most important factor deciding the longevity of the prosthesis. Various factors have influence of abutment vitality before fixed dental prosthesis cementation is done. Tooth preparation should be done minimally and vitality of the abutment should be preserved. The study concludes that most common failures of fixed metal ceramic bridges made by dental students occur during preparation of abutment teeth which can be avoided by proper treatment planning and before preparation should be done on a gypsum model.

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Dentists' Preference of Pulp Capping Agent for Indirect Pulp Capping in Primary and Permanent Molars - An Observational Study

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Abstract

One of the major challenges associated with indirect pulp capping is to prevent the exposure of the pulp and maintain its vitality. It is based on the rationale that pulpal mesenchymal cells induce dentin bridge formation. The common pulp capping agents used are calcium hydroxide and mineral trioxide aggregate both of which act based on their high pH. The aim of the study is to infer the most preferred pulp capping agent by dentists in a private dental college so as to achieve a good prognosis after indirect pulp capping of primary and permanent molars.A total of 297 restorative procedures were obtained after reviewing patient case sheets and were analysed using descriptive statistics on SPSS Software. Among 139 treatment procedures, the most common pulp capping agent used was calcium hydroxide (69.1%) in primary molars and mandibular permanent molars and MTA (30.9%) mostly in maxillary permanent molars. Within the limits of the study, calcium hydroxide was the preferred pulp capping agent for indirect pulp capping by dentists in a private dental college in both primary and permanent molars. However, with its clinical advantages, mineral trioxide aggregate shows a promising candidate as an indirect pulp capping agent in primary and permanent teeth.

Keywords: *Calcium hydroxide; mineral trioxide aggregate; indirect pulp capping; pulp capping agents*

Introduction

Dental caries is a pathologic process depending on several etiological factors which causes destruction of dental tissues and produces local and general complications [1]. It is one of the most widespread diseases in the civilized population with a prevalence of 40% at 7 years and 85% in seventeen year old boys [2]. Dental caries in primary dentition is greatly influenced by parental attitude towards restoration in deciduous teeth [3]. However, recent evidence has suggested a decrease in incidence of dental caries in children aged 5-17 years of about 36% and almost 50% of children were considered caries free in permanent dentition [4] due to inclusion of various prophylactic measures in their routine activities [5,6,7]. The inception of dental caries can be altered by components present in the saliva. It has come to light that such salivary molecules act as biomarkers in the detection of caries [8]. The consequence of pulp and near pulp exposure from caries due to intrinsic [9] or extrinsic factors[10] or trauma,[11] can be severe, resulting in pain and infection. The consequences of the treatment involve restorations, endodontics therapy, extraction which often involves considerable expense and multiple appointments [12–18].

An alternative procedure would be indirect pulp capping (IPC) where a medicament is placed over residual caries in an attempt to prevent an exposure

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and maintain pulp vitality and avoid the extensive treatment dictated by other options [19]. The pulp response is influenced by the thickness of the remaining dentin [20]. A remaining dentin thickness (RDT) of 2mm or 1.5 mm must be present to protect the pulp. However when caries extend deeper into dentin, this thickness can be distinctly compromised owing to pulpal inflammation. Some investigators found a minimum thickness of 0.5 mm to 1 mm to be sufficient for pulpal protection [21]. The success of pulp capping depends on the vitality of pulp. Teeth with a history of non-spontaneous pain and without any radiographic pathology can be ideal candidates. Unfortunately, the true state of pulp health cannot be determined by clinical signs, symptoms or radiologic appearance. The true "gold standard" of pulp status is histological analysis. Studies have shown a chronically inflamed state of pulp without any symptoms from the patient [22,23]. However, the ultimate rationale of pulp capping is to stimulate pulpal cells to form reparative dentin and arrest the progression of dental caries [24].

The ideal requirements of a pulp capping agent as suggested by Cohen are: the pulp capping agent must adhere to dentin, provide bacterial seal, resist forces, maintain vitality and must be radiopaque. However, the type of agent is less important to success than the placement of well- sealed restoration.

The gold standard of pulp capping agent is calcium hydroxide introduced in 1921. Its main advantages include excellent antibacterial activities [25] and its high pH of 12 is known to irritate the pulpal tissue and stimulate repair via an unknown mechanism [26]. Mineral Trioxide Aggregate (MTA) has generated great interest as a pulp capping agent in recent years. It's similar to calcium hydroxide in regards to antibacterial activity and biocompatibility [27]. Additionally, it provides some seal to the tooth structure [28].

The aim of the study is to infer the most preferred pulp capping agent by dentists in a private dental college so as to achieve a good prognosis after indirect pulp capping of primary and permanent molars.

Methodology

This observational study was conducted in the Department of Pediatric and Preventive Dentistry in a Private Dental College in Chennai during the period of June 2019 to March 2020. The ethical clearance for the study was obtained from the Institutional Ethical Committee.

Children of the age 3-17 years with signs and symptoms of reversible pulpitis were included in the study. The wide age range was included to include primary as well as permanent molars. Children with pre-existing medical conditions and those with special needs were excluded from the study. The two pulp capping agents included in this study were calcium hydroxide and mineral trioxide aggregate.

The pulp capping procedures were carried out by postgraduate students belonging to the Pediatric department. After appropriate diagnosis of deep carious lesions with no spontaneous pain and radiographic assessment, the caries removal was done using No.69 carbide bur under rubber dam isolation. Complete or partial carious (dark brown and hard layer) removal was carried out. After adequate drying, calcium hydroxide (Dentsply Dycal) base and catalyst pastes are taken in equal quantities, manipulated in a paper pad and placed onto the floor of the cavity. Sufficient care was taken to impart a fluid free environment so as to prevent the dissolution of the cement. This is followed by a bilayered restoration using GIC and composite resin. Similarly, mineral trioxide aggregate (MTA Angelus) was used as a pulp capping agent. A powder liquid ratio of 3:1 of MTA was manipulated on a glass slab and placed into the cavity using a carrier. This is succeeded by placement of a bilayered restoration.

The concerned data associated with these treatment procedures was obtained after reviewing 86,000 case sheets, of which those pertaining to children aged 3-17 years were analysed and the data was extracted for the purpose of this study. The data verification, collection and analysis were carried out by two persons. Cross-verification of the included data was done through photographic verification. In order to avoid sampling bias, only those data which were accustomed to the eligibility criteria were included. The internal validity was high as it was a representative sample and the external validity was high as the study results may be generalized to the Chennai population.

The extracted data was tabulated into Excel Sheet and was imported into SPSS Software by IBM for

data analysis. A total of 139 treatment procedures were included for data analysis. Any incomplete data was included. The obtained data was analyzed using descriptive statistics on SPSS software and the results were represented graphically

Results and Discussion

A total of 139 treatment procedures were analyzed using Descriptive Statistics on SPSS Software Version 23.0 based on the most preferred pulp capping agent. The study participants included 75 males (54%) and 64 females (46%). Out of 139 molars pulp capped, 43.9% were permanent molars and 56.1% were primary molars. [Figure 1] Maxillary permanent molars were 15.1%, mandibular permanent molars were 28.8%, maxillary primary molars were 12.9% and mandibular primary molars were 43.2%. [Figure 2] The most preferred choice of pulp capping agent was calcium hydroxide (69.1%) [Figure 3] irrespective of gender [Figure 4]. It was preferred in primary molars and mandibular permanent molars. [Figure 5,6] There was an inclination towards MTA (30.9%) mostly in maxillary permanent molars. [Figure 6]

Indirect pulp capping is a procedure by which some amount of caries (affected dentin) is allowed to remain adjacent to a vital pulp rather than risk pulp exposure and is covered by a cavity liner/sealer and is restored. Partial caries removal significantly reduces the chance of pulp exposure during caries excavation [19]. Several studies show restored teeth with partial caries removal have equal success compared to restored teeth with complete caries removal [29,30]. The materials used protect the pulp from noxious agents (heat, cold, bacteria) and stimulate the cell-rich zone of the pulp to lay down a bridge of reparative dentin. Dentin formation usually starts within 30 days of the pulp capping (there can be a delay in onset of dentin formation if the odontoblasts of the pulp are injured during cavity removal) and is largely completed by 130 days [31]. Studies have evaluated the fate by re-assessment of tooth in which indirect pulp capping was done. The findings include lesion colour change from light brown to dark brown, soft to dry and hard consistency, S.*mutans* and *Lactobacilli* have been significantly reduced to a limited number or even zero viable organisms, and the radiographs show either no change or even a decrease in the radiolucent zone [32,33].

Calcium hydroxide was introduced by Hermann and is considered as the gold standard of pulp capping agents. The mechanism by which calcium hydroxide (also the hydration product of MTA) may be due to its high pH which causes pulpal irritation and stimulates repair by the release of bioactive molecules. It is known that a variety of proteins are incorporated into dentin during dentinogenesis.The main proteins are Bone Morphogenic Protein (BMP) and Transforming growth factor (TGF-B1) which when stimulated by Calcium hydroxide has the ability to repair pulp and form tertiary dentin [26,34].

In our study, primary molars were likely in requirement of IPC. This may be due to the structural morphology of the enamel in primary teeth. The consequence of short time for enamel development results in formation of a thin enamel with a less organized and mineralised structure [35,36]. As a consequence, acids are able to demineralize deciduous enamel in a rampant manner [37]. Due to awareness, practise of caries preventive strategies, early detection and sealant placement, the progression of caries is reduced in the permanent dentition [4].

In this study, the indirect pulp capping procedures were more likely to be done in the mandibular arch. The studies concurrent with this fact was given by Sathe et al [38] and Tiwari and Chawla [39]. Honkala et al supplement that increased caries in primary dentition of mandibular arch may manifest as the same in the complementary set [40].

According to our study results, calcium hydroxide was highly preferred as a pulp capping agent. (69.1%). Calcium hydroxide is known for its excellent antibacterial properties [25]. One study found 100% reduction in micro-organisms within 1 hour of contact with calcium hydroxide [41]. However, the self-cure formulations are highly soluble and dissolute over time [42]. But it has been noted that a dentin bridge is formed by the time calcium hydroxide is dissolved [43]. It's been noted that calcium hydroxide can cause tunnel defects in reparative dentin allowing patency to the pulp chamber sometimes with fibroblasts and capillaries present within the defect [44]. However this finding seems to be controversial due to varying evidence.

Mineral trioxide aggregate is a new material inspired from Portland cement. It primarily consists of calcium oxide and forms calcium hydroxide on hydration [45]. Hence, the advantages of MTA are similar to calcium hydroxide, including its antibacterial and biocompatible properties, high pH, radio opacity and ability to release bioactive proteins [27,46]. A significant downside to MTA is the prolonged setting time of approximately 2 hours and 45 minutes [47]. The cost and handling characteristics are the added disadvantages.

The superior performance of MTA when compared with calcium hydroxide is elicited in two studies in which zinc oxide eugenol (ZOE) was used as the temporary restoration. Owing to the high interfacial leakage associated with ZOE,[48] MTA still showed superiority which can be attributed to its sealing properties [49,50]. Even though the success of indirect pulp capping is greatly due to a well-sealed restoration, the grander enhancement provided by MTA can be utilized for a better prognosis of a pulp capped tooth.

The limitations of the study include its small sample size , restricted geographic location and the usage of commonly marketed conventional, cost effective and readily available materials for pulp capping. A questionnaire based study which focuses on various geographic areas directed towards general practitioners, endodontists and pedodontists may provide better and generalized results for an ideal pulp capping agent.

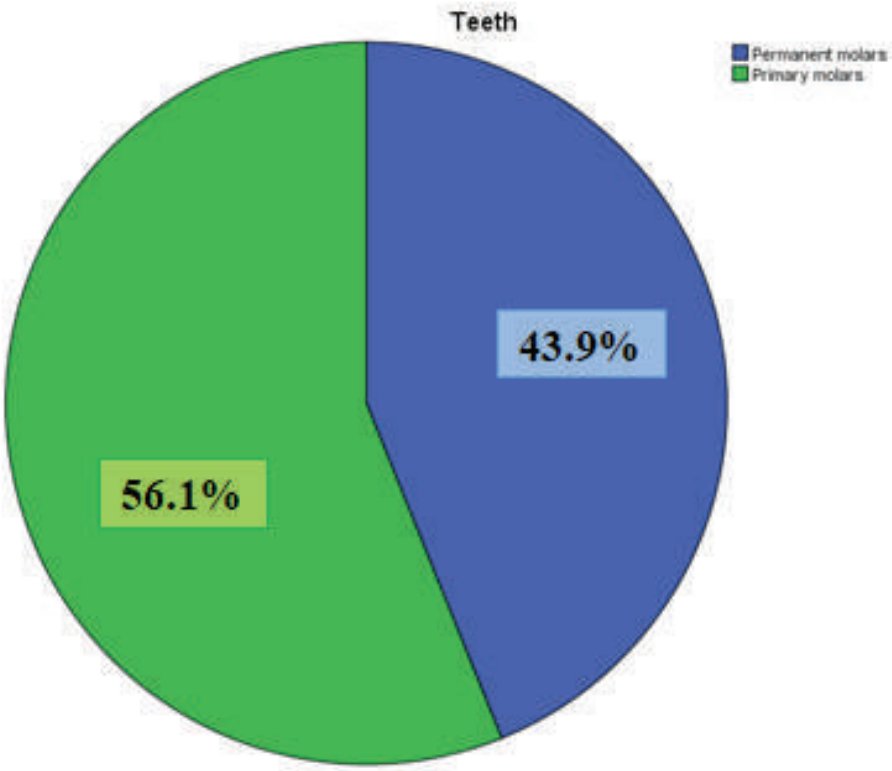


Figure 1. The pie chart represents the distribution of primary and permanent molars treated by pulp capping procedure . 43.9% were permanent molars and 56.1% were primary molars

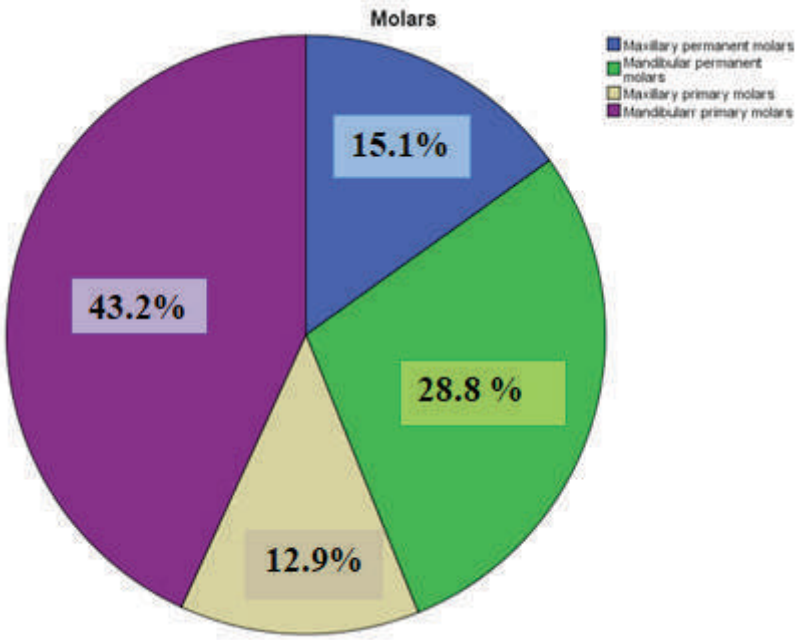


Figure 2. The pie chart represents the distribution of maxillary and mandibular primary and permanent molar teeth treated by pulp capping procedures. 15.1% were maxillary permanent molars , 28.8% were mandibular permanent molars, 12.9% were maxillary primary molars and 43.2% were mandibular primary molars.

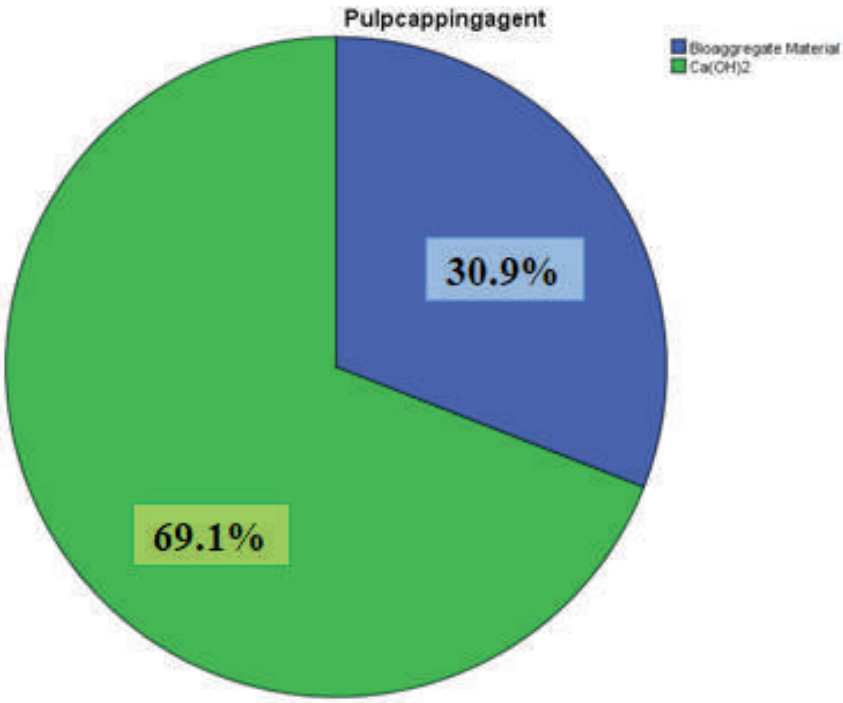


Figure 3. The pie chart represents the distribution of pulp capping procedures done according to pulp capping agents used. 30.9% used Bio aggregate material and 69.1% used Calcium hydroxide.

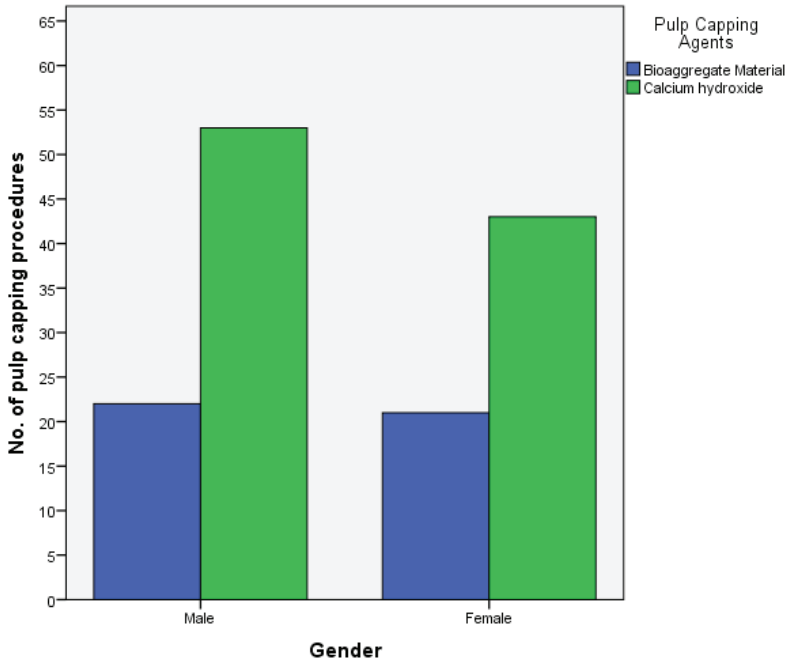


Figure 4. The graph represents the association between gender and pulp capping agents used in primary and permanent molars. X axis represents gender. Y axis represents the total number of pulp capping procedures done using calcium hydroxide denoted by green and bio aggregate denoted by blue on a scale of 0-60. Chi square test was performed. □ value = 0.65. The association was not statistically significant showing that calcium hydroxide and bioaggregate material were used irrespective of gender.

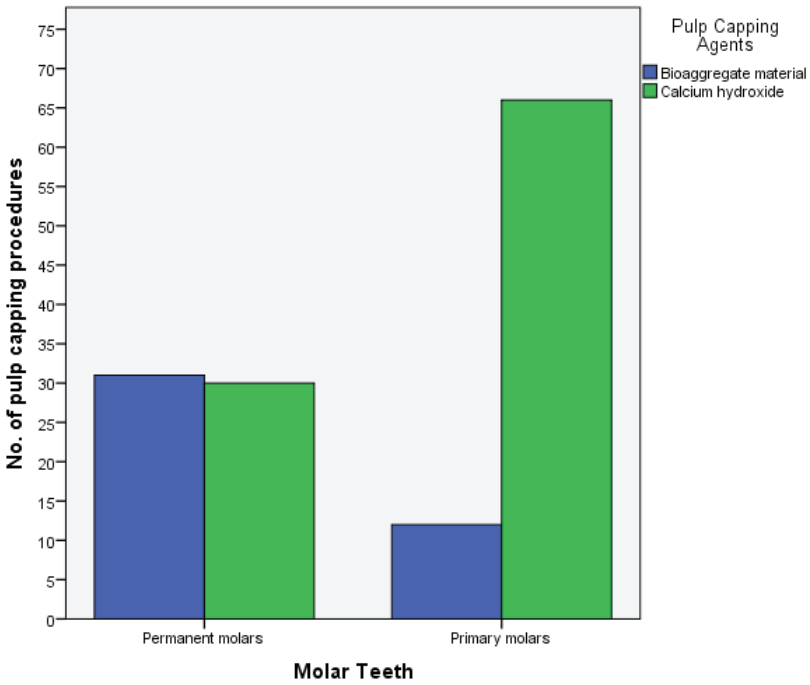


Figure 5. The graph represents the association between primary and permanent molars and pulp capping agents used to treat them . X axis represents primary and permanent molars. Y axis represents the total number of pulp capping procedures done using calcium hydroxide denoted by green and bio aggregate denoted by blue on a scale of 0-60. Chi square test was performed. □ = 0.03. The association was found to be statistically significant showing that calcium hydroxide is the pulp capping agent of choice in primary molars compared to permanent molars.

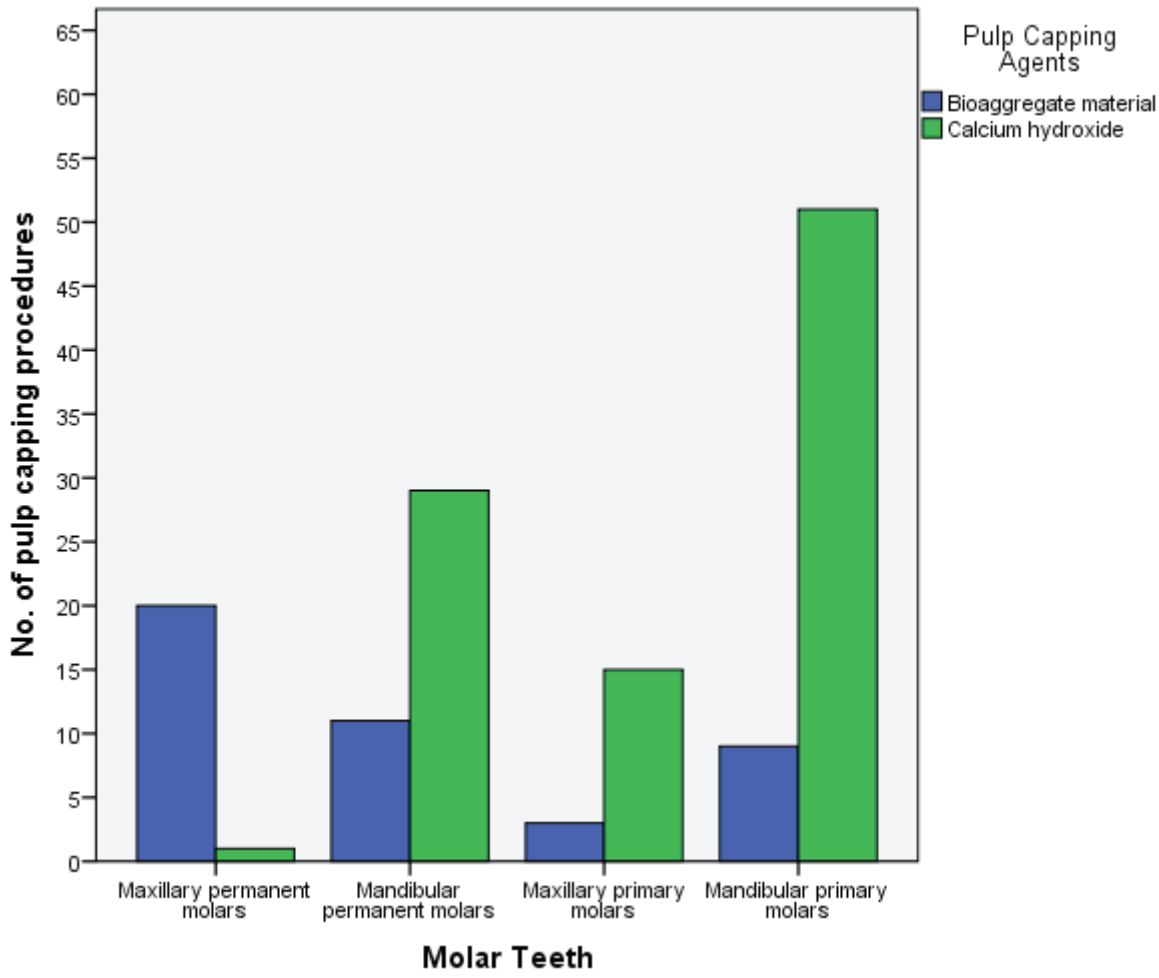


Figure 6. The graph represents the association between the maxillary and mandibular primary and permanent molar teeth and pulp capping agents used to treat them. X axis represents the maxillary and mandibular primary and permanent molars. Y axis represents the total number of pulp capping procedures done using calcium hydroxide denoted by green and bioaggregate denoted by blue on a scale of 0-60. Chi square test was performed. □ = 0.05. The association was found to be statistically significant showing that calcium hydroxide is the pulp capping agent of choice in mandibular primary and permanent molar pulp capping compared to maxillary molars.

Conclusion

Within the limits of the study, calcium hydroxide was the preferred pulp capping agent for indirect pulp capping by dentists in a private dental college in both primary and permanent molars. However, with its clinical advantages, mineral trioxide aggregate shows a promising candidate as an indirect pulp capping agent in primary and permanent teeth.

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Awareness about Common Disinfection Procedures among Undergraduate Students in Clinics

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Abstract

Dentists, dental patients and dental technicians are tasked with various dental procedures which are extremely prone to cross contamination of various infections from various pathogens usually found in blood and dental offices. A lot of attention has been directed towards proper medical care, sterilization and instrument care.

Online survey containing the questionnaire was sent to 100 undergraduate dental students. Results were tabulated and analysed. The questionnaire was based on the knowledge they gained during their student days. Among them 53% are aware of general principles of disinfection , 85% level of disinfection determines quality of clinical practice. Within the limits of the study , the awareness about common disinfection procedures among undergraduate dental students in clinics was evaluated . Upon evaluation it was found that the student dentist had a moderate level of knowledge about disinfection in clinics, which can be improved by extensive teaching about disinfection practices and improving knowledge.

Keywords: *Cross Contamination, Disinfection, Formaldehyde, Glueraldehyde*

Introduction

Disinfection is a process, which eliminates all pathogenic microorganisms on a surface. There are various types of Disinfection- namely Chemical, Radiation, Gaseous etc. Chemical disinfection is the most commonly practiced, owing to the cost effectiveness of the technique and ease of handling. Some of the more commonly used Disinfectants include Hydrogen Peroxide, Glutaraldehyde, Formaldehyde and ethanol .

Hydrogen Peroxide is an organic compound. Hydrogen peroxide is inflammable. At low temperatures it becomes solid. The amount of hydrogen peroxide in the solution is expressed in weight percentage. 35-50 % concentration is used for water treatment. It is an unstable compound in the presence of a base or a catalyst, and is typically stored with a stabiliser in a weak acidic solution. It kills pathogenic microorganisms by the action of oxidation, where it releases electrons or free oxygen, which oxidises the bacterial cell wall. Louis Jacques Thenard produced Hydrogen peroxide for the first time in 1818 by. Subsequently, the synthesis of hydrogen peroxide was improved with hydrochloric acid and sulfuric acid. This process was used from the end of the nineteenth century until the middle of the twentieth century. Pure hydrogen peroxide was first obtained through the process of vacuum distillation in 1894. The correct molecular formula for hydrogen peroxide was first described in the late nineteenth century.

Glutaraldehyde is an organic compound. It is the only aldehyde exhibiting excellent sporicidal activity. It is mildly acidic, in need of buffering. Vegetative bacteria are readily susceptible to the action of Glutaraldehyde. It is reported to be ten times more effective than formaldehyde, and less toxic. It can cause skin sensitisation when mishandled. Glutaraldehyde is commonly diluted during usage. There is a decline in its effectiveness in instruments over time. The decline is due to improper protocol and not drying the instruments properly. This raises the emphasis and the need to

ensure that semicritical equipment is disinfected with an acceptable concentration of glutaraldehyde. Studies suggest that 1.0%–1.5% glutaraldehyde is the minimum effective concentration and >2% glutaraldehyde solutions are when used as a high-level disinfectant.

Formaldehyde has a broad spectrum biocidal activity and is capable of both surface and space decontamination. Its biocidal action is through alkylation of carboxyl, hydroxyl and sulfhydryl groups on proteins and the ring nitrogen atoms of purine bases. It has various safety concerns, including its carcinogenic property. Even though Formaldehyde is a very strong disinfectant, the clinical use of formaldehyde is limited by its irritating fumes and its pungent odor even at very low levels (<1 ppm). For these reasons and others—such as its role as a suspected human carcinogen linked to nasal cancer and lung cancer. When it is used, direct exposure to employees generally is limited; however, excessive exposures to formaldehyde have been documented for employees of renal transplant units and students in a gross anatomy laboratory. Paraformaldehyde, a solid polymer of formaldehyde, can be vaporized by heat for the gaseous decontamination of laminar flow biological safety cabinets when maintenance work or filter changes require access to the sealed portion of the cabinet.

Ethanol is an alcoholic compound, containing two carbon atoms. Alcohols work through the disruption of cellular membranes, solubilization of lipids, and denaturation of proteins by acting directly on S-H functional groups. Ethyl and isopropyl alcohols are the two most widely used alcohols for their biocidal activity. These alcohols are effective against lipid-containing viruses and a broad spectrum of bacterial species, but ineffective against spore-forming bacteria. They evaporate rapidly, which makes extended contact times difficult to achieve unless the items are immersed. The optimum bactericidal concentration for ethanol and isopropanol is in the range of 60% to 90% by volume. Their cidal activity drops sharply when diluted below 50% concentration. Absolute alcohol is also not very effective. These alcohols are rapidly bactericidal rather than bacteriostatic against vegetative forms of bacteria; they also are tuberculocidal, fungicidal, and virucidal but do not destroy bacterial spores. Their cidal activity drops sharply when diluted below 50% concentration, and the optimum bactericidal concentration is 60%–90% solutions in water (volume/volume)

According to the Indian Dental Association, the following items should be sterilized or disinfected in a dental clinic:

- Instruments that penetrate oral soft tissue-Critical items like surgical instruments, periodontal knives and scaling instruments
- Instruments that are not intended to penetrate oral soft tissues or bone -Semi critical items like amalgam condensers, dental handpieces, mouth mirrors.
- Non-critical patient-care items that come in contact with unbroken skin (e.g. radiograph head/cone, blood pressure cuff, facebow, pulse oximeter)
- plastic impression trays, amalgam carriers, plastic instruments etc.

Dentists, dental patients and dental technicians are tasked with various dental procedures which are extremely prone to cross contamination of various infections from various pathogens usually found in blood and dental offices [1,2]. A lot of attention has been directed towards proper medical care, sterilization and instruments [3,4]. Receiving the right education through the right approach is the major way to enhance and develop professional skills [5,6]. The use of many dental equipment especially instruments such as cheek retractors, impression trays when such instruments are improperly disinfected can cause formation and contamination of various microorganisms and lead to spread of various diseases [7,8,9]. These instruments which are often used in the patient's mouth are immersed in disinfectant solutions [8,10,11]. Disinfection and sterilization techniques can also be used to free the instruments from various microorganisms [12,13] .Hepatitis virus poses a very large and common risk factor for dental practitioners. The commonly used disinfectants worldwide are formaldehyde and glutaraldehyde [14,15]. Disinfectants are substances mainly used in chemical form to destroy the microorganism and kill it [3,16,17]. The present research is done in order to create awareness among dental practitioners in undergraduate dental college among students about the benefits and necessity of sterilization and disinfection in clinics .

Materials and Methods

A cross sectional study based on a self administered online questionnaire was distributed through a link shared to about 100 dental students. 100 students participated and the results were tabulated in excel sheet and was analysed using SPSS windows version 2.0. The questionnaire was mainly divided into two parts , the first part contained general biological data whereas the second part of the questionnaire was about the main topic that is disinfection and its definition the various disinfectants used and the various different techniques practiced.

Results and Discussion

From the survey conducted the results obtained are as follows, about 53% of the students are aware about the general principles of disinfection practiced in dentistry .On the question based on awareness about various techniques of disinfection practiced , 52% are aware about the various techniques . The responses on the knowledge about commonly used disinfectants were 35% for formaldehyde 30% for glutaraldehyde , 24% for ethanol and 16% for hydrogen peroxide. When asked about the standards in the frequency of disinfection 51% people answered they disinfect their clinics daily , 20% said twice a week and 29% said once a week.

When asked about the better option for hand instrument 80% answered saying sterilization is the better technique. 50% of the students know the reason behind frequent disinfection practices is to reduce cross contamination.

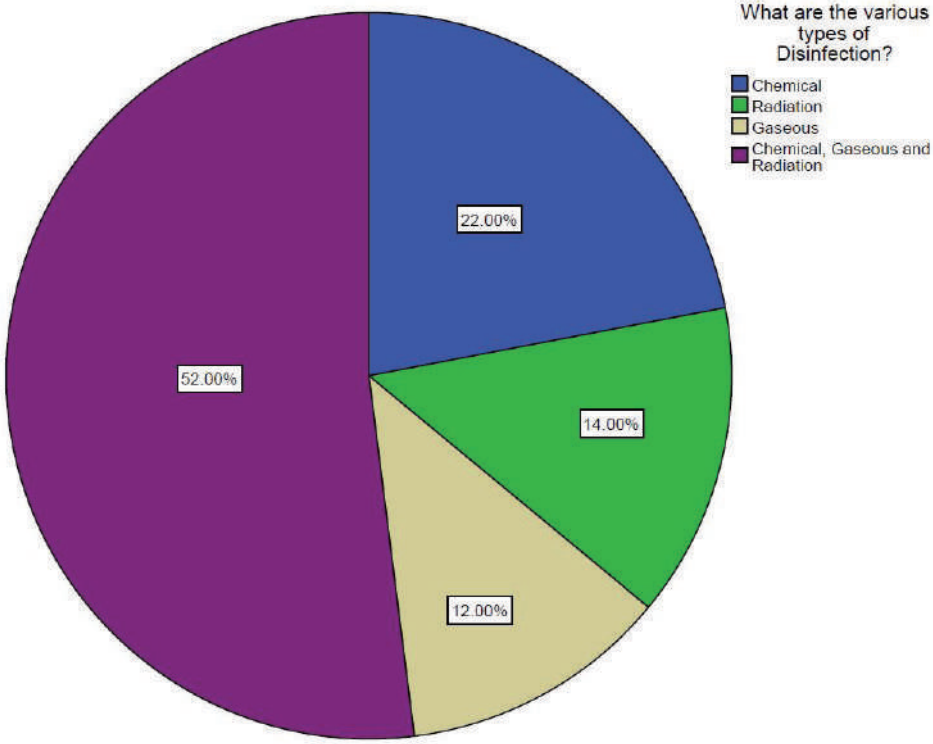


Fig. 1: Pie chart depicting the responses to the question, "What are the various types of disinfection?". In which 52% show all three chemical, radiation and gaseous types of disinfection. 12% answered gaseous only, 14% answered radiation only and 22% answered chemical only .

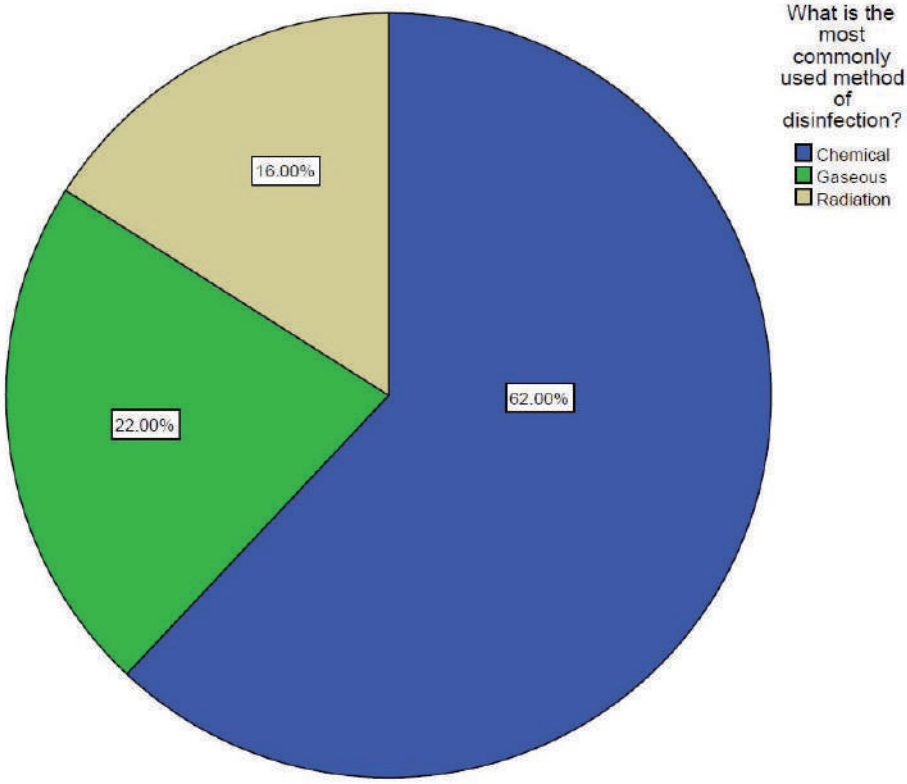


Fig. 2: Pie chart depicting the responses to the question "What is the most commonly used method of disinfection in normal practice?". About 62% students chose the chemical method to be the most common method of disinfection, followed by 22% students who chose gaseous and remaining 16% for Radiation .

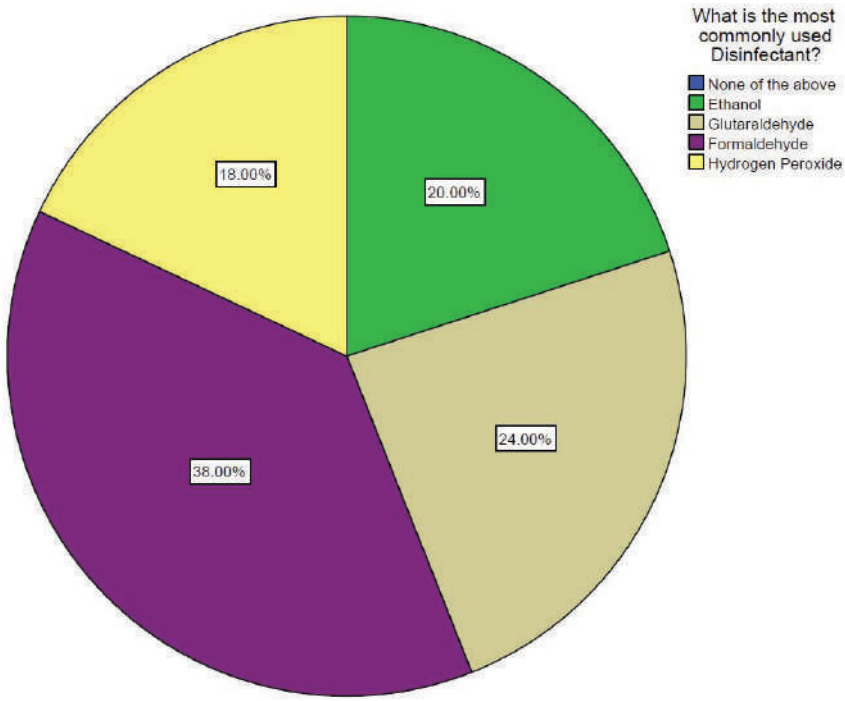


Fig. 3: Pie chart depicting the response to "Which is the most commonly used disinfectant?"

38% answered formaldehyde as the most used chemical disinfectant, followed by 24% glutaraldehyde, 20% answered as ethanol and 18% responded with Hydrogen peroxide.

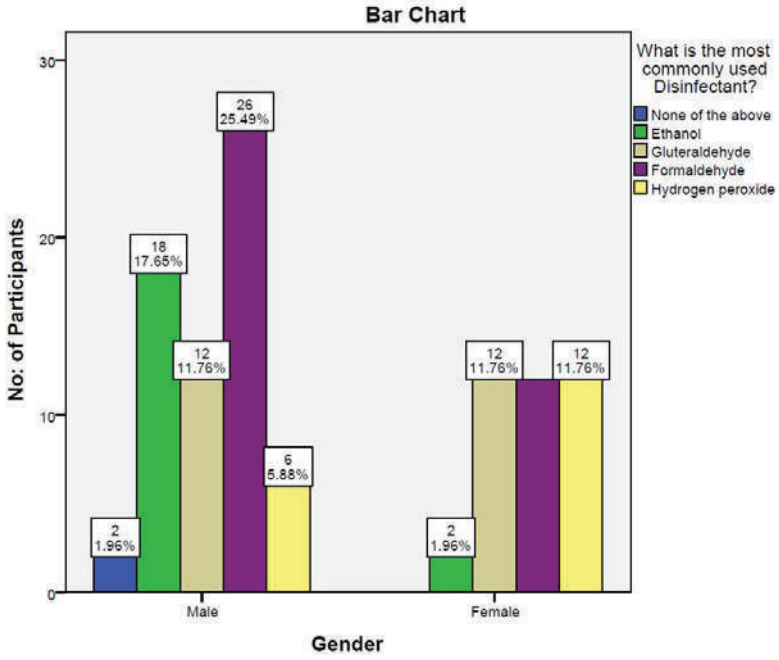


Fig. 4 : A bar chart showing the association between gender and the responses to the question

"What is the most commonly used disinfectant?". X axis represents the different types of disinfectants chosen by male and female students and Y axis denotes the number of candidates . In the male population, the highest response given was 25% as Formaldehyde. In the female population, there is no distinctive difference in the responses recorded. Hence, there is a significant difference between the responses given by the males and females. The Pearson Chi square value was found to be 16.396, and the p value was found to be 0.003 which is statistically significant.

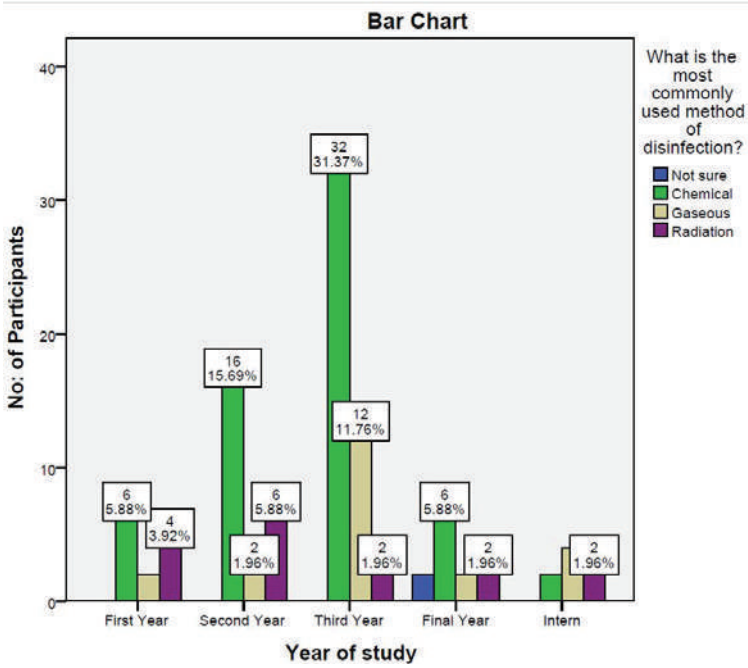


Fig. 5: A Bar chart showing the association between Year of study and the responses to the question " What is the most common method of disinfection ?". The X axis denotes the Year of Study and the Y axis denotes the number of responses. Most of the participants of the survey were Third years, and the major response was a chemical method of disinfection. The Pearson Chi square value was found to be 31.444, and the p value was found to be 0.002 which is statistically significant. There is a significant difference between the responses and the year of study.

On the question about the commonly used disinfectants in dental clinics 65-84% of them are not familiar about the four commonly used disinfectants. This lack of knowledge about the appropriate use of disinfectants during various procedures will lead to failure of complications in the patients. Based on the present study it was evident for formalin was used as a disinfectant this point consensus with the another research, in their study 10% formalin is used as immersion disinfectant [18,19,20].This tells us that formalin is an excellent disinfectant used to fumigate operation theatres [21,22]. Based on our study instruments like impression materials are mostly sterilised or if not they are immersed in disinfectant solutions [23,24].Thus one such study is an acceptance of our present study as most impression materials are most commonly either autoclaved or disinfectant spray is used [25,26]. Another case is that sterilisation is more preferred over disinfection of hand instruments; this fact is also in acceptance with our current study.The limitations of the present study is the limited sample size and unwillingness to answer certain questions. The future scope of such a study is however to improve teaching and knowledge about sterilisation and disinfection protocols in clinics.

Conclusion

Only around 50% of the participants were aware of the disinfection protocol. Only 50% of them practice the protocol. This is the reason for the iatrogenic infection in the hospital. It has been proved that success of any treatment is dependent on following sterile aseptic procedures. Students community should be trained in their learning days to get a hand on practice in such protocol, then the most important thing is the procedure and the protocol should be available to everyone, everywhere.It can be displayed at the practice area and also available as a handbook. This will improve the quality of treatment and also increase the success rate more than that it will decrease the cost of the treatment and duration of the treatment course. Similar studies on this topic (Alok Kumar Sethi et al) showed satisfactory results. However there remain few shortcomings about different teaching methodologies leading to few lapses in the adequate sterilization and ethical disposal of instruments. Hence, a disciplined training and teaching methodology in this field is the need of the hour and would go a long way in protecting themselves while learning and also would cause minimal harm to the environment

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Evaluation of Pain in Relation to Root Canal Treated Teeth Having Instrument Separation-A Retrospective Analysis

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Abstract

One of the most common mishaps during root canal treatments is the separation of an endodontic instrument during cleaning and shaping. The main problem to this is the improper debridement of the apical portion of canal and directly compromises the success of the treatment. The aim of this study was to evaluate the association of pain in root canal treated teeth having instrument separation. A total of 29 patients who have undergone root canal treatment and reported with a separated instrument were evaluated. Each of the patient's age, gender, tooth number, the association of pain, nature and the duration of pain was obtained from the patient records of the institution from June 2019 to March 2020. In this study, out of the total sample size, 15 (51.7%) of patients were females while the remaining 14 (48.3%) patients were males. It was shown that 72.4% of the patients did not experience pain with the presence of instrument separation in their root canal treated teeth, while 29.6% experienced pain. Position of the tooth in the arch did not influence the experience of pain among the patients (Chi - Square test, P- 0.35, >0.05). Within the limits of the study, an association between pain and root canal treated teeth with instrument separation was not established. Though gender and tooth did not influence the experience of pain, many female patients in the present study experienced pain. The pain was more often associated with lower posteriors.

Keywords: *instrument separation, root canal treated, pain, gender*

Introduction

An endodontic procedure is known to be a fairly predictable type of procedure that has been known to have a high success rate within the range of 86-98%.[1] In order to obtain success during an endodontic treatment, the root canal system should be thoroughly cleaned and debrided of any necrotic tissues as well as microorganisms.[2] As the carious lesion have progressed from the enamel and dentin, these microorganisms have been accepted as being the sole entity that initiates both pulpal as well as periapical pathologies.[3,4] Diseases of the pulp are both of infectious as well as inflammatory in nature. [5,6] When treatment failure has occurred; the majority of such cases have shown basic clinical signs and symptoms including radiographic findings. [7,8,9] Such lesions often originate from various etiologies and common presents as a diagnostic challenge for dental clinicians.[10]

Specifically, during an ongoing root canal procedure, there are several unwanted procedural errors that a clinician may encounter. The process of cleaning and shaping of the root canal system is considered to be one of the most fundamental aspects of a root canal treatment. [11] With the help of irrigation solutions, the number of microorganisms in the root canal system

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can also be reduced. [12] Chlorhexidine has become an appropriate adjunctive final irrigant in such cases. [13] When left untreated, further bone destruction and remodelling will contribute to more severe periapical lesions in the teeth with deep caries.[14] However, such clinical errors during cleaning and shaping particularly includes perforations, ledging as well as the separation of endodontic instruments are commonly encountered. [15,16]

The separation of an endodontic instrument inside the root canal system is one of the many mishaps that can hinder the success of an endodontic procedure. The reason behind this is because the fracture of an instrument within a root canal can result in both interference and delay for a successful root canal treatment. [17] Fractures of endodontic instrument origin are the effect of various factors which includes root canal curvatures, instrument design, dynamics of instrument usage, instrumentation techniques as well as the operator's skill set.[18,19,20] From the patient's point of view, continuous pain and discomfort that they experience is far worst if the broken instrument is not removed or bypassed from the involved tooth [(21)] As of now, such cases are treated through non-surgical measures but if the symptoms persist, then a surgical approach should be considered. [22]

In this study, the aim was to evaluate the association of pain in a root canal treated tooth with the presence of instrument separation. The purpose of it being that by determining the frequency of instrument separation and the factors associated with it, a better insight on the importance of proper instrument retrieval for the success in an endodontic treatment can be highlighted and the quality of the treatment can be improved. [23,24] It should always be of prime importance that a dentist should have the necessary knowledge as well as clinical experience to deal with such cases and especially as students, it can be understood through a variety of methods not only in practice but also in awareness. [25]

Materials and Methods

Study Setting

The study was conducted with the approval of the Institutional Ethics Committee [SDC/SIHEC/2020/DIASDATA/0619-0320]. The study consisted of one reviewer, one assessor and one guide.

Study Design

The study was designed to include all dental patients who have undergone root canal treatment with the presence of instrument separation. The patients who did not fall into this inclusion criteria were excluded.

Sampling Technique

The study was based on a non probability consecutive sampling. To minimise sampling bias, all cases sheets of patients who had a history of root canal treatment were reviewed and included.

Data Collection and Tabulation

Data collection was done using the patient database with the timeframe work of 01 June 2019 to 31 March 2020. About 86,000 case sheets were reviewed and those fitting under the inclusion criteria were included. Cross verification was done with the help of photographs and radiographic evidence. To minimise sampling bias, all data were included. The exclusion criteria was patients with systemic illness. Data was downloaded from DIAS and imported to Excel, tabulation was done. The values were tabulated and analysed.

Statistical Analysis

Descriptive statistics were performed using SPSS Statistical Analyzer (23.0 Version) by IBM on the tabulated values. Both the frequency distribution and the Chi-square tests were done.

Results and Discussion

Analysis of the presence or absence of pain in relation to root canal treated teeth with instrument separation showed that 21 (72.4%) patients reported with no history of pain in relation to the affected tooth while only 8 (27.6%) patients had a history of pain (Figure 1). Considering the gender, 66.7% of the female patients did not experience pain while 33.33% patients experienced pain. Similarly among male patients 78.6% did not experience pain, while the remaining 21.4% of the patients had pain (Figure 2).

Analysis of the duration of pain showed that the majority of the patients experienced pain for a duration of 1 - 15 days (17.2%). An equal distribution of 3.4% of the patients experienced pain for a duration of either 15-

30 days or 30 - 45 days or 45-60 days. The remaining 72.4 % did not experience pain (Figure 3). In regard to the nature of pain, the most common type of pain encountered was intermittent pain (17.2%) while 3.4 % experienced either nocturnal pain or spontaneous pain. (Figure 4)**.** In terms of the tooth region, pain was more commonly associated with the lower posteriors (50.0%) followed by upper posteriors (37.5%) and upper anteriors (12.5%) (Figure 5)

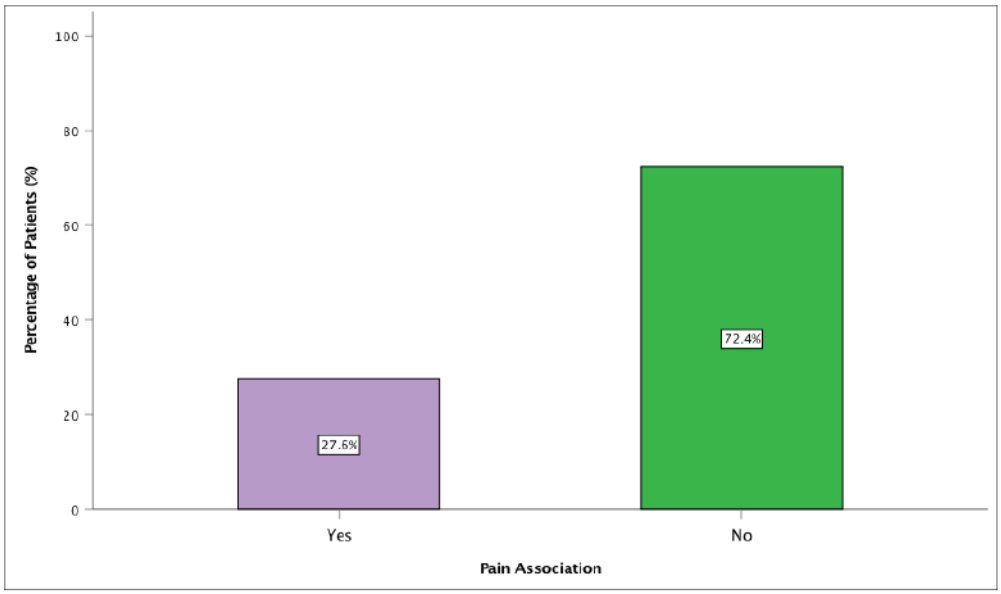


Figure 1: This bar graph represents the percentage of patients with and without pain in relation to root canal treated teeth having instrument separation. X-axis represents the presence of pain (Yes-purple) and absence of pain (No - green); Y-axis represents the percentage of patients having instrument separation in their root canal treated tooth. Majority of the patients did not experience pain in relation to root canal treated teeth having instrument separation.

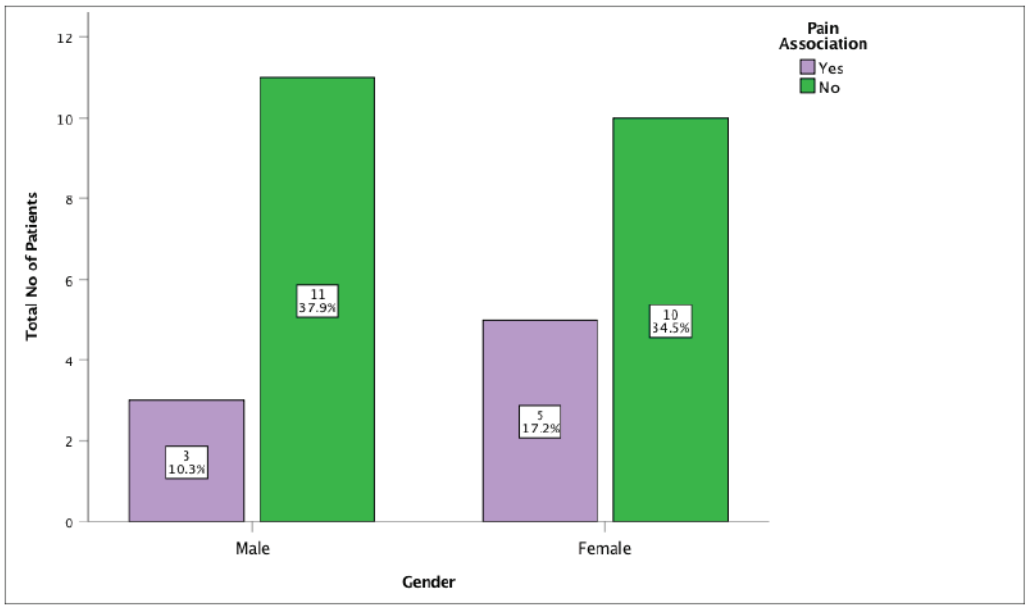


Figure 2: This bar graph represents the association between gender and presence/absence of pain in relation to root canal treated teeth having instrument separation. X-axis represents gender. Y-axis represents the number of patients with instrument separation in their root canal treated tooth. Purple bars - presence of pain (Yes);. Green bars - the absence of pain (No). More number of female patients experienced pain compared to male patients. However gender did not influence the presence and absence of pain (Chi-square test; p-value=0.47, P>0.05, not significant).

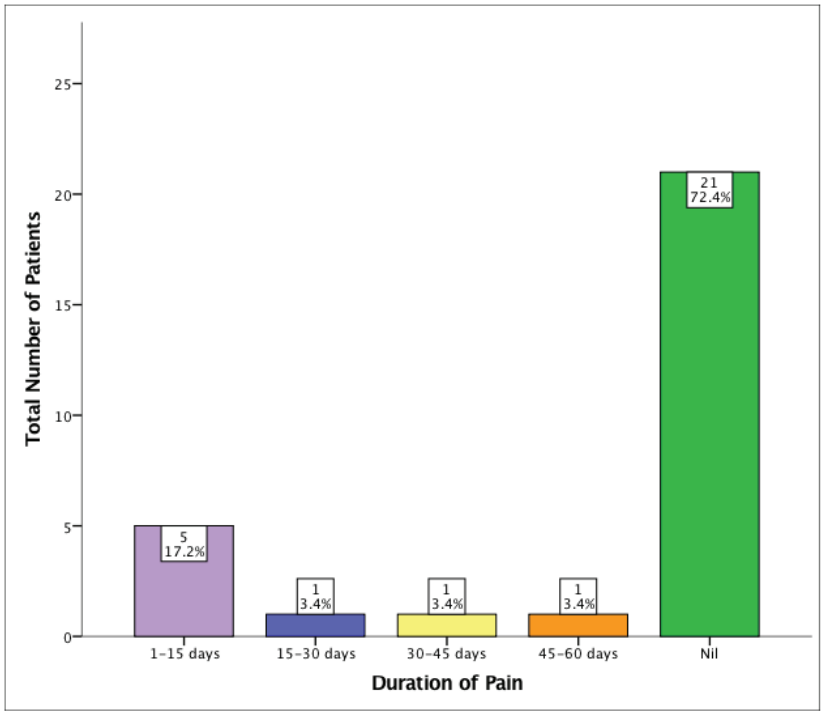


Figure 3: This bar graph represents the duration of pain experienced by the patients in relation to root canal treated teeth having instrument separation. X-axis represents the duration of pain experienced. Y-axis represents the total number of patients with instrument separation in their root canal treated tooth. Among the patients who reported pain, the majority of them experienced it for a duration of 1 to 15 days (Purple).

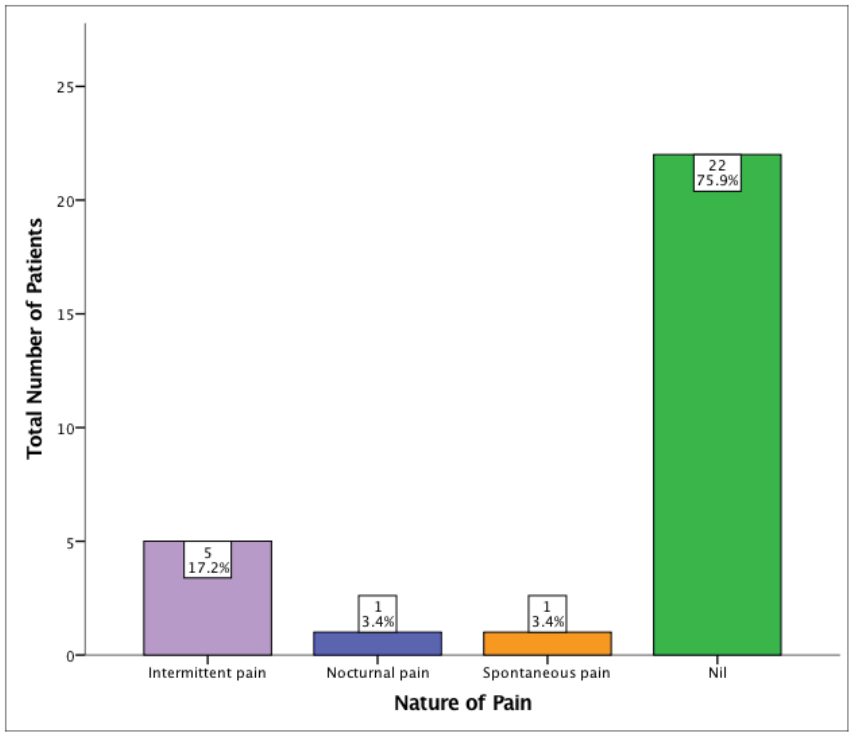


Figure 4: This bar graph represents the nature of pain experienced by the patients in relation to root canal treated teeth having instrument separation in a root canal treated tooth. X-axis represents the nature of pain. Y-axis represents the total number of patients with instrument separation in their root canal treated tooth. Among the patients who reported pain, the majority of them experienced intermittent pain (Purple).

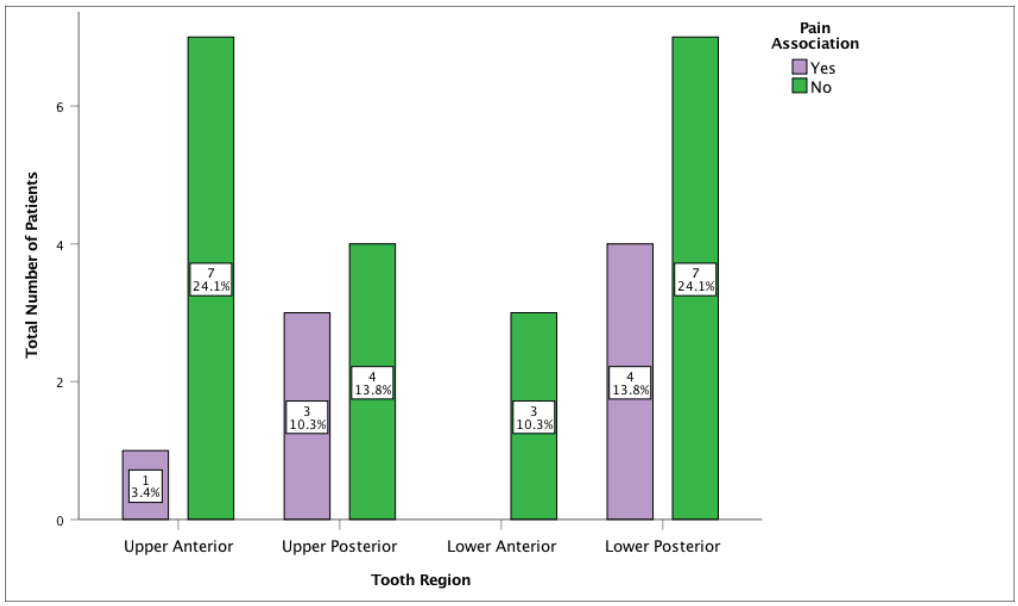


Figure 5: The graph represents the association between the tooth and presence / absence of pain in relation to root canal treated tooth having instrument separation. X axis represents the tooth region. Y-axis represents the total number of patients. Purple denotes presence of pain - Yes; and green denotes absence of pain - No. Among the teeth associated with pain, the majority of them were lower posterior teeth. However tooth involved did not influence the presence and absence of pain (Chi-square test; p-value=0.35, P>0.05, not significant)

The presence of instrument breakage in a root canal treated tooth was seen to be higher in the male population compared to females. However, when it comes to pain association, females showed a higher prevalence compared to males. Based on a study done by Lipton et al, with similar findings to our study, he found that women experienced each symptom more often than men with only two exceptions- "toothache and oral sores" and "oral sores and burning mouth". [26]

In the present study, intermittent pain was the most common type of pain experienced compared to nocturnal and spontaneous pain. Similar findings were reported by Arya et al, where they found that the patient had a history of episodic pain before reporting for the treatment. The reason behind this could possibly be because at different times, the nerve fibres can be stimulated either through mastication or even the type of food being consumed. [27,28]

Based on our study, patients experienced pain commonly at a duration of 1-15 days. A contrasting report by Arya et al showed that the patient had a history of pain for 1 month together with a history of incomplete root canal treatment. The reason behind the results of our study could be explained through the limited sample size available as well as the ability of the patient to tolerate pain.[27]

The lower posteriors were the most common tooth in which pain was associated. Shenoy et al also stated that lower posteriors were found to be the most common site for pain. However, factors other than the anatomy of the tooth that can influence the incident of an instrument separation, includes the usage of Stainless steel / NiTi File, cyclic fatigue, number of instrument usage, rotational motions/reciprocating motion, presence of apical enlargement and the sequence of filing.[21]

During the course of this study, some limitations were encountered which included the limited time frame in which the study was conducted that was only for 10 months. The study demographic was also only limited to the city population. Further multicentric studies with larger sample size must be carried out to establish the association of pain with tooth having instrument seperation.

Conclusion

In the present study, pain in the root canal treated teeth with instrument separation was recorded in 27.6% of the cases, with higher predilection in the female population. The pain was more often associated with lower posteriors. However, gender and tooth did not influence the experience of pain.

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Conflict of Interest: Nil

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Cardiovascular Diseases among Complete Denture Patients

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Abstract

Cardiovascular diseases are conditions causing blocked or narrowed blood vessels leading to chest pain, myocardial infarction or stroke. Sometimes it can be associated with dental diseases like caries, periodontal disease leading to tooth loss. In the present study, we assessed the prevalence of cardiovascular diseases among Complete denture (CD) Patients. Patient records were collected by complete analysis of data of 86000 patients between March 2019 to March 2020. The data on the patient's medical history and treatment were extracted from the patient's records and reviewed. Totally 421 case sheets were collected and used for our study. The collected data were entered in excel and transferred to SPSS for analysis by Chi square test. We found that 1.19% of complete denture patients were affected with cardiac problems and all the affected patients were males. Among them, 0.71% of patients between the age group 51 to 70 years were more commonly affected with cardiac problems. In the present study, we found that cardiac problems are statistically not significant ($p>0.05$). Though the association of cardiac problem between age and gender was found to be statistically not significant, we found that patients (especially males) between 51-70 years were more commonly affected by cardiac problems.

Keywords: *Complete denture, Cardiac, Prevalence, Tooth loss, Systemic.*

Introduction

Cardiovascular diseases are the conditions that involve blocked vessels or narrowed vessels leading to heart attack, stroke, chest pain, cardiovascular diseases including coronary heart disease, cerebrovascular disease, congenital heart diseases [1]. Cardiovascular disease can also be associated with tooth loss.

Edentulism is the condition of being toothless due to loss of tooth. Edentulousness is caused by various factors. It is caused due to periodontal problems, caries, extraction due to poor prognosis of tooth[2]. Sometimes edentulousness can also be associated with systemic disorders like diabetes, hypertension, or cardiac problems. Several studies were done to determine the association between systemic diseases and tooth loss [3,2,4].

Some studies have stated that it can be due to poor nutritional status or inflammatory responses associated with past periodontal diseases [5]. The main etiology behind tooth loss is periodontal diseases. A dental plaque marks the beginning of periodontal irritation and different elements (which include systemic and nearby factors) can affect the prevalence and severity of periodontal diseases [6]. The periodontal diseases and tooth loss can be due to systemic diseases also. Sometimes tooth loss can occur due to drug overdose [7,2].

One of the main objectives in selecting and arranging artificial teeth is to produce a prosthesis that defies detection [8]. Removable dentures replace missing natural teeth thereby restoring function and aesthetics

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it may be partial or completely edentulous patients. Complete denture is used as treatment of edentulousness [9]. Recently fixed prosthesis is more popular than removable prosthesis due to comfort, function and aesthetics [10] and the implants are getting more popular in terms of fixed prosthesis it may be complete or partial edentulousness but its success depends on many factors one of its success depends on its marginal fit [11] and abutments retaining the superstructure or prosthesis [12]. Similarly in cases with severe bony defects grafting is required before implant placements but alternatively lip bumpers help in covering up the defect [13,14]. In treatment aspect implant [15,16] and all ceramic restorations [17 18] are widely used but in case of compromised periodontium, RPDs and Complete dentures are widely used. Though so many treatment options are available, knowledge on every aspect is very much essential. In this study we are going to assess the prevalence of cardiovascular diseases among Complete denture (CD) patients.

Materials and Methods

The present study is a retrospective study. Patient records were collected by complete analysis of data of 86000 patients who visited Saveetha Dental College and Hospitals in between March 2019 to March 2020. The sample size for the present study was 421 patients who underwent CD treatment. The required data (patients wearing complete denture, their age, gender, treatment and medical history) were extracted from the patient's records and reviewed. The patients were grouped into 3 on the basis of age as 30-50 years, 51-70 years and above 71 years. The collected data were transferred to SPSS for analysis. Chi square test was used to detect the significance between age, gender and history of cardiac problems in Complete denture patients. p value less than 0.05 was considered to be statistically significant. The data was analysed and tabulated.

Results and Discussion

Patients were segregated according to their age and gender. Patients were grouped according to their age as 30-50 years, 51-70 years and above 71 years [Figure 1]. Among 421 patients, 252 were males and 169 were females [Figure 2]. We found that only 1.19% of patients were affected with cardiac problems [Figure 3] and all of them are males [Figure 5]. No female patients were affected with cardiac problems [Figure 5]. Among those 1.19% patients, 0.71% of patients between the age group 51-70 years were affected with cardiac problems [Figure 4]. We compared age with cardiac problems and found that it is not significant (p>0.05) [Figure 4]. Similarly we compared gender with cardiac problems and found that it is also not significant (p>0.05) [Figure 5]. From the results we found that cardiac problems are less prevalent in Complete denture patients.

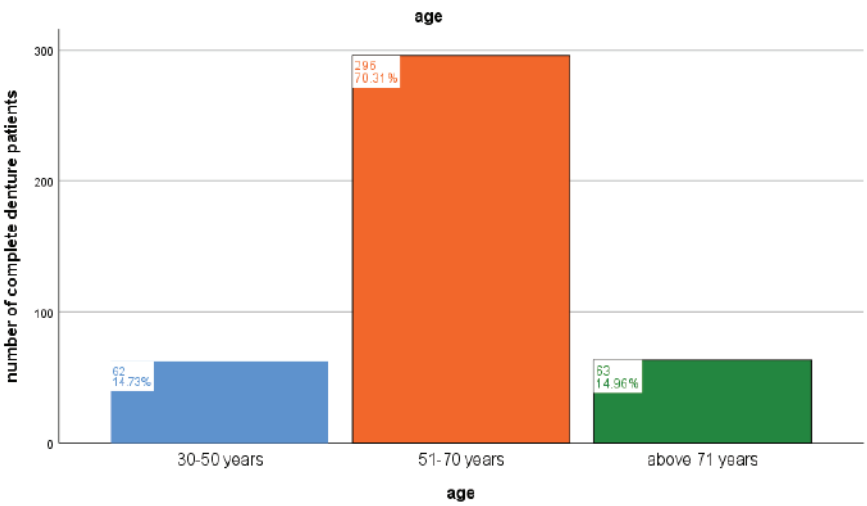


Figure 1: Frequency distribution of different age groups. X axis denotes age group and Y axis denotes number of complete denture patients. Blue denotes 30-50 years, orange denotes 51-70 years, green denotes above 71 years. 14.73% were between 30-50 years, 70.31% were between 51-70 years, 14.96% were above 71 years. Majority of the patients affected with cardiac problems were between 51-70 years of age (0.71%).

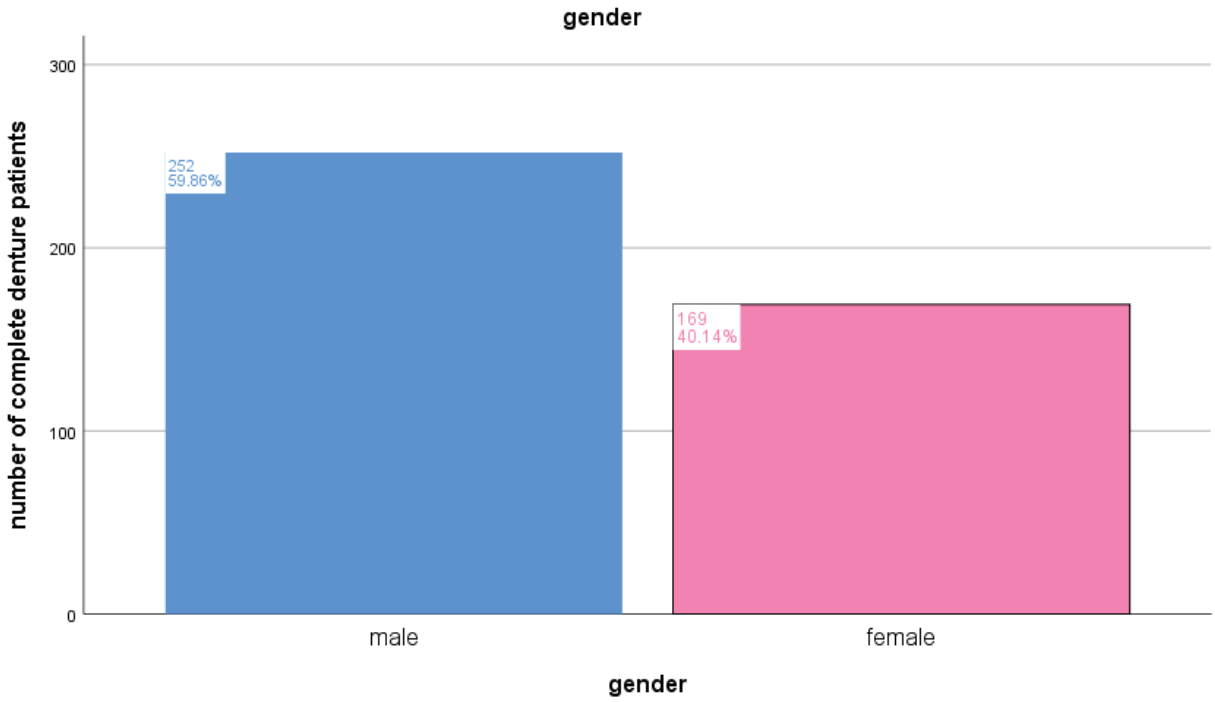


Figure 2: Frequency distribution of gender. X axis denotes gender and Y axis denotes number of complete denture patients. Blue denotes males, pink denotes females. 59.86% were males and 40.14% were females. Male population is more commonly affected with cardiac problem (1.19%).

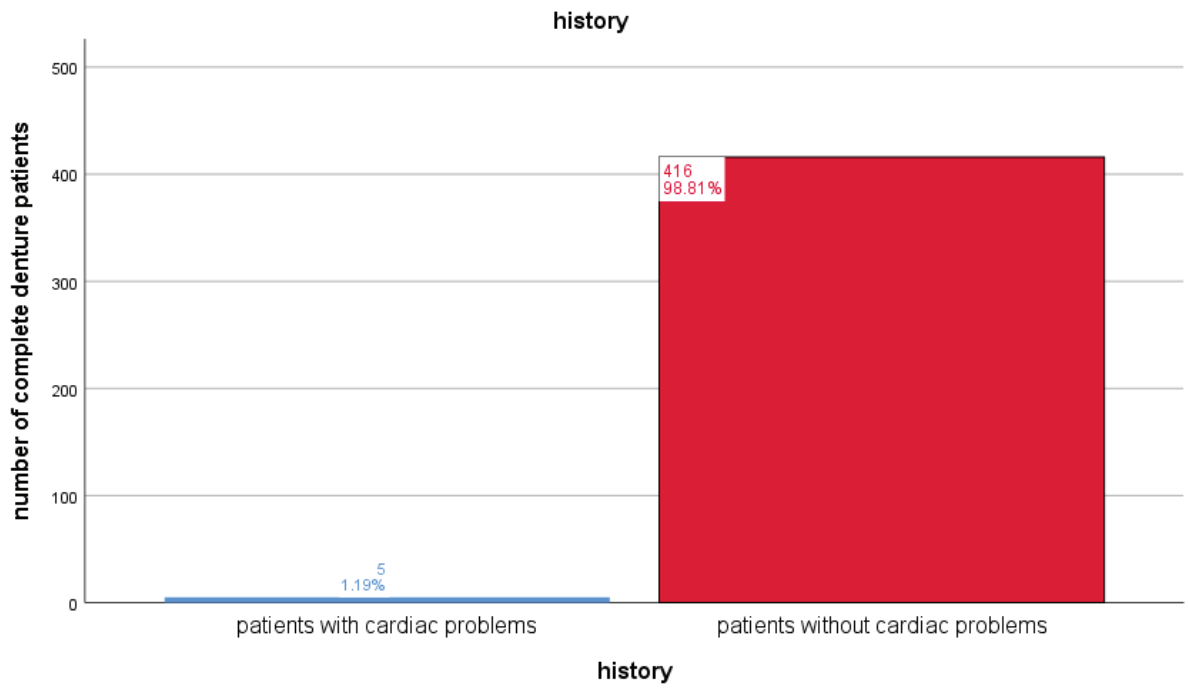


Figure 3: Frequency distribution of history of cardiac problems. X axis denotes history of cardiac problems and Y axis denotes number of complete denture patients. Blue denotes patients with cardiac problems, red denotes patients without cardiac problems. From the above graph we found that 1.19% of complete denture patients were affected with cardiac problems whereas 98.81% of complete denture patients were not affected with cardiac problems. Thus cardiac problems are less prevalent in complete denture patients.

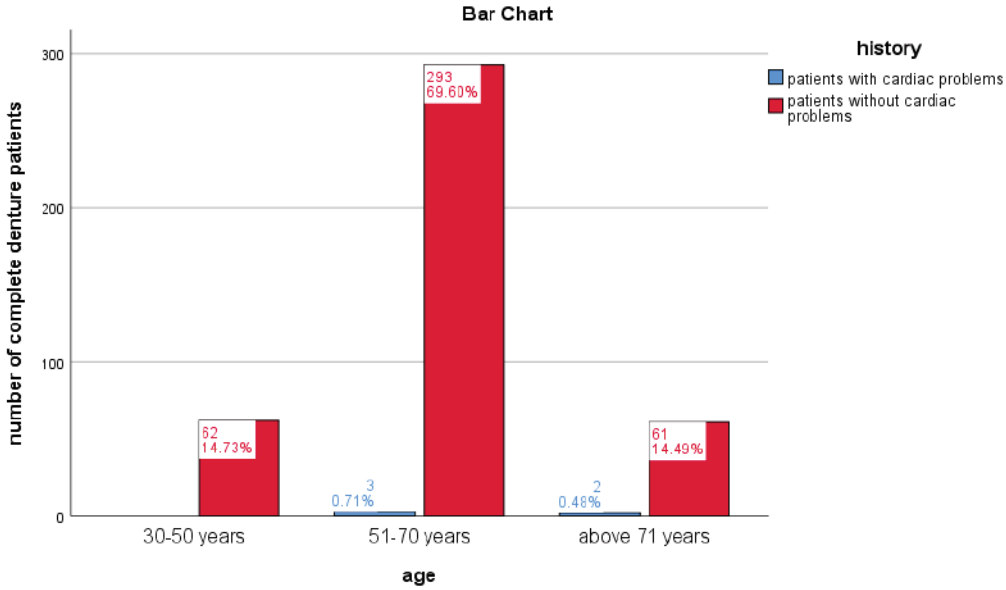


Figure 4: Bar chart representing the association between age and history of cardiac problem. X-axis denotes age and Y-axis denotes number of complete denture patients. Blue denotes complete denture patients with cardiac problems, red denotes complete denture patients without cardiac problems. Patients between the age group 51-70 years (0.71%) were more commonly affected with cardiac problems. None of the patients between the age group 30-50 years were affected. Chi square test was done and the association between age group and history of cardiac problem was found to be statistically not significant. p value obtained is 0.230 which is statistically not significant (p>0.05). Though it is statistically not significant, we found that patients between 51-70 years were commonly affected by cardiac problems.

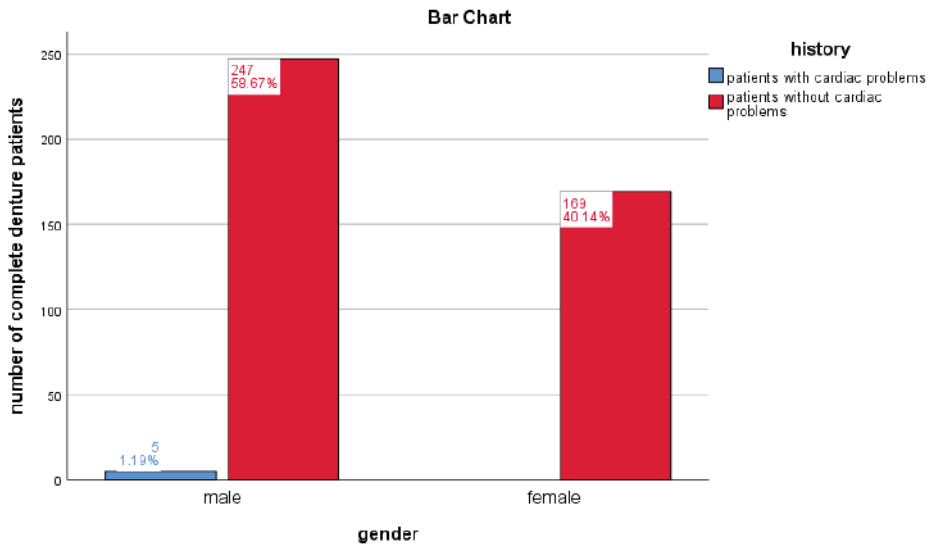


Figure 5: Bar graph representing association between gender and history of cardiac problem. X-axis denotes gender and Y-axis denotes number of complete denture patients. Blue denotes the complete denture patients with cardiac problems and red denotes the complete denture patients without cardiac problems. Males were predominantly affected by cardiac problems (1.19%) and there is no sign of cardiac problem in females. Chi square test was done and the association between gender and history of cardiac problem was found to be statistically not significant. p value obtained is 0.065 which is statistically not significant (p>0.05). Though it is statistically not significant, we found that male patients were commonly affected by cardiac problems.

Similar studies were done by other researchers. In the study conducted by Taraszkiewicz - Sulik Katarzyna et al they reported that age is not statistically significant and males are more prevalent, similar to the present study [19]. In the study conducted by Fei Cheng et al, they found a stronger association between tooth loss and cardiovascular disease, contrary to the present study; they also found that males are more prevalent, similar to the present study [20].

In the study conducted by Dr.M.Vijay Anand et al, they found that cardiac problems are second prevalent [21]. A study conducted by Hosseini et al showed that edentulism is more common in patients with diabetes and heart disease [22]. A cross-sectional study conducted by Ozturk Tonguc Maine et al showed that cardiac disorders and endocrine disorders were observed significantly higher in patients with tooth loss [23]. The study conducted by Bahekar et al showed that patients with periodontitis have higher risk of developing Coronary heart disease [24].

Tooth loss is associated with an increased risk of vascular diseases like coronary heart disease and cerebrovascular disease. Tooth loss may lead to dietary pattern change, resulting in an increased risk of hypertension because the change of dietary pattern may be associated with hypertension [25],[21]. Hypertension may be a risk factor linking tooth loss and vascular disease in postmenopausal women [26].

Tooth loss is also independently associated with aortic valve stenosis [27]. Some studies showed that edentulousness causes poor masticatory function and this may be a reason behind systemic diseases due to poor nutritional status [28]. In the study conducted by Hsin-Chia Hung et at, they found that men with tooth loss are at higher risk of coronary heart disease [29]. Some studies showed that tooth loss is associated with diabetes mellitus, cardiovascular disease or a combination of both [30]. Some studies showed that oral diseases like gingivitis, denture sore mouth can cause cellulitis which may lead to heart disease [31,32,18].

Conclusion

According to the present study, we found that cardiac problems are less prevalent in complete denture patients. Cardiac problems were mostly observed in the age group of 51-70 years and common in male population. The present study includes only a small sample size and it is a single centered study. In future, the present study can be done with a larger population with other systemic diseases included.

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Conflict of Interest : None declared.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Attrition, Abrasion, Erosion and Abfraction Among Patients Visiting A Private College Hospital in Chennai - A Retrospective Study

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Abstract

Regressive changes of the teeth include a variety of alterations in the dental tissues, mechanical tear and wear of tooth is a consequence of both pathological and physiological means. Tooth surface loss can be classified primarily as attrition, abrasion, abfraction and erosion. To assess the prevalence of attrition, abrasion, erosion and abfraction among patients visiting private college hospital in Chennai. Data of the patients retrieved was from the dental records of the hospital. Patients with attrition, abrasion, erosion and abfraction were shortlisted. Total study sample, n=7300 patients. Data was tabulated in excel and statistically analysed. From the statistical analysis, it was significant that the most prevalent type of tooth wear was attrition(49.7%), followed by abrasion(48.3%), erosion(1%) and abfraction(0.97%) (chi square test- p-value= 0.000- significant). Within the limitations of this study, it showed that there was a significant prevalence of attrition followed by abrasion, erosion and abfraction with a male predilection, predominantly between the age group of 41-60 years, reported to private college hospital in Chennai.

Key words*: attrition, abrasion, erosion, abfraction, tooth wear.*

Introduction

Regressive changes may not be necessarily related etiologically or pathologically, some of the changes may be due to general aging process or result of injury to the tissues [1]. Mechanisms most often occur together, each acting at different intensity and duration in a continuously changing salivary medium, producing immensely variable patterns and degrees of wear. Tooth wear is defined as a loss of dental hard tissue by a chemical or mechanical process not involving bacteria [2]. Tooth wear can be the result of a natural aging process [3,4] and be imperceptible to the majority of the patients. Some patients may experience tooth hypersensitivity and sometimes tooth wear may extend to the pulp. It has an impact on oral pain levels, dental appearance and function[5]. There are some other factors that contribute to tooth wear like dietary factors and environmental factors [6].

Attrition is defined as the wear process of the tooth tissue by direct tooth to tooth contact [7]. Attrition can be seen on cusps and grinding surfaces during empty-mouth grinding movements as in parafunctional habits like bruxism, several factors are reported to cause the awareness of attrition. Some of them include porcelain opposing natural teeth, occlusal collapse due to posterior support and any other abnormality [8].

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Abrasion is a type of non carious cervical lesion characterised by loss of tooth tissues with different clinical appearance [9]. It is caused by the sliding or rubbing of abrasive external objects against the tooth surface. The factors that cause abrasion include use of an abrasive toothpaste, hard bristles and a vigorous brushing technique [10]. This may also be caused by the use of toothpicks and miswaks, as well as consumption of abrasive foods. Hypersensitivity is not usually reported in case of abrasion due to the formation of a mechanical smear layer that blocks the exposed dentinal tubules [11].

Abfraction is a type of tooth surface loss believed to be caused by tensile stress generated from non-axial cyclic occlusal forces. Abfraction is the wear of hard tooth substance which is pathological, caused due to biomechanical loading forces; such loss is thought to be the result of flexure and chemical fatigue degradation of enamel and dentin at some point distant from the actual location of loading [12]. Some of the factors that cause abfraction are location, magnitude, duration and frequency of forces. The theory of abfraction is that the tooth flexure in the cervical area is caused by tensile stresses and compressive occlusal forces and results in microfractures of the hydroxyapatite crystals of the enamel and dentin with further deformation of the tooth structure [13].

Erosion is the progressive loss of dental hard tissue caused by acids from non bacterial, intrinsic or extrinsic sources. Erosion occurs due to high consumption of foods or drinks containing a variety of acids such as those from citrus and other fruits, fruit juices (citric acid) , soft drinks, urine and other carbonated drinks (carbonated acid and other acids) are the dietary causes[14]. The few cases of industrial and environmental erosion has been reported and associated to processes in the workplace (e.g. battery factories), which produce acid fumes or droplets and leisure activities such as swimming in chlorinated swimming pools [15].

Keeping in mind these implications it would be important to develop preventive strategy guidelines for clinical practise which will be useful for all practising clinicians and also to the people in society [16,17,18,19] and to create awareness about the prevalence of the tooth surface loss, causative factors, symptoms, clinical presentation and treatment [20,21,22,23]. It is imperative to understand the incidence and prevalence of disease processes in the oral cavity so that it would be an indicator of the needs of the community [24,25,26,27]. The aim of this study was to evaluate the prevalence of type of tooth surface loss like attrition, abrasion, abfraction and erosion in different groups of age, gender and tooth which are commonly affected .

Materials and Methods

In this study a total of n=7300 patients of different age groups and genders visiting the hospital were considered. This study was conducted as a university setting including predominantly South Indian population. The approval for this study was provided by the institutional ethical board. This was a retrospective study in which the data of patients from June 2019 to April 2020 of all age groups and gender were collected from the dental records. Each tooth of 7300 patients were analysed for tooth surface loss. Total number of teeth analysed was 44011. All the cases were approved and verified by an external reviewer and cross verification was done using a photographic method to eliminate the errors made while recording. Inclusion criteria included the patients with attrition, abrasion, erosion and abfraction who visited the hospital for treatment. Exclusion criteria included the patients without attrition, abrasion, erosion and abfraction. Repeated and incomplete data of patients were also excluded.

The independent variables of this study were name, age, gender, ethnicity, number of teeth, occupation, systemic conditions, external factors like brushing, abrasives, beverages etc., Dependent variables were attrition, abrasion, erosion and abfraction. The data obtained was tabulated in excel and imported to SPSS by IBM software with variables defined. Analysis of this study was done using a statistical test Chi Square.

Results and Discussion

Out of the total patients visiting the hospital, n= 7300 patients were found to have tooth wear like attrition, abrasion, erosion and abfraction. The prevalence of attrition was found to be 49.7%, abrasion was 48.3%, erosion was 1% and abfraction was 0.97%. Therefore attrition was the most prevalent followed by abrasion, erosion and abfraction was the least prevalent (graph 1)

. Out of the total patients, 67.7% were males and 32.3% were females. In which higher incidence of tooth surface loss was seen in males patients in comparison with female patients (graph 3)

Tooth wear prevalence between 7 to 20 years were 0.6%, 21 to 40 years were 22%, 41 to 60 years were 57%, 61 to 80 years were 20% and 81 to 91 years were 0.4%. Therefore higher incidence of tooth surface loss was seen in patients with 41 to 60 years of age followed by 21 to 40 years of age, 61 to 80 years of age, 7 to 20 years of age and the least prevalence was seen in 81 to 91 years of age (graph 2).

The teeth prevalence of abfraction was maxillary incisor(9.6%), maxillary canine(9.6%), maxillary premolar(32.8%), maxillary molar(15.3%), mandibular incisor(3.8%), mandibular canine(3.5%), mandibular premolar(18.9%) and mandibular molar(6.4%). From the above, it was evident that abfraction was most commonly seen in maxillary premolars (graph 4) (chi square test- p-value= 0.000- significant). The teeth prevalence of abrasion was maxillary incisor(7.4%), maxillary canine(10.1%), maxillary premolar(31.4%), maxillary molar(10.9%), mandibular incisor(2.8%), mandibular canine(3.3%), mandibular premolar(28.4%) and mandibular molar(5.8%). From the above, it showed that abrasion was most commonly seen in maxillary premolars (graph 5) (chi square test- p-value= 0.000- significant). The teeth prevalence of attrition was maxillary incisor(8.9%%), maxillary canine(4.8%), maxillary premolar(9.6%), maxillary molar(12.3%), mandibular incisor(21%), mandibular canine(9.7%), mandibular premolar(15.2%) and mandibular molar(18.5%). From the above, it showed that attrition was most commonly seen in mandibular incisors (graph 6) (chi square test- p-value= 0.000- significant). The teeth prevalence of erosion was maxillary incisor(40.2%), maxillary canine(12.2%), maxillary premolar(10.4%), maxillary molar(3.8%), mandibular incisor(10.8%), mandibular canine(5.4%), mandibular premolar(9.7%) and mandibular molar(7.4%). From the above, it showed that erosion was most commonly seen in maxillary incisors (graph7) (chi square test- p-value= 0.000- significant). The Chi Square test results showed that the study was found to be statistically significant with p value less than 0.05 (table 1).

Tooth surface loss is the dental hard tissue loss from the surfaces of the teeth. Attrition, erosion, abfraction and abrasion usually cause alterations of the tooth surface and manifest as tooth wear. These act by distinct progressions and exhibit unique clinical characteristics [28]. In the current study, prevalence of tooth surface loss based on gender was observed to have a higher male predilection. Tooth surface loss was higher in incidence, especially in males (67.7%) than females (32.3%). This is in concordance with the study done by Mithra 2018, that stated that male population showed higher tooth wear compared to the female population [29]. The study conducted by Joana 2010 also supported our study stating that high prevalence of tooth wear observed among males [30]. Hugoson also stated that men presented with more teeth with surface loss than women [31]. Our study is in concordance with the previous literature. This could be attributed to male predominance in the geographic limitation, aggressive habits and certain habits pertaining to males like increased compressive forces, industrial erosion, vigorous brushing technique, alcohol consumption , tobacco chewing [32] and parafunctional habits. Abfraction and abfraction are most prevalent in maxillary premolars, erosion in maxillary incisors and attrition in mandibular incisors in this study. According to the study conducted by Marcelle 2016, incisor and premolar are the most commonly affected teeth which is in concordance with this study and he also stated that tooth wear is more prevalent in mandibles. This is due to the increased occlusal and compressive forces in the premolars and molars and increased edge to edge contact in the anteriors [33].

In relation to the occurrence of tooth surface loss among different age group, maximum occurrence of tooth surface loss was seen in the age group between 41-60 years of age with 57%, followed by 21 to 40 years of age (22%), 61 to 81 years of age (20%), 7 to 20 years of age (0.6%) and least tooth surface loss prevalence was seen in age group greater than 81 years (0.4%). This result was in concordance with a study done by Mithra 2018, who reported that tooth wear was more prevalent in the age group of 40 to 60 years of age [29]. Vant 2009 reported an increase in prevalence of tooth wear from 3% at the age of 20 years to 17% at the age of 70 years [34]. Hugoson 1988 also reported an increase in tooth surface loss with age [31] and Nascimento 2016 reported an increase in tooth surface loss as the

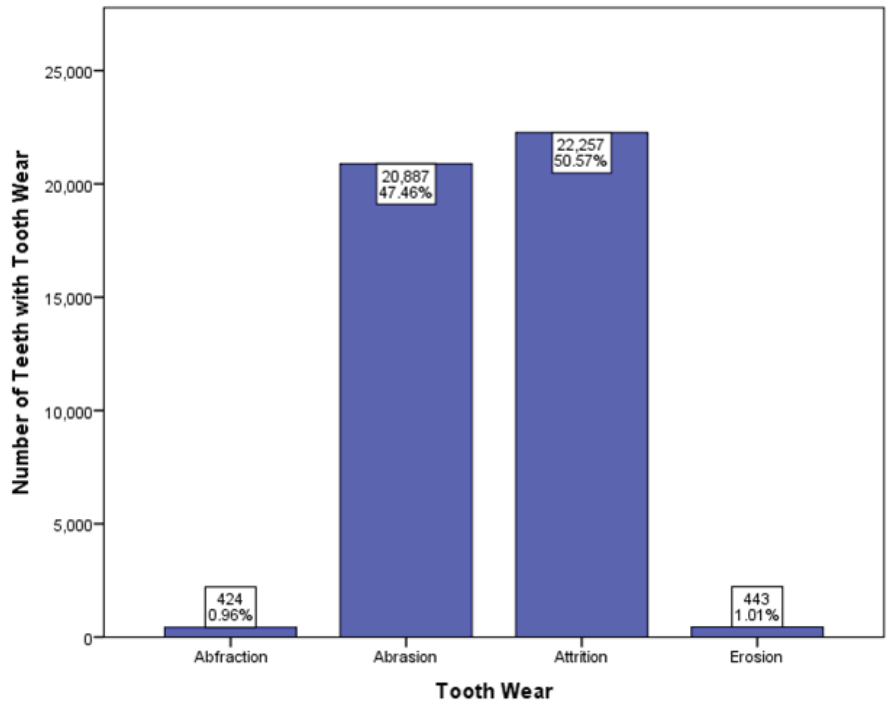
population ages [33]. Bernhardt reported that mean wear scores increased from 0.6 among 20 to 29 years to 1.4 in 70 to 79 years in a total score of 3 in which high scores indicate more severe levels of tooth wear. This shows that tooth wear score increases as the person's age increases [35]. Previous studies are in concordance with our study. This prevalence in aged people may be due to the teeth retained for a longer time but this prevalence which is increasing with age drops after the age of 60 years. This may be due to the increased missing teeth in older people and decreased number of patients seeking dental care with patients greater than 60 years. There is a study that explains the prevalence of tooth surface loss in younger people. According to Ayesha, prevalence of tooth surface is increasing and younger people are said to be at higher risk [36].

Establishing the prevalence of different types of tooth surface loss, attrition was the most prevalent with 49.7%, followed by abrasion (48.3%), erosion (1%) and abfraction (0.97%). This is in concordance with a study conducted by Mithra 2018, that revealed attrition in 29% out of 40.6% tooth wear patients, followed by abrasion in 23.7%, erosion in 4.6% and abfraction in 6.3% [29]. There are scarce studies that discuss the prevalence of type of tooth wear and it is important to analyse the prevalence of tooth wear due to the increasing population and modern lifestyle. Erosion was higher in people consuming alcohol and soft drinks with gastric regurgitation. Attrition was significantly higher among tobacco chewers [37] and in patients with parafunctional habits such as bruxism. This can be utilised for patient education and increasing the awareness regarding tooth wear which is vital for arresting irreversible disease progress and well-being. This is because most of the tooth surface loss in patients is asymptomatic and patients are unaware of this condition. The photographic method of verification plays an important role in accuracy of this study [38]. Most commonly revealed only on clinical examination by the dental practitioners.

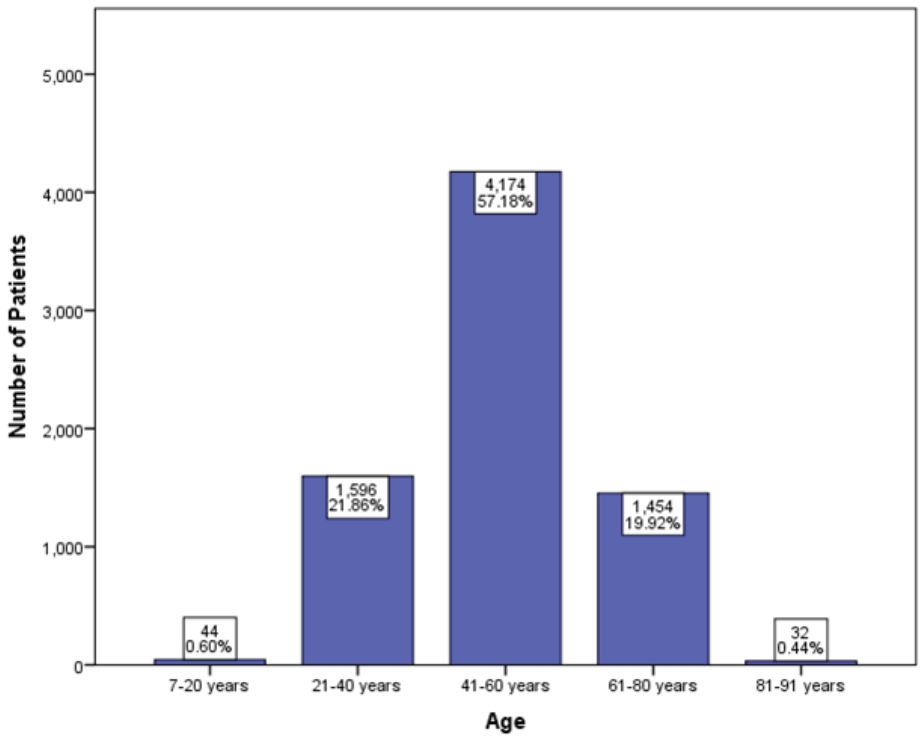
Tooth wear progression appears to occur at a relatively slow rate once the tooth wear has been diagnosed, this happens particularly where preventive advice of the practitioner has been successfully implemented. So, it is very much important for the dental practitioner to diagnose the tooth wear and implement the appropriate preventive measures [39]. Topical application of fluoride has been known to provide protection against subsequent tooth wear. A neutral sodium fluoride gel or mouth rinse can be recommended which helps to combat acid damage. Mouth rinses with less pH value should be advised. Remineralizing toothpaste such as Enamelon is recommended as it is known to increase the surface hardness of teeth exposed to acidic substances and have a greater impact. Potassium containing toothpaste is appropriate for the management of dentin sensitivity [40]. A reduction in the frequency and quantity of consumption of fruits, fruit juices, carbonated drinks or other acidic substances would be a beneficial advice. They also should be advised to limit their consumption of erosive foods or beverages. Increased intake of calcium products is known to decrease the erosion and promote re-hardening of enamel. The avoidance of aggressive tooth brushing, avoid swishing beverages in the mouth, avoid drinking acidic beverages through a wide bore straw, avoid abrasive toothpastes and refraining habits like pen or pencil biting will also help. In cases of nocturnal bruxism, a full coverage hard acrylic occlusal splint should be advised [41]. The dentine bonding agent and fissure sealant application is helpful in providing a certain level of protection and decreasing dentin hypersensitivity [42]. Medication can be used to reduce acid reflux and acid production like antacids. The primary goal for the tooth wear management of any patient is to prevent further pathological wear. In many cases of tooth surface loss, an early diagnosis followed by successful implementation of preventive measures may prove to be sufficient as a definitive management. So it is important to accurately assess and diagnose a patient presenting with tooth wear Majority of the cases can be successfully treated by preventative, passive measures which require long term maintenance and monitoring [43].

The guidelines should include the symptoms, causes, clinical presentation, preventive measures and treatment of tooth surface loss in elegant, all respective languages and distributed widely which will decrease the prevalence of tooth surface loss and this study sets an pavement for the construction of guidelines. Limitation of this study was that it covers only limited parts of the south Indian population and it was an uni-centric study. To ascertain the results of this study, a multi-centric study should be done. Future scope is to increase the population size for better ethnicity and results. This study is used to analyse the prevalence of tooth wear and

to reduce the prevalence of this irreversible progression disease.

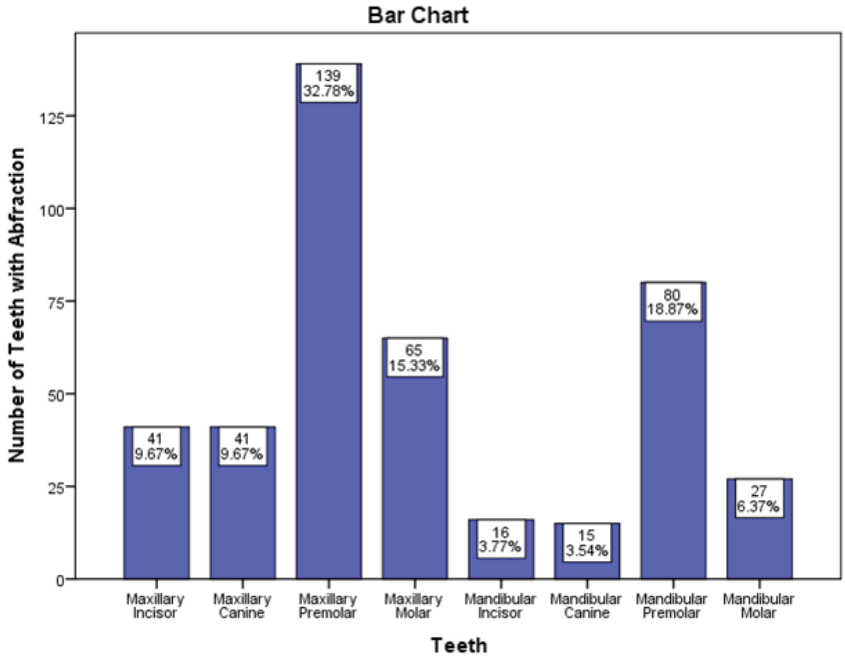


Graph 1- Bar graph representing the prevalence of attrition,abrasion, erosion and abfraction. The x-axis represents attrition, abrasion, erosion and abfraction and y-axis represents the total number of teeth with attrition, abrasion, erosion and abfraction. There is a prevalence of attrition followed by abrasion, erosion and abfraction.

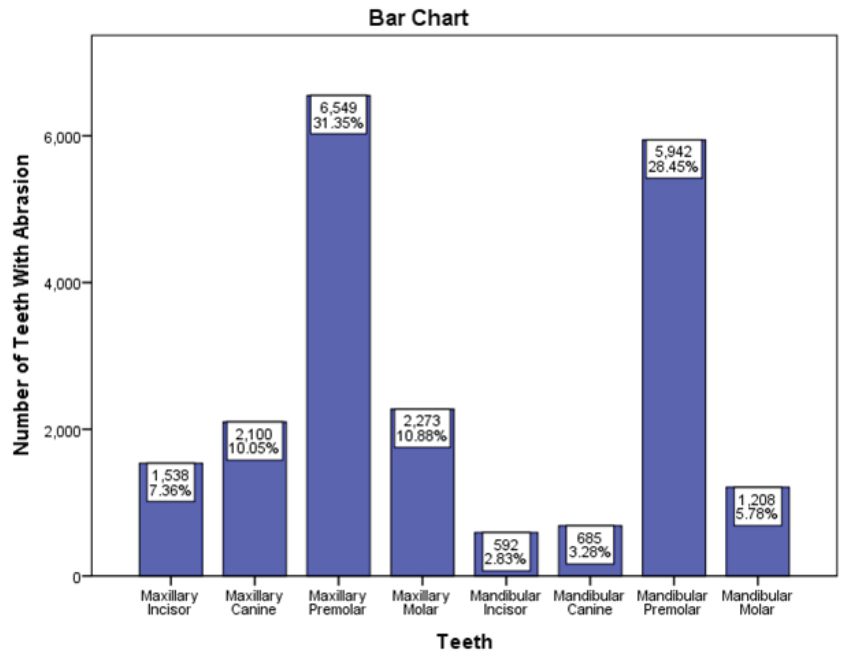


Graph 2- Bar graph representing the age distribution of this study. The x-axis represents the age group and y-axis represents the number of patients with tooth wear. There is a prevalence of tooth surface loss in the 41-60 years age group in the spacing scale.

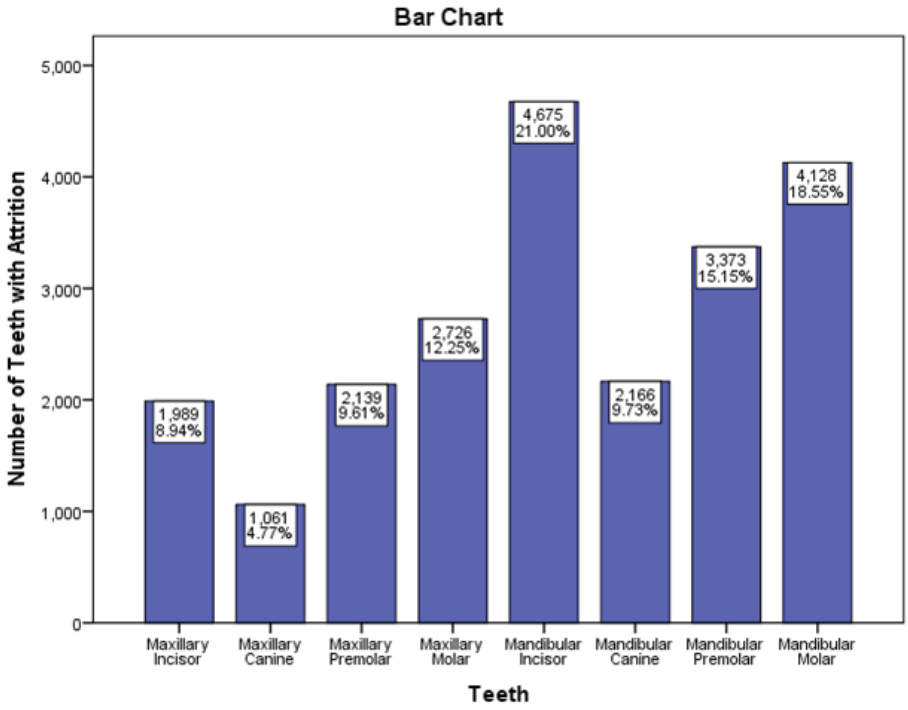
Graph 3- Bar graph showing the gender distribution of this study. The x-axis represents the gender and y-axis represents the number of patients with tooth wear. There is a prevalence of tooth surface loss in males in this study.



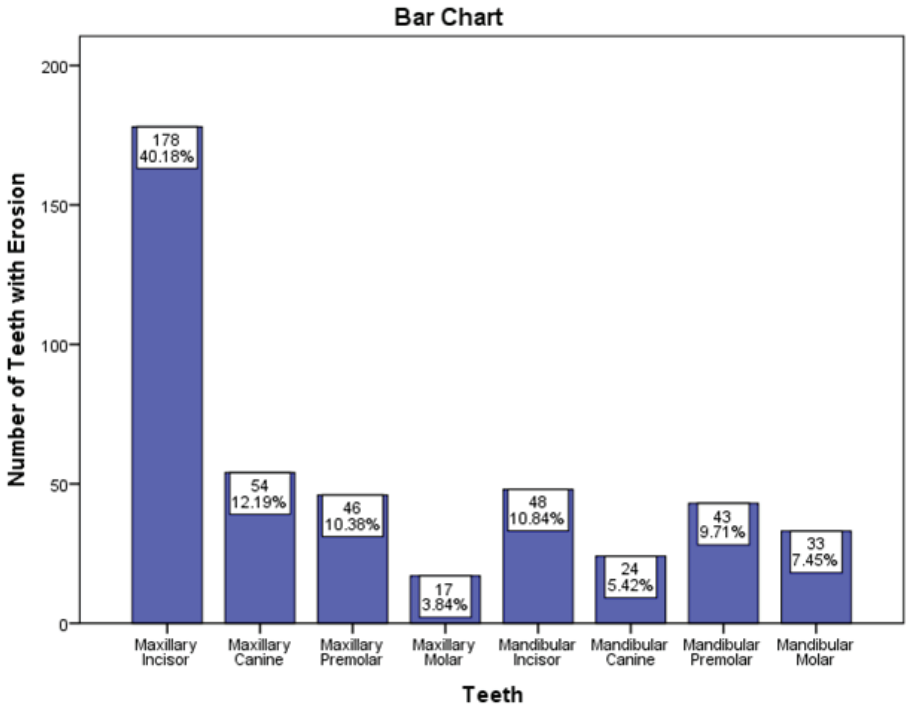
Graph 4- Bar graph representing the association between teeth and abfraction. The x-axis represents the teeth and y-axis represents the total number of teeth with abfraction. Chi square test was done and the association was found to be statistically significant. Pearson's Chi Square value: 2227.283, DF: 7, p value: 0.000(<0.05) hence statistically significant. Abfraction is most commonly seen in maxillary premolars (32.8%).



Graph 5- Bar graph representing the association between teeth and abrasion. The x-axis represents the teeth and y-axis represents the total number of teeth with abrasion. Chi square test was done and association was found to be statistically significant. Pearson's Chi Square value: 14510.210, DF: 7, p value: 0.000(<0.05) hence statistically significant. Abrasion is most commonly seen in maxillary premolars (31.4%).



Graph 6- Bar graph representing the association between teeth and attrition. The x-axis represents the teeth and y-axis represents the total number of teeth with attrition. Chi square test was done and association was found to be statistically significant Pearson's Chi Square value: 3641.525, DF: 7, p value: 0.000(<0.05) hence statistically significant. Attrition is most commonly seen in mandibular incisors (21%).



Graph 7- Bar graph representing the association between teeth and erosion. The x-axis represents the teeth and y-axis represents the total number of teeth with erosion. Chi square test was done and association was found to be statistically significant. Pearson's Chi Square value: 330.327, DF: 7, p value: 0.000(<0.05) hence statistically significant. Erosion is most commonly seen in maxillary incisors (40.2%).

Conclusion

Within the limits of this study, attrition was the most prevalent condition. From the analysis, there was a significant prevalence of attrition followed by abrasion, erosion and abfraction with a male predilection, predominantly between the age group of 41-60 years, reported to the private college hospital in Chennai.

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Prevalence and Age Related Risk of Three Clinical Variants of Aphthous Stomatitis: a Retrospective Study

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Abstract

Aphthous stomatitis is one of the most common conditions characterized by the repeated formation of benign and mouth ulcers (aphthae) in otherwise healthy individuals. The informal term canker sores is also used, mainly in North America, although this may also refer to any mouth ulcers. The case records of patients with aphthous stomatitis were assessed. The age, gender and the variant of aphthous stomatitis were recorded. The association between the gender and the variant of apthous stomatitis was assessed by chi-square test. Following the data tabulation in excel, the statistical analysis was done with SPSS.A total of 84 patients in the age range between 6 years to 73 years were recorded. From the collected data it was observed that 69% of the samples were males and 31% were females. The most common variant noted was the minor aphthous stomatitis. There was no association between the age and the occurrence of aphthous stomatitis($P>0.05$) but there was a positive correlation between the gender and clinical variant.($P<0.05$) Hence from the study it was concluded that age is not a risk factor for the development of aphthous stomatitis but males were more commonly affected.

Keywords: *Aphthous stomatitis; Minor aphthous stomatitis; major aphthous stomatitis; herpetiform aphthous stomatitis; age; canker sores*

Introduction

Aphthous stomatitis is one of the most common conditions characterized by the repeated formation of benign and mouth ulcers (aphthae) in otherwise healthy individuals. The informal term canker sores is also used, mainly in North America, although this may also refer to any mouth ulcers.[1]The etiology of the disease is not clearly understood, although an immune mediated response is seen. Among the several etiological factors pointed out a few that are well established are nutritional deficiencies, hormonal changes,allergies and a genetic predisposition. Invariably stress is implicated as a major trigger factor in the causation of aphthous stomatitis. There have been numerous cases where aphthous stomatitis has been recorded in patients during the time of examinations.[2]

These ulcers have an episodic pattern of occurrence, healing and recurring. Clinically the ulcers are classified into three major subtypes namely, minor, major and herpetiform. Irrespective of the types all the ulcers occur in the non-keratinised mucosa with the buccal and labial mucosa being the most commonest sites. The minor ulcers are small with size being less than 1 cm and heals without scarring in less than a week. The major ulcers are larger than 1 cm and heals with scarring in a span of 20 days. The herpetiform aphthous occurs in crops of ulcers and heals in a weeks time.[3]

Patients with aphthous stomatitis usually exhibit no systemic symptoms or signs.There may be prodromal

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symptoms such as burning, itching, or stinging,, which is often out of proportion to the extent of the ulceration. The pain with the ulceration may be worsened by physical contact, especially with certain foods and drinks. The pain is high in the initial days of formation of the ulcer and recedes as the ulcer heals. Lesions in the tongue have an impact on speaking and chewing which can be uncomfortable. The ulcers in the soft palate, back of the throat cause painful swallowing.[4]

The overall prevalence of the condition is 20% when compared to the general population with the onset in childhood or adolescence. The condition has a tendency to occur several times before gradually disappearing.. The ulcers typically begin as erythematous macules which develop into ulcers. The ulcers are covered with a yellow-grey fibrinous membrane that can be scraped away and surrounded by a reddish "halo". The size, number, location, healing time and periodicity between episodes of ulcer formation are dependent upon the subtype of aphthous stomatitis.[5]The treatment options have ranged from the use of topical anesthetics for the control of pain to corticosteroids.[6] Apparently corticosteroids are used in the management of several immune mediated conditions.[7,8]

Aphthous stomatitis has been associated with several other autoimmune diseases, namely systemic lupus erythematosus and inflammatory bowel diseases.[9] No clear reason could be established for this association.[5] Though the presence of ulcers in the oral cavity is a cause of concern, this ulcer has no malignant transformation potential. [10] Through genetic changes have been observed in the saliva of the patients, there is no malignant transformation potential.[11] Apparently aphthous ulcers are seen in patients with poor oral hygiene.[12,13] Several reports point to the fact of using nutritional supplements in the usage of management of aphthous stomatitis.[14]

The aim of this study is to assess the age and gender prevalence of apthous stomatitis.The objective of this study is to improve the evidence for improved understanding of the disease and plan further research and improve patient care.

Materials and Methods

The archived patient records of the department of Oral Medicine and Radiology, Saveetha Dental College were collected and the data was assessed from the time period of June 2019 - April 2020. The total patients who had visited the Dental College during this time period was 86000 and cases of aphthous stomatitis were identified and segregated. Ethical clearance was obtained from the Institutional Ethics Committee of Saveetha Dental College. The main advantages of this study were that the data was all prevalidated and the main disadvantages were that it was an unicentric study and only a single ethnicity of the population was studied.

The patient data were picked up from the case sheets and the variables recorded were the age, gender and clinical variant of aphthous stomatitis. The case sheets were reviewed by two observers. The random case sheets were verified by telephonic conversations with the patient.

The internal validity of the study was established as the data was collected from a verifiable and standardised database. The external validity is established as the data is from a clinical setup which is duplicatable.

Finally, after gathering proper information about aphthous stomatitis, the data was tabulated in Microsoft excel sheet. During analysis of the collected data, the independent variables were age, gender and dependent variable was the variant of aphthous stomatitis. Data was analysed using SPSS version24 and chi square test was done to check the association and a p value < 0.05 was said to be significant.

Results & Discussion

The study had a total of 86 patients with aphthous stomatitis were recorded during the aforementioned time period giving a prevalence percentage of 1.5% among the total case sheets reviewed. The case sheets of 2 patients had discrepancies and the case sheets were eliminated from the study and the final sample size was 84. The age range of the patients was from 6 to 73 years, with a mean age of 31.95 years. The graph in Fig1 shows the age distribution of patients, divided into smaller groups where 21-40 had the highest number of patients. A gender based distribution of the disease shows the males were most commonly affected.Fig 2. Analysing the age and the occurrence of apthous stomatitis it was found that minor was the predominant type in all age groups but it

was not statistically significant(P>0.05)Analysing the Clinical variants of aphthous stomatitis shows that the most common type of aphthous stomatitis was minor aphthous stomatitis 91.7%, followed by major aphthous stomatitis 7.1% and herpetiform 1.2%. An association between the gender and the clinical variant of apthous stomatitis reveals that in minor apthous 52 patients were males while only 25 were females. In the major variant 5 were males and 1 female patient was recorded. In herpetiform variant only 1 male patient was seen. A chi-square analysis reveals males were the most commonly affected gender.(P<0.05) Fig 4

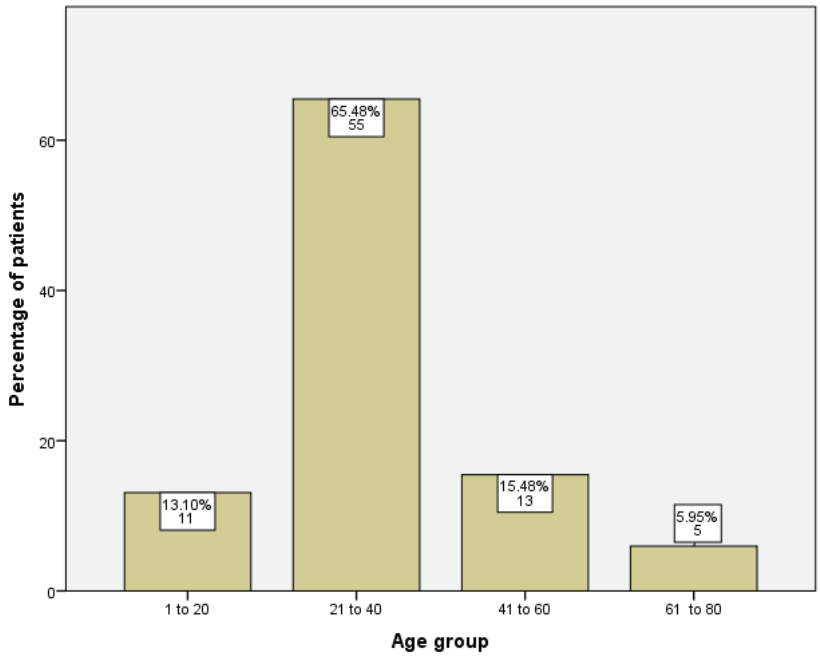


Figure 1: Graph showing the age distribution of patients.x-axis gives the age and y-axis gives the percentage The maximum number of cases were seen in the age range of 21-40 years. The mean age of the sample was 31.95 years.

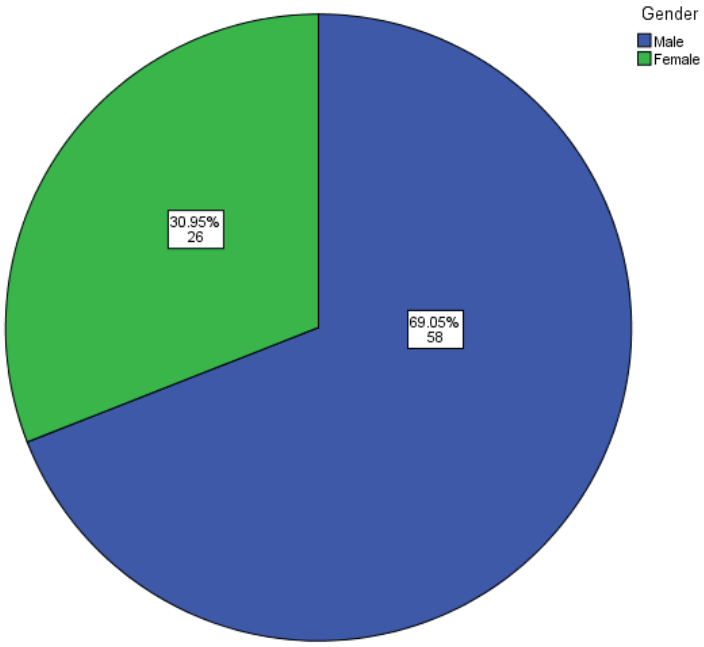


Fig 2: Pie chart showing the gender distribution of patients. Blue colour represents the males and the group had 58 patients while green represents the females which contained 26 patients.

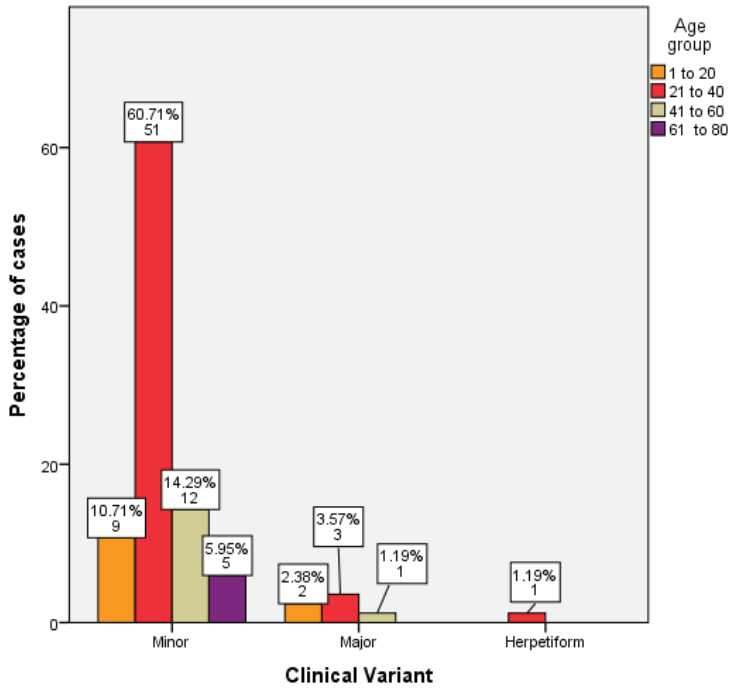


Fig 3: This Clustered bar graph represents the association between the percentage of patients according to the clinical variant and the age group. X axis represents the variants and Y axis represents the percentage. The orange colour represents the age group 1-20, red colour represents 21-40, beige colour represents 41-60 and purple represents 61-80. The age group of 21-40 had the highest number of patients(55), in which 51 were minor apthous, 3 major apthous and 1 herpetiform apthae. When the association between the variants and the age group was assessed, (chi square -3.279 , df-6, p-0.07 (p>0.05)) implying the age was not associated with the development of apthous stomatitis

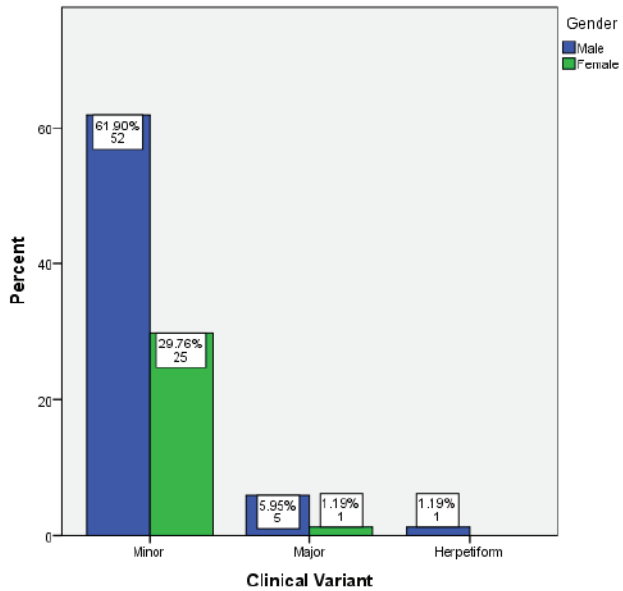


Fig 4: This Clustered bar graph represents the association between the number of patients according to the clinical variant and the gender. X axis represents the variants and Y axis represents the percentage. The blue colour represents the males and green colour the females. The minor variant had a total of 77 patients, major had 6 patients, and1 patient with herpetiform variant. Chi square test was done and it was found to be statistically significant (chi square value- 1.104, df -2;p-0.034). Males had a higher prevalence of apthous stomatitis.

Aphthous Stomatitis is the most common and painful ulcerative condition occurring in the oral cavity. The amount of pain as quantified by the pain measuring scales like visual analogue scale, numerical rating scale reveals that the intensity of pain is much higher compared to the size of the ulcer. Though clinically there are three types of ulcers, the most commonest presenting variant is the minor aphthous stomatitis also known as stress ulcers. [15] Our study also shows that minor aphthous stomatitis is the most commonest variant of apthous ulcer.

There are several episodes of ulcer occurring. With severe disease there is virtually constant ulceration and may cause debilitating chronic pain and interfere with comfortable eating. In severe cases, this prevents adequate nutrient intake leading to malnutrition and weight loss.[15,16]The reason is not fully clear, but it is thought to be multifactorial. Aphthous stomatitis is not a single entity but rather a group of conditions with various causes. There have been several attempts to identify the causative organisms but have gone in vain. But aphthous stomatitis appears to be non-contagious, non-infectious, and not sexually transmissible. The mucosal destruction is thought to be the result of a T cell mediated immune response which involves the generation of interleukins and tumor necrosis factor alpha. Mast cells and macrophages are also involved, secreting TNF-α along with the T cells. When early aphthous ulcers are biopsied, the histologic appearance shows a dense inflammatory infiltrate, 80% of which is made up of T cellsPersons with aphthous stomatitis also have circulating lymphocytes which react with peptides 91–105 of heat shock protein 65–60, and the ratio of CD4+ T cells to CD8+ T cells in the peripheral blood of individuals with aphthous stomatitis is decreased.[5]

In a study in the Swedish population, it was shown that aphthous ulcers commonly occurred in the high school and young college students which primarily means it is a disease seen in the second and third decade of life.However the study did not mention of the type of aphthous ulcers noted in the sample. The ulcers occurring in this age group self healed in about 5-8 days time which means that the ulcers belonged to the minor aphthous stomatitis.[17] The study findings are almost matching the results of our study were the minor variant of apthous seems to be the most common variant.

In an epidemiologic study analysing 20,333 people the incidence of aphthous stomatitis was 1.7%. [17] This study results are in line with the results obtained in our study. Further the study had also shown that there was a decrease in the incidence of aphthous ulcers in patients who were smokers. Our study results also seem to be matching the results of the study. The reason for the decrease in the incidence of aphthous stomatitis in smokers is due to the increased keratinization of the mucosa which renders the tissues more resilient to aphthous stomatitis.[18] There have been reports of aphthous ulcerations co-existing with other oral potentially malignant disorders.[19]

Gurkan et al. in 2015 had analysed case records from 2008 to 2019 of pediatric patients and had reported that 120 patients below the age of 18 years had developed the disease.The mean age in his exclusive pediatric study was 9.6 years. The mean duration of the disease as aphthous stomatitis has a tendency to occur, heal and recur was 3.6 years. His study also had shown that minor aphthous stomatitis was the most commonest variant. Their study had reported a significant association between aphthous stomatitis and some systemic problems. The most commonest treatment used in their study was the usage of topical anesthetics. Topical corticosteroids was the second most commonest drug used in the management of aphthous stomatitis.[20] The occurrence of minor aphthous as the commonest variant is relatable to our study where we have also arrived at the similar finding.

Most studies had reported that topical anesthetics were the commonest drugs used in the management of aphthous stomatitis, however topical steroids have been used in the management of major aphthous stomatitis. Nevertheless, corticosteroids are used in the management of immune mediated disorders to reduce the inflammation.[7,8] Apart from this several studies had pointed that the usage of nutritional supplements like Vitamin C and B complex also have a protective role in the management of aphthous stomatitis.[21] There have been no malignant transformations reported in aphthous.

Though this study is done from an Institutional database which may show that the data is from a single centre, there have been several case series and studies in the past published from this database which have proved the versatility and the high usefulness of the data set.[22–25]

Conclusion

In conclusion it is established that aphthous ulcers are one of the most common ulcers of the oral cavity which do not have any malignant transformation potential.Further the most common variant of apthous ulcer is the minor variant of apthous ulcer. Though a majority of cases of aphthous ulcers are seen in the second and third decade of life, our study results as well as literature evidence are unable to predict any sort of an association between age and apthous ulcer. However significantly a higher prevalence of apthous ulcer were seen in the males. The need of the hour would be to develop newer drugs which could probably reduce the recurrence potential of the disease.

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Age and Gender Wise Prevalence of Oral Habits - A Retrospective Study

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Abstract

Oral habits are learned patterns of muscle contraction and have very complex nature. Persistent oral habits beyonds normal age of children have been considered as a significant factor that can lead to malocclusions. A total of 86000 case records between June 2019 to march 2020 were screened and evaluated in which 18656 children less than 18 years were taken into our study. Chi square test was done to estimate the relationship between oral habits with age and gender. The results showed that the overall prevalence of oral habits among children were 3.9%. The highest prevalence rate of oral habits was tongue thrusting (43.5%) followed by thumb sucking (39.1%) and lip biting (17.4%) respectively. Chi square test was done in between age and type of oral habits and the p value was 0.025< 0.05 was termed to be statistically significant. Within the limit of our study, oral habits were detected in less than quarter of the examined population. There was a significant difference for age wise prevalence of oral habits, with younger people indulging more oral habits than elders. Among the types of oral habits, tongue thrusting was the commonest oral habits indulged among the children.

Keywords: *Thumb sucking, tongue thrusting, lip biting, malocclusion, children.*

Introduction

Oral health plays an important role in the general well-being of an individual and there is a strong relationship between dental neglect and oral health [1]. Habits can be referred to as repetitive action that is being done unconsciously. Repetitive behavior is common in the infantile period and most of the children started and finished the habits spontaneously. Oral habits also are learned patterns from muscle contraction and it has very complex nature. They can be associated with hunger, sleep, anger, tooth eruption or fear. These types of oral habits might be non-nutritive sucking such as thumb sucking, finger sucking and tongue thrusting. These habits eventually can cause damage to dento-alveolar structures [2]. The most common oral habit was thumb sucking. The prevalence for thumb sucking was high and it was between 13% to 100% in some societies but it decreased as age increased and these habits mostly should be stopped by the age of 4 years old [3]. There is correlation between child nutrition, sucking habits and level of education among patients [4].

Thumb sucking can be categorised as two types which are active and passive thumb sucking. In active thumb sucking, presence of heavy force by muscles during sucking can cause position of permanent teeth and shape of mandible to be affected if it continues for a long period of time [5]). In passive thumb sucking, no force was involved during sucking so no changes in skeletal or mandible. If this habit has stopped by the age of 5 years, dental changes would be converted automatically. These habits can lead to negative effects such as open bite, overjet and many more. Tongue thrusting normally occured due to delayed transition between infantile and

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adult swallowing patterns. Usually, it will be started around the age of 2 years and by the age of 6 years, 50% of the transition would have been completed. Tongue thrusting may also lead to open bite, cross bite, overjet and class II malocclusions[6]. Our previous research published using endodontics files for obturation quality, have followed the same methodology [7,8,9,10,11,12,13,14]. In children with habits, they are more prone for malocclusion due to increased overjet, which can result in dental trauma [15,16,17,18]. Children in this group show more prevalence of habit and dental caries, therefore habits need to be diagnosed and corrected while fluoride can help in prevention of caries [19,20,21].

Review of the previous literature on oral habits among children showed a wide range of prevalences existing between populations, races, and countries and it is yet to be believed that this difference is due to the various factors such as gender, rank of the child in the family,methods of feeding, socioeconomic status and education [22]. In addition, it has been reported that the prevalence of oral habits are rising in recent times, probably due to the change in the family and social environment. The relative prevalence of oral habits among children in India had been reported to be as low as 3% in North India and 30% in South India[2324]. Owing to this factor, the current study aims to evaluate prevalence of oral habits based on age and gender.

Materials and Methods

A total of 86000 case records between June 2019 to march 2020 were screened and evaluated in which 18656 children less than 18 years were taken into our study. Children diagnosed with some oral habits and who met our inclusion criteria were taken for detailed examination (46). Complete case reports were taken into this study. Incomplete case records were eliminated.Sampling bias minimised by verifying absence or presence of oral habits in case report. All datas was verified by a single trained examiner. A university set up was selected for this study which provided easy accessibility to data and provided a population with similar ethnicity sample population with similar ethnicity. Sample population included in this study were patients having oral habits.

Approval for this study was obtained from the institutional ethical committee (IEC) with approval number of SDC/SIHEC/2020/DIAS DATA/0619-0320. Case records were retrieved from dental hospital management systems in which records of all patients from initial to last were chronologically added. Data was verified. Data regarding age, gender, presence or absence of oral habits were retrieved and tabulated in Microsoft Excel. All data was then transferred to excel tabulation and SPSS importing were done. In SPSS software, independent variables such as gender and age were confirmed. Dependent variables were whether patients had oral habits or not. Calculations were done in Excel Sheets. Tables and bar charts were obtained. Chi square test was done in between age and type of oral habits and the p value was 0.025< 0.05 was termed to be statistically significant .

Results and Discussions

A total of 1168 completed case records which met inclusion criteria were analysed. A total of 46 patients, 26 male (56.5%) and 20 females (43.5%) showed one or the other kind of oral habits. Overall prevalence is 3.9% which is in contrast if compared to the current population, Albanese schools children and Brazilian school children with 2617 subjects [25] showed much greater prevalence of oral habits (87.4%). This finding is similar to study from [26] which reported a low prevalence of oral habits in Nigerian children with a percentage value of 9.9%. There were several other studies done within Indian subcontinent that quote a lower prevalence of oral habits in different population groups can be either due to the difference in the calibration of technicality of the population being examined or geographic location where population is based or the variation in sample size of the examined population .

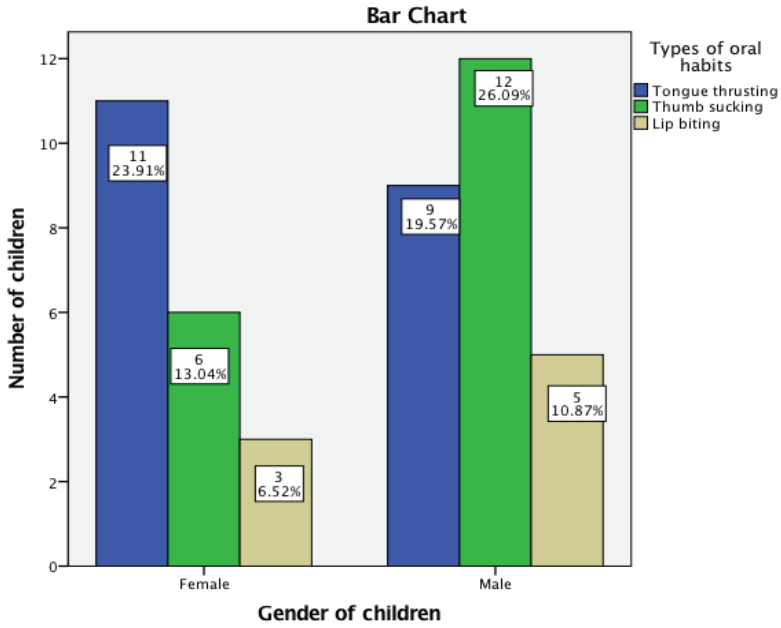
In this study, we have observed that there was statistically significant between age and type of oral habits (p value was 0.025 <0.05). Based on the relationship between age and type oral habits, it could be seen that most of the younger group of children age 7-10 years old experienced tongue thrusting more than other types of oral habits with a percentage of 28.26% . Thumb sucking and lip biting was less frequently experienced by the younger children. Elder group of children aged 11-17 years frequently having thumb sucking habits (21.74%) and tongue thrusting as the second most frequent type of oral habits [Bar chart 1]. Gender wise distribution of oral habits was depicted in [Bar chart 2]. For females,

tongue thrusting was the commonest type of oral habits with a percentage value of 23.91% followed by thumb sucking (13.04%) and lip biting (6.52%) respectively. For male populations, it can be observed that thumb sucking(26.0%) was the commonest type of oral habits developed among them followed by tongue thrusting (19.57%) as the second highest and lip biting (10.87%) as the third highest.

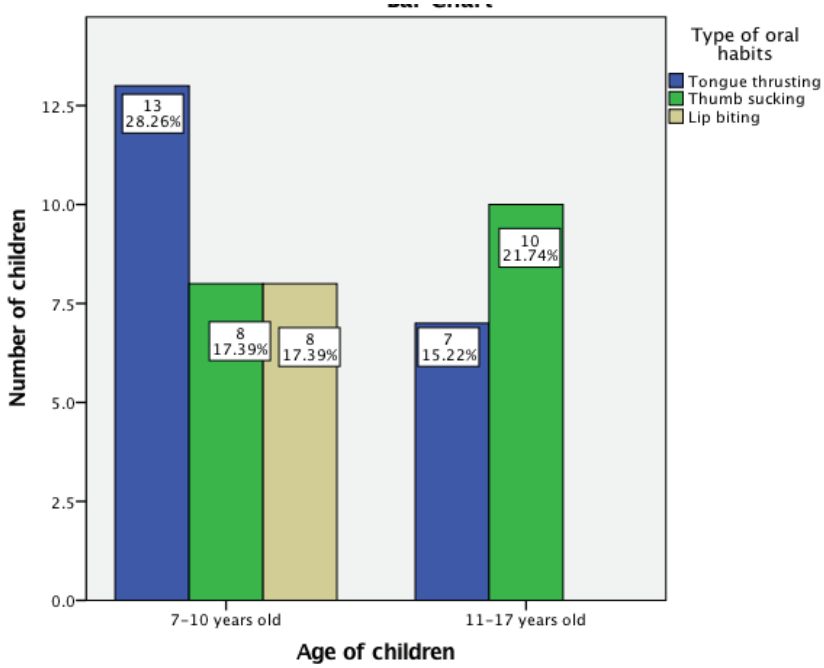
Distribution of type of occlusion based on Angle classification among gender was shown in [Bar chart 3]. There is no difference in terms of prevalent in class II division I among male and female populations . In terms of class I, it can be revealed that male populations are higher than female populations with a percentage value of 47.83% and 34.78% respectively. Based on frequency tables 1, it can be seen that younger children which are below 10 years of age showed higher prevalent towards oral habits compared to elder populations. [Figure 1] shows that male populations with a percentage of 56.5% were commonly developing oral habits compared to female populations (43.%). Based on [Figure 2], tongue thrusting habits (43.5%) were the commonest types of oral habits followed by thumb sucking with a percentage value of 39.1% and lip biting (17.4%) respectively.

The level of education or knowledge among patients plays an important role in controlling deleterious oral habits among children. Other than this factor, socio economic condition of the population also contributed to this habit. The fact that oral habits are the causation of lack of social and emotional security can be correlated to the low socioeconomic group of parents . The degree of modernisation and ability of the child to cope with such society without mental tension can be the significant factor in determining presence or absence of the habits as oral habits also can act as stress relievers. Based on the present study, the highest prevalence rate was registered for tongue thrusting (43.5%) followed by thumb sucking (39.1%) and lip biting (17.4%). This sample is similar to study from Vishnoi et al[27] who reported that tongue thrusting habits have the highest prevalence. Contrastingly, lower prevalence of tongue thrusting was noted in different population groups[28].

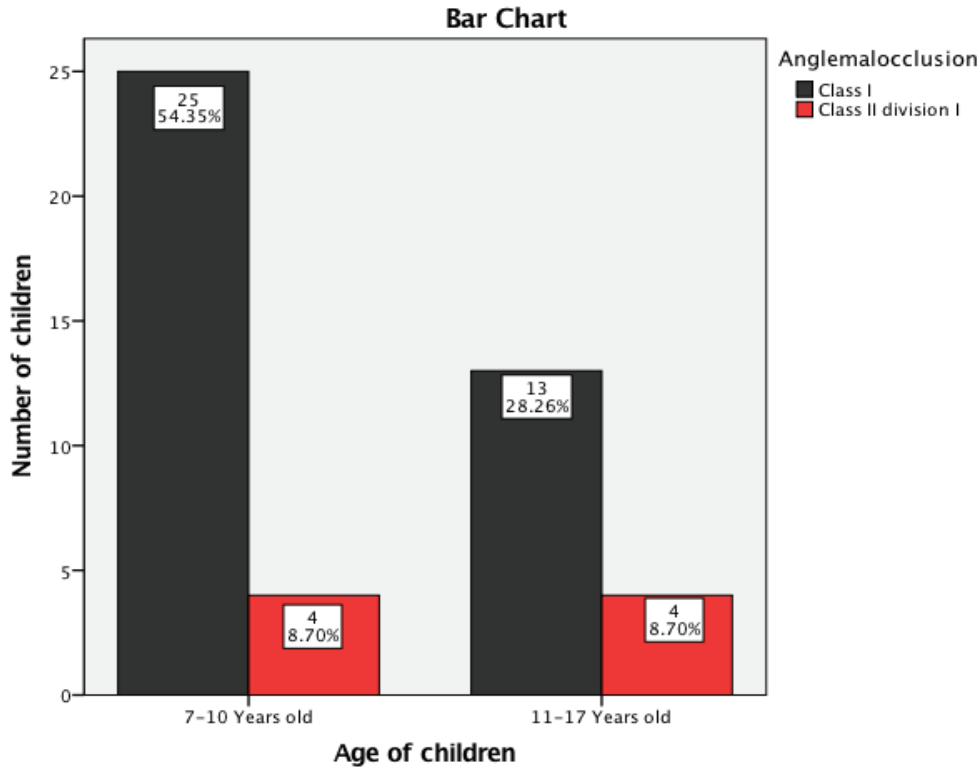
The tongue thrusting habits is known to cause functional imbalance in the oral cavity therefore possibly causing development of malocclusion [29]. The prevalence of thumb sucking in current study is 39.1% . Thumb sucking and lip biting were reported less frequently compared to the tongue thrusting and the results of current study are in agreement with this finding [26]. Even though prevalence is low but there are still warrants the need for further monitoring as thumb sucking is known to cause anterior open bite, posterior crossbite tendency in developing malocclusion. On considering the gender wise distribution of oral habits, statistically nonsignificant differences were found between male and females populations [27]. Few studies in contrast with our findings where they found greater prevalence of oral habits in females rather than male populations [30]. There was a statistically significant difference in the age wise prevalence of oral habits such as tongue thrusting, thumb sucking and lip biting . These habits were more prevalent in younger children. However this finding was in contrast with study from [27] which stated that these habits are more prevalent in the older age group children than younger one. The limitations of this study is that the sample size is small. Therefore, it does not provide results for the entire population. Since it was a retrospective study, there was also possible manual error during data collection.



Bar chart 1 represents association between gender of children and types of oral habits where blue colour denotes tongue thrusting, green colour denotes thumb sucking and light brown denotes lip biting. X axis represents the gender of the children and Y axis represents the number of children. Chi square test was done and the association between gender and oral habits were found to be not significant. P value was 0.377(>0.05). It can be concluded that males experience oral habits more than females although not a significant association.



Bar chart 2 represents association between age of children and types of oral habits where blue colour denotes tongue thrusting, green colour denotes thumb sucking and light brown denotes lip biting. X axis represents the age of the children and Y axis represents the number of children. Chi square test was done and the association was found to be statistically significant. P value was 0.025<0.05. It was observed that children aged 7-10 years old had a higher prevalence for oral habits than children aged 11-17 years old.



Bar chart 3 represents the association between age of children and Angle malocclusion where black colour denotes class I and red colour denotes class II division I. X axis represents the age of the children and Y axis represents the number of children. Chi square test was done and the association was found to be statistically not significant. P value was 0.682(>0.05). Most of the children aged 7-10 years had class I compared to class II division I although not a significant association.

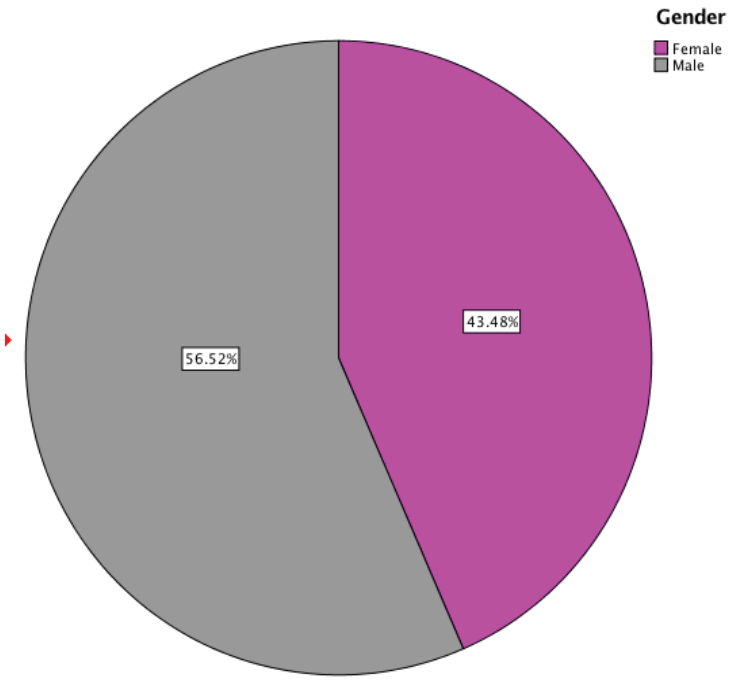


Figure 1: The pie chart shows the distribution of oral habits among different genders of population where light purple denotes female and grey colour denotes male. It can be observed that 56.52% of male populations and 43.48% female populations had oral habits. It was noted that males had a higher prevalence rate for oral habits compared to females.

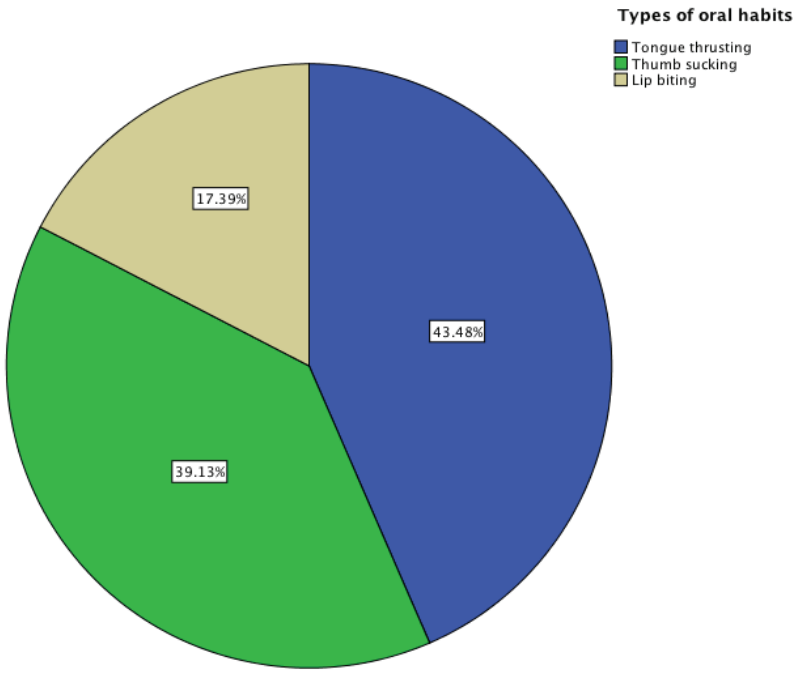


Figure 2: The pie chart shows the distribution of type of oral habits among the sample size population where blue colour denotes tongue thrusting, green colour denotes thumb sucking and light brown denotes lip biting. It can be seen that 43.48% of them had tongue thrusting, 39.13% had thumb sucking and 17.39% had lip biting. It is noted that tongue thrusting was the commonest type of oral habits indulged among the

children compared to thumb sucking and lip biting.

Conclusion

Within the limit of our study, oral habits were detected in less than quarter of the examined population. There was a significant difference for age wise prevalence of oral habits, with younger people indulging more oral habits than elders. Among the types of oral habits, tongue thrusting was the commonest oral habits indulged among the children. These findings warrant the need for educating children and parents about the negative effects from these habits.

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Conflict of Interest : None declared

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Different Types of Keratosis in Patients in a University Setting

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Abstract

The most commonly occurring type of keratosis would be tobacco pouch keratosis or smokeless tobacco associated keratosis, frictional keratosis, a chronic frictional keratosis on the buccal mucosa can lead to something called morscicatio buccarum. Although clinical features are very important and should be one of the important factors in diagnosis, epidemiology should also be considered while arriving at a concrete diagnosis. The aim of the present study was to retrospectively analyze the prevalence of different clinical variants of Keratosis in patients visiting Saveetha Dental College. Retrospective data of 195 patients was obtained and segregated. Inclusion criteria included that they should have visited the University between June 2019 and April 2020. Once the data was obtained and verified, it was statistically analyzed using SPSS by IBM version 20. Most common clinical variant was Tobacco pouch Keratosis (64.6%) followed by Frictional Keratosis (28.7%). Mean age of the patients was 37.40 + or -10.98 years. Tobacco pouch keratosis occurring most commonly in lower arch (67.46%) whereas the Frictional keratosis most commonly in relation to both arches (42.85%). All common in 21-30 years age group. Statistically significant correlation between the site of lesion and variant (p = 0.000) and between gender and variant (p=0.00) Increased Habit Prevalence as the number of Tobacco pouch Keratosis cases are greater. Further multicentered studies and awareness programs to help the society and prevent these lesions.

Key Words: *Keratosis, Oral, Tobacco Pouch Keratosis, Frictional Keratosis, Chennai.*

Introduction

Diagnosis of oral white lesions might be quite challenging. There are various recent trends [1,2]These lesions represent a wide spectrum of lesions with different etiology and various prognosis. In recent years there has been development of various lesions[3–6], developmental anomalies [7,8] and various malignancies [9]. These changing trends can be attributed to the changes in lifestyle and practices[10 11,12] . Although there are various modalities of treatment in recent times[13], [14]diagnosis is very important[15].

The diagnosis of white lesions varies from benign reactive lesions to more serious dysplastic lesions. While there are some classic features that help distinguish these lesions, similar features might give some complications in diagnosis [16]

The onset of oral white lesions can be acquired or congenital. Oral white lesions can be caused by a thickened keratotic layer or an accumulation of non-keratotic material. A gauze should be used to assess if the lesion is scrapable or non-scrapable. If non-scrapable lesions can be attributed to increased thickness of the keratin layer, which might have been induced by local factors which can lead to the formation of different types

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of keratosis [16–18]. Diagnosis is an important aspect. It is important to diagnose a case of keratosis, not as leukoplakia as it is a common error that could result in serious complication [19].

The most commonly occurring type of keratosis would be tobacco pouch keratosis or smokeless tobacco associated keratosis, frictional keratosis, a chronic frictional keratosis on the buccal mucosa can lead to something called morscicatio buccarum [20]. Although clinical features are very important and should be one of the important factors in diagnosis, epidemiology should also be considered while arriving at a concrete diagnosis.

With the recent advent of time there has been varied prevalence of lesions due to changes in lifestyle habits and other factors. It is important to have an updated epidemiological perspective about a particular lesion as there can be potential changes with time. There has not been any study that provides the epidemiological view of most common keratosis as a whole. The aim of the present retrospective cross sectional study is to evaluate the prevalence of different types of keratosis in patients that visit a dental college (Saveetha Dental College and Hospitals) in Chennai.

Materials & Methods

The present retrospective study was conducted among 195 patients who were clinically diagnosed with a variant of keratosis. The study performed in the university setting (Saveetha Dental College and Hospitals, Chennai, India) thus the data available is of patients from the same geographic location and have ethnicity. The ethical approval for the retrospective data that was collected from the dental archives of the Department of Oral Medicine and Radiology, Saveetha Dental College was obtained from the Institutional Ethics Board

(SDC/SIHEC/2020/DIASDATA/0619-0320).

The data was collected and studied for the period from June 2019 to April 2020. Once the data was obtained, the same was verified with the help of photographs by two external reviewers to limit and restrict any aspect of bias towards the present study. A well-defined elaborate inclusion criterion was laid out before the commencement of the present retrospective study. The inclusion criteria that was constructed included:

•The patient should have visited during the period of the study and treated by a resident of Saveetha Dental College (Undergraduates or Postgraduates)

•Should have been clinically diagnosed with a variant of keratosis.

On segregation of all available samples, all non specific data entries such as double entries and other types of censored data were excluded from the present study. Thus the obtained master data sheet was verified by another external reviewer. The data was tabulated and the same was analyzed using SPSS by IBM version 20. The frequencies and cross tabulations were performed followed by correlation and association using chi square test to check correlation between the different variables. The results thus obtained were analyzed.

Results and Discussion

A total of 195 patients were included as a part of the present retrospective study, out of which 178 patients were males (91.3%) and 17 patients were females (8.7%) (Graph 1). The lesion was present most commonly in the lower arch (55.4%) than being present in relation to both arches (31.8%) and upper arch (12.8%). The most commonly occurring clinical variant of keratosis was Tobacco Pouch Keratosis (64.6%) followed by Frictional Keratosis (28.7%) and least occurring was morscicatio buccarum (6.7%) (Graph 2). The mean age of the patients who had keratosis was 37.08 + or – 10.98 years. When the gender distribution of each clinical variant is observed, all three lesions were more prevalent in males than females . In males the most commonly occurring clinical variant was Tobacco Pouch Keratosis (67.97%) followed by Frictional Keratosis (26.40%). In females the most commonly occurring clinical variant was Frictional keratosis (52.94%) followed by Tobacco Pouch Keratosis (29.41%). A statistically significant ($p = 0.005$) correlation was observed between gender and clinical variant of Keratosis . When age groups are considered, Tobacco Pouch Keratosis was most common in the age group of 21- 30 years (35.71%) (Table 1), Frictional Keratosis was also most common in 21 -30 years (30.35%), similar age group common for morsicatio buccarum (46.15%) as well. statistically

significant ($p<0.05$) correlation was obtained. When location or site of the lesion was being considered, Tobacco Pouch Keratosis was present more in lower arch (67.46%) (Graph 3). Frictional Keratosis occurs most commonly in relation to both arches (42.85%) and morscicatio buccarum also occurs most frequently in relation to both arches (69.23%). Statistically significant correlation was obtained between the location of the lesion and clinical variant ($p = 0.000$).

It is surprising that even though Keratosis is one of the most commonly occurring oral lesions, yet there are cases of misdiagnosis. This is the first of its kind study where the prevalence of different types of keratosis are seen in geographic location. The three clinical variants of Keratosis that are reported in the geographic location of the present study are Tobacco Pouch Keratosis, Frictional Keratosis, Morscicatio buccarum. Out of these three, tobacco pouch keratosis is related to tobacco usage [21] whereas the other two are not directly related to tobacco usage.

In the present study it is observed that the tobacco pouch keratosis is more prevalent in males than in females. This can be attributed to the fact that there is more usage of tobacco products among the male gender than the female gender as reported by Bhawna G et al [22] in a national survey conducted on tobacco consumption. Tobacco pouch keratosis or smokeless tobacco induced keratosis develops on habitual chewing or due to snuff dipping tobacco [23]. In the present study the most common area in the oral cavity that is associated with tobacco pouch keratosis is the lower arch. This can be attributed to the fact that the most common area associated with placing tobacco in the lower vestibule. The most common age group for Tobacco Pouch Keratosis is 21-30 years as reported by Poornima Chandra et al [24]. This age group has an increase of smokeless forms of tobacco and thus this could be the reason for prevalence of the lesion in this age group. When compared with the findings in different parts of India, the following are observed. In a study conducted by KA Kamala et al [25] in Maharashtra, it is observed that the prevalence in males is greater than in females which is in agreement with the findings of the present study. Similar findings recorded by Rakhi Chandak et al [25] in a Vidharbian population which also showed a male prevalence. Manjiri Joshi et al [25,26] in a study in central Gujarat concluded that there is an increased usage of smokeless tobacco than smoking and Tobacco Pouch Keratosis was associated with the same which is in line with the findings of the present study when age grouping and area is considered. In a study conducted by Anuna Laila Mathew et al [27] in 2007, where oral mucosal lesion prevalence is seen in the South Indian population, there is no report of even one patient with Tobacco Pouch Keratosis although tobacco users are included in the study. The present study is also placed in a south Indian population, thus the results from the previous study are contradictory to that of the present one. This can be associated with the trend change in tobacco usage through the years. Thus when the trends are compared with that of the rest of the country, it is almost in agreement with the findings of the present study. Now, let us assess the prevalence on a global scale. Hakan Mornstad et al conducted a study in Sweden which also showed a male predilection for the disease. In Sudanese population, A.M Idris et Al [28] reported that the lesional site in the majority of cases with tobacco pouch keratosis was the lower labial mucosa which is in agreement to the findings of the present study. However when we consider underdeveloped countries like Africa OA Ayo Yusuf et al [29] report an increased occurrence in an older age group greater than 60 years. Even though the common site is the lower arch is in agreement with the findings of the present study, the age prevalence is in disagreement. This can be attributed to socioeconomic status, but also gives an insight into tobacco dependence in the particular region. Thus a globally uniform regimen is brought about to educate the population and prevent the occurrence of such lesions.

The next most prevalent clinical variant in the present study is Frictional Keratosis and the least prevalent is morscicatio buccarum, which is nothing but a more chronic form of frictional keratosis of the buccal mucosa [30]. Parafunctional habits whereby there is constant rubbing, chewing or sucking of the oral mucosa against the teeth can result in the formation of this clinical variant . The occurrence of Frictional Keratosis is markedly more in males than in females in the present study. This can be attributed to more rigorous chewing habits in males which may lead to local factors like tissue chewing or malposition of teeth. Usually there is however a female predilection [31]

The most common age group for the occurrence of frictional keratosis in the present study is 21 – 30 years. This can be explained by the fact that there is an increase in the prevalence of parafunctional habits during this age group [32] which may in turn lead to the development of a clinical variant of Keratosis.

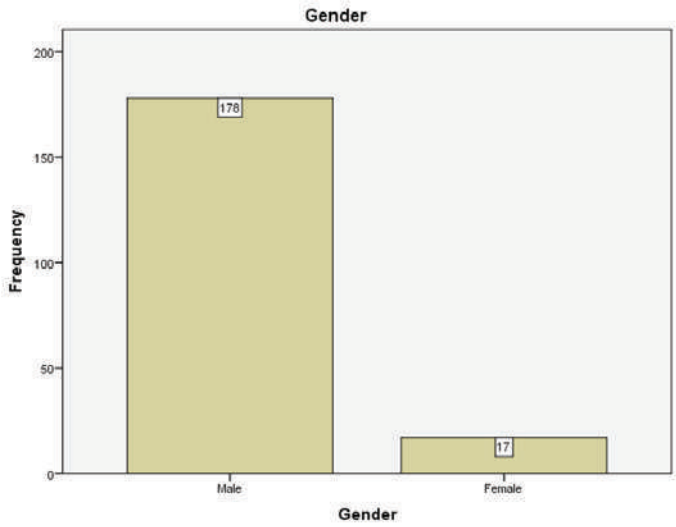
When compared with the knowledge that is available regarding the same in other parts of India, the following are observed. N C Sandeepa et al [33], in a study that was conducted on a college population observed that males are affected more commonly by Frictional Keratosis than females. This is in line with the findings of the present study. In the study of Rakhi Chandak et al [33,34] in vidharbian also there was same gender predilection. In the study by Anuna Laila Mathew et al [27] the age group in which frictional keratosis was more common was 21 – 40 years age group which is in line to the age group prevalence that is reported in the present study. However in the study carried forward by G. Sree Vijayabala et al [35] it is observed that there is an increased occurrence in older age groups, this can be attributed to further factors faulty restorations, prosthesis etc. Thus when the entire nation is concerned, the age category is in mild disagreement whereas there is clear agreement on prevalence of gender.

When we compare the same on a global level, the following observations are seen. In a study involving the population of Slovenia, Marija Kovac et al [36]reported that females are more common to present with Frictional Keratosis than men. The most common age group was reported to be in 65 – 75 years, both aspects are contradictory to the findings of the present study. This could be attributed to the geographic location and practice patterns. FC Macigo et al [37] reported in a Kenya population that males are more prone to Frictional Keratosis than females. Thus it is evident from the above discussion that there is no specific gender predilection for Frictional Keratosis. However, there is disagreement on age prevalence as well, further multicentered studies are to be done to gain concise knowledge and improve diagnosis and treatment.

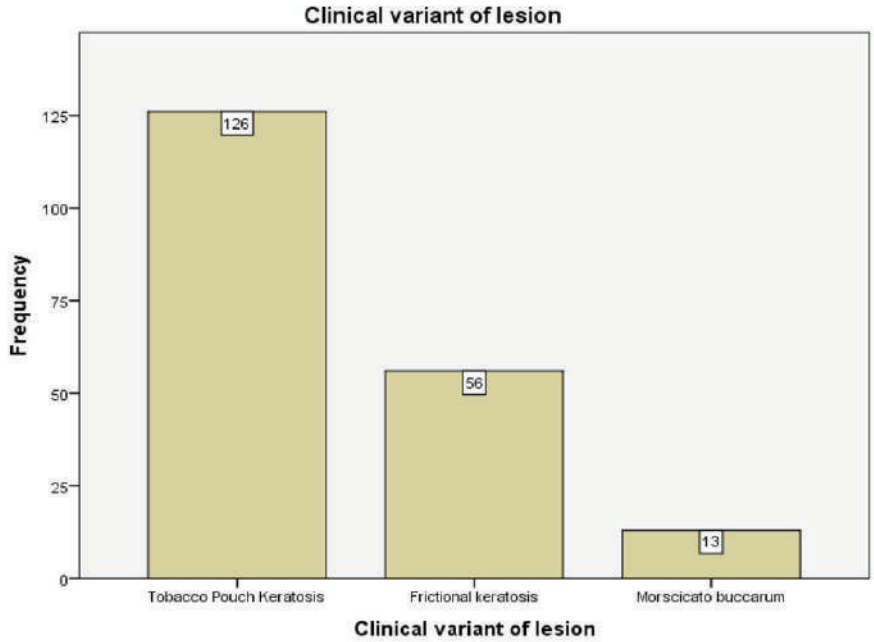
There are very few studies which compare the prevalence of Tobacco Pouch Keratosis and Frictional Keratosis. Rakhi Chandak et al [34]reported that there is an increased occurrence of Frictional Keratosis than Tobacco Pouch Keratosis. However, as in the present study it is the other way around. This raises alarm as it possibly implies that there is an increased usage of smokeless tobacco. Further studies are to be done to gain appropriate knowledge and combat the knowledge and combat the issue.

The limitations of the study included that it was single centered, geographically isolated and habit history was not taken into account.

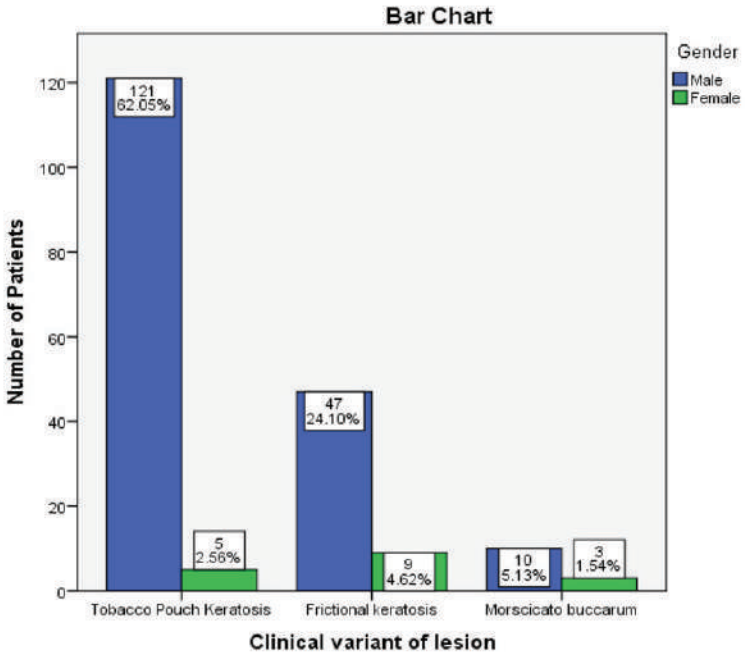
1. Tables And Graphs:



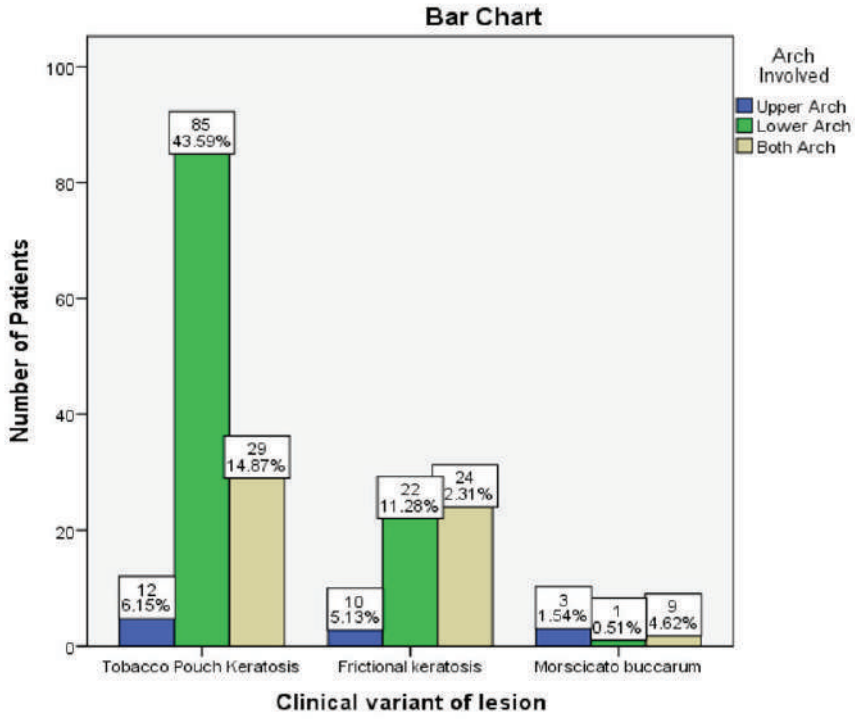
Graph 1:Bar Graph shows Distribution of keratosis in Male and females with the gender in the x axis and the frequency in the y axis. The most common gender to be affected is the male gender (91.28%)



Graph 2:Bar Graph shows Distribution of different clinical variants of lesions with the clinical variant in the x axis and the frequency in the y axis. The most common clinical variant in the present study is Tobacco Pouch Keratosis(64.61%)



Graph 3:Graph shows association between gender and different clinical variants of keratosis with the clinical variant in the x axis and number of clinical variants in the y axis. Males are represented in blue and females are represented in green. All clinical variants are seen more commonly in males than in females. In males the most common clinical variant is Tobacco Pouch Keratosis (62.05%) whereas the most common clinical variant in females is frictional keratosis (4.62%). Statistically Significant association is seen using chi square test with Value=10.745, df=2, p=0.05 (p≤ 0.05)-Statistically Significant.



Graph 3: Bar graph shows association between the location of the lesion and the clinical variant of keratosis with the clinical variant in the x axis and number of lesions in the y axis. The upper arch is represented in blue colour, lower arch in green colour and both arches in brown colour. Tobacco Pouch Keratosis is seen more commonly in the lower arch (43.59%) whereas Frictional Keratosis is seen more commonly associated with both arches (12.31%). Statistically Significant result is obtained using Chi Square test with Value= 27.087, df=4 and p=0.000(p≤0.05)-Statistically Significant.

Table 1: Table shows association between age and the clinical variant of the lesion. All three clinical variants occur most commonly in a younger age group. Statistically significant association using Chi Square test, Pearson's Chi Square Value=110.66, df=86, p=0.03

Age Groups							
Clinical Variant	**21-30 Years**	**31-40 Years**	**41-50 Years**	**51-60 Years**	**61-70 Years**	**Total**	
Tobacco Pouch Keratosis	45	39	30	11	1	126	**p value 0.03**
Frictional Keratosis	17	12	15	9	3	56	
Morsicatio Buccarum	6	2	3	2	0	13	

(p≤ 0.05)-Statistically Significant

Conclusion

The present study has certainly raised certain alarms, as in the limits of the study Tobacco Pouch Keratosis is more prevalent than Frictional Keratosis. Further multicentered studies to be done and awareness programmes to prevent the formation of such lesions and help the entire community.

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Conflict of Interest: No conflict of Interest.

Source of Funding : Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Vesiculobullous Lesions: A Hospital Based Retrospective Study

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Abstract

Vesiculobullous lesions, characterized as mucocutaneous, have a significant impact on quality of life when compared to other conditions, as they can lead to pain and difficulty in eating and speech, depending on the extent of the ulceration. The overlapping pattern of clinical presentation of vesiculobullous lesions in the oral mucous membranes makes it difficult for the clinicians to arrive at a proper diagnosis. Hence careful evaluation of clinical, histological and immunofluorescence data aids the clinician to arrive at a proper diagnosis. The aim of this study is determine the prevalence, age and gender variations of vesiculobullous lesions pertaining to oral mucosa among patients who have visited Saveetha Dental College, Chennai, India. A retrospective study was conducted and data collection was done from dental archives pertaining from June 2019 to April 2020. Data consisted of details of patients who presented with vesiculobullous lesions of the oral mucosa. Data was imported to IBM SPSS Version 20 for statistical analysis. Results were tabulated. Prevalence of vesiculobullous lesions pertaining to the oral cavity was more in females (51.8%) compared to males. (42.8%). Prevalence was more in patients belonging to the fourth decade of life (30.1%). Herpes labialis was found to be more prevalent among the vesiculobullous lesions seen in the oral cavity. Lesions were present in both the arch (44.6%) followed by lesions pertaining to only upper arch. Associations in this study were not statistically significant ($p>0.05$). The association between age and gender shows prevalence of herpes labialis to be the highest among all the age groups. There are very minimal studies done on the prevalence of vesiculobullous lesions pertaining to the oral cavity. In this study it has been found that vesiculobullous lesions of infectious origin (Herpes labialis) were found to be more prevalent compared to vesiculobullous lesions of autoimmune origin. This study was conducted in a single centre. Extensive multi centre study is to be done.

Keywords: *Vesiculobullous lesions, Mucocutaneous lesions, Herpes labialis, Vesicle, Bulla, Blisters*

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Introduction

Vesiculobullous lesions are a type of mucocutaneous disease that is characterised by vesicles and Bullae or blisters and secondary ulcers or erosions. Both vesicles and bullae are fluid-filled lesions, and they are distinguished by size, vesicles being less than 5–10 mm and bulla being larger than 5–10 mm, In the case of vesiculobullous diseases which are also immune disorders the term immunobullous is sometimes used.[1–3] These diseases, characterized as mucocutaneous, have

a significant impact on quality of life when compared to other conditions, as they can lead to pain and difficulty in eating and speech, depending on the extent of the ulceration.[4–8] Vesiculobullous disorders form a challenging domain for treating clinicians due to its varied presentation. The pattern of blistering disorders varies in different age groups. The etio-pathogenesis of vesiculobullous lesions can be categorised as inherited disorders, infectious, inflammatory, autoimmune, reactive and metabolic disorders.[9–11] Bulbous autoimmune dermatoses have a common pathogenic mechanism involving binding of autoantibodies to specific adhesion molecules in epidermal desmosomes and in some cases in the area of dermo-epidermal basement membrane zone. The binding of circulating autoantibodies and the induction of an inflammatory reaction in the area of target structures lead to loss of adhesion with subsequent intra or sub epithelial blister formation.[12–15]

The vesiculobullous lesions occurring in the oral cavity are bullous lichen planus, herpes simplex infection, pemphigus, bullous pemphigoid, bullous systemic lupus erythematosus, varicella-zoster infections, erythema multiforme, measles, mucous membranes pemphigoid, hand foot and mouth disease, paraneoplastic pemphigus, toxic epidermal necrolysis is and Stevens Johnson syndrome, dermatitis herpetiformis, herpangina, linear IgA disease and epidermolysis bullosa [1,16–18] The overlapping pattern of clinical presentation of vesiculobullous lesions in the oral mucous membranes makes it difficult for the clinicians to arrive at a proper diagnosis[19]. Hence careful evaluation of clinical, histological and immunofluorescence data aids the clinician to arrive at a proper diagnosis.[20–23] Clinicopathological correlation is always an essential prerequisite before coming to any final diagnosis.[24–27]

This study is done for epidemiological significance to check the current trends in prevalence of vesiculobullous lesions of oral mucosa among patients who visited Saveetha Dental College, Chennai, India and have undergone treatment for the same. The main aim of the study is to check the frequency distribution of different types of vesiculobullous lesions of the oral mucosa prevalent in the population. To determine the prevalence pattern of vesiculobullous lesions pertaining to oral mucosa among different age groups and gender. To determine the intra oral site (maxilla, mandible, both maxilla and mandible) that is most commonly affected. To determine if gender and age has any statistically significant association with the prevalence and type of vesiculobullous lesions involving oral mucosa. To determine if there is a statistically significant association between the type of vesiculobullous lesion and site involved – maxilla / mandible / both maxilla and mandible and also to check if there is any statistically significant association between age and gender and site involved.

Materials and Methods

Study setting:

A retrospective study was conducted in Saveetha Dental College, Chennai, Tamilnadu, India, by obtaining data from dental archives (single centre study). Ethical approval was obtained from the institutional ethics committee (Approval number: SDC/SIHEC/2020DIASDATA/0619-0320).

Sampling, data collection and tabulation:

Non probability convenience sampling method was employed. The data included records of patients who presented with vesiculobullous lesions in the oral mucosa; the type of vesiculobullous lesions and the location of the lesion. Data entries from June 2019 to April 2020 were obtained for the same and were tabulated. All the available data was included (without any sorting process) to reduce sampling bias. Data was analysed and censored data was excluded. The data was then verified by one external reviewer. A data of 83 patients (males – 48.2% ; females – 51.8%) 6 years – 66 years was obtained.

Data Analysis

The tabulated data was statistically analysed by IBM SPSS Version 20 to find the prevalence of vesiculobullous lesions among different age groups, gender, location – maxilla, mandible, both maxilla and mandible; type of vesiculobullous lesions – infectious type, inflammatory type; also find correlations between the type of vesiculobullous lesion with gender, age of the patient and location of the lesion – upper arch, lower arch and both arch. Data was imported and variables were analysed. Pearson's Chi square test was used. Results were tabulated and bar charts were plotted.

Results and Discussion

Age and gender:

Among the 83 patients, 43 (51.8%) were females and 40 (48.2%) were males. (Graph 1). 1 patient (1.2%) was less than 10 years, 1 patient (1.2%) belonged to the second decade, 14 patients (16.9%) belonged to the third decade, 25 patients (30.1%) belonged to the fourth decade, 17 patients, (20.5%) belonged to the fifth decade, 17 patients (20.5%) belonged to the sixth decade and 8 patients (9.6%) belonged to the seventh decade. (Graph 2)

Type of vesiculobullous lesion and location of the lesion:

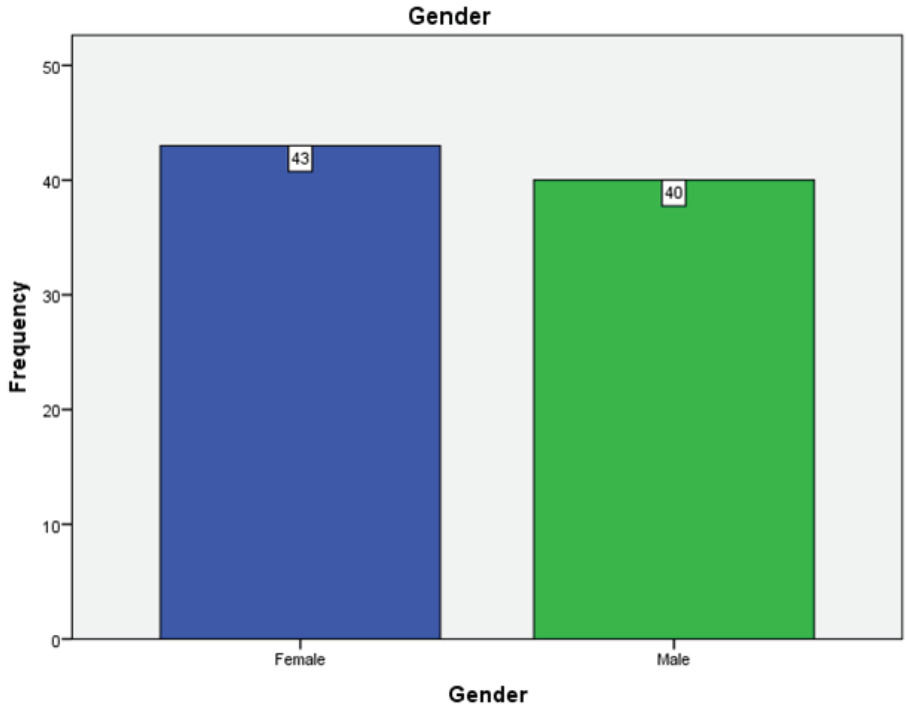
Among the 83 patients, 58 patients (69.9%) had herpes labialis, 5 patients (6%) had angina bullosa haemorrhagica, 1 patient (1.2%) had herpes zoster, 1 patient (1.2%) had bullous lichen planus, 7 patients had autoimmune vesiculobullous lesions (Pemphigus/ Pemphigoid / linear IgA disease/ dermatitis herpetiformis. 6 patients (7.2%) had primary herpetic gingivostomatitis, 3 patients (3.6%) had recurrent herpetic gingivostomatitis and two patients (2.4%) had secondary herpetic gingivostomatitis (Graph 3). 37 patients (44.6%) had lesions involving both in maxilla and mandible, 11 patients (13.3%) had lesions pertaining only to mandible and 35 patients (42.2%) had lesions involving only maxilla.(Graph 4)

Associations:

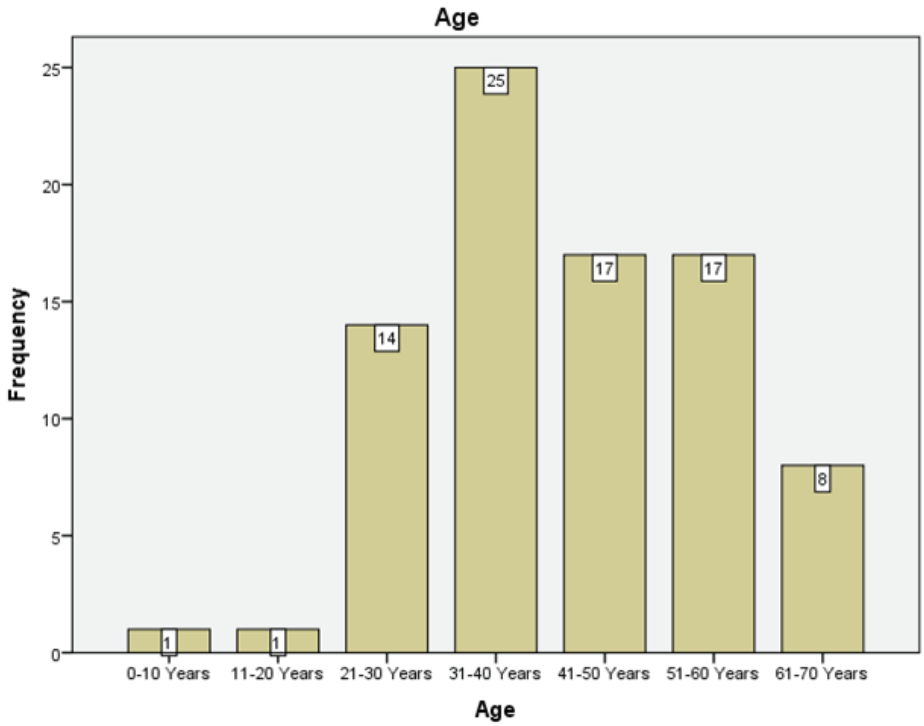
The association between gender and age is not statistically significant ($p>0.05$) but there is a female Predilection observed from the fourth decade (Graph 5). The association between age and the type of lesion shows an increased prevalence of herpes labialis in all age groups (Graph 6). The association between the location of the lesion and type of lesion shows that almost all types of vesiculobullous lesions occur in the upper arch followed by lesions occurring in both the arch. Herpes labialis most commonly affects both arch followed by upper arch. Herpes labialis in the lower arch is comparatively less (Graph 7). The association between gender and type of lesion shows that herpes labialis is the most prevalent type of vesiculobullous lesion seen in both males and females (no gender predilection) (Graph 8). All these associations are statistically insignificant ($p>0.05$).

In this study it is seen that females had more prevalence (51.8%) compared to males (48.2%) (Graph 1). This is in accordance with the studies done by Pavithra P et.al.,[28] Maharshank et.al.,[29] where females had more prevalence of vesiculobullous lesions compared to males. This may be due to genetic predisposition of female gender to vesiculobullous lesions. Hence, researching gender differences and the mechanisms of immune diseases is important to arrive at a conclusion. It is observed that prevalence was more in patients belonging to the fourth decade (30.1%) followed by the fifth and sixth decades (20.5% in each group) (Graph 2). In this study herpes labialis was found to be the most prevalent vesiculobullous lesion (69.9%). This shows that vesiculobullous lesions of infectious etiology is more prevalent than vesiculobullous lesions of autoimmune type. This is in accordance with various studies where the incidence of vesiculobullous lesions of infectious etiology was found to be ranging from 35.6% to 85.2%[30,31] The study results are in accordance with the study done by Embil JA et.al.,[32] where prevalence of herpes labialis is more in males than females. In the present study, 55.2% of patients with herpes labialis were males. This is also in accordance with several other studies.[33,34] The possible reason for male predominance may be due to habits and its role as a risk factor in prevalence of herpes labialis.[35,36] There is no significant association between gender, age; age, type of lesion; location and type of lesion; and gender, type of lesion.

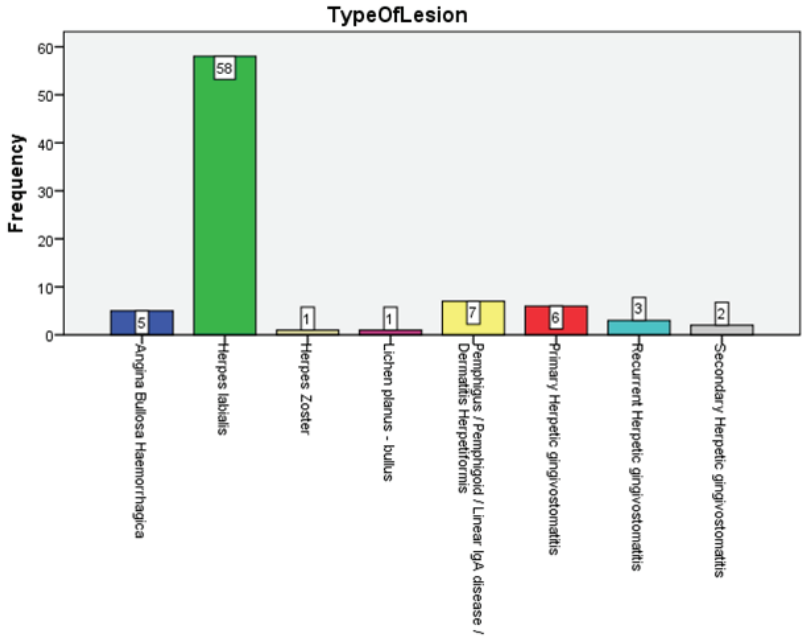
The study is a single centered study and samples were collected from a fixed time frame. Extensive research to be conducted – multi centre approach with a larger time frame to improve the scope of research. Also to evaluate the impact of geographical variations, race and habits in the prevalence, pattern and type of vesiculobullous lesions involving oral mucosa. The methodological problems faced during descriptive epidemiological studies are: case finding, using data sources such as hospital records, dental records and histopathological records often produce ascertainment bias, selection bias or both and multiple comparisons problems (i.e., the chance of occurrence of statistically significant findings). The resultant incidence and prevalence rates from studies with inadequate designs or inadequate data are limited and may be misleading.



Graph 1: Bar graph depicting the frequency of gender variations in patients with vesiculobullous lesions. X-axis shows gender and Y-axis shows the number of patients with vesiculobullous lesions. Blue colour denotes females and green colour denotes males. Prevalence was found to be more in females (51.8%) compared to males (48.2%).

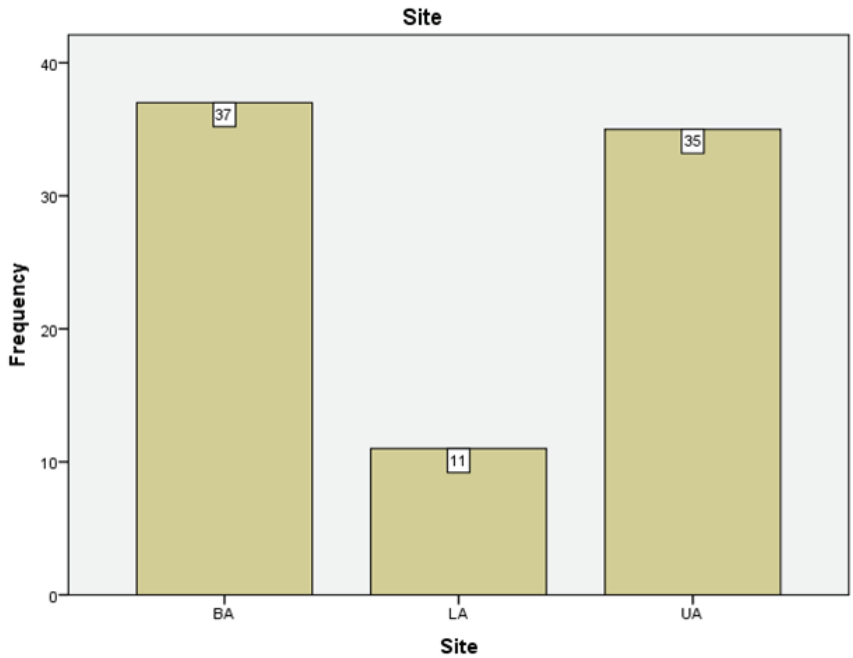


Graph 2: Bar graph depicting the frequency of vesiculobullous lesions among different age groups. X-axis shows the age groups and y -axis shows the number of patients with vesiculobullous lesions. Prevalence is more in the 4th decade (30.1%).



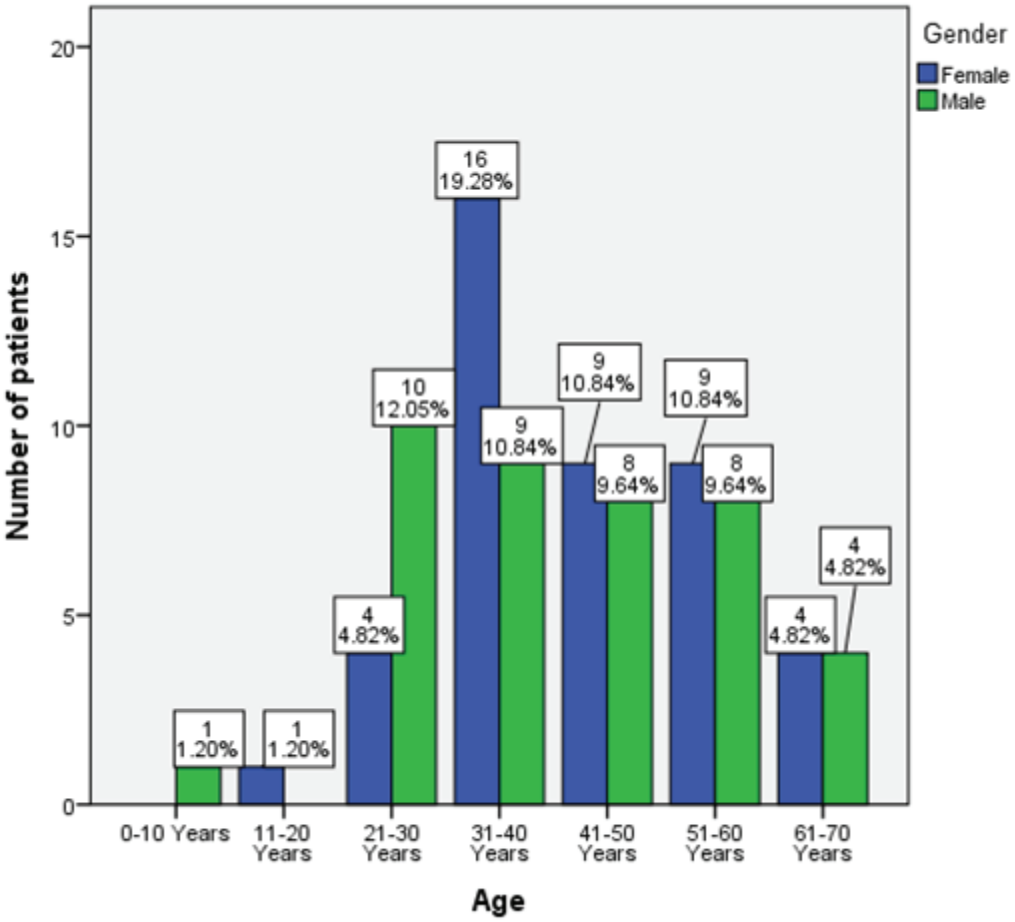
Graph 3:

Bar graph depicting the frequency of various vesiculobullous lesions in the oral cavity. X-axis shows the different vesiculobullous lesions and Y-axis shows the number of patients with vesiculobullous lesions. Blue colour denotes angina bullosa haemorrhagica, green colour denotes herpes labialis, beige colour denotes herpes zoster, purple colour denotes bullous lichen planus, yellow colour denotes pemphigus / pemphigoid / linear IgA disease / dermatitis herpetiformis red colour denotes primary herpetic gingivostomatitis, teal colour indicates recurrent herpetic gingivostomatitis and grey colour denotes secondary herpetic gingivostomatitis. Herpes labialis was found to be more prevalent (69.9%).

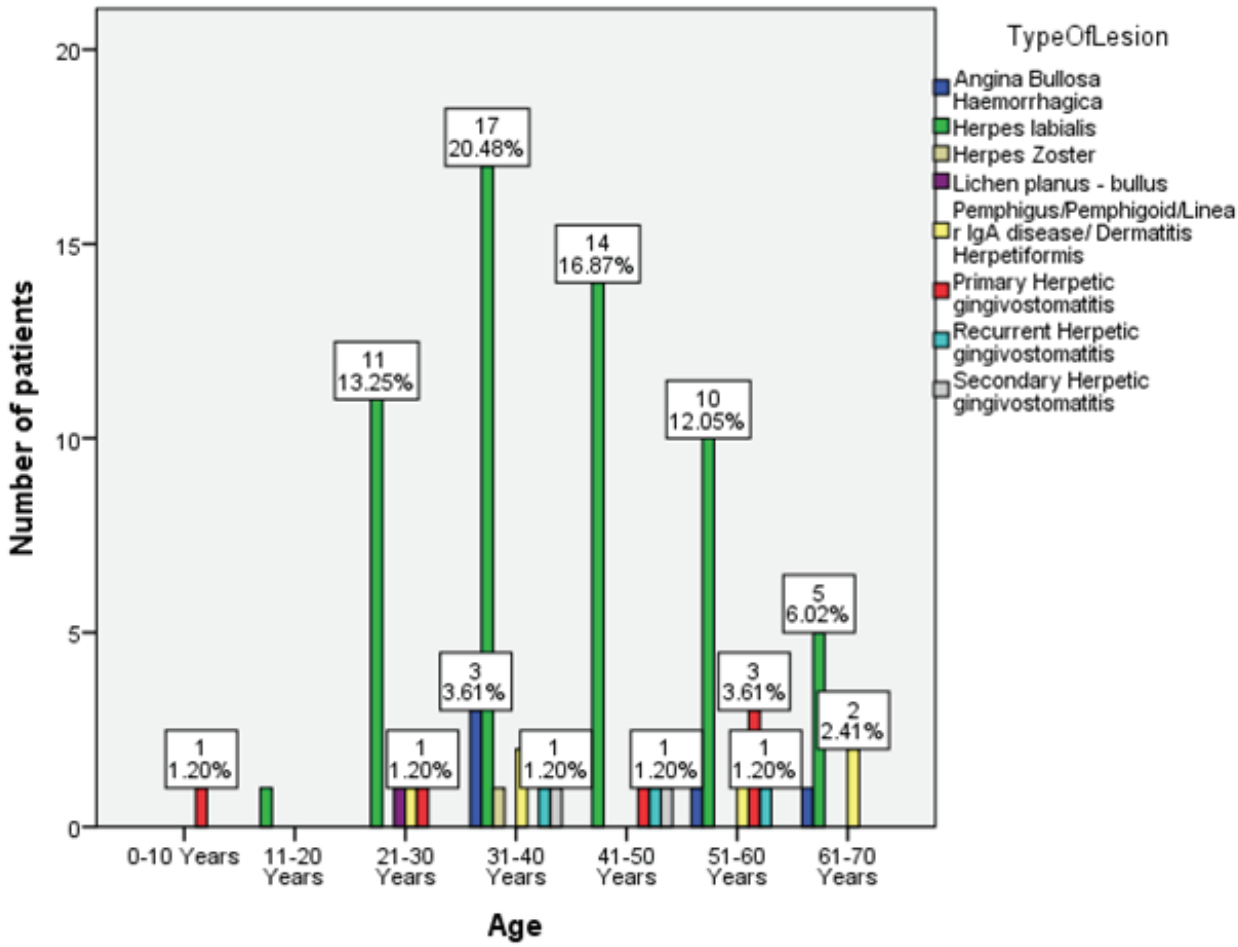


Graph 4:

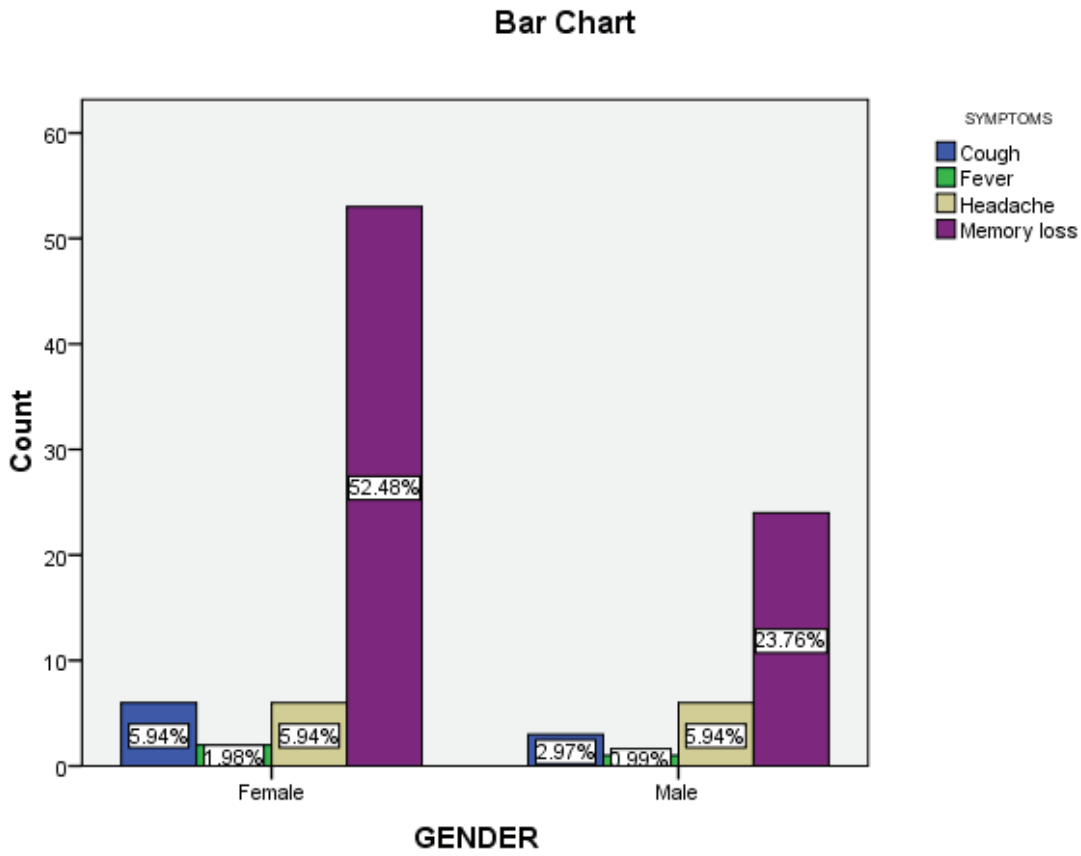
Bar graph depicting the site of prevalence of vesiculobullous lesions. X-axis shows the site of occurrence and Y-axis shows the number of patients with vesiculobullous lesions. It is commonly present in both the arches (44.6%).



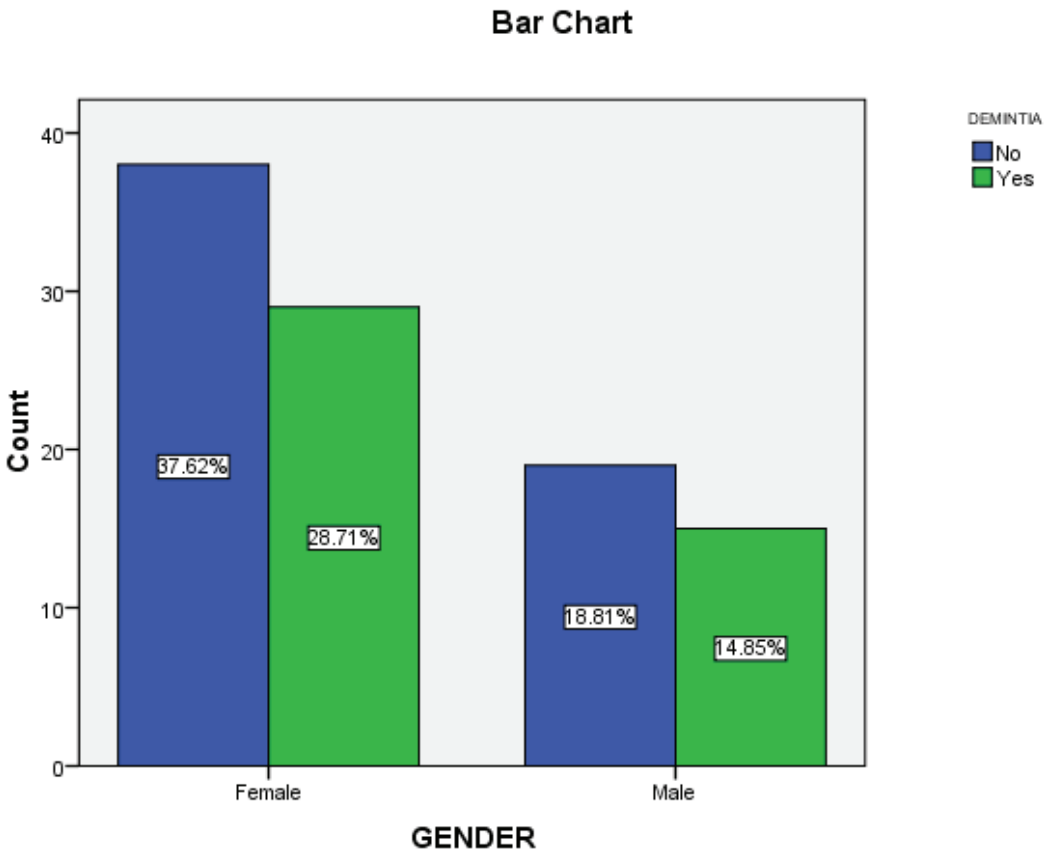
Graph 5: Bar graph depicting the association between age and gender. X-axis shows different age groups with gender variations and Y-axis shows the number of patients with vesiculobullous lesions. Blue colour denotes females and green colour denotes males. Prevalence among females was more in the 4th decade. Among males, prevalence was more in the third decade (12.50%). Prevalence was equal among both the genders in the 7th decade (4.82%) Chi-square test, P value 0.365; (>0.05 - statistically not significant).



Graph 6: Bar graph depicting the association between age and type of lesion. X-axis shows the different age groups and types of vesiculobullous lesions and Y-axis shows the number of patients with vesiculobullous lesions. Blue colour denotes angina bullosa haemorrhagica, green colour denotes herpes labialis, beige colour denotes herpes zoster, purple colour denotes bullous lichen planus, yellow colour denotes pemphigus / pemphigoid / linear IgA disease / dermatitis herpetiformis red colour denotes primary herpetic gingivostomatitis, teal colour indicates recurrent herpetic gingivostomatitis and grey colour denotes secondary herpetic gingivostomatitis. Herpes labialis is more prevalent in all age groups with highest prevalence in the 4th decade of life (20.48%). In the first decade, primary herpetic gingivostomatitis is seen (1.20%). Chi-square test, P value 0.652; (>0.05 - statistically not significant).



Graph 7: Bar graph depicting the association between site and type of lesion. X-axis shows the site of occurrence and type of vesiculobullous lesion. Y-axis shows the number of patients with vesiculobullous lesions. Blue colour denotes angina bullosa haemorrhagica, green colour denotes herpes labialis, beige colour denotes herpes zoster, purple colour denotes bullous lichen planus, yellow colour denotes pemphigus / pemphigoid / linear IgA disease / dermatitis herpetiformis. red colour denotes primary herpetic gingivostomatitis, teal colour indicates recurrent herpetic gingivostomatitis and grey colour denotes secondary herpetic gingivostomatitis. Almost all lesions are more prevalent in the upper arch. Herpes labialis is the more prevalent vesiculobullous lesion and it occurs in both the arch (32.53%). Chi-square test, P value 0.213; (>0.05 - statistically not significant).



Graph 8: Bar graph depicting the association between gender and type of lesion. X-axis shows gender and the type of vesiculobullous lesion. Y-axis shows the number of patients with vesiculobullous lesions. Blue colour denotes females and green colour denotes males. It is observed that herpes labialis (38.55%) and primary herpetic gingivostomatitis (4.82%) was more prevalent in males compared to females. All the other lesions were more prevalent in females. Chi-square test, P value 0.143; (>0.05 - statistically not significant).

Conclusion

From this study it has been found that vesiculobullous lesions are prevalent more in females than in males and in patients belonging to the 4th decade of life. Herpes labialis was found to be the most common vesiculobullous lesion occurring in the oral cavity with increased prevalence in males. The study was conducted in a single centre. Extensive multi centre study to be done.

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Conflict of Interest: No conflict of Interest.

Source of Funding: Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Graphs

Type of Study- Retrospective Study *DOI Number: 10.37506/ijfmt.v14i4.12500*

Prevalence of Different Patterns of Temporomandibular Joint Ankylosis in South Indian Population

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Abstract

The aim of this study was to identify the incidence of different patterns of temporomandibular joint ankylosis in the south Indian population. Case records of a total of 86000 patients between June 2019 and March 2020 were collected and analyzed from patient records, out of which a total of 7 cases of mandibular temporomandibular joint ankylosis cases who had undergone treatment for the same were identified and included in the present study. All these were checked retrospectively for pattern/type of ankylosis by using the radiographs with Sawhney's classification. Results show that there's a significant male predilection of about 85.7% and shows more unilateral ankylosis cases of about 57.1%. Based on Sawhney's classification it shows 14.3% of type - 1 cases, 42.9% of type - 2 cases, 28.6% of type - 3 cases and 14.3% of type - 4 cases. Within the limitations of this study, it showed that there was a male predilection, unilateral ankylosis cases were common, and among all ankylosis cases Sawhney's type 2 temporomandibular ankylosis cases were high in number.

Keywords: *TMJ, Temporomandibular joint, Ankylosis, TMJ Ankylosis.*

Introduction

The temporomandibular joint is a bilateral, diarthrodial joint in the maxillofacial region. The joint is formed by the bony articulation of mandibular condyle and the temporal bone [1]. Temporomandibular joint ankylosis is a pathologic condition where the mandible is fused to the fossa by the bony or fibrotic tissues. This interferes with mastication, speech, oral hygiene, and normal life activities, and can be potentially life-threatening when struggling to acquire an airway in an emergency. Attempting to open the mouth, stretching the periosteum can also result in pain [2].

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There are multiple factors that can result in temporomandibular ankylosis such as trauma, arthritis, infection, previous Temporomandibular surgery, congenital deformities, idiopathic factors [3], and iatrogenic causes. Trauma is the most common cause of temporomandibular joint ankylosis, followed by infection [4]. Temporomandibular ankylosis in growing patients can result in dentofacial deformity.

Diagnosis of temporomandibular joint ankylosis is usually made by clinically examining the patient and imaging studies, such as plain films, orthopantomogram, computed tomography (CT) scans, MRI, and three-dimensional reconstruction.

Ankylosis can be classified as true (intra-articular) and false (extra-articular). True ankylosis has been classified as type I, II, III, and IV by Sawhney's based on his experience with interpositional arthroplasty using an acrylic cylinder in 70 cases of bony ankylosis of temporomandibular joint [5].

Materials and Methods

Case records of a total of 86000 patients between June 2019 and March 2020 were collected and analyzed from patient records, out of which a total of 7 cases of mandibular temporomandibular joint ankylosis cases who had undergone treatment for the same were identified and included in the present study.

The radiographs were collected for all the cases and were taken for pre-treatment diagnostic purposes. This is a descriptive, cross-sectional and retrospective study in which all the radiographic images are screened by using examiner for the identification and classification of type/ pattern of temporomandibular joint ankylosis

Inclusion criteria:

- Patients with temporomandibular joint ankylosis.
- Dentulous & Edentulous patients.

Exclusion criteria:

- Patients with reduced mouth opening with etiology other than ankylosis.
- Patients with temporomandibular joint pathology other than ankylosis.

Results and Discussion

The result shows the incidence among temporomandibular joint ankylosis patient as Type 1 as 14.3%, type 2 as 42.9%, type 3 as 28.6%, and type 4 as 14.3% also the case of temporomandibular joint ankylosis which is unilateral is of 57.1% and bilateral is of 42.9 %.

It showed temporomandibular joint ankylosis cases were more common in males (85.7%) than females (14.3%) with mean age as 23.29 +- 23.5 years.

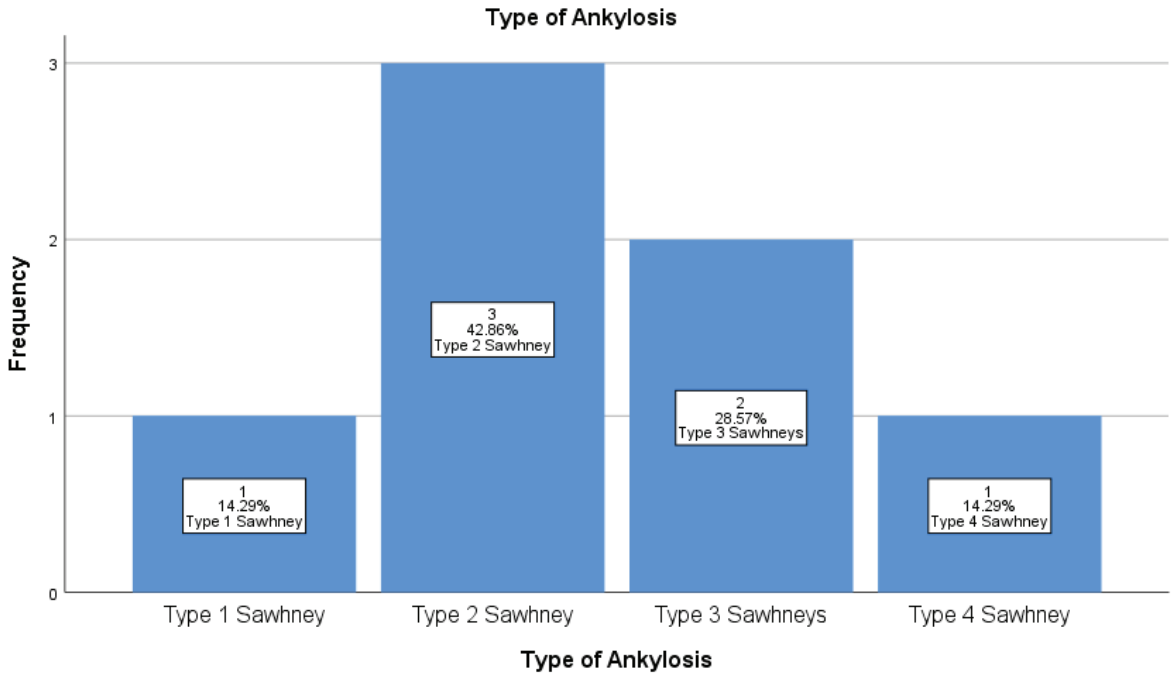


Figure 1: Bar graph shows the distribution of type of ankylosis in frequency and percentage among cases of temporomandibular joint ankylosis. The graph shows that there are more number of Sawhney's type 2 cases with 42.86% followed by Sawhney's type 3 with 28.57%, type 1 and 3 with 14.29%.

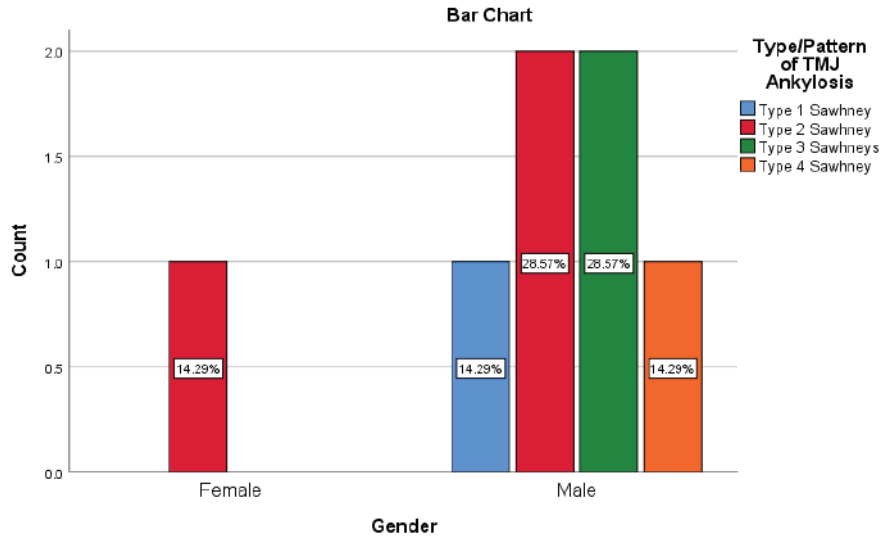


Figure 2: Bar chart representing the association between the type of ankylosis and gender distribution in temporomandibular joint ankylosis cases. The X-axis represents gender and Y-axis represents the number of patients and color-coding used for type/pattern of temporomandibular joint ankylosis with blue for type 1, red for type 2, green for type 3, and saffron for type 4 in Sawhney's classification of temporomandibular joint ankylosis. Males showed more numbers of type 2 and type 3 patterns of sawhney's type of temporomandibular joint ankylosis. Chi-square test was done and the association was found to be statistically not significant. Pearson's Chi-square value: 1.556, P-Value: 0.670 (>0.05) hence statistically non-significant, proving gender does not have any association with the type of temporomandibular joint ankylosis.



Figure 3: Bar chart representing the association between the type of ankylosis and involvement of the number of joints in temporomandibular joint ankylosis cases. The X-axis represents the involvement of the joints and Y-axis represents the number of patients and color-coding used for type/pattern of temporomandibular joint ankylosis with blue for type 1, red for type 2, green for type 3, and saffron for type 4 in Sawhney's classification of temporomandibular joint ankylosis. Chi-square test was done and the association was found to be statistically not significant. Pearson's Chi-square value: 7.000, P-Value: 0.072 (>0.05) hence statistically non-significant, proving that the unilateral or bilateral involvement of the joints does not have association with the type of temporomandibular joint ankylosis.

Dentistry comprises practices related to the oral cavity. Oral diseases are a major problem among the general population and there are various procedures carried out to prevent and treat them. Oral health has a direct impact on general health patterns as it helps to talk, eat, and feel confident [6].

Bony ankylosis of temporomandibular joint is a disabling disease that is not confirmed just to the first two decades but can occur in later stages of life also [7]. Different causes have been attributed to the condition condylar fracture with the involvement of articular surfaces, advanced arthritis, and trauma from obstetric forceps [8]. Radiography is an essential diagnostic tool for TMJ ankylosis. Current methods include panoramic radiography and CT. Recently, the value of three-dimensional CT (3D-CT) before surgery has been advocated [9].

Ankylosis can be classified into intracapsular/ articular or true ankylosis extra-articular or false ankylosis. Intra-articular ankylosis has been classified into four types as Type I, II, III, and IV [5].

Type I occurs where the condyle is medially angulated and associated with deformed articular fossa together with a mild to moderate amount of new bone formation. the head was flattened or deformed but lay closely approximated to the upper articular surface. There are injury-producing fracture-dislocation to head and neck of mandible and laceration of capsular ligaments

Type IV is found when the joint architecture is replaced completely by bone with a fusion of the fibrous adhesions all around the joint, making the movement impossible. This is probably followed by a comminuted fracture of the head of mandible/condyle.

Type II is found where there is no recognizable condyle or fossa but instead a large mass of new bone extending from the ramus to the base of the skull. The head is misshapen or flattened, but it is still distinguishable and lay in close approximation to the articular surface. There is, however; Bony fusion of the head to the outer edge of the articular surface either anteriorly or posteriorly, but this is limited to small areas. Deeper to this, the upper articular surface and articular disc are undamaged. this probably was followed by a severely comminuted fracture of the head with associated partial damage to the upper articular surface

Type III ankylosis usually results from medially displaced fracture-dislocation with bone bridging the mandibular ramus to the zygomatic arch. A bony block is seen to bridge across the ramus of the mandible and the zygomatic arch. the upper articular surface and articular disc and deeper aspect are intact. The displaced head is seen to atrophic and laying either .free are fused medial side of the upper end of the ramus. This is probably followed by a severe condyle. Sigmoid notch and coronoid process to the zygomatic arch and glenoid fossa. The Bony block was wide and deep and extended between the ramus and the upper articular surface, completely replacing the architecture of the joint. this perhaps followed. fracture of the neck of the mandible with dislocation of the head and associated injury to the capsule ligaments, Articular disc, and even the upper articular surface

It is possible to reconstruct the series of events and changes that would have taken place from infliction of trauma to the development of the bony ankylosis. Trauma leads to the development of Bony ankylosis. Trauma leads to the fracture of the head or neck of the mandible with or without dislocation of the head, disruption of the capsular ligament, Articular surface, and /or articular disc as well as adjoining periosteum, and accompanying hemarthrosis. Also, Cancer cells exhibit a wide range of genetic alterations that include gene rearrangements, point mutations, and gene amplification, leading to disturbances in molecular pathways modifying cell growth, survival, and metastasis [1011]. This is followed by a varying period of restricted mobility or complete immobility of the joint because of pain. The hematoma organizes, and the ensuring fibrosis in and around the joint leads to further restriction of mobility and gradual bone formation across the joint or between the upward displaced ramus and the zygomatic arch from the disrupted periosteum. The bone formation varies with the severity of the injury and disruption of the structures, i.e; varying from thin bony spicules across the joint to complete replacement of the joint by a bony bridge extending from mandibular ramus to upper articular and/ or the zygomatic arch. The bony bridge maybe 1 ¼ inch across and more than 1 inch in thickness [5,7].

The upward displacement of ramus reduces the apparent vertical height of the mandible. and in unilateral cases produces facial asymmetry and deviation of the chin to the affected side, which starts appearing relatively full. In bilateral cases, it causes the rescission of the chin and open bite. restriction in the range of

mouth opening and persistent efforts by depressors in the mandible (digastric mylohyoid) produces marked notching in the lower border of the mandible in front of the insertion of the masseter and medial pterygoid. This is especially marked during childhood and adolescence. The presence or absence of dental malocclusion depends on the relative position of the teeth and their fixation following trauma [1213]. With the rise of mandibular ramus by the upward pull of masseter and the pterygoid on the affected side there is relaxation of temporalis, masseter, and medial pterygoid on the affected side, which over a period of months and years become atrophic from disuse and get contracted. These changes in muscles may also occur on the normal side. This interferes with immediate restoration of the full range of mouth opening after temporomandibular joint arthroplasty because of the inability of these muscles to stretch to normal dimensions. Several factors may influence the perception of pain as it is a complex process. Dental pain and needs to be considered more often as a possible diagnosis also [14151617].

Therefore, it is recommended that coronal and axial CT are essential in identifying the relationship of these vital structures to the ankylosed bone mass. It is important for surgeons to improve their knowledge to enable the diagnosis and management of patients to have a more positive attitude toward these patients [181920]. It can be recommended that the choice of investigation can depend on the size of the lesion [2122]. These findings do not match the results of the previous literature, but this difference may be due to a change in geographical distribution which plays the role in the change in etiological factor, socioeconomic status of the patients we recommend more detailed and long term followup to include more numbers of cases.

Conclusion

Our result shows there is significant male predilection with more number of unilateral temporomandibular joint Ankylosis cases than bilateral and also higher incidence of Sawhney's type 2 temporomandibular joint followed by type 3 and type 1 & 4 respectively. And, there is no association between type of temporomandibular joint ankylosis and gender or type of temporomandibular joint ankylosis with the number of joints involved.

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An Assessment of Complete Denture Changes from Try in to Clinical Insertion- A Retrospective Study

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Abstract

Complete denture is a dental prosthesis that replaces the entire dentition and associated structures of maxilla and mandible. Its Function is to restore esthetics, mastication and speech.Various outcomes in the treatment of complete denture in edentulous patients involve a complex array of different factors which determine the accuracy of complete denture. Due to various reasons complete dentures may encounter changes from trial stage to insertion. This change may occur due to processing or handling .The present study aims to assess the differences in complete denture from try in anterior teeth position to insertion anterior teeth position with regard to midline shift and plane deviation and using chi square test statistical significance was observed. The study was carried out in Saveetha dental college, Chennai, patients data was collected from inhouse digital data bank. The data of documented evidence treated by the undergraduate students were retrieved and analysed for differences. The study reveals that there are incidences which had changes from the try in step to clinical insertion step with regard to anterior teeth.

Key Words: *Complete dentures; anterior plane; facial midline; processing error.*

Introduction

Complete denture deals with the replacement of dentition and its associated structures of maxilla and the mandible.Treating a complete denture patient is an essential resource for general dental practitioners, dental students and laboratory technicians.[1]Various outcomes in the treatment of complete denture in edentulous patients involve a complex array of different factors.[2] Prognosis of the complete denture success depends on developing an occlusion that is compatible with functional movements of the stomatognathic system. Clinically for establishment of ideal occlusion, one of the important factors that help us is the orientation of the occlusal plane. The determination of angulation of the occlusal plane is a vital clinical step in the construction of the complete dentures for the edentulous patients. The correct orientation of the occlusal plane of the complete denture will result in better denture stability. Use of stable complete dentures helps to avoid the transfer of undue stresses to the underlying residual ridges, retardation of their resorption, better aesthetics achievement in natural smile and function of final prosthesis.[3] Midline position of complete dentures is more important and it plays an important role in esthetics.[4] Computer engineered complete dentures are also used.[5] Patient dissatisfaction inadequate retention were the most common complications with computer engineered complete dentures.[6] It is important to establish a harmonious occlusion in a maxillary complete denture opposing natural occlusion.[7] One of the common problems faced in prosthetic dentistry is fracture of maxillary acrylic complete denture.[8] The commonest reason is because

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of great masticatory forces.[9] Poor laboratory techniques, use of porcelain teeth are also other causes for fracture of dentures. [10] The mandibular posture and occlusion should be recorded carefully.[11] At each visit, the soft tissues and the denture fit must be checked.[12] Retention and support of the dentures must be assessed.[13] Before giving for processing , teeth arrangement should be checked properly to avoid deviation.[14] Occlusal stability plays an important role in case of complete dentures , it is obtained by achieving maximum number of tooth contacts during artificial teeth positioning in wax.[15] There are various processing errors that occur during fabrication of complete denture.[16] It includes thickness of denture, separating media, improper ratio of monomer and polymer.[17] Before giving the dentures for processing, the occlusion should be checked.[18] The teeth movement after arrangement should not be present.[19] The movement of teeth during processing of complete dentures disturbs the harmonious occlusal scheme established at the final wax try in stage.There are two main factors responsible for occlusal discrepancies in the processed denture , change in relationship of teeth to master cast during processing as a result of investing procedure, careless packing of acrylic resins in the mould cavity or improper flask closure and wrapage of the denture base due to the inherent strains when the denture is separated from the cast.[20] The aim of the present study is to assess the complete denture from try in anterior teeth position to insertion teeth position.

Materials and Methods

The present study is done by screening the patients digital case sheets. The search for digital case sheets was from june 2019 to february 2020. The records searched close to 4068 reporting to clinics with the complaint of multiple missing teeth in upper and lower arch. The search was narrowed down for edentulous condition in upper and lower arch with documentation evidence at try in stage and insertion stage. The search led to 255 patients' details of both try in and insertion. The digital documentation was evaluated for changes in anterior plane and midline shift from trial stage to insertion stage. The details were screened by 2 expert reviewers. Those undergoing complete denture treatments and with adequate documentation details were involved in the study. This study was done retrospectively to assess at how many instances there are processing errors which tend to deviate from the trial stage. The study included only anterior teeth changes and no other changes with regard to processing error was considered. The scoring was added as try in stage anterior plane or midline shifted as yes or no, and the same parameter with scoring at insertion stage was done. The data was screened and relevant data was subjected to statistical analysis.

Results and Discussion

In the present study , the total sample size obtained is 255 out of which 153 are males and 102 were females i.e. 60.23 % were males and 40% were females. Majority of them belong to the age group of 61 to 70 years. More male patients were completely edentulous .When comparing the changes from try in step to insertion step, 241 complete dentures had no changes i.e. 94.9% of them were correct and the remaining 14 (5.1%) showed anterior plane deviation. While analysing midline shift in 210 patients 83% had no change from tryin stage to insertion whereas 45 patients 17% showed a deviation of midline from try in to insertion. (Figures 1,2,3)

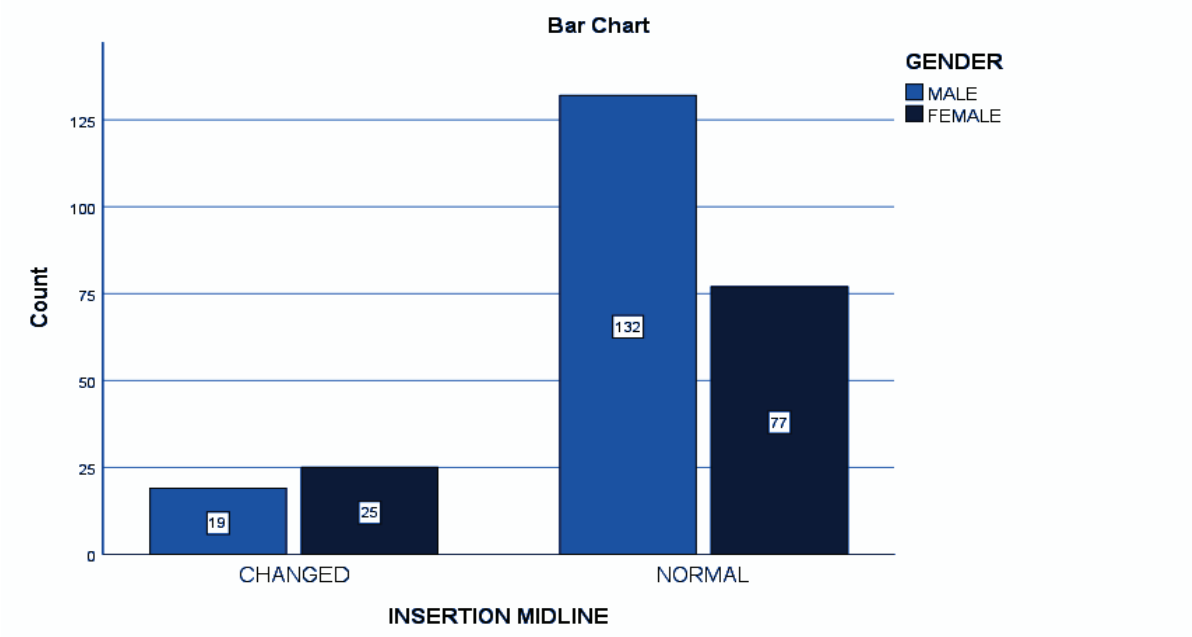


Fig 1- Bar Graph Indicating Changes or Deviation from Trial Stage to Insertion Stage In comparison to Gender. X axis denotes the Presence or absence of changes in denture and Y axis denotes the deviation. Red color represents Female gender and Blue Colour represents Male gender. Chi square association was and found to be statistically significant. Pearson chi square value - 65.392; p value= 0.020; p<0.05 which is significant. There are more changes associated with females in comparison to males

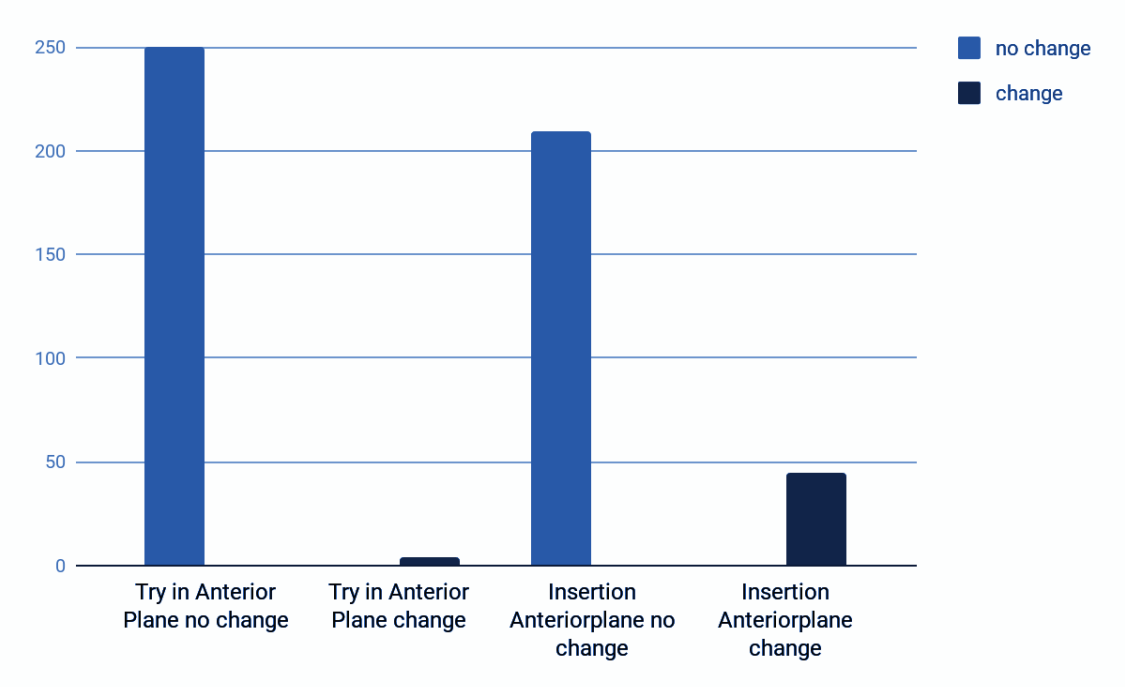


Fig 2- Bar Graph Indicating Anterior Plane Change Deviation from Trial stage to Insertion Stage.X axis denotes the Anterior Plane Evaluation and Y axis denotes the Changes. Chi square test was done and found to be statistically significant Pearson chi square value of 4.663, p=0.031(p < 0.05) significant. There is a significant change in comparison from trial stage to insertion stage with regards to Anterior Plane.

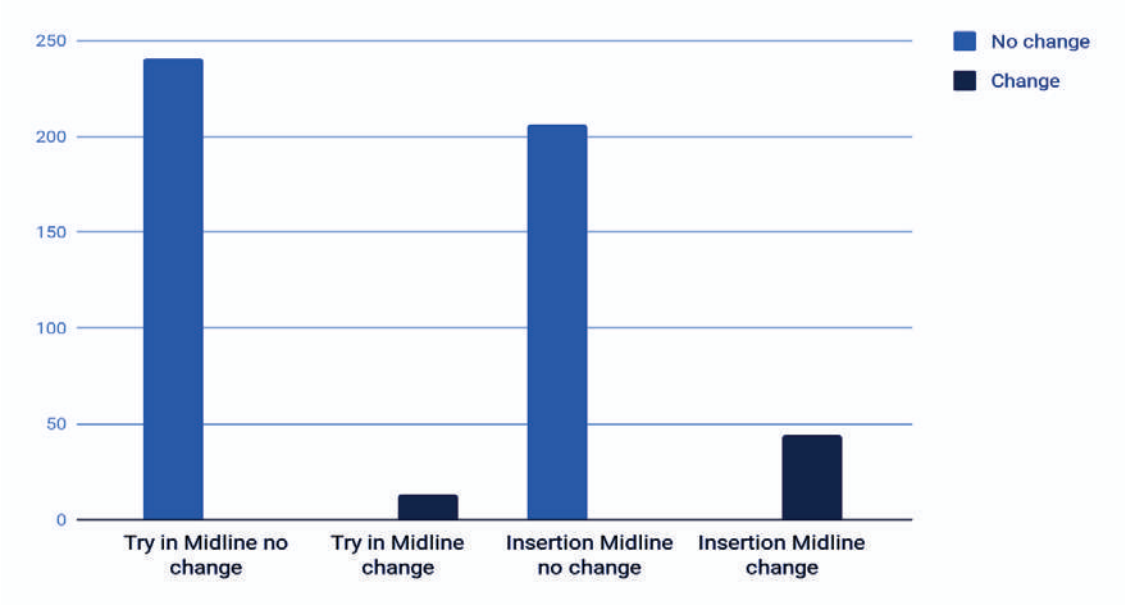


Fig 3- Bar Graph Indicating Mid Line Change Deviation At Trial Stage And At Insertion Stage. X axis denotes the Midline Evaluation and Y axis denotes the Changes. Chi square association was done and found to be significant. Pearson chi square value of 68.815, p= 0.000 (p < 0.05) - significant. Proving that there was a significant change in comparison from trial stage to insertion stage with regard to the Midline.

Komal et al conducted a study on processing induced tooth displacement and occlusal changes in complete denture.In their study, various articles were selected and assessed on the basis of various factors and variables involved in complete denture processing that could contribute to tooth movement.Various factors like flasking and type of investing material used, packing, curing and deflasking , finishing were discussed in the study.[21] Abuzar et al did a study about tooth movement during processing of complete dentures and its relation to palatal form.Tooth movement during processing of acrylic resin complete dentures was investigated in relation to palatal form.Eleven clinical casts were radiographed at four denture processing stages.Seven points were located on each cast. Radiographs were digitized and the images obtained were processed and analysed for precise measurement of tooth movement.A palatal form index was developed with respect to a relationship between tooth movement and palatal form, certain trends were found with varying degree and on the base material and the dimensional changes that occurred during processing.[22] Jamani et al did a study based on the effect of denture thickness on tooth movement during processing of complete dentures.A maxillary cast of a patients was duplicated to obtain 10 identical casts .Base plates were constructed on five casts using 1.25 mm thick wax and other 5 casts by 2.5 mm.Teeth were placed on the ridge of the main cast and TMJ pins was placed vertically upright in each tooth and radiographed before processing.The results showed that there are significant variations in tooth movement between thick and thin dentures.[23] Michelle et al did a study based on the effect of monomer content in monomer polymer ratio on complete denture teeth displacement. In this observation, there were no statistically significant differences between the group with monomer content recommended by the manufacturer groups with 25 % less monomer, in both conventional and microwave polymerisation.[24] Rafael et al did a study based on influence of storage on dimensional changes in maxillary acrylic denture bases and effect on tooth displacement.Thirty maxillary dentures were manufactured and processed using 3 different curing cycles, long , short conventional and microwaved. Distances between fixed points on the teeth were measured and the dentures stored at room temperature for 24 weeks.After storage, the distances are again measured in the dentures then stored in water at 37 degree Celsius for 24 weeks and then reevaluated. Anteroposterior distances demonstrated contraction in all acrylic resins.[25] Emel Dervis did a study about

clinical assessment of common patient complaints with complete dentures .The study investigated the relationship between patient complaints with complete dentures and several factors like age, gender, medical status and denture faults.This study included 600 patients who received new dentures were assessed three months after insertion of the denture.Patient complaints were scored according to the answers to the specific questions.It was observed that there is no significant relationship between patient complaints and age, gender and medical status. Statistically significant relationships were observed between denture construction faults or condition of patient's denture bearing mucosa.[26] Van Waas conducted a study based on the influence of clinical variables on patient's satisfaction with complete dentures. It was observed that the effect of the technical quality of complete dentures and the physical condition of the mouth on patients' satisfaction was investigated in 130 patients who received new complete dentures. A moderately positive correlation was found between satisfaction of the patient and quality of the dentures. There was no correlation found between the satisfaction of the patient and the physical condition of the mouth.[27,28] The present study indicates there is an anterior midline shift which is statistically significant $p<0.05$ and aslo anterior plane deviation at insertion in comparison to trial stage, which was statistically significant $p< 0.05$ - chi square test. (Table 1&2) The reasons elicited could vary from packing techniques, excess packing material to curing techniques or even midline shift at trial stage itself. The previous literature cited too gives similar reasons for denture insertion differences. The study gives an insight as to the anterior teeth lateral shift and plane shift seems to be present and that can be more attributed to excess packing material during flasking stages.

Conclusion

The Present retrospective clinical study indicates a definitive change in anterior teeth position from trial stages to insertion stage. The change is associated with complete dentures midline shift and anterior plane and is more commonly observed in female gender.

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Incidence of Pocket Depth More than 3 MM in Maxillary Premolars and Molars with a Class II Restoration

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Abstract

Aim: To study the incidence of periodontal pocket depth more than 3mm in maxillary premolars and molars with class 2 restorations. Introduction: A class II restoration of a tooth and its status of periodontal health are closely related. A healthy periodontium is required for the proper functioning of all restorations while the functional stimulation due to dental restorations is essential for periodontal protection. Materials and Method**s**: It is a retrospective study in which we reviewed patient records and analysed the data of 86000 patients between June 2019 and March 2020, out of these patients we collected the data of 885 patients who had class II restoration done.The data was compiled into class II restorations with pocket depth more than 3 mmm and less than 3 mm, reviewed, tabulated and exported to SPSS software for statistical analysis. Result**:** Incidence of pocket depth more than 3mm in Class II amalgam restorations was 3.42% out of 614 samples and in Class II LCR restorations was 3.69% out of 271 samples. In comparison of incidence of pocket depth more than 3 mm in both amalgam and composite class II restorations amalgam is 2.37% and composite is 1.13%. Conclusion: Within the limits of study, it can be concluded that incidence of pocket depth more than 3mm in maxillary premolars and molars with class II amalgam restoration was less (3.42%) compared with composite (3.69%). However on comparison between amalgam and composite the mean pocket depth was more in amalgam.

Keywords*: Periodontal pocket;class II restoration ; amalgam; composite; pocket depth*

Introduction

Dental restoration and periodontal health are closely related, a healthy periodontium is needed for the proper functioning of all the restorations while the functional stimulation due to dental restoration is essential for periodontal protection.[1,2] An adequate treatment must take into account to carry out the correct dental anatomy as follows[3]-Occlusal surfaces should be made in such a manner that forces are directed along the longitudinal axis of tooth.[4,5] Cuspal slopes of improper restoration in retention with the antagonist tooth can trigger enlargement of the contact point during functional movements.[6] This allows interdental impact of foodstuff with devastating conquest effects on interproximal periodontal tissues.[4,7] Marginal occlusal ridges must be placed above the proximal contact surface and must be rounded and smooth so as to allow access the dental floss. Proximal surface must be divergent,beginning from contact area towards vestibular direction orally and apically.[3,8] They must be smooth and polished and the interdental area must be correctly made,in order to prevent the settling of food in interdental spaces.[8,9]

Alteration of interproximal contact surface entails food retention, gingival inflammation, pocket formation, bone loss and finally dental mobility.[10,11] Food settling is a common cause of chronic marginal gingiva and periodontal pathologies. The reason why the contact

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surface in a lateral tooth must be situated at 1-2mm in length in occluso-gingival direction and it will measure approximately 25% of the oro-vestibular width of neighbouring tooth, in the upper arch the contact surface is situated slightly towards vestibular area, from the median mesio-distal line. These surfaces, if well proportioned, play an important role in maintaining gingival health.[10,12] Undercontoured vestibular and oral surfaces may alter the normal route of food and cause its stuffing and accumulation in the gingival groove.[10,13] Over countering will deviate food beyond the marginal mechanical action of food which can stagnate in overprotected gingival groove. Cervical extension of restorations should be placed possibly supragingival and it should present an optimal marginal closing.[14,15] The microscopical spaces at the tooth restoration interface constitute niches for plaque accumulation.[16,17]

From the periodontal point of view, the most important element is the gingival niche(embrasure).[16,17] Periodontal disease triggers tissue destruction,diminishing the level of the alveolar bone and creating greatly enlarged interdental spaces.[17,18] Restorations can be made to respect the coronal and radicular morphology,maintaining the embrasure enlarged and the interdental space open.[17,19] Teeth can be remodelled through restorations so as the gingival embrasure is replaced near the new level of gingiva.[17] This is made by modifying the contour of the proximal surfaces and by placing the contact areas more apically.[17 20,21]The interdental gingiva takes again the normal shape, filling the new embrasure, which must have adequate dimensions.[22–24]As dental restorations and periodontal health are closely related this study was aimed at finding out the incidence of periodontal pocket more than 3 mm in maxillary premolars and molars with class II restorations.

Materials and Methods

This is a retrospective study regarding the incidence of periodontal pocket more than 3 mm in maxillary premolars and molars with class II restorations in patients who visited Saveetha Dental college and Hospitals in between June 2019 March 2020. The approval for this university setting study was obtained from the Institution Ethics Board. The study was reviewed by 2 reviewers and was cross verified.

Inclusion criteria - Patients with class II restoration,Vital tooth

Exclusion criteria - Patients without class II restoration,Patients who have undergone periodontal surgeries, Patients with orthodontic appliances

Data was obtained by reviewing the patient records and analysing 86000 patients between June 2019 and March 2020, out of these the data of 885 patients who had undergone class II restorations was collected. This data was compiled into class II restorations with pocket depth more than 3 mmm and less than 3 mm. After retrieval it is tabulated in excel and then imported to SPSS(Statistical Product and Service Solutions) software by IBM. Variables were defined and selected .

The following parameter was evaluated:

Probing Pocket depth: The probing Pocket depth were carried out at six sites for every tooth (mesiobuccal, mid buccal, distobuccal, mesiolingual, midlingual and distolingual using WHO periodontal probe) The periodontal probing score on the side (mesial or distal) of the restoration was considered.

Statistical Analysis

After further verification by an external reviewer, it was imported to the SPSS Version 20 by IBM for statistical analysis.Descriptive statistics and chi square test were performed and graphs were plotted to arrive at final results. A p-value less than was of less than 0.05 was considered as statistically significant.

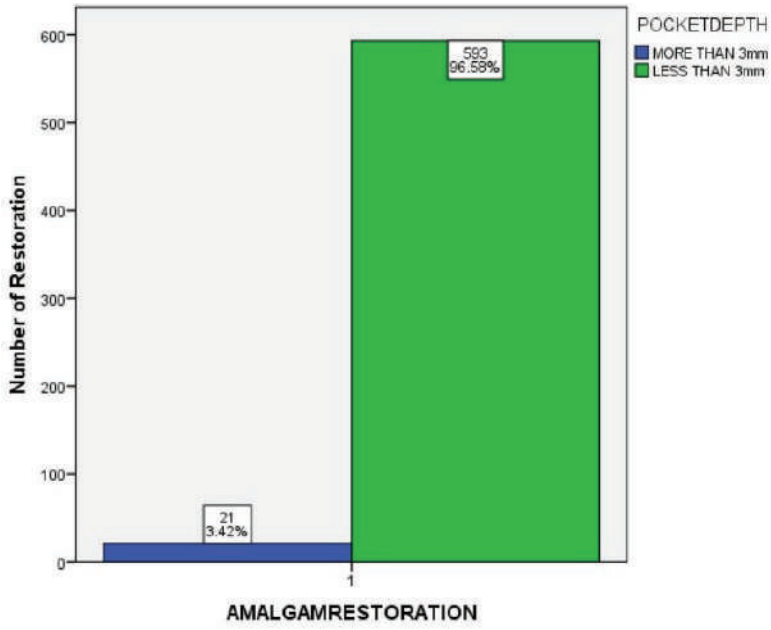
Results & Discussion

In this retrospective study we compared the incidence of periodontal pocket depth more than 3mm in maxillary premolars and molars with class II restorations. The incidence between two commonly used direct restorations amalgam and composite are compared in this study. The survival of dental amalgam is twice as high compared with composite filling, this is because of various factors like polymerisation shrinkage, deficient marginal adaptation, higher wear rates, defective contact points leading to food impactions, insufficiently converted composite at the bottom of the cavity are problems that cannot be underestimated when using resin-composite.[25,26,27] This does not imply that there is no weakness for amalgam: the need for retentive cavities at the cost of healthy tooth surface, weakening of tooth's strength by cutting through tooth's crown's ridges, the

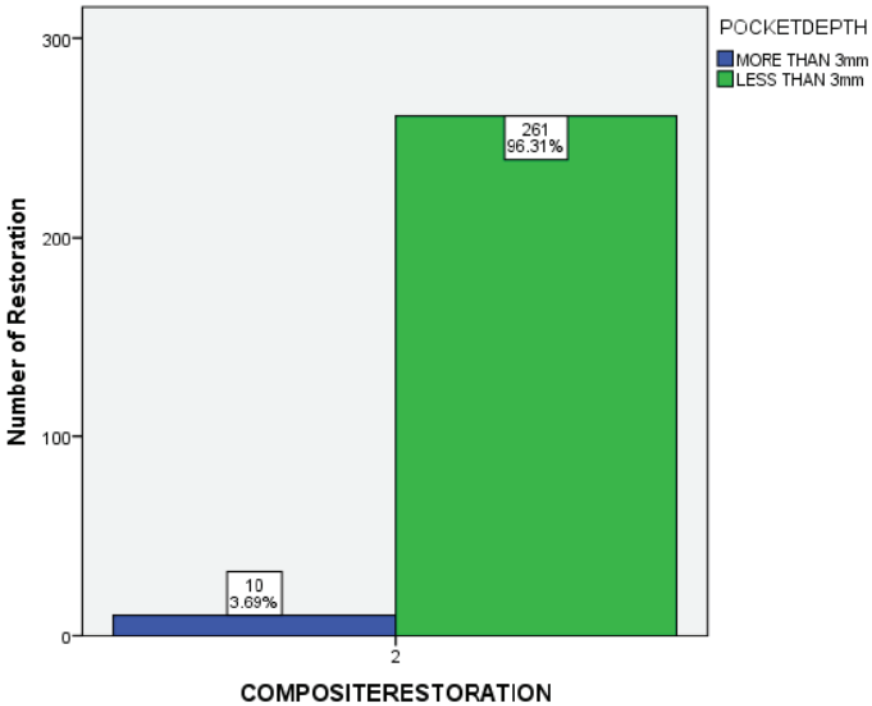
risk of fracture of remaining tooth substance as the result of cavity design and the lack of adhesion between amalgam and tooth substances.[28,29] The incidence of pocket depth more than 3mm in amalgam restorations. 614 amalgam restorations were done in a time period from June 2019 to March 2020.The incidence of pocket depth more than 3mm was found to be 3.42% in amalgam restorations(Graph 1). The incidence of pocket depth more than 3mm in composite restorations.271 composite restorations were done in a time period from June 2019 to March 2020. The incidence of pocket depth more than 3mm was found to be 3.69% in composite restorations(Graph 2). The comparison of pocket depth in both amalgam and composite restoration shows that in amalgam it is 2.37% whereas in composite it was found to be 1.13%(Graph 3). Levin L et al., and Kim K et al., stated that class II amalgam restoration has more incidence of pocket depth compared to composite.[30,31] However there are studies in which results indicated that class II composite restorations have more incidence of periodontal pocket compared to class II amalgam.[26,32-36] The other studies may have used a different diagnostic criteria..[26,32–36] As the control in other studies were normal teeth without any restorations whereas the control in the present study were the teeth with class II restoration less than 3mm pocket depth. The comparison of pocket depth of pocket depth of more than 3 mm in between maxillary premolars and molars with a class II restoration, it was found that molars had more incidence of pocket depth more than 3 mm with 21 teeth than premolars with 10 teeth(Graph 4).

The main objective of matrix systems is to reproduce the natural proximal contour of a contact that is tight enough to prevent food impaction, which is crucial for the healthy maintenance of underlying periodontal tissues.[37] Increased gingival inflammation and attachment loss, apart from the presence of overhanging restorations, have also been attributed to plaque accumulation due to loose proximal contacts.[38,39] However, alveolar bone loss is not directly attributed to open interproximal contacts but is strongly related to the overall periodontal status of the patient.[40] Annoyance and discomfort have been reported by patients who experience food impaction4 at sites of open contact.[40] On the other hand, the contact point must not be too strong, in order to avoid the shredding and impaction of dental floss fibers interproximally or periodontal trauma induced by excessive force applied during flossing.[40]

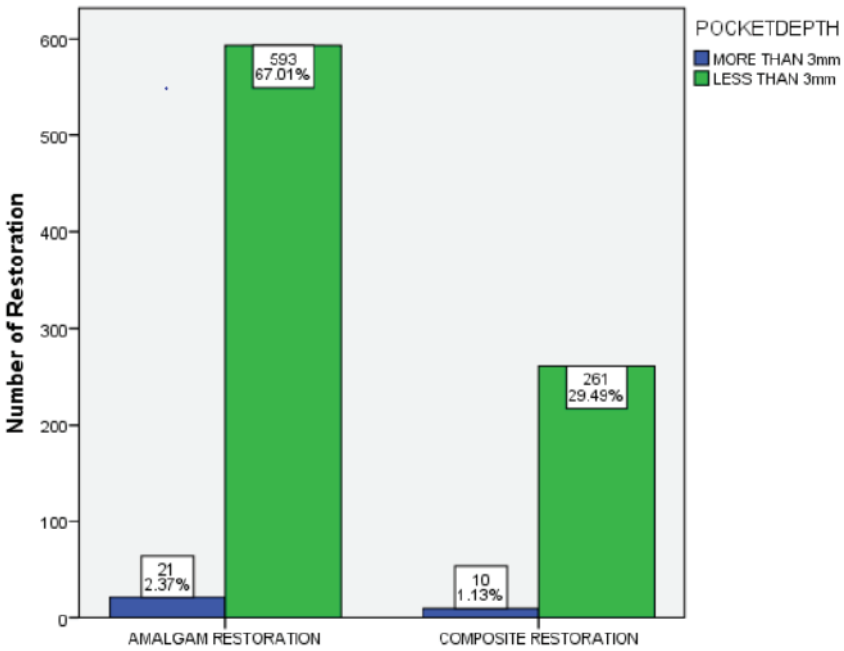
Hence, various researches were done to overcome these problems by improving material characteristics and application techniques. The choice of the matrix system, separation technique and restorative material is an important factor.[41]Conventionally Tofflemire matrix systems were used but failed to create tight proximal contacts.[41] This might also be a reason for periodontal pocket formation in class II restorations. In several in vitro and in vivo studies, sectional matrix systems in combination with separation rings showed tight proximal contacts in two surface Class II cavities.[41] There are two reasons for a successful restoration: to reproduce the natural contour with tight proximal contact and proper oral hygiene maintenance by the patient after the restoration. Further studies can be done on prognosis of class II restorations with advanced sectional matrix systems.



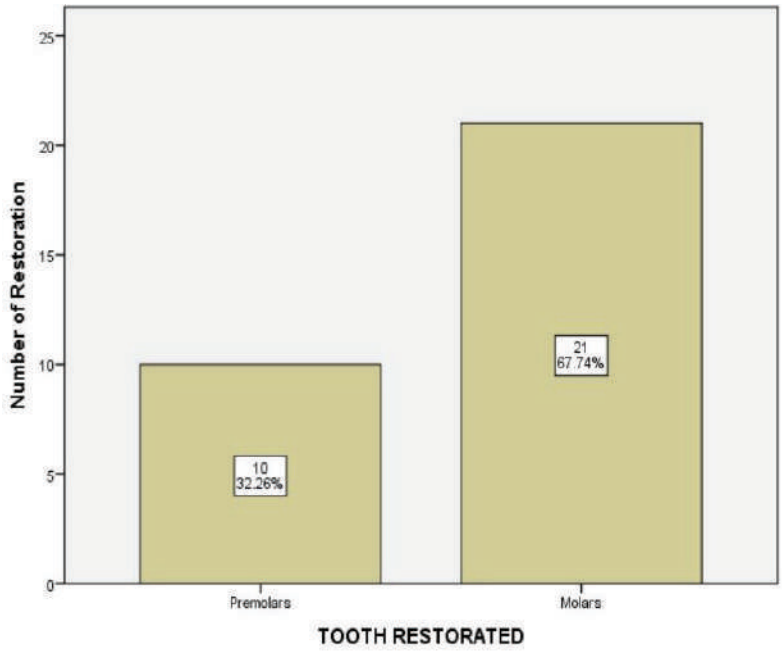
Graph 1 represents the Incidence of pocket depth more and less than 3mm in amalgam restorations. X axis represents pocket depth more and less than 3mm and Y axis shows the number of restorations where blue bar denotes the number of amalgam restoration with pocket depth more than 3mm and green bar denotes the number of amalgam restoration with pocket depth less than 3mm. Out of total 614 amalgam restorations, 21 restorations(3.42%) had pocket depth more than 3mm which is less when compared to 593 restorations(96.58%)which had pocket depth less than 3mm.



Graph 2 represents the Incidence of pocket depth more than 3mm in composite restorations. X axis represents pocket depth more and less than 3mm and Y axis shows the number of restorations where blue bar denotes the number of composite restoration with pocket depth more than 3mm and green bar denotes the number of composite restoration with pocket depth less than 3mm. Out of total 271, 261 restorations(96.31%)had pocket depth less than 3mm which is more when compared to 10 restorations(3.69%)which had pocket depth more than 3mm.



Graph 3 It represents the comparisons between the pocket depth more than 3mm in both amalgam and composite restoration. X axis shows the types of restorations-amalgam and composite restorations and Y axis shows the restorations with pocket depth more and less than 3mm where blue bars denote the number of restoration with pocket depth more than 3mm and green bar denote the number of restoration with pocket depth less than 3mm. It shows that pocket depth more than 3mm in amalgam restoration is (2.37%) which is more when compared to composite restoration (1.13%). (Chi square test was done with p value 0.841, where P value > 0.05, statistically not significant association).



Graph 4: This graph represents the incidence of pocket depth more than 3mm in maxillary premolars and molars with class II restoration. X axis shows the maxillary premolars and molars with class II restoration and Y axis shows the number of restorations with pocket depth more than 3mm. It shows that the maximum number of class II restorations with pocket depth more than 3mm was found in maxillary molars(67.74%) followed by maxillary premolars(32.26%).

Conclusion

Within the limitation of this study it concluded that class II amalgam restoration have more incidence of pocket depth more than 3mm with 2.37% than class II restoration which was 2.37% in maxillary premolars and molars.

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Conflict of Interest: There are no conflicts of interests to declare.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Radiographic Errors in IOPA

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Abstract

Radiographs are considered as a crucial aid in diagnosis, intraoral and post operative evaluation of different conditions and dental procedures. Exposing the patient to beams of x ray more than needed is time consuming and increases treatment cost. Some of the errors include cone cut, overlap, elongation, shortening, hazy image, ghost image, artifacts and reversed film.The aim of the study is to determine the prevalence of radiographic errors in IOPA. The study was aimed to investigate the correlation of radiographic errors in IOPA. 100 case reports of patients were obtained from Dental Archive Software and was tabulated in Excel. The data analysis was done using SPSS software. The graphs were obtained and the results were tabulated. Overlap type of error was more prevalent for upto 41%. There were more errors as well like elongation 16%, shortening 17%, hazy image 12%, cone cut 13% and ghost image 1%. 27% of the study population that underwent radiographic errors were between 21 to 30 years. 36% of the errors were caused by molars. Undergraduate students performed more errors in which overlap occurred for 26% while among postgraduate students they occurred for 15%(figure 5). Overlap type of error was the most common cause of radiographic error. Radiographs being one of the golden standards for diagnosis, need to be done with proper knowledge and awareness.

Keywords: *Radiographs, errors, diagnosis, IOPA, treatment planning*

Introduction

In dentistry, radiographs are an important part of diagnosis and treatment[1]. They help in clinical and anthropological diagnosis. Clinicians should be aware of the positions and processing radiographs[2]. They also help step by step management to maintain the prosthesis like implants[3]. Students should be trained in radiographic techniques in proper position and angulation[4]. IOPA can be used to assess the bone resorption levels to check periodontal health. Improvement in their performance helps reduce exposure to radiation due to faulty radiographs and is important to keep the health of the patient. With the help of the study, students can be made aware of the common mistakes that are made and this will help them improve their pattern[5].

The periapical radiograph, using paralleling technique, is considered as the gold standard for measuring the errors [2,6]. Several advanced techniques in radiographs include IOPA, CAD-CAM, MRI etc[7]. Errors like angulation disorders, film bending, reverse film, overlap and cone cut were some of the causes of errors. Anatomical variations like high narrow palate, tori,etc can also cause errors. Changes in the angle between teeth and film also have a significant effect in taking IOPA[8].

Radiographic techniques and processing errors can highly impact the radiographic interpretation. It is necessary to gather proper radiological information, hence good quality radiographs are important to be taken by avoiding errors[9]. Both paralleling and bisecting angle techniques can have both technical and manual errors. Besides the common errors, processing them incorrectly like image contrast can affect the interpretation.

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They will help evaluate the periodontal status before any prosthetics and can help decide the planning process [10,11]. Any discrepancy in interpretation causes interpretive radiological error. SLOB technique can be used to accurate positioning[12]. As a dental surgeon, they should be aware of processing radiographs. More research is needed to help avoid misinterpretation. It is also important to have radiographs as it will act as a mirror to point out any unseen errors. Radiographs can also help to determine the adaptability of prosthesis [13,14]. Besides this it is important to maintain hygiene between patients when taking IOPA to avoid infections like cellulitis [15,16,17,18].The aim of the study is to identify and quantify radiographic errors when taking an IOPA, more accurate interpretation with less patient exposure.

Materials and Methods

This is a retrospective study which was conducted by using the data collected from the educational software of Saveetha Dental College, Chennai; from June 2010 to April 2020 and it was examined by two examiners.Prior to the start of the study, ethical approval was obtained from Scientific Review Board, Saveetha Dental College, SIMATS university. The study involved a total of 100 sample sizes of patients that had to take IOPAs by undergraduate and postgraduate students. Patients attending the OPD of Saveetha Dental College were enrolled in the study by simple random sampling. 100 case sheets were reviewed of both genders and cross verification was done through radiographs taken of the oral cavity. The external validation can be generalised among the south indian population.

The data was collected from the electronic health records system used at Saveetha Dental College which was used to record and store information and oral health data of the patients reporting to the college. It helps in retrieval of data as starting from diagnosis to treatments rendered, everything is stored and can be accessed by the physicians. The inclusion criteria was patients that took IOPAs. Any other radiographic method other than IOPA were in the exclusion criteria. The data was imported to the software IBM SPSS Version 23.0 and analyzed using descriptive statistics and Pearson's correlation. Graphs were obtained and the results were tabulated. Statistical significance was set at <0.05. Ethical clearance was obtained and covered under the following ethical approval number - SDC/SIHEC/2020/DIASDATA/0619-0320.

Results and Discussion

Among the 100 study population taken for the study, it is found that 27% of the study population that underwent radiographic errors were between 21 to 30 years (figure 1), out of which 36% of the population had errors in the molar region(figure 3). 41% of the error was due to overlap which was the most prevalent type of error. Out of the other errors 16% was due to elongation, 17% was due to shortening,12% was a hazy image, 13% was due to cone cut and 1% was a ghost image due to artifacts. (figure 4). 54% of the study population were females(figure 2). In the study it was found that undergraduates performed more errors in which overlap occurred for 26% while among postgraduates they occurred for 15%(figure 5)

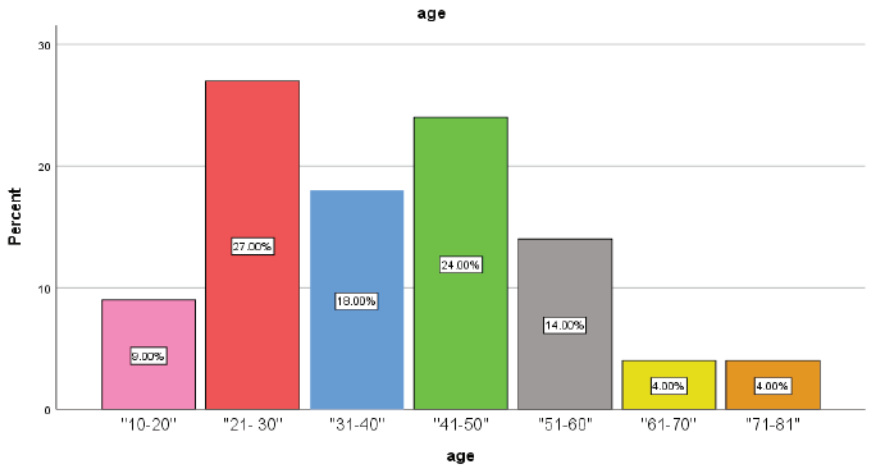


Figure 1 - Bar chart showing the age groups of the study population between 10 and 81 years between (x axis= age groups and y axis = percentage) 27% of the study population that underwent radiographic errors were between 21 to 30 years

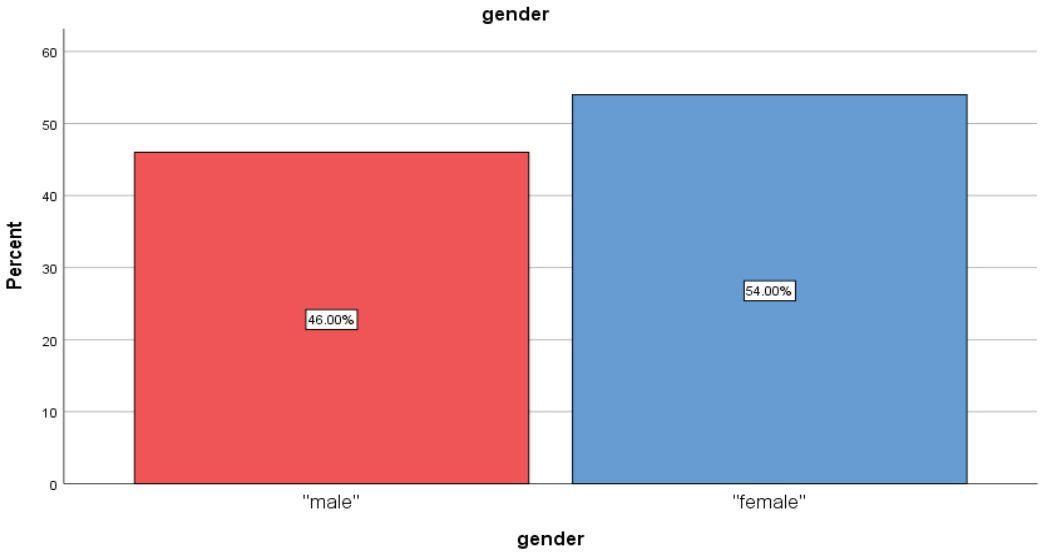


Figure 2 - Bar chart showing the genders of the study population (x axis= gender and y axis = percentage) 54% of the study population were females

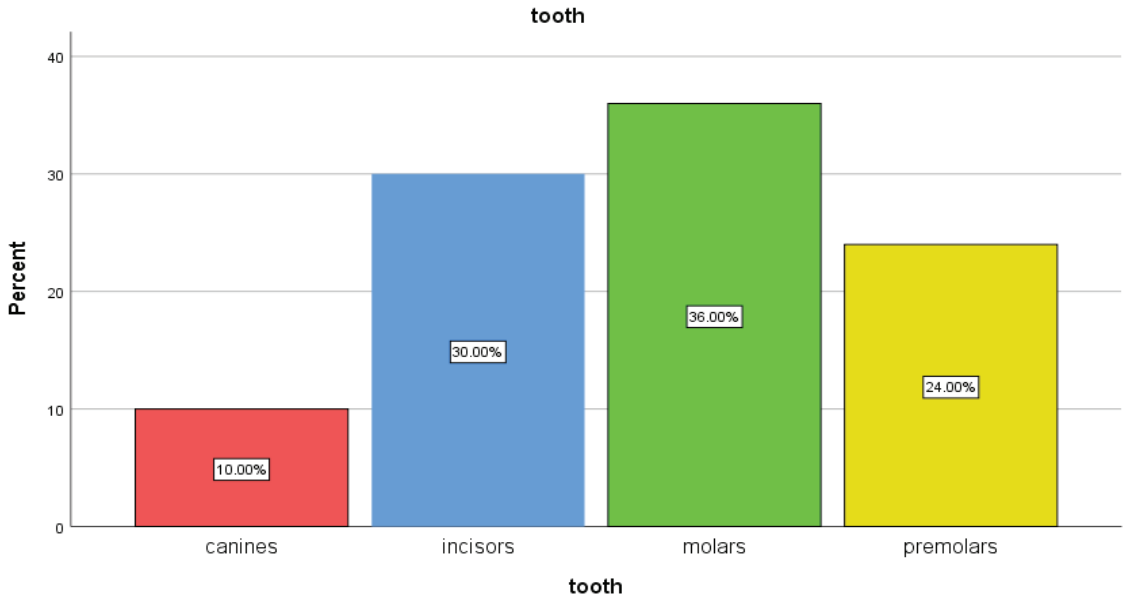


Figure 3 - Bar chart showing the affected tooth region among the study population (x axis= tooth and y axis = percentage) 36% of the radiographic errors were in the molar region

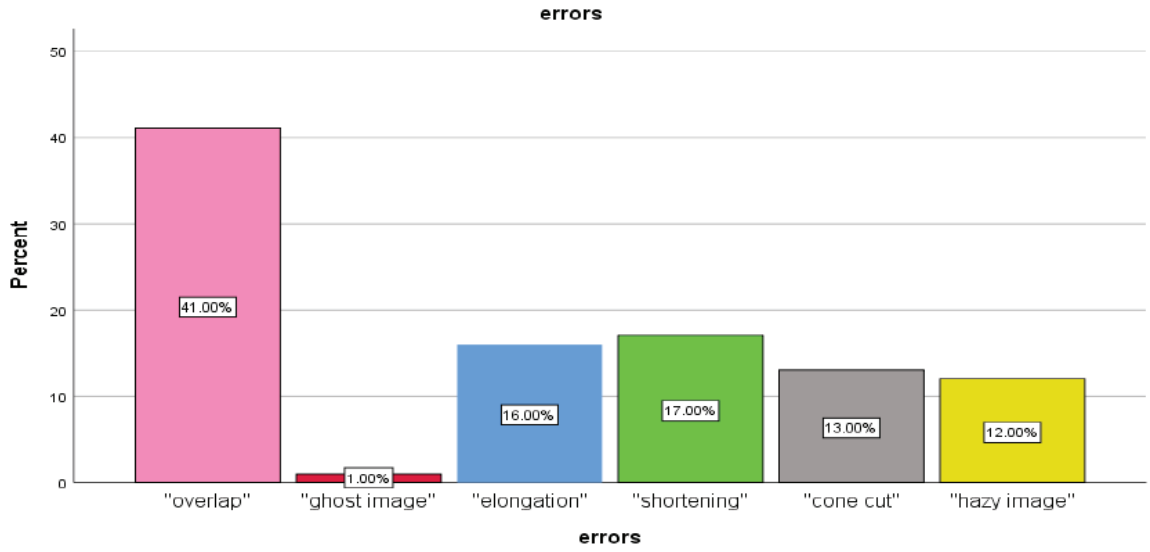


Figure 4 - Bar chart showing the different radiographic errors (x axis=radiographic errors and y axis = percentage) 41% was due to overlap, 16% was due to elongation, 17% was due to shortening,12% was a hazy image, 13% was due to cone cut and 1% was a ghost image due to artifacts.

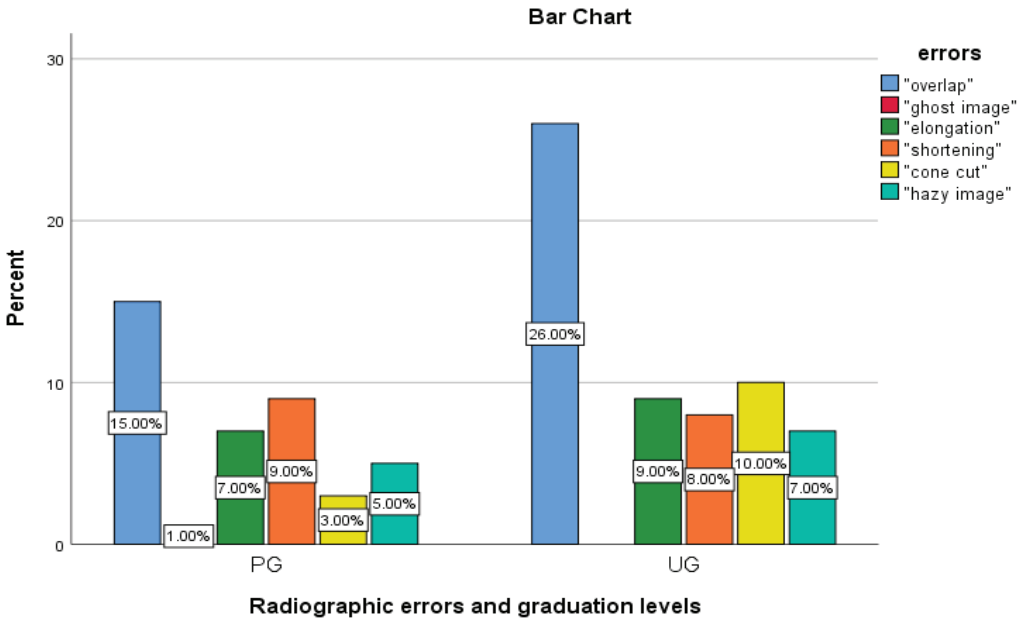


Figure 5 -Bar graph representing the correlation of radiographic errors among undergraduates and postgraduates where X axis is the radiographic errors and graduation levels and Y axis is the percentage of occurrence of the radiographic errors.The radiographic errors by postgraduates were overlap (15%), ghost image(1%), elongation(7%), shortening(9%), cone cut(3%) and hazy image(5%). The radiographic errors by undergraduates were overlap (26%), elongation(9%), shortening(8%), cone cut(10%) and hazy image(7%). The radiographic errors among postgraduates appear to be less compared to undergraduates, however there were no statistically significant differences between the two groups .(PearsonChi square test; P = 0.474.P>0.05) In the study it was found that undergraduate students were more prone to have radiographic errors than postgraduate students.

As a dental surgeon one should be aware of taking and processing radiographs. They should be aware of the different techniques and methods to obtain proper radiographs[15]. Radiography serves as a key diagnostic tool in dentistry; it renders to good and quality radiographs.

27% of the study population that had errors in radiographs were between the age of 21 to 30 years. Though in previous studies, the errors ranged between 30 to 50 years there was not much significance to age [15,16]. Some of the reasons observed due to having the need to take radiographs can be due to calcification, anatomical variations, periodontal health problems, dilacerations and other morphological problems. IOPAs are contradicted among pregnant women. Complications can also increase with age [19].

54% of the females were more prevalent to having errors in IOPA and 46% were males. In several studies, the gender prevalence is random as well, as they are caused due to random selection and less significant [20,21].41% of the errors were due to overlap as they were highly prevalent. Discrepancy in improper angulation of the receptor caused inevitable errors. Usually radiographs are taken among patients to confirm depth of caries, missing teeth and other problems in the teeth. Accordingly treatment planning is done to maintain aesthetics and maintain function[22,23,24]. Angulation arrows can be caused due to patient movement, improper arch alignment and crowding. (figure 4)

In a study by Greer, cone cut was one of the most commonly encountered radiographic faults upto 35.4% [25]. Though in the present study, cone cut happened for 13%, Elangovan et al attributes to factors such as cone cut not covering the area of interest due to minimal expertise. This will result in errors when the film is not immersed in the developing solution[26].

16% of the error showed elongation. They usually affected the maxillary molars, which causes decreased angulation. The error is due to limited skill of the personals (figure 3).

In the study conducted by S. Elangovan in 2017, stated the errors were common due to lack of experience and poor knowledge[17,26]. In the study it was found that undergraduates performed more errors in which overlap occurred for 26% while among postgraduates they occurred for 15%(figure 5).

In dentistry, radiographs have become a very essential diagnostic aid. Good quality radiographs without faults help in giving a proper treatment planning [27]. This is a serious problem, while some show a significant number of radiographs had faults. IOPAs with technical defects like cane cuts, overlaps, elongation, shortening and so on [28] . Errors on taking radiographs will require repetition and increase patients' radiation exposure. However there are fewer studies to evaluate radiographic errors in dentistry [11,29]. Patel et al evaluated the frequency of errors that will require retakes in paralleling intraoral radiographic techniques [18,30].

Conclusion

Overlap of images was the commonest radiographic error observed in this study .Accurate diagnosis to perform dental procedure, requires radiographs to evaluate success in procedures and to record dental and health status. The clinicians need to realise the value of understanding the basic knowledge of IOPAs and their accurate diagnosis.This study should help reduce the chance of facing radiographs. They should understand that theoretical approach along with practical experience always yields good response.

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Conflict of Interest : None declared

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Soft Tissue Upper Lip Thickness in Different Skeletal Malocclusion Subjects -A Comparative Cephalometric Study

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Abstract

The purpose of this study was to determine the soft tissue upper lip thickness of subjects with different skeletal malocclusions. Lateral cephalograms of 30 patients (control group : skeletal malocclusion I - 10, Case group : skeletal malocclusion II - 10 , skeletal malocclusion III - 10) were selected randomly from orthodontic records of subjects who reported to the Department of Orthodontics in Saveetha Dental College.All cephalograms were traced and upper lip thickness was measured using FACAD software. One way - ANOVA test was done, soft tissue upper lip thickness was found to be statistically significant ($p<0.05$) among different skeletal malocclusions. Post hoc test (Tukey HSD) was performed to find out the differences between each skeletal class , the upper lip thickness was found to be comparatively high in Class III compared to Class I and Class II ($p<0.001$). This study concluded that soft tissue upper lip thickness was found to be greater in skeletal class III malocclusion compared to other skeletal class I and II malocclusion.

Keywords: *Soft tissue upper lip thickness; Skeletal malocclusions; Lateral cephalograms.*

Introduction

Harmonious facial aesthetics have long been considered as the most important goal of orthodontic treatment , hence knowledge about hard tissues and its overlying soft tissue in determining facial harmony is essential for diagnosis and treatment planning[1]. Assessment of facial anatomical structures has practical applications mainly in orthodontics and evaluation of ratio of the soft tissue transition to the hard tissue change should be done especially when visualized treatment objectives are assessed in planning orthognathic surgery [2,3]. Soft tissue evaluation in patients undergoing orthodontic treatment or orthognathic surgery plays a crucial role in diagnosis and treatment planning[4]. Different thickness of soft tissue changes noted after bimaxillary surgery in patients with thick and thin soft tissues[5].The harmonious profile depends on ethnic or racial factors[6].The facial form of each individual arises from the profile and dental arches of the individual[7]. According to Riedel, the relationship of the incisors with their respective bony bases and growth pattern had a significant influence on soft tissue profile[8]. To reduce convexity more retrusive lip position is preferable for improving facial esthetics in both genders[9].

Previous research compared the lip thickness among different skeletal malocclusion showed that increased thickness was found in class III malocclusion in both genders[4,10–12].Previous studies have analyzed facial soft tissue thickness in Japanese children representing several different skeletal classes and reported that measurements differed among these various classes[13,14]. Several studies have made similar measurements in the Turkish population and found significant differences

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between genders for soft tissue chin thickness and upper lip thickness.[15–17].

The aim of the present study was to determine the soft tissue upper lip thickness of subjects with different skeletal malocclusions for diagnosis and surgical-treatment planning.

Materials and Methods

Lateral cephalogram of 30 patients of both genders (5 male and 5 female patients in each skeletal group; Control group : skeletal malocclusion I - 10, Case group : skeletal malocclusion II - 10 , skeletal malocclusion III - 10) were selected by using a random sampling method from the orthodontic records available from july 2019 to february 2020 . This retrospective cross sectional cephalometric study was conducted in Saveetha dental college in the department of orthodontics. This study got approved from the ethical review board in saveetha university.

Inclusion Criteria:

Patients with skeletal class I ,II,II malocclusion.

High quality radiographs with adequate sharpness were taken by using standard techniques and exposure conditions in natural head position.

Exclusion Criteria:

Patients of cleft lip and palate , facial asymmetry , syndromes or any other congenital anomalies and patients who underwent orthognathic surgeries were excluded.

All cephalograms were traced digitally by using FACAD software for accurate measurements by a single observer.

Skeletal malocclusion was determined based upon the ANB angle and Wits, which indicates the sagittal relationship of the maxilla and mandible. The 3 skeletal types were classified as: Class I= ANB angle 1-5 degrees (10 subjects) ,Class II =ANB angle greater than 5 degrees (10 subjects) and Class III= ANB angle less than 1 degrees (10 subjects).

Measuring variable :

Upper lip thickness:

a) the distance between point A and subnasale

b) the distance between prosthion (lowest point of the alveolar bone between two upper central incisors) and labrale superius (vermilion border of the upper lip).

The collected data were tabulated in excel and analysed by SPSS software. One-way ANOVA test was used to make a comparison between the skeletal classes. Association between gender and soft tissue lip thickness was evaluated by Pearson’s Chi-square association test.

Results and Discussion

Figure 1- represents one-way ANOVA. Table 1 represents one way -ANOVA post hoc test (Tukey HSD) to determine the significant differences in between different skeletal malocclusions. Figure 2 - Bar graph represents the association between gender and soft tissue lip thickness in different skeletal malocclusions.

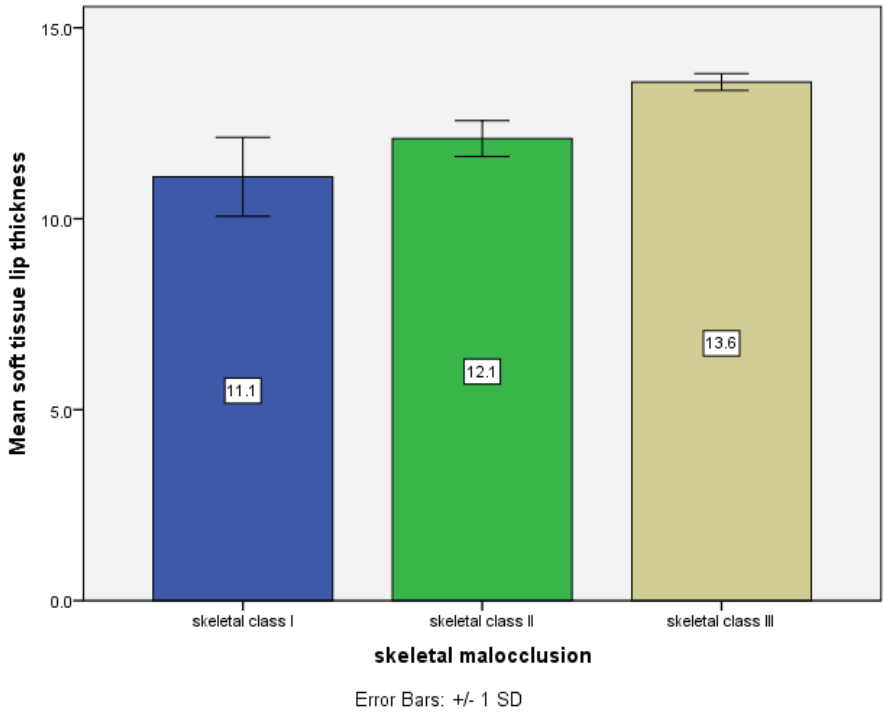


Figure 1: Bar graph represents the comparison between soft tissue lip thickness and skeletal malocclusion.X axis represents skeletal malocclusion. Y axis represents mean soft tissue lip thickness. One way ANOVA was done and the difference of mean values for upper lip thickness between skeletal class I(blue) ,II(green), III(Khaki) was statistically significant. The soft tissue upper lip thickness is higher in skeletal class III(khaki) malocclusion compared to other malocclusions.

One-way ANOVA (f value - 34.98 and p value - 0.000)..

(I) upper lip thickness		Mean Difference (I-J)	Std. Error	Sig.
Class I	class II	-1.00*	0.29	.007
	class III	-2.48*	0.29	.001
class II	class I	1.00*	0.29	.007
	class III	-1.48*	0.29	.001
class III	class I	2.48*	0.29	.001
	class II	1.48*	0.29	.001

Table 1 - one way ANOVA post hoc tests (Tukey HSD) showing differences between each skeletal class. The upper lip thickness was found to be comparatively higher in skeletal Class III malocclusion than Class I and Class II malocclusion and was statistically significant($p<0.001$).

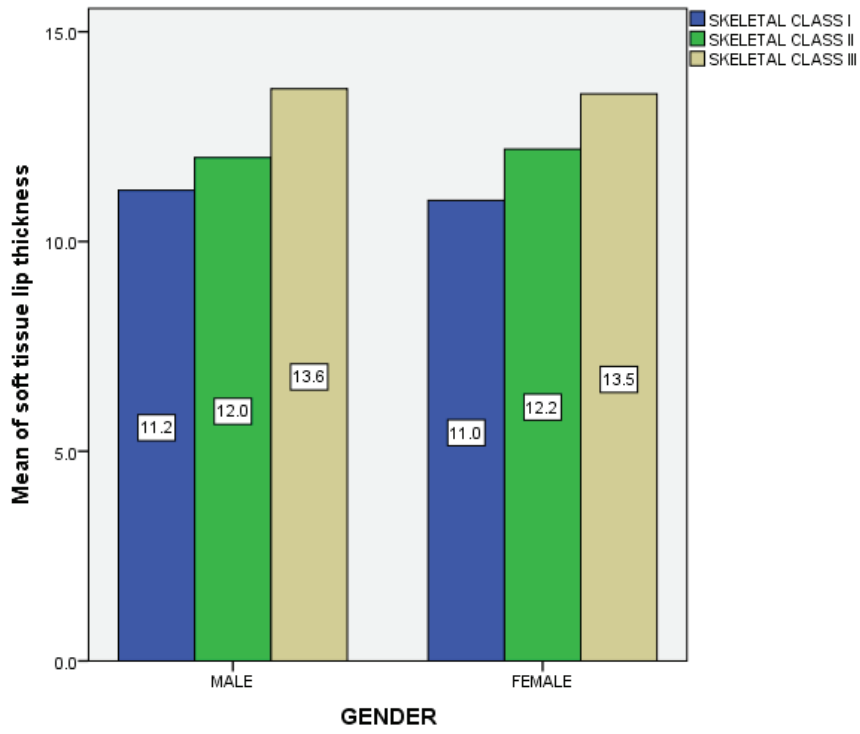


Figure 2: Bar Graph represents the association between gender and soft tissue lip thickness in different malocclusions. X- axis represents the gender and Y-axis represents the mean of upper lip soft tissue thickness in different classes of malocclusion. Blue denotes Skeletal class I malocclusions, green denotes class II and khaki denotes class III malocclusion. Both male and female have no difference with soft tissue lip thickness among various skeletal malocclusions both in percentage and also statistically..Chi-square value- 0.00 , df -2 ,p value - 1.000(p >0.05), hence it is not satistically significant.

Some considerations should be taken into account while taking lateral cephalogram such as the patient should be positioned in a relaxed lip position for evaluating the soft tissue profile since this position demonstrates the relationship of soft tissues to hard tissues without muscular compensation for dentoskeletal abnormalities[18,19]. In agreement with these studies, the relaxed lip position was used in the present study while taking the cephalograms for accurate assessment of soft tissue thickness. Since orthodontic or prosthodontic treatment may produce changes in the soft tissue profile, patients undergoing such treatment were not included in this study.

Previously our team had conducted numerous clinical trials [20–26], lab animal studies[27–31]and in - vitro studies[32–34]over the past 5 years. The idea for this study stemmed from the current interest in our community.

In the present study, we found that soft tissue upper lip thickness was found to be greater in skeletal class III malocclusion compared to other skeletal malocclusion (Figure 1 and Table 1).Gender is not associated with soft tissue lip thickness among various skeletal malocclusions(Figure 2).The present study is complemented by an another study done by Kamak et al,Tanic et al they compared the soft tissue thickness in both male and female orthodontic patients with different skeletal malocclusions and concluded that the thickness at labrale superius and stomion points among each skeletal type was significantly greatest in Class III for both males and females[4,35]. Also Uysal et al showed that statistically significant differences were determined for the thickness of the labrale superius, labrale inferius, pogonion, and menton measurements among different malocclusion[18]. Kurkcuoglu et al in a Turkish population demonstrated that highest differences were found among different malocclusions at labrale superior especially in Class III females[36]. Gungor et al in a Central Anatolian group found differences in soft tissue thicknesses at rhinion, labrale superius especially in class III males,

and at nasion[37]. Few studies comparing the lip thickness among different skeletal malocclusion showed that increased thickness was found in class III malocclusion in both genders[10–12]. According to Subtelny the thickness of subspinale region (A-A') region incrementally increased on an average of 5 mm, from 3 to 18 years old, and comparatively, there is more soft tissue covering point A region therefore soft profile tends to change more in the direction of increased thickness than in the reduction of facial convexity[38]. Aggarwal and Singla et al reported that presence of significant differences between men and women, and men had increased values for soft tissue thickness for the labrale superius, labrale inferius, pogonion and menton, compared with women, and recommended that this difference should be taken into consideration when planning orthodontic therapy[39]. On the contrary , no studies have been found against the findings of this study. Hence the overall consensus of the available literature is in agreement with the present study the upper lip thickness was found to be greater in skeletal class III malocclusion compared to other skeletal malocclusions.

This finding might be due to the angulation of the maxillary and mandibular central incisor. In skeletal class III malocclusion , maxillary incisors are tipped labially and the mandibular incisors lingually therefore, Mandibular anterior teeth might push the upper lip upward and outward[4]. The limitations of the present study was small sample size and restricted to specific race and ethnicity.

Conclusion

With this study, we conclude that soft tissue upper lip thickness was found to be greater in skeletal class III malocclusion when compared to other malocclusions. Gender has no association with soft tissue lip thickness among various skeletal malocclusions. Hence, this study will help the clinicians in diagnosis and treatment planning.

Conflict of Interest: There are no conflicts of interest declared.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Evaluation of the Lip-Tooth Relation in Various Growth Patterns- A Cephalometric Study

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Abstract

Upper incisor crown exposure is directly influenced by hard and soft tissue structures that surround and support these teeth. The amount of upper incisor crown exposure has a direct impact on dentofacial esthetics. Lip-tooth relationship during speech and smiling are important aspects of facial esthetics. Hence, the aim of this study was to evaluate the lip-tooth relations in subjects with different types of growth patterns using lateral cephalograms. Methods-This was a retrospective study wherein case records of adult subjects aged 18-35 years were involved. A total of 30 case records of patients who had reported to a university hospital were identified and included in the study. The records of subjects were divided equally into three groups Group A - horizontal growth pattern; group B - average growth pattern and group C - vertical growth pattern. All lateral cephalograms were measured using FACAD software. A chi-square test was used to determine the association of lip-tooth relationships in various growth patterns. One-way ANOVA was used to compare the incisor exposure in different growth patterns. One-way ANOVA reported a statistically significant difference in lip-tooth exposure in various growth patterns ($p=0$, $p<0.05$). Similarly, chi-square test revealed a statistically significant association between lip-tooth relationships in various growth patterns ($p=.00$, $p<0.05$) Within the limitations of this study it can be concluded that there was an increased cervico-incisal exposure of incisors in vertical growth pattern as compared to average and horizontal growth patterns.

Keywords-*Upper incisor, Lip-tooth relationship, vertical growth pattern, average growth pattern, horizontal growth pattern*

Introduction

Upper incisor crown exposure is directly influenced by hard and soft tissue structures that surround and support these teeth. Among the factors that affect upper incisor crown exposure are upper lip length and maxillary incisor inclination.[1,2] The amount of upper incisor crown exposure has a direct impact on dentofacial esthetics.[3,4] In many malocclusions upper as well as lower incisors are proclined thus influencing the facial profile of the patient.[5] Excessive incisor exposure with proclination poses a high chance of fracture due to trauma which can cause non-vitality of the teeth.[6,7]

Peck and peck classified smiles as stage I and II; Ackermann et al designated the stage I smile as the posed smile and stage II as an unposed smile.[8,9] The unposed smile is involuntary and is induced by joy. An unposed smile is natural as it expresses authentic human emotion.

In orthodontics, smile analysis is done to evaluate the posed smile on the basis of 2 major characteristics: the amount of incisal and gingival display.

It is preferred that the elevation of the lip for the posed smile stops at the gingival margins of maxillary incisors. If there is an overexposure of the gingiva during

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smile it leads to gummy appearance called as a gummy smile, in these patients mini implants are preferably used to intrude incisors followed by gingivectomy to reduce the overexposure of gingiva. [10,11] Also, orthodontically, placement of the brackets during bonding can also cause intrusion and reduce the gingival exposure.[12,13,14] Males show less of maxillary incisors and more of mandibular incisors at rest and on smile than females.[15]

Lip-tooth relationship during speech and smiling are important aspects of facial esthetics.

Hence, the aim of this study was to evaluate the lip-tooth relations in subjects with different types of growth patterns using lateral cephalometric measurements.

Materials and Methods

This was a retrospective study where in 30 lateral cephalograms of adult subjects aged 18-35 years were selected and retrieved from the case record of patients visiting the department of orthodontics and dentofacial orthopaedics, Saveetha dental college and hospitals from the time period of January 2019 to March 2019.

Inclusion criteria-

1. Adults undergoing orthodontic treatment without any missing teeth.

Exclusion criteria-

Any obvious pathology like a cyst or tumour

Presence of anterior open bite leading to inaccurate measurement of incisor exposure

History of previous orthodontic treatment.

After selecting the cephalograms they were divided into three groups. Group A consisted of 10 cephalograms of vertical growers; group B consisted of 10 cephalograms of average growers and group C consisted of 10 cephalograms of horizontal growers.

Sampling method-

Sampling method carried out for this study was Randomized sampling. To minimize sampling bias, simple random sampling was performed. The investigator, A.T, did not subject-specific demographic information of the files until the study was completed.

2.4. Incisor exposure measurement method-

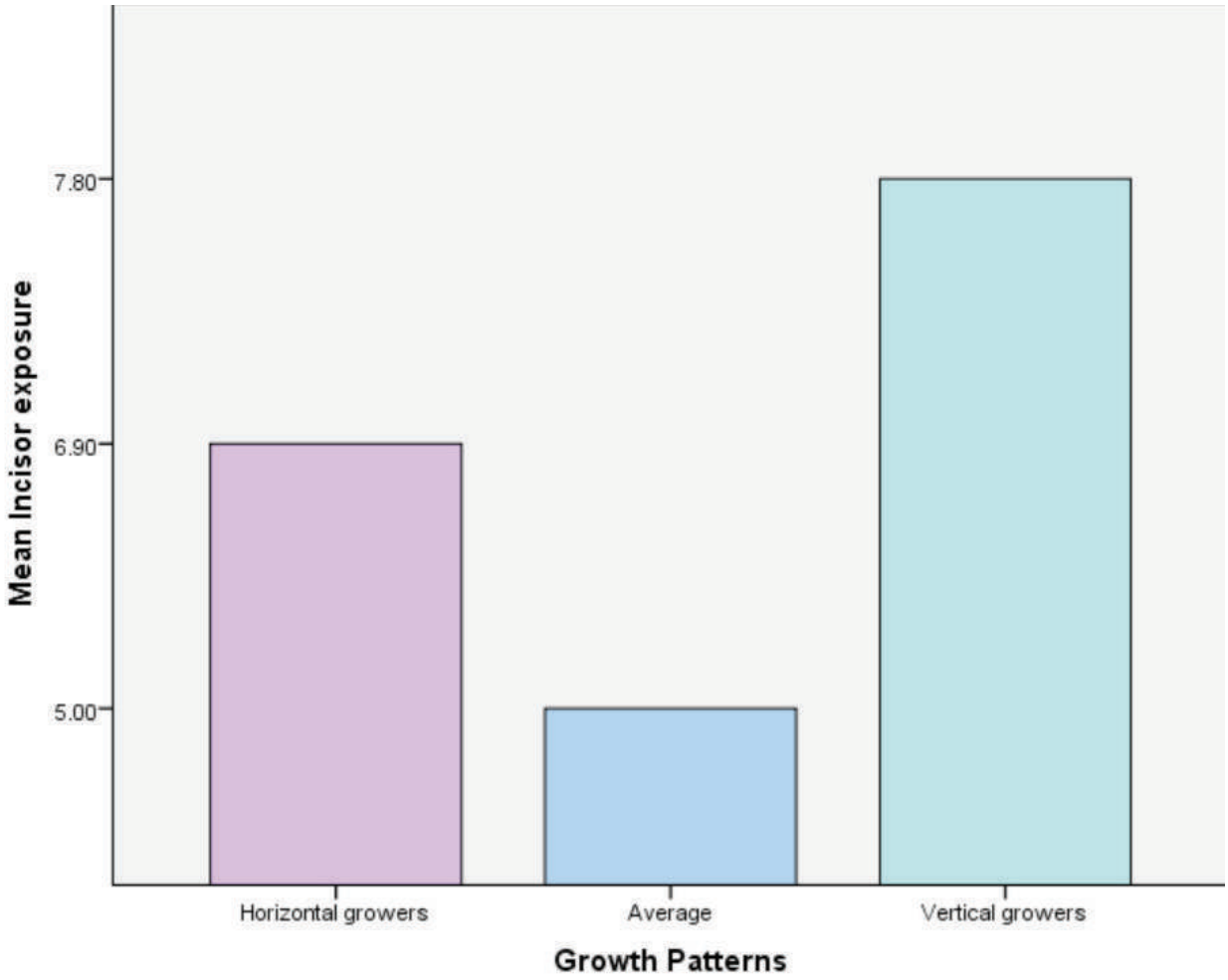
All lateral cephalograms were measured using FACAD software. The lateral cephalogram was calibrated at 10mm. To carry out the incisor exposure measurement, Burstone analysis was performed by measuring the distance from the tip of the upper central incisor to upper lip stomion.

2.5. Statistical analysis-

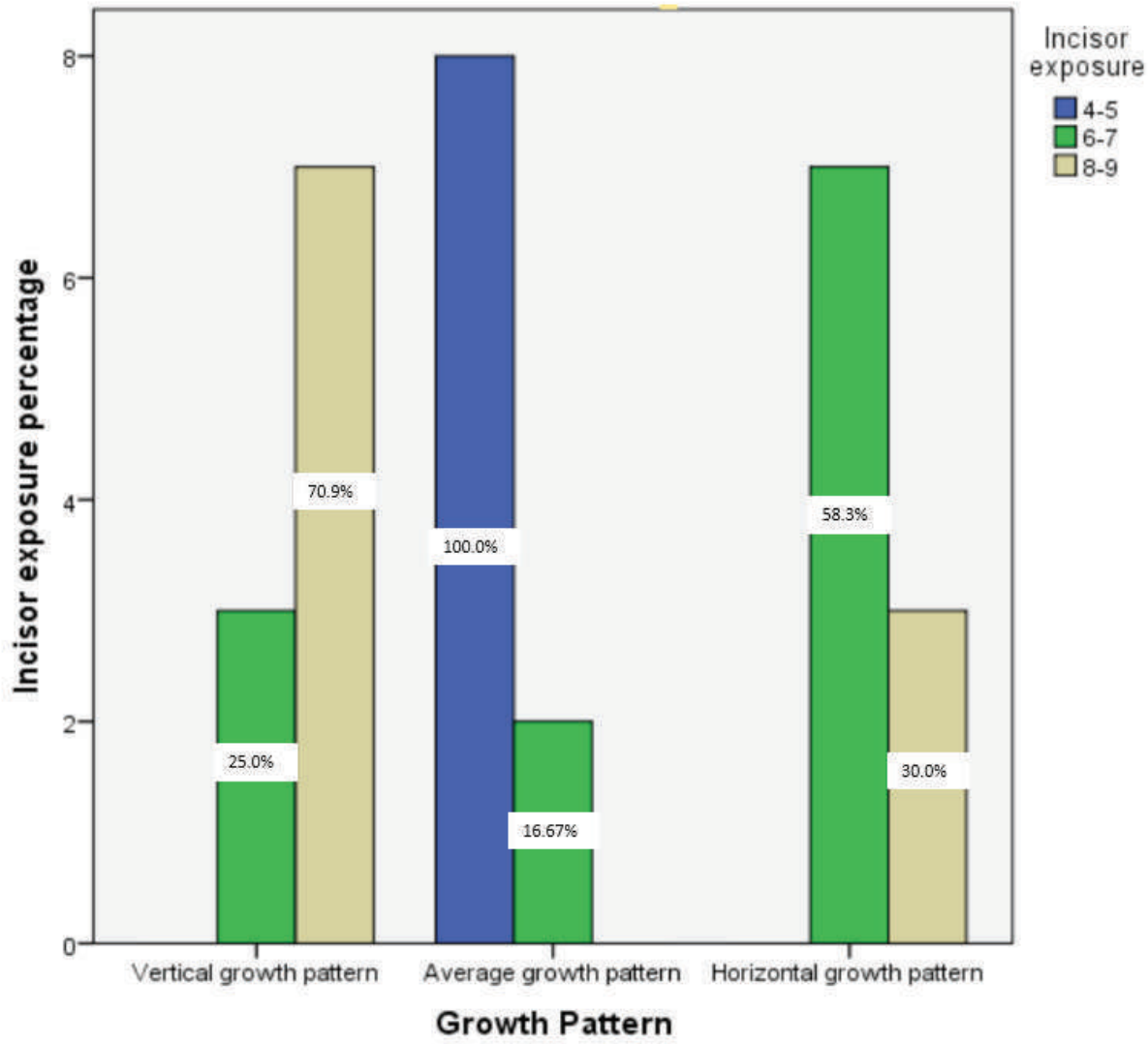
All statistical analysis was performed in SPSS. A chi-square test was used to determine the association of lip-tooth relationships in various growth patterns. One-way ANOVA was used to compare the incisor exposure in different growth patterns.

Results And Discussion

Graph 1 represents one-way ANOVA reporting a statistically significant difference in lip-tooth exposure in various growth patterns ($p=0$, $p<0.05$). Graph 2- Bar graph representing incisor exposure in different growth patterns. There was a statistically significant association between lip-tooth relationships in various growth patterns. (Chi-square value-26.9 p value=.00, $p<0.05$)



Graph 1. Bar chart representing incisor exposure in different growth patterns. X-axis represents the different growth patterns and Y-axis represents the mean of incisor exposure. Pink colour denotes horizontal growers, blue colour represents average and green colour represents vertical growers. Majority of the incisor exposure is found in vertical growth patterns(green) than the other growth patterns. Similarly, in One way ANOVA analysis, there was a statistically significant difference observed in the incisor exposure within the groups: and found that vertical growers(green) have a higher incisor exposure compared to other growers. (df=2,p=0, p<0.05)



Graph 2. Bar chart representing incisor exposure in different growth patterns. X axis represents different growth patterns and Y-axis represents the no. of patients in whom incisor exposure was measured. Blue colour denotes 4-5 mm of incisal exposure, Green colour denotes 6-7 mm of incisal exposure and brown colour denotes 8-9mm of incisal exposure. The majority of the 4-5 mm exposure(blue) is found in the average growth pattern than the other growth patterns and it is also significant statistically. (Chi-square value-26.9 p value=.00, p<0.05) proving that the incisor exposure is increased in vertical growth pattern.

Previously our team had conducted numerous studies such as an apparatus designed to measure orthodontic forces[16], quantification of intrusive forces[20], obstructive sleep apnea[22] and recycling methods[23] , comparison of intrusion on maxillary incisors[16,17] , stress distribution at mini-implant site[10] and an apparatus to measure Now we are focussing on epidemiological surveys, the idea for this study stemmed from the current interest in our community.

Incisor exposure and an attractive smile are the two major components of an attractive face, and most easily judged by a layman. Undoubtedly, patients undergoing orthodontic treatment are expected to have an attractive face with a pleasing smile and an adequate amount of incisor exposure after the treatment.[18]

In the present study, the chi-square test revealed a statistically significant association between lip-tooth relationships in various growth patterns. One-way

ANOVA reported that there was a statistically significant difference in lip-tooth exposure in various growth patterns. In subjects with vertical growth pattern, there was incisor exposure of 8-9mm whereas, in horizontal grower, incisor exposure of 6-7mm was most commonly found. Sufficient literature was not reported evaluating lip-tooth relation in various growth patterns.

Lip tooth relationships have been reported and studied extensively in various malocclusions in the past, but no studies are reported about lip tooth relationships in different growth patterns. This study was designed keeping in mind the lack of available literature in this area of interest. Some studies of lip tooth relationship in various malocclusions report that male subjects with class III malocclusion displayed slight exposure of incisors during smile than subjects with class I or class II malocclusion but did not evaluate the same in different growth patterns. [19,20,21] Factors such as age, ethnic variations affect the lip-tooth relationship in North American adolescents with class I skeletal pattern.[22,23]

Rashid et al reported there was a statistically insignificant difference in the incisor exposure in different malocclusions.[19,24]However, Arriola et al reported that there was a statistically significant difference in the upper incisor exposure in different malocclusions.[25]

The various limitations of this study are a small sample size, cephalogram based study (Lateral cephalograms are a 2-dimensional view of the 3-dimensional object).

Conclusion

Within the limitations of this study, it can be concluded that there was an increased cervico-incisal exposure of incisors in vertical growth pattern when compared to average and horizontal growth patterns. Therefore, the incisor exposure is different in various growth patterns henceforth, diagnosis and treatment planning should be carried out keeping it in mind.

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Source of Funding: Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Analysis of Root Stumps in Middle Aged Group

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Abstract

Tooth extraction is one of the dental treatments which should be considered the last option. A decrease in the number of teeth may result in poor dietary habits and deterioration of quality of life. The number of extracted teeth can serve as an indicator of socio-economic and oral hygiene level. Extraction of permanent teeth is performed for several reasons including dental caries, periodontal disease, orthodontic reasons, impacted teeth and root stumps.To assess the prevalence of root stumps in middle aged in Chennai A retrospective cross-sectional study was conducted using the patient records from the Department of Oral maxillofacial surgery, Saveetha Dental College, Chennai from February 2019 to February 2020, and patients who had root stumps were selected by nonprobability sampling. Data was collected and then subjected to statistical analysis.Microsoft Excel/2016 data spreadsheet was used and later exported to the Statistical Package for Social Science for Windows (version 20.0, SPSS Inc., Chicago Ill., USA).The prevalence of root stumps was more in males and age groups of 50-55 years showed maximum root stumps. There is no significant difference between gender and the number of root stumps in each patient P=0.562 The study concluded that the prevalence of root stumps in the middle age group seems to be high when compared to other literature. Considering the limitations of this study extensive research needs to be done to find the prevalence of root stumps in other populations and to spread awareness among the public about root stumps which are in situ.

Keywords: *Extraction;root caries;root stumps;dental caries*

Introduction

Root stumps refers to the partial root structure that remains in the jaw. Root stumps can be retained both in dentulous and edentulous patients as a preventive measure to preserve the alveolar ridge resorption. [1 2] However, retained roots also have the propensity to cause pain and discomfort [3,4,5] to patients and can be a source of infection, especially if fractured during the extraction of non-vital teeth. [6-,9] Retained roots can complicate the fabrication of complete dentures as they can potentially cause pain and numbness if the denture base or flange impinges close to the root fragment, either immediately [10,11] or after several years due to alveolar ridge resorption. [12,13,14].Historically, in the 1920s, there was a common agreement that all root stumps should be removed, and if not removed it was believed that it can cause damage to patients which would lead to pain, infection and cyst development [15,16]

Recently, Studies which carried out on the prevalence of retained roots [17–20] have shown that the majority of retained root stumps cause no harm to patients and are only detected as incidental radiographic findings.[21,22] Under certain circumstances, the study reveals that root fragments could successfully remain in situ with normal healing taking place [23] together with the formation of a cementum layer on the dentine

Loss of teeth is an unavoidable part of human life which affects many people physiologically and also

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affects the aesthetic. Tooth extraction remains a major cause of commonly performed procedures in developing countries. [24,25] Tooth loss has significant socioeconomic, quality of life, general health, and psychological consequences.[26,27] Tooth loss has become a global public health concern of immense proportion.[28,29] Despite being preventable, dental caries and periodontal disease remain the most common reasons for tooth extraction, especially developing nations. [30,31,32]

The reasons for tooth extraction and the number of teeth extracted in a population have been linked to the oral hygiene, level of education, socioeconomic status, and individual quality of life.[33,34] Degree of urbanization has also been found to affect the pattern of tooth extractions.[35,36] Moreover, oral disease and its etiological factors exhibit regional variations. [37,38] The main aim of the study is to assess the Prevalence of root stumps in the middle ages group in Chennai as there is no study on this literature.

Materials and Methods

Study Design And Setting

This retrospective study examined the records of patients during June 2019-April 2020 who were diagnosed with root stumps at Saveetha Dental College, Chennai. Ethical approval was obtained from the Institutional Ethics Committee. The study population included patients who had root stumps and were selected by means of non-probability sampling. Patients with mental or physical disability were excluded from the study.

Data Collection

Patient record was reviewed and analyzed at Saveetha Dental College and was used to identify 93 patients in the hospital database diagnosed with root stumps. Relevant data such as patient age, sex and tooth number was recorded. Repeated patient records and incomplete records were excluded. Data was verified by an external reviewer.

Statistical Analysis

Data was recorded in Microsoft Excel/2016 (Microsoft office 10) and later exported to the Statistical Package for Social Science for Windows (version 20.0, SPSS Inc., Chicago Ill., USA) and subjected to statistical analysis. Chi square test was employed with a 5% level of significance .

Results and Discussion

The final dataset consisted of 93 patients of Indian origin diagnosed with root stumps. The mean age root stump was 44.99 years (standard deviation = 6.061 years). The age group associated with greatest prevalence of root stumps was 50-55 years (50.5% N= 47), followed by 35-40 (31.2 % N=29) and the least was 41-45 (18.3% N=17) shown in [Figure 1]Most of the patients who underwent extraction was 41.9% (N=39) were female and 58.1. % (N=54) were males showing a male dominance in patients having root stumps [Figure 2]. A total of 52 male patients have 0-5 root stumps present whereas 35 female patients had 0-5 root stumps present. 1 Male patients reported to the clinic with 5-10 root stumps present whereas 3 female patients reported to the clinic with 5-10 root stumps. One of each male and female had above 10 root stumps present. There was no significant difference between the number of root stumps and age P value= 0.946 [Figure 3].Root stumps were commonly seen in the maxilla of tooth 28 and 17 which had 19 root stumps in total. The least was seen in tooth 12 and 34 which has 2 each in total shown in Figure 4

The data for this retrospective study was based on residents of Chennai seeking treatment at Saveetha Dental College. Currently, there are no existing studies investigating the prevalence of root stumps present in the middle age group in Chennai. Since all the data available was included without a sorting process, no bias was expected in the selection of patients. The current study aims to shed light on the current scenario of root stumps present in many patients which is being neglected. Despite progress in various communication and technology in today's world there is still a lack of awareness and knowledge on root stumps among common people.

In the study conducted by M.P. Santhosh Kumar et al [39] There was in increase in number of root stumps in the age group of 34-40 years(N=42) old then followed by 41-44 (N=40) and the least being 50-55 year-old (N=38) this is contraindicating to this study as the age group in the current study is associated with greatest

number of root stumps in 50-55 years (50.5% N= 47) possible reason could be change in socioeconomic status and oral hygiene practice

According to M.P. Santhosh Kumar et al [39] the study reported with 28 males having root stumps and 26 female having root stumps.In the current study males have more root stumps than female this is in accordance to the study because this study was done in the same region.However, a study done by Rashmi Saikhedkar et al [40] in a rural dental College in Indore reported that 182 root stumps were present in a male patient and 193 root stumps present in female this is contradictory to the current study as male (N=54) has more root stumps than female (N= 39) the possible reason to this could be be attributed to more of awareness and concern regarding facial aesthetics on the part of females.

According to, Simpson et al he examined a number of retained roots in humans and suggested that root fragments, which were originally unaffected, could be safely left in position [41,42] Preserving the teeth or the roots preserves the periodontal ligament [43]similarly Prabhu et al [1] reported 64% of dentist in study thinks that retained root without any pathology can preserve periodontal ligament thus this justifies as to why there is a high incidence in of root stumps present in one individual where a total of 52 male patients have 0-5 root stumps present whereas 35 female patients had 0-5 root stumps present in the current study.

In the present study molar had the majority of the root stumps. In the previous study done by M.P. Santhosh Kumar et al [39] molars were the teeth that had to be extracted more as compared to other teeth the main reason is due to early eruption of teeth predisposing to the environment conditions compared to the other teeth erupting later in which periodontitis can be seen at older age.Most of the studies claim different findings and results because of the type of methodology of the study and socio economic status of the patient.

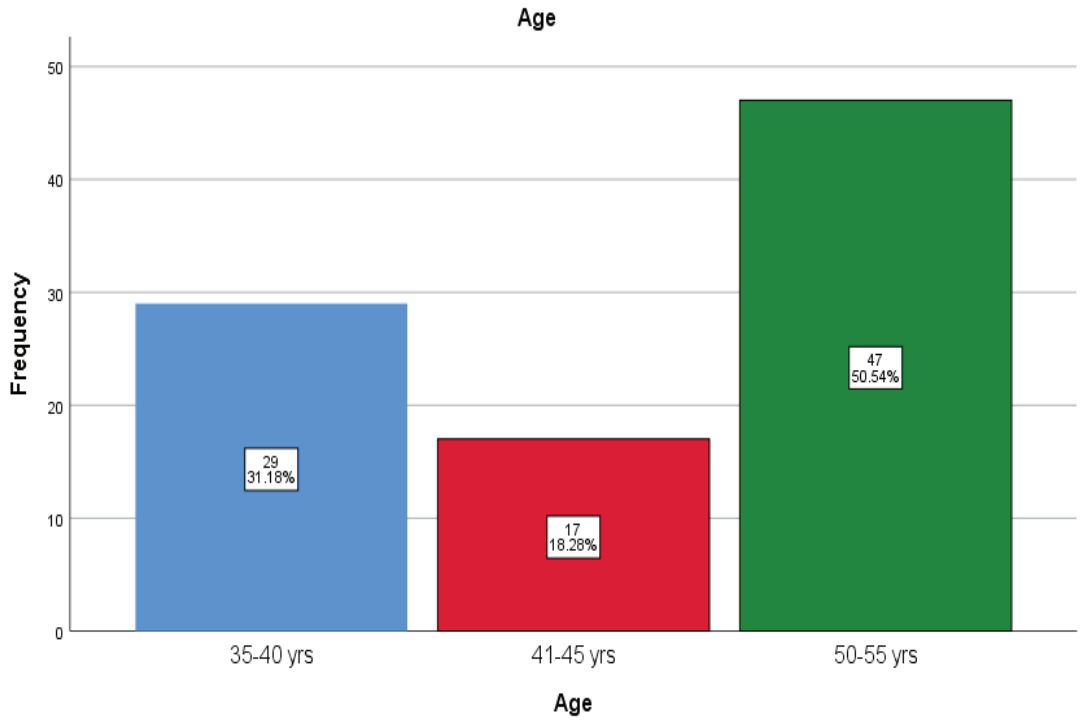


Figure 1 : Bar chart showing the distribution of age where X axis representing age and Y axis representing frequency.50.5% belonging to the age group of 50-55 years(green), 35-40(blue) (31.2 %) and 41-45years(red) (18.3%)

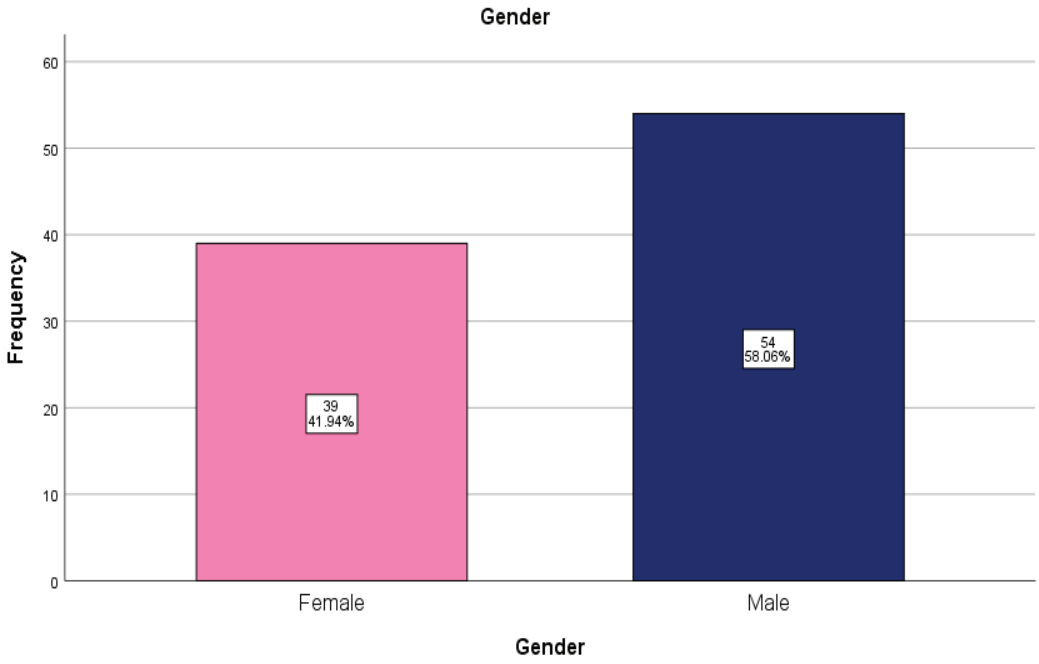


Figure 2 : Bar chart showing the distribution of gender where X axis representing gender and Y axis representing frequency.41.9% were female(pink) and 58.1. % males (dark blue)

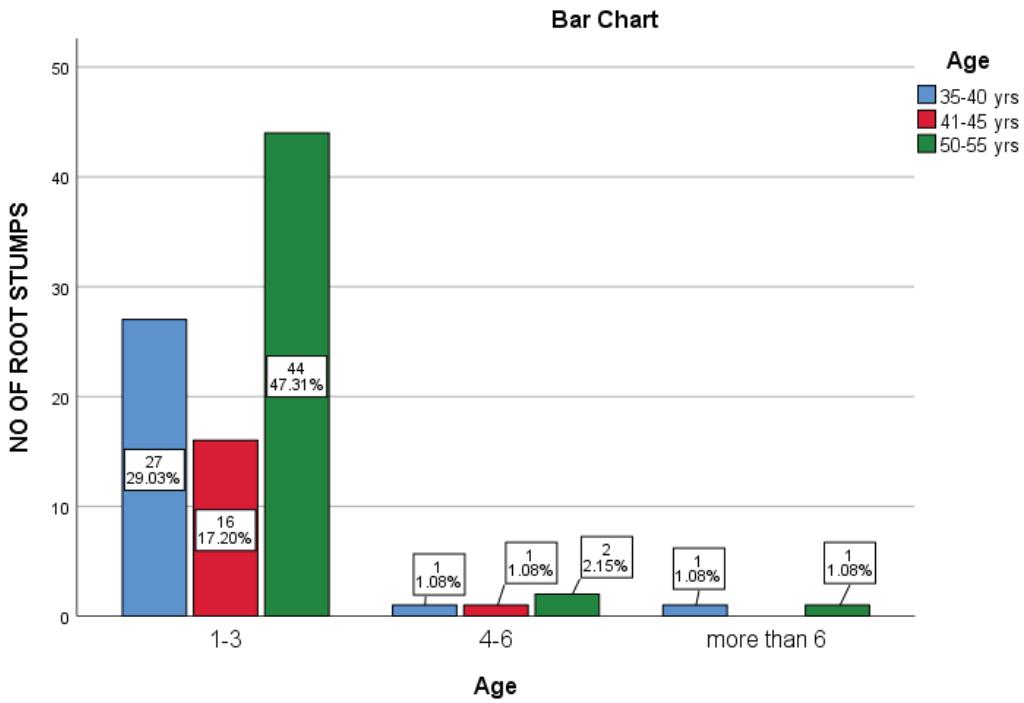


Figure 3: Bar chart showing association between number of root stumps in a patient and age. X axis representing number of root stumps in a patient and Y axis representing frequency.Blue colour denotes 35-40 years,red denotes 41-45 years and green denotes 50-55 years. Majority of the patient's with age between 50-55 years (green) have 1-3 root stumps. However, there is no association between the number of root stumps and age found statistically.Pearson chi square value was 0.742 and P value was 0.496(>0.05).

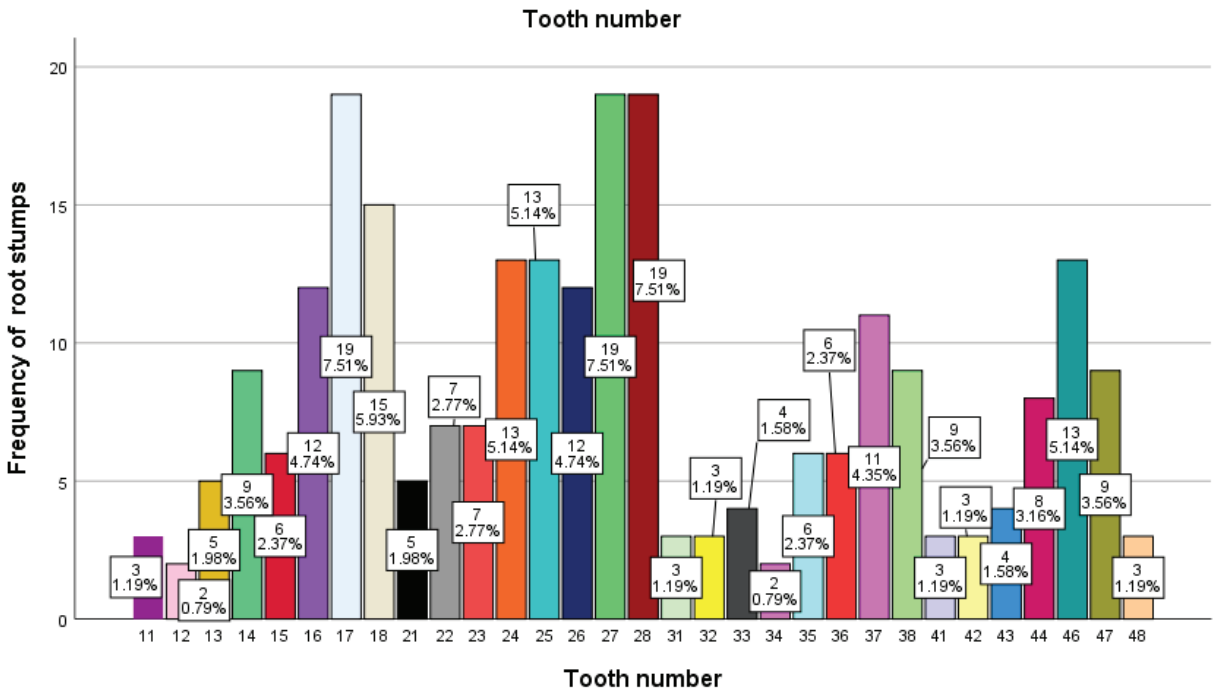


Figure 4: Bar chart showing distribution of root stumps in a patient.X axis represents the tooth number and Y axis represents frequency of root stumps. Majority of the root stumps was seen in tooth 28 (dark brown)with 7.51% and 17 (light blue) with 7.51% which had 19 root stumps in total. The least was seen in tooth 12(light pink) with 0.79% and 34(pink) which has 0.79%.This shows that the upper arch (from tooth number 18 to 28) had more root stumps than the lower arch (from tooth number 38 to 48).

Conclusion

Within the limitations of this study, it was found that the prevalence of root stumps in the middle age group seems to be higher when compared to literature from other regions. Considering ill effects of root stumps, it can be concluded that there exists a paramount need for more extensive research to find the prevalence of root stumps in all the age groups and in a different study population.

Conflict of Interest: Authors have no known conflict associated with this study

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Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Analysis of Class III Skeletal Malocclusion that Require Orthognathic Surgery for Correction as Compared to Camouflage Treatment - A Retrospective Study

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Abstract

Class III malocclusion is a complex anomaly . Not all adult class III patients will need surgical correction. In the Indian population the incidence of a class III malocclusion is lesser than a class II. Orthodontic case records were reviewed and analysed. Class III adult malocclusions were evaluated to estimate the prevalence and the treatment protocol. From this evaluation of class III patients the span on close to a year report of 44 adult class III patients out of whom 10 patients required orthognathic surgery for correction . Surgery first done in 2 cases and other cases were single jaw or bi-jaw surgery done using conventional approach.In the camouflage treatment most of the cases were treated by non- extraction. Correct classification of adult Class III malocclusion patients succeeded to a high degree. Pearson's chi-square value is 1.867 and P value is 0.1 and the results of this study are not significant. The severity of the malocclusion should be used to determine the treatment protocol.Orthognathic surgery was successful in 92% patients . Most of the camouflage studies use mini-implants and non-extraction protocols. Surgical treatment done according to severity of class III and patient compliance.Among the assessed population 44 patients had skeletal class III malocclusion and in the adult patients 8 patients were treated with orthognathic surgery . The two main deciding factors to conclude as the treatment plan was based on the severity of the malocclusion and the patient compliance. Orthognathic surgery was successful in 92% patients

Keywords: *Skeletal Class III , orthognathic surgery , camouflage, Prognathic mandible*

Introduction

Class III malocclusion has varied etiological features that involves maxilla and mandible and sometimes involves the craniofacial region.[1,2] Class III malocclusion involves anomalies of skeletal and dentoalveolar components. [1] Their primary use is to provide a means of comparison of individual dentofacial characteristics with a population average in order to identify areas of specific deviation, as well as describe the spatial relationship between various parts of the craniofacial structures.[2] Orthodontic treatment involves the application of forces that are continuous in activity on as many areas of the dentition as possible and working in the direction in which the teeth are to move, with maximum comfort to the patient.[3] [4,5]Crown lengthening with osteotomy and gingivectomy may result in varied gingival heights between adjacent teeth compromising esthetics.[6]

The class III malocclusions has the highest frequency with prognathic mandible and sometimes with a combination of prognathic mandible and retrognathic mandible.[7]

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In the cephalometric findings class III patients have a shorter anterior cranial base, longer posterior cranial base, anterior bas, shorter retrusive maxilla, proclined maxillary incisors, retroclined mandibular incisors , increase in lower anterior facial height, gonial angle is obtuse [8,9] . The presence of risk factors such as age, gender and obesity . [10] This usually results in the application of a diagonal vector of force on the maxillary anterior teeth of both sides.[11]

The envelope of discrepancy shows the limit of corrections that can be done by orthodontic treatment. [12] The side effects of maxillary dentition as an anchorage unit for protraction could lead to labio version of maxillary incisors, extrusion of maxillary molars,[6] counter clockwise rotation of palatal plane and clockwise rotation of mandible[13] Direct bonding of orthodontic brackets since its inception into orthodontics has made tremendous advances and continuous efforts are on to find better bonding materials as bracket dislodgement continues to be a problem for orthodontists. [14]

In order to establish the cephalometric criteria in adult class III patients in order to allocate them to the treatment plan move objectively.[15] Pretreatment lateral cephalograms to decide either surgical orthodontic correction by univariate statistical methods. The difference between the ratios found in ANB (Point A , Point B and nasion as viewed on Lateral cephalogram) angle, mandibular plane angle, mandibular incisor angulation , Holdaways soft tissue relationship. Skeletal and dentoalveolar deviations need to be evaluated for individual variations to explain the malocclusion. [16] It is also better to understand the relationship between craniofacial structure and occlusion using multivariate approach.[17] The approach to be used after careful considerations of limitations are the cephalometric analysis and analysis landmark identification, selection and separation of groups, sample size. [18].

Not all patients are candidates for surgical correction, patient assessment and selection are essential diagnosis and treatment planning. The aim of this study was to analyse and distinguish between surgical and non- surgical subjects presenting skeletal class III malocclusions.

MATERIALS AND METHODS

This study design is of retrospective aspect conducted in an online setting in the centre of Saveetha Dental College using the online record keeping software DIAS. Due to the not wide prevalence of class III malocclusions in the Indian population, this method of search provided a large number of the same at a given point. The inclusion of skeletal class III malocclusion in the age group of 16 years and older . This study couldn't specify a particular population and race. The number of people in this study are the investigator and guide. The study was cleared by approval from the university review board. The sampling period was obtained between June 2019 to March 2020. Orthodontic case records were evaluated. Adult patients above the age of 16 with skeletal class III malocclusion. The sample requires a minimum of 40 patients with the skeletal malocclusion of class III after estimating using G power analysis by keeping power at 90. [19]After evaluating the case records to determine and isolate class III photographic data and cephalometric values were evaluated. The data was tabulated to excel sheet. The variables in this study taken into consideration are the severity of class III malocclusion based on reverse overjet , gonial angle, wits appraisal , ANB and overbite. The statistics was done using SPSS for performing analysis , chi-square test was done and cross tabulation to obtain graphical representation.

Results and Discussion

The time period for evaluation is from June 2019- March 2020 . The evaluation of 1128 cases revealed a total of 44 adult class III patients . 10 cases were chosen for surgical correction. Surgery first approach done in 2 cases . Depending on the correction needed and the jaw at anomalous relation and the soft tissue relationship of patients. One case required only mandibular setback, other case bijaw surgery. (Figure 1)

Conventional surgical approach was done in 8 cases. 87% cases were treated with camouflage, 85% cases were treated as non - extraction protocol.

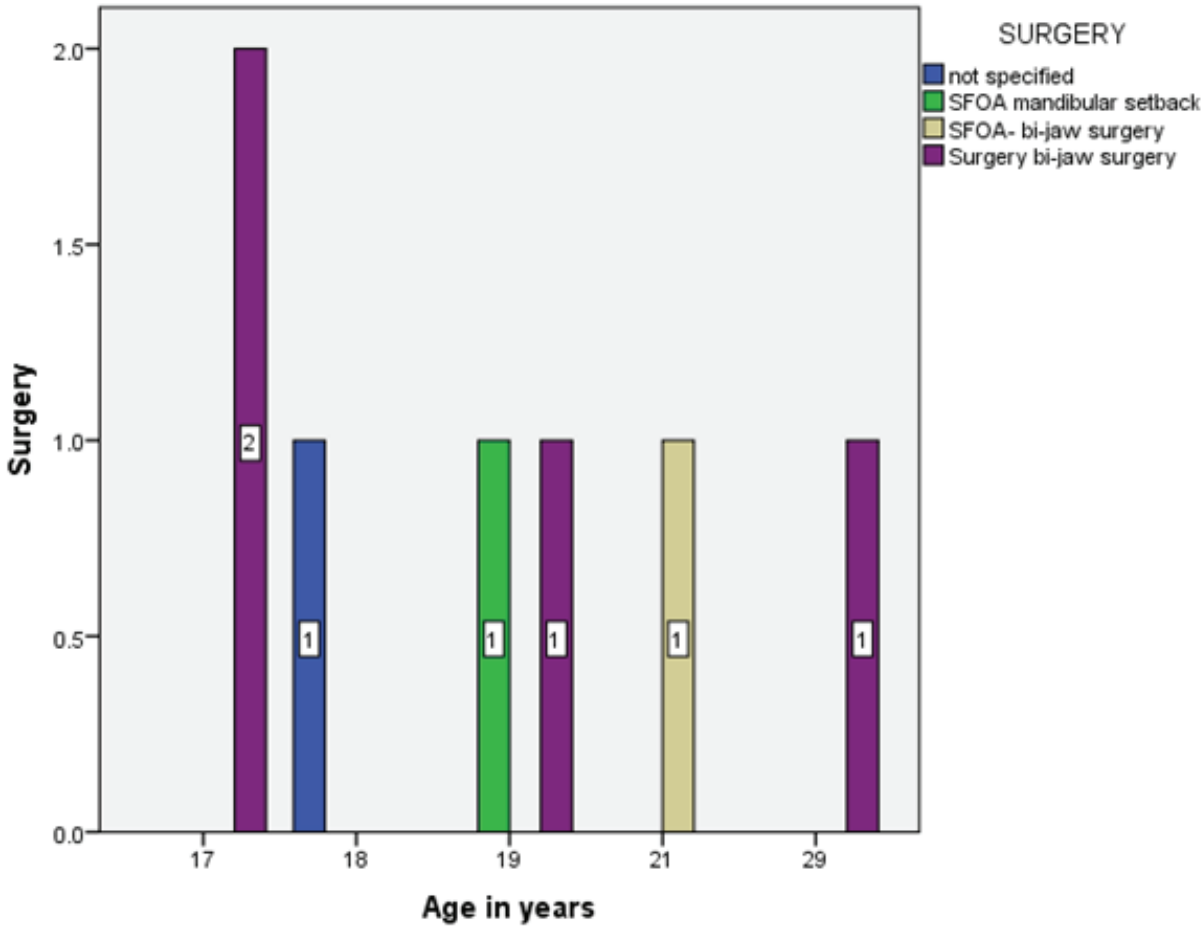


Figure 1 : Surgery done in adult class III patients

Bar graph represents the comparison between various age groups and the surgical plan options for class III orthognathic surgery. Green color represents surgery's first orthognathic approach with only a mandibular setback, blue color represents the surgical plan that isn't restricted to a particular nature, grey color represents surgery's first orthognathic approach and a bi-jaw surgery and purple color represents the conventional surgical approach.This bar graph shows that camouflage was most commonly done in all age groups and in adults above 17 years years of age surgery was done and in surgery , a bi-jaw approach was most common. However, there is no significant difference statistically. Pearson's chi-square value is 1.867 and P value is 0.1 (>0.05).

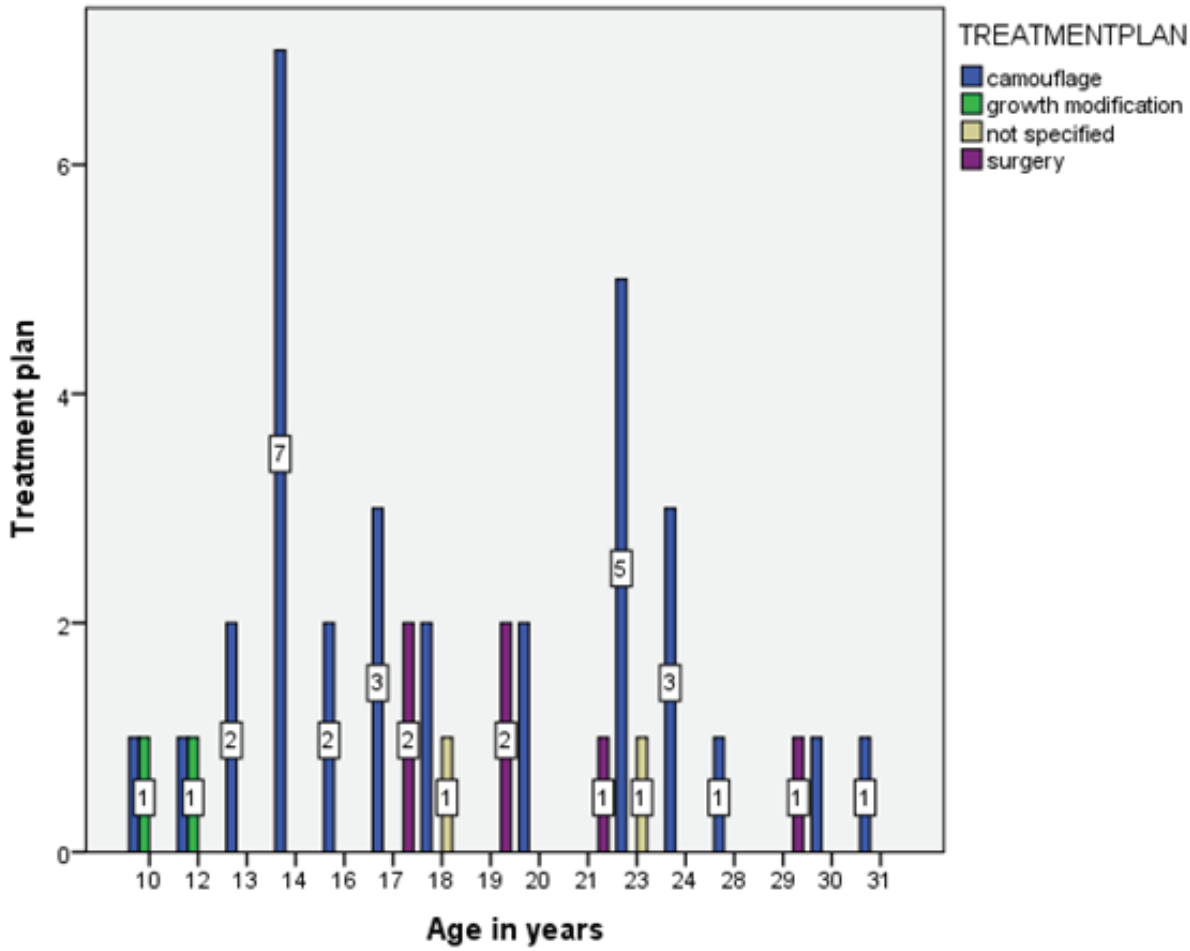


Figure 2 : Class III patient - Treatment plan . This bar graph represents comparison of all the various treatment plan options done in skeletal class III cases in comparison to various age groups of orthodontic participants. Blue color represents the camouflage as a treatment plan, grey represents an integrated treatment approach, green color the growth modification treatment plan and purple color represents the surgical plan. This bar chart shows that camouflage(blue) was most commonly done across all the age(years) groups of class III malocclusion and in adults above 17 years surgical treatment is done most commonly following camouflage. However, it is not significant statistically.

Pearson's chi-square value is 1.867 and P value is 0.1(>0.05) and the results of this study are not significant.

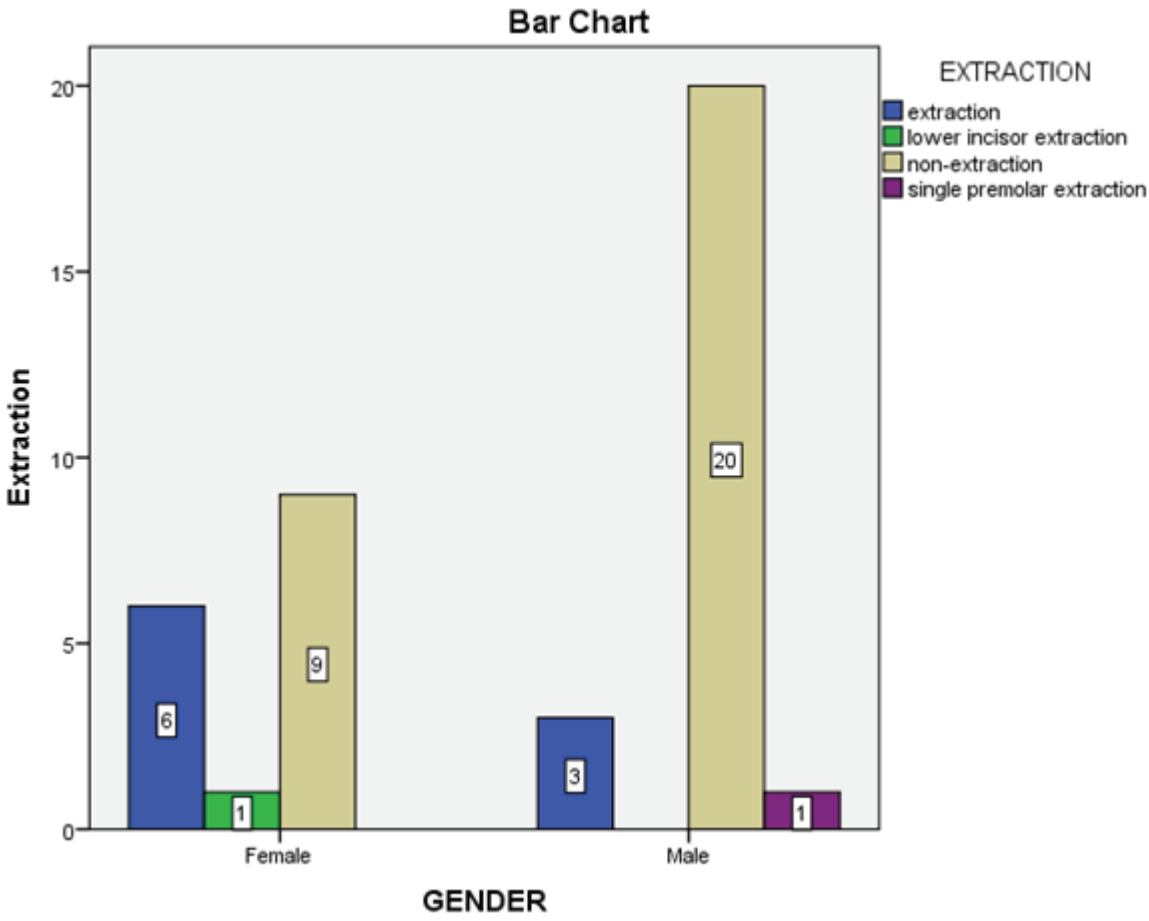


Figure 3 : The number of extraction done in Class III cases .This bar graph represents the extraction choice in skeletal class III cases in comparison to the gender (Males and Females). Green color represents lower incisor extraction, grey color represents non- extraction, purple color represents single premolar and blue color represents extraction. This bar graph shows that the majority of male patients needed extraction in skeletal class III cases of them in both the gender a non extraction approach is most commonly done. However it is not significant statistically.

Pearson's chi-square value is 1.867 and P value is 0.1(>0.05)

This study deals with the pretreatment separation of adult class III malocclusion patients into surgical and non-surgical cases. (Figure 1) Figure 2 represents the various treatment plan options for skeletal class III cases and figure 3 represents the extraction pattern chosen in class III patients. The decision as to which form of treatment was indicated to those who have degree of anteroposterior and vertical skeletal discrepancy, the inclination and the position of incisor and the dentofacial appearance. Lateral cephalometric examination to evaluate the growth pattern in Class III subjects the effect of treatment and outcome. [20] Pearson's chi-square value is 1.867 and P value is 0.1 and the results of this study are not significant. In younger individuals the camouflage or growth modification can be most commonly done whereas in adults above 19 years of age surgery and camouflage is attempted. In both the mechanics non-extraction was most commonly done as opposed to extraction pattern.

The envelope of discrepancy states that not all malocclusion can be corrected by orthodontic treatment alone , treatment with growth and surgical treatment .It is important to have clear criteria for determining patient option by established cephalometric yardsticks. While

providing absolute anchorage, these devices are used for specific periods of time and rely only on mechanical retention with the surrounding bone.[21] This initial period of treatment is also a time of acclimatization for patients because they experience restrictions concerning the food that can be masticated with abandoned appliance. [22] The fundamentals of orthodontics is that teeth move through the alveolar bone when adequate forces are delivered. Various local and systemic factors like age, nutrition, consumption of drugs, etc seem to affect orthodontic tooth movement.[23]

The results of this study indicates that the important factors that differentiate the surgery and non-surgical treatment options based on size of anteroposterior discrepancies, the inclination of mandibular incisors.[24] Discriminant model is relatively large sample so that new patient can categorize into surgical and non-surgical .[25] Miniscrews as effective temporary anchorage devices have occupied a central role in a typical orthodontic setup, since anchorage control and patient cooperation are very critical.[26]

Riedel has noted the ANB is the most commonly used cephalometric analysis to describe skeletal discrepancies between maxilla and mandible. The validity of a true indicator for anteroposterior jaw relationship is criticized due to the variations in the Nasion that is taken as a fixed point.[27].A patient judges the outcome of an orthodontic treatment by assessing the final improvement in facial esthetics. The gonial angle in patient is an important indicator for the type of growth pattern and has an influence on the treatment.[28]The binding relationship between orthodontic treatment and facial esthetics has made the facial outline as an important guideline for the treatment planning.[29] The sectioning was done to reduce the amount of time spent in optical sectioning of the tooth with the confocal microscope. The sliced disks were stored dry in amber-colored bottles until microscopic evaluation.[30] Incisor extraction can help minimize arch expansion, decrease the amount of tooth movement required, minimize facial change and reduce treatment time. When a lower incisor is extracted the canine will lie mesially beneath the upper lateral incisor and the canine tip will contact the distolingual marginal ridge of the lateral incisor rather than the mesial fossa of the canine. Magnitude of ANB angle is affected by rotations of the jaws relative to the cranial base. [31]. Because of its predictive power , discriminant analysis appears to be a particularly valuable tool for identifying class III patients in orthodontic treatment is sufficient for therapy.

Most of the camouflage studies use mini-implants and non-extraction protocols. Surgical treatment done according to severity of class III and patient compliance. Prevalence of class III patients in Indian population the class II and Class I cases . The type of surgery is not the same in all patients. Difference in choice between the surgery being done as a surgery first or conventional approach. Reason a surgical treatment is opted out for a patient also depends on patient compliance,cast and other systemic risk factors that can be better obtained from a prospective study design.Well planned randomised control trial, determine the treatment plan to give best results to compare the surgical versus the non-surgical treatment and comparison of surgery first and conventional surgery approach.

Conclusions

This study concluded that among the assessed population 44 patients(17%) had skeletal class III malocclusion and in the adult patients 8 patients(18%) were treated with orthognathic surgery . The two main deciding factors to conclude as the treatment plan was based on the severity of the malocclusion and the patient compliance. Orthognathic surgery was successful in 92% patients. In comparison, male patients needed extraction in skeletal class III cases and in both the gender a non extraction approach is most commonly done. Camouflage was most commonly done across all the age groups of class III malocclusion and in adults above 17 years surgical treatment is done most commonly following camouflage.

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Assessment of Most Common Furcation Site among Smokers with Chronic Periodontitis

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Abstract

It is generally accepted that the primary cause of periodontitis is bacterial infection of long duration. In addition there are several risk factors that may increase the probability and severity of periodontitis, one of which is smoking. This is a retrospective clinical study carried out at Saveetha Dental College, Chennai. This study involves the analysis of the most common furcation site among smokers with chronic periodontitis. The data were taken over a period of one year from over June 2019 to March 2020. The sample/data were retrieved and 265 male smokers with periodontitis were examined for grade of furcation involvement based on Glickman's classification. In this study, the most commonest site of furcation among smokers with chronic periodontitis are maxillary molars. Grade 1 furcation > Grade 2 furcation > Grade 3 furcation > Grade 4 furcation. On comparing (Chi square analysis) the grade 1, grade 3 and grade 4 furcation with different age groups, grade 1, grade 3 and grade 4 furcation was mostly seen in maxillary molars of smokers with chronic periodontitis of age group 41- 50 years. However, it was statistically not significant(p-value >0.05). On comparing the grade 2 furcation with different age groups, Grade 2 furcation was mostly seen in maxillary molars of smokers with chronic periodontitis of the age group 51-60 years and it was statistically significant. Within the limits of the study the most common site of furcation among smokers with chronic periodontitis is maxillary molars.

Keywords:*Periodontitis, molars, smokers, furcations, periodontal health.*

Introduction

Nowadays oral health and oral hygiene is one of the biggest concerns. It is generally accepted that the primary cause of periodontitis is bacterial infection of long duration. Periodontitis is a chronic inflammatory disease which results in the destruction of supporting structures of the teeth [1–3]. In addition, there are several risk factors that may increase the probability and severity of periodontitis, one of which is smoking. Tumor necrosis factor (TNF) is one such pro-inflammatory cytokine that causes periodontal tissue destruction [4]. Cigarette smokers are more likely to exhibit clinical signs of periodontitis than comparable [5].

Cigarette smoking is a major environmental factor associated with periodontal diseases [6,7]. Cigarette smoking has a deleterious effect on the periodontal tissues which is independent of plaque and causes inflammatory reactions. Interleukins comprise a large group of cytokines that are naturally occurring glycoproteins produced by the body. They help in recruitment of neutrophils and macrophages to participate and amplify the inflammatory immune reaction [8–10]. Moreover smokers are more likely to be referred for specialised periodontal treatment than non smokers [11]. Smoking adversely affects both local and systemic host systems, which may account for its deleterious effects on

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periodontal health [12,13].

Smoking cessation may restore the normal periodontal healing response[14]. At a practical level, it appears important to quantify to what extent periodontal treatment of smokers is likely to be more complex than that of non-smokers [15,16]. One measure of the severity of periodontal disease is the involvement of the molar furcations [17]. It appears considerably more difficult to stabilise the periodontal condition when it involves the molar furcation [18].The fate of molars is determined by the following factors: grade of furcation involvement, bone level, and smoking [19,20].

The morphology of the furcation region provides an environment favorable to bacterial plaque retention, which makes the professional and personal dental plaque control difficult and affects positively on periodontal health destruction [21–23]. For those reasons, teeth with furcation involvement in periodontal disease have been shown to have a poorer prognosis than teeth without furcation involvement [24,25]. Regardless of the degree of the furcation, the therapeutic approach always begins with the initial phase of periodontal treatment, which includes all the necessary guidelines for proper self-performed oral hygiene, cessation of smoking, extraction of hopeless teeth, supragingival and sub gingival debridement [(26,27)]. Moreover, furcation involvements are common in patients with periodontitis and smoking habits. The aim of this study is to analyse the most common furcation site among smokers with chronic periodontitis.

Material and Methods

This is a retrospective clinical study, carried out at Saveetha Dental College. This study involves the analysis of most common furcation sites among smokers with periodontitis visiting Saveetha Dental College that were taken over a period of one year, from June 2019 to March 2020. Ethical Approval was obtained from the Institutional Review Board (ethical approval number- SDC/ SIHEC/ 2020/ DIASDATA/ 0619-0320). The data was cross verified by 2 examiners. The data were retrieved and examined to analyse most common furcation sites among smokers with periodontitis visiting Saveetha Dental College.

Inclusion Criteria **:**

- Male patients
- Age : 30 -70 years
- Smoking habits and presence of chronic periodontitis

EXCLUSION CRITERIA **:**

- Systemic disease or conditions
- History of periodontal treatment for the past 6 months

A total of 265 patient data were collected and assessed for age, gender, smoking habits, periodontitis. The identification of furcation was carried out according to Glickman's classification and tabulated as Grade 1, Grade 2, Grade 3, Grade 4. Collected data was tabulated in the excel sheet. The data was imported and transcribed in the statistical analyses package for social sciences version 20(SPSS) IBM corporation. Chi square test was done. Analysis was based on quantitative variables and frequencies for categorical variables. P value less than 0.05 was considered to be statistically significant.

Results and Discussion

TABLE 1: Table shows the percentage distribution of various grades of furcation involvement in molars

	Maxillary molars	Mandibular molars	Both maxillary and mandibular molars
Grade 1 furcation	81.75%	17.52%	0.73%
Grade 2 furcation	74.39%	20.73%	4.88%
Grade 3 furcation	80%	20%	-
Grade 4 furcation	66.67%	33.33%	-

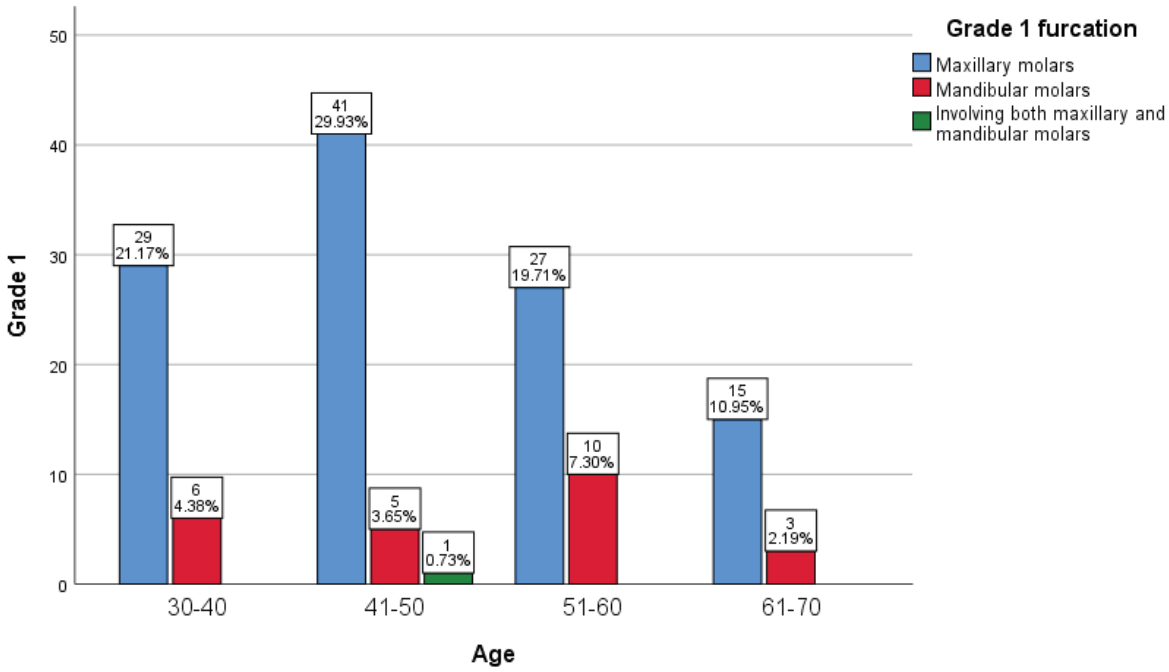


Figure 1: The graph represents the association of age and grade 1 furcation where blue denotes maxillary molars, red denotes mandibular molars and green denotes both maxillary and mandibular molars involvement. X axis denotes age and Y axis denotes count of grade 1 furcation. Grade 1 furcation is mostly seen in maxillary molars of smokers with chronic periodontitis of age group 41- 50 years (29.93%). Chi-square value = 5.637; P-value = 0.465 (> 0.05), hence statistically not significant.

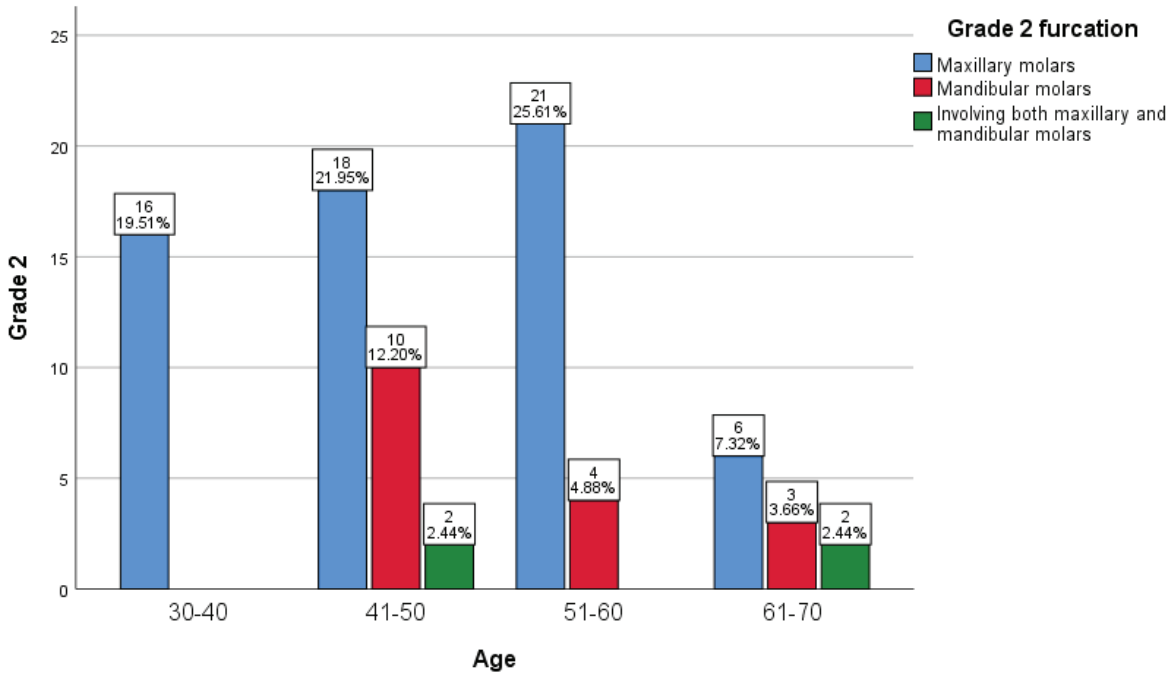


Figure 2: The graph represents the association of age and grade 2 furcation where blue denotes maxillary molars, red denotes mandibular molars and green denotes both maxillary and mandibular molars involvement. X axis denotes age and Y axis denotes count of grade 2 furcation. On comparing the grade 2 furcation with different age groups, Grade 2 furcation is mostly seen in maxillary molars of smokers with chronic periodontitis of the age group 51-60 years (25.61%) than the other age group. Chi-square value = 15.438; P-value = 0.017 (0.05), hence statistically significant.

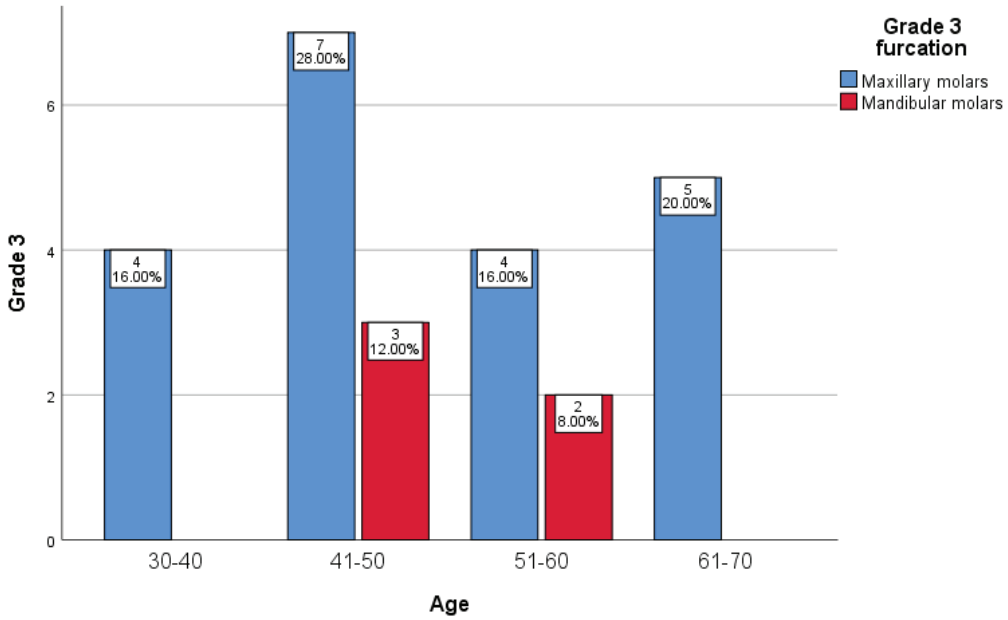


Figure 3: The graph represents the association of age and grade 3 furcation where blue denotes maxillary molars and red denotes mandibular molars. X axis denotes age and Y axis denotes count of grade 3 furcation. On comparing the grade 3 furcation with different age groups, grade 3 furcation is mostly seen in maxillary molars of smokers with chronic periodontitis of the age group 41-50 years (28%). Chi-square value = 3.542; P- value = 0.315 (> 0.05), hence statistically not significant.

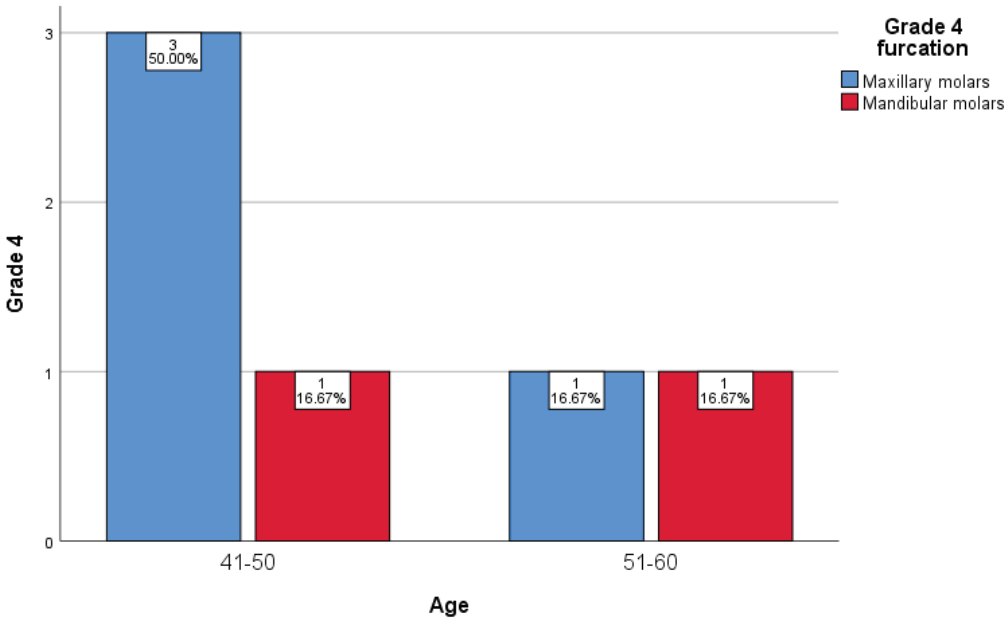


Figure 4: The graph represents the association of age and grade 4 furcation where blue denotes maxillary molars and red denotes mandibular molars. X axis denotes age and Y axis denotes count of grade 4 furcation. On comparing the grade 4 furcation with different age groups, grade 4 furcation is mostly seen in maxillary molars of smokers with chronic periodontitis of the age group 41-50 years (50%). Chi-square value = .375; P- value = 0.540 (> 0.05), hence statistically not significant.

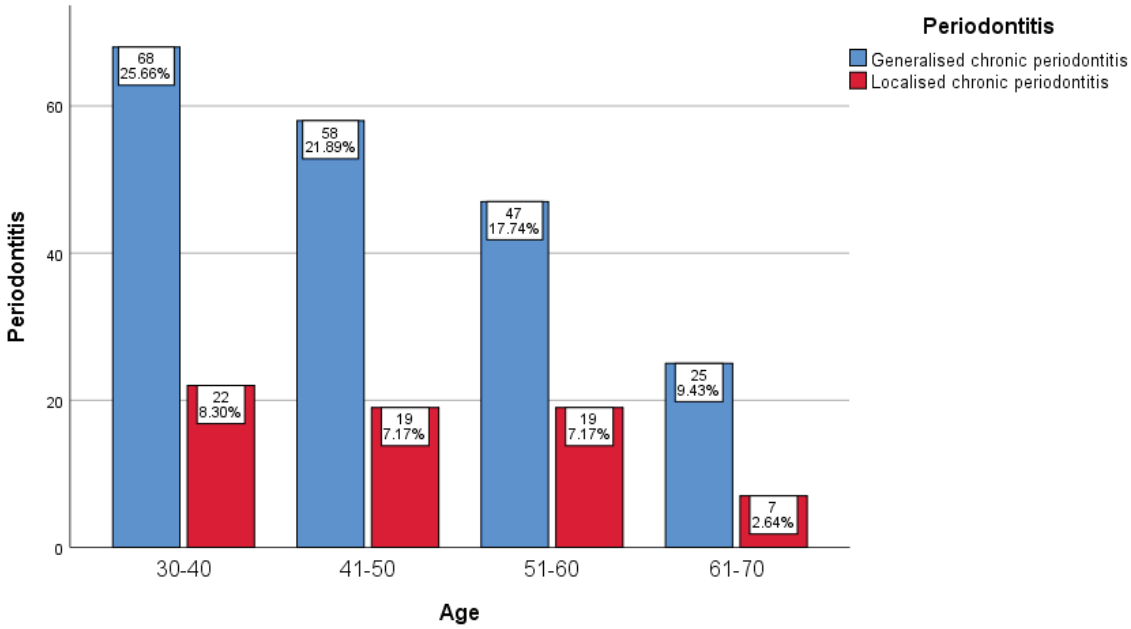


Figure 5: This graph represents association of age and periodontitis where blue colour denotes generalised chronic periodontitis and the red colour denotes localised chronic periodontitis. X axis denotes age and Y axis denotes count of periodontitis. Majority of the persons with age between 30-40 years have more prevalence of generalised chronic periodontitis than the other age groups. However, there is no significant difference between prevalence of both generalised chronic periodontitis and localised chronic periodontitis among the different age groups. Chi-square value = 0.674; P-value = 0.879 (> 0.05), hence statistically not significant.

Periodontitis disease is the leading cause of tooth loss. Periodontitis is a chronic inflammatory condition which was initiated by gram-negative organisms present in the tooth supporting structures. Disease progression occurs as a result of host-immune response to bacteria, leading to destruction of connective tissue and alveolar bone [28,29]. The pathogens present in the subgingival flora produce various endotoxins which are a prerequisite for periodontal disease. In this study, we observed that the prevalence of furcation involved molars was higher in the maxilla than in the mandible in all the smoker patients (Table 1) which is consistent with various other clinical studies [30–33]. In contrast some previous literature shows that the most common site of furcation is mandibular molars. This variation is seen due to variation in morphology of the patients, oral habits and various environment factors affecting the periodontal health.

In this study, we observed that the prevalence of furcation involved molars was higher in the maxilla than in the mandible in all the age groups (Figure 1, Figure 2, Figure 3, Figure 4) which is consistent with another study [30–33]. In the study we observed that smoking is the risk factor for furcation in the molars which is consistent with another study [34], which shows that cigarette smoking is a major environmental factor associated with periodontal diseases. Cigarette smoking has a deleterious effect on the periodontal tissues which is independent of plaque [35,36]. According to the present study generalised chronic periodontitis commonly seen in all age groups (Figure 5) which is consistent with previous study in which periodontitis is found to be common in all age groups [37,38]. On comparing the grade 1, grade 3 and grade 4 furcation with different age groups, grade 1, grade 3 and grade 4 furcation was mostly seen in maxillary molars of smokers with chronic periodontitis of age group 41-50 years. However, it was statistically not significant. On comparing the grade 2 furcation with different age

groups, Grade 2 furcation was mostly seen in maxillary molars of smokers with chronic periodontitis of the age group 51-60 years (25.61%) and it was statistically significant.

According to the present study the most common age group with furcation is 41 -50 years (Figure 1, Figure 3, Figure 4) which is consistent with another study [33,39]. Young smokers diagnosed with aggressive forms of periodontitis were shown to have more affected teeth and higher mean loss of periodontal attachment than non smokers [37,40] .

The uniqueness of this study is that it helps us to understand the most common site of furaction among the smokers. The limitations of the present study are that it cannot be generalised to a larger population and the influence of systemic disease affecting the severity of furcation was not taken underconsideration. The future scope includes study of larger populations, study of furcation involving individual teeth.

Conclusion

Within the limits of the study the most common site of furcation among smokers with chronic periodontitis is maxillary molars. Grade 1, grade 3 and grade 4 furcation was mostly seen in maxillary molars of smokers with chronic periodontitis of age group 41- 50 years. Grade 2 furcation was mostly seen in maxillary molars of smokers with chronic periodontitis of the age group 51-60 years. Smoking cessation may restore the normal periodontal healing response. At a practical level, it is important to educate the patients or the individual about the adverse effects of smoking on oral cavity which affects the periodontal health.

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Evaluation of the Knowledge Attitude and Practice of Management Skills in Laboratory Set Up among Undergraduate Dental Students

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Abstract

Managers in healthcare have a legal and moral obligation to ensure high-quality of patient care. Management skills can help in influencing the quality and safety of clinical outcomes, processes and performance.The aim of the study is to analyse the knowledge among undergraduate dental students about the managerial skills in setting up a laboratory so as to assure quality. A cross-sectional observation study was conducted among undergraduate dental students. A total of 105 participants were included in the study. The study was conducted online.The data collected were tabulated and analysed. The results showed that 87.6% agreed that management skills help in the progress of the lab and 77.1% were aware of human resource management. In our study, 91.4% agreed that high-quality laboratories have better management practises and 88.6% agreed that leadership is a major management skill. In our study we found that the undergraduate students have less knowledge and awareness about management skills. This void can be filled by including management skills and laboratory set up as a part of the curriculum.

Keywords: *management skills; undergraduate dental students; clinic; laboratory; Human resource management*

Introduction

India has a dual healthcare system comprising the private sector which is the major source of healthcare in India and the public sector which is funded by the government. Access to public health care is regardless of socio economic status whereas the private sector caters more to the Urban population. According to a National Family Health Survey, 70% of private medical sectors are urban [1]. In India one of the key constraints for achieving optimal health outcomes are due to lack of health management schemes, leadership capacity and absence of a solid structured policy framework to develop the same. Physicians have to combine their management and leadership skills to meet the challenges scenarios[2].

Human Resource Management (HRM) guarantees the effective use of knowledge, competence, individual abilities and other characteristics possessed by individuals aimed towards the achievement of an organization's goal. Effective leadership of the clinician in healthcare organisations are linked directly to superior patient care outcomes[3]. Most physicians are ill prepared to undertake responsibilities requiring organisational and management skills. This is because the medical schools and residency training facilities fail to teach them this much required skill[4]. The American College of Preventive Medicine has put forward 4 managerial competencies that are mandatory for a clinician in order

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to provide good healthcare. The competencies are the ability to manage finance, ability to manage elements of the organisation and an in depth knowledge of the legal aspects [5,6].

There is a low consensus on the basic competencies and skills required for a healthcare manager with no additional academic improvement and professional development to perform their managerial function in a healthcare set up[7]. There is limited literature on the competencies of a manager and the literature is partial in providing a mutual agreement with the role[8].This study aims to analyse the knowledge and awareness among the undergraduate dental students about the managerial skills required to set up a laboratory in order to ensure quality.

Materials and Methods

A cross sectional observational study was conducted among the undergraduate dental students. The survey was carried in a university setting, this made the study economical, easy to create with wide reach, easy to gather large data and helped in quick interpretation. The study was approved by the scientific review board of the institution. The study comprises a self administered questionnaire which was circulated using google forms among the dental students. 18 questions were included in the questionnaire. The questionnaire was divided into three parts which included questions pertaining to knowledge on management skills and HRM, six questions on attitude towards managerial skills and the rest of the questions on its benefits in dentistry. The study was conducted among 105 undergraduate dental students. All the data were collected using Google forms, uploaded in Microsoft Excel and analysed using SPSS software. Percentage analysis was done and Chi square test was done to com[are the level of awareness between the various years of study. pie charts and bar graphs were used to depict the results.

Results and Discussion

Management focuses on how to do work and the manager alliance process so that work is consistent and efficient[9]. Leadership focuses on what to do and why, where leaders articulate a vision and inspire people to work well together to do complex, innovative work[10]. In both the roles, leaders and managers influence people and work processes to achieve shared objectives[9]. The distinction between the leadership and management role is less important than achieving the right balance and enacting leadership behaviours and management structures that support efficiency and process reliable human relations, innovation and adaptability[10].

Our study revealed that out of 105 participants 66.7% were females and 33.3% were male. It was observed that 13.3% belonged to first year, 12.4% belonged to second year, 41.9% belonged to third year, 12.4% belonged to final year and 20% belonged to internship.

Human Resource Management [HRM] is the system that guarantees an effective use of knowledge, competence, ability and other characteristics possessed by individuals aimed for the achievement of an organisation's goals. After the industrial period, productivity is increasingly based on the knowledge, skills and activities of the trained human intellect [11]. In the present study, 87.6% of the participants were aware that management skills help in the progress of the laboratory and 77.1% were aware of HRM. On comparing between the groups, there is significantly increased awareness regarding managerial skills among 1st years and interns when compared to the other years of study (Figure1). On assessing awareness regarding HRM , most of the participants were aware . On evaluating their awareness on the various factors which enable HRM to achieve the organisational goal, 27.6% agreed on knowledge , 17.1% on competence, 5.7% on ability, 2.9% with other characteristics and 49.7% with all the above factors. The opinion was varied among the participants . On evaluating the awareness regarding the important managerial skills required for a manager to manage a laboratory 49.5% agreed on leadership,21.9% for planning,24.8% for organization and 3.8% for controlling. On comparing between the groups, no statistical significance was seen(Figure 2).On the other hand only 44.8% agreed that professional relationships, training employees, seeking opinion from other managers to plan and prioritise orders will be useful in developing a better manager to manage time, and on comparing the association of year of study and skills possessed for better management of laboratory, the Pearson Chi Square test shows p=0.193 which is not statistically significant(figure 3).

High-quality hospitals are more likely to have better management processes related to operations, monitoring, target setting and human resource than low quality hospitals. Management schools were significantly higher in hospitals with boards that pay greater attention to quality and were more likely to adopt effective practices related to the use of data or clinical quality metrics(Ireri et al. 2017). In our study we observed that 91.4% of the participants agree that high-quality laboratories have better management processes than the low quality ones .On comparing between the various years of study, good awareness was seen among all the years of study, but statistical significance was not found(Figure 4).

In a systematic review by Parand et al where they have compared the managerial time spent on quality and safety, managerial quality and safety activities. In that study, manager's time spent and work can influence quality and safety clinical outcomes, processes and performance. [12] In our study we found that most of the participants agreed that leadership and management influence the delivery of high-quality care. In our study 25.7% accepted leadership,21.9% accepted management, 52.4 % agreed both leadership and management influences delivery quality of high-quality care and on comparing the association of year of study and the factors that influence the delivery of high quality care ,the Pearson Chi Square test shows p=0.034 which is statistically significant(Figure 5).In ous study we observed that the participants 17.1% agreed leadership ,25.7% agreed communication ,5.7% agreed managing budgets , 2.9% agreed managing budgets and 48.6% agreed all the factors to be the essential management skills in a laboratory.

The various activities in the laboratory are diagnosis, research, teaching [13–20]. The various diagnostic methods are histopathological testing, microbiological analysis test, immunohistochemical analysis, genetic analysis, radiographic analysis [21–27]. In our study we observed that opinion among the participants regarding the major activities of clinical laboratories are administration (26.7%), patient care (15.2%), research (3.8%) and combination of administration, patient care service, research and teaching (51.4%) .

87.6% agreed that managerial skills can make work simple and easy and 72.4% agreed that only entrepreneurs should have managerial skills .76.2% of the participants agreed that holding a MBA degree prior to setting up a clinic or laboratory can help in easy management of clinical laboratory and 86.7% agreed that an expert in management can help in the development of laboratory or clinic. This shows the students are well aware of the importance of acquiring managerial and leadership skills in order to implement good healthcare.

The issues involving professional identity, lack of role awareness within the organisation, can entail poor communication with other group members[28]. In our study, 88.6% agreed that leadership is ruling and getting work done without their involvement. But on the contrary a good leader is one with proper communication, identity and awareness which can help in the progress of the organisation and thereby lead people by example and not by force.

The limitation of the study is uneven distribution of sample size, experience in clinic or laboratory were not mentioned. In future, better training and proper knowledge during training in the curriculum can help in the successful set up of laboratories or clinics.

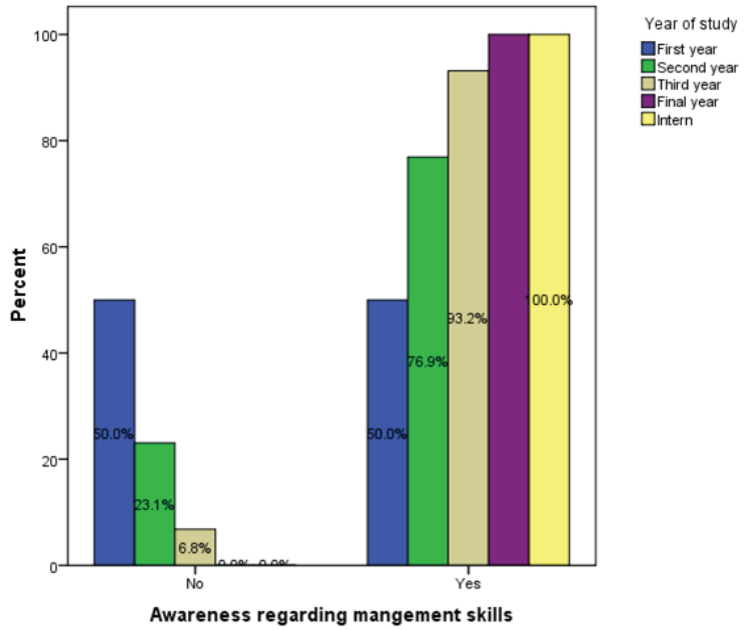


Figure1: Bar graph showing the association of awareness regarding management skills and the year of study. X axis represents the awareness on management skills and Y axis represents the percentage of responses .The interns and first year students were found to have 100% awareness regarding managerial skills.Chi square test (Chi square value = 25.694), showed statistical significance with p=0.000 (P<0.05). There is significantly increased awareness regarding managerial skills among 1st years and interns when compared to the other years of study.

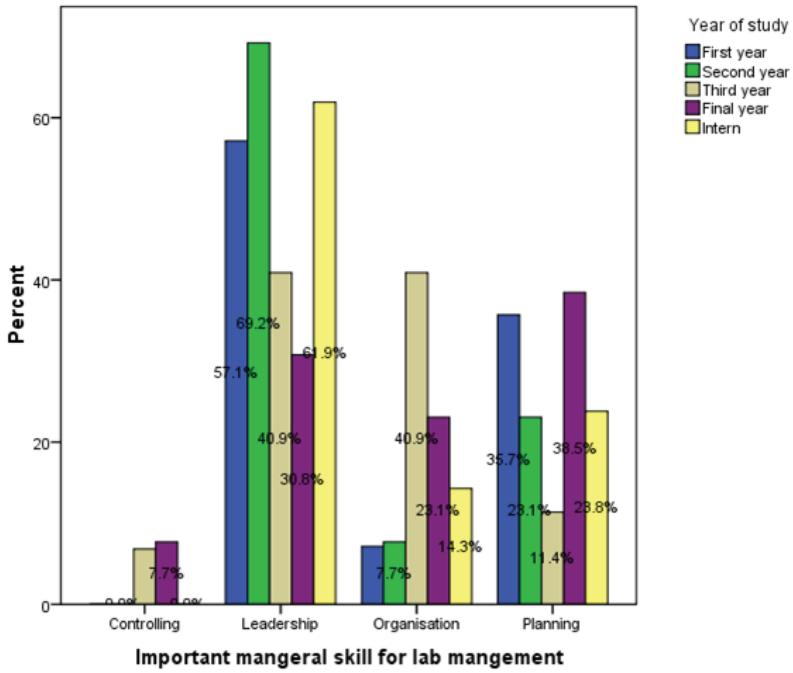


Figure 2: Bar graph showing the association of awareness regarding important managerial skills and the year of study. X axis represents the management skills and Y axis represents the percentage of responses . Most of the students agreed that leadership was the most important skill. Maximum response of 69.2% was given by the second year students.Chi square test (Chi square value = 20.789), showed statistical significance with p=0.054 (P>0.05). Increased awareness regarding managerial skills among the 2nd year students.

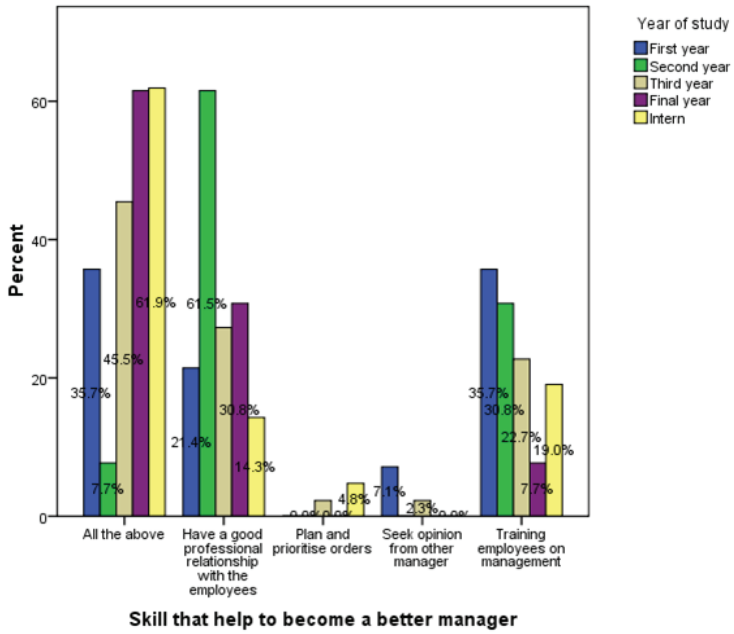


Figure 3:Bar graph showing association between skills that help to become a better manager and the year of study. X axis depicts the management skills that help to become a better manager and Y axis depicts the percentage of responses.Most of the students agreed that all the above skills are important. Maximum response of 61.9% was given by the interns.Chi square test (Chi square value = 20.789), did not show statistical significance with p=0.193 (P>0.05). Increased awareness regarding skills that make a better manager among the Interns.

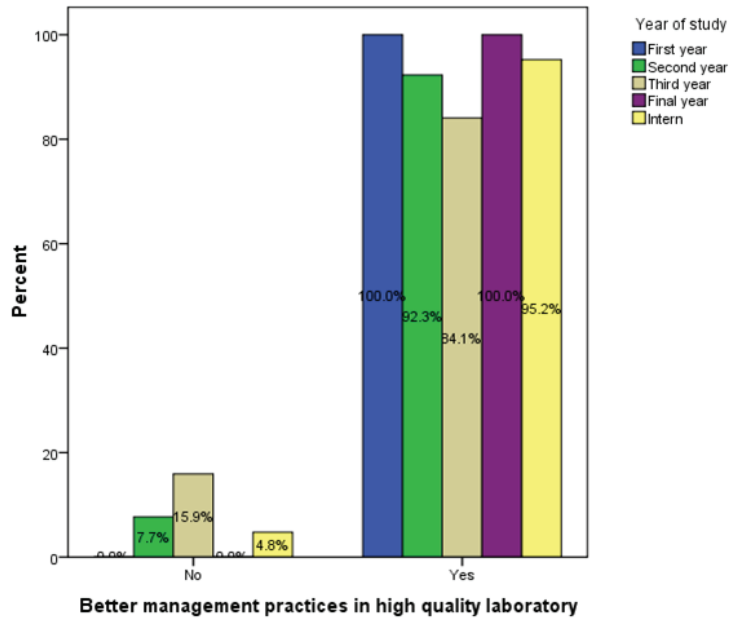


Figure 4: Bar graph showing the association of year of study to the question,"Do high quality laboratories have better management practices than low quality ones". X axis represents the response yes and no and Y axis represents the percentage of responses . Most of the students agreed that there are management practices in high quality laboratories. Maximum response of 100% was given by the first and final years. Chi square test (Chi square value =5.956), did not show statistical significance with p=0.202 (P>0.05). Good awareness among all the years regarding better management practices in high quality laboratories.

Figure 13:Pie chart showing the response for the question ,"What are the factors that influence the delivery of high quality care" where blue represents leadership ,green represents management and brown represents both leadership and management to the factors that influence the delivery of high quality care.

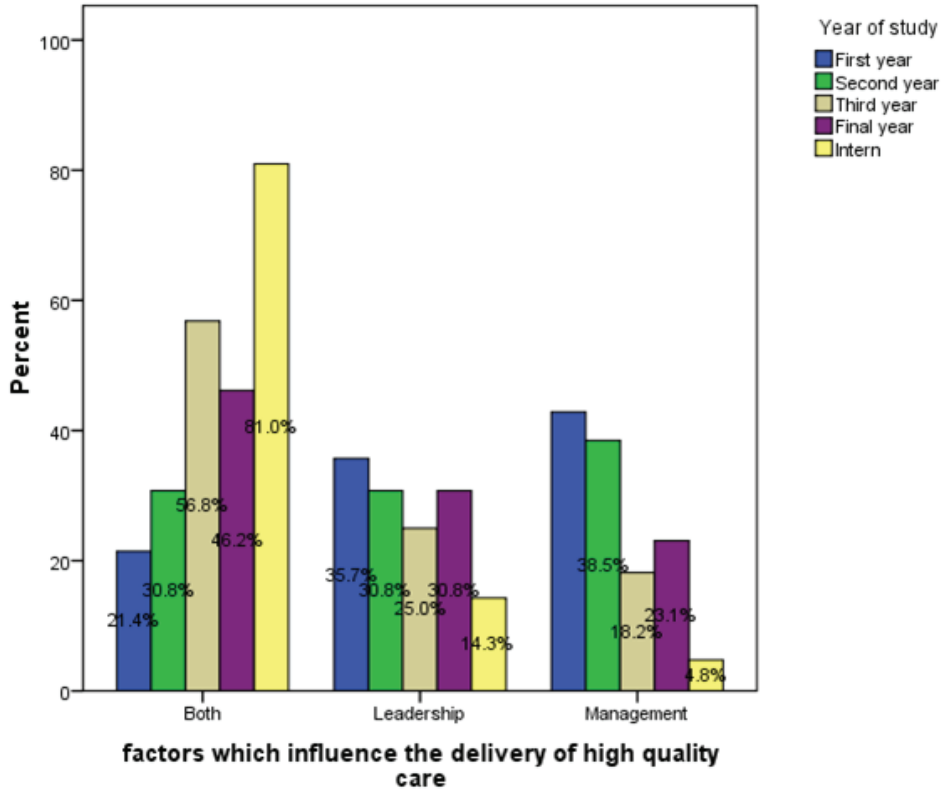


Figure 5: Bar graph showing association of the year of study and the factors that influence the delivery of high quality care. X axis depicts the factors that influence the delivery of high quality care and Y axis depicts the percentage of responses. Most of the students agreed on both leadership and management. Maximum response of 81% was given by the interns. Chi square test (Chi square value =16.669), showed statistical significance with p=0.034 (P<0.05). Significant increase in awareness regarding factors which influence the delivery of high quality care was seen among the interns.

Conclusion

In our study we found that undergraduate dental students are having less knowledge and awareness about management skills. Although students are aware that there is a need for leadership and managerial skills, there is still a lacunae in understanding its practical aspects. This void can be filled by including management skills and laboratory set up as a part of curriculum in order to equip the student to set up a smooth functioning laboratory and clinic in the future.

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Assessment of Knowledge and Awareness on Various Precautionary Measures one Must Follow While Treating Patients During Coronavirus Pandemic among Dental Practitioners - A Questionnaire based Survey

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Abstract

The corona disease is a rapidly spreading viral infection caused by COVID-19. Which is caused by the SARS family of viruses. It was discovered in the later half of 2019 after which in march 2020 the WHO declared it a global pandemic as it spread to all the major countries of the world. Due to risk of infection to the practitioner many clinics have completely closed. While some brave souls still venture outside to treat their patients. This study was done to assess the awareness of dental practitioners about the various precautions that they must follow while treating patients during coronavirus pandemic. An online survey was conducted with the help of Google forms was distributed to 100 dental practitioners. The questionnaire consisted of 15 questions that were based on the clinical protocols and guidelines one must follow when treating patients during the time of COVID-19 pandemic . After obtaining the responses, data was tabulated in excel sheet and was subjected to statistical analysis. The study shows that dental practitioners have better knowledge of the protocols they must follow when treating their patients during times of pandemic **.**The study helps to handle their patients by screening them and protecting themselves and their staff from the risk of infection.

Keywords- *Corona,pandemic, clinical protocol,dental practitioners,*

Introduction

At a conference in March 2020, Dr.Tedros Adhanom WHO's director general declared COVID-19 a global pandemic [1]. The virus was first reported in the city of Wuhan in China in December 2019. By March it spread to over 200 countries[2]. The coronavirus is a single stranded RNA virus[34]. A mutant strain of SARS virus[56].

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The incubation period of the virus is around 2-12 days and during this period the person remains asymptomatic[7]. The symptoms for one affected with COVID-19 usually present themselves a common cold like fever, tiredness, dry cough & shortness of breath 80% of individuals recover without need of treatment[89]. The remaining 20% are categorized as either severely or critically ill and there is also a chance for death amongst them they have symptoms of severe respiratory distress[10].

The virus can transmit from one infection carrier to a healthy individual.Aerosol and articles in contact with individuals can also transmit the disease. There have also been incidences of transfer of infection from asymptomatic carriers have also been reported[11].

The fact that a dental practitioner is in constant contact with oral cavity, blood & saliva and most of

the dental procedures generate aerosols from scalers & hand pieces[12]. Our profession serves as a potential risk for infection to the practitioner and his staff. There are only a few known vaccines for viruses like smallpox vaccine ,HBV vaccine , MMR vaccine etc[1314]. Hence there is no definitive cure for this disease, only treating the symptoms and use of antibiotics, antivirals and hydroxychloroquine[15161718] . Hence most dental clinics have closed themselves by doing so can indeed reduce risk of infection. But according to an article published in the USA which states that the pandemic could last for another 18 months. Hence closing clinics will increase the suffering of the patients. This study was done to assess the awareness of dental practitioners about the various precautions that they must follow while treating patients during coronavirus pandemic

Materials & Methods

The study was conducted in an online setting. It was a questionnaire survey consisting of 15 questions. The questions were designed to assess knowledge and awareness of dental practitioners on how to change their clinical protocol to safely treat patients in the time of corona pandemic and lockdown. The study was conducted among 100 dental practitioners with good experience & minimum of 5 yrs of clinical practice. The online questionnaire was circulated through google forms. The data was collected over a period of one week.

Statistical Analysis

The data was collected from google forms and tabulated in excel sheets. Then the row data was transferred to SPSS software. Chi square test was done to find the association between parameters & the level of significance.

Results & Discussion

A total of 100 dental practitioners were involved in the study. Out of which 55(55%) were male 44(44%) female, 1(1%) prefer not to say . 37(37%) were of the age group 20-30 yrs, 37(37%) were of the age group 20-30yrs, 37(37%) were of age group (31-40 yrs), 41-50 yrs age group were 16(16%), 50+ yrs age group has 10 people (10%) out of the 100 practitioners 49(49%) has history of practice of (0-5) yrs , 34(34%) had practice history of (5-10) yrs while those who practice for (10+yrs) were 17(17%).) Educational qualification of the 100 dentist were 48(48%)BDS , 40(40%) MDS, 12(12%) PHD 32(32%) of the individuals strongly agree that travel history to be included while filling their case sheet . 27(27%) Individuals strongly agree to have a triage area 34(34%) clinicians strongly feel the need to check patients temperature . For checking temperature 61(61%) dentist feel that forehead thermometer is most effective. 46(46%) Dentist feel that if patient reported to clinic with temperature and dental pain, prescribing medication and having him visit fever clinic / physician could be the course of action to prevent infection clinical office (**figure- 1**) 48(48%) of individuals have agreed to the fact that PPE set is the best choice of protective garments garment in the pandemic. Majority 71(71%) clinicians agree that surgical gloves are the best protection available. 38(38%) dentists agree that face shield is the best protective headwear (**figure-2**) 20(20%) Dentists strongly disagree to perform scaling as it produces higher aerosol emissions. 38(38%) Dentists agree that fumigation is necessary to sterilize the working area. Majority (48%0 48 dentists agree that all forms of personal hygiene measures are necessary to reduce spread of infections. 38(38%) Dentists in the study strongly recommend social distance in their waiting room.. Majority 42(42%) have agreed that autoclave is the best method to sterilize instruments. 23(23%) Individuals strongly agree that oxygen saturation is also an important screening criteria.(**figure-3**) For the final statement (27%) agreed that it's necessary to check a person's past visit to the hospital. On comparison based on gender, educational qualification and years of clinical expertise, significant results were obtained. (**Figure 4, 5, 6**)

The oral cavity is home to more than several thousands of microbes and since the introduction of aseptic surgery it falls on the clinician to prevent the spread of infection from his office. In dentistry its achieved by use of autoclaves for sterilization of instruments,use of mouth washes, intracanal medicament and many more to prevent infection spread[19,20].In this unprecedented global calamity which the world has not faced since the Spanish flu almost 100 yrs back. Hence the only measure advised by & followed by countries around the world is lockdown and decrease social interactions to reduce the spread of corona infection. But as medical professionals it falls on us to provide essential services to the populace. Due to lockdown and growing number of COVID cases

day by day many dental clinics have closed their practice till a permanent solution can be achieved. But there are still few brave souls who treat patients even in times of crises. They say that ignorance is bliss but in face of this ignorance is failal. This study is done to provide & cause awareness for those working clinics during the time of corona.

This study is a cross sectional survey involving 100 dental practitioners with varying years of practice and different educational qualifications. The practitioners seem to have better knowledge that non contact forehead thermometers are effective (61%) in checking patients temperature as any other thermometer would be in contact with the patients body hence they require to be sterilized before being used again hence decreasing the efficiency & spread in office. Majority 46% agree that providing meditation and visiting a physician clearance before starting a dental procedure for patient with high fever but 31% dentist also agree that recalling patients after 2 weeks after providing medication as if its normal fever it subsides in less than 2 weeks but of corona persists. 48% agree with guide lines that PPE suite can highly reduce infection risks can it's a very improve material to aerosol droplets. The use of chlorhexidine and other mouthwashes before treatment of the patient can also reduce infection spread[21,22] .A huge majority of 71% dentist agree that surgical gloves provide better protection & comfort while carrying out procedures 38% Individuals agree that face shield provides the highest degree of protection to facial area as it covers the enter face 35% dentist have stated N-95 also has high degree of protection but it must replaced for each patient and unlike a face shield cannot be sterilized. Due to dental treatment producing aerosols it is agreed that 38% of dentists agree that fumigation is necessary in dental offices to kill pathogens in air and on working surfaces this is done to sterilize the working environment to keep the clinician, his staff & next patient safe from infection. 48% of dentists agree that after every dental procedure it's necessary to thoroughly wash hands with soap sanitisers and alcohol scrub to prevent contaminating the work environment. 38 of dentists (38%) strongly agree that social distancing is important in the waiting area while 37% of dentists agree to the statement as it implies that they know its importance but their working environment makes it hard to implement. A majority of 42% agree that autoclave is the Best method in sterilization as dental instruments are constantly in contact with blood and other body fluids bringing it under high risk of contamination & infection hence must be sterilized using autoclave.

The 100 dental practitioners tend to show poor or insufficient knowledge for the following statement only 32 (32%) dentist of the given study size of 100 feel it's important to ask patient travel it's important to ask patient travel history part of the case sheet because he might have traveled from a region of high corona density. Only 27 dentists (27%) strongly accept the need for screening triage area before entry to the clinic. This is also due to lack of convenience and space in an individual clinic. Only 34% feel the importance to check the temperature of the patient. 20% Individuals strongly disagree that scaling is advisable during pandemic as it's a long process and produces a large amount of aerosols and it's not a pain management procedure. Patients affected with COVID-19 have respiratory distress hence their O2 saturation values would be considerably low hence it also serve as a effective screening measure for which only 23% strongly agree to it hospitals serve as sites for potential nosocomial infections and the continuous rapid spread of corona and advice from government to get one tested in testing center or visiting a hospital which has a corona patient it becomes necessary to ask his last clinical visit as he has potential risk for corona. But only 27% strongly agree to this history taking method.

There are 15 questions which were given to cases the awareness of dentists working in this pandemic and the measures they take to keep themself their staff, environment & patients safe. Overall the study reveals that the dentist has a better understanding of the situation hence they can provide safe and effective treatment.

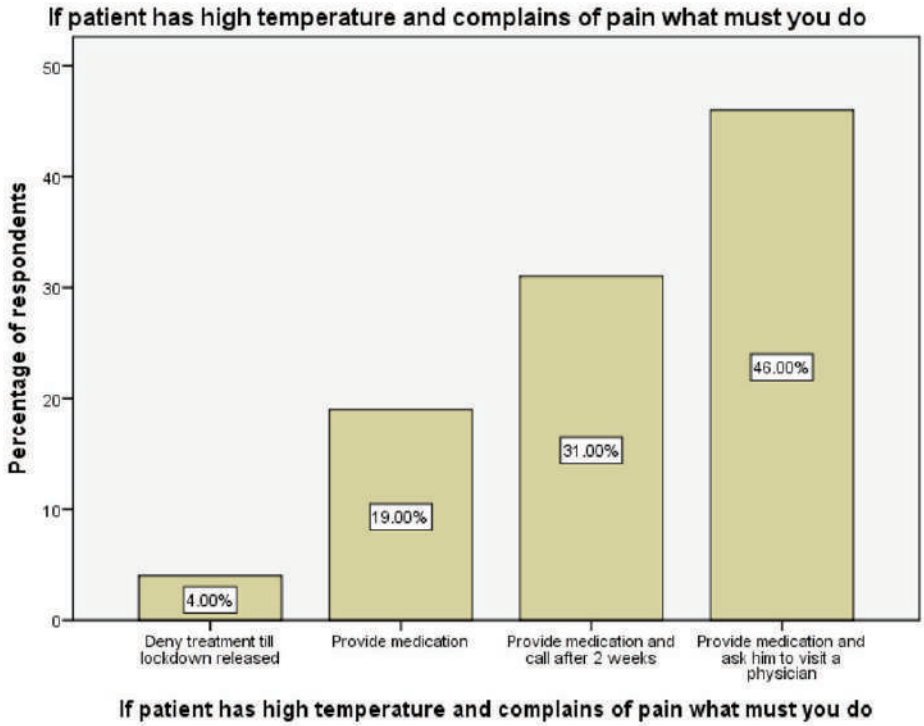


Figure 1 –Bar graph represents responses for the question. "If a patient has high temperature and complains of pain, what must you do?". X-axis shows the responses to the question, Y-axis shows the percentage of responses. Majority of the respondents (46%) have said that they provide medication to the patient and ask him to visit a physician.

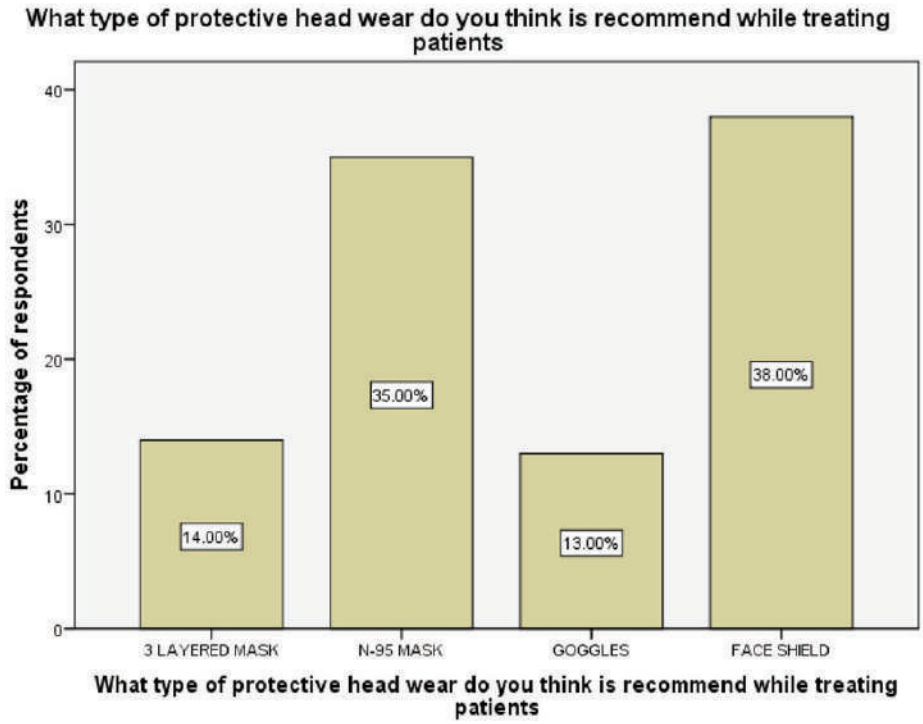


Figure 2 –Bar graph represents the responses for the question. "What type of protective headwear do you think is recommended while treating patients?" X-axis shows the responses to the question, Y-axis shows the percentage of responses. Majority of respondents (38%) have said that they use face shields.

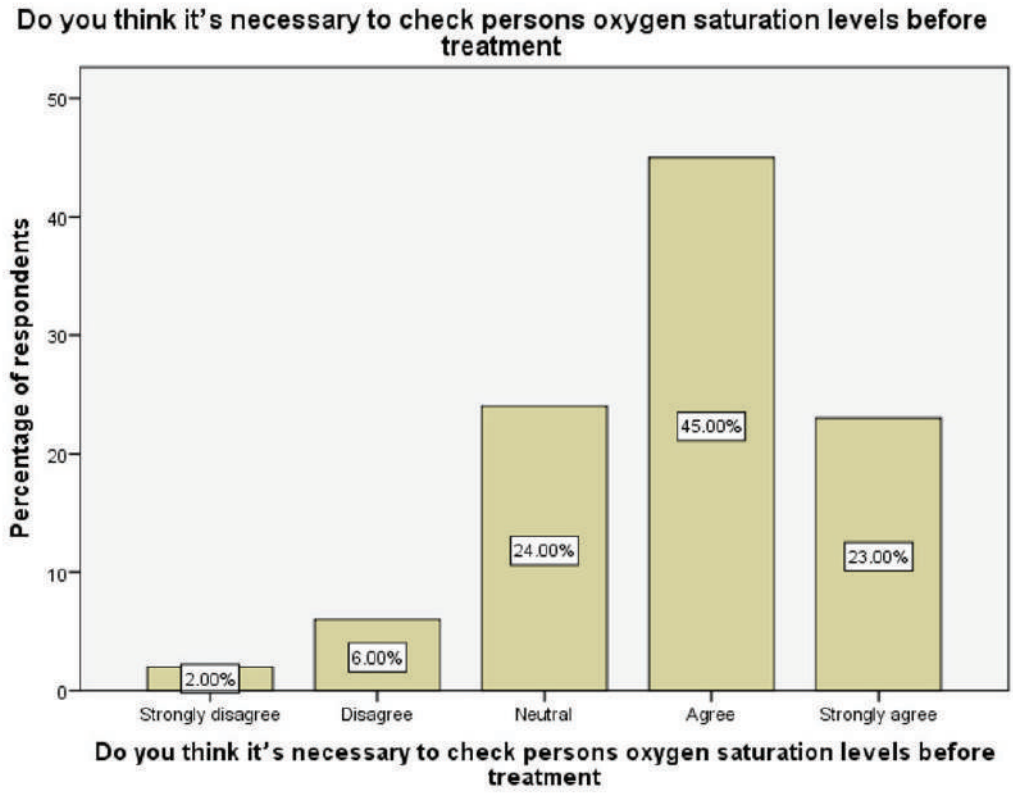


Figure 3 –Bar graph represents the responses for the question. "Do you think it's necessary to check a person's oxygen saturation levels before treatment ?"X-axis shows the responses to the question, Y-axis shows the percentage of responses. Majority of respondents (45%) have agreed with the statement .

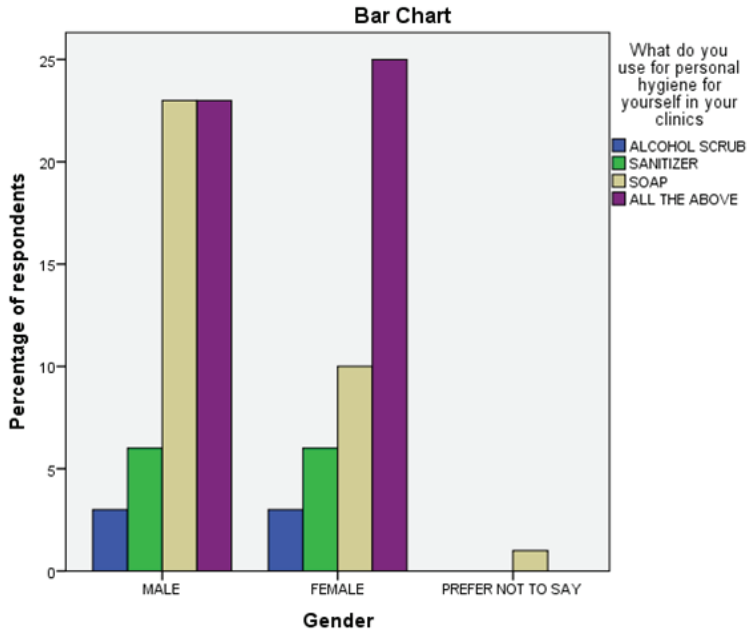
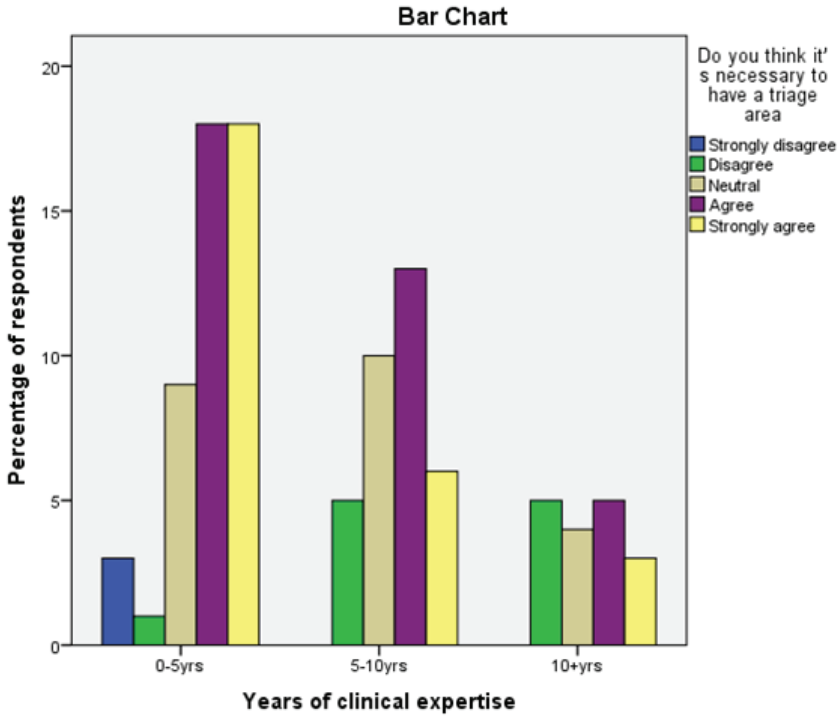


Figure -4: Bar graph showing comparison of responses based on gender to the question, "What do you use for personal hygiene for yourself in your clinics?" X-axis shows gender, Y-axis shows number of responses; blue colour represents alcohol scrub; green colour represents sanitizer; beige colour represents soap; purple colour represents all the above. Majority of females (23%) say they use all of the mentioned personal hygiene products when compared to males. Which was not statistically significant .(Chi- square test done;p-



value =0.428).

Figure -5:Bar graph showing comparison of responses based on years of clinical experience to the question on "Do you think it's necessary to have a triage area?"X-axis shows years of clinical experience , Y-axis shows number of responses; blue represents strongly disagree, green represents disagreement, beige represents neutral, purple represents agree and yellow represents strongly agree. Majority of the participants with experience of 0-5 years strongly agree with the statement (18%) when compared with other groups of (5-10yrs) and (10+yrs). (Chi square test ;p-value =0.029 ,. statistically significant)

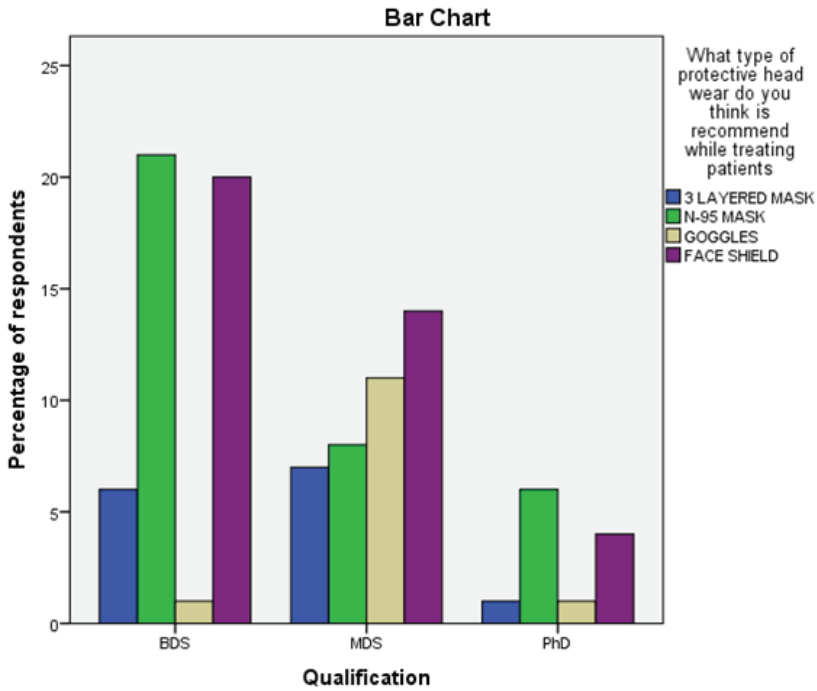


Figure 6 :Bar graph showing comparison of responses based on educational qualifications to the question "What type of protective headwear do you think is recommended while treating patients?" X-axis shows educational qualifications ,Y-axis shows number of responses; blue represents -3 layered mask, green represents N-95mask, beige represents goggles, purple represents face shield. Majority of the participants with educational qualification of having completed BDS (21%) say N-95 mask is the better protective headwear .(Chi square test; p value= 0.011, statistically significant)

Conclusion

The study reveals that dentists have better awareness on how to handle their patients by screening them and protecting themselves and their staff from the risk of infection. But more studies and larger sample size required for more accurate analysis.

Conflict of Interest: There are no conflicts of interest declared.

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Source of Funding: Self .

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Analysis of Prevalence of Oral Squamous Cell Carcinoma in Patients with History of Chronic Irritation of Oral Tissues - A Retrospective Study

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Abstract

Oral squamous cell carcinoma (OSCC) accounts for more than 90% of the reported oral cancer cases in the world. Even though the habits such as tobacco usage, smoking and old age are the main risk factors for developing oral squamous cell carcinoma some factors like chronic mucosal irritation(CMI) from mechanical and functional factors also play a significant role in the etiology of oral squamous cell carcinoma. The aim of this study was to analyse the prevalence of oral squamous cell carcinoma with history of chronic mucosal irritation and to evaluate various parameters like gender, age, site of the lesion and etiology of chronic trauma in OSCC cases with chronic mucosal irritation history. This retrospective study included a sample size of n= 78 (73 = OSCC with other etiology, 5= OSCC with etiology of chronic mucosal irritation). Data regarding the sample cases were selected after the review and analysis of records of about 86000 patients who visited our institution between june 2019 and march 2020. The retrieved data were tabulated in MS Excel and exported to SPSS for statistical analysis. Prevalence of oral squamous cell carcinoma cases with chronic mucosal irritation history was 6.41% for a duration of 8 months. Etiology associated with chronic mucosal irritation was found to be tooth related factors in all the sample cases. 80% of the oral squamous cell carcinoma cases associated with chronic mucosal irritation history were males and 20% were females and 80% of the OSCC samples with CMI were above 40 years of age. In 60% of OSCC with CMI history were found to be on buccal mucosa followed by tongue and gingivobuccal sulcus. The present study provides an overview and explains the need of considering chronic mucosal irritation as an etiological factor for the development of OSCC without the association of tobacco habit.

Key Words:*Prevalence; OSCC;chronic mucosal irritation; chronic mechanical irritation;dental factors; prosthetic factors.*

Introduction

Oral cancer is one of the most serious health problems and accounts for 2-4%of all cancer types in the world [1,2]. More than 95% of the oral cancer cases reported are oral squamous cell carcinoma (OSCC)[2]. OSCC is the 12th most common cancer in the world and for the past decades there has been a steady increase in oral cancer mortality rates of around 320,000 deaths in the world annually [3,4]. Annual prevalence of oral squamous cell carcinoma is 300,000 cases[1,5,6].

In India also the prevalence of oral cancer is high and accounts for 45% of all of the cancer cases reported in India[1,7,8]. The major etiological factors related to OSCC risk are tobacco usage, advanced age, smoking and alcohol consumption but several other factors like chronic irritation of oral mucosa from mechanical and functional factors will also play a significant role in

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etiology of OSCC [9,10,11]. Mechanical factors can be tooth related and prosthesis related with tooth related factors being sharp tooth, broken tooth and improperly positioned tooth [12,13]. In a study by Fabiana Vargus Ferreirial et al stated that almost 15% to 20% oral cancer occurs without any traditional risk factors association. Traditional risk factors include tobacco usage, smoking and alcohol consumption. Eduardo David Piemonte et al found a statistically significant association between chronic mucosal irritation and oral cancer with p value of 0.0001[14]. An australian study also assessed the gender predilection among oral cancer patients without traditional etiology stated 61% of the study population were females and in which about 30% showed some dental abnormalities in the proximity of the oral cancer lesion [15,16]. Multivariate analysis conducted in a Swedish and Brazilian study also showed the defective complete denture can be a significant risk factor for OSCC development [17,18]. Some of the past studies have also correlated the association of OSCC with CMI with the site of the lesion[19].

The aim of the present study is to analyse prevalence of OSCC with history of chronic mucosal irritation reported during 2019-2020 and to evaluate various parameters like gender, age, site of the lesion and etiology of chronic trauma in OSCC cases with chronic mucosal irritation history. This study will help to evaluate the need of the early diagnosis and treatment of CMI and to conduct institutional level interventions to improve the awareness regarding chronic mucosal irritation and its role as an etiological factor for the development of OSCC.

Materials and Methods

The present study was conducted with approval from Scientific review board, Saveetha dental college and hospitals, chennai. The ethical approval number for the present study was SDC/SIHEC/2020/DIASDATA/0619-0320. This retrospective study included n=78 samples out of which 6.41% of OSCC cases were associated with chronic mucosal irritation (Figure 1). Data regarding the sample cases were selected after the review and analysis of records of about 86000 patients who visited our institution between June 2019 and March 2020. All the retrieved case sheets were verified by an external reviewer in order to reduce errors in data recording.

Histopathologically confirmed cases of OSCC with history of CMI and proper clinical details were included in the present study. The cases with non specific histopathological diagnosis and improperly recorded case sheets were eliminated from the present study. Cross verification of retrieved data was done by photographs and by direct communication.

The data collected from n=78 were tabulated according to age, gender, history of chronic mucosal irritation, and the site of the lesion in MS excel software. This tabulated data then transported to IBM SPSS-Statistics 23 software and frequency analysis were done for age range, gender, history of chronic mucosal irritation, site of the lesion and diagnosis. Chi-square test was done to assess the correlation between variables and the results obtained after analysis were statistically represented as tables and bar graphs.

Result and Discussion

In the present study the prevalence of OSCC cases with chronic mucosal history for a period of 8 months was found to be 6.4% (Table 1). The etiology of chronic mucosal irritation was found to be tooth related in all the samples (Figure 2). Demographic data analysis stated that 80% of the sample population were males and 20% were females (Figure 3). Among OSCC patients with CMI history 80% of the population were found to be above 40 years of age (Figure 4) and the most prevalent lesion site was found to be buccal mucosa in 60% of the samples followed by tongue and gingivobuccal sulcus (Table 2). The association between gender and site of the lesion in OSCC cases with chronic mucosal irritation history was not found to be statistically significant with p value of 0.082 (Table 3 and Figure 4).

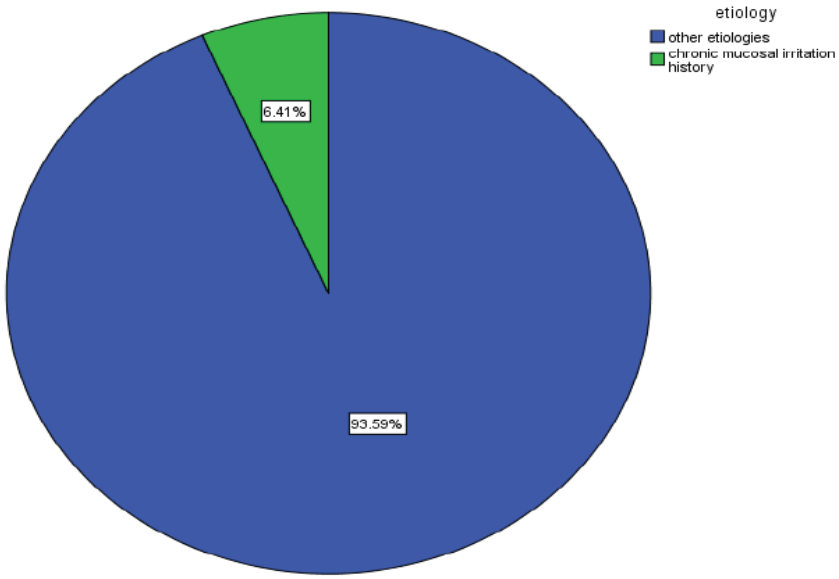


Figure 1- Pie Graph showing the overall distribution of study samples. Total samples selected= 78, and out of which the percentage of OSCC cases with the history of chronic mucosal irritation(CMI) was 6.41% and represented in green colour. OSCC cases with etiologies other than CMI was 93.59% and represented in blue colour.

Table 1 showing the prevalence of OSCC cases with CMI history during 8 months

OSCC cases reported during JUN 2019- March 2020	78
No of OSCC cases reported with chronic Irritation of oral tissues during JUN 2019- March 2020	5
Prevalence of OSCC with the etiology of chronic Irritation of oral tissues during JUN 2019- March 2020	6.41%

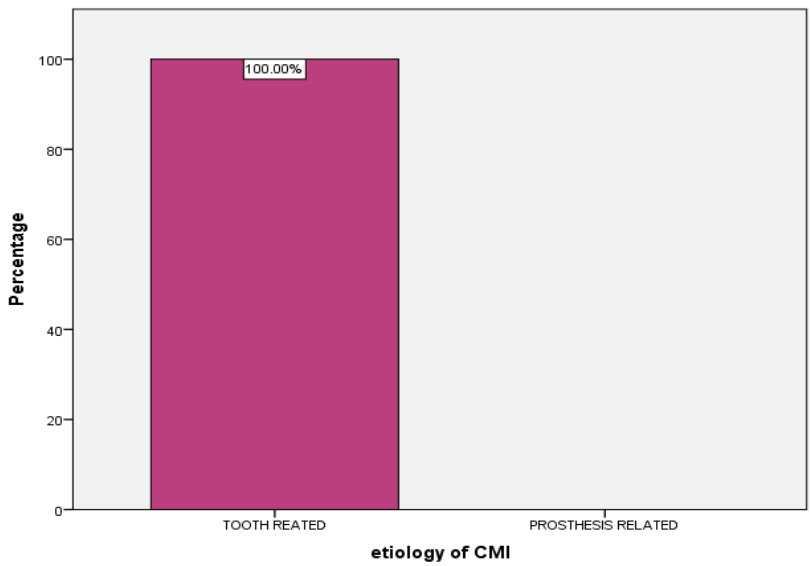


Figure 2- Bar Graph 1 showing the percentage of distribution of etiological factors of CMI in OSCC cases. X axis representing the etiology of CMI and Y axis showing the percentage. Tooth related factors are the major reason for the chronic irritation in OSCC with CMI history.

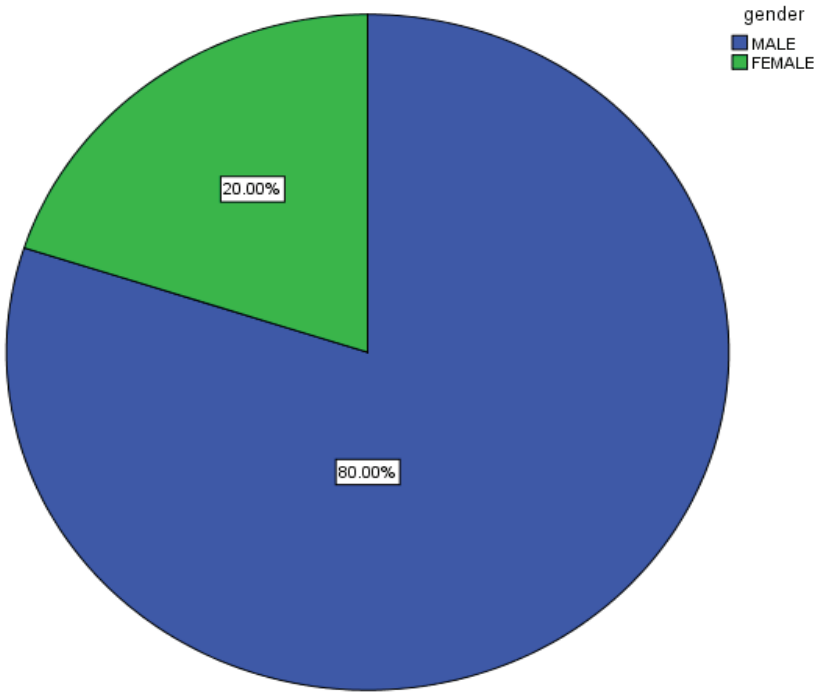


Figure 3- Pie Graph 2 showing the percentage of Gender distribution among OSCC cases with CMI history. Blue color represents Male and Green color represents Female. The male are more commonly affected by OSCC with CMI history.

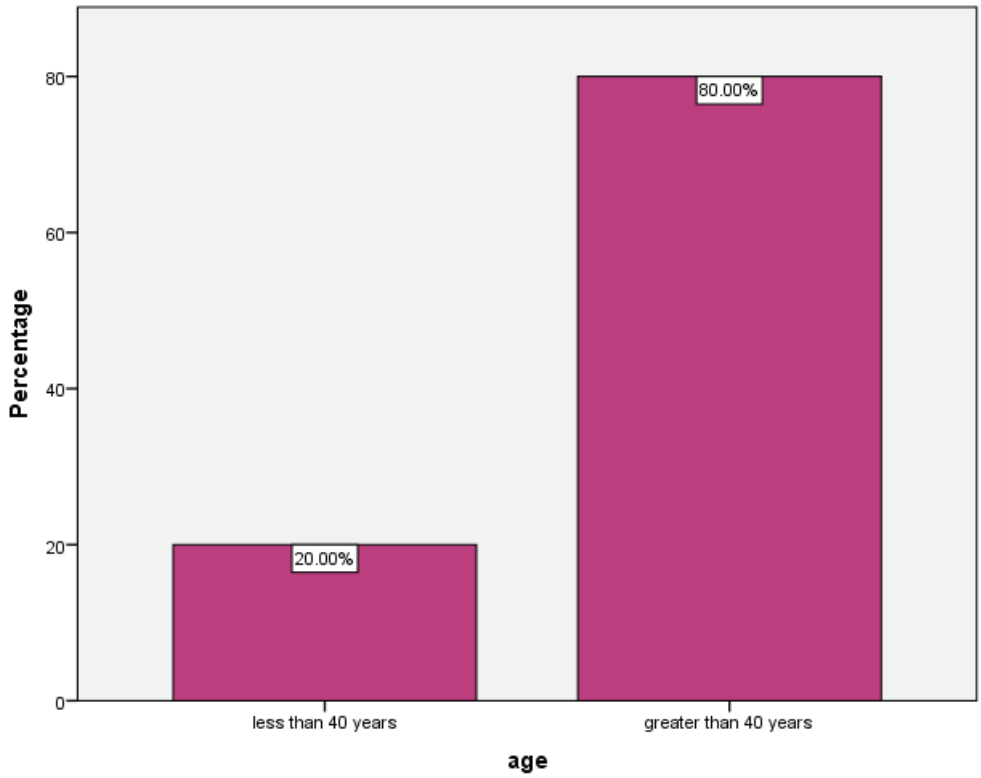


Figure 4- Bar Graph 2 depicting the percentage of distribution of OSCC cases with CMI history according to age. X axis showing the Age groups(<40 years and >40 years) and Y axis showing the percentage. The OSCC with CMI history is more commonly observed in patients above 40 years of age.

Table 2: Distribution of site of lesion among OSCC cases with CMI history. Most common site for OSCC with CMI is Buccal mucosa.

Site of the lesion	Frequency	Percent
Tongue	1	20%
Buccal mucosa	3	60%
Gingivobuccal sulcus	1	20%
Total	5	100%

Parameters compared	Statistical test	P value
Gender and Site of the lesion	Chi-square test	0.082

Table 3: The association between gender and site of the lesion among the cases of OSCC with chronic mucosal irritation history. *p value >0.05: statistically not significant. No significant association observed between the gender and site of lesion among OSCC with CMI history.

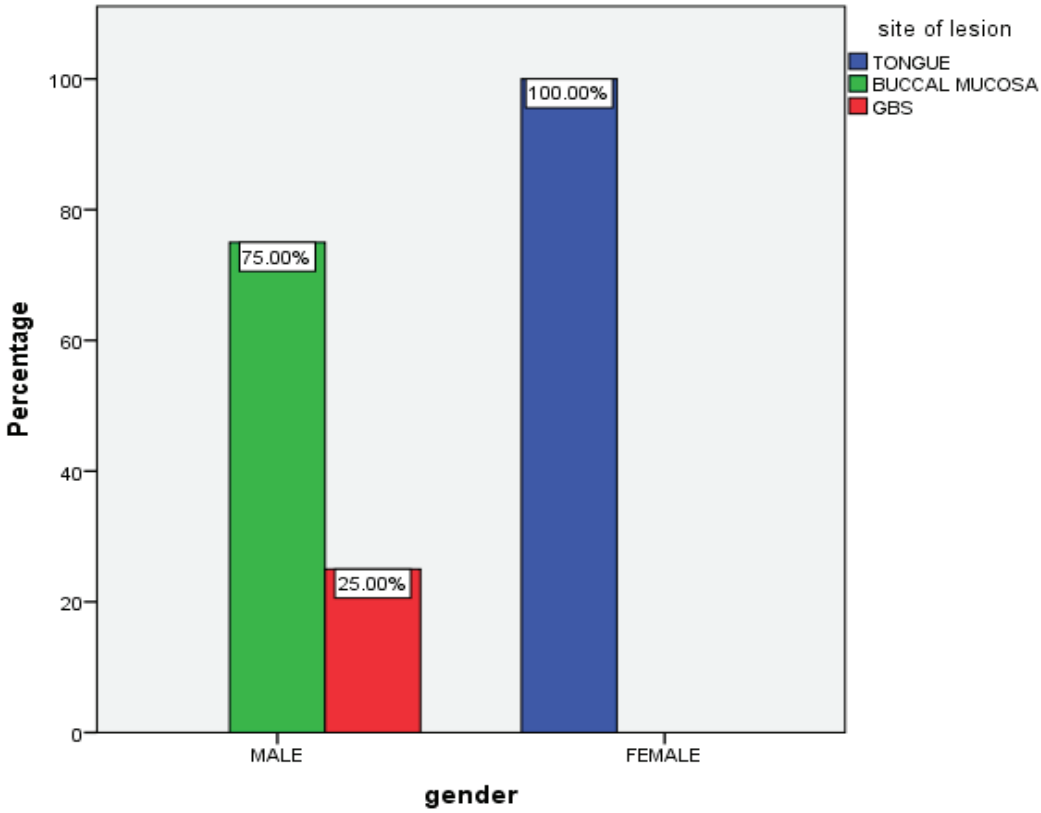


Figure 4- Bar graph depicting the association between gender and site of the lesion among the cases of OSCC with chronic mucosal irritation history. X axis depicting gender and Y axis showing percentage. Tongue is represented as blue, Buccal mucosa as green and Gingivobuccal sulcus(GBS) as red colour. Tongue is the most common site in females whereas it is buccal mucosa in males. No significant association observed between the gender and site of lesion among OSCC with CMI history (Pearson Chi-square test- P value=0.082).

Oral squamous cell carcinoma is a major health problem worldwide, especially in the developing countries[20]. Oral squamous cell carcinoma is one of the common epithelial malignancies of multifactorial etiology and with significant morbidity and mortality[21]. The significance of chronic mucosal irritation in developing malignant transformation and finally progressing into oral cancer is comparatively less studied when compared to other aetiology is like tobacco usage and alcohol consumption[22]. Some experimental animal studies suggested the mechanism of transformation of chronic mucosal irritation to malignancy[23]. They proposed that persistent mechanical irritation can cause DNA damage, which will progress into various degrees of dysplasia and finally may result in malignant transformation and this was stated by increased activity of only ADP ribose polymerase in chronic trauma cases [24-25]. Chronic mucosal trauma results in inflammation, and can release chemical mediators such as cytokine, prostaglandins, and tumor necrosis factor. The inflammation induced oxidative stress can cause genetic and epigenetic changes damaging DNA, inhibiting its repair, altering transcription factors, preventing apoptosis, and stimulating angiogenesis, thus resulting in carcinogenesis. In a nutshell, inflammation may act at different steps and result in cancer formation [26–28].

The period prevalence of oral squamous cell carcinoma cases with a history of chronic mucosal irritation for eight months visiting our institution was found to be 6.4%. This value is significant when comparing time duration. Similar results were obtained for the study conducted by Piemonte et al in 2018. He stated there exists a significant statistical association between chronic mucosal irritation and oral cancer both in cross-sectional and in case control studies [29]. Manoharan et al in 2014 and Singhvi et al in 2017 also found a significant statistical association between oral cancer and chronic mucosal irritation in relation to wearing defective prosthesis [23,30].

On analysis of etiology of chronic mucosal irritation, all the samples showed a tooth related etiology. But none of the samples showed prosthesis related etiology. This observation can be correlated with the study by Jeronimo P Lazos et al in 2017, in which he found that dental factors were the common etiological factors causing chronic mucosal irritation followed by prosthetic factors [31]. However in another study by Velly A.M. in 1998 and by Rosenquist K et al in 2005 stated that the ill fitting denture was the most common cause for Oral squamous cell carcinoma with chronic mucosal irritation history followed by sharp tooth [17,19]. Even though tooth related factors and prosthesis related factors are the major reason for chronic trauma to oral mucosa, the present study couldnt establish any relation between chronic trauma and prosthesis related factors. This could be possibly due to the small sample size and small duration of sample collection in the present study.

The gender distribution analysis of OSCC with chronic mucosal irritation history showed 80% of the samples were males and only 20% of the samples were females. Jeronimo P lazos et al in 2017 stated that the males are more affected than females in oral cancer associated with chronic mucosal irritation etiology when compared to chronic traumatic ulcer and benign irritative mechanical lesion[31]. This was in concordance with the present study, but the selection of samples, determination of adequate sample size and the duration of sample collection should be increased to get more statistically significant results.

On analysing age distribution of oral squamous cell carcinoma cases with chronic mucosal irritation history, it was found that 80% of the samples were above 40 years of age. This was also in concordance with the study by Jeronimo P Lazos et al in 2017. The possible explanation for this could be the increased incidence of teeth attrition, dental caries, fractured tooth and prosthesis usage among elderly population.

In the present study buccal mucosa was the most commonly affected lesion site among OSCC cases with history of CMI when compared to tongue and gingivobuccal sulcus. This was in contrast to the result of studies by Velly A.M in 1998 and Jeronimo. P. Lazos et al in 2017. Through their studies they both stated that tongue was the most affected site in OSCC cases with a history of chronic mucosal irritation, and they linked it with Odds ratio of 9.1 [32]. The present study results vary from the above mentioned studies and this variation could be due to the limited sample size and short duration of sample collection.

The present study couldn't find any statistically significant relation between gender and site of lesion

in OSCC cases with history of CMI. This has to be evaluated with a larger sample size and duration for more confirmatory results.

Chronic mucosal irritation is the less identified and most ignored factor in oral squamous cell carcinoma. Further research on the current topic may provide the precise understanding regarding the significance of chronic mucosal irritation in oral cancer development. Institutional level interventions like awareness programs addressing the importance of CMI as an oral cancer etiological factor can be conducted to improve the knowledge among the public, so that it will promote the early diagnosis and treatment of chronic mucosal irritation without progressing to aggressive outcomes like oral squamous cell carcinoma.

Small sample size, Geographic limitations of sampling and small duration of data collection were the major limitations of the present study.

Conclusion

In conclusion the prevalence of oral squamous cell carcinoma with chronic mucosal irritation history was found to be 6.41 % and it was significant when considering the duration for which prevalence was calculated. In the present study, the etiology of CMI was due to tooth related factors, buccal mucosa was the predominant site of lesion and OSCC with CMI was found to be more common in patients above 40 years of age. More extensive research on the current topic may provide precise understanding about the need of considering chronic mucosal irritation as an etiological factor for OSCC, without the association of tobacco and also the importance of its early diagnosis and treatment.

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Association of *Retn (Rs3219175)* Polymorphism with Oscc Progression and Development - An *In Silico* Study

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Abstract

Oral squamous cell carcinoma (OSCC) is more common with 90% of malignant neoplasm in oral cavity. Multiple factors leading to OSCC like genetic alterations, chronic exposure, micro environmental carcinogens, premalignant disease like oral epithelial dysplasia, mitochondrial membrane damage and viruses infections like HPV (human papillomavirus). *RETN* gene belongs to the family of mouse resistin-like genes. Human genetic variations are found to influence inter-individual variation observed in disease susceptibilities, drug response, response to an infectious disease. At the molecular level these sequence variations influence gene expression, protein production, function, disease susceptibility in particular individuals. The present *in silico* study determines the possible association of *RETN* polymorphism with that of oral squamous cell carcinoma in different populations. In this study the *RETN* gene polymorphism *rs321975* was analysed in comparison to the global frequencies. The present study attempts to identify the possible reason for the selection of "A" allele in the south asian population. Furthermore, genotyping analysis has to be performed in a south Indian population to ascertain the association between *RETN* polymorphism (*rs3219175*) and OSCC.

Keywords: *Polymorphism; oral squamous cell carcinoma; RETN; variant*

Introduction

Oral squamous cell carcinoma (OSCC) is more common, which accounts for 90% of malignant neoplasm in oral cavity. Worldwide, oral cancer accounts for 2% - 4% of all cancer cases, tongue as a more common sub-type[1,2]. In some regions, the prevalence of oral cancer is higher, 45% in India & 10% in Pakistan [3,4].The field cancerization has been explained as in altered telomeres and unbalanced allelic loci seen in both tumors and surrounding histologically normal tissues at distances of least 1 cm from the visible tumor margins results in the occurrence of multiple primary cancers in the head and neck region and recurrence following complete excision of the original tumor[5,6]. Multiple factors rising OSCC which are genetic alterations, chronic exposure, micro environmental carcinogens [7], premalignant disease to oral cancer can occur with alteration seen in the stromal tissue[8]. Oral epithelial dysplasia also has a malignant transformation potential [1], mitochondrial membrane damage leads to apoptosis[9,10] and viruses like HPV (human papillomavirus) also plays an important role in oral cancer[11].Numerous risk factors associated with oscc are chronic inflammation, alcohol and tobacco consumption, betel quid chewing and viral infections [12]. It is known that tumour cells on course of development undergo molecular alterations in cellular molecules including DNA, RNA, and proteins which could be attributed to the inherent biological properties of the

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cancer cell[131415]. The survival rate has reduced due to its diagnosis in the advanced stage[16]. There is a need for molecular prognostic factors to detect patients with high risk of oral cancer recurrence and treatment failure. The upregulation genes were seen involved in extracellular matrix degradation and epithelial mesenchymal transition, downregulation seen in detoxification pathways[17,18]. Expression of multinucleated giant cells shows host immune response[19,20,15].

Latest reports from the International Agency for Research on Cancer (IARC) with ICD-10 code = coo-06 : lip, oral cavity in 2018 reports shows incidence around the world is 3.54,864 and in India it is 119,992. Prevalence of 9,13,514 reported around the world and In India it is around 2,65,225. Mortality rate reported around the world is 1,77, 384 and in India around 72,616 cases. The *RETN* gene belongs to the family of mouse resistin-like genes. Hormone released suppresses insulin ability which stimulates glucose uptake in adipose cells. Studies have shown links between obesity and diabetes. It also helps promote chemotaxis in myeloid cells - gene Cards[12,21]. Single nucleotide polymorphisms (SNP) is common DNA sequence variation which influences gene expression, protein production, function, disease susceptibility in high risk individuals[7,12,21,22]. Resistin levels of plasma correlates with the inflammatory markers and coronary artery calcification, and plays an important role in the measure of coronary atherosclerosis[22–24]. Kumar et al, studied genetic variations of *RETN* associated with greater risk with various diseases like metabolic syndrome and colon cancer[22,23]. A study reported that upregulation of resistin gene expression in human breast cancer tissues[25]. The present *in silico* study determines the selection of *RETN* SNPs and its possible association with oral squamous cell carcinoma.

Materials and Methods

One of the regulatory region variants of the *RETN (*gene was selected for the study based on the literature mining process. The minor allele frequency was found to be 0.08 (A allele). Since oral cancer is a disease that is more predominant in patients with a chronic history of using smoking and smokeless tobacco use such as pan, gutka etc., genetic variants which were more closely related to environmental and habitual factors were identified in the gene and selected. As most of these factors are known to affect the inflammatory pathways leading to disease a study carried out by Yang et al was selected for comparison. The authors declared that the AA homozygous state of *RETN* polymorphism *(rs3219175)* presented a high risk of advanced tumor size when compared to GG homozygous genotype. The Ensembl database was used to acquire the frequency data of the variant in different populations. The data presented by Yang et al., was selected as a reference population (Taiwanese), which was used for further comparisons. Gene expression analysis would provide clues on the putative role or *RETN* gene in HNSCC. Hence, gene expression profile for different grades of tumor was analysed using the UALCAN database in the HNSC dataset. Survival curve analysis based on the Kaplan-Meier method was also investigated for differential expression of RETN within the patient group.[26,27] .

Results and Discussion

The phenotype database from ensembl database for *rs3219175* polymorphism showed 92% of "G" and 8% of "A" allele polymorphism reported, represented in figure 1. Individual allele frequencies of different populations are represented in figure 2. Analysis of allele frequency among different population like African (G=87% , A=13%), East Asian (G=82% , A=18%), South Asian (G=98% , A=2%) and European (G=99% , A=1%) was compared with global population (G=92% , A=8%). Allele frequency which is observed in the East Asian population showed 82% of "G" allele and 18% of "A" allele within different sub-populations such as Chinese Dai in Xishuangbanna, China(CDX), Han Chinese in Beijing, China (CHB), Southern Han Chinese(CHS), Japanese in Tokyo, Japan(JPT), Kinh in Ho Chi Minh City, Vietnam (KHV). Allele frequency observation in South-Asians population showed 98% of "G" allele frequency and 2% of "A" allele frequency which is compared among the sub-population of BEB (Bengali from bangladesh), GIH (Gujarathi Indians from Houston), ITU (Indians Telugu from UK), PJL (Punjabi from Lahore), STU (Sri Lankan Tamil from UK) among which "A" allele in higher in BEB and "G" allele is high in STU population (data not shown). To derive a more accurate picture on the deviations observed for the polymorphism in different populations, we compared the allele frequency from the Yang et al., (Taiwanese

population) study of the data acquired from the Ensembl database. The allele frequency comparison was almost similar (Figure 3 and 4). But when comparing the frequency of alleles between the south Asian and the ancestral population which is the African population, we observed marked differences in the allele frequency. The "A" allele frequency was higher in African and east Asian population when compared to other groups. Furthermore, the A allele was negatively selected in other populations such as American, south Asian and European. A negative selection of the allele in the population may be indicative of the fact that it could be deleterious in some way, but the reason for such deviation has to be analysed to derive a strong conclusion.

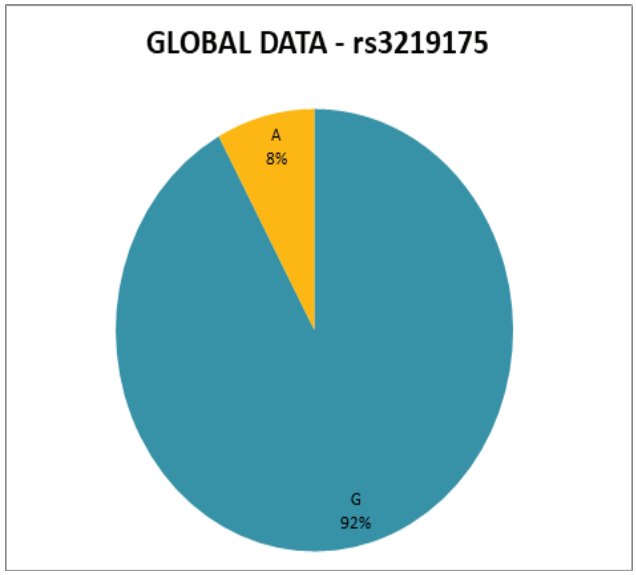


Figure 1: The global phenotype data from Ensembl database for *rs3219175* polymorphism (Yellow color denotes "A" allele frequency = 8%; Blue color denotes "G" allele frequency = 92%)

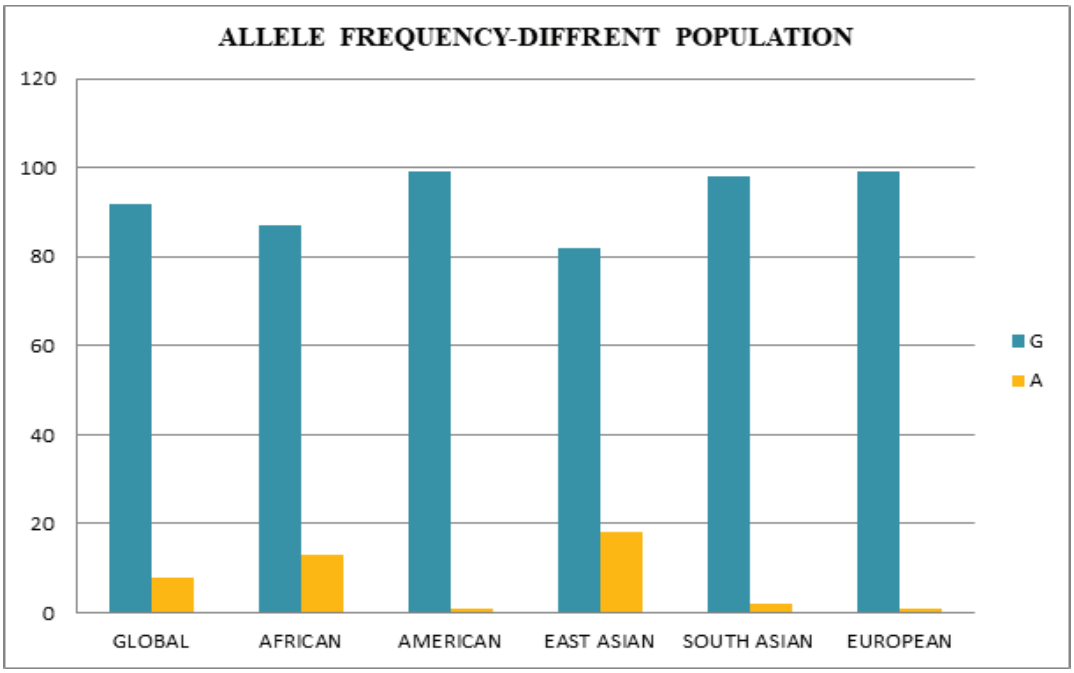


Figure 2: Analysis of allele frequency for *rs3219175* polymorphism among different populations. Yellow color denotes "A" allele frequency; Blue color denotes "G" allele frequency. [Global G - 92% and A- 8%; African - G-87% and A-13%; American -G-99% and A-1%; East Asian - G-82% and A-18%; European - G-99% and A-1%; South Asian - G-98% and A-2%].

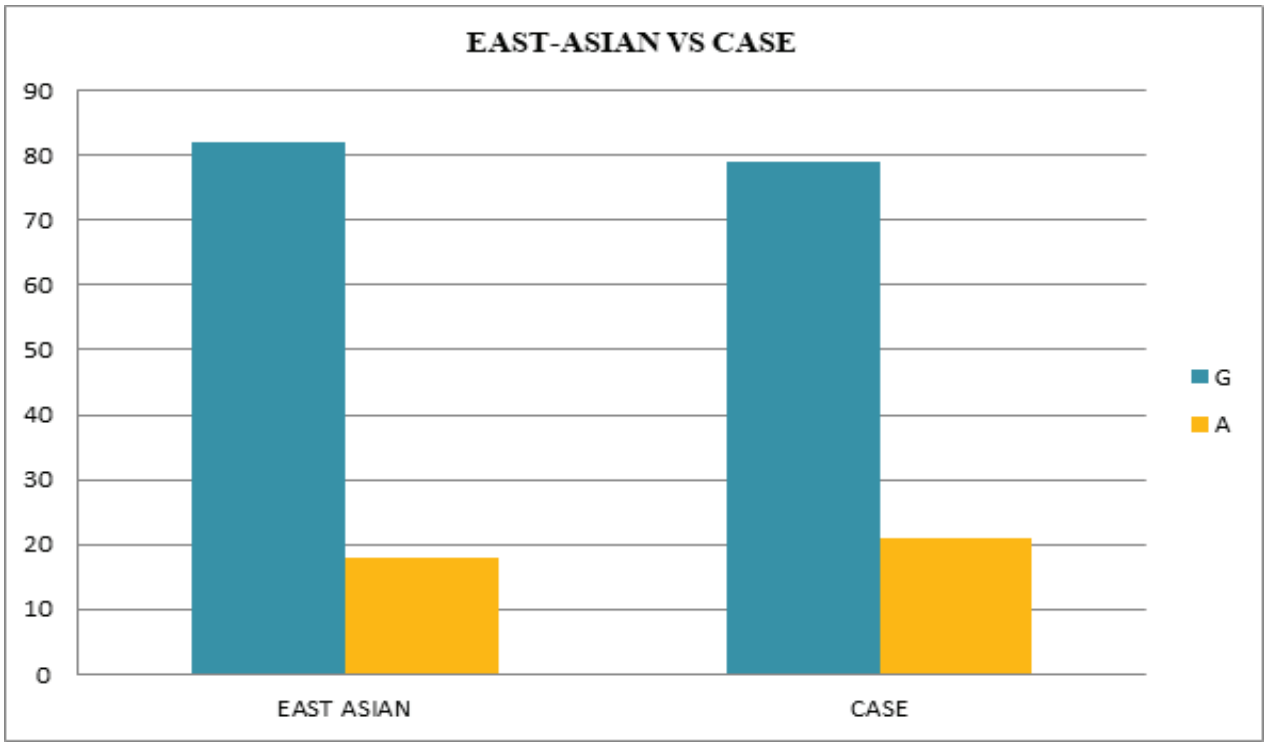


Figure 3: Comparison of *rs3219175* allele frequency case data from research study by Yang et al (G = 78% and A = 22%) with East Asian population (G = 82% and A = 12%). Yellow color denotes "A" allele frequency; Blue color denotes "G" allele frequency.

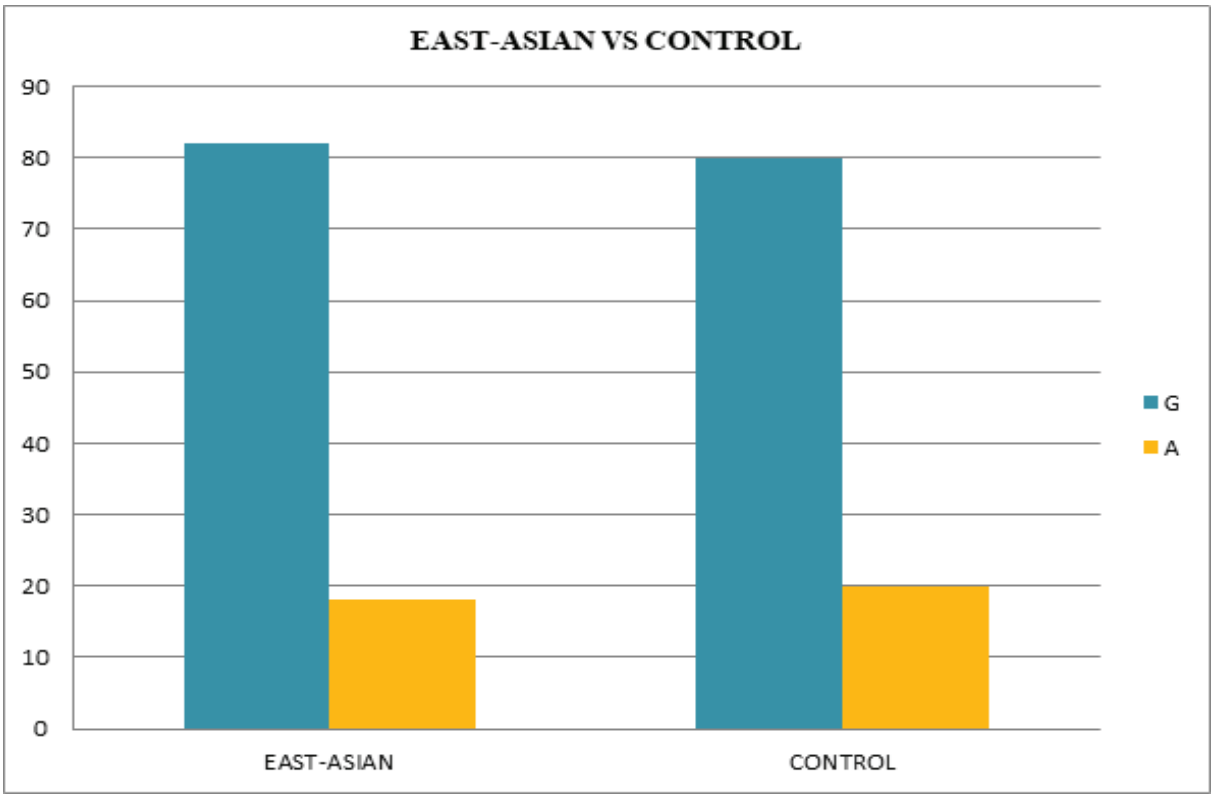


Figure 4: Comparison of *rs3219175* allele frequency of control data (G = 80% and A = 20%) from the research study by Yang et al with East Asian population (G = 82% and A = 12%). Yellow color denotes "A" allele frequency; Blue color denotes "G" allele frequency.

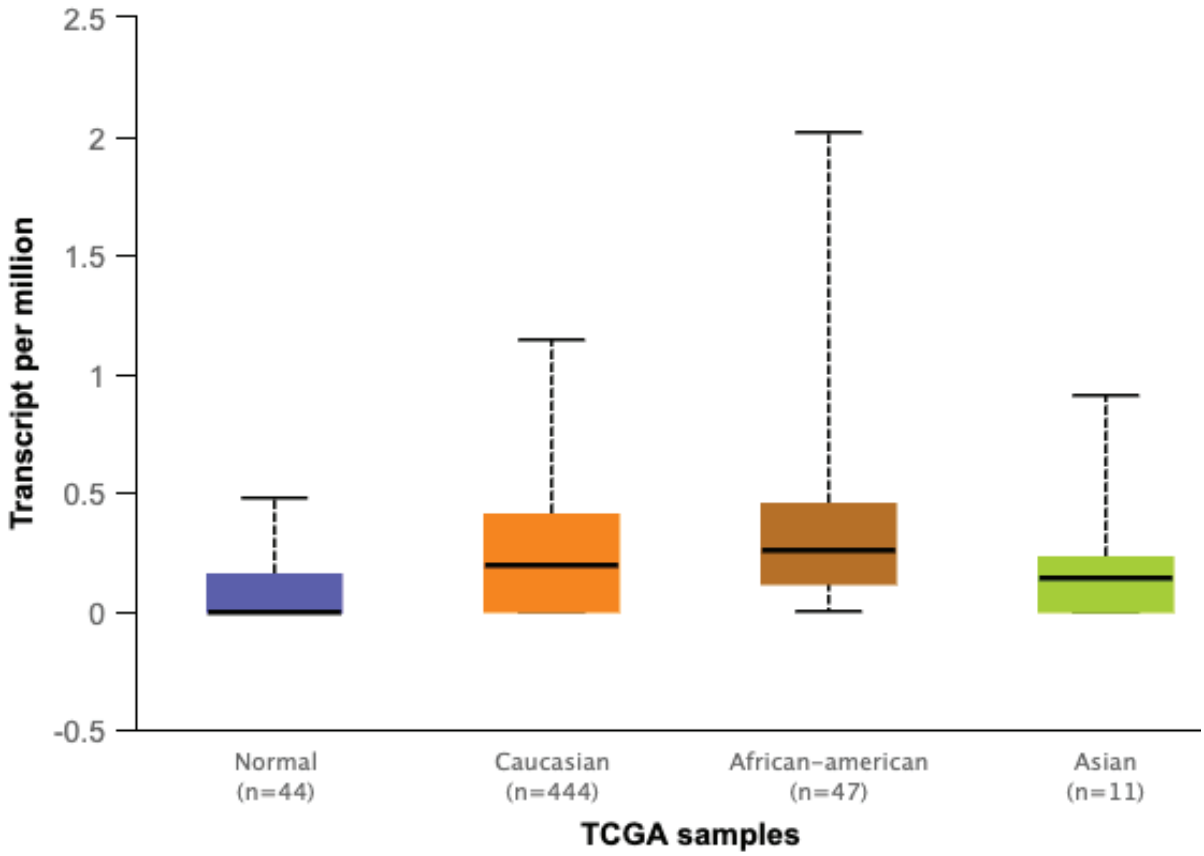
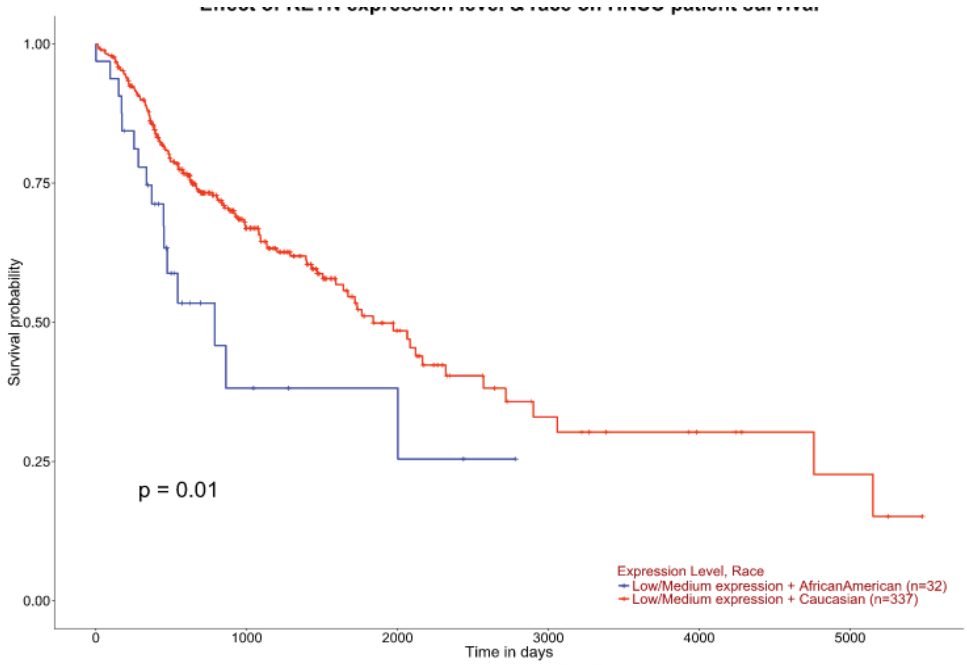
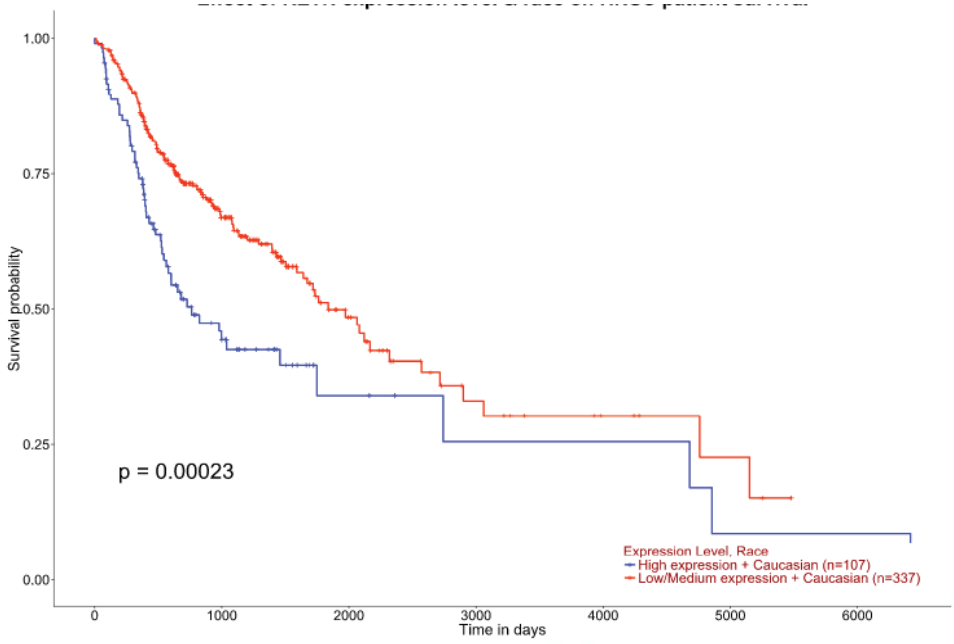


Figure 5: Expression of *RETN* in HNSC based on the patient's race. Blue color denotes normal population; Orange color denotes Caucasian population; brown color denotes African-American population; green color denotes Asian population. The comparison of gene expression between different races returned significant values between the group African-American vs Asian ($p=4.4 \times 10^{-02}$).



(a)



(b)

Figure 6: (a) Comparison of low/medium *RETN* gene expression between African American and Caucasian patients produced a significant p value of 0.01; (b) Comparison of high and low/medium *RETN* gene expression between Caucasian patients produced a significant p value of <0.01.

In addition, expression of the *RETN* gene found to differ significantly between the African American population and Asian population (Figure 5). When analysing the gene expression pattern between groups, low/medium level expression between African American and Caucasians returned a significant p value of 0.01, wherein low/medium gene expression in African Americans corroborated with poor survival of HNSC patients. Interestingly, we identified a significant difference between high and low/medium expression in Caucasian patients, where high level expression resulted in poor survival of these patients (Figure 6). These results provided preliminary evidence on the pivotal role of the *RETN* gene in the progression of HNSC. It is a known fact that nature tries to eliminate anything that is not in use[28,9]. Resistin is an adipokine that is associated with obesity, inflammation and various study cancer.[7] Resistin levels may play a role in pathogenesis of cancer cachexia[7,12]. Upregulation of serum Resistin has been detected in OSCC patients[7]. Online databases are an excellent source for identifying differential gene expression, which is a popular source for exploration of gene expression in diseases. The modern biomedical investigators can genetically profile diseases with this organised online database. There are various tools with basic designs available which are needed in experimenting *in silico* studies[7,29]. The "A" allele frequency is dramatically increased in the ancestral and east Asian population which creates an inquisitiveness to analyze the gene and the polymorphism to draw an association between the gene and the disease phenotype. The negative selection of "A" allele especially in the south Asian population is of our interest.

Conclusion

The present in silico study has provided preliminary data regarding the population wide-analysis of the *RETN* polymorphism selected. The deviation in allele frequency clearly indicates that the gene is evolving and is under constant process of natural selection. As a thumb rule, the alleles which are known to be deleterious are negatively selected, whereas a beneficial allele is positively selected in a population. The observation here denotes that the A allele could be a deleterious allele, and may confer risk of tumor progression in OSCC patients. Further experimental evidence are warranted to provide firm evidence on the association of *RETN* gene mutations or variations with that of OSCC.

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Prevalence of Posterior Cross Bite in Children Visiting a University Hospital in Chennai - A Retrospective Study

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Abstract

Posterior crossbite refers to inadequate transversal relationship of maxillary and mandibular teeth. Posterior crossbite can be unilateral left, unilateral right or bilateral. The causes of this malocclusion are deleterious oral habits, early loss of primary teeth, hereditary factors and environmental factors. It is important to know the prevalence of posterior crossbite in order to provide proper treatment as early as possible. Posterior crossbite when not treated early, can result in skeletal changes. The aim of the study was to assess the prevalence of posterior crossbite in children of chennai. The present study was a retrospective observational study conducted in a university dental hospital located in Chennai. Data of 7415 Pediatrics patients between June 2019-March 2020 were reviewed. Patients from 6 to 13 years who had posterior crossbite were noted. This was followed by Excel tabulation . Data analysis was done in SPSS software using Chi square test for comparison of groups. About 1.4% children visiting university dental hospitals among 4292 children had posterior crossbite. Children of 10 to 13 years had more prevalence of posterior crossbite (P=0.349, statistically not significant) and prevalence of posterior crossbite was more in boys than girls (P= 0.175, statistically not significant) .

Keywords: *Posterior crossbite; Malocclusion;Prevention;Children;Prevalence.*

Introduction

Posterior crossbite refers to inadequate transversal relationship of maxillary and mandibular teeth. Posterior crossbites are of two type unilateral posterior crossbite or bilateral posterior crossbite. The etiologies of this malocclusion are deleterious oral habits, early loss of primary teeth, hereditary factors and environmental factors. According to a study, posterior crossbite begins with primary canine eruption [1]. These are evidence that high prevalence of posterior crossbite is associated with pacifier-sucking habits[2]. There is an increased trend of prevalence of posterior crossbite observed in primary dentition than in permanent dentition [3].

These are only very few studies investigating the prevalence of posterior crossbite in children. If posterior crossbite is untreated it may result in development of skeletal asymmetrics, alteration of soft tissue growth and changes in the TMJ. Posterior crossbite is a common malocclusion problem in the primary and mixed dentitions.

The status of primary occlusion affects the development of the permanent occlusion. Thus a posterior crossbite is transferred from primary dentition to permanent dentition and has a long term ill effect on the patient. Posterior crossbite is due to occlusal interference which is due to deviation of mandible during jaw closure. Early diagnosis of posterior crossbite and treatment is possible only if the parents

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don't fail to fulfil the child's oral health needs [4]. Early treatment of posterior crossbites has been recommended due to the fact that these malocclusions are transferred to the permanent dentition [5,6]. Thus, Early diagnosis and treatment is very important in case of posterior crossbite.

Posterior crossbite may result in various dental problems and other health, emotional effects. If this condition is not corrected, the child's face grows asymmetrically which can result in reduction of self-esteem of the child. Dentition of Children with posterior crossbite are prone to caries . To minimize the risk of caries in such children fluoride paste can be used [7, 8]. A chewable brush can also be used for effective plaque removal and reduces S. mutans counts in saliva [9]. The levels of Malondialdehyde (MDA) in saliva of children should be checked in children with posterior crossbite to diagnose possibility of caries in children [10]. In this way incidence of caries can be prevented in patients with malocclusion such as posterior crossbite.

In a caries affected teeth pulpal exposure occurs . It is important to treat the caries infected tooth prior to orthodontic treatment for posterior crossbite. Pulpectomy is the conservative treatment used to preserve the primary tooth infected by caries [11]. Hand instrumentation technique is the conventional method for root canal preparation in pulpectomy procedure [12,13], but with recent advancements use of rotary instrumentation is used for pulpectomy [14,15]. Obturation was found to be more proper in rotary files than in hand files [16,17]. Our study aims in understanding malocclusion and alignment problems in order to prevent further complications.

Diagnosis of posterior crossbite is by proper history taking, clinical examination, study models, radiographs such as lateral cephalogram and PA view of cephalogram. It is important to examine the soft tissue and hard tissue structures properly prior to treatment [18,19,20]. Knowledge of the etiology and prevalence of malocclusion in a population is very essential for implementing preventive strategies and in determining the success of orthodontic treatment. Hence,this study aimed at assessing prevalence of posterior crossbite in children of chennai.

Material and Method

Study setting

This was a university dental hospital based retrospective , cross sectional study conducted among patients visiting a University dental hospital in Chennai. Since this was a university hospital setting, large sample size and distribution of population contributes to a major advantage for this study. Data collected was reliable and with evidence. The only limitation was that it is a single centered study.This study was approved by the Institutional Review Board, Ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320.

Sampling

The study population was 7415 Pediatric patients visiting university hospitals from June 2019 to March 2020. The sample included patients in the age group of 6 to 13 years . Sample size was 4292 children. Independent variables were demographics such as age , gender etc. Dependent variable was the presence of posterior crossbite . Incomplete dental records were excluded from the study. The parameters assessed were Age, gender, presence or absence of posterior crossbite and type of posterior crossbite. The data collected were tabulated in excel.

Data Analysis

Microsoft Excel was used for tabulation of the parameters and then the data was exported to the SPSS software version 20.0. Descriptive statistics and relation between variables was determined using the chi square test, where $p<0.05$ was considered statistically significant.

Results & Discussion

Primary teeth start to erupt at 6 months of age and complete their eruption at the age of 3 years[21]. The primary dentition in children should be ideal in order so that during future adulthood, the children may exhibit normal dental features [22]. The causative factors of malocclusion can be related to congenital, environmental, genetic, functional, or from oral habits [23]. One such type of malocclusion is posterior cross bite. Posterior crossbite is the transverse discrepancy in the arche relationship in which the palatal cusps of one or more of the maxillary posterior teeth do not occlude in the central fossae of the opposing mandibular teeth [24]. Study of posterior crossbite can help in proper early diagnosis and treatment of the malocclusion. Posterior

crossbite when not treated early, can lead to skeletal changes and also can affect the permanent dentition.

Among the 4292 children 2044 (47.62%) belongs to six to nine years and 2248 (52.38%) belongs to ten to thirteen years (Figure 1). About 2355 (54.9%) boys and 1937 (45.1%) girls were analysed in this study (Figure 2) .

About 4169 (97.1%) children did not have crossbite and only 123 (2.9%) children had crossbite (Figure 3). According to a study by Ferro.R, the prevalence of crossbite was 3.7% which was of similar evidence to the current study [25]. Similar evidence was also seen in a study by N. Venugopal Reddy according to whom the prevalence of crossbite was 14.01% [26]. The reason for such findings is that occurrence of crossbite is rare and mostly due to habits such as using pacifiers, thumb sucking etc.

Among the total study sample, 66 (1.5%) children had anterior crossbite, 28 (0.7%) children had posterior crossbite, 29 (0.7%) children had both anterior and posterior crossbite and 4169 (97.1%) children did not have crossbite (Figure 4). According to a study by Kahraman Gungor, prevalence of posterior crossbite was 15.6% which was less than no posterior crossbite establishing a similar evidence to the current study[3]. The reason is that development of any crossbite is rare compared to normal dentition. The development of crossbite can be due to deleterious oral habits, early loss of primary teeth, hereditary factors and environmental factors.

Among the total children with posterior crossbite , 4 (7.02%) children had unilateral left posterior crossbite, 21 (36.84%) children had unilateral right posterior crossbite, 32 (56.14%) children had bilateral posterior crossbite (Figure 5). Similar evidence was seen in the study of Kahraman Gungor [3] whereas different evidence was seen in the study by Da Silva Filho OG, this is mainly because only less number of children with crossbite were examined in this study whereas Silva examined a large number of children [27].

There were a higher number of children with posterior crossbite in the age group of 10 to 13 with 33.33% (only posterior crossbite-16.26%; both anterior and posterior crossbite-17.07%) (Figure 6). According to the chi square test, P=0.349 is statistically not significant. Similar evidence was seen in a study by Rajendra Reddy according to whom the prevalence of posterior crossbite was more in children aged between nine to ten years [28]. Higher prevalence of posterior crossbite was seen in boys with 26.44% (only posterior crossbite-10.74%; both anterior and posterior crossbite-15.70%). According to the chi square test, P = 0.175 is statistically not significant (Figure 7). Different evidence was seen in the study by E. Rajendra Reddy [28], this is mainly because only a smaller number of children with crossbite were examined in this study.

Development of posterior crossbite was rare but it is important to study about this malocclusion as it can cause many problems. Early diagnosis and treatment should be followed in these patients to improve the quality of their life by preventing the posterior crossbite to progress to permanent dentition and leading to temporomandibular disorders. This study helped in identifying the prevalence of the posterior crossbite and will help in formulating effective preventive strategies. The limitation of this study was that it cannot be generalised to another population since our study was a unicenter study conducted in the south Indian population.

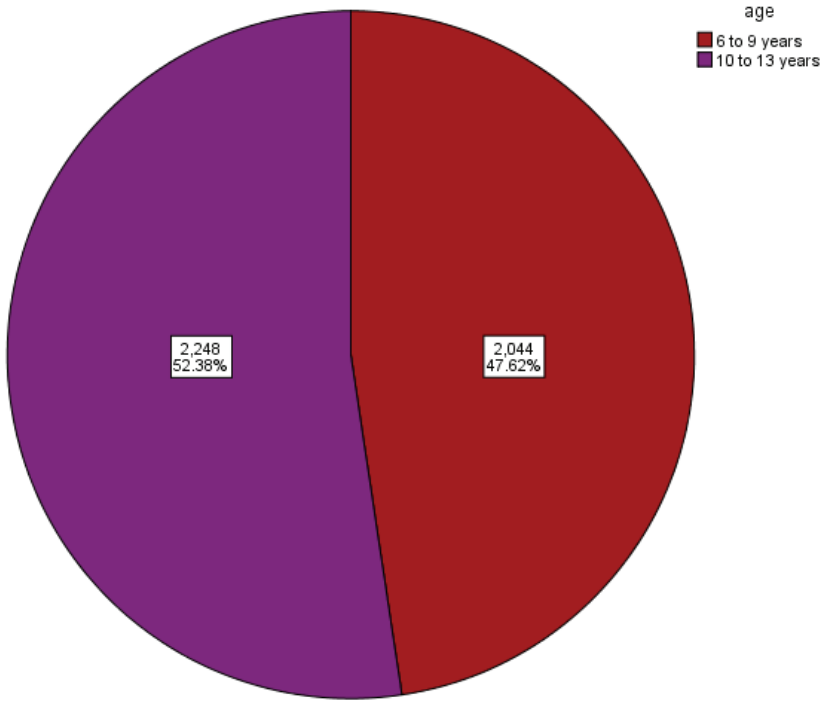


Figure 1: Pie chart showing the Age distribution among the children. Maroon colour denotes children of age group 6 to 9 years and violet colour denotes children of age group between 10 to 13 years. 52.38% children belonged to the age group of 10 to 13 years and 47.62% of the children belonged to the age group between 6 to 9 years.

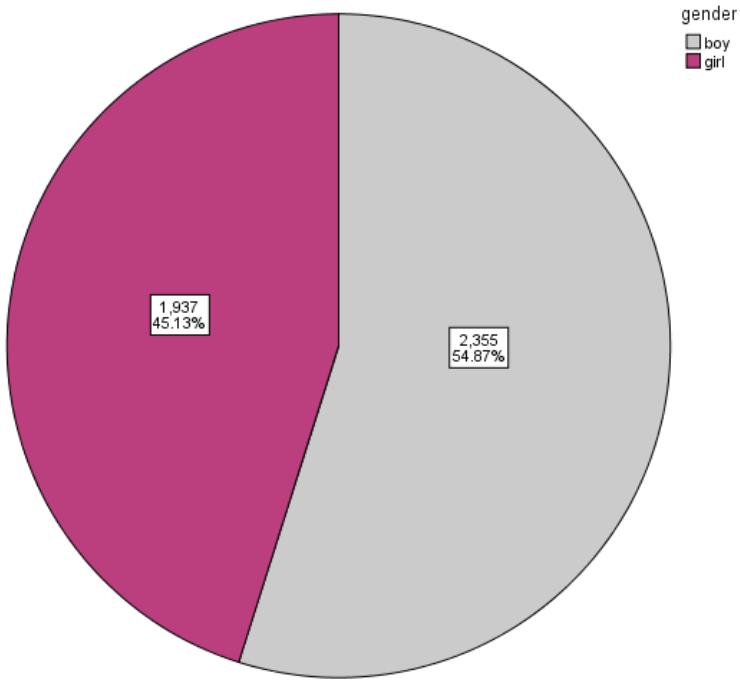


Figure 2: Pie chart showing the gender distribution among children. Grey colour denotes boy and pink colour denotes girl . 54.87% children were boys and 45.13% of the children were girls.

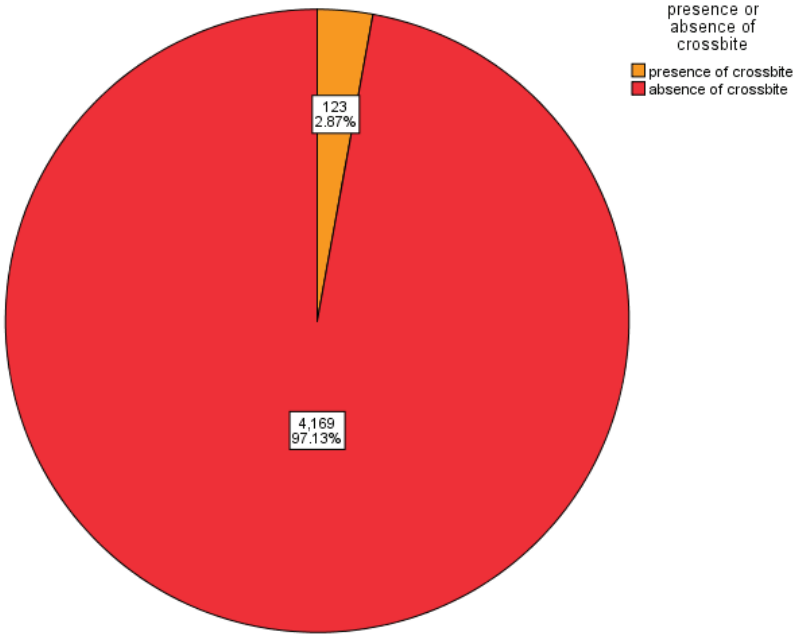


Figure 3 :Pie chart depicting the prevalence of crossbite where red colour indicates absence of cross bite and orange colour indicates presence of crossbite. Prevalence of crossbite was 2.9%.

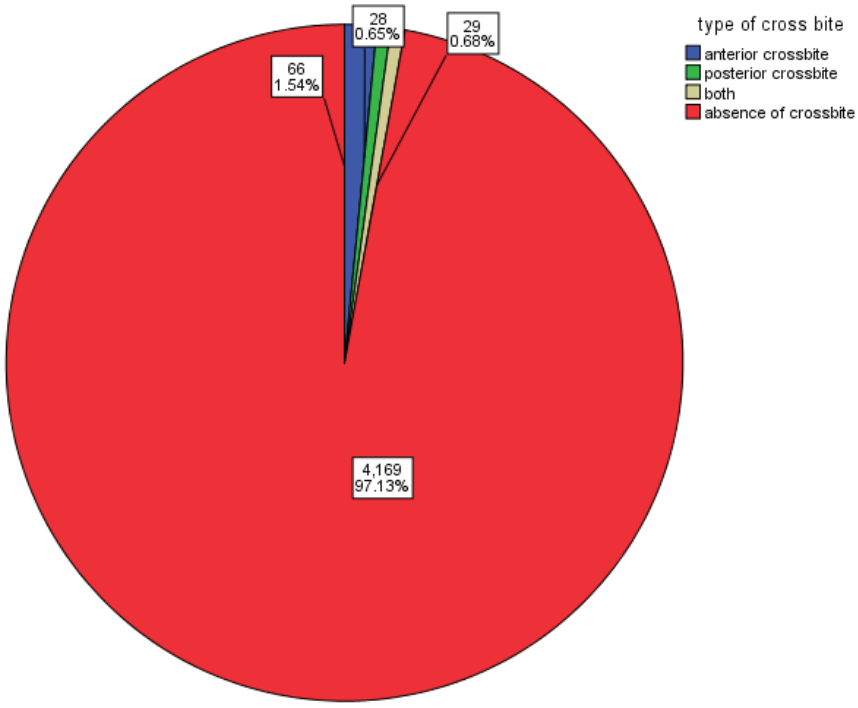


Figure 4: Pie chart showing the prevalence of different types of crossbite in the study population where blue colour denotes anterior crossbite, green colour denotes posterior crossbite , brown colour denotes both anterior and posterior crossbite and red colour denotes absence of crossbite. Prevalence of posterior crossbite was 1.4% (only posterior crossbite-0.7%; both anterior and posterior crossbite-0.7%) among the study population.

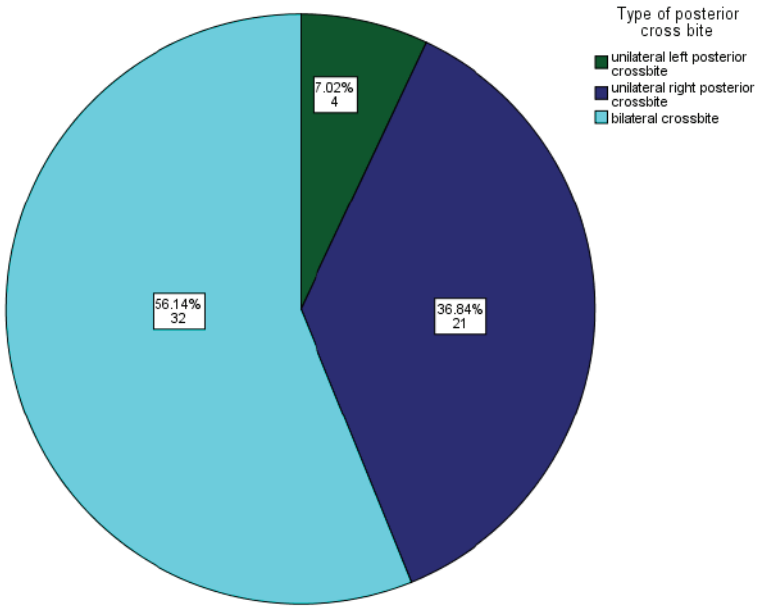


Figure 5: Pie chart depicting occurence of different types of posterior crossbite among patients with posterior crossbite. Dark green colour denotes unilateral left posterior crossbite, dark blue colour denotes unilateral right posterior crossbite and light blue colour denotes bilateral posterior crossbite . Bilateral posterior crossbite was the most frequently occurring type of posterior crossbite (56.14%) among children with posterior crossbite.

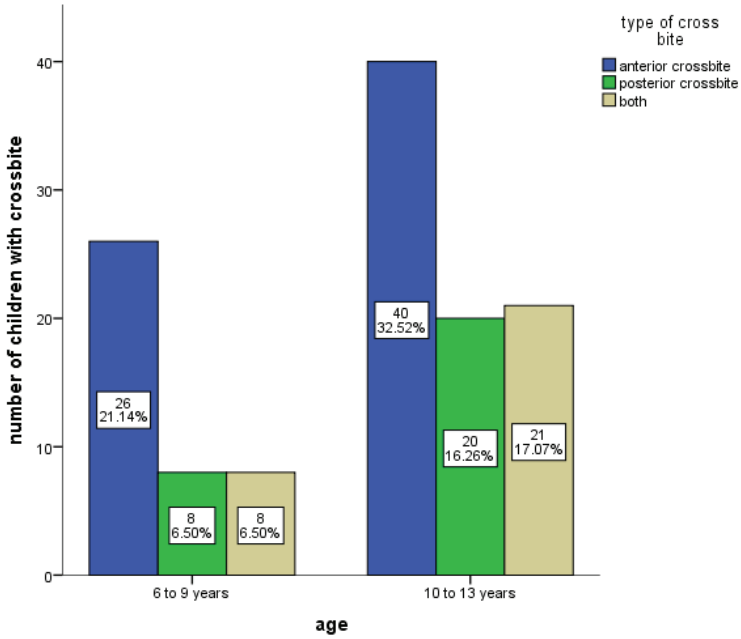


Figure 6: Bar graph shows the association between various age groups and types of crossbite where blue colour denotes anterior crossbite, green colour denotes posterior crossbite and brown colour denotes both anterior and posterior crossbite. X axis represents age groups and Y axis represents frequency distribution of type of crossbite. There were a higher number of children with posterior crossbite in the age group of 10 to 13 with 33.33% (only posterior crossbite-16.26%; both anterior and posterior crossbite-17.07%). Chi square test was done and the association was found to be not significant. (p value = 0.349 >0.05 statistically not significant).

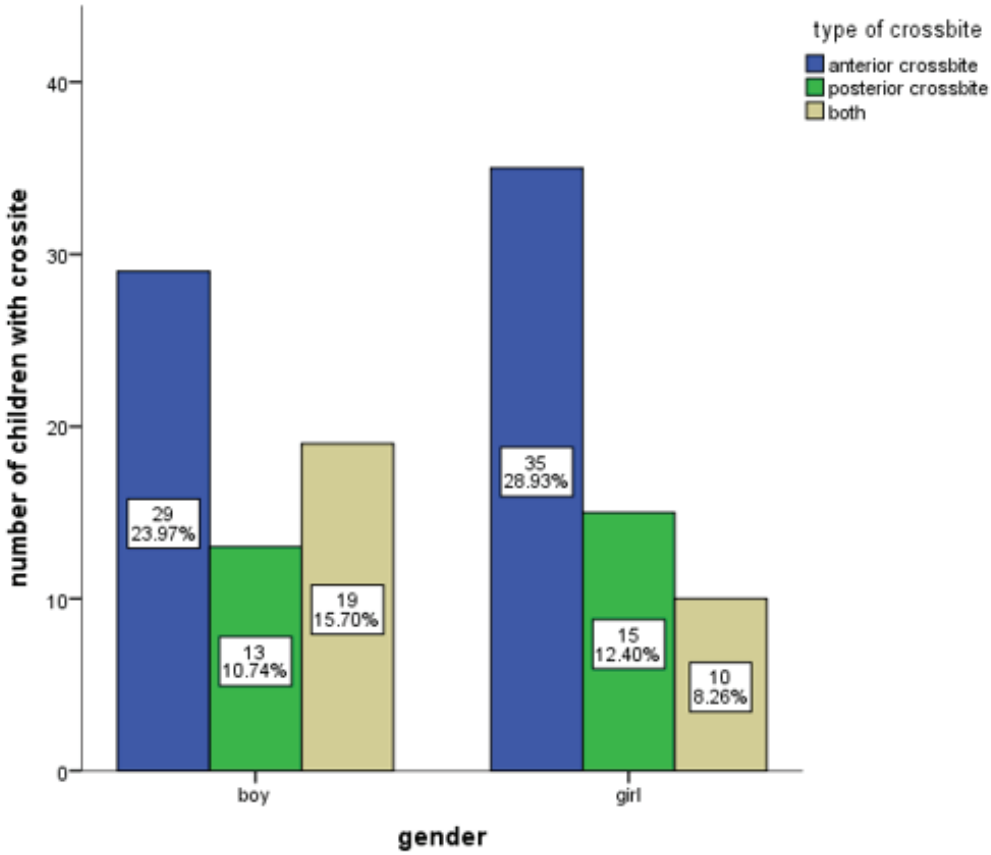


Figure 7: Bar graph shows the association between gender and types of crossbite where blue colour denotes anterior crossbite, green colour denotes posterior crossbite and brown colour denotes both anterior and posterior crossbite. X axis represents the gender and Y axis represents frequency distribution of type of crossbite. Higher prevalence of posterior crossbite was seen in boys with 26.44% (only posterior crossbite-10.74%; both anterior and posterior crossbite-15.70%). Chi square test was done and the association was found not to be significant. (p value = 0.175, >0.05 statistically not significant).

Conclusion

Within the limits of the study, About 1.4% children visiting university dental hospitals among 4292 children had posterior crossbite. Children of 10 to 13 years had significantly higher prevalence of posterior crossbite . Higher prevalence of posterior crossbite was seen in boys than in girls although the difference was not statistically significant.

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Conflict of Interest: There are no conflicts of interest .

Source of Interest: Self

Ethical Clearance- Not Required

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Awareness on the Use of Hand Sanitizer and Face Mask in Disease Spread- A Survey among College Students

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Abstract

Hand sanitizers are helpful to decrease infections in the hand. Alcohol based hand sanitizers are used in common because alcohol is an antiseptic. Face mask has many layers which removes dust and microorganism which is present in the air. The benefit of hand sanitizer and face mask is to control the further spread of disease among people. Usage of hand sanitizer is one of the hygienic practices. Face masks protect us from disease spread and protect us from pollution Hand sanitizer has 60% of alcohol content which kills microorganisms. The aim of the study is to assess the awareness about hand sanitizers and face masks in this disease spread among college students. In previous studies usage of face mask and hand hygiene are high among adults. Hand sanitizers cannot be used by people having allergies because in some sanitizers they have 90% of alcohol content which causes allergies. Alcohol based sanitizers are accepted being used by about 95% of people. This study consists of 15 self structured questionnaires. The study was approved by the Institutional Review Board. The data was collected and evaluated in the SPSS and the statistical significance was analysed in chi-square. We conclude that many are aware about the usage of hand sanitizer and face mask controlling the disease spread.

Keywords: *Awareness; Hand sanitizer; Face mask; N95 mask; Pollution; COVID-19; Alcohol content; Social distancing.*

Introduction

Hand sanitizers are another way of hand wash which kills bacteria & viruses. Usage of hand sanitizer is one of the hygienic practices. Mask prevents many communicable diseases and prevents us from pollution. Hand sanitizer is mostly made in liquid gel. Hand sanitizer has 60% of alcohol content which kills microorganisms. Face mask has many layers which removes dust and bacteria which is present in the air. The benefit of hand sanitizer and facemask to all age group people as it controls the further spread of disease among people

Proper cleanliness reduces various diseases and controls food borne illness [1-2]. Surgical masks are used to avoid contact of liquid droplets from patients [3]. When one uses a mask, that mask should not be reused by others in case usage of the same mask spreads disease [4]. Hand sanitizers cannot be used by people having allergies because in some sanitizers they have 90% of alcohol content which causes allergies. Alcohol based sanitizers are accepted being used by about 95% of people [5.] . In previous studies usage of face mask and hand hygiene are high among adults [6]. Mask use and importance of hand sanitizer was clearly known by the youngsters [7]. Usage of Hand sanitizer at home may also be considered during a pandemic[8].

This research may be needed to create awareness among people. Hand sanitizers and face masks should

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be properly used to get rid from the spreading of disease. Nowadays diseases are spread commonly among elders and children. The aim of the study is to create awareness on the role of hand sanitizers and face mask in disease spread.

Materials & Methods

This study was a self structured questionnaire administered to 100 college students through online google forms link. The advantages of this study are easy to create and reach widely, gathering large data. The study was approved by the scientific review board, Saveetha dental college, Chennai. A statistical test was done using a software SPSS. Statistical test used descriptive analysis and frequency percentage. This study is a simple random sampling. This survey contains 15 self structured questions. These survey questions have been validated based on the general population's methodology. Pie charts were used to depict the results. The association between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant. The study was approved by the Institutional Review Board, Saveetha Dental College.

Result & Discussion

Data collected were analysed using SPSS. 59% of people who had attended this survey were male and 41% were females.About 81% were aware about the importance of hand sanitizer and about 19% were not aware. About 86% were aware that hand sanitizers play an important role in preventing infection disease,14% were not aware. About 83% were aware that n95 face masks prevent COVID-19 and 17% were not aware. About 75% believe that face mask and hand sanitizer prevents microorganisms and 25% did not believe. About 75% were aware of the ingredients used in hand sanitizer and 25% were not aware. About 45% were not aware that America has the biggest production of hand sanitizer and 55% were aware. About 76% were aware that hand sanitizer prevents disease from person to person and 24% were not aware. 13% agreed that surgeons wear masks to protect themselves from water sprayer, about 41% agreed to prevent passage of germs. About 76% agreed that lifestyle was changed and there is a change in the usage protective aid. About 50% said that hand sanitizers were harmful and about 50% said that it is harmless. About 56% of people selected that hand sanitizer is better than hand washing. About 76% felt that it is safe to eat immediately after using hand sanitizer. About 86% were aware about how often we have to change the mask. About 83% are aware of the alcohol content in the hand sanitizer and about 17% are unaware (Figure 1).

We have seen the association between gender (X axis) and responses to awareness on the importance of hand sanitizer in overall hygiene (Figure 2), responses to awareness on effectiveness of the ingredients used in hand sanitizer (Figure 3), responses to awareness on Type of sanitizer which is more effective to protect against COVID-19 (Figure 4), responses to awareness on the sanitizers that it has no effect in spreading the disease from man to man (Figure 5) responses to awareness on harmfulness of hand sanitizer (Figure 6)

We have observed that association between gender and responses to awareness on effectiveness of the ingredients used in hand sanitizer was statistically significant ($p = 0.026$),association between gender and responses to awareness on sanitizers that it has no effect in spreading the disease from man to man was statistically significant ($p =0.048$) and association between gender and responses to awareness on harmfulness of hand sanitizers was statistically significant ($p=0.025$).The statistical associations between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant.

Previous studies conclude that about 58.6% agreed that hand sanitizer prevents micro organism and 41.4% disagreed [9] and 60% of them were aware that hand sanitizer has 70% to 90% of alcohol content [10]. According to the previous literature 20% of people wear masks as its surgeons tradition about 96% surgeons accepted that they wear masks and 4% wont wear [11]. 90% aware about the alcohol content in hand sanitizer [12]. 85% said hand wash is best and 15% said using hand sanitizer is easier and better [13]. According to previous studies, hand sanitizer having more than 92% alcohol content is harmful to children [14]. Recent research on silico modelling [15]. Nanoparticles [16,17]. studies on natural products [18,19, 20, 21, 22, 23, 24, 25, 26, 27.] survey on childhood obesity [28],enzymatic studies had enriched my scientific knowledge and improved my passion for research [29]. In future this study will be useful and will create awareness

about prevention of spreading disease, hand hygiene and also protection from pollution.

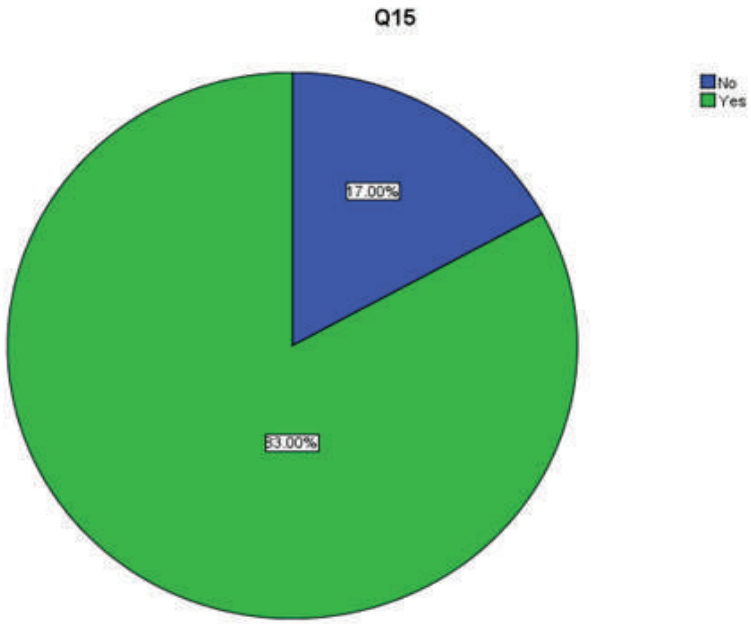


Figure 1: Pie chart represents percentage distribution of responses about awareness on alcohol content in sanitizers. About 83% Yes(green), 17% No (blue). 83% of the participants were aware that sanitizer has alcohol content.

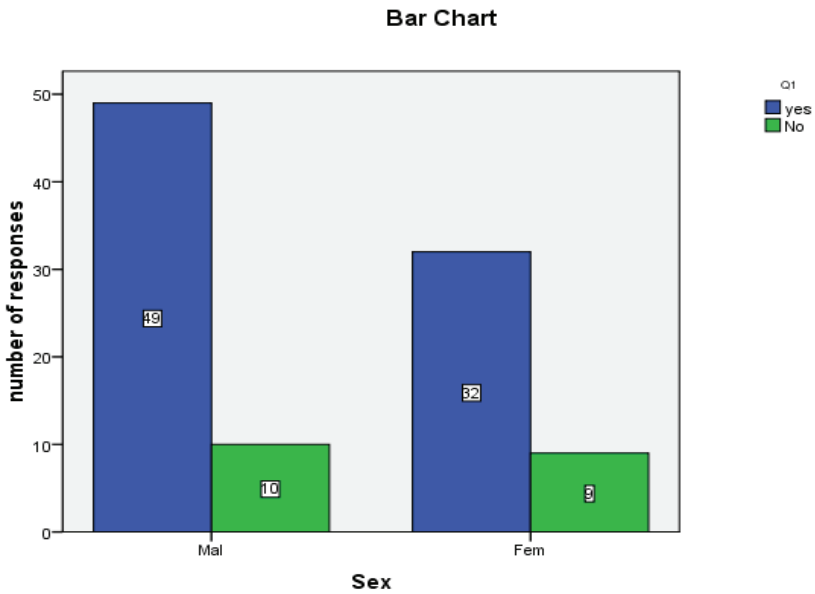


Figure 2: Bar graph represents the association of gender (X-axis) and responses to the importance of hand sanitizer in overall hygiene (Y-axis). Majority of the participants reported yes. Out of the 81 positive responses, 49 males reported yes and 32 females reported yes. Blue colour denotes yes and green colour denotes no. Majority of males were aware about the importance of hand sanitizer (49%). Level of awareness among males and females was similar, though analysis between the association of gender and responses to the importance of hand sanitizer in overall hygiene was statistically not significant. Chi square value - 0.393. p value - 0.531. (>0.05 statistically not significant).

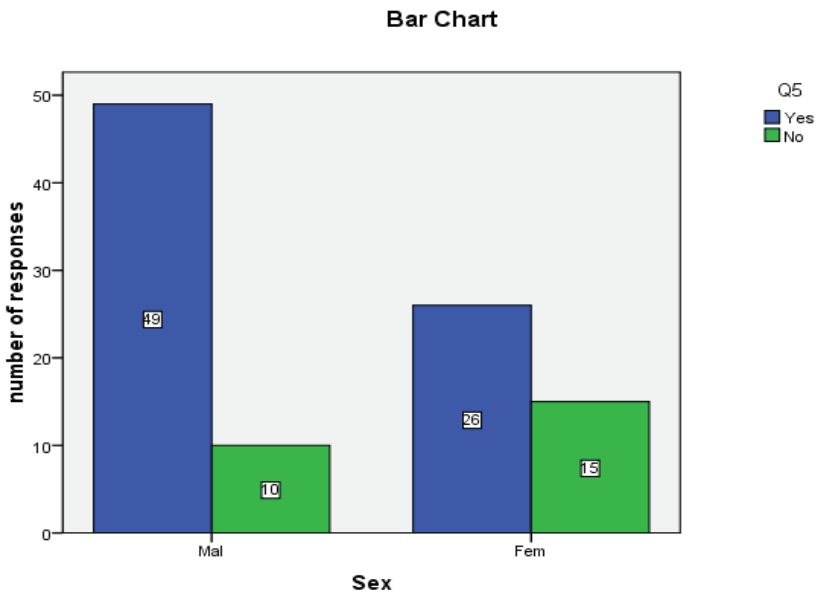


Figure 3: Bar chart represents the association of gender (X-axis) and responses to the effectiveness of the ingredients used in hand sanitizer (Y-axis). Majority of the participants reported yes. Out of the 75% of the population, 49% of male reported yes and 26% of females reported yes. Blue colour denotes yes and green colour denotes no. Majority of males (49%) perceived the effectiveness of the ingredients used in hand sanitizer. Level of awareness among males and females was similar. Chi square - 4.975. p value - 0.026. (<0.05 statistically significant).

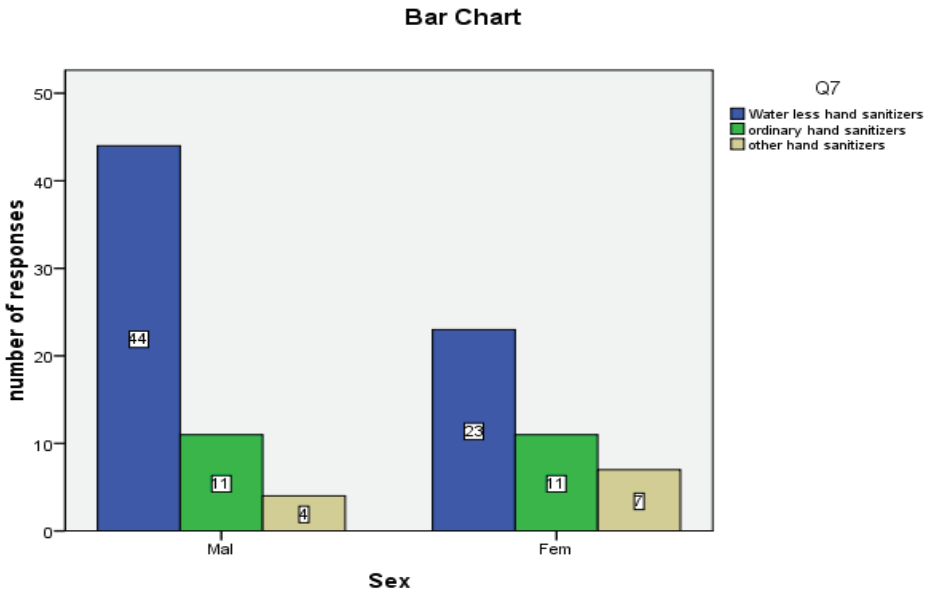


Figure 4: Bar graph represents the association of gender (X-axis) and responses to the Type of sanitizer which is more effective to protect against COVID-19 (Y-axis). Majority of the participants reported waterless hand sanitizer. Out of the 67% of the participants, 44% of males reported waterless hand sanitizer as an option and 23% of females reported waterless hand sanitizer as an option. Majority of the males (44%) were perceived about the type of sanitizer. Level of awareness among males and females was similar. Chi square value - 4.300. p value - 0.117. (>0.05 statistically not significant).

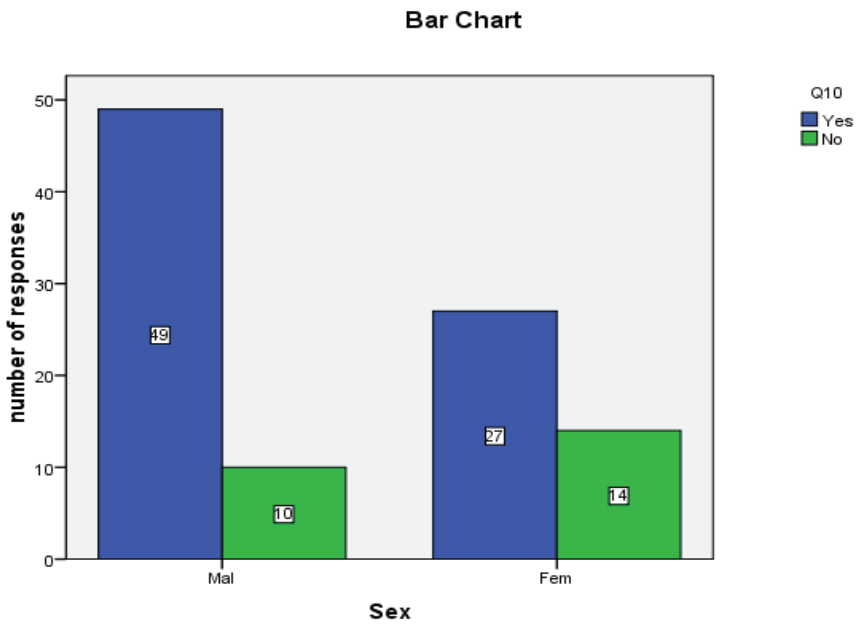


Figure 5: Bar graph represents the association of gender (X-axis) and the responses to the sanitizers that it has no effect in spreading the disease from man to man (Y-axis).Majority of the participants reported yes. Out of the 76% of the participants, 49% of males reported yes and 27% females reported no. Blue colour denotes yes and green colour denotes no. Chi square value - 3.922. p value - 0.048. (<0.05 statistically significant). Majority of males (49%) believed the use of sanitizer does not prevent the spread of disease. Level of awareness among males and females was similar.

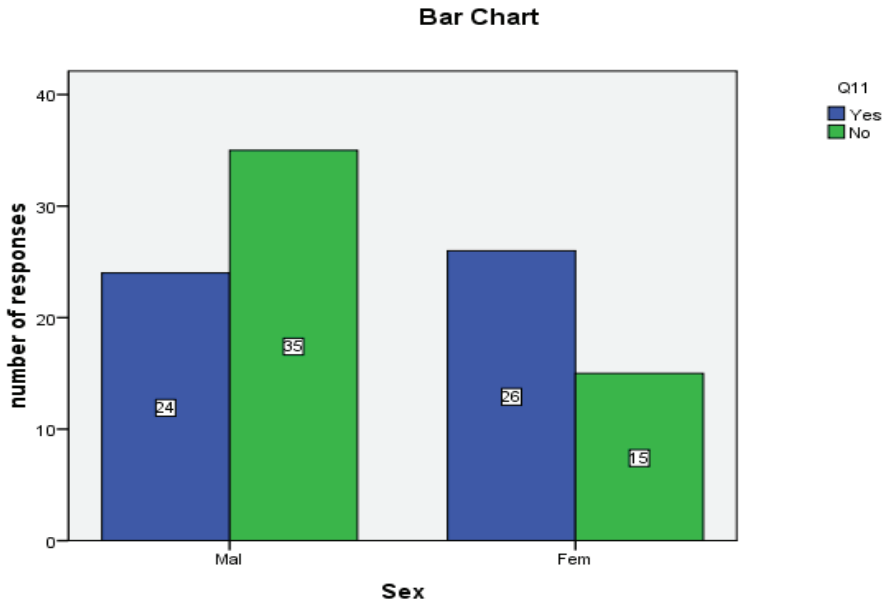


Figure 6: Bar graph represents the association of gender (X-axis) and responses of harmfulness of hand sanitizer (Y-axis). Majority of the participants about 50% of the participants, 35% males reported no and 15% females reported no. Blue colour denotes yes and green colour denotes no. Chi square value - 5.002. P value - 0.025. (<0.05 statistically significant). Majority of males believed that sanitizer does not harm the body (35).Level of awareness among males and females was similar.

Conclusion

We conclude that many college students are aware of the usage of hand sanitizer and face mask in the control of disease spread. Proper cleanliness reduces various diseases and controls food borne illness. Mask prevents many communicable diseases and prevents us from pollution. Hand sanitizer is mostly made in liquid gel. Many awareness camps, workshops may be conducted to educate the younger and older generation about the usage of face masks and hand sanitizer.

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Conflict of Interest : Nil

Source of Interest: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Knowledge and Awareness of Various Dental Biomaterials Used In Pediatric Patients - A Survey

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Abstract

Restoration of decayed primary teeth is important and significant for proper maintenance of child health and also for the development of their teeth. The materials preferred for restoration are composite, glass ionomer, compomer, resin modified glass ionomer cement, stainless steel crown. Therefore it is important to have knowledge about materials and its uses which makes the durability of restoration longer. The aim of the study is to assess the knowledge and awareness of various dental biomaterials used in pediatric patients among the undergraduate dental students. A questionnaire of 12 questions based on different dental biomaterials used in pediatric patients was prepared and it responded by 140 students. Data was collected and with the help of SPSS software data was analysed. Only 9% of students were aware that pit and fissure sealants are bound to tooth surface by both chemical and mechanical bonding. Only 33% opted correctly that stainless steel crowns are semi permanent restorations. Amalgam was the most used restorative material in primary molar by both the male and female students. The material of choice for restoring primary molars with proximal caries and in multi surface carious lesions is glass ionomer cement. The students considered ease of manipulation and durability of the restorative material while selecting it for use in pediatric cases. Within the limitations of this survey, it can be concluded that the participants have an average level of knowledge about various dental biomaterials used in pediatric patients.

Keywords: *Restoration, Caries, Pulpotomy, Pediatric Dentistry, Biomaterials, Composites*

Introduction

Pediatric dentistry is related to considering the child's feeling, to gain the child's confidence and cooperation to perform the desired treatment in a kind manner. Pediatric dentists promote dental health for children as well as serve as educational resources for the parents. It plays an important role in promoting a child's future dental health by stimulating the attitude and behaviour regarding dental care.[1] Children and young adults usually are more prone to develop deep carious lesions due to the poor oral hygiene and maintenance. These carious lesions must be treated early to prevent pulpal involvement in these teeth. Despite the modern advances in prevention of dental caries and increased understanding of the importance of maintaining the natural dentition yet still many teeth are lost prematurely. The preservation of primary tooth until the eruption of the permanent successor is very important in maintaining the integrity of the arch length and form. Pulpotomy and pulpectomy are the standard methods of pulp therapy for the treatment of carious tooth, which pulpally involved in various primary teeth, it is done to stop premature exfoliation and the main objective is total elimination of all the microorganisms from the canal and prevention of re-infection.[2,3]

One of the main challenging aspects for a pediatric dentist is endodontic treatment in primary teeth. The

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key factors for successful endodontic treatment in primary teeth are diagnosis of pulp pathology and deciding the correct treatment plan. Other factors such as instrumentation technique, working length determination, obturating materials, irrigants, root canal morphology, root resorption and follow up period plays a important role in deciding the endodontic treatment is successful or not, so that the primary tooth can be saved without any further complications. As many times, the treatment done remains incomplete either due to the lacking knowledge of the dentist or due to an uncooperative child or their parents. So many dental practitioners usually prefer to extract the tooth as the desired treatment option for the primary pulpal involved tooth because of these challenges. But knowing the importance of primary teeth in maintaining the arch length and prevention of malocclusion we have to keep the pulpal involved primary teeth wherever possible and that too free from infection.[4,5]

Therefore the pulpal therapy depends on whether the pulp is vital or non vital. It depends on clinical diagnosis of normal pulp, reversible pulpitis, symptomatic and asymptomatic irreversible pulpitis or necrotic pulp. Meticulous biochemical preparation determines the success or outcomes of root canal treatment in permanent teeth and the resorble nature and antimicrobial properties of the filling materials also determine the success of pulpectomy in primary teeth.[6,7] The aim of the study is to assess the knowledge and awareness of various dental biomaterials used in pediatric patients among the undergraduate dental students.

Materialsand Methods

A cross sectional questionnaire survey was conducted to assess the knowledge and awareness of the use of biomaterials in pediatric patients with the approval of the Institutional Review Board. The questionnaire consists of 12 questions, which were distributed among undergraduate dental students of 3rd, 4th and 5th year. The questionnaire shared on the online survey platform was responded to by 140 students. Following this data entry, verification, validation in standard computer software was done in SPSS to interpret the results. Chi Square analysis was performed. P value less than 0.05 was considered to be statistically significant.

Results and Discussion

TABLE 1: Percentage distribution of responses to questions on knowledge level about restorative materials.

Questions	Responses			
What is the most important factor we consider to select the restorative material for posterior teeth in primary dentition?	49% - Easy manipulation and durability of material	22% - Patient age and cooperation of child	9% - Cavity shape, size	20% - All of the above
Pit and fissure sealants are bound to surface by?	37% - Adhesive bond	30% - Mechanical retention	23% - Chemical bond	9% - Chemical and mechanical bond
Main reason for use of tooth coloured material for posterior primary teeth.	42% - Fluoride release	25% - Longevity as good as amalgam	23% - Aesthetic superior to amalgam	10% - Tooth does not need pulp therapy
The longevity of conventional GIC in primary molar is mostly?	33% - 3yrs	26% - 2yrs	24% - 10 yrs	16% - 5 yrs

CTABLE 1: Percentage distribution of responses to questions on knowledge level about restorative materials.

Primary function of MTA is ?	36% - Pulpotomy use	35% - Root canal obturation material in primary teeth	15% - Root canal irrigant	13% -Apexification in immature traumatised pulpless tooth
Stainless steel crown is a ?	35% - Temporary restoration	33% - Semi Permanent restoration	13% -Permanent restoration	17% - None of the above

TABLE 2: Percentage distribution of responses to questions on preferences of usage of restorative materials.

Questions	**Responses**			
Restoring proximal lesion in primary molars is better with?	46% - Resin modified glass ionomer cement	27% - Conventional glass ionomer cement	18% - Composite	10% - Amalgam
Which of the following materials is referred to as 'smart material'?	40% - Flowable composites	37% - Amorphous calcium phosphate	23% - Packable composites	-
Which of the following materials is the most ideal for indirect pulp capping?	36% - Zinc oxide powder	30% - Calcium hydroxide	25% - Zinc oxide eugenol cement	8% - Zinc oxyphosphate cement
Which is the most ideal root canal filling for primary teeth?	34% - Ferric sulphate	30% - Zinc oxide eugenol	21% - Calcium hydroxide iodine mixture	13% - Gutta-percha
Which restorative material do you use most frequently in primary molar?	45% - Amalgam	29% - Composite	15% - Glass ionomer cement	10% - Compomer
Material of choice for restoring primary molars with multi surface carious lesion?	36% - Glass ionomer cement,	32% - Amalgam	21% - Stainless steel crown	11% - Composite

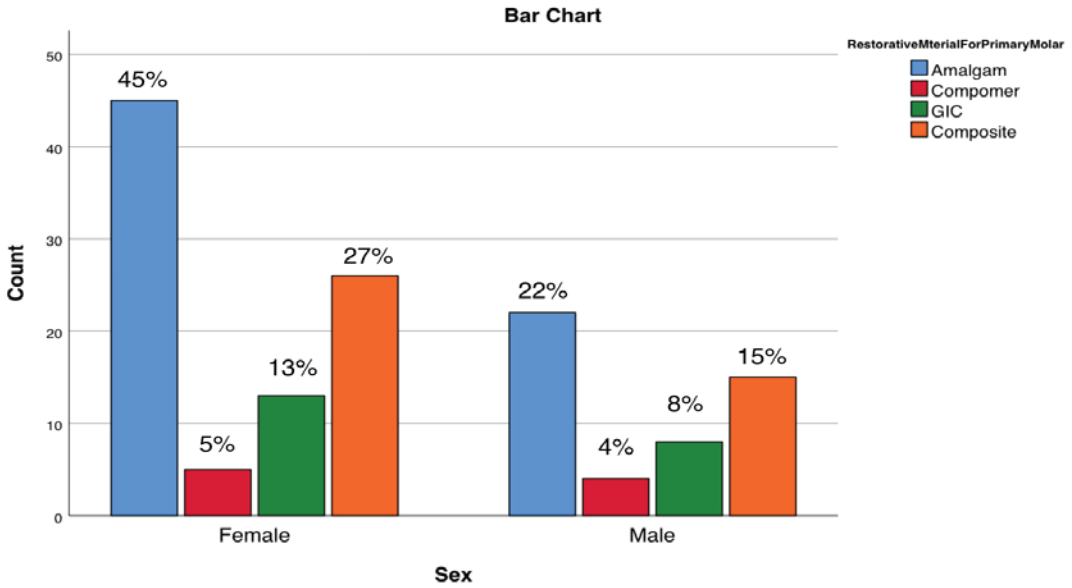


Figure 1: Bar chart represents the association between gender of students and most frequently used restorative material in primary molar. X-axis represents gender and Y-axis represents the count. Amalgam was the most used restorative material in primary molar by both the male and female students. P value = 0.897(> 0.05), hence not statistically significant.

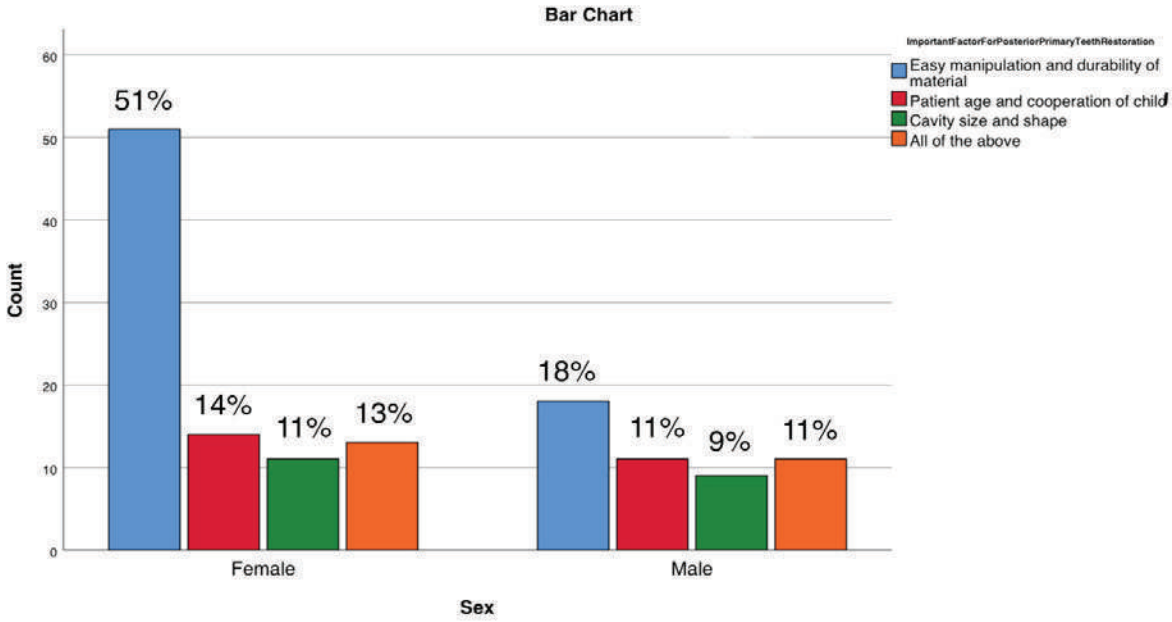


Figure 2: Bar chart represents the association between gender and important factors to consider in selecting the material for posterior teeth in primary dentition. X-axis represents gender and Y-axis represents the count. Most important factor considered was easy manipulation and durability of the material for posterior teeth in primary dentition by both the male and female students. P value = 0.144 (> 0.05), hence not statistically significant.

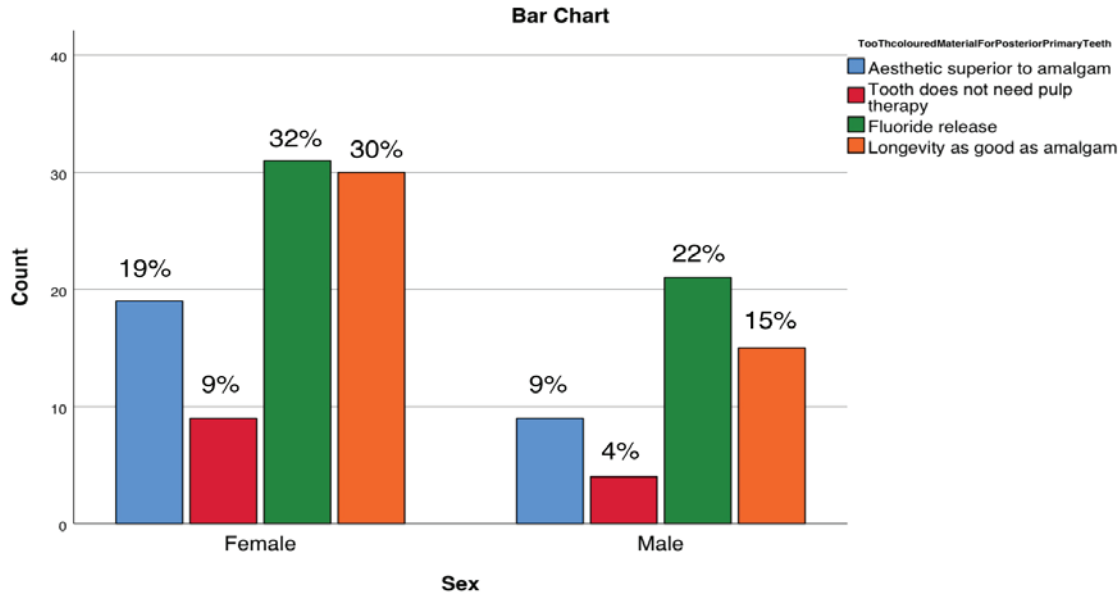


Figure 3: Bar chart represents the association between gender and reason for use of tooth coloured material for posterior primary teeth. X-axis represents gender and Y-axis represents the count. Fluoride release was the most common reason for use of tooth coloured material in posterior primary teeth by both the male and female students. P value = 0.837(>0.05), hence not statistically significant.

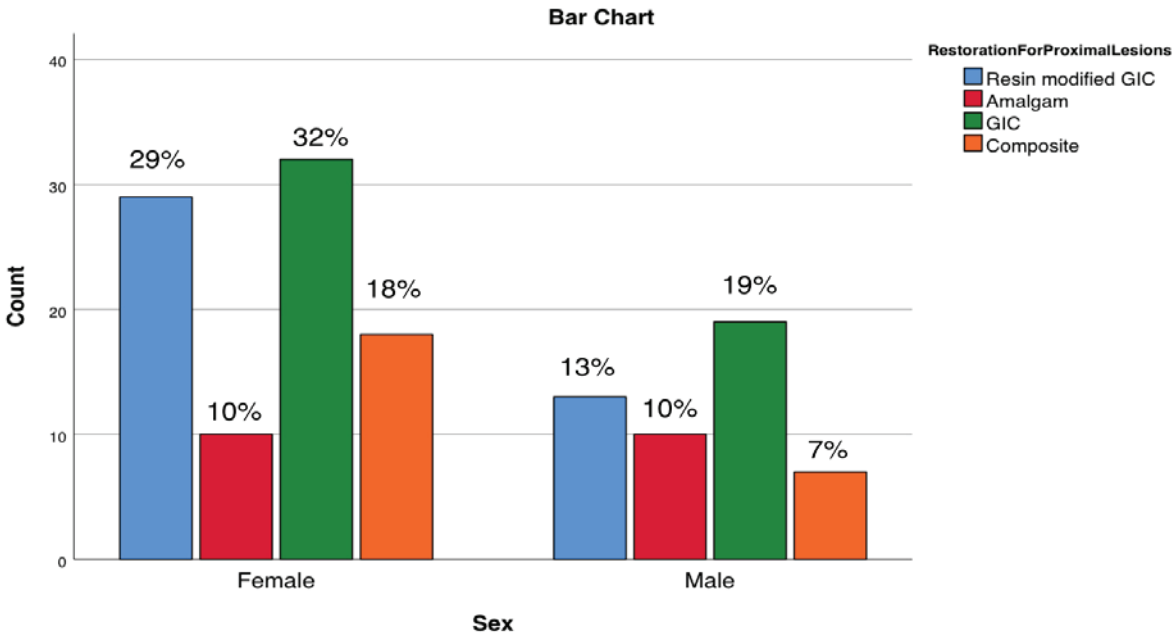


Figure 4: Bar chart represents the association between gender and restorative material used for proximal lesion in primary molars. X-axis represents gender and Y-axis represents the count. GIC was most often used for restoring the proximal lesion in primary molars by both the male and female students. Chi square analysis, P value = 0.415(>0.05), hence not statistically significant.

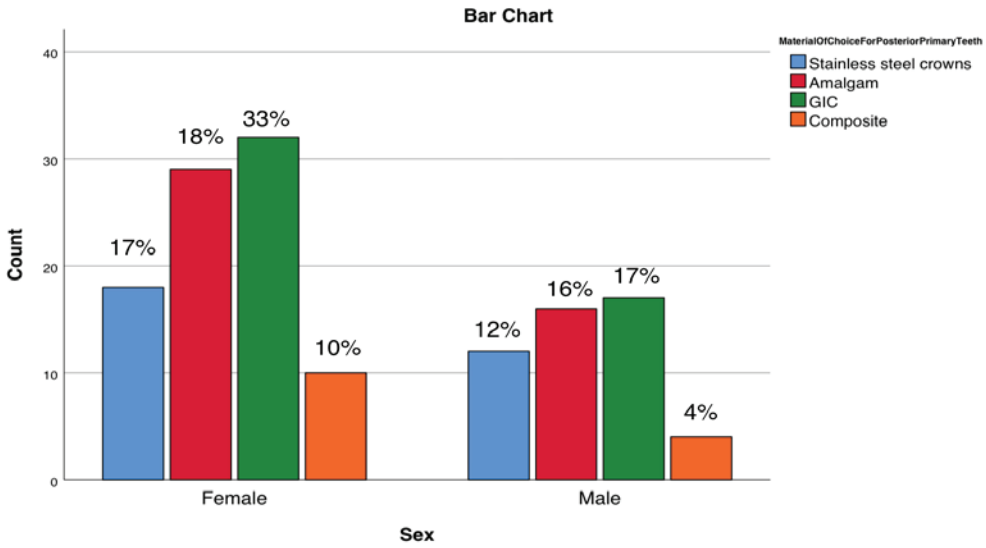


Figure 5: Bar chart represents the association between gender and material used for restoring primary molars with multi surface carious lesion. X-axis represents gender and Y-axis represents the count. GIC was the most used material for restoring primary molars with multi surface carious lesions by both the male and female students. P value = 0.901 (>0.05), hence not statistically significant.

The questionnaire was shared online and it was responded by 140 students. First set of 6 questions dealt with the knowledge level about the dental restorative materials. The results of the study have revealed that for the question about the important factors to be considered for selecting restorative material for posterior teeth in primary dentition about 49% of students chose easy manipulation and durability of material, 22% chose patient age and cooperation of child, 9% chose cavity shape, size and about 20% selected that all the above factors influence the material selection (Table 1). Only 9% of students were aware that pit and fissure sealants are bound to tooth surface by both chemical and mechanical bonding. For the reasons for the use of tooth coloured material in posterior primary teeth 42% chose fluoride release, 25% chose longevity as good as amalgam, 23% chose aesthetic superior to amalgam and 10% chose tooth does not need pulp therapy. 33% of students selected that 3 years is the longevity of conventional glass ionomer cement restorative material in primary molar, 26% selected 2 years, 24% opted for 10 years and 16% for 5 years (Table 1).

Only 33% opted correctly that stainless steel crowns are semi permanent restorations, whereas 35% think that they are temporary restoration, 13% chose permanent restoration option and 17% of study population selected none of the above. Primary function of mineral trioxide aggregate (MTA) is pulpotomy for 36% of study population, root canal obturation material in primary teeth for 35%, 15% opted as root canal irrigant and 13% as apexification material in immature traumatised pulpless tooth (Table 1).

Second set of 6 questions dealt with the preferences or the restorative materials of choice for various carious lesions, in which for restoring proximal lesion in primary molars about 46% opted for resin modified glass ionomer cement, 27% for glass ionomer cement, 18% for composite and only 10% chose amalgam. Among the total population the most used restorative material in primary molar was amalgam as 45% students selected it , 29% opted for composite, 15% for glass ionomer cement and remaining 10% for compomer (Table 2).

Material of choice for restoring primary molars with multi surface carious lesion is glass ionomer cement as 36% opted for it. 32% opted for amalgam, 21% for stainless steel crown and remaining 11% for composite. Only 37% selected correctly that the amorphous calcium phosphate is a smart restorative material. Only 30% selected calcium hydroxide as the most ideal material for indirect pulp capping. 30% selected zinc oxide eugenol as ideal root canal filling for primary teeth and 21% opted for calcium hydroxide-iodine mixture and

remaining 13% opted gutta - percha (Table 2).

The factors guiding the choice in pediatric restorations are age of the child, caries risk cooperation of the child, type of the tooth and type of material used. This is to ensure appropriate care within each materials limits because each restorative material has its advantages and disadvantages.[8] Also the cavity should be sealed so that further tooth destruction does not happen and it renders the tooth and tooth-restoration interface caries resistant. In a clinical scenario, the material selected for the procedure must survive in the extreme environment of the mouth for the period in which it is placed to be effective.[9,10]

Amalgam was the most used restorative material in primary molar by both the male and female students (Figure 1). Most important factor considered was easy manipulation and durability of the restorative material for posterior teeth in primary dentition by both the male and female students (Figure 2). Fluoride release was the most common reason for use of tooth coloured material in posterior primary teeth by both the male and female students (Figure 3). GIC was most often used for restoring the proximal lesion in primary molars by both the male and female students (Figure 4). GIC was the most used material for restoring primary molars with multi surface carious lesions by both the male and female students (Figure 5). However, all the associations were not statistically significant.

Glass ionomer cement is the restorative material which is made from calcium, strontium aluminosilicate glass powder combined with a water-soluble polymer. The components are mixed together which undergoes a setting reaction involving neutralization of the acid groups by the powdered solid glass base.[11] It has the property to stick with the tooth structure because it possesses the identical properties that will adhere to the encompassing enamel and dentin. It is used in Class I, II and V restorations.[12,13] Composite resin, the tooth colored material have completely replaced silicate cement and acrylic.[14] It is used for pit and fissure caries, occlusal surface caries extending into the dentin, Class II restorations in primary teeth, Class III, IV and V restorations in primary and permanent teeth, strip crowns in primary and permanent dentition.[15,16,17] Composite resins are the most preferred dental materials for anterior and posterior teeth. They have an important role in aesthetic and restorative dentistry. Paramount importance of composite resins is because they have an optical appearance, glossy surface and better colour stability. Different types of composite resins are conventional (Packable), flowable(Bulkfill), microfill, hybrid and fluoride releasing composites.[18] Resin-based composites are an integral component of contemporary pediatric restorative dentistry.[19,20] They can be utilized effectively for preventive resin restorations, moderate Class II restorations, Class III restorations, Class IV restorations, Class V restorations and strip crowns.[21,22,23] It has greater ability to seal the margin, which can help to prevent postoperative sensitivity and secondary caries.[24,25] The future scope is to create awareness among dental students regarding new and improved restorative materials, with better knowledge about the complex caries process and its prevention.

Conclusion

Within the limitations of this survey, it can be concluded that the participants have an average level of knowledge about various dental biomaterials used in pediatric patients. The material of choice for restoring primary molars with proximal caries and in multi surface carious lesions is glass ionomer cement. The students considered ease of manipulation and durability of the restorative material while selecting it for use in pediatric cases. Many new developments have occurred in restorative dentistry, one should develop a clear cut understanding of the unique features, strengths, weaknesses and requirements of each material available, which helps to use the right material in the right clinical situation.

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Assessment of Knowledge, Attitude and Practice based Survey on the Use of Ultrasonics in Endodontics among Practitioners

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Abstract

During the past few centuries, endodontics has benefitted from the latest technologies and new techniques, Ultrasonics being one of them. Ultrasonics in endodontics has been used for various purposes such as refining of access cavities, irrigation, removal of pulp stones/ metallic posts/ fractured instruments. It is also being used in surgical endodontics which include procedures such as root end resection and microsurgeries. Ultrasonics thus plays a key role in the successful outcome of endodontic therapy. The present survey was conducted among postgraduate students in endodontics, general dental practitioners and endodontists. The aim of the present survey is to assess the knowledge, attitude and practice regarding the use of ultrasonics in endodontics among practitioners. Questionnaire based survey comprising 19 questions divided into knowledge based, attitude based and practice based questions. The questionnaire was circulated via electronic media through mails. A 200 responses were received from the respondents,.The responses were viewed in M.S. office excel and the results were interpreted using graphs by SPSS 23.0 Version.39% of postgraduates in endodontics, 40% of Endodontist and 21% of general dentists participated in this survey.This survey enables the reader to get a better understanding of the precise and indispensable use of ultrasonics in endodontic practice. Within the limitations of the study, it shows that the knowledge with respect to ultrasonics in endodontics is moderate compared to attitude and practice among respondents.This survey indicated the need for educational intervention to improve the knowledge,attitude and practice on the use of ultrasonics in endodontics among dentists.

Key words: *Applications of Ultrasonics; Endodontics;Endosonics;KAP survey;Ultrasonics.*

Introduction

The use of ultrasonics or ultrasonic instrumentation was first introduced into dentistry for cavity preparation using an abrasive slurry, and its aids in thorough cleaning of teeth which helps in bonding of the laminates to tooth.[1]

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However a different application was introduced in 1955, when Zinner reported its use to remove deposits on the tooth surface.[2] This was improved by Johnson and Wilson into an established tool in Periodontics, the ultrasonic scaler. The concept of ultrasonics in Endodontics was introduced by Richman in 1957. However, it was Martin et al, who demonstrated the ability of ultrasonically activated k files to cut dentin to use it in preparation of root canals. The term 'Endosonics' denotes the ultrasonic and synergistic system of root canal instrumentation and disinfection. It was coined by Martin and Cunningham.[3]

Ultrasound is sound energy with a range of frequency between 25kHz and 40kHz. There are two basic methods of producing ultrasound- magnetostriction and piezoelectric. [2] The two biophysical effects of

ultrasound are acoustic streaming and cavitation. Acoustic streaming is the rapid, turbulent and circular movement of the fluid around a vibrating file producing shear stresses arising from frictional forces. Cavitation can be described as the impulsive formation of cavities or bubbles in a liquid through tensile forces induced by high speed. These bubbles expand and rapidly collapse producing a focus of energy leading to intense sound and damage.[4]

There are various applications of ultrasonics in endodontics which are as follows: [5–7]

- Access refinement, negotiating calcified canals and removal of pulp stones.

- Removal of any obstructions within the canal such as separated instruments, root canal posts, fractured metallic posts [8].

- Increased action of irrigating solutions.

- Irrigation, disinfection.

- Ultrasonic condensation of gutta percha.

- Surgical endodontics: root end resection, refinement and placement of root end obturating material.[9]

- Root canal preparation.

There are contemporary endodontic ultrasonic machines and ultrasonic tips to perform all the functions related to endodontics. The current endodontic ultrasonic tips and attachments are [10]

- Access refinement and apical microsurgery tips:pro endo tips #1 to #8 designed by Clifford Ruddle.

- Microprojection tips.

Ultrasonic action plays an important role in irrigation.[11] Basically, there are two types of irrigation-passive ultrasonic irrigation, ultrasonic irrigation and instrumentation/continuous ultrasonic irrigation. Passive ultrasonic irrigation is the technique of ultrasonic activation after the completion of instrumentation. The term passive implies only activating the irrigant but not contacting or cutting dentin.[6]

Continuous ultrasonic irrigation is achieved by simultaneously and continuously delivering irrigation during ultrasonic activation. It is more effective comparatively. The use of ultrasonics in endodontics is cropping up and many new techniques and machines have been put into use.[12]

The foray of ultrasonics into Endodontics has eased quite a few hiccups in clinical practice such as negotiating the calcified canals, removal of pulp stones or fractured instruments or dislodged posts within the canal. It has also uplifted the efficiency of the irrigation regime and ensured thorough irrigation of the root canal. This has improved the success rate of endodontics.The aim of this survey was to assess the knowledge, attitude and practice of ultrasonics in endodontics among endodontists, postgraduate students in endodontics and general practitioners.

Materials and Methods

The survey was conducted in May 2020 among General dentists, PG students in endodontics, Endodontists to assess the knowledge, attitude and practice regarding the use of ultrasonics in endodontics.

This questionnaire based survey consisted of 19 questions with multiple choice. The questionnaire was divided into demographic data, knowledge based and attitude based. The questionnaire was circulated via electronic media through mail.

Statistical analysis:The collected data was converted into excel sheets. Bar graphs and pie charts were used for pictorial representation of the result of the study.

Results and Discussion

200 responses were received from the respondents. Among the 200 responses received from the participants, 57% consisted of females and 43% males. 31.5% of the participants were of the age group 20-30 yrs. Majority of the participants were General Dentists [40%] followed by PGs in endodontics [39%] followed by Endodontists [21%]. 36% of the participants had a professional experience within 5 years whereas 64% of the participants had a professional experience of more than 5 years. (Table 1)

Based on the knowledge of the participants based on the usage of Ultrasonics in Endodontics, about 76.5% of participants are aware that both acoustic streaming and cavitation are the biophysical effects of Ultrasonics. Regarding the thread patterns used in ultrasonic units, 69.9% of participants are aware that both E&S thread patterns are the types of thread patterns. Regarding the frequency of ultrasonic irrigation it can be seen that there is lack of proper knowledge about it as 46.5% of participants find 10-15Hz as frequency of irrigation unit whereas only 30% of participants correctly knew that the frequency of irrigation was 25-30Hz [Figure 1]. Regarding the types of ultrasonic irrigation, 77.6% of participants are aware that both passive and continuous ultrasonic irrigation are the types of ultrasonic irrigation [Figure 2]. Based on the analysis done by Chi square test three responses have shown statistically significant difference between fields of practice and the responses based on their knowledge regarding ultrasonics [$p<0.05$], whereas one response has shown statistically insignificant difference [$p>0.05$].

Depending on the attitude of participants regarding the usage of ultrasonics in Endodontics, 46.6% of participants opted that sonic,subsonic,negative pressure and ultrasonic irrigation are the best adjuncts for irrigation. 69.8% of participants preferred ProEndo ultrasonic tips for access refinement. 41.5% of participants opined that ultrasonic tips aided in negotiating the calcified canals precisely [Figure 3]. 44% of the participants preferred continuous ultrasonic irrigation as the best over the others [Figure 4]. Based on the analysis done by Chi square test two responses have shown statistically significant difference between fields of practice and the responses based on their attitude towards ultrasonics [$p<0.05$], whereas two responses have shown statistically insignificant difference [$p>0.05$] .

Depending on the practice of Ultrasonics in Endodontics, 45% of participants recommended ultrasonics in pulp stone removal and locating calcified canals. 57% of the participants are aware that ultrasonic tips can be used in root end resection. 61.5% of the participants recommended ultrasonic tips in cases of access refinement and 50% of the participants recommended ultrasonic tips in root canal disinfection procedures. Regarding the types of ultrasonic tips in endodontics, 76.5% of the participants are aware of the types. 27 % of the participants recommended ProEndo #7&#8 tips for pulp stone removal and 18% of participants recommended ProEndo #1 & #2 tips for the same whereas 56% recommended both the types for pulp stone removal [Figure 5]. 84% of participants preferred ultrasonic tips in case of removal of metallic posts [Figure 6]. Most of the participants (82.6%) are willing to attend CDE programs on usage of Ultrasonics in Endodontics.Based on the analysis done by Chi square almost all responses have shown statistically significant difference between fields of practice and the responses based on their practice towards ultrasonics [$p<0.05$].

Table 1: Showing Demographic Data of participants: 39% of Postgraduates in endodontics, 40% of Endodontist and 21% of general dentists were participated in this survey, 36.5% were belong to 0-5 years of experience, 33.5% belong to 6-10 years of experience, 21% belong to 11-15 years of experience and 9% belong to >15 years of experience.

Demographic variables	Categories	No. of respondents	Percentage%
Gender	Male	86	43
	Female	114	57
	Total	200	100
Age(years)	20-30 y	63	31.5
	31-40 y	59	29.5
	41-50 y	51	25.5
	>50 y	27	13.5

Cont... **Table 1: Showing Demographic Data of participants: 39% of Postgraduates in endodontics, 40% of Endodontist and 21% of general dentists were participated in this survey, 36.5% were belong to 0-5 years of experience, 33.5% belong to 6-10 years of experience, 21% belong to 11-15 years of experience and 9% belong to >15 years of experience.**

Field of practice	PG (Endodontics) Endodontist General Dentist	78 80 42	39 40 21
Years of experience	0-5 6-10 11-15 >15	73 67 42 18	36.5 33.5 21 9

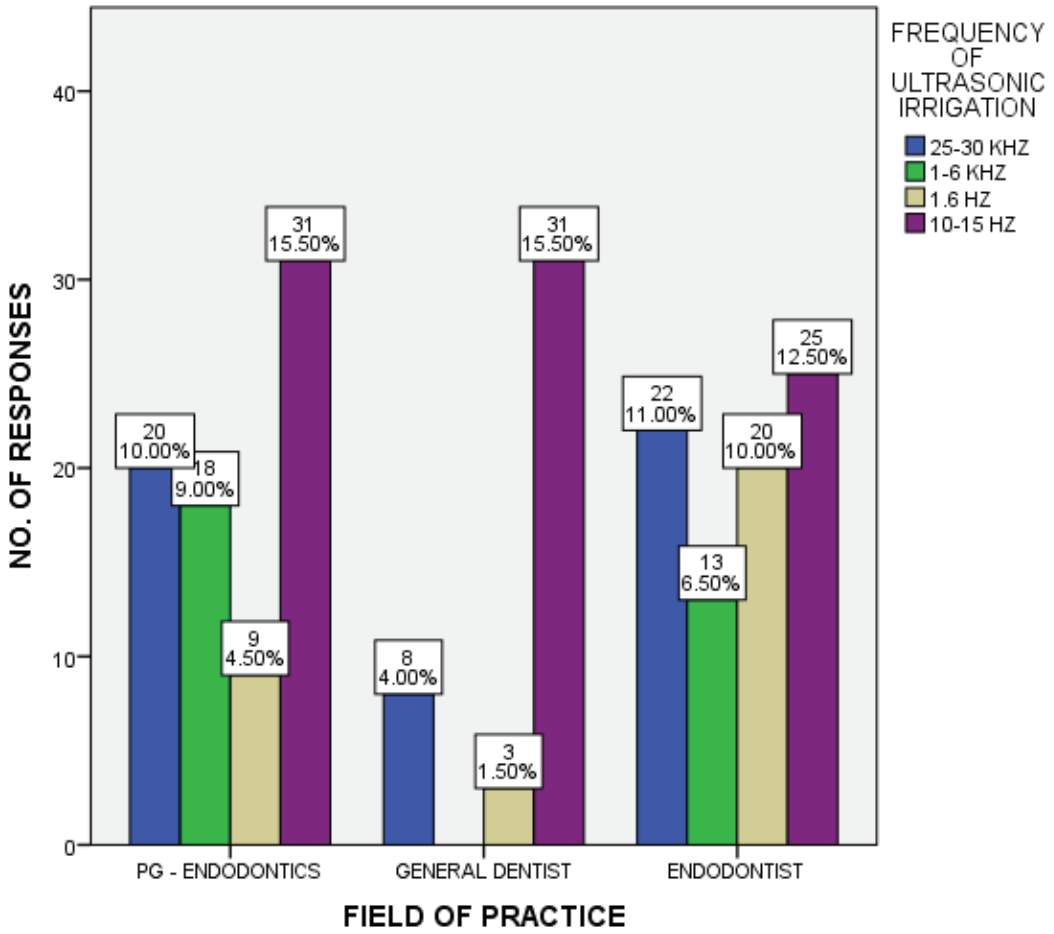


Figure 1: Bar graph showing the association between the field of practice of the participants and responses to the frequency of ultrasonic irrigation. X axis denotes the field of practice, Y axis denotes the number of responses, where blue denotes 25-30kHz, green denotes 1-6kHz, light brown denotes 1.6Hz and purple denotes 10-15 Hz. The association was analyzed using Chi square test and was found to be statistically significant,[chi square value =29.18; p value =0.00 (p<0.05)], proving that general dentists and postgraduate students have better knowledge regarding the frequency of ultrasonic irrigation compared to endodontists.

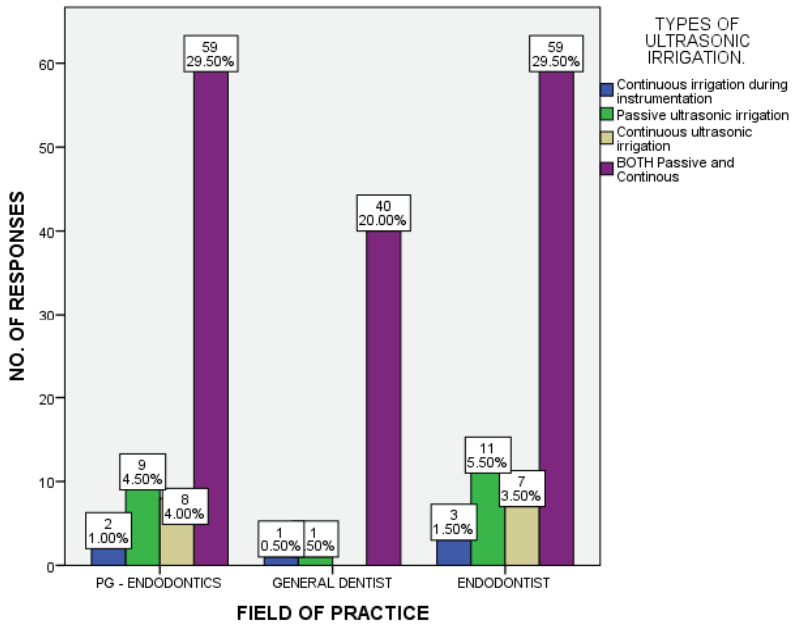


Figure 2: Bar graph showing the association between the field of practice of the participants and responses to the types of ultrasonic irrigation. X axis denotes the field of practice, Y axis denotes the number of responses, where blue denotes continuous irrigation during instrumentation, green denotes passive ultrasonic irrigation, light brown denotes continuous ultrasonic irrigation and purple both continuous and passive ultrasonic irrigation. The association was analyzed using Chi square test and was found to be statistically not significant, [chi square value=9.67; p value =0.13 (p>0.05)], proving that all the respondents have adequate knowledge regarding types of ultrasonic irrigation.

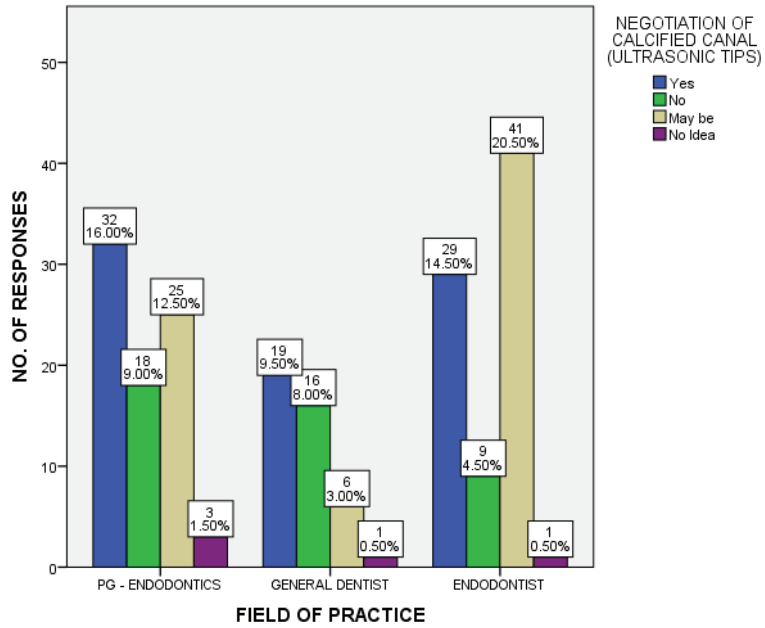


Figure 3: Bar graph showing the association between the field of practice of the participants and responses to the ultrasonic tips aiding in negotiation of the canal. X axis denotes the field of practice, Y axis denotes the number of responses, where blue denotes yes, green denotes no, light brown denotes maybe and purple denotes no idea. The association was analyzed using Chi square test and was found to be statistically significant, [chi square value=22.04; p value =0.00 (p<0.05)], endodontists (20.5%) have better awareness of ultrasonic tips aiding in negotiating the canal compared to postgraduate students and general dentists.

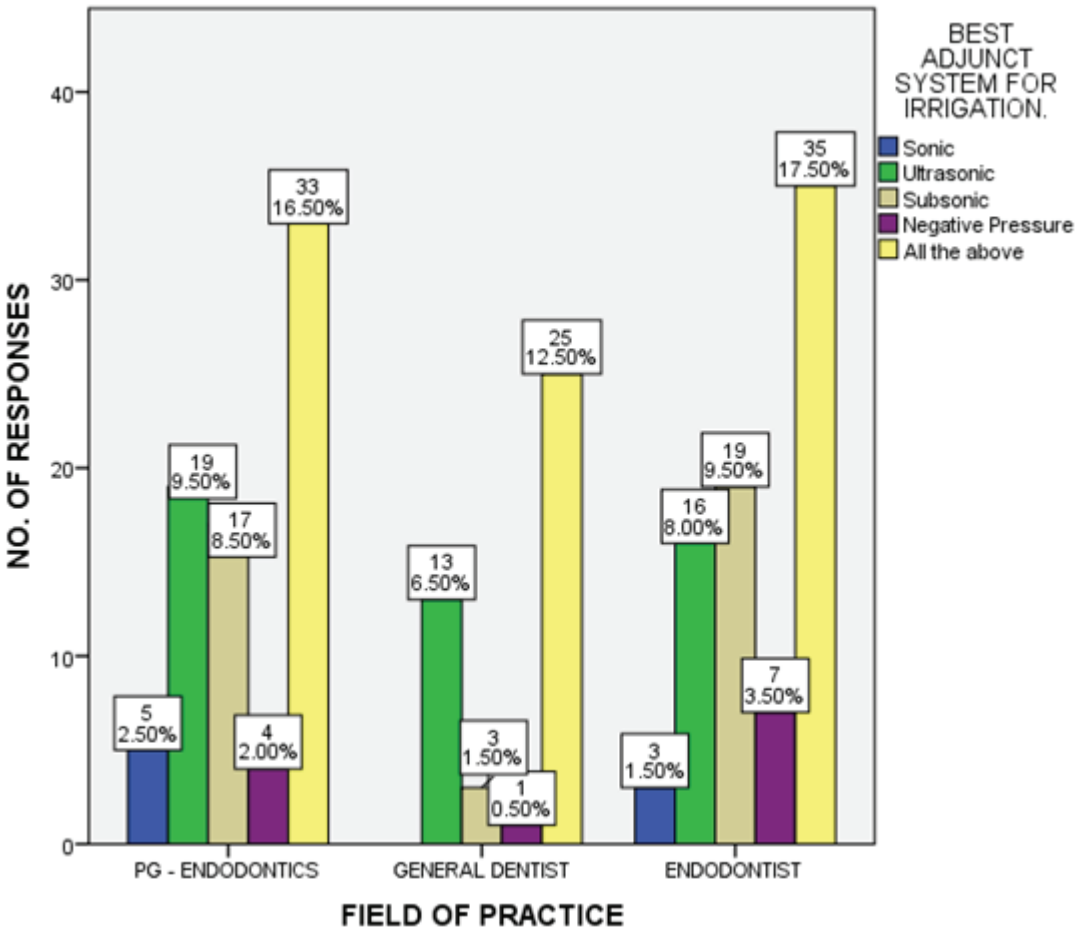


Figure 4: Bar graph showing the association between the field of practice of the participants and responses to the best adjunct for ultrasonic irrigation. X axis denotes the field of practice, Y axis denotes the number of responses, where blue denotes sonic irrigation during instrumentation, green denotes ultrasonic irrigation, light brown denotes subsonic irrigation and purple negative pressure irrigation and yellow denotes all. The association was analyzed usingChi square test and was found to be statistically not significant,[chi square value =12.43; p value =0.13 (p>0.05)], proving that all the respondents have adequate awareness about the adjuncts of irrigation.

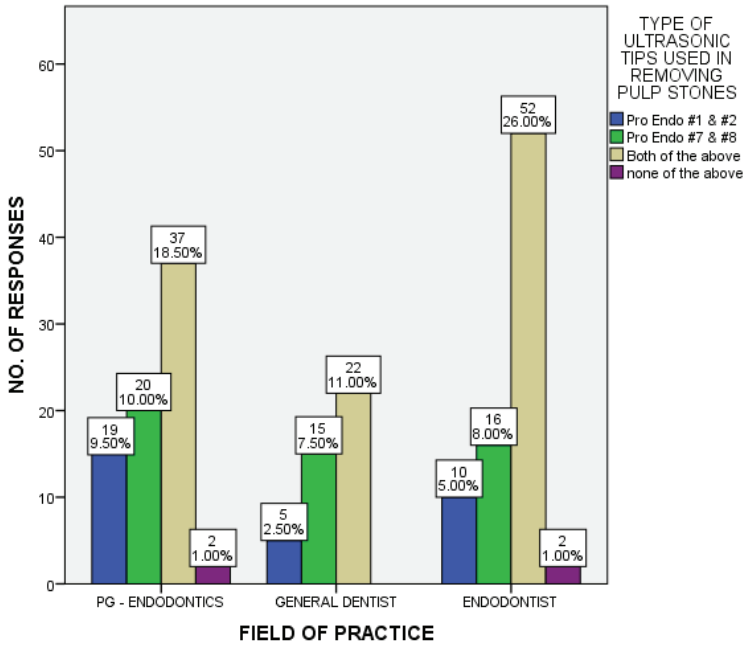


Figure 5: Bar graph showing the association between the field of practice of the participants on the usage of ultrasonic tips in case of removing pulp stones. X axis denotes the field of practice, Y axis denotes the number of responses, where blue denotes Pro Endo #1 & #2, green denotes Pro Endo #7 & #8, light brown denotes both and purple denotes none. The association was analyzed using Chi square test and was found to be statistically not significant' [chi square value=10.50;p value =0.12 (p>0.05)], proving that all the practitioners recommended both the types of ultrasonic tips in removing pulp stones.

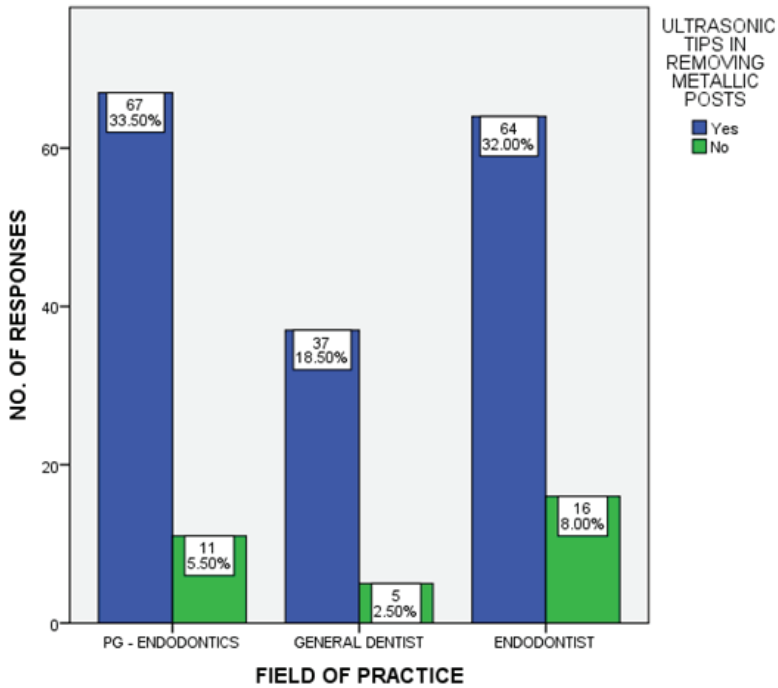


Figure 6: Bar graph showing the association between the field of practice of the participants and the recommendation of ultrasonic tips in removing metallic posts. X axis denotes the field of practice, Y axis denotes the number of responses, where blue denotes yes, green denotes no. The association was analyzed using Chi square test and was found to be statistically not significant' [chi square value=1.68;p value =0.43 (p>0.05)],proving that all the practitioners recommend ultrasonic tips in removing metallic posts.

The main objective of this study is to analyse the appropriate knowledge and usage of ultrasonics in clinical practice and dental institute among general dentists, post graduate students in Endodontics and Endodontists. This study discusses various applications and aspects of ultrasonics in Endodontics. According to literature review, ultrasonics were introduced in dentistry in conservative field to perform cavity preparation.[13] Later it was used in the field of Periodontics to perform scaling. The application of Ultrasonics in Endodontics is credited to Richman.[3] In a previous study conducted by Cesar de Gregario, he found that endodontists were more aware about disinfection with ultrasonics compared to general dentists. [14] Previous studies have reported the compromised efficacy of manual irrigation using conventional methods, as these methods could not reach the anatomical complexities and isthmuses to clean and irrigate effectively.[15,16] An in vivo study on ultrasonic irrigation in human molars conducted by Rubin Gutarts et al showed that the efficacy of Ultrasonic irrigation is superior compared to manual conventional methods.[17] He also showed that the isthmus cleanliness improved and the success rate ranges from 15 to 38%.[18] These results were in accordance with Lev et al, Goodman et al, Haidet et al,Metzler et al and Archer et al and confirmed that complete tissue removal from isthmus is impossible with hand/rotary cleaning and shaping alone. In our present study, 42.5% of the respondents also opine that ultrasonic irrigation is the best.[19,20]

In an in vitro study done by David E.Stamos et al, he compared the debridement ability of hand, sonic and ultrasonic instrumentation.[21,22] He said sonic and ultrasonic debridement was faster and superior compared to the hand/ conventional method. But he also reported that statistical analysis indicated no significant difference in canal and isthmus cleanliness at 3 mm level. However, he postulated that ultrasonics using 2.5% of NaOCl exhibited the treatest percentage of canal and isthmus cleanliness. [7,23]

In a study conducted by Anny Carine Barros et al, he demonstrated the effects of ultrasonic tips on intraradicular post removal. [24] He concluded that the most effective way in reducing force required for intraradicular post removal is to use ultrasonic vibration with elongated cylindrical shaped and activated tips. This was in accordance with the study conducted by Atiyeh Feiz et al, where he concluded that ultrasonics were effective in removal of prefabricated metal posts [24] which is in accordance with our survey where 84% of the respondents opine that ultrasonic tips are effective in removal of metallic posts.

An in vitro study on the success of ultrasonic technique in removing fractured instruments from the root canal was conducted by Hasan et al. He concluded that the success rate was 80%[25,26]. P.Jain et al conducted a study on using ultrasonic tips to remove pulp stone from the canal. In his study, he could successfully remove a 16 mm long pulp stone from the maxillary first molar.[27] But in our survey, only 45% of the respondents are aware and assume that ultrasonic tips aid in the removal of pulp stones which does not correlate with the percentage of success in the previous studies.

Ellen Paark et al said that wide array of ultrasonic tips that are currently available i.e., Pro Endo ultrasonic tips #1 # 8 are useful in access refinement and also microsurgery ultrasonic tips are effective in root end surgeries.[4,28] In our survey 57% of the respondents avail the benefits of microsurgery tips for root end resection and 61% of the respondents find ultrasonic tips better for access refinement.

However, there are certain limitations of ultrasonics [29,30] : The technique is operator dependent, Ultrasonics cannot be used for deeper structures in case of surgeries as the ultrasound waves could be potentially damaging, decreasing tactile sensitivity initially.

Surveys help in assessing the level of knowledge attained by the practitioners/participants and also enables the researchers to reach a wider group of population and assess the awareness, knowledge and practice of the practitioners especially regarding the recent advancements in the field of dentistry.

The limitations of our survey are:

- Confined to a smaller number of respondents.
- Cannot be generalized to a large population.
- Web based surveys are inaccessible to challenging respondents who may lack access to the survey.
- Sampling errors arise due to online surveys.

- Open ended questions and responses might lead to interviewer bias.

In future, a larger population, particularly for a certain speciality/age/ experience group among dentists should be studied.

Conclusion

This survey was done to assess the knowledge, attitude and practice regarding the use of ultrasonics in Endodontics. This survey enables the reader to get a better understanding of the precise and indispensable usage of Ultrasonics in Endodontic practice. Within the limitations of the study, it shows that knowledge with respect to ultrasonics in endodontics is moderate,compared to attitude and practice among respondents. It is recommended that CDE programs can be conducted for dentists to increase their awareness and to gain more knowledge about the usage of Ultrasonics in Endodontic practice.

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Conflicts of Interest: Nil.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Association of Age And Gender Distribution of Patients Reported with Open Apex in Maxillary Central Incisor

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Abstract

Usually open apex is associated with young people who develop early cavities, have a morphological anomaly or have suffered a dental trauma that prematurely halts root development The aim of this study is to assess the association of age and gender distribution of patients reported with Open apex in maxillary central incisor. The study setting is mainly a type of university based and a single centered study. This retrospective study examined the record of patients from june 2019 to april 2020 who underwent treatment at saveetha dental college, chennai. Patients' details were collected to assess the association of age and gender distribution of patients reported with Open apex in maxillary central incisor. A total of 270 patient details were evaluated. Patients were divided into 3 groups. Below 18 years, 18 to 50 years and above 50 years. Patients records were reviewed and then data was extracted. Data was recorded in excel and later exported IBM SPSS software (Version 21: IBM Corporation NY USA). Pearson chi square test was done to statistically analyze the data. Patients of all age groups were included in this study. Around 40.15% of the patients were at the age group of 10-20 years, 21.19% were between the age of 20-30 years, 22.30% were between the age group of 30-40 years and 16.36% of the patients were at the age group of 40-50 years. Around 60.2% and 39.8% reported with open apex in 11 and 21 respectively of which 51.7% male and 48.3% female patients respectively. Within the limits of this study,the age group of 10 - 20 years had been diagnosed with most number of open apices in Maxillary central incisors.Which were 65 patients (24.16%) in 11 and 43 patients (15.99%) in 22 respectively. Majority of patients with age group 10-20 years reported a higher incidence of open apex than other age groups.

Keywords: *Age; Gender; Diagnosis; Maxillary central incisors; Open apex*

Introduction

The endodontic treatment of permanent teeth with open apices and necrotic pulp is considered one of the most complex challenges for clinicians. Usually, these teeth are associated with young people who develop early cavities, have a morphological anomaly or have suffered a dental trauma that prematurely halts root development [(1)]. However, adults with completely formed roots can develop an open apex due to certain pathological (e.g., external inflammatory root resorption) [(2,3)] or iatrogenic (over-instrumentation) [(4)] factors that modify mature apical lumen diameter. Therefore, such teeth show thin radicular walls with increased susceptibility to fracture, and the wide lumen of the apical foramen makes it difficult to maintain the filling material inside the root canal system and impossible for proper mechanical cleaning to avoid excessive root weakness [(5–8)]. All of these characteristics compromise the long term health of the tooth.

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The anatomy of the apical foramen changes with age as root formation has yet to be completed when teeth erupt(9). The completion of root development and closure of the apex occurs up to three years after eruption. The causes of open apices can be due to incomplete development, Necrosis of pulp due to caries or trauma before root formation is completed,Extensive apical resorption, Root-end resection and over instrumentation. Ideal management would involve regeneration of new pulpal tissue and continued root formation(10)[1]. Novel techniques for dealing with immature apices such as apexogenesis sometimes claim to be regenerative techniques. However, assessment of the composition of this regenerated tissue has proven to be difficult and it seems that it is made up of periodontal and bone tissue rather than tissue of pulpal origin

The assessment of dental maturity is a typical strategy employed by some dental specialities (e.g. forensic dentistry) [1(11–13)], but few studies have examined the stage of root development in relation to the frequency of teeth with open apices and necrotic pulp [(14,15)]. Several classification systems for the degree of root formation and maturation have been proposed [(16,17)], although Cvek's [(18)] classification offers didactic radiographic characteristics with valuable clinical applications.

Cvek's classification describes the five stages of root development. I = < 1/2 root length, II = 1/2 root length, III = 2/3 root length, IV = wide open apical foramen and nearly complete root length and, V = closed apical foramen and completed root development.While Cvek stage V describes mature, fully formed teeth, the remaining four stages describe teeth with open apices and a lack of apical constriction development but significant morphological differences.Cvek stages I, II and III indicate wide and divergent apical openings, the root canals are significantly wider in the buccal-lingual plane than in the mesio-distal plane, the terminal portion of the root is irregular and the apical foramen diameter is higher than the root canal lumen[(19)]. In contrast, stage IV is associated with noticeable root length and convergent apical walls. Therefore, considering that endodontic procedure selection most likely depends on the maturity of the affected root.The aim of this study is to assess the association of age and gender distribution of patients reported with Open apex in maxillary central incisor.

Materials and Methods

Study design and setting:

The study setting is mainly a type of university based and a single centered study. This retrospective study examined the record of patients from june 2019 to april 2020 who underwent treatment at saveetha dental college, chennai. The study population includes patients who underwent treatment for open apex. The main advantage of this study is that flexible data can be obtained immediately and less expensively but the drawback of this study is that they have geographic limitations and involve the people of an isolated population. Ethical approval was applied to the institutional ethics community, SIMATS.

Data Collection:

Data was obtained from saveetha dental college and hospital. The record of patients were examined from june 2019 to april 2020 who underwent treatment at saveetha dental college, chennai. The study population includes patients who underwent dental post cementation and core build-up procedures. To evaluate the number of prefabricated fiber posts placed in Maxillary central incisors with 2 or more walls presen 143 case records of patients Patients records were reviewed and then data was extracted. Patients of all age groups were included in this study.Patients were divided into 3 groups. Below 18 years, 18 to 50 years and above 50 years. Repeated patient records, incomplete data without proper notes were excluded from the data. The data was recorded, tabulated and the percentage calculated.

Statistical Analysis

Data was recorded in excel and later exported IBM SPSS software (Version 21: IBM Corporation NY USA). descriptive statistics (frequencies and percentages) were calculated to explore the general features of the data. Pearson chi square test was done to statistically analyze the data. Pearson chi square test was used to identify any significant level of variation. Ethical approval was obtained from the Institutional Ethical Committee. Approval number (SDC/SHIEC/2020/

DIASDATA/0619-0320)

Results and Discussion

Patients of all age groups were included in this study. Around 40.15% of the patients were at the age group of 10-20 years, 21.19% were between the age of 20-30 years, 22.30% were between the age group of 30-40 years and 16.36% of the patients were at the age group of 40-50 years (Figure 1). 51.7% and 48.3% male and female patients respectively (Figure 2), Of which 60.2% and 39.8% Reported with open apex in 11 and 21 respectively (Figure 3). (Figure 4) shows that the age group of 10 - 20 years had been diagnosed with most number of open apices in Maxillary central incisors.Which were 65 patients (24.16%) in 11 and 43 patients (15.99%) in 22 respectively.

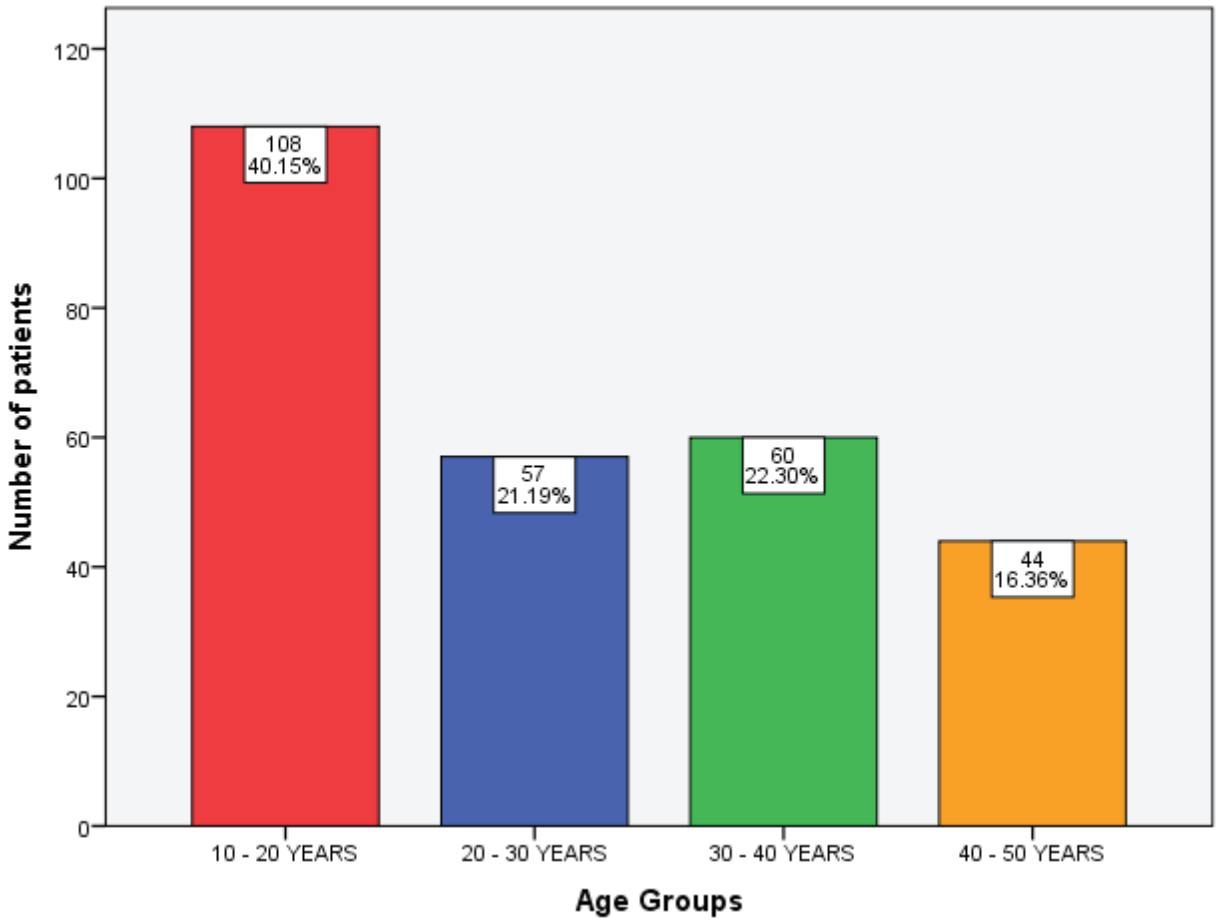


Figure 1: The bar chart depicts the distribution of study subjects based on age groups. X axis represents age and Y axis represents the number of the patient. Around 40.15% of the patients were at the age group of 10-20 years(red), 21.19% were between the age of 20-30 years(blue), 22.30% were between the age group of 30-40 years(green) and 16.36% of the patients were at the age group of 40-50 years(orange) respectively.

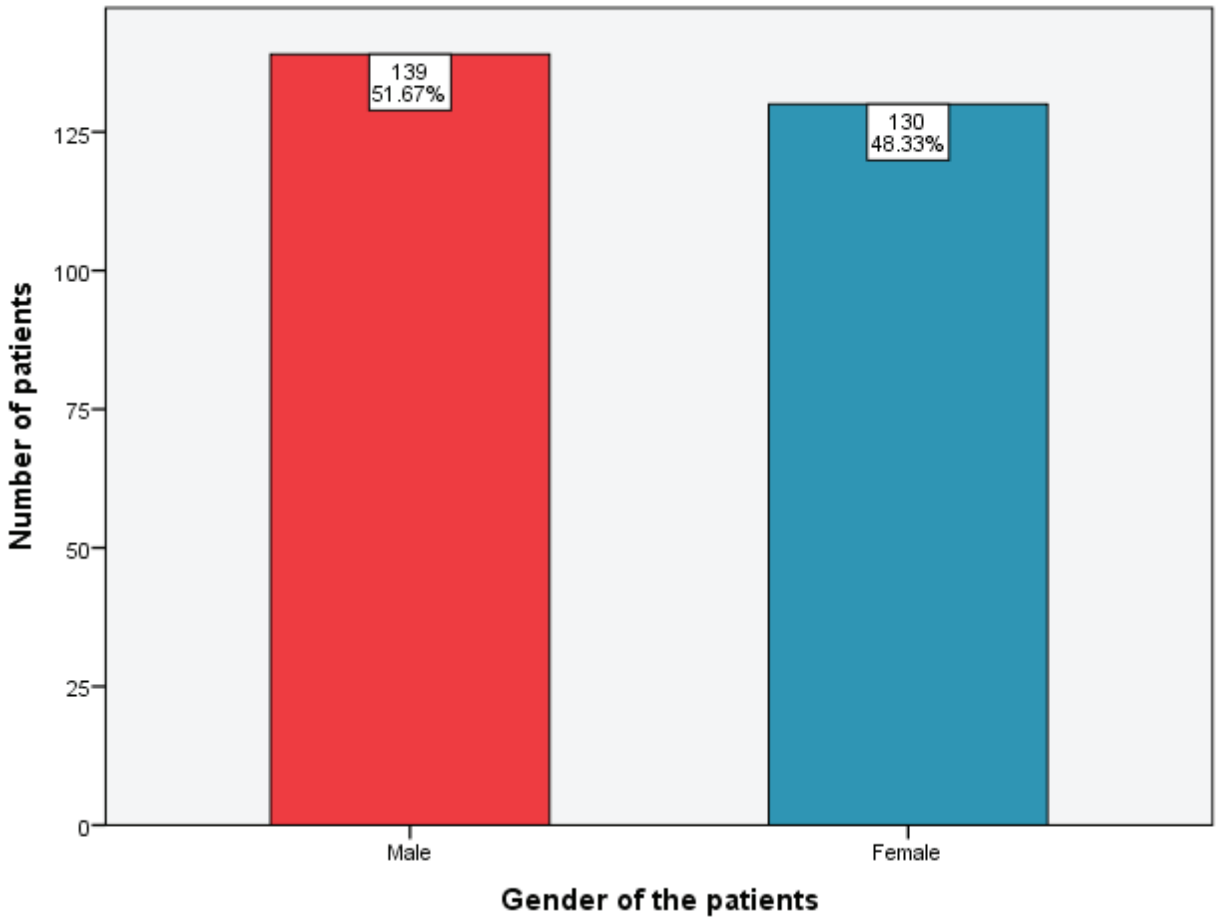


Figure 2: The bar chart depicts the distribution of study subjects based on gender. X axis represents gender and Y axis represents the number of the patient. 52% male (red) and 48% female (blue) respectively.

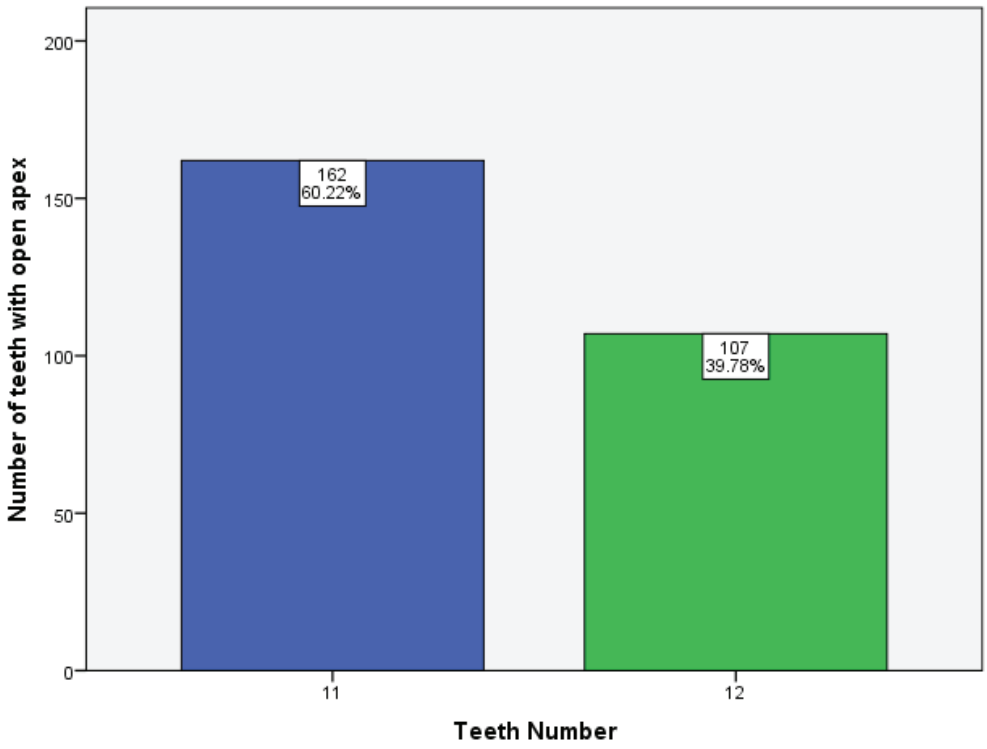


Figure 3: The bar chart depicts the distribution of teeth number which was diagnosed with open apex in Maxillary central incisors.Where the X axis represents the teeth number and Y axis represents the number of teeth with open apex. 60.2% and 39.8% reported with open apex in 11(blue) and 21(green) respectively.

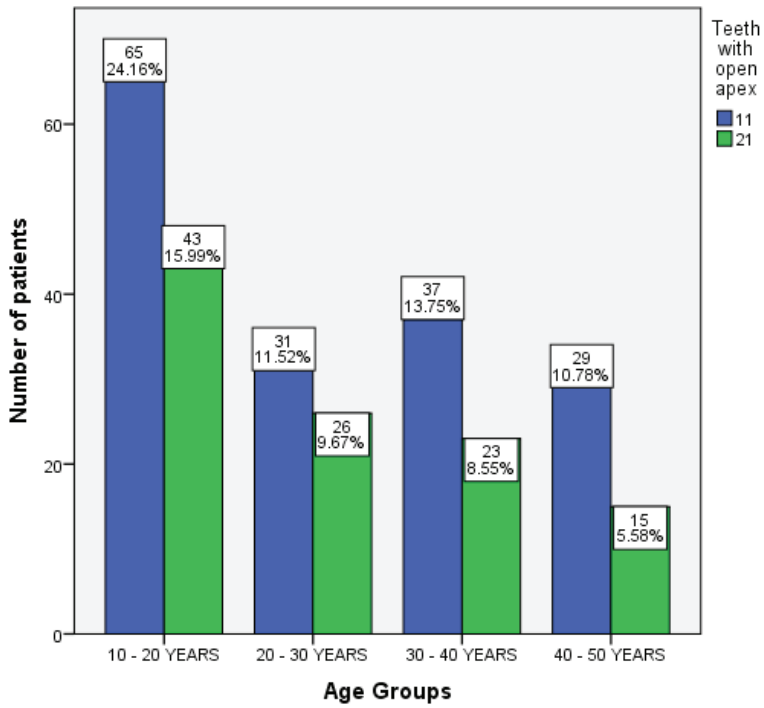


Figure 4: The Bar chart depicts the association between age groups and patients with open apex. Where the X axis represents age groups of the patient and the Y axis represents the number of patients diagnosed with open apex. Blue

denotes teeth number 11 with open apex, green denotes teeth number 21 with open apex. Majority of patients with age group 10-20 years reported a higher incidence of open apex in maxillary central incisors than other age groups. Similarly the statistical analysis also shows significant differences. [Chi square test - Pearson's Chi square value - 14.357, P Value - 0.026 (P< 0.05 which is statistically significant)].

The retrospective study analyses the association of age and gender distribution of patients reported with Open apex in maxillary central incisor. This type of study presents inherent limitations, such as limited study samples, University centric, Limited population covered and their analysis can therefore lead to biased data. Nonetheless, the obtained epidemiological information can be useful to clarify etiologic hypotheses or improve available techniques for management of these cases [20,21]. Immature permanent teeth with necrotic pulps are difficult candidates for conventional root canal fillings.

Immature permanent teeth with open apex have an increased susceptibility to root fractures after treatment [22]. In the past many different treatments have been proposed for immature permanent teeth with necrotic pulps. These include custom fitting of filling materials like gutta percha , paste fills into wide canals, and periapical surgeries[23].

The associated limitations of these procedures diverted interest towards therapies that aimed to induce a calcified barrier in a root with open apex or promote continued root development of an incompletely formed root in teeth with necrotic pulps [24]. These interventions are termed as apexification procedures [25] and were popularised by. Calcium hydroxide was the favoured choice for these procedures. The underlying principle was removal of the necrotic pulp, followed by thorough debridement of the canal and control of infection.

Typically, the clinical and radiographic diagnosis of teeth with open apices and necrotic pulp is based on a dichotomous option (yes / no). However, the term "open apex" could not be grouped as a single entity[26]. Open apices include a wide variety of cellular changes and radicular morphologies throughout all phases of root development[20]. When a tooth remains more immature, it becomes more difficult to mechanically clean the pulpal space, the risk of foreign body extrusion inside the periapical tissues increases due to the lack of apical constriction, and the presence of apical divergent anatomy [26,27].

The results of this study confirmed that the 30-50 years age group was most commonly submitted to treatment (42%) as reported by previous studies [28,29]. Completely formed teeth can suffer alteration in the terminal portion of the root by pathological or iatrogenic factors and develop open apices.

Study Limitations

Very small sample size, Geographic isolation,More parameters if assessed would give a much better quality assessment.

Future Scope

Study needs to be done on large sample size, More grading samples is required and a prospective based study would help assess the long term prognosis.

Conclusion

Within the limits of this study we conclude that the age group of 10 - 20 years had been diagnosed with most number of open apices in Maxillary central incisors. Also the occurrence of open apex was more prevalent in tooth number 11 as compared to 21. More correlations can be studied by doing similar studies in larger populations and studies can be done based on geographical location and races.

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Author Contributions

Balaji. V, acquisition of data, analysis, interpretation of the data and also in drafting the article and revising it critically for important intellectual content.

Surendar Sugumaran, contributed in the concept, contributed in the study design, correction, alignment and supervision.

Sreedevi Dharman,contributed in format and alignment and final approval of the submitted version of manuscript.

Conflict of Interest: There is no conflict of interest to this study.

Source of Funding: Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Choice of Initial Alignment Archwires in Moderate to Severe Crowding in Patients Treated with Damon and MBT Systems: A Retrospective Study

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Abstract

Initial arch wires are the firsts arch wires to be inserted into the fixed appliance at the beginning of orthodontic treatment and are used mainly for the alignment of teeth by correcting crowding and rotations. With a number of different types of orthodontic arch wires available for initial tooth alignment, it is important to understand which wire is most efficient. The purpose of this study was to evaluate the choice of initial alignment archwires in treating patients with moderate to severe crowding with the MBT and Damon appliances. Sample included 50 patients aged 18 years and above,selected from the pretreatment records of the patients reported to the Department of Orthodontics,Saveetha Dental College,Chennai.Results of the study was obtained by evaluating the initial archwires used in case of MBT and Damon appliances. This study revealed that 85.7% out of the 35 MBT cases used 0.014 NiTi as the initial archwire and all of the Damon cases started with 0.013 CuNiTi.The collected data were entered into an Excel sheet(Microsoft Excel 2007) and statistical analysis done. The association between gender and initial alignment archwires in moderate to severe crowding cases was found using Chi-square association test in IBM SPSS software, which showed a statistically not significant difference (p -value >0.05) , hence there is no gender difference in the choice of initial alignment archwires in moderate to severe crowding patients.

Keywords:- *Damon system, Conventional MBT system, moderate to severe crowding, Orthodontic therapy,initial archwire.*

Introduction

Initial archwires are the first arch wires to be used in the fixed appliance for the alignment of teeth by correcting crowding and rotations.With a number of different types of orthodontic archwires available for initial tooth alignment,it is important to understand which wire is most efficient,as well as which wire cause the least amount of root resorption and pain during initial aligning stage of treatment[1]. Over recent years a number of new materials ,various metal alloys,or mixtures of nickel and titanium(NiTi) have been developed which show a range of different properties in the laboratory and which manufacturers claim offer benefits in terms of tooth alignment. Fixed orthodontic appliance treatment uses archwires to exert force upon teeth.Treatment is carried out in stages and selection of appropriate arch wires contribute to the treatment success.There is no arch wire ideal for all stages of fixed appliance treatment. The purpose of this study was to evaluate the choice of initial alignment archwires in moderate to severe crowding cases in patients treated with Damon and conventional MBT(McLaughlin Bennett Trevisi) systems.

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Materials and Methods

Digital records from the pretreatment records of the patients who reported to the Department of Orthodontics, Saveetha Dental College were screened for patients having Moderate to severe crowding . The selection criteria included Arch perimeter - tooth material discrepancy of over 4mm. Patients above the age of 12 years with a Angles Class I or Class II malocclusion were included in the study. Patients with a history of periodontal conditions and patients undergoing retreatment were excluded.Out of 500 patient records screened from a time period of 6 months (September 2019 to February 2020) 50 case records were chosen for data analysis. Patient records were divided into Two groups - Group A undergoing treatment with the MBT appliance and Group B undergoing treatment with Damon Self ligating appliances. The data on initial arch wires used were retrieved from the pretreatment records and tabulated.

Statistical Analysis

The collected data were entered into an Excel sheet(Microsoft Excel 2007) and statistical analysis done. The association between gender and initial alignment archwires in moderate to severe crowding cases was found using Chi-square association test in IBM SPSS software version 23.0. P value was found to be 0.731, which represents an insignificant association.

Results and Discussion

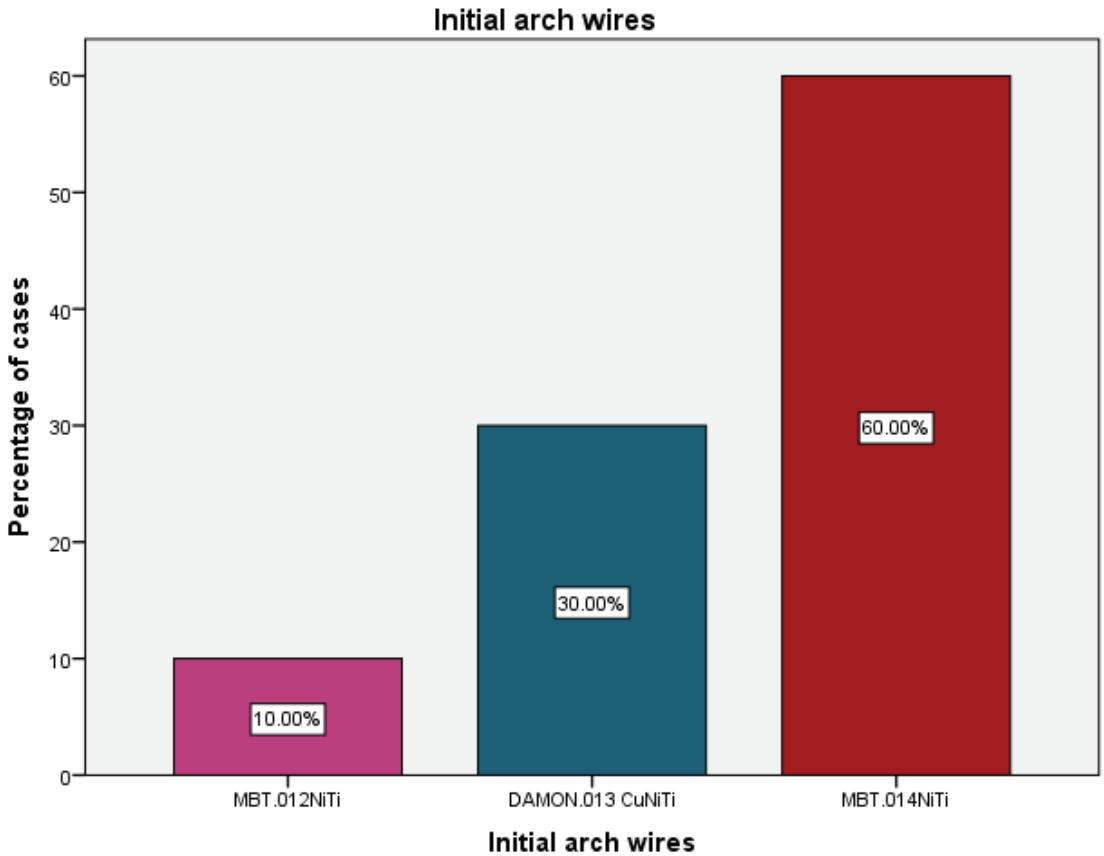


Figure 1: Bar Graph represents the choice of initial alignment archwires in moderate to severe crowding cases. X axis represents initial alignment arch wires and Y axis represents the percentage of cases. Out of the 50 moderate to severe crowding cases, 60 % used MBT0.014 NiTi (red)as the initial archwire and the rest used MBT0.012 NiTi(pink) as initial archwire. All of the Damon cases, that is, 30% of the total crowding cases used Damon0.013 CuNiTi(blue) as initial alignment arch wire. It can be inferred from the above result that MBT 0.014 NiTi was the preferred choice of initial alignment archwire in most of the crowding cases.

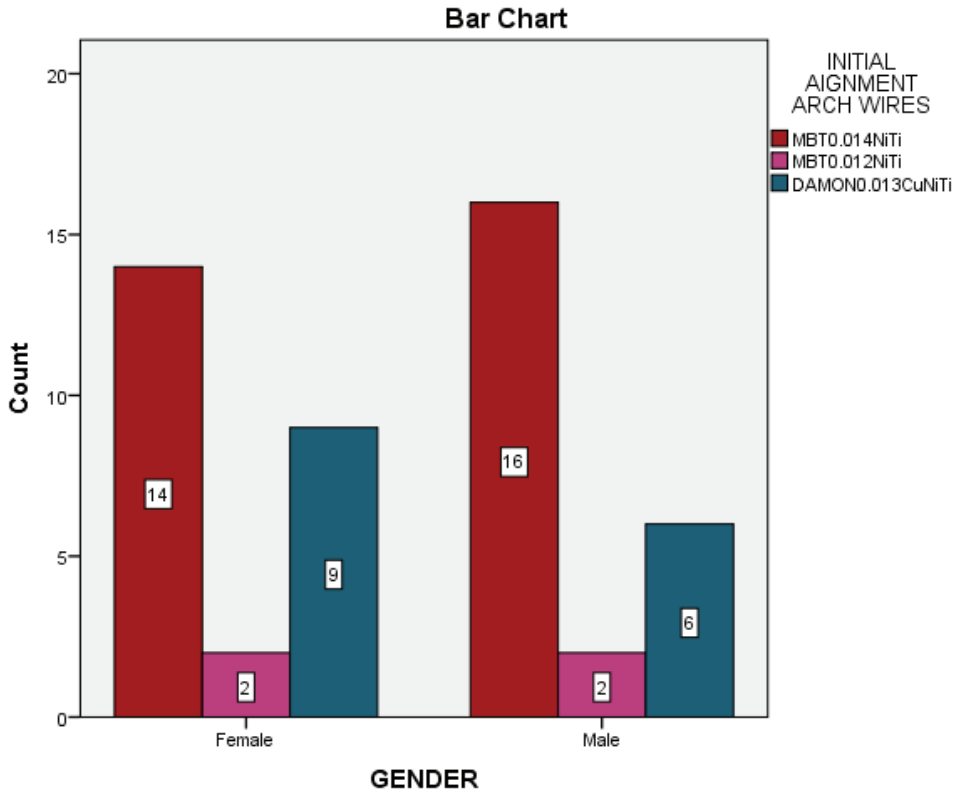


Figure 2. Cluster bar graph represents association between gender and initial alignment archwires in moderate to severe crowding cases.X axis represents gender and Y axis represents initial alignment arch wires. According to the graph among the females, 14 of them were treated with MBT 0.014 NiTi(red), 9 with Damon 0.013 CuNiTi(blue) and among males,16 were treated with MBT 0.014 NiTi(red), 6 with Damon 0.013 CuNiTi(red). A slight predominance of MBT 0.014 NiTi is found in males than in females. However, there is no significant difference found statistically. (Chi-square value- 0.713; df-2; p value=0.700).

Evaluation of the two groups showed most of the moderate to severe crowding cases treated under conventional MBT used 0.014 NiTi as initial archwire and all Damon cases used 0.013 CuNiTi.

Out of the 50 moderate to severe crowding cases; 60 % used 0.014 NiTi as the initial archwire and the rest used 0.012 NiTi as initial arch wire. All of the Damon cases, that is, 30% of the total crowding cases used 0.013 CuNiTi as initial alignment arch wire (fig 1). Association between gender and initial alignment archwires in moderate to severe crowding cases showed a statistically insignificant difference (p value=0.700). A slight predominance of MBT 0.014 NiTi is found in males than in females(fig 2). According to Proffit and Bennett and Mc Laughlin[2] the first phase of treatment deals with leveling and alignment and also correction of vertical and horizontal discrepancies by leveling out the arches, and the initial aligning wires should apply light continuous force. Conventionally,round wires are used for alignment because tightly fitting resilient rectangular arch wires produce back and forth movement of the root apices as the teeth move into alignment[3].To utilize the superelastic property of the NiTi wires,they should be deformed beyond a certain bending angle [4,5].

Results of this study shows that most of the MBT treated cases used 0.014 NiTi as the initial alignment wire; whereas all of the Damon cases used 0.013 CuNiTi as the starting arch wire.Pandis et al[6], found no difference in alleviation of mandibular anterior crowding with copper-nickel-titanium and superelastic NiTi wires.They substantiated that this may be due to differences in loading between laboratory conditions and the oral cavity resulting from free play between archwire and bracket slot. West et al[7] reported better

alignment efficiency with superelastic NiTi in the lower anterior region; which is statistically significant.Jones et al[8] found greater mean improvement in incisal alignment with superelastic NiTi.

The attempts to deform the superelastic wire to attain the superelastic plateau by selecting severe lower anterior crowding cases have led to many occasions of difficulty in engaging the single-stranded superelastic NiTi and of partial ligation with steel ties. Thus superelastic coaxial wires offer the advantage of engaging a relatively large archwire during the initial phase of the treatment with low force delivery. So a greater degree of uprighting, leveling, and rotational control is achieved than with NiTi wires other initial wires.

For the purpose of comparison on alignment and leveling, 0.013 inch NiTi arch wire used throughout the aligning phase of Damon[9]. Miles et al[10] postulated that presence of 'play' between the smaller arch wire dimension and the slot when inch archwire was fitted within 0.028 inch slot depth Damon 2 bracket would allow 8.5 degree of rotational play compared to fully engaged archwire in conventional twin brackets. Scott et al found no difference in initial alignment between Damon 3 and conventional brackets with respect to discomfort.

Previously our team had conducted numerous clinical trials [11–17] and lab animal studies [18–22] and in-vitro students [23–25] over the past 5 years. Now we are focussing on epidemiological surveys. The idea for this survey stemmed from the current interest in self ligating brackets and the rise in their usage in many practises.

Conclusion

This survey evaluated the choice of initial alignment archwires in treating patients with moderate to severe crowding with the MBT and Damon appliances The results of this study showed that the MBT appliance used 0.014 NiTi as initial alignment archwire. All the Damon appliances used 0.013 CuNiTi as the initial alignment archwire. The association between gender and initial alignment archwires in moderate to severe crowding cases was also found to have no significant difference.

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Conflict of Interest: There is no conflict of interest declared.

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Oral Hygiene in Patients with Fixed Partial Dentures

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Abstract

Fixed prosthodontics is the area of prosthodontics focused on permanently attached dental prosthesis. Patients with FPD require regular lifelong professional maintenance with repeated interventions and reinstructions regarding maintenance of proper oral hygiene around fixed prosthesis. The aim of the study is to evaluate the oral hygiene in patients with Fixed Dental Prosthesis. A total 86000 patients data were analysed and the samples were taken from the patients who checked into the hospital from June 2019 to April 2020. A total of 958 patients were taken and analysed for oral hygiene status. Data collection was done from the dental archives of the patient management software system patented by Saveetha dental college.The data was obtained from the category fixed dental prosthesis. The results were analysed using SPSS software version 20 by IBM. The results observed was that the patients with FPD were prevalent to gingival inflammation and periodontal problems. Noticeable lack of awareness about oral hygiene after the fixed partial denture is found among patients wearing Fixed Partial Denture.

Key words: *FPD patients, oral hygiene status, periodontitis, gingivitis.*

Introduction

Fixed prosthodontics is the area of prosthodontics focused on permanently attached dental prosthesis. FPD stands for Fixed Partial Denture. A partial denture that is luted or otherwise securely retained to natural teeth, tooth roots and / or dental implant abutments that furnish the primary support of the prosthesis. Many studies have investigated the influence of the fixed partial dentures (FPD s) on the health of alveolar mucosa underneath pontics [1]. The success of fixed dental prosthesis depends on many factors which should be considered during treatment planning. Tooth decay , gingival inflammation and periodontal disease are quoted as the most common biological complication of the fixed dental prosthesis.[2 3].

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Among this,tooth decay is the most frequent reason for failure of the prosthesis.[4]. Tooth decay of the abutments due to the poor oral hygiene and lack of easy access to the abutment teeth leads to the failure of the fixed partial dentures. There may be a requirement of the Endodontic treatment of the failed abutment teeth which may also lead to the failure of the fixed partial dentures.

In patients with average oral hygiene, plague accumulates more underneath pontics in comparison to axial surfaces of (FDPs) fixed partial dentures. [5]. The loose pontics designs and the spaces between the interproximal areas may be a cause of the plaque accumulation. The bacterial and plaque accumulation may lead to gingival irritations and periodontal breakdowns. Even with the desirable pontic design and favourable material partial dentures, applying the best oral hygiene measures is necessary for removing the bacterial plague and preventing mucosal inflammation [6]. In addition to regular tooth brushing the use of special aids such as superfloss, interdental brush and water flosses can improve the biological maintenance of fixed partial dental prosthesis [7 8]. The dentist also plays a major role in oral hygiene education and patient motivation. [9].

There were also studies done on our institute based on various prosthesis [10 11 12 13 14 15] and studies were also done on various health related oral problems [16 17 18 19 20 21 22 23] and studies also on prevention of oral related problems [24]. In this study we attempt to determine the presence of oral hygiene related diseases in patients with fixed partial dentures visiting The Saveetha dental college.

Materials and Methods:

This study is done under the university setting.The similar characteristics of the study is that it is done with available data under similar ethnicity of the population. The disadvantage of this study can be that the geographic location is similar. This study was approved by the institutional ethics board.Two reviewers are involved in the study. A total 86000 patients data were analysed and the samples were taken from the patients who checked in the clinic from June 2019 to April 2020. Total number of sample data includes 958 patients who were subjected to fixed partial denture treatments.The case sheets were verified with the help of photographic examination and interim procedure notes. To minimise sampling bias we included all the data available and there was no sorting of data done. Internal validity of the study included all these undergoing fixed partial denture treatment. The external validity of the measurement is to find the replication of results in different time periods.

Data collection was done from the dental archives of the patient management software system patented by Saveetha dental college. The data was obtained from the category fixed dental prosthesis.The necessary data like the name, age, gender, related oral hygiene problems were obtained and tabulated.The data was reviewed by the external reviewer.The data was imported to SPSS and the variables were defined. chi square tests were done on the data, obtained using SPSS software version 20 by IBM. The age,gender were independent and the oral hygiene related diseases were dependent variables.

Results and Discussion

The data collected from the patient management software were tabulated in SPSS and the statistics were obtained. out of 958 patients 520 were male patients, 436 were female patients and two ere transgender's. Among the 958 patients 241 patients had good oral hygiene and the remaining 717 patients had oral hygiene related pro. The frequencies calculated and tabulated and depicted as Table- chi square tests were done between the gender and the oral related problems. Oral hygiene was better among the female fixed partial denture wearers than the males (Figure1) with statistically significant difference (Chi square test ; $P = 0.00, P < 0.05$) (Figure 3). Regarding oral hygiene related problems among the FPD patients, patients aged between 20-40 years had poorer oral hygiene than the other age groups (Figure 2) with statistically significant difference (Chi square test ; $P = 0.00, P < 0.05$) (Figure 4). The most commonly prevalent oral hygiene problem was generalised chronic gingivitis. The patients were more prone to generalised chronic periodontitis than the localised chronic periodontitis.

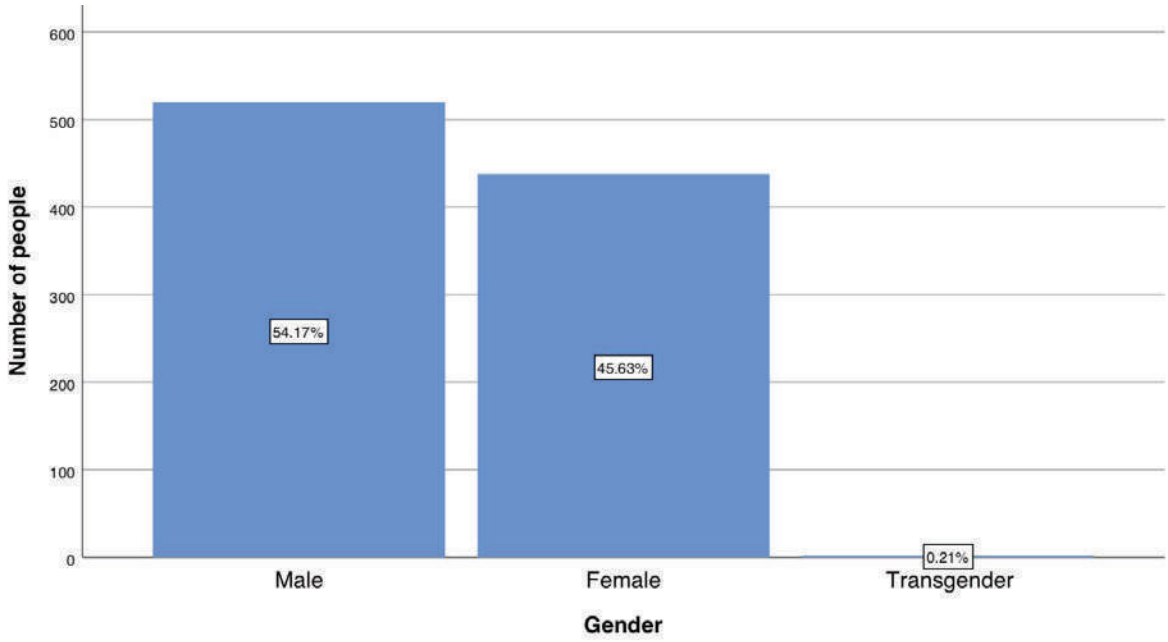


Figure1: Bar chart depicting the gender wise frequency distribution of oral hygiene related problems among the FPD patients. X-axis represents the gender and Y axis represents the number of individuals with oral hygiene related problems among the FPD patients. Oral hygiene was better among the female fixed partial denture wearers than the males.

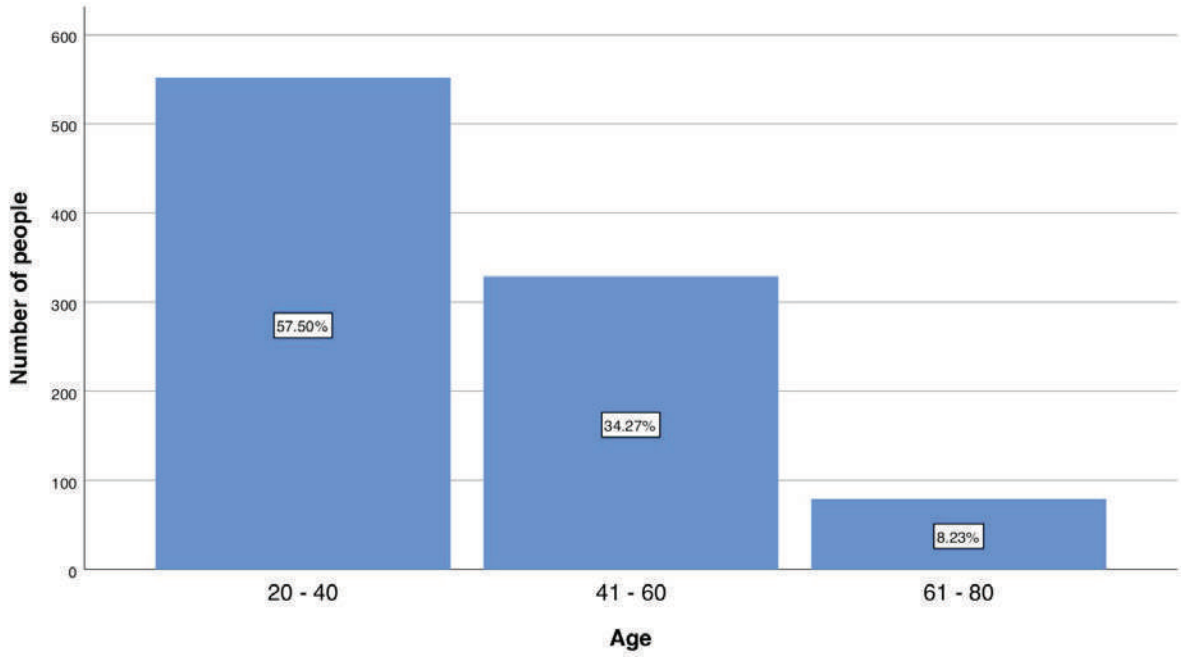


Figure 2: Bar chart depicting the age wise frequency distribution of oral hygiene related problems among the FPD patients. X-axis represents the age and Y axis represents the number of individuals with oral hygiene related problems among the FPD patients. Patients aged between 20-40 years had poorer oral hygiene than the other age groups.

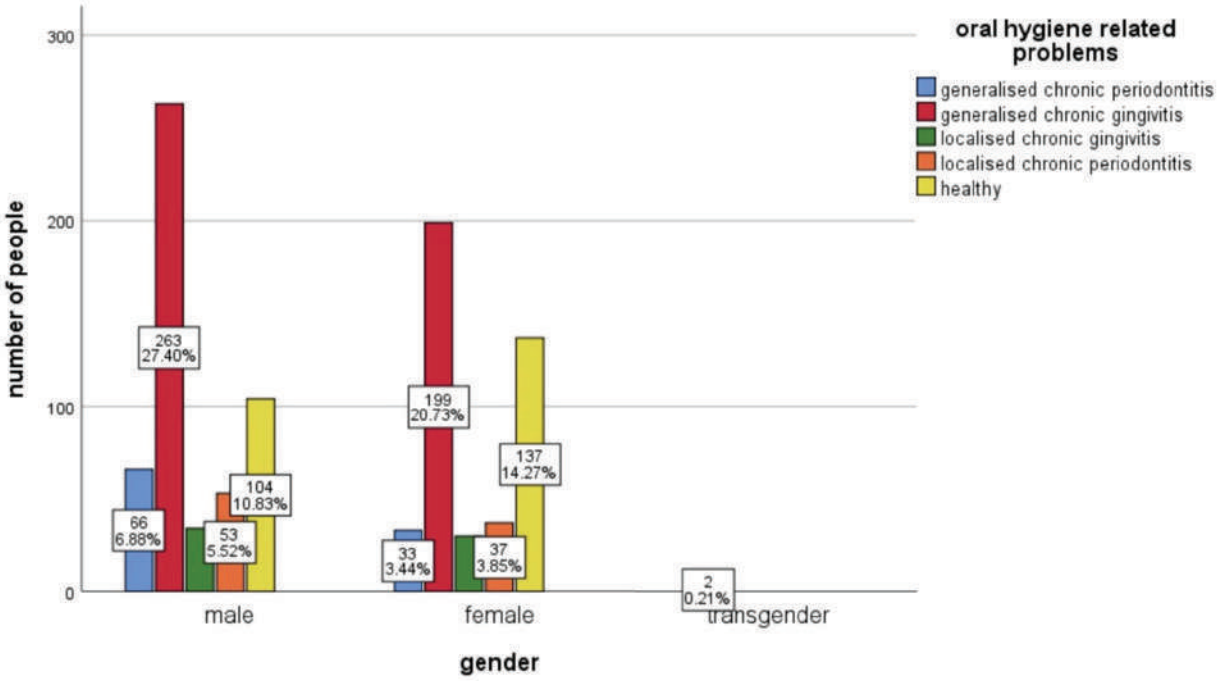


Figure 3: The graph shows the association between the gender and the oral hygiene related problems among the FPD patients. X-axis represents the gender and Y-axis represents the number of people with the oral hygiene related problems among the FPD patients. Oral hygiene was better among the female fixed partial denture wearers than the males with statistically significant difference (Chi square test ; P = 0.00,P < 0.05)

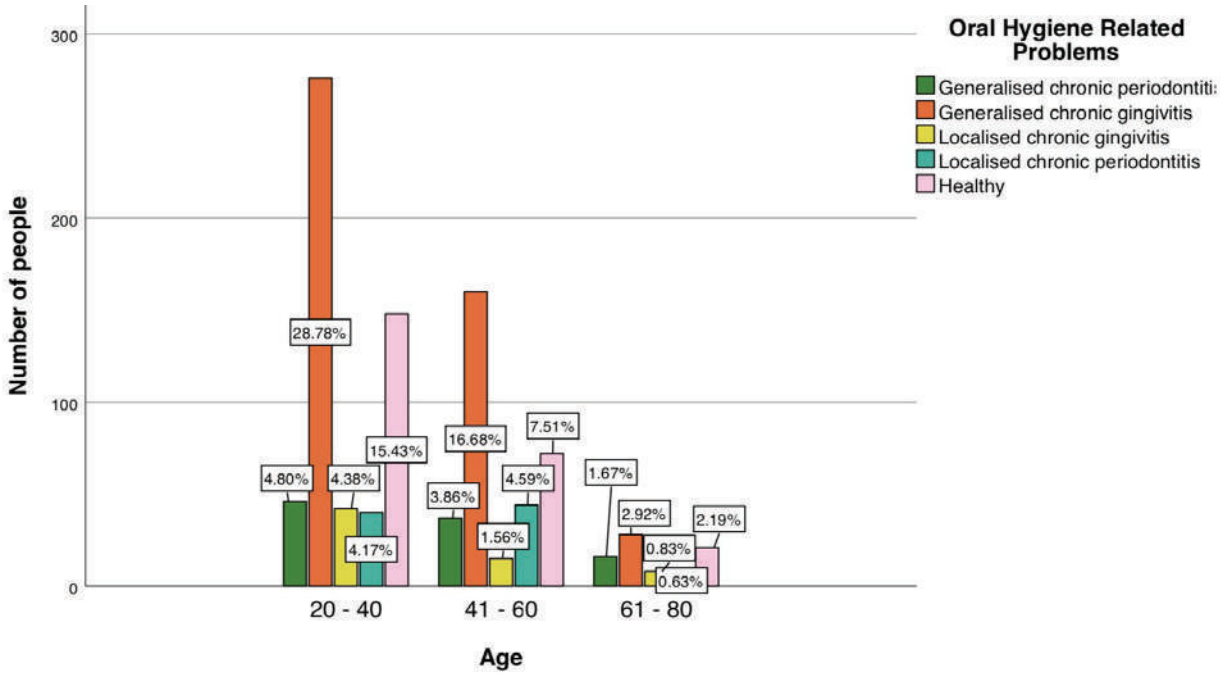


Figure 4: The graph shows the association between the age and the oral hygiene related problems among the FPD patients. X-axis represents the age groups and Y-axis represents the number of people with the oral hygiene related problems among the FPD patients. Patients aged between 20-40 years had poorer oral hygiene than the other age groups with statistically significant difference(Chi square test ; P = 0.00,P < 0.05)

A healthy oral hygiene practice is necessary to maintain a good oral hygiene in a fixed denture environment. From this the study proves that the FPD patients are prone to generalised chronic gingivitis. KC Basnyat states that poor oral hygiene leads to negative impact on gingiva.[1]. Helle Tolbert states that poor oral hygiene leads to gingival inflammation.[6] Thus the previous literature gives the same evidence. The influence of the restorations lead to plaque formation. This may be due to plaque accumulation under the fixed partial dental prosthesis. The plaque accumulation may be due to the improper care taken by the patients to maintain the oral hygiene. The patients must follow healthy oral hygiene practices to stop the accumulation of plaque and prevent the gingival inflammation. Our study states that the FPD restorations due to poor oral hygiene influence periodontal health. Keht.L. and DA.Felton has stated in their previous literature that poor oral hygiene increases probing depth and provokes periodontal inflammation.[25] [26]. This may be because of the marginal discrepancies. The marginal discrepancies may be due to the bacterial accumulation along with the plaque accumulation on the micro gaps between the margins of the fixed partial dental prosthesis. A proper brushing technique and use of mouth washes should be followed strictly as they help in prevention of the bacterial accumulations. The patients also should take up regular checkups even if they don't have a major problem regarding the fixed partial dentures. The dentists also play a major role in the oral hygiene status of the patients having fixed partial dentures. The dentists should advise the importance of maintaining a proper healthy oral hygiene to the patients. The dentists also should mention the methods by which the patients can prevent the bacterial accumulation and maintain a healthy oral hygiene. This study also reminds the dentist of the role in maintaining proper oral hygiene in patients with fixed partial dentures.

The limitations of the study includes relatively smaller data collection and doesn't represent ethinic groups.The future scope of the study includes awareness on oral hygiene in FPD patients and the clinical implications and importance of implementing preventive procedures.

Conclusion

Oral hygiene is very important in all patients with fixed partial dentures within the limitations of the current. Study. We can conclude that there is a noticeable lack of awareness among FPD patients regarding the specific measures needed to maintain proper oral hygiene underneath FPD. Dentists should also educate their patients and advise them to maintain proper oral hygiene. The dentists also should mention the methods by which the patients can prevent the bacterial accumulation and maintain a healthy oral hygiene.

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Conflict of Interest: Nil

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Analysis Into Extra Oral Features of Completely Edentulus Patients Pre and Post Complete Denture Treatment

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Abstract

Background : Edentulism has been a serious public health problem in industrialized countries due to the age of the population , life quality and nutrition intake are impaired . Which is restored by the use of complete dentures. **Aim** : To assess extra oral features of complete edentulous patients pre and post complete denture treatment .**Materials and method** :Data of complete denture patients was screened and collected from the dental archives database and assessed the documentation evidences.**Results :**According to the study in Complete denture fabrication BPS showed aesthetically better post operative extra oral features compared to the conventional method (p value < 0.05 significant [chi-square test]).**Conclusion :**Within the limitation of the study ,it is evident that BPS has shown better post operative aesthetic results compared to that of the pre op than the conventional CD. The extra oral features of a complete denture patient are enhanced while using a BPS type of a complete denture.

Keyword : *Aesthetic; extra oral features; nasolabial fold; facial wrinkles; lip support; BPS; conventional.*

Introduction

Edentulism has been a serious public health problem in industrialized countries due to population aging and in developing countries due to poor oral care.[1] The life quality and nutrition intake are impacted due to edentulism.[2] Complete dentures are the main choice for edentulous patients. Despite an anticipated decrease in the age-specific rates of edentulism, the demand for complete dentures will continuously increase in the next decades.[3–5]

Currently, complete dentures are mainly designed and fabricated using conventional methods, which involve a broad series of clinical and laboratory procedures.[6] To obtain a complete denture , edentulous patients have to makeup for a minimum of 5 sightings, including preliminary impressions, final impressions, recording jaw relations, trial placement of wax denture, and placement/insertion of complete dentures. These clinical and laboratory procedures are performed to achieve good rehabilitation. But it is very challenging to ensure the quality for the designing and fabrication of dentures due to its complex fabrication techniques.

Achieving aesthetics is a foremost objective in complete denture fabrication.[7–9] The overall appearance of the denture is more important , but only to the extent that it contributes to an aesthetic appearance and function of the face and lips. It is essential to identify the patients expectations and to explain to him or her the limitations and possibilities of fabrication of aesthetic and functional satisfactory restorations .[10] Patients' appreciation of their conventional complete dentures might be affected by the quality of the dentures.[11]

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A pleasant smile is an expression of joy. It is extraordinarily a human signal that is not normal for the scowl of lower primates.[12] It is dentist's obligation to preserve, enhance or create a pleasing smile through dental treatment without impairing function.[13] The dentition and how it influences the formation of a pleasing smile is extremely important in the emotional development of the individual.[14] Characterized dentures plays a vital role in improving one's confidence.[15]

The Biofunctional Prosthetic System (BPS) is used for the treatment of edentulous and partially edentulous patients. Today, the use of clinical protocols and simple, reliable treatment methods that can be easily adjusted to the needs of the patient is of prime importance and usually preferred by both patients and dentists . [16]

It is a system consisting of a series of steps from impression making, bite registration, Set up of denture teeth, polymerization and finishing to delivery that are carried out according to the pre defined BPS procedures using Ivoclar Vivadent's devices and materials. The BPS system provides the dentist a simple, time saving procedure based on gnathological principles of the masticatory muscles . All the steps, procedures and materials are coordinated with each other to achieve ease of denture construction. [17]

Complete denture fabrication must be done considering both the extraoral (facial muscle) and intra oral factors . [18,19] Consider the periodontal status of the patient and explore for possibilities of implant which gives better aesthetic results on a loger run [20,21,22,23] . Other modified complete dentures such as tooth supported or implant supported over dentures, magnetic retainer for better retention can also be made use for a better aesthetics .[24,25,26,27,28]

There are various studies supporting the denture maintenance and the infection that can possibly be caused due to the lack of maintenance .[29,30,31,32]

Most of the literature takes into account only the oral health and satisfaction perceived by the patient's prosthetic carrier, however, an investigation of the causes and major failure factors is necessary . Conversely, a small number of researchers sought a relationship between prosthetic quality and patient satisfaction.

This research is done to assess the extra oral features of edentulus patient pre and post treatment of complete denture (BPS/Conventional).

Materials and Method

The present study is done by screening the patients digital case sheets. The search for digital case sheets was from june 2019 to february 2020. The records searched close to 4068 reporting to clinics with the complaint of multiple missing teeth in upper and lower arch. The search was narrowed down for edentulous condition in upper and lower arch. The search led to 466 patients undergoing complete denture treatments. Further the digital documentation was evaluated for extra oral facial details at the diagnostic stage and post treatment stage. The details were screened by 2 expert reviewers. Those undergoing complete denture treatments and with adequate documentation details were involved in the study. All the complete denture patients were taken into the study with no particular age limit . Three facial features were taken into consideration for the study both pre op and post operative as follows and measured accordingly : nasolabial fold (0-prominent , 1- not so prominent), Adequate lip support (1-adequate , 0- more or less), Facial wrinkles (0- present 1- absence) .This study was done to estimate- extraoral feature of complete denture patients undergone either BPS or conventional technique denture construction. The data was screened and relevant data was subjected to statistical analysis. Chi square test is used for statistics . Statistics software used is SPSS version 26 by IBM. Independent variables are age, gender, extra oral changes ,different denture construction methods.

Results & Discussion

According to the study in Complete denture fabrication BPS showed aesthetically better post operative extra oral features compared to the conventional method. On assessment of complete denture and nasolabial fold post operative shows that the nasolabial fold is not so prominent in BPS (58.8%) - compared to conventional method.(Graph 1) ($p<0.05$ significant). On assessment of complete denture and facial wrinkles post operative , where the facial wrinkles were measured only on lower 2/3rd as the complete denture affects the facial change only on the lower third of the facial creases on which BPS(60.8%) showed better results compared to

conventional method.(Graph 2) (p>0.05 Insignificant). On assessment of complete denture and adequate lip support postoperative where the post operative of adequate lip support in BPS(88.2%) showed better results compared to conventional method.(Graph 3)(p value < 0.05 significant [chi-square test]).

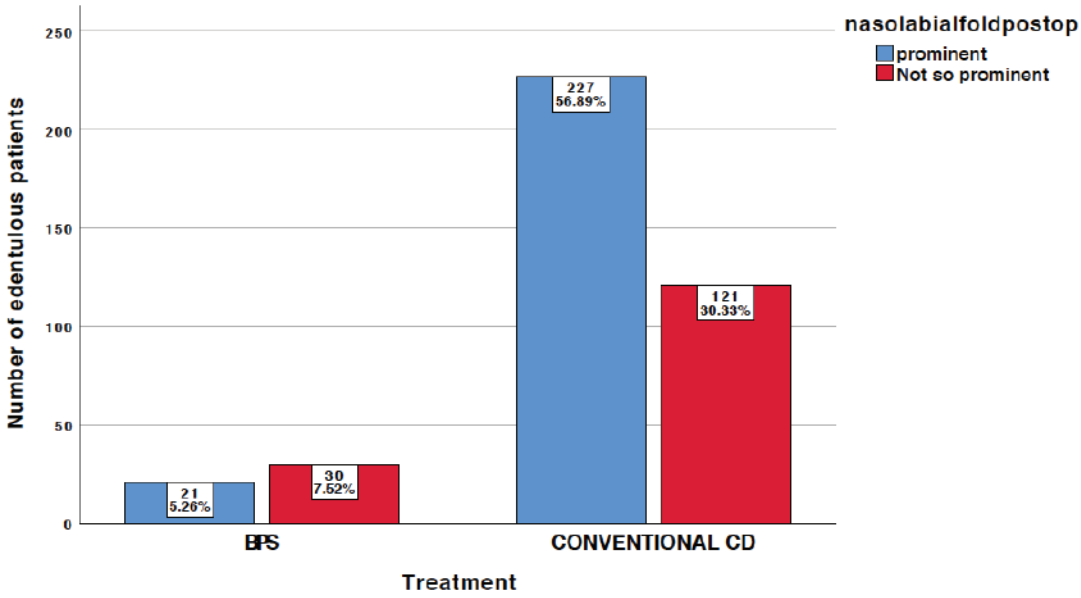
Facial ageing is an inevitable fact of life. Our genes lead our body through a series of changes ranging from growth and development to maturation, ageing and finally death. This process appears to be genetically controlled but also influenced by lifestyle and environment.[33]

The cheeks are less mobile than the lips and are supported on three sides by the zygoma above, the mandible below, and the parotid gland overlying the masseter muscle in the posterior region . Additional is provided by the subcutaneous fat and buccal fat tissues , and this support is responsible for the soft, rounded contours of the cheeks in the lower third of the face.[34]

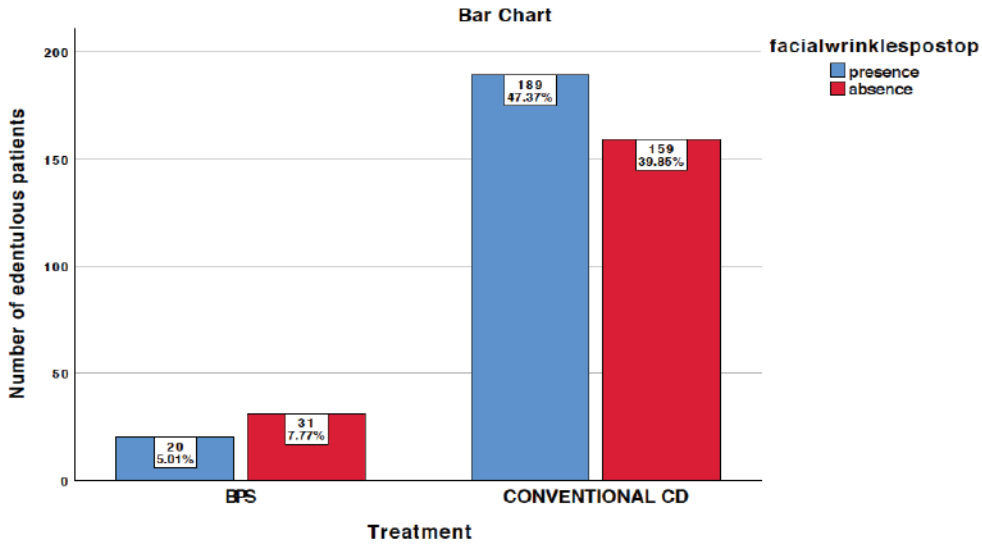
The buccal fat pads persist for a longer period compared to subcutaneous fat, even in patients with extreme thin and weak patients. The anterior parts of the cheeks are supported by the muscular framework converging into the modioli and are subject to more changes . On loss of posterior teeth, the cheek tends to collapse on varying degrees and moving medially to the laterally expanding tongue. The loss of anterior teeth and the subsequent loss of vertical dimension affects the cheek stability. Losses of subcutaneous fat and elasticity of connective tissue produces hollow cheeks. [35,36] As said earlier, loss of all the teeth is a major and an irreversible loss for the patients . So there is much concern that in completely edentulus patients , restoring aesthetics is a must by considering the extraoral features such as nasolabial fold ,lip support and facial wrinkle both in the conventional and BPS method of complete denture fabrication. It is a dentist's duty to restore the aesthetics and give confidence to the patient .

In an edentulus patients due to Complete loss of teeth the extra oral anatomical muscles of facial expressions loses their support and tonicity where the complete denture (BPS /conventional) will a important role in provide support to the facial muscles .In of the aesthetic cases BPS dentures are preferred as it has much lesser sitting and precision on fabrication aiding the better quality and confidence that the patients require.[37] In various other studies they have suggested to use radiographs to recorded changes of the facial muscle tension and change in the vertical dimension for edentulus patients. Electromyography which is excellent for diagnostic aid of muscle function and a reproducible method in detecting changes in electrical muscle activity has been suggested to be used to identify changes in isometric muscle tension.[38–40].

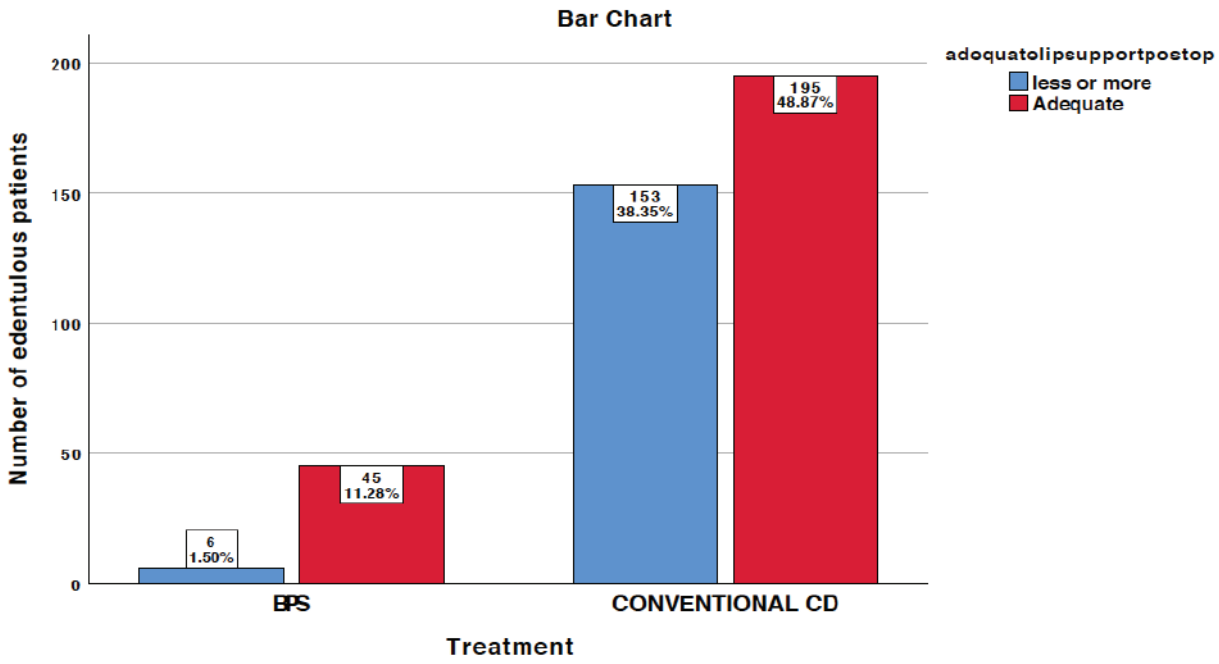
In edentulus patients basically they have difficulties with mastication and speech which is resolved by denture that gives better lip support and BPS is preferred.[41] Facial wrinkles were better resolved when a BPS denture was used compared to a conventional as far as the denture meets most exacting requirements and accuracy.[42] Not all cases are suitable for a BPS denture even though they have better stability and retention ie; better suction effect,conventional cd is preferred which can also show good results .[43] When we consider rehabilitation in a complete denture patient to achieve a pleasant smile aesthetics plays a vital role. [44,45] Future scope is a prospective study of the denture and the patient satisfactory level on a long term basis can be evaluated and can make use of Electromyography for a much precise value of the muscle activity with a review both pre op and post operative of denture placement review each month.



Graph 1 -The graph shows the assessment of nasolabial fold in post operative of complete denture patient where the x axis shows the type of complete denture treatment (BPS/Conventional denture) and the y axis shows the sample size of edentulus patients . Blue colour graph in the bar chart represents the prominence of the nasolabial fold and the red colour represents that the nasolabial fold is not so prominent. BPS type of denture fabrication showed better nasolabial fold postoperative results compared to the conventional method. Similarly, Chi - square test of association between complete denture treatment and adequate lip support post operative shows statistically significant ($p < 0.05$) where pearson chi-square = 10.941 and p value = 0.001.



Graph 2 -The graph shows the assessment of facial wrinkles in post operative of complete denture patient as presence or absence of facial wrinkles, where the x axis shows the type of complete denture treatment (BPS/ Conventional denture) and the y axis shows the sample size.Blue colour graph in the bar chart represents the presence of facial wrinkles and the red colour represents that the absence of facial wrinkles. BPS type of denture fabrication showed better facial wrinkle post operative results compared to the conventional method. However, there it is not significant statistically. Chi - square test of association between complete denture treatment and adequate lip support post operative shows statistically not significant ($p > 0.05$) where pearson chi-square =5.663 and p value =0.057.



Graph 3 - The graph shows the assessment of adequate lip support in post operative of complete denture patient as adequate / less or more than adequate ,where the x axis shows the type of complete denture treatment (BPS/Conventional denture) and the y axis shows the sample size. Blue colour graph in the bar chart represents the less or more than the adequate lip support and the red colour represents adequate lip support. BPS type of denture fabrication showed better lip support post operative results compared to the conventional method. Similarly, Chi - square test of association between complete denture treatment and adequate lip support post operative shows statistically significant ($p < 0.05$) where pearson chi-square = 19.242 and p value = 0.000

Conclusion

Within the limitations of the study it is evident that BPS has shown better postoperative aesthetic results compared to that of the pre op than the conventional CD. The extra oral features such as nasolabial fold and lip support are enhanced while using a BPS type of a complete denture.

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Conflict of Interest: None to declare.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Hypertension and Complete Denture Therapy - A Retrospective Study

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Abstract

A completely edentulous state is when the patient is in a state of complete tooth loss. In the current world, an increasing demand for complete denture prosthesis is also seen. The lifestyle, food habits etc are thought to be major factors that are influencing the oral health of the current generation. Marked evidence has been cited between the relationship of various systemic diseases like diabetes and completely edentulous state. Hypertension is the state of elevated blood pressure. An evident association between hypertension and oral diseases has been established previously. The aim of our study was to find the association between hypertension and a completely edentulous state. The study was performed in a university setting, it reviewed patient records and analysed the data of 86000 patients between June 2019 and March 2020. A total of about 450 entries were selected. Of this after removing multiple entries a total of 372 patients data were obtained. The collected data were compiled and tabulated using Microsoft Excel, then reviewed and exported to SPSS for performing statistical analysis. In the study a total of 5 parameters were considered, but only for one parameter a statistically significant data was found, and this was the demographic profile of the completely edentulous patients, p value was found to be 0.0052 (p =0.005) hence proving the data to be significant. For the rest of the parameters the study considered, a statistically significant data was not obtained, but the data has remarkable clinical significance. In the study, completely edentulous male population were, 56%, the female population represented 43%. 27% of the completely edentulous patients in the study were hypertensive and that is not a negligible count. Within the limitations of the study, it can be stated that there is a relation between hypertension and a completely edentulous state.

Keywords*: Hypertension; systemic conditions; tooth loss; complete edentulous state.*

Introduction

The size of the geriatric segment of the population is expanding in the emerging nations as the latter undergo a demographic transition, with a collateral increase in life expectancy. As a matter of fact, it is approximated that by the year 2025 the majority of the elderly people worldwide will be residing in developing countries [1,2]. Developing countries are thus likely to face an enormous burden of chronic non-communicable diseases in the near future. Of these diseases, hypertension is one of the most important treatable causes of mortality and morbidity in the elderly population [3], and accounts for a large proportion of cardiovascular diseases in the old aged population [4–6]. There are findings that provide direct evidence of an increasing burden of hypertension among the elderly population in the Indian subcontinent. A significant relationship has been obtained in regards to Hypertension, BMI, diabetes mellitus, and physical activities on a lot of studies [7–11].

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Hypertension is the synonym for high blood pressure. The normal blood pressure is below 120/80 mm Hg and above 90/60 mm Hg in an adult. When the systolic pressure is greater than 120 and the diastolic pressure is less than 80 mm Hg it is considered as the high blood pressure state. On a long run hypertension will lead to very severe health complications also it will increase the risk of heart diseases, stroke and sometimes can even lead to death of the patient. Since hypertension is considered as a lifestyle disorder, the initial line of treatment is always lifestyle modification, like regular exercise, following a proper diet stress reduction etc.

Complete edentulism can be considered as an ultimate result of various physiologic and pathologic processes that involve systemic conditions, nutritional factors and other patient-related factors. An early tooth loss can be indicative of various oral diseases or poor oral hygiene. It is proved that in 70% of the cases the early tooth loss is due to various periodontal diseases. The other reasons can be cavities or dental caries, high sucrose rich sugar intake, other systemic diseases, and personnel habits.

A marked relationship between these periodontal diseases and systemic complications has been cited. Edentulism is an enervating and irreversible condition and it has been described as the final marker of the disease burden of oral health [12]. This often affects the patient's approach to a pleasant life since it has an impact on the morale of the patient. The prevalence of tooth loss completely reduced over the last decade, but still persisting as a major disease around the globe particularly in older individuals [13–17]. The nutritional factors of edentulism are complex due to the wide range of factors that influence food initiative, nutritional status, acute and chronic disease, and financial limitations [18–24].

Many clinicians have reported that superintending hypertensive patients can be troublesome when it comes to the oral complications, dental therapy and emergency care. It has been reported that one third of clinical hypertension is not diagnosed. Some reports even suggest that though more than 70% of the patients are aware of their hypertensive state, only thirty to fifty percent among them seek medical care, and out of this less than twenty percent of the patients gain control over the hypertensive state [25]. The common drugs prescribed for hypertensive patients are thiazide diuretics, loop diuretics, angiotensin converting enzyme inhibitors. These medications are well known for their side effect, xerostomia (dry mouth) which is due to hyposalivation. A reduction in saliva secretion will result in incidence of caries, candidiasis, oral burning syndrome etc [26]. The xerostomia will in turn result in hindering the retention and stability of complete denture. So it is important to note that, for hypertensive patients who are undergoing complete denture therapy, for providing a successful treatment, artificial salivary lubricants or denture adhesives must be advised [27,28]. There are studies which have cited a higher survival rate of implant supported complete dentures in hypertensive patients who were undergoing antihypertensive therapy. The reason for this was found to be the favourable effect of the anti hypertensives in bone remodeling and as well as the lower risk of bone fracture that these medications impart [29]. The aim of this study was to determine and assess the association between hypertension and the completely edentulous state.

Materials and Methods

The study was conducted in a university setting. Being done with the entries of one university the pros regarding the study were that the data were already available and the data belonged to individuals with similar ethnicity. The cons of the study were geographic limitations, locations, and also the trends of other locations were not assessed.

The study is a retrospective cross-sectional study and the data were collected within a time period of June 2019 to March 2020. There were two reviewers for the study. Ethical approval was provided by the University ethical committee. A total of four fifty entries were assessed and after deleting multiple entries there were a total of three seventy-two entries. To ensure the validity of the data the patients were contacted through telephone. The data was tabulated by using Microsoft Excel software. It was then exported to SPSS for statistical analysis. The analysis was conducted by the Chi-square test. In the study, the independent variables were:- the ethnicity, age, gender, and the dependent variables were hypertensive, and complete denture,reason for a complete denture.

Result and Discussion

The way of living or behavioral components critically is believed to determine the level of blood pressure in individuals and the prevalence of hypertension in the population.

56% of the total study population were males and 43.01% of the respondents were found to be females. The youngest complete denture patient reported to the institution was in the age range of 31 to 40, hence we included the age range as 31 to 40, 41 to 50, 51 to 60, 61 to 70 and beyond 70. In this we found the highest number of patients in the age range of 61 to 70, and this was 37.63%. It was followed by the individuals in an age range of 51 to 60 years, and this represented 31.18%. This was followed by the individuals belonging to 41 to 50 years. (Figure 1)

In our total population as the figure 2 depicts, 72.045 of the total respondents were devoid of hypertension, and 27.96% were found to be hypertensive patients .(Figure 2)

In this female completely edentulous patients tend to show a slight predominance in having hypertension, that is 74% of the total female completely edentulous patients were reported to have hypertension and 25% of the female did not have hypertension. 70% of the male patients were reported to have hypertension and 29% of the patients did not have hypertension.(Figure 3)

Here we can observe that the patients in the age group of 31 to 40 were having the highest percent of hypertensive patients, with 91.67%. the lowest percent of completely edentulous hypertensive patients were found to be in the age group of 61 to 70.(Figure 4)

Hence in our study, we found that within the completely edentulous patients females had predominance. But in the general population hypertension has shown a male predominance.

In the general population, some studies have depicted that women have been found to be hypertensive when compared to men [30–36]. While at the same time there are other studies that have shown that women have better or equal hypertension control than men [37,38]. Another fact found in the study was the prevalence of hypertension based on the age groups, is that the maximum number of hypertensive patients were reported to be belonging to an age group of 61 to 70 in our study (Figure 4). 32% of the total population within an age range of 61 to 70 is having hypertension, and another study has stated 46% of individuals above 65 years of age being hypertensive in the general population justifying our findings [39]. Various studies have proved a remarkable relation between oral health and its possible effects on systemic health [40]. It has been found that even after being diagnosed with hypertension, if the patients oral health is not maintained properly it might lead to the deterioration in the effectiveness of the medication that the patient is undergoing in regards with hypertension, also it will increase the risk of complications which is associated with hypertension. Some findings even disclosed the fact that the individuals with healthier gums have lower blood pressure and they responded better to blood pressure lowering medications, in contrast with the individuals who have periodontal diseases. Precisely, the patients with periodontal disease were 20 percent improbable to reach healthy blood pressure ranges when compared to patients with good oral health [41]. Hence, this relationship of periodontal disease and the dietary habits to hypertension, is paving a path for us to correlate it with the increased chances of tooth loss in patients with hypertension.

Graphs

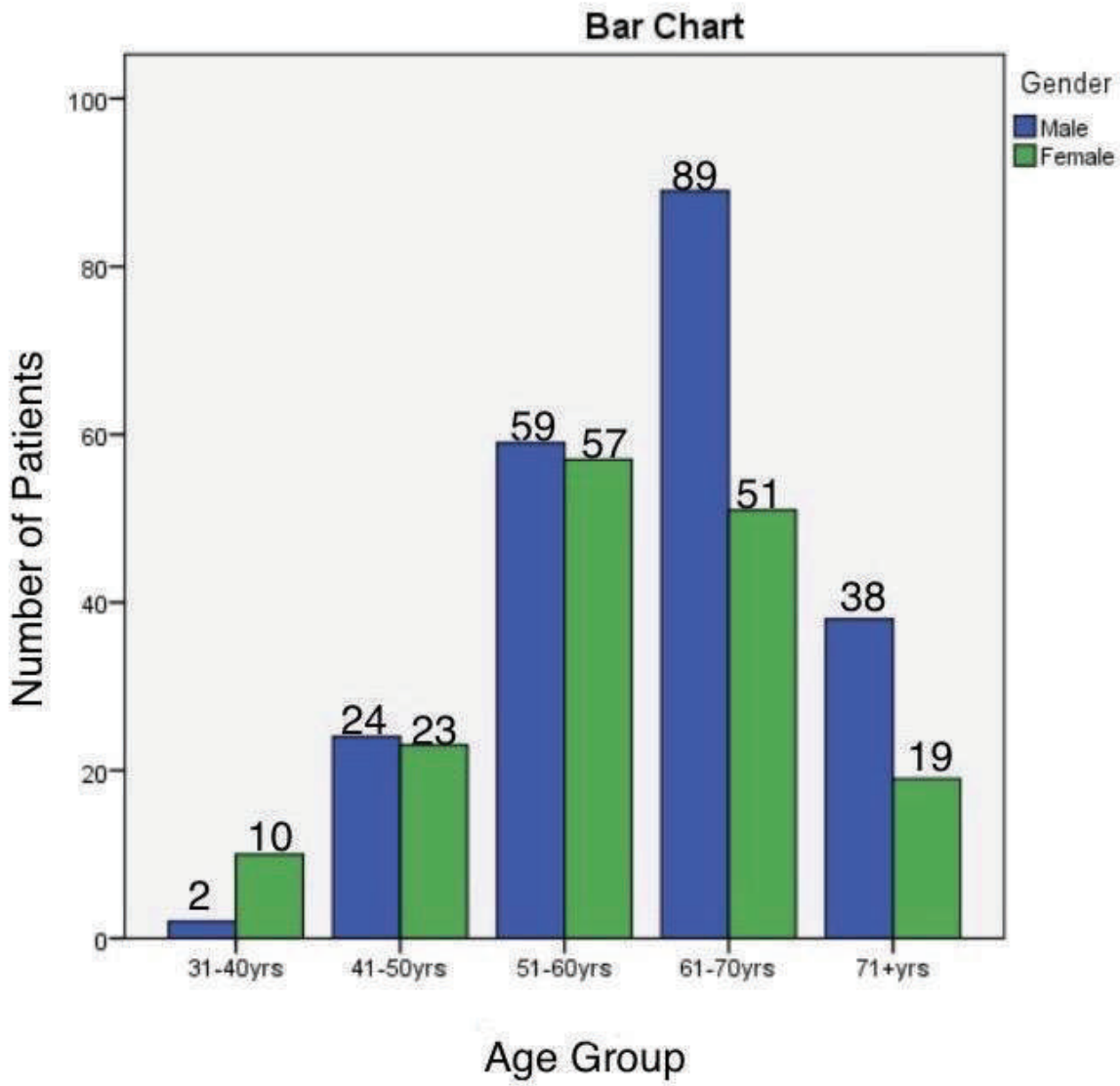


Figure 1 this is a bar chart showing the association between gender and age group of completely edentulous patients; where X axis shows the age groups which are 31 to 40, 41 to 50, 51 to 60, 61 to 70, 71+, Y axis shows the frequency of edentulousness, blue colour represents male, green represents females. A male predominance was seen when compared to the female population in having a completely edentulous state, and the maximum number of patients were seen in the age group of 61 to 70 years. Chi square test was done, Pearson Chi square value: 15.062, DF: 4, p value = 0.005, p=0.005 found to be statistically significant .

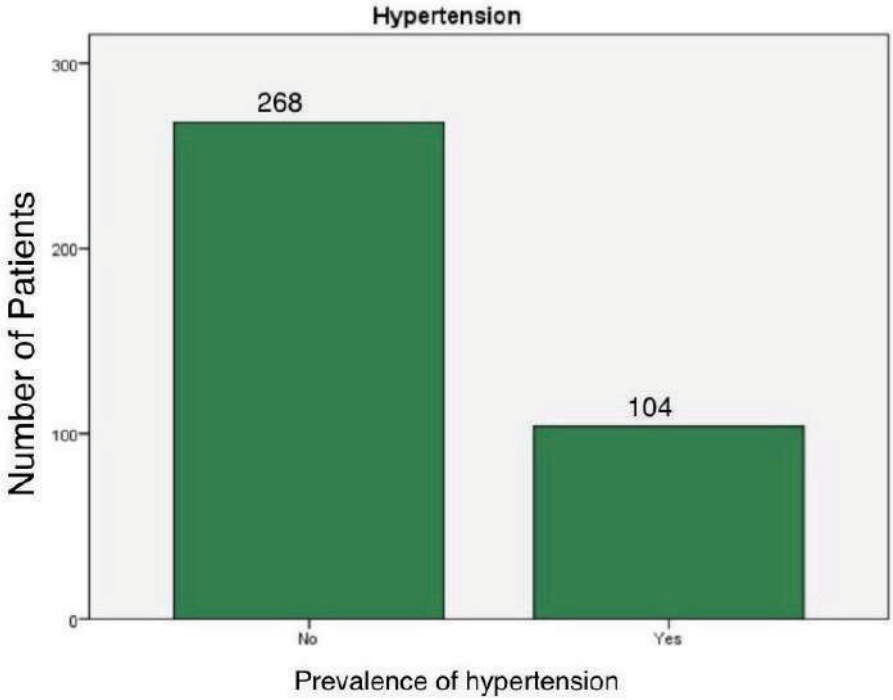


Figure 2 The graph shows the prevalence of hypertension among completely edentulous patients. 104 patients (28%) of the total population were found to have hypertension and 268 patients (72%) were devoid of hypertension.

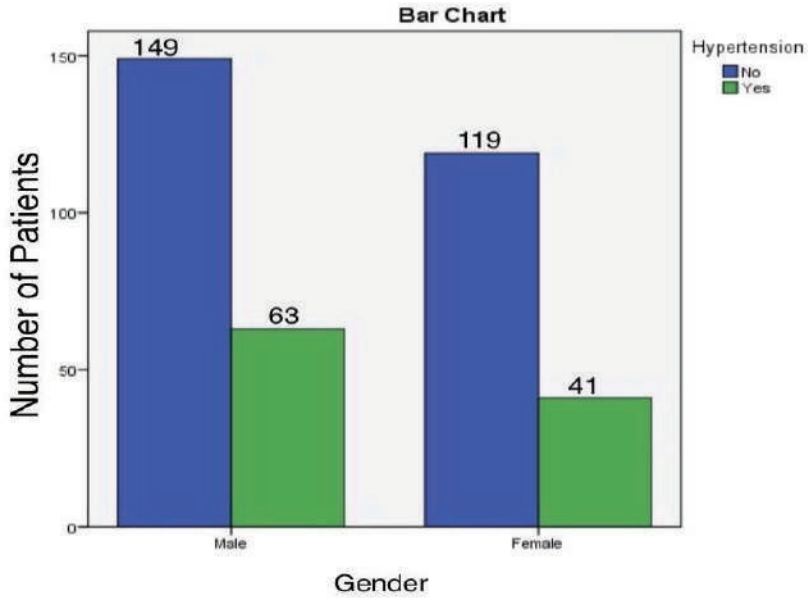


Figure 3 Bar graph shows the association between of male and female completely edentulous patients with prevalence of hypertension. X axis shows the gender distribution, Y axis shows number of completely edentulous patients with or without hypertension. Highest prevalence of hypertension was seen in males compared to females. Chi square test was done, Pearson Chi square value: 0.758, DF: 1, p value =

0.3842, p>0.05 hence and the association between gender and prevalence of hypertension was found to be statistically not significant.

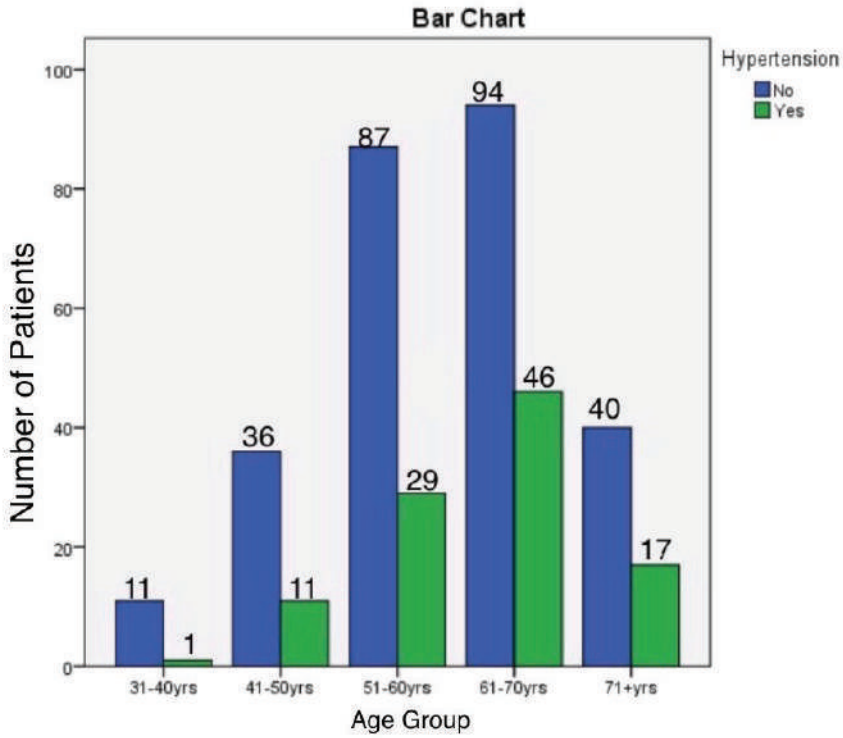


Figure 4 shows the association of age groups of completely edentulous patients with prevalence of hypertension. X axis shows the age distribution, Y axis shows the number of patients with or without hypertension. Maximum number of hypertensive patients were found to be within an age group of 61 to 70 years and the least number of hypertensive patients were found to be in an age group of 31 to 40. Chi square test was done, Pearson Chi square value: 5.049, DF: 4, p value = 0.2825, p>0.05 and hence the association was found to be statistically not significant.

Conclusion

Within the limitations of the study it can be concluded that there is a definitely possible interrelationship of hypertension and a completely edentulous state. However, the limited time period, and smaller sample size are one of the major limitations that we faced in the study.

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Association of Clinical Appearance with Histopathological Features in Oral Squamous Cell Carcinoma

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Abstract

Oral Squamous Cell Carcinoma (OSCC) is one of the most common malignant epithelial neoplasms affecting the oral cavity. The clinical appearance of oral cancer is highly variable and includes ulceroproliferative, ulcerative, leukoplakic and exophytic. These clinical variants can be graded histopathologically to be a well differentiated, moderately differentiated or poorly differentiated squamous cell carcinoma. The prognosis of the patients varies based on the histopathological grading. Hence, the aim of the study is to associate the clinical appearance of OSCC with the histopathological grading. Data was collected after going through 86,000 patients records from June 2019 to April 2020. Total sample size of the study was 44. The data was exported to SPSS and the variables were verified. Correlation analysis was carried out for the data tabulated. Highest correlation was seen between ulceroproliferative lesions and well differentiated OSCC histopathologically. Hence we can conclude that the clinical appearance of OSCC can give us some clue about its histopathological grade and the study should be extended to a larger sample size in order to give a more comprehensive outcome.

Keywords: *Oral squamous cell carcinoma; Clinical Variants; Ulceroproliferative; Histopathological grade; Oral cancer*

Introduction

Oral squamous cell carcinoma is the most common malignant epithelial neoplasm affecting the oral cavity. Oral cancer includes a group of neoplasms affecting any region of the oral cavity, pharyngeal regions and salivary glands. However, this term tends to be used interchangeably with oral squamous cell carcinoma (OSCC), which represents the most frequent of all oral neoplasms. It is estimated that more than 90% of all oral neoplasms are OSCC [1,2] .Oral squamous cell carcinoma is amongst the most prevalent forms of cancer worldwide with its predominance in the Indian subcontinent due to its etiological, behavioral pattern of tobacco consumption. Late diagnosis, low therapeutic response and aggressive metastasis are the foremost confounders accountable for the poor 5 year survival rate of OSCC. As for the oral cavity OSCCs, many authors reported frequent high-risk HPV involvement by considering the over-expression of p16INK4A as equivalent to HPV infection [3–5]. Risky oral habits (including smoking, alcohol drinking, and betel quid chewing) are major risk factors for OSCC development [6–8].

The cell of origin of OSCC is the oral keratinocyte. OSCC, as any cancer, is caused by DNA mutation, often spontaneous but increased by exposure to any of a range of mutagens – chemical, physical or microbial. The various changes in the DNA can progress from a normal keratinocyte to a pre-malignant or a potentially malignant keratinocyte that is characterised by an ability to proliferate in a less-controlled fashion than normal. The cells become autonomous and a true cancer results, characterised by invasion across the epithelial basement

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membrane and, ultimately, metastasis to lymph nodes, bone, brain, liver and other sites [9–11].

According to the recent data given by WHO, India stands in eleventh position in oropharyngeal cancer[12]. Worldwide, oral cancer accounts for 2%–4% of all cancer cases. In some regions, the prevalence of oral cancer is higher, reaching the 10% of all cancers in Pakistan, and around 45% in India[13,14]. In 2004-2020 over 300,000 new cases of oral and oropharyngeal cancer were diagnosed worldwide. During the same time period, over 7,000 affected individuals died of these cancers [5,15,16].

OSCC may manifest as the following; a red lesion (erythroplakia), a granular ulcer with fissuring or raised exophytic margins, a white or mixed white and red lesion, an indurated lump/ulcer (ie, a firm infiltration beneath the mucosa), a non healing extraction socket,A lesion fixed to deeper tissues or to overlying skin or mucosa, cervical lymph node enlargement, especially if hardness is present in a lymph node or fixation[17].

The clinical appearance of oral cancer is highly variable and includes ulcers, red or white areas, lumps, or fissures[18]. Lesions always must be palpated after inspection to detect induration and fixation to deeper tissues.Erythroplakia is a red and often velvety lesion, which, unlike leukoplakias, may not form a plaque but is level with or depressed below the surrounding mucosa. Of these lesions, 75-90% may show severe epithelial dysplasia, carcinoma in situ, or invasive changes.Red oral lesions usually are more dangerous than white oral lesions. Leukoplakia is a clinical term for a persistent adherent white patch with no histologic connotation and no implied premalignant potential. Some OSCC can also appear as a white patch [19–21]. Late OSCC may manifest as an exophytic lesion or an area of ulceration with induration. The floor of the mouth is the second most common intraoral site for cancer and more commonly is associated with leukoplakia. Carcinomas of the alveolus or gingiva can present as an exophytic mass or a persistent ulcer[22]. The underlying alveolar bone is invaded in 50% of cases, even in the absence of radiographic changes, and adjacent teeth may be loose. Carcinomas of the buccal mucosa are mostly seen at the commissure or in the retromolar area. Most are ulcerated lumps, and some arise in candidal leukoplakias [23–25].

While tobacco and alcohol use are traditionally the greatest risk factors, it is important to consider other known risk factors, such as betel quid chewing, in certain ethnic populations. Betel quid chewing is popular in Indian and Taiwanese populations and is associated with a significantly increased risk of oral cancer [26–28]. Areca nut, narcotics and cannabis use has also been found to be a risk factor for oral cancer [29–31] .

Treatment of OSCC is made challenging due to the diversity of the anatomic sites in the neck and the critical normal structures that may be near a particular tumour site. Often, the care of a patient requires a multidisciplinary team of surgeons, radiation oncologists, medical oncologists, nutritionists, gastroenterologists, speech and swallowing therapists, amongst others. Despite the availability of aggressive treatments, the 5year survival rate for oral malignancies remains relatively poor at 65%, with only modest gains in the past few years [32–3435].

Very few articles were found in the database regarding clinicopathologic features on various populations based on their ethnicity. Many studies regarding clinical features and histopathological features, molecular studies were reported in literature. The study will help in better understanding of clinical features and histopathological features of OSCC. The aim of the study is to associate the clinical features and histopathological grade of oral squamous cell carcinoma patients in Saveetha Dental College, Chennai.

Materials and Methods

The study was a retro-spective study and was done under a university setting. Total sample size of 44 patients who have undergone the treatment were included in the study. The case sheets were verified with the help of photographs. To minimise the sampling bias, we included all the data available and there was no sorting of data done. Internal validity of the study was done by non-probability inclusion. The external validity of the study includes homogenisation and replication of experimentation. One principal investigator and 2 co-investigators were involved in the study.The study was approved by the scientific review board of the institution.

Data was collected after going through 86,000 patients records from June 2019 to April 2020. The data was obtained from the category of management of Oral

Cancer, clinical features and Histopathological features were data tabulated. Data was verified by one external reviewer. Confidential details were all masked and all the censored data were excluded. The data was imported to SPSS and the variables were verified.

Chi-square test was done on the data obtained using SPSS software by IBM. Age, Gender and ethnicity were considered as independent variables. Patients diagnosed with Oral squamous cell carcinoma and their clinical and Histopathological features were considered as dependent variables.

Results and Discussion

The results showed that out of the total 44 patients, male population was greater than the female population (Figure 1). Most of the population belonged to the age group between 40-60 years old (Figure 2). The percentage of clinical variants and the histopathological grades are represented in Figure 3 and Figure 4 respectively where ulceroproliferative was the most common variant and well differentiated OSCC was the most common histopathological grade. On correlating clinical variants with histopathological grade, ulceroproliferative and exophytic lesions showed highest incidence of well differentiated squamous cell carcinoma histopathologically , Ulcerative lesions showed high incidence of moderately differentiated squamous cell carcinoma (Figure 5).

Statistically significant correlation was established between ulceroproliferative and well differentiated Oral Squamous Cell Carcinoma with $p=0.039(P<0.05)$.

Studies showing the correlation between the clinical variant and Histopathological diagnosis is not present in the literature. Many case reports have also been reported in literature. Van Zyl et al concluded that the most common clinical variant observed in their study was ulceroproliferative lesion, which was in accordance with the current study[36]. The reason associated with this similarity may be due to cultural habits associated with both the populations.

A study done by Jainkittiwong [37], reported that the population involved in the study showed high prevalence of well differentiated squamous cell carcinoma , which was in accordance with the current study. The reason for the similarity is due to the same geographic location shared by the populations involved in the study.

Similarly, on comparing the clinical variant with the histopathological grade, most of the ulceroproliferative variants were diagnosed as well differentiated squamous cell carcinoma histopathologically. This was in accordance with the study by Effiom et al [38], this correlation will help us in determining the prognosis. Compared to poorly differentiated squamous cell carcinoma, well differentiated squamous cell carcinoma are found to have a better prognosis.

Oral potentially malignant disorders are a precursor for most of the oral squamous cell carcinoma cases. Oral potentially malignant disorders (OPMD) are relatively common, showing a global prevalence from 1 to 5% and a gender, age and site predilection similar to OSCC[10,39]. Although the exact malignant transformation rate for OPMD is unknown, it is expected that leukoerythroplakic areas can be encountered in association with OSCC[40,41]. This pattern was found in few of the

patients included in the present study. As the mean size of the tumors, independent of affected gender, was lower than 4 cm and more than two thirds of the patients complained of the lesions within less than 6 months, it is acceptable to consider that surveillance directed to OPMD diagnosis and follow-up could have been important in early diagnosis of OSCC in the present population. Additionally, these results reinforce the importance of considering the possibility of OSCC when dealing with leukoplakia and erythroplakia, and the need of obtaining biopsy specimens from all lesions from this group.

OSCC age and gender profile, as well as site predilection, shows a heterogeneous pattern of distribution in different countries, in different regions from the same country and in different ethnic groups from the same region, which can be associated with both genetic factors and cultural habits/behavior[42,43]. Studies focusing on specific regions are welcome as they show the demographic and clinical profile of OSCC in restricted geographic locations, offering an enhanced comprehension of these tumors and the possibility of planning specific strategies of prevention, diagnosis and treatment.

The most representative limitations on the methods and results from the present study are associated with the retrieval of clinical and histological information from respective laboratory records and incisional biopsies. To improve the significance of the study, the study should be done extensively with a large amount of sample size, so that the results are reliable.

Figure 1: This graph shows the gender distribution where blue represents male and red represents female with X axis denoting the genders and Y axis denoting the percentage. This graph shows that most of the population in the study was dominated by male gender(76.2%) and the female gender represented a population of (23.8%).

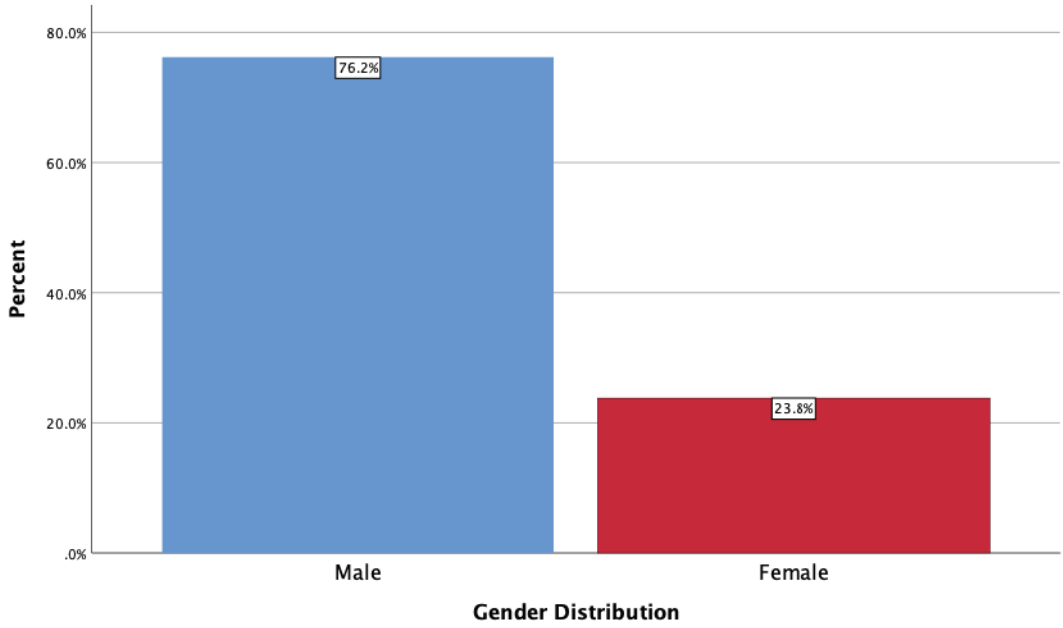


Figure 2: This graph shows the age distribution in the population studied where blue denotes the age group of 40-60 years old and red denotes the age group of 61-80 year old. X axis denotes the two age groups and Y axis denotes the percentage. This graph shows that the most of the population belongs to the age group 40-60 years(69%) and then followed by the age group 61-80 years old(31%)

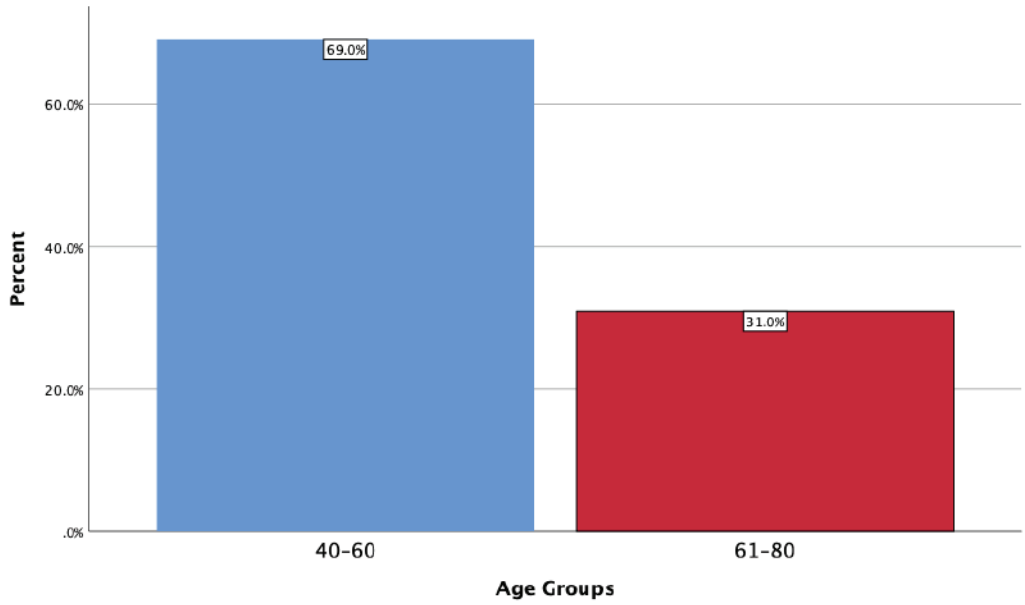


Figure 3: Bar graph representing the distribution of patients based on the clinical variants of oral squamous cell carcinoma where blue represents ulcerative, red represents leukoplakic, green represents exophytic and yellow represents the ulceroproliferative lesions.. X axis depicts the clinical variants and Y axis depicts the percentage. The most number of clinical variants involved in the study was ulceroproliferative (47.6%), followed by exophytic growth (23.8%), followed by ulcerative clinical variant (19%) and the least being leukoplakic clinical variant(9.5%).

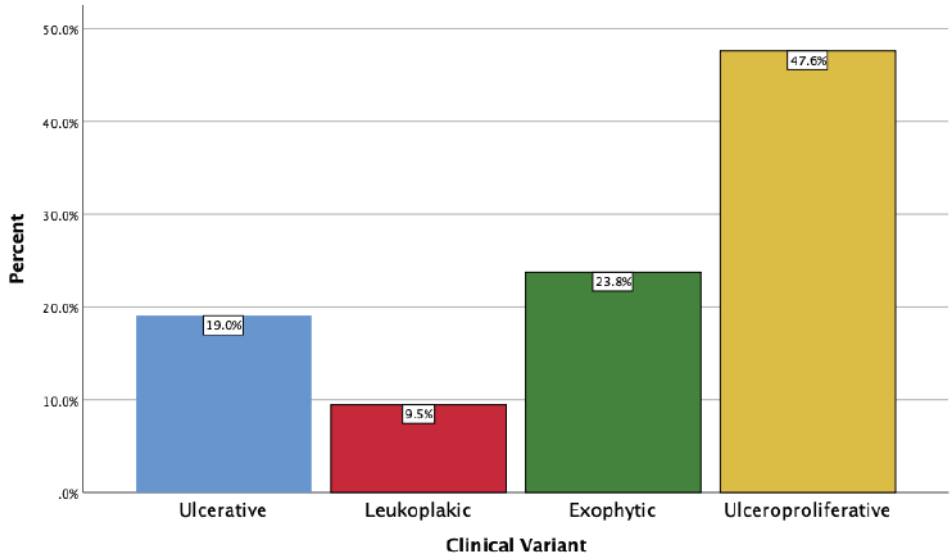


Figure 4: Bar graph depicting the distribution of the various histopathological grades of oral squamous cell carcinoma. Blue represents well differentiated OSCC, red represents moderately differentiated OSCC, green represents poorly differentiated OSCC and yellow represents Verrucous carcinoma. X axis depicts the histopathological grades and Y axis depicts the percentage. Well differentiated OSCC (61.9%) was the most common grade, followed by moderately differentiated OSCC (28.6%), poorly differentiated OSCC and verrucous carcinoma (4.8%).

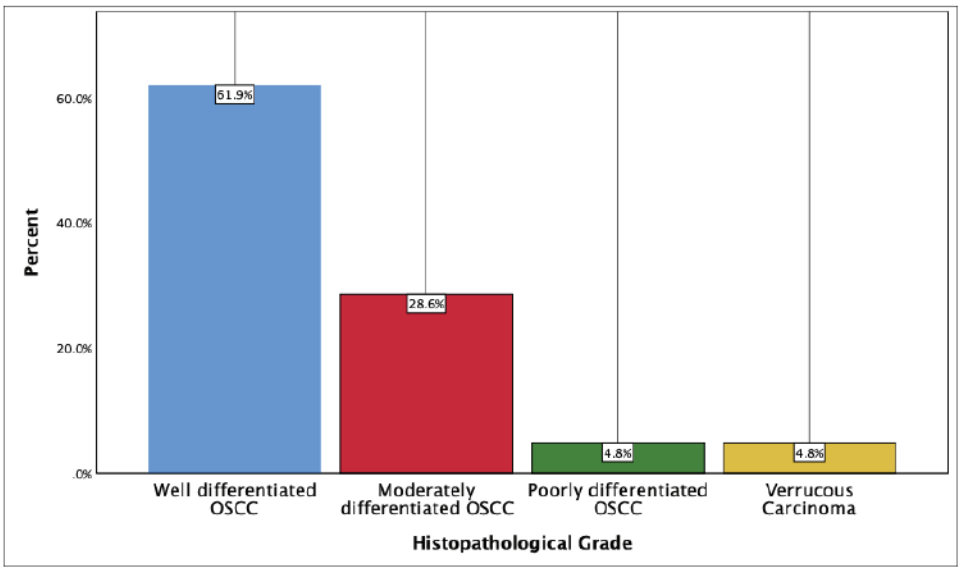
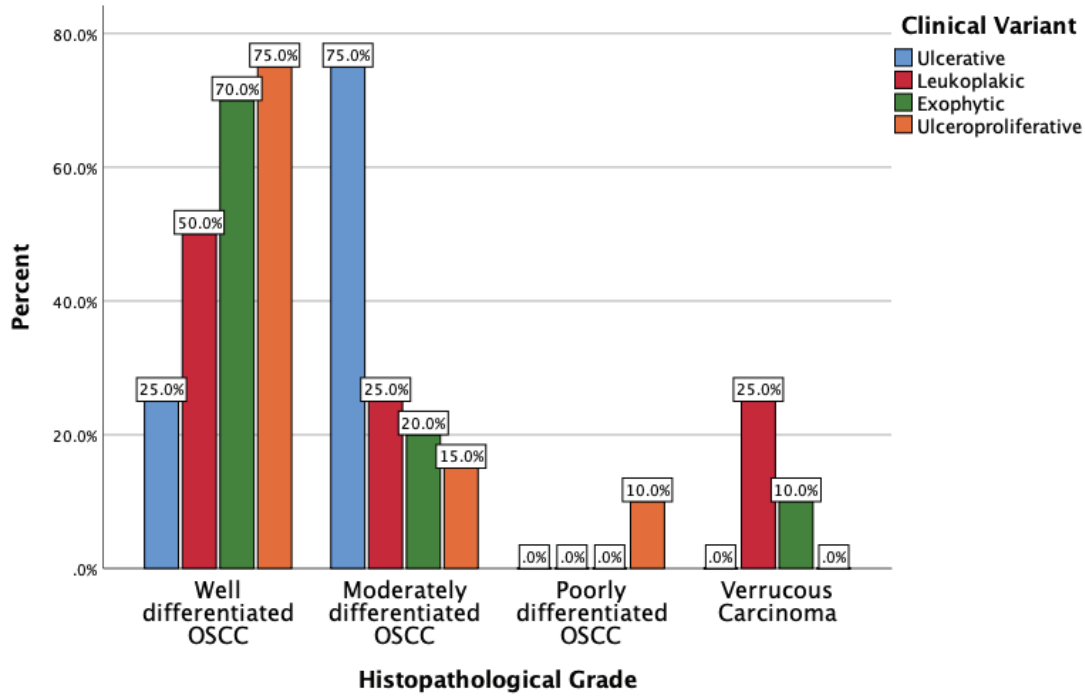


Figure 5: The bar graph represents the association between the clinical variants and the histopathological grade of oral squamous cell carcinoma, where blue denotes ulcerative lesions, red denotes leukoplakic lesions, green denotes exophytic lesions and orange denotes ulceroproliferative lesions. X axis represents the various histopathological grades and Y axis represents the percentage of clinical variants. This graph shows that the cases diagnosed with well differentiated OSCC appear clinically mostly as ulceroproliferative(75%) or exophytic(70%), whereas moderately differentiated OSCC appear clinically as ulcerative lesions(75%). Correlation between clinical variants and histopathological grades was done using Chi-square test, Pearson Chi-square value is 17.662-p value - 0.039(<0.05) was found to be statistically significant. There was a significant association of moderately differentiated OSCC with ulcerative lesions and well differentiated

OSCC with ulceroproliferative lesions.



Conclusion

Within the limits of the study, the highest correlation is seen between ulceroproliferative lesion and well differentiated Oral Squamous Cell Carcinoma. Similarly ulcerative lesions with moderately differentiated oral squamous cell carcinoma histopathologically. The results of the study will act as a guide for the clinicians and surgeons to establish the correct treatment planning and thus predict the prognosis of the patient. Hence we can conclude that the clinical appearance of OSCC can give us some clue about its histopathological grade and the study should be extended to a larger population in order to give a more comprehensive outcome.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of HIV and Hepatitis-B in the Out Patient Department of a Private Dental College Hospital Chennai, India

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Abstract

Acquired Immunodeficiency syndrome caused by the Human Immunodeficiency virus is greatly prevalent in the Indian subcontinent. Hepatitis-B is another condition that is affecting the population. Healthcare professionals are at an increased risk of being exposed to such pathogenic infections. Having adequate knowledge towards the infection and appropriate preventive equipment such as vaccinations, masks, gloves is crucial in preventing spread of infection. The current study assessed the prevalence of HIV and hepatitis-B conditions among the out patients visiting Saveetha Dental College. The study was performed in the out patient department of Saveetha Dental College, Chennai, India. Data required for the study was procured by reviewing patient records and analysed data of 86000 patients between June 2019 to March 2020. The data was sorted in excel and statistically analysed using the IBM SPSS software analysis and the results tabulated. A prevalence rate of 3.2% was observed with regard to hepatitis-B in this study. The study showed female predilection with the mean age of this study being 41yrs. Prevalence of HIV was not observed in this study.Healthcare professionals are at high stake of infection due to occupational exposure. Proper clinical examination, history of the patient, preventive gear, vaccination and sterility protocols must be ensured to minimize the spread of infection.

Keywords *: HIV ; hepatitis-B ; occupational exposure ; healthcare ; vaccination ; PPE*

Introduction

Acquired immunodeficiency syndrome (AIDS) is a chronic, contagious and debilitating disease caused by Human Immunodeficiency Virus[1]. HIV infections were first detected in Chennai, India among the female sex workers[2,3]. India harbours about 5.134million individuals affected by AIDS currently [4,5]. AIDS is mainly said to spread via the sexual contact but can also spread through body fluids. Antiretroviral treatment has aided in transforming AIDS from an inevitably fatal disease condition to a manageable disease in some aspects [6,7]. The current developments towards the field of HIV management include Enzyme Linked ImmunoSorbent Assay, Polymerase Chain Reactions etc.[8,9]. However there is an increased necessity for a powerful tool for diagnosis, management of HIV [10,11]. The risk of transmission of this disease from an affected individual to an oral healthcare professional during the course of treatment is high as they are in close proximity to the affected individual.

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Hepatitis-B is an inflammatory condition of the liver commonly caused due to a viral infection [12,13]. Other possible causes of hepatitis include autoimmune hepatitis, hepatitis that occurs as a secondary infection due to intake of drugs and toxins, alcohol. Three types of hepatitis are well identified namely, A, B, C with the B and C types having an increased rate of occurrence . About 325 million carriers of hepatitis-B, have been identified worldwide with a death rate of 1.34 billion [14].Today viral hepatitis has become a silent epidemic and is the major cause of conditions such as liver cirrhosis and liver carcinoma [15]. In a dental practice the spread of infection from an affected individual can be expedited through various routes including, direct and indirect contact with body fluids such as blood, saliva, droplet splatter, aerosols etc [16]. Studies show that patients with periodontal diseases show higher detectability of surface antigen of HBV in whole unstimulated saliva [17,18]. The magnitude of prevalence of hepatitis-B has increased ten times in the past ten years, however the prevalence of it in dental practice in India is comparatively low, about 0.3% [19]. Occupational exposures should be reported immediately to prevent further collateral damage. Dental care professionals are at an increased risk of cross infection while treating patients [20]. Aerosols are also capable of spreading pathogens from clinician to patient and vice versa [21,22]. It is important to create awareness among dental students regarding hepatitis-B vaccines, as there is possibility of exposure in dental clinics to various sources of infection [23]

Standard precautions are imperative and a prerequisite for all patients and especially in individuals having hepatitis-B/AIDS. Dental personnel should wear the barrier protective gear, which includes the gloves, mask and protective eyewear whenever there is a potential for contact with body fluids, non-intact skin, or mucous membranes [24].

The individuals affected by the above conditions tend to show features pertaining to each, hence it is imperative of the clinician to take a thorough history of the patient and examine the patient carefully if any suspicion arises.

The aim of this study focuses on the prevalence of HIV and hepatitis-B in the out patient department of Saveetha Dental College.

Materials and Methodology

The study was performed in the out patient department of saveetha dental college, under a university setting. It was a retrospective study. Ethical approval was obtained from the institutional committee (ethical approval number : SDC/SIHEC/DIASDATA/0619-0320). Data required for the study was procured by reviewing patient records and seanalysed data of 86000 patients between June 2019 to March 2020. The sample size of the study n=485. Verification of the data was done in the presence of an external reviewer, procedure notes, medication history, lesion photographs and blood test reports. Stratification and randomization was done to minimise any sampling error. Incomplete data was excluded.

The collected data was sorted, tabulated in excel and assessed for the following parameters namely Age ,Gender , presence or absence of HIV ,presence or absence of Hepatitis-B and specially referred cases.

The sorted data was then entered in the IBM SPSS software and descriptive analysis performed. The results were interpreted in graphs.

Results and Discussion

The total number of patients in this study was 485. Among them the prevalence of hepatitis-B was found to be 3.2%, that is 15 out of the study population tested positive for hepatitis-B. The prevalence of HIV was not observed in this study [GRAPH 1]. The mean age of the study population in association with hepatitis-B was observed to be around 41yrs. The gender distribution of the study shows a female predilection in association with hepatitis-B, with 60% of the tested individuals being females and 40% of the individuals being males [GRAPH 2]. The speciality referred to testing positive for such systemic diseases in this study show that the Oral Medicine department is frequented by a rate of 93.33%, followed by the Oral Surgery department at the rate of 6.67% [GRAPH 3]. Chi square statistical analysis performed and the p-value was obtained to be 0.000, which was statistically significant.

Pervasive increases in serious transmissible diseases such as hepatitis-B and HIV over the last few decades have created global concern and affected the treatment approach of all healthcare practitioners [25,26]. Serious infections such as hepatitis-B and human

immunodeficiency virus (HIV) are transmitted in dental practice through percutaneous injuries [27,28]. Infections may be transmitted in the dental operatory through several routes, most dentists believe that the diseases are acquired via a cut in the fingers contaminated by the affected individual's saliva or blood thus paving a way for the entry of the pathogenic organism [29,30].

Prevalence of HIV was not observed in this study. Previous literature cites that 0.4% chances of prevalence of HIV in healthcare professionals, claim a 0.5% rate of prevalence of HIV among dental professionals due to transmission from affected individuals [31,32]. Studies also cite that there is a high prevalence of HIV cases across all regions of India except the Southern region [33]. The focus of World Health Organisation with regard to information, education, and communication activities has been directed towards stimulating safe behaviors, reducing the HIV stigma and discrimination and demanding generation of HIV/AIDS services. Dental health care providers may be exposed to many microorganisms via blood, oral or respiratory secretions. Though the risk of transmission of HIV in dental settings is low, the repercussions of being infected are life threatening. Therefore, high standards with regard to infection control and waste disposal are essential in managing occupational contagion and cross infection. Our study findings are not in concordance with literature, this can be attributed to factors such as patient consent, sample size, uneven distribution, unicentric study, unbiased data, physician referral, patient conditions, private college study setting.

Prevalence of hepatitis-B in this study was observed to be 3.2%. Previous literature cites similar findings, 2% of the screened population is hepatitis-B positive, 3.9% prevalence rate of hepatitis-B in the North Indian population [34,35]. Our study results are in concordance with the literature.

The mean age of our study obtained in association with hepatitis-B was 41yrs. Previous literature also cites similar findings, average age of the patients screened 40years, majority of patients screened reported to be about 40yrs [36]. Our study findings are found to be in concordance with the literature citations. This may be due to various factors such as obesity with increase in age, immunosuppression with rising age thereby down in barrier for disease entry, habits such as alcoholism, smoking, lifestyle etc.

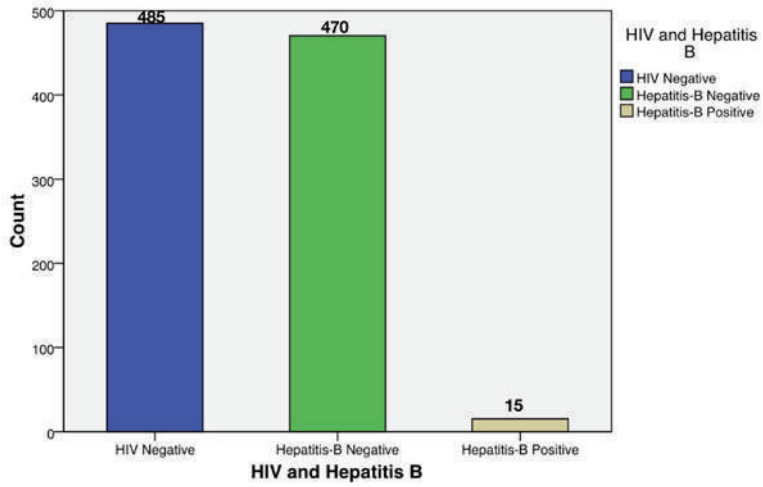
The gender distribution in this study shows a female predilection for hepatitis-B. Previous literature findings differ, cite hepatitis-B infection more frequent in males compared to females [37]. The association between gender and hepatitis-B in this study was found to be statistically significant (Chi square test, p value = 0.000) In an attempt to explain the disparity among the genders, suggestions have been made that lifestyle choices, such as drinking, smoking or even how much water a person drinks, might be the reason. Prevalence of HBV infection in males as compared to females may be due to them being employed outside their homes, wider span of exposure and contact with public, visiting salon/barber shops as well as their involvement in blood transfusion practices, while women are mostly involved in household activities, although there is an increasing number of working women in these years, based on the social, cultural and religious preferences and influence women are attributed to less contact compared to males and hence unlikely to get hepatitis-B compared to males. Our study findings are not in concordance with the literature. This can be attributed to reasons such as unbiased data, patient's consent, physician referral, unicentric study, geographical distribution and sample size.

The individuals infected with hepatitis-B are referred from the undergraduate clinics to the speciality care to assist them and narrow down the exposure spectrum across patients. Our study results point, Oral Medicine department having an exposure rate of 3% of the prevalent 3.2%, while 0.2% of the cases are referred to the Oral Surgery department. Previous literature findings report, Oral medicine department professionals are at high risk of exposure [38,39,40,41]. Our study findings are in concordance with the literature.

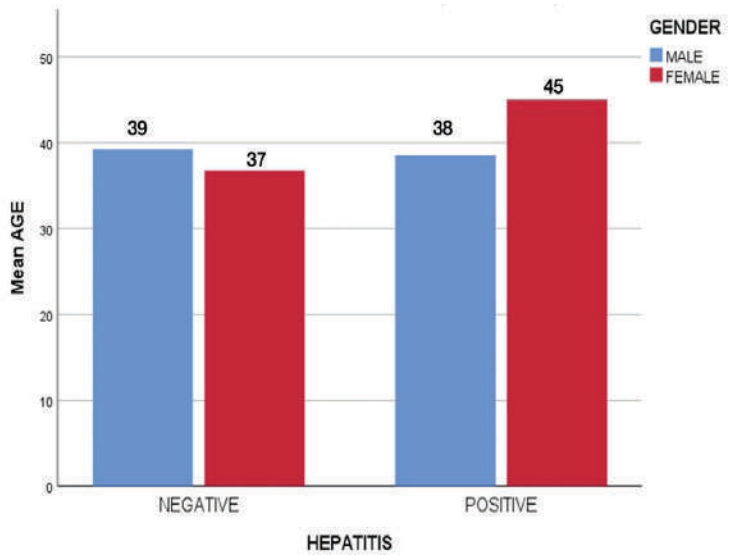
Dental surgeons have a great risk of exposure to these diseases due to the numerous encounters which involve use and disposal of sharp instruments and aerosol emitting handpieces and scalers. Every health care speciality that involves contact with mucosa, blood or blood contaminated with body fluids, must have proper protocols of ensuring compliance with standard precautions and other methods to minimise infection

risk, smaller sample size, geographical location, unequal distribution, incomplete data excluded, unicentric study.

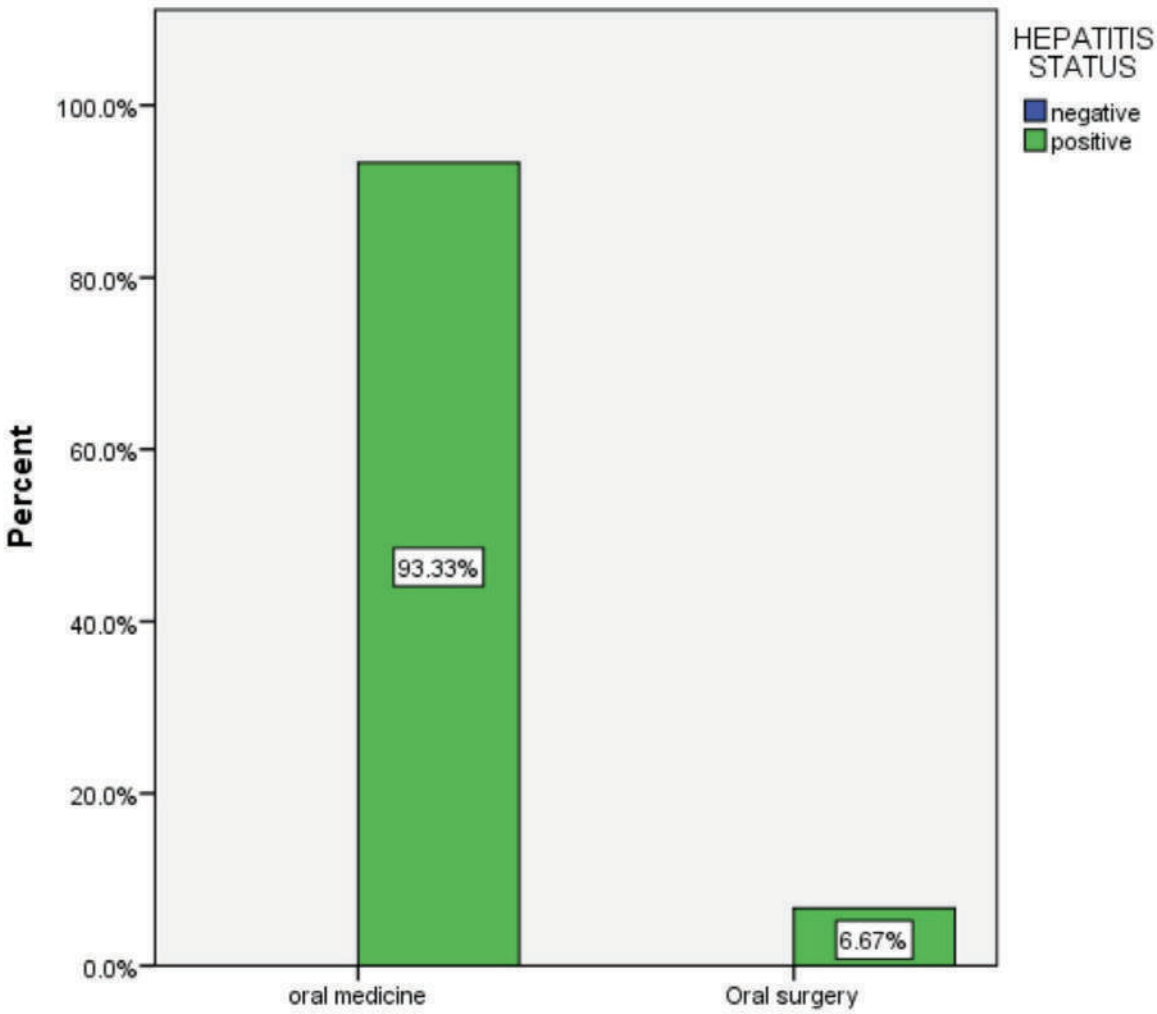
The existing rate of prevalence in dental offices is of concern. Clinical examination to reveal any lesion or oral manifestation of a disease, precautionary protective equipment, knowledge of identification of affected individuals, management of the patients and necessary vaccinations, disinfection and sterility protocol maintenance is imperative for a dental surgeon.



Graph 1: Bar chart depicting the distribution of hiv and hepatitis-b, the y-axis represents the number of individuals, x-axis represents the status of hiv and hepatitis-b as observed in this study, green denotes the number of individuals who tested negative for hepatitis-b, brown denotes the number of individuals who tested positive for hepatitis-b and blue denotes the number of individuals who tested negative for hiv. Prevalence of hiv was not observed in this study. Prevalence of hepatitis b was found to be present in this study.



Graph 2 : Bar chart depicting the gender distribution in association with hepatitis-b positive individuals, the x-axis represents status of hepatitis-b among the study population, y-axis represents the mean age of the study population, red denotes the number of females and blue denotes the number of males. An overall female predilection for hepatitis-b was observed in this study. This was statistically significant (Chi square test; p-value = 0.000 - significant)



Graph 3 : Bar chart depicts the speciality referred for dental treatment of hepatitis-b positive individuals in association with hepatitis-b positive individuals as observed in this study, x-axis represents the speciality referred to for treatment, y-axis represents the percentage of individuals referred to each speciality, green codes for positive cases referred to each speciality. Oral medicine speciality had more referred cases for treatment compared to Oral surgery speciality in this study. This was statistically significant (Chi square test; p-value = 0.000 - significant)

Conclusion

Within the limitations of this study, the prevalence of hepatitis-b was observed to be 3.2%, with the mean age of the study being 41yrs, showing a female predilection. The Oral Medicine speciality was found to have been the choice of referral for treatment of the affected individuals. Prevalence of HIV was not observed in this study. However any institution should be aware of these transmissible diseases and should ensure proper protocols and precautions when treating these patients.

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Type of study- Cross sectional study

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Utilization of Sealants and Conservative Adhesive Resin Restoration for Caries Prevention by Dental Students

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Abstract

The most prevalent dental caries is a preventable disease and established lesions can become arrested. Traditional restorative treatment has many limitations and there is a need for dentists to manage carious lesions as far as possible by preventive means. The aim of this study was to investigate the practice of dental students regarding pit and fissure sealants and conservaive adhesive resin restoration for caries prevention. Data was collected from 86000 patients' dental records in the department of pediatric dentistry to meet the inclusion and exclusion criteria. A total of 5637 records of children who had undergone either pit and fissure sealants or CARR in primary or permanent teeth were evaluated. Descriptive analysis and chi-square tests were performed. Pit and fissure sealants were done in 9637 teeth (882- primary teeth; 8755- permanent teeth) and CARR were done in 328 teeth (178- primary teeth; 150- permanent teeth) with statistically significant difference ($P<0.05$). Both sealants and CARR are more prevalent in males (5071-Sealants; 180-CARR) than in females (4566-Sealants; 148-CARR). Based on the findings of this study, sealant application is the most common treatment done than CARR for caries prevention in both primary and permanent teeth by dental students.

Key words: *Caries adhesive resin restoration; Dental students; Sealants; Prevention.*

Introduction

Dental caries is one of the most widely recognized childhood diseases worldwide which is a complex process of demineralization and dissolution of the substance of the teeth leading to cavitation.[1,2] Dental research has moved concern from reasons for dental caries to how dental caries impacts the quality of life of affected individuals.

Over the past decade, oral health has improved significantly. This improvement, however, has not been experienced similarly over the populace, being considerably greater among the better off. Oral health is consistently an indistinguishable part of general health.[3] The most recent two decades have seen an improvement in oral health among the children and youngsters in many industrialized nations, particularly as for dental caries.[4] The factors credited to this drastic change in the pattern might be adjustment in the dietary habits, improved oral hygiene, proper utilization of fluorides, and other professional practices along with the foundation of school-based preventive programs.[5,6-7,8] Additionally, a prominent improvement in the degree of oral health awareness, dental health knowledge, and attitudes among the dental professionals, children and parents has been referred to as a significant contributing factor for improved oral health.[9,10,11]

Preventive dentistry might be viewed as the summation of all endeavors to forestall dental diseases or to prevent the sequelae of a person's dental diseases

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and disorders. These endeavors incorporate primary prevention which alludes to any gauge applied in the pre pathogenic period before a preventable disease appears.[12] Therefore, there is an urgent need for oral health assessments, anticipatory guidance, prevention, and early intervention among young children for oral health care.

Prevention at the primary level is of extraordinary incentive in dentistry, particularly in pediatric dentistry. Since the utilization of preventive measures can forestall future intricacies, dental professionals share a significant obligation toward early screening, treatment, and this knowledge must be transferred into the practice of dentistry.[13] Oral health education and professional knowledge of preventive dentistry have empowered dental students to become good examples for the general population. To accomplish improved oral health in the society, dental healthcare personnel or dental students are required to have adequate knowledge and a positive attitude, towards diagnosis and treatment planning, yet in addition towards preventive oral healthcare to forestall any complications.[14–16,17]

The importance of maintaining the primary teeth in the oral cavity until physiological exfoliation has been stressed in various studies, as they act as the best space maintainer and preserve the arch integrity.[18,19,20,21,22,23,24] Thinking about the high costs of caries treatment, early execution of preventive dental care programs and clinical care management appear to be fundamental for all children.[25] In addition, for the provision of preventive dental treatment, the knowledge on the significance of preventive measures is of most extreme significance.[14]

Dental sealants can be a preventive measure used efficiently as part of a comprehensive approach to caries prevention on an individual basis or as a public health measure for at risk populations against pit and fissure caries.[26] Pit and fissure caries report for more than 80% of all caries in children and adolescents. Despite the fact that occlusal surfaces comprise only 12.5% of tooth surfaces, approximately 60% of dental caries are seen in these surfaces.[27] Due to the unique morphology of pits and fissures and lack of mechanical tooth cleaning for these sites, they are among the most susceptible sites to caries. The dental sealant material hardens after penetrating the pits and fissures, and acts as a physical barrier that inhibits the ingress of bacteria and nutrients.[28] The first clinical trial was conducted in the late 1960s and today there are multiple commercially available sealant materials that have been tired such as resin-based that are polymerized by chemical activation or light activation system; glass ionomer-based with fluoride releasing property; polyacid-modified resin sealants.[29,30]

With the proven results of etched resin techniques, there has been renewed interest in conservative cavity design with a view to the conservation of sound tooth structure.[31] Among the newer techniques indicating long-term success are preventive resin restorations (PRR), recently known as conservative adhesive resin restoration (CARR).[32] Minimal exploratory cavities in enamel are restored with pit and fissure sealants, whereas isolated carious lesions are removed without augmentation into the surrounding healthy tooth. The cavity is restored with filled resin and the unaffected pits and fissures are secured with pit and fissure sealant.[33] CARR is a secondary prevention which halts the progress of the disease at its incipient stage and forestalls further complication. Here, the pits and fissures are minimally prepared to remove demineralized enamel and dentin, and then filled with resin-based composite.[34]

Hence, the present study was conducted to investigate the practice of dental students regarding sealants and CARR for caries prevention.

Materials and Methods

This retrospective study was conducted in a university setting. The ethical clearance for the study was obtained from the Institutional Scientific Review Board. A total of 86,000 patient treatment records between June 2019 to April 2020 were assessed for the study. The data collection and analysis was done by two examiners.

The inclusion criteria were children between the ages of 3-17 years, children who underwent dental sealants and CARR treatment for primary or permanent teeth and complete records of the patient and treatment done in the case sheet with photographic evidence. Exclusion criteria for the study were patients above 17 years of age, incomplete case records and missing photographic proof of completed treatment. A third examiner reviewed the case records of the collected data

to confirm the validity of the data by confirming the data with the post operative photographs. To avoid sampling bias, simple random sampling was done. Based on the inclusion and exclusion criteria, dental records of 5637 children who had undergone pit and fissure sealants or CARR in the permanent or primary teeth from dental students were finalised for data analysis.

The extracted data was tabulated in a spreadsheet (Excel 2017: Microsoft Office) and analysed using SPSS 19.0 version software (SPSS, Inc., Chicago). Descriptive statistics and chi-square tests were performed with the level of significance at 5% (P<0.05).

Results and Discussion

In this study, based on the inclusion and exclusion criteria, dental treatment records of 5637 children were examined. Among the case records analysed, 51% were males and 49% were females within the age range of 6 to 15 years. A total of 9965 teeth were studied, of which 11% (1060) were primary teeth and 89% (8905) were permanent teeth. Table 1 shows the distribution of teeth included in the study.

Out of 9965 teeth, pit and fissure sealants were placed in 9637 teeth (9% (882)- primary teeth; (91% (8755)- permanent teeth) and CARR in 328 teeth (54% (178)- primary teeth; 46% (150)- permanent teeth). This result is statistically significant (P<0.05). Figure 1 depicts the distribution of type of teeth in relation to the type of treatment rendered.

Both sealant and CARR are predominantly placed in males (53% (5071)-Sealants; 55% (180)-CARR) than females (47% (4566)-Sealants; 45% (148)-CARR) and the result is statistically not significant (P>0.05) (Table 2).

Table 1: Distribution of teeth included in the study

Type of teeth	Number of teeth	Percentage
Primary maxillary molars	495	5
Primary mandibular molars	565	5.7
Permanent maxillary premolars	681	6.8
Permanent mandibular premolars	613	6.2
Permanent maxillary molars	3848	36.6
Permanent mandibular molars	3963	39.8
Total	9965	100

Table 2: Distribution of type of treatment based on gender

Gender	CARR	Sealants	P value
Males	180	5071	>0.05*
Females	148	4566	

*P>0.05; Not statistically significant

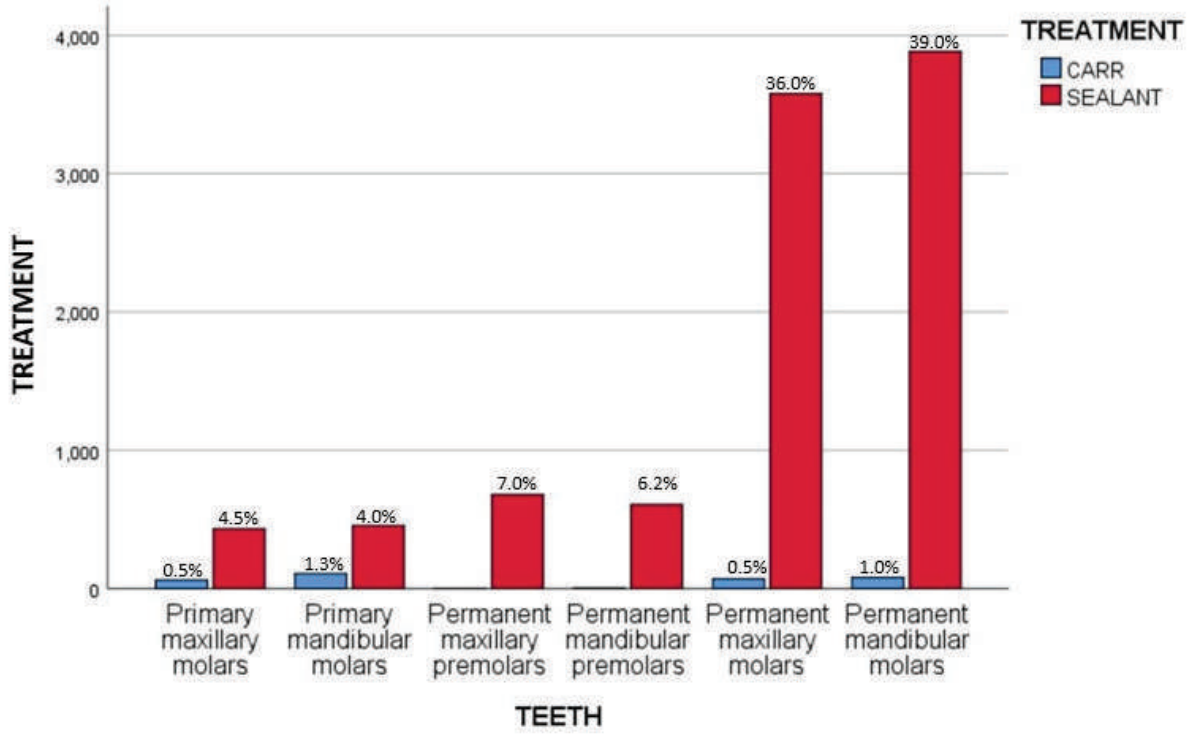


Figure 1 : The bar chart represents the correlation of type of treatment and type of teeth where X-axis denotes the type of teeth and Y-axis denotes the type of treatment. Blue color denotes CARR and red color denotes sealants. It shows that the most number of CARR was done in primary mandibular molars followed by permanent mandibular molars and most number of sealants was done in permanent mandibular molars followed by permanent maxillary molars. Sealants were performed more than CARR. Chi-square test; p value-0.001, hence it is statistically significant.

Preventive dental interventions, such as early and routine preventive care, fluoridation, and sealants are cost effective in reducing the disease burden and associated expenditures. Prevention is the backbone to avoid oral diseases and to have positive oral health. Dentists are in a key position to assist their patients with reducing the burden of oral disease and attain positive oral health behavior.[13] The attitude of dental practitioners toward preventive dentistry is a significant factor that can impact their choice to apply preventive dental care and may conceivably influence their ability to motivate patients to get preventive care measures.[15] The attitude towards improving oral health has changed with an accentuation on caries prevention. In view of current practice, non-invasive methods are preferred to invasive treatments. The current study was conducted to evaluate the practice of dental students regarding dental sealants and conservative adhesive resin restoration in primary and permanent teeth.

The sealant application was primarily indicated on the occlusal surfaces of permanent molars and premolars; and also being indicated in primary molars.[35] The disregarded carious primary teeth may further increase the risk of developing caries in permanent teeth and thus affect the quality of life. Sealants are reported to decrease the need for restoration by 75%.[36] CARR or preventive resin restoration was first suggested by Simonsen, whereby the susceptible fissures were opened up with a small tapered fissure prior to restoring the cavity with resin material. Fernandes et al.,[37] stated the benefits of sealing as the lower cost in comparison to that of restorations and nine fold decrease in the occurrence of caries in comparison to unsealed teeth. Rafatjou et al.,[27] reported CAR success rate to be 53.6% in one year for the treated teeth and Walker et al.,[38] reported a success rate of 83% over 6.5 year in pediatric patients. Pandiyan et al.,[39] compared the retention of sealants and PRR for a 2 year period and stated 10.7% complete loss and 21.4%

partial loss in sealants and 4.9% complete loss and 14% partial loss of PRR. This indicates that pit and fissure sealant has a marginally higher percentage of success in retention when compared to preventive resin restoration. Anson et al.,[40] stated that the poor placement techniques such as moisture contamination, improper sealing of all pits and fissures, inadequate etching, rinsing or drying, insufficient curing time which ultimately results in material wear as the reasons for failure. Manton et al.,[41] stated the faulty technique of sealant application to be the reason for initial loss and a secondary loss due to material wear under the forces of occlusion.

The placement of a resin is very technique sensitive and is influenced by various factors, that includes patient cooperation, operator variability, contamination of operating field, condition of included tooth. A newly erupted tooth has a lower chance of success as compared to a fully erupted tooth due to difficulty in isolation and salivary contamination.[30] In the current study, we observed that pit and fissure sealants are more commonly done than conservative adhesive resin restoration for preventing caries in both primary and permanent molars by dental students. This indicates more knowledge and positive attitude towards preventive dentistry than interceptive treatment. However, the presence of caries demands conservative preparation rather than sealant application alone.

There was no significant correlation between the application of sealants and CARR based on gender ($P>0.05$), which is in consistent with the studies by Rafatjou et al.,[27] and Memarpour el al.[42] In the present study, the maximum sealant application is done on the mandibular teeth than maxillary teeth in both primary and permanent dentition. This could be related to the higher caries susceptibility in mandibular arch due to the fissure topography of molars.[43] When comparing permanent and primary teeth, maximum sealant application is done on the permanent teeth by dental students. This can be correlated with the flatter fissures of primary teeth that do not support long-term sealant retention and also related to the uncooperative behavior of the children as the procedure is technique sensitive.[44] Whereas a report from the American Dental Association indicated that children with sealed pits and fissures in primary molars had a 76% lower risk of developing new caries than children without sealants.[45] When placed with care and then routinely maintained, sealants and CARR represent an extraordinary preventive service.[39]

A large sample size and multiple operators that improved the correlations of the study with general dental practice were the strengths of this study. The limitations of the study were, the treatment plan was not decided by a single operator and the reason for the selection of material was not included. Further research would be needed to assess the outcome and impact of such interventions. Greater effort ought to be made by the professional organization and governmental agencies to inform patients of the benefits of preventive practices.

Conclusion

Within the limits of the study, we can conclude that pit and fissure sealant application was the most common treatment done than conservative adhesive resin restoration for caries prevention in both primary and permanent teeth by dental students.

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Patients Reporting on Appointment Date After Access Opening For Further Treatment to Dental Hospital - A Cross Sectional Study

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Abstract

Failure to keep dental appointments is one of the problems affecting the outcome of treatment. Keeping dental appointments enhances patient care while failure to keep appointments can result in poor prognosis, economic cost and patient care disruption.The aim of the present study was to evaluate the number of patients reporting on appointment date after access opening for further treatment to dental hospital. 4490 met inclusion criteria, only 981 patients reported on the given appointment date. Data was tabulated into excel sheets. Then data was analyzed using SPSS software(23.0 version). Statistical analysis of the material was performed using Chi square test. Number of patients reported to the hospital on the appointment date were only 27.3% (1225) patients, 72.7% (3265) of patients did not report back on the given appointment date. In the present study 1225 patients reported for further treatment, 488 patients of 18-30 age group reported back for further treatment, more number of male patients(665) reported back for further treatment, more number of patients (132) with complaints in relation to 36,46 reported back for further treatment.Failure to keep dental appointments was high in the present study. Being busy with other activities and long distance were some reasons for failed appointments.

Keywords:- *Access opening; Appointments and schedules; Failure of treatment; Obturation; Patient compliance*

Introduction

Failure to keep dental appointments is one of the problems affecting management of patients. Keeping appointments enhances patient care while failure to keep appointments can result in poor prognosis, economic cost and patient care disruption [1].Patients failure to keep appointment has been attributed to various factors which include lack of time[2], gender, age, proximity of oral health centre[3], difficulty in transportation[3], forgetfulness[4], longer intervals between appointments, effective communication or doctor-patient relationship, religious reasons [3], cost[5]and fear[6]. Failure to attend follow up dental visits was higher in rural settings [7] than in urban settings. It also occurred more in paediatric patients [8], male[8,9], patients with multiple dental treatments[10], those from low socioeconomic background [11] and patients attending private dental clinics [8,9]. Sending SMS and text messages [12,13,14] ,educating new patients[15], providing transportation[16], and giving patients detailed information two weeks before their appointment[17] are some of the methods employed to reduce dental appointment failure. Long waiting time and previous defaulters have been shown to be the predictors of failed dental appointments[17,18].Access cavity preparation is defined as an endodontic coronal preparation which enables unobstructed access to the coronal orifice, a straight line access to apical foramen, complete control

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over instrumentation and to accommodate obturation technique [19].Patient visit to endodontist or general practitioner mostly due to tooth pain(mostly endodontic pain). In case of emergency practitioners have to perform access cavity preparation to relieve pain, once pain is relieved then the patient doesn't report to the practitioner on the given appointment date it leads to failure of the treatment after some time.Dental students and dentists are affected by the inability of patients to keep their appointments. Students have less number of patients to treat, meet clinical requirements in college while dentists have less number of patients to treat, which eventually impacts negatively on their academic progress[8,9].There is currently no study in India which evaluated the dental appointments status of patients.

The aim of the present study was to evaluate the number of patients reporting on appointment date after access opening for further treatment to dental hospital.

Material and Methods

This retrospective study included patients who attended teaching dental hospital from June 2019 - March 2020. This study was approved by the Institutional Review Board.

Inclusion Criteria - Patients above 18 years of age, patients who visited during a particular time period from June 2019 - March 2020 and patients complaining of tooth pain.

Exclusion Criteria - Patients below 18 years of age, patients who did not visit during a particular time period from June 2019 - March 2020 and patients who reported to hospital with any other complaint. Data of the patients who have undergone access opening, their appointment dates and patients who visited on a given appointment date was collected from case records. Out of 5,013 patients 4490 met inclusion criteria, only 981 patients reported on the given appointment date. Data was tabulated into excel sheets under headings age,gender,teeth no, patients reported on appointment date. Then data was analyzed using SPSS software(23.0 version). Statistical analysis of the material was performed using the chi square test. In the study group Number of patients reported to the hospital on appointment date only 27.3% (1225)of patients reported on given appointment dates, 72.7% (3265) of patients did not report back on given appointment date(Fig 1), 38.9% (1746) of included cases were 18-30 years age group, 29%(1746) were 31- 40 years age group, 18.8%(846) were 41-50 years age group, 9.2%(413) were 51-60 years age group and 4.1%(183) were above 60 years of age. Out of which 1225 patients reported for further treatment, 488 patients of 18- 30 age group reported back for further treatment and the results were statistically not significant($P>0.05$,) (Fig.2). In the present study 52.6%(2362) were male patients and 47.4%(2128) female patients, more number of male patients(665) reported back for further treatment and the results were statistically not significant($P>0.05$) (Fig.3). Teeth most commonly undergone access opening were 36,46 - 10.1%(454) followed by 16,26 - 6.6% - 6.9%,(308) least commonly involved were 18,38,48 - 0%(0) - 0.1% (1) followed by 33 - 0.6%(25). More number of patients (132) with complaint in relation to 36,46 reported back for further treatment and the results were statistically significant($P<0.05$)(Fig 4).

Result and Discussion

According to the data evaluated, a total of 5,013 patients visited dental hospital during the selected time period. Only 4490 met the inclusion criteria out of which only 981 patients reported back on the given appointment date. Statistical analysis of the material was performed using the chi square test. Number of patients reported to the hospital on appointment date only 27.3% (1225)of patients reported on given appointment dates, 72.7% (3265)of patients did not report back on given appointment date(Fig 1), Approximately 38.9% (1746) of included cases were 18-30 years age group, 29%(1746) were 31- 40 years age group, 18.8%(846) were 41-50 years age group, 9.2%(413) were 51-60 years age group and 4.1%(183) were above 60 years of age. Out of which 1225 patients reported for further treatment 488 patients of 18- 30 age group reported back for further treatment and the results were statistically not significant($P>0.05$,) (Fig.2). In the present study 52.6%(2362) were male patients and 47.4%(2128) female patients, more number of male patients(665) reported back for further treatment and the results were statistically not significant($P>0.05$) (Fig.3). Teeth most commonly undergone access opening were 36,46 - 10.1%(454) followed by 16,26 - 6.6% - 6.9%,(308) least commonly involved were 18,38,48 - 0%(0) - 0.1% (1) followed by 33 - 0.6%(25). More number of patients (132) with complaint in relation to

36,46 reported back for further treatment and the results were statistically significant(P<0.05)(Fig 4).

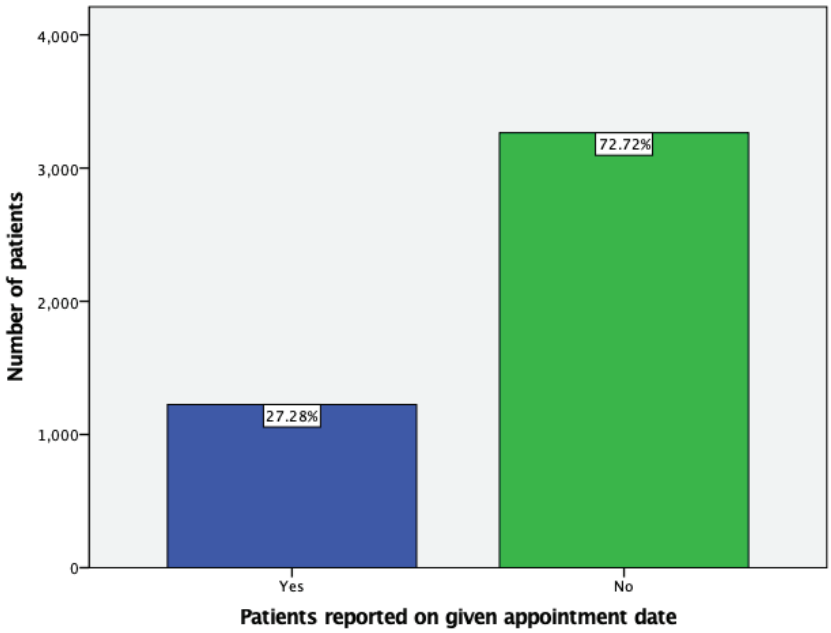


Fig.1: Depicts frequency and percentage distribution of number of patients reported on given appointment date. X axis denotes whether patients reporting on given appointment date and Y axis denotes number of patients, Yes (Blue colour), No (Green colour). 27.3% (1225) of patients reported on given appointment dates, 72.7% (3265)of patients did not report back on the given appointment date.

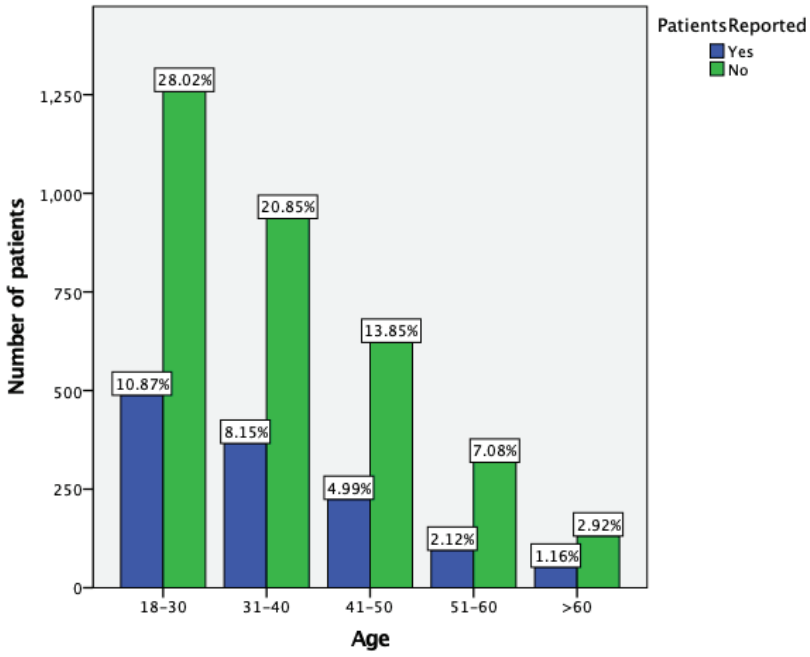


Figure 2: Bar graph shows association between age of patients and number of patients. X axis denotes age and Y axis denotes number of patients. Yes (Blue colour), No (Green colour), More number (10.87%) of 18-30 age group patients reported back for further treatment compared to other age groups. There was no significant difference between different age group patients reported on the given appointment date, chi square test p = 0.282 (p>0.05).

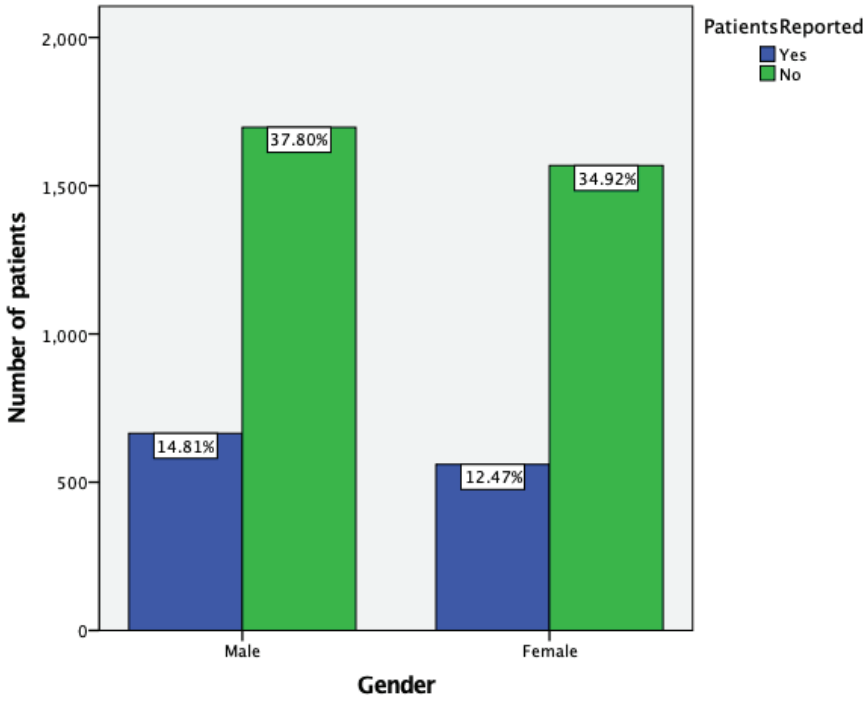


Figure 3: Bar graph shows association between gender of patients and number of patients. X axis denotes gender of patients and Y axis denotes number of patients. Yes (Blue colour), No (Green colour), More number of male patients(14.8%) reported back for further treatment as compared to female patients(12.4%).There was no significant difference between the gender of the patients, chi square test p = 0.89 (p>0.05).

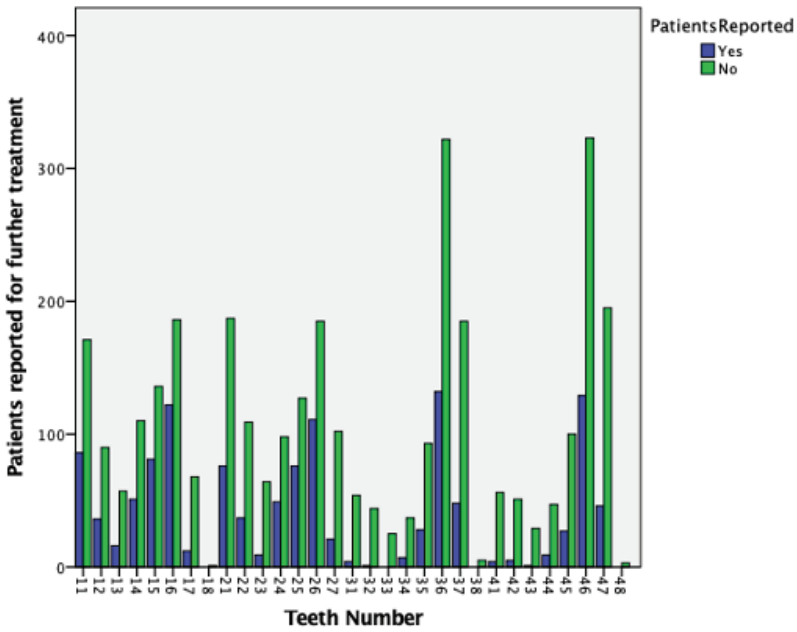


Figure 4: Bar graph shows association between teeth number and number of patients. X axis denotes teeth number and Y axis denotes patients reported for further treatment. Yes (Blue colour), No (Green colour), More number of patients (5.8%) with complaint in relation to 36,46 reported back for further treatment. There was a significant difference, chi square test p = 0.00 (p<0.05).

The aim of endodontic treatment is thorough debridement, cleaning and shaping of the root canal. So the space can be filled with an inert material thus preventing or minimizing any chances of reinfection. However, failure ensues when the endodontic treatment falls short of the standard clinical principles [20,21]. If the patient doesn't visit on given appointment date it may lead to failure of the treatment due to persistence of bacteria (intra-canal and extra-canal) , improper coronal seal (leakage), untreated canals (both major and accessory) and poor access cavity design [22].

One of the foremost causes of endodontic failure is persistent microbiological infection[20,23].The role of bacteria in periradicular infection has been well established in literature and endodontic treatment will be afflicted with a higher chance of failure if microorganisms persist in the canals at the time of root canal obturation [24,25]. Bacteria harbored in root canal areas such as isthmuses, dentinal tubules and ramifications may evade disinfectants [24,26]. A study performed by Lin *et al*. on 236 cases of endodontic treatment failures found a correlation between the presence of bacterial infection in the canals and periradicular rarefaction in endodontic failures [27,28,29].Bacteria present in the periradicular area will be inaccessible to disinfection procedures. Canals with negative cultures for bacteria are said to have higher success rates as opposed to those canals which test positive [30,31]. A well sealing coronal restoration is essential after the completion of obturation as it would prevent the ingress of any microorganisms, which are present in the ambient environment [32].Swanson and Madison [32,33] in their study stated coronal leakage should be considered as a potential factor resulting in endodontic failure. The importance of a good quality coronal restoration was also emphasized by Ray and Trope in their study[34] and later, their work was replicated by another retrospective study performed on 1001 endodontically treated teeth [34,35]. The results of this latter study showed that success rates of the teeth with poor quality coronal restorations fell in contrast to teeth with good quality obturation and coronal restorations [19,36]. However the main factor in the success of the root canal was proved to be the quality of the obturation in this study rather than the quality of the coronal restoration [37,28]. An impervious seal at the coronal area is important for a successful prognosis of endodontically treated teeth. Ng *et al*., in their meta analysis stated that the pooled success rate for teeth which have satisfactory restorations is higher than those teeth which have poor quality restorations[38]. Due temporary filling after access cavity preparation there might be ingress of bacteria.

It is not an uncommon practice to miss a canal while carrying out endodontic treatment especially in molar teeth where one root, one canal formula is frequently overruled by the fact that the number of canals is more than the number of roots [21,39]. Moreover, inadequate access opening makes it difficult for the primary dentist to locate the supplemental canals. The inability to treat all the canals is one of the causes leading to endodontic failure [40,31,41]. Bacteria residing in untreated canals lead to the persistence of symptoms. The results of one study carried out on 5616 molars showed that failure to locate the MB2 canal had resulted in a significant decrease in the long-term prognosis of those teeth[38,42]. In another prospective study carried out by Hoen and Pink[43],the incidence of missed canals were reported to be 42% of all the 1100 endodontically failing teeth. Untreated canals after access opening leads to failure of treatment.Poor access cavity design reduces the amount of dentin[44],decreases the fracture strength of teeth and increases cuspal deflection during function. The loss of dentin and anatomic structures, such as cusps,ridges and pulp chamber roof can result in fracture of the tooth after the temporary filling[45].No shows for scheduled appointments are a frequent occurrence, creating unused appointment slots, reducing patient quality of care and access to service, while increasing loss to follow up and medical cost. The study was conducted to evaluate the number of patient's visited on their given appointment date for further treatment. In our study the percentage of missed appointments was found to be 72.7% which is higher than 24.8% reported in a study conducted in Riyadh, Saudi arabia[46]. In our study 72% of 18 - 30 years age groups were highest and missed the appointment due to school or work. In our study 71.8% of males found to miss appointments due to work. These numbers are similar to study that suggested males having higher missed appointment frequency. 71.1% of patients with complaints of pain in relation to 36,46 missed appointments.In dental practice, missing appointments can disrupt the patient treatment to a large extent for example if the patient undergoing endodontic treatment does not report after access opening it may lead to apical periodontitis and granuloma after some time. If

there is prolonged infection it may be life threatening and it may progress into cellulitis and ludwig's angina. Therefore, to control missed appointments, the dentist must educate the patients on their first visit and cautiously communicate the importance of maintaining the appointment's schedule and its effect on treatment outcome. The patients should also be told how and when they can inform the clinic in case they were unable to make their appointments..Future studies should focus on the method that can reduce or eliminate missed and canceled appointments and encourage patients to report on appointment dates in order to enhance the treatment outcome in addition to improving the economics and quality of dental practice.

Conclusion

Failure to keep dental appointments was high in the present study. Being busy with other activities and long distance were some reasons for failed appointments.To control missed appointments, the dentist must educate the patients on their first visit regarding the importance of maintaining the appointments schedule and its effect on treatment outcome. The patients should also be told how and when they can inform the clinic in case they were unable to make their appointments.

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A Questionnaire based Evaluation of the Awareness among Dental Practitioners on Minimally Invasive Approach for Superficial Enamel Stains

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Abstract

The present survey was conducted to evaluate the awareness among dental practitioners on a minimally invasive approach for treatment of superficial enamel defects.The cross-sectional survey was conducted among 150 dental practitioners in India. Data was obtained by distributing a self designed pretested questionnaire which comprised 20 questions to assess the knowledge of the practitioners on various minimally invasive procedures to treat superficial enamel stains, its incorporation into routine practice and ways to improve their skills. Results were obtained in the form of graphs and percentage. A chi square test was done to determine the association of the responses with the type of practice. The results of the study revealed that a fair number of practitioners were aware of the theoretical aspects of micro and macroabrasion but the clinical incorporation into routine practice is low, 68% did not incorporate the technique in their routine dental practice. The chi square values to check the association between the responses and type of practice were <0.001 which was highly statistically significant. The endodontists had better knowledge about the techniques as compared to the general practitioners and they also incorporated the technique more routinely as compared to the general practitioners.There was a general preference to first opt for a minimally invasive technique but the treatment plan varies from person to person.There is a need for knowledge upgradations and further clinical training through continuing dental education.

Key Words: *Dental Aesthetics; Enamel Discolouration; Macroabrasion; Microabrasion; Minimally Invasive Dentistry*

Introduction

A large number of people who seek dental treatment lay emphasis on the appearance of dentition of particular cosmetic importance is the colour of their teeth. Thus dentistry demands the understanding of elements of tooth colour.

Teeth have a gradation of colours from the gingival margin to the incisal surface and one must understand the science of colour in terms of perception. Since the perception of colour is highly subjective and prone to variation among individuals, the aesthetic aspect of tooth colour becomes difficult to quantify.[1]

The crown of a tooth is formed of enamel, dentin and pulp. Any changes to these structures, alters the light transmitting and reflecting properties of the tooth which further causes an alteration in the outward appearance of the tooth. The incident light and the quantity of reflected light determine the colour of the tooth.[2]

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Discolourations of teeth are broadly classified based on their location as intrinsic or extrinsic. These discolourations may be superficial or may penetrate deep into the dentin. Further, the teeth may either be vital or non vital. Hence, careful assessment and establishing a correct diagnosis of the etiology of discolouration as well as types, extent and the location of discolouration and the status of the adjacent teeth becomes highly essential in formulating an appropriate treatment plan for a good outcome.[3]

A wide variety of management strategies exists ranging from a minimally invasive approach for superficial stains to a more invasive approach such as veneers and crowns when the defects cannot be managed by a more conservative approach. Depending on the clinical scenario, anyone in particular may be the most appropriate. One must always consider adopting a minimally invasive technique before moving on to a more invasive therapy.[4]

Enamel microabrasion and macroabrasion are two such minimally invasive approaches for removal of superficial stains. This can further be accompanied by resin infiltration for a better aesthetic outcome when the case demands.[5]

Hence the present study was conducted with the aim to evaluate the awareness among dental practitioners on a minimally invasive approach for management of superficial enamel stains and defects and to determine ways to reinforce their knowledge and skills for delivering a more proficient treatment. This study was conducted with the following objectives:

1. To evaluate the awareness among dental practitioners on minimally invasive approach for management of superficial enamel stains

2. To determine ways as to how the dental practitioners can upgrade themselves for provision of a more efficient treatment

Materials and Method

The present cross sectional questionnaire based survey was conducted among dental practitioners in India who were selected through block randomization. Lottery method was adopted for the selection of practitioners from each block to ensure a random sampling.[6] The sample size was fixed at 150. The inclusion criteria was set to include the dental practitioners willing to participate in the present survey and giving a written informed consent. The exclusion criteria of the study included those practitioners who were absent on the day of the survey and upto two rounds of follow up. Prior to the start of the study, clearance and permissions were obtained from the Institutional ethics committee (IEC) after the study protocol was sent and reviewed by two blinded reviewers.

Data was collected using a self designed pretested questionnaire that comprised 20 questions which was circulated among the dental practitioners. The questionnaire comprised four sections. Section A comprised questions on demographic details. Section B comprised questions to evaluate the knowledge among dental practitioners regarding micro and macro abrasion technique. Section C comprised questions regarding the practice protocols adopted by dental practitioners for superficial enamel stains. Section D evaluated the need for knowledge upgradation.

The questionnaire was collected upon completion by the primary investigator M.S office excel sheet was used to code and enter the collected data. A chi square test was done to determine the association of the responses with the type of practice. A p value of < 0.05 was considered as statistically significant. Descriptive statistics in the form of frequency and percentage were calculated using Statistical Package for Social Sciences (SPSS, V 2.0, IBM)[7]

Results and Discussion

The demographic details of the present study has revealed that the majority of the participants were females (57.33%) and general practitioners (68.7%) while 31.3% were Endodontists (fig 1). 42.7% of the participants were aware of the indications of microabrasion (fig 2) and 42.7% of the majority knew its advantages. 46.7% of the participants were aware that microabrasion constitutes of a low concentration acid and and an abrasive and 47.3% were aware that microabrasion acts by both abrasive and erosive action and 48.7% knew that hydrochloric acid was used. When asked about the abrasive, 32% said it was silicon carbide and 31.3% said it was aluminum oxide. The contraindications were clearly understood by 48.3% and 38.7% knew what macro reduction was

whereas 43.3% were aware of the abrasion effect. 56.75% participants knew the factors that governed the abrasive and erosive effect. The use of 12 fluted diamond bur for macroabrasion was agreed upon by 38.7%. 42% were aware of the composition of old McInnes solution and 43.3% were aware of its modification. Chi square test done to check the association of the response for knowledge based questions with type of practice gave a p value <0.001 which was highly significant. Since the majority of the endodontists opted for the right answer while the responses of the general practitioner were distributed among the various options which were not correct, it was inferred that the endodontists have better knowledge about the techniques as compared to the general practitioners. However, despite the knowledge, 68% of the participants did not routinely perform the procedure (fig 3). It was observed that the endodontists incorporated techniques like microabrasion, macroabrasion and resin infiltration more routinely in their practice as compared to the general practitioners. The reason for this could be specialised training which is received in the masters program of endodontics. When given clinical scenarios, 42% said that their choice of treatment for white spot lesions would be microabrasion followed by resin infiltration (fig 4). It was observed that it was the endodontists who mainly followed microabrasion and resin infiltration for treatment of white spot lesions as compared to the general practitioners (p<0.001). The response for treatment of localised enamel hypoplasia was veneers as opted by 31.3% respondents (fig 5). It was observed that the vital bleaching was least preferred by the endodontists for treatment of localised enamel hypoplasia while veneers was the most preferred whereas, the general practitioner did use vital bleaching more often than the endodontists and their choice of treatment was distributed among other options as well such as full veneer crowns, veneers and microabrasion (p value 0.024).

There was a general need for knowledge upgradation as agreed upon by 84% (fig 6) and they felt that seminars, symposiums and workshops were excellent ways to do so.

The results of the study has revealed that a fair number of practitioners are aware of the theoretical aspects of micro and macroabrasion but the clinical incorporation into routine practice is low. There is a general preference to first opt for a minimally invasive technique, the treatment plan varies from person to person. The study also highlighted the need to reinforce the knowledge.

Previously our team had conducted numerous clinical trials [8–10] and in vitro studies [11–15] and reviewed various aspects of endodontics and conservative dentistry [16–19] over the past five years. Now we are focusing on epidemiological surveys. The idea for this survey stemmed from the current interest in our community on minimally invasive dentistry.

Enamel microabrasion was first performed by Kane in 1926 for the removal of fluorosis white spots using 36% hydrochloric acid.[20] It was Croll et al in 1986 who recommended the use of 18% hydrochloric acid along with an abrasive due to the hazards of higher concentration of the acid.[21]

The current recommendations are the use of low concentration of HCl along with silicon carbide as an abrasive. Prema compound and Opalsture are some of the commercially available products.[20]

Dental fluorosis is the most common indication among others such as correction of surface irregularities, localized enamel hypoplasia and mineralized white stains. However, the procedure is restricted to surface defects that do not extend to more than 0.5mm into enamel.[22]

The mechanism of action of microabrasion is both erosive and abrasive. Microabrasion causes changes in the optical characteristics of enamel surfaces which is termed as an abrasion effect. Mineralized tissue gets compacted within the organic areas due to simultaneous abrasion and acid erosion of the enamel prisms, replacing the outer layer of prism rich enamel with a densely compacted prism- free region. Microabrasion along with being a conservative procedure, leaves behind a lustrous, shiny and glass-like surface of the enamel. This reflects and refracts light differently, thus helping to camouflage the remaining subsurface stains. Further, saliva helps in augmenting these optical properties.[23,24]

Studies have shown that the potential erosive and abrasive effect depends on several parameters such as type, concentration and pH of acid, abrasive medium,

time duration and force of application and the mode of application.[25]

The contraindications for microabrasion include stains involving the dentin and deeper than 0.5mm, deficient lip seal, tetracycline stains.[26]

The effectiveness and the advantages of the technique have been well documented.[27]Sundfeld et al has reported that 5 to 10 applications of micro abrasive system results in loss of 25 to 100 um of enamel, well in the acceptable range.[20] Bertoldo et al has reported that microabrasion with 6.6% hydrochloric acid and silica results in incorporation of chloride ions and silica into the enamel.[28] Chloride ions are associated with enamel rehardening and silica compound is a bioactive material that induces a new apatite layer on the acid etched enamel.[29,30,31]

Several studies have reported the lasting and stable aesthetic results of microabrasion technique. Price et al showed that enamel microabrasion could remove stains from within the outermost layer of the tooth enamel, thereby improving the appearance of teeth.[32] Yetkinet et al has shown that infiltration and microabrasion treatments were capable of diminishing the whitish appearance of white spot lesions.[33] Castro et al showed that enamel microabrasion combined with at home tooth bleaching effectively reduced staining in case of mild to severe fluorosis.[34]

Macroabrasion is the technique used when the stains are deeper (>0.4mm and no more than quarter the thickness of enamel). The technique utilizes a 12-fluted round diamond fissure bur in high speed handpiece with water irrigation.[35]

In cases where micro and macro abrasion does not produce the desired results, one can supplement it with the use of bleaching. bonding composite resin on the labial surface or resin infiltration.

Resin infiltration technique uses a low viscosity resin with high surface tension and low contact angle with the enamel as well as a refractive index similar to enamel. It is mainly indicated for white spot lesions, such as those caused by orthodontic brackets. [36–39]

McInnes solution is a bleaching agent that contains 1ml of 36% hydrochloric acid, 1ml of 30% hydrogen peroxide and 0.2ml of anaesthetic ether. Later the solution was modified to replace HCl with 20% sodium hydroxide (NaOH).[40]

Only when these techniques fail to give the desired results, should one opt for a more invasive alternative such as veneers or crowns. [41]

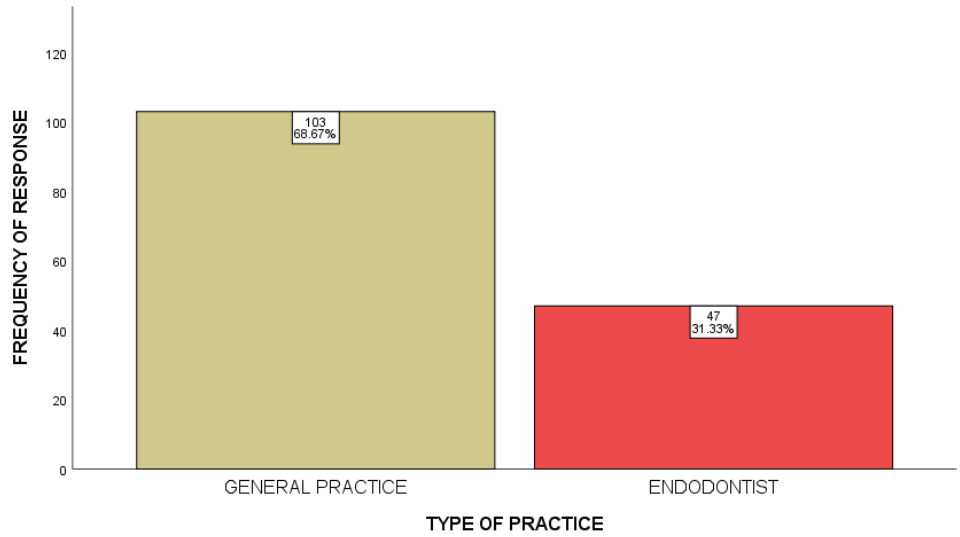


Fig 1: Distribution of sample based on type of practice. The X axis represents the type of practice and the Y axis represents the count. Yellow represents general practitioners and red represents endodontists. The number of general practitioners was higher than the number of endodontists.

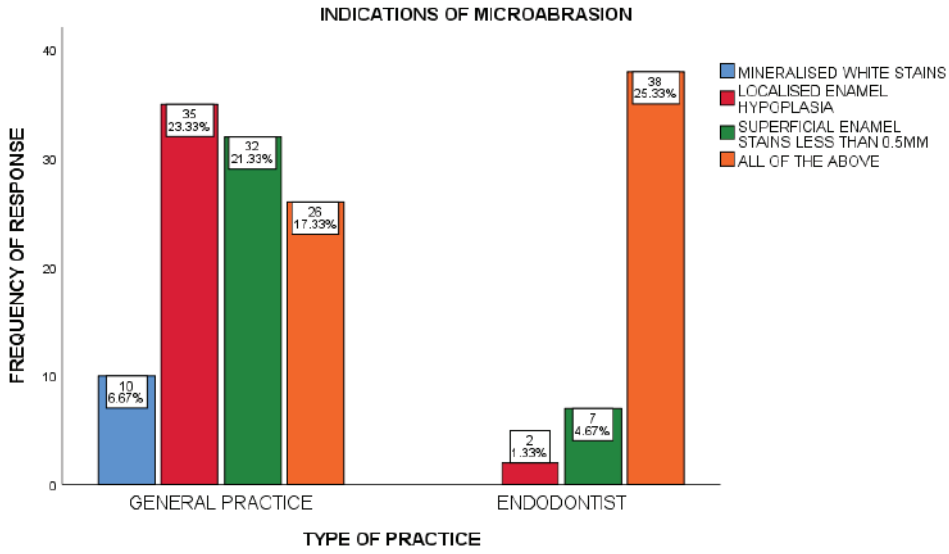


Fig 2:Distribution of responses based on type of practice for awareness of indications of microabrasion. X axis represents the type of practice and Y axis represents the count of the responses. Blue represents responses to mineralised white stains, red represents responses to localised enamel hypoplasia, green represents responses to superficial enamel stains less than 0.5mm and orange represents responses to all of the above. Chi square test done to check the association of the response with type of practice gave a chi square value of 42.761 and a p value <0.001 which was highly significant. Since the majority of the endodontists opted for the right answer while the responses of the general practitioner were distributed among the various options , it was inferred that the endodontists have better awareness about the indications of microabrasion as compared to the general practitioners.

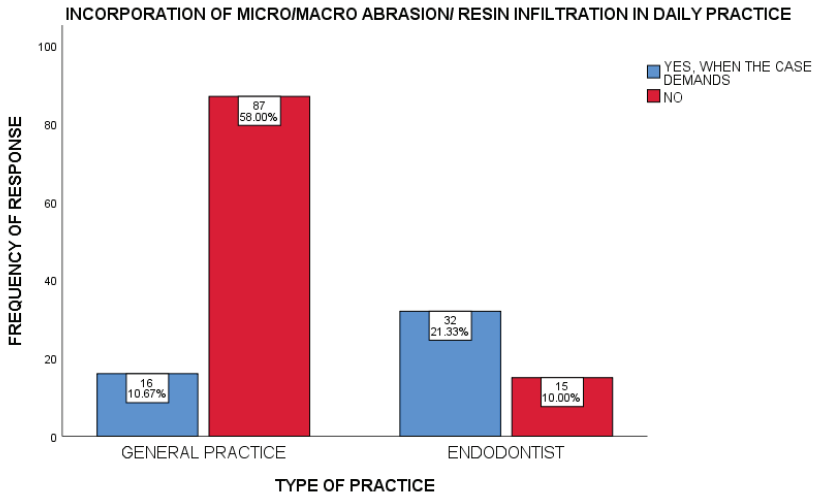


Fig 3: Distribution of responses based on type of practice for the incorporation of micro/macro abrasion technique in their routine practice. X axis represents the type of practice and Y axis represents the count of the responses. Blue represents responses to yes when the case demands and red represents responses to no. Chi square test done to check the association of the response with type of practice gave a chi square value of 40.959 and a p value <0.001 which was highly significant. Of the total number of endodontists who took part in the survey, very few responded to not incorporating the techniques in their practice while of the total number of general practitioners who took part in the survey, the majority responded to not incorporating the techniques in their routine practice. Thus it was observed that the endodontists incorporated techniques like microabrasion, macroabrasion and resin infiltration more routinely in their practice as compared to the general practitioners.

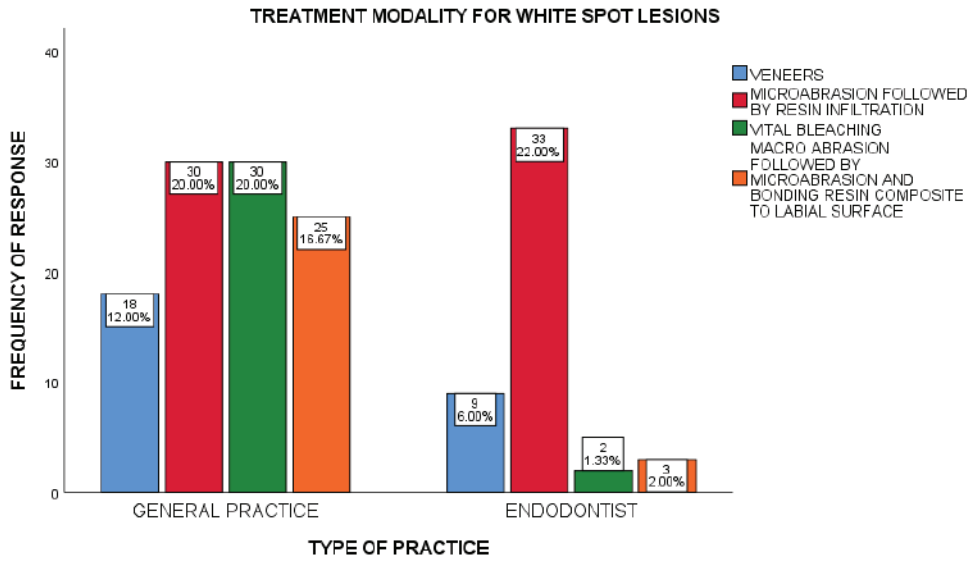


Fig 4: Distribution of responses based on type of practice for choice of treatment modality for white spot lesions. X axis represents the type of practice and Y axis represents the count of the responses. Blue represents responses to veneers, red represents responses to microabrasion followed by resin infiltration, green represents responses to vital bleaching and orange represents responses to macroabrasion followed by microabrasion and bonding resin composite to the labial surface. Chi square test done to check the association of the response with type of practice gave a chi square value of 27.912 and a p value <0.001 which was highly significant. Thus it was observed that the endodontists mainly followed microabrasion and resin infiltration for treatment of white spot lesions as compared to the general practitioners whose choice of treatment was distributed among the various options.

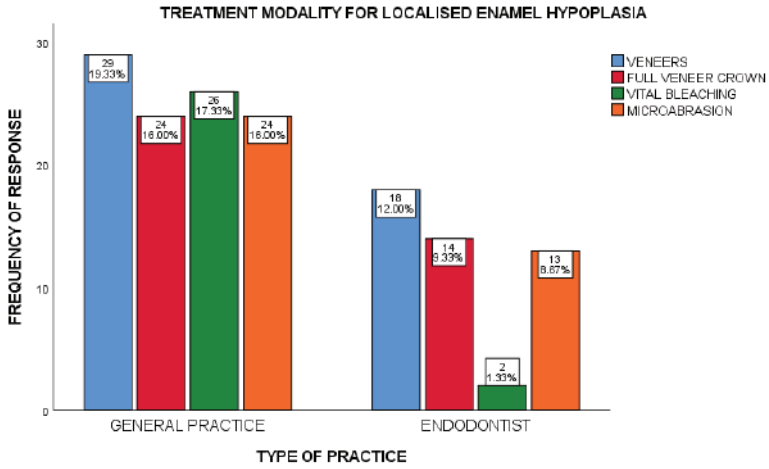


Fig 5: Distribution of responses based on type of practice for choice of treatment modality for white spot lesions. X axis represents the type of practice and Y axis represents the count of the responses. Blue represents responses to veneers, red represents responses to full veneer crowns, green represents vital bleaching and orange represents responses to microabrasion. Chi square test done to check the association of the response with type of practice gave a chi square value of 9.462 and a p value 0.024 which was statistically significant. Thus it was observed that the vital bleaching was least preferred by the endodontists for treatment of localised enamel hypoplasia while veneers was the most preferred whereas, the general practitioner did use vital bleaching more often than the endodontists and their choice of treatment was distributed among other options as well.

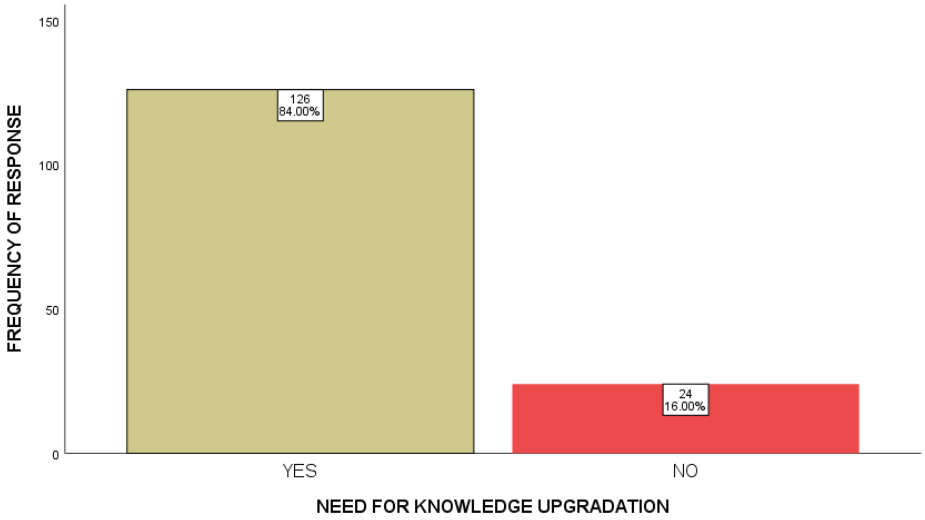


Fig 6: Distribution of responses for the need of knowledge upgradation. X axis represents the need and Y axis represents the count of the responses. Yellow represents yes and red represents no. Hence it can be inferred that the majority of the participants feel there is a need for knowledge upgradation.

Conclusion

Dental aesthetics is becoming one of the leading reasons that patients now attend a dental practice. Accumulating evidence is suggestive of the fact that enamel microabrasion and macroabrasion are effective and efficient for producing aesthetic improvements. These procedures are safe, conservative and atraumatic. The results of the study revealed that a fair number of practitioners were aware of the theoretical aspects of micro and macroabrasion but the clinical incorporation into routine practice is low, Further, it was observed that the endodontists possessed better knowledge which they were able to more frequently incorporate in their routine practice as compared to the general practitioners. However, there is a need for knowledge and skill upgradation. The implementation of these techniques into dental practice warrants further training through continuing dental education.

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Conflict of Interests: Nil

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Toxic Biological Effects Associated with Materials Used in Prosthodontics- A Survey

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Abstract

Prosthodontics practice involves contact with restorative materials, auxiliary dental materials which are broadly of different composition. The leakage and transfer of the potent allergic components from these materials is the cause of hypersensitive reactions to patients, dental professionals and the lab technicians. The biological side-effects of the materials that are in dental practice are rare. All artificial materials release substances in the oral environment which are potent allergens that might cause side effects and adverse reactions. The current study gives a statically about the dental professional knowledge and also makes the aware of toxic biological side effects of these materials, also make them aware of the reporting of the cases. A cross sectional survey was conducted among 100 dental professionals about the Toxic Biological effects associated with materials used in prosthodontics. Questionnaires with 20 close ended questions were circulated among people. Results obtained analyzed using SPSS software.In this Study, almost 80% of them were aware that some components of the dental materials might cause a hypersensitivity reaction. 57% of the participants have not experienced any allergic resin, latex, gloves etc.The prevalence of allergic reaction among Dental staff is low and most reported are due to latex, acrylate & formaldehyde. The present study shows that there is a lack of knowledge on the toxic biological effects of dental material used in prosthodontics.

Keywords: *Adverse effects; Awareness; Dental materials; Occupational hazards; Prosthodontics; Side effects; Toxic biological effects.*

Introduction

The smile is a key segment to the confidence of a person. At times, from a clinical viewpoint, it appears that ideas of denture aesthetics are being covered by mechanistic concerns for denture stability and capacity. The job of a prosthodontist is to advance oral health and dental aesthetics[12]. The ADA has defined prosthodontics as "the dental specialty pertaining to the diagnosis, treatment planning, rehabilitation, and maintenance of oral function, comfort, appearance, and health of patients with clinical conditions associated with missing or deficient teeth and/or oral and maxillofacial tissues using biocompatible substitutes."[3].Prosthodontics is an important aspect of dentistry. Prosthodontics usually deals with dentures, dental implants, oral and facial prostheses to provide solutions for the different dental issues experienced by patients.[45] Prosthodontists specialist aim to reestablish the appearance, speech , and swallowing capacity of the patients back to typical subsequent to being affected by an injury or infected condition [67]. Prosthodontics practice involves contact with restorative materials, auxiliary dental materials which are broadly of different composition. Regardless of the kind of medical procedure, there are advantages, dangers, and symptoms that might occur.[8] With dental prosthesis, the chances of issues are rather low, but

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there are still complications that can emerge [9,10] .The leakage and transfer of the potent allergic components from these materials is the cause of hypersensitive reactions to patients, dental professionals and the lab technicians.[11,1213]

An allergy is a disorder in which the body becomes hypersensitive to particular antigens which bring about characteristic manifestation whenever they are subsequently inhaled, ingested, injected, or in any case contacted [14].The biological side-effects of the materials that are in dental practice are rare. All artificial materials release substances in the oral environment which are potent allergens that might cause side effects and adverse reactions[15]. The incidence of these side effects to dental material is seen in 1 per 500 patients reported [16] which is quite high and most people don't report the incidence[17] A review article that 139 published cases of allergy to just base-metal alloy in RPD dentures [18] During dental treatment, different materials are utilized which can effectively affect patients and dental staff; henceforth it is important to utilize them with alertness.

The current study gives a statically about the dental professional knowledge and also makes the aware of toxic biological side effects of these materials, also make them aware of the reporting of the cases.There are many potential hazards & problems exist but only few are documented the adverse reaction. The clinicians are made aware to report the side-effects to the higher body.This study assesses the knowledge about the toxic Biological effects associated with materials used in prosthodontics among dental professionals, students and technicians and to create awareness on the reporting of adverse effects.

Materials & Methods

A cross sectional study conducted among 100 dental professionals, Dental students and Lab technicians in Chennai through online portal 'Google forms' TThe Participants were surveyed utilizing an organized poll including 20 close finished inquiries in regards to demographic details (age, gender, occupation) adverse effects of material, materials toxicity, biocompatibility tests etc..,(Table 1).For better understanding, occupation was put into 3 groups ,Group 1 ,Group 2, and Group 3 which represents Dentist, Dental students and Dental technicians respectively. All the collected data were then analyzed through SPSS software and pie charts were plotted with extracted data. Chi square test and pearson correlation analysis were used, with p value less than 0.05 to be statistically significant.

Result & Discussion

In the present study. A total of 100 Dental professionals were questioned out of which 39 (39%) were male and 59 (71%) female. The age range of the participants were from 18-50 & above.(table 2)For better understanding, occupation was put into 3 groups ,Group 1 ,Group 2, and Group 3 which represents Dentist, Dental students and Dental technicians respectively.

Prosthodontics application requires contact with restorative & auxiliary materials of various different compositions like metals, synthetic polymers, cements, impression material, restorative material – Dental amalgam composites & ceramics.[19,20] Different materials are used in fabricating and fitting prosthodontics appliances in patients. An association between groups and their awareness on dental materials used in prosthodontics was done [Figure 1].There was no significant difference between groups and awareness on dental materials.p=0.270;Statistically not significant. However group 2 participants are more aware of dental materials used in prosthodontics.Articles say that adverse reactions that [21,22]are related with type IV hypersensitivity reactions may be elicited by prosthodontics material but other should be considered.[23]

An association between groups and their opinion on materials used in prosthodontics are potent allergens was done[Figure 2].There is no significant difference between groups and awareness on components of dental materials which are potent allergens.p=0.154;Statistically not significant. However group 2 participants are aware that components of dental materials are potent allergens .

Data from [Figure 3] shows 57% of the participants have not experienced any allergic resin, latex, gloves etc.Association between groups and their experience of allergic reaction from dental material was done [Figure 4].There is no significant difference between groups and the experience of allergic reaction to dental materials. p=0.681; Statistically not significant. However , the majority of Group 2 participants have not experienced any allergic reaction.The prevalence of allergic

reaction among Dental staff is low and most reported are due to latex, acrylate & formaldehyde. Poly methyl methacrylate in latex triggers a delayed hypersensitivity reaction and sodium metabisulphite & nickel-immediate response.[24,25]

Patients might experience reactions when skin comes in contact with the allergen or exposure to dust, inhalation of fumes etc., [26,10,27–29,30]Most potential problems are to be brought using recommended safety protective devices[31] Removable acrylic resin partial dentures will generally ominously impact periodontal parameters when teeth are in contact with resin base. This impact is expanded with longer length of RPD wear. [3233]

Frequent dermatological reactions associated with dental materials are transient redness, irritation, blisters, soreness, pain etc..[34], caused by latex gloves, resin. Eugenol cements, impression material etc.,[35,36,37]which says most of them are not aware of the reactions as all the above mentioned were given in option. And a review conducted by subashree says that aloe vera gel can be considered a safe alternative treatment for patients with OLP[38].According to a study, Non dermatological actions include damage to eyes from UV light and visible light used in everyday practice, Which also implies, there is no proper knowledge on how the reactions are seen.[36] .an association between groups and their response on hypersensitivity reaction caused by dental materials was done [Figure 5] .There is no significant difference between groups and hypersensitivity caused by dental materials.p=0.371;Statistically not significant.However majority of group 2 says type 4.

Specialists concur similar sensitivities identified with dentistry incorporate deferred Hypersensitivity responses to explicitly include delayed Hypersensitivity reactions to specific Dental materials, true allergic reaction is uncommon[39]

Resins contain cadmium salt as a coloring agent along with other agents [10,27,28]. This poses a potential hazard to the technician while they routinely grind, finishing prosthesis made resin based materials.[40,41]Some metals used in dental alloys are biologically active & are potential hazard, includes nickel, chromium, cobalt, cadmium, beryllium [1810,27–29] most common clinical symptoms – gingivitis, stomatitis almost 25% of patient[42].Study also says eugenol is a known cytotoxic and allergic substance that causes irritation to tissues.[43]

Many preclinical biocompatibility tests are available to minimize risk test categorized basis on applicability levels primary cell culture test, hemolytic test etc., Secondary tests – implementation test, skin irritation test etc..,[45]An association between groups and their awareness on preclinical tests that are available to minimise the risk of adverse effects to dental materials among the participants[Figure 6].There is no significant difference between groups and awareness on preclinical tests.p=0.415; Statistically not significant . However , Group 2 is aware of preclinical tests more than others.

Table 1: Questionnaire of the survey

1	Age	18-30 30-50 50 and above
2	Gender	Male Female
3	Occupation	Dental Student Dentist Dental Technician
4	Prosthodontics deals with a lot of materials like resin polymer, Impression materials etc..,	Yes No

Cont... **Table 1: Questionnaire of the survey**

5	Are you aware that more than 75% of all existing dental materials are directly or indirectly used in fabricating and providing prosthodontics restoration?	Yes No
6	Some components in these might have potent allergen or leak and cause a hypersensitivity reaction	Yes No
7	Have you ever experienced any allergic reaction from the dental materials? Like acrylic resins, latex gloves, impression materials etc.,	Yes No
8	Did any of your patients / co-worker have an allergic reaction to the dental materials?	Yes No
9	Patient might experience adverse effects due to	-Contact with skin -Exposure to dust from miking, grinding, polishing -Inhalation of fumes and vapors -None of the above
10	What do you think are frequent dermatological reactions seen in hypersensitivity to materials	-transient redness -irritation -pain -None of the above
11	Non dermatological reactions includes	-damage to eyes -Respiratory reactions -others -None
12	What type of hypersensitivity reactions is more common to prosthodontics materials?	Type 1 Type 2 Type 3 Type 4
13	Studies say that 1 out of 300 patients are experiencing adverse effects reaction	Yes No way
14	Are you aware that component of polymer resin-cadmium salts is a potential hazards to the dental technicians	Yes No
15	Which metals do you think might cause an allergic reaction	Chromium Cobalt nickel Gold Nickel titanium None

Cont... **Table 1: Questionnaire of the survey**

16	Eugenol is	Cytotoxic Allergic substance Both A & B None
17	Are you aware of preclinical tests that are a available to minimize the risk of adverse effects to dental materials	Yes No
18	What do you think can be done to reduce these adverse effects	
19	Did you report the hypersensitivity reaction occurred to you or patient to certifying bodies ?	Yes No
20	Was this survey helpful	Yes No

Table 2 : Demographic details of the study participants .(n=100)

Characteristics	**Frequencies,(n%)**
Age category(years) 18-30 30-50 50 and above	 68(68%) 22 (22%) 30 (30%)
Gender Male Female	 59 (59%) 39 (39%)

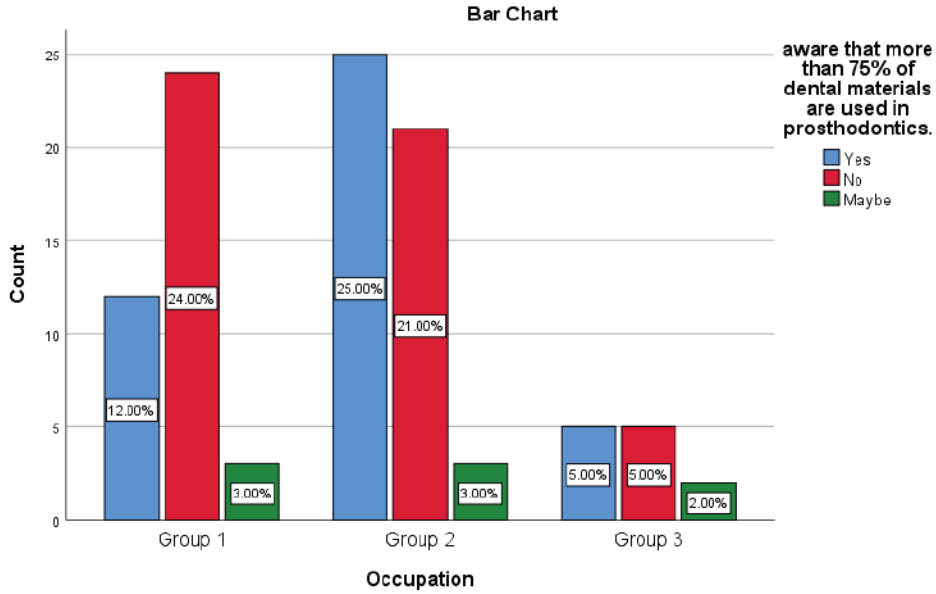


Figure 1. Bar chart showing association between groups and their awareness on dental materials used in prosthodontics. X-axis represents the groups and Y- represents the number of responses .Where blue, red and green bars denote yes, no and may be respectively. There is no significant difference between groups and awareness on dental materials. Chi square test was done ;p=0.270;p value>0.05, Statistically not significant. However group 2 participants are aware of dental materials used in prosthodontics.

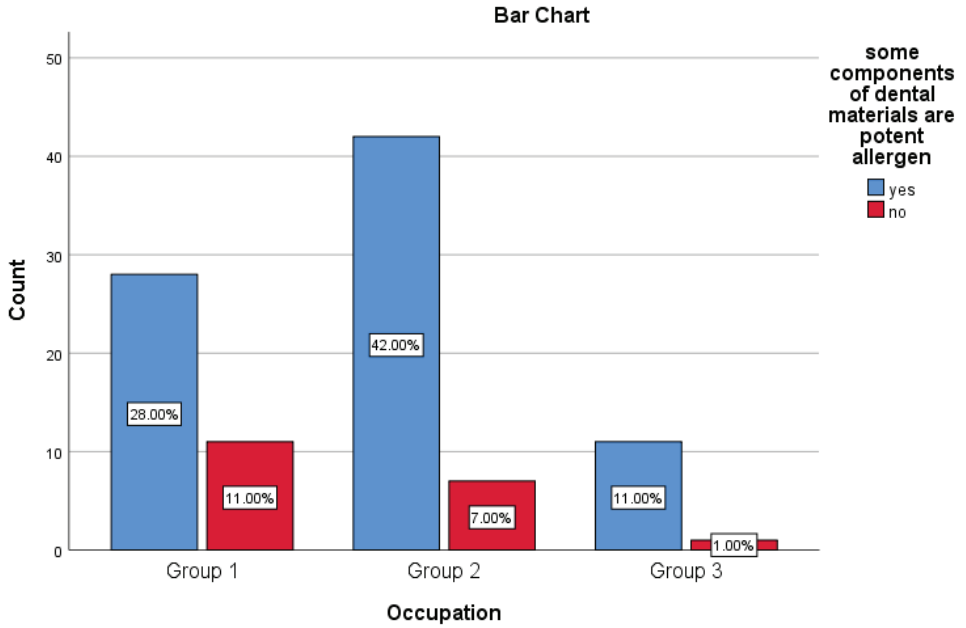


Figure 2.Bar chart depicts the association between groups and their opinion on materials used in prosthodontics are potent allergens. X-axis represents the groups and Y-axis represents the number of responses, where blue and red bars denote yes and no respectively. There is no significant difference between groups and awareness on components of dental materials which are potent allergens.chi square test was done;p=0.154;p value>0.05 Statistically not significant. However group 2 participants are aware that components of dental materials are potent allergens .

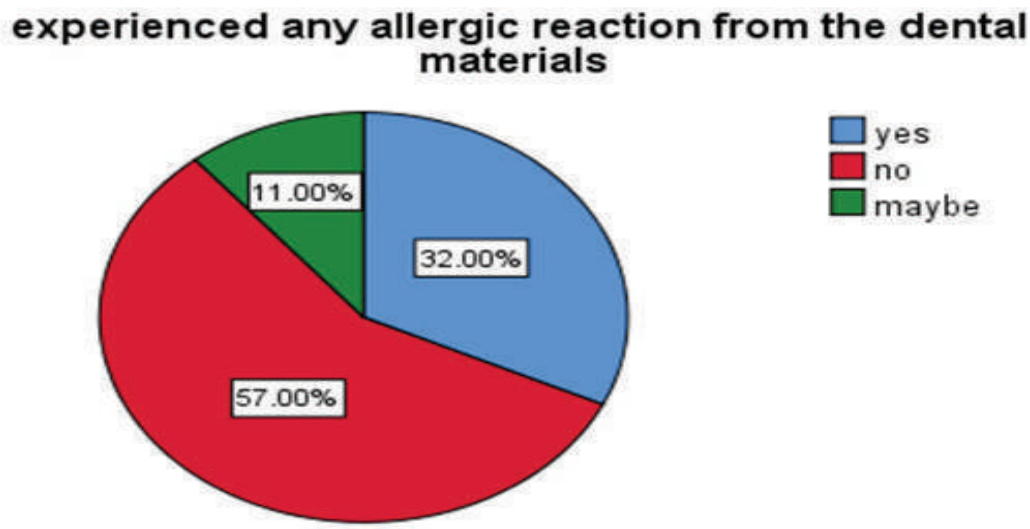


Figure 3. The pie chart shows the response to the question " experience of allergic reaction from dental material".32% said they have experience , while majority (57%) have not. Majority of them haven't experienced adverse reactions to dental materials.

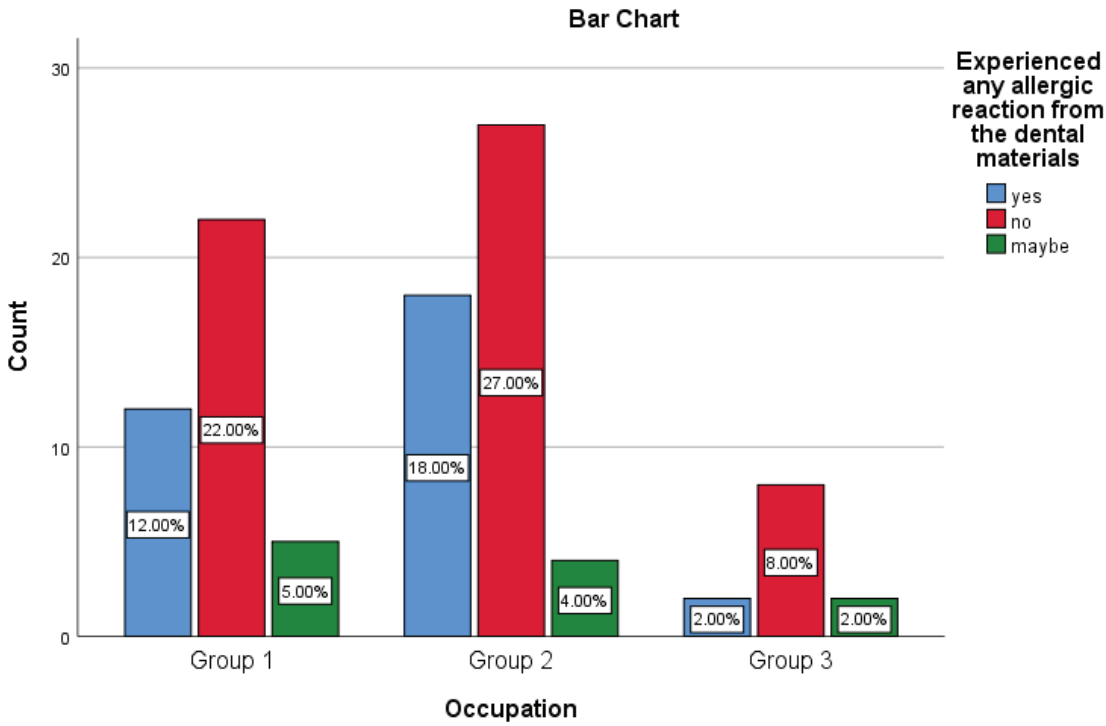


Figure 4.groups Bar chart showing association between groups and their experience of allergic reaction from dental material.X-axis represents the groups and Y-axis represents the no. Of response where blue, red and green bars denote yes, no, and may be respectively.There is no significant difference between groups and the experience of allergic reaction to dental materials.chi square test was done ;p=0.681;p value>0.05 Statistically not significant. However , the majority of Group 2 participants have not experienced any allergic reaction.

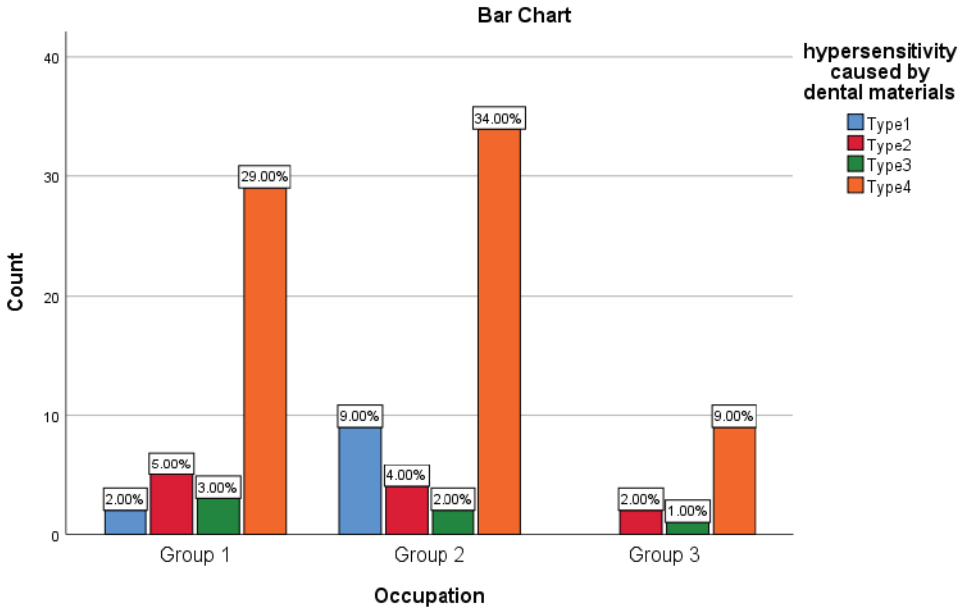


Figure 5.Bar chart showing association between groups and their response on hypersensitivity reaction caused by dental materials.X-axis represents the groups and Y-axis represents the no. Of response where blue,red,green,and orange denote Type1, Type 2, Type 3 and Type 4 respectively.There is no significant difference between groups and hypersensitivity caused by dental materials.chi square test was done;p=0.371;p value>0.05 Statistically not significant.However majority of group 2 says type 4.

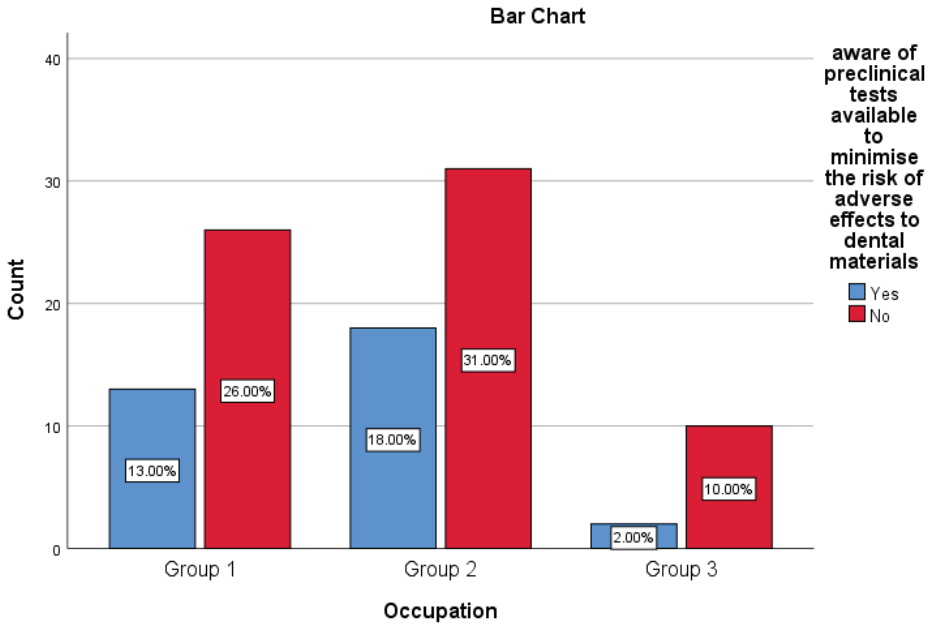


Figure 6.Bar chart showing association between groups and their awareness on preclinical tests that are available to minimise the risk of adverse effects to dental materials among the participants. X-axis represents the groups and Y-axis represents the no. Of response ,where blue and red bars denote yes and no respectively. .There is no significant difference between groups and awareness on preclinical tests.chi square test was done ;p=0.415;p value>0.05 Statistically not significant . However , Group 2 is aware of preclinical tests more than others.

The study was taken in a small population where there was difference in opinion, language communication or resource problems. The actual numbers of respondents are low compared to the total number of dental professionals and therefore might not be representative of the entire group. This study might be helpful in other similar studies.More surveys should be taken on this field so that we can assess people's awareness and knowledge on different places.

Conclusion

From the study, it can be concluded that the majority of dental professionals lack knowledge about the toxic biological effects associated with dental materials used in prosthodontics. The study also shows that dental students are more aware about dental materials and it's biohazard than dentists and dental technicians. Proper education & conferences should be conducted among the dental professionals to create awareness on the toxic effects of the dental materials.

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Assessment of Partial Edentulism Based on Kennedy's Class III Classification

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Abstract

Edentoulousness falls in a different category among the various conditions of dental origin. A partially edentulous arches classification helps to identify the possible combinations of teeth to edentulous ridges, thus facilitating discussion and communication among dental professionals. Kennedy's classification provides immediate visualisation and assessment of design features of removable partial dentures. The aim of the study was to assess the prevalence of partial edentulism based on Kennedy's class III situation in the upper arch among the patients visiting a private dental hospital. This retrospective study included 10,327 subjects who reported during June 2019 to March 2020. The sample size was collected and retrieved from the online database. Tabulation of data was done followed by statistical analysis. Out of the 10,327 subjects reported to the dental hospital, 6453 subjects were diagnosed with Kennedy's class III (62.4%). It was most commonly seen in age groups of 50-59 years (26.7%), and in males (54%). There was a significant association between gender and Kennedy's class III (p=0.000) and age and Kennedy's class III (p=0.000) Within the limits of the study, it was found that there was a significant relationship between gender and Kennedy's class III and age and Kennedy's class III.

Keywords*: Upper arch, Kennedy's class III, Prevalence, Age, Gender*

Introduction

Tooth loss has an impact on an individual's oral health related to the quality of life at biological, social and psychological levels. [1,2] The teeth in the oral cavity provide the main functional component of the oral cavity. It provides various functions such as mastication, speech and esthetics. [3,4] Therefore, absence in the teeth results in difficulty in chewing, alteration of speech and esthetics. [5,6]. According to the World Health Organisation, it was stated that an adult should have a minimum of 21 teeth which are functional for a good dietary intake. Therefore, oral health plays an important role in the quality of life [7].

Tooth loss occurs in the oral cavity by various causes like dental caries, pulpal and periradicular, periodontal disease, trauma and various systemic diseases. [8,9] Tooth loss creates a space in the oral cavity which is called as edentulous spaces. Edentulism indicates the awareness of the oral health and treatment required and provided in the population.[10,11] The variation and location of the teeth and edentulous spaces is necessary to classify the partially edentulous arches. The purpose of classification provides communication between dental colleagues, students and technicians regarding the case, for a good treatment planning. It also predicts the difficulties which occur commonly with a particular removal partial denture. [12] People living in rural and remote areas may follow a form of healthcare based on ancient traditions, beliefs and cultural habits. A major challenge for the dental profession will be to plan oral healthcare for this

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group of patients, and the attitudes of adults to healthcare and acceptance of treatment will be of fundamental interest. To organize and implement adequate strategies for the prevention and treatment of oral diseases more information is required about the reasons for extraction of permanent teeth. [13]

Partially edentulous arches have been classified by various methods. The possible combinations of partial edentulism depending upon their incidence in maxillary and mandibular arches. [14] Among the various classifications like Kennedy's, Applegates, Avant, Eicher, American college of Prosthodontics, Kennedy's classification is widely studied and most accepted in the Dental community. [15,16,17] As per Kennedy's classification, there are four main types of partial edentulous arches such as Class I, Class II, Class III, Class IV. It is known to be widely accepted due to immediate visualisation and recognition of prosthesis support. Kennedy's class III is a unilateral edentulous space which is bound with natural teeth anterior and posterior to the edentulous space. It is known as tooth-borne support to remaining natural teeth. [18,19] The frequency of partial edentulousness seems to vary widely between different countries. The prevalence and patterns of tooth loss have been studied to a certain extent in western countries and a few such studies have been carried out in this country.

The aim of the study was to assess the prevalence of Kennedy's class III situation in the upper arch among the patients visiting Saveetha dental college and hospitals.

Materials and Methods

The study population consisted of 10,327 patients who reported to the private dental hospital from June 2019 to March 2020. Online database was used for data retrieval. Ethical clearance for this study was obtained from the institutional ethical board. Both males and females of the age group 18-90 years of age were taken into account.

All the case sheets available on the online database were taken for the study. Cross-verification of the data for error was done via photographs, data evaluation and presence of additional reviewers. 10,327 patients were included to minimise the sampling bias. Relevant data such as age and gender were recorded. Patient's Kennedy class III relationship was studied. Repeated and incomplete data records were excluded. Data was verified by an external reviewer.

Data was retrieved and entered in Microsoft Excel sheet and later exported to SPSS software (version 20.0) for statistical analysis. Independent variables included age and gender. Dependent variables included Kennedy's class III in upper arch. Both frequency and chi-square tests were employed. Level of significance was set as $p<0.05$ for this study.

Results and Discussion

Edentoulous arches fall in a different category among the various conditions of dental origin. A partially edentulous arches classification helps to identify the possible combinations of teeth to edentulous ridges, thus facilitating discussion and communication among dental professionals. Kennedy's classification provides immediate visualisation and assessment of design features of removable partial dentures.

This study found that 50-59 years of age was most commonly diagnosed with Kennedy's class III situation. [20] The prevalence rate of Kennedy's class III was found to be 62.1%. In the studies reported by Kruchinin et.al, [21,22] and Fayad et.al [23,24] reported that Kennedy's class III had prevalence of around 50-55% approximately.

According to the study, it was reported that there was a significant relationship between gender of the patient and prevalence of Kennedy's class III situation. Similarly, studies reported by Prabhu et.al, [25,26] that there was a significant relationship between the gender of the patient and Kennedy's class III. Niarchou et.al [27] and Shah et.al, [28] reported that there was no significant relationship between the gender and patient's diagnosed with Kennedy's class III situation.

According to our study, it was seen that males were more commonly diagnosed with Kennedy's class III than female subjects. Similar results were reported by Niarchou et.al, but the studies reported by Sapkota et.al [29] and Caldas et.al [30] reported dissimilar results saying that female subjects were more commonly diagnosed with Kennedy's class III than male patients.

In our study, it was seen that the most common age group to be prevalent to class III situations were 50-59

years. But, the studies reported by Abdel et.al [31] reported that young individuals between 30-40 years were more commonly diagnosed with Kennedy's class III and a statistical relationship was seen.

Further studies on the topic could involve larger sample size, treatment modalities and location could be multi-centre.

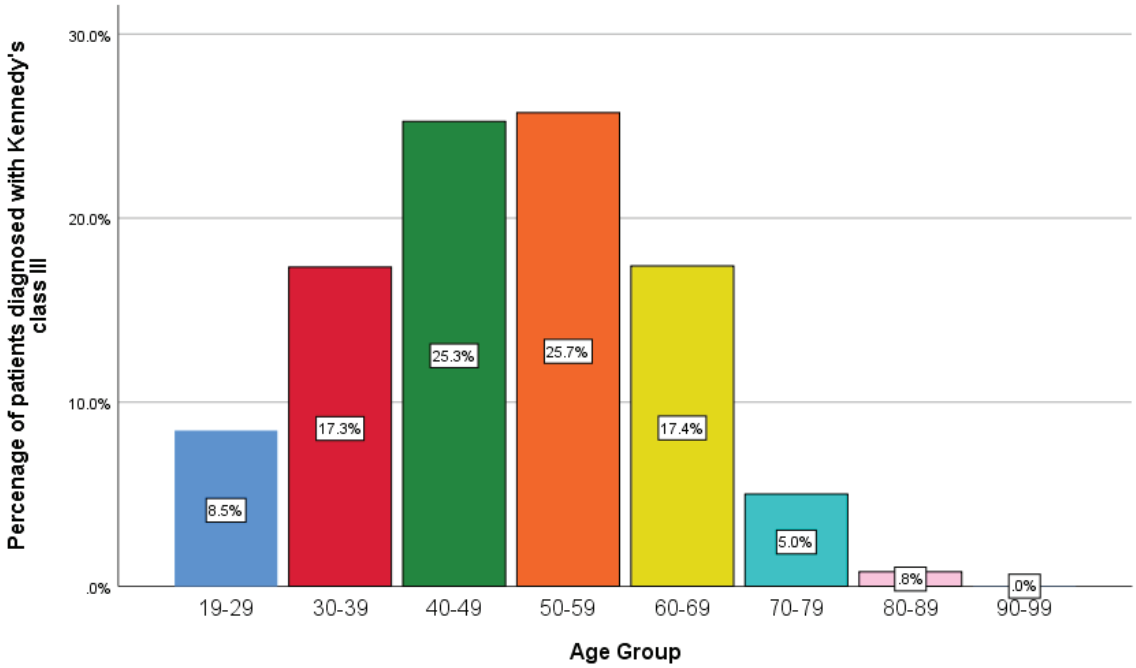


Figure 1: Bar graph showing the age distribution among patients diagnosed with Kennedy's class III in upper arch. X axis represents the age distribution while Y axis represents the count of patients. Highest number of patients seen between 50-59 years (26.7%) followed by 40-49 years (25.3%)

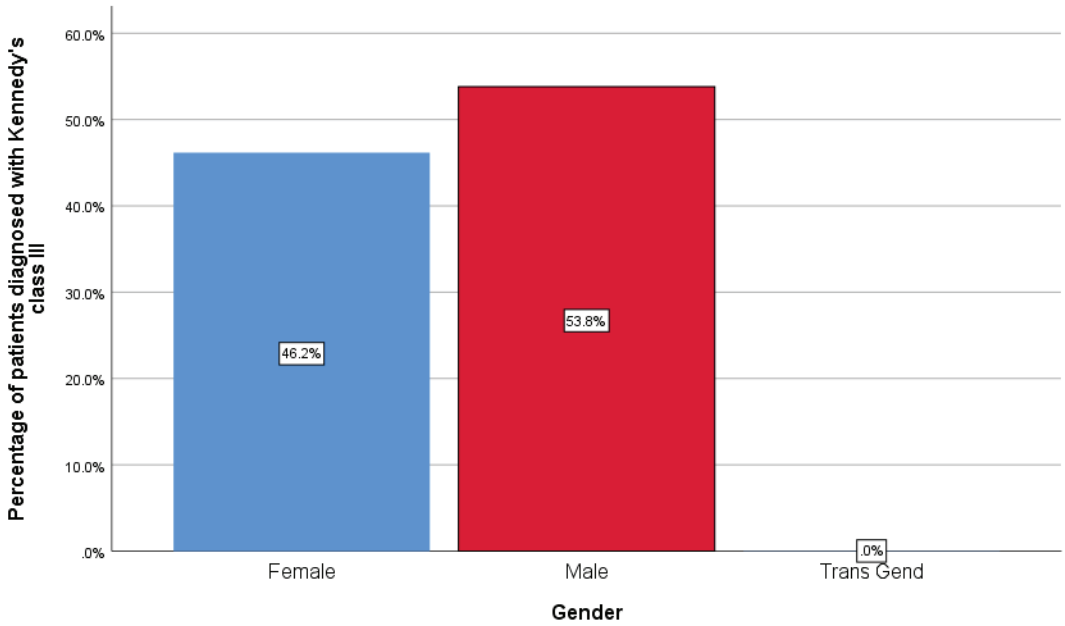


Figure 2: Bar graph showing the gender distribution among patients diagnosed with Kennedy's class III in upper arch. X axis represents the gender distribution while Y axis represents the count of patients. Highest

Kennedy's class III in upper arch was diagnosed in Males (53.8%) followed by female subjects (46.2%)

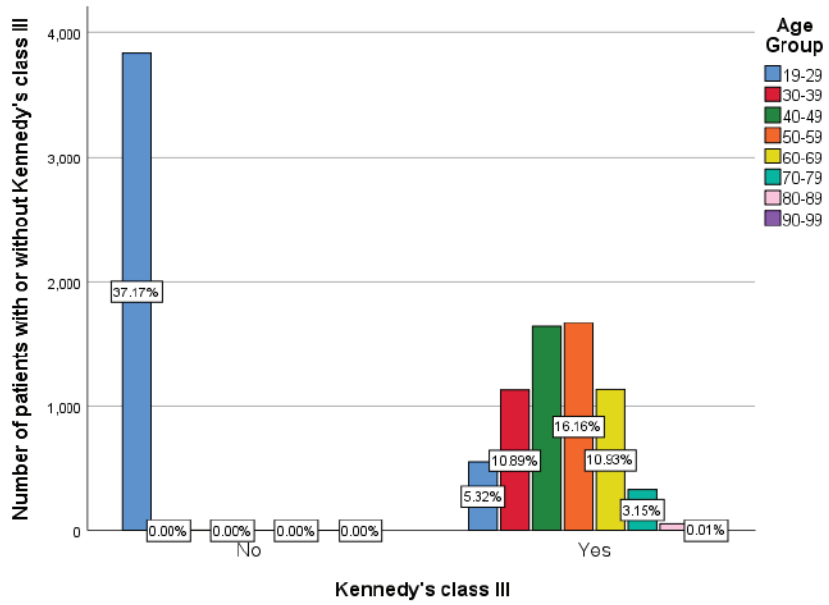


Figure 3: Bar graph showing the association between age group and Kennedy's class III in upper arch. The X axis represents the Kennedy's distribution between the age groups while the Y axis represents the count of patients. Blue represents 19-29 years, red - 30-39 years, green - 40-49 years, orange - 50-59 years, yellow - 60-69 years, light blue-70-79 years, pink - 80-89 years and purple- 90-99 years. Chi-square test was done and association was found to be statistically significant. Pearson's chi-square value: 8265.301,DF:2, p value: 0.000 (p<0.005), proving 50-59 years old were commonly diagnosed with Kennedy's class III.

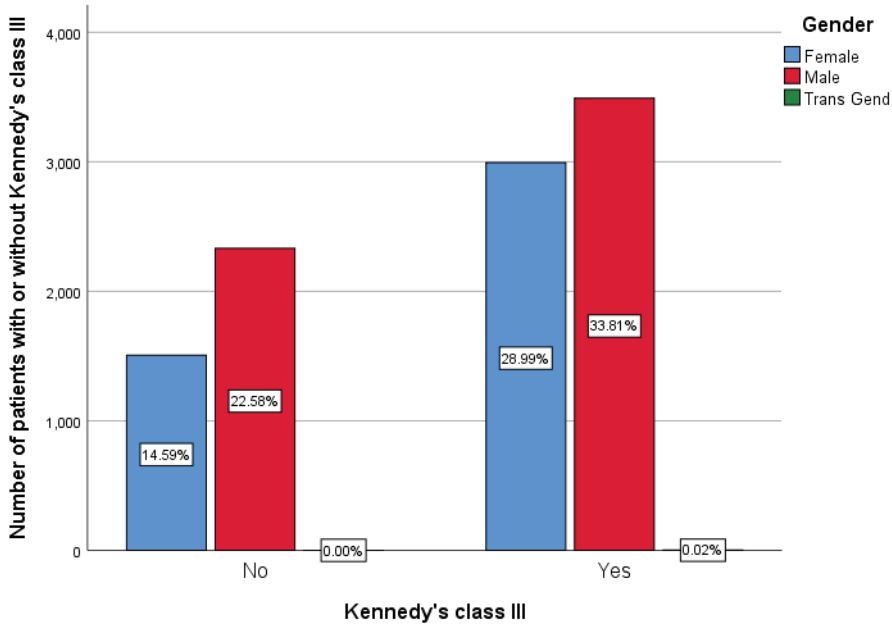


Figure 4: Bar graph showing the correlation between gender and Kennedy's class III in upper arch. The X axis represents the Kennedy's distribution between the gender while the Y axis represents the count of patients. Red represents male subjects, blu - female subjects, green - transgender. Red represents male subjects, blue- female subjects, green - transgender. Chi-square test was done and the association was found to be statistically significant. Pearson's chi-square value: 48.054,DF:2, p value: 0.000 (p<0.005), proving males were more commonly diagnosed with Kennedy's class III.

Conclusion

Within the limits of the study, the prevalence rate of Kennedy's class III situation in the upper arch was found to be 62.4% where the most prevalent age group was found to be 50-59 years with more male predilection. There was a significant association between age, gender and Kennedy's class III.

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Ethical Clearances: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Evaluation of Mandibular Third Molar Extractions Performed By Intra Alveolar Method as Opposed to Transalveolar Method

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Abstract

The aim of the present study was to retrospectively analyse the prevalence of intra-alveolar and trans-alveolar extraction of mandibular third molars in patients at Saveetha Dental college. Retrospective data of 1747 patients was obtained and segregated. The inclusion criteria included 18-90 years are group, underwent extraction and visited between June 2019 to April 2020. Once the data was obtained it was statistically analyzed using SPSS by IBM version 20. The most commonly used method of extraction was the conventional technique (90.8%) comprising the majority whereas the trans alveolar method comprised (9.2%). A high association and significance was noticed between the age of the patient and type of extraction performed(P= 0.000)Extraction being one of the most common dental procedures that is being done, it is important to approach the same with the adequate and appropriate technique to prevent further complications as a result of the procedure

Key words: *extraction, forceps, conventional, transalveolar, mandibular.*

Introduction

There are various situations in which intervention from an oral and maxillofacial surgeon is required.[1–5] . However before treatment planning there are various factors to be considered such as systemic health[67]. Before attempting a procedure a comprehensive view on the same and disposal is required [8] Extractions are routine procedures in dental surgery.[9] Within the last few decades there has been an increase in the need for the same due to poor oral health, changing habits[10] and oral hygiene practices.[11–13] Traditional extraction techniques use a combination of severing the periodontal attachment, luxation with an elevator, and removal with forceps. If the elevator fails to cause noticeable separation of the tooth from the socket, the forceps accomplish the work through intermittent apical and lateral forces [14,15]. The development of many surgical techniques when systemic effects [11,16] are considered and newer designs of instruments for the procedure as well as administration of local anesthesia [17,18]has enabled the practitioners to carry out extractions with lesser complications.

But even now extraction of third molars can be an unpleasant procedure for patients and fear and anxiety[19] are major ruling factors in the same and dentists [20–22], due to the wide anatomic variance of the teeth and poor access and visibility[23], than for other groups of teeth.There is a certain level of pain perceived while administration of local anaesthesia [24].Although there are recent approaches to reduce the pain and treat the pain [21,25.26] As root fusion is common in these cases, the extraction of the lower third molars can be more difficult than the extraction of the other molars of the

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human dentition. There are various complications that can result from Mandibular third extraction [27,28] and thus the technique for the procedure should be adequately chosen and executed with precision. It is important to take a comprehensive approach towards the same while keeping the medical history as a very important determinant for treatment planning.[29–31]

The two widely used are the transalveolar technique and the conventional technique. In conventional technique (also known as forceps,traditional or intra-alveolar technique), the tooth is extracted just like any other tooth in which an elevator to raise the mucosal tissue, severe the periodontal attachment and luxate the tooth within its bony socket and removed with the help of forceps and [32]. This technique can be followed when the tooth has reached the occlusal plane. Transalveolar extraction involves the removal of an impacted mandibular third molar often with the minor drilling of bone and sectioning of the crown under local anesthesia. [33]However the complicated rate with transalveolar extraction is much higher and there is an increased amount of postoperative pain that is produced to the patient.[33] It is important to place patient care at the focus for a practitioner. The aim of the study is to assess the Prevalence of transalveolar versus conventional technique in the extraction of lower third molar.

Materials and Methods

The present study involved a total of 1747 patients that underwent extraction of Mandibular third molar teeth. These included teeth that were extracted with forceps extraction and transalveolar extraction. The study was performed in a university setting at Saveetha Dental College and Hospitals. Thus the data obtained from the patients is of the same geographic location and ethnicity. The ethical approval for collection of retrospective data from the dental patient management archives was obtained from the Institutional Ethics Board. IRB Approval No: SIHEC/2020/DIASDATA/0619-0320).

The period of the study was between June 2019 to April 2020. Once the data was collected the same was verified by using photographs by two external reviewers who were blinded on the hypothesis from the present study. This was done to eliminate the chances of sampling bias. Before the commencement of the study a clear well defined inclusion criteria was defined. The inclusion criteria included that:

· Patients should have visited Saveetha Dental College during the study period.

· Patient has been treated by a resident of Saveetha Dental College, either an undergraduate or postgraduate student.

· Should have undergone extraction of Mandibular third molar with forceps or with transalveolar method

· Should have been within the age group of 18-90 years

Out of the study population that was chosen for the study there was no segregation process, as this would result in sampling bias. The data segregation was done according to various parameters such as speciality of clinic in which patient was treated, age of the patient, gender of the patient etc.

The data that was then tabulated was reviewed by an external reviewer and screened for internal validity of the study. The data was then exported to SPSS Software by IBM Version 20 for Statistical Analysis. Descriptive statistics was performed followed by Correlation tests to see any kind of correlation or taken in the present study.

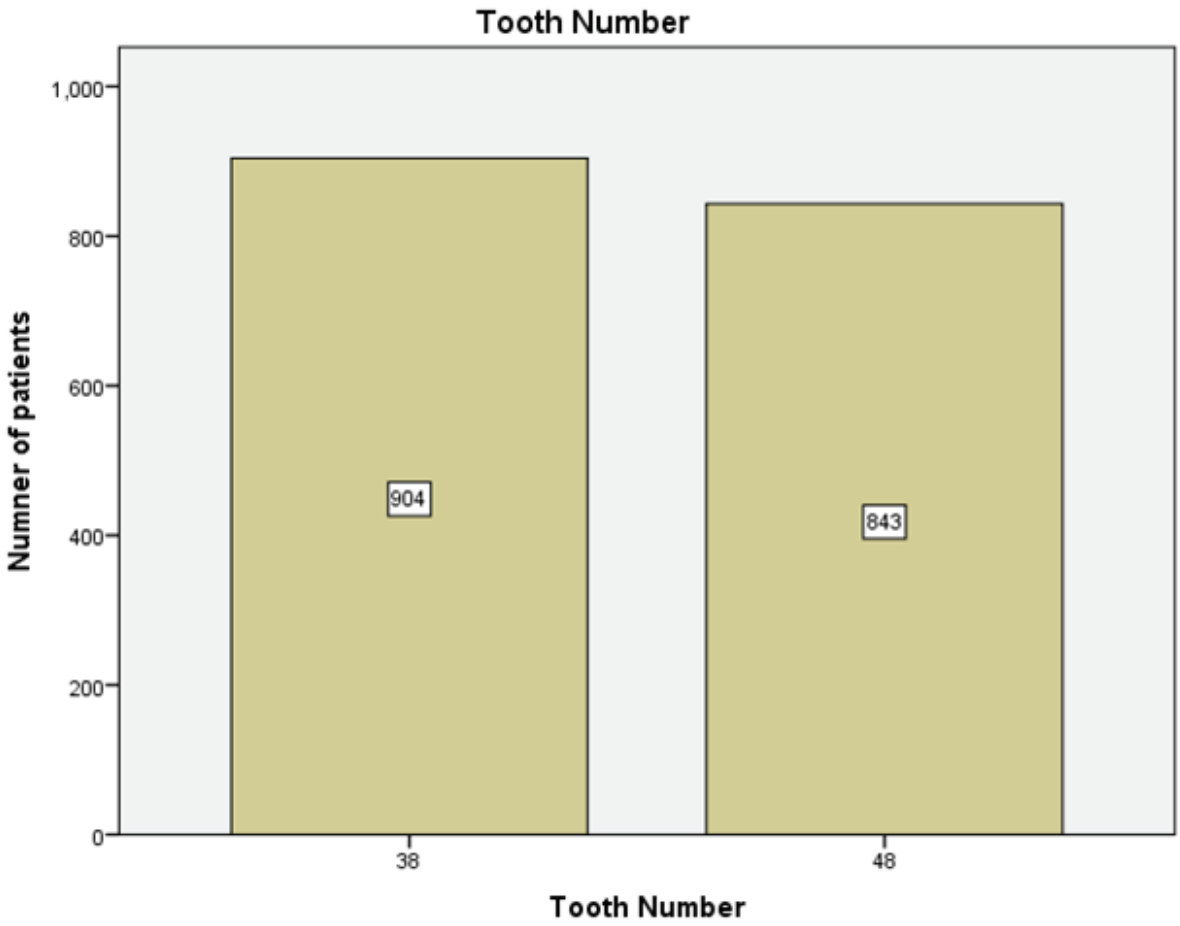
Results and Discussion

In the present study out of the total of 1747 that were included as part of the study population who underwent extraction of their third molar teeth, there was almost an equal gender predilection with males at 49.2% and females at 50.8%. The mean age of the patients undergoing extraction of their Mandibular third molar teeth is 41.84 plus or minus 14.47 years.

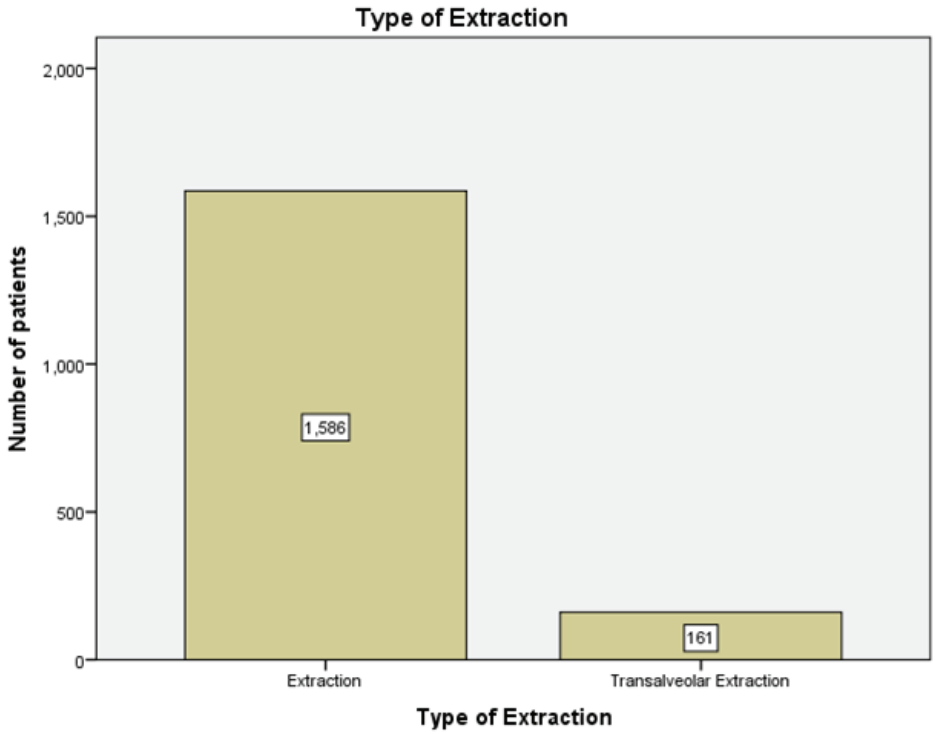
Out of the total extractions that were performed most were of tooth number 38,(51.7%) and 48.3% were tooth number 48. On the basis on the morality of treatment that was done, the major share was by conventional extraction (90.8%) whereas a smaller portion was by transalveolar approach (9.2%)

Maximum number of extractions were performed in patients between the age group of 18-30 years (27%). In the analysis of gender and the treatment modality that they have undergone it is observed that males tend to undergo a higher number of transalveolar extractions(52.1%)

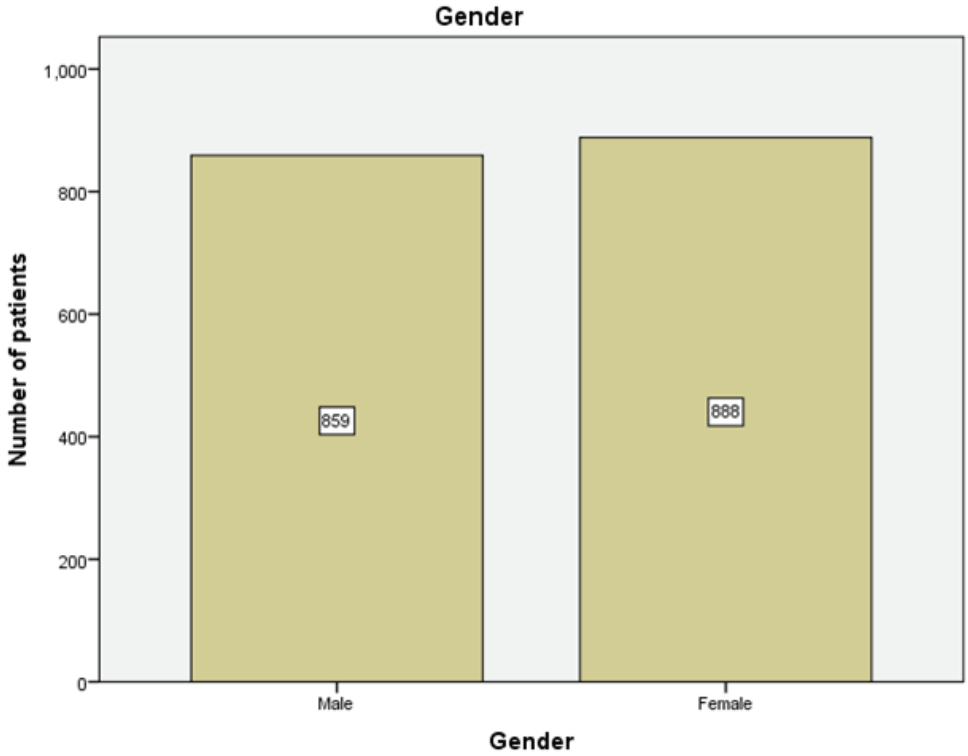
whereas females tend to undergo a higher number of conventional extractions (51.1%). There was no statistically significant association that was observed between the tooth to be extracted and the type of extraction that is followed for the same. Although there was a highly statistically significant Correlation that is observed between age and the type of extraction that is performed. (p= 0.000). Out of the total transalveolar extractions that were performed 133 of them were from impacted third molars while the rest were due to ankylosis, fracture of tooth, attempted forcep extraction etc. Out of the total 161 transalveolar extractions that are seen, 40% were seen in the age group of 31-43 years whereas 27.4% of the total forcep extractions are seen in the age group of 18-30 years.



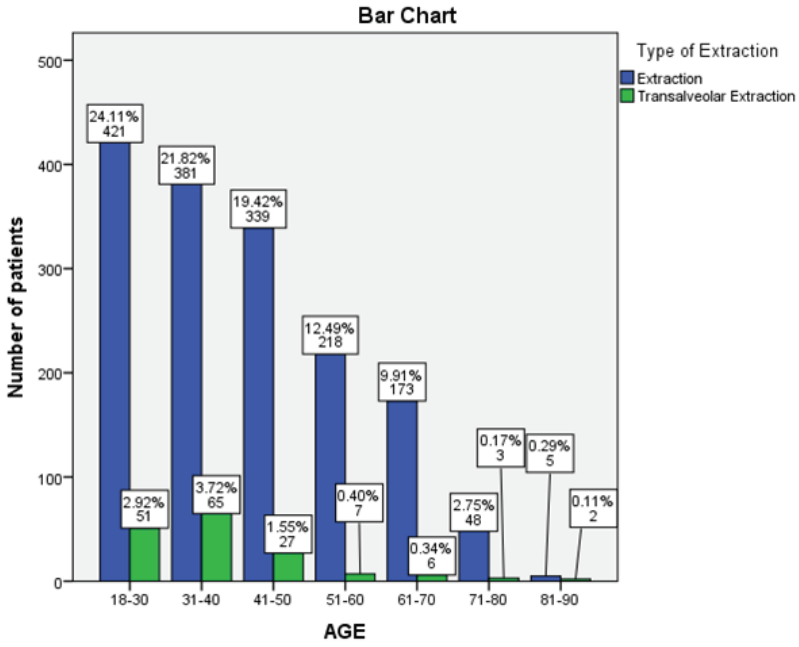
GRAPH 1: Represents the distribution of the teeth undergoing extraction with tooth number in the x axis and number of teeth extracted in the y axis. It is understood that mandibular left third molars (38) undergo extractions more often that mandibular right third molars(48).



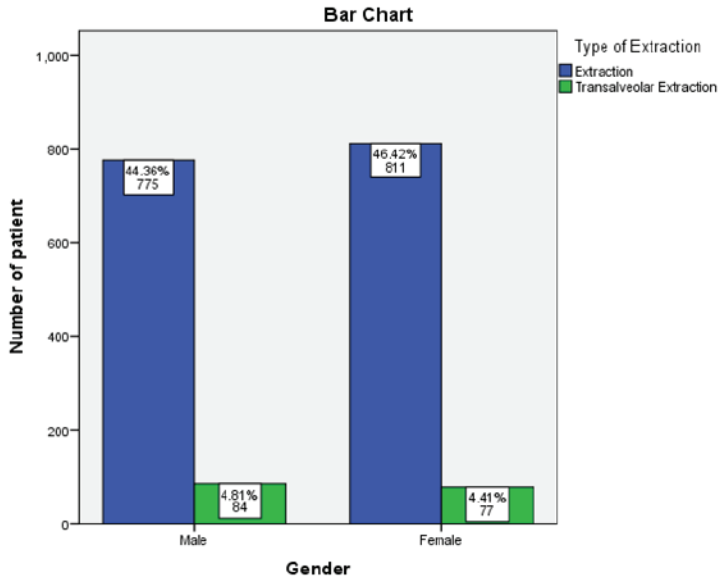
GRAPH 2: Represents the frequency of the different types of extractions performed with the type of extraction in the x axis and number of procedures in the y axis. Forceps/elevator extraction constitutes 90.8 %of all extractions performed which is greater than transalveolar for mandibular third molars



GRAPH 3: This bar chart represents the frequency of males and females who have undergone extraction with the gender in the x axis and number of patients in the y axis. It shows that both genders have undergone almost similar number of extraction.



GRAPH 4: This Bar Chart shows the association between age of the patient and type of extraction. X axis denotes the age of the patient and Y axis denotes the number of patients. The blue colour represents forceps extractions and green colour represents the transalveolar extractions. Maximum number of extractions were performed between 18-30 years, Maximum trans alveolar extractions between 31-40 years and Maximum forceps extractions between 18-30 years. Chi square test was done and association was found to be significant. Pearson Chi Square Value= 39.379, df=6,p=0.00(<0.05), hence statistically significant, proving younger individuals more often undergo extractions of mandibular third molars,



Graph 5: Bar graph shows association between type of extraction and the gender of the patient. Gender is plotted on the x axis and the number of patients is plotted against the y axis. Forceps extraction is represented by blue and transalveolar type of extraction is represented by green. It is observed that forceps extraction is performed more commonly in females whereas transalveolar type of extraction is performed more commonly in females. However there is no statistical significance for this association , Chi Square Test, Value=0.640,df=1,p=0.4(>0.05)

It is surprising that the amount of literature available towards the conventional type of extraction for Mandibular third molars is insufficient. This is the first of its kind study where the treatment modalities is compared for third molar.Although there is an increased amount of complications results from these procedures[34]

When the age group of the patients who underwent extraction of their third molars, it was observed that it was performed between the age group of 18-90 years, and the mean age group to undergo extraction is 41.84 years. Maximum number of extractions were performed between the age group of 18-30. There are various reasons for this particular aspect of the study. One of the most common reasons would be the progression and development of caries in an earlier age group as reported by Marthaler et al [35]. It is also to be remembered that this age group is prone to the eruption phases of Mandibular third molar teeth. However with evolution and recent changes in habits there has been a change in overall eruptive patterns in relation to dentition as such. Although it is expected that an older age group would have a greater count on extractions due to periodontal status, however in the present geographic location it is more prevalent in younger patients. There is also Prevalence of aggressive periodontitis [36,37] in this age group which could be attributed to the reason for extraction. Out of the total transalveolar extractions, most of them were performed within the age group of 31-44 age group. Although the ideal age reported by Al-Angudi et al [38]is 19-26 years, there could be an occurrence of trauma or any other attributed factors in the current population which could result in the same. There is no literature evidence on occurence of ankylosis and hence further studies are to be done to evaluate the same to ease the process for both clinician and the patient undergoing the same.

The most commonly performed type of extraction is conventional method as opposed to transalveolar method. It is to be noted that a more conventional type of extraction is to be done for Mandibular third molars to prevent the occurrence of various complications and pain that is associated with transalveolar extraction. It is important to tackle the entire clinical practice with a patient oriented approach and the patient should be the centre of priority. It is evident from the above discussion that extraction by conventional method would leave the patient far less painless and the chance of development of postoperative complications is also limited while using this technique. Thus further awareness programmes and studies are to be done to ensure better patient care and operatory comfort ability as well.

The limitations of the present study included that it is geographically isolated and single centered. There is no ethnic grouping that is performed in the study, this could influence the results of the study as the pattern of impaction is different in populations of different ethnicity.

Conclusion

Extraction being one of the most common dental procedures that is being done, it is important to approach the same with the adequate and appropriate technique to prevent further complications as a result of the procedure. Within the limits of the present study, simple forcep type of extraction is performed more frequently than transalveolar for lower third molars. Further multi centered studies are to be performed to assess the approach in various parts of the world and to bring about a concrete clinical practice guideline which would help the members of the dental fraternity as well as provide a better experience for the patients undergoing the procedure.

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Conflict of Interest: No conflict of Interest

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Analysis of the Association between Fast Food on Oral Health and Hygiene - A Survey

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Abstract

Health is a state of complete physical, mental and social well being of an individual and not merely the absence of disease or immunity status of an individual. An unhealthy diet is proven to implicate many risk factors for the incidence of oral diseases. Thus, there are various health education programs to educate people on the importance of a proper healthy diet, thereby reducing the incidence of oral diseases prevailing because of an unhealthy diet. Thus, this survey was done to evaluate the awareness of people towards maintaining a proper healthy diet. This study was done to check the awareness of people towards the risk factors and impacts of fast food on the oral health status of a person. This is a questionnaire based survey. An online questionnaire was shared among 100 dental students in Chennai. Based on the answers from the survey, the data was compiled and analyzed and based on the results of the survey, the responses were analysed through statistical analysis software SPSS version 20.0. The results of the study showed that 90% of the individuals who took part in the survey consumed fast food, out of which 78% of them were non-vegetarians. 91% of them were aware of the complications of having an unhealthy diet and what risk factors it possessed to the oral health of an individual. 40% of the individuals consume fast food at least once a week. 77% knew the causative reasons for the incidence of dental caries in an individual. Within the limits of the study, the study results showed that most individuals who took part in the survey consume food from fast food and they weren't aware of the impacts of unhealthy nutrition and bad hygiene measures. This survey had created an impact on the individuals by educating them on the benefits of having a healthy diet and proper hygiene.

Keywords*: Fast food; balanced diet; health; non-vegetarians; oral hygiene; dental caries.*

Introduction

Health has been falsely perceived by many people in the society.[1] Health has been merely understood as " a state of complete physical, mental and social well-being of an individual and not merely the absence of diseases and having a good immunity status"[1] There are many causes for the prevalence for diseases in man, but fast food consumptions is one of the major risk factors are increasing the prevalence of diseases associated with oral cavity and systemic diseases in man[2]. There are various programs for spreading the awareness on the prevention of oral diseases like for the maintaining a healthy diet and preventive measure like fluoride prophylaxis, periodic dental checkups, group camps and other sessions for creating the awareness for oral hygiene and secondary prevention programs[3]

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The prevention for the prevalence of oral diseases involves proper education on oral hygiene awareness and spread of its awareness to the schooling population[4,5]. The term 'bio nutrition' means important interaction between diet, nutrient status, genetic and general development of an individual[6]. Bionutrition emphasizes the role of maintaining proper health status and preventing pathologic condition by reducing the prevalence by preventive measure implementation in a society[7]. There is always a unique relationship that exists between a healthy balanced diet and health status of an individual[8].

Fast food consumption is one of the major sources for the spread or aggravation factor for diseases and malnutrition among people. High fat diet which is the most prevalent food served in fast food joints is one of the major factors for the incidence of systemic diseases and coronary heart diseases in an individual[10].

Improper intake of various nutrients can lead to many disorders involving many systems in the body. It is one of the major causes for the incidence of caries in the oral cavity. Lesser protein intake causes atrophy of lingual papillae, and degeneration of tissues[9]. Insufficient supply of lipids can lead to swelling to tissues and glands eg:parotid. Lesser carbohydrate is the major cause of the incidence of plaque, periodontal diseases and caries in the oral cavity[10,11]. Decreased vitamin and mineral intake is one of the causes for cases of enamel hypoplasia, tooth erosion. swelling of the tongue, papillary atrophy, and surface ulceration are possible in case of micronutrient deficiencies[12]. Thus, it is important to maintain healthy and a balanced diet.

The association between diet and oral cancer is very high[13]. Having a bad diet habit and increased consumption of canned and food with increased preservative content is one of the major causes for the incidence of potentially malignant lesions and malignant lesions in the oral cavity[14]. The National Cancer Institute and the American Cancer Society have established some prudential dietary recommendations for the choice of food,

- Maintain a Desirable Body Weight,
- Eat a Varied Diet,
- Include a New Variety of Fruits and Vegetables in the Daily Diet,
- Consume a Greater Quantity of Foods Rich in Fibre,
- Decrease the Total Intake of Fats (30% Less Than the Total Calories),
- Limit the Consumption of Alcohol,
- Limit the Consumption of Salted Food or Food Preserved With Nitrate [15]

Thus, this survey was done to create an awareness to the society and the dental students on the impacts fast food has on the health of an individual. Thus, this study has been done to describe the relationship between healthy eating habits and the oral health status of an individual.

Materials and Methods

This study was an online survey based study. An online questionnaire based survey was done and circulated among 100 people in chennai. The study was conducted for a period of one month and no animal testing was required for the study. The questionnaire consisted of 10 questions which were based on the awareness of the importance of the impact of fast food on oral health. The responses of the survey were tabulated with the use of excel sheets. The analysis of the responses was done with the help of a statistical software SPSS version 20.0. The dependent variables of the study were age, sex, occupation and the independent variables were stature and height. The analysis of data was done with Pearson correlation from the answers based on the questionnaire. the answers compiled were entered and the results based on the answers were evaluated.

Results and Discussion

The observations of the study showed that 90% people consume fast food of various types in which 78% of the population was non-vegetarian. 40% people consumed fast food at least once a week (Figure 1). Most of them (91%) were aware of the impacts of fast food on the health of an individual (Figure 2). 84% of the people who took part in the survey are also aware of the complication of having an unhealthy diet. 77% of the people who took part in the survey were aware

of the causative factors of the incidence of dental caries (Figure 3).

The association between age and the frequency of fast food consumption was analyzed. Age group between 20-30 years consume comparatively more fast foods than the other age group people. Chi square test was found to be statistically significant as p=0.031 (p<0.05). Hence, proving that individuals who consumed fast food once a week, daily and more than once a week were more prevalent among 20-30 year age groups than the other age groups (Figure 4).

The association between gender and the frequency of fast food consumption was made and was observed that there was no relation between them. Among both the genders, male participants consume fast food more frequently such as daily, more than once in a week, once in a week than females. Chi square test was done and was found to be statistically not significant. Pearson Chi square test, p=0.072 (p>0.05) hence statistically not significant, indicating male participants showed more frequency of increased fast food consumption than females (Figure 5).

The association between age and the awareness of the causes of dental caries was analyzed. Among the various causes of dental caries and related dental problems, the 20-30 years age group are more aware of the causes of dental caries than other age categories. Pearson Chi square test, p=0.01 (p<0.05) hence statistically significant association indicating people between the age group of 20-30 are more aware of the causes of dental caries than the young and aged groups (Figure 6).

Diet is important for maintaining the oral health of an individual. Fast food is one of the major sources for unhealthy food and lack of hygiene in certain food joints that can lead to impacts on the oral health of a person[16,17] The survey showed that most of the people consumed food from fast food joints and this alerts the people on the risk factors and the importance for the maintenance of body health.[18,19] Most of the population who took part in the survey were non-vegetarians and they had exhibited lesser frequency of increased body mass index (BMI), total cholesterol levels, etc[20,21]. this shows that a vegetarian diet has lesser impacts to the body health.[20]

Nutrition should be given the most priority when food is consumed by a person. It has also been proven that most fast food joints don't give the utmost attention to the nutrition required for the body and have higher concentration of fat in the food14. This study shows that people give more importance to taste when compared to the nutritive status of the food consumed[13,23]. This corrective thought has to be implicated in the minds of the people consuming an unhealthy diet. From this study, it has been proved that higher frequency of people consume fast food for the taste it possess[24,25]. They showed an increased incidence of caries in multiple teeth in the oral cavity, where as there was a lesser frequency of caries in the population with proper maintenance of health and hygiene[26,27]. This study has shown that 40% of the population that had taken part in the survey consume fast food and 56% consumed fast food with a frequency of at least once a week.

The presence of malnutrition is seen in unhealthy nutrition cases in a person[28]. Increased sugar and salt content in an individual diet causes an increase in the incidence of caries in the oral cavity[29]. But the incidence isn't mainly because of malnutrition but also due to a sedentary lifestyle and incidence of personal habit like smoking, alcoholism and unhealthy diet which contribute to the maintenance of body health status[30].

This study was a unicentered study where the unclear data were excluded and a different set of data was included. Thus, there are increased studies and summits conducted to spread the importance and the spread of knowledge for the importance of maintenance of oral health and its education to the respective population. This leads to a spread of awareness among people on maintaining good oral health and reducing the incidence of dental caries and other disorders in the oral cavity due to lack of proper nutrition and proper hygiene.

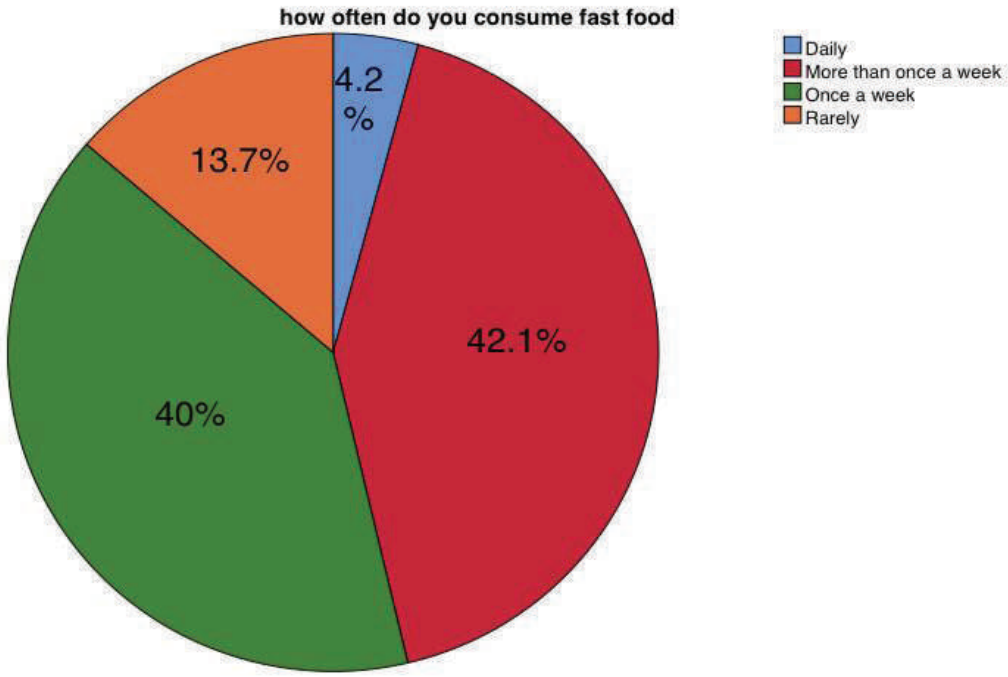


Figure 1: Pie chart showing percentage distribution of frequency of fast food consumption. 4.2% of the population consume fast food daily (blue colour), 42.1% consume more than once a week (red colour), 40% consume at least once a week (green colour) and 13.7% consume fast food rarely (orange colour).

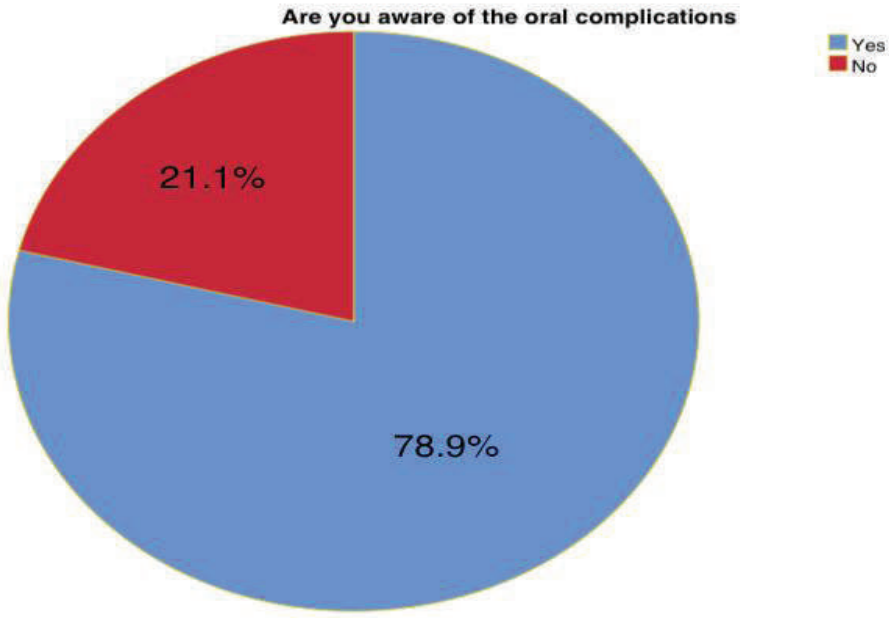


Figure 2: Pie chart showing percentage distribution of awareness on the impacts of fast food consumption. 78.9% of the individuals who took part in the survey were aware (blue colour) of the oral complications and 21.1% were not aware (red colour).

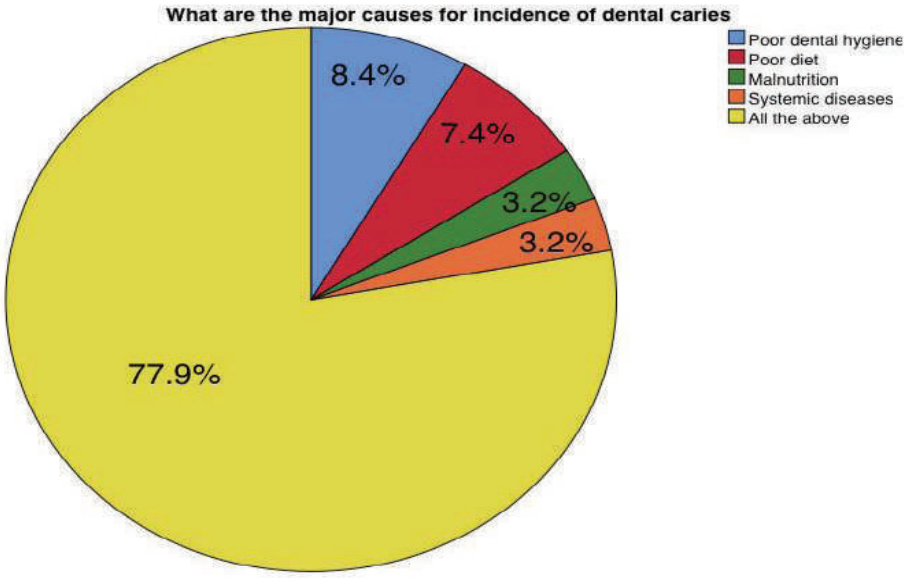


Figure 3: Pie chart showing percentage distribution of the major causes of dental caries in an individual. 8.4% thought poor dental hygiene was the cause (blue colour), 7.4% thought poor diet was the cause (red colour), 3.2% thought malnutrition was the cause (green colour), 3.2 thought the incidence of systemic disease was the major cause (orange colour), 77.9% thought all the reasons (yellow colour) described caused dental caries.

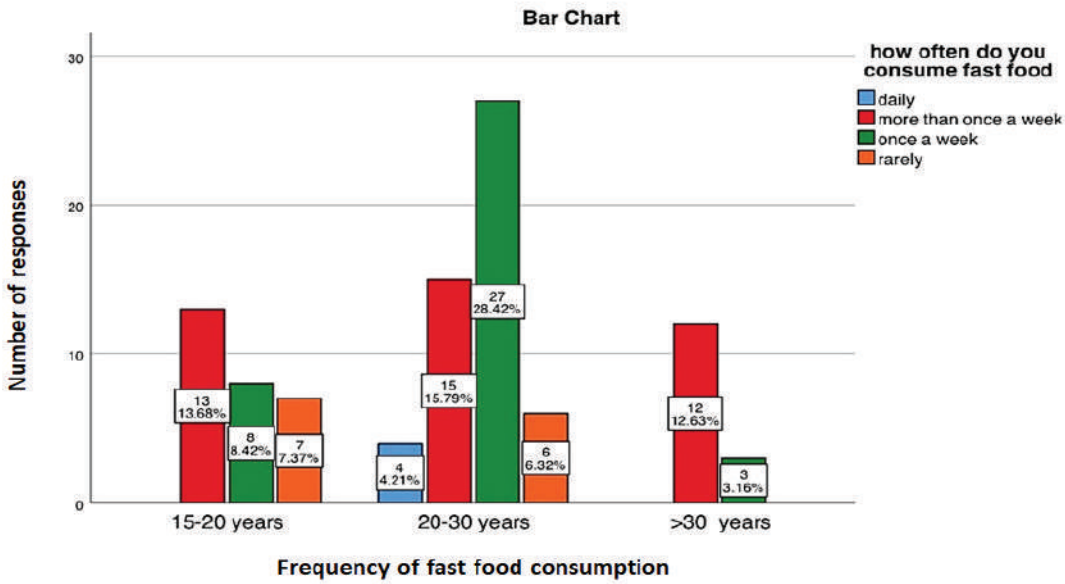


Figure 4: Bar chart shows the association between age and the frequency of fast food consumption. The X axis represents the frequency of fast food consumption among different age groups and Y axis represents the number of responses. Age group 20-30 years consume comparatively more fast foods than the other age group people. Chi square test was found to be statistically significant, p=0.031 (p<0.05). Hence, proving that individuals who consumed fast food once a week (green), daily (blue) and more than once a week (red) were more prevalent among 20-30 year age groups than the other age groups.

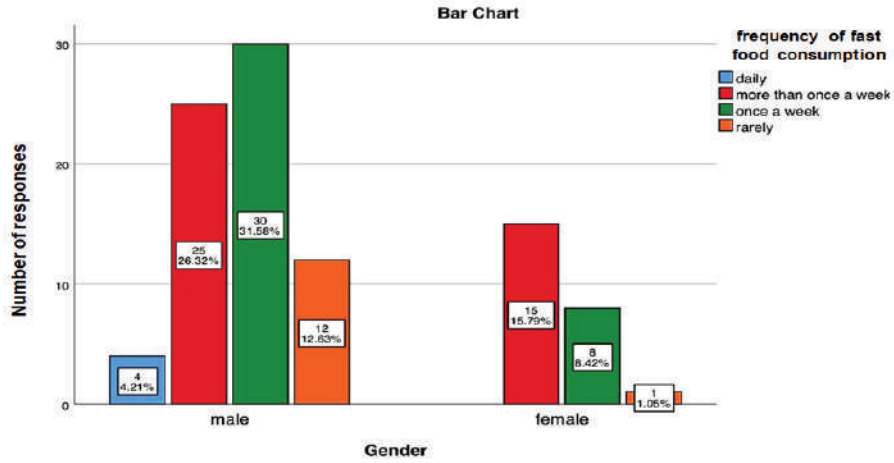


Figure 5: Bar chart shows the association between gender and the frequency of fast food consumption. X axis represents gender and Y axis represents the number of responses. Among both the gender male participants consume fast food more frequently such as daily (blue), more than once in a week (red), once in a week (green) than females. Chi square test was done and was found to be statistically not significant. Pearson Chi square test, p=0.072 (p>0.05) hence statistically not significant, indicating male participants showed more frequency of increased fast food consumption than females.

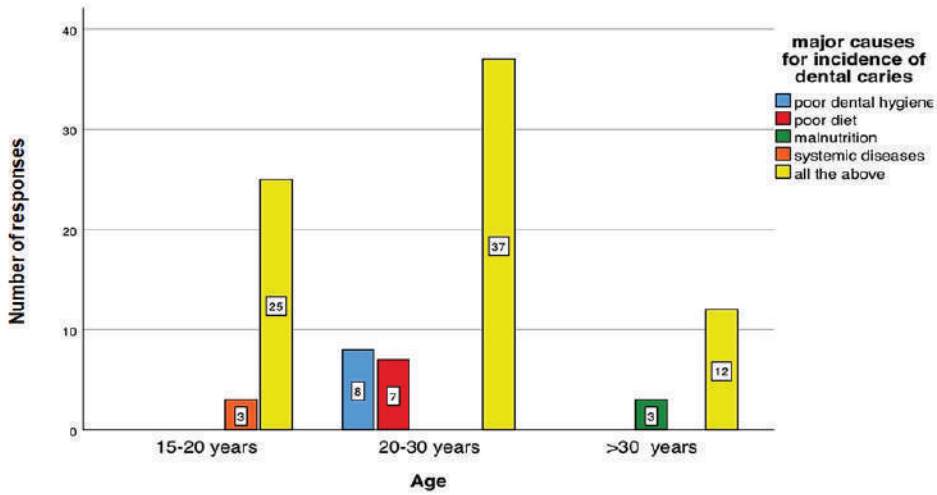


Figure 6: Bar chart shows the association between age and the awareness of the causes of dental caries. X axis represents age and Y axis represents the number of responses. Among the various causes of dental caries and related dental problems the 20-30 years age group are more aware of the causes of dental caries than other age categories. Chi square test was done and was found to be statistically significant. Pearson Chi square test, p=0.01 (p<0.05) hence statistically significant indicating people between the age group of 20-30 are more aware of the causes of dental caries than the young and aged groups.

Conclusion

Within the limitations of the study, most people consume food from fast food joints and they are not much aware of the impacts of unhealthy nutrition and oral hygiene practices. This survey has added knowledge and created an awareness of the importance of oral health and nutrition upon consumption of fast foods. Hence to have

a better oral health and to maintain good oral hygiene it is not enough if we brush or floss adequately. Instead it is necessary to avoid frequent consumption of fast foods to prevent the damaging dental reactions caused by them and its consumption should be kept under control as complications arise only if it is consumed often. Thus we conclude that there is a strong association between frequent fast food consumption and oral health and hygiene among the population.

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Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Transalveolar Extractions in Different Age Groups

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Abstract

To evaluate the prevalence of difficult extractions in different age groups. A retrospective study was done in an institutional setting. The data was retrieved from the college's patient records. All the patients who underwent transalveolar extractions were included in this study at a given time frame from june 2019 to march 2020. The patients' age, gender and method of extraction were retrieved and tabulated. The data was then analyzed using a software (SPSS). A total of 291 patients were involved in this study,majority of the patients belonged to the 31-40 years age group, 160 patients being male and the remaining 131 being female patients. Most difficult tooth which was commonly encountered during extraction was lower mandibular third molars. Lower third molars are one of the most difficult teeth to be extracted, an increased number of cases were seen in the 31-40 years age group, hence a better approach and skill to be trained in extracting lower third molars can be done in order to avoid further complications.

Keywords: *Transalveolar extraction, open method, surgical removal, exodontia,minor oral surgery.*

Introduction

Tooth extraction may represent one of the first oral surgery acts that a young dentist will face at the beginning of his/her career[1,2] .Exodontia is one of the most common procedures performed in oral surgery. Complications, unexpected clinical episodes in respect to regular operative procedures under normal circumstances, can increase morbidity.Prevention is one of the best ways of avoiding future complications. Therefore, it is fundamental that the clinician is able to evaluate the whole spectrum of complications and their implications.Complications can be wide ranging: from common ones like dry sockets or root fractures to uncommon and serious ones like displacement of a root fragment into the maxillary sinus and its consequent oro-antral fistula.Tooth extraction is a surgical procedure and several complications may arise directly connected to the operator's actions. Fracture of the alveolar bone is one the most frequent complications during tooth extraction. Incorrect hand movements can increase the possibility of tooth apex fracture.The breakage of basal bone or lower jaw dislocation, swelling, edema and bleeding are all complications related to the extraction procedure[3,4,5] .Transalveolar extraction method is the surgical technique which is employed for recovering roots that were fractured during routine extraction of teeth which could not be extracted by the routine closed methods for a variety of reasons.This method involves the reflection of a full thickness muco-periosteal flap, cutting of the bone obstructing the removal of the tooth and if required sectioning of the roots and then removal. Postoperative complications commonly include haemorrhage ,pain ,swelling. [6,7,8].The aim of this study was to evaluate the prevalence of difficult extractions in different age groups

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Materials and Methods

A retrospective study was conducted in an institutional setting. The ethical clearance was received from the institution's ethical committee. The study

involved all patients who had undergone difficult extractions in a given time frame.

Selection of subjects:All patients who had undergone difficult extractions were considered in this study. The time period of choice was from june 2019 to march 2020. A total of 86000 patients records were reviewed and analyzed.There were three people involved in this study- the guide,reviewer and researcher. All available data was collected and sorted.

Data collection:The patient's details were retrieved. Data regarding the patient's age, gender and treatment done were considered for this study. Cross verification of the data was done by the second reviewer, to avoid any missing or repetitive data. The data was manually retrieved and tabulated in excel and sorted.

Inclusion criteria:All patients who underwent transalveolar extractions were considered in this study. All age groups were considered.

Exclusion criteria:Patients with incomplete records were removed from the study. Repetitive entries were also excluded.

Statistical analysis:The tabulated data was analysed using SPSS software(IBM SPSS statistics 260). The method of analysis used was "chi square test". The analysis was done between age and type of treatment done.

Results and Discussion

Out of the 291 patients included in this study, the age group in which most difficult extractions were done in belonged to 31-40 years (35.4%) followed by 21-30 years age group(29.9%), followed by 41-50(18.9%) years age group(as seen in Figure 1). The most common teeth to undergo transalveolar extractions was found to be 48 (28.5%) , followed by 38(25.4%), followed by 36(1.7%) (as explained in Figure 2). The difficult extractions were more prevalent in males (55%) than females (45%) (as explained in Figure 3). On finding the correlation between age and tooth number,it was found that in the 31-40 years age group the most commonly encountered teeth during difficult extractions was lower wisdom teeth(as shown in figure 4). On finding a correlation between gender and tooth number it was found that female patients encountered more difficult extraction experience with respect to 48 than 38, on the other hand male patients encountered a difficult extraction experience irt 38 than 48(as shown in Figure5).

Tooth extraction requires controlled force and fineness for atraumatic extractions. Various instruments and techniques have been described to aid atraumatic tooth extraction. According to a study conducted by kautto, the mean age of occurrence of extraction was found to be 36.4 years[9,10,11].Our present study also goes in accordance with this study with a prevalence of difficult extractions in the 31-40 years age group. Various studies state that lower wisdom teeth have an increased tendency to go for open methods of extractions[12,13,14]. Our present study also goes in accordance with the study mentioned above ,having an increased number of patients undergoing difficult extractions in relation to lower wisdom teeth.Pain, inflammation, and trismus are typically observed in the postoperative period after mandibular third molar extraction[15,16,17] .factors that may affect pain include the surgeon's experience[18,19,20]. Majority of the cases were seen in male patients more than female patients.Gender is one of the predictors of difficulty of impacted third molar surgery[21,22,23].There is a statistically significant relation between gender and surgical duration which was also noted in the study undertaken by Susarla and Dodson[24] .Side of third molar tooth also has statistical significant correlation with surgical duration. This is mainly due to the advantage of right handed surgeons, which makes it easy to retract cheek and elevate the tooth while extracting.[25].

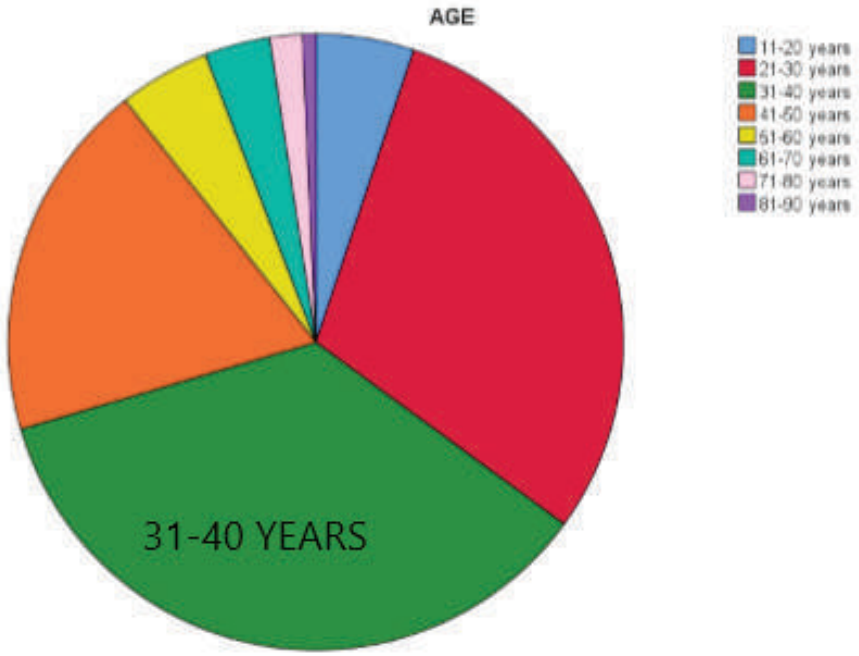


Figure 1: this pie chart depicts the different age groups who underwent transalveolar extraction. The age group between 31-40 years shows the most cases of transalveolar extraction.(dark green in color,35.4%).

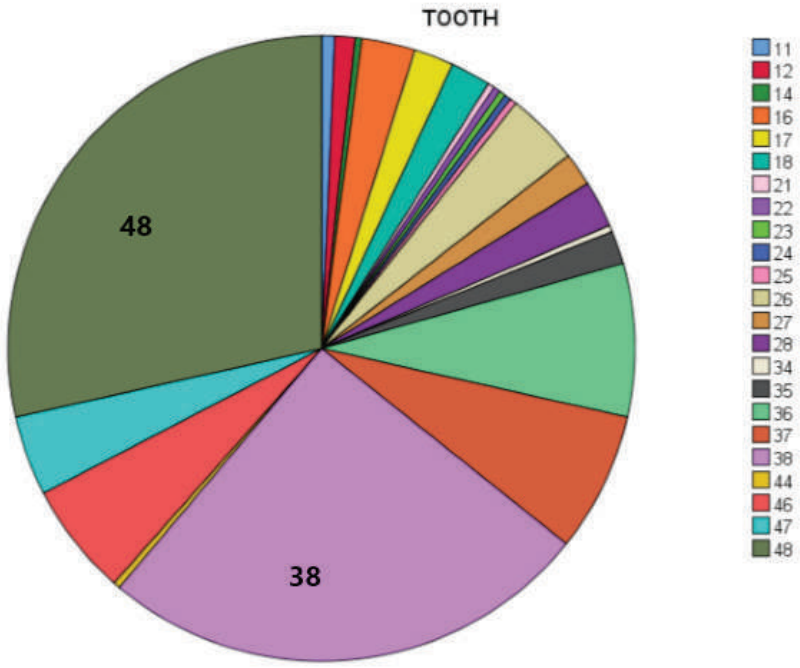


Figure 2: this pie chart depicts the teeth undergoing transalveolar extractions. Lower wisdom teeth were shown to be the most cases undergoing transalveolar extraction. Purple color depicts 38(25.4%),Grey depicts 48(28.5%).

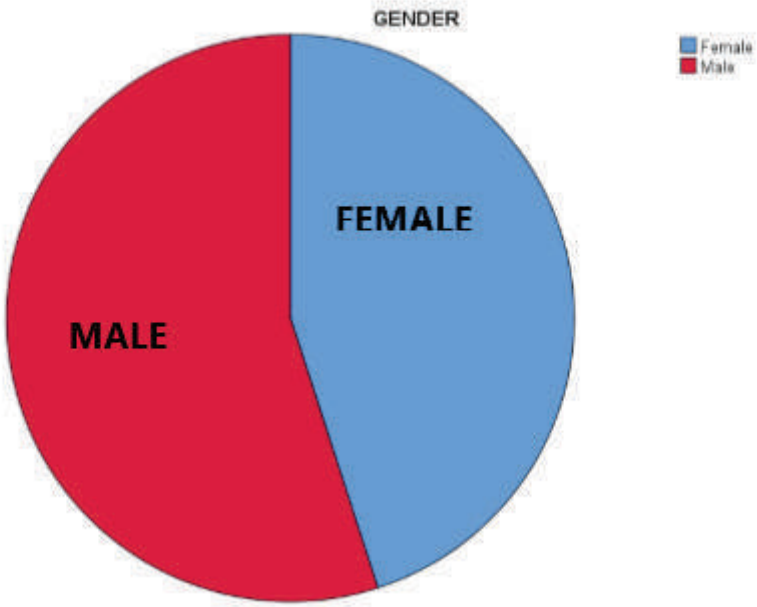


Figure 3: This pie chart depicts the gender distribution in transalveolar cases. A male predilection was seen in this study. Blue color depicts male patients(55%), red color depicts female patients (45%).

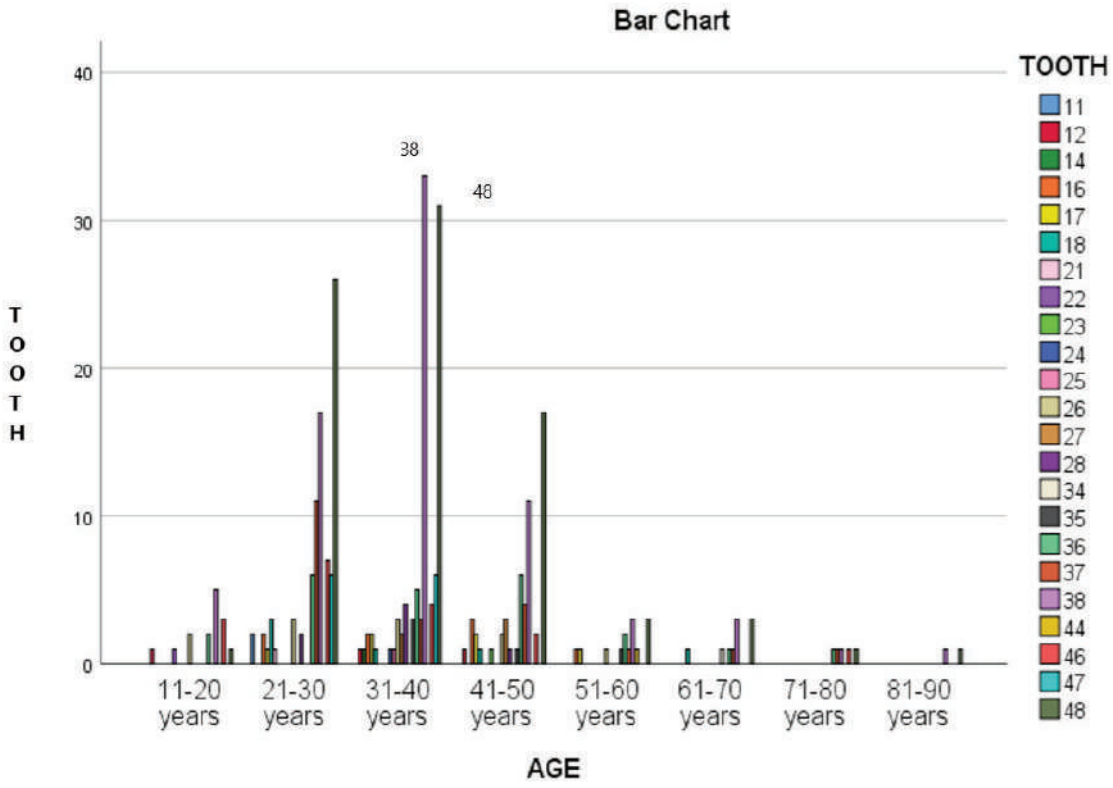


Figure 4: The bar graph depicts the correlation between age and transalveolar extraction.

X axis represents age, Y axis represents tooth number.This bar graph depicts that transalveolar extractions were prevalent in the age group 31-40 years,and the tooth involved in this age group was found to be lower third molar teeth(38 purple in color and 48 dark green in color). chi-square test was done p value is 0.38(p>0.05), it is not statistically significant.

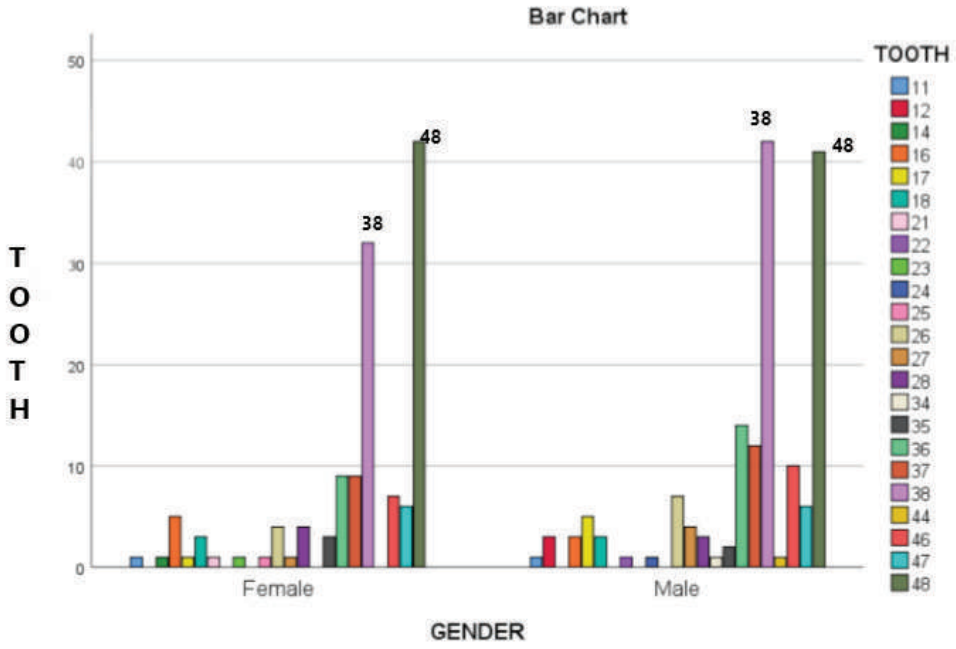


Figure 5: This bar graph depicts the correlation between gender and transalveolar extraction.

X axis- gender,Y axis- tooth number.This graph depicts that the majority of female patients underwent transalveolar extraction in relation toTooth 48,male patients underwent transalveolar extractions in relation to Tooth 38..chi-square test was done p value is 0.716, it is not statistically significant.

Conclusion

In this study we evaluated the prevalence of difficult extractions in different age groups.Transalveolar extraction method is the surgical technique which is employed for recovering roots that were fractured during routine extraction of teeth which could not be extracted by the routine closed methods for a variety of reasons. This method involves the reflection of a full thickness muco-periosteal flap, cutting of the bone obstructing the removal of the tooth and if required sectioning of the roots and then removal. Difficulty of extractions not only depend on the state of tooth but also the experience of the surgeon. An emphasis on good treatment planning and sharpening of skills can make extractions more easier to perform.

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Source of Funding: Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Incidence of Pathologies Associated with Impacted Mandibular Premolars and Third Molars

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Abstract

Impaction is the cessation of eruption or failure of a tooth to erupt caused by a physical barrier in the eruption path, abnormal positioning of tooth and loss of space are other impediments.The rising incidence of impacted teeth and their influence on the dental arches have long been of concern to the oral maxillofacial surgeons.The aim of the study is to evaluate different types of Mandibular pathologies associated with impacted third molars. This was a retrospective study conducted in the Department of Oral and Maxillofacial Surgery in Saveetha Dental College, Chennai . A total of 470 cases ranging in the age from 18 years to 45 years with the diagnosis of Impacted mandibular third molars were considered for this study. Data were analysed using IBM SPSS statistics.This study showed vertical impaction and mesioangular impaction were most frequently involved in the formation of cystic lesions among which vertical impaction occupied (1.7%) and mesioangular impaction (0.2%).Tumors with 0.6% are associated with vertical impaction of mandibular third molar followed by mesioangular and distoangular impactions.Within the limitation of the study,we observed that there exists a significant incidence of Pathologies associated with impacted mandibular third molars .

Keywords: *Cyst,impactions,mandibular third molars,tumors.*

Introduction

Most common pathologies associated with third molars are Caries of impacted teeth or adjacent tooth, molar root resorption,periodontal bone loss of adjacent tooth and odontogenic cysts and tumors[1]. Impacted third molar is a Universal problem which may be on an increased prevalence.Most epidemiological studies do not distinguish between the prevalence of one or two or more impacted third molars .The figures vary with different populations worldwide. The proponents of prophylactic removal of the third molar have used this position of possible pathologies that can be associated with the third molar teeth as evidence of early removal.Impacted mandibular third molars are common complaints among patients seen in the oral and maxillofacial surgery department[2] . Dentistry comprises practices related to oral cavity. Oral diseases are a major problem among the general population and there are various procedures carried out to prevent and treat them[3]. During dental treatment, anxiety and fear have been said to enhance pain during dental treatment. It may disturb patient's compliance during treatment resulting in poor dental and oral health[4] .

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Materials and Methods

Study setting and Data collection:

This retrospective study was conducted by collecting records of 86,000 patients from June 2019- April 2020 who had reported to Saveetha Dental College for

treatments. Patients reporting to the Department of Oral and Maxillofacial Surgery having mandibular impactions with pathologies were diagnosed and its treatment with surgical removal of the offending tooth were shortlisted from the main records based on the inclusion/exclusion criteria.So final sample,which contains 470 patients were enrolled for the study. Ethical committee approval for this study was obtained from the Institutional Ethics Committee with the following ethical approval number. SDC/SIHEC/2020/DIASDATA/0619-0320.

Sampling :

The study population included patients who underwent treatment for having mandibular impactions with pathologies Saveetha Dental College by means of Systematic Sampling.

Inclusion Criteria- Patients of all age groups and gender with impacted mandibular third molars with diagnosis of having mandibular impactions with pathologies were included.

Exclusion Criteria- Patients with impactions without any pathologies,and common dental problems were excluded from the study.

Duplicate patient records and incomplete data were excluded. Datas were reviewed by an external reviewer. Totally, n= 470 patients were included.Demographic data such as the patient's age, gender and pericoronitis, pericoronal abscess were also recorded.

Data Analysis:

The data obtained were tabulated in Microsoft Excel 2016 (Microsoft office 10) and later exported to SPSS (Statistical Package for Social Sciences) for Windows version 20.0, SPSS Inc, Chicago IU, USA) and subjected to statistical analysis. Chi-square test was employed with a level of significance set at $p<0.05$.

Results and Discussion

Position of mandibular third molar in relation to presence or absence of cysts: In 6 vertical impaction, cysts were present likewise in 1 mesioangular,1 horizontal impaction cysts were present but in contrast , no Cysts were associated with distoangular impaction.Position of mandibular third molars in relation to presence or absence of tumors.In our study we found that tumors associated with impacted mandibular third molars were common in vertical impaction,Mesio angular,and horizontal with no incidence in disto angularly impacted teeth. This study showed that vertical and mesioangular impactions were the most frequent in causing cysts, mesioangular being 0.5% and vertical impaction accounted for 1.7% and 0.6% of tumors were associated with the vertical impaction and then mesioangular and distoangular respectively. The distribution of age,gender,teeth number and position of tooth and their association with the formation of cysts and tumors and their statistics were depicted in [figures 1,2,3,4,5,6,7,8].

Third molars are usually the last tooth to erupt and most commonly impacted tooth[5]. The associated pathologies with third molar teeth may be clinically evident or hidden. When hidden, patients will frequently present vague complaints such as headache, pressure or pain that is not readily attributable to the teeth[6]. Painless dental extraction is the most common procedure carried out by dentists, and it is a common model for evaluating the efficacy of analgesics and anti inflammatories for acute dental pain relief[7].

Pain has been described to be commonly associated with the third molars ,however vague complaints may confuse the clinician leading to the misdiagnosis. Impacted third molar teeth have been reported to be common in the second and third decades of life .Surgical alterations in the position of the bony facial skeleton will inevitably affect the soft tissues[8].

The high female predilection reported may be due to difference in between the growth curve of males and females. Male jaws continue to develop during the period of eruption of third molars therefore creating more space for the third molar eruption while females of jaws cease to grow during the same time.Botulinum, is a protein and lethal neurotoxin and is one of the most potent biological substances known which is used in bioterrorism as well . It is the first toxin used for therapeutic purposes[9].

Patients having dental fear tend to go to the dentist only when they experience pain, thereby increasing the chance of fear , that their visit to the dentist will involve pain. This, in turn, results in exacerbation of their anxiety. It was found that dental anxiety was ranked fifth among the most

commonly feared situations[10] . Dental caries have been regarded as the most common dental disorder affecting the third molars because most of them will be malpositioned. Root resorption of the second molar tooth occurs as associated pathology of impacted third molars.

Sometimes dental follicular tissues can be confused with odontogenic tumors by the clinicians. Those patients who were surgically treated for their impacted teeth emphasising the need for histopathological examination of curretted tissue from the extracted socket if any suspected lesions/pathologies were present. The currected soft tissue was sent for histopathological examination. Pharmacological agents have also been used in the recent past as adjuvants in various procedures to aid in the reduction of blood loss. Among these, tranexamic acid has been proved to efficiently reduce bleeding[11]. Human immunodeficiency virus infection and acquired immunodeficiency syndrome are the globally emerging public health problems. It has profoundly affected every aspect of the public health sector[12]

In a radiographs, the dental follicular sac surrounding the tooth is interpreted as pericoronal radiolucency and width of this radiolucency is of at most important to determine the difference between a normal and abnormal follicle.A major issue related to present bio medical waste management is that many hospitals dispose their waste in an improper way, which contributes to spread of serious diseases such as hepatitis and human immunodeficiency virus[13] . The impacted mandibular third molars having follicular size measuring 2.4 mm or less radiographically and also executed pathology which emphasizes the need for the histopathological examination apart from the just clinical and radiographic examination. Bacterial endocarditis or infective endocarditis is a serious infection of the heart valves and endocardium that most often is related to congenital and acquired cardiac defects. bacterial endocarditis can commonly occur in patients with artificial heart valves[14].

There is consensus regarding removal of the third molar impactions with almost no controversy concerning its removal when pathologies associated with them. The pathological changes associated with the impact of cystic changes which includes dentigerous cyst odontogenic keratocyst,calcifying odontogenic cyst and Commonest neoplasms ameloblastoma ,myxoma and odontogenic fibroma are the components neoplasms[15] . Maxillofacial trauma is any physical trauma to the facial region, is commonly encountered by maxillofacial surgeons, and is often associated with high morbidity[16].

One of the most common postoperative complications after the extraction of permanent teeth is a condition known as dry socket. This term has been in use since 1896, when it was first described by Crawford[17] .Developmental dentigerous cysts is derived from the reduced enamel epithelium of the tooth forming organ associated with the crown of an unerupted tooth. Histologically dentigerous cyst is lined by a non keratinized stratified squamous epithelium and its cavity is filled by fluid or semi fluid content.A simple ranula which may be associated with impacted teeth can be treated by marsupialization or sclerotherapy or complete excision.[18] . Ameloblastoma contributes 10% of the odontogenic tumor.These are benign neoplasms under locally aggressive and slow growing neoplasms and infiltrative.There is equal male and female Predilection with the age range of 20 to 40 years. Ameloblastoma is classified as either intraosseous or extraosseous .Oral squamous cell carcinoma is the most frequent oral malignancy. Although it has been suggested that Oral squamous cell carcinoma arises through the accumulation of multiple genetic changes, the precise molecular mechanism and their relevance to clinicopathological variables are still unclear [19]. Oral submucous fibrosis is an insidious, chronic, disabling disease that affects the entire oral cavity, sometimes pharynx and rarely larynx[20]. Odonomas are shown growing asymptomatic with limited growth and change into Complex odontoma which are associated with the permanent unerupted mandibular teeth. These lesions have radiopaque Mineralised structures with radiolucent halo .

The cysts and neoplastic changes taking place during development and because of this,the neoplastic and preneoplastic changes, early curative measures should be taken to avoid any complication for the well being of the patient.Graph 2.Position of impacted mandibular third molar in relation to occurrence of tumours.

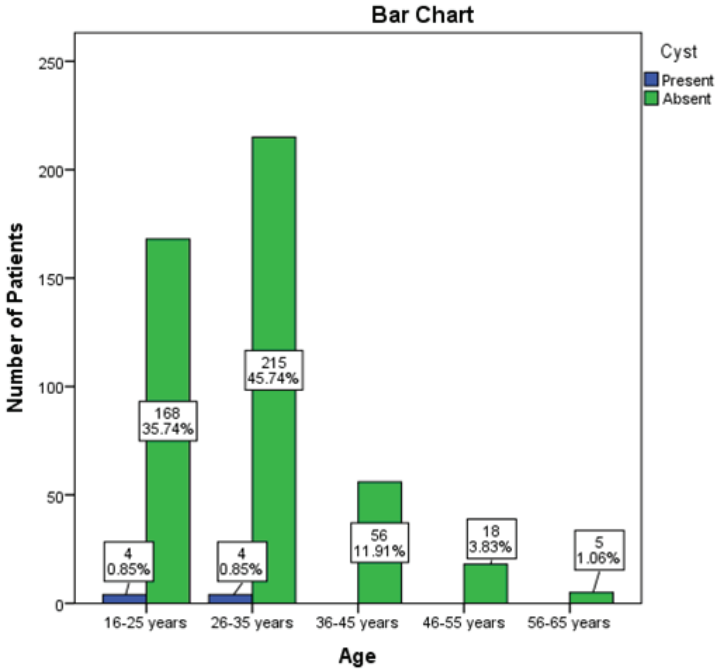


Figure 1 .Bar diagram showing the association Age with Presence or Absence of cyst.X axis represents the age group and Y axis represents no of patients affected by cyst formation.It is found that incidence of cyst formation among impacted mandibular teeth is uncommon in all age groups except very minimal prevalence in 16-35years (Blue).Chisquare test done,chi square value p-0.775[p > 0.05] which is statistically not significant.Even though, it is statistically not significant, the incidence of cyst formation among impacted mandibular tooth is more in patients with age group of 16-35 years when compared to other groups.

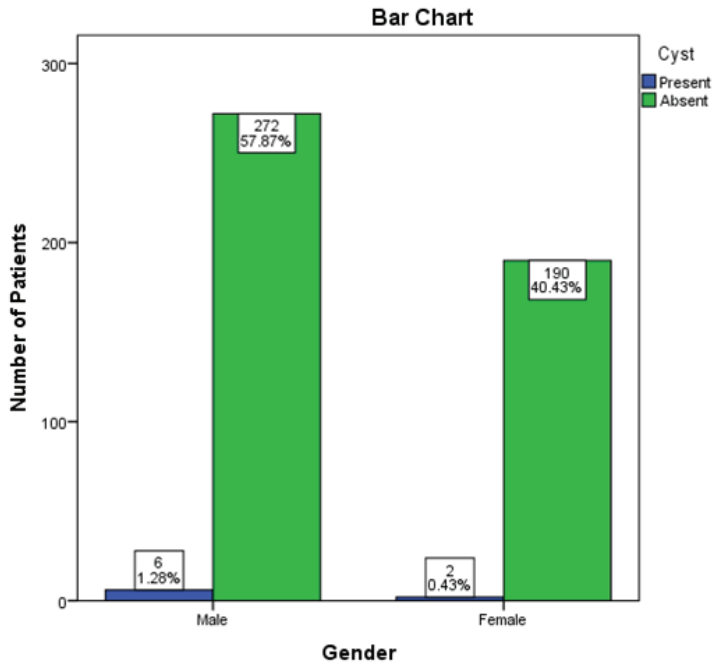


Figure 2 .Bar diagram showing the association of Gender with Presence or Absence of cyst.X axis represents the age group and Y axis represents no of patients affected by cyst formation.chi square test done (p - 0.358[p > 0.05])and was found to be statistically not significant. Even though, it is statistically not significant, the incidence of cyst formation (Blue) was found to be common among impacted mandibular tooth is more in males compared to females.

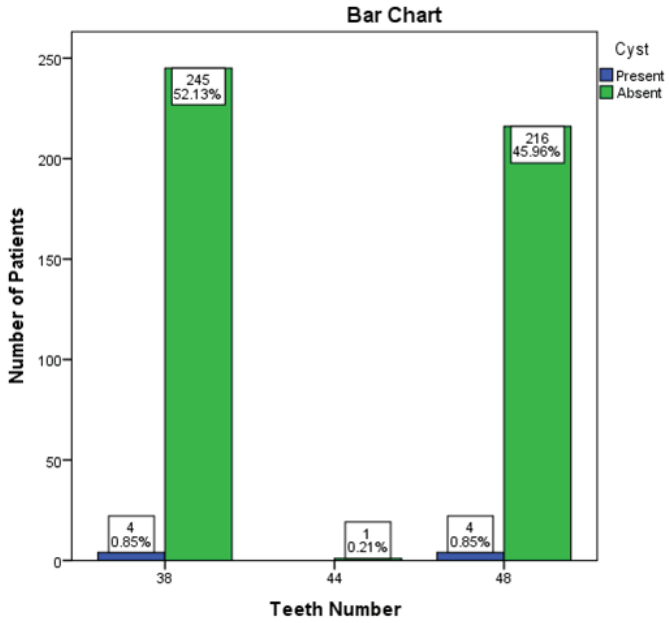


Figure 3 .Bar diagram showing the association of tooth number with Presence or Absence of cyst.X axis represents the age group and Y axis represents no of patients affected by cyst formation.Blue bar indicating presence of cyst and while Green bar represent absence of cyst,from the table,it is found that incidence of cyst formation among impacted mandibular tooth is very less in all 38,48,44 less than one percent.chi square test done,chi square value p - 0.976

[p > 0.05]and was found to be statistically not significant.Even though, it is statistically not significant, the incidence of cyst formation among impacted mandibular teeth is very low but equally distributed among mandibular molars and premolars.

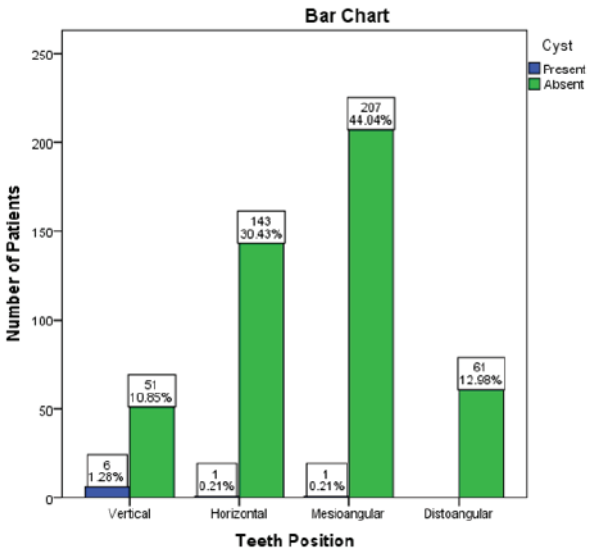


Figure 4. Bar diagram showing the association of tooth position with Presence or Absence of cyst.X axis represents the position of teeth and Y axis represents no of patients affected by cyst formation.It is found that incidence of cyst formation (Blue) among impacted mandibular tooth is very less in all type impaction maximum prevalence itself is 1.28% in vertical type and nil in distoangular.Chi square test done,chi square value p - 0.000 [p < 0.05]and was found to be statistically significant.It is statistically significant, the incidence of cyst formation among impacted mandibular tooth is more in vertical ,horizontal and mesioangular impactions.

Graphs for presence or absence of Tumor Vs mandibular impacted teeth

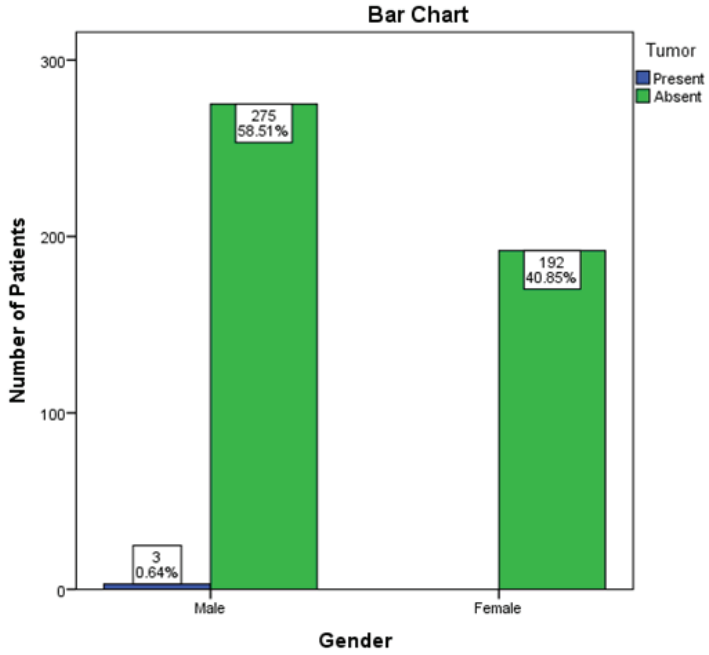


Figure 5 .Bar diagram showing the association of Gender with Presence or Absence of tumor.X axis represents the gender group and Y axis represents no of patients affected by cyst formation. It is found that incidence of tumor formation (Blue) among impacted mandibular tooth is more in males and none in females.chi square test done,chi square value p - 0.149[p > 0.05] and was found to be statistically not significant.Even though, it is statistically not significant, the incidence of tumor formation among impacted mandibular tooth is more in males compared to females.

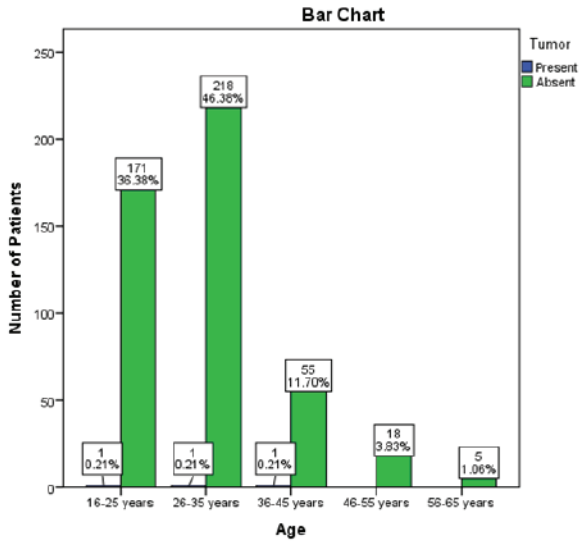


Figure 6 .Bar diagram showing the association Age with Presence or Absence of tumor.X axis represents the age group and Y axis represents no of patients affected by tumor formation.It is found that incidence of tumor formation (Blue) among impacted mandibular teeth is uncommon in all age groups except very minimal prevalence in 16-45years[0.21%].chi square test done,chi square value p-0.838[p > 0.05] and was found to be statistically not significant.Even though, it is statistically not significant, the incidence of tumor formation among impacted mandibular tooth is more in patients with age group of 16-35 years when compared to other groups.

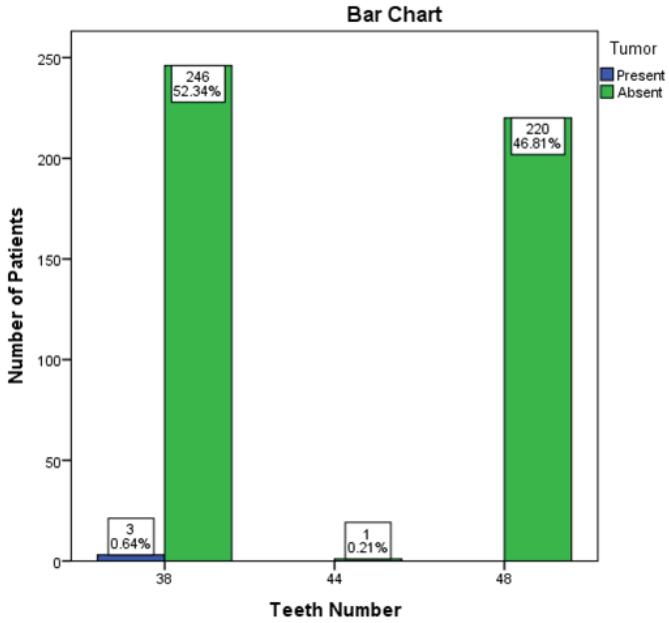


Figure 7 .Bar diagram showing the association of tooth number with Presence or Absence of tumor .X axis represents the teeth number and Y axis represents no of patients affected by tumor formation. It is found that incidence of tumor formation (Blue) among impacted mandibular tooth is very less in all 38,48,44 less than one percent..chi square test done,chi square value p - 0.976 [p > 0.05] and was found to be statistically not significant.Even though, it is statistically not significant, the incidence of tumor formation among impacted mandibular tooth is more in 38 compared to 48.

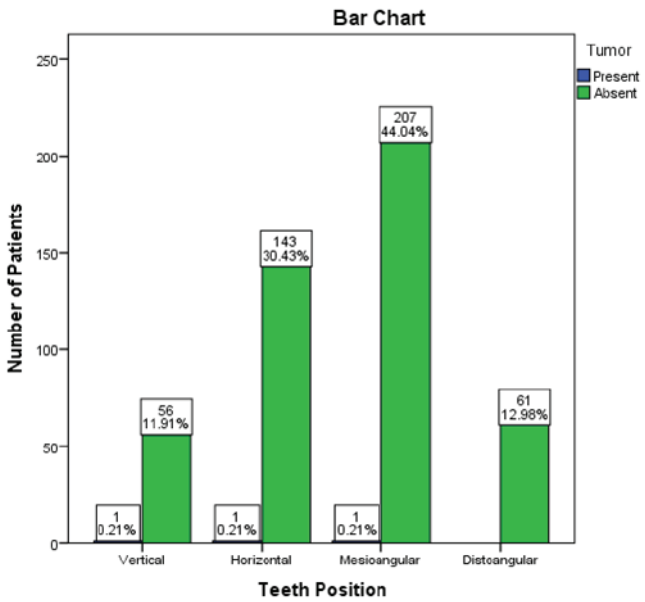


Figure 8 .Bar diagram showing the association of tooth position with Presence or Absence of tumor .X axis represents the teeth position and Y axis represents no of patients affected by tumor formation. It is found that incidence of tumor formation (Blue) among impacted mandibular tooth is very less in all 38,48,44[0.21%]..chi square test done,chi square value p - 0.659 [p > 0.05] and was found to be statistically not significant.It is statistically significant, the incidence of tumor formation among impacted mandibular tooth is more in vertical ,horizontal and mesioangular impactions.

Conclusion

Within the limitation of study there is significant finding in the incidence of pathologies associated with impacted mandibular premolars and third molars .Early diagnosis by doing biopsy is important as these cysts and tumors may be associated with morbidities and also because of chances of malignant transformation has been reported in long standing benign pathologies.

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Authors Contribution: Yandeti Srinivasulu carried out the retrospective study by collecting data and drafted the manuscript after performing the necessary statistical analysis. Abdul Wahab PU aided in conception of the topic, participated in the study design, statistical analysis and supervised in preparation of the manuscript. Senthil Murugan P participated in the selection of the topic,study design,statistics and coordinated in developing the manuscript. All the authors had equally contributed in developing the manuscript.

Conflict of Interest : No conflict of interest .

Funding for study: Nil

Limitations of the study and future scope :

This study is of shorter duration with limited population. So to ascertain the findings of our study, we have to do further studies in the future with large sample size and longer duration.This can be helpful to find out more regarding pathologies associated with impacted mandibular third molars .

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Oral Lichen Planus and Assessment of Factors Associated with It- A Retrospective Study

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Abstract

Lichen planus is a chronic inflammatory mucocutaneous disease that evolves in outbreaks, affecting the skin, mucous membranes or both. It is a recurrent disease. Etiology of lichen planus include anxiety, diabetes, autoimmune diseases, mainly chronic liver disease, intestinal diseases, increased cholesterol, medications, stress, hypertension, infections, contact with dental materials, cancer and a genetic predisposition to cancer. It mainly affects the middle aged and the elderly, with a predominance in females in a ratio of 2:1. The aim of this study was to evaluate the prevalence of Lichen planus assessment of factors associated with it. A retrospective study was conducted in the Saveetha Dental College, Chennai, India. Ethical clearance was obtained from SRB committee, Saveetha Dental College, Chennai, India. The clinical portion of this retrospective study was conducted over a 9 month period,i.e, between June, 2019 to March,2020. Some patients reported with pain while for others it was observed during routine examination. Data was collected from a total of 86000 patients who visited Saveetha dental college between Jun,2019 to March,2020. Out of this, the data of 99 patients who visited the institute were retrieved. The data obtained was tabulated in SPSS for windows, version 20. Descriptive statistics were analysed. Chi square test was done to analyse correlation. Data Analysis in the present study revealed that oral lichen planus was more predominant in females. It was more predominant between 50-59 years of age. The erosive variety of oral lichen planus was more predominant, most commonly affects the buccal mucosa and diabetes was the most predominant systemic disease in these patients. The association was found to be statistically significant between clinical variants of oral lichen planus and age (P=0.000) and gender (P=0.000). However, association was not found to be statistically significant between clinical variants of oral lichen planus and area affected (P=0.146), dental status (P=0.795) and systemic diseases (P=0.602).

Keywords: *Oral lichen planus, reticulate pattern, erosive lichen planus, autoimmune disease, precancerous lesion.*

Introduction

Lichen planus is a chronic inflammatory mucocutaneous disease that evolves in outbreaks, affecting the skin, mucous membranes or both. It is a recurrent disease[1]. Etiology of lichen planus include anxiety, diabetes, autoimmune diseases, mainly chronic liver disease, intestinal diseases, increased cholesterol, medications, stress, hypertension, infections, contact with dental materials, cancer and a genetic predisposition to cancer[2–5]. It mainly affects the middle aged and the elderly, with a predominance in females in a ratio of 2:1[6,7].

The oral lesions in Lichen planus are generally chronic, don't show spontaneous remission and have a tendency to become cancerous[8]. Even though oral lichen planus is often asymptomatic, it sometimes causes symptoms ranging from burning sensations to

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severe pain, which interferes with speaking, eating and swallowing [9–11].

An important differential diagnosis to oral lichen planus is oral leukoplakia[12]. Types of lichen planus are reticular variant, erosive variant, pigmented variant, papular variant, bullous variant, ulcerative variant, annular variant and lichenoid reaction variant. More than one form can be present in the same patient, at the same location[13].

A study by Czerninski et al states that implant placement can be done in patients with lichen planus[14]. The implants do not influence the disease manifestations. However, proper protocol and good surgical techniques need to be followed to achieve primary stability and integrity of implants [15,16]. Excess cements and marginal discrepancies must be avoided [6,7,17–20].

Known aetiologies for oral lichen planus include hepatitis C or chronic liver disease, diabetes, and stress[21]. Associations of oral lichen planus with dental material exposures, particularly dental metals, has been studied by some authors [22,23]. Allergic responses can be tested with a patch test. There is overwhelming evidence that these exogenous and endogenous factors combined with genetic factors, predispose patients to develop this disease [24–26].

The pathogenesis of Lichen planus is poorly understood. But oral lichen planus is of autoimmune origin. The mechanism is said to involve a T-cell mediated response associated with a lymphocyte-epidermal interaction. In this auto cytotoxic CD8+ T cells trigger the apoptosis of epithelial cells. This leads to antigenic alteration of the keratinocytes, which leads to an immunologic reaction. Cytokines which are released by the altered keratinocytes and the remaining associated inflammatory infiltrate, induce adhesion molecule expression. This causes further cytokine and chemokine release, causing a T-cells dominated infiltrate.

In susceptible individuals, the chronic presentation of antigen by keratinocytes and direct cell-mediated damage of the keratinocytes by CD8+ cytotoxic T cells may perpetuate the condition. The immunologic reaction begins with the activation of Langerhans' cells, which present the antigen to the CD4+ lymphocytes. These CD4+ lymphocytes promote epithelial destruction. Continuation of this process can activate CD8+ lymphocytes, leading to the chronic form of the disease [27,28].

Lundstrom et al. suggested that Salivary gland function is affected in some patients with oral lichen planus. He examined 39 patients with oral lichen planus and stated that 87% of patients exhibited low or very low unstimulated salivary rate. However, the pH and the buffering capacity of the saliva were normal[6]. Xerostomia is a subjective sensation of oral dryness, but can also be reported as a burning sensation. Structural salivary gland disorders include Sjogren's syndrome, sarcoidosis, postirradiation damage, developmental anomalies, and diabetes mellitus. The functional causes of xerostomia include chronic anxiety states, dehydration, and drug therapy[29].

The purpose of this study was to evaluate the prevalence of Lichen planus.

Materials and Methods

A retrospective study was conducted in the Saveetha Dental College, Chennai, India. Ethical clearance was obtained from SRB committee, Saveetha Dental College, Chennai, India. The clinical portion of this retrospective study was conducted over a 9 month period,i.e, between June, 2019 to March,2020. Some patients reported with pain while for others it was observed during routine examination.

Inclusion criteria:Patients diagnosed with Lichen planus, both males and females, patients of all ages.

Data was collected from a total of 86000 patients who visited Saveetha dental college between Jun,2019 to March,2020. Out of this, the data of 99 patients who visited the institute and were diagnosed with lichen planus were retrieved. The data obtained was tabulated in SPSS for windows, version 20. Descriptive statistics were analysed. Chi-square test was done to analyse association.

Results and Discussion

In the present study 40.6% were males and 57.4% were females. 13.9% were between 20-29 years, 15.8% were between 30-39 years, 18.8% were between 40-49 years, 32.7% were between 50-59 years, 14.9% were between 60-69 years, 2% were >70 years. 33.7% had reticular variant, 48.5% had erosive variant, 2% had pigmented variant, 8.9% had lichenoid reaction variant, 3% had bullous variant, 1% had ulcerative variant, 1% had annular variant. 77.2% occurred in buccal mucosa, 20.8% occurred in labial mucosa **(Figure 1)**. 26.7%

had diabetes, 17.8% had hypertension, 12.9% had other systemic diseases, 40.6% had no systemic diseases **(Figure 2)**. 24.8% had caries, 31.7% had restorations, 16.8% had crowns, 24.8% were partially edentulous **(Figure 3)(Table 1)**.

The association was found to be statistically significant between clinical variants of oral lichen planus and age (P=0.000) and gender (P=0.000). However, association was not found to be statistically significant between clinical variants of oral lichen planus and area affected (P=0.146), dental status (P=0.795) and systemic diseases (P=0.602).

Table 1 - Frequencies of gender, age, clinical variant, area affected, systemic diseases and dental status.

S.No	Variable	Options	Frequency (%)
1	Gender	Male Female	41 (40.6%) 58 (57.4%)
2	Age	20-29 years 30-39 years 40-49 years 50-59 years 60-69 years >70 years	14 (13.9%) 16 (15.8%) 19 (18.8%) 33 (32.7%) 15 (14.9%) 2 (2.0%)
3	Clinical variant	Reticular Erosive Pigmented Lichenoid reaction Bullous Ulcerative Annular	34 (33.7%) 49 (48.5%) 2 (2%) 9 (8.9%) 3 (3%) 1 (1%) 1 (1%)
4	Area affected	Buccal mucosa Labial mucosa	78 (77.2%) 21 (20.8%)
5	Systemic diseases	Diabetes Hypertension Others No systemic diseases	27 (26.7%) 18 (17.8%) 13 (12.9%) 41 (40.6%)
6	Dental status	Caries Restorations Crowns Partially edentulous Completely edentulous	25 (24.8%) 32 (31.7%) 17 (16.8%) 25 (24.8%) 0 (0%)

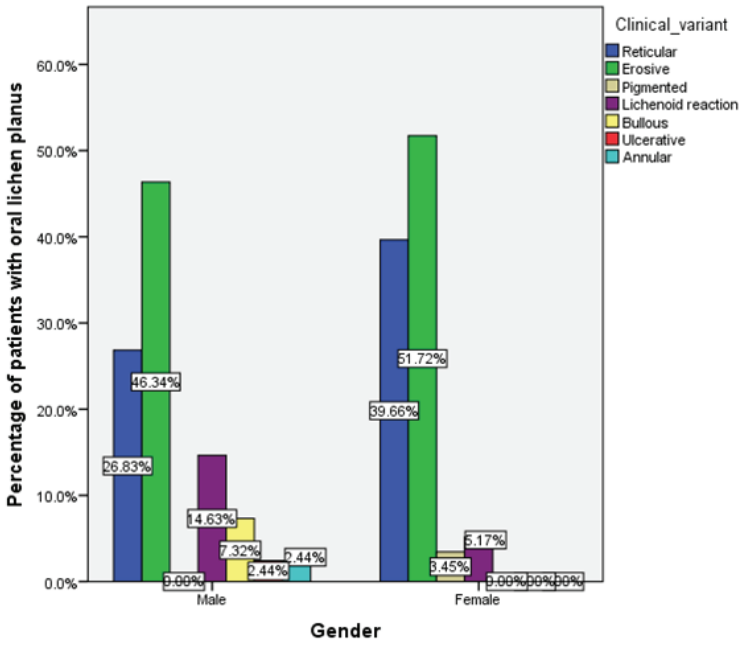


Figure 1 - Bar graph represents the correlation between clinical variants of oral lichen planus and gender. X-axis represents the gender and Y-axis represents the percentage of patients with oral lichen planus. Chi-square test was done and association was found to be statistically significant. (Pearson Chi-square Value:113.389, DF:14, P value:0.000(<0.05) hence statistically significant). There is statistically significant association between gender and clinical variants of lichen planus.

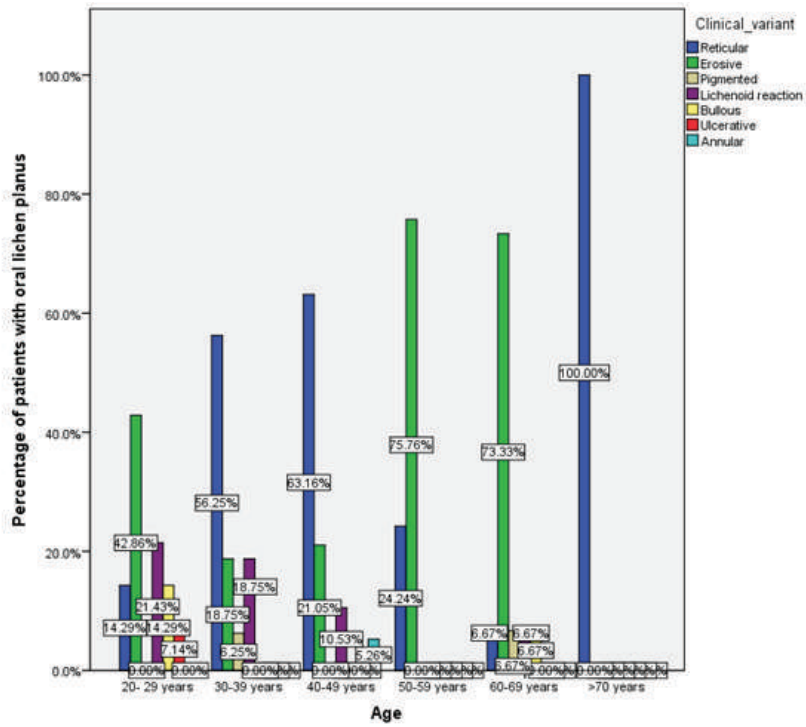


Figure 2 - Bar graph represents the correlation between clinical variants of oral lichen planus and age. X-axis represents the age of the patient and Y-axis represents the percentage of patients with oral lichen planus. Chi-square test was done and association was found to be statistically significant.(Pearson Chi-square P value:0.000(<0.05), hence statistically significant). There is statistically significant association between age and clinical variants of lichen planus.

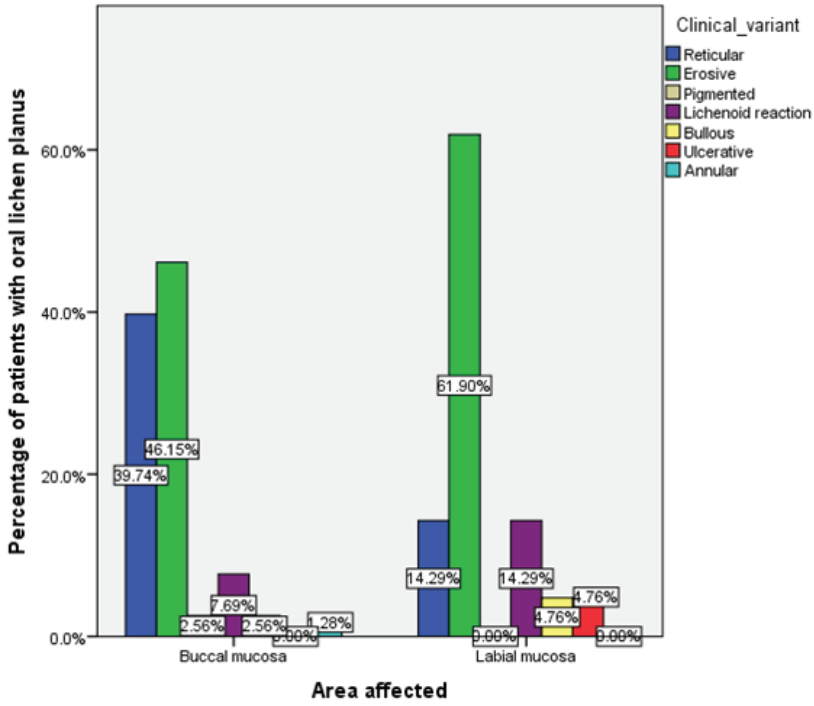


Figure 3 - Bar graph represents the correlation between clinical variants of oral lichen planus and area affected. X-axis represents the area affected in the patient's mouth and Y-axis represents the percentage of patients with oral lichen planus. Chi-square test was done and association was not found to be statistically significant. (Pearson Chi-square P value:0.146(>0.05), hence not statistically significant). There is no statistically significant association between area affected and clinical variants of oral lichen planus.

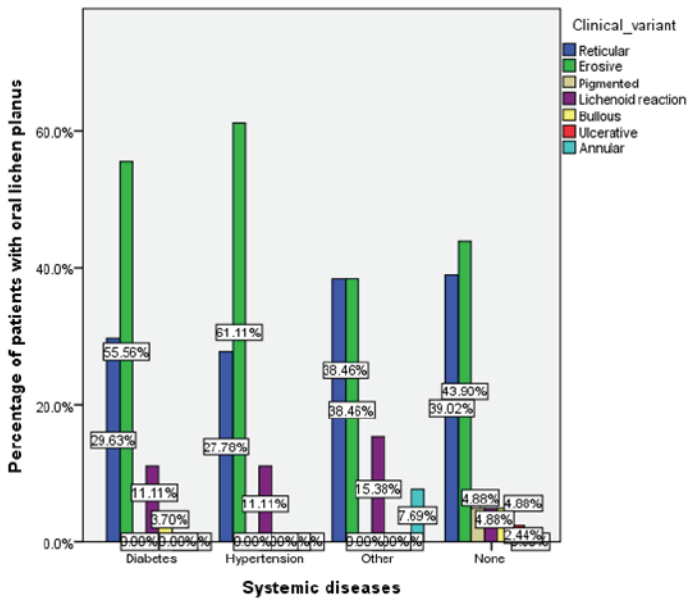


Figure 4 - Bar graph represents the correlation between clinical variants of oral lichen planus and systemic diseases that the patient has. X-axis represents the systemic diseases that the patient has and Y-axis represents the percentage of patients with oral lichen planus. Chi-square test was done and association was not found to be statistically significant.(Pearson Chi-square P value:0.602(>0.05), hence not statistically significant). There is no significant association between systemic diseases and clinical variants of oral lichen planus.

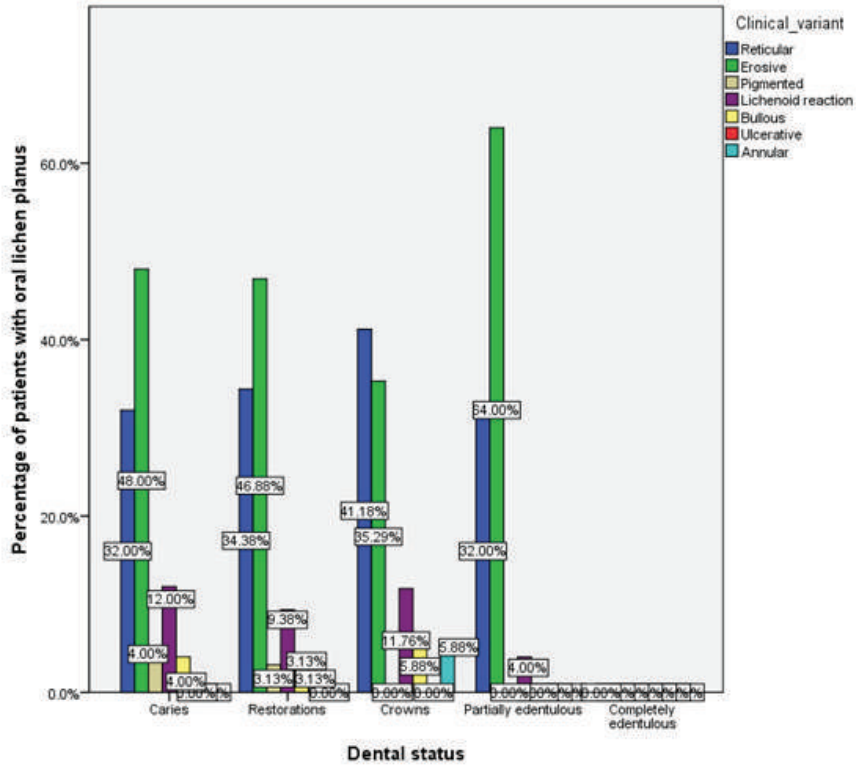


Figure 5 - Bar graph represents the correlation between clinical variants of oral lichen planus and dental status of the patient. X-axis represents the dental status of the patient and Y-axis represents the percentage of patients with oral lichen planus. Chi-square test was done and association was not found to be statistically significant.(Pearson Chi-square P value: 0.795(>0.05), hence not statistically significant). There is no statistically significant association between dental status and clinical variants of oral lichen planus.

Oral Lichen planus was more predominant in females in the present study. There was also significant correlation between the clinical variants of oral lichen planus and gender (P=.000). This was in accordance with Martin et al, Axel et al,, Colquhoun ae al, Karbach et al, Varghese et al and Mankapure et al who stated that lichen planus was more predominant in females [22,29–33]. Eisen et al stated that even though oral lichen planus was more prevalent in women, it developed at an earlier age in men [34,35]. However, Pindborg et al reported an absence of sex predominance[34]. Munde et al reported a male: female ratio of 1.61:1[36]. Bandyopadhyay et al also reported a male predominance[37].

In the present study, Oral lichen planus was more predominant between 50-59 years of age. There was also significant correlation between the clinical variants of oral lichen planus and gender (P=.000). This concurs with Gumru et al who stated that a patient's mean age was approximately 50 years[38]. Axell and Pindborg et al also stated that maximum cases were documented in patients between 55-64 years of age [30,34]. Karbach et al also stated that peak incidence of oral lichen planus occurred in the fifth decade of life[31]. However, Colquhoun et al stated that the overall median ages of men and women were 67 and 66 years old, respectively[29]. Mankapure et al stated that the mean age of oral lichen planus patients was 45.4 years[33]. Bandyopadhyay et al stated a predominance in the third decade[37].

The erosive variety of oral lichen planus was more predominant in the present study. This concurs with Gumru et al who stated that red forms were the most frequent, affecting 60.5% of patients[38]. Eisen et al also stated that the erosive form was most prevalent[35]. Axell et al, Munde et al,Varghese et al, Bandyopadhyay et al and Mankapure et al stated that the reticular type was more predominant [30,32,33,36,37].

In the present study, oral lichen planus most commonly affects the buccal mucosa. This concurs with Martin et al, Axell et al, Munde et al, Mankapure et al,

Varghese et al, Bandyopadhyay et al, Pindborg et al, and Eisen et al who stated that oral lichen planus commonly affects buccal mucosa [22,30,32–37].However, there was no significant correlation between the clinical variants of oral lichen planus and area affected (P=.146).

The incidence of systemic diseases in the present study included diabetes at 26.7%, hypertension at 17.8%, other systemic diseases like asthma, arthritis, gastric disorders at 12.9% and 40.6% had no systemic diseases. However, Munde et al stated that the incidence of systemic diseases included hypertension (11%), diabetes mellitus (2.4%), and hypothyroidism (0.78%)[36]. Varghese et al stated that hypersensitivity reaction was frequently associated with systemic illness with oral lichen planus[32]. However, there was no significant correlation between the clinical variants of oral lichen planus and systemic diseases (P=.602).

In the present study, 31.7% of the population had restorations. However, there was no significant correlation between the clinical variants of oral lichen planus and dental status (P=.795). Gumru et al stated that dentures were one of the precipitating factors for oral lichen planus[38]. Martin et al reported a significant correlation between oral lichen planus and gold and amalgam restorations[22]. Lunstrom stated that clinical signs of corrosion were significantly more frequent in the OLP group (72%) than in the control cases (28%). Patients with atrophic-erosive OLP exhibited a significantly higher frequency of corrosion (83%) than those with reticular type (46%)[6].

The limitations of this study include small sample size, sample size taken from similar geographical location and the psychological,socioeconomic status and hormonal status of the patients were not assessed. Since this was a retrospective study, the prognosis of the patient was not analysed. Further analytical studies must be done in the future to overcome these limitations.

Conclusion

The present study was conducted to understand the prevalence of oral lichen planus and assessment of factors associated with it. Within the limitations of this study, it can be concluded that oral lichen planus was more predominant in females, between 50-59 years of age. Clinically, the erosive variety of oral lichen planus was more prevalent with the most commonly affected area being the buccal mucosa and diabetes was the most predominant systemic disease in these patients. The association was found to be statistically significant between clinical variants of oral lichen planus and age and gender. However, association was not found to be statistically significant between clinical variants of oral lichen planus and area affected, dental status and systemic diseases.

Author Contributions

The primary author contributed to establish the materials and methods and analysing the results followed by manuscript writing.

The co-author verified the results and manuscript before submission.

Conflict of Interest : There are no conflicts of interest.

Source of Funding : Self.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Angular Cheilitis and Assessment of Factors Associated with It - A Retrospective Study

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Abstract

Angular cheilitis is inflammation of the skin, occurring at the labial commissure – the angle of the mouth. It is an eroded and erythematous non-vesicular lesion that can occur at one or both corners of the mouth. The point of interface for squamous epithelium of the face and oral mucosa is at the angle of the mouth . It is also a mechanically dynamic hinge for the oral aperture that endures more motion and tensile forces than the rest of the lips. Thus, the commissures are especially susceptible to certain stresses. Other names for angular cheilitis include angular cheilosis, angular stomatitis, commissural stomatitis, rhagades, or perleche. The increased prevalence is associated with human immunodeficiency virus, usage of immunosuppressants and corticosteroids, increased usage of oral prosthesis in the elderly, xerostomia, expanded usage of oral antibiotics, obesity and diabetes to name a few. In young children, angular cheilitis is associated with habitual lip licking, thumb sucking, or biting of the corners of the mouth, whereas in older patients it occurs from sagging at the commissures of the mouth. The aim of this study was to assess the prevalence of angular cheilitis and assessment of factors associated with it. A retrospective study was conducted in the Saveetha Dental College, Chennai, India. Ethical clearance was obtained from SRB committee, Saveetha Dental College, Chennai, India. The clinical portion of this retrospective study was conducted over a 9 month period,i.e, between June, 2019 to March,2020. Some patients reported with pain while for others it was observed during routine examination. Data was collected from a total of 86000 patients who visited Saveetha dental college between Jun,2019 to March,2020. Out of this, the data of 57 patients who visited the institute and were diagnosed with angular cheilitis were retrieved. The data obtained was tabulated in SPSS for windows, version 20. Descriptive statistics were analysed. Chi square test was done to analyse association of habits of the patient with age, gender,dental status and systemic diseases of patients with angular cheilitis. The results of the study indicated angular cheilitis was more predominant in males and it was more predominant between 40-49 years of age. Angular cheilitis was more predominant in completely edentulous patients and diabetes was the most common systemic disease among the patients. There was statistically significant association between gender (P=0.002) and habits of the patient. However, there was no statistically significant association between age(P=0.278), dental status (P=0.137) and systemic diseases (P=0.817) with habits of the patient.

Keywords: *Angular cheilitis, angular cheilosis, angular stomatitis, commissural stomatitis, rhagades, perleche.*

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Introduction

Angular cheilitis is inflammation of the skin, occurring at the labial commissure – the angle of the mouth. It is an eroded and erythematous non-vesicular lesion that can occur at one or both corners of the mouth[1]. The point of interface for squamous epithelium of the face and oral mucosa is at the angle of the mouth . It is also a mechanically dynamic hinge for the oral aperture

that endures more motion and tensile forces than the rest of the lips. Thus, the commissures are especially susceptible to certain stresses[2]. Other names for angular cheilitis include angular cheilosis, angular stomatitis, commissural stomatitis, rhagades, or perleche[3].

The increased prevalence is associated with human immunodeficiency virus, usage of immunosuppressants and corticosteroids, increased usage of oral prosthesis in the elderly, xerostomia, expanded usage of oral antibiotics, obesity and diabetes to name a few [4,5]. Iron deficiency anemia and other vitamin deficiencies have been cited as other predisposing factors. It usually occurs in association with oral candidiasis[6]. Staphylococcus aureus and β-hemolytic streptococci have also been associated with it.[7–9] Angular cheilitis has also been associated with diseases that cause enlarged lips, such as orofacial granulomatosis [7,10].

In young children, angular cheilitis is associated with habitual lip licking, thumb sucking, or biting of the corners of the mouth, whereas in older patients it occurs from sagging at the commissures of the mouth [8,11–14].

Initially, the corners of the mouth develop a gray-white thickening and adjacent erythema. Later, the usual appearance is a roughly triangular area of erythema, edema and maceration at either corner of the mouth[15]. Typical symptoms are soreness, pain, itching, burning and a raw feeling in the later stage. Opportunistic bacterial or fungal infection along with factors like nutritional deficiencies, decreased vertical dimension, drooling, dry mouth, persistent licking of lips can cause persistence of the lesions. Although it poses a very low mortality risk, it can be physically uncomfortable, and the patient may complain of burning mouth, dysgeusia, dysphagia, anorexia, and weight loss, leading to nutritional deficiency and impaired quality of life [16–20].

Sometimes, it is an extension of denture stomatitis. Denture stomatitis is characterised by erythema and edema in the denture bearing areas. It can occur due to candidal and non-candidal infections, allergy to denture materials, etc. The growth can also occur due to marginal inadequacies of implants [8,11–13]. This affects integrity of implants [21–23].

Although it poses a very low mortality risk, it can be physically uncomfortable. The patient may complain of burning mouth, dysgeusia, dysphagia, anorexia, and weight loss, leading to nutritional deficiency and impaired quality of life [8,11–14,24].

Ohman et al stated that the clinical appearance of angular cheilitis fell into 4 categories. A ground rhagad at the corner of the mouth involving adjacent skin in dentate patients. Among denture wearers a deep lesion following the labiomarginal sulcus was observed[25].

The purpose of this study is to assess the prevalence of angular cheilitis.

Materials and Methods

A retrospective study was conducted in the Saveetha Dental College, Chennai, India. Ethical clearance was obtained from SRB committee, Saveetha Dental College, Chennai, India. The clinical portion of this retrospective study was conducted over a 9 month period,i.e, between June, 2019 to March,2020. Some patients reported with pain while for others it was observed during routine examination.

Inclusion criteria: Patients diagnosed with angular cheilitis, both candidal and non-candidal associated, both males and females, patients of all ages, all dental status.

Data was collected from a total of 86000 patients who visited Saveetha dental college between Jun,2019 to March,2020. Out of this, the data of 57 patients who visited the institute and were diagnosed with angular cheilitis were retrieved. The data obtained was tabulated in SPSS for windows, version 20. Descriptive statistics were analysed. Chi Square test was done to analyse correlation.

Results and Discussion

The results of the present study indicated that 50.9% were males and 49.1% were females(**Figure 1)**. 14% were between 20-29 years, 12.3% were between 30-39 years, 40.4% were between 40-49 years, 12.3% were between 50-59 years, 15.8% were between 60-69 years, 5.3% were >70 years(**Figure 2)**. 24.6% were smokers, 7% were gutkha chewers, 19.3% were paan chewers, 49.1% had no habits . 21.1% had caries, 22.8% had restorations, 21.1% had crowns, 5% were partially edentulous and 26.3% were completely edentulous **(Figure 3)**. 17.5% had diabetes, 8.8% had hypertension, 5.3% had other systemic diseases, 68.4% had no systemic diseases **(Figure 4)(Table 1)**.

Table 1 - Represents the frequencies of gender, age, habits, dental status and systemic diseases.

S.No	Variable	Options	Frequency (%)
1	Gender	Male Female	29 (50.9%) 28 (49.1%)
2	Age	20-29 years 30-39 years 40-49 years 50-59 years 60-69 years >70 years	8 (14%) 7 (12.3%) 23 (40.4%) 7 (12.3%) 9 (15.8%) 3 (5.3%)
3	Habits	Smoking Gutkha Paan chewing None	14 (24.6%) 4 (7%) 11 (19.3%) 28 (49.1%)
4	Dental status	Caries Restorations Crowns Partially edentulous Completely edentulous	12 (21.1%) 13 (22.8%) 12 (21.1%) 5 (8.8%) 15 (26.3%)
5	Systemic diseases	Diabetes Hypertension Other systemic diseases No systemic diseases	10 (17.5%) 5 (8.8%) 3 (5.3%) 39 (68.4%)

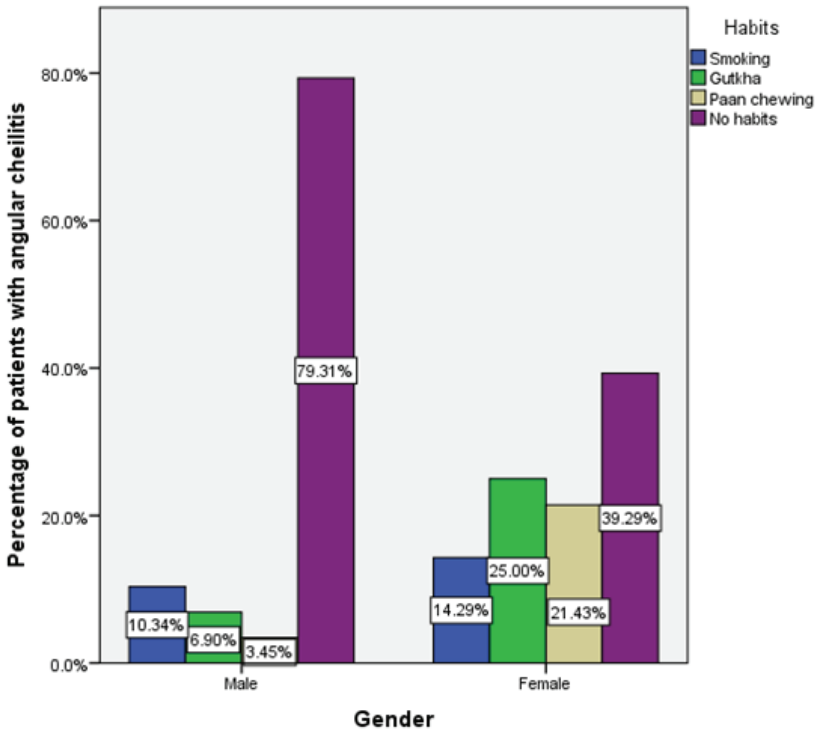


Figure 1 - Bar graph represents the correlation between gender and habits of the patient. X-axis represents the gender of the patient. and the Y-axis represents the percentage of angular cheilitis patients with habits. (Pearson Chi-square P value:0.002 (<0.05) hence statistically significant). In 79.31% of the patients with angular cheilitis, there were no associated habits and the results were found to be statistically significant in both the genders.

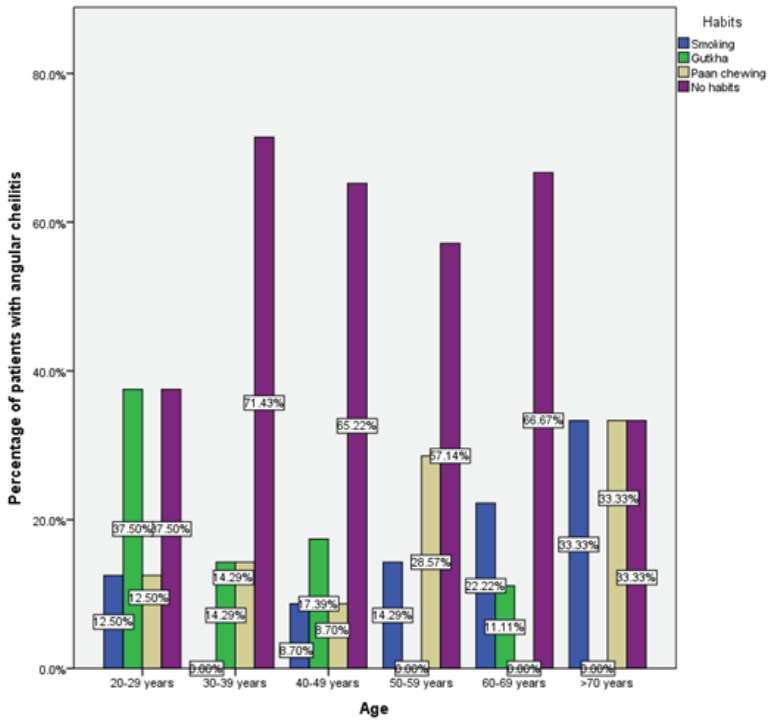


Figure 2 - Bar graph represents the correlation between age and habits of the patient. X-axis represents the age of the patient and Y-axis represents the percentage of angular cheilitis.with habits.(Pearson Chi-square test P value:0.278 (>0.05), hence not statistically significant). Even though the majority of the patients in all age groups

were found to have no habits, the results were not found to be statistically significant.

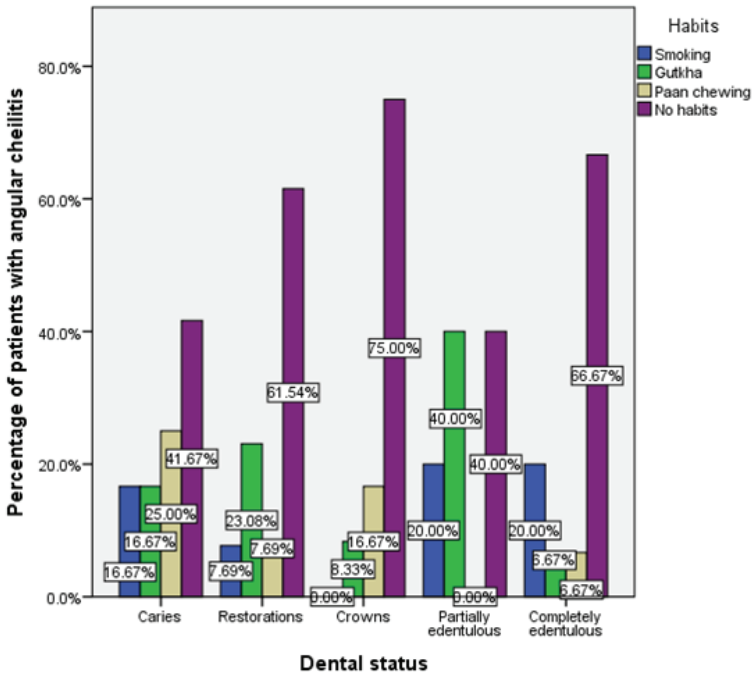


Figure 3 - Bar graph represents the correlation between habits of the patient and dental status of the patient. X-axis represents the dental status of the patient and Y-axis represents the percentage of angular cheilitis patients with habits. (Pearson Chi-square P value:0.137 (>0.05), hence not statistically significant). Even though the majority of the patients of all dental status groups were found to have no habits, the results were not found to be statistically significant.

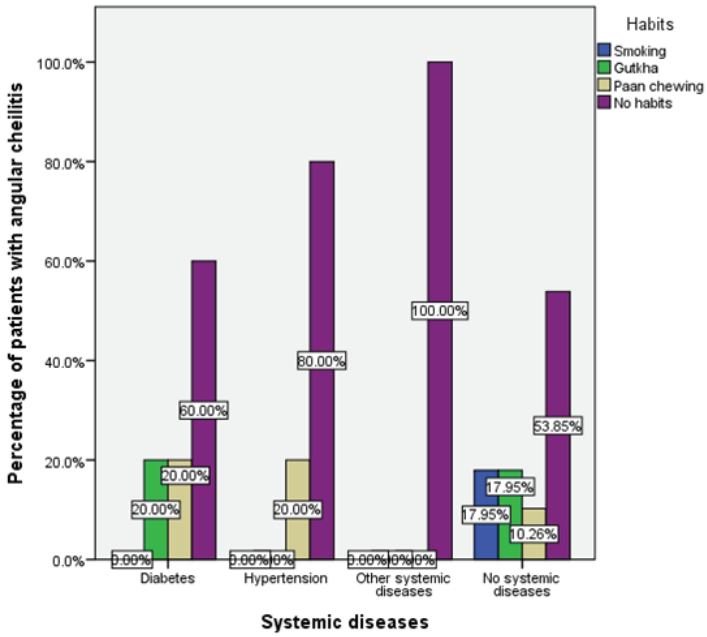


Figure 4 - Bar graph represents the correlation between habits of the patient and systemic diseases of the patient. X-axis represents the dental status of the patient and Y-axis represents the percentage of patients with angular cheilitis. (Pearson Chi-square P value:0.817 (>0.05), hence not statistically significant) Even though the majority of the patients in all systemic disease groups were found to have no habits, the results were not found to be statistically significant.

The present study had no gender predominance with angular cheilitis. This is in accordance with Shahzad et al who state that it occurs in both males and females[26]. However, Federico et al state that angular cheilitis is twice as frequent in men than in women[27]. There was significant correlation between the presence of habits and gender (P=.013) in this study.

Angular cheilitis was more predominant between 40-49 years in the present study. According to Shahzad et al, Federico et al, and Cross et al angular cheilitis predominantly occurs in adults in the third to sixth decades of life [26–28]. A study by Parlak et al in Turkey with 993 children revealed that 9% of 13–16 year olds had angular cheilitis and this was the most common oral mucosal lesion[29]. However, a larger epidemiological study by Shulman in 10,030 children aged between 2 and 17 years failed to record a single case[30]. This could be due to underlying nutritional deficiencies between these two groups, as the presence of angular cheilitis in the Turkish population was associated with anaemia[29]. There was no significant correlation between the presence of habits and age (P=.648) in this study.

In the present study, 28.1% were smokers. Some studies have stated that smoking does not affect Candida carriage significantly [31,32], while others have reported that smoking significantly increased carriage by 30 to 70%[33]. Some authors have also stated that cigarette smoking might lead to localized epithelial alterations that allow colonization by Candida. This relationship between cigarette smoke and Candida is particularly important, as the enzyme system can increase the carcinogenic activity of the hydrocarbon and candidal leukoplakia might have a higher potential for malignant changes than normal leukoplakia[34].

26.3% of the participants in the present status were completely edentulous. Federico et al stated that there is a 3-fold incidence in denture-wearers[27]. According to Scully et al, people with angular cheilitis who wear dentures may have erythematous mucosa underneath the denture. This appearance is consistent with denture stomatitis[35]. Sharon et al stated that wrinkles or at the oral commissures lead to an increased risk for angular cheilitis by creating a chronic, moist, intertriginous environment which fosters microorganisms. The long-term use of ill-fitting dentures accentuates this wrinkling. Ill-fitting dentures cause resorption of the bone on which the dentures lay thereby reducing the vertical height of the lower face. These conditions lead to saliva trapping or excessive saliva at the angles of the mouth[36]. Cross et al state that angular cheilitis was observed in patients wearing appliances with full palatal coverage slightly less frequently than expected when compared with adult complete denture wearers[28]. Ohman et al, Cawson et al and Turrell et al stated that neither loss of the vertical dimension nor the condition of the dentures seem to be a significant factor in the etiology of angular cheilitis [25,37,38]. There was no significant correlation between the presence of habits and dental status (P=.534) in this study.

In the present study 17.5% of the participants were diabetic. Sharon et al state that diabetes is a risk factor for angular cheilitis[36]. Khalid et al state that angular cheilitis occurs significantly more frequently in diabetic than in non-diabetic patients[39]. Scully et al stated that immunocompromised patients have more predilection for angular cheilitis[35]. There was no significant correlation between the presence of habits and systemic diseases (P=.602) in this study.

The limitations of this study includes the small sample size and the geographic location of the patients were not assessed. Since this was a retrospective, prevalence study the prognosis of the patients could ot be analysed. Future analytical studies need to be done with these limitations in mind.

Conclusion

Within the limitations of this study, it can be concluded that angular cheilitis was more predominant in males, between 40-49 years of age. Clinical association was seen in completely edentulous patients and in patients with systemic disease like diabetes. There was statistically significant association between gender and habits of the patient.

Author Contributions: The primary author contributed to establish the materials and methods and analysing the results followed by manuscript writing.

The co-author verified the results and manuscript before submission.

Conflict of Interest: There are no conflicts of interest.

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Ethical Clearance

It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Reliability of Profile Photography for Determining Growth Pattern and Sagittal Jaw Relationship in Different Classes of Malocclusions

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Abstract

Although the importance of facial photography has been stressed in literature, there are no studies on its diagnostic potential. This study was conducted to evaluate the diagnostic merit of photography in detecting growth patterns and jaw relationships. Standardized lateral cephalograms and profile photographs were taken for 40 patients. On cephalometry, growth patterns and sagittal classifications were determined. Diagnostic photography- based measurements for detecting departures from the norm were calculated according to the gold standard (lateral cephalometry). Independent sample t-test was used for comparing mandibular plane angles with profile photography and with lateral cephalometry. The mean value of the mandibular plane angle as determined by lateral cephalometry is 30.27+4.59. The mean value of the mandibular plane angle as determined by profile photography is 31.80+4.76. There was no significant difference seen. Profile photography is equally reliable as lateral cephalometry in detecting mandibular plane angle. Profile photography can be used as a perfectly accurate measurement tool for detecting horizontal and vertical growth patterns. It is also a rather accurate method for detecting normal growth patterns, as well as both Class I and non-Class I jaw relationships. These results highlight this non-invasive and economic method as an effective diagnostic tool. There is good association between lateral cephalometry and profile photography in detecting mandibular plane angle in different skeletal groups as well.

Keywords- *Lateral cephalograms; Profile photography; Mandibular plane angle; Growth patterns*

Introduction

One of the major components of orthodontic diagnosis and treatment planning is evaluation of patient profiles and detecting changes from the normal values[1]. Knowledge of the dentofacial pattern and sagittal relationship allows the clinician to make correct decisions and enhance treatment success in getting optimal facial harmony[2]

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The standard and widely used method for evaluating skeletal and dentofacial relationships is usage of lateral cephalograms[3]. Profile photography is used for establishing the treatment objectives and determines the soft tissue outline of the facial appearance [4,5]. It is a non-invasive method that can be easy to obtain and gives us an early impression about the complaints of the patient and our chief objectives[6]. Although the importance of facial photography in orthodontics has been recognized, correlations between photography and cephalometric measurements have been established by Zhang et al[7,8]. Furthermore there is no research on diagnostic values and accuracy of photography (compared with cephalometry) in detecting values departing from the standard values[9].

Therefore, our aim for this study was to evaluate the predictive value of photography as a diagnostic tool for detecting abnormalities in growth patterns, chin positions and sagittal jaw relationships.

Materials and Methodology

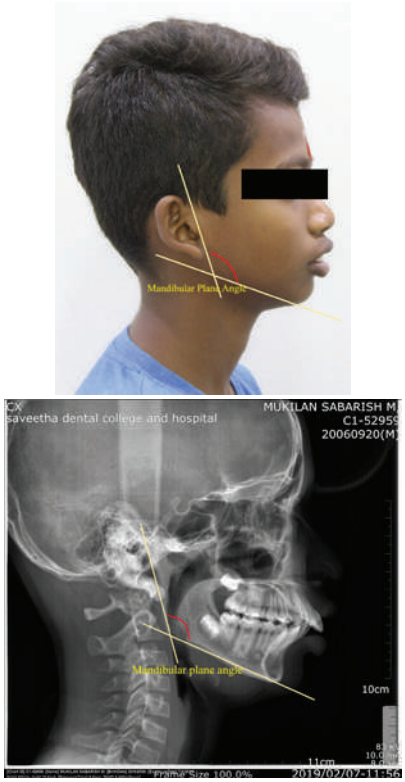


Fig 1: Anatomical landmarks utilized for tracing and determining mandibular plane angle in both lateral cephalogram as well as with profile photograph

This was a diagnostic study conducted in the Department of Orthodontics at Saveetha Dental College, Chennai. Our samples for this study comprised profile photographs and lateral cephalograms of 40 attendees each.

The inclusion criteria comprised patient's willingness to participate, necessity for lateral cephalometry and no history of any previous orthodontic treatment. A total of 40 patients were enrolled in their respective sequences

Lateral Cephalometry

Lateral cephalograms were taken from patients in the natural head position (NHP) by getting them to look at their pupils in a mirror located 1 meter away. Patients stood in a relaxed position, hands and shoulders were at rest and backs were upright without any support (Fig 1). Their Frankfurt plane was horizontal, teeth were in centric occlusion and lips were in the resting position. The best radiograph for the patient was selected and the following landmarks were traced: soft tissue menton, supramentale, tragus, soft tissue Pogonion, orbitale, subnasale, soft tissue nasion, true horizontal and true vertical planes. Analysis was done using the OneCeph software. The growth pattern was determined as average, horizontal and vertical based on the gonial angle and Jarabak index. Sagittal classification (Class I, II, III) was determined based on ANB angle and Wits analysis.

Profile Photography

All patients' foreheads, ears and necks were completely exposed. Patients stood in a marked space and made no facial expressions with lips and chin muscles at rest. Subjects' hands and shoulders were at rest and their backs were upright, without any external support. Their heads were in natural head position (NHP) with patients looking at their pupils in a mirror in a standard situation and adjusting their midface line[10]. Portrait photographs were taken of the patients right profiles with a DSLR camera with a focal length of 50mm and resolution of 10 megapixels (Fig 1). The vertical position of the camera was aligned with the subject's head, in such a way that the camera's central focus (focal spot) was set on the patient's tragus and the resulting image would include margins above the head and below the neck. Another photograph of the same patient was taken 30 seconds later in identical conditions. The best photograph of each patient was used for photographic analyses which were based on the following landmarks: soft tissue menton, supramentale, tragus, soft tissue Pogonion, orbitale, subnasale, soft tissue nasion, true horizontal and true vertical planes. Photographic soft tissue analysis were performed using FACAD software by using location of points tracing. The measurements used were the angles located, facial convexity and head position.

Statistical Analysis

Predictive values in the photogrammetric method are used for determining departures from normal values. An independent sample t-test was used to determine the mandibular plane angle and compare it between lateral cephalogram and profile photography. Presence of any significant differences was assessed between the two groups and the software used was SPSS Version 20.0 (SPSS Inc, Chicago). Pearson;s correlation test was done between the two groups to determine the correlation. Chi-square test was done to determine the association

between the two groups in different skeletal patterns.

Results and Discussion

The cases were identified appropriately by the photographic and cephalometric method. The mean value of the mandibular plane angle as determined by lateral cephalometry is 30.27+4.59. The mean value of the mandibular plane angle as determined by profile photography is 31.80+4.76. There was no significant difference between the two methods for determining the growth pattern of an individual. Levene's test for equality of variances between the two groups showed changes while detecting sagittal relationships between the two groups mostly due to changes in chin position. Pearson's correlation test was done to determine the correlation between the two groups in detecting mandibular plane angle. The correlation coefficient was 0.901 indicating good relatability between the two groups. Chi-square test was done to determine association between the diagnostic aids in different skeletal patterns. They detected a good association between different skeletal types and the type of diagnostic aid used.

Our study introduced profile photography analysis as a method for detecting different growth patterns and sagittal relationships with appropriate accuracy[11]. On analysis, we can detect that when the photography method detects a growth pattern change, it must be taken accurately in most instances[12]. When photography suggests a face has an average growth pattern, then there is 78% probability that the cephalometry method will also identify the person as having average growth pattern[13]. The reverse applied is also true in most instances with the cephalometric method being applied accurately as well. The weak point of the photographic approach is its rather low sensitivity, meaning it might miss out on one out of two cases with actual growth pattern abnormality[14].

The information given by the photographic method in detecting deviations from a non-Class I relationship is about 80 percent. Deviation in Class II and Class III relationships are much harder to detect and present with variations[15]. Visualizing bony landmarks is important when the soft-tissue and hard-tissue landmarks are situated some distance apart[16]. Zhang et al observed the highest correlations between vertical facial heights calculated according to cephalometric and photographic analysis[17].

Nasion and menton landmarks used for measuring facial height were not covered by thick soft tissues[18]. Nevertheless, they concluded that since the variations in soft tissue and bony landmark locations might have clinical implications, facial photography must be considered an adjunct to and not a substitute for cephalometry[19].

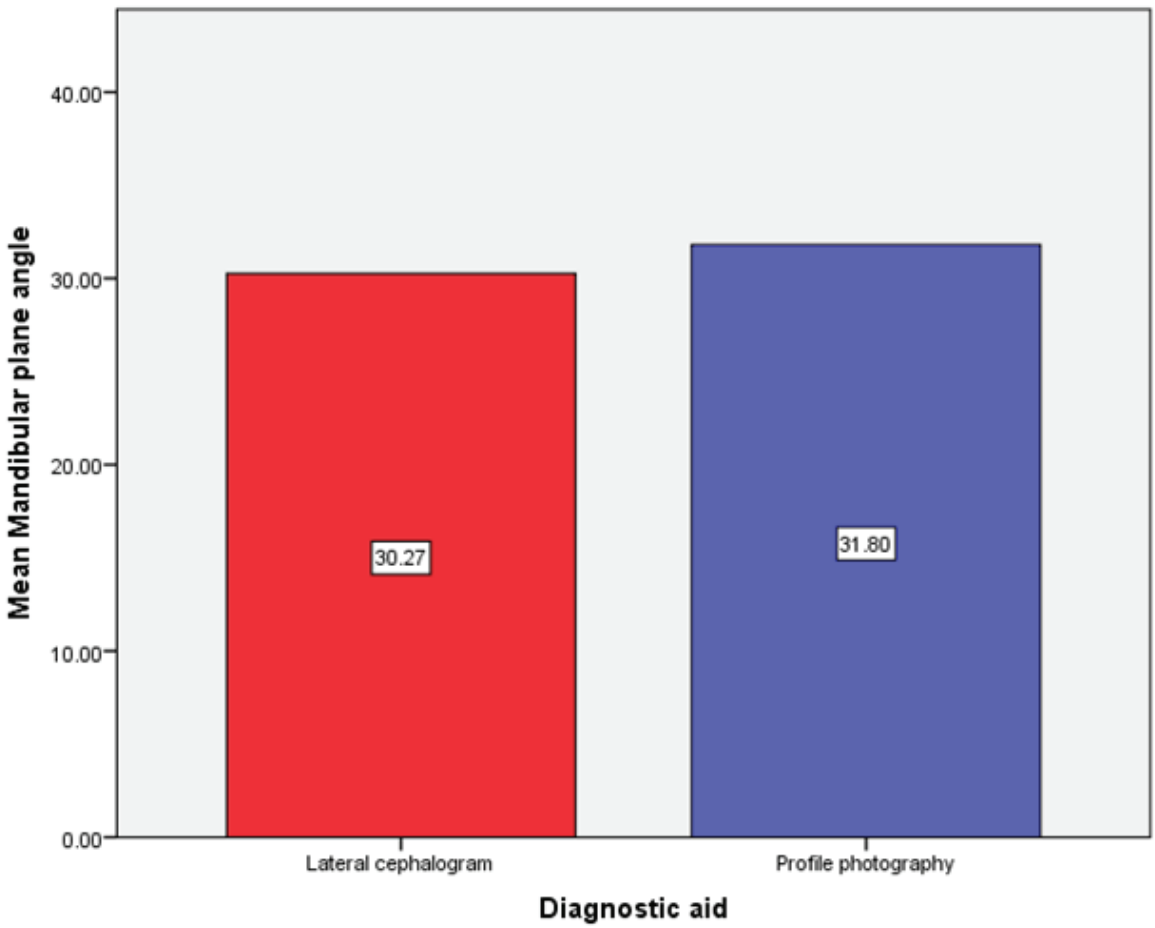
On the other hand, they approved the use of standardized photography as an epidemiologic tool as well as for evaluating hereditary facial forms in large hereditary studies regarding measurements such as facial heights which display a high correlation with the counterpart cephalometric measurements[20]. As dental practice becomes more and more computerized, digital photo documentation is also becoming a standard procedure because of its numerous advantages[21]. Digital images can be easily seen and repeated in the same session, can be stored easily and retrieved rapidly[1]. Moreover, digital images can be conveniently enhanced using digital features such as zooming and edge detection, while they take up no physical space[22].

Considering all these advantages, plus the high reliability of photographic analyses as well as the high diagnostic value of this method in detecting deviations from the normal, this approach can be recommended as an appropriate method for clinical use[23,24].

Our study was limited by a number of factors. Future studies should improve the reliability of findings by calculating intra-evaluator concordance as well[25]. Also, a sample size determination by appropriate power calculations could improve the reliability of the findings[26]

Table 1: Independent sample t-test to determine test of significance between lateral cephalogram and profile photography while determining mandibular plane angle with p-value=0.959

S.NO	N	Mean	S.D.	Sig.
Lateral Cephalogram	40	30.27	4.59	0.959
Profile Photography	40	31.8	4.76	



Graph 1: This bar graph represents the depiction of mandibular plane angle when verified by two different diagnostic aids- Profile photography and Lateral cephalometry. The x-axis of the graph represents the two diagnostic aids used in the study (Lateral cephalogram and Profile Photography) and the y-axis of the graph represents the mean of mandibular plane angles. The red bar denotes the mandibular plane angle when determined by lateral cephalometry and the blue bar denotes the mandibular plane angle when determined by profile photography method. The mean value of mandibular plane angle is greater when determined by Profile photography (31.80+4.76 degrees) than with lateral cephalometry method (30.27+4.59 degrees). There is no significant difference between the mean values and the diagnostic aids used (lateral cephalometry and profile photography). Independent sample t-test was used to determine the descriptive statistics (mean and standard deviation) of the study (p=0.959). The significant difference between the two groups is 0.959 which is not statistically significant.

Table 2: Pearson's correlation coefficient test done between the two diagnostic aids (lateral cephalometry and profile photography) used in the study. The correlation between the two is 0.901 which indicates high correlation value. Therefore, both lateral cephalometry and profile photography can be reliably used for determining mandibular plane angle.

Correlations			
		Lateral cephalometry	**Profile photography**
Lateral cephalometry	Pearson Correlation	1	.901**
	Sig. (2-tailed)		.000
	N	40	40
Profile photography	Pearson Correlation	.901**	1
	Sig. (2-tailed)	.000	
	N	40	40
**. Correlation is significant at the 0.01 level (2-tailed).			

Table 3: Chi-square test to determine association between lateral cephalometry and profile photography in Class I skeletal groups. The likelihood ratio is 83.85 indicating good association between the two diagnostic aids in Class I skeletal groups.

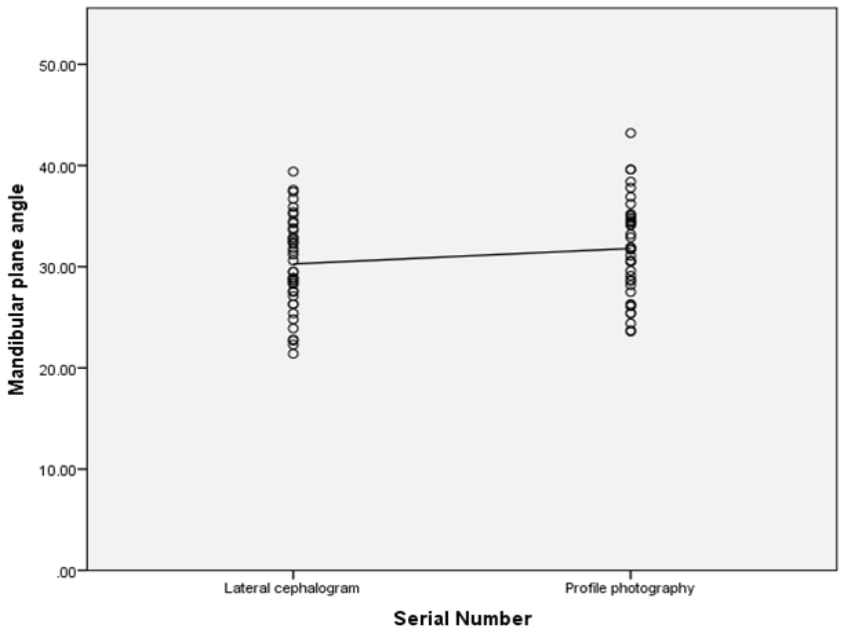
	Value	**df**	**Asymp:Sig. (2-sided)**
Pearson Chi-Square	60.667a	61	.488
Likelihood Ratio	83.857	61	.028
Linear-by-Linear Association	2.106	1	.147

Table 3: Chi-square test to determine association between lateral cephalometry and profile photography in Class I skeletal groups. The likelihood ratio is 83.85 indicating good association between the two diagnostic aids in Class I skeletal groups.

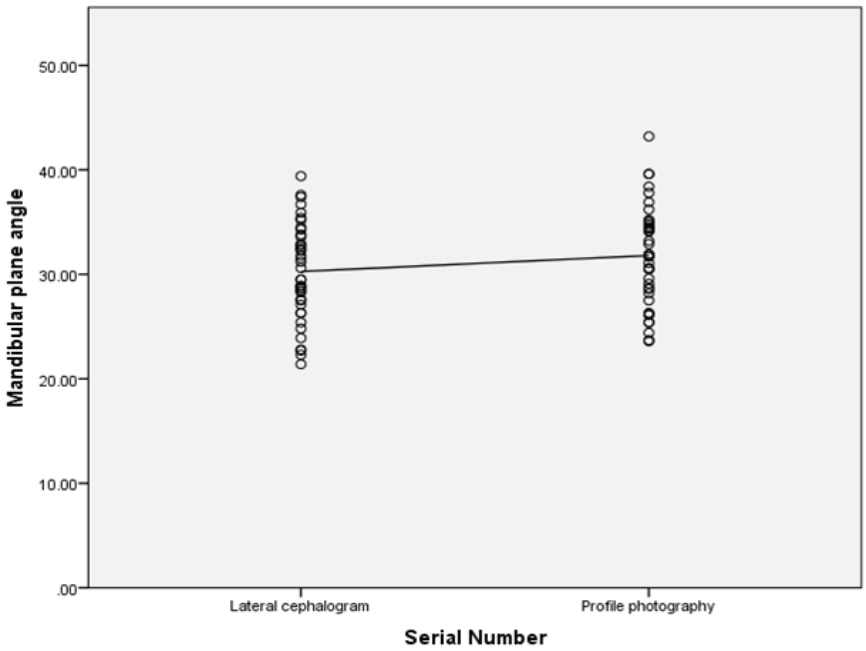
	Value	**df**	**Asymp:Sig. (2-sided)**
Pearson Chi-Square	58.646[a]	61	.625
Likelihood Ratio	79.889	61	.129
Linear-by-Linear Association	2.897	1	.169

Table 4: Chi-square test to determine association between lateral cephalometry and profile photography in Class II skeletal groups. The likelihood ratio is 79.89 indicating good association between the two diagnostic aids in Class II skeletal groups.

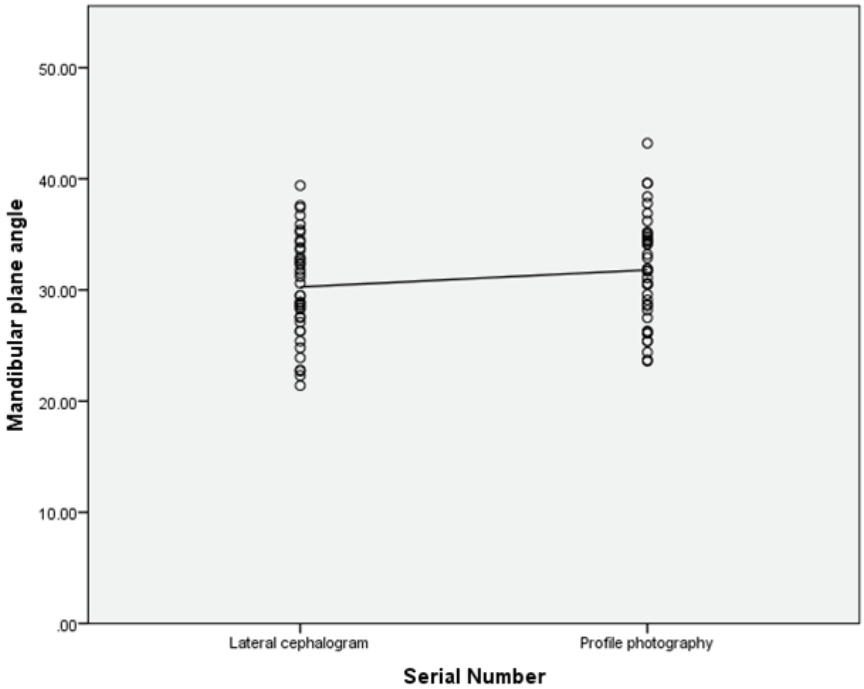
	Value	df	Asymp:Sig. (2-sided)
Pearson Chi-Square	67.822a	61	.399
Likelihood Ratio	81.369	61	.258
Linear-by-Linear Association	1.945	1	.227



Graph 2: Scatter plot to determine the association between lateral cephalometry and profile photography in Class I skeletal groups. The x axis denotes the two groups while the y axis denotes the Mandibular plane angle. The likelihood ratio is 83.85 indicating good association between the two diagnostic aids in Class I skeletal groups.



Graph 3: Scatter plot to determine the association between lateral cephalometry and profile photography in Class II skeletal groups. The x axis denotes the two groups while the y axis denotes the Mandibular plane angle. The likelihood ratio is 79.89 indicating good association between the two diagnostic aids in Class II skeletal groups.



Graph 4: Scatter plot to determine the association between lateral cephalometry and profile photography in Class III skeletal groups.The x axis denotes the two groups while the y axis denotes the Mandibular plane angle. The likelihood ratio is 81.37 indicating good association between the two diagnostic aids in Class III skeletal groups.

Conclusion

Profile photography can be used as a perfectly accurate method for detecting presence of horizontal, vertical, average growth patterns. It is fairly accurate for detecting normal growth patterns as well as both Class I and non-Class I jaw relationships. These results highlight this non-invasive and economic method as a useful clinical tool. There is good association between lateral cephalometry and profile photography in detecting mandibular plane angle in different skeletal groups as well.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Type of Study: Retrospective Study *DOI Number: 10.37506/ijfmt.v14i4.12536*

Analysis of the Cost Associated with the Treatment of Early Childhood Caries in Children Aged 2-6 Years

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Abstract

One cannot deny that cost factor plays an important role in the treatment phase of any disease and thus can widely affect the patient's acceptance to treatment. Overall cost of treatment gives an idea of the approximate cost differences that can be expected by patients. In this given study we aim to analyse the overall cost for treatment procedures done for children suffering from early childhood caries in the state of Tamil Nadu. This is a hospital based retrospective study. All data of the patients visiting Saveetha Dental College and hospitals from June 2019 to March 2020 located in Chennai were retrieved manually. Children who were diagnosed with early childhood caries between 2-6 years who had at least one procedure done were included. A total of 1210 patients were included in the study out of which 660 were males (54.55%), and 550 were females (46.45%). Out of 1210 patients: average costs for 1 to 5 procedures: Rs 231/-, 6 to 10 procedures: Rs 698/-, 11 to 15 procedures: Rs 1222/-, 16 to 20 procedures: Rs 1783/-, 21 to 25 procedures: Rs 2419/-, 26 to 30 procedures: Rs 2765/-, 31 to 35 procedures: Rs 3261/-, 36 to 40 procedures: Rs 3713/-, 41 to 45 procedures: Rs 3893/-. Out of total procedures the patient count for 1 to 5 procedures: 715, 6 to 10 procedures: 232, 11 to 15 procedures: 98, 16 to 20 procedures: 47, 21 to 25 procedures: 40, 26 to 30 procedures: 30, 31 to 35 procedures: 25, 36 to 40 procedures: 12, 41 to 45 procedures: 4. We can conclude that the most of the patients required 1 - 5 dental procedures with the frequency being highest at 5 years and mostly in male children and the overall cost had a gradual linear increase with the increase in the number of procedures.

Keywords: *dental extractions; primary teeth; early childhood caries; treatment cost*

Introduction

Dental caries is a global oral health problem with distinctive variations in its distribution. It continues to be the most common infectious disease. ECC is one of the most serious public health problems in children in developed as well as developing nations and a commonly neglected disease among children in India [1]. Early childhood caries is defined by the American Academy of Pediatric Dentistry (AAPD) as the presence of one or more decayed (cavitated or non-cavitated), missing due to caries or filled tooth surfaces in any primary tooth in a child of age 71 months or younger [2]. In rural south India, the prevalence of ECC has been reported as 40.6% among 0 to 3-y-old children, of whom 50.3% had non-cavitated surfaces and 49.7% had cavitated surfaces [3]. A high prevalence of ECC has been reported in India, with none of the states having prevalence below 40% [4] with Tamil Nadu having a prevalence of 55.73%[4–10]. Any disease having 40% prevalence is a serious health issue and requires focused attention [4]. This trend continues in young children in spite of major progress in the field of caries control over the past few decades [4,11–14].

A latest survey of National consumer usage and attitudes towards dental education in the population conducted along 150 cities in India, the dental problems seem to be due to low dental awareness and poor oral hygiene habits. Most of the rural population still used non dentifrice products like Neemstick, charcoal and ash. Many patients defer from treatment due to dental pain and also due to increase in treatment costs [13]. Few of the

barriers for seeking dental services as classified by FDI are individuals (lack of perceived need, financial issues, lack of access), dental profession (uneven distribution, not sensitive to patient's attitudes and needs), and society (insufficient support for research, ineffective support of public attitudes) [13].

Early childhood caries can give rise to sensitivity, pain and infection, difficulty in mastication, leading to systemic effects like overall malnutrition, gastrointestinal problems. Hence treatment plays a critical role in oral and overall health [15].

Treatment ranges from oral prophylaxis[16], restorations, pulp therapies[17–23]in the oral cavity [24,25] and management of trauma to the dentition[26] etc. Usage of multiple medications, laser, ultrasound equipment, radiation therapy, multiple imaging(CBCT, CT, MRI) and procedures in general anaesthesia can also significantly increase the cost.

One very important factor for the increase in cost is due to dental neglect. Dental neglect is defined as parents' persistent failure to take precautions and provide necessary dental treatment to maintain the child's oral health and to ensure their freedom from pain and infection. It is the responsibility of parents to pursue health-related necessities for their children. In this regard, the lack of parent's or guardian's attention will have a negative influence on the child's oral status. Thus, the interventions that are directed toward parents' beliefs and attitudes about oral health may be beneficial in the prevention of oral problems such as dental caries [27].

One cannot deny that cost factor plays an important role in the treatment phase of any disease and thus can widely affect the patient's acceptance to treatment. The results of the present study showed that the parents having income less than Rs15,000 and who have done secondary education showed lesser levels of positive dental attitudes which is similar to studies done by Freeman et al. and Williams et al[28,29]. Hence, there is a greater probability for the occurrence of oral diseases among children of low-income mothers[30], which is in contrast to the result of Al Gahnim et al.,[31] where it was stated that income does not play a role in attitude toward oral health.

Overall cost of treatment gives an idea of the approximate cost differences that can be expected by patients, so in this given study we aim to analyse the overall cost for treatment procedures done for children suffering from early childhood caries in the state of Tamil Nadu [32].

Materials and Methods

This is a hospital based retrospective study. It was done by two examiners. Simple random sampling was done to reduce sampling bias. Dental records of patients who had visited a private dental hospital from June 2019 to March 2020, located in Chennai, Tamil Nadu, India were retrieved manually and analysed. All information including patient ID, treatment done, bill number, cost of procedure, total number of procedures, age, and gender were retrieved.

A total 1210 case sheets were retrieved manually and compiled and tabulated in Excel. Children who were diagnosed with early childhood caries between 2-6 years who had at least one procedure done were included and children with systemic disorders, special children and children devoid of caries were excluded.

All the procedures were done by only a single operator for a specific patient. A full mouth examination with intraoral periapical radiographs of the teeth indicated for treatment was taken before the start of the clinical procedure. After confirmation of the diagnosis a parental consent in writing and video consent was obtained and then the required treatment procedures were done by adhering to strict treatment protocols.

Cross verification for incomplete and inaccurate data was cross checked with treatment bills and intraoral photographs and verified. The internal validation was achieved by making the sample representative. This was further extended to external validity due to a large sample generalized but only to the South Indian population.

Ethical aspect:

This study was conducted with ethical approval obtained from the Institutional Ethical Committee (Ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320 of Saveetha Institute of Medical And Technical Sciences.

Statistical Analyses

The collected data was entered into Microsoft office Excel 2013 datasheet transferred to SPSS version 26.0 software (SPSS software Chicago, IL, USA) for statistical analysis. They were put to descriptive analysis

and chi square test was done to assess the significance of association between the categorical variables.

The independent variables: gender, age. The independent variables: total number of procedures, cost of procedures, treatment done.

Results and Discussion

A total of 1210 patients were included in the study out of which 660 were males (54.55%), and 550 were females (46.45%) [Figure 2].

The total number of patients across various age groups 1 year: 1.08%, 2 years: 3.39%, 3 years: 12.73%, 4 years: 23.97%, 5 years: 30.99%, 6 years: 28.84% [Figure 1].

Out of total procedures the patient count for 1 to 5 procedures: 715, 6 to 10 procedures: 232, 11 to 15 procedures: 98, 16 to 20 procedures: 47, 21 to 25 procedures: 40, 26 to 30 procedures: 30, 31 to 35 procedures: 25, 36 to 40 procedures: 12, 41 to 45 procedures: 4 [Figure 3].

Out of 1210 patients: average costs for 1 to 5 procedures: Rs 231/-, 6 to 10 procedures: Rs 698/-, 11 to 15 procedures: Rs 1222/-, 16 to 20 procedures: Rs 1783/-, 21 to 25 procedures: Rs 2419/-, 26 to 30 procedures: Rs 2765/-, 31 to 35 procedures: Rs 3261/-, 36 to 40 procedures: Rs 3713/-, 41 to 45 procedures: Rs 3893/-. Chi square test was done and the association was found to be significant (p value = 0.045) [Figure 4].

The distribution of the total number of procedures for the various age groups included in the study for the various types of treatment procedures done. Chi square test was done and the association was found to be not significant (p value = 0.000) [Figure 5].

Based on gender, males underwent more treatment procedures, on an average underwent 1-5 procedures – 715 patients (59.09%) with this having the highest frequency at 5 years – 243 patients (30.99%). Overall the base minimum cost ranged from Rs.30/- to the bare maximum of Rs.3893/-.

One cannot deny the fact that early childhood caries can lead to several other problems affecting even the permanent dentition like malocclusion so the expense of treating the disease continues even after initial treatment [32,33]. This study in Alaska stated that all interventions were shown to generate a cost saving to the health care payers [34].

We see that there is a gradual increase in the cost of the overall treatment as the number of procedures increase. This can be attributed to the increase in the complexity of the treatment leading to the increase in cost. This was consistent with previous literature [34,35], it is also seen that the cost of chair side treatment was far less as compared to general anaesthesia [36–38]. At the same time we can deny that general anaesthesia was more effective than chairside treatment [36,37] and it gave an additional 4 caries free months [36].

In this study there is a gradual decrease in the number of patients as the average number of procedures increase. The most frequent type of treatment required were one surface restorations followed by extractions, pit and fissure sealants and pulp therapy [35]. In figure 3 there is a huge difference in the number of patients undergoing 1-5 procedures and 6-10 procedures. This could be due to the treatment being carried out for a temporary solution on emergency management. The increase in fallout rate also is caused due to the ability of parents to come during working hours and for children to take off from schools. Most of the patients faced severe stages of the disease mostly due to delay in proper treatment [35].

It was also observed that all sections of society lacked awareness with respect to treatment of primary teeth and had the similar misconception that primary teeth would fall off and hence do not require treatment. This leads to failure to seek or delay in seeking dental treatment, to comply with treatment plan, and failure to implement basic oral care.

We see that though in 1-5 procedures the number of patients keeps increasing, for all other procedure groups there is an increase in trend till 4-5 years and then there starts a gradual decrease. We can explain this trend as once a child enters school, proper meal times, decrease in snacking, increased school dental programmes and overall growth and development of the child further leads to better care of the oral cavity and increase in oral hygiene practices leading to a decrease in the severity of the disease, but a study by Kastenborn et al states that treatment cos of the patient is high irrespective of the age [39].

These excessive costs can be decreased by the means of early detection and practicing preventive approaches to management of dental caries [35]. Regular dental checkups must be promoted. The overall cost of all the

procedures eventually points to only one main strategy: 'prevention'. Early intervention can lead to avoidance of extensive pulp therapy and can be limited to restoration. Effective plaque control measures can significantly contribute to the prevention of dental caries. In the present day, various chemical and mechanical methods are available, of which tooth brushing persists to be the most effective method[40]. Patients who do not suffer from severe symptoms like non cavitated lesions can be treated by minimally invasive techniques to preserve tooth structure. One of the most common preventive methods range from maintenance of proper oral hygiene[40] to incorporation of fluoride in daily life in form of fluoride toothpastes, fluoridated water, topical fluoride gels, varnishes etc[41]. The World Health Organisation has recommended adding fluoride to drinking water as it has less than optimal fluoride levels [42].

Limitations of the study included retrieval and use of data from a single hospital setup which could have led to difference in costs in other hospitals/clinics. Since this study is done in a university dental setup the costs are lesser .Hence these costs of treatment to be extrapolated to the market value.The restrictions in the geographic location will not give a generalized result for the given data. Many lower sections of the society would prefer a government hospital compared to a private hospital leading to a disproportion in representation of the population.

Proper dental examination, oral hygiene maintenance and routine restorative procedures must be performed to prevent development of more serious damaging and expensive conditions. Further studies focusing on cost saving treatment of timely management of dental caries and implementation of preventive measures are necessary.

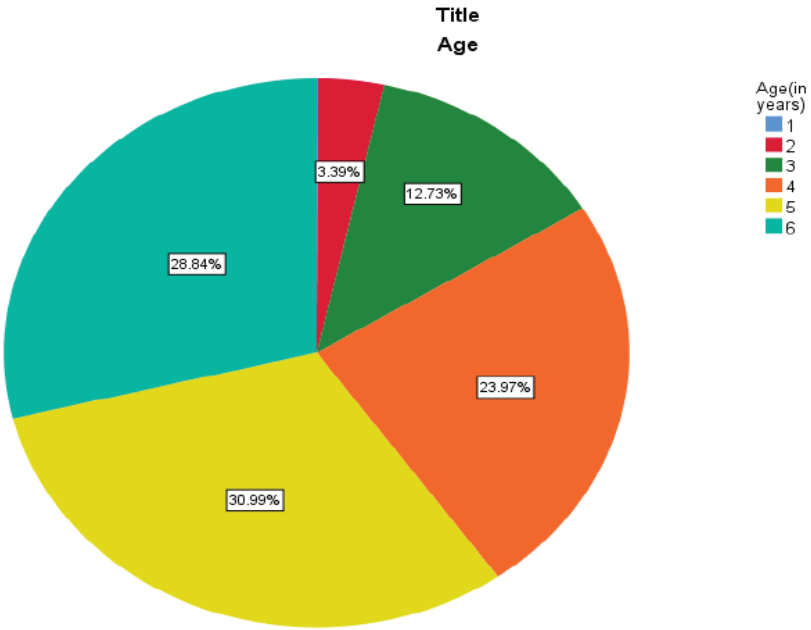


Figure 1: Pie chart showing the distribution of patients across various age groups. Blue color represents 1year olds, red color represents 2 year olds, green color represents 3 year olds, orange color represents 4 year olds, yellow color represents 5 year olds, pale green color represents 6 year olds. From this chart it is seen that the 4 year olds were the most treated (30.99%).

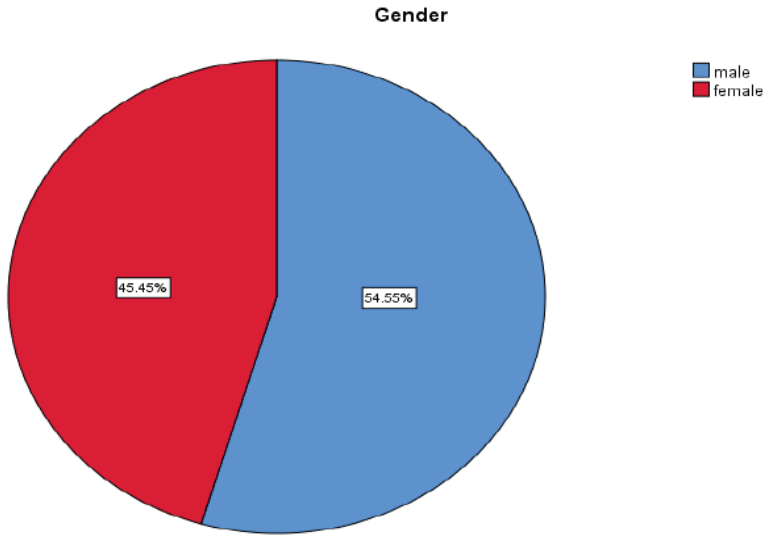


Figure 2: Pie chart showing the distribution of patients based on gender. Red color represents females and blue color represents males, The distribution of patients based on gender was 660 were males (54.55%), and 550 were females (46.45%) with the number of males being treated higher.

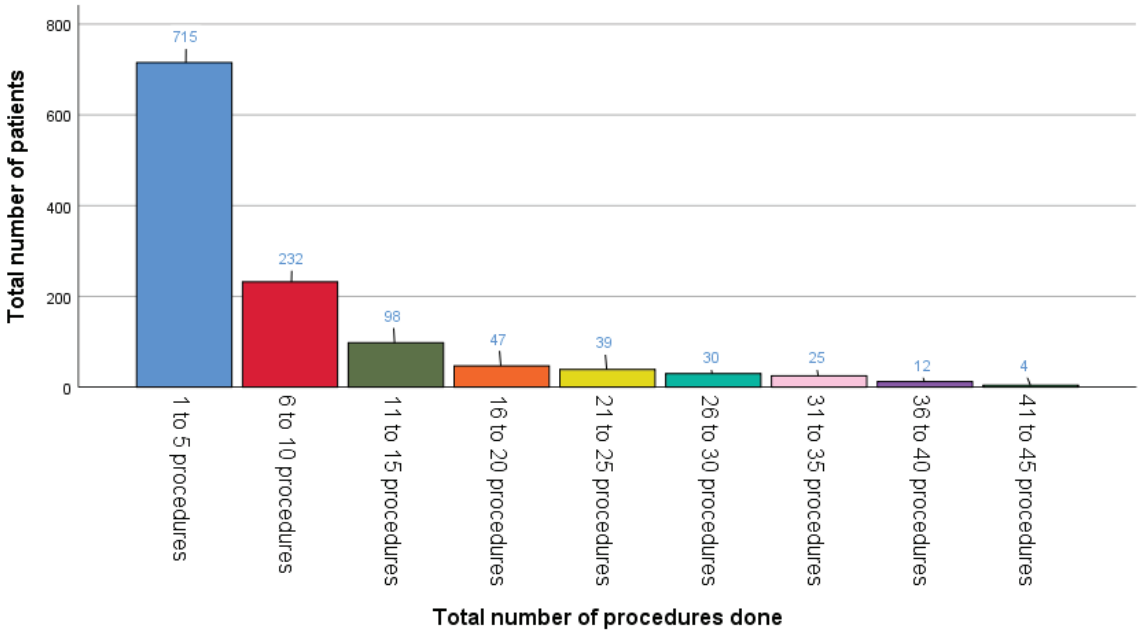


Figure 3: Bar graph showing the distribution of average number of procedures underwent by patients. X - axis represents the total number of procedures done and y axis represents the number of patients. This graph shows that most of the patients underwent only 1 to 5 treatment procedures (715).

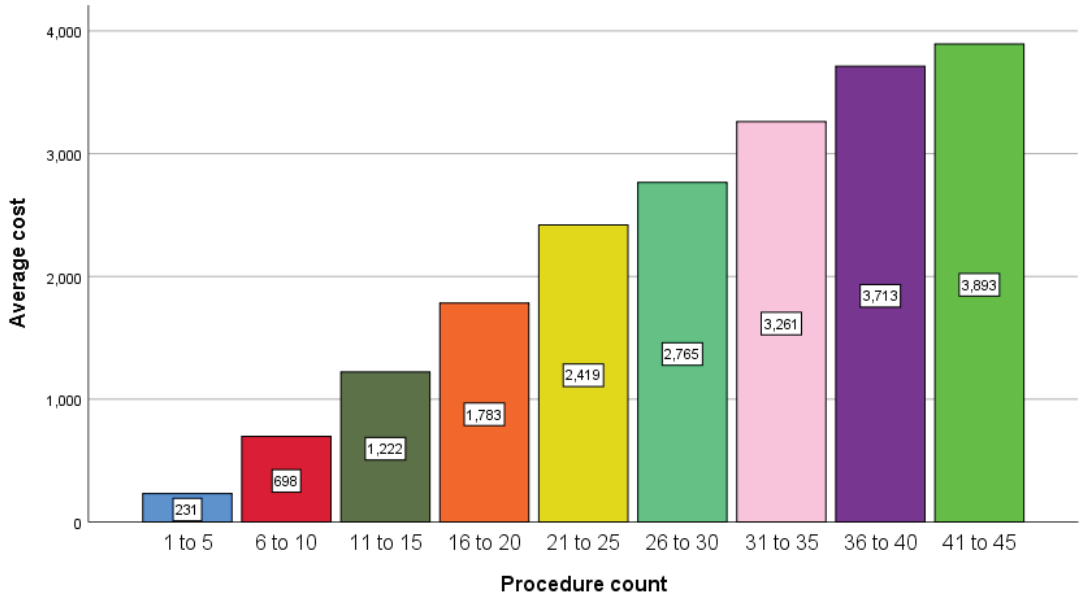


Figure 4: Bar graph showing distribution of average costs for overall treatment for all the patients according to the total number of procedures undergone by the patients. X - axis represents the total number of procedures and the y - axis represents the average cost. This graph shows that as the number of procedures increases the cost increases with the cost being highest (Rs.3893) for children in the 41 to 45 procedures group . Chi-square test was done and the association was found to be significant (p value = 0.045).

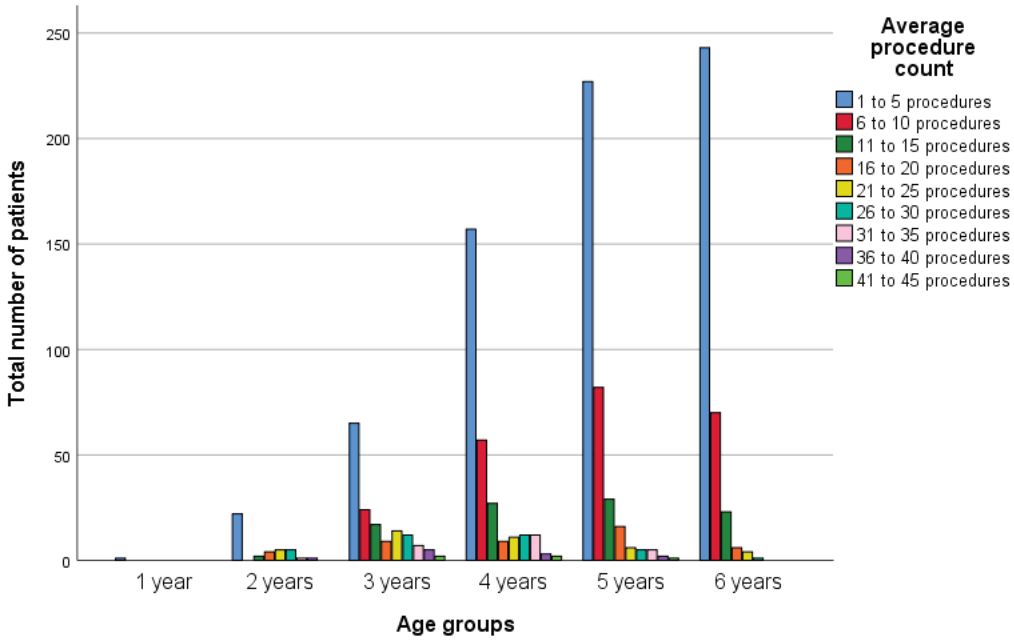


Figure 5: Bar graph showing the distribution of number of procedures for the various age groups included in the study. X - axis represents the various age groups and y - axis represents the total number of patients for the various treatment procedures. From this graph we can infer that the number of patients requiring treatment procedures decreased with an increase in age with patients requiring a minimum of 1 - 5 procedures being constantly high across all age groups. Chi-square test was done and the association was found to be not significant (p value = 0.000).

Conclusion

Within the limits of the study we can conclude that the most of the patients required one to five dental procedures with an increase in frequency with increase in age. The overall cost for a patient showed a gradual linear increase in cost with the increase in the number of procedures. This is with respect to one dental university where the charges were minimal compared to private clinic setups.

Conflict of Interest: There are no conflicts of interest.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Evaluation of Primary Central Incisors Requiring Restoration and Pulpectomy in 2 to 6 Year Old Children-An Observational Study

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Abstract

One of the first teeth to get affected by early childhood caries are the primary central incisors thereby esthetics is a main concern in restorations related to primary anterior teeth. In this study the severity of carious lesions are identified by the type of treatment performed. It is a retrospective study analyzing the need for a restoration as compared to a pulp therapy in primary central teeth. This is a private hospital based retrospective study. This is a hospital based retrospective study. All data of the patients visiting Saveetha Dental College and hospitals from June 2019 to March 2020 located in Chennai were retrieved manually. Children who were diagnosed with early childhood caries between 2-6 years who had caries in at least one of the primary central incisors (51, 61 ,71, 82) indicated for restorations or pulpectomy were included. A total of 832 teeth were treated out of which 476 were of males (57.21%) and 356 were of females (43.89%). The highest number of treatment procedures was done in 3-4 year olds – 244 procedures (58.53%). The most commonly treated tooth was primary maxillary left central incisor – 389 procedures (45.67%). Only 31.25% of the teeth underwent pulpectomy procedures. We can conclude restorations were performed in 3-4 year old common among male children in the primary maxillary left central incisor when compared to pulpectomy.

Keywords: *Pediatric restorations, Strip crowns, Composites, Glass ionomer cements, Pulpectomy*

Introduction

Esthetics is of main concern in restorations related to primary anterior teeth for parents and caretakers and children[1–4]. Treatment is usually based on opinion and clinical observation. Treatment is usually based on opinion and clinical observation.

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According to American Academy of Pediatric Dentistry usually primary maxillary central incisors erupt at 6 to 10 months and primary mandibular central incisors at 5 to 8 months of age [5] this being the initial teeth to erupt and have to face the full bacterial load and which leads to fce the full bacterial load and which leads to caries. A study shows 50% children suffered from caries involving primary maxillary anterior teeth[6].

The treatment plan shall include, development state of the dentition, caries risk assessment, patient's oral hygiene, anticipated parental compliance[7], likelihood of recall and patient's ability to cooperate for treatment, and this includes reviewing the caries risk especially in rural populations where it's high. Factors like dental trauma [8], pathologies in the oral cavity [9–11] etc can affect patient cooperation. Tooth preparation must involve removal of all caries to establish proper outline, resistance and convenience form compatible with the restoration [12].

Restorations in primary teeth need to be carefully considered due to the anatomy of the primary teeth. The mesiodistal diameter of the primary incisor crown is smaller compared to the permanent successors. They have larger pulp chambers but relatively smaller teeth [12,13].

Demineralization of primary teeth is significantly faster than that of permanent teeth, leading to requirement for early treatment[14]. Although primary incisors are among the first to exfoliate they do have a very significant lifespan and begin to naturally exfoliate at the age of 7 and 8 years for central incisors at 8 to 9 years for lateral incisors [15].

Patient cooperation plays an important role in the success of any treatment, thus age and maturity can influence treatment outcomes and changes in the clinical approach and management [16,17]. Various treatment of primary anteriors ranging from simple restorations to extractions.

The ultimate goal to restore teeth is to allow the patient to maintain it until natural exfoliation. Various treatment procedures are available for class III, class IV, class V cavities [18].

Very small cavities can be restored by using restorations, compomers or glass ionomer cements .[19]Bonded resin composite strip crowns introduced in the 1970's helped dentists provide esthetics and a promising version of restoration for primary teeth [20]. Many studies formed strip crowns to be very successful and durable [21,22].

A significant problem faced by dentists in mandibular central incisors is restoration of interproximal caries. Extremely short crowns can cause pulpal exposure. Disking the inter- proximal surface to open contacts and increase access to salivary flow. Severe caries can be restored with GIC as there are no strip crowns for lower anteriors[23].

When there is chronic irreversible inflammation or necrosis of radicular pulp pulpectomy is done. Materials like zinc oxide eugenol paste were introduced [23] but overfilling was not acceptable due to formation of mild foreign body reaction. Iodoform pastes are currently used and are successful in treatment [24].

In this study the severity of carious lesions are identified by the type of treatment performed like restorations or pulp therapy[8,25–32]. The aim of this retrospective study was to analyze the need for a restoration as compared to a pulp therapy in primary central teeth.

Materials and Methods

This is a private hospital based retrospective study. It was done by two examiners. Simple random sampling was done to reduce sampling bias. This was a hospital based retrospective study. Dental records of 832 patients who had visited a private dental hospital from June 2019 to March 2020, located in Chennai, Tamil Nadu, India were retrieved manually and analysed. All information including patient ID, treatment done, age, gender teeth treated were retrieved. Children who were diagnosed with early childhood caries between 2-6 years who had caries in at least one of the primary central incisors (51, 61 ,71, 82) indicated for restorations or pulpectomy were included. Children with systemic disorders, special children who had primary central incisors indicated for extraction and caries free children were excluded.

All the procedures were done by only a single operator for a specific patient. A full mouth examination with intraoral periapical radiographs of the teeth indicated for restoration or pulpectomy was taken before the start of the clinical procedure. After confirmation of the diagnosis for GIC restoration the tooth was isolated using rubber dam (GDC Marketing, Hoshiarpur, Punjab, India). Using a round carbide bur (MANI Inc., Japan) in a high-speed handpiece (NSK, JAPAN), all the caries were removed. The glass ionomer cement type 2 (Shofu, Shofuinc. Japan) was used to restore the cavity. For composite restoration the prepared cavity was etched using 37% phosphoric acid gel (Dtech, Pune, Maharashtra) washed, dried and cured with bonding agent (Te Econom Bond; Ivoclar Vivadent; Schaan, Liechtenstein) and then sealed using composite resin (Ivoclar Vivadent; Schaan, Liechtenstein). Light curing was done and the restoration was finished and polished.

For strip crown the tooth was prepared on all surfaces etchant and bonding agent was applied. The strip crown(3M, ESPE, USA) of suitable size was taken and a small vent hole was made on the lingual surface for the flow of excess material. The crown was filled with packable composite resin placed on the tooth and light cured. The plastic crown was then removed and the restoration was finished and polished.

For pulpectomy local anesthesia was administered using 2% lignocaine with 1:200,000 adrenaline (LOX* 2% ADRENALINE, Neon Laboratories limited, India) followed by rubber dam placement.Access cavity was made using no 4 round carbide bur (MANI Inc., Japan) which was followed by working length determination using no 15 hand K-file (Dentsply Maillefer, OK, USA). The working length was determined using the radiographic method and was kept 1-mm short of radiographic apex and rotary instrumentation was preceded by initial hand instrumentation up to no 20 K-file. The rotary instrumentation was done using the Kedo-S rotary files (Reeganz dental care Pvt. Ltd, India) for primary anterior teeth with U1. After complete instrumentation and irrigation, the canals were dried using no. 30 paper points (Dentsply Maillefer, OK, USA) and canals were obturated using calcium hydroxide iodoform paste (Metapex, META Biomed Co, PA, USA). The excess of coronal filling is removed, and initial entrance filling was given using glass ionomer cement (Shofu, Shofuinc. Japan).

The patient and the parents were blinded regarding the treatment protocol. The evaluator of the treatment procedure was blinded about the treatment protocol for each group. Since the type of treatment provided was known to the operator, the operator could not be blinded.Cross verification for incomplete and inaccurate data was cross checked with radiographs and intraoral photographs and verified. The sample was representative with specific inclusion and exclusion made it internally valid. This study had validity externally limited to the South Indian population due to limited geographic inclusion.

Ethical aspect:

This study was conducted with ethical approval from the Institutional Review Board (Ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320 of Saveetha Institute of Medical And Technical Sciences.

Statistical Analyses

The collected data was entered into Microsoft office Excel 2013 datasheet transferred to SPSS version 26.0 software (SPSS software Chicago, IL, USA) for statistical analysis. The independent variables: gender, age. The independent variables: total number of procedures, cost of procedures, treatment done. They were put to descriptive analysis and chi square test was done to assess the significance of association between the categorical variables.

Results and Discussion

A total of 832 teeth were treated out of which 476 were of males (57.21%) and 356 were of females (43.89%). Chi square test was done and the association was found to be not significant (p value = 0.517) [Figure 3]. The distribution across various age groups 1 year: 0.72%, 2 years: 10.70%, 3 years: 29.21%, 4 years: 29.33%, 5 years: 22.60%, 6 years: 7.45% [Figure 1].

Out of 832 teeth treated the distribution was namely maxillary left central incisor: 380 teeth, maxillary right central incisor: 368 teeth, mandibular left central incisor: 42 teeth and mandibular right central incisor:42 teeth. Chi square test was done and the association was found to be not significant (p value = 0.418) [Figure 2].

Out of type of procedure done the number of tooth treated by each procedure was class 3 composite (mesial): 108 teeth, class 3 composite (distal): 13 teeth, class 4 composite (mesial): 22 teeth, class 4 composite (distal): 10 teeth, class 5 composite:42 teeth, class 1 GIC: 191 teeth, class 5 GIC: 6 teeth, pulpectomy (single visit): 258 teeth, pulpectomy (multi visit): 2 teeth, strip crowns: 180 teeth. Chi square test was done and the association was found to be significant (p value = 0.000) [Figure 4].

The highest number of treatment procedures was done in 3-4 year olds with 244 procedures (58.53%). The most commonly treated tooth was primary maxillary left central incisor with 389 procedures (45.67%). Only 31.25% of the teeth underwent pulpectomy procedures.

Early childhood caries begins soon after eruption and progresses rapidly affecting infants and toddlers [18].Most of the treatment done to the children restorations(68.75%) [Figure 4]. From this we can come to the finding that though the prevalence of caries in Tamil Nadu is high (55.73%) [33]. The severity of caries is comparatively low as most of the teeth treated only required restorations.being the 1st teeth to get affected in ECC i.e maxillary central incisors and the last tooth get affected being mandibular central incisor [18] we can say that this correlated with existing studies. This is further supported by the data found in this study where the restorative rate difference between the maxillary and mandibular central incisors is highly significant.

This study shows that proximal surfaces of teeth demonstrated the highest caries rate being the majority of the restorations being class 3 and class 4 carious lesion restorations. This was consistent with previous study where maxillary teeth were more susceptible as compared to mandibular teeth. Approximal surfaces of incisors, canines have higher caries rate than other sites. The carious teeth are more common in young patients and tend to decrease with age [18,34].

We see that Figure 4 also reveals that the most preferred material was composite followed by GIC followed by strip crowns. This data was the combined data of treatments done by both undergraduates (general dentists) and postgraduate (pediatric dentists). Compared to all materials full coverage restorations are considered most durable and reliable, the earliest used being stainless steel crowns [35].

Though the procedure is very well described, it is technique sensitive and any lapses in patient selection, moisture and hemorrhage control, tooth preparation, adhesive application and resin composite placement can lead to failure [36]. This difficulty in application is reflected in a study that only 21% general dentists surveyed performed strip crowns as compared to pediatric dentists which were 73% [37].

This choice of composite also reveals that patients and caregivers concern for esthetics in anterior region. Newer materials could be used further on to give protective effect over caries, improve strength and retain esthetics like compomer which is more fluoride releasing and more moisture tolerant [38].Resin modified GIC could also be used more like normal GIC, less moisture sensitive and better strength than normal GIC [19,39].

Giomers are also bioactive materials that release and recharge fluoride similar to composite with pre reacted glass particles [40–42]. They can also resist plaque formation due to a film, the giomer filler forms on the surface when it contacts with saliva and prevents bacterial adhesion [43] and causes remineralization to adjacent surfaces [44].

The study by coll et al states that success rates of pulpotomy and indirect pulp therapy is higher compared to pulpectomy although not statistically significant[45]. Endodontic treatment is considered the last option for keeping a primary tooth that has irreversibly affected due to caries in a child [46].Early intervention can be done to prevent extensive pulp therapy.

It must be remembered that preventive measures like periodic topical fluoride therapy[47,48] or fluoride varnishes 4 times a year [49] have shown effect in remineralization of early carious lesions [50,51].

There were some limitations for the study like restricted geographic location limited to the South India population.

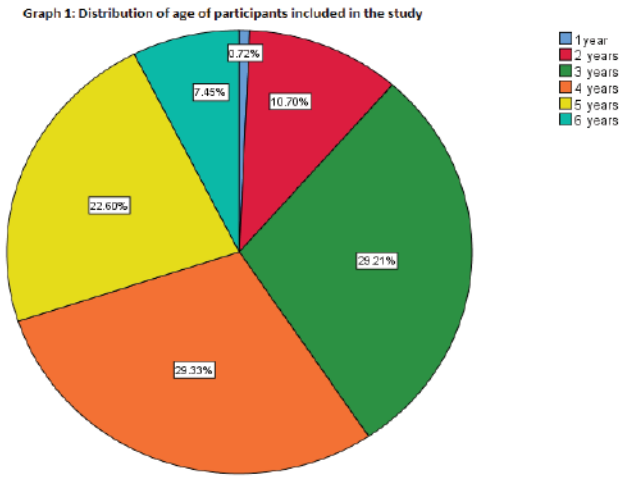


Figure 1: Pie chart showing the distribution of teeth treated across various age groups. Blue color represents 1year olds, red color represents 2 year olds, green color represents 3 year olds, orange color represents 4 year olds, yellow color represents 5 year olds, pale green color represents 6 year olds. From this chart it is seen that the 4 year olds were the most treated (29.33%).

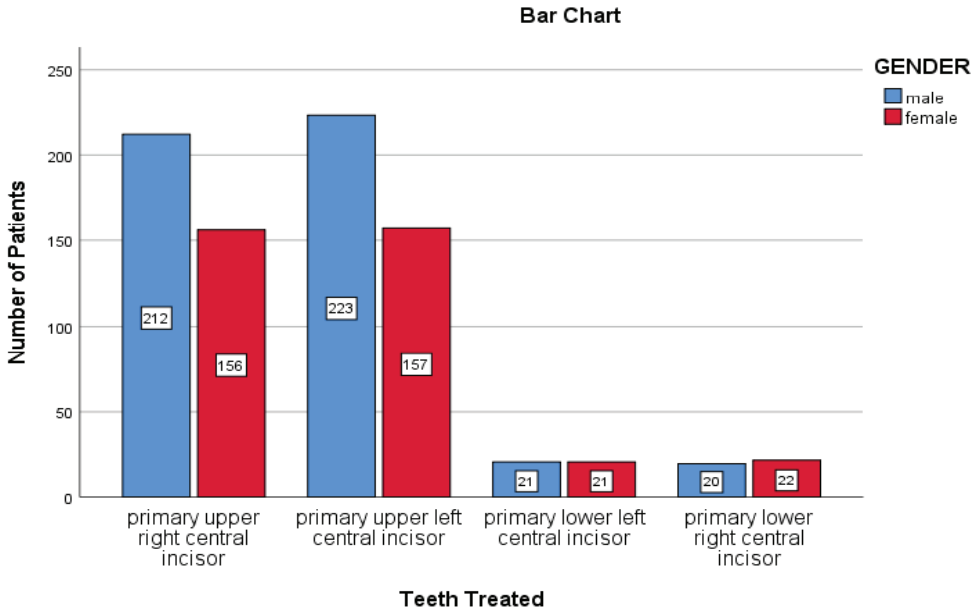


Figure 2: Bar graph showing association of gender of participants included in the study for each tooth treated. Red color represents female and blue color represents male. X - axis represents the type of tooth treated and y - axis represents the number of patients based on gender. This graph shows that the primary upper left central incisor was the most treated and this was higher in males (212) than females. Chi-square test was done and the association was found to be not significant (p value = 0.418).

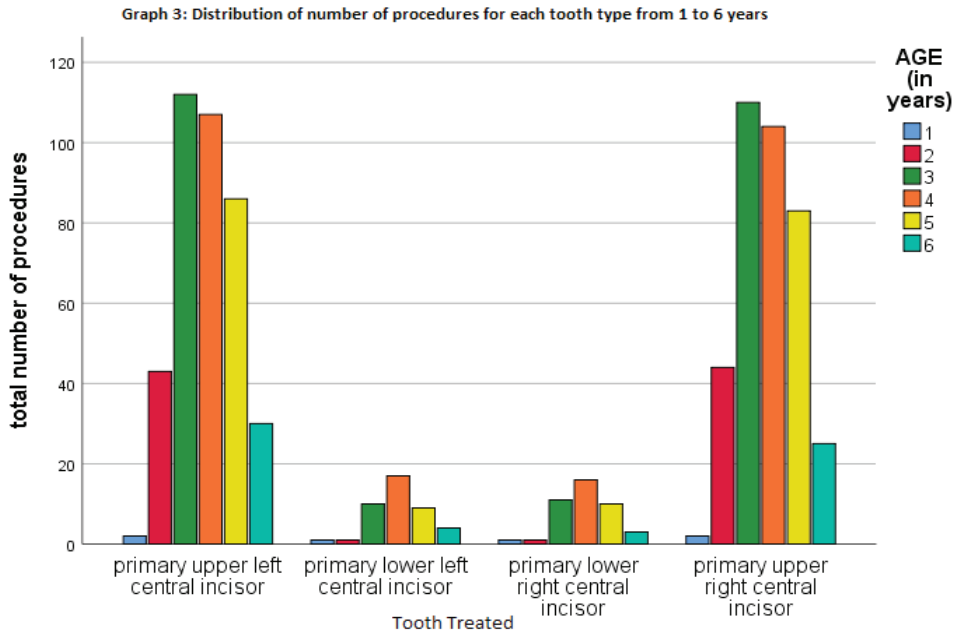


Figure 3: Bar graph showing association of number of procedures for each tooth type from 1 to 6 years. X - axis represents the type of tooth treated and y - axis represents the number of procedures done based on age. Most commonly treated tooth was the primary upper left central incisor and this was seen in 3 year old children. Chi-square test was done and the association was found to be not significant (p value = 0.517).

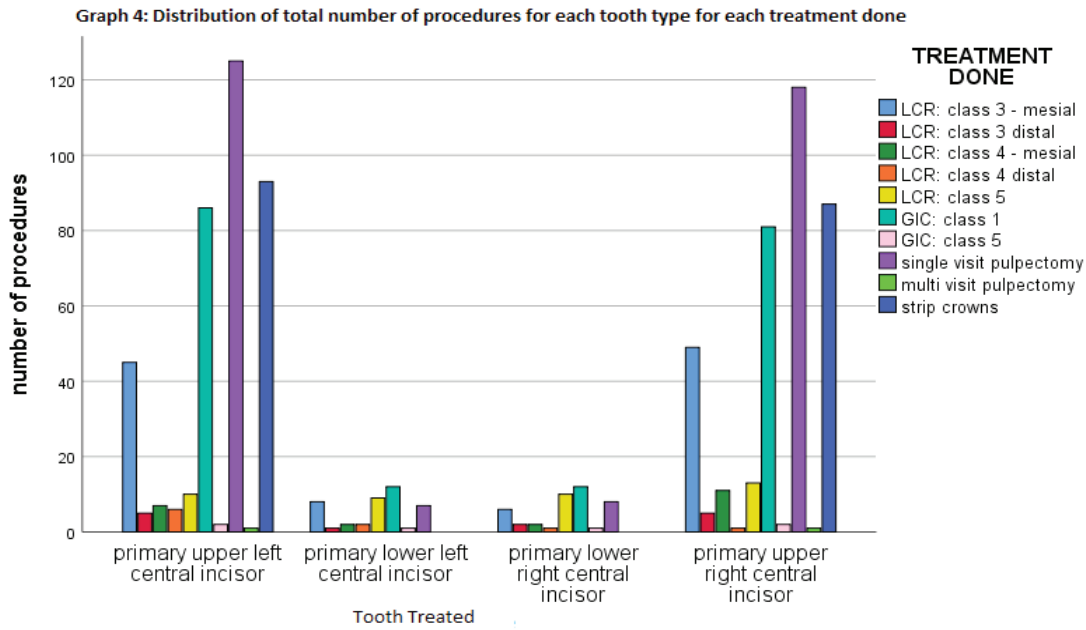


Figure 4: Bar graph showing association of total number of procedures for each tooth treated for each treatment done. X - axis represents the type of teeth treated and the y axis represents the number of teeth treated for each type of procedure. Single visit pulpectomy was the most commonly done treatment with the high frequency seen in the primary upper left central incisor. Chi square test was done and the association was found to be significant (p value = 0.000).

Conclusion

Within the limits of the study we can conclude that the most performed procedure were restorations as compared to pulpectomies with increase in restorative rate with increase in age. Adequate knowledge on the variations in tooth structure, awareness regarding the radiographic limitations and restorative technique is essential prior to commencement of any procedure. Though endodontic treatment in primary teeth has shown excellent clinical and radiographic success conservative treatment is still the most performed and most preferred choice of treatment as the pulp remains vital as the potential for recovery exists once the irritation has been removed. Also any fractures can be easily repaired by adding more material rather than repeating the entire procedure.

Conflict of Interest: There are no conflicts of interest.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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8.TABLES AND FIGURES

Type of Manuscript: *Retrospective study*

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Association between Composite Restoration in Anterior Teeth and Secondary Caries

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Abstract

Composite resin is one of the most commonly used restorative material during the past decade as it provides desirable esthetics, easy to manipulate and minimal cavity preparation required. Despite numerous benefits provided by composites, several clinical studies have reported short longevity and higher rate of failure in composite restoration. One of the main reasons for failure was due to recurrent or secondary caries. The aim of this study was to evaluate the association between composite restoration in anterior teeth and secondary caries. This study included patients above 18 years with composite restoration filled anterior teeth who reported to dental hospital from June 2019 to March 2020. Patients below 18 years, medically compromised patients and patients without anterior composite restorations were excluded from the study. A total of around 86000 patient records were reviewed and analysed for the inclusion criteria and the following parameters were extracted; (i) Patient's gender, (ii) teeth involved, (iii) presence of secondary caries and (iv) types of restoration. Data was recorded into Microsoft Office Excel (2013) and analysed using SPSS software Version 26.0. Chi-square test was done to find out correlation of presence of secondary caries between variables such as patient's gender, teeth involved and types of restoration. Significant test level was set at $p<0.05$. A total of 1062 teeth presented with composite restoration in anterior teeth. 17.6% of composite filled anteriors presented with secondary caries. Class III restoration had the highest prevalence of secondary caries (61%) and was found to be statistically significant ($p<0.05$). Females (18.3%) had higher incidence of secondary caries than compared to males (16.6%). Maxillary central incisors had the highest presence of secondary caries (64.2%). Within the limits of this study, it was observed that less number of composite filled anterior teeth had secondary caries and females presented with more number of anterior composite restoration with secondary caries compared to males. Class III restorations demonstrated higher presence of secondary caries than other types of restoration.

Keywords: *Anterior restoration, class III restoration, composite restoration, recurrent caries, secondary caries*

Introduction

Composite restoration is a tooth coloured restorative material commonly used to restore mild to moderately decayed teeth and has the benefit of improved esthetics as compared to other restorative materials in the market. Composite is known to be one of the most commonly used restorative material during the past decade [1,2]. Composite restoration not only provides desirable esthetics but is also easy to manipulate and requires minimally invasive preparation technique and has replaced amalgams which have been the standard restorative material for the past 100 years as amalgam restorations have been associated with environmental pollution [3,4] and has a negative effect on health as it releases mercury [5,6]. Moreover, composites are known to have good bonding potential to the tooth, good mechanical property and lower cost

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compared to other indirect restoration and have also replaced other types of restoration in a wide variety of clinical situations such as situations in which previously could only be treated by indirect prosthetic restoration. Composite restoration is not only used to restore traumatized or decayed teeth, but also used for direct and indirect restoration to improve esthetics.

However, despite the numerous benefits provided by composites, several clinical studies have reported short longevity and higher rate of failure in composite restorations than amalgam restorations [2,7–10]. Previous studies reported that one of the main reasons for failure was due to recurrent or secondary caries [8–10]. The term secondary caries or recurrent caries signify both new caries around a restoration and residual remaining caries [11]. Secondary caries is also one of the main reasons for replacement of restorative material [12]. Secondary caries cannot be avoided completely regardless of the material used for filling. Secondary caries may occur due to crack formation after filling or inadequate removal of caries. However, the relationship between gap and secondary craies is controversial as some studies found poor correlation [13,14] while a few studies found positive correlation [15,16]. There has not been any conclusive evidence for association between gaps and secondary caries adjacent to restoration. Nevertheless, studies have reported presence of secondary caries in complete restorations [14,17.]

One of the disadvantages of composite filling material is the inadequate sealing ability which allows the formation of micro-gaps at the composite-tooth interface followed by microleakage of bacteria and their metabolites and other oral fluids which have long been considered as an etiology of secondary caries [18]. Polymerization shrinkage is also one of the inherent shortcomings of composites, which greatly affects the sealing ability [19]. Surface roughness of composites also play a role in development of secondary caries as studies have shown that composites have greater bacterial adhesion and accumulate more plaque compared to other restorative materials [20,21]. The most common method used for detection of secondary caries around restorations is intraoral radiography [21]. In some cases, secondary caries can be detected with visual and tactile inspection for brown or black staining of margins around the composite which can be easily observed around tooth coloured restoration and marginal debonding [21]. Good operator skill is fundamental to improve clinical success of composite restorations since the application of composite materials are generally known to be very technique sensitive [22].

Over the past 5 years, innumerable clinical trials [23–28], in-vitro studies [29–34] and article reviews [35–37] had previously been conducted by our team. Currently we are focusing on analysing the prevalence of secondary caries in composite restored anterior teeth. The aim of this study was to evaluate the association between composite restoration in anterior teeth and secondary caries. This allows us to gain knowledge on the prevalence of recurrent caries in composite restorations and to understand the need to take necessary measures to improve clinical success of composite restorations.

Materials and Methods

The case control study involved adults of 18 years and above who had reported to dental hospital between June 2019 to March 2020. Both male and female adults were selected for this study. The study was conducted as a university based. This allowed flexible data retrieval, automated data collection, cost effective as well as time saving. However, the setting allowed a limited population to be covered and is subjected to researcher's personal bias. Retrieval of patient's data received approval from the Ethical Committee Board of dental hospital and will be covered by the following ethical approval number. SDC/SIHEC/2020/DIASDATA/0619-0320. Patient's informed consent was obtained from patients prior to clinical examination.

A total of 86000 patient records between June 2019 to March 2020 were reviewed and analysed for presence of composite restoration in anterior teeth and information was verified through intraoral photographs of patients uploaded into the system. Data was collected by a single calibrated examiner. The inclusion criteria was patients above 18 years with anterior composite restoration. Exclusion criteria includes patients below 18 years, medically compromised patients and patients without anterior composite restorations.The following parameters were observed and recorded: (i) Patient's gender, (ii) teeth involved, (iii) types of restoration and (iv) presence of secondary caries.

Statistical Analysis

All the data were entered into Microsoft Office Excel (2013) and analysed using SPSS software Version 26.0. Descriptive statistics were used to report distribution of gender, tooth involved, types of restoration and

presence of secondary caries. Chi-square test was further conducted to assess the association between variable factors such as gender, presence of secondary caries and types of restoration. Significant level test was set at $p<0.05$.

Results and Discussion

A total of 1062 anterior teeth was diagnosed with composite restoration. Out of which 628 were females and 434 males. 3% of anterior teeth had class V restoration, 5% had class I restoration, 45.1% had class IV restoration and 54.1% had class III restoration (Figure 1). As for the type of teeth involved, the distribution was 60% maxillary central incisors, 25% maxillary lateral incisors, 9% maxillary canine, 1.9% mandibular central incisors, 2.3% mandibular lateral incisors and 1.9% mandibular canines (Figure 2). Out of 1062 teeth with composite restoration, 187 (17.6%) of teeth were observed with secondary caries while remaining 875 (82.4%) had no sign of secondary caries (Figure 3).

Based on gender, prevalence of secondary caries was found to be higher in females (18.3%) compared to males (16.6%) (Figure 4), However, no statistically significant difference was found in presence of secondary caries between males and females ($p>0.05$)(Figure 4).

Maxillary central incisors demonstrated the highest presence of secondary caries (64.2%) (Figure 5) but this finding was not statistically significant ($p>0.05$) (Figure 5). Based on type of restoration, the presence of secondary caries was found to be higher in class III restorations compared to other types of restoration (Figure 6). Significant association was found in presence of secondary caries between types of restoration ($p<0.05$) (Figure 6)

The prevalence and incidence of secondary caries in composite restoration in the general population has not been thoroughly investigated despite secondary caries being recognized as a major issue related to composite restoration. Many studies have reported the incidence of secondary caries were used to study the longevity of composite restoration. However, there are very few studies available that deal exclusively with its incidence and prevalence in composite restoration.

Our present study found that 17.6% of composite filled anteriors were observed to have secondary caries. Hewlett et al. studied the radiographic secondary caries prevalence in teeth with restorations and reported that 14% of the restored teeth had secondary caries [17]. Previous study by Rezwani et al. Also had similar findings in which he reported that 13% of restorations exhibited secondary caries [14]. These studies confirmed the finding of the present study.

Our studies observed that secondary caries was found to be more common in class III restoration (61%) compared to other types of restoration. There is a statistically significant difference in the presence of secondary caries between different types of restoration. In agreement with our current finding was a study by Asghar et al who found that class III composite restoration had the highest prevalence of secondary caries [38]. This adds to the consensus of our finding and is to be included in clinical practice.

In a previous study done by Asghar et al reported the prevalence of secondary caries was higher in males than compared to females [38]. This study contradicted our current finding of females having a higher prevalence of secondary caries. However, this finding was not statistically significant ($p>0.05$). The possible reason may be due to difference in geographic location, oral hygiene practice and age of dental restoration.

Our current study was done to associate secondary caries with composite restoration. However, factors involved are only limited to the patient's gender, tooth involved and types of restoration. Other factors such as location of secondary caries, age of restoration and the intactness of restoration may also contribute to association of secondary caries. Many factors can contribute to secondary caries in composite restored teeth. Extensive research is needed to find out possible contributing factors which associates secondary caries to composite restoration and on a large population scale.



Figure 1. Bar chart depicts the number of anterior teeth with composite restoration and type of restoration. X-axis represents type of restoration and Y-axis represents the number of anterior teeth with composite restoration. Class III restoration was more common in anterior teeth compared to other types of restoration.

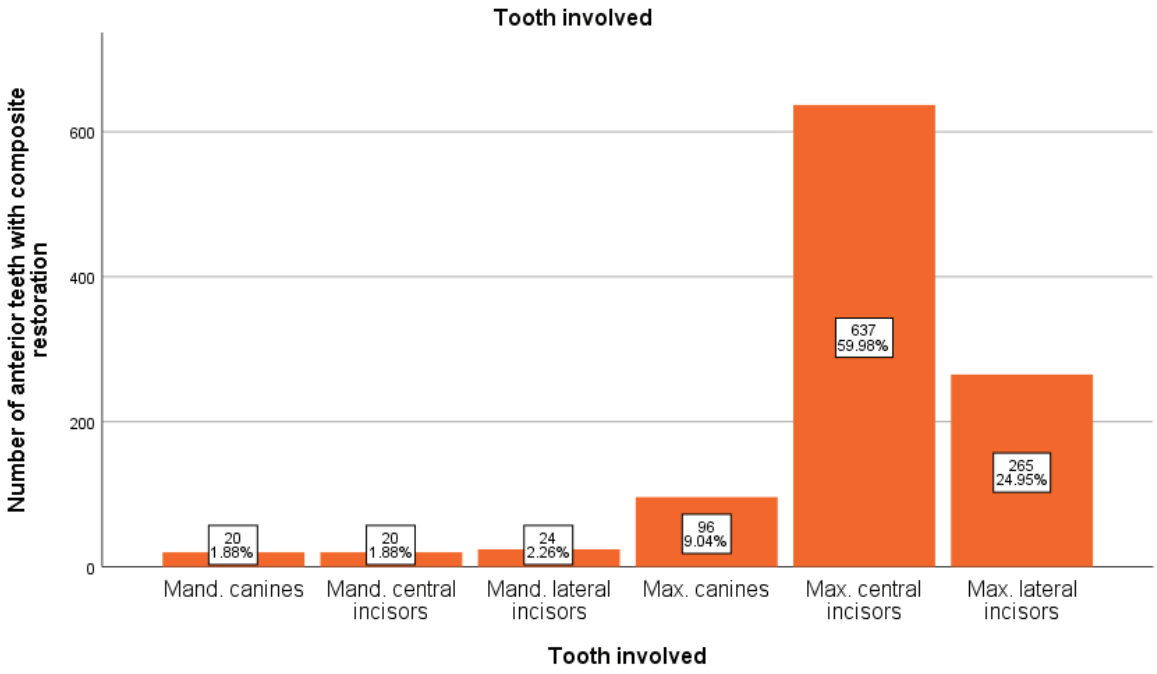


Figure 2. Bar chart depicts the number of anterior teeth with composite restoration and tooth involved. X-axis represents the tooth involved and Y-axis represents the number of anterior teeth with composite restoration. Composite restoration was more in maxillary central incisors compared to other teeth involved.

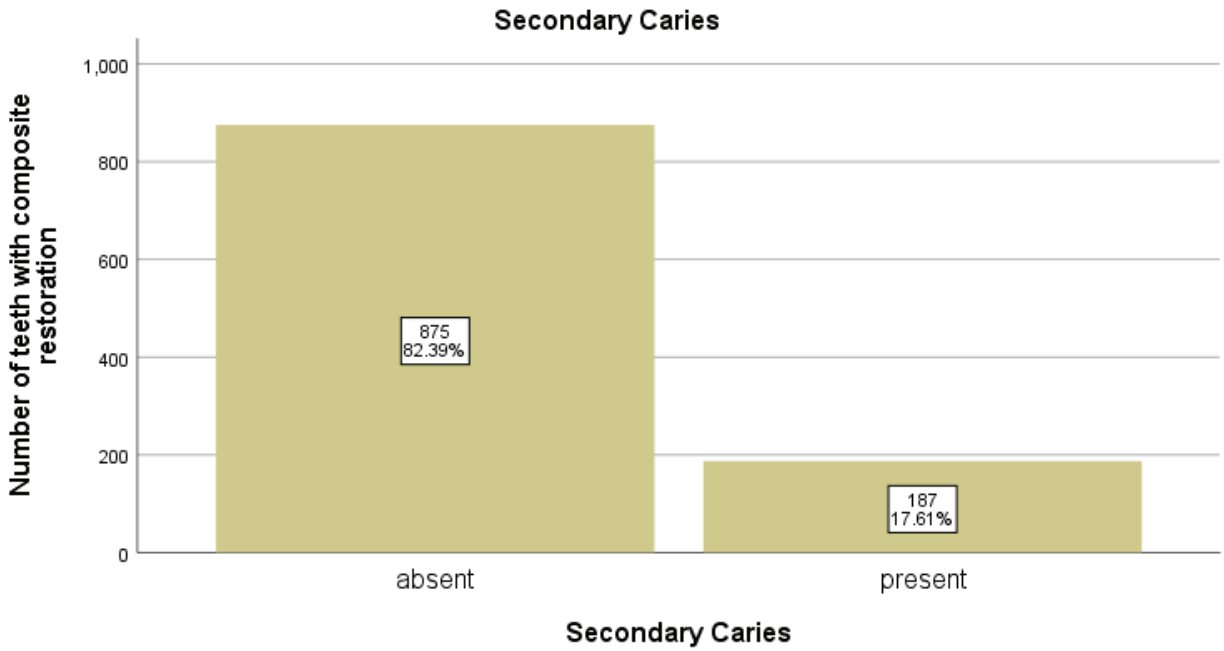


Figure 3. Bar chart depicts the number of anterior teeth with composite restoration and presence of secondary caries. X-axis represents presence of secondary caries and Y-axis represents the number of anterior teeth with composite restoration. The number of cases with secondary caries is comparatively low.

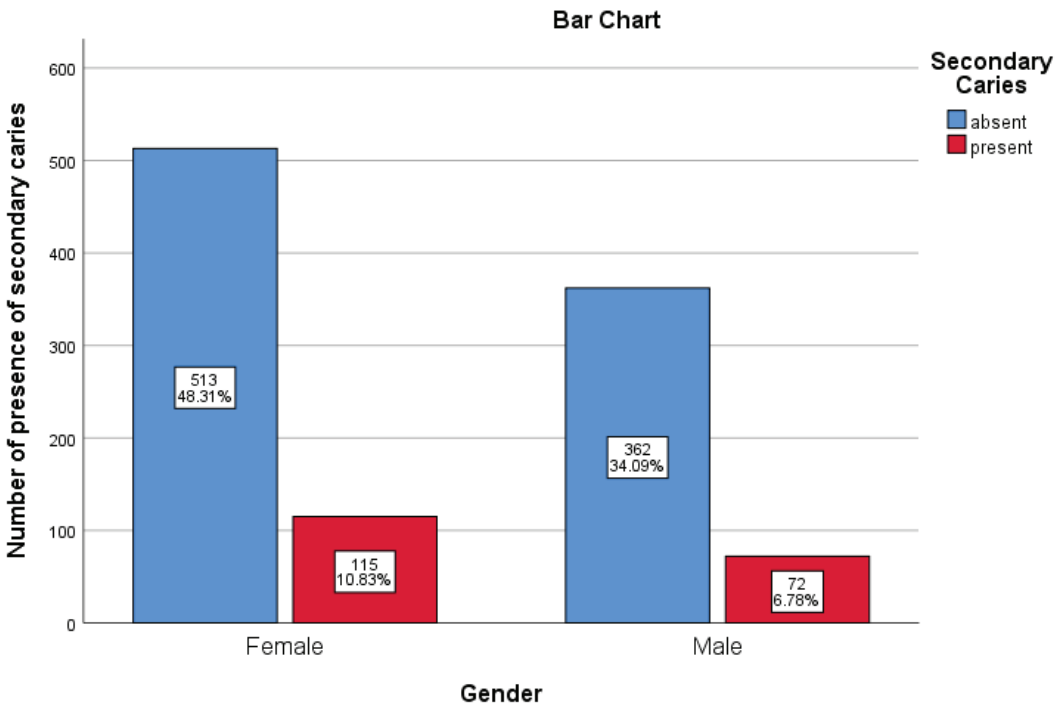


Figure 4. Bar chart depicts the association between the presence of secondary caries and gender. X-axis represents gender and Y-axis represents the presence of secondary caries (presence- red, absence- blue). Out of 187 teeth with secondary caries, 115 were females and 72 were males. Pearson chi-square value- 0.525, p value- 0.469 (p>0.05), hence, not significant. Secondary caries were observed more in females compared to males but the results were statistically not significant.

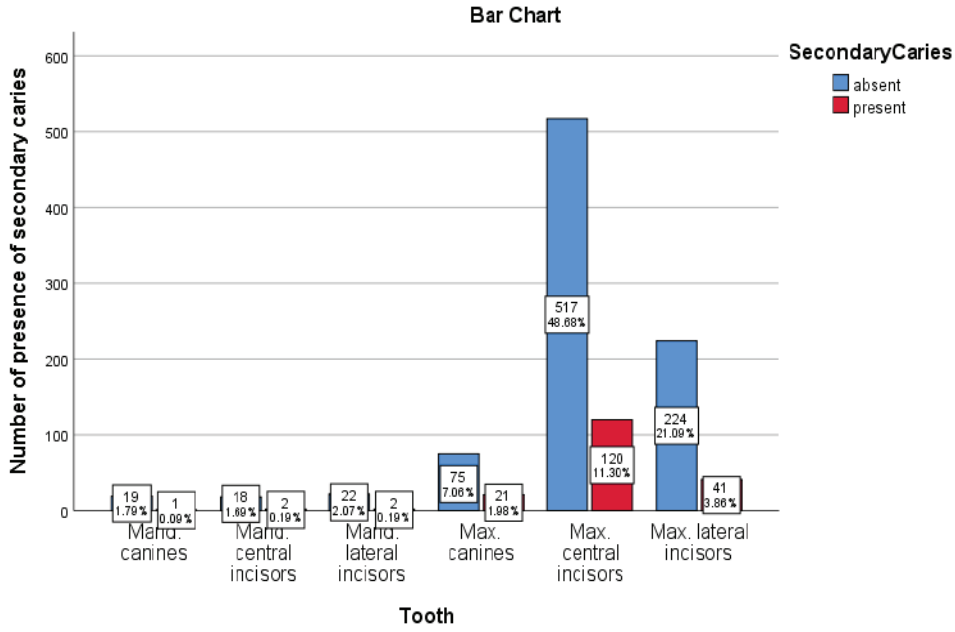


Figure 5. Bar chart depicts the association between the presence of secondary caries and tooth involvement. X-axis represents the tooth involved and Y-axis represents the presence of secondary caries (presence- red, absence- blue). Presence of secondary caries were more common in maxillary central incisors. Pearson chi-square value- 7.115 , p value-0.212 (p>0.05), hence, not significant. Maxillary central incisors demonstrated the highest presence of secondary caries but the results were statistically not significant

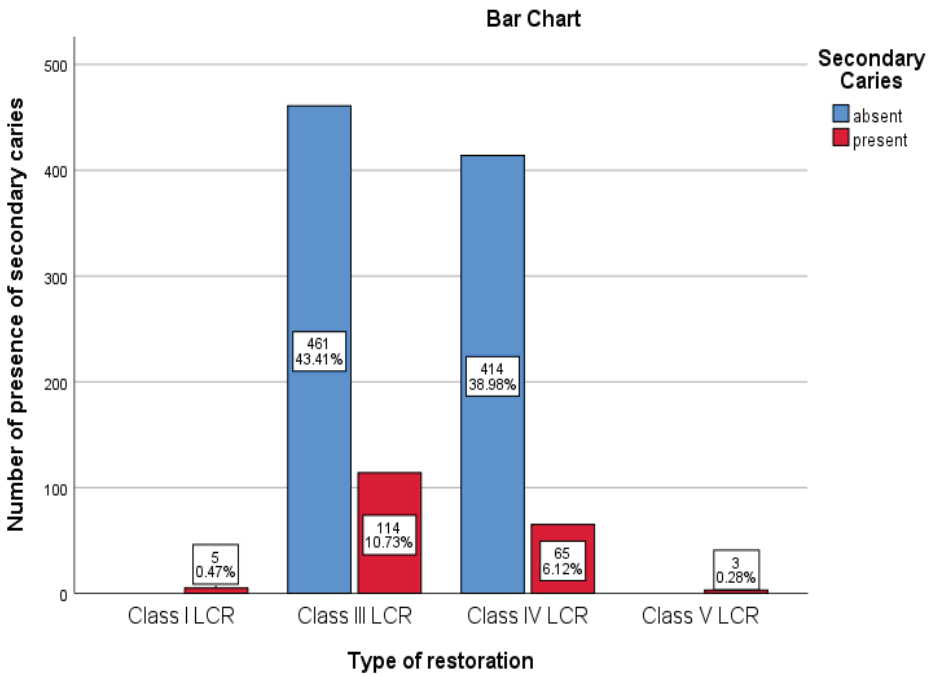


Figure 6. Bar chart depicts the association between the presence of secondary caries and type of restoration. X-axis represents the type of restoration and Y-axis represents the presence of secondary caries (presence- red, absence- blue). Presence of secondary caries were more in class III restorations followed by class IV, class I and class V restorations. Pearson chi-square , p value- 0.000 (p<0.05), hence, statistically significant. Class III restorations demonstrated higher presence of secondary caries than other types of restoration and the results were statistically significant.

Conclusion

Within the limits of this study, it was observed that less number of composite filled anterior teeth had secondary caries and females presented with more number of anterior composite restoration with secondary caries compared to males. Class III restorations demonstrated higher presence of secondary caries than other types of restoration.

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Conflict of Interest : No conflict of interest has been declared by the authors.

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Evaluation of Direct/Indirect Pulp Capping Procedures Requiring Root Canal Therapy

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Abstract

The common objective of pulp capping is to induce a physical protective barrier over the pulp to maintain the vitality and function of the tooth. Indirect pulp capping is done in cases where deep carious lesions are found in tooth and lesion is adjacent to vital pulp tissues. Pulp capping remains a controversial topic as opinions on pulp capping treatment vary from clinicians, despite new advances gained through research. Many clinicians are reluctant to opt for direct pulp capping as a treatment option in cases of carious exposed pulp may be due to conflicting data and findings reported regarding success rate of pulp capping. The aim of this study was to evaluate the number of direct/indirect pulp capping cases which underwent root canal therapy. This study included adults above 18 years who had previously undergone direct/indirect pulp capping and reported to dental hospital between June 2019 to March 2020. Around 86000 patient records were reviewed and analysed for the inclusion criteria and the following parameters were extracted; (i) Patient's gender, (ii) type of pulp capping, (iii) teeth involved and (iv) teeth which underwent root canal therapy. Data was recorded in Microsoft Office Excel (2013) and analysed using SPSS Software Version 26.0. Chi-square test was done to find out correlation between variables. Significant level test was set at $p<0.05$. A total of 218 teeth were found to have undergone pulp capping, those of which were 33.9% direct pulp capped tooth and 66.1% indirect pulp capped tooth. The teeth involved were 4.1% anteriors, 9.6% premolars and 86.2% molars. 18.8% of capped teeth underwent root canal therapy. More females (21%) underwent root canal therapy than males (17.5%). A higher percentage of premolars underwent root canal therapy compared to other teeth (42.9%) and more indirect pulp capped teeth underwent root canal therapy (20.1%) than direct pulp capped teeth (16.2%). Within the limits of this study, it was observed that the number of pulp capping cases which underwent root canal therapy is about one fifth and was more commonly seen in premolars and in indirect pulp capping cases.

***Keywords:** Deep caries management, Direct pulp capping; Endodontics; Indirect pulp capping; Root canal therapy.*

Introduction

Pulp capping is an operative procedure which attempts to maintain the tooth vitality and facilitate reparative dentin formation [1]. The common objective of pulp capping is to induce a physical protective barrier over the pulp to maintain the vitality and function of the tooth. Indirect pulp capping is done in cases where deep carious lesions are found in tooth and lesion is adjacent to vital pulp tissues [2]. Whereas direct pulp capping is a treatment option for teeth with mild cariously exposed pulp [3]. Pulp capping remains a controversial topic as opinions on pulp capping treatment vary from clinicians, despite new advances gained through research [1]. Many clinicians are reluctant to opt for direct pulp capping as a treatment option in cases of carious exposed pulp

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may be due to conflicting data and findings reported regarding success rate of pulp capping (approximately 37%-100%) [4–6]. Moreover, the tooth can develop into irreversible pulpitis and patients may experience pain and will have to go for root canal therapy [7]. Despite the issues faced in pulp capping treatment, it remains as a minimally invasive procedure that saves cost, time and effort compared to pulpectomy or pulpotomy. It can be said that such treatment options are valid from a socioeconomic point of view [8]. Many previous authors have attempted to find out prognostic factors which can influence the outcome of treatment to increase the success rate of pulp capping [4,9,10]. On the other hand, some failures may be due to previous impaired condition of the pulp rather than other factors, especially in cases of failure that occur soon after pulp capping treatment [11]. Despite pulp capping treatment being a controversial topic, some studies reported direct pulp capping to be the appropriate treatment option for carious as well as traumatic or mechanically exposed pulp [9,12].

Previous study reported the prognosis of pulp capping vary in success rate, ranging from 13-100% [1]. Paul et al. reported that the prognosis of direct pulp capping can be unpredictable and has the lowest success rate in caries exposed pulp in adult dentition [1]. Baume et al. Studied the long term clinical assessment of direct pulp capping and stated that there is an increased risk of failure if pulp is diseased before capping [13]. The same study also reported pulp capping performed by skilled practitioners have a higher success rate (90%) than compared to treatment done by students (80%) [13]. Higher risk of pulp capping failure may also be due to cases with preoperative toothache [14].

Over the past 5 years, innumerable clinical trials [15–20], in-vitro studies [21–26] and article reviews [27–29] had previously been conducted by our team. Currently we are focusing on analysing the need for root canal treatment in pulp capped teeth. The aim of this study was to evaluate the number of direct/indirect pulp capping cases which underwent root canal therapy.

Materials and Methods

This retrospective study involved adults of 18 years and above, both males and females, who have reported to dental hospital between June 2019 to March 2020. The study was conducted as a university-based study. This allows flexible data retrieval, automated data collection and saves cost. However, such setting only allows a limited population to be covered and study may be subjected to researcher's personal bias. Data retrieval was approved by the Ethical Committee Board of dental hospital and will be covered by the following ethical approval number. SDC/SIHEC/2020/DIASDATA/0619-0320. Patient's informed consent was obtained prior to clinical examination of the patients.

Around 86000 patient records between June 2019 to March 2020 were reviewed and analysed for tooth treated with direct or indirect pulp capping and the cases were cross-verified with radiographs and intraoral photographs uploaded into the system. Data was collected by a single calibrated examiner. The inclusion criteria included patient's above 18 years and patients who had undergone direct/indirect pulp capping. Patients below 18 years, medically compromised patients and patients without pulp capping were excluded from the study. The following parameters were observed and recorded: patient's gender, type of pulp capping, tooth involved and pulp capped tooth which underwent root canal therapy

Statistical Analysis

All the data obtained were entered into Microsoft Office Excel (2013) and analysed using SPSS Software Version 26.0. Descriptive statistics were used to report distribution of gender, type of indirect pulp capping, tooth involved and pulp capped tooth which underwent root canal therapy. Chi-square test was conducted to find correlation in variable factors such as gender, type of pulp capping, tooth involved and tooth which underwent root canal therapy. Significance test level was set at $p<0.05$.

Results and Discussion

A total of 218 teeth were found to have undergone pulp capping. Out of which 81 were females and 137 were males. 33.9% of those teeth were direct pulp capped teeth and 66.1% were indirect pulp capped teeth (Figure 1). As for the tooth involved, 4.1% were anterior teeth, 9.6% premolars and 86.2% molars (Figure 2). Out of 218 pulp capped teeth, 41 teeth (18.8%) underwent root canal therapy and the remaining 177 teeth (81.2%) did not undergo root canal therapy (Figure 3).

Based on gender, more females (21%) had undergone root canal therapy than compared to males (17.5%)(Figure 4). No correlation in tooth underwent root canal therapy was found between genders ($p>0.05$)

(Figure 4). More pulp capped premolar teeth (42.9%) underwent root canal therapy compared to anteriors (33.3%) and molars (15.4%) and pulp capped teeth in molars which did not undergo root canal therapy were higher compared to other teeth (Figure 5). Statistically significant association was found between teeth which underwent root canal therapy and the teeth involved ($p<0.05$) (Figure 5). More indirect pulp capped teeth underwent root canal therapy (20.1%) than direct pulped teeth (16.2%) and both direct and indirect pulp capped teeth which did not undergo root canal therapy were higher when compared to those which underwent root canal therapy (Figure 6). However, no significant association was found between teeth which underwent root canal therapy and type of pulp capping ($p>0.05$) (Figure 6).

Pulp capping is generally considered not applicable to pulp exposed by caries due to high risk of such pulp being contaminated [7,30]. However, a previous study reported that teeth with mild carious exposed pulp as well as traumatic or mechanical pulp exposure should be treated appropriately with direct pulp capping [9,12]. Despite controversial issues related to pulp capping, the treatment should not be completely contraindicated. Moreover, high success rate of pulp capping treatment should not be expected even with appropriate cases as outcome can be unpredictable.

Our study found that the overall success rate of pulp capped teeth was 81.2%. The remaining 18.8% of pulp capping cases failed and underwent root canal treatment. Similar findings were reported by Matsuo et al. and Baume et al.[9,13]. Matsuo et al. reported the success rate of pulp capping to be 81.2% [9]. Baume et al. reported a similar finding of 80% success rate in pulp capping performed by students [13]. Al-Hiyasat et al. contradicted the finding of the current study and reported a lower rate of success of pulp capping (59.31%) [31].

Our study reported more number of pulp capping cases in females compared to males but this finding was not statistically significant ($p>0.05$). A study by Al-Hiyasat was in agreement with our study. He reported that the patient's gender had no significant effect on the outcome of treatment ($p>0.05$) [31]. This adds to the overall consensus and is to be included in clinical practice.

Based on our research, 16.2% of direct pulp capping and 20.1% of indirect pulp capping had failed and underwent root canal therapy. In a previous study, the reported rate of failure of direct pulp capped tooth was 18.2% which was almost similar to our current finding [9]. However, some studies were in disagreement with our current finding, reporting a lower rate of failure of indirect pulp capping [32,33]. The difference may be due to variation in type of pulp capping material, restoration and adequacy of caries removal.

The current study was conducted to find out the number of pulp capping cases which underwent root canal therapy. Many factors play a role in the success of pulp capping such as adequacy of caries removal, state of pulp, type of restoration and interval of follow-up. The data on such information were limited and therefore were not included in our study. Extensive research needs to be done to include a large sample population. Further studies can be done with inclusion of other factors which may contribute to the success rate of pulp capping treatment.

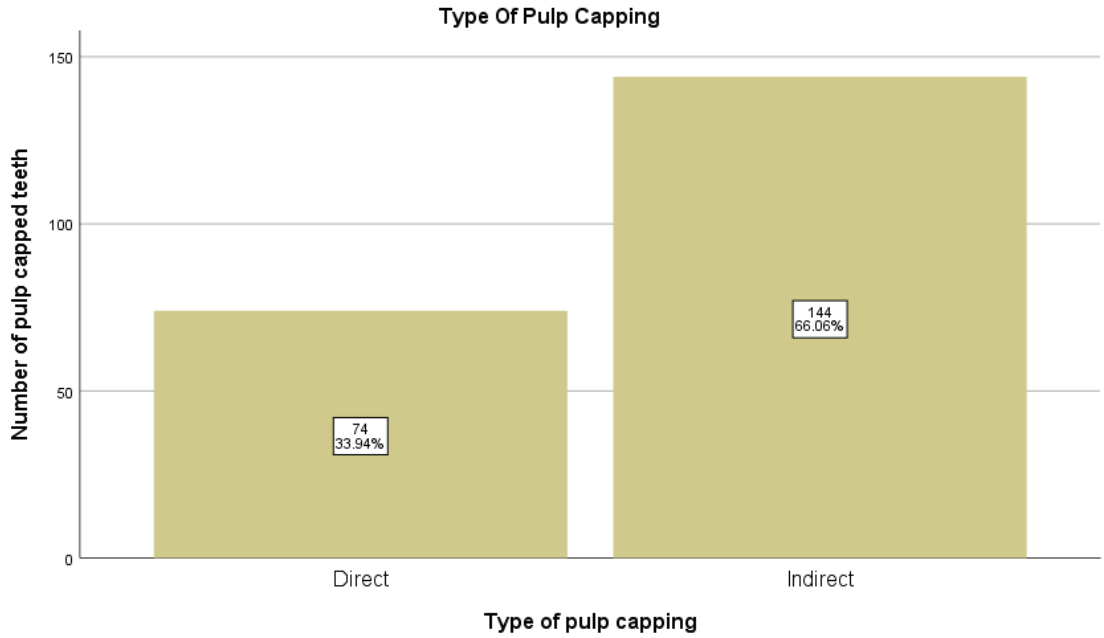


Figure 1. Bar chart depicts the number of pulp capped teeth by type of pulp capping. X-axis represents the type of pulp capping and Y-axis represents the number of pulp capped teeth. Patients presented with indirect pulp capped teeth were more compared to direct pulp capped teeth.

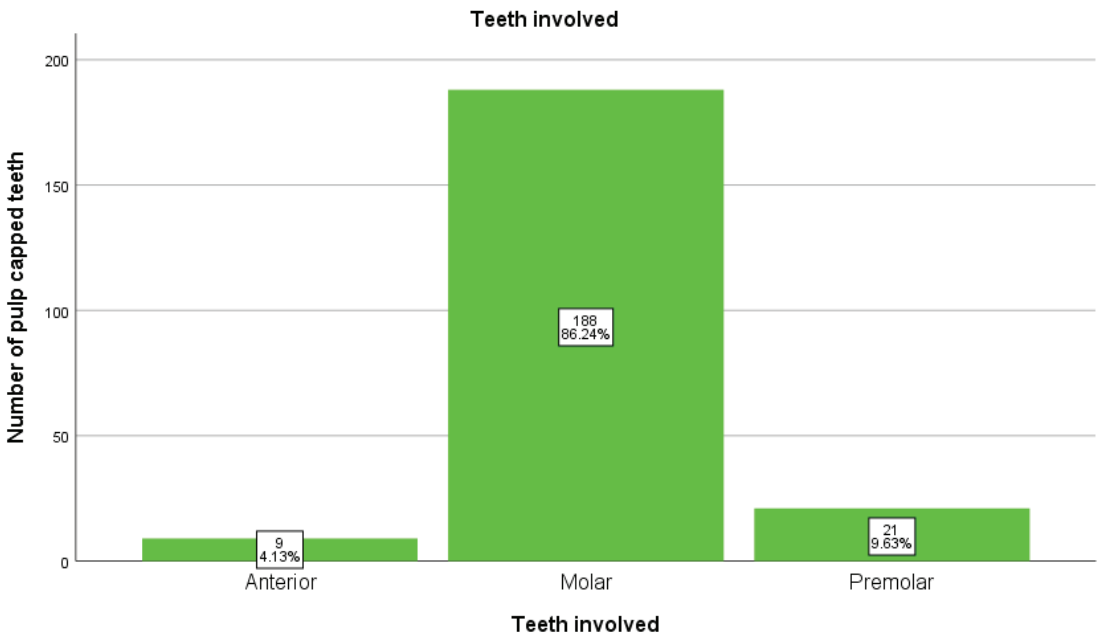


Figure 2. Bar chart depicts the distribution of pulp capped teeth by teeth involved. X-axis represents teeth involved and Y-axis represents the number of pulp capped teeth. Number of pulp capped teeth were more in molars compared to other teeth involved.

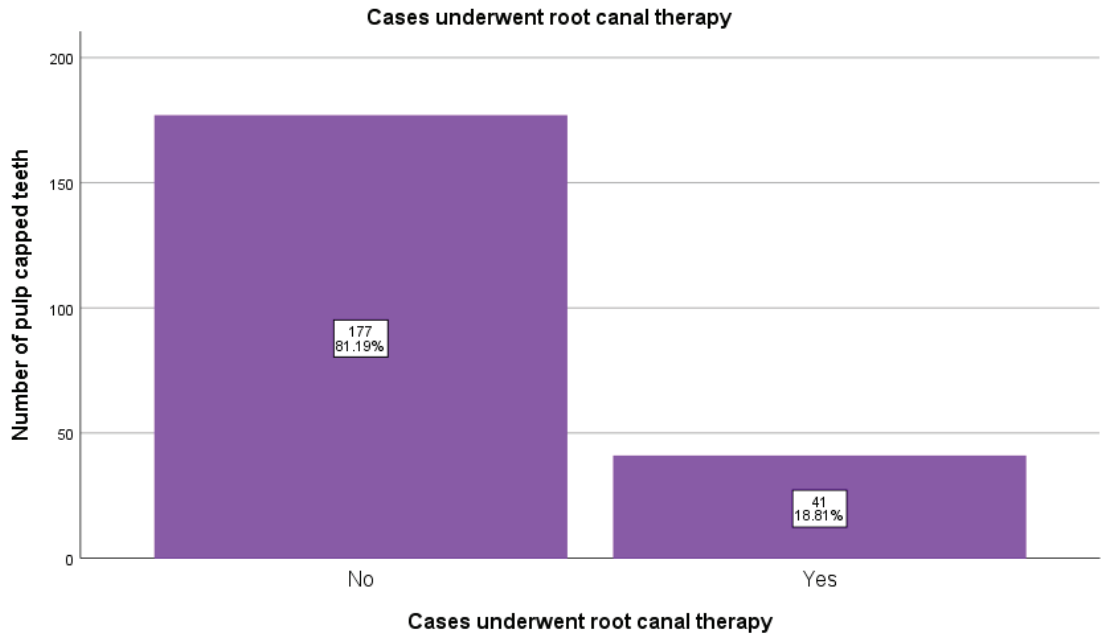


Figure 3. Bar chart depicts the number of pulp capped teeth and cases which underwent root canal therapy. X-axis represents cases which underwent root canal therapy and Y-axis represents the number of pulp capped teeth. Pulp capped teeth which underwent root canal therapy were comparatively less in number.

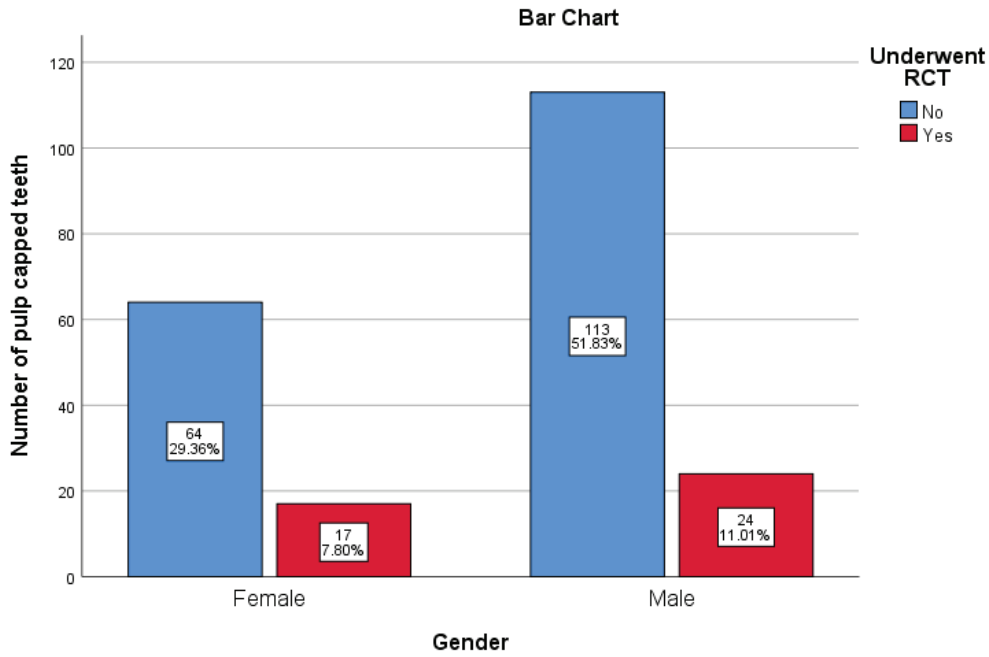


Figure 4. Bar chart depicts the association between pulp capped teeth which underwent root canal therapy and gender. X-axis represents the gender and Y-axis represents the number of pulp capped teeth which underwent (yes- red) and did not undergo (no- blue) root canal therapy. Pearson chi-square value- 0.401 , p value- 0.526 (p>0.05), hence, not significant. More males with pulp capped teeth that did not undergo root canal therapy were seen compared to females but the results were statistically not significant.

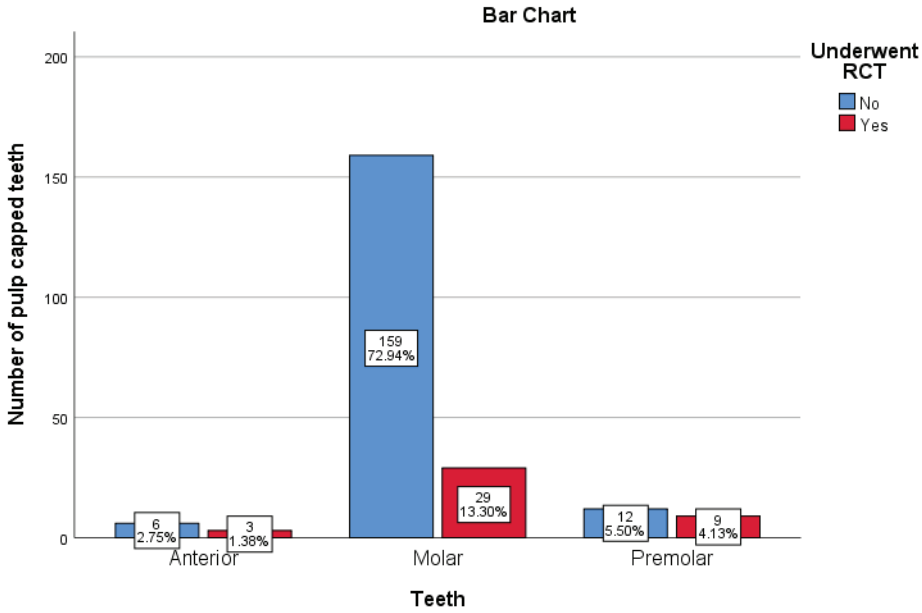


Figure 5. Bar chart depicts the association between pulp capped teeth which underwent root canal therapy and teeth involved. X-axis represents the teeth involved and Y-axis represents the number of pulp capped teeth which underwent (yes- red) and did not undergo (no- blue) root canal therapy. Pearson chi-square value- 10.606 , p value- 0.005 (p<0.05), hence, statistically significant. Pulp capped teeth (molar) which did not undergo root canal therapy were significantly higher compared to others and the results were statistically significant.

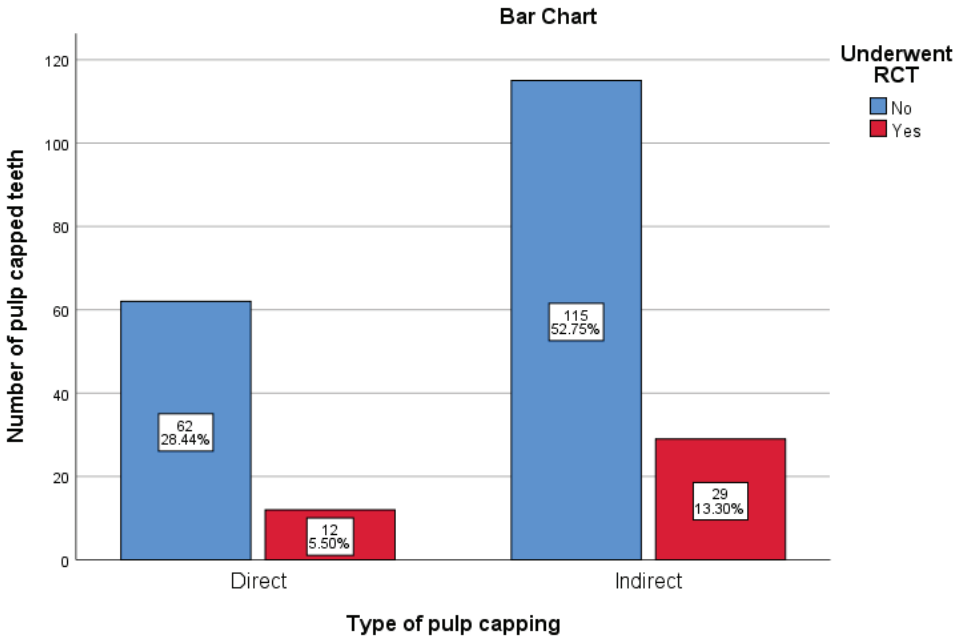


Figure 6. Bar chart depicts the association between pulp capped teeth which underwent root canal therapy and type of pulp capping. X-axis represents the type of pulp capping and Y-axis represents the number of pulp capped teeth which underwent (yes- red) and did not undergo (no- blue) root canal therapy. Pearson chi-square value- 0.493, p value- 0.483 (p>0.05), hence, not significant. Pulp capped teeth (direct/indirect) which did not undergo root canal therapy were higher when compared to those which underwent root canal therapy but the results were statistically not significant.

Conclusion

Within the limits of this study, it was observed that the number of pulp capping cases which underwent root canal therapy was about fifth of the total cases, and was more commonly seen in premolars and in indirect pulp capping cases.

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Conflict of Interest : No conflict of interest has been declared by the authors.

Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Gingival Health Status in Children with and without Cleft Lip and Palate: A Case Control Study

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Abstract

Cleft lip and palate were classified as followed by Veau in 1931. The major four groups are clefts of soft palate, clefts of hard palate, unilateral clefts of the lip, alveolus and palate, bilateral clefts of the lip, alveolus and palate. Gingival health in children is one of the important factors to be taken into a serious problem as it may progress to jeopardize the periodontium of the adult. It affects the periodontal disease in the inception of earlier in life. In dentistry, it is important for a dentist to recognize early and diagnose gingival diseases to optimize treatment outcome. To determine the gingival health status in children with and without cleft lip and palate. The purpose of the study was to analyse the gingival status in children with and without cleft lip and palate (a case control study). A study was carried out by collecting data by reviewing patients data and analysing the data of 86000 patients between June 2019 and March 2020 at the private dental institute. The sample size that was taken included 6 children with cleft lip and palate (case group) and 6 children without cleft lip and palate (control group), who came to the private dental institute for consultation. The gingival health status was evaluated using OHIS score and was compared between the groups. Data was statistically analysed using SPSS 2.0, Mann-Whitney U Test was conducted. Result was recorded. The children with cleft lip and palate showed similar OHI-S scores compared to children without cleft lip and palate. Therefore, it was concluded that within the limitations in current study, there was no difference in oral health status between children with cleft lip and palate and children without cleft lip and palate.

Keywords: *cleft; lip; palate; gingival; OHIS; health; dental*

Introduction

Cleft lip defined as a congenital deformity that occurs in the primary palate which is located anteriorly to incisive foramen. It's occurrence may be unilateral, bilateral, complete or incomplete.[1] Cleft palate is defined as a congenital abnormality that occurs in the secondary palate (soft and hard palate). It's occurrence may be unilateral,bilateral, complete or incomplete.[2] Cleft lip and palate are classified as followed by Veau in 1931. The major four groups are clefts of soft palate, clefts of hard palate, unilateral clefts of the lip, alveolus and palate, bilateral clefts of the lip, alveolus and palate. According to Koch et al in 1995 and Kernahan in 1991, suggested a new classification of cleft lip and palate and it gives a Y shape letter.[3] WHO has given the etiology factors for this condition are mainly environmental factors such as smoking and alcohol, nutritional factors, medications, organic chemicals and solvents. Whereas, another main factor is about the genetic factors.[4] Smoking and alcohol are considered as main causes of clefts where risk of developing a cleft is found in pregnancies with alcohol abuse and smoking.[5]

Moreover, nutritional status plays an essential role in developing cleft lip and palate. Vitamin B6 deficiency was the main reason for increased risk of clefts in the entire global.[6] In addition folic acid deficiency was observed as the main reason for increasing clefts. Furthermore, zinc is considered as the main element

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in the fetal development.[7] The deficiency of nutrients causes cleft lip and palate. It was observed that mother with children with clefts had lower concentrations of zincs in comparison to mother with children without clefts.[8] Clefting of the lip and palate can result from some medications such as corticosteroids, steroids in which some pregnancies take due to insomnia and anxieties. In addition, retinoids drugs are considered as one of the main reasons that cause clefts in infants because of exposing pregnant women to these drugs.[9] Finally genetic factors are influenced by family history which leads to the causative of cleft lip and palate. For example, the risk of transferring one parent having clefts to their children is 9%. The risk of transferring unaffected parents having a child is 4%.10 The occurrence of cleft lip and palate is either associated with many syndromes such as Pierre Robin's Syndrome, Treachers Collins Syndrome and Ectodermal dysplasia.[10] There are many common problems associated with cleft lip and palate, such as speech problems, hearing problems, ear infections, dental problems and feeding difficulties. In dentistry, it is known that dental problems involve orofacial clefts in the size and shape of teeth. For example, the permanent lateral incisor shows abnormalities in position and delayed eruption can be seen.[11] Moreover, many previous literatures had been reported for poor oral status in children with cleft lip and palate.[12] Dental caries and gingival health status in patients especially children with their condition are very discoordinated.[13]

Gingival health in children is one of the important factors to be taken into a serious problem as it may progress to jeopardize the periodontium of the adult.[14] It affects the periodontal disease in the inception of earlier in life. In dentistry, it is important for a dentist to recognize early and diagnose gingival diseases to optimize treatment outcome.[15] As an anatomical consideration, a dentist should analyze the gingiva in order to diagnose the health of gingiva in children.[16] The analysis should be based on the width of attached gingiva is greater in the incisor area, decreased over the cuspids and increases again over primary and permanent molars.[17] The attached gingiva increases in width with age.[18] In addition, the contact points between deciduous teeth are not as tight as those between permanent dentition provided favourable location for bacterial growth, thus leading to increased susceptibility of interdental region.[19] The gingival status can be relevantly decided based on the OHIS score for the teeth indicated in the OHIS score record.[20]

Most of the studies have evaluated the oral hygiene of children using simplified oral hygiene index (OHIS) by Green-Vermilion. It is widely used because WHO recommends this scoring criteria. OHIS contains evaluation of two genres, calculus and debris. The scoring is from 0 to 3. 0 indicated no debris or stains present.[21] Whereas, scoring 1 will be given if the soft debris covers not more than one third of the tooth surface or presence of extrinsic stains can be seen. Scoring of 2 will be given for soft debris covering more than one third, but not more than two thirds of exposed tooth. Finally, score 3 will be given in case of soft debris covering more than two thirds of the exposed tooth surface.[22]

Therefore, the current study aimed to determine the correlation of gingival health status in children with and without cleft lip and palate in a private dental institute. A case control study was planned and executed on children with cleft lip and palate from available data in the institute.

Materials and Methods

This retrospective study was conducted under a hospital based university setting. Ethical approval for this study was granted by the institute's ethical committee. Consent to use treatment records for research purposes were obtained from patient/ guardian at the time of patient entry into the university for dental needs. The retrospective data were collected by obtaining and analysing the 89000 dental case records of the university from June 2019 to March 2020. The sample size that was taken included 6 children with cleft lip and palate (case group) and 6 children without cleft lip and palate (control group), who came to the private dental institute for consultation. The inclusion criteria for the current study were children with cleft lip and palate, children between the age of 2-17 years age, complete photographic and written records regarding the complete intra-oral examination of the patient. Age and gender matched controls i.e. children without cleft palate, were taken according to the relevant cases obtained from the inclusion criteria. The exclusion criteria were incomplete and censored dental records, absence of photographic evidence and children below the age of 2 were excluded.

OHIS scores were evaluated for each of the children from both the groups. The selected case and control group were examined by three people; one reviewer, one guide and one researcher. The patients' case sheets were reviewed thoroughly. Cross checking of data

including digital entry and intra oral photographs was done by an additional reviewer and as a measure to minimise sampling bias, samples for the group were picked by simple random sampling. Digital entry of clinical examinations and intra oral photographs of selected subjects were assessed and this included the assessment of gingival status as mentioned before by the examiner based on intraoral photographs and clinical examination data for each tooth.The examiner was trained to add data of gingival status as present or absent for both case and control group by tabulation using Microsoft Excel software. The mentioned data were coded and transferred into SPSS PC version 2.0 (IBM 2019) software for statistical analysis. A correlation test, Mann-Whitney U Test was done between children with cleft lip and palate (case) and children without cleft lip and palate (control). The results were recorded. The difference was considered statistically significant as the p value was less than 0.05. ($p<0.05$)

Results and Discussion

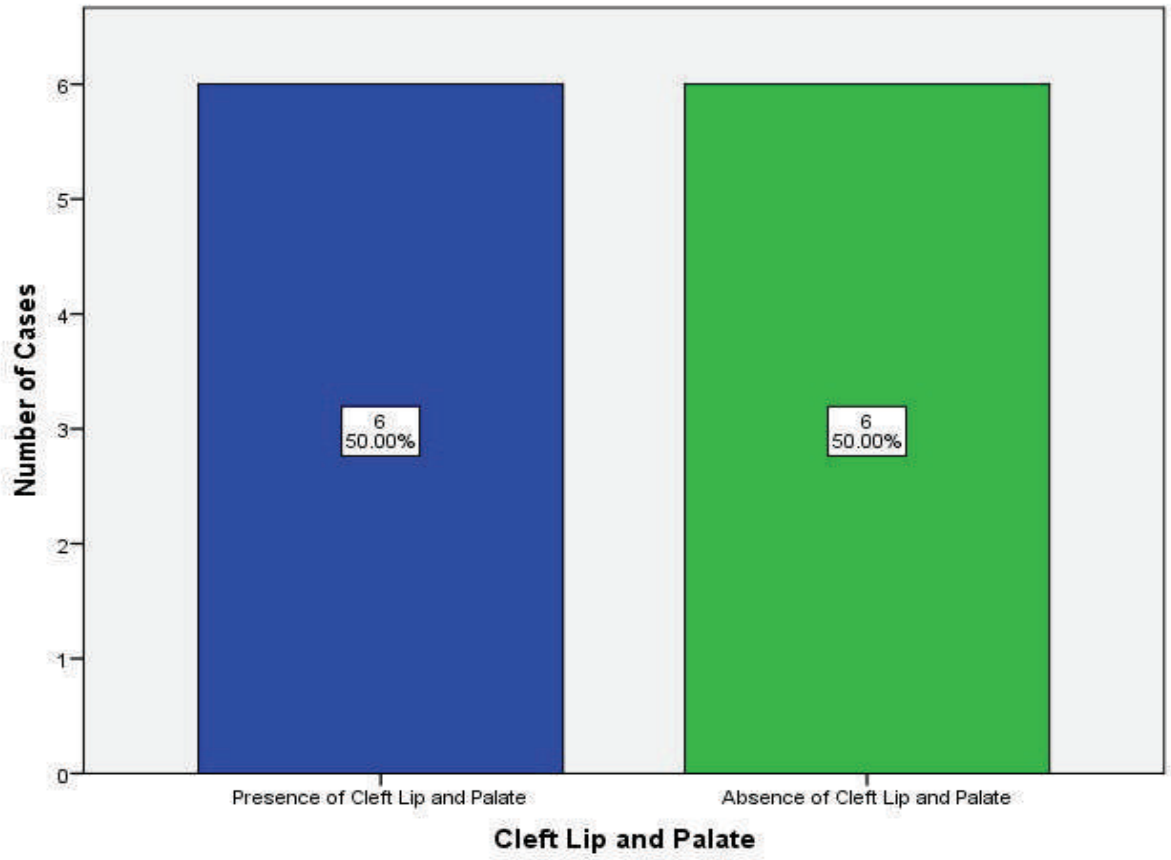


FIGURE 1 - The graph shows case distribution in case group (children with cleft lip and palate) and control group (children without cleft lip and palate). (X axis: Presence or absence of cleft lip and palate; Y axis: number of cases; Blue: Presence of cleft lip and palate; Green: Absence of cleft lip and palate). Notice the equal distribution of cases for both the groups.

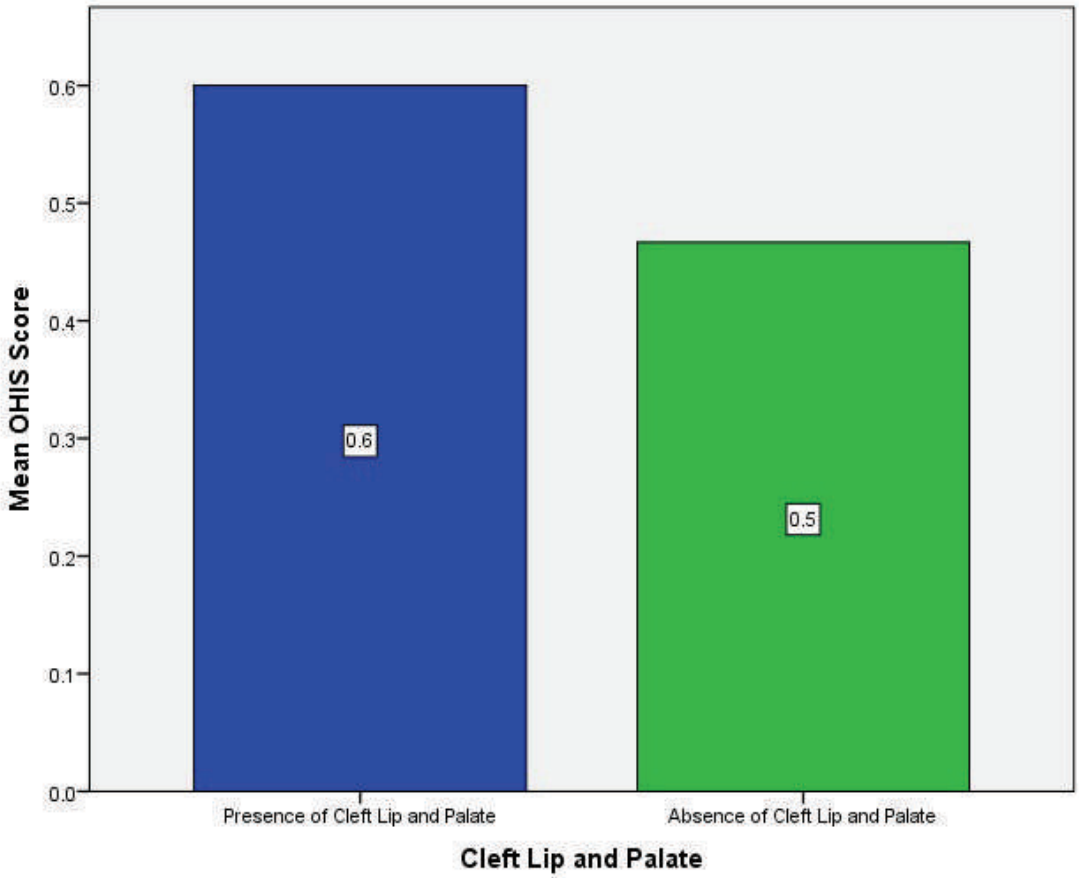


FIGURE 2 - The graph bar portrays the OHIS index score distribution for case (with cleft lip and palate) and for control (without cleft lip and palate) groups. (X-axis: Presences or absence of cleft lip and palate; Y-axis: Mean OHIS score; Blue: Presence of cleft lip and palate; Green: Absence of cleft lip and palate). There was no difference in OHI-S scores between children with cleft lip and palate and children without cleft lip and palate, which was statistically significant. (Mann-Whitney Test; p=0.039- significant).

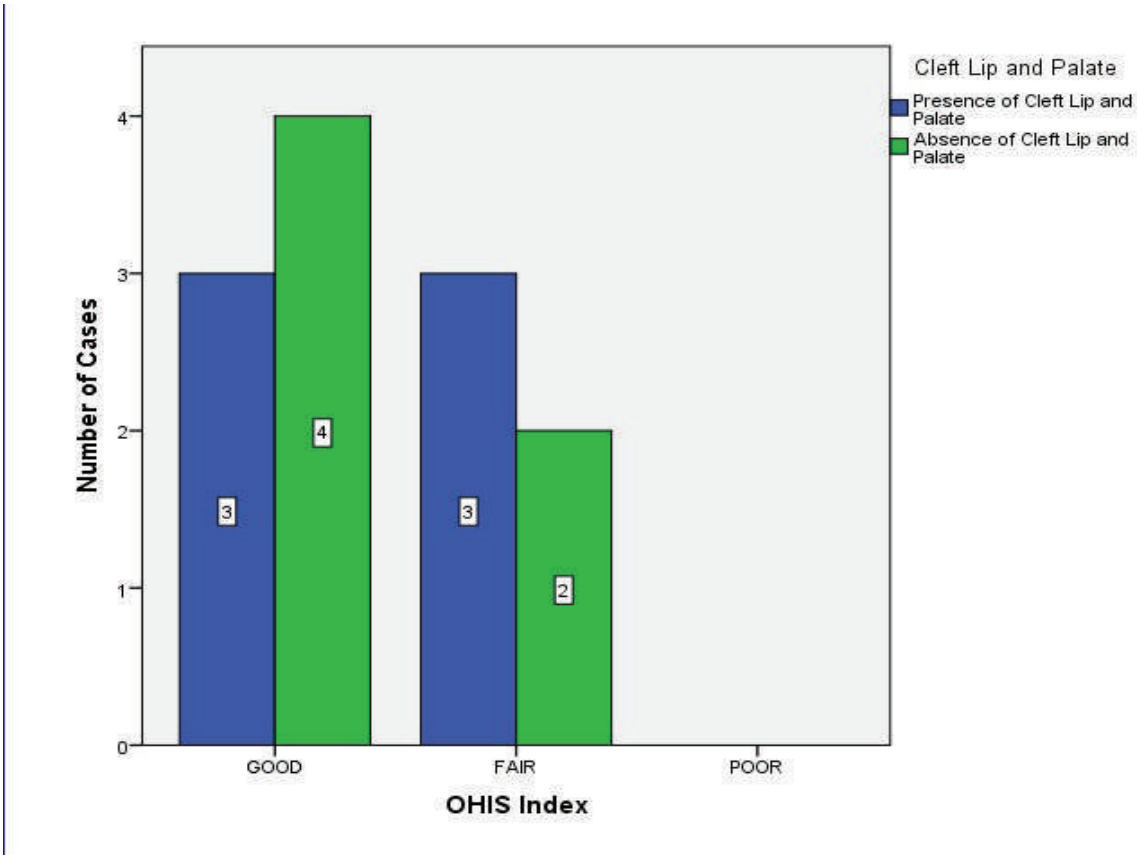


FIGURE 3: Bar graph represents the comparison of OHI-S index scores among the children with and without cleft lip and palate. (X-axis represents the different OHI-S index scores; Y-axis represents the number of cases). Although a slightly higher number of children without cleft lip and palate had good OHI-S score, there was a slight increase in number of children with cleft lip and palate had fair OHI-S score, which did not show any statistical significance (Mann - Whitney U test, p-value - 0.76 - not significant).

The final study sample size included a total of 12 patients with six patients (50%) having cleft lip and palate (case group) and six patients (50%) without cleft lip and palate (control group). It showed the equal distribution of cases in this study [Figure 1]. Among the 12 patients in both the groups, three were males (25%) and three were females (25%), respectively in both groups. The mean OHIS score was 0.6 for the case group (children with cleft lip and palate) and 0.5 for the control group (children without cleft lip and palate). There was no gross difference in the OHI-S scores between the case and control group, which was statistically significant (p=0.039).[Figure 2]. There were four children children without cleft lip and palate with good OHI-S score, while there were three children with cleft lip and palate with fair OHI-S score.[Figure 3] There was no difference in the overall gingival health status between them, which did not show any statistical significance (p = 0.76).

In a similar study by Paul et al[23], revealed that the mean of OHIS score of children with cleft lip and palate was 0.9. They concluded that children with cleft lip and palate had generally poorer oral and gingival health than those without cleft lip and palate. These results were similar to the present study. In present study, the mean score of OHIS for children with cleft lip and palate was 0.6 which was higher than those without this condition, where it showed a mean value 0.5 only. In another similar study conducted by Khijmatgar et al[24], poor oral hygiene and gingival health was observed in children with cleft lip and palate as compared to children in the control group. Again this study was accepted as it was similar to present study. Poorer oral hygiene and gingival status was observed in the case group compared to the control group.

In the present study, the higher OHIS score indicated poorer oral hygiene and gingival health status seen within the case group (with cleft lip and palate).

Statistically the difference was less than 0.05. Here, it is observed that children with cleft lip and palate had a better gingival status than children without cleft lip and palate. The results showed significant difference in the oral hygiene and gingival health status for both the groups ($p<0.05$). However, the consensus for this study was agreed but yet it has to be studied in detail as this study is limited to certain factors.

The advantages of the study were that this was a case-control study with age and gender matched control to provide better results and high internal validity. The limitations of the present study were , it is a single centered, limited sample size, unicentric study and only one ethnic group was involved. Moreover, the possible reason for children having poor gingival health status can also be the difficulties of brushing due to their anomalies and also their dietary which is rich with carbohydrates. For the future scope, the dental department should provide an assessment on the baseline of oral health status in children with or without cleft lip and palate. It is also important to emphasize the need of intensive and preventive measures in oral disease to optimize the clinical outcome in children with or without cleft lip and palate.

Conclusion

Within the limitations in current study, gingival health status of children with cleft lip and palate based on OHI-S index was similar to the gingival status of children without cleft lip and palate. Despite the presence or absence of cleft lip and palate, oral hygiene measures should be emphasized in all the children.

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Association of the Depth of Invasion wth Lymph Node Metastasis in Oral Squamous Cell Carcinoma Patients - A Retrospective Study

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Abstract

Oral squamous cell carcinoma is the most common oral malignancy representing upto 80 % to 90% of all malignancies of oral cavity. In Indian population, it ranks 3rd of all carcinomas. Dietary factors and external factors like tobacco, smoking and alcohol consumption are the major causes for progression of oral squamous cell carcinoma. Histopathologically, the tumor progression is assessed by TNM staging. It is the most common and widely practised histomorphological evaluation of tumor progression. Depth of invasion is also another tool for evaluating tumor progression in OSCC and is also used widely in every clinico histopathological slide evaluation and diagnosis. In this study, a correlation was made between depth of invasion and nodal metastasis. All the data was collected after checking 86,000 patient records. Details such as age, sex, pathological TNM staging, depth of invasion and cases with nodal metastasis were tabulated. Association was made using the IBM SPSS version 20.0. Pearson chi square test did not show any significant association between depth of invasion with nodal metastasis. Other factors like pattern of invasion, tumor differentiation, site of the lesion as well as the age of the patient has to be taken into consideration for future assessment.

Keywords: *Oral squamous cell carcinoma, depth of invasion, metastasis, Oral cancer, lymph node.*

Introduction

Oral squamous cell carcinoma is the most common oral malignancy representing upto 80 to 90 % of all malignancies [1]. In India, oral cancer represents more than 50% of all cancer cases [2]. Population of India is mostly habituated with consumption of tobacco and tobacco related products, leading to higher prevalence and even higher incidence rates of cancer. According to a study conducted, the global estimate of oral cancer accounts for 2% to 4% of all cancers [3]. Annually, incidence of oral cancer around the world is 275000 cases and 128000 deaths annually [4]. Etiological factors that lead to development of oral cancer are multifactorial but it is majority due to environmental and lifestyle factors. Consumption of tobacco in the form of smoking or chewing leads to oral cancer [5]. Tobacco is consumed in many forms. Along with habits, other etiological factors are virus infection, candida, syphilis [5 6].

OSCC has unpredictable behaviour, due to which it's difficult to correctly measure the invasion of OSCC. One such method of assessing the prognosis is by TNM staging. TNM staging is used to estimate the clinical and survival outcome of the patient. Along with these conventional methods, newer methods are in vogue. Such methods are detection of OSCC using immunohistochemistry, serum and saliva metabolomics

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[7,8 9 10 11 11,12 13 14 15 6,16] Histopathologically, OSCC is graded based on the proportion of tumor resembling normal oral epithelium and the amount of keratin production [17]. The lesions are classified as well differentiated, moderately differentiated, and poorly differentiated [18]. [66,192014]

Recent studies hypothesize that histomorphological parameters like DOI (Depth of invasion) may be used as a prognostic factor in patients with OSCC. All the cases of OSCC should be neatly photographed and documented clinically and histologically for future reference [21]. The aim of the study is to associate the depth of invasion with lymph node metastasis and to find out which method is better for cancer prognosis.

Materials and Methods

A retrospective study was conducted in the department of oral pathology. A total of 36 OSCC patient details were retrieved after assessing 86,000 patient details. On receiving approval from the scientific review board of Saveetha Dental College, the data of OSCC patients were collected. Cross verification of the cases were done by correlating reports and photographs. Both internal and external validity were applicable. Data collection was done between march 2019 till march 2020 and tabulated in the excel sheet. Demographic data was collected along with the clinical details of the tumor. Variables like clinical details, depth of invasion, TNM staging of OSCC, age of the patient, sex, site of the lesion, histopathological evaluation, depth of invasion and lymphnode metastasis were considered. The confidential details of OSCC patients were masked. After procuring the patient details, data was tabulated in excel sheets. The data was then exported to SPSS and the appropriate statistic was done. Chi square test was used to associate the depth of invasion with lymph node metastasis.

Results and Discussion

The results of the study show that the predominant number of oral squamous cell carcinoma cases belong to 40 -60 years of age (Figure1). Males were most commonly affected than females (Figure 2). The most common habit that was observed was pan chewing. Histopathological slides showed that most of the cases showed 6-10mm depth of invasion (Figure 3). On associating the depth of invasion with gender, 6-10mm depth of invasion was seen in the majority of male and female population. But > 10mm depth of invasion was more common in males than in females. (Figure 4). On assessing the lymph node involvement, 55.5% of the cases showed lymph node metastasis and 44.44% did not show any metastasis(Figure 5). On associating the lymph node involvement with depth of invasion, it was found that 60% of the cases with lymph node involvement had 6-10mm DOI. On the other hand only 20% of the cases with DOI <6mm had lymph node involvement. On the contrary 43.75% of the cases with 6-10mm DOI did not show lymph node involvement. Chi square analysis also did not show any statistical significance with $p = 0.608$ (p value > 0.05) (Figure 6).

Head and neck cancers constitute for approximately 5% of all malignant tumors in the body. According to the present study, males are affected more than females (80.5%, 19.5%). In the majority of prevalent studies relating to OSCC, almost most of the authors have found similar results. OSCC is seen to affect males six times more than the females [1].

The results of the previous studies are in accordance with the present study. The reason for higher male predilection could be due to external factors like the tobacco consumption habit, smoking and alcoholism. Males have a higher probability of consumption of tobacco, mostly in the South east Asian population where tobacco in any and all forms is easily available. Even though tobacco related products are easily available and a person doesn't need any document to procure such products, consumption of tobacco products and alcohol are comparatively lesser amongst the female population. It could be due to social stigma attached against females. Patients had a habit of tobacco consumption. Studies have proven that tobacco consumption is directly related to OSCC. Fabio et al found that young patients who did not have tobacco consumption habits suffered from OSCC [22].

The prognosis for OSCC can be predicted by various histomorphological parameters. TNM staging is determined by DOI, nodal involvement and distant metastasis. It is the most common and frequently applied parameter for assessing tumor progression. Other histomorphological parameters like depth of invasion is an independent prognostic factor in early detection of OSCC. In previous studies, authors have found the DOI of 4 mm to be common, but in the present study, 6 -10 mm was the commonest depth of invasion. In a study conducted by Inne J denn Toom et al, DOI having < 3.4 mm were having distant metastasis [23]. Meta-

analysis studies have found that DOI > 4mm leads to nodal metastasis [18]. The cut off value in Inne et al study was found to be 3.4 mm [23]. In the present study, an association was made between depth of invasion and involved and not involved lymph nodes, there was no possible significance found. Association was made between depth of invasion among males and females (*p* value = 0.608). A Chi square test was performed between the two variables. No significant association was seen (*p* value = 0.851)

In the current study, a correlation between DOI being > 4mm would lead to nodal involvement was not quantified. Presence of cases with DOI > 10 mm with no nodal metastasis is observed, whereas in cases where DOI is < 6mm with nodal metastasis is observed as well. In the present study, we have only considered DOI and pTNM to study the tumor invasiveness. In a previous study conducted by Kailiu Wu [24] along with depth of invasion > 4mm, he included other variables like neurovascular invasion, trabes pattern, poor differentiation and old age for lymph node relapse and poor prognosis. Hence it would be safe to conclude that just depth of invasion isn't sufficient enough to rule out the possibility of lymph node metastasis. Other factors like type of invasion, extent of neurovascular invasion, type of epithelial or tumor differentiation, site of the lesion as well as the age of the patient has to be taken into consideration.

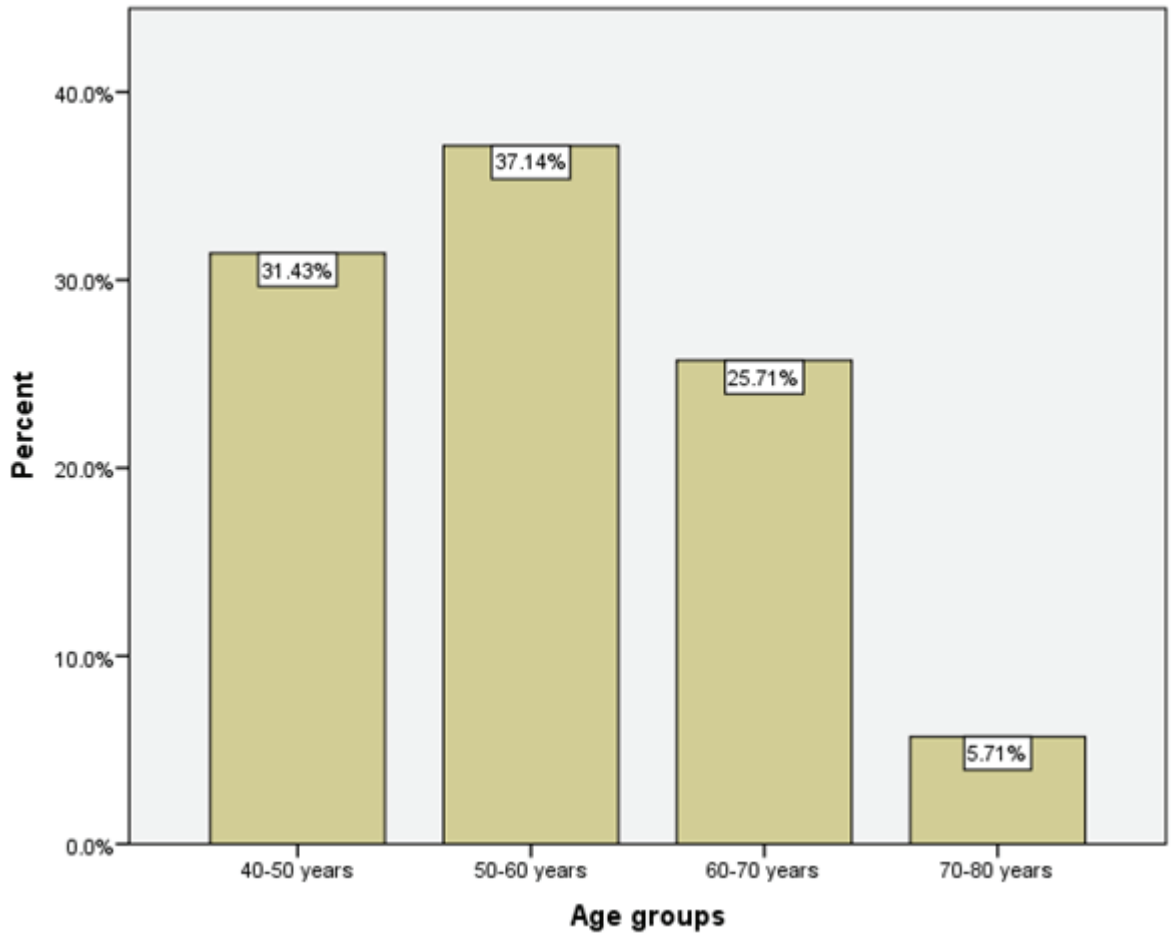


Figure 1: Bar graph depicts the age distribution of oral squamous cell carcinoma patients. X axis depicts the age groups and Y axis depicts the percent of cases. 37.14% of the cases belong to 50-60 years of age, 31.43% 40-50 years, 25.71% 60-70 years and 5.71% 70-80 years of age.

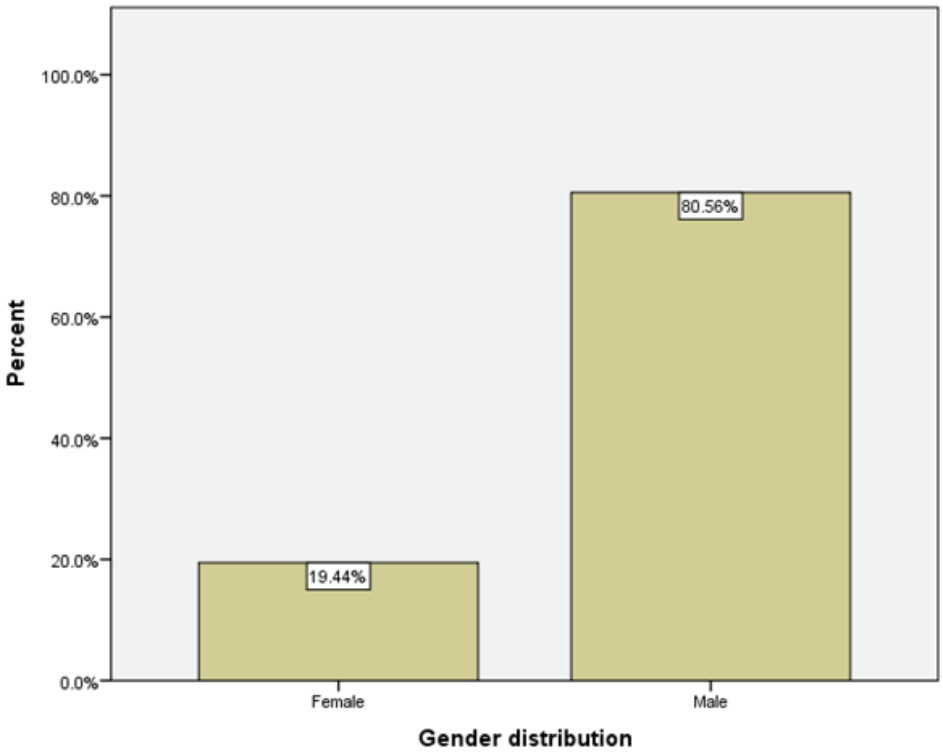


Figure 2: Bar graph depicts the gender distribution of oral squamous cell carcinoma patients. X axis depicts the distribution of males and females and Y axis depicts the percentage. 18.56% of the population are males and 19.44% are females.

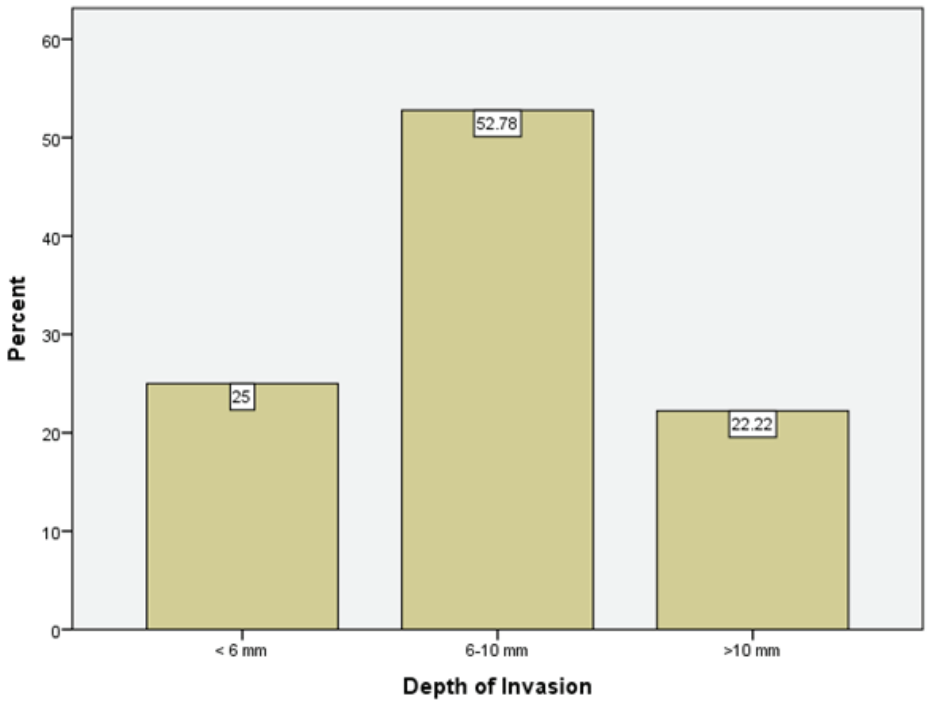


Figure 3: Graph depicting the depth of invasion in oral squamous cell carcinoma patients. X axis depicts the depth of invasion and Y axis depicts the percentage of cases. 52.78% of cases have DOI of 6-10mm, 25% have <6mm and 22.22% have > 10 mm.

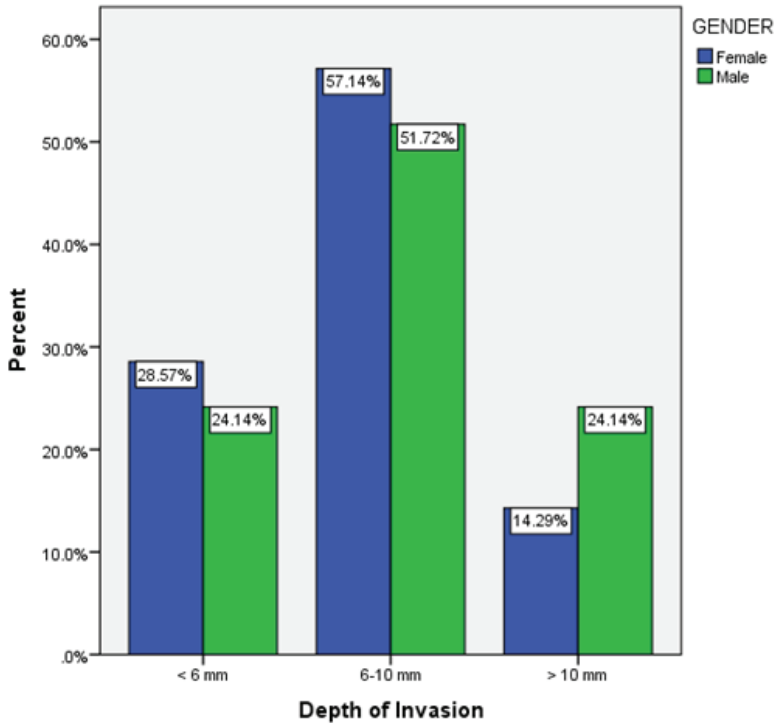


Figure 4: The graph depicts an association between gender and depth of invasion in oral squamous cell carcinoma patients. X axis depth of invasion and Y axis depicts the percentage of cases based on gender. 6-10 mm depth of invasion was seen in the majority of male and female population. But > 10mm depth of invasion was more common in males than in females. However chi square test did not show any statistical significance with $p = 0.851$ ($p > 0.05$).

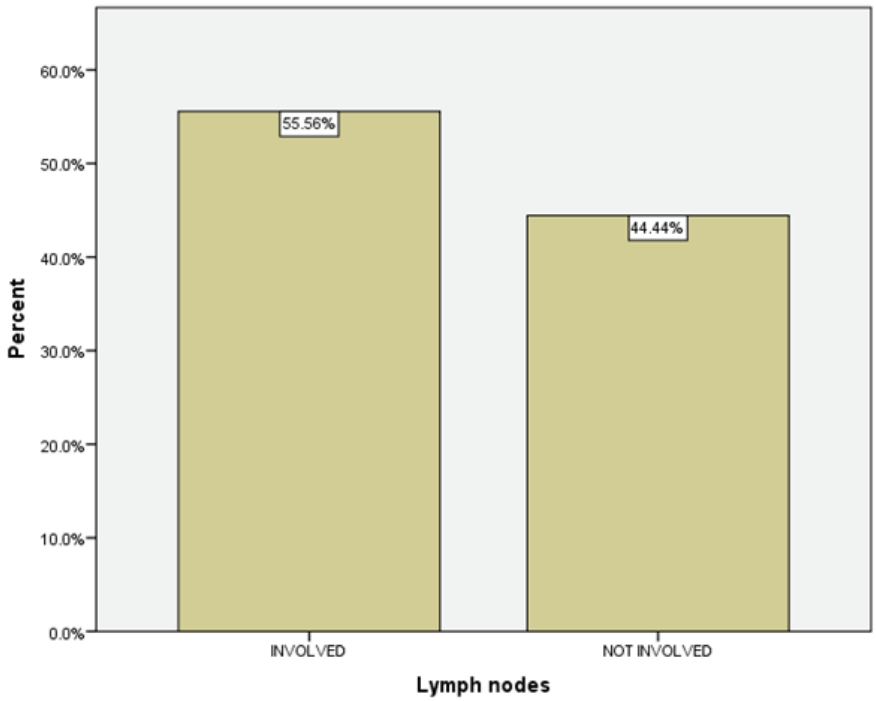


Figure 5: Bar graph depicts the frequency distribution of involved and not involved lymph nodes in oral squamous cell carcinoma patients. X axis depicts the lymph node involvement and Y axis depicts the percentage. 55.56% of the cases show lymph node involvement, whereas 44.44% do not show any involvement of lymph nodes.

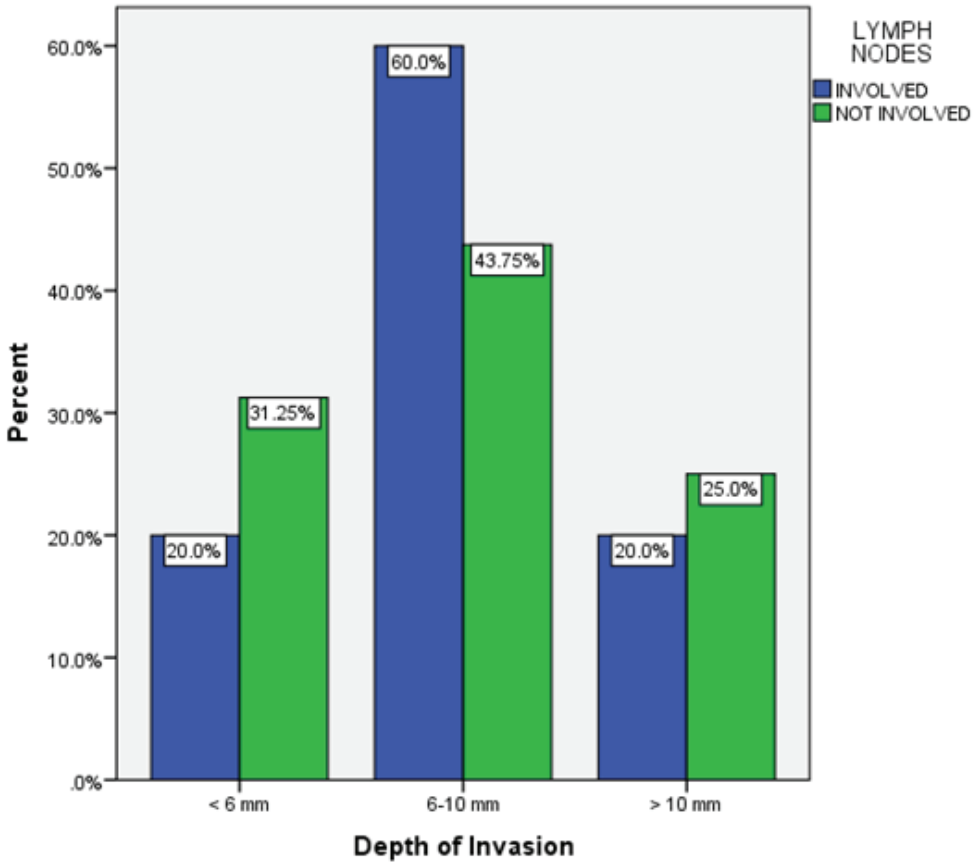


Figure 6: Bar graph depicts the association between depth of invasion and lymph node involvement. X axis depicts the depth of invasion and Y axis the percentage of lymph node involvement. Blue depicts the involved lymph nodes and green depicts the uninvolved lymph nodes. 60% of the cases with lymph node involvement had 6-10mm DOI. However , Chi square analysis did not show any statistical significance with p = 0.608 (p value > 0.05).

Conclusion

It was concluded from this study that depth of invasion is a fine histomorphological tool for assessing tumor invasiveness, but it is not enough to correlate with the nodal metastasis. Some cases with depth of invasion >10 mm had no nodal metastasis and some cases had nodal metastasis even though the depth of invasion was less than < 6mm. Other factors like pattern of invasion, tumor differentiation, site of the lesion as well as the age of the patient has to be taken into consideration for future assessment.

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Correlation Between Highly Sensitive C-Reactive Protein Level in Cases of Preeclampsia with or without Intrauterine-Growth Restriction

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Abstract

Background: Preeclampsia is one of hypertensive disorder that affects 4% of pregnant women, the exact cause cannot be identified and it is one of the major causes of both maternal and fetal morbidity and mortality , also it puts a burden on perinatal outcome as prematurity and Intrauterine growth restriction.Continuous search for predictive markers of severe PE is important and can be used to target high risk women for effective preventive treatment. **Aim of study**: to evaluate Highly Sensitive C-Reactive Protein role used as predictive factor for severity of preeclampsia and whether it can be used to predict development of Intrauterine growth restriction. **Patients and methods**: this is a cross-sectional study includes 80 pregnant women with their age 18-35 years , their gestational age range between 32- 40 weeks , admitted to AL-Zahraa teaching hospital in Najaf, whose diagnosed as PE based on blood pressure measurement and proteinuria. Full history and clinical examination were performed, venous blood aspirated for each women for biochemical analysis, Liver functions tests, Renal function tests, Highly Sensitive C- Reactive Protein, and Doppler ultrasound. **Results**:The patients divided into those with mild PE and severe PE, with or without IUGR. No significant difference between the level of Highly Sensitive C-Reactive Protein and the severity of PE as p value was[0.779], but there is significant difference between the presence of IUGR and the level of Highly Sensitive C-Reactive Protein when it is≥ 2mg/L as the p value was[0.020]. **Conclusion**: We conclude that Highly Sensitive C-Reactive Protein is not significantly associated with the severity of Preeclampsia but associated with intra uterine growth restriction.

Keywords: *Preeclampsia, hsCRP, intra uterine growth restriction*

Introduction

CRP [C-reactive Protein] is one of acute phase protein which is increased in many systemic inflammation. It is the first acute phase protein to be described by many studies and is sensitive systemic marker of inflammation in different tissues and tissue damage [1].CRP is important sensitive index of systemic inflammation that predicts adverse outcome in atherosclerotic events, including myocardial infarction, cerebrovascular accident, peripheral vascular disease, and even death [2]. Elevated CRP level are also correlated with obesity [3]. It is to be Known whether CRP and obesity are associated or predisposes to PE[4]. The response of systemic maternal inflammatory to pregnancy is responsible for endothelial dysfunction which may be responsible for the clinical and pathological picture of PE,and there is a many factors that cause abortion like bacterial infection [5]. The association between first trimester high level of CRP levels and subsequent PE supports the hypothesis that systemic inflammation may be involved in the pathogenesis of PE [6]. hsCRP introduced and can be used as one early marker of low grade inflammation and may further helping in detecting pathophysiological process

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happened early in pregnancy so as to predict possible adverse pregnancy outcome and try to use preventive therapies well in time. Normal human placenta produces hsCRP and release it mainly into the maternal blood, and also it can be found in amniotic fluid and fetal urine , its increased amount can directly relates to poor pregnancy outcome . however, the real origin of amniotic fluid hsCRP, its regulation and its exact function during human pregnancy is not clear [7].

Patients and Methods

This is a cross-sectional study including 80 pregnant women, their ages between 18-35 years, admitted to Al-Zahraa teaching hospital for maternity and pediatric over period of 8 months, their GA between[32-40] weeks , as proven by last menstrual period or early pregnancy U/S. An approval on the study were taken from gynecology and obstetrics department then ethical committee in the college of medicineKufauniversity.

We clarify the procedures included in the study for the patients and take their agreement for participation in the study and in taking information, investigations, and subsequent blood aspiration. WE classify our patients into 2 groups:

Group A: mild PE→BP systolic 140−160 and diastolic 90−109 With Albumin ≥+

Group B:Severe PE→BP systolic ≥160 and diastolic ≥110 With Albumin ≥++ or Oliguria <500ml/24hrs ,Thrombocytopenia, Epigastric pain, Pulmonary edema,Persistent Cerebral or visual disturbances. Full history was taken from the patients including obstetrical history, previous medical history, previous hypertension, previous APH ,drug used and family history. We exclude diabetes mellitus, chromic renal disease and chronic hypertension. Full examination including blood pressure[in left lateral position], pulse rate , edema , done for all patients . Venous blood had been aspirated from each patients with the following investigations: CBC, RFT, LFT, Doppler U/S. hsCRP levels were estimated from the sera of the patients using ELISA [enzyme linked immune sorbent assay] method by commercial kit SIGMA-ALDRICH

Results:

This is a cross-sectional study including 80 pregnant women having PE with age range 18-35 years.; it includes the patients with PE as follows:25 patients with mild PE with mean age of [26.84±5.22], 55 patients with severe PE having mean age of [27.84±7.28].The result was assist using T-Test at a level of significance P value ≤0.05. As Shown in Table (1) there is no significant difference between patients with mild PE and those with severe PE regarding their age, parity, GA or RBS level, but there is highly significant difference between the two groups regarding the systolic and diastolic BP as the P value is [0.000].

The result also shows that there is no significant difference between the severity of PE and presence or absence of IUGR as shown in Table (2). The result also shows that there is significant difference between patients with PE and IUGR and PE without IUGR when hsCRP ≥ 2mg/l [high risk], but there is no significant difference in those with or without IUGR when the hsCRP level < 2mg/l as shown in chart Table (3). The Result also shows that there is no significant difference between mild and severe PE when level of hsCRP< or ≥ 2mg/l as shown in Table (4).

Table [1]: Demographic and clinical characteristics of women with two group Mild & Severe PE

characteristics	Group A	Group B	P value
Number	25	55	
Age	26.84±5.22	27.84±7.28	0.489
Parity	0.911±1.7	0.777±0.849	0.646
Gestational age	37.40±2.08	36.89±2.19	0.324
Random blood sugar	93.48±7.97	96.8±11.2	0.135
Systolic blood pressure	144.00±5.77	161.6±16.4	0.000
Diastolic blood pressure	96.80±4.54	106.64±9.67	0.000

Table [2]: Patients with mild and severe PE with and without IUGR[P Value=0.210]

	IUGR	Without IUGR
Mild preeclampsia	4	21
Severe preeclamsia	17	38

Table [3] correlation between patients with IUGR and level of hsCRP.

Parameter	**PE with IUGR Number= 21**	**PE without IUGR Number= 59**	**P value**
	Mean ±SD	**Mean ±SD**	
Low risk	1.063±0. 537 mg/l	1.106±0.442mg/l	0.846
High risk	7.52±3.65 mg/l	5.28±2.21mg/l	0.020

Table [4] level of hsCRP between mild and severe PE

Parameter	**Mild preeclampsia Number= 25**	**Severe Preeclampsia Number= 55**	**P value**
	Mean ±SD	**Mean ±SD**	
Low risk	1.263±0. 523 mg/l	1.072±0.324 mg/l	0.240
High risk	8.323±4.884 mg/l	6.729±3.580mg/l	0.233

Discussion

C-reactive protein is one marker of tissue damage, necrosis and inflammation. CRP are found to be raised in pre-eclampsia, but there is still a debate about its usefulness as predictive marker for pre-eclampsia during both the first and second trimester of pregnancy [4]. In present study we found that there is significant difference in the level of hsCRP when it is ≥2 between women with PE who have IUGR baby and those without IUGR , as the mean level of those with PE and IUGR is 7.52± 3.65, and for those without IUGR is 5.28±2.21. This is similar to study from Gandevani SB 2012[8] ,who found that there were significant relationship between hsCRP level in mild and severe PE and with IUGR and birth weight. Also, Adali E. 2011[9], found that hsCRP has shown a correlation with IUGR and birth weight and Savvidou et al 2002[10], reports an elevated CRP level at 10-14 weeks of pregnancy in women who develop PE or who delivered IUGR babies. While Tjoa et al. 2003[11] reported no difference in hsCRP between PE with IUGR group and normal gestational outcome. These differences from our study may be due to the cutoff value of hsCRP used in different studies. In current study there was no significant difference in the level of hsCRP between mild and severe PE , this is similar to the result of ZohreTavana 2011 [12] who found that serum concentration of CRP did not show statistically significant changes among mild PE , severe PE , chronic HT and normal pregnancy. Hossein Ayatollah 2007 found that higher level of hsCRP in both mild and severe PE than normal pregnancy and this result suggest that hsCRP are increased more in cases of severe PE than mild PE and may be used as useful in prediction and diagnosis of degree of severity of PE. SelahattinKumru2005 , who found that hsCRP levels increase in women with PE . elevated serum levels of hsCRP in preeclampsia women are correlated well with different clinical and biochemical parameters of PE. Determination of S.hsCRP levels may be used as a marker for the severity of PE[13], and this is not an agreement with our study and alsoT.Sarala Devi 2013 who found that hsCRP may be used as good predictive marker for PE[14].It was observed that there was strong association between high TSH level and development of PE[15].

Conclusion

We conclude that hsCRP is not statistically different between mild and severe PE , while it is significantly higher in IUGR group.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Renal Function Tests in Women with Preeclampsia with and without Intrauterine Growth Restriction

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Abstract

Background: The kidney is the most likely to be affected by endothelial injury in patients with preeclampsia, so assessment of renal function is essential in the evaluation of the pregnant women with preeclampsia. **Aim of the study**: to evaluate which test of renal function in preeclamptic patients with and without intrauterine growth restriction is more useful. **Patients and method**:150 women in third trimester with preeclampsia over period of 14 months diagnosed based on measurement of blood pressure and presence of proteinuria divided into two group : group A those preeclamptic women without feature of intrauterine growth restriction by ultrasound and Doppler study and group B those with features of intrauterine growth restriction. Blood aspirated from each woman and blood urea, serum creatinine, serum uric acid, and serum cystatin-C were measured using spectrophotometer. **Results**: From 150 women with preeclampsia 120 of them had no features of intrauterine growth restriction and 30 had some features. Mean maternal age of patients included was 22±0.3 in group A and 24±0.7 in group B which is not differ statistically while the body mass index should statistically differences between the two group with P value <0.001. the blood urea, serum creatinine and serum uric acid levels found to be higher in group B but not reach statistical differences while serum cystatin C are higher in group B mean 1.23±0.45when compared to group A 1.08±0.97with p value <0.001. **Conclusion**:Renal function may be affected more in patients with intrauterine growth restriction and cystatin C level is more promising marker for renal function in preeeclamptic women.

Keywords: *preeclampsia, renal failure, urea, creatinine*

Introduction

Preeclampsia is a complex syndrome that typically starts after 20 weeks' gestation, with many risk factors and affect 5-10% of all pregnancy[1]. The exact pathophysiology of preeclampsia is poorly understood, but it mainly concentrates on dysfunction of the placenta and maternal vascular endothelium . Microscopic examination of the placenta may range from infarction, arthrosis, thrombus formation, or chronic inflammation[2]. The kidneys may play an important role, both in the adaptive physiological changes of healthy pregnancy and in the pathophysiological process of Preeclampsia [3] and some of changes found in renal function be more common at term pregnancy and Preeclampsia. Pathological lesion in PE is the pathogomonic renal lesion termed glomerular endotheliosis, which is characterized by widely spread endothelial dysfunction in the mother, that may compromise glomerular dynamical function and barrier function [4]. Assessment of renal function is, therefore, important and essential in the evaluation of the pregnant patient with different type of hypertension.

Kidneys slightly increase in size. Early in pregnancy, the glomerular filtration rate [GFR] and renal plasma flow increase. The GFR increases in about 25 percent by the second week after beginning of pregnancy and increased in about 50 percent by the start of the second trimester. Renal plasma flow also elevated. An unusual feature of the pregnancy-induced changes in renal excretion is the

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markedly increased amounts of various substances lost in the urine. There is loss of greater amounts of amino acids and water-soluble vitamins in pregnancy. Serum creatinine levels decrease during normal pregnancy from a mean of 0.7 to 0.5 mg/dL. Creatinine clearance in pregnancy approximates 30 percent higher than the 100 to 115 mL/min in women who are not pregnant. Glucosuria during pregnancy also may be encountered. The remarkable elevation in glomerular filtration, with impaired tubular resorptive ability for filtered glucose, resulted in normal pregnancy there is no proteinuria and albumin excretion is minimal and usually ranges from 5 to 30 mg/day.

Pathophysiology of renal system in preeclampsia

Renal perfusion and glomerular filtration are reduced in pregnancy complicated with preeclampsia as compared with normal pregnancy. Diminished glomerular filtration may be due to a reduced plasma volume which cause serum creatinine values to rise to 1 mg/mL [as in non-pregnant], but sometimes even higher. The level of plasma uric acid is markedly elevated in preeclampsia.The diagnosis of preeclampsia-eclampsiacan be established by presence of some degree of proteinuria. This proteinuria may develop late, and some women may develop eclampsia before the appearance of proteinurea [5].Cystatin C is a non-glycosylated, Low Molecular Weight [LMW] protein. Its reabsorption and catabolism take place by the proximal convoluted tubules. Serum cystatin C risesmarkedly in patients with renal impairemnet, and even small decline in GFR can significantly increase serum cystatin C levels [6,7]. Pre-eclampsia is usually characterised by a decrease in GFR and changed in renal handling of cysteine protease inhibitors [8,9].

Intrauterine growth restriction [IUGR]

It refers to a fetus whose growth has been disrupted by some pathologic mechanisms.Fetal growth can be divided into two phases: before 20 weeks ofgestation, growth is mainly due to cellular hyperplasia [increasing number of cells], and after 20 weeks, which is primarily due to hypertrophy [increasing cell size]. Any insult before 20 weeks of gestation may result in symmetric growth restriction, while after 20 weeks will causeasymmetric growth.Maternal risk factors includeanemia, hypertension, antiphospholipid antibody syndrome,chronic renal disease, severe malnutrition and systemic lupus erythematosus,. Long standing diabetes with vascular disease mayalso lead to IUGR. Placental factors like; placenta previa, velamentous and marginal cord insertion, and placental thrombosis with or without infarction leading to diminished placental blood flow can lead to IUGR [10,11].

Diagnosis of IUGR: Identification of growth-restricted fetus remains a challenge, however; early estimation of gestational age, identification of risk factors, careful measurement of uterine fundal level and good evaluation of maternal weight gain during pregnancy will identify many cases of IUGR in low-risk pregnancy.[5]

Patients and method:

This is cross sectional study from December 2018 till February 2020 in Najaf city, 150 women in reproductive age group included in the study after taking their agreement to participate in this study. All women in the third trimester of pregnancy[based on last menstrual period and dating ultrasound] diagnosed as preeclampsia based on measurement of blood pressure [more than 140/90 on 2 accossion] and presence of proteinuria on urine exam, ultrasound done for each woman to calculate gestational age and compare to early dating ultrasound, measure amniotic fluid index to identify presence of oligohydramnios, then Doppler for umbilical artery done to measure RI, PI, S/D ratio to detect changes in blood flow in order to diagnosed cases of IUGR.

We divide the patients included in the study into two groups, group A those without IUGR and group B those preeclamptic with IUGR, for both groups blood aspirated [5 ml], centerfuged, then serum used to measure blood urea, serum creatinine, and serum cystatin C.

Results

From 150 women with preeclampsia enrolled in the study, 120 of them had no clinical or radiological feature of preeclampsia belong to group A, while 30 women had features of IUGR.

The mean age for both groups showed no statistial differences, for group A the mean age 22±0.3 years and in group B 24±0.7 years. Body mass index[BMI] was measured and there is stastical differences [p value

<0.001] between the two groups in group A the mean BMI was 25±1.1Kg/m² while in group B 27±0.96Kg/m² as shown in table 1

Table1: Comparison between age and body mass index

parameters	Group A	Group B	P value
Age [years]	22±0.3	28±1.2	0.12
Body mass index kg/m²	25±1.1	27±0.96	<0.001

Renal function tests were made and blood urea, serum creatinine, and serum uric acid were higher in group B but statistically not significant between the two group while there is significant difference in level of serum cystatin C, in group A 1.08±0.97 while group B was 1.17±0.4

Table 2: differences in parameter of renal function between the two group

Parameters	Group A	Group B	P value
Blood urea mg/dl	38±2.3	42±1.2	0.04
Serum creatinine mg/dl	1.07±0.85	1.3±0.9	0.05
Serum uric acid mg/dl	6.8±0.95	7.2±0.8	0.04
Serum cystatin C mg/dl	1.08±0.97	1.23±0.45	<0.0005

Discussion

Preeclampsia and intra uterine growth restriction are significant causes of fetal and women morbidity and mortality[12,13].The pathophysiology of both Preeclampsia and IUGR is incompletely clarified, but a process of defective endovascular trophoblastic invasion and changes of spiral artery earlier in pregnancy due to an exaggerated inflammatory response and later on endothelial cell stimulation may plays a vital role in the pathogenesis of this disease [14]. Recent studies focus on screening tests by various maternal serum markers including: serum pregnancy-specific beta-1-glycoprotein [sp1], which is associated mainly with SGA.[15] The preform of eosinophil major basic protein [proMBP], which is associated essentially with PIH, IUGR and PE [16] and glucose regulated protein 78 [grp78] [17], cystatine C [18], matrix metalloproteinase-9 [19], which are also associated with PE.

The limitation of the widely use of serum markers has manifested by the introduction of specific antibody assays, which is a time consuming and more expensive procedure. As PE and IUGR without preeclampsia seem to have genetic basis, understanding their genetics might help to find most useful predictive markers for screening high risk women. Most of maternal serum markers have been identified and their relationship with poor pregnancy outcome has been evaluated [14].

An association was found between preeclampsia and maternal body mass index [BMI], but whether BMI has an effect on preeclampsia of all severities is controversial. Yet, an association between maternal height andpreeclampsia is undetermined. In a study done by Sara Sohlberg, Stephansson, Sven Cnattingius and Anna-Karin Wikström1 et al 2011, they found that not only maternal BMI, but also maternalheight is associated with preeclampsia of all severities. A short

maternal stature was associated with increased risks of the different types of preeclampsia, while overweight and obesity were closely associated with the milder forms of preeclampsia [20].One previous study has shown an association between maternal height and the risk of development of severe preeclampsia, but only in multiparous and not in primiparous women [21]. Infants born small for gestational age may have an increased risk of short final stature, although most of them catch up in height during childhood[22].

Preeclampsia manifests by proteinuria, hypertension, and resulting impaired renal function. Serum creatinine only is an unreliable measure of kidney function in pregnancy. Cystatin C measurment has been proposed as one measure of kidney function in population studies and in pregnancy. Cystatin C in general is an endogenous low molecular weight protein [13kDa] synthesized by nearly all nucleated cells and produced at a constant rate. It is freely filtered in the healthy kidneys and then completely metabolized by the proximal tubular cells. Elevated cystatin C concentrations is mainly associated with cardiovascular outcomes in population studies. Many previous studies have shown increased in cystatin C levels in preeclampsia. However, these studies have not quantified different preeclampsia risk with varying cystatin C levels, nor have they adjusted for any potential confounders. A case control study by Nora Franceschini, ChunfangQiu, David A. Barrow & Michelle AWilliams et al 2009 to evaluate the relation between plasma cystatin C and preeclampsia in pregnant women without pre-pregnancy comorbidities. This study shows an increased risk of developing preeclampsia aassociated with a high cystatin C plasma levels among women without preexisting renal disease, diabetes, and different type of hypertension. The increased risk of preeclampsia was independent of maternal age, pre-pregnancy BMI, and physical inactivity, and remains after adjustments many cofounders like gestational age, maternal smoking, and corticosteroid exposure, which have all been associated with eleveted cystatin C levels. This study had some limitation by its cross-sectional design, and therefore unable to document whether this increased concentrations of cystatin C levels precede the clinical diagnosis of preeclampsia. Finally they conclude that there is an association between high plasma cystatin C levels and preeclampsia, which may reflects the acute kidney dysfunction that associated with preeclampsia. A large prospective cohort studies are needed to clarify the temporal relationship between changes in cystatin C concentrations and the later development of preeclampsia, and to determine whether elevated cystatin C concentrations in early pregnancy can be used as predictive of poor maternal and fetal outcomes in women with preeclampsia.[23]

Another study that was done by Magna Manjareeka, Sitikantha Nanda et al; 2013 to measure and compare the level of serum uric acid, serum creatinine and serum urea in pre-eclamptic and normotensive women. In this study, though the serum uric acid level was higher in pre-eclamptics when compared to the normotensives, the elevated levels of uric acid did not correlate closely with the raised systolic blood pressure or the raised diastolic blood pressure. The differences in each of the mean SUA or SCr concentrations between the preeclamptic and the normotensives respectively were not statistically significant and that these parameters may bo of little value in the prediction of preeclampsia. Few studies[9,16] observe insignificant change in serum creatinine level in the two studied groups. On the other hand, an early study showed increased serum creatinine level but conclude to be of no predictive value in preeclampsia. Certain aspects of this study had few limitations like :Small sample size and a lack of urinary measurements of uric acid level, creatinine and urea. [24]

A study that was done by Cimona Lyn Saldanha*, ShabnumAra, Tabassum Parvez et al 2017 found thatserum creatinine has been routinely used for monitoring of renal functions. In this study, we have found a significant increase in serum creatinine in pre-eclamptic patients in comparison with healthy control group. Same results were found in other studies as well. Kidneys play a important role in the metabolism of uric acid and it is an important predictor to understand the outcome of pregnancy in terms of maternal and fetal morbidity and mortality. Similar results for serum creatinine and uric acid are noticed in all groups. There was significant difference in the mean values of serum uric acid between normal controls women and pre-eclamptic patients. There is conflicting evidence regarding the efficacy of this test but recent data is showing favour for the usefulness of cystatin C assay in the diagnostic armamentarium of renal decline in preeclampsia. Cost is a major obstacle , especially in

the low resource setting and among patients with lower socioeconomic status [25].

Study done in 3rd. trimester of pregnancy, using several tests for measuring renal function including GFR but the substance frequently used to assess renal function impairment is serum creatinine .Cystatin C measurement is more promising ,especially in cases of mild impairment of kidney function compared to the use of serum creatinine. This may be explained as Cystatin C does not pass the placental barrier and there is no significant correlation between maternal and neonatal levels,unlike creatinine level [26].

In a study done by Tam M. Le1, Long H. Nguyen Nam L. Phan Duong D. Le Huy V.Q. Nguye|Vinh Q. Truong Thanh N. Cao1 There were 205 women enrolled in this study. Serum uric acid at a cutoff value of 393 μmol/L can be used as a good predictor for fetal and neonatal complications [AUC 0.752], with 64.4% sensitivity and 79.5% specificity. High uric acid level [≥393 μmol/L] resulted in increased risk of complications like preterm labor [OR 6.367, 95% CI 3.009–13.084], low Apgar scores and intrauterine growth restriction [OR 7.188, 95% CI 3.592–14.382], and even early neonatal death [OR 7.818, 95% CI 1.614–37.867]. There was no significant relationship between uric acid level and fetal death [OR 1.803, 95% CI 0.355–9.168][27].

A German study which describes relationship of maternal serum uric acid level [SUA] and serum cystatin C with maternal and neonatal cardiac and metabolic risk markers and with birth weight and risk of small-for-gestational age [SGA] as well as large-for gestational age [LGA] shows a positive association of cystatin C with birth weight and obvious increased risk for LGA with maternal increased cystatin C values in a population with fairly normal kidney function. In this study, most of the mothers were of German nationality [85%] and were between 26 and 35 years of age [69%]. Maternal SUA changes was associated with maternal age, body mass index, alcohol consumption and history of hypertension as well as with many other maternal and neonate cardiovascular risk markers. Cystatin C was associated with parity. No clear association of SUA with SGA and LGA was observed. However, cystatin C was found to be negatively associated with SGA with an odds ratio [OR] of 0.35 [95% CI: 0.16±0.77]. [28]

Another case–control study was performed by SK Srinivas, AG Edlow, PM Neff, MD Samme, CM Andrela1 and MA Elovitz et al . Cases were patients with preeclampsia. Controls were patients presenting for delivery at term [≥ 37 weeks]. IUGR prevalence by case–control status, or severity of disease was evaluated using Pearson w2 tests. Multivariable logistic regression was used to control for most confounders. In patients with CHTN and superimposed preeclampsia, the pathogenesis of IUGR may be related by various factors including the use of different medications, aggressiveness of treatment of hypertension, or willingness to deliver at a clinically earlier stage of the disease. Their findings conclude that preeclampsia is associated with IUGR independent of CHTN. The lack of synergy between preeclampsia and CHTN in the development of IUGR [as these patients did not have the higher rates of IUGR] suggests that the pathways leading to the development of IUGR may be disparate and not all IUGR develops from the same pathways. IUGR is more frequent in women with lower BMI [29].

Another study has declared that women of advanced maternal age [AMA] develop more preeclampsia [9.4%] than younger women[6.4%]. They had more prior terminations of pregnancy [<0.001], were more likely to have a body mass index [BMI]>25 kg/m2 [<0.001], more with in vitro fertilization [IVF][<0.001] and other fertility treatments [<0.001] and a higher incidence of maternal diseases like diabetes [<0.001] and chronic hypertension [<0.001]. Multivariate logistic regression indicated that women of AMA had higher rates of: preterm labor before 37 weeks 19.2% [OR 1.39 CI 1.24 to 1.56] and before 34 weeks8.7% [OR 1.68 CI 1.43 to 2.00] low Apgar scores at 5 min. 7.1%, Small-for-Gestational Age[SGA] 26.5% [OR 1.42 CI 1.28 to 1.57], birth Asphyxia 12.1% [OR 1.54 CI 1.34 to 1.77], Caesarean delivery 50% [OR 2.02 CI1.84 to 2.20] and the need for admission to a Neonatal Intensive Care Unit [NICU] 31.6%[30].

Other study in India demonstrates the association of serum UA, creatinine and cystatin C with maternal and fetal outcomes. The adjusted odds ratio [OR] was 3.73 [95% CI: 1.18-11.75; P=0.024] for UA; 15.79 [95% CI: 3.04-81.94; P=0.001] for serum creatinine and 2.03 [95% CI: 0.70-5.87; P=0.192] for cystatin C in different hypertensive disorders of pregnancy. All the three renal

parameters were not significantly associated with birth weight, gestational age at delivery and mode of delivery after adjusting for the other confounding factors. They conclude that serum creatinine and uric acid are independent risk factors for hypertensive disorders of pregnancy. High serum uric acid is associated with low birth weight and more delivery by caesarian section whereas high serum creatinine with preterm delivery only before adjustment for confounding factors and not after adjustment. But Serum cystatin C was not significantly associated with the maternal and fetal outcomes[31].

Another study was conducted at 2006 to examine whether preeclampsia, gestational hypertension, and unexplained IUGR [the latter often assumed to be due to placental insufficiency], could be linked together by comparing their different risk factors and perinatal outcome in a large collected data base from 4 distinct ethnic populations. They found similarities between preeclampsia and gestational hypertension, but the results suggest that unexplained IUGR [e.g, those not associated with smoking, under nutrition, secondary to hypertensive conditions, or congenital malformations] is obviously different entity from preeclampsia. Furthermore, we have confirmed that preeclampsia is a major risk factor for severe perinatal morbidity and mortality, but that gestational hypertension without proteinuria also independently may increases perinatal risk. [32].

A study that was done in Egypt has recruited twenty women normal primigravidae with singleton pregnancy and another 40 with severe preeclampsia recruited from preeclamptic unit of El-Shatby Maternity University Hospital, in Egypt in the third trimester of gestation without any medical disease. They are selected and full history taken, complete clinical examination, laboratory investigations including serum uric acid, serum creatinine and serum cystatin C, obstetric abdominal ultrasound and Doppler. The difference in the mean serum uric acid level [3.55±0.58 versus 6.76±1.06mg/dl] was significantly higher in the preeclamptic women [p=0.001], the specificity was 100% and sensitivity was 97.5%, the difference in the mean serum level of creatinine [1.55±0.89 versus 0.66±0.12] was significantly higher in preeclamptic group [p=0.001], the specificity of the test was 100% and the sensitivity was 60%. Serum cystatin C level has a mean value [0.98±0.29 versus 0.70±0.06] which was significantly higher in preeclampsia [p=0.001] with a specificity 100% and sensitivity 72.5%. They found a positive correlation between serum concentration of cystatin, and PI of umbilical artery Doppler [p=0.05]. Also, a significant correlation was found between serum level of cystatin and both PI and RI of middle cerebral artery Doppler [p=0.05]; this may indicates the relation of serum cystatin C level with the severity of preeclampsia , resulting in increasing poor reproductive outcomes [33].

Another study was done to compare between creatinine and cystatin C value in preeclampsia regarding severity and neonatal outcome. In this study, creatinine, cystatin C, and neonatal outcomes were assessed in 17 normotensive pregnant , 17 cases of mild preeclampsia and 17 samples with severe preeclampsia.Analysis of data with statistical tests of ANOVA and t test differences between 2 proportions. The mean levels of creatinine in the normotensive group, mild preeclampsia, severe preeclampsia are 0.56 mg/dL, 0.67 mg/dL, and 0.75 mg/dL respectively, p=0.138; While on cystatin C are 0.82 mg/L,1.03 mg/L and 1.32 mg/L, p=0.000. The adverse neonatal outcome wasn't recognised in the normotensive group. In mild preeclampsia obtained 1 preterm birth and 1 intrauterine fetal death[IUFD], whereas in severe preeclampsia obtained 3 babies born preterm, one IUFD, and 1 intrauterine growth restriction [IUGR]. It was concluded that levels of cystatin C was increased significantly parellel with increased severity of preeclampsia, whereas creatinine was not increased significantly with severity of the disease. Cystatin C is better than creatinine as a marker of renal dysfunction in preeclampsia patients. There was significat increase in adverse neonatal outcomes in the group of preeclampsia. [34].

Conclusion

We conclude that Cystatin C may be promising marker for renal function in patients with preeclampsia complicated by IUGR.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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5-Years Prevalence Study of HBV and HCV infections in Babylon Province: Forensic Helping Data

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Abstract

HBV and HCV infections are serious global health care problem because they may end with chronic hepatitis, cirrhosis, and hepatocellular carcinoma. HBV infection results in approximately 2 billion human infections while HCV approximately 160 million individuals. Raw database were collected from General health department at Babylon Health directorate for 715505 investigated person during a period from Jan. 2015 to Dec. 2019. Blood samples was drawn in Gel tube from suspects and centrifuged to separate the serum. Serum submitted for investigation of HbsAg and HCV Ab by both rapid immunochromatographic test and ELISA test. Actually the database taken from Babylon Health directorate and the results analysis depends upon dividing of data records from four health sectors, blood bank record lab, health care checking lab, pre-surgery checking, Marriage lab, thalassemia lab, Hemodialysis lab Prisoner and foreigners lab. The results revealed that , total positive cases were 4284 and 2260 for "HBV and HCV" respectively. The highest no. of cases for both "HBV and HCV" were recorded in 2018 (976 and 467) and (961 and 557) for "HBV and HCV" respectively. The incidence rate of "HBV and HCV" were varies worldwide. The results revealed that the occurrence rate (Mean ± SD) of "HBV" was (0.627±0.134) while for HCV was (0.327±0.084). The results revealed that, there is no defined month or semester for high rate infections. The high prevalence percentage were showed for prisoner-foreigner category (48.53 and 45.95 for HBV and HCV respectively) followed by surgery (22.66 and 19.03 for HBV and HCV respectively), blood bank (14.03 and 13.95 for HBV and HCV respectively) and in married (7.79 and 3.92 for HBV and HCV respectively. The current study conclude the high prevalence percentage of HBV (duplicate) than HCV with no seasonality of occurrence and the Prisoners and foreigners, Surgery, blood donor and hemodialysis were mostly involved with HBV and HCV infection.

Keywords: *HBV, HCV, Healthcare worker, HBV vaccine*

Introduction

Hepatitis is" the term used to describe inflammation of the liver. It's usually the result of viral or damage" . The hepatitis B virus (HBV) is a major global public health problem, affecting more than 2 billion people worldwide [1]. HBV and HCV infection results in approximately 2 billion and 160 million human infections respectively [2]. HCV super infection in patients with chronic HBV infection was the most common clinical features of coinfection in Asia–Pacific countries[3]. The chronically infected persons are at high risk of death from cirrhosis of the liver and liver cancer, diseases that kill about 1 million persons each year'[4]. It is assessed that more than 240 million people are recurrently infected with "HBV" and, therefore, are at risk for the development of cirrhosis, hepatic decompensating, and hepatocellular carcinoma (HCC)[5]. HCV disease advances to chronicity in 70% of cases, a condition that may prompt liver cirrhosis and hepatocellular carcinoma [6,7]. As per a WHO report, 130-150 million individuals are incessantly tainted with HCV. HCV the study of disease transmission shows impressive territorial differences[8]. There is no antibody to restrict the dispersion of HCV

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contamination while all medical services laborers ought to be considered for HBV immunization and ought to carefully apply the general prophylactic measures to forestall introduction to HBV and HCV.There is a need to calculate the liability of chronic "HCV" infection at the state level [5,9]. The study aims to study the prevalence rate of HBV and HCV infections for last 5 years (2015-2019) in Babylon Province.

Materials and Methods

Samples:

Raw database were collected from General health department at Babylon Health directorate for 715505 investigated person during a period from Jan. 2015 to Dec. 2019.

Specimens:

Blood samples was drawn in Gel tube from suspects and centrifuged to separate the serum.Serum submitted for investigation of HbsAg and HCV Ab by both rapid immunochromatographic test and ELISA test.

Results

The present study was showed to see the distribution & incidence rate of "HBV" and "HCV" infection among screened patients in Babylon province for past 5 years (January 2015 to December 2019). Actually the database taken from Babylon Health directorate and the results analysis depends upon dividing of data records from four health sectors, blood bank record lab, health care checking lab, pre-surgery checking, Marriage lab, thalassemia lab, Hemodialysis lab Prisoner and foreigners lab. Total of (715505) cases were investigated for HBV and HCV using rapid test and ELISA assays to confirm infections (Figure 1). The results revealed that , total positive cases were 4284 and 2260 for HBV and HCV respectively. The highest no. of cases for both HBV and HCV were recorded in 2018 (976 and 467) and (961 and 557) for HBV and HCV respectively. The incidence rate of HBV and HCV were varies worldwide. Our results revealed that the incidence rate (Mean ± SD) of HBV was (0.627±0.134) while for HCV was (0.327±0.084) (Table 1). Concern the categories from which infections were recorded, the results revealed that Prisoners and foreigners (48.53%, 45.95%), Surgery (22.66%, 19.03%), blood donor (14.03%, 13.95%) and hemodialysis (1.27%, 11.63%) for HBV and HCV respectively (Table 2). Concern the seasonality or weather relationship with HBV and HCV infections the results showed that there is no association between sessions and infections (figure 2). HVB vaccination were displayed in table (3).

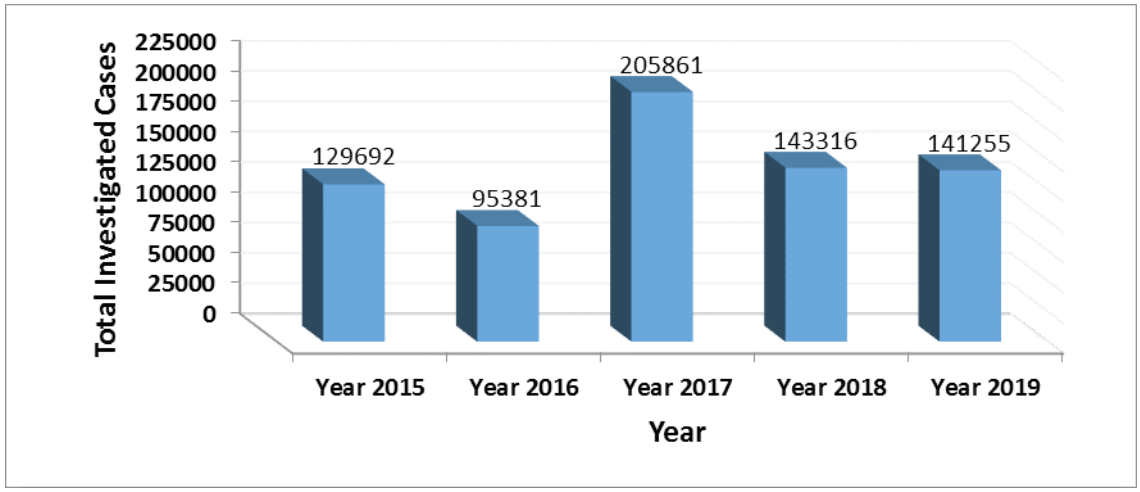


Figure (1): Total Cases of HBV and HCV infections in Babylon Province (2015-2019)

Table (1): Distribution of incidence % of HBV and HCV infections in Babylon Province (2015-2019).

Year	Tested	HBV cases	HBV %	HCV cases	HCV %
2015	129692	790	0.609	534	0.412
2016	95381	721	0.756	288	0.302
2017	205861	836	0.406	414	0.201
2018	143316	976	0.681	467	0.326
2019	141255	961	0.680	557	0.394
Total	715505	4284	Mean±SD 0.627±0.134	2260	Mean±SD 0.327±0.084

Table (2): Distribution of HBV and HCV infections cases among categories in Babylon province (2015-2019).

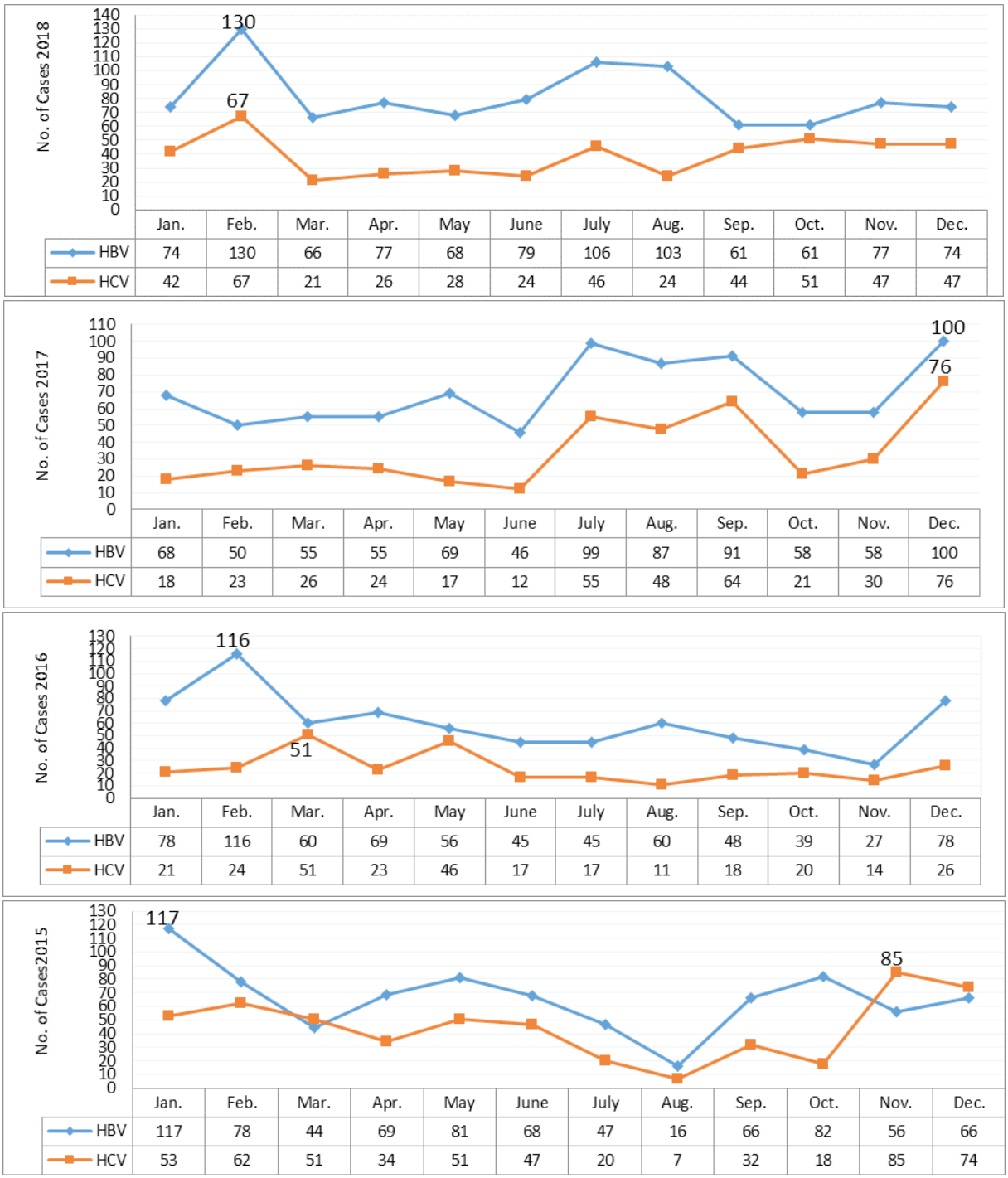
Categories	Total (2015-2019)			
	HBV	%	HCV	%
Blood Bank	596	14.03	313	13.95
Healthcare Worker	35	0.82	26	1.16
Contacting	178	4.19	43	1.92
Surgery	963	22.66	427	19.03
Married	331	7.79	88	3.92
Thalassemia	30	0.71	55	2.45
Hemodialysis	54	1.27	261	11.63
Prisoner and Foreigner	2062	48.53	1031	45.95
Total	4249	100	2244	100

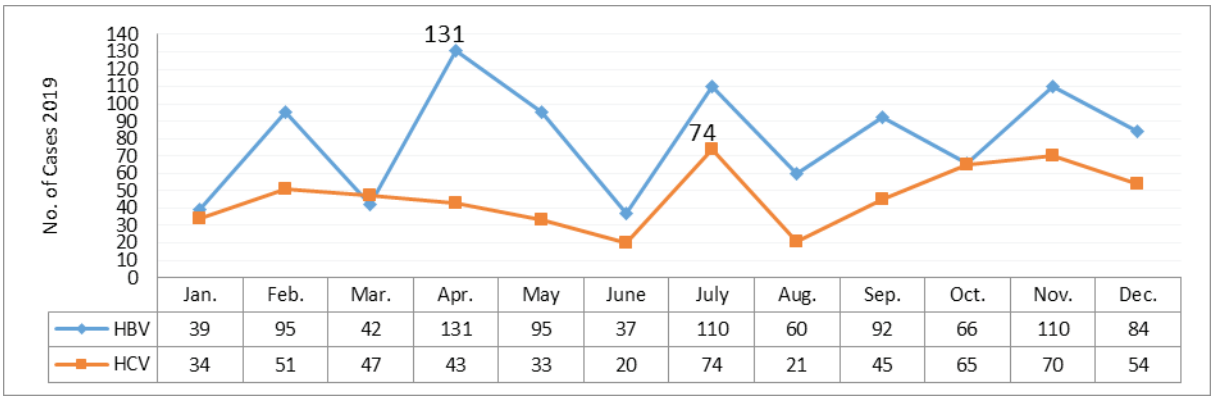
Table (3): HBV vaccine distribution in Babylon province (2015-2019)

Year	HBV vaccine				Total
	first Dose	second Dose	Third Dose	Child Dose	
2015	11711	9184	13805	36748	71448

Cont... **Table (3): HBV vaccine distribution in Babylon province (2015-2019)**

2016	14436	6722	19642	38466	79266
2017	12863	6519	9346	39520	68248
2018	11672	6290	8028	37529	63519
2019	12996	5598	4827	38778	62199





	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
HBV	39	95	42	131	95	37	110	60	92	66	110	84
HCV	34	51	47	43	33	20	74	21	45	65	70	54

Figure (2): Distribution of HBV and HCV infections in Babylon Province according to month at 2015-2019.

Discussion

It seem that our results were in accordance with some of the Iraqi studies like [2] who found that out of 4851 patients, (1.79%) were positive for hepatitis B and (0.14%) were positive for hepatitis C. Salehi-Vaziri et al., (2016)[1] stated that, in several areas of the world, the occurrence of "HBV" infection diverse from 0.1 to 20%. Our results in accordance with [10] who found that the incidence rate of HBV was double of HCV. Predominance of HCV disease In 2015, 71 million people were living with ceaseless HCV contamination. Contrasted and HBV, the commonness of HCV contamination is lower, however more heterogeneously conveyed, with contrasts across and inside WHO districts and nations. Spread through breaks in disease control practices or infusion drug use may clarify this example. In general, in 2015, the worldwide predominance of HCV contamination was 1.0%. The Eastern Mediterranean Area had the most noteworthy pervasiveness (2.3%) trailed by the European District (1.5%).In 2015, viral hepatitis led to 1.34 million deaths. The results revealed that the HBV frequency is double or more than HCV. Many studies were in accordance with our results whose show the high percentage of HBV versus HCV among blood bank donors[11-15].

The results of (figure 2) revealed that, there is no defined month or semester for high rate infections. This is may be due to fact that , both HBV and HCV not respiratory virus (respiratory viruses are highly spread viruses especially during winter semester), not food-borne diseases and their transmission may be limited percutaneous and per mucosal or blood transfusion, sexual transmission, and mother-to-child transmission [16-21]. (Salehi-Vaziri et al., 2016) [1] found that, "HBV" is really spreadable and comparatively easy to convey from infected persons to others through some ways like birth, unprotected sex, blood transfusion, and also sharing needles. Jansen et al., (2015) [10] found that, the incidence of HBV showed no trend over time, did not differ between study participants living in rural or urban area. Concerning the prevalence or registry of HBV and HCV positive persons among categorized patients were displayed in table (2).

The high prevalence percentage were showed for prisoner-foreigner category (48.53 and 45.95 for HBV and HCV respectively) followed by surgery (22.66 and 19.03 for HBV and HCV respectively), blood bank (14.03 and 13.95 for HBV and HCV respectively) and in married (7.79 and 3.92 for HBV and HCV respectively). Though most of HBV contaminations among detained people are obtained in the network, transmission has been distinguished in jail, and rate rates have extended from 0.8 to 3.8% every year [18,19]. Our results was in agreement with many studies who is establish high ratio of HBV and HCV among prisoners like those documented in Iran [24,25], Sweden [26] and France [27]. Elective surgeries were recorded as good monitoring of HBV and HCV surveillance due to fact that the person need to be checked for HBV, HCV and HIV prior to surgery especially in delivery [28], eye surgery [29,30], oral and maxillofacial [31] and dental surgeries [32-34].

Conclusion

The current study conclude the high prevalence percentage of HBV (duplicate) than HCV with no seasonality of occurrence and the Prisoners and

foreigners, Surgery, blood donor and hemodialysis were mostly involved with HBV and HCV infection.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Cytotoxic Effects of Al-Yohedia Municipal Waste Water

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Abstract

Household waste water represent an hazardous reservoir for wide range of medically important pathogens than can push an alarm on public health threatening diseases. It contain toxic materials, hazardous heavy metals and different pathogens. The study includes investigation of microbial, environmental, pathological and molecular effects of waste water. The results of dissolved heavy metals concentration revealed high Ni concentration (Mean±SD of four sites were 119.675±0.778 mg/L), high Mn concentration (Mean±SD of four sites were 108.2±1.095 mg/L), high Fe concentration (Mean±SD of four sites were 119.675±0.740.475±1.228 mg/L), high Cd concentration (Mean±SD of four sites were 13.75±1mg/L), high Cu concentration (Mean±SD of four sites were 5±0.755mg/L) with no significant differences in concentration among four sites for Ni and Cu while difference noted among Mn, Fe and Cd. Results of microbial study revealed that the Mean±SD of CFU/ml were $1.97x10^{10}$±0.543 and the microbes were *E. coli, K. pneumoniae, P. aeruginosa, V. cholerae, V. parahaemolyticus, S. aureus, E. faecalis, C. albicans, C. krusei and C. glabrata* with MAR index ranged from (0-1). DNA damage results using comet assay for revealed that high level of damage to DNA of rat dosed with waste water compared with those dosed with fresh water in both tail length (11.35±2.1, 2.7±0.9) and tail moment (1.193±0.11, 0.2±0.1) respectively. Immunohistochemistry (IHC) assay was used to detect of 8-OHDG among liver tissues of rat dosed with waste water (study) and fresh water (control) and the results revealed strong signal (high expression) in all liver tissues of rat dosed with waste water (study) compared with those dosed with fresh water (control) whose gave negative results. Histopathological results of eat dosed with waste water compared with those dosed with fresh water (control) were observed after 60 days of dosing. the results displayed that the control was normal where mild chronic hepatitis showed in samples of site 1, 2, 3 while site4 had moderate chronic hepatitis and Fasciola hepatica. The current study conclude that, Al-Yohedia Municipal wastewater is very dangerous and have different clinically important pathogens along with different cytotoxic materials.

Keywords*: wastewater, Vibrio cholerae, Candida spp., Hepatitis, Fasciola hepatica*

Introduction

Municipal wastewater is a collection of both domestic and industrial waste water. Al-Yohedia is fresh water stream pass through Hilla city but unfortunately it serve as municipal waste water reservoir. Sewage water, healthcare clinics, butchers waste, diesel engine waste and farm animal waste were delivered directly to this stream rendering it dangerous spot of pollution [1,2]. The compartments of wastewater includes biological and non-biological parts. The biological part includes bacteria, fungus, viruses and parasites while non-biological includes chemicals (organic and inorganic compounds like hydrocarbons and heavy metals) which pose dangerous impact on aquatic environments and human health [3-8]. Enterococci and enterobacteriaceae members like Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp., Shigella spp., Enterobacter spp. were common among waste water while Pseudomonas spp., and Vibrio spp. push an alert of clinically important bacteria that cause life threaten diseases [9-13]. The presence of medically important yeast like Candida spp. among waste water were also stated. Human and animal waste water can be loaded with viruses like hepatitis and parasite like Fasciola

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hepatica and this proposing biohazard when reach to human being [6,14].

Cytotoxicity of waste water can be reflected and oxidative damage to biomolecules like to lipids of cellular membranes, proteins, and DNA which may lead to carcinogenesis. 8-hydroxy-2' -deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for oxidative stress and carcinogenesis. 8-OHdG used to estimate the DNA damage in humans after exposure to cancer-causing agents, such as tobacco smoke, asbestos fibers, heavy metals, and polycyclic aromatic hydrocarbons. In recent years, 8-OHdG has been used widely in many studies not only as a biomarker for the measurement of endogenous oxidative DNA damage but also as a risk factor for many diseases including cancer. Oxidative DNA damage is common in various forms of chronic liver disease suggesting a possible link between chronic inflammation and hepatocarcinogenesis. Heavy metals and some metalloids are known to be carcinogenic to humans. The oxidative concept in metal carcinogens were involvement of redox mechanisms production of hydroxyl radicals can attack DNA causing hydroxylation and oxidative DNA damage these products of DNA damage (including 8-OHdG) induce mutations leading to neoplastic transformations [15-18]. The current study aimed to investigate the cytotoxic effects of Al-Yohedia municipal waste water by IHC of 8-OHdG and histopathology of Rat lab animals.

Materials and Methods

Sampling:

Four waste water samples were collected from Al-Yohedia Stream. The study area included 4 different sites from Al-Yohedia stream in Hilla city (site 1 Al-karama quarter (N 32 0 28.944 E044 0 25.057), Site 2 Al-Jameia quarter (N32 0 28.383 E044 025.067), Site 3 Al-Iskan quarter (N32 0 27.545 E044 025.058), Site 4 Al-Askari quarter (N32 0 25.827 E044 024.394).

Waste water Culture:

All samples mixed well and 1 ml of waste water sample transferred to 9 ml of normal saline (diluent) (Stock 10-1) and from it prepare five serial dilutions (10-2, 10-3, 10-4 and 10-5) were prepared. 10 µl from the last dilution (fifth dilution) were spreaded on nutrient agar plate by disposable spreader and the plates were incubated upside down in 37°C for 24 hr. Triplicates were performed to each sample to increase the accuracy and only plate that has (30-300) colonies will be chosen for count. The exact number of colonies per original sample will calculated according to the following equation: CFU/ml = No. of colonies x Inverse of the dilution x 100[19].

Microbial Isolations and Identification:

Immediately after sample collection and along with total plate count the samples inoculated UTI chromogenic agar, Pseudomonas chromogenic agar, m-EI chromogenic agar Vibrio chromogenic agar and Candida chromogenic agar to investigate the presence of common pathogens among wastewater samples. The sample mixed well and from each sample a triplicate of 100 ml will filtrated on (0.45 µm) cellulose nitrate membranes (Sartorius/Germany) using Sartorius filtration system and then the filter content resuspended in sterile normal saline then took 5 µl and cultured on the above mentioned agar, incubated at 37°C for 48 hr [20-22].

Dosing:

The experiment includes (20) Rats (Albino Rats) of males and females have (same of the age ,weight and conditions) treated from Al-Yohedia stream by drinking at need for two months whereas control groups were treated with (RO) tap water and divided into five groups: first group dosed with site 1 sewage samples , second group dosed with site 2 sewage samples , third group dosed with site 3 sewage samples , forth group dosed with site 4 sewage samples and fifth group dosed with tap water was control and after complete the period of dosing rats anatomized by anesthesing rats with chloroform and then with drawing 5ml blood from heart area by syringe for detection molecular biomarker by comet assay and take tissue (liver) for histopathology and immunohistochemistry (IHC) tests [23].

Comet Assay:

This assay has been done by Comet assay Kit, Malgene-Italy (Single cell gel Cat. No. 0905-050- K) according to (Steinert, 1996) [24].

Immunohistochemistry (IHC):

Immunohistochemistry assay include using of specific monoonoclonal /polyclonal antibody and detection kit directed toward this antibody. In this study we used Anti-8 Hydroxyguanosine antibody [N45.1] (8-OHDG) (cat. # ab48508, Abcam /England). The immunohistochemistry detection kit is a reliable and convenient tool to identify specified gene expression on tissues. A biotinylated, cross – adsorbed and affinity purified primary anti-mouse and rabbit specific HRP/ DAB is used to detect primary antibody –antigen complexes adhered to a glass microscope slide, following reaction with an enhanced detection reagent. This kit is compatible with mouse and rabbit specific HRP/DAB secondary antibodies and can be used for paraffin – embedded tissue sections (Cat. Number: Ab80436, Abcam, England).

Histopathological Study:

Specimen with dimension 1x1x1 cm was taken from inside organ together with liver. The tissue have been fastened in (10%) buffer formaldehyde re at this time later removing wards 72 hr of the fixation , specimen was washed with tap water after which processing was robotically carried out with a collection of upgrading alcoholic focus from (70%) to genuine (100%) for two hr in every focus to eliminate water from the tissue , then clearance was executed by xylol , then the specimen was infiltrated with Simi-liquid paraffin wax at(58ºC) on two phases then blocks of specimen was made with paraffin wax and sectioned by rotary microtome at 6 µm for tissue , tissue was stained with haematoxylin and eosin and the Histopathological vary was noticed beneath mild microscope [25].

Quantitation of Dissolved Heavy Metals:

Concentrated hydrochloric acid (5 ml) was added to 1 L of the waste water filtrate and concentrated by oven (800C) until evaporate to 100 ml and then measured by Flame Atomic absorption - spectrophotometer model (7000) Aa type (Shimadzu/Japan) was used to determine the concentrations (mg/L) of the elements [26].

Results and Discussion:

The results of dissolved heavy metals concentration revealed high Ni concentration (Mean±SD of four sites were 119.675±0.778 mg/L), high Mn concentration (Mean±SD of four sites were 108.2±1.095 mg/L), high Fe concentration (Mean±SD of four sites were 119.675±0.740.475±1.228 mg/L), high Cd concentration (Mean±SD of four sites were 13.75±1mg/L), high Cu concentration (Mean±SD of four sites were 5±0.755mg/L) with no significant differences in concentration among four sites for Ni and Cu while difference noted among Mn, Fe and Cd (table 1). All values were higher that standard permissible limits of WHO [27-30].

Table (1): Concentrations of dissolved heavy metals of municipal wastewater.

Dissolved mg /L	Site1	Site2	Site3	Site4	Mean±SD
Ni(Nickel)	120.7 ±0.1 a	119±1 a	119±0.01 a	120±2 a	119.675±0.778
Mn(Manganese)	116.6±2.08 d	100.1±0.1 a	110.2±0.2 c	106±2 b	108.2±1.095
Fe(Iron)	30.6 ±0.4 a	55±3 c	32 ±0.01 a	44.3±1.5 b	40.475±1.228
Cd(Cadmium)	12 ±1 a	12±0.01 a	16±2 b	15±1 b	13.75±1
Cu(cupper)	6±0.01 a	5 ± 2 a	4± 1 a	5±0.01 a	5±0.755
Pb(Lead)	2.7±0.6 a	3 ± 2 a	3±2 a	0.8 ± 0.01 a	2.375±1.152

$p<0.05$

The main sources of lead contamination are industrial threw battery in sewage, run off from contaminated land areas, atmospheric fall out and sewage effluents. The sources of heavy metal also the reason in the high concentration of nickle in Al-Yohedia stream may be attribute to the high numbers of small engine oil exchange and electricity station near to stream, disposal of tires of cars in the stream, generator located at the side of stream .The exchanged oil and waste of generators delivered directly to stream in addition to the nickel not use and not consume by microorganism [31]. Cd was classifies as a first category of carcinogenic factors in human cancers because cadmium ions have a configuration of electrons analogous. Pb responsible for oxidative stress and genotoxicity [32-35].

Results of microbial study revealed that the Mean±SD of CFU/ml were $1.97x10^{10}$±0.543 and the microbes were *E. coli, K. pneumoniae, P. aeruginosa, V. cholerae, V. parahaemolyticus, S. aureus, E. faecalis, C. albicans, C. krusei and C. glabrata* with MAR index ranged from (0-1) (table 2). The results agreed with Yang et al., (2009) [36] who found that the clinical isolate of *E. coli, K. pneumoniae, S. aureus and P. aeruginosa* were common among sewage water. Concern presence of Candida spp. it supported by Filipkowska et al., (2008)[37], Biedunkiewicz and Ozimek, (2009)[38] and Korzeniewska et al., (2011)[39] whom found the different species of Candida in municipal wastewater. It is important to state that recovering mentioned microbes from high concentration of heavy metals waste water reveal their ability to live with such toxic concentrations may be due to their efflux pump or plasmid coding genes. The heavy metals and antibiotic resistance were closely related and may be carried on same plasmid [40-44].

Table (2): Microbes with resistance phenotypes and MAR index.

Pathogen	Resistance Phenotype	MAR index
E. coli	AmoxiclavR/CefotaximeR	0.22
K. pneumoniae	AmoxiclavR/CeftazidimeR	0.22
P. aeruginosa	AmoxiclavR/CefotaximeR/CeftazidimeR/AmikacinR	0.44
V. cholerae	NON	0
S. aureus	ClarithromycinR	0.1
E. faecalis	NON	0
C. albicans	KetoconazoleR/MiconazoleR	0.33
C. krusei	KetoconazoleR/MiconazoleR/EconazoleR/FluconazoleR /ItraconazoleR/ClotrimazoleR	1
C. glabrata	KetoconazoleR/MiconazoleR/EconazoleR/FluconazoleR /ItraconazoleR	0.83

DNA damage results using comet assay for revealed that high level of damage to DNA of rat dosed with waste water compared with those dosed with fresh water in both tail length (11.35±2.1, 2.7±0.9) and tail moment (1.193±0.11, 0.2±0.1) respectively (table 3).

DNA damage based on the comparison with the control according to the pictures that were taken DNA damage can be classified into three classes (class 1, no damage; class 2, low damage; class 3, medium damage and class 4, high damage) as shown in figures (1). The observed DNA damage may attributed to heavy metals which have a role in high percentage of DNA damage and ability to produce bulky DNA adducts. The heavy metals can produce chemical or physical modification to DNA measured by means of DNA fragmentation [45].

Table (3): Comet Assay Parameter among rat dosed with waste and fresh water.

Comet Parameter	Mean±SD	
	Rat dosed with waste water	Rat dosed with fresh water
Tail Length (µm)	11.35±2.1	2.7±0.9
Tail Moment (%)	1.193±0.11	0.2±0.1

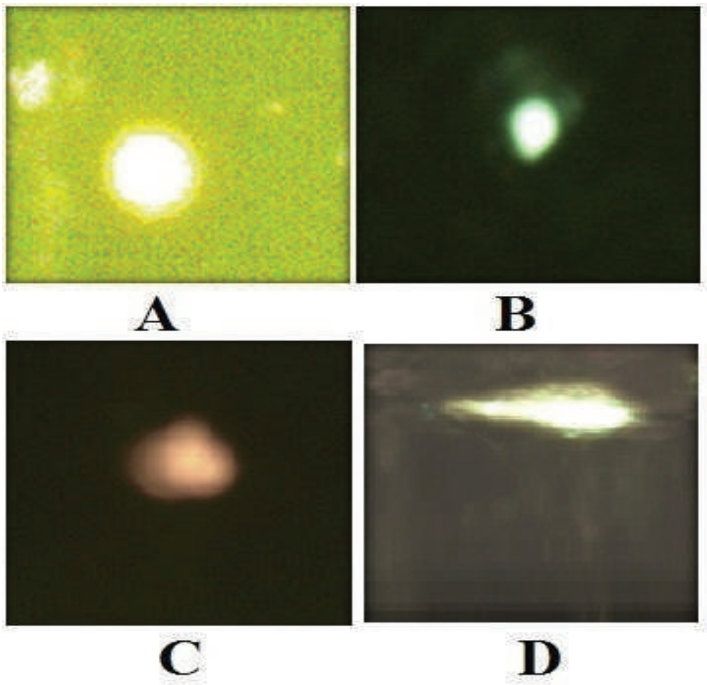


Figure (1): Classes of DNA damage in rat sample (blood) oral administrated from wastewater of four sites according to comet assay: Class (A) Control, Class (B) low damage, Class (C) Medium damage, Class (D) High damage.

Immunohistochemistry (IHC) assay was used to detect of 8-OHDG among liver tissues of rat dosed with waste water (study) and fresh water (control) and the results revealed strong signal (high expression) in all liver tissues of rat dosed with waste water (study) compared with those dosed with fresh water (control) whose gave negative results (figure 3). High expression of 8-OHDG revealed high oxidative stress caused by pollutants especially heavy metals like Cd and Pb. liver tissues used to understanding biotransformation and toxicity in municipal wastewater, this was agreement with Herman and Geraldine, (2009)[46] who considered that the liver is one of the target organs affected by lead toxicity owing its storage in the liver after lead exposure. Also it is organ of detoxification [47,48].

Figure (3): Infiltrative rats liver tissues showing the results of immunohistochemical staining of 8-OHDG protein over expression using biotinylated -labeled anti-8-OHDG protein antibody, stained by DAB-chromogen (Brown) and counter stained by Mayer's haematoxylin (Blue): A. Positive 8OHDG –IHC reaction with high score and strong signal intensity (10X) B. Liver tissues with negative 8-OHDG-IHC reactions (10X)

Histopathological results of eat dosed with waste water compared with those dosed with fresh water (control) were observed after 60 days of dosing . the results displayed that the control was normal where mild chronic hepatitis showed in samples of site 1, 2, 3 while site4 had moderate chronic hepatitis and *Fasciola hepatica* (figure 4 and 5). Many studies of mammalian tissues noted that the histopathological alterations induced by different heavy metals in the tissues of liver organ. Human and animal wastewaters play role important as sources of parasitic pathogens infecting human [49,50].

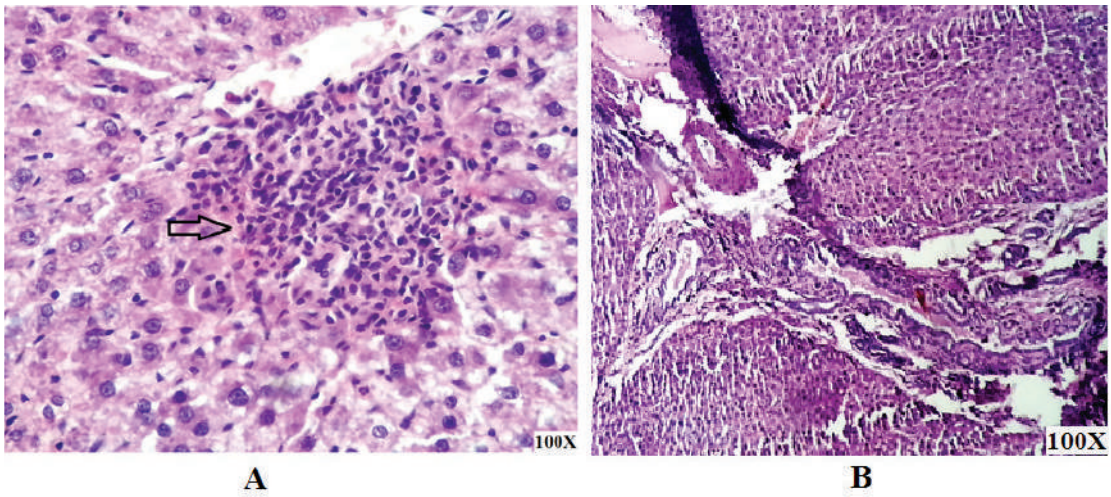
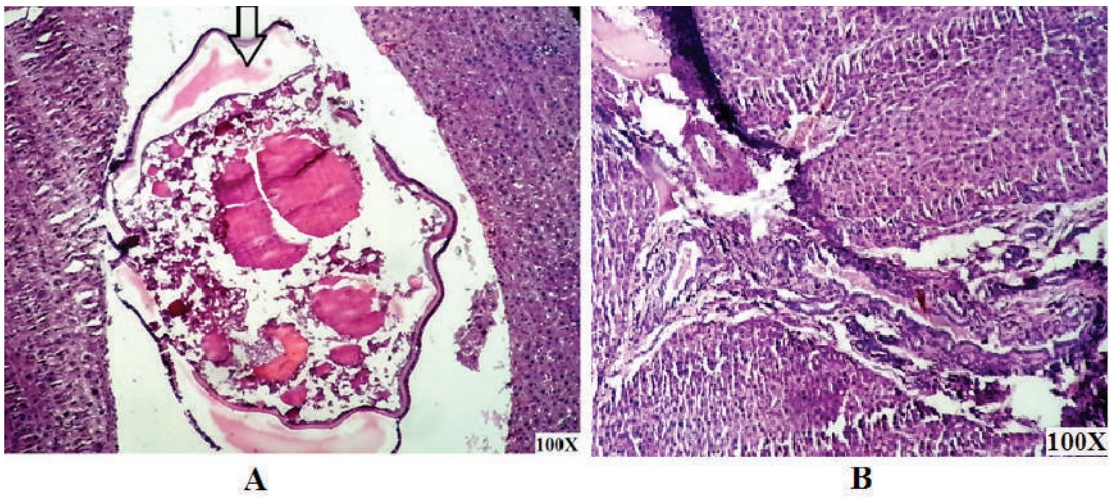


Figure (4): Show the histopathology of rat Liver sections after 60 days of dosage with wastewater: A:Show liver tissue with moderate chronic hepatitis, intralobular and periportal chronic inflammatory cell infiltrate (arrows) (100X) (H&E). B:Show normal Liver (Control) (100X) (H&E).



Figure(5): Show the histopathology of rat liver sections after 60 days of dosage with wastewater: A: Show the liver tissue with Fasciola spp. (Fasciolosis) (arrow) (100X) (H&E). B: Show normal Liver (Control) (100X) (H&E).

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Antibacterial Effect of Virgin and Refined Coconut Oils on Pathogenic Bacteria: A Review

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Abstract

Virgin coconut oil (VCO) is the most oldest edible oil, extracted from fresh and mature kernel using wet or dry methods, therefor the resulting oil is a pure, clear, and colorless with a scent of fresh coconut, these characteristics make VCO has the preferableness in medical and health applications, over refining coconut oil (RCO) that is extract from kernel of coconut after drying by methods makes the oil refining, bleaching and deodorizing. In spite of that, the chemical compositions particularly the fatty acids in both VCO and RCO are the same with no significant difference. MCFAs which are classified as MCTs, are the basic components in both VCO and RCO, these MCFAs particularly lauric acid (LA) following by capric and caprylic acids playing a great role in antimicrobial activity of coconut oil, therefor the extraction of these fatty acids are show more antibacterial activity on pathogenic bacteria more than lipolyzed VCO or VCO itself that may not show inhibition effect. Gram positive bacteria is more sensitive than gram negative one towards LA and other fatty acids, therefor these fatty acids at high concentrations showed inhibition effect on gram negative bacteria, while at low concentration showed resistance. This study attempts to review the chemical components in both VCO and RCO based on their extraction methods, also the antibacterial activity and the mechanism of action, also suggests that RCO has the same effect as antibacterial as VCO based on their chemical compositions.

Keywords: *Virgin coconut oil, Refined coconut oil, Medium chain fatty acids, Antibacterial activity.*

Introduction

Coconut nucifera commonly known as Coconut, is a member of the Arecaceae family, coastal tropical countries like Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam are considered the native of coconut tree cultivation [1,2], "tree of life" referred to the tree of coconut and all parts of this tree could be consumed by humans or animal and transformed into many products like brushes, ladle, spoon and house furniture [3]. The oval rough coconut fruit consist of the outer hard thin skin (exocarp), fibrous thick layer (mesocarp), hard core (endocarp), white kernel (endosperm), and a big cavity suspended with a thick, sweet albumen liquid called coconut water, the development of endosperm layer will give arise the flesh meat edible coconut or kernel, after drying of flesh coconut it would be called copra, which is a source of coconut oil extraction. Coconut oil is an oldest edible oil that was used in tropical countries for thousands years ago, which is extract from the fresh kernel or meat of coconut after maturation, that make coconut oil can be easily homemade that would be give natural oil and chemical free treatment, and with its low oxidation point making coconut oil stable for two years of storage without any oxidation process could be occur as the presence of saturated fat also it has long validity, stable in high heat and melting point from 23º to 26ºC, that makes coconut oil very healthy for backing and deep-fry [4-8].

The natural creamy and soft texture of VCO is well known for skin and hair care using [9], and also used in

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cosmetics industry such as prepare natural shampoo, scents making, soaps, massage oils, aromatherapy and many other beauty and cosmetic products [10,11]. VCO has very strong benefits in health and medical fields that make it very useful in weight loss, digestion, healing and infection, aid in prevention liver disease, dissolve stone of kidney, pancreatitis treatment, diabetes treatment and the most important fields include antibacterial, antiviral, antiparasitic, antifungal, anticancer, antidermatophytic, antioxidant, anticaries and disinfectant activities [12,13].

Coconut oil

Coconut oil can be classified into VCO and RCO. According to Philippine National Standard VCO the definition of VCO is the oil that obtained from fresh and mature kernel of the coconut, either by natural or mechanical methods in the presence of heat or not, without chemical refining, bleaching and deodorizing, and which does not lead to the changing the oil nature [14]. VCO is pure oil, clear and colorless, with a scent of fresh coconut, which depends on the method of production oil. Two mains procedures can be used to extract VCO, which are wet and dry procedures, in wet procedure VCO is obtained by pressing fresh and mature coconut by manual or mechanical methods with water addition or without, that forming the milky fluid coconut mixture from this coconut milk VCO can be obtained, in wet method it should be avoiding dry of coconut kernel, after that VCO is produced by three mains methods; modified kitchen, modified natural fermentation and fresh-wet centrifuge methods. In dry method VCO is obtained immediately from fresh coconut kernel, after dried the kernel partially under controlled heat for reaching the moisture to 10-13% and then mechanically pressed the kernel for obtaining the oil, dry method including; low pressure oil extraction, high pressure expeller and fresh-dry centrifuge methods [15-18], these methods that is used in VCO extraction makes it very useful without losing the nature of VCO or the active natural components like vitamins especially vitamin E, polyphenols and antioxidants [17,19]. On the other hand RCO is extracted from dried coconut kernel ‘’copra’’, the drying process could be by using sun, smoke, or hot air, the obtained oil should be purified to make it appropriate for consumption, therefor the oil is must be chemical refined, deodorized and bleached, the heat is using for deodorizing the oil and for bleaching and the oil is filtered by clays to separate impurities, while using of sodium hydroxide to separate free fatty acids that make coconut oil has long shelf life, these processes will remove some characteristics of the oil [20], and that makes the resulting RCO is yellow color, no odor and no taste [21], whereas VCO which has not any refining, deodorizing or bleaching processes, makes it is the unique form and more useful which can be used in many applications in health and medical fields as compared with RCO that has traditional use [17,22].

Composition of VCO and RCO

One tablespoon of coconut oil supply 121 kilocalories, 13.7 gram fat, no protein, no carbohydrate, and very few amount of vitamins or minerals, that was reported by the National Nutrient Database published by the United States Department of Agriculture. About 99.9% of coconut oil is fatty acids (FA) and 91.9% of these fatty acids are saturated fatty acids (SFA), followed by 6.4% for monounsaturated fatty acid (MUFA) acids and 1.5% for polyunsaturated fatty acids (PUFA), and there is no dietary cholesterol [23]. The most predominate (SFA) is Lauric Acid (LA) about 50% and could be reached 56% depending on the variety of the coconut [17], this is followed by other fatty acids [12,24,25]. The standard quality of FAs compositions for coconut oil was reported by Codex Alimentarius [26], while for VCO was reported by Asian and Pacific Coconut Community (APCC) [27], it was also reported the value of iodine, percentage of free fatty acid (FFA), moisture, volatile matter and the value of peroxide as shows in Table 1.

VCO cannot be differentiated from RCO by the compositions of FAs as reported by, even the VCO was obtained from different methods for extraction, their no significant differences in fatty acid profile for various types of coconut oil when compared with the results of the Codex standards for coconut oil and the APCC standards for VCO [28,29],that means, the processing methods does not affect the profile of Fas[30], while the deferens are being that VCO has higher FFA, volatile matter, moisture, and lower peroxide value compared to RCO, which also comes with the Codex and APCC standards[29], these values determine the quality of VCO in addition to the color, odor and taste[17,31], FFA is the most crucial and important characteristic in the VCO producing and sales of products [32].

The saturation degree and fatty acid carbon chain length in any oil or fat will assist in determination of its characteristics, uses and impacts on human health, and VCO contains about 91.9% of SFAs 50% of it is LA as mention previously in this study, LA is a medium chain free fatty acids (MCFAs), and classified as medium chain triglycerides (MCTs), with 12 carbons chain (C12), followed by other MCFAs such as like caproic (C6), caprylic (C8), capric (C10), including lauric acid (C12), and long chain fatty acids (LCFAs) myristic (C14), palmitic (C16), palmitoleic (C16:1), stearic (C18), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) [33,34]. About two thirds of fatty acids in coconut oil is a saturated MCFAs, while LCFAs which is also saturated comprise fewer than the third and about tenth for the unsaturated one [35]. Coconut oil is the unique oil from other vegetable oils because it's the one that 50% of fatty acids in it is LA [24,25].

Table 1: Standard parameters of quality of Codex Alimentarius for coconut oil and APCC for VCO compared with VCO and RCO values.

Parameters	Codex standard for coconut oil	APCC standard for VCO	VCO products (Dayrit et al, 2007)	RCO products (Dayrit et al, 2007)
% C6:0 Caproic acid	ND–0.7	0.4–0.6	0.24–0.56 (0.40)	0.32–0.59 (0.41)
% C8:0 Caprylic acid	4.6–10.0	5.0–10.0	4.15–9.23 (7.23)	5.32–8.83 (6.61)
% C10:0 Capric acid	5.0–8.0	4.5–8.0	4.27–6.08 (5.21)	4.56–6.03 (5.00)
% C12:0 Lauric acid	45.1–53.2	43.0–53.0	46.0–52.6 (48.66)	46.7–49.4 (48.14)
% C14:0 Myristic acid	16.8–21.0	16.0–21.0	16.0–19.7 (17.82)	16.2–19.6 (17.88)
% C16:0 Palmitic acid	7.5–10.2	7.5–10.0	7.65–10.1 (8.51)	7.80–9.73 (8.88)
% C18:0 Stearic acid	2.0–4.0	2.0–4.0	2.73–4.63 (3.50)	2.94–3.69 (3.26)
% C18:1 Linoleic acid	5.0–10.0	5.0–10.0	5.93–8.53 (7.16)	7.24–8.04 (7.63)
% C18:2 Linolenic acid	1.0–2.5	1.0–2.5	1.00–2.03 (1.52)	1.82–2.36 (2.19)
Iodine value	6.3–10.6	4.1–11.0	5.6–10.3 (7.28)	6.81–8.91 (8.00)
% FFA, as lauric acid	No standard	≤0.5	0.047–0.337 (0.131)	0.008–0.076 (0.021)
% Moisture	No standard	0.1–0.5	0.05–0.11a (0.08)	0.01–0.10a (0.05)
% Volatile matter	0.2	0.2	0.07–0.18c (0.13)	0.00–0.08c (0.03)
Peroxide value, meq/kg oil	<15	<3	0.00–1.86 (0.56)	0.27–3.39 (0.98)

a By Karl–Fisher titration.

b Mass loss at 105 °C.

c Mass loss at 120 °C.

Antibacterial activity of VCO

MCFAs that are found in coconut oil make this oil has potential role as antibacterial, antiviral, and antifungal activities [36], particularly LA and its other derivatives of MCFAs with carbon chains C6 to C12 gave the coconut oil its antimicrobial activity [37] against gram positive, therefor many studies showed the antibacterial effect of LA on *Staphylococcus aureus* and *Bacillus cereus*, while the activity of MCFAs and LCFAs with various carbon chain length (C8-C18) could not show inhibition effect against a number of gram negative bacteria [38-40] on the other hand studies reported the antibacterial effect of LA against gram negative bacteria such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis, Helicobacter pylori* [41-44]. But still antibacterial activity of LA has great effect against gram positive bacteria when compare with gram negative bacteria, that is reported by a study performed by Abbas *et al*., 2017[45] that the esterified LA from coconut oil at different dilutions showed the antibacterial activity at highest dilution with largest inhibition zone for *Staphylococcus aureus* and *Streptococcus pneumoniae* followed by lowest inhibition zone for *Salmonella spp.*, *Mycobacterium tuberculosis* and *Escherichia coli*, while at lowest concentration, *Staphylococcus aureus* and *Streptococcus pneumonia* showed lowest inhibition zone compared with *Mycobacterium tuberculosis*, *Escherichia coli* and *Salmonella spp.*, that are showed resistance at the same lowest concentration, other study also reported the antibacterial the largest inhibition zone of extracted LA at different concentration for *Staphylococcus aureus*, *Streptococcus spp*. and Lactobacillus and the lowest inhibition for *Escherichia coli* which is also showed the resistance at the lower concentration of LA, in this study the same species showed resistance towards the coconut oil, which mean the extracted LA is more effect than coconut oil as antibacterial agent [46].

Antibacterial mechanism of VCO

The specific mechanism for the VCO as antibacterial agent is not detected and stilled unknown, the suggestion was that the metabolizing of the VCO makes it release its component of MCFAs, like caprylic, capric, and lauric acids, which are have the antimicrobial action [47], and LA is the most effected one, the integration of MCFAs of VCO into the bacterial cell membrane induce the solubilization lipids of the membrane which lead to disintegrate the bacteria cell wall [47,48], the disruption of bacterial cell membrane was indicated by the studies of electron microscope, which demonstrated the lysis of *Streptococcus agalactiae, S. aureus Clostridium albicans, C. perfringens,* and *C. trachomatis* when are exposed to the fatty acids of VCO [49,50]. VCO and its fatty acids showed the antibacterial effect on *Clostridium difficile* and the most action was belong to LA (C12) which is the predominant one, and followed by capric (C10) and caprylic (C8) acids, which are represented about 9% and 6% for each respectively according to Codex and APCC, these two fatty acids (Capric and caprylic acid) were showed less antibacterial action, while the activity of VCO towards *C. difficile* was abcent, but when coconut oil was lipolyzed showed the inhibition action on *C. difficile* [51]. The lipolyzing of MCTs by lipase and water leading to glycerol, diglycerides, monoglycerides and FFAs forming [52], the antimicrobial action belongs to monoglycerides and free fatty acids [49,50,53] and the less activity belongs to triglycerides, diglycerides and glycerol [48], therefor when VCO is lipolyzing by lipase to release its fatty acids and exposed the cells of *C. difficile* to this lipolyzing VCO had the inhibitory effect on the growth of bacteria at 0.15–1.2% of lioplyzing VCO while no effect for non-lioplyzing one, there were inhibitory effect of lauric, capric and caprylic acids individually for each, the most effect was for LA at concentration 1000 μM, these fatty acids integrate with lipid bacterial membrane works on disruption of bacterial cell membrane [48,51].

Conclusion

Many studies were reported the chemical components of in VCO and RCO with no significant difference in both, whatever the extraction methods were used. In the other hand the antimicrobial activity was also reported of the extracted fatty acids from VCO especially LA and followed by capric acid and caprylic show while no activity for caproic acid because it comprise about 0.5%, this low content of it makes it shows no antimicrobial activity. Coconut oil fatty acids were showed the antibacterial activity particularly on gram positive bacteria and in low effect for gram

negative in specific concentrations for several species, that indicate using fatty acids in high concentration shows antibacterial activity on gram negative bacteria, which makes the antibacterial action was no longer for gram positive but also for gram negative bacteria, and as the both types of VCO and RCO have the same chemical compositions with no significant difference as mentioned previously, that suggests the RCO has the same mechanism as antibacterial activity as VCO.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Recent Advancements in Pulp Testing Devices

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Abstract

Pulp vitality test is very crucial in monitoring and regulating the state of health of a dental pulp, especially after the case of traumatic injuries. The dental pulp is considered to be the innermost part of the tooth. It is surrounded on all sides with dentine except near the apical foramina. This hard dentine casting around the pulp protects it from infection by microbes present in the mouth. The traditional pulp testing methods involve thermal and electric pulp testing methods. This conventional method basically depends on the innervation and often yields or records either false positive or negative response. The newer pulp testing devices, some of which are still under developmental stage, detect the blood supply of the pulp, enumerate pulpal vasculature, check the vitality of the tooth etc. These newer technologies function by light absorption and reflection methods in. They are considered to be more accurate and non-invasive methods. The review article attempts to discuss the recent advancements in various pulp testing devices related to pulp vitality testing in clinical endodontic practice. It also describes the principle on which these newer pulp testing devices are basically based on its mode of working. The aim of this review paper is to highlight the difference between sensitivity testing and vitality testing.

Keywords: *Pulp vitality, pulse oximetry, laser doppler flowmetry, pulpal circulation, revascularization, light transmission.*

Introduction

One of the greatest diagnostic challenges in dental clinical practice is to accurately assess pulp status in different age groups of people. This may be further more complicated in many cases of pediatric dentistry where the practitioner is faced with a developing dentition, traumatized teeth, etc[1].

The dental pulp is considered to be an unmineralized oral tissue that is composed of certain soft connective tissue, vascular, lymphatic, and nervous elements that occupy the central part of the pulp cavity of each tooth of the dental pulp [2]. These nerve vessels enter and leave the tooth through this foramen. Dental pulpal testing devices are used in clinical as well as in diagnostic purposes in the field of dentistry to help establish the health of the dental pulp, within a pulp chamber and root canals of any tooth in the oral cavity [3]. These pulp testing devices can function in two manners as "Vitality testing" which assesses the blood supply to the tooth, while the "sensitivity testing" assesses the sensory supply to the tooth [4]. The assessment of pulp vitality is a very crucial diagnostic procedure in the practice of dentistry.

Current routine methods which include the conventional or the traditional practices are based on the stimulation of nerve fibers and thus giving no direct indication of blood flow within the pulp chamber [5]. These include certain constituents like thermal stimulation, electrical, or direct dentine stimulation. These testing methods are considered to have the potency to produce an unpleasant and occasionally painful sensation to the

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tooth. The main disadvantages revolve with inaccurate results which can be either false positive or even negative which can be obtained in many instances [6].

Recent studies have proved that blood circulation inside the pulpal chamber is the most accurate determinant in assessing pulp vitality [7]. This factor helps in providing an objective which enables to differentiate between necrotic and vital pulp tissue. This review article highlights tests depending on the passage of light through the control tooth to detect pulp vitality of that tooth with greater objectivity in the longer run. They also depend either on the detection of changes in case of light absorption as it passes through the tooth, as in photoplethysmography devices [8]. pulse oximetry and dual-wavelength spectrophotometry. Also if there is a shift in light frequency it gets reflected from a tooth which is in case of laser Doppler flowmetry [9]. This review paper attempts to illustrate various advancements in pulp testing devices. Ehrmann has proposed mainly three key uses of pulp testing in clinical practice[10]. They are-

BEFORE OPERATIVE PROCEDURES

Pulp testing may be indicated for a selected tooth before certain restorative or orthodontic interventions, particularly where pulpal health or pulpal vasculature may be in question. The absence of all the symptoms or radiographic changes alone may not be considered as conclusive evidence of pulp vitality in the case of teeth, because pulpal degeneration can occur without accompanying symptoms[11].

DIAGNOSIS OF PAIN

The origin of most oral pain is due to pulpal tissues. case reports have illustrated the value of pulp testing in identifying crucial pulpal pain from other conditions such as myofascial pain dysfunction syndrome and referred pain [12].

INVESTIGATION OF RADIOLUCENT AREAS

Radiolucent areas at the apices of teeth may be the result of any periapical extension of pulpal pathology, but may also arise due to certain pathological processes. If pulpal pathology is not responsible for the underlined lesion, the associated teeth would be expected to give a normal response to vitality testing. Periodontal lesions, cysts, fibrous lesions, congenital abnormalities, and even neoplastic processes may all produce periapical radiolucencies similar to those associated with pulp degeneration. The mental foramen and the incisive canal are two normal structures which may present as periapical radiolucencies.

Materials and Methods

All the articles collected have complete relevance to the topic, which is a recent advancement in pulp testing. They have been collected from search engines such as PubMed, Google Scholar, Elsevier, etc. All the articles collected had information about pulpal tissue, pulpal sensitivity, principles of newer pulp testing devices, pulpal vasculature, etc. All the articles which were irrelevant to the topic were excluded from the list. The results for this review article are based on previous studies done by other esteemed and honored authors and journals.

Ethical clearance is not essential as this is a review.

Result and Discussion

For this review, various articles from google scholar, PubMed and Elsivier were collected. The result of this review depicts the recent advancements and technologies in pulp testing devices. Majority of the article collected mainly emphasis on the dental pulp, pulpal vitality, pulpal vasculature, and different pulp sensibility tests as well as pulp vascularity tests. The data collected from different articles shows that there are still many pulp testing devices under developmental stage.

Previously our team had conducted numerous clinical trials [13 14 15], in vitro studies [16 17 18 19 20], surveyed [21 22] and reviewed [23 24 25 26 27] various aspects of endodontics and conservative dentistry over the past five years. Now we are focusing on review studies, the idea for which has stemmed from the current interest in our community. This review study was conducted acknowledging the importance of the pulp and enabling to differentiate between vital and non-vital pulp, also about different pulp testing devices which contributes as a factor to the outcome of endodontic therapy.

DENTAL PULP

The pulp is the part in the center of a tooth which is made up of living connective tissue and cells termed as odontoblast [28]. The pulpal tissue is the neurovascular bundle central to each tooth, in case of permanent or primary dentition. This dental pulp consists of a central pulp chamber along with the pulp horns, and radicular canals. Because of the continuous deposition of dentine, the pulp chamber starts becoming smaller with age[29]. This factor is not uniform throughout the coronal pulp but progresses at a faster rate on the floor than on the roof or sidewalls.

Radicular pulp canals start extending down from the cervical region of the crown to the root apex of a tooth. These canals are not straight most of the time but vary in different aspects like shape, size, and number. They are continuous with the periapical tissues through the apical foramen. The mean volume of a single adult human pulp is 0.02cc[30]. There are four layers of pulp from innermost to the outermost aspect of the tooth [31]. They are-

PULPAL CORE

This is present in the center of the pulp chamber with many cells and an extensive vascular supply.

CELL RICH ZONE

It consists of fibroblast and undifferentiated mesenchymal cells.

CELL FREE ZONE

Also termed as (zone of Weil) this zone is rich in both capillaries and nerve networks together.

ODONTOBLASTIC ZONE

It is an outermost layer that contains odontoblasts and lies next to the predentin and mature dentin.

Cells found in the dental pulp include fibroblast (that constitutes the principal cell), odontoblasts, defense cells like histiocytes, macrophages, granulocytes, mast cells, and plasma cells. The nerve plexus of Raschkow is located central to the cell-rich zone [32].

PULP INNERVATION

Within the coronal pulp, nerve bundles start diverging and branching out towards the pulpo-dentine border, and emerge from their myelin sheaths[33]. Nerve divergence keeps continuing until each bundle loses its integrity and smaller fiber groups travel towards the site of dentine. This course is relatively straight in the axis until the nerve fibers start to form a loop and result in the formation of mesh termed the nerve plexus of Rashow [34]. Terminal axons exit from their Schwann cell investiture and pass between the odontoblasts as free nerve endings. This nerve plexus is most well developed in the case of the peripheral pulp. Two types of sensory fibres are present in the pulp: the myelinated (A fibres) and unmyelinated C fibres[35] .

The A fibres predominantly innervate the dentine (entering the dentinal tubules) and are sub-grouped according to their diameter and conduction velocities into Aβ and Aδ fibres[36]. The C fibres innervate inside the body of the pulp. Aδ fibres mediate acute, sharp pain and are excited by hydromechanical events in dentinal tubules such as drilling or air-drying. C fibres have a high threshold value and can be activated by intense heating or cooling of the tooth crown. Once activated, the pain initiated by C fibres can start to radiate throughout the face and jaws. C fibre pain is associated with tissue injury and is modulated by inflammatory mediators, vascular changes in blood volume and flow, and increases in pressure result in a progressive increase in the sensory response [37]. It has been assessed that the highest concentration of neural elements was in the pulp horn region (pain sensation is felt more in this region). There is a progressive decrease in the number of nerve fibres in the cervical and radicular areas [38]. It is considered that the direction of dentinal tubules is also important in establishing pulp test responses in various parts of the tooth crown. The dentinal tubules have an almost straight course which extends from the incisal edge of anterior teeth in the oral cavity to the pulp horn. In multi-cuspal teeth, the course of tubules is somewhat curved and resembles an 'S' shaped structure [39]. It is principally the fluid that is present in the dentinal tubules that allow the conducting of electrical impulses from the pulp tester electrode to the pulp. If the distance is shorter between the electrode and the pulp, then there will be lower resistance to the flow of current [40].

TECHNIQUES OF PULP TESTING DEVICES

The accurate diagnosis of the true histological status of the pulp is of significant importance for treatment planning. This is indirectly done by assessing the neural sensitivity of the pulp. The more accurate method would be to assess the vascularity of the pulp. However, most common methods clinically employed assess neural sensitivity, while the recent researches and developments are trying to find a clinical method of vascularity assessment. Various methods for tooth vitality assessment are given below-

Pulp sensibility tests

- Thermal tests
- Heat testing
- Cold testing
- Electric pulp tester (EPT)
- Anesthetic test
- Test cavity

Pulp vitality/vascularity tests

- Pulse oximetry
- Laser Doppler flowmetry
- Recent technologies
- Dual-wavelength spectrophotometry
- Thermography
- Crown surface temperature
- Transmitted light photoplethysmography

PULP SENSIBILITY TESTS

Dental pulp testing is a very useful and essential diagnostic aid in endodontics treatment. Pulp sensibility tests investigate the extrapolate pulp health from the sensory response [41].

MECHANISM

Currently, the most widely used pulp vitality testers are able to assess the integrity of the Aδ nerve fibers inside the dentine–pulp complex. This can be done by briefly applying a stimulus to the outer surface of the tooth. If the Aδ nerve fibres are successfully stimulated, then the patient will respond by acknowledging a very short, sharp sensation/tingling from the tooth. A positive response indicates that the nerve fibres are functioning (to some degree), but does not give any indication of pulpal blood flow. If there is no vascular supply to the pulp, it will rapidly become anoxic and the Aδ fibres will cease to function [42].

THERMAL TEST

These tests involve the application of cold and heat stimuli to a tooth, to determine sensitivity to thermal changes in a tooth. Response to cold stimulus given to the tooth usually states about the vital pulp, regardless of whether that pulp is normal or abnormal in condition. In contrast to the context, an increased response to heat stimulus is suggestive in the case of pulpal or periapical pathology which may require endodontic intervention.

Thermal tests involve the application of cold and heat to a tooth to determine its sensitivity to thermal changes. The results of the thermal test should be correlated with the results of other tests to ensure validity[43].

HEAT TEST

By using different techniques which deliver different degrees of temperature. It uses-

- Electrical heat carrier
- Hot gutta-percha stick
- Others
- Hot water under rubber dam isolation
- Hot burnisher, hot compound
- Dry rubber polishing wheel

The heat test can be performed in the posterior as well as anterior tooth [44]. The area to be tested is isolated and dried and suitable lubricant (Vaseline) is applied and heat is directed to the exposed surface of the tooth, the patient's response is noted. A heat carrier instrument that can deliver a controlled temperature to the tooth is preferable, or a hot GP stick can be used.

Mechanism Of Heat Test

The mechanism of the heat test is explained in figure 1.

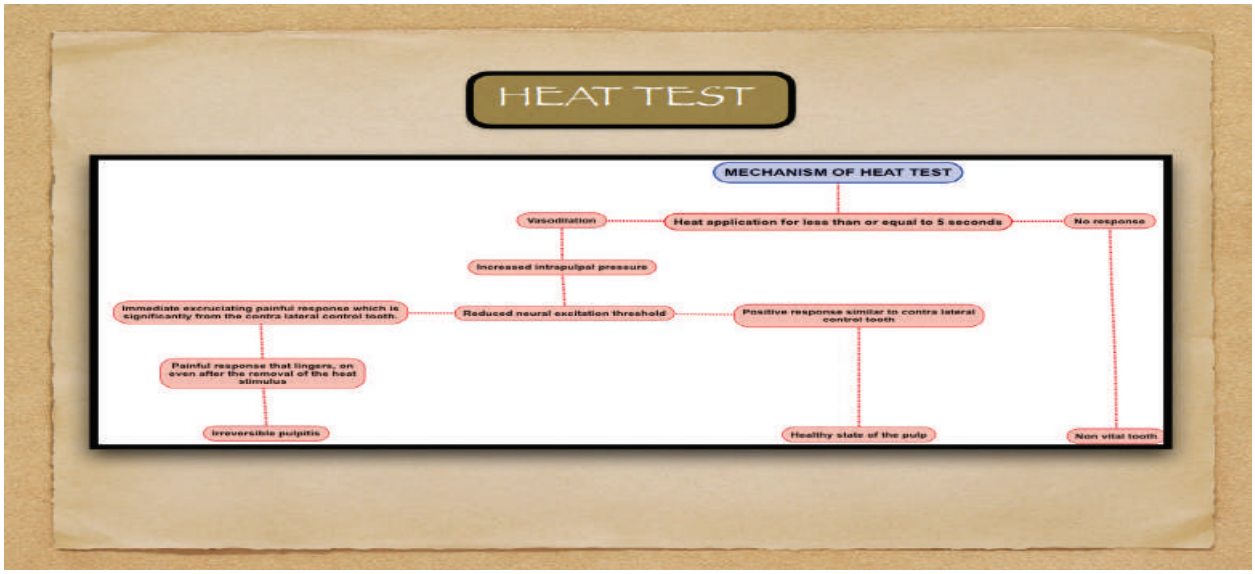


Figure-1: Mechanism of heat test, works by the process of heat application for less than or equal to 5 seconds to the affected tooth.

COLD TEST

Cold thermal testing results in contraction of the dentinal fluid within the dentinal tubules of the dentine, resulting in a rapid outward flow of fluid within the patent tubules [45]. This rapid movement of dentinal fluid inside the dentine results in 'hydrodynamic forces' acting on the Aδ nerve fibres within the pulp–dentine complex, leading to a sharp sensation lasting for the duration of the thermal test[46]. A variety of cold tests can be done; the major difference between all these is the degree of cold that is applied to the tooth. The most common pulp testing method employed by clinical practitioners is to seek a response to cold stimuli. Ideally, cold testing should along with the conjunction of an electric pulp tester so that the results from one test will verify the findings of the other test. If a mature, non-traumatized tooth in the oral cavity does not respond either to EPT or cold, then the tooth may be considered non-vital. However, care should be taken along with proper procedure while testing multi-rooted teeth, as they may respond positively to a cold stimulus, even though only one of the roots consists of the vital pulp tissue. The cold test may be used to differentiate between reversible and irreversible pulpitis can be achieved[47].

ELECTRIC PULP TESTER

The electric pulp test is one of the tests used to determine pulp vitality. The electric pulp tester, when testing for pulpal vitality, uses nerve stimulation. The objective of this electric pulp tester is to stimulate a pulpal response to the tooth by subjecting it to go through an increasing degree of electric current [48]. A positive response is an indication of vitality and helps in determining the normality or abnormality of that pulp. No response to the electrical stimulus can be an indication of pulp necrosis [49].

TEST CAVITY PREPARATION

This test may be served as a last resort in testing for pulp vitality. It is only considered to be used when the results of all other tests have proved inconclusive [50]. Its value in dental clinical practice has been largely anecdotal as there is no evidence base to support its effectiveness. The test cavity is made by drilling through the enamel–dentine junction by following preventive measures to eradicate any dilemma. This is done in case of an unanesthetized tooth along with good isolation of the tooth. This may be achieved under a rubber dam with a small round diamond bur in a high-speed handpiece with adequate coolant. The patient is asked to respond

immediately if any painful sensation is being felt during the drilling procedure of the tooth. If the patient feels certain pain that persists once the bur contacts the sound dentin, then the procedure is terminated completely and the cavity is restored

LOCAL ANESTHETIC TEST

When dental symptoms are poorly localized or referred most of the time, then the prediction of an accurate diagnosis is extremely difficult. Sometimes, patients may not even be able to specify whether the symptoms are from the maxillary or mandibular arch. In such cases, and where pulp testing has proved inconclusive, an anesthetic test may be helpful. The technique involved in this local anesthetic test includes certain procedures that are being followed in a stepwise manner. In the beginning, there is a use of either infiltration or an intraligamentary injection along with which the most posterior tooth in the area suspected of causing the pain sensation is being anesthetized. If the pain persists and prolongs once after the tooth has been fully anesthetized, the tooth immediately mesial to it is then completely anesthetized, and so on routine goes by, until the pain completely disappears and subsides. If the source or origin of the pain sensation cannot be even localized to the upper (maxilla) or lower (mandible) jaw, an inferior alveolar nerve block injection is given; cessation of pain indicates the involvement of a mandibular tooth. This approach has an advantage over a test cavity, which may incur iatrogenic damage.

PULP VITALITY/ VASCULARITY TESTS-

PULSE OXIMETRY

It is considered to be a non-invasive oxygen saturation monitoring device widely used to record blood oxygen saturation level [51]. Pulse oximeter is a piece of standard equipment in operating rooms, including intensive care units, emergency rooms, etc. where sedation & analgesia are provided [52]. This device is currently under investigation in case of dental practice to detect pulpal blood circulation by its non-invasive design.

The principle of this is based on Beer's law which relates the absorption of light; by a solute to its concentration and optical properties at a given wavelength. In the red region, oxyhemoglobin absorbs less light than deoxyhemoglobin and vice versa in the case of the infra-red region[53]. The system has a probe which contains a diode that limits light in two different wavelengths.

1. Red Lights (660mm)

2. Infra-red light (850mm)

There is a silicon photodetector diode which is placed on the opposing surfaces of the tooth. This is connected to a microprocessor. The ideal placement of the probe is in the middle third of the crown.

Many dentists have approved pulse oximetry to be an effective manner of pulp testing. It is also very evident in the time of "impact injury" where the blood supply remains intact but the nerve supply is being damaged. Gingival circulations can also be used to detect the pulse based on this device [54].

Limitations include the background absorption associated with venous blood and tissue constituents. In addition to this there are chances for refraction and reflection of light that may add on to its disadvantage.

DUAL WAVELENGTH SPECTROPHOTOMETRY-

DWLS is a method that is independent of a pulsatile circulation. This method completely ensures the measure of oxygenation changes in cases of the capillary bed rather than in the supply vessels and hence does not depend on the pulsatile blood flow [55]. DWLS depends on the presence or absence of oxygenated blood at 760mm and 850mm. DWLS helps to differentiate reproductive readings between a pulp chamber of a vital and non-vital tooth.

In young children, where there are avulsed and replanted teeth with open apices, the blood supply is regained within the first 20 days after replantation but nerve supply lags. Repeated readings are taken in such cases up to 40 days to ensure an increase in blood oxygenated level that means the healing process has occurred; the pulp of such teeth has also started to recover [27] [56]. The instrument is comparatively small, portable, relatively inexpensive, and suitable for usage in clinical practice.

LASER DOPPLER FLOWMETRY

It is considered to be a noninvasive, electro-optical technique which allows the semiquantitative recording of the pulpal blood flow[57]. The technique is based on the Doppler principle whereby light from a laser diode is incident on the tissue which is scattered by moving the RBC's; thus the frequency broadened[58].

The frequency broadened light along with the laser light scattered from static tissue is photo detected and the resulting photocurrent processed to provide a proper blood flow measurement. It helps to locate the revascularization property accurately of a tooth.

THERMOGRAPHY/CROWN SURFACE TEMPERATURE

Thermography is a test that uses an infrared camera to detect blood flow in the pulpal body tissue. It is effective in recording heat patterns as wheels. It has become an important diagnostic tool because of the accurate measurement of regional temperature and differentiation of temperatures while doing the dental procedure[59].

It is a non-invasive, and highly accurate method of measuring the body's temperature. The teeth that need to be assessed must be isolated using rubber dams. The technique is complex and requires the subjects to be at rest for 1 hour period to test[60].

TRANSMITTED LIGHT PHOTO THERMOGRAPHY(TLP)

It is a non-invasive technique used to monitor pulpal blood flow and has been successfully applied in human studies. It has been suggested that TLP incurs less signal contamination from the periodontal blood flow than the other devices[61].

Conclusion

An accurate assessment of tooth vitality is of the utmost paramount importance in clinical practice. It is required to establish devices with better materials for easy handling. These devices are used in dental clinical practice on a large scale, thus it should be less expensive. Rapid advancements in the knowledge and applied technology related to the pulpal blood flow as well as pulp vasculature may lead the way to a more objective, accurate, and predictable means of pulp vitality assessment.

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Ethical Clearance

Not Required

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Knowledge and Awareness of Basic Life Support (BLS) among Dental Students

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Abstract

Basic Life Support is an important life skill which should be taught to everyone. An emergency can happen at any time and any place and it is important that the people around the happening are trained to handle the emergency with utmost efficiency. This can be very helpful in reducing the number of deaths that occur. It is a must that all healthcare professionals have access to knowledge about BLS. In our study we aim to find out the knowledge and awareness of BLS among dental students. A well structured, self-prepared questionnaire was circulated among dental students within the university of Saveetha Dental College. The students were split into three groups such as BDS students, Interns and MDS students. The confidence level was 95% and the data was considered statistically significant if P value < 0.05. The results obtained were collected and analysed to find out the awareness levels among the students and to find out which group had the highest knowledge on BLS. The data obtained was analysed and it was found that among the three groups the MDS students(42.86%) had the overall highest knowledge on BLS and CPR. The knowledge and awareness of BLS among dental students was found to be moderate and not sufficient. Knowledge should definitely be improved and more knowledge must be created.

Key words: *Basic Life Support ; emergency; knowledge ; awareness ; dental students.*

Introduction

Basic Life Support (BLS) refers to maintaining airway patency and supporting breathing and the circulation, without the use of equipment other than a protective device[1]. BLS, a key component of the chain of survival decreases the arrest- cardiopulmonary resuscitation interval and increases the rate of hospital discharge[2]. Healthcare professionals are expected to have a knowledge of current basic and advanced life support (BLS/ACLS) guidelines to revive unresponsive patients[3]. Life threatening emergencies can occur at any time, anywhere, to anyone[4]. It is very important that every member of the community be trained in effective BLS technique to save lives. Atleast doctors including medical, dental and paramedical staff should be trained in CPR, as it is a basic medical skill which can save many lives if implemented timely [5].

The theoretical knowledge and practical skills of Basic Life Support and Advanced Life Support are among the important determining factors of Cardiopulmonary Resuscitation success rates [6]. Cardiac arrests and accidents are the most common emergencies with grave consequences but there is high mortality associated with them [7]. For decreasing the mortality rate and increasing the survival ratio it is crucial for a healthcare provider to have a firm grip on basic cardiac life support knowledge and practices. Globally, about

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92% out-of-hospital cardiac arrest subjects lose their lives due to limited availability of CPR facilities [8]. BLS is an important component of CPR, which includes adequate maintenance of ventilation and circulation in case of respiratory/ cardiac arrest [9]. It involves recognition of signs of sudden cardiac arrest (SCA), heart attack, stroke and foreign-body airway obstruction (FBAO), defibrillation with an automated external defibrillator (AED) [10]. Viability is maintained until full CPR can be commenced. Early access to emergency medical care has saved thousands of lives around the world [11]. The European Resuscitation Council (ERC) last issued guidelines for BLS in 1998. These were based on the 'Advisory statements' of International Liaison Committee on Resuscitation (ILCOR) published in 1997 [12].

Previously studies have been conducted among various populations to find out the knowledge and awareness of BLS and responses given were seemingly unsatisfactory. This indicates the need for teaching and training even non-healthcare professionals and laymen to provide effective and safe resuscitation prior to hospital care [13]. Looking into history, early records from Egyptian mythology and Bible suggest that mouth-to-mouth and mouth-to-nose respiration were among the earliest resuscitation efforts using artificial respiration. This technique has also been used by midwives for many centuries to revive apparently still born infants [7]. The life of an individual is influenced by various factors including condition of health, education, occupation, socioeconomic status, etc. However, it is the condition of health which influences life to the greatest extent. Emergencies like these can occur even within the confines of a dental clinic. Effective management is ultimately the dentist's responsibility. Hence, even dentists must be well prepared to manage medical emergencies [4]. Previously our team had conducted numerous original studies [14-20] and surveys [21-28] over the past 5 years. Now we are focussing on epidemiological surveys. The idea for this survey stemmed from the current interest in our community. In this study, we aim to spread as well as find the awareness levels, knowledge and attitude of dental students towards Basic Life Sciences (BLS) among the South Indian population.

Materials And Methods

A questionnaire-based cross sectional study was conducted among dental students within the University. The study was conducted in 2020. Approval was received from the Institutional Review Board. The well structured questionnaire was passed on among 154 participants (sample size). The participants were grouped based on their year of study as BDS, interns and MDS students. The sampling method used was convenient sampling.

The questionnaire was self-structured and modified from previous similar study questionnaires. Other than age, all the questions were closed ended. Data collection software used to collect the responses was Google forms. From Google forms, the data was analysed and cleaned up to excel sheet. The responses were represented using pie charts and bar graphs. The statistical software used was SPSS version 22. The statistical analysis used are descriptive statistics to summarise demographic data and chi-square test to analyse survey data. The confidence level was kept at 95%. P value less than 0.05 was considered statistically significant.

Results and Discussion

The data obtained was analyzed. It was found that 18.18% participants were interns, 38.96% BDS and 42.86% MDS students.

56.49% have heard of BLS previously. When asked what BLS stands for, 57.14% answered correctly with Basic Life Support. 62.34% seemed to have taken up BLS training earlier. When asked whether BLS training should be mandatory in curriculum, 66.88% replied yes. Only 18.83% knew that both 911 and 112 are Indian numbers to be called in case of an emergency. 33.12% were aware of the correct CPR sequence. 27.27% were aware of the correct ratio of chest compression to rescue breaths. 28.57% knew that the carotid artery is to be felt for pulse in adult patients. 42.21% knew that the correct expansion of AED. 46.75% were aware of the immediate action in response to choking. Only 20.78% were aware of the fact that mid-chest is the correct location of chest compression. 31.17% knew the full form of EMS. Only 9.09% were aware of the chest compression to ventilation ratio in newborns. 14.94% knew that both mouth-to-mouth and nose breathing can be used for rescue breathing in infants. 57.14% were

aware of the correct chest compression rate during CPR. 18.18% knew the depth of chest compression in children during CPR. Only 15.58% were aware of the depth of CPR in adults. 64.94% felt that their knowledge on BLS is sufficient. When asked to self-grade their knowledge on BLS ,11.04% rated themselves poor, 24.68% rated themselves good, 51.95% felt themselves average and 12.43% thought they had excellent knowledge. (Table 1)

Upon comparing the levels of awareness among BDS (60), Interns(28) and MDS(66) for all the above questions, the following were the results.

43 MDS students had heard of BLS previously. 41 MDS students knew the correct expansion of BLS. 51 BDS students had BLS training before. 47 MDS students feel that BLS training should be mandatory in the curriculum. 11 MDS students knew that both 911 and 112 could be contacted in case of emergency in India. 19 interns knew the correct sequence of resuscitation. 22 MDS students knew the correct ratio of chest compression to rescue breaths in adults. 19 MDS students knew that the carotid artery has to be felt for pulse in adults. 36 MDS students knew the correct expansion of AED. 41 MDS students knew the correct response to a choking person. 13 MDS students knew that midchest is the correct location of chest compression. 13 BDS students knew the correct expansion of EMS as Emergency Monitoring Services. 11 MDS students knew the correct ratio of chest compression to ventilation ratio in children. 11 MDS students knew that both nose and mouth-to-mouth breathing can be used as rescue breaths in infants. 43 BDS students knew chest compression rate as 100/min during CPR for adults and children. 10 MDS students knew the correct depth of chest compression in children. 14 MDS students knew the correct depth of compression in adults. 44 BDS students felt that their BLS knowledge was sufficient. 8 BDS students and Interns rated their knowledge as excellent. Hence, we can infer from these results that dental students' knowledge on BLS was between poor to moderate. MDS students had maximum awareness upon comparing the three groups.

Table 1: Table showing percentage of responses

1. Have you ever heard of BLS previously?	Yes	56.49%
	No	43.51%
2. What does BLS stand for?	Basic Life Support	57.14%
	Basic Life Sciences	42.86%
3. Have you had previous BLS training?	Yes	62.34%
	No	37.66%
4. Do you think BLS training should be mandatory in the curriculum?	Yes	66.88%
	No	33.12%
5. What is the number to be called in case of an emergency in India?	911	35.06%
	112	46.10%
	Both	18.83%

Cont... **Table 1: Table showing percentage of responses**

6. What is the correct sequence of resuscitation in BLS?	Compressions, Airway, Breathing	33.12%
	Airway, Breathing, Compressions	50.65%
	Breathing, Airway, Compressions	16.23%
7. The correct ratio of chest compression to rescue breaths is	30:2	27.27%
	15:2	38.96%
	30:1	31.17%
	25:2	2.60%
8. Which artery is to be felt for a pulse in an adult patient?	Carotid	28.57%
	Femural	13.64%
	Brachial	57.79%
9. What does abbreviation AED stand for?	Automated External Defibrillator	42.21%
	Arrhythmia Extradural Defibrillator	57.79%
10. Recommended action in response to a choking person.	Start CPR	38.96%
	Attempt abdominal thrusts	46.75%
	Keep patient in Trendelenburg position	14.29%
11. What is the location of chest compression ?	Left chest	25.32%
	Mid chest	20.75%
	Right chest	53.90%
12. Abbreviation EMS stands for	Emergency Medical Services	31.17%
	Electrical Monitoring System	49.35%
	Emergency Monitoring Sciences	19.48%
13. In a newborn, chest compression to ventilation ratio is	2:1	32.47%
	3:2	58.44%
	3:1	9.09%

Cont... **Table 1: Table showing percentage of responses**

14. Types of rescue breathing used for infants	Mouth to mouth	23.38%
	Nose	61.69%
	Both	14.94%
15. Rate of chest compressions in adults and children during CPR	50/min	42.86%
	100/min	57.14%
16. Depth of compression in children during CPR	1-1.5 inches	18.18%
	2-2.5 inches	67.53%
	3 inches	14.29%
17. Depth of CPR in adult	½ to 1/3rd depth of chest	15.58%
	1/4th depth of chest	66.88%
	2/3rd depth of chest	17.53%
18. Do you feel your knowledge about BLS is sufficient?	Yes	64.94%
	No	35.06%
19. What would you grade your knowledge on BLS after taking up this questionnaire ?	Poor	11.04%
	Average	51.95%
	Good	24.68%
	Excellent	12.34%

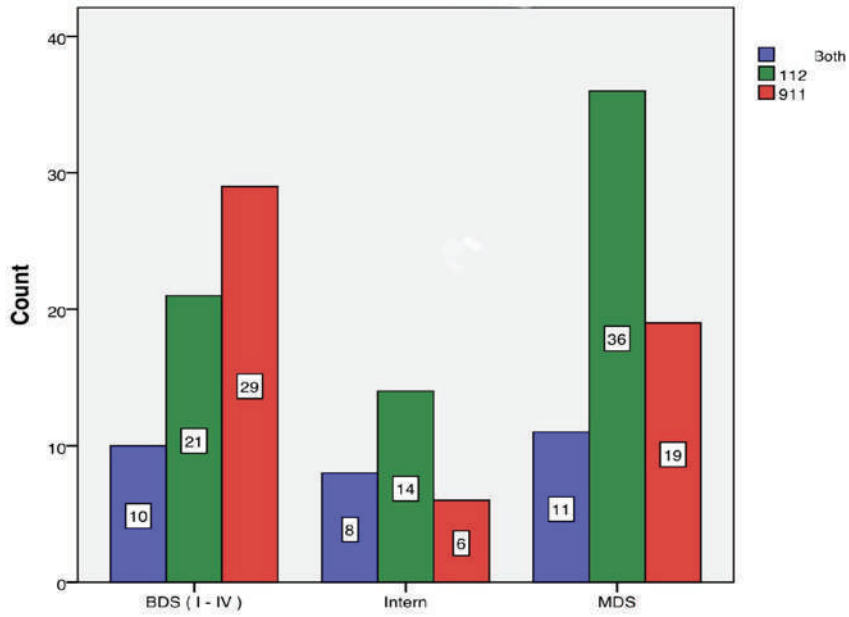


Figure 1: Bar graph showing comparison between course of study of participants and the awareness of emergency phone numbers among students. x-axis represents the course of study of students while y-axis represents the count of students. Red denotes 911, green denotes 112 and blue denotes both. MDS students answered with the right answer 112 highest and this was statistically significant (Chi-square test p=0.046 - indicates statistical significance)

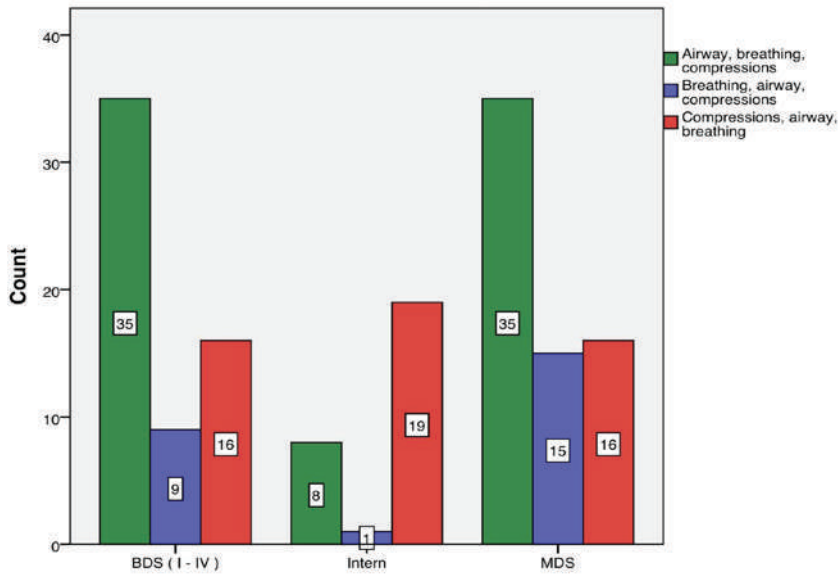


Figure 2: Bar graph showing comparison between course of study of students and their responses to sequence of resuscitation. x-axis represents the course of study of students while y-axis represents the count of students. Blue denotes breathing, airway, compressions. Red denotes compressions, airway, breathing. Green denotes airway, breathing, compression. Interns answered the correct answer highest and this was statistically significant (Chi-square test p=0.000 - indicates statistical significance)

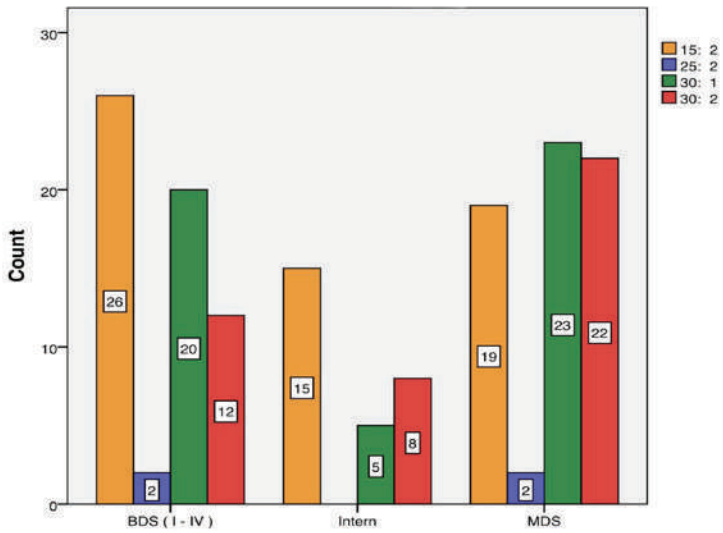


Figure 3: Bar graph showing the comparison between course of study of participants and their answers to ratio of chest compressions to rescue breaths. x-axis represents the course of study of students and y-axis represents the count of students. Blue denotes 25:2, red denotes 30:2, green denotes 30:1 and orange denotes 15:2. MDS students answered correctly 30:1 highest and this was statistically significant. (Chi-square test p=0.202 -indicates statistical insignificance)

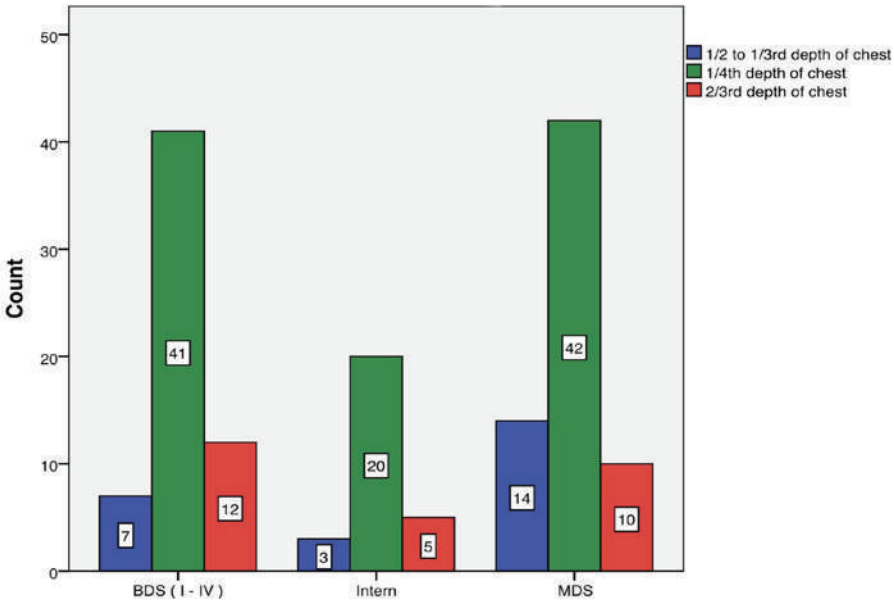


Figure 4: Bar graph showing the comparison of course of study of students and their responses to depth of CPR in adults. x-axis represents the course of study of students and y-axis represents the count of students. Blue denotes ½ to 1/3rd depth of chest, red denoted 2/3rd depth of chest and green denotes 1/4th depth of chest. MDS students answered correctly highest, but this was statistically not significant (Chi-square test p=0.559 - indicating statistically not significant).

In our study, the participants were questioned whether they had heard of BLS previously. It was seen that 56.49% of dental students had heard of BLS before. We find that 27.9% of MDS students had heard of BLS previously, which is the highest among the 3 groups. The p-value was found to be 0.112 indicating statistical insignificance. According to Sahiti Reddy et al,2013 [11], 17.8% interns, 17.2% PG students and 53% BDS students had heard of BDS previously.

57.14% had answered correctly the expansion of BLS as Basic Life Support. MDS students' % of correct response was highest (26.6%). The p-value for data was 0.037 which indicated statistical significance. According to Sahiti Redy et al [11], 53% BDS, 17.7% interns and 15.9% PG answered correctly. This is opposite to our finding. This may be due to the fact that experience and knowledge gained by MDS students has increased with their medical practice.

62.34% of dental students had BLS training previously, the % of BDS students being the highest,i.e, 33.15%. The p-value was found to be 0.000 which indicates statistical significance. According to Nouf Al Harbi et al, 2020 [13], only 10.8% of participants had taken BLS training before. This finding is opposite to our finding. This is because the study population taken was different. It was conducted among female secondary students.

66.88% of participants felt that BLS training should be mandatory in the curriculum. In our study,,MDS students responded positively highest with 30.5%. The p-value was found to be 0.109, indicating statistical insignificance. According to Nouf Al Harbi et al, 2020 [13], 38.5% felt the course should be included. This differs from our study as the study population differs.

18.83% dental students knew that both 911 and 112 could be dialed in case of an emergency . We see that 7.14% MDS students knew this [Figure 1]. The p-value was found to be 0.046 which indicates statistical significance. 70.4% answered correctly according to Shahabe A Squib et al, 2019 [9]. This is because the study was conducted among various healthcare professionals who have expertise in BLS and CPR.

33.12% knew the correct sequence of resuscitation. 12.34% interns were highest to answer correctly [Figure 2]. P-value was found to be 0.000 which indicated statistical significance. According to Shahabe A Saquibe et al, 2019 [9], 28.5% answered correctly. In Avabratha et al, 45.2% interns had inadequate CPR knowledge. Sahithi Reddy et al [11] recorded that only 3% interns had inadequate knowledge on CPR.

We see that 27.27% knew the correct ratio of chest compression to rescue breaths as 30:2 in CPR. 14.28% MDS students answered correctly [Figure 3]. P-value was 0.202 indicating that data is statistically insignificant. According to Raghava Sharma et al, 2012 [29] - 49% answered correctly. In Shanta Chandrasekaran et al,2020 [10], 25.5% answered correctly.

28.57% knew that the carotid artery should be felt for checking pulse in adults. 12.34% MDS students were highest with the answer carotid artery. The p-value was found to be 0.033. Data is statistically significant. According to Shahabe A Saquib et al, 2019 [9], 71% answered correctly. This finding opposes our findings as the study was carried out among a different population (healthcare professionals).

42.21% answered that AED stands for Automated External Defibrillator. 23.38% MDS students answered correctly, which was highest among the 3 groups. The p-value was 0.000 which signified that data was statistically significant. According to Babar Irfan et al, 2019 [8], 49.3% answered correctly.

46.75% knew the recommended action in response to a choking person. 26.62% MDS students answered right. P-value was 0.000 which indicates that the data was statistically significant. In Babar Irfan et al, 2019 [8], 22.1% answered right.

We find that 20.78% knew that midchest was the correct location of chest compression. 8.44% MDS students were highest with the answer midchest]. The p-value was found to be 0.016 indicating statistical significance. According to Sahiti Reddy et al [11], 24.2% MDS students answered right.

31.17% knew the expansion of EMS is Emergency Medical Services. We see that 17.5% BDS students were highest in answering. The p-value was 0.001. Data is statistically significant. According to Shanta Chandrasekaran et al [10], 56.1% know the expansion.

Babar Irfan et al [8] has 65% correct responses and in Sahithi Reddy et al [11] 53% BDS answered right. We have two articles opposing our finding as the study populations taken in each are different.

9.09% dental students knew the correct compression to ventilation ratio as 3:1. 7.14% MDS students were highest in answering correctly. According to Shaithi Reddy et al, 11.2% MDS students answered right. According to Shanta Chandrasekaran et al [10], 25.52% answered right. In present study, p value was 0.000 indicating statistical significance.

14.94% knew that both mouth-to-mouth and nose breathing was used in infants for rescue breathing. 7.14% MDS students were highest in answering with the right option. P-value was found to be 0.005. Hence data is statistically significant. Babar Irfan et al [8] states 22.1% answered correctly.

57.14% knew the correct ratio of chest compression in adults and children during CPR. 27.92% BDS students were highest in answering 100/min. P-value was found to be 0.004. Data is statistically significant. In Shanta Chandrasekaran et al [10], 35.1% answered right. In Sahithi Reddy et al [11], 44.3% BDS students were right with their answers.

18.18% total students knew the depth of chest compression in children during CPR. 6.49% MDS students were highest in answering right. P value was 0.006. Data is hence statistically significant. Sahithi Reddy et al [11], Shanta Chandrasekaran et al [10] and Babar Irfan et al [8] support our findings.

15.58% knew the correct depth of CPR in adults. We see that 9.09% MDS students were highest in answering correctly [Figure 4]. P-value was found to be 0.559% indicating statistical insignificance of data. Babar Irfan et al [8] and Sahithi Reddy et al [11] support our findings while Shanta Chandrasekaran et al [10] opposes them.

We find that 64.94% felt their BLS knowledge was sufficient. 28.57% BDS students gave the highest correct response. P-value was 0.159, indicating that data is statistically insignificant.

12.34% of students self-graded their knowledge on BLS as excellent. 5.19% interns and BDS students were highest in rating themselves as excellent. P-value was found to be 0.025. Hence data is statistically significant. Shahabe A Saquib et al [9] says 15.6% self graded their knowledge as good. Raghava Sharma et al [29] says that 0% graded themselves as excellent.

3.1 Limitations

The limitations of our study include limited sample size, homogenous population and regionalism, overcoming which we can get more accurate results.

3.2 Future Scope

This study creates awareness on BLS and emphasizes the need for knowledge on BLS during emergencies and also setting up awareness programmes.

Conclusion

Knowledge and awareness on BLS among BDS, Interns and MDS students was found to be moderate. The MDS students have a higher knowledge on BLS and its procedures. Interns and BDS students have lesser awareness of BLS. With a greater sample size we can get even more reliable and accurate results. However, we can conclude that there is definitely a need to create more awareness on BLS.

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Ethical Clearance

It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Prevalence of Anemia among Women Patients Visiting A Dental Hospital

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Abstract

Anemia is the decreased oxygen carrying capacity of the blood due to reduction in the concentration of Hemoglobin or RBC. Anemia has a high prevalence and it is considered as a public health issue affecting developed and developing countries. It occurs most commonly in women and children.Prevalence of Anemia worldwide suggests the need for substantial improvement in the nutritional status of women.The aim of the study is to assess the prevalence of Anemia among women patients visiting a Dental hospital. Patient records of a total of 86000 patients who visited the hospital between June 2019 to March 2020 were reviewed and the laboratory values of Hemoglobin levels and RBC count of women patients undergoing complete hemogram were collected from the case sheets of . The age, Hb% and RBC values were collected. The collected data was tabulated in Excel. It was then imported to SPSS software. Descriptive statistics was performed for the obtained results and comparison between groups were done by using Chi square tests. $p<0.05$ was considered as statistically significant. The prevalence of Anemia among the total women patients was 39.1%. Among the 39.1% women who were diagnosed with anemia, it was found to be more prevalent in the women of age group 40-60 years with a frequency of 17.8%. The most commonly observed range of Haemoglobin is 12-13g/dl with a frequency of 26.6% . 0.6% of women had RBC count less than 3 million cells/mm^3. A significant number of female patients were tested with abnormal results. The most common age group diagnosed with Anemia are women belonging to 40-60 years of age. Routine hemoglobin testing in Dental practice of population at risk may be beneficial. Screening of Anemia is essential to avoid late diagnosis.

Keywords: *Anemia;Haemoglobin; Investigations; Diagnosis;Treatment*

Introduction

Anemia is a condition which causes the deficiency of red blood cells or haemoglobin in the blood, which results in low oxygen carrying capacity of the blood and produces signs and symptoms like pallor and weakness.

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[(1)] Anemia affects one third of the global population and is a major factor that causes increased morbidity, mortality, decreased productivity of work, and impaired physiological and neurological development. The normal range of hemoglobin for men is 13.5-17.5g/dl whereas for women it is 12- 15.5g/dl[(2)]. There is a need to understand the different and sophisticated etiology of anemia to emphasize on the prevention and control programmes of anemia[(3)]. It is considered as a largely preventable and treatable public health issue if detected on time and strategies for its prevention are well documented[(4)(5)].

Anemia needs to be correctly defined and identified by establishing appropriate Hemoglobin thresholds, and thereby preventing its negative impact on health. For the

development of the required interventions that address the causes of anemia which are content specific and to monitor the success rate of anemia prevention programs, understanding the etiopathology of anemia is essential[5,6]. The concentration of Hemoglobin varies naturally by gender, age, pregnancy status, genetics, environmental factors, economic status and geographical factors [7]. The clinical features of anemia are predominantly non-specific which includes conjunctival pallor, fatigue, dyspnea, and tachycardia. After the diagnosis of anemia, the mean corpuscular volume (MCV) should be checked to differentiate between microcytic, normocytic, and macrocytic anemia which thereby determines the successive steps in diagnosis and treatment. Reticulocyte count can also be checked to evaluate any abnormalities of the bone marrow. Treatment for anemia depends on the type of anemia present and the underlying condition. In cases of acute/severe anemia , blood transfusion may be required

The gender differences in Hemoglobin concentrations usually begins at puberty. It is due to the effect of menstruation, depletion of iron stores and which subsequently causes anemia. This continues throughout the reproductive phase of the individual.[8] In females, during pregnancy, there is expansion of blood volume which consequently has a dilution effect. Therefore the concentration of Hemoglobin declines naturally during the first and second trimesters of pregnancy [9]. Social behavior and environmental conditions like smoking, altitude, also affects the concentration of Hemoglobin. During their reproductive phase and pregnancy women are at a high risk of anemia, which consequently leads to maternal morbidity and mortality [10][11]. Both anemia and lower BMI are indications of poor nutritional status. Poor diet quality may end in the lower bioavailability of dietary iron, thus increasing the danger of developing anemia[12][13].

Anemia is diagnosed typically by the measurement of Hemoglobin and Hematocrit(HCT)[14][15].Reducing the burden of anemic disorders in women includes understanding risk factors, making rapid, accurate diagnoses when symptoms occur, implementing appropriate therapies, and monitoring treatment [16,17,18] Since anemia is typically detected through routine blood tests, laboratory diagnostic testing plays an integral role in caring for ladies throughout the continuum of anemia treatment [19,20]

This study aims to assess and study the prevalence of Anemia among women patients visiting a Dental hospital and the possible factors associated with it. In a Hospital setting patients undergo routine blood investigations before procedures[21]. This study helps in identifying anemic patients who are otherwise not diagnosed and those who do not visit physicians with symptoms of Anemia as a chief complaint. Identifying and treating the patients is the best way to address the disease that is most often unreported and treated despite wide prevalence[22]. This study explores the prevalence of anemia among women who were the outpatients of a Dental hospital and the significant role played by the hospital in identifying and addressing the diseases.

Materials and Method

Study setting:

This study was conducted as a retrospective study in a hospital setting. The sample taken for the study are predominantly South Indians. The study setting had certain advantages like flexibility in data collection and less expenditure. The ethical approval for the current study was obtained from the Institutional Review Board.

Study sample:

We reviewed the patient records and analysed data of 86,000 patients between June 2019 to March 2020. All the case sheets of female patients were collected and reviewed. The laboratory values of Hemoglobin levels and RBC count of women patients undergoing complete hemogram were collected from the case sheets of patients undergoing Complete Blood Count in the In-house laboratory.

Diagnostic criteria and data Collection:

The inhouse laboratory uses an automatic analyser (Sysmex 3 part XP 300) for Complete blood count analysis from venous blood. The diagnostic criteria for Anemia is based on Hemoglobin levels and RBC count. The necessary data such as Age, Hb level, RBC count were collected. State For hemoglobin, the cut-off criterion indicating anemia was the WHO cut-off of 12 g/dL for females and RBC for females was considered normal in the range of 4200–5800/mm3. Values below

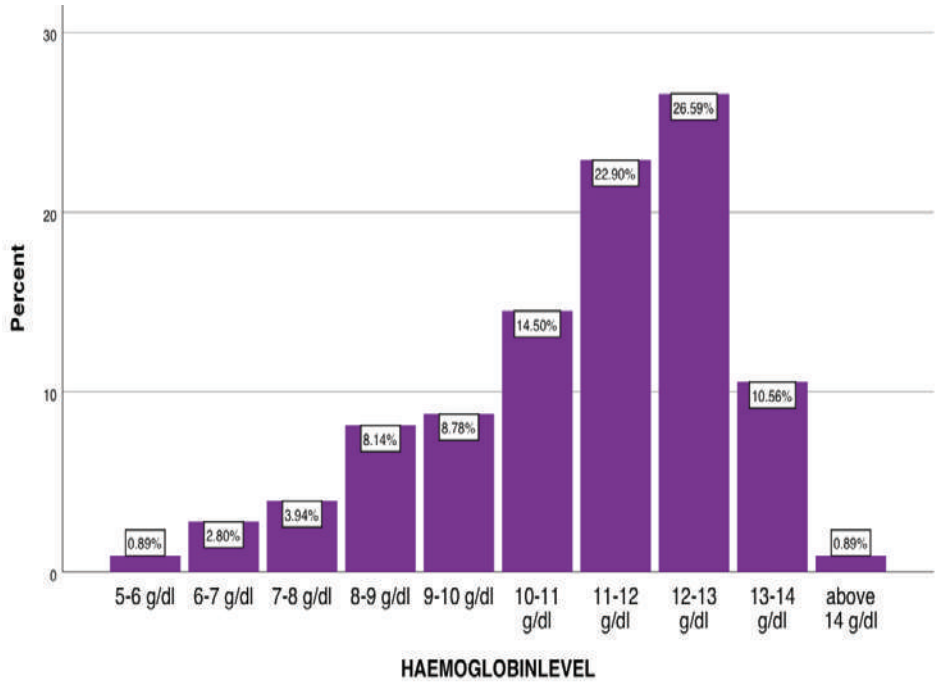
this were considered anemic[23]. The data was tabulated in Microsoft Excel 2016. The Data was cross verified by the analyser. Incomplete data of patients was excluded from the study to reduce errors. The tabulated data from Excel is imported to SPSS software for Windows (Version 20.0, SPSS, Inc, Chicago, USA) for statistical analysis. Descriptive statistics was performed for the obtained results and comparison between groups were done by using Chi square tests. $p<0.05$ was considered as statistically significant.

Results and Discussion

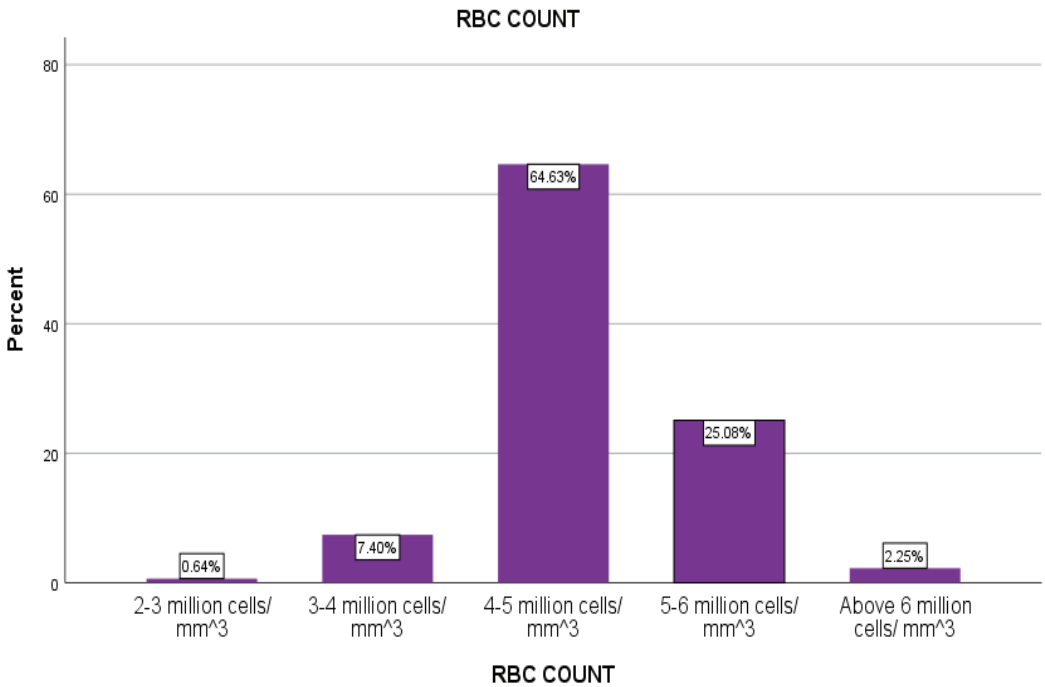
The total study sample consists of 786 female patients undergoing blood investigations.The prevalence of Anemia in the total population is 39.1% of the women since their Hemoglobin level was less than 12 g/dl. In the total study, 18.3% belong to 1-20 years age group, 34.2% belong to 20-40 years age group , 37.9% belong to 40-60 years and 9.6% of them belong to the age group above 60 years (Table 1) .The most commonly observed range of Hemoglobin level is 12-13g/dl with a frequency of 26.6% followed by 11-12g/dl with a frequency of 22.9%(Graph 1). In case of RBC count, the commonly observed range was 4-5 million cells/mm^3 with a frequency of 64.6% followed by 5-6 million cells/mm^3 with a frequency of 25.08%. 0.6% of the population had RBC count less than 3 million cells/mm^3(Graph 2). The lowest value of Hemoglobin level was 5 g/dl found in the age group of 60-70 years. Anemia was found even in children< 10 years. Women in the age group of 10-30 years had as low as 6-7g/dl. 0.9% of the female patients had the lowest hemoglobin level of 5-6 g/dl.However there is no statistically significant difference in Hemoglobin levels among different age groups(Graph 3). RBC count as low as 2-3 million cells/mm^3 were found in the age group of 50-70 years with a prevalence of 0.6%. RBC count above 6 million cells/mm^3 were most prevalent in the age group 40-50 years (1%) (Graph 4). Anemia was found to be more prevalent in the age group 50-60 years with a frequency of 9.41% followed by 40-50 years with a frequency of 8.40% (Graph 5).

Age	Percentage
1-10 years	12.8%
10-20 years	5.5%
20-30 years	14.0%
30-40 years	20.2%
40-50 years	23.7%
50-60 years	14.2%
60-70 years	7.8%
70-80 years	1.8%

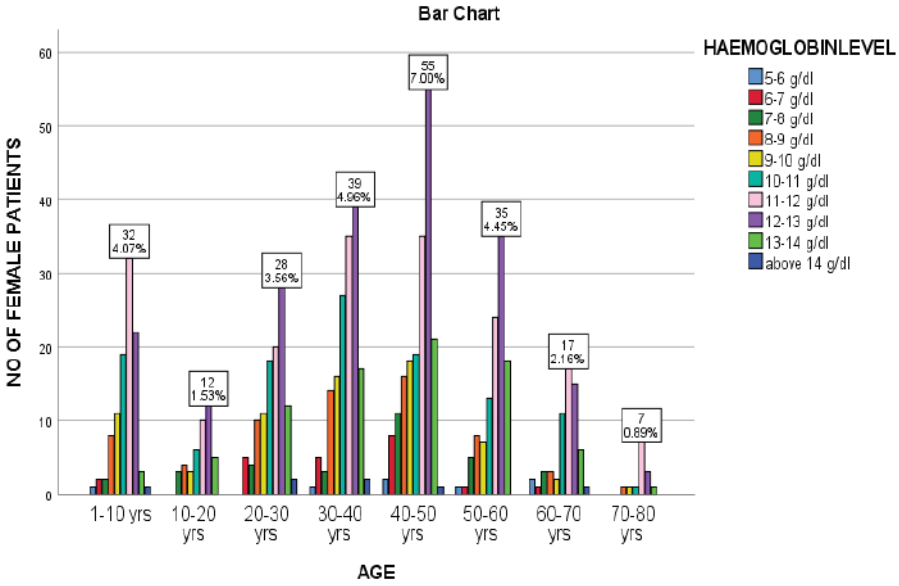
Table 1 - Table showing the percentage of age distribution of female patients taken for the study. 18.3% belong to the 1-20 years age group, 34.2% belong to the 20-40 years age group , 37.9% belong to the 40-60 years and 9.6% of them belong to the age group above 60 years.



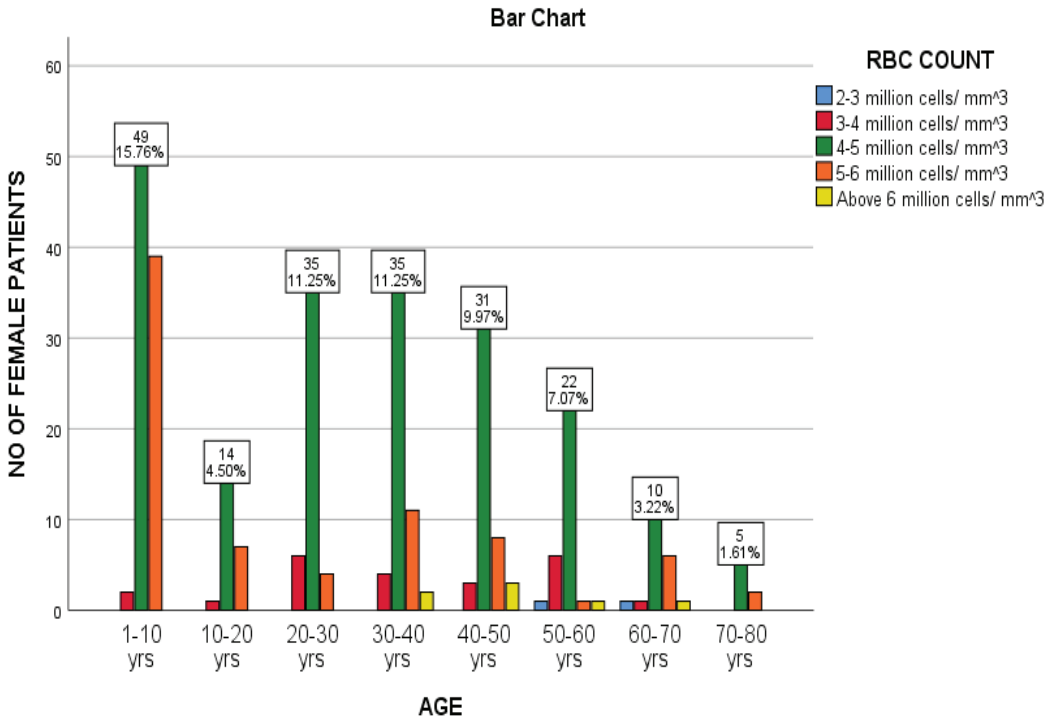
Graph 1 - Bar chart showing the frequency of Hemoglobin levels of Female patients. X axis denotes the range of Hemoglobin levels and Y axis denotes the percentage of distribution. The most commonly observed range of Hemoglobin level is 12-13g/dl with a frequency of 26.6% followed by 11-12g/dl with a frequency of 22.9%. The mean hemoglobin value was found to be 11.22g/dl.



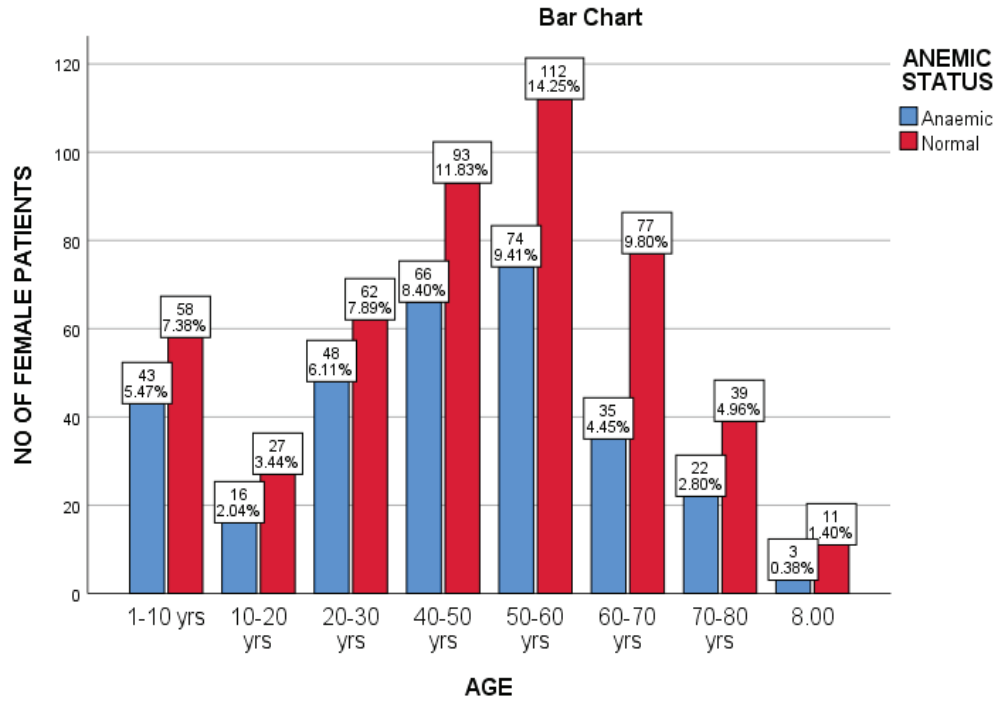
Graph 2 - Bar chart showing the frequency of RBC count of Female patients. X axis denotes the range of RBC count and Y axis denotes the percentage of distribution. The commonly observed range of RBC count was 4-5 million cells/mm^3 with a frequency of 64.6% followed by 5-6 million cells/mm^3 with a frequency of 25.08%. 0.64% of the population had RBC count less than 3 million cells/mm^3



Graph 3 - Bar chart showing the comparison of Hemoglobin levels in different age groups among the female patients. X axis denotes the age group of the patients and Y axis represents the number of patients. The lowest value of Hemoglobin level was 5 g/dl and was found in the age group of 60-70 years. 0.9% of the female patients had the lowest hemoglobin level of 5-6 g/dl. There is no major difference in Hemoglobin levels among different age groups. Chi square test was done and p value was found to be 0.696 (p value > 0.05, statistically not significant)



Graph 4- Bar chart showing the association of age groups and RBC count in females in the study population . X axis denotes the age group of the patients and Y axis represents the number of patients. RBC count as low as 2-3 million cells/mm^3 were found in the age group of 50-70 years with 0.6%. RBC count above 6 million cells/mm^3 were mostly seen among the age group 40-50 years (1%). Chi square test was done and p value was found to be 0.000 (p value < 0.05 , statistically significant)



Graph 5- Bar chart showing the association of age groups and Anemia in females in the study population. X axis denotes the age group of the patients and Y axis denotes the number of patients.Blue colour represents anemic patients and Red colour represents normal patients. Anemia was mostly seen in the age group 50-60 years with a frequency of 9.41% followed by 40-50 years with 8.40%. There is no major difference in anemic status among different age groups. Chi square test was done and p value was found to be 0.437 (p value >0.05, statistically not significant)

The purpose of this study was to identify anemic patients who are otherwise not diagnosed and those who do not visit physicians with symptoms of Anemia as a chief complaint. This study explores the prevalence of anemia among women visiting a Dental hospital and the possible role played by the institution in identifying and addressing the diseases. This study shows that a significant number of female patients were diagnosed with anemia. The prevalence of anemia among the women patients visiting the Dental hospital was found to be 39.1%. This can be considered as a moderate public health issue. Poverty in India is a major barrier to positive health outcomes for both men and women,but poverty tends to yield a higher burden on women and girls' health due to malnutrition and unsafe fuels. The prevalence was found to be higher than some previous studies[24]. According to the World Health Organization, 54.8 % of women around 15-49 years and 50.4% of children under 5 years of age were reported to be anaemic in Tamil Nadu by a National Family Health Survey. This had reflected even in our present study. The possible reasons could be lack of awareness, Geographical variations, poor socioeconomic status and nutrition deficiency such as Iron deficiency and Vitamin B-12 deficiency [25,26,27].

In this study anemia was found to be more prevalent among the middle aged women belonging to 40-60 years of age with a frequency of 17.8% followed by the age group of 20-40 years. The possible reason could be Iron deficiency which can be associated with Cardiovascular disease in middle aged women. Also in post menopausal women, there is an increased risk of Anemia.

Iron deficiency anemia in post menopausal women occurs due to gastrointestinal bleeding or malabsorption [27]. The prevalence of anemia in younger age groups is less compared to some of the previous studies.[28]The difference could be due to geographic variations and socioeconomic status which affects their nutrition and health. Women of reproductive age have an additional risk of developing anemia because of periodic menstrual blood loss. [29] Also women have extra iron requirements

especially during pregnancy for the proper development of the foetus. In fact, pregnant women need 50 percent more iron than usual (27 mg per day rather than the standard 18 mg per day). There is also loss of blood in women during childbirth. It is essential for females who are pregnant to keep their Hemoglobin and Iron levels checked and to intimate their caretakers if they have one or more symptoms of anemia.[30] Iron and Vitamin B12 supplements can be taken regularly which may help prevent low iron levels and pregnancy-related anemia. In the study, the most commonly observed range of Hemoglobin level is 12-13g/dl with a frequency of 26.6% followed by 11-12g/dl with a frequency of 22.9%. [31] The mean hemoglobin value was found to be 11.22g/dl which was found to be related to previous studies conducted in Tamil Nadu due to the same geographical location and economic conditions of the people. [32]

The normal range of RBC count for females is 3.5-4.5 million cells/mm^3 . In this study, 0.6% of women had RBC count less than 3 million cells/mm^3. . The commonly observed range of RBC count in this study was 4-5 million cells/mm^3 with a frequency of 64.6%. In many cases the RBC count may be normal but the Hemoglobin level may be decreased. [33]This situation occurs with iron-deficiency anemia, during which red blood cells have less hemoglobin than normal. Iron deficiency anemia is additionally mentioned as hypochromic anaemia .[34] Decrease in RBC count may be caused due to Anemia, Bone marrow failure, Erythropoietin deficiency, Hemolysis or destruction of RBCs , Internal or external bleeding and leukemia. Assessment of both RBC count and Hemoglobin level is essential for the diagnosis of Anemia. Anemia is associated with imapired survival and health related quality of life. It may cause adverse effects in the health of an individual especially women, which hinders their day to day activities[35]. It may also cause complications in their later stages of life. A pan European study concluded that anemia affects the quality of life and their testing awareness is quite low. [36] So, in order to reach out to the patients in a populated country like India, a Dental hospital might function along with primary health care facilities to improve the health status and quality of life of these patients thereby contributing to women health.[37,38]Hence the early diagnosis and treatment of Anemia is essential for a healthy living. The study is limited by a few factors. The sample size can be expanded and it is a short duration study. The quality of life is not assessed. Some of the clinical features of anemia may not be known and may be left undiagnosed . The study also has geographical limitations as it was done in a hospital setting. A large sample size of people from different ethnicities would give better results for the study. Further study can be done to classify the anemic patients as mild, moderate or severely anemic.

Conclusion

A significant number of female patients were tested with abnormal results. The most common age group diagnosed with Anemia are middle aged women. Routine Hemoglobin testing in Dental practice of populations at risk or diagnosed with Anemia may be beneficial. Screening of Anemia is essential to avoid late diagnosis and better treatment options. An overall higher prevalence of Anemia suggests the necessity of developing appropriate nutrition intervention . Further research studies on anemia are recommended to estimate the prevalence and determinants of Anemia on a large scale. High prevalence of anemia in Indian population, especially females may cause a burden for them, their family, and for the economic development and productivity of the country. Iron supplementation programs, for a spread of reasons, haven't been effective in reducing anemia prevalence and operational research on how best to enhance existing iron supplementation programs is required .New and innovative strategies are needed, particularly people who improve the general health and nutrition status of adolescent girls before they enter their reproductive years.

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An Estimation of Haemoglobin Levels in Children with Early Childhood Caries- A Retrospective Study

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Abstract

Aim: This study aimed to evaluate Haemoglobin (Hb) levels in children with Early Childhood Caries (ECC) and to find out whether there is any variation from normal values in Hb level in children with early childhood caries.

Materials & Methods: In this retrospective study the Hb levels and dmft status of 281 children with ECC in the age group of 0 to 6 years were evaluated. The mean Hb values calculated were compared with the suggested normal levels by the World Health Organisation for the particular age group. Kruskal Wallis test and post hoc test comparisons were done to compare differences in Hb levels in 4 different groups based on dmft values in children with ECC.

Results: Overall mean value of Hb in children with ECC was 10.93 ± 1.73 g/dl and mean dmft was 7.19 ± 4.3. Group 1 with less than 5 dmft value showed significant difference in Hb values compared to children in groups with higher dmft values ($p \leq 0.05$).

Conclusions: Half of the study population had low haemoglobin levels i.e. below that of WHO criteria. Children with more than 5 dmft score had significantly lower Hb levels compared to children with less dmft values ($p \leq 0.05$). There was no significant difference noticed in the Hb levels in between boys and girls with ECC. This descriptive study results point that ECC can result in low Hb levels in children, however authors suggest that an analytical study with a control group should be done to test the hypothesis.

Key Words*: Anaemia, Children, Early Childhood Caries, Haemoglobin Levels, dmft.*

Introduction

Early childhood caries (ECC) is a major health problem affecting children all around the world. The impact of early childhood caries on childrens' health is enormous, affecting general development, nutritional intake, growth of the child etc. According to American Academy of Pediatric Dentistry "Caries is a biofilm (plaque)-induced acid demineralization of enamel or dentin, mediated by saliva. The disease of early childhood caries (ECC) is the presence of 1 or more decayed (non cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger. In children younger than 3 years of age, any sign of smooth-surface caries is indicative of severe early childhood caries (S-ECC). From ages 3 through 5, 1 or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces constitutes S-ECC" [1] . Oral health should be of utmost importance in any age but it is very significant in early

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childhood. Since it is the phase during which most of the oral and overall development of the child takes place.[2] Dental caries is usually referred to as a multifactorial disease which affects the hard & soft tissues of a tooth. It is otherwise known as tooth decay.[3,4]

Early childhood caries, a serious dental health issue affecting infants and toddlers is prevalent all around the world[5] . Reported prevalence of ECC in various countries was, in USA it's 11% - 53.1%, England it's 6.8% - 12%, Asia (Far East) it's 36% - 85%, and in India it's 44%.[6–10] Early detriment of oral health due to early childhood caries may cause permanent damage to the underlying permanent dentition. Also when left untreated it could affect the nutritive intake of the child ultimately affecting the development of the child.[11] Nutritional impact in children with ECC can be a reason for low Hb levels in children.

Diet and nutrition being an important risk factor for ECC, iron deficiency i.e. lack of iron is one of the most frequently observed dietary deficiency all around the globe.[12,13] World Health Organisation -WHO states that anaemia in children less than 6 years of age as Haemoglobin (Hb) levels less than 11g/dl[14]. WHO also states that prevalence of anaemia is the greatest in preschool children which is 47.4%.[15]

Many studies show correlation between early childhood caries and nutritional status [16–18] India being a developing country is still coping with malnutrition, poverty, lower socio- economic status among the population which is undeniable.

ECC can influence the nutritional intake of children due to tooth pain and sensitivities. There are very limited studies conducted among the south Indian population evaluating Haemoglobin levels and its relation with early childhood caries.

Our department is passionate about child care, we have published numerous high quality articles in this domain over the past 3 years[19,11,20–32]. With this inspiration we planned to pursue research on Haemoglobin (Hb) levels in children and to find out whether there was any relation with early childhood caries.

Materials and Methods

Study Design & Study Setting

This retrospective descriptive cross sectional study evaluated the records of patients who visited the university dental hospital situated in Tamilnadu, India from June 2019 - March 2020. Study was carried out after obtaining approval from the institutional ethical review board, ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320 and informed consent from the parents. The study population included patients age ranging from 0 to 6 who visited the pediatric dentistry department and underwent blood tests for pre anaesthetic evaluation before treatment for ECC under general anaesthesia. Special children and children under any medication which can influence Hb levels such as iron supplements or with any systemic condition were excluded from the study.

Data Collection

After analysis of 86000 records by two clinicians, 1519 patients' records of age ranging from 0-6 were obtained. Out of 1519 , 304 patients with early childhood caries who underwent haematological investigations for treatment of ECC under general anesthesia were shortlisted. Record of 304 children underwent blood tests were retrieved. After exclusion of 23 records which did not match the inclusion criteria a final sample of 281was obtained. Two clinicians collected and analysed the data after which it was cross verified and examined by another examiner to prevent any discrepancies. The required data such as age, gender, Hb levels, dmft status etc were collected.

Statistical Analysis

Data was obtained and filtered using (Microsoft Corporation. Microsoft Excel [Internet].2018.) and was then exported to (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) for further statistical analysis. After data collection descriptive statistics such as calculation of mean Hb levels, dmft values were performed. The mean values of Hb levels obtained were compared with the suggested normal levels by the World Health Organisation for the particular age group of the study. Point biserial correlation analysis was done to find out the relationship between Hb levels of children with ECC

and gender. Based on dmft values children with ECC were categorised into 4 groups to evaluate the severity of ECC in influencing the Hb values . The 281 participants were categorised into various groups based on dmft values for severity of ECC into, first group (n=124) had a dmft ≤5, the 2nd group (n=79) had dmft values between 6 to 10, the 3 rd group (n= 67) had dmft values between 11 to 15 and the 4th category (n=11) dmft ≥16. After homogeneity testing of the Hb values it was found that there were outliers in observations hence the Kruskal Wallis test and Post Hoc test were done to find out the differences in Hb levels between groups based on dmft values & severity of ECC. The confidence interval was set at 95 % at 0.05 level of significance.

Results

This retrospective study included 168 males (59.79%) & 113 (40.21%) females based on the inclusion and exclusion criteria , the study population had patients who had early childhood caries and were undergoing treatment of age ranging from 1-6. Frequency distribution of the age groups are as follows, age group 3 was the most frequently occurring age group in the given study population (42.35%) followed by, age group 4 patients (29.18%), age group 2 (15.66%), age group 5 (9.25%), age group 6 (2.55%) and age group 1 (0.71%).

Hb level ranges in males 5.1-15.4 g/dl,in females 5.6-14.1 g/dl. Overall range 5.1-15.4 g/dl. Mean value of Hb in Males 10.85 ±1.75 g/dl,in Females 10.87 ±1.65 g/dl. Overall mean value of Hb 10.93 ± 1.73 g/dl. WHO states Hb levels below 11g/dl are considered to be anaemic in children below 6 years of age [17]. 50 percentile of the participants had Hb levels below 11g/dl, 25 percentile had Hb values <10g/dl, 10 percentile had Hb levels below 8.5g/dl and 5 percentile had levels below 7.5 g/dl. It was found that almost half of the study population had Hb levels below that of WHO criteria. There was no correlation or difference in the Hb levels in boys and girls with ECC [Figure:2, 3].

The decayed, missing and filled tooth index (dmft) of all the study participants were assessed, mean value of the DMFT index was found to be 7.19 ± 4.33. Mean Hb levels of different groups based on severity of ECC was found to be, group 1 (dmft ≤5) was 11.92 ± 1.3 g/dl, group 2 (dmft values between 6 to 10) was 10.63 ± 1.53 g/dl, group 3 (dmft values between 11 to 15) was 9.64 ± 1.68 g/dl and of group 4 (dmft ≥16) was 9.74± 1.94 g/dl (table 1). Kruskal Wallis test showed a significant difference between the groups ($p \leq 0.05$) and a post hoc test was done to find out which group differed significantly. It was found that group 1 differed significantly ($p<0.05$)compared to groups 2, 3 & 4 (table 2). This showed that children with higher dmft values had significantly lower Hb levels compared to children with dmft values ≤5 .

Table 1 : Categorisation of children with ECC into groups based on dmft values and mean Hb and dmft values of each group shown.

Groups	Mean dmft value of each group	Mean Hb level of each group (g/dl)
Group 1 (dmft ≤ 5) n=124	3.23 ± 1.35	11.92 ± 1.20
Group 2 (dmft 6 to 10) n=79	7.67 ± 1.25	10.63 ± 1.53
Group 3 (dmft 11 to 15) n=67	12.43 ± 1.34	9.64 ± 1.68
Group 4 (dmft ≥16) n=11	16.63 ± 1.03	9.74 ± 1.94
Total Sample : Children with ECC(n=281)	Overall mean dmft value 7.19±4.33	Overall mean Hb Value 10.93±1.73

Table 2: Post Hoc Test Comparison of Hb levels of children with ECC categorised into different groups depending on dmft values observed. Kruskal Wallis test showed a significant difference between the groups ($p < 0.05$). Results showed that Hb levels of group 1 i.e of children with dmft level ≤ 5 differed significantly in there Hb levels compared to other group children with higher dmft values.

Post Hoc Comparisons	Significance value ($p \leq 0.05$ considered significant)
Group 1 vs Group 2	0.000
Group 1 vs Group 3	0.000
Group 1 vs Group 4	0.000
Group 2 vs Group 3	0.006
Group 2 vs Group 4	.907
Group 3 vs Group 4	1.000

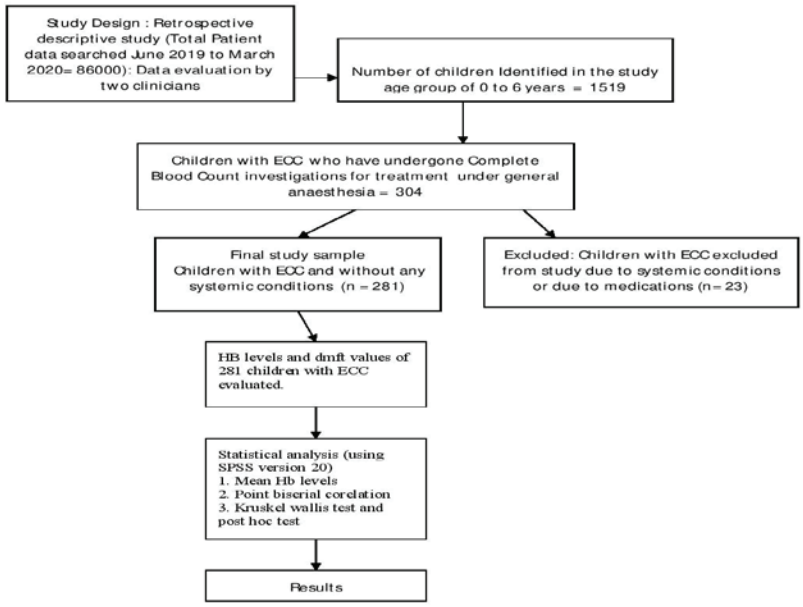


Figure 1 : Flowchart showing the study methods and design.

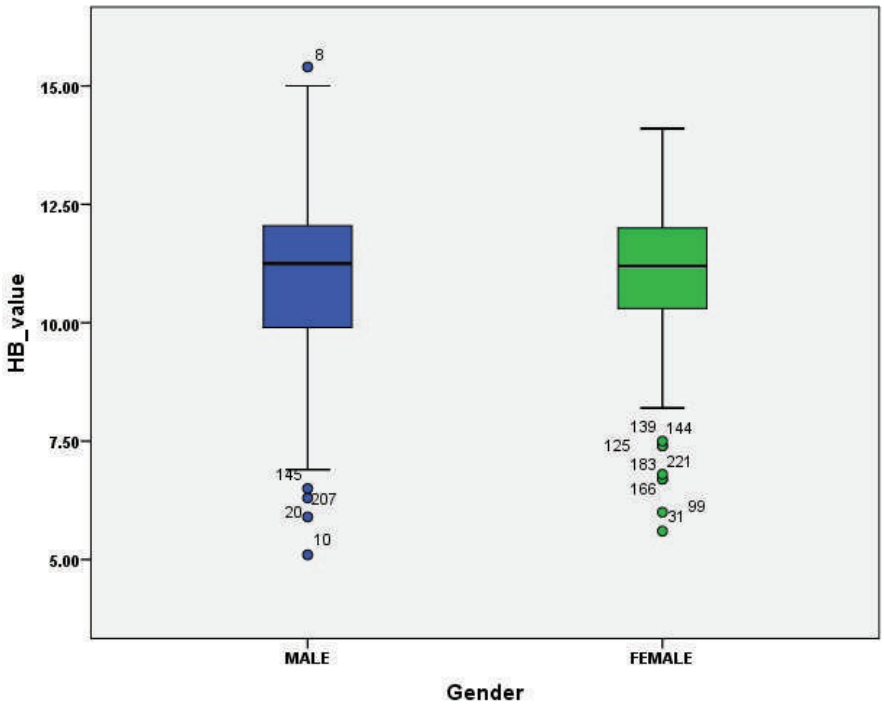


Figure 2: Box plot depicting correlation between Hb levels in boys and girls with ECC. X axis depicts the gender and the Y axis represents the Hb levels of the participants. Multiple outliers can be noticed as dots in the plot suggesting unusual low levels of Hb in some participants. Point biserial correlation coefficient was elicited and value was found to be .007 (close to zero) suggesting no significant differences between Hb levels of boys and girls with ECC.

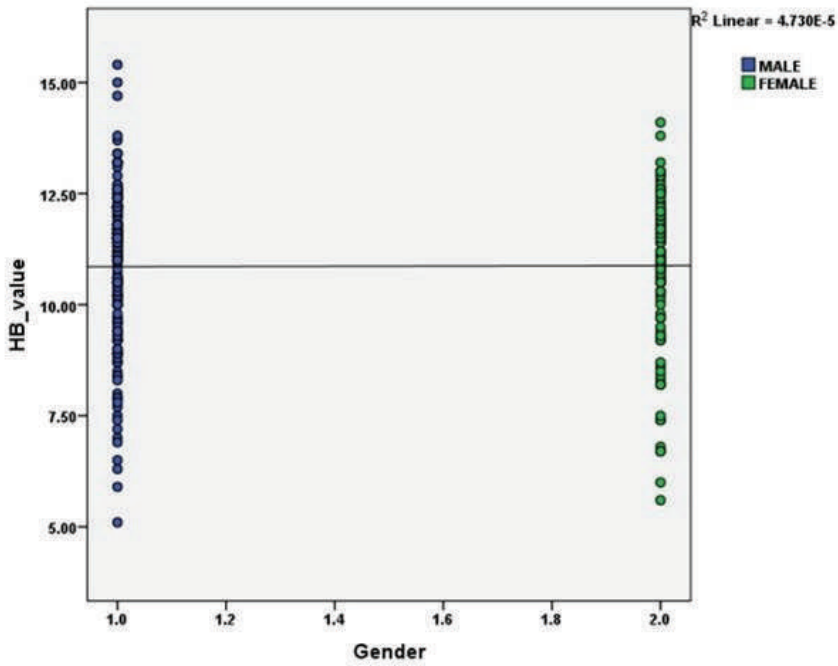


Figure 3: Scatter plot depicting the correlation between gender and Hb levels. X axis denotes gender and Y axis denotes the Haemoglobin level in g/dl.Blue represents male gender and green represents female gender. Point biserial correlation coefficient was elicited and value was found to be .007 (close to zero) suggesting no significant differences between Hb levels of boys and girls with ECC

Discussion

This study was based on the south indian population who visited a university dental hospital in Chennai, Tamil Nadu. To our knowledge this was the first study done in Chennai to evaluate Hb levels in children with early childhood caries.

Oral health and hygiene is often neglected among children in the south indian population.[27] Half of our study population with early childhood caries had Hb levels below that of 11g/dl which is the suggested normal level for the age group of study by WHO. This could be due to various factors such as improper diet and nutrition which plays a major role in Hb level deficiency. Diet & nutrition plays a major role in relation to early childhood caries.[33] India being a developing country, factors such as the socioeconomic status might affect the diet, nutrition and food habits leading to iron deficiency anemia in children aged 5 and below.[34]

Mean value of Hb in Males 10.85 ± 1.75 g/dl, in Females 10.87 ±1.65 g/dl .Overall mean value of Hb 10.93 ± 1.73 g/dl. It was noticed that half of our study population had Hb levels lower than what is suggested for the age group by WHO. There was no relation found between the gender of children with ECC and Hb levels. However male population had a higher frequency of 59.79% and this is similar to another study done in Puducherry which assessed age, gender, weight and height association with early childhood caries.[35]

Age group 3 was the most frequently occurring age group in the given study population 119 patients (42.35%) followed by age group 4, 89 patients (29.18%) and as such. Age group 1 had the least mean Hb value of 10.15 g/dl, however this value was not statistically significant to show that this age group was more prone to have low Hb levels, thus depicting age is not directly associated with Early childhood caries & Hb Levels and is supported by few similar studies.[35,36]

A study done in Canada by Clarke et al reported that almost 28% of their study population with early childhood caries had low Hb concentration levels.[37] Another study done by Bansal K et al depicted strong association of anaemia due to iron deficiency[38]. A study done by Koppal P et al. showed that there is no direct relation of Early childhood caries with Hb levels but are interrelated. These above study results are in accordance with our study.[39]

Another study done by Schroth et al depicted that children with ECC have lower haemoglobin levels and are most likely to have Iron deficiency anaemia. He found that 75% of the children with Early childhood caries had low Hb levels, which is also confirmed by another study by Janaki Ram et al [40,41] . In another study done by Hashemi et al it was found that early childhood caries could be a risk factor for low Hb levels.[42]

From the above discussion it is very evident that early childhood caries & low Hb levels are interrelated but it is still inconclusive whether early childhood caries is the reason for low Hb levels. The evaluation of dmft score and categorisation of children into 4 groups based on dmft score in this study showed that children with higher dmft values had significantly lower haemoglobin levels compared to children with dmft values less than 5. This clearly indicated that early childhood caries is influencing the Hb levels in children. However our study is a descriptive study without a control group due to ethical reasons. Hence an analytical case control study will be more beneficial in establishing a clear consensus.

It was noticed that half the children with ECC had Hb levels below the WHO criteria for the age group. Since both Hb levels & early childhood caries play a major role and might affect development of a child, it is essential to conduct further case control or longitudinal studies in future to understand the association between Hb levels and ECC.

Anaemia in children with Early childhood caries will not only affect the primary dentition but as well as can affect the overall systemic health. Proper awareness and knowledge regarding the relationship between oral health and systemic health is important. Low levels of Hb in children with ECC can affect the systemic health which in turn can affect the quality of life. This study throws light to the fact that oral health in children cannot be neglected, if neglected can have systemic health implications. Hence adequate preventive and therapeutic measures against ECC should be taken by health providers for overall well being of children.

Our study had few limitations such as unequal male/female ratio,unequal age ratio, control group of normal

children were not available due to ethical reasons, and also the reference value for comparison is based on generalised criteria by WHO which is not derived from the same south indian population.

Conclusion

Despite the limitations of the study it can be concluded that half of the study population had low haemoglobin levels i.e. below that of WHO criteria. Children with more than 5 dmft score had significantly lower Hb levels compared to children with less dmft values ($p \leq 0.05$). There was no significant difference noticed in the Hb levels in between boys and girls with ECC. This descriptive study results point that ECC can result in low Hb levels in children, however authors suggest that an analytical study with a control group should be done to test the hypothesis.

Conflict of Interest: None declared.

Source of Funding : Self

Ethical Approval: Study was carried out after obtaining approval from the institutional ethical review board, ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320 and informed consent from the parents

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Novel Gene Mutations Associated with Crouzon Syndrome

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Abstract

Crouzon syndrome exhibits consideration of phenotypic heterogeneity, within the aetiology of which genetics play a crucial role. The *FGFR2* gene mediates extracellular signals into cells and mutations within the *FGFR2* gene cause Crouzon syndrome. The review summarizes the genetic phenotype study and genetic evaluation related to Crouzon syndrome (CS) which frequently determines the degree of complexity, guide management, guidance and intervention related to this craniofacial defect. CS is a disorder characterized by early fusion of certain skull bones (craniosynostosis). This prevents normal growth of the skull, which may affect the form of the top and face. Signs and symptoms of Crouzon syndrome may include wide-set, bulging eyes; strabismus (misalignment of the eyes); "beak-shaped" nose; and an underdeveloped upper jawbone. Other features may include dental problems, deafness, and/or harelip and palate. The severity of signs and symptoms can vary among affected people, even within a family. Intelligence is typically normal, but intellectual disability could also be present. Crouzon syndrome is caused by changes (mutations) within the *FGFR2* gene and is inherited in an autosomal dominant manner. Treatment may involve surgeries to stop complications, improve function, and aid in healthy psychosocial development.

Keywords: *Crouzon syndrome; genotype; molecular pathology; phenotype.*

Introduction

Craniosynostosis is a birth defect characterized by premature fusion of one more of the calvarial sutures before the complications of brain growth and development, leading to restricted growth of the skull, brain, face and central nervous system development[1]. Among craniosynostosis are valued to comprise 15% of all cases. Crouzon syndrome (CS) is a genetic disorder characterized by the premature fusion of certain skull bones (craniosynostosis)[2]. Many features of CS result from the premature fusion of the skull bones. Abnormal growth of those bones results in wide-set, bulging eyes and vision problems caused by shallow eye sockets; eyes that don't point within the same direction (strabismus); a beaked nose; and an underdeveloped upper jaw. In addition, people with CS[3] may have dental problems and hearing loss, which is sometimes accompanied by narrow ear canals. A few of the CS patients have an opening in the lip and at the root of the mouth[4]. The severity of those signs and symptoms varies among affected people. Individuals with Crouzon syndrome usually have normal intelligence [5,6].

Frequency of CS is seen in about 16 per million newborns. It is the most common craniosynostosis syndrome. Crouzon syndrome cs is a most commonly seen syndrome. It is a genetic disorder related to multiple fibroblast growth factor receptor 2 (*FGFR2*) mutations[7,8]. *FGFR2* modulates the extracellular signals into the cells and several mutations in the *FGFR2* gene have been associated with CS. *FGFR2* along with the other genes of the family *FGFR, FGFR1* and *FGFR3* are related to signaling function in cranial sutures and play a crucial role in embryonic development of the limbs[9].

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This syndrome was first reported by Louis Edouard octave crouzon in 1912 when a triad of Calabrian deformations with craniofacial dysostosis, exophthalmos and facial anomalies was defined in a mother and her son[10]. Inheritance pattern of this condition is inherited in an autosomal dominant pattern, which means one copy of the altered gene in each cell is sufficient to cause the disorder.[11]

Materials and Methods

By reviewing various available studies, datas were collected through search engines, PubMed, google scholar, journals. The period of duration consideration of collected articles from 2000 - 2020. The dependent variables involved in this were about gene mutations associated with Crouzon syndrome.

Inclusion Criteria :

- Crouzon syndrome prevalence
- Novel mutations associated
- Pathways involved
- Pattern of inheritance
- Management of cs

Exclusion Criteria :

- other than crouzon syndrome were excluded from this study.

Normal Function *FGFR2* Gene :

The *FGFR2* gene encoded for a protein known as the fibroblast growth factor receptor 2 (FGFR2).[12] Fibroblast growth factor receptors governs the processes of cell growth and division (proliferation), cell maturation (differentiation), formation of blood vessels (angiogenesis), wound healing, and embryonic development.[13,14] The FGFR2 protein serves in the outer membrane of cells which are characterized by an extracellular, transmembrane and an intracellular domain.[15] Such a positioning allows the FGFR2 protein to bind to specific growth factors from outside the cell and transduce the signals which aids the cell to respond to its environment. The FGFR2 protein plays a crucial role in the development of bones before birth (embryonic development).[16] Early signals by the protein directs immature cells in the developing embryo to become bone cells and form the head, hands, feet, and other tissues.[17] Several isoforms of the FGFR proteins have also been identified in different tissues, whose patterns may change throughout growth and development.[18] The cytogenetic location of *FGFR* gene is 10q26.13.

Clinical Report of Crouzon Syndrome:

The first description about CS was given by Crouzon in 1912, who described it as a hereditary syndrome or craniofacial dysostosis in a mother and son. He described the calvarial deformities, facial anomalies, and exophthalmos. CS is characterized by premature closure of calvaria and cranial base sutures also as those of the orbit and maxillary complex (craniosynostosis). Other clinical features include hypertelorism, exophthalmos, strabismus, beaked nose, short upper lip, hypoplastic maxilla, and relative mandibular prognathism. Prevalence is 1 per 60,000 (approximately 16.5 per 1,000,000) live births. CS makes up approximately 4.8% of all cases of craniosynostosis.[19]

Genetics or Basic Defects Involved in Crouzon Syndrome

Inheritance:

CS is inherited in an autosomal dominant manner. This means that having a change mutation in just one copy of the responsible gene in each cell is enough to cause features of the condition. There is nothing that either parent can do, before or during a pregnancy, to cause a toddler to change state with CS. In some cases, an affected person inherits the mutated gene from an affected parent. In other cases, the mutation occurs for the primary time during a person with no case history of the condition. This is called a de novo mutation. When an individual with a mutation that causes an autosomal dominant condition has children, each child features a 50% (1 in 2) chance to inherit that mutation.[20]

Pathophysiology:

Premature synostosis of the coronal, sagittal, and occasional lambdoidal Suture. begins in the first year of life completed by the second or third year. Degree of deformity and disability determined by the order and rate of suture fusion. After fusion of a suture growth perpendicular to that suture becoming restricted. fused

bones acting as a single bony structure. Compensatory growth occurring at the remaining open sutures to allow Continued brain growth Multiple sutural synostosis often extend to premature fusion of the skull Base sutures causing the following effects such as midfacial hypoplasia, shallow orbit, a foreshortened nasal dorsum, maxillary hypoplasia,occasional upper airway.[21,22] Several *in vitro* studies carried out in our institution formed the basis for the present review on Crouzon syndrome.[23–36]

Recent Gene Mutations Associated With Crouzon Syndrome :

A plethora of mutations have been identified in several genes with close association with craniosynostosis. A case study reported by Luong et al., on a Vietnamese family of three generations identified a heterozygous *FGFR2* missense mutation (c.1012G>C, p.G338R) which was proposed to have been the candidate gene mutation associated with CS. The mutation was further screened in about 200 unrelated control subjects from the same population. The entire coding region or exon 10 of the *FGFR2* gene was amplified and sequenced. The heterozygous mutation was identified only in the patient with CS, while the other family members or controls did not show any such mutations in their gene. In addition to it a novel heterozygous mutation in the exon 11 of FGFR2 was seen in the CS patient which was considered to be a silent mutation.[39] In a case study reported by Korakavi et al., biallelic loss-of-function variant in the interleukin receptor 11 alpha gene (IL11RA) was identified in a patient with Crouzon-like craniosynostosis syndrome with associated dental anomalies. A total of six missense mutations were identified of which four were found to be novel, including 2 in the Ig-like C2-type domain.[37]

Similarly Keupp et al., [38]demonstrated that mutations in the *IL11RA* gene caused an autosomal recessive Crouzon-like craniosynostosis. The homozygosity mapping procedure followed by targeted next-generation sequencing identified a c.479+6T>G mutation in the interleukin 11 receptor alpha gene (IL11RA) on chromosome 9p21 in a family from Antakya, Turkey. The patients presented with features such as multiple suture synostosis, midface hypoplasia, variable degree of exophthalmos, relative prognathism, a beaked nose, and conductive hearing loss. [39]The segregation of mutations were further identified in five families: a German patient of Turkish origin and a Turkish family with three affected sibs all of whom were homozygous for the previously identified *IL11RA* c.479+6T>G mutation; a family with pansynostosis with compound heterozygous missense mutations, p.Pro200Thr and p.Arg237Pro; and two further Turkish families with Crouzon-like syndrome carrying the homozygous nonsense mutations p.Tyr232 and p.Arg292[40].

A report by Nur et al.,demonstrated several mutations in a cluster of patients with different craniosynostosis syndromes. Mis-sense mutations which leads to the substitution of one amino acid to the other viz., p.Trp290Arg, p.Cys342Tyr, p.Cys278Phe, p.Gln289Pro were identified along with a novel p.Tyr340Asn mutation. Ohishi et al., [41]reported a novel mutation in the *FGFR2* gene (p.Asn549Thr) in a CS patient. The team also identified p.Ser252Leu mutation is a CS patient who had phenotypically normal father. The evidence gathered through the present review of literature clearly identifies the *FGFR* family of genes playing a crucial role in craniofacial development.[42,43,44]

Conclusion

The advent of advanced molecular techniques have aided in understanding the genetic basis of craniofacial disorders. The genotype analysis in connection with a disease phenotype is required for proper evaluation of the diseases, to determine the pattern of inheritance and to ascertain the degree of complexity of the disorder. A clear outline about the genetic disorder would further help the clinicians to guide the process of disease management by providing genetic counseling and start with an appropriate treatment intervention.

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Knowledge, Attitude, Practice and Barriers Towards Research Among Dental Undergraduates: A Questionnaire Based Survey

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Abstract

Research in the field of medicine heightens the extent of understanding and also inculcates evidence-based practice and further enhances clinical skills. The impact of research ranges from prevention to newer and alternative treatment modalities. However undergraduate student research experience is plagued by a number of obstacles. This study aims to evaluate the knowledge, attitude, practice, and the barriers towards research among dental undergraduate students. An online questionnaire consisting of 16 questions which included demographic details, and questions regarding their knowledge and perception of barriers towards research. The study was conducted among undergraduate students of a private dental institute. Data was collected from filled questionnaires and analysed using SPSS software. A total of 169 undergraduate dental students participated in the online survey. A majority of students (78.3%) expressed a positive attitude towards willingness to conduct research. Barriers for research were lack of time being overburdened with education/ exams (30.7%), lack of allocated research time (25.5%), difficulty in following up study subjects (22.6%), inadequate financial support (21.9%%) and difficulty in obtaining approval (22.6%). Only 9.4% of the study population chose lack of interest as a barrier towards conducting research. Majority of the participants had sufficient knowledge regarding research and expressed a positive attitude toward participation in research activity. Major barriers were lack of time, difficulty in patient follow-up and inadequate funds.

Keywords: *Attitude; awareness; research experience; research barriers*

Introduction

Research is a systematic process that helps acquire new knowledge, science or invention by the use of standard guidelines.The improved understanding of scientific principles and methods is necessary for the conduction of research. The students should be acquainted with the methods in carrying out research as this will enable them as future doctors to practice evidence-based medicine in patient care. Scientific research can prove or disprove theories and hypotheses through a systematic approach [1]. Research in the field of health care is useful in the development of prevention, diagnosis and treatment of diseases and also affects healthcare policies. The healthcare curriculum has integrated scientific research as one of the important subjects [2]. Both developing and developed countries have expressed increased concern toward scientific research as research in the biomedical field can significantly influence medical care [3]. Medical research involves basic, applied, or translational research in the field of medicine conducted to assist and contribute to the development of knowledge [4]. Early exposure to health training research is recommended by the recent undergraduate education system. Studies have identified the factors which critically affect research as knowledge, attitude, experience, and barriers toward research. [5–8] .

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Research, not just in the medical field but also in every other field, is a way through which students and scholars apply the information they obtained through books onto tests and find new conclusions or confirm pre-established facts. Research assures understanding of the subject and improves the knowledge of students. Understanding the concept of evidence-based medicine needs strong research knowledge, and that can only be obtained through experience. Though nobody denies the importance of research in the medical field, it is still not given much importance when it comes to the undergraduate Indian medical curriculum [9,10].

Dental research in the Indian setting is in the developing stages even though we have more than 300 dental colleges, which is higher than many countries in the world. This comprises an extensive number of oral health care professionals, who can make a considerable impact in the advancement of the dental research situation in India [11]. Unfortunately, the dental research representation by India on the international stage is almost infinitesimal. Advancement of research in the dental field is at a remarkable pace worldwide. Research in dentistry inculcates evidence-based practise and helps in better understanding of the subject. It has explored areas in dentistry such as diagnostic aids for early detection of caries [12], use and safety of fluoride [13] [10]. Research in dentistry has also investigated dentists' attitudes towards certain treatment modalities [14] [15] and knowledge regarding various instrumentation techniques [16] [17]. Research of course primarily focuses on dental caries, the importance of maintaining oral health [18] and efficacy of newer tooth brushing systems [19]. Research in the dental field can help improve quality of treatment and enhance clinical skills to ensure optimal treatment. Research to improve treatment includes advancement in instruments ,comparative analysis of various instrumentation techniques and treatment outcomes [20] [21] [22] [23] [24] [25] .

Research serves as the foundation of improving health care. Research experience is an integral part of the curriculum of many postgraduate dental programs and some undergraduate courses in India[26]. Active involvement in research results in students being more likely to complete specialty training, become faculty members, and also contribute to future research.[27]. A number of strategies have been recommended to increase the involvement of undergraduate dental students in research.[28]. Undergraduate involvement in research is comparatively lower due to their extensive curriculum, lack of familiarity with research methodology, and insufficient time. When these students get into their post graduation program, their capability in writing a protocol or proposal is not satisfactory. Grants are being provided by some of the regulatory bodies to encourage undergraduates and postgraduates students to carry out research. Even though these facilities are provided by the regulatory organizations and the institutions to expand the research by undergraduate students, there is deficiency in the research quantity and quality. Increase in research participation requires sufficient knowledge and positive attitude toward research as an essential component [29]. Identification of the factors that hinder the participation of students in conduction of research remains a critical issue in dental education. The objective of this study was to evaluate the knowledge, attitude, practise and perceived barriers towards research among dental undergraduate students.

Materials and Methods

This was a cross-sectional questionnaire-based study which was conducted among the undergraduate students from a private dental college, Chennai. The study was approved by the Institutional Ethics Committee. Students who were willing to participate in the study were included. A total of 138 undergraduate students from a private dental college participated in this study. A predesigned validated questionnaire consisting of 16 questions which included demographic details, and questions regarding their knowledge and perception of barriers towards research was employed. Questions were taken from previous similar studies [29]. Questionnaire validation was based on content validity and logical reasoning.

First part of the questionnaire was regarding the demographic details of the study participant such as age, gender, year of the study and number of indexed publications till date. The second part consisted of 14 questions on knowledge and attitude towards research, such as various study designs, hypothesis, protocol writing, and willingness to participate in a research methodology workshop and willingness to conduct clinical and community related research. The third part

assessed the motivational factors that help in conduction of research. The fourth part assessed various barriers deterring students from conducting research. A total of 23 probable factors were listed as barriers that addressed the concerned limitations on conducting research such as poor knowledge, lack of interest, insufficient funds, inadequate of encouragement by faculty, time constraints, and follow-up difficulties and the responses were evaluated by a 5-point Likert scale rating ranging from strongly disagree (score 1) to strongly agree (score 5).

The data was collected from the filled questionnaires and was entered in Microsoft Excel spreadsheet. The statistical program SPSS was used for data management and analysis. Descriptive statistics was computed. The frequency for each domain of knowledge, attitude, and practice was calculated after giving weightage for each option. Statistical test t-test was used to analyze the difference in the knowledge, attitude, and practice between various factors. A $P < 0.05$ was considered to be statistically significant.

Results and Discussion

A total of 138 students participated in this study and the response rate was 76.8% among female students and 23.2% among male students. The respondents were of the age group of 18-22 years with the mean age of 20.(Graph 1) Majority of the respondents were third year undergraduate students (53.6%)(Table 1). Majority of the respondents had between 1-5 publications (76.03%) (Table 2).

Questioning on attitude towards research 68.84% were willing to participate in a workshop on research methodology, 78.26% were willing to conduct clinical and community related research, 88.6% have participated in research, 50.72% have read journals related to dentistry regularly, 86.23% have publication in a journal and 89.86% have presented a poster or research paper at a conference.

Majority of respondents have contributed in proposal writing (21.01%) and data entry (18.84%). Majority of respondents have participated in clinical trial (21.01%) and case series (20.29%).

Questioning on preference for research 89.86% agreed that research should be a part of UG curriculum, 49.29% agreed that it will help in better understanding of subject, 52.17% agreed that research will help in clinical practice, 42.02% neither agreed or disagreed that research is an extra burden and 44.20% neither agreed or disagreed that research is not waste of time.

Questioning on what motivates you to conduct research 86.23% said it was compulsory (part of curriculum, 56.52% said that it is to fulfil their interest in research, 75.91% said that it would facilitate their acceptance to future education, 76.64% said that it shows positive accomplishment in their job resume, 71.74% said that it improves their research skills and 81.88% said that it is to have their research published.

On questioning about barriers about conducting research, 30.43% equally agreed and were neutral for lack of time due to educational activity, 38.41% strongly disagreed and were neutral that it was due to gender of the resident, 44.53% totally disagreed that it was other personal commitments and 35.04% totally disagreed that it was their lack of research curriculum.

Majority neither agreed or disagreed to the reasons of lack of interest (48.55%), inadequate facility for research(33.33%), difficulty in transportation(36.23), lack of interest by program/guide(38.69%), lack of motivation or reward(34.31%), inadequate support by mentors or assistance(31.39%), difficulty in obtaining a research supervisor(33.58%), inadequate training(29.2%), lack of statistical support(35.04%), inadequate financial support (38.69%), lack of allocated research time (35.77%), difficult in obtaining approval (42.34%), unavailability of samples/patients (40.15%), difficulty in following-up patients (37.23%), poor accessibility to data base (38.69%), difficulty in presenting research (40.86%), difficulty in publishing (45.26%) and acknowledgment for contribution(47.45%).

On comparison of responses based on gender, year of study and number of publications, only a few significant associations were noticed. Males had a higher number of publications (68.84%) when compared to females, which was statistically significant (Chi squared test; p-value = 0.035 - statistically significant). Although a higher percentage of III year undergraduates were willing to participate in a workshop on research methodology, a higher percentage of them denied it, which was not statistically significant (Chi square test; p-value = 0.33

- not statistically significant). Respondents with 1-11 publications had a higher chance of presenting a paper or poster at a conference when compared to respondents without any publication. This difference was highly significant (Chi square test; p-value = 0.000 - statistically significant)(Graph 2-4).

Research is an integral element in the improvement and growth of the health-care system which is attainable to the general population [30]. Appropriate knowledge, a positive attitude and adequate skills are required to carry out research in good standards [31,32]. In this study we intended to evaluate the knowledge, attitude, practice, and barriers to research among the undergraduate dental students. The total knowledge regarding research among them was encouragingly good and this finding was in accordance to another study done on undergraduates in Saudi Arabia [5] and among dental undergraduates in bengaluru city which showed good knowledge regarding research [5,33]. Majority of the students felt that research was important to clinical practice and helped in better understanding of the subject. This finding was found to be similar to a study done in South Africa that observed the importance of research interest by most of the participants [34]. The paper presentation (89.9%) and publication (86.2%) was higher among our study population which is in stark contrast with a study among medical undergraduates in Kolar as they had only a meager presentation and publication by students [29]. This response was owing to their progression academically which ends in a change in priorities, so most of them do not complete their project. A study done in India on resident doctors had similar findings among postgraduate students [35]. Many universities in India have made paper presentation and publishing of manuscript mandatory for postgraduates as a part of curriculum, however, it is not the case for undergraduates to be involved in research in many institutions.

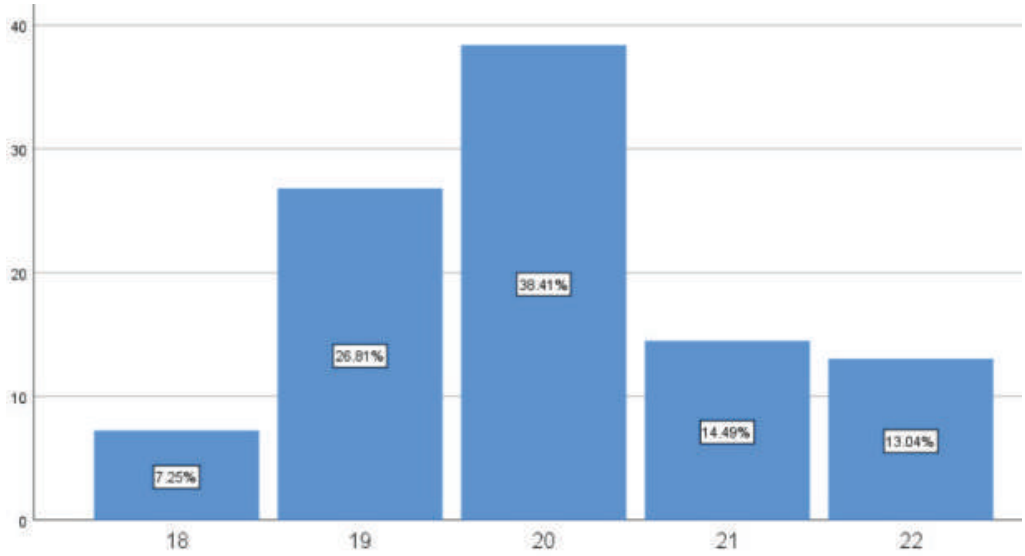
Even though students expressed a positive attitude toward research, the participation in research, presentation of paper, and publication were mostly motivated due to research being a compulsory part of the curriculum (87.7%). A few studies have shown that the prime motive of conducting research was due to research being mandatory part of their curriculum [36–38]. Barriers for research were lack of time (25.5%) , inadequate financial support (21.9%), lack of time being overburdened with education/ exams (30.7%), difficulty in following up study subjects (22.6%), difficulty in obtaining approval (22.6%), lack of interest (19.6%), lack of reward or motivation (19%). In this present study, the main barrier as opined by the students were lack of time and funds. Similar studies from Arabia, Canada, and Pakistan have reported findings similar to the current study regarding barriers of research [4,7,29,38–40]. The studies in India regarding the opinion of undergraduate dental students on research is very limited. This survey was made as an effort to evaluate their attitude, perception and perceived barriers regarding the same.

Our study reports that despite having sufficient knowledge and the right attitude towards dental research, the major motivation to participate in research was research being a mandatory part of the curriculum. However more than half the study population believed research should be included as part of the dental curriculum. The students should be acquainted with research methodology at the undergraduate level and at regular intervals. The students should be encouraged to present in undergraduate conferences and publish their completed work by providing incentives as lack of reward and motivation seems to be one of the prime barriers towards research. Training of dental faculties in research methodology and consequent motivation of students to take up research projects is recommended. In the present study, males had a higher number of publications when compared to females. Although a higher percentage of III year undergraduates were willing to participate in a workshop on research methodology, a higher percentage of them denied it. Both these results could be due to the uneven sample size that was noticed in the study. However respondents with 1-11 publications had a higher chance of presenting a paper or poster at a conference when compared to respondents without any publication. This could be due to the fact their respondents pursue their research to the next level by publishing them in various journals.

This study discussing research among dental undergraduates is one among a handful of studies. The present study results are limited to this particular university and corresponds to that of a small population. These findings should be relayed to the students so as to make them aware of their knowledge and perception. The barriers faced by the students need to be discussed

at the administrative level to bring about reforms in the dental curriculum so as to reduce the obstacles faced by students and also make the research experience uncomplicated.

8. TABLES AND GRAPHS



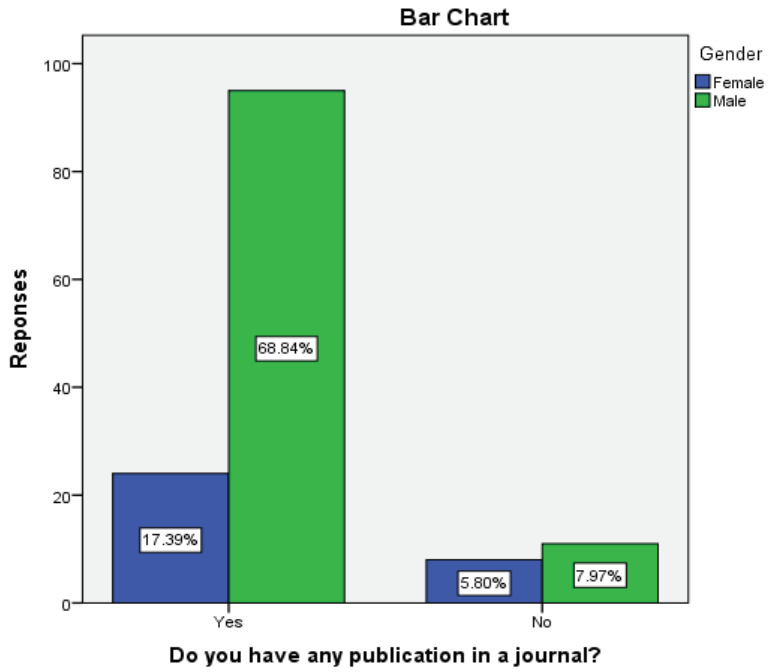
Graph 1: This bar graph represents the distribution of respondents based on age. X-axis denotes age group of participants and Y-axis denotes the percentage of participants. The respondents were between the age group of 18-22 years.

Table 1: Table showing representing demographic profile of study participants

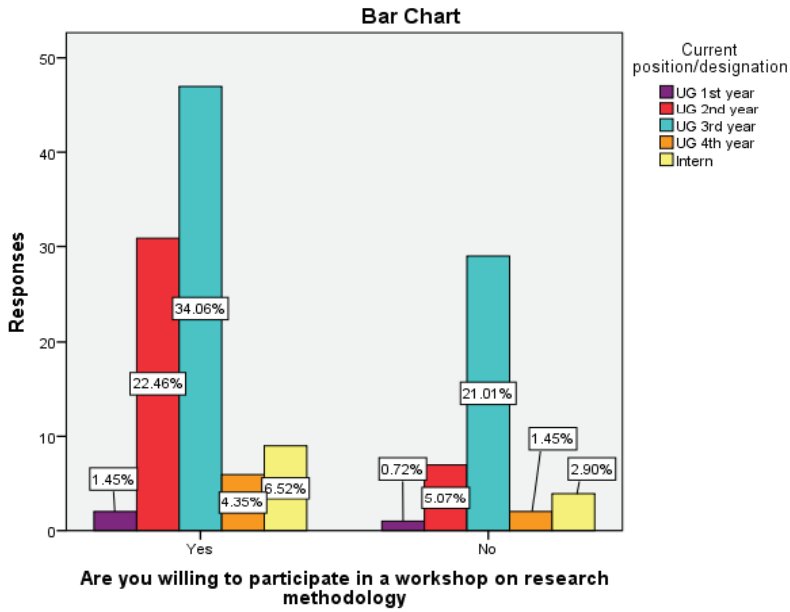
Designation	
UG 1st year	0.7 %
UG 2nd year	23.9 %
UG 3rd year	53.6 %
UG 4th year	4.3 %
Intern	9.4 %

Table 2: Number of publications

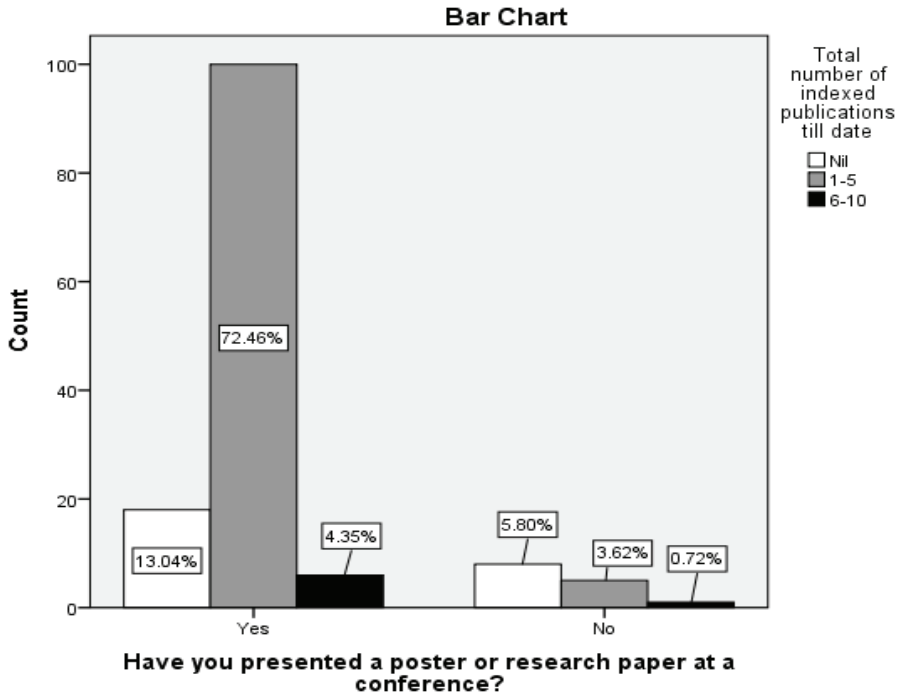
Total number of indexed publications	
Nil	18.9%
1-5	76.03%
6-10	5.07%



Graph 2: Bar graph representing comparison of responses based on gender to presence of publication in a journal. X- axis denotes the responses yes and no, Y-axis denotes the number of responses. Color blue and green represents female and male participants respectively. Males had a higher number of publications (68.84%) when compared to females, which was statistically significant. (Chi squared test; p-value = 0.035 - statistically significant)



Graph 3: Bar Graph representing comparison of responses based on year of study to the statement "Are you willing to participate in a workshop on research methodology". X- axis denotes the responses yes and no, Y-axis denotes the number of responses. Color violet represents 1st year UG students, red represents 2nd year UG students, blue represents 3rd year UG students, orange represents 4th year UG students and yellow represents interns. Although a higher percentage of III year undergraduates were willing to participate in a workshop on research methodology, a higher percentage of them denied it, which was not statistically significant. (Chi square test; p-value = 0.33 - not statistically significant)



Graph 4: Bar Graph representing comparison of responses based on number of publications to the statement "Have you presented a poster or research paper at a conference". X- axis denotes the responses yes and no, Y-axis denotes the number of responses. Color white represents no publications, grey represents 1-5 publications and black represents 5-10 publications. Respondents with 1-11 publications had a higher chance of presenting a paper or poster at a conference when compared to respondents without any publication. This difference was highly significant. (Chi square test; p-value = 0.000 - statistically significant)

Conclusion

Within the limitations of the current study, most of the undergraduate students had adequate knowledge regarding research and exhibited a positive attitude toward participation in research activity. Some of barriers that impede students in their involvement in research were inadequate time, insufficient funds, and difficulty in patient follow-up. Addressing the said barriers and subsequent changes by faculty and institutions can improve research involvement by students.

Conflicts of Interest: There were no conflicts of interest.

Source of Funding : Self

Acknowledgement: The authors acknowledge the undergraduate students, who participated in the study.

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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An Evaluation of Marginal Fit and Aesthetics in FPD Is Achieved During Graduate Training

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Abstract

Background : The survival of fpd depends on the state of the marginal adaptation.The longevity of fpd depends on the condition of marginal fit and esthetics is also major concern during restoration of anterior partial edentulous areas.Hence, adequate marginal fit and esthetics is a goal of a conscientious prosthodontists. The aim of this study is to evaluate the marginal fit and aesthetics in fpd, achieved during graduate training. **Materials and methods :** A Retrospective study was conducted using the records of the patients. 3178 case sheets were reviewed which were dated between june 2019 to march 2020.the data was collected by the patient records of saveetha dental college and hospitals.**Results:** The total sample size of this study was 603, in which 4.3% were 3rd years, 40.1% were 4th years and 55.5% were interns. Tables were distributed based on USPHS criteria in which Marginal adaptation were smooth margin 63.3%, all margins closed 26.5% and obvious crevice at margin 10%.Based on Colour match, very good colour match were 33% ,good colour match were 54% , slightly mismatch of colour were 10% , obvious mismatch were 1% and gross mismatch were 0.2%. **Conclusion :** Therefore from this study, we observed that better Marginal adaptation and Colour match in fpd was achieved by the interns and the most frequent anatomical site was upper anterior teeth.

***Keywords:** Esthetics ; Marginal adaptation; Fixed Prosthesis*

Introduction

The Role of dental professionals is to promote oral health and dental esthetics.[1] Increased life expectancy and increase in population, creates high demand in dental care especially in the field of prosthodontics.[2] Nowadays, the problem of missing teeth continues to be more prevalent.[3,4,5] Fixed partial dentures have been the treatment of choice for the replacement of missing teeth. [6] Missing teeth can damage mastication,self-esteem and also social interactions due to its effects on appearance and the ability to have a conversation. [7] Recently fixed prosthesis is more popular than removable prosthesis due to comfort, function and aesthetic. [8,9] The survival and longevity of fixed prosthesis mainly depends on the state of marginal adaptation. Marginal gaps can create favourable conditions for biofilm deposition, which contributes to the development of caries and periodontal diseases.[10] Marginal fit in fpd is one of the significant factors influencing the clinical acceptability.[6,11]Inadequate marginal fit in fpd will lead to marginal leakage , plaque accumulation , bone loss that will end up in fpd failure.[12,13] Various local intervention methods suggested for preventing oral diseases and suggested in prosthetic associated maintenance phase.[14] The best method to measure marginal gaps is still a controversial topic .Several techniques have been described like replica technique, light and scanning electron microscopy, micro computed tomographic evaluation.[15] Few investigations revealed that the most common and adequately used treatment for replacement

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of missing teeth is removable partial dentures but more than 90% of patients were satisfied with having missing teeth replaced with FPDs from a functional and aesthetic point of view .[2,3]

Esthetics is a major concern during restoration of any anterior partial edentulous areas. When overall dental appearance is considered , several factors are of significance especially of anterior teeth that are colour match, shade, position , restoration quality and arrangement of dentition.[16] All ceramic dental materials claim to provide optimal mechanical and aesthetic characteristics mainly in anteriors , namely glass ceramics and polycrystalline zirconium dioxide ceramics. Zirconia ceramics provide a natural looking appearance compared to metal ceramics ,as zirconia is highly biocompatible metastable material and can hinder crack propagation[17]. Several studies investigated the maximum clinically acceptable marginal gap width and different values were proposed according to the type of restoration used for both lithium disilicate and zirconia copings showed clinically acceptable accuracy of fit.[18] The long term success of fpds depends on the crowns longevity which is strongly related to the quality of marginal fit[19]. Marginal openings of no more than 100mu are considered clinically acceptable. Francesco Riccitiello et al, reported that zirconia and lithium disilicate crowns showed better marginal adaptation and good esthetics.[20, 21] This study is designed in assessing the success rate of fixed prosthesis treatment based on marginal adaptation and aesthetics treated by undergraduates.

Materials and Methods

Study design and setting:

A Retrospective study was conducted using the records of the patients.603 case sheets were reviewed which were dated between june 2019 to march 2020.The data was collected by the patient records of saveetha dental college and hospitals. After screening the search was narrowed to 603 fixed dental treatments. Relevant data such as year of training, teeth number ,colour match, marginal adaptation were collected. Based on United States Public Health Services (USPHS criteria), the marginal adaptation and colour match was graded.

Inclusion criteria:

The study included only patients who underwent fixed partial dentures replacing single or double units treated by graduate trainee students.

Exclusion criteria: Complex multi unit fixed partial dentures were not considered in the study. Incomplete data without notes and photographs that did not give expected details were excluded.

Statistical Analysis

Data was recorded in Microsoft Excel and later exported to IBM SPSS (version 20.0 Chicago USA) and subjected to Statistical analysis. Chi Square test was then employed with a level of significance set at $P<0.05$.The statistical analysis between clinic type, teeth number, colour match, marginal adaptation was carried out in SPSS software. Chi square test was done to compare the parameters. The outcome was represented in a form of tables and bar charts.Ethical clearance was obtained. Ethical approval number SDC/SIHEC/2020/DIASDATA/0619-0320.

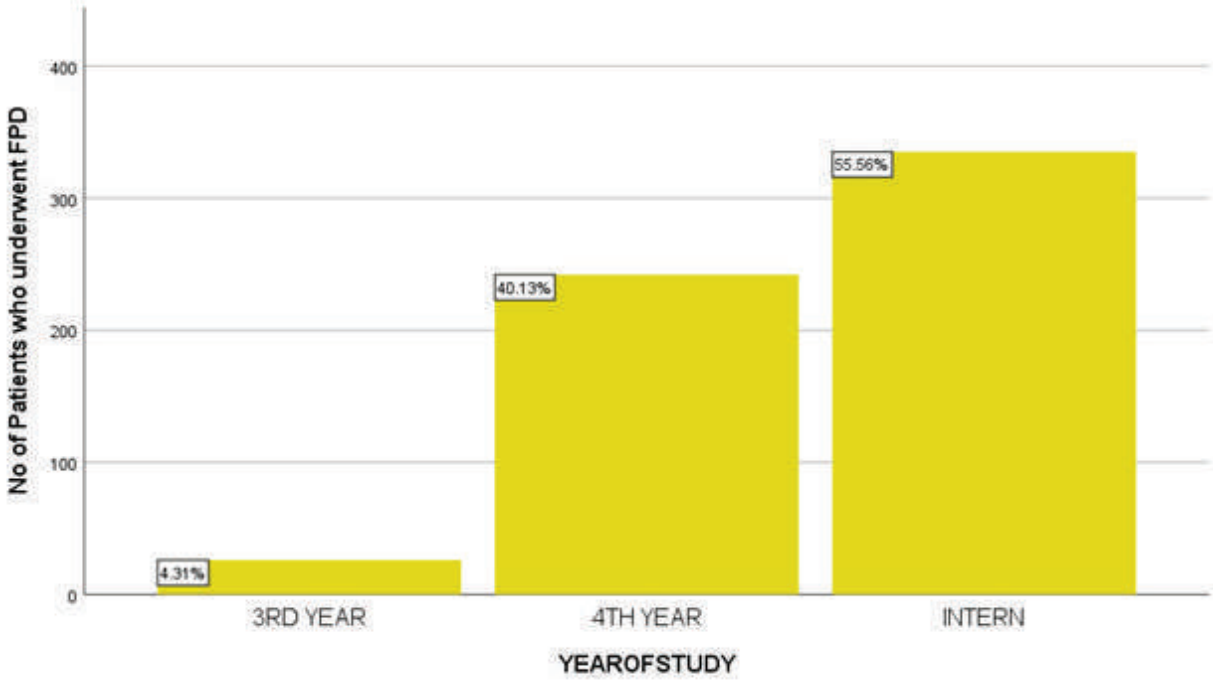
Results & Discussion

The total sample size of this study was 603, the Year of study (UG) were divided into 3rd years, 4th years and interns which was 55.5% (n=335) followed by 4th years 40.1% (n=242) and 3rd years 4.3% (n=26) as shown in **(graph 1)**.Distribution of teeth number and number of fpds performed by the undergraduates was given in **(graph 2).**It shows that the most common anatomical site performed by the undergraduates was upper anteriors (sextant 2). Correlation between the year of study and frequency of marginal adaptation based on USPHS criteria as shown in **(graph 3)**.Marginal adaptation in fpd were divided into smooth margin in which interns 35.82%,4th years 25.3% and 3rd years 2.16%, all margin closed in which interns 14.7%, 4th years 10.9% and 3rd years 0.83% and Obvious crevice at margin in which interns 4.9%, 4th years 3.8% and 3rd years 1.33%.This shows that better marginal adaptation in FPD was achieved by the interns.Chi square test was done and association was found to be statistically significant. Correlation between the year of study and frequency of marginal adaptation based on USPHS criteria as shown in **(graph 4)** Colour match were divided into Very good colour match in which interns 18.5%, 4th years 13.2% and 3rd years

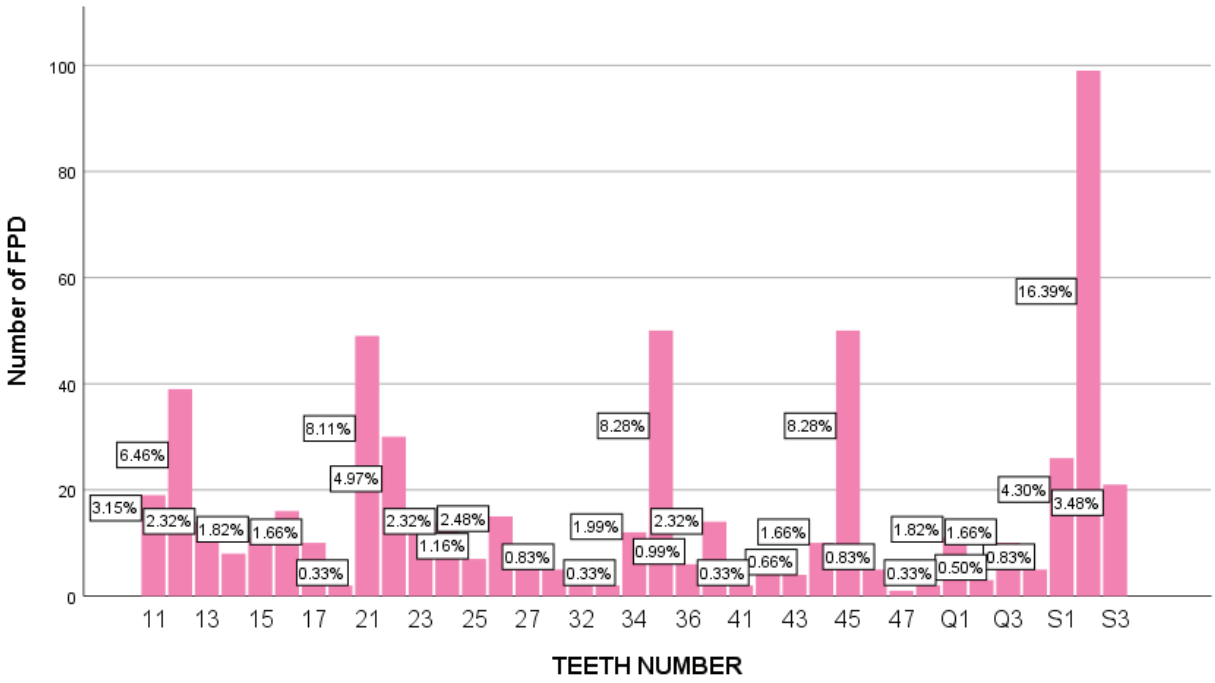
1.82%,Good colour match in which interns 30.4%,4th year 21,8% and 3rd years0.6% ,Slightly mismatch of colour in which interns 5.31%,4th years 4,96% and 3rd years 1%,Obvious mismatch in which interns 0.1% and Gross mismatch in which interns 1%**.**This shows that good colour match in FPD was achieved by the interns. Chi square test was done and association was found to be not statistically significant.The study was aimed at finding out by evaluating the marginal fit and aesthetics in fixed prosthesis treated by undergraduates in Saveetha Dental college and Hospitals. The present study reveals that although operators are novice they tend to achieve accuracy with more exposures, similar reports were found in other studies too.

A study conducted by Sharanya et al, reported that out of 100 dental interns , 33.18% were confident about fpd procedures , 20.5% were very confident , 16% were not confident about fpd procedures. Though fpd has become an important part of undergraduate teaching programs, the level of understanding and awareness among graduate training students regarding fpd is important.[22]According to Ranganathan et al,reported that the veneers fabricated using pressable ceramic processing systems exhibited less marginal discrepancy than the veneers fabricated using CAD/CAM milling technique. Cervical marginal discrepancy was observed to be lesser than the incisal marginal discrepancy in all the experimental groups. [23,24] A study conducted by Baiget al, reported that crown longevity is strongly related to quality of marginal fit, there were significant differences in marginal values between chamfer and shoulder margins.[25] Nakamura et al., 2005, evaluated the marginal and internal adaptation of all-ceramic crowns fabricated using the CAD/CAM system. A master die of maxillary first bicuspid was prepared, and experimental crowns were fabricated. The marginal gap for all experimental crowns met the clinically acceptable criteria.[26] Quintas et al. have reported an increase in the marginal discrepancy following luting with resin cements. [26,27] Borges et al.also evaluated in vitro marginal fit of three all-ceramic crown systems before and after cementation and observed that both resin-modified glass ionomer and resin cements induce increase in marginal discrepancy.[23]

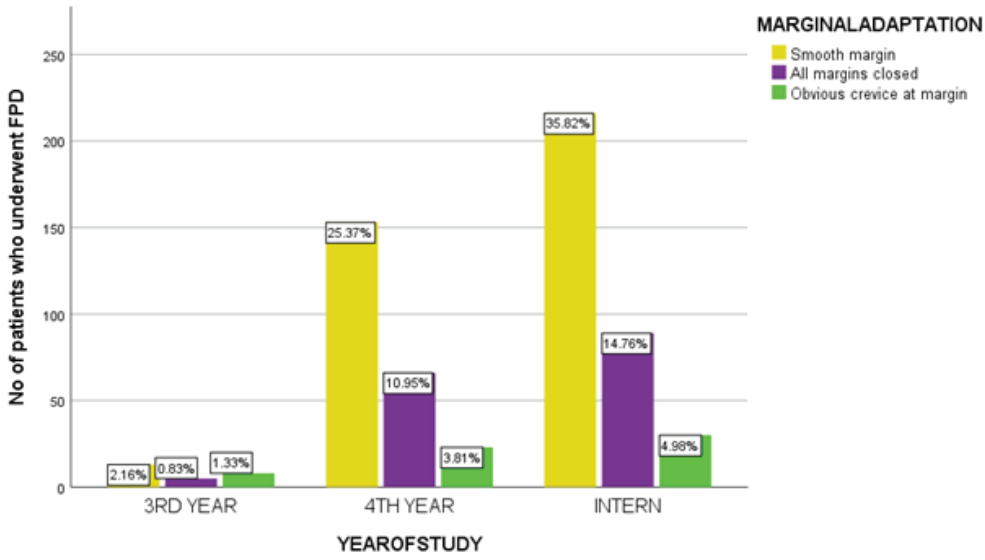
A study conducted by bronson et al, reported that surfaces associated with greater margin gaps tended to have a greater proportion of ratings of "clinically unacceptable." The proportion of prosthodontists and predoctoral students rating a margin "clinically unacceptable" were highly correlated.[28]In 2016, Ghada et al, reported that shade and colour match in fpd play an important role in patient satisfaction in which 805 of patients are pleased with the esthetic outcome of their treatment and 20% of the patients reported that they are not being satisfied with esthetic results. The highlights the importance of esthetic parameters in fpd design and construction.[25,29]Sakrana et al, reported that a good marginal fit seems to be one of the most important technical factors for the long term success of any restoration, clinicians should strive to minimise the marginal misfit.[30] A study conducted by Smitha et al, reported that shade matching was found to be the most challenging for undergraduate students and they have to understand the various aspects to consider before selecting a shade for a patient.[30,31]



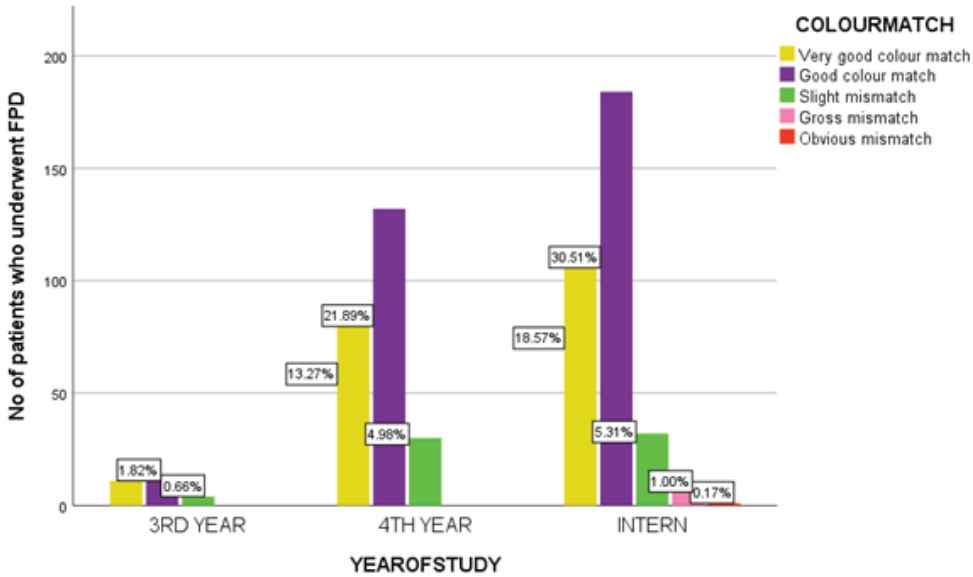
Graph 1 :This graph represents the distribution of year of study (UG), where X-axis denotes the year of study and Y-axis the number of patients who underwent FPD.



Graph 2:Bar graph represents the distribution of teeth number, X-axis denotes the teeth number and Y-axis denotes the number of FPDs performed by the undergraduates.The graph shows that the most number of FPDs performed by the undergraduates was upper anteriors(sextant 2).



Graph 3:Bar graph represents the association between the year of study and frequency of marginal adaptation based on USPHS criteria, where yellow denotes the smooth margin, violet colour denotes all margins closed and green colour denotes the obvious crevice at margin.X axis represents the year of study and Y axis represents the number of patients who underwent FPD(Marginal Adaptation).Chi square test was done and association was found to be statistically significant.Pearson Chi square value:12.871,DF:4, p value:0.01(<0.05)hence statistically significant,proving that better marginal adaptation in FPD was achieved by the interns.



Graph 4:Bar graph represents the association between year of study and frequency of Colour match in FPD based on USPHS criteria, where yellow colour denotes the very good colour, violet colour denotes the good colour match,green colour denotes the slight mismatch,pink colour denotes the obvious mismatch and orange colour denotes the gross mismatch.X axis represents the year of study and Yaxis represents the number of patients who underwent FPD(colour match). Chi square test was done and association was found to be not statistically significant.Pearson Chi square value:8.452,DF:8,pvalue-0.39(>0.05) not statistically significant,proving that the FPDs done by interns were better in terms of color matching.

Conclusion

Within the limits of this current study, we observed that better marginal adaptation and colour match based on USPHS criteria in fixed partial denture was achieved by the interns when compared to the 3rd years and 4th years of undergraduate study. The most frequent anatomical site treated by the undergraduates was upper anterior teeth replacement.

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Conflict of Interest: The authors would like to declare that there is no conflict of interests.

Source of Funding: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Awareness on Ebola Virus among Undergraduate Dental Students in a Private Dental Institution, Chennai - A Questionnaire Survey

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Abstract

Ebola virus disease (EVD) is a deadly disease with occasional outbreaks that occur primarily on the African continent. EVD most commonly affects people and nonhuman primates (such as monkeys, gorillas, and chimpanzees).The aim of the study to determine the awareness of Ebola virus among the undergraduate dental college students.This study involved the awareness of Ebola virus in the age group of 18 to 25 years. A well structured questionnaire comprising 14 questions covering socio demographic information, knowledge, attitude, perception was framed and administered to the participants through an online google forms. Survey was conducted among 100 college students. In that 30.39% male students and 69.61% of female students have taken this survey. The association between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant. We have observed that association between gender and responses to awareness on the mode of spread of Ebola virus was statistically significant ($p = 0.020$). The awareness on ebola virus was analysed among the few college students by this survey and more awareness should be created among the population about the ebola virus.

Keywords: *Ebola virus, online survey, knowledge, diagnosis*

Introduction

Ebola virus disease, formerly known as a haemorrhagic fever. It is rare but very severe for every patient it often leads to fatal and death of the ill person. The virus is transmitted from person to person or person from the wild animals. Symptomatic treatment improved survival but no treatment is proven to control the virus but a range of the blood, immunological and drug therapies are under development. When it is untreated it leads to death.Symptoms of Ebola virus at the beginning stage is fever fatigue, muscle pain, headache, sore throat, and severe symptoms include vomiting, diarrhoea, rash, impaired kidney, and liver function both internal and external bleeding [1]

The use of the syrian hamster as a new model of Ebola virus disease and for the viral hemorrhagic fever.[2] there is a suppressing effect of Ebola virus on T-cell proliferation in vitro[3].Systematic analyses of monoclonal antibodies against a Ebola virus [4].Ebola virus is tested on infected pigs[5]. Ebola virus in that one has recently demonstrated durable immunity within the nonhuman Primates[6].vaccines potential of Ebola virus VP24, VP30, VP30,VP35,VP40 proteins are also capable of protective immune responses[7].

No effective vaccine and drugs are developed so far because of the coded nature of the virus but many of

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them are in trial and error method[8]. In order to promptly identify the diseased person and prevent the further spreading of the disease. The diseased person should be kept in the incubation to avoid the spread of disease[1]. The aim of this study is to create an awareness on Ebola virus among the college students.[9]

Materials And Methods

This study involved the awareness of ebola virus in the age group of 18 to 25 years . A well structured questionnaire comprising 14 questions covering socio demographic, knowledge, attitude, perception was framed , circulated among 100 undergraduate students and administered through an online google form link.

In this study setting is prospective observational study and the pros are economical, easy to create, gather larger ,quick interpretation,wide reach and cons is response, survey fatigue, homogeneous population and the study is approved by the scientific review board of Saveetha dental College Chennai. a simple random sampling method was done. measures taken for minimising the sampling is based on the internal and external validity, minimizing error in questions and avoiding the leading questions. internal validity is based on risk factors, complication, ebola virus among the college students. External validity is based on the awareness, research conclusion and study outcome. output variable is associated with demographic information, social media, via ebola virus, contagious virus, blood product, contaminated surface, precaution, avoid contact with infected people and frequent hand wash. output variables are represented using pie charts for each output.Each output variable was collected as ordinal data and the collected data were represented as pie charts and bar graph.A statistical test was done using SPSS software.Statistical test used descriptive analysis and frequency percentage.

Result and Discussion

Sample size for the study was 100. In that 30.39% male students and 69.61% females students have taken this survey. 68.63% of the students were aware about the ebola virus and 31.37% were not aware about the ebola virus.

67.65% of students said that ebola virus leads to death. 15.69% of the students said yes it is spread by blood products. 9.80% of the students said that the symptom of Ebola virus was fever, 19.61% of the students said that it cause headache, 23.53% of the students said that it cause pain and vomiting and 3.92% students said that it cause coughing and 43.14% of the students and that it cause all the above symptoms.

45.10% of the students said that Ebola virus is transferred from person to person.13.73% of the students said that it will affect adult, 24.51% of the students said that it will affect children and 31.37% of the students said that it will affect more older people and 30.39% of the students said that every group of the people will be affected.

41.18% said that every group of people is at most risk. 51.96% said that many people survived with ebola virus and 48.04% say no one is survived with ebola virus. 58.82% of students said that Ebola virus is contagious. 52.94% of the students said that Ebola virus vaccines are available and 47.06% of the students said that they Ebola virus vaccines are not available.

65.69% of the students said that ebola virus is curable and 34.31% of the students said that it is not curable. 10.78% students said that bleach will kill ebola virus, 11.76% said that detergents will kill ebola virus. 18.63% said that alcohol will kill them, 15.69% said that ammonia will kill the Ebola virus and 29.41% said that none of the above the chemical agent does not kill ebola virus, 13.73%Of the students said that every chemical agent will kill ebola virus.

We have seen the association between gender (X axis) and responses to awareness on Ebola virus responses to awareness on Ebola virus which leads to death (Fig1), responses to awareness on the mode of spread (Fig 2), responses to awareness symptoms (Fig 3), responses to awareness on contagious ebola virus(Fig 4), responses to awareness on vaccine availability of ebola virus(Fig 5), responses to awareness on chemical agent of ebola virus (Fig 6).

We have observed that association between gender and responses to awareness on mode of spread of Ebola virus was statistically significant ($p = 0.020$) and association between gender and responses to awareness of symptoms was statistically significant ($p =0.00$).The statistical associations between groups was assessed

by Chi Square test where $p < 0.05$ was considered statistically significant.

The previous literature said that the clinical features of the Ebola virus include weakness, extreme fatigue, vomiting, diarrhoea, mental symptoms bleeding and a loss of appetite [10,11]. said that it is transmitted through human apes [12]. 45.10% said that it is transmitted by person to person and 26.47% of the students said that it is not transmitted by person to person.

In a previous study the author said that vaccines are available to fight against the Ebola virus disease[13]. among the junior doctors in England among them 119 doctors participated in that survey[14]. Among 153medical MBBS student surveys was conducted about their knowledge about the ebola virus[15].

In Hungary among 267 dental students a survey was conducted about the ebola virus[16]. Due to the improper diet and improper food habits there are many chances to cause ebola virus.so they need to intake proper food to prevent them from the disease[17].

Advanced studies on enzymes[9] , in silico modelling[17], role of natural products in health and disease [18,19,20,21,22,23,24], carcinomas [25,26,27,28], liver cirrhosis [29], obesity[30] kindled my passion for research and motivated me to initiate my work with awareness studies.

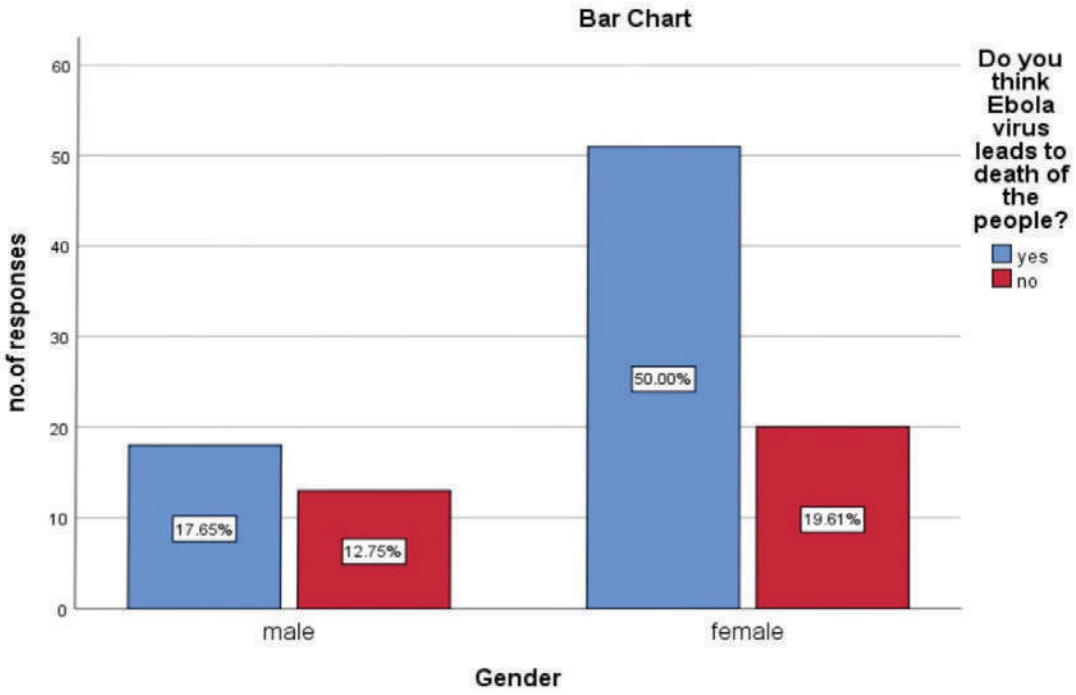


Figure 1: Bar chart represents the association between gender and ebola which lead to death of people. X- axis represents gender and Y- axis represents ebola which led to death of people.Blue colour denotes yes, and red colour denotes no. In this 50% of females reported that it leads to death of the people whereas only 17.65% of males reported that it leads to death. Hence females were more aware about the death of the people than males, through an analysis it is found to be statistically not significant. (p-value=0.172, df value=1,chi square value-1.869)(p>0.05 statistically not significant).

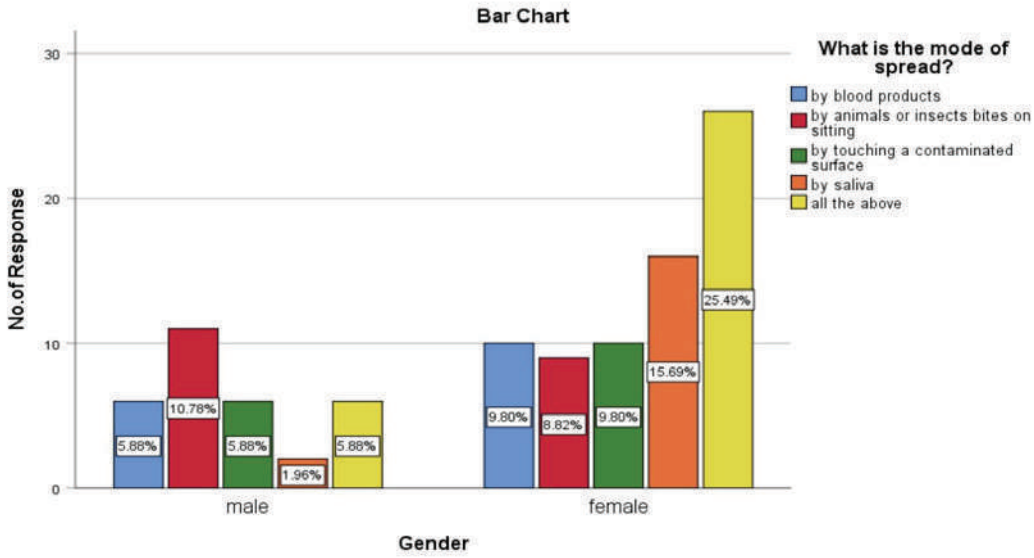


Figure 2: Bar chart represents the association between the gender and the mode of spread. In that X- axis represents the gender and Y- axis represents the no.of responses on mode of spread. In that15.69% of the females reported that it spread through the saliva but 2% of the male reported that it is spread through the saliva. Majority females were more aware about the mode of spread than males, through an analysis in chi square it was found to be statistically significant. In this blue colour denotes blood products, red colour denotes animals or insects bites on sitting, green colour denotes touching a contaminated surface,orange colour denotes the saliva and yellow colour denotes all the above.

(p- value 0.020, df value=4 chi, squarevalue-11.702).(p<0.05 statistically significant).

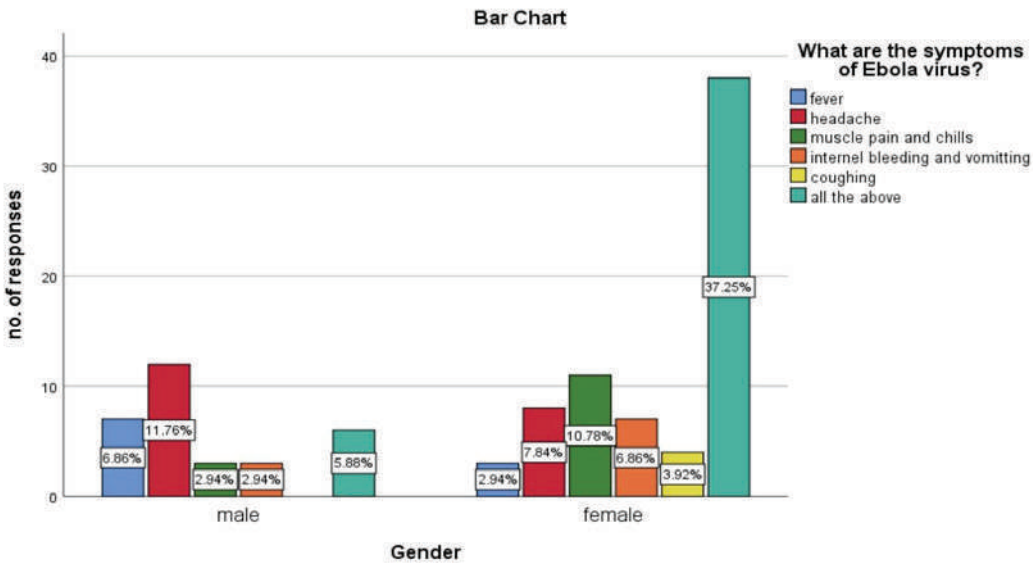


Figure 3: Bar chart represents the association between the gender and symptoms. X- axis represents the gender and Y-axis represents no.of responses on symptoms of Ebola virus. 37.25% females were aware about all the symptoms of Ebola virus. Majority of females(37.25%) were aware about the symptoms of ebola virus(aqua blue) than male, through an chi square analysis it was found to be statistically significant. Blue colour denotes fever, red colour denotes headache, green colour represents muscle pain and chills, yellow colour denotes coughing and aqua blue represents all the above symptoms. (p-value=0.00,df value=5

chi square value-23.821) (p<0.05 statistically significant).

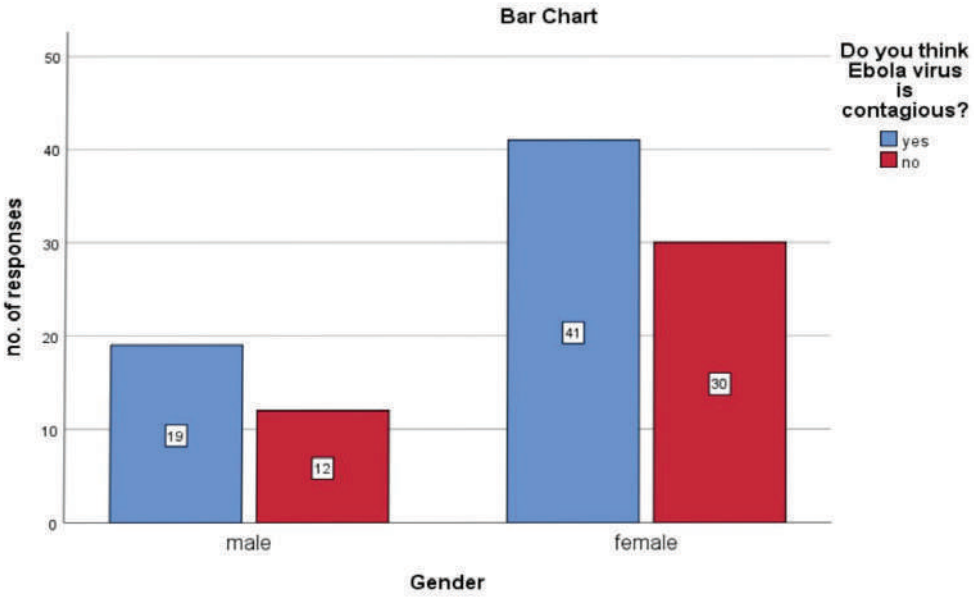


Figure 4: Bar graph represents the association between the gender and contagious ebola virus. X- axis represents the gender and Y-axis represents contagious ebola virus. 41% of the female population were aware about the contagious ebola virus(blue) than male. In chi square analysis it was found to be statistically not significant. Blue colour represents yes and red colour represents no. (p- value= 0.738, df value=1,chi square value-112) (p>0.05not statistically significant).

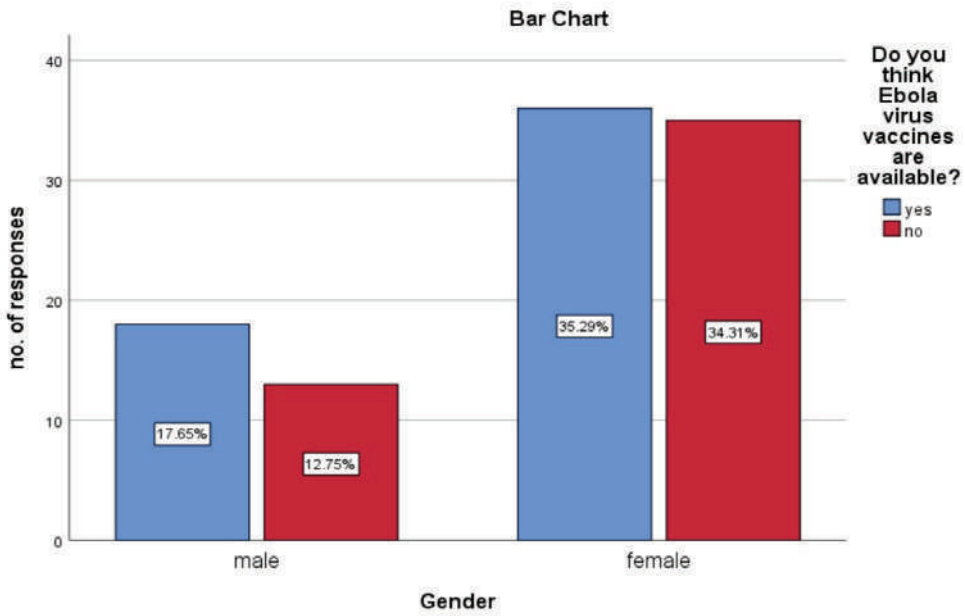


Figure 5: Bar chart represents the association between the gender and the vaccines availability of the ebola virus. X- axis represents the gender and Y- axis represents the vaccine availability. 35.29% of the female population were aware about the availability of vaccines(blue)than male. Through an analysis it is found to be not statistically significant. In this blue colour represents yes and red colour represents no. (p-value=0.493,df value=1, chi square value-0.469). (p>0.05 not statistically significant).

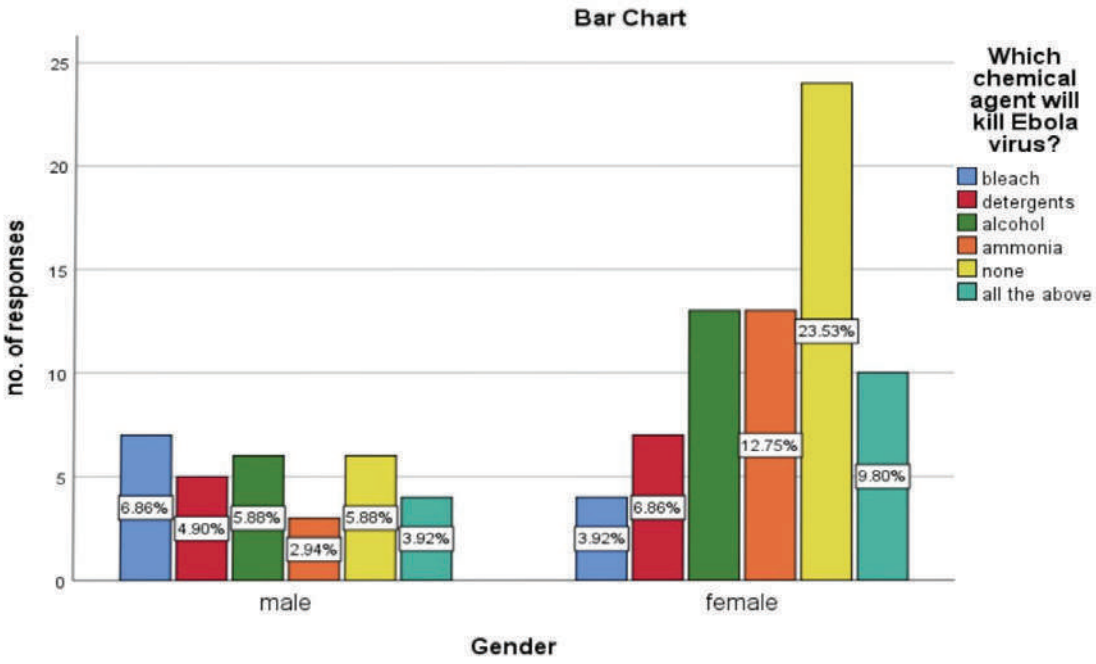


Figure 6: Bar chart represents the association between the gender and the chemical agent that kills the ebola virus. X-axis represents the gender and Y-axis represents the chemical agent. Majority of the females (23.53%) population reported that none of the chemical agents will kill the ebola virus(yellow). Through an analysis it was found to be statistically not significant. Blue color denotes Bleach, red color denotes detergents,green color denotes alcohol,orange color denotes ammonia, yellow colour denotes none and aqua blue represents the all the above chemical agents which kill the Ebola virus. (p-value=0.107, df value=5, chi square value-9.059)(p>0.05 not statistically significant).

Conclusion

From this survey we may conclude that the awareness on Ebola virus is important for current and future generation it is essential to conduct awareness camp to

educate the public about the risk factors, complications, diagnosis and treatment of the disease.

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Retrospective Study of Pattern of Mandibular Third Molar Impaction in South Indian Population

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Abstract

The aim of the study is to assess prevalence and pattern of mandibular third molar impaction in south indian population. Numerous Patient records were analysed and data was recorded The data that was collected was tabulated in excel and then imported into SPSS software. Incomplete or censored data was excluded from the study. The Statistical test that was run was the chi sequence test using the statistical software SPSS by IBM.In this study the results obtained can be summarized, presence of statistical significance between the Correlation of winters classification and gender of the study population (p=0.005), presence of statistical significance between the Correlation of winter's classification and age (p=0.002), presence of no statistical significance between the Correlation of winter's classification and tooth number (p=0.089). In the present study the Most prevalent gender to have impacted mandibular 3rd molar was males (58.36 %), Most prevalent impacted 3rd molar based on teeth number teeth number is 38 (54.1%), Most prevalent age group to be diagnosed with impacted 3rd molar was between the year 20-29 year (54.86 %). Most prevalent angulation Winters classification was found to be mesioangular (42.16%). Within the limitation of the present study, correlation between tooth angulation and age, sex and tooth number was found to be statistically significant , further studies to be conducted to asses the treatment difficulty and option for impacted tooth .

Keywords: *Impaction, Mandibular third molar, Mesioangular, Distoangular, Mandible*

Introduction

The definition of impaction has gone through several modifications by several authors, In the year 1954 Mead defined an impacted tooth as a tooth that is prevented from erupting into its positioning because of malposition, lack of space, etc. Later Peterson characterized impacted teeth as those teeth that fail to erupt into the dental arch within the expected time which is widely accepted [1]. In 2004 Farman [2] wrote that impacted teeth are those teeth that are prevented from eruption due to a physical barrier within the path of eruption. The advent of civilization, the use of a soft and refined diet has completely eliminated the need for a large and strong jaw for mastication and so with evolution, human jaws have shrunk from its large ape size to a smaller one and therefore there is no space in our mouths in order to accommodate all 32 teeth [3]. Some of the common causes of impaction are inadequate space in the dental arch, insufficient development of the retromolar space, Mandibular ramus growth -resorption at its anterior surface ,deposition at its posterior surface, in case of imbalance, the mandibular third molar impacted [4] . Proper history has to be taken while performing these minor surgical procedures. Patients under Antiplatelet monotherapy or even antiplatelet dual therapy need not be altered or stopped before minor oral surgical procedures. Most of the post-operative bleeding can be easily controlled by local hemostatic measures

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However, patients on combined anticoagulant and antiplatelet therapy or dual antiplatelet therapy appear to be at increased risk for postoperative bleeding complications, hence care should be taken [5 6] . It is also important to obtain the cardiac history and physician fitness letter before starting treatment for cardiac patients . Since surgical dental procedures are common and risk for cardiac diseases is on the rise, use of antibiotic prophylaxis before the start of the treatment in susceptible patients is highly recommended [7].

Local anesthesia forms an integral part of the extraction processes . A local anaesthetic (LA) is a drug that causes a reversible local anaesthetic change in the oral cavity , generally for the aim of having a local analgesic effect, that is, inducing absence of pain sensation by inhibiting nerve conduction during a variety of dental procedures [8]. Hypotensive anaesthesia is now an established method for the same, as it is said to reduce blood loss by up to 40%.6 Pharmacological agents have also been used in the recent past as adjuvants in various procedures to aid in the reduction of blood loss [9]. The required armamentarium may be chosen according to the patient's needs. Dentists must be well aware of these newer delivery systems, their usage and must have up-to-date knowledge, so as to provide the benefits of latest technology to their patients [10]. Even with proper planning the occurrence of medical emergencies are common, it can be prevented by taking a thorough medical history, examining the patient and formulating a comprehensive treatment plan with appropriate alterations to dental treatment as required [11].

The location and arrangement of impacted third molar, surrounding bone, mandibular canal and adjacent tooth are significant in imaging diagnosis for the proper surgical treatment planning. The most efficient radiographic technique for the assessment of 3rd molar impaction is the panaromic radiographic technique. CBCT can also be used for this purpose With CBCT, it was possible to perform the scanning with the patients sitting upright [12]. Based on the radiographic method the classification by winter was given. The angle of impacted teeth is measured using the Winter's classification system, by measuring the angle formed between the two longitudinal axes of the second and third molars [13]. He classified the impaction based on the angulation of impaction. He classified the 3rd molar into Mesioangular, Horizontal, Distoangular , Vertical, Inverted.

Several studies assessing the prevalence of 3rd molar impaction based on winters classification. The most common angulation noticed was mesioangular . A study conducted in a population in Oman showed results where mesioangular angulation was the most common occupying around 35% of the study population [14]. Another study conducted in the north Indian population also revealed that the mesioangular angulation was the most common comprising about 49.2% of the study population with the mandibular arch being the most common arch for impaction of 3rd molar [15]. Studied conducted always come with several limitations , the challenges faced by other articles include Many research done failed to show much difference between male and female population ,Not many population studies done for pattern of 3rd molar impaction in South Indian population , Improper radiographs taken for assessment , Inter examiner variability

The need for such a study is to full fill the gap between the clinical practitioners and the researchers. To figure out an Effective method of surgical removal of impacted tooth that can be formulated based on a particular population and the need to increase awareness among dental professionals.

The aim of the study is to assess prevalence and pattern of mandibular third molar impaction in south Indian population

Material and Methods

The study was performed to assess prevalence and pattern of mandibular third molar impaction in south indian population conducted in a university setting. Ethical approval was obtained from the institutional ethical committee (ethical approval number: SDC/SIHEC/2020/DIASDATA/0619-0320). The number of people involved in the study included two researches and the data and results were reviewed by two reviewers. The data collected was carried out during the period between July 2019 to march 2020. 564 patient reports were viewed and data was collected. Numerous Patient records were analysed and data was recorded . The data that was collected was tabulated in excel and then imported into SPSS software. Incomplete or censored

data was excluded from the study.The Statistical test that was run was the chi sequence test using the statistical software SPSS by IBM.

Results and Discussion

The following results were obtained after importing and analyzing the data in SPSS

The p value obtained after running the chi-square analysis for the comparison of winter's classification of impacted 3rd molar and gender was statistically significant (p=0.005) (Refer table 1, figure 5). The p-value obtained after running the chi-square analysis for the comparison of winter's classification of 3rd molar impaction and age was statistically significant (p=0.002) (Refer table 1, figure 6). The p- value obtained after running the chi-square analysis for the comparison of winter's classification of 3rd molar impaction and tooth number was not statistically significant (p=0.089) (refer table 1, figure 7). Based on the study population of the males occupied about 58.38% of the population and females occupied about 41.64 % of the population (Refer figure 4). Based on this study population mesioangular angulations occupied about 42.16% of the population, distoangular angulation occupied about 14.26% of the population, horizontal angulation occupied about 34.48% of the population and vertical angulation occupied about 9.091% of the population (Refer figure 1). Based on this study population the age group where most of the impactions were diagnosed was between 20-29 years occupying about 54.55% of the population, followed by 30-39 years which occupied about 29.15 % of the study population, 40-49 years occupying about 7.680 % of the population , 17-20 years occupying about 6.583 % of the population and 50-59 occupying about 2.038 % of the population (Refer figure 2). Based on the tooth number the most common mandibular 3rd molar impaction was noticed in tooth 38 occupying about 54.86% of the population and tooth 48 occupied about 45.15 % of the population (Refer figure 3).

The third molars are the last teeth to erupt in the oral cavity. Since they erupt at about the time when the youth goes off into the world to become wise, they are referred to as wisdom teeth [16]. Impacted 3rd molars are very troublesome , retained, unerupted mandibular third molars are often associated with varied pathologies which are Pericoronitis , Dental caries , Cysts and tumours associated with the tooth , Periodontitis , Root resorption [17] . untreated mandibular third molar can sometimes even cause fracture of the mandible , unfortunately not much data is available on the etiology and fracture patterns seen in the South Indian region [18] , it can also cause temporomandibular disorders, recent studies have shown that BTX injections can be the least invasive mode, which can provide relief of intractable symptoms in patients who have failed to show any improvement with the conventional modalities of treatment [19]

Surgical removal of impacted third molar is the single most commonly performed operation by oral and maxillofacial surgeons. Dental anxiety and fear are common among patients, and dental extractions are one of the most feared procedures [20]. Klingberg and Broberg described dental anxiety as a state of apprehension that something dreadful is going to happen in relation to dental treatment or certain aspects of dental treatment [20] . The reduction of anxiety can be done using pharmacological methods . newer and more innovative techniques have come into use . VR is an effective distraction tool to alleviate the anxiety of the patient. It is easy to use, affordable, and avoids the need for the use of pharmacological agents to reduce the anxiety level of the patient [21].Pain associated with surgical removal of teeth ranges between moderate and severe during the first 24 hours after surgery, with the major pain intensity occurring between 6 and 8 hours when a conventional local anaesthetic is used.Dental pain is largely inflammatory, and evidence based medicine has shown that non-steroidal anti-inflammatory drugs (NSAIDs) are the best analgesics for dental pain [22]

A surgeon's lack of experience could also be a major factor in the development of postoperative complications [23]. The surgical removal of many impacted mandibular third molars which have been asymptomatic for years are often carried out to prevent any future complications and pathologic conditions that might occur [24]. Regular dental checkups will help with early identification of pathogenesis and early treatment [25 26] . Patient awareness about common dental procedures is very important so that they can seek early treatment. In a survey conducted it was noticed that the awareness level about basic dental procedures were appropriately good among the general population [27].

Extraction of Mandibular third molar was performed when the following indication proposed by Koerner [28] where present which include existing pathology or pain due to pericoronitis, periodontitis, periapical abscess, cysts or neoplasms , resorption of adjacent roots, and inflammation of the opposing soft tissue leaving the impacted tooth being can even lead to oral carcinoma. Cancer cells exhibit a wide range of genetic alterations that include gene rearrangements, point mutations, and gene amplifications, leading to disturbances in molecular pathways modifying cell growth, survival, and metastasis [29]**.** Treatment of patients sometimes even puts the operator at risk this occurs due to improper asepsis Dental professionals are at a greater risk for acquiring cross-infection while treating patients. This is evident from the fact that most of the human pathogens have been isolated from oral secretions [30] [31]. There are also very prone to needle stick injuries which puts them at greater risk for exposure to deadly infections due to greater exposure to microorganisms and viruses that cause blood borne diseases, such as the human immunodeficiency virus (HIV) and the hepatitis B (HBs) and hepatitis C viruses [32]. It is important for dental students to improve their knowledge to enable diagnosis and management of HIV/AIDS and patients with other diseases to have a more positive attitude toward these patients. Furthermore, as their knowledge improves, dental students may understand methods of infection control and how to prevent transmission [33] [33]

Pain, trismus and surgical edema dry socket are the most common complications that occur following impacted lower third molar surgery . Inferior alveolar nerve injury , lingual nerve injury have also been noted to be complications to surgical removal of impacted teeth [34] the formation of Simple ranula is common during the first and second decade of life while plunging ranula occurs frequently during the third decade of life this can complicate the treatment of 3rd molar impaction removal [35]. It has also been noted that alveolar osteitis is also a common complication that occurs following extraction of 3rd molar. In a study conducted it was noted that a usage of eugenol post extraction showed no occurrence of alveolar osteitis [36]. Complication can occur that will make it difficult for execution of the extraction like the presence of oral sub mucous fibrosis which causes limitation in mouth opening leading to increase in difficulty in extraction [37]. High consumption of tobacco has been noticed in the south east asia region by the population belonging to the age group of 20-30 which is common occurrence age for impaction also. Attainment of homeostasis is an integral part of any surgical procedure should also be done for impaction surgeries , the 3 basic steps are (1) Vasoconstriction, (2) formation of a platelet plug, and (3) coagulation (secondary hemostasis) [38].

In this study the results obtained can be summarized, presence of statistical significance between the Correlation of winters classification and gender of the study population (p=0.005), presence of statistical significance between the Correlation of winter's classification and age (p=0.002), presence of no statistical significance between the Correlation of winter's classification and tooth number (p=0.089). In the present study the Most prevalent gender to have impacted mandibular 3rd molar was males (58.36 %), Most prevalent impacted 3rd molar based on teeth number teeth number is 38 (54.1%), Most prevalent age group to be diagnosed with impacted 3rd molar was between the year 20-29 year (54.86 %). Most prevalent angulation Winters classification was found to be mesioangular (42.16%).

In the present study there was statistical significance present between Correlation of winters classification and gender of the study population similar results were obtained by study conducted by Eshghpour M et al [39] It was also noted that there was statistical significance between the Correlation of Winter's classification and age of the patients in the study population similar findings was noted in a study conducted by Mahmoud Al-Dajani et al [40] . It was also noted that there was no statistical significance between Correlation of winter classification and tooth number , dissimilar findings were noted in a study conducted by Krishna Gopal Bhuju et al [41], whereas similar findings were noted in a study conducted by Aveek Mukherji [42]. We found that the prevalence of mandibular third molar impaction was significantly higher in males population in comparison to females. This is in contrast with the study of Hashemipour *et al* [43], Quek *et al* [44] Hugoson and Kugelberg [45] Ma'aita and Alwrikat [46] and Kim *et al* [47]. They reported a gender predilection for females. However, Brown *et al* [48] in their study said that s no sexual predilection gender-

wise for incidence of mandibular third molar was found . The pattern of occurrence of angulation of impacted third molars in our study showed that mesioangular impaction was the most common. Our findings are in accordance with previous studies who have reported that mesioangular impaction was the most prevalent type of impaction in the mandibular third molars of African American, Singaporean, American, Arabian, and Iranian populations [49]). We also noted that the most common age to be diagnosed to have impaction was between 20-29 years which was similar to a study conducted by Gopal Bhuju et al [41].

The Limitation of the present study included Unequal distribution of cases, Ethnic distribution was not accessible and Treatment complication was not assessed.Future scope of this study will include more studies to be conducted to verify results based on different populations treatment difficulty assessment.

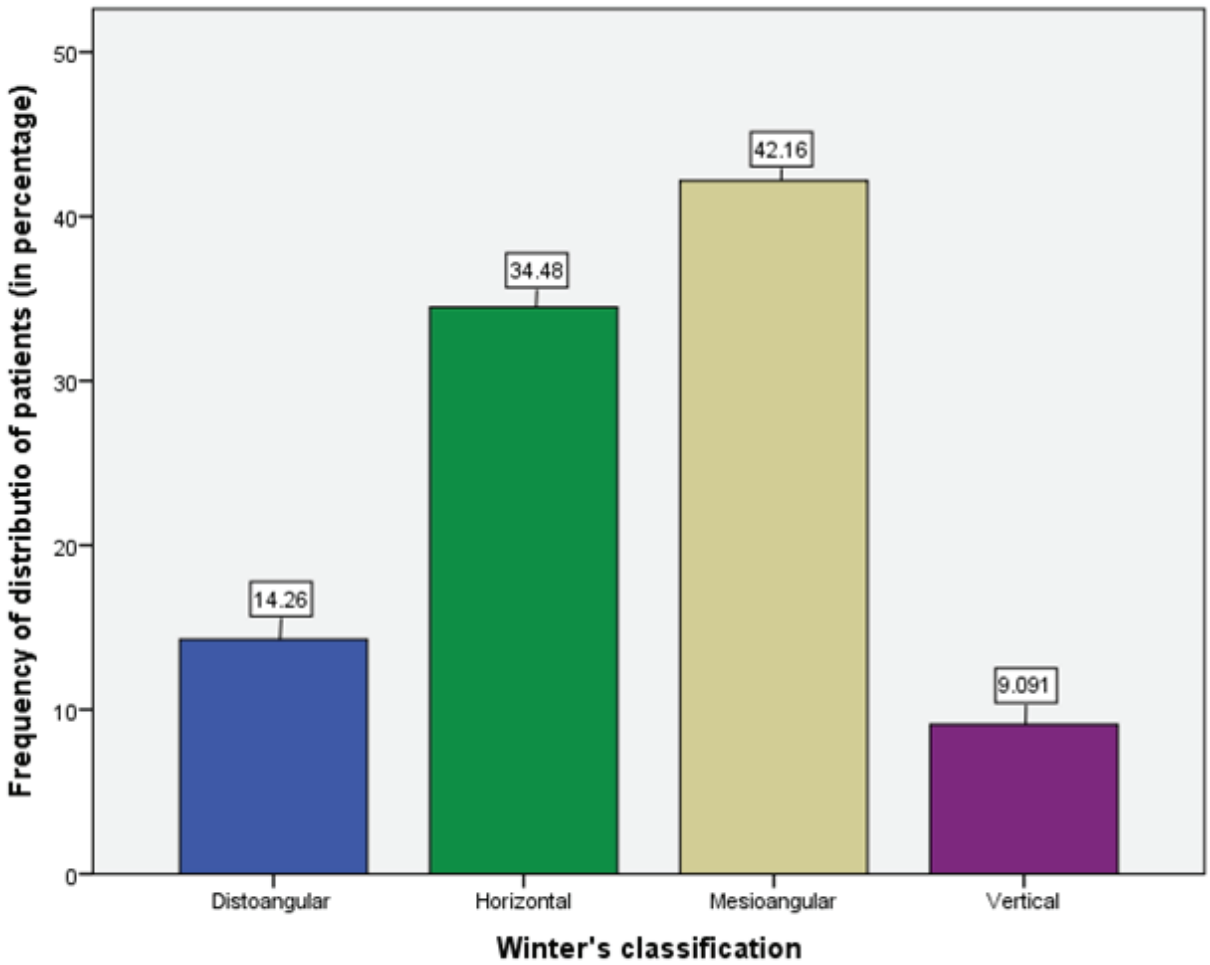


FIGURE 1: This graph represents the represents the of distribution of angulation of impacted mandibular 3rd molar based on Winter's classification (x-axis represents Winter's classification , y axis represents frequency of distribution of patients in percentage) where blue represents distoangular angulation, green represents horizontal angulation and grey represents mesioangular angulation and purple represents vertical angulation . The graph shows that about 42.16% of the population had mesioangular angulation of impacted 3rd molar, 34.48% had horizontal angulation, 14.26% had distoangular angulation and 9.091% had vertical angulation. It can be inferred that the most common angulation noticed was Mesioangular angulation

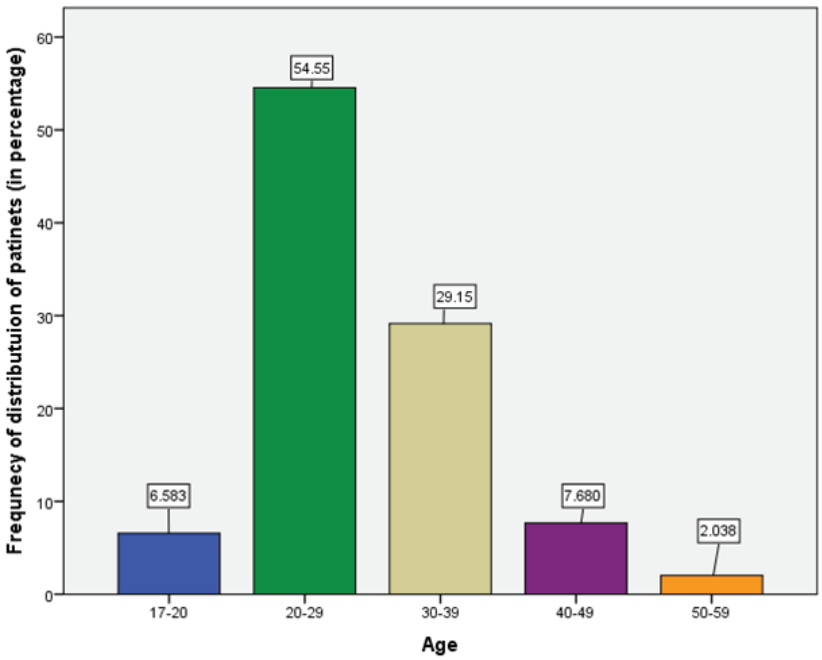


FIGURE 2: This graph represents the distribution of patients with impacted mandibular 3rd molars based on age (x-axis; age , y axis: percentage) where blue represents age group 17-20 years , green represents age group 20-29 years, grey represents age group 30-39 years, purple represents age group 40-49 years and orange represents age group 50-59 years . The graph shows that the(54.55% of the study population belong to the age group of 20-29 years , f(29.05% belong to the age group of 30-39 years , 7.680% belong to the age group of 40-49 years , 6.583% belong to the age group of 17-20 years and 2.038 belong to the age group of 17-20 years. It was noted that the most common age group where impacted mandibular 3rd molar was present was in the age group of 20-29 years.

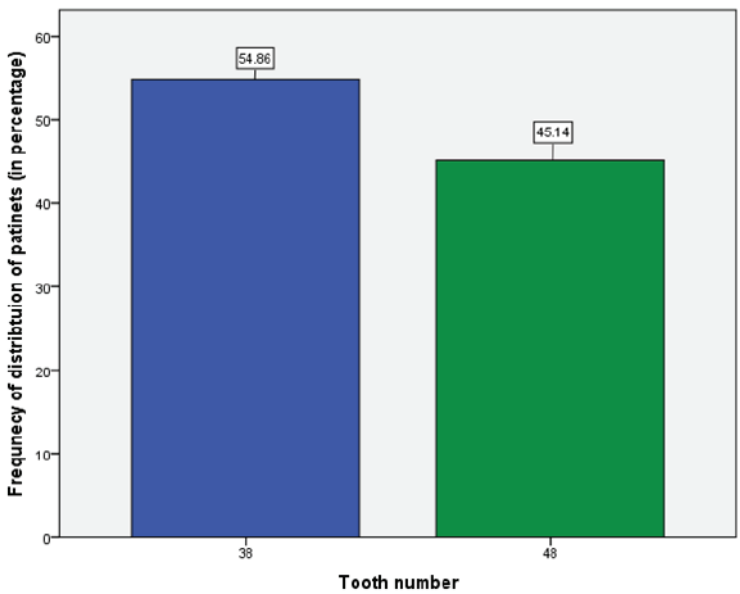


FIGURE 3: This graph represents the distribution of patients with impacted mandibular third molar based on tooth number (x-axis; tooth number , y axis: percentage) where blue represents tooth number 38 years and green represents tooth number 48. The graph shows that about 54.86% of the patients had impacted teeth in 38 and 45.14% had impacted teeth in 48. It was noted that the most common impacted mandibular teeth was 38.

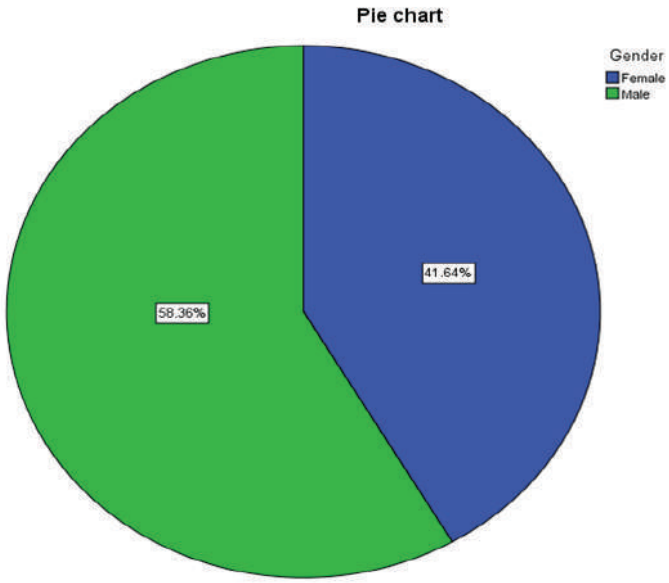


FIGURE 4: This pie chart represents the distribution of patients with impacted mandibular third molar based on gender where blue represents female patients and green represents male patients. The chart shows that 58.36% were male patients and 41.64% were female patients. It can be inferred that the mandibular impacted 3rd molar was more commonly seen in males than in females.

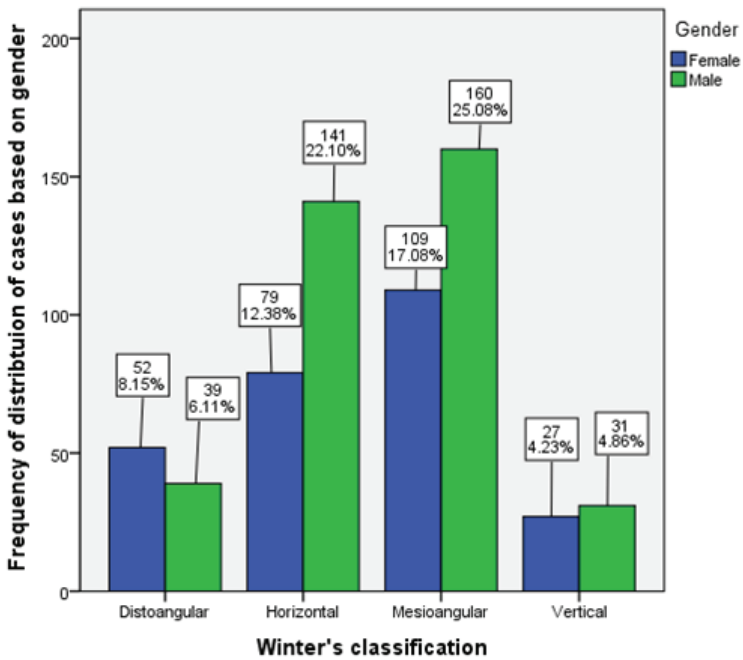


FIGURE 5 : This bar chart represents the correlation between Winter's classification and gender of patients. X-axis represents Winter's classification , Y-axis represents frequency of distribution of cases based on gender where blue represents female and green represents male.Chi- square test was done and association was found to be statistically significant . Pearson's Chi-square value:12.658, DF: 3, P value - 0.005 (p<0.05) hence statistically significant , showing that mesioangular angulation was most commonly associated with males.

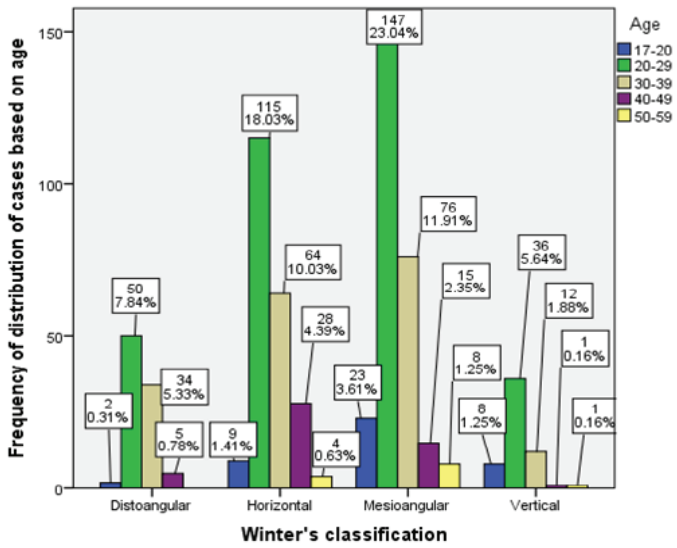


FIGURE 6: This graph represents the correlation of the Winter's classification and age of patients. X-axis represents Winter's classification , Y-axis represents frequency of distribution of cases based on age) where blue represents age group 17-20 years and green represents age group 20-29 years, grey represents 30-39 years , purple represents age group 40-49 years and yellow represents 50-59 years. Chi- square test was performed and association was found to be statistically significant . Pearson's Chi-square value:30.496, DF: 12, p value - 0.002 (<0.05) hence statistically significant , showing that mesioangular angulation was most commonly associated with patients in the age group of 20-29.

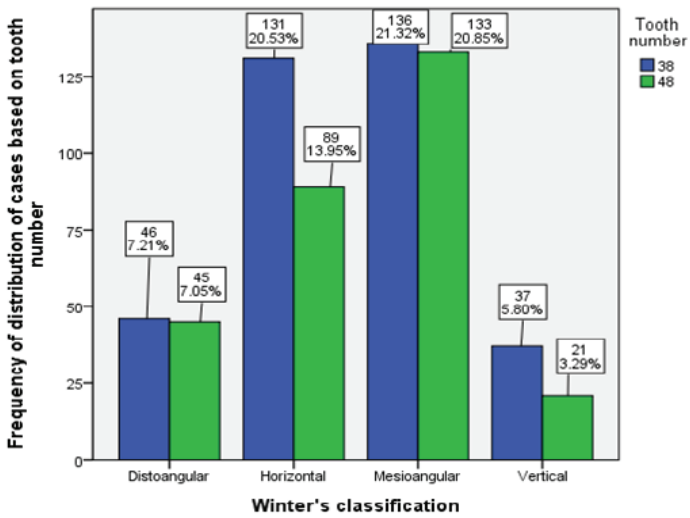


FIGURE 7: This graph represents the correlation of the Winter's classification and tooth number . X-axis represents Winter's classification , Y-axis represents frequency of distribution of cases based on tooth number) where blue represents tooth number 38 and green represents tooth number 48 , Chi- square test was performed and association was found to be statistically insignificant. Pearson's Chi-square value: 6.513, DF: 3, P value - 0.089 (>0.05) hence not statistically significant , showing that there is no association present between Winter's classification of angulation mandibular 3rd molar and tooth number.

Conclusion

Within the limitation of the present study, Correlation between tooth angulation and age, sex was found to be statistically significant, similar findings were also noted in studies done in other populations. These studies will be helpful in optimising treatment for a specific population.Further studies to be conducted to assess the treatment difficulty and option for impacted teeth.

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Incidence of Dry Socket after Extraction Done in Private College and Hospital-A Retrospective Study

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Abstract

Dry socket is a most commonly seen complication of extraction. Dry socket is also known as fibrinolytic osteitis. It is an inflammation of the alveolar bone around the extracted tooth and characterized by severe pain, breakdown of clot formed in the socket results in the socket empty. The main causes of dry socket is due to increased local fibrinolysis which leads to breakdown of clot. The aim of the present study is to evaluate the incidence of dry socket after extraction done in private college and hospital. We reviewed patient records and analysed the data of 86000 patients between June 2019 and March 2020. In that, a total of 1300 patients performed extraction. Out of the total population 0.5% has a complaint of dry socket. The study was done in a private dental college and hospital. Data envy about dry socket. Excel tabulation is done and statistics is done using SPSS software. Approval of the ethical committee was taken before the start of the study. A total of 1300 extractions were done in private dental college and hospital. The overall frequency of dry socket was 0.5%. Incidence of dry socket among females(50%) is higher than the male (44.4%). Incidence of dry socket with smoking is more than non-smoking. Patients age, gender, reason for extraction, onset of symptoms, tooth number, and treatment given play no role in the occurrence of dry socket. Surgical trauma and smoking are the major factors in the occurrence of dry socket. High incidence of dry socket is seen in mandibular molars than the maxillary molars. Treatment with zinc oxide eugenol dressing and irrigation of socket is the most common dry socket management technique. Thus the incidence of dry socket after extraction done in private college and hospital is estimated.

Keywords: *Dry socket, fibrinolytic osteitis, extraction, wound healing, irrigation of socket, ZOE pellets*

Introduction

The postoperative complications following the extraction of a third molar is a condition called dry socket[18,19]. Irrigation removes the debris and bacteria from the denuded bone in the dry socket and use of powdered sodium perborate, heated saline solution, cotton gauze with iodoform can also be done. The prescription of codeine and irrigation with a concentrated solution of sodium perborate is a Home instruction for maintenance of proper oral hygiene. Dry socket is the most common surgical complication following extraction of grossly decayed, impacted and decayed teeth. Dry socket is caused due to reduction of blood clot and exposure of alveolar bone. It is most commonly seen in the mandibular third molars. Many factors result in a dry socket. Factors like-low level of experience. Gender, preoperative infections, use of oral contraceptives in women, smoking and use of local anesthesia with vasoconstrictor [1,2,3,4].

Although dry socket is a self limited complication after extraction of tooth.dry socket is also known as a fibrinolytic osteitis. The incidence of dry socket is more commonly seen in mandibles than the maxilla occurring upto 10 times more often for molars compared with maxillary molars[5,6]. The reason is because of the dense bone[7]. Dry socket clinically characterised by a severe throbbing pain, halitosis[8], foul odour[9], difficulty in opening of mouth and greyish look. Dry socket may also be caused due to extraction of tooth after antiplatelet therapy[10].Every procedure may result in some level of

postoperative bleeding and inflammation, which may result in dry socket complication[11]. The treatment of dry socket depends on each professional experience mainly due to etiology[12]. Most commonly seen management of dry socket are irrigation of socket, zinc oxide eugenol pellet is placed, wound debridement done using betadine saline[14], socket irrigated[15]with betadine saline[13]. 0.2% chlorhexidine gel[16]and a eugenol-based paste is a control group on the postoperative incidence of dry socket in patients having third molars extracted[17] and gentle warm saline rinses help in the healing of the dry socket[20,21,22,23] . Placement of medicated dressing is suggested as surgical intervention due to the occurrence of local complications[24]. The removal of sutures with saline solution under local anesthesia before the application of a medicated dressing which contains zinc oxide eugenol mixed into a semisolid consistency applied to an iodoform ribbon gauze [25,26,27,]. Medicated dressings commonly used are antibacterials agents, topical anesthetics[28] and obtudents like ZOE cotton pellets[29], dentalone, bismuth subnitrate and iodoform paste (BIPP) and lidocaine ointment. Therefore preventive is more effective in dry socket [30,31]. Indication of risk factors and eliminating them along the pharmacological prophylaxis[32]resulted in reduced incidence of dry socket[33] Recently literature has become very sparse on this condition. The aim of study is to evaluate the incidence of dry socket after extraction done at private colleges and hospitals.

Materials and Methods

The study was done in private dental colleges and hospitals, chennai india. We reviewed patient records and analysed the data of 86000 patients between June 2019 and March 2020. These datas were cross verified with photographs. Total sample data was 20 and minimized by inclusion of all available data. Both internal and external validity is available. Data collected with following parameters like age, gender, smoking habits, reason for extraction, sign and symptoms, tooth number and treatment given. Approval of the ethical committee was taken before the start of the study.

Records of total number of patients were studied who underwent extraction between june 2019-february 2020. There were both male patients as well as female patients. Information regarding reason for extraction, onset of system, management given and its outcome were analysed. Data were analysed using SPSS statistical software. Data analysis done using chi-square test. p value was set as 0.05 as level of significance.

Results and Discussion

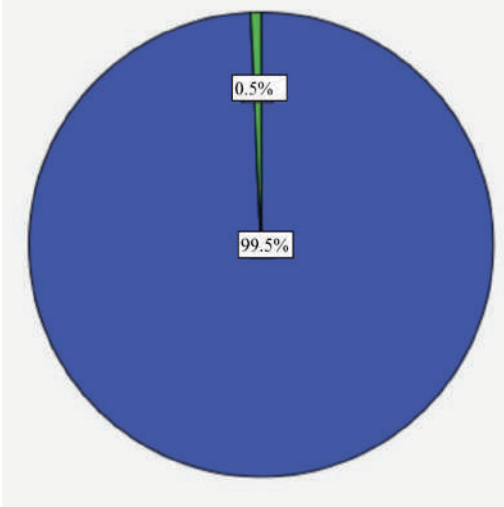
Out of the total number of extraction done, only 0.5% of patients were found with an incidence of dry socket. There were male(44.4%) and female (50%). Age of patients ranged from 20-40 years (27.9%), 41-60 years (34%), 61-75 years(27.9%). A total of 20 patients, among those smokers are 3 patients(11.8%) and non smokers(88.2%). The proportion of smokers was noticeably higher in the male than the female. The most common reason for the extraction is grossly decayed tooth(29.4%), poor prognosis with furcation involved(29.4%), other reasons were impacted tooth (5.9%), root stump(23.5%), dental caries with chronic pulpitis(11.8%). Mandibular teeth are most commonly extracted and result in dry socket complications(54.6%-mandibular teeth). All patients undergo dry socket management, most commonly done treatment is a placement of zinc oxide eugenol pellet in the socket(29.4%), irrigation of socket(11.8%), wound debridement(5.9%), socket irrigation with betadine saline(11.8%). Prevalence of dry socket, the overall incidence of dry socket was 0.5%(20 dry socket, 1300 extractions)(Graph 1)**.** Association of smoking habits and gender. Incidence of dry socket in male is (44.4%) and females is (50.0%)females are more prone to dry socket than the male. This difference was statistically not significant (P>0.05)(Graph 2). Association of gender with dry socket and reason for extraction. More commonly patients above 40 years underwent extractions. Most common cause of dry socket is due to grossly decayed teeth (29.4%). This difference was found to be statistically not significant (P>0.05)(Graph3).

Dry socket is an important clinical complication after extraction. It is characterised by severe throbbing pain after 2 to 3 days of extraction[34]. The main causes of dry socket is due to increased local fibrinolysis which leads to breakdown of clot. Since the formation of blood clots is an important part of healing, when the healing of the extraction site is disrupted this leads to a condition called as dry socket. Although there are risk factors that result in a dental patient's to the formation of a dry socket, knowing who will actually develop one is totally

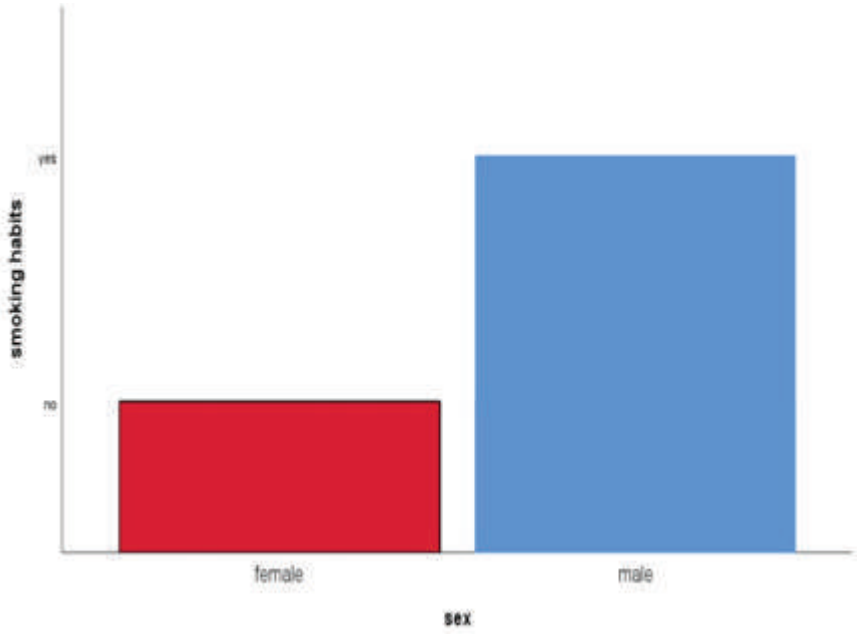
unpredictable. Dentists should give proper advice after extraction of the tooth.

The study performed by Al khateeb et al in 1991[7]and vezeau PJ et al in 2000[35], stated female has a high chance of dry socket than the male - this study coincide with our study where results showing females(50.0%) higher that the males(44.4%) the possible reason is women who take birth control pills may be at a greater risk for getting dry sockets. Prevalence of dry socket highest to be in fourth and fifth decades with a peak incidence of 41-60 years. This study coincided with Oginni FO et al in 2003[36], stated - females were more prone to dry socket with the age group of middle adolescence. The possible explanation of this difference in age group is presence of well developed alveolar[37]one and relative infrequency of periodontal disease[38]. The Most common causes for extraction of teeth are due to grossly decayed.[29]. The reason is due to various microbial infections which lead to dry sockets. Dental caries with chronic periodontitis are also a commonest indication for extraction. Indication for extraction was not stated in 13% of the cases and this was probably due to the fact that it was one main tooth that was causing severe pain. Such pains commonly seen in acute pulpitis or irreversible pulpitis. Most common tooth to be extracted is mandibular molars, This result coincides with the Chaudhary M et al n 2012, stated-mandibular third molar most commonly affected and possible reasons could be difficulty of extraction was significantly associated with the development of dry socket[39].

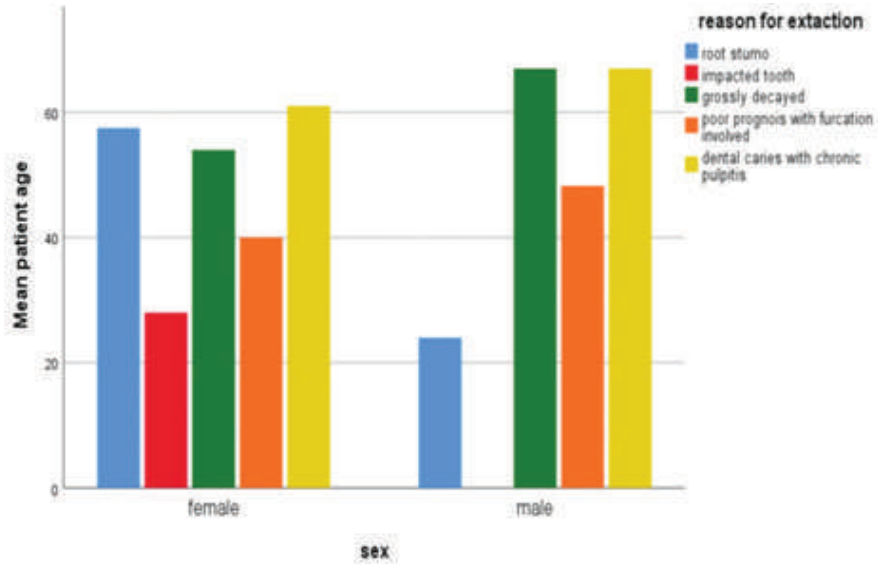
In our study results show smokers are more prone to dry sockets than non-smokers. Field EA et al in 1985, have shown that smokers are more likely to get a dry socket as compared to non smokers. The possible reason is when the person smokes-sucking action creates which disrupts or dislodge the blood clot that has formed at the tooth sockets. The carbon monoxide that enters bloodstreams when they smoke limits the amount of oxygen to the site of healing tissues. Nicotine interferes with the normal healing process. Prevalence of dry socket can be managed by a proper dry socket management and zinc oxide eugenol pellet(29.4%), this finding was acceptable with Bowe D c et al on 2007[40], he concluded that the dry socket can be managed by using a zinc oxide eugenol pellet[41]. But according to modern trends and recent strategy in private colleges and hospitals only less number of patients were found to be developed with dry sockets out of the total number of extractions. Limitation of our study was that some data were unclear of certain reporting parameters, such data were not considered and it is a unicentered study. Reason for minimal dry socket complication in private college is due to Good Quality of treatment, proper oral health education, insisting the patients to follow up the post operative instructions promptly and most importantly best method of sterilisation technique. Dry socket was seen in patients after extraction of grossly decayed teeth as well as mandibular teeth due to multifactorial reasons.



Graph 1: Pie chart showing the percentage of dry socket from the total number of extraction. Patient complaints of dry socket are represented in green colour and total numbers of extraction are represented in blue colour. Very less number of patients complaints of dry socket after extraction is done.



Graph 2: Bar graph showing the association of gender and smoking habits who had complaints of dry sockets. Y-axis shows smoking habits and X-axis shows prevalence of smoking habits among male and females with dry sockets. Blue color represents male and red colour represents females. The incidence of smoking habits in male is higher than in females. From the graph, it is evident that dry sockets have a male predilection. However, this is statistically not significant. Chi-square test;p-value-0.110; (p>0.05)- not significant



Graph 3 : Bar graph showing the association of gender and reason for extraction. Y-axis shows percentage of age of the patients and X-axis shows reason for extraction of tooth among male and female patients. Blue color represents extraction due to root stump,red color represents extraction due to impacted tooth, green color represents extraction due to poor prognosis, orange color represents extraction due to poor prognosis with furcation involved and yellow color represents extraction due to dental caries with chronic pulpitis. Most common cause of extraction is mainly due to grossly decayed teeth and dental caries with chronic pulpitis. Males are more affected than the females. However, this is statistically not significant. Chi-square test;p-value-0.464; (p>0.05)-not significant

Conclusion

Our study shows that incidence of only 0.5% of patients undergoing extraction have got dry socket conditions at private college and hospital. High incidence of dry socket is seen in mandibular molars than the maxillary molars. Thus the incidence of dry socket after extraction done in private college and hospital is estimated.

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The Assessment of Longevity and Efficacy of Different Types of Splints Used in Periodontal Therapy: A Single Center Epidemiological Retrospective Study

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Abstract

Splints are routinely used for occlusal force dissipation from the long axis of periodontally compromised teeth towards adjacent healthy teeth. This process causes forces generated from traumatic occlusion to be separated from underlying periodontium favouring regeneration of lost tissue. Splinting periodontally compromised teeth together causes the redistribution and redirection of functional and parafunctional forces to bring them within tolerance of the supporting tissue and to reduce mobility. However the common drawback of using splints include the lifespan of the splint without periodic modifications. The present study tries to relate the reduction of mobility in periodontitis cases from grade II to grade I using different splinting materials and the lifespan of the same. The present study was a single centered retrospective university based design, using patient records of Saveetha dental college, from 1st June 2019 till 1st March 2020 . The included patients were individuals with generalised/ localised chronic periodontitis, who had undergone non surgical periodontal therapy along with the use of provisional splints.The parameters assessed for two hundred and fifty eight patients included in the present study was longevity/lifespan of splints and the reduction in the degree of mobility. Our observations revealed that stainless steel wire composite splints were used 1.96 time more frequently than mesh reinforced composite splints, reduced grade II mobility to grade I mobility in teeth affected by periodontitis to a greater than composite resin wire mesh.($p<0.05$). Within the limits of this study splints were found to reduce mobility as an adjunct to periodontal therapy from grade II mobility to grade I mobility while using stainless steel wire composite resin splints among age groups of 20-40 years with a lifespan of 6 months 24% of the time .

Keywords- *attachment loss, composite resins, longevity, periodontitis, periodontal splints*

Introduction

Periodontal disease is of a chronic inflammatory nature characterized by gingival inflammation, loss of connective tissue attachment and destruction of alveolar bone. Progressive attachment loss around involved teeth eventually results in the increased mobility.[1,2] Increased tooth mobility adversely affects functions , aesthetics and patient comfort. Clinical management of such periodontally compromised teeth creates a dilemma in the operators hands to splint teeth or extraction followed by the use of dental prosthesis.

Periodontal literature suggests the prime reason for using splints includes primary or secondary trauma from occlusion, progressive mobility, migration or drifting due to periapical lesions.[3] Splinting is defined as the process of joining of two or more teeth into a rigid unit by

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means of a fixed or removable restorative device.[4] There are several types of splint and the choice of splint to be used depends upon the severity of mobility, the stage of treatment involved, and the anticipated outcomes. The types of splints can be broadly classified into removable-external, fixed- internal, cast metal resin bonded fixed partial dentures, combined splints with a fixed and removable component and endodontic splints. The materials commonly used include ligature wire, enamel bonded material like composite resin, welded bands, continuous clasps and night guards. [5] The objectives of splinting include the ability to provide occlusal rests, redirect occlusal forces, preserve the integrity of arch, stabilization of mobile teeth during surgery, prevent extrusion of unsupported teeth and most importantly to improve patient comfort around teeth. [6,7] Splinting is used routinely in both periodontal as well as restorative dentistry as a provisional means of managing mobility while simultaneously attempting to reduce its underlying cause.

Studies done by Clement Azodo showed an increase in the number of newly splinted cases in attempts of favouring regeneration for future periodontal and restorative dentistry using newer materials such as growth factors, plasma rich in growth factors, platelet rich fibrin and a future role of stem cells in regenerative therapy .[8–14]Considerable advances in the field of diagnosis of periodontal disease has also occurred adding a molecular biology as a factor to prior microbiological evidence alone.[15,16]Conventional research done on this topic usually involves case reports and case series justifying newer methods of splinting and aesthetic splints to retain teeth. [17,18] Periodontal literature has fewer clinical studies comparing different types of splints however studies assessing the longevity of splints justified their findings using a sample of 39 patients with 162 teeth and 57 patients with 227 teeth.[19,20] Considering the only source of comparison of clinical benefits from splints is not in the form of clinical trial with randomization it creates a large void in literature in terms of comparative benefits of different splints. The lack of such studies also creates difference in opinion in the usage of splints all together.

Thus the aim of this study was to compare two different modalities of splinting (stainless steel wire reinforced composite resin splints and composite resin mesh splints) and the clinical scenarios where they had maximal lifespan without modification and reduction of mobility compared to demographic data over a period of 6 months.

Methodology

The current study was performed as a single centered retrospective university based design, using patient records of Saveetha dental college and hospital in Chennai from 1st June 2019 till 1st March 2020. Data was screened by two blinded investigators, where inter-examiner agreement was reached prior to inclusion of individuals into the study. The segregation of data was initiated after ethical approval from Saveetha university scientific review board.The included patients were individuals with generalised/ localised chronic periodontitis (chronic periodontitis diagnosed as per criteria of Armitage classification of periodontal diseases 1999). These patients had undergone non surgical periodontal therapy followed by splinting.

Individuals excluded from this study were based on (i)pregnancy or lactating mothers(ii) patients currently smoking (iii) patients with uncontrolled systemic diseases (iv) Incomplete data collection in terms of radiographic /periodontal status or broken appointments (v)Aggressive periodontitis as per Armitage classification of periodontal diseases 1999.

A total of two hundred and fifty eight patients were categorized into two groups based on the use of stainless steel wire composite splints and the other using Composite resin reinforced splints.Among these patients 211 cases were treated using stainless steel wire composite resin mesh and 47 cases were treated using composite resin mesh splints.

Age groups of patients included were subdivided for analysis and interpretation into group A (21-40 years) and groups B (41-60 years). Similarly the longevity of the splints were assessed over a period of 6 months and individuals included were subdivided into subgroups based on lifespan of 1 month, lifespan of 3 months and lifespan of 6 months.

The parameters assessed in this study included longevity/lifespan of splints and reduction in grade of grade II to grade I mobility as per Miller`s tooth mobility

index on subsequent review appointments recorded in the clinicians notes.

Statistical Analysis

Statistical package for social sciences version 23, software from the University of Stanford (Norman H. Nie, C. Hadlai, Hull and Dale H) was used for all statistical analysis of the present study.Chi square test was used to assess statistical significance which was found to be significant while comparing the reduction in grade of tooth mobility using stainless steel wire composite resin splints ($p<0.05$).(graph 1)The association between type of splint used and lifespan of the splint was also found to be statistically significant.(graph 2)

Results & Discussion

In the present study we observed that a total of two hundred and fifty eight cases were reviewed. Among these subjects, 211 were treated using stainless steel wire composite splints while remaining 47 were treated with composite resin mesh splints. (Table 1, Graph 1) Stainless steel wire composite splints were used 1.96 times more frequently than mesh reinforced composite splints.(Table 2) A total of 63 cases among 211 stainless steel wire composite splints had a lifespan of 6 months while 21 among 47 composite resin reinforced mesh splints cases had a longevity of 6 months.(Table 1,Graph 1)There was reduction in the grade of mobility using stainless steel wire composite resin splints in 85 cases and 12 cases in composite mesh reinforced splints.(Table 1) In the remaining cases it was found that no reduction in the degree of mobility was seen in 126 out of 211 cases using stainless steel wire composite resin splints and 35 out of 47 composite resin reinforced composite resin mesh splints.(Table 1) Similarly longevity of the splint for upto 3 months was seen in 160 cases between the age groups of 20 to 40 years, while 66 cases had a survival rate of splints for upto 6 months between the age group of 41-60 years.(Table 1, Graph 2) Stainless steel wire composite splints reduced grade II mobility to grade I mobility in teeth affected by periodontitis to a greater extent as compared to composite resin wire mesh.($p<0.05$) (Table 1)

Observations obtained from the present study showed a higher tendency of reduction of grade II mobility to grade I mobility in periodontitis patients where stainless steel wire composite resin mesh over a period of 6 months.(Graph 1) It was also observed that Reduction of tooth mobility from Miller`s grade II mobility to grade I mobility was seen 1.578 times more frequently after using splints.(Table 2) Similar findings were seen by Forsberg and Hagglund in 1958, where reduced mobility was seen after splinting upto 90 days using stainless steel wire composite resin splints.[21]

Goldberg on the other hand performed measurements on 10 patients using modified muhlemann periodontometer (custom instrument patented by Dr. Goldberg) to assess mobility after scaling and root planing. His results revealed 25% decreased mobility immediately after scaling and root planing, while there was a decrease of 40-50% within 3 months, 50% within 6 months of occlusal adjustments.[22] Rateitschak et al suggested that additional incorporation of splints or orthodontic appliances increased mobility in the first month followed by a gradual decrease after 2-3 years.[23]

Studies comparing splint materials and thickness on tooth mobility have shown significantly less tooth mobility with direct composite splints and no difference between stainless steel wire composite splints and nylon based splints.[24,25] A possible explanation for these findings obtained could be due to custom manipulation of stainless steel wire along buccal contour of teeth while composite resin mesh splints on the other hand may set without appropriate adaptation to tooth surface leaving voids conventionally filled with flowable composite resin acting as a weak link in splints. A consensus based on available literature can be summarised as splinting done provisionally while treating the underlying periodontal cause for tooth mobility. The available literature also suggests stainless steel wire composite resin splints reduce mobility to a greater extent than composite resin mesh splints but is used less frequently due to unaesthetic appearance. A similar explanation was given by Lindhe and Nyman in 1975 stating tooth mobility with splints remained unaltered or slightly reduced only after periodontal therapy was done for periodontal pockets.[26,27]However since the advent of subperiosteal implants in comprehensive treatment plans,the use of splints have been questioned as an unnecessary additional load on periodontally compromised teeth abutment teeth.[28] Splints and removable prosthesis are kept as a last alternative in case of proximity to vital structures

such as arteries and nerves aside from aesthetics.[29,30]

Splinting does not completely eliminate occlusal stress , functional stimuli exerted in the long axis of the teeth supply functional stimulation.[31] The function of a splint is to distribute and redirect functional and parafunctional forces to bring them within tolerance of the supporting tissue and to reduce mobility.[5]

Limitations of the present study include lack of correlation of the severity of periodontitis and extent of bone regeneration resulting in reduction of mobility in periodontitis patients from grade II to grade I. No biomarkers were used to assess extent of healing and inflammation in periodontal tissue such as fibroblast growth factor 2, endothelin-1, cytokines.[32–35]

Future scope in the present study are towards newer techniques in splinting teeth or newer biomaterials such as quartz, nylon, ethylene fiber which can be used as a substitute with both mechanical properties and aesthetics related to composite resin.[36–38] Longitudinal clinical trials with standardised treatments like scaling alone, scaling and root planing alone with and without the use of splints are required to confirm their role in periodontal therapy and not just as an adjunct to conventional periodontal therapy.

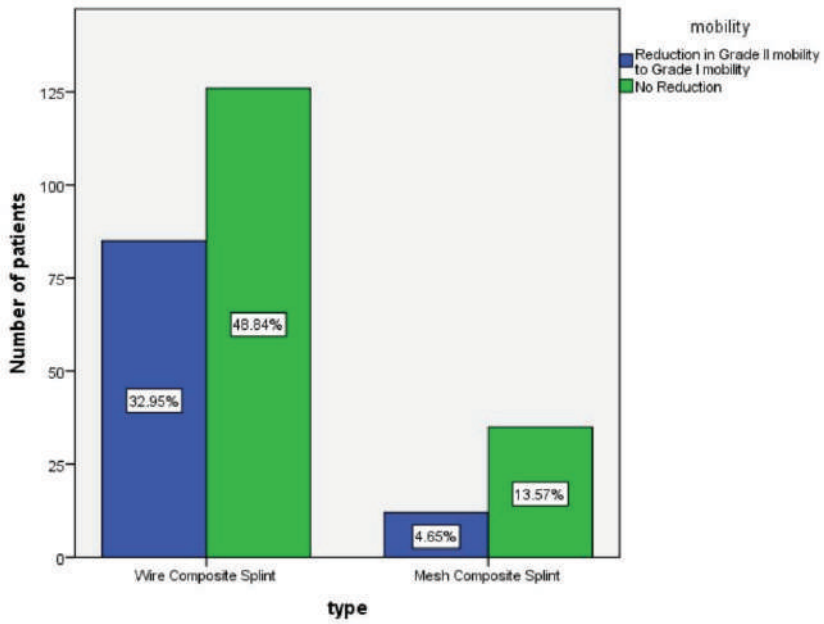
Legends:

Age groups	**Lifespan of 1 month without modifications**	**Lifespan of 3 months without modifications**	**Lifespan of 6 months without modification**	**Total**
20 - 40 years	14	160	18	192
41-60 years	0	0	66	66
Total	14	160	84	258
Type of splint				
Wire composite splint	8	140	63	211
Mesh composite splint	6	20	21	47
Mobility				
Type of Splint	**Reduction in Grade II mobility to Grade I mobility**	**No Reduction in mobility**	**Total**	
Wire composite splint	85	126	211	
Mesh composite splint	12	35	47	

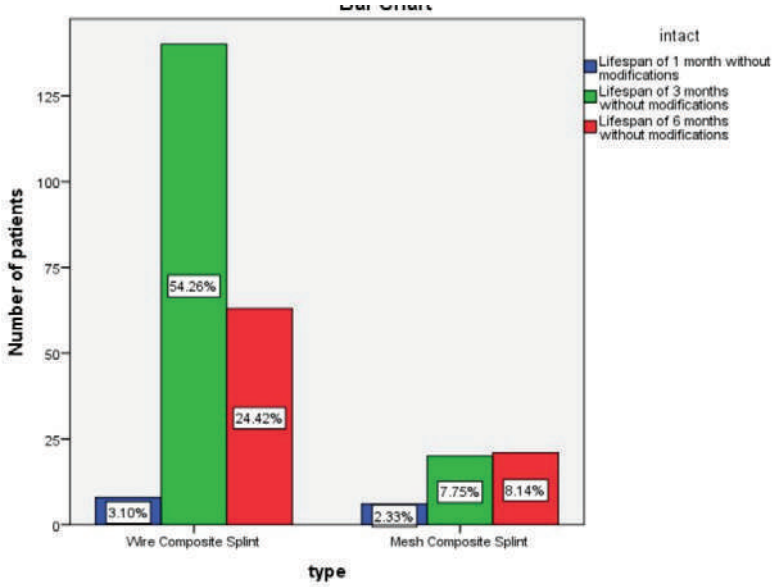
TABLE 1- Tabulation of all Descriptive data collected from the study based on age of the patient and lifespan of the splint, type of splints and age groups of patients, type of splint and reduction of mobility.

Feature	Value	95% Confidence Interval	
		Lower	Upper
Odds ratio for type of splint (stainless steel wire composite splint/ mesh reinforced composite splint)	1.968	0.966	4.006
For the cohort of Reduction in grade II tooth mobility to grade I tooth mobility using splints	1.578	0.943	2.641
For the cohort of No reduction in tooth mobility using splints	0.802	0.656	0.980

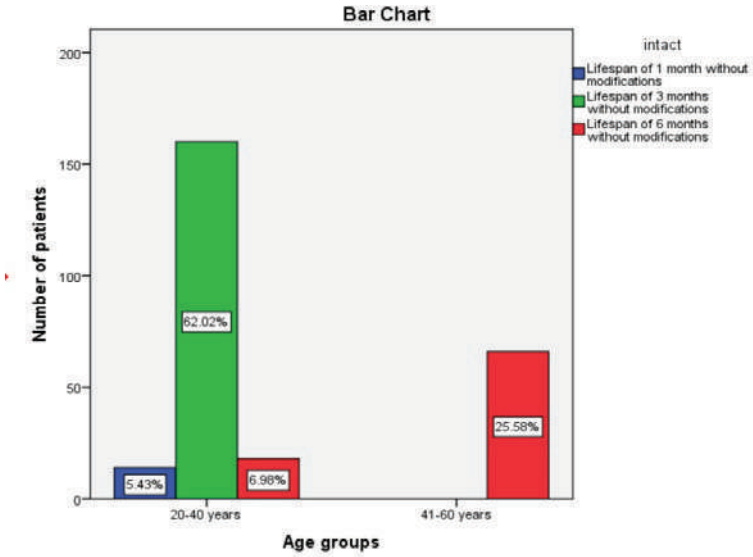
Table 2- Odds ratio calculated for independent findings; Stainless steel wire composite splints are used 1.968 times more frequently then mesh reinforced composite splints; Reduction of tooth mobility from Miller`s grade II mobility to grade I mobility is seen 1.578 times more frequently after using splints; No reduction in Millers mobility is seen 0.802 times after using splints.



Graph 1- Bar graph illustrates reduction of mobility and type of splint used in the present study, where X-axis denotes type of splint used to treat patients namely stainless steel wire composite splints and mesh reinforced composite splints; Y axis denotes number of patients treated with splinting; Blue colour in legends denotes cases with reduction of Miller`s grade II tooth mobility to grade I tooth mobility while green colour denotes no reduction in tooth mobility. Chi square test was used and the association between type of splint and reduction of mobility was found to be statistically significant. (X^2 value 3.56, p value 0.041($p<0.05$)). The reduction in tooth mobility was higher in the patients where stainless steel wire composite was used than composite reinforced mesh type of splints.



Graph 2- Bar graph illustrates the type of splint used and lifespan of splints without modification at different periods of time. X-axis denotes type of splint used to treat patients namely stainless steel wire composite splints and mesh reinforced composite splints; Y axis denotes number of patients treated with splinting; Blue colour in legends denotes cases where the splint was modified less than 1 month after fabrication, green denotes cases where the splint was modified within 3 month of fabrication, red denotes cases where the splint was modified within 6 months of fabrication. Chi square test was used and the association between type of splint and lifespan of splint without any modifications over 1 month, 3 months and 6 months was found to be statistically significant. (X^2 value 11.809, p value 0.003($p<0.05$)) The longevity of the splints for upto 6 months after fabrication without any modification was higher in the stainless steel wire composite splints.



Graph 3- Bar graph illustrates age of patients and type of splint used in the present study, where X-axis denotes age group of patients from 20-40 years and 41-60 years; Y axis denotes number of patients treated with splinting;Blue colour in legends denotes cases where the splint was modified less than 1 month after fabrication, green denotes cases where the splint was modified within 3 month of fabrication, red denotes cases where the splint was modified within 6 months of fabrication. Chi square test was used and the association between age of patients and lifespan of splint was found to be statistically significant. (X2 value 189.044, p value 0.00 ($p<0.05$)). There was a higher lifespan of splints among patients belonging to the age group of 41 to 60 years which was highly statistically significant.

Conclusion

Within the limits of this study splints were found to reduce tooth mobility as an adjunct to periodontal therapy from grade II mobility to grade I mobility using stainless steel wire composite resin splints among age groups of 20-40 years with a lifespan of upto 6 months 24% of the time.

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Conflict of Interest: All authors involved in the present study declared No conflict of interest.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Incidence of Impacted Canines in Maxillary Arch

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Abstract

The aim of this study is to determine the incidence of impacted canines in maxillary among the patients visiting a Dental College.This study was conducted to assess the incidence, frequency, side and associated symptoms of impacted canines in maxillary arch.This study was conducted on a hospital based ,matched case - control study among the patients visiting saveetha dental college. Data was collected from record management software.700 case sheets were reviewed from the dental treatment records for presence of impacted canines from June 2019-March 2020.Final sample size consist of The above mentioned data was coded and transferred into a statistical package for social sciences(SPSS) software for statistical analysis.A correlation test and chi square analysis was done.The results were recorded.The difference was considered statistically significant.Impaction of maxillary canines most commonly occurs in females than in males, mostly buccally placed and most commonly in left arch.

Keywords: *Impaction,Canines,maxillary arch,Incidence and frequency, gender*

Introduction

Impacted tooth is the one that is prevented from erupting into the position due to lack of adequate spaces,malposition and other impediments.Maxillary canines corresponds the most common impacted tooth following third molars(1).Dental anxiety and anxiety-related avoidance of dental care creates significant problems for the patients and dental practitioners(2) The etiology of impacted maxillary canines is still unknownTheir occurrence is considerable and its frequency increases with other genetically associated dental anomalies(3).Notwithstanding the opinions of some researchers in favour of an exclusively genetic etiology for its occurrence,there are many and various reasons for impacted canines(4).The maxillary canines are one of the most important teeth in terms of aesthetic and function(5).In dentistry, various treatment procedures are done to treat dental problems.(6).Treatment of impacted maxillary canine is a common challenge faced by dental professionals in daily practice (7).Alignment of impacted canines with minimal morbidity of adjacent teeth and periodontium is the main challenge being faced. Peck et.al.,suggested palatal canine is genetic in origin,whereas labial impaction is due to inadequate arch space,common in females, with a wide variation in different racial population(3).Also similar studies have been like Effectiveness of 0.2% chlorhexidine gel and a eugenol-based paste on postoperative alveolar osteitis in patients having third molars extracted(8), assessment of the knowledge, awareness,attitude, and practices among the undergraduate dental students of our institution regarding biomedical(9), Comparison of Pterygomaxillary Dysjunction With Tuberosity Separation in Isolated Le Fort I Osteotomies(10). Canonical Wnt Pathway Gene Expression and Their Clinical Correlation in Oral Squamous Cell Carcinoma(11), Patterns and Etiology of Maxillofacial Trauma in South India(12),antibiotic prophylaxis for infective endocarditis (IE) (13), Evaluation of Three-Dimensional Changes in Pharyngeal Airway Following Isolated Lefort One Osteotomy for the Correction of

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Vertical Maxillary Excess[14].

The aim of this study is to determine the incidence of impacted canines in maxillary among the patients visiting for Saveetha Dental College, Chennai.

Materials and Methods

This study was conducted on a hospital based ,matched case - control study among the patients visiting saveetha dental college.Data was collected from record management software from June 2019- March 2020.It consist of a retrospective analysis of record for up to 800 patients to reveal any evidence of impacted maxillary canines.Presence of impacted canines was evaluated for each of the patient.The analysis was done by analysing the pre operative photographs and radiographs from the record management software.Those collected data was entered into Microsoft excel with parameters as follows -PID number, Patient Name, Gender, Age, presence of impaction, side of placement, presence of any infections or cyst..The above mentioned data was coded and transferred into a statistical package for social sciences(SPSS) software for statistical analysis.A correlation test and chi square analysis was done.The results were recorded.The difference was considered statistically significant.

Results and Discussion

Among 800 patients, 17 patients had impacted maxillary canines, out of which 10 of them were Male patients and 7 of them were female patients.On analysing the results it can be concluded that there is an increased incidence of maxillary canine impaction when compared to females.This is in contrary with the other study, which suggest the higher incidence of maxillary canine impaction in females,than in males.Maxillary canines erupt at an average age from 11-12 yrs, erupting earlier in females than in males[5].Buccally placed canines are more common than palatally placed canines.Buccally and palatally placed impacted canines have different etiology,where buccally placed canines had sufficient place for eruption whereas palatally placed canines had insufficient place[5].Almost every patient had unilateral impaction,except for one of the patients with bilateral impacted teeth.

The diagnosis and localisation of impacted teeth is the most important step in management of impacted teeth.Surgical exposure and orthodontic alignment was chosen for the choice of treatment[4].

There are three types of imaging to specify the position of the impacted canines : a retroalveolar x ray, occlusal film and three quarters of profile image[6]. Radiographs are required to view impacted canine in three dimension.The most common means of radiographic localisation currently In use are parallax and CBCT.

For the management of impacted canines,usually involves five treatment options-No active treatment I.e., leave in situ and monitor radiographically for cyst formation, Interceptive treatment,surgical exposure, orthodontic alignment,surgical repositioning and extraction[7].A study has also found diagnosis of ranula can be made through clinical characteristics, fine needle aspiration and various imaging techniques such as Magnetic Resonance Imaging (MRI) or Computed Tomography (CT). [15]. Pre-operative dental anxiety is a major predictor of pain experienced by patients during dental extractions. [16].The pain of tooth extraction varies among individuals, and each extraction of an individual may be quite different. [17].Botulinum,It is the first toxin used for therapeutic purposes helps in curing many post operative pain[18].A similar study was also conducted assess the knowledge, awareness, and attitude of dental students toward human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS) patients[19].

The surgical techniques to expose the impacted teeth , include open eruption and closed eruption techniques [5].A study has evaluated the application of extended nasolabial flap versus buccal fat pad graft in the surgical management of oral submucous fibrosis[20].

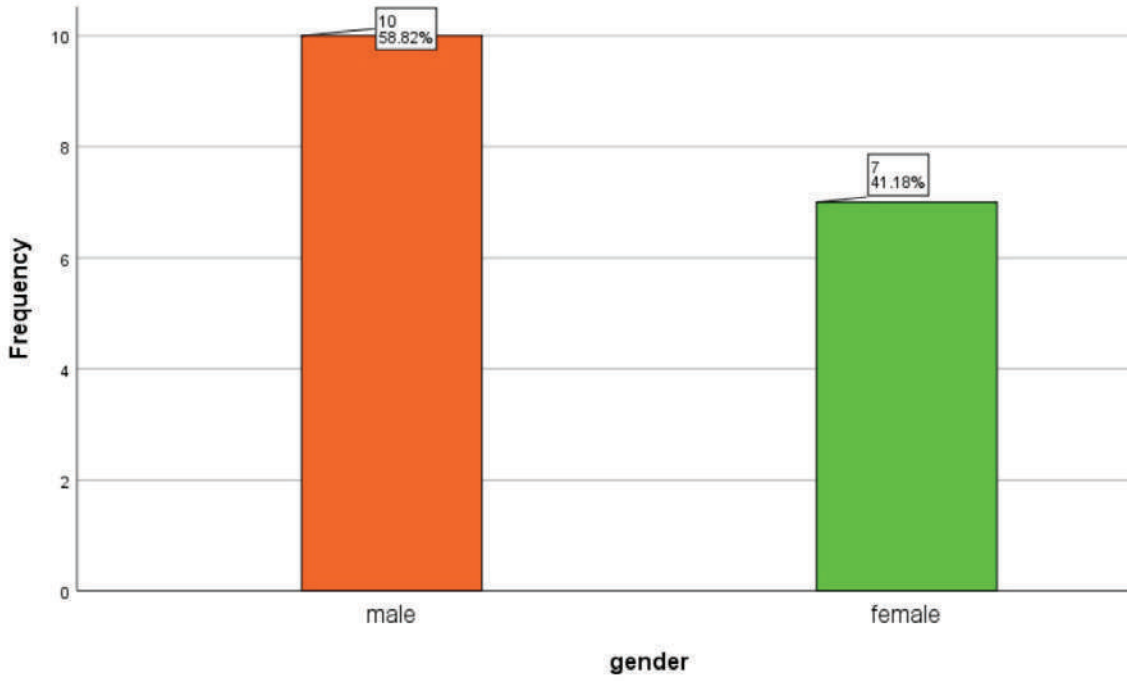


Figure 1: This Bar graph depicts the frequency distribution of patients' gender. X axis represents the gender of patients and Y axis represents the frequency of patients.58.82% of the patient's were males(orange) and 41.18% of the patients were female patients(green) with impacted canines.Impacted canines are more prevalent in males when compared to females.

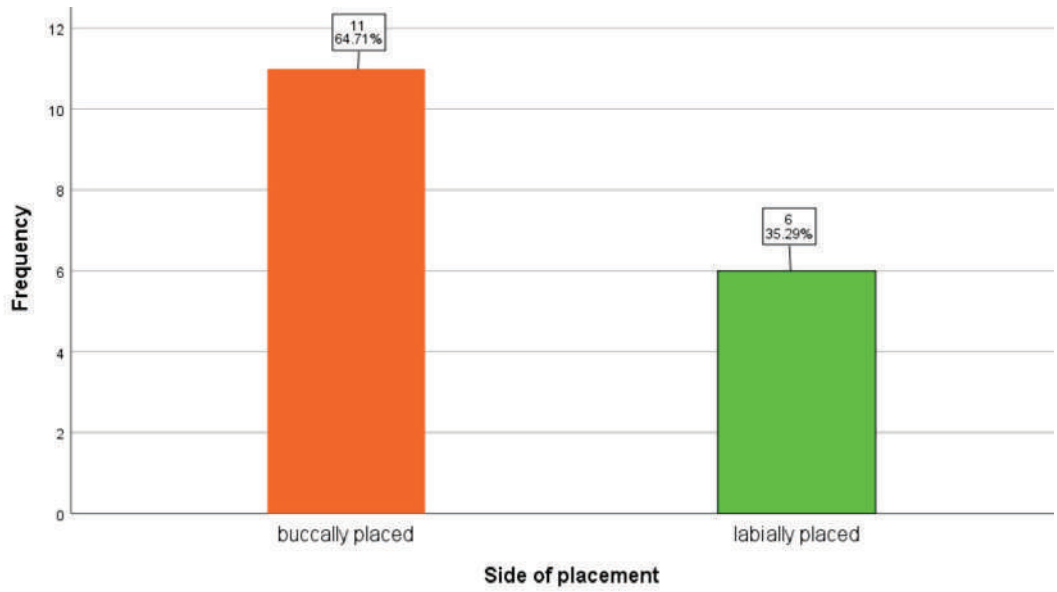


Figure 2: This Bar chart depicts the frequency distribution of side of placement of impacted canines.X axis represents the side of placement of impacted canines and Y axis represents the frequency of patients. 64.72% of the patients had buccally placed impacted (Orange) canines.35.29% of the patients had labially placed impacted canines(Red). Impacted Canines are more common on the buccal side of the arch.

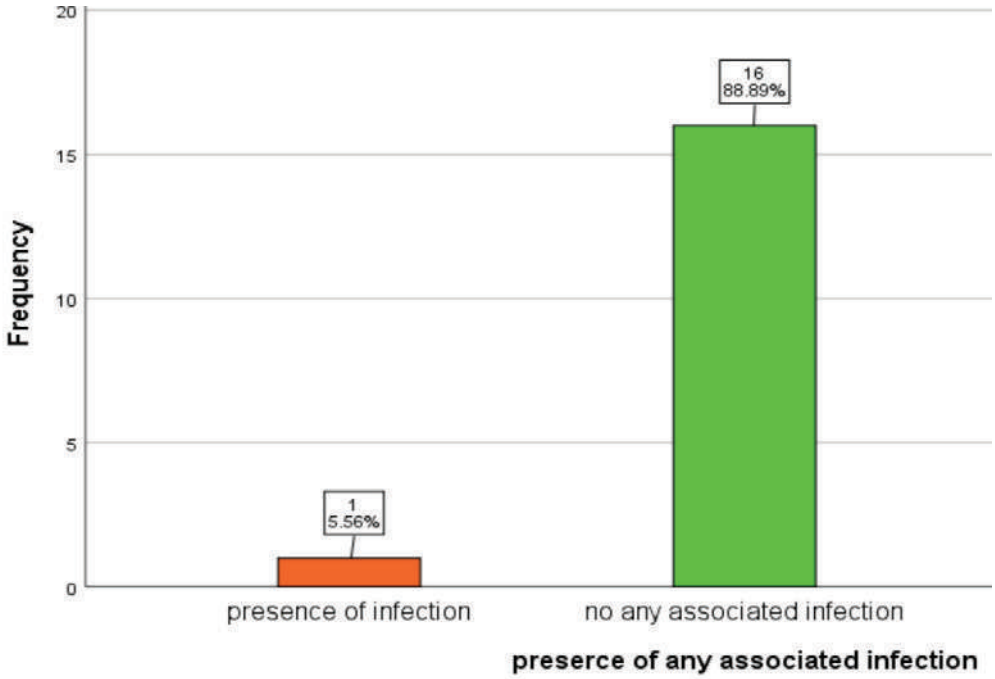


Figure 3:This Bar chart depicts the frequency distribution of associated cyst/ infections in relation to the impacted tooth.X axis represents presence/ absence of any associated infections and Y axis represents the frequency of patients. 5.56% of the patients had impaction associated infection(Orange colour)and 88.89% of the patients did not have any associated infection(Green colour). There was no any significant association of infection in relation to impacted canines.

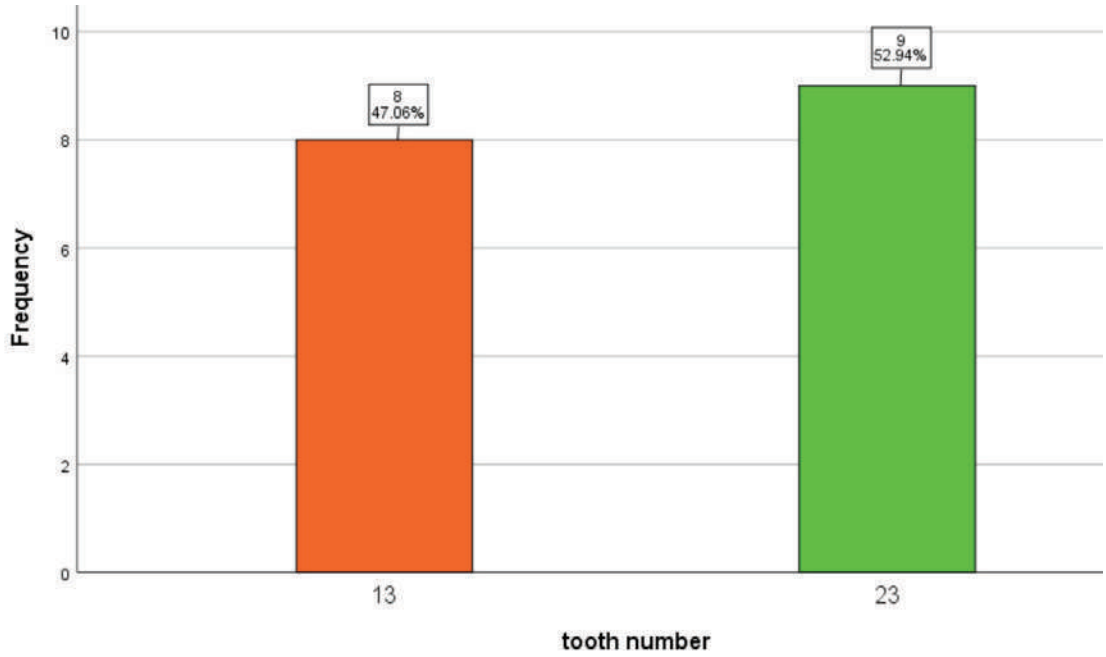


Figure 4: This Bar chart depicts the frequency distribution of the side of placement of impacted teeth.X axis represents the tooth number and Y axis represents the frequency of patients. 47.06% of the patients had impacted canines on the right side of maxillary arch(Orange). 52.94% of the patients had impacted canines on the left side of the maxillary arch(Green).Impaction of canines more commonly occurs on the left side of the arch.

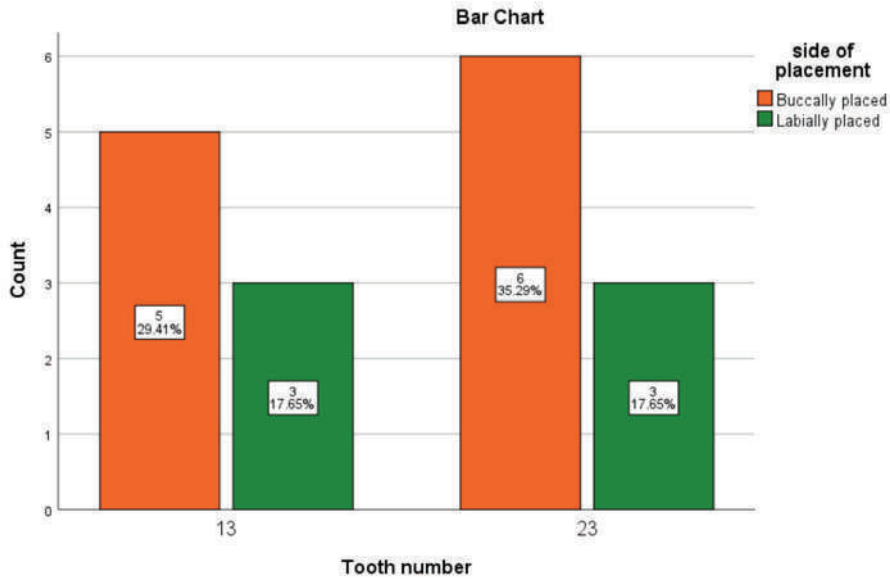


Figure 5: This Bar chart represents the correlation between side placement of impacted canine and tooth number. X axis represents the tooth number and Y axis represents the frequency of patients. Impacted canines on the right side of the arch(Orange) and impacted canines on the left side of the arch(Green). On the right side of the maxillary arch(13) ,29.4% of the Patients had buccally placed canines and 17.64% of the patients had labially placed canines.On left side of the maxillary arch (23), 35.2% of the patients had buccally placed canines and 17.64% of the patients had labially placed canines. Chi square analysis was done and p value 0.092 (p >0.05), hence not statistically significant. In males the impacted canines are mostly seen on the left side of the arch.

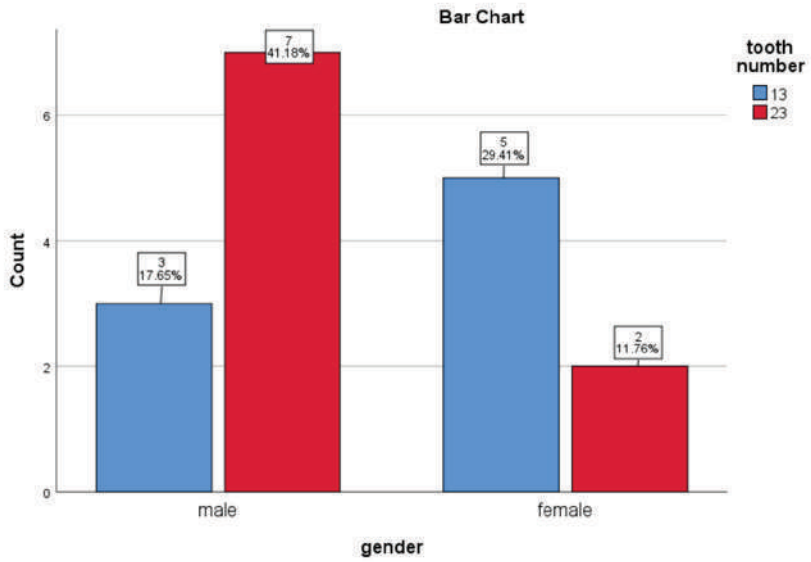


Figure 6: Bar chart representing the correlation between the tooth number and gender distribution of patients.X axis represents the gender of patients and Y axis represents the frequency of patients.Impacted canines on the right side of the arch(Blue) and impacted canines on the left side of the arch(Red).17.65% of males have impaction of canines on the right side of the maxillary arch and 41.8% of the male patients have impaction on left side.For female patients 29.41% of the impacted canines is on the right side and 11.76% of the impacted canines is on the left side of the arch.

Conclusion

From the results it can be concluded that impaction of maxillary canines most commonly occurs in males than in females.It mostly occurs on the buccal side of the maxillary arch and most commonly in the left side of arch.Some of the findings of this study is in contrary with other similar studies which can be attributed to various limitations of the study.

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Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Type of Study: *Original Research*

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Most Frequently Extracted Primary Tooth in Maxillary Arch of Children Visiting A University Hospital In Chennai: A Retrospective Study

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Abstract

Early or premature loss is defined as the loss of a deciduous tooth before its time of natural exfoliation.The disruption in the normal physiological process of deciduous tooth exfoliation and eruption of its successor may cause a series of changes in the dental arches ranging from a change in arch length to malocclusion of successive teeth. Premature loss of teeth can adversely affect oral health-related quality of life in children . The main aim of this study was to investigate the most frequently extracted maxillary primary tooth in children aged 1-15 years in Chennai, Tamil Nadu. This retrospective study was conducted among pediatric patients who visited the OP of a university teaching hospital in Chennai during the period of June 2019 to March 2020. The collected data was then subjected to statistical analysis using Statistical Package for Social Science (SPSS). Descriptive statistics and Chi square tests were used. The most frequent age group was the 7-12 years age group with 61.5% of the total population. Exodontia was most frequently seen in boys with 53.9% of the total population.The most frequently extracted teeth was maxillary first right primary molar with 17.8% followed by maxillary first left primary molar with 16.1%.Maxillary central incisors was the most frequently extracted teeth in younger age group 1-6 years with right primary maxillary central incisor 8.33% and maxillary left primary central incisor 7.95% of total population. Within the limits of the study,Most frequently extracted tooth in the maxillary arch was the first primary molar. Exodontia was more prevalent in the age group of 7-12 years. The most frequently extracted tooth in the younger age group (1-6 years) was found to be the central incisors.

Keywords*: Maxillary primary teeth; extraction; Children; Dental Caries; Retrospective study*

Introduction

Oral diseases continue to be a major public health concern across the world despite the number of efforts being made to improve oral health [1–3]. Interestingly, recent reports suggest an increasing prevalence of oral diseases; the number of tooth extractions carried out in children are alarmingly on the rise. Primary teeth are equally important as the permanent teeth. These teeth are functional from the age of 6 months to 12 years until their successors start to erupt in the oral cavity [4]. Deciduous teeth help in mastication, speech, aesthetics and also act as a template for the erupting permanent teeth to assume a proper position in the dental arch [5]. Time has come when diagnosis and treatment planning must be based on clinical research and not just on the basis of convenience, experience or clinical preference of clinicians. Even though there are various literature on advancements in pediatric endodontic therapy such as rotary files to save deciduous teeth, premature loss of

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teeth still continues to exist [6–12].

Early or premature loss is defined as the loss of a deciduous tooth before its time of natural exfoliation [13]. The disruption in the normal physiological process of deciduous tooth exfoliation and eruption of its successor may cause a series of changes in the dental arches ranging from a change in arch length to malocclusion of successive teeth [14,15]. Premature loss of teeth can adversely affect oral health-related quality of life in children [16]. Loss of teeth can affect the nutritional intake of a child, thus tooth loss can be considered as a good indicator of oral health status of an individual or community. It is therefore important to understand the most frequently extracted primary tooth and develop strategies to prevent it.

Fluoride in optimum quantities can prevent caries [17–20], and is widely used in preventive dentistry. Many studies reveal that dental diseases such as caries, periodontal diseases, and trauma remain as the primary etiological factors early loss of teeth among all age groups which can lead to space loss [13,21–23] . Evidence suggests that the most frequently extracted tooth in the maxillary arch is the maxillary primary incisor in 2–5 years age group and first primary molar in [6–15] years age group [24]. Similar findings have been reported in literature suggesting the most frequently extracted tooth to be the first primary molar in the mixed dentition period [25].

It is believed by many that deciduous teeth need no treatment as successor teeth will erupt and may be attributed to the fact that the parents lack in knowledge and attitude towards dental treatment in deciduous teeth [26]. In order to expedite planning for dental health services and to develop strategies against premature tooth loss it is important to identify the factors which caused such loss. Despite the abundance of studies documenting reasons and rates of exodontia of permanent teeth , very little literature is available describing the rate of extraction of primary teeth in the state of Tamil Nadu, India. In order to develop strategies to reduce or prevent primary tooth loss, it is important to understand the frequency and factors which lead to such loss as reasons may vary geographically.

Hence, the main aim of this study was to investigate the most frequently extracted maxillary primary tooth in children aged 1-15 years in Chennai, Tamil Nadu.

Materials and Methods

Study Setting

This university hospital-based retrospective study was carried out by reviewing the dental records of pediatric patients aged 1-15 who had visited a university teaching hospital in Chennai. Since this was a university hospital setting the large sample size and distribution of population contributed a major advantage for this study. Data collected was reliable and with evidence. The study was conducted after obtaining approval from the Institutional Ethical Review Board. Ethical approval no. SDC/SIHEC/2020/DIASDATA/0619-0320.

Sampling

Data was reviewed and collected from 86,000 patient records over a ten months period from June 2019 to March 2020. Data of those patients who underwent extraction of a primary tooth in the maxillary arch were collected. From 6347 children belonging to the age group of 0–15 years, patients who had undergone extraction of the maxillary primary tooth under local anesthesia or general anesthesia were included in the study while those with incomplete hospital records were excluded from the study. Cross verification was done using photographs and radiographs.

Data Collection

The following patient data were recorded as follows: hospital record number, gender, age at which extraction was done, radiographic/dental diagnosis and type of the maxillary primary tooth extracted and age and date of extraction.

The Total Population of the Study was 1345 patients who had undergone extractions in maxillary arch under 15 years of age.

Data collected was then exported to Microsoft Excel 2010.

Data Analytics

The acquired data was subjected to statistical analysis. Microsoft Excel 2010 data spreadsheet was used for tabulation of parameters and later exported to the Statistical Package for Social Science (SPSS version 20.0) for Windows. Descriptive statistics was applied to

the data and chi-square tests were applied at a level of significance of 5% ($P < 0.05$).

Results and Discussion

The present retrospective study consisted of a sample population of 1345 paediatric patients. The collected data was subjected to descriptive statistical analysis and chi square tests in SPSS to find the frequencies of age and gender and bar graphs were charted. Exodontia was most frequent in the age group 7-12 years with 61.5% of the total population (graph 1).Exodontia was most frequently seen in boys with 53.9% of the total population (graph 2). The most frequently extracted teeth was maxillary first right primary molar with 17.8% followed by maxillary first left primary molar with 16.1% (table 1). Maxillary central incisors were the most frequently extracted teeth in the younger age group 1-6 years with right primary maxillary central incisor 8.33% and maxillary left primary central incisor 7.95% (graph 4) (chisquare test; p=0.000).

Premature tooth loss in a selected region or population gives us an insight into the knowledge and attitude towards oral health. Therefore, It is essential to understand the contributory factors such as age, gender and frequency of extracted tooth to plan and develop effective strategies [27].

Dental records of patients aged between 0 and 15 years over 1 year period were analyzed in the present retrospective study. Statistically significant differences were observed in the primary maxillary tooth type extracted according to age. A similar observation was reported by Alsheneifi and Hughes [22].

From this study; primary exodontia was more frequent in the 7-12 age group with 61.5 % of total population (graph 1). Studies by Bansal M and Mukhopadhyay S et al reveal that the majority of the population lie in a similar age group, presumably because prevalence of mutans Streptococci acquisition by infants increases with age leading to caries formation and children are more prone to trauma in this active age group [28].

Findings in this study demonstrated a prevalence in boys than in girls with 53.9% of the total study sample (graph 2) and is in concordance to findings reported by Mukhopadhyay S et al and Cavalcanti AL et al [16,29]. The reason for such findings may be because of cultural differences in Indian society where male priority is predominant leading to increased DMFT scores due to diet. It was also observed that the most frequently extracted teeth in boys and girls was the maxillary right primary first molar followed by maxillary left primary first molar and maxillary right primary central incisor (graph 3).

The most common tooth type extracted in the present study was the Maxillary first primary molar, similar to the reports of Ahamed et al and Alsheneifi and Hughes [22]. This study found a higher percentage of extraction of maxillary primary first molars (Maxillary right primary first molar- 17.8% followed by Maxillary left primary first molar- 16.1%) (table 1) similar to findings reported by Ahamed et al. and Alamoudi [25,30].This could be due to the chronological age difference between first primary molar and second primary molar, in addition, the success rate of pulp therapy for the primary first molar is generally considered to be less while the second primary molar is often preserved to maintain space [30].

Maxillary central incisors were the most frequently extracted teeth in the younger age group 1-6 years with right primary maxillary central incisor 8.33% and maxillary left primary central incisor 7.95% (graph 4). Similar results were observed in literature reported by Zeng L et al and Taiwo et al. This may be postulated to be due to the high prevalence of nursing bottle caries in anterior tooth in addition to retained incisors and trauma [31,32].

Despite the tremendous advances made in prevention and management of oral diseases in children, dental extraction, the sine qua non of failed preventive efforts, continues to be a common occurrence for many children. Therefore it is essential to understand the contributory factors such as age, gender and frequency of extracted teeth to develop effective strategies.

The results in this study provides an insight into primary tooth mortality among Indian children in the 0-15 age group. The high prevalence of premature loss of maxillary primary teeth in children of age group 7-12 years reflects on the lack of awareness regarding the importance of primary teeth among parents. Therefore, there is a need to initiate pediatric oral health programs,

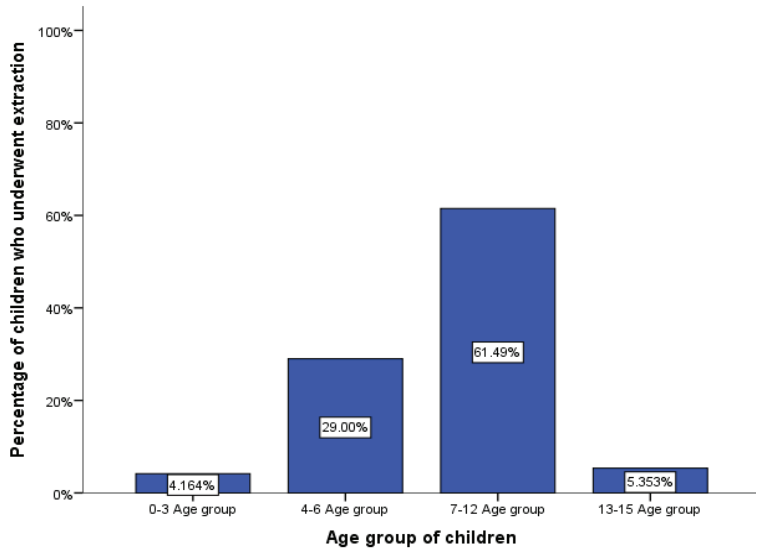
effective school and community dental programs throughout India to create awareness about premature loss of teeth and its influence on the oral health of children.

Limitations of the study includes that it is a retrospective study and therefore cannot be generalised to a larger population. Subjects were not available for direct examination.

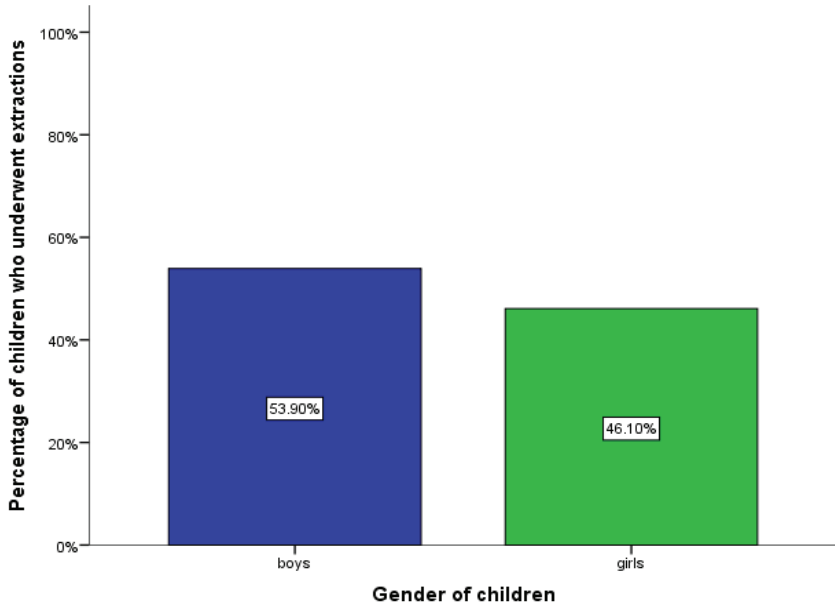
Graphs and Tables:

Tooth number. (FDI SYSTEM)	Frequency	Percent
51	164	12.2%
52	92	6.8%
53	61	4.5%
54	240	17.8%
55	147	10.9%
61	159	11.8%
62	89	6.6%
63	49	3.6%
64	217	16.1%
65	127	9.4%

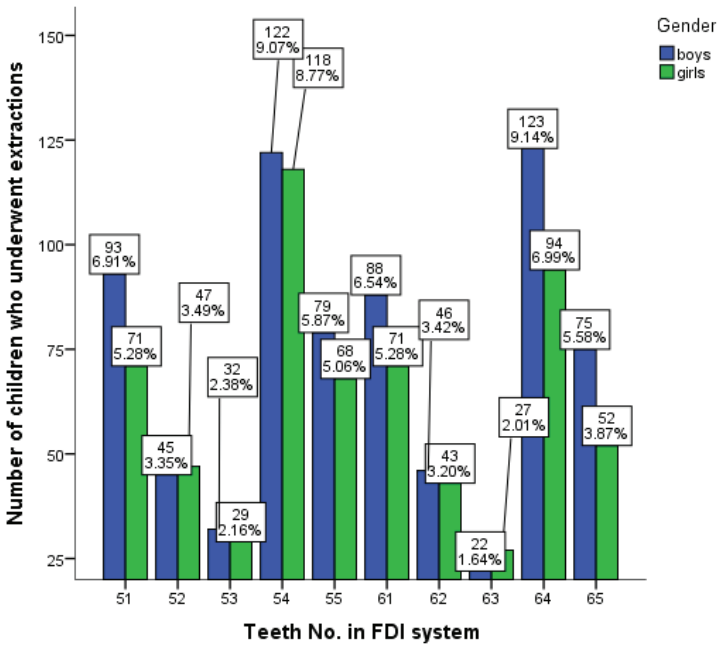
Table 1: Table depicting the frequency and percentage of extracted maxillary primary teeth. The most frequently extracted teeth (teeth number in FDI system) was maxillary first right primary molar with 17.8% followed by maxillary first left primary molar with 16.1%.



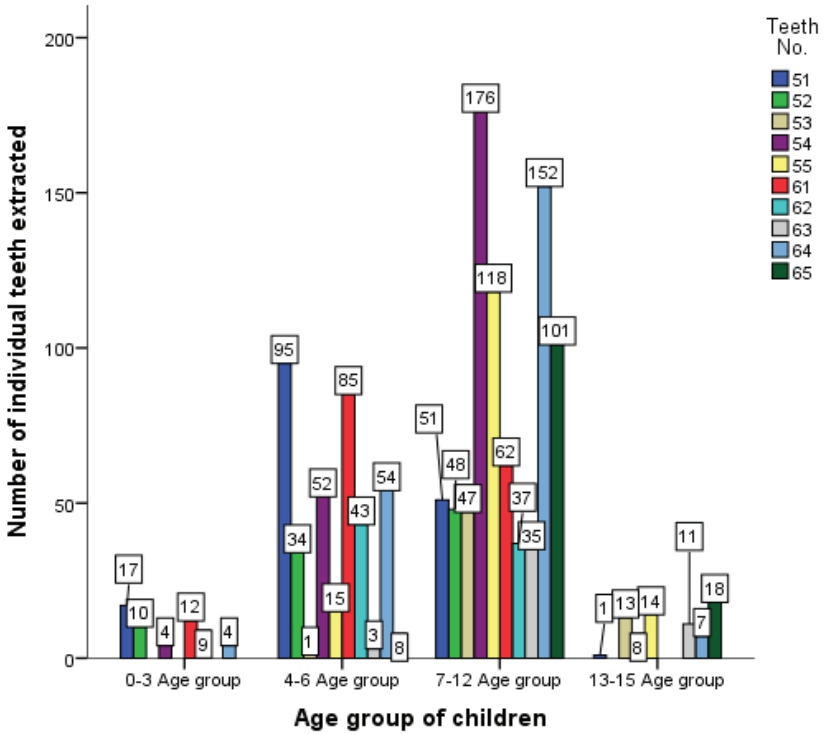
Graph 1: Age distribution of children who underwent extraction. It was noted as 0-3 age group: 4.2%, 4-6 age group :29%, 7-12 age group: 61.5% and 13-15 age group: 5.4%. X-axis represents the age group and the y-axis represents the percentage of patients. Exodontia was most frequent in age group 7-12 years with 61.5% of the total population.



Graph 2: Gender distribution of children who underwent extraction. Distribution was 53.9% boys and 46.1% girls.X-axis represents the gender and the y-axis represents the percentage of patients. Exodontia was most frequently seen in boys with 53.9% of the total population.



Graph 3: Bar graph depicting association between individual tooth extracted and gender. X-axis represents the individual teeth extracted in the FDI system of notation with gender in the X cluster and Y-axis represents the number of children who underwent extractions.Blue colour denotes boys and green colour denotes girls. Chi-square test was done and the association was found to be statistically not significant. Pearson chi square value:6.344; df: 9; p value: 0.705 (<0.05), hence statistically not significant proving there was no significant association between individual teeth extracted and gender. However, it was noticed that the most frequently extracted teeth in boys and girls were the maxillary right primary first molar followed by maxillary left primary first molar and maxillary right primary central incisor.



Graph 4: Bar graph depicting association between individual tooth extracted and age group. X-axis represents the age group with tooth number in the X cluster in the FDI system of notation and Y-axis represents the number of individual teeth that were extracted. Chi-square test was done and the association was found to be statistically significant. Pearson chi square value:402.724; df: 27; p value: 0.00 (<0.05), hence statistically significant, proving that there was an association with age and individual tooth extracted. The most frequently extracted tooth was maxillary right first primary molar followed by maxillary left first primary molar and maximum number of extractions were observed in the age group of 7-12 years.

Conclusion

Within the limits of the study, Most frequently extracted tooth in the maxillary arch was the first primary molar,maxillary exodontia was most prevalent in the age group of 7-12 years. The most frequently extracted tooth in the younger age group (1-6 years) was found to be the central incisors.

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Conflict of Interest: The authors declare no conflict of interest.

Source of Interest: Self

Ethical Clearance: It is taken from "Saveetha Institute Human Ethical Committee" (Ethical Approval Number- SDC/SIHEC/2020/DIASDATA/0619-0320)

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Awareness on Oral Hygiene and Practices among General Population

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Abstract

India is the sixth biggest country by area and is the second-most populous country. Factors contributing to the steady rise in prevalence of oral disease include poor oral health awareness and practice among fellow people. Oral health knowledge is considered to be an essential prerequisite for health-related behavior, although only a weak association exists between knowledge and behavior. Keeping a healthy oral profile requires joint efforts from the dentist as well as the patient himself. The study aims to analyze knowledge and awareness of oral hygiene and practice among the general population A descriptive cross sectional survey was conducted among 100 patients, visiting a private dental institution, Chennai, through a self administered questionnaire. The responses were collected, tabulated in excel sheet and analysed using SPSS software. 97% of the participants were using brush with hard bristles. 81% changed their toothbrush. 40% of the subjects visited a dentist when they were in pain. This shows very less care about oral hygiene which leads to the future undesirable outcome. This study concludes that there is an urgent need for comprehensive educational programs to promote good oral hygiene and impart education about oral hygiene practices.

Keywords: *Oral hygiene; oral health; awareness; oral profile;oral hygiene, Knowledge*

Introduction

Oral disease is a major public health concern owing to their high prevalence and their effects on the individual's quality of life [(1)]. The possible etiological factors leading to these oral diseases, developmental problems, poor oral hygiene, and traumatic incidents. [(2),(3)] Oral hygiene behavior and oral health care regimens should be positively reinforced. Lack of knowledge is one of the reasons for the lacunae in following oral hygiene practices. Keeping a healthy oral profile requires joint efforts from the dentist as well as the patient himself. One of the most important factors that decide the dental health of a population is the outlook of its people toward their dentition

We keep reading about studies done to judge the oral health and oral hygiene practices at so many places scattered all across the globe to know about how much the people are aware of the knowledge and awareness of oral hygiene and how they are practicing oral hygiene. Furthermore, even the people living in cities, despite having easy access to dental care, fall prey to dental diseases due to their negligence in dietary habits and an unhealthy lifestyle [(4).] Globally, with increasing age there is also an associated increase in the calculus among children and adolescents. Oral hygiene maintenance may be a totally ignored practice in people of the low socioeconomic class of developing countries. Good oral hygiene is the foundation of a healthy mouth and prevents 80% of all dental problems. Primary prevention through tooth brushing has paramount importance in the prevention of dental health problems.

One of the most important factors that decide the dental health of a population is the outlook of its people toward their dentition [(5)], Keeping a healthy oral profile requires joint efforts from the dentist as well as the patient himself which works in joint venture of both dentists. Previously our team had conducted numerous original studies [(6–13)] and surveys [(14–20)] over the past 5 years. Now we are focussing on epidemiological surveys. The idea for this survey stemmed from the current interest in our community. Therefore the study aims to analyze knowledge and awareness of oral hygiene and practice

among the general population.

Materials and Methods

Study Design:

A cross sectional study was conducted among 100 participants. A simple random sampling was used to select the study participants.

Ethical Considerations:

Returning the filled questionnaire was considered as implicit consent with no need for signing a written consent. Ethical approval for the study is obtained from the Institutional Review Board (IRB) .

Study Method:

Self administered questionnaire of 13 close ended questions was prepared and it was distributed among undergraduate dental college students of private dental institutions through online survey forms "google forms". The collected data were checked regularly for clarity, competence, consistency, accuracy and validity. Demographic details were also included in the questionnaire.

Statistical Analysis

Data was analysed with SPSS. Descriptive statistics as number and percent were calculated to summarise qualitative data. Chi square test was used to analyze and compare the level of knowledge on oral hygiene and practises among the general population. The confidence level was 95% and of statistical significance $P < 0.05$. Finally, the result was presented by using bar charts and frequency tables.

Results and Discussion

The survey included 100 patients of which 97% were male and 3% were female(Table 1).

27% people brush their teeth occasionally, 24% of them were brushing once a day, 22% were brushing twice a day, and none of them brushed more than 2 times a day which is very less as compared with 75% of the elderly Chinese adults in urban areas in a study by Zhu et al(21).

Around 52% of the subjects brush their teeth in vertical direction, which is the most dangerous method of brushing but the results are in consensus with the study done by Zhu et al (21). where 60% of the sample did the same.Approximately 97% of the participants were using brush with hard bristles and only 3% of the sample used a soft toothbrush, which is less than that observed among Zhu et al [21] study where 27% of the sample uses the soft bristles. Chi square test was used to compare gender and the type of brush used, where P value= 0.604(statistically not significant)(figure 1). 81% changed their toothbrush and the remaining 19 % answered that they don't change their toothbrush regularly. 45% change their toothbrush once in 3 months, and surprisingly reamning 32% change their brush only when it is useless. 88% of the participants were aware of interdental aids. 57 % of the participants washed their mouths after eating . Chi square test was used to compare gender and mouth washing after eating, where P value= 0.272(statistically not significant)(figure 2). 93% of the participants used dental floss and remaining 7% did not use flossing. The results show lacunae in the knowledge on interdental aids.

Only 66% of the studied population said that they clean their tongue and the remaining 34% don't clean their tongue . Chi square test was used to compare responses based on gender to the question on tongue cleaning, where P value= 0.319 (Figure 3) (statistically not significant). The results from the present study clearly indicate missing lack of awareness of oral hygiene among study participants. 58 % of the participants used a mouthwash for cleaning their mouth after eating. Chi square test was used to compare gender and awareness on using mouthwash, where P value= 0.119(statistically not significant) (Figure 4). Furthermore, 88% reported halitosis. This study is in contrast with that of an epidemiologic survey by Miyazaki H, et al [22] in Japan 24% of the individuals complained about bad breath. Our study showed that 40% of the subjects visited a dentist when they had a dental pain, which is similar to the study done by Nabil Al-Beiruti (69.5%) [23].

Knowledge and awareness of oral health is very poor in India, due to poor socio – economic conditions. In addition to this, two thirds of people have never seen a dentist. Lack of awareness about the role of regular dental checkups in detecting and preventing dental diseases necessitates the need for public education. As dentists, it is our responsibility to educate and motivate people to visit a dentist.

Table 1: Depicts percentage of responses on Knowledge and awareness on Oral Hygiene and

Practices among General Population.

S.NO	QUESTION	CHOICES	PERCENTAGE
1.	Gender	● Male ● Female	● 97% ● 3%
2.	Frequency of cleaning the teeth	● Once daily ● Twice daily ● More than twice ● Occasionally	● 24% ● 22% ● 18.3% ● 27%
3.	Technique used for brushing	● Horizontal ● Vertical ● Circular ● combined	● 24% ● 52% ● 18% ● 6%
4.	Type of toothbrush used	● Hard ● Soft	● 97 % ● 3%
5.	Knowledge On changing the tooth brush	● Yes ● No	● 81% ● 19%
6.	Frequency of changing the toothbrush	● When Useless ● Once in 3 months ● Every 6 months	● 32% ● 45% ● 23%
7.	Knowledge on use of interdental aids	● Yes ● No	● 88% ● 12%
8.	Knowledge on rinsing the mouth after eating	● Yes ● No	● 57% ● 43%
9.	Type of interdental aid used	● Floss ● Interdental Brush	● 93% ● 7%
10.	Awareness of cleaning the tongue	● Yes ● No	● 66% ● 34%
11.	Awareness on using mouthwash	● Yes ● No	● 56% ● 44%
12.	Odour from mouth after eating	● Yes ● No	● 88% ● 12%
13.	Frequency of visiting dentists	● Never ● Only in problem ● Once in 3 months ● Once in 6 months ● Any other	● 11% ● 40% ● 11% ● 36% ● 2%

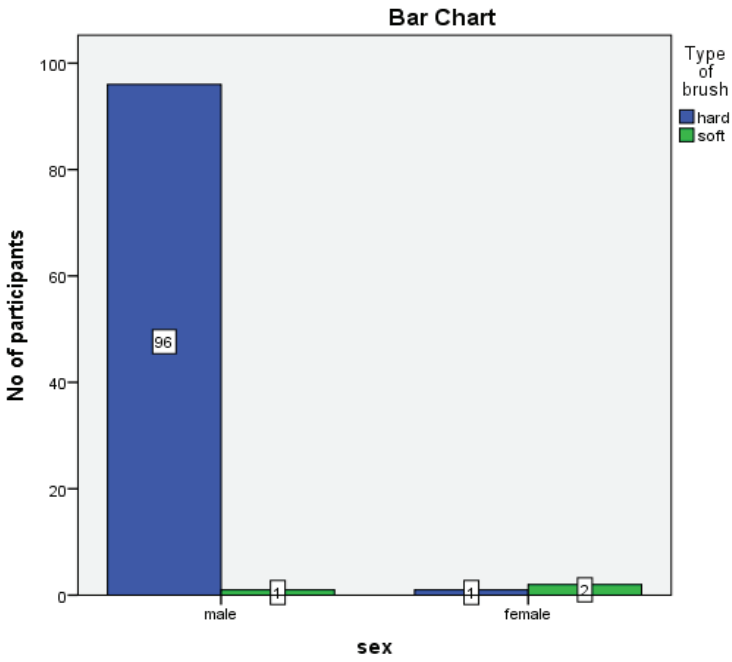


Figure 1:Bar graph showing association between gender and type of brush used, where (blue) hard, (green) soft. X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response (96-blue) was among males. Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.604 (p>0.05)hence not statistically significant.

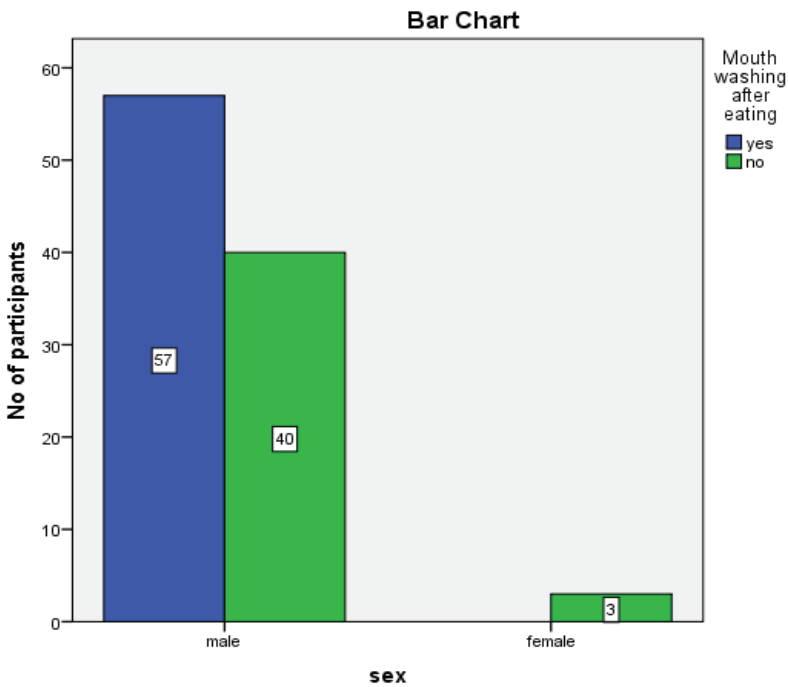


Figure 2: Bar graph showing association between gender and habit of mouth washing after eating, where yes(blue),No(green) X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response was among males(53). Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.272 (p>0.05) hence statistically not significant.

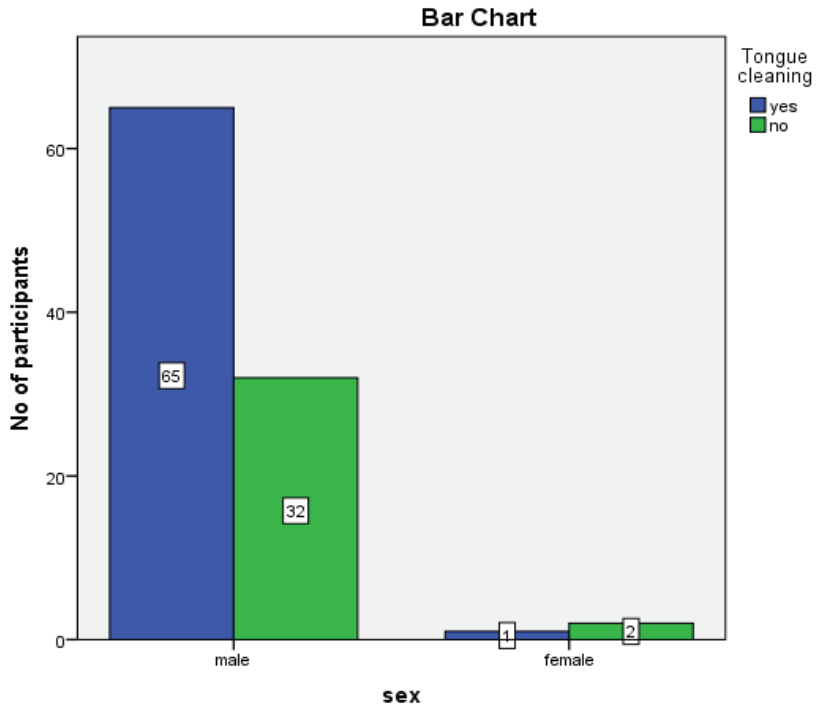


Figure 3: Bar graph showing association between gender and tongue cleaning, yes(blue),No(green). X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response (65) was among males. Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.319 (p>0.05) Hence not statistically significant

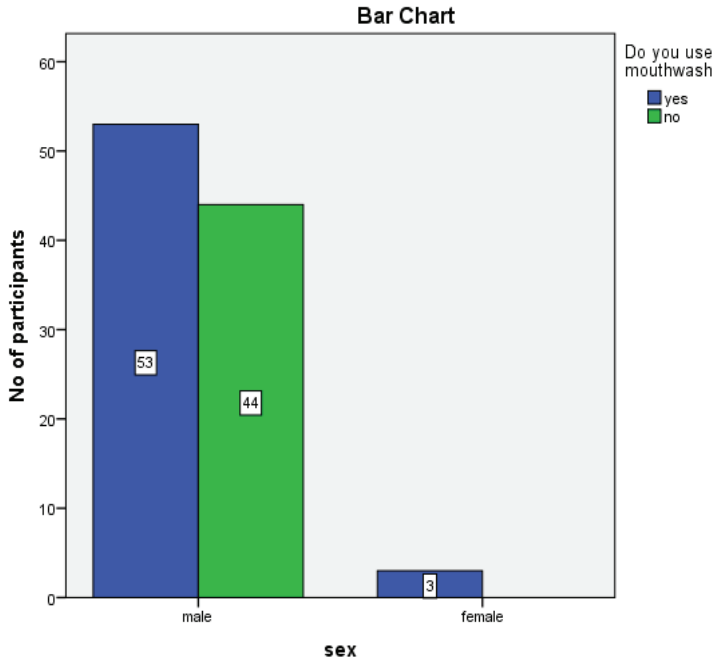


Figure 4:Bar graph showing association between gender and use of mouthwash, where denotes yes(blue), no(green). X-axis represents the gender and Y-axis represents the Number of participants. Maximum awareness response was among males(53). Bar chart shows an unequal distribution of participants. However the difference is statistically not significant. Chi square test P value= 0.319 (p>0.05) Hence not statistically significant

Conclusion

The results of the present study indicates that the study participants have good knowledge on oral hygiene and practices.From the present study it can be inferred that among the general population both males and females had adequate knowledge on oral hygiene and practices.

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Conflict of Interest: Conflict of interest none declared.

Source of Funding: Self.

Ethical Clearance: Not Required

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Semiological Discourse Analysis of the Editorial Cartoons of International Newspapers on COVID-19

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Abstract

The present study aimed to find the hidden ideological approaches used by the newspaper editors in the portrayal of their cartoons in the respective newspapers. The target of this research was to achieve a broad spectrum comparison of semiotics as depicted in international newspapers as Dawn based in Pakistan and The Economist based in Britain. The objectives of the research were achieved by dividing the study into two parts. At first, the written parts of the cartoons were analyzed, and secondly, the semiotics was analyzed. An integrated framework of analysis was used and the researcher blended the CDA model by Norman Fairclough and the semiological framework of Roland Barthes. The study revealed that Dawn presented the domestic problems of the health care system. Further Dawn presented the severe attacks of Covid-19 and locusts and the drastic effects of locusts on the agriculture-based economy. The Economist presented an American cultural disorder of racism that has been part of its earlier history. The Economist also highlighted the precarious state of Brazil and the inefficiency of the government to tackle the spread of Covid-19. It was concluded that the semiotics presented in the newspapers portrayed the situation related to Covid-19 and its long-lasting effects have been discussed in the context of culture and social practice. A comparison of semiotics was presented at the end of the study.

Keywords: *Covid-19, International media, Third-world, Western media, Semiotics, CDA.*

Introduction

Since the advent of civilization art and paintings have contributed to mass education. the earlier cave men even loved paintings and the evidence have been collected from the caves and later, when people started living in city-states they started entertaining each other with music and art. Initially, these were the enactment of the religious subject which resulted in Modern Greek drama and stage and slowly liberal art developed. The western renaissance started with art. While in Asia especially in Indus valley there were religious ceremonies where the stage and art were a prominent part of religious and cultural festivals. Art contributed to the spread of knowledge and information. Cartoons became a prominent part of newspapers since the advent of journalism as a field that now enjoys a position of stakeholder in state. Cartoons provide concise, sarcastic, and clever impressions of issues on the situation pertaining at a specific time. The first political cartoons appeared in Mesopotamian Civilization (modern-day Egypt) in 1360 BC. A painter ridiculed political leaders of the time. Later on, it was developed as a form of criticism.

The cartoon may highlight the issues related to religion, economy, law and order, and international emergencies. Socio-political cartoons bring humorous criticism and questions to the existing order. The biases of newspapers and editors are the real questions that are often neglected. Cartoons and discourse together work as motivators. The cartoons are considered a valuable part of the newspaper as the cartoons present the existing problems differently.

Van Dijk explained the idea of critical discourse analysis is being critical in scientific thinking. He asserts that CDA and CL (critical linguistics) share common perspectives on carrying out discourse analysis, semiotic analysis, and linguistic analysis. The subject matter of critical discourse analysis is the investigation of social inequalities, class conflict and class struggles, cultural disorders, and social practices.[11]

The study argues that the discourse of newspapers is ideologically loaded which employs a language replete with multiple meanings and plays an important role in the opinion the building of masses. The media plays a vital role in public perception.

The cartoonists use analogy, labelling, humour, satire, and exaggeration for expression of their opinion and ideology to readers.

It is argued that in literature writer's satire the situation and art, artists create it in cartoons. Satire hits human follies and prejudices through written and spoken discourse while in art satire is painted or sketched. Cartoons provide an escape from lengthy discourses and their explanations. The area of semiotics is very significant as it highlights the existing problem with pictorial imagery. It may highlight culture, cultural disorders political disorders, the role of language in nation-building and use of language at public places, etc. The study analyzes the semiotics of two leading English newspapers like The Economist and Dawn. The CDA model of Fairclough and semiotics model of Roland Barthes has been integrated to carry out the study.

Significance of the Study

The study has focused on socio-economic and geopolitical constructions of countries and their possible response to international emergencies in their premises. Moreover, the drastic economic effects of Covid-19 have been discussed. Besides, the analytical framework used in the study has been a blend of three-dimensional Fairclough's model of critical discourse analysis and Barthes' model of semiotics study. The data have been collected from international newspapers as Dawn based in Pakistan and The Economist based in Great Britain. This factor makes it interesting that how western media and Eastern Media depict the same issue in their respective newspapers, keeping in view the existing socio-economical problems of the country.

Research Questions

1. How did daily dawn present the international medical emergency of Covid-19 through its' semiotics?

2. How did The Economist present the international medical emergency of Covid-19 through its' semiotics?

3. How did the newspapers' semiotics differ in portraying the same news?

Literature Review

Conceptual Definitions

To make the subject of the study comprehensible, the following terminologies and concepts are briefly discussed.

Cartoons and Caricature

Oxford Advanced Learner's dictionary defines the cartoon as "an amusing drawing in a newspaper or magazine, especially the one about politics or events in news". The cartoon is often called a non-realistic or realistic type of drawing. The concept of cartoons came into being during the middle ages or dark ages and it was considered a piece of art painting, fresco, tapestry, etc. but later on, it was considered as a means of satire and humor. In the years 1843 cartoon was considered as an ironic or comical picture in periodicals and newspapers. In the early 20th century it was considered as animated films like printed cartoons. While in the case of the socio-political cartoon it is considered as a medium of graphic representations of public figures with the expression of the editor's opinion. The cartoons typically contain artistic skills to portray an image of public interest ranging from religion, philosophy, and history, economy to politics, health, and law. This cartoons satire the existing norms of society and cultural practices (directly or indirectly). These cartoons were analyzed based on the semiotic model of analysis in this study.

Semiotics

The study of signs and symbols is called semiotics. Signs can be symbolic, linguistic, and artificial, or any other thing. The writer divides signs into three particular categories as symbols, icon, and index.[10] Ferdinand de Saussure proposed the concept of signifier and signified. Saussure asserted that signs and language are arbitrary.[3] Roland Barthes introduced the idea of the significance of signs as denotative, connotative, and mythical. Roland Barthes proposed that a symbol or sign can be analyzed on three levels. These levels are denotative, connotative, and mythical respectively.[2]

Written and Visual Discourse

The literal meaning of the word 'discourse' is an argument. Levin and Hovav asserted that the most commonly used type of discourse used in academic, legal, and writings of the press are expository discourse.[7] The discourse was primarily the subject of written

and spoken language but later in the 18th century as scientific knowledge was elaborated with visuals rather than written codes. This was the breakthrough in systemic and logical patterns of language. Now language was science and its' evolutionary aspects were discussed. Morgan said that linguistic and paralinguistic features of language constitute discourse collectively.[8] Historical shreds of evidence as well as existing issues are discussed in the critical discourse analysis. Now it is argued that comprehension of discourse can be increased by visual representation. Print media relies on the semiotic or visuals for concrete issues as well as the subjects of common life.

Albers argued that there is VDA as a visual discourse analysis in which image is analyzed by using the rules of written discourse.[1]

Covid-19

Coronavirus disease of 2019 abbreviated as Covid-19 has been declared as an international health emergency by the United Nations and it has spread large scale deaths and large scale economic recession for consumers and producers that are recognized first from China in November 2002 and later on in Vietnam in 2003.

In 2020 in December it was reported from Chinese Wuhan that individuals have been affected by this disease. And then it spread around the globe with drastic effects. The countries are facing problems in the economy and health sector. The case of third world countries is very critical. The incapacity of the health department in these countries has been affected severely. Paramedical staff and doctors are losing their lives daily. The western countries are of no exception. Italy has been a major victim of this pandemic and Brazil is exhausted. International media is reporting with heavy hearts as the death toll keeps on increasing. The economic impacts of covid-19 are shaping world affairs in another dimension. The linguists are calling the future as the post-covid-19 era. Newspapers are also trying to depict the situation in their cartoons. Editorial articles are published and expert opinions are published for the control of pandemic on the individual level. This study explores the use of semiotics in international newspapers. And comparison will be drawn at the end.

Review of different researches

Namibian newspapers were analyzed by Mwetulundila, and Kangira. The researchers explored the use of hectic and humour in Dudley's political cartoons. The descriptive study was carried out qualitatively for the analysis of semiotics and content. Criteria sampling technique was used to collect the data. The study was carried out by using the concepts of Aristotle on rhetoric persuasion, which include logos, ethos, and pathos. it was concluded that ethos was used for higher figures in societies. Pathos was used to accelerate the feelings of readers to earn sympathy for discussion and logos were used to convey that cartoons brought the truth to surface.[9]

Idid and Chang explored the mass agenda of two major groups and media in 2008 elections by using tools of survey and content analysis. The concepts of rank-order correlation were used to analyze the impact of the media campaign in the election. It was found that Chinese media had a great affiliation with the public agenda and maintained that Chinese newspapers affected what should people think.[6]

The research was conducted by Sheikh Et.al. 2013 on Pakistani newspapers that how newspapers used communicative cartoons in the 2013 election. The researcher implied the Quanto-qualitative method was used for semiotic analysis. Persuasion techniques were used by cartoonists. It was explored that persuading techniques were used with irony, exaggerations, and symbols to produce desired outcomes from readers.

El Falaky, examined the editorial cartoons extracted from an Egyptian newspaper named AL Ahram. The study explored how cartoons and visuals were used to create the desired ideologies. The researcher used the visual grammar method of Kress and Leeuwen. The study revealed that with the use of multimodal texts the editors of the newspapers inscribed their desired political and ideological stances on the readers in the critical times of social and cultural unrest.

This study relies on the semiotics of two English newspapers like The Economist and Dawn based in Britain and Pakistan respectively.[4]

Methodology

The research methodology is a systemic way to carry out a particular study at hand. In the field of language and literature different techniques, theories and models have been introduced. The field of linguistics deals with the research on scientific terms, definitions and processes

for the conduction of the research. This study has been carried out by blending the CDA model and Semiotic Model. In the field of critical discourse analysis, different techniques have been at hand. Van Dijk's model of CDA, Foucault's discourse analysis model in terms of sociology and there is another prominent name in the field of CDA and it is Norman Fairclough introduced three steps method to carry out the research. In the field of semiotics two major names of great importance. The first is Saussure who introduced the concept of signifier and signified in the field of semiotics. And second is Barthes who introduced the three levels of analysis. CDA and its model of analysis have been elaborated and Barthes' model follows CDA in the order.[11, 5]

CDA

Critical discourse analysis is elaborated as follows along with its critiques. The first rule of critical discourse analysis is that it talks about social problems and arising disorders as its resultant. CDA focuses on language, use of language, and existing social and cultural problem which affects it. CDA makes a critical approach to find political relationships in the power systems. According to Fairclough and Wodak, CDA tries to extract outcomes that have relevance to the social, cultural, and economical perspectives.[5]

Further, it deals with the idea that relations of power are discursive. It explores how multiple cultural relations related to power are exercised and how these relations are negotiated through discourse. CDA mediates the link between society and text. The main concern of CDA is to make a connection between the properties of text on one hand and socio-cultural practices on the other hand.

Critical discourse analysis also explores ideological practices. It is often said that ideologies are often practiced through discourse. In discursive practice, the effects of discourse practice are observed.

Discourse practice is a subject of historical conventions. Comprehension of discourse can be made through historical practice. Extra linguistic elements like ideology in historical terms, culture, and society are included in the study.

The CDA model by Fairclough includes the following steps:[5]

Critical discourse analysis model of Fairclough:

1. **Text (descriptive level)**

The first level in this model deals with textual analysis of a given piece and particular aspects of the text are dealt with the analysis of text as the use of vocabulary, grammatical structures, lexical choices, etc. These aspects are described for further explanation to the next level.

2. **Discursive practice (interpretive level)**

It deals with the production and consumption of the text. The source of text and mode of its production are discussed at this stage. The text is interpreted as social practice and social ideologies of text are interpreted.

3. **Social practice (explanation level)**

In this stage factors of power and ideology are created to explain the relationship between socio-cultural context and production of the text and its use in a particular context. The linguistic and meta-linguistic features of the text are analyzed.

Semiotics

According to Ferdinand de Saussure, language is a system of signs. Society responds to these signs in a predictable way. According to Saussure sign is made up of a signifier which may be an acoustic form of the word, and a signified which may be a mental concept. Signifier and signified contribute to the understanding of meaning. Saussure asserts that semiotics is a way to unlock many cultural phenomenons.

Barthes's contribution to semiotics is marvellous. He helped to formulate the science of semiotics on modern linguistic principals of structuralism. He introduced the idea of the myth that he described in his mythologies**.** According to him, myth is a type of speech and hence anything can be a myth. The semiotic model of Barthes is discussed below.[2]

Barthes' model of Semiotic Analysis

This model has three levels as:

1. Denotative level: it discusses the literal or dictionary meaning of a given word or sign.

2. Connotative level: this level deals with meaning which may vary from speaker to writer and vice versa.

3. Mythological level:

This level deals with the concepts which are culturally flexible and cultural variables are applied in

the limited context of culture.

Data Collection

This research decodes the semiotic and linguistic discourses of editorial cartoons published in international newspapers on covid-19 and other existing problems. The samples were extracted from the *Dawn* and *the Economist.* The purpose of the study is how the editors select and publish the cartoons with a verbal discourse that propagates their desired meanings. These newspapers (Dawn and The Economist) were selected to bring about a comparison between Asian and European media and their selections of contents.

Sampling

The data for analysis was collected from the newspapers (Dawn & The Economist). The semiotics of Dawn was published on May 21, 2020, and June 11, 2020, while the semiotics of The Economist appeared on June 4, 2020, and on July 9, 2020. The role of print media has been very important as it reaches everywhere around the world and anyone can access it easily. And the study aims at finding the fact that print media has been playing its role in portraying the times of pandemic around the globe.

Framework of Analysis

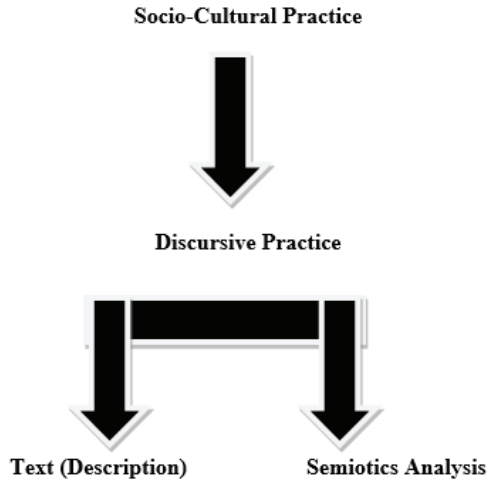
A combined framework has been devised for the study. The researcher selected the Barthes model of semiotic analysis with its levels combining it with three dimensional of discourse analysis proposed by Fairclough. Both models have been discussed earlier.

Limitations and delimitations of the study

The analysis contains the critical discourse and semiotic analysis of cartoons or semiotics published in two newspapers as Dawn and The Economist. No regional language newspaper's semiotics has been added. It is only English newspapers. This research is limited to the semiotics of two particular newspapers published in Pakistan and the United Kingdom.

Scheme of analysis

For data analysis, an integrated model was used by combining two models of semiotics and critical discourse analysis. It is shown in the following figure.



It has been established that the integrated model operates from bottom to top. There the analysis is as follows.

Data Analysis

Barthes' model of semiotics and Fairclough's model of critical discourse analysis provides a comprehensive framework for the analysis of semiotics and discourse in the existing socio-cultural as well as economic and political processes. The study uses the integrated framework as mentioned in figure1. The three-dimensional models of Fairclough has been used in the bottom-up scheme integrating it with the semiotic model of Roland Barthes on parallel lines. The data analysis practice starts from a broader perspective to a narrow one. Then cultural and social context is explained initially and discursive practice and text analysis with semiotic analysis follows it at the micro-level.[5]

Socio-Cultural Practice (Explanation)

Norman Fairclough proposed that during the analysis of the socio-cultural practice of an event, the researcher can explore different contexts as a context of frame of society, institutional practice context, and immediate situational context. The semiotics is given to discuss the events of contexts. The analysis of relationships has been carried out by keeping in view the particular conditions of society in the time of the pandemic.[5]

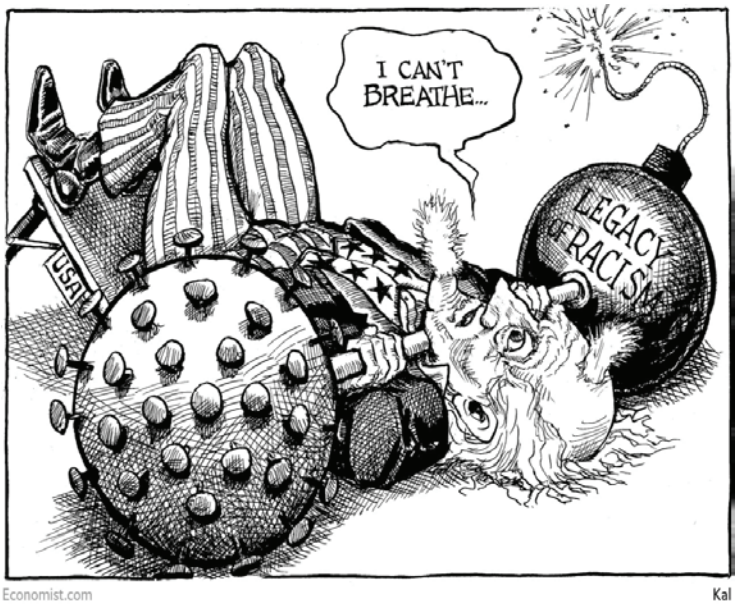


Figure 1. KALS Cartoon

It contains the semiotics as well as written discourse as "I can't breathe". Taking into view the immediate situational context it comes to light that a black American was killed by a white policeman in America. While the public faces drastic effects of coronavirus on their lives another act of racism was reported and the world responded to it as ' black lives matter '. The event attracted the attention of people around the globe and protests erupted everywhere in Europe. The contextual analysis of institutional practices refers to the historical events which are part of that particular culture. American history is full of stories which concern the persecution of black Americans from the time of slave trades. Black has been marginalized as untouchables (idea taken from the Indian caste system that was abolished by the government after the partition of the subcontinent, but still this is practiced in other parts of the subcontinent partially or completely) and their rights were neglected. This institutional practice refers to the subjugation of blacks in the 21st century. The written discourse has the phrase ' I can't breathe'. This is layered meaning as George Floyd was killed by a white policeman in daylight. Another meaning refers to the social practice of Americans about Blacks as there is no freedom for Black people as the White enjoy. So this shows the moral suffocating environment produced by white supremacists in America.



Figure 2. The Brazilian government

This figure highlights the attitude of the Brazilian government on the pandemic considering it the mere flu. The semiotics shows that Brazilians are playing football surrounding by covid-19 moving and attacking freely. The idea comes to mind as if they are playing while viruses as the highlighted discourse say ' play on'. This is the state of affairs in Brazil risking the lives of citizens. The social and cultural practice shows negligence and there is no protective measure to be taken for the control of the pandemic. People are advised to be safe and stay home rather they are allowed to have a party or outdoor get-together. The immediate situational context refers that there is no conscience of society and the public on the gravity of matter which has jammed the global wheel of economy and politics.



Figure 3. Pakistan & Covid-19

This figure contains the semiotics as well as discourse. In mid of the year, Pakistan faced critical problems of covid-19 and locust. Locusts were destroying the villages and covid-19 attacked the major cities severely where people lost their lives and the toll continued till mid-July. The situation was worse for the whole country and people suffered economically and psychologically. Living under the poverty line Pakistan is placed in a third world according to ONE NATION PROJECT index that Pakistan lays on number 42 for its GDP. The institutional practice by the state has been a great burden on taxpayers and leaving a loan of trillions on the public. While talking about social practices from a historical perspective it is revealed that from the day of its independence from the imperialist rule of British colonizers it couldn't stabilize its economy due to multiple reasons. This semiotics can be considered as a classic picture of socio-political practices in the country.



Figure 4. Zahoor's Cartoon

This figure discusses the health care system of the country. It is obvious that pandemic is hovering over the heads of the family but the health care system is not supporting. The bold discourse ' PANDEMIC' refers to the spread of the pandemic in the country while there is a lack of appropriate infrastructure. It refers to the idea that how society works and how institutions provide services to people.

Discursive Practice (Interpretation)

The production of semiotics discourses in the wake of covid-19 have described as a discursive practice. The semiotics of Dawn and The Economist has been selected for data analysis. The coverage of The Economist seems to be of an international kind as it portrayed the racism in America along with the situation of covid-19. It is of the view that secular Americans are unable to breathe in these circumstances. They have to face racism on one hand and covid-19 on the other. The Economist also portrayed the situation of Brazil that the virus has spread everywhere such that there is seems no difference that every object seems infected. Dawn discussed the domestic issues of agriculture as locusts had destroyed the seasonal crops badly and how covid-19 along with these locusts is pressing down the common man. It portrayed the health care system with its shortcomings and lack of modern ways of protection from pandemics.

Text and Semiotics (Description)

This part of the analysis contains the explanation of semiotics and the description of the text. For the semiotic analysis, four cartoons have been selected for analysis (Two from Dawn and two from The Economist). The cartoons have been analyzed one by one.

Table 1. Semiotic Analysis of figure 1

Sign	Denotation	Connotation	Myth
Bomb	Bomb in exploding position	Bomb symbolizes the extortion power	Related to the use of military power and modern weapons to suppress the public
Coronavirus	The devastating effect that it pushes down the man	Destroyed and questioned the idea of human relations	Representing the symbol of threat and total blackout
An old bald man with the beard	The senior citizen	This refers to the idea that old citizens are suppressed or have been suppressed	It may refer to the notion that senior citizens are more affected by racism which is still being practiced and coronavirus as it targets old age severely.

Table 2. Semiotic analysis of figure 2

Sign	Denotation	Connotation	Myth
A half-naked man shouting	This image may refer to the Brazilian government	This refers to the idea of chaos and anarchy	Man shouting 'play on' shows that he is concerned with the outrageous pandemic.
Coronavirus	The novel virus spread in the world	A threat to the world	Covid-19 was declared as an international health emergency by the United Nations. This may refer gravity of the pandemic

Table 3. Semiotic analysis of figure 3

Sign	Denotation	Connotation	Myth
Rain	Pandemic	The virus is coming like raindrops	The intensity of covid-19has been portrayed as if the virus is a raindrop that drops everywhere.
Tattered umbrella	Health care system in Pakistan	Criticism of the health care system	This refers to the devastating effects and precious situation of the health system of Pakistan. That it is unable to provide necessary measures to the public

Table 4. Semiotic analysis of figure 4

Sign	Denotation	Connotation	Myth
Gun	Use of power	This may refer to the powerful attack of locusts and pandemic	In the mid-year period in the country, Pakistan faced massive attacks of covid-19 and locusts. This pointed gun refers to the use of force.

Findings and Discussion

Critical discourse analysis is a disciplined way of text analysis. Fairclough devised a way to systematically analyze the data at hand with a scientific approach. It refers to the idea that language is also a scientific field. Three stages of analysis provide an insight into the socio-political context of text production. The text is a product of cultural processes that result in a great discourse.[5] Barthes's model of semiotics provides a systematized way to analyze text for better understanding and getting the real editorial ideologies being presented in the semiotics. [2]

The research at hand analyzed the semiotics of international newspapers from Pakistan and Britain. The Economist focused on pandemic and international

affairs. It discussed the hazards of covid-19. The recent incident of racism was also presented in the semiotics. This incident took the life of a black American. The discourse 'I can't breathe' is of much importance. This shows the suffocating practice of racism in the time of pandemic going parallel. This brought a real of great America as it is practicing the outdated version of racist practices. It is the time when people are trying to help each other and reconcile with one another. But in the case of America, the case is different. This shows how much trump administration is concerned with the health, safety, and civil rights of the public. Other semiotics taken from The Economist is in Brazil.

Brazil didn't pay serious heed to the pandemic. Brazil crosses the number of 3 million cases of patients infected with the virus. The casualty of seventy thousand patients has been reported. Brazilian president was tested positive with the infection. The semiotics referred to the idea that they have no problem with covid-19 and as seems it is the president of Brazil in the cartoon urges to play on and he is controlling the spread. The excessive spread of the virus has been portrayed in the way that players are playing with the virus instead of football. This is a serious situation and alarms the travelers to decide once again who are planning to visit Brazil.

The semiotics of Dawn portrays the domestic situation of the country. One semiotics refers that the public has been attacked by the locusts on one hand and covid-19 on the other hand. Locusts attacked in the mid-year as the covid-19, the attack was at its peak. The semiotics was published on May 21, 2020, when the country was facing massive blows from both sides. And the economy faced tremendous problems as the lockdown was imposed to tackle the situation. This directly affected the common man who was not ready for such outcomes of covid-19. Another semiotics referred to health care as a tattered one in Pakistan. These semiotics was published on June 11, 2020. The month of June in Pakistan had been categorized as the month of a severe attack of covid-19. The hospitals were full of covid-19 infected persons and doctors were also facing problems with lack of infrastructure. Hospitals were lacking in PPE's and doctors were forced to work in these circumstances. This entire devastating situation was portrayed in semiotics. It was also observed that Dawn portrayed the situation without the risks of establishment and forced censorship as it is often practiced invisibly. The Economist grasped the racism and situations of covid-19 in two countries boldly. This is how the semiotics was presented.

Conclusion

The research briefed on the published semiotics of newspapers in the wake of covid-19 and other cultural practices in recent times around the world. The study relied on two different aspects of linguistic analysis. First was the semiotic analysis model proposed by Roland Barthes and three-dimensional critical discourse analysis model by Norman Fairclough. The researcher devised an integrated model of analysis and applied it with a bottom-up approach. The findings revealed different editorial tactics to produce a piece of semiotics. It also revealed the incident of racism and its socio-cultural impacts were discussed and its historical perspectives were also discussed. [2'5]

The Economist portrayed the effects of covid-19 in Brazil and its spread all around. The Economist also presented the social practice of racism and its consequences were discussed. Dawn presented the socio-economical effects of covid-19 and locusts. It also presented the precarious situation of the health care system.

Recommendations

The role of media in educating the world and spreading the ideologies has been a contributing factor in nation-states. A critical comparison of the ideological biases of newspapers by readers would make an analytical atmosphere for future readings. It is recommended that a careful study of coverage style should be the subject of the decision.

Conflict of Interest: I declare that there is conflict of interest.

Ethical Approval: The research approved by the scientific and ethical committee in my department.

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5-Years Prevalence Study of HBV and HCV infections in Babylon Province: Forensic Helping Data

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Abstract

HBV and HCV infections are serious global health care problem because they may end with chronic hepatitis, cirrhosis, and hepatocellular carcinoma. HBV infection results in approximately 2 billion human infections while HCV approximately 160 million individuals. Raw database were collected from General health department at Babylon Health directorate for 715505 investigated person during a period from Jan. 2015 to Dec. 2019. Blood samples was drawn in Gel tube from suspects and centrifuged to separate the serum. Serum submitted for investigation of HbsAg and HCV Ab by both rapid immunochromatographic test and ELISA test. Actually the database taken from Babylon Health directorate and the results analysis depends upon dividing of data records from four health sectors, blood bank record lab, health care checking lab, pre-surgery checking, Marriage lab, thalassemia lab, Hemodialysis lab Prisoner and foreigners lab. The results revealed that , total positive cases were 4284 and 2260 for "HBV and HCV" respectively. The highest no. of cases for both "HBV and HCV" were recorded in 2018 (976 and 467) and (961 and 557) for "HBV and HCV" respectively. The incidence rate of "HBV and HCV" were varies worldwide. The results revealed that the occurrence rate (Mean ± SD) of "HBV" was (0.627±0.134) while for HCV was (0.327±0.084). The results revealed that, there is no defined month or semester for high rate infections. The high prevalence percentage were showed for prisoner-foreigner category (48.53 and 45.95 for HBV and HCV respectively) followed by surgery (22.66 and 19.03 for HBV and HCV respectively), blood bank (14.03 and 13.95 for HBV and HCV respectively) and in married (7.79 and 3.92 for HBV and HCV respectively. The current study conclude the high prevalence percentage of HBV (duplicate) than HCV with no seasonality of occurrence and the Prisoners and foreigners, Surgery, blood donor and hemodialysis were mostly involved with HBV and HCV infection.

Keywords: *HBV, HCV, Healthcare worker, HBV vaccine*

Introduction

Hepatitis is" the term used to describe inflammation of the liver. It's usually the result of viral or damage" . The hepatitis B virus (HBV) is a major global public health problem, affecting more than 2 billion people worldwide [1]. HBV and HCV infection results in approximately 2 billion and 160 million human infections respectively [2]. HCV super infection in patients with chronic HBV infection was the most common clinical features of coinfection in Asia–Pacific countries[3]. The chronically infected persons are at high risk of death from cirrhosis of the liver and liver cancer, diseases that kill about 1 million persons each year'[4]. It is assessed that more than 240 million people are recurrently infected with "HBV" and, therefore, are at risk for the development of cirrhosis, hepatic decompensating, and hepatocellular carcinoma (HCC)[5]. HCV disease advances to chronicity in 70% of cases, a condition that may prompt liver cirrhosis and hepatocellular carcinoma [6,7]. As per a WHO report, 130-150 million individuals are incessantly tainted with HCV. HCV the study of disease transmission shows impressive territorial differences[8]. There is no antibody to restrict the dispersion of HCV contamination while all medical services laborers ought

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to be considered for HBV immunization and ought to carefully apply the general prophylactic measures to forestall introduction to HBV and HCV.There is a need to calculate the liability of chronic "HCV" infection at the state level [5,9]. The study aims to study the prevalence rate of HBV and HCV infections for last 5 years (2015-2019) in Babylon Province.

Materials and Methods

Samples:

Raw database were collected from General health department at Babylon Health directorate for 715505 investigated person during a period from Jan. 2015 to Dec. 2019.

Specimens:

Blood samples was drawn in Gel tube from suspects and centrifuged to separate the serum.Serum submitted for investigation of HbsAg and HCV Ab by both rapid immunochromatographic test and ELISA test.

Results

The present study was showed to see the distribution & incidence rate of "HBV" and "HCV" infection among screened patients in Babylon province for past 5 years (January 2015 to December 2019). Actually the database taken from Babylon Health directorate and the results analysis depends upon dividing of data records from four health sectors, blood bank record lab, health care checking lab, pre-surgery checking, Marriage lab, thalassemia lab, Hemodialysis lab Prisoner and foreigners lab. Total of (715505) cases were investigated for HBV and HCV using rapid test and ELISA assays to confirm infections (Figure 1). The results revealed that , total positive cases were 4284 and 2260 for HBV and HCV respectively. The highest no. of cases for both HBV and HCV were recorded in 2018 (976 and 467) and (961 and 557) for HBV and HCV respectively. The incidence rate of HBV and HCV were varies worldwide. Our results revealed that the incidence rate (Mean ± SD) of HBV was (0.627±0.134) while for HCV was (0.327±0.084) (Table 1). Concern the categories from which infections were recorded, the results revealed that Prisoners and foreigners (48.53%, 45.95%), Surgery (22.66%, 19.03%), blood donor (14.03%, 13.95%) and hemodialysis (1.27%, 11.63%) for HBV and HCV respectively (Table 2). Concern the seasonality or weather relationship with HBV and HCV infections the results showed that there is no association between sessions and infections (figure 2). HVB vaccination were displayed in table (3).

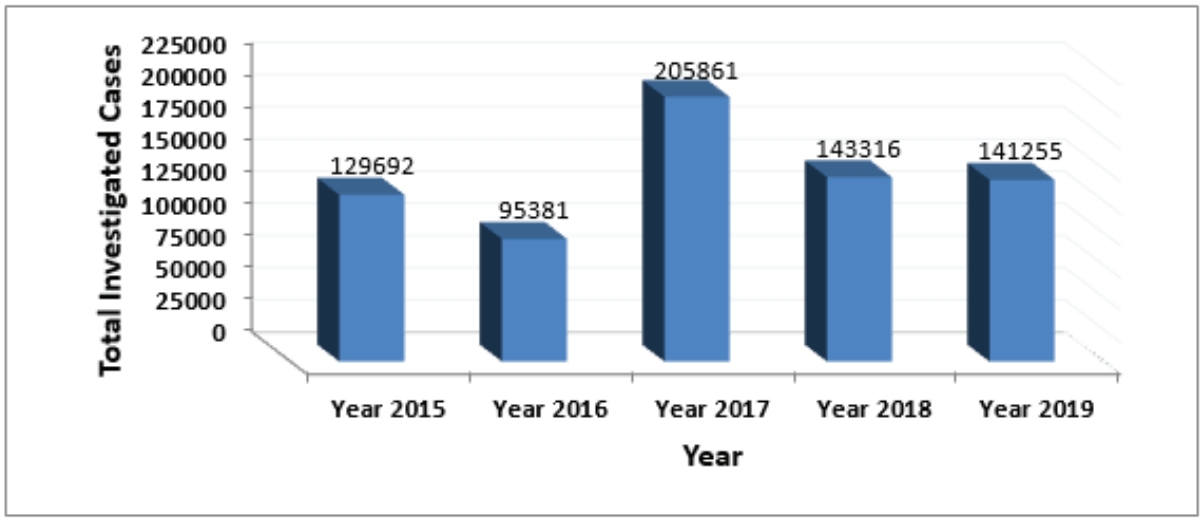


Figure (1): Total Cases of HBV and HCV infections in Babylon Province (2015-2019)

Table (1): Distribution of incidence % of HBV and HCV infections in Babylon Province (2015-2019).

Year	Tested	HBV cases	HBV %	HCV cases	HCV %
2015	129692	790	0.609	534	0.412
2016	95381	721	0.756	288	0.302
2017	205861	836	0.406	414	0.201
2018	143316	976	0.681	467	0.326
2019	141255	961	0.680	557	0.394
Total	715505	4284	Mean±SD 0.627±0.134	2260	Mean±SD 0.327±0.084

Table (2): Distribution of HBV and HCV infections cases among categories in Babylon province (2015-2019).

Categories	Total (2015-2019)			
	HBV	%	HCV	%
Blood Bank	596	14.03	313	13.95
Healthcare Worker	35	0.82	26	1.16
Contacting	178	4.19	43	1.92
Surgery	963	22.66	427	19.03
Married	331	7.79	88	3.92
Thalassemia	30	0.71	55	2.45
Hemodialysis	54	1.27	261	11.63
Prisoner and Foreigner	2062	48.53	1031	45.95
Total	4249	100	2244	100

Table (3): HBV vaccine distribution in Babylon province (2015-2019)

Year	HBV vaccine				Total
	first Dose	second Dose	Third Dose	Child Dose	
2015	11711	9184	13805	36748	71448
2016	14436	6722	19642	38466	79266
2017	12863	6519	9346	39520	68248
2018	11672	6290	8028	37529	63519
2019	12996	5598	4827	38778	62199

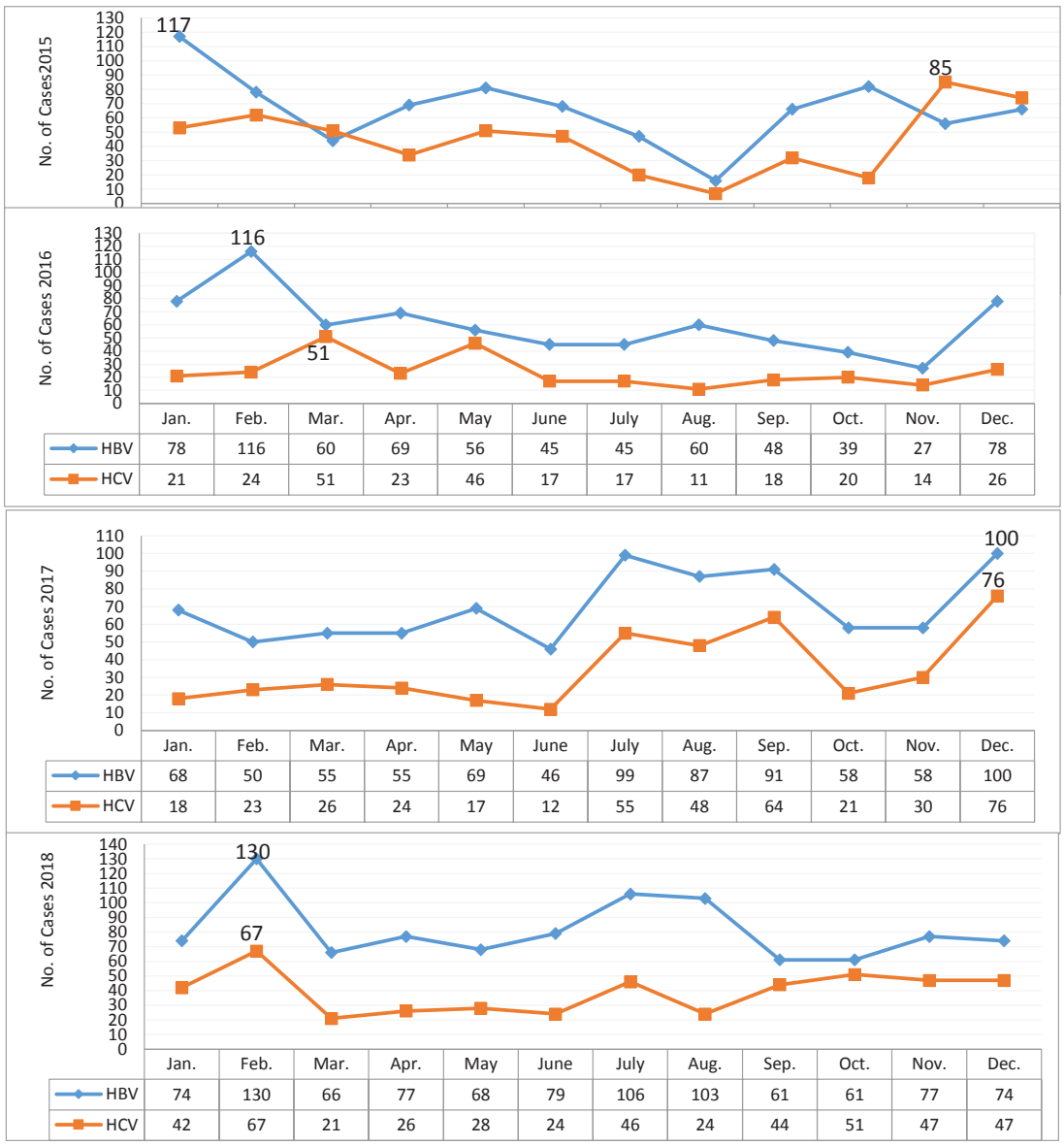


Figure (2): Distribution of HBV and HCV infections in Babylon Province according to month at 2015-2019.

Discussion

It seem that our results were in accordance with some of the Iraqi studies like [2] who found that out of 4851 patients, (1.79%) were positive for hepatitis B and (0.14%) were positive for hepatitis C. Salehi-Vaziri et al., (2016)[1] stated that, in several areas of the world, the occurrence of "HBV" infection diverse from 0.1 to 20%. Our results in accordance with [10] who found that the incidence rate of HBV was double of HCV. Predominance of HCV disease In 2015, 71 million people were living with ceaseless HCV contamination. Contrasted and HBV, the commonness of HCV contamination is lower, however more heterogeneously conveyed, with contrasts across and inside WHO districts and nations. Spread through breaks in disease control practices or infusion drug use may clarify this example. In general, in 2015, the worldwide predominance of HCV contamination was 1.0%. The Eastern Mediterranean Area had the most noteworthy pervasiveness (2.3%) trailed by the European District (1.5%).In 2015, viral hepatitis led to 1.34 million deaths. The results revealed that the HBV frequency is double or more than HCV. Many studies were in accordance with our results whose show the high percentage of HBV versus HCV among blood bank donors[11-15].

The results of (figure 2) revealed that, there is no defined month or semester for high rate infections. This is may be due to fact that , both HBV and HCV not respiratory virus (respiratory viruses are highly spread viruses especially during winter semester), not food-borne diseases and their transmission may be limited percutaneous and per mucosal or blood transfusion, sexual transmission, and mother-to-child transmission [16-21]. (Salehi-Vaziri et al., 2016) [1] found that, "HBV" is really spreadable and comparatively easy to convey from infected persons to others through some ways like birth, unprotected sex, blood transfusion, and also sharing needles. Jansen et al., (2015) [10] found that, the incidence of HBV showed no trend over time, did not differ between study participants living in rural or urban area. Concerning the prevalence or registry of HBV and HCV positive persons among categorized patients were displayed in table (2).

The high prevalence percentage were showed for prisoner-foreigner category (48.53 and 45.95 for HBV and HCV respectively) followed by surgery (22.66 and 19.03 for HBV and HCV respectively), blood bank (14.03 and 13.95 for HBV and HCV respectively) and in married (7.79 and 3.92 for HBV and HCV respectively). Though most of HBV contaminations among detained people are obtained in the network, transmission has been distinguished in jail, and rate rates have extended from 0.8 to 3.8% every year [18,19]. Our results was in agreement with many studies who is establish high ratio of HBV and HCV among prisoners like those documented in Iran [24,25], Sweden [26] and France [27]. Elective surgeries were recorded as good monitoring of HBV and HCV surveillance due to fact that the person need to be checked for HBV, HCV and HIV prior to surgery especially in delivery [28], eye surgery [29,30], oral and maxillofacial [31] and dental surgeries [32-34].

Conclusion

The current study conclude the high prevalence percentage of HBV (duplicate) than HCV with no seasonality of occurrence and the Prisoners and foreigners, Surgery, blood donor and hemodialysis were mostly involved with HBV and HCV infection.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Cytotoxic Effects of Al-Yohedia Municipal Waste Water

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Abstract

Household waste water represent an hazardous reservoir for wide range of medically important pathogens than can push an alarm on public health threatening diseases. It contain toxic materials, hazardous heavy metals and different pathogens. The study includes investigation of microbial, environmental, pathological and molecular effects of waste water. The results of dissolved heavy metals concentration revealed high Ni concentration (Mean±SD of four sites were 119.675±0.778 mg/L), high Mn concentration (Mean±SD of four sites were 108.2±1.095 mg/L), high Fe concentration (Mean±SD of four sites were 119.675±0.740.475±1.228 mg/L), high Cd concentration (Mean±SD of four sites were 13.75±1mg/L), high Cu concentration (Mean±SD of four sites were 5±0.755mg/L) with no significant differences in concentration among four sites for Ni and Cu while difference noted among Mn, Fe and Cd. Results of microbial study revealed that the Mean±SD of CFU/ml were $1.97x10^{10}$±0.543 and the microbes were *E. coli, K. pneumoniae, P. aeruginosa, V. cholerae, V. parahaemolyticus, S. aureus, E. faecalis, C. albicans, C. krusei and C. glabrata* with MAR index ranged from (0-1). DNA damage results using comet assay for revealed that high level of damage to DNA of rat dosed with waste water compared with those dosed with fresh water in both tail length (11.35±2.1, 2.7±0.9) and tail moment (1.193±0.11, 0.2±0.1) respectively. Immunohistochemistry (IHC) assay was used to detect of 8-OHDG among liver tissues of rat dosed with waste water (study) and fresh water (control) and the results revealed strong signal (high expression) in all liver tissues of rat dosed with waste water (study) compared with those dosed with fresh water (control) whose gave negative results. Histopathological results of eat dosed with waste water compared with those dosed with fresh water (control) were observed after 60 days of dosing. the results displayed that the control was normal where mild chronic hepatitis showed in samples of site 1, 2, 3 while site4 had moderate chronic hepatitis and Fasciola hepatica. The current study conclude that, Al-Yohedia Municipal wastewater is very dangerous and have different clinically important pathogens along with different cytotoxic materials.

Keywords*: wastewater, Vibrio cholerae, Candida spp., Hepatitis, Fasciola hepatica*

Introduction

Municipal wastewater is a collection of both domestic and industrial waste water. Al-Yohedia is fresh water stream pass through Hilla city but unfortunately it serve as municipal waste water reservoir. Sewage water, healthcare clinics, butchers waste, diesel engine waste and farm animal waste were delivered directly to this stream rendering it dangerous spot of pollution [1,2]. The compartments of wastewater includes biological and non-biological parts. The biological part includes bacteria, fungus, viruses and parasites while non-biological includes chemicals (organic and inorganic compounds like hydrocarbons and heavy metals) which pose dangerous impact on aquatic environments and human health [3-8]. Enterococci and enterobacteriaceae members like Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Salmonella spp., Shigella spp., Enterobacter spp. were common among waste water while Pseudomonas spp., and Vibrio spp. push an alert of clinically important bacteria that cause life threaten diseases [9-13]. The presence of medically important yeast like Candida spp. among waste water were also stated. Human and animal waste water can be loaded with viruses like hepatitis and parasite like Fasciola

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hepatica and this proposing biohazard when reach to human being [6,14].

Cytotoxicity of waste water can be reflected and oxidative damage to biomolecules like to lipids of cellular membranes, proteins, and DNA which may lead to carcinogenesis. 8-hydroxy-2' -deoxyguanosine (8-OHdG) is one of the predominant forms of free radical-induced oxidative lesions, and has therefore been widely used as a biomarker for oxidative stress and carcinogenesis. 8-OHdG used to estimate the DNA damage in humans after exposure to cancer-causing agents, such as tobacco smoke, asbestos fibers, heavy metals, and polycyclic aromatic hydrocarbons. In recent years, 8-OHdG has been used widely in many studies not only as a biomarker for the measurement of endogenous oxidative DNA damage but also as a risk factor for many diseases including cancer. Oxidative DNA damage is common in various forms of chronic liver disease suggesting a possible link between chronic inflammation and hepatocarcinogenesis. Heavy metals and some metalloids are known to be carcinogenic to humans. The oxidative concept in metal carcinogens were involvement of redox mechanisms production of hydroxyl radicals can attack DNA causing hydroxylation and oxidative DNA damage these products of DNA damage (including 8-OHdG) induce mutations leading to neoplastic transformations [15-18]. The current study aimed to investigate the cytotoxic effects of Al-Yohedia municipal waste water by IHC of 8-OHdG and histopathology of Rat lab animals.

Materials and Methods

Sampling:

Four waste water samples were collected from Al-Yohedia Stream. The study area included 4 different sites from Al-Yohedia stream in Hilla city (site 1 Al-karama quarter (N 32 0 28.944 E044 0 25.057), Site 2 Al-Jameia quarter (N32 0 28.383 E044 025.067), Site 3 Al-Iskan quarter (N32 0 27.545 E044 025.058), Site 4 Al-Askari quarter (N32 0 25.827 E044 024.394).

Waste water Culture:

All samples mixed well and 1 ml of waste water sample transferred to 9 ml of normal saline (diluent) (Stock 10-1) and from it prepare five serial dilutions (10-2, 10-3, 10-4 and 10-5) were prepared. 10 µl from the last dilution (fifth dilution) were spreaded on nutrient agar plate by disposable spreader and the plates were incubated upside down in 37°C for 24 hr. Triplicates were performed to each sample to increase the accuracy and only plate that has (30-300) colonies will be chosen for count. The exact number of colonies per original sample will calculated according to the following equation: CFU/ml = No. of colonies x Inverse of the dilution x 100[19].

Microbial Isolations and Identification:

Immediately after sample collection and along with total plate count the samples inoculated UTI chromogenic agar, Pseudomonas chromogenic agar, m-EI chromogenic agar Vibrio chromogenic agar and Candida chromogenic agar to investigate the presence of common pathogens among wastewater samples. The sample mixed well and from each sample a triplicate of 100 ml will filtrated on (0.45 µm) cellulose nitrate membranes (Sartorius/Germany) using Sartorius filtration system and then the filter content resuspended in sterile normal saline then took 5 µl and cultured on the above mentioned agar, incubated at 37°C for 48 hr [20-22].

Dosing:

The experiment includes (20) Rats (Albino Rats) of males and females have (same of the age ,weight and conditions) treated from Al-Yohedia stream by drinking at need for two months whereas control groups were treated with (RO) tap water and divided into five groups: first group dosed with site 1 sewage samples , second group dosed with site 2 sewage samples , third group dosed with site 3 sewage samples , forth group dosed with site 4 sewage samples and fifth group dosed with tap water was control and after complete the period of dosing rats anatomized by anesthesing rats with chloroform and then with drawing 5ml blood from heart area by syringe for detection molecular biomarker by comet assay and take tissue (liver) for histopathology and immunohistochemistry (IHC) tests [23].

Comet Assay:

This assay has been done by Comet assay Kit, Malgene-Italy (Single cell gel Cat. No. 0905-050- K) according to (Steinert, 1996) [24].

Immunohistochemistry (IHC):

Immunohistochemistry assay include using of specific monoonoclonal /polyclonal antibody and detection kit directed toward this antibody. In this study we used Anti-8 Hydroxyguanosine antibody [N45.1]

(8-OHDG) (cat. # ab48508, Abcam /England). The immunohistochemistry detection kit is a reliable and convenient tool to identify specified gene expression on tissues. A biotinylated, cross – adsorbed and affinity purified primary anti-mouse and rabbit specific HRP/ DAB is used to detect primary antibody –antigen complexes adhered to a glass microscope slide, following reaction with an enhanced detection reagent. This kit is compatible with mouse and rabbit specific HRP/DAB secondary antibodies and can be used for paraffin – embedded tissue sections (Cat. Number: Ab80436, Abcam, England).

Histopathological Study:

Specimen with dimension 1x1x1 cm was taken from inside organ together with liver. The tissue have been fastened in (10%) buffer formaldehyde re at this time later removing wards 72 hr of the fixation , specimen was washed with tap water after which processing was robotically carried out with a collection of upgrading alcoholic focus from (70%) to genuine (100%) for two hr in every focus to eliminate water from the tissue , then clearance was executed by xylol , then the specimen was infiltrated with Simi-liquid paraffin wax at(58ºC) on two phases then blocks of specimen was made with paraffin wax and sectioned by rotary microtome at 6 µm for tissue , tissue was stained with haematoxylin and eosin and the Histopathological vary was noticed beneath mild microscope [25].

Quantitation of Dissolved Heavy Metals:

Concentrated hydrochloric acid (5 ml) was added to 1 L of the waste water filtrate and concentrated by oven (800C) until evaporate to 100 ml and then measured by Flame Atomic absorption - spectrophotometer model (7000) Aa type (Shimadzu/Japan) was used to determine the concentrations (mg/L) of the elements [26].

Results and Discussion

The results of dissolved heavy metals concentration revealed high Ni concentration (Mean±SD of four sites were 119.675±0.778 mg/L), high Mn concentration (Mean±SD of four sites were 108.2±1.095 mg/L), high Fe concentration (Mean±SD of four sites were 119.675±0.740.475±1.228 mg/L), high Cd concentration (Mean±SD of four sites were 13.75±1mg/L), high Cu concentration (Mean±SD of four sites were 5±0.755mg/L) with no significant differences in concentration among four sites for Ni and Cu while difference noted among Mn, Fe and Cd (table 1). All values were higher that standard permissible limits of WHO [27-30].

Table (1): Concentrations of dissolved heavy metals of municipal wastewater.

Dissolved mg /L	Site1	Site2	Site3	Site4	Mean±SD
Ni(Nickel)	120.7 ±0.1 a	119±1 a	119±0.01 a	120±2 a	119.675±0.778
Mn(Manganese)	116.6±2.08 d	100.1±0.1 a	110.2±0.2 c	106±2 b	108.2±1.095
Fe(Iron)	30.6 ±0.4 a	55±3 c	32 ±0.01 a	44.3±1.5 b	40.475±1.228
Cd(Cadmium)	12 ±1 a	12±0.01 a	16±2 b	15±1 b	13.75±1
Cu(cupper)	6±0.01 a	5 ± 2 a	4± 1 a	5±0.01 a	5±0.755
Pb(Lead)	2.7±0.6 a	3 ± 2 a	3±2 a	0.8 ± 0.01 a	2.375±1.152

$p<0.05$

The main sources of lead contamination are industrial threw battery in sewage, run off from contaminated land areas, atmospheric fall out and sewage effluents. The sources of heavy metal also the reason in the high concentration of nickle in Al-Yohedia stream may be attribute to the high numbers of small engine oil exchange and electricity station near to stream, disposal of tires of cars in the stream, generator located at the side of stream .The exchanged oil and waste of generators delivered directly to stream in addition to the nickel not use and not consume by microorganism [31]. Cd was classifies as a first category of carcinogenic factors in human cancers because cadmium ions have a configuration of electrons analogous. Pb responsible for oxidative stress and genotoxicity [32-35].

Results of microbial study revealed that the Mean±SD of CFU/ml were $1.97x10^{10}$±0.543 and the microbes were *E. coli, K. pneumoniae, P. aeruginosa, V. cholerae, V. parahaemolyticus, S. aureus, E. faecalis, C. albicans, C. krusei and C. glabrata* with MAR index ranged from (0-1) (table 2). The results agreed with Yang et al., (2009)[36] who found that the clinical isolate of *E. coli, K. pneumoniae, S. aureus and P. aeruginosa* were common among sewage water. Concern presence of Candida spp. it supported by Filipkowska et al., (2008)[37], Biedunkiewicz and Ozimek, (2009)[38] and Korzeniewska et al., (2011)[39] whom found the different species of Candida in municipal wastewater. It is important to state that recovering mentioned microbes from high concentration of heavy metals waste water reveal their ability to live with such toxic concentrations may be due to their efflux pump or plasmid coding genes. The heavy metals and antibiotic resistance were closely related and may be carried on same plasmid [40-44].

Table (2): Microbes with resistance phenotypes and MAR index.

Pathogen	Resistance Phenotype	MAR index
E. coli	AmoxiclavR/CefotaximeR	0.22
K. pneumoniae	AmoxiclavR/CeftazidimeR	0.22
P. aeruginosa	AmoxiclavR/CefotaximeR/CeftazidimeR/AmikacinR	0.44
V. cholerae	NON	0
S. aureus	ClarithromycinR	0.1
E. faecalis	NON	0
C. albicans	KetoconazoleR/MiconazoleR	0.33
C. krusei	KetoconazoleR/MiconazoleR/EconazoleR/FluconazoleR /ItraconazoleR/ClotrimazoleR	1
C. glabrata	KetoconazoleR/MiconazoleR/EconazoleR/FluconazoleR /ItraconazoleR	0.83

DNA damage results using comet assay for revealed that high level of damage to DNA of rat dosed with waste water compared with those dosed with fresh water in both tail length (11.35±2.1, 2.7±0.9) and tail moment (1.193±0.11, 0.2±0.1) respectively (table 3).

DNA damage based on the comparison with the control according to the pictures that were taken DNA damage can be classified into three classes (class 1, no damage; class 2, low damage; class 3, medium damage and class 4, high damage) as shown in figures (1). The observed DNA damage may attributed to heavy metals which have a role in high percentage of DNA damage and ability to produce bulky DNA adducts. The heavy metals can produce chemical or physical modification to DNA measured by means of DNA fragmentation [45].

Table (3): Comet Assay Parameter among rat dosed with waste and fresh water.

Comet Parameter	Mean±SD	
	Rat dosed with waste water	Rat dosed with fresh water
Tail Length (μm)	11.35±2.1	2.7±0.9
Tail Moment (%)	1.193±0.11	0.2±0.1

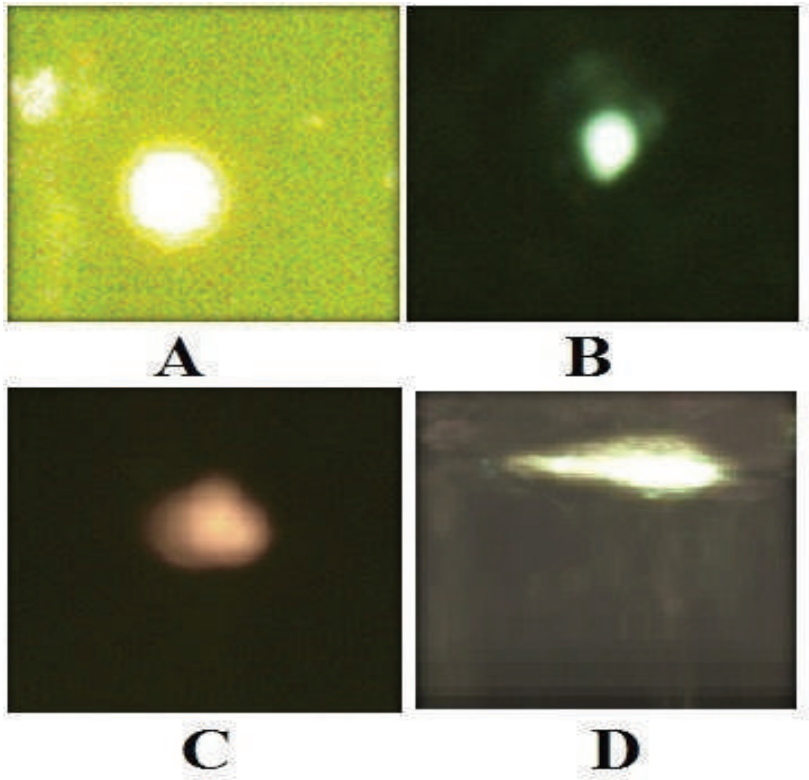


Figure (1): Classes of DNA damage in rat sample (blood) oral administrated from wastewater of four sites according to comet assay: Class (A) Control, Class (B) low damage, Class (C) Medium damage, Class (D) High damage.

Immunohistochemistry (IHC) assay was used to detect of 8-OHDG among liver tissues of rat dosed with waste water (study) and fresh water (control) and the results revealed strong signal (high expression) in all liver tissues of rat dosed with waste water (study) compared with those dosed with fresh water (control) whose gave negative results (figure 3). High expression of 8-OHDG revealed high oxidative stress caused by pollutants especially heavy metals like Cd and Pb. liver tissues used to understanding biotransformation and toxicity in municipal wastewater, this was agreement with Herman and Geraldine, (2009)[46] who considered that the liver is one of the target organs affected by lead toxicity owing its storage in the liver after lead exposure. Also it is organ of detoxification [47,48].

Figure (3): Infiltrative rats liver tissues showing the results of immunohistochemical staining of 8-OHDG protein over expression using biotinylated -labeled anti-8-OHDG protein antibody, stained by DAB-chromogen (Brown) and counter stained by Mayer's haematoxylin (Blue): A. Positive 8OHDG –IHC reaction with high score and strong signal intensity (10X) B. Liver tissues with negative 8-OHDG-IHC reactions (10X)

Histopathological results of eat dosed with waste water compared with those dosed with fresh water (control) were observed after 60 days of dosing . the results displayed that the control was normal where mild chronic hepatitis showed in samples of site 1, 2, 3 while site4 had moderate chronic hepatitis and *Fasciola hepatica* (figure 4 and 5). Many studies of mammalian tissues noted that the histopathological alterations induced by different heavy metals in the tissues of liver organ. Human and animal wastewaters play role important as sources of parasitic pathogens infecting human [49,50].

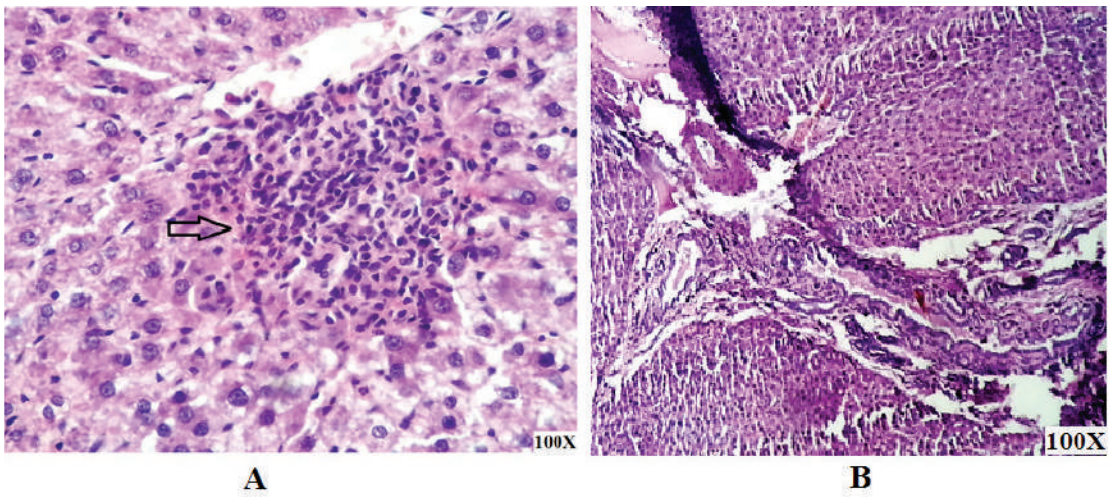
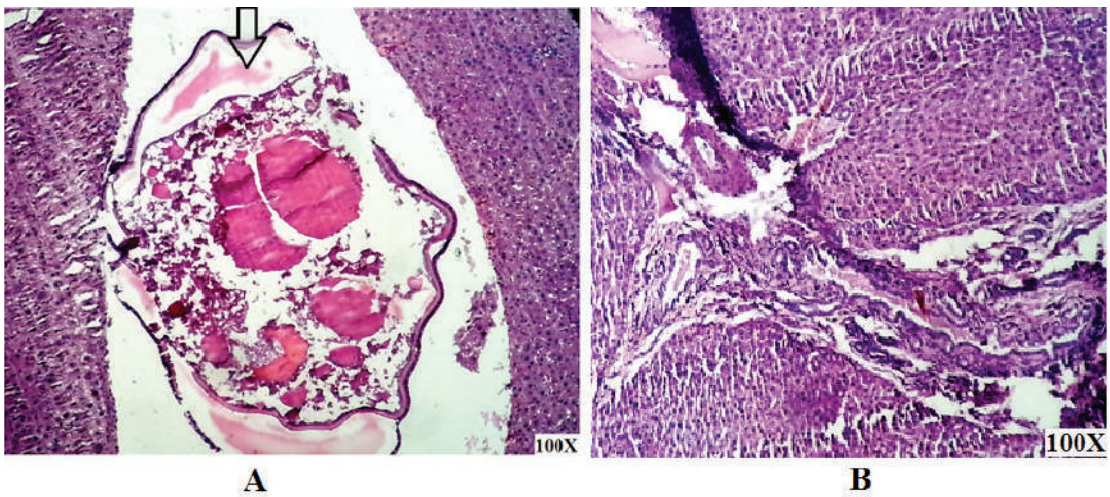


Figure (4): Show the histopathology of rat Liver sections after 60 days of dosage with wastewater: A:Show liver tissue with moderate chronic hepatitis, intralobular and periportal chronic inflammatory cell infiltrate (arrows) (100X) (H&E). B:Show normal Liver (Control) (100X) (H&E).



Figure(5): Show the histopathology of rat liver sections after 60 days of dosage with wastewater: A: Show the liver tissue with Fasciola spp. (Fasciolosis) (arrow) (100X) (H&E). B: Show normal Liver (Control) (100X) (H&E).

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Antibacterial Effect of Virgin and Refined Coconut Oils on Pathogenic Bacteria: A Review

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Abstract

Virgin coconut oil (VCO) is the most oldest edible oil, extracted from fresh and mature kernel using wet or dry methods, therefor the resulting oil is a pure, clear, and colorless with a scent of fresh coconut, these characteristics make VCO has the preferableness in medical and health applications, over refining coconut oil (RCO) that is extract from kernel of coconut after drying by methods makes the oil refining, bleaching and deodorizing. In spite of that, the chemical compositions particularly the fatty acids in both VCO and RCO are the same with no significant difference. MCFAs which are classified as MCTs, are the basic components in both VCO and RCO, these MCFAs particularly lauric acid (LA) following by capric and caprylic acids playing a great role in antimicrobial activity of coconut oil, therefor the extraction of these fatty acids are show more antibacterial activity on pathogenic bacteria more than lipolyzed VCO or VCO itself that may not show inhibition effect. Gram positive bacteria is more sensitive than gram negative one towards LA and other fatty acids, therefor these fatty acids at high concentrations showed inhibition effect on gram negative bacteria, while at low concentration showed resistance. This study attempts to review the chemical components in both VCO and RCO based on their extraction methods, also the antibacterial activity and the mechanism of action, also suggests that RCO has the same effect as antibacterial as VCO based on their chemical compositions.

Keywords: *Virgin coconut oil, Refined coconut oil, Medium chain fatty acids, Antibacterial activity.*

Introduction

Coconut nucifera commonly known as Coconut, is a member of the Arecaceae family, coastal tropical countries like Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam are considered the native of coconut tree cultivation [1,2], "tree of life" referred to the tree of coconut and all parts of this tree could be consumed by humans or animal and transformed into many products like brushes, ladle, spoon and house furniture [3]. The oval rough coconut fruit consist of the outer hard thin skin (exocarp), fibrous thick layer (mesocarp), hard core (endocarp), white kernel (endosperm), and a big cavity suspended with a thick, sweet albumen liquid called coconut water, the development of endosperm layer will give arise the flesh meat edible coconut or kernel, after drying of flesh coconut it would be called copra, which is a source of coconut oil extraction. Coconut oil is an oldest edible oil that was used in tropical countries for thousands years ago, which is extract from the fresh kernel or meat of coconut after maturation, that make coconut oil can be easily homemade that would be give natural oil and chemical free treatment, and with its low oxidation point making coconut oil stable for two years of storage without any oxidation process could be occur as the presence of saturated fat also it has long validity, stable in high heat and melting point from 23º to 26ºC, that makes coconut oil very healthy for backing and deep-fry [4-8].

The natural creamy and soft texture of VCO is well known for skin and hair care using [9], and also used in cosmetics industry such as prepare natural shampoo, scents making, soaps, massage oils, aromatherapy and many other beauty and cosmetic products [10,11]. VCO

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has very strong benefits in health and medical fields that make it very useful in weight loss, digestion, healing and infection, aid in prevention liver disease, dissolve stone of kidney, pancreatitis treatment, diabetes treatment and the most important fields include antibacterial, antiviral, antiparasitic, antifungal, anticancer, antidermatophytic, antioxidant, anticaries and disinfectant activities [12,13].

Coconut oil

Coconut oil can be classified into VCO and RCO. According to Philippine National Standard VCO the definition of VCO is the oil that obtained from fresh and mature kernel of the coconut, either by natural or mechanical methods in the presence of heat or not, without chemical refining, bleaching and deodorizing, and which does not lead to the changing the oil nature [14]. VCO is pure oil, clear and colorless, with a scent of fresh coconut, which depends on the method of production oil. Two mains procedures can be used to extract VCO, which are wet and dry procedures, in wet procedure VCO is obtained by pressing fresh and mature coconut by manual or mechanical methods with water addition or without, that forming the milky fluid coconut mixture from this coconut milk VCO can be obtained, in wet method it should be avoiding dry of coconut kernel, after that VCO is produced by three mains methods; modified kitchen, modified natural fermentation and fresh-wet centrifuge methods. In dry method VCO is obtained immediately from fresh coconut kernel, after dried the kernel partially under controlled heat for reaching the moisture to 10-13% and then mechanically pressed the kernel for obtaining the oil, dry method including; low pressure oil extraction, high pressure expeller and fresh-dry centrifuge methods [15-18], these methods that is used in VCO extraction makes it very useful without losing the nature of VCO or the active natural components like vitamins especially vitamin E, polyphenols and antioxidants [17,19]. On the other hand RCO is extracted from dried coconut kernel ''copra'', the drying process could be by using sun, smoke, or hot air, the obtained oil should be purified to make it appropriate for consumption, therefor the oil is must be chemical refined, deodorized and bleached, the heat is using for deodorizing the oil and for bleaching and the oil is filtered by clays to separate impurities, while using of sodium hydroxide to separate free fatty acids that make coconut oil has long shelf life, these processes will remove some characteristics of the oil [20], and that makes the resulting RCO is yellow color, no odor and no taste [21], whereas VCO which has not any refining, deodorizing or bleaching processes, makes it is the unique form and more useful which can be used in many applications in health and medical fields as compared with RCO that has traditional use [17,22].

Composition of VCO and RCO

One tablespoon of coconut oil supply 121 kilocalories, 13.7 gram fat, no protein, no carbohydrate, and very few amount of vitamins or minerals, that was reported by the National Nutrient Database published by the United States Department of Agriculture. About 99.9% of coconut oil is fatty acids (FA) and 91.9% of these fatty acids are saturated fatty acids (SFA), followed by 6.4% for monounsaturated fatty acid (MUFA) acids and 1.5% for polyunsaturated fatty acids (PUFA), and there is no dietary cholesterol [23]. The most predominate (SFA) is Lauric Acid (LA) about 50% and could be reached 56% depending on the variety of the coconut [17], this is followed by other fatty acids [12,24,25]. The standard quality of FAs compositions for coconut oil was reported by Codex Alimentarius [26], while for VCO was reported by Asian and Pacific Coconut Community (APCC) [27], it was also reported the value of iodine, percentage of free fatty acid (FFA), moisture, volatile matter and the value of peroxide as shows in Table 1.

VCO cannot be differentiated from RCO by the compositions of FAs as reported by, even the VCO was obtained from different methods for extraction, their no significant differences in fatty acid profile for various types of coconut oil when compared with the results of the Codex standards for coconut oil and the APCC standards for VCO [28,29],that means, the processing methods does not affect the profile of Fas[30], while the deferens are being that VCO has higher FFA, volatile matter, moisture, and lower peroxide value compared to RCO, which also comes with the Codex and APCC standards[29], these values determine the quality of VCO in addition to the color, odor and taste[17,31], FFA is the most crucial and important characteristic in the VCO producing and sales of products [32].

The saturation degree and fatty acid carbon chain length in any oil or fat will assist in determination of its characteristics, uses and impacts on human health, and VCO contains about 91.9% of SFAs 50% of it is LA as mention previously in this study, LA is a medium chain free fatty acids (MCFAs), and classified as medium chain triglycerides (MCTs), with 12 carbons chain (C12), followed by other MCFAs such as like caproic

(C6), caprylic (C8), capric (C10), including lauric acid (C12), and long chain fatty acids (LCFAs) myristic (C14), palmitic (C16), palmitoleic (C16:1), stearic (C18), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) [33,34]. About two thirds of fatty acids in coconut oil is a saturated MCFAs, while LCFAs which is also saturated comprise fewer than the third and about tenth for the unsaturated one [35]. Coconut oil is the unique oil from other vegetable oils because it's the one that 50% of fatty acids in it is LA [24,25].

Table 1: Standard parameters of quality of Codex Alimentarius for coconut oil and APCC for VCO compared with VCO and RCO values.

Parameters	Codex standard for coconut oil	APCC standard for VCO	VCO products (Dayrit et al, 2007)	RCO products (Dayrit et al, 2007)
% C6:0 Caproic acid	ND–0.7	0.4–0.6	0.24–0.56 (0.40)	0.32–0.59 (0.41)
% C8:0 Caprylic acid	4.6–10.0	5.0–10.0	4.15–9.23 (7.23)	5.32–8.83 (6.61)
% C10:0 Capric acid	5.0–8.0	4.5–8.0	4.27–6.08 (5.21)	4.56–6.03 (5.00)
% C12:0 Lauric acid	45.1–53.2	43.0–53.0	46.0–52.6 (48.66)	46.7–49.4 (48.14)
% C14:0 Myristic acid	16.8–21.0	16.0–21.0	16.0–19.7 (17.82)	16.2–19.6 (17.88)
% C16:0 Palmitic acid	7.5–10.2	7.5–10.0	7.65–10.1 (8.51)	7.80–9.73 (8.88)
% C18:0 Stearic acid	2.0–4.0	2.0–4.0	2.73–4.63 (3.50)	2.94–3.69 (3.26)
% C18:1 Linoleic acid	5.0–10.0	5.0–10.0	5.93–8.53 (7.16)	7.24–8.04 (7.63)
% C18:2 Linolenic acid	1.0–2.5	1.0–2.5	1.00–2.03 (1.52)	1.82–2.36 (2.19)
Iodine value	6.3–10.6	4.1–11.0	5.6–10.3 (7.28)	6.81–8.91 (8.00)
% FFA, as lauric acid	No standard	≤0.5	0.047–0.337 (0.131)	0.008–0.076 (0.021)
% Moisture	No standard	0.1–0.5	0.05–0.11a (0.08)	0.01–0.10a (0.05)
% Volatile matter	0.2	0.2	0.07–0.18c (0.13)	0.00–0.08c (0.03)
Peroxide value, meq/kg oil	<15	<3	0.00–1.86 (0.56)	0.27–3.39 (0.98)

a By Karl–Fisher titration.

b Mass loss at 105 °C.

c Mass loss at 120 °C.

Antibacterial activity of VCO

MCFAs that are found in coconut oil make this oil has potential role as antibacterial, antiviral, and antifungal activities [36], particularly LA and its other derivatives of MCFAs with carbon chains C6 to C12 gave the coconut oil its antimicrobial activity [37] against gram positive, therefor many studies showed the antibacterial effect of LA on *Staphylococcus aureus* and *Bacillus cereus*, while the activity of MCFAs and LCFAs with various carbon chain length (C8-C18) could not show inhibition effect against a number of gram negative bacteria [38-40] on the other hand studies reported the antibacterial effect of LA against gram negative bacteria such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis, Helicobacter pylori* [41-44]. But still antibacterial activity of LA has great effect against gram positive bacteria when compare with gram negative bacteria, that is reported by a study performed by Abbas *et al*., 2017[45] that the esterified LA from coconut oil at different dilutions showed the antibacterial activity at highest dilution with largest inhibition zone for *Staphylococcus aureus* and *Streptococcus pneumoniae* followed by lowest inhibition zone for *Salmonella spp.*, *Mycobacterium tuberculosis* and *Escherichia coli*, while at lowest concentration, *Staphylococcus aureus* and *Streptococcus pneumonia* showed lowest inhibition zone compared with *Mycobacterium tuberculosis*, *Escherichia coli* and *Salmonella spp.*, that are showed resistance at the same lowest concentration, other study also reported the antibacterial the largest inhibition zone of extracted LA at different concentration for *Staphylococcus aureus*, *Streptococcus spp*. and Lactobacillus and the lowest inhibition for *Escherichia coli* which is also showed the resistance at the lower concentration of LA, in this study the same species showed resistance towards the coconut oil, which mean the extracted LA is more effect than coconut oil as antibacterial agent [46].

Antibacterial mechanism of VCO

The specific mechanism for the VCO as antibacterial agent is not detected and stilled unknown, the suggestion was that the metabolizing of the VCO makes it release its component of MCFAs, like caprylic, capric, and lauric acids, which are have the antimicrobial action [47], and LA is the most effected one, the integration of MCFAs of VCO into the bacterial cell membrane induce the solubilization lipids of the membrane which lead to disintegrate the bacteria cell wall [47,48], the disruption of bacterial cell membrane was indicated by the studies of electron microscope, which demonstrated the lysis of *Streptococcus agalactiae, S. aureus Clostridium albicans, C. perfringens,* and *C. trachomatis* when are exposed to the fatty acids of VCO [49,50]. VCO and its fatty acids showed the antibacterial effect on *Clostridium difficile* and the most action was belong to LA (C12) which is the predominant one, and followed by capric (C10) and caprylic (C8) acids, which are represented about 9% and 6% for each respectively according to Codex and APCC, these two fatty acids (Capric and caprylic acid) were showed less antibacterial action, while the activity of VCO towards *C. difficile* was abcent, but when coconut oil was lipolyzed showed the inhibition action on *C. difficile* [51]. The lipolyzing of MCTs by lipase and water leading to glycerol, diglycerides, monoglycerides and FFAs forming [52], the antimicrobial action belongs to monoglycerides and free fatty acids [49,50,53] and the less activity belongs to triglycerides, diglycerides and glycerol [48], therefor when VCO is lipolyzing by lipase to release its fatty acids and exposed the cells of *C. difficile* to this lipolyzing VCO had the inhibitory effect on the growth of bacteria at 0.15–1.2% of lioplyzing VCO while no effect for non-lioplyzing one, there were inhibitory effect of lauric, capric and caprylic acids individually for each, the most effect was for LA at concentration 1000 μM, these fatty acids integrate with lipid bacterial membrane works on disruption of bacterial cell membrane [48,51].

Conclusion

Many studies were reported the chemical components of in VCO and RCO with no significant difference in both, whatever the extraction methods were used. In the other hand the antimicrobial activity was also reported of the extracted fatty acids from VCO especially LA and followed by capric acid and caprylic show while no activity for caproic acid because it comprise about 0.5%, this low content of it makes it shows no antimicrobial activity. Coconut oil fatty acids were showed the antibacterial activity particularly on gram positive bacteria and in low effect for gram negative in specific concentrations for several species, that indicate using fatty acids in high concentration shows antibacterial activity on gram negative bacteria, which makes the antibacterial action was no longer for gram positive but also for gram negative bacteria, and as the both types of VCO and RCO have the same

chemical compositions with no significant difference as mentioned previously, that suggests the RCO has the same mechanism as antibacterial activity as VCO.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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Awareness on Alzheimer's Diseases among College Students

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Abstract

Alzheimer's disease is the common form of dementia and possibly contributes about 60-70%. of Cases .There is no cure for Alzheimer's disease and drug therapy for disease is still in its infancy. Approved medication for the treatment of probable Alzheimer disease helps control the Symptoms of Alzheimer's disease **.** This study involves college students in the age of 17 to 20 years. A well Structured questionnaire comprising questions covering socio-demographic information , knowledge, altitude, perception was framed and adminstrested to these participants through an Online survey. The results were collected and were represented in pie charts. The association between groups was assessed by Chi Square test where $p < 0.05$ was considered statistically significant. More awareness may be created on the risk factor and complications of Alzheimer's diseases among the younger generation.

Keywords*: Awareness; Alzheimer's disease; dementia; online survey*

Introduction

Alzheimer's disease is a common form of dementia that possibly contributes about 60-70% of Cases. Other types of vascular dementia, dementia with Lewy bodies in which a group of diseases that contribute to frontotemporal dementia. The boundaries between subtypes are in distinct and mixed forms mostly co-exist [1]. There is no cure for Alzheimer's disease and drug therapy for the disease is still in its infancy . Approved medication for the treatment Alzheimer's disease, helps control Symptoms of Alzheimer's disease but does not slow down the process progression or reverse [2]. Alzheimer's disease is characterized with mild cognitive impairment and dementia· Pathological changes that underlie Alzheimer's disease begin to accumulate for years or even decades, before emotional, physical or cognitive Symptoms occurs, at which the onset of a gradual and progressive decline in cognition occurs [3] Many physicians do not screen for cognitive problems in their practices unless they receive complaints from either the patient of their family [4] previous studies has demonstrated that quality care of the person with dementia is feasible in acute care setting. When attention to the special needs of patients with dementia is given [5]Currently, only symptomatic treatment can be given to patients with Alzheimer's diseases, and concentrate on potential prophylactic strategies[6]. Recent studies explain that dementia,mainly in men, may be declining in western countries. it is unclear which is the reason behind the dementia that are declining and this could be underpinned by better management of vascular risk [7]

Alzheimer's disease is substantially increased among aged 15 years and more with a progressive decline in memory, thinking , language and learning capacity etc. Alzheimer's diseases should be different compared to normal age - related decline in cognitive function, Which is more graduate and associated with less disability. Disease often begins with mild symptoms and ends with severe brain damage. People with dementia lose their ability at various rates [1] . The German psychiatrist Du. Alois Alzheimer was credited for the first time a dementing condition which later becomes known as Alzheimer's disease In landmark 1906 conference lecture and subsequent 1907 article put alzheimer's described the case of Auguste D, a 51-year old women with a peculiar disease of cerebral corter who had presented with progressive and language impairment, disorientation , behavioural symptoms like

hallucinations, delusions, parabola and psychosocial impairment [2]. Alzheimer's disease (AD), a very common dementia in elders is a chronic neurodegenerative disease affecting approximately 30 million people worldwide in 2015 [8] Previous studies state that someone in the country will develop Alzheimer's disease every 66 sec . By the year 2050, every case of Alzheimer's is expected to develop within 33 seconds, concluding nearly 1 million new cases per year. In the year 2013, official death certificates recorded 84,767 death cases of Alzheimer's disease, making it the sixth largest cause of death in the United States .The fifth largest cause of death in Americans who age more than 65 year [9] The treatment is currently targeted towards symptomatic therapy although trials are underway Alzheimer's diseases [10]. Researchers indicates the that people are very concerned about the status of their cognitive health and development of AD [11]

In the past, Alzheimer's disease dementia was difficult to differentiate from other dementia causing pathogens, however with the advent of advanced medical imagéng techniques, including molecular imaging have given clinicians and researchers much greater insight into the neuropathological process of their patients aiding in diagnosis. Regardless of these advances, the recognitication of Alzheimer's disease remains one primarily formed on the clinical history and presentation of the patients as examined by the physician , According to the most recent diagnostic Criteria , the clinical onset of Alzheimer's disease dementia one can be divided into 3 broad periods : preclinical, MCI and Alzheimer's disease can be divided dementia and the lengths of time elapsed during these different periods of disease is on the order of decades [12]. Several of these pathologic changes may occur decades before symptom onset, leaving ample time for implementing prevention strategies that target the earliest stages of the disease. [13] .The current population has seen the rise of unaging and other biomarkers to characterize preclinical disease before the development of significant cognitive decline . At last, we suggest future directions and predictions for dementia - related research and potential therapeutic interventions. One of the biggest challenges faced by neuropsychologists over the past oo years is to understand the cognitive and behavioral manifestation of dementia and at their relationship between brain pathology [14]

Patients who have subjective cognitive decline have been found out as a useful population in whom we need to look for preclinical Alzheimer's disease [15] Recent research on childhood obesity [16],natural products in health and diseases [17,18,19,20,21] ,nanoparticles [22,23,24,25], studies on cancer cells [26,27,28,29,30] improved my passion for research and chose this topic to create awareness on Alzheimer's diseases in the society.The aim of this study is to create awareness and knowledge and to determine the attitude and practice towards Alzheimer's disease among College Students

Materials and Methods

This study involved both male and female in the age group of 19-23 years among College students. A well-structured questionnaire comprises 15 questions. This was conducted through an online survey. The online survey Software used was Google forms software. This is a cross sectional descriptive survey that is conducted among 100 students College Students . The results were collected and Tabulated. The descriptive statistics were done using the SPSS software. The results were analysed using statistical analysis.

Results and Discussion

In the survey conducted among 100 college Students 66.3% were female and 33 .7% were males. 81.2% were aware of Alzheimer's disease whereas 18.8% were not aware of Alzheimer's disease. 45.5% of the participants think Alzheimer's disease is curable and about 54.5% say it not curable. 76.2% think memory loss is the symptom for Alzheimer's disease. 43.6% think Alzheimer's disease and dementia are the same and about 56.4% think it is not the same. 47.5% thinks there is no cure for Alzheimer's disease.

37.6% agreed that they have disorientated time and place, often forgetting days of a week. About 50.5% agreed that they have memory loss and 49.5% agreed that they don't have memory loss. 36.6% agreed that memory loss get's worse day by day and 63.4% agreed that it doesn't get worse day by day. 79.2% agreed that they gain information from this survey.

We have seen the association between gender and awareness on symptoms of Alzheimer's disease [Fig 1], awareness on differentiation between Alzheimer's disease and dementia [Fig 2], awareness on treatment

for Alzheimer's disease [Fig 3], awareness on Alzheimer's disease [Fig 4], awareness on memory loss getting worse everyday [Fig 5], awareness on gaining information about Alzheimer's diseases after doing this survey [Fig 6].

In the Study done by Wendy Smyth et al 2012 regarding the opinion of the participant thinking if Alzheimer's diseases and dementia are the same 62% agreed that yes and 38% agreed that No [5]. In the Study done by Miri Kim et al, 2020 majority of the participants agreed that younger people are affected more [8] And in Study done by Maria .S. Sabbagh et al, 2011 , 45.7% agreed that younger people are affected more[4].

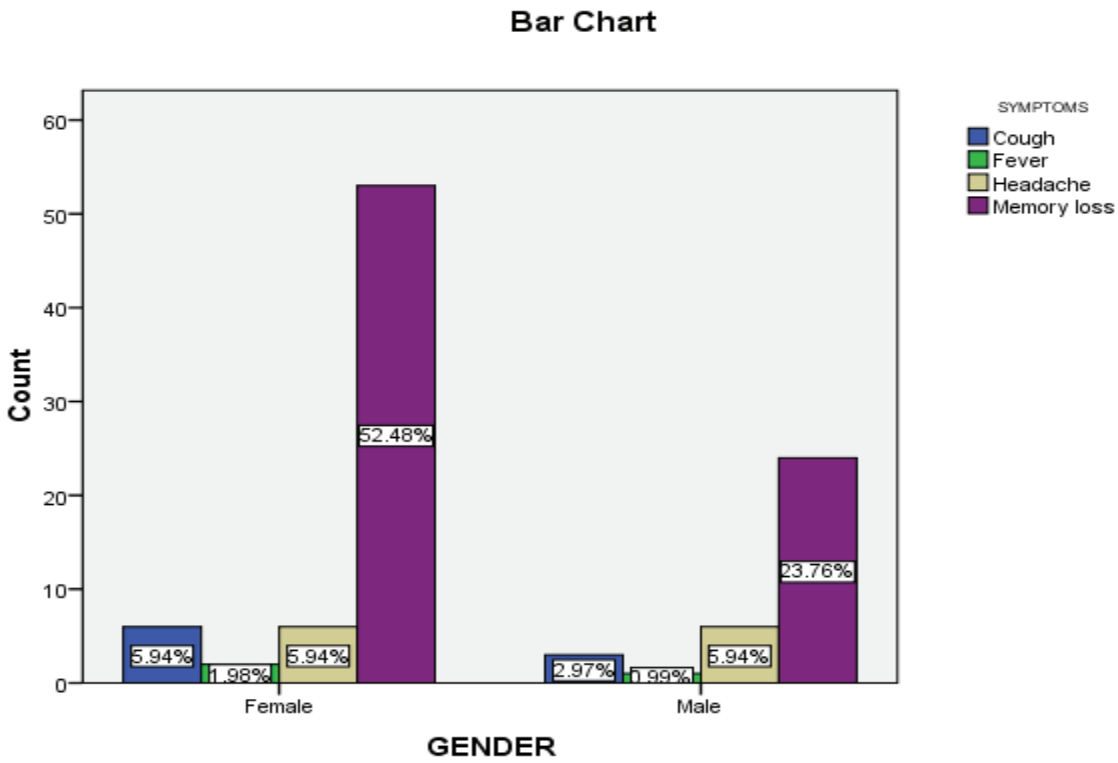


Figure 1:Bar Chart depicts the association between gender (X axis) and responses of awareness on symptoms of Alzheimer's disease (Y axis). 52.48% of female and 23.78 % of male agree that memory loss is a symptom . Blue colour denotes Female and green colour denotes Male .The majority of the female population (52.48%) agree that memory loss is a symptom of Alzheimer's disease . The association was found by chi square test (Chi square value= 1.649) which shows (p value=0.648) no statistical significance.

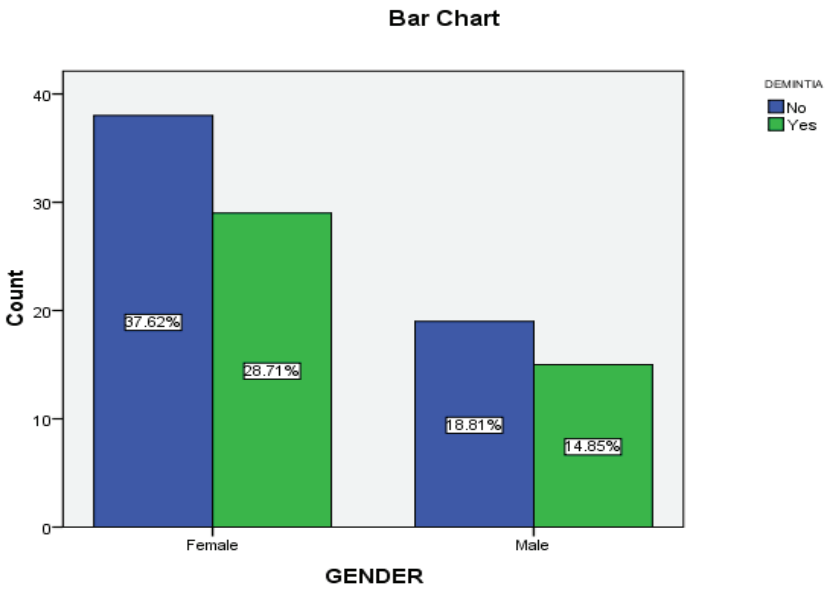


Figure 2:Bar Chart depicts the association between gender (X axis) and response of awareness on differentiation between Alzheimer's disease and dementia (Y axis). 28.71% of female and 14.85% of male accept dementia and Alzhemier's disease are the similarity .Blue colour denotes Female and green colour denotes Male. The majority of the female population (37.62%) agree that dementia and Alzheimer's disease are not similar. The association was found by chi square test (Chi square value= 0.004) which shows (p value = 0.936) no statistical significance.

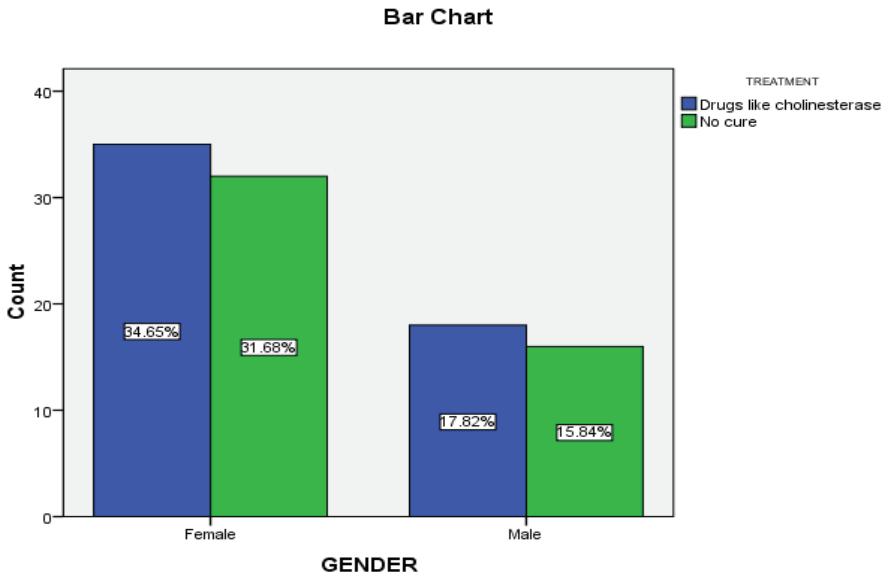


Figure 3 : Bar Chart depicts the association between gender (X axis) and responses of awareness on treatment for Alzheimer's disease (Y axis).34.65% of female and 17.82% of male admit cholinesterase as one of the best treatments for Alzheimer's disease .Blue colour denotes Female and green colour denotes Male. The majority of the female population (34.65%) responses that drug like cholinesterase is the best treatment for Alzheimer's diseases .The association was found by chi square test (Chi square value= 0.004) which shows (p value = 0.947) no statistical significance

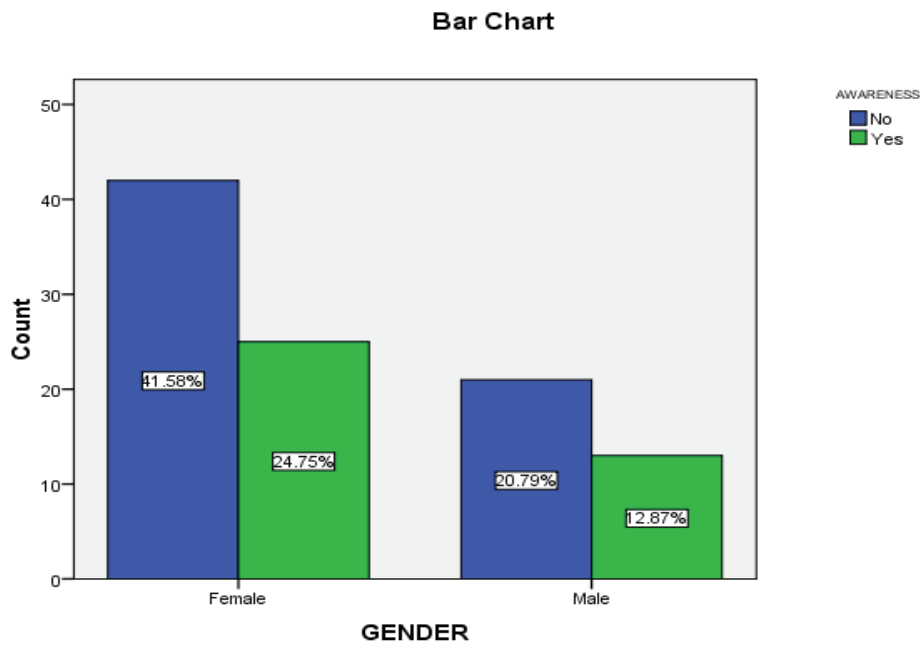


Figure 4 :Bar Chart depicts the association between gender (X axis) and responses of awareness on Alzheimer's disease (Y axis). 24.75% of female and 12.87% of male have enough awareness about Alzheimer's disease.Blue colour denotes Female and green colour denotes Male .The majority of the female population (34.65%) agrees that there is no enough awareness on Alzheimer's diseases . The association was found by chi square test (Chi square value= 0.008) which shows (p value = 0.928) no statistical significance.

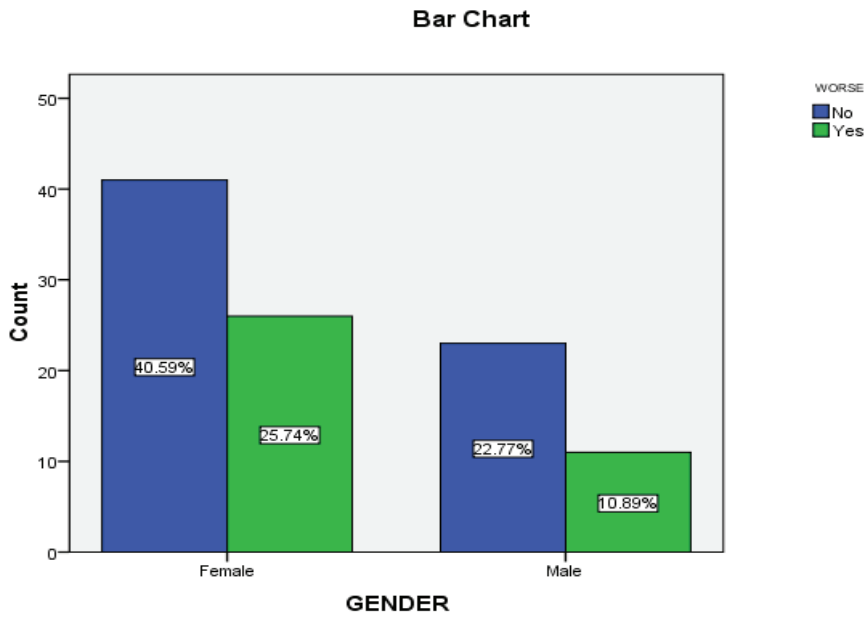


Figure 5:Bar Chart depicts the association between gender (X axis) and response of awareness on memory loss getting worse everyday (Y axis). 25.74% of female and 10.89% of male population says memory loss gets worse day by day. Blue colour denotes Female and green colour denotes Male.The majority of the female population (34.65%) responded that the memory loss doesn't get worse . The association was found by chi square test (Chi square value= 0.242) which shows (p value = 0.525) no statistical significance.

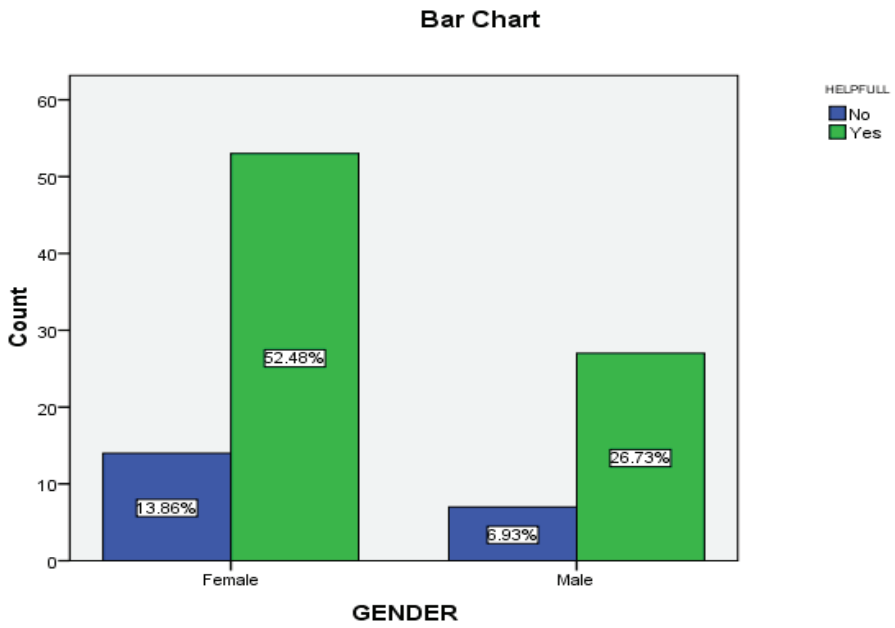


Figure 6 :Bar Chart depicts the association between gender (X axis) and response of awareness on gaining information about Alzheimer's diseases after doing this survey (Y axis).52.48% of female and 26.73% of male agree that this survey was helpful.Blue colour denotes Female and green colour denotes Male .The majority of the female population (52.48%) respond that this survey was helpful to learn more about Alzheimer Diseases . The association was found by chi square test (Chi square value= 0.405) which shows (p value = 0.971) no statistical significance.

Conclusion

From our study we can conclude that students are having good knowledge and awareness of Alzheimer's disease and its symptoms, treatment etc, but still few participants are not fully aware of Alzheimer's disease. Seminars in educational institutions, active lifestyle modifications and memory workshops may be required to bring awareness on Azheimer's disease among the community.

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Conflict of Interest - All the authors declare no conflict of interest in the study.

Ethical Clearance: Not Required

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Surgery for Gastric Adenocarcinoma with Hospital Stay: A Prospective Study

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Abstract

Background: With the new changing lifestyles and the control if infectious diseases, cancer is soon emerging to be one of the leading causes of deaths in developing countries like India. Surgical procedure shows improvement in survival period for a patient at stage II and III after correcting the nutritional status of the patients. There is a decline in post-operative complication with patients who underwent surgery. Hence this study was undertaken to study the curative and palliative surgeries in the patients of gastric adenocarcinoma and their hospital stay.

Methodology: This was a prospective study in which 50 of gastric adenocarcinoma patients who were admitted in the surgery department at Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur in collaboration with Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi Meghe, Wardha, Maharashtra were studied.

Results: Majority of patients presented with Stage II (88%) while 12% presented with stage III. 82% patients underwent curative surgeries while 18% underwent palliative surgeries. The duration of hospital stays for Gastric Adenocarcinoma surgery for Stage II ranges from 6-8 days (median 7 days) whereas for Stage III it is 7-8 days (median 7.5 day). A total of 50 patients who underwent surgery for histologically proven gastric adenocarcinoma. Out of 50 patients a majority i.e. 88% presented in stage II while 12% presented in stage III.

Conclusion: On the basis of our observations we concluded that the patients presenting earlier in stage II undergo curative surgeries most often and mean hospital stay post-surgery around a week.

Keywords: *Curative surgery, gastric adenocarcinoma, palliative surgeries, cancer and infectious diseases.*

Introduction

With the new changing lifestyles and the control if infectious diseases, cancer is soon emerging to be one of the leading causes of deaths in developing countries like India. Solid tumors arising from lung, stomach, colorectum, liver, esophagus, breast, cervix and prostate form the major causes of cancer related deaths in India[1]. There are various screening programmes which may detect early stages of cancer and thereby resulting in higher cure rates of the cancers in early stage. Gastric carcinoma is increasing becoming commoner day by day.

The most common site for metastases is the peritoneum, in two thirds of cases synchronous and in

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one third metachronous. In recurrent disease, 50%of the patients with GC have peritoneal metastases (PM) and in patients who undergo resection with curative intent for GC it is 10-20%. However, the true incidence, prognosis, treatment and outcome of patients with PM in G Care not well-known. The most common treatment for advanced GC is palliative chemotherapy, with a median overall survival (MOS) of 8-17 months 12-15. In cases with non-curable GC (distant lymph node metastases, liver metastases, remnant tumor or PM) treated with palliative resection, a MOS of 7-8 months has been observed and in patients not actively treated, it is shorter[2]. This poor outcome of PM from GC underlines the importance of further investigation and the evaluation of a new treatment method that may improve survival rates.

For a locally advanced gastric cancer neoadjuvant chemo-radiation can be given to achieve RO resection. Diagnosis of gastric cancer in early stages gives good curative resection and less mortality & morbidity for patients. In patients morbidity and mortality considerably decreased, for those who were hospitalized at stage II and III. Surgical procedure shows improvement in survival period for a patient at stage II and III after correcting the nutritional status of the patients. There is a decline in post-operative complication with patients who underwent surgery. Curative surgeries like endoscopic or surgical resection can be done in patients in which lesions are superficial or present early while for advanced cancer, last resort is palliative surgery.[3] Also the mean duration of stay of patients post-surgery of gastric adenocarcinoma was about 7-8 days which is less than other cancers. Hence this study was conduced to study the curative surgeries and hospital stay of the patients with gastric adenocarcinoma post surgery.

Methodology

Patients admitted in the surgery department in a tertiary hospital were included in the study. This was a prospective descriptive study conducted on a total of 50 patients at the department of general surgery in a tertiary hospital from July 2012 to December 2013. The information obtained included patients date of admission, demographics, clinical history, investigations, and type of surgery, type of anesthesia, duration of surgery, date of discharge and return to work. The patients were further enquired and clinically examined. Patients were further reviewed on subsequent clinical visit.

Inclusion Criteria: The study population includes 50 patients who underwent surgery for gastric adenocarcinoma (histopathologically proven gastric adenocarcinoma) by endoscopic biopsy at the Department of General Surgery.

Exclusion Criteria:

1. Patients with gastro intestinal stromal tumor or Gastric Lymphoma or Gastric carcinoid's tumors are excluded from the study.
2. Patient only received palliative chemo radiotherapy without going any forms of surgery are excluded from study.
3. Patient who is unfit for surgery excluded from study.

All patients underwent a detailed medical history and clinical examination.

Clinical examination comprises of General examination, systemic examination and Digital rectal examination. Diagnosis of patients based on UGI Endoscopy, biopsy, CT scan, PET scan and assessment for H. Pylori. Staging based on TNM Classification system according to the AJCC Staging. From July 2012 to Dec 2013 data entered prospectively in to a methodology specifically designed for the study.

Statistical analysis: The categorical data were represented by frequency with percentage and chi-square and Fisher exact test were used for analysis. The continuous data represented by mean (SD) for normal data and Median (Range) for abnormal data was analyzed by using Independent t-test and Mann- Whitney U test. SPSS version 16 was used for analysis and P value less than 0.05 were considered as statistically significant.

Results

A total of 50 patients who underwent surgery for histologically proven gastric adenocarcinoma. Out of 50 patients a majority i.e. 88% presented in stage II while 12% presented in stage III. (table 1)

82% of the surgeries done were curative surgeries while only 18% were palliative surgeries.(table 2 and figure 1)

The duration of hospital stay for Gastric Adenocarcinoma surgery for Stage II ranges from 6-8 days (median 7 days) whereas for Stage III it is 7-8 days (median 7.5 day). The p value is <0.16 which is not significant. (table 3)

Table 1 showing stage of tumour:

Stage of tumour	Number of patients	Percentage
I	0	0%
II	44	88%
III	6	12%
IV	0	0%

Table 2 showing the number of curative surgeries done:

Type of surgery	Number	Percentage
Curative surgeries	41	82%
Palliative surgeries	9	18%

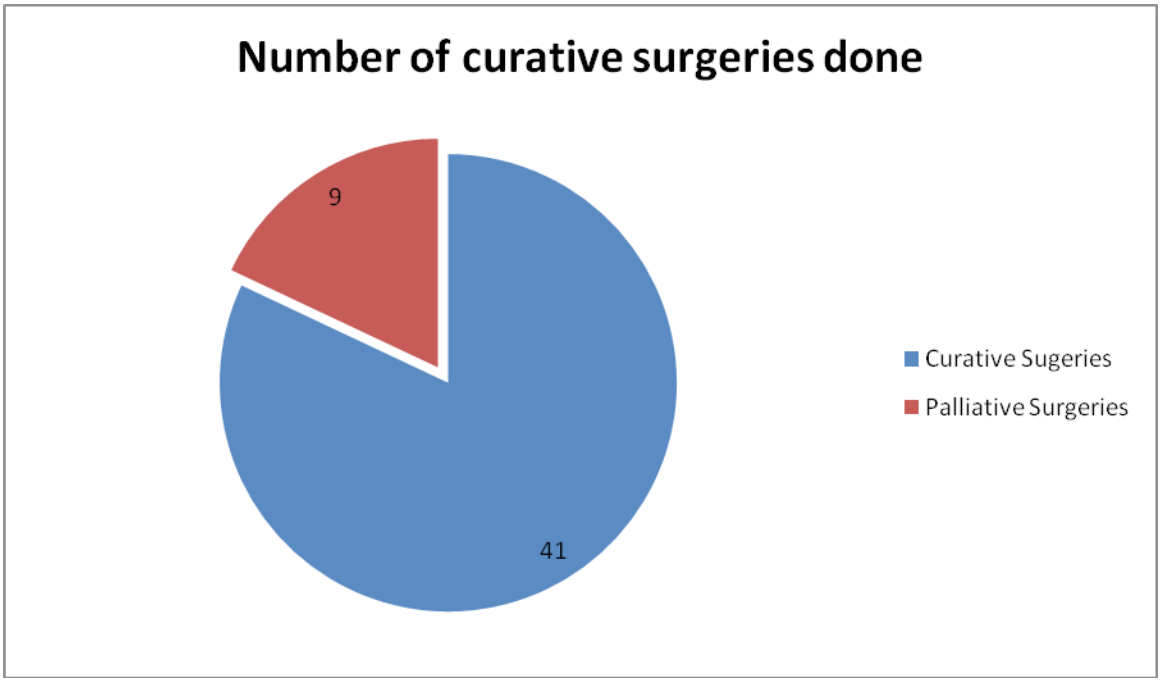


Figure 1 showing number of curative surgeries done:

Table 3 showing duration of hospital stay:

	Variables	Median	Standard Deviation	P. Value
Duration of hospital stay	Stage II	7	1.25	0.16
	Stage II	7.5	0.5	

Discussion

In our study 50 patients underwent surgery out of which 41 had a curative surgeries and 9 had a palliative surgeries. The Indian health system may promote early detection of cancer helpful in decreasing morbidity & mortality of patients to give good survival rate; In this study most of patients came to hospital at stage II & III undergoes surgery and having good survival rate up to follow up.

Improvement in treatment modality for a gastric cancer patient give good survival rate to the patient; and Decreased in post-operative complication rate for the patients who undergoes surgery In our study population total number of 41 patients undergoes curative resection

it means that Patients undergoing curative surgery shows Histopathological/Microscopic Macroscopic aberrations are showing negative in RO resections; total number of patients undergoes palliative surgery were 9. It means that there histopathology was showing R1 resection which means microscopic residual tumor. Hospital stay is one of the factors used for assessment of outcome of various surgical method. When compare to open literature studies, in our study hospital stay is significantly lower than others studies. Many factors are related to length of hospital stays apart from nature of surgery; In our study patient presented with advanced stage IV gastric adenocarcinoma are referred to medical oncology and radiotherapy department for palliative chemo radiotherapy. In our study patients presented with similar symptoms and signs of gastric cancer most of them showed gastric obstructive symptoms; In my study 88 % of patients found to have gastric cancer in distal part of stomach. Patients undergoing curative surgery– Total gastric showing good tolerance to surgery; H. Pylori are a well-recognized risk factor for gastric cancer, a slightly lower prevalence & infections were observed in this study.

Curative surgeries can be done in early stage in which tumour has not metastasize which comprises of a complete excision of the tumor with margins free of tumor and microscopic tumor cells (R0), combined with adequate lymphadenectomy (the removal of more than 15 lymph nodes).[4].

Treatment of gastric carcinoma is mostly surgery and chemotherapy. Curative surgery in the form endoscopic resection appears the most effective. Surgical resection implies the removal of the primary tumor and regional lymph nodes with resection margins free of tumor. Gastric carcinoma is not very sensitive to radiotherapy and often needs used of chemotherapy to limit the size of tumour[5]. Laparoscopic staging prior to surgical resection helps to make preoperative treatment decisions and thus has positive outcome [6].Surgery is better in cases of endoscopic closure failure [7].

We have 41 curative surgeries and only 9 palliative surgeries. Palliative surgeries are needed in advanced disease-causing gastric outlet obstruction as it allows patients to consume liquid diets, preventing dehydration and frequent hospital admissions. Stent migration and occlusion are however possible complications. The Surgery for the treatment of gastric outlet obstruction aims to remove the obstruction. Gastric outlet obstruction resulting from gastric carcinoma should be resected by distal partial gastrostomy or subtotal gastrostomy with lymphadenectomy. Yonemura Y et al[8] in their study of 53 patients with gastric or small-bowel obstruction performed endoscopic gastrostomies for decompression and found that decompression was successful in 89% of these cases with low complication rates[9]. Few articles reflected on effective diagnostic tools and evaluation of abdominal malignancies[10-11]. Number of articles reflected on various aspects of different types of carcinoma prevalent in this region[12-24.] Some articles on prevalent, potentially pre-malignant lesions and conditions were reviewed[25-36].

Conclusion

On the basis of our observations we concluded that the patients presenting earlier in stage II undergo curative surgeries most often and mean hospital stay post-surgery around a week.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Study between Fracture Fixation of Patella with Cerclage and Tension Band Wiring Technique

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Abstract

Introduction: In human body among the skeletal system patella is the largest sesamoid bone in the quadriceps tendon in the body. Surgery is required for about one third of patella facture that indicated damage to the extensor mechanism. Regarding treatment of patellar fracture there is a controversy since earliest time. TBW is consider as current gold standard for the displaced transverse patella fractures whereas some surgeons recommend augmentation of TBW with circumferential cerclage wiring to enhance the strength of the fixation.

Aim: The main aim was study between fracture fixation of patella with cerclage and Tension Band Wiring technique.

Material and Method: Total 62 patients with fracture of patella were included in this study with the age more than 20 years. Almost for all the patients surgery was performed and tourniquet was also used. By using a midline longitudinal incision surgery was done over anterior aspect of the knee. By using tension band wiring with or without augmented cerclage with stainless steel wire open reduction and internal fixation were done. After the surgery as post operative knee brace was given so that patients could start range of motion at knee without pain. To make sure that whether proper fixation was done or not Post-operative radiographs were also done.

Result: In study total 50 patients were included in which 34(68%) were male and 16(32%) were female. Among 50 patients was included different age group in which more patients were in the age group of 40-50 years old as 36%.

Conclusion: In the facture of patella modified TBW was better than encirclage wiring in treatment probably because of good stability of implant and easier postoperative rehabilitation. Hence use of circumferential cerclage wiring along with tension band wiring for fractures of patella has no added advantage over fixation with tension band wiring alone.

Keywords: *Patella fracture, Cerclage wiring, Tension band wiring.*

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Introduction

In human body among the skeletal system patella is the largest sesamoid bone in the quadriceps tendon in the body. The main function of patella is to improve the efficiency of quadriceps muscle by improving the mechanical leverage of the quadriceps muscle[1]. In the

human body, facture occurring in all skeletal facture about 1% occur as patella facture[2]. Usually with direct trauma there is occurring of patellar facture. Surgery is required for about one third of patella facture that indicated damage to the extensor mechanism[3]. In the facture of patellar with displacement less than 3mm, conservative treatment was suggested as the best option[4,5]. Due to small bone fragments comminution extra articular fracture of the distal pole of patella are difficult which reduce and maintain reduction due to the extension mechanism of the knee[6,7,8 & 9].

Common procedure to attach patellar tendon to the patella by transosseous pull out suture after removal of bone fragments[10]. In the facture of patella most of the facture are displaced and associated with failure of extensor mechanism at knee. Such kind of cases treatment as open reduction and internal fixation (ORIF) is the recommended which restore the extensor mechanism and augment fracture healing to ascertain early knee movements[11,12]. There are various method used for ORIF of these fractures tension band wiring (TBW) technique using wires and most commonly used is stainless steel wires[13,14]. In the treatment of TBW which works by converting tensile forces into compressive forces when movements occur at the knee Joint. However TBW is consider as current gold standard for the displaced transverse patella fractures whereas some surgeons recommend augmentation of TBW with circumferential cerclage wiring to enhance the strength of the fixation[15,16,17 & 18]. The main aim was study between fracture fixation of patella with cerclage and Tension Band Wiring technique.

Material and Method

This study was carried out in the Department of Orthopedics in Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardha in collaboration with Datta Meghe Medical College, Nagpur, Maharashtra, during the period of 1 year. Total 62 patients with fracture of patella were included in this study with the age more than 20 years. Among total patients were divided into two groups as group I (n=30) include facture treated with tension band wiring (TBW) along with augmented circumferential cerclage wiring whereas in group II (n=32) included fractures treated by TBW along. From all the patients for the records of data as documentation was collect by physical examination, past and present history and medical assistance and investigation such as Mode of Injury, surgery, radiographies, type of fracture and pattern of fracture were taken.

Patients with facture of patella, extensor mechanism lag at knee and with closed injury were included in this study. Almost for all the patients surgery was performed and tourniquet was also used. By using a midline longitudinal incision surgery was done over anterior aspect of the knee. By using tension band wiring with or without augmented cerclage with stainless steel wire open reduction and internal fixation were done. Using two K wires and stainless-steel wire Tension band wiring was also done. After the surgery as post operative knee brace was given so that patients could start range of motion at knee without pain. To make sure that whether proper fixation was done or not Post-operative radiographs were also done.



Figure 1: (A) Pre-operative radiograph (AP view). (B) Pre-operative radiograph (lateral view).



Figure 2: (A) Post-operative radiograph in (B) Post-operative radiograph in group 1 (AP view). group 1 (lateral view).

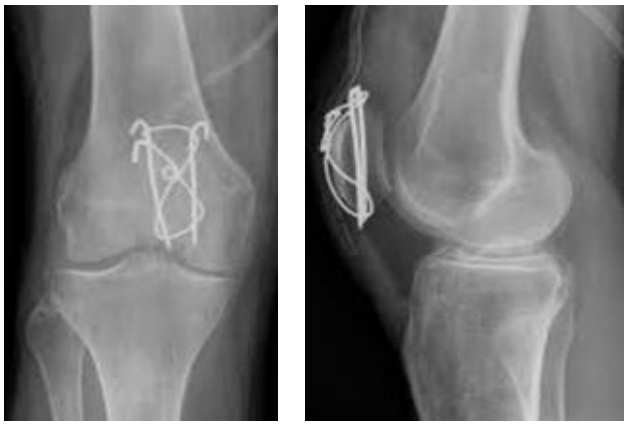


Figure 3: (A) Post- operative radiograph (B) Post-operative radiograph in group 2 (AP view). group 2 (lateral view).

For every patients followed up were also done every 2 weeks for first two months and after that once in a month. At the time of followed up each patients were examined for both subjective symptoms and objective signs which were noted. Mobilization of the knee was started at the fourth week in TBW patients, whereas the sixth week in encirclage patients.

Table No: 1 Showing Scoring of outcome.

Score	4	3	2	1
Knee pain	No pain	Mild	Moderate	Severe
Knee stiffiness	No Stiffness	Mild	Moderate	Severe
Quadriceps wasting	0-1.5cm	1.6-2.5cm	2.6-3.5cm	>3.5cm
Knee extension loss	0°-5°	6°-10°	11°-20°	>20°
Knee flexion loss	0°-10°	11°-20°	21°-30°	>30°

Table No: 2 Showing grading of outcome.

Result	Overall score
Excellent	16-20
Good	12-15
Fair	08-11
Poor	04-07

The patients were followed every monthly for 6 months post-operatively. Outcome was studied, graded and compared on the basis of knee pain, knee stiffness, quadriceps wasting, loss of flexion and loss of extension as shown in table no 1 and 2.

Result

In this study total 50 patients were included in which 34(68%) were male and 16(32%) were female as shown in table no 1 below.

Table no: 3 Showing gender wise distribution

Gender	No.	%
Male	34	68
Female	16	32
Total	50	100

Among 50 patients with different age group were included. In this study, more than 19 years to 80 years old patients were included as shown in table no 2 below. In this study more patients were in the age group of 40-50 years old as 36%.

Table no: 4 Showing age wise distribution (in years).

Age wise distribution	No	%
20-30	5	10
30-40	12	24
40-50	18	36
50-60	9	18
More than 60	6	12
Total	**50**	**100**

In this study group I showed excellent in 3 patients (14.3%), good in 12 patients (57.1%), fair in 5 patients (23.8%) and poor in 1 patient (4.8%) and in group II showed excellent in 4 patients (13.8%), good in 16 patients (55.2%), fair in 7 patients (24.1%) and poor in 2 patient (6.9%) as shown in the table below. In this study patients also has to suffer from few complications post operatively including infection, fixation failure and nonunion among both the groups. In these cases infections were managed with daily debridement and dressings were done along with proper antibiotics. Fixation failure and nonunion were managed with revision surgery with bone grafting. After revision surgery union was achieved in all the cases of fixation failure and nonunion.

Table no: 5 Showing results based on clinical grading of outcome.

Result	Group I		Group II	
	Patients	%	Patients	%
Excellent	3	14.3	4	13.8
Good	12	57.1	16	55.2
Fair	5	23.8	7	24.1
Poor	1	4.8	2	6.9
Total	**21**	**100**	**29**	**100**

Discussion

In the body facture of the patella are managed with tension band wiring (TBW) which is considered as the gold standard. Some researchers showed that when circumferential cerclage wiring added to the TBW has been regarded to improve the strength of the construct[19,20 & 21]. In the studied of Curtis et al showed that addition of cerclage wiring to the TBW significantly adds to the strength of the construct[22]. In this study total 50 cases were included with facture of patella in which all the facture were closed injuries. In this study total 50 patients were included with the age range from 20 – 80 years old. Most of the patients were aged more than 40 years. Among 50 patients 34(68%) were male and 16(32%) were female. Total patients were divided into 2 groups as group I (n=21) included patients which were managed with TBW along with circumferential cerclage wiring whereas in group II (n=29) included patients which were managed with TBW alone. All the patients were advice for the followup and followed every monthly for a period of 6 months. At the time of followup clinical outcome were recorded with grading to compare the results in the two groups. Among 21 patients in group I showed 71.43% and in group II showed 68.97% excellent to good results. Fixation failure and necessary for revision surgery among group I and group II was found to be 8.2% and 9.5% respectively. In some patients infections and nonunion also occurred which were managed with daily debridement and antiseptic dressings along with proper antibiotics whereas Fixation failure and nonunion were managed with revision surgery with bone grafting. After revision surgery union was achieved in all the cases of fixation failure and nonunion. According to studied of Srinivaslu et al[23] union was seen in all cases at 8-12 weeks in

TBW and 10-14 weeks in Encirclage group as compared to this study. In the series of Rudolph and Rosenberg showed 84% of cases excellent to good results and remaining showed poor results[24].

Conclusion

Patella is important for effective function of quadriceps and proper biomechanics of knee joint. Thus, it should be preserved wherever possible. Conservative treatment is only in undisplaced fractures. For the facture of patella it is important for operative treatment to carry out repair of torn expansion of quadriceps and gives good results. Therefore, in the facture of patella modified TBW was better than encirclage wiring in treatment probably because of good stability of implant and easier postoperative rehabilitation. Hence use of circumferential cerclage wiring along with tension band wiring for fractures of patella has no added advantage over fixation with tension band wiring alone.

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Conflict of Interest: Nil.

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Critical Review of Asthikshaya and its Management: Ayurvedic Perspective

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Abstract

The state of equilibrium of Dosha, Dhatus, Malas is health and its disequilibrium is known as disease. This disequilibrium may either be Vriddhi or Kshaya. Asthiis thefifth Dhatu among the seven Dhatus .It is the seat of vatadosha and carrying the function of dehadharana. Asthikshayais one of the condition described by Acharya Charak under 18 types Kshaya in which there is kshayaof Asthi Dhatu. According to the principle of Ashraya-ashrayee Bhava, Asthi & Vata are inversely proportional to each other regarding Vriddhi and Kshaya. Vriddhvata leads to Kshaya of Asthi Dhatu. The symptoms of Asthikshaya resemble to Osteoporosis which is major health problem of ageing population. It is a skeletal disease characterized by low bone mass and micro architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. Fracture is the most common complication of Osteoporosis which increases the risk of morbidity as well as mortality. Vatais the major factor in the Samprapti of Asthikshaya, Sampraptiof Asthikshaya occurs in two ways- Dhatukshayajanya and Srotorodhajanya. In Srotorodhajanya, the obstruction can be cleared by the use of Deepana and Pachana Dravyas. Nidanparivarjana, Shodhanachikitsa including Basti, Shamana Chikitsa including use of Swayoni Dravyas, Sudha Vargiya Dravyas, Guggulukalapa are the major treatment modalities for Asthikshaya. They help in Vatashamana and as well as improving the bone health. By following Dincharya, Rutucharya and diet regulation, this disease can be prevented. This review article highlights on etiopathogenesis of Asthikshaya and its measures as per Ayurvedic perspective.

Keywords: *Asthikshaya, Osteoporosis, Nidanaparivarjana.*

Introduction

Human body is made up of Panchamahabhutas. The state of equilibrium of Dosha, Dhatus, Malas is health and its disturbance isknown as disease.[1] This disequilibrium may either be Vriddhi or Kshaya. According to Ayurveda, Balyavastha is a period of Dhatunirman or anabolic phase; Yuvavastha maintains Samavastha (equilibrium) of different Dhatus; but in old age all Dhatus decrease gradually as catabolism speeds up. Asthikshayais a condition described by Acharya Charakunder 18 types Kshaya.[2] Asthi & Vata are inversely proportional to each other regarding Vriddhi and Kshaya. Vriddhvata leads to Kshaya of Asthi.[3] The symptoms of Asthikshaya are similar to Osteoporosis, in which there is a decrease in bone mass leading to increased bone fragility and susceptibility to fractures. By 2050, the global osteoporosis sufferers will reach 6 million (including both males and females), 3/4 of who will reside in developing countries.[4] Fractures of vertebral bodies, ribs, proximal femur, humerus, distal radius with minimal trauma are the most common complications.[5] Advanced age, female sex, sex hormone deficiency, a diet having low calcium, protein and vitamin-D, smoking, alcoholism, prolonged corticosteroid therapy, low Body Mass Index(BMI) are some of the risk factors of Osteoporosis.[6] But it is seen that people consuming nutritious diet are also suffering from the disease. If the symptoms are present before menopause, they grow rapidly after menopause. Here an effort is made to study

the etipathogenesis of Asthikshayaand its management according to Ayurveda.

Aims and Objectives:

1. Study the Asthikshaya and Osteoporosis in Ayurvedic and modern perspective.
2. Study the management of Asthikshayain light of Ayurveda.

Materials and Method

The concept of Asthikshayais studied from various Ayurvedic Samhitas while the concept Osteoporosis is studied from modern books, by searching various databases like PubMed, google scholar and other research articles.

Asthikshaya: Asthisaushirya is another condition explained in Majjadhatukshaya.[25] Saushirya is Sarandhratvam means porous bones.[7] Asthikshaya is the first stage of the disease, which is mainly characterized by different kinds of pain and deformities of Upadhatus and Malas of Asthi. Dantha, Nakha, Kesha etc. As the disease progresses, as per the principles of Anulomakshaya, it will affect Majjadhatu and manifest as Asthisoushirya, so Asthisaushirya is increase in porosity of bones which may lead to Bhagnaafterwards.

Asthi Dhatu Guna and Karma: Asthi is a Pitruja Avayava (paternal tissue). Guru (heavy), Khara (rough)[26] Kathina (hard), Sthula (bulkiness),Sthira (stable) and Murtimandare the Gunas of Asthi Dhatu. Its function is Dehadharana (supports the body and gives perfect shape to the body), Majjapushti (acts as reservoir of Majjadhatu) and it is the seat of vata.[8]

The etiological factors for Asthikshaya: The etiological Factors of Asthikshaya are not explained separately in the text. On the basis of Ayurvedic principle of Ashrayashrayee Bhava,The increase or decrease of Asthi and Vata are inversely proportional to each other. Hence the factors vitiating Vata will cause decrease in Asthi Dhatu. Acharya Charakhad explained Samanya Nidana (general etiological factors) leading to the Kshaya of 18 types which includes mostly the Vataprakopak Nidana.[9] The factors provocating Vata are excessive exercise, intake of dry vegetables, irregular dietary habits which includes excessive fasting, dieting and limited foods, excess of food also, excess of worry, grief, fear, hunger, waking at nights, letting out excess of blood, Dosha, Dhatu Mala and time factor (Adanakala and Vridhavastha).

Majjadhatu is the next to Asthi Dhatu which is present inside the Asthi Dhatu and very closely related to each other. Hence the factors responsible for the vitiation of Asthivaha and Majjavaha Srotas are also responsible for Asthikshaya. The vitiating factor of Majjavaha Srotassuch as intake of Abhishyandi and incompetentdiet vitiatesVata due to Margavarodha (obstruction).[10] Vitiation ofAsthivaha Srotas directly leads to aggravation of Vata,resulting in Asthikshaya.[11]Acharya Dalhana described the Asthidhara Kalaas Purishdhara Kala.[12]So the causative factors responsible for vitiation of Purishvaha Srotas are also responsible for Asthikshaya. It includes suppression of urge for stool, consumption of large quantity of food, eating during indigestion, eating before digestion of previous meal, person having weakAgni and emaciation.[13]

Symptoms of Asthikshaya according to different Samhitas[14-19]:

Symptoms	Charaka	Sushruta	Ashtang Sangraha	Ashtang Hrudaya	Bhavaprakash	Harita Samhita
Kesha vikara	+	-	+	+	-	-
Loma vikara	+	-	+	+	-	-
Nakhavikara	+	+	+	+	+	-
Smashruvikara	+	-	-	+	-	-
Dantavikara	+	+	+	+	+	-
Shrama	+	-	-	-	-	-
Asthi Toda	-	-	+	+	-	-
Ruja	-	-	-	-	-	+
Asthi Shula	-	+	-	-	+	-

Symptoms	Charaka	Sushruta	Ashtang Sangraha	Ashtang Hrudaya	Bhavaprakash	Harita Samhita
Ruja	-	-	-	-	-	+
Sandhishaithilya	+	-	+	-	-	-
Rukshata	-	+	+	-	+	-
Parushya	-	-	+	-	-	-
AsthiBaddha Mamsabhilasha	-	-	+	-	-	-
Angabhanga	-	-	-	-	-	+
Atimandacheshta	-	-	-	-	-	+
Medakshaya	+	-	-	-	-	+
Viryasyamandya	-	-	-	-	-	+
Vikampana	-	-	-	-	-	+
Vamana	-	-	-	-	-	+
Visangnata	-	-	-	-	-	+
Shosha	-	-	-	-	-	+
Kathorata	-	-	-	-	-	+
Shopha	-	-	-	-	-	+

Samprapti: Samprapti of Asthikshaya is not explained in Ayurvedic texts. Vitiation of Vatais the main factor in Samprapti of Asthikshaya. Vitiation of Vata occurs by two ways; Dhatukṣaya and Margarodha.[20] Therefore the Samprapti can be explained by two ways, one is Dhatukṣhayajanyaand another is Margavarodhajanya. *Apatarpan* i.e. taking Vataprakopak Ahara (diet) and *Vihara* (living habits) leads to vitiation of Vata due to decrease in Dhatus *and the Srotasbecome Rikta (devoid of unctuousness)*. Vitiated Vata fills the Rikta Srotas. The walls of Srotas got stretched by the force of Vata leading to weaken them and vitiated Vataoverpower the weak Srotas to create disease.

Osteoporosis: The remodelling of bone is a continuous process throughout the life, for many years after closure of the epiphyses, skeletal mass remains constant and the rates of bone formation and resorption are approximately equal. In Osteoporosis the rate of bone resorption accelerates that of bone formation.[21]

Women are more prone to Osteoporosis than men. The bone loss starts from the age of 30–40 years in both men and women. In women, menopause is followed by an immediate decrease in bone mass and density within a year. This increased rate of bone loss reaches steadiness approximately 10 years after menopause and then merges into a continuous age-related loss.[22] Thus due to age-related bone loss in addition to menopausal bone loss women suffer more from Osteoporosis.

Changes in calcium metabolism occur with the age. As age advances, there is decrease in absorption of calcium from gut and increase in calcium loss by kidney and skin. It has been reported that with aging and after menopause, fractional calcium absorption decreases on average by 0.21 percent per year after 40 years of age.[23] Osteoporotic fracture can turn out to be life threatening; 1 in 5 persons die during the first year after a hip fracture, whereas nearly one third need nursing home placement after hospital discharge, and fewer than one third recover their prefracture level of physical function.[24,25]

Classification of Osteoporosis.[26]: Primary Osteoporosis & Secondary Osteoporosis Symptoms[27]: Fracture after minimal trauma, Joint pain, Low back pain, Neck pain, Stiffness of joints, Bony tenderness, Deformity of joint, Deformity of spine (Kyphosis).

Diagnosis as per Modern Medicine

Bone Densitometry: It is indicated in 1) premenopausal women with long term steroid therapy, Primary or secondary amenorrhea, post oophorectomy,

organ transplantation 2) post-menopausal women who are not on estrogen therapy, vertebral or hip fractures and 3) men with unexplained fractures, hypogonadism and history of recurrent falls.[28]

Bone Mineral Density (W.H.O. criteria for Osteoporosis)[29]: Bone density measurements of healthy young adults in a population are taken as the reference measurement (called a T-score). Osteoporosis is diagnosed when a person's BMD is equal to or more than 2.5 standard deviation below this reference measurement. Osteopenia is diagnosed when the measurement is between 1 and 2.5 standard deviation below the young adult reference measurement.

As per this criteria, T- score value determines the bone health. It is graded as follows:

T-score

Normal	=	-1 & Above
Osteopenia	=	Between-1 to-2.5
Osteoporosis	=	less than or equal to-2.5
Severe osteoporosis	=	less than-2.5 with fracture

Prevention and Management: Old age, sedentary life style and unwholesome diet and behavior and stress are the causative factors of this disease. Old age is inevitable, so by avoiding later factors and following Ayurvedic principles and medications the disease can be managed.

Nidanparivarjana[30]: It is the first and foremost method of managing the disease. The factors responsible for aggravation of Vata i.e. VataprakopakaAhara, Vihara, stress should be avoided. Aggravating factors for Osteoporosis should be avoided e.g. Smoking, alcohol, inactivity, poordiet. Stoppingthe indulgence of factors will increase the chances of getting disease or progression of disease.

Shamana: Vitiation of Vata takes place due to both Santharpana and Apatharpana. Apatharpana directly causes Vatavridhi, while Santharpana leads to Margavarodha which in turn causes Vatavridhi. Most of the metabolic diseases occur due to disturbance of Agni. When Agni is diminished, it leads to Ajeerna and further formation of Ama takes place. Ama further deteriorates the Agni leading to blockage of channels. Therefore, management of disease should start from the level of Jatharagni and Dhatwagni. It should be corrected by administration of Deepana and Pachana Dravyas in the form of Churna or Ghrita processed with these dravyas[27] such as TrikatuChurna, Panchkola Churna,PippalyadiGhrita, ArdrakaGhrita.

Sudha Varga: For the treatment of diminished Dhatus, use of drugs which are similar to diminished Dhatus are indicated. These drugs bring about increase in that Dhatu[28] these include Pravalbhasma, Pravalpishti, Mukta shuktibhasma, Shankhabhasma, Kapardikabhasma, Kukkutandatwakbhasma.

Guggulukalpa: Lakshadi Guggulu, Abhadi Guggulu, Trayodashang Guggulu, Yograj Guggulu.

Shodhana-Basti Chikitsais the major treatment modality for Vatadosha. Pakvashaya is the place of Purishdhara Kala which resembles Asthidhara Kala where BastiDravya reaches & it is also the main seat of Vata Dosha. Hence it acts on AsthiDhatu. Thus, Basti plays an important role in strengthening the AsthiDhatu and act as preventive measures for Asthikshaya.

Vaghbhatta had mentioned Tiktaksheera Basti in the treatment of Asthikshaya. For Asthipradoshaja Vikara, Charakacharya has given the similar line of treatment which includes Panchakarma, especially Basti which contains Kshira, Ghrita and Tikta Dravya.

Discussion

In today's perspective, due to sedentary life style, faulty food habits and excessive stress metabolic diseases are occurring commonly. Asthikshaya is one among these diseases. The symptoms of Asthikshaya are similar osteoporosis in which there is decline in the bone tissue. A condition known as Osteopenia is considered as pre stage of osteoporosis. In this condition also, there is decrease in bone mineral density but it is not as notable as compared to Osteoporosis. Asthikshaya is the earlier stage characterized by different kinds of pain and deformities of Upadhatus and Malas of Asthi. As the disease progresses, as per the principles of Anulomakshaya, it will affect Majjadhatu and manifest as Asthisoushirya characterized by porosity of bone. So it is better to compare Osteopenia with Asthikshaya and Osteoporosis with Asthisoushirya.

Vitiation of VataandAsthi, Majja Dhatu are the main factors in Samprapti of Asthikshaya. The Samprapti may follow two patterns, one is Margarodha and another is Dhatukṣaya. In old age as there is predominance

of Vata Dosha and decrease in Dhatus, the disease occurs due to Dhatukṣayajanya Samprapti. When the disease occurs in obese people, in middle aged people who are taking calcium rich diet, nutritious diet, it follows Margavarodhajanya Samprapti. Menstruation is considered to be SrotasShodhana. Early menopause leads to Srotorodha, disturbs the AnulomaGati of Vata and vitiates Vata.

In Srotorodha, the obstruction can be cleared by the use of Deepana and Pachana Dravyas. It helps in improving the Jatharagni and Dhatwagni. Any symptom related to upper or lower gastrointestinal tract persisting for longer duration should not be taken frivolously. It should be corrected by use of Deepana and Pachana Dravyas. These medicines also correct the Dhatwagnimandya.

Acharya Sushruta mentioned SwayoniDravyas in the treatment of Asthikshaya. Sudha Vargiya Dravyas are Samana Dravya of the Asthi Dhatu. These drugs bring about increase in Asthi Dhatu.

Major content of Lakshadi Guggulu, Abhadi Gugguluand Trayodashang Guggulu is Guggulu. Guggluis Ushnaviryatmaka, Tridoshahar, Rasayana, Asthisandhanakar, Deepana, and Vatanashaka in properties. By its Vatashamaka Gunaand Ushnavirya, it relieves pain in Asthikshaya. When its Kalpas with dravyas such as Laksha, Baboolare used, they help in binding of broken bones and filling the empty bones with calcium and helps in deposition of calcium.

The Kshaya: Vriddhi of earlier Dhatus like Rasa, Rakta, Mamsa, Meda are measured clinically and laboratory findings, but deeper Dhatus like Asthi, Majja,Shukra are difficult to measure clinically. A person used to see the doctor for checking his blood sugar, blood pressure, lipid profile etc., but he rarely sees a doctor to know his bone health. In Osteoporosis, most of times there is a long latent phase before clinical symptoms develop. So it is important to go to the doctor if any risk factors are relevant to the person.

Ayurveda strains more on the prevention of diseases. By following proper Dincharya and Rutucharya, the disease can be prevented.

Abhyanga followed by Atapasevanshould be incorporated in daily routine for improving bone health. Dantadhavana and Tailagandusha will help to maintain dental cleanliness and health. Shiroabhyanga will prevent excessive hair fall and graying of hairs. Nasya is useful to maintain the health of all Urdhwajatrugata Vikaras.

Food articles having Madhura, Amla, Lavana rasa, Guru, Snigdha, Ushnagunas which are Vatadoshashamaka and Asthimajjaposhaka should be included more in Ahara. Intake of milk, cheese and other dairy foods, green leafy vegetables, legumes and dried fruits help to improve calcium level.

Conclusion

Osteopenia andOsteoporosis can be better correlated with Asthikshaya and Asthisoushirya respectively. Along with the factors responsible for vitiation of Vata,factors vitiating Asthivaha, Majjavahas and Purishavaha Srotas are considered as the causative factors for Asthikshaya. Samprapti of Asthikshaya occurs in two ways i.e. Dhatukshayajanya and Margavarodhajanya. In old age, many therapies are not convenient for patients, so it is better to prevent Osteoporotic changes right from early age by following Dincharya and Rutucharya. In case the disease ispresent, by making optimistic lifestyle changes and following appropriate treatment including Basti, makinguse of Swayoni Dravyas and herbomineral preparations, it can bemanaged at early stage.

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A Self-Controlled Prospective Study between Methylprednisolone and Dexamethasone Inter-Laminar Epidural Steroid Injection in Radicular Pain for Prolapse Intervertebral Disc in Lumber Vertebrae

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Abstract

Background: Previous studies have shown varying results with trans-foraminal approach epidural steroid injections performed with particulate versus non-particulate corticosteroids. The purpose of this study was to investigate the difference in pain relief and functional improvement between particulate and non-particulate lumbosacral (MIL) in patients who had undergone both injections through intra-laminar approach. Method: This was a self-controlled, prospective study of 40 patients who underwent both a methylprednisolone and a dexamethasone intra-laminar injection to the same vertebral level and side. Primary outcomes included pain relief according to the visual analogue scale (VAS) and functional improvement determined by a yes/no answer to questions regarding mobility and the activities of daily living. Post-injection data was recorded at 6, 12, and 24 weeks. Results: A decrease in VAS scores of-3.6 +/- 3,- 3.2 +/- 3.2,-3 +/- 3.1 is noted in methyl prednisolone group as compared to better VAS score projected in patients with dexamethasone group as suggested-3.9+/- 3.2,-3.1 +/- 2.9,- 2.2 +/- 3.2 at 6 weeks, 12 weeks and 24 weeks respectively. Conclusions: Findings positively supports the use of non-particulate steroids for lumbar prolapse inter-vertebral disc with radicular pain in contrast of safety use of particulate steroids for one of treatment modality in dealing with localized or radicular pain.

Keywords: *Corticosteroids; Methylprednisolone; Dexamethasone; Epidural Injections; Low Back Pain; Radiculopathy.*

Introduction

Lower back pain in considered to be the most commonest complains of patients coming to the orthopaedic OPD. Pain also impairs day to day living causing economical loss and lessen the quality of life. For lower back pain many treatment modalities have been come into implementation to over-come this problem. Epidural steroid injection has been considered as one the modality for pain management. Steroidal base in both particulate and non-particulateforms are used for pain relief. By local infiltration of steroids in epidural format shows a promising result in past. Epidural injections shows an effective treatment for radicular pain[1-6]. Particulate corticosteroidshas a larger size ranging from

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(0.5-100 μg)[7] and therefore it had lesser water solubility and they potential aggregate in crystalline hydrophilic environments. Some of these are prednisolone Acetate, methylprednisolone acetate, acetonidetobetamethasone to triamcinolone acetate. Where as non particulate corticosteroids like dexamethasone are of profound smaller in size (0.5 μg)[7] and water soluble due to their easy solubility property and hence do not aggregate. The safety use of particulate corticosteroids for epidural steroid injections is still uncertain because of many complications reported in past referral studies due to blockage of radiculomedullary arteries[8,9] causing paraperesis or quadriparesis, paraplegia or quadriplegia, stroke, and even death[10-17]. Pathophysiology behind these absurd complications is still under evaluation and thought to be due to larger size molecules and less water solubility. U.S. Food and Drug Administration (FDA) in 2011 stated in public safety the use of drug triamcinolone acetonide is hazardous causing various complications like spinal cord infarction,paraparesis/ paraplegia, quadriparasis/quadriplegia/stroke and even death in certain cases reported in association with steroid administration through epidural and intrathecal routes.

Some surgeons still the use of particulate corticosteroids for epidural steroid against cases reported by FDA complications arising. Some studies suggest amblozationtheoryand attributing complication of poor spinal needle placement [18] Inspite of above complication few studies suggested in pain relief for a longer duration with particulate corticosteroids[19-21]. The aim of study is to detect superior pain relief at follow up of 24 weeks post epidural injection with use of both types of corticosteroids

Materials and Method

It is a self control prospective study conducted in the department of Orthopaedics Datta Meghe maghe Medical College at Shalinitai Meghe Hospital and Research Centre, Nagpur in collaboration with JNMC DMIMS,Between July 2019 to April 2020. All the patients with single level PIVDwith radicular pain had under-gone intra laminar epidural steroid infiltration. Inclusion criteria for this study includes patients ages more then 18 years of age,sign and symptoms suggestive of PIVD with radicular pain confirmed on MRI scan, history of no past epidural steroid infiltration given within 6 months. Patients with any past spinal surgery were excluded from this study. All patients undergone epidural steroid infiltration randomly basis with use of either particulate or non-particulate steroid which later have been followed up at 6 weeks, 12 weeks, 24 weeks. At follow up functional data is collected from patients with the help of VAS score in view of pain assessment and simple yes/no questionnaire basis to assess the patients over all mobility and daily functional activity with use of either type of medication.

Procedure is performed by single doctor by safe triangle approach for epidural infiltration. A 12.7 cm long 22 gauge spinal needle used. After local infiltration with 4ml of 1% lidocaine hydrochloride, spinal needle placed at 4 o'clock position. A total mixture of 3 ml consisting of 2ml of 1% lidocaine mixed with either 40mg methyl predniosolone acetate or 1 ml of 10mg dexamethasone sodium phosphate is injected. Post op patients is hydrated with 2 units of ringer lactate or normal saline and later patients were kept under observation to watch out any development of complications for 24 hours post infiltration.

Results

A total of 40 patients were included in this study according to the inclusion criteria. All patients had come to orthopedic opd with complains of lower back pain with lower limb radiculopathy. All cases were diagnosed on MRI were central and paracentral disc with foraminal stenosis. On basis of stenosis patients were divided in mild (4/40), moderate (16/40), severe (20/40) groups. Patients with age group ranging from 63 +/- 16 years (methyl prednisolone group) versus 65 +/- 16 years (dexamethasone group) is included. Out of 40 patients, 28 were males and 12 were females with an average duration of symptoms of ranging from 5 years +/- 1. Pre injection VAS noted for both groups with mean values of 8.2 +/- 0.8 for methyl prednisolone group in comparison to 7.9 +/- 1.1 for dexamethasone group. Post injection opioids with was given for 3 days for extended pain relief.

The presentation of pre epidural and post epidural mean scores according the VAS score. The slop of the particulate steroid at 12 week was lower as compared to non-particulate steroid, but at 24 week both steroid show almost similar VAS score mean value.

Results shows changes in mean VAS score for both the groups at different intervals of follow up. A decrease of-3.6 +/- 3,- 3.2 +/- 3.2,-3 +/- 3.1 is noted in methyl prednisolone group as compared to better VAS score in patients with dexamethasone group as suggested-3.9+/-

3.2,-3.1 +/- 2.9,- 2.2 +/- 3.2 at 6 weeks, 12 weeks and 24 weeks respectively. As suggested by the figures calculated the mean VAS scores is nearly equivalent in both group types of medication at any given point of time of follow up. Both the group shows improved functions at final follow up as compared to pre injection state.

Discussion

Aim of study to differentiate in terms of pain relief and function outcome by using corticosteroids i.e. particulate and non-particulate by using epidural steroid infiltration by intra laminar route. The primary outcome of our study show similar result in term of pain relief and functional betterment at end of 24 weeks. Few studies in past showed similar result between the two group in literature. One of the study conducted by Dennis et al[22] concluded in his double blind randomized control trail(RCT) that there is no significant difference in functional outcome when patient treated with 7.5 mg of dexamethasone and 6 mg of betamethasone. Study showed significant change in VAS score and showed the promising result on basis of Oswestry disability index (ODI) showing statistically improvement by patient treated with dexamethasone group at 6 months follow.

Kennedy et. Al[23] also conducted a double blind RCT in 78 patient grouped in two treated with 15 mg of dexamethasone and 60mg of traimcinolone acetonide given by trans-foraminal route suggested no significant difference in terms of VAS score which showed clinical improvement in pain relief at the end of 6 months. Park et al[24] also conducted an RCT in 106 patients treated with either 40mg traimcinolone acetonide or 7.5mg dexamethasone showed clinical improvement on VAS score and McGill pain questionnaire as well as functional improvement as per ODI scoring. Study showed better results in particulate group as compared to non particulate group in accordance to VAS score at final follow up.

El-Yahchouchi et al[25] conducted a retrospective study in 2,634 patients who has been admitted with radicular pain and divided these patients into three groups treated with trans-foraminal epidural injection of 10mg dexamethasone, 80mg traimcinoloneacetonide and 12 mg betamethasone respectively. When patients follow up conducted showed a better pain relief and functional improvement by Numerical Rating scale (NRS) and Roland Morris disability questionnaire (R-M) in group treated with dexamethasone.

As our study conducted to compare the effectiveness of particulate and non particulate epidural steroid infiltration through inter laminar route shows a promising result in pain relief for radiculopathy. With few study in past literature have better functional outcome and clinical improvement from pain when treated with trans-foraminal epidural infiltration. Though the result of studies may show a difference in pain relief or duration of relief at any follow up it is important to note a variedclinical improvement in daily living and activity in subsequent follow ups post injection when compared to pre injection state. In this study statistically we noted there is no major significant difference in pain relief at final follow up done at 6 months but dexamethasone group showed a upper hand in relieving patient from pain when compared mean values of VAS scores and functionally assessing clinically showed a better daily living activity. But while considering safety of non – particulate steroid infiltration noted in literature, our study strongly suggest the use of non particulate steroid for treating radicular pain for upto 6 months and further follow up is needed to certain the final outcome of use of this treatment modality.

Few limitations of this study is small sample size and increased rise of selection bias is inherited. And also use of any statistical analysis is not taken into account for assessing functional outcome but considering any modality for this purpose would not solve any change in result even after enrolling a larger sample size. This study demonstrate a good efficacy with use of 10mg dexamethasone injected for relief of radicular pain and shows a good functional result in pleasuring daily living activity at 24 weeks of follow up. These findings positively supports the use of non-particulate steroids for cervico- lumbar prolapse inter-vertebral disc with radicular pain in contrast of safety use of particulate steroids for one of treatment modality in dealing with localized or radicular pain.

Conclusions

Findings positively supports the use of non-particulate steroids for lumbar prolapse inter-vertebral disc with radicular pain in contrast of safety use of particulate steroids for one of treatment modality in dealing with localized or radicular pain.

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Conflict of Interest: Nil.

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A Rare Case Report of Isolated Tuberculous Caecal Perforation Presented as Acute Appendicitis

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Abstract

Background: Koch's abdomen is a common problem in India, with significant morbidity and mortality. A tuberculous caecal perforation without the involvement of adjacent ileum and ascending colon is very rare.

Case Presentation: We report the case of a 23 years old man with clinical features of acute appendicitis. On laparotomy showed a caecal perforation with normal ileum, ascending colon and appendix. A caecal resection with end to end ileoascending anastomosis done. Histopathology confirms tuberculous granulomas of the caecum.

Conclusions: We report this extremely uncommon case of caecal tuberculosis. This is very important as the incidence of tuberculosis is increasing in developing countries as well as developed.

Keywords: *Caecal resection, ileoascending anastomosis, intestinal tuberculosis*

Introduction

Perforation peritonitis is a quite common surgical emergency. Colonic perforations are rare, caecum being rarer. Caecal perforation is uncommon in adulthood. It may be secondary to several disorders like distal colonic malignancy, diverticulitis, and inflammatory bowel disease[1]. We report a 23-year young adult man presented with acute appendicitis on clinical examination and ultrasound but have caecal perforation without appendicitis. Intestinal tuberculosis is still endemic in India. Any part of the intestine may be affected. However, a primary caecal perforation due to tuberculosis is rare, with normal ileum and ascending colon[2].

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Case report: A 23-year-old male patient presented with a chief complaint of right lower abdominal pain from 3 day's duration. He was having a fever of 2 days. On examination, he had fever and tachycardia. There was localized tenderness and positive Blumberg sign in the right iliac fossa. The bowel sounds were absent in the right iliac fossa. The total leucocyte count was raised (13000/Cu mm). No e/o free air under the diaphragm on the chest X-ray. Ultrasound of the abdomen was normal except marked probe tenderness in the right iliac fossa.

A preoperative diagnosis of acute appendicitis was done, and the patient was taken up for emergency laparotomy. A gridiron incision was made to approach the caecum. On inspection, there was caecal perforation with the formation of a localized abscess. An appendix was normal macroscopically. Perforation was of size 4cm by 3 cm with marked mesenteric lymphadenopathy(see figure 1). Ileocecal resection along with appendix with a hand-sewed end to end ileo-ascending anastomosis was performed. The largest mesenteric lymph node was sent along with a resected specimen for histopathological examination.

The patient's postoperative course was uneventful.

Histopathological examination reveals tuberculous granulomatous lesion of the caecum. (see figure 2)



Figure 1. Shows large caecal perforation with normal appendix and ileum

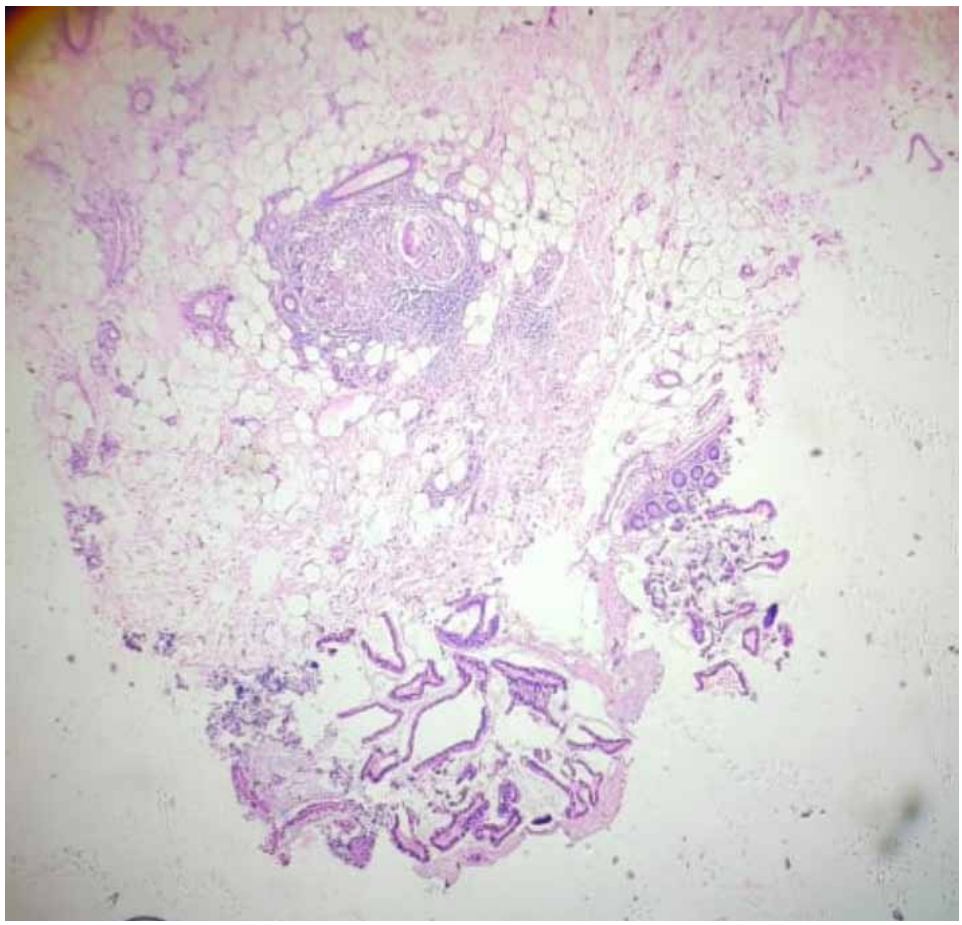


Figure 2: Shows a tuberculous granulomatous lesion of the caecum.

In a view of the histopathology report, his chest x-ray and the Mantoux test was negative for tuberculosis. Erythrocyte sedimentation rate (ESR) was 20 mm at end of 1 hour. Anti-tuberculosis chemotherapy was prescribed for six months. (isoniazid, rifampicin, pyrazinamide, and ethambutol for two months, and isoniazid and rifampicin for the remaining 4 months), with good response and 8 kilograms of weight gain.

Discussion

Isolated tubercular caecal perforation, is a very rare disorder[1]. Even caecal perforation of any etiology is very rare and as per Wong and Naqvi et al (2011)[2], he quoted only nine case reports. One more case was reported by Sharma et al[1] in 2018 and hence only 11 cases published to date including a case report of Wong and Naqvi et al (see Table 1). The most common surgery performed was right hemicolectomy for caecal perforation. The simple closure is having less morbidity. But there is no sufficient evidence is available to support this[3].

Table 1: Past case reports of caecal perforation with management

Sr.No.	Past case reports	Surgical management	References
1	Multiple (two) perforations of caecum	a limited resection of the diseased caecal wall with primary closure was done along with a diverting loop ileostomy	Sharma et al 2018[1]
2	Appendicular base perforation	Omental patch repair	Wong and Naqvi:2011[2]
3	7 case reports of caecal diverticular perforation by Papapolychroniadis C. et al (2004) Ghoneim et al (1995), Dorfman et al (1990) Mosca et al (1997). Vitali et al (1998) Cole et al (2009), Mauvais et al (1999)	Right hemicolectomy was done in most of the cases	Wong and Naqvi:2011[2]
5	Perforation of the cecum with cesarean section. Wesch et al (1980)	The perforation was oversewn	Wong and Naqvi:2011[2]
6	Caecal perforation secondary to tuberculosis	A right hemicolectomy with an ileostomy	Jain et al (2010)[3]

As per from above table Jain et al (2010)[3] report a case of primary tubercular caecal perforation similar to the present case.

Calista. Harbaugh et al[4] present a case of a 16-year-old boy diagnosed with acute uncomplicated appendicitis on ultrasound, had idiopathic cecal perforation with a normal appendix on histopathology. Presentation-wise is similar to the present case but not due to tuberculosis[33].

Hakan Guven et al[5] found in his series of 48 patients (without caecal perforation) of right hemicolectomy (32) and Ileocecal resection (16), only six cases of tuberculous granuloma of ileocaecal part of the intestine but not associated with perforation. The present case was having similar histopathology of tuberculous granuloma but with caecal perforation.

Yatndra kashid[6] report a case of acute appendicitis. At operation found to have multiple perforations in the caecum secondary to ileocaecal tuberculosis for which right hemicolectomy was performed. This case is similar to the present case except for ileum is also affected by tuberculosis.

Amad N. Khan et al[7] also present a case of caecal perforation due to tuberculosis of caecum but it was along with tuberculosis of the lung. Similar to the present case but it was not having pulmonary tuberculosis. A few other studies reflected on different aspects of TB and abdominal surgeries[8-13].

Conclusion

Isolated tuberculous caecal perforation is a very rare disease process. Hence for diagnosis of it requires a high index of suspicion. Surgical intervention with appropriate anti-tubercular chemotherapy is the definitive management.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Rare Presentation of Systemic Lupus Erythematosus with Juvenile Dermatomyositis

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Abstract

The systemic lupus erythematosus (SLE) is a rare type of autoimmune disease, which is associated with the involvement of multiple systems. It is characterized by the production of autoantibodies. Usually, it has a relapsing and remitting course. Juvenile dermatomyositis is the most prevalent chronic inflammatory muscle disease among children and adolescents with SLE. This disease predominantly involves the skin and skeletal system. Its most common symptoms include distinctive skin rashes and inflamed muscles. The insufficient disposal of apoptotic cells may result in the stimulation of T cells and B cells by the antigens in the patients with SLE. At the surface of the dying cell, fragments of cellular material develop during the cycle of cell death. We presented the case of a 15-year-old female, who complained of erythema and generalized rash present on the face, markedly present over her cheek, bridge of the nose and the forehead since 7 months. She developed erythematous, scaly and crusted lesions on the scalp, back, trunk and upper limbs along with the vesiculobullous lesion of the oral mucosa. She also developed a progressive generalized muscle weakness for the past 3 months. To a large extent, intravenous glucocorticoids are helpful, but standardization of tests and treatment schemes are required to enhance the awareness of this rare case.

Keywords: *SLE, lupus erythematosus, juvenile dermatomyositis.*

Introduction

Lupus is a chronic autoimmune inflammatory condition, presenting with a vast range of clinical presentations, which arises due to the involvement of multiple systems[1,2]. The systemic lupus erythematosus (SLE) is the prevalent form of lupus, which is usually referred to as "lupus," but it is distinguished from other types of lupus due to its effects on the multiorgan systems. It is a chronic autoimmune disease, which has clinical and serological heterogeneity[2,3]. SLE is a rare autoimmune disease that involves multiple organ systems, characterized by inflammation and production of antibodies to nuclear and cytoplasmic antigens. It has a relapsing and remitting course[4]. The prevalence of SLE ranges from 0.03% in Caucasians to 0.2% in Afro Carribeans. Its incidence in females is 90% or more, and the most common age is 20-30 years[5]. SLE's most important feature is the development of auto-antibody, which is specific to a wide variety of targets but often attacks the antigens present inside the cell or nucleus [4]. SLE accompanies a sustained production of immune complexes and autoantibodies. The antigens cause the release of cytokines, vasoactive peptides, chemokines, proteolytic enzymes and oxidants due to the activation of complement. This causes activation and influx of multiple tissue cells into target tissues of T and B cells, monocytes or macrophages, and dendritic cells[4]. It is believed that SLE may arise as a result of programmed

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cell death or inequitable clearance of these cells. It corresponds to inherited mutations in complements C1q, C2 and C4.

Juvenile dermatomyositis is the most common chronic inflammatory muscle disease in children and adolescents with SLE. It predominantly involves the skin and skeletal system[4,6]. Distinctive skin rashes, as well as inflamed muscles, are the most common symptoms. It can be monocyclic or polycyclic, with periods of remission and relapse. The findings include proximal muscle weakness and violaceous discolouration of eyelids, also called as heliotropic rashes [3)]. 10-20% of cases can be ulcerative, while 30% of cases can progress to calcinosis later on. In many cases, intravenous methylprednisolone and methotrexate can induce a rapid response. Cyclophosphamide can be used for the treatment of Lesional ulcerations. Cutaneous manifestations are mostly attributed to juvenile cases than adults. SLE commonly involves the skin, and many skin eruptions can be due to exposure to UV light.

The main types include the following[2,4]:

- The classical facial rash is elevated, erythematous and painful, and appears on the cheeks. It can be itchy. The nasolabial folds are usually spared.
- The discoid rash is hyperkeratosis with alopecia which is of scarring type especially on the scalp.
- Non-scarring alopecia
- Urticarial eruptions
- Livedo reticularis

Case report: A 15-years-old female born of secondary consanguineous marriage presented with the complaints of erythema and generalized rash on the face, markedly over her cheek, bridge of the nose and forehead for the past 7 months. She developed vesiculobullous lesion of the oral mucosa and erythematous, scaly and crusted lesions on the scalp, upper limbs and back, which healed with hypopigmentation. Similar lesions were found on the trunk and the extensor surface of the upper limbs. She also complained of painful oral ulcers, which resulted in significant difficulty in eating.

She also presented with progressive generalized muscle weakness for the past 3 months and complained of the inability to perform household chores. She had difficulty getting up from the bed and lifting the arm while changing clothes. She complained of easy fatiguability and tightening of limb, with more involvement of the lower limb in comparison to the upper limb.

She later developed low-grade intermittent fever associated with joint pain, particularly in the large joints like knee joints and hip joints. She complained of a reduction in vision in the eyes, with more involvement of the left eye, which gradually progressed. She also complained of dryness in the eyes. She complained of alopecia for the past 5 months, more on the temporoparietal regions, presenting in patches with sustained frontal margin. She showed a decrease in appetite and weight loss for the past 7 months. She complained of irregular menstrual bleeding with the decreased flow, which lasted for 1-2 days over a gap of 35-40 days. It was not associated with pain or blood clots.

On general examination, the patient was thin-built and malnourished. On cutaneous examination, there was a diffused loss of hair with patchy alopecia at some areas along with few hyperpigmented scaly lesions, erythematous rashes over the face including both cheeks, bridge of nose and forehead, and scaling and crusting accompanying the lesions. Local examination revealed oral ulcers in the buccal mucosa. Systemic examinations were found normal.

Laboratory investigations were performed. Complete blood count (CBC) revealed Haemoglobin (Hb) = 8.9 g/dl, White blood cells (WBC) = 2600 cells/ mm3 (Monocyte = 4, Granulocyte = 34, Lymphocyte = 60, Eosinophil = 02, Basophil = 00), platelets = 75,000/mm3, erythrocyte sedimentation rate (ESR) = 70 mm/hr, C Reactive Protein (CRP) = Positive, Serum creatinine = 0.73, direct Coomb's test = positive and serum creatine kinase = 400 unit/litre. The CBC showed a raised erythrocyte sedimentation rate (ESR), which suggested of thrombocytopenia, thereby pointing towards an infectious cause. Liver function tests (LFT), renal function tests (RFT) and Urine routine were found within the normal range. Specific tests were carried out, and anti-*double stranded DNA* (anti-*dsDNA*) was found positive (4.16) and anti- antinuclear antibody (anti-ANA) was positive (102.44). The ophthalmologic test was suggestive of anterior subcapsular cataract of the left eye. An autoimmune etiology was suspected as per the pattern of rashes and cachexia in the adolescent female. Further investigations were performed, and the skin biopsy reported of scleroderma. USG pelvis revealed hypoplastic uterus.

According to Bohan and Peter classification, this patient fit in the criteria of Juvenile Dermatomyositis. According to the American College of Rheumatology (ACR) criteria of SLE, the diagnosis was confirmed. The diagnosis was 'SLE with juvenile dermatomyositis'.

Discussion

SLE is a chronic disorder involving multiple organ systems, predominantly due to the development and deposition of immune complexes and autoantibodies, resulting in subsequent impairment in the organs[1,7].

The antigens, which cause the stimulation of T cells and B cells in patients with SLE may be due to the insufficient disposal of apoptotic cells[4,5]. The fragments of cellular material develop at the surface of the dying cell during the cycle of cell death. A higher concordance is seen in the monozygotic twins, and the disease is associated with polymorphic variants at the HLA locus. In a few instances, SLE is linked with inherited mutations. [8]. Genome-wide association studies have identified polymorphisms near other genes, which can lead to SLE; these genes are involved in regulating immune cell function.[9] The fact that environmental factors, which can cause flares of lupus such as UV light and infections, can increase the oxidative stress and cell damage supports the apoptotic theory.[4] Autoantibodies are prevalent in Juvenile Dermatomyositis. They can be categorised into two groups, as follows: myositis specific antibodies (MSA), which target the synthesis of proteins, cytoplasmic or nuclear components, and myositis-associated antibodies (MAA), which are usually present in many autoimmune disorders and syndromes that overlap. Approximately 70 per cent of children with Juvenile Dermatomyositis have one or more detectable MSA or MAA[11]. Balwani et-al reported progressive renal failure in Lupus patient.[12]

Conclusion

SLE with juvenile dermatomyositis is a rare disorder. Since the international consensus regarding diagnosis and treatment is currently unavailable, the management is thus variable. So, precise recommendations would help physicians in the proper management of patients with juvenile dermatomyositis. Specific recommendations at present involve examination of muscle disease, skin disease, calcinosis, biomarkers, and autoantibodies. Management with intravenous glucocorticoids is helpful to some extent. It is suggested that the standardization of tests and treatment plans with collaborative research studies would acknowledge the profundity of rare disorders like this.



Erythematous, scaly and crusted lesions on the scalp, upper limbs and back, which healed with hypopigmentation

Conflict of Interest: There is no conflict of interest among the authors.

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Ethical Approval: Necessary approval was taken from the Institution and the patients for carrying out this work.

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A Prospective Study of Etiology and Clinical Presentation of Gastric Adenocarcinoma

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Abstract

Background: Dietary factors, Smoking, Alcohol, H. Pylori all have been linked to gastric carcinoma and most of them are modifiable which can reduce the risk of cancer hence this study was undertaken to study the etiology of gastric adenocarcinoma and its clinical presentation.

Methodology: This was a prospective study in which 50 patients of gastric adenocarcinoma admitted in the surgery department at a tertiary hospital were studied.

Results: Out of 50 patients studied, 36 were males and 14 were females. The risk factors like H. pylori biopsy, heredity, salt and preserved food were found less in the cancer patients while alcohol was found to be more in them. Smoking was present equally in both the groups. Vomiting (100%) and loss of weight (100%) were the most common presenting feature. 46% had a Karnofsky score more than 80.

Conclusion: Alcohol is a risk factor for gastric adenocarcinoma and loss of weight and vomiting are common clinical presentation.

Keywords: *Gastric adenocarcinoma, etiology, clinical presentation.*

Introduction

Among all cancer related deaths, Gastric Carcinoma holds the 2nd spot. Though incidence of cancer in age less than 30 years is very rare[1, 2], It still causes nearly 900,000 new diagnosed cases and upto 700,000 deaths each year[4]. The decline in the incidence of gastric cancer is unknown as it occurred before the discovery and eradication of H. pylori. Gastric cancer incidence and mortality rates show a large variation across different countries the highest incidence rates being in Japan and China [5]. Portugal in Europe has the highest incidence rates (31.9/100,000) and gastric carcinoma is the top cause of cancer related deaths in this country [11]. Gastric carcinoma incidence is known to increase with age most commonly occurring between 60-80 years.

The pathology of gastric adenocarcinoma is a sequential process which is started by Helicobacter pylori- decisively involving bacterial virulence factors and host body's immune response along with possible involvement of other environmental factors[3]

Symptoms usually develop at a late stage, and the incidence of advanced GC (Stage IV) is 20-30% from GC in general. Median survival in Stage IV cancer is up to 9 months with no long-termsurvival5 6. For patients with PM from GC, median overall survival (mOS) is3 months,

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if patients treated with an aggressive approach (intra-peritoneal chemotherapy or crypto-reductive surgery (CRS) followed by intra-peritoneal chemotherapy) are excluded. Hence this study was conduced to study the etiology and the clinical presentation of the patients with gastric adenocarcinoma.

Methodology

This was a prospective descriptive study conducted on a total of 50 patients admitted in the Surgery department in a tertiary hospital from July 2012 to December 2013.

Inclusion Criteria: The study population includes 50 patients who underwent surgery for gastric adenocarcinoma (histopathologically proven gastric adenocarcinoma) by endoscopic biopsy at the Department of General Surgery.

Exclusion Criteria:

1. Patients with gastro intestinal stromal tumor or Gastric Lymphoma or Gastric carcinoid's tumors are excluded from the study.
2. Patient only received palliative chemo radiotherapy without going any forms of surgery are excluded from study.
3. Patient who is unfit for surgery excluded from study.

All patients underwent a detailed medical history and clinical examination.

Clinical examination comprises of General examination, systemic examination and Digital rectal examination. Diagnosis of patients based on UGI Endoscopy, biopsy, CT scan, PET scan and assessment for H. Pylori. Staging based on TNM Classification system according to the AJCC Staging. From July 2012 to Dec 2013 data entered prospectively in to a methodology specifically designed for the study.

Statistical analysis: The categorical data were represented by frequency with percentage and chi-square and Fisher exact test were used for analysis. The continuous data represented by mean (SD) for normal data and Median (Range) for abnormal data was analyzed by using Independent t-test and Mann- Whitney U test. SPSS version 16 was used for analysis and P value less than 0.05 were considered as statistically significant.

Results

A total of 50 patients who underwent surgery for histologically proven gastric adenocarcinoma were studied out of which 36 were males and 14 were females. The risk factors like H. pylori biopsy, heredity, salt and preserved food were found less in the cancer patients while alcohol was found to be more i.e. 28 as compared to 22, in them. Smoking was present equally in both the groups. (table 1 and figure 1).

The most common clinical features were vomiting (100%) and loss of weight (100%) while 12% patients had melena and 10% patients had dysphagia. 46% patients presented in early stage. (table 2 and figure 2).

The Karnofsky Performance Status was calculated for all patients and it was found that a majority of the patients i.e. 46% had a score more than 80. (table 3 and figure 3).

Table 1 showing distribution of Risk factors in the study population

Risk Factor	Present	Absent
Helicobacter Pylori Biopsy	23	27
Heridity	13	37
Salt and preserved food	13	37
Alcohol	28	22
Smoking	25	25

Table 2 showing clinical features in study population:

Clinical feature	Number	Percentage
Vomiting	50	100%
Loss of weight	50	100%
Dysphagia	5	10%
Lump in epigastric region	0	0%
Hematemesis	0	0%
Melena	6	12%
Early stage	23	46%

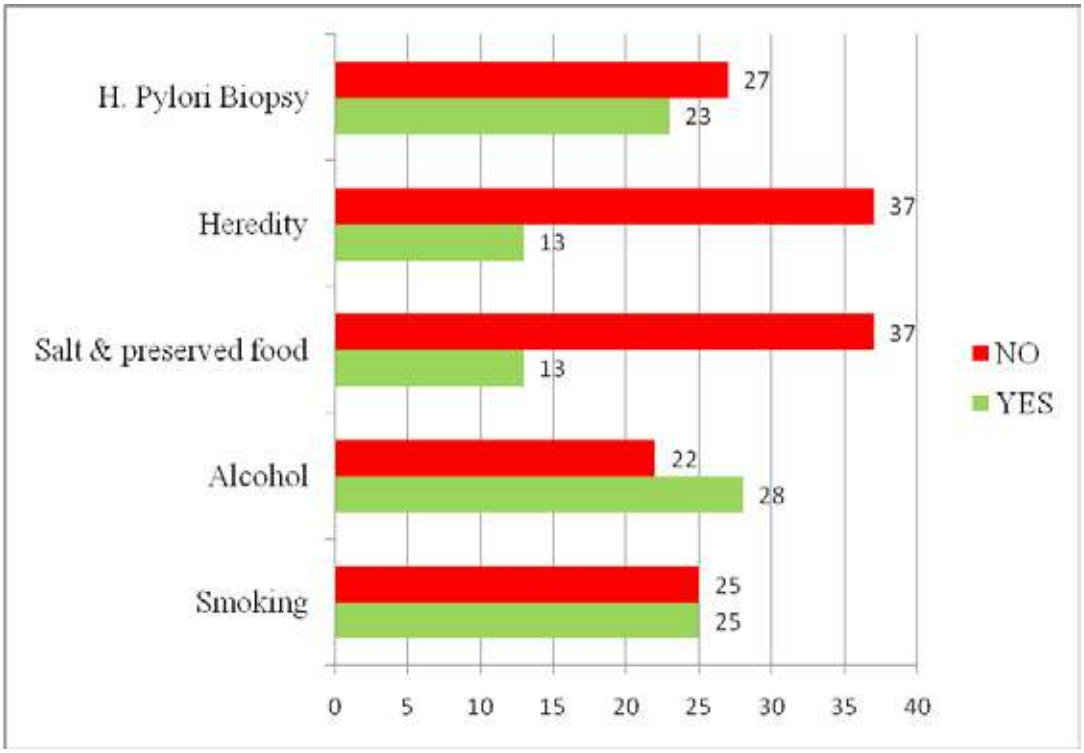


Figure 1 showing distribution of risk factors in the study population:

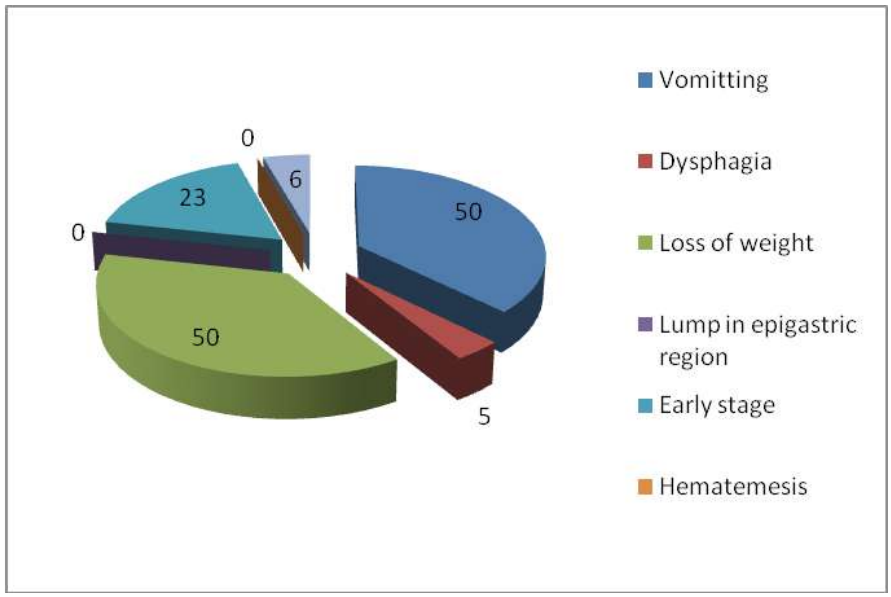


Figure 2 showing clinical feature of gastric carcinoma in the study population:

Table 3 showing Karnofsky Performance Status of the study population:

Karnofsky performance status	Number	Percentage
<40	8	16%
40-80	19	38%
>80	23	46%

Discussion

A total of 50 patients of gastric adenocarcinoma were studied out of which 28 patients showed alcohol as more common risk factor while helicobacter pylori on biopsy, diet rich in higher salts and heredity were found to be less commonly present.

Becker K[17] found that heredity plays an important role in gastric cancer. There is an increased risk of developing lobular breast cancer among women in those families who have a germ line mutation of CDH1 and hence should be screened accordingly. The family members of young patients with the diffuse-type of gastric cancer are advised to undergo Genetic screening.

Adachi Y[6] in their study found that the most important cause of distal gastric adenocarcinoma isChronic H. pylori infection as It commonly causes chronic gastritis and may induce mucosal atrophy after few decades causing possibility of development of cancer[7-10]. An important role in the severity of gastritis and intestinal metaplasia is played by bacterial virulence factors, such as CagA of the so-called pathogenicity island and Vac A (vacuolating enzyme). [11-12]

Causes of gastric carcinoma incudediet as a major risk factor which by providing a number of elements and vitamins reduce the formation of carcinogens, but also at the same time acts as the source of well-established carcinogenic molecules or precursors of them. Diet high in fruits and vegetables may protect against gastric carcinoma while diet rich in salted foods, processed foods could increase the risk of this malignancy[15-16]. In our study however it was not found.

Smoking too is an important risk factor and byincreasing the role of H. pylori infection by consumption of high salted or preserved foods and smoking, gastric carcinoma risk is increased[13,14]. In our study smoking as a risk factor was distributed exactly equally in both groups.

Alcohol is an important risk factor and it increases risk of gastric carcinoma by 20% per 50g+ (6+ units) per day of alcohol consumed. Though in meta-analysis there was a lack of association found between moderate alcohol drinking and gastric cancer risk we found an increased association with heavy alcohol drinkingin our study.

Symptoms usually develop at a late stage and include vomiting, loss of weight, hematemesis, melena, lump in the abdomen etc and the incidence of advanced GC (Stage IV) is 20-30% from GC in general. Median survival in Stage IV cancer is up to 9 months with no long-termsurvival 5 6. For patients with PM from GC, median overall survival (mOS) is 3 months, if patients treated with an aggressive approach (intra-peritoneal chemotherapy or crypto-reductive surgery (CRS) followed by intra-peritoneal chemotherapy) are excluded. In our study almost 100% patients had vomiting and loss of weight while a remarkable 46% presented in early stage. A majority of patients had a Karnofsky score >80 which means that they could do normal activity with effort and had some symptom of the disease which shows that majority of our cases presented early[22].

.Conclusion: Alcohol was more commonly found as a risk factor for gastric adenocarcimoma, vomiting and loss of weight were most common clinical features and majority of cases presented early hence the Karnofsky score was above 80.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Study of Functional Outcome of Calcaneal Fractures with Percutaneous Cancellous Screws Fixation in Central Rural India

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Abstract

Background: Calcaneum fractures are nearly 2% of all adult fractures. Calcaneum is the main weight bearing bone in the foot. Mostly these fractures are caused by high energy trauma/fall from height leading to various patterns of fractures. There are various method of treating such fractures but due to high rate of complications by open reduction and internal fixation hence middle path regime shall be discovered for treating most important weight bearing bone. Inevitable malunion and arthritis due to conservative management can be avoided by percutaneous fixation.

Material and Method: A prospective retrospective review of 12 patients with 16 calcaneal fractures who had percutaneous reduction and screw fixation between January 2019 to April 2020 was done at at Shalinitai Meghe Hospital and Research center Nagpur. These cases were managed with 3-4 percutaneous cancellous screws.

Results: The results were assessed with Calcaneal fracture scoring system and Maryland foot scoring system and showed good to excellent functional results of complex intra-articular calcaneal fractures.

Conclusion: Percutaneous method of fixation with Cancellous screws is a far superior modality of treatment of calcaneal fractures in order to get better functional outcome with minimal complications.

Keywords: *CALCANEAL FRACTURES, traumatology, joystick.*

Introduction

Calcaneum is the main weight bearing bone in the foot. It bears and transmits 90% of the weight of body in different phases of gait cycle of human being. So, it plays very important part of lower limb function. So the fractures of calcaneum are most important when it comes to its functional results. The 3rd and 4th decade males (younger age group) are more affected due to their job profile[1] of all tarsal fractures 60% fractures are calcaneum. Calcaneum fractures are nearly 2% of all adult fractures[2]. Among fractures, 75% are intra-articular, and most proposed mechanism of injury is axial loading while fall from a height or a road traffic accident. . The important association of these calcaneal fractures is peculiarly with spinal fractures of thoraco-lumbar region

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which accounts for approximately 10%[3]. The bilateral calcaneum fracture injuries are 10% and open injuries are less than 5%.. The disability with calcaneal fractures leads to severe physical as well as financial burden. So these fractures are considered as most problematic since many decades [4]. Historically, treatment of displaced calcaneal fractures has varied from nonoperative management with or without closed reduction, to open reduction with internal fixation by various surgical approaches, to primary arthrodesis[5-10] Historically, most fractures were treated non-operatively because open reduction and internal fixation were associated with high complication rates and did not result in significantly improved outcomes. It's been reported that the infection rate is very high when open reduction and internal fixation is indicated leading to chronic osteomyelitis and permanent disability[11]. However, Conservatively treated patients have difficulty in maintaining fracture reduction leading to local complications like restricted movements, broadening of heel, moderate to severe pain, peroneal impingement and subtalar arthritis causing permanent disability and impaired gait[12-20]. Therefore, joystick reduction percutaneously by k wires and followed by percutaneous fixation could be the ideal option for displaced calcaneal fracture addressing both the above issues. Thus, we present a study to evaluate the results of percutaneous reduction and fixation with cancellous screws of displaced fracture calcaneum.

Materials and Method

A prospective retrospective review of 12 patients with 16 calcaneal fractures who had percutaneous reduction and screw fixation between January 2019 to April 2020 was in Dept. of Orthopedics at Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi, Wardha in collaboration with Datta Meghe Institute of Medical Sciences, Shalinitai Meghe Hospital and Research center Nagpur. The patients were assessed with plain radiography. In developing countries like India, cost of medical treatment is often a concern, so computed tomography scan was not done as most of the patients were poor and did not afford the treatment. Standard calcaneal views were performed along with other views of the ankle and foot to find out other concominant fractures. Closed displaced calcaneal fracture were included in the study. All open calcaneal fracture and concomitant fractures were excluded from this study. The fractures were classified according to Essex-Lopresti classification system

- Essex-Lopresti 1952 (X-ray-based classification-lateral view foot)
- Extra-articular
- Intra-articular – Tongue type/Articular depression type.

As per our protocol, we operated all patients specifically in between 7–14 days, this golden period is due to subsidence of oedema around calcaneum till the wrinkle sign appeared. We have given RICE treatment for our patients. All Surgeries were performed without tourniquet under spinal anaesthesia, all patients were given injection ceftriaxone with sulbactam for antibiotic prophylaxis 20 min before starting surgery. Unilateral cases were done in lateral position and bilateral cases were done in supine position with 'figure of 4' leg. Using the image intensifier, percutaneous reduction of fracture fragments was done using 4mm k-wires, and fixation was done with 6.5 mm cancellous cannulated screws.

Post-operatively, a posterior splint given in neutral position of foot. Patients were kept non weight-bearing for minimum of 6 weeks and later ankle physiotherapy started after removal of splint. Follow up of patients were done on regular basis The patients were followed up at 6 weeks, 12 weeks and 24 weeks in the OPD. We allowed partial weight-bearing stand/walk at 6 weeks time and full weight-bearing at 12 weeks when radiological union is noticed. Functional assessment was done by calcaneal fracture scoring system by Kerr *et al*.[21] and functional outcome noted with Maryland foot scoring system[22] on questionnaire basis by questioning patients level of activity and pain control. All patients were listed in various subordinates on scoring basis of 90–100 (excellent), 80–89 (good), 65–79 (fair), and below 64 (poor).

Patient 1-28 years male
Pre op xray (lateral views) showing calcaneal fracture.

Right side-Intra-articular



Left side-Extra-articular + Intra-articular extension





Post op xray (axial views) showing Percutaneous fixation with Cancellous screws.

Right side



Left side





Results

Our study consists of patients aged between 21-65 years with mean age group of 43 years. Out of 12, 10 patients were male and 2 were female. The right calcaneum involved in 8 patients and 4 were left calcaneum with bilaterally involved in 2 patients. The mode of injury were due to fall from height. Among 12 patients,4 patients had associated spinal injuries in which 3 patients had wedge compression fracture of dorso-lumbar junction which were managed with rest, medications and LS brace and 1 patient underwent spinal fixation. All calcaneal fractures cases were listed on fracture pattern which was classified on the basis of Essex-Lopresti classification. 10 fractures were Tongue type and remaining 6 were Joint depression type. The average time of surgery was 7 days (ranges from 4–14 days). Mean follow-up was 24 weeks (range 12–36 weeks). No major complication were noted in our study. 2 patients developed superficial wound infection which was managed with regular wound care and antibiotic medications. Time to union was judged both radiologically and clinically, at an average of 12 weeks (range 10–14 weeks). The outcome assessed on 'Calcaneal fracture scoring system' is ranging from 62 to 92 with mean score of 77. The mean calcaneal fracture score was 87 (72–91) for all tongue- type fracture group and 73 (52–83) for all joint depression type group (P = 0.0034). The mean score is 73 on 'Maryland foot scoring system', with 86% of patients have achieved fair to excellent results. The mean functional score was 83 (42–91) for the tongue-type group and 61 (52–73) for the joint depression type group (P = 0.002). A significantly better functional outcome is noted in group with tongue-type fracture fixation when compared to joint depression type fracture fixation.

Discussion

In our study, 86% patients were between 21 and 65 years of age. Four patients had associated spine fracture which accounts for around 33%, which is higher when compared to other studies, but this can be explained by mode of injury being fall from height in all the 12 patients. As fall from height is also the commonest cause of wedge compression fracture in dorso-lumbar spine. Essex-Lopresti classified displaced intra-articular calcaneal fractures as either tongue type or joint depression type, based on secondary fracture line pattern passing through the posterior facet. He suggested that nondisplaced fractures be treated nonsurgically. Displaced tongue fractures are best treated by percutaneous pin fixation, using a large posterior reduction tool for closed reduction. Joint depression fractures require surgical reduction because of fracture impaction. Essex-Lopresti classification is X-ray-based classification system for calcaneal fractures, and we assess the fractures only on X-rays due to economic constraints. Furthermore, we compared the outcome of both tongue-type and articular depression-type fracture managed with percutaneous reduction and fixation. Kitaoka *et al,*[23] in his study treated conservatively 16 of 27 patients with plaster, most of these patients showed difference in gait pattern which was observed while walking specially on uneven ground. So this study states the conservative management of calcaneal fractures gives fair to poor results in most of their patients. O'Farrell *et al*[24] compared equal number of surgical and conservatively managed patients which suggested superior results in surgically treated patients. Similar study with long follow up of 3 years done by Si Leung *et al*. [25] showed superior results with surgical outcome then patients who are managed with conservative method.

The most authors suggest that the surgery is advisable after occurence of wrinkling which usally expected between 7- 10 days post injury when oedema subsidence occurs. According to study conducted by Sander[26] the results were fair to poor if surgery is executed after 2 weeks post injury and optimal outcome seen in cases which were operated in between 7 and 10 days injury26 The study by Weber *et al*[27] in which 50 cases of displaced intra-articular calcaneal fractures manged with different modalities of treatment, which showed complications like deep soft tissue infection and wound dehiscence due to loss of vascularity. Damian Griffin *et al*[28] in his study of 151 cases with calcaneal fractures showed the ate of complications and re-surgeries and wound complications were much higher after treatment of displaced intra-articular calcaneal fractures with open reduction. Similar results were also seen by authors like Essex-Lopresti and Tim Schepers *et al*. [29] who concluded that percutaneous cancellous screws fixation after joystick reduction is a good modality. Though the results were not similar in tongue-type and articular depression-type of calcaneal fractures, the percutaneous fixation shows optimal results in displaced intra-articular fractures of calcaneum. The tongue-type fractures showed much better results than articular type calcaneal fractures with Maryland foot score as well as calcaneal fracture scoring system . The joint depression

type fracture group depicted mean calcaneal fracture score of 73, whereas the mean score was 86 for the tongue-shaped type fracture group (P = 0.0034). The mean functional score was 83 for the tongue-shaped type fracture group and 61 for the joint depression type fracture group (P = 0.002).

CONCLUSION

Percutaneous cancellous screw fixation after joystick method of reduction under image intensifier in displaced closed calcaneal fractures both intra articular as well as extra articular gives good to excellent results. This method proves to be beneficial in terms of soft tissue management and functional outcome as compared to open reduction and internal fixation which showed wound dehiscence in many cases and conservative method which also showed poor functional outcome because of inevitable malunion.

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Principles of Agnikarma and its Advances for Shonitsthapana (Hemostasis)

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Abstract

Agnikarma has been one of the peculiar parasurgical procedures described in *Sushrut Samhita*. The most important indication of *Agnikarma* is when there is bleeding from vessel or diseases in which bleeding is expected. Technology and instruments used for hemostasis now a days differ from ancient ones. However fundamental principle of heating the tissues remains same which is widely used in present era. Earlier rods (*shalaka*) made from different metals were used for burning out bleeding veins. Red hot *shalaka* was applied on tissues and bleeders so that contraction of vessels occurs. So hemostasis was achieved by cauterization of tissues at high temperature. With advancement of technology in bio-medical engineering various machines like Electrocautery, Infrared coagulation, Electrosurgical unit (high frequency and low frequency) and LASERs were invented for similar purpose.

In Electrocautery electrical current is used to heat a metal wire to burn or coagulate the specific area of tissue. In Electrosurgical unit alternating current is passed through tissue and heat is created by the resistance of tissue to current. Electrodes are used to cut, coagulate or even fuse the tissue. IRC involves the use of infrared light as a heat source to coagulate vessels supplying blood. Lasers are used to ablate the veins in which amplified light energy is used to generate heat. Tissue dehydration, protein coagulation and vaporization are the processes involved in achieving hemostasis.

Working principles and energy sources used in these devices vary from each other; however the hemostasis is achieved by common fundamental principle i.e. generating heat which is similar to *Agnikarma*.

Keywords: *Agnikarma, Hemostasis, Electrocautery, Lasers, BEIM.*

Introduction

Sushrut Samhita is the core literature of *Shalyatantra* in which many principles of ancient surgery have been mentioned in *sutra* (concised) form. The concept of these *sutras* can be applied at various disease conditions and stages. *Agnikarma* has been one of the peculiar parasurgical procedures described in *Sushrut Samhita*. In which heat is used for intentional burning and therapeutic purpose.Diseases and conditions in which *Agnikarm*a can be done are mentioned in *Sushrut Sutrasthana*[1]. However *Agnikarma* now a days is mostly used only in varrious pain of musculoskeletal origin such as cervical spondylosis, tennis elbow, chronic plantar fasciatis, heel pain, sciatica, osteoarthritis of knee joint, calcaneal spur, frozen shoulder, lumbar spondylosis[2,3]. One of the important indication of *Agnikarma* mentioned in *Sushrut* Samhita is when there is bleeding from blood vessels[1] or diseases in which bleeding is expected like haemorrhoids*(Arsha).* Bleeding from vessel can be due to any type of external trauma e.g. lacerated *(Chhinna*) or punctured(*bhinna*) wound which tends to bleed more[4] or during an elective surgery.

In ancient times *Agnikarma* i.e. cauterization[5] was used in these conditions for Hemostasis (*Shonitsthapan)*[6]. In present era also this principle is used for hemostasis. But with the advancement of technology, devices and machines used now a days are different. Researches especially in field of bio-medical engineering have lead to the development of various machines like Electrocautery, Infrared coagulation, Electrosurgical unit (high frequency and low frequency) and LASERs. Application of principles of physics along with basic principle of *Agnikarma* have resulted in these inventions. Hence use of these advanced instruments for hemostasis in refference with classical agnikarm is to be studied[7].

Review of Literature: A procedure in which heat is applied or generated is called *Agnikarma.* Earlier *shalaka* made from metal was used for *Agnikarma*[8] while now electrodes, optic fibres supplied by various energy sources are used to generate heat at the point of cauterization.

In classical *Agnikarma* there is constriction of bleeding vein due to heat and thus hemostasis is achieved.[9]

Role of this principle is evident in modern era, as not a single surgical procedure is performed without use of electrosurgical unit. Claim of similarity in fundamental principles of *Agnikarma* and that of current electrocoagulation machines can be understood after knowing basic mechanism of these machines.



Fig. 1. Agnikarma Shalaka

Electrocautery: Electrocauterization is a process in which a metal probe heated by electric current and heat conduction is used to cut, coagulate and desiccate (dry) tissues and small bleeders. In this electric current is not passed through tissue but electrode heated by alternating current is directly applied on target tissue area. (Fig. 2) Electrocautery can be used in various minor surgical procedures in dermatology, ophthalmology, plastic surgery, urology etc.[10]



Fig. 2. Electrocautery

Electrosurgery: Electrosurgery uses radio frequency (RF) alternating current to heat the tissue. In this, current induced intracellular oscillation of ionized molecules results in elevation of intracellular temperature. With rise in intracellular temperature simultaneous processes of tissue desiccation (dehydration) and protein coagulation occur (coagulation). If the intracellular temperature increases rapidly, the intracellular contents undergo a liquid to gas conversion, massive volumetric expansion, and resulting explosive vaporization i.e. cutting.

Coagulation is performed with lower average power, generating slow heat insufficient for explosive vaporization, but producing a thermal coagulum instead.

There are two method of *electrosurgery*:

1. Monopolar
2. Bipolar procedures.

- In monopolar electrosurgery, a complete electrical circuit consists of-
  (a) Electrode
  (b) Patient
  (c) Return Electrode (patient plate)
  (d) Electrosurgical generator [ESU] (fig.3).

With the *monopolar* technique, the electrical current travels from an electrode through the patient until it reaches a grounding pad (return electrode) placed in proximity on another location on the patient's skin and then the energy returns to the generator. Prolonged or repeated application of current to the tissue induces heat accumulation and undesired tissue destruction.[11]



Fig. 3. Electrosurgical unit

- The bipolar technique uses an electrode in the form of a forceps (fig.4) that serves as the electrode and return electrode, whereby the electrical current flows from one tip to the other, with the desired tissue positioned between them. The main difference between the two is that monopolar uses a patient plate to direct the current, while bipolar uses opposing electrode points to do the same.[12]



Fig. 4. Bipolar Forceps



Fig. 5. Bipolar Clamp

A bipolar clamp (Fig.5) can also be used instead of a forceps which can seal the vessels up to diameter of 7mm. Currently BEIM (Fig.6) machines are making a buzz in the market. They are modified form of vessel sealer which automatically senses and deliver the energy required to seal the tissue and stops itself. So tissue charring and lateral heat spread is reduced.

There are various modes in electrosurgical unit like fulguration, desiccation, spray etc. according to need of procedure.



Fig. 6. BEIM Machine

Infrared coagulation (IRC): It is a non-surgical outpatient procedure for the treatment of hemorrhoids. IRC involves the use of infrared light as a heat source to quickly coagulate, or clot, vessels supplying blood to the hemorrhoids (Fig.7). A small probe is used to deliver a few short bursts of infrared light to the hemorrhoid. The infrared coagulation causes the enlarged hemorrhoidal tissue to shrink and recede.[13]

Laser: 'Laser' is anacronym for Light Amplification by Stimulated Emission of Radiation. A laser is a light beam that can be focused on a very small area (Fig.8). The laser heats the cells in the area being treated until they burst.[14] Laser increases the temperature of cells and it results in denaturation of proteins and collagen that leads to coagulation of tissue and it can necrotize cells. [15] The intense beam of light interacts with tissue and can be used to 'Cut, coagulate or ablate the tissue. The laser-tissue interaction depends on the type of laser i.e. the wavelength of laser. Commonly used surgical lasers are- CO2 laser, Nd : YAG laser, Diode laser, Argon laser. Depending on purpose and type of laser, various modes are used.[16]

Laser ablation is the process of removing unhealthy tissue from a solid (or occasionally liquid) surface by irradiating it with a laser beam. At low laser flux, the tissue is heated by the absorbed laser energy and evaporates. [17] Laser ablation is used in a variety of surgical specialties including ophthalmology, general surgery, neurosurgery, ENT, dentistry, oral and maxillofacial surgery.

In proctology, lasers specially Diode lasers are very much useful for In situ coagulation of hemorrhoids in which the hemorrhoid is shrunk from within and ablation of hemorrhoid in which tissue is destroyed. Fistula tract or pilonidal sinus are also ablated using lasers.[18] (Fig.9)

In esophageal varices endoscopic laser ablation is done. In varicose veins endovascular laser ablation (EVLA) is done. In which abnormal veins are heated by a laser. The heat kills the walls of the veins and the body then naturally absorbs the dead tissue and the abnormal veins are destroyed.[19]

Radio surgery: It is different from electrosurgery or electrocautery. The principle of radio surgery involves using high-frequency radio waves at 4.0 MHz, delivered at low temperature through radiofrequency microfiber electrodes; Unlike electrocautery the electrode used in radiofrequency surgery remains cold because of the use of the very high-frequency current of 4 MHz. (Fig.10)

Coagulation with radiofrequency energy occurs through the application of a high-frequency current which, when applied to tissue with sufficient current intensity, dehydrates the cells and coagulates their organic contents. This action is evidently self-limiting, because the surface coagulation helps protect the underlying tissues.[20]

Discussion

From above all information it is clear that, working principle and source of energy used in above devices are different from each other. But some sort of heat directly or indirectly is generated in tissues or veins to coagulate or ablate it. *Agnikarma* done by metal *shalaka* burnout the tissue and bleeders and also cause significant damage to surrounding stuctures by lateral heat spread. So tissues injury is more. In electrocautery, heated metal wire or electrode will be less traumatic to tissues but it can stop bleeding from small vessels only. In Electrosurgery heat generated depends upon tissues resistance which is specific and for fraction of seconds thus less violent than electrocautery. In high frequency Radiowaves and Lasers thermal injury is further minimum. With advancement of these technologies easy and sophasticated modes are available for heamostasis, coagulation or ablation.

Symptoms of ideal *Sira Dagdha* i.e. cauterization of vein are *Krushna Vranata* (blackish colouration), *Stravsannirodh* (arrest of bleeding). These are also found after achieving hemostasis on bleeding vein. However blackish discolouration is due to charring of tissues in attempt to achieve complete coagulation. Sound and smell of burning the tissue, contraction of tissues are also observed in cut-coagulation process by any of the devices, which are quite similar to symptoms of ideal *Tvak* and *Maans Dagdha.*[21] Even in endovenous laser ablation and laser haemorrhoidoplasty sound and smell of burning of tissue is observed.

Conclusion

The similarity proves, though tissue injury was more in conventional agnikarm method for achieving hemostasis, fundamental principle of ...*Dah sankochayet sira* is still evident in this era. Electrocautery, electrosurgery, radiofrequency, infrared coagulation, BEIM, Lasers etc. are new advancement in *Agnikarma* with wide range of indications and more sophasticated approach.

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Cardiovascular Risk Assessment and Profiling among Employees of Tertiary Care Rural Hospital: Central India

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Abstract

Background: Cardiovascular risk factors are the major cause of mortality and morbidity. WHO STEPS instrument is a standard tool proposed for surveillance of Non-Communicable Diseases (NCD) risk factors. The study was aimed to assess risk factors for cardiovascular diseases in the employees of tertiary care hospital using WHO STEPS instrument.

Materials and Method: WHO "STEPwise approach to surveillance of non-communicable diseases" methodology was used to conduct a cross-sectional survey among 290 employees aged 20-60 years.

Results: The mean age of the study population was 39.05 out of which men constituted 52.06%. There were the almost equal proportion of males and females with a slight male preponderance (52.06% vs. 47.93%). Family history of hypertension 22.07%, diabetes 20%, history of CVD deaths 10%, current smokers 15.52%, alcohol intake 38.28%, tobacco consumption 41.03 %, obese & overweight (≥23 kg/m^2) 44.49% were the prevalent major cardiovascular risk factors. 3.08% had a >10% risk of developing CVD in next 10 years.

Conclusion: High prevalence of cardiovascular risk factors was seen which can lead to increased morbidity and mortality. Regular health checkup camps should be conducted to combat the disease burden and create awareness.

Keyword: *Cardiovascular Risk, Hospital employee, Tertiary care.*

Introduction

Cardiovascular diseases (CVDs) are the number one cause of death globally. An estimated 17.7 million people died from CVDs in 2015, representing 31% of all global deaths. Out of this, 82% are in low- and middle-income countries.[1] According to the 2012 Global Health Observatory report for India, the age-standardized mortality rate for CVD was 306.3/100 000 population.[2] Currently India experience CVD deaths at least a decade earlier than their counterparts in countries with established market economies (EME).[3] It is predicted that by 2020, 2.6 million people will die in India due to coronary heart disease constituting 54.1 % of all CVD deaths.[3,4] With the current burden of CVD, India would lose $237 billion over the period of 10 years due to loss of productivity and spending on health care.[5]

Much of this burden is already evident due to the increased lifespan and adaptation to the adverse lifestyle in urban as well as in semi-urban population. This can be attributed to inadequate preventive health behavior

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among the Indian population. Along with this, lack of health awareness and low detection and control rates plays a major role.[6] Most of the behavioral risk factors such as poor nutrition, stress, physical inactivity, smoking, and alcohol consumption are potentially modifiable and preventable, cessation of which have been shown to reduce the risk of cardiovascular disease.[7-9]

Nowadays, health promotion and preventive health activities are increasingly being carried out in workplaces. These have dual benefits as they not only help to prevent occupational diseases but also helps in assessing and improving worker's overall health.[10,11] Such types of initiatives create conducive environments for motivating people to adopt and sustain healthy behaviour. Such initiatives for health promotion have been proposed by the World Health Organization for improving the population health. One of the components of this approach is to conduct risk factor surveillance.[12]

Tertiary care hospitals with adequate healthcare infrastructure are ideal settings for initiating these preventive and promotional activities and various studies have already been conducted so far in employees of urban setups.[6,13,14] On the similar ground we planned this study to assessed risk factors for cardiovascular diseases amongst the employees of rural tertiary care hospitals using the WHO STEPS approach (STEPs 1, 2 and 3) to understand the prevalence of risk factors and risk factor profiling.

Material and Method

A cross-sectional study was conducted in the 290 employees at Acharya Vinobha Bhave Rural Hospital (AVBRH), Sawangi (M), Maharashtra during a period of 2018 to 2020. Written informed consent was taken from all employees prior to their enrollment. The study was approved by the Institutional Ethics Committee. All the employees of both gender of 18 to 60 years of age, in administrative and non-administrative positions like electricians, plumbers, carpenters, typists, clerks, computer operators, engineers, civil contractors, ground staff, public relation officers and willing to give informed consent were included in the study. Medical and paramedical employees and those who were not willing to give consent were excluded from the study.

Participants of the study were approached individually and the purpose and conduct of the study was explained to them. The data for the study was collected using the adapted STEPS instrument.[15] STEPS instrument is a standard tool proposed by WHO for surveillance of non-communicable diseases (NCD) risk factors. It consisted of 3 steps. STEP 1 (Questionnaire) involved the implementation of a validated questionnaire that is suitably modified for the easy understanding of the subjects. It seeks information on core socio-demographic information (education, occupation, and income); core behavioral measures (self-reported tobacco use, alcohol intake, diet, and physical activity). STEP 2 (Physical measurements) involved the recording of blood pressure, heart rate and anthropometric measurements like height, weight, hip, and waist circumference as per standard protocols. STEP 3 (Biochemical measurements) involved biochemical measurements such as blood glucose, blood lipids, urinary sodium and creatinine, triglycerides and HDL cholesterol levels.

Data were collected using "Cardiovascular Disease Risk Factor Surveillance Questionnaire" which consisted of four sections (i) Demographic information section (ii) behavioral measurement section (iii) physical measurement section and (iv) biochemical measurement section.

To complete STEP 1 participants were interviewed as per the questionnaire and the data was entered in the data sheet. The subjects were interviewed under adequate privacy. To complete STEP 2, anthropometric and clinical measurements were taken. All the equipment (like weighing machine, electronic blood pressure machine) used for measurements were calibrated. Weight was measured (to the nearest 0.5 kg) with the participants standing motionless on a bathroom weighing scale without shoes or any heavy outer garments, and weight equally distributed over each leg. Height, waist circumference and hip circumference were measured (to the nearest 0.1 cm) using a standard non-elastic tape. Height was measured with the participant standing erect against a wall, without shoes, and the head looking straight. To measure waist circumference participant was asked to stand with the arms by the sides and to breathe out normally. Standing to the side of the participant, the inferior margin (lowest point) of the last rib and the crest of the ileum (top of the hip bone) was located and marked with a fine pen. The midpoint between the two was marked and measurement for waist circumference was then taken at the level of this midpoint. Hip circumference was measured around the maximum circumference of the hips. Clinical parameters like sitting blood pressure were measured using an electronic blood pressure machine (to the nearest 1 mm

Hg). Two readings were taken at an interval of 10 min. If the difference between the two readings was more than 10 mmHg, a third reading of BP was also recorded. The mean of 2 (or 3) readings was taken as the final measurement.

To complete STEP 3 date and time was fixed with the participant as per his/her convenience for biochemical investigations. Blood and urine samples were collected and sent to a central laboratory for further investigations. Urinary sodium and creatinine were analysed in XD Electrolyte Analyser and blood glucose, lipids, triglycerides and HDL cholesterol levels were analysed using RX Daytona and RX Imola.

Risk factor profiling of the individuals above 40 years of age was done using WHO/ISH risk prediction chart. **(Figure 1)** WHO/ISH risk prediction charts indicate 10-year risk of a fatal or non-fatal major cardiovascular event (myocardial infarction or stroke), according to age, gender, blood pressure, smoking status, total blood cholesterol, and presence or absence of diabetes mellitus. The charts provide approximate estimates of CVD risk in people who do not have established coronary heart disease, stroke, or other atherosclerotic diseases. [14, 16]

Statistics: Statistical analysis was performed using the IBM SPSS statistics version 17.0. Quantitative data was expressed in Mean and Standard deviation and qualitative data was expressed in number and percentage. Chi-square test and Fisher's exact test were used to analyse qualitative data. The p values <0.05 were considered as statistically significant.

Results

Total of 290 employees participated in the survey and completed all the three steps of STEPS program.

Demographics: Mean age of the participants in our study was 40.08 ± 8.94 years (range 20-60 years), with a maximum number of participants in the age group of 30 to 50 years. 52.07% were male and 47.93% were female. Most of the participants had completed secondary school (37.59%) or high school (31.03%). 76.55% of participants were married. Detailed demographics are given in **Table 1**.

Prevalence of major cardiovascular risk factors:

Behavioural measurements: 15.52% of participants had a habit of regular smoking, 41.03% had a habit of tobacco chewing and 38.28% had a habit of alcohol consumption. All these habits were observed in male participants of the study. Only one female participant had a habit of chewing tobacco (**Table 2**).

There was an inadequate intake of fruits and vegetables in the diet. 22.76% did not eat fruits at all in a week while 3.45% of the subjects ate it on all days. On an average, participants consumed fruits for 1.52±1.48 days and vegetables for 6.43±0.79 days. 13.79% participants reported that they took extra helpings of salt in their meals.

56.12% women participants in the study were physically inactive as compared to 37.75% of male participants. A family history of; hypertension was present in 22.07%, diabetes mellitus in 20%, hypercholesterolemia in 2.41%, and CVD in 10%. 7.59% of the participants were already a known case of hypertension, 1.72 % was known case of diabetes, 6.55% were known case of hyperlipidemia and 2.41% were known cases of cardiovascular diseases (**Table 2**).

Physical measurements: On blood pressure measurement, 16% of the studied participants were found to be hypertensive and 30% to be pre-hypertensive with a slight preponderance in male participants (**Table 3**). BMI measurements show that 26.20% of participants were obese with BMI ≥25 kg/m^2, 18.28% were overweight with BMI of 23-24.9 kg/m^2 and 8.97% were underweight with BMI of <18.5 kg/m^2. Central Obesity was noted in 62% males and 58% of females. The range and mean of various bio-physiologic parameters were as elaborated in **Table 4**.

The mean body mass indices of both males and females were greater than 25 (the cut-off limit for obesity as per Indian guidelines). The mean waist circumference and mean waist-hip ratio in both male and female groups, i.e. 96.3 cm and 1.0 respectively in males, and 89.2 cm and 0.9 respectively in females, were greater than the cut-off limits for abdominal obesity as per Indian guidelines.

The mean height, weight, waist circumference and waist hip ratio in both male and female are 66.10 ± 3.40, 65.68 ± 11.52, 87.02 ± 10.93, 0.92±0.05 and 62.13 ± 2.92, 55.96 ± 9.64, 82.09 ± 9.65, 0.86±0.05 respectively (**Table 5**).

Biochemical measurements: Biochemical measurements demonstrated that blood glucose, total cholesterol, serum sodium, serum creatinine and

triglycerides were within normal limits for both male and females except for HDL cholesterol which were lower for both male and female (**Table 5**).

Risk Profiling: CVD risk was assessed by using WHO risk assessment chart in 48.9% participants who were above 40 years of age. Accordingly, it was observed that 95% of participants had CVD risk below 10%, 2.81% had CVD risk of 10 to 20% while only 2% participants had risk level above 20% (**Table 6**).

Table 1: Demographic characteristics of the study subjects

Demographic characteristics	Males n(%)	Females n(%)	Total n(%)
Age group (years)			
20-29	31(20.53%)	15(10.79%)	46(15.86%)
30-39	53(35.10%)	48(34.53%)	101(34.83%)
40-49	52(34.44%)	50(35.97%)	102(35.17%)
50-59	15(9.93%)	26(18.71%)	41(14.14%)
≥60	0(0%)	0(0%)	0(0%)
	151 (52.06%)	139 (47.93)	290 (100%)
Educational Qualification			
Illiterate	0(0%)	14(10.07%)	14(4.83%)
Primary school	1(0.66%)	14(10.07%)	15(5.17%)
Secondary school	47(31.13%)	62(44.60%)	109 (37.59%)
High school	70(46.36%)	20(14.39%)	90 (31.03%)
College	22(14.57%)	15(10.79%)	37 (12.76%)
Post-graduation	11(7.28%)	14(10.07%)	25 (8.62%)
Religion			
Hindu	127(84.10%)	105(75.54%)	232(80%)
Other	24(15.89%)	34(24.46%)	58(20%)
Marital status			
Married	118(76.15%)	104(74.82%)	222(76.55%)
Separated/Divorced/widowed/unmarried	33(21.85%)	35(25.17)	68 (23.44%)

Table 2: Prevalence of major cardiovascular risk factors and morbidities

Risk factor	Males n (%)	Females n(%)	Total n(%)
Smoking	45(29.80%)	0(0.00%)	45(15.52%)
Chewing Tobacco	118(78.15%)	1(0.72%)	119(41.03%)
Alcohol	111(73.51%)	0(0.00%)	111(38.28%)
Physical Inactivity	57(37.75%)	78(56.12%)	135(46.55%)
Family history of Hypertension	39(25.83%)	25(17.99%)	64(22.07%)
Family history of diabetes mellitus	29(19.21%)	29(20.86%)	58(20.00%)
Family history of Cholesterol	4(2.65%)	3(2.16%)	7(2.41%)
Family history of CVD deaths	18(11.92%)	11(7.91%)	29(10.00%)
K/c/o hypertension	14(9.27%)	8(5.76%)	22(7.59%)
K/c/o diabetes	2(1.32%)	3(2.16%)	5(1.72%)
K/c/o Cholesterol	14(9.27%)	5(3.60%)	19(6.55%)
K/c/o CVD	5(3.31%)	2(1.44%)	7(2.41%)

Table 3: Blood pressure of the study subjects

Blood Pressure (mmHg)	Males n(%)	Females n(%)	Total n(%)
Normal	69(45.70%)	88(63.31%)	157(54.14%)
Pre-Hypertension	60(39.74%)	27(19.42%)	87(30%)
Grade I	10(6.62%)	14(10.07%)	24(8.28%)
Grade II	8(5.30%)	6(4.32%)	14(4.83%)
Grade III	4(2.65%)	4(2.88%)	8(2.76%)

Table 4: BMI and central obesity of subjects

BMI (kg/m^2)	Males n(%)	Females n(%)	Total n(%)
Underweight (<18.5)	9(5.96%)	17(12.23%)	26(8.97%)
Normal (18.5-22.9)	71(47.02%)	64(46.04%)	135(46.55%)
Overweight (23-24.9)	23(15.23%)	30(21.58%)	53(18.28%)
Obese (≥25)	48(31.79%)	28(20.14%)	76(26.20%)
Total	**151 (52.06%)**	**139 (47.93%)**	**290 (100%)**
Central Obesity (using W-H ratio)	93(61.59%)	80(57.55%)	173(59.66%)

Table 5: Physical and biochemical measurements of subjects

Parameters	Males (mean ± SD)	Females (mean ± SD)	Total (mean ± SD)
Physical measurement			
Systolic BP (mmHg)	123.52 ±13.54	121.92 ±14.53	122.76 ± 14.02
Diastolic BP (mmHg)	82.95 ± 10.97	79.39 ±10.64	81.24 ± 10.94
Height (Inches)	62.13 ± 2.92	62.13 ± 2.92	64.20 ± 3.74
Weight (kg)	65.68 ± 11.52	55.96 ± 9.64	61.02 ± 11.70
Waist Circumference (cm)	87.02 ± 10.93	82.09 ± 9.65	84.66 ± 10.61
Hip Circumference (cm)	94.64 ± 10.44	95.16 ±10.21	94.89 ± 10.32
Heart Rate (bpm)	78.79 ± 12.42	80.71 ± 12.00	79.71 ± 12.24
Biochemical Measurements			
Random Blood glucose (mg/dl)	108.47 ± 22.09	113.88 ± 36.68	111.07 ± 30.05
Total cholesterol (mg/dl)	169.75 ± 28.88	173.55 ± 32.34	171.57 ± 30.59
Serum sodium (mmol/l)	141.26 ± 2.83	141.39 ± 3.40	141.33 ±3.11
Serum creatinine (mg/dl)	0.97 ± 0.15	0.89 ± 0.15	0.93 ± 0.15
Triglycerides (mg/dl)	148.02 ± 62.81	147.06 ± 68.67	147.56 ± 65.57
HDL Cholesterol (mg/dl)	35.74 ± 5.21	37.40 ± 5.97	36.53 ± 5.64

Table 6: 10-year risk of cardiovascular disease according to WHO/ISH risk prediction chart

Risk Level (% risk)	n(%)
<10%	135(95.07%)
10 to <20%	4 (2.81%)
20 to <30%	1 (0.7%)
30 to <40%	1 (0.7%)
≥40	1(0.7%)
Total	**142 (100%)**

Discussion

This cross-sectional survey was planned to study the prevalence of cardiovascular risk factors and to study the health practices among the administrative and non-administrative employees of the tertiary care rural hospital. These employees were chosen for the study as they constitute the least health-conscious population in a hospital set up in spite of having proximity to health care facilities, health care programs and even to healthcare professionals.

The survey revealed the prevalence of various CVD risk factors in the study population which comparatively belongs to the young age group. Nearly 30% of male in our study were smokers and 74% were consuming alcohol. This prevalence was higher compared to other studies conducted in a similar hospital setting.[11,12] Tobacco chewing was found to be a very common practice among the study participants with nearly 80% male chewing it on a regular basis. This can be attributed to the customary habit of people of this part of India.

A higher number of women were physically inactive as compared to men. This may be because they were more involved in table jobs. Prevalence of overweight and obesity including abdominal obesity were observed in 18 to 26% of our participants. Though these levels were lower as compared to other similar studies,[11, 12] some studies done in Asians had observed a higher susceptibility to cardiovascular risk factors at lower BMIs.[17] Only 7.59% of the participants knew that they were hypertensive and only 20% knows that they had a family history of hypertension and diabetes. On the measurement of their blood pressure during the study, 30% of the study participants were found to be pre-hypertensive and 16% as hypertensives. Surprisingly, they all were unaware of this fact before participation in the study. Blood sugar levels, total cholesterol, and triglyceride levels were found to be in normal range on measurement, but mean HDL levels were found to be below 40 mg/dl in the study participants which indicates increase risk of CVD in them. Findings of our study were comparable to similar cross-sectional studies conducted in other hospital set-ups.[15, 16]

Risk profiling done in our participants shows that 95% belongs to the low-risk group and only 5% participants had >10% risk of developing cardiovascular diseases in the next 10 years. But as our study population is very small, 5% participants with >10% risk becomes an alarming finding. Low risk does not really mean that these participants would not develop the disease in near future and hence preventive and conservative management focusing on lifestyle and dietary modifications should be advised to all irrespective of the severity of the risk[18].

This study helped us to understand the knowledge and attitudes of the employees towards cardiovascular diseases and their implementation of preventive health behavior. Findings suggest that our participants, who are employees in a hospital-setups constitutes the least health-conscious population. They were not concern about the regular check-ups and screening programs which can help them in the early detection and initiation of steps to curb the morbidity and mortality related to CVDs.

Necessary steps have to be initiated to increase awareness among the employees by implementing low-cost programs at workplaces. Regular check-up camps need to be organized for employees as part of hospital policy. Health check-up in these camps can reveal the risk factors, management of which help lowering the risk of cardiovascular diseases. Routine medical appointments should strictly include measurements of body weight and blood pressure which can help in early detection of risk factors of CVDs. Also, policy measures that restrict chewing and smoking of tobacco at work should be implemented. Employees should be encouraged to involve more in physical activity and to have a balanced and nutritious diet. Health education programs should be implemented to increase awareness of healthy lifestyle. As workplaces can serve as an effective environment for conducting similar studies and spreading awareness such studies should be planned in more numbers. This can help in bringing down the morbidity and mortality rates of CVDs by early detection of risk factors.

The study has few limitations; the study subjects were employees, so they were not aged above 60 years. Sample size was small (n=290) and hospital based hence it does not represent the general population. This was a cross sectional survey, data collected here is only useful for characterizing the prevalence of a risk factor in a study population and is not able to infer any causation.

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Conflict of Interest: Nil.

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To Determine the Role of Clonidine as an Adjuvant to Intrathecal Bupivacaine in Patients Undergoing Lower Abdominal Surgery

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Abstract

Background: Clonidine is an alpha-2 adrenergic agonist with various actions, one of which enhances the effectiveness of local anaesthetics. For patients undergoing lower abdominal surgery, we examined the effect of adding intrathecal clonidine to hyperbaric bupivacaine on postoperative pain relief and side effects, if any.

Aim: To assess the efficacy of intrathecal clonidine along with hyperbaric Bupivacaine in patients undergoing lower abdominal surgeries.

Method: It was a retrospective study carried out under spinal anaesthesia on 80 patients undergoing lower abdominal surgery. The research population was assigned randomly to two groups; group B- received 15 mg bupivacaine+ 0.4mi Normal Saline and group C received 15 mg bupivacaine + 60mcg clonidine.

Results: Addition of 60mcgs of intrathecal clonidine to 0.5% hyperbaric bupivacaine significantly prolonged the duration of motor & sensory blockade along with the duration of effective postoperative analgesia. The demand for rescue analgesic was earlier in group B as compared to group C. Both groups were matched for the side-effects like hypotension, bradycardia, nausea, vomiting, and shivering.

Keywords: *Spinal Anaesthesia, Clonidine, Lower Abdominal Surgeries, Post-op Analgesia.*

Introduction

Neuraxial blockage is more preferred mode of anaesthesia for lower abdominal surgeries. It has gained prominence since the introduction of spinal anaesthesia by "August bier" in 1898 due to its ease, minimal implementation ability, optimal operating environment, reduced risk of aspiration, low intraoperative blood loss, prolonged postoperative analgesia and minimal postoperative morbidity. Thus it was mostly used in sub-umbilical surgeries such as orthopedic lower extremity, arthroscopic, lower abdominal surgery[1]. Sub-arachnoid block (SAB) is one of the earliest known types of regional anaesthetic techniques.[2] This technique, however, suffered a significant setback shortly after it was described,[3] is now commonly used by anaesthetists[4] and clonidine has been introduced with newer hopes. Amide local anaesthetic, hyperbaric bupivacaine is most widely used for spinal anaesthesia. A small dose of hyperbaric bupivacaine produces a short lasting spinal anaesthesia,

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which may be clinically useful in ambulatory surgical procedures[5].

One drawback with spinal anaesthesia using bupivacaine alone is the relatively short duration of action, which means the postoperative period required early analgesic intervention. Potentiation of the effect of subarachnoid block and prolongation of postoperative analgesia can be achieved by using adjuvants to local anaesthetic agents such as Midazolam, Ketamine, Phenylephrine, Neostigmine, Clonidine, and Opioids.[6-12] out of which opiods are the most popular one's. Nevertheless, addition of opioids as adjuvant to local anaesthetic agent is associated with side effects[13] such as nausea, vomiting, pruritus, urinary retention, herpes labialis activation, and respiratory depression which directed the research in favour of non-opioid adjuvant and resulted in the introduction of clonidine as adjuvant to local anaesthetic agent.

Clonidine Hydrochloride is an imidazoline derivative with á2- adrenergic agonistic activity with a selectivity ratio of 200:1 in favour of á2 receptors. Intrathecal clonidine is demonstrated to potentiate the effect of subarachnoid block as well as reducing the local anaesthetic agent requirement.[14] Intrathecal clonidine also offers prolonged postoperative analgesia,[15,16] reduced shivering associated with subarachnoid block, and is devoid of side effects associated with intrathecal opioids. This research was therefore conducted to determine the degree of postoperative analgesia in patients undergoing lower abdominal surgery by intrathecal clonidine admixed with bupivacaine compared to bupivacaine alone.

Materials and Method

A cross sectional, prospective, observational study was conducted in the department of Anaesthesiology at Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, (DMIMS), Hingna Nagpur, in collaboration with Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi Meghe, Wardha, Maharashtra after obtaining permission from the Institutional Ethical Committee and written informed consent.

The study was carried out in the department of Anaesthesiology, Shalinitai Meghe Hospital and Research Centre Annexed to, DMMC, Wanadongari, Nagpur. Eighty patients with ASA physical status I or II in the 20-60 year age range, scheduled for lower abdominal surgery under spinal anaesthesia, have been enrolled in the study. Exclusion requirements included rejection of patients, age less than 20 years and older than 60, any contraindication to spinal anaesthesia or any patient with history of allergy to local anaesthetics. Patients were assigned randomly to two groups of 40 each to obtain either 15 mg of Bupivacaine (H) + 60mcg of clonidine (measured with insulin syringe) (group C) and 15 mg of Bupivacaine (H) + 0.4ml of regular saline in (group B). All of the research drugs were intrathecally added, and the total amount of administered agents was 3.4 ml. Preloading was performed with 10–15 ml/kg of ringer lactate. Monitoring parameters such as heart rate, ECG, oxygen saturation, and blood pressure were recorded. Under all aseptic precautions, subarachnoid block was given with 25 gauge Quinckie needle in L3-L4 space in sitting position and depending upon the groups, either 60 mcg clonidine or 0.4ml normal saline admixed with 3.0ml of 0.5% hyperbaric bupivacaine resulting in total volume of 3.4ml.

The primary outcome of this randomized observational study is to determine the time required for the first analgesic supplement and total analgesic intake post-operatively in the first 24 hours.

The secondary findings included the assessment of sensory block onset time, onset of motor block, duration of blockade, hemodynamic variables, the incidence of hypotension, mephentermine requirements, bradycardia, and adverse events such as and postoperative nausea, vomiting and shivering. Sensory and motor block was monitored every 5 mins for first 15 mins. Pinprick method was used to check the sensory block. The motor block was measured according to the modified Bromage scale: Bromage 0: Patients able to move hip, knee, and ankle, Bromage 1: Patients unable to move hip but able to move the knee and ankle, Bromage 2: Patient unable to move hip and knee but able to move the ankle, Bromage 3: Patient unable to move hip, knee, and ankle.[17] The onset of sensory block was taken from the time of intrathecal injection untill loss of pin prick sensation at T10. Sensory block length was taken as time from maximum height of block till regression to Level 1. The onset of motor block was described as time from intrathecal injection to motor blockade Level 2 in Bromage scale. Duration of motor blockage was taken as time from intrathecal injection untill no motor weakness (Bromage 0). Analgesia duration was described as time from intrathecal injection till administration of first rescue analgesic. The pain score

was measured postoperatively by using VAS between 0 and 10 (0 = no pain, 10 = extreme pain).[18] Injection diclofenac (75mg) was intramuscularly administered as rescue analgesic when VAS was >5. The time when the first dose of rescue analgesic was administered was noted. Data analysis was performed and analyzed using statistical package for social sciences (SPSS version 17.0) and GraphPad Prism 5.0 and $p<0.05$ is known as the degree of significance ($p<0.05$).

Observations And Results

Both groups were comparable in terms of demographic data, hemodynamic parameters. The observations are recorded in following tables:

Table 1: Comparison of initial block features in both categories

Initial Block Characteristics	Group C (n=40)	Group B (n=40)	t-value	p-value
OSB(sec)	87.75±14.40	96.25±16.66	2.440	<0.0169, S
DSB (min)	340±28.69	250.62±30.98	-13.388	<0.0001, HS
OMB(sec)	110.75±23.46	122.25±18.04	2.458	0.0162, S
DMB(min)	262±27.36	219.12±28.34	-6.885	<0.0001, HS
T2SR(min)	156.50±20.45	110.9±23.75	-9.202	<0.0001, HS
TDA(min)	270.75±29.71	222.87±32.38	-6.891	<0.001,S

Statistically significant difference was observed in total duration of analgesia between both groups. In group C it was 234.75±29.71 (min) while in group B it was 170.87±32.38 (min), (p value 0.000,S).

Table 2: Comparison of Time for First Rescue Analgesia in both the groups

Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
Group C	40	357.00	54.26	8.57	9.19	0.000 S,p<0.05
Group B	40	259.50	39.35	6.22		

The above table and graph show the mean time required for rescue analgesia in both groups. Mean time for rescue analgesia in clonidine group was 357.00 minutes which was significantly higher compared to plain Bupivacaine group which was 259.00 minutes (p-value>0.05, NS).

Table 3: Total number of rescue analgesic in both the groups

	Clonidine (Mean)	Plain Bupivacaine (Mean)	t-value
Mean	1.5	2.15	2.51 p=0.045 S, p<0.05
SD	0.64	0.23	

The above table and graph shows the mean number of rescue analgesics that were required in both the groups. In clonidine group mean no of rescue analgesics that were required were 1.5 and in plain Bupivacaine group mean number of rescue analgesics that were required were 2.15. The difference was statistically significant. (p value 0.045, <0.05, S).

Table 4: Comparison of side effects in both the groups

Complications	Clonidine		Plain Bupivacaine		χ^2-value
	No	%	No	%	
Bradycardia	12	30	3	7.5	15.72 P<0.0001,S
Hypotension	4	10	2	5	0.68 P=0.40,NS
Nausea/Vomiting	0	0	5	12.5	13.90 P=0.0002,S
Shivering	0	0	12	30	12.77 P=0.0007,S

The above table and graph shows the incidence of side effects that were observed in both groups. 30% patients in clonidine group had bradycardia while only 7.5% patients in plain Bupivacaine group had bradycardia. The difference was significant (p-value <0.05, S). 10% patients in clonidine group had hypotension while only 5% patients in plain Bupivacaine group had hypotension. The difference was non-significant (p-value 0.40, NS). None of the patients in clonidine group suffered from shivering while 30% patients in plain Bupivacaine group had shivering. Difference was statistically significant (p-value 0.0007, S). None of the patients in clonidine group suffered from nausea and vomiting while 12.5% patients in plain Bupivacaine group had nausea and vomiting. Difference was statistically significant (p-value 0.0002, S).

Discussion

Clonidine is an α_2-agonist that block the conduction of Aδ and C fibers, thus prolonging the local anaesthetic action. It stimulates the postsynaptic α_2-receptors in substantia gelatinosa of spinal cord when used intrathecally and induces analgesia[15,19]. Roh et al.,[20] recently indicated that its ability to modulate spinal cord N-methyl-D-aspartate receptor activation through the suppression of NR1 phosphorylation is one of the mechanisms for enhanced intrathecal clonidine administration in a rat model of neuropathic pain.

Our observational research results highlight some main findings. First, the administration of intrathecal clonidine was associated with an increase in postoperative analgesia, as demonstrated by the significant decrease in rescue analgesic intake of Inj diclofenac 75 mg IM in 24 hours and the extension of time to the first analgesic application. Tuijl et al[21] observed the total duration of analgesia up to 120mins by using 75 μgs of clonidine in their study while in our research we found that it was 270.75±29.71mins. The mean time in the clonidine group for two segment regression (T2SR) was also 156.50 minutes, while in the control group it was 110.9 minutes. This was in line with Rajan et al's analysis where similar results were found as T2SR was 137.00 ± 10.42.[22] in the clonidine group.

Second result that should be noted is that the length of both the sensory block and the motor block is clearly enhanced by intrathecal clonidine. The mean length of sensory and motor block with clonidine group was 340±28.69 minutes and 262±27.36 minutes respectively, and 250.62±30.98 minutes and 219.12±28.34 minutes in simple Bupivacaine group. The disparity was extremely statistically significant. This result is also consistent with previous studies.[(11),(23),(24)] The mechanism of sensory block potentiation induced by clonidine in spinal anesthesia is stated to be based on the action of presynaptic (decrease in transmitter release) and post-synaptic (increase in hyperpolarisation)[25,26] Bhure et al. demonstrated that adding clonidine, morphine, and midazolam to bupivacaine substantially enhances the onset and length of sensory and motor block with greater hemodynamic control, prolongs the length of surgical analgesia and successful post-op anaesthesia, and decreases the intake of systemic analgesics compared to bupivacaine alone. They concluded that clonidine is an excellent addition to bupivacaine in spinal anaesthesia and offers extended analgesic time with no deleterious effects on mother and baby when used in the delivery of cesarean section[27.]

Third result that we found was that in the plain Bupivacaine group the mean time for rescue analgesia was 259 minutes while in the clonidine group it was 357 minutes. Rescue analgesic was issued at a score of 5 and more than 5 in the VAS.

Fourth thing we found was linked to intrathecal clonidine-induced side effects. We found in our analysis that systemic side effects such as bradycardia and hypotension were more in the clonidine group relative to the plain Bupivacaine group. Although bradycardia was more important than hypotension in the clonidine population, there is no other obvious explanation available except for the sympatholytic action of clonidine and profound analgesia which also reduces sympathetic function. Sethi et al.[15] and Shah et al.[16] reported very little occurrence of hypotension and bradycardia using 1 mcg/kg of intrathecal clonidine in non-obstetric surgery.

One major side effect that we observed in our study was shivering which is common during spinal anaesthesia. It is observed that around 30% patient under regional anaesthesia develop shivering either intra or post operatively[28]. Surgery and anaesthesia induce shivering as a compensatory mechanism due to thermal dysregulation, which is exacerbated by spinal anaesthesia vasodilatation that redistributes core body heat. We also found in our sample that the number of patients complaining of shivering in the simple Bupivacaine group was significantly high compared to the clonidine group[29]. Few studies also reflected on anaesthetics used lower abdominal surgeries [30-32].

Conclusion

We concluded by this that intrathecal clonidine 60 mcg with bupivacaine prolonged intraoperative anaesthesia and postoperative period for first analgesic request compared to plain Bupivacaine, as well as postoperatively less total analgesic intake in the first 24 hr in clonidine community.

Limitation of the study:

Need to study a larger sample size

The side effects like bradycardia & hypotension need more e attention & need to be evaluated more

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Psychosocial Impact of Lock Down among College Students in Nagpur

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Abstract

Background: The deadly COVID 19 has brought the life to a standstill in many parts of the world. The uncertainties in lives of people are increasing along with the spread and increasing death tolls due to disease. College students, the predominant youth are maximally affected by this crucial stage. **Aim:**The current study aims at assessing the psychosocial impact of lock down among the college students.

Study Design: Online survey.

Study Population: College students in the age group of 17 to 25 years both male and females.

Sample Size: 337.

Material: Psychosocial Impact Scale for students developed by investigators (r=0.85).

Results: The mean age of the students is 19.93+1.14. 96.4% students were living with their parents during the period of lock down. 36% experienced borderline negative impact and 20.17% reported mild to moderate negative impact of lockdown in just 15 days of lock down period. Most surprisingly despite being with parents; 20.18% had been out on roads during lock down, 1.48 for their job, 36.80% for purchasing medicines, 39.17% for grocery and vegetables, 4.75% to meet their friends, 2.37% to attend a funeral, 2.97% attended family function, 9.50% to complete their academic assignments, 13.65% to refresh themselves, 12.76%to help neighbour/relatives.

Conclusion: Lockdown has adversely affected the psychosocial status of the college students. While equipping for any disaster, a nation will have to find out strategies for rebuilding mental strength of its people after the disaster is over so as to enhance the return to normal functioning.

Keywords: *Psychosocial impact, Lock down, students.*

Introduction

The COVID 19 spread compelled many countries to impose complete lockdown. Naturally the schools and colleges stopped functioning. Youth are the topmost priority of any country. India was not exception either. Since 16th march 2020 all the colleges in Nagpur are closed down. It is in the months of January to April the colleges, schools and students also take their studies seriously. The portion completion targets, preliminary examination and working on formative assessments, completion of assignment are geared up at both ends; the students and the teachers; during this period. The investigators, both being from the field of mental health and academicians were contacted by parent of many student and students themselves expressing worries and concerns about their studies and examinations. Some expressed concerns regarding teaching of portion to be covered while some others thought that they will not be able to cope up with studies of their own through self-

study. On the other hand, the institutes also anticipated the needs of students and were thinking and planning on terms of completing the portion for all the students through sharing the study material and online lectures wherever possible. It was challenging as many students did not have android phones or PCs to attend interactive lectures online. Some others who had necessary phones but the internet facility was not available in their areas. Apart from these technological hurdles, many were not techno-savvy enough to handle the interactive lectures virtually. Thus one can say it is chaos in education front in many cities marked with red zone for corona infection including Nagpur[1].

Material and Method

Aim of the study: The study aims at assessing the level of psychosocial impact of lock down on the college students.

Objectives of the study: The objectives of the study are

1. To identify the reasons for breaking the lock down by students.
2. To assess the nature and extent of psychosocial impact of lock down among the college students.

Study setting: The study was conducted in Nagpur city of Maharashtra state which was declared as red zone for Corona infection. The students were from all the disciplines of study.

Study design: Online survey with the Google form of questionnaire

Study population: College students from all disciplines of study in the age group of 17 to 25 years both male and females were included in the study.

Study duration: From 6th April to 9th April 2020 (Initial respondents are included)

Sample size: 337

Tool for data Collection: Investigator prepared psychosocial impact of lock down scale. The tool had three parts. The Part I caters to the demographic information of the students and includes, age, residence, course of study, year of study, types of family, number of family members, during lock down living with, etc. Part II of the tool evaluated the reasons for breaking the lock down and number of times the lock down was broken by the student in past 15 days. The Part III of the tool was a self-rating scale with 30 statements for which response was elicited on 5 point scale. The response was to be graded based on how often the student felt that way in last 15 days. Higher the response higher was the impact. Each item had minimum score of 0 and maximum score of 4. The responses ranged from minimum 0 to maximum 120. The response scores were categorized into five categories of reactive depression. The score range of 0 – 20 was considered as normal response to the event of lock down. 21 – 40 score was considered as borderline negative impact. Scores between 41 – 70 was treated as mild negative impact, 71 – 100 was put into moderate negative impact category and scores between 101- 120 were put into severe negative impact category.

Method of data collection: The students were contacted through their functional emails and students Whatsapp groups. The link of the Google form was shared with the students. The participation in the survey was purely on voluntary basis. The investigators were accessible through phone for clearing the doubts of the students. Only the initial respondents are included who responded within 72 hours of sharing the forms. The self-report forms were shared with the students after 15 days complete lockdown on 6th April 2020.

Plan for Data Analysis: The collected data was coded and analysed based on the objectives of the study. Descriptive statistics of mean, standard deviation, frequency percentage was used to describe the students according to their responses to different items on the scale.

Results

The observation and analysis section deals with the description of the study participants according to their demographic information, behaviour during lockdown in terms of number of times going out of the house and the reasons for it, and the depression level is assessed. Further analysis of association of depression levels with demographic characteristics is also done to identify the predictors of depression among the demographic characteristics.

Table 1: General Information of study participants

S.N.	Demographic Characteristics	Category	Frequency	Percentage
1	Age	17	2	0.6
		18	25	7.4
		19	98	29.1
		20	117	34.7
		21	70	20.8
		22	19	5.6
		23	4	1.2
		24 & more	2	0.6
2	Course of Study	Nursing	15	4.5
		BPTh	103	30.6
		BAMS	43	12.8
		BE	165	49.0
		Others	11	3.3
3	Year of study	1	96	28.5
		2	111	32.9
		3	100	29.7
		4	30	8.9
4	Residence	Rural	84	24.9
		Urban	204	60.5
		Semi-urban	49	14.5
5	Religion	Hindu	201	59.6
		Muslim	5	1.5
		Christian	1	0.3
		Jain	5	1.5
		Buddhist	15	4.5
		Other	110	32.6
6	Type of Family	Nuclear	249	73.9
		Joint	88	26.1
7	No. of family members	2	5	1.5
		3	62	18.4
		4	142	42.1
		5	57	16.9
		6	22	6.5
		7	13	3.9
		8 And more	36	10.7
8	During Lock down living with	Alone	5	1.5
		Parents	325	96.4
		Relatives	6	1.8
		Friends	1	0.3

The above table shows the distribution of students according to their demographic characteristics. The mean age of the students is 19.93+1.14. The students belong to Nursing (4.5%), Ayurvedic sciences (12.80%), Physiotherapy (30.6%), Engineering (49) and other streams of education (3.3%). The participants were studying in first year (28.5%), second year (32.9), Third year (29.7%) and final year (8.9%) of their respective course of study. They lived in rural areas (24.9%), urban areas (60.5%) and semi-urban areas (14.5%).Among the participants 59.6% were Hindu, 1.5% each were Muslims and Jain, 4.5% were Buddhist 0.30% were Christians and 32.6% belonged to other religions. The students belonged to Joint families (26.1%) and nuclear families (73.9%). 19.9% students had 2 to 3 members in their family, 42.1% had 4 members I their families whereas 38% had more than 4 members in their families. 96.4% students were living with their parents during the period of lock down, 1.8% lived with their relatives, 0.30% lived with friends and 1.5% were living alone.

Table 2: Movement during lock down

S.N.	Statements	0	%	1	%	2	%	3	%	4	%
1	Been out on roads during lock down.	269	79.82	45	13.35	117	34.72	2	0.59	3	0.89
2	For job.	332	98.52	5	1.48	0	0.00	0	0.00	0	0.00
3	For medicines.	213	63.20	98	29.08	21	6.23	1	0.30	4	1.19
4	For grocery and vegetables.	205	60.83	97	28.78	22	6.53	5	1.48	8	2.37
5	To meet friends.	321	95.25	15	4.45	0	0.00	0	0.00	1	0.30
6	To attend a funeral.	329	97.63	7	2.08	0	0.00	0	0.00	1	0.30
7	To attend family function.	327	97.03	8	2.37	0	0.00	0	0.00	2	0.59
8	To complete academic assignments.	305	90.50	27	8.01	3	0.89	0	0.00	2	0.59
9	To refresh myself.	291	86.35	38	11.28	6	1.78	0	0.00	2	0.59
10	To help neighbour/relatives, etc.	294	87.24	31	9.20	5	1.48	3	0.89	4	1.19

The above table shows the number of times the students left their house and the reasons for which they went out of their house during the period of lockdown. Among the participants 20.18% had been out on roads during lock down. 1.48 had to go out for their job. 36.80% had to go out for purchasing medicines. 39.17% had to go out for grocery and vegetables. 4.75% went out to meet their friends. 2.37% went out to attend a funeral. 2.97% attended family function during lock down. 9.50% had to go out to complete their academic assignments. 13.65%went out to refresh themselves. 12.76%went out to help neighbour/relatives, etc.

Table 3: Level of Negative impact of Lockdown

Level of depression	Score Range	% range	Frequency	%
Normal Response	0-20	0-17	144	42.73
Borderline impact	21-40	18-33	124	36.80
Mild impact	41-70	34-58	54	16.02
Moderate impact	71-100	59-83	14	4.15
Severe impact	101-120	84-100	1	0.30

The above table shows the intensity of negative impact on students due to lock down. 42.73% students had normal response to the lock down conditions. 36.80% showed borderline negative impact of lock down. 16.02% showed mild negative impact, 4.15% showed moderated negative impact and 0.30% showed severe negative impact due to nationwide lock down.

The lock down impact scores were found significantly associated with age (p=0.028) at 5% level of significance and with course of study (p=0.062) at 10% level of significance. This association can be attributed to most participants, having their age 20 years and most participants belonging to engineering course of study respectively.

Discussion

The study aimed at assessing the psychosocial impact of lock down among college students in Nagpur. The findings reveal that the mean age of the students was 19.93+1.14. 96.4% students were living with their parents during the period of lock down**.** 36.80% experienced borderline negative impact and 20.17% reported mild to moderate negative impact of lockdown in just 15 days of lock down period. One out of 337 students reported severe negative impact of lock down. Despite being with parents; 20.18% had been out on roads during lock down, 1.48 for their job, 36.80% for purchasing medicines, 39.17% for grocery and vegetables, 4.75% to meet their friends, 2.37% to attend a funeral, 2.97% attended family function, 9.50% to complete their academic assignments, 13.65%to refresh themselves, 12.76%to help neighbour/relatives. The lock down impact scores were found significantly associated with age (p=0.028) at 5% level of significance and with course of study (p=0.062) at 10% level of significance. This association can be attributed to most participants, having their age 20 years and most participants belonging to engineering course of study respectively. This also indicates that engineering students fear that the existing poor employability in engineering may be aggravated by the lockdown conditions[2].

COVID 19 Psychological Research Consortium was founded in UK to study the psychological impact of COVID 19 in March 2020. A group of psychologists lead by Prof. Richard Bentall of Sheffield University and in collaboration with psychologists from Ulster University published their initial research findings on COVID 19 and Mental Health in UK. This study is the first of its kind reporting on COVID 19 and mental health of people. The authors report that 20.58% males and 23.40 females experienced depression due to COIVD 19 spread in UK. 17.90% male and 25.12% females experienced anxiety and 18.93% males and 14.90% females felt stressed due to COID 19 in UK. The study was conducted on general population. The authors also report that a spike in depression and anxiety was observed after the Prime Minister's declaration of nation–wide lock down to control COVID 19 spread. The investigators found that higher rates of anxiety and depression were associated with young age, urban living and living alone during the pandemic[3-14]

Conclusion

The present study findings reveal that COVID 19 and resulting lockdown has adversely affected the psychosocial status of college students. The uncertainties due to lock down have been overwhelming to the extent that despite being with their parents, a significant number of students are negatively affected. The higher rated responses like privacy being challenged, do not want to see the same faces day and night, expected to study all the time, being instructed all the time; are of concern for the parents. There is need to study the interaction between disease and its psychological impact on people of different age groups.Such studies are rarely done in the past. While equipping for any disaster, a nation will have to find out strategies for rebuilding mental strength of its people after the disaster is over so as to enhance the return to normal functioning.

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Significance of Hematological Parameters in Uncomplicated Diabetes Mellitus

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Abstract

Background: Diabetes mellitus leads in chronic metabolic syndromes worldwide. The physiochemical changes in the blood bought about by persistent hyperglycemia are reflected in the hematological parameters on complete blood count.

Introduction: Diabetes Mellitus is a smouldering global pandemic destroying mankind slowly and silently. The associated detainable microvascular and macrovascular complications are responsible for much of morbidity and mortality. With ever increasing incidence and prevalence, it is important to device economical method to easily monitor, predict and prevent untoward health outcomes in these patients. Complete blood count being used as a basic and easily available investigation can be utilized for this purpose.

Material and Method: A case control study was carried out at Datta Meghe Medical College and Shalini Tai Meghe Hospital and research center in association with Acharya Vinobha Bhave hospital & research center and Jawaharlal Medical College, both a tertiary care hospital for a duration of 3 months. Study group of 100 diabetic patients and control group of 100 nondiabetic patients were evaluated using questionnaire, clinical findings, case records and hematological findings. Hemogram was analysed on Beckman coulter five part cell counter. Leishman stained peripheral smears were studied. The data thus collected was analysed using test of significance to find out relevance of changes in hematological parameters in diabetic patients.

Results: Anaemia along with higher leucocyte and platelet counts were observed in diabetic group.

Conclusion: Monitoring changes in hematological parameters can be a useful tool in better management of diabetic patients.

Keywords: *Diabetes mellitus, anaemia, erythropoiesis.*

Introduction

Diabetes Mellitus is a global pandemic with associated significant morbidity and mortality. Due to urbanization and adverse lifestyle changes like undesired food habits and sedentary life style, the incidence is constantly increasing with younger population becoming victim[29]. In 2015 International Diabetes Federation reported around 415 million people with diabetes and predicted this number to rise by 642 million by 2040[30]. India is known to be diabetic capital of the world and by 2030, it is estimated that about 80 to 87 million people of India will be diabetic[31,32]. Consistent hyperglycemia can be associated with functional and structural changes in hemoglobin molecule, cytoplasmic

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viscosity and osmotic disturbances within red blood cell and affects cellular metabolism by oxidative stress, which are reflected in various hematological parameters. This is depicted as changes in the various hemogram parameters. Glycation of haemoglobin molecule with resultant physiochemical changes in the red blood cells are reflected in parameters like MCV, MCH, MCHC and RDW[33]. Anemia is a common hematological finding in diabetic patients. Role of renal impairment in etiopathogenesis of anemia is well established, however, research studies have suggested association of anemia with diabetes in patients even prior to development of renal insufficiency[34],[35].Patients with diabetes mellitus show significant derangement in various haematological parameters. In fact, several haematological changes affecting the red blood cells (RBCs), white blood cells(WBCs), platelet and the coagulation factors are shown to be directly associated with DM.RDW is now being considered as an inflammatory marker and an elevated RDW value is shown to be significantly associated with diabetic nephropathy in type2 diabetes patients independent of traditional risk factors including diabetes duration and glycemic control[36]. Elevated WBC count is a basic inflammatory marker which can be used to measure the oxidative stress at cellular level. Endothelial dysfunction can be predicted by parameters like platelet count and platelet to lymphocyte ratio[37]. Complete blood count being a basic routinely advised investigation can be used to predict the glycemic control and in turn various degenerative complications of DM[38]. Hence these haematological parameters can prove to be easily available and economical method to monitor and predict health outcome in these patients. However, limited studies are available on this subject and exact relationship of changes in these parameters in predicting glycemic control and associated morbidity is not well established. Hence to have a better understanding of this, we propose to undertake this study with the objective to determine the hematological profile among type 2 Diabetes mellitus in comparison with non-diabetic controls.

Aims and Objectives:

Aim: To determine utility of hematological parameters in uncomplicated diabetes mellitus.

Objectives of study

1. To collect data on hematological parameters by using Beckman Coulter five part cell counter.
2. To collect further data by clinical history and previous case files.
3. To analyse all data thus collected to find out the differences in the two study groups.
4. To establish the role of hematological parameter as early preventive strategy.

Material and Method

A case control study was carried out at Datta Meghe Medical College and Shalini Tai Meghe Hospital and research center in association with Acharya Vinobha Bhave hospital & research center and Jawaharlal Medical College, a tertiary care hospital under Datta Meghe Institute of Medical Sciences, for duration of 3 months. Hundred subjects were included in each of the groups mentioned below. Subjects were evaluated using detailed history, clinical findings, previous case papers and hematological findings.

Patients were selected from out patients visiting central clinical laboratory. The data was analysed using test of significance to find out relevance of differences in hematological parameters in the two groups.

Study groups are outlined below:

- Known Diabetic Patients with history of diabetes mellitus for at least 2 yrs.
- Non diabetic healthy individuals.

Inclusion Criteria:

Group 1: All uncomplicated type 2 diabetic patients visiting out patient department.

Group 2: Healthy individuals visiting out patient department for routine health check up or for minor ailments and hospital staff.

Exclusion Criteria:

Group 1: Diabetic patients with micro or macrovascular complications, patients on insulin/aspirin or any antiplatelet drugs and patients with any other acute unrelated illness were not included in the study.

Group 2: Individuals with history of any chronic illness or any acute infective illness were not included in the study.

Sample Collection Process: 2 ml of venous blood was collected under aseptic conditions in EDTA bulbs

and hemogram was analysed on Beckman coulter five part cell counter. Peripheral smear stained with leishman stain were studied.

Results

In our study we included 100 subjects in each group. The mean age of patients in group 1 was 48.7 years with youngest subject being 38 yrs and oldest 59 yrs. There were 64 males and 36 females. The mean age of subjects in control group was 46.9 yrs, with youngest being 35 yrs and oldest 60 yrs. There were 58 males and 42 females (Table 1). There was no significant difference between age and sex distribution of the two groups. In group 1, patients had history of diabetes from 2 to 10 yrs duration, with average duration being 5.3 years.

Among the diabetics 48% of subjects were anaemic, with 33%, 12% and 3% having mild, moderate and severe anaemia respectively, whereas among non diabetics 25% were anaemic, with 15%, 09% and 1% having mild, moderate and severe anaemia respectively. Haemoglobin was significantly low in patients with diabetes (9.8±2.5g/dl) compared to controls (12.1±1.5 g/dl). Among other RBC parameters PCV and RBC count, were significantly lower in diabetic patients, whereas, RDW-CV, MCHC and MCV were significantly higher. MCH did not show any significant difference.

Mean WBC count with mean lymphocyte count was significantly higher in diabetic patients compared to non diabetics.

Mean Platelet count and MPV were significantly higher in diabetic patients compared to non diabetics.

Table 1 Age and Gender distribution of study population

	Diabetics	Non Diabetics	p value
Males	64%	58%	0.385
Females	36%	42%	0.385
Mean Age	48.7±7.5 yrs	46.9±8.2 yrs	0.106

Table 2 Comparision of various haematological parameters in the two groups

Parameter	Diabetics	Non Diabetics	p value
Haemoglobin (g/dl)	9.8±2.5	12.1±1.5	<0.0001
RBC count (10^6/µl)	4.01±1.52	4.82±1.14	<0.0001
PCV (%)	29.4±5.28	36.3±4.82	<0.0001
MCV (fl)	98.53±15.33	82±14.82	<0.0001
MCH (pg)	29.7±5.8	28.3±4.9	0.066
MCHC (g/dl)	37.1±5.3	34.1±6.2	0.0003
RDW (%)	18.8±5.3	16.4±4.2	0.0005
WBC count ($X10^3$/µl)	8.5±3.3	7.3±2.8	0.0061
ALC (/cmm)	4200±502	3400±600	<0.0001
ANC (/cmm)	6000±840	5800±750	0.077
AMC (/cmm)	650±150	600±250	0.087
AEC (/cmm)	450±270	390±180	0.065
Platelet count ($X10^3$/µl)	270±150	210±150	0.005
MPV (fl)	13.5±2.3	11.5±2.2	<0.0001

*ALC- Absolute lymphocyte count, ANC- Absolute neutrophil count, AMC-Absolute monocyte count, AEC- Absolute eosinophil count

Table 3 Comparision of grades of Anemia in the two groups

Grade of Anemia	Diabetic	Non Diabetic	p value
Mild	33	15	0.0008
Moderate	12	09	
Severe	03	01	

Table 4 Peripheral Smear findings in the two groups

PS finding	Diabetic	Non Diabetic	p value
Normocytic Normochromic	18	05	0.004
Microcytic Hypochromic	10	13	0.507
Macrocytic	20	07	0.007
Giant platelets	12	01	0.001
Neutrophilia	02	00	0.156
Lymphocytosis	50	02	<0.0001
Eosinophilia	07	01	0.030
Monocytosis	01	00	0.3
Polychromasia	17	02	0.0003
Fragmented RBCs	24	05	0.0001
Burr cells	01	00	0.3

Discussion

Diabetes Mellitus is a complex metabolic multisystemic disorder with steadily increasing prevalence. Persistent hyperglycaemia results in non enzymatic glycation of various proteins in blood such as haemoglobin, prothrombin and fibrinogen, which in turn changes viscosity, flow, red cell deformability, surface charge, erythrocyte aggregation and other physiochemical properties of blood[39]. Also the pro inflammatory milieu associated with diabetes brings about various changes in haematological parameters. The resultant RBC, WBC and platelet dysfunction is reflected in the hemogram[8].

In our study 100 subjects were included in each of the two groups. Mean age of diabetics was 48.7 yrs and of non diabetics 46.9 yrs, which was lower as compared to studies by Ravi Patel[40] and Harish Kumar[3].

Hemoglobin along with RBC parameters like RBC count, PCV and MCHC were significantly lower in diabetic group, whereas MCV and RDW CV showed significant increase. Diabetes with its associated hyperglycemia induced changes in the body's metabolic and physiochemical composition has been shown to be independent risk factor for Anaemia in previous studies[41,42,43]. The etiopathogenesis of anaemia is complex and multifactorial in diabetes. The assosiation of renal failure and development of secondary anaemia through erythropoietin pathway is well known, however, it has been observed that diabetics develop anaemia much earlier in the course of disease and also the severity of anemia when linked to stage of renal impairment was much more compared to non diabetic patients with renal impairment due to various other causes[44]. These findings point towards the complex multifactorial etiopathogenesis of anaemia in diabetes. Other pathways contributing to development of anaemia in these patients are inflammatory meliu with asssosiated increase in cytokines like IL 6 having detrimental effect on erythropoiesis, decreased responsiveness to erythropoietin, direct toxic effect on hematopoiesis, defect in feedback loop of peripheral blood cells and bone marrow and accelerated ageing and destruction of RBCs due to membrane protein glycation, rigidity and increased viscosity[45,46,47,48,49]. Drugs like metformin and ACE inhibitors also contribute to anaemia in diabetic population[18,50]. Decreased hemoglobin concentration along with accelerated ageing of RBCs interfere with HbA1C estimation, thus creating conflict in effective pharmacotherapeutic control of hyperglycemia[51]

.Anaemia in turn hastens the development and progress of micro- and macrovascular complications of diabetes, adding to the morbidity caused by these. Hence, correction of anaemia in diabetic patients can act as a preventive measure, avoiding much of morbidity and in turn improving quality of life[52,53].

MCV was found to be significantly higher in diabetic patients. This reflected megaloblastic anemia in 20 patients, which could be due to use of metformin and/ or alcoholism[54]. Also polychromasia seen in 17 patients contributed to the increased MCV on the whole[55].

RDW showed significant increase in diabetic patients reflecting impaired erythropoiesis and inflammatory mileu[7,56,57]. RDW is being considered as an independant inflammatory marker by some[1,7,58]. Barbieri M et al found RDW to be elevated in patients with macro and microvascular complications in diabetes[27].

Although normal in value, total count along with absolute lymphocyte count showed positive corelation with diabetes. Absolute neutrophil, eosinophil and monocyte count were higher in diabetic patients but they didnot show any significant corelation. Proinflammatory mileu along with oxidative stress present in diabetes is linked to the higher WBC count along with lymphocytosis in these patients, which in turn is linked to development and progress of various angiopathic complications assosiated with diabetes[59,60,61]. Epidemiological workers have suggested role of inflammation in inducing diabetes[4,62,63]. Leucocyte function is derranged in diabetics, making them prone to recurrent infection[64]. Even though absolute eosinophil count did not show any significant corelation, the number of diabetic subjects with eosinophilia was higher than non diabetics. This could be due to asymptomatic/subclinical fungal infections they are prone to[65,66].

Platelet count along with MPV was higher in diabetic subjects compared to control group. Due to microhemorrhages in the atheromatous plaques, the bone marrow is signalled to release reserve and immature giant platelets[67]. Swelling of platelets due to hyperosmalirity of plasma and degranulation of platelets contribute to increase MPV and increased variation in size of platelets reflected in PDW[68]. Platelet function is also affected in diabetes contributing to thrombotic complications[69,70]. Reduced effectiveness of antiplatelet drugs in diabetes has been studied by Mortensen SB et al[71]. Development and progression of atherosclerosis is reflected in platelet parameters, particularly raised MPV[72]. Karthikeyan et al suggested a possible relation between MPV and pathophysiology of cardiovascular disease[73]. Further platelet parameters are shown to be associated with HbA1C levels and cholesterol levels, which in turn is a major determinant of cardiovascular morbidity[9,74,47,48].

Conclusion

To conclude periodic monitoring and careful assessment of haematological parameters can prove to be of utmost help in foreseeing, preventing and delaying many diabetes associated complications.

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Intubating Conditions Comparison, After Administration of Propofol with Muscle Relaxant and Profofol without Muscle Relaxant-An Observational Study

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Abstract

Introduction: General anaesthesia is a condition where generally neuromuscular blocking agents in the form depolarizing or non-depolarizing agents are used for intubation of trachea. A quest for a safer and suitable intravenous induction agent has led to the development of Propofol, a 2, 6, di-isopropyl phenol after a series of investigations. Propofol also reduces hypertension and tachycardia which occurs response during intubation. Thus this technique protect against the potential adverse effects of tracheal intubation like intra-cranial, intraocular hypertension and tachycardia.

Material and Method: 100 adult patients of American Society of Anaesthesia (ASA) grade I & II of both the sexes belonging to the age group of 18-55 years, from various surgical specialties and undergoing surgery under general anaesthesia were divided in to two groups of 50 each. In both the group intubation was graded and scored according to ease of laryngoscopy, position of vocal cord, coughing, jaw relaxation and movement of limb. Score of 5 was classified as excellent, 6-10 good, 11-15 poor and 16-20 as bad. Total score of <=10 was considered as acceptable and score of >=10 as unacceptable.

Results: Youngest patient was of 18 years in group I and 20 years in group II. Eldest patient was of 54 years in group I and 55 years in group II. Maximum number of patients were in 20-30 years of age 31 (62%) in group I and 23 (46%) in group II. Excellent intubating conditions were seen in 32 (64%) patients of group I and 49 (98%) patients of group II**.** Before induction baseline readings of mean arterial pressure, systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate were noted in both the groups values. Similarly reading above parameters were just after induction, after intubation, one minute after intubation, two minute after intubation and 5 min after intubation. In both the groups slight decrease in all the parameter were noted just after induction. In both the groups slight decrease in all parameters were noted just after induction .However slight increase in value of all the parameter were noted after intubation. Difference which was noted was not statistically significant. Also after 5 minute of intubation parameter in both groups were approximately similar that of baseline ($p>0.05$).

Conclusion: 2.5 mg/kg body weight of Propofol with adequate doses of opioids and inhalational agent can produce acceptable intubating condition when compared with Propofol 2.5mg/kg along with succinylcholine.

Keywords: *Intubation without the use of muscle relaxants, Propofol-Sevoflurane induction.*

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Introduction

After induction of general anesthesia, neuromuscular relaxants provide the best conditions for laryngoscopy and tracheal intubation. Due of the fast onset and

short duration of succinylcholine is widely used as an effective muscle relaxant. However, it is proposed that succinylcholine should not be used regularly due to the risk of certain complications, such as rhabdomyolysis and hyperkalemia, as well as the risk of masseter spasm and malignant hyperthermia.[75] James and Glen presented a description of the discovery of this and other alkyl-phenol anesthetic activity in 1977.[76] Clear headed, rapid and symptoms free recovery was observed in Propofol particularly in day case surgeries[77]. Propofol's most common side effect is peripheral vasodilation which results in hypotension. The primary cause of peripheral vasodilation is a reduction in the sympathetic outflow from the central nervous system[78]. Recent studies have shown that tracheal intubation with hypnotics and short-acting opioids such as alfentanil or remifentanil can be performed effectively in a patient with normal anatomy of the airway without the need for muscle relaxants[79,80]. Studies have also shown that Propofol offers better condition for tracheal intubation as opposed to thiopental or etomidate[81]. Nondepolarizing neuromuscular blocking agents are an option, but they are slow acting, have a prolonged neuromuscular blockade, and paralysis cannot be reversed easily if mask ventilation or tracheal intubation fails to control the airways[82,83,84]. Muscle relaxants are given traditionally to facilitate the tracheal intubation. of all the available muscle relaxants, succinylcholine, with a rapid (approximately equal to 1 min) onset and a fast recovery is still the most commonly used muscle relaxant for rapid-sequence induction despite its side effects[85]. In this study we compared the intubating conditions and intubation response with muscle relaxant, and without muscle relaxant.[86]

Material and Method

Present study was conducted in the department of Anaesthesiology at Datta Meghe Medical College and Shalinitai Meghe Hospital and Research Centre Nagpur, in collaboration with Jawaharlal Nehru Medical College Datta Meghe Institute of Medical Sciences, Sawangi Meghe, Wardha. 100 patients were randomly divided into two groups of 50 patients each. In the preanaesthesia room, an intravenous (IV) cannula of 20G was inserted and patient were shifted into the operating theatre and preinduction monitoring of NIBP, SPO2 and pulse rate was done. In both the groups patients oropharynx was sprayed with 10% lignocaine spray 3 times before induction of anaesthesia, premedication in the form of injection midazolam 1mg IV, injection fentanyl 2µgm/kg was used.

In Group I: Induction of anaesthesia was done with injection Propofol 2.5 mg/kg slowly over 20 seconds and Sevoflurane with MAC 1.2 with 100% oxygen, laryngoscopy and intubation was attempted 150sec after induction.

In Group II: Induction of anaesthesia was done with injection Propofol 2.5 mg/kg slowly over 20 seconds. After loss of eye lash reflex, ventilation was checked and then injection succinylcholine 2mg/kg body weight was injected, laryngoscopy and intubation was done after 60 second.

All cases included were monitored throughout the procedure with E.C.G. using cardiac monitor on lead II, PaO_2 with Pulse Oxymeter. Blood pressure and pulse were recorded. In both the groups laryngoscopy and intubation was performed by an experienced anaesthesiologist having at least 3 years of experience using Macintosh blade and appropriate size oral endotracheal tube. During laryngoscopy and intubation every patient was assessed for variable like ease of laryngoscopy, position of vocal cord, coughing, jaw relaxation and movement of limb and scoring was done as mentioned in table no 3.

Statistical analysis was done. All the observation were recorded and tabulated in MS Excel Windows Version 2013. Results were analyzed statistically by X^2 test and 'P' value was less than 0.05 the difference of the two sets of observation was considered significant and P>0.05 as not significant.

Results

Youngest patient was of 18 years in group I and 20 years in group II. Eldest patient was of 54 years in group I and 55 years in group II. Maximum number of patients were in 20-30 years of age 31 (62%) in group I and 23 (46%) in group II. Intubating condition are classified as mentioned in table**.**

Table 1: Steyn's modification of Helbo-Hansen intubation scoring system (Score of 5 was classified as excellent, 6-10 good, 11-15 poor and 16-20 as bad. Total score of <=10 was considered as acceptable and score of >=10 as unacceptable.)

Variable	Acceptable		Unacceptable	
	Excellent	Good	Poor	Bad
	1	2	3	4
Jaw relaxation	Relaxed	Not fully	Stiff	Rigid
Vocal cord position	Open	Moving	Closing	Closed
Laryngoscopy	Easy	Fair	Difficult	Impossible
Coughing	None	Slight	Moderate	Severe
Limb movement	None	Slight	Moderate	Vigorous

Excellent intubating conditions were seen in 32 (64%) patients of group I and 49 (98%) patients of group II.

Table 2: Overall intubating conditions

S.No.	Intubating conditions	Group I		Group II		Group I Vs Group II		Significance
		No.	%	No.	%	X^2	P-value	
1	Excellent	32	64	49	98	18.591	< 0.0001	S
2	Good	17	34	1	2	17.171	< 0.0001	S
3	Poor	01	02	00	00	_	_	NS

S: Significant, NS: Not significant.

Statistically significant difference was observed in group I & group II**.** Intubating conditions were excellent in 32 (64%) patients of group I and 49 (98%) patient of group II, although less in group I but they were acceptable for intubation. Good intubating conditions were found in 17(34%) and 1(2%) in patients of group 1 and group 2 respectively which is also statistically significant.

From above finding although, intubating condition were excellent in 98% in group II compared to 64% in group I, good intubating condition were found in 34% in group I and 2% in group II. Poor intubating condition was found in only 1 patient of group I. However, there was no failed intubation noted in our study.

Table 3: Mean arterial pressure change as compared to baseline

Sr. No.	Mean arterial pressure	Group I		Group II		T-value	p-value	
		Mean	SD	Mean	SD			
1	Before Induction	93.25	7.68	94.78	9.52	0.884	0.3786	NS
2	After Induction	83.26	6.78	82.58	6.89	0.497	0.6200	NS
3	After relaxant	-	-	80	6.07	-	-	
4	Just after intubation	94.20	7.17	94.48	7.26	0.194	0.8465	NS
5	One min. after intubation	87.40	5.42	84.83	6.89	-2.073	0.040	S
6	Two min. after Intubation	88.54	5.48	85.87	6.73	-2.175	0.032	S
7	Five min. after intubation	92.48	4.79	91.55	5.98	-0.858	0.3928	NS

Before induction mean arterial pressure (MAP) (baseline) was 93.25±7.68 and 94.78±9.52 in group I and group II respectively (p=0.3786, T value=0.884). There was slight decrease in mean arterial pressure initially after induction with mean 83.26 ±6.78 and 82.58±6.89 in group I and group II respectively which was statistically not significant (0.6200). There was slight increase in mean arterial pressure just after intubation with mean 94.20±7.17 and 94.48±7.26 in group I and group II respectively .These result were also statistically not significant (P=0.8465) when compared in both group.

One min. after intubation in group I and II responses were 87.40±5.42 and 84.83±6.89 respectively (p=0.040). Two min. after Intubation in group I and II responses were 88.54±5.48 and 85.87±6.73 respectively. Both these value were statistically significant (p=0.032). After 5min result of MAP wer92.48 ±4.79 and 91.55±5.98 in group I and II which is also statistically not significant (p=0.3928), also these value are nearer to baseline value as before induction.

Table 4: Systolic Blood Pressure (SBP) change as compared to baseline

Sr.No.	Systolic Blood Pressure (SBP)	Group I		Group II		T-value	p-value	
		Mean	SD	Mean	SD			
1	Before Induction	126.30	12.68	128.78	11.24	1.03	0.11	NS
2	After Induction	121.45	9.56	119.08	9.56	1.13	0.25	NS
3	After relaxant	-	-	120.89	9.77	-	-	
4	Just after intubation	131.09	12.87	130.09	13.07	-0.385	0.70	NS
5	One min. after intubation	120.96	6.78	117.08	8.08	-2.601	0.01	S
6	Two min. after Intubation	114.09	6.98	110.76	7.97	-2.223	0.02	S
7	Five min. after intubation	124.43	8.96	127.98	9.76	1.8	0.06	NS

Similarly Table 4 indicates before induction SBP (base line) value as 126.30±12.68 and 128.78±11.24 in group I and II. There was slight decrease in SBP just after induction with mean 121.45±9.56 and 119.08±9.56 in group I and II respectively (p=0.25 whisch is statistically not significant). However, there was slight increase in SBP just after intubation with mean 131.09±12.87 and 130.09±13.07 in group I and group II respectively (p=0.70). These results were not statistically significant (P=0.70). After One minute and two minute slight decrease SBP was observed P value of which were 0.01 and 0.02 respectively which were statistically significant. After 5min result of SBP were 124.43 ±8.96 and 127.98±9.76 in group I and II which were statistically not significant (P=0.06), also these value are nearer to baseline value as before induction.

Table 5: Diastolic Blood Pressure (DBP) change as compared to baseline

Sr.No.	Diastolic Blood Pressure (DBP)	Group I		Group II		T-value	p-value	
		Mean	SD	Mean	SD			
1	Before Induction	85.45	6.54	86.54	4.85	0.94	0.34	NS
2	After Induction	83.14	5.77	82.13	5.98	0.85	0.39	NS
3	After relaxant			83.01	6.97	-	-	
4	Just after intubation	89.08	5.98	88.86	5.05	-1.97	0.84	NS
5	One min. after intubation	84.65	6.80	81.98	5.65	-2.1	0.0352	S
6	Two min. after Intubation	84.76	6.96	82.01	5.25	-2.079	0.0403	S
7	Five min. after intubation	84.89	3.34	85.85	4.14	1.2	0.2049	NS

Table 5 indicates before induction value of DBP (base line) value as 85.45±6.54 and 86.54±4.85 in group I and II respectively (p=0.34, T value=0.94). There was slight decrease in DBP just after induction with mean 82.13±5.98 and 83.14±5.77 in group I and II respectfully. . However, there was slight increase in DBP just after intubation with mean 88.86±5.05 and 89.08±5.98 in group I and group II respectively. These results were also not statistically significant (P=0.84). After One minute and two minute slight decrease in DBP was observed. After 5min result of DBP were 84.49 ±3.34 and 86.65.98±5.34 in group I and II which is also statistically not significant (P=0.01), also these value are nearer to baseline value as before induction.

Discussion

Anaesthesia induction with short-acting hypnotic medications is facilitated by the simultaneous administration of a depolarizing muscle relaxant such as succinylcholine for endotracheal intubation. Even non-depolarizing muscle relaxants may be associated with undesirable side effects such as prolonged neuromuscular blockage, the need to reverse neuromuscular blockage, or the inability to reverse paralysis rapidly if necessary, especially in context with difficult airway. For these purposes, many researchers have found a method of providing good intubating conditions rapidly without the use of muscle relaxants[87].

In a study by McNeil IA[88] it was observed that patients who were intubated after remifentanil had dose-dependent intubating conditions, similar to those developed with succinylcholine at 4 micrograms/kg. Post-induction mean arterial pressure in remifentanil 2 micrograms/kg, remifentanil 4 micrograms/kg and succinylcholine 1 mg/kg group has been observed to be decreased from baseline values by 21 percent, 28 percent and 8 percent (P > 0.05).Similar results were observed in the present study like, there was slight decrease in mean arterial pressure initially after induction with mean 83.26 ±6.78 and 82.58±6.89 in group I and group II respectively.

The pre induction mean arterial pressure (MAP) (baseline) was 93.25±7.68 and 94.78±9.52 in group I and group II respectively (p=0.3786, T value=0.884). One min. after intubation in group I and II responses were 87.40±5.42 and 84.83±6.89 respectively. Two min. after Intubation in group I and II responses were 88.54±5.48 and 85.87±6.73 respectively. These were statistically significant (p=0.040, p=0.032). However, after 5min result of MAP were 92.48±4.79 and 91.55±5.98 in group I and II which is also statistically not significant, also these values were nearer to baseline value as before induction.

Before induction SBP (base line) values were 126.30±12.68 and 128.78±11.24 in group I and II. There was slight decrease in SBP just after induction with mean 121.45±9.56 and 119.08±9.56 in group I and II respectively (p=0.25 not significant). However, there was slight increase in SBP just after intubation with mean of 131.09±12.87 and 130.09±13.07 in group I and group II respectively. These results were not statistically significant (P=0.70). After One minute and two minute slight decrease SBP was observed p value of which were 0.01 and 0.02 respectively. After 5min result of SBP were 124.43±8.96 and and127.98±9.76 in group I and II respectively which is also statistically not significant (P=0.06), also these value are nearer to baseline value as before induction.

Similarly before induction value of DBP (base line) value were 85.45±6.54 and 86.54±4.85 in group I and II respectively (p=0.34, T value=0.94). There was slight decrease in DBP just after induction with mean 83.14±5.77 and 82.13±5.98 in group I and II respectively (p=0.39). However, there was slight increase in DBP just after intubation with mean 89.08±5.98 and 88.86±5.05 in group I and group II respectively. These results were also not statistically significant (P=0.84). After One minute and two minute slight decrease in DBP was observed p value of which were 0.0352 and 0.0401 which were statistically significant when compared in either group. After 5min result of DBP were 84.49 ±3.34 and 85.85±4.14 in group I and II which is also statistically not significant (P=0.2049), also these value are nearer to baseline value as before induction.

Conclusion

From above study we can conclude that, our technique of using Propofol with inhalational agent for intubation proves to be promising in difficult airway situations and in cases where use muscle relaxant of both classes are contraindicated for intubation of trachea e.g. neuromuscular disorder like myasthenia gravis. Use Propofol plus opioids and lignocaine spray without the use of muscle relaxant to achieve similar intubating conditions without much change in hemodynamics.

Limitation of our study is present trial was designed for elective cases and emergency cases were excluded, ASAIII, ASAII, MMTIII, MMTIV were excluded from our study. This study in future can be applied for patients where use muscle relaxant of both classes are contraindicated for intubation of trachea such as neuromuscular disorder like myasthenia gravis.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Study of Semen Parameter Contributing Infertility in Vidarbha Region

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Abstract

Introduction: As per WHO 2010 guidelines it states that, after one year of unprotected sexual relation if couple unable to achieve pregnancy can be labelled as infertility. The European societies for human reproduction and embryology (ESHRE) defines infertility as, the failure of pregnancy occur within two year of regular coital exposure. The data reveals in an average 50% of all infertile couple, infertility could be caused by male partner. Infertility affects both partner equally and on an average 10% to 15% of couples (Either male or female or both) in developed country. The aim of this study is to understand abnormal semen parameters of infertile male partner visiting infertility clinic and to study insight of the prevalence of abnormal semen parameters in vidarbha region, also to study cause infertility in male.

Aim and Objective: To study the prevalence of male factor contributing infertility with semen parameter in vidarbha region.

Material and Methodology: This is been carried out from September 2019 to February 2020. This is retrospective study of patients visited to our infertility clinic Wardha Test Tube Baby Centre, AVBRH, JNMC for infertility reason.

Observation and Result: It is observed that, 77 (64.16%) patients were from 31-40 years of age group. Out of total 120 patients, 71(59.17%) partner showed abnormal semen parameter and remaining 49 (40.83%) showed normal seminogram. This indicates that contribution of male pattern infertility was 59.17% in infertile couple who enrolled for the study. Out of 71 patients, 50.70% patients were showed abnormality as oligozoospermia, 19.72% azoospermia, 12.67% asthenozoospermia, 11.26% Cryptozoospermia and 5.63% teratozoospermia.

Conclusion: The endocrinologist/androloger's role is primary and critical for full diagnostic evaluation and etiological therapeutic potential as reported in a wide range of pregnancies. We also confirm the importance of research on the semen parameters that leads to understanding between fertile and infertile people.

Keywords: *Semen, Infertility, ESHRE, ICSI.*

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Introduction

As per WHO 2010 guidelines it states that, after one year of unprotected sexual relation if couple unable to achieve pregnancy can be labelled as infertility. The European societies for human reproduction and embryology (ESHRE) defines infertility as, the failure

of pregnancy occur within two year of regular coital exposure. The data reveals in an average 50% of all infertile couple, infertility could be caused by male partner [1]. Infertility can affect patient by means of big financial losses and with mental stress also, currently it is affecting approximately 4.9 to 7.2 crore couple all over the world. Infertility affects both partner equally and on an average 10% to 15% of couples (Either male or female or both) in developed country [2]. Male factors accounted for infertility are greater then in female[3] Semen analysis is very important tool to diagnose male infertility, it gives long range of information about the status of infertility in male, WHO have given various guidelines of semen analysis. As per WHO 2010 guidelines oligozoospermia, asthenozoospermia, azoospermia, teratozoospermia, and mixed multiple abnormality (eg. oligoasthenoteratozoospermia) are term used for abnormal semen parameters, that can contribute infertility in male. In half of infertile male partner, cause of infertility is abnormal seminogram, approximately in 30% to 40% infertile male, causes of infertility are hidden. When we are unable to correlate Infertility with abnormal semen parameter it can be called as idiopathic infertility, it may occur due to several reasons like as failure of endocrine, ROS (Reactive oxygen species), and genetic defects[4], also in recent years functional defects in male partner like erectile dysfunction and ejaculatory dysfunction have increased. Infertility in male can also occur due to defects in spermatogenesis and it can partly be attributed to neurogenic factor, genital tumour, germ cell aplasia, obstruction in transport of sperm, varicocele in testes or toxins present in environment[5]. Primary infertility can be observed in 1 of 8 couple as well as secondary infertility can be observed in 1 in 6 couples [4]. As infertility is not life threatening disorder it received very less attention than other complex diseases. In an current scenario ICSI (Intra cytoplasmic sperm injection) is the treatment of choice for unknown cause of male infertility[6] also pregnancy rates higher if frozen-thawed embryos are used for transfer[7]. The aim of this study is to understand abnormal semen parameters of infertile male partner visiting infertility clinic and to study insight of the prevalence of abnormal semen parameters and cause of infertility in male at Vidarbha region.

Aims and Objective:

1. To study the prevalence of male factor contributing infertility in Vidarbha region.
2. To correlate prevalence of male infertility with semen parameter.
3. To identify possible reasons contributing infertility in male.

Material and Methodology

This is been carried out from September 2019 to February 2020. This is retrospective study of patients visited to our infertility clinic Wardha Test Tube Baby Centre, AVBRH, JNMC for infertility reason.

Inclusion Criteria:

Infertile couple with male factor

Patient with Primary infertility

Patient giving consent for study

Exclusion Criteria:

Patient with secondary infertility

Patient having infection like HIV, HbsAg etc

Patient having female factor for infertility

Patient not giving consent for study

History obtained from patient specific questionnaire (Demographic, age, Duration of infertility, BMI, frequency of sexual activity data obtained) format and medical history of patient was obtained from their previous medical records. All the recruited couples had regular and unprotected sexual activity from more than one year still they did not achieved desired pregnancy. Semen analysis of all patients was performed at Wardha Test Tube Baby Centre. Semen analysis carried out after minimum 3 days of abstinence period. Semen analysis was done according to the WHO 2010 laboratory manual[8]. The data collection were made according to their age, duration of infertility, BMI, semen quality and sperm morphology as oligozoospermia, asthenozoospermia, azoospermia, teratozoospermia, and mixed multiple abnormalities (eg. oligoasthenoteratozoospermia).

Observation and Result

It is observed that, 77 (64.16%) patients were from 31-40 years of age group. Out of total 120 patients, 71(59.17%) partner showed abnormal semen parameter and remaining 49 (40.83%) showed normal seminogram. (Table 1 and Figure. 1) This indicates that contribution of male pattern infertility was 59.17% in infertile couple who enrolled for the study. Out of 71 patients, 50.70% patients were showed abnormality as oligozoospermia,

19.72% azoospermia, 12.67% asthenozoospermia, 11.26% Cryptozoospermia and 5.63% teratozoospermia (Table 2 and Figure.2). In our study maximum patients were from 30-40 years of age group. The infertility duration was lesser than or equal to 5yrs in 47% patients, this shows that maximum couple came for treatment within 5 years of infertility. It is also observed that patient with higher BMI has lower number of sperm count, most of oligozoospermia patient were suffering from overweight or obesity problem.

Table 1: Patient with Normal and Abnormal semen parameter

Sr No	Quality of semen	Number of patient
1	Abnormal Semen	71 (59.17%)
2	Normal semen	49 (40.83%)

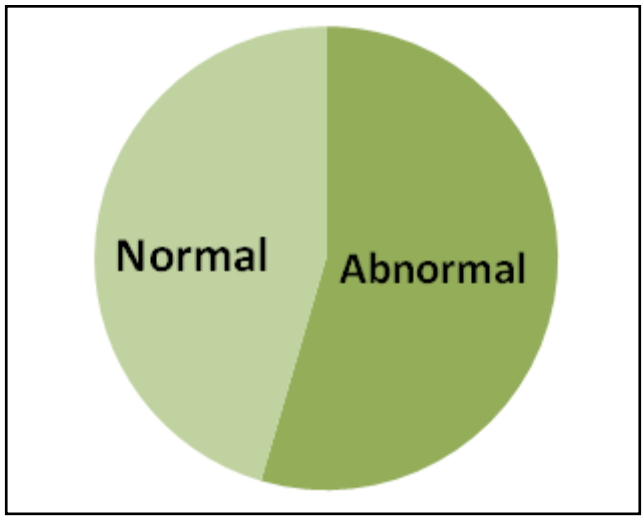


Figure. 1 Shows Normal and Abnormal semen parameter

Table 2: Proportion of abnormalities present in semen

Sr.No.	Abnormality	Number of patient	Percentage
1	Azoospermia	14	19.72%
2	Oligozoospermia	36	50.70%
3	Asthenozoospermia	09	12.67%
4	Teratozoospermia	04	5.63%
5	Cryptozoospermia	08	11.26%
Total		**71**	

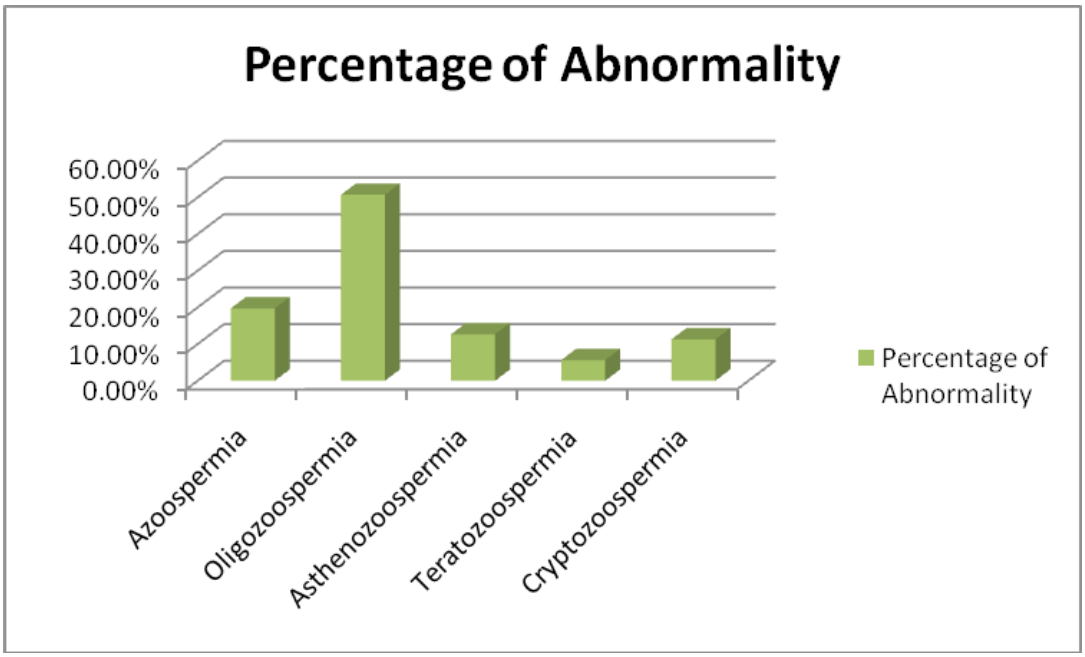


Figure. 2 Shows abnormalities present in semen

Discussion

There were very few literatures present on male infertility in vidarbha region. Similar study done by Khan et al[9] in Pakistan, Joshi et al[10] in Bangalore, Jain et al[11], Jajoo et al[12] and by P. R. Pant[13] in Nepal, they also found oligozoospermia to be the most common cause of infertility in male. In african country study done in a nigerian by Ugwuja et al[14], they found motility defects were most common. In our study we found only in 12.67% cases. Literature shows the prevalence rate in azoospermic man was 10 to 20% or 1% man in the general population[15] that is almost akin with our observation. Seda Karabulut et al[15] revealed that the incidence of cryptozoospermia amongs turkey population was 8.73% that is close with the our finding. Kruger et al[16] study revealed that approximately 4 % of infertile men have isolated teratozoospermia as indicated by Tygerberg's strict criteria (< 5 percent of normal forms) this is also nearly akin to our observation. However, this is now documented that even though sperm concentration or motility is good, a morphological defect may be the dominant factor reflecting the actual sperm fertilization capacity[17]. Results variability may be seen due to local ethnic differences, environmental variations, dietary disparity, social life and evolving lifestyle factors. Currently very few therapies are available to treat male infertility, proper diagnosis of idiopathic cause and treatment to reverse the disorder would be the game changer role to promote male partner fertility[18].

Limitation: The limitation of this study is that, we took only patient age and BMI into consideration, there may be many other factors like occupation, and drugs, etc. Hence, we can work in many directions to prevent these conditions that can affect fertility in males.

Conclusion

The endocrinologist/androloger's and dietician role is primary and critical for full diagnostic evaluation and etiological therapeutic potential as reported in a wide range of pregnancies. We also confirm the importance of research on the semen parameters that leads to understanding between fertile and infertile people.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Outcome of Infero Temporal Suture Less and Glue Free Conjunctival Autograft in Patients with Pterygium at a Tertiary Care Hospital of Vidarbha Region

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Abstract

Introduction: Pterygium is a common degenerative condition of the conjunctiva specially in the dry and arid climate. There are numerous treatment options for pterygium. Gluefree & Sutureless conjunctival autografting is one of the popular method to reduce its (Pterygium) recurrence.

Purpose: To study outcome of inferior temporal conjunctival autografting which is sutureless & gluefree in Pterygium patients.

Material and Method: A Prospective study was carried out on 104 out of 123 patients, which met Inclusion criteria. Pterygium surgery procedure include, pterygium excision followed by inferior conjunctival autografting without any glue and suture. The patients were followed up post-operatively on day 1, 1 week, 15 days, 1 month, 2 months. They all were examined for complications such as graft displacement, recurrence, haemorrhage along with routine post- operative examination.

Results: The age of Patients varied between 21 to 70 years. 62.50 % of which were Male. Common age group in our study was 41-50 years. Redness & watering was most common symptoms in our study. Recurrence was seen 09 patients & graft displacement was seen in 7 patients. Recurrence was most commonly seen in patients having grade 3 Pterygium.

Conclusion: Pterygium excision followed by inferior conjunctival grafting has an advantage of leaving the superior conjunctiva (commonly used in conjunctival autografting procedure) for glaucoma or cataract surgery if need arises . its also economical in context to our Country. The study aims at exploring options to improve the quality of life in cosmetically and visually challenged patients

Keywords: *Pterygium, Inferior conjunctival autografting, glue-free and suture less, superior conjunctival autografting, Recurrence, graft displacement.*

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Introduction

Pterygium is a common degenerative condition of the conjunctiva specially in the dry and arid climate. Pterygium has high prevalence rates all over the world including India which is part of " Pterygium belt" described by Cameron[1]. Nasal side pterygium is more common than Temporal side of Pterygium. There are numerous treatment options for pterygium, surgery

being the mainstay. Kenyon and colleagues proposed the use of free Conjunctival autograft for preventing recurrence, which is main complication, after surgery [2]. Conjunctival superior autografting is a commonly used procedure nowadays. Other option include Fibrin glue but carries potential risk of prion and viral transmission and anaphylaxis [3]. The latest techniques include glue free and suture less inferior conjunctival autograft placement

Aims and Objective: To study the efficacy and outcome of glue free and suture less inferior conjunctival autograft placement for treatment of pterygium.

Inclusion Criteria:

Both genders

Age above 20 years

Exclusion Criteria:

Patients with history of recurrent pterygium.

Severe dry eye patients

Pseudo pterygium

Material and Method

This was a prospective non comparative study duration from august 2019 to January 2020, in the Dept. of Ophthalmology at Datta Meghe Medical College, SHMRC Hospital Nagpur in collaboration with Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi, Meghe Wardha. Primary pterygium patients managed by surgical interventional procedure include excision of pterygium with infero conjunctival autograft which was glue free and suture less from the same eye. An informed consent from all patients was taken before the procedure and concerned patients were told to instil plain antibiotic eyedrop moxifloxacin four times a day two days prior to a surgery.

Surgical procedure: Patients who were enrolled undergo pterygium excision followed by infero conjunctival autografting under peri bulbar block.

Confirmation of the eye of the patient which is to be operated done by the operating surgeon and then only peri bulbar block was given along with on table instructions. Betadine painting and draping done under aseptic conditions. the pterygium was excised by using Bard Parker knife with number 11 blade which was continued up to the limbus. The surgeon tried to clean and free the pterygium which had encroached the cornea. Then by holding the pterygium at the apex and by stretching it a little, it was cut with the help of conjunctival scissors and bare sclera was created. Scleral bed was cleaned by cauterization and we made sure that it would free from Tenon's capsule. Castor – Vejjo calliper was used to determine the size of the scleral bed. The graft was marked approximately (1mm) larger than scleral bed on the inferior conjunctiva (mostly temporal side). After giving a nick the thinnest possible continuous graft was obtained which was free from tenon's capsule. Once the limbus was reached the graft was flipped over and on to the cornea. The graft was meticulously dissected from tenons attachment. The graft was then placed on the bare sclera by maintaining the orientation i.e the limbal side of the graft to the limbal side of the bare sclera. The graft's position was checked after 4 to 5 mins by using Macphearsons's forceps. A single drop of an antibiotic eye drop was put in the operating eye, which was then pad bandaged. Patient was kept as an indoor patient for a day and then discharged after day 1 post op examination. Patient was asked to follow up after 7 days, 15 days, I month and two months from the day of surgery. All surgeries were performed by the same surgeon.

Scope of the study: Vidarbha region and its surroundings have dry, hot & dusty climate.

Main occupation of the people in this place is farming and daily labourers, mostly outdoor occupation. The study aims at exploring options to improve the quality of life in cosmetically and visually challenged patients

Observations and Results

Out of 123 patients registered in our pterygium surgery OT register. 104 cases were analysed which met the Inclusion criteria. Cases were divided into four arbitrary grades as shown in table 1

Table No. 1: Slit-Lamp Gradation

Grades of pterygium	Patients
Grades 1 Pterygium- invading the limbus	20
Grades 2 of Pterygium- midway between limbus & pupil	56
Grade 3 of Pterygium- Reaching up to pupillary margin	24
Grade 4 of Pterygium- Crossing pupillary margin	04

Above table shows no. of patients enrolled in different grades of pterygium most common patient in our study were having grade 2 pterygium

Table No. 2: Distribution of cases according to sex

Males	Females	Ratio
65	39	1.66 : 1

Above table shows us sex (gender) wise distribution in our study. Males are in majority compared to females.

Table 3: Distribution of patients according to age.

Age group in years	Male	Female	Total cases
21-30	03	06	09
31-40	12	02	14
41-50	21	18	39
51-60	16	11	27
61-70	13	02	15

Above table shows us different age group and total no. Cases enlisted in it. 39 out of 104 cases belong to 41 to 50 yrs of age group.

Table 4: Complaints of the Patients at the time of admission.

Complaints	No. of patients
Redness & watering	54
Foreign body sensation	35
Cosmetic blemish	10
Diminution of Vision	05

Table no 4 shows us what symptoms braught them to eye opd. Redness with watering was most common symptom in our study.

Table 5: Post operative complications in Cases.

Complication	No. of Cases
Graft displacement	07
Granuloma formation	03
Conj cyst	02
Recurrence	09
Sub. Conj haemorrhage	03

Above table shows of different complications after surgical intervention. Recurrence was troublesome & common complication

Table 6: Recurrence rate according grade of Pterygium.

Grade of Pterygium	No. of patients
Grade 1	nil
Grade 2	02
Grade 3	06
Grade 4	01

Above table shows us grade of pterygium in which recurrence was seen. Patients i.e. out of 9 cases of recurrence 6 were having grade 3 Pterygium. Grade 1 pterygium patients had no recurrence

Discussion

Out of 104 cases which we enrolled in our study, we found 56 cases belong to grade 3 pterygium, according to Slit lamp gradation. In percentage wise maximum ie 53.84%. Distribution of cases according to Gender, Male predominance seen . 62.50% male & 37.50% female. Male to female ratio was 1.66 : 1, according to TABLE NO 2. These finding are not similar to a study done by Priya VR et al[4] showed that 69% were female and 31% were male. But our findings are replicated in study done by Poonam Bhargava et al[5] where male predominance is seen. According to age wise distribution, We found that highest no. patients were seen in age group 41- 50 yrs, total 39 cases (21 male patient and 18 female patient) . 37.5 % of total cases. Which is replicated in the study done by Thangjam Amit Singh & et al found to be more marked in the 4th-5th decade of life6. Next common age group was 51- 60 yrs. Total cases 27 (16 male and 11 female patient) 25. 96 % of total cases.

One interesting observation was seen in age group 21-30 yrs, out of 9 cases 6 female and only 3 male. Female predominace seen in only this age group. Subjective pre-operative complaints were also taken under consideration, we found that in our region redness with watering is there main symptons which mostly braught them to hospital.

In this Study, Recurrence remain to be most common post operative complication . It was seen in 9 patients which was approx. 8.65 % of total cases. Recurrence occurred in 3 out of 9 patients after 1month post operatively & remaining 6 patients after 1.5 – 2 months after Surgery, which is more than a study done by Vichare N et al[7] showed that at the end of final follow up at 6 months, 3 cases (10%) from suture group and

1 case (3.33%) from fibrin group had recurrence. Also more than another study done by Thangjam Amit Singh & et al[6] where recurrence rate was 1.28% after pterygium excision with conjunctival autografting using fibrin glue (group A) and 2.24% after excision and autografting with suture (groupB). Recurrence of pterygia was more common in patients younger than 40 yrs.

Displacement of the graft was 2 nd most common complication seen in our study, as we are procuring inferior graft, sometimes it was difficult to get ideal graft cause surgeons handedness and surgical maneuver compared to superior conj .grafting . this complication was marginally higher in our study compared to study done by Poonam bhargava et al[5](in 3oo cases 16 cases show graft displacement ie 5.33%) . in our study 7 cases found to have graft displacement ie 6.73 %

According to Table no. 6, recurrence was more common in grade 3 pterygium. TAN DTH & et al, suggested that fleshy Pterygium had a greater chance of recurrence than thin pterygia [8]

India is a country of 1.32 billion. The World Health Organization recommends, one doctor to serve 1,000 people, across all levels of care[9]

In India, there are >100 million elderly people who often have comorbidities that are often neglected[10]. Vision 2015 of MCI emphasizes on 'Competency-based learning' and insists for assessment of newer learning experiences, competencies, along with certification of essential skills[11]. Due attention is needed on patient specific systemic conditions like diabetes[12-17], hypertension[18-26], respiratory and renal disorders[27-33].

Conclusion

Pterygium excision followed by inferior conjunctival grafting has an advantage of leaving the superior conjunctiva (commonly used in conjunctival autografting procedure) for glaucoma or cataract surgery if need arises. it's also economical with minimum to moderate complications such as recurrence & graft displacement considering existing ways of management of Pterygium.

Limitations: Shorter follow up time may underestimate the recurrence rate.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Incidence of Post Dural Puncture Headache in Our Set Up with Quincke Spinal Needle: An Observational Crossectional Study

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Abstract

Introduction: Post-dural puncture headache (PDPH) is an iatrogenic complication associated with spinal anaesthesia. The occurrence of PDPH is determined by different factors. They are sex, age, pregnancy, past PDPH history, needle tip shape and needle size, bevel orientation, number of attempts, and approach used for lumbar puncture. We conducted the present study to find the incidence of PDPH.

Material and Method: After ethics committee approval, we observed 500 patients who had undergone surgery for lower abdomen and lower limb. Patients were randomly divided in Group A and Group B 250 patients in each group and received spinal anaesthesia via 23 and 25 quincke spinal needle respectively. They were followed post operatively regularly for development of any signs and symptoms of post dual puncture headache, which was end point of study.

Results: Out of 500 patients 6 patient developed PDPH. The incidence was 1.2%. There was no statistically significance with sex, number of attempts and guage of spinal needle.

Conclusion: In our series the incidence was 1.2% which is far less than other studies. Taking into consideration cost effectiveness & success rate we feel that in our setup we can continue with use of 23 & 25-gauge quincke needle.

Keywords: *Spinal Anaesthesia, Post dural puncture headache, Quinke spinal needle.*

Introduction

Spinal anaesthesia is one of the commonest anaesthetic technique used in lower abdominal and lower limb surgeries[1]. Variables of the needle design, such as needle size and needle shape have been adjusted to allow intrathecal injection and rapid flow of cerebral spinal fluid (CSF), while at the same time restricting Dural damage and loss of CSF[2,3] .Post dural puncture headache (PDPH) is an iatrogenic complication associated with spinal anaesthesia due to persistent leakage of cerebrospinal fluid (CSF) from the site of the puncture resulting in meningeal stretch. The occurrence of PDPH is determined by different factors. They are sex, age, pregnancy, past PDPH history, psychological stress of invasive procedure, needle tip shape and needle size, bevel orientation, number of attempts, and approach used for lumbar puncture[4,5,6].

A headache that occurs within 5 days of lumbar puncture and aggravates when standing or sitting and relives when lying flat is classified as postdural puncture

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headache (PDPH) on the grounds of the 3rd edition of the International Classification of Headache Disorder[7]. PDPH is a well-known iatrogenic complication of spinal anaesthesia that remains a major problem[8-11]. It is the downside to the use of spinal anaesthesia or diagnostic lumbar puncture resulting from the loss of CSF and the resultant strain on meninges excited by the hole formed in the dural tissues[2,12]. PDPH is normally moderate, without PDPH any movement restrictions, and no treatment needed when patients with extreme PDPH were confined to bed. An epidural blood patch (EBP), which injected the patient's own blood into the epidural space to cover the hole produced in the dural tissues, was used to treat severe PDPH[13]. PDPH's modifiable risk factors included needle size, needle shape, bevel and insertion angle orientation, stylus replacement, and operator experience[14]. Needle size could be the most significant factor in PDPH development[8,15,16]. Spinal needles commonly used today are 22 to 27 G, but sizes ranging from 19 to 30 G are available[1]. The incidence of PDPH following spinal anesthesia done with Quincke, a cutting needle, is 36% with a 22 G needle, 25% with a 25 G needle, 2% to 12% with a 26 G needle, and less than 2% with a 27 G needle[8,16-19]. The smaller needle diameter decreases the risk of PDPH[17,20]. 29 G needles will minimize the complication of PDPH but they are too thin to use[17].

Extremely thin spinal needle (29 G or smaller) will increase the probability of spinal anaesthesia failure. And multiple dural punctures resulting from unsuccessful puncture will increase the PDPH rate[15,21-24]. And occasionally CSF is too viscous for a small needle to get through[10]. As for the tip style, the cutting point needles were easier to insert through the skin and ligaments, while the dura mater was easier to identify by the pencil-point needles[25,26]. Some studies argued that the incidence of PDPH was not significantly different between cutting-point and pencil-point needles[27,28]. Some, opposed arguing noncutting needle lead to lower rate of headache[29-31]. Zhang D et al published a meta-analysis in 2000 had compared the frequency of PDPH between Quincke and pencil-point spinal needles which suggested that pencil-point spinal needle will significantly reduce PDPH rate compared with Quincke spinal needles[29]. However in this meta-analysis only Quincke spinal needle were included, while other cutting spinal needles were ignored. Zhang D et al in 2016 done a meta-analysis which showed Whitacre spinal needle was better than Quincke spinal needle.[24]

As the general assumption has been that the pencil point needle is better than cutting-point spinal needle for preventing PDPH. The cost of pencil point needle is much higher than cutting point spinal needle. This factor is of very much concern in our country. We decided to conduct an observational crossectional study to know the Incidence of post dural puncture headache in our set up with quincke spinal needle.

Material and Method

Methodology: Present study was conducted in the department of Anaesthesiology at Datta Meghe Medical College and Shalinitai Meghe Hospital and Research Centre Nagpur, in collaboration with Jawaharlal Nehru Medical College Datta Meghe Institute of Medical Sciences, Sawangi Meghe, Wardha. Ethical committee approval taken. All patients underwent routine pre anaesthesia check up with relevant investigations. We selected 500 patients who are posted to undergone surgery for lower abdomen and lower limb. Patients were randomly divided in Group A and Group B 250 patients in each group. Group A patients were received spinal anaesthesia with 23 gauge quincke spinal needle.

Group B patients were recieved spinal anaesthesia with 25 guage quincke spinal needle.

Inclusion criteria:

- Age 18yrs – 60yrs
- No Pre-existing headache
- ASA grade I and II.

Exclusion Criteria:

- Pre-existing headache.
- Age below 18yrs and above 60 yrs.
- ASA grade III and IV
- Contraindications to spinal anaesthesia.
- All were monitored routinely according to standard ISA guidelines. They were followed post operatively for development of any sign and symptoms of PDPH.

Patients were observed for

- Headache in frontal/occipital area,
- Increasing with sitting and standing
- Reliving with lying down.

- Any neck muscle spasm by
- Allowing them to describe themselves

Followed on day 0,1st, 2nd, 3rd and after discharge telephonically conversation with the concerning surgeon regarding the above symptoms for 4th and 5th day. Assuming that no feedback means no complaints. This we considered as end point of our study.

Those with signs and symptoms suggestive of PDPH were treated with bedrest, adequate hydration, IV Fluids, and analgesics like aspirin, codeine, paracetamol and sphenopalatine ganglion block twice daily for 2 days. Patients had complete relief of headache with above treatment. In sphenopalatine ganglion block patient were placed supine and bilateral nostrils anaesthetised with lignocaine jelly. Each nostril then inserted by swab stick soaked by 4% lignocaine in upward and backward direction till resistance is felt. After 5 minutes swab stick is removed.

Data collected and incidence of post dural puncture headache calculated.

Results

In this study it was observed that Mean age of participants was 30.1+9.5 years and Mean weight of participant's was 56.7+ 8.71 kg.

Table 1: Distribution of Study Group Based on Sex

Sex	Frequency	Percentage
Male	278	55.6%
Female	222	44.4%
Total	**500**	**100%**

Of the total 500 participants 278(55.6%) were male and 222 (44.4%) were female

Table 2: Distribution of study group according to gauge of quincke spinal needle

Guage of Quincke Needle	Frequency	Percentage
23 Guage	250	50%
25 Guage	250	50%
Total	**500**	**100%**

250 participants were received spinal anaesthesia by 23 gauze needle and rest 250 by 25 quaze needle**.**

Table 3: Distribution according to the frequency of headache:

Headache	Frequency	Percentage
Absent	494	98.8 %
Present	6	1.2%
Total	**500**	**100%**

In our study 6(1.2%) participants complained headache, while no headache was recorded in 494 (98.8%) cases.

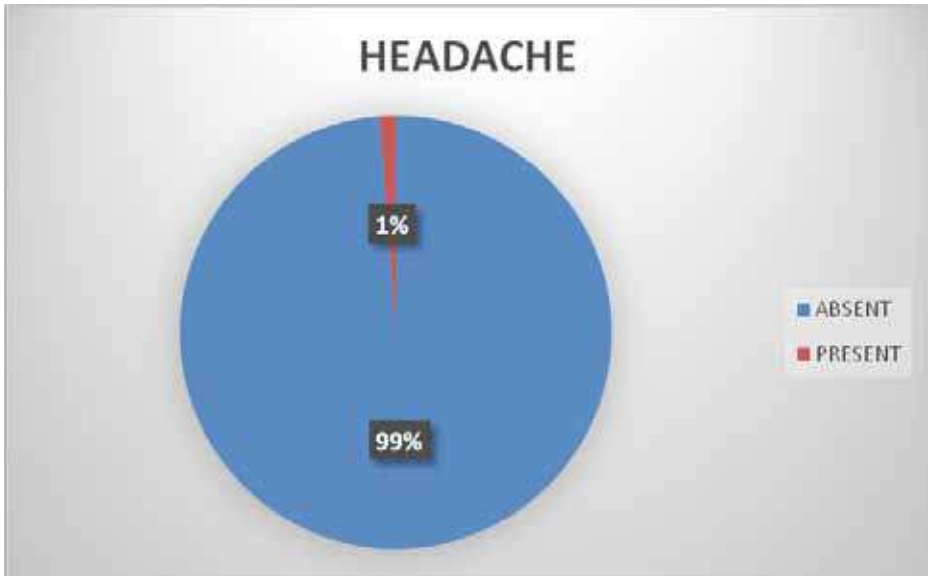


Figure 1: Headache

Table 4: Frequency of headache depending on type of needle:

Headache (p/a)	Gauge of Quincken Spiinal Needle				Total	P value
	23		25			
	N	%	N	%		
Absent	247	98.8	247	98.8	494	0.7, NS
Present	3	1.2	3	1.2	6	
Total	**250**	**100**	**250**	**100**	**500**	

NS: Not Significant

The incidence of headache is 1.2% for both 23 and 25 gauze needle (P=0.7,NS).

Table 5: Statistical analysis of headache based on sex

Sex	Headache				Total	CHI Square	P
	Absent		Present				
	N	%	N	%			
Male	276	55.2	2	0.4	278	1.2	0.269
Female	218	45.6	4	0.8	222		
Total	**494**	**98.8**	**6**	**1.2**	**500**		

The incidence of headache in males is 0.4% and females is 0.8%. (P=0.269, NS)

Table 6: Statistical relationship between no of attempts and incidence of headache

No of Attempts	Headache				Total	CHI Square	P
	Absent		Present				
	N	%	N	%			
1	396	79.2	4	0.8	400	0.675	0.411, NS
2	98	19.6	2	0.4	100		
Total	**494**	**98.8**	**6**	**1.2**	**500**		

0.8% of incidence was there for spinal on 1st attempt and 0.4 on second attempt.

Discussion

Regional anaesthesia particularly spinal anaesthesia is well established popular technique. Because of its safety profile, ease of administration and cost effectiveness it continues to be the technique of choice in most of below umbilical surgeries unless contraindicated. Never the less like other techniques it is also associated with complications. Post-dural puncture headache (PDPH) is one of the complication which needs concern and attention. Incidence of PDPH depends upon number of variables such as age of patient, sex, size, type & orientation of needle.[2,4,5,7-29] The frequency of PDPH ranges from 0% to 36%.[11,19-22]

In our study 6 patients out of 500 (1.2%) developed headache. Among this 3 (0.6%) patients belonged to 23 NO quincke group and 3 (0.6%) belonged to 25 NO QUINCKE group. Study done by Malik et al found out the incidence of PDPH was 5 percent with 25 G Quincke Babcock spinal needle.[32]

Large spinal needles will produce bigger defects in dura so chance of dural puncture headache is more in comparison with smaller needles which produce small dural defects & less incidence of headache[14,20]. This large bore needle was associated with an incidence of 70 percent whereas advanced small size needles are less likely to produce higher incidence. In a study done by

Weasel observed 12.8% incidence in pregnant patients with 27G Quincke needle was used.[33]

In contrast in our study we observed overall 1.2% incidence with both 23 and 25 gauze quincke spinal needle. We divided our patients in two groups, 250 in each based on the quaze of quincke spinal needle 23 and 25. We haven't found any significance of quaze of spinal needle associated with PDPH, p value is 0.7.

Number of puncture attempts can be reduced by the experience of the Anaesthesiologist. Experienced anaesthesiologist is likely to get success in first pass. In our study spinal anaesthesia was performed by the anaesthesiologist with atleast 3 years of experience in single puncture in 400 patient out of which 4(0.8%) patient had PDPH and in second attempt in 100 patient out of which 2(0.4%) patient had PDPH. We haven't found any association with the no of attempts and associated PDPH, p value is 0.4. Wail N khraise et al conducted a study in 2015 showing that the incidence of PDPH is higher with the repeated no of dural puncture headache.[34]

In our study we haven't found any significance regarding the sex of patient for PDPH. The p value is 0.26. Total 4(0.8%) female patients and 2 (0.4%) male patient had headache. Faramarz Mosaffa et al conducted a study in 2007-8 stating that females have higher incidence of PDPH after spinal anaesthesia.[35]

Treatment options for Post dural puncture headache includes simple measures such as adequate hydration, NSAID"S to complex procedures as epidural blood patch. Simple measures are very effective in managing most cases of PDPH. In our study NSAID"s hydration & adequate rest and sphenopalatine block relieved headache in patients[36]. Studies on other anaesthetics and related aspects in this region are available [37-44].

CONCLUSION

PDPH is not a rare complication associated with spinal anaesthesia. Though incidence can be reduced it's not preventable. In our series the incidence was 1.2% which is far less than other studies. Taking into consideration cost effectiveness & success rate we feel that in our setup we can continue with use of 23 & 25-gauge quinckie needle.

Limitation of the study:

Though the results are satisfactory with the finding, we need to increase the data base for needle guaze, sex, no of attempts and continue with the follow up may be with formulation of post-operative questionnaire which will be filled by the patient regularly.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Effectiveness of Mind Body Intervention on Subfertile Females: An Approach to Accesses Oocyte Quality

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Abstract

Introduction: In recent times, there is a much greater emphasis on the physical causes of infertility so that psychological effects can be ignored. There are, of course, a number of physical and emotional factors that can induce infertility. Mind and body interventions are efficient in lowering psychological distress thereby increases the ART success rate, fetal outcome by improving the psychological and physiological stress. The purpose of the current study is to assess the relationship between effect of mind body intervention and serum cortisol level and oocyte quality.

Aim and Objectives: To study the effectiveness of mind body intervention on oocyte quality and serum cortisol level in subfertile females.

Materials and Method: This study is done in wardha test tube baby centre AVBRH (SAWANGI) WARDHA Relevant data on the demographics and treatment history as well as the indications for IVF treatment will be recorded. Serum cortisol level of all participant were analysed and then stimulated using a routine short antagonist protocol.

Observation and Result: From total recruited patient, one didn't attended intervention program at all. Four patients attended intervention program from 4 to 6 week in discontinuation manner. Eight patients attended up to 70% to 90% of intervention program and remaining 24 patients attended complete 10 week mind body intervention program. In group of 7 to 9 week intervention 6 patient out of 8 showed decrease in serum cortisol level. Patient who attended complete 10 weeks intervention they are showing significant decrease in serum cortisol level. From 40 patient, 24 attended complete 10 week mind body intervention, among them 16(66%) gave positive response to mature oocyte retrieval.

Discussion: In this research, a mind body intervention on subfertile females was created and to see their response to mature oocyte in IVF study. Study revealed that, there were very few studies examined the role of cortisol in relation to M II oocyte and finally clinical pregnancy outcomes. Two studies reported that pregnancy rates were enhanced by adopting the MBI. This is in support to our study.

Conclusion: This study concludes that, there is direct correlation of serum cortisol level and oocyte quality and Mind-Body intervention is key factor to improve oocyte quality.

Keywords: *Infertility, oocyte, cortisol.*

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Introduction

In recent times, there is a much greater emphasis on the physical causes of infertility so that psychological effects can be ignored. There are, of course, a number of physical and emotional factors that can induce infertility. Many infertile couples encounter conflict over the social significance of pregnancy and negative emotions, current medical research focuses on the diagnosis and treatment of infertility, the degree of psychological and social support is minimal. That may be caused by issues with either spouse and may be of a primary or secondary in nature. [1-3] study revealed that anxiety and, in particular, depression prior to IVF/ICSI therapy were positively correlated with adrenaline levels of urine during treatment. They also found an association of lower adrenaline at oocyte extraction with an increased risk of pregnancy.[4] Cortisol is a steroid hormone produced by the adrenal gland. It is the key hormone in stress and fight or flight response. It is a well-established stress biomarker and is controlled by adrenocorticotropic hormone from the pituitary gland. Its levels can be affected by physical stress, emotional stress, and illness. In case of high level of stress, serum cortisol value is bound to increase, thus it is a reliable indicator in patients undergoing stressful events in life.[5-7] A series of studies have looked at stress during the IVF cycle.[8] But the role of anxiety and stress and the effects of psychological stress on the outcome, it is important to examine these dimensions more closely in women undergoing IVF therapy.[9] Mind and body interventions are efficient in lowering psychological distress thereby increases the ART success rate, fetal outcome by improving the psychological and physiological stress.[10] The purpose of the current study is to assess the relationship between effect of mind body intervention and serum cortisol level and oocyte quality. The perception that psychological stress may prevent a woman from attaining and maintaining a pregnancy has become widely accepted. Mind-body interventions (MBIs) may be an effective tool to help women manage the demands of infertility diagnosis and treatment.

Keywords: Cortisol, Mind-body interventions, IVF, oocyte quality

Aim and Objectives: To study the effectiveness of mind body intervention on oocyte quality and serum cortisol level in subfertile females.

Materials and Method

This study is done in wardha test tube baby centre AVBRH (SAWANGI) WARDHA Relevant data on the demographics and treatment history as well as the indications for IVF treatment will be recorded. Serum cortisol level of all participant were analysed and then stimulated using a routine short antagonist protocol.

Infertile Patients:

↓

Serum cortisol level

1st OPU

↓

Anlysis of oocyte

↓

After 1 month of recovery

↓

8 to 10 week mind body intervention

↓

Serum Cortisol level

↓

2nd OPU

↓

Analysis of oocyte quality

Sample Size: 40 sub fertile female patient.

Inclusion Criteria: Couples suffering from primary and secondary infertility and registered for two

1. OPU cycle
2. Patient from age group 25-35.
3. Patient with normal Anti-Mullerian Hormone

Exclusion Criteria:

1. Patients registered for single IVF cycle.
2. Patients having AMH <1.5
3. Patients not fit for IVF having viral infections like HIV, HbsAg etc.
4. Patients who want to undergo fresh embryo transfer.

Observation and Result

From total recruited patient, one didn't attended intervention program at all. Four patients attended intervention program from 4 to 6 week in discontinuation

manner. Eight patients attended up to 70% to 90% of intervention program and remaining 24 patient attended complete 10 week mind body intervention program. [Table 1, Pie Diagram 1].

Table .1: Duration wise intervention distribution of patient number

Intervention Period Group (Weeks)	No of patient
0	01
01-03	03
04-06	04
07-09	08
10	24
Total	**40**

Patient who attended 4 to 6 week intervention program among them 50% showed positive response in terms of decrease in serum cortisol level. In group of 7 to 9 week intervention 6 patient out of 8 showed decrease in serum cortisol level. Patient who attended complete 10 weeks intervention they are showing significant decrease in serum cortisol level. [Table 2, Pie Diagram 2].

Table. 2: Week wise intervention distribution of patients and their response in terms of change in Serum cortisol value

Intervention Period Group (Weeks)	No of patient	Number of Patient with positive response	Number of Patient with Neutral response	Number of Patient with Negative response
0	01	00	00	01
1-3	03	00	01	02
4-6	04	02	00	02
7-9	08	06	01	01
10	24	17	01	06
Total	**40**	**25**	**03**	**12**

Patient who attended 4 to 6 week intervention they showed slightly positive response in terms of mature oocyte retrieval. In group of 7 to 9 week intervention 50% patient showed positive response and 25% gave negative and neutral response respectively. From 40 patient, 24 attended complete 10 week mind body intervention, among them 16 (66%) gave positive response to mature oocyte retrieval. [Table 3].

Table 3: Week wise intervention distribution of patients and their response in terms of no of retrieved metaphase II oocyte

Intervention Period Group (Weeks)	No of patient	Number of Patient with positive response	Number of Patient with Neutral response	Number of Patient with Negative response
0	01	00	00	01
1-3	03	00	00	03
4-6	04	01	01	02
7-9	08	04	02	02
10	24	16	03	05
Total	**40**	**21**	**06**	**12**

It is observed that after mind body intervention 26 patient shows decreased serum cortisol level and along with them 20 patient also shows improved in their M II oocyte retrieval. Correlation of decrease in Serum cortisol level and M II oocyte retrieval after Mind Body intervention. [Table 4, Pie Diagram 3].

Table 4: Correlation of decrease in Serum cortisol level and M II oocyte retrieval after Mind Body intervention

Number of patient decreased serum cortisol level	26
Number of patient increased MII oocyte retrieval	20
Number of patient increased serum cortisol level	11
Number of patient decreased MII oocyte retrieval	15
Number of patient retained same serum cortisol level	03
Number of patient retained same no of MII oocyte retrieval	05

Discussion

In this research, a mind body intervention on subfertile females was created and to see their response to mature oocyte in IVF study. Study revealed that, there were very few studies examined the role of cortisol in relation to M II oocyte and finally clinical pregnancy outcomes.[11,12] literature also reported that there is no significant differences in cortisol between pregnant and non-pregnant groups report data derived from blood, saliva, or urine sampling.[9,13] The systematic review described two non-blinded RCTs that looked at the effect of MBI on infertile women (N = 566). Outcome results for both non-blinded RCTs was incomplete due to high attrition rate.[14] the study revealed that the impact of MBI on the marital behavior of couples cannot be concluded with respect to effectiveness in pregnancy outcomes.[15] Two studies reported that pregnancy rates were enhanced by adopting the MBI. [16,17] this is in support to our study. Overall, it is fair to believe that anxiety, depression and fertility-specific quality of life have improved over time in tandem with MBI for women undergoing IVF treatment.[18] Outcome of this research i.e. analysis of serum cortisol provides a good parameter for the assessment of the oocyte quality and further on IVF outcome.

Conclusion

This study concludes that, there is direct correlation of serum cortisol level and oocyte quality and Mind-Body intervention is key factor to improve oocyte quality. This small scale study gives favorable results. In future, large scale samples if studied, will be a revolution in the field of mind body treatment and assisted reproductive technique for the women facing problem of lesser and immature oocyte retrieval.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Relevance of MCV and Knuckle Pigmentation in Early Diagnosis of Vitamin B12 Deficiency in Children

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Abstract

Background: Vitamin B12 deficiency is common in children, especially who take strict vegetarian diet. Limited data is available on relevance of knuckle pigmentation and elevated MCV for early diagnosis in central India, especially in pediatric population.

Aim: To study association of knuckle pigmentation and elevated MCV with vitamin B 12 deficiency in children in central India

Method: This was hospital based cross sectional study, carried out from March 2019 to February 2020. 41 children with anemia (Hb < 11 gm%) or knuckle pigmentation or elevated MCV (>100 Fl) or any combination of these, were enrolled. The association of knuckle pigmentation and elevated MCV with vitamin B 12 deficiency was studied. Data was analyzed by Chi square test. P value < 0.05 was considered to be statistically significant.

Results: 29 out of 41 subjects had vitamin B 12 deficiency, maximum of which were strictly vegetarian. There was statistically significant association between vitamin b12 deficiency with elevated MCV but not with knuckle pigmentation.

Conclusion: Knuckle pigmentation and elevated MCV value may not be seen in every child with vitamin B 12 deficiency, but their presence gives important clue to early diagnosis and hence leads to early treatment

Keywords: *MCV (Mean Corpuscular Volume), knuckle pigmentation, vitamin B12 deficiency.*

Introduction

Vitamin B12 deficiency is common in India, as a majority of the population is vegetarian. Awareness about malnutrition, and anaemia is also poor among Indian mothers.[1] Vitamin B 12 deficiency is an under recognised, easily correctable cause of anemia, fatigue and neurological symptoms[2]. In children, the most common cause of megaloblastic anemia is vitamin B12 deficiency[3]. Hence childhood vitamin B12 deficiency is common in some parts of India where people follow strict vegetarian diet. Exclusively breastfed infants of vegetarian mothers are at a higher risk[4] Effective public policies are needed to support appropriate infant and young child feeding (IYCF) to ensure adequate child growth and development, especially in low and middle income countries[5]

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A vitamin B deficiency in children (under 19 years) is defined as a serum B12 value of < 229 pmol/L or a MMA value > 0.26 to 0.29 µmol/L[6]. Dermatological manifestations of B12 deficiency include cutaneous and mucosal hyperpigmentation[7] About 19.0 % patients with megaloblastic anemia show knuckle hyperpigmentation and sometimes may be the only marker of vitamin-B12 deficiency[8] Various mechanisms have been suggested to explain hyperpigmentation associated with vitamin B12 deficiency like decreased glutathione levels activating tyrosinase and increased melanogenesis**,** defect in the melanin transfer between melanocytes and keratinocytes, resulting in pigmentary incontinence[9,10] Appropriate treatment results in dramatic clinical and laboratory response in most patients [11]

Several reports state that the incidence of macrocytosis varies from 1.7 to 3.6%. The etiology and demographic profile varies among various western and Indian studies.[12,13,14,15] Higher prevalence of macrocytosis suggests that it is one of the unrecognized and undiagnosed in routine clinical practice[16] Macrocytosis is not accompanied by anemia in upto 60% of cases; however, isolated macrocytosis should always be investigated. Macrocytosis without anemia may indicate early folate or cobalamine deficiency.[17] Macrocytosis is reported in terms of mean corpuscular volume (MCV). Normal MCV values range from 80 to 100 femtoliters (fl) and vary by age and reference laboratory.[18] In the era of automated blood cell counters, macrocytosis is a relatively common finding, with prevalence of 1.7% to 3.6%.[19,20,21] However, the sensitivity and specificity of MCV as a test for detection or exclusion of vitamin B12 deficiency is not well known

The peripheral blood smear is considered to be more sensitive than RBC indices to identify early macrocytic changes, however, MCV may underestimate macrocytosis in over 30% of cases compared to the peripheral blood smear. [22] In patients with folate or vitamin B12 deficiency, macrocytosis is the earliest abnormality seen in complete blood counts. In those patients with elevated MCV values, laboratory tests for vitamin B12 and folate deficiencies are routinely ordered . The lower limits of normal for vitamin B12 levels are not well defined, especially in pediatric age group.[23] There is paucity of data on early recognition of vitamin B12 deficiency in pediatric population in central India. Limited information is available regarding use of MCV and knuckle pigmentation for diagnosis of vitamin B12 deficiency in suburban area, in resource limited setting, hence this study is planned.

Method

The present hospital based cross sectional study was conducted in pediatric department from March 2019 to February 2020, at Shalinitai Meghe Hospital & Research Centre in collaboration with Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences Sawangi Meghe, Wardha, Maharashtra. Ethical clearance was sought for from institutional ethical committee before start of the study. Children of either sex, aged between 6 months to 18 years, presented with Hb < 11 gm % or knuckle pigmentation or MCV > 100 fL or any combination of these, were enrolled in the study after taking informed consent of parents. Children with congenital bone marrow failure syndromes, leukemia, severe protein energy malnutrition, anemia due to surgical cause, infants (i.e babies < 6 month of age) were excluded from the study. In the present study, Anemia is defined as Hb < 11 gm% and categorized as mild (Hb : 9 – <11 gm%), moderate (Hb : 7- < 9 gm%) and severe (Hb: < 7 gm%). Vitamin B 12 deficiency was defined as serum vitamin B12 ≤ 180 pg/ml and furthet classified as Mild (≥ 100- ≤ 180 pg/ml), moderate (≥ 50 – < 100 pg/ml) and severe (< 50 pg/ml).

After enrolling subjects, detailed present history, relevant past history, diet history and family history was obtained. Those subjects were further investigated with CBC, peripheral smear, retic count and serum vitamin B12 level. The association of knuckle pigmentation, elevated MCV with serum Vitamin B12 level was studied. Population based variation in knuckle pigmentation and abnormally elevated MCV was observed. Appropriate treatment was started as per the standard protocol. The information collecte was recorded in a master chart. Data analysis was done using Epi info version 2.3 and percentage, mean, $\chi 2$ and P values were calculated. P value less than 0.05 was taken to denote significant relationship.

Results

Out of 41 subjects enrolled, maximum number of subjects (22, 53.6%) belonged to the age group of 12 years to 18 years, however mean age of presentation was 11.43 years. Proportion of males and females was 51.2% and 48.8 % respectively. Maximum 32 (78.04%) subjects were taking non vegetarian diet. Out of 41 enrolled subjects, 31 (75.60) subjects had anemia, of which 13 (31.7 %) subjects had mild anemia, 15 (36.5 %) had moderate anemia and 3 (7.3 %) had severe anemia. Anemia was detected accidentally in 26 (83.87%) subjects.

Vitamin B12 deficiency was observed in 29 (70.73%) subjects. 14 (34.1%) subjects had mild deficiency, 12 (29.2%) subjects had moderate deficiency and 3 (7.3%) subjects had severe deficiency. Mean value of vitamin B12 was 159 pg/ml. Severe vitamin deficiency was noted predominantly in 12 to 18 years of age group. Out of total 31 anemic subjects, 25 (80.64%) subjects were deficient in vitamin B 12. The commonest presentation of vitamin B 12 deficiency was fever (15, 51.72%) followed by generalized weakness (10, 34.4%) and paleness of body(8, 27.58%). Only one child was presented with decreased scholastic performance.

Knuckle pigmentation was present in 18 (43.90%) subjects, out of which maximum subjects 6 (33.33%) had mild vitamin B12 deficiency. Only 14 (45.16%) anemic subjects had knuckle pigmentation. The association between vitamin B12 deficiency and presence of knuckle pigmentation was not found to be statistically significant by applying chi square test with P value of 0.426.

Table 1: Table showing association of knuckle pigmentation and vitamin B12 deficiency

Knuckle pigmentation	Vitamin B 12 deficiency		P value	X^2 value
	Present	Absent		
Present (n = 18)	13	5	0.426	0.034
Absent (n = 23)	16	7		
Total (n= 41)	29	12		

Elevated MCV (> 100 Fl) was documented in only 6 (20.68%) subjects with vitamin B12 deficiency. Mean MCV value was found to be 82.26.Fl. The association between vitamin B12 deficiency and elevated MCV was found to be statistically significant with P value of 0.0015.

Table 2: Table showing association of MCV and vitamin B12 deficiency

Vitamin B 12 Deficiency	MCV		P value	X^2 value
	< 100 Fl	≥ 100 Fl		
Mild (n= 14)	12	02	0.0015	12.97
Moderate (n= 12)	11	01		
Severe (n= 3)	00	03		

On peripheral smear, macrocytes were found in only 5(17.24%) subjects. The commonest Peripheral smear finding was hypochromic microcytic with anisocytosis.

Discussion

Megaloblastic anemia is not uncommon in the Indian subcontinent as well as other parts of Asia with females and vegetarians being more susceptible to B12 deficiency. Various studies in the past have shown that occult B12 deficiency may be rather prevalent among Indian urban and rural population.[24] The diagnosis should not be dependent on abnormal blood values. Macrocytosis for example is not specific to a vitamin B deficiency in children and also anaemia is not always present – as was previously assumed. In our study, 4(9.75%) subjects were deficient in vitamin B12, but not anemic. It is estimated that if the values for haemoglobin, haematocrit and MCV are normal, more than 30% of vitamin B12 deficient patients will be missed. [25] In a Mexican study done on varied etiology of megaloblastic anemia with pre-school children, no cases had folate deficiency, while 41% had low B12 levels.[26] In a study on 45 patients with megaloblastic anemia by Bay A et al found that, 93% cases had vitamin B 12 deficiency and only 7% had folate deficiency.[27] In our study we found 70.73% subjects were vitamin B12 deficient.

Anne-Lise Bjorke Monsen et al in 2003 studied cobalamin status and its biochemical markers methylmalonic Acid and Honmocysteine in 3766

children from the age of 4 days to 19 years. They found that lowest value of serum vitamin B12 was in children with age group of 12-19 years, which is in accordance with our study in which sever vitamin B 12 deficiency (< 50 pg/ml) was predominantly seen in the age group of 12 years to 18 years. [28]

Pooled data from different studies shows that a considerable number of B12-deficient patients will remain unnoticed when the MCV is used as the only parameter to rule out the diagnosis of B 12 deficiency.[29] In our study**,** elevated MCV > 100 Fl was seen in only 6 (20.68%) subjects with vitamin B12 deficiency but the association between vitamin B12 deficiency and elevated MCV was found to be statistically significant with P value of 0.0015 and x^2 of 12.97.

A clinical study was done on 1752 children by Haken Sarbay et al.on Evaluation of children with macrocytosis found that macrocytosis in 2017. They found that macrocytosis was detected in only 14 (19.4%) in vitamin B 12 deficient children which is in accordance with our study where only 5 (17.24%) children with vitamin B12 deficiency showed macrocytosis.[30]

A retrospective analysis was conducted by S Srikant on Clinical Spectrum and a Hematological Profile of 21 children with age group from 2 months to 15 years with Megaloblastic anemia. There were no patients in the age group of 3-13 years in contrast to our study. The most common symptoms were pallor, fatigue, and neurological involvement . In another study by Katar et al.,most frequently reported clinical signs and symptoms of nutritional megaloblastic anemia were paleness, hypoactivity, attention deficit and stomatitis in young Turkish children**,** whereas the commonest presentation in our study was fever, followed by generalized weakness and paleness of body. [31,32]

Conclusions

- Knuckle pigmentation and elevated MCV value may not be seen in every child with vitamin B 12 deficiency, but their presence gives important clue to early diagnosis and hence early treatment
- Vitamin B-12 deficiency has varied manifestations and its possibility should be thought of even in absence of anemia, especially in teenage group of children.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Prospective Clinical Study to Evaluate Recurrence and Infection in Laparoscopic Hernia in a Tribal Area

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Abstract

Background: Safe surgical service is mostly inaccessible to most of the poor tribal population of the Gadchiroli district of Maharashtra. Temporary multidisciplinary surgery camps can be the answer, but its safety and cost-effectiveness were always questioned.

Method: This is a prospective interventional study included 172 laparoscopic ally operated cases of inguinal hernia at Hemalkasa camp from 2015 up to 2020. Along with a study of clinical parameters like demography, infection and recurrence were evaluated.

Results: Most of cases in an age group of 20- 40 years and belong to the male gender with a presentation as indirect inguinal hernia. The right side was outnumbered than left. Mesh was not fixed and a single dose of antibiotic prophylaxis was administered. A promising zero infection rate was observed with recurrence was only in 4(2.3%) cases. Most of the patients were operated by the TEP method. Clinical presentation was reducible swelling in groin in maximum patients. The average operative time was 60 minutes. There were no intraoperative (neurovascular, visceral) and any post-operative complications. 4 days early return to work time was noted with very high acceptability laparoscopic surgery in tribals was observed.

Conclusions: Laparoscopic hernia repair in tibal people in multi diagnostic camps is found to have acceptable and good results. It is a safe as having zero infection and minimal recurrence rate. This procedure is also having less postoperative pain and discomfort, cosmetically good with early return.

Keywords: *Inguinal, Mesh, Prophylactic, Madias.*

Introduction

The word "hernia" means torn in Latin and branch or bud in Greek[1]. In the medieval era, up to the 19th century, inguinal hernia surgery had poor results. At the end of the 19th century there was a fast understanding of anatomy, surgical asepsis, and anesthesia leads to development of improved techniques of hernia repair. Bassini introduced his landmark technique on tissue repair for hernia surgery in 1887[2]. Actual preperitoneal mesh repair by the laparoscopic way was reported by Arregui and Dion in the 1990s was an actual throughgoing idea in hernia repair[3]. Dr. Prakash and Dr. Mandakini had first come to Hemalakasa with some co workers after becoming medical graduates. The couple started a type of open hospital, in which tribal patients were admitted for the proper administration of medicines. This was because there had been a few instances of over-medication by the patients. To overcome hurdle of the availability of modern health facilities, the team of Datta Meghe

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Institute of medical sciences has got an opportunity to serve tribal by participating in laparoscopy surgery camps from 2015, operating around 172 hernia patients by laparoscopic method with Lok Biradari Project . Today, the Lok Biradari Project hospital treats thousands of patients. Dr. Prakash not only care for humans but also loves wild animals. The Amte couple look after many such wild animals. He started a school for tribal children. Dr. Prakash Amte was awarded a Padma Shri, and both Dr. Prakash and Dr. Mandakini Amte are recipients of the prestigious Ramon Magsaysay Award, besides many others.[4]

It is of our great surprise that even tribal peoples are having great favor about the laparoscopic hernia surgery. The first camp was conducted in 2015 at Hemalkasa tribal area was lasted for four days. Most of the hernias were operated by open method but few by laparoscopic method. It was of surprise that in next camp onwards almost all operated by laparoscopic method on request of these tribal patients. Laparoscopic technique getting favor because of less postoperative pain and hospital stay and more cosmesis probably. The results of mesh repair, both open and laparoscopic are encouraging.

Method

This is a prospective interventional study conducted in the Dept. of General Surgery at Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Nagpur in collaboration with Jawaharlal Nehru Medical College,Datta Meghe Institute of Medical Sciences Wardha; included 172 laparoscopically operated cases of inguinal hernia at Hemalkasa camp from 2015 up to 2020. A surgeon's team from, Datta Meghe Medical College Hingana, Nagpur, in collaboration with Jawaharlal Nehru Medical College, Datta Meghe institute of medical science (DMIMS), Sawangi, Meghe, Wardha, Maharashtra India, participating in the camp and operate various types of laparoscopic surgeries including hernia surgery. Ethical approval for the study was obtained from the DMIMS ethical clearance committee. Patients were given both the option of open or laparoscopic repair after taking informed consent. Those who opted for laparoscopic hernia repair were included in the study and underwent laparoscopic TEPP and TAP repair.

Inclusion criteria: Patient of all ages and both gender including unilateral or bilateral inguinal hernia and epigastric hernia.

Exclusion Criteria: Pregnant females, uncorrectable coagulopathy and patients unfit for general anaesthesia with a past history of abdominal surgery below umbilicus.

Preoperative Assessment: Proper assessment with necessary investigations done along with careful physical examination.

Preoperative check-list of the patient:

- The type of surgery and anaesthesia is explained to the patient. To see that the patient has consented for surgery including, conversion to open method, if necessary
- To identify correctly, side of the hernia.
- The patient was kept nil by mouth for 6 hours before surgery
- A prophylactic preoperative single dose of injection Ampicillin was given IV 30 min before surgery.
- To see that, the patient is shaved from Xiphoid to Groin and mid-thigh

Intraoperative: Patients are routinely catheterized before an operation. Operative time, and intraoperative complications, if any are noted.

Post-operative: Feeding is resumed soon after full regain of consciousness. A catheter is removed after 24 hours. After the surgery, injection Diclofenac sodium 75 mg IM BD given for 24 hours for pain relief. Postoperative pain is assessed at regular intervals using the VAS scale.

Post operative surgical site infection noted.

Discharge: The patients were discharged when fit and asked to come for regular follow up after 7 days, 1 month, 3 months, 6 months, and 1 year postoperatively. Oral analgesic is advised if needed. Asked them to resume to normal activity when they felt comfortable.

All the patients were encouraged to return to the pre hernia lifestyle except lifting heavy weights (till 4 weeks). All were followed up for chronic pain, interference with activities of daily living, use of analgesics, recurrence, seromas, or any other complications. All the data was entered in the proforma and analysis was done manually.

Parameters studied: Operative time

Operation time was noted from the skin incision

to the last suture. All-time measurements were approximated to the nearest multiple of 5 minutes.

Post-operative pain: A visual analog scale (VAS) was calibrated from 0 to 10 as per severity of pain. VAS was used to assess pain for 24 hours after surgery

Complications during and after surgery: Complications like intestinal or urinary bladder tear, if any were noted.

Infection: Superficial surgical site infection[5] (SSI) was identified by the basis of these standard benchmarks: Infection in 30 days after surgery affecting the cutis and subcutaneous tissue and have one out of below:

- Pus.
- Positive culture.
- Any one out of : pain or tenderness; localized swelling; redness; or heat.
- Identification of superficial infection by the treating surgeon.

Deep was identified by the basis of these standard findings: Infection in 30 to 90 days after surgery affecting deep tissues and have one of the following:

- Pus from the deep tissue.
- Burst abdomen or bursting of all layers of incision. Has any one out of: fever (>38o C); localized pain or tenderness.
- Collection of pus in deeper part by palpation or imaging.

Post-operative hospital stay: The total number of nights spent in the hospital after the operation was defined as a hospital stay. Return to daily activity in days and follow up at 7 days, 1 month, 3 months, 6 months, and 1 year.

Recurrence: Defined as the presence of lump clinically diagnosed as hernia by physician ai operated site.

Statistical analysis: All data were analyzed using SPSS software.

Results

In present study, 80 cases (51%) were in (40-60 years) age group followed by 54 cases (34%) in (20-40 years) group, 18 cases (11%) in > 60 age group and 6 cases (4%)in less than 20 years . The minimum age was 5 years and the maximum age was 76 years.

The present study, included 168 men (98%) and women (2%). The men to women ratio was 49:1. In our study, there 102 cases (59%) were right-sided and 58 cases (34%) were left-sided and 11 cases (6%) were bilateral and 1 (1%) case is of an epigastric hernia. 161 cases (94%) were indirect type, 10 cases (6%) were of direct type. Around 18 cases were in the age group of more than 60 years of age. Out of these patients, 12(67%) were of right indirect inguinal hernia,4 (22%) of left indirect inguinal hernia and 2 (11%) were of bilateral indirect inguinal hernia.

Mode of clinical presentation: In our study, 171 patients with the presentation of swelling in the groin and One female patient was having swelling epigastrium. 12 patients of them had complaints of swelling along with pain 150 patients have reducible swelling rest were partially reducible.

Possible risk factors/precipitating factors: In the present study, all cases had occupations involving strenuous activities like farming, manual labor, and hunting. In only 2 cases there were symptoms of prostatism and 5 had a history of chronic cough (COPD).

Time is taken for an operation: In our study, the mean operating time was 61.2±16.85 min (range 50-110 min).

Post-operative pain: The mean VAS score at 12 hours after surgery was 2.70±1.54 (range 0-6) median = 2.50. In the present study, 112 cases out of 172 cases (%) experienced mild pain post-operatively at 12 hours. Moderate pain occurred in 60 cases (33.33%) up to 24 hours after surgery. None of them had severe pain. Controlled with a single dose of diclofenac sodium.

Fixation of mesh: Out of 172 patients except 4 all patient operated laparoscopically undergone fixation of mesh.one patient was having an epigastric hernia and the other 3 patients needs fixation were bilateral hernia. No tucker was used to fix the mesh, it was fixed by laparoscopic suturing.

Surgical site infection: No single case of infection was observed in the present study. There were no major intra-operative (neurovascular, visceral), post-operative, and local complications.

Recurrence: Recurrence was observed in only 4 (2.35%) patients.

Other complications: Seroma, hematoma, intraoperative complications, cord related injuries, anesthetic complications not observed in a study population.

Type of surgery: TEP was performed in 168 patient and TAPP in 3 patients and one was with ventral hernia repair.

Hospitalization and discharge: In the present study, the mean duration of hospital stay was 2. + 0.56 days (range 1-3 days) and the average hospital stay was 2 days. Time taken for ambulation was 4 ±2.62 hours (range 3-8 hours).

Return to work: All patients were given similar advice at discharge and encouraged to return to work as early as possible and requested to note down the progress in their activities. Heavy work/straining was advised after 1 month postoperatively. Actual all these tribal people start their work very early. the average return to work was 4 days and the range was 3- 5 days.

Discussion

Age at presentation: In the present study, 80 cases (51%) were in (40-60 years) age group followed by 54 cases (34%) in (20-40 years) group, 18 cases (11%) in > 60 age group and 6 cases (4%)in less than 20 years (see figure 1), and similar to the studies of Singh S et al[1], Ira M Rutkow et al[6].

The present study, included 168 men (98%) and women (2%). The men to women ratio was 49:1. (see figure 2). Gender presentation of various studies is compared in the following table 1.

Table No. 1: Comparison of gender presentation of various studies

Sex	Present study	Jain R et al[8]	Gurjar[7]	Ira M. Rutkow[6]	Sing S et al[1]
Male	98%	97%	97.33%	90%	96.30%
Female	2%	3%	2.26%	10%	3.70%

In the present study, there 102 cases (59%) were right indirect sided and 58 cases (34%) were left indirect sided and 11 cases (6%) were bilateral and 1 (1%)case is of an epigastric hernia. 161 cases (94%) were indirect type, 10 cases (6%) were of direct type (see figure 3).comparison of gender presentation of various studies is depicted in table 2.

Table No. 2: Comparisons of gender presentation of various studies

Type	Present study	Rutkow IM[6]	M. Sangwan 18[9]	Sing S et al[1]
Right- indirect	102 (59%)	36 (36%)	72 (28.92%)	19 (35.19%)
Right- direct	5 (2.5%)	15 (15%)	67 (26.91%)	12 (22.22%)
Left- indirect	15 (27.78%)	28 (28%)	53 (21.29%)	15 (27.78%)
Left- direct	5 (2.5%)	13 (13%)	37 (14.86%)	5 (9.26%)
Bilateral	11 (6%)	2 (2%)	20 (8.03%)	2 (3.70%)
Pantaloon	0	6 (6%)	0	0
Others	1 (0.5%)	0	0	1 (1.85%)

Occupation: In the present study, all 172 patients were tribal and (38.89%) had occupation (farmer, laborer, player) involve in hunting and other strenuous work. In a study by Rahul et. Al[10], the maximum number of a patient were from laborer class related to strenuous physical activity from south India.

Mode of presentation: In a study done by Sing S et al[1] 2/3 of patients had pain or discomfort in the groin along with swelling. In a study by Liem MSL et al[11], also reveals more than 90% presented with swelling in the inguinal region. Almost all patients present with groin swelling but very few with pain.

Duration of surgery: In our study, the mean operating time was 61.2±16.85 min (range 50-110 min). The study by Sing S et al[1] had average operating time was around 90 minutes.

Return to work: In our study, all the patients were given similar advice at discharge and encouraged to return to work as early as possible and requested to note down the progress in their activities. Heavy work/ straining was advised after 4 weeks post-operatively. The mean time for the return to work was 8.93± 2.66 days (range 3-15 days) and the median was 9 days. It is comparable to other studies as shown in table 3.

Recurrence: In our study, there were four cases of recurrences detected in the first week of surgery. However, there was no recurrence up to one year follow up. This recurrence is comparable with other studies as shown in table 4.

Table No. 3: Return to work after surgery comparison among various studies

Laparoscopic repairs	Return to work (days)
A Eklund et al[12]	15
Hamaza Y et al[13]	14.87
SCUR trial[14]	15
Sing S[1]	8.93±2.66
Present study	4

Table 4. Recurrences observed in various studies

Laparoscopic repair	Recurrence rate
Liem et al[11] (n = 487)	4.9% (4 years)
Yassar Hamaza et al [13](n = 50)	1 (4%) 1 year
Sing S et al[1] (n = 41)	2(3.57%) 2.43 months
Present study (n = 172)	4 (2.3 %) 1 year

Surgical site infection: In a present study, a single prophylactic dose of antibiotics 30 minutes before surgery leads to a promising zero infection rate. Another reason may be the efforts of hospital staff for strictly following principals of asepsis. A comparison with various studies is given in table 5.

Table 5. Comparisons of SSTI in various studies.

	Total infection	Superficial surgical site infection	Deep surgical site infection	Number of a patient requiring mesh removal
	N (%)	N (%)	N (%)	
Present study	0	0	0	0
Maheshwari MK et al[5].	11 (11)	11 (11)	0 (0)	0
V Gomathi Shankar et al15	29 (6.4)	27 (6)	2 (0.4)	1
Sing S et al[1]	2(3.7%)	2(3.7%)	0	0

Old age and indirect inguinal hernia: Contrary to the common belief of association of direct hernia with old age, we found indirect hernia in all patients having age more than 60 years (see figure 4).

Conclusions

Laparoscopic hernia repair in tribal people in multi diagnostic camps is found to have acceptable and good results. It is a safe as having zero infection and minimal recurrence rate. This procedure is also having less postoperative pain and discomfort, cosmetically good with potential of early return to work.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Glimpses of Eugenics in Ayurveda WSR to Preconceptional and Antenatal Remedies

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Abstract

Every parent wish to have a good progeny. Improvement is basic human nature. Hence eugenics emerged in 20th century as a movement in many countries which had its own pros and cons with serious ethical concerns. Though conception is a natural and physiological phenomenon, Ayurveda advocates that conception should not be accidental, rather it must be planned. The planning includes preparation of body as well as mind. To fulfil the wish of getting a good progeny Ayurveda texts mention a detailed framework to be followed. It includes pre-conceptional preparation to be executed by the couple and anti-natal care of pregnant lady. This is uniform and open to all aspirant parents without any selective bias. It is purely aimed at achieving a healthy and intelligent future generation irrespective of any race, cast and creed. The unbiased holistic approach of Ayurveda brings out positive aspect of eugenics to the society.

Keywords: *Eugenics, Ayurveda, planned pregnancy.*

Introduction

Pregnancy is a beautiful phase of every woman's life who wishes to be a mother. Giving birth to a healthy and intelligent child is the desire of all aspiring parents. This desire gave rise to principles and practices of eugenics. The word Eugenics come from Greek word *Eugenes* (eu = well, genes = born). The term means improving the human race by the bearing of healthy offspring. The word was first used by Francis Galton (Charles Darwin's cousin) in 1883 to describe the science of biological improvement of human race. He believed that human traits physical, mental and moral are inherited. Therefore he proposed that progress of human race depends on selective transmission of inheritable qualities. [1]

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The thoughts of Galton could not gain momentum due to lack of scientific records. In the early 1900s Mendel's laws of inheritance were rediscovered and applied to genetics, giving rise to a new dimension to inheritance. It reinforced the idea that human beings are made up of their germ plasm. In Germany, this gave impetus to formulation of eugenics policies supported by medical community. The ideas of racial hygiene were incorporated by Hitler in his book "Mein Kamf.[2] Similarly, in USA, the eugenics theory gained attention due to undesirable social circumstances that were thought to be related to genetics and the scientists believed that they could be controlled with eugenics. Hundred years ago, eugenicists claimed that all of man's diseases were caused by inferior inherited characteristics. This gave rise to positive and negative eugenics. It resulted in Johnson immigration act of 1924, Involuntary sterilisation act in USA[3] It resulted in racial as well as individual abuses and the dark side of eugenics came to the fore front. Eugenics as a belief system promoted the systematic destruction of those deemed inferior. Starting with the killing of infants and children with congenital defects and mental retardation, gradually it also included

disabled and mentally ill adults. Further it expanded to include adults and children with "antisocial behavior" and those who had minor handicaps and were considered inferior in the society. Thus it took the form of a drive for social improvement by elimination of inferior persons.

The new eugenics: The modern eugenicists present the old eugenics as unscientific and authoritarian, concerned with improving the race. The new eugenics promotes individual human enhancement and undesirable trait elimination not improving the species. Robertson claimed that parents should be allowed to use reproductive technologies as they see fit.

One of the major limitations of the eugenics theory is that one person's definition of "disabled" or "impaired" may be drastically different from another person's. For example, in some cultures thick lips are considered as good while they may not be appreciated by another culture. The new eugenics supporters claim that human enhancement through genetic and other reproductive technologies is the new liberal eugenics which considers that it is the parent's moral duty to produce the best children possible. Although science-based, new eugenics still pursue the same goal as the old eugenics, the development of a superior individual [4]

Aims: The article is aimed to have a brief outlook of pre conceptional and anti natal measures for a healthy offspring described in Ayurveda texts.

Objectives:

After reading the article, the reader will be able to understand:

1. That Ayurveda advocates well planned pregnancy
2. That Ayurveda describes a detailed nine months plan for good progeny
3. Ayurveda eugeny principles are not biased and differ from other modern thought schools of eugenics

An overview of Ayurveda principles for good progeny:

I. Pre-conceptional preparation: Ayurveda describe the process of conception as a ceremonial rite named as *Garbhaadhaan samskaar* i.e. sacrament of impregnation. *Samskaar* is a Sanskrit word which indicates measures taken to improve the quality of something. Likewise, the aim of this measure is to gain a good progeny[5] According to Charaka,prior to this ritual, the man and woman willing for conception are advised to undergo proper *snehan* (oleation) and *swedan* (fomentation), followed by *shodhan (Cleansing of body from within)*. Thus, the couple have to go through body detoxification treatment with *Vaman* and *Virechan.* These two processes are part of *Panchakarma,* one of the branches of Ayurveda. After the cleansing process, couple is directed to follow a special diet regime for a specified time period. Then, gradually they are to be shifted to normal diet. Once this is done, the couple is advised to be administered two types of *basti* (enema) viz. *Anuvaasan* and *Aasthaapan*. Then, the male partner should consume milk and Ghee boiled with herbs of *Madhur gana* while the female partner should consume black gram and oil[6]

Implementing above set of instructions, the couple becomes ready for conception.

II. Garbhadhaan Sanskar (Ritual for conception): Charaka further elaborated that after detoxification of body, the couple should try for conception in the next menstrual cycle of female partner. On the fourth day of cycle the couple should take bath and wear white clothes, eat easily digestible food, converse with each other and then proceed to intercourse in a fragrant room with good ambience[7] Charaka has specifically described the coitus position best suitable to maintain the balance of three doshas during intercourse[8] At this time, some hymns are to be chanted as prayer to the deities to bless with good offspring. This ritual is described in detail along with the measures to be followed by the lady and the man. The woman is advised to wear the clothes and behave in the same manner as she imagine her child to be[9]

III. Masanumasik Garbhini Paricharya (Anti natal care): *Garbhini paricharya* is the unique contribution of Ayurveda in the field of Obstetrics. Differernt *acharyas* have prescribed their own regimen for the mother and fetal wellbeing. The monthly regimen to be implemented by mother for the well being of foetus as per Charaka[10] Sushruta[11] Vagbhata[12] Harita[13]

IV. Garbhopghatkara Bhava: Ayurveda experts have also mentioned the factors that are supportive as well as harmful for fetal growth and development. The beneficial ones are *Garbhasthapak* and the harmful ones are termed as *Garbhopghatkara bhava*. These factors are summarised as:

According to Charaka[14]: Ushna and Pungent drugs, violent activities, coitus, exercise, less intake of food, alcohol, indulging in fights, unpleasant news, excessive consumption of any one rasa.

According to Sushruta [15]: Coitus, exercise, excessive emaciation, sleeping in day, awakening in night, grief, riding onvehicle, fear, squatting, oleation, bloodletting, suppression of natural urges etc.

According to Vagbhat[16]: Pungent drugs, exercise, coitus, excessive emaciation, sleeping in day, awakening in night, grief, riding on vehicle, fear, squatting, oleation, bloodletting, Suppression of natural urges, indigestion, prolong stay in hot sun or near fire, Anger, grief, fear, terror, fasting, squatting, looking or hearing of unpleasant things etc.

IV. Garbhasthapak Dravyas[17]: These are the herbs which are said to be useful for good implantation of fetus and its proper growth and development during pregnancy. They are *Aindri (Bacopa monnieri), Brahmi (Centellaasiatica), Shatavirya (Asparagus racemosus), Sahasravirya (Cynodon dactylon), Amogha (Stereospermum suaveolens), Avyatha (Tinospora cardifolia), Shiva (Terminaliachebula), Arishta (Picrorhiza kurroa), Vatyapushpi (Sida cardifolia), Vishwasenkanta (Collicarpa macrophylla) etc.* These herbs are said to support the products of conception. These should be taken with milk or ghrita.

Discussion

With increasing discussions on human rights at global level and opposition on the basis of ethical issues, the eugenics policies had to face a back foot. It has been shown that many mental disabilities are not linked with genes; that those that are not simply a result of genetic make-up; and that most human behaviours (including deviant ones) are at least as much shaped by environment as by biological inheritance. These findings also had a negative impact on eugenics progression. Suppression of some and boosting others on the basis of superiority –inferiority complex is another flaw in eugenics as it interferes with individual rights.

Ayurveda's approach towards good progeny have a different stand. According to the aim of Ayurveda prevention comes ahead of treatment. Therefore, Ayurveda start from advice of not marrying in close relation. Marriage within clan, tribe, caste, or biraderi has been a long-established tradition in many parts of the world. Acharya Charak in *Atulya Gotriya Adhyaya* clearly states that the marriage should be in distant families not in close kinship so as to procure good progeny. [18] Modern science also accept that the frequency of genetic disorders among such children is around twice than in children of non-related parents. Consanguineous unions lead to increased expression of autosomal recessive disorders. One cross sectional study showed that there was a significant difference in the prevalence between the offspring of consanguineous versus non-consanguineous for all cases viz. cancer, blood disorders, anaemia, mental disorders, heart diseases, asthma, hypertension, gastrointestinal diseases, hearing deficits, common eye diseases and diabetes mellitus. All reported diseases were more frequent in consanguineous marriages.[19]

Thus choosing a life partner from non Consanguineous community is the first step in elimination of some genetic disorders. Ayurveda advised this principle many thousand years ago for all, without limiting it to a specific class of society. This practice may play an important role in maintaining gene diversity. The lack of genetic diversity is often associated with a decrease in the adaptive response of a population. [20]

The couple willing for conception is advised purification of body through Panchakarma . Vaman is a body cleansing measure which improves appetite, regulates bowel habits and improves sleep pattern. It decreases LDL and serum cholesterol level[21] After detoxification of body with vaman and virechan, male partner has been advised to consume madhur dravya siddha milk and ghrita .A clinical study showed that administration of cow ghee after performing *Virechana* provided statistically highly significant improvement on seminal parameters.[22] The texts mention purificatory effect of virechan on Beeja (sperm and ovum).

The minute description of intercourse in a good ambience with peaceful and spiritual mindset indicates that intercourse at this point of time is not meant for pleasure, but aimed purely at conception. Mental, spiritual and environmental influence is given importance at the time of conception. This may contribute to positively accepting the process of conception .

Ayurvedic philosophy is based on *tridosha*. Their balance is said to be the state of health. Even during the intercourse for conception, the ideal position has been advised to keep the tridosha in balanced state.

Thought of maintaining balance of *tridoshas* during sex for conception shows medical and clinical concerns. Because the predominance of a dosha in body at the time of conception is responsible for creating the *prakriti* (body type). And the susceptibility of these body types to diseases varies according to the dominance of *doshas*.[23] *Prakriti* has an impact on physical and mental build up of an individual. Once formed at the time of conception, *Prakriti* do not change throughout life. Therefore, Ayurveda has advised measures for laying the foundation of balanced Prakriti, before birth itself. Charaka Samhita, mentions that the pregnant women should be treated like an oil filled pot. The slightest oscillation of such pot can cause spilling of oil. Similarly, a little stimulation or excitement to the pregnant women can lead to Garbhapata (abortion).Thus utmost care of the pregnant lady should be taken to prevent miscarriage.[25]

By the usage of regimens from first to ninth month, women gains strength and complexion and she delivers easily at proper time as desired, with excellent healthy child possessing all qualities and long life.[26] The dietary recommendations for pregnant lady has been advised so as to achieve mother and fetal nourishment and strength . It is useful in reducing the month wise complains like nausea & vomiting by liquid diet in initial months, oedema by using Gokshura as a diuretic during second trimester & Constipation by Asthapan Basti in third trimester. Also, the use of various herbs is advised to facilitate conception and pregnancy. Number of studies are available on various aspects of pregnancy, maternal and child health [27]Thus, Garbhini Paricharya offers a complete package for overcoming fetal complications, removing anxiety of mother related to pregnancy and delivery and facilitating growth and development of fetus.

Ancient Ayurveda scholars probably knew that there is a connection between mother's diet and behaviour to epigenetic changes that could be transmitted to the next generation. Hence along with clinical management of pregnancy they laid emphasis on environmental factors, too.

Conclusion

Most of the traits the earlier eugenicists were interested in actually result from interactions between genetic and environmental factors. Therefore, even today predictions of traits amaze scientists despite the progress of science and technology. Epigenetic changes are also being studied keenly by the researchers recently. Ancient Ayurveda theories covered both the aspects genetics as well as environmental factors. Therefore, Ayurveda not only advised physical detoxification of body before conception, but also mental and spiritual wellbeing of mother and father to be. Further, the nine months advisory for anti natal care of fetal and mother wellbeing includes dietary as well as behavioural dos and don'ts. This indicates that Ayurveda experts were aware of the fact that apart from paternal genotype, other factors are also responsible for promotion of eugenics. Thus, Ayurveda principles of good progeny seems to be more broad spectrum in terms of eugenics.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Comparative Study of Three Min Respiratory Exercise Test & Six Min Walk Test as a Means to Score Breathlessness in COPD Patients and Normal Healthy Individuals

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Abstract

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2011 update recommends that the management and treatment of COPD be made to combine the impact of disease and future risk of exacerbation.

Objectives: The aim of the study was to compare 3MRET & 6MWT as a means to score breathlessness in COPD patients & normal healthy individuals along with to analyse and compare POD index of 3MRET & walking POD index of 6MWT in COPD patients, normal healthy individuals and to analyse whether POD index of 3MRET or walking POD index of 6MWT is a better means to score breathlessness in COPD patients when compared with normal healthy individuals.

Material and Method: It was observational comparative study conducted in respiratory Physiotherapy set up on 45 Normal healthy individuals and 45 COPD patients between the age group 30-50 years.

Results: Study found that there is a statistically highly significant difference between POD index of 3MRET and walking pod index of 6MWT in COPD patients. There is a statistically highly significant difference between the POD index of 3 MRET and walking pod index of 6MWT in normal healthy individuals. MRET is a better means to score breathlessness in COPD patients as compared to normal healthy individuals. It was found that 3MRET is a better means to score breathlessness as compared to 6 min walk test.

Keywords: *Perception of dyspnoea, COPD, Normal subjects, Incentive spirometry, 6 min walk test.*

Introduction

COPD represents a major social & economic burden for worldwide health system, patients with COPD experience period of dyspnoea, fatigue & disability which impact on their life. COPD is highly prevalent. Usually progressive illness associated with disability and early death (WHO2008).[1] According to Donald A Mohler 2015, Dyspnoea is the most prevalent symptoms among patients with respiratory disease. It is an independent predictor of mortality in patients with heart disease, COPD & elderly. In 2012 American thoracic society recommended that dyspnoea can be considered across three different constructs sensory intensity, affective (distress) & impact on daily activity. In 2013, Gold (Global initiative for chronic obstructive lung disease) recommended a treatment for patients with COPD based on medical research council Dyspnoea score.[2] Various exercise tests are used to evaluate the problem of dyspnoea and provide an opportunity to measure the severity of dyspnoea. According to LoL C, Jayaram

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et al, measurement of disability and breathlessness is important so, he developed 3MRET (3 min respiratory exercise test) by using an incentive spirometer to score MBC (maximal breathing capacity) & POD (Perception of dyspnoea) for a period of 3 min[3].

Methodology

1. **Study design:** Observational comparative study.

   It is an observational comparative study in which 3 min respiratory exercise test & 6 min walk test will be administered to COPD patients & normal healthy individuals and the results will be compared.

2. **Study set up**- Respiratory Physiotherapy set up

3. **Selection criteria:** COPD patients and the normal healthy relatives accompanying them to the respiratory OPD of the hospital will form the sample population for the experimental & control group. g

Inclusion criteria:

(a) COPD patients and normal healthy individuals in the age group of 35-50 yrs

Exclusion criteria:

(A) For normal healthy individuals:

Any acute or chronic respiratory conditions

(B) For COPD

- Any acute exacerbation

(C) Common for both groups are:

(a) Any musculoskeletal conditions

(b) Neurological disorder

(c) Cardiac conditions

(d) Psychological conditions

Materials used: -Incentive spirometry, Measuring tape, stopwatch, staws and cone

Procedure: Approval has been obtained from the ethical committee. The subjects who fulfill the inclusion criteria were explained about the study in detail, inform consent was taken in consent form in a language they understand. The subjects were explained about 3 MRET & 6MWT until they mastered the test technique. In a random allocation by chit system the order of the two tests were decided for each subjects. Both the groups of subjects underwent either 3min respiratory exercise test or 6 min walk test as per the chit picked After completion of one test, the subjects were made to rest for a period of 30 mins, so as to recover from any after effect of the previous test, and he asked to rate the POD and after that POD index were calculated, then the subjects were made to perform the next test .

3 min respiratory exercise test (3MRET): The subject is asked to hold the mouthpiece of the incentive spirometry in his mouth and inhale as deep as possible with maximum inspiratory effort to score MBC (maximal breathing capacity),so that all the three balls reach the top of the coloumn & hold it for 3 seconds and release.

6 min walk test (6MWT): The subjects were asked to perform a 6min walk test. The subject was asked to walk on a flat, long &, hard surface as fast as possible in the departmental corridor which is a 100 ft hallway with turn around points at each end clearly marked with cones for a period of 6 min. The distance covered during 6 min is measured & recorded as walking distance, after that the subject is made to score POD (perception of dyspnoea) by VAS(visual analogue scale).

Outcome Measures:

1. **POD index of 3 MRET is:** The POD was calculated by dividing POD VAS score with the MBC (maximal breathing capacity) ant then multiplying it by 1000.To standardize, POD VAS score zero was replaced by a standard value of 0.125,to enable the calculation of POD index The formula for MBC (MAXIMAL BREATHING CAPACITY) is as follows:

   MBC= (number of times that all three ball reach the top of column) X 2+(no of times that two ball reached the top of column) X1.5+(no of times that one ball reach the top of column)X1

   The formula for POD index is as follows:

   POD index = POD VAS X1000/MBC

2. **Walking POD index of 6 MWT was:** The walking POD index will be calculated by dividing POD VAS score with the walking distance and multiplying it by 1000.To standardize, POD VAS score zero was replaced by a standard value of 0.125, to enable the calculation of POD index.

   The formula for walking POD index is as follows:

Walking POD index = POD VAS x 1000/Walking Distance

Results

45 COPD and 45 normal healthy individuals were taken who volunteered to participate in our study according to inclusion and exclusion criteria. Out of 45 COPD patients 29 were male and 16 were female and the relatives accompanying them to respiratory department were taken as normal healthy individuals of the same sample size i.e 45 out of which 23 were male and 22 were female 3MRET and 6MWT were performed in normal healthy individuals and COPD patients,the variables taken in both the test groups were MBC (maximal breathing capacity), walking distance, and POD index (perception of dyspnoea), the MBC and POD index of 3 min respiratory exercise test was considered as equivalent to walking distance & walking POD index of 6 MWT in both the test groups. The POD index of both the test were compared for strength of association and significance. Out of 45 COPD patients 29 were male and 16 were female and the relatives accompanying them to respiratory department were taken as normal healthy individuals of the same sample size i.e 45 out of which 23 were male and 22 were female 3MRET and 6MWT were performed in normal healthy individuals and COPD patients**.** Mean MBC score in normal healthy individuals is 151(108-194) and in COPD patients 109.8(66-149), we found that mean MBC score was highest in normal healthy individuals and it was very lowest in COPD patients, there is statistically significant difference between normal subjects and COPD patients.

Table No. 1: Shows mean maximal breathing capacity MBC (109.8) and walking distance (381.33) in COPD patients

Parameters	MBC	Walking distance
Mean	109.8	381.33
SD	19.69	58.83
Median	112	360
Ranges	66-149	240-480

Table no 1 shows that mean MBC score in normal healthy individuals is 151(108-194) and in COPD patients 109.8(66-149), we found that mean MBC score was highest in normal healthy individuals and it was very lowest in COPD patients, there is statistically significant difference between normal subjects and COPD patient.

Table No. 2: Shows correlation of MBC and walking distance in walking distance normal subjects.

	r s-value	p-value	Significance
Normal subjects	0.1349	0.3771	Not significant
COPD patients	0.4958	0.0005	Highly significant

Table no 2 shows correlation of MBC with the walking distance in normal healthy individuals and in COPD patients, we also found that there is not significant difference between mean MBC 151.26 (108-194) and mean walking distance 432 (300-540) in normal healthy individuals (p value) (0.3771) but there is statistically highly significant difference between mean MBC 109.8(66-149) and mean walking distance 381.33 (240-480) in COPD patients(p value) (0.005).

Table No. 3: Shows comparisons of mean POD index of 3MRET and mean POD index of 6 MWT in normal healthy individual

	3 MRET	6MWT	t-value	p-value
Mean	23.0	4.81	22.0954	<0.0001, HS
SD	6.38	2.20		
Median	22.38	4.16		
Range	12 – 35.7	2.08-11.11		

Table no 3 shows that comparisons of POD index of 3 min respiratory exercise test with the POD index of 6 min walk test in normal healthy individuals and in COPD patients,observed that mean POD index of 3 min respiratory exercise test was highest as compared to POD index of 6 min walk test in normal healthy individuals and COPD patients, we found that there is statistically highly significant difference between POD index of 3MRET and POD index of 6 min walk test in normal healthy individuals and COPD patients.

Table No. 4: Shows comparisons of mean POD of 3 MRET and mean POD index of 6MWT in COPD patients

	3 MRET	6MWT	t-value	p-value
Mean	45.56	8.48	32.8903	<0.0001, HS
SD	8.68	2.96		
Median	45.8	8.3		
Range	29.4-66.03	2.7-16.6		

Table no 4 shows that comparisons of POD index of 3 min respiratory exercise test with the POD index of 6 min walk test in normal healthy individuals and in COPD patients,observed that mean POD index of 3 min respiratory exercise test was highest as compared to POD index of 6 min walk test in normal healthy individuals and COPD patients, we found that there is statistically highly significant difference between POD index of 3MRET and POD index of 6 min walk test in normal healthy individuals and COPD patients.

Table No. 5: Shows correlation of POD index of 3 MRET and POD index of 6 MWT in normal healthy individuals & in COPD patients

	rs-value	p-value	Significance
Normal subjects	0.5314	0.0002	Highly significant
COPD patients	0.5641	0.0001	Highly significant

Table no 5 shows Correlation of POD index of 3MRET & POD index of 6MWT in COPD patients and Normal subjects. it shows that there is statistically highly significant difference between POD index of 3 MRET and POD index of 6 min walk test.

Table No. 6: Comparisons between normal subjects and COPD patients by 3MRET

Groups	3MRET (Min Respiratory Exercise Test)
	Mean Value
Normal Healthy Individuals	23.0
COPD Patients	45.56
t-value	114.0481
p-value	<0.0001.HS

Table no 6 shows the comparisons between normal healthy individuals and COPD patients by 3 min respiratory exercise test, as comparisons was made between two tests we came to know that 3min respiratory exercise test is superior as compared to 6 min walk test, then we compared 3 MRET between normal subjects and COPD patients. We come to know our conclusion that 3 min respiratory exercise test is a better means to score breathlessness in COPD patients as compared to normal healthy individuals.

Discussion

As 3MRET and 6MWT were performed in normal healthy individuals and COPD patients, the variables taken in both the test groups were MBC (maximal breathing capacity), walking distance, and POD index (perception of Dysnpoea),the MBC and POD index

of 3 min respiratory exercise test was considered as equivalent to walking distance and walking POD index of 6 MWT in both the test groups. The result of study, shows mean MBC score in normal healthy individuals is 151 (108-194) and in COPD patients 109.8(66-149), we found that mean MBC score was highest in normal health individuals and it was very lowest in COPD patients, there is an highly statistically significant difference between normal subjects and COPD patients. This is supported by **LI-Cher loh, Pek-Ngor the 2005**[3] Correlation of MBC with the walking distance in normal healthy individuals and in COPD patients, it was found that there is not significant difference mean MBC 151.26(108-194) and mean walking distance 432 (300-540) in normal healthy individuals p value (0.3771) but there is statistically highly significant difference between mean MBC 109.8 (66-149) and mean walking distance 381.33 (240-480) in COPD patients (p value)(0.005). This is supported by the study **Amorim, Stelmach et al 2014**[4].

The current study shown comparisons of POD index of 3 min respiratory exercise test with the POD index of 6 min walk test in normal healthy individuals and in COPD patients we observed that mean POD index of 3 min respiratory exercise test was highest as compared to POD index of 6 min walk test in normal healthy individuals and in COPD patients, We found that there is statistically highly significant difference between POD index of 3 MRET & POD index of 6b min walk test in normal healthy individuals and COPD patients. This result is supported **by American thoracic society guidelines**[5] **& Li-cherloh,pek-ngor et al**[6]**.**

We had compared POD index of 6MWT between normal healthy individuals and COPD patients. The result of our present study shows that POD index was lowest in normal healthy individuals and it was highest in COPD patients by 6 min walk test, we found that there is statistically highly significant difference between normal healthy individuals and COPD patients by 6 min walk test. This result is supported by **Golpe R1,Perez-de 2013**[7].

Therefore, from the above all discussion it is clear that 3 min respiratory exercise test as superior as compared to 6 min walk test in COPD patients and in normal healthy individuals. We come to know that 3 min respiratory exercise is a better means to score breathlessness as compared to 6 min walk test in normal subject and in COPD patients. Comparisons between normal healthy individuals and COPD patients by 3 min respiratory exercise test conclude that 3 min respiratory exercise test is a better means to score breathlessness in COPD patients as compared to normal healthy individuals.

Conclusion

There is statistically highly significant difference between POD index of 3MRET and walking POD index of 6MWT in COPD patients & normal healthy individuals as well as 3MRET is a better means to score breathlessness in COPD patients as compared to normal healthy individuals

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

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Popliteal Artery Diameter: A Cadaveric Study

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Abstract

Introduction: Femoral artery proceeds as a popliteal artery from the osseo-aponeurotic opening of the adductor magnus to the caudal margin of the popliteal muscle. It passes through the adductor canal, the adductor hiatus then through the popliteal fossa ends at the lower margin of the popliteal muscle, in further course it divided into the anterior and posterior tibial arteries. Popliteal aneurysm i.e. abnormal dilation of the whole or part of the popliteal artery causes edema and pain in the popliteal fossa. The popliteal artery serves as bypass grafts in specific location i.e. above or below-knee. It is also often affected by penetrating and blunt trauma of the lower limb. As a consequence, the exposure of this artery is frequently and needed exploration of either in emergency or in elective vascular procedures.

Material and Method: The present Study was carried out in the department of anatomy at Jawaharlal Nehru Medical College in Collaboration with department of anatomy and Datta Meghe Medical College, Nagpur Maharashtra. Total 102 lower limbs of 51 cadavers were included in the study.

Observation and Results: In males the mean diameter of popliteal artery at it origin is 6.7 ± 0.7mm on right and 6.6 ± 0.6 mm on left respectively, whereas in females the mean diameter on right is noted as 6.4 ± 0.5 mm and on left it is 6.2 ± 0.3mm. At proximal to termination the mean diameter of popliteal artery in males is observed to be 6.9 ± 0.7mm on right and 6.8 ± 0.7mm on left, while in females it is noted to be 6.6 ± 0.5mm on right and 6.4 ± 0.4mm on left respectively.

Conclusion: To conclude the data from the present study is useful for the vascular surgeons, radiologists.

Keywords: *Popliteal artery, popliteal aneurysm, popliteal artery diameter.*

Introduction

Femoral artery proceeds as a popliteal artery from the osseo-aponeurotic opening of the adductor magnus to the caudal margin of the popliteal muscle. It passes through the adductor canal, the adductor hiatus then through the popliteal fossa ends at the lower margin of the popliteal muscle, in further course it divided into the anterior and posterior tibial arteries. The artery may divide just proximal to popliteus muscle in its terminal branches, in this case the anterior tibial artery sometimes descends anterior popliteus muscle[1-4] The popliteal artery is palpable and one can easily able to locate it and count the pulse in the back of the knee. [5] If the knees are slightly bent to approximately 45 degrees, the pulse will be easier to locate. Pulsations are best experienced in the lower part of the fossa, where the popliteal artery is close to the tibia. [6] The weakening or failure to palpate the popliteal pulse is a sign of femoral artery obstruction. [7] Popliteal aneurysm i.e. abnormal dilation of the whole or part of the popliteal artery causes edema and pain in

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the popliteal fossa.[8] The popliteal artery serves as bypass grafts in specific location i.e. above or below-knee. It is also often affected by penetrating and blunt trauma of the lower limb. As a consequence, the exposure of this artery is frequently and needed exploration of either in emergency or in elective vascular procedures.[9,10] Knowledge of the normal popliteal artery diameter is important to assess popliteal artery dilation, as it is known sites for aneurysm formation. Knowledge of popliteal artery is valuable not only for anatomists, but also for radiologists and vascular surgeons. Considering the clinical significance, this study was conducted to assess the circumferential diameter of the popliteal artery.

Material and Method

The present Study was carried out in the department of anatomy at Jawaharlal Nehru Medical College in Collaboration with department of anatomy and Datta Meghe Medical College, Nagpur Maharashtra. Total 102 lower limbs of 51 cadavers were included in the study. Out of 51 specimens 40 cadavers were males and 11 were female. All the cadavers were age group ranging between the 25-70 years.

Incision was taken and exposes the region of adductor hiatus which is the origin of popliteal artery and into the popliteal fossa. The following landmarks have been identified i.e. at the adductor hiatus and the termination of popliteal artery at the level of lower border of popliteus, the circumferential diameter of the popliteal artery was measured at its origin, and at its proximal to termination.

Observation and Results

In males the mean diameter of popliteal artery at it origin is 6.7 ± 0.6 mm on right and 6.6 ± 0.5 mm on left respectively, whereas in females the mean diameter on right is noted as 6.3 ± 0.4 mm and on left it is 6.2 ± 0.3 mm. Near just proximal part to its termination the mean diameter of popliteal artery in males is observed to be 6.9 ± 0.7 mm on right and 6.8 ± 0.6 mm on left, while in females it is noted to be 6.6 ± 0.5 mm on right and 6.4 ± 0.4 mm on left respectively.

Table 1: Mean diameter of popliteal artery at origin

	Total Specimen	Mean diameter of right popliteal artery	Mean diameter of left popliteal artery	P value
Male	40	6.7 ± 0.6 mm	6.6 ± 0.5 mm	0.42
Female	11	6.3 ± 0.4 mm	6.2 ± 0.3mm	0.49

Table 2 : Mean diameter of popliteal artery at proximal to its termination

	Total Specimen	Mean diameter of right popliteal artery	Mean diameter of left popliteal artery	P value
Male	40	6.9 ± 0.65mm	6.8 ± 0.6mm	0.47
Female	11	6.6 ± 0.5mm	6.4 ± 0.4mm	0.21

Table 3: At origin

	Male (n = 40)	Female (n = 12)	P value
Mean diameter of right popliteal artery	6.7 ± 0.6 mm	6.3± 0.4 mm	0.03
Mean diameter of left popliteal artery	6.6± 0.5 mm	6.2± 0.3mm	0.01

Table 4: At proximal to its termination

	Male (n = 40)	Female (n = 12)	P value
Mean diameter of right popliteal artery	6.9±0.65mm	6.6±0.5mm	0.04
Mean diameter of left popliteal artery	6.8±0.6mm	6.4±0.4mm	0.03

Mean difference in diameter of popliteal artery was significantly higher in males compared to females on either side at origin. There is statistically significant difference between mean diameter of popliteal artery of males and females at just proximal to its termination.

Discussion

Popliteal artery is having considerable importance in various diagnostic and therapeutic procedures in the world of healthcare such as bypass surgery, trans-luminal angioplasty and sub intimal angioplasty. Popliteal aneurismal and vascular injuries proceeded to be extremely dangerous leads to high percentage of leg amputations[11,12] Various researchers like Thomas Sandgren et al.[13] Zuhal et al[14] Morris-stiff et al[15] and Debasso et al[16] measured the mean diameter of the popliteal artery was 7.4 mm ± 0.9., 7.5 mm ±1.3, 7.4 ± 1.3 mm and 7.4 mm respectively in their study. This was the higher value in comparison to our study. Crawford et al[17] and Wolf YG et al[18] Dhaval K Patil et. Al[19] found that the mean diameter was 6.7 mm 10 and 6.8 ± 0.8 mm and 6.3 ± 0.6 mm respectively in their study. This result is almost comparable with our study. One more study by Anjali Vishwanath Telang et. al. observed that the mean diameter was 4.7± 0.9, 4.4± 0.7 and which was lower than what we get in our study. The variation in the measurement may be due to the difference in live and cadaveric spacemen or due to the method of measurement used by the researchers on the basis of i.e. whether they take inner or outer diameter.

Conclusion

To conclude the data from the present study is useful for the vascular surgeons, radiologists. The variation in the measurement may be due to the difference in live and cadaveric spacemen or due to the method of measurement used by the researchers on the basis of i.e. whether they take inner or outer diameter.

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A Prospective Study of Dry Eye in Patients after Manual Small Incision Cataract Surgery

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Abstract

Introduction: Dry eye is a multifactorial disease of the tears and ocular surface with symptoms of discomfort, visual disturbance, etc. with potential damage to the ocular surface. Additionally, it is followed by increased tear film osmolarity and ocular surface inflammation. Schirmer's I and tear film breakup time is performed to determine the dryness level as mild, moderate, and severe.

Aim : The research aims to establish the incidence of dry eye in patients after small incision cataract surgery.

Material and Method: 100 patients who met inclusion criteria were enlisted for cataract surgery. Every patient was asked about symptoms related to dry eye and then they underwent Schirmer's test 1 and tear film breakup time test. After this, patients were subjected to surgery with a 6-7 mm superior incision. On 1st, 7th and 30th day after operation schirmer's and tear film breakup time test was again performed. The dryness of the eyes was then analysed and graded in accordance with the 2007 DEWS classification.

Result: Out of 100 samples, 16% had dry eye preoperatively which increased to 89% postoperatively on 1st day. Out of these 89 patients having dry eye postoperatively, 35 were male and 54 were female. Gradually it reduced after 7th day and 30th day postoperatively. The mean age of the patients was 66.15 years with SD 6.10. The schirmer's test I and tear film breakup time p values were clinically significant< 0.0001.

Conclusion: After manual small incision cataract surgery, moderate to severe dryness was observed. This dryness stabilized gradually after 1st week of surgery and improved significantly further after one month with the use of lubricants, antibiotic and steroid combination.

Keywords: *Dry eye, SICS, Tear film, post –operatively, ocular Surface, Cataract.*

Introduction

Cataract is defined as any opacity in the lens or its capsule whether congenital, developmental or acquired which causes visual impairment. In India, cataract is one of the leading cause of visual impairment. Small incision cataract surgery (SICS) with corneoscleral tunnel is the most routinely performed surgery for the cataract in countries such as India .Many patients have reported of dry eye and post-operative pain symptoms following

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surgery[1]. One or more of the above contributing factors may be implicated in dry eye pathogenesis after cataract surgery .In addition, the use of topical eye drops, reduced corneal sensitivity and surgical inflammation such as a large incision created in the eye and exposure to light from microscope during surgery are considered to be responsible for post-operative dysfunction of the tear film[2,3] This study analyzes changes in tear films and production of tears after cataract surgery.

Material and Method

- **Settings:** All the examination was conducted at the Department of Ophthalmology in Shalinitai Meghe Hospital & Research Center, Wanadongri, Nagpur.
- **Research Design:** Prospective observational study.
- **Duration:** 6 months
- **Sample Size:** A total of 100 patients undergoing small incision cataract surgery.
- The study will be adhered to the tenets of the declaration of Helsinki, and it will be approved by the institutional ethics committee of SMH & RC.
- Informed consent will be obtained from all subjects after the nature and possible consequences of the study is explained to them.
- **Inclusion criteria:** Patients having unilateral or bilateral age-related cataract with or without dry eyes symptoms.
- **Exclusion Criteria:**
    1. Cataract other than senile cataract
    2. Glaucoma
    3. Sjogren's syndrome
    4. Uveitis
    5. Contact lens users
    6. Patients with constant ocular medication
    7. Patients who have had previous eye surgery
    8. Patients suffering from pterygium, ocular allergies and blepharitis.
    9. Disorders of lid and naso lacrimal pathway.

Pre-operatively all patients were asked about symptoms related to dry eye such as eye discomfort, which include soreness, itchiness, gritty sensation, blurring of vision, redness improving with blinking, and excessive tears. After this, observations were noted according to slit lamp examination, Schirmer's test 1, tear film breakup time. After all the inquiries, with a superior incision of 6-7 mmall the patients underwent cataract surgery.

Schirmer's test I: This test tests the secretion of tears over a given time span. Schirmer test I is performed with standardized stripes without topical anesthesia (total tear secretion). The strip is folded at the notch and placed at the junction of the lower eyelids in the middle and lateral thirds and allowed to remain there for 5 minutes with patient's eyes gently closed. The amount of wetting is measured after the filter paper is removed. More than 10 mm of wetting after 5 minutes is considered normal, 8-10 mm of wetting is considered mild dryness, 5-7 mm of wetting is considered moderate dryness, and less than 5 mm of wetting is considered severe dryness at the end of 5 minutes.

Tear film break up time: With minimal stimulation, fluorescein strips (Omni Strips Fluro; Ophthalmic Strips U.S.P.) are moistened with non-preservative saline and are inserted in the conjunctival sac, undetected by patients. Individuals will then be instructed to blink for a few seconds several times to ensure an appropriate mixing of stain. The tear film is then examined with a broad beam and a blue cobalt filter. The interval between the last complete blink and the appearance of the first corneal black spot or line is measured using a stopwatch. A TFBUT value more than 10 seconds is considered normal, a value of 8-10 seconds is considered mild dryness, a value of 5-7 seconds is considered moderate dryness, and a value less than 5 seconds is considered severe dryness.

The eye drops, 'Tropicacylplus' which consists of tropicamide (0.8%) plus phenylephrine hydrochloride (5%) were instilled 3 times over an hour before surgery for the dilatation of pupil.With4ml of Lignocaine (2%) with 1:100000 adrenaline plus 2ml of Bupivacaine (0.75%) plus 150 units of hyaluronidase, a peribulbar block was given and then a superior partial thickness incision of 6-7 mm was made. Then a self-sealing tunnel was made and side port incision was taken at 9'o clock. At the end of the surgery, side port was hydrated and subconjunctival Gentamycin and dexamethasone were administered at lower fornix and eyes patched for one day. The surgery lasted for about 20-30minutes. After surgery all patients received topical 4quinD eye drops (Moxifloxacin + dexamethasone) hourly for 1 week

which was then tapered weekly over a duration of 1month and Nepatop eye drops (Nepafenac) 3 times a day for 1 month and carboxymethyl-cellulose 4 times a day for 1 month. Slit lamp examination, Schirmer's-1, tear film breakup time were repeated at 1st, 7th, 30th day after surgery. The dryness of the eyes was analysed and graded in accordance with the 2007 DEWS classification.

Results

Mostly the patients were from rural population of Wanadongri in Nagpur district . 100 patients were between 55- 80 years age. The mean age of the patients was 66. 15 years with SD 6.10. Among these patients, 26 (26%) patients had dry eye and 74 (74%) patients didn't have dry eye before surgery. Out of these 26 patients having dry eye 9 were male and 17 were female.

After implementation of the grading system of eye dryness, 89 (89%) patients had dry eyes postoperatively. Among them 25% had mild grade, 22% had moderate grade and 42% had severe grade of dryness. Out of these 89 patients having dry eye postoperatively, 35 were male and 54 were female.

Table 1: Grading of dryness of eyes among the patients (preoperative & postoperative)

Grading	Number of the patients		Percentage	
	Pre op	Post op	Pre op	Post op
Mild	16	25	16 %	25 %
Moderate	8	22	8 %	22 %
Severe	2	42	2 %	42 %
Total	26	89	26 %	89 %

The mean Schirmer's 1 value was 16.78 mm with SD 5.54 mm preoperatively. After 1st day of surgery, the mean Schirmer's test value decreased to 7.03 with SD 5.17. After 1 week of surgery, the mean Schirmer's test value improved to 7.15 mm with SD 5.08mm. After one month of surgery, the mean value of Schirmer's test became 10.45mm with SD 5.97 mm. The Schirmer's test minimum value was 4mm and maximum value was 30mm. The difference of values between preoperative Schirmer's test with that of post-operative 1st day, 1st week and 1 month is significant with p value less than 0.0001.

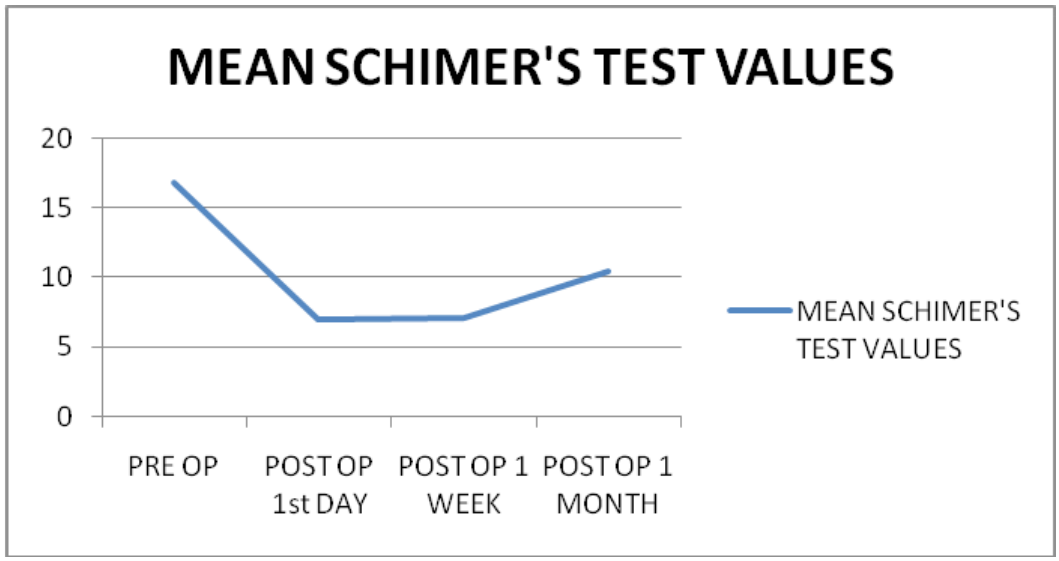


Figure 1: Line graph showing mean Schimer's test value (preoperative & postoperative)

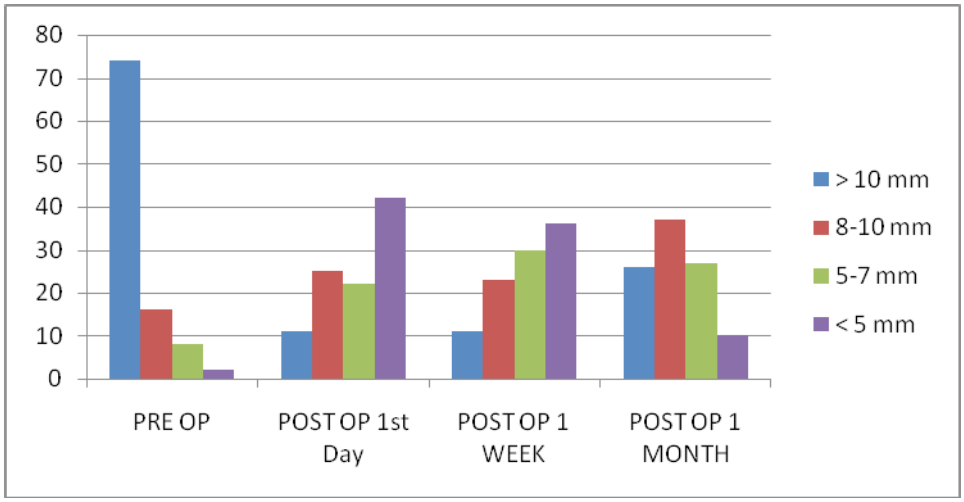


Figure 2: Bar graph showing Schimer's test value among patients preoperatively & postoperatively (1st day, 1 week & 1 month) in percentage

In this study, Schirmer's 1 value>10 mm was taken as normal, 8-10 mm as mild grade and 5-7 mm considered as moderate and < 5 mm as severe. The Schirmer's test analysis shows that 74% patients had normal value, 16% patients had mild grade of dryness, 8% patients had moderate grade of dryness and 2% patients had severe grade of dryness preoperatively. After 1week of surgery, 36% patients had severe grade of dryness, 30% had moderate grade and 23% had mild grade. After a period of one month, the Schirmer's test analysis shows that 26% patients had normal value, 37% had mild, 27% had moderate and 10% had severe grade of dryness.

In TBUT study, the mean tear breakup time (TBUT) preoperatively was 17.89 seconds with SD 6.98. The mean TBUT decreased to 7.24 seconds with SD 5.40 at 1st post-operative day and after 1week postoperatively the mean TBUT increased to 7.84seconds with SD 5.22. After one month postoperatively, the mean TBUT further increased to 13.23 seconds with SD 7.48. The maximum value of mean TBUT was 28 seconds and minimum value was 2 seconds. The difference of values between pre-operative TBUT with that of post-operative 1st day, 1st week and 1 month is significant with p value < 0.0001.

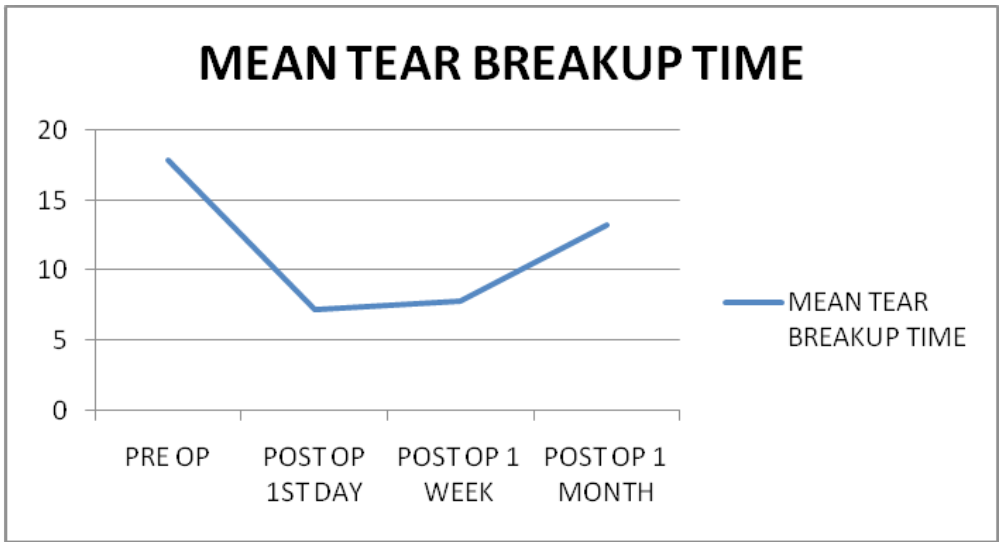


Figure 3: Line graph showing mean TBUT (preoperative & postoperative)

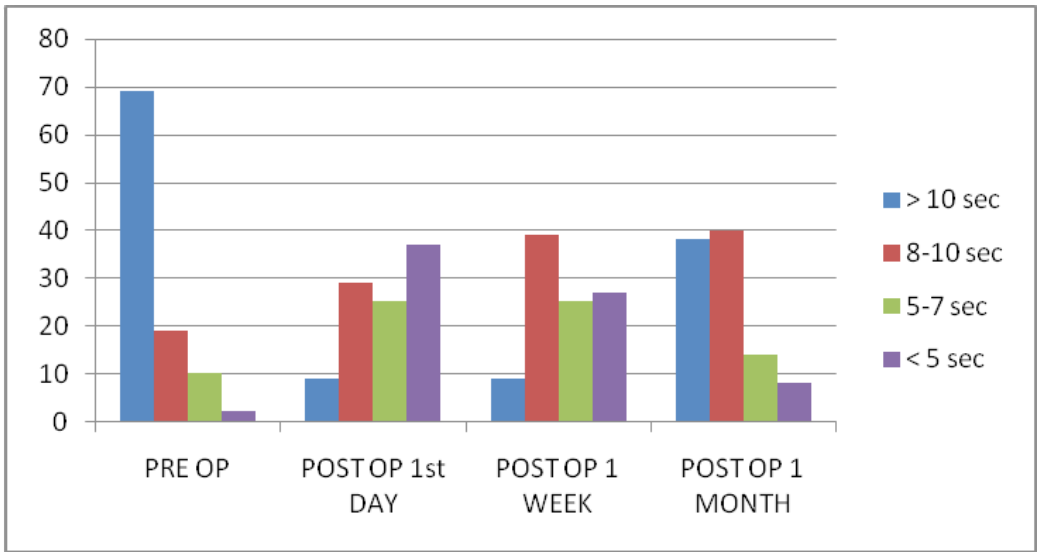


Figure 4: Bar graph showing TBUT among patients preoperatively & postoperatively (1st day, 1 week & 1 month) in percentage

In this study, tear breakup time of more than 10 seconds was taken as normal, between 8-10 seconds as mild, 5-7 seconds as moderate and less than 5 seconds as severe. Preoperatively 69% patients had tear breakup time more than 10 seconds, 19% patients had between 8-10 seconds, 10% patients had between 5-7 seconds and 2% patients had less than 5 seconds. However postoperatively after 1 week, only 9% patients had normal value that is more than 10 seconds, 39% had between 8-10 seconds, 25% had between 5-7 seconds and 27% had less than 5 seconds. After one month postoperatively, 38% patients had normal TBUT, 40% had between 8-10 seconds, 14% had between 5-7 seconds and 8% had less than 5 seconds.

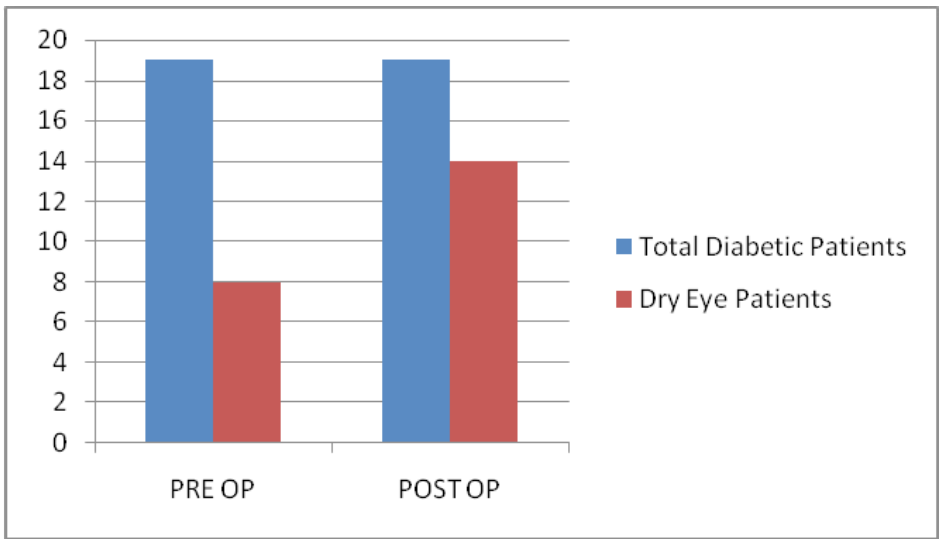


Figure 5: Bar graph showing increase in dry eye patients having diabetes mellitus

In our study, we found that out of 100 patients, 19 were diabetic. 8 of these 19 patients had dry eye pre-operatively which increased to 14 patients post-operatively. Hence it can be seen that diabetic patients are more prone to have dry eye[14].

Discussion

In this study, after manual small incision cataract surgery, the dry eye syndrome was assessed. The causes of dryness after small incision cataract surgery are as follows:

1. Damage to corneal nerve due to the act of cutting during cataract surgery via keratome leads to dry eye syndrome.[4]
2. Damage to corneal epithelium as a result of topical anesthesia and eye drops containing preservatives like benzylkonium chloride.[5]
3. Post operative dry eye on account of exposure to light from the operating microscope during surgery.[5]
4. Irregularity of the ocular surface after surgery contributing to faster tear film breakup time.[6]
5. Destabilized tear film because of decrease in mucin production.
6. Injury to the long ciliary nerve causes reduction of corneal sensation leading to decreased tear secretion.[7]

Here in this study, the Schimer's test was used for measuring tear secretion and TBUT was used for testing tear film instability. Tear secretion and tear film instability were significantly reduced in the 1st post-operative day. After one week of surgery, there was a gradual improvement in dry eye syndrome and it improved significantly after one month of surgery[8-10.] Jayshree MP et al[11], also found the same results as she also had seen significant improvement in dry eye after one month post-operative. We observed that this improvement in dry eye syndrome could have been because of the use of a combination of antibiotic and dexamethasone (0.01%), nepafenac and carboxymethyl-cellulose in patients post-operatively. Jae Kyeong Song et al. and Henriksson JK et al., in their respective studies found that there is improvement in dry eye by the use of carboxymethy-cellulose and dexamethasone (0.01%) respectively [12,13]

Kasetsuwam N et al., had studied the symptoms of dry eye after surgery. He found reduction in dry eye symptoms 7 days post-operatively and significant improvement 30 days after surgery [15] Similar results were seen in our study as well.

The main reason behind decreased tear secretion and tear film instability after 1st post-operative day was the recovery process of corneal nerves [4] The cornea has about 44 corneal nerves bundles entering the cornea and is one of the most highly innervated organs [11] Distortion of the corneal nerves reduces the tear flow and blink rate and induces hyperosmolarity of tear film and tear film instability. After surgery, new neurite cells emerge releasing neural growth factors to regenerate the corneal axon [11] This explains the dry eye syndrome that was observed after the cataract surgery and it improved after 1 month.

The other reasons for decreased tear secretion and tear film instability are exposure to microscopic light, ocular surfaces irritation, post-operative topical eye drops containing preservatives, reduction in mucin production, reduction of corneal sensation [6]Owing to vigorous irrigation of the tear film and intraoperative manipulation of the ocular surface, the goblet cell density and mucin production are reduced.[6]

Limitation: Shorter follow up time may affect incidence in our study.

Conclusion

After small incision cataract surgery with corneoscleral tunnel, moderate to severe dryness was observed on 1st day post–operatively. This dryness stabilized gradually after 1st week of surgery and improved significantly further after one month. Thus to prevent post-operative dry eye, the use of lubricants, antibiotic and steroid combination is recommended.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Analysis of Caesarean Section Rate in a Hospital of Central India: According to Robson's 10-Group Classification

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Abstract

Introduction: Caesarean section is most common surgery performed worldwide, but increasing rate of caesarean birth has become an international concern of public health. According to WHO (World Health Organisation), rate of caesarean section should not be more than 15%.

Aims and Objective: The main aim of this study was to classify the caesarean section according to Robsons's citeria and to identify the rising causes of caesarean section in our scenario.

Material and Method: This was a cross sectional observational study and data was collected over a period of six months from every single patient, who underwent caesarean section in our hospital.

Results: During the study period, total number of deliveries in this hospital was 519, out of which there were 300 caesarean section, thus, we have 57.80% rate of caesarean section. Maximum rate of caesarean section was in group 5 i.e. Multiparous with prior caesarean section, singleton, cephalic, ≥37 weeks followed by group 2 i.e. Nulliparous, singleton, cephalic, ≥37 weeks, induced labour or caesarean section before labour.

Conclusions: This attempt of classifying the caesarean section by Robson's criteria helped to audit and identify the problem and to think about modifying the hospital protocols for reduction of rate of caesarean section.

Keywords: *Caesarean section, Central India, Robson's classification.*

Introduction

Caesarean section is most common surgery performed worldwide and crude rate of caesarean section is considered an important indicator for measuring the accessibility of obstetric services to people[1]. However, increasing rate of caesarean birth has become an international concern of public health. Currently the rate of caesarean section in world is around 30%[1] whereas according to WHO (World Health Organisation), it should not be more than 15% because increasing rates did not show any evidence of improvement in maternal and neonatal health[2] . But on the contrary, it increases maternal morbidity like adherent placenta, uterine rupture, converting the normal pregnancy into a high risk one[3-5]. For evaluation of the different indication of caesarean sections, MS Robson proposed the standardized classification of cesarean section in the year 2001 which is known as Robson's classification. It is a 10 group classification and it was then appreciated

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and approved by WHO (2014) and FIGO (2016)[6,7]. In this classification, all women who underwent caesarean section were categorised into 10 groups based on their obstetric characteristics like parity, gestational age, previous CS (caesarean section), fetal presentation, mode of onset of labour, and number of fetuses. These groups are structured in such a way that they are mutually exclusive and totally inclusive[8].

Robson's classification of cesarean section[6,9]:

1. Nulliparous, singleton, cephalic, ≥37 weeks, spontaneous labor
2. Nulliparous, singleton, cephalic, ≥37 weeks, induced labor or cesarean section before labor
3. Multiparous without previous cesarean section, singleton, cephalic, ≥37 weeks, spontaneous labor
4. Multiparous without previous cesarean section, singleton, cephalic, ≥37 weeks, induced labor or caesarean section before labor
5. Multiparous with prior cesarean section, singleton, cephalic, ≥37 weeks
6. All nulliparous breeches
7. All multiparous breeches (including previous cesarean section)
8. All multiple pregnancies (including previous cesarean section)
9. All pregnancies with transverse or oblique lie (including those previous cesarean section)
10. Singleton, cephalic, ≤36 weeks (including previous cesarean section)

Aims and objectives: The main aim of this study was to classify the caesarean section based on their indication according to Robsons's citeria and objective of this study was to identify the rising causes of caesarean section in our scenario.

Materials and Method

This was a cross sectional observational study and data was collected over a period of six months from November 2019 to April 2020 in Obstetrics and Gynecology department of our hospital. All data collected were entered in a preformed structured performa.

Inclusion criteria: Patients delivered by caesarean section during the period of November 2019 to April 2020 in our hospital were considered for study and they were classified according to Robson's 10 group classification system.

Parameters considered for classification were:

- Parity (with/without previous CS);
- Gestational age (>37/<36 weeks),
- Onset of labour (spontaneous/induced/prelabour CS).
- Fetal presentation (cephalic/breech/abnormal lie)
- Number of fetuses (singleton/multiple)

Exclusion criteria:

- Women delivered normally or by instrumental vaginal delivery.
- Women delivered outside this hospital (either vaginally or by caesarean section).

This study was conducted after approval by the ethical committee of our institute. Simple statistical measures like percentage and proportion were used for analysis of data. Descriptive statistical analysis was done.

Results

During the study period, total number of deliveries in this hospital were 519, out of which there were 300 caesarean section. Thus, we have 57.80% caesarean section rate in our hospital. After analysis of data, we have maximum rate in group 5 i.e. Multiparous with prior caesarean section, singleton, cephalic, ≥37 weeks (93.97%) followed by group 2 i.e. Nulliparous, singleton, cephalic, ≥37 weeks, induced labour or caesarean section before labour (63.86%). In group 1, where nulliparous patients went into spontaneous labour, 50.4% had caesarean section. Caesarean section rate in multiparous patients in group 3 and 4 were 27.94% and 39.02% respectively. All the patients in group 6 to 9 undergone caesarean section (100%). In group 10 i.e. Singleton, cephalic, ≤36 weeks (including previous cesarean section), 57.14% women underwent ceasarean section. The major contribution of caesarean section in our hospital were from group 2 and 5, i.e. (2)Nulliparous, singleton, cephalic, ≥37 weeks, induced labor or cesarean section before labor and (5)Multiparous with prior cesarean section, singleton, cephalic, ≥37 weeks, respectively.

Table: 1 Classification of Cesarean Section Rate By Robson's Citeria

Robson's criteria	Total no. of deliveries in each group	Total no. of caesarean in each group	Caesarean section rate present	Contribution of each group to caesarean section rate
1	123	62	50.4%	20.66%
2	119	76	63.86%	25.33%
3	68	19	27.94%	6.33%
4	82	32	39.02%	10.66%
5	83	78	93.97%	26.0%
6	9	9	100%	3.0%
7	6	6	100%	1.66%
8	3	3	100%	1.0%
9	5	5	100%	1.66%
10	21	12	57.14%	4.0%
Total	**519**	**300**	**57.80%**	

Discussion

In our hospital, we tried to classify the caesarean section into different groups based on some obstetrical parameters according to Robson's classification. In the present study, we had caesarean section rate of 57.8%, which is quite higher than the standard rate recommended by WHO[2]. In the study of Kant A et al[3], rate of caesarean section was also similarly high i.e 53.86%, whereas in the study of K.Tanaka et al[8], rate was 23.5%. In the WHO global survey (2004-2008) and WHO multi country survey (2010-2011), rate of caesarean section were 26% and 31% respectively[1]. This higher rate of caesarean section in our hospital could be because we had maximum number of patients reffered from primary health centre in view of high risk pregnancy. All the patients from group 6-9 underwent caesarean section (100%) as these criteria were considered as indications for caesarean section and neither of the patient were given trial of vaginal delivery. Majority of caesarean sections were seen in group 5 i.e. Multiparous with prior cesarean section, singleton, cephalic, ≥37 weeks (93.97%). In the study of Kant A et al[3], rate of caesarean section in group 5 was 94.49%, while in the study of K.Tanaka et al[8], it was 76.5%. Majority of patients from group 5 who delivered vaginally were already in active labour with cervical dilatation of more than 4 cm and 80% effacement. But the other patients with previous caesarean section were not ready for TOLAC(trial of labour after caesarean section), either because they did not want to take risk for their baby and also they wanted procedure of tubal ligation to be done simultaneously during the same surgery. In group 2 and 4 where labour was induced or decision of caesarean section taken prior to labour, rate of caesarean section was more i.e 63.86% and 39.02% when compared with the patients of group 1 and 3 who were allowed spontaneous labour i.e 50.4% and 27.94% respectively. Similar higher rates were seen in group 2 and 4 in the studies of Kant A et al[3], K.Tanaka et al[8],Jacob KJ et al[10], where induction was done. Every hospital has different policy regarding induction of labour (IOL) depending on the obstetrical parameters of patients, fetal parameters and its prognosis and available institutional resources, but the unindicated induction surely increases the rate of caesarean section specially primary caesarean section which ultimately increases the rate of group 5. The studies of Pandey D[11], Mbaye M et al[12] were of similar views. However, the Robsons criteria did not consider the antenatal factors for induction of labour, and also regarding the fetal distress and failure of induction, in its classification, we could not held the procedure of IOL solely responsible for increasing cesarean section rate because some studies like S Wood et al[13] and E. Mishanina[14] did not show co-relation of IOL and increasing caesarean section rates. In our study, in group 10, rate of caesarean section was 57.14%, whereas in the study of Kant A et al[3], rate was 50.9% and in K.Tanaka et al[8] it was 24.2%. This group 10, consist of preterm pregnancy where head of fetus is not engaged, it has unstable lie resulting in prolonged labour and increasing incidences of fetal distress due to

fetal prematurity resulted in higher caesarean section rate.

Conclusion

This study showed higher rate of caesarean section in our hospital, where majority of contribution is from previous caesarean group and induction group. This attempt of classifying the caesarean section by Robson's criteria helped to audit and to identify the problem and to think about modifying the hospital protocols for reduction of rate of caesarean section. Since the aim of every obstetric unit is achieving good fetal outcome without jeopardising the maternal health, this study will help to improve the obstetrical techniques and protocols for right selection of patient for induction of labour and also encouraging the trial of labour after caesarean section.

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To Study the Prevalence of Areca Nut Induced Oral Submucous Fibrosis in Patients Visiting Dental OPD of Shalinitai Meghe Hospital and Research Center Nagpur: A Demographic Study

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Abstract

Background: Oral submucosal fibrosis (OSMF) is now recognized internationally as an Indian disorder. This has one of the highest percentages of malignant transition between oral lesion and condition potentially malignant, Consequently, it is a source of concern for oral medical practitioners. The goal of the present study is to assess the frequency of OSMF among betel nut chewers in various group of age.

Research Methodology: Collectively 80 OSMF sufferers in dentist OPD of Shalinitai Meghe Hospital and research center, Nagpur over a period of 4 months (1 December 2019 to 21 March 2020) Chosen for the analysis. A comprehensive history of the case and a clinical test was carried out under displayed illumination. OSMF diagnosis was based on mouth opening troubles and blanched oral mucosa, marked with noticeable fibrous bands. Many diagnostic features included sensation of pain, salivation, protrusion of the tongue, behaviors and related malignant changes. The study was conducted based on age group, period of the habit, habit frequency and habit form. Simple correlation analysis was performed.

Results: of OSF's 80 cases searched, 37 (46.25%) cases were in stage II, 27 (33.75%) were stage III, 9 (11.25 %) stage I, and 7 (8.75 %) stage IV. Based upon age group, group III (30--40 years) Had been more prominent than the others. Areca nut (gutkha) was an important etiologic agent (55.8%) as compared with other etiological agent.

Conclusion: The high incidence of OSMF in the general population requires a substantial understanding and treatment of these lesions. Primary health care practitioners and dentists should be informed and familiar with these lesions, including etiology and pathogenesis, clinical appearance, treatment and management.

Keywords: *Areca nut; clinical fibrosis; prevalence. staging; oral submucous.*

Introduction

Shushrutha defined a disorder in ancient medicine, "vidari" below mouth and throat disorder. He observed gradual narrowing of the mouth, oral mucosa discoloration and difficulty in taking the food. Such features align specifically with the oral submucosal fibrosis symptom severity.[1] A case of "atrophica idiopathica tropica mucosae oris" that occurs in Indians in East Africa was first mentioned by Schwartz (1952). That disorder was first mentioned in India by Lal and Joshi (1953). The word "oral submucous fibrosis (OSMF)" discovered by Joshi.[2] Histopathologically, the 4 successive stages of the OSMF were mentioned by Pindborg and Sirsat (1966).[3]

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Seedat and Van Wyk (1988) reported on the permanent existence of the disease, that is, once OSMF was caused by the addiction of chewing betel nut, reversal of the disease could not appear after termination of habit.[4]

The severity of the condition can be pucked by facts published in a 2004 study that India ranks the maximum among all world records for oral cancer rates with 75,000-80,000 cases recorded per year. This disorder had been confined to countries such as India, Pakistan, Bangladesh, etc. for many years, but now this condition is stated to be due to increased levels of immigration.[5-7]

This outbreak is partly due to the lot quicker in the variety of industries engaged in efficient and easy manufacturing and intense promotion of items such as gutkha and pan masala that was launched professionally in India in 1980. Major steps even by Governments to counter this important health problem are lacking mainly because of the apprehension of impacting farmers' living standards and others. Karnataka, an indian state, develops about 65 percent of the overall areca nut consumed in the country, but a restriction was imposed on gutkha only recently after many other states restricted the commodity there under Food Safety and Standards Act.Farmers have to be informed about the adverse affects, and allowed to grow other crop that can bring benefits to them .[8-11]

The World Health Organization says that by 2020, tobacco death in India will reach 1,5 million per year. Oral cancer develops by the development of normal oral mucosa exposed to nicotine into a pre- malignant lesion that gradually transforms to carcinoma. OSMF is now recognised internationally as an indian disease.it has one of the highest levels of malignant transformation among pre- malignant oral disease. [12,13]

Considering its historical nature, medical service physicians may play a very important role in rapid recognition of OSMF. It is a severe oral cavity disease with unknown etiology, with areca nut chewing the most popular. The occurrence of this harmful addiction in India is already on the rising, so this once rare disease has become really widespread, so knowledge of clinical characteristics, diagnosis, and management is the main stay to counter this threat. Thus the position of healthcare provider becomes essential, becoming the first line of interaction with the general population. The current study aimed to assess the incidence of OSMF amongst betel nut chewers among north Indian population in different demographics.

Materials and Method

The present study was conducted in the Dept. of dentistry at Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre in collaboration with Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences, Wardha, a cross- sectional survey was carried out for 4 months. Approval was obtained from the Institute's ethical committee and written consent was received from the participant. Patients with OSMF had been separated by gender. It screened out a total of 80 patients with OSMF. All oral exams were carried out by expert investigators who knew about oral mucosal defects in the surrounding area. A clean mouth mirror was used for tissue retraction, and the oral cavity test was conducted using exam gloves. The patients selected were divided into four groups, based on their clinical grade:

Grade I: Interincisal mouth which opens up to or above 35 mm, stomatitis and blanching of mucosal surface.

Grade II: Interincisal mouth openings between 25 and 35 mm, with/without stomatitis,

Grade III: Interincisal mouth opening from 15 to 25 mm; development of visible fibrous bands in buccal surface and/or pharynx, and in every other portion of the oral cavity. .

Grade IV: Interincisal mouth with less than 15 mm of width.

A. For eg, some other grades along with other pre-malignant lesion, oral leukoplakia, oral erythroplakia, etc

B. Any additional stage associated with an oral carcinoma.

Based on class of age the OSMF patients were divided into four categories:

- Class I: 10–20 years
- Class II: 20–30 years
- Class III: 30–40 years
- Class IV: 40–50 years.

OSMF incidence was also reported based on the duration of the habit, and divided into three class:

- Class A: 2–5 years
- Class B: 5–10 years
- Class C: More than 10 years.

This research was performed on the basis of the habit type and broken down into three classs:

- Class 1: Guthka, pan masala.
- Class 2: Betal quid.
- Class 3: Tobacco, smoking.

Results

A number of 2,570 subjects were tested up, 80 subjects were discovered to be suffering from the OSMF. Out of 80 subjects, 78 were males (97.33%) and 02 were females (2.33%). Accordion to mouth opening, OSMF was divided in four Stages. Table 1 shows more prevalence in Stage II 37 (46.74%), followed by Stage III 28 (34.66%), Stage I 08 (10.47%), and Stage IV 07 (8.14%). Table 2 displayes the incidence on the basis on age of group, more prevalence of OSMF was found in Group III (34.42%), followed by Group II (30.70%), Group IV (27.91%), Group I (4.65%), and Group V (2.32%). In Group I, more incidence was discovered in Stage II (75%) OSMF than Stage I (25%). In Group II, approximately same incidence was found in Group II (34.90%) and Stage II (34.90%). Minimum incidence was recorded in Stage I (30.70%) under Group II. In Group III, maximum incidence was discovered in Stage II (54.73%) OSMF than Stage III (45.27%) OSMF. No prevalence was found in Stage I and Stage IV. In Class IV, maximum incidence was discovered in Stage II (50%) OSMF followed by than Stage III (29.17%) and Stage IV (20.83%) OSMF. Finally, in Group V, all 02 patients were found in Stage IV. ($P < 0.001$).

Table 1: OSMF incidence according to clinical stage

OSMF stage	Number	Percentage	Mean
Stage I	08	10.47%	20
Stage II	37	46.74%	
Stage III	28	34.66%	
Stage IV	07	8.14%	
Total	**80**		

Table 2: OSMF frequency according to group of age.

Age group (in years)	Total	Percentage	Stage	Number	Percentage	Mean
10-20 years (Group I)	04	4.65%	Stage I	01	25%	01
			Stage II	03	75%	
			Stage III	00	-	
			Stage IV	00	-	
20-30 years (Group II)	25	30.70%	Stage I	08	30.20%	06
			Stage II	08	34.90%	
			Stage III	09	34.90%	
			Stage IV	00	-	
30-40 years (Group III)	27	34.42%	Stage I	00		07
			Stage II	15	54.73%	
			Stage III	12	45.27%	
			Stage IV	00	-	
40-50 years (Group IV)	22	27.91%	Stage I	00	-	06
			Stage II	11	50%	
			Stage III	06	29.17%	
			Stage IV	05	20.83%	

Age group (in years)	Total	Percentage	Stage	Number	Percentage	Mean
50-60 years (Group V)	02	2.32%	Stage I	00	-	01
			Stage II	00	-	
			Stage III	00	-	
			Stage IV	02	100%	
P<0.05	80					

Table 3 Displays the frequency of OSMF according to addiction length. Group A was comprised of people with a 2–5 year addiction. Group B consists of persons with a habits period of 5–10 years, and group C consists of individuals with a habitual period of even more then 10 years. Group C (48.84 percent) showed a high prevalence compared with Group B (47.67 percent) and Group A (3.49 percent). This incidence had been significant Stastical [*P*<0.0001].

Table 3: OSMF incidence depends on the timing of the habit.

Duration of habits	Total	Percentage	Stage	Number	Percentage	Mean
2-5 years (Group A)	03	3.49%	Stage I	30	100%	0.1
			Stage II	00	-	
			Stage III	00		
			Stage IV	00	-	
5-10 years (Group B)	38	47.67%	Stage I	06	14.63%	09
			Stage II	14	38.05%	
			Stage III	16	42.44%	
			Stage IV	02	4.88%	
More than 10 years (Group C)	39	48.84%	Stage I	0	-	10
			Stage II	22	58.57%	
			Stage III	12	29.52%	
			Stage IV	05	11.90%	
P<0.05	80					

Table 4 Displays habit intensity (per day) and was split into 3 groups. Group A had such a habits intensity of 2-5 times/day; group B had such a habits intensity of 5-10 times/day and group C had such a habits intensity of even more over 10 times/day. For group C (45.81 percent) the incidence has increased than even in group B (41.86 percent) and group A (12.33 percent). Significant statistical incidence was (P < 0.001).

Table 4: OSMF incidence according to habit level per day.

Frequency habit/day	Total	Percentage	Stage	Number	Percentage	Mean
2-5 (Group A) Times/day	10	12.33%	Stage I	06	56.60%	02
			Stage II	04	43.40%	
			Stage III	00	-	
			Stage IV	00	-	

Frequency habit/day	Total	Percentage	Stage	Number	Percentage	Mean
5-10 times/day (Group B)	33	41.86%	Stage I	03	8.33%	08
			Stage II	19	58.33%	
			Stage III	10	29.17%	
			Stage IV	01	4.17%	
More than 10 times/day (Group C)	37	45.81%	Stage I	0	-	09
			Stage II	14	37.05%	
			Stage III	18	48.98%	
			Stage IV	05	13.96%	
P<0.05	80					

Discussion

Yang showed the effect, distribution of gender, age, incomes and urban growth status of patients with OSF in Taiwan. During the duration 1 January 1996 to 31 December 2013 people were identified with OSMF. It found that OSMF frequency substantially increased from 8.3 (per 10 (5)) in 1996 to 16.2 (per 10 (5)) in 2013 ($P < 0.0001$). Males had a substantially greater incidence of OSMF than females. [14] Sinor et al . found higher prevalence in OSMF case in India. [15] In the current study, male prevalence could be more common than females allows for easy availability for males to use areca nut and its items. Male patients was higher than females, with an OSMF incidence of 97.67 per cent compared to 2.33 per cent in females.

Mehrotra performed a research in Allahabad, North India, to determine the prevalence levels of oral mucosal lesions at this hospitals from 1990 to 2001. With relation to age, sex, site involving and histological results, data has been collected year wise. This found that pre-malignant and malignant oral lesions in patients visiting the hospital in this area were prevalent[16] Likewise, in a population-based case-control study in various socio-economic Lucknow, patients have used pan masala have been observed to be at an increased risk of getting OSMF. [17]

Babu et al. ‘s findings amongst Hyderabad OSF patients found that people were more susceptible to gutkha than in any other associated areca nut and tobacco products like pan, pan masala, and raw areca nut. They found a clear correlation between gutkha chewing and OSMF, and pointed out that gutkha eating resulted in OSMF [18] Nigam et al. established OSMF incidence and seriousness between common gutkha, areca nut, and Moradabad, India pan eating. OSF incidence was 6.3 per cent and gutkha eating was the most common harmful habit in the study of OSF patients. [19] Likewise, traditional gutkha chew with tobacco was more predominant in this sample than gutkha.

Throughout this study the age demographic of 80 patients was 15–60 years, with a highest prevalence in 30-40 years. (34.42%), followed by 20–30 years (30.70%).Therefore, it can be assumed that the incidence of OSMF is most often seen in age category 30--40 years influenced by 20-30 years. The youngest patient is 16 and the oldest were 60. The finding of the current study were similar to that of Nigam, who recorded the highest limit of OSMF cases in the 36--40 year age category. [19] This may be attributed to increased social interactions and economic freedom in a quickly developing country like India that they are getting at this age. Therefore, they indulge in various chew habits throughout that period, such as betel nut, guthka, pan masala, smoking, alcohol, etc., either to alleviate tension, as a trend, or because of social pressure.

Shah discovered OSMF ‘s relationship to different chewing and smoking behaviors. These are discovered that eating of areca nut/quid or pan masala (a promotional preparation of areca nuts, lime, catech and unconfirmed colouring agents, flavoring agents and sweetening agents) was closely applicable to OSMF and eating intensity has been highly linked with OSMF instead of the duration of the habit. [20] Ali et al. assessed the impact of the intensity, length and form of areca nut products on OSMF occurrence and progression. This found that the length and intensity of its use and the form of isca nut material has had an impact on OSMF occurrence and progression. Gutkha and pan masala have quicker

and more deleterious effects on the intestinal cavity. The gutkha-chewing addiction together with the other habits has no major impact on the occurrence rate and the frequency and intensity of the OSMF . [21] Current study demonstrated significant impact on the incidence and severity of OSMF by the period and duration of the use of areca nut items..

Clinical staging of all 80 OSMF patients has been evaluated in the present report. Stage II was discovered to see highest patients (46.42%) and stage III (34.52%), stage I (10.73%) and stage IV (8.33%). In his study of 1,006 OSMF patients, Kumar discovered that there were 422 (41.94 per cent) cases in stage II. 226 (22.29 percent) were stage IV, stage III 184 (18.29 percent) and stage I 174 (17.29 percent) which varies slightly from the current analysis. [22] This may be on the basis that major changes in particular are not used in the cases resulting, restricted opening of the mouth, and unless there is a considerable adoration of the body of the patient's functions, patients will not response the doctor, and moreover a lack of data about the disease can attach to this as well.

The conversion from premalignant to malignant varies between 3% and 19%. A recent Indian study indicated that 25.77 percent of OSF cases were transformed to oral squamous cell carcinoma (OSCC) indicating the troubling malignancy possibility of OSMF.[23]

Accumulated epidemiological studies across a large geographical area will assist assess the total current prevalence rate and implement suitable measures to prevent. epidemiology data accumulated across a wide geographic area can help determine overall incidence and prevalence levels and formulate appropriate preventive and control measures. Highly risky members of the community of tobacco use will be focused and Community involvement should be made. Policymakers interested need to laid down legislation to counter this ever-growing threat. Primary healthcare practitioners and dentists should play an significant part in preventing and managing lesions caused by tobacco, as they are typically the first line of interaction for patients at elevated risk.

Conclusion

The widespread accessibility and marketing of these areca nut product, especially gutkha and pan masala in social sites, has affected the average person in India, leading to an increased incidence of pre-malignant OSMF. In this study, the incidence of OSMF in gutkha eaters is much more rapid and extreme compared to other types of areca nut eaters. Intensity of habit displayed statistically significant, suggesting that the seriousness of the disease also grew as intensity of habit increases. OSMF's increasing rate requires a considerable assessment and acceptance of such lesions amongst these normal community. Primary health care practitioners, even dentist, must be associated with etiology and pathogenesis, diagnostic criteria, prevention and rehabilitation of these diseases.

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Usefulness of Aarogya Setu App to Fight with Covid19

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Abstract

Like most other countries in the world, India has been battling the Covid-19 pandemic for the past few months. To limit the spread of this virus, Prime Minister Narendra Modi called for a complete nationwide lockdown from March 25th and April 14th. However, due to the rising number of cases, this lockdown was extended until the first week of June and we have now moved towards Unlocking. While addressing the nation to inform billions of Indians about this extension, the Prime Minister also asked every citizen to download and use a mobile-based application, named Arogya Setu app. Social distancing has become the new norm for most Indians today, amidst this pandemic. At such a time, people need to stay updated regarding containment zones, virus hotspots and more related information. This Arogya Setu app is a government initiative to ensure the utmost safety for its citizens from this deadly virus. In essence, this application connects Indian health services to its people at this unpredictable time. Through contact tracing, the app can notify a user of any hotspot or 'high-risk' areas near his or her residence. It can assist a person to adopt adequate measures to avoid infection.

Keywords: *Arogya Setu, Lockdown, Covid-19.*

Introduction

COVID-19, a pandemic disease as declared by WHO has marked its presence all over the world. As a result many people unaware of the COVID-19 patients were coming in contact with each other and as a result the disease was spreading. So, in order to help people and in ensuring the safety of all citizen in India, a mobile phone application AAROGYA SETU has been developed. Aarogya Setu, a mobile phone application developed by the National Informatics Centre (NIC) under the Ministry of Electronics and Information Technology in India has been made mandatory to download by all people and use it so in a way to fight against novel COVID19.



Aarogya Setu App Logo

In order to control and restricting the spread of COVID-19, government of India has adopted the strategy of contact tracing using the Arogya Setu app. Aarogya Setu in Sanskrit means "bridge to health" which was launched on 2nd April 2020. Since then it has been made mandatory to download on every device. Aarogya Setu helps in tracing the contact by using the Bluetooth and GPS coordinates of the user to track its location as well the location of the other users so as to helping to know

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whether the other user have come in touch with any of the COVID-19 patients and making the user aware of the area or person having COVID-19[1].

Working of Aarogya Setu app: Aarogya Setu app is available for both android and iphone users. It can be downloaded directly from the play store or app store.To understand the working of this app, we need to register it after the downloading of app is done on mobile devices.

When registering on the app, users name, sex,age, profession and the countries (if visited any in the last 30 days) are collected. These collected data is then stored on the server which is run by the Government of India. A unique ID is allocated to every user which is used for the identification of users in case of app related transactions. This digital Id is then used to upload or update any other information.



Also, while registering on the app the users location details are tracked and collected from the device and is stored on the server. One of the most important necessity of this app to function properly is that the user must keep the location and Bluetooth features always ON.

After uploading all the details for the registration of app, it starts its functioning when the other registered user come at a distance less than 10 meters i.e within Bluetooth range of each other. After these, the apps, irrespective of the devices on which is installed will exchange the unique Id(digital Id) and will record the GPS location at which the contact took place.

This information which will be collected at this point is stored locally on the device. If any of those registered users tests positive for COVID-19, the information will stored on the server from their mobile device.

As per the policy of Aarogya Setu app, it keep on continuously collecting data of the users location and stores it on the device. The data usually consist of all the places visited at 15-minutes intervals.

Everytime the user conducts his assessment on the app, on the basis of tick-marking the symptons, the user is marked as Yellow, Orange or Green depending on the result of self-assessment. Green means the person is safe[1].

"According to the information technology ministry,despite fears over privacy, Aarogya Setu app has been downloaded 100 million times."

Aarogya Setu app consist of four section:

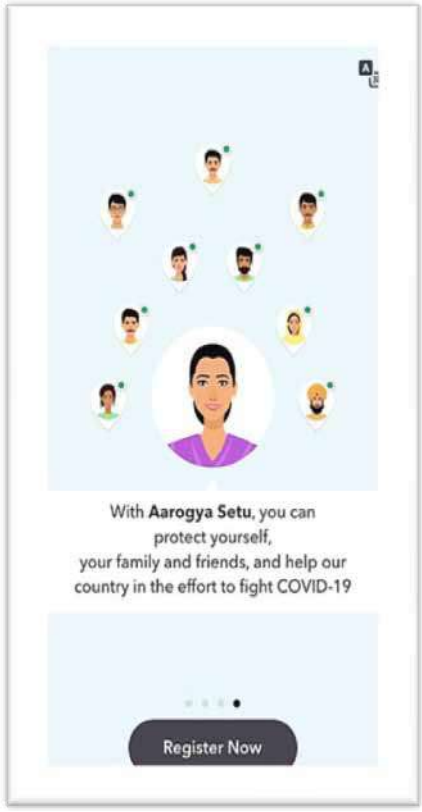
1. **Your Status section:** These section in the app tells the user status and the risk of getting infected by COVID-19.
2. **The Self Assess section:** These section is used to tick

marking the self assessment question regarding the covid-19 symptons. It is used to do self assessment of user and know the probability of the infection if any.

3. **The COVID Updates section:** It is used to provide updates and notification regarding the COVID-19 cases locally as well as internationally.
4. **The E-pass section:** These section is not yet live and will be functioning soon[2].

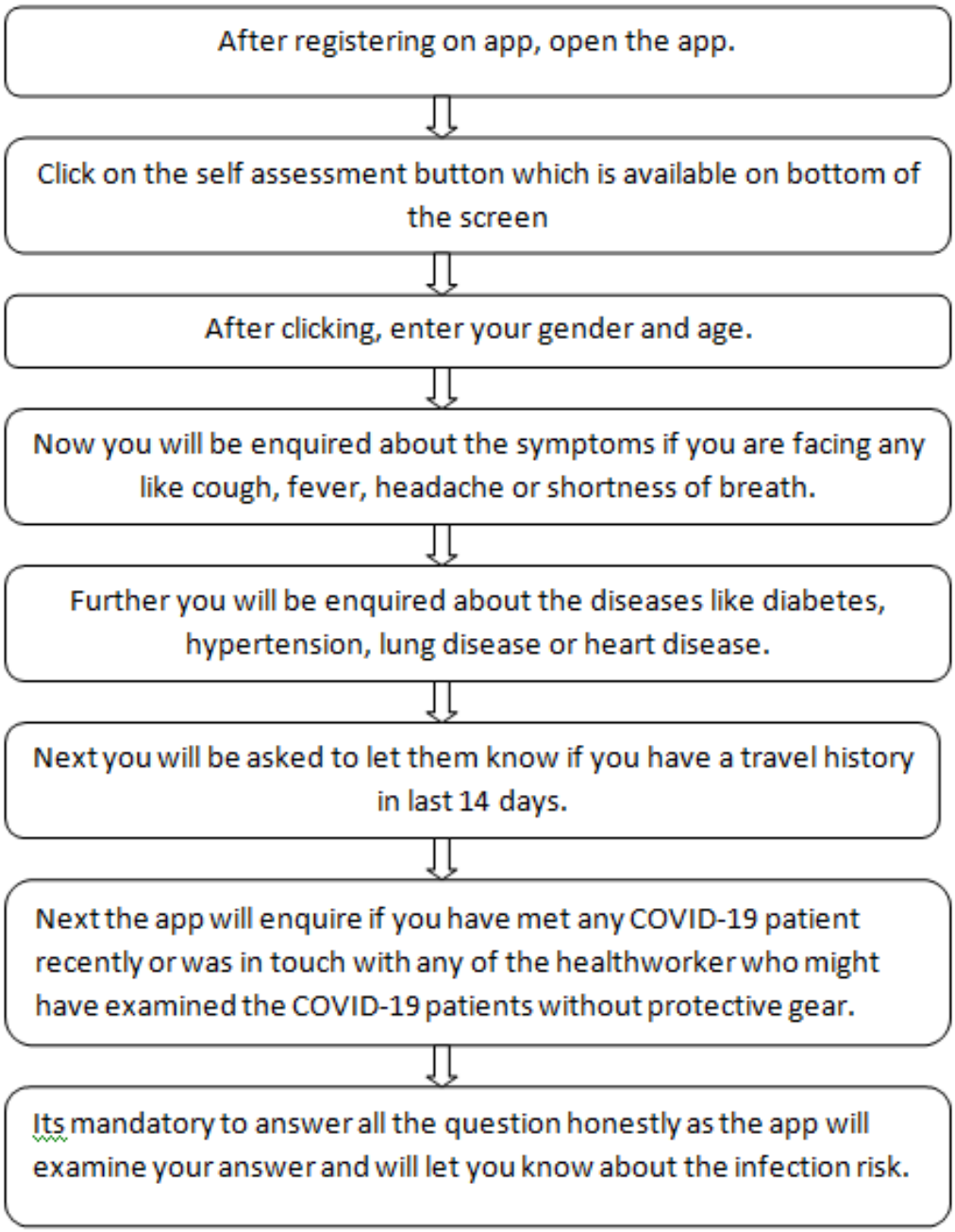
Procedure to download the Aarogya Setu App: India is taking all the measures to fight against these globan pandemic. As a measure to stop spread of this virus, an app called Aarogya Setu has been developed. This app can be downloaded in your mobile devices in following way:

1. For android user download the app from Google Play Store and for apple iphone user download it from Apple app store.
2. Language selection (available in 11 languages).
3. From the settings tab of the device, switch on the location and Bluetooth.
4. From the Phone settings, set location sharing as "Always". Location should be always On to track the area live.
5. A pop will asked to register than click on Register.
6. After registering, click on "I agree" button.
7. Now user will be asked to enter the mobile number.
8. After entering the mobile number, user will receive the OTP. Enter the OTP and click on verify.
9. Now the registration is done and user can access the app. Do the self assessment and check the status of getting infected on regular basis.



Using Aarogya Setu App to find out if you have any COVID-19 symptoms: COVID-19 a global pandemic has haunted the whole world very badly. India, a developing nation is taking all the measures to fight against these desease. It has provided so many guidelines and advisories has been set up so as to be successful in winning against these deadly disease. The most important thing is to stop spreadness of this virus for which certain guidelines have been issued by Ministry of Health & Family Welfare. Also, spread of this virus can be stopped by visiting the nearest testing centre if in case any symptoms is observed[5].

Below is the step by step procedure to use the app in your mobile device and do the self assessment:



Benefits of using Arogya Setu App:



The Aarogya Setu app is available in around 11 languages i.e English, Telugu, Hindi, Kannada, Tamil, Malayalam, Punjabi, Oriya, Bengali, Marathi and Gujarati. It is expected to be available in other Indian languages soon. The benefits of using Aarogya Setu App are described below:-

1. The app is used to determine the risk based on the user location as it works on Bluetooth based technology.
2. It is also determine the risk of infection based on the users location as the location is always need to kept ON while using this app.
3. It helps the user keep informed if he/she has come in contact with any of the COVID-19 patients within 6 feet of distance.
4. Several measures is recommended by these app to users such as Social distancing, Self Assessment Test, do's and don'ts.
5. It also provides users with all the precautionary measures to be taken and the need of maintaining social distancing in these pandemic.
6. In case of the user having symptons of COVID-19 or is at a higher risk, these app will advice him to go to the nearby testing center and to make a call to toll free number 1075 immediately.
7. A chatbot is also there in the app itself which will provide answers to all the questions regarding Coronavirus disease.
8. It can also help in providing epass for travelling.
9. For each state of India, users can find the helpline number through this app.

Is it mandatory to download the app?: On 5th May 2020, it has been mandatory by centre for all the private and government employees to download these app on there smartphones . Prime Minister Narendra Modi had also urges all the people living in containment zone to download it as well as to all the working employees and has been mandatory.

As the cases in Delhi is rising day by day,Delhi has made it compulsory to download it and the person if found any without this app in his device can be even jailed for 6 months.

Zomato and swiggy has also made it mandatory for there staff to download it[3].

Reception of the Aarogya Setu app: As it has been made mandatory for Government and private sector employees to download it. But these initiative has been questioned by some:

- How does it track contact and help in tracing. Also, is my mobile data safe.
- In an interview to The Indian Express newspaper, BN Srikrishna, a former supreme court jude was quotted as saying "utterly illegall" against the app.
- MIT Technology Review's that there are around 25 contact tracing app around the world and there is concerned about its functionality.
- Ethical hacker Robert Baptise of French was quoted as saying regarding the app that "Forcing people to install an app doesn't make a success story. It just means that repression works"[3].

Privacy Concerns regarding Aarogya Setu app: No doubt the government has rubbishes all the privacy

concerned and has said that it has been made by keeping privacy rule in mind . Still many concern has been raised over the safety of using users data which include gender, location details etc as the data is stored in cloud. SFLC a Delhi based software company has raised an issue regarding collection of sensitive information and storing on cloud and has highlighted the clause 2 (a) of the Privacy Policy and wrote, "This clause enables the Government to share personal information uploaded to the cloud with "such other necessary and relevant persons" in order to "carry out necessary medical and administrative interventions". This is problematic as the clause is broadly worded allowing the data to be shared with practically anyone that the Government wants."[4]

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

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The Study of Profile of Autopsy Cases of Hanging with Respect to Educational Status, Family Status and Place of Hanging of Victims in a Rural Region of Central India

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Abstract

The increase crime rates with stress in the day to day life is responsible for vide range of medico legal autopsies in the society. The violent asphyxial deaths are responsible for the large number of medicolegal autopsies out of which hanging is a very common in society. Hanging is the cheapest and painless form of self-destruction. It has been employed as the method of suicide since time immortal. The study under consideration is a cross sectional study which was carried on the dead bodies of either sex. The deceased were brought for medico legal autopsy with the history probably revealing the cause of death due to hanging in the autopsy room at a medical college in Central India from 1st August 2011 to 31st July 2017. The number of hanging cases during the study period were 101,the majority of victims 66 (65.34%) belong to the nuclear family while the 31 (30.69%) cases belong to the joint family. The family status of 4 (3.96%) cases cannot be ascertained as the bodies were unidentified. The majority of victims 70 (69.30%) cases, preferred indoor place for hanging while about 31 (30.69%) cases preferred outdoor place for hanging. Considering their educational status about 24.75% cases had educated upto primary school, 10.89% had upto secondary school, 24.75% upto higher secondary, 22.77% upto graduate or more and 11.88% were uneducated. Also in 4.95% cases, the educational status was unknown.

Keywords: *Hanging, Nuclear family, Educational status, indoor.*

Introduction

Although the tradition of hanging can be traced back to millennia earlier, the well-reported documents indicate that it was practiced in Anglo-Saxon Britain as a form of practice until the 5th century. The first person in Britain to have been hanged by state officials on charges of treason, Mr. William Fitz Osbert. This is one of the widely used high-mortality suicide strategies. Often it is found that the partially conscious person may be killed and suspended around the neck in order to induce a suspicious by certain means. If the victim is found to be hanging, the question arises whether it is a suicide or a murder? In addressing this question, post mortem review and crime scene visits are key. An assessment by the World Health Organization in 56 countries in 2008 found the suspension to be the most commonly

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used suicide tool, contributing 53% of males with 39% of female suicides.[1] Moreover, it has been found that the adolescent age group which has the highest impact of electronic media on them are more prone for substance abuse ad adapt hanging as a method of suicide.[2]

Hanging could be typical or atypical depending upon the features of the ligature mark around the neck. Also it can be complete or partial depending upon the degree of suspension. If the body is completely suspended such that none of the body parts touches the ground, it is called as complete hanging and if any part of the body is touching the ground then it is called as partial hanging [3]. The pattern of neck injuries differs in cases of judicial hanging with that of the routine suicidal hanging. Rarely the manner of death in the cases of hanging can be accidental e.g. auto erotic deaths. Such cases are of paramount importance as the manner of death can be established by the pornographic photographs and the partially naked dead body at the scene of crime. Even though hanging is suicidal in the majority of cases, the post-mortem suspension of the body is also very common. In situations like the allegations of post mortem suspension, the internal findings of the neck structures play an important role to assess the manner of death of the individual. Regardless of the material used to form the noose, suspension hanging will kill its victims in three ways: compression of the carotid arteries, the jugular veins and the airway [4, 5]. A 5 kg of pressure is required to compress the carotid artery; 2 kg for the jugular veins and at least 15 kg for the airway [6]. The amount of time it takes to die and lose consciousness is difficult to predict accurately and depends on the several factors. It took a man who filmed his hanging, 13 seconds to become unconscious, 1 minute and 38 seconds to loose muscle tone and 4 minutes and 10 seconds for muscle movements to cease[6]. The post mortem lividity is found on the dependent parts of the body during hanging from which time since death can be assessed.[7] The ligature mark is a vital evidence of hanging deaths. The source and direction of ligature mark helps in differentiating the death as hanging or strangulation. The ligature mark is an abraded contusion which changes its colour as time passes and has age related changes.[8] An inverted V shaped mark is often seen in hanging. Because of the pressure on the jaw, the tongue is sometimes protruded, causing it to dry[4]. In hanging, the ligature mark is commonly located in the upper part of the neck resulting in the compression of the neck structures. The damage caused by the ligature mark to the internal neck structures depends on various factors like nature of the ligature material, weight of the body and the length of time of suspension. [3]

Aims and Objectives:

1. To study the summary of hanging cases with respect to the educational status, family and the economic status of the victims.
2. To device and promulgate the plan for the prevention of hanging in such cases by target based approach.

Materials and Method

The study is a cross sectional study type based on the bodies of either sex brought for medico legal autopsy with the history of death due to hanging in the autopsy room at Datta Meghe Medical College in collaboration with Jawaharlal Nehru Medical College (Datta Meghe Institute of Medical Sciences) Sawangiand Mahatma Gandhi Institute of Medical Sciences Sevagram, Wardha, Maharashtra, from 1st August 2011 to 31st July 2017. The study is having prospective and retrospective nature with a total of 101 cases with definitive history of hanging is included in the study. All the information gathered from relatives of the deceased, from the police inquest, crime scene reports and photographs taken from the police and post-mortem reports of the department were studied.

Inclusion Criteria: Autopsy on all medicolegal cases of asphyxial deaths due to hanging conducted at Mortuary, situated in central India are included in the current study.

Exclusion Criteria:

1. All violent asphyxial deaths other than hanging.
2. Birth asphyxia.
3. Sudden natural deaths.
4. Presence of advanced decomposition state.

Statistical Analysis:

1. The software for graphs and calculation of statistical values is – SPSS.
2. The software used during creation or modification of some of the diagrams.
   (a) Adobe Photoshop (R) 7.0.
   (b) Corel Draw X3.
   (c) Windows-10.

Observations and Results

The present study is a cross sectional study (both prospective and retrospective) carried out on 101 cases of hanging for a time period of 6 years. All the cases of hanging autopsied are being studied. of the total medicolegal autopsied cases of the above tenure, the hanging cases were precisely selected based on the objectives of the study, inclusion and the exclusion criteria's.

Table No. 1: Showing Educational status of hanging cases.

Type of Education	No. of cases	Percentage
Primary	25	24.75
Secondary	11	10.89
Higher Secondary	25	24.75
Graduate or more	23	22.77
Uneducated	12	11.88
Unknown	05	4.95

Out of the 101 cases, 25 (24.75%) cases were educated upto primary school, 11 (10.89%) cases upto secondary class education, 25 (24.75%) cases upto higher secondary class, 23 (22.77%) cases upto graduate or more and 12 (11.88%) cases were uneducated. Also in about 5 (4.95%) cases, the educational status was not known. Thus the majority of victims were educated.

Table No. 2: Showing profile of cases with respect to place of hanging.

Place of Hanging	No. of cases	Percentage
Indoor	70	69.30
Outdoor	31	30.69

Out of the 101 cases of hanging, the majority of victims 70 (69.30%) cases, preferred indoor place for hanging while about 31 (30.69%) cases preferred outdoor place for hanging.

Table No. 3: Showing profile of hanging cases with respect to type of family.

Type of family	No. of cases	Total	Percentage
Nuclear	66	101	65.34
Joint	31	101	30.69
Unknown	04	101	03.96

Out of the 101 cases, the majority of victims 66 (65.34%) belong to the nuclear family while the 31 (30.69%) cases belong to the joint family. The family status of 4 (3.96%) cases cannot be ascertained as the bodies were unidentified.

Discussion

1. **Educational status of victims of hanging:** Out of the 101 cases, 24.75% cases had educated upto primary school, 10.89% had upto secondary class, 24.75% upto higher secondary, 22.77% upto graduate or more and 11.88% were uneducated. Also in 4.95% cases, the educational status was unknown. Thus the majority of victims were educated.

   The above findings are consistent with that done by authors Udhayabanu R et al[9] and Chandegara Paresh Kumar et al [10]. The findings are in contrast to the observations done by author Samanta Ashok Kumar et al[11] at Bhubaneswar where he had found that in 45.7% of cases, hanging is common in illiterate people which is in contrast with our study where we had observed the same percentage to be 11.88%. The reason might be the fact that though the study area of ours is situated in a rural set up but it is having more educational infrastructure with the government and private colleges as well as schools in the vicinity of the medical college and professional courses in the maximum field of education. It is considered as the educational hub in central India.

2. **Family status of the victims of hanging:** Out of the 101 cases, the majority of victims (65.34%) belong to the nuclear family while the 30.69% belong to the joint family. The family status of 3.96% cases cannot be ascertained as the bodies were unidentified.

   The findings are in harmony with the study conducted by Ali E et al[12] in Dhaka, Bangladesh where observed that the 76% victims of hanging belong to a nuclear family. The reason might be the fact that in the joint family there is good cooperation between people resulting in the establishment of tremendous socio economical and psychological backing from the various family members in different matters of life as compared to nuclear family. There is always a psychosocial rapport among family members especially in crisis and disastrous incidences occurring in family. Hence there is a need to reconstruct the concept of joint family in our society which is always pushing towards the nuclear family concept.

We did not find the observations of any other study in contrast to our findings.

3. **Profile of hanging victims with respect to the place of hanging:** Out of the 101 cases of hanging, the majority of victims 69.30% preferred indoor place for hanging while 30.69% preferred outdoor place for hanging. Thus the most preferred area for doing the act of hanging is indoor and lonely place.

   The findings of our study are in accordance with the study done by authors Udhayabanu R et al[9], Samanta Ashok Kumar et al[11], Pal SK et al[13], Singh Pradipkumar K H et al[14],Vinita V E et al[15], Bhosle S H et al[16], Meera T H et al[17] and Kumar Chetan et al[18]. The reason might be the fact that the victims did not want to get rescued during the attempt of hanging and also they want to be alone during the a goning hours of hanging and further they are so determined to do the act that they don't want their act to get interfered by any other person.. We did not find the observations of any other author in contrast to our study.

Conclusion

From the above observations it can be summarized that hanging is more common in educated people who are having nuclear family in the majority of cases with the preferred site being indoor for hanging. The government should target such parameters by constructive planning including chapters like psychosocial stress management in educational studies right from primary education. The importance of concept of joint family should be borne in mind of the new generation so that they will be able to share thoughts with their elderly people who are well versed with the strain and stress of life. The above active interventions will boost their morale thereby reducing the death due to hanging in our society.

Ethical Clearance: Taken from institutional ethics committee.

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Morphometry of Scapular Acromion Process Pattern in Vidarbha Region

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Abstract

Introduction: The shoulder joint is the most important joint although most commonly dislocated joint in human body. The most common problem of shoulder joint replacements is the osteoarthritis, post-traumatic arthritis, rotator cuff tear arthropathy, rheumatoid arthritis, avascular necrosis (osteonecrosis) and comminuted fractures of the shoulder joint. The aim of the current study was to acquire morphometric data for the acromion process variations in shape and various dimensions of the acromion process of the scapula.

Material and Method: Total 152 subjects' scapula according to their side which was equally divided into 76 right and 76 left. The morphometric analysis of the acromion process of scapulae was recorded according as per Bigliani-Morrison et al. classification and dimensions were measured by using vernier callipers.

Observation and Results: It is observed that the mean length of acromion on either side does not differ significantly, while the mean width of acromion and its mean thickness if found to differ statistically significantly on both sides. The acromio-coracoid distance shows no significant difference on either side.

Discussion: There are many studies i.e. by the Singroha R et al (north India), Gosavi et al (Maharashtra), Singh et al (Rajasthan), Mansur et al (Nepalese), Paraskevas et al (Greek) revealed that the mean of the scapular length, width, thickness and coraco-acromial distance in centimeter found almost the same in correspondingly with our study.

Conclusion: To conclude acromion process knowledge are useful for orthopaedic surgeon during surgery of shoulder joint with associated pathology of the rotator cuff.

Keywords: *Scapula, acromion process, shoulder joint, Morphometry.*

Introduction

The shoulder joint is the most important joint although most commonly dislocated joint in human body. It has a acromion process, that projects almost at right angles from the lateral end of the spine of the scapula. Coraco-acromial ligament extends between the tip of the acromion and the coracoid process, forming a Coraco-acromial arch. The space below the arch gives way to the tendons of muscles forming the 'SITS' muscle or rotator cuff muscles.[1-2] The glenoid cavity is small and shallow, the vertical diameter of the glenoid cavity is longer but wider below than above. The most common problem of shoulder joint replacements is the

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osteoarthritis, post-traumatic arthritis, rotator cuff tear arthropathy, rheumatoid arthritis, avascular necrosis (osteonecrosis) and comminuted fractures of the shoulder joint.[3,4] Any abnormality that disturbs the relationship of the subacromial structures may lead to impingement syndrome and the treatment used for the of impingement syndrome are acromioplasty or acromionectomy.[5-8] The aim of the current study was to acquire morphometric data for the acromion process variations in shape and various dimensions of the acromion process of the scapula which will be important for the orthopaedic surgeons.

Aim and objective: To study the morphometric analysis of the acromion process of scapulae

Material and Method

The present Study was carried out in the department of anatomy at Jawaharlal Nehru Medical College in Collaboration with department of anatomy and Datta Meghe Medical College, Nagpur Maharashtra. Total 152 subjects' scapula according to their side which was equally divided into 76 right and 76 left. The morphometric analysis of the acromion process of scapulae was recorded according as per Bigliani-Morrison et al.[9] classification and dimensions were measured by using vernier callipers. Statistical analysis was done by using SPSS 21.0 software.

Observation and Results

A total of 152 dry scapulas were included in the study. Two groups were made and were equally divided into 76 right and 76 left scapulas each. It is observed that the mean length of acromion on either side does not differ significantly, while the mean width of acromion and its mean thickness if found to differ statistically significantly on both sides. The acromio-coracoid distance shows no significant difference on either side. [Table 1].

Table 1: Various parameters of right and left scapulae

Parameters	Right Mean/SD (cm) N=76	Left Mean/SD (cm) N=76	P value
Length of acromion	4.51± 0.54	4.46±0.44	0.53
Width of acromion	2.46±0.09	2.41±0.07	0.0002
Thickness of acromion	0.73±0.01	0.71±0.01	<0.01
Acromio-coracoid distance	3.08 ± 0.41	3.01 ± 0.21	0.18

Table 2: Types of acromion process

Type	Left(%)	Right(%)	P value
Type I	38%	37%	0.89
Type II	41%	43%	0.80
Type III	21%	20%	0.87

Type I scapulae in our study were 38%, type II as 41% and 21% were type III showing no significant difference on either side. [Table 2].

Discussion

Number of studies has been done previously on morphometry of the acromion process of the scapulae and its known association between shoulder impingement and the rotator cuff tears.[9] There are many studies i.e. by the Singroha R et al[10] (north India), Gosavi et al[11] (Maharashtra), Singh et al[12] (Rajasthan), Chandni Gupta et al[13] (Karnataka), Mansur et al[14] (Nepalese), Paraskevas et al[8] (Greek) revealed that the mean of the scapular length, width, thickness and coraco-acromial distance in centimeter found almost the same in correspondingly with our study. El-Din et al[15] study revealed that in Egyptian population the mean of the scapular length, width, thickness and coraco-acromial distance found to be larger than in our study.

Natsis *et al.*[16] observed that subacromial impingement syndrome and rotator cuff tears were found mostly in type III acromion's more in cases having enthesophytes formation. Nicholson et al[17] found type I (flat) 32%, type II (curved) 42%, and type III (hooked) 26% of cases in their study and this is almost similar to our study. We found type III acromion process was approximately 20 to 21%. It was almost half in comparison with the study conducted by Neslihan Boyan et al[18], one more study Mythraeyee Prasad et al[7] observed that type III acromion process was approximately 3% that was less then what we found in our study and it may be because of racial/ traits variations seen with social dimension.

Presence of systemic disorders like hypertension[18-24], diabetes[25-30] also play a key role in bone development and osteological deformities. Few of the other related studies on bone and joint disorders are available[31-44].

Conclusion

To conclude acromion process dimension knowledge are useful for orthopaedic surgeon during surgery of shoulder joint with associated pathology of the rotator cuff.

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Conflict of Interest: Nil.

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Physico-Chemical Analysis of Pure and Impure *Bhallataka*

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Abstract

Bhallataka, geeru beeja and marking nut is common Semecarpus anacardium. Washer men used this herb to mark clothes before washing. This versatile herb is used in ayurvedic system to cure various diseases by pacifying kapha dosha in human body. *Bhallataka* is mentioned under *Upavisha* group in *Ayurvedic* classics. All the parts are poisonous mostly seeds are hazardous to human due to its irritant property. By shodhan sanskara, *Bhallataka* has most beneficial effects. Fruit of *Bhallataka* is used either as a single drug or as an ingredient in many compound formulations of Indian systems of medicine to cure many diseases. *Bhallataka* grows throughout India in hot weather. *Bhallataka* used by externally and internally in both ways. *Ashuddha* (impure) *Bhallataka* containes oil in it which is removed after *Shodhana* process. It is observed that *Shodhana* (purification procedures) of the fruits should be carried out before its internal administration. There are different *Shodhana* methods mentioned in Ayurveda. This research paper deals with impact of *Shodhana* on *Ashuddha* (impure) *Bhallataka* and to compare the physico analytical parameters of *Ashuddha* (Impure) *Bhallataka* and *Shuddha* (Pure) *Bhallataka.*

Keywords: *Impure Bhallataka, Pure Bhallataka, Shodhana, Physico-chemical Analysis.*

Introduction

In Ayurveda *Bhallataka* is described as vanaspatij visha and in modern science, it is irritant organic vegetable poison. *Bhallataka* is described as a poisonous medicinal plant in Drugs and Cosmetics Act (India), 1940. Tarry oil present in the pericarp of the fruit causes blisters on contact. The major constituent of the tarry oil is anacardic acid and bhilawanol.[1]. Juice from seeds mixed with lime is used by Dhobi to mark clothes. Seed is almost black, roughly cone shaped and 2.5*1.5cm.[2] Analytical study of the drug means its physical and chemical analysis. It is carried out to check quality, identity and purity of the drug. For this purpose some analytical tests are designed and their results should be comparing with standard parameters established by C.C.R.A.S. in "Pharmacopoeial Standards for Ayurvedic formulations". If a drug fulfilling these criteria can be taken as standard drug and used for therapeutic purpose. Till recently *Ayurvedic* medicines used to be prepared and practicing by physician himself for the use of the patients. He was well qualified for identifying the single drugs and trained in the various processes of preparing compound formulations because of his training in the *Gurushishya Parampara*. The physician was at liberty to modify the composition of any preparation according to prevailing local conditions and with a view to serve the needs of any individual patients.[3]

Aims and Objectives:

- *To collect the literature review of Bhallattak.*
- To evaluate the impact of *Shodhana* on *Ashuddha Bhallataka*.
- To compare the physico analytical parameters of *Ashuddha* (Impure) *Bhallataka* and *Shuddha* (Pure) *Bhallataka*

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Synonyms:

Sanskrit Name:

Bhallat, tapan, agni,	Bhallatak, agni	vatari, spotbijak,
Krumighn, vatari1.	dahan, tapan,	dhanurbeej,
Arushk arushkar, agnik,	aruskar, anal,	bhallat,
Agnimukhi, bhalli,	krimighnha,	veejpadap,
Veervruksha, shophkrut[4]	tailbeej,	vanhi, vartaru[5]

Classification[6]

English Name - Marking Nut

Botanical Name - Semecarpus anacardium

Family - Anacardiaceae

Hindi **-** *Bhilawa*

Marathi - *Bibba*

Ayurvedic - *Sthavar vanaspatij visha*

Modern - *Irritant Organic vegetable poison*

Chemical Composition : Semecarpol, Bhilwanol[7]

Ayurvedic Properties*:*

Rasa - *Katu, Tikta, Kashaya[8, 9]*

Guna - *Snigdha, Laghu, Tikshna*

Virya - *Ushna*

Vipaka - *Madhur, Katu[10, 11]*

Doshkarma - *kaphavatshamak and pittvardhak*

Local action - *Sphotjanana, shitprashaman, vishagnha[12]*

Internal action [13]:

No.	System	Karma
1	Pachanvah sansthan (Digestive)	Dipan, pachan, bhedan krumighna
2	Swasansanshtan (circulatory)	Hrudayuttejit, shothnashan
3	Mutravah sansthan (excretory)	Mutrajanan (diuretic)
4	Prajanan sansthan (Reproductive)	Vrushyya, garbhashauttejak
5	Tapakram (Fever)	Jwaraghn
6	Twaka	Swedjanam, kushtagnha
7	Nadisansthan	Medhya, balya

Vishakt lakshane:

1. Dah, vran, mukhshoth, visarp[14]
2. Kandu, dah, twakvaivarnya, atisar, jwar, raktmeh, unmad[15].
3. Guda-shishna kandu, swedatipravrutti, trushna, alpamutrapravrutti, twakdah-kandu [16.]

Vishakt chikitsa:

1. Til tail, nariyal tail, ghee or ral malahar – local application.[17]
2. Narriyal dugdha, chincha patra swaras, nariyal, lead lotion.[18].

Post mortem appearance: Blisters in the mouth, throat, mucous membrane in the stomach, congested and inflamed, fatty degeneration of liver.[19]

Medico-legal aspect:

1. Juice of an bhallataka is used as abortificient by means of its application to the os uteri by means of an abortion stick, applied to the genitals for the punishment for adultery, juice has been thrown on the face with an evil intensions. Used by malingerers to produce as artificial bruises, internal administration by quacks –due to accidental poisoning. [20].
2. Apllied externally produces irritaton and blisters containing acrid serum, administration of juice by hakim and vaid due to accidentally. Homicidal poisoning is rare[21]

Materials and Method

Physico-chemical analysis of *Bhallataka*: In Analytical study, the following parameters were used. Parameters were taken as per Ayurvedic pharmacopoeia.

Physico-chemical analysis:

1. Loss on drying
2. Total Ash value
3. Acid insoluble Ash values
4. Acid soluble ash values
5. Water soluble Ash values
6. Water soluble extract
7. Alcohol Extractive values
8. PH of Aqueous Extract of 1% of 10% ofsolution.
9. Conductivity of Aqueous Extract of 1% & 10% of solution.

Physico-chemical Analysis:

1. Loss on Drying:

Procedure: About 2 gm sample was taken in a tarred china dish, dried at 105 °C, cooled it in desiccators and weighed. Again the procedure of heating, cooling and weighing was repeated until the constant weight was obtained. The loss on drying was calculated.

Importance: Loss on drying gives information about moisture content of the samples.

2. pH of 1% and 10% solution: pH is the measure of acidity or alkinity. It is the logarithm of the reciprocal of the hydrogen ion concentration or is equal to the logarithm of hydrogen ion concentration with negative sign.

This method has the advantage that all scales of acidity and alkality between those of solution molar (or normal) with respect to hydrogen or hydroxyl ions, can be expressed by a series of positive numbers between 0 to 14.

The plant material, in general contains many alkaline or acidic compounds.

Procedure: 5 gm sample was suspended in 100ml of chloroform water in conical flask with their mouth closed. The flask was shaked for 1 hr and then left for 6hrs and filtered. pH of filtrate (1% and 10% of aqueous extracted) was measured by Ph meter calibration of electrode. (Toschon-Toshniwal Instrument)

3. Conductivity of Aqueous extract of 1% and 10% of solutions

Procedure: 5 gm of sample was suspended in 100ml of conductivity water in conical flask with their mouth closed. The flask was shaked for 1 hr and then left for 6 hrs, and filtered. Conductivity of each filtrate (1% and 10% of aqueous extract) was measured by conductivity meter.

4. Total Ash: About 2 gm sample was taken in silica dish incinerated at temperature not exceeding 450°C until become free from carbon(for 1.5 HR), cooled in dessicator and weighed.

Again sample was heated, cooked and weighed till the constant weight was obtained.

$$\text{Total Ash} = \frac{(100 \text{ x weight of Ash})}{\text{Weight of sample taken for the test}}$$

5. Acid Insoluble Ash: Acid Insoluble Ash gives information about silicate or sand present in the sample.

About 2 gm sample was taken in silica dish. Incinerated at a temperature not exceeding 450°C until become free carbon, cooled in dessicator and weighed samples were heated, cooled and weighed till constant weight was obtained.

Thus obtained ash of sample was treated with dil. HCL The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate becomes neutral. Then this filter containing insoluble matter was transferred to original crucible dried on hot plate and ignited to constant weight.

$$\text{Acid Insoluble Ash} = \frac{(100\text{x wt. of acid insoluble ash})}{\text{Wt. of sample taken}}$$

6. Water Soluble Extractive: The sample was taken about 2 gm in a flask. 100ml of chloroform water added to it; the mouth of flask was closed. It was then shaken frequently for 6 hours then allowed to stand for 10 hours and filtered. 25 ml of filtrate evaporated in a tarred flat bottom shallow dish, dried at 105 °C in oven to constant weight.

7. Alcohol Soluble Extract: The sample weighing

about 2 gm was treated with 100ml of alcohol in closed flask for 24 hours, shaking frequently during 6 hours and allowed to stand for 18 hours. Then it was filtered 25ml of filtrate evaporated in tarred flat bottomed shallow dish at 105 °C until constant weight obtained. The presence of alcohol soluble extract was then calculated.

Bhallataka Shodhana[22,23]:

References: Rasatantrasara and Siddhayogasangraha (Part –I), (Page No.38)

Equipments - Gas stove, *Mritpatra, Dolayantra,* steel plate, knife, etc.

Ingredients

1.	*Ashuddha Bhallataka*	-	500 gm
2.	*Ishtika choorna*	-	q.s.
3.	*Buffelo* dung	-	q.s
4.	*Gomutra*	-	q.s.
5.	*Godugdha*	-	q.s.
6.	*Narikela Jala*	-	q.s.
7.	Hot water	-	q.s.

Procedure:

1. With *Ishhtika Churna:*

- The fruits which were submerged in the water, were selected for *Shodhana Sanskar* while the floating fruits were discarded.
- Receptacles of selected *Bhallataka* fruits were drawn out after three days and all fruits were cut near receptacle under water with sharp cutter in two pieces. The weight of *Bhallataka* fruit was 350 gms.
- These fruits were rubbed with brick powder (5 times more than *Bhallataka* fruits).
- This cloth bag was kept under observation for next 24 hours. After 24 hours colour of brick powder turned black.
- *Bhallataka* fruits were then separated and further rubbed with same quantity of brick powder.
- The process was repeated on second and third day, where the change in colour of brick powder was dark maroon and as original brick powder respectively.
- Hence the process was stopped here. *Bhallataka* fruits sorted, rinsed with water and dried.

2. *Shodhana* in *Buffelo dung* mixed with water:

- Receptacles of selected *Bhallataka* fruit were drawn. *Bhallataka* fruits total weight was near about 325 gm.
- *Dolayantra* was assembled in earthen pot having capacity 5 liter.
- The *Pottali* of *Bhallataka* was suspended to a iron rod on the mouth of pot in such a way that it did not touch the bottom of pot and swinging and submerged in liquid media.
- The pot was heated to boil gently in *buffelo dung* mixed with water for 3 hours.
- *Buffelo dung* mixed with water was added frequently to maintain level.
- This in turn increase the heating by ½ hour as it decreases the temperature of *Shodhana dravya.*
- Hence total 3 ½ hours heating was given. After this *Bhallataka* fruits were drawn out, washed with hot water and dried.

- Same method was applied for the *Bhallataka Shodhana* by using *Gomutra, Godugdha and Narikela Jala* as a *shodhana dravya*.
- At last they were washed with hot water and dried in sunlight. Dried fruits of *Bhallataka* crushed to make powder and filtered through piece of cloth to get fine powder.
- All above mentioned processes detoxified *Bhallataka* fruits by removing its poisonous oil and making it more suitable for medicinal use.

Observation:

- Consistency - Soft
- Colour - Black powder
- Odour - *Gomutragandhi*
- Weight after *Shodhana* - 275 gm
- Total loss of Weight - 225 gm

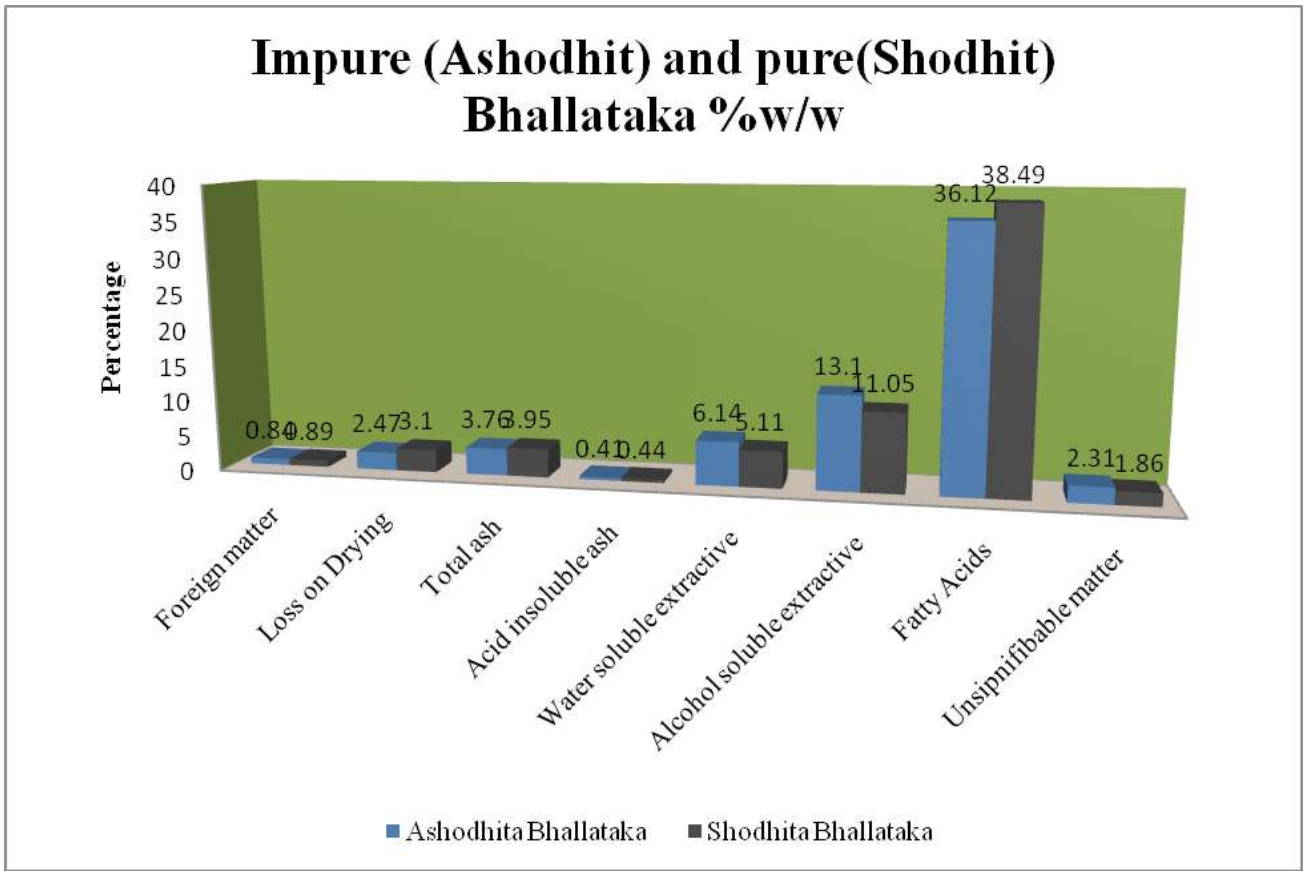
Causes of weight loss:

- Floating *Bhallataka* fruits was not taken for *shodhana.*
- Receptacles of *Bhallataka* fruits was removed during *shodhana.*
- Because of removal of external layer.
- While cutting, the damaged portion was removed.
- maximum amount of oil excretion takes place during *shodhana* in *Ishtika choorna and Gomutra.*

Precautions:

- *Pottali* should be dipped completely in each liquid media, but should not touch the bottom.
- The level of *shodhana dravya* in *Dolayantra* should be maintained as it goes down during heating.
- *Shodhana* was done on *mandagni*.
- All body should be covered during *shodhan*

Observations and Results



Sr.No.	Analytical Tests	Impure sample of Bhallataka	Pure sample of Bhallataka
1	Foreign Matter (%w/w)	0.84	0.89
2	Loss on drying (%w/w)	2.47	3.10
3	Total Ash (%w/w)	3.76	3.95
4	Acid Insoluble Ash (%w/w)	0.41	0.44
5	Water soluble extractive (%w/w)	6.14	5.11
6	Alcohol soluble extractive (%w/w)	13.10	11.05
7	Fatty Acid (%w/w)	36.12	38.49
8	Unsaponifiable matter of oil by weight (%w/w)	2.31	1.86
	Identification Test for Phenolic Constituents (%w/w)	Positive	Negative

Discussion

Analytical study of the drug means its physical and chemical analysis. It is carried out to check quality, identity and purity of the drug. For this purpose some analytical tests are performed. Till recently *Ayurvedic* medicines used to be prepared and practicing physician himself for the use of the patients. He was well qualified for identifying the single drugs and trained in the various processes of preparing compound. *Shodhana of Bhallataka* was done in Ishtika choorna, Buffelo dung, Gomutra, Godugdha and Narikela jala which removes thetoxic oil percentage in Bhallataka and make it therapeutically useful.When such *shodhana* sanskara carried out, Bhallataka posses properties like Rasayana, Yogavahi, Tridoshaghna. After *Shodhana Bhallataka* becomes soft, *Gomutra Gandhi* and black in colour. The fatty acids in *impure Bhallataka* were found in 36.12%w/w which was within normal limits. Maximum weight loss of *Bhallataka* takes place during *shodhana* in *Ishtika choorna* and *Gomutra.* The water soluble, alcohol soluble extractive and unsapnifibable matter was less in pure *Bhallataka* than *impure Bhallataka*. Identification Test for Phenolic Constituents(%w/w) positive in impure sample of Bhallataka which gets negative after purification of Bhallataka.

Conclusion

Nowadays there are various complaints about market prepared Ayurvedic Herbal Preparations . Indian market as well as global market also suffering by this thought that the market prepared drugs are not prepared well or not following the norms of Ayurvedic text described by our *Acharyas.* It affects the drug quality and patients also due to adulteration and unscientific process of preparing any Herbal preparation. In recent time, adulteration becomes very serious problem, the requirement of *Ayurvedic* formulization increased so that the quality gets decreased to fulfill the demand. To prepare quality drugs, authentification and analysis of drugs needed. In analytical study chemical configuration of raw material, physio-chemical changes during process and effect of different *Shodhana Sanskara* and configuration of *Bhallataka* were studied in terms of *Ayurvedic* as well as Modern Science. Fatty acids increases by 2.37 % in pure *Bhallataka* than impure *Bhallataka* concludes that, the *Shodhana* carried out in *Godugdha* successfully. It concludes that the principle of *Vishadravya shodhana* is to reduce its poisonous properties up to needed extent, where it does not show any unwanted and hazardous effect therapeutically.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Management of Lumber Canal Stenosis through Panchakarma: Case Study

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Abstract

Lumbar canal stenosis is a narrowing of spinal canal and compressing the nerves travelling through the lower back into the legs. The symptoms includes pain, weakness and tingling sensation or numbness of legs which radiate down to the feet along with psuedoclaudication, typically worsens with standing or walking and improves with sitting and is often related to posture and lumbar extension. It will hamper daily activity of patient along with quality of life. In modern science there is no appropriate treatment except surgical intervention which is having complication and also the chances of recurrence. *Ayurveda* can provide a suitable answer through appropriate *Panchakarma* modalities useful in Lumber Canal Stenosis. According to *Ayurveda* it can be related to *Katigata Vata* but it is not a separate disease entity. In Ayurvedic literature it is explained as one of the *Vatavyadhi.* The present case study will analyse effectiveness of *Panchkarma* in Lumber Canal Stenosis.

Keywords: *Lumber Canal Stenosis, Panchakarma, Katigata Vata, Vatavyadhi.*

Introduction

Lumbar canal stenosis is the medical condition in which the spinal canal narrows and compresses the nerves and blood vessels at the level of the vertebrae of lumber area. Lumbar spinal stenosis causes pain in the low back or buttocks, abnormal sensations and the numbness in the legs, thighs, feet or buttocks or loss of bladder and bowel control. The annual incidence of Lumber canal Stenosis is reported to be five cases per 100,000 individuals which is fourfold higher than the incidence of cervical canal stenosis.[1] A population-based study in Japan[2] found that the LSS incidence was increased by age, about 1.7–2.2% in 40–49 years old population, and 10.3%–11.2% in 70–79 years old population. Acquired lumber canal stenosis occurs due to degenerative joint disease related with vertebra and spinal cord. A community based cross sectional study in Wardha it was observed that most (82%) of the workers, who performed the activities of brick making experienced musculoskeletal symptoms any time during last one year in shoulder (s) followed by lower back (71%)[3]. Therefore causes of lumber canal stenosis are occupational over use of joint, metabolic diseases (hyperparathyroidism, hemocromatosis, and ochronosis). Lumber canal stenosis can also be caused by osteophytes, osteoporosis, tumour, trauma or various skeletal dysplasia, such as with pseudoachondroplasia and achondroplasia. The most common form of degenerative canal stenosis generally becomes symptomatic during or after the seventh decade of life[3]. It is more common in women than in men[4]. In a study population of 100 patients, Amundsen et al. found the occurrence of back pain and sciatica in 95%, and claudication in 91% of the patients. Obesity is a major risk factor[5]. Conservative treatment of lumbar canal stenosis includes physiotherapy, anti-inflammatory medications, lumbar corsets and epidural injectables[6]. It is generally accepted that surgery is indicated if well-conducted conservative management fails[7].

So there is a need and scope of *Ayurveda Panchakarma* treatment which can improve the quality

of life hampered due to associated pain and to limit the further degeneration of spine. The description about *Katigata Vata* is not a separate disease entity in any Ayurvedic text. It is mentioned as one of the *Vatavyadhi* by *Charaka*[8]. In case of *Katigata vata, dhatu kshaya* (direct stress induced factor at the *Kati* region and loss of nutritional support to the *asthis, sandhis* and surrounding *snayus* together lead to *vata prakopa* in *asthivaha srotasa.* This lead to the symptoms of numbness (*suptata*), stiffness (*stambha*) and restriction in movement (*gati avrodh*). *Ayurveda* can provide miraculous effect to such patient with the help of *panchakarma*. *Basti* procedure is main treatment used for the vitiated *vata*[9]. In the management of '*Asthi Prodoshaj Vyadhi' Charaka* stated '*Kshir Basti*'and '*Sneha Basti*[10].

Aims: Management of Lumber Canal Stenosis through *Panchakarma*: Case Study.

Objectives::

1. To observe the symptoms of lumbar stenosis in patient.
2. To prepare an Ayurvedic line of treatment according to samprapti of disease.
3. To analyze results.

Methodology

To fulfil the aims and objectives of the study this work has been carried out in the following phase wise manner.

1. Case study.
2. Discussion.
3. Conclusion and summary

Plan of Work: The clinical study was conducted in the I.P.D. of Datta Meghe Ayurveda College & Hospital, Nagpur.

1. Patient was suffering from the lumbar canal stenosis with reference to *Katigata Vata* admitted in I.P.D. of Datta Meghe Ayurveda Hospital.
2. *Panchakarma* treatment protocol was planned which includes *Sarvanga Patra pottali sweda*, *Sarvanga Shashtika pinda sweda, Katibasti*, two courses of *Kalabasti* for 33 days.
3. First course of *Kalabast*i has *Erendamooladi Niruha Basti* 450 ml and *Sahacharadi tailam Matra Basti* 60 ml.
4. Second course of *Kalabasti* has *Panchatikta ksheera Basti* 300 ml and *Sahacharadi tailam Matra Basti* 60 ml.
5. VAS (Visual analogue scale), An Owestry low back pain disability Index[11], 6MWT (6 min walking test), Pin prick test to assess the patient.

Case Study: A 55 yr old Female Indian, married consulted in Out-Patient Department of *Panchakarma* at Datta Meghe Ayurveda Hospital presenting with complaints pain in lumber region radiating to both lower limbs, Tingling sensation in both lower limbs below knee joint for 3 years. Patient was unable to walk or lie straight and was not able to perform his daily personal work, chappal slip from right foot. But from 15 days there is severity in symptoms. She was also suffering from constipation since long duration. The patient was diagnosed as Lumber canal stenosis in Goverment hospital Nagpur and advised surgery but patient was not willing to operate. So the patient was admitted in *Panchakarma* female ward of Datta meghe Ayurveda Hospital on 2nd September 2019.

On examination:

1. General condition of patient was moderate and a febrile.
2. PULSE: 82/min
3. Blood pressure: 140/80 mm of hg
4. Pallor: present
5. Systemic examination:

   CVS: S1 S2 Normal

   CNS: Well oriented, conscious.

   RS: AE=BE, clear.

   P/A: Soft, non tender; Liver, Kidney,

   Spleen: Not palpable.

Ashta Vidha Parikshan[12]:

1. Nadi- Pitta Vata
2. Mala-Malavshtambha (occasionally)
3. Mutra – Samyak Pravrutti
4. Jivha- Sama
5. Shabd-Spashta.
6. Sparsha- Anushna Sheet

7. Druk-Panduta

8. Akriti- Madhyam.

Vikrut Strotas Parikshan: Rasavaha Strotas: Ubhaya Pindiko Dveshtan (cramps in both legs), Panduta (pallor).

Asthivaha Strotas: Katishool (pain in lumbar region), Kati to Vama Pada Shool (radiating pain from lumbar region to left legs).

Majjavaha Strotas: Tingling and numbness in both upper and lower limb, difficulty in walking, slipping of chappal from right foot.

Present Illness: Patient was having above complaints from three year. She was consulted and admitted at Goverment hospital Nagpur for treatment and But there was no improvement in any symptoms during treatment and so they advised surgery. So that patient came to the Datta Meghe Ayurveda Hospital for further treatment.

Past History: No H/O- DM/HTN/IHD/PTB/BA/ Jaundice/Typhoid or any other major medical illness.

No H/O-Any major surgical illness.

No H/O- Accident

H/O- fall from 5 foot height before 5 years.

Clinical examination of spine

Inspection: No scoliosis, no lordosis, no kyphosis were found in the patient. No any other major abnormality was seen in spinal examination of patient. No any swelling and surgical marks were seen in spinal examination of patient.

Gait: waddling gait.

Palpation and other clinical examinations

SLR -right + at 45°.

Left + at 50°.

VAS score –10.

6MWT- 50 m

Owestry low back pain disability Index- 45%

Investigation:

Haemoglobin %- 9.2gm %

Total Leucocyte Count- 8200 cu/mm

Differntial Leucocyte Count-Polymorph-55%, Lymphocyte-47%, Eosinophil + Monocyt-3%

Urine examination

Albumin-Nil

Sugar-Nil

Microscopic examination-NAD

RBS (random blood sugar)-106 mg/dl.

MRI of Lumbar Spine: MRI on dated 2/12/2018 reported that Marginal osteophytes at multiple level. L3-L4 disk reveals diffuse posterior bulge compressing thecal sac without neural compression. L4-L5 dick reveals broad based posterocentral protrusion compressing thecal sac and bilateral L5 nerve root. Facetal arthropathy and ligamentum flavum thickening is noted at this level, contributing to central canal stenosis.

Panchakarma chikitsa[13]: for *Katigata vata* case Table No. 1.

***Panchakarma* procedures**	**Method of preparation**	**Method of administration**	**Duration of treatment**
Sarvanga PPS	Leaves of *Erenda*, *Nirgundi*, *Amlika, Shigru, Karanja* etc *vatahara dravya* cooked with *methika* and *shatpushpa churna* and tied in cotton cloth in the form of *pottali*.	The *pottali* is dipped in warm *tila tailam* and applied all over body in 7 position of *abhyanga*.	14 days
Katibasti	Ring shaped cavity made at centre of low back by dough prepared by black gram flour.	Warm *tila tailam* and *sahacharadi tailam* in equal quantity is filled in the ring repeatedly upto 45 minute.	22 days
Sarvanga SPS	500 gm *shashtika* rice cooked in 1.5 liter *Balamoola kashaya* and 1.5 liter of milk and boiled till it becomes semisolid. Tie in cotton cloth in the form of *pottali*.	The pottali is dipped in warm milk *balamoola kashaya* mixture and applied all over body in 7 position of *abhyanga*.	14 days

***Panchakarma* procedures**	**Method of preparation**	**Method of administration**	**Duration of treatment**
Erendamooadi Niruha basti	*Erendamooladi kashaya* 300ml + *Shatapushpadi kalka* 30 gm + Sahacharadi tailam 50 ml + Honey 50 ml + Gomutra 30 ml + *Saindhava* 15 gm	Administred in left lateral position with *basti yantra* before meal.	Total 6 *basti* for first round of *kala basti* manner.
Panchatikta ksheera basti	*Guduchi, Nimba, Vasa, Patola, Kantakari ksheera kashaya* 200 ml + honey 40 ml + *Sahacharadi tailam* 60 ml	Administred in left lateral position with *basti yantra* before meal.	Total 6 *basti* for second round of *kala basti* manner.
Anuvasana Basti	60 ml of warm *Sahacharadi tailam*	Administred in left lateral position with *basti yantra* before meal	Total 20 *basti* for two round of *kala basti*

Table No.2 First round of Kala basti

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Type of Basti	A	A	EN	A	EN	A	EN	A	EN	A	EN	A	EN	A	A	A

A = *Anuvasana basti*, EN= *Erendamooladi Niruha basti*

Table No.3 Second round of Kala basti

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Type of Basti	A	A	PN	A	PN	A	PN	A	PN	A	PN	A	PN	A	A	A

A = *Anuvasana basti*, PN= *Panchatikta kasheera basti*

Table No.4 BT and AT comparison of Lumber canal stenosis

Domain	**BT**	**AT**
VAS	10	4
Owestry disability Index	45%	20%
6MWT	50m	320m
Pin prick test for both lower limb	Numbness	Normal

Discussion

Lumbar canal stenosis is the most common condition associated with lumbar spine now days and causes changes in the locomotor system. It is highly prevalent in old age, mostly advised for surgical correction. Surgical treatment has excellent effects and remission in symptoms is seen in 64% of patients[14]. But mostly symptoms reappear after surgery and no complete cure so patients are seeking for alternative treatments such as *Ayurveda* can be useful. *Doshas* in this case found *kha vaigunya* at *kati Pradesh, sthana Samshraya* occurred in the *asthi, sandhi, snayus* of *kati* region. Hence, we have followed line of treatment of *katigata vata* for this case. *Ayurvedic* interventions relieved the symptoms by doing *samprampti bhang*. After *Panchakarma* treatment, the patient got its symptoms relieved.

Patra pinda swedana (PPS) made of *vatahara dravyas* and *sarvanga abhyanga* with *Tila tailm* which when applied locally increases the permeability of the skin. *Patra Pinda Sweda* is an unparalleled treatment in painful conditions caused predominantly by *Vata Dosha*, mainly in degenerative diseases. *Patra Pinda Sweda* is mainly performed to relief from pain, inflammation, swelling and stiffness associated with bone, joint and or musculoskeletal pains[15].

***Shashtika pinda sweda*(SPS)** made of *shashtika shali* is cooked with milk and decoction of *balamoola kwatha*. In SPS heat, massage and pressure causes nourishment of joints, tendons and stimulate nerve ending. *Shashtika pinda sweda* will help to improve circulation, nourishment to the body and it improves the strength of the tissues of bones and muscles[16].

Katibasti involves retention of warm medicated *tailam* over the lower part of spine for specific period. It

reduces pain, numbness, strengthens the back muscles, reduces stiffness, and increases blood circulation of the local region. It also have nourishment property for muscle of spinal column[17].

Basti: *Pakwashya* is the *moolasthana* of *vata dosha. Basti* by its action on *moolasthana* get control on *vata* all over body. *Basti* is most widely used and highly effective treatment for spine and neurological disorder. *Anuvasana basti* has *Balya, Brimhana, Vatarogahara* property and also provides deeper nourishment to deep *dhatus*[18].

Conclusion

From the above case study we can say that *Ayurveda* can be opted as alternative treatment in lumber canal stenosis. The main *dosha* vitiated is vata. In this case *Katigata vata* line of treatment has to be considered. In *Ayurveda* point of view if proper *Panchakarma* protocol performed the *samprapti bhanga* can be achieved with significant relief from the disease symptoms. From this study we can suggest that *Panchakarma* therapy have effective role in lumber canal stenosis and can be done in large population.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: nil.

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Role of Oestrogen and Testosterone in Male Infertility

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Abstract

Introduction: About 15% of couples are infertile. This suggests they can't think about a child, in spite of the way that they've had visit, unprotected sex for a year or more. In over 33% of these couples, male barrenness accept a role. Male infertility is a direct result of low sperm creation, unusual sperm limit or blockages that forestall the movement of sperm. Afflictions, wounds, interminable clinical issues, lifestyle choices and various parts can expect a job in causing male barrenness. Testosterone and Estrogen have been viewed as male and female sex hormones, separately. However, estradiol, the dominating type of Estrogen, likewise assumes a critical role in male sexual capacity. Estradiol in men is fundamental for modulating libido, erectile capacity, and spermatogenesis.

Aim: Role of Eestrogen and Teststerone in Male Infertility.

Material and Method: The present study includes total 60 subjects that include 30 infertile male and 30 fertile male. Blood samples collected from the subjects were obtained for serum estrogen and testosterone estimation, from OPD, general medicine and OBGY wards.

Result: As we found the level of oestrogen in infertile male was 19±1.2 as compared to fertile male 37.1±2.7pg/ml that is statistically highly significant. The degree of testosterone in infertile male was 4.94±0.98 while in fertile male it was 5.01±1.31 and it is statistically non-significant.

Conclusion: concentrations of serum estradiol in infertile patients are reduced significantly. It remains to be explained why estradiol decreased. Based on our results, however, there is little benefit to be gained from routine estradiol measurement as part of investigating men who complain about infertility. Low testosterone does not cause stomach discomfort. In fact, sperm production is stimulated by hormones other than testosterone.

Keywords: *Infertility, oestrogen, Testosterone, estradiol, gametogenesis.*

Introduction

Human male barrenness is the significant medical issue around the world. In most recent ten years there has been colossal logical development in the field of reproductive medication. Its prevalence in Western nations has been assessed with 20%. According to WHO study, rate of barrenness in India is 10 to 15%. In 15% barren couples, about 30% cases are because of

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guys only and in another 20% the two accomplices have perceptible variations from the norm. Subsequently a male factor assumes a huge job in about half of infertile couples.(1)

Infertility is characterized as inability to conceive following one year of standard unprotected intercourse with a similar accomplice. Barrenness has been credited to number of elements, for example, anatomic imperfections, endocrinopathies, immunologic issues, gene mutation, radiation presentation, chemotherapy, ejaculatory disappointments and ecological exposures. Causes cannot be found in over 25% of infertile guys, and these cases are alluded to as idiopathic infertility.(2)

Hypogonadism characterized as lacking gonadal capacity showed by insufficiencies in gametogenesis or hormone discharge, is a typical clinical condition influencing men's fertility and sexual health.In the United States, practically 39% of men beyond 45 years old were found to have low serum testosterone (T) levels. (3)

Estrogen is viewed as the 'female' hormone, while testosterone is considered the 'male' hormone. In any case, the two hormones are available in both genders.(4) Estradiol in men is basic for regulating libido, erectile capacity, and spermatogenesis. Estrogen receptors, just as aromatase, the enzyme that changes over testosterone to Estrogen, are inexhaustible in mind, penis, and testis, organs significant for sexual capacity. In the cerebrum, estradiol union is expanded in regions identified with sexual excitement.(5)

Testosterone production is central to the spermatogenesis. Typical levels of intratesticular testosterone are 50 to several times the testosterone concentration present in serum. Interference with this important amount of intratesticular testosterone is one reason why exogenous testosterone can either weaken or close spermatogenesis altogether extraordinarily. Similarly, hypogonadal endogenous states may be a cause for impaired spermatogenesis, and patients with hypogonadism that give oligozoospermia or azoospermia.(6)

Estradiol effect at low testosterone levels: To discern the effect of estradiol, it is important to evaluate its effect on libido at both low and normal levels of circulating testosterone. Decreased testosterone is clearly associated with low libido in males.(7) In men with diminished testosterone, the administration of exogenous estradiol has been shown to increase libido.(8)

Estradiol (E2) levels have not been concentrated broadly in infertile guys. There is proof that plasma concentration of E2 in a group of infertile men with azoospermia because of seminiferous tubule harm was essentially higher than men with obstructive azoospermia.(9)

Association between Estradiol and Erectile Function: Erectile capacity is multifaceted with an important blend of nerve, vessel, and endocrine activities that cooperate to deliver resulting penile basic changes in a planned manner. Smooth muscle, endothelium, and cell-to-cell interchanges by means of hole intersections are fundamental to erectile capacity, and accordingly pathology in any of these can prompt ED. The pathophysiology and clinical job of testosterone in erectile capacity have been concentrated widely. Androgens are vital for the penis to develop and create, and furthermore add to the physiology of erections. (10)

Testosterone is a pleiotropic hormone that assumes numerous physiological functions in, and during, the formation of male genitalia in utero. Classically, testosterone is a masculinity-related hormone. Testosterone is used as a therapy for males with late onset hypogonadism, a disease in men with symptoms triggered by a drop in serum testosterone. Low testosterone related symptoms can include reduced libido, diminished muscle mass, depression and/or erectile dysfunction.(11)

Testosterone development in normal healthy men is strictly controlled by the axis of the HPG. Higher cortical centers throughout the brain signal the hypothalamus in a pulsatile manner to secrete the GnRH. GnRH in turn stimulates the release by the Sertoli cells of LH and FSH from the anterior pituitary which modulates the development of testosterone from the Leydig cells and spermatogenesis, respectively. As the levels of testosterone increase, negative feedback suppression is introduced to the androgen receptors in the hypothalamic neurons and pituitary gland, thereby inhibiting the release of GnRH, FSH and LH.(12)

Material and Method

Study Area: The study was conducted in the Department of Biochemistry and Department of Medicine at Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Hingana Nagpur

in collaboration with Jawaharlal Nehru Medical College & AVBRH (Datta Meghe Institute of Medical sciences) Sawangi (Meghe) Wardha, Maharashtra.

Study Population:

Group I: 30 infertile male.

Group II: 30 fertile male

Patients Selection: Total 60 subjects were enrolled and were grouped as mentioned ahead.

Study Type: A cross-sectional study

Sample Collection: All experiments were performed from 8:30 to 9: OO a.m. in an ambulatory environment. 5ml venous blood will be collected under strict aseptic conditions in plain bulb for serum. Serum will be used for estimation of Oestrogen and testosterone.

Biochemical Analysis: Serum Oestrogen were analyzed by radioimmunoassay (RIA)[13] Serum Testosterone were analyzed by T RIA method.[14]

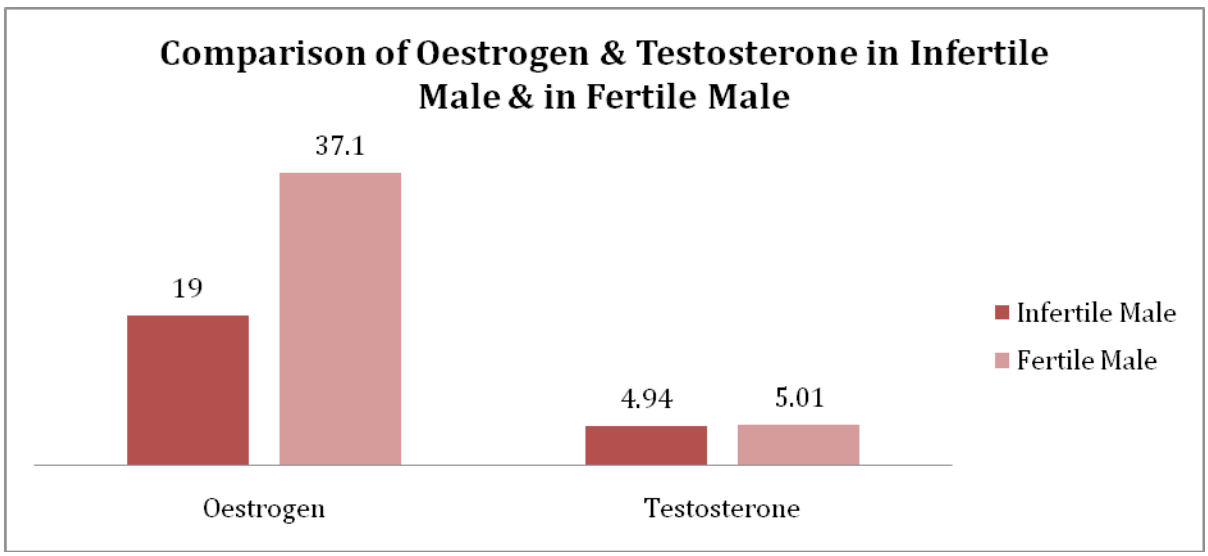
Statistical Analysis: Data collected was entered into Microsoft Excel Worksheet and statistically analysed by using SPSS (Statistical Package for Social Sciences) version 20. For quantitative data mean, standard mean, standard deviation, t-test and Karl Pearson's Coefficient of Correlation were calculated. P value < 0.05 (0.01) will be considered as statically significant (highly significant) at 95% confidence interval.

Result

Table 1: Comparison of Oestrogen & Testosterone in Infertile Male & in Fertile Male

Parameters	Infertile Male	Fertile Male	P-Value
Oestrogen	19±1.2	37.1±2.7	P < 0.0001
Testosterone	4.94±0.98	5.01±1.31	0.8155

Table shows the mean value of oestrogen and testosterone in infertile male and fertile male. As we found the level of oestrogen in infertile male was 19±1.2 as compared to fertile male 37.1±2.7pg/ml that is statistically highly significant. The degree of testosterone in infertile male was 4.94±0.98 while in fertile male it was 5.01±1.31 and it is statistically non-significant.



Graph 1: Shows the Comparison of Oestrogen & Testosterone in Infertile Male & in Fertile Male

Discussion

Roughly 15% of couples have barrenness, however just 1 of every 5 of these couples look for assessment and treatment.[15] In 30% of infertile couples, a noteworthy male factor alone is found as the reason for infertility. An extra 20% of couples have both male and female elements; in this way, male elements add to one-portion of every single non-fertile relationship.[16]

The mean serum oestrogen rates in infertile males were significantly reduced in the present study as opposed to fertile males, and our research was confirmed by Masanori Yamamoto et al.(1995) showed that serum estradiol concentrations in azoospermic or oligozoospermic patients were significantly reduced than those in normozoospermic men.[17] Hargreave et al. verified that infertile men had low estradiol level in azoospermic or oligozoospermic patients. [18]

In the present study the difference in the mean serum testosterone levels between fertile and infertile men were insignificant. Similar observations were made by Subhan et al.[19] Smith et al.[20], Rege N.A et al.[21] and S. Ramesh Babu et al.[22]

The testosterone levels were found to be common in infertile men with Sertolicell only syndrome. S. Ramesh Babu et al.[22] studies documented normal testosterone levels with Sertoli-cell only syndrome in infertile people.

Conclusion

In general, concentrations of serum estradiol in infertile patients are reduced significantly. It remains to be explained why estradiol decreased. Based on our results, however, there is little benefit to be gained from routine estradiol measurement as part of investigating men who complain about infertility. Low testosterone does not cause stomach discomfort. In fact, sperm production is stimulated by hormones other than testosterone. Testosterone is needed for sperm production, but there are several times higher levels in the testes where sperm is produced than in the blood. Even men with low or borderline T levels may have enough T levels to produce sperm.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Glomus Tumour: Uncommon Entity But Commonly Remain Undiagnosed

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Abstract

Glomus tumor is a benign and vascular hamartoma that originates from the neuromyoarterial cells of the normal glomus apparatus in the reticular dermis. It accounts for 1–5% of soft tissue tumors of the hand. This tumor typically presents with cold hypersensitivity, pain, tenderness, and sometimes nails deformities or nail discoloration. Although the precise cause of glomus tumors is unknown. We report an atypical case of a patient with painless glomus tumor.

Keywords: *Glomus Tumour, neuromyoarterial cells, hamartoma.*

Introduction

Glomus tumor are rare tumor of hand.[1] Originating from neuromyoarterial cells of normal glomus apparatus in reticular dermis they are the benign and vascular hemartoma.[2] It accounts for 1 to 5 percent of total soft tissue tumor of hand[3] Commonly presenting in hand [5] of which 90 % are located in subungal tissue of fingers[6] . It can occur at any age but commonly seen in 3rd to 5th decade of life[7] . Pain, point tenderness, cold hypersensitivity is the classical triad, Clinically commonly presents as solitary blue or purple nodular lesion .

Case Presnetation: 35 years female came with the complaint of pain in the left distal ring finger for the last 6 years which aggravated in the winter season. The patient visited multiple specialists but the pain was partially relieved by pain-relieving drugs. Before consulting us she was being treated for Reynaud's phenomenon by a physician with no relief in symptoms. History of paroxysmal pain exacerbation during cold exposure and tenderness with bluish discoloration. On clinical examination there was severe pain when pressure was applied over the proximal nail (love's sign was positive), On careful examination, the proximal nail was bluish and there was fullness near proximal nail fold and the angle was obliterated but the nail plate was not deformed.

On nail ultrasonography, a small well defined hypo echoic shadow was present in the proximal nail bed. MRI study of left ring finger reveals-well defined elongated homogeneously enhancing soft tissue lesion in the subcutaneous plane over the dorsal aspect of the ring finger in the shaft region of distal phalanx (Fig 2) and differentials of Glomus tumor and tendon sheath Giant cell tumor were kept. We considered differentials of glomus tumor, hemangioma, arteriovenous malformation, tendon sheath tumor. We performed therapeutic as well as a diagnostic biopsy. Under all aseptic precaution and ring block procedure was performed. The first eponychium was lifted and retracted backward than the nail plate was removed then using a 5mm biopsy punch, the bluish mass was excised. Histopathology finding was suggestive of glomus tumors. The patient was called after 7 days to look for any complications. Two months post-procedure there was partial regrowth of nail plate and there were no complaints of pain or tenderness. Excised tissue was sent for histopathology examination which shows feature suggestive of glomus tumor.



Figure 1–Glomus tumor at left ring finger showing fullness near proximal nail fold

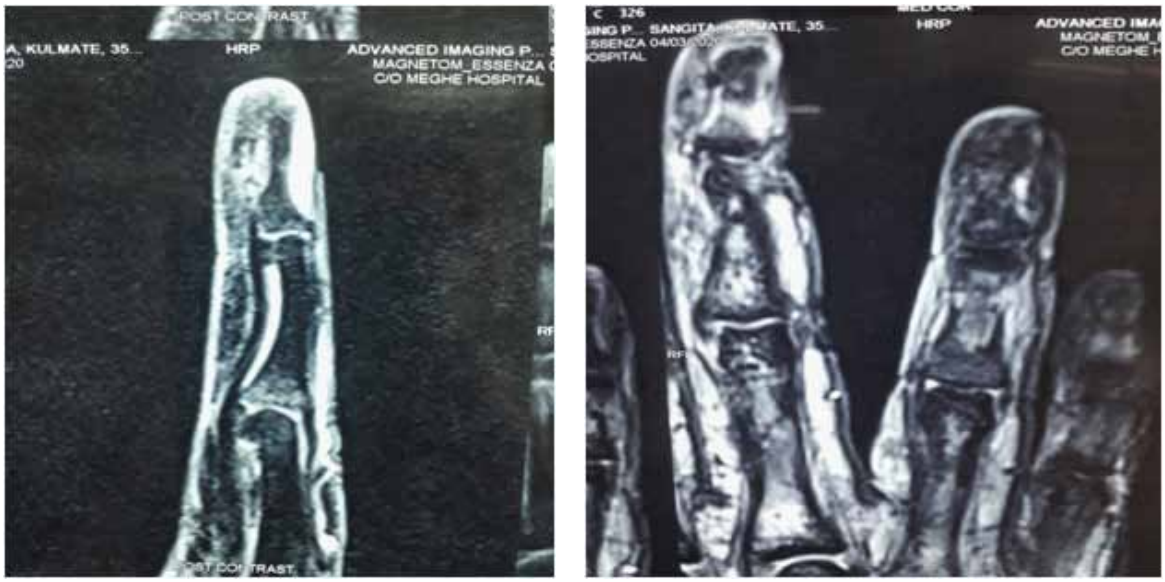


Figure 2–MRI showing well defined elongated homogeneously enhancing soft tissue lesion in the subcutaneous plane

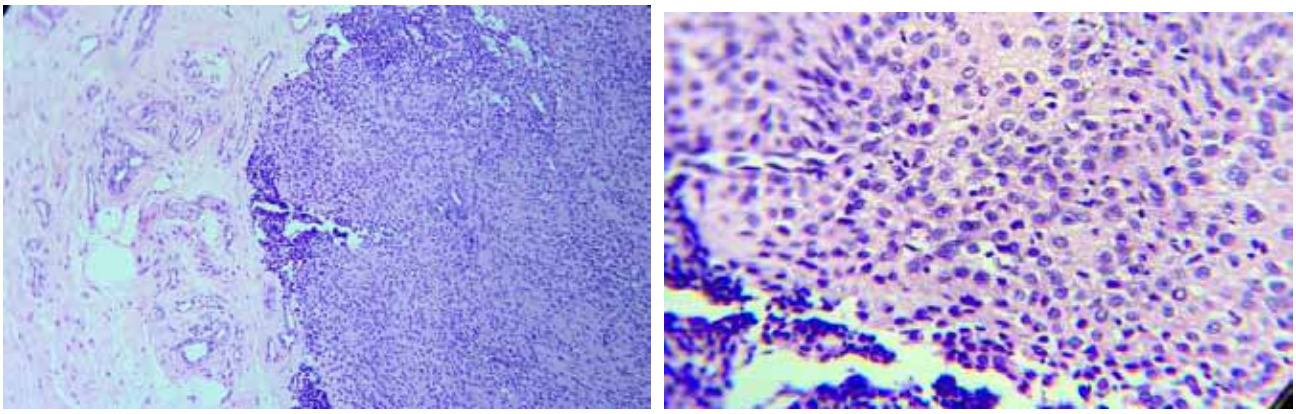


Fig 3a – 10x resolution Fig 3b – 40x resolution

Figure 3–Photomicrograph showing well-circumscribed dermal tumor composed of glomus cell



Figure 4–Nail plate removed with 5 mm biopsy punch

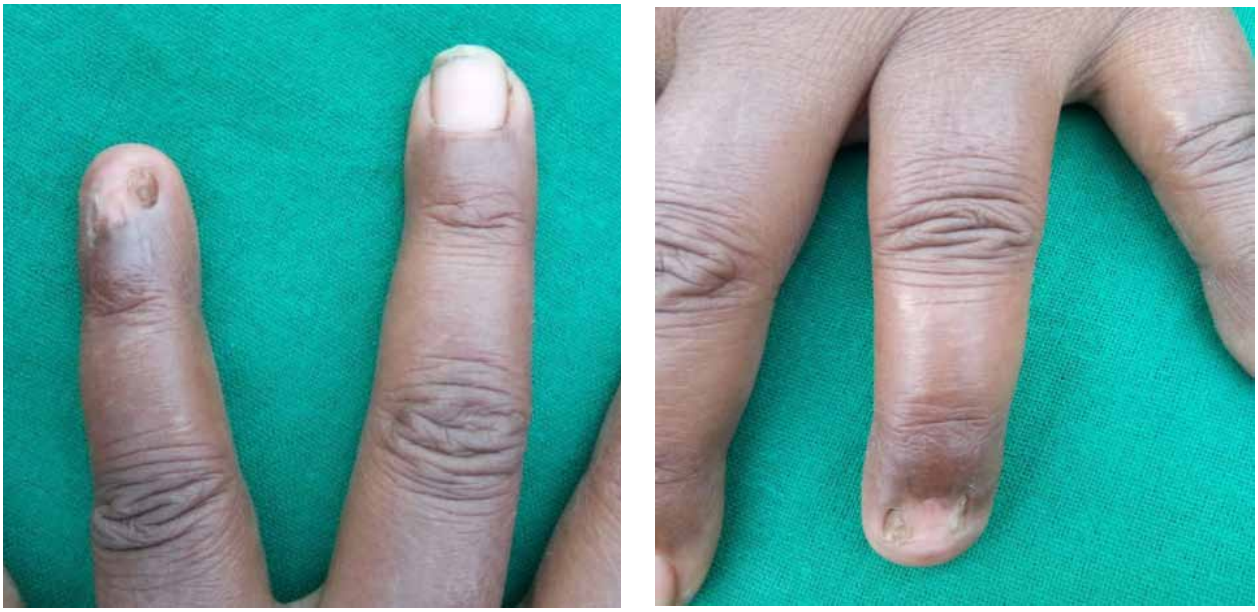


Figure 5–Post Operative Image After 2 Months

Discussion

Benign vascular hamartoma containing neuromyoarterial cells of normal glomus apparatus is known as a glomus tumor.[2] Glomus body comprises an afferent arteriole, an anastomotic Suquet–Hoyer canal, an efferent venule, the intraglomerular reticulum, and its capsule. The Suquet–Hoyer canals are lined by endothelial cells, which are surrounded by smooth muscle cells[8-10]. Interspersed in the smooth muscle are the large cuboid glomus cells. Their primary function is thought to be thermoregulation.[15]

Glomus bodies are therefore composed of three main structures: glomus cells, the vasculature, and smooth muscle cells. Glomus tumor is a result of hyperplasia of any of this structure. Accordingly, they can be classified as a solid glomus tumor, it is most

common and accounts for 75 % cases, a glomangioma (20%), and glomangiomyoma which is the rarest that is only 5 % cases. Other variants of the glomus tumor are glomangiomatosis and malignant glomus tumors.[14]

The etiology of glomus tumor is unknown but sex, age, trauma or inheritance could play a role, familial variant had been linked to chromosome 1p21-22[3] Young adults, mostly women are primarily affected[10] In hand fingertips and pulp are most common site [11] Commonly presents as small, slightly, raised, bluish or pinkish-red, painful nodule which when subungual located can lead to elevated, deformed and discolored nail[3]

Severe lancinating pain, extreme pain on touch, and intolerance to change in temperature is the classical triad for glomus tumor[12], our case had all the features of the classical triad. The diagnosis of glomus tumor should involve positive results for Love's test, Hildreth's test, and Cold hypersensitivity[5] all these tests were positive in our case.

Though diagnosis can be made on the clinical ground imaging modalities like X-ray, ultrasonography, and MRI can help in confirming the diagnosis. MRI and Ultrasound are valuable tools visualizing and diagnosing glomus tumor and ruling out other possibilities. Cortical thinning or erosive changes in adjacent bones are common radiographic findings. Ultrasound helps know the size, site, and shape of the tumor, in our case small well defined hypoechoic shadow was seen.[3] MRI shows typical characteristics of glomus tumor-like low signal intensity on T1-weighted images, marked hyperintensity on T2-weighted image & enhancement on T1-weighted images after gadolinium injection, MRI in our case reveals 8.0 x 3.1 x 4.7 mm well defined elongated homogeneously enhancing soft tissue lesion in the subcutaneous plane over the dorsal aspect of a finger in the shaft region of distal phalanx [FIG 2] Angiography and color Doppler are among the other imaging modalities.

Histopathological findings of glomus tumor include glomus cell, blood vessel smooth muscle, and in our case well-circumscribed dermal tumor composed of glomus cell with a round to oval basophilic nucleus and scanty eosinophilic cytoplasm was seen adjacent to blood vessel. (Fig 3).

Complete surgical excision is the treatment of choice for glomus tumor, complete excision of the tumor was done under local anesthesia . There was no recurrence of tumor even after 4 months post-surgery and the patient was free from local site pain during follow up period. We aimed to emphasize by detailed history, proper physical examination along with classical finding on MRI imaging we can significantly decrease the mental agony and improve the surgical outcome of patient suffering from glomus tumor.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Antimicrobial Resistance Pattern of Bacterial Pathogens in Intensive Care Unit (ICU) of Tertiary Care Hospital

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Abstract

Introduction: In ICU the spread of drug resistance has been realized that is related to widespread of antibiotics uses. In ICU the rate of antimicrobial resistance is higher than in general hospital setting. The emergence of MDR bacteria is an increasing problematic cause of health care associated infections in ICUs, not only due to increased morbidity and mortality, but also due to increased treatment costs as result of frequent empirical failure and lengthy hospital stay. Aim: The main purpose of this study was to know prevalence of the drug resistance pattern for the patients admitted in ICUs. Material and Method: The study was conducted in the Department of Microbiology of a tertiary care hospital in Maharashtra during December 2019 to May 2020. In this study patients admitted in ICUs of the hospital who were clinically suspected of having any infection were included. Depending on the clinical suspicion from the patients samples were collected. Samples were subjected to testing for identification and antibiotic susceptibility testing. Result: *E.coli* was the most common organism isolated and shows highly resistance to Amoxcillin+Clavulanic acid, Ciprofloxacin. ESBL producer were 20.17% out of all isolate and 29.07% out of total Gram negative organism. Among all Gram positive organism 13(61.90%) were *Staphylococcus aureus* in which 8(61.54%) were MRSA. *Enterococcus* showed 100% resistance to Erythromycin and Penicillin whereas *Staphylococcus aureus showed* 84.62% and 69.23% resistance to Penicillin and Erythromycin respectively. Conclusion: This study concludes general overview of the incidence and antimicrobial resistance of bacteria isolated from ICU. Gram Negative Organisms are causing more infection than Gram Positive Organisms in ICU patients. Hence gram negative organisms are more resistance to higher antibiotics. So antibiotics are used carefully. Therefore antibiotics policies should be formed for ICU and other critical care facilities.

Keywords: *Intensive Care Unit (ICU), Antimicrobial Resistance, ESBL, MRSA, E.coli, Staphylococcus aureus.*

Introduction

Antibiotics resistance has emerged as a major factor in predicting outcomes and overall use of resources in intensive care units (ICU) following infections. In ICU the spread of drug resistance has been realized that is related to widespread of antibiotics uses. In ICU the rate of antimicrobial resistance is higher than in general hospital setting[1,2]. Globally ICUs are encountering emergence and spread of antibiotic resistant pathogens and for some pathogens there are few therapeutic options available[3]. Previous epidemiological studies have focused primarily on 2 common Gram positive antimicrobial resistant organisms; Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus* (VRE)[4]. Spellberg *et al* showed that multi drug resistance (MDR) among Gram-negative

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bacteria is becoming even a greater problem in health care facilities[5]. The emergence of MDR bacteria is an increasing problematic cause of health care associated infections in ICUs, not only due to increased morbidity and mortality, but also due to increased treatment costs as result of frequent empirical failure and lengthy hospital stay[6]. Key factors in the management and prevention of MDR bacteria include rational use of antibiotics, hand hygiene, single-use items for individual patients, isolation of patients infected with resistant isolates, environmental cleaning, surveillance, active patient and resource management and education[7,8]. These surveillance programs help to maintain current knowledge of local susceptibilities and relevant treatment options. The family members of Enterobacteriace are the most important human bacterial pathogens which account for the majority of bacteria isolated from clinical samples[9]. It is a matter of concern that this Gram Negative Bacilli (GNB) is rapidly acquiring resistance to one or more traditionally used antimicrobial agent for treatment. Prompt institution of effective empirical antimicrobial therapy is the most important determinant in the successful management of ICU patients infections; inappropriate empirical theraphy affects both patients mortality rates and patient time spent at ICU[10,11]. Staphylosoccus aureus is one of the most common organism causing Nosocomial infections. (i.e. acquired in hospital) and dangerous human pathogen since publication of its role in sepsis by Ogston in 1880 and 1882[12]. Despite the introduction of antimicrobial agent and improvements in Staphylococcal disease frequency and morbidity in the twentieth century, Staphylococci have persisted as an important hospital and community pathogen[13].

The source for multi drug resistance bacteria may include repeated use of higher antibiotics, poor hand hygiene, reuse of items used for individual patients, lack of environmental cleaning, resource management and education and surveillance.

The main purpose of this study was to know prevalence of the drug resistance pattern for the patients admitted in ICUs. This study may provide that might improve the overall management of ICU.

Material and Method

This study was prospective study conducted in the Department of Microbiology of Jawaharlal Nehru Medical College (Datta Meghe Institute of Medical Sciences), Wardha, in collaboration with Datta Meghe Medical College, Wanadongri; Nagpur; Maharashtra from December 2019 to May 2020. In this study patients admitted in ICUs of the hospital who were clinically suspected of having acquired any infection were included.

In this study was done for total 124 isolates from ICU of 100 patients. Specimens for the culture were taken from ICU patient, depending upon the infection like sepsis, ventilator associated pneumonia, respiratory tract infection and post operative patients. Multiple specimens from different site as well as in different time were taken from the patient having multiple infections.

Samples like blood, urine, pus and CSF etc. were taken depending upon the type of infection. Samples were received in the microbiology laboratory for the culture in sterile universal container. Initially strains were identified based on the morphological behavior of the isolates on various differential media.

The specimens were inoculated on 5% sheep blood agar, MacConkey agar and incubated aerobically at 37ºC for 18 to 24 hours. Chocolate agar plates were incubated at 37ºC in 5% CO_2 for 18 to 24 hours. For blood culture 5-10 ml of blood for adult were collected and processed using the BACTEC 9050 blood culture system (Becton Dickinson, Maryland, USA). If growth is displayed as positive, then it is sub-cultured on appropriate media. Organism's identification was done by using routine biochemical test like catalase, Oxidase, citrate, indole, urease etc. Antibiotic susceptibility testing was done by Kirby-Bauer method using Muller Hinton Agar (MHA). Clinical Laboratory Standards (CLSI) interpretive criteria were used for susceptibility results[14]. Quality control was performed by using reference strains of *Staphylococcus aureus* ATCC 25923*, Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 to confirm consistency of materials, methods, and results[14].

Results

In vitro susceptibility data of 124 isolates obtain from ICU over the period of the study. Among 100 samples of 100 patients from ICU 62(62%) male and 38(38%) females with age ranging from 20 years to 75 years (average 40-50years old) were studied.

Table no 1. Showing Percentages of the different cases in the ICU

Cases	Percentage (%)
Respiratory tract infections	23
Urinary tract infection	20
Road traffic accidents	3
Sepsis	37
Those undergoing surgery for complicated diseases	17

Table no 2. Microorganisms isolated from 100 samples of 100 patients from ICU according to the site of infection

Microorganism	Urinary tract no. (%)	Respiratory tract no. (%)	Bloodstream no. (%)	Wound no. (%)	Other sites(*) no. (%)	Total no. (%)
E.coli	12(41.38)	5(17.24)		5(17.24)	7(24.14)	29(23.38)
Pseudomonas spp	8(40)	3(15)		1(5)	8(40)	20(16.13)
Klebsiella spp	4(23.53)	8(47.09)		1(5.88)	4(23.53)	17(13.71)
Acinetobacter spp	5(29.41)	2(11.76)	1(5.88)	2(`11.76)	7(41.18)	17(13.71)
Staph.aureus	4(28.57)	3(21.43)	1(7.1429)	2(14.29)	3(21.43)	14(11.29)
Candida non albicans	5(41.67)	4(33.33)	1(8.33)	2(16.66)		12(9.677)
Enterococcus	7(100)					7(5.645)
Candida albicans		2(50)			2(50)	4(3.226)
Proteus mirabilis				1(50)	1(50)	2(1.613)
Providencia Spp	1(100)					1(0.806)
Aspergillus spp		1(100)				1(0.806)
TOTAL						124

(*) Cerebrospinal fluid, Peritoneal fluid, Asitic fluid, Pleural fluid, Abdominal fluid, Endotracheal Secretion, Endotracheal tube and Tip.

Out of 124 isolates 86(69.35%) were Gram negative organisms, 21(16.94%) were Gram positive organisms and 17 were Fungus (13.71%). Among all Gram positive organism 13(61.90%) were *Staphylococcus aureus* in which 8(61.53%) were MRSA and 5(38.46%) were CONS among all gram positive organism isolates respectively. The most frequent species isolated from infections in ICU was *E.coli,* followed by *Pseudomonas spp, klebsiella spp, Acinetobacter spp, Enterococcus, Proteus mirabilis* and *Providencia spp* which were 23.38%, 16.13%, 13.71%, 13.71%, 5.65%, 1.61% and 0.81% respectively. ESBL producer were 25(20.17%) out of all isolate and 29.07% out of total Gram negative organism. The most frequent species isolated from infections in ICU was E.coli as shown in table no 2 and table no 3. .

Table no 3. Showing distribution of organisms in ICU

Name of organism	Number(%)
E.coli	29(23.38)
Pseudomonas spp	20(16.13)
Klebsiella spp	17(13.71)
Acinetobacter spp	17(13.71)
Staph.aureus	13(10.48)
Methicillin-resistant Staph. aureus (MRSA)	8 (6.45% among all isolates and 61.54% among all Staph isolates)
Candida non albicans	12(9.68)
Enterococcus	7(5.65)
Candida albicans	4(3.23)
Proteus mirabilis	2(1.61)
Providencia Spp	1(0.81)
Aspergillus spp	1(0.81)
Total	**124**

Table no 4. Showing the resistance pattern of common antibiotics against isolated Gram positive pathogens

Antibiotics	Staphylococcus aureus (%)	Enterococcus (%)
Erythromycin	69.23	100.0
Penicillin	84.62	100.0
Cortimoxazole	61.54	28.57
Linezolid	15.38	00.00
Oxacillin	61.54	85.71
Vancomycin	00.00	00.00
Levofloxacin	30.77	85.71
Gentamicin	61.54	85.71
Doxycycline	30.77	71.43

Table no 5. Showing resistance pattern of common antibiotics against isolated Gram negative pathogens

Antibiotics	E.coli (%)	Klebsiella spp(%)	Acinetobacter spp(%)	Proteus mirabilis (%)	Providencia spp(%)	Pseudomonas spp(%)
Amoxicillin + Clavulanic Acid	100.0	94.12	94.12	50.00	100.0	-
Gentamicin	65.52	82.35	88.24	50.00	100.0	35.29
Amikacin	37.93	52.94	70.59	50.00	100.0	50.00
Cefuroxime	100.0	88.24	88.24	50.00	100.0	-
Cefepime	96.55	82.35	88.24	50.00	100.0	65.00
Cefotaxime	96.55	70.59	94.12	50.00	100.0	-
Ciprofloxacin	100.0	70.59	82.35	00.00	100.0	70.00
Imipenem	00.00	00.00	35.29	00.00	00.00	00.00
Cotrimoxazole	93.10	82.35	88.24	00.00	100.00	-
Ceftazidime	-	-	-	-	-	60.00
Piperacillin	-	-	-	-	-	45.00
Piperacillin + Tazobactam	-	-	-	-	-	45.00
Aztreonum	-	-	-	-	-	75.00

Note: (-) = Antibiotics not used.

Discussion

This study is based on the antimicrobial resistance and multidrug resistance of the organism isolated from ICU. Antibiotics are most commonly prescribing drugs in ICU which producing the strength of resistance to the organism.

Reduction of antimicrobial resistance in ICUs has been a target for all ICUs because it increases outcomes and cost for patients in terms of expenses for costly antibiotics as well as length of stay in ICUs. This study shows Imipenem was the drug which was increasing resistance to *Acinetobacter spp* and Vancomycin has 0% resistance in case of gram positive organism.

Most isolates recovered from the urine specimens followed by the respiratory specimens and other specimens. Total 100 clinical isolates were analyzed. The most common isolates observed in this study were *E.coli* (23.38%), *Pseudomonas spp* (16.13%), *Klebsiella spp* (13.71%), *Acinetobacter spp* (13.71%), *Staphylococcus aureus* (10.48%), *Candida non albicans*(9.68%), *Enterococcus* (5.65%), *Candida albicans* (3.23%), *Proteus mirabilis* (1.61%), *Providencia Spp*1(0.81%) & *Aspergillus spp*1(0.81%). The incidence of Gram-

positive and Gram-negative bacilli in this study was 15% and 85% respectively. The *Acinetobacter spp* show the increasing resistance in Imipenem (35.29%) where as other shows 100% sensitive.

In the studied of Zaveri Jitendra et.al[15] showed *E.coli* was the commonest isolated organism in the study showing commonly sensitive to Amikacin. In the study *Citrobacter species* (66.7%) was most common multidrug resistant organism followed by *Proteus* and *Enterococcus* (33.3%, 33.3%) respectively.

Another studied of Trivedi TH et.al[16] showed ICU from Mumbai found enteric gram negative organism as the commonest isolates (61.9%) followed by *Staphylococcus aureus* (29.8%).

Similar studied of Singh AK et .al[17] from Varanasi showed that the enteric gram negative bacilli were uniformly resistance to beta lactam- beta lactamase inihibitors combinations. Ciprofloxacin and Ceftriaxone showed resistance that range from 50-100% and 25-83.3% respectively. *Staphylococcus showed* 100% resistant to Penicillin and Tetracycline, 80% resistant to Cotrimoxazole, 60% resistant to Erythromycin and Gentamicin and 40% resistant to Amikacin. *Acinetobacter spp* showed highly resistant to most of the antibacterial agents except Gentamicin while *Pseudomonas spp*. showed 75% resistance to it.

In another studied of Azad Jammu et at[18] showed Ceftazidime resistance was decreased in *Pseudomonas* and other non-fermenting gram-negative bacteria (NFGNB). In NFGNB third generation resistance of Cephalosporin seemed to be reducing; however resistance to Carbapenem appeared to be increasing, possibly due to their increasing in use.

Studied of Nidhi Goel Et al[19] showed *Pseudomonas aeruginosa as* commonly isolate bacteria which followed by *Acinetobacter baumanni* and *Klebsiella pneumoni.* Gram Negative Bacteria showed high rate of resistance to ciprofloxacin, ceftazidime, co-trimoxazole and combination of amoxycycillin/clavulanic acid. meropenem and doxycycline was noted as least resistance.

Limitations: This investigation has certain limitations. There was major shortcoming resistance rates calculated from isolates recovered exclusively from blood cultures were essentially identical to rates derived as isolates from other sites. Patient demographic information like primary source of infection, history of antibiotic used was not available as result so analysis could not be performed which could take these important factors into account. Test isolates were not routinely available for ancillary molecular characterization of either resistance determinants or clonal relationships. Not with standing these shortcomings, it is believed that this study provides a unique, objective, and systematic view of the scope and magnitude of the problem of antimicrobial resistance among GNB as well as GPC in ICU patients today.

Conclusion

This study concludes general overview of the incidence and antimicrobial resistance of bacteria isolated from ICU. *E.coli* was the most common organism isolated and shows highly resistance to Amoxcillin+Clavulanic acid, Ciprofloxacin whereas Erythromycin and Penicillin shows highly resistance to *Enterococcus*. This studied also shows the emergence and rates of Multi Drugs Resistance (MDR) organisms and emphasizes the importance of timely clinical and bacteriological monitoring among patients in a critical care situation. Infection control programs should focus on preventing infections in patients who are at highest risk of infection because of exposure to certain procedures and medical devices. Frequent hand washing and good aseptic technique should be reinforced for all health care personnel. Hence gram negative organisms are more resistance to higher antibiotics. So antibiotics are used carefully. Therefore antibiotics policies should be formed for ICU and other critical care facilities.

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Study of Maternal and Fetal Outcome Following Term Prelabour Rupture of Membrane in a Peri Urban Tertiary Care Centre

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Abstract

Background: Prelabour rupture of membrane at term accounts for 2-10% incidence. It is associated with significant maternal and fetal complications if not timely managed. Early complications include cord prolapse, cord compression and placental abruption and delayed complications include chorioamnionitis, maternal and fetal sepsis. The present study was undertaken to study the labour outcome following active management of term prelabour rupture of membrane and associated maternal and fetal outcome.

Method: Cases of spontaneous rupture of membranes with singleton pregnancy with gestational age >37 weeks with confirmed PROM by a speculum examination were selected. A detailed history was taken and gestational age confirmed, general, systemic and obstetric examinations were done. Non stress tests and blood investigations were sent to rule out sepsis. All patients received prophylactic antibiotics. After taking consent, induction of labour was done with oral misoprostol and maternal vitals were recorded four hourly and fetal heart rate was recorded for variability. Induction to delivery interval was recorded and maternal and fetal outcome following PROM was noted.

Results: PROM was more common in primigravida. Majority of women were admitted within 12 hours of PROM (87.83%). Recurrent episodes of urinary tract infection was found a significant cause for PROM (33.78%). Patient delivered vaginally with 55.40%. Post delivery complications included post partum sepsis in 12.16%. No maternal mortality was noted. Neonatal sepsis was seen in 6 babies (8.10%).

Conclusion: PROM is associated with many maternal and fetal complications which can be reduced by educating the women to have regular antenatal check up and early recognition of urinary and genital tract infection, and treat appropriately and to report at the earliest in case of rupture of membrane for timely management to reduce maternal and fetal morbidities and mortalities.

Keywords: *PROM, chorioamnionitis; Caesarean section; maternal morbidity; fetal morbidity; mortality.*

Introduction

Premature rupture of membranes (PROM) at term refers to a patient who is beyond 37 weeks' gestation and has presented with rupture of membranes (ROM) prior to the onset of labor that is active uterine contactions.[1] Spontaneous rupture of membranes (ROM) is a normal component of labour and delivery[2], but the pre-labour rupture of membranes (PROM) is not. Preterm premature rupture of membranes (PPROM) is ROM prior to 37 weeks' gestation. Spontaneous preterm rupture of the membranes (SPROM) is ROM after or with the onset of labor occurring prior to 37 weeks[3,4,5]. Prolonged ROM is any ROM that persists for more than 24 hours

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and prior to the onset of labor.[1] Term PROM occurs in 5-20% of all labours. Indian studies (Swati Pandey and Bandi S. 2000) [6] reported an incidence of PROM in 7-12% of all labours. These patients are more prone to cord compression, cord prolapse, placental abruption and higher risk of chorioamnionitis. The longer the time interval between rupture of membranes and onset of labour, the greater the risk of ascending infection and chorioamnionitis.[7,8]. PROM is associated with increased risk of dysfunctional labour, increased cesarean rates, postpartum haemorrhage and endomyometritis in the mother. In the fetus, there is increased occurrence of hyaline membrane disease, intraventricular haemorrhage, sepsis, cerebral palsy, fetal distress and mortality.[9,10.] Management of PROM has always been controversial regarding conservative management and waiting for spontaneous onset of labour which has more chances of ascending infection with maternal and fetal morbidity, or active induction of labour which causes more failure of induction with higher cesarean rates. The role of tocolytics, antibiotics and steroids has also been controversial in cases of preterm PROM. Almost 80% of patients close to term with PROM begin labour within 24 hours and 95% deliver within 72 hrs. Urinary tract or vaginal Infection, Cervical incompetence, Trauma, uterine anamolies, Antepartum haemorrhage, polyhydramnios, Multiple gestation and coitus in pregnancy, contribute to PROM.[9,10]. It is often not possible to pinpoint the exact cause in an individual case. For women with previous pregnancy history of preterm birth early detection and treatment of the cause can reduce the chances of preterm PROM in this pregnancy. **[11]** Jiwane (1991) observed that the incidence of PROM increased 4.4 fold in women undergoing routine pelvic examination in the third trimester of pregnancy. Kodkany and Telang (1991) **[12]** observed that coitus in the last trimester led to a six-fold increase in PROM. Cases needs to be individualized and all parameters should be considered while dealing with term PROM. All cases needs to be individualized considering maternal and fetal parameters so as to avoid maternal and fetal morbidity and mortality while dealing with term PROM. This study was also intended to decrease the complications associated with term PROM by actively managing labour induction and timely intervention and observing the maternal and fetal outcomes and complications associated with it.

Material and Method

The study was a cross sectional study. The study was conducted at Datta Meghe Medical College and Shalinitai Meghe Hospital and Research Centre, Wanadongri, Hingna, Nagpur during a period of 6 months from January 2020 to June 2020 where the patients who presented with term PROM and who fullfilled the criterias were included in the group and analysis of labour outcome in 74 cases with premature rupture of membranes after 37 completed weeks were done.

Inclusion Criteria:

1. Gestational age of >37 weeks confirmed by dates, clinical examination or ultrasound.
2. Primigravida and multipara
3. Pervious cesarean section patients and malpresentations.
4. Cervical dilatation of <3 cms.
5. Lack of uterine contractions for atleast 1 hour from PROM.
6. Single live pregnancy in vertex presentation.
7. PROM confirmed by Direct visualization

Exclusion Criteria:

1. Gestational age <37 weeks
2. Cervical dilatation of >3 cms.
3. Women in labour or with uterine contractions within 1 hour of rupture of membrane.
4. Multifetal gestation.
5. Women not giving consent.

Depending upon the Bishop's score, labour was induced with prostaglandin (misoprostol oral) or accelerated with oxytocin. The labour of each case was closely monitored and assessment of progression was monitored 4 hourly. Induction to delivery interval and PROM to delivery interval were noted. Maternal pulse, blood pressure, fetal heart rate and its variations were checked frequently. The onset of any complications like fetal distress, fetal heart rate variations, chorioamnionitis (clinical) were looked for. Progress of labour was monitored. If case of non reassuring NST, fetal distress or any other obstetrical complications, labour was cut short by instrumental delivery or cesarean section as required. Patients of previous lower segment cesarean section or malpresentation were directly taken for LSCS after proper investigations and consent.

Observations: Total patients who full-filled the inclusion criteria were 74: The majority presentation of PROM observed were of age group 26- 30 (39.43%), followed by 21-25 yrs (32.43%). Most of the patients in the study were unemployed doing house hold moderate activities accounting to 75.67% which was significant. Presentation of PROM was more in primigravida (56.75%) as compared to multigravida (43.24%). Majority of the patients were primigravida (56.75%) with no prior abortions, followed by previous 2 abortions in 16 cases (21.62%) and previous 1 abortion (17.56%). Majority of the patients presented within 12 hrs of PROM i.e 87.83%, in which 46 patients presented within 6 hours and 19 between 6-12 hours. Remaining 9 patients presented after 12 hours.

Table no.1: Probable causes of PROM.

Causes of PROM	N=74	%
Previous PROM	11	14.86%
Recurrent UTI	25	33.78%
Vaginitis	13	17.56%
Cervical incompetence	04	5.40%
Polyhydramnios	08	10.81%
Unknown	13	17.56%

Table no. 1 showed: The causes of PROM are endless but looking into the cases 33.78% cases presented in opd previously with recurrent history of urinary tract infection as per culture report and were treated with antibiotics which was a significant cause followed by vaginal infection observed in 13 patients. Other causes included polyhydramnios (10.81%), cervical incompetence in 04 cases and patients who had previous pregnancy with PROM were 11. Patients in whom no cause was found were 13.

Table no. 2: Associted other complications

Comorbidities	N=74	%
Pregnancy induced hypertension	16	22.97%
Polyhydramnios	08	10.81%
Hypothyroidism	11	14.86%
Breech	03	4.05%
Oligohydramnios	05	6.75%
Previous 1 lscs	06	8.10%
Previous 2 lscs	03	4.05%
No complications	22	29.72%

Table no. 2 showed: Cases of PROM were screened for other causes which can indirectly be a cause for PROM. It was found that 16 patients had coincidental pregnancy induced hypertension (21.62%) which was found significant followed by polyhydramnios in 08 cases, oligohydramnios in 05 cases. Patients with previous 1 and 2 klscs and with breech presentation associated with PROM were taking directly for LSCS. Those patients with no other complications were 22 (29.72%).

Table no. 3: Mode of delivery

Mode of delivery	N=74	%
Full term vaginal deliveries	41	55.40%
Cesarean section	33	44.59%

Table no. 3 showed: Patients showed good result with oral prostaglandin and normal delivered patients were 41 (55.40%). Primigravida comprised of only 17 (22.97%) cases and multigravida were 24 (32.43%). Most of the primigravida went for lscs 25 (33.78%) cases, n multigravida undergoing lscs were 8 (10.81%).

Table no.4: Indications of lower segment cesarean section.

Indications	N=33	%
Failed induction	07	21.21%
Fetal distress	14	42.42%
Previous LSCS	09	27.27%
Breech	03	9.09%

Table no.4 showed: Indications of lscs included fetal distress in 42.42% patients following induction with misoprostol which was significant and second cause was failed induction in 21.21%. Patient not progressing into active labour for 12 hrs even after induction were considered failure of induction.

Table no. 5: Post operative maternal complications

Maternal complications	N=74	%
Post operative pyrexia	09	12.16%
Wound discharge	02	2.70%
Episiotomy gape	01	1.35%
Post partum haemorrhage	04	5.40%
Endomyometritis	00	0
Maternal mortality	00	0

Table no. 5 showed: Post operatively 9 mothers had pyrexia on day 2-3, fever profile were sent, where their white cell counts were raised, patients were managed conservatively and recovered uneventfully. LSCS woung gape was seen in 2 patients and episiotomy gape in 1 patient. Post partum haemorrhage was seen in 4 patients who were medically managed. No cases of endomyometritis and maternal mortality were noted.

Table no. 6: Neonatal complications

Neonatal complications	N=74	%
ICU Admission	21	28.37%
Hyperbilirubinaemia	13	17.56%
Low birth weight babies	22	29.72%
Respiratory distress	02	2.7%
Neonatal sepsis	06	8.10%
Neonatal deaths	00	0%

Table no 6 showed: Hyperbilirubinaemia were noted in 13 babies (17.56%), they were admitted for phototherapy in ICU and discharged without any complications, 2 babies had post delivery respiratory distress and were managed in ICU, later were discharged. Post delivery sepsis were noted in 6 babies who were managed with injectable antibiotics, all these babies of hyperbilirubinaemia, respiratory distress and sepsis were shifted to ICU. (21 babies). No cases of neonatal deaths were noted.

Discussion

Prelabour rupture of membranes is an important problem in obstetric requiring active management so as to avoid maternal and fetal complications. There are various tests to diagnose PROM such as fern test, ph test, nitrazine test along with ultrasonography to see for the amniotic fluid index. All these tests are of value in managing preterm PROM along with blood investigations,vaginal swab,urine culture and clinical symptoms and signs to look for chorioamnionitis. Conservative management in cases of preterm PROM includes steroids, tocolytics and antibiotics.

The etiology of PROM is multi-factorial and in some cases yet unclear. Infection is one of the exogenous etiologic factors thought preventable in some cases with proper antenatal screening and treating of genito-urinary infections. Management of pregnancies with pre-labour rupture of membranes varies depending on gestational age and obstetric status. Screening of high risk patients who has previous history of PROM, cervical incompetence, cervical surgeries, polyhydramnios, genitourinary infections should be done to avoid PROM.

In the study done by Anjana Devi[13] done at J.I.P.M.E.R, Pondicherry (1996), 76.9% of cases belonged to age group of 20-29 years. . In Piya Ray's[14] study done at R.G. Kar Medical College, Calcutta (1997), 62% belonged to the age group of 20-25 years and 20% to 26-30 years group. In another study done by B.S. Kodkany[12] at J.N. Medical College, Belgaum (1990), 46% of cases were in age group of 21-25 years. In the present study, the PROM was common in 26-30 yrs age group, (39.18%) followed by 32.43% between 21-25 yrs of age group.

In the study by Umed Thakor[15], 53.2% were primigravidas. In the study by Swati Pandey[6], 62% were primigravidas. In current study, 56.75% were primigravida which is comparable to other studies.

As the duration of PROM increases, problems in the mother and the baby also increases. In the study by Umed Thakor,[15] the duration from PROM to admission was 12.06±6.04 hours. In the study by Anjana Devi,[13] the duration of PROM ranged from 3 hours to 5 days, mean being 16 hours. In present study, 87.82% patients reported within 12 hours of PROM which is comparable with the study by Umed Thakor [15].

In the present study, 44.59% had cesarean section, being comparable to the study by Piya Ray[14]. Rate of cesarean section was higher in the studies by Anjana Devi [13] and Singhal[16] and lower in the studies by Jayaram VK[17] compared to the present study.

Maternal morbidity included pyrexia in 12.16% in the present study which was comparable to studies conducted by Anjana Devi (20.19%)[13] and Singhal (17.5%)[16]. PPH was seen in 5.40% which was higher as compared to that of studies by Anjana Devi (1.92%)[13] and Singhal (1.5%)[16].

In Anjana Devi's[13] study, perinatal mortality rate was 4.8%. In study by Sanyal[18] perinatal morbidity was 32% and mortality was 5%. In Piya Ray's[14] study it was 2.5%. Kodkany[12] perinatal morbidity was 39.8% among which birth asphyxia was responsible for 29.5%.

In the present study perinatal morbidity was 28.37% which is comparable with other studies and mortality rate was 0%.

Conclusion

Pregnancies complicated with term PROM should have early induction and supervised labor preferably in an institution. Management of each case has to be individualized. A combined effort of obstetrician and neonatologist is necessary. A good neonatal intensive care unit back up can be helpful in reducing the perinatal morbidity and mortality. Timely diagnosis of PROM by patient and early approach to hospital along with vigilant monitoring and acceleration of labour can help in significant reduction in the maternal and fetal morbidity and mortality.

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Single Dose Methotrexate in Successful Evacuation of Second Trimester Placenta Accreta

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Abstract

Morbidly adherent placenta is a serious and life-threatening condition. High index of suspicion for placenta accreta can save patient from undergoing hysterectomy. Timely and appropriate treatment modality is key in reducing morbidity of the patient Reporting herewith a case of 22-24 weeks intrauterine fetal demise in a primigravida patient who was diagnosed with Placenta accreta. Ultrasonography and MRI confirmed the diagnosis. Primigravida with no live issue, no active bleeding and stable vital parameters favored the option of conservative management in the form of "single dose methotrexate regime". Patient was then given Injection methotrexate 50 mg IM on day 1. Regularly monitored with CBC(complete blood count),LFT(Liver Function Test),KFT(kidney Function Test), beta HCG, USG and pelvic doppler. On day 7 piecemeal removal of complete placenta accrete was successful under USG guidance.

Keywords: *Placenta accreta, methotrexate, USG, HCG.*

Introduction

Morbidly adherent placenta is a condition in which, due to myometrial invasion by chorionic villi, all or part of the placenta adheres to the uterine wall. This may occur when the deciduas basalis and the Nitabuch layer are either primarily deficient or secondary compromised[(1).] Abnormal trophoblast invasion has been associated with up regulation of the endothelial vascular growth factor (VEGF) and angiopoietin-2 and down regulation of the VEGF-R and Tie-2 receptors[(2)].

Adherent placenta complicates approximately 1 in 2500 deliveries and the risk of placenta accreta is approximately 0.1% with previous one caesarean section and close to 0.4%, 0.7%, 2-2.2% and nearly 3.5% with previous two, three, four and five or more cesarean section respectively. The wide range of complications may include severe postpartum hemorrhage, postpartum curettage, perforation of the uterus, shock, infection, fertility loss, and even death. In developing countries the most common treatment is hysterectomy[(3)]. Some studies have, however, identified specific therapies[(4)], which resulted in the survival of the uterus and fertility. Reported treatments include methotrexate medical therapy[(5)], uterine embolization, and expectant management.[(6,7)] Life-threatening hemorrhage, embolism, damage to neighboring organs with secondary injury, transfusion-related complications, reoperation and multi-organ failure are only a few of placenta accreta's potential implications, making optimal clinical management highly essential.

Here is the case report of adherent placenta in a 22-

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24 weeks intrauterine fetal demise (IUFD) which was treated conservatively with single dose methotrexate.

Case Report:

Clinical profile: A 26 years old primigravida woman with 26 weeks of gestation age presented in the ANC OPD for routine checkup. On general examination, vitals were stable. Per abdominally, uterus was relaxed, and uterine height was corresponding to 22 weeks. However, on auscultation fetal heart sound was absent. Ultrasonography confirmed intrauterine fetal demise of 22-24 weeks.

Patient was hospitalized and induction of labour was done with tablet Misoprost 50 ug, repeat dose every 4 hours. After 4 doses, abortus was delivered. However, Placenta was not delivered spontaneously and there was no active bleeding. Patient was administered injection Oxytocin (20 units) over1-2 hours, followed by second dose of injection Oxytocin (20 units) after a gap of 2 hours. Despite 40 Units of Injection Oxytocin, there was no separation of placenta. Urgent ultrasonography showed completely retained placenta with loss of the hypoechoic space between uterus and placenta at some places and extension of placenta into superficial part of myometrium suggestive of Placenta Accreta. MRI was done which confirmed the presence of focal placenta accreta.

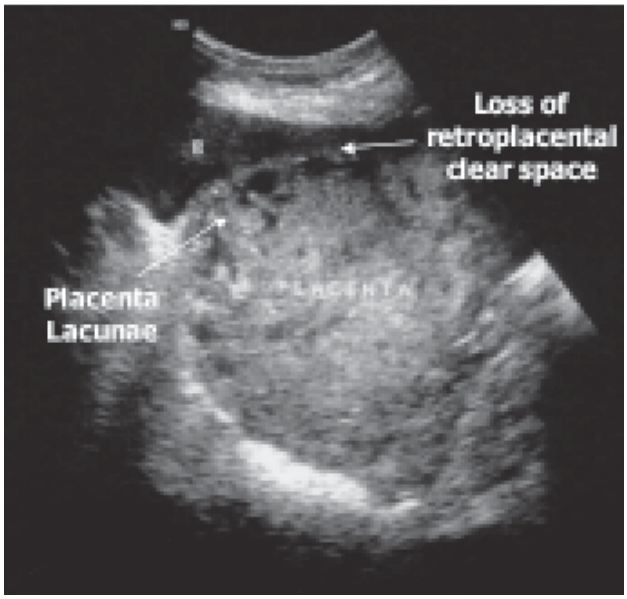


Image 1: Ultrasonography suggestive of placenta accreta

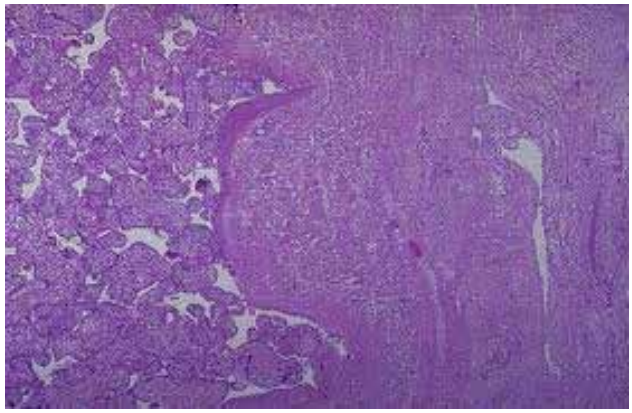


Image 2: Histopathological feature of placenta accreta

Management: After the detailed discussion and explaining management options to patient and her relative, a decision was taken for conservative management of Placenta accreta. Primigravida with no live issue, no active bleeding and stable vital parameters favored the conservative management of the case.

The Serum beta-HCG was 4,080mIU/mL. After taking informed written consent, single dose injection methotrexate 50 mg intramuscular was given to the patient. Alternate day Complete Blood Count(CBC), Kidney Function Test (KFT), Liver Function Test (LFT) was done. The patient was followed up regularly with clinical, laboratory and ultrasonography investigations. On Day 3, repeat beta HCG was 1,430mIU/mL (Table 1).

Review USG with color Doppler was done on day 6 which showed reduced placental perfusion and gradual signs of detachment in the affected area. Patient and relatives were explained the risk of bleeding, sepsis and spontaneous expulsion and was given option for dilatation and curettage with due risk. After the consent of patient and relatives, under USG guidance piecemeal removal of placenta was done. Complete removal of placenta with no active bleeding was ensured on USG. Patient was monitored for post operatively for any complications. As post-operative, patient was stable without any complications, patient was discharge after 48 hours. Weekly follow ups were advised. Serum beta HCG was repeated at 4 weeks (<5 mIU/mL). The couple was counselled and advised to avoid conception for 6 months.

Table 1: Lab profile of patient with placenta accreta

Parameter	DAY 0	DAY 1	DAY 3	DAY 5	DAY 7	Day 10
bHCG (mIU/ml)	4080	2035	1430	1260	980	910
Hb (gm%)	13 gm	11gm	11 gm	10 gm	10gm	10
Platelets (Lac)	2	1.9	1.8	1.8	1.7	1.6
TLC	8300	9800	11000	9600	8700	8800
Blood Urea	28	26	30	27	28	26
Sr Creatinine	0.8	0.7	0.8	0.8	0.9	0.9
Sr Bilirubin	1.0	0.9	0.8	0.8	0.8	0.8
SGOT	18	20	18	20	20	21
SGPT	24	28	21	22	22	18
Remarks	Diagnosis	Inj methotrexate 50 mg IM			USG guided removal	Discharge

Discussion

Methotrexate has been identified as an adjuvant therapy for preservative placenta accreta management[8]. Tong, *et al* pioneered the conservative form of systematic methotrexate administration[9]. The outcome differs widely from placenta removal at 7 days to progressive resorption in approximately 6 months[10]. Methotrexate is suspected to function by causing placental necrosis and speeding up a more rapid placenta involution. This contradicts the assumption that methotrexate operates on rapidly dividing cells, as the proliferation of trophoblasts does not occur at term. There is no consensus on the appropriate dosage, duration, or route of administration. In this patient methotrexate was administered in single 50 mg intramuscular dose and follow-up to ensure placental tissue resolution was done with a combination of clinical evaluation, ultrasound examination, and serum beta HCG assay. USG is useful for assessing placental involution and combined with Doppler color imaging to assess placental vascularity[9,10, 11].

Conclusion

The case described here shows that in selected patients diagnosed with placenta accreta during peri-partum, even after vaginal birth, conservative expectant management with methotrexate is possible. With patients closely monitored, conservative management tends to be a healthy alternative to extirpative management and is a reasonable choice in well-selected, haemodynamically stable patients desirous of potential reproductive function of adherent placenta.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: nil.

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Case Report on Marfans Syndrome

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Abstract

Marfan syndrome (MFS) is one of the most common inherited disorders of connective tissue. It is an autosomal dominant condition with a reported incidence of 1 in 3000 to 5000 individuals. There is a wide range of clinical severity associated with MFS with classic ocular, cardiovascular and musculoskeletal abnormalities, while some patients demonstrate significant involvement of the lung, skin and central nervous system. A 11 years old female child came to the ophthalmology OPD with chief complaints of diminution of vision in both eyes, more in right eye since last 2 months. Patient also complains of pain in right eye since last 3 days. On examination patient had a tall stature, arachnodactyly with positive wrist and thumb signs, right eye had superotemporal subluxation of lens,the left eye had temporal subluxation with iridodonesis on autorefractometer patient was having high myopic reading

Keywords: *Marfan syndrome, Connective tissue disorder, radiological and vascular abnormality, case report.*

Introduction

Marfan syndrome is an autosomal dominant systemic disorder of connective tissue[1,2]. Children affected by the Marfan syndrome carry a mutation in one of their two copies of the gene that encodes the connective tissue protein fibrillin-1 (FBN 1).[3] It mostly affects skeleton, lungs, eyes, heart, and the aorta[4]. Affected individuals often are tall and slender, have arachnodactyly, scoliosis, and either a pectus excavatum, pectus carinatum, or ectopia lentis in eyes[5]. The incidence of mitral prolapse in such patients is essentially equal in children and adults of the same sex [6]

Worldwide, the incidence of Marfan syndrome is approximately 7–17/100,000. The incidence of aortic dilatation and mitral prolapse in patients with Marfan's syndrome was essentially equal in children and adults of the same sex.

Material And Method

A 11 years old female child came to ophthalmology OPD with chief complaints of diminution of vision in both eyes since 2 months. Patient also complains of pain in right eye since 3 days.

History of glare present in both eyes.

No history of

- Trauma
- Floaters
- Flashes of light
- Diplopia

Family history: No known family members with Marfan Syndrome.

On general examination

- Blood pressure: 110/80 mmHg
- Stature: tall

On neurological examination patient was fully alert and orientedand had fluent speech. There were no signs of meningeal irritation.

- All cranial nerve examination was normal.
- High arched palate +

Ophthalmic Examination:

	Right Eye	**Left Eye**
Vision	CF 1M-No improvement	6/60p-no improvement
conjunctiva	Normal	Normal
Cornea	Bright	Bright
Anterior chamber	Deep	Deep
Iris	Normal colour pattern	Normal colour pattern
Pupil	Normal size reacting to light No anisocoria and no relative afferent pupillary defect	Normal size reacting to light No anisocoria and no relative afferent pupillary defect
lens	Supero temporal subluxation of lens	Temporal sublaxation +
Extraocular movements	Free and full in all gazes of direction	Free and full in all gazes of direction
Fundus examination	Normal	Normal
Intra-ocular pressure (GAT)	18	20



Pic showing superotemporal sublaxation of lens in right eye



Pic 2. showing high arched palate .



Pic 3 . showing dysmorphic fascial features and also temporal sublaxation of lens in left eye

Plan of Management:

- Lens extraction for ectopia lentis with Contact lens or IOL (anterior chamber, sutured to sclera/iris)
- Regular screen for myopia, amblyopia, strabismus, keratoconus, and glaucoma
- Chest x-ray and 2D- ECHO advised

Discussion

Marfan syndrome is a autosomal dominant genetic condition that causes weakening of connective tissue in the cardiovascular, musculoskeletal and ocular systems. The major ocular abnormality in Marfan syndrome is ectopia lentis (lens subluxation or dislocation). While relatively little is known about the exact mechanism of this ocular pathology in Marfan syndrome, a number of theories have been suggested.

Al (1995) noticed that while fibrillin is located on the surface superior to the capsule and on the ciliary epithelial surface when zonules are attached to normal eyes, Marfan patients lack such position and have irregular ciliary processes with missing or extremely disorganized zonules. This pathology was found to be positively correlated with lens subluxation.

Clinically, ectopia lentis is bilateral in 60-87% of Marfan patients and is stable from childhood. Symptoms consist of blurring of vision, diplopia, and pain. On examination, patients show refractive instability with myopia and astigmatism, iridodonesis, phacodinesis, and a recessed angle. The most common direction of dislocation on examination is superotemporal. In addition, there may be secondary complications from lens movement such as phacolytic uveitis from posterior subluxation of the lens to the vitreous. Retinal tears and detachments are also quite common in patients with Marfan syndrome.

Other ocular manifestations of Marfan syndrome include flattened cornea (causing astigmatism), keratoconus, increased globe length (causing myopia), iris coloboma, cataracts, glaucoma, strabismus, amblyopia, and vascular malformations. Central to each of these findings is the ubiquitous abnormality of fibrillin in the ocular connective tissue of the Marfan patient.

The systemic manifestations of Marfan syndrome are well-studied, the most obvious of which are the musculoskeletal abnormalities. Again, the common thread in the variety of Marfan phenotypes is the weakness or incompetence of the connective tissues due to defects in fibrillin. The Marfan patient is often very tall with long, flexible extremities and marked scoliosis. They exhibit arachnodactyly (spider fingers) with the ability to dramatically encircle the wrist (Walker-Murdoch sign). In addition, they often have pectus excavatum, a high-arched palate, and facial abnormalities. The cardiovascular findings range from mild mitral valve prolapse to severe aortic aneurysm or dissection; the severe cardiovascular complications are the primary causes of mortality among Marfan patients. Pulmonary diseases include apical blebs or spontaneous pneumothorax. Articles on few other rare syndromes[7-9] are available.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: nil.

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Anaphylactic Reactions to Blood Transfusion in a Patient with Iron Deficiency Anemia: A Case Report

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Abstract

Obstacles of transfusion of blood are uncommon but can endanger the health. Anaphylactic reactions associated with the blood transfusion (BT) of PRC blood components presents as bradycardia followed by tachycardia and urticaria within 15 minutes of transfusion. In suchcases, clinicians should strictly monitor the patient's condition at least while the patient receives blood transfusion.

Here we are reporting a case of 28 year old pregnant female presented with Iron deficiency anemia (IDA), a LSCS (caesarean section) delivery was performed and blood was transfused, who was affected by an acute anaphylactic reaction following red cell transfusion. This case highpoints the need to examine potential risk of transfusion of the blood component against the alleged advantage.

Keywords: *Anaphylactic reaction, Caesarean, Iron Deficiency anemia, blood component transfusion.*

Introduction

Anaphylactic transfusion reactions are unusual complications accompanying blood transfusions.[1] Knowledge with reference to a variety of better prognosis can be provided by the clinical characteristics of acute and delays in transfusion reactions that allow severe reactions to be evaluated in time. [2] The intravenous administration of the blood components complement or accompanies these reactions. The severity varies, depending on blood transfusion, form of treatment, and general health of the person, from mild (temperature and chills) to severe (acute kidney failure or complete vascular collapse and death).[3]

Types of transfusion reactions:[4]

Non Hemolytic:

- Bacterial infection
- Febrile non-hemolytic transfusion reaction
- Allergic reaction
- Anaphylactic reaction
- Transfusion-related acute lung injury
- Transfusion-associated circulatory overload

Hemolytic:

- ABO incompatibility (immediate intravascular red celldestruction)
- Other red cell incompatibility e.g. Rhesus, Kell (extravascular red cell destruction)[5]

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The incidence of anti-IgA mediated anaphylactic transfusion reactions is extremely low. As, in 2015, about 86 out of 2,5 million blood transfusions occurred per year in the UK in acute and extreme anaphylactic or hypotensive transfusion reactions (excluding hemolytic reactions and transfusion-related lung injury)[6]. Sensitization of IgA-deficient patients by IgA exposure and subsequent reactions to anti-IgA-mediated anaphylaxis are the expected mechanism for anaphylaxis.[5]

Our patient as per past history has never received blood transfusion, and also had provided all care to expose him to IgA, for example Immunoglobulin or immunoglobulin intravenous tetanus. There are also anti-IgA antibodies. of the 39 blood donors with detectable anti-IgA antibodies only 9 naturally, in one study, had history of Transfusion or pregnancy.[7] IgA red cells or platelets deficient donors not regularly are available. Pre-to issue red cell and platelets may be washed to reduce the plasma in the blood component of the donor.[5]

Urticarial reaction: The manifestation of urticaria throughout and right away after blood component transfusion, is seen approximately in 1% of the recipients. Generally, a slight reaction is harmless. In conjunction with others like fever, may be suggestive of evaluation for a hemolytic reaction.[8]

In such cases of mild urticarial reaction blood specimens should not be submitted after transfusion.

One can reinitiate the blood transfusion.[9]

Case Report: Authors report a case of 28-year-old pregnant female patient second gravida (G2P1L1) with IDA and history of amenorrhea since 9 months was admitted in the OBS GYN maternity ward I/V/O previousscar in labor. Her family history was not significant. She had blood pressure (112/68) and anemia (hemoglobin-7.2g/dl), Spo2- 99%, respiratory rate 20/min.

In the Summary of anemia, the patient had to be admitted in ward and posted for lower segment cesarean section (LSCS) on 20th April, and blood transfusion of PRC blood components was planned to be given. On the same day whilein OT, while the patient was anaesthetized, she started developing blood transfusion reaction after 10 minutes i.e. approx. 30ml of blood transfusion after attaching the blood bag.

Following clinical signs of BT reaction wereas noted:

- Bradycardia followed by tachycardia
- Blood pressure (BP) 170/110 mmHg,
- Pulse rate 58/min,
- Respiratory rate 18/min, and
- Body temperature 98°F,
- SPO2 86 %
- Urticaria
- B/L crepts.

The transfusion was stopped and a quick respiratory, and circular assessment was carried out. The patient's details were verified against blood bag and no inconsistency were found. The IV saline, hydrocortisone 100 mg and Inj. Avil (1amp) i. v. was administered immediately

A diagnosis anaphylactic reactions to blood transfusion was made.

Rapid onset of pain or swelling of the lip or throat or hives indicates anaphylactic reactions[5].

Parameters of the laboratory:

The laboratory features at the time of admission were as follows:

RBS -140mg/dl; Fasting blood sugar- 90mg/dl; RBC- 3.17, Hb- 7.2gm %, WBC- 11400/ cu.mm, Platelet-1.07 lac/cu.mm, MCV- 68, MCHC 33.6%, sickling negative.

Treatment: She was treated with 100 mg injection hydrocortisone and Avil 1ampule I.V in single doses in stat. Rashes subsided over 4 hours. Later on Tab Atrax 25mg BD and tab Allegra 120mg BD were given for 3 days. Patient recovered and was discharged. Other drugs given in this period were Cap Becasule 1 OD, Cap Autrin 1 OD and Tab Albendazole 400mg. Other drugs for her anaemia were continued.

For acute transfusion reaction the patient was examined. Normal experiments were conducted, including total blood count, renal and liver function tests and hemolysis tests. As the fever persisted, the unit was returned for repeated reliability checks to the laboratory and the patient was screened for red cell antibodies. The unit was sent to cultivate bacteria.

The call manager for the blood transfusion service was contacted. The transfusion reaction was documented in the Haemovigilance Monitoring System for serious hazards of transfusion (SHOT).

No serological incompatibility between the patient and the donor red cells was verified by repeated compatibility checking. It was a negative repetition screening. Culture did not produce any bacterial growth.

Discussion

In an emergency that threatens life, if the risk of delaying in the waiting for washed cells outweighs the need for an urgent transfusion, then standard cells should be used. The incidence of transfusion reaction, as discussed above, is extremely low.[5] Any transfusion must be conducted in an environment where staff have been trained and access to adrenaline[10,11] in anaphylaxis management.

The transfusion with standard components should be given to patients with IgA deficiency who never had previously received transfusions or who received standard blood components without reaction. [12]

Points to Learn:

- Early treatment is separate from clinical test findings for an immediate transfusion reaction.
- Anaphylactic transfusion reaction control requires timely assistance and IM adrenaline steps.
- The degree of IgA should be assessed in patients with anaphylactic transfusion reactions.
- Blood is typically really healthy to transfuse. There has been a chance of serious harm

(Even by extreme transfusion or infection) 1 in 15,000 and death chance 100,000 in the United Kingdom[4].

- Annual transfusion in the UK reaches 2.5 million units of blood drug.

The risk minimization includes checking the transfusion indication for each patient.

It is the combined liability of the blood transfusion advisor and their clinical complement to create awareness about safe transfusion services so that proper haemovigilance system can be achieved providing patient care.[13]

Conclusion

Authors hereby would like to conclude that blood transfusion of PRC blood components

bear a slight chance of life-threatening adverse reactions if not properly treated. The benefits of the components ignore the dangers of anaemia therapy.

Clinician's must carefully observe the patient's condition at least during the 6 h while the patient gets blood transfusion. I.V drugs should be provided only when well-trained professionals are available with resuscitation facilities to diagnose and treat hypersensitivity reactions.

This could be mistrust worthy that a test dosage is not sufficient. For signs of adverse events (HSR), patients should be closely observed at least 60 minutes following each administration.

Patients with documented allergies, extreme atopy, or inflammatory systemic diseases (e.g., systemic lupus erythematosus, rheumatoid arthritis) should be given special precautions.

Products should only be administered, when well trained staff to assess and manage hypersensitivity reactions, with resuscitation facilities, are available.

A test dose is not appropriate as it may give false assurance.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

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Assessment of Pulmonary Functions in Obese Young Adults

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Abstract

Introduction: Obesity, especially, adolescent obesity is specially a matter of concern and the causes are manifold that includes lack of regular exercise, sedentary life style, over consumption of high caloric foods; genetic, endocrine and metabolic mechanisms and perinatal and early life factors. Pulmonary function testing is the most commonly performed lung function test and are considered to be the initial screening tool for pulmonary diseases. Medical students of M.B.B.S. course have a hectic schedule along with inappropriate diet and sedentary lifestyle of can cause them to gain weight. Since obesity in these young adults is associated with various pulmonary function alterations, it becomes necessary to assess the respiratory functions of these medical students.

Aim: To assess the pulmonary functions in obese young adults.

Objectives: To compare the effects of central obesity versus peripheral obesity on pulmonary functions as compared to the normal.

Settings: Department of Physiology Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Wardha and Department of Medicine, TNMC & BYL Nair Ch. Hospital, Mumbai Central, Mumbai.

Method: After taking informed written consent, anthropometric data was recorded from each participant. Obese medical students were divided into central and peripheral obese groups on the basis of waist hip ratio and waist circumference. On brief general and clinical examination, pulmonary function tests (FVC, FEV1 and FEV1/FVC ratio) of central obese, peripheral obese and healthy subjects were recorded using MIR Spirometer with Winspiro-Pro 4.4 software after one practice attempt and report was interpreted. Comparison of data among various study groups was done by using Chi square test and one way Analysis of Variance (ANOVA) – Tukey's post hoc test. The level of significance was set at 5%. The P-value <0.05 was considered as statistically significant.

Interventions: Not applicable.

Results: There was a statistically significant difference of mean values of FVC, FEV1 and FEV1/FVC % between normal and peripheral obese groups and between normal and central obese groups and also between peripheral obese and central obese groups.

Conclusion: This study shows that young obese adults have lower pulmonary function tests values as compared to normal individuals whereas the detrimental effect is more in the central obese than peripheral obesity. Hence early identification and initiation of preventive measures to prevent the deterioration of lung functions in susceptible young adults is crucial.

Keywords: *Medical students, central obese, peripheral obese, pulmonary function test, young adults.*

Introduction

Obesity is a medical condition in which excess body fat accumulates to the extent that it may have an adverse effect on the health leading to reduced life expectancy and increased health problems. Adolescent obesity is specially a matter of concern and the causes are manifold

that includes lack of regular exercise, sedentary life style, over consumption of high caloric foods; genetic, endocrine and metabolic mechanisms and perinatal and early life factors.[1,2] Co-morbidities of adolescent obesity encompass both short and long term health concerns and can cause various deleterious effects on the respiratory function.[3] Pulmonary function testing is a method of assessing lung function by measuring the volume of air that the subject is able to expire from the lungs after a maximal inspiration. It is the most commonly performed lung function test and are considered to be the initial screening tool for pulmonary diseases. The test can be performed at the bedside, in a physician's office or in the pulmonary laboratories. Students of M.B.B.S. course have a hectic schedule consisting of lectures, practicals and examinations due to which they get less time for exercise. Moreover inappropriate diet and sedentary lifestyle of medical students can cause them to gain weight. Since obesity is associated with various pulmonary alterations, it becomes necessary to assess the respiratory functions of these obese young adults. The current study looks into the association of pulmonary function tests with body fat distribution in normal weight and obese students in the age group of 18-25 years.

Aim: To assess the pulmonary functions in obese young adults.

Objectives: To compare the effects of central obesity versus peripheral obesity on pulmonary functions as compared to the normal.

Materials and method: The study was conducted in the Department of Physiology in collaboration with Department of medicine in a tertiary care hospital in city of Mumbai. The Ethics Committee of the hospital and institution approved the protocol employed for the study. All tests are standardized and have been used in medicine department of institute for assessing pulmonary functions in patients.

80 Male and Female medical students having age between 18 and 25 years and BMI between 25 and 29.9 kg/m^2 as per Asian standards were included in study group. (4) 40 physically healthy male and female medical students, without any symptoms, having age between 18 and 25 years and BMI between 18.5 and 24.9 kg/m^2 as per Asian standards were included in control group. [4] Medical students of age less than 18 years & greater than 25 years, with BMI less than 18.5 & more than 29.9 Kg/m^2, with acute or chronic respiratory diseases taking treatment for the same, having other comorbidities, pregnant females, were excluded from the study after a detailed history and a detailed physical examination of the subjects to exclude the respiratory diseases. Weighing machine, stadiometer, non-elastic measuring tape, Lloyds hand held body composition monitor, MIR Spirometer with Winspiro-Pro 4.4 software. (P.F.T. machine) [5], nose clip and disposable mouthpiece were used for the data collection.

All cases and controls were selected according to the inclusion and exclusion criteria. Subjects were instructed to not to have food or drinks 2 hours prior to the test, not to smoke before coming for pulmonary function tests (PFT) examination. Subjects were instructed to have sufficient hydration and light meal in the previous night and Light breakfast in the morning and also to report acute respiratory episodes

On arriving, Cases and controls were explained in detail about the procedure to be performed in their vernacular language to their satisfaction. A brief explanation regarding the purpose of study and the tests to be performed was given to the subjects. The subjects were registered and their information recorded. Written informed consent was obtained. Anthropometric measurements like body weight, height, waist circumference, hip circumference, body mass index, waist-to-hip ratio, total Body fat percentage were taken.

The subjects were divided into normal non obese subjects (control) and obese subjects (cases) based on body mass index (BMI) values.[4,6]. Subjects having BMI between 18.5 to 23 Kg/m^2 were normal subjects (control) and 25 and 29.9 kg/m^2 were obese subjects (cases). The subjects who fell under obese category were divided into central obese and peripheral obese using waist circumference and waist to hip ratio (WHR). Females with waist circumference $\geq$80 cm and WHR $\geq$ 0.8 were centrally obese subjects and those with waist circumference $\leq$ 80 cm and WHR $\leq$ 0.8 were peripherally obese. Males with waist circumference $\geq$ 90 cm and WHR $\geq$ 0.95 were centrally obese subjects and those with waist circumference $\leq$ 90 cm and WHR $\leq$ 0.95 were peripherally obese.[7,8,9]

Pulmonary function tests were performed with subject in sitting position with legs uncrossed. A nose clip was put on his/her nose. The subject was asked to perform tidal breathing through mouth and after that

to execute forceful expiration as fast as possible at the end of a deep, full expiration. One practice attempt was given.[10]

Forced vital capacity (FVC), Forced expiratory volume at the first second (FEV_1) and FEV1 / FVC ratio were assessed in cases and controls, calculated and analyzed on MIR Spirometer with Winspiro-Pro 4.4 software. MIR Spirometer was calibrated every morning.

Statistical analysis: The data entry was done in Microsoft – EXCEL programme and the results were tabulated and statistically analyzed by Statistical Package for the Social Sciences (SPSS) version 20.0 software. Descriptive statistic (mean and standard deviation) was used for numerical data. Comparison of data among various study groups was done by using Chi square test and one way Analysis of Variance (ANOVA) – Tukey's post hoc test. The level of significance was set at 5%. The P-value <0.05 was considered as statistically significant.

Results

The percentage distribution of males and females in the study and control group was statistically non-significant and hence we concluded that the three groups were comparable. (Table No. 1)

On applying ANOVA – Tukey's Post-hoc test, the groups it was observed that the mean values of Age were statistically not significant and hence the groups are comparable. (Table No. 2).

But It was observed that there was a statistically significant difference of mean values of BMI between groups were statistically insignificant, thus indicating proper selection of the groups. (Table No.3)

On applying ANOVA - Post-hoc Tukey test to groups it was observed that there was a statistically significant difference of mean values of **FVC** between normal and peripheral obese groups (P value - 1.926E-03) and between normal and central obese (P value - 1.876E-08) and also between peripheral obese and central obese (P value - 0.0146).Thus groups were statistically significant. (Table No. 4)

It was observed that there was a statistically significant difference of mean values of **FEV1** between normal and peripheral obese central obese (P value - 7.201E-08) and between normal and central obese (P value - 5.100E-09) and also between peripheral obese and central obese (P value - 0.0014) were statistically significant. (Table No.5)

Also It was observed that there was a statistically significant difference of mean values of **FEV1/FVC %** between normal and peripheral obese (P value - 4.619E-04) and between normal and central obese (P value - 5.100E-09) and also between peripheral obese and central obese (P value - 5.1003E-09) were statistically significant. (Table No. 6).

Table 1 - Distribution of study groups as per sex

Sex		Group			Total
		Normal	Central Obese	Peripheral Obese	
Females	Count	20	20	20	60
	Percent	50.0%	50.0%	50.0%	50.0%
Males	Count	20	20	20	60
	Percent	50.0%	50.0%	50.0%	50.0%
Total	Count	40	40	40	120
	Percent	100.0%	100.0%	100.0%	100.0%
Chi Square Test		P value		Association	
Pearson Chi-Square test.		1.000		Not significant	

Table 2 - Comparison of Age (Years).

Parameter	**Normal**			**Central Obese**			**Statistical Parameters**	
Age (Years)	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	20.63	1.444	20.0	20.55	1.449	20.00	0.971	Not significant
	Normal			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	20.63	1.444	20.0	20.98	1.459	21.0	0.529	Not Significant
	Central Obese			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	20.55	1.449	20.00	20.98	1.459	21.0	0.3924	Not Significant

Table 3 - Comparison of BMI (Kg/m^2)

Parameter	**Normal**			**Central Obese**			**Statistical Parameters**	
BMI (Kg/M^2)	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	20.72	1.768	20.0	26.55	1.339	26.00	5.100E-09	Significant
	Normal			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	20.72	1.768	20.0	26.95	1.131	27.0	5.1003E-09	Significant
	Central Obese			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	26.55	1.339	26.00	26.95	1.131	27.0	0.4296	Not Significant

Table 4 - Comparison of FVC (Litres)

Parameter	**Normal**			**Central Obese**			**Statistical Parameters**	
FVC (Litres)	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	3.08	0.572	3.00	2.35	0.483	2.00	1.876E-08	Significant
	Normal			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	3.08	0.572	3.00	2.68	0.474	3.0	1.926E-03	Significant
	Central Obese			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	2.68	0.474	3.0	2.35	0.483	2.00	0.0146	Significant

Table 5 - Comparison of FEV1 (Litres)

Parameter	**Normal**			**Central Obese**			**Statistical Parameters**	
FEV1 (Litres)	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	3.00	0.641	3.00	2.00	0.000	2.00	5.100E-09	Significant
	Normal			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	3.00	0.641	3.00	2.38	0.490	2.0	7.201E-08	Significant
	Central Obese			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	2.38	0.490	2.0	2.00	0.000	2.00	0.0014	Significant

Table 6 - Comparison of FEV1/FVC (Percentage)

Parameter	Normal			Central Obese			Statistical Parameters	
FEV1/FVC Percentage	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	95.75	4.081	96.0	83.05	3.809	84.0	5.100E-09	Significant
	Normal			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	95.75	4.081	96.0	92.63	2.696	93.0	4.619E-04	Significant
	Central Obese			Peripheral Obese			Statistical Parameters	
	Mean	SD	Median	Mean	SD	Median	P- value	Significance
	92.63	2.696	93.0	83.05	3.809	84.0	5.1003E-09	Significant

Discussion

The fat content of the human body exhibits the most striking variations in the state of nutrition varying along the emaciation-obesity continuum than perhaps any other body constituents. This study sheds light on the effect of pattern of fat distribution (depending on WC and WHR) on some pulmonary functions in obese young adults.

A. Effect of central obesity on FVC FEV1 and FEV1/FVC ratio: Following mechanisms can be responsible for the decrease in **FVC FEV1 and FEV1/FVC ratio** seen in central obesity.

1. Physical compression of lungs and alveoli due to fat deposition in mediastinum, around the heart, pleural space and above the diaphragm.
2. Fat deposition in the abdomen raises the diaphragm due to increased intra-abdominal pressure and reduces the functional residual capacity.
3. Thoracic kyphosis and lumbar hyperlordosis in central obesity reduces chest wall compliance.
4. Airway inflammation and reduced lung volume by lateral compression causing the narrowing of the airway increasing the respiratory resistance. Closure of the dependent airway may result in small areas of atelectasis ultimately leads to decreased lung compliance.
5. Excessive mechanical load of deposited fat in the respiratory and non-respiratory muscles imposes great burden on inspiratory muscles and overstretching of the diaphragm and decreased skeletal muscle glycogen synthesis results in the decreased isokinetic respiratory muscle strength and endurance.
6. Increased blood flow due to increased metabolic demands by the muscles and the lungs results in increased pulmonary blood pressure. This leads to decreased lung volume and airway inflammation thereby narrowing the airway. Also airway caliber of these obese persons is reduced possibly by remodeling of the airway. These together probably result in reduction in FVC, FEV1and FEV1/FVC ratio in central obese individuals.

B. Effect of peripheral obesity on FVC FEV1 and FEV1/FVC ratio:

1. Interleukin 6 act as myokine in muscle and released in response to increased cost of breathing in obese person. Interleukin 8 is produced by airway smooth muscle cells. It induces chemotaxis for neutrophils and other granulocytes. It increases the oxidative stress and inflammatory and autoimmune processes affecting the lung functions.
2. TNF α induces inflammation and proteolysis. This results in decreased strength of smooth muscles and decreased elasticity of airway and, in turn, increased airway resistance and impaired lung functions.
3. Adiponectin, an anti-inflammatory substance, is exclusively secreted from adipose tissue in the bloodstream. A decreased level of adiponectin results in adipose tissue formation thereby increasing the secretion of pro-inflammatory substances.

4. Also adipokines, either directly or indirectly, adipokines affect inflammation of small airway resulting in premature closure of the inflamed and edematous small airways and result in reduction in FVC, FEV1and FEV1/FVC ratio in peripheral obese subjects.

Studies are available on Metabolic healthy Obesity, Pulmonary Artery Hypertension in young adults and related problems among medical students.

Conclusions

This study shows that young obese adults have lower pulmonary function tests values as compared to normal individuals whereas the detrimental effect is more in the central obese than peripheral obesity. Hence early identification of the subjects who are at risk and the risk factors leading to excessive weight gain is important to initiate preventive measures to prevent the deterioration of lung functions in susceptible young adults. Further studies are needed in this regards.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Effect of Covid-19 Lockdown on Perceived Stress Scale in Medical Students

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Abstract

Background: Stress is defined as the physiological and psychological state which could overburden an individual's ability to appropriately respond to it. In India,the corona virus pandemic had created a sudden closure of educational institute which leads to shift of conventional classes to virtual classes because of which the medical students were in a stage of dilemma. The present study aimed to assess perceived stress during the COVID 19 lockdown, among the medical students.

Aim and Objectives: To assess the perceived stress of medical students in regard to corona pandemic lockdown.

Materials and Method: A cross-sectional online survey study was done on 300 medical students whose perceived stress were assessed using an online questionnaire. The stress test was done with the help of Perceived Stress Scale (PSS) which has got 14 items.

Results: In 300 medical students (151- boys and 149 girls), the mean perceived stress score was observed as 27.60. In boys the PSS score was found to be 27.85 and in girls it was 27.52. When the score were compared with the mean validated score the students were having moderate amount of stress (p value < 0.05).

Conclusion: The present study concluded that the medical students perceived the moderate level of stress. The study thus concludes that lockdown has affected the psychological well being of medical students.

Keywords: *COVID 19, lockdown, Perceived Stress Scale, Medical Students.*

Introduction

Stress is considered as a structured series of physiological, neuro-hormonal and psychological response to situations that threatens or challenges us and that require some kind of adjustment. Stress primarily signifies condition of disturbed normal functioning due to imbalance between individual's interactions with the environment. Stress is an adaptive response to noxious stimulus causing disturbance in normal functioning.[1]

Corona virus pandemic lead to the global lockdown and all activities were forced to close. Normal daily routine was disturbed with many of people was forced to stay in the house. Meeting with friends, dining out, worshiping and other daily routines were nearly halted as nations took drastic steps to try to stop the corona virus pandemic. All educational Institutes and Universities were closed and students were sent to their respective hometowns during lockdown period. The whole country was staying indoors and hoping that corona pandemic will slow down. During this lockdown period which continued for more than 100 days many people suffered from psychological disturbances. These psychological disturbances may be due to academic problem, lack of adjustment to stay indoors for long period, financial insecurity, job problems, family problems, future planning problems etc.

Medical education which starts with rigorous

competitiveness in selection process is very stressful affair. Also the medical course is of long duration and syllabus is vast so this makes students more vulnerable for higher level of stress. Delay in learning process, lack of conceptual knowledge and hands on training due to lockdown gives stress to the medical students because of uncertainty of their future. The COVID-19 pandemic has affected mental health of individual due to many reasons like economic loss, confusion, emotional isolation, school/work closure, insecurity, etc. Because of this people are more prone to emotional disturbances & psychological instability like stress, irritability, frustration which leads to psychiatric disorders like behavioural changes, anxiety depression and trauma related disorders. [2,3,4]

We conducted the study using Perceived Stress Scale (PSS) questionnaire which is associated with direct changes to both physiologic and psychological processes.[5] The PSS is the most widely used psychological instrument for measuring the perception of stress. It is a measure of the degree to which situations in one's life are appraised as stressful. The scale includes a number of direct queries about current levels of experienced stress.

The aim of this study was to see the Perceive Stress scale (PSS) score in medical students during the lockdown period of Covid-19 pandemic. This study would help to evaluate the amount of stress perceived by the medical students during lockdown.

Material and Method

The cross sectional study was performed on randomly selected 300 medical students (N= 151 boys and N= 149 girls) with age group of 18- 25 years studying in Datta Meghe Institute of Medical Sciences (DMIMS) Sawangi (M), Wardha. The study was conducted during the COVID -19 pandemic lockdown period in April and May 2020. All the students gave consent for the study and they were provided with PSS questionnaire which contains 14 items (questions). The questionnaire was made available online in English and Hindi language. PSS is associated with direct changes to both physiologic and psychological processes. This scale helps to measure the perception of stress by an individual. The scale includes a number of different questions about present levels of experienced stress. In this scale there are 14 items which are easy to understand, and the response alternatives are simple to understand. The questions ask about feelings and thoughts during the last month. Out of the 14 items, 7 items (item number on PSS as 1,2,3,8,11, 12,14,15) are neutrally stated questions which indicate that if the stress score is more then more is the stress perceived by the subject .Remaining 7 questions (item number on PSS as 4, 5,6,7, 9,10 & 13) are positively stated questions means scores get reversed i.e. more is score; less is the severity of stress perceived. [5] Average score all 14 items of 300 subjects were calculated. Statistical analysis was done by descriptive statistics.

Results

A total of 300 medical students (Boys – 151, Girls – 149 students) included in the study and had completed the whole PSS questionnaire. Table no 1 gives gender wise and overall average PSS score findings. The mean perceived stress score among the students was 27.60 ± 8.13 (Mean ± Standard deviation) . When the data is compared with the validated data of PSS- 14 scale which suggest average score of 19.62 showed significant difference ($p < 0.0001$) between the gender wise and average distribution.

Table no 2 shows average score of 300 students for each item.

Table No. 1: Total and Gender wise average PSS score

Gender wise distribution	Mean PSS score ± standard deviation (SD)	t test
Boys (n- 151)	27.85 ± 8.32	p value < 0.0001
Girls (n- 149)	27.52 ± 8	
Total (n - 300)	27.6 ± 8.13	

Table No. 2: Average score for each item of PSS

Item number as per Perceived Stress Scale (PSS) having neutrally stated questions	Average score of each item (n-300)	Item number as per Perceived Stress Scale (PSS) having positively stated questions	Average score of each item (n-300)
1	2	4*	1.9
2	1.8	5*	2
3	2	6*	2.4
8	1.9	7*	1.7
11	2.1	9*	1.9
12	2	10*	1.6
14	2	13*	2.3

Discussion

In this study we assessed perceived stress on medical students during covid-19 lockdown period. Pandemic which is a health emergencies can have psychological impact on college students such as stress, anxiety etc. The PSS is stress assessment instrument. The scale is useful to understand how different situations affect our feelings and our perceived stress. The questions in the scale ask about your thoughts & feeling in the last month[5].

The corona disease has high transmission rate and has dangerous effect on health. To prevent this spread of infection Government authorities undertook various norms of lockdown which included social distancing, no mob gatherings, no classroom teachings.[6] The lockdown leads to changes in daily routine life which causes stress, anxiety and depression among the peoples.[7] The news headlines, watsapp updates, information from colleagues and news reports have also leads to fear and anxiety.[8] Students had been residing at home and teaching has been online since then which causes lack of classroom environment,assignments which adds to the stress.

In current study it was found that, average 14 item PSS scale score is 27.60. The results were compared with the previous evaluation of PSS scale in healthy populations. From the validation studies, the mean score for 14 item PSS scale was 19.62 in healthy population.[9,10] The average score of the PSS and gender wise distribution showed significant difference ($P< 0.0001$) when compared with the validated score which suggest the moderate amount of stress in medical students. However, when average PSS score of boys and girls were compared there was no significant difference in stress between the two.

Similar findings were found in the other study conducted by Qiu et al., which showed that there was significant psychological distress due to COVID 19 pandemic using a novel Career and Personal Development Institute scoring.[11] Senturk et al (2018) in Turkey determined in a descriptive study done on the nursing students that the level of stress faced by them was above moderate level.[12] Sheroun D et al study indicates moderate level of perceived stress with mean perceived stress score of the students nurses being 21.88.[13] So the present study highlights that the moderate level of stress is experienced by the medical students due to this pandemic during lockdown period.

Conclusion

This study concluded that the moderate amount of stress is experienced by the medical students in lockdown period and that it can be reduced by providing mental support, counseling & assurance to perform better in their academics.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Bilateral Spontaneous Twin Tubal Ectopic Pregnancy: Case Report

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Abstract

Ectopic pregnancy is a condition where the fertilized oocyte gets implanted outside the uterus. Tubal ectopic pregnancy accounts for 95 % of the ectopic gestation. Remaining 5 % involves the ovaries, cervix, cornua, abdominal and scar site pregnancies. Spontaneous ectopic pregnancy occurs when there is no prior infertility treatment taken. Bilateral spontaneous tubal ectopic pregnancy is a rare form of ectopic gestation. Hereby I would discuss a case of spontaneous bilateral ectopic pregnancy and its outcome. Case: A 35 years old gravid 2 abortion 1 with secondary infertility of 13 yrs came to emergency casualty with complaints of lower abdominal pain, spotting per vagina with giddiness and syncopal attack.

Diagnosis: Based on clinical findings of acute anaemia due to internal blood loss and free fluid in the lower abdomen with fullness on forniceal palpation and bulky uterus. Trans abdominal ultrasonography was confirmatory in diagnosing bilateral adnexal mass with free fluid in pouch of Douglas.

Intervention: Emergency exploratory laparotomy with adequate blood transfusion. Patient recovered uneventfully.

Conclusion: Women in reproductive age group with history of amenorrhoea with irregular spotting and acute abdominal pain with syncopal attack should be managed vigorously keeping in mind ectopic pregnancy so as to have less morbidity and chances of good future pregnancy. Salphingotomy as an alternative method should be practiced.

Keywords: *Ectopic pregnancy, exploratory laparotomy.*

Introduction

Implantation outside the uterus is considered ectopic and accounts for 0.5 to 1.5 percent of all first trimester pregnancy[1]. Ectopic pregnancy is a potentially fatal emergency condition if early diagnosis is missed[2,3]. Incidence of ectopic pregnancy is about 1 in 725 to 1 in 1580 ectopic pregnancies and 1 in 2 lac intrauterine pregnancies[4-8]. Around 200 cases of bilateral tubal ectopic pregnancy have been reported in the literature till date, twin tubal pregnancy with both embryos in same tube or with one in each tube has been reported[9]. However, there has been a significant increase in its incidence over the past three decades[10]. Ovulation stimulation typically occurs as in artificial reproductive methods, pelvic infection and tubal surgery[11,12]. Few cases of preoperative diagnosis of bilateral ectopic pregnancy are reported[4,13].

Anything that interferes with the passage of the ovum through the tube increases the risk of implantation at an ectopic site. Common sites for ectopic pregnancies are the fallopian tube (approximately 95%), of which 70% is ampullary which is the most common site followed by isthmus (12%), fimbrial (11%) and interstitial (2%), [14] with 3% being ovarian in location and the rest (<1%) abdominal or cervical, prior cesarean scar or in the cornua. Even bilateral tubal ectopic pregnancies have

been reported. Hence, one must always meticulously inspect the other tube also.[15)] Heterotrophic pregnancies account for 1/30,000 cases.[16]. However, 9 of 10,000 pregnancies are due to ART.[17]

Abnormal tubal anatomy underlies many cases of tubal ectopic pregnancy. Surgery prior to tubal pregnancy for fertility restoration or for sterilisation has increased the risk to 20 fold.(18). Previous one ectopic pregnancy the chances increases 2 to 7 fold[18]. Prior sexually transmitted diseases or other tubal infections, which can distort the normal tubal anatomy, can also cause ectopic pregnancy. Single episode of salphingitis can be followed by ectopic in 9% of women. [19]. With successive pelvic inflammatory disease the chances of ectopic increases as 13% after 1 episode, 35% after 2 episodes and 75% after 3 episodes.[20]. Prior recurrent abortions increases the risk to 4 times for ectopic pregnancy[21]. Salphingitis isthmica nodosa also increases the chances. [22.] Congenital fallopian tube anamolies secondary to use of diethylstilbestrol also predisposes to ectopic pregnancy[23]. 10 years cumulative incidence of acute tubal pregnancy after sterilisation procedure is 7.3/1000[24]. Smoking is also associated with increase chances of ectopic also the mechanism is unclear[25]. With any form of contraception the chances of ectopic decreases as the fertility rate is decreased but the contraception failure can increase the risk.

Outcome of ectopic pregnancy includes rupture, abortion or pregnancy failure with resolution. If the fimbrial end is occluded, tube gets distended with blood and forms haematosalphinx. Acute ectopic pregnancies are associated with increased beta HCG levels and rapid growth, leading to timely diagnosis. They have increased risk of rupture.[26].

Classical triad of delayed menstruation, pain and vaginal bleeding or spotting can be present in majority of the cases of ectopic gestation. In rupture, there is severe lower abdominal pain, cervical motin tenderness, posterior fornix may be filled with blood and a tender boggy mass is felt beside uterus.[27,28].

In our case, patient reported to hospital for the first time with acute rupture. Treatment of an ectopic pregnancy depends on its clinical presentation, size and beta hcg levels. Serum beta hcg levels and trans vaginal sonography allows early diagnosis as a result of which the maternal survival rates and conservation of the reproductive capacity are improved. Initial Beta hcg levels above the discriminatory zone of 1000 – 2000 uIU/ml with non visualisation of the G sac strongly supports ectopic gestation[29]. Repeat beta hcg should be performed after 48 hours to see for doubling.

Single value of srum progesterone level > 25 ng/ml excludes ectopic pregnancy with sensitivity of 92% [30]. Transvaginal ultrasonography with appearance of trilaminar endometrium is diagnostic.[31]. Bilateral tubes with ovaries and extrauterine yolk sac, embryo or fetus is diagnostic [32]. Other findings include hyperechoic halo or tubal ring surrounding anechoic sac[33].

Surgical management is done in acute ruptured ectopic pregnancy, in haemodynamically unstable patient or in those who have failed medical treatment or have contraindications to medical treatment. Laproscopy is the preferred treatment as it is associated with lower cost, less operating time, shorter hospital stay and faster recovery. Salphingectomy is the recommended treatment, however, salphingostomy can be considered for women with one tube who are wishing to preserve the fertility.

Methotrexate treatment has also been given by few. Management depends on the initial low beta hcg levels, small size of sac and absent fetal cardiac activity.[34,35]. After an ectopic pregnancy, there are 8 – 15 % chances of recurrent ectopic pregnancy with single dose methotrexate conferring the lowest risk and linear salphingostomy associated with the highest risk. [36] Uterine artery embolisation for management of interstitial twin ectopic pregnancy has also been reported[37].

Our patient presented as a case of acute ruptured ectopic with haemodynamic instability. This case is reported for its rarity and also signifies the need of early ultrasound for diagnosing ectopic pregnancy even in low risk women.

Case:: A 35 yrs old gravida 2 para 0 live 0 abortion 1 with previous spontaneous abortion, 13 yrs back of 2 months with secondary infertility of 13 yrs, no treatment taken for conception married for 15 years arrived in the institute's gynecological emergency with two months of amenorrhea, excruciating pain abdomen 1 day and 5-6 episodes of vomiting 1 days. She had not used contraception. Her preceding menstrual cycles were thirty days regular with 3 days bleeding period. She did not follow up in hospital for registration due to COVID 19 pandemic after she missed her periods and a healthy

UPT at home. As stomach pain became debilitating she was admitted to hospital. Its past medical and family history was not important. Maternal tachycardia (pulse 128 per minute), hypotension (BP = 90/60 mmHg), RR = 20 / min, and pallor were her general condition on admission. Her abdomen was tender with healthy rebound tenderness and protecting, and absent bowel sounds on palpation. Bimanual pelvic examination revealed a large cervical excitation uterus with bilateral adnexal fullness and tenderness that was more marked in the right fornix. A transabdominal ultrasound scan showed an empty uterus with a thickened endometrium and a right adnexal mass that suggested a good tubal pregnancy with a fetal pole and heart activity (figure 1,2) with a moderate amount of free fluid in the pouch of Douglas. Her investigations revealed Hb 8.6 gm%, TLC 14,200, DLC N88 L12, platelets 2.2 Lac, PT, PTTK normal, blood urea 18, creatinine 0.6, Na 138, and K 3.7. After taking high risk consent and explaining the probability of salphingectomy and guarded future pregnancy prognosis, she was resuscitated and taken up in emergency for exploratory laparotomy . Preoperatively 1.5 litres of hemoperitoneum was drained. There was a ruptured right sided ampullary ectopic pregnancy ~5 *3 cm; left tube revealed multiseptated organized hematoma seen in ampullary region with left fimbrial stenosis. Uterus and bilateral ovaries were normal. Pouch of douglas had flimsy adhesions which suggested pelvic inflammatory disease which could be a reason for ectopic pregnancy to occur. A bilateral salpingectomy was hence performed as left sided upruptured mass could not be milked out from the tube due to fimbrial stenosis as we wanted to save one tube due to nulliparity of the patient, after informing patient's husband regarding requirement of in vitro fertilization for future child bearing. Two units of packed red blood cells were transfused to her intraoperatively. She had an unremarkable postoperative rehabilitation and on the fifth day of postoperative discharge. Histopathological examination in both tubes showing chorionic villi and trophoblasts confirmed the diagnosis of bilateral tubal ectopic pregnancy with evidence of rupture on the right.



Figure 1&2: A transabdominal ultrasound scan showing an empty uterus with a thickened endometrium and a right adnexal mass

Discussion

Ectopic means "out of place". In an ectopic pregnancy, fertilized egg has implanted outside the uterus. Tubal pregnancy, if not detected and treated earlier, fallopian tube can rupture and cause serious problems and sometimes death. The highest rate of ectopic pregnancy occurs in women aged 35-44 years. One proposed explanation involves myoelectric activity in fallopian tube, which is responsible for tubal motility. Aging may result in progressive loss of myoelectric activity along the fallopian tube.

According to a study by Dr. Samiya Mufti(2008), in a multicentre case control study in India, the incidence of ectopic pregnancy is 3.12 per 1000 pregnancies or in every 250 pregnancies. Peak age of incidence is 26 to 36 years and primi were the most common suffers. Most patients were diagnosed as ruptured tubal pregnancy, which is 60.52 per cent. As in this case study the patient was 28 years old and had been diagnosed with tubal pregnancy ruptured.

While only 40-50 percent of patients with ectopic pregnancy with vaginal bleeding may have abdominal tenderness, 50 percent may have visible adnexal mass with 75 percent. At the initial presentation, nearly 20 percent of patients with ectopic pregnancy are haemodynamically compromised, which is strongly predictive of rupture. Most ectopic pregnancy may be detected before rupture, thanks to advanced diagnostic techniques. Early intervention carries much better prognosis. If management starts before tubal rupture and haemodynamic instability even surgery can be avoided. Approximately 43-55 per cent of ectopic pregnancy has no typical triad of lower abdominal pain, amenorrhoea duration and vaginal bleeding. Any sexually active women with lower abdominal pain, bleeding or spotting with variable-period ammenorrhoea should therefore be carefully screened with ectopic gesstation in mind..

In order to prevent unnecessary care, such as removing the contralateral drain, preoperative and intraoperative assessments are required. Color Doppler transvaginal ultrasonography improves the diagnostic sensitivity for early detection of irregular and normal intrauterine pregnancy and low extra-uterine masses. (38,39,40). Many articles related to pregnancy are available from this region (41-47). Currently, the main management of ectopic pregnancy has become more conservative in order to save the tube instead of salpingectomy.

Conclusion

Adequate clinical experience and skills are essential preconditions for successful individualized tubal pregnancy treatment. Simple dictum of “think ectopic” should not be forgotten, particularly when conceiving infertility. Careful history taking along with proper clinical examination allows us to diagnose ectopic gestation even before it ruptures . Timely diagnosis and management of ectopic can decrease the rate of maternal mortality with reduced morbidity. More concern should be towards the restorative tubal surgeries which will conserve the reproductive capacity of women with better future pregnancy outcome.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Comparative Study of Effect of Specific Yogasanas and Exercise in Obesity

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Abstract

The reason for the study is to analyze the impact of chosen Yoga-Pranayama on obese individual of influenced people, in Mahatma Gandhi Ayurvedic college Hospital and Research Centre, and Datta Meghe Institute of Medical Sciences Wardha, Maharashtra. Sixty selected participants were arbitrarily separated in to two groups of thirty each, out of which group I (N-30), underwent Yogasan and group II-aerobic exercise (N-30). Pre-test and post test were directed for every one of the two groups on Weight, Basal Metabolic Index (BMI), chest circumference, abdominal circumference and waist circumference. The test group took part in their separate chosen yogasana schedule for a time of about two months. In short the Post-test concentrate obviously demonstrates that there is a critical distinction of Weight, BMI, chest circumference, abdominal circumference and waist circumference on pre-test and post test.

Keywords: *Yoga, Aerobics, Obesity, BMI.*

Introduction

The widespread need and significance of day by day Yogasanas efficiently arranged can't be overlooked in the present day. Yoga gives perhaps the best mean of personal development and increasing maximum capacity of one's body, mind and soul. It has been demonstrated certain that pranayama and certain Asana's are a significant methods for forestalling and relieving numerous ailments.[1] Yoga speaks to a group of practices and is increasing expanding prevalence in numerous nations around the globe, comprising of different stances (Asana), breathing and contemplation methods (Pranayama).[2] Yoga is accounted for to lessen pressure and nervousness, improves autonomic capacities by activating neurohormonal components by the concealment of thoughtful activity.[3] The physiological impacts of Yoga preparing that have been recently detailed incorporate the hindrance of body weight gain, decrease in cholesterol levels and circulatory strain alongside progress in invulnerable capacity just as helpful mental effects[4-9]. Yogasanas utilizes different stances to create physical quality, adaptability and continuance which can be utilized as a moderate-power practice for patients with constrained oxygen consuming limit or confined capacity to exercise.[10] Yoga has been appeared to diminish hypertension and heart function improvement, balance out the thoughtful sensory system, and improve mental wellbeing and the cardiovascular function.[11-13].

Material and Method

60 Patients were shortlisted for the study. Ethical clearance was taken from the Institutional Ethical Committee before starting the study. Written Consent was taken from all participants before admitting in the study. Participants were divided into two groups. First group labeled as a study group and second one control group. Each group consists of thirty participants. The study group suggested asanas specially for 3 months and control group was asked to do only aerobic exercises for three months.

Group I:

Yogasanas for study group (duration- 90 minutes)

Purva asanas:

- Sun Salutations:- 6-12 full rounds in 20 minutes

Yogasanas:

- Uttanpad Chakrasana (3 rounds),
- Akarna Dhanurasana (1 minute on each side),
- Ushtrasana (2 minutes)
- Udarsanchalan (3 rounds)
- Trikonasana (1 minute on each side)
- Matsyasana (1 minute)
- Paschimottasana (1 minute)
- Ardhamatsyendrasana (2 minutes on each side)
- Shavasana (when needed)

Group II: Aerobic exercises

The follow up of the patients was taken for every one month. The results were noted time to time in the CRF (Case report form).

Table 1: Assessment parameters

S.No.	Parameters
1	Height
2	Weight
3	BMI Body mass Index
4	Abdominal circumference
5	Chest circumference
6	Waist circumference

Observation and Results

Table 2: Comparison and analysis of weight

Time Period	Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
Before Treatment	A	30	72.73	5.47	0.99	1.18	0.24**,NS**
	B	30	74.33	4.96	0.90		
30 days	A	30	71.63	5.20	0.94	1.67	0.10**,NS**
	B	30	73.86	5.14	0.93		
60 days	A	30	70.33	5.10	0.93	2.31	0.024**,S**
	B	30	73.33	4.95	0.90		
90 days	A	30	68.96	5.27	0.96	2.93	0.005**,S**
	B	30	72.90	5.12	0.93		
100 days	A	30	68.53	5.59	1.02	2.94	0.005**,S**
	B	30	72.60	5.08	0.92		

Table 3: Comparison and analysis of Body Mass Index (kg/m2)

Time	Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
Before	A	30	28.41	1.65	0.30	0.005	0.996,NS
	B	30	28.40	2.23	0.40		
30 days	A	30	27.98	1.59	0.29	0.482	0.631,NS
	B	30	28.22	2.20	0.40		
60 days	A	30	27.48	1.62	0.29	1.079	0.285,NS
	B	30	28.02	2.26	0.41		
90 days	A	30	26.93	1.51	0.27	1.874	0.066,NS
	B	30	27.85	2.24	0.41		

Table 4: Comparison and analysis of Abdominal Circumference

Time	Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
Before	A	30	107.00	3.68	0.67	1.00	0.32,NS
	B	30	105.96	4.27	0.78		
30 days	A	30	105.46	3.15	0.57	0.25	0.80,NS
	B	30	105.23	3.92	0.71		
60 days	A	30	103.36	3.21	0.58	1.03	0.30,NS
	B	30	104.30	3.77	0.69		
90 days	A	30	101.33	2.92	0.53	2.51	0.015,S
	B	30	103.50	3.71	0.67		

Table 5: Comparison and analysis of Waist Circumference

Time	Group	N	Mean	Std. Deviation	Std. Error Mean	t-value	p-value
Before	A	30	113.26	3.32	0.60	0.08	0.93,NS
	B	30	113.33	2.89	0.52		
30 days	A	30	111.00	3.12	0.57	1.45	0.15,NS
	B	30	112.13	2.89	0.52		
60 days	A	30	109.16	3.05	0.55	2.96	0.004,S
	B	30	111.43	2.87	0.52		
90 days	A	30	106.56	3.22	0.58	4.79	0.0001,S
	B	30	110.33	2.84	0.51		

Discussion

The changes in parameters were noted from time to time. It was observed that mean weight of all the thirty study participants was reduced as compare to control group. The BMI of the control group was reduced more than study group. There were significant changes in waist and chest circumference of study group as compare to control group.

Weight: In a study done by P. B. rishikesan et al on Yoga Practice for Reducing the Male Obesity and Weight Related Psychological Difficulties-A Randomized Controlled Trial, because of yoga practice weight was reduced from 82.63 ±10.05 to 80.47 ±9.59.

Waist Circumference: There is significant reduction in waist circumference. Similar results were reported by rishikesan et al reduction in waist circumference from 99.58 ±7.37 to 95.09 ±6.76 . [14] In a study by S. Dhananjai et al, on reducing psychological distress and obesity through yoga shows reduced waist circumference from 102.08±10.25 to 83.01±33.0 [15]

Basal Metabolic rate (BMR): Present study shows significant reduction in BMR from 28.41 to 26.93. In a study by Dr Annapurna K. and Dr. Vasantlakshmi K. on effects of yoga therapy on quality of life in woman, after three months of yoga practice shows decrease in Basal Metabolic Index from 30.79 ±4.12 to 28.03 ±3.95.[16,17]

Conclusion

Present study highlighted importance of yoga and pranayam in day to day life. Yoga helps to keep biological values under control if compared to modern era exercises like aerobic exercise.

Ethical Clearance: Taken from institutional ethics committee

Source of Funding: Self.

Conflict of Interest: Nil.

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Role of Vrishyashatavarighrit on Sukrakshaya (Oligospermia) by Studying Siddhant

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Abstract

Ayurveda is oldest tradition addressing major healthcare issues in sophispticated way. There are number of modern medicines giving relief to the patients but having adverse effects at highest level. Ayurveda smartly not only addresses the issues but having less side effects. Current study focuses the use of Shatavarighritin shukrakshay (oligospermia) patients. The study comes to a conclusion with a promising results in the patients of oligospermia. The study result to reduce the burden for patients and giving light who having infertility.

Keywords: *Sukrakshay, Shatavari, Vrushya, Samanya Siddhant.*

Introduction

Siddhant means, the principle which is examine by many experts via different ways and then proved or established. The use of drugs having similar properties brings unity and increases of similar dhatus in body.[1] Application of similarities of all substances is the always cause of increasing of body tissue. Ayurveda is the richest heritage of world whose principle and guidelines are laid by Vedas, which are consider as the first available literature of world.

Infertility is one of the important medical problem which affects sexual life, social function and psychological problems in couple. Vajikarana a branch of Ashtang Ayurveda mainly deals with the drugs and therapies which are aphrodisiac in nature[2,3,4].

These Vajikaran drugs are of 3 types viz.

1. Shukravriddhikara
2. Shukrastrutikara
3. Shukrastrutivriddhikara

Shatavarighrut is described in Mashparnbhritiyaadhyaaya 3 rdpaad of vajaikaranaadhyaaya. All the drugs described in this 3 rdpaada are of Balaprada and Shukraprada in nature and hence Shatavarighrut is selected for study. As Shukrakshaya affects the prakrut karma of Shukradhatu and ultimately leads to infertility, Ayurveda gives very much importance to Dhatu Samanya Chikitsa and describes ultimate aim of treatment for infertility cause by Shukradhatukshaya (oligospermia). Repeatedly use of any thing is known as Abhyas, here use of vrishyashatavarighrit for one month is in bid dose is important for increasing of shukradhatu.

Samanyasiddhant is one of the basic principle in ayurveda which deals with increase of dhatu which were decreased by mitthya-viruddhaaahaarvihaar or other cause. For practical assessment of this siddhant in the patients of Shukrakshaya the vrishyashatavarighrit which is Gun Samanya to Shukradhatu is selected for study. This Shatavarighrit is given in 10 ml bid dose for one month with anupaan of Godugdh which results into satisfactory increases of semen volume and sperm count in patients[5,6,7].

Aims: To study the siddhant "Saman Gunabhyaso Hi Dhatunam Vriddhikaranamiti" w.r.t. Shukrakshaya (Oligospermia) by using Vrishya Shatavari Ghrit.

Objectives:

1. To study "Saman Gunabhyaso Hi Dhatunam VriddhikaranamIti" siddhant

2. To assess the "Saman Gunabhyaso Hi Dhatunam Vriddhikaranamiti" siddhant by practical assessment using gun samanyavriddhikaranamsiddhant for shukradhatu.
3. To study effect of Vrishya Shatavari Ghrit on Shukrakshaya (oligospermia).

Material and Method

30 Male patients suffering from infertility caused due to oligospermia and accidentally found patients who were oligospermic we examined, only those qualifying for selection criteria were selected for the trial, They were explained the objectives and methodology along with the advantages and disadvantages of trial. Those patients who are interested and agreed were involved in the study.

1. **Written consent:** Written consent was taken from the patient for his legal guardian.
2. **History:** Detail history of patient was taken about duration of infertility, marital status, occupation, previous pregnancies, sexual habits like erection of penis, ejaculation, frequency of coitus, history of masturbation and exposure. History of exposure to heat, radiations and toxic environment was ruled out. History of alcohol consumption, smoking, tobacco, gutkha chewing was included in study. Whole history of childhood illness like post pubertal mumps, testicular trauma and torsion, tuberculosis was obtained.
3. **Examination:** Patient was examined for major systemic or surgical illness. For this strotoparikshan was done. Patients who are found to be suffering from major systemic illness were rejected. Similarly, patients suffering from hydrocele, varicocele and other obstructive disorders, inhibiting proper ejaculation of sperm were rejected.
4. **Laboratory investigations:** Laboratory investigations such as semen analysis particularly sperm count and volume of ejaculation was done. VDRL and other investigations were done if necessary.
5. **Selection of patients:** Patients were selected randomly. After screening those found to be oligospermic (sperm count less than 20 million) were selected. Total 30 patients were selected in trial group. Patients included in trial group were treated by Vrishya Shatavari Ghrit 10 ml BD with milk for one month.
6. **Record of observations:** Observed parameters were recorded on first visit and after thirty days by semen analysis (which was done after 3 days of abstinence).
7. **Parameters:**
   a. Sperm count measured in millions.
   b. Volume of ejaculation measured in ml.
8. Treatment procedure:

For the patients of trial group, Vrishya Shatavari Ghrit 10 ml bid was given with anupaan of Godugdh (100 ml) for one month. Patients were asked for follow up after one month. Then again semen analysis was done after 3 days of abstinence.

9. Advice and instruction given to patient:
   - Avoid tight and warm undergarments.
   - Cold scrotal bath at least twice a day for 5 minutes each time.
   - Avoid smoking and alcohol consumption.
   - Avoid shukranashakdravyas and karma's.
   - Avoid excess katu, tikta and kashayarasatmakaahar.
   - No use of any other medicine during treatment.
10. **Statistical Analysis:** After collection of data it was analyzed statistically by applying proper test such as paired t test.

Observation and Result

To study siddhanta 'samanyamvriddhikaranam' with respect to shukradhatu following observations were made and result was calculated. According to age maximum 12 (40%) patients were belonging to age group 21-25 years. The higher number of Hindu indicates demography of region. Maximum 63.33 % patients were with mixveg diet and 36.66 % patients were with pure veg diet. Maximum 63.33 % patients were with mixveg diet and 36.66 % patients were with pure veg diet. Maximum 56.66% patients were addicted and 43.33% patients were non-addicted.

1. Prakruti wise distribution:

Prakriti	No. of patients	Percentage
Vataj	8	26.66%
Pittaj	5	16.66%
Kaphaj	3	10%
Vatapittaj	7	23.33%
Vatakaphaj	4	13.33%
Pittakaphaj	3	10%

Max

26.66% patients were having vatajprakriti. Maximum 26.66% patients were having vatajprakriti.

2. Samhanan wise distribution

Samhanan	No. of patients	Percentage
Pravara	08	26.66%
Madhyam	06	20%
Alpa/Avar	16	53.33%

Above table shows maximum of 53.33% patients were of Avar samhanan and 20% patients of madhyamsamhanan and remaining 26.66% patients were having Pravarasamhanan.

3. Volume of Ejaculation:

Mean volume of ejaculation in trial group as per visit i.e. before and after was as follows:

Visit	Before trial	After trial
Mean	2.2166	2.5833
SD	0.52	0.732

Paired t-test:

Volume of ejaculation	Before to after trial
Mean	0.3667
SD	0.29165
SE	0.05325
t value	6.8861
t table	2.05
p	P < 0.05

Above table shows mean increase in volume of ejaculation of total 0.366 ± 0.29 ml which is significant at p=0.05 level.

4. Sperm count:

Mean sperm count in Trial group as per visit was as follows:

Visit	Before trial	After trial
Mean	11.6699	13.42
SD	4.038	4.8532

Paired t–test

Volume of ejaculation	Before to after trial
Mean	1.76
SD	1.12728
SE	0.20582
t value	8.5513
t table	2.05
p	P < 0.05

Above table shows increase in sperm count of total 1.76 ± 1.12 million which is significant at P=0.05.

Discussion on Observations

In clinical study, maximum numbers of cases were in between the age group 21-25 years, reflecting marriage age. After getting proper education one has to get on establishment in occupation. Man is struggling physically and mentally to get into an occupation which posses social status and other fulfilling criteria. Fear, mental tension, unwholesome food habits, improper regimen (mithyaaaharvihar) are come in way and this leads to vitiation of biological humors. Younger stage is the time of pitta doshaadhikya and along with above factor vatadosha is also vitiated. This factor can be attributed to maximum number of patients in younger age group[8].

In the observation of age wise distribution 40 % of the patients occur of age group 21 to 25, and 20% of patients occurs of age group 26 to 30. According to aacharyaCharak the age distribution for yuvavastha as 30 years and madhyam/praudhavastha is 31 to 60. In my study the shukrakshaya occurs in 60% of patients of yuvavastha this may be due to aparipakvadhatuavastha. The no. of patients occurs in praudhavastha/madhyamaavastha are 40%. By taking maximum sample size this observation can be calculated again very accurately. As per religion wise distribution in my study the maximum no. of patients are of Hindu religion i.e. 70%. This does not mean the occurrence of shukrakshaya is maximum found in Hindu religion but this is due to Demography of region. This need again maximum sample size to get more accurate observation. In my study pure vegetarian patients are 36.66% and mix vegetarian are 63.33%. As per the Rasatmakaahar variation, maximum patients taken Shukrakshayakaraahar. In the observation of Addiction wise distribution, 56.66% of the patients were addicted. This addiction was commonly about 3 to 6 years of addiction mostly. For the period of medication I advised them to stop their addiction. These addictions were of Alcoholism and of Tobbaco chewing/smoking or of both types. In patients who have the Vat as pradhanprakriti the percentage are 63.33%. In which the vat prariti patients are 26.66%, vat-pittajprakriti patients are 23.33%, and vat-kaphajprakriti patients are 13.33%. While the patients having Pitta as pradhanprakriti are 26.66% in which mainly pitta prakriti patients are 16.66% and pitta-kaphprakriti patients are 10%. Kaphprakriti patients are only 10 % occurred. This suggest that,As per Samhita granthas, the Vat prakritipurush has the Rukshata, laghuta, parushata, vishadata in their body as wel as they are having naturally Alp Bala, alp apatya (own childs), alp aayushya (age). These all snigdh, shlakshn, mrudu, madhur, saandra, guru etc are the gunas which are the gunas similar to gunas of Shukra. So the Kaphprakritipurusha are comparatively less in percentage for Shukrakshaya.

Shatavari: Guru and snigdhaguna of shatavari is similar to that of shukra. Madhur rasa of shatavari is also similar to shukra. The veerya of Shatavari is Sheet in nature and the Vipak of Shatavari is madhur, both these are as equall of ShukraGunas, so they all are helpful for increasing the Shukradhatu by Saman gun siddhant. As per modern experiments Shatavari has the contain Sugar, glycosides, saponin, sitosterol. According to web search- pure 9, 10 dihydrophenanthrene has been shown to interact with androgen receptors and may therefore inhibit androgen dependent prostatic growth; the 25 shatavrin (the steroidal saponin) may be responsible for the hormonal like effect of Shatavari for traditional use as Reproductive tonic.

Goghrita: Madhur rasa, guru and snigdhaguna of goghrita are similar to shukraguna, which result in increasing of Shukradhatu by Saman gun siddhant. Shatavari, goghrita and godugdha does not have

any opposite guna to that of shukra. According to samanyasiddhanta when samanguna meet together they increases the saman gun dhatu, Hence gunasamanya is responsible for increase in vyaktashukra. This shukravriddhi has occurred in 30 days and shukra is the last dhatu to form. Thus shukravriddhi occurs after the nourishment of all dhatus. ksheerdadhinyaya may be responsible for this vriddhi. According to modern science it indicates that all the patients may have temporary oligospermia. There is Aashraya - Aashrayi relationship between shukra and kapha. Hence the dravya which increases kapha also increases shukra. Most of the properties of vrishyashatavarighrit are similar to kapha and this may be responsible for increase in shukradhatu. Honey is yogvahi in Vrishya Shatavari Ghrit and yogvahidravya when mixed with drug increases the action of that drug. Honey also contains fructose and fructose is the source of energy required for the metabolism of sperm it may helpful in maintaining healthy environment in sperm and also nourishes sperm thus honey may increases lifespan of sperm. All the drugs in shatavarighrit are madhurrasatmak which is formed by prithvi and jalamahabhuta. As spermatozoon has definite shape and size it has predominance of prithvimahabhuta. The jaliyamahabhuta increases jaliya part of shukra (seminal plasma) and parthivmahabhuta increases parthiv part of shukra (sperm count). Vrishyashatavarighrit is madhur and causes bruhan in the body hence can be called as vajikarana. All drugs in vrishyashatavarighrit are sheeta (formation of cold atmosphere in body) which is useful for spermatogenesis. Oja is described as essence of shukradhatu and formation of oja increases immunity of the body. It indicates that formation of pure shukradhatu is important for general health. Ghrita contains vitamin E (Tocopherol) which has antioxidant properties. Under normal circumstances, oxidants potential of male reproductive tract and seminal fluid is maintained by adequate levels of enzymatic and nonenzymatic antioxidants, vitamins and minerals. When the production of oxidant is high, it may lead to oxidative damage of spermatozoa, hence it may decrease sperm count. Ghrita may neutralize oxidant present in male reproductive system. Hence less damage to spermatozoa occurs. All ingredients of vrishyashatavarighrit have good nutritional values; hence it is useful in nourishing all body tissue including sperms. Proper nutrition of sperm and seminal vesicles helps in their proper functioning and therefore adequate secretion.

Conclusion

Shukra can be correlated with sperm and seminal plasma both. Shukrakshaya can be correlated with oligospermia. Predominance of vata and pitta dosha in predisposing disease shukrakshaya is collaborated clinically by its mode of presentation i.e. most of the patients were from madhyamvaya (period of pitta pradhanya) and maximum were possessing vatapittaprakriti. Vrishya Shatavari Ghrit was found to be beneficial in increasing sperm count and volume of ejaculation significantly.The composition of gunasamanyaaushadha the Vrishya Shatavari Ghrit has effectively contributed for the increase of shukradhatu.

Samanyasiddhanta (samangunabhyaso hi dhatunamvriddhikaranam) was proved by the Vrishya Shatavari Ghrit on shukradhatu.

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

Abbreviations:

CH.SU.	Charaka Samhita Sutrasthan
SU.SU.	Sushruta Samhita Sutrasthan
A.S.SU	Ashtanga Sangraha Sutrasthan
A.H.SU.	Asthanga Hrudaya Sutrasthan
SHAR.PU.	Sharangadhar Samhita Purvkhand
B.T.	Before Treatment
A.T.	After Treatment
M.D.	Mean difference
S.D.	Standard Deviation
S.E.	Standard Error
E.G.	Experimental group
P.V.	Pure – veg diet
M.V.	Mix – veg diet
A	Addicted
NA	Not Addicted
R.No.	Registration No.
USNDB	United states nutrient database.

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Review on Effect of *Sarshap Taila* and *Til Taila* in Prevention of *Atiyog* of *Karnendriya* with Special Reference to *Karnanaad*

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Abstract

Lifestyle changes has created lot of hurdles for simple *upkramas* of *Dincharya* like *Karnapuaran* in daily routine, leading to commonly occurring disease i.e *Karnanad* (Tinnitus). It has remained as a formality in Hindu festivals like Diwali & Dashera. In some parts of the country to follow rituals like applying Taila on the body or procedures like *Abhyanga* and in that case people done karnapuran as well. So, problems related to Ear like Karnanad are increasing day by day in *atiyog* of *karnendriya* and old aged people in modern era. There are different treatment modalities available for the same, but these are costly. These treatments are not affordable by low socio economic people. So this review focused with *Sarshap Taila* and *Til Taila* to prevent *atiyog* of *Karnendriya* given in *Ayurved.*

Keywords: *Atiyog, karnapuran, karnanaad, abhyanga, karnendriya.*

Introduction

Ayurveda aims at maintaining the health of "*Swastha Purush*" and also cures the *Vyadhi* of '*Atur Purush'*. Lifestyle changes & poor hygiene leads to chronic disease, ear disease and like *atiyog* of *karnendriya. Karna* is considered as one among the *panchendriyas.* It is known as *shrotrendriya.*[1]. Main function of ear is the perception of Sound. Along with function of hearing, it maintains the equilibrium of the body. Nowadays there is a lot of change in the life styles of peoples. Lots of people pay attention to different food habits and way of living. Very cold foods like ice-cream, cold drinks have an increasing demand. Consumption of such products will leads to vitiation of *doshas.* Also due to increasing number of vehicles and electronic sound conductor as head phones with their different types of sound pollution which affects the ear. Thus due to different *apathyakar Ahar vihar* there will be development of different types of *karnarogas.*[2] *Acharya Sushruta* described *28 karnarogas* in *sushruta samhita*.[3] Among these "*Atiyog of Karnendriya*" causes major symptomatic condition which may leads to *Karnanad*. Which is one of the major serious disease which will found common in most of the peoples.

According to modern science due to Atiyog of karnendriya Vertigo, Headache, Karnashuskata, karnadaha, Tinnitus are commonly available diseases in most of the peoples now a days. This karna-atiyog resembles with vertigo, headache, karnadaha, and karnanad. In karnanad there is vitiating of tridosha. In this vat will leads to dry of kapha which produces khaph in the external auditory canal.[4] Vata associates with pain in this disease. So mainly tridoshagha treatment is required. Sarshap tail and til tail are very much beneficial in karnanad. It has different ingredients which will definitely reduce the symptoms.

These drugs are easily available and also low cost and act as a home remedies. So the persons who are living in low middle class family they can also be prevented from this symptoms.

Karna sharir described in Ayurvedic texts: Karna is made up of panchamahabhutas in which there

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is dominance of akash mahabhut mention in Sushrut Sutrasthan 6/14 and charak sutrasthan 6/11 . It is also adhisthan of shabda tanmatra.[5]

Karna Uttpati: In the third month of garbhavastha there is division of head by neck, after which various parts of head are expressed in minute form. Thus in third month karna is expressed in minute form is described in Sushrut sharir sthan 5/20.[6]

Karna Indriya Srotas: While decribing srotasas, karna indriya is included in bahirmukh srotas described in Sushrut sharir sthan 5/10.[7]

KARNA–ASTHI: According to sushruta each of ganda, karna and shanka has one asthi. Karna also has tarunasthi in it described in Sushrut sharir sthan 5/19 and 5/28.[8]

Karna Dosha: VATA The main dosha present in karna is vata described in Ashtang Hriday Sutrasthan 12/01.

Pranavayu: Among the types of vata doshas pranavayu is present in karna. Pranvayu does the shabda vahan in karna. Indriya dharan is karma of pranavayu described in Ashtang Hriday Sutrasthan 12/04..[10]

PITTA:

Sadhak Pitta: Sadhak pitta is present in karna. Indriyarthagrahana is karma of sadhak pitta in karna described in Ashtang Hriday Sutrasthan 12/01.

KAPHA: Tarpak kapha is present in karna described in Ashtang Hriday Sutrasthan 12/17[12]

Shleshak Kapha: Sleshak Kapha is present in karna sandhi. sleshak kapha does sandhishoshan described in Ashtang Hriday Sutrasthan 12/17[12]

Karna Mala: Mala present in karna is khamala which is mamsa Dhatu mala. Karna mala is the collection of secretions from glands situated in karna.[12]

Karna-Roga Sankhya: According To Different Acharyas.

Sushruta Samhita: According to sushruta samhita there are 28 karna rogas. Karnakandu, Karnashula, Karnapak, Karnavarchas, karnanad, Karnasrava is few disease from these rogas mention in Sharirsthan 3/5.[13]

Karna Roga--Sharangdhar Samhita: There are 18 karna rogas descried in sharangdhar samhita Prathan Khanda 7/142 [16]

Ashtang Hridya: There are 25 karna rogas described in Ashtang Hridya Uttarsthan 17/2.[17]

Ashtang Sangraha: Ashtang sangraha also describes 25 karna rogas.[18]

Charak Samhita: According to Acharya charak, karnarogas are divided into 4 types. He described karnarogas according to doshas Vataj, Pittaj, Kaphaj and Sannipatik karnarogas.[19]

Yogaratnakar: In yogaratnakar 28 karnarogas have been described by acharya.[20]

Bhavprakash & Gadanigraha: In Bhavprakash and Gadanigrah, 28 karnarogas have been described.[21]

Bhel samhita: Bhel samhita describes 23 karnarogas.[22]

Karnaroga:

HETU: Living in damp climate, Swimming in water, Itching in ear and improper and overuse of shalaka in ear cause vataprakop. This vataprakop causes karnashula mention in Vadghbhat Uttartantra 17/1,2.[23] In this way, above factors are responsible for karnaroga

Karna Naad: There is explaination in Ashtanghridaya sutrasthan 22/32 in Dinacharya atiyog. Yogaratnakar explained the same as sushruta. Due to different aetiological factors such as Trauma to the head-blood stained discharge .Swimming. Due to excessive Head phone users causes headache, karnashuskata, karnadaha, karnanaad .Excessive head phone user or high intencity sound or DJ Runner Boys Causes karrnanad[24]

Sarshap Tail And Tila Tail For Karnapuran: Acharya Vaghbhat mentioned the use of Sarshap Taila and Til taila Karnapuran and its definition and whole process mention in Sutrasthan Gandushadi Vidhi adhyay karnpuran vidhin in Shlok no. 22/32 in atiyog of karnendriya. He has advised to instill 10 drops(2ml) of tail in ear for 100 matra. (3 minutes)[25] Sarshap [26]

Botanical Name: Brasica compestris Linn.

Family: Cruciferae

Properties Rasa: Katu,Tikta

Virya: Ushna

Vipak: Katu

Guna: Teeksna, Ruksha

Til Tail[27]

Botanical Name: *Sesamum indicum* Linn.

Family: Pedaliaceae

Properties: Rasa: Madhur, kashay, Katu,Tikta

Virya: Ushna

Vipak: Madhur

Guna: Guru, Snigsha

Doshakarma: Vatshamak, Kaphapittaprakopi

Rogaghnata: Snehan, Vednasthapan, sandhaniya,

Dosha karma: Kaphavatghna, Pittakar

Rogaghnata: Shonitotkleshkarak, Vednasthapak, Snehan

The science which deals with the treatment of diseases of upper part ear, eyes, mouth and nose is called as shalakya tantra.

Ayurved samhita contains the anatomy of urdhva jatrugata avayavas. While dealing with knowledge and treatment of diseases we should know the anatomy & physiology of respected avayavas.

Discussion

Karna is one of the most important dhnyanendriya in body. It's important function is hearing. It is also important organ in maintaining balance of body. Review of ayurvedic literature shows that there are 28 karnarogas (according to Acharya Sushruta). Karnanaad is important karnaroga. Tridosha vitiation occurs in this disease. The common symptoms produced by Atiyog of karnendriya causes inconvience to the patients in daily routine. So it is necessary to have very effective treatment without side effects and economical drug on it. Review of modern literature shows the structure, physiology, mode of examinations and investigations, because of advancement in instrumentation. Various antibiotics and anti-inflammatory drugs are used but there effect is found to be poor as well as effect is very low recurrence rate is high. In some patients aggressive treatment like surgical intervention is adviced but it is uneconomical to the patients. So effort is made to have effective and economical medicine. After study it is observed that the patients having the habits of who exposed to noise pollution and excessive headphone users are more prone to this disease. In the types karnarog dry ear, headach, vertigo are more seen. The laxanas of karnanad can be corelated with tinnitus . In agewise distributation of the patients 20-30years of age group are prone to this disease because of excessive head phone users. In occupation wise distribution of patients excessive head phone users are more prone to this symptoms. In locality wise distribution of patients urban area peoples are more affected studied by Dr. Abasaheb Lahankar in national magzine.[28]

Sarshap i.e Brasica compestris Linn. Having Properties like Rasa Katu, Tikta, Virya –Ushna, Vipak : Katu, Guna :Teeksna, Ruksha and Til Tail i.e *Sesamum indicum* Linn.Properties like Rasa-Madhur, kashay, Katu, Tikta Virya :Ushna Vipak: Madhur Guna: Guru, Snigdha works on kapha dosha related to karnendriya to prevent all comlications.

In a previous done studies like to study the efficacy of sarshap taila karnapuran in atiyog of karnendriya w. s. r. to continuous head phone users shows that sarshap tail karnapuran reduces all the symptoms like larnakandu, Shiroshula karna shushkata, karna daha and karnanaad.[29]

In another study by Dr. Abasaheb M. Lahankar already done a work on tinnitus and give literature in National health Magzine Arogya sanjeevani October 19.[30]

Probable Mode of Action of Karnapurana: In Purva Karma, Snehana and Swedana were performed. Generally the medicated oil is used in Karnapurana, Taila is having virtue of Vata Shamana but do not aggravate Kapha. In this way Karnapurana eliminates disease of ear which occurs due to vitiation of Vata. In addition medicine is used luke warm so Vata is eliminated by Ushna Guna also. These properties do not only have beneficial effect on Vata but also removes Avarana of Kapha. As medicine for Karnapurana is made by Snehapaka method, all fat soluble active ingredients of drug get enclosed. Fat soluble active principle can be easily assimilated through nerve endings. As a combine effect of all these Karnapurana is useful in various diseases.

Karnapurana is conducive to the nutrition of the skin and the softness of the muscles. It penetrates into the

skin quickly and enters the blood stream, through the capillaries & supply nutrition to nerves. It has a unique quality of getting absorbed easily by the pores in the skin and there by acts as a catalyst.[31]

Conclusion

The literature review is devoted to supplementation of Ayurvedic knowledge with Modern Medical concepts, where Anatomy and Physiology of ear is discussed. Under the topic 'Drug review' the detail explanation of the drugs from formulation are described By applying Student's paired and unpaired 't' test, efficacy of the therapy was analyzed statically. Sarshap taila better acts on different symptoms of Atiyog of karnendriya like Headach, vertigo, karna shuskata and karnanad. By sarshap tail relatively than til taila. People who exposed to noise pollution & excessive head phone users are maximum prone to this disease. Symptoms & signs of atiyog of karnendriya can be corelated with karnand. This taila used for longer duration may prove more effective in atiyog of karnendriya. This taila is easily available & cheap conservative line of treatment for atiyog of karnendriya.

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

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Clinical Outcome of Supracondylar Femoral Fractures Managed Locking Plate Osteosynthesis in a Rural Hospital

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Abstract

Introduction: Distal femur fractures in the supracondylar region accounts for approximately seven per cent of all femur fractures and are very challenging to manage. These fractures occurs majorly due to severe grade of injury in young age groups and low grade injury in geriatric people with significant soft tissue injury with joint surface involvement and is bimodally distributed.

Material and Method: This prospective study consisted of 45 patients of supracondylar femur fracture surgically managed by locking plate osteosynthesis in Department of Orthopaedics at our institute within the study period of 2 years from June 2016 to June 2018. Functional outcome was measured using Neer's scoring. Patient were followed up regular intervals of 12 weeks and last follow up was done at the end of 1 year.

Results: 45 patients were studied i.e 36 males and 9 females with a mean age of 45.46±17.55SD years. Road accident was the most common cause of injury (71.1%),AO classification was used to classify fracture configuration and most common was type "33 C3" (26.67%). 39 patients (86.66%) were treated with distal femur locking plate osteosynthesis and 6 patients (13.33%) with external fixation which was later converted into plate osteosynthesis after infection control. Knee stiffness (17.78%) was a common complication and shortening was seen in 6 patients (13.33%). Neer's scoring was used to calculate outcome of patients in which 31(68.89%) patients had excellent to good outcome, 9 patients had fair and 5 patients had poor outcome.

Conclusion: Distal femoral locking plate can be considered as a suitable choice for achieving adequate fixation in distal femoral fractures as there was significant progressive increase in Neer's outcome scores in majority cases

Keywords: *Distal femur fractures; clinical outcome; Neer's score*

Introduction

Distal femur fractures in the supracondylar region accounts for approximately seven per cent of all femur fractures and are very challenging to manage[1]. These fractures occurs majorly due to severe grade of injury in young age groups and low grade injury in geriatric people with significant soft tissue injury with joint surface involvement and is bimodally distributed. Multiple fracture patterns are observed with this type

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of fracture and these are often associated with open wounds, fractures shaft or neck of femur, tibia fractures etc. Thus, it is challenging for a surgeon to treat these kind of fractures.

The major cause of such fractures are vehicular accidents, fall from high altitude, assault, etc. Historically, these fractures were managed conservatively with traction and often resulted in complications but in recent times, surgical management is the treatment of choice. Surgical aim is anatomical restoration of joint surface, restoration of leg length, bone alignment and correction of rotation of fracture fragments.

The study was done to evaluate clinical outcome of distal femur fractures treated with locking plate osteosynthesis and to evaluate complications associated with it.

Material and Method

The study was aimed to analyse clinical outcome of patients having supracondylar femoral fractures. This prospective study consisted of = 45 patients of supracondylar femur fracture treated by distal femur locking plate osteosynthesis in Department of Orthopaedics, at our institute during the study period of 2 years (June 2016 – June 2018). Inclusion criteria was all patients more than 15 years of age having open or closed supracondylar femur fractures. Exclusion criteria were Patients of age below 15 years, pathological fractures, polytrauma patients, patients with polyneuropathy disorders and patient not willing to consent for study.

In every case a detailed history was taken regarding the mode of injury, duration of injury and nature of injury. A thorough examination of the affected limb with examination of neighbouring joints was done. Special attention was given regarding the neurovascular status of the limb. Detailed general and systemic examination of the patient was done. Immediate posterior splinting of the affected limb was done with plaster of paris (POP) slab or Thomas splint with traction or skeletal traction on Bohler Brown splint was given till surgery.

All the patients were subjected to radiological evaluation to know the type of fracture. Radiological assessment was done using Anteroposterior and X rays of distal thigh with knee joint. Fractures were classified in accordance with AO classification and treatment modality decided accordingly keeping in mind the type of fracture (Open/Closed) and severity of fracture.

Comminuted fractures with gross swelling were given proximal tibial skeletal traction. Haematological, biochemical and other radiological investigations were carried out as per the requirement. Closed supracondylar femur fractures were surgically reduced and fixed with distal femur locking plate osteosynthesis and open supracondylar fractures were primarily stabilized with knee spanning external fixator application till the infection control and wound management. After wound healing and infection control, open reduction and plate osteosynthesis was done. Static quadriceps strengthening exercises started immediately from day 1 and active knee range of motion was started as soon as soft tissue healed. After clinic radiological union of the fracture, weight bearing on the affected limb was initiated.

Neer's Functional Scoring[2] was used to evaluate surgical outcome for distal femoral fractures. It is combination of two units: Functional (70 units) and Anatomic (30 units).

The results were evaluated by taking into consideration the following factors:

1. Pain – 20 points
2. Function – 20 points
3. Motion – 20 points
4. Work – 10 points
5. Gross Anatomy – 15 points
6. Roentgenograms – 15 points

Functional outcome and radiological assessment evaluated postoperatively, 12 weeks, 6 months and 1 year.

Table 1: Showing interpretation of outcome using Neer's score

Outcome	Score
Excellent	Above 85
Good	70-84
Fair	55-69
Poor	< 55

Post operatively physiotherapy in bed was started on the 2ndpost-operative day or according to the tolerance of patient and associated injuries. The physiotherapy of patients were initiated with quadriceps strengthening exercises, knee and ankle active range of motion exercises. Walking with weight bearing on unaffected

limb was initiated depending upon the intra operative stability of fracture.

Observations and Results

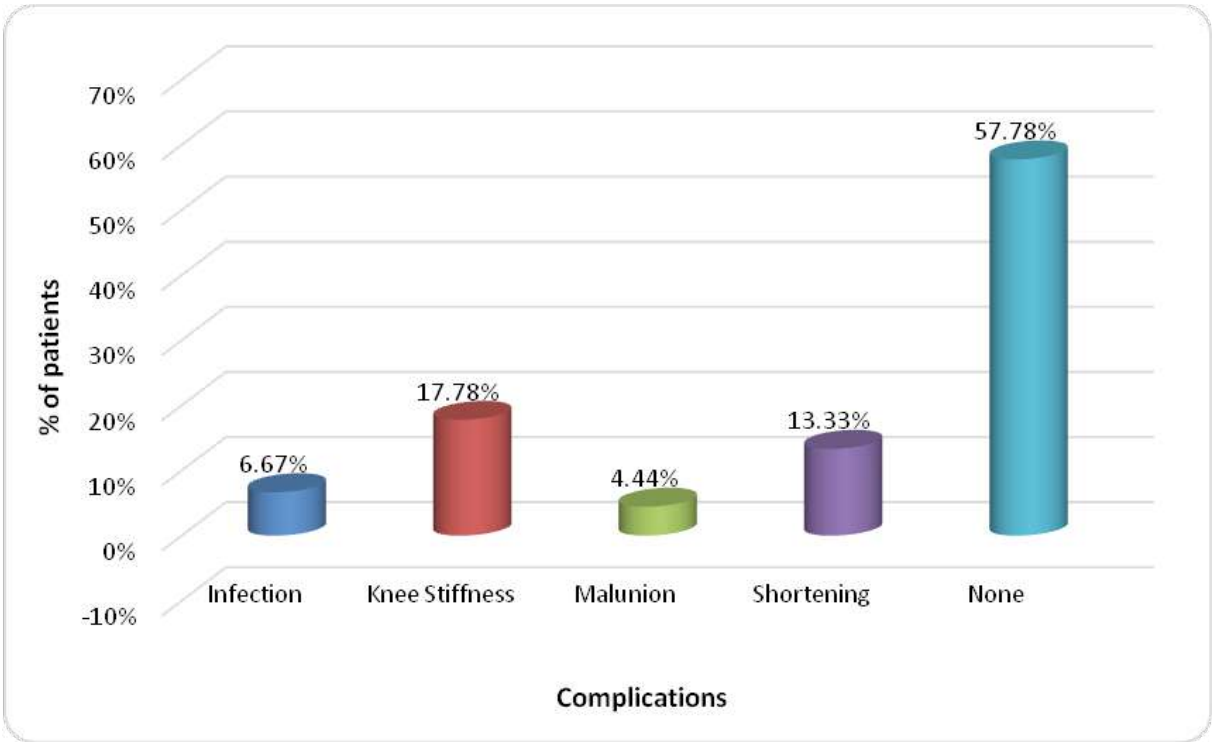
The demographical assessment of the study population was done. The mean age being 45.46±17.55 SD (standard deviation) years. 36 (80%) patients were male and 9 (20%) patient were female. In 21 patients, right side of femur was involved and 24 patients had left side involvement. Vehicular accident (71.11%) was the mode of injury in majority number of cases. Femur fracture were classified in accordance with AO classification of distal femur fracture with maximum being type 33 C3 (26%) followed by 33 A1(22%). 13 (28.8%) patients sustained an compound fracture and 32 (71.1%) cases sustained a simple supracondylar femur fracture.

39 (86.66%) patients were managed by surgical reduction and locking plate fixation and 6 (13.33%) patients were managed initially with application of external fixator, which was later converted to open reduction and plate osteosynthesis after wound management and infection control.

Table 2: Showing Neer's functional outcome scoring at follow-up period of 1 year.

Final Outcome	No. of patients	Percentage (%)
Excellent	7	15.56
Good	24	53.33
Fair	9	20
Poor	5	11.11
Total	45	100

68.89 % patients had excellent and good final outcome score, 20% had fair outcome and poor outcome was seen in 11.11 %. 19 (42.2%) patients had complications with knee stiffness (17.78%) being the most common, followed by limb length shortening (13.33%).



Graph 1: Showing distribution of complication in the study population.

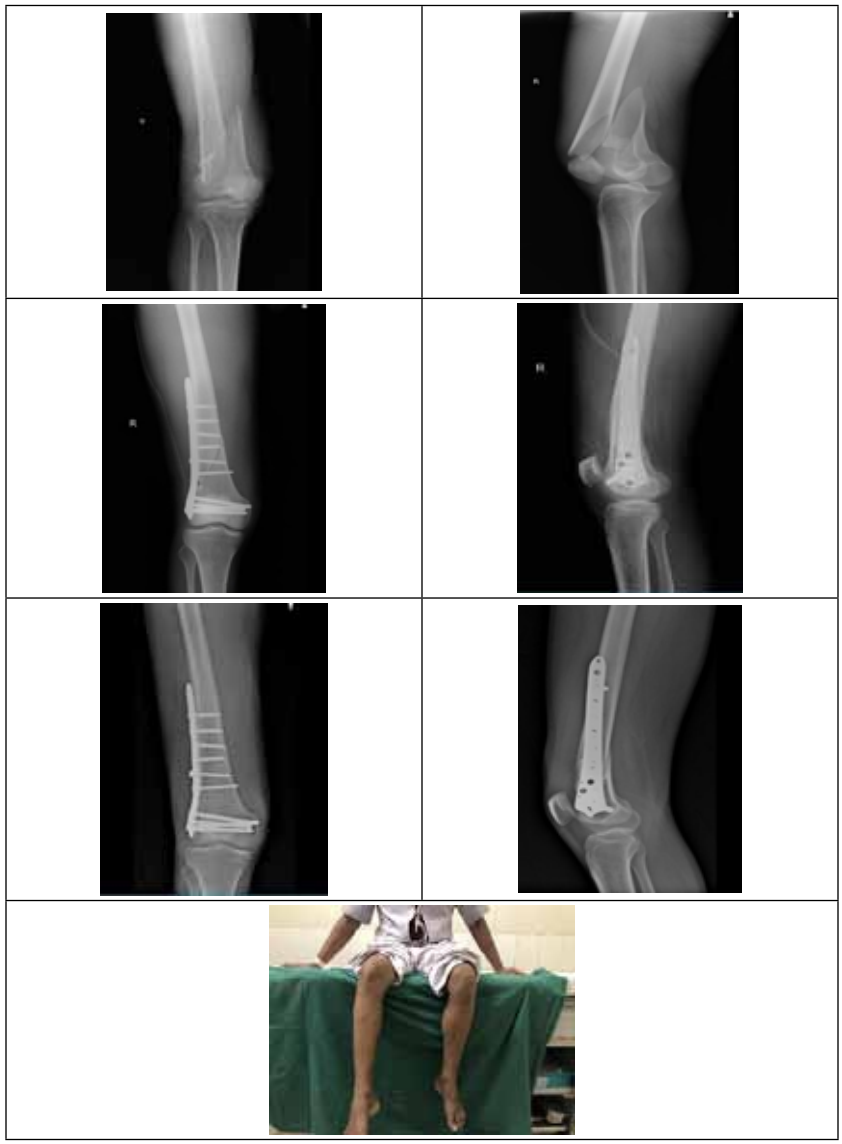
Patients with shortening were given shoe raise accordingly. All the fractures were united between 16-24 weeks with a mean duration of union being 18.93±2.15 SD weeks.

Table 3: Showing Progresion in Neer's score at sequential follow ups.

Neer's Score	Mean	N	Std. Deviation	P value
Preoperative score	22.84	45	10.61	-
Postoperative Score	37.55	45	9.64	0.0001
At 6 months	45.24	45	10.58	0.0001
At 1 year	71.13	45	12.95	0.0001

Neer's score was evaluated at sequential follow ups. with mean score before surgery being 22.84±10.61 SD which increased post-operatively to a score of 37.55 ± 9.64SD, and at 1 year follow up score was 71.13±12.95 SD. Thus, a statistically significant increase in the mean functional score was observed post operatively, at 6 months follow-up and at 1 year follow-up as shown in Table 3.

On comparison of age with outcome scores, it was found that there was no statistical significance between age and outcome scores. Gender and outcome scores were also correlated and it was observed that 88.89% females had good outcome scores and the result was statistically significant.



Closed fracture treated with plate osteosynthesis directly had significantly better outcome scores as compared to open fractures which were treated primarily with external fixator and eventually plate osteosynthesis (P value 0.024) and more number of complication were seen in patients of compound fractures as compared to closed fracture(p value 0.001) .

Type of fractures were correlated with time of union and it was observed that (87%) closed fractures were united in 16-20 weeks whereas 61% open fractures united in the same time and results were statistically significant.(P value : 0.03)

Clinical Photographs and Radiographs: 46 year old male with closed supracondylar femur fracture managed with surgical reduction and distal femur locking plate osteosynthesis.

Figure 2 showing Pre operative, post operative x rays and final follow up radiographs and clinical photographs showing post operative range of motion.

Discussion

This prospective study had a study population of 45 patients. We evaluated the clinical outcome of supracondylar fracture of femur surgically managed by open reduction and locking plate osteosynthesis. Supracondylar fractures of the femur are complex fracture that are difficult to treat and causes morbidity for long period of time. Thus, surgical management with open reduction and internal fixation became necessary. Surgical treatment of intraarticular fractures, especially in weight bearing joint, requires anatomic reduction and maintenance of the congruity of joint surfaces.[3]

The locking plate (LCP) system has advantages in fracture fixation as it provides angular stability through the use of locking screws. This angular allows extra periosteal fixation of the plate to bone.[3]

In our study, mean age was 45.46 years. Comparable Mean ages of different studies are shown in Table 4.

Table 4: Comparable Mean ages of different studies

Study	Mean age (years)
Our study	45.46
EJ Yeap et al.4	44
Srinath et al.5	47
Wong et al. 6	75
Schütz et al. 7	52

80% study population were males and 20% study population were females. Studies done by Tapi Nalo et al.[8], Srinath et al.[5] and Wong et al.[6] also showed male preponderance. In 46.67 % right femur of the patient was affected and in 53.33 % patients, left side involvement was observed. Sabarisree M et al.[1] stated that, bilaterally equal involvement was seen in his study and in study done by Srinath et al.[5], right femur was more commonly involved.

The distal femur fractures were categorised in accordance with AO classification and it was found that type 33 C 3 (26.67%) was most common, followed by 33 A1 (22.22%), 33 A2 (15.56%), 33 C2 (15.56%)33 C1 (08.89%). Srinath et al.[4] observed 33 C2 (45%) in maximum study population. Sabarisree M et al.[1] also observed 33 C3 (37.3%) to be a major type.. 86.66 % patients in this study were treated with locking plate, 13.33% were initially treated with external fixation which was later converted to plate osteosynthesis after wound management and infection control, whereas in study done by Rijal et al.[9], 47.8% patient were fixed with distal femur locking plate, 15.2% with closed reduction and CC screw fixation and 2.2% with externa fixation.

In this study, clinical outcome was evaluated using Neer's Scoring. In 68.89 % patients excellent to good outcome was seen, 20% had fair outcome and 11.11% had poor outcome. The findings of other studies were shown in Table 5.

Table 5: Showing outcome scores of different studies.

Study	Excellent %	Good %	Fair %	Poor %
Our Study	15.56	53.33	20.00	11.11
Sabarisree M et al.[1]	58.4	20.8	12.5	8.35
Tapi Nalo et al.[12]	83.33	13.33	3.33	-
EJ Yeap et al.[8]	36.36	36.36	18	9
Srinath et al.[9]	65	15	20	-

On correlation of age and outcome, 75% of young age patients had excellent and fair outcome whereas 25% of patients had poor outcome. In middle aged patients (31-50yrs), 85.72 % had excellent and fair outcome and 14.29% resulted in poor outcome. In geriatric age group (>50 yrs), none of the patients had poor outcome scores. The correlation of age and clinical outcome score was statistically insignificant. Sabarisree M et al.[1] studied that 11(91.7%) patients with age smaller than 40 years had excellent and good outcome and only 1(8.3%) patient resulted in poor outcome, similarly in age more than 40 years 8(66.7%) patients showed excellent or good outcome and 4(33.3%) resulted in poor outcome.

Correlation of gender and functional outcome was tabulated. It was seen that 63.88% males had excellent or good outcome and 88.89% females had good outcome and the result was statistically significant as number of fractures in females were less as compared to males. Sabarisree M et al.[1] witnessed, good outcome scores in males as compared to female population.

On correlating fracture type and clinical outcome of the patient, it was evaluated be a statistically significant correlation (p– 0.024) i.e. 30.77% of all open fractures resulted in poor outcome whereas 86.89% of closed fractures showed good outcome. Thus, closed fractures had better outcome scores as compared to open fractures.

On crosstabulation of modality of treatment with outcome, association was significant (p - 0.0001). 76.47 % patients managed fixation with locking plate had excellent and good outcomes, 83.33% patients managed initially with external fixation resulted in poor outcome scores.

Mean time of union of fracture was 18.93 weeks. Average time of union in comparison with other studies is shown in table 6.

Table 6: Average time of union in comparison with other studies

Study	Time of Union (weeks)
Our study	18.93
Sabarisree M et al.[1]	13.5
Tapi Nalo et al.[12]	14.47
EJ Yeap et al.[8]	18
Srinath et al.[9]	18
Wong et al.[10]	16.6

Complications: Knee stiffness was commonest complication (17.78%) which was followed by Infection (06.67%) and malunion (4.44%). Tapi Nalo et al.[12] observed infection in 2 % cases and Srinath et al.[9] in 10 % patients respectively.

2 (04.44%) patient had malunion and 6 (13.33%) patients had shortening on the affected limb. Shortening was managed with the use shoe raise while walking on the affected limb. Sabarisree M et al.[1] also encountered shortening in 2 patients which was managed with shoe raise.

Conclusion

Distal femoral locking plate can be considered as a suitable choice for achieving adequate fixation in distal femoral fractures as there was statistically significant progressive increase in Neer's outcome scores in majority cases.

To conclude, clinical outcome in supracondylar femur fractures was found to be dependent on type and complexity of fractures i.e. closed fractures resulted in excellent and good outcomes and were united in lesser time and with lesser complications as compared to open fractures. AO Type 33 C fractures had maximum poor outcome scores due to comminution of fragments. Complications like Infections, knee joint stiffness and shortening occurred commonly in compound fractures. Although study was not free of complications, but management with distal femur locking plate provides satisfactory functional outcome in supracondylar femur fractures.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Investigate the Effect of Role Play on the Skill among 2nd Basic B.Sc. Students if SRMMCON Regarding Selected Community Procedure

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Abstract

Background: Home visiting is a service offered to pregnant mothers, children from birth to eight years old and their families in their homes. Home visits were found to enhance parenting, school readiness and health. Review of Home Visiting's history indicates that home visitors' roles and responsibilities have changed with the passage of time. Today's emphasis on family rather than individual, and on family control rather than a directive style means home visitors need more expertise, experience and versatility than ever before.

Objectives: Short term- To enhance students learning experience by using role play.

Intermediates: To develop innovative teaching learning approaches.

Long Term:

1. Using innovation in the health care settings by future nursing educators.
2. Apply skills in Futuristic Research.

Material and Method: Presents study is descriptive study conducted among 80 Students of Basic B.Sc. Nursing 2nd year by using non probability convenient sampling technique. The Checklist OSCE and feedback form was selected as tool for data collection.

Result: The mean score of Role play group is 9.32 and Non role play group 6.27 the essential skills are increased with use of role play. The comparison between two groups shows that calculated value is more than table value tcal = 16.51 and t tab = 1.98 p=0.0001 p<0.05 hence it is significant.

The Major: The analysis of the data of the study samples gives an idea about the general information about effectiveness if using role play.

Conclusion: After the detail analysis of the study, following conclusions were drawn. They require new innovative teaching learning interactive method to enhance their knowledge and create more interest in learning.

Keywords: *Role play, home visiting, skill, community procedure.*

Introduction

The viewpoint of educating new nurses should not necessarily be based on the readiness to think. Students must have the courage to address circumstances in which human relationships are of great importance. At the same time, the capacity to discern the motives of other peoples plays an important role and is linked to the understanding of actual circumstances.

Nursing teachers need to redefine their teaching-learning dream. By encouraging them to research, argue and experiment with the challenges they will contribute to advancing nursing as a profession and discipline, if they emphasize the development of critical thinking and help students discover their analytic, critical, and reflective abilities. Role Play, developed as an experience-based form of learning, helps one to be more informed about the concerns posed above.

Home visiting is an evidence-based approach that is introduced in response to results from a need assessment that involves home visiting as a key service delivery approach that is delivered on a voluntary basis to pregnant women or children from birth to 5 years of age targeting participant outcomes in legislation that enhanced maternal and under five child health.

Nurse home visiting has been used for over 100 years as a care delivery method to improve family safety and well-being. Public health family home visiting is a voluntary maternal and child health intervention, ideally delivered prenatally and often beginning with a comprehensive public health nursing assessment. Trained home visitors provide health education and care giving support to families and connect them to community resources as needed. Families receive information on prenatal and infant care, child growth and development,effective parenting approaches, home safety, disease prevention and preventing exposure to environment hazards such as lead and second-hand smoke.

The aim is to help parents and their kids simultaneously. Evidence-based, voluntary home visit programs show potential benefits for kids, families, and the state.[1]

Role play is a common stable, active learning technique commonly used in higher education. There are several different forms of role play for example simulation for teaching debates and court cases. The other styles are metaphorical role playing, in which students represent a real object such as biological molecule, and presentations in which students comment on a task or procedure.[2]

In the humanities and social sciences, simulation role play is often used to bring historic events to life, creating mock business environments and sparking ethical debates. Metaphorical role-playing, in which students represent specific parts of a process and interact with each other, was particularly adopted in science to visualize abstract concepts such as respiration, photosynthesis and cell division.[3]

Home visits have been demonstrated to be effective in helping families, improving health status, achieving economic self-sufficiency, improving positive parenting, reducing child abuse, achieving goals such as child spacing, education and employment, and establishing links with community resources.

A home visit by a nurse is a family-nurse contact that enables the health worker to evaluate the home and family situations in order to provide the necessary nursing care and related health activities. In carrying out home visits, it is essential to prepare a visiting plan to meet the customer's needs and achieve the best outcomes desired.

Hypothesis:

H1: There is a significant increase in the skill regarding selected community procedure among Basic B.Sc. Nursing 2nd year students.

Objectives:

Short term: To enhance students learning experience by using role play.

Intermediates: To develop innovative teaching learning approaches.

Long term:

1. Using innovation in the health care settings by future nursing educators.
2. Apply skills in Futuristic Research.

Material and Method

The study involves nursing students of SRMM college of nursing, Sawangi (Meghe). The sample consists of 80 students. Non-Probability Convenient Sampling was the sampling technique used in this analysis. Objective structured clinical examination (OSCE) checklist and Feedback form were used as tool for data collection. Inclusion criteria included participants who gave consent, participated in the role play and present at the time of data collection. Spearmen Brown's Formula was used for reliability. Initially the investigator gave them Didactic lecture, and showed them demonstration. After the lecture, she provided them with checklist OSCE score and feedback form. The second

group was given didactic lecture and then role play was used for this group. After session OSCE checklist was administered on both the groups separately.

The Major Findings: The analysis of data of the study samples gives an idea about the general information about effectiveness if using role play. Study results were discussed with respect to research objective and study assumption.

Essential Skills Variables: Comparison of checklist OSCE score between role play and non-role play group

Group	N	Mean pretest score	Mean post score	Std. Deviation	Std. error Mean
Role play	40	3.11	9.32	0.58	0.093
Non role play	40		6.27	1.00	0.158

The table above shows a Mean RPG score of 9.3 and a Non RPG group of 6.27. So this means there is significant increase the essential skills of role play group.

Comparisons between two groups:

Students unpaired t test with both groups:

Calculated t value tcal	Df	p-value	Mean difference	Std. error difference
16.51	78	0.0001 S,p<0.05	3.02	0.18

The table above shows that the calculated value is more than the table value i.e. calculated t value, t cal – 16.51 and tabulated t value, t tab = 1.98 p=0.0001 i.e. p<0.05, hence it is significant.

Hence the hypothesis H1,that there will be a significant increase in the skill is accepted.

Implications: The study findings have implications in nursing practice, administration of nursing education as well as nursing research.

Practice in Nursing: Nurses can expand and extend their role in the primary health center and a direct care provider in nursing practice, thereby providing direct care. To prepare a nursing care plan, it is important that the person, primary health center and other health professional understand and implement this level of expertise. It would be beneficial for the community to do knowledge-based work.

Nursing Education: The nursing curriculum should focus on new teaching modalities, and both the teaching staff and nursing students will help improve their knowledge and practice in their area.

In Nursing Administration: The results of the study should be used by the nursing administrator in the creation of strategies and initiatives that would require all nursing staff to engage actively in health education programs in their respective centre.

Nursing Research: The study would contribute to new innovative practice, problems solving in different difficulties faced. Research study may be conducted to bring awareness, and formulation of strategies and standard care for a number of prevalent public health, communicable and non-communicable entities[4-8.]

Nursing research is essential aspect of nursing as it uplifts the profession, develop new norms, and enhance the body of nursing knowledge. It also enhances the image and perception of nursing in society which is urgently needed for the nursing future.

The findings of the research can be further used in other research studies.

Recommendations: Based on the study findings, it is recommended that you conduct the following studies:

1. To replicate with a control group and a larger sample size.
2. To see the improvement in skill of nurses in relation to practice of health survey.

3. Periodic follow-up of the same study participants will assess the impact of time on trainees' retention of adopted skills, positive attitudes and practice.

Conclusion

From the results of this analysis the conclusions were drawn. Following the study's thorough review the conclusions were drawn. They need new creative interactive teaching method to develop their awareness and build greater interest in learning. WILCOXON Rank Test was applied for the comparison of role play group and non-role play group (OSCE score). This work has shown that the use of role play has a beneficial impact on the understanding of students about improving their motivation for learning. As discussed in this paper, the lectures followed the process of implementing role play and received positive feedback from the students throughout the process. This paper stresses the need for an active participative method of learning. The challenge is to balance this simplicity with our needs, to cover complex materials, to cater for different learning styles, to be accessible to students, to work within our often limited role and even to be entertaining. Teaching learning using Role play is highly challenging, often frustrating, and extremely time consuming, but it can have a dramatic impact on student learning when it is done well.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Domestic Violence: A Silent Panendemic on the Rise During Covid-19

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Abstract

Staying at home is the best way to beat COVID -19. But nobody ever bothers to think about the victims who are stuck up in abusive relationships with their partners. Naturally, lockdown has forced them to suffer even more. Their sufferings have increased in numerous ways. The data around the globe also shows that domestic violence has been on the rise as the entire world was staying indoors. The National Commission for Women (NCW), which is the government body that receives complaints of domestic violence from all parts of India, has recorded more than twofold rise in gender-based violence during this lockdown. Domestic violence cases can go across social, racial as well as economic boundaries. Shattered dreams, bruised mentally and physically, these victims are either shy or afraid to raise their voices against domestic abuse. Attention and care is needed not only for the visible physical injuries, but also for the psychological and emotional hidden trauma. The foregoing article takes stock of domestic violence during COVID-19 lockdown.

Keywords: *COVID-19, silent pandemic, abusive relationship,domestic violence, lockdown.*

Introduction

'Home' is a place where you get comfort, warmth and safety, and an escape from the busy working schedule of the day and other environmental stressors, but this is not true for all. Some do not get the stability, love, respect, support and happiness which is expected from a place called'home'. A moment of peace is what any human being would want at home. But for victims of domestic violence, it is not so. Especially with COVID-19 crisis and subsequent lockdown, there has been a huge surge in cases of domestic violence in India as well as in other countries. According to the National Coalition Against Domestic Violence (NCADV), domestic violence is defined as the intentional physical assault, intimidation, battery, sexual assault, and/or use of other threatening behavior by one member of a household against another. Other forms of the abusive behavior include stalking, the use of threatening looks or gestures, attempts to control the reproductive health of an intimate partner and displays of psychological aggression such as putting down, humiliating, or isolating an intimate partner.[1] The Protection of Women from Domestic Violence Act, (2005), expands the definition of domestic violence to include any act (omission or commission) which harms, injures, or endangers the physical or mental well-being of a woman and includes physical, verbal, emotional, sexual or economic abuse.[2] Several other classifications of domestic violence include murder, rape, stalking, assault, battery, and assault with a dangerous weapon, and aggravated assault.[3]

With a sharp increase in domestic violence cases across the world, Phumzile Mlambo-Ngcuka, Executive Director of the UN Women, called this increase in domestic violence as a "shadow pandemic".As per the report in Economic Times the National Commission for Women received 587 complaints from March 23 to April 16, 2020, out of which 239 were related to domestic violence. As per the data given by NCW, 123 cases of domestic violence were received between February 27 and March 22, 2020. In the last few days, the commission received 239 more such complaints. NBC News had requested for data on domestic violence from law enforcement agencies across United States, from those who responded 18 departments confirmed that they had seen a rise in March 2020. Houston police received about 300 more domestic violence calls in

March than they did in February, a roughly 20 percent increase during the COVID-19 crisis.

An analysis by UN Women of the gendered impacts of COVID-19 in the Palestinian territories found an increase in gender-based violence, and warned that the pandemic will likely disproportionately affect women, exacerbate preexisting gender specific risks and vulnerabilities, and widen inequalities.[4] In Latin American countries such as Mexico and Brazil, a spike in calls to hot lines in the months of February, March 2020 also suggests an increase in domestic abuse. Arussi Undafrom Mexican feminist organization Brujas del Mar working for women in abuse cases, said initially many calls to the group had come from neighbors hearing fights in other houses. There has been a reported rise in cases of "digital violence" and women were looking simply to escape.[5]

Psychologist Josie Serrata, PhD, a research and evaluation consultant and co-owner of Prickly Pear Therapy and Training, has found in her research that stress and social isolation can raise the risk of domestic violence.[6] The figures, published by the UNFPA and its partners Avenir Health, Johns Hopkins University in the US and Victoria University in Australia, also predict a 20% increase in violence during an average of three-month lockdown in altogether 193 UN member states. Wendo Aszed, the founder of Dandelion Africa, which runs economic empowerment programmes in Rift valley of Kenya, said that from mid-March to mid-April, more than 40 cases of violence have been reported to her organization. That was more than the total number of cases it dealt with last year.[7]

Reports from China, the United Kingdom, the United States, and other countries suggest an increase in domestic violence cases since the beginning of COVID-19 outbreak. According to one report, the number of domestic violence cases reported to a police station in Jingzhou, Hubei Province city, tripled in February 2020, as compared to the same months the previous year.[8]

The reason behind this sudden surge is that lockdown has brought the couples in close contact 24/7, which was not the case earlier. Where already rifts were there, this forced home stay has increased the level of frustration in them. Sometimes violence is a way men assert their false notion of masculinity. Being stuck at home, they take out their anger, at the same time refusing to share responsibility of domestic chores. Women cannot go out and share their grief either. As per NCW most of the complaints are coming via email as people are scared of going to the police. *With alcohol being scarce at such times of lockdown, frustration could lead to increase in domestic violence.*[9]

During lockdown even children are under stress due to their daily lives being affected, no schools and colleges, no friends to meet, only being at home with the social media. They are forced to see domestic violence within the family, especially their mother or siblings being subjected to violence. Their psychological distress increases. Some of the children themselves are victims of violence. Such episodes harm their future psychological development and create fear and anxiety in their minds, also affecting their other aspects of life like studies, peer relationships, confidence and self-esteem. According to Gosselin, 2005, if as a child, men witnessed child abuse or battering behaviors, they were more likely to abuse their partners when in adult domestic relationships.

The biggest ever question in the circumstances is; "when the lockdown ends, would their lives return to normal?" The answer is "no". Domestic violence will not stop on its own. The victims will have to take action and come out of such situation. Then only the life of their children will improve.

Discussion

Domestic violence has a huge psychological impact on the victim. Other aspects are also affected like - physical, social, spiritual. The victim may have low self-esteem, guilt, hopelessness and may go into tension, anxiety, depression[10]. Seeing the abuser, the victim may suffer from panic attacks. Suicidal thoughts are common. But some victims bear this as they see no way out and some have futile hope that the days and the abuser may change. Due to prestige issues, they may not complain or take help. Some would rather hide behind a false smile than letting others know about it. As children may be affected due to a police complaint or divorce, many suffer at the hands of the abuser.Many a victims, women in particular, isolate themselves from friends and family to keep it a secret. Though mostly women are the sufferers, it can be the other way round also. Men can also be the victims of abuse. It is seen that some men may take to addictions due to the mental trauma. Thus the psychological and emotional wounds of domestic violence are totally devastating. They can haunt victims

for many years to come, thus cutting short their future dreams of a beautiful life.

Courts and government agencies are doing a commendable work, but only these efforts may not be enough. A recent report indicates that only about 38% of women in India own a mobile phone and so it might not be easy for a victim of domestic violence to use the available helplines. Those who own a mobile may be able to use it for personal safety.

Creating awareness in schools and colleges to raise voice against such injustice should be cultivated in the minds of future generation at a young age. A victim should not blame self or feel guilty. The victims are not wrong, the other person, the abuser is mentally ill, and needs treatment. The treatment can be from a psychologist/psychiatrist or a police or an NGO activist. Violence is never accepted in a society which bows their heads in front of Goddess Saraswati, Goddess Laxmi, and DurgaaMaa. There are also a number of helplines for domestic violence and free online counseling websites as available options. A number of NGOs are already providing support to victims of domestic violence, but due to the lockdown restrictions, it is vital that the government should take the lead. The task is huge; but the lives are precious.

Conclusion

Viruses may come and go, lockdowns may be there in future too. But the silent panendemic of domestic violence shouldn't accompany. Let us start taking action now. Recognize the signs of domestic violence. Look around you. Unexplained bruises, partner putting them down in front of other people, jealous partners, depressed or anxious women who prefer to isolate themselves from social life. Be alert! If you are aware of such victims, approach them in a safe place, not in their homes. Trick them or coax them to come out with you. Be tactful and honest to your motive of helping the victim. Take them to a safe environment to talk. Approach the subject of violence with concern. Be genuine, listen and be empathetic. Then suggest ways in which they can be helped. Let us all keep our eyes open to such cases around us, identify, refer or help them. Let us work to reduce the stigma attached to victims of such violence. Respect women. Let's practice the age old Sanskrit verse and live it ourselves, train our near and dear ones-

Yatranaryastupujyante, Ramantetatra Devata, Yatraitaastunapujyante, Sarvaastatrafalaahkriyaah.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Analysis of Nidan (Etiological Factors) of Metabolic Syndrome

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Abstract

Metabolic syndrome is a clustering of at least three of these-Abdominal (central) obesity, Elevated blood pressure, Elevated fasting plasma glucose, High serum triglycerides and Low high-density lipoprotein (HDL) levels. In recent years, there is an alarming increase in metabolic syndrome. The overall prevalence rate of metabolic syndrome is 33.5%. It is 24.9% in males and 42.3% in females. The terminology 'Metabolic syndrome' represents its relation with metabolism. It can be said that disequilibrium of metabolism causes metabolic syndrome. The word metabolism can be correlated with 'concept of Agni' described in Ayurveda because entire range of digestive and metabolic activities of the body take place with the help of the biological fire of the body called Agni. This study was conducted to explore the *nidan* (Etiological factors) of metabolic syndrome based on basic concepts of *Ayurveda* and it suggest to think about overcoming mental stress (as one of the prominent etiological factor) while managing Metabolic syndrome.

Keywords: *Achar, Agni, Ahar,Metabolic syndrome, Nidan and Vihar.*

Introduction

Metabolic syndrome, is a clustering of at least three of these -Abdominal (central) obesity, Elevated blood pressure, Elevated fasting plasma glucose, High serum triglycerides and Low high-density lipoprotein (HDL) levels. In recent years, there is an alarming increase in metabolic syndrome.[1] The overall prevalence rate of metabolic syndrome is 33.5%. It is 24.9% in males and 42.3% in females. The factors which increase the risk of metabolic syndrome are older age, female gender, general obesity, inadequate fruit intake, hypercholesterolemia, and middle–to-high socioeconomic status. The treatment of it is symptomatic because of unknown aetiology. It is generally accepted that the current food environment contributes to the development of metabolic syndrome.[2,3]

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There is no any resembling term in Ayurveda for metabolic syndrome. But there is a quote related to genesis of any disease is '*loZjksxssssfieansXukS*[4]. The terminology 'Metabolic syndrome' represents its relation with metabolism. It can be said that disequilibrium of metabolism causes metabolic syndrome. The word metabolism can be correlated with 'concept of Agni' described in Ayurveda because entire range of digestive and metabolic activities of the body take place with the help of the biological fire of the body called *Agni.* There are three types of *agni-Jatharagni*, *Bhutagni* and *Dhatvagni*. The *Jatharagni*is the most important *Agni* which digests all types of food and transforms them into *Rasa* and *Mala. Jatharagni* is the master *Agni* which controls and regulates other two types of *Agni* viz. *Bhutagni* and *Dhatvagni.* It is primarily situated in *Mahastrotas* (Gastro intestinal tract). *Acharya Charak*has described that *dirghayu, smruti, medha, arogya, tarunavaya, prabha, Varna, swara, aoudarya, deha-indriyabala* are all dependent on the status of *Agni.* When this *Agni* becomes abnormally weak, it leads to production of improperly processed by-products of digestion and metabolism. It is called as '*Aam*'. This

Aam acts as a toxic material. The presence of *Aamdosha* in the body produces many types of abnormalities in various *Strotas*as (Micro-channels) and pathogenesis of disease process may start[5].

Need of Study: Metabolic syndrome is major lifestyle disorder. Its prevalence has increased drastically over a past few decades. It has no radical cure. So it is an urgent need to discover its etiological factors so that it can be prevented and controlled by using treatment principle of Ayurveda i.e. '*Nidanparivarjan* '[6].

This study was designed to find out the etiological factors of metabolic syndrome based on *ahar*, *vihar* and *achar* described in Ayurveda.

Aim and Objectives:

Aim: Exploration of *Nidan* (Etiological factors) of Metabolic syndrome based on basic concepts of Ayurveda.

Objectives:

- To study the etiological factors related to *Ahar* (diet)
- To study the etiological factors related to *Vihar* (Daily activity).
- To study the etiological factors related to *Achar* (Intuitive behaviour).

Material and Method

Place of Study–OPD of kayachikitsa and peripheral OPDs attached to institute.

Study design-Cross-sectional.

Study type-Analytical case control study.

Sample size-Total 300 (each group 150).

(The sample size was calculated as per the prevalence of metabolic syndrome in India.)

Grouping-Two groups (case and control)

Inclusion Criteria:

1. Age group of 30-50 years irrespective of sex.
2. Known patients of metabolic syndrome for case group.
3. Healthy person from patient's family for control group.

Exclusion Criteria:

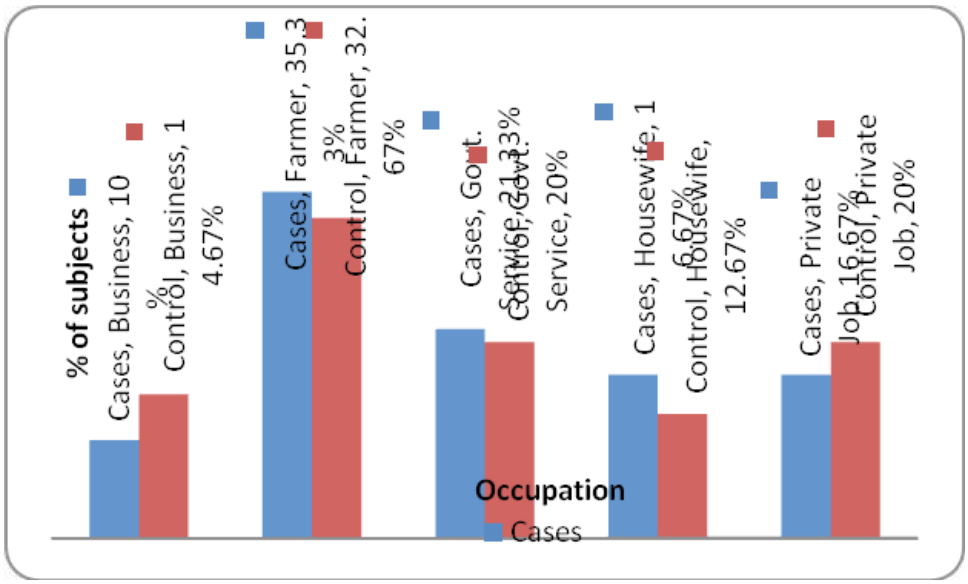
1. Bed ridden patients.
2. Subjects those who are unable to answer the questionnaire.

Selection of subject - Subjects were selected from above said study places by organizing special health check-up camps. Subjects were selected according to above mentioned criteria to case and control group. The approval from Institutional ethics committee was taken before started the study (IEC no. DMIMS(DU)/ IEC/2017-18/6393) (Annexure 1) before commencement of study, consent was taken from each subject. (Annexure 2). All the subjects were exposed to structured proforma prepared to note their *dinacharya*. Case proforma (Annexure 3) was designed in such a way that the factors related to *Ahar*, *Vihar*and*Achar* were covered.

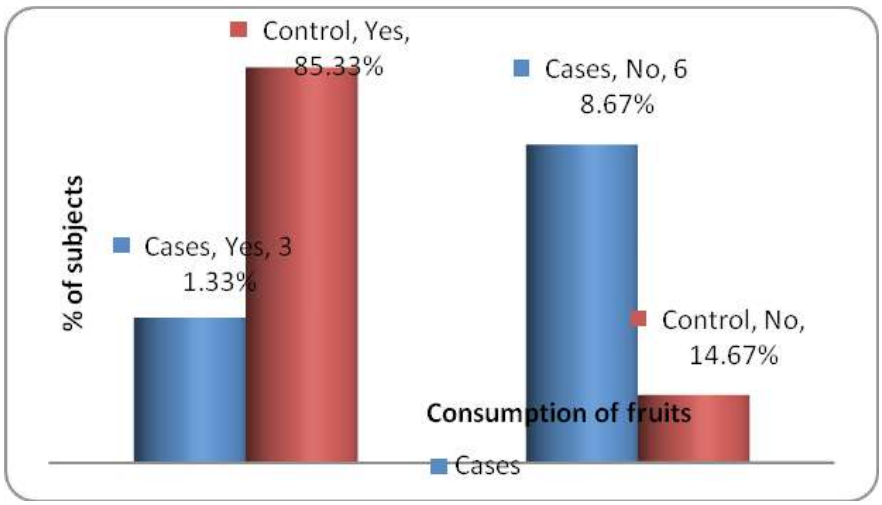
Observation and Results

This study entitled "*Analysis of nidan* (Etiological factors) of metabolic syndrome." was conducted in institute.

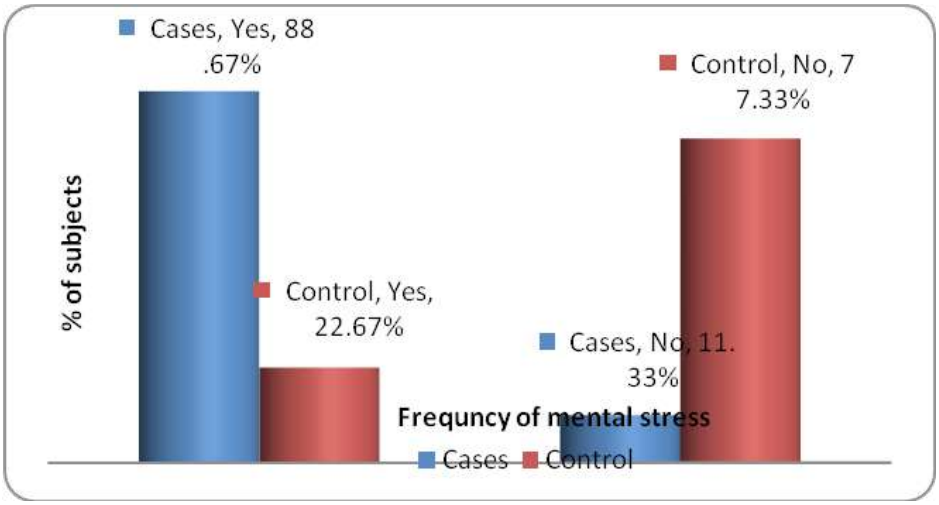
Total 300 volunteers were included in the study. The data obtained was coded and entered into Microsoft Excel Worksheet. Statistical analysis was done by using descriptive and inferential statistical using chi-square test and student's unpaired t test and software used in the analysis were SPSS 22.0 version and Graph Pad Prism 7.0 version and $p<0.05$ is considered as level of significance.



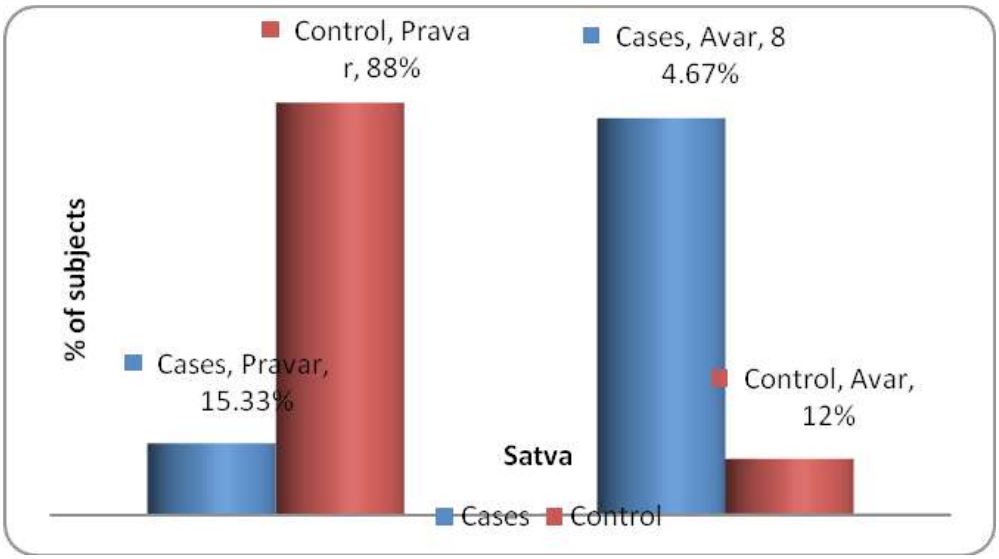
Graph 1: Distribution of subjects in both groups according to their occupation



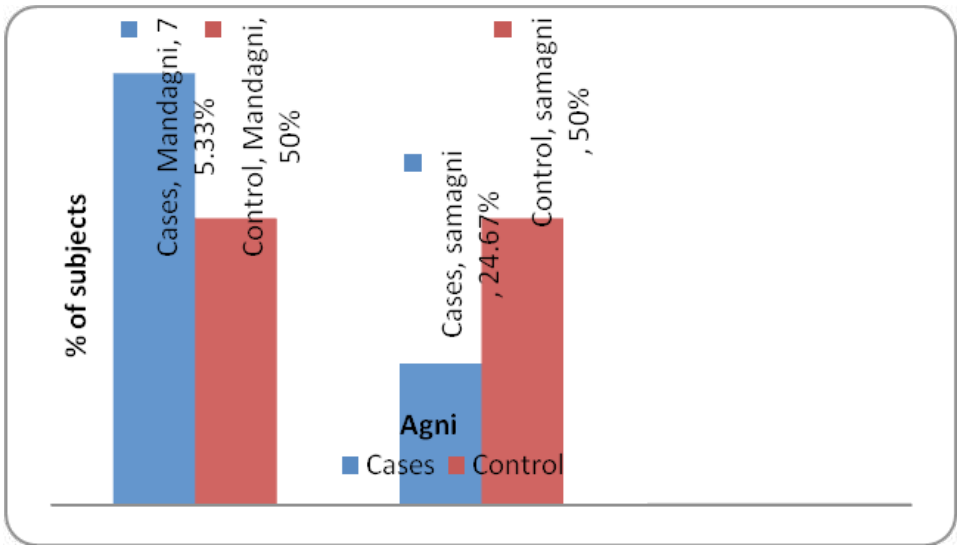
Graph 2: Distribution of subjects in both groups according to consumption of fruits (fruits consumption minimum thrice a week)



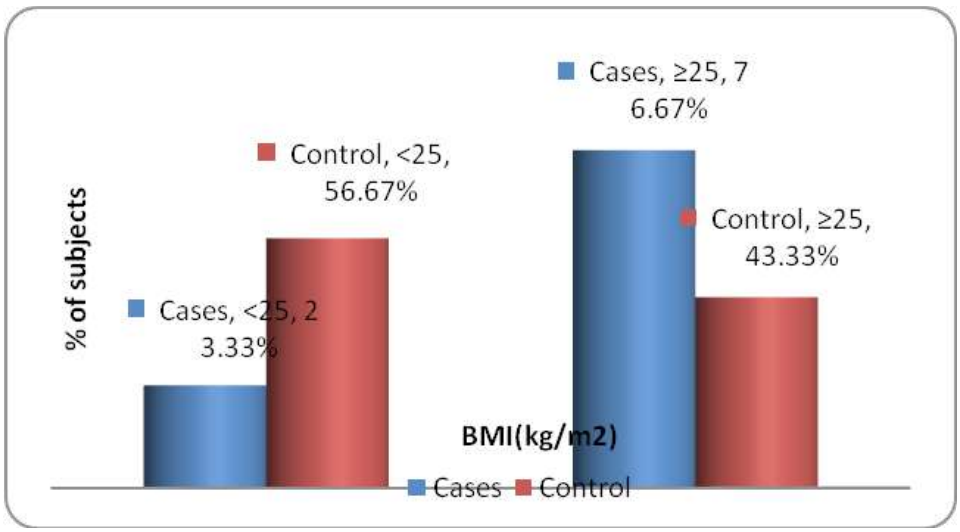
Graph 3: Distribution of subjects in both groups according to frequency of mental stress



Graph 4: Distribution of subjects in both groups according to type of *satva*



Graph 5: Distribution of subjects in both groups according to *Agni*



Graph 6: Distribution of subjects in both groups according to BMI

Discussion

In this study total 300 subjects were included. Out of that 150 were in case group and remaining were in control group. The patients of 30-50 years of age were included in this study because above 50 years of age, most of the components of metabolic syndrome are established due to age related changes. In our study, Most of the patients were in the age group of 41-50 years.

In the study conducted by D.S. Prasad et al. they also found maximum number of patients in above said age group.[7] This study supporting to the findings of our study so it can be said that the prevalence of metabolic syndrome is increasing in middle age also. Our study reveals no specific gender differences. It is supported by NHANES III data. According to their data, the prevalence was differed very little among men (24.0%) and women (23.4%)

However in many worldwide studies and the studies of Indian subcontinent, women had higher prevalence of metabolic syndrome.[8] According to Indian Diabetic Federation (IDF)-defined metabolic syndrome. The prevalence is 29.6% in men and in women it was 30.3%.[9]

It is observed that the patients having various occupations are suffering from metabolic syndrome but the proportion of farmers and government employee were comparatively more as this was a hospital based study and hospital comes under rural area so the proportion of farmers may be more. In detail case history it was found that, though they were farmers by profession their routine was sedentary. The government employees were also having sedentary life style.

In another study by Andrea Bankoski it was found that the proportion of sedentary time was strongly related to metabolic risk.[10]It is observed that most of the patients of metabolic syndrome were having irregular meal while healthy persons were having regularity in meal time.

The study by Justo Sierra-Johnson et.al showed meals regularity is inversely associated with metabolic syndrome.[11] These findings suggest that meal irregularly may be associated with metabolic syndrome. In another study by Wennberg M, it was found that irregular eating of meals at age 16 years is associated with higher prevalence of the metabolic syndrome at age 43.[12]

According to *ayurveda*, *aniyamitahar* (irregularity in meal) disturbs the *agni* leads to low metabolism which may be one of the factors of metabolic syndrome.[13] It is observed that in the group of metabolic syndrome, patients were consuming fewer amounts of fruits in comparison with control group.

In one study it is said that the Fruits are high source of vitamins and minerals along with low calories. Eating lots of fruits and green vegetables control weight and blood pressure. Fibre rich diet is the form of unrefined whole grain, water soluble dietary fibres can be incorporated into diet & result in significant lowering of cholesterol.[14]

It is observed that patient suffering from metabolic syndrome were used to sleep just after meal at day time .Sleep just after meal at day time is given as the one of the causative factor of *sthoulya* and *medadhatuvikruti*[15] In another study by Martica H. Hall, their data suggested that sleep duration has a significant correlation with metabolic syndrome.[16]

This study showed that those who had habit of frequent anger, frequent stress were suffering from metabolic syndrome. They were also not involved in any spiritual activity. The subjects who enjoyed their work are found more in control group and the subjects of *avarsatva* were significant in case group.

In the study by James A. Dunbar, metabolic syndrome was found to be associated with depression. Participants with the metabolic syndrome had higher scores for depression than individuals without the metabolic syndrome.[17]

In another study, it seems that prolonged exposure to work stress may affect the autonomic nervous system and neuro-endocrine activity directly, contributing to the development of metabolic syndrome. Cortisol is an insulin antagonist, and cortisol output is increased in the metabolic syndrome.[18]

According to *Ayurveda*, *satva* plays an important role in maintaining health.[19] In *Charak* and *Shusrutasamhita, krodha* (anger) is described as one of the etiological factors in *paittikaprameha* and *shoka* (grif), *bhaya* (fear), *udvega* (anxiety) and *chinta* (worry) are depicted as the causative factor for the *vatikaprameha*[20]

In this study, significant numbers of patients were having *mandagni*. In *Ashtang Hriday*, *Mandagni* is described as the basic factor for all kind of diseases.[21]. As there is direct correlation of *agni* with metabolism, any disturbance in *agni* causes metabolic dysfunction. In

another study by Sharma arjunkumar et.al. It was found that *agnimandya* is the causative factor of metabolic syndrome.[22] In this study, most of the patients of metabolic syndrome were having BMI more than 25.

The study of G Rodrı´guezet.al. Showed the similar findings of more BMI in patients of metabolic syndrome.[23] In one study it was found that effect of electronic media on diet, exercise, sedentary behavior, and sexual activities has mixed effects on lifestyle and disorders.[24]

Limitations: This study was hospital based so only limited population was covered.

Recommendations:

1. This study can be continued on large population to confirm the etiological factors.
2. Further study can be conducted to assess the effect of mental stress on *agni* and metabolism.
3. In our study, mental stress was found to be significant factor in Metabolic syndrome. So another study can be conducted to evaluate the effect of stress relieving measures on Metabolic syndrome.

Conclusion

This study was conducted to explore the *nidan* (Etiological factors) of metabolic syndrome based on basic concepts of *Ayurveda*. From the study observation, it can be concluded that

- In *Aharaj* factor, irregular timing of meal and stale food were prominently observed in patients of Metabolic syndrome
- In *Viharaj* aspect, sleeping habit (Day sleep just after meal) was significantly found.
- In the view of *Achar*, mental stress related to work, family, financial, personal and unfulfilled expectations were observed as high in metabolic syndrome.
- In comparison with all above factors, *manasnidan* i.e. mental stress (*Achar*) was found to be more significant than other factors.

So this study suggest to think about overcoming mental stress (as one of the prominent etiological factor) while managing Metabolic syndrome.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Review of Advance Directives as Per Mental Health Care Act 2017

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Abstract

The year 2017 is surely a milestone year for the professionals of mental health and the mentally ill patients. The year marks the historic moment of the Mental Health Care Act (MHCA). The utmost significance of this act cannot be ignored because it has come as a ray of hope because, for the first time, people with mental illness have been granted few rights, such as the right of the mentally ill person to make advance directives. Hence, it can be said that its arrival is an important accomplishment and a ray of hope for the millions of mentally ill people. It is an important critical step in addressing mental illness and focuses the public's attention on the current status of the mentally ill patients. The attempts to have its implementation would surely face several challenges. Understanding the values relevant to ADs is important for clinicians because they have a major role to act in coping with severe and chronic mentally ill person. The object of this article is to gather the brilliant flashes and the boring aspects as well as drawbacks of the act. To start recognizing the strengths and anticipating challenges in their implementation and the future solution.

Keywords: *Mentally ill patient, Mental Healthcare Act 2017, Advance Directives, Nominate representatives, Central authority.*

Introduction

Advance Directives (ADs) are the legal and the explicit rights of any adult person who has the mental capacity to decide while making ADs, regardless of the previous medical condition as set out in the Mental Health Care Act (MHCA) 2017.[1] In ADs, one can state in written form one's desires for care and/or deny and/or authorize/nominate a delegate (NR) who may take any kind of future treatment decisions on his behalf if he/she lacks the intellectual capacity to do so. Once the person can make the decision, it is worth remembering; then at any time, or if one wishes, one can override the ADs previously written. Following the definitive regulations of the Central Authority (MHCA, 2017), this can of course be withdrawn, changed or cancelled by the person who made this. The person must take care of existing laws during the formation or revocation of ADs, and follow the absolute regulations established and enforced by the central authority. The person writing ADs and his NR is responsible for making sure the treating organization and the practitioners have the right to access to it whenever it is possible. It is the

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obligatory responsibility of every in-charge medical officer/physician in charge of a person's treatment to provide medication upon appropriate ADs. If the person concerned has reason not to follow the ADs, whether he/she or the caregiver/NR; if so, the appropriate board can order it (MHCA, 2017). For example, while MHCA 2017[1] provides any insurer the option of offering medical insurance for mental illness treatment on the same basis asphysical illness coverage is provided. Within the same vein, in 2000, Anil Gumber found that "over 90% of the Indian population and nearly all the elderly are not covered by any kind of health insurance scheme of government or private." Their healthcare needs are met mainly by direct cash expenses spending on public and private sector facilities.[2] This also prevents individuals from an insufficient, unwanted or possibly harmful treatment; and helps prevent crises and the resulting use of involuntary medication or preventive measures such as isolation or seclusion. It also helps prevent untoward outcome by retaining greater control over their own mental health services; avoids compulsory medication and promotes person-centered care and shared decision-making.[3] Many a times the physical abuse has been used as a method to teach discipline in such mentally ill children in rural area for which there was a need for Mental Health Care act. [4] The use of Psychomotor and Affective Domains has been found to be an effective way of integrated teaching in such mentally ill persons. [5] The use of electronic media has been found to affect the psychological development of mentally ill children with some of them predisposing to substance abuse.

Criteria's for making or assessing ADs:

Three criteria should be considered for making ADs:

1. **Assessment of age:** Any adult, who is a major, has the right to make an advance directives.
2. **Assessment of mental status:** A person can offer an advance directive under subsection (1), regardless of his or her past mental illness or treatment as per MHCA, 2017[1]. Nonetheless, determining whether the person actually suffers from a mental condition that may involve an acute confusion, delirium, delusion or drug dependence condition is critical; as it may be insensitive or even incompetent to let someone go home with poor memory, impaired judgment, and little risk awareness.
3. **Assessment of Mental capacity:** A leading move to tackle the punitive nature of mental health legislation is to replace the test which clinically evaluates with the mental ability assessment (in) as a basis for incarceration and mandatory care.[7,8] A decision-making capacity assessment can be carried out, especially where the lack of capacity to decide on treatment/admission for care is concerned. When evaluating a person's skill, it should be borne in mind that any kind of active psychiatric impairment due to any kind of pathological problem impairs this capacity, and subjective evaluations of physicians 'abilities without scientific tools are often unreliable.[9] Chapter 3 of MHCA (2017), [1] i.e., "Advance Directive" consists of 9 Sections (5-13) are presented in the important points for the effective understanding and its applications as:-

I. Eligibility for making and executing the Advance Directives (ADs): In India "advance directive" stands for the advance directive of an individual in compliance with the section 5(1) of the MHCA 2017. The ADs are the form of legal and valid rights of any adult person who has the intellectual ability to make a written decision and thought at the time of making ADs. It is irrespective of one's any kind of previous mental illness, unless it is contrary to any existing legislation in force. In ADs, one can nominate its care wishes or deny any kind of treatment, and authorize/name a Nominated Representative (NR) who can execute any kind of future decisions; in the situation on one's behalf and where the one lacks the capacity to do so.

Simply put, ADs are the executionable powers of any adult who is major (18 years of age) and mentally capable of making decisions, whereby a number of desires may be expressed in writing, for its futuristic care, including care preferences and refusals, and the appointment of a person (s) capable of making treatment decisions on his or her behalf, in the event the person lacks the decision-making capacity. It should be further noted, however, that a person who is a minor is not capable of making ADs, but in compliance with Mental Health Care Act 2017 his "legal guardian shall have the right to make an advance directive in writing for a minor and all provisions relating to the advance directive shall extend to such minor until such time as he reaches a majority."

The United Kingdom Mental Capacity Act[10]grants British citizens an additional right that can be made in a written or in an oral form, with the exception of any conditional decisions to refuse or to deny any form of

life-sustaining care which ought be written, signed and had to be witnessed for confirmation.

II. **Invoking of ADs and its validity:** The MHCA 2017 states soundly that 'An advance directive made pursuant to subsection (1) shall only be enforced if the person stops to have the ability to take decisions on his/her mental health or any kind of treatment and remains valid until amount of time that the person regains the ability to make decisions on mental health care or treatment.

III. **To over-ride the ADs:** Under the MHCA 2017 if a person has the ability to make a decision, then it is possible, at any time, to bypass the previously written ADs or, if desired, ADs can, of course, be removed, altered or subdued by the patient by following the regulations established by the central authority. Consequently, any ADs which are actually in effect contrary to any statute shall be deemed unconstitutional from the outset. Simply put, it is to be presumed that having mental ability makes any written ADs ineffective beforehand. The person can change it if he/she wishes to change it, given he/she has the ability to do so.

IV. **Procedure to formulate an Advance Directive?:** The Mental Health Care Act, 2017 stipulates that 'Advance Directives shall be constructed in the manner defined by the Central Authority's regulations.' It should be noted that legal ADs in certain Western countries must adhere to a particular format involving witness identification and notarization;Mental Healthcare Rules 2017 state the rules for the creation of ADs as below: a) An AD shall be in written form in the format as CR-A; b) if a NR (Nominated Representative) is mentioned in the AD it shall sign the AD indicating its willingness to function as an NR; c) all forms of Ds shall be signed by two witnesses attesting to the fact that the AD was signed by the person producing the AD in their physical presence.; d) The person making an AD shall obligatory keep a copy of the executed AD with himself and will also share a written copy of the same with the NR.

V. **Maintaining the ADs with an E-register:** The MHCA 2017 stipulates that [clause (a) of sub-section 91] "each board shall maintain an electronic record of all the advance directives recorded with it and make them available to the mental health practitioners concerned as and where applicable." It is beyond doubt that a simple and secure access to medical data through the internet has many advantages; and it is accepted fact that our country will also benefit from those facilities as set out in the 2017 Mental Health Care Rules that ADs must be made display online within the time period of 14 days of registration with the board of importance. Such register will be maintain by the concerned in the dedicated software by the concerned board online.

VI. **Procedure to amend or cancel the ADs:** The MHCA2017 makes it a transparent fact that the person making it may revoke, change or cancel ADs at any point of time. The process for amending or canceling anykind of advance directives shall be as that for executing an advance directive under section 6 (i.e. the Central Authority's regulations on ADs). The Mental Capacity Act of the United Kingdom[10] states that this can be amended at any time when one has the ability to do so.

VII. **Circumstantial validity of ADs:** The MHCA 2017 states that the ADs is not extendable to a person who has made the ADs for any kind of an emergency treatment which is included under section 103 (e.g., Code of Criminal Procedure, 1973, etc., i.e., an offender is involved in any kind criminal court proceedings or under trial). However, the Mental Capacity Act, 2005 makes it transparent that there are some circumstances where, for example, if any kind of threat is present to themselves or othersor if one is placed under the UK Mental Health Act, this may be overridden.[10]

VIII. **Need of doctors to follow ADs:** According to the MHCA 2017 "It is the responsibility of each medical officer in charge of a mental health facility and of the psychiatrist in charge of the treatment of a person to prescribe or treat a person with a mental disorder in compliance with his or her lawful advance directive." [1] It should be remembered that mental health professionals are not responsible for non-observance of a valid advance directive until they have obtained a readable copy of the relevant advance directive.

IX. **Duties of the executioner of ADs and their NRs:** The 2017 MHCA[1] lays out the various obligations on the person who made the ADs as well as his or her NR. The person who wrote the ADs and their corresponding NRs will be absolutely responsible

for ensuring that the providers of mental health will have access to the ADs where applicable. Additionally, if any, the person concerned or the NR shall provide necessary information to the mental health professional of the new ADs. The Act also gave the authority to the legal guardian the right to make ADs in writing for a minor, and before achieving a majority all laws relating to such ADs shall be applied to the minor in question.

X. **The power to review and modify in any ADs making procedure:** According to the 2017 MHCA[1] the Central Authority periodically and yearly tracks the use of ADs. It also provides the prescription for the same and lay down the specifications the procedure for making and executing the ADs. It also addresses the legal procedure to safeguard the absolute rights of the people with psychiatric disorders. This may also adjust the ADs process or make additional regulations relevant to the ADs process.

XI. **Liability of health professionals to follow ADs:** Mental health employees and professionals must obey the ADs. If there are any sort of reasons not to do so, a report must be lodged with the board concerned. The MHCA 2017[1] makes it very transparent that no mental health worker and professional shall bemedicolegally held responsible for any adverse effects in case if one follows valid ADs; nor shall anyone be held liable for failure to follow valid and clear ADs, unless a desired copy of the same is given.

XII. **Are there any additional regulations, regarding the procedure of advance directive?:** Recently, MHCA 2017 refer to several other regulations relating to the ADs process, which can be stated as a) All forms of ADs are registered with the Mental Health Review Board, which has jurisdiction where the individual resides; b) for registering an AD no fees is paid with the Mental Health Review Board; c) all ADs are made available online within 14 days of registration; (d) The individual concerned or the NR shall brought to notice to the mental health professional about the new ADs if any after modification; (e) information contained in the AD shall not be revealed to any unauthorized person or media.

Conclusion

Bridging the gap between the demand for care and the treatment facilities has become a major challenging problem from generation for healthcare professionals and finding and addressing barriers to the efficient provision of the ethical practice is critical. The information on medical knowledge and the available care option, we will be in a stronger and effective position to take the systematic, coordinated and appropriate treatments that are in the best cause of the individual health. The recent acts on mental health from both within and outside our home country have acted as an absolute reminder of ADs need.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Management of Lumber Canal Stenosis Through Panchakarma: Case Study

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Abstract

Lumbar canal stenosis is a narrowing of spinal canal and compressing the nerves travelling through the lower back into the legs. The symptoms includes pain, weakness and tingling sensation or numbness of legs which radiate down to the feet along with psuedoclaudication, typically worsens with standing or walking and improves with sitting and is often related to posture and lumbar extension. It will hamper daily activity of patient along with quality of life. In modern science there is no appropriate treatment except surgical intervention which is having complication and also the chances of recurrence. *Ayurveda* can provide a suitable answer through appropriate *Panchakarma* modalities useful in Lumber Canal Stenosis. According to *Ayurveda* it can be related to *Katigata Vata* but it is not a separate disease entity. In Ayurvedic literature it is explained as one of the *Vatavyadhi.* The present case study will analyse effectiveness of *Panchkarma* in Lumber Canal Stenosis.

Keywords: *Lumber Canal Stenosis, Panchakarma, Katigata Vata, Vatavyadhi.*

Introduction

Lumbar canal stenosis is the medical condition in which the spinal canal narrows and compresses the nerves and blood vessels at the level of the vertebrae of lumber area. Lumbar spinal stenosis causes pain in the low back or buttocks, abnormal sensations and the numbness in the legs, thighs, feet or buttocks or loss of bladder and bowel control. The annual incidence of Lumber canal Stenosis is reported to be five cases per 100,000 individuals which is fourfold higher than the incidence of cervical canal stenosis.[1] A population-based study in Japan[2] found that the LSS incidence was increased by age, about 1.7–2.2% in 40–49 years old population and 10.3%–11.2% in 70–79 years old population. Acquired lumber canal stenosis occurs due to degenerative joint disease related with vertebra and spinal cord. A community based cross sectional study in Wardha it was observed that most (82%) of the workers, who performed the activities of brick making experienced musculoskeletal symptoms any time during last one year in shoulder (s) followed by lower back (71%)[3]. Therefore causes of lumber canal stenosis are occupational over use of joint, metabolic diseases (hyperparathyroidism, hemocromatosis and ochronosis). Lumber canal stenosis can also be caused by osteophytes, osteoporosis, tumour, trauma or various skeletal dysplasia, such as with pseudoachondroplasia and achondroplasia. The most common form of degenerative canal stenosis generally becomes symptomatic during or after the seventh decade of life[3]. It is more common in women than in men[4]. In a study population of 100 patients, Amundsen et al. found the occurrence of back pain and sciatica in 95% and claudication in 91% of the patients. Obesity is a major risk factor[5]. Conservative treatment of lumbar canal stenosis includes physiotherapy, anti-inflammatory medications, lumbar corsets and epidural injectables[6]. It is generally accepted that surgery is indicated if well-conducted conservative management fails[7].

So there is a need and scope of *Ayurveda Panchakarma* treatment which can improve the quality of life hampered due to associated pain and to limit the further degeneration of spine. The description about *Katigata Vata* is not a separate disease entity in any Ayurvedic text. It is mentioned as one of the *Vatavyadhi* by *Charaka*[8]. In case of *Katigata vata, dhatu kshaya* (direct stress induced factor at the *Kati* region and loss of nutritional support to the *asthis, sandhis* and surrounding *snayus* together lead to *vata prakopa* in *asthivaha srotasa.* This lead to the symptoms of numbness (*suptata*), stiffness (*stambha*) and restriction in movement (*gati avrodh*). *Ayurveda* can provide miraculous effect to such patient with the help of *panchakarma*. *Basti* procedure is main treatment used for the vitiated *vata*[9]. In the management of *'Asthi Prodoshaj Vyadhi' Charaka* stated '*Kshir Basti*'and '*Sneha Basti*[10].

Aims: Management of Lumber Canal Stenosis through *Panchakarma*: CASE STUDY

Objectives:

1. To observe the symptoms of lumbar stenosis in patient.
2. To prepare an Ayurvedic line of treatment according to samprapti of disease.
3. To analyze results.

Methodology

To fulfil the aims and objectives of the study this work has been carried out in the following phase wise manner.

1. Case study.
2. Discussion.
3. Conclusion and summary

Plan of Work: The clinical study was conducted in the I.P.D. of Datta Meghe Ayurveda College & Hospital, Nagpur.

1. Patient was suffering from the lumbar canal stenosis with reference to *Katigata Vata* admitted in I.P.D. of Datta Meghe Ayurveda Hospital.
2. *Panchakarma* treatment protocol was planned which includes *Sarvanga Patra pottali sweda*, *Sarvanga Shashtika pinda sweda, Katibasti*, two courses of *Kalabasti* for 33 days.
3. First course of *Kalabast*i has *Erendamooladi Niruha Basti* 450 ml and *Sahacharadi tailam Matra Basti* 60 ml.
4. Second course of *Kalabasti* has *Panchatikta ksheera Basti* 300 ml and *Sahacharadi tailam Matra Basti* 60 ml.
5. VAS (Visual analogue scale), An Owestry low back pain disability Index[11], 6MWT (6 min walking test), Pin prick test to assess the patient.

Case Study: A 55 yr old Female Indian, married consulted in Out-Patient Department of *Panchakarma* at Datta Meghe Ayurveda Hospital presenting with complaints pain in lumber region radiating to both lower limbs, Tingling sensation in both lower limbs below knee joint for 3 years. Patient was unable to walk or lie straight and was not able to perform his daily personal work, chappal slip from right foot. But from 15 days there is severity in symptoms. She was also suffering from constipation since long duration. The patient was diagnosed as Lumber canal stenosis in Goverment hospital Nagpur and advised surgery but patient was not willing to operate. So the patient was admitted in *Panchakarma* female ward of Datta meghe Ayurveda Hospital on 2nd September 2019.

On Examination:

1. General condition of patient was moderate and a febrile.
2. PULSE: 82/min
3. Blood pressure: 140/80 mm of hg
4. Pallor: present
5. Systemic examination:

CVS: S1 S2 Normal

CNS: Well oriented, conscious.

RS: AE = BE, clear.

P/A: Soft, non tender; Liver, Kidney,

Spleen-not palpable.

Ashta Vidha Parikshan[12]:

1. Nadi- Pitta Vata
2. Mala-Malavshtambha (occasionally)
3. Mutra – Samyak
4. Jivha- Sama
5. Shabd-Spashta.

6. Sparsha- Anushna Sheet
7. Druk-Panduta
8. Akriti- Madhyam.

Vikrut Strotas Parikshan:

Rasavaha Strotas: Ubhaya Pindiko Dveshtan (cramps in both legs), Panduta (pallor).

Asthivaha Strotas: Katishool (pain in lumbar region), Kati to Vama Pada Shool (radiating pain from lumbar region to left legs).

Majjavaha Strotas: Tingling and numbness in both upper and lower limb, difficulty in walking, slipping of chappal from right foot.

Present Illness: Patient was having above complaints from three year. She was consulted and admitted at Goverment hospital Nagpur for treatment and But there was no improvement in any symptoms during treatment and so they advised surgery. So that patient came to the Datta Meghe Ayurveda Hospital for further treatment.

Past History: No H/O- DM/HTN/IHD/PTB/BA/ Jaundice/Typhoid or any other major medical illness.

No H/O-Any major surgical illness.

No H/O- Accident

H/O- fall from 5 foot height before 5 years.

Clinical examination of spine:

Inspection: No scoliosis, no lordosis, no kyphosis were found in the patient. No any other major abnormality was seen in spinal examination of patient. No any swelling and surgical marks were seen in spinal examination of patient.

Gait: waddling gait.

Palpation and other clinical examinations

SLR - right + at 45°.

Left + at 50°.

VAS score –10.

6MWT- 50 m

Owestry low back pain disability Index- 45%

Investigation:

Haemoglobin %- 9.2gm %

Total Leucocyte Count- 8200 cu/mm

Differntial Leucocyte Count - Polymorph-55%, Lymphocyte-47%, Eosinophil+Monocyt-3%

Urine examination

Albumin-Nil

Sugar-Nil

Microscopic examination-NAD

RBS (random blood sugar) - 106 mg/dl.

MRI of Lumbar Spine: MRI on dated 2/12/2018 reported that Marginal osteophytes at multiple level. L3-L4 disk reveals diffuse posterior bulge compressing thecal sac without neural compression. L4-L5 dick reveals broad based posterocentral protrusion compressing thecal sac and bilateral L5 nerve root. Facetal arthropathy and ligamentum flavum thickening is noted at this level, contributing to central canal stenosis.

Panchakarma chikitsa[13]-for *Katigata vata* case Table No. 1

***Panchakarma* procedures**	**Method of preparation**	**Method of administration**	**Duration of treatment**
Sarvanga PPS	Leaves of *Erenda*, *Nirgundi*, *Amlika, Shigru, Karanja* etc *vatahara dravya* cooked with *methika* and *shatpushpa churna* and tied in cotton cloth in the form of *pottali*.	The *pottali* is dipped in warm *tila tailam* and applied all over body in 7 position of *abhyanga*.	14 days
Katibasti	Ring shaped cavity made at centre of low back by dough prepared by black gram flour.	Warm *tila tailam* and *sahacharadi tailam* in equal quantity is filled in the ring repeatedly upto 45 minute.	22 days

***Panchakarma* procedures**	**Method of preparation**	**Method of administration**	**Duration of treatment**
Sarvanga SPS	500 gm *shashtika* rice cooked in 1.5 liter *Balamoola kashaya* and 1.5 liter of milk and boiled till it becomes semisolid. Tie in cotton cloth in the form of *pottali*.	The pottali is dipped in warm milk *balamoola kashaya* mixture and applied all over body in 7 position of *abhyanga*.	14 days
Erendamooadi Niruha basti	*Erendamooladi kashaya* 300ml + *Shatapushpadi kalka* 30 gm + Sahacharadi tailam 50 ml + Honey 50 ml + Gomutra 30 ml + *Saindhava* 15 gm	Administred in left lateral position with *basti yantra* before meal.	Total 6 *basti* for first round of *kala basti* manner.
Panchatikta ksheera basti	*Guduchi, Nimba, Vasa, Patola, Kantakari ksheera kashaya* 200 ml + honey 40 ml + *Sahacharadi tailam* 60 ml	Administred in left lateral position with *basti yantra* before meal.	Total 6 *basti* for second round of *kala basti* manner.
Anuvasana Basti	60 ml of warm *Sahacharadi tailam*	Administred in left lateral position with *basti yantra* before meal	Total 20 *basti* for two round of *kala basti*

Table No.2 First round of Kala basti

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Type of Basti	A	A	EN	A	EN	A	EN	A	EN	A	EN	A	EN	A	A	A

A = *Anuvasana basti*, EN= *Erendamooladi Niruha basti*

Table No.3 Second round of Kala basti

Days	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Type of Basti	A	A	PN	A	PN	A	PN	A	PN	A	PN	A	PN	A	A	A

A = *Anuvasana basti*, PN= *Panchatikta kasheera basti*

Table No.4 BT and AT comparison of Lumber canal stenosis

Domain	**BT**	**AT**
VAS	10	4
Owestry disability Index	45%	20%
6MWT	50m	320m
Pin prick test for both lower limb	Numbness	Normal

Discussion

Lumbar canal stenosis is the most common condition associated with lumbar spine now days and causes changes in the locomotor system. It is highly prevalent in old age, mostly advised for surgical correction. Surgical treatment has excellent effects and remission in symptoms is seen in 64% of patients[14]. But mostly symptoms reappear after surgery and no complete cure so patients are seeking for alternative treatments such as *Ayurveda* can be useful. *Doshas* in this case found *kha vaigunya* at *kati Pradesh, sthana Samshraya* occurred in the *asthi, sandhi, snayus* of *kati* region. Hence, we have followed line of treatment of *katigata vata* for this case. *Ayurvedic* interventions relieved the symptoms by doing *samprampti bhang*. After *Panchakarma* treatment, the patient got its symptoms relieved.

Patra pinda swedana (PPS) made of *vatahara dravyas* and *sarvanga abhyanga* with *Tila tailm* which when applied locally increases the permeability of the skin. *Patra Pinda Sweda* is an unparalleled treatment in painful conditions caused predominantly by *Vata Dosha*, mainly in degenerative diseases. *Patra Pinda Sweda* is mainly performed to relief from pain, inflammation, swelling and stiffness associated with bone, joint and or musculoskeletal pains[15].

***Shashtika pinda sweda* (SPS)** made of *shashtika shali* is cooked with milk and decoction of *balamoola kwatha*. In SPS heat, massage and pressure causes nourishment of joints, tendons and stimulate nerve ending. *Shashtika pinda sweda* will help to improve circulation, nourishment to the body and it improves the strength of the tissues of bones and muscles[16].

Katibasti involves retention of warm medicated *tailam* over the lower part of spine for specific period. It reduces pain, numbness, strengthens the back muscles, reduces stiffness and increases blood circulation of the local region. It also have nourishment property for muscle of spinal column[17].

***Basti*:** *Pakwashya* is the *moolasthana* of *vata dosha. Basti* by its action on *moolasthana* get control on *vata* all over body. *Basti* is most widely used and highly effective treatment for spine and neurological disorder. *Anuvasana basti* has *Balya, Brimhana, Vatarogahara* property and also provides deeper nourishment to deep *dhatus*[18].

Conclusion

From the above case study we can say that *Ayurveda* can be opted as alternative treatment in lumber canal stenosis. The main *dosha* vitiated is vata. In this case *Katigata vata* line of treatment has to be considered. In *Ayurveda* point of view if proper *Panchakarma* protocol performed the *samprapti bhanga* can be achieved with significant relief from the disease symptoms. From this study we can suggest that *Panchakarma* therapy have effective role in lumber canal stenosis and can be done in large population.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Efficacy of Median Nerve Mobilization Versus Upper Trapezius Muscle Stretching on Extensibility of Median Nerve in Young Healthy Individuals: A Randomized Controlled Trial

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Abstract

Objective: The purpose of this study was to compare the effect of upper trapezius muscle stretching and median nerve mobilization on median nerve extensibility.

Design: A randomized controlled trial. **Participants**: 80 healthy participants with mean age range () were randomized to two groups who received trapezius muscle stretching & median nerve mobilization.

Outcome Measures: Right and left Elbow Extension Deficit Angle (EEDA) in degrees.

Intervention: Passive stretching of upper trapezius was done 4 times per week, 3 repetitions, 30 sec hold bilaterally. Median nerve mobilization was done 4 times per week, 3 sets of 10 cycles each; 30 sec rest in between bilaterally. Supervised self stretching and nerve mobilization was done 3 times per week, with number of hold and repetitions being the same.

Results: Baseline characteristics including demographic, anthropomorphic and EEDA between groups were comparable. Intragroup comparison of right and left Elbow Extension Deficit Angle (EEDA) were done using Repeated Measure Multivariate ANOVA. Inter group comparison was done using post hoc test Bonferroni. The level of significance was set at $P < 0.05$.

Conclusion: The study concludes that Upper trapezius muscle stretching is effective in improving median nerve extensibility. Both treatment techniques are equally effective.

Keywords: *Median Nerve Mobilization, Trapezius Muscle Stretching, musculoskeletal dysfunction.*

Introduction

Pain is central to the practice of physiotherapy and is the most common symptom for which patients seek health care and is most common cause of physical dysfunction. The musculoskeletal system is composed of 3 subsystems i.e. muscular, articular and neural systems. Janda has asserted that it is impossible to have damage or change in any one system without its being reflected in each of the other 2 systems[1]. The examination of normal compliance to movement should not only involve treatment of muscles and joints but also abnormal responses to mechanical provocation of neural tissue. The physical examination of patients with upper quadrant musculoskeletal dysfunction includes testing for normal movement and extensibility of mobile tissues of articular, muscle and nervous system. Many of these structures have close anatomical relationships and a test for one structure will definitely move adjacent structure or stress them. For e.g, the anatomical locations and orientation of upper trapezius muscle and nerves of brachial plexus. The upper trapezius from its origin at

occiput, ligamentum nuchae and seventh cervical spinous process has vertical and more oblique to transverse fiber orientation to its insertion into lateral one third of clavicle, spine of scapula and acromion. The trunks of brachial plexus follow an oblique to transverse course from their origin at C4 to T2 spinal nerves in their path through thoracic inlet region to their emergence from the axilla as peripheral nerves of upper limb[2]. The tests for the movement and length of upper trapezius muscle and neural tissues of upper quadrant share common components of shoulder girdle depression and cervical contralateral lateral flexion. Those with decreased neural extensibility had significantly less measured length of upper trapezius muscle[3].

Need for the Study: The upper trapezius muscle length and upper quadrant neural extensibility is interdependent is proved. But very little literature is available to evaluate whether upper trapezius muscle stretching will influence median nerve extensibility. The asymptomatic individuals were selected for the study as it eliminates commonly encountered confounding variables associated with clinical conditions including concomitant physical therapy interventions (e.g., exercise or modalities) and pain medications. Hence, the need for the study arises.

Aims: To compare the effect of upper trapezius muscle stretching and median nerve mobilization on median nerve extensibility.

Objectives: To assess elbow extension deficit angle (Elbow Extension Deficit Angle)

a. After giving upper trapezius muscle stretching bilaterally.

b. After giving median nerve mobilization bilaterally.

Materials and Method

1. A plinth
2. A universal goniometer
3. A wedge
4. Measuring tape
5. Pressure biofeedback
6. Chair
7. Mirror
8. Stop watch
9. Marker

Study Design: It is a randomized controlled, single rater blinded trial.

Place of Study: Physiotherapy Department of Tertiary care hospital

Sample Size: 80 young healthy individuals (40 in each group).

Inclusion Criteria:

1. Normal young individuals, 15–24 years, 32 having

a. Elbow Extension Deficit Angle [ELBOW EXTENSION DEFICIT ANGLE] in Median Neurodynamic test [MEDIAN NERVE TENSION TEST 1] (600 – 00).10

b. Cervical lateral flexion of 200 – 400 indicating reduced trapezius flexibility.

Exclusion Criteria:

1. Pain and deformity of upper limb, cervical spine or similar history in past.
2. Neurological or vascular pathology in upper limb, cervical spine.
3. Spinal surgery, joint surgery in upper limb.
4. Individuals performing regular stretching exercise

Subjects were recruited from physiotherapy department according to inclusion and exclusion criteria. The purpose and procedure of the study was explained to subjects. An informed consent form was signed by them.

80 subjects were randomly divided into 2 groups, 40 in each, using computer generated randomization chart.

Group 1: Upper trapezius muscle stretching group

They received upper trapezius muscle stretching bilaterally for 1 week.

Group 2: Median Nerve Mobilization group

They received median nerve mobilization bilaterally for 1 week.

Assessment:

Elbow Extension Deficit Angle (Elbow Extension Deficit Angle)[4]: Intra rater and inter rater reliability for Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE) assessment was done using ICC on

30 subjects with 10 minutes break in between. The intra rater reliability was 0.895 with 95% confidence interval ranging from 0.833 to 0.941. The inter rater reliability was 0.883 with 95% confidence interval ranging from 0.813 to 0.935

Treatment:

- Passive- Static stretching of upper trapezius muscle
- Supervised self -stretching of upper trapezius muscle
- Median nerve mobilization by therapist
- Supervised self-mobilization of median nerve

Results

Data was analyzed using SPSS software version number 16. Normality for all the parameters was tested using Kolmogorov- Smirnov test. One way ANOVA was used for the comparison of age distribution in all 3 groups and Pearson chi square test for sex distribution. Intragroup comparison of right and left Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE) were done. If normally distributed, Repeated Measure Multivariate ANOVA was used and if not normally distributed Friedman's test was used.

In the presence of statistical significance further post hoc analysis was done. For intergroup comparison, if normally distributed then one way ANOVA was used and if statistically significant then post hoc test Bonferroni was applied and if not normally distributed Kruskall Wallis test was used and if statistically significant then post hoc test Dunn's Multiple Comparison was done. The level of significance was set at $P < 0.05$.

Table 1: Intra group comparison of right Elbow Extension Deficit Angle

Right Elbow Extension Deficit Angle	Day 1 pre Median (range)	Day 1 post Median (range)	Day 7 post Median (range)	P value	Significant
Group 1	35(9,60)	30(0,60)	22(0,52)	<0.001	Significant
Group 2	35(5,52)	24(0,44)	15(0,40)	<0.001	Significant

At Repeated Measures Multivariate ANOVA Group 1 and 2 showed significant improvement in right Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE) post treatment.

Table 2:

Post hoc test Right Elbow Extension Deficit Angle	Day 1 pre Median (range)	Day 1 post Median (range)	P value	Significant
Group 1	35(9,60)	30(0,60)	<0.001	Significant
Group 2	35(5,52)	24(0,44)	<0.001	Significant

Table 3:

Right Elbow Extension Deficit Angle	Day 1 pre Median (range)	Day 7 post Median (range)	P value	Significant
Group 1	35(9,60)	22(0,52)	<0.001	Significant
Group 2	35(5,52)	15(0,40)	<0.001	Significant
Right Elbow Extension Deficit Angle	**Day 1 post Median (range)**	**Day 7 post Median (range)**	**P value**	**Significant**
Group 1	30(0,60)	22(0,52)	<0.001	Significant
Group 2	24(0,44)	15(0,40)	<0.001	Significant

Both Group 1 and 2 showed significant improvement in right Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE) after single session of upper trapezius muscle stretching and median nerve mobilization.

Also group 2 and 3 showed further significant improvement in right Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE) when treatment was continued for a week.

Table 4:Inter group comparison of right Elbow Extension Deficit Angle

Right Elbow Extension Deficit Angle	Group 1 Median (range)	Group 2 Median (range)	P value	Significant
Day 1 post- Day 1 pre	-6.5(-30,0)	-6.5(-25,3)	<0.001	Significant
Day 7 post- Day 1 pre	-12.5(-40,0)	-18(-37,-2)	<0.001	Significant

One way ANOVA: Both group 1 and 2 showed significant improvement in right Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE)

Table 5:Inter group comparison of left Elbow Extension Deficit Angle

Left Elbow Extension Deficit Angle	Group 1 Median (range)	Group 2 Median (range)	P value	Significant
Day 1 post- Day 1 pre#	-5(-25,5)	-7.5(-23,3)	<0.001	Significant
Day 7 post- Day 1 pre#	-15(-38,1)	-16(-29,38)	<0.001	Significant

Kruskall Wallis test

Group 1 and 2 showed significant improvement in left Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE) after single session of upper trapezius muscle stretching and median nerve mobilization.

After 1 week of treatment of upper trapezius muscle stretching and median nerve mobilization, both group 1 and 2 showed significant improvement, but there was no significant difference between group 1and 2.

Discussion

The results obtained showed statistical significant improvement in elbow extension deficit angle bilaterally in group 1 and 2.

Upper trapezius muscle stretching improved median nerve extensibility in group 2 by following mechanism: During movement, the musculoskeletal system exerts non uniform stresses and movement in neural tissues, depending on local anatomical and mechanical characteristics and pattern of body movement. This leads to mechanical and physiological responses in neural tissues. These responses include neural sliding, elongation, tension and changes in intaneural micro circulation, axonal transport and impulse traffic[5].

The neurodynamics during scapular depression and contralateral lateral flexion of neck are as follows: Scapular depression increases tension in the peripheral nerves of the upper limb by producing an increase in the distance between the neck and arm. The cervical nerve roots move distally with this movement. Proximal sliding of brachial plexus relative to the glenohumeral joint is likely to occur. From the sonographic recordings on the movements of the median nerve in forearm and wrist in vivo, scapular depression moves the nerve proximally[6].

Median nerve mobilization improved neural extensibility in group 2 by following mechanism: All the 80 subjects showed covert response either in terms of asymmetry in Elbow Extension Deficit Angle (ELBOW EXTENSION DEFICIT ANGLE) or site and quality of symptoms bilaterally. Covert response is differentiated to be neural. It is a comparable sign worth treating[7].

C type nerve fibers i.e. substance P and calcitonin gene related peptide exist in the epineurium of peripheral nerves and act as nociceptors in several forms. Strong stretch of connective tissues around the nerve roots activates sensory fibers in related dorsal nerve root. Hence, nociception and stretch through application of forces may arise directly from the nervous system[8].

The nerve distal to adhesion has to compensate for the loss of stretching ability. This can place tension on the nerve by the combination of movements performed during MEDIAN NERVE TENSION TEST 1. As the

connective tissues of the nervous system are innervated, movement of neural tissues against adhesions may generate a pain response in the nervous tissue. Two ended slider consisted of elbow and wrist movements in standard[9] MEDIAN NERVE TENSION TEST 1.

Neurobiomechanics during above movements are as follows: Glenohumeral abduction causes increased tension in the brachial plexus and the more distal peripheral nerves of the upper limb. The C5 nerve root slides in a distal direction in the intervertebral foramen. The median nerve at the level of shoulder joint moves as much as 1 cm. Convergence of neural structures occur towards the shoulder joint. The median nerve at wrist slides 8 – 9 mm proximally with abduction[11].

Elbow extension increases the length of the bed of median nerve by 20% which result in an increase in length of the median nerve by 4-5%. The principle of convergence i.e. the neural structures slide towards the joint being moved occurs. Supination produces a small amount of effect. However when it occurs as a part of MEDIAN NERVE TENSION TEST 1 it produces significant effect on nervous system as it is already under tension[12].

Wrist flexion decreases the tension in median nerve and wrist extension increases the tension. The total change in strain in median nerve at the elbow between wrist flexion and extension is 14.8%. At the wrist the total change in strain of the nerve reaches 9.6%. The total longitudinal sliding of the nerve at the elbow between flexion and extension is 5.6mm and at wrist it can reach 19.6mm. Transverse motion of the median nerve at the wrist occurs and has been measured between 1.5 mm and 3 mm[13].

With shoulder in 90 degree abduction, wrist extension combined with elbow flexion cause an increase in tension in distal part of median nerve. The nervous system must undergo mechanical events such as elongation, sliding, cross sectional change, angulation and compression. If these dynamic protective mechanisms fail, the nervous system is vulnerable to neural edema, ischemia, fibrosis and hypoxia which may cause altered neurodynamics.

When neural mobilization is used for treatment of adverse neurodynamics, the objective is to restore dynamic balance between the relative movement of neural tissues and surrounding mechanical interfaces. The nerve trunk slides longitudinally in its bed when the limb is moved. When longitudinal movement of a peripheral nerve is restricted, continual trauma results from the normal movements of the limb[14].

Conclusion

Upper trapezius muscle stretching is effective in improving median nerve extensibility. Median nerve mobilization is effective in improving median nerve extensibility. Both treatment techniques are equally effective.

Clinical Implication: Normal length of upper trapezius muscle will help in maintaining median nerve extensibility.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Simultaneous Ipsilateral Distal Interphalangeal and Metacarpophalangeal Dislocation of the Fifth Finger: A Rare Case Report

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Abstract

There has been no case of simultaneous ipsilateral distal interphalangeal and metacarpophalangeal dislocation of little finger in the literature. We report a case of A 61 years old male patient with traumatic ipsilateral dislocation of the Dorsal Interphalangeal (DIP) joint with dislocation of the metacarpophalangeal (MP) joint of the little finger. Both the dislocations were reduced by traction and closed reduction.

Keywords: *Metacarpophalangeal Dislocation, Dorsal Interphalangeal Dislocation, Rare Simultaneous dislocation.*

Introduction

This report was aimed to discuss a rare injury that is simultaneous ipsilateral dislocation of metacarpo-phalangeal joint (MP) and Distal Interphalangeal joint (DIP) of little finger. Although simultaneous dislocation of MP joint and Carpo-Metacarpal joint of same digit and MP joint and Proximal Interphalangeal joint dislocation of same digit has been reported in previous studies [1,2]. But, simultaneous dislocation of MP and DIP joint in the same finger was not found in the Medical Literature.

Case Details: A 25 years old, female patient reported to OPD with history of fall on hand while walking on the road. Patient reported with complains of pain and swelling over the little finger of left hand and was unable to flex the finger and make a fist with left hand. On local examination, the swelling and tenderness was at MP joint and DIP joint of the 5th Finger. Deformity was present in littlr finger. Sensory and Neurovascular status of little finger was intact.



Figure 1



Figure 2

Figure 1 and 2 showing swelling and deformity of the little finger. Figure 2 showing the patient is unable to flex little finger.

Patient was advised Anteroposterior and Oblique Radiographs of hand (Figure 3 and 4) which depicted Dislocation of Metacarpophalangeal joint and Dorsal interphalangeal joint of little finger of left hand with no evidence of fracture.

Figure 3 showing AP view of Hand	**Figure 4 showing Lateral view of Hand**
	
AP and Oblique Radiographs of hand shows Dislocation of Metacarpophalangeal joint and Dorsal interphalangeal joint of little finger of left hand with no evidence of fracture	

Closed reduction of MP joint was achieved first by giving gentle traction to proximal phalanx under ring block anaesthesia with 2% xylocaine. Thereafter, closed reduction of DIP joint was achieved by gentle traction. The clinical confirmation of reduction was suggested by correction of deformity of the little finger and patient was able to flex both MP and DIP joints. To verify the reduction radiologically, AP and Oblique radiographs of hand were done which showed reduced MP and DIP joint of little finger of left hand. (Figure 5,6).

Figure 5	Figure 6



X rays shows reduced Metacarpophalangeal and Distal Interphalangeal Joints of Little Finger of left hand.

Discussion

No case of simultaneous dislocation of an ipsilateral distal interphalangeal and metacarpophalangeal joint of the fifth finger was found in literature. However, a case of simultaneous dislocation of Proximal interphalangeal joint (PIP) and Metacarpophalangeal Joint (MP) of little finger was reported in which PIP joint was reduced by closed reduction and open reduction was needed to reduce MP joint. Another case of ipsilateral dislocation MP joint and Carpometacarpal joint was also reported in literature[1,2] in which carpo-metacarpal joint required closed reduction and fixation with K wire and MP joint required surgery using Volar approach. Metacarpophalangeal dislocations which are irreducible by closed reduction are rare injuries due to buttonholing of the metacarpal[2,3,4]. It is the volar plate which causes hinderance in reduction. However, in our case, the fracture was easily reduced by traction and reduction only and no such difficulties were encountered. The patient regained all the movements of the finger by 6 weeks.

Conclusion

We believe that result and functional outcome of this case was satisfactory. Although, it was a surprise that no such case has been reported in orthopaedic literature till date.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Current Treatment Approaches for Pandemic Corona Virus Disease 2019 (Covid 19)

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Abstract

Coronavirus disease 2019 (COVID-19) is a respiratory disease, spread through personal contact or contact of person with the surface containing virus. First patient of COVID-19 was found in Wuhan, China which is now been spreading all over the world.Coronaviruses belongs to the Coronaviridaefamily. Genomic characterization has shown that probably bats and rodents are the gene sources of Coronaviridaefamily. Today, more than 10 drugs are being undergonein clinical trials for the treatment of COVID-19. The present review article includes the causes of COVID-19 spread, symptoms of infections and their complications involved, current treatment strategies which various countries have been applying including plasma therapy, treatment using combination of antiviral drugs. It also comprises of ongoing clinical trials of vaccines on COVID 19 patients. Overall, the present article gives the global current scenario of the COVID 19.

Keywords: *COVID-19, SARS-CoV, CORONA.*

Introduction

Today, more than 18,53,168 people are infected with pandemic COVID 19. Among all 200 countries, United States of America leading at the top position with more than 13 million corona positive cases. COVID-19 is a respiratory infectious illness that can spread from person to person. Corona virus Infected person shows mild to moderate respiratory illness which can be treated without any special strategic treatment. Older person and those who are having already medical complications like cardiovascular disease, diabetes, chronic respiratory disease, kidney problem and cancer are more vulnerable to get serious development of illness. The best way to prevent and stop the transmission of coronavirus infection are to maintain 6 feet distance from infected person wash your hand with soap or 70%(V/V) alcohol based sanitizer and at regular interval of time and avoid touching of your hand to your face, mouth and Eyes. Coronavirus spreads through droplets of saliva or liquid discharge from the nose whenever an infected person cough or sneeze he should cover his mouth with tissue paper or handkerchief or he should cough or sneeze into his flexed elbow[1,2] So far, there is no potential medicine or vaccine for the treatment of corona virus infection or covid-19. However, many clinical trials are going on to evaluate the potential vaccine or strategic treatment[3-5]. The dynamics of SARS-Cov-2 are currently unknown, but there is speculation that it also has an animal origin and that is Bat.Novel Corona virus has potential to become a pandemic infectious disease and a threat to the public health. Worldwide it is seen that many countries are in the stage of human to human transmission and there are lakhs of people are infected and thounds of deaths are reported and observing this situation WHO declared the COVID-19 a pandemic[6].

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Etiology: Coronavirus positive-sense single-stranded RNA genome and a nucleocapsid of helical

symmetry, when it is observed under an electron microscope it shows the cahracteristics of club-shaped spikes which are glycoprotein that project from its outer surface and it look like a crown. (coronam is the Latin term for crown). Coronaviruses constitute the subfamily Orthocoronavirinae belongs to the family Coronaviridae and it classifies into four genera of CoVs: Alphacoronavirus (alpha CoV), Betacoronavirus (beta CoV), Deltacoronavirus (delta CoV) and Gammacoronavirus (gamma CoV). Genomic characterization has shown that probably bats and rodents are the gene sources of alpha CoVs and beta CoVs. On the contrary, avian species seem to represent the gene sources of delta CoVs and gamma CoVs[7,8]. Other human CoVs that is beta CoVs can cause epidemics with variable clinical severity featuring respiratory and extra-respiratory manifestations[9,10,11]. Coronavirus positive-sense single-stranded RNA genome contains 29891 nucleotides, encoding for 9560 amino acids. However from which species it is originated it is not confirmed till now but the study of genome of the novel corona virus comes out with the findings that COVID 19 probably evolved from a strain found in bats but the intermediate species between bats and humans is not known[12-14].

Transmission: Analysis of the data related to COVID-19 shows that Virus gets transmitted from human to human only when the individuals comes in contact with infected person. It is seen that initially the family members, Health Care professionals gets infection only when they comes in contact with the infected person. Studies comes out with the findings that the incubation period of the novel coronavirus is 3 to 14 days. Studies also showed that this novel corona virus swiftly transmitted from human to human and Gets doubled in about one week[15-18].

Treatment: After studying we have identified around 15 drugs which are undergoing clinical trials of different phases. After medical intervention. It was found that COVID 19 or Corona virus could be treat by using drugs like Chloroquine, Hydroxychloroquine, Remdesivir, Ribavirin, Ribavirin plus Interferon, Camostat Mesilate, Lopinavir/Ritonavir, Darunavir/Cobicistat, Favipiravir, Umifenovir, Oseltamivir, Baloxivirmarboxil, Tocilizumab, Sarilumab Eculizumab. Chloroquine drug molecule and hydroxychloroquine drug molecule not only shows antimalarial activity but also have antiviral effect against (HIV) Human Immuno Deficiency Diseases virus by showing mechanism of action against the virus by preventing the virus to enter into the host cells. Hydroxychloroquine drug have shown the positive effect in order to treat Acquired Immuno Deficiency Syndrome (AIDS). In the ongoing clinical trials for the treatment of COVID 19 come out with the results that Hydroxychloroquine drug if given with macrolide anti-biotic azithromycin shows that 100% of Corona positive patients are cured as if only Hydroxychloroquine drug given alone then only 57.1 % Corona positive patients are cured. Now the clinical trails are undergoing to test weather the Hydroxychloroquine drug can use for treatment of COVID 19 positive patient those having Pneumonia[4, 23-26].

There are 2 clinical trails are undergoing using the drug Remdesivir in the mild to severe respiratory viral infection by COVID 19. Remdesivir is a nucleotide analog inhibitor of the EBOV RNA-polymerase RNA-dependent (RdRp). Dyer et al. 2019 depicted the primary outcomes the mortality rate of 33 % in 499 patients treated with Remdesivir drug against the EBOV diseases in initial stages. Also noted that a mortality rate of 75 % in 1900 patients of non treated infected patients during the same epidemic time period. Wang et al. 2020 depicted the alalysis indicating that Remdesivir is giving effective results in the treatment of COVID 19 in Vero E6 cells (EC90 1.76 μM). He also implied the mechanism of remdesivir drug involves the inhibition of viral application[19, 27].

China already approved the Umifenovir in order to treat influenza viral infectious diseases since it has not shown any adverse effects while treating the SARS. Umifenovir involves the mechanism of anti- viral action against the influenza virus A and B by virul fusion inhibition with targeted membrane in order to block the entry of virus into the cell.

Remdesivir: Remdesivir drug is a antiviral drug which has already used in the treatment of Paramyxo virus, Filo virus, Pneumo virus and Corona Virus. In vitro it is observed that Remdesivir shows the potent to control the clinical symptoms in SARS- CoV-1; MERS-CoV and in recent studies it is also shows against COVID 19 and comes out with the result that Remdesivir drug can be act as a SARS- CoV-2 inhibitor which is replicate in nasal and bronchial airway epithelial cells. However, in COVID 19 the clinical symptoms improvement and antiviral efficacy of remdesivir drug remains to be established. Gilead Sciences Inc., developed the Remdesivir on which the Adaptive COVID 19 Treatment Trial or ACTT was conducted by National Institute

of Allergy and Infectious Diseases (NIAID) at NIH in to order to study the infection causes and to design a effective standard procedure to cure COVID -19[4].

Placebo controlled randomised trial of remdesivir in the sever patients of COVID-19 with the method of double –blind trial in order to check the efficacy and adverse effect of remdesivir drug in adults (aged ≥18 years). In this trial only men and non- pregnant women who are positive with SARS-CoV-2 and suffering from Pneumonia and having oxygen saturation of 94% or lower on room air or a ratio of arterial oxygen partial pressure to fractional inspired oxygen of 300 mm Hg or less and were within 12 days of symptom onset were eligible. For this trial patients were given Remdesivir intravenously that is 200 mg on first day and 100 mg followed by second to tenth day in single infusion or infused placebo for same time period. Laboratory parameters were regularly monitored and assessed the adverse effects.

From the time period 6 February 2020 to 12 March 2020 trial was done on 236 eligible patients of whom Remdesiver were given to 158 eligible patients and 78 patients were given placebo. Mostly patients was suffering from common disease like Coronary heart disease, hypertension and diabetes and maximum patients of these disease were in the Remdesivir group as compared to the placebo group. Some patients were given Lopinavir–Ritonavir drug in the trial. It was observed that the patients in placebo group shows the symptoms for 10 days and in case of Remdesivir group the patients had shown the respiratory rate of more than 24 breaths per minutes and those patients administered with Remdesivir drug shows the significant improvement in the clinical symptoms and laboratory parameters within 10 days after the trial starts. In patients with use of remdesivir within 10 days after symptom onset, 28-day mortality was not significantly different between the groups, although numerically higher in the placebo group; by contrast, in the group of patients with late use,

Adverse effects were noted during the trial in both the group and it was observed 102 patients in Remdesivir group and 78 patients in placebo group reported the common adverse effects. In Remdesivir group common adverse effects like constipation, anaemia, hypoalbuminaemia, thrombocytopenia, hypokalaemia and increased total bilirubin were reported. In placebo group common adverse effects like constipation, anaemia, hypoalbuminaemiahypokalaemia increased aspartate aminotransferase, increased blood lipids and increased total bilirubin were reported. All deaths during the observation period were judged by the site investigators to be unrelated to the intervention.

Vaccine: Till date 8 april 2020 the COVID-19 vaccine Reasearch and Development are doing by 115 Vaccine making entities, of which 78 as an active project in which 73 are currently at the stage of exploration and preclinical trial and remaining 37 entities are unconfirmed (development status cannot be determined from publicly available or proprietary information sources). The most progressive entities have recently undergone into development of clinical studies which includes mRNA-1273 from Moderna, Ad5- nCoV from CanSino Biologicals, INO-4800 from Inovio and LV- SMENP-DC and pathogen- specific aAPC from Shenzhen Geno-Immune Medical Institute. Many vaccine developers entities have shown their plans to start the human clinical trials in 2020. This COVID-19 vaccine developers is evaluating the different technologies like nucleic acid (DNA and RNA), virus- like particle, peptide, viral vector (replicating and non- replicating), recombinant protein, live attenuated virus and inactivated virus approaches. Many of these technologies are not the basis for licensed vaccines but the Oncology study experience convinces vaccine developers to explore the novel alternative resources in the new world in order to swift the development of vaccine[29].

Plasma Therapy: Covalescent Plasma Therapy (CP) has been used since 100 years for treating several infectious diseases including viral diseases. CPis an adaptive immunotherapy which helps in improving the patients immunity to fight against the specific infection. CP therapy was reported to be used for the treatment of SARS (write the long form), H1N1 (write the long form) and MERS (write the long form) with no adverse effect. However it was also reported that the CP therapy fails to decrease the mortality rate due to Ebola Virus disease. Because there is no data available for neutralising antibody titration for stratified analysis (It is not connecting to the previous sentence). Virological Studies and clinical studies comes out with findings that MERS, SARS and COVID 19 shows similarity and therefore CP therapy might be useful for the treatment of COVID 19. In the CP therapy, neutralizing antibody titer from COVID 19 recovered patients can be used for the affected individual.

For clinical trail of CP therapy 10 critical patients

were identified with COVID 19 positive. 200 Ml single dose of convalescemt plasma derived from recently recovered healty donors with neutralizing antibody titers above 1:640 and this plasma was transfused to these patients with the combination of antiviral drug Lopinavir and Ritonavir and extra supportive care.

Conclusion

Overall, the present review article summarizes the history of COVID 19, its etiology, transmission and various treatment strategies so far have been tried by various medical experts. Considering the work progress for the treatment or prevention of COVID 19, it is expected that vaccine will soon be available in the market.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Selfcare Management and Strategies of Drug Development for COVID 19

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Abstract

Corona virus disease (COVID 19) is a recently discovered pandemic. Coronavirus infectious disease originate from Wuhan, China and widely and quickly spread throughout the world. WHO pronounced it as Pandemic. As the spread of disease is through contact and the rigidity of virus in term of its lifespan outside the human body its threat human beings is alarming with no thearapy to treat. As per the WHO guidelines the only way to control is to prevent the disease by following selfcare method. Though the Scientific community started research on the vaccine as well as newer drugs the development of which may require certain time to reach at the people at large. Thus selfcare management is found to be the only strategy to combat the infection by COVID 19.

Introduction

Novel corona virus(COVID-19) is rapidly spreading worldwide and its mitigation is threat to human life and declared as pandemic. It is caused by virus belonging to family Corona viradae initially originated from Wuhan China and having size of 65-125 nm in diameter.[1] The virus that causes COVID-19 is transmitted mainly through droplets produced when a person who is infected coughs, sneezes, or exhales. Contact with someone who has COVID19 or touching a contaminated surface and then eyes, nose, or mouth,be infected with the virus by breathing. These droplets are too heavy to hang in the air and quickly fall on floors or surfaces. People with COVID-19 generally develop signs and symptoms, including mild respiratory symptoms and fever, on an average of 5-6 days after infection (mean incubation period 5-6 days, range 1-14 days). At times experience additional symptoms, such as: runny or stuffy nose, sore throat, headache, body aches and pains,diarrhoea, some observations suggest that respiratory symptoms may worsen in the second week of illness. This appears to occur after 8 or 9 days. According to the World Health Organization (WHO), about 1 in 5 people with COVID-19 become seriously ill. Most people infected with COVID-19 virus have mild disease and recover. Older people and those with underlying health conditions such as cardiovascular disease, diabetes, chronic respiratory disease and cancer are more likely to experience serious illness.[2,3]

The number of patients worldwide is increasing even though the virus is not that fatal. Total number of affected patients by COVID 19 Globally, as of 14:00 pm CEST, 8 June 2020, there have been 7103804 confirmed cases of COVID-19, including 406303 deaths, reported to WHO. In India till 14:00 pm CEST, 8 June 2020, there have been 258090 confirmed cases of COVID-19, including 7207 deaths. India is a country which ranks second in population, the seventh-largest country by area and the democracy with largest population in the world. In country like India, where the population is 135 Crores and literacy is low risk of COVID-19 virus is very evitable and can lead to dangerous situation challenging life and healthcare system. Covid pandemic has affected a lot of developed countries and likewise India will also be affected. A lot of check has to be done

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in administrative and clinical level in India to combat the healthcare post pandemic where social distancing is only vaccine available.[4] To know how population is affected by spread of virus COVID 19 is illustrated by a very true example in central India is mentioned in Fig 1.

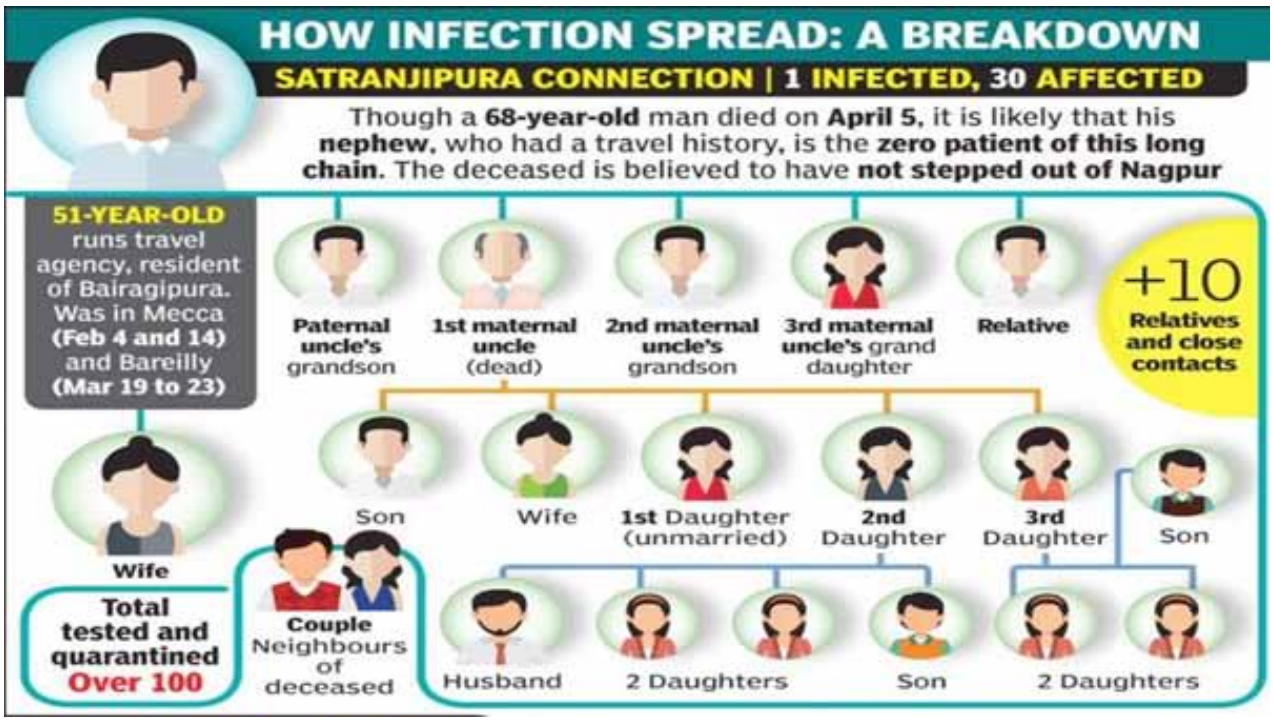


Fig 1: The 68-year-old male deceased from Satranjipura, Nagpur, India was the First case of COVID 19 in Nagpur with affected 44 other people in family and relatives[5]

India is a social country, where mostly daily life of Indians may include social gathering, meeting, celebrations, hoteling, weddings and for some conferences. Therefore to deal with COVID 19 would be difficult. More over most of these occasions revolve round food items and maintenance of hygiene in bulk food will be the big query.

Moreover for people who have travelling as their job profile will face difficulty in maintaining social distancing during their travel due to population and sanitization of such big travel network on daily basis will be challenging.

Worshiping in religious place is also big tradition in India, visiting temples, mosques and church is a part of daily routine for many in India. Buying groceries,visiting mall, cinemas, salons and spas, taking domestic help are required to be done with due care.

The population can face very dangerous situation in case of COVID 19 spread and thus in India management of this virus is very crucial and important.

There are various measures through which novel corona virus can be fought back and will be discussed further.

In topsyturvy times of global pandemic it is important to remember that self care is farmost important to keep safe, there are several ways to selfcare issued by WHO

1. As per the guidelines wearing mask is needed
2. No hugging and shaking hands anymore
3. Distancing from others in crowdy places
4. Washing hands with soap regularly protects health of everyone
5. Avoid touching eyes, nose and mouth.
6. Using alcohol based sanitizers, cleaning and disinfecting high-touch surfaces regularly is an important precaution to lower the risk of infection

7. Cleaning laundry at home
8. Removing shoes before entering home and changing clean clothes after being in crowded places and washing hands with soap and water immediately
9. Washing Groceries properly with running water
10. Informing people about importance of hygiene
11. Keep surroundings clean, sanitising frequently touched objects
12. Providing sanitation of Toilets used by COVID 19 patients at home
13. Many health co-benefits observed by safely treating water and sanitation facilities and by applying good hygiene practices.
14. Special care for safety of children, elderly, patients, caregivers and staff of organisations giving service to COVID 19 patients or ward.
15. Leaving home only if very necessary
16. Testing and Visiting doctor if symptoms occur[6-9]

Other Self Care Measures includes:

1. Exercise: Exercising at home can be helpful and improve physical as well as mental health
2. Yoga: stressful times can be managed by yoga and meditation
3. Making meal at home can be safe for prevention of outside food contamination
4. Nutrition plays a vital role in immunity building which inturnplays a key role against diseases
5. Hopeful: Being hopeful can give positive wibes moreover keeping self-busy as well as learning something new can be helpful
6. Maintaining routine on daily basis
7. Taking breaks from media and news
8. eHealth tools such as applications and websites can be used to support self-care behaviors and maintain chronic illness stability

Strategies for development of new drugs for treatment of corona virus[12-14]

The stages of new drug development:

- Phase 1: Discovery and Development.
- Phase 2: Preclinical Research.
- Phase 3: Clinical Research.
- Phase 4: FDA Review.
- Phase 5: FDA Post-Market Safety Monitoring

The parameters which are considered in the drug repurposing

1. Possible targeting
2. Knowledge of small molecules
3. Data reported against viruses
4. Search of substance that interact with viral proteins
5. Identification of right drug
6. Repurposing of drugs that have already been reported
7. Novelty in work to produce results
8. Drugs that increase the pace to save more lifes in COVID19
9. Host directed therapies
10. Bioinformative tools
11. Computer aided drug design
12. Computational studies
13. Drug data management and patient data management
14. *Invivo* models
15. ACE Blockers
16. Points which are lesser danger
17. Amino acid targeters
18. Look for existing molecules
19. Biological affecting points
20. Consider genetic mutations points
21. Find for functional group
22. Uniform data manner
23. Defined route of administration
24. Pharmacokinetics and dynamics
25. Electronic health record
26. Screen all chemical class of drugs
27. Preventing antibiotic reuse in repurposing
28. Emergence of antimicrobial resistance
29. Emphasis on small molecules

30. Intellectual property and economic consideration
31. Clinical trials of all the phases
32. Patentability of the proposed work
33. Consideration of crucial protein protein interaction
34. Finance management of research and pricing of drug in developing country like India
35. Multi institutional efforts
36. Industry sponsoring for development and research
37. Drugs which can be active against other species of Corona virus
38. Drugs that can be used in future pandemic

R & D Blueprint and COVID-19[15-16]: The current COVID-19 pandemic is new, however the worldwide response attracts on the teachings learned from epidemic outbreaks over the past many decades.

As a part of WHO's response, the R & D Blueprint was activated to accelerate nosology, vaccines and medicine for this novel coronavirus. The Blueprint aims to enhance coordination between scientists and world health professionals, accelerate the analysis and development method and develop new norms and standards to find out from and improve upon the worldwide response.

On thirty January 2020, following the recommendations of the Emergency Committee, the WHO agency Director-General declared that the occurrence constitutes a Public Health Emergency of International Concern (PHEIC). The scientists of all over world on COVID-19 met at the World Health Organization's Geneva headquarters on 11–12 Feb 2020 to assess the present level of data regarding the new virus, agree on vital analysis queries that require to be answered desperately and to seek out ways that to figure along to accelerate and fund priority analysis to curtail this occurrence and indurate those within the future. The discussion PROVED TO BE CRUCIAL agreement on 2 main goals. the primary was to accelerate innovative analysis to assist contain the unfold of the epidemic and facilitate look after those affected. The second was to support analysis priorities that contribute to world analysis platforms in hopes of learning from the present pandemic response to higher indurate ensuing unforeseen epidemic.Building on the response to recent outbreaks of Ebola virus, SARS-CoV and MERS-CoV, the R & D Blueprint has expedited a coordinated and accelerated response to COVID-19, together with associate degree new program to develop a immunizing agent, analysis into potential pharmaceutical treatments and reinforced channels for data sharing between countries. The current world COVID 19 public health emergency underscores the necessity to accelerate the event of COVID 19 candidate vaccines. The unit for immunizing agent prioritization aims to supply aspirational support to immunizing agent developers from a public health perspective likewise on grade immunizing agent platform approaches and/or candidates to be thought-about for additional development and probably take into account for later stage of analysis within the context of the worldwide COVID 19 occurrence.

The objectives are:

1. To review the present pipeline of candidate vaccines for COVID 19
2. To review the present pipeline of candidate vaccines for different coronaviruses and discuss their price in protective against the COVID 19
3. To create preliminary recommendations on whether or not the event of COVID 19 candidate vaccines ought to be prioritized over the event of different coronaviruses candidate vaccines.

This Consultation presents associate degree initial step towards the analysis of vaccines against this novel Coronavirus. There are currently efforts to spot further vaccines and to expand the support of proof on the market on every of the candidates.

The COVID-19 (Technology) Access Pool are voluntary and supported social commonality. That gives a one-stop buy knowledge domain, information and data to be shared equitably by the worldwide.

The aim is to accelerate the invention of vaccines, medicines and different technologies through open-science analysis and to fast-track development by mobilizing further producing capability. This may facilitate guarantee quicker and additional evenhanded access to existing and new COVID-19 health merchandise.

There are a unit 5 key parts to the initiative:

- Public speech act of factor sequences and data;
- Transparency round the publication of all clinical test results;

- Governments and different funder's area unit inspired to incorporate clauses in funding agreements with pharmaceutical corporations and different innovators regarding even distribution, affordability and the publication of trial data
- Licensing any potential treatment, diagnostic, immunizing agent or different health technology to the Medicines Patent Pool - a United Nations-backed public health body that works to extend access to and facilitate the event of, life-saving medicines for low- and middle-income countries.
- To promote and enhance support to innovative models and technology transfer that increase national income and provide capability, together with through connection the Open Covid Pledge and therefore the Technology Access Partnership (TAP).

With worldwide help and initiation, C-TAP can function as important part to the access COVID-19 Tools (ACT) Accelerator and different initiatives to support efforts to fight COVID-19 globally.

WHO, Costa Rica and every one the co-sponsor countries have conjointly issued a "Solidarity decision to Action" asking relevant stakeholders to hitch and support the initiative, with suggested actions for key teams, like governments, analysis and development funders, researchers, trade and civil society.

To date, the COVID-19 Technology Access Pool is currently supported by the subsequent countries: Argentina, Bangladesh, Barbados, Belgium, Belize, Bhutan, Brazil, Chile, state, Ecuador, Egypt, Central American country, Honduras, Indonesia, Lebanon, Luxembourg, Malaysia, Maldives, Mexico, Mozambique, Norway, Oman, Pakistan, Palau, Panama, Peru, Portugal, St. Vincent and Grenadines, African nation, Sri Lanka,Sudan, European nation, Timor-Leste, Uruguay, Zimbabwe.

Conclusion

COVID 19 is dangerous, but with above approaches and united efforts with government such pandemic can be overcome. Giving priorities to healthcare and following the guidelines can increase lifes expectancy in COVID 19.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Physiological Effect of Yogic Procedures on *Pranvahastrotas* (Respiratory System) in Healthy Individuals

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Abstract

The present research aimed at researching the physiological effect of yogic procedures in healthy subjects on *Pranavahastrotas*. The objective of the study was to test *Pranvahastrotas'* physiological functions by evaluating Herat Rate (HR), Respiration Rate (RR), Tidal Volume (TV), Inspiratory Reserved Volume (IRV), Expiratory Reserved Volume (ERV), Forced Vital Capacity (FVC), MVV (Maximum voluntary ventilation), Ratio-FEV1/FVC (Forced expiratory volume in one second). Total thirty healthy participants were selected by lottery method with prior written consent. After institutional Ethical committee clearance selected participants performed schedule of *Yogasanas* and *Pranayama* for 9o minutes daily for two months and they were again assessed for all the above parameters after completion of 60 days. After 60 days heart rate and respiration rate decreases to significant level whereas there is significant rise in IRV, ERV, FVC, MVV and FEV1. In conclusion, yogic exercises and *Pranayama* done regularly improve pulmonary function test, there helps in improving the health of *Pranvahastrotas*

Keywords: *Asan, Pranayam, Pranvahastrotas.*

Introduction

According to Chhandogyopanishad, one of the most ancient Upanishads, a human being may live without eyes, ears, legs, etc but cannot live without breathing and nourishment, for life derives from these two elements and depends entirely on them. This is the vital energy within them or vital force. *Pranvayu* (vital air) is responsible for breathing, regulating manas, sensory organs and activities such as circulation of blood, sneezing and belchingare vital functions for life. *Pranvayu* staying at *Murdha* (head region) and moving in neck and throat area conducting all these things is therefore called *Prana* life[1]. This *Pranvahastrotas*is vitiated by waste, repression of natural desires, indulgence in dry things, exercise during hunger and other *Strotasa* (systems) disability.[2]

Pranvahastrotas is among the body's most important structures. It is mainly responsible for providing oxygen and removing carbon dioxide. It facilitates speaking, too. Oxygen is the source of life and lack of supply of oxygen in the body may mean death. The oxygen that our respiratory system produces is used by the hundred billion cells that make up our body, in particular the Brain [3] The respiratory system include the nose, the nasal cavity, the pharynx, the larynx, the trachea, the bronchi and their smaller branches and the lungs which contain the terminal air sacs or alveoli. [4] The diseases developed due to impairment in the functions of *Pranvahastrtotas* may develop diseases like *Shwas*(asthma),kasa(cough) and also point out the problems of respiratory system.[5]

Asanas (physical postures) and *Pranayam* (respiratory exercises) are essential steps of *Ashtanga Yoga* (eightfold yoga), *Asanas*are the stable postures of the body, helps to improve and promote muscle health. *Pranayama* is a procedure of controlling mind by controlling the *Vayu* (air) in inspiration and expiration. These yogic procedures are not only having psychological benefits but they are having many physical benefits also[6,7] Lung volumes and capacities express the functional status of respiratory system in physiological as wellas pathological situations.[8]

All *Pranayama* forms include deep inspiration, with holding of air and deep expiration. Respiratory activity thus greatly affects the lung capacities and volumes.[9]

Pranayam can be described as to inhale, toretain and to exhale the additional quantity of *Pranvayu* or fresh air. As the additional quantity of air is inhaled and retained for an additional period in the lungs, its gets more time to exchanged by alveoli and the vital capacity of the lungs is increased. The amount of airthatisflushed in and out during aspiration is measured by spirometer.[10] Normal volumes and capacities are as follows.-

Tidal volume (T.V.): 500 mlper inspiration. 360 ml reaches the lungs while 140 ml remains in bronchi.

Inspiratory reserved volume (IRV): The air which can be forcibly inspired beyond the tidal air volume (2100- 3200ml)

Expiratory reserved volume (ERV): The amount of air that can be evacuated after tidal expiration. (1000-1200ml)

Respiratory capacities: The following features are covered in inhaled after normal expiration.

Inspiratory capacity (IC): Maximum amount of the air that can be inhaled after normal expiration.

Vital capacity (VC): Maximum amount of the changeable air that can be expired after normal inspiration. It includes tidal inspiratory reserve and expiratory air volumes. In healthy youths it is about 4800ml.

Functional residual capacity (FRC): Volume of the air remaining in the lungs after a normal tidal volume expiration.[11]

Methodology

Participants fulfilling inclusion criteria were selected for study. Thirty healthy subjects were enrolled in the study (n = 30). All participants were between 20–50 years of age and were recreationally physically active. After detailed oral and written information regarding all procedures and possible consequences, each subject signed a written consent prior to participation. The study was approved by the Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences.

Selection of participants for the study: All the participants were selected by lottery method. The health of the participants was assessed by noting the present, past, family and personal history and also by thorough general and systemic examinations.

Inclusion criteria:

- Healthy individuals between the age group 20-50 years
- With normal lung function
- Donot smoke or take any medications
- Do not suffered from acute or chronic diseases

Exclusion criteria:

- Had acute injuries
- Did participants take part in any activities that could possibly influence respiration such as singing or playing a wind instrument on a regular basis
- Subjects who were trained in yoga.
- Subjects with history of any systemic illness.
- Subjects who were not able to perform respiratory function tests
- Subjects who smoked, consumed alcohol, or any drugs

Procedure: Before starting yogic procedures, on first day following respiratory parameters of all the subjects were recorded to assess the functions of *Pranavaha Strotas*.

- Herat Rate (HR)
- Respiration Rate (RR)
- Tidal Volume (TV)
- Inpiratory reserved volume (IRV)
- Expiratory reserved volume (ERV)
- Forced Vital Capacity (FVC)
- MVV (maximal voluntary ventilation)
- Ratio-FEV1/FVC (Forced expiratory volume in one second)

All the subjects were under uniform yoga training under the supervision of yoga expert for a period of 60 days for 90 minutes daily from 6am to 7.30am.

The schedule ofyogic procedures:

1. Prayer - 5 min.

2. Yogasan -30 min
3. Break - 10 min.
4. Pranayama - 40 min.
5. Prayer - 5 min.

The squence of Yogasanas was:

- Sandhi sanchalan
- SharirSanchalan
- Bhujangasan
- Dhanurasan
- Matsyasan
- Ardhmatsendriyasan
- Shavasan

The squence of Pranayama was:

- Nadishuddhi
- Anulonmaviloma
- Bhrastrika
- Anulonmaviloma
- KapalabathiKriya
- Anulonmaviloma
- Bhramari
- Shitali
- Shitakari
- Omkar

The session was started and concluded by prayer.

Observations:

Table No. 1: Observations of Heart rate and Respiration rate before and after yogic procedure

Sr.No.	HR (/min)		RR (/min)		Sr.No.	HR (/min)		RR (/min)	
	Before	After	Before	After		Before	After	Before	After
1	76	74	16	14	16	76	76	18	17
2	75	74	18	16	17	76	74	14	14
3	78	76	17	16	18	75	74	15	14
4	80	76	19	16	19	74	72	16	15
5	82	78	20	18	20	78	76	14	12
6	78	76	15	14	21	78	76	15	13
7	74	72	16	14	22	80	78	14	14
8	75	75	19	18	23	78	76	16	14
9	76	78	16	14	24	76	74	15	14
10	70	72	15	14	25	74	72	16	13
11	72	74	16	14	26	75	73	18	16
12	71	73	14	12	27	74	74	16	14
13	72	74	18	16	28	75	72	16	14
14	70	72	16	14	29	79	78	15	13
15	78	76	20	18	30	80	78	18	16

Table No. 2: Observations of Tidal Volume and Inspiratory reserved volume before and after yogic procedure

Sr.No.	TV (in ml)		IRV (in ml)		Sr.No.	TV (in ml)		IRV (in ml)	
	Before	After	Before	After		Before	After	Before	After
1	478	488	2560	2660	16	502	523	2950	3150
2	490	496	2600	2660	17	506	506	2540	2700
3	488	496	2700	2800	18	510	506	2590	2800
4	492	516	3200	3250	19	486	496	2960	3100
5	502	514	3210	3300	20	482	496	3100	3300
6	502	520	2800	3000	21	484	498	3200	3300
7	482	502	2950	3100	22	486	490	3210	3300
8	489	505	2900	3000	23	488	496	3250	3320
9	488	520	3300	3400	24	487	496	3200	3330
10	478	486	3200	3300	25	489	490	2500	2700
11	476	492	3200	3290	26	492	526	2490	2600
12	454	478	3250	3400	27	494	521	2590	2700
13	480	492	3120	3300	28	496	513	2650	2800
14	492	510	3160	3210	29	498	526	2580	2600
15	506	521	3020	3200	30	502	530	2600	2800

Table No. 3: Observations of Expiratory reserved volume and Forced expiratory volume before and after yogic procedure

Sr.No.	ERV (in ml)		FVC (in lit)		Sr.No.	ERV (in ml)		FVC (in lit)	
	Before	After	Before	After		Before	After	Before	After
1	820	920	3.8	3.9	16	860	860	4.0	4.1
2	880	950	3.9	4.1	17	780	850	3.9	4.1
3	950	970	3.7	3.9	18	790	850	3.8	3.9
4	960	970	3.9	4.1	19	840	950	4.2	4.2
5	880	900	4.1	4.2	20	860	950	4.1	4.1
6	860	910	4.0	4.2	21	860	910	3.9	4.2
7	790	820	4.3	4.5	22	900	950	3.8	3.9
8	900	950	4.2	4.3	23	740	820	3.6	3.8
9	980	1020	3.5	3.6	24	750	770	3.7	3.9
10	890	920	3.9	4.1	25	760	860	3.9	4.0
11	760	820	4.1	4.3	26	820	890	4.0	4.2
12	780	820	4.2	4.2	27	860	860	4.0	4.1
13	780	800	4.3	4.4	28	780	850	3.9	4.1
14	850	890	3.5	3.8	29	790	850	3.8	3.9
15	840	940	4.1	4.2	30	840	950	4.2	4.2

Table No. 4: Observations of maximal voluntary ventilation and Ratio-FEV1/FVC (Forced expiratory volume in one second) before and after yogic procedure

Sr.No.	MVV (L/min)		FEV1/FVC		Sr.No.	MVV (L/min)		FEV1/FVC	
	Before	After	Before	After		Before	After	Before	After
1	110	120	72	73.2	16	100	120	72.3	74.8
2	100	100	73	74.5	17	110	120	71.6	76.5
3	90	100	74.4	75.2	18	80	110	72.6	76.4
4	140	150	75.5	76.5	19	90	100	73.2	74.5
5	130	140	72.5	73.5	20	100	110	73.6	75.8
6	110	120	71.4	72.4	21	150	150	70	75.8
7	120	140	70.2	72.5	22	140	160	70.4	76.5
8	110	120	71.9	72.9	23	120	120	72.2	74.5
9	100	120	76.4	79	24	110	`120	72.4	76.5
10	80	100	74.2	76.5	25	130	140	72.2	76.2
11	90	90	74.9	78	26	100	110	75.8	76.2
12	100	120	75.2	76.8	27	80	90	75.2	76.8
13	110	140	74.2	78.5	28	90	100	74.3	75.9
14	80	110	72.2	76.4	29	110	120	73.2	76.2
15	90	100	71.3	76.2	30	80	90	71.2	76.2

Results and Analysis

Results were presented as Mean ± SD. Student t test (two tailed, independent) has been applied using software (SPSS 15.0, Stata 8.0, MedCalc 9.0.1 and Systat 11.0) to find the significance of study parameters. P value less than 0.05 was considered as significant.

Descriptive Statistics:

Parameters	Duration	Mean	N	Std. Deviation	Std. Error Mean
HR	Before	75.83	30	3.02	0.55
	After	74.76	30	2.04	0.37
RR	Before	16.36	30	1.75	0.31
	After	14.70	30	1.64	0.30
TV	Before	489.96	30	11.31	2.06
	After	504.96	30	14.13	2.58
IRV	Before	2919.33	30	286.19	52.25
	After	3045.66	30	279.35	51.00
ERV	Before	836.66	30	62.49	11.41
	After	890.33	30	60.19	10.99
FVC	Before	3.97	30	0.22	0.04
	After	4.11	30	0.20	0.03
MVV	Before	104.82	29	19.57	3.63
	After	117.58	29	19.20	3.56
FEV1	Before	72.98	30	1.717	0.31
	After	75.69	30	1.63	0.29

Student's paired t test:

Parameters	Paired Differences					t	df	p-value
	Statistical results			95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
HR	**1.06**	**1.79**	**0.327**	**0.39**	**1.73**	**3.24**	**29**	**0.003,S**
RR	1.66	0.71	0.124	1.40	1.93	12.83	29	0.0001,S
TV	-15.00	9.49	1.734	-18.54	-11.45	8.65	29	0.0001,S
IRV	-126.33	53.20	9.71	-146.20	-106.46	13.00	29	0.0001,S
ERV	-53.66	29.41	5.37	-64.65	-42.68	9.99	29	0.0001,S
FVC	-0.14	0.08	0.01	-0.17	-0.11	9.60	29	0.0001,S
MVV	-12.75	8.40	1.56	-15.95	-9.56	8.17	28	0.0001,S
FEV1	-2.71	1.62	0.29	-3.32	-2.10	9.14	29	0.0001v

Discussion

In the present study, respiratory rate decreases from 16.36±1.75 to 14.70±1.64 after two months of yogic practices with t-value 12.83 and $p < 0.0001$ which is highly significant. Similar finding was observed by Joshi (1992), Srivastava (2005), Jain (2005) and Makwana (1988).[12-14]

Because of pranayamic breathing by prolonged inspiration and expiration, bulbopontine complex is adjusted to a new pattern of breathing which is slower than basal breathing[12], Decline in respiratory rate is influenced probably by hypocapnea on medullary respiratory centre and persistent voluntary breathing producing inhibition of rhythmic spontaneous breathing by a phenomenon akin to over drive suppression [13]

In the present study, mean value of FVC (L) increases from 3.97±0.22 to 4.11±0.20 after 2 months with t-value 9.6 ($p<0.0001$) which is highly significant in study. However, Khanam (1996) reported no significant change in FVC. In the study, there is significant increase in mean FVC (litre), may be because of strengthening of respiratory muscle due to regular practice of pranayamic breathing during which lung and chest inflate and deflate to the fullest possible extent and muscles works to the maximumextent[14]

The study shows significant increase in IRV from 2919.33±286.19 to 3045.66±279.35 and ERV also increased significantly from 836.66±62.49 to 890.33±60.19 after two months. Mandanmohan et al who reported that, six months yoga training causes significant increases in IRV and ERV values in subjects of 12-15 age groups.

The study shows significant improvement in FEV1 from 72.98± 1.717 to 75.69±1.63 after yogic practices. In accordance to our findings, Joshi et al and Madanmohan et al showed statistically significant increase in FEV1 after yoga training which were statistically significant Makwana K et al also found significant increases in FEV1 after yoga training of 10 weeks Kadu P. Pet al concluded in their study that yogic exercise and *Pranayam* done regularly and for long term, improves respiratory efficiency.

Conclusion

The increased IRV and ERV after yogic practices indicate *Yoga* training improves the strength of expiratory as well as inspiratory muscles. *Kapalbhati* (expiratory strokes) included in the present training program involves powerful strokes of exhalation which trains the subjects to make full use of diaphragm and abdominal muscles. Slow, deep and full inspiration and expiration in *Pranayama* also trains the respiratory muscles and increases the strength of respiratory muscle.

Considering the observation, results and analysis, it is fair to conclude that yogic procedures can be beneficial in the promotion of the health of *Pranavahastrtas*.

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

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The Association between Flat Feet and Body Mass Index in Various Age Groups

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Abstract

Background/Objectives: The arches of the feet serve as an adaptive support for the entire body to drive away the forces of weight bearing and cause less energy consumption during the gait cycle. The arches of the feet are a tough, elastic network of ligaments, tendons and fascia between the forefoot and the hind foot. Though the flat feet are usually asymptomatic, but it can alter the biomechanics of the lower extremities and even the lower spine causing an increased risk of pain and injury. There are various studies who claim the association between obesity and flat feet in children's. To know the association of flatfeet and BMI in other age group,this study was carried out.

Methodology: Observational research with a cross sectional design was carried out at the physiotherapy OPD on 90 subjects which was selected by inclusion and exclusion criteria in the duration of 1 year. Group wise distribution was done by using purposive sampling. 3 groups were formed accounting their age.

Result: There is significant association between gender and Flat foot, where males in age groups 10-18 yr and 19-30 yr are more affected. BMI and flatfoot also showed significant association in above 2 age groups. When the analysis of variance (ANOVA) of average BMI was compared to the flat foot, there was a significant result in both same groups A. No significant results were found in chi square for association between age and Flatfeet.

Conclusion: Present study concludes that there is a association between BMI and Flat Feet in children's between 10-18 years of age and adults between 19-30 years of age.

Keywords: *Flat Feet, BMI, WHO, Obesity, ANOVA.*

Introduction

Flatfeet is a very regular term that is been used in daily routine. Flat feet" pes planus", it refers to the loss of the medial longitudinal arch of the foot where it contacts or nearly contacts the ground[1]. Though it is common problem in various age groups,negligence is often observed. Flat feet are always associated with heel pain, feet pain or even lower extremity pain. Altered biomechanics of arches of the feet often give rise to compromised functioning of lower extremities. These leading to altered posture and gait mechanism, the above all reason are sufficient enough to make difference in individual's quality of life.

In normal individual arches are been developed

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during the first five year of life span. If not develop, it can lead to flat feet which may be congenital or acquired[2].

Several studies have proven the association between the flat foot and obesity, and also obesity as a cause for the occurrence of flat feet. Increased weight of body places higher stress over the feet causing the arches to drop[3,4]. Obesity is seen as the first wave of defined cluster of non communicable diseases called NEW WORD SYNDROME, creating an enormous socio economic and public health burden in poorer countries[5,6]. Obesity is associated with other systemic problems like Diabetic, hypertension[4,7]. It is one of the listed cardiovascular risk factors[8], has significant effect on peripheral nerves[9] and microvasculature when associated with diabetes[4,10-12]. WHO has described Obesity as one of today's most neglected public health problem, affecting every region of the globe contributing to 1,12,000 deaths each year[13] occupational, a vocational & actions of daily living in adults involve adequate amount of time weight-bearing on our feet, such as while walking, running, roaming in public transport or standing at workplace. Flat arches may expose the affected individual to undue stress & faulty mechanism leads to pain & discomfort. Thus it is essential to their quality of life to and extent of their difficulty in functional activity. Presently specific to those individuals who travel or work on a daily basis, i.e., the age group of 19-30 years as they are more susceptible to occurrence of pain, limitation and social obstacle to foot problems. There is an growing need to endorse foot health amongst various age groups including adults, old age and childrens which is an issue that is common but frequently ignored as being inconsequential. Quantifying the impact of flat feet on quality of life in such individuals therefore makes it possible to identify the impact and plan preventive and rehabilitative strategies in these patients.

Who Classificatiuon of Weight Status	
Weightr Status	**BMI.KG/m**
Under Weight	<18.5
Normalrange	18.5-24.9
Over Weight	25.0-29.9.
Obese	>30
Obese Class 1	30.0-34.9
Obese Class 2	35.0-39.9
Obese Class 3	> 40

Previous studies in literature determined the prevalence of flat foot footedness among them, there is a study echarri and forriol 7 who reported 70% prevalence of flat foot in children 3-4 years of age and 40% between 5-8 years of age[14]. Foot problem appear in approximately 70%-80% of adults and 30% of children. The commonly appearing problem concern deformities of medial longitudinal arch, either because it excessively high or excessively low and have a significant impact on foot function and the development of musculoskeletal pathologies[15-21]. Even weakening of the calf muscle causes the drop of navicular bone resulting in flat foot.

Thus increased BMI causing increase incidence of flat feet in various ages will be targeted in a present study. Also, any gender different in will be monitored.

Objectives: The study was carried to find out Flat feet in obese individuals in various age groups. Again the study was targeted to observe any association between obesity and flat feet. Also any age and gender association with the flat feet were monitored.

Procedure of Study: The ethical clearance was obtained from the institutional ethical committee, to carry out the study. The institutional physiotherapy OPD was targeted to obtain the subjects for carrying out the study. The inclusion criteria was to get the willingly participating subjects between 10-45 years of age including both genders, whereas the subjects with recent lower extremities surgeries, recent trauma, any limb length discrepancy were excluded from the study. All the subjects included in study were then divided into 3 different groups according to their ages. The BMI was then calculated in all the 3 groups and Flat feet were seen by using DENIS method. The data was then collected and statistical analysis was done[1].

Results

In the present study 90 subjects were included according to the inclusion and exclusion criteria within the span of 6 months. In group A 53%male and 46% female between the age 10-18 yrs were analysed for their comparison of BMI with Flat feet, similarly Group B (19-30 yrs) and Group C (31-40yrs) were having 53% male,46% female and 43.33%male,56.67% female respectively. The study represents association of BMI with flat foot, using Chi square test showing p-value 0.015 significance in 10-18 years of age and 0.01 significance in 19-30 yrs of age. ANOVA test shows significant result on average BMI comparison to flatfoot in Group A and B.

Table 1: Association-BMI vs Flat Foot

Variable	Groups	Flat Foot			Chi Square	d.f.	p value	Significance
		Normal	FF1	FF2				
A BMI	Normal	3	3	0	15.83	6	0.015	Significant
	Overweight	5	5	0				
	Obese 1	4	6	2				
	Obese 2	0	0	2				
B BMI	Normal	4	6	0	16.99	6	0.01	Significant
	Overweight	1	10	1				
	Obese 1	1	1	3				
	Obese 2	1	0	2				
C BMI	Normal	3	3	2	1.52	4	0.82	Not Significant
	Overweight	7	7	1				
	Obese 1	3	3	1				
	Obese 2	0	0	0				

Table 2: ANOVA-Average BMI comparison according to Flat Foot

Variable	Groups	N	Average BMI	SD	F value	d.f.	p value	Significance
A Flat Foot	Normal	12	26.83	3.78	7.69	2,27	0.002	Significant
	FF1	14	28.21	3.44				
	FF2	4	34.75	2.75				
B Flat Foot	Normal	7	23.85	6.59	10.05	2,27	0.001	Significant
	FF1	17	25.52	2.62				
	FF2	6	32.83	2.78				
C Flat Foot	Normal	13	23.00	4.00	0.42	2,27	0.66	Not Significant
	FF1	13	27.46	4.53				
	FF2	4	27.50	5.06				

Discussion

In the present study we investigated flat foot among various age groups by using DENIS method which was carried out in cross sectional population, in that we have found correlation of flat feet with obesity and Gender in age between 10-40 years. The various factors give rise to the flat foot and the bottom of the foot, including the age, sex, weight, race and some other anthropometric factors that make it one of the most serious and common problems in the 21st century[15-16]. Meanwhile, Body Mass Index (BMI) is an indicator of functional and easily estimated relative weight and is largely linked to most direct obesity measures. The BMI depends to a large extent on the physical composition. BMI is a tool for evaluating healthy body weight. BMI is calculated by measuring the ratio of individual weight to height squared and is used to determine obesity and overweight. Several reports have shown that overweight and obesity may affect foot structure in children and adults[17-19]. Fattahi and Goodarzi (1395) showed that there is a significant relationship between BMI anthropometric index and flat foot posture[20]. A study conducted by Suciatiet. al in 2019 to find out the correlation between flat foot an BMI in primary school students of Indonesia, concluded significant relationship between both; with PR value of 2.36, indicating obese people have 2.36 times risk for flat foot compare to people who have normal BMI.

Shyamala Shree et.al in January 2018,conducted a

prevalence study to check whether the obesity can cause flat foot or not. They found a strong correlation between obesity and flat foot and no association of gender and age with flat foot also shows.[21] Our study also support the finding of the study conducted by Heba H. Hazaaa. al who found a significant correlation between obese children and flat foot when they performed a cross sectional study on 150 children between age 8 to 14 years.[22]

Adult flat foot may categorize as either residual flat foot deformity from a developmental cause or as an acquired flat foot. Acquired flat foot is associated with tight triceps' sure or isolated gastronomies tightness, posterior tibial tendon dysfunction, mid foot laxity, abduction of fore foot,external rotation of hind foot, subjugation of the talus, traumatic deformities, rupture planter fascia and neuro muscular imbalance[23].

Some persons with flat foot may never face any sort of problem while others may find it hard to engage in activities such as running or sports. Pain in the arch and heel area with or without swelling around the ankle, sometimes difficult to stand on some positions areall signs of symptoms of flatfoot. Getting shoes with arch and foot support is helpful in training the feet to develop an arch[24] arches give a functional support to the feet to stand on mare unstable surface and sturdy archmeans good support to your foot different joint in loer limb is work like a chain if one is in problem other cant work well. The analysis of data for 100 mtr sprint, 600 mtr run and 12 mi walk/run test revealed. That the normal foot performs better in 100 mtr sprint and 12 min run test but no difference was found for 600 mtr run test. 100 mtr sprints is an explosive activity which requires more forceful reaction from the ground and normal arched foot must be given help than a flat foot due to its springing action and strong muscles for the performance of 100 mtr sprint. Again 12 min run is a long distance run and weak foot might get fatigued in this run. Our findings correlate withy the authors (Cameron Thompson) who showed decrease in ankle muscle strength in flat foot. This might be the reason for hampered performance. Again 600 mtr run is neither explosive activity nor an endurance event, so the flat foot might had not fatigued and performed equally as normal foot[25].

We found that variability in plantar pressure does not follow the same U-shaped curve relationship with speed, as do other lower limb step kinematic parameters. The strength of long-range correlations for each gait pattern consistently follows U-shaped curves, centred on the subjects preferred speed.[26,27] Study by Chang-Ryeol Lee, Myung-Kwon Kim et al[26] said the sub talar joint excessively phonated, causing a rear foot valgus posture,in which the calcaneous is everted away from the midline. In this position, the fore foot is usually adducted, and talus and navicular bones a new depressed. Furthermore, although the anterior/posterior index,medial/lateral index,and overall satiability index increased after the exercises, the increases were not significant flat foot has been associated with increased planter peak forces,increased forefoot supination,and increased knee internal rotation.

Fatigue of intrinsic muscle did not affect the hip,knee and ankle strategy which would have had a direct impaction balance. Walking for a long time and impact on the foot reduce the ability of people with flatfoot to support the medial longitudinal arch, which increases the pressure on the middle fore foot area and mid foot medial area[4,27-29].

Conclusion

The present study concluded that there is significant association between Flat feet and obesity in Age group 10-18 yrs and 19-30 yr. Present study shows non-significant association between age and Flat feet and also no gender discrimination in the occurrence of Flat feet. Male obese individuals in age 10-30 yrs should be given more attention as they are more prone for the occurrence of flat feet. Recently one study at Navi Mumbai by Twinkle Dabholkar, Anushka Agarwal,et al which stated that the presence of flat feet in an individual in the age group 20-40 years impacts his/her quality of life in the form of foot pain, difficulty to do daily living tasks such as walking on uneven ground, walking fast, running and maintaining balance. Also, a high proportion of our participants reported having trouble in walking on uneven ground and running (79%). Continuous and proactive measures for screening and prevention of flat foot are recommended in this population.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

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Experimental Evaluation of Talisadi Suspension for Anti-Histaminic and Anti-Inflammatory Activities in Animal Models

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Abstract

Talisadichoorna is a routinely used formulation among ayurvedic practitioner in disorder of *Pranavaha, Annavaha* and *Rasavahastrotasvyadhi's*. As *choorna* has some drawbacks, as a dosage forms *talisadichoorna* was converted into a modern dosage form i.e. suspension. It is better than *choorna* in aspects like ease in administration, longer shelflife and faster absorption. Standard Operating Procedure was followed for its preparation. No work was reported regarding its pharmacological action except for anti microbiological study. This study aimed to assess its anti inflammatory and antihistaminic actions, which is clinically seen. This study helped in assessing mode of action of the formulation. The disease *Tamak shwas* can be correlated with Bronchial Asthma on the basis of pathology, signs and symptoms. Bronchial Asthma is characterised by dyspnoea, excessive cough, wheeze, chest tightness etc caused by airway inflammation and increase bronchial responsiveness. To study antiinflamtoamry activates model selected was "Granuloma pouch model" using rats as experimental animals. To study antihistaminic activity experimental animal guinea pig was used and model used was "Protection against histamine induced brochospasm model". It was evident that Talisadi Suspension exhibit significant anti-inflammatory and antihistaminic activities.

Keywords: *Talisadi Choorna [TC], Anti-Histaminic study, Anti-inflammatory study, Tamak Shwas, Bronchial Asthma, Talisadi Suspension [TS].*

Introduction

The branch of Ayurveda which deals with preparation of medicines is called as Bhaishajya Kalpana. It does not appear as a separate branch in Ashtang Ayurveda[1] yet it is the backbone of ayurvedic science. It is inexistence since time immerioaland is flourishing till date. References from primitive era and Vedic period[2] elaboratemaking of different dosage forms and formulations along with its usage in different disease conditions. Sharangdhar samhita[3] in 14 th century for the first time compiled various formulations and explained their manufacturing process in detail. Hence it can be termed as the first book of Ayurvedic pharmaceutics and pharmalogy. Twenty five different dosage forms such as sneha, parpatikalpa, choorna, asavaaristha, khalvirasakalpa etc are in routine use.

These are mainly derived from basic dosage forms i.e. panchvidhkashayakalpanaand formulated by applying the different principles of ayurvedic pharmaceutics of natural drugs. In modern era by adopting newer techniques and with the application of principles of ayurvedic pharmaceutical science one can develop various formulations and new dosage forms having longer shelf life,better palatability and ease in administration.

With this view, work was carried out previously in the department of RSBKV, BVDU, Pune for a routinely

used and effective formulation i.e. Talisadi Choorna[4] and a standard operating procedure was developed to change the dosage form to prepare Talisadi Suspension[5]. There are various formulations under the name of Talisadi Choorna wherein some variations in its ingredients are noticed. To prepare suspension the contents must disperse in water and it should be possible to collect its extracts. As Vansshlochan, one of the ingredients does not fulfil this parameter; the formulation which does not contain vanscholan was selected to prepare talisadisus pension. Talisadi suspension has many benefits over choorna, discussed as follows;

- Its shelf life is longer than choorna
- It is easy to administer in all (children and elderly)
- More absorbent and ease in dosage calculation,since in liquid form
- It is palatable
- Choorna may clog the trachea sometimes, which is not with the suspension.

Talisadichoorna possesses actions like deepen (stomachic), Pachan (digestive), Rochan (relishing)[6] and it is very frequently used in the management of Kaas (Cough), Shwas (Asthma), Pandu (Anemia), Jwar (Pyrexia), Chardi (Emesis), Atisaar (diarrhoea).Thus we can say choorna is a drug of choice in the treatment of various respiratory diseases including Tamakshwaas

Tamakshwaas is a disease of Pranavaha Strotas described in all the basic text of Ayurveda that can be correlated with Bronchial Asthma, which is a chronic inflammation of bronchioles causing narrowing and hence obstruction of air passage. This leads to difficulty in breathing. Thusactivities that mark improvement in bronchial asthma are anti inflammatory and mast cells stabilisation[7].

To generate evidence of activities of talisadichoorna, animal experiments were carried out in the department. Studies regarding Anti inflammatory[8&9] and Antihistanmic[8&9] activities of talisadichoorna containing natural[8] and synthetic[9] Vanscholan have been completed in which it was evident that TC possesses Anti inflammatory and Anti histaminic activites. Talismadi suspension has been tested only for its stability[10] and anti-microbialactivity. No further work was done.

Need of Study: Asantihistaminic and anti-inflammatory of the formulation in its suspension form has not been studied. Hence it was proposed to study these two activities of talisadi suspension and validate whether this modified dosage form possesses same mode of action as choorna or change in dosage form will affect its action.

Aim and Objectives: Aim: To study antihistaminic and anti-inflammatory activities of Talisadi Suspension in Experimental Animals.

Objectives:

- To prepare Talisadi Suspension by following SOP
- To assess antihistaminic activity of Talisadi Suspension on Guinea pigs
- To assess the ant inflammatory activity of Talisadi Suspension on Rats.

Material and Methodology

Preparation of Talisadi Suspension[5]: To prepare Talisadi Suspension, Standard Talisadi Choorna[4] was taken of 10 mesh size and Excipients Carboxyl Methyl Cellulose Sodium Salt (CMC),Methyl Paraben (MP) Pharmaceutical grade and Propyl Paraben (PP) Pharmaceutical grade was taken.

For 100 ml of Talisadi Suspension with concentration 2 gm TC per 10 ml suspension

- 95 ml freshly collected sterile distilled water was taken in sterile conical flask.
- 250 mg (0.25 %) CMC was dissolved in distilled water by constant agitation with glass rod at room temperature .
- 20 grams of TC was added to this solution and shook properly till all the particles were dispersed uniformly.
- MP and PP (0.2 gm each) were dissolved in 5 ml of DW and the solution was mixed to above suspension. These are preservatives used in the suspension.
- This TS was then packed in bottles.

Organoleptic tests: On preparing the suspension it was tested for its organoleptic characters and was found to be standard as it was identical with that of previous.

- Taste: Sweet, Pungent
- Odour: Aromatic
- Consistency: Turbid

- Colour: Light brown
- Redispersion pattern: Particles Redispersed easily
- Viscosity: 4500CPs

Experimental Studies: Permission from ethical committee was taken before starting the animal experiments.

Antihistaminic study[11]:

- Animal used for study: Guinea pig[12]
- Animal model used for study: Histamine Induced Bronchospasm

The study was conducted at Department of Pharmacology BVU medical college, Pune. Total 12 animals were used for study. 2 groups each of 6 animals was made, 6 for test drug and other 6 as control group. Adult male guinea pigs were used of 250-300gms. As per recommended adult dose of 10ml/day suspension in human beings the drug dose[12] was extrapolated in animals.

Methodology[13]: To study anti histaminic activity Guinea Pigs were pre-treated for 21 days with single dose of test and control drug orally. Daily care of animal was taken. On 2[2nd] day animals were subjected to Histamine Aerosol challenge and time required to produce pre-convulsive dyspnoea [PCD] was noted.

Anti-inflammatory study[12]

Animal used: Rat[12]

Animal model used: Cotton Wool Granuloma[14]: The study was conducted at Department of Pharmacology BVU medical college, Pune in collaboration with Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences. Total 12 animals were used for study. 2 groups each of 6 animals was made, 6 for test drug and other 6 as control group. Adult male Wistar Rats were used of 200-300gms. As per recommended adult dose of 10ml/day suspension in human beings the drug dose[12] was extrapolated in animals.

Table 1: Extrapolation of Dose in Rats

Group	For 70kg man in 24 hrs	Dose calculating factor	For 200mg Rat	For 1kg Rat
Talisadi suspension	10ml	0.181	1.81ml	9.05ml

Table 2: Dose calculation for Rat:

Group	Test drug	No. of Animals	Dose calculation in ml
A	Talisadi Suspension	6	Wt. of animal x 0.00905
B	Base of suspension	6	Wt. of animal x 0.00905

Methodology[14]: Wistar Rat of average wt of 200 gm was anaesthetised and back skin was shaved and disinfected. Incision was made in lumbar region and subcutaneous tunnel was formed. Sterile cotton pellet of 10mg was placed and sutured back. Inflammation took place and granuloma pouch was formed at incision site. Animals were treated with test drug orally for 7 days. On 8[th] day animals were sacrificed and granuloma pouch were removed, weighed and then dried at 400°C for 24hrs and weighed till constant weight was obtained.

Observations and Results

Anti- Histaminic activity:

Table 3: Time required for PCD

Group	Animal marking	Time required in sec
A. Experimental	Head	24.4
	Back	24.57
	Tail	25.67
	Right fore limb	27
	Left fore limb	24.65
	Right hind limb	32.85

Group	Animal marking	Time required in sec
B. Control	Head	20.99
	Back	19.22
	Tail	13.41
	Right fore limb	22
	Left fore limb	18.3
	Right hind limb	20.36

Result

Table 4: Values obtained on applying unpaired t-test

Sr. No.	Group	Mean	SD	T table	T cal	P value
1.	Group A	26.5233	2.9669	-4.03	-15.25	0.0000109
2.	Group B	19.0466	2.7863			

When mean of time taken for Pre Convulsive Dyspnoea was compared between control and Talisadi Suspension group by unpaired t-test at 1% level of significance. P value calculated was less than 0.01 which denotes significance difference between mean. Thus it was interpretated that Talisadi Suspension is effective against histamine induced bronchospasm.

Anti-inflammatory activity:

Table 5: Weight of Wet and Dried Granuloma

Group	Animal	Prior weight of granuloma pouch [mg]	Final weight of dried granuloma pouch [mg]
Talisadi Suspension	RP1	227	42.3
	RP2	199	42.6
	RP3	240	44
	RP4	195	47.3
	RP5	244	38.1
	RP6	395	35.8
Control	RQ1	245	53.5
	RQ2	284	47.8
	RQ3	275	54.9
	RQ4	248	57.6
	RQ5	191	50
	RQ6	181	62

Result

Table 6: Values obtained on applying unpaired t-test

Sr. No.	Group	Mean	SD	T table	T cal	P value
1.	Group A	41.6833	3.77	2.57	5.13	0.0018
2.	Group B	54.3	4.688			

When mean weight of dried granuloma was compared between control and Talisadi Suspension group by unpaired t-test at 1% level of significance. P value calculated was less than 0.01 which denotes significance difference between mean. Thus it was interpretated that Talisadi Suspension is effective as an anti-inflammatory agent.

Discussion

Talisadichoorna is one of the widely used formulations among Ayurvedic community. However it has some drawbacks found through experimental studies and to overcome such deficiencies it was converted into talisadi suspension. TS was tested in animal models to check activities i.e. anti-histaminic and inflammatory to study antihistaminic activity the model used was protection against histamine induced brochospasminguineapigs. The result showed that TS has antihistamicactivity. To study anti-inflammatoryactivity, model used was granuloma pouch model in rats. The result showed that TS has anti-inflammatory activity.

Conclusion

It was possible to prepare similar quality of TS by following SOP of previous study. TS matched the standard parameter from the observation recorded in the experiment carried out. It can be concluded that mode of action of TS is anti-inflammatory and antihistaminic. Talisadi Suspension works on the same mechanism as that of Talisadi Choorna. Absence of vanscholchan does not hamper the activity of formulation.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Attendance and Academic Performance of First BAMS Students in Subject Rachana Sharir: A Retrospective Cohort Study

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Abstract

Student's absenteeism is a continuous problem in medical education despite of mandatory attendance policies introduced by the universities. Aim and objective of the study was to correlate the class attendance and academic performance in theory examination in Rachana Sharir. Hence a retrospective cohort study was conducted at *Rachana Sharir* Department, DMAMCHRC Nagpur. Total 100 students from batch 2018 who were appeared for both internal assessment examination and final University examination of first BAMS were included in the study. Three groups were formed based on theory attendance percentage, Group I with more than an equal to 75%, Group II less than 75% and more than an equal to 50% and Group III with less than 50%. Data of first and second internal assessment theory examination as well as University theory examination of these 100 students was taken. Average marks of the students of these three groups were compared with their attendance percentage. Gender wise and residence wise (Hosteller -non hosteller) correlation studied. Female students and non-hostellers have more attendance and higher score in internal assessment and University examination. Significantly higher marks in internal assessment and University examination were observed in students with high attendance percentage. There was significantly high pass percentage in University examination in students with high attendance. Performance in internal assessment and University examinations were significantly affected by the attendance percentage of students.

Keywords: *Attendance, Internal assessment Examination. University examination, Rachana Sharir, academic performance.*

Introduction

Duration of Bachelor of Ayurved in Medicine and surgery (BAMS) course was four and half year, among which first BAMS duration was one year. After twelfth passing students entered in professional course where teaching pattern, examination pattern and course contents are vast different than HSC course. Hence to understand new terminology, to cope up with studies students should attend the classes regularly. Regular attendance in theory and practical, help the students to get clear idea about the subject as per course curriculum, which is essential for performing better result in examinations. Attendance during their undergraduate curriculum plays an important role to extrapolate their knowledge in the later professional life.[1]

BAMS Curriculum is vast and emphasis has been laid down by the Universities and regulatory bodies on attendance policies. Maharashtra University of Health Sciences has kept attendance minimum 75% for theory and 80% for practical as eligibility to appear in the

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university exams. Student attendance is an important part of professional development and it is measured as an evidence of professionalism.[2]

Several researches on class attendance established that on average, student with high attendance achieves higher academic performance in both coursework and examination than student with poor attendance[3-6]. Sarode et al conducted a study on Psychological Pain as Predictor of Impulse Control among BAMS New Entrants[7]. Quality of sleep has profound effect on Student's attendance in college. Rathi et al conducted a study on Quality of Sleep among Medical Students[8]. Goyal et al conducted the Assessment of Competency Based Medical Internship Training with 'Cumulative Grade Points Average System'[9]

Rachana Sharir is an essential subject as it contributes the basic knowledge of structural, histological aspect of body. Thorough understanding of anatomy is necessary for effective and safe treatment of patients. The classroom lectures and dissection sessions are the primary teaching method in subject Rachana Sharir for undergraduate students. The lecture-based learning enhances the cognitive, affective and psychomotor skills of the students.[10] The lectures provide benefit for learning despite its didactic nature and poor feedback. The dissection sessions (practical)also are most effective tool for improving student's knowledge, goes by hand with theory classes for better understanding and concept building.[11] If the student misses these classes, generally it will lead to incomplete learning and poor academic performance.

However, the studies showing relationship between separate theory and practical attendance and their comparison with assessment are limited. In Ayurveda profession there may be hardly any study on this issue. Hence, this retrospective cohort study is done to know whether attendance had any correlation with academic performance.

Material and Method

This retrospective cohort study to assess the effect of class attendance on academic performance in theory examination of first professional BAMS students who have passed examination in July 2019 in the Rachana Sharir Department of Datta Meghe Ayurvedic Medical College, Hospital and Research Centre, Wanadongri, Nagpur, affiliated to Maharashtra University of Health sciences (MUHS), Nasik.

Students have to appear for two internal assessment examinations which were conducted in January and April 2019 as per University policy. Final University examination was conducted by University in May-June 2019. Universal sampling all 100 students of first BAMS (Batch 2018-19) who had appeared in both internal assessment examinations and final University examination were taken as study subjects. This study was an observational study so there was no ethical issue.

The data of attendance and marks in internal assessments was obtained from department record and University theory examination marks were obtained from MUHS result records. The data was compared to know whether there is any correlation between attendance and performance of students. Three groups were formed based on attendance percentage, Group I with more than an equal to 75%, Group II less than 75% and more than an equal to 50% and Group III with less than 50%. These groups were formed as per student's original attendance.

However after second assessment examination, to make students eligible to appear University examination extra classes were conducted.

Data entered in Microsoft Excel as attendance percentage and marks obtained by the students. Also gender wise and residence wise (Hosteller -non hosteller) data entered in Microsoft excel sheet. Results were tabulated; the quantitative data was analyzed by average, proportions and Chi-square test for significance. $P< 0.05$ significance was expressed.

Result

This study was undertaken to see the attendance and academic performance of BAMS students in subject Rachana Sharir. Total 100 admissions in the study institute and all 100 students included in the study. The average attendance of the students was 68.31±11.95% ranging from 34.0 to 90.67%. The average internal assessment marks in theory was 44.84±14.15% with range 3.89-78.89. The average Final University marks scored in theory was 56.12±9.39% ranging from 26.67 to 80.0. To compensate the less attendance of the students, Rachana Sharir department had conducted 45 extra classes so as to make the students eligible for university examination.

There were 63 female students and 37 male students, their attendance and performance in internal assessment and final University examination marks are as shown in table No. 1.

Table 1: Gender wise distribution of study subject as per attendance and marks score.

Gender	Female	Male	P value
No. of students	**63**	**37**	
Attendance in percentage	70.43±10.64	64.71±13.29	0.01, S
Int. Assessment Marks in percentage	46.97±14.72	41.23±13.71	0.02, S
Uni. Marks in percentage	57.81±9.55	53.24±8.47	0.009, S

The average attendance of female students was 70.43±10.64% and that of male students was 64.71±13.29%. Female students have more attendance than their counter part. This difference was statistically significant (p=0.01). Internal assessment marks scored in theory by female students was 46.97±14.72% and that of male students was 41.23±13.71%. This difference in marks scored in theory examination was statistically significant (p=0.02). Final University examination marks scored by female students was 57.81±9.55% and that of male students was 53.24±8.47% and this difference was statistically significant (p=0.009).

There were 74 students who were staying in hostel and 26 students who were staying at home (non-hosteller),their attendance and performance in Theory internal assessment and final University examination marks are as shown in table No. 2.

Table 2: Residence wise distribution of study subject as per attendance and marksscore.

Residence	Non Hostel	Hostel	P value
No. of students	**26**	**74**	
Attendance in percentage	72.29±11.45	66.92±11.87	0.02, S
Int. Assessment Marks in percentage	49.08±15.37	43.36±14.06	0.04, S
Uni. Marks in percentage	57.80±9.11	55.53±9.47	0.14, NS

The average attendance of non-hosteller students was 72.29±11.45% and that of hosteller students was 66.92±11.87%. Non-hosteller student's attendance was more than that of hosteller students. This difference was statistically significant (p=0.02). Internal assessment marks scored in theory by non-hosteller students was 49.08±15.37% and that of hosteller students was 43.36±14.06%. This difference in marks scored in theory examination was statistically significant (p=0.04). Final University examination marks scored by non-hosteller students was 57.80±9.11% and that of hosteller students was 55.53±9.47% though the marks scored by non hosteller was more but this difference was not statistically significant (p=0.14). Attendance and performance in theory examination of internal assessment and final examination were shown in table no 3.

Table 3: Distribution of study subject as per attendance and marks obtained in theory

Group Percentage	Group I (≥75)	Group II < 75 and ≥ 50	Group III < 50	Total	ANOVA P Value	Post hoc Results
No. of students	30	62	08	100		
Attendance	81.00±4.37	65.55±6.52	42.18±5.61	68.31±11.95		

Group Percentage	Group I (≥75)	Group II < 75 and ≥ 50	Group III < 50	Total	ANOVA P Value	Post hoc Results
Int. Asse Marks in percentage	50.11±10.85	44.87±13.88	24.93±16.02	44.84±14.55	0.000	Gp I vs Gp II: NS Gp II Vs Gp III : S Gp I Vs Gp III : S
Uni. Marks in percentage	58.15±6.79	56.21±10.03	47.78±9.06	56.12±9.39	0.000	Gp I vs Gp II: NS Gp II Vs Gp III : S Gp I Vs Gp III : S

There were 30 students whose attendance was more than or equal to 75%, were in group-I. 62 students have attended more than or equal to 50 and less than 75% were in group - II and 8 students attended the classes less than 50% were in group - III. The average attendance was 81.0± 4.37, 65.55±6.52 and 42.18±5.61 in group I, II and III respectively. The marks scored in internal assessment in theory were 50.11±10.85, 44.87±13.88 and 24.93±16.02 in group I, II and III respectively. The difference was statistically significant between group II verses III and group I verses III but not significant in group I verses II. The marks scored in final theory examination in percentage were 58.15±6.79, 56.21±10.03 and 47.78±9.06 in group I, II and III respectively. The difference was statistically significant between group II verses III and group I verses III but not significant in group I verses II.

Table No. 4 shows marks scored in internal assessment theory examination by students from different groups of attendance in subject Rachana Sharir.

Table 4: Distribution of study subjects as per attendance and marks obtained in Internal Assessment Exam

Group Percentage	No. of students	Marks less than 50 %		Marks more than and equal to 50%	
		No.	%	No.	%
Group I ≥ 75	30	17	56.67	13	43.33
Group II < 75 and ≥ 50	62	44	70.97	18	29.03
Group III < 50	08	08	100.00	00	00.00
Total	100	69	69.00	31	31.00

Chi square = 3.04, d.f. = 1, (^{3}75 Vs <75), P = 0.08, NS

17(5 marks and 13 (43.33%) students scored more than an e6.67%) students scored less than 50% qual to 50%, in theory internal assessment examination, whose attendance was more than 75 %(group I). From group II, 44(70.97%) scored less than 50% marks and 18(29.03%) scored more than an equal to 50%. All 8(100%) scored less than 50% were among group III. However the marks scored in these different groups were not statistically significant.

Table No. 5 shows marks scored in final University theory examination by students from different groups of attendance in subject Rachana Sharir.

Table 5: Distribution of study subject as per attendance and marks obtained in University Exam

Group Percentage	No. of students	Marks less than 50 %		Marks more than and equal to 50%	
		No.	%	No.	%
Group I ≥ 75	30	03	10.00	27	90.00
Group II < 75 and ≥ 50	62	16	25.81	46	74.19
Group III < 50	08	04	50.00	04	50.00
Total	**100**	**23**	**23.00**	**77**	**77.00**

Chi square = 4.089, d.f. = 1, (^{3}75 Vs <75), P = 0.043,S

Less Only 3 (10.0%) students scored than 50% marks and 27 (90.0%) students scored more than an equal to 50%, in final university theory examination, whose attendance was more than 75 % (group I). From group II,. III equal no. of students i. e. 4(50.0%) scored less than as well as more than and equal to 50%. This difference was statistically significant (p = 0.043). 16(25.81%) scored less than 50% marks and 46(74.19%) scored more than an equal to 50% and in group.

Table no 6 shows the University result in subject Rachana Sharir by attendance in different groups.

Table 6: Distribution of study subject as per attendance and University Result In subject

Group Percentage	No. of students	Pass		Fail	
		No.	%	No.	%
Group I ≥75	30	29	96.67	01	3.33
Group II < 75 and ≥ 50	62	49	79.03	13	20.97
Group III < 50	08	05	62.50	03	37.50
Total	**100**	**83**	**83.00**	**17**	**17.00**

Chi square=5.67, d.f. = 1, (^{3}75 Vs <75), P = 0.017, S

From group I, 29(96.67%) students passed in final University Examination in subject Rachana Sharir and only 1 (3.33%) student failed. 49(79.03%) students passed and 13(20.97%) students failed from group II. Whereas from group III, 5(62.50%) students passed and 3 (37.50%) failed. It indicates that higher the percentage of attendance, more number of students gets through the examination and vice versa and this difference is statistically significant.

Discussion

Student's absenteeism is a continuous problem in medical education despite mandatory attendance policies introduced by the universities. Professional courses like medical education require high attendance in theory and practical classes for better understanding of the subject and for acquiring skills for better performance in their later career life. There is no better alternative of attending the classes for better academic performance in medical profession.

The average attendance of female students was 70.43±10.64% and that of male students was 64.71±13.29%. Internal assessment marks scored in theory by female students was 46.97±14.72% and that of male students was 41.23±13.71%. Final University examination, marks scored by female students was 57.81±9.55% and that of male students was 53.24±8.47%. Average attendance, internal assessment examination and University examination marks were more in female students than male students and this difference was statistically significant. This indicates that female students are more sincere and studious.

The average attendance of non-hosteller students was 72.29±11.45% and that of hosteller students was 66.92±11.87%. Internal assessment marks scored in theory by non-hosteller students was 49.08±15.37% and that of hosteller students was 43.36±14.06%. Average attendance and internal assessment examination marks were more in non-hosteller students than hostel students and this difference was statistically significant. Hostellers are from other places and initially may be homesick. They may require more time to get adjusted with professional course environment.

The average attendance was 81.0± 4.37, 65.55±6.52 and 42.18±5.61 in group I, II and III respectively. The marks scored in internal assessment in theory were 50.11±10.85, 44.87±13.88 and 24.93±16.02 in group I, II and III respectively. The marks scored in final theory examination in percentage were 58.15±6.79, 56.21±10.03 and 47.78±9.06 in group I, II and III respectively. The difference was statistically significant between group I verses group III and group II verses group III. Similar findings were observed by Sangeeta M Varalakshmi K[12], Richard P Deane and Deidre J Murphy[13], Lima Koruthara Mohanan et al[14]

From group I, 90% students score more than 50% marks in University examination while in group II and III score 74.19% and 50% students score more than 50% marks. This difference was statistically significant. Similar observation was found by Priya Dhengre and Ashok Jadhao(1), Roy SS and Chadalawada J[15], Biswas SS and Jain V(6)

From group I, 96.67% students passed in final University Examination in subject Rachana Sharir, 79.03% group II. 62.50% students passed from group III. It indicates that higher the percentage of attendance, more number of students gets through the examination and this difference is statistically significant. Similar observation by Lima Koruthara Mohanan et al(14), Varul M, Vegad A et al[16], Chilwant KS and Hundekari JC[17].

Attendance and performance are closely related with each other. Assessment is an integral part of education system. The goal of assessment is to monitor student's academic performance [18]. To improve the performance of students we can apply remedial teaching method stated by P. Dandekar through which performance can improve which helps to create student's interest in the studies. Assessment improves students approach towards learning [19]. It also helps in attendance improvement. Applying Objective structured practical examination (OSPE) assessment system used by Anita et al could improve students' performance and attendance [19.]

Here in this study an effort had been taken to find out the correlation between attendance & academic performance based on formative and summative assessment.

Conclusion

This study demonstrates a significant association between attendance percentage and academic performance in internal assessment theory and final University examinations of first BAMS students in subject Rachana Sharir. It was also observed that gender wise and residence-wise correlation between class attendance and academic achievement. Further studies require identifying the reasons for student absenteeism and measures to overcome these factors for improving the quality of education in Ayurved colleges.

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Forensic Odontology: A Potent Tool for Human Identification

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Abstract

Throughout the last 100 years, Forensic Odontology is becoming an integralpart of Medico Legal research which uses dental as well as oro-facial studies to aid in the investigation for judicial system. It can be used in legal investigations which involve the identification of human remains, bite marks and age estimation & the list goes on. Dental practitioners have a significant responsibility of keeping correct dental records and supplying the relevant details to law enforcement authorities'. This paper will attempt to sum up the current positions of Forensic Medicine dental experts.

Keywords: *Age Estimation, Bite Mark, Rugoscopy, Cheiloscopy.*

Introduction

Forensic Dentistry is a fresh increasing segment of Forensic Medicine. The history of Forensic Odontology begins in 49 A.D. with Agrippina, the mother in Roman Emperor Nero. She identified the discoloration of front teeth of her rival, Lollia-Paulina[1]. In 1970, Keiser Nielson identified Forensic Odontology as well as Forensic Dentistry as "branches of Forensic Medicine which, in the interest of justice, dealing with the safe handling and analysis of dental proof & with the proper assessment and presentation of dental results." Forensic science applies to fields of research that can be used in the judiciary and approved by courts and the basic scientific panel to distinguish reality from outright lie[2].

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Individual's identity is the foundation of civilization and has always been of essential value for humanity. It is not only necessary to recognize the individual to ensure proper identification but there are also concerns such as criminal inquiries, insurance claims and legal trials that could only be addressed with a successful identifications. Identifying a missing person will aid family and friends enormously in their dispute resolution process. Finally to sum up, in a current scenario; forensic dentists play a significant role in victim identification, study of biting marks, maxillofacial damage and negligence and so on.

Identification: Dentistry plays a key role in identifying human remains whenever severe tissue damage or loss of a fingerprints database invalidate the use of traditional method. Whenever the dead person is skeletonized, decomposed, burned or disemboweled, the recognition of dental remains is of prime importance. The key benefit of the dental evidence is that it is always retained after death, like many hard tissue. Even a human's teeth status changes during lifetime and the texture of declined, missed and filled tooth is observable and equivalent to any fixed time mark. Teeth seem to be the strongest sections of a bodies and it can be heated to higher temperatures without even any noticeable loss of micro–structural[3,4]. Teeth may remain relatively intact

even after deterioration or decomposition has damaged many soft tissues and skeletal tissues.

Species Identification: Species identification generally does not pose any problems because only uneven evidence is discovered at crime scene. Fragment of lower jaw, impacted teeth or, at the very worst, a tiny portion of a single tooth with no more than a few mm in size may exist. It's been shown recent times which dentinal fluids generate special information about the species. Such fluids can be contrasted with artificial antisera utilizing counter - current electrophoresis. The whole method is capable of identifying species up to twelve months afterdeath[5].

Gender and race determination: Parentage can be obtained by examining a facial anatomy by matching the characteristics of three ethnic groups' with primary features: Mongoloid, Negroid and Caucasoid[6]. Even before ethnic group is described, the sexuality of a person is the most vital of variables to be determined from a skeletal system. If a bone's sex is correctly determined, the identification process automatically removes almost 50 per cent of the total bias. In particular, gender identification is not a complicated question when the full skeleton is present but there are not always bony remains were found in this state. For eg, bones can indeed be fractured into many fragments during aircraft accidents but only a tiny proportion can be made usable for recognition. Techniques, such as DNA analysis of genetic material or molecular DNA for genetic mutation identification and a limited repeated series of DNA to fit such results with presumed serologic research results, are becoming very common.[7,8]

DNA research of dental forensics: Utilizing DNA testing, the sample which is derived from biological matter like blood, semen, hair root, skin, teeth, bone and saliva may be determined. DNA was successfully isolated on both saliva including salivary-stained content in 1992.[9] Saliva includes sloughed epithelialcell from the internal layer of a lip and mucous membrane; It is probable DNA reference.[10] Multiple enzymes are found in saliva from a multitude of outlets. There are Streptococcus salivarius & Streptococcus mutans in the saliva as well as on the teeth. In the science of polymerase chain reaction (PCR), a streptococcal DNA sequence gives us a way of distinguishing the bacterial composition through bite mark and it can be compared specifically with those in the teeth.[11] The dental pulp taken from given tooth specimen may be used to test DNA. Latest tooth test may be considered to have better sources for blood group determinations. The involvement of ABO blood categorizing antigens in hard and soft oral tissues allows us to determine the typing of the blood group and help identify a highly decapitated body. However, The impact for proteolysis, starvation and lack of pulp antigens or even a higher amount of overseas antigen-borne defects on root canal teeth that result in variations throughout the analysis.[12] Whittaker as well as Rawle endorse that even for a duration of 1-6 months after removal, the antigenicity of crushed dentin as well as cement of extracted human teeth remains unchanged regardless of social circumstances.[13]

Facial Reconstruction and facial superposition: When the post mortem description doesn't really elicit tentative recognition of the deceased, it may even be necessary to recreate the image of a person throughout lifetime. That's the duty of forensic experts who are using the oral image to support recreate their facials. The need for pre mortem images to enable facial superposition of skeletal and dental characteristics has been used for verification purposes. The procedure demands that appropriate perimortem images showing the dental be made available. Assigned work and distortion also place problems in placement of a pictures[14].

Determining the age based on the dental records: The assessment of age forms essential portion of forensic dentistry. Human dentition continues to follow the consistent and sustainable pattern of development. Once all the permanent teeth are fully developed,use of X rays is reflective of processes involving findings from distinct cell mineralization stages. These determinations are often used mostly on level of roots & crown structure development, eruption stages.[15] Children's age could be obtained by evaluating the growth of the tooth as well as consequent compare with the growth graphs, generally to a consistency of about 1.6 year. Usage of the third molar attrition and growth has been proposed as a means for aging for all those persons over the age of 18 and both are ineffective. The use of attrition and development of the 3rd molar have been suggested as means of ageing for those individuals over 18 years of age, but both are unreliable. New strategies such as aspartic racemization and transparent dentin have also been suggested and also have proven highly effective in the assessment of adulthood.[16,17] Two factors which can be used to determine age in adult are the evaluation of a pulp cavity quantity as well as the 3rd molar of growth. The decrease in the volume of the pulp cavity arising

from either a deposit of secondary dentin with ageing as measured by radiology can be used as a guideline for estimating an individual's personal age.[18,19,20]

Teeth image matching via cone beam computational tomograph & X-ray micro-focus CT: Beam of the cone[21] Computed Tomograph(CT) and X-ray micro- focus CT can also be used on the proportion of pulp as well as teeth volume.[22] X-ray offers low-resolution, two dimensional information whereas cone-beam CT technique enables the extracted tooth's root canal to be constructed in three dimensions. It appears to work with much the same monitoring concept as used in medical computed tomography (CT); even then, order of magnitude greater was its high resolution. A coronal pulp cavity proportion is indeed a useful measure for age assessment in the forensic context, particularly in persons with different datasets. The coronal pulp cavity ratio is a reliable biomarker for age study in the forensic control, especially in the living individuals of unknown information.[22]

Radiographic Examination: Radiograph and dental cast are valuable medico legal recording devices. Through diligent documenting and written explanations of a tooth structure as well as radiographs, the forensic odontologist provides the post mortem documents. Radiograph must be labelled with holes to prevents ambiguity-one gap in ante mortem film with double holes in post-mortem films.[23] By recording a dental identity a variety of solutions have been proposed. The US forensic dentistry board recommends that some of these are restricted to just the four main conclusion:[24]

- **Accurate identity:** Ante- as well as post-mortem information fit in great detail, without any unexplained differences, to determine that they have been identical to each other.
- **Probable identity:** Ante-as well as post-mortem information have similar characteristics however it is not able to determine the identification confidently because of its consistency from either the post-mortem remain or even the ante mortemproof possible to establish the identity positively.
- **Lack of evidence:** The information available is inadequate to provide the foundation for just a summary.
- **Exclusion:** The information from ante-mortem and post-mortem are obviously incoherent.

Bite mark evidence: Biting is always a symbol of an offender trying to ensure maximum dominant position.[25] Bite mark consist mainly of surface deformation, and/or subsurface hemorrhage, or skin bruise due to biting.[26] Bite mark analysis is one element of forensic dentistry which requires the forensic dentist to respond immediately. The bite mark may result from either a physical alterations in a channel triggered by teeth interaction, or from a representational sequence remaining during an entity or tissue by either a human or animal's tooth structure. The markings, individual or numerous in nature, can range in intensity, varies from minor tissue creating to extreme epidermis and dermis disruption.[27] To prevent inconsistency and improve the quality of bite marks analysis, the American Board of Forensic Odontologist (ABFO) has developed a manual on bite mark technique to acquire and maintain the mark. The advice is not really a directive but a compilation of approaches universally acknowledged.[28] An exact digital file with a one to one move to clear sheet or acetate paper are invaluable for capturing a bite marks. Bite trauma rinsing is critical in recovering proof of residue. Wherever necessary, stain of saliva or human tissue must be obtained for just a DNA evidence.[29] The investigation of an identification of bite mark and lip print, that can be created in sexually and nonsexual attacks, murders, as well as in non biological material and artifacts left in scene of crimes, includes the use of advanced photographic technique or even electron microscopy. In these procedures, careful selection and handling of information is necessary in order to ensure that the chain of evidence meets legal criteria for its appropriateness of court evidence.[17] Process of Healing

The recovery process of bite mark may be helpful in assessing a period of a bite marks, is applied prior to the time of deaths in situations where suffocation has caused death. The discoloration of a bite marks compared to the redness of the bruises also on neck suggests the duration of the imposition of a bite marks prior to the assassination.[30]

Recent advances in forensic dentistry:

Rugoscopy: Palatal rugae comprise about three to seven ridges radiating out tangentially from the incisive papilla. These ridges can be classified as curved, straight, wavy and branched. The pattern of these rugae is considered unique to an individual.[31] Identification is not possible, as in edentulous mouths, palatal rugae can be used as a supplement. In case of teeth loss, due

to reasons such as trauma, palatal rugae pattern serves as an alternative method for identification because of its uniqueness. As rugae is internally placed in the oral cavity and is protected by tongue and buccal pad of fat, it remains undisturbed from heat and other assaults. Rugae patterns change with age and other environmental influences such as orthodontic movements, tooth extraction, cleft palate surgery, periodontal surgery and impacted canine eruption. Materials and Method used to analyze the rugae patterns includes, photographs and impression of maxillary arch, computer software programs (for e.g., RUGFP-ID), calcorrugoscopy or overlay print, stereoscopy (through which three-dimensional [3D] image of palatal rugae can be made), stereophotogrammetry (which is comparatively accurate).[32,33]

Cheiloscopy: Cheiloscopy is a forensic investigation technique that deals with identification of humans based on lips traces. Lip prints have to be obtained within 24 hours of time of death to prevent erroneous data that would result from post mortem alterations of lip. Lip print pattern depends on whether mouth is opened or closed. In closed mouth position lip exhibits well defined grooves, where as in open position the groves are relatively ill defined and difficult to interpret. The foundations of cheiloscopy, however, are the same as that of dactylography, that is to say, lip prints are invariable, permanent & allow establishing a classification[34,35].

Ameloglyphics: [Study of patterns of enamel rods] Ameloblasts lay down the enamel rods in an undulating and intertwining path. This is reflected on the outer surface of the enamel as patterns of the ends of a series of adjacent enamel rods. This study of the enamel rod end patterns is termed as ameloglyphics by Manjunath et al[36].and could aid as an identification tool in decomposed or burned bodies as enamel can resist decomposition.

Conclusions

Forensic dentistry plays an important task in identifying certain individuals those are not visibly or otherwise recognizable. The special design of our tooth development and the positioning of design restorements guarantee precision whenever the rules are employed correctly. Sadly, forensic dentistry also isn't practiced in countries such as India up to the global standards and facilities of forensic dentists haven't been used. The policy will order the Indian Dental Association and other provided by government to guide the country's dental surgeons to preserve the dental data and debris of teeth substance in the case of dental surgeon-treated patient restoration work. Therefore the stored content may be used to identify deceased individual.

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Management of Coronary Artery Disease Through Yoga

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Abstract

Cardiovascular diseases are the leading cause of morbidity & mortality accounting for approximately 30% global burden of death per year. Disorders & in turn diseases due to gradual shifting towards sedentary lifestyle diseases like type 2 diabetes mellitus, hypertension, stroke, obesity etc are the major menacing factors for the progression of coronary artery disease. With speedy development of commerce and increased inclination towards lifestyle influenced by western countries in the past many years, prevalence of such diseases has reached frightening proportions among Indian population. Apart from physical consequences of cardiovascular diseases which are prominently looked for and taken care of, mental aspects of such patients is always overlooked. Suchpatients mentally become vulnerable & always remain worried & keep on feeling in their subconscious mind that soon they may die due to their heart condition. This in turn results into stress and anxiety which has become common amongst all the Indians. Medicines are commonest curative remedy in India but through this article, I am proposing some remedies through Yoga. This article doesn't guarantee 100% result but it is preventive remedy for Coronary Artery Disease.

Keywords: *Coronary artery disease, Yoga, lifestyle disorders.*

Introduction

When the major blood vessels (aorta, right coronary, left coronary, left anterior descending & circumflex) that supply heart get pathologically damaged, coronary artery disease develops. The most common cause for such pathological assault on coronaries is development of atheromatous plaques over their inner walls. Gradually it will lead to narrowing which in turn lead to partial or total block of the vessels. These blocks will limit or obstruct the blood supply to heart which will ultimately lead to ischemia to the heart. There are varieties of clusters of symptoms that will give indication of coronary heart disease. They are pain or discomfort (arms, left shoulder, back, neck, jaw or upper abdomen), breathlessness, palpitations, sweating, extreme weakness or anxiety. Various risk factors for coronary heart disease are:

- **Age:** As it advances, coronaries get narrowed and hardened.
- **Sex:** Comparatively women are protected but their risk increases after menopause.
- **Family history:** Person is more susceptible if any close relative got any history of coronary heart disease.
- **Smoking:** Smokers are at higher risk for developing coronary heart disease.
- **High blood pressure:** Uncontrolled blood pressure always result in hardening and narrowing of coronaries arteries.
- **High blood cholesterol levels:** There are higher chances of development of plaques which result in furtherance of atherosclerosis.

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- **Diabetes:** Type 2 diabetics are more prone.
- **Obesity:** Excess weight typically worsens other risk factors.
- **Physical inactivity & High stress:** They will worsen the situation.
- **Unhealthy diet:** High amounts of saturated fat, trans fat, salt and sugar can increase your risk of coronary artery disease.

Prevention: The same lifestyle habits that can help treat coronary artery disease can also help prevent it from developing in the first place. Leading a healthy lifestyle can help keep your arteries strong and clear of plaque. To improve your heart health, you can: Quit smoking, Stay physically active, Eat a low-fat, low-salt diet that's rich in fruits, vegetables and whole grains, Maintain a healthy weight, Reduce and manage stress. Yoga is giving a new lease of life to many, so to tackle chronic diseases it is providing remedies which will be lifelong. Yoga will provide support to patients having coronary artery disease through its various relevant postures (asana's) which can be done very easily. Yoga does not send a message that if you do it regularly you will be relieved from a particular disease. If it is performed every day it will add vital energy for smooth life even if you are suffering from any chronic disease. In this article we will be providing 6 postures (asana's) which can be performed at any time in the day but early morning will always be preferred (empty stomach) to get good result. Secondly this postures must be implemented after due consultation with your physician. If your physician disapproves don't do these postures as it will be harmful for your body[1-3].

Asanas:



1. **Utkatasana:** It is also called as Chair Pose. Chair Pose clearly works the muscles of the arms and legs, but it also stimulates the diaphragm and heart. The steps are being given and it should be done maximum 10 times or as per your strength.

Step 1 - Stand in Tadasana. Inhale and raise your arms perpendicular to the floor. Either keep the arms parallel, palms facing inward, or join the palms.

Step 2- Exhale and bend your knees, trying to take the thighs as nearly parallel to the floor as possible. The knees will project out over the feet and the torso will lean slightly forward over the thighs until the front torso forms approximately a right angle with the tops of the thighs. Keep the inner thighs parallel to each other and press the heads of the thigh bones down toward the heels.

Step 3- Firm your shoulder blades against the back. Take your tailbone down toward the floor and in toward your pubis to keep the lower back long.

Step 4- Stay for 30 seconds to a minute. To come out of this pose straighten your knees with an inhalation, lifting strongly through the arms. Exhale and release your arms to your sides into Tadasana.

2. **Bhujangasana:** Bhujangasana or Cobra Pose is a reclining back-bending asana in hatha yoga and modern yoga as exercise.



Step 1- Lie flat on your stomach on the floor. Stretch your legs back, tops of the feet on the floor. Spread your hands on the floor under your shoulders. Hug the elbows back into your body.

Step 2- Press the tops of the feet and thighs and the pubis firmly into the floor.

Step 3- On an inhalation, begin to straighten the arms to lift the chest off the floor, going only to the height at which you can maintain a connection through your pubis to your legs. Press the tailbone toward the

pubis and lift the pubis toward the navel. Narrow the hip points. Firm but don't harden the buttocks.

Step 4- Firm the shoulder blades against the back, puffing the side ribs forward. Lift through the top of the sternum but avoid pushing the front ribs forward, which only hardens the lower back. Distribute the backbend evenly throughout the entire spine.

Step 5- Hold the pose anywhere from 15 to 30 seconds, breathing easily. Release back to the floor with an exhalation.

3. Makarasana:



Step 1: Lie down on the floor on your stomach.Fold your hands and keep the tip of the elbows on the ground with your fingers facing upwards. Keep your elbows shoulder distance apart.

Step 2: Now, raise your shoulders and head. Keep your neck straight and look ahead.Bend your head a little forward and place your chin in your palms.

Step 3: Stretch out your legs with the toes facing outwards. Feel your body touching the ground.Breathe normally and slowly and relax your muscles.

Step 4: Stay in the asana for a few minutes until you feel completely relaxed.To release from the position, gently remove your palms from the chin, bring your shoulders and head down and roll over.

4. Padmasana:



Step 1: Sit on the floor with your legs straight in front. Bend your right knee and bring the lower leg up into a cradle: The outer edge of the foot is notched into the crook of the left elbow, the knee is wedged into the crook of the right elbow and the hands are clasped (if possible) outside the shin. Lift the front torso toward the inner right leg so the spine lengthens (and the lower back does not round). Rock your leg back and forth a few times, exploring the full range of movement of the hip joint.

Step 2: Bend the left knee and turn the leg out. Rock your right leg far out to the right, then lock the knee tight by pressing the back of the thigh to the calf. Next swing the leg across in front of your torso, swiveling from the hip and not the knee and nestle the outside edge of the foot into the inner left groin. Be sure to bring the right knee as close to the left as possible and press the right heel into the left lower belly. Ideally the sole of the foot is perpendicular to the floor, not parallel.

Step 3: Now lean back slightly, pick the right leg up off the floor and lift the left leg in front of the right. To do this hold the underside of the left shin in your hands. Carefully slide the left leg over the right, snuggling the edge of the left foot deep into the right groin. Again swivel into position from the hip joint, pressing the heel against the lower belly and arrange the sole perpendicular to the floor. Draw the knees as close together as possible. Use the edges of the feet to press the groins toward the floor and lift through the top of the sternum. If you wish, you can place the hands palms up in *jnana mudra,* with the thumbs and first fingers touching.

Step 4: Padmasana is the sitting asana par excellence, but it's not for everybody. Experienced students can use it as a seat for their daily pranayama or meditation, but beginners may need to use other suitable positions. In the beginning, only hold the pose for a few seconds and quickly release. Remember that Padmasana is a "two-sided pose," so be sure to work with both leg crosses each time you practice. Gradually add a few seconds each week to your pose until you can sit comfortably for a minute or so. Ideally you should work with a teacher to monitor your progress.

5. Shavasana:



Step 1: Lie on your back with your legs straight and arms at your sides. Rest your hands about six inches

away from your body with your palms up. Let your feet drop open. Close your eyes. You may want to cover your body with a blanket.

Step 2: Let your breath occur naturally.Allow your body to feel heavy on the ground.

Step 3: Working from the soles of your feet up to the crown of your head, consciously release every body part, organ and cell.

Step 4: Relax your face. Let your eyes drop deep into their sockets. Invite peace and silence into your mind, body and soul.Stay in Shavasana for five minutes for every 30 minutes of your practice.

Step 5: To exit the pose, first begin to deepen your breath. Bringing gentle movement and awareness back to your body, wiggling your fingers and toes. Roll to your right side and rest there for a moment. With an inhalation, gently press yourself into a comfortable seated position. Let your head be the last thing to come into place. Carry the peace and stillness of Shavasana with you throughout the rest of your day.

6. Pawanmuktasana:



Step 1: Lie flat on your back on a smooth surface, ensuring that your feet are together and your arms are placed beside your body.

Step 2: Take a deep breath. As you exhale, bring your knees towards your chest and press your thighs on your abdomen. Clasp your hands around your legs as if you are hugging your knees.

Step 3: Hold the asana while you breathe normally. Every time you exhale, make sure you tighten the grip of the hands on the upper shins and increase the pressure on your chest. Every time you inhale, ensure that you loosen the grip.

Step 4: Exhale and release the pose after you rock and roll from side to side about three to five times. Relax.

Benefits of Yoga in coronary heart disease:

1. It tones your digestive organs and heart.
2. Stretches chests and lungs, open the heart and lungs.
3. It relaxes your body completely and keeps you rejuvenated.
4. It calms the brain, increase awareness and attentiveness.
5. It helps to lower blood pressure, calms the brain and helps relieve stress and mild depression, lastly relaxes the body.
6. Strengthens the abdominal muscle, massages the intestine and internal organs of digestive system and improves digestion.

Conclusion

Current research on coronary artery disease and its implication concludes that Yoga plays a vital role in maintaining your health. It also gives an impetus to your body to keep moving. Any disease basically sends a negative signal and to counter it,Yoga plays a major role[4-6]. The study gave an opportunity to understand the reasons for coronary artery disease and subsequently how Yoga can provide benefits to a common man. Everybody should take out time from there regimen to focus on Yoga as it will give an added energy to your vital organs and keep you healthy and fit[7,8]. Secondly the postures recommended by the researcher is very simple and can be done by any age group person. The various Yoga poses will help you in leading a happy and stress-free life. Keep doing it and stay healthy and keep your heart strong[9].

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Management of Renal Calculus (Mutrashmari) with Hydronephrosis by Ayurvedicformulations: A Case Study

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Abstract

Background: Stones that grow in the urinary tract (recognized as nephrolithiasis or urolithiasis) form when the urine becomes excessively supersaturated with respect to a mineral, leading to crystal formation, growth, aggregation and retention within the kidneys[1].Worldwide, approximately 80% of kidney stones are composed of calcium oxalate (CaOx) mixed with calcium phosphate (CaP). Stones composed of uric acid 9%, struvite 10% and cystine of 1% are common[2].

Mutrashmari (Renal calculi/Kidney stones) is one among the Ashtamahagada (eight fatal conditions) and is Kaphapradhan Tridoshaja Vyadhi. It is correlated with urolithiasis. The prevalence of symptomatic urolithiasis is approximately 10% in men and 5% in women. Many treatment modalities have been adopted in medical sciences, but it is quite expensive and also the recurrence of development of stone cannot be avoided. Hence, it is necessary to find out easily available, an cost effective medicinetotreat Mutrashmari.

Aim: To evaluate the efficacy of Shaman Chikitsa in Mutrashmari

Materials and Method: It is a single case study. A 37-year-old woman who approached to outpatient department of Kayachikitsa of Datta Meghe Ayurved Medical College Hospital and Research Centre with complaints of pain in abdomen, low backache, which was radiating from loin to groin region; burning micturition; and dysuria. The patient was administered with Shaman Aushadhi.

Results: The patient got 100% results in chief and associated complaints and during and after the completion of therapy there was an improvement in the quality of life of the patient.

Conclusion: Satisfactory relief in symptoms was seen in patient after 7 days of Shaman Chikitsa.

Keywords: *Mutrashmari, Shaman Chikitsa,urolithiasis, hydronephrosis, Ashmari.*

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Introduction

Stones that grow in the urinary tract (recognized as nephrolithiasis or urolithiasis) form when the urine becomes excessively supersaturated with respect to a mineral, leading to crystal formation, growth, aggregation and retention within the kidneys[1]. Worldwide, approximately 80% of kidney stones are composed of

calcium oxalate (CaOx) mixed with calcium phosphate (CaP). Stones composed of uric acid 9%, struvite 10% and cystine of 1% are common[2]. A series of studies by Balwani et al have reported correlation of lupus[3] parathyroid adenoma[4] and psoriasis[5] with progressive renal failure. Studies are also available showing effects of Guillain Barre Syndrome[6], Kimuras disease[7] and Kikuchi's disease[8] on renal health.

Renal calculi (Kidney stones) are mineral densification in the renal calyces and pelvis that are found free or attached to the renal papillae[9].

In our country kidney stone comprise one of the commonest diseases. and pain due to kidney stones is recognized as worse than that of labour pain. The information regarding Ashmari[10] is available in almost all samhita of Ayurveda. In India, approximately 5-7 million patients suffer from stone disease and at least one from 1000 patients of Indian population needs hospitalization due to kidney stone disease. Thus, the disease is as common as it is old, particularly in countries with hot and dry climate. These are "stone belt regions". The incidence of calculi varies as per geographical distribution, sex and age group. The reappearance rate is 60 to 80%. Males are more commonly affected than the female with their ratio is 4:3. The incidence is higher in the age group between 30-45 years and incidence diminishes after age of 50 years.

Many medicinal formulations mentioned in Ayurvedic literature for the management of Mutrashmari, are cost effective, devoid of complications and provide wide scope for the successful treatment of Urolithiasis.

Important measures in preventing recurrence of kidney stones are Fluid intake and dietary changes. Many trials have shown that increasing urine volume to at least 2 L/day OR 2 lit/day can reduce the recurrence of stone disease by up to 40–50%. Fluid intake mainly should include water. As tea and coffee contain oxalate, milk (which binds free oxalate) should be added to them. However, increasing the urine volume has a disadvantage of reducing urinary citrate.

Formation of calcium oxalate stones has been found significantly reduced by a small reduction in urinary oxalate. Hence, oxalate-rich foods like cucumber, beetroot, spinach, soya bean, chocolate, popcorn and sweet potato should be avoided.

Many studies have established calcium restriction increase the risk of stone disease; therefore, dietary calcium restriction is not suggested.

Aims and Objectives: The aim of this study was to assess the efficacy of Shaman Chikitsa in the management of Mutrashmari (urolithiasis).

Materials and Method:

It is a single case study and the informed consent of the patient is taken in his own language.

- **History of Present Illness:** A 37-year-old woman was in a healthy condition before 2 months, then she started complaining of abdominal pain and it was found that the pain was intermittent and colicky in nature and it was present on right side of the abdomen, which was radiating from loin to groin region; difficulty in urination normally at the beginning of urination which is of pricking type; burning micturition sometimes; and occasionally smoky urine since 1 month. These chief complaints are briefly mentioned in Tables 1 and 2.
- **Past History:**

Not Significant: Patient had taken allopathic medicine Tab. Zerodol twice a day for Seven days and Tab. Levofloxacin once a day for seven days.

- **Family History:**

Not significant.

Demographic Details: Patient named Mrs.ABC, aged 37 years (female) was visited to our hospital on 21/06/2018.

OPD Registration no.- 1806210004

Address - Wanadongri, Hingna, Nagpur

Chief complaints with Duration:

Table No. 1:

Sr.No.	Complaints	Duration
1	Abdominal pain – radiating pain from loin to groin region	2 months
2	Nausea with Vomiting on and off	1 month
3	Burning Micturition	1 month
4	Dysuria	15 days
5	Feverish with mild rigors	15 days

Examination of patient:

Table No. 2:

Pulse	70/min
Bp	110/70 mmhg
Respiratory rate	17/min
Temperature	98.70 F
Pallor	No pallor
Icterus	No icterus
Urine	Burning and painful micturition
Stool	Unsatisfactory bowel habits

Treatment Advised: By analyzing the above pathogenesis of disease in this patient following Shaman Chikitsa treatment plan was prescribed.

Intervention:

Table No. 3:

Sr. No.	Intervention	Dose	Time	Anupan	Duration
1	Chandraprabhavati	500 mg BD	After meal	Water	For 7 days
2	Ayurvedic Formulation Tablet	2 tab TDS	After meal	Water	For 7 days
3	Varunadi Kashaya	3 t.s.f. BD	After meal	Lukewarm water	For 1 month

BD–twice a day, TDS–thrice a day

Assessment of Patient: Overall assessment of the therapy was made on the basis of the improvement in pain, Mutra Pravrutti and ultrasonography (USG) finding.

Assessment parameter:

Objective Parameter: USG of KUB was done before (0 day) and after intervention (7^{th} Day) for the assessment of change in size of urinary calculi.

Subjective Parameter: Assessment was done before (0 day) and after intervention (7^{th} day) based on grading of symptoms as follows.

Assessment of overall effect of therapy: Overall effect of the therapy was assessed by adopting the following criteria.

Complete Remission: 100% relief in Chief complaints and absence of calculi in USG of KUB.

Marked improvement: >75% and <100% improvements in chief complaints and decrease in size of stone was recorded as marked improvement.

Moderate improvement: <75% and >50% improvement in chief complaints and decrease in size of stone was recorded as moderate improvement.

Mild improvement: <50% and >25% improvement in chief complaints and reduction in size of stone was considered as mild improvement.

No improvement: <25% improvement in chief complaints and reduction in size of stone was recorded as no improvement.

Observations and Result

In the first follow-up; the patient informed the stone was expelled out through urine, and she experienced moderate pain and disturbance in the urine flow on 5th day. The patient got complete relief from pain in abdomen and did not experience dysuria.

Other assessment of before and after observation is mentioned in Table no. 4, Fig. (A and B), Table No. 5 and no. 6.

Table No. 4:Assessment of Patient

Complaints	Days	
	0 day [Before Treatment]	7th day [After Treatment]
Abdominal Pain [bastishool]	Severe	Nil
Renal angle tenderness	Severe	Nil
Burning micturition [mutradaha]	Severe	Nil
Generalized weakness	Moderate	Nil
Dysuria [mutrakruchra]	Moderate	Nil
Feverish	Mild	Nil

Table No. 5: USG findings

Sr.No.	Date	Clinical Findings	USG (Ultrasonography) Findings
1	22 June 2018	Generalized weakness Nausea/vomiting Burning micturition Dysuria Feverish	Mild Hydronephrosis noted on Right side with a calculus of about 5 mm in Right Mid Pole Calyx
2	29 June 2018	No any complaints	No sonic evidence of calculus or Hydronephrotic changes seen

Table No. 6: Investigation

Sr.No.	Investigation	Before Treatment	After Treatment
1	Epithelial cells	4-5/hpf	1-2/hpf
2	Pus cells	3-5/	1-2
3	Erythrocytes	0-4 p.v.f.	0-4 p.v.f.
4	Urine colour	Turbid	Clear
5	ESR	38/1 st h	15/1st h

Discussion

The intervention was found to be very effective in calculus calculus of about 5 mm in Right Mid Pole Calyx. This combination of drugs found to be very effective in reducing the symptoms of mutrashmarii. ebastishoola, mutrakrichrata and mutradaha within seven days.

Chandraprabha Vati is a potent anti-inflammatory Ayurvedic remedy used for the treatment of diseases of the urinary tract, kidney, pancreas, thyroid gland, bones and joints.

'Chandra' signifying 'moon' and 'prabha' denoting 'glow', Chandraprabhavati brings a glow to your body and promotes strength and immunity.

Ayurvedic Formulation Tablet contains Shilapuspha (*Didymocarpuspedicellata*) 130mg,

Pasanabheda(*Saxifagalgulata* Syn.*Bergenialigulata/ ciliata*) 98 mg,Manjishtha (Rubiacordifolia) 32 mg, Nagarmusta (Cyperusscariosus) 32 mg, Apamarga (Achyranthesaspera) 32 mg, Gohija (Onosmabracteatum) 32 mg, Sahadevi (Vernoniacinerea) 32 mg,Shilajeet (Purified) 26 mg, Punarnava, Ushira, Shweta Parpati and Hajarulyashood Bhasma which is used in urinary retention, dysuria, renal calculi, hematuria and burning in urination due to acidic urine.

Varunadi Kshayais capable of lithotryptic action, reducing pain intensity, dysuria and is also capable of reducing haematuria.

Conclusion

The result revealed that renal calculi can be cured with ayurvedic Shaman Chikitsaand lithotripsy and other surgical interventions can be avoided. Kidney stones present as an important and challenging clinical problem. Medical therapy, when used judiciously in conjunction with dietary measures, can help in preventing recurrence and in expulsion of small size (<10 mm) stones. It's really great that mid calyx stone expelled out **within seven days** with this **Ayurvedic Shaman Chikitsa**.

Till date there is no need to patient to undergo any surgical intervention as well as no recurrence of symptoms. This study is the management of the single case study only. An attempt must be made for additional investigation of effect of this Shaman therapy in large population for establishing standard treatment protocol. With the obtained results it can be concluded that the **Chandraprabha Vati**, **Ayurvedic Formulation Tablet**, **Varunadi Kshaya**can be safely and effectively carried out in patients of Mutrashmariwith good results.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Clinical Study of Perinatal Outcome and its Relation to Oligohydramnios in Pregnant Women at Term

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Abstract

Background: Oligohydramnios is one of the major causes of antenatal and perinatal morbidity and mortality.

Objective: Aim of the study is to find out the perinatal outcome in terms of LSCS, meconium staining, Apgar scores, fetal distress, birth weight, NICU admission in gravid women with oligohydramnios.

Method: The present study was done prospectively carried out on 110 pregnant women with AFI <5 cm with intact membranes were analysed for perinatal outcome atour hospital, during period of July 2019 to June 2020. Olighydramnios was confirmed by measuring AFI on USG.

Results: In this study, oligohydramnios was mainly seen in primigravida (60%). There were 80% of females in between 20-30 years. 66.7% underwent for caesarean section, because of fetal distress. 11.8% neonate had low APGAR score in 1 minute were 11.8% and 8.2% were APGAR score in 5 minute. In this study 22.7% of babies were admitted to NICU.2(1.81%) babies expired due to septicaemia and meconium aspiration syndrome in 2.72%.

Conclusion: AFI is important screening test for fetal surveillance method. Oligohydranmios is associated with more LSCS rate due to fetal distress, low birth weight babies, NICU admission, so that timely detection of this condition required proper antenatal care to reduced perinatal morbidity and mortality.

Keywords: *Amniotic fluid volume (AFV), Amniotic fluid index AFI, Intrauterine death (IUD), Fetal distress, Oligohydramnios, Perinatal outcome, APGAR.*

Introduction

Amniotic fluid is the yellowish, clear protective liquid contained in the amniotic sac of gravid uterus. This fluid serves important role as a cushioning the growing fetus, maintaining temperature within the uterus, prevention of cord compression, allowing fetal movements and also aids the digestive and respiratory systems of the fetus, but also facilitate the exchange of nutrients, water, biochemical product between mother and fetus [1]. Amniotic fluid volume (AFV) progressively increases from 30 ml at 10 weeks to 200ml by 16 weeks and reaches 800 ml by mid third trimester. As the pregnancy continues post term, further fluid reduction to about 200ml at 43 weeks[2].

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Oligohydramnios is defined as decreased amount of amniotic fluid volume related to gestational age, incidence approximately 1 to 2 percent of pregnancy. The oligohydramnios measures by ultrasonographic diagnosis is usually based on an amniotic fluid index (AFI) ≤5 cm or a single deepest pocket of amniotic fluid ≤2 cm^3 oligohydramnios is associated with preterm premature rupture of membrane, post-term, utero-placental insufficiency or intrauterine growth restriction, genitourinary tract abnormality and chromosomal abnormality in fetus[4,5,6]. Oligohydramnios has been correlated with increased maternal morbidity by increasing the rate of caesarean section, blood loss and perinatal morbidity by causing risk intrauterine growth retardation, meconium aspiration syndrome, severe birth asphyxia, low APGAR scores and congenital abnormalities[6-8].

Oligohydramnios depends upon the stage of pregnancy at which it accurs, in 1st trimester serious sequel like birth defects, in 2nd trimester there can be miscarriage, Potter's syndrome, club foot, premature birth, intrauterine death (IUD) and in last trimester there can be sequel like pulmonary hypoplasia, Compression of the umbilical cord between the fetus and the uterine wall may occur during contractions causes Fetal Heart Rate (FHR) decelerations which are associated with low APGAR score and acidosis at birth, meconium staining, still birth, IUD[9-11].

Material and Method

Present study was done prospectively in department of obstetrics and gynaecology, from July2019 to June 2020. 110 patients inthird trimester of pregnancy with Oligohydramnios selected randomly after satisfying inclusion and exclusion criteria.

Inclusion Criteria: All singleton pregnant women who completed 34 weeks with intact membranes, postdate pregnancy, IUGR, AFI ≤5cm,

Exclusion Criteria: Antenatal patients with gestational age between <34 to > 42 weeks having heart diseases, Polyhydramnios, premature rupture of membranes, twins and multiple pregnancies, AFI ≥6 cm, fetal congenital anamaly, PIH.

On admission, after taking an informed written consent, a detailed history was taken including personal, menstrual, obstretrics history and thorough general physical, systemic examination, clinical examination was performed. Ultrasound was done by ultrasonologist with Logiq P3 USG machine and Amniotic fluid index was determined by using Phelan's technique[4]. The uterus was divided in to four quandrants, ultrasound transducer kept perpendicular to abdomen with no pressure, no limbs, no cord, were observes in measured pocket. The AFI (Amniotic Fluid Index) which is the sum of four quadrants, was calculated as per Phelan as very low/low/ normal/poly which coincides with <5/5-8/>8/18 cm.

All oligohyadramnios women were subjected to Doppler for determination of fetal wellbeing. Cardio-tocography (CTG)was done in these cases routinly. Abnormal CTG and abnormal Doppler studies at the time of diagnosis or any time during fetal surveillance were considered for termination of pregnancy Management was decided after doing P/V examination (LSCS/ Vaginal). After birth, APGAR was noted 1 and 5 min and babies getting admitted in NICU were recorded.

Result and Observations

In this study 110 patients were recruit in our study, out of 110 around 80% were in 20-30 years age group. Out of 80 % women 49.4% delivered vaginally and 14.6% delivered by forceps. 36% were delivered by caesarean section. 7.2% patients were less than 20 years of age. Out of these patients 62.5% patients were delivered by caesarean section. 11.8% patients were more than 30 years of age, 61.5% patients delivered by caesarean section. Rate of caesarean was highest in patients of age group 20-30 years.**(Table No. 1).**

In this study 60% women were primigravida, out of which 45% were delivered by caesarean section and 34.8% were delivered by vaginally. Out of 40% multigravida patients were 52.27% delivered vaginally, 34.09% were delivered by caesarean section. **(Table No. 2**) In this study, operative morbidity was significantly higher in NST non-reactive (85.3%) group than NST reactive (14.5%) group. 63.7% women with reactive NST delivered vaginally, 21.7% were delivered by instrumental. **(Table No. 3)** In our study, most common reason to perform LSCS was (66.7%) fetal distress which was due to cord compression or IUGR. In our study, most common reason to perform LSCS was (66.7%) fetal distress which was due to cord compression or IUGR and 8.9% women were in meconium stain liquor. **(Table No. 4**).

As shown in table no. 5, there was poor fetal outcome with oligohydramnios. Out of 110 babies, 32 (29.1%)

baby were in less than 2.5 kg weight and 25(22.7%) baby were admitted to NICU similar finding was observed by shadabkhalid et.al. 6. In this study, babies had APGAR score <7 in 1 minute were13 (11.8%), 9 (8.2%) had APGAR score <7 in 5 minutes. In NICU admission 3(2.72%) were expired due to Meconium aspiration syndrome, In NST Reactive group 2(1.81%) babies expired due to septicaemia. **(Table No. 5).**

Table No. 1 Age and perinatal outcome of labour

Age	Vaginal Delivery	Instrumental Vaginal Delivery	LSCS	Totle
< 20 Years	1 (12.5%)	2 (25%)	5 (62.5%)	8 (7.2%)
20-30 Years	44 (49.4%)	13 (14.6%)	32 (36%)	89 (80%)
>30 Years	1 (7.6%)	4 (30.7%)	8 (61.5%)	13 (11.8%)
	46 (46.8%)	19 (17.2%)	45 (40.9%)	110 (100%)

Table No. 2 Parity and Maternal Outcome of Labour

	Vaginal Delivery	Instrumental Vaginal Delivery	LSCS	Totle
Primigravida	23 (34.8%)	13 (19.7%)	30 (45%)	66 (60%)
Multigravida	23 (52.27%)	6 (13.63%)	15 (34.09%)	44 (40%)
	46	19	45	110 (100%)

Table No. 3 Non-Stress Test (NST)

NST	Vaginal Delivery	Instrumental Vaginal Delivery	LSCS	Totle
R	44 (63.76%)	15 (21.7%)	10 (14.5%)	69 (65.5%)
NR	2 (5%)	4 (9.75%)	35 (85.3%)	41 (34.5%)
	46	19	45	110 (100%)

Table No. 4 Indication of Caesarean Section

Indication	Totle
Fetal distress	30 (66.7%)
Meconium stain liquor	5 (8.9%)
IUGR	3 (6.67%)
Breech	2 (4.5%)
Others	5 (8.9%)

Table No. 5 Neonatal outcome

Neonatal outcome		Totle
Baby Weight	Aga (>2.5 kg) SGA (<2.5 kg)	78 (70.9%) 32 (29.1%)
NICU admission	Present Absent	25 (22.7%) 85 (77.3%)
APGAR SCORE <7	1 min 5 min	13 (11.8%) 9 (8.2%)
Neonatal death	Septicaemia	2 (1.81%)
	Meconium aspiration syndrome (MAS)	3 (2.72%)

Discussion

In our study, 80% women were in 20-30 years age group. In Vidyasagar V et al.80.49% women were in age group of 20 to 30 years [12]. In Casey et al, the mean maternal age was 23.9 years which is comparable to our study[13]. In this study 60% women were primigravida. In Donald D et al.[14] the incidence of oligohydramnios was 60% in primigravida, Reddy P et al.[7] reported 60% in primigravida and 40% in multigravida which comparable to our study. Charu J et al. 60% in primigravida, Kolsoum R et al. 49% in primigravida, Patel P et al. reported 58.75% in primigravida, Enas M et al. reported 58.2% in primigravida. 15-18. Incidence of oligohydramnios was maximum in primigravida

In present study 60.1% vaginal delivery and 40.9% were LSCS. Indication for LSCS was mostly fetal distress followed by meconium stain liquor. Regarding other study which was comparable to our study were Reddy P et al. 38.67% delivered vaginally, 61.33% delivered by caesarean section and fetal distress was the major indication for LSCS (42.39%)[6], Vidyasagar V et al[12] reported 62.6% delivered vaginally and LSCS in 35.3% in women with AFI <5, Enas M et al.[16] reported that 63.69% performed LSCS in study group than 28.8% in control group, Dwivedi R et al.[19] 40% patients were performed caesarean.

In our study, found that non-reactive NST was present in 34.5% and caesarean section was done in 85.3% patients were non- reactive NST as seen inCharu J et al. study[15] and in Dwivedi R et al.[19], 32% were non-reactive NST which was comparable to this study. In present study 29.1% babies had weight<2.5 kg as seen in Kolsoum R et al. 29%[16]. The incidence of low birth weight babies is higher in women had AFI <5 suggesting correlation of IUGR with oligohradramnios. In our study 70.9% AGA and 29.1% SGA, as seen in Casey et al.[13] 75.5% AGA and 24% SGA,in Raj Sariya et al[20] 83.4% AGA and 16.6% SGA. In our study 22.7% babies were admitted in NICU and 1.81% babies died due to septicaemia. Krishna J et al reported 22 % NICU admissions and 1 % neonatal death due to septicaemia[21]. According to Patel P et al NICU admissions was 20% in women had less AFI[17], 16% inCharu J et al [15]. In this study APGAR score <7 in 11.8% at 1 minute and 8.2% in 5 minute. In other study Enas M et al.[18] reported 5.59% at 1 minute and 2.05% at 5 minutes in Kolsoum R et al.[16] reported 4.7% in both groups at 5 minutes in oligohydramnios group. There was 2.72% babies had meconium aspiration syndrome.

Conclusion

According to our study,Amniotic fluid is an important predictor of fetal tolerance in labour, increased risk of fetal distress when liquor volume decrease. Pregnancy associated with oligohydramnios was adverse antenatal outcome as well as perinatal outcome, because of intrapartum complication and high perinatal morbidity. Oligohdramnios in pregnancy is frequently associated with meconium stained liquor, development of fetal distress, the rate of LSCS, low Apgar score, low birth weight, NICU admission therefore maternal morbidity can be prevented by proper prenatal care, careful antenatal evaluation and fetal monitoring, timed pregnancy termination. AFI detection is a valuable screening test for predicting perinatal outcome.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Prospective Comparative Study to Assess-Post Operative Analgesic Effect of Intraperitoneal Instillation of Bupivacaine with Dexmedetomidine to Bupivacaine with Tramadol in Patients Undergoing Laparoscopic Cholecystectomy

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Abstract

Objective: Tocompare post operativeanalgesic effect of intraperitoneal instillation of bupivacaine with dexmedetomidine, to bupivacaine with tramadol in patients undergoing laparoscopic cholecystectomy.

Method: Sixty patients belonging to age group 20 to 50 years, from both sexes posted for laparoscopic cholecystectomy under general anesthesia were enrolled in our study. They were randomized in two groups, Group A and Group B with 30 patients each. ASA1 & ASA2 physical status were selected. A written and informed consent was taken from all the patients. The group A received 35 ml of 0.25% Bupivacaine with 50 mcgdexmedetomidine and Group B received 35 ml of 0.25% bupivacaine with 50 mg tramadol before removal of trocar. The sites selected for instillation were hepato-diaphragmatic space (10ml), sub hepatic (10ml), on gall bladder bed (10ml) and near and above hepatoduodenal ligament (5ml), at the end of surgery. Intraoperative hemodynamic monitoring was done. Postoperative pain relief was assessed hourly for first 6 hours by VASscore. Time to requirement of first dose of rescue analgesia was noted and this was the endpoint of this study, although the patients were monitored for 24 hours. Sideeffects like postoperative nausea, vomiting and respiratory depression were noted.

Results: We noted that intraperitoneal instillation of bupivacaine with dexmedetomidine prolonged the duration of post operative analgesia compared to bupivacaine & tramadol combination. The requirement of first dose of rescue analgesic is delayed more inbupivacaine dexmedetomidine group as compared to bupivacaine tramadol group. Also the incidence of post operative nausea and vomiting was found to be less in group Ai.e. bupivacaine dexmedetomidine group.

Conclusion: From this study it can be concluded that the use of intraperitoneal instillation of bupivacaine with dexmedetomidine significantly reduces the requirement of post operative analgesics in patients undergoing laparoscopic cholecystectomy with better compliance.

Keywords: *Bupivacaine, Tramadol, Intraperitoneal Instillation, Laparoscopic cholecystectomy.*

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Introduction

Pain in post laparoscopic procedure is common complaint which prolongs the duration of hospital stay and morbidity. Use of local anesthetics for post operative pain relief is a growing practice forlaparoscopic surgeries in recent times. Various studies are available in medical

literature on the different types of anesthetics and adjuvant drugs. Rajan et al studied about the Intrathecal Clonidine and Dexmedetomidine on Characteristics of Bupivacaine Spinal Anesthesia[1]. Jejani et al conducted a study of Intrathecal Buprenorphine for Postoperative Analgesia after Cesarean Section[2]. Singh et al conducted a Comparative Evaluation of Dexmedetomedine versus Clonidine as an Adjuvant in Supraclavicular Brachial Plexus Block[3]. Traditionally, opioids or NSAID'S in various combinations have been used for post operative analgesia after laparoscopic procedures[4,5,6]. Intraperitoneal instillation of local anesthetic agents alone or in combination of opioids[2,3], alpha-2 agonists dexmedetomidine and clonidine have been found to reduce post operative pain following laparoscopic cholecystectomy[7,8]. Pain due to laparoscopic surgeries is because of, stretching and peritoneal inflammation. Shoulder pain is due to irritation of phrenic nerve. Parietal pain is due to incision. Peritoneal origin of pain suggests that analgesia delivered locally to the peritoneal cavity may be of benefit post operatively[9,10].

Methodology

This is a prospective observational study to compare the post operative analgesic effects of intraperitoneal instillation of bupivacaine with dexmedetomidine to bupivacaine with tramadol in patients undergoing laparoscopic cholecystectomy. This study was conducted from Oct 2019 to April 2020 at SMRHC Wanadongri in collaboration with the data collected from AVBRH, JNMCSawangi and some private practitioners. Ethical committee clearance was obtained. Written and informed consent was taken. Group of 60 patients were evaluated in the age group of 20 to 50 years of either gender with ASA1 and ASA2 physical status. Two groups were made;Group A -B upivacaine with Dexmedetomidine and Group B -Bupivacaine with Tramadol.

The patients in whom the procedure was converted to open cholecystectomy, where abdominal drain was put and the patient who did not give consent for the study were excluded from the study.

A detailed preoperative evaluation was done a day prior to surgery pertaining to a detailed general and systemic examination and biochemical tests for each patient and the patients were categorized as ASA1 and ASA 2 physical status accordingly. A written and informed consent was taken from all the patients and VAS scale was explained. The patient was given Tab Alprazolam 0.25 mg on the night prior to surgery and was kept nil by mouth since 10 pm. On the day of surgery after entering the operation theater,standard monitors as ECG, Pulseoxymeter and NIBP were attached, 20G iv access was secured. Prior to induction all the patients were preoxygeneted with 100% oxygen for 3 minutes. After this as premedication, inj glycopyrrolate 0.2 mg IV, injondenseteron 4 mg IV and inj fentanyl 2mg/kg was given. Induction was done with inj Propofol 2 mg/kg. Inj vecuronium 0.01 mg/kg was given to facilitate laryngoscopy and intubation. Adequate depth of anesthesia was maintained with O2:N2O 50:50 with sevoflurane in the range of 1.5 -2% dial concentration. Relaxation was maintained with intermittent doses of 1mg vecuronium ½ hourly. Monitoring of vitals was done. Minute ventilation and respiratory rate were adjusted to maintain the Etco2 in between 35-38. Intra abdominal pressure post Co2 insufflations was maintained in between 12-14 mm of Hg. The patients were randomly placed in two groups of 30 each. Group A - 35 ml of 0.25%bupivacaine with 50 mcg/kg dexmedetomidineand Group - B35 ml of 0.25% bupivacaine with 50 mg Tramadol. Towards the end of the procedure,Co2 was slowly released and the surgeon was asked to instill the drug intraperitoneally; into the hepato-diaphragmatic space (10ml), sub hepatic area (10ml) on gall bladder bed (10ml) and near and above hepatoduodenal ligament (5ml). Patient was reversed with inj glycopyrrolate 0.5mg IV and inj neostigmine 2.5 mg. The patient was shifted to postoperative recovery room and monitored for vitals, pain & any side effects like nausea vomiting, excessive sedation, shivering & pruritus. The intensity of post-operative pain was recorded for all the patients using VAS score at 1, 2, 4, 6 hours after surgery and over all VAS score (mean of all VAS scores) was recorded. (VAS score 0 - no pain, VAS score 10 - worst possible pain) Patients who reported VAS >3 were given Inj paracetamol 1g intravenously as rescue analgesia. Though time of rescue analgesia was considered as the end point of study all patients were followed up for 24 hours.

Statistical Analysis: The statistical analysis was performed by statistician by using Windostat Version 9.2*T-tests* to analyze differences between two groups.

Differences in parameters such as VAS score,time of 1st request of analgesia,any adverse effects over time were analyzed using ANOVA Test.

Observation and Results

The demographic data pertaining to Age, Sex, Weight,duration of surgery and ASA physical status was comparable in both groups and was not significant. ANOVA is applied for age and duration of surgery .P value<0.05 is considered significant t test applied.

Table 1: Demographic Parameters of patients

Parameters	Group A	Group B
Age in years	43.56±9.8	45.44±7.9
Weight in kgs	66.11±7.8	62.7±7.1
Duration of surgery (min.)	63.4±4.5	66.04±4.1

Table 2: Comparison of ASA physical status and gender

Parameters	Group A [n=30]	Group B [n = 30]
Sex (M/F)	11/19	13/17
ASA 1/ASA2	19/11	20/10

Table 3: Comparison of mean VAS Score over 6 hrs

Time [hrs]	Group A (Mean VAS score)	Group B (Mean VAS score)	p Value
1	0.5±0.49	1.5±0.5	0.0009
2	0.93±0.66	1.8±0.45	0.0008
3	1± 0.4	2.3± 0.6	0.0007
4	1.39±0.5	2.5±0.7	0.009
5	1.5±0.5	2.5± 0.5	0.000
6	1.6±0.5	2.7±0.8	0.0089

The above table shows comparison of mean VAS score at specified time intervals that is at 1,2,3,4,5 and 6 hours. p value was <0.05 and is significant.

Table 4: Comparison of VAS score in 6 hours, Time of 1st request of analgesics.

Variable	Group A (Mean±SD)	Group B (Mean±SD)	P value
Overall VAS score in 6 hours	1.16 ± 0.56	2.08 ± 0.63	0.007
Time of first dose of analgesics (min) inj paracetamol 1 gm	315.6±33.3	211.6±23.6	0.009

P value < 0.05 is significant.

Time required for first dose of rescue analgesia was longer in group A (315.6±33.3 min) than in group B (211.6±23.6 min), indicating better and longer pain relief in group A as compared to group B.

Table 5: Showing comparision of heart rate in between two groups at various time intervals during the study.(p value is >0.05)and is not significant.

Heart Rate	Group A (N=30)	Group B (N=30)	P value
PreOp	78.7	80.7	0.6617
Induction	82	78	0.6513
Start OF procedure	81	82	0.5812
IntraOp 15 min	79.7	79	0.712
IntraOp 30 min	82	81	0.618
IntraOp 45 min	81	80	0.762
IntraOp 60 min	78	79	0.823
PostOp	76	73	0.612
PostOp 1 hour	65	70	0.423
PostOp 2 hour	64	74	0.222
PostOp 3 hour	63	72	0.245

Heart Rate	Group A (N=30)	Group B (N=30)	P value
PostOp 4 hour	64	70	0.355
PostOp 5 hour	66	74	0.185
PostOp 6 hour	65	78	0.155

Table 6: showing comparision in systolic blood pressure in between two groups at various time intervals during the study.(p value is >0.05 and is not significant.

Systolic Blood Pressure	Group A (mm Hg)	Group B (mm Hg)	P Value
PreOp	113	114.4	0.5896
Induction	124	124.4	0.9847
Start OF procedure	110	120.4	0.722
IntraOp 15 min	111	118.4	0.7419
IntraOp 30 min	119.4	116.4	0.6493
IntraOp 45 min	113	121.4	0.9103
IntraOp 60 min	115.5	114.4	0.623
PostOp	120	115.4	0.8287
PostOp 1 hour	116	110.4	0.6841
PostOp 2 hour	118	117.4	0.5823
PostOp 3 hour	117.6	119.4	0.7423
PostOp 4 hour	124	122	0.8774
PostOp 5 hour	118	120.4	0.7455
PostOp 6 hour	120	119.6	0.6551

Discussion

Good postoperative analgesia aims to produce long-lasting, continuous effective analgesia with minimum side effects. Laparoscopic cholecystectomy is considered superior to open cholecystectomy with reduced post-operative pain, morbidity and duration of convalescence[6]. The intensity of pain usually peaks during the initial postoperative period.

In this study we compared intraperitoneal instillation of dexmedetomidine and tramadol as an adjuvant to study their analgesic efficacy when added with 0.25% bupivacaine in patients undergoing laparoscopic cholecystectomy.

Dexmedetomidine, an imidazole derivative, a highly selective α2-adrenoreceptor agonist, decreases systemic adrenaline and noradrenaline production and has negative chronotropic and inotropic effect.[13]

Dexmedetomidine when added to local anaesthetic or given intravenous prolongs the duration of sensory block of local anaesthetic during peripheral nerve block.[14]

Dexmedetomidine has antinociceptive effects at dorsal root neurons, decreasing substance P release and stimulating the inhibitory G protein, which increases potassium channel conductivity.[15]

Tramadol also has sensory blockade and similar mechanism of action to that of local anaesthetic in the form of blocking of voltage gated sodium channels[11]. So we used tramadol as the adjuvant in our study.

Shukla et al compared the analgesic effects of dexmedetomidine and tramadol with bupivacaine to bupivacaine alone intraperitoneally after laparoscopiccholecystectomy. Dexmedetomidine group had remarkable less shoulder pain than tramadol group., Time to first request of analgesia (min) was (128 ± 20, & 118 ± 22) in Dexmedetomidine & Tramadol group respectively which is similar to the result of our study[11].

Golubovic et al reported that analgesic effects of intraperitoneal injection of bupivacaine and tramadol in patients with laparoscopic cholecystectomy were effective for treatment and management of pain after surgery[8].

Memis et al studied effects of tramadol and clonidine added to intraperitoneal bupivacaine on post operative pain in Total abdominal hysterectomies and found combination of tramadol or clonidine with intraperitoneal bupivacaine to be more effective than bupivacaine alone.

Ahmed et al compared the analgesic effect of intraperitoneal instillation of Ropivacaine alone to Ropivacaine with dexmedetomidine. In this, the time of requirement of first request of analgesia is longest that is 487± 40.96(min) in ropivacaine dexmededtomidine combination and 242.5 ± 19.85 min with ropivacaine alone.

In a study conducted by Radhe Sharan,Manjit S & Rohit Kadian, 20 ml of 0.5% bupivacaine was compared to 0.5% ropivacaine after intraperitoneal instillation in laproscopic cholecystectomy patients[16].In this group side effects as arrythmia and hypotension were noted in group 0.5% bupivacaine group as compared to 0.5 % ropivacaine group.

As a result we took a lower concentration of bupivacaine that is 0.25% in our study.

Rapolu et al studied the antinociceptive effect of 0.25% bupivacaine with saline to 0.5% bupivacaine with 1 mcg/kg dexmedetomidine after intraperitoneal instillation in patients of laproscopic cholecystectomy and named them as Group B and Group BD respectively. They found that the time to first dose of analgesia was longer in group BD as compared to Group B and also the side effects were not significant[17,18].

In our study we found that dexmedetomidine group has significantly lower VAS score at all points of time((1.80 ± 0.36)P< 0.05. and statistically lower than tramadol which was in agreement with the study done by Shukla et al and Ropulo et al.

In our study minimal side effects in the form of nausea and vomiting were observed and that too were statistically insignificant(p value=0.171).There were no major side effects like respiratory depression.

This study results show that a combination of bupivacaine with dexmedetomidine was superior to the combination of bupivacaine with tramadol in terms of post operativepain, time to the first request of analgesia and adverse effects. General demographics such as age,sex,weight,ASA status and duration of surgery between the two groups had no statistical difference.

The results of our study are consistent with above studies. Time to first rescue analgesia in post operative period was significantly delayed in group A as compared to Group B in our study. There were few post operative complications like nausea and vomiting in both the groups. The incidence was more in the group B than the group A. We have found this to be statistically insignificant (P = 0.171).

Conclusion

We conclude that intraperitoneal instillation of dexmedetomidine 50µg in combination with 35ml of 0.25% bupivacaine in elective laparoscopic cholecystectomy significantly reduces the post operative pain and significantly reduces the analgesic requirement in post-operative period as compared to 35ml of 0.25% bupivacaine combined with tramadol. This is simple to use with minimal side effects and discomfort to the patient and hence can be suggested for routine use.

Limitation: End point of our study was the time for first requirement of rescueanalgesia. VAS score over 24 hours was not taken into consideration. Also the total dose of analgesics was not calculated. We need to take a larger sample size & include a control group of plain bupivacaine without any adjuvant to have a better comparison. Last but not the least cost of dexmedetomidine can not be ignored. We need to compare it with the cost of total analgesics required post operatively.

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

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Review Article on Etiopathogenesis (Samprapti) of Tamak Shwasa W.S.R. Kriyakala

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Abstract

The *Kriyakala* describes the different stages or steps of the evaluative process of disease, ithelps to recognize the stage (*Avastha)* of the disease thus help in early diagnosis, easy cure and prevent further complications. All the three *Acharya* give stress on the need to recognize the disease in its *Chayavasta* itself and adopt measure to interfere the disease *Samprapti* to abort the subsequent stages. *Tamakashwasa* is mentioned as a one of the type of *Shwasa Roga* having *Vata*and *Kapha* dominancy in *Samprapti*. It is considered as *Yapya Vyadhi* (incurable but manageable, persists for a long time) Acute attacks of *Tamak Shwasa* need urgent management. Otherwise it can prove life threatening also. The chroniciy carries a bad prognosis. Early diagnosis with understanding the etiopathogenesis (*Samprapti*) in all aspect can show excellent prognosis by *Ayurvedic* medications. So there is need to understand the etiopathogenesis of *Tamaka Shwasa* w.s.r. *Kriyakala.*

Keywords: *Tamakashwas, Shwas, Bronchial Asthama, Kriyakala, Samprapti.*

Introduction

The prevalence of respiratory disorders like *Tamaka Shwasa* is increasing due to excessive pollution; overcrowding and poor hygiene. It is a *'Swantartra' Vyadhi* having its own etiology, pathology & Management. It is mentioned as *Yaapya Vyadhi*[1] (incurable but manageable, persists for a long time). It is well known for its episodic and chronic course which comes under the life threatening disease which afflicts the human race. It is analogous to Bronchial asthma due to similarity in symptoms, pathogenesis, onset, causes and precipitating factors. Bronchial Asthma is a chronic inflammatory disease of airway. It leads to recurrent episodes of wheezing, breathlessness,tightness of chest and cough particularly at night or early morning. Asthma is a globally significant non-communicable disease with major public health consequences for both children and adults, including high morbidity and mortality in severe cases.[2]

AsAacharya said before going to treat any disease, physician should understand and examine disease in all way,the detailed study of disease is very important

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which ensures the curability of disease[3]. To describes the general Pathogenesis of disease *Aacharya* state the concepts of *Kriyakala* and it is based on the doctrine that, disease is a 'Process' and not a 'state'. The phases of natural history of disease described in modern sciences which parallel the ideas implicit in the concept of *Kriyakala* which describes the natural history of the development of disease, terms of the *Chaya,Prakopa, Prasara, Sthansamshraya, Vyati* and *Bheda*.[4]furnishes the knowledge so necessary to make attacks at various levels of its developments, with a view to achieve complete cure of the disease or at least, to ensure the limitation of disabilities and bring about a rehabilitation. Dhar et al in their article Bronchiectasis in India detailed about theresults from the EMBARC and Respiratory Research Network of India Registry[5].A Systematic Review was conducted by Gaidhane et al on effect of Electronic Media on Diet, Exercise and Sexual Activity among Adolescents[6]. In India, the allopathic treatment modalities and manpower are limited compared to the demand of respiratory problems reported [7]. Here attempt is made to describe the *Samprapti* of *Tamak Shwas* according to six stages of *Kriyakala* which will help to understand disease *Samprapti* in all way and for its further management.

Aim and Objectives: To study Etiopathogenesis of *Tamak Shwas* w.s.r. *Kriyakala*.

Material and Method

Available Ayurvedic literature,article, journal, monograph related to disease *TamakShwasa*

Disease Review: *Shwasa* is main Vyadhi of *PranavahaStrotasa*. The general features of *PranavahaStrotodushti*are related to abnormal respiration which is closely suggested symptoms of *Shwasa* Vyadhi [8]also samanya *chikitsa* sutra given for *Pranavaha* Strotasa vyadhi isShwaski chikitsa[9]Thus Etiopathogenesis (Samprapti) of shwasa vyadhi can be applicable for all Pranavaha Strotasa Vyadhi(Shwas Kasa Hikka etc) The same etiological factors (Nidana) are described for hikka shwas kasa vyadhi[10]Also site of origin is same i.e pittasthanasamudbhava[11]Normal *Gati* of *Vata* (mainly *Pranavayu* and *Udanavayu*) is hampered and becomes pratiloma (opposite) due to ostruction by *kapha* which is main incidence in above mention *vyadhi*. Vitiation of *Vata* and *Kaphadosha*occurs in all these three disease, *Pitta* remains as associated *Dosha.* The etiological Factors for *Shwas* can be classified as*Strotovaigunyakar*factors,*Vata* provocating factors *(VataprakopakaNidana)* and *Kapha* provocating factors *(Kaphaprakopaka Nidan)*[12]

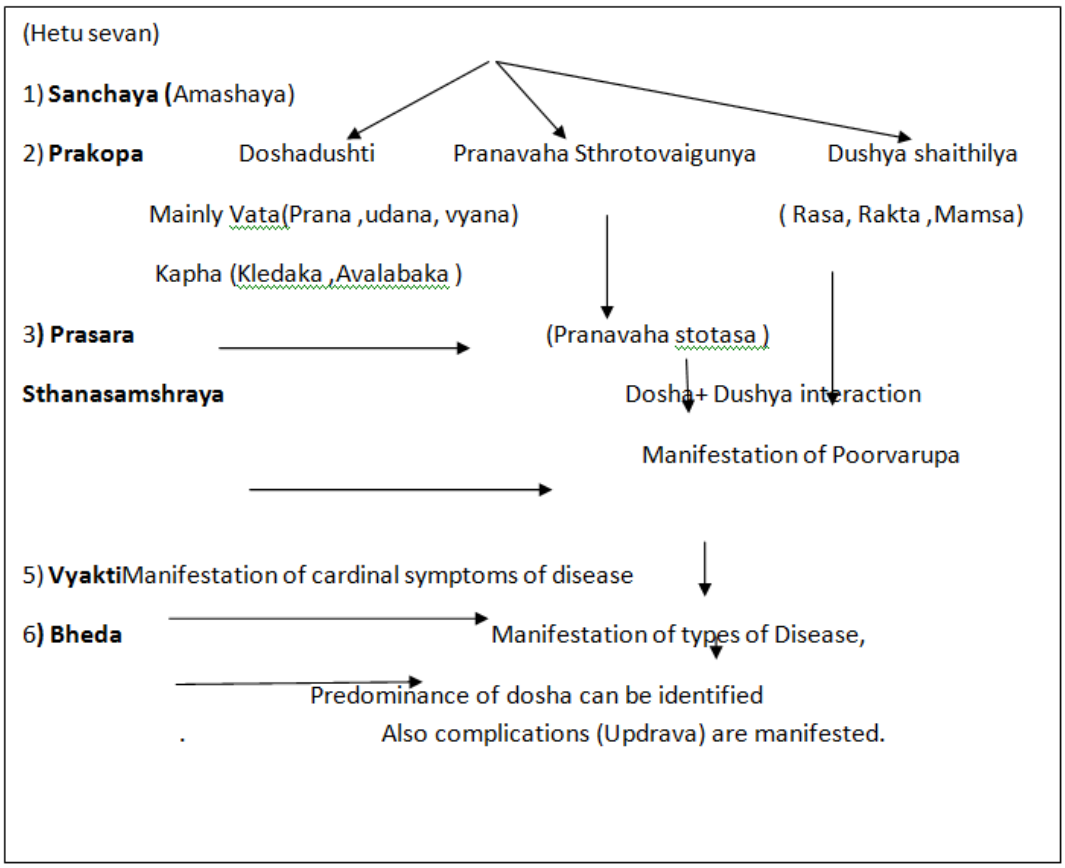
1. Environmental factors: Exposure to dust, pollen, fumes, smoke and wind, residing in cold place, Injury to throat and chest act as *StrotovaigunyakarNidana*.
2. Excessive physical activity, excessive sexual intercourse, excess walking, Intake of dry food, *Vishamashana* (food in excessive or less quantity at irregular time), act as *Vata* provocating factors *(VataprakopakaNidana)*
3. Accumulation of *ama, anaha* (constipation associated with flatulence), dryness in the body, excessive depletion (*Apatarpana*), weakness, injury to *marmas* (vital points), rapid change in exposure to heat and cold, diarrhea, fever, vomiting, rhinitis, *Kshata* (injury), *Kshaya* (wasting), *Raktapitta* (bleeding disorder), *Udavarta* (upward movement of *Vata*), *Visuchika* (enteritis), *Alasaka* (sluggish bowel), *Pandu* (anemia) and intake of poisons can result in *Hikka* and *Shwasa*.
4. Regular intake of *Nishpava* (beans), *masha* (black gram), *pinyaka* (oil cake), *Tila taila* (sesame oil), *pishta* (cakes and pastry), *shaluka* (lotus stem), *Vishtambhi anna* (food aggravating *vata*), *vidahi* (food causing burning sensations), Guru anna (heavy to digest food), flesh of aquatic and marshy animals, curd, raw milk, *Abhishyandhi* (ingredients leading to obstruction of channels), Food,use of cold water,Various types of *Vibandha* (obstructions) act as *Kapha* provocating factors *(Kaphaprakopaka Nidan)*

- When the *Prana Vayu* is not performing its normal physiological functions (vitiated) and become defiles (*Viguna*); obstructed by *Kapha* and moves upwards i.e., loss of natural function of *Shwasa kriya*, then the condition is known as *Shwasa Roga*.This describes all the aspects of dyspnoea.[13] Acharya Charak described *SamanyaSamprapti* of *Shwasa,* According to him due to *Nidanasevana*, the vitiated *Vata* enters in the *PranavahaStrotas* (Respiratory Channels) and provokes the *UrasthaKapha* (*Kapha* staging in chest).This provoked *Kapha* obstructs the *PranavahaStrotas* (Respiratory Channels) and gives rise to five types of *Hikka* and *Shwasa*.[14] Vitiated *Kapha* which lodges in the *Pranvahastrotas* produce the obstruction to the normal functioning of *Vayu* is considered as the one of the factor to initiate the

pathology[15] According to *Vagbhat* vitiated *kapha* is responsible for obstruction, so that *Vayu* is vitiated. Vitiated Vata *dosha* which is *Ruksha*, *Shuska* and *Laghu* produces *Rukstha*, *kathinnyata* and *sankocha* in *Pranvahasrotas*. *Udakavaha* srotas & *Annavaha* srotas also deranged. [16]

- *ShwasaVyadhi* is classified on the basis of clinical features into five types as *Maha Shwasa, Urdhva Shwasa, Chinna Shwasa, Tamaka Shwasa*, and *Ksudra Shwasa*.[17] On the basis of Prognosis it is classified as *Sadhya* (curable) - *Kshudra Shwasha, Krichra Sadhya* or *Yapya (*Palliable*)* - *Tamaka Shwasha*, *Asadhya (*Incurable) – *MahaShwasha and Urdhva Shwasha.*

The general Etiopathogenesis of Shwas vyadhi according Kriyakala is as follows:



Etiopathogenesis (Samprapti) of Tamak shwas: *Tamasa* means darkness .Darkness in front of eyes is produced during attack of this type *Shwasa*. It is one of type of *Shwasa* so *Nidana* of Shwasa *Roga* in general are applicable for tamak *shwasa*. *Tamaka* Shwasa is an episodic disease. So, role of Vyanjaka Hetu in this disease is more. *Vyanjaka Hetu* is stimulating, precipitating or aggravating factors. These also cause aggravation of the symptoms in an already generated disease or these cause the precipitation of the *Samprapti* of a disease. The knowledge of these factors is useful in preventing the actual formation of diseases by taking care to avoid such factors. *Megha* (Cloudy weather), *Pragvata* (East sided wind), *Ambu* (Rainy season), *Kapha* aggravating factors, *Shitasthana* (winter season or Cold atmosphere) can be considered as *Vyanjaka Hetu*.[18]

Etiopathogenesis of Tamak Shwas According to Kriyakala:

Sanchaya: The inceptive phase of the disease when, the *Dosha* is stated to have accumulated in its own place, instead of freely circulating, as in its normal *Avastha* or state.[19] Due to *StovaigunyakarNidana* like exposure to dust, fumes, smoke, previous external trauma, genetic

factor etc causes *Pranavaha Strotovaigunya* in body. In such condition after taking *Vata* provocating factors like Excessive physical activity (*Ativyayama*), excessive sexual intercourse (*Ativyavaya*), exposure to cold air (*Sheetvayusevan*), excess walking, intake of dry food (*Rukshanna*) Vitiates *Vata* by its *Ruksha* and *Sheeta Guna*.On the other hand due to *Kapha* provocating factors like *Guru* Anna (heavy to digest food), flesh of aquatic and marshy animals, raw milk, *Abhishyandhi* (ingredients leading to obstruction of channels) Food, vitiates *Kapha* in *Aamashaya* and *Nidana* like *Vishamashana*, *Amapradosha*, *Vishtambhi Ahara* leads to *Agnivaishmya* (disturbed digestive fire). This stage is characterized by Vague and ill defined symptomatology such as the dislike a factors responsible for increase of *Dosha* i.e. and the desire for factors or substances possessing qualities opposite of the dosha involved.[20]

Prakopa: If above said *Nidanasevan* remain continue, then the previously accumulated and stagnated *Dosha* in *Aamashaya* tends to become swollen and excited.[21]

Prasara: *Vayu* which possesses the power of motion and which is extremely mobile is stated to supply the motive force for the expansion, overflow and spread of the Doshas to one place to other. The medium for the spread of the *Doshas*, either singly or in two or all of them put together, is *Rakta* which is also stated to be involved in the process, resulting in their spread in all directions, in the body.[22] Due to vitiated *Vata*, Vitiated *Kapha* goes (*Vinmargagamana*) in *Pranavaha Strotasa* where already *khavaigunya* is there.

General symptomatology which may be expected to manifest in the *Prasara* stage, according to *Dosha* are to be considered from the point of view of the location of the '*Arambhaka Dosha'* in the sites of other *Doshas* . In *Vata*, when spreading to places other than its own, causes *Vinmargagamana* (upaward movement instead of its normal pathways) and for *KaphaPrasara* manifestation of *Arochaka* (anorexia) *Avipaka* (impairment of digestion of food), *Angasada (*inertness of the limbs) and *Chardi* (vomiting) occurs.[23]

Sthanasamshraya: In this stage the *Arambhaka Dosha*is stated to interact with the *Sthanika Dosha* as well as the *Dhatus*, including *Malas* in the place of its localization, due to obstruction caused by pathological involvement of the related *Strotamsi* a process described as '*Doshduushya Sammurchana*.'[24]

Aggravated *Vata* and kapha *doshas* circulating all over body localizes on the place of *Khavaigunya i.e.*in *Pranavaha Strotasa* where *Doshas* react with local *Dhatu* i.e. *Rasa, Rakta, Mamsa* leading to *Dosha-Dushya Sammurchana* (Interaction of *dosha* and *dhatu*). Vitiated vata by its Ruksha and Shita Guna. Producing *Sankocha* (contraction), *Kharata* (roughness) *Rukshata* (Dryness) in the *pranavaha Strotasa* which is similar to the pathophysiology of asthma with reference to bronchospasm. and vitiated *kapha* leads to *Strotorodha* (obstruction) in the path of *Vata* (Prana vayu) thus leading to the disease . The symptomatology of the oncoming disease, i.e. *Poorvarupa* is stated to manifest in this stage.[25] *Poorvarupa* of *Tamaka* Shvasa have not described separately, so the *Poorvarupa* of *Shwasa Roga* may be considered as the *Poorvarupa* of *Tamaka Shwasa*. *Anaha*, *Parshvashula*, *Pidanam Hridayasya*, *Pranasya Vilomata*, *Bhaktadvesha*, *VadanasyaVairasyata*, *Arati*, *Adhmana*, *Shankha Nistoda*, *Shula* are *Poorvarupa* of *Tamakshwasa*.[26]

Vyakti: This stage represents the clinical phase- the outcome of *Dosha-Dushya Sammurchana* where the characteristics symptomatology of the disease and the nature of the *Dosha* involved, become manifest.[27] The cardinal *signs* and symptoms of *Shwasa Vyadhi* become manifest Abnormality in the breathing pattern, which is episodic, is the cardinal symptom of *Tamaka Shwasa*. Cardinal features of *Tamaka Shwasa* mentioned in the texts are:[28]

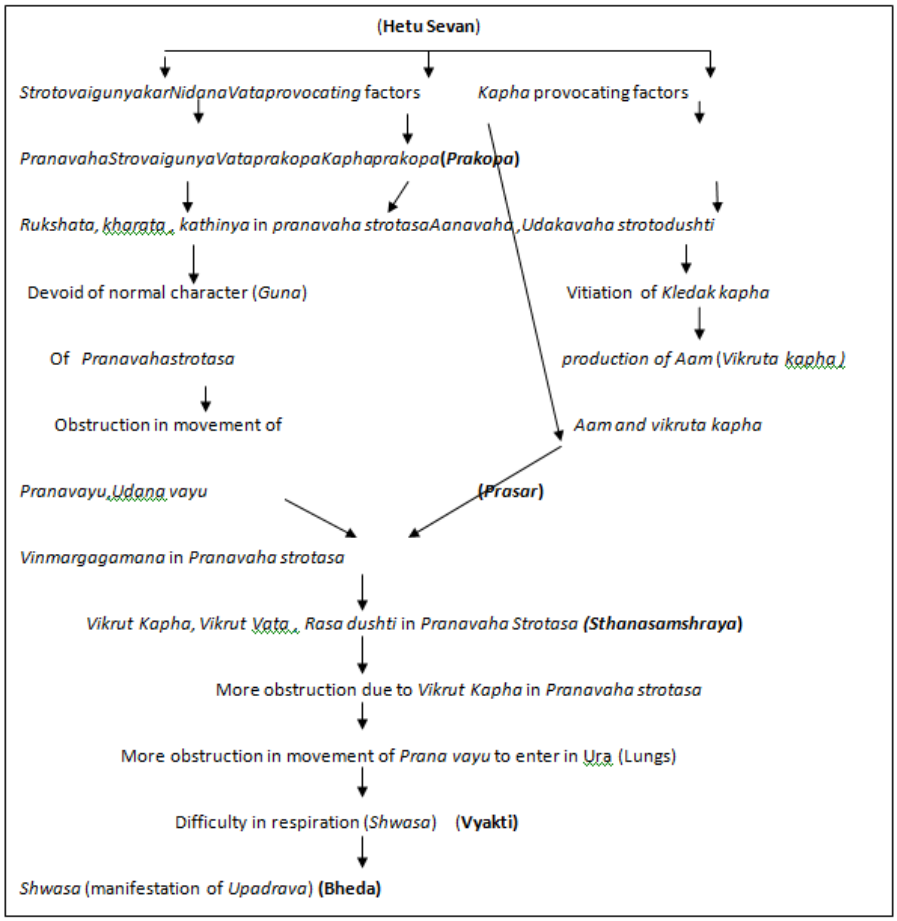
1. Obstruction in the *Pranavaha Strotasa* due to accumulation of *Kapha* renders the phenomena known as *Pranavilomata* .Patient is likely to experience (*HridayaPidana*) tightness in the chest.
2. Respiration becomes difficult due to obstruction (*Ruddha Shwasa*).
3. Disturbed respiration results in audibility of respiration in the form of abnormal wheeze (*Ghurghurukam*).
4. Respiration also becomes rapid and will be much fasterthan thenormal rate-16-20/minute.
5. Breathlessness worsens on walking, speaking or any otherphysical exercise, with bouts of paroxysmal cough.
6. Expectoration of sticky sputum gives some temporary relief (*Shlesma Vimokshante Muhurtam Labhate Sukham*).

7. During thesevere attack of *TamakaShwasa*, breathlessness increases in lying position (*ShayanaShwasapidita*).
8. Patient even may not be able to speak (*KrichchhernaBhasitum*),
9. Perspiration may be seen in the forehead (*LatateSveda*), dryness of mouth (*Vishushkasyata*), rolling of eyes in upward direction (*Uchchhritaksha*) and even canbecome unconscious (*Pramoha*).
10. The patient feels more ease for breathing in the sitting position (*AsinoLabhate Saukhyam*), expectoration of sticky sputum gives some temporary relief (*Shleshma Vimokshante Muhurtam Labhate Sukham*), taking of hot things (*Ushnabhinandati*)

Bheda: This is the final stage of the evolutive process of the disease when it may take one of the following turns: 1) It may resolve and the patients may proceed to convalescence. 2) It may become chronic. 3) It may become complicated or serve as the *Nidana* (cause) for the other disease. I.e. *Nidanarthakara Roga*.[29] In this stage if disease not treated at the earliest, disease may become incurable.

Regarding the *Samprapti* of *Tamaka Shwasa Charaka* narrates that the *Gati* (movement) of vitiated *Vata* due to obstruction in *Pranavaha Strotasa* becomes *Pratiloma* i.e. normal *Gati* of *Vata* is hampered and becomes reverse in its course. *Vata*spreads with in *Pratiloma Gati* and involving the neck and head region, which produces *Pratishyaya* by excitation of *Kapha Dosha*. This *Kapha* causes obstruction at the site of the throat region and produces *Ghurghurukam Shabda* when *Vata* passes through the same region. This results into an increase in the respiration rate resulting in disease of *Shwasa*, which includes pain in the chest. Hence there is a great parlance of pathogenesis between ancient and modern concepts. Here the vitiated *Prana Vayu* produces bronchospasm and the vitiated *Kapha* makes to swelling of the mucous membrane and excessive secretion of mucus.[30] The symptoms are presented firstly with upper respiratory tract involvement. *Pinasa* is the prior symptoms followed by wheezing sound (*Ghurghurukam*

The Etiopathogenesis of Tamak Shwasa vyadhi is given below in terms of Kriyakala:



Discussion

The general outlook of the concept of *Kriyakala* which can be summed up as preventive, curative, prognostics, in keeping with the main objectives of the science of Ayurveda itself, i.e. 1) The promotion and preservation of health, strength and longevity in the *Swastha* or the healthy person and 2) the cure and/or relief in the case of those, who are ailing and afflicted. *Kriyakala* describes the different stages or steps of the evaluative process of disease, in terms of the *Chaya,Prakopa, Prasara, Sthansamshraya, Vyati* and *Bheda*. The disease *Tamaka Shwasa* is predominantly caused by *Pranavaha Sroto Dushti. The Etiological factors are given can be dividedas Strotovaigunyakar* factors*, Vata provocating* factors*, Kapha provocating* factors. *Vata* as well as *Kapha Dosha, Rasa Dhatu and Pranavaha Srotasa* are the predominant factors involved in the pathogenesis of *Tamaka Shvasa*. These factors determine the course and clinical manifestation of the disease. Some of the cardiovascular risk factors also need to be given special attention[31,32]. *Pratiloma Gati* of *Vata* plays an important role along with *Strotorodha* produced by *Kapha*. In *Tamaka Shwasa*, two types of *Samprapti* occur:

1. (Kaphapradhana Samprapti) - *Vata* is in normal state and *Kapha* is either vitiated with kapha provocating factors like *Shita, Guru, Dadhi, Amakshira* etc. or *Vishamashana, Vishtambhi Bhojana* etc. can produce *Mandagni* and *Mandagni* produces *Ama* and this Ama produces *Malarupa Kapha*. This vitiated Kapha *vinmargamana* with vayu in the *Uraha Pradesha* causes the obstruction in the normal path of *Vata* (*Prana*) it further leads to *AvaranajanyaVata Prakopa* and *Gati* of *Vata* becomes *Pratiloma* .
2. *Vatapradhana Samprapti* - *Vata* is vitiated through its own etiological factors like *Apatarpana*, *Raja, Dhuma, Vegavarodha* etc. and by *Dhatukshaya* (due to chronic disorders), vitiated *Vata* causes contraction of *Pranavaha Srotasa*, which further produces *Pratishyaya* by excitation of *urastha Kapha Dosha* and obstruction in movement of *Vata*Thus, leading to the disease *Shwasa*.

According to *Vaidya Ranjeetrai Desai Tamaka Shwasha* can be categorized into *Kaphapradhan* and *Vatapradhan* on the basis of *Doshaja* predomince in *Rogotpatti* and *Lakshnas*. It is mentioned in his book *Nidan ChikitsaHastamlaka* vol-4.Clinicaly we can observe these two types of patients There are other literature also available to support this; *Kaphaja* and *Vataja* type. Clinical features are as outcome of samprapti appear in the Vegavastha of Tamaka Shvasa and some remain present in between the two attacks. Acharya Charaka has given a detailed description of *Tamaka Shvasa Lakshana* in whichhe has emphasized more on the *Vegavastha Lakshana* while *Sushruta* has described both type of *Lakshana*.*Charaka* has mentioned two different stages of *TamakaShwasa* Pratamaka and *SantamakaShwasa*. When a patient of *Tamaka Shvasa* suffers with fever and fainting, then the condition iscalled as *Pratamaka Shwasa*. It is suggestive of involvement of *Pittadosha*. When the patient of *Tamaka* Shwasa feels submerged in darkness, the condition is calledas *SantamakaShwasa*. This can be taken as the severe stage of *Pratamaka*. These both conditions are aggravated by *Udavarta,* dust, indigestion, humidity in body and suppression of natural urges. Though cooling regimen is one of the causative factors of *Tamaka Shwasa* but in *Pratamaka* and *SantamakaShwasa*, the patient gets relief by administering cooling agents due to *Pitta Dosha*.[33]

Conclusion

Tamak shwasa is the disease condition of respiratory system affect personal and professional life of affected individual. On the basis of their clinical manifestation, the disease can be compared with Bronchial Asthma. Etiological factors mainly *Vata* and *Kapha* provocating with *Strotovaigunyakar* factors responsible for *Vatapradhana Samprapti* and *Kaphapradhana Samprapti* in disease. Involvement *Pranvaha, Udakavaha* and *Annavaha Strotas* with their specific clinical features show the broad approach to disease. The origin of *Tamaka Shwasa* from *Amashaya* (stomach) has a great importance. For the better management of *Tamak shwasa* every physician must have detail knowledge of disease according to stages of disease i.e. *Kriyakala* both Ayurvedic and modern perspective. Because disease should not be overlooked under any circumstances whatsoever. The course of treatment given either at an inappropriate time or not resorted at the proper time, as well as, over or under medication proves useless, even in a curable disease.

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Evaluation of Treatment of Tinea Corporis and Tinea Cruris with Oral Terbinafine Using Direct Microscopic Examination with Special Stains

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Abstract

Terbinafine is a fungicidal allylamine drug and was considered a drug of choice to treat dermatophyte infection. However recently there was an increase in the incidence of clinical failure and relapses with terbinafine.

Aims and Objectives: This study was undertaken to assess the effectiveness and safety of oral terbinafine 250 mg given in patients suffering from tinea cruris and tinea corporis for 6 weeks.

Materials and Method: In a prospective, open-label randomized controlled comparative trial 40 patients suffering from tinea cruris and tinea corporis were recruited. Only patients with tinea cruris and tinea corporis, confirmed through positive microscopy before the start of treatment were eligible for inclusion in the trial. The patients were distributed into two groups and randomly allocated into group A and group B of 20 patients each. Both groups received the same treatment i.e., oral Terbinafine 250mg once daily for 6 weeks. Patients of both groups were followed up at weeks 2, 4, 6 during treatment and at the end of 8 weeks. Mycological control test for group A and group B patients were done by KOH mount at baseline and each visit. Patients of group B underwent mycological control testing in the form of KOH mount with the concurrent use of DMSO and CSB at baseline and each visit. By the end of treatment clinical and mycological cure were evaluated.

Results: In group A (mycological testing with only KOH was done), fungal elements were detected in 100%, 40%, 20%, 0% and 0% of baseline patients, 2 weeks, 4 weeks, 6 weeks and 8 weeks respectively. In group B (mycological testing with KOH and DMSO+CSB was done), fungal elements were detected in 100%, 75%, 50%, 10% and 15% of baseline patients, 2 weeks, 4 weeks, 6 weeks and 8 weeks respectively. Even after 2 weeks of completion of oral terbinafine 250 mg for 6 weeks,the clinical cure was not seen in 40% and 35% patients in groupA and group B.

Conclusion: Although mycological cure was achieved in more than 90% of patients in group A and group B after 8 weeks clinical cure was achieved in only 60% and 65 % patients in group A and group B respectively. Implying that we need to continue oral terbinafine for a longer period to achieve mycological as well as a clinical cure.

Keywords: *Tinea cruris, tinea corporis, dermatophytosis, terbinafine 250 mg, relapse.*

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Introduction

Dermatophytes are hyaline fungi of genera, Trichophyton, Microsporum and Epidermophyton which infect keratinized tissues such as skin, hair and nails.[1]

In tropical and subtropical countries dermatophytosis is common.[2] The superficial mycotic infections are the most frequent form of infection seen in clinical practice nowadays, which affects more than 20–25% population of the world.[3-6] Tinea corporis (contributing 36-59%) and tinea cruris (contributing 12-27%) are the most common type of dermatophytosis in India.[7] Directmicroscopic examination, culture and molecular studies are used to diagnose superficial dermatophytosis. The most common test employed to diagnose dermatophytosis is potassium hydroxide (KOH) wet mounts. Although rapid, direct microscopic examination with the KOH wet mount lacks a contrasting color and needs considerable interpretative ability. To increase the accuracy of KOH wet mount different stains are used. Chicago sky blue (CSB) stain is a rapidand reliable diagnostic method for diagnosingdermatophytosis.[8] Terbinafine is the orally available allylamine antifungal having fungicidal activity against dermatophytes. Oral terbinafine was considered to be a drug of choice for the treatment of tinea cruris and corporis.Terbinafine inhibitsthe synthesis of ergosterol, which disintegratesthe fungalcell wall.[9,10]. Oral terbinafine 250 mg/day is effective in treating tinea cruris, tinea corporis,andpedis, typically achieving mycological cure in 80 to 90 percent of patients treated for two weeks.[11]

However, resistance to common antifungal agents used in the treatment of patients with tinea infections is being reported with an increase in the incidence of clinical failure and relapses with terbinafine.[12,13,14]

Both clinical and microbiologic resistance, either occurring singly or in tandem, as well as primary and secondary resistance to antifungal agents have been reported.[15]

Terbinafine resistance frequency from patients with tinea pedis or tinea unguium having T. rubrum and T. interdigitale infection is alarmingly high, about 1%. Increased antifungal drug exposure favors the development of resistant strains.[16] The key reasons for this may include low plasma concentration and incomplete cure which are very frequent after 2 weeks of Terbinafine therapy with 250 mg/day.

The increased use, improper prescription and counter sale ofantifungal drugs have also created resistance to these drugs. Therefore different approaches were required when using terbinafine. We conducted this analysis to assess the effectiveness of terbinafine 250 mg given over 6 weeks.

Materials and Method

A prospective, open-label randomized controlled comparative research was carried out in patients sufferingfromtinea corporis and tinea cruris. On the first visit, informed written consent was taken. Demographic data along with clinical history was taken, followed by clinical examination.

The study was done in the dermatology OPD of a tertiary care hospital in central India. Patients between the age of 18 to 70 years suffering fromtinea corporis with or without tineacruris on clinical examination were taken up for the study. The number and body sites involved were recorded and the worst affected area was chosen for specific measurement and observation. The study included only KOH smear-positive patients and KOH smear-negative patients were excluded from thestudy. Patients who had taken topical or oral therapy in the past 1 month for dermatophytosis, patients withthe previous history of intolerance to the terbinafine,had any abnormality in the laboratory investigation, patients unwilling to participate in the study or who were pregnant or lactating, immunocompromised were omitted from the study.

Forty patients who attended the dermatology OPD of tertiary care hospital in central India and fulfilled the inclusion criteria and the clinically evaluable exclusion criteria underwent laboratory evaluation to rule out other exclusion criteria and then were recruited for the analysis.

The samples were collected from the worst affected area and two approaches tested for the presence of fungal elements first by 10% Potassium hydroxide mount (KOH) only and another approach by using 10% Potassium hydroxide (KOH) with Chicago sky blue (CSB) stain and Dimethyl Sulfoxide Mount (DMSO). Only tinea corporis and tinea cruris patients confirmed by positive microscopy before the start of treatment were eligible for inclusion in the trial. The patients were divided randomly into two groups of 20 each (groups A and B) based on a random allocation sequence generated by the software.

Pretreatment evaluation and advice: On the first visit, an informed written consent will be taken. Clinical history will be taken, followed by clinical examination, as per the proforma. Baseline laboratory investigations viz. Complete blood count,renal function test,and liver function test were done.

Treatment Protocol: Patients were split into 2 groups at random with, A and B having 20 patients in each group. Both groups received the same treatment i.e., oral Terbinafine 250mg once daily for 6 weeks. Patients of both groups were followed up at weeks 2, 4, 6 during treatment and at the end of 8 weeks. Mycological control tests were done at each visit for each patient of both groups. Mycological control test for patients of group A was done in the form of KOH mount at baseline and each visit. Patients of group B underwent mycological control testing in the form of KOH mount with the concurrent use of DMSO and CSB at baseline and each visit.

Efficacy Evaluation: The clinical and mycological cure were evaluated after completion of treatment.

Based on the improvement in pruritus, erythema and scaling grading of clinical response was done and categorized into 4 groups (Grade I - was very good response with>75% improvement,Grade II - was good response with 51 to 75% improvement Grade III - was poor response with 26% to 50% improvement Grade IV - response ≤25%improvement). The patients were declared clinically cured if clinical improvement was more than 75%. and if potassium hydroxide (KOH) with and without DMSO/CSB examination is negative patients were considered as mycologically cured. All adverse events (AEs) were assessed for severity and relationship to Terbinafine at each visit. Multiple occurrences of the same adverse effects were only counted once for each patient. Laboratory measurements such as liver function tests were also included for safety evaluation.

Statistical Test and software used: Statistical analysis was performed using descriptive statistics and inferential statistics by using the chi-square test and software used in the study were SPSS 24.0 version and GraphPad Prism 7.0 version and p-value <0.05 is considered as the degree of significance.

Results

A total of 40 patients (20 patients in each group) were included in this study. Both groups were matched for age and sex and were thus, comparable. The disease was found to be more common in males, with a male: female ratio of 1.67 (25 males and 15 females). The mean age of the patients was 30.04 ± 11.24 years (18-63 years). The majority of patients (64.78%) were in the age group of 20-30 years. The most common clinical type seen was tinea cruris et corporis (39.7%), followed by tinea cruris (31.88%) and tinea corporis (28.42%). Other infectionsonychomycosis (0.75%), tinea barbae (0.5%), tinea manuum (0.5%). The commonly involved sites were the inguinal region, waist, axillae and the inframammary areas (in females). In group A (mycological testing with onlyKOH was done), fungal elements were detected in 100%, 40%,20%, 0% and 0% patients at baseline, 2 weeks, 4 weeks, 6 weeks and 8 weeks respectively. In group B (mycological testing with KOH and DMSO+CSB was done), fungal elements were detected in 100%, 75%, 50%, 10% and 15% patients at baseline, 2 weeks, 4 weeks, 6 weeks and 8 weeks respectively.

Table 1: Demographic data

Total number of patients	40	
Males	25	
Females	15	
M:F	1.67:1	
Mean age	30.04 ± 11.24 years	
Clinical types	Tinea cruris et corporis Tinea cruris Tinea corporis	39.7% 31.88% 28.42%
Other infections	Onychomycosis (0.75%), tinea barbae (0.5%), tinea manuum (0.5%)	
Sites	Inguinal region Waist Axillae Inframammary areas (in females)	
The average number of body sites involved	2	

Table 2: Mycological testing at baseline and at follow up visits

		Baseline	2 Weeks	4 Weeks	6 Weeks	8 Weeks
Positive Results	Group A	20(100%)	8(40%)	4(20%)	0(0%)	0(0%)
	Group B	20(100%)	15(75%)	10(50%)	2(10%)	3(15%)

Table 3: Group A

	Baseline	2 Weeks	4 Weeks	6 Weeks	8 Weeks
Mycological detection	20 (100%)	8(40%)	4(20%)	0(0%)	0(0%)
Patients not having clinical cure	20(0%)	20(0%)	18(90%)	7(35%)	8 (40%)

Table 4: Group B

	Baseline	2 Weeks	4 Weeks	6 Weeks	8 Weeks
Mycological detection	20(100%)	15(75%)	10(50%)	2(10%)	3(15%)
Patients not having clinical cure	20(100%)	20(100%)	18(90%)	6(30%)	7 (35%)

Discussion

The most common superficial mycoses worldwide are dermatophytosis. A variety of antifungal drugs have been developed to treat this condition and more are underway. Terbinafine is one of the most widely used antifungal medications used in the treatment of superficial fungal infections because of its broad-spectrum fungicidal activity. The drug has shown consistent efficacy against dermatophytes achieving more than 90% cure rates when prescribed daily 250mg for 2 weeks. However, recently, clinical relapses and failure in patients with tinea infections with an increased incidence of terbinafine resistance have been identified with terbinafine. Although terbinafine resistance in dermatophytosis is not usual in clinical practice, it has been reported in clinical isolates by few authors. Mukherjee et al. published the first documented evidence of resistance to terbinafine in dermatophytes in 2003. In their study, Majid et al. reported that there were only 43 cases at the end of 12 weeks out of the total 100 cases enrolled, who maintained a long-term clinical and mycological cure after two weeks of oral terbinafine therapy. The authors concluded that incomplete mycological cure, as well as relapse,were very frequent in patients with tinea cruris and tinea corporis after standard terbinafine therapy (2 weeks). (5) In our study incomplete mycological cure was seen in 4 out of 20 patients in group A and 10 out of 20 patients in group B even after 1 month of terbinafine therapy which was statistically significant (p value = 0.023).

One of the principal mechanisms of antifungal resistance is a decrease in effective drug concentration, which in the case of Terbinafine is quite a known feature following standard dosing regimen of 250 mg daily because of substantial skin and adipose tissue build up.This clearly shows that the current standard terbinafine therapy with 250 mg/day dose is not sufficient in the current scenario where fungal resistance is further aggravated by increased use, unsuitable prescription and counter sale of antifungal drugs.

Even though there is no clear proof as to which strategy to use to best avoid resistance, the most commonly suggested measures in the past include rational use of antifungal agents and adequate dosing with particular focus on avoiding low antifungal dosage treatment and for the inadequate duration.

We conducted this survey to find out the safety and efficacy of terbinafine 250 mg/day in patients suffering from dermatophytosis. In our study, 65% of patients in group A, 70% group B patients,achieved clinical cure after 6 weeks of terbinafine 250mg (The clinical cure was described as Grade I response in improvement). Mycological cure was 100% in patients of group A and 90% in patients of group B after 6 weeks of daily terbinafine 250mg. In this survey, Terbinafine was well tolerated by the patients with only 12% of patients reporting the adverse effects. All the adverse effects were of mild to moderate severity and due to any incident, none of the patients interrupted treatment. Adherence to the medication was good across all the groups.

Limitations: Studies with more sample size to resolve the deficiencies of the present study are needed. The probability of observer bias due to the methodology and nature of study while interpreting the results cannot be ruled out.

Conclusions

While terbinafine resistance is uncommon in clinical practice, its occurrence is on the increase.

Relapses and incomplete cure and are usually seen with standard terbinafine therapy. Our analysis indicates that Terbinafine 250 mg/day should be given for more than 6 weeks for clinical and mycological cure or till complete clearing of lesions. Concurrent use of DSMO + CSB with KOH is more sensitive than KOH alone for determining the presence of fungal elements.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Comparative Study of Efficacy of *Hemidesmus Indicus* (L.) R. Br. and *Decalepis Hamiltonii* Wight & Arn. in Acne Vulgaris (*Mukhadushika*)

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Abstract

Hemidesmus indicus (L.) R. Br. (*Sariva*) commonly known as *Anantmool* with its unique attributes is used in therapeutics in Ayurveda. From few decades due to heavy demand and extreme commercial collection from natural habitat of this plant its natural population is decreasing with higher rate and has resulted in extinction of population. In various Ayurvedic raw drug market and in the leading Ayurvedic pharmacies, the roots of *Decalepis hamiltonii* which is considered as *Sariva* species is used in place of the roots of *H. indicus* (*Sariva*) in Ayurvedic formulations. *H. indicus* though is cultivated by special method, still it is very time consuming and expensive process. *D. hamiltonii* is easily cultivated with high yield and less expensive as compared to *H. indicus*. The present study was undertaken to compare the efficacy of *Hemidesmus indicus* (L.) R. Br. and *Decalepis hamiltonii* Wight & Arn. In Acne vulgaris (*Mukhadushika)*and compare its efficacy. On the basis of the observation obtained both drugs relieved symptoms burning sensation (*Daha),* pain *(Vedana),* itching *(Kandu),*erythema*(Lalima)* and Global acne grading scale (GAGS) of Acne vulgaris significantly. As per study it is concluded that tablet (*Ghana Vati*) and Gel of *Hemidesmus indicus* and *Decalepis hamiltonii* are equally effective in the management of Acne vulgaris. Hence *D. hamiltonii* can be used as substitute drug of *Hemidesmus indicus* in the management of Acne vulgaris.

Keywords: *Acne vulgaris, Decalepis hamiltonii, Hemidesmus indicus, Mukhadushika, substitute.*

Introduction

Sariva- Hemidesmus indicus (L.) R. Br. commonly known as *Anantmool*, belonging to family Asclepiadaceae, is a slender, twining or prostrate perennial shrub with cylindrical stems and aromatic roots[1]. They are common in the open deciduous and scrub forest hedges and on degraded sites all over India and Srilanka[2]. The root and root bark are considered as *raktashodhak* (blood purifying), *shothahar* (demulcent), *rasayana* (tonic) and *mutrajanana* (diuretic)[3]. It is used in various skin diseases and in several well known Ayurvedic formulations[4]. *Acharya Charaka* described *Sariva* in six (6) *mahakashayas*. This plant is in heavy demand and natural population is decreasing with higher rate due to extreme commercial collection from natural habitat[5]. *H. indicus* propagation is usually practiced through seeds and has tedious method of collection of root, expensive harvesting and very low yield inspite of adopting special method of cultivation in Bamboo[6].

Decalepis hamiltonii Wight & Arn. belonging to Apocynaceae family, is a climbing shrub with jointed

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branches and cylindrical, fleshy, aromatic roots, found in peninsular India up to an elevation of 1400 m; common in the forests of Western Ghats[7]. The aromatic roots of *D. hamiltonii* are highly prized for their role in preparation of natural cold drinks and Ayurvedic drugs [8]. The *D. hamiltonii* shows the presence of bioactive compounds such as Alkaloids Flavonoids, Phenols, Tannins, Terpenoids and Glycosides[9].

In various Ayurvedic raw drug market *Sariva* species *D. hamiltonii* is sold as *Sariva* instead of *H. indicus*[10]. Nayar and others gathered that in the leading Ayurvedic pharmacies in South India, the roots of *D. hamiltonii* which is considered as *Sariva* species is used in place of the roots of *H. indicus* (*Sariva*) in Ayurvedic formulations[11]. Though *H. indicus* is cultivated by special method, still it is very time consuming and expensive process, *D. hamiltonii* is easily cultivated with high yield and less expensive as compared to *H. indicus*. The essential oil of these two herbs showed a great similarity with chemical components and the actions [12].

Acne is prevalent disease to affect 9.4% of the global population and commonest disease distressing humanity having significant impact on quality of life[13].

In *Ayurveda*, acne has been elaborated as *Mukhadushika* one of the *Kshudra Rogas* (minor ailments) in various texts. Ayurved texts quote that due to vitiation of *Vata* and *Kapha Doshas* and vitiation of *Rakta Dhatu*, *Tarunyapitikas* or *Mukhadooshikas* are produced[14]. Ayurvedic text described *Mukhadushika* with *Saruja* (pain), *Ghan* (firm on touch), *Medogarbha* (filled with oil/sebum) and it has shape like *Shalmali Kantaka* (thorn of *Salmalia malabarica*)[15]. Acharya Sushruta quoted that *Mukhadushika* must be treated by *Vamana (emesis)* and *Lepana*[16].

As per modern science the main causes for acne vulgaris are hormone, bacillary interference and allergic manifestation which directly affect the skin or the pilosebaceous unit so as to produces excess amount of sebum[17]. Most common skin disorder of pilosebaceous unit is Acne vulgaris, which is caused by bacteria *Propionibacterium acnes*, *Staphylococcus epidermis* and *Malassezia furfue*[18].

The roots of *H. indicus* showed strong inhibitory effect on *P. acne* and *S. epidermis* in a study conducted by Kumar and coworkers[19].

Due to the similarity in the chemical components, actions and Ayurvedic properties and the non-availability of the roots of *H. indicus* in large quantity, the roots of *D. hamiltonii* are used in South India as a substitute for the roots of *H. indicus*[20]. Today it is the need to evaluate efficacy of these substitute drugs in clinical trial and use the substitute to get evidences in the Ayurvedic therapeutics. In view of these facts the present study was undertaken to compare the efficacy of *H. indicus* and *D. hamiltonii* on *Mukhadushika* (Acne vulgaris) in view to research in further direction to generate clinical evidences and use it practically in the emerging market.

Methodology

Collection of Field Samples: Field samples of roots of *Hemidesmus indicus* (L.) R. Br. and roots of *Decalepis hamiltonii Wight* & Arn. were collected.

Identification and Authentication of Plant: The field samples collected were identified and authenticated from FRLHT (Foundation of Revitalization of local Health traditions), Bangalore, Karnataka.

Pharmaceutical preparation of drug: The root of *H. indicus* and *D. hamiltonii* was used to prepare *Ghana Vati* (Tablet) and Gel.

Ethics committee approval: After approval from institutional ethics committee (reference no. DMIMS (DU)/IEC/2017-18/6368) on dated 30th March 2017 informed consent was taken from each patient participated in the study.

Clinical Source: The Patients were randomly selected from Kayachikitsa OPD & IPD, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha

Study Design: Single blind interventional study.

Sample Size: Sixty (60) patients.

Materials and Method

Collection of Field Samples: Roots of *Hemidesmus indicus*(L.) R. Br. was collected on from Dapoli, Ratnagiri, Maharashtra and Roots of *Decalepis hamiltonii Wight* & Arn. was collected from Agasti Agroved farms, Sarola Kasar, Ahmednagar, Maharashtra.

Identification and Authentication of Plant: Sample A was identified and authenticated as *Hemidesmus indicus* (L.) R. Br. (Voucher specimen no.-FRLH COL. No. 123401), Sample B as *Decalepis*

hamiltonii Wight & Arn. (Voucher specimen no.-FRLH COL. No. 123403).

[Plate no. 2 & 3]

Pharmaceutical preparation of Drug:

Preparation of Bharad: Field samplecollected of root of *H. indicus* and *D. hamiltonii.* Washed under tap water to remove its physical impurities and dried under the shade. Cut into small pieces and pulverized under no. 10 sieve. *Bharad* (coarse powder) obtained and stored in air tight containers.

Preparation of Ghana Vati (Tablet): Coarse powder of the crude drug was soaked in 8 parts of water overnight. Then heated continuously on low flame until it was reduced to one fourth of its initial quantity. The *Kwatha* (decoction) was filtered through single folded cotton cloth and collected in a separate vessel. Then, the decoction was boiled again over low flame, till a semisolid consistency was obtained. The viscosity of the extract increases, as the water evaporates, resulting in *Ghana* (solid mass).the *Ghana* was mixed with the powder of *Sariva* (up to 10% of *ghana*) further forming a solid mass and it was dried. After drying, the solid mass (*Ghana*) was crushed and granules were prepared and dried in a hot air drier at 40°C. The dried granules were sifted through a no. 20 sieve. Gum acacia (binding agent) and talc powder (lubricant)was used. The formulation was later compressed in a 16 STN single rotor punch tablet press with a target weight of 500mg.

Preparation of Gel:

Phase I: Extraction-The *bharad(coarse powder)* of the crude drug were soaked overnight in sufficient amount of water and extracted by continuous hot extraction method using water (100%).Required quantity of extract was achieved and required amount of sodium benzonate, propyl paraben and methyl paraben were later added. Phase II: Required quantity of Carbomer 934 was soaked in some amount of distilled water for 2 to 3 hrs.

Phase I and II were mixed and adjusted to a pH of 6.8 to 7 with drop wise addition of triethanolamine. The remaining quantity of distilled water was added to make up the final 2000g weight. The formulation was stirred with mechanical stirrer to homogenize and stored in well closed air tight tubes as containers and kept in cool place. (Waghmare N *et al*, 2011).

Inclusion criteria:

1. Patients fulfilling the diagnostic criteria of *Mukhadushika*- Acne vulgaris (facial acne) in 15 to 40 year age group of either sex.

Exclusion criteria:

1. Patients having other dermatological or any other medical problem such as, Rosacea, Folliculitis and boils, Sycosisbarbae, Milia, Peri-oral dermatitis, Acneiform eruption.
2. Patients using any topical acne treatment, topical retinoid, topical and systemic antibiotics, topical any systemic steroids, medicated facial cleansers from 2 weeks prior to the baseline visit and throughout the duration of the study.
3. Patients using facial cosmetic producers (such as laser resurfacing, chemical peel, dermabrasion, etc.) from 6 months prior to the baseline visit and throughout the duration of the study.
4. Patients with any immune compromised disease, uncontrolled diabetes, any systemic illness and structural deformities.

Study Duration: 60 days. Including follow-up period. Assessment of patient was done on the day of Enrollment i.e. 0 day.

Follow Up: On 10^{th}, 20^{th}, 30^{th} day during treatment and on 45^{th} and 60^{th} day after completion of treatment.

Screening Investigation:

1. HB %
2. RBS

Clinical Methodology: The Patients attended Kayachikitsa OPD & IPD, Mahatma Gandhi Ayurved College, Hospital and Research Centre, Salod (H), Wardha with clinical features of *Mukhadushika* were selected for the study. The patients were grouped by simple random sampling method irrespective of their sex, religion and occupation. The informed consent was obtained from each patient before participation in the study. Total 64 patients were registered as per the inclusion criteria and randomly divided into group A and group B by lottery method. Total 60 Patients completed the study, one (01) patient from Group A and three (03) patients from group B withdrawn during study as these patients were not attending regular follow up of scheduled treatment.

Diagnostic Criteria: The parameters for diagnosis of patient include symptoms like *Burning sensation (Daha), Pain (Vedana),* Itching *(Kandu),* erythema *(Lalima)*were selected for the study. Overall Acne (*Pidika*) were assessed by GAGS (Global Acne Grading Scale)[21]. A special proforma was prepared and patients were examined on the basis of available sign and symptoms.

Criteria of Assesment: The patients were assessed on the basis of Subjective and Objective parameters. Severity of disease was assessed by gradation of each criterion. Gradation was done as follows -

Subjective Parameters:

Gradation: 0-None, 1-Mild, 2-Moderate, 3-Severe

1. ***Daha*** **(Burning sensation)**
2. ***Vedana*** **(Pain)**
3. ***Kandu*** **(Itching)**
4. ***Lalima*** **(Erythema)**

Objective Parameter:

Overall rating of Acne was done by GAGS (Global Acne Grading Scale)

Each type of lesion is given a value depending on severity:

No lesions = 0, comedomes = 1, papules = 2, pustules = 3 and nodules = 4.

The score for each area (Local score) is calculated using the formula:

Local Score = Factor x Grade (0-4)

The global score is the sum of local scores and acne severity was graded using the global score. A score of 1-18 is considered mild; 19-30 – moderate, 31-38 – severe and >39 – very severe.

Note: As acne on chest and upper back was not included in the study the local score of the region was not calculated and considered.

Assessment of Results: The assessment of progress was observed after 60 days, after completion of the course of treatment. An assessment scale was framed to assess the rate of improvement. At the end of treatment, the percentage of relief was calculated and classified under the following headings:

1. **Maximum improvement:** More than 75% improvement of the subjective and objective criteria.
2. **Moderate improvement:** 50-75% improvement of the mentioned subjective and objective criteria.
3. **Mild Improvement:** 25-50% improvement of the mentioned subjective and objective criteria.
4. **No Improvement:** 0-25% improvement of the mentioned subjective and objective criteria.

Table No. 1: Intervention for Group A & B

Group	Group A (Standard Control Group)	Group B (Experimental Group)
Sample Size	30 patients	30 patients
Intervention	Internally Tablet (*Ghanavati)* of *H. indicus* (Hi)	Internally Tablet (*Ghanavati)* of *D. hamiltonii* (Dh)
	Externally *Sariva* Gel of *H. indicus* (GHi)	Externally *Sariva* Gel of *D. hamiltonii* (GDh)
Dose	Tablet (*Ghanvati*)-500mg BD after meal Gel- q.s. local application on infected area at bed time	Tablet (*Ghanvati*)-500mg BD after meal Gel- q.s. local application on infected area at bed time
Duration	30 Days	30 Days
Follow up period	On 10^{th}, 20^{th}, 30^{th} day (During treatment) On 45^{th} and 60^{th} day (After treatment)	On 10^{th}, 20^{th}, 30^{th} day (During treatment) On 45^{th} and 60^{th} day (After treatment)
Total Duration	60 days	60 days

Results

In this study 60 patients who fulfilled inclusion criteria were randomly selected from Kayachikitsa, OPD & IPD of Mahatma Gandhi Ayurved College Hospital and Research Centre, Salod (H), Wardha, Maharashtra. The selected patients were categorized in two Groups of 30 patients each. As per proforma prepared, observations were made and statistically presented as follows.

Comparative efficacy of drug on subjective parameters of patients:

Table No. 2 Comparative efficacy of drug on *Daha* (Burning Sensation) of patients:

Daha	Median		Wilcoxon Signed Rank W	P-Value	% Effect	Result
	BT	AT				
Group A	1	0	-3.782[a]	0.000	91.4	Significant
Group B	1	0	-3.568[a]	0.000	88.6	Significant

Table No. 3 Comparative efficacy of drug on *Vedana* (Pain) of patients:

Vedana	Median		Wilcoxon Signed Rank W	P-Value	% Effect	Result
	BT	AT				
Group A	2	0	-4.761[a]	0.000	92.2	Significant
Group B	2	0	-4.565[a]	0.000	90.6	Significant

Table No. 4: Comparative efficacy of drug on Kandu (Itching) of patients:

Kandu	Median		Wilcoxon Signed Rank W	P-Value	% Effect	Result
	BT	AT				
Group A	1.5	0	-3.999[a]	0.000	90.0	Significant
Group B	1.5	0	-3.808[a]	0.000	90.0	Significant

Table No. 5: Comparative efficacy of drug on *Lalima* (erythema) of patients:

Lalima	Median		Wilcoxon Signed Rank W	P-Value	% Effect	Result
	BT	AT				
Group A	2	1	-4.388[a]	0.000	60.0	Significant
Group B	1.5	1	-4.099[a]	0.000	56.5	Significant

Comparative efficacy of drug on Global Acne Grading Scale (GAGS) Score of patients:

Table No. 6: Comparative efficacy of drug on Global Acne Grading Scale (GAGS) Score of patients:

GAGS Score	Median		Wilcoxon Signed Rank W	P-Value	% Effect	Result
	BT	AT				
Group A	12.5	4	-4.794[a]	0.000	70.3	Significant
Group B	13	4	-4.791[a]	0.000	66.9	Significant

Discussion

Comparatively in all subjective and objective parameters there was no significant difference between group A and B. (Table No. 4 to 9).

Comparative overall effect of drug in 60 *Mukhadushika* patients, a majority of 51.7% patients were having marked improvement in *Mukhadushika*. While 40% patients benefited moderate improvement and 8.3% had mild improvement in *Mukhadushika*. The entire enrolled patients were benefited with improvement in *Mukhadushika*(Graph no. 1). This shows a strong effectiveness of both the drugs in *Mukhadushika*.

Probable mode of action of drug on Acne vulgaris (Mukhadushika): *Sariva is* having *madhura* (sweet taste), *tikta rasa* (bitter taste), *guru and snigdha guna, sheeta veerya* (cold potency), *madhura vipaka,vata pitta dosha samaka* and t*vakdosahara* (reduce skin disorder), *raktasodhaka* (blood purifier), *varnya* (improving the complexion), *dahaprasamana karma* (reduce burning sensation).

Sushruta explained, *Rakta dhatu Dushti* (blood impurities) is one of the main pathogenic factors of acne formation with other local and systemic pathogenic components related to sexual changes during adolescence.[22] Many authors elaborated that vitiation of *kapha, vata* and *raktadhatu* are main pathophysiological components in the development of *Mukhadushika* (acne). The primary site for acne formation is *Twaka* (skin) so it is included in '*Twagdosha*'.[23]

*Madhura rasa, sheeta veerya, madhura vipaka a*nd *guru - snigdha guna* of *sariva* balances the vitiated *vata dosha.* These attributes helps to minimize the burning sensation andpain in acnes. *Tikta Rasa* acts upon the *pitta dosha* and *rakta dhatu* by performing *raktasodhaka karma* and *twakdosahara karma* which reducesitching over the acnes and helps to detoxify the skin. Though it is *sheeta veeryatmak,* it does not elevate the *kapha dosha*. The *varnya karma* helps to diminisherythma over the acnes. These unique properties present in *sariva* helps in breakdown of pathophysiology of acne vulgaris.

In a study conducted by Kumar and coworkers, the roots of *H. indicus* showed strong inhibitory effect on P.acne and S.epidermis organisms responsible for acne vulgaris.

This clinical study evaluated similar effectiveness of both drugs which is needed to prove *D. hamiltonii* can be used as substitute to *H.indicus*.

Conclusions

The concept of substitute prevailed ages back and in Ayurveda there is need of analysis of this concept with the present trend of substitution so that we can adopt the proper substitute. In case of *Hemidesmus indicus*, comes under the category of substitution of different species, though it is abundantly available naturally in specific region of India, its root collection method is very tedious, time consuming and having low yield, leads to the scarcity of root of *Hemidesmus indicus* in raw herbal market and ultimately use of *Decalepis hamiltonii* which can be easily cultivated having high yield as compare to *Hemidesmus indicus*.

The most essential criteria for substitute is the pharmacological activity rather than morphological. Substitution of herbs achieves many goals through basic idea to provide similar, easily available, cost effective, most appropriate for the clinical condition. In our study *Decalepis hamiltonii* is appropriate drug used as a substitute of *Hemidemus indicus*.

On the basis of study both drugs relieved symptoms burning sensation (*Daha),* pain *(Vedana),* itching *(Kandu),* erythema *(Lalima)* and Global acne grading scale (GAGS) of Acne vulgaris significantly.

As per all observations it is concluded that *Ghana Vati* and Gel of *Hemidesmus indicus* and *Decalepis hamiltonii* are equally effective in the management of *Mukhadushika* (Acne vulgaris). So we can use *D. hamiltonii* as substitute drug of *Hemidesmus indicus* in the management of Acne vulgaris.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Subacute Oral Toxicity Study of Arsenic Compound Sameerpannag Rasa in Albino Rats

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Abstract

Background: Despite the long tradition of thousands of years and acceptance in common public, Ayurvedic medicines of mercurial, metallic and mineral origin are seen with fear and doubt because in toxicology mercury, metals etc. are known as toxic to the human body. The main reason for dispute lays in the method of analysis of the Ayurvedic metallic and mercurial medicines. The usual method of analysis of medicines for heavy metals is usually atomic absorption spectro-photometry etc. where elements can be detected but the molecular form is not detected. Arsenic in Sameerpannag Ras is more than a thousand times higher than the permissible limit of Arsenic in medicines, still successful use of this medicine is Ayurveda practice brings the hypothesis that it is non-toxic in therapeutic dose.

Method: Sameerpannag Ras is a medicinal compound made with three Arsenic ores and Sulphur. Proper animal ethics committee approval was taken, 150 Albino rats were divided into five groups viz control, vehicle control, therapeutic dose, 2X dose and 5X dose. Subacute oral toxicity study was conducted as per OECD guidelines, general behaviours, blood analysis and histopathology study of vital organs were done to access toxicity.

Results: Histopathology study reveals that the consumption of Sameerpannag Rasa in therapeutic dose as well as in five times the therapeutic dose for 28 days did not cause any significant changes in tissues of liver, kidney, lungs, heart or brain. The behaviour of animals was unchanged during the study, also there was no mortality during the study period.

Conclusion: Sameerpannag Ras prepared as per classical Ayurvedic pharmaceutical procedure consumed in therapeutic dose is non-toxic in acute toxicity study. Irrespective of the Arsenic as its major constituent, the pharmaceutical method makes it not-toxic, hence re-establishing its safety and relevance in Ayurveda system of medicine.

Keywords: *Sameerpannag Ras, Ayurveda, Arsenic, Toxicity, heavy metal.*

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Introduction

Ayurveda believes that even a fatal poison when used with skill and knowledge proves to be a good medicine and a medicine used ignorantly acts as a poison. Accumulated toxicity data on the hazardous effects of heavy metals have made health scientists afraid of heavy metals. As a result, renewed interest in the beneficial effects of metals and minerals is often viewed

with skepticism. Hence it is high time that instead of blindly following the text and prescribing the medicine, one should test it thoroughly about its side effects, dose duration and toxicity in the target organ of body. These tests will allow us to form those guidelines regarding contra-indications of our drug and also unravel the myths and ambiguities about mercurial and metallo-mineral medicines[1]. The contents of this drug include mercury and minerals containing Arsenic and sulfur. The dose and vehicle of the drug is mentioned but the duration of dose is not found anywhere and whether it can be used for repeated dose or not is also not mentioned. So the topic of present study was testing of sub-acute oral toxicity of Sameerpannag Rasa in albino rats. This study helps us to know the safe dose and dose duration of Sammerpannag Rasa .Its effect on general behavior, food consumption, body weight, hematology and blood-biochemistry and histology of albino rats[2,3].

Aim and objectives:

- To study the effect of Sammerpannag Rasa on hematological parameter of Wistar albino rats.
- To study the effect of Sammerpannag Rasa on blood biochemical picture of Wistar albino rats.
- To study behavioural changes induced if any, in Wistar albino rats due to feeding of Sammerpannag Rasa for a period of 28 days.
- To study the effect of Sammerpannag Rasa on histo-structural changes if any, in Wistar albino rats.
- To determine the sub-acute oral toxicity of Sameerpannag Rasa.

Materials and Method

1. **Testing of sub acute oral toxicity in albino mice:** The objective of the study was to determine the sub-acute oral toxicity of Sameerpannag rasa in albino rats, following the OECD guidelines, 407 adopted on 27th July 1995. In the assessment and evaluation of the toxic characteristics of a chemical or a drug, the determination of oral toxicity using repeated doses may be carried out after initial information on toxicity has been obtained by acute testing, which has been already carried out, as mentioned earlier(prior to commencement of sub-acute oral toxicity studies). The test material i.e. Sameerpannag Rasa is orally administered by gavage daily 7 days per week in graduated doses to three experimental groups of Wistar albino rats. One dose level, per group, for a period of 28 days. As per, Aushadhigunadharma shastra betel leaf is mentioned as a vehicle so fresh Juice of betel leaf prepared was selected as suitable vehicle for Sameerpannag Rasa and the same was administered in the animals from control group as vehicle control group to test whether it has got any toxicity on its individual use. Also plain control group without any dose was kept to compare the observations with experimental group. During the period of drug administration the animals from all 5 groups were observed closely, each day for signs of toxicity, body weight, food consumption per day, were recorded weekly and the dose volume was changed accordingly. Blood samples were collected at the end of sub-acute toxicity study for Hematological and clinical Biochemical analysis. All animals were autopsied and their liver and kidneys were tested for Histopathology.

Observations and Statistical analysis:

Table 1: Data showing comparison of body weight of test groups to control group from 0 to 4 weeks in male & female albino rats

Group	Mean diff	SD-1	SD-2	SE	T	P
V-I male	41.5	15.67	4.481	9.4	4.41	<0.05
V-II male	30.16	19.32	4.481	11.45	2.633	>0.05
V-III male	25.5	14.54	4.481	8.78	2.902	<0.05
V-IV male	9.667	12.20	4.481	7.154	1.286	>0.05
V-I female	1.167	4.726	6.062	4.421	0.262	>0.05
V-II female	26.833	14.28	6.062	8.951	2.995	<0.05
V-III female	23.167	9.07	6.062	6.288	3.677	<0.05
V-IV female	6.167	4.041	6.062	4.188	1.466	>0.05

Table 2: Hematological data (unpaired t test) in males

Parameter	Mean diff	SD-1	SD-2	SE	T	P
V-I						
WBC	5.533	4.09	0.624	2.38	2.3	>0.05
Lymph	3.5	3.65	0.611	2.13	1.6	>0.05
Moncyt	0.133	0.152	0.173	0.13	1.0	>0.05
Grancyt	1.8	0.49	0.057	0.86	6.2	<0.05
RBC	0.12	0.135	0.545	0.32	0.3	>0.05
Hb	0.4	0.43	0.866	0.56	0.7	>0.05
Group-V-II						
WBC	4.9	2.835	0.624	1.67	2.9	<0.05
Lymph	1.833	1.81	0.611	1.11	1.6	>0.05
Moncyt	0.266	0.15	0.173	0.13	2.0	>0.05
Grancyt	2.7	0.98	0.057	0.56	4.7	<0.05
RBC	1.5	1.16	0.545	0.98	1.5	>0.05
GroupV-III						
WBC	3.4	3.29	0.624	1.93	1.7	>0.05
Lymph	2.1	2.34	0.611	1.39	1.5	>0.05
Moncyt	0.0	0.1	0.173	0.11	0.0	>0.05
Grancyt	1.2	0.86	0.057	0.49	2.4	>0.05
RBC	0.6	1.13	0.545	0.72	0.8	>0.05
Group V-IV						
WBC	4.63	8.81	0.624	5.1	0.9	>0.05
Lymph	3.86	7.26	0.611	4.2	0.9	>0.05
Moncyt	0.03	0.32	0.173	0.1	0.1	>0.05
Grancyt	0.6	1.2	0.057	0.71	0.8	>0.05
RBC	2.29	2.26	0.545	1.34	1.7	>0.05

Table 3: Hematological data (unpaired t test) in female

Parameter	Mean diff	SD-1	SD-2	SE	t	P
Group V-I						
WBC	0.86	1.19	3.19	1.96	0.4	>0.05
Lymph	1.26	0.83	2.49	1.51	0.8	>0.05
Moncyt	0.00	0.11	0.11	0.09	0.0	>0.05
Grancyt	0.4	0.28	0.75	0.46	0.8	>0.05
RBC	0.65	2.03	3.04	2.1	0.3	>0.05
Hb	1.63	3.71	6.09	4.1	0.3	>0.05
Group V-II						
WBC	1.9	1.55	3.19	2.04	0.9	>0.05
Lymph	1.4	0.85	2.49	1.15	0.9	>0.05
Moncyt	0.0	0.05	0.11	0.11	0.0	>0.05

Parameter	Mean diff	SD-1	SD-2	SE	t	P
Grancyt	0.46	0.66	0.75	0.46	0.8	>0.05
RBC	1.56	2.0	3.04	2.11	0.3	>0.05
Group V-III						
WBC	0.26	4.6	3.19	0.32	0.8	0.05
Lymph	0.43	3.05	2.49	2.27	0.1	0.05
Moncyt	0.06	0.2	0.11	0.13	0.4	0.05
Grancyt	0.86	0.4	0.75	0.49	0.7	0.05
RBC	0.78	1.61	3.04	1.99	0.3	0.05
Group V-IV						
WBC	1.36	2.08	3.19	2.20	0.6	>0.05
Lymph	0.6	2.56	2.49	2.06	1.5	>0.05
Moncyt	0.0	0.05	0.11	0.07	0.0	>0.05
Grancyt	0.86	0.40	0.75	0.49	1.7	>0.05
RBC	0.29	2.3	3.04	2.2	0.1	>0.05

Table 4: Biochemistry data in males

Parameter(Sr)	Mean diff	SD-1	SD-2	SE	t	P
Group V-I						
BUN	5.0	8.86	6.8	6.49	0.7	>0.05
T.Protein	0.86	0.88	0.513	0.59	1.4	>0.05
Al.phos.	66.33	36.5	54.19	37.8	1.7	>0.05
SGPT	13.00	13.3	7.76	8.89	1.4	>0.05
Group V-II						
BUN	0.36	8.8	6.8	6.05	0.5	>0.05
T.Protein	0.3	1.7	0.513	1.07	0.2	>0.05
Al.phos.	43	31.0	54.19	36.0	1.1	>0.05
SGPT	50	17.1	7.76	10.8	4.5	<0.05
Group V-III						
BUN	11	1.52	6.8	4.0	2.6	>0.05
T.Protein	1.6	0.15	0.513	0.3	5.1	<0.05
Al.phos.	93.0	28.3	54.19	35.9	2.6	>0.05
SGPT	14.0	13.6	7.76	9.06	1.5	>0.05
Group V-IV						
BUN	0.758	10.2	6.8	7.0	0.3	>0.05
T.Protein	0.76	0.2	0.513	0.3	2.4	>0.05
Al.phos.	48.6	95.9	54.19	35.9	0.7	>0.05
SGPT	35.6	21.19	7.76	13.0	2.6	>0.05

Table 5: Biochemistry data in females

Parameter(Sr)	Mean diff	SD-1	SD-2	SE	T	P
Group V-I						
BUN	8.0	12.66	7.5	8.49	0.9	>0.05
T.Protein	0.1	0.305	0.404	0.29	0.3	>0.05
Al.phos.	35	44.2	14.9	26.9	1.2	>0.05
SGPT	6.3	8.18	18.23	11.5	0.5	>0.05
Group V-II						
BUN	1	3.05	7.5	4.6	0.2	>0.05
T.Protein	1.6	0.5	0.404	0.47	2.1	>0.05
Al.phos.	90.3	52.7	14.9	31.6	2.8	<0.05
SGPT	9.33	0.57	18.23	10.5	0.8	>0.05
Group V-III						
BUN	9.66	3.05	7.5	4.37	2.2	>0.05
T.Protein	0.33	0.5	0.404	0.27	1.2	>0.05
Al.phos.	5.33	52.73	14.9	30.8	0.1	>0.05
SGPT	15.66	0.577	18.23	12.1	1.3	>0.05
Group V-IV						
BUN	7.3	8.5	7.5	6.54	1.1	>0.05
T.Protein	0.4	1.0	0.404	0.67	0.6	>0.05
Al.phos.	144.6	81.9	14.9	48.1	3.0	<0.05
SGPT	17	8.32	18.23	11.5	0.2	>0.05

Discussion and Results

As per principles of medicine, the histological, biochemical, haematological and clinical changes have to be significantly relevant to reach up to certain diagnosis.[1-4]

- The test group animals H, 2H and 5H showed normal and healthy weight gain and food intake throughout the study. The mean percent body weight gain of animals of control male group was 19.4%. While of vehicle control group, H, 2H and 5H was 29.4%, 44.5%, 37.66%and 33.86% after the completion of 4 weeks. In females the mean percent body weight gain of control group was 6.1%. In vehicle control group H, 2H and 5H it was 3.12%, 6.56%, 21%and 21%.
- All survived animals were observed healthy and showed no abnormal behavioral changes. This particularly indicates the safety of Sammerpannag Rasa.
- The RBC count is normal in all the groups. This shows that there is no toxic effect of Sameerpannag rasa on the test groups. The Hemoglobin levels are within normal limits in all animals The test group 5H female rats showed leukopenia but it do not has any relevance with its biochemical, clinical and histological changes. But possibility of initiation of an allergic drug reaction cannot be denied clearly[4].
- In the present study SGPT of all animal lies within normal level, except in Group2H animal no 1, 2 and 3 which show significant rise in SGPT levels. but histological picture shows no evidence of cell necrosis of liver. Similarly the SGPT levels of 5H group are normal with normal picture of liver histology. There were no associated clinical symptoms like dark stool, diarrhea, tiredness etc. seen in the animals. Regardless of this they show increased food consumption and body weight gain. Hence the rise of SGPT level is not significant from toxicity point of view[5].

- The Sr. Alkaline phosphatase values lie with in normal limits in all the groups.
- In present study the levels of total protein are raised in 5H group which may be due to higher dose of Sammerpannag Rasa . The histology of liver is normal in 5H group, but it shows evidences of primary cellular changes to the kidney. Hence it can be said that High doses of Sammerpannag Rasa minutely though, has affected the renal function .No mortality occurred in any group. No clinical signs like dark stool, diarrhea, or fatigue etc. The biochemical parameters of H and 2H groups were within normal limits as compared to control and vehicle control groups.
- Histology of liver in 5H group shows NAD with mild lobular and periportal mononuclear cells, which is of no toxicological significance.
- However in renal pathology the animals in 5H group shows focal tubular necrosis, which indicates damage to the renal cells, this change is not seen in control or vehicle control group. These changes may be due to Sameerpannag rasa in high dose. The Sr. protein levels are also raised in males of this group, but females show normal values. Because cells are constantly adjusting structure and function to accommodate changing demands and extracellular stresses. Cells tend to preserve their immediate environment and intracellular milleu with a relatively narrow range of physiological parameters, they maintain normal homeostasis. The cells encounter pathologic stimuli, they can undergo adaptation, achieving a new steady state and preserving viability. The principle adaptive responses are atrophy and hypertrophy. If the adaptive capability is exceeded, cell injury develops. Within certain limit, injury is reversible and cells resume to a stable baseline. However, in severe or persistent stress results in irreversible injury. Indeed, the observed changes in these animals are subjected to persistent increased load on glomerular filtration, ultimately the interstitial tubular cells, which were undergone adoption by hypertrophy (an increase in the size of individual cells) to generate required filtration rate[6]. Thus, due to this adaptation of cell the pathological stimuli are observed without hampering clinical, haematological and biochemical values. Hence, BUN values were not affected during this course. Particularly, tubular necrosis and cloudy changes observed in these animals are reversible.

The study shows pathological changes in kidney in animals given 5 times higher dose and all normal values in H group, hence it should be concluded that Sammerpannag Rasa is safe to be given for repeated dose at dose level of 60-120mg.

Conclusions

- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of food consumption data.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Body weight gain data.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Behavioural changes.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Clinical signs observed.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Haematological parameters considered.
- Prepared Sameerpannag Rasa has not showed sub-acute toxicity by oral route in albino rats on the basis of Biochemical parameters.
- Prepared Sameerpannag Rasa was found safe at therapeutic dose levels when administered for a limited duration of time.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Experimental Evaluation of Wound Healing Activity of Various Dosage Forms of Tridax Procumbens

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Abstract

Introduction: *Tridax procumbens Linn.* (Tridax) is a common weed found all over India. It is commonly known as Peet Bhringraj, Jakhamjod and Kambarmodi etc. It is traditionally being used since centuries for wound healing and haemostasis and also for the treatment of diarrhea, dysentery, bronchial catarrh etc.

Need of the Study: Tridax is traditionally being used in its crude form. Despite of the marked utility of Tridax in wound management, in Ayurvedic literature neither there is much description of Tridax nor its dosage forms are mentioned. Exploring the various dosage forms

Objective: Thus the study is designed to find out an effective Ayurvedic dosage form of Tridax that is more effective and on the same hand is holistic in approach as per principles of Ayurveda.

Materials and Method: An animal experiment is conducted on Albino rats using excision wound model. Five dosage forms of Tridax were prepared such as Ash, Kalka, Oil, Ghana and Ethanolic extract and were compared with control to evaluate rate of contraction and period of epithelialization.

Results and Conclusion: The results were outstanding. Different dosage forms of Tridax exhibited excellent wound healing property. Few dosage forms exhibited typical behavior in different stages of wound healing. Hence Tridax can be seen as a potent drug to develop a good wound healing agent of future.

Keywords: *Tridaxprocumbens, wound healing, dosage forms, herbal medicine.*

Introduction

Tridax procumbens Linn. (Tridax) is a common weed found all over India up to 2400 m above sea level and in all hot countries. The plant is native of tropical America and naturalized in tropical Africa, Asia and Australia. In English it is popularly called 'coat buttons'. It is known for its various medicinal properties. Native healers of India are using this herb since ages for various treatment purposes. Bapalalvaidya in Nighantu Adarsha has mentioned Tridax by the name 'Pardesi Bhringraj'[(1)]. Shodhal Nighantu has mentioned Tridax by the name Avanti[(2)].

Tridax is known by different names in various parts of India, such as, Kambarmodi, Jakhamjod, Ekdandi, Brahmadandi, Dagdipala, Tan tani, Man modi in Maharashtra; Moliamehandi, Pathhargada, Raktrodhi, Raktashoshi in Rajasthan[(3)]; Tumbi, Vettu-kkaya-

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thalai in Tamilnadu; Raavanaasuraaditalakaai, Kampu Chemanti in Andhra; Gabbu sanna savanthi, nettu gabbu savanthi, Tikki kasa, Tikki toppala, Gayad toppala, Kulae puduga, Kulae buskad, Hochod tasad, Robrobpuru in Karnataka; Vranaropini, Raktarodhi, Tikki kasa, Dandotpala, Sanjivani, Peet Bhringraj in north India[4].

It is traditionally known for its various medicinal properties, especially wound healing, haemostatic, Antiseptic, pain relieving. In tribal region, application of Tridax fresh leaves paste for haemostasis and wound healing is a common practice. Tridax has a good application for wound management in traditional medicine from centuries.

It is also used in dysentery, diarrhea, bronchial catarrh, sore throat etc. It is eaten as vegetable in some parts. It is also fed to cattle as a nutritive feed. In Kenya the leaves are chewed followed by a drink of water by the Masai for stomachache and malaria and in Tanganyika a decoction of the whole plant in water is taken for persistent low backache and a root decoction for infantile diarrhoea. Traditionally local Yoruba population of Western states of Nigeria uses the leaf of the plant as treatment to reduce blood pressure, conjunctivitis, diarrhoea and dysentery to wound healing and related inflammatory conditions[5].

Need of the Study: One cannot always use the herb in its native form due to different reasons like undesirable test, smell, physical form, mal absorption etc. Also, drug cannot be obtained fresh in all seasons of the year. Hence, man started converting drug into some processed form that possesses desirable durability, good test, smell, form, potency, prolonged shelf life etc. This science then developed and called Bhaishajya Kalpana Vigyana.

Tridax is traditionally being used in its crude form. The most frequently used form of Tridax for medicinal purpose is Paste, fresh juice and rarely decoction. A few research works has been carried out to evaluate the wound healing activity of Tridax. But most of the times organic solvent extracts of Tridax were used. Ayurvedic system of drug manufacturing does not support the use of extracts derived using toxic and carcinogenic solvents. In Ayurvedic system all the things used are in edible form as a holistic approach. But Despite of the marked utility of Tridax in wound management, in Ayurvedic literature neither there is much description of Tridax nor its dosage forms are mentioned. Thus the study is designed to find out an effective Ayurvedic dosage form of Tridax that is equally or more effective than solvent extracts and on the same hand is holistic in approach as per principles of Ayurveda.

Method And Material

Materials: For experimental study, the materials required are,

Albino rats: 36

Diethyl Ether, Scissors, Mosquito forceps, Povidone iodine, BP blades, BP handles, surgical gloves, Sterile surgical gloves, sterile gauze pads, sterile eye towels, scientific measuring scale, Rat cages, feeding bottles, standard rat feed, Tridax fresh leaf paste, Tridax Ghana solution, Tridax oil, Tridax Ash, Tridax Ethanolic extract etc.

Method:

Animal Ethics committee approval: Proper approval of animal ethics committee of Apollo college of veterinary medicine, Jaipur was taken for the preclinical study.

Selection of animals: Healthy albino rats of either sex weighing between 150-200 gm were selected randomly for study. The rats were housed in the animal house of Apollo College of veterinary medicine, Jaipur. The rats were fed with standard rat pellets and water ad-libitum.

Grouping of animals: Thirty six Albino rats were selected randomly from the animal house and were divided into six groups each containing six albino rats. The rats were housed in individual cages and kept in a well ventilated room under hygienic conditions.

Table I: Table showing grouping of animals

Group	Group drug	No. of rats	Purpose
C	No drug (control)	6	To serve as prophylactic control
A	Tridax Ash (Bhasma)	6	To serve as prophylactic effect
K	TridaxPaste (Kalka)	6	To serve as prophylactic effect
O	Tridax Oil (Taila)	6	To serve as prophylactic effect
G	Tridaxaqueous extract (Ghana)	6	To serve as prophylactic effect
E	Tridax Ethanolic Extract	6	To serve as prophylactic effect

Experimental Study: The wound healing property of the trial drugs, 5 dosage forms of Tridax procumbens was analyzed by Excision Wound Model. This study included following stages.

- Pre operative stage
- Operative stage
- Post operative stage

Pre Operative Stage: The selected albino rats were fasted overnight and water was given ad-libidum. Each rat was hydrated with 5 ml of water and was kept in individual cages.

The part to be operated was shaved with scissors and razors and painted with povidone iodine 5% solution.

The animals were anesthetized using Diethyl ether inhalation anesthesia in a semi-aseptic condition. A stage 3 anesthesia was achieved.

Operative Stage: This was conducted as per the method developed by Morton and Mallon. The anesthetized animals were secured to the dissection tray in prone position. A circle of 15 mm in diameter was marked with surgical marker pen on dorsal region approximately 5 cm away from the ears of the anesthetized rat. Full skin thickness from the marked area was excised in a circular fashion with the help of forceps and surgical scissors. The approximate area of wound thus formed was 177 mm square. After achieving full haemostasis rats were placed in individual cages.

Post operative stage: The control group was dressed with 5% povidone iodine solution, while other trial groups were applied with respective drugs locally once a day. Such as

Tridax Ash (Bhasma)–Dusting locally

Tridax Paste (Kalka)–Painting Locally

Tridax Oil–Local application

Tridax Aqueous extract (Ghana)–1gm/ml solution local application

Tridax ethanolic extract–50mg/ml solution local application

The rats were observed till the recover from anesthesia. Each post operative day, the wounds were cleaned with normal saline water and applied with trial drugs. The rats were given normal feed and water.

Assessment Criteria: Wound contraction and epithelialisation were the parameters employed to study excision wound model and this was achieved using planimerty.

1. **Wound Contraction:** The factor which contributes mainly to wound healing (closure) is contraction. This was done by tracing the wound margins and measuring the area of wound. Later the percentage of contraction was calculated arithmetically from the measured area. This measurement was done till the wound completely closes.

2. **Period of Epithelialisation:** It was measured in terms of days required for the falling of the scar. Falling of scar leaving no raw area behind was taken as the end point of complete epithelialization and the time was noted in all animals.

Observations and Results

To monitor the change in wound shape, the wound

diameters in two perpendicular planes were measured. The area of the oval was calculated as per the formulae '$A = \pi X r_1 Xr_2$' where A stands for the area of the ellipse, $\pi = 3.14$, r_1 = longer radius and r_2 = shorter radius. The observations of percentage of wound closure were made on 3rd, 6th, 9th, 12th, 15th, 18th and 21st post wounding day. The wound was also observed for the period of epithelialisation in term of days.

Table II: Table showing comparative closure of wound area on 3rd post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 7.5456	< 0.0001	A > Cextremely significant
2	Control C & O	t = 3.4374	=0.0064	O > C very significant
3	Control C & K	t = 13.2686	<0.0001	K > Cextremely significant
4	Control C & G	t = 2.4709	= 0.0331	G > C significant.
5	Control C & E	t = 0.0703	= 0.9453	Not significant.

Table III: Table showing comparative closure of wound area on 6th post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 11.2320	<0.0001	A > Cextremely significant
2	Control C & O	t = 0.1733	=0.8659	Not significant
3	Control C & K	t = 9.6743	<0.0001	K > Cextremely significant
4	Control C & G	t = 5.3336	=0.0003	G > C extremely significant.
5	Control C & E	t = 1.8691	=0.0911	Not quite significant.

Table IV: Table showing comparative closure of wound area on 12th post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 3.5979	=0.0049	A > Cvery significant
2	Control C & O	t = 0.2116	=0.8367	not significant
3	Control C & K	t = 7.1164	<0.0001	K > Cextremely significant
4	Control C & G	t = 2.2653	=0.0469	G > C significant.
5	Control C & E	t = 2.8618	=0.0169	E > C significant.

Table V: Table showing comparative closure of wound area on 18th post operative day

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 3.6576	=0.0044	A > Cvery significant
2	Control C & O	t = 3.6576	=0.0044	not significant
3	Control C & K	t = 3.6576	=0.0044	K > Cvery significant
4	Control C & G	t = 3.6576	=0.0044	G > C very significant.
5	Control C & E	t = 0.6458	=0.5329	Not significant.

As represented in above tables, gross findings about wound contraction are as follows.

1. All the dosage forms of Tridax showed statistically significant results compared to control group in wound contraction.
2. In first 3 days Kalka and Ash were extremely significant in contracting wound. Though in two cases Oil failed in wound contraction, statistically oil was very significant. Ghana was also significant where Ethanolic extract was not significant.

3. On 6th post wounding day Ash, Kalka and Ghana were extremely significant whereas Alcoholic Extract and Oil were insignificant.
4. On 9th post wounding day Kalka was extremely significant in wound contraction, whereas other dosage forms were not significant.
5. On 12th post wounding day Kalka was extremely significant, Ash was very significant, Extract and Ghana were significant But Oil was not significant.
6. On 15th day the wounds of group Kalka and Ash were completely contracted and healed with extremely significant results. Ghana also showed extremely significant results whereas Oil and Extract were not significant.
7. On 18th day wounds of all the group were healed except that of Extract group that was also non significant in contraction.

Period of epithelialisation: Falling of scar crust leaving no raw area behindwas taken as the end point of complete epithelialisation

Table VI: Table showing comparative epithelialization time of wound.

No.	Groups	t- value	p- value	Remark
1	Control C & A	t = 12.7279	<0.0001	A < C extremely significant
2	Control C & O	t = 1.3815	=0.1972	not significant
3	Control C & K	t = 12.4581	<0.0001	K < C extremely significant
4	Control C & G	t = 10.1419	<0.0001	G < C extremely significant.
5	Control C & E	t = 2.6769	=0.0232	E < C significant.

As clear from above table, followings were the results about epithelialisation of wounds.

1. All the dosage forms of Tridax significantly reduced the period of epithelialisation as compared to the control group.
2. Ash was the best in reducing period for epithelialisation with statistically extremely significant results. It was followed by Kalka and Ghana with extremely significant results. Extract was also significant whereas Oil was statistically insignificant in epithelialisation.
3. It was observed with group Kalka that, on 18th day the healed scar showed hair growth whereas no other group showed hair growth on scar even after 21 days after wounding.

Discussion

As reviled from this experiment, different preparation of Tridax had shown different pattern of activity in initial, intermediate and late phase of wound healing. Such as Ash and Kalka has shown rapid wound contraction in initial stage whereas oil failed to contract wound. On contrary Oil caused to dilate the wound area in some cases in initial stage. Ethanolic extract of Tridax promoted wound contraction in intermediate stage of wound healing whereas it delayed the last stage of wound healing. This typical behavior may be due to the phytochemical contents of Tridax in that particular dosage form, may be due to physical nature of dosage forms such as Ash is in an absorbent powder form, Kalka itself contracts and along with contracts the wound as it dries, Oil prevents the wound from drying and contracting, etc. or it may be the combined action of phytochemicals, vehicles and physical form of the dosage form. A separate study of each dosage form may unwind the mystery[6,7].

Another interesting observation was that, the Tridax Kalka promoted hair growth after healing the wound. Whereas other dosage forms and control group showed absence of hairs on the scar area. The Kalka might have got some novel property that promotes healthy epithelialisation and formation of hair follicles. Generally excised wounds heal through collagen and fibrous tissue formation that lack hair follicles. Further histological investigation is necessary to find out exact pattern of tissue development[8].

Finally the traditionally used form of Tridax that is Kalka is found to be most useful for wound healing purpose. This might be one of the reasons why other dosage forms of Tridax could not evolve in the course of time. Another most useful dosage form of Tridax is found as its Ash. Also other dosage forms may be purposefully used as they promote wound healing at different stages of wound healing. Further detailed study including histological and histochemical investigation will be beneficial in order to develop a good wound healing agent of future from Tridax[9].

Conclusion

Tridax is traditionally being used for wound management in India. Ayurvedic textquotes no reference about different dosage forms of Tridax. The experimental trial of different dosage forms of Tridax on wound healing in Albino rats reviles that, Tridax has got highly significant improvement in wound healing including wound contraction and epithelialization, except the oil that is not significant in reducing time for epithelialisation. Kalka the most famous traditional dosage form of Tridax and Ash were found to be best in wound healing, followed by the Ghana. Different preparation of Tridax shows different pattern of activity in initial, intermediate and late phase of wound healing. Tridax Kalka also promoted hair growth on scar after healing the wound. Further detailed study including phytochemical, histological and histochemical investigation will be beneficial in order to develop a good wound healing agent of future from Tridax.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Various Dosage Forms of Tridax Procumbens and their Antimicrobial Activity Against Specific Pathogens

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Abstract

Background: TridaxProcumbens Linn, (Tridax) is a herb traditionally used in Indian traditional medicine for different ailments like cough, cold, amoebiasis and most importantly for seizing the bleeding and wound management. Practically it is observed that the wound treated with Tridax was found to be very rarely infected and healed properly. Researched suggests that the different dosage form of the Tridax have different potential in wound healing. On these two backgrounds, the hypothesis was made that the Tridax has got antimicrobial property and the antimicrobial potential varies as per the dosage form.

Method: To examine the hypothesis, in the present experiment, seven Ayurvedic dosage forms of Tridax viz. fresh leaves juice, fresh flower juice, fresh root juice, aqueous decoction, medicated oil, medicated Ghee, ash, alkaline aqueous water-soluble fraction of the Ash and six organic solvent extracts of Tridax viz. ethanol, methanol, chloroform, ethyl acetate, benzene and petroleum ether were tested for their antimicrobial activity against eleven different disease-causing microbes viz. Bacillus subtilis, Proteus vulgaris, Shigellaflexneri Staphylococcus aureus, Enterobacter aerogenes, Chryseobacteriumgleum, Klebsiella pneumoniae, Aspergillus fumigatus, Candida albicans, Aspergillus niger and Aspergillus flavus. Antimicrobial experiment was carried out with appropriate nutrient media and disc diffusion method.

Results: Leaf juice showed microbial inhibition zone of 28mm against B. subtilis. Flower juice showed 17mm against C. albicans, aqueous decoction showed 14mm against C. gleum, Ethanol extract, Methanol extract, Chloroform ext. and Ethyl act. Extract showed microbial inhibition zone of 17mm, 14mm and 14mm against respectively.

Conclusion: The different dosage form of Tridax have a different and significant antimicrobial potential against different microbes. It supports the importance of different processing suggested in classical Ayurvedic text for obtaining different results from aparticular herb.

Keywords: *Tridaxprocumbens, Antimicrobial, Dosage forms.*

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Introduction

Tridaxprocumbens Linn. (Tridax) is a herb found everywhere in India, especially in rocks and mountains. This plant is called Avanti[1] in Ayurveda and Peet Bhrigraj, Kambarmodi, Dagadphul, Gabbu Chemanti[2,3,4] as different vernacular names. Tridax is traditionally used for wound healing, seizing bleedings, cough, cold,

amoebiasis etc. It is observed that the wound treated with Tridax heals properly without developing any infection or sepsis. Research regarding the wound healing activity of different preparations of Tridax shows that the different preparation has different wound healing activity and analgesic activity[5]. These two studies suggest that the Tridax may have antimicrobial potential and the potential may be different in different dosage forms.

Hypothesis: The different dosage forms of Tridax like Juice, Paste, Decoction, Oil, a water-soluble fraction of ash and organic solvent extracts like alcohol soluble extract, chloroform extract, petroleum ether extract etc. have different antimicrobial activity on specific pathogenic microorganisms

Objective: To identify the antimicrobial potential of different dosage forms of Tridax against different eleven microbes.

Materials and Method

Preparation of dosage forms: Different dosage forms of Tridax were prepared as per general guidelines laid for herbal preparations from classical Ayurvedic treaty Sharangdhar Samhita[6]

Table 1: Dosage Form

Sr.No.	Dosage form
1	Fresh juice- leaf
2	Fresh juice- flower
3	Fresh juice- root
4	Reduced aqueous decoction
5	Alkali- water-soluble fraction from ash
6	Oil- medicated sesame oil with Tridax
7	Clarified butter (Ghrita) medicated with Tridax

Organic solvent extracts were also prepared and included in the study for comparison. As follows

1. Ethanolic extract 10% w/w
2. Methanolic extract 10% w/w
3. Chloroform extract 10% w/w
4. Benzene extract 10% w/w
5. Ethyl acetate extract 10% w/w
6. Petroleum ether extract 10% w/w

Microbial Strains: The trains of pathogenic microbes[7] involved in the study were obtained from the Institute of Microbial Technology and Sawai Man Singh Medical College and Hospital, Jaipur #.

Table: 02: Microbial strains

No.	Species and MTCC No.
1	Bacillus subtilis (441)
2	Proteus vulgaris (744)
3	Shigellaflexneri (1457)
4	Staphylococcus aureus (7443)
5	Enterobacter aerogenes (111)
6	Chryseobacteriumgleum (1916)
7	Klebsiella pneumoniae (109)
8	Aspergillus fumigatus#
9	Candida albicans (3017)
10	Aspergillus niger (1344)
11	Aspergillus flavus#

Microbiological techniques adopted

Culture media was prepared and set in Petri dishes to form media plates. Sterile 5mm disks for disk diffusion method were prepared from standard Whatman paper No. 1. These disks were soaked in individual trial drug 10 ml and dried for the antimicrobial study. 24-hour microbial subculture was prepared and 100 ml of it was spread over the media plate with a spreader. Trial disks were placed and marked on media plate and incubated in an incubator for 24 hours at 37°C. Solvent used for extraction was used as a control. Media plates were observed for inhibitions zones of microbial growth after 24 hours.

Observations and Results

Table: 3: Observations and Results

	Drug and their inhibition zone in mm	B. subtilis	P. vulgaris	S. flexneri	S. aureus	E. aerogenes	C. gleum	K. pneumoniae	A. fumigatus	C. albicans	A. niger	A. flavus
1.	Leaf juice	28	6	6	0	7	11	0	8	9	8	9
2.	Flower juice	7	7	0	6	0	10	0	10	17	0	9
3.	Root juice	8	8	6	8	7	13	6	9	11	0	8
4.	Kwatha Ghana	8	7	6	13	0	14	9	8	0	0	0
5.	Kshara	13	9	0	11	11	12	8	10	12	7	13
6.	Taila	8	0	8	0	8	9	0	0	11	7	0
7.	Ghrita	6	9	0	9	7	6	0	0	7	0	0
8.	Ethanol ext.	7	6	11	9	8	13	0	0	17	0	8
9.	Methanol ext.	7	6	13	7	6	8	6	12	14	10	7
10.	Chloroform ext.	7	6	12	7	0	13	8	14	13	12	8
11.	Benzene ext.	7	9	9	9	7	9	7	9	8	7	8
12.	Ethyl act. Ext	8	0	19	7	6	6	0	8	8	7	7
13.	Pet. Ether ext.	10	10	11	12	10	10	7	7	11	7	9

Note: Nil/0 = No Inhibition, Insignificant = 6 mm-9 mm, Significant= 10mm-13mm, Highly significant = 14mm and above.)

Image I

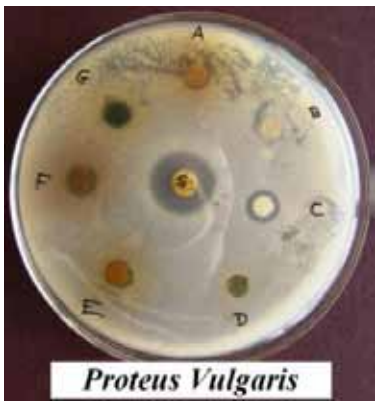


Image II



Image III



Results

1. **Fresh juice (Leaf):** Demonstrated highly significant activity of microbial growth inhibition against B. subtilis. Significant inhibition of growth against C. gleum and insignificant activity against other microbes.
2. **Freshjuice (Flower):** Demonstrated highly significant inhibition of microbial growth against C. albicans. Significant inhibition against C. gleum and A. fumigates. and insignificant activity against other microbes.
3. **Fresh Juice (Root):** Demonstrated significant inhibition against C. gleum and C. albicans. and insignificant activity against other microbes.
4. **Reduced aqueous decoction:** Shows highly significant inhibition against C. gleum. Significant inhibition against S. aureus. and insignificant activity against other microbes.
5. **Alkali (water-soluble fraction from Ash):** It did not show any significant activity against any pathogenic microbe.
6. **Medicated oil:** It has significant inhibition against C. albicans. and insignificant activity against other microbes.
7. **Clarified butter medicated with Tridax (Ghrita):** Shows significant inhibition against S. aureus. and insignificant activity against other microbes.

8. **Ethanolic extract**: Shows significant inhibition against S. flexneri, C. gleum and C. albicans. and insignificant activity against other microbes.
9. **Methanolic extract**: Shows highly significant inhibition against C. albicans. Significant inhibition against S. flexneri, A. fumigates and A. niger. and insignificant activity against other microbes.
10. **Chloroform extract**: Shows highly significant inhibition against A. fumigates. Significant inhibition against S. flexneri, C. gleum, C. albicans and A. niger. and insignificant activity against other microbes.
11. **Benzene extract**: Failed to show any significant activity against any pathogenic microorganisms.
12. **Ethyl acetate extract**: Demonstrates highly significant inhibition against S. flexneri. Failed to show significant activity against any other pathogenic microorganisms.
13. **Petroleum ether extract:** Demonstrates significant inhibition against P. vulgaris, B. subtilis, S. flexneri, S. aureus, E. aerogenes, C. gleum and C. flavus. Failed to show significant activity against any other pathogenic microorganisms.

Discussion

Nowadays solvent extraction industry has developed to a great extent. Solvent extracts are preferred since they provide maximum yield. In Ayurveda, different preparations of the same herbs are employed for a specific condition. Not only the fraction of herbs diffused into the solvent but also the solvent itself like oil and Ghee etc. plays a role in the pharmacological action, prevention etc. of the drug. The organic solvent does not hold a good reputation due to its carcinogenic and other adverse effects. Ayurveda believes that the medium used for the processing of any medicines leaves its impression and adds its attributes to the drug being processes. In that sense, if we are processing herbs with organic solvents, definitely, the drug is going to carry the trace and attributes of the toxic solvents in their constitution whereas the use of natural holistic solvents not only adds to the bioavailability but also eliminates the possibility of unforeseen adverse effect[6]. The only disadvantage is that all the phytochemicals may not be extracted in the process thus affecting the yield of industrial processed. In the current study, we need to learn that when a adopt a formula from traditional medicine, not only the herbs involved but also the processing and methodology adopted should be taken into consideration in favour of utilization of the traditional knowledge in a safe as well as effective way.

Conclusion

Tridax is a traditionally used herb in India for various ailments. Antimicrobial study of this herb shows that its various classical preparations and dosage forms demonstrated selective and distinct antimicrobial activity against different pathogenic microbes. fresh juice of leaves, aqueous decoction and flower juice show highly significant inhibition of Bacillus subtilis, C. gleum and Candida albicans respectively. Alkali derived from water-soluble ash and other organic extracts have inhibited about 50% of the tested microbes. Tridax may act as a potent antimicrobial agent for specific strains and may have potent utility in the pharmaceutical industry. Further study is necessary to explore the potential to more depth.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Development of Dose Sipping Technology, a New Design Approach for Improving Drug Delivery of Acyclovir in Pediatric Medication

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Abstract

Background: In quest of developing a dosage form having increased focus on the development of novel technologies which combines the advantages of liquids as easy swallowing with that of solid dosage forms as storage stability and exact unit dosing as well as taste masking which may improve compliance in children or elderly, dose sipping technology tends to be a better alternative and hence preferred for the present experimental part.

Objectives: The aim and objective of the present work are to study one such new design approach (Dose sipping technology) in order to improve drug delivery of antiviral drugs for pediatric medication.

Method: The proposed dose sipping (DS) administration system consists of Acyclovir drug-loaded pellets formed by the sequential method, further packed in a system of a drinking straw-like tube closed on the bottom end by a filter plug called a controller.

Results: Cumulative drug release data for formulated Acyclovir loaded spheres against marketed Acyclovir tablet using Prototype model forstudyingin-vitro drug release per sip, considering the suction process,achieved 99.70 % release within 11 minutes as compared to 60 minutes for 99.45% with improved patient compliance.

Conclusion: Dose Sipping (DS) Technology is a dosage form which combines the advantages of liquids as easy swallowing with that of solid dosage forms as storage stability and exact unit dosing as well as taste selection in the patient favorite beverage might improve compliance in children or elderly.

Keywords: *Antiviral agent, Dose sipping technology, pediatric medication, new design approach.*

Introduction

Multiparticulate drug delivery systems are composed of a number of discrete units such as granules, pellets or minitablets. Multiparticulate products are expected to provide improved patient acceptability over single-unit solid dosage forms (i.e., tablets and capsules) by dint of their reduced size and thus improved ease of swallowing plus the increased dose flexibility provided by their multi-unit composition.

Multiparticulate can be directly administered into the patients' mouth or dispersed in a vehicle prior to administration as preferred[1,2]. Water, milk, juice or coconut water is potential vehicles commonly proposed. In quest of developing a dosage form having increased

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focus on the development of novel technologies which combines the advantages of liquids as easy swallowing with that of solid dosage forms as storage stability and exact unit dosing as well as taste masking which may improve compliance in children or elderly, dose sipping technology tends[3] to be a better alternative and hence preferred for present experimental part. The aim of the study is to improve the drug delivery by using dose sipping technology for pediatric medication. The objective of this study is study of novel technologies for the preparation of age-appropriate formulations and development of a dosage form which combines the advantages of liquids as easy swallowing with that of solid dosage forms as storage stability and exact unit dosing as well as taste masking which may improve compliance in children or elderly, dose sipping technology tends to be a better alternative and hence preferred for present experimental part. Among various diseases occurring in pediatric patients, HIV infection and their associated opportunistic infections is major cause for mortality and is issue of concern globally. Hence Acyclovir drug used for treating opportunistic infections was preferred for present work.

Experimental Work and Evaluation Data: Present study involves formulation of an Acyclovir drug loaded pellets by sequential method. Following steps were involved: Formulation of Sugar Beads; Formulation of Acyclovir coated beads; Color coat and Filling of straw.

Experimental:

Formulation of Sugar Beads:

Sugar spheres were prepared and evaluated in NM Pharma at Navsal

Formulation of Acyclovir coated beads: The composition of the spray liquid comprises of Acyclovir drug, 0.1 N HCl solution and ethanol. Concentration of 16 % (w/w) was used, which was close to its maximum solubility (18 % w/w in water). Acyclovir was added slowly under continuous stirring to get a uniform dispersion. Drug loading[5] was carried out in R & D coater having speed of around 25 RPM with an optimum temperature of about 60°C. Coating was continued until 22 to 23 % weight gain was achieved. After achieving desired weight gain the coated beads were dried by rotating in pan for 10 minutes. The coated beads thus formed are gently passed through sieve with mesh size 10 and 20**.** Granules retained on 10 # (larger size) and passed through 20 # (smaller size) were discarded. Beads retained on mesh size 20 were even in size and were used to fill in the straws. The Beads were stored in suitable air tight container. Yield was calculated and is recorded.

Color Coat: Prepared Acyclovir coated beads were coated by different colors or flavors like Amaranth, D & C Green-5, D & C Yellow-8, D & C Orange-4 and FD & C Blue-1 by simultaneous method in R & D coater, with temperature 60˚c and 25 RPM.

Filling of Straw: Beads retained on mesh size 20 were even in size and were used to fill in the straws. Twins retained on the sieve were removed. Quantity equivalent to desired dose was accurately weighed and transferred to straw after removal of cap and later closed. The straw was packed in zip lock bag to assure storage stability.

Evaluation:

Evaluation of formulated Sugar Beads: Sugar beads using mentioned tools were evaluated at NM Pharma - **Description** (using lense), **Identification (**using iodine solution), **Particle size (**using sifter analysis method), **Loss on Drying (**using LOD analyser) **Residue on ignition** (using furnace), **Heavy metal (**using AAS), **Assay (on dried basis), Microbial Limit test (**Total aerobic microbial count, Salmonella species determination, Escherichia Coli determinations, Staphylococcus aureus determination and Pseudomonas aeruginosa determination), **Friability and sphericity (**Oscillating Friability Apparatus-Erweka GTA 120). The results are enclosed in table no.1.

Behaviour of sugar spheres in water and different buffer solutions: Sufficient quantity (1g) was placed in beaker containing 10 ml of solutions having pH 7, 6.8 and 1.2 were recorded at different time intervals. The results are enclosed in table no.2.

Table No. 1: Sugar Beads testing parameters and results obtained

Sr.No.	Test	Specifications	Results
1	Description	A white to off-white spherical pellets	A white spherical pellets
2	Identification	A 1:10 suspension give a violet to deep blue colour with iodine TS	A violet colour is produce with iodine TS
3	Specific Rotation	Between + 410 to +610	+52.450
4	Particle size	NLT 90% passes through mesh sieve size (2.36 mm) NMT 10% pass through mesh size (2.00mm)	98.45% 0.60 %
5	Loss on drying (at 1050C for 4 hours)	Not more than 4.0 % w/w	1.28 % w/w
6	Residue on ignition	Not more than 0.25% w/w	0.15 % w/w
7	Heavy metals	Not more than 5 ppm	Complies
8	Assay on (dried basis)	NLT 62.5 and NMT 91.5 %	82.59 %
9	**Microbial Limit test:** (a) Total aerobic microbial count (b) Salmonella species (c) Escherichia Coli (d) Staphylococcus aureus (e) Pseudomonas aeruginosa	 NMT 100 cfu/g Should be absent Should be absent Should be absent Should be absent	 35 cfu/g Absent Absent Absent Absent
10	Friability*	NMT 20 %	0.12 %
11	Spherocity	NLT 0.9	0.98

Table No. 2: Behavior of sugar spheres in water and different buffer solutions:

Sr.No.	pH	Time required to get complete solubalised
1	7.0	1 Min 45 Second
2	8.2	1 Min 42 second
3	1.2	1 Min 15 Second

Evaluation of Acyclovir loaded beads:

- ***In vitro* drug release study of Acyclovir coated beads (Percent drug content determination):** Percent drug content determination was done using UV spectroscopic method. Validated method was developed and applied to Acyclovir coated spheres at 252 nm. Drug content was computed using a calibration curve ($R^2 = 0.9993$).

Drug release study considering suction process:

- **Amount of drug dose sucked per sip:** Dose sipping technology is quite new to regulatory world to get central designed process for studying release pattern[3,4]. Process considering release per suction was also designed and is explained here with. Considering the viscosity parameter and resistance provided by drug coated sugar spheres present in straw, a study was designed. Based upon suction capacity study of group of pediatric volunteers, peristaltic pump was set at around 10-12 RPM. Samples were collected per suction and percent release study was performed using a spectrophotometer at 252 nm. Studies performed revealed that per sip around 6 ml of milk is sucked in 30 seconds i.e 12 ml of milk is sucked per minute and for water 8 ml was sucked in 30seconds i.e 16 ml per minute. Considering this fact we recommend use of milk, water, carbonated drink or coconut water as beverage for fluid to be sucked through drug containing straw.

Comparison with marketed dosage form preparation (Tablet): No product on Dose Sipping is available in market so it was decided to compare the dosage form. Marketed preparation ACIVIR-800 (DT)

was procured from market and drug release study was carried out in triplicate using a USP II tablet dissolution test apparatus (model TDP-06P, Electro Lab, Mumbai, India) at 37±0.5 ºC and 50 rpm. The tablet was place in enzyme-free simulated gastric fluid (900 mL, 0.1N HCl, pH 1.2). A 5 mL aliquot of the dissolution fluid was withdrawn at regular intervals of time and replaced immediately with the same volume of fresh media. The aliquots, following suitable dilution, were analyzed for drug content using a spectrophotometer at 252 nm.

Appearance of drug coating was improved using various colour dyes. Under this 0.5 % weight gain was assisted. Appropriate dosing amount of active coated sugar spheres were filled manually in the straw by removing the cap and leakage from filters after mechanical shaking were evaluated.

Conclusion

During experimentation critical aspect of this technology were observed. It can be concluded that ability to form in-situ suspension with a good mouth feel, attainment of appropriate particle size so that it should not be too small to block the tube (no fine powder), nor too big as to avoid gritty feeling in the mouth (<<1mm). Apart from this neutral taste or in case of bad tasting active ingredients a taste masking for at least the holding time in mouth is important. It can be concluded that in quest of developing a dosage form which combines the advantages of liquids as easy swallowing with that of solid dosage forms as storage stability and exact unit dosing as well as taste masking which may improve compliance in children or elderly, dose sipping technology tends to be a better alternative. Acyclovir one of the important antiviral drug can be successfully delivered using this technology**.** Higher percentage of drug can be easily loaded by this method.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

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Spectrum of Respiratory Involvement in COVID 19 Era; An Overview

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Abstract

Introduction: COVID 19 is novel coronavirus which first struck the world 5 months ago and became pandemic in such a short time. China suffered first and then this virus spread to more than 180 countries. Primary organ involved is lungs and the commonest cause of fatality is respiratory failure[1]. Although, its new virus, related to two earlier deadly viruses e.g. SARS and MERS but has already caused tremendous morbidity and mortality in nations heavily affected by it. This short review highlights about lung involvement due to this virus and to make our healthcare professionals aware about what to expect by learning from the experience of the countries where it has already affected thousands of civilians.

Methodology: This review article was written with systematic literature review with the help of data search machine like Pubmed, Scopus, Web of Sciences and google scholar. In this article observational study wand case reports were included. Most of the data were taken from China, Italy and Spain.

Review Findings: COAVID 19 affects lung parenchyma in moderate to severe disease causing pneumonia which starts as unilateral lower lobe, commonly on right side, peripheral ground glass infiltrates and rapidly spreads to involve both lungs with bilateral, multifocal consolidations[2]. Pleural effusion and pericardial involvement may occur. In severe cases the disease progresses to Acute Respiratory Distress Syndrome accounting for >90% of mortality. Pathology of lungs showed diffuse alveolar damage with organization and fibrosis as disease progresses.

Conclusions: COVID 19 a novel coronavirus which is presently a pandemic has affected the world in manner reminiscence of 1918 Spanish flu. Lung involvement as community acquired pneumonia is determinant of mortality, which spreads exponentially to develop sever respiratory failure.

Keywords: *COVID 19, Lymphopenia, Pneumonia, Respiratory failure,Acute lung Injury, Diffuse alveolar damage.*

Introduction

Since December 2019, in Wuhan province of China there was spate of cases of community acquired pneumonias where no etiological agent could be identified. Later, a novel enveloped RNA betacorona virus was isolated currently labelled as SARS CoV2 (Severe Acute Respiratory Syndrome Coronavirus -2) being ~80% similar in structure to earlier seen during Severe Acute Respiratory Syndrome(SARS CoV 2) with mortality ~ 25% in 2002. Clinical presentation mimicked creating a public health emergency followed by WHO declaring it as a pandemic on March 11,2020. Disease though appears to have better survival as compared to SARS CoV-2 (now called COVID 19: Corona Virus Disease 2019) but respiratory involvement & failure

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appears to be the driver for mortality and significant residual effects[1].

COVID 19 has swept various nations over a short span of 3 months. In this pandemic, various countries are at different parts of the epidemic curve. Some has seen the worst while other are at the beginning of the curve. India is still at early part with < 10,000 positive cases and fewer than 300 deaths (at the time of going to print), our physicians need to be aware of the implications of the disease which is driven by lung involvement. Review of this nature would not only enhance our medical professionals but also help them to enhance their skills to manage these patients better, translating into less poorer outcomes of this disastrous illness.

Methodology

We review available literature on lung involvement in COVID 19 available on Medline and PubMed data search engines, but little is reported so far considering that its new virus and has crippled the healthcare system of most of the countries where it has reached in significant numbers. More data will emerge when clinicians get time to publish their experiences but at present only few research papers from China and Italy formed source of this review.

Review Findings: Clinical presentation of COVID 19 include, fever, cough are common symptoms as in other viral illnesses but GI symptoms though uncommon but indicate propensity of virus to affect gut, makes it distinct from influenza prevailing at this time in most of the countries including India. Lung involvement on CXR was rarely absent in severe cases at the time of presentation (3%) to the hospital but was also present in 18% of patients with non-severe disease and 15% patients admitted with non-severe disease did progress to severe COVID19[1]. However, during hospitalization >91% of COVID 19 patients received physician diagnosis of pneumonia followed by ARDS in 3.4% and shock in 1%. It's also clear from early experience with COVID 19 cases in Wuhan that chances of missing pneumonia diagnosis were higher in cases admitted with non-severe illness. Hence, its recommended to have high suspicion of pneumonia in view of propensity of disease to cause it and fatal outcome associated with it. CT chest done at the time of admission revealed abnormalities in 86% of patients.

Therefore, it's clear that lung involvement in COVID 19 is the denominator for severity of the disease and subsequent mortality and morbidity.

Epidemiological Features of Lung Involvement in COVID 19: Pneumonia is the most common lung manifestation of COVID 19. Classically pneumonia is divided as community acquired vs hospital acquired pneumonia and COVID 19 causes community acquired pneumonia. However, transfer of infection from a case of CAP in hospital to another non-infective patient can occur (unconfirmed reports from Italy & Spain) but still too early to comment on at present. Approximately 24% patients had underlying comorbidities e.g. Diabetes mellitus, Chronic kidney disease, hypertension, stroke, etc. but reached 37% in cases with severe disease. Recent found lesser survivors in hypertensives vs normotensives (34% vs 52%) patients, study postulated that hypertensives on Angiotensin Converting Enzyme (ACE) inhibitors could be the underlying factor predisposing them to higher mortality as ACE-2 receptors besides being vasopeptidase are also coronavirus receptor and hence, are more likely become sicker.

In last 2 decades, there is shift in etiology of community acquired pneumonia, indicating rise in viral infections particularly by the spurt of Influenza infections including H1N1. COVID19 is the next etiological agent where the transmission was initially from animals to human and later human to human by respiratory droplets and direct contact. Potential route of transmission via gut or salivary secretions is under investigation. Interestingly, 8.9% patients of COVID 19 had no pneumonia or did not develop pneumonia later too, raising the role asymptomatic carriers in community.

Clinical Features of COVID 19 Lung Involvement: COVID 19 pneumonia study showed that mean age of patients was 52.8+ 12.2 years with 62% being males. All had symptoms at the diagnosis of disease (this could be due to the study design where inclusion criteria was diagnosis of pneumonia on CT scan). Depending upon clinical presentation, pneumonias are classified as *Typical or Atypical* depending upon whether the presentation is typical with fever, cough, sputum, pleuritic chest pain, breathlessness and blood tinged sputum or atypical with fever, myalgia, headache and minimal or no respiratory symptom, delaying the diagnosis of pneumonia in such cases. The retrospective studies showed that COVID 19 patients with severe disease and pneumonia had presentation with fever, cough with sputum and breathlessness, more like typical

pneumonia but other non-specific symptoms ofmuscle pains and fatigue were also common (1/3rd) and rarely patients had abdominal pain and diarrhea, where early CXR would make diagnosis clear. Worsening vitals and oxygenation at room air predict progression to pneumonia and can be used monitor patients with mild disease.

Laboratory Features of COVID 19 lung Involvement: Patients with COVID 19 pneumonia showed leukopenia in 20%, however, even if total counts are normal or raised, 80% show lymphopenia in differentials while 50% had low percent lymphocytes. ESR was raised in 2/3rd of patients but hs-CRP was elevated in all (100%).

Radiological Features of COVID 19 Lung Involvement: Chest imaging is an essential tool in diagnosis, assessment of severity, clues to etiology, allocating site of care, prognosis and monitoring the progress of pneumonia. CXR is the first imaging modality however can miss about 10% of pneumonia due to poor resolution of bedside films as well as some areas of lung can't be visualized e.g. retro-cardiac and retro-diaphragmatic regions, CT chest is considered better modality to pick up lung infiltrates[2].

Based on lung involvement on chest imaging, pneumonias are labeled as Lobar, bronchopneumonia or interstitial pneumonia. Progression to ARDS(Acute Respiratory Distress Syndrome) leads to development of imaging s/o acute lung injury. COVID 19 pneumonia. Based on CT scan findings in COVID 19 patients, single lesion (lobar/segmental infiltrate) was seen 16% while 84% had multiple lesions (bronchopneumonia). It was observed that multiple lesions were seen when CT scans were done late after the onset of symptoms (6.6+4.0 days vs 2.2+1.8 days), suggesting that it's the evolution of disease and COVID 19 lung disease progresses from single infiltrate to widespread multiple infiltrates in both lungs (Fig 1).



Fig 1 CXR showing bilateral multifocal airspace shadows

Single lesions were seen in 90% in right side with right lower lobe being the commonest to be involved and left lingular segment was the only case in left lung. When multiple lesions were on imaging, there was no case with central region involvement as all cases showed either peripheral (78%) or combined peripheral and central infiltrates in rest of the cases. CT scan study showed that lower zones as well as posterior areas were more frequently and severely involved in comparison to the rest of the lung fields.

CT patterns of Lung Involvement in COVID 19: CT scan involvement of lungs in COVID 19 may show various changes in lungs but may involve all compartments in thorax except mediastinal lymph nodes[3,4]:

A Lung parenchymal involvement: Air space changes (Fig. 2 A to G).

- Ground glass abnormalities (GGO's) – 40%
- Consolidations – 34%
- Reticular opacities with GGO's
- Vacuolar sign
- Microvascular dilatation sign
- Fibrotic streaks
- Sub pleural line of involvement or sparing

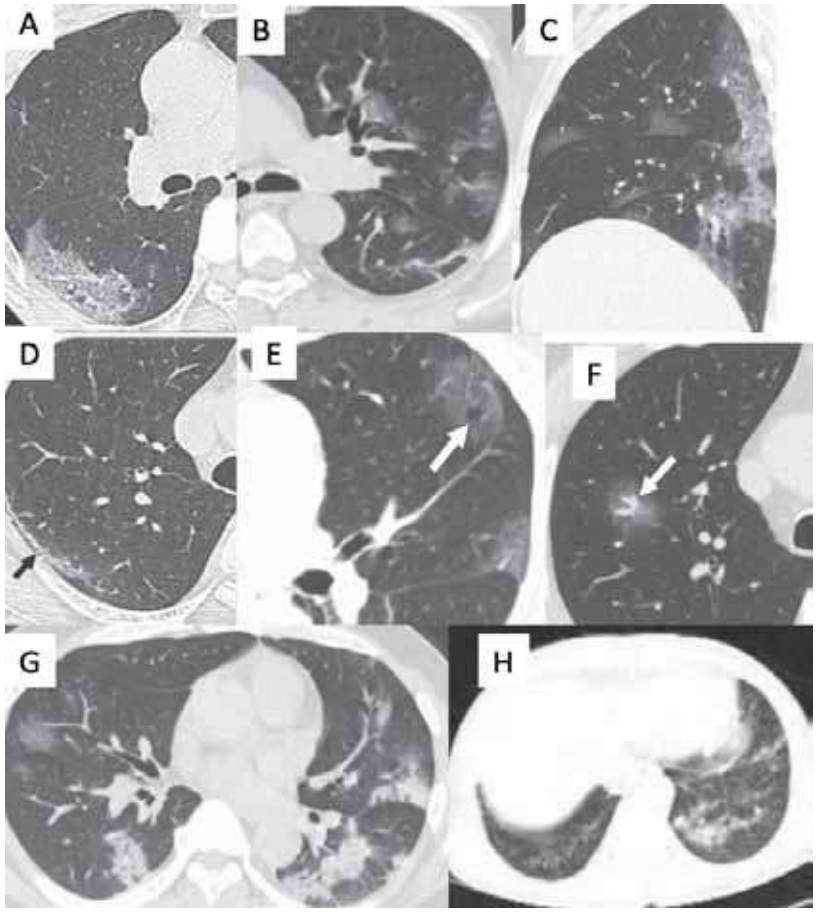


Fig 2: A GGO, B Fibrotic lesion, C Peripheral & posterior distribution, D Sub pleural spared lesions, E Vacuolar sign, F Microvascular dilatation sign, G Consolidation with multifocal widespread lesions H GGO's with linear streaks

B Airway involvement:

Bronchial changes: seen as air bronchogram – 73%

- Dilatation
- Distortion
- Air trapping

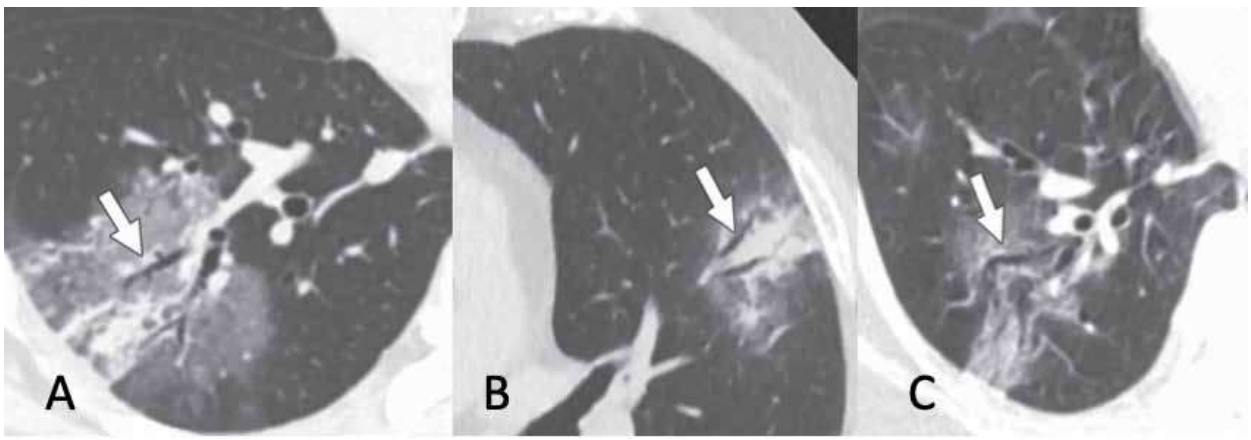


Fig 3 A Airway dilatation, B Air bronchogram, C airway distortion marked with arrow

C Pleural Involvement:

- Pleural effusion – 10%
- Pleural thickening – 48%
- Pleural retraction

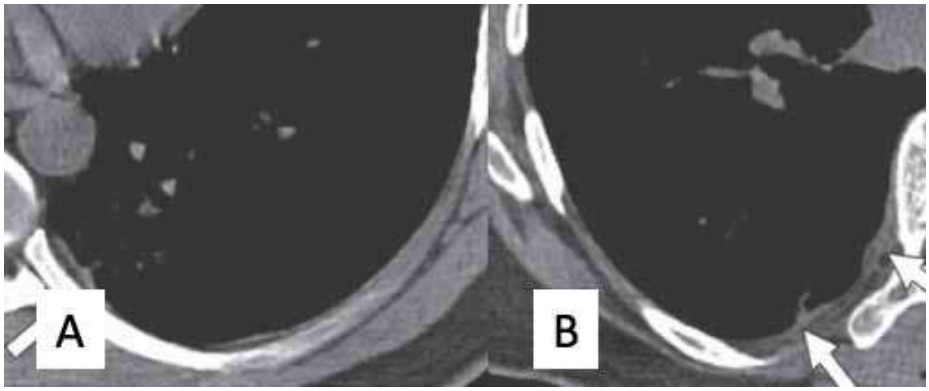


Fig. 4: A pleural effusion, B pleural thickening and retraction marked with arrow

As the disease progresses, it was found that GGO's, airway and pleural abnormalities were less prominent (after 7 days of illness)

CT scans showed that COVID 19 on imaging may show diverse and mixed findings of pneumonia and a clinician needs to be aware of these findings and should realize that such findings are evaluating very fast with time becoming multi focal and diffuse. Bilateral infiltrates or rapidly progressive lung lesions indicate poor prognosis.

Lung Pathological Findings in COVID 19 Pneumonia: Autopsy studies in COVID 19 patients showed features similar to the cases earlier reported with SARA CoV and MERS pneumonias as lungs showed picture of ARDS with desquamation of Type 2 pneumocytes and formation of hyaline membranes (Fig 5). Since, lung may show different stages of ARDS, interstitial mononuclear infiltrates may be seen in early phase while interstitial fibrosis in later stage. Intra-alveolar organization with multinucleated syncytial cells with enlarged atypical pneumocytes showing viral cytopathic changes[5]. In late stage disease, interstitial thickening with intra-alveolar fibroblastic plugs seen. In some casesthrombo-emboli was also detected and Microthrombi were regularly found within small lung arteries, occasionally within the prostate, but not in other organs.[6]

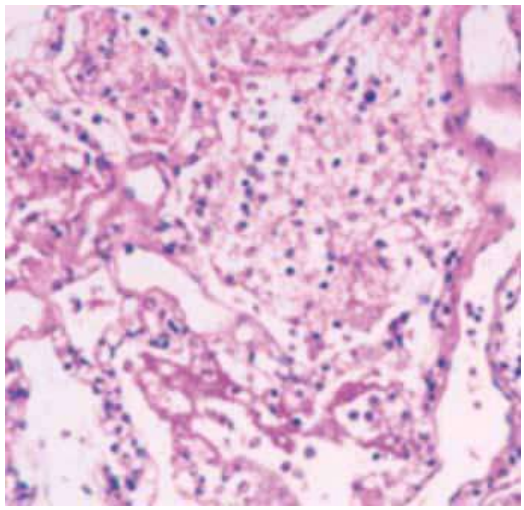


Fig 5: Intra-alveolar organization with lymphocytic infiltration of Interstitium and multinucleate syncytial cells

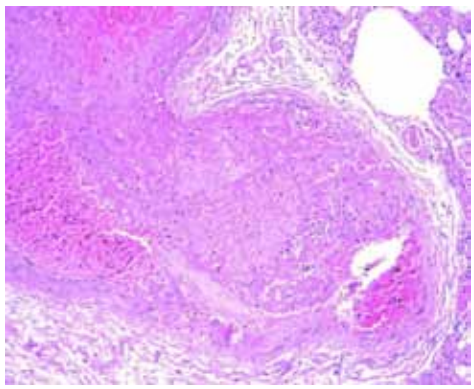


Fig 6 Pulmonary thromboembolism in a patient of Covid 19

Treatment & Outcome with COVID 19 Pneumonias: Follow up data from patients with COVID 19, shows that nearly 50% patients needed oxygen therapy while 6% requires mechanical ventilation. Non-invasive ventilation has been used in less severe cases while systemic corticosteroids have been used mostly in severe cases. Hydroxychloroquine (HCQ) and Azithromycin has been used by some centers to treat severe pneumonia but trails are still ongoing. [7]

Once patient develops ARDS they are managed on standard guidelines for ARDS management. Rarely ECMO (Extra Corporal Membrane Oxygenation) has been used in severe cases with yet undefined success. However, latest communications from Italy suggested to use early mechanical ventilation, low PEEP, prone ventilation in refractory hypoxemia. NIV in contrast led to higher mortality which could be delay in initiation of respiratory support or mechanical injury produced by large volumes of tidal volume in lungs. High frequency nasal cannula for providing higher fiO2 with led to some success in cases with moderate hypoxemia.

Frequentreasons for admittingpatients of COVID 19 to the Intensive Care Unit were hypoxemic respiratory failure leading to mechanical ventilation, hypotension requiring vasopressor treatment, or both.[8]

Melatonin is also being studied as a potential adjuvant treatment due to its anti-inflammatory effects.[9]

Even though physiological models of SARS-CoV infection show a theoretical benefit of using ACEI/ ARB these findings are yet to found in the studies done practically.[10] Research efforts regarding production of vaccine is being conducted increasingly and are already being reported in various distinct studies.[11,12,13.]

Various factors like smoking also effect the progression of the disease as smoking is most likely associated with the negative progression and adverse outcomes of COVID-19.[14]

Rather than specific pattern on CXR or CT scans with CT scans being much more sensitive to diagnose pneumonia·Studies show that More Consolidation in Upper Lungs on Initial CT Increases the Risk of Adverse Clinical Outcome in COVID-19 Patients .[16]Wheras progressive stage on CT is the most common stage with negative co relation with Spo2 and lymphocyte count.[17]Vital signs monitoring is key to early diagnosis of pneumonia and effectively use triage**.** Human CoVs remains a great threat for global public health. However, human beings have not gained enough experience in previous battles with SARS and MERS and thus appropriate measures and studies need to be conducted to control this lethal pandemic with a great destructive potential.[18,19,20]

Conclusions

Pneumonia is the most common determinant of severity and outcome in COVID 19 and at present evidence favors its presentation as typical, community acquired pneumonia with constitutional features suggesting viral etiology. Radiologically it manifests as progressive bilateral, multifocal pneumonia, though may initially begin as localized GGO peripheral infiltrates. Such findings may be non-specific and at present h/o travel or contact with COVID 19 may be more indicative of underlying etiology.

Ethical Clearance: Taken from institutional ethics committee.

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COVID-19 Pandemic: Inducing Pan-Hysteria and Panic Disorder; An Overview

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Abstract

Summary: Wuhan Municipal Health Commission, China, gave an account of a few cases of pneumonia which were later established as cases of the Novel Coronavirus Disease by the end of December 2019. By 4th of January, WHO reported these cases on social media and by the beginning of March WHO had declared it as a pandemic which was preceded and followed by lockdowns in various countries and had led to a scenario of fear and panic amidst the minds of all.

The coronavirus disease 2019 (COVID-19) pandemic has challenged the society in many aspects, one of them being the psychosocial aspect. The stress due to the pandemic could produce worry and fear about health for oneself, family members and friends. Even changes in sleeping or eating patterns, the worsening of chronic health problems,unavailability of alcohol, tobacco or other drugs in the market due to shutting down of manufacturing units and problems in concentration are among the possible etiologies of a precipitating panic disorder. Containment measures such as lockdown and isolationcan also precipitate psychiatric illnesses and morbidities such aspanic disorder, depression, anxiety and suicidal tendencies.COVID-19 may cause or exacerbate even the existing psychiatric illnesses or existing panic disorder in individuals which could lead to physiological symptoms like palpitations, sweating, shortness of breath and a decreased sense of wellbeing. It gives a feeling of impending doom or danger and is followed by intense fear which can all take place when one is facing such a pandemic.

Conclusion: The COVID-19 pandemic has not only had physical but even mental and psychosocial impacts. New mental health challenges may arise owing to this pandemic.

Keywords: *COVID-19, Panic Disorder, Anxiety Disorders, Psychosocial, Novel Coronavirus.*

Introduction

In December 2019, a life-threatening disease, the Novel Coronavirus disease 2019 came into play, which has now become a massive global health concern. It had commenced in Wuhan, China and has now escalated and taken almost every country under its wing. It has affected nearly 10 million people till date and has taken the life of approximately 500 thousand people across the nations. Not only has it affected health physically but mentally as well. It has gripped the mind of every human with fear, anxiety and panic. From the moment of declaration of this as a pandemic, to lockdowns being imposed, all of it has seized people with hysteria and panic attacks.[2,8] The psychosocial impact of this whole pandemic is huge and could lead to various disorders that could be short term or long term like anxiety disorder, major depressive disorder, mysophobia, acute stress disorder, panic disorder etc.[1,2]

Panic disorder is classified underanxiety disorder and it is characterized by repeated and unexpected panic

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attacks. During this, a feeling of terror develops and may trigger severe physical reactions combined with alterations in behavior or persistent worry over having further attacks. The fear of an impending panic attack can hamperroutine day-to-day life. Panic disorder is more common in women as compared to men and thus women are more susceptible to such an attack. It could also possibly lead to Agoraphobia, which is a fear of going into places that are crowded. This is because it can cause an individual who has already had a panic attack to circumvent from places and situations where an attack has previously taken place because the individual experiences anticipatory anxiety. Diagnostic Criteria of Panic Disorder [300.01 (F41.0)] is given under DSM5 and says that at least 4 out of 11 physiological symptoms need to be present for an attack to be categorized as a panic disorder. Even though these anxiety symptoms may not be advanced enough to cross the diagnostic threshold of DSM-5, assistance and comfort from experts and specialists may help the people to get over their current anxieties.[1]

Literature Review:

Panic Disorder Correlated to Pandemic: As COVID-19 was spreading across the nations, the people of these countries were mostly not aware of coronavirus and so ignored it. As soon as it was declared a pandemic, reality came crashing down on everyone as they realized there is no more escaping and ignoring what is going on around the world. The countries that had already been affected and had an increased number of cases had already gone through the realization phase, but knowing that almost all countries are affected by the same was still harsh on them as now they could see the same helplessness everywhere.

The disease was not known to many at the time as to what it was, how it spread and why it was the cause of death for many which thus created fear and panic amongst everyone. They did not know who to approach, who to ask and were completely ignorant on the topic. Non- availability of vaccines and uncertainty at the time regarding the treatment further aggravated the problem.[1,3]

All individuals who were quarantined for 14 days post-travelling and those who were contacts and contact of contacts were also very scared as they feared the outcome of their test results. They had to wait for a period of 14 days where they were completely isolated and had to stay without their family, after which testing was done one final time before releasing them from the quarantine facilities. This caused unease and increased panic.[1,3]

Cases of stigmatization, racism and xenophobia against targeted communities were also being highlighted in the news across the globe. WHO highlighted in their mental health outline that beratement and victimization of individuals affected by the disease, frontline health care professionals combating the disease and government officials is a predictable occurrence in epidemics and tragically this trend is still alive during this ongoing pandemic of COVID-19, which has caused stress among the individuals affected by the disease as well as among the healthcare professionals which could precipitate in even them having panic attacks. The healthcare professionals are already under stress due to the increasing cases of COVID-19, increased working hours, less rest when they are relentlessly working, which leads to an increase in their exposure towards the disease. This means that they do not get to spend much time with their family, worrying about themwhile at the same time trying to efficiently work with reduced resting period. Even for those who are affected by the COVID-19, it very difficult to cope as they are internally panicking. They get respiratory symptoms which they don't know much about and wonder what is happening to their body. They can't meet their family and are completely isolated which further aggravates the fear and anxiety already brewing in them.[1,3]

Hence, it is very important for the mental health professionals to take an initiative and educate the public so as to decrease their fear and stigma and counsel those affected by COVID-19. Certain psychosocial guidelines were also delivered by the WHO for diminishing the increasing stigma against COVID-19.[3,4]

Panic Disorder Correlated to Social Media: Over the last few years, social media has proven to be a platform for circulating awareness and imparting knowledge regarding health. But, misuse of social media has also been done time and again. In a time like this, where already very little was known about the Novel Coronavirus disease, misinformation spread like wildfire all across the social media platform, from supposed cures to false information about the spread of the disease and gave rise to more panic and anxiety. This fear and anxiety could lead to people trying various supposed cures which could be life endangering. Social

media also became a platform where fake sanitizers and N95 masks were being sold and since the people were going through so much at the time, they were vulnerable and so fell for such schemes.[5,6,7]

Panic Disorder Correlated to Lockdown: As the cases kept rising, countries with so many cases eventually had to declare a lockdown to contain the spread of the virus. In the past outbreaks, we have seen that quarantine, lockdown and isolation has had a psychosocial impact whichvaries from overnight and concerning effects like agitation,worry over acquiring the disease, worry over transmitting the disease to members of their family, anger, panic, denial, loneliness, despair, anxiety, insomnia, depression, hysteria, to extreme effectslike suicide. It has also affected individuals into over thinking the entire pandemic situation and has led to mysophobia and obsessive-compulsive disorder resulting in the individuals feeling a constant need to use a sanitizer and washing their hands.[1]

The lockdown was a very unforeseen,but necessary event. When the lockdown was announced,it created panic among the people as they realized that it's better to stock and pile up on household items to avoid going out for fear of contracting the virus. Thus, began the panic buying all across the globe as countries started going under lockdown. Not just food but even sanitizers and masks were soon out of stock as people began hoarding these up and the manufacture had to be boosted to get them back in stock as soon as possible. Even repeated reassurance to the people that there were enough supplies did little to soothe the minds of the people and lead to empty isles in the markets which further aggravated the panic and motivated panic buying.

All the people hailing from various countries who had gone elsewhere for studying and work purposes got stuck in the lockdown and could not go back home to their families and were constantly worrying for their family members. The students who had been preparing for years for various exams are unable to give it and time is slipping away which could potentially lead to anxiety.

Lockdown created a crash in the economy and further the shutdown of manufacturing units and non-essential commodity related trades has led to lack of income in a lot of economic sectors.It means that there is a feeling of insecurity and powerlessness due to the lockdown. We need to understand that poverty is dependent on income,which is importantduring emergency lockdowns. Poverty means reduced capacity of these individuals to effectively participate in society. Feeding and providing a roof over the head is not enough, but also increased access to school or clinic, land for growing food, even a job is equally important. Poverty means, one is living in a delicate environment, without access to clean water or sanitation.[8]

Not just the poor but it created confusion even amongst people who had small-businesses, artists and everybody who did not have a steady income and job. They did not know how to deal and process the lockdown as there was too much uncertainty of what the future held. This again led to anxiety and panic regarding how they will feed themselves and their families and maintain a roof over their heads while their sources of income were cutoff and they were being forced to stay at home. In the job sector, people are being laid off by the companies that can no longer support as manyemployees due to losses during the lockdown and there is an air of panic amongst the employees owing to the uncertainty.[8]

Methodology

Literature review is a review that is more than just the analysis of findings from primary studies,rather it also allows researching in other dimensions. Thus, Literature review helps to develop an overview regarding a topic from secondary data. Literature reviews concentrate on gathering knowledge about a certain topic under study and allows the formation of a summary of the available data on a topic such as"Covid-19 Pandemic: A source of pan-hysteria inducing Panic Disorder". It grants generic conclusions ona research topic while answering a targeted question, which we know based on other articles and highlighting the key points. Thus, once the question is framed, data cumulation for the literature review would be defined. In this study, the topic "Covid-19 Pandemic: A source of pan-hysteria inducing Panic Disorder" has been investigated to identify and highlight this problem.[8] The guiding query of this literature is "Covid-19 Pandemic: A source of pan-hysteria inducing Panic Disorder". Crossetti (2012) had proposed an integrated review method which has been used to construct this review. This method allowed the formation of a thorough assessment of the literature in qualitative and quantitative aspects. Thus, it completely assesses the research question or topic which is being addressed. A literature search was doneon this topic using the following electronic databases: Google Scholar, PubMed, Scopus etc. Keywords were utilized to search for the relevant articles for the same.[8]

Discussion

The COVID-19 pandemic has not only had physical but even mental and psychosocial impacts. Thus, psychological and social wellbeing during such a time carries universal importance. Paranoia and hysteria as a result of the pandemic could lead to panic disorders. COVID-19 may cause or exacerbate the existing psychiatric illnesses or existing panic disorder in individuals which could lead to palpitations, sweating and a decreased sense of wellbeing. In a pandemic such as this, which has affected so many and taken so many lives, psychosocial impact could take a backseat especially in developing countries and thus, it is very important to shed light on this topic. This is now our new normal and we need to build our strategy to combat this pandemic while keeping this in mind. The COVID-19 has impacted both developed and developing economies, manufacturing units were shut, a significant reduction happened in the agricultural activities, tourism, trade and even in the travel industry. In developed countries for example Europe, the panic and fear are more regarding the consequence of the pandemic leading to loss of jobs. The European Commission took steps to help make recovery guidelines for the problems due to COVID-19 outbreak.[8]

The government should focus on themental wellbeing and also the related comorbidities of COVID-19 and evaluate the mortality associated sequelae. Beratement and accusations which are addressedto a certain section of societyhit by the pandemic, may hamperglobal trade, relationships, financeresulting in further havoc. Thus, there is a need to eliminate the discrimination against those associated with the disease and racism. The mental health impact on those suffering such discriminations is eliminated by counselling with specialists and healthcare professionals and building a team of healthcare professionals who are directly a part of healthcare delivery systems.[8]

To manage the economic crisis, countriesneed to aspire to reduce the adverse effects of COVID -19 by focusing on the communities that are underprivileged. This is because, in such situations, the underprivileged and the poor communities suffer the most. They get neglected, which gives rise to starvation, health deterioration, low quality of life and even deaths due to improper hygiene and starvation. Thus, separate measures for lockdowns should be made to make sure it addresses the needs of the less privileged in the future. The Officials of such countries and the WHO should make sure that the underprivileged do not have to worry about food or whether they will be able to survive the lockdown before surviving the pandemic. It highlights the need for constructing more powerful health systems for the underprivileged so that we can help the underprivileged communities to live in a healthy manner. An example for this kind of a model is the Brazilian Unified Health System (SUS), where there was an instant expansion and redistribution of the income amongst the Bolsa Familia. This modelgave way for expansion and urgent payments for everyone who had informal jobs or for those who were unemployed.[8]

Regarding control of Misinformation: Social media needs to be used responsibly so that people know what the disease is, how it is transmitted, what are the signs and symptoms, what can be done to avoid it and when to reach out to a consultant. To protect social media from misinformation regarding COVID-19, strict government laws need to be implemented against fake news, rumors, misinformation.[5.6.7]

Thus, it is very important right now to harness social media platforms to spread accurate information regarding all aspects of the disease and how to avoid it. We need to battle against the social media pandemic. We need to rapidly identify and provide an answer to the fake news, rumors, behaviors and various perceptions around COVID-19 and how we can control and eliminate the misinformation that has been and is still being spread.[5.6.7]

The most important element for the beneficial impact of social media during the pandemic is focusing on making accurate data available to the public so that the public is well informed and thus, can help to stop the transmission of COVID-19. Moreover, some expertshad suggested that a digital tool can be built. Keeping this in mind, WHO made a webpage to set forth right information and also to correct misinformation that was being spread about COVID-19. The social media pandemic can only be eliminated with accurate information. Social media needs to be used wisely, for example,using social media to communicate and keep in touch with what is going on across the globe, for conveying the importance and cause for quarantine and also in providing relief to the pre-empt panic and misinforming rumors.[5.6.7]

Conclusion

Constructing a plan of action on how to deal

with mental health issues and making mental health organizations specific for pandemics can be the first step to approaching the problem. Arranging awareness programs regarding this at personal as well as community level is very important. Building websites and toll-free helpline numbers for the people for easy access to psychologists for communication and assistance during this pandemic when faced with such situations of panic is also something that can be promoted.[1,3,9,10]

First thing to focus on can be to change loneliness to solitude. Loneliness is an emotion that is filled with fear and dejection. To gain mental strength to combat and overcome it is to engage in hobbies, focus on neglected passions, cherish bonds that we have with our friends and family. Great works of philosophy, literature and even great works of arts have been achieved from solitude and thus can be used to motivate others.[11-12]

As mental health professionals, it is important that we are sensitized to the needs of those who are in quarantine and are able to address those needs. Their psychological and even personal needs should be addressed. Digital communications with their loved ones need to be arranged and encouraged. Similar protocols were followed in China in the first stage of the disease and it was seen that it had significantly impacted the quality of life of those who were quarantined. Also, in a research it was seen that even weekly counselling sessions over the phone can help reduce fear and stress during such times. These sessions should be brief and should focus on the solution. Social integration is another significant aspect, in which integration of individuals around us matters. Caring and looking out for thehousehold helps, security guards, vendors, etc. is important in such stressful times.Amere greeting to neighbors or even passersby can strengthen the feeling of unity that we are all in on this together. The pandemic will in the end teach us two important lessons: preparation for solitude during a crisis such as this and that the physico-psychological well-being forms the cornerstone of public health.[11]

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COVID-19 Pandemic: A Positive Influence on Lifestyle, Economy, Administration & Environment; An Overview

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Abstract

Summary: On the December 31st 2019, World Health Organization (WHO) was alerted about a bunch of newly arrived pneumonia cases in city of Wuhan, China. The Severe Acute Respiratory Syndrome (SARS) later became well known as COVID-19. The causative virus was isolated on 7th January, which was the novel coronavirus 'SARS-CoV-2. Initial evidences suggested it is a zoonotic disease originating from bats which was then transmitted to humans. As of June 27 2020, the number of Identified COVID-19 cases globally were **9,815,826** of which active number of cases were **4,018,892** and total number of deaths were **494,118.** To contain the spread of this disease many nations around the world introduced partial/complete lockdown. India declared nationwide lockdown on 25 march 2020, which is considered as the world's largest lockdown. This led to too much distress in the society and nation. But every coin has 2 sides, today let's flip the coin and have a look at the unnoticed head of the coin. This article is the review of numerous articles available online highlighting the disguised positive impacts under the following domain:

- Lifestyle and health related positive impact includes hygiene practices, attitude of good health and immunity.
- Positive Impact on Administration & Health sector indicating the lacunas of poor health infrastructure.
- Social & Personal impact leading to better personality development.
- Impact related to Lockdown which includes good environmental changes, trade and work-related positivity

Keywords: *COVID-19, Positive impact, Improved hygiene, lock down, Reduced Pollution, Self-sufficient Economy.*

Introduction

On December 31 2019, in the City of Wuhan (China) a cluster of new pneumonia cases were reported to 'World Health Organisation (WHO)' . This later became well-known as COVID-19. It was caused by a New RNA Virus of the Corona Viridae Family, SARS CO-V 2 (NOVEL CORONAVIRUS.) WHO declared COVID-19 as pandemic on March 11, 2020[1][2]

It is spread via Air in the form of droplets and via contact routes. The incubation period of COVID-19 is 5-14 days with the median of 5.2 days. Sometypical symptoms include fatigue, dry cough, fever whereas sore throat, diarrhoea, headache, loss of taste or smell are the atypical ones, serious ones include difficulty in breathing and chest pain.[3][4][5]

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The R_0 (Reproductive Number) is between 2.2–3.6 to 4.1–6.5 depending on the country of study. R_0 → measures secondary transmission potential. To break the chain of transmission lockdowns were imposed in many parts.[6]

WHO says mild or asymptomatic cases account 80%, severe infections are 15%, 5% cases are critical (COVID-19 Situation Report 46.)The death rate of COVID 19 as of June 30 2020 was 8% (data of worldometers.info June 30 2020 @17:11:30 IST). Among the dead were mainly elderly, immuno compromised patients with cardiac co-morbidities, diabetics etc.[7][8]

Due to non-availability of vaccine and any definitive treatment, supportive care remained the mainstay which includes oxygenation, ventilators and other symptomatic care for fever and cough.[2][9]

Methodology

The Article was prepared using various journal articles, news articles and guidelines outlined by various institutes highlighting the positives of COVID 19 pandemic (all the reference articles are mentioned in the end). This was an effort to bring together most of the available literature available at the time of preparing this article. A comparison of before impact and post impact scenario of COVID-19 in attitude, administration, healthcare sector is included. A comparative illustration of positive changes in environment, crime rates and road traffic accidents arealso shown using news articles about pre-lockdown and lockdown.

Positive Influence of Covid-19 on Public Lifestyle & Health:

- **Hygiene Practices:** COVID-19 led to increased awareness about personal hygiene amongst people which includes properhand hygiene practices like washing hand with soap for 40 sec to 50 sec following all appropriate steps of hand washing, hand rubbing with alcohol-based (60%) sanitizer after returning to their homes, before having meals. These dropped the fomite-based pathogen propagation.[10][11][12][18]

Respiratory hygiene like covering face while coughing or sneezing use of shoulder or elbow to cover face while sneezing or coughing. Wearing acloth mask or surgical mask to avoid infection and its spread. Avoidance of contacting the face with contaminated hands. Following no touch practices to avoid contacting contaminated surfaces has led to decreased risk of communicable infection manifold.[11]

Regular Hygiene practices can reduce common infection rate by 50 %and decrease antibiotic need by 30% (American Journal of Infection Control) which also reduces the risk of Multi Drug Resistant (MDR) Infection which causes~2000 deaths/day worldwide.

Avoidance of unnecessary overcrowding and following social distancing norms inpublic places along pre-sanitisation before entering shops has become new normal.

- **Health & Immunity:** With rising awareness of Basic Health, immunity, knowledge of complication of co-morbidities humans became more vigilant about their diet, health and lifestyle. Humans started following ways and method to increase immunity with natural immunity boosters, yoga and exercise strengthening primary prevention.

Mass media and social media also helped a lot in spreading awareness about identification of common signs and symptoms and seeking medical assistance whenever alarming symptoms are experienced.

- **Idea of Isolation and self-quarantine:** Isolation of infected people & self-quarantine on occasion of outbreak of communicable diseases have gained an importance to cut the chain of transmission. For e.g. people of a far-off village in East Siang of Arunachal Pradesh independently constructed huts using bamboo plants for quarantine of the migrant workers returning back to villagethis shows positive impact on people's sense of responsibility for their friends and society.

Impact on Administration & Health sector:

- **Administration:** The introduction of nationwide lockdown was a very prompt monumental step which indicates the idea that life is more important than economy. An impressive score of '100'was given by "Oxford COVID-19 Government Response Tracker (OxCGRT)" which compares the government's response to the novel coronavirus outbreak. Honourable Prime Minister Shri Narendra Modi said that "Every crisis is an opportunity."

India realised that it has enough resources to protect the country from external war but not enough resources to fight an outbreak of disease. India ranks 145 of 195

nations in terms of quality and accessibility of health care services. The pandemic of COVID-19 has shed light on the poor Health Sector of the country due to which the government is aspiring to increase the spending on health care services from 1.4%-2.5% of GDP in the next 2-3.

There were stimulus packages introduced around the globe to fulfil the deficiencies of the health-care sector and to fight the pandemic-led economic slowdown. A similar stimulus package was introduced by Government of India (GOI) as well, to boost the micro, small and medium enterprises (MSME) to tackle the dying economy. GOI also gave loan security to banks for funding the MSME which paved a way for the birth of various start-ups

Steps were taken to become self-reliant for manufacturing of indigenous goods so as to not depend on other nations for its availability. An important e.g. that can be given is that India was not manufacturing a single PPE kit leading to shortage of PPE kits for health care workers but by 2nd may 2020 India started manufacturing 2.06 lakh PPE kit/day.

Govt. encouraged several non-medical based companies and home-based establishments to manufacture medical equipment and supplies, as a result several companies transiently converted themselves for manufacture of medical supplies and equipment using their available resources, this shows flexibility and efficiency of countrymen. Several households started making masks just by using 4 cloth strips, scissors and sewing machines to fulfil the increasing demands of masks. Several liquor manufacturers started making sanitizers using available resources.

Steps were taken for regular cleanliness and sanitation of public places by the government as well as at local level, cleanliness has become a priority which is a good sign.

Among every ten infectious diseases in people six are zoonotic in origin and 70% new viruses originate from animals mostly wild. Evidence suggests that the ground zero for COVID-19 was Hunan Wet Market, Wuhan. Initial evidence suggests SARS CO-V 2 originated from abat with pangolins being the intermediate, due to this ban was imposed on many wet markets around the world to reduce animal and human contact. Many of the wildlife species have come to verge of extinction due to illegal wildlife trade, this ban may cause significant decrease in illegal poaching.

- **Health Sector:** There has been heavy investment in the healthcare sector since the advent of COVID-19.

There were a smaller number of beds and doctors available for the rising number of patients, there were 7,13,986 total government beds in India i.e. 0.55 beds per 1000 people. Govt. has taken appropriate steps to increase the number of beds and doctors to tackle not only this pandemic but also to fight against similar outbreak in future.[24]

The Union health ministry's data suggests that the number of ventilators were 8,432 ventilators in the public sector in India in February 2020 whereas there were in all 40,000 ventilators in the country of which 75% are imported ventilators. The need of ventilators was far more which led to increase in demand and thus, medical device manufacturers increased their manufacturing capability, eventually we shall overcome the pandemic but these devices shall stay.[25]

The death of several medical professionals and security personnel has thrown light on the importance of safety of Medical professionals and paramedic staff and also the importance of PPE kit and its availability.

Health care professionals including doctors and paramedical staff are trained to identify, diagnose and isolate infected patients efficiently and also trained regarding the use of PPE Kit effectively.

Use of telemedicine and increased use of technology has gained importance. Ministry of Health and Welfare (MoHW) along with Niti Aayog issued Tele-Medicine Practice Guidelines enabling Registered medical practitioners around the nation to provide healthcare services in remote settings, which is not only a faster accessible method but also affordable. Tele-medicine also enabled online training sessions for paramedical staff and PHC staff educating them about the recent updates related to healthcare.

Pharmaceutical companies of India are amongst the major producers of generic medicines, which are gaining popularity and respect around the globe. Indian Pharma industry has a 20% share in global supply of generic medicine and 50% of total vaccine demand around the globe.

Various institutes around the globe are raising their standards and using advanced tools and trying to create

an efficient COVID-19 vaccine. They are also preparing for the mass production of vaccines once invented, to prevent delay in supply of vaccines.

Social & Personal Impact:

- The risk of complications of COVID-19 skyrockets with increasing age due to associated comorbid conditions which are prevalent amongst elderly. People beyond the age of 65 are at very high risk and it is highest beyond 85 years of age. This generates a sense of responsibility for Elderly, their health has become a priority.
- The Pandemic has also generated the sense of respect for the health care workers, police personnel, blue-collar workers and everyone whoare risking their livesand working round the clock in this period of crises. This helps them stay enthusiastic and work efficiently.
- The attitude of financial, personal and social preparedness for the period of crisis has taken root in people's mind. Loss of jobs was the immediate direct effect of the lockdown; India's Unemployment rate rose to 24% by 19 April 2020 compared to 6%-7% in 2019 i.e. 100-120 million people lost their jobthe worst hit was amongst the migrant labourer. Preparedness will help all of us to sail the tides of future crisis with minimal distress.
- The sense of responsibility and gratitude towards society's healthcare has risen due to the pandemic and we have seen people helping the ones in need, this has created an air of positive energy.

Lockdown related positive Impact:

- **Impact on Environment:** The colossal positive impact was seen on the environment with reduction in our ecological footprints.

o Air Quality-In November 2019, the quality of air in Delhi was so bad that a public health emergency was declared, when the AQI (Air Quality Index) was coded Red. With the shutdown of factories, travel banand empty roads the air pollution level dropped drastically. The AQI was in Code Green in most parts. As per a news article Dauladhar mountain range (Himalaya) could be seen from Jalandhar which is 250 km away for the first time in three decades.

Drastic drop in levels of PM2.5, PM10 and NO_2 was observed.

- **PM 2.5** – Particulate Matter 2.5 is particulate matter having size less than 2.5 μm.Combustion of fossil fuel is the major contributor in PM 2.5. With cessation of mining activities, lockdown of factories and restricted motor vehicle activity, lowest daily average dropped to 20 $\mu g/m^3$ (2017-2019 → 100-140 $\mu g/m^3$.) As per 'Central Pollution Control Board (CPCB)'there was 46% reduction in PM 2.5 compared to pre-lockdown period.
- **PM 10** – Resuspended dust on road and construction dust is the major contributor in this category. With cessation of traffic flow and construction activity, lowest day wise average dropped to 35 $\mu g/m^3$ (2017-2019 → 200 $\mu g/m^3$.) as per CPCB there was 50% reduction in PM 10 compared to pre-lockdown period.
- **NO_2** – The fall in Nitrogen Dioxide was dramatic. Its main source is vehicular emission. With the travel ban the level fell by 56%.[28][29]
- The adjacentgraph shows spatial pattern of National Air Quality Index (NAQI) in Delhi NCT comparing pre-lockdown and lockdown period between 3 March 2020 and 14 April 2020. The PM 2.5 being small can enter lungs and bloodstream the cut off of these particulate matters has been beneficial for human health.
- Data Collected by Moderate Resolution Imaging Spectroradiometer (MODIS) aboard NASA's Terra and Aqua satellites between 31 Mar 2020 and 5 April 2020 that measures optical depth & size distribution of ambient aerosol over the globe on an hourly basis. Aerosol Optical Depth concentration was estimated within a week of lockdown. [Brown Colour Denotes High AOD]

o **Water Quality**–With shutting down of factories, shutting of fishing practices, shipping industries shut down imprints were seen in water quality as well.

As per the study in Thoothukudi in southern Tamil Nadu in India in the initial phase of lockdown (19 April 2020 – 20 April 2020) for ground water quality compared to pre-lockdown period, changes were seen in chemical as well as biological parameters.

- Physico-chemical parameters have shown significant reduction in TDS (Total Dissolved Solids.) A 50% reduction in lead, 60 % drop in iron values, 51%

dip in arsenic and 42% reduction in selenium was observed.

- Reduction was also seen in NO_3, in the water sample about 56% reduction in NO_3 was seen compared to pre-lockdown water sample.
- Total coliforms and faecal coliforms have shown steep drop of 52% and 48% respectively compared to 10 February 2020, which is mainly due to lockdown on fisheries leading to transient reduction in exploitation of ocean's living resources.
- As per the study of Delhi Pollution Control Committee (DPCC) on Yamuna River there is 18% to 33% reduction in Biological Oxygen Demand (BOD) and 2.3 mg/l –4.8 mg/l increase in Dissolved Oxygen (DO) compared to April 2019. BOD is theoxygen requirement of microbes to decompose organic matter. Increase in BOD corresponds to increased pollution.

o **Global Warming** – lockdown was triggered in numerous parts of the world and in others there were economic slowdown which caused steep 17% dip in global Carbon emission and 26% dip in India in April 2020 compared to April 2019. As per National Climate Change Journal (United Kingdom) study annual carbon emission could fall by 4.4% to 8% by the end of 2020, which would be the "largest drop in average annual carbon emission since second world war" as per some researchers.

o **Wildlife** – With restricted human mobility and most of the people staying inside their houses, wildlife started recuperating their forgotten territory. E.g. Leopards were spotted on several occasions on the streets of suburban Maharashtra, Navi Mumbai saw flocks of flamingos after a long time. Migratory birds started revisiting their once abandoned water bodies, due to transient reduction in pollution the water bodies were habitable again. Because of the lockdown and economic slowdown poaching of wild animals also reduced.[19]

• **Impact on Personal and Family Life** – In this race of survival,humans have somewhere forgotten to enjoy their lives with their loved ones.With the trigger of lockdown, fast running lives of people in the cities suddenly lost speed enabling them to spend time and enjoy with their family and friends. Slow pace lives have drawn their attention from the distraction of stressful lives towards their personality development and rejuvenation of their relationships.

Many people rediscovered their deeply buried hobbieslike singing, painting, cooking. No stress of pending files and workload also improved the mental health, lockdown triggered the sleeping innovations of many. Humans started paying attention to small things which they earlier ignored like the importance of decreasing pollution and global warming for healthy living.

Many of us learned new things this lockdown period. Internet literacy increased as we could spend more time without distraction for e.g. earlier people would read news and agree to whatever they read without anticipating due to lack of time, now people became aware about fake news and the need to reverify the news before blindly sharing it.

The closure of alcohol factories, liquor shops and unavailability of cigarettes may have helpedpeople trying to quit smoking and drinking.

• **Impact on Work and Economy:** Due to the dip in the economy many citizens lost their sources of income, which forced them to think about alternatives for income and use this opportunity innovatively.

Due to social distancing and lockdown there was an increasing demand of virtual communication services giving importance to Indian Blue-Chip companies.

The idea of work from home had a deep impact. Working from your own residence was a win – win situation for both the employee and the employer. This not only reduced the need for fancy work places which require a huge investment but also increasedt he ease of working.'Work from Home' also overcame the problem of physical distance barrier empowering many employees to work from their own city/state/nation and need not migrate.

A big impact was on migrant workers. Total number of migrants in India is 45.36 crore (37%) (according to the 2011 census). Due to migration of workers, their respective states understood the need for creating jobs in their own states so that people don't have to migrate to another state in search of jobs Creating local jobs would not only reduce the hassle of migration but also reduce overcrowding of cities (as most of the migrant workers migrate from villages who migrate to cities) thereby reducing communicable diseases.

- **Self-sustained Nation**– Before pandemic more attention was given on internationalisation of trade but as the pandemic took a bad picture importance shifted towards localisation. With cessation of trade & supply chain cut-off we realised our dependence on other countries. This gave birth to the need of being self-sustained. E.g. India is a leading country in the pharma world, but still we are dependent on China for API (Active Pharmaceutical Ingredients), shortage of API was a limiting factor in medicine output efficiency giving rise to needs of finding alternatives. As a result,govt. has announced a $394 million scheme for development of 3 bulk drug parks for manufacture of API and Bulk drugs inthe next 5 years.

Dependence on China for COVID-19 testing kit delayed the detection of cases in India during the initial period, now India Manufactures 75% COVID-19 test kits domestically. TransAsia Bio Medicals (Mumbai Based) has set up the largest Asian factory for manufacture of COVID 19 diagnostic test kit in Visakhapatnam capable of manufacturing 10 lakh COVID 19 diagnostic test kit per day as claimed

- **Decrease in Road Traffic Accidents(RTA) and Crime Rates** – As the restriction of free movement were imposed, there was a steep drop of 68% in the number of Road Traffic Accidents between 21 March 2020 – 31 May 2020 compared to 21 March 2019 – 31 May 2020

During the lockdown, decreased crime rates were observed. Delhi Police registered ~2000 cases only between 15 March 2020 and 12 May 2020 i.e. a drop of 42% in crime rates, this was mainly attributed to strict enforcement of police personnel around the city.

Conclusion

The COVID-19 pandemic has claimed lives worldwide and has caused havoc. It is yet very important that we shed some light on thepositive influences it has had and acknowledge the wisdom that it has imparted. Not only has it resulted in brighter skies and cleaner lives it has made individuals mentally strong and changed their personality for the better.

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Gastric Outlet Obstruction: A Case Report

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Abstract

Introduction: Gastric outlet obstruction is an obstruction in the pylorus of stomach it is also known as a pyloric obstruction. It is a common condition but sometimes accurate cause could not be found in these cases it can be diagnosed on the basis of signs and symptoms and on diagnostic evaluation i.e endoscopic examination, barium meal, ultrasonogaphy, computed tomography and chest x-ray.

Case Presentation: Here we are mentioninga 50- year'sold male visited our hospital with the complaints of severe abdominal pain, nausea and vomiting since 2 days, weakness since 5 months, has passed stool 2 days ago, weight loss for 2 months. All routine laboratory tests were done in this case and all the results were within normal range except decreased hemoglobin level, increase in white blood cells also increased creatinine level and decreased albumin level. In Abdominal ultrasonography it was mentioned that severely edematous mucosa with erosive patches and places, Pyloric canal deformed scope could not be negotiated across the pylorus. Barium meal study depicted dilated stomach where greater curvature is below the level of iliac crest. Finally a client case was diagnosed as a gastric outlet obstruction. Primarily as per priority Blood transfusion and all conservative treatment was started by surgical team but client was not relieved of obstruction by the medical management. Diagnostic exploratory laparotomy with repair of duodenal perforation with gastrojejunostomy under general anesthesia was done. Vital signs and cardiac monitoring was done. Maintained intravenous fluid and colour of drainage was observed, propped up position and antibiotic, analgesic, anti emetic given and ryle's tube aspiration was done 4 hourly also abdominal girth was also measured 4 hourly. A positive response to treatment was observed by patient. Patient was discharged with full recovery without any postoperative complication.

Conclusion: In this study, we mainly focus on expert surgical management and quality nursing care due to which the patient was discharged without any postoperative complications and satisfaction with full recovery.

Keywords: *Edematous mucosa, Gastric outlet obstruction, Pylorus, Stomach.*

Introduction

Gastric outlet obstruction is also known as a pyloric obstruction. It is a common condition which that requires a good intervention as early as possible. Gastric outlet obstruction is an obstruction of the channel of the pylorus and duodenum i.e part of small intestine through which the stomach empties. The cause of the obstruction may be benign or malignant disease.

The incidence of gastric outlet obstruction is exact unknown. But after detection proton pump inhibitors and H_2 blockers, the major cause of gastric outlet obstruction was peptic ulcer disease

The Helicobacter pylori and developing an effective treatment for the same has resulted in fever cases of peptic ulcer disease presenting with gastric outlet obstruction (<5%); in the modern era, the major cause is known to be malignancy, especially in the developed world. 80% of population which is highly affected with Helicobacter.

Pyloribacteria and the prevalence of peptic ulcer disease and gastric carcinoma near about 8.0/100,000 and 3.0/100,000, respectively. Many books and various articles suggest that the benign causes continue to be the major cause of gastric outlet obstruction in developing country[2].

Mostly benign causes of gastric outlet obstruction are peptic ulcers diseases i.e age factor, gastric polyps means masses of cells that form on the lining of the stomach, pyloric stenosis, ingestion of caustics, gallstone obstruction, bezoars, congenital duodenal webs, ulcers within the pyloric channel and first portion of the duodenum usually responsible for outlet obstruction[1].

The commonest cause of gastric outlet obstruction is pyloric stenosis i.e narrowing of the opening from stomach to the first part of the duodenum which is the part of small intestine. It is secondary to peptic ulcer disease or gastric carcinoma[1].

Transitional cell carcinoma is one of the main and common malignancy of the urinary tract of urinary system and is the second most common reason for death in genitourinary tumors. Near about 90% of all bladder cancers is related to transitional cell carcinoma. After it will spread towards lungs, liver and bone. In late stage metastases are seen towards brain and it is common[3,4].

In our client's case it was found that a novel presentation of gastric outlet obstruction because of

Severely edematous mucosa with erosive patches and places, pyloric canal deformed scope could not be negotiated across the pylorus. Barium meal study depicted dilated stomach where greater curvature is below the level of iliac crest.

Case History: A surgical case was taken by Acharya Vinoba Bhave Rural Hospital, DMIMS, Sawangi, Wardha, Maharashtra. After operation, this complicated case was taken care nicely by the hospital because of expert surgical team management and quality nursing care.

Patient Information: Mr. XY 50years old male was admitted in hospital with the complaints of severe abdominal pain just like a last stage, nausea vomiting since 2 days, weakness since 5 months, passed stool before 2 days, passed flatulence in morning, weight loss for 2 months. Along with all these complaints patients was admitted in hospital in emergency situation. He received treatment from others hospitals also but he was unsatisfied. Otherwise no any history of hypertension and diabetes mellitus and other communicable disease.

Patient belongs to low socioeconomic status; he likes to take vegetarian and non vegetarian diet. He maintained good interpersonal relationship with family members and relatives also.

Physical Examination: Patient's Physical examination was done and on examination he looked undernourished and his Body mass index was 15.22. Temperature 38^0F, Pulse rate was 82/min, Respiratory rate was 20/min and systolic and diastolic blood pressure was 120/70 mm of Hg. Conjunctiva looked pale due to deficiency of Hemoglobin. During percussion fluid collection was present due to perforation peritonitis. Otherwise no any abnormality detected during physical examination

Diagnostic Assessment: Laboratory test revealed that hemoglobin level was decreased i.e 7.3 gm/dl and mean corpuscular volume was 54fl it was decreased, white blood cell count was increased i.e 28300 micro/lit and platelet was increased with 4.91 and Lymphocytes was decreased with 8%, Granulocytes was 88% it was increased, creatinine level was increased with 2.6meq/l. and albumin level was decreased with 2.7gm/dl and random blood sugar level was 67 mg/dl. Otherwise all routine tests were normal.

Radiological examination was done and it was found in Barium meal report that dilated stomach where greater curvature is below the level of iliac crest and Barium does not passed into duodenum. Gastroscopydone to rule out stomach cancer. Electrolyte study was done for the correction of electrolyte imbalance. X-ray chest erect-free gas under right diaphragm was depicted. Electrocardiogram done to check for hypokalemia. Ultrasonography was done and report was depicted severely edematous mucosa with erosive patches and places, Pyloric canal deformed scope could not be negotiated across the pylorus.

The patient was admitted in our hospital and he was treated for gastric outlet obstruction conservatively. But after that treatment the patient was not relieved of the obstruction through medical treatment. Finally surgeon planned for surgery and under the expert surgeon supervision and the presence surgery was performed.

Pre-operative Care: Nasogastric tube intubation

was done as per doctor's order and Ryle's tube aspiration was done 2 hourly. Abdominal girth was recorded 2 hourly. Bladder was catheterized as per doctor's orders and maintained intake and output strictly. Doctors tried to treat this condition with the help of conservative management i.e. Inj Ceftriaxone 750mg antibiotic intravenously, inj. Metronidazole 500ml antimicrobial intravenously, Inj tramadol pain killer in IV drip, Inj neomol 100ml antipyretic intravenously given, but only the operation was next choice for surgeon to handle this case and before surgery written consent was taken by patient and inform to family members. Physical and psychological preparation was done.

Finally Diagnostic exploratory laparotomy with repair of duodenal perforation with gastrojejunostomy under general anesthesia was done.

Post-operative Care: Post-operatively patient shifted in surgery ICU; propped up position was given,4 hourly Ryle's tube aspiration and abdominal girth was measured. Post operatively continuous cardiac monitoring was done, maintained intake and output of the client. Oxygen inhalation given, asthalin nebulization given to the patient. Inj Ceftriaxone 750mg antibiotic intravenously, Inj. Metronidazole 500ml antimicrobial intravenously, Inj tramadol 2cc in 100ml pain killer in IV drip, Inj neomol 100ml antipyretic intravenously given, Inj.albumin 20% in dextrose 5% in 500ml for 3 to 4 hours given.

Nursing Management: Postoperatively patient was under strict observation of on duty nursing staff. Intravenous fluid administered as per calculated. Observed and recorded the character of the drainage postoperatively. Care of wound and daily dressing was done. Drainage care was taken and maintains intake and output 2 hourly. Vital signs were recorded strictly. The drain output became less and was removed after 4 days. Then patient was shifted in surgical ward from surgical ICU after recovery. Excellent nursing care was given and patient himself reported to nursing staff that, he was very satisfied about nursing care. Complete discharge procedure was explained by nursing staff to the patient and his family members along with medication prescribed at home as advised by surgeon. Explained dietary menu plan to be followed at home. The patient was discharged from ward after 10 days post surgery without any complications.

Patient visited regularly at surgery OPD with his family members, during this period routine checkup was done and he had nocomplaints, therefore no furthermore evaluation was found.

Discussion

Gastric outlet obstruction is a common surgical condition. It is common in male as compared to female. Management of gastric outlet obstruction is a one type of surgical challenge in developing country because of low setting. It is a condition in that gastrointestinal surgery is needed during its study period. On an average nearly about 10 cases per year found of incidence cases.

Sometimes gastric outlet obstruction causes because of effect of others disease process. That produces a mechanical blockage gastric emptying. Gastric outlet obstruction cause due to benign obstruction as the stomach one hear, the stomach can see and stomach can feel also[5] the cause of obstruction benign or malignant disease.

Gastric outlet obstruction develops for very short period of time. Never produce dilatation or distension of the stomach. This condition can be managed by surgical treatment.

Conclusion

Gastric outlet obstruction is very surgical common problem in our setting but if it is notmanage as early as possible this condition will be threat to the patient's life. and make a demand of advanced diagnostic evaluation and therapeutic challenges. Gastric outlet obstruction is very common in males with malignant causes being more prevalent. But it is more found in young patients while malignant. Causes in elder age group.

Overall this condition was very complicated for patient. But due to surgeon's expert team and quality nursing care his condition was improved. In such type of cases, if patient diagnosed at early stage we can prevent mortality and morbidity. Otherwise it will be very serious issues for the patient and patient life can be spoiled. On observation it shows that after exact treatment on cause, patients recover well and no further medical intervention was required.

On admission patient XY's condition was life threatening. It was complicated to handle because of all these symptoms patient was so scared and tensed, hence it was difficult to handle but after treatment and surgery slowly his condition was improved. Patient and his

family members had multiple doubts regarding over all condition and their each and every doubt were cleared. Quality nursing care was given and he had given positive feedback to nursing staff he felt better after surgery and he was satisfied, he always talked to every nursing personnel very nicely and given full co-operation to nursing personnel while giving therapeutic care and he was discharged from hospital with full recovery without any post operative complication and satisfaction, with happy face along with his family members.

Patient in form Consent: While preparing case report patient's informed consent has been taken.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Thalassemia in Children: A Case Report

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Abstract

Introduction: Congestive Cardiac failure (CCF) is an anomalous clinical condition involving insufficient heart pumping and filling Cardiac failure causes the heart to be unable to provide enough blood to meet the tissue's oxygen needs. Heart disease is the most common. Why older people are admitted to hospital This puts a huge economics stress on the health care system.

The dynamic, progressive nature of heart failure also leads to poor results, with hospital readmissions being the costliest About half of those patient die within 5 years after diagnosis.[3] *Case presentation:* A male patient of 62 years from Arvi Naka was admitted to Medicine Intensive Care Unit (MICU), AVBRH on 11th January 2020 with a known case of Ischemic Cardiomyopathy which was diagnosed itself at AVBRH on 22nd October 2018 and a known case of diabetes and hypertension for 10 years. My patient was brought to AVBRH Emergency Unit on 11th January 2020 with a chief complaint of breathlessness for 2 days, sweating over both feet for 5 days and generalize weakness for 2 weeks. He was having difficulty in breathing for about 2 days which eventually become severe on 11th January 2020 evening and was brought immediately to AVBRH and got admitted on the same day. The patient was delirious and vomit two times on admission.

Keywords: *Congestive Cardiac Failure, dyspnoea, arrhythmia, tachycardia, ischemic cardiomyopathy.*

Introduction

Congestive Cardiac Failure (CCF) is a chronic progressive condition that effects on involves inadequate pumping and filling of the heart. It means that the heart cannot provide enough blood to fulfill the oxygen needs of the tissues. Since all heart failure does not include pulmonary congestion, heart failure is commonly called congestive cardiac failure.[1]

Cardiac Failure is associated with various cardiovascular diseases, particularly untreated elevated blood pressure, coronary artery disease and myocardial infarction. Unlike other cardiovascular disease, incidence and prevalence of cardiac failure is on the rise. This is attributed to increased survival chance after heart attacks and rising population ageing.[2]

Heart is unable to pump when the myocardium cannot maintain a cardiac output adequate to satisfy the body's metabolic needs. Cardiac failure is extensively classified into: - right-sided heart failure and left-sided insufficiency. At the same time, congestive cardiac failure can be left sided and right sided. The disease typically starts in the left side and then spreads to the right side if the left side remains untreated. Right-sided heart failure occurs when it becomes difficult for the right ventricle to pump blood to the lungs, blood pools up in the blood vessels, causing fluid accumulation in the lower extremities, abdomen and other vital organs. Left side cardiac insufficiency is categorized as systolic dysfunction and diastolic dysfunction. Inadequate blood drainage from the ventricles results in systolic dysfunction. Diastolic dysfunction (stiff heart syndrome) occurs when diastole does not adequately fill the ventricle.[1,5]

Case History:

Patient Information: A male patient of 62 years from Aarvi Naka was admitted to Medicine Intensive

Care Unit (MICU), AVBRH on 11th January 2020 with a known case of Ischemic Cardiomyopathy which was diagnosed itself at AVBRH on 22nd October 2018.

Present Medical History: A male patient of 62 years old was brought to AVBRH Emergency Unit on 11th January 2020 with a chief complaint of breathlessness for 2 days, sweating over both feet for 5 days and generalize weakness in the last 2 weeks. The patient was having difficulty in breathing for about 2 days which eventually become severe on 11th January 2020 evening and was brought immediately to AVBRH and got admitted on the same day. The patient was delirious and vomit two times on admission.

Past Medical History: My patient had his first heart attack 5 years ago that is 2015 back in Mumbai, he was taken to hospital and got his second heart attack on 3rd October 2018 and got admitted to AVBRH and was diagnosed as Ischemic Cardiomyopathy and advised to do Coronary Artery Bypass Gas (CABG). He is a known case of diabetes and hypertension and is on medication for 10 years.

Family History: There are 3 members in the family, my patient, his wife and his son. The other family members do not have any communicable or hereditary disease except for the patient himself having diabetes and hypertension in the last 10 years back. The type of marriage of the patient parents and he himself and his wife is non-consanguineous marriage. The other family members are healthy.

Past interventions and outcome: My patient had his first heart attack 5 years back and his second heart attack on 3rd October 2018 and was diagnosed with Ischemic Cardiomyopathy at AVBRH and had done Coronary Artery Bypass Gas (CABG). My patient is also on medication for hypertension and diabetes for a period of 10 years. My patient is able to do daily activities of living without much interruption after his surgery on 2018 till the past few weeks of his admission to AVBRH. He went for regular medical check-up and is taking medicines regularly.

Diagnostic Assessment: Blood study shows: Haemoglobin-13.4%, TLC-54200cumm, Platelets-2.2lac/cum, Sodium-125mg/dl, Potassium-4.5mg/L, Creatinine-2.4gm%, Urea-70mg%. Chest X-Ray and Echocardiogram shows dilated left ventricle with a reduced ejection fraction.

Management:

1. **Medical management:** Patient was treated with Tablet Cardivas/Carvedilol (Antihypertensive α/β adrenergic blockers) orally once a day with a dose of 3.125mg. oxygen was administered at 2l/hour till my last day of care.
2. **Nursing Management:** Observed for cardiac rhythm, monitor vital signs closely. Keep patient safe from falls at risk of weakness. Provide pain management. Assessment of complications of CCF like arrhythmia, kidney damage, heart valve and rhythm problem and liver damage. Give health Education to patient and family members.

Discussion

A male patient of 8 years old from Aarvi Naka was admitted to Medicine Intensive Care Unit, AVBRH on 11th January 2020 with a complaint of breathlessness for 2 days, sweating over both feet for 5 days and generalize weakness for about 2 weeks. The patient is a known case of hypertension and diabetes mellitus and is on medication for 10 years. He was diagnosed with Ischemic Cardiomyopathy on 22nd October 2018 after his second heart attack at AVBRH and had undergone CABG. After admission to AVBRH he was then diagnosed with congestive cardiac failure and is under appropriate treatment and investigations were done. The patient is conscious and does not shows symptoms of gastric problem which he had at the time of admission after his hospital stay[6,7,8.]

A study was done on, “Discharge Education Improves Clinical Outcomes in Patients with Chronic Heart Failure”. It was done to assess whether there is improvement in patient condition after their hospital discharge related to health education given during their hospital stay and at the time of discharge. For this study, 223 systolic heart failure patients were selected using randomised controlled trial and they compared the effects of a one-hour, one-on-one teaching with a nurse educator to the standard discharge process. The samples were then contacted through telephone after 30, 90 and 180 days to assess any change or specifically improvements on the clinical events, symptoms and their self-care practices. The result shows that samples who were receiving health education during the hospital stay were having fewer hospitalised days and the death rate was much more reduced as compared to the others who were not receiving health education to about 75

percentage. Also, samples who are receiving education have lower risk of rehospitalization and their cost of care is much more reduced to 95 percentage. This conclude that the result of the addition of one-hour, one-on-one health education given to the patients with congestive heart failure by the nurse educator during their hospital stay and at the time of discharge has resulted in improved clinical outcomes, improved their self-care measures and reduced their chance for rehospitalization and cost of care to a high amount[9,10.]

Conclusion:

The congestive heart failure is a chronic progressive disease that inhibits the functioning of theheartmuscle. It is common among obese, hypertensive, diabetes, congenital heart disease and history of heart attack. If it is treated and diagnosed early, it can be controlled and the patient could live a normal life and practice the daily activities of living without much interruption. My patient does not have very much profound improvement after admission but his deliriousness and gastric symptoms at the time of admission were subsiding and the treatment was still going on till my last date of care.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Ischemic Heart Disease: Case Report

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Abstract

Introduction: Ischemic Heart disease is one of the leading causes of mortality in the world. Advancement in the diagnosis and treatment of this disease available, but still they were considering as major health problems in the world.

Case Presentation: The purpose of this report only to reduce the major risk factor that is obesity and provide preventive base Health care treatment which has the greatest effect in the shortest period. The main symptoms of this disease are chest pain (Angina) and pain mostly occur on the middle or left side of chest and pain sharp and radiate towards the neck, arm or back, other symptoms like shortness of breath. Obesity is the one of risk factors in heart diseases. The main diagnose of ischemic heart disease is includes the complete medical history,electrocardiogram, bloodanalysis, chest x-ray, echocardiography, coronary computed tomography and coronary angiography. Therapeutic interventions or treatment for ischemic heart disease includes that pharmacological therapy, lifestyle modifications and regular exercises are the most cost-effective interventions. Cardiac rehabilitation is for all patients with coronary artery disease is required.

Outcome: The effective interventional strategies to help the patient to change the lifestyle and it helps to prevent risk factors such as obesity and also helps to the patient in identifying the symptoms.

Conclusions: Considering the patient's recovery process regarding physical strength it seems that the combination of pharmacological treatment and lifestyle changes as a control on obesity,regular exercises and cardiac rehabilitation helps to treat coronary heart diseases.

Keywords: *Ischemic heart disease, Obesity, Prevention and treatment, cardiac rehabilitation.*

Introduction

Ischemic heart disease is the most common cause of mortality in various societies; the occurrence of heart diseases varies with culture and tradition and differs from country to country. In the Middle East and Western Europe have the highest mortality rates of cardiovascular diseases.(1) Ischemicheart disease is the firstcause among the most common causes of death;Ischemic means the reduced blood supply. The coronary arteries that supply blood to the heart muscle, so due to that blockage in the coronary arteries reduce the blood supply to heart muscles. Most ischemic heart diseases are caused due to atherosclerosis. The narrowing or closure is caused by atheromatous plaque on heart walls and artery rupturing and it leads to heart attacks it is due to only the narrowing of the artery. A heart attack is the cutting of blood supply to heart muscles.(2)

Risk factors for cardiovascular diseases include Hypertension,diabetes, hyperlipidemia,obesity and smoking. According to United States data,more than 6 million people admitted for an emergency each year with chest pain and they diagnosed with myocardial infarction and are admitted in cardiac care units and also 500,000 deaths each year are due to Ischemic heart disease. Various therapeutic approaches, including medicine treatments using thrombolytic drugs, anti-thrombus,

beta-blockers, calcium antagonists and invasive treatments like angioplasty and open heart surgery have also been used in the treatment of this disease, etc. Most hospitalized patients can experience anxiety due to heart problems before coronary angiography. Due to increasing heart diseases,cost of treatment also increase so it put pressure on health system, so prevention, its complication and treatment is necessary.(1)

Case History:

Patient Information: A 78 years old male patient admitted in emergency department with complained of chest pain. Since two week, he was suffering from cough and mucus expectorant and chest pain and abdominal distension before five days of hospitalization. He was diagnosed as angina with acute coronary diasese after coronary angiography and admitted in cardiac ward for management. The patient was not having family history and genetic history of heart disease. patient was past history of Coronary artery disease since 6 month ago .

Clinical Findings:

Medical History and Physical Examination: The patient was not having any past history of heart diseases and high blood pressure but the patient was having past history of obesity since 5 years.; the patient did not use proper nutrition for the prevention of disease. and he did not follow the advice of doctors and not keep hygiene. The patient's sleep status was disturbed. Due to the health problem he was not working for family. The Body Mass Index of patient was 33.13 kg/m2 (weight - 105 kg and height -178 cm.). In physical examination some clinical findings were obtained i.e The patient was on chest pain since five days and patient chest pain was radiate towards back and cough with mucus expectorant and cold sweating, distension of abdomen also found,the patient had a hot temperamental and patient's pulse was full and felt warmer when touching the skin. The patient had the following vascular risk factors: 1) body mass index of 33.13kg/m2, in other words, very obese; suffering from hyperlipidemia and fatty liver for over 2 years treated with Atorvastatin, 40 mg once a day, clopitab 75 mg once in a day, Ramipress 2.5 mg once a day, 2) lack of sufficient physical activity, 3) Stressduring this period, the patient was treated with modern method. He was anxious as a result of being hospitalized in cardiac care and undergoing angiography.

Timeline: Coronary heart disease was initially thought to be a disease of modern humans, but the disease is not a new .article in 2013 that shows atherosclerosis may be very ancient. The clinical syndrome of angina pectoris existed in ancient Egyptbut there is no record of any anatomical and clinical correlation. But currently, from this episode prevention and lifestyle modification, it is important aspects related to heart diseases.(3)

Diagnostic Assessment: The patients when admitted to the hospital, the patient suffered from chest pain for five days, the patient suddenly experienced persistent pain at the back of the chest six hours before his visit. This pain increased until the time of admission to the Emergency department . The patient history taking and physical examination done. Electrocardiogram were done. Coronary angiography was done and after all based on investigation patient diagnosed as Ischemic heart disease and shifted to the cardiac ward for treatment . The patient received coronary artery angiography at the time and by the diagnosis of cardiovascular obstruction and other diagnoses available like computed tomography of heart, Cardiac biochemical markers, Biopsy of heart, etc.

Sr.No.	Diagnostic Test	Findings	Remark
1	Pathology – Total WBC	16400/cumm	Increased
2	**Livet function test:** • SGPT • SGOT • Alkaline phosphate	 52 IU/L 61 IU/L 54 IU/L	 Increased Increased Decreased
3	Ultrasonography	Grade I fatty liver, Gasious distention of the abdomen	
4	2D–Echo	Left atrium is normal ang enlargement of left ventricle, Hypokinesia of anteroseptal wall of left ventricle.	

Prognosis: After the treatment such as combination of therapeutic method and lifestyle modification practices, regular exercises, cardiac rehabilitation, continuation in pharmacological therapy, modification in diet pattern, the patient's general condition improved after the second week, so that the patient's stress and anxiety were not observed or recorded during that period and the patient's vascular obstruction was not improved in his clinical report, at the moment; the patient was only treated with atorvastatin, 40 mg once a day,and Ramipress 2.5 mg once a day for his blood pressure and used health and nutritional measures.

Therapeutic Interventions

Pharmacological,dietary,surgical, Preventive management are very effective in Ischemic Heart Disease .

*Pharmacological:*For this patient, due to his condition of disease, lifestyle modification and pharmacological drugs were used. Pharmacological treatment treatment was used.to treat patient. The client was only treat with Atorvastatin, 40 milligramsonce a day and Ramipress 2.5 mg once a day for his blood pressure and clopitab 75mg once a day used health and nutritional measures.

Surgical:The doctors advised the patient for surgical treatment, but the patient and relatives said that they will plan after a few days.because the patient belongs to poor class family and medical treatment was not affordable to them.

Preventive measures: The patient's advice to reduce weight because obesity having risk for the further development of Ischemic heart disease. Advised the patient regarding diet that is salt restriction diet, low cholesterol diet, avoids spicy fast food and use the garlic is one of the herbs used to treat cardiovascular diseases (especially vascular obstruction). For men, especially those who are warm in temperament, use more protective foods like fruits, vegetables,whole grains, fish,nuts limitation or avoids sweets,change the lifestyle practices such as sedentary lifestyles,regular physical exercises it helps to keep healthy body weight, Aerobic exercise strengthens the heart muscles, It helps in reduction of stress with a relaxation technique.

Self-care: Coronary heart diseases require self-care including diet restriction, use of medication on regular, monitoring of symptoms and instructed to the patient to engaged in routine management,self-care including self -management, self-regulation, self-monitoring, etc[4]

Follow–up: At the time of discharge, given the proper information to patients and relatives regarding follow-up treatment. Inform the patient after 8 days or 15 days visit to the hospital and if any emergency or complication or any sudden incidence happened with patients that time consults with doctors. and inform the health prognoses to the physician. Follow-up after discharge is very important to monitor health status with a laboratory test, physical assessment it helps to know the health status of patients.

Outcome: Effective interventional strategies help the patients to change the lifestyle and it helps to prevent risk factors such as obesity and it also helps the patient in identifying the symptoms improve function and quality of life and prevent coronary disease events. After discharge, the patient visited the hospital to follow up so that time improvement in patient health status was seen.

Discussion

On each year, due to cardiovascular diseases many deaths in the world, such that these type of diseases occur in males and females, due to coronary disease the mortality rate increases, heart disease death rates are declined in men and increased in women since the past decade[5]. It has been studied, in the year 1965 - 1990,cardiac-related death fell by near 50% in the United States and by 60% in Japanese.[1] Burdens of disease in developing countries It has been estimated that 5.3 million deaths attributable to CVD occurred in the developed countries in 1990, whereas the corresponding figure for the developing countries ranged between 8 to 9 million (ie, a relative excess of 70%).[6]

Cardiovascular diseases are multifactorial illnesses and so far many risk factors have been identified. Also, various studies have been carried out on these risk factors at home and abroad. In 1990, the world's population was about 3.5 billion and cardiovascular disease was responsible for 3.34 million deaths, which equaled 28.4% of the world's 50 million deaths, of which 3.6 million died from coronary artery disease including 44% of deaths due to cardiovascular diseases. At the beginning of the 20th century, only 10% of the world's deaths were related to cardiovascular diseases, but it reached 28% by the end of the 20th century and today it accounts for about half the deaths of developed countries and 25% of the deaths in developing countries. It is predicted that

by 2020 these diseases will become the leading cause of mortality in the world with an estimated 36% of the total deaths in world [1]. The increasing use of tobacco in several developing countries will also translate into higher mortality rates of CVD, lung cancer and other tobacco-related diseases. Globally the availability of cheapest vegetables, mixed oil and fat product in low-income countries are reported as increased fat consumption among people and this also responsible for the development of heart diseases.(6)

In most developing countries reported as there are increase the number of people of tobacco use According to Recent information of health organization suggest that, 2020 this year is the single most cause behind death due to tobacco near range accounting for 12.0 % of worldwide mortality.[6] In India only,mortality rate in near about range due to the tobacco use 1.4% in 1990 to 13.0 % in recently 2020. Coronary artery inflammation with oxidative stress causes lipid accumulation and as a result, leads to arterial injury called atheroma. Also, high levels of lipid in the plasma lead to endothelial activation and increase the adhesion of immune cells to the endothelium, resulting in impaired functioning of the endothelium. The conditional risk factors associated with an increased risk of coronary artery disease include triglyceride, homocysteine, lipoprotein, coagulation factors,and inflammatory markers(1). Despite the significant, diagnostic and treatment advancement availability of these diseases in the last three decades, a high amount of treatment of this disease imposes a significant burden on the individual and the health system of the community .

The patient studied in this report had ischemic heart disease that was treated with medical treatment. In this report, an attempt was made to use the most effective and least complicated method .In general, there is no specific definition of modern medicine in the description of traditional medicine for coronary artery disease. To treat the disease and its complications. Over the past decade, new solutions have been proposed for the treatment of ischemic heart disease and this has reduced mortality. With creativity and innovation, which includes the use of physiopathology of modern medicine, the definitions of traditional medicine in Iran were used in the study of temperament, nutritional reform, strengthening of the patient, radiotherapy, herbal therapy,etc. This is referred to as targeted effective medicine. Therefore, the patient needs therapeutic interventions including behavioral therapy, physical exercise, nutrition, herbal medicine. Various studies have shown that lifestyle modification toward a healthy lifestyle, including the use of healthy eating, proper nutrition, exercise, non-smoking and good mental health can provide health indicators even in an individual with ischemic heart disease. It also significantly reduces risk factors and complications.

Conclusion

Effective medicine as a combined treatment, modern medicine and traditional preventive treatment such as regular exercises such as aerobic that strengthing the heart muscles and lower cholesterol diet, cardiac rehabilitation that improve the functional activity and improve quality of life. It also helps in reducing the cause of heart disease. It provides effective clinical findings in the treatment of certain diseases, such as ischemic heart disease. Therefore, in the treatment of this disease, the use of effective medicine method will cost less and improve the quality of life of patients. Therefore, according to modern medicine principles and other preventive treatments, it can be evaluated as a non-invasive therapeutic method that is very useful in the treatment of coronary heart diseases with the highest effect in a short time.

Patient perspective: The after discussing with the patient, patient verbalize that about their health at the time of follow up .He gave thanks to medical team for providing the best care to patient .He explained that the passive role of patient but most active role of health care team and he told that regular use of treatment, physical exercises,weight reduction, preventive care this all are helping to prevent further complications and a good attitude, beliefs and behaviors are most important factors.

Informed Consent: The patient's informed consent was taken and signed by the Patient before writing a case report.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Case Report on Systemic Lupus Erythematosus

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Abstract

Background: Systemic Lupus Erythematous (SLE) is a multi-system chronic but often episodic, autoimmune condition defined by widespread inflammation of connective tissues and immune complex-mediated vasculitis as well as the involvement of Antinuclear Antibodies.

Case Presentation: The 11 years old female patient who was apparently alright 2 months back admitted in "Acharya Vinoba Bhave Rural Hospital Sawangi (M), Wardha," Maharashtra on date 22/02/2020 with the chief complaint of high-grade fever on and off since 2 months and facial puffiness since 6 days, ascites, constipation and pain in joints. The patient started experiencing fever 02 months back which was high grade and she took treatment on an OPD basis but there was no relief. After that she was admitted at GMC Chandrapur for the complaint of cough, cold, fever and oral ulcer and symptomatic treatment was given and got discharged after 12 days. Fever, facial puffiness, pain in knee joints. The patient had undergone various investigations like complete blood count, urine analysis, lipid profile, anti-nuclear antibodies (ANA), ANTI-DSDNA (Anti-Double Stranded DNA). The patient was treated with antibiotics, corticosteroid, antacid, DMARD (Disease-Modifying Anti-Rheumatic Drug), a loop diuretic, antiemetic and oral paste to treat mouth ulcers. Monitor all vital signs, checked and recorded intake and output, administered medication as prescribed.

Conclusion: The patient was admitted to AVBRH with chief complaints of fever on and off, facial puffiness, ascites, constipation and pain in joints. Immediate treatment was started by health team members and now the patient's condition is satisfactory.

Keywords: *Antinuclear antibodies, autoimmune disease, Systemic Lupus Erythematous.*

Introduction

'Lupus' is the Latin word is 'Wolf'.'Erythematosus' means 'Red Rash'.In 1851, Dr. Cazenave found red rashes that looked like wolf bites on a patient's face and he named that rash is Discoid Lupus Erythematosus (DLE).[1]

In 1885, Sir William Osler recognized that numerous individuals with Lupus had including skin as well as numerous organs or system and he named the disease Systemic Lupus Erythematosus (SLE).[1]

Systemic Lupus Erythematosus (SLE) is a long-term disease characterized by systemic inflammation in connective tissues, like cartilage and blood vessels lining, which give strength and flexibility to structures all through the body. SLE is an autoimmune disorder that occurs when the immune system attacks the body's own tissues and organs.[2]

Incidence: Systemic Lupus Erythematosus is rare in India.[3] Systemic Lupus Erythematous is relatively uncommon in children under the age of 9.[4]In the pediatric population, the overall prevalence of SLE is 10 to 25 cases per 100,000 children.[4] Childhood Systemic Lupus Erythematosus affects girls more often than boys

(8:1 ratio) and more commonly affects Asians, Blacks and Hispanics than Caucasians.[5]

Patient Information

- **Patient present history-** The 11 years old female patient who was apparently alright 2 months back admitted in AVBRH on dated 22ndFebruary, 2020 with the complaints of high-grade fever on and off since 2 months, facial puffiness since 6days, ascites, constipation and pain in joints.
- **Past history-** The patient started experiencing fever 02 months back which was high grade and took treatment on an OPD basis. There was no relief. After that she got admitted to GMC Chandrapur for cough, cold, fever and oral ulcer and they gave symptomatic treatment and got discharged after 12 days.
- **Birth History:**

Prenatal History:

- ✓ **Nature of marriage:** Non-consanguineous
- ✓ **Exposure to radiation:** None
- ✓ **Antenatal checkup:** Not done

Perinatal History:

- ✓ **Type of delivery:** Full Term Normal Delivery
- ✓ **Place of delivery:** Home delivery
- ✓ **Mother condition following delivery:** mother condition was good and she did not have any complication following delivery.

Postnatal History:

- ✓ **Child condition at birth:** normal
- ✓ **No NICU stay.**
- **Immunization History:** The patient received all immunization according to her age.

Diagnostic Assessment: Physical examination, patient history and other investigations reveal different outcomes through clinical evaluation.

Physical Examination:

Vital signs:

- **Temperature:** 39°C
- **Pulse:** 84 beats/min
- **Respiration:** 26 breaths/min

Anthropometry Measurement:

- **Height:** 142 cm
- **Weight:** 20 kg
- **Head circumference:** 50 cm
- **Chest circumference:**58 cm
- **Mid upper arm circumference:**14 cm
- **BMI (Body Mass Index):** 9.9 (underweight)

Head to Foot Assessment:

- **Nourishment:** Undernourished
- **Body built:** Thin
- **Skin:** Rashes were present in the hands and lower extremities.
- **Face:** Pale and puffiness was present
- **Conjunctiva:** Pale
- **Upper and lower extremities:** Pain while movement
- **Hemorrhoid:** Present

Blood Investigation report

- ✓ Hb% was 6 gm% (severe anemia), monocyte count was decreased 03%, total RBCs count was 1.73 million/cu.mm (decreased), total platelet count was 1.34 lacs/cu.mm (decreased), HCT was 17.8% (decreased), MCV (mean corpuscular volume) was increased 102 cub. Micron, MCH (mean corpuscular hemoglobin) was 34.3 picogram (increased), ESR (Erythrocyte Sedimentation Rate) was 90/1st hour (increased).
- ✓ **Peripheral Smear:** RBC mass reduced. RBC – Predominantly normocytic mild hypochromic with few macrocytic RBC has seen. Platelet- reduced on smear, APC- 1,34,000 cells//mm3
- ✓ **Liver function test:** AST (SGOT) was increased to 285 U/L, alkaline phosphate was increased to 607 U/L and albumin was 2.4 g/dl.
- ✓ **Lipid profile:** HDL was 19mg/dl, triglyceride was 382 mg/dl and verylow-density lipoprotein was 76 mg/dl.
- ✓ **Antinuclear antibodies (ANA):**Positive
- ✓ **ANTI-DSDNA:** Positive
- ✓ **USG:**

- It shows bilateral minimal pleural effusion,
- Ascites
- Hepatomegaly
- Spleen is mildly enlarged.

Therapeutic Intervention:

The following treatment was given to the patient:

Name of Medicine	Dose	Frequency	Route
Tab. Cefpodoxime	100 mg	BD	Oral
Tab. Flucon	150 mg	OD	Oral
Tab. Cetirizine	10 mg	SOS	Oral
Tab. Hydroxychloroquine	200 mg (1/2 tab)	BD	Oral
Tab. Neurokind	1500 mg	OD	Oral
Tab. Folinine	1500 mg	OD	Oral
Syp. Gelusil	5 ml	BD	Oral
Tab. Lasix	20mg	BD	Oral
Tab. Prednisolone	20 mg	BD	Oral
Tab. Zincot	850 mg	OD	Oral

Nursing Management: As per criteria, the nursing care was given to maintain the health status and to prevent further complications.

- Provided a comfortable position to the patient
- Monitored vital signs of the patient.
- Administered all the prescribed medications.
- Monitored intake and output.
- Assisted in doing the daily activity of the patient.

Nursing Diagnosis:

Nursing diagnosis according to patient complaints are as follow:

1. Acute pain related disease condition.
2. Constipation related to low fiber diet as well as medication use.
3. Fluid volume excess related to steroid therapy.
4. Disturbed sleeping pattern related pain.
5. Knowledge deficient related to the treatment regimen.

Prognosis: The survival rate is currently 90 percent at 5-10 years after initial diagnosis with the latest treatment. The significant reasons for deaths are an infection that secondary to immunosuppression), renal failure and Central Nervous System complicationtoo many long-term trials are underway to determine long-term survival (50 years) in children diagnosed with systemic lupus erythematosus.

Follow up and outcomes: At the time of discharge, the patient's condition was satisfactory. The relatives were informed about the prognosis of the disease, drug therapy and personal hygiene and the importance of taking medication in time. It is also told that they should come after 7 days for routine follow to see the disease outcome.

Discussion:

SLE is essentially the same disease in children as in adolescents with incredibly comparative etiologies, pathogenesis, clinical symptoms and laboratory diagnosis. Nevertheless, because of the effect of the disorder and its treatment on physical and psychological growth and development, the care of children and adolescents with SLE varies significantly from that of adults.[5] Children typically have more malar rash, severe onset renal disease and higher rates of involvement with other organs than adult SLE. SLE diagnosis are made with the presence of signs and symptoms and investigations. It is triggered by the immune system which erroneously attacks the body's own tissue, leading to inflammation of the skin, joints, kidneys, lungs, nervous system and other organs. Balwani et al had reported a case of lupus having rapidly Progressive Renal Failure. Usually, systemic lupus erythematosus starts to be seen in adolescents average age is 12. It's rarely seen with children before age 5. It is more common among females and some ethnic groups, such as African-American, Hispanic, Southeast Asian and Native American.[7] Pediatric onset of systemic lupus erythematosus (SLE) is not rare and the ratio between female and male varies. Pediatric SLE patients undergo more serious disease at the onset, higher organ involvement rates and more rapid clinical progress than adults. There is no known specific cause for SLE. This is assumed to be a combination of many factors. This may involve the environment (e.g., reactions to a virus, sun or drug reaction), hormones (puberty), and/or hereditary predisposition to an overactive immune system. Nobody knows how to prevent it because the origin of SLE is unknown. Taking calcium and vitamin D will reduce the risk of osteoporosis.

Strength: An 11 years old female patient tolerates all medication and responded well within 7 days to the therapeutic treatment.

Informed consent: Before taking this case, information was given to the patient and their relatives and informed consent was obtained from the patient as well as relatives.

Conclusion

The systemic lupus erythematosus (SLE) is an autoimmune disease affecting different parts of the body. Lupus flares can be reduced by avoiding exposure to the sun (wearing strong sunscreen, hats, long-sleeved shirtson sunny days), getting adequate sleep and taking recommended medicines.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Case Report on Primary Infertility

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Abstract

Introduction: All and sundry has aspiration to become a mother and who wants to become a mom, substance impotent draw up little one is an elegant hurting fact. Several of us disburse fragment of existence to avert haphazard gestations also trust that if she is prepared to bear a child it will occur with miniature trouble. One be prone to ponder that convey cogwheel from gestation safeguard to fertilization and planning of parturition will make one's way in comparably flush and neat way. Therefore, inability to conceive is a big life stressor, which can be caused by otherwise well-adjusted pairs.

Patient History: 26 Years old Mrs. Rajeshree Chaudhari is nulligravida since 2½ year with primary infertility and anxious to conceive. C/O pain in abdomen during menses since 1 year. No irregular menses.

Past History: Client has no history of diabetes, TB, hypertension, Asthma, epilepsy, thyroid disease. She has no other history of hospitalization other than obstetrics. No history of blood transfusion.

Clinical Finding: Pain in abdomen. Ovarian dysfunction causing the egg development to be absent or diminished.

Investigations: Serological Investigations, Sonography, Semen analysis in her Husband and Laparotomy in my client.

Surgical Management: The laparotomy was done and the cyst was removed by surgical intervention.

Medical Management: Patient was treated with antibiotics, antacids, anti- cholinergic, anti inflammatory, IV fluids and analgesics.

Nursing Management: Nurse need to obtain history as prenatal, family and other relevant history. Nurse has to perform primary physical examination and collect other relevant information regarding reports of patients. Give psychological support throughout the counselling. Collect other information about tests, reports and documents. Establish plan of care with family and co-ordinate care with other health care professionals. Maintain privacy and confidentiality of all cases. Ensure follow up and supportive services to individual and family during counselling.

Conclusion: Infertility is an important social and medical problem that affects couples all over the world. Factors both female and male are equally responsible. Evaluation is essential for both partners. Treatment depends on the cause of infertility and can vary from ovulation to surgery to ART. My patient come to the hospital as nulligravida with primary infertility and anxious to conceive. As a treatment, cause of infertility detected and exploratory laparotomy is done as a treatment regimen

Keywords: *Primary infertility, laparotomy, Tumor marker, breathing exercises.*

Introduction

This case is very unique because my patient married before 2½ years. As she belongs to the middle class family, we all know it is very difficult for one women specially who belongs to a middle class family because she has to answer members in her family specially her mother in law. She is nulligravida since 1 ½ years, so the couple have to think about the family planning. The pair are keen to conceive. Barrenness arise to be a crucial well being issue by various cliques. Elevated incidence of the affair magnified its relevance. Large piece of sterility has exist to be associated with environs circumstances as well as in addition earned peril strands. Enormous surrounding situations have stressed necessity to learn unlike seeds of barrenness in single sector. More research now position infertility within broader social contexts and structures of social science, while clinical emphases remain. Methodological issues remain but significant improvements are also apparent.

Clinical findings: Pain in abdomen.

Ovarian dysfunction causing the egg development to be absent or diminished.

1. **Diagnosis:** Pain related incision due to exploratory laprotomy.

Intervention: - Assessed the level of pain and intensity too. – Provided comfortable devices such as pillows. – Given diversional therapy by asking about the daily activities. – Teached deep breathing exercises. – Administered analgesics as per Physician order.

Outcomes: Pain will be reduced to some extent.

2. **Diagnosis:** Fluid volume deficit related to surgical procedure done.

Intervention: - Assessed patient hydration status. – Provided frequent oral and skin care. - Urinary output observed, reviewed (Hb/HCT) serum electrolyte. – Given plenty of oral fluids & assisted in IV infusion.

Outcome: Fluid volume will be restored.

3. **Diagnosis:** Activity intolerance related to incision given.

Intervention: - Assessed patient activity level. – Advised and encouraged to do self care activities like hygienic care. – Paced the activities as per tolerance level. – Advised to take adequate rest and sleep.

Outcome: Daily activities will be restored.

Conclusion: Inability to conceive carries a stigma in many cultures. Infertility can have deep psychological consequences. Being a nurse, we can play a vital role in it by counselling the couple. We can tell them about the various preventive measures and management of infertility.

Introduction: In anthropoids, infecund is an in capacity to obtain parturiency following 1 yr. of lovemaking, unescorted by a genesis including both colleague. So many roots of barrenness incorporating these cathartic arbitration can nursed. 1997 Figures show that around 5% of all heterosexual couples worldwide have a problem with unresolved infertility**.**

However, for at least one year, far more people have been suffering from unintentional childlessness: figures vary from twelve to twenty eight percent. Masculine sterility accounts for twenty – thirty percent of infecund sufferers, whereas twenty–thirty five percent is attributed to woman barrenness and twenty five –forty percent is attributed to joined complications in either parties.In ten-twenty per cent of victims, there is neither reason. Bulk of reason of feminine sterility is ovulatory disorders that are typically evidenced by irregular. Masculine sterilityis probably often caused by insufficiencies in milt and trait of semen is utilized as a proxy compute of masculine fecundity.

Patient Information:

Specific Information:

Patient

Name of the patient: Mrs. Rajeshree Sushant Chadhari

Age: 26 years. Sex: Female

IPD No. – 6051480 OPD No. – 3112397

Address: At Thakur Dada Villlage, Bhiwandi, Mumbai

Education: Graduation (B.A.)

Religion: Hindu (OBC)

Marital Status: Married

Duration of marriage: 2.5 yrs

Date of admission: 29/12/2019 at 12:10 pm

Diagnosis: Primary Infertility

Husband

Name: Sushant Ramdas Chaudhari

Age: 29 yrs Sex: Male

Occupation: Job in private sector

Income: 30,000/-

Presenting Complaints: 26 Years old Mrs. Rajeshree Chaudhari is nulligravida since 2½ year with primary infertility and anxious to conceive.

C/O pain in abdomen during menses since 1 year.

No irregular menses.

Symptoms

- unable to get pregnant
- an inability to stay pregnant
- the impossibility of carrying a pregnancy to a live birth

Medical History: History of pain during menses since 1 year. She has no history of diabetes, TB, hypertension, Asthma, Epilepsy, Thyroid disease. She has no other history of hospitalization other than obstetrics. No history of blood transfusion.

Family History: Mrs. Rajeshree lives in nuclear family with her husband and father-in-law. Name of her father in law is Ramdas Chaudhari, 65 years old, studied upto 4th standard, retired person, no health issues. Her husband name is Sushant Ramdas Chaudhari, 29 years old, graduated one, having job in private sector, no significant health problem. Patient name is Rajeshree Sushant Chaudhari, 26 years old, she is also graduated, homemaker having primary infertility.

Ramdas Sushila Chaudhari

Chaudhari

65 years

Sushant Chaudhari Rajeshree Chaudhari

29 years 26 years

Key:

Male

Death

Patient

Psychosocial History: Mrs. Rajeshree and her family celebrates all the festivals. She believes in God. She maintains good relationship with her neighbor and her family members. She does not have any kind of psychological problem as such. She belongs to middle class family. Earning source is her husband and father in law. Monthly income is about 35- 37,000/- In her house, toilet, bathroom, tap water is available. Also closed drainage system is present.

Past Interventions: There is no specific past interventions that had been done.

Clinical Findings

Physical Examination:

Date: 28/12/2019 Time: 11 am

General appearance-

Body built – Thin

Health- Good Health

Activity- Less active

Mental Status- Patient is conscious and well oriented, patient looks anxious and weak

Head- Normal

Scalp- Clean

Face – Normal

Skin condition- No jaundice, no cyanosis

Eyes –

Eye brow – Normal

Eye lashes- Normal

Eye lids- Normal, no edema, no lesion

Eye ball – Normal

Conjunctiva- No pallor

Sclera – Normal

Cornea and Iris- Normal

Pupil – Normal reaction to light

Lens – Normal

Vision- Normal

Nose

External nares -No crust or discharge; normal

Nostrils – Normal

Ears

External ears- Normal

Hearing- Normal

Mouth

Lips – dryness present

Odour of mouth- Normal, no foul smell

Mucous membrane – Pallor present, no ulcer, no bleeding

Teeth – No dental caries

Neck

Lymph node – Normal

Thyroid gland – Normal

Range of motion – Normal

Chest

Heart sound – Normal, no crept on wheezing

Heart – S_1 S_2 normal

Pulse rate – 96 beats/min, tachypnea

Liver spleen – No spleenomegaly

Upper extrimities

Normal range of motion, no edema

Abdomen

Linea nigra – Absent

Straie gravidarum – Absent

Gastrointestinal system

Loss of appetite

Uterus – feels firm

Urine output – 1500ml, transparent, clear

Lower extrimities

Edema on the ankle or feet absent

General parameters

Height – 155cm

Weight – 52 kg

Vital signs

Temperature – 98.6^0F

Pulse – 96 beats/min

Respiration – 28 beats/min

Blood Pressure – 130/90 mmHg

Clnical Findings: Ovarian dysfunction causing the egg production to be absent or decreased

Historical and Current Information: 26 years old Mrs. Rajeshree is Nulligravida since 2 ½ year with Primary Infertility and is anxious to conceive. C/O pain in abdomen during menses since 1 year. No irregular menses. She had menarche at 15 years of age. Duration of menstruation is 30 days cycle and duration of cycle in days is 4-5 days. Menses is regular, average flow, pain during menses. Last date of menstrual history is 13/12/2019 and Expected date of delivery is 20/09/2020. The couple not using precaution since 1 year.

Diagnostic Testing:

Diagnostic Testing: WBC count is 19.23 UL, while normal range is 4.00-10.00 10^3/UL, so it is raised. Neutrophil level is 16.79 UL, while normal range is 2.00-7.00 10^3/UL, so it is raised. Normal value of neutrocytes is 2.00- 7.00 10^3/UL, while in my patient it is 87.2 %, it is slightly increased. Lymphocytes normal level is 20.0 – 40.0 % and in patient it is 6.5 %, it is decreased one. MCV normal range is 77.0 while in patient it is 80.0-100.0%, slightly decreased. MCH level is 25.4 in patient on other hand normal range is 27.0 – 34.0, so it is slightly decreased. Hemoglobin level is 7gm% and the normal range is 12.0-16.0gm%, so patient is Anemic. Tumor marker CA is 58.71 and the normal range is less than 35.0 unit, so inference is she is having ovarian cancer.

8b. Diagnostic challenges

Semen Analysis:

Test

Physical Examination

Quantity - 2.5 ml

Colour – Opaque, grey

Consistency – Semi- viscous

Self liquification time within abstellience – 3 days

Chemical Examination

Reaction – Alkaline

Fluorescence test – Positive

Microscopic Examination

Sperm count semen – 23.2 millions/ml, 60 -110 millions/ml

Mobility(within ½ hrs) - 78 %

Motility (2 hrs) – 63%

Puscells – 4-6 h.p.f.

RBCs – 1-2/h.p.f.

Epithelial cells – 2-3/h.p.f.

Abnormal sperm – Amorphous head sperm seen.

Sonography

Name- Rajeshree Sushant Chaudhary

Reffered By – Wadia hospital (R – 218)

Date: 24/12/2019

HCG study was performed by Inj. 76% Urograffin using precaustion.

The uterus is normal in size and contour no intrinsic or extrinsic filling defect seen. Uterine margins are normal in course caliber. No evidence of any hydrosalpinx seen. Free peritoneal spill noted bilaterally.

Impression: Uterus and both tubes are normal.

Diagnosis: Primary Infertility

Prognosis: Infertility is an important social and medical problem that affects couples all over the world. Factors both equally responsible are the females and males. For both partners assessment is critical. Treatment depends on the cause of the infertility and can differ between ovulation and surgery and ART. My patient come to the hospital as nulligravida with primary infertility and anxious to conceive. As a treatment, cause of infertility detected and exploratory laparotomy is done as a treatment regimen. and my patient prognosis is good.

Therapeutic Intervention:

Pharmacologic Intervention:

1. Tab. Fasigeph, 500mg, oral route frequency is HS, action is antibiotic, side effects are -Vaginal itching, Nausea, Vomiting, Loss of appetite, Constipation, Diarrhea, Headache, Dizziness, a metallic or bitter taste in your mouth, INDICATION- Anerobic infection, Bacterial septicaemia, Upper & lower respiratory tract infection. CONTRAINDICATION-Hypersensitivity,Blood dyscrasia, First trimesterof pregnancy. Nurses Responsibility is hold back medicine, apprise S/S & Central Nervous System decayed physician, monitor INR/PT frequently, praepostor agglutinoid cast iron extent with coincidingemploy,monitor the toxicity of diphenylhydantoin with coincident IV phenytoin
2. Tab. Restyl, 0.5 mg, route is oral, frequency is HS, action is exact mechanism of action not understood, main sites of action may be limbic system and reticular formation, side effects include CNS-Transient mild drowsiness, sedation, depression, CV-bradycardia, tachycardia, hypertension, hives, rash. GI- constipation, nausea, vomiting, incontinence INDICATION- Anxiety disorders, panic attacks, social phobia, premenstrual syndrome, depression CONTRAINDICATION-Hypersensitivity to benzodiazepines, impaired liver or kidney function. Nurses Responsibility is -Use as a taper dosage gradually.Should not use grapefruit juice for administration. Stop alcohol, sleep – or the counter drugs.
3. Tab. Diamol, 40mg, oral route, frequency is TDS, action is Prevents the formation and accumulation of gas. Side effects are Itching, diarrhea, regurgitation, vomiting, heartburn, constipation. INDICATION-Antigas, bloating,

intestinal disorder, gastrocardiac syndrome. CONTRAINDICATION-Hypersensitivity to simethicone, intestinal obstruction, intestinal perforation. Nurses responsibility is do not exceed recommended dosage, do not take the tablet in an empty stomach, increase of missing dose, use it as soon as you notice.

4. Inj. Avil, 25 mg(2cc), route is IM, Frequency is stat, action is antihistamine with anticholinergic, non steroidal anti- inflammatory drug.Confusion and serious allergic side effects, blurred vision, reaction, irregular heartbeat, urinary retention. INDICATION-allergic reaction, runny nose, itching skin, skin rash, inner ear disorders. CONTRAINDICATION- Do not use if sensitive to any ingredient in this product. Nurses responsibility is take with or soon after food, it can cause drowsiness, which may decrease with time,take only as instructed by the doctor

Surgical Intervention

Laproscopic Findings

Left chaudate cyst 8*8cm identified: Wall pealed and cyst evacuated with tooth forcep grasper. Written consent checked. Patient given lithotomy position with general anesthesia. Parts painted and drapped. Bladder evacuated with k-90 catheter foley's catheter inserted. Sim's speculum inserted and anterior cervical lip held with vulsellum. Hysteroscope introduced. Cyst punched and contents aspirated. Cyst wall retrieved and sent for HPR. Hemofasis checked. Bleeding could not be controlled using bipolar artery forceps from the cyst and ovarian edges. Decision of exploratory laparotomy taken. Laproscope and parts retrieved under ventouse. Skin incision closed with vicryl 2-6. Incision taken, abdomen opened in layers and uterus is identified. Bleeding points traced, bleeding controlled using hemostatic sutured with vicryl no. 1. Right sided endometrious identified 3*3 cm two fibroid cyst 0.5*1 cm identified, 1*2 cm simple cyst identified. Cystectomy done cyst wall enated and sent for HPR. Contents of simple cyst atropoted and sent for fluid cytology. Hemoplasis checked and peripheral wash given. Hemistasis confirmed. Intraperitoneal drain kept. Abdomen closed in layers using vicryl no. 1. Skin closed with vicryl no. 1 subcuticular. Dry sterile disting given. Patient tolerated procedure well. Approximated blood loss was less . 10 PCV given intro- OP.

Preventive Intervention: Most infertility medications are given by injections. We understand that this aspect of fertility treatment causes many couples anxiety. You may want to make an appointment to meet with sister for hands-on injection training. Sister will instruct and demonstrate the proper technique for patient and her partner. Also return demonstration of the technique guided . nurse are committed to providing patient with the skills and confidence patient need to complete this part of treatment.

Self-Care: Women should do something that makes her feel attractive- No matter what messages she may have internalized, dealing with infertility makes her no less of a women. Sehe could be putting herself on her favorite outfit, sprizing on some expensive perfume or cologne, breaking out favorite lipstick.

She should do something tha makes her body feel good- when she is dealing with infertility, her body can feel like a war zone that never quite does what she want it to do. Make up with her body by doing something that makes it feel fantastic. She should go a hike up her favorite nearby hill, she should take a dip in the hot tub.

She should do something that makes her feel erotic-when she is trying to conceive, sometimes sex feels like a means to an end and her pleasure takes a back seat. She should get back in touch with her erotic nature by spending some one-on-one time with herself . she may light some candles, put on some Enya, grab her favorite toy and explore her own body.

She may practice daily affirmations like she should think that she is complete the way she is, she can achieve her goals or she is worthy of love. She may practice yoga or mindfulness, meditation. May she is into adult coloring books or hugging her pet cat under a blanket fort.

Administration of Therapeutic Intervention: Clomiphene citrate persuades rescue of gonadotropins.

Gonadotropin releasing hormone analogs in form similar to natural GnRH, induce massive release of GnRH into circulation.

Gonadotropins –Human gonadotropin menopausal (HMG), which contains equal amounts of FSH and LH.

Bromocriptine – suppress production of prolactin.

Changes in Therapeutic Intervention: There were no specific changes in therapeutic intervention.

Follow up and outcomes

Clinician Patient Assessed Outcomes: There are various outcomes to be performed for the patient with primary infertility.

General and sexual history has to be taken. Visual evaluation and pelvic exam for women.

Fertility evaluation of female partner – evidence of evaluation.

Diagnostic evaluation like Hysterosalpinogram (HSG), Laparoscopy to determine state of uterine cavity.

Assessment of male partner fertility-semen analysis.

Intrauterine insemination

Assisted Reproductive Technology (ART)

ART- Fertilization In Vitro

ART – Gamete Intra-casualty Transfer(GIFT)

ART – Zygote Intra-casualty Transfer (ZIFT)

ART –Intracytoplasmic Injection of Sperms (ICSI)

In my patient, Laparoscopy is performed.

Important Follow-Up Diagnostic and Tolerability Test Results & Intervention Adherence

Semen Analysis: Semen analysis was done in which no any kind of abnormality was found and amorphous head sperm seen in analysis.

Sonography: No evidence of any hydroscopinx seen. Free peritoneal spill noted bilaterally.

Uterus and both tubes are normal. No intrinsic and extrinsic filling defect seen.

Laparoscopic Findings: Left chaudate cyst 8*8 cm identified, which was corrected by laparoscopy, cystectomy done, cyst wall enatedand sent for HPR, contents of simple cyst atropoted and sent for fluid cytology.

Adverse and Unanticipated Events: There were no adverse and unanticipated events as such found during follow up.

Discussion

A scientific discussion of the strengths and limitations associated with this case report: As this is a very unique case and most of the couple nowadays face with infertility problem, it is very important to study the case in every aspect. Primary infertility can be treated now, many medical and also surgical interventions are available for the treatment. Now, in many hospitals well equipped ART Centers and also In – Vitro Fertilization Centers are available.

Research related to prolificacy with CAH among virile is disputed. The fertility in CAH males was assumed to be natural and the therapy might not be appropriate for fertility[1-4]. 18 out of 20 people in that patient series find apparent natural fertility, demonstrated by paternity and regular sperm counts. The authors also identified two untreated patients and two patients who had not received care many years before assessment, all of whom beared 1-3 babies. On physiological assessment we had natural claret levels of gonadotropin and T. Accompanied recognition of hellenic Congenital and Hyperplasia and glucocorticoid therapy prolificacy estimate was decreased among population of all adult Finnish males. The reason for that is the lack of sperm tests. It remained available, but considered trivial emotional alteration to long term sickness or inadequate replacement of glucocorticoids. Otherside, lofty prevalence of cullions deformities and irregular milt sum up has been presently identified in category of thirty males (age 15 yr) who have been regularly supervised at one institution. Nine out of 16 fertility patients tested using either milt add up; seven men had stock fecundity. Fecundity issues were either cause of subduing of the hypothalamicpituitary-cullions axis with subsequent tiny tests as well as reduced development of spermatozoa, or due to uprooted bullocks suprarenal reposes, which encounter suit overgrowth under beneath dreadful Adrenocorticotropic hormone invigoration. This concludes that stated buds sequentially enlarge either destroy cullions substrate, evolving in low T development, sterility too. Lately, Murphy et al. identified client along oligozoospermia cause of obstacles by suprarenal recline stuff, dicisively located at the funicle of the testes.Tumors of suprarenal recline, also linked to barrenness. The distinction between the two pathogenetic pathways can be made by computing agglutinin gonadotropin degree, which are even elevated for testicular rest tumors.

Discussion of the relevant medical literature with references: In one case the very high circulating concentrations of T.(5) showed serum gonadotropin repression. It was also associated with increased serum estrogens, which originated from

Straightly amid adrenal transference of the suprarenal testoids also from acting through gray matter flat to impede Gonadotropin Releasing Hormone expel. Its agglutinogen estriol and T in our patient were typical. Regrettably, we haven't tested serum estronol. Cyclopental may be extra effective stilboestrol forerunner relative T and our patient has developed it in high quantities. It appears probable that an increase in the production of hypothalamic estrogen could occur in male CAH patients cause of confined cyclopentanal sweeten to estriol and be responsible for a hypothalamic release inhibition of GnRH(6). Following the use of antiestrogen clomiphene (6), the conjectureexpanded flush Follicle Stimulaing Hormone and Leutinizing Hormone. It has been shown recently that mestranol chiefly governers of Follicle Stimulating Hormone excretion in masculine in terms of sex steroid input and that T primarily strives its comentary sequel on FSH by aromatizing to oestriol. Consequently, in the present case, the bit part of postulated enlarge in regional hexestrol synthesis comprise put up longer to FSH repression than to the Leutinizing Hormone emission. Bearing note that he generally had go round T concentration, Follicle Stimulating Hormone lack solitary may have been the key explanation for his sterility.

The scientific rationale for any conclusions: Infertility among young adults materialize on the rebel in US and growing aggregate of pompous adults of all ages are searching for solutions. In vitro fertilization tactic also their dissimilarities append to extra conventional operative and therapeutic therapies for this disarray since 1978. However, modern strategies for generative non-observance own uplifted communal and executive bother over attributed benchmark, communal surveillance and commercial in quickness of their maturing and accretion. Arguments built beside the discrete health centres regarding their achievement ratio are therefore unbolted to exploitation[7,8].

Conclusion

Infertility is a significant social and medical issue that affects couples all over the world. Equally responsible are both the female and male factors. Assessment of both partners is vital. Treatment depends on the infertility cause and ranges from ovulation to surgery to ART. My patient come to the hospital as nulligravida with primary infertility and anxious to conceive. As a treatment, cause of infertility detected and exploratory laparotomy is done as a treatment regimen.

Patient perspective: Patient was telling that she is nulligravida since 2.5 years. So she is eager to conceive. She had not taken any kind of treatment brfore but now she is anxious to conceive. As she is not having irregular menses, or any other problem, she is anxious to know the exact problem and wanted to treat it.

She came to the hospital. All the tests had been done like blood tests, sonography, semen analysis of her husband and laparotomy was also done. Blood tests, sonography and semen analysis were normal. Only in laparoscopic findings, left chaudate cyst 8*8 cm was identified that was corrected by surgical intervention. So her prognosis is good and now she is satisfied with the treatment she received.

The patient did not give any informed consent.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Overview of Sanitizer Usability in COVID-19 Pandemic Scenario

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Abstract

Hand cleanliness is the most significant thing as it might be tainted effectively from direct contact with microorganisms. In the circumstances of COVID-19, its essential to intrude on the chain of the infection with the use of appropriate hand cleaner. The motto is accomplished by keeping up excellent hand cleanliness in emergency clinic settings and indoor and outdoor patient departments. The compelling hand purification relies upon the utilization of legitimate hand cleaning operators defined in different sorts and structures, for example, alcohol-based hand sanitizer, antimicrobial cleansers and so on. However, frequent utilization of sanitizer or ill-advised usage of antimicrobial operator prompts show a reaction to human well-being. This orderly audit corresponded with the information accessible in the literature search. It is exploring available hand disinfectants and their effectiveness just as the antagonistic effects and there are some suggestions to get the upgrade for the human well-being.

Keywords: *Alcohol, Antimicrobial, Hygiene, Sanitizer.*

Introduction

It is antimicrobial, which irreversibly inactivates and kill near about 99.9% of bacteria, viruses and fungi present on a surface. It is available in the form of liquid, gel, or foam. There are two types of sanitizers i.e., Alcohol-based and Alcohol-free. Alcohol-based sanitizers may contain various types of alcohol. It applied on the surface to irreversibly inactivates or kill bacteria, viruses and fungi present on a surface. It acts quickly and effectively on microbes and destroys it without needing water. But its effect is for short duration and not so active on protozoa, bacterial spores and non-lipophilic viruses.[1] Alcohol-free sanitizer made from chemicals which has antiseptic properties. It is safer to use, especially among children, because they are not inflammable and prepared at low concentrations.[2,4]

Alcohol-based sanitizer contains ethanol, isopropanol, N-propanol, hydrogen peroxide, other type sanitizer contains chlorhexidine, chloroxylenol, iodine, iodophors, quaternary ammonium compounds, triclosan[5,7]

Alcohol-based sanitizer contains either n-propanol, ethanol, or isopropanol. The optimum bactericidal activity required a concentration of 60 to 95 % of alcohol[8,9]. It dissolves the lipid membranes of microbes and also deprived the natural qualities of the proteins in it. They have broad-spectrum antimicrobial activity against enveloped viruses (HIV,herpes simplex virus,etc.), fungi, bacteria (Mycobacterium tuberculosis,etc.). Routine use of alcohol-based sanitizer manifests dryness, redness, cracks, etc. In rare conditions, it causes blisters that cause itch and pain. Hydrogen peroxide

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acts as antiseptic and comes in different concentrations according to the purpose of its uses. 3% for household, 6 to 10% for hair bleaching, 90% for industrial purposes. It is effective against bacterial spores found in raw materials were ethanol, isopropanol, or n-propanol based sanitizer ineffective. They have side effects as eye irritation, vomiting, bleaching of the hair or skin. It required handling with caution during production due to its corrosive nature. [10]

Chlorhexidine destroyed cytoplasmic membranes,which leads to precipitation of cell contents.[11] It is highly effective against gram-positive bacteria and show moderate action against gram-negative bacteria and enveloped viruses[12]. It is cationic and avoids using soap and cream, which contain anionic agents as they cause inactivation and reduce efficacy [13]. Chlorhexidine gluconate 0.12% have antiviral activity against the coronavirus[14] and they are having side effects like redness, itching, severe burning or irritation.

Chloroxylenolis used as an antimicrobial agent in soap or as a preservative in cosmetics. It can alter cell wall synthesis in microbes by deactivating enzyme systems. It effectively destroys bacteria and enveloped viruses but is less effective against Pseudomonas aeruginosa[15,16] and has side effects such as skin rash, angioedema, itching and redness.

Iodine or Iodophorsis an effective antiseptic. It can penetrate the cell wall of microbes and impaired the synthesis of cellular components. It causes discoloration and irritation of skin [17]. It has polyvinyl pyrrolidone, which acts as a polymer carrier and improves the iodine solubility with sustained release. So,the irritation of skin lessened[18].

Quaternary ammonium is related to nitrogen atom i.e., acetylperidium chloride, benzethonium chloride, benzalkonium chloride. Thus, causing leakage of the constituents' act by adsorbing to the cytoplasmic membrane. They are effective against lipophilic viruses and gram-positive bacteria.[19] but may irritate the skin.

Triclosanis bacteriostatic at low concentration, but with high intensity, it destroys bacterial membranes. It is more activelywork on gram-positive bacteria. Its efficacy affected by pH, ionic nature of individual skin formulations and use of emollients but causes dermatitis and allergic reaction.[20].

Worldwide use of sanitizer: The development of the COVID-19 pandemic is spread worldwide and it is generally concerned for well-being and hence prompted for broad utilization of disinfectants[21,22]. COVID-19 is an irresistible illness brought about by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), which survive on various surfaces around nine days[23,24]. Transmission of SARS-CoV-2 is conceivable as vaporized and fomite. The infection can stay feasible and irresistible in pressurized canned products for a considerable length of time and on surfaces up to a few days[25]. Consequently, it is essential to interfere with the chain of transmission through the disconnection of communicable things and the intervention of disease controlling instruments[26]. Regular use of face mask and shield covers, suitable hand cleaners are significant to avoid direct contact with patients.

By any means, respiratory droplets encourage the transmission and increase the chances of infection[27–29]. The 2003 extremely intense SAR Soutbreak was brought about by a novel human coronavirus that survived 24 to 72 hours[30]. The investigations on this episode indicated that efficient hand washing and cleanliness at offices decreased transmission [31]. Consequently, the government advanced and energized hand cleanliness through handwashing or utilizing hand sanitizer [32]. Hand disinfectants are accessible in different kinds and structures, such as alcohol-based hand sanitizers, hostile to microbial cleansers and so forth utilized in medical clinic settings. WHO recommended alcohol-based hand sanitizer on account of fast activity and a wide range of antimicrobial action offering insurance against microbes and infections[27,33,37].

Effect of sanitizer: The most generally revealed skin responses with the utilization of alcohol-based sanitizer are aggravation contact dermatitis and unfavorable susceptible contact dermatitis[38,39]. The side effects of aggravation contact dermatitis can go from gentle to crippling with signs like dryness, erythema, pruritus and dying, if severe. Concerning unfavorably susceptible contact dermatitis, the indications may restrict extreme types of contact dermatitis in case of respiratory trouble or other side effects [40,41]. Occasionally, it might not be so easy to recognize the kind of dermatitis because of the cover and likenesses of side effects. Hand cleanliness items, for example, sanitizer and cleansers can be harming to the skin through a few instruments: change of intercellular lipids, denaturation of the layer corneum proteins, decrease of layer corneum water-restricting limit and decline in corneocyte union[42,43]. The greatest

worry is the consumption of the lipid obstruction, particularly with repeateduse of lipid-emulsifying cleansers and lipid-dissolving alcohols for progressively visiting colonization of microscopic organisms, might beentered into the layers of the skin and tends to change the flora[44–46]. Among the alcohol-based sanitizers, ethanol has minimumskin-damaging properties [47]. Alcohol-based sanitizershad hand drying property, which can break the skin[48]. Then again, unfavorably susceptible responses towards specific operators are noted with chlorhexidine, iodophors, chloroxylenol, triclosan and alcohols. People with hypersensitive reactions to alcohol-based disinfectants may have genuine sensitivity to alcohol or sensitivity to aldehyde metabolite or different chemical aroma, parabens and benzyl alcohol

Solutions to minimize side effects of sanitizer: The antagonistic effects brought about by sanitizer can be handily anticipated by distinguishing with the triggering and countering factor with suitable strategies like saturating skin after hand sanitation, choosing items with a less disturbing specialist and evading tendencies that may cause or bother skin aggravation. At the point when continuous hand purging is regular, for example, among social insurance laborers, it is desirable over select items having a decent harmony between effectivity, well-being and similarity with various types of skin. Only worries about drying effects of alcohol and similar effects might delay the acknowledgment and extreme utilization of sanitizers. Subsequently, to decrease this issue, alcohol-based sanitizer containing emollients can be utilized. Considering the temperature and moistness as significant supporters for dermatitis, the maintenance of skin moistness is more extended in tropical nations and areas with relative humidity compared tocold and dry conditions. This perspective requires a changing need of emollients concerning separate natural terms and atmospheres as indicated by topographical regions. A few people who are regularly wearing gloves, are prone to develop dry skin condition. So, these persons recommend using lotions containing fats and oils to upgrade skin moisture.

Discussion

Appropriate hand cleaning with alcohol-based sanitizer is one of the standard procedures to forestall immediate or unusual spread of the COVID-19 as it lessens the quantity of virus infection on dirty hands. As per literature search, five occasions are identified where hand cleanliness should be monitored: when having direct contact with patients, before taking care of obtrusive gadgets for understanding consideration, after contact with body fluids, waste or discharges, before, during and after contact with clinical machines inside vicinity and beginning of any procedure where aseptic precautions are required. The researchers suggested that washing the hands with cleanser at whatever point is conceivable because handwashing diminishes the measures of a wide range of germs and synthetics on sides. On the off chance that cleanser and water are not accessible, utilization of a hand sanitizer at any event ethanol or isopropyl alcohol deactivates infections like COVID-19. The time to time handwashing can precisely expel the microbes; however, the expulsion of resident pathogenesis is progressively successful when cleanliness is preferably done through competent microbial experts, as per WHO health policy suggestions length for the whole handwash system ranges between 20 to 40 seconds with the advance method. Nearly, sanitizer with 60% alcohol is more effective in devastating the microorganisms than other handwash. The alcohol-based handwash may not be comparably effective in case of messy or oily hands, so handwashing with cleanser and water is favored under certain conditions.

Conclusions

Legitimate hand cleanliness is one of the standard disease control methodologies as it can verifiably bring down the probability of immediate transmissions of microorganisms. The utilization of alcohol-based hand sanitizers is becoming increasingly regular because of their quick activity and efficiency in slaughtering microbes. For the most part, handwashing utilizing cleanser and water isn't useful.

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Source of Funding: Self.

Conflict of Interest: Nil.

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To Assess the Effectiveness of Planned Teaching Regarding Domestic Accident among the Elderly People

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Abstract

Background: In most developed countries accidents at home are a significant public health problem since they are a common cause of injury and death. Also, because a large portion of these incidents includes the elderly, it is important to determine their social effects in this community. Most incidents will result in a bruise or scratch, but elderly people falling will cause even more severe injuries like hip fractures, head trauma or even death.

Aim: The study aimed to explore the knowledge regarding domestic accidents among elderly people.

Objective:

1. To assess the knowledge regarding domestic accidents among elderly people.
2. To evaluate the effectiveness of planned teaching regarding domestic accidents among elderly people.
3. To correlate the findings with selected demographic variables.

Method and Material: This was a Cross-sectional research design. The study was undertaken in selected rural areas of Wardha district. Sample size was 100 and Convenient sampling technique was followed.

Results: Majority of samples 44% of the age 60-64 years, 54% of them were male, 62% were married people, 75% were educated up to primary education, 62% of them belong to the farmer, 82% of the people living in a Pakka house, 90% people it is known their occurrence of domestic accident and 27% got information from the newspaper.

Keywords: *Domestic accidents, elderly, knowledge.*

Introduction

The domestic accident is an incident that happens at home or in its immediate vicinity and more commonly, all incidents not related to travel, automobiles and sports. Can accident in the home inflict detrimental effects on the physical and mental health of the victim, loss in earning ability and efficiency. Especially children are more prone to domestic injuries. In various age groups the frequency of injuries is different due to their vision failure, slow movements, osteoporosis and osteoarthritis. Women who are having pregnancy, anger, anxiety or stress often suffer from burn, suffocation, electrocution and cut injury. Domestic accidents are a worldwide public health issue.(1) Public health authorities used the term 'Modern Day Epidemic' for accidents. Though majority of the accidents and associated morbidity & mortality information about their distribution, pattern, predisposing factors are hardly known to the epidemiologists. Most accident-related research concentrates on The few cross-sectional surveys that concentrate on rural regions including INDIA, indicating that domestic injuries present a possible danger to the

public health field [2]. Most incidents will result in a bruise or scratch, but elderly people falling will cause even more severe injuries like hip fractures, head trauma or even death. In 2010 the CDC reported that throughout emergency rooms, 2.3 million nonfatal fall injuries to older adults were treated. [3]

It was found that the rate of domestic accidents was 1.7%. The most frequent reported accident was a fall. Falls occurring were found to be associated with aging and overcrowding. Many noted incidents include burns, scalds, electrocution, explosions and accidental poisoning. Accidents were identified in severe age groups to a substantially higher proportion. Lower accident rates occurred during morning and evening hours.[4]

Material and Method

Plan for Data Analysis: Data were analyzed using inferential descriptive statistics.

Major Findings: The analysis and interpretation of the data are organized under 3 sections as per the objective of the study.

Table No. 1: Distribution of samples with regards to selected demographic variables (n =100)

Demographic variable	Frequency	Percentage (%)
Age (Years)		
60-64 yrs	44	44.0%
65-70 yrs	31	31.0%
71-75 yrs	13	13.0%
Above 75 yrs	12	12.0%
Gender		
Male	54	54.0%
Female	46	46.0%
Marital Status		
Married	62	62.0%
Unmarried	9	9.0%
Widow	22	22.0%
Widower	7	7.0%
Educational Status		
Primary education	75	75.0%
Secondary education	15	15.0%
Higher secondary education	10	10.0%
Graduate	0	0.0%
Post Graduate and above	0	0.0%
Occupation		
Farmer	62	62.0%
Labour	28	28.0%
Private job	6	6.0%
Government job	4	4.0%
Type of house		
Kaccha house	18	18.0%
Pakka house	82	82.0%
Occurrence of domestic accidents		
Yes	90	90.0%
No	10	10.0%
Source of information		
Friends	25	25.0%
Mass media	26	26.0%
Book	22	22.0%
Newspaper	27	27.0%

Table 1. Show percentage-wise distribution of sample with regards to age, gender, marital status, educational status, occupation, area, type of house, the occurrence of domestic accidents and source of information regarding the prevention of domestic accidents among elderly people.

- Distribution of elderly people according to age in years was 44% (60-64) years, 31% (65-70) year, 13% (71-75) year and 12% (75)year of them belong to age.
- Distribution of elderly people according to their gender shown that 54% of them were male and 46% were female
- Distribution of elderly people according to their marital status shown that 62% were married people, 9% were unmarried people, 22% were widow people and7% were widowed people.
- Distribution of elderly people according to their educational qualification shown that 75% were educated up to primary education, 15% were educated up to secondary education and 10% were educated up to higher secondary education.
- Distribution of elderly people according to their occupational status shown that 62% of them belong

to farmers, 28% of them belong to labour, 6% of them belong to the private job and 4% of them belong to a government job.

- Distribution of elderly people according to the type of house shown that 18% of the elderly people were living in a Kaccha house and 82% of the people living in a Pakka house.
- Distribution of elderly people according to their occurrence of domestic accidents shown that in 90% of people it is known and 10% not known.
- Distribution of elderly people according to their source of information shown that 25% people were getting information from friends, 26% people were getting information from mass media, 22% people were getting information from books and 27% people were get information from

Assessment of existing knowledge regarding prevention of domestic accidents among elderly people:

Table No. 2: Assessment of existing knowledge regarding prevention of domestic accident among elderly people (n-100)

Level of knowledge score	Score range	Percentage score	Pre-Test	
			Frequency	Percentage
Poor	0-4	0-20%	3	3%
Average	5-8	21-40 %	60	60%
Good	9-12	41-60%	35	35%
Very good	13-16	61-80%	2	2%
Excellence	17-20	81-100%	0	0%
Minimum score	3			
Maximum score	14			
Mean score	7.94± 2.024			
Mean %	39.7%			

Table No. 2 deals with the existing knowledge regarding the prevention of domestic accidents among elderly people. The level of knowledge is divided under poor, average, good, very good and excellent.

The samples show that 3(3%) of them had a poor level of knowledge score, 60(60%) of them had an average level of knowledge score, 35(35%) of them had a good level of knowledge score, 2(2%) of them had a very good level of knowledge score and none of them had an excellent level of knowledge score. The minimum score was 3 and the maximum score was 14, the mean score was 7.94± 2.024 with a mean percentage score of 39.7%.

Assessment of post test knowledge regarding prevention of domestic accident among elderly people:

Table No. 3: Assessment of post-test knowledge regarding prevention of among domestic accident elderly people (n=100)

Level of knowledge score	Score range	Percentage score	Post Test	
			Frequency	Percentage
Poor	0-4	0-20%	0	0%
Average	5-8	21-40%	0	0%

Level of knowledge score	Score range	Percentage score	Post Test	
			Frequency	Percentage
Good	9-12	41-60%	0	0%
Very Good	13-16	61-80%	12	12%
Excellent	17-20	81-100%	88	88%
Minimum score	14			
Maximum score	20			
Mean score	18.13 ± 1.440			
Mean %	90.65%			

Table No. 3 Deals with evaluating post-test awareness of domestic accident prevention among elderly people. The level of knowledge is divided under following the hearing of poor, average, good, very good and excellent.

The samples had a poor, average and a good level of knowledge score, 12(12%)of them had a very good level of knowledge and 88(88%) had an excellent level of knowledge score respectively. The minimum score was 14 and the maximum score was 20, the mean score was 18.13±1.440 with a mean percentage score of 90.65%.

The effectiveness of structured teaching regarding prevention of domestic accident among elderly people:

Table 4: Percentage-wise distribution of Effectiveness of planned teaching regarding the prevention of domestic accidents among elderly people. (n=100)

Tests	Mean	SD	't'-value	Degree of freedom	p-value	Significant
Pre-Test	7.94	2.024	42.792	99	0.00	S, $p<0.05$

Table No. 4 shows that there is a significant difference between pretest and post-test knowledge scores interpreting domestic accidents among planned teaching on knowledge regarding the prevention of domestic accidents among elderly people. The mean value of the pre-test is 7.94 and the post-test is 18.13 and the standard deviation values of the pre-test are 2.024 and the post-test is 1.440. The calculated t-value is 42.792 and tabulated p-value is 1.98. Therefore, the proposed teaching about the prevention of domestic accidents among elderly people is statistically interpreted. Thus, the H_1 is accepted and H_0 is rejected in this study.

Discussion

The results of the study were explored about the above objectives along with the outcomes of the other research in this section. The present study was undertaken to assess the effectiveness of planned teaching regarding domestic accidents among elderly people in the elected area of Sewagram. Increasing significance is attached to the study of domestic injuries, which involve incidents at home and in institutions. Examination of the causes of injuries at home helps the preparation of ways to avoid them. The principal responsibility lies with the general practitioners and their colleagues in the primary health care team[5].

The study population was 5,682 among them the majority were in the age group of 0-15 years (31.3%), gender-wise 51.6% were males. The total no of accidents found was 115, so the prevalence of domestic accidents for all age groups was 2.0%. Similar findings were observed by Haniff et al. Devroey et al. reported an incidence of 2.7% in their study done in Belgium. In contrast to this in a study in Karnataka they found a prevalence of 9.6% in the rural community. The most common accidents reported were injuries from sharp or

pointed instruments (32.2%) followed by falls (26.9%). Similar findings were observed in the LARES survey of the WHO regional office for Europe.[5]

Conclusion

This research leads to the following result, after a thorough review. Since the implementation of the teaching curriculum, there was a substantial increase in the awareness of subjects. To find the training program's effectiveness. To find the efficacy of the student's expected teaching test was applied and the value of 't' was determined, the post-test score was slightly higher at 0.05 than the pre-test score. Thus it was concluded that the planned home accident teaching program among elderly people was found to be successful. Demographic variables did not show a major role in influencing the score of knowledge among elderly people on the pre-test and post-test. Therefore, based on the above-mentioned observations, it was certainly concluded that such a program enabled the elderly to develop their domestic accident awareness.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Screening of Adolescents for Selected Mental Illness and to Identify its Risk Factors

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Abstract

Background: Mental health can be disturbed among adolescents at any time. One in five adolescents are affected by mental illness age between 14-24 years. 10-20% of adolescentsis affected by mental illness by the age of twenty all over the World.

Aims: The study aims to Screening of adolescents for schizophrenia and depression and to identify its risk factors.

Objectives: 1. To identify the risk factors of specific mental illness among adolescents. 2. To screen and identify the adolescents for depression through BECK depression scale. 3. To screen and identify the adolescents for neurotic disorders through Brief psychiatric rating scale. 4. To associate the risk factors of specific mental illness with their demographic variables.

Method: Research approach: Exploratory approach, Research design: Descriptive design.

Sample: Adolescents aged between 10-20 years. Sample size: 300. Sampling technique: Non probability Purposive sampling Tools: BECK depression inventory to identify depression, Brief psychiatric rating scale: To identify for neurotic disorders. Setting of study: Selected Schools of Wardha district.

Results: The main risk factors identified are: Most of the adolescents was having enmeshment relationship towards their parents and 2% were having disengagement relationship; 15.67% family members of adolescents were diagnosed with psychiatric illnesses and on psychiatric treatment. In BECK depression inventory, most of the adolescents were adopted everyday ups and down are considered normal whereas 20% of them were having moderate depression.

Conclusion: More interventions are required to deal with mental disorders among adolescents.

Keywords: *Depression, Mental illness, Enmeshment, Disengagement, Neurotic disorders.*

Introduction

Mental disorders are a major public health concern. Lifetime prevalence of mental disorders among adults around 12.2%-48.6% According to the 2001 World Health Report, most of the adolescents are going to be affected by mental disorders and 50% of the mental disorders will start by 14 years of old. According to World Health Organization reports many suicide happening always associated with mental illness and they from low and middle income countries.(1) Screening of mental disorders among adolescents, school and colleges can be a more suitable place because they can be more free to ventilate everything to the investigator and the identification of mental illness will be easy and feasible to conduct the study.(2) The investigator have more insight because the adolescents are the future citizens, they should be mentally healthy concern to prevent mental disorders and suicidal thoughts and abnormal behaviors. (3) The adolescents in India are affected by depression, anxiety disorders, bipolar disorders, schizophrenia and

eating disorders. Depression and eating disorders are higher among females than males.[4] Mental illness is one of the main causes where the students are affected by social maladjustment, academic stress which leads to suicide in the universities.[5]

The reason for mood disorders among children and adolescents are due to chronic disturbance in sleep, genetic factors like microbiome and various types of stress in the life and environment. [6] Therefore attention towards adolescents is emergent factors to identify the risk factors and to prevent mental illness and early intervention is required.[7]

Behavioral symptoms identified in mood disorders are mainly depressed mood, anxious behavior increased energetic behavior, abnormal perception and suicidal/ homicidal ideation.[8] The adolescents suffering from severe distress and expressed suicidal ideation immediate intervention is required to prevent suicide.[9] The community health workers should educate the students at school and colleges and to prevent mental health problems to occur.[10]

Anxiety had a significant effect on adolescents developmental status where they pass through one stage to another.[11] One of the risk factors in adolescents is abnormal parental relationship towards their children.[12] Another risk factors of mental illness among adolescents is academic pressure with increased amount of curriculum and expectation from the teachers and parents.[13]

Method and Materials

The researcher has adopted quantitative research design to identify risk factors of mental illness and screening of adolescents for depression and neurotic disorders. It provides the best framework for the study. The setting for this study was in Wardha district, at school. Criteria were: Adolescents in the age group of 10 to 20 years, both males and females, who were cooperative and willing to participate in the study. The adolescents who was suffering from any systemic diseases, physically and mentally challenged was excluded. Materials used by the investigator was structured questionnaire to identify risk factors, BECK depression inventory in Marathi language to identify the level of depression and brief psychiatric rating scale to identify neuropsychiatric disorder. The investigator explained about the nature and purpose of study to the participants. A written consent was obtained from the participants prior to their recruitment in the study. They were assured about their confidentiality of the data.

Ethical Considerations: The permission to conduct the study was obtained from Institutional Ethical Committee Board. First, Investigator approached the school principal to gather them in a designated place. The nature of the visit was explained to the principal and teachers and an informal gathering was arranged. To begin with, the purpose and objectives were announced and any doubts of the participants were clarified. The participants were assured about the confidentiality of their information and its applications for this study only. A written consent of participation in the study, protecting their identity was taken from each participant. Both local vernacular Marathi and Hindi languages were chosen as the medium of conversational exchange and information.

Data Extraction: References are collected from PUBMED, Cochrane database, CINAHL and etc.

Results

Table 1: Percentage wise distribution of adolescents according to their demographic characteristics (n=300)

Demographic Variables	Frequency	Percentage (%)
Age (yrs.)		
10-12	2	0.7
13-15	93	31
16-17	87	29
18-20	118	39.3
Gender		
Male	89	29.7
Female	211	70.3
Education		
Primary	5	1.7
Secondary	90	30
Higher Secondary	89	29.6
Under Graduate	116	38.7
Family Income (Rs)		
3000-5000 Rs	61	20.3
5001-7000 Rs	42	14
7001-9000 Rs	54	18
>9001 Rs	143	47.7
Religion		
Hindu	231	77

Demographic Variables	Frequency	Percentage (%)
Christian	9	3
Muslim	7	2.3
Buddhist	51	17.1
Others	2	0.6
Type of family		
Nuclear	248	82.7
Single Parent	8	2.7
Extended	10	3.3
Step Family	4	1.3
Grand Parent Family	30	10
Residence		
Urban	157	52.3
Rural	114	38
Semi Urban	29	9.7

The above table no 1 shows that distribution of adolescents according to their age was 0.7% of adolescents are belongs to 10-12 years of age, 31% of adolescents were belongs to 13-15 years of age,29% of adolescents was belonging to 16-17 years of age and 39.3% of adolescents were belongs to 18-20 years of age group.

Distribution of adolescents according to their gender reveals that most of the participants were females and it was 70.3% and 29.7% were males.

Distribution of adolescents according to their educational qualification shows that 38.7% adolescents were undergraduate, 29.6% of adolescents were having higher secondary education, 30% of them had secondary education and only 1.7% were having primary education.

Distribution of adolescents according to their family income shows that 47.7% were having more than 9001 Rs, 20.3% were having family income between 3001-5000 Rs, 18% were having family income between 7001-9000 Rs and 14% of adolescents were having income between 5001-7000 Rs.

Distribution of adolescents according to their religion shows that 77% of adolescents was Hindu, 17.1% of adolescents belongs to Buddhist, 3%of the adolescents belongs to Christian, 2.3% of them were Muslim and 0.6% was of other religion.

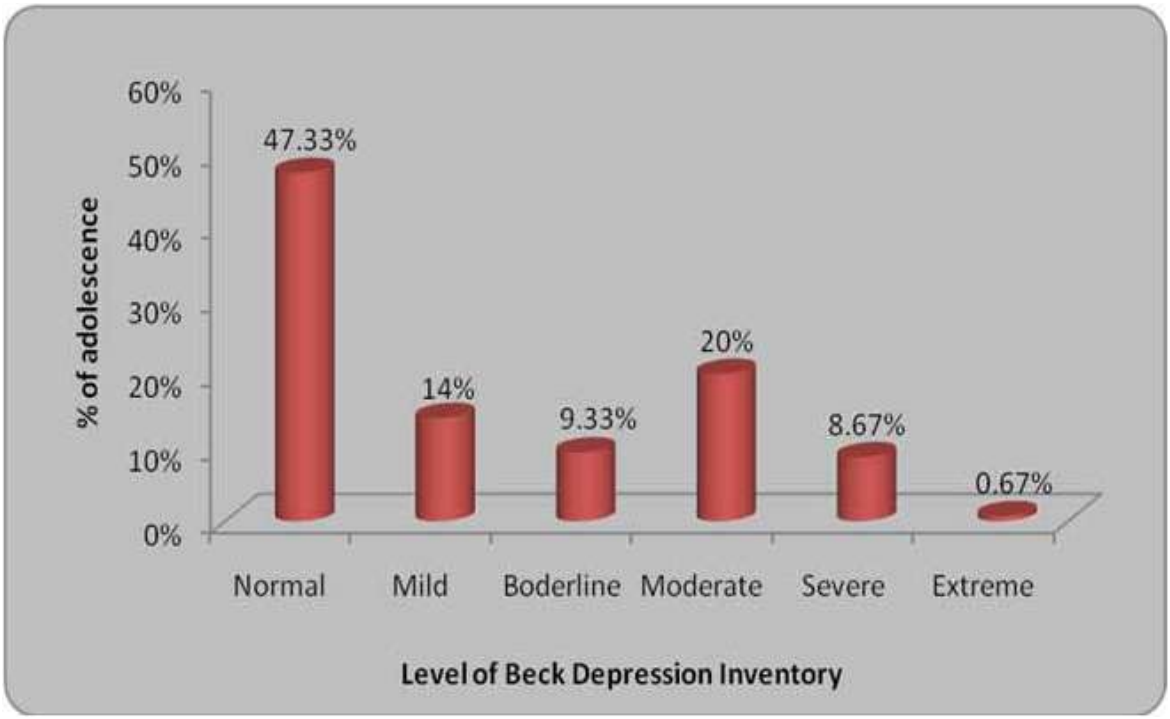
Distribution of adolescents according to their type of family reveals that 82.7% of adolescents was living with nuclear family, 10% were living with grandparent family, 3.3% were living extended family, 2.7% of adolescents had single parent family and 1.3% was having step family.

Distribution of adolescents according to their residence 52.3% of adolescents living in urban area, 38% of adolescents living in rural area and 9.7% of adolescents living in semi urban.

Table 2: Identified risk factors among adolescents of mental illness

Risk factors	Frequency	Percentage
Family history of mental illness	47	15.7
History of Addiction	120	40
History of accident in the past	32	10.7
Special attachments towards the parents (Enmeshment)	287	71
Hatred relationship towards the parents (Disengagement)	06	2
Prenatal, natal and postnatal exposure of viral infections	09	3
Pathological family	55	18
Gambling behavior	95	31.6

Table 2 reveals that 15.7% of adolescents family members were suffering from mental illness, 40% of adolescents were addicted to alcohol or tobacco, 10.7% of them met with an accident, 71% adolescents were having special attachment towards their parents and 2% of adolescents were having hatred relationship towards their parents 3% of adolescents mothers were suffered from viral infections during prenatal, natal and postnatal, 18% of the adolescents were having pathological family like violent, lack of resources etc and 31.6% of them were having gambling behavior .



Graph 1: Screening of depression among adolescents through BECK depression inventory.

The above graph shows that 47.33% of the adolescent had normal level of beck depression inventory, 14% had mild, 9.33% had borderline, 20% had moderate, 8.67% had severe and 0.67% had extreme level of beck depression inventory. The minimum beck depression inventory score was 0 and the maximum score was 42, the mean score was 11.75±11.03.

Table 3: Screening of neurotic disorders through Brief psychiatric rating scale. (n=300)

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
Somatic concern	300	1.00	7.00	1.99	1.37	1.616	0.141
Anxiety	300	1.00	7.00	2.54	1.52389	0.761	0.141
Depression	300	1.00	7.00	2.12	1.45523	1.345	0.141
Suicidality	300	1.00	7.00	1.49	1.15226	2.888	0.141
Guilty	300	1.00	7.00	2.15	1.53547	1.448	0.141
Hostility	300	1.00	7.00	1.84	1.23538	1.593	0.141
Elated mood	300	1.00	7.00	2.00	1.37828	1.744	0.141
Grandiosity	300	1.00	7.00	1.86	1.39291	1.927	0.141
Suspiciousness	300	1.00	7.00	1.81	1.30207	1.708	0.141
Hallucination	300	1.00	7.00	1.54	1.10717	2.726	0.141
Unusual thought content	300	1.00	7.00	1.94	1.41426	1.829	0.141
Bizarre behavior	300	1.00	7.00	1.59	1.19443	2.447	0.141
Self –neglect	300	1.00	7.00	1.82	1.36075	1.735	0.141
Disorientation	300	1.00	7.00	1.65	1.28270	2.288	0.141

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error
Conceptual disorganization	300	1.00	7.00	1.67	1.23445	2.139	0.141
Blunted affect	300	1.00	7.00	1.90	1.42011	1.835	0.141
Emotional withdrawal	300	1.00	7.00	2.09	1.59576	1.606	0.141
Motor retardation	299	1.00	7.00	1.81	1.34145	2.013	0.141
Tension	300	1.00	7.00	2.59	1.85259	.957	0.141
Uncooperativeness	300	1.00	7.00	1.96	1.46020	1.634	0.141
Excitement	300	1.00	7.00	2.68	1.91568	.934	0.141
Distractibility	300	1.00	7.00	2.28	1.74544	1.436	0.141
Motor hyperactivity	300	1.00	7.00	1.90	1.33765	1.712	0.141
Mannerism and posturing	300	1.00	7.00	1.95	1.46913	1.629	0.141

Mean score for somatic concern was 1.99±1.37 (sk=1.61), Mean score for Anxiety was 2.54±1.5 (sk=0.76), Mean score for Depression was 2.12±1.45 (sk=1.34), Mean score for suicidality was 1.49±1.15 (sk=2.88), Mean score for guilty was 2.15±1.53 (sk=1.44), Mean score for hostility was 1.99±1.37 (sk=1.61), Mean score for elated mood was 2.00±1.37 (sk=1.74), Mean score for grandiosity was 1.86±1.39 (sk=1.92), Mean score for suspiciousness was 1.81±1.30 (sk=1.70), Mean score for hallucination was 1.54±1.10 (sk=2.72), Mean score for unusual thought content was 1.94±1.41 (sk=1.82), Mean score for bizarre behavior was 1.59±1.19 (sk=2.44), Mean score for self-neglect was 1.82±1.36 (sk=1.73), Mean score for disorientation was 1.65±1.28 (sk=2.28), Mean score for conceptual disorganization was 1.67±1.23 (sk=2.13), Mean score for blunted affect was 1.90±1.42(sk=1.83), Mean score for motional withdrawal was 2.09±1.59 (sk=1.60), Mean score for motor retardation was 1.81±1.34 (sk=2.01), Mean score for tension was 2.59±1.85 (sk=0.95), Mean score for uncooperativeness was 1.96±1.46 (sk=1.63), Mean score for excitement was 2.68±1.91 (sk=0.93), Mean score for distractibility was 2.28±1.74 (sk=1.43), Mean score for motor hyperactivity was 1.90±1.33 (sk=1.71), Mean score for mannerism and posturing was 1.95±1.46 (sk=1.62).

Discussion

One article has reported alcohol is another risk factor among adolescents and others are conduct problems, alcohol and drug use in the family and friends' substance use[14]. Many of them does not approach to mental health services at all because of their negligence and lack of resources.[15]

Another article has reported that low parental attachment leads to mental illness; addiction to cannabis, alcohol use and cigarette smoking is having independent association to mental disorders among adolescents.[16]

Another study reported that negative functioning behavior and gambling behaviors are having greater association with mental illness among adolescents.[17]

Presence of mental disorders during adolescence may be a greater predictor point for mental illness later in their life time.[18] Increased rates of emotional and behavioral problems are very common among youth nowadays.[19] There is extensive evidence that association of addicted to smoking, alcohol and other drugs and mental health problems like depression and anxiety with the indicators of low income level and educational attainment.[20]

One of the article reported clearly and vigorously that low social maturity, mental energy and emotional stability among late adolescents are associated with non affective psychotic illnesses.[21] Chronic stress also mediates with the growing brain and produce significant changes in the neural circuits which predominantly causes mental illness among adolescents.[22] Another author has reported, depression is more among adolescent girls and the present study also noted moderate level of depression among adolescents

Adolescents with behavioral problems frequently have difficulty in expressing their emotions and to understand others emotions also. There are evidence that relations exist between depression and smoking among adolescents. Parental separation also one of the reason

for adolescents to have mental disorders. There is a temporal relationship between selected mental disorders and having chance of addiction in later life. Aryal et al reported similar findings.Gaidhane et al have reported effects of electronic media among adolescents.

Conclusion

The adolescents are facing lots of stress in their academic because of growing competition and inequalities in the educational system, the parents should be more supportive for their children.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Comparison of Functional Outcome of Fracture Proximal Humerus Treated with Open Reduction and Plate Osteosynthesis v/s Closed Reduction and Wire Fixation

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Abstract

Introduction: Fractures of the proximal humerus constitute about 4% of all fractures and 26% of humerus fractures. Treatment of fracture proximal humerus has always been controversial. Many studies indicate that the majority of favourable results of these fractures are achieved by conservative method. Whereas some other studies suggest that surgical management is better.

Aim: The aim of this study was to compare functional outcome between Open Reduction and Plate Osteosynthesis and Closed Reduction with wire Fixation of Fracture of proximal humerus.

Method and Materials: 30 patients with fracture of proximal humerus were first categorized according to Neer's classification and were divided alternatively into Proximal Humerus Interlocking System (PHILOS) group which is named Group A and wiring group which was called Group B according to randomized control study design. These patients were then followed up for 12 months. The results were compared using Constant-Murley score.

Results: In our study, the favourable outcome was seen in 12 patients out of 15 in group A (80%) whereas in group B the favourable outcome was seen in 9 out of 15 patients (60%) which is statistically insignificant. At the final follow up after 1 year, the final mean Constant and Murley's score was 78.4 for group A while it was 70.13 for group B which is statistically significant showing better results with Philos.

Complications: Complications seen in group A were 3 shoulder stiffness, 1 varusmalunion and 1 subacromial impingement, 1 superficial infection while in group B, 5 shoulder stiffness, 2 varusmalunion, 3 superficial infection, 2 wire migration. There were no incidences of non-union & osteonecrosis of the head of proximal humerus.

Conclusion: Based on the analysis of the results, we recommend that ORIF with PHILOS is desirable for optimal outcome in patients of fracture proximal humerus. However for low demand patient, closed reduction and wire fixation is also an option.

Keywords: *Proximal humerus interlocking system, Constant-Murley score, subacromial impingement.*

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Introduction

Fractures of the proximal humerus constitute about 4% of all fractures and 26% of humerus fractures.[24] These are the second most common upper extremity fractures and the third most common fracture, after fractures in the hip and distal radius. The risk factors for proximal humerus fractures are mainly associated with

osteoporosis due to low bone mineral density and higher risk of falling. In young and middle age population, this injury is of great importance because it may cause temporary or permanent disability and therefore, loss of precious working hours. Paramount importance is given to restoration of the function of the limb and minimising disablity and pain.

The care of proximal humerus fractures has always held diversity of opinion, with regular controversies, infact achievement of some good anatomical reduction after surgical treatment may result in unfavourable outcomes unless thorough post operative rehabilitation is instituted, which is often more demanding in these fractures than the surgical technique itself.[25,26,27]

Many studies indicate that the majority of favourable results of these fractures are achieved by conservative method. Whereas some other studies suggest that surgical management is better, depending on the quality of the bone and type of fracture pattern. Operative treatment of these fractures may be associated with some morbidity and unwanted sequelae. They also include complication like avascular necrosis, non-union, malunion, infection, loss of movements of shoulder due to adhesive capsulitis, neurovascular injury, elbow stiffness and atrophy of the soft tissues due to prolonged limb immobilization causing significant disability during healing and in the rehabilitation phase.

The aim of our study was to compare functional outcome between Open Reduction and Plate Osteosynthesis and Closed Reduction with wire Fixation of Fracture of proximal humerus.

Materials and Method

It is a prospective and observational study carried out in a tertiary hospital between August 2017 and December 2019. The study was done after the clearance from Institutional Ethical Committee. 30 patients with fracture of proximal humerus were first categorized according to Neer's classification and were divided alternatively into PHILOS group which is named Group A and wiring group which was called Group B according to randomized control study design. These patients were then followed up for 12 months. Informed consent was taken from all the patients.

All the patients above 18 years of age who were admitted to this hospital with proximal humeral fractures (Neer's classification: 2 part, 3 part, 4 part) were included in the study with the exclusion of undisplaced fractures, compound fractures, pathological fractures and isolated greater tuberosity fractures.

Both the groups were compared clinically using Constant Murley score at regular follow up intervals.

All the patients were operated using delto-pectoral approach in Group A. Fractures were reduced in anatomical position and fixed with Proximal Humerus Interlocking System (PHILOS) using cortical screws of size 4.5 mm and cancellous screws of size 6.5mm for fifteen patients. Fifteen patients underwent fixation with K-wires or J-wires. Fixation rigidity was checked on table. All the patients were given Universal Shoulder Immobilizer for 1 month. Physiotherapy in the form of pendulum exercises was encouraged in all the patients in the first post-operative week. Suture removal was done on 12th day or later post operatively. Per-operatively, compatible blood was transfused as per patient's requirement.

Results

The mean age incidence in our study of 30 patients analyzed, ranging between 27 to 85 years is 52.3 years and the gender ratio is 1:0.57, 19 of 30 patients were males. The most commonly seen mode of injury in our study was simple fall on ground accounting for 46.67% of all the cases. Many among them were over 50 years old depicting the role of osteoporosis in these fractures. 26.67% patients had road traffic accident which was the second most common cause in our study and other causes included blunt trauma, fall from height and history of electric shock. Types of fracture in our study revealed 15(50 percent) were 2-part fractures, 09(36.67 percent) were 3-part fractures and 04 (13.33 percent) were 4-part fractures of proximal humerus. In our study, the favourable outcome was seen in 12 patients out of 15 in group A (80%) whereas in group B the favourable outcome was seen in 9 out of 15 patients (60%) which is statistically insignificant.

	Group A	Group B	Total
Excellent	2	1	3
Good	10	8	18
Moderate	2	4	6
Poor	1	2	3
Total	15	15	30

At the final follow up after 1 year, the final mean Constant and Murley's score was 78.4 for group A while it was 70.13 for group B. The p-value using unpaired t-test comes out to be 0.04 between Group A and B which is statistically significant showing better results with PHILOS.

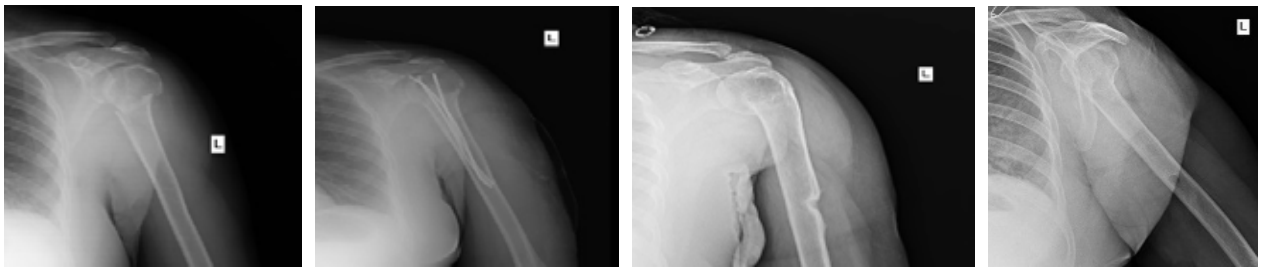
In ages less than 50 years, all the 14 patients had favourable outcome where as in age group more than 50 years of age, favourable outcome was seen in only 7 out of 16 patients. The result is better in group A as 3 out of 6 patients (50%) showed favourable results while in Group B, 4 out of 10 patients showed favourable outcomes. This may be because open reduction helps in better restoration of anatomy and thus helps in early physiotherapy of elder patients.

The results showed insignificant statistical results when these procedures were compared among groups of 2-part, 3-part and 4-part fracture. This may be because the study was done on a smaller scale as significant difference was seen clinically in favour of Group A among 3 and 4 part fractures.

	2 Part		3 Part		4 Part	
	Group A	Group B	Group A	Group B	Group A	Group B
Excellent	1	1	1	0	0	0
Good	5	5	4	3	1	0
Moderate	1	1	1	2	0	1
Poor	0	0	0	1	1	1
Total	7	7	6	6	2	2



Case 1: Radiographs and clinical pictures of patient managed with PHILOS





Case 2: Radiographs and clinical pictures of patient managed with wiring

During the period of follow up, in group A, 3 patients developed shoulder stiffness out of which 1 patient developed varusmalunion and subacromial impingement and screw perforation was seen in 1 patient each. 1 patient had superficial infection which was treated with iv antibiotics only and implant removal was not required. While in group B, shoulder stiffness was present in 5 patients and 2 went into varusmalunion, 3 patients developed superficial infection. I.V. antibiotics were started and k-wires were removed after the union. 2 patients developed the complication of wire migration. There were no incidences of non-union & osteonecrosis of the head of proximal humerus.

Complication	Group A	Group B
Stiffness	3	5
Malunion	1	2
Superficial Infection	1	3
Wire Migration	-	2
Impingement	1	-
Screw Perforation	1	-

Discussion

Due to changes in lifestyle and an increase in motor vehicle accidents, the incidence of fractures of proximal humerus have increased in recent years. In these fractures, the optimal treatment is still not conclusive. Studies show that conservative and operative treatments, both give positive outcomes and hence, the uncertainty remains. Most of the undisplaced fractures can be managed non-operatively. However, displaced proximal humerus fractures do not show good results with conservative treatment, hence they require surgical management for better outcome. The goal of the treatment should be to achieve pain free shoulder with full Range of movements. Different techniques of fixation are used for these complex fractures like tension band, non-absorbable bone sutures, T-plate, K-wire, Intramedullary nailing and hemiarthroplasty. Every technique has its own advantages and disadvantages and has a constant rate of mechanical failure[5,6.] Proximal humerus fractures occur mostly in patients with older age group, showing osteoporosis as a cause of these fractures. In the fractures of proximal humerus, union occurs in almost all the patients due to cancellous nature of the bone in proximal humerus unless the articular part or anatomical neck is involved as it compromises the blood supply[7]. In our study, there were no cases of delayed or nonunion. Open Reduction and Internal Fixation with PHILOS provides better reduction anatomically with rigid fixation to the metaphysis, thus consequently allowing for early mobilization. Another advantage of PHILOS is that it provides better reduction of greater tuberosity fracture fragment. Hence, there were more favourable results in plating group as compared to wiring group. The Neer's score and Constant-Murley score was also higher and better range of movements were seen in plating group due to same reasons. Two part fractures showed good results in both the groups but for three and four part fractures, better results were seen in plating group as compared to the wiring group. In the patients with age more than 50 years, PHILOS showed better results as compared to wiring whereas in patients less than 50 years of age, both groups showed significant results. The proximal locking screws produce angular stable construct with PHILOS and enhance the gripping in osteoporotic bones in elderly patients and in patients with multi-fragmentary fractures[8]. The purchase in head of proximal humerus improves with alternate diverging and converging screws and it also increases the pullout strength. Impingement can be prevented by proper reduction of all the fragments in anatomical position before applying the plate. Acceptable fracture reduction with proper placement of plate and perforation

of screws should be checked intra-operatively using image intensifier for better results. Medial comminution should be fixed properly using additional inferomedial screws. To prevent stiffness and obtain good range of movements, early physiotherapy is of paramount importance. Careful surgical dissection and medial periosteal hinge prevent damage to posteromedial vessels present at the posteromedial aspect of the neck of humerus, thus the incidence of Avascular Necrosis can be reduced. The results of K-wires or J-wires was not appreciable in our study in patients with 3-part and 4-part fractures. This is likely to be due to poor bone quality in elderly and comminution at the fracture site along with less stable fixation which could lead to wire migration and loss of reduction. Furthermore, threaded k-wires, terminally threaded Schanz screws, transosseuos sutures and cannulated cancellous screws were not used in our study which could have helped in better reduction of the tuberosities when used along with wiring and thus providing a better outcome. The advantages of wiring is that it helps in retaining the vascularity of the head of humerus. It can be used in two and some three part fractures in young adults without comminution. In older population, this technique can be considered if the bone quality is good and there is less comminution to shorten the time duration of the surgery. In this study, the follow up of the patients was taken for 1 year but longer duration follow up helps in providing the broader spectrum of complication rates like secondary osteoarthritis and avascular necrosis of proximal humerus which appear late after surgical intervention.

Conclusion

We recommend that ORIF with PHILOS is desirable for optimal outcome in patients of fracture proximal humerus. However for low demand patient, closed reduction and wire fixation is also an option.

Limitations and Recommendations:

1. Surgeon related bias due to surgeries performed by various surgeons.
2. Sample size was small.
3. Follow up duration was shorter.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

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Efficacy of Role Play on Ill Effects of Alcoholism among College Going Students

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Abstract

Introduction: Alcoholism is the most serious type of substance addiction which includes drinking habits and unable to control them. It is also called as Alcohol Dependence Syndrome or Alcohol use disorder.

Aim: To determine the efficacy of role play on ill effect of alcoholism among college going student.

Materials and Method: Purposive sampling was used to collect data from college going students by using structured knowledge questionnaire regarding ill effects of alcoholism with their selected demographic variables.

Results: The mean pre-test knowledge score was 12.27 and mean post-test knowledge score was 25.19. There was a statistically significant improvement in the level of knowledge regarding ill-effects of alcoholism among college going students. The tabulated value for n=100-1 i.e. 99 degrees of freedom was 1.98. The calculated 't' value i.e. 58.96 are much higher than the tabulated value at 5% level of significance for overall knowledge score of college going students which is statistically acceptable level of significance and noassociation was found between post-test knowledge score with their selected demographic variables.

Conclusion: The demand of increase knowledge regarding ill effects of alcoholism is important among college going students because they are more prone to consuming alcohol due to recreation, experience and specially peer pressure.

Keyword: *Effectiveness, psychodrama, side effects, Alcohol use disorder, College Student.*

Introduction

The word alcoholism and alcoholic were first used by Dr. Magnus Huss, a Swedish physician.[1] Alcoholism is defined as the consumption of or preoccupation with alcoholic beverages to the extent that this behavior interferes with the alcoholic's normal personal, family, social, or work life.[2] Chronic alcohol use can contribute to both psychological and physiological disorder.[3] Alcohol abuse can have harmful effects that can endanger life. There may be increased threat to certain cancers-especially liver, esophagus, throat, larynx and breast cancers. Excess alcohol consuming can also lead to cirrhosis of liver, gastro intestinal disease, anemia, immune system difficulty, pancreatitis, brain damage and damage to the fetus during pregnancy. WHO has reported that around the world 6,000 people are killed every day. Although 28% of these deaths are caused by accidents such as traffic collisions, self-harm and crime, 21% are caused by digestive disorders, 19% are caused by cardiovascular diseases and the remainder are caused by infectious diseases, tumors, psychiatric disorders and other conditions of health. Each year in India nearly 1 lakh deaths occur on roads indirectly linked to drug misuse. About 30,000 deaths can also be attributed from drug use by cancer patients each year. The most serious health issue posed by alcohol use is liver cirrhosis with 4.5 lakh deaths per sunlight hours

Materials and Method: A research design was used in this study is pre-experimental–one group pre testpost test. The study was conducted during 30 December 2019 to 14 January 2020 and the setting was selected in Yashwant Mahavidyalayaand the Vidyabharti College Seloo, Wardha after getting ethical permission (Ref No. DMIMS(DU)/IEC/Sep-2019/8501). By using purposive sampling technique, 100 college going students were selected based on the calculation. where, z=standard normal variate, which is 1.96 at 5% type 1 error, SD=standard deviation of knowledge score, d=absolute error or precision . On the basis of the previous study, p-values are considered significant below 5%, hence, 1.96 is used in formula. Considering 95% Confidence Interval (CI) and 20% allowable error, the sample size was calculated to include 92 respondents. However, the researcher decided to include 100 college going students (data collection phase was completed within one month only to avoid sample mortality).Students who were going to college were told and explained about the study intent. The registered, informed consent was received dully signed by them individually. The inclusion criteria were: i. College of arts, commerce, science and electronics students studying in Wardha City. ii. College going students who were willing to participate in the study. iii. College going students who were accessible at the time of the data collection. iv. College going students who know the language like Marathi and English. Students who are participated in similar type of project in last 1 year have excluded.

Demographic variables were collected in terms of Age (in years), Gender, Residence, Religion, Types of family, Family income (monthly), Have you taken alcohol anytime? If yes, Duration…Frequency… Quantity…, Type of alcohol, Do you have any information about ill effects of alcoholism? If yes, what the source of information? A structured knowledge questionnaire,which is attached in Annexure 1, has 30 multiple choice questions. The questionnaire was prepared based on the existing literatures, which were discussed in previous sections and clinical experiences of handling of Alcoholism clients. Each right answer carries one mark and total score is 30. Prepared tool was validated by nine experts, out of them four were from nursing department,four were from the Department of psychiatry and one was from statistics department. Split half method was adopted for reliability testing and it was found as r=0.86. Hence it was reliable. Researchers were planned to gather demographic information and the knowledge on ill effects of alcoholism.

The role play was organized on ill effects of alcoholism. Role play was shown to 100 college going students. Role play was shown to 25 students on first day. 2nd day next 25 students, 3rd day next 25 students and 4th day last 25 students for duration of 30-45minutes. After seven days of each group from the day of role play shown, each of college going students was asked individually for his/her responses by structured knowledge questionnaire i.e. 30 questions. The responses, as collected, were arranged in tabular form to conduct statistical analyses, which are mentioned in the following sections.

Statistical Analysis: Demographic data, collected in pre-test point, was analyzed in terms of frequency and percentage, on nine closed-ended questions. The paired t-test was used to compare the scores of information before and after the test. To find out the relation between selected variable and Post-test, unpaired t test and one-way ANNOVA were applied.

Results

Hundred college going students randomly identified as sample to achieve the purpose of present study. The demographic variables of samples are depicted in the [Table/Fig-2] which shows that the maximum (i.e. 37%) of college going students belonged to 17 years of age group. Majority of college going students (i.e. 70 %)were male. Out of 100 college going students, majority (i.e., 52%) belonged to rural area and almost (65 %) were Hindu religion, (58 %)belonged to nuclear family, (33 %) had 5001-10000 Rs. family income per month, only 7 % college going students consumed alcohol, 87 % of had information regarding ill effects of alcoholism and out off 87 college going students (29.9 %) got information regarding ill effects of alcoholism from medias i.e. face book, whatsApp, newspaper etc. depicts that 50 % of college going students had average knowledge was in the range of (07 -12) and 50 % had good knowledge regarding ill effects of alcoholism was in the range of (13 -18)in pre test. Where 66 % of college going students had excellent knowledge was in the range of (19-24) and 34 % had very good knowledge regarding ill effects of alcoholism was in the range of (25-30) in post test.

Table 1: Demographic Variables

Sr. No.	Demographic Variables	No. of college going students	Percentage (%)
1	**Age (yrs)**		
	17 yrs	37	37
	18 yrs	33	33
	19 yrs	30	30
2	Gender		
	Male	70	70
	Female	30	30
3	**Area of residence**		
	Rural	52	52
	Urban	48	48
4	**Religion**		
	Hindu	45	45
	Muslim	19	19
	Christian	13	13
	Sikh	2	2
	Others	21	21
5	**Type of family**		
	Nuclear	58	58
	Joint	40	40
	Extended	2	2
6	**Monthly family income**		
	<5000 Rs	28	28
	5001-10000 Rs	33	33
	10001-15000 Rs	18	18
	>15000 Rs	21	21
7	**Have you taken alcohol any time**		
	Yes	7	7
	No	93	93
8	**Information about ill effects of alcoholism**		
	Yes	87	87
	No	13	13
9	**Source of information**		
	Health Personnel	17	19.5
	Media	26	29.9
	Neighbours	23	26.4
	Friends	8	9.2
	Parents	13	14.9

Table 2: Frequency percentage of knowledge score (N=100)

Level knowledge	Score Range	Percentage range %	Pre test		Post Test	
			Frequency	Percentage %	Frequency	Percentage %
Poor	1-6	0-20 %	0	0	0	0
Average	7-12	21-40 %	50	50	0	0
Good	13-18	41-60 %	50	50	0	0
Very Good	19-24	61-80 %	0	0	34	34
Excellent	25-30	81-100 %	0	0	66	66

The effectiveness of Role play was analyzed as follows:

H_1: Mean post-test knowledge score was significantly higher than mean pre-test knowledge score represented that mean post-test knowledge score i.e., 25.19 was higher compared to Pre-test knowledge score (i.e.,12.27) with mean difference of 12.92. This was suggested that there was significant improvement of knowledge due to Role play. It was also observed that calculated t value (i.e., 58.96) was greater than tabulated t value (i.e., 1.98) at df 99 and 0.001 level of significance.

Table 3: Statistical analysis of pre-test post-test knowledge score (N=100).

Overall	Mean	SD	Mean Difference	t-value	p-value
Pre Test	12.27	1.97	12.92±2.19	58.96	0.0001 S,p<0.05
Post Test	25.19	1.36			

t (at α = 0.001 and 99 df) = 1.98; df = degree of freedom

Thus, null hypothesis was rejected and alternative hypothesis was accepted, i.e., knowledge was significantly increased after Role play. Therefore, Role play was effective in provisions of increase in knowledge.

Table 4: Association between post-test knowledge score with their selected demographic variables (N=100).

Sr. No.	Demographic variables	Frequency	Percentage %	Mean post test knowledge score	F-value	p-value
1	Age in years			F value		
	17 yrs	37	37	25.43±1.34	1.03	0.36 NS,p>0.05
	18 yrs	33	33	25.12±1.51		
	19 yrs	30	30	24.96±1.18		
2	Gender			F value		
	Male	70	70	25.28±1.39	1.07	0.28 NS, p>0.05
	Female	30	30	24.96±1.27		
3	Residence			F value		
	Rural	52	52	25.19±1.54	0.01	0.98 NS, p>0.05
	Urban	48	48	25.18±1.14		

Sr. No.	Demographic variables	Frequency	Percentage %	Mean post test knowledge score	F-value	p-value
4	Religion					
	Hindu	45	45	25.06±1.37	0.62	0.64 NS, p>0.05
	Muslim	19	19	25±1.33		
	Christian	13	13	25.30±1.10		
	Sikh	2	2	25±1.41		
	Others	21	21	25.57±1.53		
5	**Type of family**					
	Nuclear	58	58	25.32±1.31	2.02	0.13 NS, p>0.05
	Joint	40	40	24.92±1.36		
	Extended	2	2	26.50±2.12		
6	**Monthly Family Income**					
	<5000 Rs	28	28	24.96±1.13	0.94	0.42 NS, p>0.05
	5001-10000 Rs	33	33	25.30±1.46		
	10001-15000 Rs	18	18	24.94±1.43		
	>15000 Rs	21	21	25.52±1.40		
7	**Have you taken alcohol any time**			F value		
	Yes	7	7	25.85±1.34	1.35	0.18 NS, p>0.05
	No	93	93	25.13±1.35		
8	**Information about ill-effects of alcoholism**			F value		
	Yes	87	87	25.19±1.40	0.10	0.91 NS, p>0.05
	No	13	13	25.15±1.06		
9	**Source of Information about ill-effects of alcoholism n= 87**					
	Health Personnel	17	17	25.82±1.23	1.72	0.15 NS, p>0.05
	Media	26	26	25.23±1.70		
	Neighbors	23	23	25.13±1.21		
	Friends	8	8	24.37±1.06		
	Parents	13	13	24.92±1.25		

[Table/Fig-6] illustrats that the that there was no significant correlation between post-test knowledge score about ill effects of alcoholism of college-going students and their selected demographic variables such as Age (in years), gender, home, religion, family forms, (monthly) family income, Have you ever taken alcohol? If yes, length, quantity, frequency, form

Discussion

This research sponsored the presentation of a report and it was supported to present study that a descriptive study to determine the level of awareness about alcoholism and its hazards in selected school among adolescents in order to create an information pamphlet. The main finding was that 63.0% of students had average knowledge of alcoholism and its risks while 27.0% and 10.0% had poor and good knowledge. It has been proposed that nurses ought to teach adolescents to prevent drinking and its health risks.

This study also supported to present study that intend to assess the effectiveness of structured teaching program on knowledge regarding alcohol use and its harmful effects among high school children at Municipal Corporation School in Tirupati."In this study research design was used i.e. pre-experimental one-group pre-test post-test design.The populations had been high school

children. The study findings exposed that among 60 high school children 15 (25%)had inadequate knowledge, 24 (40%) had moderate knowledge and 21 (35%) had adequate knowledge in pretest.After administration of structured teaching program, 8 (13.3%) had inadequate knowledge 28 (46.7%) had moderate knowledge and 24 (40%) had adequate knowledge in post test.The value of the pre-test means and standard deviation scores was 15.40 + 2.499 and the value of the post test means and standard deviation was 24.08 + 2.499 and the calculated t-value was 15.846 that was statistically significant at p=0.01 It showed that after intervention, there was a significant difference between pre-test and post-test scores among high school children so it was fulfilled that after the standardized teaching programs the high school children had improve their knowledge of alcohol use and its adverse effects.[7]

This study also supported to present study which was conducted to assess the effectiveness ofrole play on knowledge regarding substance abuse among adolescents. Quasi experimental, one group pre-test post-test design was research design with interventional approach. Sample size was 60 selectedfrom 8thstd., 9thstd and 10thstdby probability sampling technique i.e. simple random sampling with lottery method.The resultreveled that pre testscore was 11.51 + 3.55 and post testscore was17.5 + 2.89 it means the post test knowledge score was increased after showing role play to participants. The paired't'test value were 15.363 ($p < 0.0001$) showedasignificant increase in knowledge regarding substance abuse8. There was an association of post test knowledge score of substance abuse with their demographic variables i.e. type of family and general information on substance abuse, education of father with commonly abused substances and the total score on substance abuse, education of mother with commonly abused substances and prevention and management of substance abuse and number of children in the family so it concluded that the role played on substance abuse has been effectively increased adolescent understanding and thus encouraging adolescents to be aware of the harmful effects of substance abuse and to take effective action to handle it.[9,10.]

Limitation: The study was limited to sample size i.e. 100, which might be inadequate to generalize the study findings. More time duration would give more relevant results with variations of any research, but the investigator planned to complete the research work within 21 days to get more feasibility of getting sample and avoid sample mortality. Therefore, sufficient number of sample and time duration was required to establish the effect of Role play, in general.

Conclusion

Based on the above results, it was concluded that role play is an effective teaching tool to provide information about the negative effects of alcoholism on individuals, families and society. We can reduce the incidence of negative effects of alcoholism and motivate people to stop drinking. We found no correlation between selected demographic variables and post test knowledge score after study.

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Correlation of hsCRP with Edmonton's Obesity Staging System: A Cross Sectional Study

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Abstract

Background: Obesity comes with a huge number of co-morbidities such as type 2 diabetes mellitus (T2DM) and cardiovascular diseases. The most usually used factor for segregating different degree of adiposity is Body Mass Index (BMI) and Waist Circumference (WC).Despite being a convenient and commonly used method it doesn't show the occurrence of essential obesity-related co-morbidity & deplete quality of life. Hence, Edmonton's Obesity Staging System has been proposed which incorporates such co-morbidities and functional status of individual. Serum levels of high-sensitivity CRP (hs-CRP) can be calculated using highly sensitive assays and has ability to detect augmented inflammation in vasculature. Thus, chronic systemic inflammation linked to the metabolic syndromes. The Study aims to correlate such factors.

Aims: To estimate hsCRP levels in obese population.

Material and Method: After thorough anthropometric and biochemical evaluation obese individuals will be classified accordingly into Edmonton's obesity staging system and their hs-CRP will be evaluated and then correlation between two will be done.

Result: A linear correlation between hs-CRP and Edmonton's Obesity Staging System is expected.

Conclusion: With the help of correlation between hs-CRP and EOSS co-morbidities of obese individuals can be tracked efficiently.

Keywords: *Obesity, hs-CRP, Edmonton's, Co-morbidity.*

Introduction

There has been a dramatic increase in occurrence of overweight and obese, which is of a major concern to most of the countries.[1] Obesity comes with a large number of cardiac and other systemic co-morbidities. Communal prejudice against fatness has grave impact for psychosocial health.[2]

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The most frequently used restriction for segregating various degree of adiposity is Body Mass Index (BMI). [3]WHO has proposed race specific cut off values for Asians[4-8]. According to revised guidelines overweight is BMI of 23.0–24[9] and obesity BMI≥25.[10] According to WHO cutoff points for waist circumference: 90 cm for men and 80 cm for women. [11]

A new clinical staging system;Edmonton Obesity Staging System (EOSS), describes obese phenotype on a 5-point ordinal scale in order to determine obesity treatment.[12] Therefore, it has been proved to be animproved tool in predicting morbidity and mortality than BMI.[13] It consist of 5 stages from 0-4.[14]

Studies through various modes have now shown

a state of low-grade chronic inflammation in obese individuals, making them vulnerable for various co-morbidities, which is visible through various inflammatory markers in serum.[15] C-reactive protein (CRP) is a typical acute-phase protein that is formed in the liver under the incentive of cytokines such as tumor necrosis factor, interleukin-1, 2, 4, 6 & 7.One of the most important study conducted to assess Cardiovascular risk in middle aged individual males at high risk with help of hs- C reactive protein was the **Multiple risk factor Interventional trial (MRFIT)**[16-23]. A major study involving 17,800 individuals (ages ranging from middle age – elderly age) who are at low to intermediate risk, the trial was known as the **JUPITER randomized trial,** suggested that to estimate the benefit of statin therapy is best to utilize hs-CRP as an indicator marker. [24]The present study aims to find association between hsCRP and Edmonton's staging.

Aim:

To estimate hsCRP levels in obese population.

Objective:

1. To classify obese according to Edmonton's Obesity Staging System (EOSS).
2. To correlate hsCRP with Edmonton's Obesity Staging System (EOSS).

Method and Material

Study design:

Cross sectional study

Selection of Cases: All cases will be randomly selected from the university students, staff, workers, subjects attending the health camps, patients attending OPD and inpatients of medicine having minor illnesses.

Informed consent will be taken: History of Diabetes mellitus, systemic hypertension, anxiety and depression will betaken. Detailed drug history will be obtained. Detailed physical examination and anthropometric measurement in form of BMI, Waist circumference will be calculated. Biochemistry analysis including fasting Blood sugar (FBS), serum triglyceride (TG), high density lipoprotein (HDL) and HSCRP will be done after fasting for 10-12 hours.

Classification in Edmonton's Obesity Staging System: Based on factors such as FBS, LDL Cholesterol, HDL cholesterol and Total cholesterol, triglycerides, history of liver disease, kidney disease, osteoarthritis and physical health, subjects will be classified as mentioned in the table, the score of the higher side will be considered in case an individual presented with multiple co-morbidities.[25-32]

Inclusion Criteria:

All people falling into criteria of:

- Metabolic syndrome
- BMI >23
- Age group 18-70 years

Exclusion Criteria:

- BMI within normal range
- Subjects with no indicators of metabolic syndrome
- Major infections/sepsis/critically ill
- Rheumatologic and Collagen vascular disorders
- Women on oral contraceptive pills
- Persons with hs-CRP levels more than 10 mg/ L(presuming occult infections)
- Subjects not willing to consent

Procedures: Anthropometric measures including weight, height, BMI, waist circumference (WC) will be measured by standard method.

i. Weight will be estimated according to protocol. [33]

ii. Height will be measured according to protocol. [33]

iii. Body mass index will be calculated according to Quetelet formula [Weight (in Kgs)/Height (in m^2)]

iv. Waist circumference (in cms) will be estimated.[33]

v. Blood pressures (BP): The WHO protocol will be used.[34]

Estimation of Glucose in Plasma: Fasting plasma glucose will be estimated by the GOD/POD method by the machine Robonic Semi Automatic Chemical Analyzer.

Estimation of Serum Lipids: PAP method by machine RobonicSemi Automatic Chemical Analyzer

Direct Enzymatic Method will estimate serum HDL.

Method of measuring hs-CRP: A solid phase ultra-sensitive EIAwill be used.

Expected Results: Through this study we expect linear correlation bet hsCRP and Edmonton's obesity staging system. Implying a raised hs-CRP for patients with higher EOSS.

Discussion

Ju-Yeon Yu et al, in their study related hsCRP to visceral obesity and cardio-metabolic diseases.[35]

In study by Gregory Pavela et al, hs CRP was increased in Obesity.

There was conclusion of Edmonton's obesity staging system being better than BMI for the health service use and polypharmacy, in study done by Evan Atlantis et al.

Jennifer L Kuk, et al. and Raj S Padwal et al. in their study stated that EOSS provides clinicians a powerful tool for detecting obese phenotypes at the risk of mortality.

Moreover Soinja Chiappetta, et al. in their study proposed that patients with EOSS more than 3 shows increased complication post operatively and high mortality rate.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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An Experimental Study on *Mahehwar Dhoop* to Evaluate Fumigation Effect in Comparison with Bacillocid

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Abstract

Background: Ayurveda advocates fumigation as a method of sterilization and therapeutic tool for different infective diseases and mental disorders. However, it is not popular and proven scientifically hence there is great scope and prospects for research. For patient safety, fumigation is a regular protocol.

Aim and Objectives: It is the need of the hour to search a safe, cheap, effective and easily available drugs for fumigation hence *Maheshwar Dhoop*-MD was chosen and compared its efficacy with control- Bacillocid.

Material and Method: Study was performed in Shalakya (eye-ENT), *Panchkarma* procedure rooms and minor operation theatre by pre-post swab and Agar plate exposure on 2nd (MD) and 4th week (Bacillocid) monthly once each for total three months. The microbial load was estimated for different micro-organisms like bacteria, fungus etc and subjected to statistical analysis.

Observation and Results: The study revealed that the effect of *Dhoopana* was significant in comparison with Bacillocidto control both during study period and follow up period up to 6 days against bacterias and fungus. **Conclusion**: It was found that MD is safe, cheap and equally efficient as Bacillocid ($p<0.0001$) with one-week residual effect.

Keywords: *Bacillocid, Maheshwar dhup, fumigation, Sterilization, Dhoopan kalp, microbes, Agar plate and swab.*

Introduction

Ayurveda is a life science which is mainly well known for disease prevention[1]. *Dhoopan*imply fumigation or sterilization which is one of the approaches of prevention[2]. The decontamination of enclosed environment is an important consideration for the control of pathogens. Ayurveda recommends fumigation as a method of sterilization and therapeutic procedure for various human diseases including microbial infections and psychological disorders. However, due to the lacking scientific validation, these Dhoop kalpas are not popular, which brings attention to research opportunities and understand its safety and efficacy, anti-microbial activity with safe, economical and eco-friendly potential.[3] Fumigation is an age-old method of sterilization. There are few examples mentioned in Ayurveda compendia specially in *Kashyap samhita, Dhupkalpadhyay* for the use as *Rakshoghna* (antimicrobial*)* in treatment of various diseases. For the purpose of prevention of neonatal diseases, which are prone during neonatal period, *Kashyap* emphasized to fumigate the clothes of newborn after bath by antimicrobial drugs[4]. The soiled clothes with faeces, urine, sweat or dirt should be used only after

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washing, drying and getting them fumigated by Yava, Sarshap, Atasi, Hingu, Guggulu, Vacha etc. with ghee[5]. *Yogratnakar* advice that the child who is fumigated with the feathers of cock dipped with cow's ghee for seven days need not be afraid of any evil demons[6]. Probably the fumigation has disinfectant properties and protects the child and place by making them sterile. *Vagbhat* has suggested fumigation with feathers of a normally dead crow mixed with *Trivritta*, includes both disinfectant as well as deodorants. Various amulets, jwels, horn tip, *Vacha* are made into lockets to be tied to hands, neck for the longevity, increase intelligence and ward off the evil demons[7].

In Hindu mythology the concept of '*Raksha' and 'Bhuta'* is very important which can be compared with infectious agent having impact on health due to known or unknown causes. The scientific intelligence of ancient scholars regarding the infections gives importance to *Rakshoghna vidhi*. '*Dhupkalpa'* prepared by combination of several drugs which may have anti-infective properties producing fumes with aroma. *Dhoopn* is carried out by healthcare worker to prevent nasocomial diseases to society. There are three types of fumigation on the basis of action i. e. *Dhoop, Anudhoop and Pratidhoop* and on the basis of origin, two types are mentioned as *Sthavar* (Herbal) & *Jangam* (animal origin)[8]. *Maheshwar Dhoop* has been described in *Kashyap samhita* in *kalpasthana*. It is indicated for treatment of malaria and also for *Graharogas* (infectious diseases)[9]. There is overabundance of microbes everywhere in atmosphere hence, it is a protocol to fumigate operation theatres, minor procedure rooms prior surgery to prevent hospital acquired infections (HAI)[10]. Fumigationrequires to be performed as per the protocol to ensure patient safety[11]. Formalin fumigation which is outdated now, owing to its harmful and carcinogenic effects which was required 300 ml of 10% Ammonia to neutralize formalin vapours and open the OT to start surgery. Such formalin fumigation is required at weekly intervals. There are other disinfectants like Phenol-carbolic acid 2%, Bacillocid, Ethylene oxide, ultrasonic cleaner or ultra-violate, gamma radiation which are costly. It is the need of the hour to search a better option to make environment sterile. Standard fumigation should overcome all these lacunae and prove as a safe, cost effective, quick, easy method to avert hospital acquired infections (HAI) also useful to prevent vector-borne diseases, bugs, mites/ arachnida, insects etc.

Recently, *Dhoopn* is seldom used as fumigation method, therefore, this study aimed to evaluate the efficacy *of Maheshwar dhoop* for fumigation purposeas *Rakshoghna* (antimicrobial/sterile) with safe and instant actioncompared with Bacillocid control. Currently, Bacillocid (liquid concentrated chemical) is in use for fumigation for sterilization[12].

Materials and Method

The study was carried outat Mahatma Gandhi Ayurved College, Hospital and Research centre, Salod after ethical clearance from institutional ethical committee. The pre-post swabs were taken from Shalakya (ENT, Ophthalmic) procedure room, Minor OT-operation theatre, male and female *panchkarma* procedure rooms and *Balpanchkarma* room. Main surgical operation theatre was kept away from this study to avoid the interference in protocol of sterilization.

Materials: Herbsrequired for *Dhoopan*/fumigation were collected and authenticated from taxonomist. Then *Dhoop* was prepared at institutional pharmacy and standardized at analytical lab of institute- M.G.A.C.H. & R.C. to see the fumigation effect by swab analysis which was tested by Microbiologists at Jawaharlal Nehru Medical College, Sawangi, Wardha.

Method of *Maheshwar Dhoop (MD)* preparation and Fumigation: The raw drugs were cleaned and made in to coarse powdered form (Mesh size 40-60)separately and homogenous mixture was prepared by mixing all the ingredients. Pre-swabs were taken to check the presence of organisms. Agargel plates were kept in each room. Then the coarse MD powder of 25 gms was sprinckled on burned cow-dung cake of size around 250 gms.,medicated fumes were produced till the area became full of fumes. Dhoopan/fumigation area of five rooms of 10x10 ft size was cleaned first and then window, doors were kept closed, AC, fans switched off till the process has been carried out[13]. *Maheshwardhoopan karma* was carried out like regular fumigation in OTand Panchkarma therapy rooms. Thereafter,post-swaband agar gel plates were received after 2 hours of *Dhoopan* to check the anti-microbial activity of MD on 2nd week of a month for four consecutive months after procedures at afternoon hours. Again regular fumigation by Bacillocid 500ml: water 1000ml for an area of 10x10 feet was done on every 4th week of four consecutive months. Fumigation was done after procedures and operative minor work as usual as per protocol. In all four months, fumigation by MD and

Bacillocid, the timings were same in the afternoon (2-4 pm). The swabs were taken from five sites (Procedure and preparation Table top, floor, east and west wall) from each therapy room and OT. Single agargel plate was kept in the center of the room. Every time swabs were taken and plate was kept at same places. Then *Maheshwar dhoop* efficacy was checked as compare to regular fumigation with Bacillocid by separate pre-post swabs and agar plates after Bacillocidfumigation also. Duration of *dhoopan* was continued up to filling of smoke in OT room and therapy rooms according to area and quantity calculation. It was observed that only one cow dung cake of around 250 gms and 25 gm coarse powder of MD formulation was sufficient to produce full of smoke in the room of 10x10 feet size. Initially sterlium (hand sanitizer) was used to burn the cake later due to ghrit the procedure of dhoopan was smoothly carried out without any problem.

Frequency: Total four consecutive months, *dhoopan* was done and same for Bacillocidfumigation for comparison by taking pre-post swabs and agargel plates exposure.

Laboratory Investigations: Pre-post swab and Agar gel plates culture reports were done at Microbiology Dept. JNMC, Wardha.

Action of above drugs has been proved the efficacy for maintenance of health as well as prevention of diseases by the action of anti-microbial[14-18]. In original text of Kashyap Samhita (from where *Maheshwar dhup* reference has chosen), there is *Nameru* drug also mentioned along with above drugs but it is not taken due to not clear identification and availability of it.

Assessment Criteria: For standardization of *Dhoopan dravyas*, following Specific criteria were assessed to check each drug's quality separately and together in combination of *MD dravyas*.

Table No. 1: Organoleptic parameters of Maheshwar Dhoop

Parameters	Pharmacopoeia standard	Committee standard	Observations	Inference
Color	Not available	Brown	Brown	Acceptable
Odor	Not available	Aromatic and pleasant	Aromatic and pleasant	Acceptable

Table No. 2: Physico-chemical parameters of Maheshwar Dhoop

Parameters	Pharmacopeia standard	Committee standard	Observations (Avarage of three batches)	Inference
Loss on drying at 105°C	Not available	Not more than 13%	8.25%	Acceptable
Total ash	Not available	Not more than 9%	6.6%	Acceptable
Acid insoluble ash	Not available	Not more than 2%	1.2%	Acceptable
Alcohol soluble extractives	Not available	Not less than 13%	16.4%	Acceptable
Water soluble extractive	Not available	Not less than 11%	14 %	Acceptable
pH	Not available	-	5.2 (10% aqueous solution)	Acceptable
Hardness	Not available	4–6 kg	4.2 kg	Acceptable
Tablet Disintegration time	Not available	5 to 20 minutes	14 minutes	Acceptable

Table No. 3: Microbiological specification of Maheshwar Dhoop formulation

Parameters	Pharmacopeia standard	Observations	Inference
Total viable count	Maximum 105/gm	No growth	Acceptable
Enterobacteriaceae	103/gm	Absent	Acceptable
Total fungus count	Maximum 103/gm	Absent	Acceptable
E-coli	Maximum 10/gm	Absent	Acceptable

Parameters	Pharmacopeia standard	Observations	Inference
Salmonella	None	Absent	Acceptable
Staphyliococus aureus	Absent	Absent	Acceptable
Psudomonas aueruginosa	Absent	Absent	Acceptable

Organo-leptic characters such as Color, Odor, Touch then **Identification** was done by Microscopy. **Physico-Chemical Parameters:** were assessed by loss on drying at 110°C, Total ash, Acid soluble ash, Water soluble extractive, pH and particle size with Microbial contamination also noted. Assessment was carried out on the basis of swab reports by microbiologist with the help of comparison in mean pre - post swab reports and statistics.

The cow dung two cakes and MD formulation coarse powder 100 gms including ghrit were required to fill up the full of smoke of 10x10 feet area rooms. The cakes were kept opposite to each other to spread the smoke fast. Observations were noted and inference drawn after swab reports of pre and post *Dhoopan* versus Bacilosid fumigation from *Shalakya* procedure room, Minor OT, procedure room*s* of *Balrog,* male and female *panchkarma*. Alternate Dhoopan and fumigation was done on every fortnight up to three months in all these rooms and OT. Then average % with statistical reports, the efficacy of substantial changes in bacterial and fungal count were seen after Dhoopan up to 15 days, when compared with control which is depicted in table no.2. This indicates that the effect of *Dhoopan* was reserved up to one week. On the basis of the result of the present study, it may be said that the microbial concentration will be under control by procedure of *Dhoopan*.

Table No. 4: Statistical analysis of microorganisms pre vspost Maheshwar Dhoopan

Parameter	Group Name	Mean	Std Dev	SEM	t value	P value
Shalakya	Pre MD	28.00	3.367	1.683	8.981	P = <0.001
	Post	6.00	3.559	1.780		
Shalya OT	Pre MD	65.500	35.351	17.675	2.627	<0.039
	Post	16.000	13.038	6.519		
Male PK PR	Pre	46.250	5.965	2.983	8.648	<0.001
	Post	12.250	5.123	2.562		
Female PK PR	Pre	20.250	6.898	3.449	2.535	0.044
	Post	9.500	4.933	2.466		
Balrog PKPR	Pre	25.500	5.802	2.901	4.800	0.003
	Post	11.250	1.258	0.629		

Table No. 5: Statistical analysis of microorganisms Bacillocid before & after study

Parameter	Group Name	Mean	Std Dev	SEM	t value	P value
Shalakya	Pre fumigation	33.750	5.315	2.658	5.974	<0.001
	Post	10.500	5.686	2.843		
Shalya OT	Pre	62.750	31.700	15.850	2.751	<0.001
	Post	18.750	4.272	2.136		
Male PK PR	Pre	47.500	3.512	1.756	7.289	<0.001
	Post	21.750	6.131	3.065		

Parameter	Group Name	Mean	Std Dev	SEM	t value	P value
Female PK PR	Pre	25.500	5.745	2.872	1.436	0.201
	Post	14.500	14.201	7.100		
Balrog PKPR	Pre	31.00	7.348	3.674	4.477	0.004
	Post	13.00	3.266	1.633		

AS per the data is shown in Table no 2 and 3, both the groups post counts are significant as compared to pre colonies count of micro-organisms but the post MD count when compared by Paired t test with post Bacillocid group, it was observed that in some procedural rooms, MD count was not significant over Bacillocid group count as depicted in table no. 4.

Table No. 6: showing statistical outcome of post MD vs post Bacillocid fumigation

Parameter	Number	Mean difference	Std Dev	SE	t value	P value
Shalakya	04	4.500	6.952	3.476	1.295	P = 0.286
Shalya OT	04	2.750	8.995	4.498	0.611	P = 0.584
Male PK PR	04	9.500	1.915	0.957	9.922	P = 0.002
Female PK PR	04	5.000	18.074	9.037	0.553	P = 0.619
Balrog PK PR	04	1.750	3.096	1.548	1.131	P = 0.340

It was observed that the post fumigation, residual effect was good than Bacillocid and it remained up to one week.

Discussion

There are 3 types of *Dhoopan- Dhoop, Anudhoop & Pratidhoop*, total *Dhoopas* mentioned by *Acharya Kashyap* are 40 for different indications such as to, progeny to human beings, to cure all diseases & to eradicate grahas means *Rakshoghna*efficacy i.e. bacteriocidal or antimicrobial function8,9. Inspite of age-old method of fumigation, traditional *Dhoopan* is a neglected method for sterilization thus taken for this study as comparative experiment with popular Bacillocidinstead of prior reputed formalin which is banned recently. In this study, both the groups were highly significant in pre-post comparison of individual group but when compared to post MD with post Bacillocid control group, MD which is a trial group was not much significant.

Probable Action of MD: All ingredients of MD are showing the evidence of *Krimighna* properties, so after burning the ingredients of MD, smoke was filled in the tight closed room and later room was kept closed overnight to disinfect the microbial load by its *Rakshoghna* characteristic. As per the study by Ananthakumar and Dahikar in 2009 the same result was achieved. According to Tambekar in 2010, Sumitha et al -2015 and Rathi et al in 2020 all these ingredients are having virucidal, bactericidal, fungicidal, mycobactericidal and non-toxic compounds. On the other hand, Bacillocid is a broad spectrum microbicidal, corrosion inhibitor and cleanser. It has long residual effect,used diluted solution and microorganisms are non-resistant to it but the main concern is that it contains 30 % formaldehyde which is a carcinogenic chemical. Therefore, in spite of not much significance with bacillocid, MD is safe, cost-effective and having a good residual action also.

Conclusion

In the nut shell, the study performed up to four consecutive months, MD is more effective as compared to Bacillocid fumigation in different procedure rooms and minor operation theatre as both groups are highly significant ($P<0.001$) but post study residual effect was more than Bacillocid. Also, MD is safe, economic and very effective solution in sterilization to protect from microbial load as well as nosocomial infection. Time has come to change the chemical based synthetic and toxic agents with herbo-mineral or animal origin safe

compounds for sterilization the high-risk area of hospital. The double benefit is that these *Dhoop dravyas* are also beneficial in therapeutic aspect.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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The Study Protocol of "Pharmaceutico Analytical and in Vitro Antimicrobial Activity of *Gunjadya Taila* Prepared by Using *Murchchhita* and *Amurchchhita Tila Taila*"

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Abstract

Background: In this modern era life is full of glamour and faced many health related problem. In modern science there are huge numbers of drugs said for treatment of diseases but these drugs get resistance to that particular disease. Traditionally medicinal plants have been used for many years as topical and internal administration in the treatment of fungal and bacterial diseases. In *Rastarangini Gunjadya Taila* mentioned in *Kushta and Kandu*. In Ayurveda all skin diseases are involved in *Kushta Roga*. Skin diseases mainly caused by microorganism as per modern science such as *Staphylococcus* aureus, streptococci and Candidiaalbican etc. Hence to provide scientific data and statistical validation the study on pharmaceutical analysis and in vitro antimicrobial activity of *Gunjadya Taila* prepared by *Murchchhita* and *Amurchchhita Tila Taila* is undertaken.

Objective: To study antimicrobial activity of Gunjadya Taila prepared by using *Murchchhita* and *Amurchchhita Tila Taila*.

Material and Method: *Gunjadya Taila* will be prepared in *Rasashastra* department by two method one with *Murchchhita Tila Taila* and other with by using *Amurchchhita Tila Taila*. The prepared drug *Gunjadya Taila* will be evaluated for organoleptic characters and physicochemical parameters. *Gunjadya Taila* will be assessed for antimicrobial effect on gram positive bacteria,gram negative bacteria and fungal group. Inhibition of microbial growth will be investigated by diffusion method.

Observation and Results: The data will be assessed for its antimicrobial and antifungal effects by using ANOVA test & unpaired 't' test.

Conclusion: This research work on *Gungadyataila* will show all analytical parameters within range and significant effects on gram positive and gram negative bacteria.

Keywords: *Gunjadya Taila, Kushta, Skin Disease, Antimicrobial activity.*

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Introduction

Traditionally medicinal plants have been used for many years as topical and internal administration in the treatment of fungal and bacterial diseases. Medicinal plants are considered as new resources for producing agents that could act as alternative to antibiotics in the treatment of fungal and bacterial diseases. Skin disease is a common ailment affecting all age groups[1]. Skin conditions such as purities, scabies impetigo, eczema

and molluscumcontagiosum ranged from the 2nd to 11th leading cause of disability in India. Skin conditions were account the fourth leading cause of nonfatal disease burden at global level and affect nearly 900 million population throughout world[2].Skin infections categorized as primary or secondary. Primary infections have specific characteristic morphologies, are originated by lone organisms and routinely occur in normal skin. They are often caused by *Staphylococcus aureus*, *Streptococcus pyrogenes* and coryne form bacteria and responsible to develop erythrema, impetigo, boils and folliculitis. Secondary infections initiate in diseased skin as a superimposed form. e.g. Impetigo and toe web infections[3].There are various topical and systemic synthetic drug available in the market but they have various adverse effect.

Ayurveda is a complete and holistic traditional health-care system of India that contains both preventive and therapeutic aspects. Bhaishajya (drug) is the pride and part of Ayurveda and recognized as one of the piller of therapy used to conquer the deadly diseases[4]. In modern science there are huge number of drugs said for the treatment of diseases but these drug get resistance to that particular diseases. There are large number of drugs said in Ayurvedic literature.In *RastaranginiGunjadya Tail*is mentioned for the treatment of*Kushta and Kandu*[5].In ayurveda all skin diseases are involveed in *KusthaRoga*. As per modern science skin disease caused by micro-organisms such as stapphlococcus aureus, strepto- cocci and candidiaalbican. Hence to provide scientific data and statistical validation the study on pharmaceutical analysis and antimicrobial activity of *Gunjadya Taila* prepared by *Murchchhit*and *Amurchchhittilataila* is undertaken.

Rationale of the study: Skin diseases commonly occur in children and adult scan direct to disability. At global level, it accounts for the 4th leading source of nonfatal disease burden. Various research studies conducted proved that skin diseases can have a major impact on the quality of life of those suffered. Skin cancer and infections are some of the potentially life-threatening skin diseases and involve high costs for the individual and society. In Ayurveda and in modern science,several theories have been put forward describing this disorder. As in modern medical science its treatment is symptomatic with bothersome side effects, there is an ample possibility to work out on its etio-pathology and management aspect of the skin diseases. In Ayurveda, *Gunjadya Tail* is described in skin diseases but its preparative and analytical standards are not established yet .This study will fulfill this gap. This study is an attempt to prepare and standardise *Gunjadya Taila*with *Murchchhita* and *Amurchchhita Tila Taila* and to test its efficacy through antimicrobial activity.

Material and Method

A Pharmaceutical study: This study is related to drug preparation in which three different batches of *Gunjadya Taila* will be prepared to establish pharmaceutical standardization. Pharmaceutical study will be done in following steps;

I. **Procurement of Raw materials:** All required raw materials will be procured from field and authentic reliable sources.

II. **Authentication of Raw materials:** Raw drugs will be indentified and authenticated by Taxonomist. Raw drug will be standardized as per A.P.I.

III. **Process of Taila Murchchhana[6]:** *TailaMurchchhana* will be done as per SOP . Ingredients required for *Taila Murchchhana* mentioned in Table No. 1.

Standard operative procedure of *Murchchhana* of *Tila Taila:* All the collected and authenticated drugs will be cleaned (to remove foreign matter).

Individual

↓

The individual drugs will be then powdered using pulvelizer.

↓

All individual drugs will be sieved through mesh no. 80.

↓

The required quantity of powder will be taken.

↓

Powder of drugs will be mixed with sufficient quantity of distilled water to prepare *Kalka*.

↓

Kalka (1/4th part), *Tila Taila*(1part) and distilled water (4 parts) will be taken in clean and wide mouth steel vessel.

↓

The above mixture will be heated on *Mandagni*(low flame - gently boiling) with frequent

Stirring till *SnehasiddhiLakshanas*appears.

⬇

*Taila*will be allowed to get *SwangSheeta.*

⬇

After cooling *Taila*will be filtered through doubled muslin cloth and stored in air tight glass

Bottle.

⬇

Murchchhita Tila Taila

IV. Purification of GunjaBeejain Godugdha[7]: *Gunjabeeja* (seeds of *Abrusprecatorius*) will be purified as per reference with the help of *Dolayantra* in *Godugdha***.**

V. Preparation of *Bhrungaraja Swaras*[8]: Fresh *Bhrungaraja* leaves will be taken and will be crushed to a paste and finally expressed through white cloth to obtained clear liquid.

VI. Preparation of *Gunjadya Taila*[5]:

Ingredients required for preparation of *Gunjadya Taila* mentioned in Table no 2.

Preparation of *Gunjadya Tail* by using *Murchchhita Tila Tail*

Kalka from *Gunja Beeja & Bhrungaraja Swaras* will be prepared from fresh plant

⬇

Obtained *Gunja Beea Kalka 1part+Murchchhita Tila Taila* (4 part) + *Bhrungaraja Swaras* 16 part

⬇

Will be taken in a wide mouth steel vessel.

⬇

Above mixture will be heated on *Mandagni* (low flame-gently boiling) with frequent stirring till the *Snehasiddhi Lakshans* appears.

⬇

Taila will be allowed to get *Swang Sheeta* (self cooled)

⬇

After cooling *Taila* will be filtered through doubled muslin cloth and stored in air tight glass

Bottles

⬇

Gunjadya *Taila* prepared by *Murchchhita Tila Taila*

Preparation of *Gunjadya Taila*by using A*murchchhita Tila Taila:*

Kalka from *Gunja Beeja & Bhrungaraja Swaras* will be prepared from fresh plant

⬇

GunjaBeeja Kalka 1part + *Amurchhita Tila Taila* (4 part) + *Bhrungaraja Swaras* 16 part will be taken in a wide mouth steel vessel.

⬇

Above mixture will be heated on *Mandagni* (low flame-gently boiling) with frequent stirring till the *Snehasiddhi Lakshans* appears.

⬇

Taila will be allowed to get *Swang Sheeta* (Self cooled)

⬇

After cooling *Taila* will be filtered through doubled muslin cloth and stored in air tight glass Bottles

⬇

Gunjadya *Taila* prepared by *Amurchchhita Tila Taila*

The research drug will be prepared in 3 batches with same quantity of ingredients and same condition one with *MurchchhitTila Taila*and other with *AmurchchhitTila Taila.*

B. Analytical study

a. Subjective Parameters of finished drugs:

Description (Organoleptic characters)[9]: The prepared drug Gunjadya Taila will be evaluated by organoleptic characters.

1. *Sparsha.*(Sensation)
2. *Rupa*.(Colour)
3. *Gandha.*(Smell**)**

b. Objective Parameters of finished drugs:

Description (Physicochemical parameters)[10]

1. Specific Gravity
2. Refractive index at 25^0C
3. Viscosity
4. Acid value
5. Saponification value
6. Iodine value
7. Peroxide value
8. Unsaponificable matter
9. HPTLC or GC-MS (Quantitative)

C. Antimicrobial study[11-13]: Antimicrobial activity is tested byvarious method but in present study Agar well diffusion method will be used.

Principle: The antimicrobial present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organism. The resulting zone of inhibition will be uniformly circular as there will be a confluent lawn of growth . The diameter of zone of inhibition can be measured in millimeter as shown in Table no 3,4,5.

C1: Selection of test microorganism: The bacterial strains used for the experiment will be procured from dept. of microbiology JNMC, Wardha

Selection of test microorganism

(a) Gram Positive bacteria:

1. Staphylococcus aureus
2. Streptococcus pyrogen

(b) Gram Negative bacteria:

1. Escheria coli
2. Klebsiellapneumoni

(c) Fungal group: 1 Candida albicans

C2: Positive control group drugs[14]

For gram positive bacteria - Azithromycin 50ug/6ul

For gram negative bacteria - Ciprofloxacin 30ug/6ul

For candida albicans - Clotrimazole 50ul/6ul

Sample Size:

1. *Tila Taila*-1sample
2. *Gunjadya Tail* aprepared by *Murchchhit Tila Taila*-1 sample
3. *Gunjadya Taila* prepared by *Amurchchhit Tila Taila*-1 sample
4. Gram positive bacteria-2 samples
5. Gram negative bacteria-2 samples
6. Fungal group -1 sample
7. Doses of oil -3 samples
8. Positive control group – 3 samples

Method of data analysis: Statistical analysis will be carried out by institutional statistician. Statistical assessment will be done throughunpaired 't' test and ANOVA test.

Results

Gunjadya Taila will show antimicrobial activity against gram +ve, gram -vebacteriaand fungal group

Table 1: List of ingredients used for *Tila Taila Murchchhana*

Sr No	Dravya	Part to be used	Proportion
1	*Manjishtha (Rubiacordifolia* Linn*)*	Stem	¼ part
2	*Haridra (Curcuma longa.*Linn*.)*	Rhizome	1/4 part
3	*Lodhra (Symplocosracemosus*.Roxb*)*	Stem bark	1/4 part
4	*Musta (Cyperusrotundus* Linn)	Tuber	1/4 part
5	*Amalki* (*Emblica officinalis* Gaertn)	Pericarp	1/4 part
6	*Bibhitaki* (*Terminalia bellirica* Roxb)	Pericarp	1/4 part
7	*Haritaki* (*Terminalia chebula* Retz)	Pericarp	1/4 part
8	*Vatankur (Ficusbengalensis* Linn*)*	Leaf bud	1/4 part
9	Tila Taila		1 part
10	Water		4part

Table 2: Contents of *Gunjadya Taila*

Sr No	Contents	Proportion
1	Gunja Beeja Kalka	1 part
2	Tila Taila	4 part
3	Bhrungaraja Swaras	16 part

Discussion

To treat infections with plants and their extracts is known for centuries and has been practiced all overthe world. As modern medicines are costlier and associated with side effects, herbal medicines are gaining growing interest among the people due to its cost effective and all time availability. In the last three decades, even though pharmacological industries have manufactured number of new antibiotics, resistance to these drugs by microorganisms has been increased. Hence, more and more studies with respect to the use of herbal drugs as therapeutic agents is the need of the hour, especially those related to the control of antibiotic resistant microbes. Hence, this study is proposed to asses pharmaceutico analytical and in vitro antimicrobial activity of *'Gunjadya Taila'* prepared by using *Murchchhita* and *Amurchchhita Tila Taila.*

Conclusion

In vitro study on *Gunjadya Taila* will show significant antimicrobial effect on gram positive and gram negative bacteria.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Ayurvedic Lifestyle Prescription for Autoimmune Disorder W.S.R. Pemphigus Folliculitis: A Review

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Abstract

Summary: Pemphigus folliculitis or Pemphigus foliaceous is one of the autoimmune disorders in which superficial blisters are induced by IgG (mainly IgG4) auto-antibodies which are directed against desmoglein i.e. molecule of a cell adhesion, which occurs in the granular layer of the epidermis in Pemphigus folliculitis, this special response as a result of pathogenic & non-pathogenic antibodies produces specific blisters in this layer of skin. Though it found to be rare in the world's population, it doesn't respond satisfactorily with current established treatment. Considering its pathophysiology, lifestyle modification plays an essential role in its prevention & management. Therefore, this review study is planned with the aim & objective to review the lifestyle modification w.r.s. pathya & Apathya (*Ahara & Vihar*) for Pemphigus folliculitis with *Ayurvedic* perspective. Pemphigus folliculitiscan be correlated with *Visphotaka* (type of *Kshudra Kustha Roga*) in the *Ayurvedic* perspective. The concerned material related to topic has been reviewed from various modern as well as *Ayurvedic* literature,including *Samhita* and numerous review articles in different journals. *Ayurveda* i.e. ancient system of medicine described the psychosomatic platform for the healthy lifestyle including the pharmacological and non-pharmacological measures, even for the prevention as well as management for Kustha i.e. dermatological disorders,which are always found successful in checking over the pathogenesis of this disease. More clinical research studies are expected to prove their efficacy on scientific clinical ground.

Keywords: *Auto-immune; Pemphigus folliculitis; review, lifestyle; Pathya, Apathya; Visphota.*

Introduction

Immunity is an ability of the body to resist the entry of different pathogenic agents like viruses, bacteria, or toxic substances. Layer of skin (Dermis)contains mast cells that secrete histamine and other vasoactive amines and lymphocytes that play an essential immunologic role. Autoimmune disorders are defined as the immune system, which acts against one's own (self) cells where lymphocytes cannot recognize bodies own cells & starts attacking or destroying the cells. Lymphocytes are a part of WBCs, which play a vital role in the defense mechanism of body & protecting the cells from invading organisms. B-Lymphocytes & T-Lymphocytes (types of Lymphocytes) are responsible for the development of cellular & humeral immunity, respectively[1,2].

There are a total of more than 80 autoimmune diseases. Among them, Pemphigus folliculitisis such an autoimmune disease where Bullae/blisters formation takes place on the scalp, which are attacked & destroyed by its antigen.

Need of Study: Pemphigus Foliculitis is a group of chronic autoimmune skin diseases characterized by loss

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of intracellular adhesion of keratinocytes in the upper part of the epidermis, forming superficial blisters of skin & mucous membrane. It is clinically seen that the formation of blisters appears on the skin when rubbed. Dr. Piotr Nikolsky was the first who described this sign in 1896.[3] Pemphigus foliaceous (PF), the reported incidence ranged from 0.6 to 6.8 cases per million persons per year[4].

The conservative treatment of Pemphigus Foliaceus includes steroid drugs like prednisone, Immunosuppressant, Antibiotics, Anti-inflammatory drugs, mainly a drug called Rituximab has shown significant result on Pemphigus folliculitis. However, steroids can trigger dangerous side effects, such as weight gain, mood changes and liver problems. They may also experience drug-related side effects, anxiety or depression and self-consciousness about their skin[5]. Ascauses of this disease are genetic or environmental in nature, therefore It is the need of the hour to search an alternative, effective & safe measures of lifestyle for this disease to avoid environmental factors which trigger the pathology of the disease.

Aim of the study: This article aims to compile & analyze the data concerning the concept of Ayurvedic lifestyle for pemphigus folliculitis.

Material and Method

This articleis based on a review of Ayurvedic literature regarding *Visphotaka* (pemphigus foliaceous) from *Charaka Samhita, Sushrutasamhita* & Some modern textual or books- Prena Sembulingam, Ross & Wilson, etc.

Observations and Results

- **Concept of Pemphigus foliaceous with Ayurvedic perspective:** *Kushta* is the broad spectrum found in *Charaka Samhita* used to describe all types of skin disorders. It is classified as 07 types of *Mahakushta* & 11 types of *Kshudrakustha*[6]. In ancient science, there is narration regarding varieties of causative factors responsible for dermatological disorders including environmental factors, genetic as well as psychological factors. *Acharya Charak* has described,*tvacha* is considered as *'ChetahSamvayi'* i.e.there is a strong positive correlation between *Twak*(skin) and *manas*(mind). *Visphota* is the type of *kshudrakustha*that can be correlated with Pemphigus folliculitis characterized by the formation of small, red, itchy, blisters or pustules or nodular lesion in chest, head & later all overthe skin.
- **Pathology of *Visphotaka:*** *Acharya Sushruta* describes that *Doshaja* and *Karmaja Hetus* in *Visphota,* which is a type of *Kshudra Kushta,*involving vitiation *Pitta* and *Kapha Dosha* which obstructs the *Vata Dosha* by making *Avarana* over it leading to its further aggravation. As a result Vitiated *Vatadosha* get lead to *Tiryaka Siras* with rest of two *Doshas,* & spreading all over the body. At last, all three vitiated humors induces *Kleda* formation in *Tvaka, Rakta, Mansa,* and *Lasika* & produces blister formation .It these vitiated *Doshas* remains untreated, it becomes difficult to treat as pathogenesis enters into the deeper tissues[7].
- ***Ahara-Vihara Hetu* (Etiological factors) of Pemphigus Folliculitis with *Ayurvedic* perspective:** The dietic & behavioral etiological factors of *Kustha* mentioned in table no.1.[7,8].
- **Diet (*Ahara*) recommendations for *Visphotaka* w.s.r. Pemphigus Folliculitis:**

Following therapies includes in the treatment protocol of *Kushtha Roga*in addition to *Yuktivyapashayara Chikitsa* i.e. Pharmacological therapy[10,11,12]:

- *Daivavyapashraya Chikitsa*
- *Satvavajaya Chikitsa*
- Lifestyle modification

As healthy & balanced diet with proper quantity or dietary supplementation (*Hitahara*) is the integral part of the *Ayurvedic* system of medicine which is vividly elaborated by our ancient *Acharyas* to prevent as well as to managea wide range of disorders,especially variousSkin disorders. Such type of wholesome & unwholesome diet i.e. *Ahara & Vihara Pathya* for *Visphotaka* w.s.r. Pemphigus Folliculitis is mentioned in table no. 2 & 3 [13].

Discussion

- **Probable mode of action of lifestyle as mentioned earlier:** According to modern, there is a complex interplay between genetic, immunologic and environmental factors involved in most of the pathogenesis of dermatological disorders and also,significant culprits involved in the etiopathogenesis of dermatological diseases are Psychological stress and related factors. *Mansika*

(psychological factors), i.e., *China, Shoka, Bhaya,*various sinning,anti-rituals and anti-social behavior or activities have a negative impact on the psyche/mind.

Aharaja (diet & dietetic patterns) H*etu of Vishotaka*includes ***Snigdha & Guru Ahara,*** which produces *Dushti* of *rasavahastrotas*. *Acharya Charaka* also described '*GurubhojanamDurvipakanam'* while excessive intake of ***Dravaahara,*** i.e., liquid food, leads to dushti of *Raktavahastrotas*. ***Viruddhaahara*** vitiates *Rakta* and obstructs the body channels (*Srotorodha)* and also forms'*Ama'* due to the state of *Mandagni* (low digestive power).In the patho-physiology of various dermatological disorders,vitiated Rakta is considered as an common & essential factor due to which formation of *Ama* takes place which lead to formation of specific unwanted immunological reaction which support the etiopathogenesis of many skin disorders. The combination of a rich source of protein i.e. both milk and fish, may causea new type of protein molecules, which may result in molecular simulation and generate autoimmune diseases.

According to ancient science, *Nidana Parivarjana* i.e. avoidance of causative factors is half of the treatment responsible for the vitiation of doshas, which slowly progress & forms a disease. If this progression of disease is clogged, then half of the procedure is done. Therefore, It is highly essential to know the fundamental cause of any disease. Complete cure incurable diseases and management of such conditions can only be achieved after *Nidana Parivarjana.*

Considering above said etiological factors & involvement of vitiation of *Pitta* & *KaphaDosha* in the pathogenesis of *Visphoaka*,it is necessary to avoid diet & lifestyle, which vitiates these two doshas.As Ayurveda emphasizes on threefold therapeutic regimes in which *NidanaParivarjana*(avoidance of causative factors) plays a crucial role. *Pitta-Kaphahara*regime helps to maintain the *Dosha* and *DhatuSamvayi* i.e to maintain the homeostasis & modulates the body immunity, which ultimately results into the repairing and regeneration of the different body cells & tissues. It also cleanses the body channels by removing toxins out of the body, which brings equilibrium between *Tridosha & Manasdosha (Raja & Tama)* to obtain beneficial effects for long term.

Proper dietic regimen helps to protect the *Strotas* (channels & ducts) of the body, in a way compatible to the whole system, each cells & tissues of the body to maintain the equilibrium state of body & mind. The main origin of *Kushtha Roga* is from the absence of the harmony between *Doshas*, *Dushya,* as a result of unwholesome diet & lifestyle through *Paapkarma* (sinful activities), i.e., not following *Achararasayana* which is an integral part of *Dincharya*. All activities mentioned in *Achararasayana* exert a positive impact on the mind, which leads to a reduction in stress. Unwholesome *Arthas* (object), i.e. unwholesome *Ahara & Vihara,* which withdrawals or restrain the mind is *Sattvavajaya* (Psychotherapy). Thus, lifestyle modification helps in the prevention & management of the *Visphotaka.*

Table No. 1: Ahara-ViharaHetu of Kustha w.s.r. Pemphigus Folliculitis

S.N	Aharaj Nidana	Viharaja Nidana	Manasika Nidana
1.	Snigdha & Guru Ahara	Papakarma	Chinta
2.	*Drava Ahar,* i.e., liquid food	*Vegadharan* (suppression of natural urges)	Shoka,
3.	Viruddhaahara	*Atapasevan* (excessive exposure of sun)	Bhaya
4.	Frequently intake of *Gramya, Anupa* & *Jaliyamamsa* with milk.	Excessive exposure to air-conditioned	-
5.	Asatmya Ahar	Contradicting with a hot and humid environment	-
6.	Adhyasana	*Ativyayam* (over-exercise) & *Atishrama* (over exertion)	-
7.	Intake of cold water	*Diwaswapa* (day sleep)	-
8.	Praklinnaanna	*Ratrijagaran* (Late night sleep)	-
9	-	Complications of *Panchakarma* therapy	-
10	-	Various sinful, anti-social & anti-rituals behaviors	-

Table No. 2 : Diet (Ahara) recommendations for *Visphotaka* w.s.r.Pemphigus Folliculitis

S.N.	(Pathya)	*(Apathya)*
1	Diet rich in *Tikta Rasa*.	Amla, Lavana and Katu Rasa Pradhana Dravya
2	Purana Dhanya Godhum,Yava, tandoola Moonga, & Masoor	Dairy product, e.g., milk, curd
3	Arhar, Madhu	Guda (Jaggery)
4	*Triphala*, old Ghrita	Heavy diet
5	Single drugs like Patola, Neem, Garlic.	Diet, which aggravated *Kapha dosha.*

Table No. 3: Wholesome behavioral regime (Vihara) recommendations for *Visphotaka* w.s.r.to Pemphigus Folliculitis

S.N.	(Pathya)	(Apathya)
1	Proper sleep	*Vegadharan* (Suppression of natural urges)
2	Avoid stressful activities	*Atapasevan* (Excessive exposure to sunlight)
3	Achara Rasayana	Excessive exposure to air-conditioned environment
4	Dincharaya & Rutucharya Palana	Alternate exposure to excessive hot and excessive humid environment
5	Proper dietic regimen	*Atishrama* (Overexertion) and *Ativyayam*(over exercises)
6	Yoga	*Diwaswapa* (Day sleep)
7	-	Chinta
8	-	Shoka,
9	-	Bhaya
10	-	*Ratrijagaran* (Late night sleep)
11	-	Avoid sinful activities such as anti-rituals and anti-social activities

Conclusion

Shaman Chikitsa, lifestyle modification, dietic regimen, etc. play an essential role in curing any disease and maintain a healthy life. Lifestyle-related factors like proper sleep, surrounding environment,the discipline of food intake, state of mind, energetic activities, etc. play a significant role in the positive & negative impact of health and disease, respectively. Various extrinsic factors such as financial and social status, literacy,social awareness about rights & regulations, family backgrounds, social & family supporting systems, sanitation etc. are some lifestyle-related factors that strongly affects the psychosomatic health of any person. Therefore, such lifestyle modification is significant for the management of any skin disorders, including Pemphigus folliculitis.

Maintaining proper dietic regimen, regular exercise, breaking the causative factor of the disease is the most effective treatment to be followed to suppress the Pemphigus folliculitis and maintainthe normal state of an individual. Various pharmacological & non-pharmacological i.e. *Dincharya, Ritucharya, AcharaRasayana, Rasayana, Sadvratta & Yoga* recommendations of Ayurveda are excellent lifestyle modification for healthy & disease-free life. Ayurveda has exclusively describesthe proper implementation of *Trayaupastambha,* i.e., *Ahara* (diet), *Nidra* (sleep) and *Brahmacharya* (celibacy) in daily life, which are three basic pillars for the promotion of somatic as well as psychological health and better living in patients of Pemphigusfolliculitis.

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Clinical Importance of *Vamana Karma* in *Tamakshwasa*: A Review

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Abstract

Vamana Karma (medicated emesis) is the act of eliminating the vitiated *Kapha* through the mouth. *Vamana Karma* is the internal purificatory practice described in Ayurveda which helps to clean the body of excess amount of the vitiated *Kapha, Pitta Doshas* consecutively. *Tamak Shwas* means a state in which a sense of darkness prevails due to movement of *Prana Vayu* in wrong direction. It is a disease in which the patient experience severe symptoms of respiratory distress resemble like Bronchial asthma. Bronchial asthma is a syndrome of variable airflow obstruction. *Vamana* is the first line of treatment for *Utkleshitavastha* of *Tamakshwasa.* In Ayurveda, *Shaman* and *Shodhana* treatment is mentioned. We can't deny the importance of *Vamana Karma* in *Tamakshwasa*, because *Kapha* is the foremost *Dosha* that needs to be taken care of than others. In this article an attempt has been made to search the research works conducted on *Vamana Karma* related to *Tamakshwasa* and to establish its role in *Tamakshwasa*

Data Source: The related data were collected from various research article from the years 2011 – 2019 and from the classical texts Charak samhita, Sushrut samhita and Astang hrudaya Observations: Total 16 various reaserches found and overall outcome is *vamanakarma* is effective in *tamakshwasa. Vamanakarma* is very effective in *tamakshwasa* in *utkleshavastha* because *kaphadosha* can easily eliminate from body

Keywords: *Ayurveda, Tamakshwasa, Sadyovamana, Bronchial Asthama, Vamana, kapha dosha, therapeutic emesis.*

Introduction

The aim of Ayurveda is to maintain and promote the health of healthy as well as to treat the disease of the diseased person.[1] The five procedures of biopurification *Vamanna* (medicated emesis), *Virechana* (induced purgation), *Basti* (medicated enema), *Nasya* nasal insufflation, *Raktamokshana* (bloodletting) purify the body system by removing vitiated *Dosha* from the body.[2] As far as the *Ritucharyaadhyaya* is concerned in Ayurved *Samhitas,* all the sages have advised *Vamana* as *Samshodhana Karma* in *Vasantritu* for healthy and diseased person.[3] *Vamana Karma* (emesis) is the act of eliminating the vitiated *Kapha* through the mouth.[4] It is indicated in many diseases viz. *Kasa, Putinasa, Shwasa, Kushtha, Amlapitta, Sleepada, Apasmar*a, *Agnimandya, Unmada, Visha, Granthi, Garavisha, Arbud*a, *Stanyadosha, Medo-roga, Atisara, Prameha, Pandu*etc.[5] *Tamakashwasa* constitutes of two words i.e. *Tamaka*and *Shwasa*. The word *'Tamaka'* is a specified source of Dhatu "*Tamglanou*" which means Sadness.[6] The name of *Tamakashwasa* is due to the fact that, the symptoms or attack of this disease hapeen

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suddenly at night and also during the time of attack, the breathing difficulty is so severe that patient feels entering into the darkness (*Tama Pravesh*). Bronchial asthma is a highly prevalent disease and in most of the etiopathogenesis and symptomatologyit is the one of distressing disease and quite common in all age groups. It is a chronic, inflammatory and episodic in nature. It can be severe, sometimes fatal. As per the W.H.O. by the year of 2020, asthma along with COPD will become the third leading cause of death.[7] *Tamakashwas Tamakashwas viz*. dyspnoea, hack and chest snugness are like that of bronchial asthma. Additionally a portion of the etiological elements like presentation to smoke, dust are comparable. As indicated by Ayurveda, rehashed and long haul introduction to different etiological variables. Causes vitiation of the elements of *Pranavayu (*breathing*),* which over some undefined time frame starts basic change *(Sthanvaigunya)* in the lungs. Flexibility and perfection *(Mruduta* justas *Masrunatva)* at the site begin changing to hardness and unpleasantness *(Kathinatva, Kharatva)* which hampers the ordinary narrowing and dilatation process *(Sankocha and Vikasaprakriya).* To battle the change, emission of mucous *(Shleshma)* increments, however further dries due to *Rukshaguna* and close the bronchial spaces *(Srotas avarodh)* diminishing the lumen of bronchus. Breathing gets troublesome and worked *(Alpalpa, Kupita, Sashabda, Sashoola).* After serious hacking a modest quantity of thick and clingy mucous *(Avila, Tantula and Styana shleshma)* is expectorated. This clears the aviation route and patient feels better as he can relax. These sorts of rehashed assaults for a time of over one yearrealize a lasting or irreversible change in the structure of lungs *(Sthanvaigunya).* Subsequently the sickness becomes *'Yapya'* which implies quiet can't be relieved off by prescription.[8] In Ayurveda *Shaman* and *Shodhana* treatment is mentioned. We can't deny the importance of *Vamana Karma* in *Tamakshwasa*,because *Kapha* is first than other humors *Vamana Karma* detoxifies the body & enhance the elasticity of lung tissue & develops natural immunity of the body. Hence, it is the need to review the topic. In this article an attempt has been made to search the researchers conducted on *Vamana Karma* related to *Tamakshwasa* to establish its role in *Tamakshwasa.* Hence the presence review of study was undertaken to critically analyze the roll of *Vamana Karma* in *Tamakshwasa*.

Need of Study: According to the WHO disease will become the third leading cause of death. In Ayurveda *Shaman* and *Shodhana* treatment is mentioned. We can't deny the importance of *Vamana Karma* in *Tamakshwasa*,because *Kapha* is first than other humours *Vamana Karma* detoxifies the body & increases the elasticity of lung tissue & develops natural immunity of the body. Hence, it is the need to review the topic. The present review is aimed at analyzing the clinical importance of *Vamana Karma* in *Tamakshwasa*. All the classical reffrences regarding *Vamamana Karma* & *Tamakshwasa* are taken for the present study. The publish articles regarding *Vamana Karma* and *Tamakshwasa* are from pubmed and scopes are taken

Observations: The observations in the study are broadly divided into two parts. First observation deals with understanding the role of *Vamana Karma* in *Tamakshwasa* based upon the description in various classical literatures and the second is the role of *Vamana Karma* in *Tamakshwasaas* per the data of various research work conducted till date.

Charaka Samhita: Both (Hikka and Shwasa) are overseen indistinguishably because of their indistinguishable etiology area. The patient of *Tamakshwasa* ought to be overseen first with unctuous sudation by the cylindrical, bed and bolus strategy after he is dealt with salted oil. With this, the mucus gets condensed, to move into the channels, goes to the *Amashya* because of this the entry or channels get mollified and *Vayu* come back to its typical course. After full sudation patient ought to be given the eating routine comprising of rice with unctuous substance alongside meat, soup of fish or pig or with the supernatant greasy layer of curd. Along these lines when *Kapha* is expanded, he ought to be encouraged to take emesis blended in with *Pippali*, rock salt and nectar.[9]

Sushrut Samhita: In Sushrutchikitsa Sthan 33rd chapter indications for Vamana is mentioned. [10] Butprocedureof*Vaman*ain*Tamakshwasa*isnotmentioned in detailed.

Astang Hrudaya: In Astanghrudaya Samhita *Mrudu Vamana* is mentionedin Shwasa. After full sudation patient should be given the diet consisting of unctuous substance i.e. meat of boarand the supernatant fatty layer of curd.[11]

Table 1: Studies conducted on *Vamana Karma*

Year	Title of study	Author	Outcome	Type of vamanadravya used
2011.[12]	A comparative study between the efficacy of *Vamana* & *Virechana Karma* in the management of *Tamakashwasa* (Bronchial Asthma).(Mar-2011)	Kundan Gadhvi	*Vamana Karma* is having comparatively better results followed by *Shaman Chikitsa.*	Madanphaladadhi yoga
Sept, oct. 2013.[13]	Scope of *Vamana Karma* in the management of *Tamakshwasa* –A critical review with clinical evidences.	Pillai Vishnu, M Ashvini Kumar.	*Sadyovamana* is very effective in *Vegavastha.*	Madan phalachurna
2015.[14]	A study on the role of *Vamanam* on serum bilirubin and calcium levels.	Vijaya lakshmi Narra	*Vamana Karma* can improve the functional and metabolic activities of the body.	Madanaphala pippali Churna
30.05.2016.[16]	A Holistic Approach of Ayurveda Towards Broncial Asthma.	Kshiteeja Choudhary and Alok kumar Srivastava.	*Vamana Karma* is effective and quick responding in *Tamakshwasa.* So *Vamana Karma* can be best option for *Tamakshwasa* management.	Not mentioned
6.6.2016.[17]	An Ayurvedic Review on management of *Tamakashwasa*	Kimmiseth, Nitesh Anand	*Vamana Karma* is very effective During *Vegavastha.Due to Srothorodha* patient carves for free flow of *Prana Vayu* so it can removed by *Vamana Karma* and free flow of *pranavayu* may occur.	Madanaphalachurna
2017.[18]	Efficacy of *Vamana Karma* with *Ikshvaku Ksheera Yoga* in the management of *Tamakashvasa* (bronchial asthma)	Shweta G. Patil, Anup B. Thakkar	*Ikshvaku Ksheera Yoga* is safe, efficacious and best emetic yoga in bronchial asthma due to its *Samshodhana*, *Bhedaka* and *Kaphanissaraka* properties. Its clinical efficacy on bronchial asthma also suggested that the *Ikshvaku KsheeraYoga* is extremely potent emetic drug to induce self, projectile *Vega* which relieves the symptoms of the disease immediately and it also provides prevention by reducing frequency and severity of symptoms of *Tamakashvasa* for longer duration.	Ikshvakuksheer yoga
1.6.2017.[19]	*Vamana Karma* in *Tamakshwasa*: A case stuey	Aparna Bagul	*Vamana* therapy has substantial role in treating *Tamakshwasa,*not only symptomatically but also to cure the disease from root.	Madanaphala yoga
March 2017[20]	Management of *Tamakshwasa* with *Shodhana* and *Rasayana*: A case study.	Tribhuvan pareekh	*Vamankarma* followed by *Rasayana* is good line of treatment proved in *Tamakshwasa.*	Madanaphala yoga
2017[21]	A Review on Ayurvedic Management of *Tamakshwasa* (Bronchial Asthma)	Dharmaveer Choudhari	*Vamana* therapy is effective in adult patients. *Vamana* with *Snehahana Swedana* liquefies the thick sticky bronchial secretion and help in expectoration (Removal of *SamaKaphaDosha*)	Not mentioned
2018[22]	Evaluation of Efficacy of *Jimutaka* and *Madanaphala Vamana* in *Tamakashwasa*-A Comparative Study	Mayuri Garge and Shweta Parve	*Jimutaka Yoga* is highly significant So,it can be better option for *Madanaphala.*	Jeemutaka and Madanphala
10.07.2018[23]	A Comparative Randomized Clinical Study to Evaluate the Effect of *Sadyo Vamana* and *Simhyadi Kwatha* in the Management of *TamakaShwasa*/Bronchial Asthma	Priyadarshini A.M., Shrilatha Kamath T and G. Shrinivasa Acharya	*Sadyo Vamana* and *Simhyadi Kwatha*, both have shown improvement in almost all the parameters of *Tamakshwasa*.But comparatively *Sadyovamana* have shown better results in *Tamakshwasa*.	Not mentioned

Year	Title of study	Author	Outcome	Type of vamanadravya used
Nov. 2018[24]	*Arkapatri (Tylophoraasthmatica*(Burm. F.) Merrill) in *Tamakashwasa*asa *Vamaka Dravya*: A Case Study	Kavyashree M.R, Hegde Prakash L, Harini A	*Arkapatri Patra Swarasa* has showed significant improvement as an emetic single drug in reducing the symptoms of *Tamakashwasa.*It reduces the requirement of bronchodilator puff.	Arkapatri swarasa.
Dec. 2018[25]	Management of Bronchial Asthma Through Ayurveda: A Critical Review	Pawle Tribhuwansingh, Shukla H.M., Chhajed A.	*Vamana Karma* is having significant result in Balwan (strong) patient of *Tamakaswasa*.	Not mentioned
2.02.2019[26]	*Sadyovamana* in *Tamakshwasaan Atyaikchikitsa* – A case report	Ghanshyam jadhav	*SadhyoVamana* is having considerable importance because its role in treating*Tamakshwasa* is significantly effective.	Lavana jala
Jan. 2019[27]	A Comparative Clinical Study of *Vamana* and *Virechana* in the management of *Tamakashwasa*	Akhilanath Parida, Satyasmita Jena	*Vamana* with *Pippali Churna* statistically significant in *Tamakshwasa.*	Pippali Churna
1.4.2019[28]	The Study of the Efficacy of *Vamana* in *Swastha* and *Rugna* in *Vasant Rutu*	Aparna Bagul	*Vamana Karama* is effective in *Tamakshwasa.*After *Vamana Karma* signs and symptoms of *Tamakshwasa*wasa reduced in that entire *Vasant Rutu.*	Madanaphala yoga

Discussion

Role of *Vamana Karma* in *Tamakshwasa* is described in various classical literatures. Out of *Bruhatrayee,* in Charak *Samhita Adhyaya* 17th there is adetailed description of *Vamana* procedure in *Tamakshwasa.* But in Sushrut *Samhita* it is not mentioned. In Sushruta *Samhita adhyaya* 33rd of *Purvardha Chikitsa sthan* while describing the indication of *Vamana* there is description of *Shwasavyadhi.* However, the process of *Vamana Karma* not mentioned separately *Tamakshwasa*. In Ashtangh hrudayam *Mrudu Vamana* is mentioned briefly.

16 studies have been conducted on the role of *Vamana Karma*in *Tamak Shwasa*, out of 16 studies 7 are clinical studies, 4 are case studies and 5 are review studies. In this studies, different type of *Vamana Dravyas* are used such as *Madanphala dadhi*, *Madanphalachurna*, *Ikshwaku ksheer Madanaphala yoga,Jeemutaka churna Arkapatri swaras, Lavana jala, Pippalichurna* as an emetic drug. *Vamana Karma* is radical treatment to treat *Kapha* malady just as *Tamakashwasa. Vamana karma* treats the *Tamakshwasa* by dispensing with causative factor *Kapha* from its fundamental site of amassing. *Vamana* scrubs the various sorts of harmful materials from the body. *Vamana Karma,* one of the purging treatments reestablishes the *Agni* (impeded digestion) by acting at cell level.[29]

Vamaana Karma or *Sadyo Vamana* can be the choice treatment or procedure in *Vegakalinavastha*. In *Vegagavastha doshas* are in excited stage means ready to expell out from the body. So *Abhyanga and Swedana* followed by *Vamana Karma* are useful for *Vilayana* of this *Grathitha Kapha*, thereby removing the *Sanga* (Obstruction of airway). Once the *Kapha* is removed from airways, it flows back to its base in *Amashaya* from where it i sexpelled out by *Vamana*.[30] Susruta also explained the *Chikitsa* of *Shwasa* is *Urdhva Shodhana* in *balvan* patient that is *Mrudu Vamana* after *Abhyanga*and *Swedana.* Whereas Vagbhata explains that *Utpattisthan* or the place of origin of this *Vyadhi* is *Amashaya*, srotas involved here are *Prana, Anna* and *Udakavaha* and the *Vyaktasthana* being *Ura*which happens because of obstruction of normal movements of *Vata* by *Kapha*.

Both *Sadhyo Vamana* and *Sneha Poorvaka Vamana* are havingequally good effect in relieving the signsand symptoms of *Tamakashwasa* but *Sadhyo Vamana* can be planned only when *Utkleshkshana* is present in the patient. Though the immediate relief rate was almost equal in both *Sadhyo Vamana* and *Sneha Poorvaka Vamana* it can be clearly noted from the above available data that there lief rate obtained just after *Vamana Karma.*

A central emetic drug induces vomiting by directly stimulation of the vomiting centre. These drugs also

stimulate gastric muscles, diaphragm, G.I.T. and also increase peristalsis. Their action is more prompt. Instead of these factors, some other factors also play role in the process of emesis. During the underlying episodes of emesis, the substance of the upper gastro-intestinal tract up to the pylorus alone comes out. Rehashed emesis makes a negative weight inclination towards the lumen of the upper gastro intestinal tract while hypertonic saline water causes a positive osmotic slope towards the lumen. The two reason a constrained extraction of effectively separable substances from the cells of the lumen and those containing waste metabolites arranged close by incidentally. Along these lines, unreasonable lipid that was devoured during the preliminary days blebs into the lumen with the water-solvent substances, which are separated out because of the osmotic slope. Rehashed emesis likewise causes the age of the negative weight slope up to them.

Limitation of the study: Present work includes data obtained from researches conducted in last 9 years i.e. 2011 to 2019 only

Conclusion

From this review it can be concluded that *Vamana Karma* is having significant clinical importance in *Tamakshwasa*. *Sadyovamana* is also having very much importance without any *Snehana Karma* because of already *Utklisthavastha* of the *Dosha* in the disease.

Future studies can be conducted on large number of patients of *Tamakshwasa* to establish its efficacy and mechanism of action.

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The Prime Role of Preventive Approach of Ayurveda in Autoimmune Disorder with Special Reference to Vitiligo

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Abstract

Vitiligo is a skin disease with unknown etiology and has unpredictability on the extent and rate of spread. It is considered an autoimmune disorder that destroys melanocytes in the skin characterized by depigmentation or hypopigmented patches. Vitiligo patients feel distressed and suffer social stigma as it has a significant impact on the quality of life of patients. Treatment available in contemporary medicine has its limitations and side–effects. Hence, maintenance of health and prevention of disease is of utmost importance. *Ayurveda* has incorporated this condition into broad terms of *Shwitra,* which is described under the heading of *Kushta*. *Ayurveda* states the causes of *Shwitra* may be *Chhardivegadharan*, *Atisewan* of *Amla–Katu-Ushna–Lavan Aaharsewana, Virrudha Ahar* and *Atimatra Bhojan* along with *Navanna Sewana* and *Dadhi Sewana*. Hence, a multidisciplinary approach is needed to treat such conditions with lifestyle medications and dietary interventions. *Ayurveda* has a holistic and scientific method in health management. It emphasizes much more on diet and regimen, along with medicines. *Ayurveda* deals with *Pathya –Vyavastha* in a very scientific way. Here an attempt has been made to compile the knowledge of ancient holistic science to throw light upon the concept of *Ayurveda* regarding the modification of lifestyle and the preventive measures effective concerning Vitiligo which will be a subject of benefit to humankind along with the awareness of the fact that the judicious use of food and healthy lifestyle interventions are the real medicines for health, so early responses are necessary for better results.

Keywords: *Shwitra, Autoimmune Disorder, Vitiligo.*

Introduction

Vitiligo is a dermatological disorder with unknown etiology in which skin loses its color in blotches and develops white patches causing cosmetic imbalance, mainly leading to asocial stigma. It is considered an autoimmune disorder that destroys the melanocytes of the skin. The factors responsible for the healthy skin color are hemoglobin, carotenoids and melanin pigments. It is an autoimmune disease in which due to the insufficiency or complete absence of melanin in the skin, hypopigmentation occurs along with the autoimmune destruction of melanocytes in the epidermis resulting in the progression of the disease with hypo-pigmented areas. Though the exact cause is unknown,factors like stress trigger the illness, including the increased levels of catecholamines and neuropeptides[1].

Need of the current era: The numbers of patients suffering from Vitiligo are not only affected physically but also suffer from social stigma and are psychologically

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under stress. However, it affects approximately about 0.5% to 2% of the worldwide population, with an overall prevalence of about 1%. It appears to be equally in men and women. The extent and rate of spread is unpredictable[2].

The present-day lifestyle has a high impact on the disease condition. It affects the quality of life of an individual as it leaves behind cosmetic disfigurement. The treatment modalities for Vitiligo in conventional medicine involve topical and systemic steroids, topical and systemic PUVA, which brings forth side effects such as phototoxicity, hyperpigmentation and solar elastosis. Other safer alternatives are highly expensive and thus have limitations to use.

Aim and Objective: Considering this scenario & considering its increasing incidence & prevalence rate due to the current stressful lifestyle, it is necessary to focus on a healthy, stress-free lifestyle. Therefore, the prime of this paper is to throw light upon the concept of *Ayurveda* regarding lifestyle modification and preventive measures practical concerning Vitiligo, which will be a subject of benefit for humanity.

Material and Method

Textual references are compiled from the *Ayurvedic* texts and *Samhitas*, moderntexts, journals and websites.

Observations and Results

- **Concept of Vitiligo with Ayurveda perspective:** In *Ayurveda*, Vitiligo can be correlated with a group of a skin disorder i.e., *Shwitra. Acharya Kashyapa* defines *Shwitra* as *Shweta Bhava* Micchanti[3]. As per *Acharya Sushruta*, it means a reflection of white color. and mentioned it as *Kilas* instead of *Shwitra*[4].
- **Role of *Nidana Parivarjana* in prevention & management of Vitiligo:** The ancient classical texts have mentioned the etiological factors of *Shwitra* as that of *Kushta*. The treatment of *Shwitra* needs a holistic approach which involves *Nidan Parivarjan,* which means avoidance of the etiological factors. It has been given prime importance behind the success of many treatments in *Ayurveda.* The basic principle-‘ prevention is better than cure’ is the first step in the management of *Shwitra*. Therefore, the first step is to avoid the *Nidana*. Various *Hetu* related to *Ahara* & *Vihara* depicted in *Ayurvedic* literature in *Shwitra Nidana* are mentioned in table no. 1[5,6,7].

Acharya Charaka has mentioned some specific causes which might cover the idiopathic aspects such as-*Vachamsi Asatyani* (telling lies)*, Krutaghnabhava* (un-grateful), *Suranam Ninda* (abusing gods), *Guru-Gharshanam* (abusing and disrespecting the teachers and elders), *Papakriya* (being involved in sinful acts), *Purvakruta Karma* (sins done in a previous birth) and *Virodhi Anna* (incompatible foods). The current faulty lifestyle compromises all of these *Hetu,* which are highlyessential to avoid the occurrence of *Shwitra.*[8].

- **Role of lifestyle in the prevention & management of Vitiligo:** *Ayurveda* ensures the lifestyle that depicts the bundle of behavioral and mental aspects of well–being. If anyone follows a healthy diet and lifestyle, no medicine is required to tackle the disease. The controlled diet itself works as a medicine. On the contrary, if someone is not following such diet & lifestyle, no medication will work because improper diet and lifestyle are the prime cause of diseases. In the present era, man has less time to cook and eat food and has replaced it with fast food, which is considered *Mithya Ahar* in *Ayurveda*, a significant cause of disease formation.

The prevention & treatment of Vitiligo needs a holistic and scientific approach that emphasizes diet and regimens and medicines. *Ayurveda* deals with *Pathya Vyavastha* (planning of food) in a very scientific way. Even healthy lifestyle interventions have gained scientific evidence in the treatment of diseases that seem to be as effective as medications and are considered safe without side effects.

- **Importance of *Pathya Apathya:*** The chief foundation upon which an individual is raised depends on *Ahara* and *Vihara. Ayurveda* focuses on diet and regimen as leading causes for diseases. In our *Ayurveda Samhitas,* the phenomenon is implied to *Pathya-Apathya*in terms of *Ahar*and *Vihara*. Once the treatment is adopted, it becomes essential to utilize the dietary rules for efficient results. *Ayurveda* mentions about *Pathya- Apathya* regarding *Ahar* and *Vihar*, which are suitable to the mind and body when followed appropriately. These measures help in correcting the morbid conditions of the *Dhatus* and bringing them to a hemostatic state. Hence *Dhatu Samyata*is established and the disease can be cured, avoiding further complications. *Pathya–Apathya Ahhara* & *Viharas* applicable for *Shwitra*[9] have compiled in table no. 1 & 2.

Table No. 1: *Samanya & Vishishta Nidana* of *Shwitra*

S.N.	Samanya Nidan	C.S	S.S	A.S
A	Aharaj Nidana			
1	Haviprashana	+	-	-
2	Intake of *Drava, Snigdha, GuruAhara*	+	-	-
3	Mithya Ahara	-	+	+
4	Ahitashana	-	+	-
5	Asatmya Bhojana	-	+	-
6	Navanna Atisewana	+	-	-
7	DadhiAtisewana	+	-	-
8	MatsyaAtisewana	+	-	-
9	Lavana, Amla, Atisewana	+	-	-
10	Virrudha Ahara	+	+	+
11	Intake of *Gramya, Anupa, Audak Mamsa* along with milk	-	+	-
12	Intake of *Mulak* with milk	-	+	-
13	Intake of *Guda* with milk	+	-	-
14	Intake of *Madya, Amla Dravya* with milk	+	-	-
15	Intake of *Matsya, Nimba* along with milk	+	-	-
B	Viharaja Nidana			
1	Chhardi Nigraha	+	+	-
2	Vegavarodha	+	+	-
3	Sheetoshna Vyatyasa	+	-	-
4	*Sheetambu Snana* after *Atapa Sewana*	+	-	-
5	Mithya Vihara	-	+	+
6	Diwaswapna	+	-	-
7	Ratrijagarana	-	-	-
8	*Vyavaya* after *Vidahi Ahara Sevana*	-	-	-
9	Dharma, Shrama, Bhayartanam Dritam Sheetambu Sewana	+	-	-
10	*Vyavaya* in *Ajeerna*	+	-	-
C	Anya Nidana			
1	Vipram Gurun Gharshayatam	+	-	-
2	Papakarma	+	+	+
3	Brahamana, Stree, Sajjana Vadha	-	+	-
4	Purvakrita Karma	-	+	-
5	*Sadhu Nindaand Vadha*	-	-	+
6	Go-Hatya	-	-	-
D	Chikitsa Sambandhinidana			
1	Snehashchaticharato	+	-	-
2	Santarpana–Apatarpana Vyatyaya	+	-	-
3	Panchakarma Apacharinam	+	-	-
4	*Snehapeetasya, Vantasya Va Vyayama* and *Gramyadharma Sevana*	-	+	-
5	Snehadeenam Ayatharambha	-	+	-

*C.S- Charak Samhita, S.S.-Sushrut Samhita, A.H.- Ashtang Hridaya

S.N.	Specific causes of *Shwitra*	C.S	S.S.	A.S
1	*Vachamsatathyani Kritagnabhava* or *Sinful Deeds Related* to *Vak* and *Manas*	+	-	-
2	Sura Ninda	+	-	-
3	Gurugharshana	+	-	-
4	Papakriya	+	-	-
5	Poorvakrita Karma	+	-	-
6	Virodhi Anna	+	-	-

*C.S- Charak Samhita, S.S.-Sushrut Samhita, A.H.- AshtangHridaya

Table No. 2: *PathyaAhara–Vihara* in *Shwitra*

S.N.	Pathya Ahara–Vihara	C.S	S.S.	A.S
A	PathyaAhara			
1	Shashtikashali	+	+	+
2	Yava	-	+	+
3	Godhuma	-	-	+
4	Shyamaka	-	-	+
5	Koradusha	-	-	+
6	Uddalak	+	-	-
7	Mudga	-	+	+
8	Adhak	-	+	-
9	Masoora	+	+	+
10	Nimbapatra	-	+	+
11	Arushkara	-	-	+
12	Priyangu	-	-	+
13	Mandooka Parni	+	-	+
14	Khadira Kashaya Pana	+	-	+
15	Patola	-	+	+
16	Brihati Phala	-	+	-
17	Chakramarda	-	+	-
18	Meshashrungi	-	+	-
19	Koshataki	-	+	-
20	Tila	-	+	-
21	Punarnava	-	+	-
22	Go, Khara, Ushtra, Ashwa Mootra	+	+	-
23	Sarpi	+	-	+

S.N.	Pathya Ahara–Vihara	C.S	S.S.	A.S
24	Tikta Shaka	+	-	-
25	Bhallataka	+	-	-
26	Triphala	-	-	+
27	Jangala Mamsa	-	+	-
B	Pathya Vihara			
1	KhadiraJalaParisheka	+	-	-
2	Virechana	+	-	-
3	Aragvadhadi Kashaya, Udwartana	+	+	-
4	KhadirodakAvagaha	-	-	+
5	VajrakTailabhyanga	-	-	+
6	Vrata	-	-	+
7	Yama	-	-	+
8	Dwija, Sura Pooja	-	-	+
C	**Seasonal regimen (**Regular *Shodhana)*			
1	Vaman	Given at every 15th day		
2	Virechan	At every 1 month		
3	AvapidanNasya	At every 3rd day		
4	Raktamokshan	At every 6 months		
5	Yogic practices	Daily		

*C.S- Charak Samhita, S.S.-Sushrut Samhita, A.H.- Ashtang Hridaya

Table No. 3: *Apathya Ahara–Vihara* in *Shwitra*

S .N.	Apathya Ahara–Vihara	C.S	S.S.	A.S.
A	Apathya Ahara			
1	Guru anna	+	+	-
2	Drava anna	-	+	-
3	Guda	+	+	+
4	Dadhi	+	+	+
5	Dugdha	+	+	+
6	Matsya	-	+	-
7	Masha	-	+	+
8	Tila	+	+	-
9	Navanna	-	+	-
10	Anoopa Mamsa	+	-	+
11	Maricha	-	-	-
12	Amla, Lavana Ras	-	-	+
B	Apathyavihara			

S .N.	Apathya Ahara–Vihara	C.S	S.S.	A.S.
1	Papa Karma Sinful Acts	+	+	-
2	Diva NidraSleeping During Day Time	+	+	-
3	Virrudhashana	+	+	-
4	Vyayama Physical Exercises	-	+	-
5	Vegavarodha	-	+	-
6	Vyavaya	-	+	-

*C.S- Charak Samhita, S.S.-Sushrut Samhita, A.S.- Ashtang Sangraha

Discussion

The healthy lifestyle and dietetics are to be followed to create a balance by restoring a uniformity in the skin tone, texture & color.

- **Avoidance of *Ahara Nidana***

- ***Viruddhaahara*:** Certain diet and wrong combination, which interrupts the tissues' metabolism and has different properties, is referred to as *Viruddha Anna* or an incompatible diet. The food in the false conjunction with wrong processing and being consumed in the incorrect quantity at the wrong time is *Virrudha*[10].
- ***Guru, Snigdha, Drava Ahara*:** It includes *Santarpanjanya, Krimikara Ahara.* Theguna of *guru Ahara Dravya*are opposite to that of *Agni*. Hence it is *Agnihrasakara* according to *Hrasahetu Vishesh Siddhanta*[11].
- ***Kshira*:** Acts as *Viruddha Ahara* with various combinations like fish[12].
- ***Kulattha:*** *Ushna, Amlapaki,* causes *Kapha–Pitta* aggravation[13].
- ***Matsya*:** *Bahudoshkara*, vitiates *Rakta*[14].
- ***Mulak*:** Riped *Mulak* is *KatuVipaki, Ushna Virya*. *Tridoshkara,Guru*, and *Abhishyandi*[15].

- **Avoidance of *Vihara Nidana***

***Papakarma*[16]:** Behavioural misconduct,anti-social activities,sinful activities bring about psychogenic stress, which is important in pathogenesis as the stress triggered Vitiligo include the increased levels of catecholamines and neuropeptides.

- **Yogic practices (therapy):** Vitiligo is a condition in which the body status reaches a stage called autoimmunity where the melanocytes get in the arena of dormancy or become dead. Yoga detoxifies body and mind and copes up with the condition of autoimmunity, thereby maintaining healthy melanocytes.

Ayurveda has a different approach to the understanding and treatment of *Shwitra*, which requires further researches. However, lifestyle modification and healthy dietetics can contribute significant benefits to the patients of Vitiligo. Thus, the holistic approach of *Ayurveda* for treatment, including its lifestyle prescriptions, is pooled to the mainstream of world medicine today. It can bring a transformation in the quality of health care for the suffering of humanity all over the world.

Conclusion

In today's lifestyle of hurry and worry,stress is increasing day by day. Modern medicines are suitable for short term treatment but have their limitations. It is the need of an hour to have researched and advance the physical and psychosocial aspects of Vitiligo.

Ayurveda is practiced in daily life; it fulfills both the preventive as well as curative aspects. In *Ayurveda*, the treatment is highly emphasized to achieve *Dhatu Samyata*. It can be concluded that by avoiding the use of incompatible diet,faulty lifestyle,and *Pathya Ahar Vihar* and *Shodhan Chikitsa*, mainly *Vaman* & *Mridu Virechan*, it can be managed along with this *AchaaraRasayan* and *Sadvritta Palan* which have high relevance in modern society.

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Management of Single Rooted Mandibular Second Molar with Single Canal: Two Case Reports

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Abstract

Background: The root canals of mandibular molar teeth show a wide variation in its system ranging from single to multiple canals. Thorough understanding of the variations present in internal anatomy of teeth is imperative for favorable outcome of the endodontic treatment. The insight of the tooth morphology, variations present related to its root structure, shape and information regarding root canal, with number of orifices and canal configuration, all this should be assessed prior to commence the endodontic treatment

Case Presentation: The present case report describes the variation in second mandibular molar, which is infrequently mentioned in literature. Variation present may not always be in the form of extra canal, it can be in the form of fewer or lesser canal than what is usually seen. On inspection of the pulpal floor with endodontic explorer, a single round orifice was seen at the centre of the floor of pulp chamber. Numerous angulated radiographs were obtained to assure the presence of independent single canal. Individual canal preparation was carried out using ProtaperNiTi instruments (Maillefer, Dentsply) till F5. Using lateral condensation technique along with sealapex sealer, obturation was carried out.

Conclusion: Knowledge of the number of root canals present within the tooth is imperative for successful root canal treatment. Some teeth may show extra canals, while other may show fewer than normal number of canals present. Mandibular second molar may present with single canal and this should be promptly recognized. Single canal identification helps to conserve the tooth structure by eliminating the need for removal of excessive tooth structure in search of extra canal.

Keywords: *Mandibular second molar, Root canal variations, Single canal, Single Root, One canal.*

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Introduction

The root canal system presents one of the most complex anatomies in the human body. The thorough understanding of tooth anatomy is prerequisite for favorable long term prognosis of the root canal therapy. The insight of the tooth morphology, variations present related to its root structure, shape and information regarding root canal, with number of orifices and canal configuration, all this should be assessed prior

to commence the endodontic treatment.[1] The routine intraoral periapical radiographs, radiovisiography and recently three dimensional imaging modality such as Computed beam tomography helps us to visualize the otherwise hidden root canal system.[2] The Endodontic literature has constantly discussed the variations in the form, configuration and number of canals present. The variations present in multirooted teeth are most commonly noticed in the number of canals present. The mandibular molars are one of the most common teeth to present with this variation. According to Vertucci FJ, the second mandibular molar is nearly same as the first mandibular molar with only difference observed in root length (shorter) and canal curvatures (more curved).[3] Mandibular second molars generally present with two clearly distinct roots, mesial and distal root. Mesial root mostly presents with two canals and a distal root with one or two canals. Any variation from this should be promptly recognized as it may affect the outcome of the endodontic treatment.[4]

Maggiore C et al. (1998) reported that lower second molar could range from one to three roots.[5] Manning SA (1990) studied the morphology of second molar and reported incidence of single root in 22%, two roots in 76% and three roots in 2 % of the teeth examined.[6,7] Weine F.S et al (1988) studied extensively the mandibular second molar for canal variation and he stated that amongst the entire molar, this tooth is most common to show the anatomical variation. He reported single canal in only 1.3% of the cases. Single Root and Single canal, though present, is an unusual and rare occurrence in the mandibular second molar.[8]

The purpose of this case report is to report occurrence of single canal in single rooted mandibular second molars that required endodontic therapy.

Case Report 1: A 27-year-old female reported to the dental OPD with complaint of pain in the lower right back region of jaw from past 10 days. Pain was localized, sharp and constant type. It got aggravated on intake of cold and associated with posture and relieved on intake of medication. There was no history of swelling, pus discharge and fever. Medical history was not significant. On examination of the area of complaint, occlusal caries was seen with 47. Tooth was tender on percussion and non-mobile. Radiographic and neural sensibility investigations were carried out. On radiographic examination of tooth 47, caries was found to be involving enamel dentin and pulp chamber and fused root with single canal was appreciated. (Fig -1)

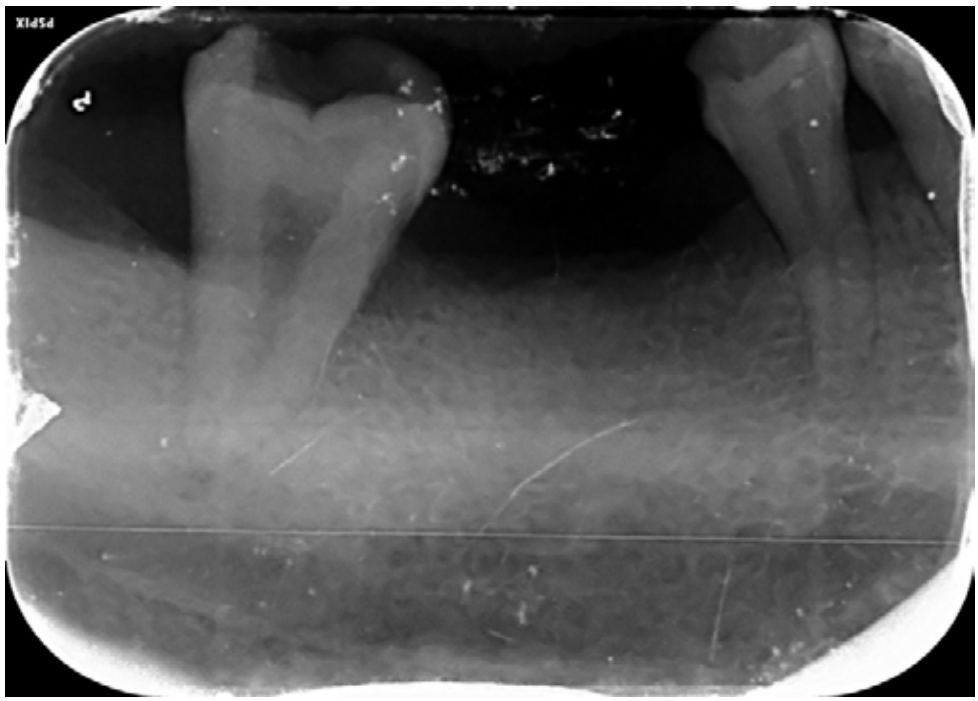


Figure 1- Pre-operative Radiograph

Periodontal ligament space around the roots was found intact with widening of the ligament. On neural sensibility test, the affected tooth showed an exaggerated lingering response to pulp testing. The clinico-radiographic diagnosis was symptomatic Irreversible Pulpitis with Apical Periodontitis with 47, indicating the need of endodontic therapy. The treatment was then initiated; right IANB anesthesia was injected with 2% lignocaine with 1:100,000 adrenaline. Rubber dam was used to isolate single tooth. Caries excavation was done with 47. Access cavity preparation was done with BR-45 (Mani Inc, Japan) round bur and modified using EX-24 (Mani Inc, Japan). Pulp tissue in the floor was removed and canal was copiously irrigated with 5.25% NaOCl(Chloraxid 5.25%, Cerkamed, Poland) for hemostasis. On inspection of the pulpal floor with endodontic explorer, a single round orifice was seen at the centre of the floor of pulp chamber. (Fig 2).



Figure 2: Access opening showing single canal

Cavity walls were extended slightly to check for any other canal, but only single canal was present. The canal patency was obtained using a no. 10 K- file. Numerous angulated radiographs were obtained to assure the presence of independent single canal. Working length was determined using apex locator and confirmed with the help of radiograph.(fig 3)

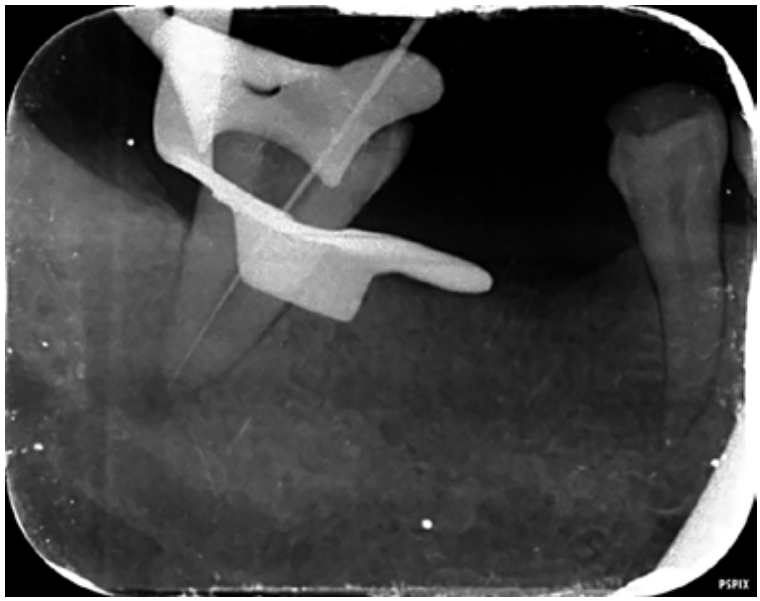


Figure 3: Working length determination

Individual canal preparation was carried out using ProtaperNiTi instruments (Maillefer, Dentsply) till F5. Profuse irrigation was done with 5.25% NaOCl and EDTA alternatively with normal saline. Calcium Hydroxide as an intracanal medicament was placed for 5 days. Intracanal medicament was removed using ultrasonic tip. Canal was again irrigated with 5.25% NaoCl and EDTA alternatively with normal saline. Final flushing with chlorhexidine was done followed by drying of canals using absorbent points. Master cones were selected. (Fig 4).

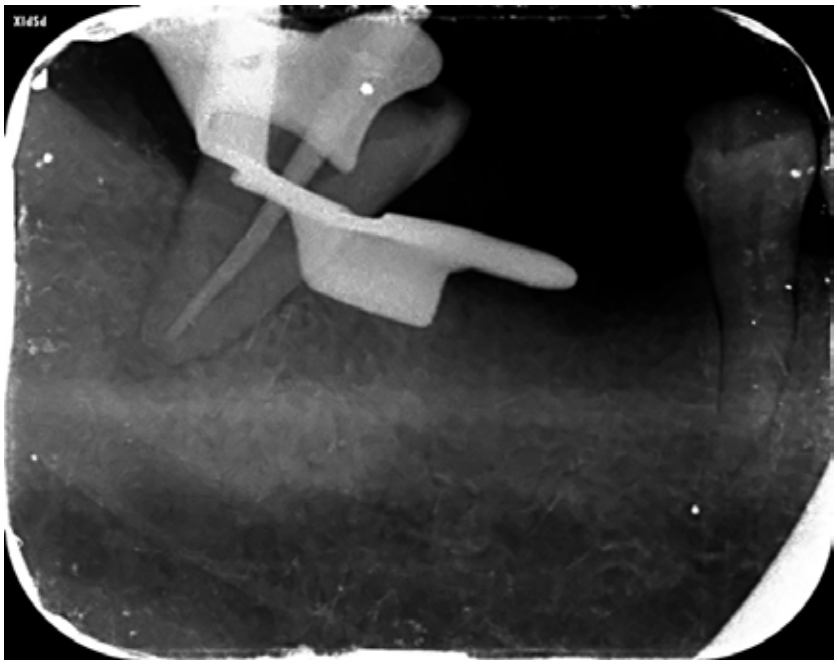


Figure 4: Master cone selection

Using lateral condensation technique along with sealapex sealer, obturation was carried out. (Fig 5 and 6). The post-endodontic restoration was done using composite resin restoration.

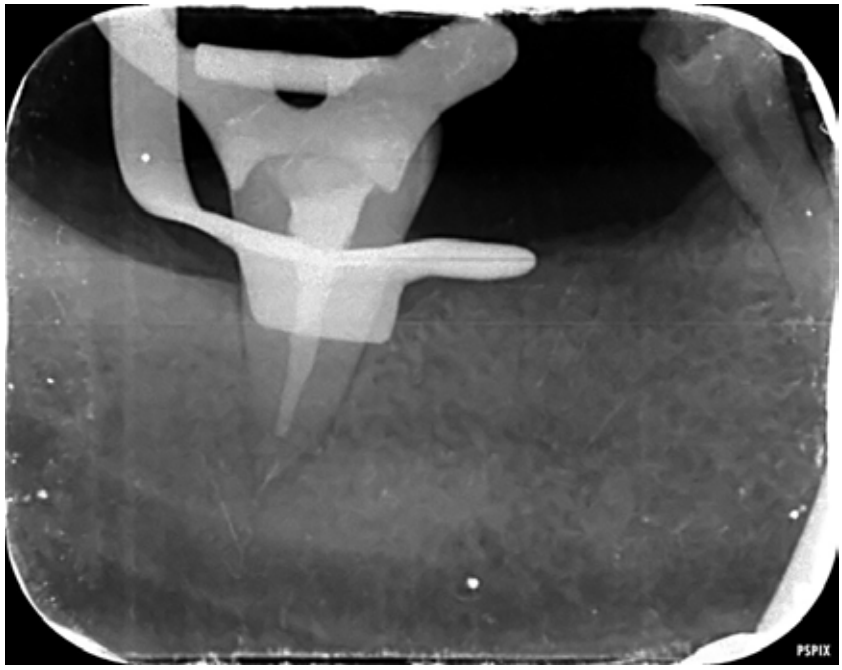


Figure 5: Obturation

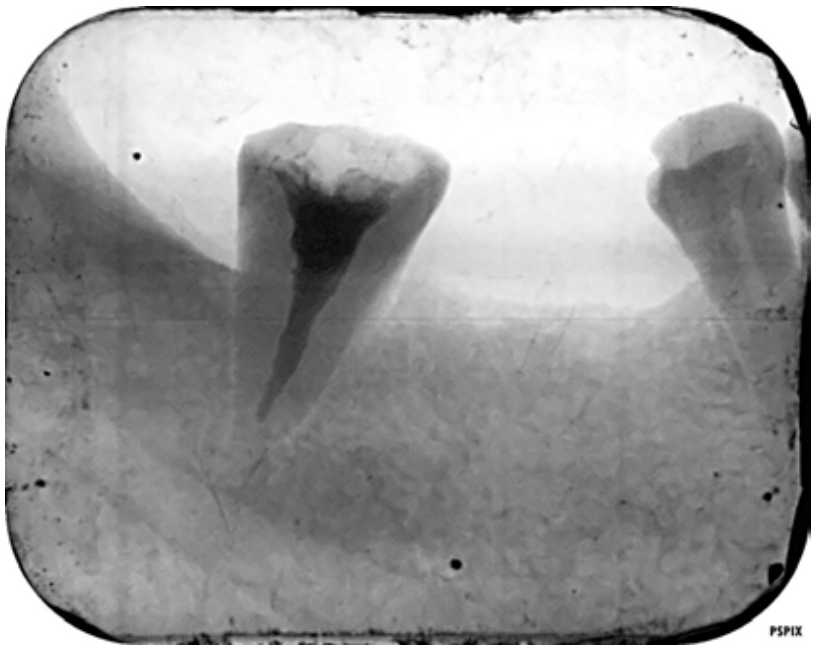


Figure 6: Inverted image showing complete filling of canal

Case report 2: A 30-year-old male reported to the dental OPD with complaint of pain in the lower right back region of jaw since 5 days. On examination of the area of complaint, mesio-proximal caries was seen with 47. Tooth was tender on percussion and non-mobile. Radiographic and neural sensibility investigations with 47 were carried out and root canal therapy was suggested for same. On radiograph, fused root with single canal was appreciated. (Fig 7). After access cavity preparation similar single canal configuration was seen. The further technique performed was similar as discussed above. (Fig 8).



Figure 7: Pre-operative radiograph



Figure 8: Obturation

Discussion

The endodontic therapies of multi-rooted teeth are often challenging, mainly due to existence of countless variations of root canal system. The knowledge of this complex root canal morphology is essential for success of endodontic therapy since missing of even one canal can lead to failure.[9] The diversities in multi-rooted teeth are seen in terms of number of roots, root canals, ramifications, apical deltas.[10] The pattern of such complexity is determined by age, sex and ethnicity of individual.

In case of mandibular molars, mandibular 2nd molar shows more variation than mandibular 1st molar. One of the deviations includes fusion of roots. The external morphology of mandibular 2nd molars seems to be conical or square shaped. The buccal or lingual aspect of fused roots shows presence of radicular grooves. The extensions and depth of grooves decides the internal canal anatomy. If groove is shallow, single wide canal is expected whereas if groove is deep, C-shaped anatomy is expected.[11]

In single rooted mandibular 2nd molar, the root canal may have following presentation: single wide canal, two canals that may or may not join and c- shaped canal. Pansiera & Milano found 6 out of 102 (5.88%) mandibular 2nd molar having single root and single root canal.[12] The present two case also showed single root and single canal configuration. Sabala et al. stated that "the more rare the aberration, the more probable that it is bilateral."[13] Hence suspecting similar morphology on contralateral side, periapical radiograph was evaluated. But no such configuration was found on contralateral side. Fava et al reported rare existence of a single canal in all four 2nd molars.[14]

For diagnosis of such cases periapical radiograph is commonly used. However, limitation of such 2D vision is well documented. The 3D radiography such as CBCT allows studying external and internal morphology. With aid of CBCT, the differentiation of shallow groove with single wide canal and deeper groove with c-shaped canal is possible. In search of canal in fused molar, possibility of perforation is higher. This can be eliminated with proper illumination and magnification and minimally invasive technique.[15,16,17] Since the canal is wider and tapering, the chemo-mechanical preparation should be such that it should be able to eliminate all the debris. The obturation technique used can be cold lateral condensation, warm lateral, thermo-plasticized technique. The thermo-plasticized gutta percha offers better adaption to canal irregularity however, in present case conventional gold standard lateral condensation has been used for obturation.

Conclusion

Knowledge of the number of root canals present within the tooth is imperative for successful root canal treatment. Some teeth may show extra canals, while other may show fewer than normal number of canals present. Mandibular second molar may present with single canal and this should be promptly recognized. Single canal identification helps to conserve the tooth structure by eliminating the need for removal of excessive tooth structure in search of extra canal.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Management of Calcified Root Canal: A Case Report

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Abstract

Background: Calcification of root (calcific metamorphosis) is usually seen as defensive reaction of pulp to trauma. Intensity of trauma/hit, time span since trauma, pulp response to trauma are multiple factor deciding degree of calcification. Calcification may be seen partially or entirely in root canal. Usually calcified tooth remain asymptomatic, but when tooth becomes symptomatic endodontic treatment need to be done. Treatment of calcified canal is challenge to dentist.

Case report: A female patient reported to the department of conservative dentistry and endodontics at sharad pawar dental college with chief complain of pain in upper front region of jaw. On radiographic examination calcified root canal was associated with upper anterior teeth. Root canal treatment was planned for calcified tooth. Access opening was carried out using BR45. To achieve patency within canal file#8, 10 were used, but initally patency could not be achieved due to calcified canal. With help of chelating agents 17% EDTA liquid and gel and small size K file #8,10 canal negotiation was carried out to achieve patency. Working lengh was then determined and canal was instrumented. Naocl, EDTA was used as intermittent irrigant. Final rinse was done using 2%CHX. Canal was obturated using cold lateral condensation technique followed by permanent restoration. Entire procedure was carried out under magnification.

Conclusion: Calcified canal treatment is bit tedious then routine endodontic procedure. But it can be managed if procedure is carried out under proper illumination, magnification, CBCT, understanding root canal anatomy, use of dye, modified access bur, files, chelating agent, irrigants and irrigation activation system.

Keywords: *Calcified canal, Negotiation, Glide- path, chelators.*

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Introduction

Goal of endodontic treatment is to completely eradicate micro-organisms and disinfect root canal. This is obtained by biomechanical preparation, use of irrigants, intracanal medicament.It is difficult to access complex root anatomy, calcified canal for complete disinfection. Calcification of root (calcific metamorphosis) is usually seen as defensive reaction of body to trauma. Intensity of trauma/hit, time span since

trauma occurred, body's response are multiple factor deciding degree of calcification.[1] Calcification may be seen partially or entirely in root canal. Locating root canal in such teeth is a tedious job. As there are chances of perforation, excessive dentin removal. Present case report enlighten about endodontic management of calcified canal in upper central incisor.

Management: Local anesthesia was administered "2% lignocaine with 1:80,000 epinephrine".[2]Access preparation was done with high speed round bur (BR 45) and modified using EX24 bur. No canal orifice was visible upon access opening initially. On probing catch was present more towards lingually. File. No. 6 K file was inserted to confirm the opening of canal. File was in canal but not upto working length. File was 5 mm short of working length due to calcification of canal. No. 6 K file got bind at middle third of root. so to negotiate the canal till apex, filling with file # 6 along with use of chelator 17% EDTA gel and EDTA liquid was done. Same procedure was done in canal to create glidepath with No. 8 & 10 K Files & Path Files. working length was recorded using apex locator and confirmed on PSPIx, WL- !7mm .Complete Chemico-Mechanical Preparation was done using Step - back technique by hand K-files upto 80 No. K file, Master apical file was 55 No. k file. 3.5% sodium hypochlorite (Prime dental) and saline was used for irrigation during RCT. Final rinse by 17% EDTA (Prime Dental) followed by 2% CHX (Neelkanth). Master cone fit was taken. Sealapex sealer was coated in canal using lentulospiral and obturated using cold lateral condensation technique.[3,4,5,6] Post endodontic restoration using GIC (Fuji II) was done.[7,8]

Discussion

Mechanism behind canal calcification is not clear. It is believed when trauma occurs it leads to disruption of blood vessel. Clot formation occurs which acts as source for calcification in root canal. 4 - 24% teeth show varying degree of calcification due to trauma. Radiographically loss of pulpal space, discoloration of teeth is suggestive of calcification in teeth. [9] If such teeth is symptomatic treatment of following tooth come into high difficulty class according to " American Association of Endodontists Case Assessment criteria".[10] Pulp chamber and root canal is usually shifted more towards lingually in calcification of upper anterior teeth. Access cavity preparation should be made such that excessive loss of dentin is avoided.[11] Law of color change, i.e pulp chamber will be more darker compared to root dentin. This will help to identify entry in pulp chamber. [12]Use of CBCT, dental operating microscope for treatment of calcified canal is boon. ultrasonic tips to negotiate calcified root canal, use of bur with long shank are used for troughing and to locate calcified canal. Detection of orifice by use of methylene blue dye or champagne bubble test under microscope are useful to locate canal orifice. [13]Multiple angulated radiograph should be taken in deep access preparation to ensure central alignment and no loss of excessive dentin or perforation. ([14] Negotiation of calcified canal, narrow constricted canal is a challenge toward dentist. Usually negotiation is done with use of file #6,8,10 which has small diameter therefore used as pathfinder files. Due to small diameter these files lack rigidity and may fracture if excessive watch winding force is applied. So to prevent fracture of this file and further complication alternate use of file # 6,8,10 should be done.[15,16] Gentle watch winding motion to file with slight push pull motion should be used. Files should be checked before insertion and discarded when sign of distortion appears. Many different file system for negotiation of calcified canal have been introduced. Such file has quadrangular cross section to increase rigidity of file to prevent its breakage.[17] Decalcifying, chelating agents and irrigants should be used during biomechanical preparation to soften canal dentin and facilitate passage of filelike 17 % EDTA gel, 17% EDTA irrigant .[18] Calcification usually occurs from corona to apical direction. Therefore if file is negotiated well at coronal and middle third, instrumentation in apical third becomes easier.[19] Schindler & Gullickson stated if negotiation of canal is not achieved and tooth is symptomatic. root end resection under microscope should be treatment of choice.[20,21,22]

Conclusion

Prognosis, success of root canal treatment rely on complete disinfection till apex. In case of calcified canal to achieve negotiation use of various file system, irrigants, chelating agents, modified bur and procedure under dental operating microscope renders successful treatment of calcified tooth.

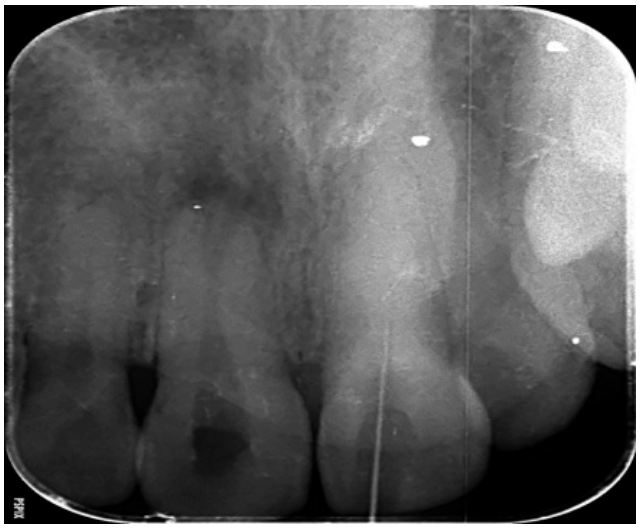


Fig 1- File #8 Short of apex 6-7mm

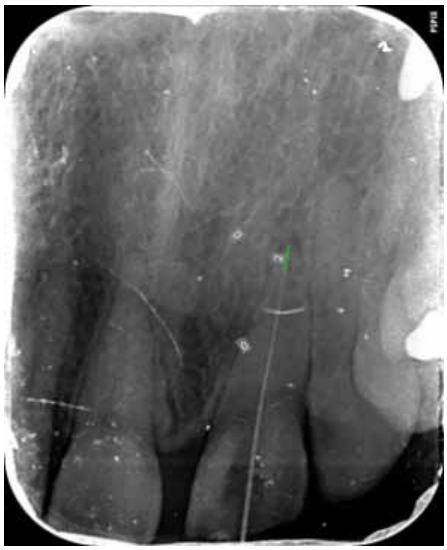


Fig 2- File #8 short of apex 2mm



Fig 3- File #8 negotiated till apex



Fig 4- GP fit with 21



Fig 5- Obturation with 21

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Management of Deep Carious Lesion with Single Visit Indirect Pulp Capping: A Case Report

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Abstract

Background: Preservation of pulp vitality is of utmost importance during management of deep caries cases. There remains a possibility of pulp tissue to repair upon excavation of caries with no pulpal exposure. However, in case of exposure of pulp, such type of minimally invasive management becomes doubtful & impulsive. Indirect pulp capping(IPC) treatment plays an essential role in these cases to prevent pulpal exposure.

Case Presentation: This case report describes the single visit indirect pulp therapy in mandibular molar. Patient reported with a chief complaint of food lodgement and mild intermittent pain. There was no history of night pain and/or spontaneous pain. The clinical and radiological evaluation revealed no peri-radicular pathosis and no pulpal involvement. The tooth was examined for vitality using electric pulp test and revealed vital pulp. The treatment was done with the protocol of Single Visit Indirect Pulp therapy using light cure calcium hydroxide and cavity was disinfected with 2% chlorhexidine. Cavity was lined with Glass Ionomer Cement and final restoration was done using Composite resin restoration. The patient was followed up till 6 months and showed satisfactory outcome eliminating the need for more invasive approach as root canal therapy.

Conclusion: Indirect pulp therapy is a safe, economical and proven treatment option in the teeth that have not involved pulp and with sound peri-radicular tissues. The success of Indirect Pulp Capping relies on proper case selection and diagnosis, complete isolation, caries removal and disinfection with selection of suitable pulp capping material.

Keywords: *Indirect pulp capping, indirect pulp therapy, single visit indirect pulp capping, calcium hydroxide, pulp capping, deep carious lesion.*

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Introduction

Deep carious lesion has always been the major topic of attraction.[1] The diagnosis of actual condition of pulp is complicated by the overlying caries, hence it is difficult to precisely diagnose the tooth vitality.[2] Preservation of pulp vitality is of utmost importance during management of deep caries cases. There remains a possibility of pulp tissue to repair upon excavation

of caries with no pulpal exposure. However, in case of exposure of pulp, such type of minimally invasive management becomes doubtful & impulsive. Also there will be a significant decrease in favorable outcome in situations of pulp becoming infected & pulp exposure during excavation.[3]

Indirect pulp capping (IPC) treatment plays an essential role in these cases to prevent pulpal exposure. This type of management could be single step or 2 step procedure or stepwise caries excavation.[4,5] Indirect pulp capping, in cases where removal of entire caries would result in pulp exposure, it requires infected dentin removal & leaves behind affected dentin layer. Both IPC & final restoration of teeth can be carried out in a single appointment.[6] This was recommended from the data from some earlier studies which checked the tooth which undergone indirect pulp capping & was again reopened. The studies came to a conclusion that there was hard demineralized dentin showing no signs of progression of caries.[7] A superfluous risk for pulp exposure might be present during re-entering the cavity for entire removal of caries in 2nd appointment.[8] Therefore single visit indirect pulp capping is indicated wherein the entire procedure in carried out within single appointment and tooth is not revisited again. For pulp protection, remineralization can be induced by placing calcium hydroxide(bactericidal or bacteriostatic liner) on carious dentin, which is considered as a traditional procedure for indirect way of pulp treatment.

Calcium hydroxide is white, odorless powder, less soluble in water & has increased ph.[9] If remaining thickness of dentin is minimum of 0.5mm without any pulpal exposure, the relevance of acidic conditioner on either enamel or dentin would not lead to irreversible pulpitis. [10,11]

Case Report: A 30 year old male patient came to dental OPD with complaint of food lodgment in lower left back region of jaw since 1 month which was associated with intermittent pain. The pulpal status & periradicular tissues were suspiciously examined. When clinically examined, adjacent gingival tissue was normal in appearance & occlusal caries involving enamel as well as dentin was noticed. No peri-apical radiolucency was observed and no periodontal ligament widening was seen. (fig 1) Pulp sensitvity was found well-matched with reversible pulpitis which was confirmed by using thermal test. On application of cold stimulus pain disappeared quickly.



Figure 1 Pre-operative Radiograph

The steps followed during treatment was: The procedure began with the scaling of teeth, subsequently IAN block was given (2% lignocaine 1:2,00,000). Rubber dam isolation was done in the operative field. #6 Round diamond bur along with water coolant was used to remove the infected carious dentin.(fig 2).



Figure-2 Caries removal

Spoon excavator was used to remove the peripheral carious dentin which was followed by using a low-speed round carbide bur which was well-matched in accordance to the size of cavity. Careful caries removal was performed such that only the wet infected dentin was removed with the dry affected dentin left. Careful inspection of the cavity was done so as to remove the complete carious tissue and check for pulpal exposure, if any. Further cavity was disinfected by using 2% chlorhexidine (Consepsis, Ultradent) for 60 seconds. Then the placement of a layer of light cured $Ca(OH)_2$ over the affected dentin was done & cured for 40 sec. (fig 3).



Fig-3 Placement of calcium hydroxide

After that mixing and placement of a layer of GIC, GC Fuji II was over light cured calcium hydroxide was performed. Excess material was removed from the walls & margin.(fig-4).



Figure- 4 Placement of GIC

Sterile dry cotton pellet was placed in the cavity & patient was asked to wait for 15 min for GIC to set. After removing the cotton pellet, tooth was etched with 35% phosphoric acid gel (Scotchbond™ Etchant, 3M/ ESPE) for 30 seconds followed by rinsing and drying. The application of bonding agent (Single Bond™, 3M/ ESPE) was done as per manufacturer's instructions. Incremental technique was used for placing composite increments (Filtek Z250™, 3M ESPE) and each increment light cured for 40 second. (fig5)Occlusion was checked followed by removal of rubber dam.



Figure 5 Composite restoration

Follow-up: After 1 month follow up, on clinical examination & evaluation on radiograph,the results were constant with regular vital pulp. 6 months and 1 year followed up also showed satisfactory result.

Discussion

Indirect pulp capping treatment is opted after the thorough clinical examination and radiographic assessment. The clinical examination should reveal no frank pulp exposure, sensibility test showed positive response and radiograph excluded the peri-radicular pathosis. In the present case, the radiographic images & clinical assessment pointed towards the indirect pulp capping treatment.

Material which could be placed on the dentin still remains a topic of discussion. Materials having bacteriostatic or bactericidal action are applied over affected dentin, for example: zinc oxide eugenol, calcium hydroxide & glass ionomer cements. The use of calcium hydroxide has demonstrated to arrest caries lesion by its action on bacterial activity and growth.[12] Kiranmayi G et al conducted a systematic review and meta-analysis and concluded that calcium hydroxide proved similar results as MTA when used for Indirect Pulp treatment. MTA and calcium hydroxide both demonstrated favorable outcome for this indirect pulp modality.[13] In deep cariesthe success rates of indirect pulp capping using $Ca(OH)_2$ liner ranges from 92%-97%.[14] It was found that the IPC with $Ca(OH)_2$ liner and a consequent high-quality sealing of margins decreased the substrate for micro-organism, reduced progression of lesion and allowed a physiological response in the pulp-dentin complex.[15]

Glass Ionomer cement was placed over calcium hydroxide. It has been suggested that ionomer cement helps to arrest caries and provide antimicrobial effect. Glass Ionomer release fluoride ions and it has thermal expansion variable very close to that of tooth structure and is biocompatible.[16] The permanent restoration can be done with composite resin restoration so as to provide adequate strength at the time of mastication and esthetic results. The restoration with resin composite should be placed so as negligible or minimum amount of microleakage will be anticipated.[17]

Conclusion

Indirect pulp therapy is a safe, economical and proven treatment option in the teeth that have not involved pulp and with sound peri-radicular tissues. The key determinants for successful indirect pulp capping are accurate case selection, adequate isolation, disinfection of the cavity, caries restriction and selection of suitable pulp capping material.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Case Report-Primary Renal Lymphoma: A Rare Entity

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Abstract

Background: Kidney involvement is an under-recognized complication of both Hodgkin and non-Hodgkin lymphoma (NHL).[1,2] Many patient presents with acute kidney injury in newly diagnosed NHL, but patient being diagnosed with primary renal NHL is very rare. As it rarely occurs, it may not be considered initially during patient presentation. In patient presenting with idiopathic kidney failure along with non-specific pain in abdomen, primary NHL must be kept in mind. Below we present a patient who presented with pain in abdomen and was later diagnosed as a rare case of primary renal NHL.

Case History: A 65 year old male presented with the chief complaints of pain in abdomen since 2 months and history of nocturia 3-4 times at night and dribbling of urine since 1 month. There were no significant findings on general and systemic examination. CT scan revealed a solitary renal mass of 90 × 85 × 61 mm in upper pole of left kidney. Patient underwent Radical Nephrectomy and on histopathology specimen was diagnosed as - Infiltration of Non-Hodgkins lymphoma in kidney. Primary renal NHL.

Conclusion: Primary involvement of kidney in NHL is extremely rare entity which should be always kept as differential diagnosis in case of radiological suspicion of primary renal mass. NHL is diagnosed on histopathological grounds and can be first proven by performing renal biopsies in order to start chemotherapy which is the main modality in treatment of PRL. Recent immunohistochemical tests allow correct diagnosis and also helps in classifying renal lymphomas.

Keywords: *Non-Hodgkin lymphoma, NHL, Primary renal lymphoma, renal lymphoma, B cell lymphoma.*

Introduction

Kidney involvement is an under-recognized complication of both Hodgkin and non-Hodgkin lymphoma (NHL).[2] Various lymphoma-related renal manifestations makes diagnosis difficult: while abrupt worsening of kidney function may be the first sign of malignant disease, renal effects can be subtle or even silent. The etiologies of renal involvement are similarly varied. Primary renal lymphoma (PRL) is rare and is B cell NHL.[3]

There are many patients who presents with acute kidney injury in newly diagnosed NHL, but patient being diagnosed with primary renal NHL is very rare. As it rarely occurs, it may not be considered initially during patient presentation. In patient presenting with idiopathic kidney failure along with non-specific pain in abdomen primary NHL must be kept in mind. Below we present a patient who presented with pain in abdomen and was later diagnosed as a rare case of primary renal NHL.

Case History: A 65 year old male presented with the chief complaints of pain in abdomen since 2 months

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and history of nocturia 3-4 times at night and dribbling of urine since 1 month.

He stated that the pain was present in the umbilical region, continuous dull aching type which aggravated after heavy meals and was non-radiating.

During general examination, the patient was afebrile, moderate GC with all normal vital signs.

There were no palpable cervical, axillary and inguinal lymph nodes. No was no pallor, icterus, clubbing, cyanosis or pedal edema.

On systemic examination, cardiovascular, respiratory, central nervous system were within normal limits.

On per abdomen examination- the abdomen was soft, non-tender, no palpable lump was felt with normal bowel sounds.

Per rectal digital examination was done which revealed enlarged prostate of grade II with normal anal tone. Different laboratory tests and ultrasound examination were carried out in view of above history and examination. Serum urea, creatinine, electrolytes and hemogram were within normal limits. Urine microscopy was normal with no albuminuria and glycosuria.

On ultrasound of abdomen and pelvis well-defined hypoechoic lesion measuring 81 x 68 x 66 mm was seen in left hypochondriac region medial to upper and mid pole of left kidney with good internal vascularity. Suggestive of Renal cell carcinoma arising from upper pole of left kidney or Retroperitoneal mass lesion (Lymphoma). Mild Prostatomegaly (32 cc with median projection noted)In view of the sonographic findings, contrast-enhanced computed tomographic (CECT) scan of the abdomen and pelvis was done.

Routine CECT abdomen and pelvis along with CT Urogram for the evaluation of lymphoma was performed which revealed a solitary renal mass of 90 × 85 × 61 mm was seen in upper pole of left kidney also involving the suprarenal region with distortion of the normal renal architecture lesion demonstrated moderate contrast enhancement and the left adrenal mass was not seen separately. Left renal artery is seen traversing through the mass without any infiltration. Renal vein was normal. No thrombus was seen in IVC. No hydronephosis or mass effect was noted. Enlarged left para-arotic lymph nodes were also seen. Suggestive of retroperitoneal lymphoma/renal cell carcinoma.

Diagnostic laproscopy was done where, enlarged left renal mass with para- aortic lymphadenopathy was seen intraoperatively.

Radical Nephrectomy was planned and specimen was submitted for histopathological examination.

On gross examination left kidney containing diffuse greyish white mass of size 92 x 91 x 65 mm sparing the peripheral portion (0.5 cm) of kidney.

On cut section, diffuse infiltrative greyish white mass seen involving the major portion of kidney.

On microscopy it shows small round cells with condensed chromatin with round to slightly irregular nucleoli of size 6- 12 micron and scanty cytoplasm admixed with mature lymphocytes, arranged in diffuse fashion infiltrating interstitium around the renal tubules.

Diagnosis- Infiltration of Non-Hodgkins lymphoma in kidney. Primary renal NHL.

Discussion –

Our patient presented with abdominal pain and symptoms of benign Prostatomegaly. His histopathological reports of radical nephrectomy concluded to be Primary NHL of kidney or Primary renal lymphoma (PRL).

Primary renal lymphoma accounts for <1% of cases of extranodal lymphoma. [4]

The tumour is mostly a B cell NHL of intermediate or high grade.[5]

PRL is an infiltrating tumour defined as NHL which is originating from parenchyma of kidney and not invading from an adjacent lymphomatous mass. It does not causes disruption of architecture or function. [6]

Its origin is uncertain and debatable as kidneys normally lack lymphatic tissue. In the development of PRL different pathological mechanism have been proposed which suggests the origin from renal capsule having rich in lymphatics or parenchymal invasion from perinephric fat, or the origin can be from lymphocytes existing in chronic inflammation which may be responsible for causing lymphomas.[1,5,7–9]

It is important to differentiate a primary renal lymphoma with a secondary renal involvement from a

primary retroperitoneal lymph node lymphoma. Primary renal lymphoma on histopathological findings show diffuse lymphomatous infiltration of monomorphous population of lymphocytes in the renal interstitium. While secondary involvement of kidney is frequent in NHL, generally presenting with features of renal failure owing to different histopathological renal pathologies such as membranoproliferative glomerulonephritis, crescent glomerulonephritis, minimal-change disease, intraglomerular large B-cell lymphoma e.t.c.[5]

It affects patients of middle age or older age group. The diagnosis of PRL is confirmed on histopathological examination. Mostly the disseminated cases of lymphoma are seen to have a renal involvement as secondaries. However, the frequent findings seen in PRL is acute renal failure, abdominal pain and mass in renal area. [6]

Renal metastasis can resemble renal lymphoma. Renal cell carcinomas have hypervascular enhancement pattern which helps in differentiating it from renal lymphoma. Transitional cell carcinoma and severe pyelonephritis can be confused with diffuse renal infiltration by lymphoma.[10]

Therefore, lymphoma must be kept as a differential diagnosis whenever there is a tumour seen around renal pelvis or ureter, even if there is no abnormality in the renal parenchyma or abdominal lymphadenopathy.

Nephrectomy is considered as the mainstay of treatment modality in patients of renal mass. In contrast, chemotherapy is considered as a standard therapy in cases of primary renal lymphoma. Thus, despite of its rare occurrences, it is very essential to differentiate PRL from carcinoma.

Conclusion

We conclude that the diagnosis of PRL is generally done by radiological assessment, however it is important to have a histological confirmation in order to start chemotherapy which is the main modality in treatment of PRL. Primary involvement of kidney in NHL is extremely rare entity. NHL is diagnosed on histopathological grounds and can be first proven by performing renal biopsies. Recent immunohistochemical tests allow correct diagnosis and also helps in classifying renal lymphomas.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Gastrointestinal Stromal Tumourmetastasis in Lymph Node: A Case Report

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Abstract

Background: GIST are the tumours originating in the gastrointestinal tract with a high malignant potential and the chance to metastasize and recur. They are widely studied due to their distinctive biologic behavior, clinicopathological manifestations, molecular mechanisms and treatment modalities. They can affect any part of gastrointestinal tract or extraintestinal sites. Most GISTs stain positive for KIT, CD117 andanoctamin-1(DOG-1). Tumorresection along with Imatinib therapy is said to be mainstay of the treatment.

Case Presentation: Here by presenting a case of a male aged 60 years operated for cholecystectomy and intraoperatively found to have a solitary nodule over the stomach, which was later found out to be GIST metastasis to a lymph node on histopathology.

Conclusion: GIST has a low incidence and rare tendency to metastasize to lymph node; therefore it is important to study clinical importance to lymph node in this malignancy. Lymph node dissection should therefore be kept in mind during resection of GIST.

Keywords: *Gastrointestinal Stromal Tumour, Interstitial cells of Cajal, Metastasis*

Introduction

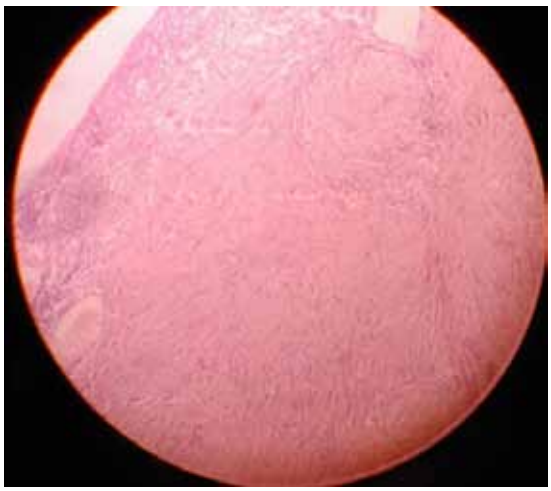
The Gastrointestinal Stromal Tumor (GIST) is a rare mesenchymal tumor of gastrointestinal (GI) tract. The tumour has a peak inclination towards metastasis to liver along with recurrence in the peritoneum. Primary lymph node involvement or metastasis is rare.[1] It can affect any part of the gastrointestinal tract and may also be seen to involve the extraintestinal sites.[2] The origin of this tumour is the Interstitial Cells of Cajal. The incidence of GIST is about 10-20 patients per million and occurs mostly in patients aged more than 5th decade of life.[1] Mainstay of the treatment being tumor resection; lymph node dissection being rarely done due to the rarity of its involvement.[1]

Case Presentation: A 60 year old male presented to surgery OPD with the chief complaints of pain in abdomen since 15 days, progressive in nature. Clinical findings revealed pain in right hypogastric and epigastric region. CECT Abdomen was suggestive of calculus cholecystitis with empyema for which pig tail catherization was done. Elective Cholecystectomy was planned considering the possibility of Chronic Cholecystitis. A solitary nodule on the greater curvature of stomach was accidently discovered intraoperatively while Cholecystectomy procedure, for which wide local excision was done. Gall bladder and nodule specimens were submitted for histopathological examination. The nodule was well delineated, globular, brown, tissue piece measuring 2 X 2 cm. On cut section, white, homogenous areas were seen.

On microscopy, lymph node architecture is

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completely effaced by neoplastic element with smooth muscle differentiation arranged into whorl like pattern showing moderate nuclear atypia, pleomorphism and hyperchromasia and also mitosis with condensation of lymphoid follicle towards the periphery.



Discussion

GIST are rare tumours of the gastrointestinal tract. These tumours are mostly seen in 6th and 7th decades of life; mainly in the stomach.[1] Majority of the patients stay 'silent' until the tumor attains a larger size.[2] Common manifestations being pain in abdomen, anorexia, weight loss, vomiting manifesting depending upon the size of tumor.[2]

80% GISTs are located in the gastrointestinal; remaining seen in omentum, mesentry, gall bladder and urinary bladder.[2] Out of these, 60% are found in the stomach.

The diagnosis of GIST are made up by histology and immunohistochemistry findings. The histological variants of GISTs include spindle (70%), epitheloid (20%) and mixed (10%).[6]

Most GISTs stain positive for KIT, CD117 and anoctamin-1 (DOG-1). The major oncogenic pilot for GISTs are mutations in KIT and PDGFRA.[5]

The main factors determining the malignant ability of the tumor are the size of the tumor, site, rate of mitotic activity, C-Kit and PDGFRA genotype; also predicting the prognosis.[4] Tumor resection has been said to be the mainstay of the treatment along with Imatinib therapy.[4]

Even after resection, metastasis and reccurence are not uncommon in these patients. Most common sites being liver and peritoneum.[1]

Kubo N et al; reported a case of 68 year old male with weight loss and anorexia and gastroscopy disclosed a tumor mass with ulceration over the upper body of the stomach. On histopathology, GIST was confirmed. Computed Tomography (CT) findings revealed primary gastric tumor, right mediastinal tumor and a left axillary mass. On dissected and histopathological examination, it showed spindle cells and also positive immunohistochemical stains for C-Kit, said to be a metastasis of GIST.[4,5,6]

In our study, we have seen a 60 year old male being operated for Cholecystectomy and accidently diagnosed to havesolitary nodule over the curvature of stomach. The histopathological findings revealed GIST metastasis to lymph node. The patient was later given Imatinib therapy and follow-up was done for recurrence.

Conclusion

GIST has a low incidence and rare tendency to metastasize to lymph node; therefore it is important to study clinical importance to lymph node in this malignancy. Lymph node dissection should therefore be kept in mind during resection of GIST.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Rhinoscleroma: A Case Report

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Abstract

Background: Rhinoscleroma is a chronic granulomatous disease caused by gram negative bacteria; Klebsiellarhinoscleromatis. Respiratory mucosa is predominantly affected involving mainly the nasal cavity and nasopharynx and further extending to the respiratory tract. Systemic involvement is rare. The bacilli can be stained for histopathological examination by Hand E, Gram's stain and Giemsa stain. Treatment being antibiotics and anti-inflammatory drugs.

Case Presentation: Hereby presenting a case of 52 year old male with nasal blockage; clinically found to have a mass encasing the bilateral chonchae and nasopharyngeal wall being investigated for malignancy, but proved to be diagnosed with rhinoscleroma on histopathology.

Conclusion: Rhinoscleroma, usually difficult to diagnose, may be confused with granulomatous disease, carcinoma, tuberculosis and leprosy. Seen majorly affecting the respiratory tract, adequate biopsy material sent for histopathology may prove to be useful in the diagnosis; along with a proper clinical history. The presence of inflammatory background containing Mikulicz cells are highly suspicious of rhinoscleroma. The disease often presents with nasal mass therefore clinically mimics with neoplastic mass. Due to its chronic course, it often proves challenging to treat. Early diagnosis thus ensures timely treatment further avoiding relapses and complications.

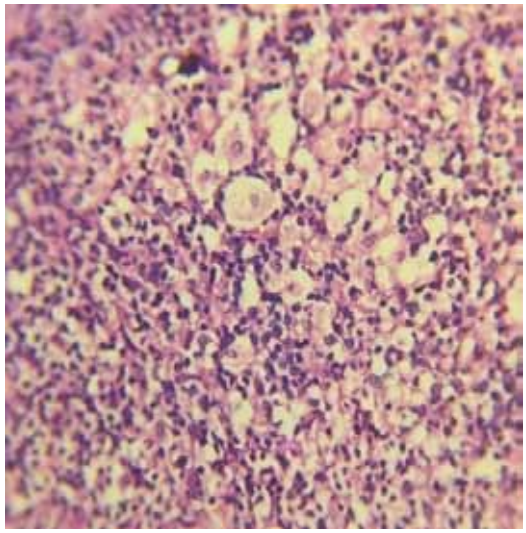
Keywords: *Rhinoscleroma, Mikulicz cells, Klebsiellarhinoscleromatis, Microscopy.*

Introduction

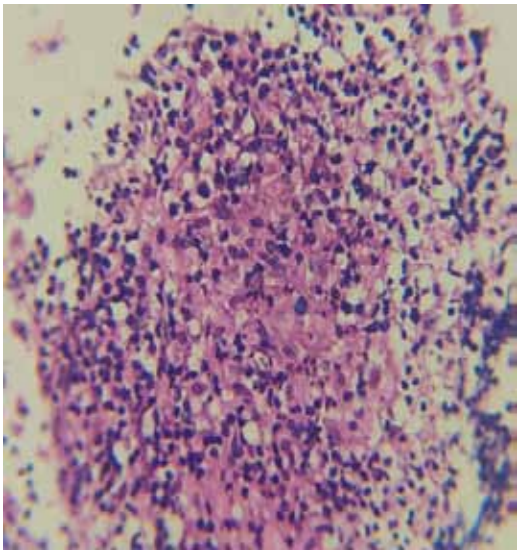
Rhinoscleroma is a slowly progressive infectious disorder caused by gram negative rod shaped bacilli; Klebsiellarhinoscleromatis.(1) The bacilli mainly involves the upper respiratory passage, with further extension into the lower respiratory passage. Rarely the involvement of other systems have been identified.(2) The disease has been divided into three clinicopathologial stages: Catarrhal-atrophic, granulomatous and sclerotic.(3) Usually the patients present with nasal blockages, foul smelling purulent discharges, crust formations and repeated epistaxis with progression to permanent defects of nasal deformities, anosmia, dysphonia and stridor.(4)

Clinical Case Presentation: A 52 year old married male came to Otorhinolaryngology OPD with the complaints of bilateral nasal blockage since 6 months. On clinical examination, it was found that the patient had bilateral inferior turbinate hypertrophy and congested mucosa; along with a mass encasing the bilateral chonchae and nasopharyngeal wall on both sides. The probable clinical diagnosis were nasopharyngeal carcinoma or hemangiopericytoma of nasopharynx. Radiological investigations were carried out. Computed Tomography (CT) of Paranasal Sinuses findings were suggestive of Nasopharyngeal carcinoma. A biopsy specimen with multiple, whitish brown tissue pieces aggregating 1 x 1 cm were sent for histopathological examination.

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40X microphotograph showing foamy macrophages (Mikulicz cells) in the background of inflammatory infiltrate.



40X microphotograph showing epitheloid granuloma in the background of inflammatory infiltrate.

Discussion

Rhinoscleroma is a chronic granulomatous disease; commonly affecting the respiratory tract. [1] Most common site being the nose (95-100%), followed by nasopharynx, larynx, trachea and bronchi. [1,3] The disease is caused by a immotile, short and encapsulated gram negative organism known as Klebsiellarhinoscleromatis.[1] The only host for this organism are the humans.[1] Inhalation of nasal droplets of the infected person is the mode of transmission.(1) Opportunistic disease in immunocompromised people and must be differentiated from other granulomatous diseases.[2] It is seen to affect majorly the people with low socio-economic status and with poor hygiene. [2]

It is categorized as non-neoplastic mass of the respiratory tract. (5) The progression of the disease is seen in three stages: (A) Catarrhal stage- starting with rhinitis and progressing to rhinorrhoea, crust formation and nasal obstruction; can last uptoweeks or months. (B) Hypertrophic stage- It involves granulation tissue formation with nasal pyramid widening and destruction of nasal septum. Symptoms involve epistaxis, anosmia, reduced sensations of the soft palatefollowed by others. (C) Sclerotic phase- Involves extensive scarring and stenosis of the nasal vestibule.[2]

Histopathology plays a key role in the diagnosis of rhinoscleroma. The bacilli can be demonstrated by H and E, Gram's stain, Geimsa stain, PAS. (1) Diagnostic features seen in rhinoscleroma are the dense inflammatory infiltrate, Mikulicz cells and the granulomas.[6] K. rhinoscleromatis is positive in routine cultures on blood or MacConkey agar in 50%-60% of patients in the granulomatous stage.[7] Detection of Klebsiella antigen, CD68 and altered CD4:CD8 ratio are useful in the diagnosis.[1]

Malkud S, et.al; (2017) documented a case of 34 year old female with complains of swelling of lips and gums since 9 months. On clinical examination, a swelling of the peri-oral area, hypertrophy of gingival and crust formation with fissures was observed over the lips. The nose and throat were found to be uninvolved. The biopsy specimens were sent to histopathology for further examination. The slides stained by H and E, Gram and PAS stains were observed under microscope. Presence of inflammatory infiltrates of plasma cells with Russel bodies and Mikulicz cells were the diagnostic findings that were seen. The patient showed significant improvement with Ciprofloxacin and Doxycyclin and advise of follow-up.[1]

Ahmed A, et.al; (2015) conducted a retrospective study to spot the potential diagnostic features of nasal rhinoscleroma when mikulics cells are absent. Predominance of plasma cells with Russell bodies and neutrophils with squamous metaplasia along with nasal crust formations was found to be diagnostic. This also helped in early diagnosis and treatment of the cases.[4]

In our case, male aged 50 years presented with blockage in the nasal cavity and a nasal mass was found on examination. The biopsy specimens sent for

histopathology examination were stained with H and E, Giemsa and Gram's stains. On H and E stain, Mikulicz cells along with epitheloid granuloma was observed which confirmed the diagnosis of rhinoscleroma. The patient was treated indoor with antibiotics and advised for follow-up.

Conclusion

Rhinoscleroma, usually difficult to diagnose, may be confused with granulomatous disease, carcinoma, tuberculosis and leprosy. Seen majorly affecting the respiratory tract, adequate biopsy material sent for histopathology may prove to be useful in the diagnosis; along with a proper clinical history. The presence of inflammatory background containing Mikulicz cells are highly suspicious of rhinoscleroma. The disease often presents with nasal mass therefore clinically mimics with neoplastic mass. Due to its chronic course, it often proves challenging to treat. Early diagnosis thus ensures timely treatment further avoiding relapses and complications.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Signet Ring Cell Carcinoma

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Abstract

Signet ring cell carcinoma is very rare carcinoma of colon and aggressive subtype of colorectal carcinoma that is mostly diagnose inadvance stage. 5-year survival rate is very poor as compared to other adenocarcinoma of the colon. First case reported in 1951 but clinical literature on this subtype of adenocarcinoma is very limited. In this report a 32-year old male presented with SRCC of colon. Our literature review describes the many factors that contribute to the poor outcome and high recurrence in SRCC of the colon. This study prove that survival rate is increased by earlydiagnosis and surgical resection. However, the rarity of this cancer and its propensity for insidious onset and aggressive course prove a challenge to the development of such guidelines.

Keywords: *Colorectal carcinoma, Signet ring cell carcinoma, Proctoscopy, Poor prognosis.*

Intoduction

Colorectal cancer (CRC) is third largest cause of cancer in men and second most common cause of cancer in women [1]. In young adults CRC mostly found in the left side[2]. Some studyprove that CRC inyoung age have more dangerous histological features, like perineural and vascular invasion and positive margins after resection[3], inflammatory bowel disease association and positive family history[4]. SRCC is very uncommon type of CRC and occur less than 1% of CRC cases[5]. It is poorly differentiated adenocarcinoma and show more aggressiveness than other adenocarcinoma of colon because of diagnosed in advance stage.[6]

SRCC is a type of mucinous carcinoma, which is histologically characterized by cancer cells with increase intracytoplasmic mucin, which pushed nuclei to peripherally and giving them their "signet ring" appearance. SRCCs mainly arise in stomach, with rest occurring in other organs like breasts, gallbladder, urinary bladder, pancreas and colorectum[7]. Colorectal SRCC, is different from other colorectal adenocarcinomas in terms of early onset, higher rate of peritoneal seeding, higher distant metastasis, lower rate of liver metastasis and the poor prognosis[8]. SRCC has higher tendency of spreading inintrmucosal with sparing of mucosa. That why SRCC is rarely detected in some cases in regular biopsies[9,10]. Here, we describe a case of SRCC of rectum in which definite diagnosis done byproctoscopic biopsies.

Case History: 32 year old male come with chief complaint of bleeding per rectum while passing stool, since 02 months with history of loss of appetite. He has no positive family history of GIT cancer or any disease. No history of vomiting and weight loss and no association with pain.

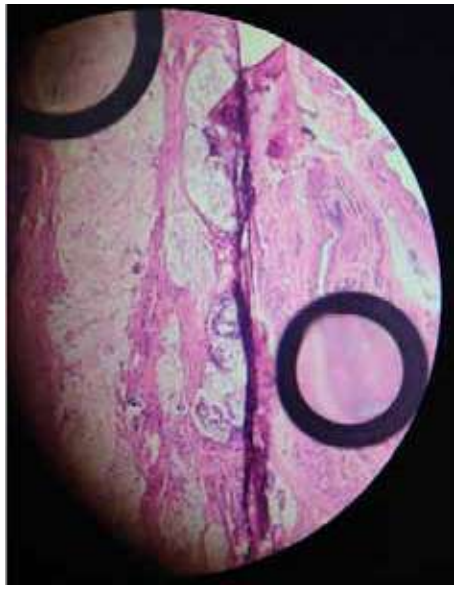
On clinical examination, abdomen was soft and nontender. No clinical finding of external skin tag, fissure, fistula but ulcerative growth present 1 cm from anal verge on per rectal examination.

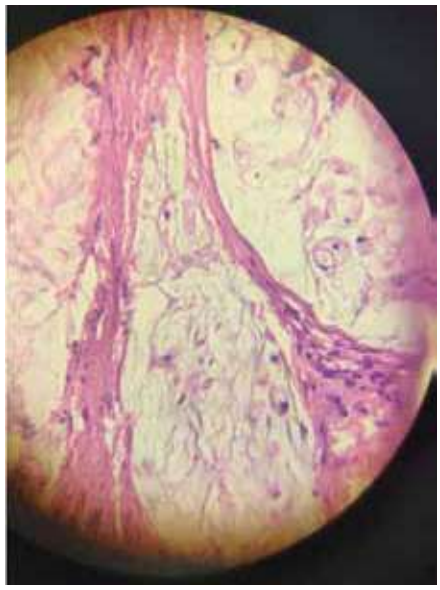
CECT finding were enhancing soft tissue density, polypoidal mass lesion arising from the posterior right lateral wall of the anorectal region suggestive of rectal carcinoma with lymphadenopathy.

Proctoscopy was performed and biopsy was submitted for histological examination.

Gross Examination: Multiple, irregular, whitish brown fibrofatty tissue piece aggregating 1 x 1 cm.

Microscopic Examination: Low power view showing mucin lake containing signet cells of adenocarcinoma. High power view showing signet cell in mucin background.





Discussion

According to various study more than 96% cases are found in stomach.SRCC is very uncommon type of colorectal carcinoma, which is 0.1%–2.6% of all colorectal cancer carcinoma[8] and have poor outcome, compared to other adenocarcinomas because it have high tendency of diffusion in intramural, lymph node, peritoneal and distant metastasis[11]. some study shows that survival rate of SRCC is significantly lower than other adenocarcinomas due to advance stage at time of diagnosis and distant metastasis[6]. In this case patient come with bleeding per rectum while passing stool and loss of appetite. CECT and proctoscopy finding were suggestive of diffuse infiltrative carcinoma of rectum[10,11]. With the help of Proctoscopy, sufficient specimens was taken from deeper tissue and confirmed diagnosis was found.

Rectum is most commonly involve, with a incidence of 32.4% to 46.8% bone metastasis from colorectal cancers[13,14,15]. Considering that patient had no lung or liver metastasis. In summary, SRCC diagnosed by proctoscopy biopsies and SRCC is a malignancy of glandular lining of gastrointestinal tract with signet rings like appeared cells under microscopy. The signet ring-like appearance of cells due to the abundant mucin within cells, pushing the nuclei to the peripheries. These cells are mostly associated with pools of extracellular mucin.

Conclusion

SRCC, auncommon histopathologic type of CRC that is more commonly found in younger and has a distinct clinical presentation.SRCC in our study of a patient come with prolonged gastrointestinal symptoms, as SRCC is an aggressive type with poor prognosis when detected in an advanced state. Rule out a primary GI malignancy that has metastasized to other location in the colon is very important.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Homocysteinemia: Cause of a Rare Case Coexisting DVT and Recurrent Foetal Loss in Pregnancy

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Abstract

Deep vein thrombosis and venous thromboembolism is a major health problem and one of the leading causes of maternal morbidity and mortality Recurrent pregnancy loss either early or late is a serious problem and has both psychological and physical impact. Thrombophilia are one of the most important causes of DVT as well as Recurrent pregnancy loss as it worsens the physiological hypercoagulable case which exists in pregnancy. Homocysteinemia is rare but an important cause of deep vein thrombosis and recurrent pregnancy loss. Serum homocysteine levels in pregnancy have been linked to preeclampsia,recurrent abortions and low birth weight. Diagnosis of this condition is missed on routine basis due to extremely less frequency of the evaluation of serum homocysteine levels on a routine basis. Here we report a case of hyperhomocysteinemia as underlying cause of bad obstetric history and DVT which are few of the classic presentations of the entity seen in the single patient. the condition was diagnosed by multidisciplinary approach.

Keywords: *Homocysteinemia, Recurrent pregnancy loss, Deep vein thrombosis, Preeclampsia.*

Introduction

Deep vein thrombosis during pregnancy is associated with high mortality and morbidity.(1) The incidence of venous thrombosis in pregnancy is approximately 1 in 1000-2000 pregnancies. Pregnancy being a hypercoagulable state is one of the important factors contributing to venous thrombosis during pregnancy a history of hereditary or acquired thrombophilia or a history of previous DVT has been determined to be most important risk factors. homocysteinemia remains a risk factor for DVT after wellestablished risk factors are excluded. Due to rare association of the condition with deep vein thrombosis serum homocysteine levels are not included in the routine investigations carried out for diagnosis of deep vein thrombosis or recurrent pregnancy loss hence leading to early and specific management of DVT.

Case Report: A 22 years old patient with a h/o spontaneous abortion in second month of her pregnancy presented to casualty at 28 weeks of gestational age with c/o acute abdominal pain and bleeding PV since 6 hrs. patient was drowsy and afebrile on touch. On general examination patient was pale and bilateral pedal oedema was present. on admission Patient had a weak and thready pulse with PR 112/min, bp was recorded to be 150/100 mm hg and albuminuria +2. On abdominal examination patient uterus was tense and tender and was disproportionately larger than the average gestational age. foetal heart rate was absent following which an emergency obstetric ultrasound was done. Obstetric ultrasound showed single foetus in transverse lie with no foetal cardiac activity and hypoechogenic retroplacental clot with intraplacental anechoic areas suggestive of intrauterine foetal demise owing to abruptio placenta. Patient's haemogram and biochemical laboratory tests

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had the following values: hb-7 gm%, TLC-13200/ cumm, platelets-1.23 lakhs/cumm,S. LDH-1017/ cumm,S .uric acid 0.66/cumm. After evaluating the patient's condition,lab investigations and assessing the risk of complications of preclampsia leading to placental abruption and foetal demise of duration more than 6 hrs, an emergency caesarean section was done intraoperatively a hematoma covering 60% of the placenta was observed. a male baby who did not cry was delivered placenta was extracted and sent for histopathological examination [image 1]. 2 units of whole blood were transfused to the patient postoperatively. Patient was started on tab Labetolol 50 mg bd following which her raised BP was well controlled. Owing to history of two unexplained pregnancy losses, patient was evaluated for structural and endocrinal abnormalities, chromosomal anomalies, antiphospholipid antibody panel test which came out to be normal. The lab investigations were not suggestive of any acute or chronic infection in the patient her postoperative period in the hospital was uneventful and the patient was discharged on 5th postoperative day patient had c/o pain,swelling and redness in right lower limb on 9th day of puerperium [image 2]for which she visited medicine OPD and was advised a colour doppler of bilateral lower limbs in view of symptoms suggestive of DVT .thrombosis of femoral, superficial femoral, popliteal, posterior tibial veins was noted in colour doppler of right lower limb. The patient was admitted in medicine ICU following which all the risk factors contributing to DVT were assessed and a battery of investigations was done to rule out the specific cause of DVT in this case. the investigations also included serum homocysteine levels which are not a part of the routine investigations and are rarely considered as a causative factor. However in this case patient's lab investigations came out to be normal except plasma homocysteine level which was elevated[26.58 micromole/l]. patient was started on low molecular weight heparin (enoxaparin) for the management of DVTalong with oral vitamin B6 and B12 tablets once and folic acid 500 mcg twice daily for the treatment of hyperhomocysteinemia. patient had a hospital stay of 7 days and was discharged after relief of symptoms. she conceived spontaneously and visited the obstetric OPD for her regular visits. She was advised to continue low molecular weight heparin as a prophylactic measure for DVT and folic acid tablets supplementation once daily in order to maintain plasma homocysteine levels well within a normal range. A multidisciplinary approach involving physicians and obstetricians helped her in having an antenatal period without any undue complications. Patient presented to casualty at 35 weeks of gestation with c/o appreciation of decreased foetal movements persistently. USG OBS findings were normal and colour doppler showed normal colour flow and spectral waveform. keeping in mind a bad obstetric history of patient an elective caesarean section was performed and the patient delivered a healthy female child of birth weight 2.5 kg. Her intraoperative and postoperative period was uneventful. This case is unique as a simple investigation and treatment of the cause in this case could help the patient in having a favourable pregnancy outcome.

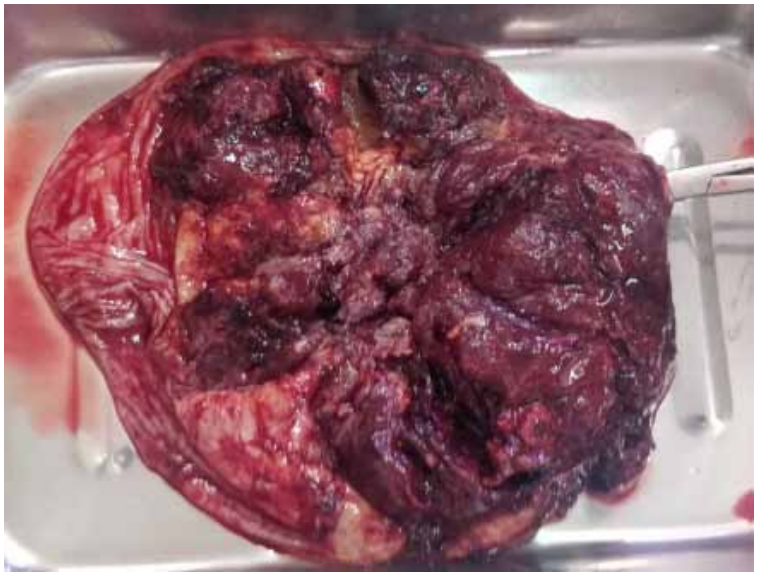


Figure 1: Gross appearance of placenta extracted post LSCS at 35 weeks of pregnancy



Image 2: Unilateral pedal edema in rightcalf seen in patient on 9th day of puerperium

Discussion

Pregnancy and the puerperium are well-established risk factors for venous thromboembolism (VTE). The likelihood of women developing DVT is 5 times more as compared to nonpregnant state. The risk of DVT is accentuated when pregnancy is associated with some specific comorbidities like inherited or acquired thrombophilias, a previous history of thrombosis, antiphospholipid syndrome, lupus, heart disease and sickle cell disease.(2)

Homocysteinemia has been reported as one of the risk factors contributing to recurrent pregnancy loss.Insufficient supplementation of vitamin B12 and folic acid and inherited disorders within the methionine homocysteine metabolic pathway such as MTHFR C677T gene polymorphism can lead to hyperhomocysteinemia(3).

Homocysteine is a sulphur-containing amino acid which is formed by demethylation of methionine. Transsulphuration is important step in homocysteine metabolism wherecystathione beta-synthase catalyses the conversion of homocysteine to cysteine. CBS is vitamin B6 (pyridoxine) dependant enzyme where B6 is an essential cofactor.(4)

Increased incidence of preeclampsia has been noted in women who have raised plasma homocysteine levels in early pregnancy compared to women who have normal levels of blood pressure throughout pregnancy. The incidence can be three times more than that in normotensive pregnant women. Endothelial dysfunction which is pathophysiology implied in preeclampsia can be brought about by homocysteine.

V Stoĭkova *et al* conducted a study and concluded that the women with a severe preeclampsia had a significantly higher serum homocysteine levels than those with mild form (F = 0.025). The study finds a link between the serum homocysteine as an endothelial dysfunction marker and the development of preeclampsia and a relation between the severity of preeclampsia and the degree of the elevation of the serum homocysteine levels.(5)

Nutritional deficiency and/or inherited disorder of methionine metabolism have been a causative factor of hypercystinemia which in turn might be a risk factor for recurrent foetal loss. Hence in such patients a probable association between raised serum homocysteine levels and vitamin B6 and folic acid should be lookedinto so that a nonteratogenic regimen to restore the normal metabolic is begun and there a successful maternal and perinatal outcome.(6) Gris et al reported an association between increased levels of Hcy and a first early pregnancy loss. Del Bianco et al found 25% of women with recurrent loss to have hyperhomocysteinemia or at least a pathological methionine loading test.(7)

Inadequate vascular supply to placental vascular bed might lead to Placental insufficiency,rupture of placenta, recurrent pregnancy loss and pre-eclampsia. A systematic literature review was carried out by JG et al to evaluate the risk of placental pathologies in the presence of metabolic disorders. It was observed among four studies that folic acid deficiency, though statistically insignificant was a prominent risk factor for placental insufficiency and rupture. There was also an association between placental rupture and infarction in the presence of homozygosity of methylenetetrahydrofolate reductase gene and hyperhomocysteinemia with or without methionine loading test. A wide number of observational studies had a common consensus that folate deficiency, hyperhomocysteinemia and homozygosity for methylenetetrahydrofolate reductase thermolabile variant are probable risk factors for placenta-mediated diseases.(8)

Substantial and vast amount of data obtained from the research has shown significant association between mild to moderate hyperhomocysteinemia and vascular disease. Hyperhomocysteinemia is contributing factor

for both arterial and venous thromboembolic events. Langman *etal* conducted a retrospective case control study and observed that fasting hyperhomocystinemia is a remarkable risk factor for venous thromboembolic disease.(9)

The intricate pathway of homocysteine metabolism is regulated by vitamin B6 and Folic acid. vitamin B12 and folic acid are and effective contributors to neural and global development of foetus and embryo. It is not feasible to assess vitamin B12 levels on a routine basis There is very poor relation between levels of intracellular methylfolates (2)active form of folate]and total plasma and/or folates in the red blood cells. Rise in homocysteine levels beyond the normal range has been seen in case of pyridoxine and intracellular methylfolate deficiency(10)

Extensive planning and implementation regarding the management of vitamin B12 deficiency is required but minimal measures have been taken by clinical practitioners and policy makers in India. A reduction of total plasma homocysteine levels has been noted after vitamin B12 supplementation in women in late pregnancy who previously had normal folic acid reserve but had deficiency of vitamin B12.Hyperhomocystinemia is typically managed with vitamin B6,B9 and B12 supplementation.(11)

Conclusion

Homocysteinemia should be considered as a possible diagnosis in pregnant woman presenting with recurrent pregnancy loss and deep vein thrombosis. Early diagnosis and a simple regimen of vitamins supplementation can prevent pregnancy loss especially recurrent pregnancy loss which is be physically and emotionally taxing for couple.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Plasma Cell Gingivitis: A Rare Perpetuating Entity

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Abstract

Background: Plasma cell gingivitis is a rare unexpected condition in an individual inconsiderate to be diagnosed for. Although many research has shown the light on the treatment and diagnosis of the disease again exclusion criteria are most considerate. In the current situation patient presented with typical features of inflammation after the confirmatory histopathological diagnosis only plasma cell gingivitis came into contemplation. Properly applied medications were provided to the patient and improvement was seen in follow up. Being congruent with a number of conditions, its diagnosis is served quite succour for mankind.

Case Presentation: A 35-year-old female presented with swollen and occasional painful gums, with intermittent gum bleeding. Her medical, dental, family history and personal history were non-contributory. There was no history of any allergy or Para functional habits such as mouth breathing. No abnormalities were detected in her general physical and extra oral examination. On examination, there was erythema and inflammation of gums suggestive of gingivitis, with normal CBC and PS report. She has been refracted any of the treatment given earlier. Thereafter, a punch biopsy of gums was done and the tissue was sent for histopathological examination.

Conclusion: PCG is a remarkable condition, most likely allergic in nature. While being a purely benign, the clinical appearance and localization may conceal much more detrimental conditions Therefore, each lesion requires due attention.

Keywords: *Plasma cell, gingivitis, rare, inflammation, histopathological.*

Introduction

It is a rare benign inflammatory condition of gingiva characterized by sharply demarcated erythematous and oedematous, non-ulcerative gingivitis often extending to the mucogingival junction occasionally accompanied by cheilitis and glossitis[1,2]. Various synonyms used in literature are idiopathic gingivostomatitis, plasma cell gingivostomatitis, atypical gingivostomatitis and allergic gingivostomatitis and many more[3]. It is been considered as a hypersensitive and immunological reaction to some antigen often chewing gum, toothpaste, or other foreign objects[4]. The exact etiology is unknown but considered mostly due to immunological reaction because of the presence of plasma cells [5]. The allergens identified are mostly cinnamon aldehyde and cinnamon used as flavoring agents. There is no particular sex or age predilection. The diagnosis needs clinical, histopathological and hematological screening to make differential diagnosis other clinically similar conditions[3].

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Case History: A 35-year-old female presented with swollen and occasional painful gums, with intermittent gum bleeding. Her medical, dental, family history and personal history were non-contributory. There was no history of any allergy or parafunctional habits such as mouth breathing. No abnormalities were detected

in her general physical and extraoral examination. On examination, there was erythema and inflammation of gums suggestive of gingivitis, with normal CBC and PS report. She has been refracted any of the treatment given earlier. Thereafter, a punch biopsy of gums was done and the tissue was sent for histopathological examination.

Radiological Findings: On radiographic examination, Orthopantomograph (OPG) revealed interdental bone loss with ill-defined radiolucency of apical teeth [figure 1].

Gross findings [Figure 2]:



Histopathological findings [figure 3, 4]:

The section shows squamous mucosa with oedematous squamous acantholytic separation of squamous cells. Submucosa shows diffuse as well as nodular aggregates chiefly composed of mononuclear cells and abundant plasma cells. No vesico-bullous lesion is identified. The plasma-lymphocytic infiltrate at places shows histiocytes but no granuloma or giant cells seen.

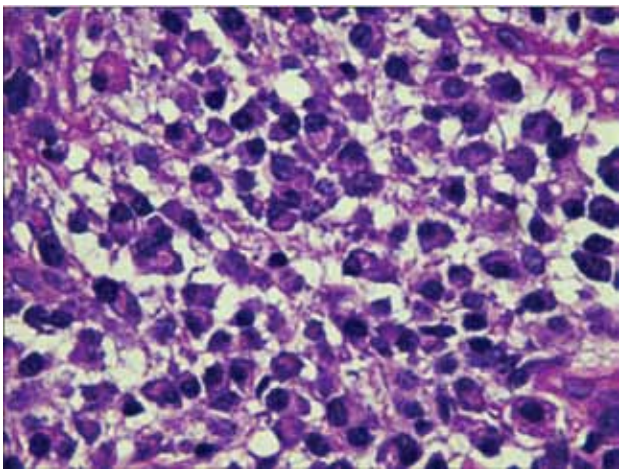


Figure 3

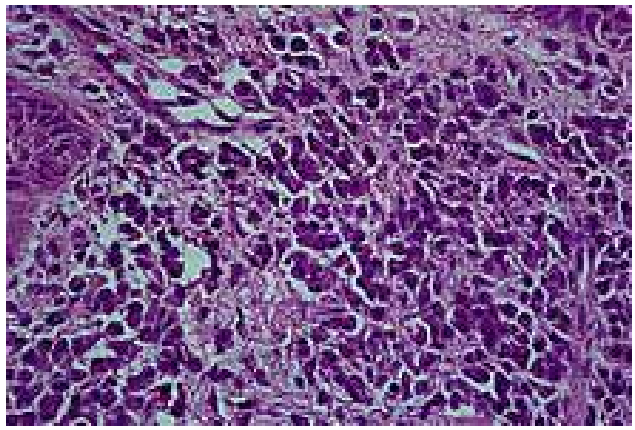


Figure 4

Discussion

The enigma of PCG considered a clinical condition for several years, with many reports appearing in the literature. Clinically, PCG appears as a diffuse erythematous & edematous lesion of gingiva along with a sharp demarcation along the mucogingival junction. It frequently bleeds with little trauma or touch[6]. As seen here the patient also presented with a similar complaint. The exact etiology of PCG is not clear but considered as a hypersensitivity reaction to allergens present in the toothpaste, chewing gums and certain food and oral care products, strong spices and some herbs such as chili, pepper and cardamom. Other than allergic etiology it can also present as a neoplastic lesion or as lesions of unknown cause.[7, 8, 9].

Based on etiology, plasma cell gingivitis is categorized as three types: (1) lesions of unknown cause, (2) lesions owing to some allergen and (3) lesions due to neoplastic origin [10]. Clinically areas of whitening, epithelial sloughing and erosions also have been observed[11,12]. Laboratory investigations usually are within normal limits and sometimes an elevated erythrocyte sedimentation rate can be seen. Patch tests to identify an allergen could be helpful. Also, a biopsy would be of great help in differentiating other gingival conditions [13].In most cases, the first-line therapy should commence with the exclusion of all known potential allergens, as this may result in improvements. Also, oral hygiene improvements and professional periodontal care usually result in the reduction of the marginal gingivitis. excision biopsy of the lesion, wherever applicable, including the case presented here, followed by histological analysis might be not only the best diagnostic approach but also the most beneficial therapeutic option.

Conclusion

PCG is a remarkable condition, most likely allergic in nature. While being a purely benign, the clinical appearance and localization may conceal much more detrimental conditions Therefore, each lesion requires due attention.

Clinical differential diagnosis may include any of the desquamative gingivitis, such as erythematous/ erosive lichen planus, autoimmune vesiculo-bullous diseases, such as mucous membrane pemphigoid and pemphigus Vulgaris, pubertal or pregnancy-induced gingivitis and leukemia-associated gingivitis should be taken into consideration.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Critical Appraisal of e-learning through Mobile Devices in Medical Education

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Abstract

Summary: In COVID-19 pandemic, e-learning is the essential component of education system. All the schools, colleges, universities implemented e-learning program for the purposes of teaching and learning. Syllabus of new session is started for the said academic year through e-learning to minimize the academic loss and to coverup the academic session. Nowadays, medical education is full off with information technology solutions, computer--assisted learning has facilitated teaching of a wide range of topics, including basic science and advanced surgical procedures. The e-learning is totally depending upon the expertise in the handling of technology, electronic devices as well resource material available for the conduction of e-classes and one of the most important factors i.e. internet connectivity. In due course, mental status of students and teachers along with motivation from the teaching institute is also important factor for e-learning program. There were various challenges and disadvantages reported in previous research studies regarding e-learning, suggested for periodic need assessment of educational program, emphasize upon breach in between expected and existing system, resources management system, technical expert team, financial budget, mindset of the teachers and students and training sessions for development of e-learning culture.

Keywords: *COVID-19, E-learning, medical education.*

Introduction

E-learning is the need of the COVID-19 pandemic situation to overcome the educational loss. But students and the teachers are facing problems while conduction of e-learning sessions. Learning is the process which involves variouspsychomotor activities like critical thinking and ability to solve the problems. The precise aim of education system to provide the developers with the best learning tools, so learner can gain thorough understanding, skills and relevant information required for their career(1). In the field of education, technology is not latest revolution but today education system is shifting towards techno based education modalities like app based learning, simulation and in COVID-19 pandemic online teaching(2).

Information technology facilitate medical education and assist teaching-learning with wide range of topics, like basic science and advanced surgical procedures, online learning. Forthe development of field of medical education one should have to consider teaching strategies through advance technology(3).

Nowadays students are more technology friendly, they search and learn most of the required information through various search engines. Up-to-date technologies is required for the upgradation of field of health care and medical education. Consequently, mobile phones are increasingly being used for various purposes as like video conferencing, chatting, entertainment, simulations which enhances the accessibility of information. Thus,

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the upcoming of health care and medical education exceedingly depend upon technological innovations.[4].

Mobile Apps facilitate various learning activities to engage and motivate to the students. Simultaneously, it increases the learner's attainment which eventually reflected into the retention of knowledge[5].Presently various educational programs or applications are available in the cloud that helps the learner in learning process. Newer technologies are the blessing for today's teaching and learning progression.

Medical Education and Information Technology: Introduction of medical students and residents to information technology solutions is the need of the situation which familiarize the students with newer systems presents in clinical environments. The technology solutions can help undergraduate and postgraduate students to study clinical cases, simulation, decision making and management of electronic record[6]. Portable technology for medical education has been evaluated from several perspectives. Personal Digital Assistants became popular, predominantly due to their small size and ease of use for the mobileuser[7]. Recent studies have further explored the use of mobile tablets in medical education. A survey among radiology residents found that 81% of the residents believed that they would spend more time learning if they were provided with mobile tablets for learning difficult cases[8].

Introduction to mobile application: A mobile application is a designed computer program, which can be run on a mobile or tablet. Mobile applications can be run on desktops with windows version and desktop applications that run in mobile with the help of web application through mobile web browsers[9]. The term "app" is the short form of the word "software application". It was listed as "Word of the Year" by the American Dialect Society because of its popularity[10]. These newer smartphones could be named as "app phones" to differentiate them from older version of smartphones[11].Apps are the software designed programs that run on a computer or mobile device to accomplish and facilitate difficult as well as vast task.It is well equipped with faster processors, enhanced memory, advance technology batteries and extremely competent operating systems that easily perform multifaceted functions in least duration [12].

Necessity of Mobile App for E-learning: E-learning is the experience of learning through mobile or wireless computing devices which promoting learning in such a way that knowledge content is always available and accessible anytime, anywhere for the learner. The development of smartphones, tablets and small size laptops with improved capacity and functionality, upgraded memory, bigger screens, the facility to access the highspeed internet and freely downloadable software facilitate e-learning through wide range of uses which enhances communication, diagnostics, self-monitoring with the help of several apps[13]. Users like students and faculty members of various are progressively using mobile devices at the workplace[14,15]; appears to be the part of a technological revolution in medical as well as other sectors [16].Recognizing and keeping up-to-date with developing technology that support workplace as well as distance learning in today's pandemic situation, but it is a key challenge for medical educators[17]. Technology time to time provides help to the learner for enhancement of the knowledge and the skillduly offer a form of dynamic scaffolding[18]. The medical educators need both the factors that gain the knowledge and take part in learning processes, by recognizing and integrating the spirit of learning through participation as well as the distinct attainment of knowledge[13].So, Mobile App is the need of the era to acquire medical education with conventional teaching methodologies.

Mechanisms of e-learning: E-learning that comprises of three key components as follows[19].

1. Technological flexibility- it is the portablefeature of installed hardware and software that enable wireless net connection.
2. Learners' mobility- it means learners can gain knowledge through various sites at a time.
3. Mobile learning processes-it is the process which includes both the technology and the learners in mobile mode.

E-Learning Approach: Mobile learning approach comprises of three important factors-Active instructor or teacher, Active learner and Creative pedagogy for effortlessnesse-learning process[20].

1. Active Instructor: the personfacilitates students, engage them throughout learning process in side or outside the classroom via teaching, assigning work or with continuous assessment.
2. Active learner: the student engage him/herself with self-study, home assignments, self-assessment,

group discussion at digital platform, access course content anytime as he/she want, share as well as exchange knowledge or resulted findings with collogue companion

3. Creative pedagogy: the learning process where instructor and learner mutually agree for teaching content, which create encouragement in the students and keep them active throughout the learning process bycollective learning, discussion on genuine issues, emphasis on challenging themes.

Role of student's in E-learning: Students playsimportant role in four modes- Performance, Satisfaction, Behavior and Motivation.

Performance: E-learning may help the students for learning at anywhere as per ease of learning environment. It facilitates quick learning with new activities, creative thoughts, actions and required skills. Thus, the e-learning allow the learners to improve their study and personal skills through numerous tasks as like communication, critical analysis, problem solving and challenges in learning environment [20].

Satisfaction: The learners satisfaction is the very most important thing depends upon usefulness of the technology, instructors skill as well as quality content learning material, that perceived usefulness directly reflected in to higher satisfaction.

Behaviors: Learners behaviors changes with the content, ease of technology, layout, design of mobile applications. Learners good performance also have the positive effect on their behaviors which boost the moral and increase the positivity. Comfort in using technology also effect on learners' behavior. The appealing interface, layout, colors and overall appearance also influence the learner behaviors.

Motivation: learners performance, satisfaction and positive behaviors motivate the learner for further learning process and also encourage for personal needs, desires and determination.

Advantages of E-learning: Laptops, tabs or mobiles playing an important role in medical education, students and techno savvy faculty using various app as well as software for teaching-learning purpose. Most of the medical students using mobile devices for sharing experiences or notes, access the information, learning new things, clinical case discussions. Nowadays, medical practitioners also using mobiles or various apps for learning or updating themselves with evidence-based information in the competitive era. In the such pandemic situation and distance mode education; mobile devises playing vital role of teaching and learning in medical curriculum.

Considerations for learning Mobile Apps: The learning mobile app should be enough readable and consistence about the contrast colors, type and font of the text. Outlet of the app should be attractive, appealing and noticeable as compared to routine apps. Minimum distractors should be used in Apps for learning purpose except navigators. Make the application in simple format and comfortable as common users can use it easily. Soothing colors should be used to reduce irritation and strain on the eyes for curtailing of visual fatigue.

Challenges of E-learning through mobile app: As per researches regarding e-learning, commonest obstacle of implementing e-learning program is insufficient groundwork of the teachers. So, faculty development program or sufficient training as well as attitude of adoption regarding newer technics of teaching and learning is very important.

Familiarity of power point presentation is the major issue influencing on e-learning interface. In the recent research study, students reported that technical dysfunction in the mobile during online learning is the common scenario found to be triggering factor. Due to small screen of mobile; they were not comfortable to learn science portion which totally depend upon images, analysis. Poor internet connectivity is also the interrupting issue in the e-learning program. There are various problem in e-learning like technology, system quality, data security, self-efficacy of teacher or students and traditional attitude.

Discussion

The technology is the part and parcel of the life in today's scenario, the medical education needs to cope up with the challenges in the education system during COVID-19 pandemic. The researcher suggested that the integration of e-learning into curricular to facilitate learning and learning practice is the need of the education system. The challenges for e-learning are the poor motivation, connectivity, undue expectations, lack of resources, skill and suitability for the content, mere viability of resources is not sufficient but preparedness of the faculty also important. E-learning link the various resources in several variable formats in efficient way due

to its ease and flexibility to use at any time anywhere. On one click learner can connect with the teacher for continuous teaching-learning process. To mitigate the challenges and disadvantages of the online learning, one should have to work on policies of university or institution, design and delivery of the curriculum, learning outcomes in the form of assessments and feedback of the student.For successful implementation of e-learning program in developing countries, it is recommended that effective evaluation and controlling of the e-resources and maintenance of hardware is necessary. Proper training of the teaching staff as well as students to access the advance technology should be incorporated in the university academic calendar as a part of curricula. Partial assignments should be conducted through e-learning mechanism so as to minimize the future discomfort for assessment and evaluation system. Management should motivate the teachers and students through some incentives and competition respectively for the purposes of e-learning. University should allot the separate budget for e-learning mechanism considering a parallel system of teaching-learning and assessment-evaluation.

Conclusion

Periodic need assessment and evaluation of educational system is very important fact. The teaching learning strategies plays important role in the education system. For implementation of newer teaching method like e-learning needs piloting in the same setting to avoid upcoming hitches. So, to sidestep future challenges and expected disadvantages, one should emphasis on need assessment, gap in between present and expected system, resources management system, technical expert team, financial budget, mindset of the teachers and students and adequate training sessions to develop comfort during the teaching and learning.

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Vit D Status and Osteoporosis in Tobacco Consuming Men in Rural Region Surrounding Wardha City, Maharashtra, Central Part of India

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Abstract

Background: Awareness towards tobacco consumption has increased and it is well accepted too but will it help to cease the consumption. With widespread menace of tobacco consumption in developing country like India, not much data of study is available currently about tobacco consumption and osteoporosis around the rural region of Wardha a central part of India.

Aim: Vit D status and osteoporosis in tobacco consumers and non consumers.

Objective: To asses influence of tobacco consumption on Sr. Vit D, Sr. ionized Ca, Sr. Phosphate, Sr. parathyroid hormones the biochemical markers of bone turnover and Bone mineral density in a cohort of 214 healthy men in rural area surrounding Wardha region of Maharashtra.

Design: A cross sectional study

Subjects: 214 healthy men aged around 30-50 years with no disease history were included in the study.

Results: Tobacco consumers had significantly reduced levels of Sr 25 OH Vit D ($P<0.02$), PTH ($P<0.001$). There was no significant difference in Sr ionized Calcium between tobacco consumers and Non tobacco consumers. We got negative effect of tobacco consumption on Sr Osteocalcin ($P<0.01$).

Conclusion: Calcium and Vit D metabolism is significantly deranged by habit of tobacco consumption and cannot be explained by life style factors. Vitamin D- PTH system depression among tobacco consumers represent a other mechanism for the deleterious effects of tobacco consumption on the bones and contribute to reported risk of osteoporosis among tobacco consumers.

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Keywords: *Tobacco consumers, 25- Hydroxy Vitamin D, Parathyroid Hormone, Osteocalcin, Bone Mineral Density.*

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Introduction

Tobacco chewing, smoking and other means of consuming tobacco are rampant in rural parts throughout India. Two third of worlds smokers live in only ten countries. 40% live in India and China alone. India counts for 10% of total tobacco consumers[1,2,3].

Vit D is an essential molecule for the development of bone and growth. Tobacco smoking is in most studies found to be associated with a low bone mass and an increased risk of osteoporotic fracture[3]. An increased bone loss has been registered in smokers[4]. The life style habits are more causative factors among smokers compared to non-smokers poor nutrition, less physical activity and more intoxication all of which play a role. Smoking tobacco also has direct toxic effect on bone cells is also a possibility[5]. Hormonal metabolism of pituitary, thyroid and glucocorticoids may also be affected by smoking[6-8]. Metabolites of Vit D and Parathyroid hormone are important in bone and calcium metabolism. Few studies have done investigation on effect of smoking on 25OH Vit D and PTH. [9-15].

This cross sectional study was undertaken to investigate the impact of tobacco smoking and chewing on bone metabolism and compare the circulating levels of Serum calcium and Serum Vit D metabolism of healthy 214 rural men who are in the age group of 30 to 50 years.

Aim: Vit D status and osteoporosis in tobacco consumers.

Objectives:

1. To measure the serum levels of ionic calcium, Phosphate and alkaline phosphatase for screening.
2. To measure the height, weight and BMI
2. To measure the Serum level of total Vit D
3. To measure the Serum level of Para Thyroid Hormone
4. To measure the Serum level of Osteocalcin
5. To measure the Bone mineral Density

Materials and Method

Study Design: A cross sectional study

Study Setting: Department of Biochemistry, JNMC Wardha, under DMIMS DU

Duration of study: 1 Year

Subjects: On the basis of previous pilot study on prevalence of tobacco consumption in rural area around Wardha city 214 subjects were selected by 19 cluster sampling method total 15 different villages around Wardha city.

Materials and Method

Inclusion Criteria: Men in the age group of only 30-50 years were selected. Healthy men who do not having any disease or disorder or on any treatment specially hormones were included in study. Only men around the Wardha from rural area were only included in the study. Females were not included in the study because we wanted to exclude the study of menopause and disorders related to menopause like hormonal and osteoporotic effects.

Exclusion Criteria: Men with no age match are excluded. Men with any disease or disorder excluded.

Biochemical screening was done before inclusion that ensured that all subjects had Sr levels of Ca, PO4 and alkaline phosphatase within normal range.

Sample Size: 214

Sampling Method: By cluster sampling method. It was a cross sectional study, results on pilot study of prevalence of tobacco use in rural region of Wardha was taken into consideration for sample size calculation. 214 men were selected by 10 cluster sampling method from 9 different villages around the Wardha city. The study was carried out during January 2019 to December 2019,

Observation and Results

Of the total tobacco consumers 47 were found to be bidi smokers. They were smoking more than 10 bidis per day, 41 were consuming raw tobacco with calcium carbonate more than 7 times per day, 13 were consuming Gutka/khainy more than 7 occasions per day, none was found using snuff. The age span of the study was kept between 30-50 years and age factor was not counted for any observations. All tobacco consumers those were selected, they have a habit of consuming tobacco at least continuous for 3 years in past. Non tobacco consumers who have left the habit of smoking at least for the past 3 years were also included.

The baseline parameters for inclusion in the study and differences between tobacco consumers and non tobacco consumers are depicted in Table No. 1.

Table No. 1: Base Parameters

Sr.No	Parameters	Non tobacco consumers (n=112) Mean ± SD	Tobacco consumers (n=101) Mean ± SD	P value
1	Age (Yeats)	44±2.9	43±2.1	<0.001
2	BMI (Kg/m^2)	26.63± 4.12	26.76 ±5.72	NS
3	Ionic Calcium (mmol/l)	1.16 ±0.10	1.14± 0.09	NS
4	Sr. Phosphate (mg/dl)	3.54 ± 0.60	3.76 ± 0.61	NS
5	Sr. Alkaline Phosphatase (IU/L)	341.48 ± 106.29	387.26 ± 125.84	<0.006

BMI was just similar for the two groups. No significant difference was observed. No significant differences were found in Sr ionized calcium amongst two groups. Increased Sr phosphate level among tobacco consumers was noted. There was significant difference observed between levels of Alkaline phosphatase amongst two groups.

Table No. 2: Comparison of Non Tobacco consumers versus Tobacco consumers

Sr.No	Parameters	Non tobacco consumers (n=112) Mean ± SD	Tobacco consumers (n=101) Mean ± SD	P value
1	Vit D (ng/ml)	19.51 ± 8.58	16.22 ± 6.23	<0.05
2	PTH (pmol/l)	1.73 ± 1.96	1.281 ± 2.72	<0.039
3	Osteocalcin (ng/l)	15.27 ± 3.7	13.20 ± 3.3	0.1
4	BMD spine (g/cm^2)	1.039 ± 0.13	1.004 ± 0.14	0.05
5	BMD Total hip (g/cm^2)	0.994 ± 0.11	0.816 ± 0.11	0.03

Hypovitaminosis D means Serum levels of 25(OH) D below 20ng/ml was seen in 22.7% of the tobacco consumers and 15.2% in Non tobacco consumers. No linear relationship was observed between number of bouts of tobacco consumption and Total Sr Vit D or PTH.

A non significant decrease of mean serum total Vit D levels was observed when tobacco consumers were classified as Bidi smokers, Raw tobacco chewers and Gutka/Khainy chewers. Low levels of Vit D and PTH in tobacco consumers the mechanism for which could not be explained properly. Many toxic chemicals are present in tobacco and tobacco smoke which must be causing the lower values of Vit D and PTH.

Serum total vit D was not correlated with serum PTH in tobacco consumers. PTH and Serum total vit D were negatively related to each others in Non Tobacco consumers (P<0.04).

Results showed there was positive correlation between BMI and PTH (P<0.001) in both the groups. Body weight was lower in tobacco consumers as compared to non tobacco consumers.

Tobacco consumption was still anindependent predictor of PTH in multiple regression analysis (p<0.005).

Tobacco consumers showed lower levels of Osteocalcin than non tobacco consumers.Osteocalcin and Total Vit D were highly correlated but multiple regression analysis showed smoking was still an independent predictor of Osteocalcin (P<0.01).

To assess the influence of possible confounding factor like alcohol intake on parameters like PTH and total Vit D, we did multiple regression analysis with BMI and smoking. Alcohol consumption was not significant predictor of PTH and Vit D. Smoking was positively correlated with Vit D and with BMI negatively.

Tobacco consumers had decreased age adjusted BMD when compared to non tobacco consumers all the time. Relationship of Total Vit D and Bone density among tobacco consumers and non tobacco consumers was seen. There was inverse relationship between Sr Vit D and bone density. It was of not much significance for the hip bone in non tobacco consumers.

Discussion

As per data of NFHS-III, in India 55.8% male and10.8% female of the age group of 12-60 years were tobacco consumers in different forms. of the males 32.7% were reported to be smokers and 36.5% were tobacco chewers, while in females it was 1.4% and 8.4% respectively[2]. In India tobacco consumption is widespread; it is also prevalent in the central part of India Maharashtra. Our study region and population was not unusual and was suitable for our present study to see the effects of tobacco consumption. In our study population of cohort of normal men, there was significant negative association between tobacco consumption, total Vit D and PTH. William B. Ogunkolade et al also found that there is negative correlation of Vit D and PTH with betel nut and also tobacco consumption in the form of chewing tobacco in their cross sectional study of British Bangladeshi subjects [17].A similar study got the results of 10% lower values of 25(OH)Vit D and PTH in smokers in the control group mean difference was 2.8ng/ml. results correlate well with our study [11]. In one of the Swedish study also it was reported lower values of PTH in young women smokers [12]. In another Swedish study also reported lower levels of PTH in a population based study of men and women aged 25 ± 64 years [13]. Another studdy carried out by Scragg et al 1995 observed no association between smoking and serum 25 (OH) D over elder English women. However these studies of negative findings were consisting of small number of subjects. Lower levels of PTH found in tobacco consumers can't be explained on the differences in ionized calcium or Serum phosphate. We think that the lower levels of PTH are due to corresponding decrease of total vit D levels. In rural set up there was no such noticeable difference among tobacco consumers and non tobacco consumers but confounding factor like alcohol intake. Tobacco consumers can have difference with non tobacco consumers that means tobacco consumption and tobacco non consumption in intake of caffeine, intake of calcium and vitamin D, Exercise and alcohol intake, which also acts as confounding factors and shows association between smoking and vit D and PTH levels[18]. Our study could establish that alcohol consumption by tobacco consumers aggravates the condition and shown lowered levels of VitD. Further more it was observed that tobacco consumption was still associated significantly with serum levels of total vit D and PTH.

Establishment of mechanism by which tobacco consumption lowers the PTH and VitD could not be explained properly. Tobacco itself and its smoke contains various toxic compounds like tars and nicotine. Also contains various heavy metals like nitrosamines, hydroxyquinones, cadmium, thiocynates and others[20,21,22]. Intracellular calcium is increased by hydroxyquinone and it also affects theliver [23]. There may be altered metabolism of Vit D as tobacco consumers have more hepatic degradation of estrogen [24]. The lower values of PTH in tobacco consumers may be due to lower secretion or more degradation of estrogen. We didn't get correlation between lower PTH who has half life of few minutes and,25(OH)2 D who has half life of few hours. Other studies also failed to detect such correlation[25]. There might be an impaired 1-alpha hydroxylation of the tobacco consumers. Accumulation of cadmium in kidney may also cause the lower levels of 25(OH)2 [26,27]. Low levels of serum calcium may be due to the reduced serum 1,25(OH)2 D[28]. Calcium levels in serum are unchanged may be due to the hormones acting for maintaining serum calcium in normal values. Though levels of PTH and 1,25(OH)2 D are low the plasma level of calcium remains unchanged this could be due to decreased uptake of calcium in bone. In our study we observed low levels of Sr. Osteocalcin in tobacco consumers. Low Osteocalcin levels in tobacco consumers shows decreased osteoblastic activity[29,30].In our study there was no significant difference for the Sr. Alkaline phosphatase activity/. There was no difference in serum alkaline phospatase activity in tobacco consumers and non tobacco consumers. A recent study demonstrated the male smokers have lowered parameters such as PTH, Vit Dstatistically significant over non smokers. Our study suggests that tobacco consumption compared to normal males has an inhibitory effect on bone formation. Sensitive bone markers and studies on RNAs also suggest the toxic content of tobacco effect on bone formation. In our study we got decrease in Bone Mineral Density in tobacco consumers compared to non tobacco consumers. Association between Bone Mineral Density and circulating concentrations of 25(OH) D has been demonstrated in number of studies. Non tobacco

consumers may compensate for such as low calcium intake leading to decreased bone mineralization but this compensation is not as much effective in tobacco consumers.

Conclusion

Our study concludes that tobacco consumption decreases total vitamin D and PTH. Tobacco consumers have decrease in serum levels of vit D and decrease in PTH levels. These differences were not explained only by other confounding factors like low socio economic status and intake of alcohol. Lower levels found couldn't be explained with proper mechanism involved. But it is explained only on the basis of observations obtained. The important finding in study was suboptimal levels of Vit D i.e. serum levels of Vit D below 15 ng/ml was 50% higher in tobacco consumers. The trend was higher in age group of 40-50 years. Though the difference found is small and though the size of sample is not large enough the findings are clinically important to correlate among tobacco consumers for the decreased bone mass and for treatment of fractures and osteoporosis.

Ethical Clearance: Taken from institutional ethics committee.

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Role of Frozen Section in Neck Dissection of Oral Cancer Patients

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Abstract

Introduction: Oral carcinoma is emerging as growing problem in many areas of the World and 4th most common cancer among women in India. Globally, over 3,00,000 people are diagnosed with oral cavity cancer each year. Radical or modified radical neck dissection is usually a choice of surgeon in node positive patients. Frozen section when performed along with supra-omohyoid neck dissection provides solution to this dilemma of surgeon by detecting the metastasis in cervical lymph nodes intra-operatively during neck dissection. This study was planned to establish usefulness of frozen section examination in decision of correct plan of management of patients of oral cancer with clinically nonodal metastasis in neck (N0).

Objectives: To study appropriate plan of management for clinically neck examination with lymph node status N0 in patients of oral cancer and to determine efficacy of frozen section examination in detection of occult nodal metastasis in neck in patients of oral cancer.

Method and Materials: In 102 cases, excision of lesion, followed by supra-omohyoid neck dissection was primary mode of treatment and lymph nodes at various levels were sent to frozen section for assessment of metastasis by cancer cells. Intraoperatively during frozen section,if any of the lymphnode between levels I to levels III is found positive for the metastatic disease then the plan of surgery was changed to a more comprehensive Modified radical neck dissection.

Results: Maximum cases were carcinoma tongue followed by cases of carcinoma of gingiva-buccalsulcus. Carcinomas of stage 1,stage 2,stage 3 are 43.8%, 52.2% and 3.9%. Histopathological grading of oral squamous cell carcinoma was done by Broader's grading system as Well differentiated SCC (52 cases), Moderately differentiated SCC (42 cases), poorly differentiated SCC (08 cases). Supra-omohyoid neck dissection was initial plan of management in cases but it changed to modified radical neck dissection in 42 cases as lymph nodes between level 1 to 3 were positive for metastasis of SCC.

Conclusion: Oral cancer should be surgically managed intra-operatively by frozen section examination of alllymph nodes to avoid short term recurrences and institution of post-surgicalchemo/radiotherapy. Frozen section examination of yielded lymph nodes in these surgeries canmodify the extent of dissection of neck.

Keywords: *Oral carcinoma, frozen section, supra-omohyoid neck dissection (SOND), modified radical neck dissection (MRND).*

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Introduction

Oral cancer is an emerging as an growing problem in India, with prevalence of 60%[(1,2)]. Men are 2 – 3 times more commonly affected than women. 98% of cases occur in patients aged more than 40 years and are linked to tobacco chewing or smoking[(3-7)]. The spread of oral cancer from primary site to cervical lymph nodes has

been well addressed [8]. The presence of occult metastasis ranges between 10 – 52%. Lymph node metastasis has been found to be one of the most important factors in prognosis of oral squamous cell carcinoma and successful treatment depends on the management of neck. When compared to Node negative disease, the presence of even a single micro-metastasis in a lymph node is associated with a significance difference in recurrence and survival. Supra omohyoid neck dissection (SOND) has been described as a quality staging procedures for OSCC in clinically N0 nodes by some authors[9-11]. Radical neck dissection/MRND is usually a choice of surgeon in Node positive patients.Frozen section when performed along with SOND provides solution to the surgeon by detecting the metastasis in cervical lymph nodes intra-operatively during neck dissection. Incase frozen section detects occult nodal metastasis then SOND get modified to RND/MRND[12-14].

Aim and Objective:

The aim of our study was:

1. To study appropriate plan of management for clinically N0 neck examination in patient of oral cancer
2. To determine efficacy of frozen section examination in detection of occult nodal metastasis in neck in patients of oral cancer

Materials and Method

It was a Prospective study of 2 years conducted in 102 patients of oral malignancy with clinically N0 neck in Pathology department, Jawaharlal Nehru Medical College, Sawangi, (M) Wardha.

Inclusion Criteria:

- Biopsies of patients in OPD of oral and maxillofacial surgeries with ulcer or growth over tongue, lip or buccal or gingivobuccal sulcus were collected with no palpable nodes in neck.

Exclusion Criteria:

- Patients with carcinoma of thyroid and oropharynx
- Patients with diagnosis other than SCC
- Patient with recurrent malignancies
- Patients with lymph node status other than N0

Materials required for frozen section:

- Cryostat (Leica)
- Materials for rapid H and E staining
- Lymph-node specimen
- Tissue freezing medium
- Compound light microscope
- Glass slides and slide marker
- Scalpel and blade
- Normal saline
- Filter paper, forceps and gloves

Method:

Procedure[15]: During the neck dissection lymph nodes at various levels of neck were sent to frozen section for assessment of metastasis. All the resected lymph-node submitted for intra-operative frozen section were grosslyexamined and the respective areas were sectioned and embedded in optimal cutting temperature compound and were freezedat -20 °C to -30 °C in cryostat. The sections were cut approximately 4- 5 µm thick and picked on glass slides whichwere stained with rapid Hematoxylin and Eosin .

Observation: The total of 102 cases in IPD of oral and maxillofacial surgery meeting the inclusion criteria were included and studied for defined objectives. The observation of the study is described in the following tables.

Table 1: Sitewise distribution

Site	No. of Cases and Percentage
Tongue	41(40.19)
Gingivo-buccal sulcus	28 (27.45)
Buccal mucosa	22 (21.57)
Alveolus	07(6.87)
Lip	04 (3.92)

The present study has observed maximum number of cases belonging to carcinoma tongue followed by GB sulcus, by buccal mucosa, alveolus and lip.

Table 2 :Stage wise distribution (n=102)[16]

Stage	No. of Cases and Percentage
T1N0M0 (Stage I)	44 (43.84)
T2N0M0 (Stage II)	54 (52.24)
T3N0M0 (Stage III)	04 (3.92)

TNM staging (AJCC) was adopted for staging of oral squamous cell carcinoma. Stage of oral squamous cell carcinoma defines aggressiveness and prognosis of the tumour .In the study 54 cases belong to stage II followed by 44 cases belonging to stage I category. Only 4 cases of high grade tumour were observed.

Histopathological grading of oral squamous cell carcinoma was done by broader's grading system.[17]

Table 3: Distribution of cases diagnosed on biopsy prior surgery (n=102)

Diagnosis on Biopsy	No. of Cases and Percentage
Well differentiated SCC(WDSCC)	52 (50.99)
Moderately differentiated SCC (MDSCC)	42 (41.16)
Poorly differentiated SCC (PDSCC)	08 (7.85)

In the present study, maximum cases were observed of Well differentiated grade i.e. 52 cases followed by Moderately differentiated grade i.e. 42 cases and only 8 cases belong to poorly differentiated category.

Table 4. Distribution of Lymph node status on frozen section [18]

Initial Surgery Planned	Diagnosis of Lymphnode on FS	No. of Cases and Percentage	Final Surgery Performed
SOND	Negative for metastasis	60(58.82)	SOND
	Positive for metastasis	42(41.18)	MRND

The initial plan of management of all cases was SOND . The level of lymph nodes resected in SOND were level I, IIand III. These lymph nodes were sent for frozen section study. Incase the frozen section detected nodal metastasis, the neck was further dissected upto the level V, which meant that neck dissection was converted from Supra Omohyoid Neck DIssection (SOND) to Modified Radical Neck Dissection (MRND).

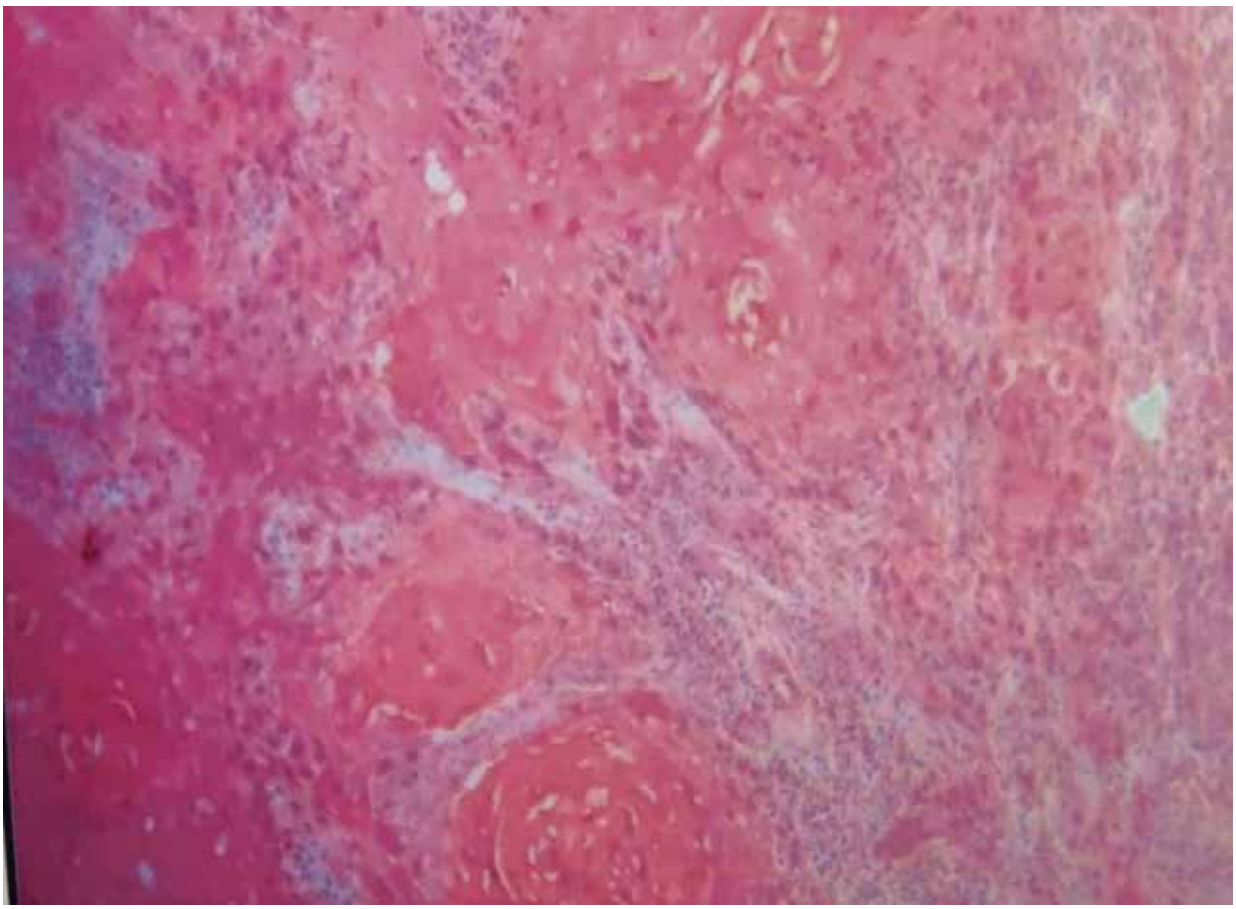


Fig. 1: 10x view of metastasis of WDSCC in lymphnode in FS

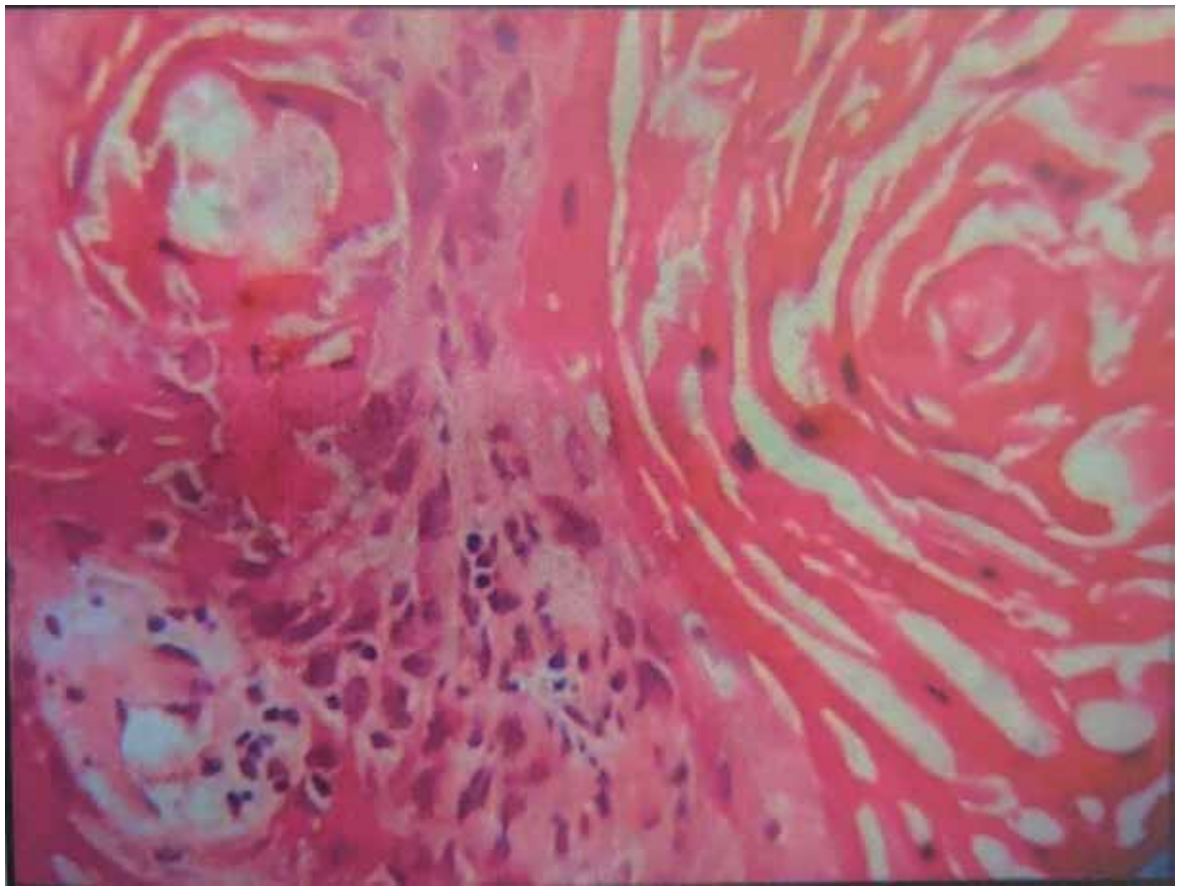


Fig. 2: 40x view of metastasis of WDSCC in lymphnode on FS

Discussion[19-22]

The word "Cancer" derived from a greek word "KARKINOS". According to WHO, cancer is a generic term for large group of diseases characterized by growth of abnormal cells beyond their usual boundaries which can invade other parts or spread to other organs. World is moving towards various non-communicable diseases (modern epidemics) of which cancer is most common cause of mortality in developed countries. Environment and lifestyle play an important role in causing cancers. These days addiction among young population is rising resulting to advance stage disease at an early age. This study intends to access the usefulness of frozen section for the decision of neck dissection management in oral cancer patient with nodal status as N0of neck.

Manni et al.[19] conducted a study in 57 patients with oral squamous cell carcinoma out of which 10 cases were detected as metastasis disease. The initial plan was SOND. After the study they concluded that frozen section biopsy of lymph node suspected to be metastatic during SOND proves a valid staging procedure.

Hoogen et al.[20] also performed a retrospective analysis in 57 patients with an average age of 59 years, performed SOND as an initial treatment in all cases. Amongst them 10 cases showed positive metastasis in lymph node.

Manni and Hoogen et al have suggested SOND as an appropriate staging procedure to manage cases of oral SCC with N0 neck. In the present study, out of 102 cases frozen section examination detected lymph node metastasis in 42 cases. This finding is in concordance with the study done by ***Rassekh et al.***[21]

Chaturvedi et al.[22] in their study on 877 patients used frozen sections to detect intraoperatively and were able to detect 188 cases for metastasis. In studies done by Manniet, Hoogan et al and Chaturvedi et al, the plan of management changed from SOND to MRND in 10, 10 and 188 cases respectively.

In the present study, also the cases which were found in level I, II and III were dissected upto level V i.e. MRND. Out of 102, 42 cases underwent such conversion of dissection of SOND to MRND in the present study which is similar to above mentioned studies.

Conclusion

In clinically N0 nodes of a oral squamous cell carcinoma patients the initial surgical plan preferred is Supra omohyoid neck dissection, however if neck nodes (level I to level III) are found positive for metastasis on FS during surgery then the SOND could get modified as a RND/MRND .

Thus, Frozen section play a major role in the decision on surgery of oral cancer patients, is a easy,possible and life saving technique.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

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A Study on Designing, Development and Testing of an Innovative External Jig for the Percutaneous Screw Fixation of the Acetabular Column Fractures

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Abstract

Background: Operative treatment of acetabulum fractures often requires open surgical fixation. Minimally invasive surgery (MIS) with percutaneous screw fixation can be done is some acetabular column fractures which are amenable to closed reduction. This can be done free hand using intraoperative fluoroscopy or with the help of advanced 3D navigation system. Free hand technique exposes the operative staff to considerable intraoperative fluoroscopy,has a steep learning curve and there is a risk of screw malpositioning. Even though 3D navigation is highly accurate, the affordability of the navigation system in a rural set up is not feasible. Use of an external universal modular jig which has been routinely used for the fixation of long bone fracture, can give similar results in the fixation of undisplaced/minimally displaced acetabular column fractures amenable to close reduction. Currently such jigs are not available for the management of acetabular fractures.

Objectives: With this study we aim to design a modular jig for the percutaneous fixation of the acetabular column fracture fixation. Also we will validate in vitro efficacy and safety on cadaveric models. To test the clinical efficacy on minimally displaced acetabular column fractures.

Methodology: It will be an observational study conducted at the Department of Orthopaedics, JNMC, Sawangi and Wardha, India. A 3 D model of the pelvis will be made based on the CT scan data of 40 patients using Mimics 10.01 software (Materialize, Leuven, Belgium) software. After the desired jig is made,its accuracy will be checked on the saw bone and cadaveric model. Once the accuracy of the jig has been established, it will be used on the suitable patient population and the results will be compared with the matched patient population who have been operated using the traditional free hand technique under fluoroscopy control.

Results: The results will be compared based on the operating time,radiation exposure,hospital stay and postoperative outcome. During follow up,patients in both the groups will be evaluated clinically with Harris hip score (HHS) and radiologically with Matta outcome grading. To evaluate functional outcome patients the patients will be categorized into excellent (HHS, 90-100), good (HHS, 80-90), fair (HHS, 70-80) and poor (HHS, <70).

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Conclusion: This study will help in designing and development of an universal external modular jig for the MIS and percutaneous fixation of acetabular column fracture.

Keywords: *Acetabulum column Fracture, Minimally invasive surgery (MIS) Universal external modular Jig, Percutaneous screw Fixation.*

Introduction

Minimally invasive surgery (MIS) for fixation of acetabular fractures is possible with the advances in intraoperative fluoroscopic imaging.[1] Percutaneously inserted screws in medullary pubic ramus, iliac wing and iliosacral bone can stabilize pelvic or acetabular disruptions directly mean while can diminish operative blood loss, shorten operative time and allow patient's early activity.[1–3] Complications associated with open surgical procedures are similarly avoided by using percutaneous techniques. Several biomechanical studies have shown that screw fixation gives good stability as obtained with plate fixation for certain varieties of acetabular fractures with the added advantage of being minimally invasive thereby reducing blood loss,operative time and patient morbidity.

Stable and safe percutaneous fixation techniques depend on accurate closed reduction, excellent intraoperative fluoroscopic imaging and detailed preoperative planning.[4–6] A thorough knowledge of pelvic osseous anatomy, injury patterns, deformities and the related intraoperative imagery techniques are essential for the surgeon to fulfil the operation of percutaneous acetabular fixation.[7,8]

Although percutaneous screw fixation has several advantages over open surgical procedure in minimally displaced acetabular fractures, there is always a risk of injury to the surrounding neurovascular pelvic structures.[9,10] The learning curve for free hand percutaneous fixation is steep, requires expertise and the intraoperative fluoroscopic exposure to the surgeon is more as compared to open procedure. Image guided Orthopaedic surgery has the potential to be a widely used, minimally invasive and intelligent option.[9] But cost is a major issue for their use on a larger scale in Indian scenarios.

Background: Management of the acetabular fractures is a challenging situation for the orthopaedic surgeon worldwide. Percutaneous fixation is a preferred modality of treatment for certain varieties of minimally displaced acetabular fractures which are amenable to closed reduction. Traditionally percutaneous fixation is done using free hand technique,which requires considerable expertise,is time consuming and requires significant radiation exposure.Navigation guided percutaneous fixation is emerging as a promising treatment option but cost is a major issue for its use in Indian setup.Use of a modular universal jig can significantly overcome the current difficulties,will be cost effective and can improve the postoperative outcome in this patient population. Currently there is no commercial jig available for the percutaneous fixation of acetabular column fractures.

Background/Rationale: Surgical jigs are being traditionally used in the management of long bone diaphyseal fracture of femur, tibia and humerus. They reduce the operative time, intraoperative fluoroscopic exposure and also the surgeon-based errors thereby improving the surgical safety and assuring more predictable outcomes. Use of a modular external jig in the percutaneous fixation of the acetabular column fracture can give similar results. Such jigs are currently not available for the surgical fixation of acetabular column fracture.

Objectives:

1. To design an external jig with modularity for the percutaneous fixation of anterior and posterior acetabular fracture fixation, facilitating accurate insertion of antegrade and retrograde lag screws in the fixation of minimally displaced acetabular column fractures on pelvic specimens.
2. To develop the designed jig into a model through 3D printing (CAD).
3. To validate in vitro efficacy and safety on cadaveric models.
4. To test the clinical efficacy on minimally displaced acetabular fractures.

Method

Study Design: Observational Case Control Study

Setting: The study will be conducted at the Department of Orthopaedics, JNMC, Sawangi, between June 2020 to June 2022. The complete helical CT data will be collected from 40 adult patients (20 males and 20 females) aged between 18 to 75 years without any bone lesions or anatomic abnormalities,who have been advised CT abdomen pelvis for other any medical reason apart from Orthopaedic trauma at JNMC Hospital. The data collected will be used for the reconstruction of 3 D Pelvic model. The entry site for screw insertion will be determined using Mimics software.

Participants: Informed consent will be obtained from all participants. After the jig has been devised and it's validity and accuracy has been checked on cadaveric models,it will be checked on the appropriate patient population.

Eligibility Criteria: All the patients with fresh (less than 05 days old) minimally displaced acetabular column fracture amenable to closed reduction will be included in the study.

Exclusion Criteria:

1. Both column fracture with intra articular component requiring open reduction
2. Old fractures(more than 02 weeks old) not amenable to closed reduction

Variables: We will be checking the accuracy of the jig on the Pelvic Saw bone models and cadavers. Accuracy will be checked based upon the deviation between planned and executed screw at respective screw entry and tip.

Once accuracy has been identified then it will be checked on suitable patient population and the results will be compared with matched patient population in terms of radiation exposure to Operation theatre personnel, operative time and hospital Stay.

Data Sources/Measurement: During follow up,patients in both the groups (MIS using Jig vs MIS using free hand technique and conventional fluoroscopy) will be evaluated clinically with Harris hip score (HHS) and radiologically with Matta outcome grading. To evaluate functional outcome the patients will be categorized into excellent (HHS, 90-100), good (HHS, 80-90), fair (HHS, 70-80) and poor (HHS, <70).

Bias: None

Study Size: Because of the novelty of the current research and as there are no prior previous published similar studies, the sample size cannot be calculated methodically. So purposive or convenient sampling method will be used and the efficacy of the jig will be first checked on 05 cadavers (10 Hips) first. If found to be suitable and accurate then the jig will be used on all the suitable cases as per the inclusion criteria of the current study.

Quantitative Variables: The Student's t-test will be used in evaluating the statistical significance of differences in means. The unpaired t-test will be used in the comparison of mean surgery time between the two groups.

Statistical Method: Data will be imported to Microsoft excel sheet. The level of significance of the Universal modular jig as compared to conventional fluoroscopy for percutaneous fixation, will be measured using Chi square test.

Discussion

Acetabular fracture accounts for 10–22% of all fractures. They are traditionally managed surgically using open reduction internal fixation (ORIF) using plate osteosynthesis.[13,15]

ORIF is a preferred but requires extensive surgical exposure,have higher incidence of soft tissue complications, prolongs hospital stay and increases risk of heterotopic ossification and avascular necrosis of femoral head due to exposure per se. Minimally invasive surgery (MIS) and percutaneous screw fixation is an equally effective alternative in the management of certain variety of acetabular fractures which are amenable to close reduction.[13,16,17]

In the pelvi acetabular surgery,there is a very narrow pelvic corridor with the neurovascular structure in close proximity of the fracture surface and also the overlapping bowel shadow makes the intraoperative fluoroscopic visualization difficult.[14] MIS and percutaneous screw fixation can be done with the traditional free hand method using intraoperative 2D fluoroscopy or with the help of 3D navigation system in the management of certain varieties of minimally displaced pelvi acetabular fractures. Free hand technique is technically

demanding, has a steep learning curve, has a high risk of screw malplacement and exposes the operative staff to prolonged radiation.[17] 3D navigation based MIS is an excellent upcoming option with encouraging results, high safety margin but is very expensive and not a cost effective option in a rural setup.[17]

External jig aided intramedullary interlocking nailing has been used in the management of long bone fractures in Orthopaedic trauma routinely. The advantage of the use of an external jig is that it makes the surgery reproducible, is relatively cheap and decreases the operative time and complications.

We have hypothesized that the use of an universal modular jig would give similar benefits in the management of indicated acetabular column fractures which are amenable to closed reduction and percutaneous screw fixation. Also the use of this new jig would help in decreasing the operative time, surgical exposure, blood loss and would improve the patient outcome and reduce the hospital stay. Such jigs are not available for the fixation of acetabular columns fractures. So through this study we aim to design and develop an innovative external jig for the percutaneous screw fixation of the acetabular column fractures and then check its accuracy first on saw bone and cadaveric specimen. Once its accuracy and safety is established we will use the jig on selected patient population which are suitable for percutaneous acetabular fracture fixation and will compare the outcomes on the matched patient population treated with the current free hand technique using intraoperative fluoroscopy in terms of operative time, functional outcome and radiation exposure.

Key Results: This study will help in the designing, development and testing of an innovative external jig for the percutaneous screw fixation of the acetabular column fractures.

Limitations: It will be a single centre study so reproducibility of the current device would not be possible to check during the current study. A multicentric study comparing the efficacy of the jig will be required subsequently.

Interpretation: This will be the first study to design, develop and test the efficacy of a universal modular jig for the fixation of the acetabulum column fracture. We hypothesize that the development of this jig will help in successful management of the acetabular column fractures in terms of decreased operative time, reduced radiation exposure and improved patient functional outcome.

General is Ability: Once developed and validated a multicentric study will be done to determine the reproducibility and accuracy of the jig.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Exploring the Etiological Evidences at Avascular Necrosis of Femoral Head by Investigative Approaches of Histopathology, Clinical Assessment and Radiology

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Abstract

Background- The avascular necrosis of the femoral head includes interference of the blood flow to the femoral head. This ischemia leads to death of the bone cells and bone marrow followed by the collapse of the bone femoral head, which finally leads to the destruction of the bone tissue and arthritis,

Objectives:

1. Histopathological analysis of the tissue obtained after core biopsy or sections of head after head excision while doing Arthroplasty.

2. Correlation between histopathological evidence with clinical and radiological findings.

Methodology: The tissue will be sampled from site of AVN during surgical procedure that is Core decompression or hip arthroplasty. Tissue material obtained will be transfered to regular histological fixative of 10 % formalin. It will be fixed for a day, later to be reprocessed for decalcification. Formalin fixed tissue will be subjected for treatment of running water to remove excess of formalin. Later the tissue will be examined for its gross appearance of any changes of autolysis The process of decalcification adopted for present work is chemical one. The decalcification tissue will be subjected for histokinette cycle. The tissue is now ready for paraffin block. These paraffin blocks will be subjected to microtomy for obtaining 5 micro milimeter sections. These paraffin sections will be stained for H/E and Van. Giesson stain. Histopathological assessment of the tissue will be carried out for the evidence and grading of avascular necrosis.

Expected Results: Correlation between histopathological evidence with clinical and radiological findings.

Conclusion: This study will be helpful in developing Histopathological staging in correlation with clinical, radiological findings.

Keywords: *Avascular necrosis, Head of femur, Histopathology.*

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Introduction

The avascular necrosis of the femoral head includes loss of blood supply to the femoral head. This leads to death of the bone cells and bone marrow followed by the collapse of the femoral head. Which finally leads to the destruction of the bone tissue, arthritis and loss of function[1]. Avascular necrosis of the femoral head is a disease, which affects young adults, in their 3rd, 4th and

5th decade. This disease affects men more frequently as compared to women. ONFH in North Indian patients[2] is a disease of young individuals, male more than females. This disease affects specially young adults, who are socially and professionally active[3]. These patients are initially without symptoms but eventually need hip arthroplasty surgery until their 5th decade [4].

Nowadays, there are more than 16 different classification systems used to classify and describe avascular necrosis of the femoral head[5]. Classification system introduced by Ficat and Arlet is the most commonly used. The Ficat and Arlet classification system consists of five stages (0 to IV): The first three describe the events that occur before the collapse of the femoral head and the last two stages describe the post-collapse changes.

Stage – 0: Only suggestive on symptoms

Stage – 1: Radiograph is normal except minimal osteoporosis, blurring of bony trabecule

Stage – 2: Femoral head contour is maintained but there is sign of bone remodelling such as cystic subchondral lucency beneath articular surface (crescent sign)

Stage – 3: There is subchondral sequential collapse or flattening of femoral head (crescent sign and eventual cortical collapse).

Stage – 4: Joint space is narrowed and secondary degenerative changes seen in acetabulam along with marginal osteophytes formation.

Other classification is international ARCO staging system[6].

Even after collapse occurs, subsequent cessation of collapse can be expected in certain percentage of hips [7].Collapse of femoral head does not necessarily indicate a poor prognosis In such patients use of bisphosphonates[8][9][10] in the treatment of AVN is encouraging. This is relatively new option.[11][12].

Similarly use of Enoxaparincan prevent progression of stage I and II osteonecrosis of the hip [11].

Avascular necrosis of the femoral head is either primary (idiopathic) or secondary may be due to post trauma, alcohol consumption, smoking, steroid induced and following sickle cells disease.

Background/rationale

There is a paucity of histopathological evidence with specific etiopathogenesis in AVN of femoral head. The proposed study is first of its kinds to explore potential of histopathological analysis and attempting to establish it's clinical and radiological correlation.

Objectives:

1. To analyse clinical, radiological and histopathological correlation in avascular necrosis of femoral head"
2. Histopathological analysis of the tissue obtained after core biopsy from different sectors of femoral head while doing core decompression or sections of head after head excision while doing Arthroplasty.
3. Radiological analysis of X-ray and MRI findings
4. Correlation between histopathological evidence with clinical and radiological findings.
5. Secondary objective of the correlation between haematological investigations and histopathological evidence.

Materials and Method

Study plan:

Study design: This will be a Prospective Observational Studyat DMIMS (DU) Jawaharlal Nehru Medical College,Department of Orthopedics, Sawangi Meghe Wardha, during the period from July 2020 to JULY 2022.We will include all patients suffering from non traumatic Avascular necrosis of femoral head. Age group will be from 20 to 60 yrs.

Data Collection: The tissue will be sampled from site of AVN during surgical procedure that is Core decompression or hip arthroplasty. Tissue material obtained will be transferred to regular histological fixative of 10 % formalin. It will be fixed for a day, later to be reprocessed for decalcification. Formalin fixed tissue will be subjected for treatment of running water to remove excess of formalin. Later the tissue will be examined for its gross appearance of any changes of autolysis The process of decalcification adopted for present work is chemical one. The decalcification tissue will be subjected for histokinette cycle. The tissue is now ready for paraffin block. These paraffin blocks will be subjected to microtomy for obtaining 5 micro millimetres sections. These paraffin sections will be stained for H/E

and Van. Giesson stain. Histopathological assessment of the tissue will be carried out for the evidence and grading of avascular necrosis.

Participants: Patients suffering from non traumatic Avascular necrosis of femoral head. Age group will be from 20 to 60 yrs,of both sex

Inclusion Criteria:

1. Patients suffering from non traumatic Avascular necrosis of femoral head attending orthopaedic OPD.
2. Those who are ready to undergo this study with written consent

Exclusion Criteria:

1. **Patients with metabolic disorders:** Variables: Participants who are included in the study having avascular necrosis of femoral head will have a differentetiology like Sickle cell disease,Alcohol induced,Steroid Induced and Idiopathic.

Data sources/measurement: The Histopathological data obtained from this study will be critically analysed according to etiological factors and compared accordingly for each variable of interest.

Data will be collected from the pathologist and tabulated according to findings across various etiological factors. Source of data and details of method of assessment (measurement) will be described Comparability of assessment method will be judged in different groups.

Bias: Measures will be taken to control the selection bias.

Statistics: Raw data processing will be done by using convenient sampling. we will include 10 patients in each group during the study period.

Discussion

Avascular Necrosis of the femoral headis a disease characterized by loss of blood supply to the affected part of the head of the femur which ultimately leads to it's death. Hence it is also called as coronary disease of the hip. Head of femur receives blood supply from 3 main sources-reticular branches from the lateral circumflex femoral artery (supplying majority of the head) which anatomises which branches from medial circumflex femoral artery (supplying area around greater a trochanter) and third source is obturator artery which gives rise to artery of ligamentumteres (supplying small portion of the head.[1] The condition usually involves patients in their early adult hood and middle ages i.e. 30 to 40 years.[2]Every year 10to 20 thousand cases of avascular necrosis of femoral head[3] are diagnosed in the united states, out of which approximately 10%undergo replacement surgeries.[4] The condition is usually unilateral but it gradually progresses to become bilateral. Studies have shown that the etiopathogenesis of avascular necrosis of femoral head is varied and very confusing. Usually the term avascular necrosis refers to the non traumatic, non infective or idiopathic . Broadly the term can be divided into two main groups that is one group with directly identifiable causative factors and other group without any directly identifiable causative [3]factors. Excessive steroid usage for longer duration and chronic alcoholism accounts for approximately more than 80% cases of avascular necrosis of femoral head .exact pathogenesis of this varied aetiologies is not yet established. Various studies are being under taken to establish the exact stapes in the pathogenesis.

Generally following Factors are thought to be associated with the pathogenesis of avascular necrosis of femoral head. End effect all the aetiologies lead to hindrance of blood supply to the femoral head, which in term may be due to occlusion of vessels supplying to the femoral head, stenosis of the blood vessels supplying the femoral head, atherosclerosis involving the arteries supplying femoral head, hyercoagulation, increases intra-osseouspressure, mechanical forces disturbing vascular supply to femoral head (hip dislocations and displaced fracture neck of femur) or various metabolic diseases which hampers blood supply to femoral head.

Due to variability of etiopathogenesis, the clinical, histopathological and radiological pictures also vary. Various staging system[5] have been developed to categorize various patients in groups and then to develops suitable management protocols for the groups. But there has not been single ideal staging system for avascular necrosis of femoral head till date. The diagnosis of avascular necrosis of femoral head is routinely done with the help of detailed history, clinical examination, assessment of the gait, bony palpation of hip and surrounding structures, presence or absence of deformities, limb length discrepancy. Clinical findings start to appear in the patients in late stages of avascular necrosis that is after the collapse[6] of femoral head. After the collapse of the femoral head, the modalities meant

for salvage of femoral head do not work and patient will required total hip arthroplasty.

Radiological staging is based on various radiological modalities like plain radiographs (plain anterioposterior radiograph of pelvis with both hip joints and frog leg or lateral view), CT scan and MRI scans. There are more than 16 various classification[5] and staging system base on radiological modalities out of which two most commonly used are Ficate and Arlet classification and University of Pennsylvania classification. Most of these imaging modalities based staging systems take into account exact site of the lesion, exact size and the extent of the lesion and presence of subchondral fracture or collapse. The exact site of the lesion is best visualised using plain radiograph (cost effective, simple and easily available but not useful in early stages) exact size and extent of the lesion is best visualized with help of MRI scan (high sensitivity and specificity, modality of choice for screening) and subchondral fractures are best studied with the help of CT scan (most sensitive for subchondral fractures). There are various staging systems which consider all these radiological modalities.

There is no staging system which takes into account all the three aspect that is clinical, histopathological and radiological pictures of the patients. Although various studies have shown that histological staging done by taking biopsy from the affected lesion helps in differentiating early stage disease from late and it thus helpful in delaying the replacement surgeries. Histological changes start to developed after few weeks of the starting of the disease. Usually biopsy is done once the disease has been diagnosed. By that time irreversible changes might have occurred in the femoral head. So early biopsy should be promoted. In our study we have tried to correlate histological staging of the patient with clinical and radiological findings. This will help in developing a new staging system which incorporates all the three modalitiesthat is histopathology, clinical and radiology. Thus it will help in developing ideal staging system for the patients suffering from avascular necrosis of the femoral head. It will in terms help in better understanding of the disease, proper categorisation of the patients in various groups and then in determining the ideal treatment modality for those groups.

Various treatment modalities available for treatment of avascular necrosis of femoral head ranges from non surgical modalities like observation and non weight bearing mobilization, various pharmacological agents like bisphosphonates[7][8][9][10] Enoxaparin are shown to be useful in some studies, hyper baric oxygen therapy (all these non surgical modalities are useful in early stages of avascular necrosis of femoral head). Surgical modalities like core decompression with or without additional procedures that is bone grafting, biologicadjutants– Platelet Rich Plasma, stem cells etc. In advance stage of AVN rotational osteotomies or replacement surgeries are indicated. For such a complex disease with varied aetiopathological factors and varied treatment modalities a proper staging system is must. Our study will help in proper staging and thus selecting the appropriate treatment modality for a particular patient especially in cases of early stage of the disease. This will also help in prediction of Prognosis.

Conclusion

This study will be helpful in developing Histopathological staging in correlation with clinical, radiological findings.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Role of Anion Gap as a Prognostic Indicator in Acute Coronary Syndrome (ACS)

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Abstract

Background: Cardiovascular diseases are the leading cause of mortality in India and acute coronary syndrome (ACS) is one of its anomalies, affecting mainly the coronary arterial blood supply and further the functioning of heart muscle. Its subtypes are Non-ST elevation myocardial infarction (NSTEMI), unstable angina, and ST-elevation myocardial infarction (STEMI). There are various diagnostic tools available to detect ACS like; electrocardiography (ECG), Cardiac stress test, Coronary CT angiography, Coronary angiogram and cardiac biomarkers. Yet, tools for assessment of prognosis are scanty and new prognostic tools are always welcomed, one such is anion gap, which can be detected by assessment of certain ions present in the serum, urine or plasma, etc. In this study we will assess the role of anion gap, in the assessment of prognosis in ACS.

Objective:

1. To estimate anion gap (AG) in cases of acute coronary syndrome (ACS) at the time of diagnosis;
2. To evaluate association of anion gap (AG) with the prognosis of acute coronary syndrome (ACS)

Methodology: The study will be conducted in the Department of Medicine of a Rural Tertiary Care Hospital. Estimation of anion gap (AG) in cases of acute coronary syndrome (ACS) will be evaluated at the time of admission and after five days of treatment. Anion gap will be calculated or measured as difference between measured cations (Na+ + K+) and the measured anions (Cl- + HCO3-) in serum. Any complications during the management will be noted (Heart failure arrhythmias, Hypotension, Reinfarction, Death) will be correlated with anion gap (AG). Chi square test will be applied to evaluate statistical difference between among anion group quartile groups.

Results: The study will play a key role in identifying and assessing various other prognostic tools like anion gap, in assessing the severity of disease to some extent in patients with acute coronary syndrome.

Conclusion: High value of serum anion gap levels is associated with poorer prognosis of the disease outcome in patients with acute coronary syndrome.

Keywords: *Acute coronary syndrome, anion gap, cardiovascular disease, prognostic tool.*

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Introduction

India is the second most populated country of the world with a high mortality rate caused due to various medical conditions and diseases, especially cardiovascular diseases (CVDs) becoming the leading cause of mortality in India.[1] In 2017, CVD caused an estimated 17.8 million deaths worldwide.[2] Coronary

artery disease(CAD) is the most common type of heart disease caused due to reduction in oxygen and blood supply to the heart muscle due to build up of plaque in the arteries of the heart i.e. the coronary arteries. Epidemiologic studies have shown that there are at present over 30 million cases of CAD in the country.[3] Furthermore, acute coronary syndrome(ACS) is a subtype of CAD. Itis a group of diseases in which blood supply to any of the coronary arteries is decreased partially/completely which causes the heart muscle either to function improperly or its death. The following are the subtypes of ACS are:- Non-ST elevation myocardial infarction (NSTEMI), unstable angina, and ST-elevation myocardial infarction (STEMI) .There are various tools for diagnosing these conditions like- ECG, Cardiac stress test, Coronary CT angiography, Coronary angiogram, Blood Tests, etc.

Presently used prognostic tools for acute coronary syndrome are various biochemical markers like estimation of cardiac troponin, natriuretic peptides, growth-differentiation factor-15, etc. These are some of the mose efficient prognostic biochemical markers.

New prognostic tools in CAD patients are always welcomed. The serum anion gap (AG) is a mathematically derived parameter that has been used for more than 50 years.[4] Till now AG has been used as a prognostic indicator in studies related to advanced kidney disease,[4] hypertension,[5] insulin resistance[6] & cardiac deaths.[7] It is yet unknown whether AG can alter the course of prognosis of ACS. Therefore dearth of medical evidence relating to association of AG & ACS. So, this study will evaluate AG & its prognostic implication in case of ACS.

Aim: To assess the anion gap as a prognostic indicator or tool in patients with acute coronary disease.

Objective:

1. To estimate anion gap (AG) in cases of acute coronary syndrome (ACS) at the time of diagnosis
2. To evaluate association of anion gap (AG) with the prognosis of acute coronary syndrome (ACS)

Methodology

Study Design: Cross sectional study

Study Population: Will be carried out in 50 consecutive patients

Study period: Two-Months

Study setting: This cross-sectional study will be conducted in the Department of Medicine of a Rural Tertiary Care Hospital. Consecutive patients or subjects with diagnosis of acute coronary syndrome (Ischemic chest pain, ECG changes, and Cardiac biomarkers) admitted in the intensive care unit (ICU) of a tertiary care hospital. The study will begin after clearance from institutional ethical committee. Detailed history of Rural or Urban backgrounds, Physical activity levels, Addiction (smoking, alcohol intake), Family history of Blood Pressure and Diabetes will be recorded and Clinical Examination will be done.

Estimation of anion gap (AG) in cases of acute coronary syndrome (ACS) at the site of diagnosis:- Anion gap will be evaluated at the time of admission and after five days of treatment. Assessment of severity of coronary artery disease (CAD) will be done on the basis of severity of coronary artery stenosis (in coronary angiography), Killip's classification and cardiac function grades based on left ventricular ejection fraction (LVEF). Grades of cardiac function compromised normal (defined ≥50%), preserved (40%-49%), and reduced (<40%) LVEF.[8] Significant coronary artery stenosis was defined as ≥ 75% narrowing of the diameter of at least one major epicardial vessel.[9]

Evaluation of association of anion gap (AG) with the prognosis of acute coronary syndrome: Any complications during the management will be noted (Heart failure arrhythmias, Hypotension, Reinfarction, Death) will be correlated with anion gap (AG).

Calculation of Anion gap (AG)**:** Anion gap is calculated or measured as difference b/w measured cations (Na+ + K+) and the measured anions (Cl- + HCO3-) in serum.

Formula: The formula used will be

ANION GAP= (Na+ + K+) – (Cl- + HCO3-)

The concentrations will be expressed in the following units i.e. of milliequivalents/litre (mEq/L) or in millimoles/litre (mmol/L).

Selection Criteria:

Inclusion Criteria: Adults ≥ 18 years admitted to intensive care unit (ICU) with diagnosis of acute coronary syndrome

Exclusion Criteria:

1. Heart failure due to other causes
2. Acute coronary syndrome with cardiogenic shock
3. Any infection at the time of presentation
4. Subjects not giving consent
5. Other causes of high anion gap levels like advanced kidney disease, metabolic acidosis, etc

Implications: It is a rural tertiary hospital-based study carried out through assessment of anion gap levels in patients of acute coronary syndrome. High anion gap levels will be observed in patients with worsening cardiac functions. We can interpret prognosis of individual patients based on their anion gap levels along with its severity to some extent; allowing to give better treatment & management based on individual prognostic values; allowing better life value.

Methodology in PICOT format:

P (Population): 50 consecutive patients attending Medicine OPD and ICU of Rural Tertiary Care Hospital in the backdrop of Central India.

I (Intervention): Assessment and understanding of anion gap as a useful prognostic indicator in a group of cardiovascular diseases i.e. acute coronary syndrome.

C (Comparison): Not applicable.

O (Outcome): Knowledge of a prognostic indicator i.e. anion gap to assess and predict severity level of the disease in individual patients

T (Time): Study will be conducted over a duration of 2 months

Measurements: Anion gap is calculated or measured as difference b/w measured cations (Na+ + K+) and the measured anions (Cl- + HCO3-) in serum.

The formula used will be: ANION GAP = (Na+ + K+) – (Cl- + HCO3-)

Quantitative Variables: Serum albumin land phosphate levels and unmeasured ions

Statistical Method: Chi square test will be applied to evaluate statistical difference between among anion group quartile groups. Ordinal logistic regressions will be used to evaluate between anion gap and severity of acute coronary syndrome. P values of <0.05 will be considered statistical insignificant. Software used in analysis will be SPSS 25.0 version.

Results

The study will play a key role in studying the association of serum anion gap in prognosis of acute coronary diseases along with determining its severity to some extent.. It will be observed that in patients who are severely affected, high values of serum anion gap will be obtained. This is similar to high values of anion gap observed in patients with metabolic acidosis and kidney failure. This will enable us to understand a unique yet somewhat efficient prognostic tool for study i.e. anion gap.

Discussion

Cardiovascular diseases are one of the leading causes of death in our country. Acute coronary syndrome is one of the heart anomalies. Though there are many diagnostic tools for detection of the disease but prognostic tools are being developed every day. One such tool is anion gap which can be assessed to determine and give prognosis based on individual patients, thus providing somewhat efficacy in treatment and management options.

In a study done by Yang, Shi-Wei, et al. in the year 2017, it concluded that higher levels on anion gaps were associated with worsening cardiac functions and cardiovascular diseases [10], similar to the results expected results in this study.

A study by Taylor, Eric N., John P. Forman, and Wildon R. Farwell in 2007 and another study by Abramowitz, Matthew K., Thomas H. Hostetter, and Michal L. Melamed in 2012 on determinants like hypertension[11] and cardiorespiratory fitness[12] respectively;these determinants were associated with cardiovascular diseases which lead to increase values of anion gap levels.

Another study by Ahn, Shin Young, et al. in 2014 showed that higher levels of serum anion gap were associated with an increased risk of cardiac deaths or mortality.[7] This indicates a poorer prognosis if the serum anion gap levels are found to be high.

Similarly, higher value of serum anion gaps are obtained in diseases like metabolic acidosis and kidney disease. A study done by Cheng, Bihuan, et al. in 2020 showed that high levels of anion gap were associated with poor prognosis in patients with acute kidney

injury[13]. Few studies on related aspects of this issue are available[14-18].

The results will be concluded and determined from this study, and will help to indicate the role of anion gap as a prognostic indicator in acute coronary syndrome.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Comparative Evaluation of Effective Route of Administration of MASAM Oil in *Vishwachi* (Cervical Radiculopathy): A Study Protocol

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Abstract

Background: Ayurveda proposed a lot of *Vatavyadhi* treatments. The cervical problems mimic the disorders in the *vata*. Thus in the present study, the *vata* management of medicated oils by rectal is better management for the identified clinical condition *Vishwachi* (Cervical Radiculopathy). Among such disorders Ayurvedic classics in the name of *Vishwachi* have described a disease. In Harrisons Principles of Internal Medicine and Davidsons Principles of Internal Medicine and Golwalla Medicine for Students, the symptoms of Cervical Spondylosis with Radiculopathy, such as a neck pain that radiates to the upper limbs, restricted movements of the neck have been explained. Due to today's modern lifestyles, food, behaviors, stress and anxiety, more people face these problems and the incidence of the *Viswachi* disease increases dramatically everywhere.

Aim and Objectives: To evaluate and compare the effect of MASAM oil for improving the Nerve Conduction Velocity in patients of *Vishwachi*(Cervical Radiculopathy) and to compare the MASAM oil through different routes of administration (*Pana*, *Abhyanga*, *Basti*) for improving Nerve Conduction Velocity in patients of *Vishwachi* (Cervical Radiculopathy).

Methodology: The proposed study, 120 subjects will be randomly divided into three groups in every group 40 patients will be including. In Group A (*Pana*) –with MASAM OIL dose of 24 ml will be administered orally a day in empty stomach for 21 days and Group B (*Abhyanga*) – local massage on affected side with MASAM OIL dose of 24 ml a day at morning after breakfast for 21 days and Group C (*Basti*) MASAM OIL 24 ml a day will be administered through rectal route after meal for 21 days. The record of assessment will be taken at zero day 21st day and after 21st for followup.

Results: On the basis of objectives outcome changes will be observed. Changes will be observed in objective outcomes.

Conclusion: It may conclude that the parameters included in this study will be reducing pain, tingling sensation and improvement in Nerve conduction parameters.

Keywords: *Vishwachi, Matrabasti, Abhyanga, Pana, Nerve Conduction Study, Cervical radiculopathy.*

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Introduction

Cervical Radiculopathy disease occurs if an irritated nerve in the neck enters the spinal canal. Cervical radiculopathy, commonly known as "pinched nerve," is typically a herniated disk or spurt of the bone that

pushes the inflamed nervous root into one. These are usually a consequence of degenerative neck changes. The significant cause of neck pain and disability may be cervical radiculopathy (CR). 83.2/100.000 people report an annual CR incidence[1]while the reported prevalence is 3.5/1000 persons[2]. In today's world, cervical radiculopathy is more common with more cervical problems. We are used to electronic and/or work-related instruments. Therefore it is not possible to avoid it and to find a better healing area. The characteristic of *Vishwachi* may be correlated with cervical radiculopathy. Under *vatavyadhi* (disorders of *vata*), *Vishwachi* is identified because *Vishwachi* can be explained as disease starting from posterior part of hand and prakoshtha, hands, and fingers, manifest due to flexion and extension of the nerves which gives strength for the movements[3]. Ayurveda proposed a lot of *Vatavyadhi* treatments. The *vata* conditions are imitated by cervical problems. Therefore, the *vata* management of orally, rectal and surface (external) medicated oils is better controls in the current study of the clinical condition of *Vishwachi* (Cervical Radiculopathy).

Rationale of the study: *Vata* diseases are commonly expressed in musculoskeletal and neurological disorders. The selected disease condition *Vishwachi* is a neuro-musculoskeletal disorder. The nerve conduction test or demylination and degeneration are not attributed to *vatavyadhi* with any measurable parameters. As there were no studies in relation are found to determine the nerve conduction in terms of *vata* here an attempt is made to evaluate and compare the effect of MASAM oil through *Basti*, *Pana* and *Abhyanga* for the improvement of nerve conduction. The main *Chikitsasutra* (principle) of *Vatvyadhi* is *Pana*, *Abhyanga* and *Basti*[4]. Yet, this tool of Nerve Conduction Velocity study didn't use in Ayurvedic research. There are no such evidences in*Vishwachi* (Cervical Radiculopathy) regarding Nerve Conduction Velocity (NCV)

Hence, we selected NCV studies to find out the better route of administration and effectiveness of MASAM oil in *Vishwachi* (Cervical Radiculopathy).

Aim and Objectives

Aim: To evaluate and compare the effect of MASAM oil for improving the Nerve Conduction Velocity in patients of *Vishwachi* (Cervical Radiculopathy).

Objectives:

1. To evaluate the effect of MASAM oil for improving Nerve Conduction Velocity in patients of *Vishwachi* (Cervical Radiculopathy).
2. To compare the MASAM oil through different routes of administration (*Pana*, *Abhyanga*, *Basti*) for improving Nerve Conduction Velocity in patients of *Vishwachi* (Cervical Radiculopathy).

Case Definition: A diagnosed *Vishawachi* subject with *Bahuprusthatha* symptoms (the back aspect of the upper limb) and with tingling sensation to the left or right, along with positive spurling test and a cervical distraction test along with range of motion.

Research Question: Whether MASAM Oil through different routs of administration (*Pana*, *Abhyanga*, *Basti*) is effective in improving Nerve Conduction Velocity in patients of *Vishwachi* (Cervical Radiculopathy)?

Research Hypothesis: MASAM oil is effective in *Vishwachi* (cervical radiculopathy) because of its composition qualities.

Null Hypothesis: There is no effect on MASAM oil in *Vishwachi* (cervical radiculopathy).

Study Type: Interventional

Trial Design: A Randomized Comparative clinical trial.

Methodology

Place of Study: This study will be plan in M.G. Ayurved College Hospital & RC Wardha.

Registration Number: CTRI/2020/01/023022.

Composition of the trail drug: MASAM (Manjishta, Ashwgandha, Sahachar, Atmagupta, Masha) oil

Table no. 1 Contents of MASAM oil

Sno	Sanskrit name	Botanical name	Parts used	Quantity
1	Manjishta	Rubia cordifolia Linn	Root	1 part
1.	Masha	Vingna Mungo	Seed	1 part
2.	*Atamagupta*,	Mucuna pruriens Hook	Seed, root, pod hair.	1 part
3.	Sahachar	Barleria prionitis	Panchanga,leaf	1 part
4.	Ashwagandha	Withania somnifera	Root	1 part
6	Til	Sesamum indicum.Linn	Seed,oil,leaf,root	4 part

Eligibility Criteria: A 20 yrs to 60 years of both sexes, A diagnosed *Vishawachi* subject with Cervical compression.

Diabetic neuropathy, Post operated cervical radiculopathy and Indicated for Surgery and Severe giddiness and loss of function are excluded.

Table no. 2. Interventions table

Group	A	B	C
Sample size	40	40	40
Intervention	Abhyanga	Pana	Basti
Dose	24 ml	24 ml	24 ml
Duration	21 days	21 days	21 days
Follow up period	21 day	21 days	21 days
Total duration	42 days	42 days	42 days

Criteria for discontinuing or modifying allocated interventions: Subject will be pulled back from the investigation if any untoward rate, highlights of medication affectability or some other malady or issue emerges, Free treatment will be offered to the patients till the issues dissolves.

Follow up: After 21 days

Primary Outcomes: We will observe the efficacy of MASAM oil through *Pana*, *Basti* and *Abhyanga*on pain and tingling sensation before and after management. Data will be expressed as mean and standard deviation at 5% significance level. It may assumed that MASAM oil will be more effective through *Pana*, *Basti* or *Abhyanga* and we will compare the effect between the *Pana*, *Basti* and *Abhyanga* in pain, tingling sensation with no any adverse effects.

Secondary Outcomes- We will see the effect of MASAM oil through *Pana*, *Basti* and *Abhyanga* on Nerve Conduction Parameters like Latency, Amplitude and Velocity.

Discussion

Numerous studies established for *vatavyadhi* like *Panchakarma* procedure and shaman *chikitsa* too. The essential driver of cervical radiculopathy is age-related degeneration. In any case, there are a few special cases where spinal wounds to the plate can enlarge the degenerative procedure in the more youthful patient. An optional appearance of spondylosis is identified with the pressure of the vascular and neural structures brought about by a misfortune in the plate tallness and impinging osteophytes that add to the deadness, stun like sensations, pain, and interminable motor and tangible effects, which if not revised may prompt lasting incapacities[5]. Local application of MASAM oil may help to correct the *vata* vitiation. Moreover according to Ayurveda pain was caused by because of *vata* vitiation. All drugs have property of *vatahara* (anti inflammatory) and all drugs have property of nerve protector or working as a nervine tonic. So selected for this study.

Manjishta-is Anti-inflammatory, antioxidant[6]. ***Aatmagupta***–is Neuro-protective and Neuro-restorative[7]. L-DOPA characterized by a deficiency in the synthesis of the neurotransmitter dopamine in nerve cell[8]. ***Sahachar*** –is commonly used in Neurological disorders and *vatakaphahara*[9]. ***Masha***-is Anti-inflammatory and Nervine tonic[10]. The response involved in inflammatory as complex array of enzyme activation, mediator release, cell migration, fluid extravasations, tissue breakdown and repair. ***Ashwagandha***–is Neuroprotective, Immunomodulatory and Anti – inflammatory.

Statistical analysis: Data will be analysed on the basis of appropriate statistics paired unpaired test and ANOVA by using SPSS software.

Time duration till follow-up: The treatment duration for the patients is 21days and after that follow-up for 21 days.

Followup period: 0 day, 21 day and followup 21 day

Time schedule of enrolment, intervention: *Matrabasti*, *Pana* and *Abhyanga*

Recruitment: 40 (every group) sample recruited by single randomizing sampling.

Method: Literature review search, plane of work, Blue print in the form of flow chart, preparation of medicine, Data collection, treatment and its effect, will be observe which route of administration is best and statistical analysis.

Data collection Method: Randomized sampling

Objective Criteria: Assessment of numerical pain scale, neck disability index, cervical distraction, range of motion, Nerve conduction velocity, all the variables will be assessed before and after intervention of different route of administration.

Data management: The data coding will be done by principle investigator.

Statistical Method: Paired and unpaired for objective criteria, non parametric for subjective criteria, ANNOVA for comparing between three groups.

Ethics and dissemination: Research ethical approval: After critical evaluation and presentation the ethical committee has approve the research topic reference No – DMIMS (DU)/IEC/2018 -2019/7352.

Consent assent: Patient will be given detail information regarding intervention in his own language. Then written consent will be taken from patients before starting the study.

During study the confidentiality will be maintain.

Dissemination policy: The data will be spreading in the form of paper publication and Monograph. Authorship eligibility guidelines and any intended use of professional writers

Informed consent materials: Patient will be given all consent material in the form of hard copy, and related documents will be given to the participants.

Strengths: MASAM oil will be work for the nerving tonic and to find out best route of administration. If projected work results in the positive outcome then it will be established new mode of management for the cervical radiculopathy.

Limitation: Specific geographical area, convincing the patients for Nerve conduction study.

Conclusion

Conclusion will be mentioned after the deliberate and analyzing data.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Concise Review of Gingival Depigmentation Techniques and Re-Pigmentation

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Abstract

Gingiva pigmentation has an impact on patient's esthetics and formulates pscyhological negativity. Gingival pigmentation is most predominant and endogenous pigmentation among oral mucosa. The gingival pigmentation can be physiologic and pathologic. It can be a genetic trait in some population. The physiologic melanin pigmentation does not possess a medical problem, it is mainly associated with individuals with high smile line and excessive gingival display. Pigmentation of gingiva is due to deposition of melanin in basal and suprabasal layer of epithelium. The gingival depigmentation is a esthetical correction technique where hyperpigmentation is removed. The treatment depends on patient need and skin complexion. Proper treatment method is required for treating the unesthetic gingival pigmentation. Perio-esthectic is focused on proper equilibrium of both pink and white(teeth) tissue. To overcome this problem wide array of method are put forth which include scalpel method, cryosurgery, gingival ablation method, electrocautery, chemical method, lasers and grafts. Each technique has its own advantages and disadvantages.

Keywords: *Gingival pigmentation, depigmentation, repigmentation, various techniques of gingival depigmentation.*

Introduction

"The science of beauty is nothing but aesthetic" as rightly quoted in the literature, which makes any living or non living object attractive to the eyes.(1) Aristotle correctly said as "Beauty is a greater recommendation than any letter of introduction."(2) A harmonious smile is described by various factors which include shape of the teeth along with the proper positioning. Other factors which should be considered are the color of the tooth as well as how well it goes with the gingiva. The major component of an aesthetic smile is definitely gingiva which is in its healthiest form. Other gingival factors which are responsible for a healthy smile are removal of gingiva which shows pigmentation which is required for a smile which boosts confidence and oozes aesthetics.(3) One of the most commonly tissues which are seen with pigmentation is gingiva which is dependent on several factors like epithelial thickness, amount of keratinization, amount of vascularisation and existence of pigments within the gingival tissues like melanin(4) ''Melas,'' is a Greek word which means black from which the word melanin was derived.(5) One of the most common pigment secreated endogenously, Melanin, is present in the basal and suprabasal layers of the epithelium.(6) Melanin is secreted by Melanocytes which were first isolated by Dr Becker et al in the year 1927 in the oral epithelium(7) After a few years, Melanocytes isolation was carried out from samples tissues of gingiva by Dr Laidlaw et al. ***Dummett***in his study stated that presence of pigmentation oral cavity observed in black race is:

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"gingiva 60%; hard palate, 61%; mucous membrane, 22%; and tongue, 15%".[8] Evidence of first melanin pigmentation occur 3 hours after birth. Pigmentation are mainly caused by five primary pigments:- "Melanin, Oxyhemoglobin, Melanoid, Carotene, Reduced haemoglobin".[9,10]

1. **Pigmentation seen in gingiva due to physiologic factors(ethnicity/race):** Colour variation may be seen on one side, or both the sides, it can beuniform, or have a macular distribution, Mottling or blotching can also be seen. It can be seen on gingival papillae. or the whole in general and into other oral structures. Pigmentation due to normal physiology can be clinically manifested as being multifocal or diffused general. More pigmentation occur in attached gingiva.
2. **Various pathologic causes of gingival pigmentation[12]:**

Endocrine disease	Addison's disease Acromegaly Nelson's disease Albright's disease
Drug-induced	Quinine Zidovudine Chloroquine Minocycline Cyclophosphamide
Heavy metals	Lead Bismuth Arsenic
Mucosal conditions	Lichen planus Blue nevus HIV oral melanosis Hemangiomas Oral melanoacanthoma Inflammatory mucosal lesions
Malignant neoplasms	Melanoma Kaposi's sarcoma
Idiopathic melanin pigmentation	Peutz-Jegher's syndrome Leopard syndrome Carney syndrome
Tobacco associated	Smoker's melanosis
Others	Amalgam tattoo Gingival tattoo

Depigmentation of Gingiva: The definition of gingival depigmentation can be given as "a periodontal plastic surgical procedure whereby the gingival hyperpigmentation is removed or reduced by various techniques."When the patient desires better aesthetics the procedure of depigmentation can be carried out and is not a clinical indication.

Bur abrasion method: The first case of depigmentation with bur was documented by Ginwalla *et al. in 1966.*

Technique: A medium grit football shaped diamond bur is used at high speeds to denude the epithelium. It takes around 45 min to 1 hour to complete.[10]

Advantages:

1. Easy to perform
2. Simple, non-aggressive and safe
3. Less discomfort to patient
4. Good esthetic outcome

Disadvantages:

1. Difficult in controlling the depth of de-epithelialisation
2. Technique sensitive
3. Post operative pain
4. Longer treatment time
5. High recurrence rate
6. Periodontal dressing required

Complications:

1. Harm to underlying periosteum and bone
2. Gingival recession
3. Wound healing is delayed
4. Enamel loss can occur

Scalpel surgical technique:

Synonyms: Surgical stripping and split thickness epithelial excision

Technique- Pigmented gingival epithelium is removed along with a layer of underlying connective tissue with the help of a surgical blade and allow it to heal by secondary intention.[10]

Advantages:

1. Economically feasible
2. Does not require any extensive armamentarium

3. Rapid wound healing

Disadvantages:

1. More bleeding during and after surgery
2. Periodontal dressing required
3. Re-pigmentation can occur by migration of active melanocytes from adjacent untreated area {Dummett and Bolden (1963)}

Contraindication:

1. Thin gingival biotype
2. Narrow papillary areas

Electro-surgery:

Technique: After administration of local anesthesia, the needle electrode is used in a sweeping motion in the operated area. Feather-light brushing strokes with high frequency electrical current in the range of 1.5-7.5 Megahert are used for de-epithelization. The procedure is performed in a cervico-apical direction and operated area is cleaned with the help of sterile gauze soaked in 1% normal saline solution.[13]

Advantages:

1. It controls hemorrhage
2. Permits adequate contouring of tissue
3. Lesser operating time
4. Less scar formation after surgery
5. Less discomfort to patient

Disadvantages:

1. More expertise required
2. More post operative pain
3. Causes undesired tissue destruction
4. Periodontal pack required

Complications:

1. Intense inflammation occured
2. Loss of crestal bone height seen

Cryosurgery: Technique- freezing of gingiva is carried out with variousagents which include liquid nitrogen. Cryoprobe which is cooled to a minimum of -81 °C is the basic necessity used for application in the areas which show pigmentation for a oeriod of about 10 seconds. A little degree of erythema occurrs within a time span of 1 minute. The following method uses the idea of rapidly freezing the water & followed by slowly melting slow .This leads to the deterioted tissue formation . The Dehydration of Cell, denaturation of protein, enzyme inhibition,including the death of cell which occurs due to the shock due to thermal activity are few of the many effects which occur due to the process of cryotherapy. Cryotherapy leads to changes in the vascular supply and also affects the immunity of the individual leading to cell death.[14]

Advantages:

1. Easy and rapid to apply
2. Done in less time
3. No anesthesia required
4. No suturing needed
5. No bleeding encountered
6. No scar formation seen

Disadvantages:

1. Post operative swelling
2. Increased soft tissue destruction
3. Difficult to control depth of penetration

The Procedure of Cryosurgery: Tissue type, size and depth of the lesion are used to decide the dose and delivery method. Additionaly underlying structures, thickness of the epidermis, local blood flow and the water content of the skin can be considered.[10]

1. Dipstick method
2. Spray technique
3. Cryoprobe technique

Spray technique using spray tip: This is also known as Open-spray technique. The hand held or table top cryosurgical unit filled with liquid nitrogen is used, Select a spray tip that sprays within the border of the lesion. For single short freeze, no local anesthesia is required . But if the lesion is large and requires more freeze time, then 1% lignocaine is given . The spray should be done 1 cm away from the area and is administered at the centre of the lesion. The lesion is allowed to thaw slowly i.e. come back to room temperature. Thaw time is usually double of freeze time .

Cryoprobe technique: In this method, liquid nitrogen is circulated to cool the tip of cryoprobe. Hence,

freezing occurs by conduction. This technique is slower than spray technique.[10]

Disadvantages:

1. Excessive tissue damage
2. Depth of penetration difficult to control
3. Burning sensation or pain
4. Stinging sensation

Lasers: Lasers are introduced by Maiman in 1960, since than it is used widely in medical field. This method has been conceded as one of the pleasant, reliable and effective method.

Types	Wavelength
Carbon dioxide (CO2)	(10,600 nm)
Neodymium-doped: Yttrium, Aluminum, and Garnet (Nd:YAG)	(1064 nm)
Diode	(820 nm)
Erbium (Er)-doped:YAG	(2940 nm)
Erbium- and chromium-doped: yttrium, scandium, gallium, garnet (Er,Cr: YSGG)	(2780 nm)

Advantages:

1. No periodontal dressing required
2. Minimalpost operative pain and swelling
3. Less discomfort (protein coagulum formation)
4. Blood less field (sealing nerve endings)

Disadvantages-

1. Thermal damage
2. Delayed wound healing
3. Expensive treatment
4. Bone exposure and gingival fenestration can occur[15]

Diode laser: This laser is a solid-state semiconductor that utilizes light energy from electric energy. Flexible quartz fiber optic hand piece can be used. Wavelength spectrum of 800 to 980 nm is used to absorbed by water, soft tissue and chromophores, such as oxyhemoglobin and melanin.

Advantages:

1. Good visibility
2. Excellent hemostatic agent
3. Better patient comfort
4. Less damage to periosteum and underlying bone
5. Shorter operating time

Disadvanatges:

1. Slight discomfort
2. Dry and itchy feeling during procedure

Er: YAG laser: It is manufactured with a wavelength of 2940 nm, which is ideal for absorptionby water and hydroxyapatite.

Advantages:

1. Less thermal damange
2. Less pain
3. Rapid wound healing

Disadvantages:

1. Wavelength of this laser cannot absorb melanin

Nd: YAG laser: This laser is set at 60 mJ per pulse, 6 W and 100 pulses per second. A 320 micron diameter fiber optic is used in the pigmented area with a contact mode.[10]

Advantages:

1. No complications
2. No bleeding
3. No pain

Radiosurgery: It is the most advanced form of electrosurgery. It is used for removal of soft tissue with the aid of radio frequency energy.“Frequencies of 3.0 MHz (MHz) to 4.0 MHz is being used where 4.0 MHz being the optimal frequency”.(10)

Advantages:

1. Produce coagulation in the treated site
2. Less thermal damage
3. Faster healing (4MHz wave technology)
4. Self sterilising method
5. Little or no scar formation seen
6. Better visibility due to bloodless field

Disadvantages:

1. Atleast two appointment required

2. Cannot be used in pacemaker patients
3. Uncomfortable to patients due to foul smell

Chemical method: Chemical agents like 90% phenol along with 95% alcohol is used.

Disadvantages:

1. Tissue necrosis
2. Pain
3. Burn the gingiva

Free gingival graft: Free Gingival Grafts are used extensively in root coverage procedure and to increase the width of attached gingiva. According to *Tamizi, Taheri (1996), Kumar et al., 2012 and Fowler et al.(2000),* a free gingival graft is required after depigmentation procedure for better esthetic outcome. It showed no evidence of repigmentation even after 4.5 years. ***Vikas V Pakhare etal (2017)*** in their case report, used free gingival autograft for treating gingival melanin hyperpigmentation and no repigmentation was seen even after 6 months.[16]

Advantages:

1. No reoccurrence
2. Esthetically appreciated

Disadvantages:

1. Second surgical site needed
2. More discomfort to patients
3. Extensive procedure
4. Poor colour matching of tissue
5. Healing is slow and painful
6. Limited donor area available

Acellular dermal matrix allograft: Surgical blade no. 15 is used for two vertical incisionon the non-pigmented tissue both mesial and distal to the pigmented area. In the pigmented area, partial thickness flap is reflected and excised by a horizontal sulcular incision. The graft is then trimmed according to the treated area and secured with sutures. This technique yields better result compared to gingival abrasion method.[14]

Advantages:

1. Less time compared to free gingival graft
2. Less post operative discomfort
3. Less post operative complications

Disadvantages:

1. Clinical expertise required
2. Expensive procedure
3. Contraction of graft can happen

Reasons for repigmentation: "Reappearance of melanin pigmentation after a certain period of depigmentation is called repigmentation".[14]

- ✓ Migration of melanin pigments from untreated area to treated area causes repigmentation.
- ✓ Repigmentation can occur by the left over melanocytes during surgery
- ✓ Hormonal changes, Excessive sunlight exposure genetic and ethnic factors(17)

Prevention of repigmentation: Complete removal of melanin pigment including free gingiva, interdental gingiva and marginal gingiva.[14]

Repigmentation based on evidence[15-23]:

Kaur et al 2010	Higher reoccurance of pigmentation in the dark-complexioned patients seen as compared to wheatish- or brown-complexioned patients in a 9 month followup.
Hegde et al 2013	Er:YAG-laser-treatedsites resulted more repigmentation than the surgically or CO2-laser-treated sites.
Kumar et al 2013	3 out of 10 cases showed mild areas of repigmentation on the 30th day where other 7 cases didnot show any repigmentation till 2 years.
Ribeiro et al 2014	Slight reapperance of pigmentation was found in five of the patients using Nd;YAG laser and scalpel technique.
Grover et al 2014	In 11 cases, four laser and seven scalpel, a patchy repigmentation was observed at the end of 3 months
Giannelli et al 2014	Er:YAG and diode lasers gave satisfactory clinical results, with no recurrence of gingival pigmentation in a six month study.

Gupta et al 2014	7 sites in the scalpel excision group showed recurrence, whereas only 4 site showed recurrence in the electrosurgically treated group.
Basha et al 2015	Repigmentation appeared in the interdental and marginal tissues in both surgical stripping method and laser technique.
Suragimath et al 2016	At the end of one year, slight melanin repigmentation was observed in three subjects treated with scalpel depigmentation procedure.
Bakutra et al 2017	At 12 months followup study repigmentation is significantly lesser in surgical stripping compared to laser ablation method.
Narayankar et al 2017	Recurrence of pigmentation was observed in five cases in scalpel group and two cases in TFE group in 6 month followup.
Gholami et al 2018	After 12 months, surgical stripping and Er, Cr:YSGG laser treatment techniques showed similar trends in re-pigmentation where as one laser treated site, a short melanin strip recurrence seen.
Kamboj et al 2020	Repigmentation was observedin four cases out of 16 sites treatedwith electrocautery technique.
Chandra et al 2020	At 6 month, nearly 62.5% of cases showed postsurgical repigmentation of gingiva in both scalpel and laser techniques

Conclusion

The patients which show excessive gingiva should be advised for the procedure of Depigmentation thickness of gingiva, the level of experience the clinician holds, the preference of the patient, and rate with which pigmentation recurs,are the major factors which influence treatment selection the available studies in the field of depigmentation are mainly case reports, so Randomised control trials with longer follow up period are required to establish better treatment needs for gingival depigmentation.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Artificial Intelligence: A Dental Odyssey

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Abstract

Electronic globalization with such a gigantic technological advancement during last few decades have made a huge impact on our everyday life. Artificial intelligence has been a breakthrough in hi-tech progression and has entranced the brains of scholars worldwide. It is field of technological science dealing with computer science and ability of computers to mimic the function of human brain to perform the tasks readily. Ever since its commencement in the field, dental science has witnessed modernization. From patient data records preservation, diagnosis and treatment planning to robots performing surgeries in supervision of clinician has now become possible because of artificial intelligence. In current scenario, it has become necessary for dental professionals to get oriented with clinical as well as technological advancement to provide easy, quick, relatively cost effective and excellent patient care. Though artificial can't replace all the traits of human mind, it has made a huge contribution in reducing a dentists efforts and assisting him in decision making. It is of prime importance for a practioner to be alert of the potentials of artificial intelligence to assimilate this technology for a enhancing his practice in forthcoming years.

Keywords: *Artificial intelligence, technological advancement, computer science, diagnosis, treatment planning, dental professionals.*

Introduction

In the era of hi-tech world, with such a speedy advancements in the field of technology, machines have become a big part of human life. In an attempt to reciprocate the functions of a human brain containing some traits of its mind,technological advancement have been witnessed over time. Advanced technology provide specific tools to unravel problems encountered when decision making and investigation is done. Hence, the involvement of computers in health industry has lately became a much of significance. The main object of this paper is to enlighten the readers about present-day accomplishments of artificial intelligence in clinical science.

Artificial intelligence is defined as "the study of intelligent agents, any device that perceives its environment and takes action that maximize its chance of successfully achieving its goals (Russell and Norvig 2003)"[(1)]. Artificial intelligence signifies nothing but machines that performs human tasks and so often

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referred as machine intelligence. The AI research have been significantly based on reasoning, natural knowledge processing, planning, learning, interpretation, logistics, ability to move and ease of knowledge representation.

Artificial intelligence has been classified into two subdomains; machine learning and deep learning. Machine learning (ML) was first referred by Arthur Samuel[2] which is based on set of rules to learn the arithmetic frameworks and edifices in data which permits prediction of concealed data. ML model are neural networks (NNs) which can outdo conventional ML systems specifically on dynamic figures.

Deep learning is precisely useful for complicated data because of their skill of image representation and its organizedtopographies. It is a machine learning method where the models are trained using artificial neural networks (ANN).ANN is a group of numerous organized processing elements whiche valuatedata by their computerized potent response to external inputs. It has established substantial results in many non-health and wellness programby means of computer imaging and natural language processing. Deep neural networks are considered universal approximation machines (Hornik 1991)[3].

History: AI was founded in the year 1956 at a conference but it gained much recognition from 1980s with the arrival of neural networks[4]. The beginning of AI can be traced back to more distant past and typically linked with the discovery of first functioning computers after the Second World War, or in the year 1956 when John McCarthy used the term in its full meaning for the first time[1]. Hence, known as father of Artificial intelligence[5]. Philosophers and mathematicians have offered explanations of the development of anthropological intellectual and decision making, determined to describe overall forms and guidelines underlying the process since prehistoric times. The first real successes to create 'intelligent machine' have arisen with the encounter of digital electronic computers and scientific papers of a British mathematician, Alan Turing who laid the foundation of artificial intelligence and created the Turing test which is "a test to measure a machine's aptitude to reproduce human intelligence" in 1950[6,7]. Electronic diagnostics & decision making codes extemporize the course of evidence based guidelines, apt clinical medical assessment and be a foundation for education and investigation.[8,9]

Application of Artificial Intelligence in dentistry[10-23]:

Author	Year	Inference
Hammond et al	1993	Growth of design assistant, RaPiD for use in prosthodontics.
Speight et al	1995	Risk assessment of oral cancer
Käkilehto et al	2009	To compare the life expectancy of different dental restoration material by analysing the electronic dental records using data mining AI techniques.
Miladinović et al	2010	Finding and linking the major factors that may have effect on the reasons of tooth extraction.
Bas et al	2012	Diagnosis of subtypes of TMJ disorders by using ANN as a diagnostic tool.
Kikuchi et al	2013	Evaluate how VRS affected students knowledge and skills associated with porcelain fused to metal crown (PFM) preparation.
Jung and kim	2016	Diagnosis of the need of orthodontic extraction
Thanathorwong	2018	AI clinical decision support
Patcas et al	2018	Treatment outcome analysis
Lee et al	2018	Diagnosis and interpretation of periodontally compromised teeth.
Feres et al	2018	Differentiate between chronic and aggressive periodontitis.
Lee et al	2018	Diagnosis of caries
Kim et al	2018	Prediction of medication related osteonecrosis of jaw.
Wenzhe et al	2020	Detection of dental plaque on primary teeth by deep learning based AI technique.

General dentistry: AI has been significantly used toper form different tasks in routine dental practice. Because of this the need of man power has been reduced and fewer errors with increase in precision in comparison to human has been achieved. From the date of admission of patient, case history recording, arriving at clinical diagnosis of a disease, interpreting X-rays and then making a detailed treatment plan for patient has now become easier with the help of these computer systems[24].

Endodontics: Endodontics has always been the most rapidly developing branch in dentistry. With the use of AI, devices has been made to measure the pain level of patient and thus providing proper medication. Studies have been done in an attempt to make accurate diagnosis of caries. AI has contributed to test the vitality of tooth which has always been a crucial factor of treatment planning by introducing various method such as electric pulp testing, pulse oximetry, laser dopplemetry etc. The operating microscope and ultrasonics has heightened the quality of treatment and represent a modern aide in treatment of tough cases[25].

Prosthodontics: Fabrication of prosthesis has been most technical process. It requires accuracy, time and effort from the dentist and even the patient. With the increasing need of prosthesis various technology has been used to make the task easier. AI in combination with the designing software's can help the clinician to make the finestpossible and aesthetic prostheses bearing in mindnumerous factors like facial form, colour, patient desire, etc[26].Since 1980, 'digitizing the impression procedure'and 'computer aided design and computer aided manufacturing (CAD/CAM) technology' was made known todentistry[27]. To minimize the error in impression making, digitalized impression recording was introduced. CAD/CAM is the most enormously emerging part in dentistry. In the current scenario there are two possible method for using the CAD/CAM system in fabrication of dental crowns, laminates, veneers electronically: the direct and the indirect method[28]. Laboratories are using CAD/CAM to mechanically produce unconventional dental restoration, planned to seamless fit and supreme function beyond aesthetic outlooks [29].

Forensic odontology: It is the appropriate handling andinspectionwith thoroughevaluation of dental evidence which can be presented as a evidence in the interest of justice. Age estimation is the main area of practice for dentist in forensic odontology. Recent studies has shown that along with the tooth eruption pattern and tooth wear the deposition of cementum has shown the annual pattern of deposition. Age estimation using facial pattern has been under research since a long time.With arrival of AI, there are numerous software design by using ANN whichcommands the computers to inevitably estimate the age.[26]

Oral medicine and radiology: Computer-based diagnosis has become much popular and reliable because of its capability to spot and diagnose lesions which are difficult to diagnose and difficult to catch by the human eye[30]. Breakthroughs in image interpretationwith the addition of artificial intelligence Systems has been transformed from a myth to reality in the radiology practice within a short span of time. In the context of head and neck and imaging, AI offers additional power with its inimitable skill to learn.Ultrasonography helped in differentiating benign and malignant tumors, cystic and non-cystic lesions and also helped in on screen nodal measurement[31]. 3D imaging systems such as 'Cone Beam Computed Tomography (CBCT)'along with 'Magnetic Resonance Imaging(MRI)' has provided advantage in determining the extend and progression of lesion that may have gone unnoticed to human eye. The introduction of digitialized radiographs have eliminated the use of the manual processing of radiographs thereby reducing the chances of errors in processing of radiographs. This has also helped to keep the dental records digitalized making it accessiblefor future reference. Additional 3D system has been used in dental radiology such as intraoral scanners. These intraoral scans are then examined by dental inspection software. In order to locate minor apical foramen, artificial neural networks has been found to improve the working length determination for root canal treatment by radiographs[32], cephalometric analysis and diagnosis of caries[21].It also given adequate sensitivity, specificity, and accuracy with digital radiographs in detection of vertical root fracture [33].Study has shown use of artificial neural network (ANN) help to establish a diagnostic tool to diagnose subtypes of temporomandibular joint (TMJ) disorders[14]. Artificial intelligence has helped to establish relation of oral cancer to genetic factor significantly in large population and to differentiate between premalignant and malignant lesion helping us to categorize the disease and providing treatment appropriately.

Orthodontics: Movement of the teeth in the position where maximum function and esthetic can be achieved

has always been difficult to achieve. How much force to be applied for tooth movement keeping in mind the anatomic landmarks and the outcome of treatment is now done using AI [34]. Orthodontic diagnosis, treatment planning and long term monitoring of treatment is being done by analysing digital radiographs and facial photographs records (35).A model was trained to evaluate the craniofacial skeletal and dental anomalies in cephalometry and the same was compared with the expert opinion. The analysis results of both were found to be equal. Furthermore, the model also showed inconsistencies offered in the datawhich were missed by the clinicians, thus stressing on the importance of AI in orthodontic analysis [36].

Oral Surgery: Introduction of Robotic surgery in oral and maxillofacial surgery has been the greatest contribution of AI where human body motion and intelligence is imitated by robots while performing surgery in the guidance of expert [37]. AI s has abetted the surgeon in planning surgeries by preserving the vital structures around the lesion with higher intra operative precision with reduced operation time there [38]. The oral soft and hard tissue structures when lost due to some pathology or accidental reasons can be reformedthroughan advanced AI technique known as "bio-printing" through which organs and living tissues are formed in consecutive thin layers of cells in the future [39].

Periodontics: Deep learning AI is used in early detection, diagnosing and treatment planning of periodontal diseases using intraoral radiographs by aiding the initial finding of periodontal changes[40], furcation involvement, bone loss, and scoring bone density. Peri-implantitis recognition also help in primary intervention during placement of implant.[41]With help of electronic toothbrush now it has become easy for patient to asses the level of plaque in their oral cavity on their mobile screen.

Conclusion

Artificial intelligence has made a huge contribution in the health care sector. It has certainly helped us in improving the patient health care system. Technologies have helped clinicians and research workers to assimilate different arenas of knowledge. That day is not far, when dentistry will totally be revolutionized with the advancement of artificial intelligence based technology and we will be familiarized to errorless clinical procedures with greater accuracy and increase in patient care. Though it can never replace a dentists judgment, but it will surely aid the dentist in decision making. Therefore, to understand the countless notions and the system involved in artificial intelligence will have a strong benefit in the future. AI may assist in addressing the weaknesses harshly criticized in conventional dental care (Watt et al. 2019).

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Review on Covid-19 Disease: Its Outbreak and Current Status

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Abstract

Summary: Coronavirus (COVID-19) is diversely found in human beings and wild animals. It is an enveloped RNA virus. There are a total six species which cause disease in humans. They commonly infect respiratory, neurological, enteric, and hepatic systems. There is history of endemic outbreaks in past in the form of Middle East respiratory syndrome coronavirus (MERSCoV) and severe acute respiratory syndrome related coronavirus (SARS-CoV).We have seen another outbreak due to a new strain called the SARS-CoV-2 virus which presented as a pneumonia of unknown etiology in patients in Wuhan, China. The epicenter of infection was linked to seafood and exotic animal wholesale markets in the city. It is highly contagious and has resulted in a rapid pandemic of COVID-19. As the number of cases continue to rise, it is clear that these viruses pose a threat to public health. This review will describe clinical features and treatment of COVID-19 patients and raise awareness among healthcare workers and general population during the current pandemic. This review article aims at giving up-to-date information about the disease and counselling of patients.

Keywords: *RNA virus, COVID-19, SARS-CoV-2, MERSCoV, Coronavirus, Pneumonia.*

Introduction

Coronavirus (CoV) is a single-stranded RNA virus that belongs to the Nidovirales order. The order includes Roniviridae, Arteriviridae, and Coronaviridae families which are subdivided into Torovirinae and Coronavirinae subfamilies.[1] Coronavirinae is further subclassified into alpha-, beta-, gamma-, and delta- COVs.[1] Viral RNA genome ranges from 26 to 32 kilobases in length and can be isolated from different animal species such as birds, livestock, and mammals such as camels, bats, masked palm civets, mice, dogs, and cats.[2] Human pathogenic subtypes of CoV are associated with mild clinical symptoms. In 2012, Middle East respiratory syndrome coronavirus (MERS-CoV) was first detected in Saudi Arabia and was responsible for 2,494 confirmed cases, which led to 858 deaths.[2] In 2002, a severe acute respiratory syndrome related coronavirus (SARS-CoV) rapidly spread across Guangdong, China which resulted in 8,000 infections and 774 deaths in 37 countries.[2] The outbreak in 2020 has presented in the form of pneumonia of unknown etiology in Wuhan, China. Deep sequencing studies and lab investigations have identified the culprit as a new strain of COV.[3] Initially, this virus was designated as 2019-nCoV. However, the International Committee on Taxonomy of Viruses designated it as the SARS-CoV-2 virus.[4] On February 11, 2020, the World Health Organization (WHO) announced the disease caused by this novel virus as coronavirus disease-2019 (COVID-19). The repeated emergence and outbreaks of CoVs indicate a public health threat. This suggests the possibility of animal-to-human and human to-human transmission of newly emerging CoVs. The ongoing changes in ecology and climate make future emergence of such infections more likely.[3]

History of Coronavirus: In 1960 corona virus was first identified as a causative agent for common cold. One study was carried out in Canada in 2001, flu-like symptoms were present in more than 500 patient. Virological analysis by polymerase chain reaction (PCR)

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of these patients showed that 3.6% of these cases were positive for the HCoV-NL63 strain. By 2002 Corona virus was considered as a relatively simple, nonfatal virus.

After the outbreak in 2002-2003 in Guangdong province in China, which resulted in spread to many other countries, including Vietnam, Hong Kong Singapore, Thailand, Taiwan and the United States of America, caused severe acute respiratory syndrome (SARS) and high mortality, microbiologists and infectious disease experts focused on the understanding the pathogenesis of the disease and discovered that this infection was caused by a new form of corona virus. Corona virus is not a stable virus demonstrated by the evolution of this virus and can adapt to become more virulent, even lethal to human. In 2012 in Saudi Arabia, it resulted in many deaths and spread first to other countries within the Middle East and then worldwide.[5]

Types: Corona viruses are the members of subfamily Coronavirinae in the family Coronaviridae. Uptill now seven types of coronaviruses are recognised that can infect humans.

Common types:

1. 229E (alpha coronavirus)
2. NL63 (alpha coronavirus)
3. OC43 (beta coronavirus)
4. HKU1 (beta coronavirus)

MERS-CoV strain causes more severe complications like Middle East respiratory syndrome (MERS), and SARS-CoV, leads to severe acute respiratory syndrome (SARS). In 2019, a new dangerous strain was discovered called SARS-CoV-2, causing COVID-19 disease.

Corona virus life cycle:

Steps:

1. Attachment and entry
2. Replicase protein expression
3. Replication and transcription
4. Assembly and release.

Epidemiology: As of April 27, 2020, the WHO has confirmed 28,78,196 cases globally and of these confirmed cases,1,98,668 (6.9%) patients have succumbed to the virus.[6] In the Europian region out of 13,59,380 confirmed cases,1,24,525 (9.2%) patients,in the region of America out of 11,40,520 confirmed cases,58,492 (5.1%) patients, in Eastern Mediterranean Region out of 1,65, 933 confirmed cases 6991(4.2%) patients, in Western Pacific Region out of 1,44, 121 confirmed cases 5958 (4.1%) patients, in South-East Asia Region out of 46,060 confirmed cases,1824 (3.9%) patients, in African Region out of 21,470 confirmed cases 865 (4.1%) patients have succumbed to the virus. [6] The majority of cases and deaths have been reported in USA. of the total number of cases, 9,31,698 (32.4%) patients have been identified in USA.[6] Likewise, the majority of fatalities (47,980 [24.2%]) have also been reported in USA.[6] It is important to note that confirmed cases are clinically diagnosed and laboratory-confirmed. Due to the ongoing nature of the pandemic, the number of cases and involved countries are expected to vary.

Table 1 Provides a comparison of the epidemiological characteristics of SARSCoV, MERS-CoV, and SARS-CoV-2.

Features	SARS-CoV-2	SARS-CoV	MERS-CoV
Estimated R0	2.68	2.5	>1
Host of virus	Bats are natural hosts,pangolins are Intermediate hosts, and humans are terminal hosts	Chinese horseshoe bats are natural hosts, masked palm civets are intermediate hosts, and humans are terminal hosts	Bats are natural hosts,dromedary camels are intermediate hosts, and humans are terminal hosts
Transmission Mode	Human-to-human through fomites, physical contact, aerosol droplets, nosocomial transmission, zoonotic transmission	Human-to-human through aerosol droplets,opportunistic airborne transmission, nosocomial transmission, fecal-oral transmission,zoonotic transmission	Respiratory transmission, zoonotic transmission, nosocomial transmission, limited human-to-human transmission,aerosol transmission
Incubation Period	6.4 days (range:0-24 days)	4.6 days	5.2 days

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SARS-CoV, severe acute respiratory syndrome coronavirus; MERSCoV, Middle East respiratory syndrome coronavirus; R0, reproduction number

Table 2: The number of confirmed cases and death of COVID – 19 outbreak according to WHO as of 27 April 2020

Reporting Country	Total confirmed cases	Total deaths
Region of America		
United States of America	931698	47980
Brazil	58509	4016
Canada	45778	2489
Peru	25331	700
African Region		
South Africa	4546	87
Algeria	3382	425
Cameroon	1621	56
Ghana	1550	11
Eastern Mediterranean Region		
Iran	90481	5710
Saudi Arabia	17522	139
Pakistan	13328	281
United Arab Emirates	10349	76
South-East Asia Region		
India	27892	872
Indonesia	8882	743
Bangladesh	5416	145
Thailand	2931	52
European Region		
Spain	207634	23190
Italy	197675	26644
Germany	155193	5750
The United Kingdom	152844	20732
France	123279	22821
Russian Federation	87147	794
Western Pacific Region		
China	84341	4643
Singapore	13624	12
Japan	13385	351
Republic of Korea	10738	243
Australia	6713	83
New Zealand	1122	19

Table 3: State/UT wise list of COVID confirmed cases (As on 28 April 2020 at 05:00 PM).

State/UT	Total Confirmed	Cured/Discharged/Migrated	Death
Andaman & Nicobar	33	11	0
Andhra Pradesh	1259	258	31
Arunachal Pradesh	1	1	0
Assam	38	27	1
Bihar	346	57	2
Chandigarh	40	17	0
Chhattisgarh	37	32	0
Delhi	3108	877	54
Goa	7	7	0
Gujarat	3548	394	162
Haryana	296	183	3
Himachal Pradesh	40	22	1
Jammu & Kashmir	546	164	7
Jharkhand	103	17	3
Karnataka	520	198	20
Kerala	482	355	4
Ladakh	22	16	0
Maharashtra	8590	1282	369
Manipur	2	2	0
Meghalaya	12	0	1
Mizoram	1	0	0
Madhya Pradesh	2368	361	113
Odisha	118	37	1
Puducherry	8	3	0
Punjab	313	71	18
Rajasthan	2185	518	41
Tamil Nadu	1937	1101	24
Telengana	1002	280	26
Tripura	2	2	0
Uttar Pradesh	2043	400	31
Uttarakhand	51	33	0
West Bengal	697	109	20

Etiology: CoVs are a large family of RNA viruses that are found diversely in animal species and known to cause diseases of the respiratory, hepatic, nervous system, and gastrointestinal systems in humans.[3] They have crown-like appearance under the electron microscope, due to the presence of envelope spike glycoproteins.[4] CoVs belong to the Roniviridae, Arteriviridae, and Coronaviridae families.[3] The Coronaviridae family can be classified into four genera of alpha-COV, beta-COV, delta-COV, and gamma-COV.[4] Furthermore, beta-COV can be sub-divided into 5 lineages.[7] Bats and rodents are the gene source of alpha-COV and beta-COV, while avian species are the gene source of delta-COV and gamma-COV.[4] CoVs causes about 5-10% of acute

respiratory infections.[3] Some common human CoVs include HCoV-OC43, HCoV-HKU1, HCoV-229E, and HCoV-NL63.[4] CoVs clinically present with self limitimg respiratory infections and common cold in immunocompetent individuals.[4] In the old age and immunocompromised idividuals, they can involve the lower respiratory tracts.[4] Other human CoVs such as MERS-CoV, SARS-CoV, and SARS-CoV-2 present with pulmonary and extra-pulmonary features.[4]

SARS-CoV-2, which is responsible for the COVID-19 pandemic, is a type of beta-COV. Genomic characterization studies of the new strain have indicated an 89% nucleotide match with bat SARS-like CoVZXC21.[3,8] and 82% nucleotide match with the human SARS virus. [8] Therefore it is to be called SARS-CoV-2. SARS-CoV-2 is sensitive to ultraviolet light and heat.[4] The Virus binds to their target cells through angiotensin-converting enzyme 2 (ACE2), which is expressed in the lungs. These viruses can be destroyed with the use of ethanol (60%), ether (75%), and chlorine-containing disinfectants.

Transmission: The initial cases of Covid 19 were associated with direct exposure to infected animals (animal-to-human transmission) at a seafood market in Wuhan, China. But human-to-human transmission of the virus is also possible and human-to-human transmission is now considered the main form of transmission. Asymptomatic individuals could also transmit the virus.[4] However, the symptomatic people are the most common source of infection .Most commonly transmission occurs from the spread of respiratory droplets through coughing or sneezing.[4] But close contact between individuals can also result in transmission.[9] There are more chances of transmission in closed spaces due to elevated aerosol concentrations.[4]

SARS-CoV-2 has a basic reproduction number of 2.2.[4] This suggests that a patient can transmit the infection to two other individuals. Current data suggest that the virus has an incubation period of three to seven days.[10] These findings are based on initial cases. Therefore, further studies are needed to address transmission dynamics and incubation times.

Clinical features: COVID-19 manifests as a spectrum ranging from asymptomatic patients to septic shock and multiorgan failure.[4] COVID-19 is classified on the basis of severity of presentation as mild, moderate, severe, and critical.[4,11] The most common symptoms of patients include fever (98.6%), fatigue (69.6%), dry cough, and diarrhea.[11]

Mild Disease: These patients may present with symptoms of an upper respiratory tract viral infection which includes dry cough, mild fever, nasal congestion, sore throat, headache, muscle pain, and malaise.[4] Dyspnea is not present in such cases. The majority (81%) of COVID-19 cases are mild in severity.[4] Radiological changes are also absent in such cases.[11] But these cases can quickly deteriorate into severe or critical cases.

Moderate Disease: These patients present with respiratory symptoms of cough, shortness of breath, and tachypnea.[4] However, signs and symptoms of severe disease are absent.

Severe Disease: These cases present with severe pneumonia, acute respiratory distress syndrome (ARDS), sepsis, or septic shock[4]. These cases are diagnosed on the basis of clinical findings and radiographic studies. Clinically these cases are presented with severe dyspnea, tachypnea (respiratory rate > 30/minute), respiratory distress, $SpO2 \leq 93\%$, $PaO2/FiO2 < 300$, and/or greater than 50% lung infiltrates within 24 to 48 hours.[4] Fever can be absent or moderate even in these cases.[4]

About 5% of patients can develop respiratory failure, RNAaemia, cardiac injury, septic shock, or multiple organ dysfunction.[4,11] Chineese Centres data for Disease Control and Prevention (CDC) suggest that the case fatality rate for critical patients is 49%. [4] Patients with pre-existing co-morbidities like diabetes (7.3%), respiratory disease (6.5%), cardiovascular disease (10.5%), hypertension (6%), and oncological complications (5.6%) have a higher case fatality rate and those without co-morbidities (0.9%).[11]

Acute Respiratory Distress Syndrome: It occurs within one week as a complication of known infection which indicates worsening respiratory failure. PaO2/FiO2 values are used to distinguish ARDS based on varying degrees of hypoxia. $PaO2/FiO2 \leq 100$ mm Hg is indicative of severe ARDS, between 100-200 mm Hg are diagnostic for moderate ARDS and between 200-300 mmHg indicative of of mild ARDS.[4] Higher levels of AST (aspartate transaminase) and ALT (alanine transaminase) at the time of admission result in rapid clinical deterioration to ARDS.

In addition to this chest imaging modalities such as chest X-ray, computed tomography (CT) scan, and

lung ultrasound can be used to support the diagnosis. The most frequent finding on CT scan includes ground-glass opacity (86%), consolidation (29%), crazy paving (19%), bilateral disease distribution (76%), and peripheral disease distribution (33%).[12] It is important to note that a chest X-ray has a lower sensitivity (59%) to detect subtle opacities. A CT scan can further detect mediastinal lymphadenopathy, nodules, cystic changes, and pleural effusion.

Sepsis and Septic Shock: Sepsis and septic shock are considered to be the most critical of them all. The accompanying multi-organ dysfunction results as a consequence of dysregulated host response to infection. Signs of multi-organ dysfunction include severe dyspnea, low oxygen saturation, reduced urine output, tachycardia, hypotension, cold extremities, skin mottling, and altered sensorium.[4] Laboratory evidence of other homeostatic dysregulation includes acidosis, high lactate levels, hyperbilirubinemia, thrombocytopenia, and evidence of coagulopathy[4].Patients with septic shock are persistently hypotensive despite volume resuscitation. They may also have an accompanying serum lactate level of >2 mmol/L.

Laboratory Features: Laboratory findings include elevated prothrombin time, LDH (lactate dehydrogenase), D-dimer, ALT, C-reactive protein (CRP), creatine kinase and the early stages of the disease shows marked reduction in CD4 and CD8 lymphocytes.[11] Patients in the intensive care unit have shown higher levels of interleukin (IL) 2, IL-7, IL-10,GCSF (granulocyte colony-stimulating factor), IP10 (interferon gamma-induced protein 10),MCP1 (monocyte chemotactic protein 1), MIP1A (macrophage inflammatory protein alpha),and TNF-α (tumor necrosis factor-α).[13] They also displayed other abnormal findings indicative of coagulation activation, cellular immune deficiency, myocardial injury, renal injury, and hepatic injury.[11] In critical patients, amylase and D-dimer levels are significantly elevated and blood lymphocyte counts progressively decreased.[4,13] Common to non-survivors are the elevations in serum ferritin, neutrophil count, D-dimer, blood urea, and creatinine levels.[14] Elevations in procalcitonin levels are not a feature of COVID-19. Therefore, an elevated level of procalcitonin may suggest an alternative diagnosis such as bacterial pneumonia. Levels of CRP correlate directly with disease severity and progression.

Diagnosis: The U.S. CDC has developed criteria for persons under investigation (PUI).[4] If a person is considered a PUI, immediate prevention and infection control measures are undertaken. Epidemiological factors which are used to assess the requirement of testing include close contact with a laboratory-confirmed patient within 14 days of symptoms or travel history to an infected area within 14 days of symptom onset.[4]

According to WHO recommendations the samples should be collected from both the upper and lower respiratory tracts which includes expectorated sputum, bronchoalveolar lavage, or endotracheal aspirate.[4] By using polymerase chain reaction (PCR) these samples are then assessed for viral RNA. If the test is positive then it is recommended to repeat the test for re verification purpose and if the test is negative with a strong clinical suspicion, CDC recommends repeat testing.

Management: Isolation and social distancing remains the most effective measure for containment of COVID-19. Neither specific antiviral medication nor vaccine is currently available for treatment.[4] Hence, the treatment consists of symptomatic care and oxygen therapy. Patients with mild infections require early supportive management which includes use of acetaminophen, external cooling, oxygen therapy, nutritional supplements, and anti-bacterial therapy.[11] Critical patients require high flow oxygen, extracorporeal membrane oxygenation (ECMO), glucocorticoid therapy, and convalescent plasma.[11] The use of systemic corticosteroid is not recommended to treat ARDS.[4] Unnecessary administration of antibiotics should also be avoided. ECMO should be considered in patients with refractory

hypoxemia despite undergoing protective ventilation.[4] Intubation, mechanical ventilation, high-flow nasal oxygen, or non-invasive ventilation is recommended in patients with respiratory failure. Hemodynamic support with the administration of vasopressors is requires in patient of septic shock. Patients with multiple organ dysfunction require Organ function support. [4]

Therapeutically, suggested drugs are aerosol administration of alpha-interferon (5 million units twice daily), chloroquine phosphate, and lopinavir/ ritonavir. [4] Other suggested anti-virals include ribavirin and abidor.[11] The use of three or more anti-viral drugs simultaneously is not recommended. Ongoing clinical studies suggest that remdesivir (GS5734) can be used

for prophylaxis and therapy.[4] The antiviral drug, called favipiravir or Avigan, has been used in Japan to treat influenza, and the drug was approved as an experimental treatment for COVID-19 infection. So far, reports suggest that the drug has a high degree of safety and is clearly effective in treatment. Furthermore, a fusion inhibitor targeting the HR1 domain of spike protein is reported to have the potential to treat COVID-19.

Prevention: Preventive measures must focus on optimizing infection control protocols, self-isolation, and patient isolation during the provision of clinical care. The WHO has advised against close contact with patients, farm animals, and wild animals.[4] Patients and the general public must cover their mouth while coughing and sneezing to help prevent aerosol transmission. Frequent hand washing with soap and water is also required and hand sanitizers can also be used. It is very important to avoid public gatherings by immunocompromised individuals. Strict hygiene measures for the control of infections must be applied by emergency medicine department. Healthcare personnel must use personal protective equipment (PPE) such as N95 masks, FFP3 masks, gowns, eye protection, gloves, and gowns.

Conclusions

The COVID-19 pandemic is spreading across the globe at very fast rate. It is considered as more infectious than SARS or MERS and responsible for more infections and deaths as compared with SARS or MERS. Old age and immunocompromised patients are at the greatest risk of infection. Due to rapid spread of disease there is need for intense surveillance and isolation protocols to prevent further transmission. Neither confirmed medication nor vaccine has been developed. Currently treatment strategies include symptomatic care and oxygen therapy. For the future prevention of COV-related epidemic or pandemic prophylactic vaccination is required.

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Role of Ayurveda with Multi Modal Therapy in Prevention and Management of Autism Spectrum Disorders (ASD): The Whole System Approach

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Abstract

Introduction: Autism is a neuro-developmental ailment seen in the preschool age. It is a lifelong condition and requires long term and multiple treatment interventions.

Aim and Objective: To review and present a possible prevention and treatment protocol on Autism Spectrum Disorder (ASD).

Material and Method: The present study is based on the literature search from Ayurveda compendia, recent pediatric textbooks, articles published in research journals from 1983 to 2019.

Observations and Result: ASDs are the complex diseases with imbalance *of Tridosha* and *Dwimanasdoshas* (fundamental principles of body and mind), based on typical clinical features which match with DSM-V (Diagnostic and Statistical Manual) that can be correlated with *Unmada* (Psychosis/insanity). ASDs can be classified into Autism, Asperger's and Rett's syndrome. The probable etiopathogenesis may be defective organogenesis, brain damage, metabolic or genetic changes.

Discussion: *Acharya* emphasized on *Yuktivyapashray* (rational therapy), *Daiva vyapashray* (non-pharmacological spiritual therapy) and *Satvavajay chikitsa* (Ayurveda psychotherapy) altogether in *Unmad* treatment due to its complex nature. Recently developed therapies such as play therapy and cognitive behavioral therapy-CBT can be a good adjuvant with Ayurvedic management of ASDs for better quality of life.

Conclusion: As no satisfactory treatment is available in modern medicine till now, multi-modal treatment such as Ayurveda, behavioral therapy, physiotherapy, occupational therapy and early educational interventions might be beneficial to treat ASDs effectively. This review paper will be a torchbearer in the prevention and management of ASDs with the whole system approach and proposed management algorithm.

Keywords: *Autism Spectrum Disorder, Autism, Ayurveda, Multi-modal Therapy, Unmada, whole system approach, management algorithm.*

Introduction

ASDs are the third most common developmental disability which are inadequately understood. They are easily diagnosed by the main differentiating features like lack of interpersonal and communication skills, poor eye contact, delayed speech with pervasive abnormal body movements or posture.[1]Till 1980, they were uncommon disorders but recently then umbers are increasing drastically, maybe due to adoption of western lifestyle, prenatal stress, maternal complications, faulty diet and daily regimen. The definite etiology is not known but many environmental predisposing factors may be responsible for the occurrence of these disorders.[2] ASDs are neuro-behavioral and neuro-developmental

ailment[3] which can be correlated with *Unmad* described in Ayurveda. *Unmada* is characterized by perversion of mind,memory, desire,behavior and conduct with five varieties.[4] Management of *unmad* and ASD has need of utilizing multiple interventions including various behavioral and educational strategies[2,4] repeatedly for long duration. Present review is an effort to interpret and analyze the concept of Autism through Ayurveda perspective along with other multiple interventions which play a synergistic role in its prevention and management.

Material and Method

A retrospective clinical review to evaluate the effect of Ayurveda modalities, modern medicines, association of biochemical parameters, other interventions in ASDs. Method of data Source: Google scholar, MEDLINE (Pub med), Cochrane and Ayurveda were searched from 2010 to 2019. Manual search was also done to access the articles or thesis wherever possible. Electronicsearch included the following key words search to find the clinical studies with use of MeSHterms-Double blind study, "Ayurved*" AND|OR autism, Autism Spectrum Disorder, clinical trials, RCT, meta-analysis, systematic review, the diagnostic tests and interventional studywhich were reviewed thoroughly. Inclusion Criteria a) randomized clinical trial. b) Published in English accessible in full text. c) Evaluated on the effect of autism prevention and management or treatment.

Observations and Result

Many complex signs and symptoms are present in clinical features of ASD such as behavioral problems, screaming, developmental delay in all milestones i.e. gross motor, fine motor, personal-social and language. Such patients have strong interests in irrelevant things (like rope), mood-anxiety disorder, obsessive-compulsive disorder, gastro-intestinal upset, mental retardness, phobia, sleeplessness, etc. Imaginative play, communication and social interaction severely lack in ASDs.ASDs are classified into Autism, Asperger syndrome, Rettsyndrome, Pervasive developmental disorder (PDD) and Childhood disintegrative disorder (CDD) mentioned in table no 1. [2]

Some investigations such as Karyotyping, DNA, IQ test etc. help to rule out differential diagnosis and hidden associated features. In particular, diagnosis of ASDs are based on DSM -V criteria, typical clinical features, examination and history with so many other assessment scales such as ADI-RAutism diagnostic interview-Revised; ADOS-G-autism diagnostic observation schedule generic; CARS- childhood autism rating scale; DISCO-10: diagnostic interview for social and communication disorders-tenth revesion; GARS-2: Gilliam autism rating scale; 3 Di: Developmental, dimensional and diagnostic interview. [17]

Samprapti: The probable etiopathogenesis is based on faulty organogenesis, brain damage, trauma, obstructed labour, metabolic changes in intra-uterine life. ASDs may be caused due to genetic predisposition or defects, radiation, pollution, *Dushivishasevan* (food with toxic elements),improper use of food and medicines. Environmental triggers like stress during ANC, perinataland postnatal insults, *Vega Dharna* (suppression of natural urges)and other known or unknown reasons may provoke the disorder. It is a *Tridoshajvyadhi* with variation in their single or mix dominance, also vitiates *Mansik Doshas.* They together affect *Buddhi*-intellect *(Dhee*-short time memory *Dhruti*-act with dedication,*Smruti*-memory*)*and create various clinical features especially abnormal pervasive movements, screaming suggesting ASD[4,18] An attempt has been made to correlate treatment of unmad with features of autism in table no. 2

Table no 1: *Doshaj* variance matching with ASD features with Ayurveda management [2,4,19]

SN	Type	Characteristic features	Resemblance to Autism	*Shaman* therapy/ Pharmacotherapy	*Shodhan* (bio-purification Therapy)
1	Vataj Unmada	Sudden abnormal laughing, crying, talking, body movements	Repeated movements of eye brow, lips, chin, hands-feet and other organs, Frothing of saliva, Excessive screaming, dancing, singing, Frequent utterance of uncontrolled sound and voice	*Deepan-pachan-Anuloman, Snehpan, Medhyakalp, Sandnyaprabodhan, Ashwasan*/assurance *Saraswatarishta, Unmdgajakesariras* with milk, *Ghrit*	Mrudurechan, Basti, Nasya

SN	Type	Characteristic features	Resemblance to Autism	*Shaman* therapy/ Pharmacotherapy	*Shodhan* (bio-purification Therapy)
2	Pittaj Unmada	Impatient, irritable, aggressive, demanding food and water	Aggressive behavior, Irritable, recurrent fever, Harsh high pitch screaming	*Trasanchikitsa* & above all	*Mrudu-rechan, Basti*,
3	Kaphaj Unmada	Limited talk & movements, sleepy, drooling of saliva	Stay in one place or spot, less -talkative, clumsiness, less mobility. Drooling of saliva, running nose, dislike for bathing and toileting	Same asabove	Snehan-Swedan, Vaman, Virechan
4	Sannipataj Unmada	Mixed features	Mixed features	Same asabove*Doshaj* treatment	As per dominance
5	Agantuj Unmada	Extraordinary behavior in knowledge & strength	Sudden grief, loss/separation of close relative, brain trauma etc. Features seen of *Vataj* and *PittajUnmada* mainly	*Ghritkalpa, Nasya, Ashwasanchikitsa,* counselling sessions	--

Prevention:*Acharya Charak* has suggested implementation of pre-conceptional *Shodhan* and *Rasayan* (rejuvenation therapy) for newly wedded couple. He also advised genetic counselling, consanguinity check, pursue dailyregimen, seasonal regimen and *Yoga.* While during pregnancy, adoption of *Garbhiniparicharya* (antenatal checkup), fulfillment of *Dauhrida* (desires during pregnancy), avoidance of *Garbhopaghatakara Bhavas,* stress (detrimental factors affecting fetus) are some measures that play the pivotal role in prevention of ASDs.[20,21] *Pathya* (dietary restrictions) and *Nidanparivarjan* (restricting causative factors) are dynamic in etiology with abidance of *Satvavajaychikitsa* (mind control therapy), *Achar Rasayan*and *Sadvritta* (good conduct)to control *Niyamak Buddhi.* These factors are essential for prevention of *Pradnyaparadha* (intellectual blasphemy), *Asatmendriyarthasanyog* (incompatible contact of sense organs with their objects) and *Parinama* (time factor for chronological error)[22] which are three basic causes of all diseases. Prevention is easy and better dealt with Ayurvedic interventions than management.

Algorithm of ASD Treatment proposal: As both *Sharirika*s well as *Mansik Doshas* are involved in etio-pathogenesishence multi modal treatment approach is necessary for the whole system correction. [23] Initiating with *Yuktivyapashraychikitsa-YC* (rational therapy), medicines having *Deepan* (appetizer), *Pachan* (digestive) and *Anulomak* (mild laxative) properties must be added because *Agneemandya* (poor appetite, digestion & metabolism) is essentially present with *Vatadushti*. Coherent use of *Yuktivyapashraychikitsa* which mainly comprise *Shaman* (pharmacotherapy), *Mrudu Shodhan* (purify and detox therapy) and *Rasayan* (Rejuvenation therapy) along with multimodal treatment approachcanplaya vital role in the management of ASD.[15, 24-25]

Medhyaghritkalp: As the main involved site is brain hence any *Ghritkalp* fortified with *Medhyadravyas* (Ghee with nootropic/intelligence boosting medicines, such as *Bramhighrit, Kalyanak*or *Guduchyadi-Bhringrajadighrit* etc.[26]) is very important in prevention and treatment. *Medhyashamanaushadh/* nootropic medicine's action is synergistic to *ghritkalpa* with *Balya- brihan & Rasayan* (General tonic for rejuvanation) efficacy.[27]

Panchakarma-Poorva Karma: Pre-procedures such as *Rukshan, Deepan, Pachan* and *Snehan-swedan* are important for facilitation of *Pradhankarma* (main procedures).Long term and repeated use of some procedures like *Talapothichhil/Shirolepa, Shirodhara, Udvartan, Udsadan, Annalepan, Patrapottali, Pindasweda, Kwathdhara, Takradhara, Kanjidhara* or

*Tailadhara*etc are beneficial to pacify ASDsfor better efficacy. Multiple sittings of these procedures are required to treat ASDs due to its complex nature.

Pradhan Karma: *Nasya, Basti-Niruh, Matrabasti* are mainly beneficial in all types of Autism.As per *Doshaj* predominance *Vaman* and *Virechan* can be used.

Paschat Karma: S*ansarjankrama* (light diet therapy), followed by *Balya-brihan & Rasayan* therapy (anabolic action) is recommended.[28]

Satvavajayachikitsa: It comprises the act of controlling mind which can be achieved by doing *Buddhiniyaman* to avoid committing basic causes of all diseases[29], *Sadvritta*, *Achar Rasayan* and *Trasan* (act to deviate mind by frightening or eliciting painful stimulus).[19] *Yog*a, meditation and its other modalities are also helpful in achieving positive results in autism.[30]

Daivvyapashraychikitsa: Itincludes non-pharmacotherapy with spiritual belief which provides assurance and due to its psychological impact on mind, such measures can be useful to treat psychiatric illness to some extent.[19]. It synergistically acts with or without main treatment for a particular disease like Mantra chanting, use of amulets, holding therapy etc.

Multi-modal treatment such as, counselling of parents for tackling children affected with ASD, play therapy, Cognitive behavioral therapy, applied behavior analysis, Yoga, Speech therapy, Occupational therapy, Physiotherapy, Music therapy, Hydrotherapy, Reiki etc. can be incorporated as whole system approach[23].

There is no comprehensive management available in any system but synergistic action of these therapies altogether may prove better quality of life. Hence, a hypothesis of treatment guideline is framed to resolve this issue with the whole system approach for ready reference to Ayurveda physicians to treat ASDs effectively.

Discussion

(a) Key of preventing ASDs: It is necessary to prevent ASDs by strictly adapting daily and seasonal regimen, CAM-complementary and Alternative medicine-Yoga-meditation, recreational activities to keep away stress, pre-conceptional *Shodhan* and no consanguineous marriages to prevent mutational genetic changes *Beejdushti*, *Vatadushti,* and *Agneemandya*[15,25] All other etiological factors should be avoided with happy gestational period to boost *Satva*and balance *Raja-Tama.*

(b) Rationale of Shodhana Therapy: *Shodhan* therapy aims at removal of the vitiated *doshas* and correction of *Agni* and metabolic derangement which are the prime causative factors of all diseases. *It also facilitates the link between sense organs and brain efficiently.* [39,24] In Autism, mainly *Nasya* and *Basti* are more important procedures as *nasya* stimulates the function of brain and all *urdhvajatrugat* organs. [28] *Basti* is the main pathway of the gut-brain axis to pacify the vitiated *Vata* and stimulates brain function.[32] Other pre-*Panchkarma* procedures and all previously narrated therapies support motor function by strengthening muscles, joints, ligaments and facilitate in achieving day to day activities on their own.[33] In table no 4, there is a description of pre and main panchakarma procedures with their mode of action and references.

(c) Rationale of *Shamana* therapy (Internal medication): The process by which vitiated *Dosha*s ubside and return to normal without creating an imbalance of other humours is known as *Shamana*. This treatment is achieved by the use of appetizers, digestives, Rasayanas, symptomatic treatment, exercise etc.[19]

(d) Rationale of Diet therapy: It comprises *Pathya* (indications) and *Apathya* (contra indications) with respect to diet and eating habits. This is done to enhance the effects of therapeutic measures and to impede the pathogenic processes.[40]

(e) Role of *Nidan Parivarjan*: It is to avoid the known disease-causing factors with respect to diet, environmental issues and lifestyle of the patient. It also encompasses the idea to refrain from precipitating or aggravating factors of the disease. [19]

(f) Role of *Rasayana* therapy: It deals with the promotion of strength, vitality, immunity against the disease.[38]

(g) Role of *Satvavajaya:* It is an Ayurvedic psychotherapy. *It* concerns mainly with the area of mental disturbances which includes restraining the mind from desires for unwholesome objects and intellectual blasphemy in *collaboration* with *good conduct.[29]*

(h) Role of *Daivavyashray Chikitsa*: It was a proven

therapy since time immemorial but since medieval period it declined drastically due to the influence of modern medicine. Not manystudies have found to prove the scientific role of its various components. Some randomized controlled trials (RCTs) of music interventions for ASD have reported positive effects of music on emotional engagement, social interaction, communication and parent–child relationship.[13,40]

(i) **Role of Multi-modal Treatment and Whole System Approach:** It aims to evaluate the outcome of disease related to multiple variables individually.[23] It is an emerging approach for a complex disease management. It is not possible to fully understand the role of each component individually of whole system approach when implemented together.

Conclusion

Multi-modal treatment facilitates synergistic action for good outcome in ASD.Long duration is required to correct the whole system which gets impaired due to multifaceted nature of the disorder. Each course of these therapies either separate or together as per the symptoms and tolerance, magnify steady results in terms of sensory, motor integration, immunomodulation and ability of verbal communication. *Satvavajaychikitsa* is a broad term which may comprises all other multi-modal treatments those are developed separately nowadays. Thus, the three main *Chikitsa* modalities of Ayurveda-*Yuktivyapashray, Daivavyapashray* and *Satvavajay* are stepping stones in the management of ASDs (which is a life-long condition), giving a ray of hope and indicating the need towards more research in this arena. *Agneemandya* and *Vatadushti* are most important cause of ASD and should be eliminated. By implementing need-based modalities with multi-dimensional and wholistic approach, Ayurveda plays a fundamental role in not only its prevention but also in management, with improvement in quality of life to much extent.

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Nanotechnology in Periodontal Regeneration: A Review

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Abstract

Periodontitis involves destruction of the periodontal ligament and supporting structures. It ranges from a simple inflammation of gums to major damage resulting in loss of bone. Multiple conditions like trauma, periodontal disease, reconstructive surgery, ageing, osteoporosis and neoplastic pathology, have a common characteristic feature being loss of bone and tissue. The regenerative procedures that are currently practiced aim to regenerate the lost tissue via means of grafting, placement of membranes and many more, but have been accompanied with a set of limitations and major disadvantage being graft rejection. Periodontal regeneration still endures to be a partially unsolved algorithm. In dentistry, a new arena of development is being exploited to set a new approach for regeneration that is Nanotechnology. This branch involves tissue engineering which aims to regenerate the lost tissues via manufacturing scaffolds which transport signaling cells and molecules. These scaffolds possess magnificent physical as well as chemical properties and biomimetic features that act as a substitute of the highest standard offering innumerable benefits for promoting cell growth and tissue regeneration via tissue engineering.Nanomaterials because of their refined physiochemical characteristics and biomimetic attributes serve as a scope worthy substitute that offers several advancements for stimulation of cell growth and tissue regeneration. This review provides an insight to basic information about nanotechnology, the various nano particles having application in periodontal regeneration, the advanced regenerative procedures fabricated on the principles of nanotechnology, their applications, the clinical studies highlighting the achievements so far, supremacy over the conventional method and limitations.

Keywords: *Nanotechnology, periodontal regeneration, nanomaterials, tissue regeneration, bone augmentation*

Introduction

Periodontal tissue regeneration serves to be the pioneer for the lost tooth and the supporting structures. The regenerative and augmentative procedures are multiplex in nature and therefore challenging for the maxillofacial and periodontal surgeons. Variousailments liketrauma, periodontal disease, reconstructive surgery, ageing, osteoporosis and neoplastic pathology, have a common characteristic feature being loss of bone and tissue. Due to increased consumption of tobacco and betelnut, the prevalence rates of periodontitis are quite high in India.[1] Currently, many approaches exist in tissue regeneration and bone augmentation, but they are not efficient enough to deliver consistent results.[3] This is where nanotechnology comes into picture as it has manufactured and processed an entity called nano scaffolds. These scaffolds possess magnificent physical as well as chemical properties and biomimetic features

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that act as a substitute of the highest standard offering innumerable benefits for promoting cell growth and tissue regeneration via tissue engineering. Hence, the paramount of this literature-based article is to bring for than outline of diverse nanoscale scaffolds as vital elements in tissue engineering and regenerative medicine prototype used for alveolar bone regeneration and bone augmentation.

Tissue Regeneration and Bone Augmentation: Present view: At present, autografts, allografts, xenografts and synthetic graft materials are currently used for tissue regeneration and bone augmentation.[4,5] But they have their own set of limitations.[6–8]Tissue engineering is a process where the wound healing is manipulated to achieve tissue regeneration.[9] This process broadly covers three key components: the signaling molecules, scaffolds and cells.[10,11] Tissue engineering shows improved results with the use of advanced materials such as "enamel matrix derivatives (EMD)" for treatment of infra-bony defects, "platelet derived growth factor (PDGF)" and "transforming growth factor-β (TGF-β)" are the growth factors that are effective for periodontal regeneration."Guided tissue regeneration (GTR)"and "laser-assisted new attachment procedure (LANAP)" can also be considered for new attachment, but requires further clinical trials.[12,13]

Tissue engineering demands the cells to stay alive inside the human body and continue to perform the function, which becomes difficult due to poor biomechanical and physico-chemical properties of the traditional scaffolds.[14] Hence, nanotechnology set the ball rolling on the evolution of tissue regeneration and bone augmentation.

What is Nanotechnology?: Nanotechnology is an arena of scrutiny and novelty concerned with fabricating things on the extent of atoms and molecules and has reached to all areas of science. A nanometer is one-billionth of a meter that is ten times the diameter of a hydrogen atom. This field offers advanced alternative solutions for scientific and medical problems. It involves manipulation of atoms and molecules to form larger nano structures, this is called the bottom approach. Whereas, larger structures or pieces are broken down to generate nanostructures from them, this is the top-down approach.[15,16] Nanotechnology has been exploited on a large scale over the last twenty to thirty years in various fields. For example, in dentistry use of submicron grained size ceramics for production of all-ceramic restorations improves its appearance, aesthetics and mechanical properties, increase the resistance to wear and increase strength.[17]

Nano biomaterials for tissue engineering and bone augmentation: In oral biology, the main purpose of practicing these procedures is to restore the functionality of the lost tissue and the tissue itself that take place during alveolar and periodontal tissue genesis, with the help of scaffolds carrying signaling molecules and cells.[2,14] This technology has outlooked to integrate scaffold matrix technologies with the regenerative potential of stem cells which are in close proximity, mainly to dental pulp, periodontal ligament (PDL) and alveolar tissue.[18] Therefore, an ideal biomaterial prospective should involve a scaffold that is functionally categorized where the chemical constituents and 3-D construct of each recess should match the detailed framework, biochemical components and mechanical attributes. Additionally, the matrices are supposed to degrade at a suitable rate so as to meet the regeneration pace of the tissues.[19,20]A study stated that there has been development of a scaffold which is a "3-layered construct" for concurrent regeneration of hard and soft tissue structures of the periodontium, like cementum, alveolar bone and PDL.[21]

Nano scaffolds: The current developments in nanotechnology have been cutting edge because of their ability to cause osteogenic enhancement.[22]It has acknowledged remarkable advancement of these scaffolding materials to propose distinctive 3-D matrix conditions for cells and tissues.[23] Nano scaffolds show superior most attributes in every dimension than various conventional approaches that are practiced for the purpose of tissue regeneration and bone augmentation.[24,25] The existing examples of scaffolds like "3-layered construct" suggest the nanomaterials that are used often should be multi-layered for multi tissue regeneration as this helps in exacting reproduction of even the minute characteristics of the structures that are to be regenerated.[26]

Nanoparticles, due to their reduced size, aid in quick reaction to extraneous stimulants such as ultrasounds, magnetic fields, X-ray and pH exposure. Delivery by nanoparticles which is controlled, depends mainly upon their reduced size and subsequent high surface area. The topography of nanoparticles is such that it imparts definitive physicochemical signals to cells such that it becomes easy for the cells to adhere and differentiate.

Besides, various active molecules and biological proteins are usually amalgamated along with the scaffolding materials to employ therapeutic efficiency and structurally control the cellular activity at nanoscale level. This is how nano tissue engineering succeeds in achieving structures that mimic their native tissue and develop structures of equivalency to the dentin, cementum, pulp, PDL and alveolar bone.

Role of extracellular matrix [ECM] in tissue regeneration: Extracellular matrix is an elaborate molecular complex made of three major constituents: protein, glycoconjugate and glycosaminoglycans. These elements along with cell adhesion receptors interact to form a complex in which cells live in tissues and organs. These receptors send cues to the cells from ECM which regulates manifold and paramount procedures like, proliferation, metastasis, growth and differentiation.

The ECM mainly contains collagen fibers which are produced via self-computing of smaller subunits. These self-computed fibers of collagen result in the mineralization of hydroxyapatite nanocrystals in a highly organized way. This bottom-up approach creates a structure of fine architecture that mimics the native tissue with accurate details. This can provide an engineered entity with distinctive mechanical properties. This enables amelogenesis and dentinogenes is induction in the matrix protein.

Hence, manipulation of nano construct of native tissue has been the matter of contention in current research era of tissue engineering scaffolds.

How are nano scaffolds produced?: Nano scaffolds are commonly produced by approaches such as: phase separation, peptide synthesis and electrospinning approach. Nanofibrous scaffolds which are built of poly-l-lactide acid (PLLA) can be incorporated through the "phase-separation" as well as "porogen leaching method." These scaffolds, in human dental pulp cells, manifest the stimulation of odontogenic differentiation in contrast to solid-walled PLLA scaffolds.

In recent times, scientists have invented scaffolds made up of biopolymers in combination with bioactive glass, known as nanocomposite nanofibrous scaffolds. These nanoparticles play a key role in upgrading mineralization and remineralization of mutilated dentin matrices. Furthermore, a study reported incorporation of magnesium phosphate into nanofibrous scaffolds, where they helped in enhancing the rate of differentiation of pulp stem cells.

Nanoparticles: A nanoparticle (NP) is a minuscule particle which usually ranges between 1 to 100 nanometers in diameter. They have peculiar physicochemical properties to their material counterparts of comparatively larger size. They are classified as nanospheres and nanocapsules. Nanospheres are those nanoparticles that have a matrix type of construct where a drug is dispersed. Nanocapsules have a membrane wall construct with an oil core in the center containing the drug. Several applications use nanospheres in delivery of drugs, genetic material and growth factors. These include controlled drug delivery using porogens for reinforcement of a mechanically weak scaffold as bioreactors of microscopic nature helping in the formation of hydroxy apatite crystals, followed by mineralization using microspheres for providing the sites for cell adhesion and preparation of formulations like suspensions, colloidal gels and injectables to be used in minimal invasive surgery.

Inorganic Nanoparticles

i. Synthetic Polymers: The ones customarily used are "poly-α-hydroxyesters [poly-glycolic acid (PGA), poly-lactic acid (PLA), poly-lactic-glycolic acid (PLGA) and poly-caprolactone (PCL)]" for biomedical applications. They are available at reasonable cost, easy to adapt and have a high safety index. Major disadvantage is hydrophobicity and degrade via autocatalysis.Various combinations of TGA/PLA/PLGA/PEG/PCL have been tested for alveolar bone regeneration and have shown positive results.

ii. Ceramic NPs: Ceramic materials are "synthetic crystalline, solid, inorganic non-metallic materials". Bioceramics include "bioactive glass, bioactive glass–ceramic, calcium phosphate groups and alumina." These have been used to refine the morphology and performance of the scaffolds. For example, calcium phosphate, mineral trioxide aggregate (MTA) and bioactive glass.

iii. Silica NPs: These can be integrated as bioparticles, either core or shell silica NPs and mesoporous silica nanoparticles (MSNPs). They have been used in biomedical field and for drug delivery because of their unique structure and increased pore volume and surface area. The limitation of silica NPs is variable toxicity.

iv. Metallic NPs: They are the safest and most commonly used is gold NPs because of their ability to be drawn into variable shapes such as rods, cages or spheres. Thus, can be implemented in drug delivery, bio-imaging, photo-thermal therapy and biosensing. The combination of gold and silver NPs along with chitosan have shown successful outcomes for bone regeneration, mineralization and osteointegration of implants. They are biocompatible NPs with less cytotoxicity.

v. Magnetic NPs: They show paramagnetic properties of long duration and non-invasive tracking. Therefore, in biomedical field, cobalt, nickel, iron and their subsequent oxides have been used for cell tracking, drug delivery, as biosensors, 3-D cell organization and imaging procedures.

Organic nanoparticles:

i. Liposomes: Liposomes are mostly used in imaging and drug delivery. They are bilayer structures made up of phospholipids. Their properties vary because of their size, composition, surface charge and preparation and are used in imaging and drug delivery.

ii. Natural Polymeric NPs: These are classified as proteins and polysaccharides. They are biocompatible and bio-degradable materials that possess bulk physical characteristic features.NPs hold high capability for drug loading,have a flexible synthesis and their structure constitutes of polymer chains which show a broad variety in terms of composition and characteristics. Collagen, gelatin, alginate, chitosan and fibrin are some of the natural polymericNPs.

iii. Carbon:

a. Carbon nanotubes (CNTs)- these are cylindrical structures made of carbon allotropes. They are mechanically and chemically stable with conducive electrical properties.Usually come eitherin the form of a single or multi-walled tubular architecture. CNTs show effective performance on bone and tissue regeneration, also aid in multiplication of cells and bone integration.

b. Graphene and its derivatives: A film which is single layer,1 atom thick and is arranged with carbon atoms in 2-D hexagonal construct which shows similarity to honeycomb.They constitute large variation of graphene derivatives like, graphene oxide(GO) and reduced form of it, called as reduced graphene oxide(rGO).

Composite Scaffolds: Combinations of various organic as well as inorganic particles have been evaluated as composite scaffolds, which in turn improve the properties and decreases the limitations of singular components. Many researchers have experimented with varied composite scaffold designs of different combinations such as, nHA with high molecular-weight polymers, nHA with PLA, PCL[88], PLGA, chitosan, collagen, coralline and polyamide. Most of these combinations have come up with ameliorated mechanical strength and scaffold biocompatibility.

Hazards of Nanotechnology: Although there are many potential advantages of using nanotechnology, it ought to be countervailed against the risks. The physical and chemical properties have enormous ramifications in many ways via which these nanoparticles interrelate with biotic constituents, their uptake, accretion and excretion through the body and interact with environs at large. The interplay between nanoparticles and human tissue can be physical or chemical. The physical interactivity mostly leads to disintegration of membranes and its activities such as "protein folding, aggregation and various transport processes."Whereas, the chemical interactions result in oxidative damage and formation of reactive oxygen species. Besides these, environmental reactions also determine the nanomaterial toxicity.

It is difficult to avoid the exposure to nanoparticles, being airborne in nature. Humans are exposed to these, knowingly through nanotherapy or unknowingly through natural/human-induced particles leading to multiple entry routes due to high reactivity, which aggravates the condition.[98]Interaction of nanoparticles with proteins, enzymes, molecular structures DNA, RNA in a reversible or irreversible manner may lead to devastation in biological systems resulting in emergence of new diseases. This warrants detailed studies to evaluate the toxicity of nanoparticles before using them for therapeutic purpose.

Future prospects: This multidisciplinary domain of science is undergoing colossal development and has grown immensely in the past few years, thus exhibiting a step aheadof existing practice in the healthcare sector. The research and development has paved its way in the field of nanoengineering which will change the practice of dentistry, medicine and day to day life, moulding it into

a better future.Like other technologies, nanotechnology also has the aptitude to be exploited and misused on a large scale.

In the field of dentistry, nanotechnology will give a superiorperception to preventive management, which is the latest trending treatment approach along with advantages like early detection of the disease and treatment of oral cancer.

Conclusion

One of the recent exploitations in periodontology has been the use of nanoparticles in oral biology. Periodontal regeneration still endures to be a partially unsolved algorithm. The purview of periodontal tissue regeneration in nanotechnology is to rejuvenate the lost structure using tissue engineering, in conjunction with materials called "nanoscaffolds" which manipulate the physiological and biological processes for an improved performance of the objective. This will revolutionize the province of periodontal tissue regeneration. In the subsequent wave of research and innovation, the underlying fundamental mechanism of interactions conducted in vivo between nanoparticles and cells at a molecular scale will remarkably facilitate the evolution of this domain.

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Tongue Prints: A Forensic Review

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Abstract

Forensic odontology is a branch of forensic sciences which proof to be very helpful during investigation of criminal cases and during person identification by providing the dental expertise in the court and to the legal system so that proper justice can be done based on evidence. The tongue is a crucial organ very much encased inside the oral cavity and shielded from nature. Tongue print is the subtleties present on uncovered section of tongue when the surface and shape are assembled. The morphology and surface highlights are attributes of each person, and these characteristics can be utilized for criminological distinguishing proof. It has unique shape and surface textures in each individual. The shape, colour and texture of tongue are different in identical twins. And hence it can be used as a new method for personal identification with further elaboration. The unique features make it a valuable tool in forensic odontology. The exposed portion of tongue contains information with visible differences from one individual to another therefore it is helpful in identification of suspect during forensic investigation. Tongue becomes the forensic evidence for establishing the cause of death. It is an evolving science in the field of forensic and there is greater scope for its further development. The use of tongue print as forensic identification is at budding stage but now its use as a biometric authentication system is gaining a lot of popularity. This review is aimed at describing the distinctive features of tongue, application of tongue prints in personal identification and as biometric system and the challenges faced in its use.

Keywords: *Tongue print; Forensics; Biometric; Identification; Forensic odontology.*

Introduction

Forensic science or forensics is a multidisciplinary branch of a science which uses the scientific concepts in issues of law[1]. Forensic odontology can be characterized as a part of dentistry which manages the suitable taking care of and assessment of dental proof and with the correct assessment and introduction of dental discoveries in light of a legitimate concern for equity[2]. Forensic odontology is a branch of forensic sciences which proof to be very helpful during investigation of criminal cases and during person identification by providing the dental expertise in the court and to the legal system[3]so that proper justice can be done based on evidence.Forensic dentistry or forensic odontology includes various zones; the general definition might be the mix of the science and craft of dentistry and lawful

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framework, a junction of dental science and law[4]. It is an evolving science in the field of forensic and there is greater scope for its further development. As the dental tissues are often preserved even after death of person, whether the person's body is decomposed, skeletonized, burnt, or dismembered[5]. Therefore, it has established a great popularity in medico-legal matters and in the identification of the dead person.

With advancement in technology, the tools have evolved as well. The various tools currently used in forensic odontology include bite mark analysis[6], Cheiloscopy[7,27], Tongue print[8], Rugoscopy[9], Odontometric[10], Tooth print[11], Radiographs[12], DNA analysis[6], comparison with past dental records[6] etc.

Therefore this review aims to throw light on new tool- tongue prints which has evolved in recent times owing to its uniqueness. It is an effort to summarise the distinctive features of tongue, its usefulness in personal identification, use as a biometric tool, its limitations and future scope so as to understand its implication in forensic odontology.

Tongue Print: Tongue print is the subtleties present on uncovered section of tongue when the surface and shape are assembled[13]. Tongue can be effortlessly exposed for inspection but at the same time it is well protected from any kind of environmental influences and therefore it is quite difficult to manipulate or forge the tongue print[14]. However, the use of tongue print as forensic identification is at budding stage[5] but now its use as a biometric authentication system is gaining a lot of popularity[13].

Overview of Tongue: The tongue is an exceptionally movable set of muscles, which is richly supplied with blood and nerves[15].Tongue is joined to the mandible, hyoid bone, pharynx, sense of taste and styloid process by means of muscles. It comprises two sections; a foremost 66% and a back 33% which is partitioned by V-formed sulcus terminalis[16]. The root of the tongue is securely anchored to the floor of the mouth[15]. The mucous layer of tongue has three kinds of papillae. The dorsum of the tongue show filiform papillae. Fungiform papillae are seen close to the surface of tongue, are marginally redder and increasingly at tip and edges of the tongue. Circumvallate papillae are approximately 8 to 12 in number[17]. They are bigger in size and are positioned at the intersection of oraland pharyngeal tongue[17]. It is pink in colour and moist with neither localized nor diffuse discoloration nor ulceration[17]. The human tongue is known as the mirror of the oral and general health[18].

Unique Features of Tongue: Tongue is the main organ that is available inside which can be ordinarily and easily be uncovered for investigation purposes in forensic odontology[19]. The process of tongue inspection gives a reliable confirmation of life[20]. It has unique shape and surface textures in each individual[19]. The shape, colour and texture of tongue are different in identical twins[21]. And hence it can be used as a new method for personal identification with further elaboration[21]. It performs various functions such as articulation of speech, perception of taste, and formation of bolus[22].

When the morphology of the tongue is examined it displays a "U-shape", with slightly round off tip. The other commonly seen shape of the tongue was trapezoidal, which have the large base at the oral commissures level and a smaller base at anterior side[8]. In the dorsum surface of the tongue there is presence of fissures which is the most common morphological feature[13]. A preliminary study stated that geographic tongue and sharp tips are commonly found in female subjects, whereas a septate tip is a common feature in males[18]. Gender wise differences are observed in width and length of the tongue with Males depicting an increased width and length of tongue as compared to females[23].

Recording Tongue Print: There are different ways that can be used to obtain tongue prints. Some of the basic features of tongue like the colour of the tongue, variations in surface texture, mobility and any other special characteristics if present they can be inspected by simple visualisation.

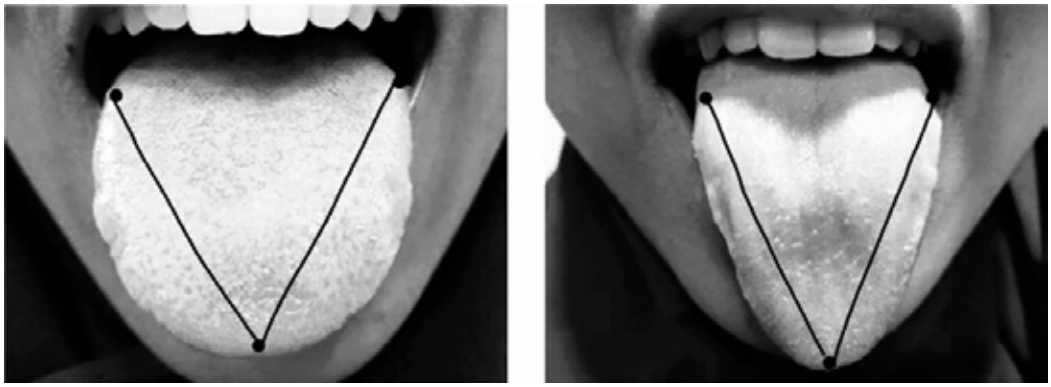


Figure 1: Reference points on tongue for shape determination 24

Determination of the shape of tongue is done by utilizing three reference focuses. The reference focuses incorporate the area of the tongue in contact with the commissure of the lips when distended outside the mouth and the tip of the tongue, as depicted in Figure 1[24]. The most viable option for tongue assessment is a three dimensional analysis.

Alginate impressions followed by cast preparation can be used for analysis. The unique features of tongue can be captured and those can be reproduced on cast for study purpose[18]. Digital images can also be used for analysis. Digital software autocorrects the colour and hue along with positional alterations and later analyses the tongue for it to match with data base for positive identification. However, there are a number of researches going on to prepare an exact algorithm for analysis of tongue images[25].

There are various other method that are been used to capture the video of tongue and to pull out the images from it as this organ is non-rigid. The most common technique used in tongue diagnosis is sublingual valve analysis. An ultrasound transducer is introduced that is positioned in sublingual area for the analysis of the tongue function[26]. Another method that is helpful for the examination of tongue is the Histological examination. Tongue dorsal surface is different in each person. Even between identical twins the characteristic features of tongue are different[21]. The most useful proof in forensic odontology is taking impression of the dorsal surface of tongue including lateral borders when it is used in combination with Cheiloscopyand Rugoscopy[10,27].

Application of Tongue Print in Forensic Odontology: It has unique features like colour, shape, and surface features which are different from person to person. They are different between identical twins also making it a tool for personal identification. Both texture and shape of the tongue is used for identification purpose. The exposed portion of tongue contains information with visible differences from one individual to another therefore it is helpful in identification of suspect during forensic investigation.

Tongue becomes the forensic evidence for establishing the cause of death. Bitten tongue in addition to protruding eyes or anus and swollen abdomen is important evidence in drowning. Protrusion of tongue is evidence in detecting the death by hanging whereas dark tongue becomes the evidence in case of poisoning.

Tongue Print as Biometric System: There are various biometric systems that are used for recognition of person's identity which includes fingerprint, face, palm print, iris, hand shape, gait, voice, and signature. However, this traditional biometric system has some restriction as they can be easily forged[19]. Each biometric system has some advantages and some disadvantages. The disadvantages of these systems make it vulnerable to use in forensic sciences. Fingerprints cannot be trusted because of their biggest disadvantage that they can be eroded, altered by surgery, and are subjected to injuries due to which finger print can be altered. The disadvantage with the voice is that it can get affected by sickness such as cold and cough. The most sensitive being the retinal scan. But cataract and astigmatism can be a down fall in case of retinal scans. Yet another type of biometric system is skin colour, but it is subjected to stability problems due to the differences that are seen with age, burns, diseases, and use of skin creams or medications.

Lately, tongue print is increasing a great deal force as a significant apparatus in biometric verification. A study was done in which Dual tree complex wavelet transform (DT-CWT) was used to draw out some unique features of tongue (size, shape, textures). This revealed that use of the sensors and some other contributed resources in real time environment, with DT-CWT, tongue would be definitely a stepping stone in personal identification and authorization process by means of security[30]. Though the research is in budding stage the results are promising.

Future Scope of Tongue Print: Various technologies are being used to capture and analyse tongue prints including spectral analysis, Gabor filter and wavelet transformers, all these technologies yield different results. The tongue print verification procedure can be done by tongue images, which goes to pre-processing for shape analysis and texture analysis of tongue. After matching the score of shape vector and texture code, fusion and decision is taken placed for verification and matching of the tongue. Therefore, the complexity involved in capturing and analysis for achieving accurate results becomes a limitation as it requires expensive resources and expertise. Rapid advancement in technological field will overcome these limitations and enable easy use of this tongue prints in forensic odontology and therefore the future looks promising.

Conclusion

The uniqueness of tongue prints makes it a good tool for personal identification and biometric system. Dentists can adopt the technique for tongue prints on a regular basis. The lingual photographic image which taken by dentist can serve as a guide for personal identification. Tongue prints hold a promising future in this digitalized word as a tool in forensic odontology.

Ethical Clearance: Taken from institutional ethics committee.

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Impact of Covid 19 Pandemic on Tuerculosis

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Abstract

Summary: Covid19 is the most dreaded pandemic of the century causing wide spread and on the other hand Tuberculosis is an age old disease killing billions. Tuberculosis is an air borne infection while Covid spreads via droplet spread and through fomite as well. Tuberculosis and complications related to it may lead to increased mortality due to poor lung compliance. Moreover Tuberculosis control programmed may get affected due to the ongoing pandemic. To decrease the adverse outcome of Covid19 on Tb strict measures like proper social distancing, hand hygiene, compliance to Anti Tubercular drugs, domiciliary Tb care and strengthening immune system may prove important.

Keywords: *Covid19, Tuberculosis, Pandemic, fomite, Social distancing.*

Introduction

The novel coronavirus-19 (nCoV-19) also known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new coronavirus recently discovered in 2019. The disease has expanded to more than 150 countries with around 45 lack cases and more than 3 lack deaths around the globe.(1)

According to WHO, "TB is a worldwide pandemic. TB has killed around 1 billion people between 1800 and 2000. It attacks the human body more slowly than viral diseases like flu or COVID-19. It kills around 4000 thousand people every day in under developed and developing countries."

In this article we would like to discuss the possible impact of Tuberculosis during the Covid 19 pandemic and vice a versa and an observation on what Measures are being taken by a Rural Hospital in Central India for Control of Covid19 and Tuberculosis.

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Pathogenesis of Covid19: Virus Structure: Coronavirus virus has a simple structure, it is a round and pleiomorphic, 80 to 120 nm in diameter in size.(2) It is club-shape with spike projections emanating from the surface.Nucleocapsid is in the envelope containing positive-strand RNA. There are four main structural proteins in coronavirus particles. These are the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins.(3) Spike protein with ACEII receptors helps in adhesion to the target cell.

COVID-19 infection induces IgG antibodies against N protein that can be detected by serum as early as day 4 after the onset of disease and with most patients seroconverting by day 14. Laboratory evidence of clinical patients showed that a specific T-cell response against SARS-CoV-2 is important for the recognition and killing of infected cells, particularly in the lungs of infected individuals.(4) T cell response is also seen in patients of Tuberculosis.(5) BCG vaccine (vaccine given to prevent complication of Tb) is predicted to protect from Covid infection. This could be because of "off target effects" of and increases the ability of immune system to fight infections other than Tb. Though further studies are being done to prove such claims. The virus binds to carbohydrates and hinds behind it (Carbohydrate Camouflage) makes it difficult for body's immune system to detect it. Moreover it has a very high secondary attack rate compared to SARS (SARS Cov 1) and MERS, which makes it more fatal.

Spread of Covid 19: Covid spreads via droplet infection when a person is in in close contact (within 1 meter) with patient having respiratory symptoms (e.g., coughing or sneezing) and the respiratory droplets can infect the exposed mucosae (mouth and nose) or conjunctiva (eyes). Transmission also occur through fomites. The virus can spread by direct contact with infected people and indirect contact with surfaces and objects[1]. Transplacental and feco-oral spread are also reported.

Spread of Tuberculosis: Airborne spread from one person to another. The TB bacteria are suspended in the air when a person with TB coughs. People nearby may breathe in these bacteria and become infected.[6] Unlike Covid19 TB is NOT spread by shaking hand, touching bed linens or toilet seats. Reactivation is common in Tb which has not been reported in Covid19.

Comorbidities, like hypertension, diabetes and coronary heart disease, have shown poor outcomes.[7] It is not clear whether having underlying lung disease like TB, will increase the severity of COVID-19. Both COVID-19 and TB cause respiratory symptoms – cough, fever and shortness of breath. One of the biggest differences is the progression of onset of symptoms. TB symptoms do notoccur immediately, butdevelop over a period of weeks, unlike COVID-19, where symptoms can occur within a few days.

Possible Impact on Tuberculosis in Covid19 Pandemic: The majority of TB hospitals in China have been converted to COVID-19 hospitals because of their roles in communicable disease. Likewise India could face a similar problem of lack of staff for management of Tb as most of the Pulmonary Physicians, technician and other staff are shifted towards Covid19 management and screening. Due to lockdown the patients might not be able to visit hospitals for treatment or follow up. This might lead to delay in diagnosis and initiation of treatment. Lack of availability of Anti Tubercular drugs due to lock down. Active Tb cases might be at increased risk for Covid19 due to poor lung functions, or patient of Tb with HIV the severity and mortality of Covid might be higher. Covid patient might develop Tb due to reactivation due to temporary weakened immune system due to Covid or corticosteroid therapy, few such cases were reported during the SARS outbreak. Patients with post tubercular complications like destroyed lung, bronchiectasis, and extensive fibrosis might also show increased complications and higher mortality due to poor lung compliance. This may lead to poor outcome and increased overall mortality in the ongoing Pandemic. If the situations arises in a country of high Tb prevalence like ours where the Covid cases goes very high, then the Tb patients might not get ventilators or ICU support due to poor outcome of these patients.

These conditions may lead to increased neglect of the tubercular patients moreover the relative/caretaker of Tb patients are at higher risk for Covid19 due to frequent hospital visit.

Due to Covid19 pandemic the Tb eradication program might take a hit back. There can be chances of increase in Tb spread during or after the Covid pandemic. Due to above reasons WHO on 20th March 2020 has stated- "COVID-19: Considerations for tuberculosis (TB) care services-As the world comes together to tackle the COVID-19 pandemic, it is important to ensure that essential services and operations for dealing with long-standing health problems continue to protect the lives of people with TB and other diseases or health conditions. Health services, including national programmes to combat TB, need to be actively engaged in ensuring an effective and rapid response to COVID-19 while ensuring that TB services are maintained".

Measures to be taken by people with TB to reduce their risk for COVID-19: Social distancing with "reverse-quarantine": Remain at home and avoid contact with people as much as possible. Wash hands frequently with soap and water. Avoid touching face, nose, eyes with unwashed hands. Avoid close contact with those who are unwell. Strictly adhere to your TB treatment. Avoid hospital visits as much as possible and keep in touch with your doctor/nurse/health facility by phone. Strengthening of the domiciliary care for Tb should be done. Admissions of Tb patients and management of complications should be done as per protocol. Use masks, and take extra caution to maintain hygiene such as - disinfection of hands, used surfaces, proper disposal of used tissues, etc.

Measures taken in a Hospital in Central Rural India to fight Covid19 and Tuberculosis: Measures taken to control Covid19: A separate section of the Hospital has been assigned where suspected patients are admitted. The block consist of a separate ICU with ventilators, separate ward with designated rooms for dawning and dwarfing of PPE (Personal Protective Equipment) and designated staff. The entry and exit

of the block is kept separate from the Hospital's entry. Personal Protective Equipments including N-95 mask, gowns, helmets etc is being provided to the staff present in the block. Patients who falls under following criteria- 1. All asymptomatic patients with history of foreign travel 2. All contacts of laboratory confirmed cases 3. Health care workers managing respiratory distress/ Severe Acute Respiratory Illness should if they become symptomatic 4. All Hospitalized patients with Severe Acute Respiratory Illness or previously hospitalized patients showing signs of atypical pneumonia and 5. Patients with flu like symptoms arriving from districts where cases of Covid19 are reported. Those patient's are being admitted and oropharyngeal and nasal swabs are been send for testing.

For the screening purpose a separate OPD(Out Patient Department/Unit) has been set up, all the patients as well the at tenders visiting the hospital are being screened before entering the wards and OPDs and their temperature is being measured by infrared (gun) thermometers. All the suspects are advised either home quarantine or hospital admission accordingly. Multiple hand washing units with washbasins and soaps have been set up and hand washing is mandatory for everyone entering the Hospital premises. The use of masks and hand hygiene is being promoted extensively. Assemblage of people is being discouraged. Limited number of people are being allowed to enter the hospital. Barricading has being done to avoid gathering of people. A separate screening unit has been set up in front of the Emergency (casualty) department for proper screening triage and management of the patients.

Measures taken to control Tuberculosis: Tuberculosis treatment and management of complications due to tuberculosis is practiced according to the RNTCP (Revised National Tuberculosis Control Programme) according to Government of India guidelines. Patients of Tuberculosis are being admitted in the TB Isolation ward with designated staff. Gloves and masks has been provided to the staff. Patients of Multi Drug Resistant tuberculosis and tubercular patients with comorbidities like Diabetes Mellitus, Hypertension etc. are given special attention and nutritional care. The visit of attendants and relatives have been restricted or allowed for limited time with due permission. The supply of Anti Tubercular Drugs is being maintained. Hospital's DMC (Designated Microscopy Center) Laboratory is kept functional for diagnosis and management of Tuberculosis patients. Domiciliary Tuberculosis treatment is being encouraged. Those patients which are unable to visit hospital due to lockdown or restricted transportations are being closely monitored and managed telephonically. The use of mask and repeated hand washing with soap or alcohol based sanitizers is advocated to the patients and the relatives.

On the contrary we have seen a sharp fall in Tb cases in our hospital in last 2.5 months. The number of Tb have fallen less than half in month of March and April. The possible reasons could be – As the Covid19 cases spread from China to other parts of the world in later half of February and March, it made big news on media and social media and other platforms as well. This lead to the people becoming more aware regarding the coughing and hand hygiene. Practices like splitting/coughing in the public have reduced significantly. Social Distancing could play a major factor in decreasing the spread of Tb. Pan India lockdown might also have played a major role in breaking the chain of Tb transmission. Lack of transport could also be a reason. But to prove this extensive research needs to be undertaken on this with more data collection. Anything to comment regarding this would be too early.

Conclusion

Both Covid-19 and Tb are global pandemics, while one is a newer disease the other is centuries old. Both disease spread can be halted by proper cough hygiene, hand wash and proper isolation. Breaking the chain of transmission is important to stop the spread of both the diseases. Mortality related to Covid19 in Tuberculosis may be more as compared to normal individual. Domiciliary care in Tb may prove beneficial in management of Tuberculosis. Strengthened immune system and absence of co-morbid conditions like diabetes, hypertension leads to lesser complications and mortality. The factors like social distancing, hand hygiene,cough hygiene etc. which break the chain of transmission for Covid19, those factors might as well reduce Tuberculosis in the country but this needs further research and time.

Ethical Clearance: Taken from institutional ethics committee.

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Case Report on Acute Lymphatic Leukemia

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Abstract

Background: Leukemia are the most common cancer in children, the malignant neoplasms are the cancer of the blood or bone marrow produce blood cells. Leukemia can happens when there is a problem with the production of blood cells. About 30 to 40 percentage of childhood malignancies include leukemia. There is higher proportion of T- cells. Linkage. Acute lymphocytic leukemia ALL.

Case Presentation: The female patient 6yr old who was apparently admitted in AVBRH on date 16/09/2019. Patient has fever since 1 month of continuous fever spike with high grade fever, swelling over neck since one month and pain in the leg. Patient was diagnosed with Acute lymphatic leukemia one year back, was brought here with complaint of one complaint of one month of continuous fever, they checked for the complaint in two hospitals of Yawatmal, they referred to AVBRH for further management.

Investigations: Complete blood count, history and physical examination, complete blood count and differential count, peripheral smear examination and platelets count, bone marrow aspiration and Patient doesn't have surgical history. Patient was treated with tab prednisolone, Induction therapy,chemotherapy Nursing management: patient care was given according to patient complaint, Keep patient safe from falls at risk of weakness or paralysis. Provide pain management, protection from infection, check lab reports,

Conclusion: Patient was admitted to hospital for further treatment of acute lymphatic leukemia and he was admitted in AVBRH and got treatment and now the patient condition is satisfactory.

Keywords: *Acutelymphatic leukemia, ALL.*

Introduction

Leukemia are the most common cancer in children, the malignant neoplasma are the cancer of the blood or bone marrow produce blood cells. Leukemia can happens whenthere is a problem with the production of blood cells. Leukemia is one of the blood cancers which affect WBC of body. WBC are they playing a major role in our body like they acts as fighting mechanism and help the body from harmful foreign bodies.[1]

But in case of leukemia there will be the fighting mechanism are disturbed because the white blood cells it changes their ability to fight with infection and it changes into cancerous cells form in spongy tissues and bones.[2]

Incidence: About 30 to 40 percentage of childhood malignancies include leukemia. There is higher proportion of T- cells. Linkage.[3] Acutelymphatic Leukemia in children aged between 0 to 4 year with 7.55 and 3.32 cases per 100,000 patients in year for male and female child respectively[4,5].

Objective:

- To know general idea regarding disease condition
- To explore knowledge regarding surgical, medical and nursing management.

Present history: The female patient 6yr old who was admitted in AVBRH on date 16/09/2019 and she is known case of Acute lymphatic leukemia. Patient had

fever since 1 month of continuous fever spike with high grade fever, swelling over neck since one month and pain in the leg.

Past history: Patient was diagnosed with Acute lymphatic leukemia one year back, was brought here with complaint of one complaint of one month of continuous fever,they checked for the complaint in two hospitals of Yawatmal, they referred to AVBRH for further management

Birth History

Pre-Natal History

Nature of pregnancy- Normal

Nature of marriage – non consanguineous

History of drug and tobacco- Not present

Exposure to radiation-Not present

Tetanus toxoid vaccines - taken

Natal history

Type of delivery- normal vaginal delivery

Place of delivery- Home delivery

Birth Weight- 3kg

Post Natal History: A newborn baby born immediately and healthy. There was no complication and infection in both the child and the mother

Surgical history: There is no surgical management of acute lymphatic leukemia. In my patient doesn't have surgical history

Clinical findings: Fever, generalized body pain and headache was present

Investigations

Blood tests

- Total WBC Count was increased 11330/cu.mm
- Platelet count 0.25 lac/cm decreased
- Total RBC count 2.18 million decreased

Physical examination

Temperature**-100 0 C**

Pulse – 86beats/min

Respiration Rate **– 22breaths/min**

Bone marrow aspiration: Use to detect leukemia and the responses of treatment, reports not available

Management: Multidisciplinary health care team are involved in care of patient with Acute lymphatic leukemia

Patient complaints like:

- Pain in upper and lower limb
- Swelling
- Fever
- Headache
- Generalized body ache

The Treatment for Acute lymphatic leukemia patients is divided into four stages:

Induction Therapy: The goal of this phase is to eradicate leukemia such that at the end of this phase is L5 percentage leukemia blasts in the bone induction therapy with the regimen marrow induction therapy with the regimen

Chemotherapy: The aim of the chemotherapy to eradicate the malignant cell and restore the normal bonemarrow function

Maintenance Therapy: It is given for 2.5 to 3 yr with mercaptopurine and MTY method of upper recommended dose

Reinforcement therapy: It is provided to vincristine and prednisone every four week

CNS Prophylaxis: In case of CNS involvement triple therapy i.e. Methotrexate, hydrocortisone cytosine are administered once a week during induction and every 8 week for 2 yr

Supportive and symptomatic management

To prevent the complication like

- Infection
- Bleeding
- Anemia
- Pain

Intravenous Antibiotic therapy blood and platelets transfusion,bone marrow transplantation

- Multidisciplinary health care team
- Pediatrician

Prescription of medications

Tab. Prednisolone -40 mg- once in a day orally

Syrup Gelusil – 5ml/TDS

Nurses Responsibility

- Monitored vital signs closely
- Assessed the condition of patient location, onset and severity of pain
- Evaluate the therapy responses
- Monitored the weight of the patient
- Assessed motor strength
- Checked lab reports
- The platelets counts are low child was observed for hemorrhage
- Maintained oral hygiene
- Observed the episodes of convulsions
- Assessed in bone marrow aspiration
- Assessed radiation therapy

Dietician:

- Provide balanced meal
- Low fat dairy products they prescribe the patient
- Protein rich food
- Whole grains and legumes

Discussion

Case of female patient 6yr old who was apparently admitted in AVBRH on date 16/09/2019. Patient has fever since 1 month of continuous fever spike with high grade fever, swelling over neck since one month and pain in the leg . Patient was diagnosed with Acute lymphatic leukemia one year back, was brought here with complaint of one complaint of one month of continuous fever,they checked for the complaint in two hospitals of Yawatmal, they referred to AVBRH for further management there clinical signs of patient is fever, generalized body pain and headache was present undergone the diagnostic evaluation are complete blood count, history and physical examination, complete blood count and differential count, peripheral smear examination and platelets count, bone marrow aspiration . Patient doesn't have no present surgical history. Patient was treated with tab prednisolone, Induction therapy,chemotherapy Nursing management for acute lymphatic leukemia Monitor vital signs closely, Keep patient safe from falls at risk of weakness or paralysis. Provide pain management, protection from infection,check lab reports, observed the side effects of chemotherapy drugs, maintained oral hygiene, observed for convulsions, associated with bone marrow aspiration. Patient was admitted to hospital for further treatment of acute lymphatic leukemia and she was admitted in AVBRH and got treatment and now the patient condition is satisfactory.

The condition of patient was improved after giving the care by various multidisciplinary health care team and supportive management . Pain is slightly reduced as per evidence by patient verbalization, patients nutritional pattern is improved as per evidenced by her health condition.

The patienth as reduced the swelling, pain over leg and fever was reduced as giving all the management the symptoms of the patient reduced as verbalize by the patient and their parents. Now the patient condition is satisfactory.

A study by Deshpande et al reflects on biomarkers of human cancers 6. Khatib et al have focused on therapeutic options for cachexia in cancer patients.

Conclusions

If we provide proper care with the help of various multidisciplinary health team and there supportive management,it helps to improve the condition of the patient .Now the patient is conscious, alert improved his condition

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Case Report on Thalassemia in Children

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Abstract

Introduction: Thalassemia which is also called as Cooley's anemia or Mediterranean anemia is a group of hereditary hemolytic anemia characterized by reduction in the synthesis of hemoglobin. The word 'thalassemia' is derived from the Greek word 'Thalassa' which means the great sea.

Clinical Findings: Abdominal pain, fever (Temperature - 101°F), Anemia (Hb – 6.4gm%).

Diagnostic Evaluation: Blood test: Hb – 6.4gm%, Total RBC count – 2.3 millions/cu mm, RDW – 18.2%, HCT – 20.2%, Total WBC count – 3200/cu mm, Monocytes – 02%, Granulocytes – 20%, Lymphocytes – 77%, AST(SGOT) – 112 U/L.

Peripheral Smear: RBC – mild hypochromic with mild cytosis which show few microcytic and mildly hypochromic. Platelets – Reduced on smear, APC – 62,000 cells.

Ultrasonography: Splenomegaly.

Therapeutic Intervention: Blood transfusion, Inj. Cefotaxime 750mg IV x BD, Syr. Azee 4ml x OD, Tab. Folic Acid 5mg x OD, Tab. Udiliv 150mg x BD, Cap. Hydra 500mg x OD, Tab. Prednisolone 10mg x BD.

Outcome: After treatment, the child show improvement. His fever and abdominal pain were relieved and his Hb% increased from 6.4gm% to 11gm% after blood transfusion.

Conclusion: My patient was admitted to Pediatric Ward No – 14, AVBRH with a known case of Thalassemia and he had complaint of fever and abdominal pain. After getting appropriate treatment his condition was improved.

Keywords: *Thalassemia, Cooley's anemia, Mediterranean anemia, hereditary, hepatosplenomegaly.*

Introduction

Thalassemia produces hypochromic microcytic anemia due to defective hemoglobinization of RBCs, hemolysis and ineffective erythropoiesis. It can be considered as hemolytic and hypo - proliferative anemia related to abnormal hemoglobin.

It was first noticed in patients, originating from the littoral countries of the Mediterranean Sea. At present the disease has been found in several countries all over the world. The prevalence of the disease in India is high among Gujaratis, Sindhis and Punjabis. Millions of people are found to be carriers of thalassemia gene and every year thousands of thalassemic children are born in our country.[1]

Progressive pallor, anemia, fatigue or weakness, jaundice, hepatosplenomegaly, recurrent respiratory infection, failure to thrive, facial bone deformities are common symptoms of thalassemia.[2] Blood examination, Bone marrow study, Osmotic fragility test, radiological screening are common investigations done in thalassemia.[1]

Mild thalassemia might not be needed to be

treated but severe thalassemia required regular blood transfusions along with symptomatic treatment.[2]

Thalassemia is classified into three main types, the classification depends on the number of gene mutation and the part of hemoglobin molecules which are affected either alpha or beta.[3] The different types are: *Thalassemia major* – Here thalassemia genes (beta) are inherited from both the parents and it is associated with homozygous state. Synthesis of beta chain is markedly reduced. *Thalassemia intermedia*: It is a chronic hemolytic anemia caused by alpha or beta chain synthesis. It is also a homozygous form. *Thalassemia minor*: It is a mild form of illness and produced by heterozygosity of either alpha or beta chain.[1]

Management of thalassemia is mostly done by Repeated blood transfusion, iron chelation therapy, splenectomy, folic acid supplementation, bone marrow transplantation, gene therapy and gene mutation, supportive management.[1]

Patient Identification: A male child of 8years from Shirajgam admitted to pediatric ward no 14, AVBRH on 23rd January 2020 with a known case of Thalassemia major. He is 25kg and his height is 126cm.

Present medical history: A male child of 8 years old was brought to AVBRH on 23rd January 2020 by his parents with a complaint of abdominal pain (left hypochondriac region) and fever and he was admitted to Pediatric ward no 14. He is a known case of Thalassemia Major and his Hemoglobin level at the time of admission was 6.4gm%. The child is weak and inactive on admission.

Past medical history: My patient was diagnosed to have thalassemia at the age of 8 months when he was admitted to hospital due to fever. Till then, he was admitted to hospital time to time for the purpose of blood transfusion.

Family history: There are four members in the family. My patient was diagnosed to have Thalassemia major and his parents were diagnosed to be carrier of thalassemia. Type of marriage of the parents is non – consanguineous marriage. All other members of the family were not having complaints in their health except for my patient who was being admitted in the hospital.

Past interventions and outcome: My patient was diagnosed with thalassemia when he was 8 months old, from that time onwards he was admitted to hospital time to time for treatment of the disease mostly blood transfusion. It was found effective as the patient does not develop complications till then.

Clinical findings: Abdominal pain, fever (Temperature - 101˚F), Anemia (Hb – 6.4gm%)

Etiology: Thalassemia occurs when there is mutation or any abnormality in anyone of the gene which take part in producing hemoglobin. When there is genetic abnormality, it can be pass – on from parents to children. When only one of the parents is a carrier, there is chance of developing a mild form of thalassemia that is thalassemia minor where symptoms might not be seen but the person will be a carrier. When both the parents are carrier, there is a great chance of developing severe form of thalassemia like thalassemia major or thalassemia intermedia.[4]

Physical examination: There is not much abnormality found in head to toe examination, the child is lean and thin and having dull look. He is weak and not so cooperative. Though it is found that the child is having splenomegaly from ultrasonography, it is not palpable.

Diagnostic assessment: Blood test: Hb – 6.4gm%, Total RBC count – 2.3 millions/cumm, RDW – 18.2%, HCT – 20.2%, Total WBC count – 3200/cumm, Monocytes – 02%, Granulocytes – 20%, Lymphocytes – 77%, AST(SGOT) – 112 U/L. **Peripheral Smear**: RBC – mild hypochromic with mild cystosis which show few microcytic and mildly hypochromic. Platelets – Reduced on smear, APC – 62,000 cells. **Ultrasonography**: Splenomegaly.

Therapeutic Intervention: Blood transfusion, Inj. Cefotaxime 750mg IV x BD, Syr. Azee 4ml x OD, Tab. Folic Acid 5mg x OD, Tab. Udiliv 150mg x BD, Cap. Hydra 500mg x OD, Tab. Prednisolone 10mg x BD.

Discussion

A male child of 8 years old from Shirajgam was admitted to pediatric ward no 14, AVBRH on 23rd January 2020 with a complaint of abdominal pain, fever and Hb% less than normal limit. He is a known case of thalassemia major which was diagnosed when he was 8 months old. As soon as he was admitted to hospital investigations were done and appropriate treatment were started. After getting treatment, he shows great

improvement and the treatment was still going on till my last date of care.

A study was done on, "A clinico - epidemiological study of thalassemia cases in India". It was done to assess the clinical presentation and managements practices in thalassemia. For the study, patients case sheets were collected between 2005 - 2014 which is 10 years. These case sheets were being examined and recorded in a specially made proforma for the study. The result from the study were - a total of 183 cases are recorded and among those, 179 (97.8%) were beta thalassemia major, 3 (1.6%) were beta thalassemia intermedia and 1 (0.6%) was beta thalassemia minor. Most of the case were diagnosed at age of 1 year, and one fourth of the case were diagnosed in first 6 months. Fever present in 34 patients (18.6%), pallor found in 179 patients (97.8%), hepatomegaly seen in 172 patients (94%) and bone deformity found in 13 patients (7.1%). One third of the under five patients were found to be underweight and more than half of the patients were found to be stunted. Mean post - transfusion value of hemoglobin after 1 year of transfusion among cases was 10±1.6g%, 51 patients (27.9%) were given desferrioxamine as iron chelation therapy and the mean age of starting this therapy was 11.1±8.2 years. In 4 cases of beta thalassemia major splenectomy was done at a mean age of 10.7±4.8 years. On treatment of thalassemia with desferrioxamine lenticular opacity was found in greater proportion. The study concluded that among thalassemic patient different kinds of complications were found so it is important to involve different specialization in care of thalassemia patient to control the problems.[5] Baliga et al studied about the Malondialdehyde Levels in Serum and Saliva of Children Affected with Sickle Cell Anemia[6]. Jaiswal et al conducted a comparative study on peripapillary retinal nerve fiber layer thickness in patients with iron-deficiency anemia to normal population[7]. Garg et al studied about Dengue serotype-specific seroprevalence among 5- to 10-year-old children in India[8]. Puri et al [9] and Uddin et al [10] analyzed on infants and young child nutrition. Taksande et al reported on 3 cases of children with rare diseases[11]. Tendolkar et al reported about physical abuse of children whereas Khatib et al reviewed the effect of electronic media on children. All these studies have some aspects related to thalassemia, anemia and other nutritional disorders.

Conclusion

Thalassemia is one of the most common case found among children, its is very important to diagnose in early stage so that the child will not develop complications from the disease. It is also very important to take preventive measures like antenatal screening and giving genetic counseling are very important. My patient show great improvement after getting the treatment and the treatment was still going on till my last date of care.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Effect of Minerals on Markers of Risk of Pre-Eclampsia in Pregnant Women: A Hospital Based Study

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Abstract

Introduction: Pregnancy specific complication characterised by hypertension, proteinuria, after 20 weeks of the gestation, and along with various signs of damage to organ systems, haemolysis, elevated levels of liver enzymes and low platelet count (HELLP syndrome) is collectively termed as eclampsia.

Method: Cross-sectional study with 3 groups of pregnant women, were taken followed by inclusion and exclusion criteria for characterisation. The study was approved by ethical board of DMIMSU. Minerals and their relationship were estimated using different method. Preeclamptic group with and without supplementation and pregnant women without preeclampsia were included in the study. Significant differences were seen in Na+, K+, Ca, Mg, Se, and Mn when compared with each group.

Result and Observation: The levels of K were increased significantly ($P < 0.001$) in pre-eclamptic group as compared to control group. There was significant change in levels of Mn when pre-eclamptic group was compared to control. Mn was also found to decrease significantly ($P < 0.001$) in cases as compared to control. However, the levels of Se, Mg, Ca and Na between cases and control were not highly significant. In supplementation and without supplementation group, Significant differences were seen in levels of Na, K, Ca and very significant difference was seen in levels of Mn. ($p<0.0001$).

Conclusion: Pre-eclamptic pregnant women have higher levels of serum potassium and our finding on Mn, an essential trace mineral, provides new insight into a potentially modifiable way to prevent preeclampsia. In multi-ethnic, predominantly urban and low-income pregnant women high level of Mn is associated with pre-eclampsia.

Keywords: *Mn, Na, K, Se, Ca and Preeclampsia.*

Introduction

Pregnancy specific complication characterised by hypertension, proteinuria, after 20 weeks of the gestation, and along with various signs of damage to organ systems, haemolysis, elevated levels of liver enzymes and low platelet count (HELLP syndrome) is collectively termed as eclampsia.[1]

Eclampsia is simply convulsions of pregnancy due to rise in blood pressure. Pre-eclampsia maybe seen before a eclampsia, condition where swelling can be seen on feet, face and urine usually consists proteins.[2]

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WHO, recognise eclampsia as major cause of fetal and maternal morbidity and mortality.[3]

Prevalence of pre-eclampsia stands around 3 to 10% in world population. Whereas factor such as obesity, diabetes, calcium deficiency advanced maternal age, oxidative stress, placental ischaemia, genetic factors and immune maladaptation have been implicated. [4] In addition, the American heart association recently added preeclampsia as a risk factor for future cardiovascular diseases in women. [5]Also, modified lipid profile has a possible role in the development of endothelial dysfunction and preeclampsia expression. Early detection of such lipid parameters will benefit pregnant patients in preeclampsia by avoiding maternal and fetal complications, which can also enhance preeclampsia management.[6] Only low dose of aspirin maybe effective in preventing the pre-eclampsia here in women at high risk.[7]

Some of the evidences from various line for examples the essential mineral manganese (Mn) and Selenium (Se) toxic heavy metals such as cadmium, lead and mercury. These minerals have opposing roles in the development of the preeclampsia.[8-10] and for early detection of PE and eclampsia serum uric acid, serum creatinine and serum urea are better diagnostic and predictive marker. With the help of these parameters most cases are detected early in the pregnancy before they can progress to eclampsia.[11] Due to limited number of studies result on epidemiologic studies on Mn have varying results. Se in pregnancy, reduce oxidative stress and also improve preeclampsia features in rats. Although need of large-scale studies in human is required.[12-17]

Aims and Objectives: The present study was undertaken in the Department of Biochemistry and Dept. of OBGY at Jawaharlal Nehru Medical College, and AVBRH (Datta Meghe Institute of Medical Sciences) Sawangi, Meghe, Wardha, in collaboration with Datta Meghe Medical College and Shalinitai Meghe Hospital and Research Centre Hingana, Nagpur, Maharashtra India with the following aims and objectives: -

Aim of Study: To estimate role of minerals, in patients with pre-eclampsia.

Objectives of study:

1. To estimate sodium, potassium, magnesium, calcium, manganese and selenium in pregnant women with pre-eclampsia and eclampsia.

2. Find out correlation of sodium, potassium, magnesium, calcium, manganese and selenium in pre-eclampsia and compare with normal pregnant women.

Material and Method

Study Area: The present study was undertaken in the Department of Biochemistry of Jawaharlal Nehru Medical College, and AVBRH (Datta Meghe Institute of Medical Sciences) Sawangi, Meghe, Wardha in collaboration with Datta Meghe Medical College, Shalinitai Meghe Hospital and Research Centre, Hingana, Nagpur, Maharashtra India.

Study Population: 250 subjects were included in the study and were grouped as under.

Group 1: 100 pre-eclampsia diagnosed subjects without any mineral supplementation.

Group 2: 50 pre-eclampsia diagnosed subjects with mineral supplementation.

Group 3: 100 Normal pregnant women.

Study Type: Cross sectional interventional study

Data collected was entered into Microsoft Excel Worksheet and statistically analysed by using SPSS (Statistical Package for Social Sciences) version 20. For quantitative data mean, standard mean, standard deviation, t-test and Karl Pearson's Coefficient of Correlation were calculated. P value < 0.05 (0.01) will be considered as statically significant (highly significant) at 95% confidence interval. The proposed study was undertaken subject to approval by Institutional Ethical Committee.

Observations and Results

Table 1. Serum minerals levels in pregnant women with preeclampsia and norm tension.

Serum minerals	Group III	Group I	'P' Value
Potassium	4.38 ± 0.80	4.95±0.99	P<0.001
Sodium	140±1.12	139±1.95	p<0.01
Calcium	7.27±1.46	7.96±0.22	P<0.05
Magnesium	1.37±0.22	1.42±0.19	P<0.05
Manganese	0.13 ±0.077	0.072±0.068	P<0.001
Selenium	62.33±9.42	58.23±8.44	P<0.0014

Table no 1 showed the levels of serum trace elements in control and preeclamptic women are seen in table 1. Analysis of trace elements found that mean values of K, Na, Ca, Mg, Mn and Se were 4.38 ± 0.80, 140±1.12, 7.27±1.46, 1.37±0.22, 0.13 ±0.077 and 62.33±9.42. in control group and 4.95±0.99, 139±1.95, 7.96±0.22, 1.42±0.19, 0.072±0.068, and 58.23±8.44 women with pre-eclampsia respectively. The levels of K were increased significantly ($P < 0.001$) in pre-eclamptic group as compared to control group.

There was significant change in levels of Mn when pre-eclamptic group was compared to control. Mn was also found to decrease significantly ($P < 0.001$) in cases as compared to control. However, the levels of Se, Mg, Ca and Na between cases and control were not highly significant.

Table 2. Serum minerals level in pregnant women with preeclampsia and eclampsia without and with supplementation.

Serum minerals	Group I	Group II	'P' Value
Potassium	4.95±0.99	4.23±0.98	P<0.001
Sodium	139±1.95	141±1.40	P<0.001
Calcium	7.96±0.22	8.53±0.21	P<0.001
Magnesium	1.42±0.19	1.39±0.21	P<0.3803
Manganese	0.072±0.068	0.131±0.058	P<0.0001
Selenium	58.23±8.44	60.48±7.63	P<0.1144

Table no 2 showed the levels of serum minerals in cases with pre- eclampsia with and without any supplementation are depicted in table 2. On the analysis of serum minerals among patients with pre-eclampsia without supplementation the levels for K, Na, Ca, Mg, Mn and Se were 4.95±0.99, 139±1.95, 7.96±0.22, 1.42±0.19, 0.072±0.068 and 58.23±8.44 respectively. And the levels for K, Na, Ca, Mg, Mn and Se in patients with supplementation were 4.23±0.98, 141±1.40, 8.53±0.21, 1.39±0.21, 0.131±0.058 and 60.48±7.63 respectively. Significant differences were seen in levels of Na, K, Ca and very significant difference was seen in levels of Mn. (p<0.0001).

Table no 3 showed the levels of serum minerals in cases of pre- eclampsia with supplementation and normal pregnant women are depicted in table 2. On the analysis of serum minerals among patients of pre-eclampsia with supplementation the concentration of K, Na, Ca, Mg, Mn and Se were 4.23±0.98, 141±1.40, 8.53±0.21, 1.39±0.21, 0.131±0.058 and 60.48±7.63. In normal

pregnant the concentration of K, Na, Ca, Mg, Mn and Se were 4.38 ±0.80, 140±1.12, 7.27±1.46, 1.37±0.22, 0.13 ±0.077 and 62.33±9.42 respectively.

Table 3. Serum minerals level in pregnant women with preeclampsia and eclampsia without and with supplementation.

Serum minerals	Group II	Group III	'P' Value
Potassium	4.23±0.98	4.38 ± 0.80	P < 0.3177
Sodium	141±1.40	140±1.12	P < 0.0001
Calcium	8.53±0.21	7.27±1.46	P < 0.0001
Magnesium	1.39±0.21	1.37±0.22	P < 0.5950
Manganese	0.131±0.058	0.13 ±0.077	P < 0.9355
Selenium	60.48±7.63	62.33±9.42	P < 0.2303

No Significant differences were seen in levels of K, Mg, Mn and Se (P < 0.3177, P < 0.5950, P < 0.9355 and P < 0.2303) respectively, but in levels of Na and Ca significant difference was seen. (p<0.0001).

Discussion

Calcium as well as magnesium are very important for metabolism at the cellular level and are mandatory for muscle contraction, also for neuronal activity making it very essential in pregnancy[18,19]. **Mitchell et. al (1990)**discovered that eclampsia was in arrears to maternal deficiency in calcium through foetal absorption and held that good results were achieved by calcium administration. He stated that the hypocalcaemia of eclamptic patients led to an accumulation of fat in the liver and therefore impairment of its function resulting into sudden attacks[20]. **Knight and Keith (1992)** studied calcium supplementation on normotensive and hypertensive pregnant women. A significant inverse relationship was observed between dietary calcium intake and blood pressure[21]. **Jama (1997)** observed that pre-eclampsia still remains a dangerous entity with an elusive pathogenesis and causes significant morbidity in both, the mother and foetus[22]. It has been found that women on a high calcium diet have a low incidence of pre-eclampsia. It is also known that pre-eclamptic women have reduced urinary excretion of calcium, so there is an inverse relationship between the dietary consumption of calcium and events of eclampsia. **Walker (2000)** stated that calcium supplements lower the risk of pre-eclampsia and eclampsia because calcium is required for maintaining tone of muscles[23].

Present study finding showed no change in serum sodium levels in preeclamptic as compared to normotensive pregnancies. It also showed that serum magnesium level was significantly depleted in mothers with pre-eclampsia as compared to healthy control group. This finding suggests that hypomagnesemia may be one of the aetiologies of preeclampsia.

Serum Potassium levels were highly significantly increased in preeclamptic pregnant women as compared to normal. These findings are in close conformity with the findings of **Agyei Frampong et al**[24] who demonstrated that there was only slight correlation between the plasma sodium levels and diastolic blood pressure with no significant difference between normotensive and pre-eclamptic subjects. Serum potassium levels however, showed a highly significant increase in pre-eclamptic women. These results were consistent with earlier study by **Handwerker et al**[25] where the study group had significantly higher levels of serum potassium than control group. Normal pregnancy has been associated with a decrease in mean serum potassium possibly related to a physiologic increase in Na+/K+ adenosine triphosphatase activity in the cell membranes, causing a shift of K+ into cells with extrusion of Na+.

Seydoux J et al. study concludes that serummagnesium leveldecreases with progress in pregnancy.[26] These results are were consistent with earlier study by **Seydouse et al, Handworker et al and Zhao F**[27-28] where the study group has lower serum magnesium than control group.Our study on preeclamptic women with and without supplementation shows remarkable differences in calcium levels p<0.001.

Thus, our findings of higher extracellular K+ levels in toxaemia may be of interest and our study also concludes that minerals supplementation also decreases K+ levels in preeclamptic women after supplementation.

Furthermore, in a prospective cohort study of 620 pregnant women in Iran, **Goodarzi Khoigani et al**[29] reported that Mn intake in the third trimester, estimated using 48-hour dietary recalls, was significantly lower among women who developed preeclampsia compared with those who did not. In contrast, in a case–control study of 396 participants (31 preeclampsia cases) in Iran, **Vigeh et al**[30] reported that Mn concentration in umbilical cord blood, but not maternal blood, was associated with higher odds of preeclampsia after adjustment.

Conclusion

In the light of the above interpretations it can be determined that pre-eclamptic pregnant women have **higher levels of serum potassium**, **decreased level of serum calcium** and **magnesium** and **no significant difference in sodium** levels as compared to normotensive pregnant women in their pregnancy during third trimester.

Our finding on Mn, an essential trace mineral, provides new insight into a potentially modifiable way to prevent preeclampsia. In multi-ethnic, predominantly urban and low-income pregnant women high level of **Mn is associated with pre-eclampsia**. Our study also concluded thatserum **Se have no contribution in pre-eclampsia** and eclampsia.

Moreover, study of serum lipid profile in pre-eclampsia showed by **Shreya Yadav et. al**.[6]with our study helpful to early detection and used in better management of preeclampsia which is important to improve the maternal and fetal outcome. Further research with information on characteristic features of preeclampsia (such as severity of blood pressure and proteinuria) and clinical subtypes of preeclampsia (e.g. early-onset versus late-onset) is needed to elucidate the potential heterogeneity of mechanisms that are responsible for the effect of trace minerals.

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

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The Effects of Multi Vitamins Supplementation in the Prevention of Preeclampsia

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Abstract

Introduction: Preeclampsia is a multifactorial pregnancy-specific disorder which is symbolized by the development of hypertension and proteinuria after 20 weeks of gestation. With the greatest morbidity and mortality, preeclampsia affects 5% to 7% of all pregnant women.

Material And Method: 390 subjects were included in the study and were grouped.

Results: Mean maternal age of cases in years was observed as 26.68±4.2, mean gestational age in weeks was 36.2. Systolic and diastolic BP in mmHg was 159.4 and 104.8 respectively. Mean weight was observed as 72.6 Kg and Height was 158.4 cm. There was statically significant correlation observed between vitamins in Group I and Group II, but Vitamin E and Vit B 12 does not show any significant correlation between them. No Stastical significance were found in the vitamins level in Group III and Group II. Statistically significant difference was observed between group I and Group III, for vitamin B12, vitamin D, vitamin C and vitamin A. No statistical significance difference was observed in vitamin E levels.

Conclusion: The intended intervention of Vitamins has a protective effect against recurrent preeclampsia. Vitamins supplementation therapy in pregnancy could help in reducing the incidence of gestational hypertension/preeclampsia.

Keywords: *Pre-eclampsia, eclampsia, Vit B12, VitA, Vit C, Vit D and Vit E.*

Introduction

Eclampsia is a serious disorder affecting women during pregnancy. Early symptoms are known as pre-eclampsia, which further develop to eclampsia and can land up in complications during pregnancy. Thus, eclampsia requires immediate medical treatment. Most cases are detected early in the pregnancy before developing eclampsia.[1]

Preeclampsia is a multifactorial pregnancy-specific disorder which is symbolized by the development of hypertension and proteinuria after 20 weeks of gestation.[2] Hypertensive disorders is a common complication of pregnancy that put women and their foetuses at disproportionate risk for further complications, as well as life-long sequelae.[3]

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PE is a very notable disease which complicates from 2% to 5% of pregnancies in Europe and America and can reach up to 10% of pregnancies in developing countries, mainly due to the lack of incompetence of emergency care. [3] According to the WHO its incidence is seven times higher in developing countries (2.8% of live births) than in developed countries (0.4%).[4] PE is associated with an increased risk of placental abruption, preterm birth, foetal intrauterine growth restriction (IUGR), acute renal failure, cerebrovascular and cardiovascular complications, disseminated intravascular coagulation, and maternal death.[5]

With the greatest morbidity and mortality, preeclampsia affects 5% to 7% of all pregnant women but it is responsible for over 70 000 maternal deaths and 500 000 fetal deaths worldwide every year. [6]

Preeclampsia progresses in 2 stages:

A. Abnormal placentation early in the first trimester followed by

B. Maternal syndrome in the subsequentially second and third trimesters characterized by an excess of antiangiogenic factors.[7]

The end stage of pre-eclampsia is eclampsia which is defined by generalized seizures, with or without raised blood pressure and proteinuria, occurring during or after pregnancy with or without other identifiable cause. The cause is usually multifactorial including cerebral vasoconstriction, ischemia, vasogenicedema, or other pathology. In the first stage, abnormal placentation and deficient spiral artery remodeling leads to placental ischemia. Placental ischemia in turn influence on the clinical manifestations of preeclampsia, including the development of hypertension and proteinuria. Although eclampsia occurs mostly in women with severe pre-eclampsia, there is no convincing test for predicting its onset.[8-11]

Aim: Correlation of Vitamins levels before and after supplementation in pre-eclampsia

Objective: To determine serum vitamin A, C, D, E, B12 levels in pregnant women attending maternity clinic at AVBRH Wardha and SMHRC Nagpur.

Material and Method

The present Study was carried out in the Department of Biochemistry and Dept. of OBGY at Datta Meghe Medical College, Shalinitai Meghe Hospital & Research Centre, Nagpur in collaboration with Jawaharlal Nehru Medical College and AVBRH (Datta Meghe Institute of Medical Sciences), Sawangi (Meghe) Wardha Maharashtra.

Study Population: 390 subjects were included in the study and were grouped as under.

Group 1: 90 pre-eclampsia diagnosed subjects without any supplementation.

Group 2: 90 pre-eclampsia diagnosed subjects with supplementation.

Group 3: 210 Normal pregnant women.

Study Type: Cross sectional interventional study

Statistical Analysis: All estimated results were expressed as mean±SD. Mean values will be assessed for significance by unpaired student –t test. A statistical analysis will be performed using the Statistical Package for the Social Science program (SPSS, 24.0). Frequencies and percentages will be used for the categorical measures. Probability values $p < 0.05$ will be considered statistically significant.

Observation and Results

Table 1: Baseline Characteristics

Variables	Cases (n=90)
Maternal Age (years)	26.68±4.2
Gestational Age (weeks)	36.2
Systolic BP(mmHg)	159.4
Diastolic BP (mmHg)	104.8
Weight(Kg)	72.6
Height (cm)	158.4

Table no. 1 shows that mean maternal age of cases in years was observed as 26.68±4.2, mean gestational age in weeks was 36.2. Systolic and diastolic BP in mmHg was 159.4 and 104.8 respectively. Mean weight was observed as 72.6 Kg and Height was 158.4 cm.

Table 2: Levels of Vitamins before and After Supplementation.

Vitamins	Group I Mean±SD (n=90)	Group II Mean±SD (n=90)	Group III Mean±SD (n=210)
Vitamin B12	149±40.09	195.67±104.34	264.4±269.57
Vitamin D	10.73±3.46	12.43±4.43	14.67±17.82
Vitamin A	0.325±0.143	0.63±1.10	0.92±2.144
Vitamin E	5.44±7.725	5.7±7.80	6.49±9.13
Vitamin C	0.38±0.172	0.53±0.531	0.77±1.63

Table no 2 shows the mean values in Group I, Group II and Group III of Vit B 12, Vit D, Vit A, Vit E and Vit C were (149±40.09, 195.67±104.34, 264.4±269.57), (10.73±3.46, 12.43±4.43, 14.67±17.82), (0.325±0.143, 0.63±1.10, 0.92±2.144), (5.44±7.725, 5.7±7.80, 6.49±9.13), (0.38±0.172, 0.53±0.531, 0.77±1.63) respectively.

Table 3: Correlation of Vitamins levels in pre-eclamptic and after supplementation.

Vitamins	Group I	Group II	t-value	p-value
Vitamin B12	149±40.09	195.67±104.34	-3.93	0.50
Vitamin D	10.73±3.46	12.43±4.43	-2.84	0.0024
Vitamin A	0.325±0.143	0.63±1.10	-2.73	0.0034
Vitamin E	5.44±7.725	5.7±7.80	-0.27	0.390
Vitamin C	0.38±0.172	0.53±0.531	-2.81	0.002

P value <0.05 Significant

Table no 3 shows there was statically significant correlation observed between vitamins in Group I and Group II, but Vitamin E and Vit B 12 does not show any significant correlation between them.

Table 4: Correlation of Vitamins levels in normal with supplementation.

Vitamins	Group III	Group II	t-value	p-value
Vitamin B12	264.4±269.57	195.67±104.34	-2.343	0.0198
Vitamin D	14.67±17.82	12.43±4.43	-1.176	0.2405
Vitamin A	0.92±2.144	0.63±1.10	-1.780	0.0716
Vitamin E	6.49±9.13	5.7±7.80	-0.716	0.4744
Vitamin C	0.77±1.63	0.53±0.531	-1.365	0.1733

P value <0.05 Significant

Table no 4 shows no Stastical significance were found in the vitamins level in Group III and Group II.

Table 5: Correlation of Vitamins levels inpre-eclamptic with normal.

Vitamins	Group I	Group III	t-value	p-value
Vitamin B12	149±40.09	264.4±269.57	-4.038	0.001
Vitamin D	10.73±3.46	14.67±17.82	-2.079	0.0385
Vitamin A	0.325±0.143	0.92±2.144	-2.628	0.0090
Vitamin E	5.44±7.725	6.49±9.13	-0.954	0.3408
Vitamin C	0.38±0.172	0.77±1.63	-2.262	0.0244

P value <0.05 Significant

Above table no 5 shows that statistically significant difference was observed between group I and Group III, for vitamin B12, vitamin D, vitamin C and vitamin A. No statistical significance difference was observed in vitamin E levels.

Discussion

The prevalence of PE has been reported to be 5-7% of all pregnancies, mainly due to the lack of or inadequacy of emergency care. PE is associated with an increased risk of placental abruption, preterm birth, foetal intrauterine growth restriction (IUGR), acute renal failure, cerebrovascular and cardiovascular complications, disseminated intravascular coagulation, and maternal death. PE is a leading cause of maternal mortality it affects both mother's and foetuses health during pregnancy.[12-16] Epidemiological research suggested that preeclampsia has a multifaceted etiology that includes an immunogenetic component [17].Established risk factors include nulliparity, family history of preeclampsia–eclampsia, and preeclampsia in a previous pregnancy, obesity, increased insulin resistance, hyperlipidemia, multiple gestation, molar pregnancy. Protective factors include a prior completed pregnancy and a terminated pregnancy for women conceiving again with the same partner [18-21], therefore, PE prevention is highly crucial. Several factors may increase homocysteine levels in women with preeclampsia. Metabolism in the kidney is the major route by which homocysteine is cleared from plasma and this route of elimination may be affected by pre eclamptic changes in the kidney.[22] Similarly, in a systematic review by Mignini et al., folic acid and vitamin B12 concentrations were lower in pre eclamptic women when compared with those of normotensive women.[23] From early pregnancy placenta assumes a state of oxidative stress arising from increased placental mitochondrial activity and leads to the production of reactive oxygen species (ROS), mainly superoxide anion.[24,25] In preeclampsia, an increased level of oxidative stress is seen. The source had been allocated to the placenta, where free radical synthesis occurs, with maternal leukocytes and the maternal endothelium likely contributors. [26,27] The superoxide–producing enzyme NADPH oxidase, shown to be present in placental trophoblast. Women with early onset of preeclampsia have been found to have increased superoxide production compared with those with late-onset disease.[21] However, clinical trials of antioxidant therapy with vitamins C (1000 mg) and E (400 IU) have been dismayed and were associated with an increased number of low–birth weight babies in the treatment.[28] So in this study we are trying to see the role of Vitamin in PE, as preeclampsia remains a challenge to diagnose. Therefore, the ability to provide an early diagnosis of PE is vital for health of both mother and fetus. A study conducted by **A. P. Mackay, C. J. Berg et. Al**[6] studied that Mortality from preeclampsia and eclampsia increased with increasing maternal age. A study conducted by **Azami M, Azadi T, Farhang S et. al**[7] studied that the incidence of preeclampsia in group A was significantly lower than the control group (p=0.03), while there was no significant difference between group B and controls (p=0.50), as well as groups A and B (p=0.063). The incidence of neonatal complications in the group A was significantly lower than the control group (p=0.01), while there was no significant difference between group B and control (p=0.48). The present study concluded that multimineral and vitamin D during pregnancy can be a low-cost and affordable way to reduce the incidence of preeclampsia in women who are at high risk of preeclampsia. A study done by **Rudra P, Basak S, Patil D, Latoo MY**[8] concluded that out of 8,524 deliveries, 104 cases of pre-eclampsia were documented giving an overall prevalence of 1.2%. The prevalence in 2009, 2010, and 2011 was 0.7%, 1.2%, and 1.5%, respectively. Fifty-nine case notes were available for analysis (56.7% retrieval). The mean age of the patients was 27 ± 4.9 and majority; 25 (42.4%) were in the age group 25-29 years. The mean parity was 1.1 ± 1.4 and 26 (44.1%) were nulliparous. 11 (18.6%) had family history of hypertension, 2 (3.4%) had personal history of hypertension, and 2 (3.4%) had a personal history of pre-eclampsia. The slight increase in the prevalence rate over the years underscores the need for close surveillance. A study done in 2016 by **Hariharan N, Shoemaker A, Wagner S**[9] concluded that preeclampsia remains a complex disease process with multiple components intertwined in its development including angiogenic and endothelial dysfunction. A study done by **Mayrink J, Costa ML, Cecatti JG**[10] figured that preeclampsia is still one of the main causes of death and severe maternal morbidity. A case control study was carried out in the Neonatal Division, JNMCH, in 2018 by **Prasad D, Smita KS, Nisha S**.[19] In the study 208 mothers were divided into two groups with newborns birth weight <2.5 kg (cases) and 2.5 kg or more (controls). Maternal vitamin D levels were estimated. Maternal vitamin D statuses of both groups were compared. Study done by **Raijmakers MT, Dechend R, Poston L**[20] concluded that Preeclampsia remains a frequent

and potentially dangerous complication of pregnancy. A study done by **Bastos Maia S, Rolland Souza AS et. Al**[21] concluded that adequate levels of vitamin A during pregnancy are of critical importance for the health of pregnant women and their fetuses. Study done by **Mujawar SA, Patil VW, Daver RG**[22] evaluated the occurrence of association between homocysteine, folic acid and vitamin B_{12} in patients with preeclampsia. A study conducted by **Mignini L, Latthe P et. al**[23] concluded that Homocysteine concentrations are slightly increased in normotensive pregnancies that later develop preeclampsia and are considerably increased once preeclampsia is established, because of a lack of consistency in data, dose-response relationship, and biologic plausibility, the observed association cannot be considered causal from the current literature.

Conclusion

Our study findings suggest that levels of Vitamins are altered in Pre-eclampsia patients. Maternal vitamins deficiency can lead to serious complications. Supplementation with vitamin C and E may be helpful in reducing the risk of preeclampsia. The intended intervention of Vitamins has a protective effect against recurrent preeclampsia. Vitamins supplementation therapy in pregnancy could help in reducing the incidence of gestational hypertension/preeclampsia.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Prevalence of Depression in College Students During Covid-19 Pandemic

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Abstract

The outbreak of Covid-19 has caused major amount of suffering around the globe. Along with the physical symptoms in affected cases, it has severely spoilt the mental health of general public. College students too are facing multiple challenges in the stir of this COVID-19 outbreak. The present research is an attempt to assess the prevalence of depression among students residing in Nagpur region. A total 433 students responded to the online 9-item questionnaire (PHQ 9) based survey. Our survey showed that 96% of college students were affected with varied levels (minimal to severe) of depression resulting from the COVID-19 pandemic. of these, about 9% experienced severe depression, about 13% experienced moderately severe depression, about 21% experienced moderate depression, about 29% experienced mild depression and about 23% experienced minimal depression. It is suggested that a timely concern should be given to students' psychological health as they are flagbearers of country's success and future.

Keywords: *Covid-19, questionnaire, psychological health, Depression, mental burden.*

Introduction

Covid-19 believed to be originated from Wuhan, China has spread worldwide, with high mortality rate.[1] At present, the situation has turned troublesome as medicine or vaccines are yet to be available for Covid-19.[2] In order to restrain the spread of the virus a lockdown has been imposed in many provinces worldwide.[3] Residents have been strictly advised to obey the instructions of maintaining physical distance, using hand sanitizers and mask.[4] As a result along with a risk to life, mental burden has also increased.[5]

India has similar scenario, when it comes to there strains of lockdown.[6] Studies have shown increased mental burden in the form of fear, anxiety, stress and depression in the times of epidemic[8] and COVID 19 being a pandemic may definitely have a larger impact. Thus along with evident physical symptoms, mental health has also been affected. Stress may be put into words as an emotional feeling raising from any occurrence that seems to be hazardous to our daily life or routine.[9] On the other side of the coin, anxiety is the fear of the untold[10] whereas lack of concern in daily activities is considered as Depression. It is inferred that people fronting a pandemic without a vaccination or assured drug would have anxiety and psychological affection. Evidently, during pandemics such as SARS mental health issues (stress, depression, and anxiety) had raised.[11] Moreover, the World Health Organization has also delivered certain guidelines regarding psychological concern that may ascend during this Covid-19 pandemic.[3] In India, the present lockdown has affected people with regards to their gender, profession, physical health, socioeconomic status and their place of residence[3] Various reports of people emptying malls and supermarkets and panic purchases indicates anxiety in times of this crisis.[12]

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Methodology

The study was conducted on students in various colleges across the Nagpur region. Students from various courses viz. Engineering, Medical Sciences, Arts and Commerce, were invited to take part in the study. The survey was conducted by creating and circulating a Google form questionnaire link on various online stages. The respondents were sampled by virtual snowball sampling technique. The survey took approximately 5-10 minutes to complete and remained open for 8 days. The questionnaire was kept anonymous, thus ensuring the privacy and reliability of the information.

Total 433 participants completing the online questionnaire were incorporated in the study. By clicking on the link provided, the participants were first informed about the nature, purpose and confidentiality of survey. After approving the consent to participate in the survey, they were directed to the next page of the survey. The first section of the survey comprised of the demographic data (Name, Age, and Gender) and name of college and course.

The second section of the survey consisted of Patient Health Questionnaire 9 (PHQ-9). The Patient Health Questionnaire (PHQ) is a tool widely used to diagnose depression and other mental disorders. PHQ-9 consist of the actual nine items or symptoms which forms the basis for DSM-IV depressive disorders diagnosis.[13] It is a self-administered questionnaire consisting of total 9 items that enquired the frequency of symptoms experienced by the participant during the previous 2 weeks. Respondents reported their symptoms as 0- Not at all, 1-Several days, 2-More than half the days, 3- Nearly every day. The total score ranged from 0 to 27 points. Among adults, PHQ-9 score have been used for grading the severity for depression as: a score 1-4: Minimal depression, 5-9: Mild depression, 10-14: Moderate depression, 15-19: moderately severe depression, 20-27: severe depression. In adult population, PHQ-9 is found to have high sensitivity (73%) and high specificity (98%) in diagnosing major depression. Also, The PHQ-9 is a dual-use instrument that, with its 9 items, can diagnose a depressive disorder and also grade its severity.[14]

Results

A total of 433 responses were received throughout the study duration through the online questionnaire survey.

Table 1: Demographic data of the students

Mean age of students (in years)	Male participants	Female Participants
20 ± 2	121	242

The above table shows the age gender distribution across the study population. The mean age of participants was 18–22 years. Among the 433 students, 121 were male and 242 were female.

Table 2: Distribution of severity grade of depression according to PHQ 9

Depression Severity grade	Total Number of Students
No depression (0)	17
Minimal depression (1-4)	102
Mild depression (5-9)	127
Moderate depression(10-14)	90
Moderately severe depression (15-19)	58
Severe depression (20-27)	39

The above table shows that 17 (4%) students were found to have no depression, 102 (24%) had minimal depression, 127 (29%) were categorised as having mild depression, 90 (21%) students have moderate depression, 58 (13%) had moderately severe depression and 39 (9%) had severe depression.

Table 3: Distribution of students with severe depression among different courses.

Name of course	Number of students with severe depression	Percentage of students with severe depression
Engineering	24	9.4 %
Medical Sciences	11	9.8 %
Arts and Commerce	4	6%

The above table shows that approximately 9% of the engineering students, approximately 10% of the medical sciences students and 6% of arts and sciences students were suffering from severe depression when assessed on PHQ 9.

Table 4: Percentage of severe depression among male and female students.

Gender	Percentage of students with severe depression
Male	9.04 %
Female	9.09 %

The above table shows that 9.04% of male students and 9.09% of female students suffered from severe depression when assessed on PHQ 9

Discussion

The present research intended to inspect the levels of psychological suffering in the form of depression in college students during this pandemic. Depression is one of the most prevalent diseases worldwide and a chief factor in problems of mental health[15] Researchers suggest that global health emergencies can have many psychological effects in the form of mental distress on college students, expressed in the form of anxiety, fear, worry and depression **[16]**An Indian study showed that about 33.2% respondents of the online survey had significant psychological impact regarding COVID-19.[15]

Our survey showed that 96% of college students were suffering from depression because of the corona virus outbreak. of the total students, about 9% experienced severe depression, about 13% experienced moderately severe depression, about 21% experienced moderate depression, about 29% experienced mild depression and about 23% experienced minimal depression. In our study we also found that medical college students were slightly more depressed (psychologically distressed) as compared to engineering college students. Our findings are in line with various other studies showing similar results. A study done to compare psychological strain, depression and anxiety among medical and engineering students showed that overall prevalence of stress, anxiety & depression was higher among medical college students in comparison with engineering college students.**[17]** Also females were found to be more severely depressed as compared to males. A number of studies linking university students, stress, and gender [18-19] noted that female students had higher levels of depression than male students [20-21] A study done by Anbumalar C showing gender differences in perceived stress level revealed females reporting higher feeling of anxiety and depression as compared to males.[22-25]

Also Cornine et al intheir study have stated that depression among college students during this pandemic might be in relation with the effect of corona virus on studies and uncertainty of future jobs or employment.[16,26] Unavailability of educational resources such as online network, cell phones and laptops may also lead to stress and anxiety. In addition, owing to technological issues online learning may be troublesome.**[16]** Getting acquainted with fluent use of them may require some time as well, leading to unconscious fear and stress. A recent study exploring the blow of COVID 19 on undergraduate education and welfare showed that approximately one fourth of their samples reported experiencing psychological distress, certainlycorrelating with raised apprehension about academic interruptions and delays, economic out turn and aftermath of the pandemic. **[16]**

In addition, a rise of psychiatric symptoms and of mental health problems is expected in the general population.[27] Common responses representing eminent risk factors for several mental disorders particularly, frustration, loneliness, and worries about the future are evident.[28] Hawryluck et al. studied 129 quarantined persons during previous epidemic revealing high prevalence of psychological distress. Symptoms of depression were found in 31.2% study population. Longer duration of quarantine was more associated with more symptoms[29]

Also the detrimental impact of quarantine and physical distancing on the mental health has been studied in the current pandemic. Qiu et al. in their study found that slightly more than one third of the study population experienced psychological distress, particularly women and individuals aged between 18 and 30 years. The same could be one of the reasons of students' anxiety & depression. It has been identified that the absence of interpersonal communication may trigger and worsen anxiety disorders.

Loneliness and mental health are evidently associated the strongest association being with depression. Adding to the straight effects of imposed separation and quarantine, loneliness as an unintentional consequence of outbreak has become troublesome for youth. Cutting off from others and feeling debarred may result in affective depression.

Furthermore, some previous studies have shown, not living with parents or living away from parents is linked with emotional and psychological distress. Due

to lockdown many students could not reach their native places and had to live away from their family leading to psychological stress. Friends or relatives being diseased with corona virusis considered as an independent predisposing factor of anxiety in collegiate, which might be in relation to the soaring infectiousness of the new coronavirus pneumonia

Additionally, the financial stability of family may also be a notable factor of psychological affection during this crisis. Some studies have indicated that the financial system of the country and residents will be significantly swayed in addition to the health crisis. Because of the spread of the virus and subsequent lockdown, some families may have lost their job or employment, and students might be worried about paying their coaching charges.[16]

Conclusion

COVID-19 crisis has given rise to a lot of ambiguity in the students' lives and its convoying effects will continue affecting college students' mental health and wellbeing profoundly. Many studies have shown that there are significant adverse effect of epidemics and pandemics on the psychological health of collegiate. The main aim of our survey was to explore levels of depression in the college students of Nagpur region. About 9% college students are suffering from severe depression because of this COVID-19 outbreak. The research outcomes direct that students need superior attention because of the higher psychological distress. As students represent tomorrow's future, it is recommended that the college authorities and parents should team up to solve this problem in order to provide finest psychological services to college students.

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Combination of Misoprostol with Transcervical Foley's Catheter Compared to Misoprostol Alone for Cervical Ripening at Term and Labour Induction in Tertiary Care Hospital: A Randomized Trial

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Abstract

Background: Induction of labour is a very common in obstetrics, for that both mechanical and pharmacological method used for induction. Mechanical Foley's with misoprostol is an effective method to cut out course of labour in safe timely manner, for safe neonatal and maternal outcome.

Aims and Objective: The aim of this study was to compare combination of transcervical Misoprostol with Foley's catheter balloon to misoprostol alone for induction of labour and cervical ripening in uncomplicated pregnancy, with regards to safety and effectiveness.

Result: A prospective, randomized controlled study was conducted on 220 term singleton women admitted in our hospital for induction of labour were randomly allocated to Group 1 (combination of Foley's and misoprostol) and Group 2 means (misoprostol) each 110. Combination of Foley's and vaginal misoprostol was much more effective in nulliparous female than multiparous women. Induction delivery interval was shorter in combination group;14.4±5.70 hours than misoprostol group; 17.1±5.62 hours; p value is 0.007.

Conclusion: This study showed that the use of misoprostol with a trans cervical Foley's is an effective method to shorten the course of labour compared with misoprostol alone. This study suggest that combination may be useful to achieve safe and timely delivery in unripe cervix.

Keywords: *Transcervical Foley's, Misoprostol, LSCS, Induction.*

Introduction

The incidence of labour induction has continued to rise over the past decades. The World Health Organization Global Survey on Maternal and Perinatal Health, conducted in 24 countries which included nearly 3, 00,000 observations, showed that 9.6% of them were delivered by labour induction.(1) Mostly labour starts spontaneously near term, but in high risk antenatalcases, labour induction is often required. There are many method of IOL such as mechanical, surgical, pharmacological (prostaglandins (PG) E1 and E2, oxytocin, relaxin) and combined method. Previously for induction of labour, mechanical method were used for dilatation of cervix as well as cervical ripening in women with unfavourable cervix. At term, Osmotic dilators are effective, but they are associated with an increase in maternal infection.(2,3)

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Induction of labour is a common obstetric procedure;it is defined as the stimulation of uterine contractions during pregnancy before initiation of labour before its spontaneous onset for the purpose of delivery of the foeto-placental, which ultimately aims at achieving vaginal delivery depends on the Bishop's score at the time of induction.(4) The patients with a poor Bishop's score have unacceptably higher rates of failure of induction.(5)

Mechanical cervical dilatation is an old method that was used routinely.(6) Transcervical Foley's catheter balloon found to be effective and a safe method for unripe cervix. Mechanical method leads to separation of membrane from lower uterine segment, leads to release of lytic enzymes which stimulating the production of prostaglandins.(7) Misoprostol is a prostaglandian E1 analogue (methyl- 11a 16 - dihydroxy- 16 methyl- 9-oxoprost- 13 enloate), it has uterotonic and cervical ripening action. It is widely used for termination of pregnancy, management of incomplete abortions, induction and augmentation of labour and treatment of postpartum haemorrhage.(8) It is used by sublingual, oral, buccal, vaginal and rectal route. Oral route of misoprostol has several publications been shown to be effective method for induction of labour comparable to oxytocin.(9,10) The main disadvantage of prostaglandins is uterine hyperstimulation contributing to arisk for foetal hypoxia and may leads to operative intervention. As mechanical Foley'sleads to dilation of the cervix and misoprostol result in soften and efface the cervix.(11)

Aims and Objective: The aim of this study is to assess effectiveness and safety of transcervical Foley's catheter with vaginal misoprostol combination for cervical ripening and induction of labour in term as compare to misoprostol alone.

Material and Method

A prospective, randomized controlled study was conducted on 220 women admitted to labour room for induction of labour with term singleton pregnancy, cephalic presentation, viable gestation, intact membranes, and having poor bishops score were included in this study for cervical ripening and induction of labour over a period of one year. Informed consent was taken from Patients who were admitted to hospital for induction of labour. The women with History of prior uterine surgery (LSCS or myomectomy), multiple gestations, malpresentation, heart disease in mother, contraindication to prostaglandins, vaginal bleeding (placenta previa, active herpes infection), anomalous foetus, foetal demise, patients not giving consent were excluded from the study.

Patients were select randomly in to group 1 (misoprostol alone) and group 2 (cervical ripening using misoprostol with Foley's bulb). Simple Randomization with one to one was achieved through a computer-generated assignment with 110 patients in each group. A detail history was taken of all admitted patients along with detailed general and systemic examination was done. In group 1, women were received with F16 Foley's catheter with balloon filled with 50 ml normal saline inserted intra-cervical. The catheter was taped to the patient's inner thigh under gentle traction. Simultaneously they received 25 micrograms of misoprostol per vaginum in posterior vaginal fornix which was repeated every 4 hours for a maximum of 6 doses. Catheter was removed after 24 hours if women didn't go into labour. In group 2 women who were received, misoprostol (prostaglandin E1) 25 micrograms inserted vaginally into the posterior fornix every 4 hours, up to maximum of six doses under all aseptic precautions as per our standard hospital protocol. If cervix became favourable or patients goes in active labour then oxytocin was started after titrating dose, Partograph was maintained throughout the labour. If the cervix was not favourable for artificial rupture of membranes after the stipulated six doses, the induction was considered to have failed. A further dose was withheld in case of tachysystole, hypertonus or hyperstimulation or if patient was in progressive active labour and spontaneous rupture of membranes. Patients were monitored till patient delivered vaginally or by Caesarean section. The two groups were then compared with respect to indication of induction, induction to delivery interval,mode of delivery, maternal side-effects and foetal outcome.

Statistical Analysis: The data collected was organised in a separate sheet programme (MSExcel) and analysed with the help of Statistical Package for Social Science for testing. At the end of the study, the data was collected and analysed by using Student t-test and Chisquare test. A p value of < 0.05 was considered significant.

Result and Observations:

Total cases of 220 patients were taken for studied 110 were divided to each group and result as follows:

As shown in this study, maximum numbers of patients were in the age group of 26to 30 years in both the groups, in (Foley's plus Misoprostol) group 1 26.4±3.80 and in (Misoprostol) group 2, 25.9±3.81. In this study, maximum primigravida were 81.9% women in Group 1 and 71% in Group2 (Table 1) In our study most common indication for induction of labor were 26.8% postdated pregnancy, 22.7% GDM, 20.9% oligohydramnios and 19.1% preeclampsia. (Table 2)

Table 1: Demographic data:

Variables	Group 1	Group 2	P value	Significance
Age	25.9±3.81	26.4±3.80	0.90	Not significant
Primigravida	81.9%	71%	0.056	Not significant
Bishop score	3.0700±.76877	3.5600±.68638	0.124	Not significant

Table 2: Indications of induction of labor.

Indications of induction	Group 1	Group 2	Total
Postdate	35.5%	18.1%	26.8%
PIH	10%	28.2%	19.1%
Oligohydramnios	14.5%	27.3%	20.9%
GDM	29.1%	16.4%	22.7%
IUGR	10.9%	10%	10.45%

In group 1, 78.1% delivered vaginally, 21.9% underwent caesarean section due to meconium stain liquor and foetal distress, tachysystole. In Group 2, 76.4% delivered vaginally, 23.6% women underwent caesarean section due to foetal distress and meconium stain liquor.

In our study, women were delivered in 6-12 hours in Group 1 55.8%, whereas 33.1% in Group 2. In Group 1 majority of the cases (93%) delivered within 24 hours and only 7% of the women needed > 24 hours to deliver whereas in other group, 16.7% delivered in >24 hours. The mean induction delivery interval (IDI) in Group 1 came out to be 14.4± 5.70 which was shorter than Group 2 duration17.1±5.62.

Table 3: Indications of LSCS.

Indications of LSCS	Group 1	Group 2
Meconium stain liquor	37.5%	38.4%
Fetal distress	21%	19.3%
Tachysystole	21%	26.9%
Failure of Induction	16.6%	11.5%
Abruption	4.1%	3.9%
Totle	100%	100%

Table 4: Duration of induction to delivery interval.

Time in hours	Group 1		Group 2	
6-12 hours	48	55.8%	28	33.4%
>12-24 hours	32	37.2%	42	50%
>24 hours	6	7%	14	16.7%
Total	86		84	
Mean ± S.D.	14.4± 5.70		17.1±5.62	
P value	P-value 0.007			
Significance	Not significant			

Neonatal complications in the form of mild birth asphyxia were seen in 8.1% of the neonates in Group 1 and 12.7% of the neonates in Group 2. Neonatal jaundice occurred in 5.45% of the newborn in Group 1 and 7.27% in Group 2. Around 1% of the newborn in both groups had hypoglycemia. In maternal side-effects, hypertonic uterine action was observed in 5.45% of the cases in Group 1 and 6.36% of the cases in Group 2. The other less common side-effects were postpartum hemorrhage, shivering, nausea and vomiting. No case of chorioamnionitis was seen in our study (Table 5).

Table 5: Maternal and neonatal outcomes

Variables		Group 1	Group 2
Neonatal complications	Birth Asphyxia	8.1%	12.7%
	Neonatal jaundice	5.45%	7.27%
	Hypoglycaemia	1.1%	1%
	Uterine hypertonic action	5.45%	6.36%
Maternal outcome	PPH	1%	2.7%
	Nausea & Vomiting	6.3%	4.54%
	Shivering	2.7%	1.1%
	Rupture uterus	0	0
	Chorioamnionitis	0	0

Discussion

In this study, showed that in misoprostol group 56.3% and in Foley's plus Misoprostol group 54.5% in the age group of 26 to 30 years. The mean age group were25.9±3.81 and 26 ±3.80 and in combination group and misoprostol alone respectively. Others study was similar to our study, in *Agrawal M et al.[12]the mean age group of the participants was 24.72 and 24.12 in group 1 and group 2 respectively.* In Noor et al, in misoprostol mean age were *25.1 ± 2.8 and in Foley's catheter mean age were 25.6 ± 4.1[13].*

In this study, 76.4% women were primigravida and 23.6% were multigravida. Other study which was comparable to our study were Agrawal M et al.[12] In our showed that vaginal delivery was 78.1% and 76.4% while 21.9% and 23.6% delivered by caesarean section in group 1 and group 2 respectively. Others study which was comparable our study were Charaya E at el [14] and Baron B at el [15] whereas Chung JH et al [16] and Carbone JF et al [7] studies have shown higher caesarean rates. The studies by Kehl S at al[17] and Ande AB at al[18] showed lesser LSCS rate in combination group.

In this study showed that rate of success means induction to delivery interval was shorter in (14.4±5.70) Foley'scatheter plus misoprostol than misoprostol alone (17.1±5.62). Others study which were similar to our study that Carbone et al. [7],Hussein et al[14],Ande et al, showed that combination of *Foley's* with vaginal misoprostol for induction takes significantly shorter duration of time in cervical ripening and induction to delivery time. In Charaya E at elInduction to delivery interval was 11.76±5.89 hours in combination group and 14.54±7.32 hours in misoprostol group with difference of 2.78 hours. The study by Levine et al showed that shortening in the induction to delivery time by 4 hours 30 minutes with the combination group as compared with the misoprostol-alone group. In Hill JB et.al, the time from induction to delivery in group Foley's catheter and oral misoprostol (12.9 hours) was significantly shorter than that in group vaginal misoprostol (17.8 hours). Maternal and neonatal outcome were maximum in misoprostol group than combination group.

Conclusion

The results of this prospective, comparative study showed that the combination of Foley's with vaginal misoprostol results in a shorter duration of labour as well as induction to delivery interval compared with vaginal misoprostol alone. These results also suggest that the combination of Foley's with vaginal misoprostol may be useful to achieve timely and safe delivery in the presence of unripe cervix mainly with nulliparous. So, all nulliparous women with poor bishop should be offered induction with combination of Foley's bulb with vaginal misoprostol.

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Conflict of Interest: Nil.

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Online Teaching in the Time of Covid 19-Effectiveness and Challenges

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Abstract

India is facing COVID 19 Education Crisis many students have been affected by the closure of schools and colleges. This has caused a dramatic change in the education with a distinctive shift to e-learning where the teaching is done on digital platforms. Most of the schools and colleges are conducting online classes to cope up with the situation and to save the students from the loss. However it has its own drawbacks like issues of internet connectivity, availability of required resources, understanding the software application and lack of personal attention, less student attentiveness, etc. This paper studies the effectiveness of online students classes conducted to evaluate the effectiveness of online teaching. It also includes the software applications that can be used to conduct online classes. Online teaching is the future of students.

Keywords: *Online Teaching. Software application, digital platforms, Education Crisis.*

Introduction

The corona pandemic has led to the education crisis; all the schools and college have been closed till further notice of the government. To ensure that the students do not lag behind due to this temporary closure many of the schools and colleges have started online classes[1,2,3.] So that students can continue their education from the comfort zones of their home. The Schools and colleges are using software applications like Zoom, Google Classroom, Webex Meet to conduct online lectures. These applications are having many features to make online teaching more engaging and effective. However it has many drawbacks like availability of resources, internet connectivity & speed, usage of software application. In online lectures it is very difficult for the faculty to monitor all the students and also to ensure students attentiveness[4,5]. If the students are having proper access to right technology learning online can be effective in many ways. Thus the Institutes are making continuous efforts to make online teaching effective. Most commonly used Software Applications used for conduction lectures by schools and colleges is google class room and Zoom. While the shift is difficult for the students to adapt even the faculties are facing difficulties understanding the features of the applications and conducting classes[6,7,8.]

Objectives of the Study:

- To evaluate the effectiveness of online classes conducted
- To analyse the problems faced in conducting online classes
- To study and analyse the areas of concern in online teaching

Significance of the Study: Due to the present scenario of COVID 19 there has been an education crisis

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and a major shift from traditional classroom teaching to online teaching. The instant shift has been a challenge for the education sector and the situation is such that the schools & colleges have no other choice left than to conduct online classes. As the infrastructure is a problem it is not possible to conduct classes maintaining the social distancing norms. Also it is not right to make the children open to the risk of getting infected. The study helps to identify the effectiveness of online teaching it also address the major challenges involved in it.

Research Methodology

For the purpose of research, data was collected from 500 students who attended online class conducted. An online Class was conducted for Undergraduate final year students on digital platform. Primary data was collected from the participants via questionnaire. The data was collected via questionnaire from the participants of the online class after the session.

Hypothesis: There is a lack of effectiveness in online teaching

Data Collection & Analysis:

The data collected using a questionnaire and analysed as below:

Table 1: One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Digital platform used	500	1.6905	.46099	.02062
Login issues	500	1.6710	.43547	.01948
Session audible	500	1.7455	.46426	.02076
Power point presentation visibility	500	2.2170	.35272	.01577
Disturbances	500	1.7365	.46972	.02101
Interaction Internet connectivity	500	1.7015	.44432	.01987
Number of participants	500	1.7755	.47241	.02113
Overall Satisfaction	500	2.0360	.43166	.01930
Recommended in future	500	1.6790	.45428	.02032

Table 2: One-Sample Test

	Test Value = 1.5					
	t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	**Upper**
Digital platform used	9.240	499	.000	.19050	.1500	.2310
Login issues	8.780	499	.000	.17100	.1327	.2093
Session audible	11.824	499	.000	.24550	.2047	.2863
Power point presentation visibility	45.454	499	.000	.71700	.6860	.7480
Disturbances	11.258	499	.000	.23650	.1952	.2778
Interaction Internet connectivity	10.141	499	.000	.20150	.1625	.2405
Number of participants	13.040	499	.000	.27550	.2340	.3170
Overall Satisfaction	27.766	499	.000	.53600	.4981	.5739
Recommended in future	8.811	499	.000	.17900	.1391	.2189

The mean value obtained in all the above cases is more than 1.5 and the significance (2-tailed) value obtained is also less than 0.05 ($p<0.05$) hence it can be concluded that there is lack of satisfaction in online teaching among the students. Thus from the above, it is concluded that the hypothesis "There is a lack of effectiveness in online teaching" is accepted.

Conclusion

The digital platforms are not user friendly to start with for the students. The students need assistance in understanding the software application initially. Most of the audience faced the issue understanding the application it delays the session and cascading effects are on the further schedule of the day[9,10]. The students also need assistance to login into the software application. Though this is only a one time problem faced as once the login id is created further the same is used. Audibility is a big concern as students unmute the audio and the background noises makes it difficult to hear. Also many students don't understand the basic functions so cannot understand & follow the instructions given. The visibility is proper if the laptop is used by the presenter in case of smart phone the visibility is not up to the mark. Audio and video is disturbed in case the internet bandwidth is low. Thus many participants have issue in the audibility & visibility. Interaction during the session is disturbed due to the issue of low bandwidth internet or connectivity. Even the attentiveness of students is an issue in case of online teaching. It is difficult to monitor their presence and attentiveness[11].

Many students have internet connectivity issues due to which they often leave the class and login again. Thus the students miss the important content delivery in class and cannot understand the complete concept.If the number of participants is more then it impacts the effectiveness of the online teaching negatively. If the number of participants is less in class it is possible for the faculty to monitor the students and engage them well. In case of larger audience it becomes difficult and thus negatively impacts the effectiveness. Overall satisfaction is not there as the online classes had issues related to audibility, visibility and interaction. As the students have internet connectivity problem therefore they do not understand the concepts delivered in class. Due to the various issues faced by the participants there is no willingness to attend the classes online and it further makes it ineffective.

The transition from traditional offline teaching to online teaching is not easy and is impacting the effectiveness of teaching. The online classes are not that effective due to the above mentioned reasons but the pandemic has left no other option. As if the schools and colleges open the children will be open to the risk of getting infected. Till the scenario improves education of students is going to be a big question in front of the academicians and government.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Estimation of Stature from Foot Anthropometry in Central Indian Population

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Abstract

Out of many parameters for human identification, stature is a principal anatomical variable. Stature estimation, especially in post-mortem cases where only some parts of an individual is available or cases where only footprints are available, foot anthropometry will be extremely useful. The objective of the current study is to find out interrelationship between the stature & foot length & formulating a regression equation for determination of height from foot length among individuals from Central India. This cross sectional study was conducted at the department of Forensic Medicine & Toxicology of Datta Meghe Medical College, Nagpur from June 2020 to August 2020. Collected data was entered in Microsoft Excel sheet & analysed using SPSS software. Mean stature in male was 165.8 cm with SD of 6 cm, while in female, it was 159.9 cm with SD of 6.7 cm. Gender wise difference in mean stature & foot length was statistically significant. This research found remarkable positive correlation between stature & foot-length in both sexes. Regression formula was derived which will be extremely helpful to forecast the height of an individual from known foot-length in cases of medicolegal importance.

Keywords: *Stature, Foot length, Central India, Anthropometry, Regression equation.*

Introduction

Establishment of human identification especially in cases of decomposed bodies, mutilated remains, amputated bodies, charred bodies etc has become of paramount importance in homicidal investigation. Also identification of human remains is extremely important in mass disasters (both natural & man made) like floods, earthquakes, cyclones, aircraft accidents, wars, blast injuries & the list goes on. Persons involved in medicolegal investigation are well aware of the fact that in many such cases at the death scene or crime scene, only some bony remains or human remains are available & they have to utilize their knowledge regarding various anthropometric techniques for human identification. In forensic anthropology, there is a long list of parameters for human identification, out of which height of an individual is of immense importance. Many researchers have done studies regarding estimation of stature from evaluation of both upper limb & lower limb but in extreme mutilated cases, even complete limbs are not available[1]. In such cases, it has been found that the reliability of prediction of stature from foot anthropometry has shown promising results[2,3,4].Epiphyseal union & maturation in foot occurs earlier than long bones and this fact will be of immense help in cases of adolescents requiring medicolegal attention[5].

Aims & objectives: To find out relationship between height & foot length and to develop a regression equation for estimation of stature from foot length among Central Indian population.

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Methodology

This is a cross sectional study was conducted at the department of Forensic Medicine & Toxicology, Datta Meghe Medical College,Nagpur during the period of June 2020 to August 2020. 140 domicile individuals from Nagpur, who are born & brought up here were registered for this study. Informed written consent was taken from each individual & their participation was voluntary. Socio-demographic parameters for each individual were noted. The study participants who are having any disease, physical deformity, any history of injury or fracture or any surgical intervention were not included in the study.

The stature of an individual was measured between the vertex & floor in standing & erect position & head in Frankfurt plane. It was measured using stadiometer. As per recommendations of International agreement for paired measurements at Geneva (1912), left foot should be selected for measurements but in our study, both sides were included. Foot length was measured as a direct distance from the most prominent point of the back of the heel to the tip of the hallux or to the tip of the second toe when second toe was longer than hallux using spreading callipers (nail extensions were excluded in the measurements). Measurements were taken during the fixed time duration of the day i.e between 11:00 AM to 2:00 PM to exclude any diurnal variation & single observer took all the measurements to avoid bias. The obtained data was entered in Excel sheet and was analysed using SPSS (statistical package for social sciences).

Results:

Table 1: Gender-wise distribution of study subjects:

Sex	Number
Male	70
Female	70
Total	140

In this study, 140 healthy subjects were included with 70 males and 70 females. All subjects were grown adults with age ranging from 23 years to 50 years.

Table 2: Descriptive statistics for foot length & stature with respect to gender (all readings are in cm):

	Male			Female			Combined		
	Left foot length	Right foot length	Stature	Left foot length	Right foot length	Stature	Left foot length	Right foot length	Stature
Mean	24.5	24.5	165.8	23.4	23.3	159.9	24.2	23.9	162.9
SD	1.5	1.4	6.0	1.7	1.7	6.7	1.8	1.6	7.0
Minimum	22.3	22.3	155.3	20.8	20.9	148.4	20.8	22.3	148.4
Maximum	27.4	27.3	181.4	26.7	26.7	172.4	26.7	27.4	181.4

Left and Right foot length as well as height were noted for males and females. It was observed that the left foot length in males varied from 22.3cm to 27.4 cm with mean (SD) of 24.5 (1.5) cm. The mean (SD) right foot length in males was found to be 24.5 (1.4) cm varying between 22.3 cm to 27.3 cm. In females, left foot length ranged from 20.8 cm to 26.7 cm while the right foot length varied from 20.9 cm and 26.7cm with mean (SD) of 23.4 (1.7) cm and 23.3 (1.7) cm respectively. Mean (SD) combined left foot length was 24.2 (1.8) cm whereas for right foot length it was 23.9 (1.6) cm. The stature in males varied from 155.3 cm to 181.4 cm with mean (SD) stature of 165.8 (6.0)cm and for females the reading was noted to be 159.9 (6.7) cm ranging from 148.4 cm to 172.4 cm.

Table 3: Paired t-test for left & right foot length comparison:

	Left foot length (cm)	Right foot length (cm)	p – value
Male	24.5 ± 1.5	24.5 ± 1.4	>0.05
Female	23.4 ±1.7	23.3 ± 1.7	>0.05

There was no significant difference observed between left and right foot length in males as well as females. Hence, regression equations for estimation of stature were obtained with left foot length only.

Table 4: Regression statistics:

Stature (Dependent variable - DV)	Left Foot length (Independent variable-IV)		
	Male	Female	Combined
Intercept	73.37	77.3	74.43
Regression coefficient	3.71	3.52	3.66
Correlation coefficient	0.91	0.92	0.93
R^2 (coefficient of determination)	0.83	0.85	0.87
Significance	<0.01	<0.01	<0.01

It is observed that there is a strong positive correlation between dependent variable stature and in dependent variable foot length. The correlation coefficient for foot length of male and stature is 0.91, foot length of female and stature is 0.92 and correlation of foot length with stature of any person irrespective of gender is 0.93. The R^2 (coefficient of determination) shows how good fit is the linear regression between the independent and dependent variable. In this study it is noted that the foot length of male explains 83% of variability in male stature while for females it explains 85% of stature variability. R^2 is maximum for foot length irrespective of gender, it explains 87% of variability in stature.

Linear regression equation is expressed as:

DV = Intercept + Regression coefficient*IV

In this study, stature is DV and foot length is IV, thus the estimation of stature can be done by following equations.

1. Estimated stature of males

Est. Stature (M) = 73.37 + 3.71 (Length of left foot print)

2. Estimated stature of females

Est. Stature (F) = 77.3 + 3.52 (Length of left foot print)

3. Estimated stature irrespective of gender

Est. Stature = 74.43 + 3.66 (Length of left foot print)

Discussion

Sr. No.	Author	Region	Year	Mean foot length(cm)		Mean Stature(cm)		Correlation Coefficient		R^2 (coefficient of determination)	
				Male	Female	Male	Female	Male	Female	Male	Female
1	SanliS et al[1]	Turkey	2005	26.25	23.47	175.05	159.96	0.716	0.699	0.512	0.488
2	Krishan K et al[2]	Himachal Pradesh	2007	24.7	22.6	168.24	155.72	0.741	0.734	0.549	0.538
3	Kanchan T et al[3]	Punjab	2010	26.01	23.78	166.39	159.56	0.704	0.536	0.496	0.287
4	Rajesh D et al[4]	Puducherry	2011	22.05	20.89	166.03	157.24	0.787	0.876	0.619	0.767
5	Kanchan T et al[5]	New Delhi	2013	24.89	-	167.08	-	0.575	-	0.33	-
6	Khairulmazidah M et al[6]	Malaysia	2013	24.8	22.3	166.56	156.08	0.659	0.662	0.434	0.438
7	Atal D et al[7]	Uttarakhand	2015-2016	26.51	23.83	172.85	158.2	0.525	0.65	0.276	0.422
8	Jat et al[8]	Rajasthan	2016-2017	25.47	24.05	162.1	150.5	0.94	0.93	0.88	0.86
9	Lalwani R et al[9]	Central India	2019	25.99	23.23	169.52	154.75	0.731	0.686	0.534	0.47
10	Karan Jain	Central India	2020	24.5	23.4	165.8	159.9	0.91	0.92	0.83	0.85

Current study was done to find out the correlation between foot length & stature and in turn to formulate a regression equation for the population of Central India. It will be of immense help in medicolegal cases requiring anthropometric assessment. When we compare our study with the other studies done in the past we found out that, our study is in total agreement with study conducted by Jat et al[8] in Ajmer region (Rajasthan). Like our study which is from Central India, Lalwani et al[9] conducted similar study in this region in 2019, where the researchers concluded that, mean foot length (25.99 cm) in males & (23.23 cm) in females along with that, height in males (169.52 cm) & in females (154.75 cm) are similar to our findings. Also in the same study, the correlation is positive (high) but in our study, it is 0.91 (for males) & 0.92 (for females) which indicates strong correlation. Similar study was conducted in Puducherry[4] which is in Southern part of India in 2011. When we compare our study with this study, it is in agreement with correlation between height & stature. Studies conducted by Kanchan et al[3], Atal et al[7] & SanliS et al[1] showed low degree of correlation between height & stature, hence the estimate for stature would be poor.

Conclusion

Our study found significant positive correlation between height & foot length in both genders. Regression equation was formulated for Central Indian population, which will be of tremendous help in determining the height of an individual from foot length especially in medicolegal cases. When we go through all the studies which we have taken into consideration, there is a lot of variation in correlation coefficients between height & foot length which may be due to improper sampling or sampling error. So to study this discrepancy in correlation more & more studies from different regions of India must be conducted to check whether it is sampling fluctuation or the difference is actually variable for the different regions. Also the studies must include larger sample size which will result in reduction of sampling variability & will provide stronger estimates.

Ethical Clearance: Taken from institutional ethics committee.

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Conflict of Interest: Nil.

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Radiological Evaluation of Innominate Bone for Age Estimation: A Cross Sectional Study from Central India

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Abstract

Age is cardinal in both civil & criminal cases as it not only affects the way an individual is treated, both in court and with regards to bail, but it also influences the defence under Indian Penal system. The defence for children is easier to establish than that for an adult. This study focuses on investigating the relationship between the stage of fusion of secondary ossification centres of innominate bone & biological age in Chhattisgarh population. This study was carried out in 140 healthy subjects (70males and 70 females) aging from 17 to 24 years. The obtained results revealed that the complete fusion of both epiphyses of innominate bone is seen at 21-22 years. Comparatively, females were developing epiphysealunion at a younger age than their male counterparts. Results also suggest that the age of epiphyseal fusion at iliac crest & ischial tuberosity is found to vary greatly all over the India, indicating the need for separate standards for separateregions.

Keywords: *Epiphyseal union, iliac crest, ischial tuberosity, innominate bone.*

Introduction

Evaluation of age by radiological study of union of ossification centers is helpful to solve many civil & criminal cases like capital punishments, sexual offences, abductions, kidnappings, matrimonial disputes, contract agreements, consent for medical examination, admission & eligibility for various institutions etc. Also, age determination at the time of death is of supreme importance for identification purpose. It is also true for bio archaeological studies, where the reconstruction of health and disease patterns or demography of past societies is evaluated. The fusion of the various epiphyses occurs at a specified order and period of time in life and their correlation is useful to establish an age in both antemortem & post-mortem cases. By taking into account the radiological analysis of epiphyseal union at iliac crest & ischial tuberosity, this study will be of immense help in further understanding the details of accurate assessment of age in Central Indian population.

Aims and Objectives:

1. To evaluate age specific difference in epiphyseal union at iliac crest & ischial tuberosity.
2. To estimate age from epiphyseal union at iliac crest & ischial tuberosity.
3. To compare bisexual difference in epiphyseal union at iliac crest & ischial tuberosity.
4. To compare the findings of epiphyseal union at iliac crest & ischial tuberosity in Central Indian population with other parts of India.

Material and Method

The present study was carried out in the Department of Forensic Medicinein collaboration with Department of Radiology, SSIMS, Bhilai, Chhattisgarh. A total of 140 individuals participated in this study. The subjects included were individuals of 17-24 years of age from Bhilai & Durgcity. They are born to parents domicile

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from Central India & have lived here since birth. The subjects do not have any disease/deformity related to bones or chronic disease affecting the general health. An informed consent was taken from all the subjects prior to each procedure. Skeletal maturity was evaluated according to the Jit & Kulkarni's classification[1] of four stages: Appearance, Non fusion, Partial fusion & Complete fusion. X-Rays showing clear gap between the epiphyseal and diaphysial ends & showing saw tooth like appearance end were designated as "Non-fusion" (NF) X-rays. X-Rays showing a line replacingthe hiatus between the epiphyseal & diaphysial ends & not showing saw tooth like appearance were designated as "Partial Fusion" (PF) X-rays. X-Rays showing the same bony architecture in the diaphysis and epiphysis & showing scar of the previous stage were designated as "Complete Fusion" (CF). It was classified, analysed and compared with known standards. At the end conclusions were drawn, which were compared with available results of various previous studies.

Results

Table 1: Epiphyseal fusion in males:

Age (years)	Iliac crest			Ischial tuberosity		
	NF	PF	CF	NF	PF	CF
17-18	4	6	0	7	3	0
18-19	2	8	0	3	7	0
19-20	0	10	0	0	10	0
20-21	0	2	8	0	7	3
21-22	0	0	10	0	0	10
22-23	0	0	10	0	0	10
23-24	0	0	10	0	0	10
Total	**70 (100%)**			**70 (100%)**		

Table 2: Epiphyseal fusion in females:

Age (years)	Iliac crest			Ischial tuberosity		
	NF	PF	CF	NF	PF	CF
17-18	2	8	0	6	4	0
18-19	0	10	0	0	10	0
19-20	0	5	5	0	8	2
20-21	0	2	8	0	6	4
21-22	0	0	10	0	0	10
22-23	0	0	10	0	0	10
23-24	0	0	10	0	0	10
Total	**70 (100%)**			**70 (100%)**		

In males, iliac crest shows: non fusion in 6 (8.57%) cases in age group of 17-19 years, partial fusion in 26(37.14%) cases in age group of 17-21 years & complete fusion in 38 (54.28%) cases in age group of 20-24 years. Complete fusion is seen in all the subjects in age group of 21-24 years. In females, iliac crest shows: non fusion in 2 (52.85%) cases in age group of 17-18 years, partial fusion in 25 (35.71%) cases in age group of 17-21 years & complete fusion in 43 (61.42%) cases in age group of 19-24 years. Complete fusion is seen in all the subjects in age group of 21-24 years.

In males, ischial tuberosity shows: non fusion in 10 (14.28%) cases in age group of 17-19 years, partial fusion in 27 (38.57%) cases in age group of 17-21 years & complete fusion in 33 (47.14%) cases in age group of 20-24 years. Complete fusion is seen in all the subjects in age group of 21-24 years. In females, ischial tuberosity

shows: non fusion in 6 (8.57%) cases in age group of 17-18 years, partial fusion in 28 (40%) cases in age group of 17-21 years & complete fusion in 36 (51.42%) cases in age group of 19-24 years. Complete fusion is seen in all the subjects in age group of 21-24 years.

Discussion

Table 3: Comparison of Age of union (years)

Sr. No	Researcher	Region/population	Iliac crest		Ischial tuberosity	
			Male	Female	Male	Female
1	Galstaun[2]	Bengalis	19-20	17-9	20	20
2	Pillai[3]	Madrasis	14-18	14-18	-	-
3	Flecker[4]	Australia	18	15-16	20	-
4	Davies & Parson[5]	English	23	23	-	-
5	Dasgupta et al[6]	Uttar Pradesh	19-20	19-20	-	-
6	Parikh[7]	Indian	20-21	18-19	23-24	21-22
7	SankhynS et al[8]	Himachal Pradesh	$21^{1/2}$	$21^{1/2}$	-	-
8	Bennet KA[9]	USA	23	23	24-25	24-25
9	BhiseS et al[10]	Mumbai	21-22	20-21	21-22	21-22
10	Devraj N et al[11]	Maharshtra	-	20-21	-	20-21
11	Sangma W et al[12]	Manipur	-	21	-	21
12	Sharma Y et al[13]	Rajasthan	21-22	20-21	21-22	21-22
13	Singh P et al[14]	Punjab	-	-	20-21	20-21
14	Naik M et al[15]	Andhra Pradesh	15-17	15-17	17-18	17-18
15	Present study	Chhattisgarh	21-22	21-22	21-22	21-22

Iliac Crest: The observations of present study for males, matches with work of Parikh[7], SankhynS et al[8], BhiseS et al[10] & Sharma Y et al[13]. For females, the observations match with the findings of SankhynS et al[8], Bhise S et al[10], Devraj N et al[11] & Sharma Y et al[13].

Ischial Tuberosity: The observations of present study for males, matches with work of BhiseS et al[10], Sharma Y et al[13] & Singh P et al[14]. For females, the observations match with the findings of Parikh[7], Bhise S et al[10], Devraj N et al[11], Sangma W et al[12], Sharma Y et al[13] & Singh P et al[14].

Summary and Conclusion:

1. This study was conducted exclusively on the young indigenous population of Central India.
2. The epiphyseal union at iliac crest in both males & females is completed in all instances (100%) at the age of 21-22 years.
3. The epiphyseal union at ischial tuberosity in both males & females is completed in all instances (100%) at the age of 21-22 years.
4. Central Indian inhabitants are of diverse type comprising of various castes, religions & tribes. The opinion about age should always be given in the range.
5. From this study, range of 1-2 years of margin of error can be concluded.
6. For age estimation, pertinent joints should be radiologically examined for different centres and opinion should be arrived considering the status of multiple centres.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Age Estimation among Adolescent Individuals from Fusion of Tri-Radiate Cartilage: A Radiological Study from Central India

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Abstract

Age determination in living individuals, dead persons & in human remains may shed light on issues with notable legal and social consequences for individuals as well as for the community. In the age determination of the deceased individual; the post-mortem circumstances can change the body parts and make age estimation difficult. In such cases, skeletal remains are extremely helpful for age determination. This study focuses on investigating the relationship between the stage of fusion of tri-radiate cartilage of innominate bone & biological age in population of Central India. This study was carried out in 120 healthy subjects (60 males and 60 females) aging from 12 to 18 years. The obtained results revealed that the complete fusion of tri-radiate cartilageis seen at 16-17 years. Comparatively, females were developing union at a younger age than their male counterparts. Results also suggest that the age of fusion of tri-radiate cartilage is found to vary greatly all over the India & world, indicating the need for separate standards for separate regions.

Keywords: *Tri-radiate cartilage, innominate bone, Central India.*

Introduction

Preciseage determination of an individual is foremost in matters with respect to law and medicine. The word 'Adolescent' literally means 'passing from childhood to maturity'. WHO defines 'Adolescents' as individuals in the age group of 10-19 years. However, among this age group, individuals of 14 to 16 years are considered immensely important in routine medico-legal practices as they are more pivotal in matters (civil or criminal)with reference to sexual offences, kidnapping,juvenile delinquency, substance abuse, child marriages, factory employment & so on. All over the world, parameters like secondary sexual characters, skeletal ossification progression, time of eruption of teeth etc are admissible in court as unparalleled scientific evidence in deciding disputes in adolescent age determination. All these parameters at their best can tend to fix the age of the person (either live or dead) only at a near approximate range and not the exact age. It is also true for bio archaeological studies, where the reconstruction of health and disease patterns or demography of past societies is evaluated. The fusion of the various epiphyses occurs at a specified order and period of time in life and their interrelationship is useful to establish an age in both living & dead cases. By taking into account the radiological analysis of fusion of tri-radiate cartilage, this study will be of enormous help in further understanding the details of accurate assessment of age in Juvenile population of Central India.

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Aims and Objectives:

1. To evaluate age specific difference in fusion of tri-radiate cartilage.

2. To estimate age from fusion of tri-radiate cartilage.
3. To compare bisexual difference in fusion of tri-radiate cartilage.
4. To compare the findings offusion of tri-radiate cartilagein Central Indian population with other parts of India & the world.

Material and Method

The present study was carried out in the Department of Forensic Medicinein collaboration with Department of Radiology, DMMC, Nagpur. A total of 120 individuals participated in this study. The subjects included were individuals of 12-18 years of age from Nagpurcity. They are born to parents domicilefrom Central India & have been residing here since birth. The subjects do not have any disease/deformity associated with bones or chronic disease affecting the general health. Age was confirmed from history and from documents like drivinglicense, passports, ration card, voter's cardaadhar card etc. An informed consent was taken from all the subjects prior to each procedure. Xrays of Tri-radiate cartilage, AP & lateral views were taken & five differentstages of union were graded. The 5 stages are: F1 (Non union): epiphysealcartilage did not begin to decrease in thickness, F2 (Commence of union): thickness of epiphyseal cartilage was found tobe reduced appreciably (1/4th united), F3 (Incomplete union): epiphysis has begun to fuse with shaft and complete union was well underway (1/2 united), F4(Complete union): epiphyseal cartilage was bony in architectureand its density indistinguishable from the epiphysis & diaphysis in its neighbourhood butan epiphyseal line called epiphyseal scar couldstill be distinguished. (3/4 united) & F5 (Complete union): absence of epiphyseal scar. The appearance and fusion of Triradiate Cartilage was assessed radiologically and the results were compared with the previous known standard studies.

Results

In males, Triradiate cartilage shows: commencement of fusion in12(20%) cases in age group of 12-14 years, Incomplete fusion in 9(15%) cases in age group of 13-15 years, complete fusion with epiphyseal scar in 14 (23.33%) cases in age group of 13-16 years & complete fusion without epiphyseal scar in 25 (41.66%) cases in age group of 15-18 years. Complete fusion is seen in all the subjects in age group of 16-18 years.

Table 1: Extent of fusion of Triradiate cartilage in males:

Age (Years)	F1	F2	F3	F4	F5
12-13	0	10	0	0	0
13-14	0	2	6	2	0
14-15	0	0	3	7	0
15-16	0	0	0	5	5
16-17	0	0	0	0	10
17-18	0	0	0	0	10

Table 2: Extent of fusion of Triradiate cartilage in females:

Age (Years)	F1	F2	F3	F4	F5
12-13	2	8	0	0	0
13-14	1	9	0	0	0
14-15	0	2	6	2	0
15-16	0	0	0	3	7
16-17	0	0	0	0	10
17-18	0	0	0	0	10

In females, Triradiate cartilage shows: non fusion in 3 (5%) cases in age group of 12-14 years, commencement of fusion in 19 (31.66%) cases in age group of 12-15 years, Incomplete fusion in 6 (10%) cases in age group of 14-15 years, complete fusion with epiphyseal scar in 5 (8.33%) cases in age group of 14-16 years & complete fusion without epiphyseal scar in 27 (45%) cases in age group of 15-18 years. Complete fusion is seen in all the subjects in age group of 16-18 years.

Discussion

Table 3: Comparison of Age of union (years):

Sr.No	Researcher	Region/population	Triradiate cartilage		
			Male	Female	Mixed
1	Davies & Parson[1]	English	-	-	16
2	Flecker[2]	Australians	15	13	-
	Pillai[3]	Madrasis	-	-	11-14
3	Galstaun[4]	Indians	15-16	14	-
4	Chaurasia[5]	Indians	-	-	16-17
5	Parikh[6]	Indians	-	-	15-16
6	Krishnan Vij[7]	Indians	-	-	15-16
8	Bangal R et al[8]	Mumbai	16-17	16-17	-
9	SingiY et al[9]	Karnataka	16-17	15-16	-
	Sankyanet al[10]	Himachal Pradesh	15.5-17.5	13.5-15.5	-
	Kumar A et al[11]	Uttar Pradesh	15-16	15-16	-
	Shilajiya Det al[12]	Gujarat	16-17	14-15	-
	Bhore D et al[13]	Mumbai	15-16	16-17	-
	Naik M et al[14]	Andhra Pradesh	15-16	14-15	-
	Tailor C et al[15]	Surat	-	-	14-15
	Present study	Nagpur	16-17	16-17	-

The observations of present study for males, matches with work ofDavies & Parson[1], Galstaun[4], Chaurasia[5], Parikh[6], Krishnan Vij[7], Bangal R et al[8], SingiY et al[9], Sankyanet al[10], Kumar A et al[11], Shilajiya Det al[12], Bhore D et al[13] & Naik M et al[14]. For females, the observations are in agreement with the findings of Davies & Parson[1], Chaurasia[5], Parikh[6], Krishnan Vij[7], Bangal R et al[8], SingiY et al[9], Kumar A et al[11] & Bhore D et al[13].

Summary and Conclusion

1. This study was conducted exclusively on the young indigenous population of Central India in the adolescent age group.
2. The union at Triradiate cartilage in both males & females is completed in all instances (100%)at the age of 16-17 years.
3. Central Indian inhabitantsare of diverse type comprising of various castes, religions & tribes. The opinion about age should always be given in the range.
4. From this study, range of 1-2 years of margin of error can be concluded.
5. For age estimation, pertinent joints should be radiologically examined for different centres and opinion should be arrived considering the status of multiple centres.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest-Nil.

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Comparison between Immediate Effects of MET and Passive Stretching Techniques on Hamstring Flexibility in Patients with Hamstring Tightness: An Experimental Study

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Abstract

Background: Muscular flexibility is an important part of functioning of human being and hamstring muscle is prominently found to be tight in human body. Hamstring muscle flexibility can be improved by a variety of stretching method and other techniques, yet very little work has been done to compare muscle energy technique (MET) and passive stretching techniques. The objective of our study was to compare the immediate effectiveness of muscle energy technique (MET) and Passive stretching on hamstring flexibility in individuals with hamstring tightness.

Method: 42 participants who met the inclusion criteria were randomly divided into two equal groups, Group A – MET and Group B – Passive stretching. The range of motion (ROM) of knee extension was measured before and after both the techniques by AKET using universal goniometer.

Results: There was a significant improvement in hamstring flexibility in both the groups following the application of MET and Passive stretching.

Conclusion: From the present study we can conclude that both MET and passive stretching techniques have an immediate effect on reducing hamstring muscle tightness. From the mean difference values it is visible that MET (12.81) is slightly more effective than passive stretching (6.38) although statistically significant difference is not seen.

Keywords: *Muscle energy technique, Passive stretching techniques, hamstring tightness.*

Introduction

Flexibility means the ability to elongate skeletal muscle and tendons. In rehabilitation of any muscle, flexibility is a significant component. Static flexibility can be measured through Range of Motion (ROM), which is equivalent to tolerance threshold toward stretches, and dynamic flexibility which implies necessary resistance toward stretch is measured through stiffness. Muscle stiffness means the ratio of torque changes to muscle length changes.[1] There are multiple factors and varied reasons for decrease in joint ROM and one of which is muscular tightness. Muscle "tightness" is defined as an increase in tension from active or passive mechanisms. Passively, muscles can become shortened through postural adaptation or scarring and actively, they can become shorter because of either spasm or contraction. This type of tightness will limit range of motion and may create a muscle imbalance.[2]

Hamstring muscles have a great tendency to shortening,due to their multi-joint function and their tonic postural character. It is very well known that the length of the hamstring muscles is known to play an essential role in both the effectiveness and efficiency of human movements like running, walking etc. It has been proved in the literature that reduced hamstring flexibility is a common clinical finding in the general populationas

well as athletic populations which result in major muscle imbalances, predisposing athletes to muscle injuries,patellar tendinopathy, and patellofemoral painas also it may lead to low back pain.[3]

There are variety of techniques used in physiotherapy for improving joint muscle flexibility in different set ups. Some of them include- Muscle energy techniques, passive and active stretches, positional release technique, active release technique,static stretching etc. Out of which we will be considering two techniques here -MET and passive stretching. Passive stretching includes the stretching through the use of mechanical devices, the assistance of gravity, or use of a partner. In the process of passive stretch, an external force is applied, usually through a person in a rehabilitative setting and that person holds that stretch for a period of time and this is how the muscle gains new length. Passive stretching is also widely used in conjunction with active stretches as a part of warm up and cool down programs, apart from being a main intervention.[4]

Muscle energy technique (MET) is a procedure in which there is voluntary contraction of a patient's muscle in a precisely controlled direction with different levels of intensity. In this particular technique, the client provides the initial effort while the practitioner will facilitate the process. The benefits of MET are restoring normal tone in hypertonic muscles, strengthening weak muscles, preparing the muscle for subsequent stretching, improved joint mobility.[5] It is hypothesized that one of the efficient treatments to increase hamstring flexibility is Muscle Energy Technique (MET), which includes controlled isometric contraction in the targeted muscle. The results of various studies have shown that MET was effective in improving the flexibility as well as reduction of pain and discomfort and caused more immediate and long term changes.[1] There are multiple studies showing the effectiveness of MET and Passive stretching in hamstring tightness.[6-9]Apart from this, both the techniques have proved to be safe and easy for achieving hamstring flexibility. So in order to compare which of these techniques has more immediate effect on hamstring flexibility, this study was carried out on individuals with hamstring tightness.

Materials and Methodology

Study design: An experimental study

Sampling method: Simple random sampling

Sample size: Total 42

- **Group A:** 21 subjects received MET treatment
- **Group B:** 21 subjects received passive stretching treatment

Study setting: DattaMeghe College of Physiotherapy, Nagpur

Study duration: 2 months

Inclusion Criteria:

- Patients with Hamstring tightness of 15 degrees or more on AKET
- Gender – male and female both
- Patients willing to participate in the study

Exclusion Criteria:

- Patients with any low back pathology.
- Patients with history of any recent fracture or surgery of lower limb
- Patients with of knee joint pain or hypermobility
- Patients with any neurological disorder.
- Patients with history of recent hamstring injury

Material used: Plinth, Stabilising belt, Universal goniometer, Stop watch

Outcome measure: For measuring the hamstring flexibility, the active knee extension test was used in both the groups.

Active knee extension test (AKET): The patient lies in supine lying with hip flexed to 90 degrees. The patient is asked to perform active knee extension movement and stop when he/she feels strong resistance to the movement. The range of motion of knee joint is measured with the help of goniometer at this end range.[10]

Procedure: The study was carried out at the DattaMeghe College of Physiotherapy, Wanadongri, Nagpur. Ethical approval was obtained from Institutional Ethical Committee & an informed consent was obtained from the participants. Total 47 patients were assessed for the study. Inclusion and exclusion criteria were applied and 5 patients were excluded from the study. The study was conducted on 42 patients fulfilling the inclusion criteria. The participants were randomly divided into two groups by computer generated randomized table into groups of 21 each. Group A received Muscle energy

technique treatment and Group B received passive stretching treatment. The pre and the immediate post reading of AKET were measured

Group A - MET For Hamstrings tightness

- The contralateral leg may be either flexed or may lie straight on the plinth.
- The affected leg was flexed at both the hip and knee joints, and then slowly straightened by the therapist until the restriction barrier was identified
- The limb was moved a little away from the restriction barrier and the isometric contraction against resistance was introduced.
- The patients were asked to resist the movement with no more than 25% of strength. The contraction was held for 7-10 seconds followed by complete relaxation of the limb.
- On exhalation, the knee joint was straightened (extended) towards its new barrier and through that barrier a stretch was applied and maintained for 30 seconds.
- 3 repetitions of this process were done[11]

Group B - Passive stretching For Hamstrings tightness: The patient were in supine lying with a small pillow below the contralateral knee. The patient were asked to maintain the hip in 90 degrees of flexion, with the help of both hands. The ankle joint was in a relaxed position. The patient's foot was placed on therapist's shoulder and the hamstring muscles were maximally stretched from this position. The stretch was maintained for a period of 30 seconds and the procedure was repeated for a total of three times.[12,13]

Results

The software MYSTAT 12 was used in this study. Active knee extension test (AKET) was used as an outcome measure. Descriptive statistics such as mean and standard deviations were calculated to describe all the variables. The paired t-test and the unpaired t-test were used to verify differences between pre- and post-intervention. The statistical analysis was conducted at 95% confidence level, and $p<0.05$ was considered statistically significant.

The gender ratio was 13:8 (13 females and 8 males) in Group A – MET group and 11:10 (11 females and 10 males) in Group B – Passive stretching group. The difference in the mean age of both the groups was statistically not significant (Table 1)

Comparison of the pre and post intervention values (student's paired t test) of the outcome measure showed that there was statistically significant difference in AKET values in both the groups. (Table 2 and table 3) Inter group comparison (student's unpaired t test) showed that there was no statistically significant difference between the two groups in terms of AKET (Table 4).

Table 1: Baseline demographic data of both the groups

Group	Group A	Group B	t value	p value	Inference
Age (years)	38.19±14.12	38.38±13.81	2.02	0.96	Not significant
Gender ratio (M:F)	13:8	11:10			

Table 2: Mean and SD scores of Group A(Paired t test)

Group A - MET				
			Paired t test	
	Pre intervention	Post intervention	Mean Difference	p value
AKET	144.14 ± 18.33	156.95 ± 15.44	12.81	0.000

Table 3: Mean and SD scores of Group B (Paired t test)

Group B - Passive stretching				
			Paired t test	
	Pre intervention	Post intervention	Mean Difference	p value
AKET	146.81 ± 18.19	153.19 ± 15.93	6.38	0.000

Table 4: Result of post-intervention values of both the groups (Unpaired t test)

	Group A	Group B	Unpaired t test	
	Post intervention	Post intervention	p value	Inference
AKET	156.95 ± 15.44	153.19 ± 15.93	0.44	Not Significant

Discussion

In our study we found out that there was an immediate positive effect on hamstring tightness in terms of AKET in both the groups. Various studies have shown similar effects of MET on hamstring muscle tightness.[5,14] Desai Sonali et al conducted a study to compare effectiveness of Muscle Energy Technique (MET) & Static Stretching on hamstring tightness in healthy young individuals and found that both MET and static stretching showed significant improvement in reducing Hamstring Tightness in agreement to our study [9]

Bandy et al in their research have stated, the optimal duration for an effective stretch as a duration of 30 seconds.[13] During MET, muscle elongation is maintained for this duration thus leading to increased muscle length with a combined effect of creep and plastic changes occurring in the connective tissue.[15,16] Holuskova et al stated that the variations in the connective tissue show mechanical properties associated with both elastic and viscous constituents. Creep is the temporary lengthening of the connective tissue during the stretch period (viscoelastic property).[17] Fryer et al in their study have explained that the reasons of increased flexibility after MET may be the result of biomechanical or neuro-physiological changes or increased stretch tolerance.[15,18]

Ivan et al affirmed MET as an effective and non-traumatic manipulative technique. MET is said to have an inhibitory effect on motor activity acting through the muscle spindles or the Golgi tendon organs. [19]Post isometric relaxation technique reduces of the tone of the muscles. The afferent nerve impulses entering the dorsal route links up with the inhibitory motor neuron, stopping the efferent motor neurons impulse discharge, thus preventing further contraction and decreasing the muscle tone. This in turn relaxes the agonist muscle. [20] During this period, as a result of reduced tone, lengthening becomes easier. Kuchera et al in their study discussed similar reasons.[9,21] They explained that the inhibitory golgi tendon reflex is said to be activated during isometric contraction of muscles leading to reflex relaxation of the muscle.[22]

June-Su Yua et al, on 51 healthy individuals with tight hamstrings compared the immediate effect of hamstring stretching techniques and found that static stretching and PNF-hold relax showed a significant effect on ROM measured by active knee extension (AKE) test.[23] Phil Page in clinical commentary on current concepts in muscle stretching for exercise and rehabilitation has also stated that Static passive stretching is effective at increasing ROM. A static stretch of 15-30 seconds applied 2 to 4 times showed increase in ROM not because of increased length (decreased tension) of the muscle but simply due to an increased tolerance of the individual to stretching.[24] Patrick G De Deyne in his perspective on Application of Passive Stretch and Its Implications for Muscle Fibers explained that the increase in the range of motion immediately after application of static passive stretch can be because of the viscoelastic behaviour of muscle and short-term changes in muscle extensibility. Passive stretching leading to positive changes in range of motion often may involve biomechanical, neurological, and molecular mechanisms.[25] Static stretching leading to increased range of motion might be due to increase in the number of sarcomeres in series (muscle length) due to prolonged exposure to the stress. In addition, stretching causes increase in viscoelasticity and decrease in stiffness of muscular and connective tissues that results in improved muscular extensibility.[26] Also, during passive stretching it is assumed that the Golgi Tendon Organ, monitoring tension resulting from stretch of a muscle-tendon unit, may result in muscle elongation by intervening any assisting impulses from the primary afferents of the muscle spindle (Ia afferent fibers) and may contribute to muscle relaxation by inhibiting tension in the contractile units of the muscle being stretched. [4]

Maryam Azizi et al in their pilot study concluded that a single session of MET (3 repetitions) resulted in significant immediate improvement in flexibility and reduction in stiffness of hamstring.[1] Yuichi Nishikawa et al in their study on Immediate effect of passive and active stretching on hamstrings flexibility found that both active and passive static stretching had significant effect on

improving hamstring flexibility immediately however, passive stretching elicited greater improvements in hamstring flexibility than active stretching.[27] Shadmehr et al. in their study related to passive stretch and MET on hamstring flexibility concluded that both techniques relatively had the same effect on increasing the flexibility in healthy young females which is similar to our results aswell. Thus the results of our study corroborates the previous findings about a significant immediate effect of single session of MET and passive static stretching (3 repetitions each) on improving flexibility of hamstring.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

Conclusion

From the present study we can conclude that both MET and passive stretching techniques have an immediate effect on reducing hamstring muscle tightness. From the mean difference values it is visible that MET (12.81) is slightly more effective than passive stretching (6.38) although statistically significant difference is not seen.

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Role of Prolactin and β-hCG in Female Infertility

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Abstract

The World Health Organization (WHO) describes infertility as a disability for couples in childbirth to attain pregnancy within 12 months of daily sexual intercourse. Infertility affects 13–20 per cent of couples worldwide, regardless of race or ethnicity. Human chorionic gonadotropin (hCG), also known as the "pregnancy hormone," plays a significant role in human reproduction. The present study was conducted in the departments of biochemistry and dept. of obstetrics and gynaecology. The study was conducted on two groups of 40 infertile Females and 40 healthy controls. Blood sample was collected for the estimation of prolactin and hCG was assessed by dry chemistry analyzer. Serum prolactin levels in infertile females is 33.96±11.46 and hCG levels was86.38±12.45 which was higher in infertile female as compare to healthy control with a p value of<0.05. Present study concludes that hyperprolactinemia is a major contributing hormonal factor in infertility among infertile women and, as such, prolactin and hCG should be measured in infertile women.

Keywords: *Prolactin, female infertility, hCG.*

Introduction

The reproductive years of women begin when she starts her menstrual cycle during puberty (about 13 years of age, and the capacity to have a child typically ends about age (45) years, although it may be possible for a woman to become pregnant before her cycles end with menopause (about 51 years of age)[1]. Already born girl bears around (400000) immature eggs (oocytes) in her body. These are stored in small, fluid-filled sacs, called follicles, in her ovaries. Upon reaching her reproductive years, she begins to have one egg (or, less generally, more than one) monthly, which can join a male mobile sperm cell during fertilization and become pregnant[2].

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The production and release of the egg is largely dependent on hormone balance (chemicals that signal the body organs to perform a particular task). Some of these hormones are produced in the ovaries, while others are derived from the two brain glands, the hypothalamus and the pituitary[3]. Primary infertility is a term that describes a pair who has never been able to conceive through unprotected sex after a minimum of one year of attempting to do so. Primary infertility causes include a wide array of both physical and emotional factors[4]. Infertility is described as the failure of a couple to achieve conception after one year of unprotected and adequately timed intercourse (regardless of cause) [5]. It may be primary i.e. if a couple never conceived following cohabitation and exposure to sexual activity over a two-year span or secondary infertility i.e. if a couple had previously achieved pregnancy but frequent unprotected sexual intercourse did not result in a second pregnancy[6]. Human infertility is a complicated issue that has various implications depending on the context of society and culture, gender, lifestyle, the sexual history of the people it affects. Infertility is a major problem for

public health, partially due to its importance in etiology as well as its difficulty in preventing, diagnosing, and treating it[6]. Although some find infertility primarily a woman's problem, men also contribute to it and are also affected[7]. Essentially, infertility is graded into two primary infertility and secondary infertility. Predominant infertility in a couple who have never had a child is infertility. Secondary infertility means the inability to conceive following a previous pregnancy. Infertility can be caused by male or female infection, Infertility is often perceived as a predominantly female disorder, although male-factor infertility is equally prevalent, and half of the infertile couples fail to reproduce due to man fertility problems. Prolactin (PRL) is a 198 polypeptide amino acid hormone secreted from the anterior pituitary and synthesized by lactotroph cells. It weighs 23 kDa and many factors affect its secretion including stress, sleep, pregnancy, food ingestion, and stimulation of the chest wall (trauma, or pain). Prolactin plays an important role in controlling reproductive functions. Although it's level increase beyond normal (hyperprolactinemia) can be either physiological, pathological, or idiopathic, its clinical manifestations can vary from extreme to none [8]. Endometrial cells were found to have prolactin receptors. Endometrial prolactin secretion helps to preserve endometrial receptivity and has been shown to provide an ideal environment for implanting blastocyst transferred during periods of in-vitro fertilization (IVF). A sufficiently high level of prolactin may inhibit the proliferation of luteinizing granulosa cells, and may also interfere with the function of the corpus luteum resulting in luteal phase defects as well as irregular implantation and embryo growth. Specific studies have shown the presence of a state of hyperprolactinemia during cycles of IVF/Intra cytoplasmic Sperm Injection (ICSI)[9,10,11]. In the early stages of pregnancy, hCG is luteotropic, preserving progesterone development and endometrial support. In maternal semen, hCG can be detected eight days after ovulation[12] and in blastocysts as early as seven days after fertilization [13]. HCG levels in the maternal blood steadily rise in early pregnancy until peak levels are reached at seven to nine weeks.[14] They progressively decrease until about 20 weeks when plasma levels remain relatively small and steady until the end. Episodic fluctuation in maternal hCG levels occurs in the first trimester, indicating pulsatile secretion, with a nadir at 1900 hours and peak at 0700 hours. The daily variation in concentrations of maternal serum hCG can be up to 20 percent. Early in pregnancy, there is no link between hCG rates and fetal sex or birthweight. Higher rates of hCG are therefore associated with female fetuses in late pregnancy [15,16].

Aim and Objective:

Aim: To study the levels of prolactin and beta hCG in female infertility.

Objective: To correlate the levels of prolactin and beta hCG in infertile womens and healthy controls (age matched) attending AVBRH Wardha and SMHRC Nagpur.

Material and Method

The present Study was carried out in the Dept. of Obstetrics and Gynecology and Department of Biochemistry at Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi (Meghe) Wardha Maharashtra in collaboration with Datta Meghe Medical College, Shalinitai Meghe Hospital & Research Centre, Nagpur.

Total 80 subjects were selected for study. Out of which 40 infertile female and 40 age and gender matched healthy control.

Sample Collection: Blood sample was collected, prolactin and beta hCG level was measured in Dry Chemistry Analyzer.

Inclusion Criteria: Women with primary and secondary infertility.

Exclusion Criteria:

- Urogenital tract anomalies
- History of thyroid disease/thyroid surgery/thyroid medication.
- Women unwilling to participate or sign the informed consent

Statistical Analysis: All estimated results were expressed as mean ±SD. Mean values will be assessed for significance by unpaired student –t test. A statistical analysis will be performed using the Statistical Package for the Social Science program (SPSS, 24.0). Frequencies and percentages will be used for the categorical measures. Probability values $p < 0.05$ will be considered statistically significant.

Observation and Results

Table 1: Age Distribution

Groups	Number	Age	P value
Cases (Infertile Female)	40	31.67±9.05	0.2531
Control (Fertile Female)	40	29.62±6.70	

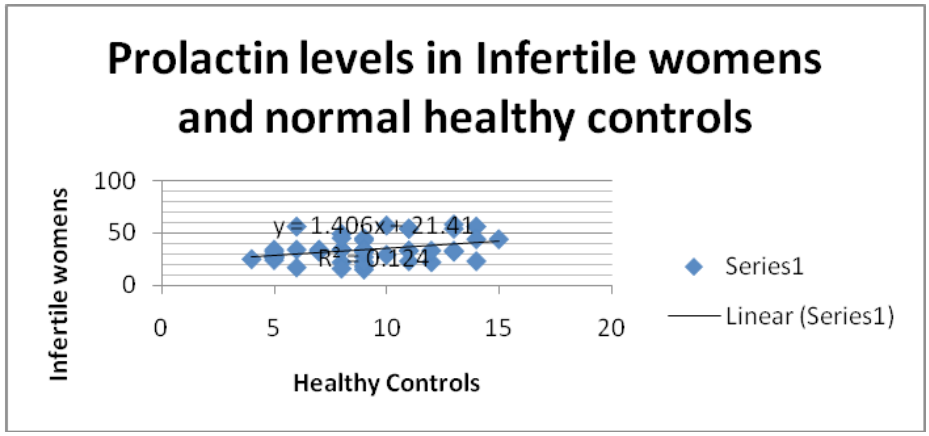
The above table depicts the age distribution in cases and controls, the mean age in cases is 31.67 and in control 29.62, no significant correlation was seen in between cases with a p value of 0.2531.

Table 2: Levels of Prolactin and beta hCG in infertile Female and Healthy control.

	Infertile Female Mean±SD (n=40)	Healthy Control Mean±SD (n=40)	t Value	P Value
Prolactin	33.96±11.46	8.92±2.87	-13.405	<0.0001
Beta hCG	86.38±12.45	6.6±1.988	-40.021	<0.0001

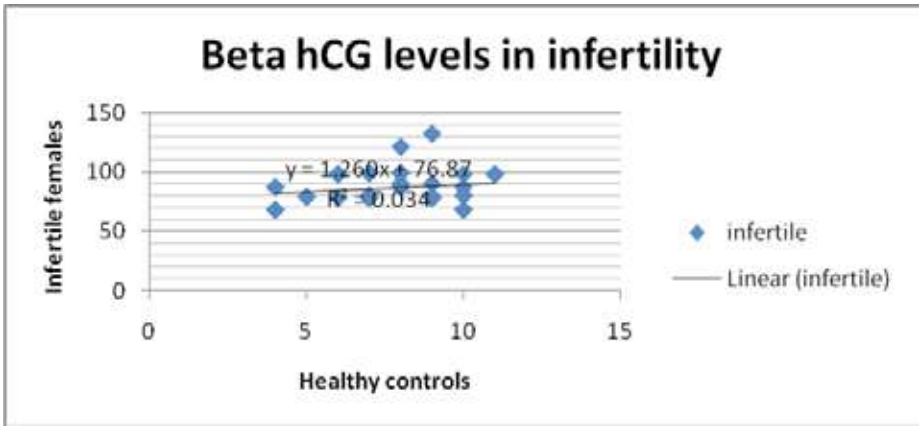
$P < 0.05$

The levels of Prolactin and beta hCG was increased in infertile female as compared to healthy control. In Infertile females the levels of prolactin was 33.96±11.46 and in healthy controls the levels was 8.92±2.87 with a p value of <0.0001 which a significantly indicating Hyperprolactinemia. Serum beta hCG levels was also increased in infertile women's with a p value of <0.0001



Graph 1: Scatter graph showing prolactin levels.

The above scatter graph depicts the levels of prolactin in infertile womens and normal healthy controls.



Graph 2: Scatter graph showing beta hCG levels.

The above scatter graph depicts the levels of beta hCG in infertile womens and normal healthy controls.

Discussion

Human chorionic gonadotropin (hCG), also known as the "pregnancy hormone," plays a significant role in human reproduction. Serum hCG has a circulating half-life of 24 hours. Female infertility accounts for 37% of all infertile couples and most of them are attributed to ovulatory dysfunction and are also correlated with dysregulation of the hormonal network.[17] The existence of abnormally high prolactin values is considered hyperprolactinemia, which is one of the most common endocrinological disorders of the hypothalamopituitary axis affecting fertility.[18-20] Hyperprolactinemia is one of the fertility-influencing endocrinological disorders most severe. The perception that hyperprolactinemia is not only expressed as galactorrhea and amenorrhea but also induces gonadal dysfunction and infertility has contributed to prolactin estimation in infertile females. Hyperprolactinemia influences the reproductive capacity by impairing pulsatile GnRH secretion and interferes with the function of ovarian-level gonadotropins so as to interfere with ovulation.[21,22] Hyperprolactinemia triggers galactorrhea together with menstrual and ovulatory disorders. It is present in females with both galactorrhea and amenorrhea in two thirds. Thus, serum prolactin levels should be estimated in unexplained infertility, any menstrual abnormalities with or without hirsutism, galactorrhea with or without amenorrhea, luteal phase defects, and anovulation.[23] Mild hyperprolactinemia may cause infertility even with normal menstruation.[24] Women with galactorrhea and hyperprolactinemia may be predominantly hypothyroidism. Hypothyroidism induces increased TRH secretion that induces thyrotrophin and lactotrophs, causing both TSH & prolactin levels to rise[25]. Hyperprolactinemia decreases the pulsatile release of the GnRH and impairs the secretion of FSH, LH. It also affects ovarian follicle steroidogenic activity and induces insufficient luteal-phase progesterone secretion[26]. These all result in luteal phase defects, inconstant ovulation, and chronic anovulation. Luteal insufficiency impairs endometrial development and inhibits embryo implantation.[27] It accounts for 3–10 percent of infertility and two-thirds of these women have hyperprolactinemia.[28] In hyperprolactinemic infertility, reduced prolactin secretion with dopaminergic drugs is the best therapy for raising the risk of conception[29].

Conclusion

For nonpregnant people, HCG in the serum rises with age. Consequently, it is concluded that hyperprolactinemia is a major contributing hormonal factor in infertility among infertile women and, as such, prolactin and hCG should be measured in infertile women. Even with normal menstruation, moderate hyperprolactinemia can cause infertility. Hyperprolactinemia is one of the most common female infertility endocrinological disorders and is often associated with hypothyroidism. Present study concludes that all infertile women should be offered serum prolactin estimation at an early stage of infertility checkup rather than going for more costly tests or invasive procedures.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Chondroid Chordoma: An Uncommon Bone Neoplasm

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Abstract

Chordomas are rare, slowly growing, locally aggressive neoplasms of bone that arise from embryonic remnants of the notochord. Chondroid-chordoma is an uncommon variant of chordoma. These tumors typically occur in the axial skeleton. Here we report a rare occurrence of chondroid variant of chordoma at sacrococcygeal region in a 60 year old female. Magnetic resonance imaging revealed expansile mass involvingsacrococcygeal region.Gross and microscopic examination confirmed chondroid chordoma.Later patient underwent radiotherapy, doingwell,on a one year follow up. Chondroid chordoma is discussed due to its predilection for occurrence at sacrococcygealregion and its more favorable prognosis compared with that of conventional chordoma.

Keywords: *Chondroid-chordoma, notochord, sacrococcygeal.*

Introduction

Chordoma is rare tumor that grows slowly,locally aggressive, invades surrounding soft tissue and metastasizes infrequently. It accounts for 1%– 4% of all primary malignant bone [(1,2)] Chordoma usually occurs in the sacrococcygeal (50%), spheno-occipital region (35%) and (15%) in the true vertebrae. [(3,4)] Nearly, 33% chordomas in the base of the skull are chondroid in type. [(5)] It is only malignant tumor arising from notochordal elements, more frequent in fifth and sixth decades, affect much more often men than women. Chondroid chordoma is slower growing than conventional chordoma and shows foci of chondroid (cartilaginous) differentiation. It has a better prognosis than classic (non-chondroid) chordoma. Heffelfinger *et al*. described this variant of chordoma indistinguishable from hyaline type chondrosarcoma.[(6)]

Case Report: A 60-year-old female was admitted in orthopaedicward of this hospital with a complaint of numbness and pain in left hip and has increased since 4-8 months period. Magnetic resonance imaging (MRI) findings are showed aapproximately 5.8x4.2x4.8cm, expansile mass involving S4-S5 bodies and coccyx protruding in presacral space. Operative findings revealed tumor mass eroding sacrum below S2 level. It was pink, friable and soft to firm and vascular mass. We received multiple grayish-white to brown pieces which had soft to hard consistency. Hematoxylin and eosin stained sections studied from multiple bits show tumor cells arranged in sheets, cords and nest embedded in myxoid to chondroid matrix. These are small round cells having eosinophilic to vacuolated cytoplasm giving bubbly appearance known as physaliferous cells and round nuclei. Areas of hemorrhage and necrosis seen.

Discussion

Chordomas are usually relatively slow-growing, low-grade malignancies. They arise from the sacrum in approximately 50%–60% of cases, skull base region (spheno-occipital/nasal) in approximately 25%–35% of cases, cervical vertebrae in approximately 10% of cases, and thoracolumbar vertebrae in approximately 5% of cases.[(2)]Genetic studies performed on chordomas include chromosome analysis, telomere reduction and telomere

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activity, DNA microsatellite, loss of heterozygosity (LOH), and clonality studies.Clonality studies on eight cases of sacral chordomas indicated a polyclonal origin of the tumor.[7]

Clinical presentation is usually with pain as the cardinal symptom, whereas neurologic deficits tend to vary based on the location of the lesion. Chordomas are divided into conventional, chondroid, and dedifferentiated types. Conventional chordomas are the most common. They are characterized by the absence of cartilaginous or additional mesenchymal components. Chondroid chordomas contain both chordomatous and chondromatous features. The cartilaginous foci of chondroid chordomas resemble those of chondroma or low grade chondrosarcoma. Histologically, they display lobules and vacuolated (physaliphorous), moderately atypical, neoplastic cells across a myxoid stroma separated by fibrous bands.[8]

They have an ectodermal origin, chordomas are not sarcomas; however, they are traditionally classified and approached as sarcomas on the basis of being a primary bone tumor.[9]

In case of diagnositicdelimma, immunohistochemistry stain demonstrates that tumor cells of chondroid chordoma are reactive to epithelial markers like epithelial marker antigen (EMA) and cytokeratin (CK). Chondroma and chondrosarcomas are negative for CK and EMA and are positive for vimentin.[5]

MRI and computed tomography scan are important for pre-operative planning and staging of disease. Tumor can be visualized as midline, lobular, osteolytic foci or an expansile concomitant soft-tissue mass. Technetium-99m bone scan demonstrates hot areas within the tumor showing destructive changes with sclerotic rim and calcification in pre or paraspinal soft-tissue mass.[10]

Though chordoma has been considered of low metastatic potential; but distant metastasis to lung, bone, soft tissue, lymph node, liver, and skin has been reported in up to 43% of patients.[11,12,13] Metastatic disease may be considered of adverse prognostic significance because the median survival time was reported to be <12 months in a series of 28 chordoma patients after the development of distant metastasis.[9]

Surgery continues to be the primary modality in the management of chordomas. Chordomas are considered radioresistant tumors and require doses in excess of 60 Gy. Patients with spinal and sacral chordomas at the Massachusetts General Hospital, with a 5-year local control rate of 53%.[14]Chordomas are not reported to be sensitive to chemotherapy, similar to many other low-grade malignancies. Accordingly, chemotherapy response has been reported in patients with high-grade dedifferentiated chordomas, which represent <5% of all chordomas.[15]

Conclusion

Chondroid Chordomas are uncommon primary bone tumors with a high risk for local recurrence and modest propensity for distant metastasis. Surgery is the primary modality to achieve the best long-term control therapeutic value. Newer techniques and charged particle radiotherapy allow for better dose delivery, and hence better disease control. Chondroid chordoma has better prognosis than conventional chordoma.

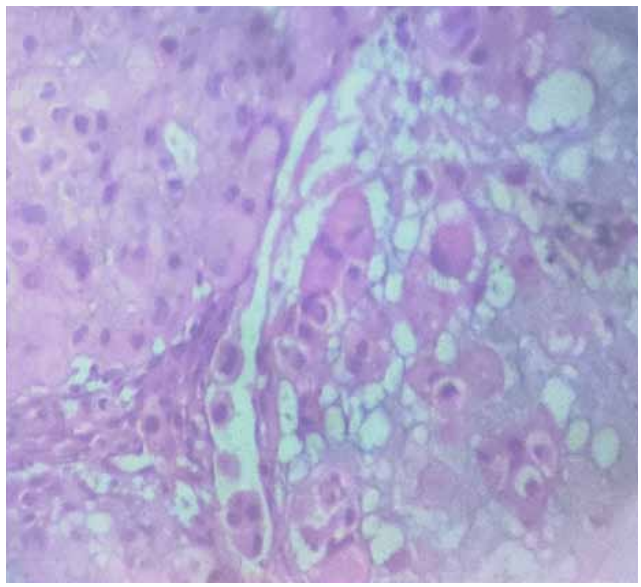


Figure 1: High power view showing small round cells have eosinophilic to vacuolated cytoplasm giving bubbly appearance known as physaliferous cells and round nuclei embedded in myxoid to chondroid matrix. (H and E).

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Comparison between Immediate Effects of Post Isometric Relaxation and Reciprocal Inhibition Techniques on Hamstring Flexibility in Patients with Hamstring Tightness: An Experimental Study

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Abstract

Aim: There are studies comparing the effects of post isometric relaxation technique & reciprocal inhibition technique on hamstring muscle flexibility but to the best of our knowledge there is hardly any study that has reported comparative immediate effect of these two techniques. Thus the aim of our study was to evaluate the immediate effect of these two techniques on hamstring muscle flexibility.

Method: The research design used was Comparative Experimental study design. Total 46 participants were screened and those meeting the inclusion criteria (n=40) were included in the study. The participants were randomly divided into two groups- group A - post isometric relaxation and Group B - reciprocal inhibition of 20 each. Pre and immediate post intervention scores were measured in terms of AKET

Findings: Inter group comparison (student's unpaired t test) showed that there was no statistically significant difference between the two groups in terms of AKET. The comparison of pre and post intervention values of the outcome measure in both the groups showed a statistically significant difference.

Conclusion: It can be concluded from the present study that both post isometric relaxation technique & reciprocal inhibition technique have an immediate effect on reducing hamstring muscle tightness. From the mean difference values it is visible that PIR (13.20) is slightly more effective than RI (5.45) although statistically significant difference is not seen.

Keywords: *Post isometric relaxation, Reciprocal inhibition, Immediate effect.*

Introduction

Hamstrings are important muscles of the human body. They contribute to posture stabilization of the body as also to the control of the pelvis region. Hamstring muscles possess the ability to generate high forces rapidly through their eccentric and concentric contractions. A hamstring muscle's length can be changed up to one third as a result of either eccentric or concentric contraction. Usually hamstring muscle is subject to high forces in closed and open kinetic chain activities.[1] Hamstring tightness not only hampers the activities of daily living like running, walking etc. but also causes postural issues and low back pain. Thus treating the hamstrings and relieving the tightness is of utmost importance. Many interventions are available to treat hamstring tightness like passive and active stretches, positional release technique, active release technique, muscle energy techniques etc. Multiple studies have shown the immediate and prolonged effectiveness of Muscle energy techniques in Hamstring tightness [2-4]

Devised by an osteopathic physician, Fred Mitchell, Sr, and polished by Fred Mitchell, Jr Muscle energy technique has been used for the treatment in various aspects. Muscle energy technique involves voluntary contraction of target muscles against a counterforce provided by operator, in a precisely controlled direction.

MET is useful in reducing pain, stretching tight muscles, decreasing muscle tone, improving local circulation, strengthening weak musculature and mobilizing joint restrictions.[6]

Different variations of MET are used in daily practice such as Isometric contraction using antagonist or agonist (with or without stretching), isotonic (concentric or eccentric) contraction, slow eccentric isotonic contraction and isokinetic variation (combined isotonic and isometric contractions) depending on the need.[7]A common explanation proposes that a voluntary agonist muscle contraction, prior to stretching of the muscle, has the effect of reducing reflexive components of that contraction thus causing relaxation, increased muscle length and range of motion of the joint that is in other words causing post isometric relaxation.[7]The second is reciprocal inhibition (RI) mechanism which states that when a muscle contracts isometrically, its antagonist is inhibited, and there is reduced tone immediately after this. Thus the isometric contraction involves two neurological components: Postisometric relaxation (PIR) will follow contraction of the agonist, as a result of Golgi tendon organ mediation and Reciprocal inhibition (RI) whichaffects the antagonist, as a result of spindle mediation. So,once the stretching has actually commenced, biomechanical effectis seen as low intensity force is applied to lengthen the tissues and 'creep' stage begins.[8]However, there is limited documented evidence to find out which technique is the best MET technique on Hamstring function. Thus this study was conducted to find out which of the two widely used MET techniques has a better immediate effect on hamstring muscle function.

Methodology

Study design: A Comparative Experimental study

Sampling method: Simple random sampling

Sample size: Total 40

- **Group A:** 20 subjects received MET (PIR)treatment
- **Group B:** 20 subjects received MET (RI) treatment

Study setting: DattaMeghe College of Physiotherapy, Nagpur

Study duration: 2 months

Inclusion Criteria:

- Patients with knee flexion angle of 15 degrees or more on AKET
- Gender: Male and female both
- Patients willing to participate in the study
- Age group: 25-60 years.

Exclusion Criteria:

- Patients with any low back pathology.
- Patients with history of any recent fracture or surgery of lower limb
- Patients with of knee joint pain or hypermobility
- Patients with any neurological disorder.
- Patients with history of recent hamstring injury

Material Used: Plinth, Stabilising belt, Universal goniometer, Stop watch

Outcome measure –For measuring the hamstring flexibility, the active knee extension test was used in both the groups.

Active knee extension test (AKET) – The patient lies in supine lying with hip flexed to 90 degrees. The patient is asked to perform active knee extension movement and stop when he/she feels strong resistance to the movement. The range of motion of knee joint is measured with the help of goniometer at this end range.

Procedure – The study was carried out at the DattaMeghe College of Physiotherapy, Wanadongri, Nagpur. Ethical approval was obtained from Institutional Ethical Committee & an informed consent was obtained from the participants. Total 46 patients were assessed for the study. Inclusion and exclusion criteria were applied and 6 patients were excluded from the study. The study was conducted on 40 patients fulfilling the inclusion criteria. The participants were randomly divided into two groups by computer generated randomized table into groups of 20 each. Group A received post isometric relaxation technique and Group B received reciprocal inhibition technique. The pre and the immediate post reading of AKET were measured.

Group A – MET (PIR) for Hamstrings tightness: The contralateral leg may be either flexed or may lie

straight on the plinth. The affected leg was flexed at both the hip and knee joints, and then slowly straightened by the therapist until the restriction barrier was identified. The limb was moved a little away from the restriction barrier and the isometric contraction against resistance was introduced. The patients were asked to resist the movement with no more than 25% of strength. The contraction was held for 7-10 seconds followed by complete relaxation of the limb. On exhalation, the knee joint was straightened (extended) towards its new barrier and through that barrier a stretch was applied and maintained for 30 seconds. 3 repetitions of this process were done.[7]

Group B – MET (RI) For Hamstrings tightness: The supine patient was asked to fully flex the hip and knee on the affected side. The affected leg was then slowly straightened by the therapist until the restriction barrier was identified. The calf of the affected leg was then placed on the practitioner's shoulder that stands facing the head of the table on the side of the affected leg. Then the patient was asked to attempt to straighten the lower leg utilizing the antagonists to hamstrings, employing approximately 20 percent of the strength of the quadriceps. This was resisted by the practitioner for 5 to 7 seconds. Appropriate breathing instruction were given. On exhalation, the knee joint was straightened (extended) towards its new barrier and through that barrier a stretch was applied and maintained for 30 seconds. 3 repetitions of this process were done.[7]

Results

MYSTAT 12 software was used in this study. The outcome measure used was Active knee extension test (AKET). Descriptive statistics such as mean and standard deviations were calculated to describe all the variables. The paired t-test and the unpaired t-test were used to confirm differences between pre- and post-intervention. The statistical analysis was conducted at 95% confidence level, and $p<0.05$ was considered statistically significant.

The gender ratio was 12:8 (12 females and 8 males) in Group A – MET PIR group and 10:10 (10 females and 10 males) in Group B – MET RI group. The difference in the mean age of both the groups was statistically not significant (Table 1)

Comparison of the pre and post intervention values (student's paired t test) of the outcome measure showed that there was statistically significant difference in AKET values in both the groups (Table 2 and table 3). Inter group comparison (student's unpaired t test) showed that there was no statistically significant difference between the two groups in terms of AKET (Table 4).

Table 1: Baseline demographic data of both the groups

Group	Group A	Group B	t value	p value	Inference
Age (Years)	39.05±13.91	35.65±13.26	0.76	0.45	Not Significant
Gender ratio (M:F)	8:12	10:10			

Table 2: Mean and SD scores of Group A (Paired t test)

Group A - MET PIR group				
			Paired t test	
	Pre intervention	Post intervention	Mean Difference	p value
AKET	143.10 ± 18.15	156.30 ± 15.54	13.20	0.000

Table 3: Mean and SD scores of Group B (Paired t test)

Group B - MET RI group				
			Paired t test	
	Pre intervention	Post intervention	Mean Difference	p value
AKET	146.75 ± 15.02	152.20 ± 14.68	5.45	0.000

Table 4: Result of post-intervention values of both the groups (Unpaired t test)

	Group A	Group B	Unpaired t test	
	Post intervention	Post intervention	p value	Inference
AKET	156.30 ± 15.54	152.20 ± 14.68	0.39	Not Significant

Discussion

A study done by Bandy et al concluded that the optimal duration for an effective stretch as duration of 30 seconds.[10] In both PIR and RI techniques stretch is maintained for this duration thus leading to increased muscle length with a combined effect of creep and plastic changes occurring in the connective tissue.[5,11] Various studies have shown a positive effect of post isometric relaxation technique on hamstring muscle flexibility.[1,12] A study done by Sambandham et al showed an immediate positive effect of MET on hamstring tightness which upholds the result of our study.[13]

Similarly a study conducted in 2018 by Desai Sonali et al showed considerable improvement in AKET ranges on application of MET on hamstring muscles which supports our study.[4] Ivan et al has stated MET as an effective manipulative technique. MET is said to inhibit the motor activity from the muscle spindles or the Golgi tendon organs.[14]The technique of post isometric relaxation leads to reduction of the muscle tone. The afferent nerve impulses entering the dorsal route associate with the inhibitory motor neuron, stopping the efferent motor neuron impulse discharge. This prevents further contraction and decreases the muscle tone resulting in relaxation of the agonist muscle.[15] In this period lengthening becomes easier as a result of reduced tone. Similar reasons have been discussed by Kuchera et al in their study. [16] In addition Shellock F G et al explained that the inhibitory golgi tendon reflex is said to be activated during isometric contraction of muscles leading to reflex relaxation of the muscle. [17] A pilot study done by Azizi M et al concluded that post isometric relaxation technique can be used to increase the flexibility of hamstring muscle. Visco-elastic changes in muscles or increased tolerance threshold toward stretch are thought to be some of the several possible mechanisms of immediate effectiveness of MET.[18] Furthermore Lederman also explained the viscoelastic changes of the connective tissue to be responsible for the increased flexibility. Thus it can be stated that any reduction in hamstring stiffness after application of MET can be resulted from the viscoelastic changes in musculotendinous unit. In addition Fryer et al in their study have explained that the reasons of increased flexibility after MET may be the result of biomechanical or neuro-physiological changes or increased stretch tolerance.[5]

In the present study we found that there was significant improvement in RI group as well. The amount of literature available on effect and mechanism of working of RI is not as much available. A comparative study of PIR and RI techniques on flexibility of hamstring muscles done by Agrawal S also showed similar results which is in accordancewith our study.[20] The nerve impulses discharged from muscle spindles exciting the agonist muscles' afferent nerve fibers synapse with their excitatory motor neuron in the spinal cord. Simultaneously there occurs inhibition of the motor neuron of the antagonist muscle leading to prevention of its contraction, thus resulting in antagonist relaxation [15]

A study done by Nasiri et al concluded that both PIR and RI techniques are equally effective in increasing the flexibility of hamstring muscle which correlates with our study. Moreover it has been proposed that by virtue of an isometric contraction of antagonist muscle, a muscle can be inhibited prior to stretching.[7]

Conclusion

It can be concluded from the present study that both post isometric relaxation technique & reciprocal inhibition technique have an immediate effect on reducing hamstring muscle tightness. From the mean difference values it is visible that PIR (13.20) is slightly more effective than RI (5.45) although statistically significant difference is not seen.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Prospective Analytical Study of Burn Deaths in Infant and Children Below 6 Year

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Abstract

Introduction: Burn injury is not only leading health problem in adult but it also cause significant mortality and morbidity in children.Burns can be devastating injuries for children, the immediate effect of which is compounded by ongoing pain, cosmetic and physical disfigurement, impairment, multiple dressing changes and surgical procedures. This prospective analytical study was planned to study the pattern of burn deaths in infant and children.

Material and Method: This Study was carried out in department of forensic medicine and Toxicology, B.J. Medical College, Ahmedabad over the period of 2 year during which 30 cases were studied and analysed. Data was maintained using Microsoft Office Excel and analysed suing test of proportions.

Result: Out of 30 cases studied during this period 18 (60%) cases were males and rest 12 (40%) were females. Most common type of burn was scald in 25 (83%) of cases, followed by electrocution in 3 (10%) cases and most common cause of death was septicaemia in 70% of cases followed by shock due to burns in 6 (20%) cases.

Conclusion: Burn injuries caused to them are because of their curiosity and innocent nature and unable to understand the extent of damage that will be cause by it. As these injuries are accidental and mostly occur in home, they are preventable.

Keyword: *Infant, Children burns, scald, pattern.*

Introduction

Burn injury in infant and children is considerable health problem not only in India but throughout the world. Paediatric burn injury constitutes about one-fourth of the total burn accidents in India.[1] Burns can be devastating injuries for children, the immediate effect of which is compounded by ongoing pain, cosmetic and physical disfigurement, impairment, multiple dressing changes and surgical procedures.[2] Burn injuries have been ranked as second most common cause of accidental death in children younger than 6 years and the most common cause accidental death in the home.[3] It is also called as a man's first double edged sword evidenced throughout the history; it has served as well as destroyed mankind.[4] Burn is a injury which is produced by the application of dry heat such as flames, radiant heat or heated substance over the body surface.[5] Burn injuries have been described as among the most serious injuries that may afflict a human being.[6] A child below 3 years is

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too young to understand the danger in the proximity of burn area, so an identification of the risk factors leading to burn injuries is crucial. So this study was planned with aim to study the pattern of burn deaths in children under the age of 6 years brought for post-mortem examination.

Material and Method

This prospective analytical study was carried out in department of Forensic Medicine and Toxicology, B.J. Medical College, Ahmedabad over a period of 2 years from January 2017 to December 2018. A total of 30 cases of burns brought for post-mortem examination at mortuary of civil hospital, Ahmedabad between 0-6 years was included in our study whereas deaths due to chemical burn, lightening, radiation and post-mortem burns were excluded. Detail history of the case was obtained from the patient close relatives, police and other person who were available at the time of incident and those who accompanies the victim at the time of post-mortem examination.

Data was maintained in Microsoft Office Excel and analysed for age, sex, type of burns, percentage of burn and cause of death using tests of proportion.

Result and Observation

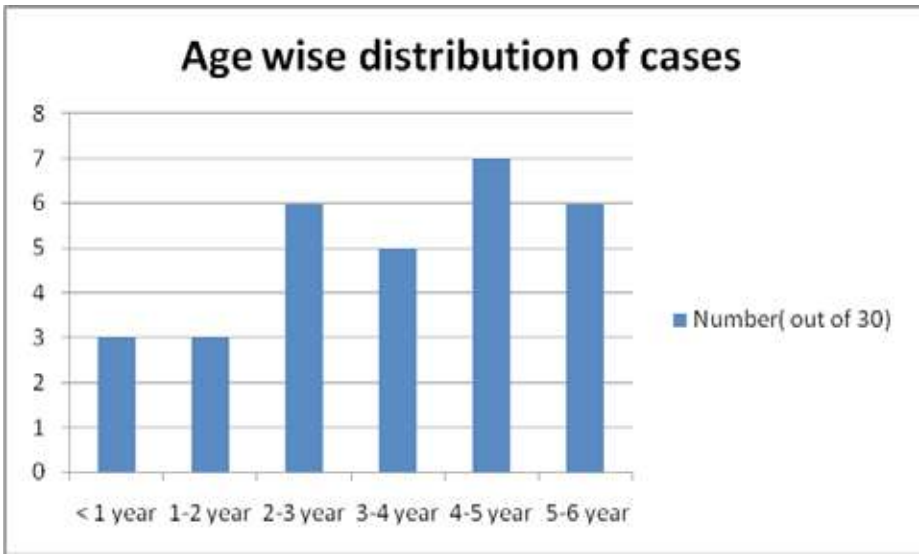


Fig. 1: Showing Age wise distribution of cases included in study

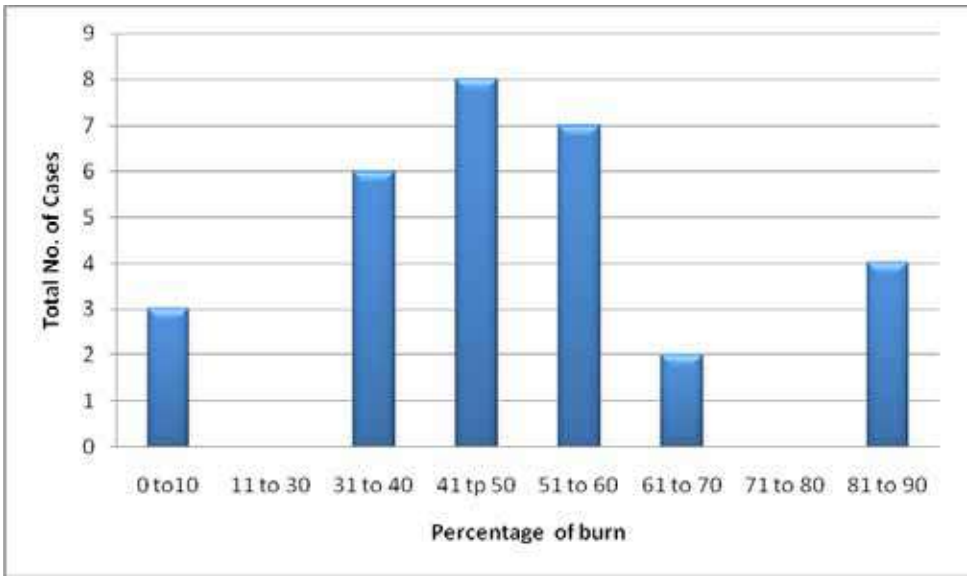


Fig. 2: Showing distribution of cases as per percentage of burns

During the study period total 30 cases were studied, 18/30 (60%) were males and rest 12/30(40%) cases were female showing male predominance. 7/30(23%) cases were found in age group of 4-5 years followed by 6/30 (20%) in age group of 2-3 years and 5-6 years. Age group 1-2 year and <1 year shows 3/30(10%) cases each. (Fig-1).

In majority of cases, 25/30 (83.30%) death was due to scald burns while in 3/30(10%) cases death was due to electrocution where as in 2/30 (6.66%) cases death was due to flame burn.

In our study maximum number of cases i.e. 8/30 (26.66%) cases shows 41-50% burns followed by 7 (23.33%) showing 51-60% burn. Whereas 4/30(13.33%) cases and 2/30(6.66%) cases showed 61-70 % and 81-90% of burns. 3/30(10%) cases shows burns less than 10%. In between 11-30% and 71-80% there were no burns cases. (Fig-2).

Cause of death was septicaemia in 21/30 (70%) cases followed by 6/30 (20%) and 3/30(10%) cases because of shock due to burns and shock due to electric burns respectively.

Discussion

Fire has been known to mankind for about 400,000 years. Although the use of fire was known to ancient man, it is probably the potential fury of an unharnessed fire that made man bow before it. India has an ancient culture where fire is worshiped since the civilization started. Along with water (jal), air (vayu), earth (prithvi), fire (agni) is perceived as one of the four basic components of universe.[7] As per ministry of health and family welfare, Government of India database[8], approximately 70 lakh people sustain moderate to severe burns in India every year. More than 7 lakh burn injuries require admission every year. About 80% of admitted cases are due to accidents at home i.e. kitchen related mishaps. Out of which 1.4 lakh peoples are succumbed to death. Amongst all traumas, burns have highest duration of hospital bed occupancy.[8]

In our study female were less affected than male, as 60% cases are male and 40% cases are female similar findings are also seen in other studies.[9,10] Over activity of male babies as compared to female babies may be the reason of male preponderance. In the study scald burn is more common seen in 83% of cases followed by electrocution in 10% cases and in 7% cases death was due to flame burn. Our this result is comparable with the study done by Lal St et al.[11] in which 54% cases shows scald burn. Scald is caused by boiling water, hot tea or milk. Young children usually sustain this injury due to their curious nature and unsteady movement. Electrocution is probably caused by naked wire carelessly kept in home. Out of two flame burn one was caused by accident while in other case mother burn herself with suicide intention keep baby along with her. All of the case in our study, incident occur in home that too mainly in kitchen. Home remains a dangerous place for children particularly in India due to lack of space to play, cook and storage of hot liquid.

Burns are the frequent cause of injury among children. Pediatrics burn injury can lead to severe physical, social, and psychological impairment. Burn injury results in longer hospitalization and a substantial cost of treatment. Pediatric burn injury can be more severe in comparison to the adults, because the skin is thinner. Even trivial burns in children can lead to dense scarring and contractures.[11]

Conclusion and Suggestions

This study shows that burns injuries can cause major mortality and morbidity to the children. Burn injuries caused to them are because of their curiosity and innocent nature and unable to understand the extent of damage that will be cause by it. As this injury mostly occurs in home, they are preventable. Preventive measures such as

Provision of better housing conditions and reduction of overcrowding, especially in the kitchen or around cooking area.

Improvement of cooking facilities and with provision of raised cooking platform at least 3 feet above the ground level, so that the children cannot reach up to hot utensils.

Burning and lighting agent, kerosene wick, Deepak, candles, should not be left unguarded and should be kept away from the reach of children.

By applying such measures and proper educating the people we can reduce the mortality and morbidity caused by burns in children.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Effect of Exposure of Smartphone Use on Visually Evoked Potential (VEP) in Healthy Adults

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Abstract

Aims: To assess and compare VEP parameter (amplitude and latency of P100) in healthy adults (MBBS students) using smartphone.

Objectives:

- To record P100 (latency, amplitude) in 1st year MBBS students using smartphone for less than three hours and for more than three hours after sunset
- To record P100 (latency, amplitude) MBBS students using smartphone for duration of less than three years and for more than three years
- To Compare P100 (latency, amplitude) 1st year MBBS students using smartphone for less than three hours and for more than three hours after sunset
- To Compare P100 (latency, amplitude) MBBS students using smartphone for duration of less than three years and for more than three years

Settings and Design: Neurophysiology Lab at the department of Physiology, JNMC, Sawangi (Meghe), Wardha.

Method and Material: A cross sectional study was done in 50 healthy MBBS studentsfrom JNMC.

Results: There is significant difference in the mean values of P100 latencies of right and left eye and in the mean values of P100 amplitude of right and left eye in individuals with exposure of less than three and more than three hours per day after sunset.

Conclusions: We conclude that nocturnal use of mobile phones especially in the bright light exposes an individual to the blue light resulting in various deleterious effects.

Keywords: *Nocturnal smartphone use, VEP (visually evoked potential), Latency.*

Introduction

Use of mobile phone as a token of electronic century and digital world has risen to a very high scale in humans and, especially nowadays, has become an integral part of the young generation. The technology has transformed mobile phones extensively since its inception as analogue phones and later being replaced, over the years, by digital ones, more precisely, the smartphones. Smartphone has voided the tools like calculator, mp3 player, Microsoft office, dextop browsers, book readers etc., and has taken over their places. Thus using smartphone has indirectly become the essential commodity.

The operating power of analogue phones is higher than digital phones, emitting more electromagnetic radiation (EMR). Operating frequencies of mobile phone are 450- 900MHz (analogue systems), 1800-1900MHz (digital systems) and 1900-2200MHz (Universal Mobile Telecommunications System).(1) Peak power output of

mobile phone antenna ranges from 8-15 W (450 MHz), 2W (900 MHz) and 1W (1800 MHz).[2] The "Specific Absorption Rate" (SAR) is the rate at which energy is absorbed by the human body. In the USA and India, the maximum level of SAR for smartphones set by governmental regulating agencies is 1.6W/Kg, averaged over a volume of 1g of tissue.[3,4]

Younger generation has become habitual to the smartphone and the usage starts from the morning till the person goes to sleep. Thus the smartphone is used almost continuously throughout the day and also after the sunset. During the use of smartphone, especially in the dark condition than the illuminated environment, electromagnetic radiations from antenna during a phone call and electromagnetic waves and bright light (especially the blue light) from the screen of smartphone during browsing causes more photo-mechanical damage, photo-thermal damage, and photo-chemical damage[5,6] make eyes more vulnerable to adverse effects because of their anatomy and composition (they are having a few blood vessels).[7,8] Blue light is an important factor emitted from smartphone and is known to generate poisonous molecules in the light sensitive cells of the eye and leads to macular degeneration and age related changes.[9] So it becomes a need of time to determining the deleterious effects of electromagnetic radiations generated from smartphone on the human visual system.

P100 latency prolongation appears to be the most reliable indicator of clinically significant abnormality, being least affected by technical factors and degree of patient co-operation. It appears to be generated by the pyramidal cells in layer IV of area 17.[10] Imaging studies point to the source of the early phase of the P100 peak as being in dorsal extrastriate cortex of the middle occipital gyrus, whereas the late phase of P100 appears to be generated by the ventral extrastriate cortex of the fusiform gyrus.[11] These results suggest the cortical generation of VEP waveforms. Thus VEP is useful in detecting the site of lesion when the conduction disturbance is in the anterior visual pathway.

The present study was planned to assess the effect of duration of usage of MP on human visual system by recording of pattern reversal visual evoked potentials (PRVEP).

Aim: To assess and compare VEP parameter (amplitude and latency of P100) in healthy adults (MBBS students) using smartphone.

Objectives:

1. To record P100 (latency, amplitude) in 1st year MBBS students using smartphone for **less than three hours and** for **more than three hours** after sunset
2. To record P100 (latency, amplitude) MBBS students using smartphone **for duration of less than three years and for more than three years**
3. To Compare P100 (latency, amplitude) 1st year MBBS students using smartphone for **less than three hours and** for **more than three hours** after sunset
4. To Compare P100 (latency, amplitude) MBBS students using smartphone **for duration of less than three years and for more than three years**

Materials and Method

Study Set-up: The present study was undertaken in the Neurophysiology Lab at the department of Physiology, JNMC, Sawangi (Meghe), Wardha.

Study Design: It was a cross sectional study, in which effect of exposure of smartphone was assessed in MBBS students using visually evoked potential (VEP).

Sample Size: A total of 50 subjects were studied.

Study Protocol: The subjects were selected by random method of sampling and studied after obtaining the approval from institutional ethics committee to carry out the research work at Jawaharlal Nehru Medical College (JNMC) and Acharya Vinoba Bhave Rural Hospital (AVBRH), Sawangi (Meghe), Wardha. The study group consisted of MBBS students from JNMC. Every Subject included in the study was examined in details after taking a detailed clinical history and brief general examination.

Inclusion Criteria:

- Apparently healthy MBBS students including Males and females.
- Age group between 18-23 years.
- Subjects with normal visual acuity or corrected by the glasses.

Exclusion Criteria:

- Presence of any illness that could influence visual evoked potentials.

- Best corrected visual acuity worse than 6/60.
- Extreme pupil sizes.
- History of major illness like diabetes, hypertension, etc.
- Any evidence of diabetic retinopathy, optic atrophy, glaucoma, etc.
- Alcoholics, smokers, tobacco users or with any other addictions.

Subjects were grouped in four categories based on duration of exposure of smartphone as

Group IA: Duration of exposure of smartphone less than three hours after sunset

Group IB: Duration of exposure of smartphone more than three hours after sunset

Group IIA: Duration of exposure of smartphone less than three years

Group IIB: Duration of exposure of smartphone more than three years

All the subjects selected for study were subjected to a detailed history taking by means of planned questionnaires including special enquiries regarding complaints of dimness of vision, tingling, paresthesia, addiction to alcohol, smoking, tobacco chewing. All the subjects underwent brief ocular examinations like general examination of eyes, recording of visual acuity and ocular tension. Subjects underwent careful systematic neurological examination for the evidence of peripheral neuropathy. Motor power and reflexes were checked for motor neuropathy. Previous reports, if any, for any ailment were assessed.

For the best results of VEP testing, subjects were explained about the test in a language he/she can understand to ensure the full co-operation. The usual glasses were, if any, were asked to put on during the test. The results of ocular examination were reviewed before starting the test. Subjects were advised to come without applying oil to the scalp. Preparation of the scalp skin was done with 'Nuprep® skin prep gel' before the study. The subjects were instructed to avoid any meiotic or mydriatic drug, if any, 12 hours before the test.

Running the Test: VEP was performed on Neuro-**MEP-4 : 4-channel NCS, EMG and Multi-modality EP system Neurosoft** machine.

Stimulus parameter (Pattern Reversal Stimulus):

- **Type and method of generation** – Computer generated patterns on a video monitor.
- **Type of pattern** – Black and white checkerboard pattern
- **Size of pattern element** – 32 minutes
- **Size of stimulus field** – 20 degrees
- **Stimulus type** – Full field stimulation with fixation point in the centre and pattern extending to both the sides equally.
- **Mode of pattern presentation** – Mode of stimulus presentation was pattern reversal in which dark and light pattern elements were alternately reversed
- **Stimulus rate** – 1.71 Hz (cycles per second)
- **Mono-ocular versus Binocular stimulation** – Mono-ocular stimulation was performed with each eye tested separately
- **Distance between the subject and the screen** – 100cms

Recording Parameters:

System Bandpass – 2-200 Hz

Analysis Time – 250 ms

Amplifications- 20,000-1,00,000

Recording Electrodes – Standard disc surface electrodes

Electrode placement – International 10-20 system placement with Active electrode (Oz), Reference electrode (Fz) and Ground electrode (Fpz)

Number of Epochs – 100

Replications – Two responses were recorded and superimposed for the reproducibility of the waveform. The replicated response measurements with P100 latency, within a 2.5 ms difference and peak-to-peak amplitude of N75-P100 or P100-N145, within 15% difference were accepted. (12, 13)

Methodology

The subject was explained about the procedure. After the preparation of scalp skin by abrading and degreasing properly, the electrodes were placed at O, Fz, and Fpz sites using the 'Nuprep® skin prep gel' as

per 10-20 international system. Uniform illumination was maintained for every subject studied. Distance between the TV screen and the subjects eye was kept constant at 100 cms. Subject was instructed to fix his gaze at the central spot on the TV screen. Mono-ocular stimulation was done and each eye tested separately with an eye-patch covering the other eye. With the preset stimulus and recording conditions as mentioned above and keeping the electrode impedance <5 KΩ, the recording procedure was started. Two responses from each eye were recorded and superimposed to ensure the reproducibility.

Statistical Analysis: All the data was expressed as mean ± S.D. The significance of difference between groups was calculated by Fisher's Exact Test of analysis on SPSS software version 24.

Observations and Results

Table 1. Comparison of Latency and amplitude in individuals with exposure of smartphone for less than three and more than three hours per day after sunset

Parameters	Duration of exposure in Hours per day		Significance
	<3Hrs/day Exposure	>3Hrs/day Exposure	
P100 Latency (Right Eye)	98.41	109.56	0.000
P100 Latency (Left Eye)	98.72	110.12	0.000
P100 Amplitude (Right Eye)	7.96	4.56	0.004
P100 Amplitude (Left Eye)	7.96	4.13	0.000

Table 2. Comparison of Latency and amplitude in individuals with exposure of smartphone for less than three and more than three years.

Parameters	Duration of exposure in years		Significance
Duration of exposure	<3Years of Exposure	>3 Years of Exposure	
P100 Latency (Right Eye)	103.41	104.24	0.696
P100 Latency (Left Eye)	103.86	104.72	1.000
P100 Amplitude (Right Eye)	6.67	6.00	0.741
P100 Amplitude (Left Eye)	6.48	5.72	1.000

Table 3. Inter-ocular differences in Latency and amplitude

Parameters / Duration of exposure	P100 Latency (Right Eye)	P100 Latency (Left Eye)	P100 Amplitude (Right Eye)	P100 Amplitude (Left Eye)
<3Hrs/day Exposure	98.41	98.72	7.96	7.96
>3Hrs/day Exposure	109.56	110.12	4.56	4.13
<3Years of Exposure	103.41	103.86	6.67	6.48
>3 Years of Exposure	104.24	104.72	6.00	5.71

From the results, it is obvious that there is significant difference not only in the mean values of P100 latencies of right and left eye as well as in the mean values of P100 amplitude of right and left eye in individuals with exposure of less than three and more than three hours per day after sunset. Our study is consistent with the findings of a study done to assess the effect of exposure of smartphone for less than three per day after sunset.[14-18] But our study couldn't find any significance in comparing the mean values of P100 latencies of right and left eye as well as in the mean values of P100 amplitude of right and left eye in individuals with exposure of less than three and more than three years of exposure.

Also we could find the inter-ocular differences in the values P100 latencies and P100 amplitude and the findings are consistent with many studies. [19-23]

Discussion

VEP stands to be an important procedure for assessing visual function. Though VEP can detect abnormality along the visual pathway ranging from the eye, retina, the optic nerve, optic radiations, upto the occipital cortex,[24] it appears to be highly sensitive to lesions of the optic nerve and anterior chiasm[25] *i.e.* to optic neuropathies (26), demyelination[27], multiple sclerosis [28, 29]. The activation of visual cortex primarily occurs by the central visual field.

The latency of VEP components is indicative of extrastriate source of defects rather than primary visual pathways. For instance, difficulties in motion detection, or "optic flow", seem to be especially prominent and may be explained by parallel defects in primary magnocellular stream and extrastriate visual processing. [30-31] The commonest cause of prolonged P100 latency is demyelination in the optic pathways.[32] A power density dependent decrease in latency of some of the late components of thalamic evoked potentials by affecting multisynaptic neural pathways. The amplitude of P100 has a wide inter-individual variability reducing its clinical utility.Conditions leading to axonal loss such as ischemic optic neuropathy produce decreased amplitude.

The inter-ocular asymmetry can be attributed to small differences in visual acuity, dominance of eye, changes in the alertness, and eye movement during the recording. These differences are usually small. Intraocular amplitude and peak latency analysis increases the sensitivity of the VEP to monocular diseases since each subject serves as his own control and may reveal abnormality.[19,20] The disparities between the dominant and the non-dominant eyes seem to be the presence of lateralization in the Central Nervous System [21] and neuro-anatomic asymmetries of the human striate cortex. [22] Proximity of the right eye to the cell phone and feeling greater heat and more radiation power also has been found to be the reason for inter ocular asymmetry.[23] (Table no. 3).

That blue light has a greater tendency to affect living organisms by disrupting biological processes that rely on natural cycles of day and night Therefore, light rich in short-wavelength (blue) light should be avoided in the late evening to promote a healthy night-time lighting environment. Apoptosis plays a major role in retinal visual cell loss.[38-44] The caspase-dependent as well as caspase-independent pathways, over stimulation of Poly [ADP-ribose] polymerase 1 (PARP-1) through oxidant-induced DNA damage, cleavage of PARP-1 inhibiting the enzyme responding to DNA damage along with Retinal endo-illuminator toxicity can induce apoptotic cell death.

Conclusion

Thus we conclude that nocturnal use of mobile phones especially for more than three hours per day for more than three years at least and especially in the bright light exposes an individual to the short wavelength light (blue light) resulting in various deleterious effects. Hence, combating the unhealthy effect of blue light from smartphone displays at night does not simply mean turning off the smartphone, but rather using well designed surge protection devices (SPDs), using smartphone at the proper distance, optimally adjusting the brightness setting in a dark room and the proper intensity of smartphone displays to see better where and when required while protecting people's health and circadian rhythm. Similarly if the blue light in smartphone displays in the night, if controlled finely, can have a greater impact on reducing the unhealthy effect like disturbed circadian rhythm due to decreased melatonin secretion. As this is a pilot study and sample size is small, further research in this regards is necessary.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Metabolic and Cardiovascular Responses to External Weights During Treadmill Walk in Overweight Young Adults

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Abstract

Aim and Objective: Compare the physiological responses within bouts of aerobic exercise varying in intensity and the presence of wearable weights.

Study Design: Interventional study

Settings: Exercise Physiology Lab at the department of Physiology, JNMC, Sawangi (Meghe), Wardha.

Method and Material: Forty (18 females, 22 males, mean age = 22.23 ± 1.32) healthy volunteers were tested for aerobic fitness on a treadmill to determine VO2. Participants completed eight 30-minute walking trials on a treadmill and oxygen consumption (VO2) and heart rate (HR) were monitored while walking at different speeds and with varying combination of upper and lower body external weights. The design included two intensities (slow walking and brisk walking) and four conditions (no weights, arm weights, leg weights, and arm and leg weights) for a total of eight experimental trials.

Statistical Analysis Used: ANOVA and pair-wise comparisons on SPSS software version 24.

Results: VO2 was significantly lower without the wearable weights in comparison to wearing both upper and lower weights in the slow walk trial ($P < 0.001$; ES = 0.74) and also during the brisk walk trial ($P < 0.001$; ES = 0.35). HR was significantly higher during the brisk walk trials with external weights on both the arms and legs (P=0.018, ES=0.37).

Conclusions: Findings suggest that the metabolic responses were significantly increased wearing external weights on both the arm and leg during walking exercise on treadmill and increasing energy expenditure (burning additional calories) over the 30 minute session and have minimal impact on heart rate.

Keywords: *External weights, VO_2Max, Heart rate, treadmill.*

Introduction

Obesity has become one of the major health issues over the world. Worldwide obesity has nearly tripled since 1975. In 2016, more than 1.9 billion adults, 18 years and older, were overweight. Of these, over 650 million were obese. 39% of adults aged 18 years and over were overweight in 2016, and 13% were obese.[1]

Overweight and obesity have become the major health problem, in addition to physical inactivity, in almost all the age groups particularly in urban areas with serious medical and psycho-social consequences, replacing the more traditional public health concerns. Though low prevalence of obesity among Indian children (3-2%) may be surprising, but the relatively high prevalence of overweight (14-9%) is alarming.[2]

Overweight children often become overweight adults[3] and overweight in adulthood is a health risk. Large cohorts from various populations have demonstrated the important influence of childhood BMI on future cardiovascular health. Juvenile obesity is particularly alarming because it is considered to be a key predictor for obesity in adulthood[4], and its prevention

and treatment have a remarkable clinical relevance [5]. Several disorders have been linked to overweight and obesity in childhood. Obesity results in considerable morbidity and mortality, of which cardiovascular disease and type 2 diabetes mellitus remains one of the principal causes.[2]

Physical activity has been the integral part of human body and it should naturally be part of our everyday life. Because of a more modern and demanding lifestyle, physical activity has declined, becoming less important than other activities such as work, family, or social responsibilities. Regular physical activity is associated with various health benefits. Active individuals can lower their risk of coronary heart disease, hypertension, colon cancer and diabetes by being active for thirty minutes a day. Exercise also helps maintain a healthy weight, stronger bones, muscles and joints, reduce body fat and increase lean muscle. Also regular exercise can decrease anxiety and depression while improving mood[6].

Methodology

Study design: Interventional study

Settings: Neurophysiology Lab at the department of Physiology, JNMC, Sawangi (Meghe), Wardha.

Participants: 40 participants (22 boys and 18 girls) ranging from 18-25 years were recruited. Each participant was asked to walk on the treadmill to determine the exercise intensities for subsequent exercise trials.

Familiarization with the external weights included instruction on proper size, location, and fit for the ankles and wrists. The purpose of this portion of the trial was to provide exposure to the external weights prior to the experimental manipulation to limit the perceptual impact of wearing a novel device.

Following setting workload and familiarization, the participants was informed about the exertion assessment scale that was to be used throughout the research study. Borg's 6-20 Rating of Perceived Exertion scale (RPE) [7] was explained in full detail to each participant, and the participants were then required to confirm that they understood the tool clearly.

Sample size: Sample size was calculated as fourty (40) using formula $4PQ/L^2$ where prevalence of overweight adults has been taken as 11.3% [8]

Description of Laboratory Visits:

Visit Description:

1. Screening to include a physical exam, informed consent, and resting assessments
2. Maximal treadmill test
3. Setting Workload and Familiarization with treadmill and external weights
4. 4-7 Experimental exercise trials

Screening (Visit 1): The screenings included a comprehensive health history, pre-participation physical exam administered by a physician, completion of the informed consent document, and assessment of resting heart rate, weight, height, and blood pressure.

Maximal Exercise Testing (Visit 2): Each participant was complete a graded exercise maximal treadmill test that included measurements of heart rate, blood pressure, perceived exertion, and metabolic gas exchange. The "Health and Exercise Science (HES)" protocol[9] was used for this test consists of a starting speed of 3.0mph on the treadmill with speed increases of 0.5 every minute afterward. Heart rate and RPE were recorded every minute; blood pressure was recorded every three minutes. When participants reached a speed of 7.0 (females) or 8.0 (males) on the treadmill, the incline was then increased by 2% every minute thereafter, with no additional increases in speed. Participants were encouraged to go until maximal effort and exhaustion was achieved.

Setting Workload and Familiarization (Visit 3): Each participant was asked to walk on the treadmill to determine the exercise intensities for subsequent exercise trials.

One workload corresponds to a "slow walk" which was designed to replicate walking that was associated with activities of daily living.

The second workload corresponds to a "brisk walk" which was designed to replicate walking that was purposeful and associated with fitness. Workload establishment of the two separate speeds lasted approximately 30 minutes with 15 minutes designated to each walking speed. Collectively, the two workloads were self-selected and were intended to reflect public health recommendations related to lifestyle physical activity.

Familiarization with the external weights included instruction on proper size, location, and fit for the ankles and wrists (3 Kgs, **AURION** Wrist/Ankle External weights Home Gym Weight Bands commercially available on **AMAZONE**) [10, 11]. The purpose of this portion of the trial was to provide **exposure to the external weights prior to the experimental manipulation to limit the perceptual impact of wearing a novel device**. Following setting workload and familiarization, the participants were informed about the exertion assessment scale that was to be used throughout the research study. Borg's 6-20 rating of perceived exertion scale (RPE) was explained in full detail to each participant, and the participants were then required to initiate that they understood the tool clearly.

Experimental Exercise Trials (Visits 4-7): The four experimental trials were allowed for both exercise intensities to be tested across four equipment conditions. The two exercise intensities examined were the "**slow walk**" and "**brisk walk**". The four equipment conditions included: no external weights, ankle and wrist external weights, wrist external weights only, and ankle external weights only (see Table).

Prior to each exercise trial, the metabolic cart was properly calibrated by the research team. A heart rate monitor was supplied for each participant upon arrival to the laboratory. A warm-up of 30 seconds was preceded every exercise trial at a speed of 2.5 mph on the treadmill. At the conclusion of the warm-up, the speed was adjusted to either to a self- selected "slow" or "brisk" walking pace as previously determined, and was dependent upon what condition the participant will be assigned for that day. Every six minutes heart rate and rate of perceived exertion for the ankles, chest/breathing, and overall were assessed. At minute 24 of the exercise trial maximal heart rate achieved and perceived exertion of the ankles were recorded from minute 24 to minute 29 at an interval of 1min.

At 30 minutes, a 30 second cool down at 2.5mph on the treadmill was transpired. Once the treadmill was stopped by the research team, immediate post exercise perceived exertion was taken and again ten minutes afterwards.

Thus Heart rate and oxygen consumption were investigated across self- selected two intensities (slow and brisk) with four different combinations of External weights (Table 1) and eight balanced experimental trials (walking for thirty minutes at a self- selected slow or brisk speed with either no togs, arms only, legs only, or both arm and leg togs) were incorporated.

Table 1: Experimental Trial Conditions

Intensity		External weights			
Slow Walk	Brisk Walk	None	Wrist & Ankle	Ankle Only	Wrist Only

Inclusion Criteria:

- Age between 18-25 years
- BMI 23 – 24.9 kg/m^2 [12]
- Participants not doing regular physical exercise
- Participants giving consent

Exclusion Criteria:

- Age between <18 and >25 years
- BMI between < 22.9 and >25 kg/m^2
- Participants having cardiovascular, metabolic, pulmonary diseases and related symptoms
- Participants doing regular exercise
- Patients not following the guidelines of this study
- Participants not giving consent

Outcome Measures:

- Cardiovascular – Heart rate
- Metabolic - VO_{2max}

Statistical Data Analysis: The research design utilized two intensity namely slow walk and vigorous walk and four conditions namely no external weights, wrist and ankle-external weights, ankle-external weights only, wrist-external weights only. Repeated measures ANOVA test for statistical analysis was applied. Each participant served as their own control. Each p-value will be<0.05.

Results

Table 2: Demographic profile of the participants (n = 40)

	Minimum	Maximum	Mean ± SD
Age	18	25	22.23±1.32
Height (inch.)	59	71	64.84±2.11
Weight (lbs.)	116	194	161.0±23.9
BMI	23.82	24.87	24.35±0.65
Slow speed	1.8	3.1	2.45±0.55
Brisk speed	3.2	4.6	3.91±0.70

Table 3: $VO2_{max}$ Data: Descriptive statistics (n = 40)

Treadmill speed–external weights	Mean ± SD
Slow-none	12.11 ± 1.43
Slow-arms	12.81 ± 1.45
Slow-legs	11.98 ± 1.81
Slow-both	12.83 ± 1.42
Brisk-none	14.97 ± 2.12
Brisk-arms	15.64 ± 2.32
Brisk-legs	15.92 ± 1.98
Brisk-both	16.12 ± 2.69

Table 4: Follow-up Comparisons (VO2)

Variable 1	Variable 2	P-value	Effect Size
Brisk-none	Brisk-both	<0.001	0.35
Brisk-none	Brisk-arms	0.876	0.39
Brisk-none	Brisk-legs	0.006	0.34
Brisk-both	Brisk-arms	0.001	0.56
Brisk-both	Brisk-legs	0.372	0.98
Brisk-arms	Brisk-legs	0.001	0.76
Slow-none	Slow-both	<0.001	0.74
Slow-none	Slow-arms	0.546	0.09
Slow-none	Slow-legs	0.008	0.33
Slow both	Slow-arms	<0.001	0.34
Slow-both	Slow-legs	0.006	0.87
Slow-arms	Slow-legs	0.328	0.45

The results showed that comparison between "VO2 during the "slow" walk with and without external weights on the **arms**." (p=0.546); "VO2 during the "brisk" walk with and without external weights on the **arms**" (p=0.876); "VO2 during the "brisk" walk with external weights on both the **arms and legs** and that with external weights on the **legs only**" (p=0.372); and "VO2 during the "slow" walk with external weights on the **arms** that with external weights on the **legs**" (p=0.328) had no significant difference.

But the results showed that comparison between "VO2 during the "slow" walk with and without external weights on the **legs**" (p=0.008); "VO2 during the "brisk" walk with and without external weights on the **legs**" (p=0.006); "VO2 during the "slow" walk with and without external weights on both the **arms and legs**" (p<0.001); "VO2 during the "brisk" walk with and without external weights on both the **arms and legs**" (p<0.001); "VO2 during the "slow" walk with external weights on both the **arms and legs** that with external weights on the **arms only**" (p<0.001); "VO2 during the "brisk" walk with external weights on both the **arms and legs** and that with external weights on the **arms only**" (p=0.001), and lastly "VO2 during the "slow" walk with external weights on both the **arms and legs** and VO2 during the "slow" walk with External weights on the **legs only**" (p=0.006) showed a significant difference.

Heart Rate:

Table 5: HR Data: Descriptive statistics (n = 40)

Condition	Mean±SD
Slow-none	96.87±10.34
Slow-arms	97.23±12.01
Slow-legs	108.89±8.25
Slow-both	103.26±14.980
Brisk-none	116.87±11.89
Brisk-arms	117.12±10.87
Brisk-legs	119.11±14.68
Brisk-both	119.95±13.48

Table 6: Follow-up Comparisons (Heart Rate)

Variable 1	Variable 2	P-value	Effect Size
Brisk-none	Brisk-both	0.018	0.37
Brisk-none	Brisk-arms	0.656	0.23
Brisk-none	Brisk-legs	0.002	0.26
Brisk-both	Brisk-arms	0.044	0.65
Brisk-both	Brisk-legs	0.621	0.81
Brisk-arms	Brisk-legs	0.035	0.19
Slow-none	Slow-both	0.003	0.21
Slow-none	Slow-arms	0.629	0.96

Variable 1	Variable 2	P-value	Effect Size
Slow-none	Slow-legs	0.041	0.65
Slow both	Slow-arms	0.034	0.21
Slow-both	Slow-legs	0.214	0.36
Slow-arms	Slow-legs	0.023	0.73

The results showed that comparison between "heart rate during the "slow" walk with and without external weights on the **arms**." (p=0.629); "heart rate during the "brisk" walk with and without external weights on the **arms**" (p= 0.656); "heart rate during the "brisk" walk with external weights on both the **arms and legs** and that with external weights on the **legs only**" (p= 0.621); and "heart rate during the "slow" walk with external weights on the **arms** that with external weights on the **legs**" (p=0.083) had no significant difference.

But the results showed that comparison between "heart rate during the "slow" walk with and without external weights on the **legs**" (p= 0.041); "heart rate during the "brisk" walk with and without external weights on the **legs**" (p= 0.002); "heart rate during the "slow" walk with and without external weights on both the **arms and legs**" (p<0.003); "heart rate during the "brisk" walk with and without external weights on both the **arms and legs**" (p<0.019); "heart rate during the "slow" walk with external weights on both the **arms and legs** that with external weights on the **arms only**" (p< 0.034); "heart rate during the "brisk" walk with external weights on both the **arms and legs** and that with external weights on the **arms only**" (p= 0.044), "heart rate during the "slow" walk with external weights on **arms only** and that with external weights on the **legs only**" (p=0.023) and lastly "heart rate during the "brisk" walk with external weights on **arms only** and that with external weights on the **legs only**" (p=0.035) showed a significant difference.

Discussion

The present experiment is designed to examine the cardiovascular and metabolic responses of walking with external weight worn on the wrists and ankles. Results showed a significant increase in oxygen consumption while wearing External weights during exercise. Wearing these additional weights while walking elicited a linear rise in oxygen consumption, and more specifically, the largest responses were seen during trials with External weights on both the arms and legs.

An approximate 13% increase in energy cost was seen during the "slow" walk when both sets of external weights were added to the body while about 16 extra calories were burned throughout the walking trial. Furthermore, about 12% increase in energy cost was also seen when adding arm and leg external weights during the "brisk" trials. When walking at a "brisk" speed with no external weights, participants burned, on average, about 184 calories. When arm and leg External weights were added, it resulted in an average of 23 more calories burned over thirty minutes. But there was a little change in heart rate while wearing External weights. From resting heart rate to maximal heart rate, heart rate increased about four times, and the resting oxygen consumption had increased up to sixteen times during the brisk walking trials. Thus the significant increase in heart rate is relatively difficult to see. It still suggested the metabolic adjustments produced by the addition of External weights. [10] There was significant increase in heart rate and oxygen consumption with wrist weights, explaining that walking with wrist weights increased the energy cost more than the ankle weights.

Wearing external weights during exercise by an overweight individual can easily overcome the discouragement he/she experiences from the amount of maintenance required to stay healthy and fit leading to higher self-efficacy in the exerciser. This also prevents further weight gain by making small increases in physical activity.

As we are solely looking at a low-risk healthy population of young adults ranging 18years to 25years, therefore the findings cannot be generalized to other groups such as the elderly, adolescent or hypertensive. Thus we recommend for further research on individuals with different fitness levels and using a more intense exercise modality like running or cycling. Also, the External weights implicate small changes for those individuals who are sedentary, and may not provide a large metabolic response due to the little amount of weight that they add. Therefore, it is difficult to consistently report such a sensitive amount of change in oxygen consumption.

Conclusion

The metabolic responses were significantly increased wearing external weights on both the arm and leg during walking exercise on treadmill and burn additional calories over the 30 minute session, eliciting an approximate 12% improvement over exercise sessions

when no external weights were worn. Significant increase in oxygen consumption and caloric expenditure was observed by wearing the external weights, but the cardiovascular responses were not significant at both the slow and brisk speeds. External weights can thus be a practical and promising tool to be implemented into the fitness industry and rehabilitation centers.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Assessment of the Financial Status in Adult Diabetic Patients Attending the Diabetic Clinic in Bankura Sammilani Medical College, Bankura

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Abstract

Introduction: Diabetes and its complications impose significant economic consequences on individuals, families, health systems and countries. People living with diabetes and their families feel the impact of diabetes most directly, often through the expenses of diabetes treatment and loss of family income when diabetes interferes with work.

Objective: To assess the financial status of adult diabetic patients attending the diabetic clinic of Bankura Sammilani Medical College, Bankura.

Materials and Method: It is an Institution based, Cross-sectional, descriptive Study. Complete enumeration of all patients attending Diabetic clinic of Bankura Sammilani Medical College and hospital, Bankura, West Bengal was done. Eligible consenting of adult patients (> 18 years of age) who were diagnosed as a case of diabetes at least 1 year back and on treatment were interviewed with review of medical record and Out-of pocket expenditure information was collected in terms of INR. It views the cost of NCDs as the sum of several categories of direct and indirect costs. The study was preceded after obtaining ethical clearance. Data were entered in MS Excel spread sheet. Calculation was done with the help of software SPSS 22.0 free version.

Results: Majority of annual treatment expenses of study subjects were in Drugs and consumables (66.73%) followed by Investigation cost 15.19% and others. The average INR for annual total diabetes care was 13,733.17±13,389.86.

Conclusion: Drugs and consumables, investigation and transportation cost leads to more than 90.0% of out-of pocket expenditure causing financial burden on both adult diabetic patient and its family.

Keywords: *Adult diabetic, direct cost, indirect cost, out-of pocket expenditure.*

Introduction

All types of diabetes are on the increase, type 2 diabetes in particular: the number of people with diabetes will increase by 55% by 2035. In human as well as financial terms, the burden of diabetes is enormous, provoking 5.1 million deaths and taking up some USD 548 billion dollars in health spending (11% of the total spent worldwide) in 2013. In India the total number of diabetic population in 2013 is 65.10 millions and estimated around 109.00 millions in 2035 (20-79 years)[1].

Diabetes affects all segments of the population and

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is one of the leading causes of premature morbidity and mortality, and requires life-long healthcare services. Poverty, low education, and gender discrimination adversely affect women with diabetes. About 90% of all respondents did not test their blood glucose regularly and suffered from several complications with low medication adherence and poor clinic attendance. Information on the availability, cost, and quality of medical care for diabetes is generally not available[2].

Diabetes and its complications impose significant economic consequences on individuals, families, health systems and countries[3]. People living with diabetes and their families feel the impact of diabetes most directly, often through the expenses of diabetes treatment and loss of family income when diabetes interferes with work[4].

Besides the health expenditures, other measurements have also been used to assess the economic impact of diabetes. First, diabetes also imposes a large financial burden on people with diabetes and their families. The size of this burden depends on their economic status and the social insurance policies of the countries in which they live. Second, at the societal level, diabetes leads to loss in productivity and economic growth. Finally, the largest economic burden caused by diabetes is the monetary value associated with disability and loss of life as a result of the disease itself and its related complications, including heart, kidney, eye and foot disease[5].

The National Rural Health Mission[6] (NRHM) launched in 2005 and the new pilot National Programme for prevention and control of Diabetes, Cardiovascular diseases and Stroke[7] (NPDCS) offer opportunities for improving care for diabetes and other non-communicable diseases through service provision at the primary and secondary levels of care.

Availability and affordability of anti-diabetic medication is another problematic aspect, in spite of "The National List of Essential Medicines" identifying glibenclamide, metformin, and insulin (soluble and lente) as anti-diabetic drugs that need to be available universally[8]. The availability of glibenclamide in public health facilities, for example, varied from 100 percent in Karnataka to 3.8 percent in West Bengal[9]. Given the uncertainty of availability of medicines, and the lack of pricing control over the private sector, compliance with medication becomes a serious issue[10-14].

Materials and Method

An Institution based, Cross-sectional, descriptive Study was done in Diabetic Clinic of Bankura Sammilani Medical College and hospital, Bankura, West Bengal which runs once in a week on every Thursday and approximately 20 to 30 patients attend the OPD day per week. Total duration of study was of 20 months from May 2014 to December 2015. Patients who were diagnosed as a case of diabetes in private clinic or other department of the B.S.M.C and hospital and/ or on treatment on anti-diabetic drugs were enrolled as a new patient on first visit of the patient in Diabetic Clinic. Adult patients (> 18 years of age) attending Diabetic clinic and who were diagnosed as a case of diabetes at least 1 year back and was on treatment are study subjects. All eligible patients who give informed or verbal consent attending the Diabetic clinic are taken in the study during data collection period. On an average of 5-6 patients were newly enrolled in register per week on Diabetic clinic. During the data collection period all eligible patients attending the diabetic clinic, were taken as study population and 150 study subjects were taken. The study was proceeded after obtaining ethical clearance from the institutional ethics committee and Diabetic clinic under Medicine Department. Pretesting of Schedule was done in 20 patients attending medicine OPD and were excluded from the final data collection. Any difficulty that arose during pretesting was corrected in the final questionnaires.

Result

84.0% of subjects did not have to pay for annual Consultation charges. 16.0% of subjects had to payfor Consultation charges at private clinic. Even 2.7% of them had to pay INR of ≥2000/-. The Mean INR for annual consultation charges was 221.61±593.65 with arange of 0.00-3500/-INR.

Majority of the subjects (43.3%) had to pay 1000-2000 rupees every year for investigations (outdoor annually) due to diabetes and associated conditions. The Mean INR for annual investigation cost was 1972.33±1788.43/- with a range of 0-12000/-INR.

One fourth of study subjects had to (27.3%) pay for more than 10,000 rupees in a year for Drugs and Consumables(outdoor annually). The Mean INR for annual cost on Drugs and Consumables was 8931.93±10554.39/-and a range of INR for annual cost on Drugs and Consumables was 0-96000/-.

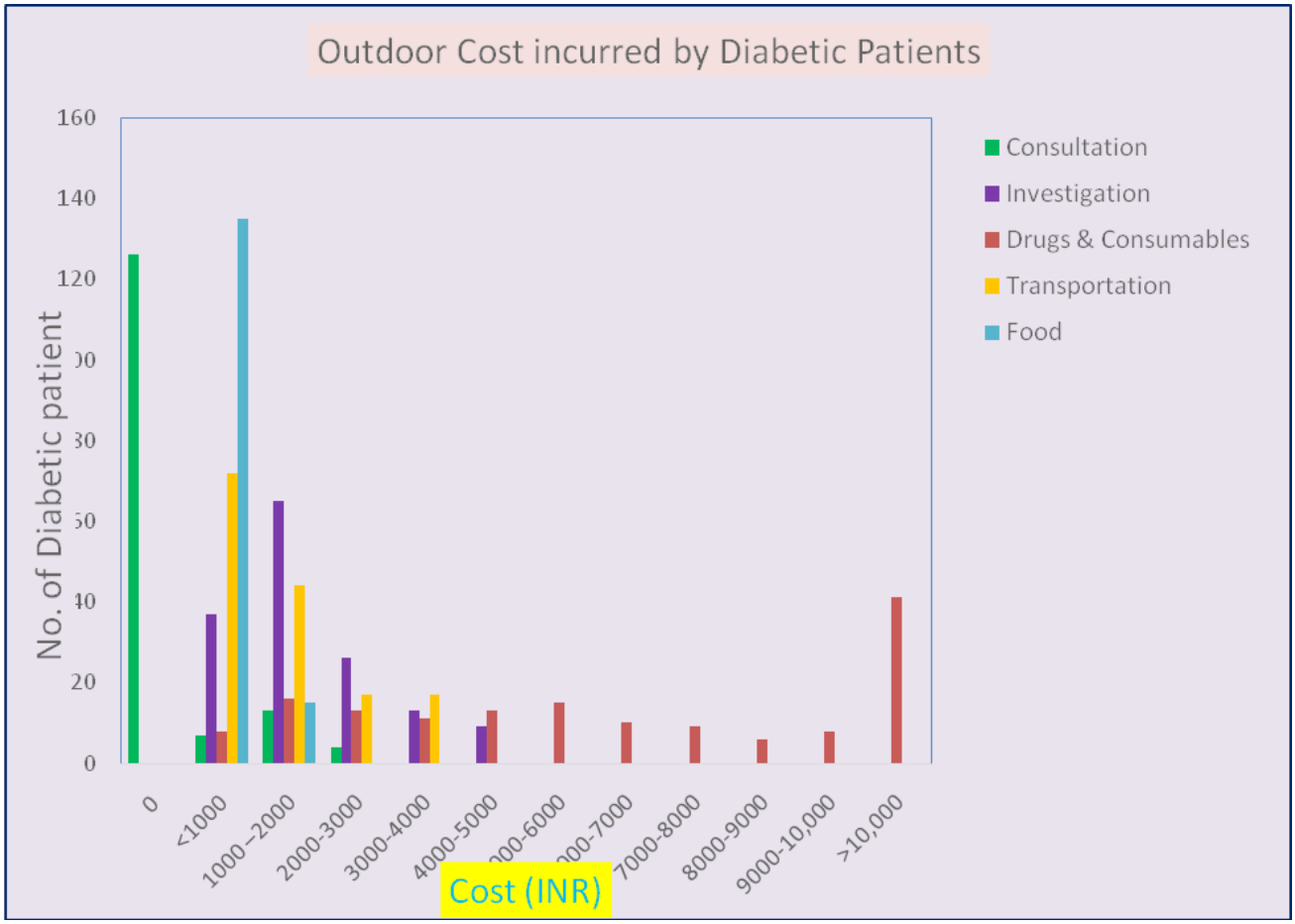


Figure 1: Distribution of study subjects according to annual expenditure on different outdoor cost by Adult Diabetic Patient (n=150)

Table No. 1: Distribution of study subjects according to Total annual cost (outdoor) (n=150)

Treatment	Outdoor Cost (INR)	Indoor Cost (INR)	Total Cost (INR)	Percentage
Doctor Consultation cost	33,020/-	0.0	33,020/-	1.59
Investigation cost	2,95,850/-	19,250/-	315100/-	15.19
Drugs and consumables cost	13,39,790/-	43,670/-	1383460/-	66.73
Transportation cost	2,42,676/-	20,870/-	2,63,546/-	12.72
Food cost	59,220/-	17,990/-	77,210/-	3.72
Surgical cost	0.0	1000/-	1000/-	0.05
Total cost	19,70,556/-	1,02,780	20,73,336/-	100.0

Majority of annual treatment expenses of study subjects were in Drugs and consumables (66.73%) followed by Investigation cost 15.19%, Transportation cost 12.72%, Food cost was 3.72% and least by Consultation cost was 1.59%. The mean INR for annual total diabetes cost was 13,733.17±13,389.86/-, the median INR was 10,520.00±9,682.50/- and the range of INR for annual total diabetes cost was 520-1,21,200/-.

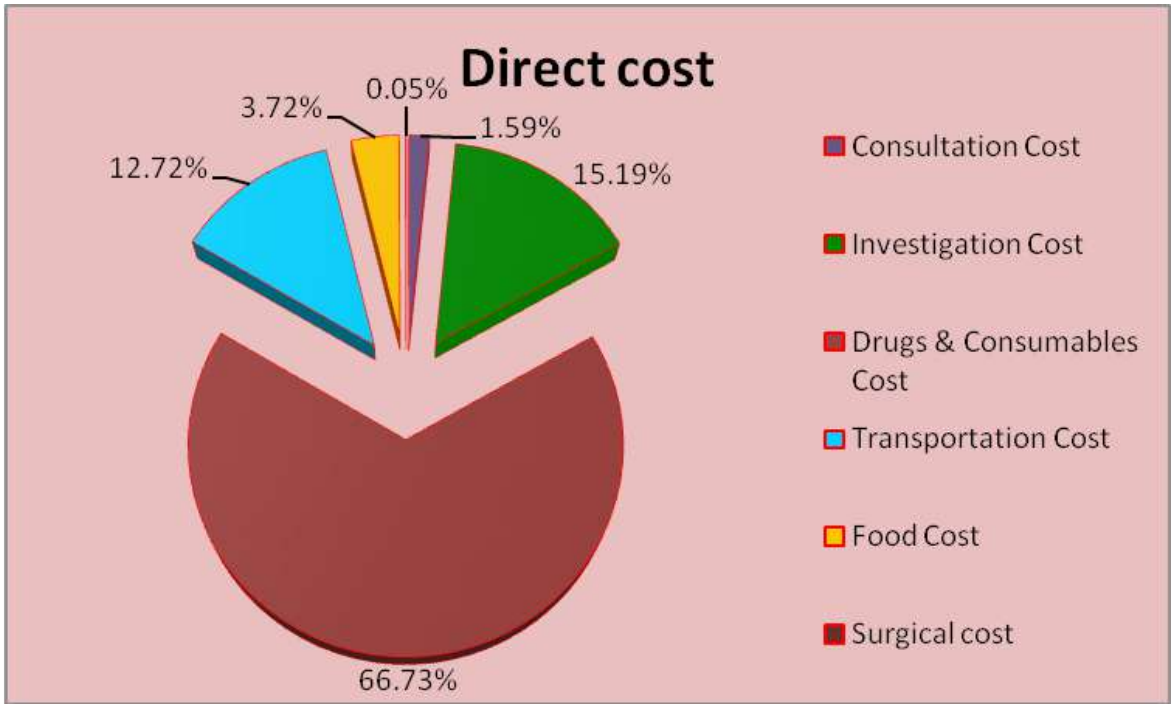


Figure 2. Distribution of study subjects according to Total Direct cost (n=150)

Majority of treatment expenses were in Drugs and consumables followed by Investigation cost 15.01% and then Transportation cost was 12.31%. Only 10.0% of subjects were hospitalised during the last one year for diabetes associated complications. According to duration of stay in hospital, 66.7% of subjects were admitted to hospital for 1-7 days in the last one year.

Table No. 2: Distribution of study subjects according to Total Hospital admission cost in last one year (n=15)

Treatment	Cost (INR)	Percentage
Investigation cost	19,250/-	18.72
Drugs and consumables cost	43,670/-	42.48
Transportation cost	20,870/-	20.30
Food cost	17,990/-	17.50
Surgical cost	1,000/-	1.0
Total cost	1,02,780/-	100.0

During hospitalization due to diabetes and associated complication, 42.48% of cost was spend on Drugs and Consumables followed by 20.30% in transportation and 18.72% for investigation and 17.50% for Food cost. The mean INR for hospitalization due to diabetes and its complication was 643.33±2411.06/-, the Range of INR for hospitalization was 0.0 -17,000/-.

Majority of 58.67% subjects had Man days lost of them and their companions in last one year. Total Man days lost of them and their companions in last one year was 1538 days. Mean of Man days lost of them and their companions in last one year was 17.48±11.79 days. Range of Man days lost of them and their companions was 4-72 days. Majority of subjects had loss of more than 2,000 rupees in last one year from Wages of them and their companions due to diabetes. Total INR wages lost of study subjects and their companions annually was 1,30,750/-. Mean INR wages lost by study subjects and their companions annually was 2,563.73±2,792.59/. Range of INR for wages lost of study subjects and their companions annually was 240-18000/-.

Table No. 3: Distribution of adult diabetic patients according to Total Direct Cost and diabetic complications (n=150)

Cost	Variables		N	Median±IQR	Mean rank	Mann-Whitney U Test (p value)
Total Direct cost (INR)	Diabetic Complications	Present	51	10,200.0±9,760	74.15	2455.5 (0.784)
		Absent	99	10,600.0±10,570	76.20	
	Comorbid conditions	Present	98	10,300.0±10850	73.81	2382.5 (0.513)
		Absent	52	11,212.0±33180	78.68	
	Absenteeism	Present	51	10,320.0±10445	76.45	2644.0 (0.749)
		Absent	99	10,840.0±9810	74.15	

The median score of Total cost was less when Diabetic complications was present than when it was absent and the difference was statistically not significant (p >0.05).The median score of Total cost was less when Comorbid conditions was present than when it was absent and the difference was statistically not significant (p >0.05).The median score of Total cost was less when Absenteeism was present than when it was absent and the difference was statistically not significant (p >0.05).

Discussion

In this study 150 adult diabetic patients attending the diabetic clinic were interviewed along with review of medical records. In this study physical assessment was done by comparing with diabetes complication, comorbid conditions and financial assessment of annual expenditure was done by collecting data of recent three months expenditure on diabetes care by the study subjects for both direct and indirect costs. Physical and financial status association was seen with socio-demographic characteristics and health services related factors, utilization of health facilities and among themselves also.

Proportion of female (51.3%) was more than male (48.7%) in present study and similar finding was found in study done in Saudi Arabia[17].

In present study the mean age of female was 50.00±12.69 years and the mean age of male was 52.40±12.64 years whereas the overall mean age of the participants was 54 ± 12 years in a study conducted in Oman[18].

In present study 80.6% of study subjects had good drug compliance whereas 19.4% had poor drug compliance whereas a multicentric study in India, revealed 79.4% were compliant with their medication[19]. But other studies revealed poor drug compliance[20-23, 9].

79.3% of study subjects skipped the drug when it was finished. Only 5.3% of study subjects had supply of drug from hospital fully but rest of them had to buy the drug from the shop. 50.0% had to buy all the antidiabetic drugs from the shop and 44.7% were dependent both on shop and hospital for anti-diabetic drug. 52.0% of study subjects had irregular supply of drug from hospital whereas 48.0% of subjects had regular supply of drugs.

Diabetes was diagnosed in 6.0% of study subjects below 20 years of age. The proportion of diagnosed case of diabetes increased up to 50 years of age and then decreased. The Mean age of diagnosis of diabetes was 45.92±13.58 years in study subjects, Median age of diagnosis was 47.0±21.0 years and Range was from 14-87.5 years. In Diab-Care Asia, a multi-country study in Asia, the mean age of diagnosis among Indian respondents was 43.6 years[24].

Majority of study subjects attending the diabetic clinic had 1-5.99 years (67.3%) of duration of diabetes and only 13.3% of subjects had more than 11 years of duration of diabetes. In present study Median year of duration of diabetes was 3.0 (1-32) years whereas a study in Oman showed a median year of duration of diabetes was 7 (4-10) years[25].

Majority of annual treatment expenses of study subjects were in Drugs and consumables (66.73%) followed by Investigation cost 15.19%, Transportation cost 12.72%, Food cost 3.72% and Consultation cost was least at 1.59%. In present study drug costs accounted for more than half of the total direct costs which was similar to other studies conducted in India[26-33].

Conclusion

Antidiabetic drug were not regularly available in hospital so all most all had to purchase drug from the shop when not available at hospital.

Drugs and consumables, investigation and transportation cost leads to more than 90.0% of out-of pocket expenditure in both in-door and out-door care causing financial burden on both adult diabetic patient and its family. Majority of study subjects had Man days lost of them and their companions in last one year was approximately 4 years.

Total INR wages lost of study subjects and their companions annually was also a financial constraint for follow-up of hospital by subject.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Assessment and Comparison of Knowledge and Awareness about Hand Hygiene among Health Care Workers from PHC and Medical College

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Abstract

Objectives: Present study was conducted to assess and compare the knowledge of hand hygiene (HH) according to WHO guidelines among healthcare workers (HCW) from medical college hospital and primary health care centre (PHC). Improvement in knowledge of HH was assessed after training.

Method: Cross-sectional study was conducted in two groups comprising of 167 HCW from a medical college hospital (GROUP A) and from PHC (GROUP B). The WHO "Hand Hygiene Knowledge Questionnaire" was used to collect data. Multiple choice questions were given to participants before and after training program for HH.

Results: Eighty & eighty seven HCW were recruited in group A & B respectively. Before HH training program, both the groups had very few people with good knowledge of HH (8.7% in group A & 5.7% in group B). After attending the HH training program, there was significant increase in the number of individuals having good knowledge of HH practise (52.5% & 35.6 % in group A and B respectively, $p<0.001$ for the groups).There was significant increase in the mean scores in both groups after HH training from 57.638+ 12.52% to 75.66 + 12.45%, $p <0.001$ in GROUP A & 46.66 +18.7% to 64.59 + 18.97%, $p<0.001$ in Group B.

Conclusions: There was low level of awareness about HH practices in studied population. Regular awareness program must be conducted to improve the knowledge of HH. Medical colleges should consider conducting such program for all HCW including PHC HCW at regular interval.

Keywords: *Hand hygiene, PHC, HCW, HH, WHO.*

Introduction

Healthcare workers (HCW) play a significant role in transmission of nosocomial pathogen.[1] Improper hand hygiene by health care worker is responsible for about 40% of nosocomial infections.[2] Health care associated infections (HCAI) are major cause of increasing morbidity, mortality and health care cost among hospitalized patients. Hand hygiene (HH) is an effective way of preventing the spread of infectious diseases and antimicrobial resistance.[3] Improved compliance with HH, with proper use of alcohol-based hand rubs (ABHR) can reduce the nosocomial infection rate by as much as 40%.[1] Practicing HH, either washing the hands with water and soap or using ABHR, is a simple effective way to prevent infections.

Compliance with recommended HH procedures for HCWs has been poor, with mean baseline rates as low

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as 5% and an overall average of 38.7%.[4] In our study, HCW's awareness and knowledge regarding hand hygiene before and after educating them with WHO guidelines was studied with the help of questionnaire. HCWs working in a medical college & those working in primary health care centres in Vidarbha (India) were randomly recruited for the study. Aim of our study was to assess and comparison of knowledge of hand hygiene according to WHO guidelines among healthcare workers from medical college hospital and primary health care centre workers. Also comparing the knowledge of HH among HCW before and after educating them by comprehensive awareness and training programme.

Material and Method

Research design and setting: This cross-sectional study was conducted in two groups. Group A comprised of HCW from medical college hospital and Group B comprised of HCW from primary health care centres (PHC). Both the groups included doctors and nurses.

Ethics: Ethical committee clearance was taken from institute. Health care workers were explained about the content and nature of the study.

Sample size and sampling: As per previous studies, 8-10.6% HCW had a good knowledge of hand hygiene practice (i.e. score ≥75%).[5,6] Considering 9% prevalence of good knowledge of HH practice in our population and 95% confidence level, we calculated sample size of 80 in each group.

Exclusion Criteria: HCWs who refused to give consent for participation were excluded. Incompletely answered questionnaire were also excluded from final analysis.

Methodology and Data Collection: The World Health Organization (WHO) "Hand Hygiene Knowledge Questionnaire"-revised 2009 edition was used to collect data. The questionnaire contained questions on the participant's profession, years of the experience working in hospital, attendance in previous HH programme and awareness about 7 steps and 5 moments of HH as described by WHO. Twenty one multiple choice questions were given to participants in English & Marathi. These questionnaires were filled by participants before and after taking educational hand hygiene training programme. Educational hand hygiene programme was conducted by medical college and primary health care centre workers between March to May 2020. All the participants were randomly assigned to 30 batches each comprising of 5-6 HCW for training. Training was conducted in the form of audio visual presentation and HH demonstration. To identify the level of knowledge, scores were given to responses from the participants. For the scoring, "1" point was given for a response that indicated a "good level of knowledge" and "0" point was given for a response that indicated a "poor level of knowledge".[3] A score of 75% and above was considered good, 50-74% was considered moderate, and below 50% was considered as poor knowledge of HH practice.[5-6]

Statistical Analyses: Statistical analysis was done with the help of SPSS version 16. Paired T test was used for analysis of pre & post test scores of individual respondents. Unpaired T test & Mann Whitney U test were used for comparing mean scores of two groups. Chi square test was used to compare categorical variables. P value of < 0.05 was considered significant.

Results

Total 167 HCWs which included doctors and nurses were enrolled in the study. HCWs were divided into two groups, 87 health care workers working at primary health care centre and 80 healthcare workers working at medical college. The demographic characteristics of the HCW are shown in Table 1. In group B, 42 were doctors and 45 were nurses while in group A42 were doctors and 38 were nurses. Median experience of HCWs was 3 years in group A & 4 years in group B. Out of 167 participants 115 had attended hand hygiene training in last three years.

Table 1: Demographic parameter of Group A and Group B Participants

Profession	Group A (%)	Group B (%)
Doctor	42(52.5%)	42(48%)
Nurse	38(47.5%)	45(51%)
Median experience working in hospital (years)	3	4
Attended any hand hygiene programme in last 3 years	55 (68.8%)	60 (68.9%)

There was significant increase in the mean scores in group B after HH training (from 46.66 +18.7% to 64.59 + 18.97%, $p<0.001$) Table 2.

There was significant increase in the mean scores in group A after HH training 57.638 %(+ 12.52) 75.66 % (+ 12.45) Table 2.

Table: 2 Comparison between pre-test and post test score for awareness and knowledge about Hand hygiene among group A and B participants.

	Group A		Group B	
	Mean±SD (%)	P Value	Mean±SD (%)	P Value
Pre Test Score	57.638 +12.52	< 0.001	46.66 +18.7	< 0.001
Post Test Score	75.66 +12.45		64.59 +18.97	

Mean increase in the scores after attending the HH program was comparable in both the groups. (18.62+19.89 % vs. 18.02+11.40 % group B vs. group A respectively, p= 0.733) Table 3.

Table: 3 Comparison between growths in percent score between both groups

Groups	Growth in Percent (Mean±SD)	P value
Group A	18.02±11.40	0.733
Group B	18.62±19.89	

Before HH training program, both the groups had very few people with good knowledge of HH (8.7% in group A & 5.7% in group B). After attending the HH training program, there was significant increase in the number of individuals having good knowledge of HH practise (52.5% & 35.6 % in group A and B respectively, p<0.001 for the groups).

Before HH training, poor score i.e. < 50% was found in 19(23 %) and 40 (45 %) participants in group A and B respectively. There was significant reduction in the number of participants having poor score after HH training program. 2(2.5 %) and 19(21.8%) participants in group A and B respectively) Table 4.

Table: 4 Comparison of the participant's knowledge level in both groups.

Hand Hygiene Knowledge	Group A		P value	Group B		P value
	Pre Test N (%)	Post Test N (%)		Pre Test N (%)	Post Test N (%)	
Good (≥75%)	7(8.7%)	42(52.5%)	p<0.001	5(5.7%)	31(35.6%)	p<0.001
Poor (< 50%)	19(23%)	2(2.5%)	p<0.001	40(45%)	19(21.8%)	p<0.001

Discussion

Nosocomial infections constitute a major challenge of modern medicine. On an average, infections complicate 7% to 10% of hospital admissions.[7] Transmission of microorganisms from the hands of HCW is the main cause of nosocomial infections, and hand washing remains the most important preventive measure.[8] There is evidence that hand antiseptics reduce the transmission of health care associated pathogens and the incidence of Health Care Associated Infection (HCAI).[9]

Several hand hygiene studies have been conducted among medical students and nursing students and HCW including doctors and nurse from tertiary health care centre.[5-6, 10-14] In developing country like India, primary health care centre is the first point of health care contact for many patients. There are no previous studies conducted on knowledge and awareness about hand hygiene among health care workers from PHC. In our study we tried to assess the awareness and knowledge of HH in HCW working in a medical college & PHCs.

Rezaee et al had carried out an interventional study to assess the effect of an educational program on HH to medical students. The medical students were participating in a one-day teaching workshop about the importance of HH. The participants filled out the questionnaire before attending, on finishing, and 3 months after the

workshop. There was a significant difference between the pre-test and the late post-test scores in knowledge and performance categories.[10]

Ansari et al compared the level of knowledge attitude and practice (KAP) regarding hand hygiene among doctors and nurses, training sessions were conducted for 100 healthcare workers (56 doctors and 44 nurses) and their assessment for knowledge, attitude and practice was done through pre-test and post-test questionnaire. They found that there was a significant improvement in the KAP score for both doctors and nurses after the training session.[11]

In our study we conducted training session for health care workers from medical college and primary health care canters. Pre-test and post-test questionnaire were given to assess knowledge about HH. At baseline, very few HCW had good knowledge about HH (< 10% of the participants in both the groups). Our findings are similar to those observed in previous similar studies.[5]

We also observed that mean scores at baseline were significantly higher for the HCW working in medical college as compared to those working in PHC. This may be attributed to repeated training programs in medical colleges & presence of signage highlighting importance of hand hygiene being prominently displayed in the wards. There has been no published study comparing HH awareness between HCW in medical college & PHC.

After attending the training program, there was significant improvement in scores in both the groups. There was also a significant increase in the proportion of HCW having good knowledge of HH. Mean increase in the scores after training program was comparable in both the groups. This shows that, conducting HH awareness program in medical college as well as PHCs will lead to better awareness of HH practices in healthcare settings. Improving HH practices in HCW working in PHC can result in reducing HCAI at primary healthcare level. This can help reduce the spread of MDR organisms in the health care setting ultimately leading to reduced healthcare expenditure.[15]

Hence our study shows that training program is needed for both HCWs from medical college and PHC. A well conducted program can bring about remarkable improvements in HH awareness among HCW. We also suggest that such program should be conducted more frequently by medical colleges for HCWs. Regular reinforcement of HH practices among HCW will result in improved compliance and reduction in rate of HCAI.

Conclusions

All HCW should be educated about hand hygiene. Regular awareness program must be conducted to improve the knowledge of HH. Emphasis should be given on HH training programs for PHC HCW. Medical colleges should consider conducting such program for all HCW at regular interval.

Limitation: We conducted this study in one hospital and PHC workers from a small area. A larger study in wider geographical location may give better insights on the subject. Awareness of HH practice should be reassessed at longer time interval after training program to assess the long term retention of HH practices.

Ethical Clearance: Taken from institutional ethics committee.

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Myositis Ossificans Traumatica of the Wrist in Elderly: A Description of an Unusual Case

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Abstract

Myositis ossificans commonly involves muscles and subcutaneous tissues in people of younger age group. The myositis ossificans is commonly seen around the muscles of elbow joint but occurs less commonly in muscles around hands and wrist. We report a case of Myositis ossificans involving the wrist in a 65 year old lady.

Keywords: *Myositis Osificans, Wrist Joint, Myositis in Elderly.*

Introduction

Myositis ossificans commonly involves muscles and subcutaneous tissues in people of younger age group.[1] The disease is usually found in muscles but less commonly changes are seen in subcutaneous tissues, tendons, fascia and periosteum. The myositis ossificans is commonly seen around the muscles of elbow joint but occurs less commonly in muscles around hands and wrist. This is a case report of myositis ossificans in a 65 year old women involving muscle and tendons around wrist joint.

Case Report: A 65 years old lady came to Out-patient department with a history of injury to right wrist 4 year earlier. She had fall of a heavy object over her right wrist sustaining an injury to right wrist. The patient was advised massage from a practitioner for 5–6 weeks, there-after mobilization of wrist was started. She then incidentally noticed lump around her right wrist after 6 months which was of the size of a pea. The swelling gradually increased for next 12 months, also there was wrist joint movement was restricted. Since past 1 year, patient has pain localised to right wrist and has aggravated since past 6 months. On presentation, patient now has restricted right wrist movements. No history of constitutional symptoms. Single spherical swelling of size 3x3cm was present on the dorsum of right wrist along the ulnar border, with smooth surface, ill defined edges, tender on touch, hard in consistency, attached to the underlying bone was seen. Movements of involved wrist were reduced to 10 degrees of dorsiflexion and palmar flexion. Movements at Distal radio-ulnar joint like supination and pronation were restricted to 0°-30°only. Visual analogue scale was 8/10 and Mayo wrist score was poor (52). Distal vascular status of hand was preserved.

Antero-posterior (AP) and lateral radiographs of the right wrist (As shown in Fig. 1) revealed ossification around dorsal aspect of distal end of ulna in the areas of the extensor tendons. This radiological finding was in favour of traumatic myositis ossificans.

Clinically, the patient had history of trauma followed by massage and radiographically there was ossification around distal ulna. Based on above 2 findings, a provisional diagnosis of myositis ossificans (secondary to trauma) was made. This was confirmed by subsequent Magnetic resource imaging of right wrist joint (Fig. 2) which showed lobulated calcified mass around anterior, posterior and lateral border of distal ulna. The mass was surgically excised (Fig. 3) and histopathological examination confirmed the diagnosis. There was an improvement in the Post-operative Visual analogue scale and Mayo wrist score which was 2/10 and good (84) respectively.



Fig. 1: Antero-posterior and lateral radiographs of the right wrist showing ossification around dorsal aspect of distal end of ulna.

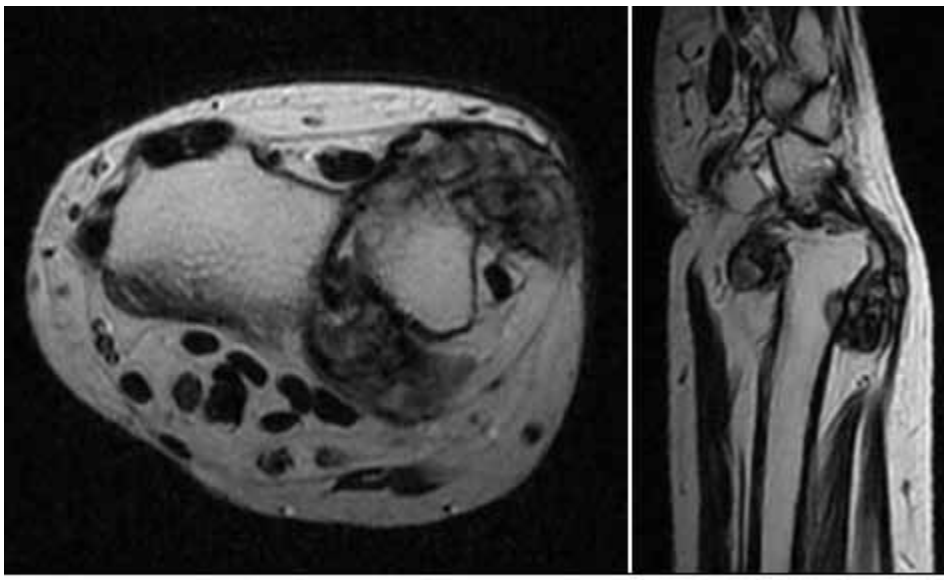


Fig. 2: MRI of right wrist joint showing calcified mass around anterior, posterior and lateral border of distal ulna.



Fig. 3: Intra-operative picture of the mass

Discussion

The term "tendonitis ossificans traumatica", a broad term for calcification or ossification in or around tendons was coined by Rothberg[2]. The majority cases of myositis ossificans, mentioned in literature are of non-traumatic cause involving in the muscle and subcutaneous tissues of the hand often mimicking as a neoplasm[3]. In this case, the patient had myositis ossificans which was secondary to trauma and involving extensor tendon sheaths around wrist making it as a rare presentation.

The hypothesis behind ossification can be proliferation of mesodermal tissues as a reaction to traumatic stimulus[4]. Increased vascularity and metabolism with calcium brought to the injured part by the hyperaemia of the injury results in new bone formation. It is difficult to differentiate between calcification and ossification in a tendon radiologically. The histopathological examination is considered to be accurate in these cases.

Foreign-body granuloma, osteoclastoma, atypical fibroxanthoma and nodular fasciitis are some of the differential diagnosis with similar features. Kai Y [5] et al reported pseudo-malignant myositis ossificans of the hand in 35 years old male without any history of trauma. The histopathological examination revealed central zone of fibroblastic tissue with multinucleated giant cell and intermediate zone of mixture osteoblasts and fibroblasts.

The benign and self-limiting nature of this disease, the treatment of choice can be conservative for a majority cases. However, severe pain with compressive symptoms and loss of function of the joint may require aggressive surgical intervention.[6]

Conclusion

In immediate post traumatic phase, hyperaemia due to massage should be prevented and there is definitive role of cryotherapy[2] in prevention of post traumatic myositis ossificans.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Prevalence of Different Resistance Pattern in Staphylococcal Aureus Isolates From Tertiary Care Hospital

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Abstract

Introduction: Staphylococcus aureus is common isolated pathogen from clinical specimen with increasing trend of antimicrobial resistance. Staphylococcus aureus and Coagulase-negative Staphylococci (CoNS) infections have become common among both hospitalized and non-hospitalized patients.

Aim: The aim of this study was to determine the inducible clindamycin resistance among the MRSA and MSSA.

Material and Method: This study was prospective study which includes the entire sample obtained in the Department of Microbiology laboratory from Tertiary Care Hospital with bacterial infection and confirmed by positive culture. The standard microbiological protocol for detection of inducible clindamycin, Disc approximation method (D–test) was performed.

Result: Out of 124 Staphylococcus aureus isolates, 17 (13.7%) were inducible clindamycin resistant; 6 (35.29%) from (cefoxitin susceptible) MSSA and 11 (64.71%) from (cefoxitin resistance) MRSA.

Conclusion: The incidence of inducible clindamycin resistance among the Staphylococcus aureus was high. This high rate screen among the MRSA and MSSA provokes the necessity of performing the D- test before starting the antibiotic therapy.

Keywords: *Inducible Clindamycin Resistance (ICR), Staphylococcus aureus, MRSA, MSSA, D-test.*

Introduction

Staphylococcus aureus and Coagulase-negative Staphylococci (CoNS) infections have become common among both hospitalized and non-hospitalized patients. Staphylococcus aureus is one of the pyogenic bacteria most often infecting humans[1]. The development of Staphylococcoi resistance to antimicrobial agents is a growing concern.Staphylococcus aureus is known for acquiring antimicrobial resistance promptly after the introduction of new antibiotics[2].Reports of methicillin resistance in Staphylococcus aureus followed rapidly upon the introduction of methicillin in 1961. MRSA are present worldwide today, and most are resistance to multidrug[3]. Study of early isolates of MRSA showed that a key genetic component responsible for resistance, *mecA,* is not native to the *Staphylococcus aureus* genome. The cassette mec (SCCmec) of Stapylococcal chromosome has been characterized as a novel, mobile

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resistance factor which differs from both transposons and bacteriophages[4].

MRSA usually spreads by clones; however, the mec gene is known to have been transmitted between Staphylococcus aureus strains, and probably between other staphylococcal species. Nevertheless,it is noteworthy that some forms of SCCmec bear various additional genetic elements (Tn554, which encodes resistance to macrolides, clindamycine and streptogramine B; and pT181, which encodes resistance to tetraclines) which may confer resistance to additional groups of antibiotics; these genetic elements are especially common in HA-MRSA[5]. Additionally, strains that are resistance to erythromycin will easily become Clindamycin resistance due to inducibility of resistance. Clindamycin, a protein synthesis inhibitor, is a frequent therapeutic choice for staphylococcal infections, especially infections of the skin and soft tissue, and as an alternative in patients with penicillin allergies[6].

As for the central nervous system, it does have exceptional tissue penetration[7]. The drug accumulates in abscesses, and no changes to renal dosage are needed. Good oral absorption makes it convenient for outpatient prescription or as follow-up drug after intravenous therapy [6]. Resistance to this drug, however, is yet another problem. Staphylococcal resistance to clindamycin can be either inducible or constitutive (iMLS $_B$ - inducible Macrolide-Lincosamide-Streptogramin B resistance). It is known that treatment with clindamycin in patients harboring iMLS B Staphylococci leads to the production of constitutive resistance, which in turn leads to therapeutic failure[8]. Inducible clinamycin resistance test can be detected by simple test known as Disk approximation test or D-test[9]. The optimal inter disk distance between the antibiotics is not yet clear, and the Clinical and Laboratory Standards Institute (CLSI) suggests a range of 15 to 20 mm separation of the disc[10].

Aims and Objective: The aim of the present study is to know the inducible clindamycin resistance among the MRSA and MSSA isolates from Tertiary Care Hospital.

Material and Method

Study Site and Population: This study was conducted in Department of Microbiology at Shalinitai Meghe Hospital and Research Centre, Wanadongri, Nagpur in collaboration with Jawaharlal Nehru Medical College (Datta Meghe Institute of Medical Sciences), Wardha, from November 2019 to April 2020.

This study was prospective study which includes all the sample obtained in the Department of Microbiology laboratory from the patients attending OUT patient Department and IN patient Department of Shalinitai Meghe Hospital and Research Centre with bacterial infection and confirmed by positive culture.

Isolation, Identification and AST of Staphylococcus aureus: The samples received in the microbiology lab were inoculated on Blood agar and Mac Conkey's agar, incubated for 24 hours at 37^0C for staphylococcal isolation.

Patients with clinical infection but organisms not grown in the culture or organism expect Staphylococcus auresus, who has taken antibiotic before sample collection, were excluded from the study. Total of 124 Staphylococcal species were isolated and confirmed by gram stain and various biochemical tests like catalase test, oxidase test, coagulase test, mannitol salt agar, etc. Antibiotic susceptibility test (AST) was examined on Mueller-Hinton agar (MHA) plateby using modified Kirby-Bauer disc diffusion method following CLSI guidelines[11] with commercial antibiotic discs (Hi Media).

Detection of MRSA and MSSA: Screening of Methicillin Resistant was performed by Cefoxitin (30µg) disc diffusion method and interpreted according to CLSI guideline[11]. The isolates which gave zone of inhibition(ZOI) $\leq$ 21mm were Methicillin Resistant (MRSA) and isolates with ZOI $\geq$ 21 mm were Methicillin Susceptible (MSSA).

Detection of inducible Clindamycin (D-Test): For detection of inducible clindamycin, Disc approximation method (D – test) was performed as ruled by CLSI 2019. Clindamycin disc was placed at a measured distance (between 15-26 mm) from erythromycin disc and incubated.Appearance of flattening of the clindamycin zone adjacent to the erythromycin disk ['D' shape] was classified as D-test positive.[9]

Quality Control: Quality of each test was performed by using standard protocol Staph *aureus* ATCC 25923 an *Escherichia coli* ATCC 25922 were respectively used as positive and negative control

Result

Prevalence of Inducible Clindamycin-resistance: The prevalence of Inducible Clindamycin Resistance

was found to be 13.7% among the *Staphylococcus aureus* isolated.

Frequency of Clindamycin Resistant isolates: There was no any observation of constitutive clindamycin resistance among the *Staphylococcusaureus* isolates. However, erythromycin-induced clindamycin-resistance (ICR) was observed among 17 isolates (13.7%): six (35.29%) of those ICR positive or D-effect positive (D+) isolates were susceptible to cefoxitin (MSSA) and eleven (64.71%) were resistant to cefoxitin (MRSA). However, distribution of inducible clindamycin resistance among MSSA and MRSA was statistically insignificant (p value > 0.05).

Table No. 1: Distribution of Inducible clindamycin resistance among MSSA and MRS

Methicillin-resistance	ICR screening		Total	P value
Screening	D+	D-		
MSSA	6	62	68	0.018
MRSA	11	45	56	
Total	**17**	**107**	**124**	

Discussion

Staphylococcus aureus is one of the common human pathogens capable of causing a large variety of infection.The incidence of invasive infections has been raising with emergence of community acquired (CA) and hospital-acquired (HA) methicillin-resistant Staphylococcus aureus (MRSA)[12]. In particular in penicillin-allergic patients, clindamycin is an alternative option for mild to moderate MRSA infections. Sub inhibitory concentration of erythromycin,however, is a common resistance inducer to inducible clindamycin resistance (ICR)[13,14].

The recent studies reveal that clindamycin has become an excellent drug of choice for the staphylococcal infections specially skin and soft tissue infections. It has been used as alternative drug for the penicillin allergic patients[6].

However, the differentiation of inducible-clindamycin resistance (iMLSb phenotypes) from other type of resistance is a critical issue because of the therapeutic implications of using clindamycin to treat a patient with an inducible clindamycin-resistant *Staphylococcus aureus* isolate. Clindamycin resistance may be either of *Erm*-mediated or M*srA*-mediated. *MsrA-mediated* resistance is due to efflux-pump mechanism. *ErmA-mediated* resistance of clindamycin may be either constitutive or inducible[6].

But when induction test was performed, 17 (13.7 %) of the isolates showed D-effect, i.e. they were inducible clindamycin resistant strains (iMLSB phenotype). Sixof those strains were MSSA and eleven were MRSA. Absence of constitutive resistance but still presence of 13.7 % of inducible resistance to clindamycin provokes the importance of D test, if clinical failure is not anticipated[15]. The observation of D-effect to be more among MRSA in comparison to MSSA indicate that clindamycin may not be suitable to be used as empirical therapy against.

In the study of Michael Z. David et al[16] showed prevalence of MRSA among adult 32.81% and among children 17.49% which is greater than this study as shows prevalence of MRSA was 13.7%. Iraj Sedighi et al[17] studied reported Inducible clindamycin resistance of 37.5% of CA-MRSA isolates at the time of admission and 22.2% of HAMRSA isolates at discharge whereas in this study showed 13.71% Inducible clindamycin resistance of MRSA AND MSSA.

Another study of Pal N et al[18] showed Inducible clindamycin resistance of 43.56% in MRSA (Methicillin resiatant staphylococcus aureus), 6.93% MSSA (Methicillin sensitive staphylococcus aureus), 43.56% MRCNS (Methicillin resiatant coagulase negative staphylococcus aureus) and 6.0% MSCNS (Methicillin sensitive coagulase negative staphylococcus aureus) whereas this study showed 35.29% MSSA and 64.71% MRSA out of total Inducible clindamycin resistance. According to the study of Naima Fasih et al[19] showed Inducible clindamycin resistance of 62% among MRSA staphylococcal isolates which was greater than this study.

A studied done by Mukesh Patel et al[20] showed the presence of MLSBi in 212 (52%) of the isolates overall, with 139 (50%) MRSA and 73 (60%) MSSA isolates exhibiting MLSBi which was greater than this study as this study showed showed 35.29% MSSA and 64.71% MRSA out of total Inducible clindamycin resistance[21].

Conclusion

The incidence of inducible clindamycin resistance among the Staphylococcus aureus was high. This high rate screen among the MRSA and MSSA provokes the

necessity of performing the D- test before starting the antibiotic therapy. The observation of D-effect to be more among MRSA in comparison to MSSA indicate that clindamycin may not be suitable to be used as empirical therapy against.

Ethical Clearance: Taken from institutional ethics committee.

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A Unusual Case of Charcot's Arthropathy of Elbow Caused by Syringomyelia and Chiari Malformation Complicated with Scoliosis

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Abstract

Neuropathic joints, which is also known as Charcot's Arthropathy, is a destructive joint pathology resulting from loss or decrease in proprioception, pain and temperature sensations. Patients suffering from diseases associated with neuropathy like diabetes mellitus, syringomyelia and syphilis are said to be prone to the disease. Patients with syringomyelia commonly suffer with arthropathy involving shoulder. We report a case of 35 year old male patient with syringomyelia and charcot's arthropathy involving left elbow.

Keywords: *Charcot's Arthropathy; Syringomyelia; Elbow.*

Introduction

Charcot's arthropathy which was first reported by Jean-Martin Charcot in 1868[1], is a destructive joint pathology resulting from loss or decrease in proprioception, pain and temperature sensations[2-4]. The etiological factor of Charcot arthropathy includes Tabes dorsalis, syringomyelia, traumatic spinal cord injury, leprosies, congenital pain insensitivity, diabetes mellitus, chronic alcoholic intoxication, and so on. Although Charcot arthropathy associated with above mentioned factor has been mentioned in the literature, but a very less number of cases describing Charcot arthropathy of elbow caused by syringomyelia and Chiari malformation associated with scoliosis have been reported.

Case Presentation: A 35 years old male patient reported to our hospital complaining of weakness in right hand since the last 12 years and swelling over his left elbow since 3 years. Weakness was associated tingling and numbness in left upper limb. Swelling over the elbow joint was painless, diffuse and was gradually progressive. Patient underwent incision and drainage 2 years back carried out by a local practitioner and after the drainage, swelling did not subside and a gradual increase in size was noted. Patient gave a history off and on fever with loss of appetite and weight loss of around 15 kg in 3 years. Patient had no history trauma, comorbidities or any surgical history in the past.

On clinical examination, Diffuse swelling of size 5 x 6 cm approximately, non-adherent to bone or underlying structures, smooth over the lateral surface and irregular over the medial surface was present around the elbow joint and valgus deformity was present. Wasting of left forearm and hand muscles was seen. Elbow range of motion was 0-130 degrees and pain-free with full supination and pronation with medial and lateral laxity at the elbow joint. Bony crepitus was noted while performing elbow range of movements. On clinical examination of cervical spine, there was weakness in finger grip of left hand with sensory hypoesthesia over the dorsal aspect of left hand. Motor power of bilateral shoulder, elbow and wrist joints were normal. Examination lumbar spine and other limbs was normal.

Haematological examination in the form of complete blood count with Erythrocyte sedimentation rate, fasting and post meal blood sugar levels, C reactive protein levels, veneral disease research laboratory tests, uric acid, liver function test and kidney function test were carried out and found to be within normal limits except ESR level was slightly raised.

Joint aspiration was also done to rule out infection but culture did not find any organism growth.

Radiographs of Antero-posterior and lateral view of left elbow jointshowed degenerative changes in left elbow with decrease in joint space, flattening of articular margins of olecranon with marginal osteophytes at the olecranon process with periarticular sclerosis was seen. Loose bodies are seen near the radial head. Radiographs of Anteroposterior and lateral views of cervical spineshowed scoliosis at the cervico-dorsal spine with primary curve having convexity towards left side and secondary curve having convexity towards right side.

MRI of cervical spine showed a syrinx from the level of upper border of C1 vertebra till lower border of C2 vertebra. Sagittal section of T2 weighted image was suggestive of Chiari malformation with tonsillar herniation.

Patient was sent to neurosurgery department and patient was treated with posterior fossa decompression. From Orthopaedic point of view, swelling was aspirated and patient was immobilized with above elbow slab for 15 days and non-steroidal anti-inflammatory drugs were started. Swelling gradually subsided after 15 days of conservative treatment and above elbow slab was removed. Rather than pain, instability was the chief complaint. Hence, Patient was given customised Dynamic Hinged Elbow Braceand Elbow was mobilised using the brace. At the end of 1 year, patient remained asymptomatic and able to do his day to day activities.

Discussion

Charcot's Arthropathy of elbow is usually rare condition with few case report and case series being reported in literature.[6-17] Only 3% to 8% neuropathic joints, reports involvement of elbow joint. In the literature, two theories were postulated by the researchers that describe the pathogenesis of this condition, they are the neurotraumatic and neurovascular theory. As described Johnson in 1967, the neurotraumatic theory suggest the cause to be repetitive trauma to the affected joint which has lost sensations. The neurovascular theory described by Allman et al suggests active bone resorption due osteoclasts after sympathetic dysfunction and hyperaemia leading to joint destruction.[4]

On the basis of Underlying aetiology, joint involvement pattern can be identified like patients with Diabetes Mellitus are prone to develop this pathology in the ankle and foot while in Patients with syphilis, hip and knee joint involvement is common. In cases with syringomyelia, the upper limbs joints are seen to get commonly involved. Thus, in our case report underlying etiology and pattern of joint involvement resembles the literature findings.

Syringomyelia is characterized by cavitation of the spinal cord which can translates into a progressive clinical syndrome and can thereby lead to loss of neurologic functions. 16 one of the underlying aetiology of syringomyelia can be Chari malformation which was first described by H. Chiari in 1891. It was described as herniation of inferior lobe of cerebellum with elongated tonsils into the spinal canal. Thorough neurosurgical examination and investigation for consideration of surgical management should be obtained, as was carried out in our case.[18,19]

The treatment of choice for charcot's arthropathy has not been very clearly described in literature. The recent study derived guidelines suggests analgesia, elbow immobilization till swelling subsides followed by physiotherapy, and management of patient expectations.

Surgical Arthrodesis with external or internal fixation has resulted in variable surgical outcomes to some researchers.[15,16,17]. Kwon et al[15] reported a case series of 6 neuropathic elbows, out of which 3 cases were managed surgically developed post-operative complications which required additional surgery. A septic neuropathic elbow was managed with surgical debridement and external fixation resulted in instability in further follow-ups.[7] Elbow arthrodesis which was a treatment of choice after tubercular elbow is rarely performed today[20]. Arthrodesis is suggested only when there is no other reasonably reconstructive option available.[20]

As there was lack of valid guidelines for neuropathic elbow in the recent literature and patient wanted to have some function at elbow for daily activities, a trial of conservative management was considered in our case. Immobilisation of elbow with above elbow slab for 15 days was advised and as swelling was reduced, elbow was mobilised with dynamic hinge elbow brace for 3 months. At the end of 1 year follow-up, patient was asymptomatic and patient was able to perform daily activities.

The case of neuropathic elbow joint secondary to syringomyelia with chiari malformation with scoliosis has rarely been reported in the recent literature.

We conclude that the maintenance of function of a joint, rather than immobilization, is the 'motto' of treatment of neuropathic joint. We managedour patient conservatively, as suggested by the majority of theliterature, and the patient was satisfied with the treatment.

After a thorough literature search, we can say that, this is the first report of neuropathic joint of elbow, secondary to syringomyelia with Chiari Malformation with scoliosis on the Indian subcontinent.

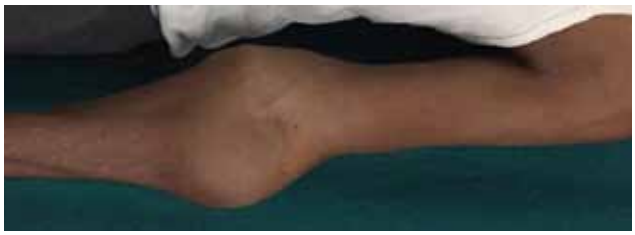


Figure 1: Showing clinical photographs depicting gross swelling over the elbow joint with wasting of muscles of forearm and hand.



Figure 2: Antero-posterior radiographs of the Left Elbow Joint showing decrease in joint space, flattening of articular margins of olecranon with marginal osteophytes at the olecranon process.

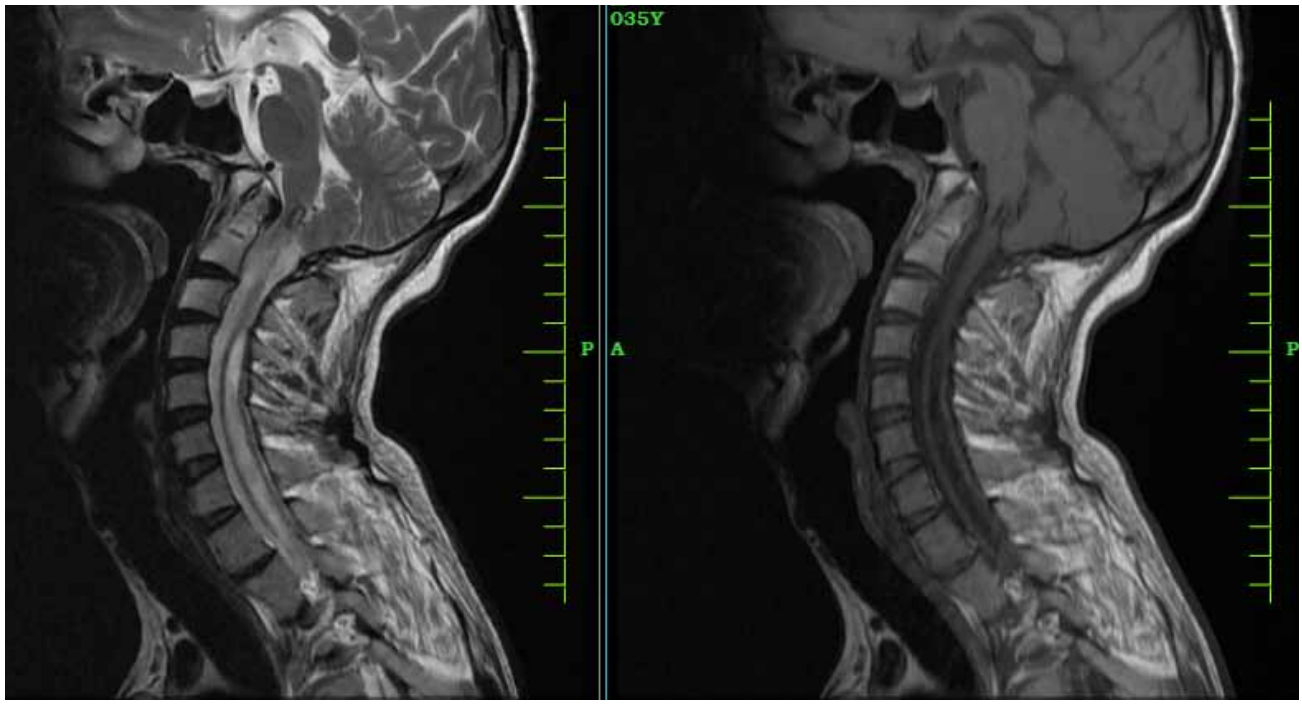


Figure 3 & 4 showing T2 and T1 weighted images of MRI showing a syrinx from the level of C1 vertebra to C2 vertebra. Sagittal section of T2 weighted image was suggestive of Chiari malformation with tonsillar herniation in the spinal canal.



Figure 5 showing clinical photograph of patient's elbow mobilised with hinged dynamic elbow splint.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Influence of Anxiety and Claustrophobia on Blood Pressure and Heart Rate during MRI Scan

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Abstract

Aim: To assess the effect of anxiety and claustrophobia on human blood pressure and heart rate during MRI scan.

Method: Samples of 48 patients who need MRI scan service were chose. A questionnaire and scales were used to evaluate the patients' anxiety group and claustrophobia. Blood pressure and heart rate were determined in before and after the scan in the same circumstances and method.

Results: The majority of the patients were female. The relation between anxiety scale and gender revealed that majority of the female have the high anxiety scales (68.57%). Alternatively, the minority of male have high anxiety level (40%). The relation between anxiety and claustrophobia with the systolic pressure before and after MRI scan, approved a statically significant difference with t-test = 3.007 and P value <0.05. No significant association was found of diastolic pressure and heart rate at the same time and circumstances with anxiety levels and claustrophobia.

Conclusion: This study represents significant evidence for an association of high blood pressure with high anxious and claustrophobic patients, measured before and after the MRI scan. While diastolic blood pressure and heart rate did not change during MRI exam.

Keywords: *Claustrophobia, blood pressure, heart rate, diastolic.*

Introduction

Magnetic resonance imaging (MRI) is vital medical equipment that has been steadily used as it gives image with high quality by using non ionizing radiation. For this procedure, the patients need to be scanned by a long narrow tube for approximately several minutes without movement, because technical issues which need to provide image free artifacts[1,2]. This period of MRI procedure which includes fear, noises, stress, nervousness, stability and unfamiliar environment could make challenge to manage the scan typically with anxious or claustrophobic patients. These are common problems which occurs with the patients during the MRI scan[3]. Anxiety is a indistinct and still vague[4]. It has been reported that 25% to 37% of the patients undergoing MRI scan experienced substantial anxiety[5,6]. What is more, the fear of enclosed spaces is called claustrophobia. Which is a common specific phobia with a prevalence of 1.0% to 15% in the general patients [7]. The American Cardiology Association emphasize that it should be important for the physician, dentist and medical staff to deal with complications that may take place in patients who have hypertension [8]. In addition to the light-headedness, nausea, fainting, fears of actual harm, the blood pressure and heart rate increasing

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may present with anxious and claustrophobic patient. [9]. This leads to necessity to avoid possible adverse reaction for cardiovascular disease like hypertension which could be occur with such psychological status. Recently the Global Health Risks shows that arterial hypertension remains the primary cause of mortality and morbidity in the world [10,11].

This study aims to evaluate the influence of anxiety and claustrophobia on the blood pressure and heart rate before and after the MRI scan. For this purpose, a questionnaire based on a rating scale was designed to capture the emotions feeling before the MRI scans. Blood pressure and heart rate recorded for the patients ten minutes before and after the imaging procedure in the same conditions.

Method

This experiment was to assess the potential for claustrophobia, anxiety and clinical relevance with MRI. All patients who agreed in participating received information about the study procedure, with free to withdraw from trail at any time before or after the MRI scan.

Eighty four patients with an age ranged between 25 to 50 years were selected in this experiment in Baghdad hospital during the period from May to October in 2015. The patients with cardiac disease were excluded to avoid solely based on arterial hypertension changes which found with their history previously[11]. For this reason, it is noteworthy that subjects who had any of the hypertension were included for the evaluation of the HR and PB.

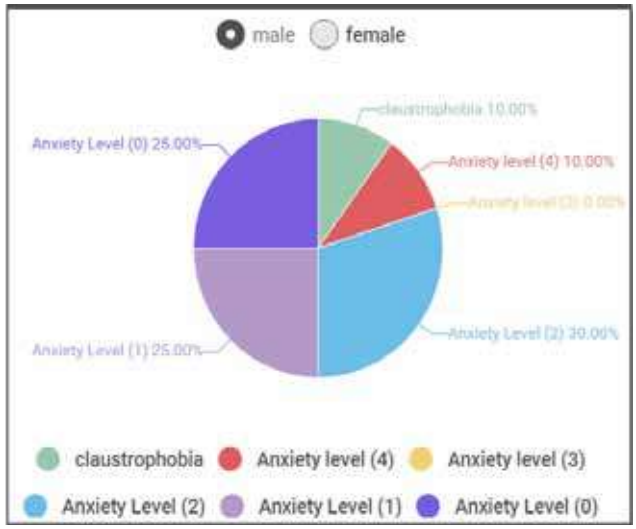
All patients were interviewed and evaluated face to face in the next room of the MRI scan. Then they asked to answer a general health questions by the same examiner to exclude any blood pressure disorders, previous history to the hypertension or any cardiac diseases following the instructions and any drugs which were taken by the patients through 24 hours before the scan that would affect the sensitivity of results were excluded [12].

The blood pressure and heart rate measured by a digital cardiologic stethoscopein two separate times. First measurement, when the patient in the waiting room, trying to characterize the BP and HR two minutes before the MRI scans. The second measurement is taken two minutes after the scan in the same place and techniques of the first measurement. To evaluate the patients' phobia and anxiety level, before the MRI examination, patients were informed with a description of how to rate the intensity of anxiety and claustrophobia if present by using four points numerical scale; the following values were established: A=0, B=1, C=2, D=3, E=4.

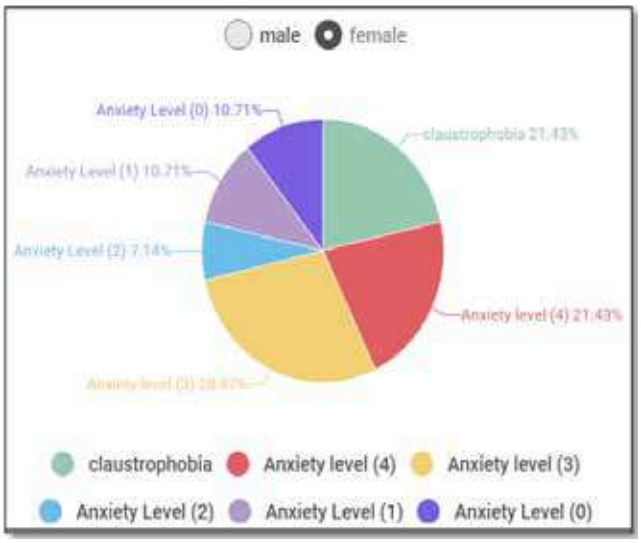
The scale was used and consists of four questions with five possible answers, which characterize the patient anxiety level in response to different situations that involve a visit to the MRI exam room and it is applied before the scan.

The sum of the values assigned to each answer, the possible interval score may vary between 4-20 points. The level of anxiety classified in naught, mild, moderate and exacerbated groups. The collected data were firstly stored in an Excel® worksheet and then we held a descriptive statistical analysis using the SPSS®, accompanied by a form containing issues about patient identification, harmful habits, diseases, frequency to MRI visit and cardiovascular parameters. For the claustrophobic group which was detected by using a claustrophobia questionnaire according to (CLQ)[13].

Results



(a) Female patients

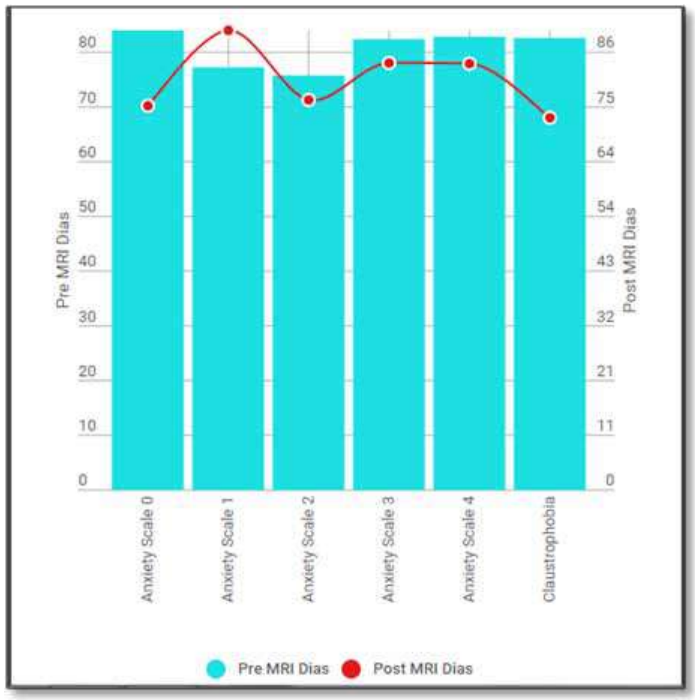


(b) Male patients

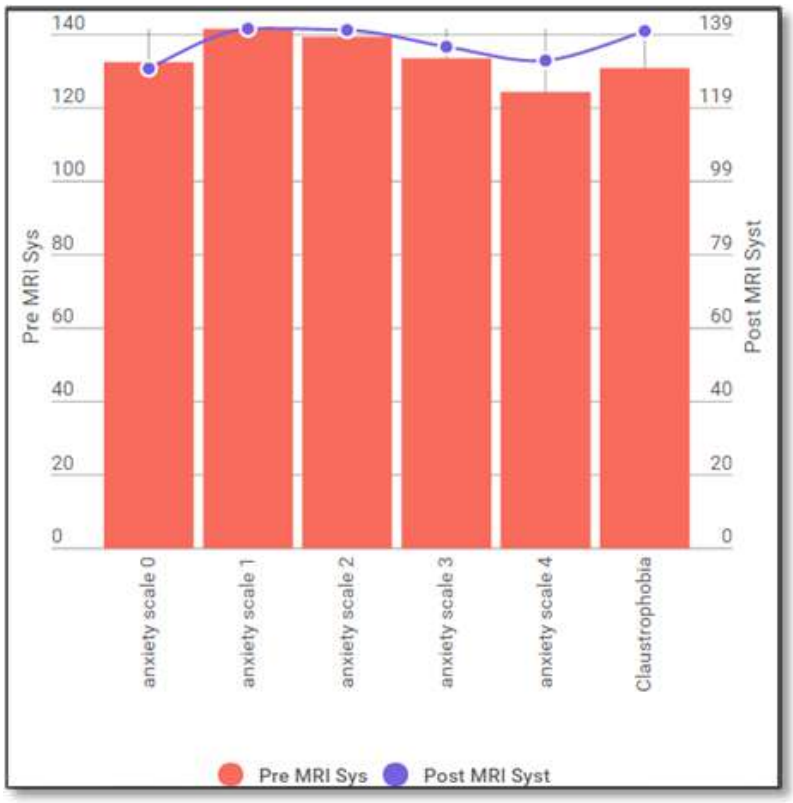
Figure (1a, 1b) anxiety scale percentages and claustrophobia across sex and age groups where

Figure (1) shows 48 volunteers participated in this research, (58.3%) belonged to the female with a mean age of 38 years and incomplete high education, (41.7%) belonged to male with a mean age 39. According to the aspects related to health, most of them did not have the habit of smoking, but presented a family history of systemic diseases, such as diabetes Mellitus, hypertension, cancer, heart disease, pulmonary emphysema. During the period of the patient's follow-up no systemic change that could interfere with the results of the study was detected. The relation between anxiety scale and gender revealed that majority of the female have the high anxiety scales with Claustrophobia. On the otherhand, the majority of male have mild or no anxiety level (25%), female had mild anxiety only (10%). The data obtained from the relation between anxiety scales and the age group revealed thereno significant relation could be detected.

It can be clearly seen that first and second anxiety scales groups show no functional relation between there systolic pressure and the experiment. While patients with higher anxiety scale with level 3 there are obvious upwards. Figure (2, a) Illustrates the statistically significant difference concerning to systolic BP with patients with high anxiety levels and claustrophobic group with (mean ± standard deviation) in the pre and post MRI scan whereas levels of anxiety and claustrophobia t-test=3.007 and P value <0.05). Figure (2,b) shows the difference (mean ± standard deviation) of diastolic BP in accordance to the anxiety scale and claustrophobia pre and post the MRI scan, in different measures, considering the different anxiety level observed.



(a)



(b)

Figure (2): Association of blood pressure with anxiety levels and Claustrophobia. (a) The relationships of pre- and post MRI scans systolic BP with 5 groups (b) the relationships of pre- and post MRI scans diastolic BP with 5 groups.

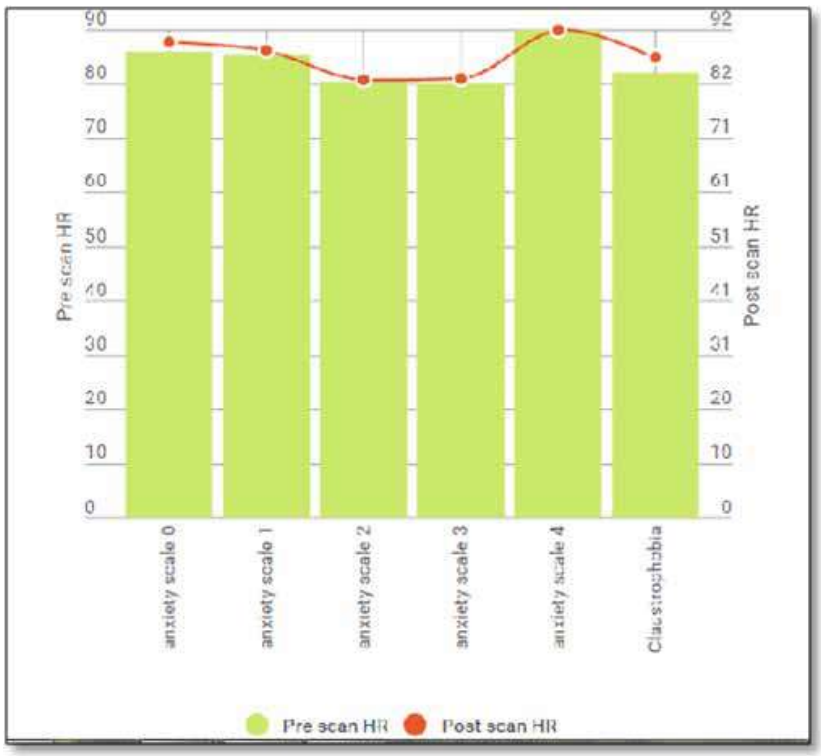


Figure (3) Association of heart rate with anxiety levels and Claustrophobia plots with pre and post MRI scan

Figure (3) shows the HR of volunteers in the two mentioned periods. We can detect that the HR virtually does not change as a function of claustrophobia or anxiety scales. There is a minor alteration after MRI scan for the non-noxious with increasing the difference till plunge in the group 3 and returning to rise again with higher anxious and claustrophobic patients. This is non-significant changes according to the difference of (mean ± and standard deviation).

Discussion

The purpose of this research was to study the relationship between the influence of anxiety and claustrophobia with the bloodpressure and heart rate before and after MRI scan. This section discusses the findings that were observed with the psychophysical questionnaire and vital signs correlation. The study shows three major results about the influence of the anxiety and claustrophobia on; systolic, diastolic blood pressure and heart rate. The authors made this MRI developed anxiety scale in relay on Corah NL. to diagnose the state of anxiety in dental patients [14]. This scale was developed to our study because of the capability to be applied in count for helping as a predictor for the workers in the MRI room in compare with other anxiety scales. Patients during MRI scan are commonly undergoing negative emotions. The unfamiliar environment, unknown staff and noises can incite fear and anxiety as result they cannot complete the scan or motion artifacts will appear in the images[15].

The description provided by radiological technologist and researcher before and after MRI scan is efficient for those patients who presented with mild and non-anxiety level, because we noticed reduction of the patients expectation in relation to the scan performed. This finding reinforces the importance of MRI questionnaireto verbalize so early and safe to the patient about the procedures to be performed in each visit, thus avoiding the fear of the unknown procedures of the scan [6,16].

Keen patients to MRI scan with no anxiety or mild level are found more with younger or educated patients. The result suggests that it is associated with the fact the patients relate the MRI scan to the feeling of fear and anxiety, perhaps to have previous negative experience[17]. Most of the previous studies on MRI associated anxiety and claustrophobia focus on psychological aspects [18]. So this pilot study as our knowledge is the first in related with main vital signs in MRI procedures. Thus, the questionnaire had been prepared to evaluate the patient reactions and to place psychological strategies to reduce the anxiety of the same. In this study, the volunteers received information about the steps and the procedures that were performed, as recommended by other authors[5,12].

The percentages in Figure (1) of this study were approved by other researchers which explained this variation in relation to different reasons for example: the age, gender and circumstances, background and even gene defect [19]. The different individuals have different thresholds for the anxiety sensitivity[20]. Similarly, the difference claustrophobic reactions of MRI patients are affected by body part examined, gender and positioning in the MRI tube [21].

The pie charts illustrate the primary percentages of having different anxiety scale and claustrophobia. It is clear that the main factor influencing their various percentages is the gender difference.

Femalehad higher anxiety levels about 21 % for claustrophobia and anxiety level scale 4; also around 28 % have anxiety scale 3. This result was also found by other study [22]. Whilethe male have lower anxiety level but, the total percentage of less anxious and feared female are fewer than 25%.

In comparison, male percentage shows proportion of the high anxiety scale 4, 3 and claustrophobia of 10%, 0% and 10% respectively. The reasons might belong to anxiety and phobia occurs more often in female than in male [23]. Reckelhoff,et al have shown that BP is less in woman than in men at similar ages. After menopause period, conversely, the BP increases in female more than in male[24].

Sledge and Vinger-hoets; approved interoperation which the first phase of reaction with anxiety and specific types of phobia which leads to increase the systolic pressure and heart rate [25]. Supporting to this study, figure (2-a) illustrates the actual (mean±) values in the five levels groups. Where after the third anxiety level there were significant change between the systolic pressure and the anxiety and rising gradually with moderate, exacerbated levels and claustrophobia. In result, the systolic mean values of the groups raising influence by two factors: the increase in anxiety scales and the present of claustrophobia.

In contrary, significant changes in the diastolic and HR were not found in relation to the five groups during the MRI examination. These results were in the same line, with previous pilot study were found the phobia, anxiety and panic attack do not associated with diastolic and heart rate [26]. In spite of the gigantic improvement in the aspects of arterial hypertension detectionnumerous fields of blood pressure measurement require further rigorous study, such as blood pressure measurement in distinct clinical situation and population [10].

Conclusion

The study results revealed that there was a substantial relation between systolic to anxiety and claustrophobia. On the other hand, the diastolic and heart rate did not change before and after the MRI scan in relation to the anxiety levels recorded with the patients. It is recommended that further research should be conducted in this field. Therefore, according to these results, patient with high blood pressure and/or heart diseases measures his blood pressure before the scan and calm he down should be a mandatory technical step before MRI.

Conflict of Interest: None

Source of Findings: Self-findings.

Ethical Clearance: None

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Molecular Study of Antibiotic Resistance *Mycobacteriumtuberculosis* Isolated from Clinical Samples in Baghdad City

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Abstract

Background: Drug-resistant TB is the most important issue that threatens to interrupt the improvements achieved in tuberculosis control. Multidrug-resistant (MDR) tuberculosis is defined as resistance to both rifampicin and isoniazid.

Objectives: To find molecular feature via DNA extraction for genes in charge of drug resistance in pulmonary tuberculosis patients with hyperglycemia and role of the Xpert MTB/RIF Ultra molecular test efficacy in DR-TB cases detection.

Method: 106 pulmonary TB patient sputum and blood samples were collected in the Institute of Chest and Respiratory diseases in Baghdad and Medical City Hospital from December 2018 - July 2019, 52 were drug resistances TB patients. All patients were examined by conventional method such as direct examination (AFB test), Gene-Xpert test, FBS and HbA1c test were carried out for all cases.

Results: GeneXpert/ultra results revealed 43 cases were Rif resistance in which it was highly significant. The GeneXpert/ultra quantity was mostly medium in the bacillary load. The Rif's resistance cases were mostly registered in the age group (20-59) and in male population. The most frequent patient's type was New MDR. The GeneXpert/ultra quantity and HbA1c levels results showed that most elevated HbA1c cases had a medium to high bacillary load. The DST results were significant in which 95.3% were Rif's resistance and 62.8% were STR's resistance. The LPA on 23 DR cases showed the following; 13 patients were MDR cases in which a deletion in the band WT8 and mutation in the MUT3 band of the gene rpoB and deletion in the inhA WT1 band of the inhA gene were the most notice.

Conclusions: Molecular method had showed that mutations in the *rpoB* gene especially 530 and S531L codon are responsible for majority of RMP resistance in MTB while mutations at the -15/-16 *InhA* codon and 315 of *KatG* codon responsible for most INH-resistance cases.

Keywords: *Antibiotic resistance; Mycobacteriumtuberculosis: clinical samples.*

Introduction

Tuberculosis (TB) is the leading cause of death due to an identifiable infectious pathogen worldwide[(1)]. TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). Millions of people continue to fall sick with TB each year[(2)]. Drug resistance is a natural phenomenon increased by man. Resistance is initiated by a genetic mutation that makes a drug unsuccessful against the mutant bacteria. There is an increased risk of developing resistant bacteria in patients with a large bacillary load because more spontaneous mutations arise in a big population. Then the inadequate treatment permits for

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the selection of a drug-resistant strain to become the dominant strain in patients infected with TB, which can spread from person to person in the same manner as drug-sensitive TB[3]. Multidrug-resistant (MDR) TB is defined as disease due to M. tuberculosis resistant to at least rifampin (R) and isoniazid (H), the vital elements of TB treatment(4). MDR-TB progresses in otherwise curable TB when the course of antibiotics is interrupted and the levels of the drug in the body are inadequate to kill the bacteria 100%[6]. An estimated 600,000 individuals infected with multidrug-resistant (MDR) forms of tuberculosis (TB) in 2016, including 110,000 with rifampicin-resistant TB (RR-TB). Only 153,000 cases of MDR-TB were identified and only 130,000 people began MDR-TB treatment. An expected 240,000 individuals deceased from MDR/RR-TB in 2016[7]. Multi drug resistant TB is a serious public health problem, as it has been associated with higher rates of failure and mortality than drug-susceptible TB, especially in human immunodeficiency virus (HIV) confected patients[8].

Method

Study was conducted at National Reference Laboratory of Tuberculosis/Baghdad from December 2018 to July 2019. 106 TB specimens were collected in this study, 54 of them were new cases and sensitive for RIF and INH; while the other 52 of specimens were DR, 30 of those 52 were hyperglycemia. Their history was taken including the sex, age, history of the symptoms.

The direct examination (AFB) was done by Ziehl-Neelsen stain. The stain was prepared according to the guidelines of laboratory manual guidelines in Iraq[11], the culturing was done on Lowenstein-jensen medium (L.J) solid media, while drug susceptibility test was also carried as instructed in the Laboratory manual guidelines in Iraq(11). Molecular Method for Screening Drug-Resistant TB [12] included **Gene Xpert System MTB/ RMP or Xpert MTB/RIF Ultra**(13-16). and **Line Probe Assays**[17] the Genotype MTBDR *plus* test (Hain Lifescience GmbH, Nehren, Germany)[18] is based on the DNA-strep technology[19] in which they were carried outaccording to the guidelines of laboratory manual guidelines in Iraq.[11]

Result

106 TB specimens were collected in this study, 54 of them were new cases and sensitive for RIF and INH; 19 female and 35 male, while the other 52 of specimens were DR cases 14 female and 38 male, 30 of those 52 were hyperglycemia. The patients were arranged according to age into four groups.

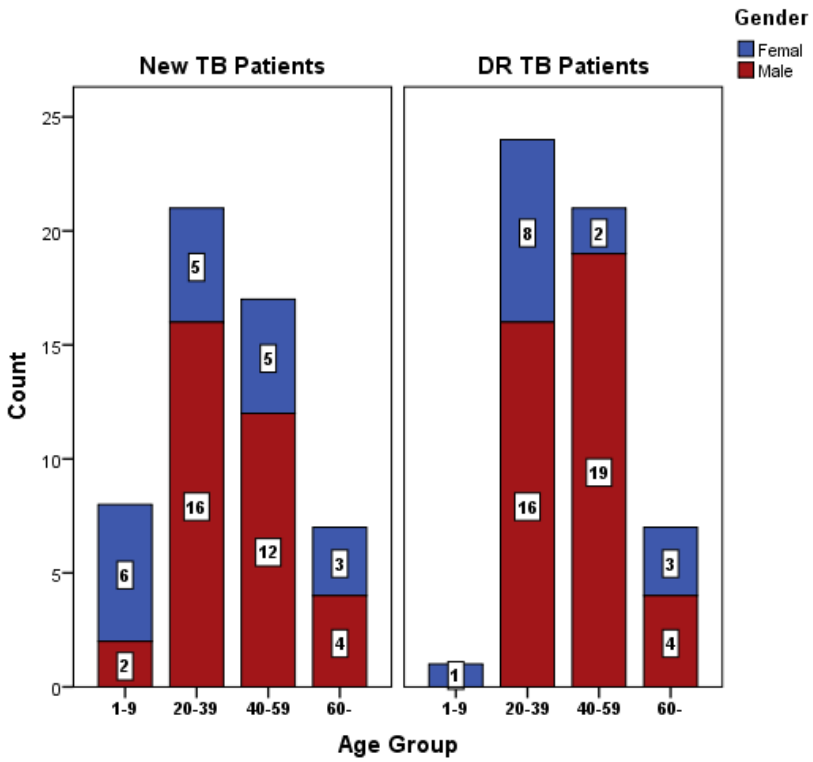


Figure (1): The age group and gender distrubution acording to the TB type

In this study the most recorded cases were in the age groups of (20-38)y in which (38.9%) patients were new TB cases while (46.2%) were DR TB cases and the age group of (39-57)y in which (33.3%) patients were new TB cases while (38.5%) patients were DR TB cases. The P value of age was (0.126) and the mean age of patients was (40.4) years old. Males subjects were more innumerable than female in TB cases according to results, which showed that males were 35 (64.8%) in New TB cases (control), 19 (35.2%) in DR TB cases.

Table (1): Distribution of DR-TB patients according to their DST results:

		New DR		Retreat DR		New MDR		Retreat MDR	
		N=5	100%	N=10	100%	N=21	100%	N=16	100%
Streptomycin	Resistant	2	40.0%	3	30.0%	12	57.1%	12	75.0%
	Sensitive	3	60.0%	7	70.0%	9	42.9%	4	25.0%
Isoniazid	Resistant	1	20.0%	9	90.0%	21	100.0%	16	100.0%
	Sensitive	4	80.0%	1	10.0%	0	0.0%	0	0.0%
Rifampicin	Resistant	4	80.0%	1	10.0%	19	90.5%	16	100.0%
	Sensitive	1	20.0%	9	90.0%	2	9.5%	0	0.0%
Ethambutol	Resistant	2	40.0%	3	30.0%	11	52.4%	12	75.0%
	Sensitive	3	60.0%	7	70.0%	10	47.6%	4	25.0%

Out of the 52 cases ((21/52) (40.38%) new MDR, (16/52) (30.76%) retreated MDR.

The 5 new DR cases (4/5) (80%) RIF's resistance. While the retreated DR cases showed that (9/10) (90%) were INH resistance. The new MDR cases were 21 in which (21/21) (100%) were INH resistance, (19/21) (90.5%) were RIF's resistance. The 16 retreated MDR cases (16) (100%) were INH resistance, (16) (100%) were RIF resistance.

Table (2): The Gxpert/Ultra results distribution according to age, gender, type, HbA1c Level, Zeheel Nelson and Gxpert/Ultra Quantity:

	GeneXpert/Ultra Result				P value
	RR		RS		
Variables	N=43	100%	N=63	100%	
Age Group					0.256
< 20 y	1	2.3%	8	12.7%	
20-39	20	46.5%	25	39.7%	
40-59	15	34.9%	23	36.5%	
≥ 60y	7	16.3%	7	11.1%	
Gender					0.869
Female	13	30.2%	20	31.7%	
Male	30	69.8%	43	68.3%	
Type					<0.001
New	0	0.0%	53	84.1%	
Retreat	0	0.0%	1	1.6%	
New DR	4	9.3%	1	1.6%	

	GeneXpert/Ultra Result				P value
	RR		RS		
Variables	**N=43**	**100%**	**N=63**	**100%**	
Retreat DR	2	4.7%	8	12.7%	
New MDR	21	48.8%	0	0.0%	
Retreat MDR	16	37.2%	0	0.0%	
HbA1c Level					<0.001
Normal(4.6-6.5)	18	41.9%	58	92.1%	
Abnormal	25	58.1%	5	7.9%	
Zeheel Nelson					0.147
Positive	43	100.0%	60	95.2%	
Negative	0	0.0%	3	4.8%	
GeneXpert/Ultra Quantity					0.016
High	17	39.5%	10	15.9%	
Medium	17	39.5%	35	55.6%	
Low	8	18.6%	10	15.9%	
Very low/Scanty	1	2.3%	8	12.7%	

The study is showing that the most age group cases with RR lies in the (20-39) group as 20(46.5%) patients followed by the (40-59) group of TB patients by 15(34.9%) The male patients are also dominant in number in the both RR cases as well as the RS cases, males 30 (69.8%) out of 43 cases with RR results. The 43 RR cases present most patients are new MDR with 21 (48.8%). While the 63 RS cases mostly are new TB patients with 53(84.1%), 8 (12.7%) retarded DR cases and 1(1.6%) retreated and new DR respectively.

Table (3): The Gxpert/Ultra results distribution according to the TB patients symptoms:

	GeneXpert/Ultra Result				P value
	RR		RS		
Variables	**N=43**	**100%**	**N=63**	**100%**	
Productive cough					0.011
Present	26	60.5%	52	82.5%	
Absent	17	39.5%	11	17.5%	
Night Sweating Fever					<0.001
Present	4	9.3%	41	65.1%	
Absent	39	90.7%	22	34.9%	
Weakness					0.134
Present	31	72.1%	53	84.1%	
Absent	12	27.9%	10	15.9%	
weight loss					0.609
Present	28	65.1%	44	69.8%	
Absent	15	34.9%	19	30.2%	
Hemoptysis					0.343
Present	3	7.0%	8	12.7%	
Absent	40	93.0%	55	87.3%	

The night sweating fever were the most symptom with significant P < 0.001 followed closely by productive cough symptom the P value being 0.011. While the most common symptoms were weakness present in 31 (72.1%) RR cases and 53 (84.1%) RS cases, P value 0.134.

Table (4): Gexp/Ultra distribution according to culture on LG and DST (SIRE) results

Variables	GeneXpert/Ultra Result				P value
	RR		RS		
	N=43	100%	N=63	100%	
Culture on LG					---
Positive	43	100.0%	10	100.0%	
Negative	0	0.0%	0	0.0%	
Streptomycin					0.014
Resistance	27	62.8%	2	20.0%	
Sensitive	16	37.2%	8	80.0%	
Isoniazid					0.884
Resistance	38	88.4%	9	90.0%	
Sensitive	5	11.6%	1	10.0%	
Rifampicin					<0.001
Resistance	41	95.3%	0	0.0%	
Sensitive	2	4.7%	10	100.0%	
Ethambutol					0.108
Resistance	25	58.1%	3	30.0%	
Sensitive	18	41.9%	7	70.0%	

All the 53 samples culture result were positive except with 3 of them were subjected to contamination and had to be re-cultured. DST culture results for RIF resist showed 41 (95.3%) positive results on LG out of the 43 RR Gexp/Ultra cases with highly significant P value < 0.001.

Table (5): The LPA results distribution according to the TB patients types:

LPA	Retreat		New DR		Retreat DR		New MDR		Retreat MDR	
	N=0	100%	N=3	100%	N=7	100%	N=7	100%	N=6	100%
rpoB	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT1	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT2	1	100.0%	2	66.7%	7	100.0%	7	100.0%	6	100.0%
rpoBWT3	1	100.0%	2	66.7%	7	100.0%	7	100.0%	6	100.0%
rpoBWT4	1	100.0%	2	66.7%	7	100.0%	7	100.0%	6	100.0%
rpoBWT5	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT6	1	100.0%	3	100.0%	7	100.0%	7	100.0%	6	100.0%
rpoBWT7	1	100.0%	3	100.0%	7	100.0%	7	100.0%	5	83.3%
rpoBWT8	1	100.0%	2	66.7%	5	71.4%	1	14.3%	2	33.3%

LPA	Retreat		New DR		Retreat DR		New MDR		Retreat MDR	
	N=0	100%	N=3	100%	N=7	100%	N=7	100%	N=6	100%
rpoBMUT1	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
rpoBMUT2A	0	0.0%	1	33.3%	0	0.0%	0	0.0%	0	0.0%
rpoBMUT2B	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	16.7%
rpoBMUT3	0	0.0%	2	66.7%	2	28.6%	4	57.1%	4	66.7%
katG	1	100.0%	3	100.0%	7	100.0%	7	100.0%	5	83.3%
katGWT	1	100.0%	3	100.0%	4	57.1%	6	85.7%	3	50.0%
katGMUT1	0	0.0%	0	0.0%	3	42.9%	1	14.3%	2	33.3%
katGMUT2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	1	16.7%
inhA	1	100.0%	3	100.0%	6	85.7%	7	100.0%	6	100.0%
inhAWT1	1	100.0%	2	66.7%	3	42.9%	2	28.6%	3	50.0%
inhAWT2	1	100.0%	2	66.7%	5	71.4%	5	71.4%	5	83.3%
inhAMUT1	0	0.0%	0	0.0%	1	14.3%	4	57.1%	2	33.3%
inhAMUT2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
inhAMUT3A	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
inhAMUT3B	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Discussion

The current study showed a broad range of patients' age with tuberculosis which suggests that all ages can be susceptible to the infection with MTB. These results were compatible with that being reported by studies in Baghdad by AL-Khafaji[23] and in Sulaimaniyah, Iraqi Kurdistan (24) and another which involve almost seven countries[13].

The results also showed that the most prevalent age groups are (20-38) and (39-57) years old of TB patients. and this was also found in the other studies in Iraq such as Kareem *et al.,*[25] and study from 2009- 2012 by Al-khazraji.; et al[26], also The results of study expressed by AL-Khafaji in [23] displayed a large-scale age range of TB patients (> 10 to more than 60 years old) and reveal that the predominant age groups were (21-30) and (41-50) years old of TB patients, the low percentage of TB cases among age group less than 14 years may be due to the BCG vaccine which is given at young age, vaccination give good protection for children however this immunity decline with age.

The Difference results showed in this study of TB cases between men and women may reveal physiological differences (i.e. sex differences) in the epidemiology of TB, differences in the social positions of female and male that affects the risk of exposure and/or gender differences in access to care.

The results of the Genexpert/ultra assay with the HbA1c were of highly significant and it goes with results of the study by Ali RH.; et al which conducted in the developed countries, that the most TB cases were found in Europe; it was more prevalent in elder people due to diabetes mellitus and among immune-compromised patients.[34].

Conclusion

Molecular method had showed that mutations in the *rpoB* gene especially 530 and S531L codon are responsible for majority of RMP resistance in MTB while mutations at the -15/-16 *InhA* codon and 315 of *KatG* codon responsible for most INH-resistance cases. Also Diabetes Mellitus is a recognized risk factor for TB (DR-TB) cases in Iraq. TB patients with diabetes mellitus had shown a rather high bacillary load in molecular test. Some DM-TB patients (with uncontrolled DM) may not respond to the anti-TB treatment and more likely to develop into drug resisting (DR) cases.

Conflict of Interest: None

Source of Findings: Self-findings.

Ethical Clearance: None

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Assessment of Risk Factors of Asthma in Health Institutions in Maysan Governorate, Iraq

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Introduction

Background: Asthma is a common chronic lung disease in which the airways (bronchi) become inflamed and are abnormally sensitive to certain triggers. Asthma can affect people of all races and ages and although there is no known cure, there are many ways to control it. The symptoms of asthma include coughing, shortness of breath, wheezing and chest tightness.

Materials and Method: This is A case-control study conducted in Consultation Center for Chest and Respiratory Diseases at Al-Sadr Teaching Hospital in Maysan Governorate Data was collected over a period of five months between the 1st of November 2019 to the 1st of April 2020.

Results: The commonest risk factors that had been found causes asthma in cases was family history of asthma with OR=5.127, CI=(2.77-9.49), upper respiratory tract infection with OR=5.059, CI=(2.29-8.76), active smoker with OR=2.145, CI=(1.18-3.91)Occupational exposure to dust with OR = 4.660, CI= (2.68-8.10), skin allergy with OR =2.77, CI=(1.28-6.03).

Conclusion: Significant association was found between **(**Family history of asthma, upper respiratory tract infection, active smoking,occupational exposure to dust, chemical, fumes and gases, allergies and the used of NSAIDs before the onset of asthma) and asthma in adults in maysan governorate.

Keywords: *Risk Factors; Asthma; chronic lung disease; Maysan.*

Introduction

Asthma is a common chronic respiratory disease and is a major public health problem worldwide and affects people of all races and genders. Reports indicate that the number of asthma sufferers worldwide may exceed 334 million, according to a report published by the World Asthma Network in 2014**(1)**. Many studies have shown that the prevalence of asthma varies between countries and within countries and is directly proportional to different allergies, where different lifestyles are adopted and societies become civilized and this trend is expected to continue during the next two decades**(2)**. For people who suffer from asthma, it can cause them a major disability and affect the quality of life significantly**(1)(2)**. The significant increase in the prevalence of asthma and other allergies (for example, eczema) cannot be explained over the past several decades by relying on genetic factors alone and this leads to an increased focus on other risk factors such as environmental exposure, for example and it is expected that cases will rise Asthma to over 400 million cases globally in 2025**(3)**. due to climate change, increased exposure to air pollution, urbanization, changing the immune response and changing lifestyle**(3)(4)**. in the UK receiving approximately 4.3 million adults treating from asthma**(5)**.

Aim of Study: To assess the major risk factors of asthma in adult in health institutions in maysan governorate.

Patients and method

A case-control study was conducted in Consultation Center for Chest and Respiratory Diseases at Al-Sadr Teaching Hospital in Maysan Governorate(one of the 18 Iraqi governorate located in south of Iraq the number of persons 1 million and characteristics with Marshes and oil) It is a center for the diagnosis and treatment of

respiratory diseases and is located in the center of the city of Amara and provides care and treatment to respiratory patients from all its districts and aspects of Maysan Governorate. The center provide services to average 50 patients daily with respiratory diseases of them about 2-3 patients with asthma daily for 5days in a week. The daily center activities include history taking, clinical examination, laboratory investigations, management, the follow-up to asthma patients, health education, also different respiratory cases for management and follow up. Data was collected over a period of five months between the 1st of November 2019 to the 1st of April 2020.

Statistical Analysis: Analysis of data was carried out using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25).

Finding: The results in table 1 showed the distribution of age and gender among cases and controls and there was no statistically significant difference between the two groups (p >0.05) the higher percentage of cases was in age groups (40-49) years (27.5%). the higher percentage of controls was in the age group (30-39) years (30.8%). Predominantly males (36.7%) and females (63.3%) in both cases and controls.

Table (1): Distribution of cases and controls according to age and gender.

		Asthmatic patients		Controls		P value
		No	%	No	%	
Gender	Male	44	36.7	44	36.7	
	Female	76	63.3	76	63.3	
Age (Years)	<20 Years	7	5.8	4	3.3	0.356
	20---29	12	10.0	21	17.5	
	30---39	30	25.0	37	30.8	
	40---49	33	27.5	27	22.5	
	50---59	20	16.7	18	15.0	
	=>60 Years	18	15.0	13	10.8	

The results in (table 2) shows persons that used electrical heating system protected from asthma with low significant association OR=0.553, CI=(0.32-0.95). Persons that use oil heating system at risk (5 times) to have asthma with highly statistical significant OR =4.846, CI=(2.40-9.80). Regarding dampness on the wall, the percentage of the cases was 73.3% and controls were 65.0%, with no statistical differences between the two groups (p-value= 0.162).). Regarding household pets, the percentage of cases was 19.2% and controls were 15.0% with no statistical significant P-Value =0.162. persons that allow pets in bed at risk 5 times to have asthma with significant association OR=5.46, CI=(1.24-24.09).

Table (2): Distribution of cases and controls according to heating system inside the house, dampness on the wall and household pets.

Type of heating system inside the house		Asthmatic patients		Controls		P value	OR (95% CI)
		No	%	No	%		
Electrical	Yes	34	28.3	50	41.7	0.030*	0.553 (0.32-0.95)
	No	86	71.7	70	58.3		
Oil	Yes	108	90.0	78	65.0	0.0001*	4.846 (2.40-9.80)
	No	12	10.0	42	35.0		

Type of heating system inside the house		Asthmatic patients		Controls		P value	OR (95% CI)
		No	%	No	%		
Is there dampness on the wall	Yes	88	73.3	78	65.0	0.162	1.481 (0.85-2.57)
	No	32	26.7	42	35.0		
You have household pets	Yes	23	19.2	18	15.0	0.391	1.344 (0.68-2.64)
	No	97	80.8	102	85.0		
Allow pets be in bed	Yes	12	52.2	3	16.7	0.019*	5.46 (1.24-24.09)
	No	11	47.8	15	83.3		

This table shows the persons that have a family history of asthma at risk 5 times to have asthma compared with none with highly significant association OR=5.127, CI=(2.77-9.49). the high percentage of family history in cases was 18.3% in mother and in controls was 7.5% in sister.

These results shows persons that have upper respiratory tract infection at risk five times to have asthma with highly significant association OR=5.059, CI=(2.29-8.76). this table shows persons that have an infection from 6-11 months at risk 8 times to have asthma with highly significant association OR=8.25, CI=(1.80-37.91), persons that have infections =>12 months at risk 4 times to have asthma with highly significant association OR=4.29, CI=(1.48-12.41).

Table (3): Distribution of cases and controls according to family history and upper respiratory tract infection.

		Asthmatic patients		Controls		P value	OR (95% CI)
		No	%	No	%		
Family history of asthma	Yes	57	47.5	18	15.0	0.0001*	5.127 (2.77-9.49)
	No	63	52.5	102	85.0		
The relationship with the person in the family has asthma	Brother	3	2.5	3	2.5		
	Father	17	14.2	2	1.7		
	Grandfather	4	3.3	-	-		
	Mother	23	18.3	3	2.5		
	Sister	3	2.5	9	7.5		
	Sons	-	-	1	0.8		
	Uncle	7	5.8	1	0.8		
Have URT infection before asthma onset	Yes	86	71.7	40	33.3	0.0001*	5.059 (2.29-8.76)
	No	34	28.3	80	66.7		
Duration of infection	<6 months	40	46.5	33	82.5	0.001*	-
	6---11	20	23.3	2	5.0		8.25 (1.80-37.91)
	=>12 months	26	30.2	5	12.5		4.29 (1.48-12.41)

The results in table 4 show persons that are an active smoker before the onset of asthma at risk 2 times to have asthma from non-smoker with significant association OR=2.145, CI=(1.18-3.91) the high percentage of cigarettes packet/day was 56.4% of cases and 72.7%of controls were in 1 packet/day, With statistically no significant (p-value = 0.433). the high percentage of the duration of smoking of cases was 43.6% in duration

(=>15 years), for controls were 40.9% in duration (10---14 years). Regarding second-hand smoke, the percentage of cases was 50.8% and controls were 49.2% with statistically no significant (p-value = 0.796).

Table (4): Distribution of cases and controls according to smoker and second hand smoke.

		Asthmatic patients		Controls		P value	OR (95% CI)
		No	%	No	%		
Smoker before onset asthma	Yes	39	32.5	22	18.3	0.012*	2.145 (1.18-3.91)
	No	81	67.5	98	81.7		
Cigarettes packet/day	1	22	56.4	16	72.7	0.433	
	2	15	38.5	5	22.8		
	3	2	5.1	1	4.5		
Duration of smoking	<5 years	5	12.8	1	4.5	0.530	
	5---9	7	17.9	4	18.2		
	10---14	10	25.6	9	40.9		
	=>15 years	17	43.6	8	36.4		
Second hand smoke	Yes	61	50.8	59	49.2	0.796	1.069 (0.64-1.77)
	No	59	49.2	61	50.8		

The results in (table 5) show persons that are overweight that have BMI(25-29.9) at risk 1 time to have asthma but with no significant association OR=1.42, CI=(0.78-2.58). Persons that are Obese I that have BMI (30-34.9) at risk 3 times to have asthma with significant association OR=2.54, CI=(1.15-5.63). persons that are Obese II that have BMI (=>35) at risk 5 times to have asthma with significant association OR=4.89, CI=(1.77-13.53).

Table (5): Distribution of cases and controls according to BMI.

		Asthmatic patients		Controls		P value	OR (95% CI)
		No	%	No	%		
BMI (Kg/m^2)	Normal (18.5-24.9)	33	27.5	51	42.5	0.005*	-
	Overweight (25-29.9)	45	37.5	49	40.8		1.42 (0.78-2.58)
	Obese I (30-34.9)	23	19.2	14	11.7		2.54 (1.15-5.63)
	Obese II (=>35) Total	19120	15.8100	6120	5.0100		4.89 (1.77-13.53)

Discussion

Regarding the age, the results in this study demonstrated that the higher percentage was in the age group between (40-49) years with no significant association (p-value =0.356). it disagrees with the study that reported by Abdulhussian, 2015 in Basrah that found most of the study samples were among age group (30-44) years with no significant association (p-value =0.730)[6]. In the current study, this results demonstrated that the higher percentage of gender in females 63.3% in compared with males 36.7%.which is in agreement with the result that done by Abdulhussian, 2015 in Basrah that found most of the study samples were among females 62.5% in compared with males 37.5%[6].

Regarding the type of heating system inside the house the results of current study shows that the higher percentage was 90.0% in cases and 65.0% in controls were oil, which it disagrees with the result of study done by Esmaeil Idani,2019 in Iran which found that the higher percentage of cases was 98.3% and controls were 98.4% were Gas[7] . This disagreement may be due to the type of fuel available in Iraq compared to other countries, Also, it may be due to economic and environmental matters.

In this study regarding dampness on the wall, this result demonstrate that the percentage of the cases was 73.3% and controls were 65.0%, with no statistical differences between the two groups (p-value= 0.162). which is in agreement with the result of study done by Esmaeil Idani,2019 in Iran that found that the percentage of cases was 51.1% and controls were 49.2% with no statistical differences between the two groups (p-value= 0.433)[7].

regarding household pets, the current study demonstrate that the percentage of cases have household pets was 19.2% and controls were 15.0% and this results shows the percentage of allowing pets in bed was 52.2% in cases and 16.7% in controls with statistical significance (p-value = 0.019).which is in agreement with the results of study done by Esmaeil Idani,2019 in Iran that found the percentage of cases have household pets was 8.5% and controls were 7.9% and the percentage of allowing pets in bed was 2.8% in cases and 0.9% in controls with statistical significance (p-value <0.001)[7].

The present study demonstrate that the percentage of cases have family history of asthma was 47.5% and for controls 15.0%,which is in agreement with results of study that done by Elfaki NK, 2017 in Saudi Arabia. That found that the percentage of cases have family history of asthma was 66.8%. and for controls 40.2%[8].

The current study demonstrate that the percentage of cases have upper respiratory tract infection was 71.7% and for controls were 32.5% with highly statistically significant (OR= 5.253, CI=3.03-9.11) . which is in agreement with the results of study that reported by Al-Mazam, A., 2001 in Saudi Arabia that found that the percentage of cases have upper respiratory tract infection was 61.8% and for controls were 13.6% that found to be an independent risk factor for bronchial asthma (OR=10.5, CI=5.11-21.89)[9].

Regarding active smoker this study shows that the percentage of the active smoker was 32.5% among cases and 18.3 among controls,which is in agreement with the results of study done by Elfaki, N. K., 2017 in Saudi Arabia. that found the percentage of cases are active smokers was 26.6% and for controls were 22.8%[8].

Regarding second-hand smoke, this study shows that the percentage of cases was 50.8%,and for controls were 49.2%. which is in agreement with the results of study done by Elfaki, N. K., 2017 in Saudi Arabia. that found the percentage of cases was66.3% and for controls were 56.0%[8].

Regarding BMI(Body Max Index) this study shows that the higher percentage of cases was 37.5% were overweight (25-29.9) and the lower percentage of cases was 15.8% were obese II(=>35). which is in agreement with the results of study done by Muhammed, S. M et al,2012 in Baghdad which found that the higher percentage of asthmatic patients was45.7% were overweight (25-29.9). and the lower percentage of cases was 2.3% were obese II(=>35)[10]. But, it is disagreed with the results of study done by Rönmark, E.et al,2005 in northern Sweden that found that the higher percentage of cases was 42.1% were normal (20-24.9)[11]. This disagreement may be due to a different lifestyle in Iraq than in other countries.

Conclusions

Statistically significant was found between the type of heating system inside the house and asthma in adults. Also, between patients who had a family history of asthma and asthma in adults so Positive association was found between upper respiratory tract infection and asthma in adults.

Conflict of Interest: None

Funding-self or other Source: None

Ethical Clearance: None

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Atherogenic Index of Plasma Levels in Iraqi Diabetic and Diabetic Nephropathy Patients

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Abstract

The study aimed todetermine the Levels of HbA1c %, urea, createnine, albumin,total cholesterol, triglyceride,high density lipoprotein (HDL-c), low density lipoprotein (LDL-C) and very low-density lipoprotein (VLDL) in diabetic and diabetic nephropathy patients and to compare the results with that of control group. Then, to compare Atherogenic Index of Plasma levels in these groups that may be predict prone of patients to cardiovascular illness. This study has selected one hundred fifty participates who were consideredto be enrolled in this research with aged ranged (40-65) years that divided into three groups as follows: group one (G1) consists of 50 healthy individuals as a control group, group two (G2) consists of 50 patients with diabetes and group three (G3) consists of 50 patients with diabetes and nephropathy as complication.

This study illustrated significant increased were found in Total Cholesterol and low-density Lipoprotein in G2 comparing to G1, while there was highly significant increase in total cholesterol and low-density lipoprotein in G3 comparing to G2 and in G1. Results, also, revealed highly significant increase in triglyceride and very low density in G2 and G3 comparing to G1. Data demonstrated highly significant decrease in high density lipoprotein in G2 and G3 comparing to G1. Also, demonstrated highly significant increase in Atherogenic Index levels in G2 and G3 comparing to G1, while no significant increased observed in G3 comparing to G2. Sothe study concluded that Atherogenic Index is elevated in diabetic and nephropathy diabetic patients who could be used as a marker for predict cardiovascular disease in patients groups.

Keywords: *Diabetic Nephropathy, Lipid profile and Atherogenic Index (AIP).*

Introduction

Diabetic nephropathy is the most common cause of chronic kidney disease and represents a large and ominous public health problem. Patients with diabetic kidney disease have exceptionally high rates of cardiovascular morbidity and mortality. In fact, the excess mortality among patients with diabetes appears to be largely limited to the subgroup with kidney disease and explained by their high burden of cardiovascular disease [(1)].

Obesity increases the risk of many diseases such as heart disease, type 2 diabetes, obstructive sleep apnea, cancers and osteoarthritis**[(2)].** There is a seven times greater risk of diabetes in obese people in contrast with healthy weight, with a threefold increase in risk for overweight people. Also, body fat distribution is an important determinant of increased risk of diabetes **[(3)].**

The atherogenic index of plasma (AIP), defined as logarithm [log] of the ratio of plasma concentration of triglycerides to high-density lipoprotein (HDL) cholesterol, has recently been proposed as a predictive marker for plasma atherogenicity and is positively correlated with cardiovascular disease risk. Also, AIP correlates with the size of high-density lipoprotein and

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low-density lipoprotein particles and with the fractional esterification rate of cholesterol by lecithin: Cholesterol acyl transferase in plasma. This ratio accurately reflects the presence of atherogenic small low-density lipoprotein and high-density lipoprotein particles, is a sensitive predictor of coronary atherosclerosis and cardiovascular risk and a useful surrogate for insulin resistance [4].

Materials and Method

One hundred fifty individuals with age ranged between (40-65) years were enrolled in this study. They divided into three groups as follows:-

1. Group (G1) that consists of 50 healthy individuals as control group.
2. Group (G2) that consists of 50 diabetic patients.
3. Group (G3) that consists of 50 diabetic nephropathy patients.

Blood samples were collected from all groups after a period of fasting 12-14 hours. The study was conducted between March 2020– June 2020 in the diabetic & endocrinology center in Al- Yarmouk Teaching Hospital/Iraq.

Whole blood was used in determination of HbA1c. The HbA1c was determined by HPLC method. A hemolyzed whole blood was mixed with a weakly binding cation-exchange resin. The nonglycosylated hemoglobin (HbA0) linked to the resin, leaving (HbA1) free to remove by a resin separator. The percent of HbA1 was measured by evaluation of the absorbance values at 415nm of HbA1 fraction and of the total Hb fraction,by calculating the ratio of absorbances (R). This ratio comparing was compared to the ratio of a glycohemoglobin standard that obtained from the same procedure[5]. The urea level was estimated after enzymatic hydrolysis by urease enzyme. Indophenol compound was generated from Salicylate and Hypochlorite as shown in below . The intensity of the green complex is proportional to the urea found in the sample[6]. Creatinine in the sample was reacted with picrate in alkaline medium forming a coloured complex (Jaffe method)[7]. The method for determination of albumin was based on the specific binding of bromocresol green (BCG), an anionic dye and the protein at acidic pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed was proportional to the concentration of albumin in the sample[8]. Serum glucose was measured by using kits from (Randox Company, United Kingdom) which based on the PAP enzymatic determination of glucose[9]. Total cholesterol[10],triglyceride [11]and HDL-c [12]were measured by enzymatic method from (Human Gesellschaft fur biochemical and Diagnostica mbH, Germany.). The levels of LDL-C and VLDL-C were analyzed by using Friedewald equation as follows[13]:

$$\text{LDL} - \text{C}\left(\frac{mg}{dl}\right) = Total\ cholesterol - HDL - \frac{TG}{5}$$

Serum VLDL-C can be calculated by:

$$\text{VLDL} - \text{C}\left(\frac{mg}{dl}\right) = \frac{TG}{5}$$

The atherogenic index of plasma (AIP) was calculated according to Milada formula equation as display below[14]:

$$\text{AIP} = \frac{logTG}{HDL}$$

Statistical Analysis: The results expressed as mean± SEM. Students t-test was applied to compare the significance of the difference between DN, Diabetic patients and control groups. p-value ($P \geq 0.05$), ($P \leq 0.05$), ($P \leq 0.001$) considered statistically nonsignificant, significant and highly significant respectively. The correlation coefficient (r) test is used for describing the association between the different studied parameters.

Findings: Results in table (1) illustrated levels of (FBG, HbA1C%, urea, creatinine and albumin) in G1, G2 and G3. Results in table (1) reveled a significant elevation in FBG, HbA1c%, urea and creatinine levels in G2 comparing to G1. Results, also, showed a highly significant elevation in these parameters in G3 comparing to G1 and G2. While results display a significant decrease in albumin levels in G2 and G3 comparing to G1 and in G3 comparing to G2.

Table 1: Descriptive Parameters for G1, G2, G3

Parameters	(G1)	(G2)	(G3)	T-Test G1 vs G2	T-Test G1 vs G3	T-Test G2 vs G3
FBG (mg/dL)	90.62±7.223	177.62±25.742	230.67±50.974	S	S	S
HbA_{1C} (%)	5.32±0.475	8.42±1.068	11.77±1.47	S	S	S
Urea (mg/dL)	20.31±3.4	33.67±4.62	107.37±57.2	S	S	S
Creatinine (mg/dL)	0.47±0.11	0.92±0.164	3.94±2.42	S	S	S
Albumin (mg/dL)	4.029±0.3	3.97±0.215	2.4±0.836	NS	S	S

Table (2) shows the levels of total cholesterol, triglyceride, high density lipoprotein (HDL-c), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL) and AIP levels for G1, G2 and G3. Results revealed a significant elevation in levels of total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein in G2 and G3 comparing to G1 while a significant decrease was found in HDL levels in G2 and G3 comparing to G1. Results, also showed a significant elevation in (TC, TG, LDL-c and VLDL-c) in G3 comparing to G2,while nosignificant was found in G3 comparing to G2 in HDL levels. Result in this table, also, demonstrated highly significant elevation in Atherogenic Index levels in G2 and G3 comparing to G1, while no significant increase observed in G3 comparing to G2.

Table (2): Lipid profile (TC, TG, HDL-c, LDL-c, VLDL-c) and AIP levels for all studied groups.

Parameters	(G1)	(G2)	(G3)	T-Test G1 vs G2	T-Test G2 vsG3	T-Test G1 vsG3
TC (mg/dL)	187.4±6.1	193.5±6.6	271.47±5.5	N.S	S	S
TG (mg/dL)	105.04±20.111	177.01±52.8	228.5±6.8	S	S	S
HDL-c (mg/dL)	48.166±4.444	35.167±4.32	35.2±4.211	S	NS	S
LDL-c (mg/dL)	82.2±14.37	110.1±32.47	195.95±5.8	S	S	S
VLDL-c (mg/dL)	22.475±6.34	34.42±9.21	53.62±12.41	S	S	S
AIP	0.54±0.017	0.82±0.024	0.89±0.017	S	N.S	S

Diabetic dyslipidemia comprises a triad of raised triglycerides, decrease HDL-c and excess of small, dense low density lipoproteinparticles, which is in agreement with the present study[15]. The dyslipidemia are found in diabetes patients due to insulin resistance or deficiency affect key enzymes and pathways in lipid metabolism[16].

In individuals with type 2 diabetes, metabolic syndrome and the combined dyslipidemia, cardiovascular risk is elevated by a clustering of risk factors such as abdominal obesity, impaired fasting glucose, increased blood pressure, low HDL-cholesterol (HDL-C), elevated triglycerides (TGs) and an elevated in small, dense LDL particles. The current increase in the incidence of type 2 diabetes in the population perhaps poses the most urgent cardiovascular risk. Although insulin resistance is crucial to the pathogenesis of the disease, the associated atherogenic lipoprotein phenotype considerably enhances the risk. Hence there is an ongoing intense search for a medication capable of modifying the atherogenic lipid profile as well as lowering glucose. Medications of the thiazolidinedione class traditionally used for glycemic control in patients with type 2 diabetes seem to hold promise in this respect[17].

Atherogenic Index may be an important tool for analyzing the results of clinical trials. The association of triglycerides and high density lipoprotein in this simple ratio theoretically reflects the balance between risk and protective lipoprotein forces and both triglycerides and high density lipoprotein are widely measured and available[18].

In summary, Atherogenic Index provides

information about the atherogenicity of plasma and quantifies the response to therapeutic intervention[19].

Conclusion

The study concluded that Atherogenic Index is increased nephropathy diabetic patients more than diabetic and control group that could be used as a marker for predict CVD in patients' groups.

Conflict of Interest: None

Funding-self or other Source: None

Ethical Clearance: All studies were conducted in accordance with the ethical committee of Al-Farahidi University/Iraq.

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Evaluation of Serum Level of Serum Catalase and SOD as a Predictive Factors in Preterm Labor Patients

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Abstract

The study is a comparative case control study conducted on 45 preterm delivery between (28-37 week) of gestation as cases group and 45 term delivery between (37-42 week) of gestation as control group, at obstetrical and gynecology department of Salah Alden general hospital in Salah Alden city from 20th of November 2019 to 15th February 2020. The blood sample taken from participated women and the oxidative stress parameter (F2-8-isoprostan and protein carbonyl) was measured by ELISA technique. The cases were Single and twin pregnancy with cervical dilatation >3 cm and fully effaced cervix, and/or rupture membrane, Uterine contraction 3per 10 min and Indicated labor pregnancy like bleeding, DM, hypertension, pPROM, fetal destress, fetal death. But uncertain gestational age, elective term cesarean section, liver disease, renal disease and hepatitis infected women were excluded.

Result: Mean age of participants was (26.05±6.59 years) with a range of (14-45years) and mean weight was (74.84±6.728 Kg), range of (60-95 Kg) and mean height of participated (156.14±5.3 cm) and range of (150-170 cm).Superoxide dismutase and catalase showed significant decrease in preterm labor (mean±stander error) (529.161 ± 35.87 U/ml) (1.637 ± 0.12 pg/ml) respectively in compare with (mean± stander error) (1.637± 0.12U/ml) (529.161± 35.87 pg/ml) in preterm labor.

Conclusion: This study showed that catalase and superoxide dismutase significantly decrease in preterm labor group.

Keywords: *Serum catalase; SOD; Predictive Factors; Preterm Labor Patients.*

Introduction

A preterm birth (PTB) defined as a labor occurring before 37 weeks (259 days) of gestation. PTB are usually classified into three categories: medically induced (25% of all PTB), spontaneous preterm labor (50% of all PTB) and preterm premature rupture of membranes (pPROM) (25% of all PTB). Preterm birth are divided into four degrees of prematurity: extremely preterm (less than 28 weeks), very preterm (28 – 31^{+6} weeks), mild preterm (32 – 33^{+6} weeks) and moderate preterm (34 – 36^{+6} weeks)[(1)]. Preterm birth, depending on one's point of view, may be considered as an adverse gestational outcome (where a fetus is incapable to achieve its in utero growth potential) or a favored outcome (e.g., where an abortion or non-viable prematurity has been successfully prevented). Even healthy women with low-risk pregnancies, a fraction of babies can be probably born preterm[(2)].

The most popular definition of free radicals is {molecules or molecular fragments that contain one or more unpaired electrons in the atomic or molecular orbitals}. The free radicals are uncharged, short-lived molecules and very reactive. Human body contain about 10,000–20,000 free radicals which attack each individual cell of human body. Numerous of these radicals are beneficial in that they effort for immune cells responsible

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for bacterial cells killing and toning the smooth muscles, which in order regulate the normal function of blood vessels and the internal organs. Uncontrolled generation of the free radicals in human body may cause various harmful effects like heart and neurodegenerative diseases, autoimmune diseases, cancers, etc.[3].

Preterm labor is a complex condition resulting from multiple etiologic factors, but it is well documented and accepted that both inflammation and infection represent highly significant risk factors for PTB. However, despite the public health and clinical significance of PTB and recent research achievements, the relations between infectious agents, immunological or molecular pathways and triggering factors for spontaneous PTB remain poorly understood reflecting the complexity of this obstetrical problem. It has been widely acknowledged that OS may involve in the pathogenesis of human disease and represent a major topic in all areas of medicine [4]. In this regard, there is emerging evidence that an imbalance between oxidants and antioxidants may play a role in PTB [5].

This study is designed to evaluate the association of catalase and SOD with preterm labor.

Patient and Method

This study is case control study carried out in Salah Alden city from 20th of November 2019 to 15 February 2020. The study included 90 pregnant women attend Salah Alden general hospital department of obstetrics and gynecology for labor, 45 of them are preterm (between 28 and 37 weeks) as cases and 45 of them complete term (between 37 and 41 weeks) as control, the patients included Single and twin pregnancy, cervical dilatation >3 cm, fully effaced cervix, and/or rupture membrane, uterine contraction 3per 10 min and indicated labor like bleeding, DM, hypertension, pPROM, fetal destress and fetal death. The exclusion criteria include uncertain gestational age, elective term cesarean section, liver disease, renal disease and hepatitis infected women. Ablood sample was taken from them and an interview was carried out with these patients using questionnaire form designed by the investigator including their demographic characteristics, age, weight, length. etc. The antioxidant parameter (catalase and SOD) was measured by ELISA technique and colorimetric methodrespectively.

Results

The results showed that mean age of participants was (26.05±6.59) years with a range of (14-45) years and mean weight was (74.84±6.728) Kg, range of (60-95)Kg and mean height of participated (156.14±5.3)cm and range of (150-170) cm. The participants include 32 urban females and 58 rural and they deliver 51 male babies and 39 females. 22 of the participants are prim gravitas and the other 77 are multigravida.

The study groups include 15 prim gravida women and 30 multigravida in preterm group and 7 prim gravida and 38 multigravida in term group.

The study groups include 10 women with positive history of abortion and 35 women with negative history in preterm group and 8 women with positive history of abortion and 37 women with negative history in term group.

Catalase Activity: Comparison between study groups regarding serum catalase activity level was performed using anova test. Cases were compared with controls. There was a statistically significant difference in the serum mean catalase activity level for cases group (mean±S.E) (529.161± 35.87) pg/ml and control group (mean±S.E) (739.284± 17.60) pg/ml, p value <0.001. Table (1).

Table (1): Serum catalase activity level comparison between term and preterm groups

Catalase (pg/ml)						
Group	**N**	**Mean**	**S.E**	**Minimum**	**Maximum**	**P value**
Term	45	739.28	17.60	365.13	992.08	*<0.001
Preterm	45	529.16	35.87	27.78	902.08	

*The result was highly significant.

Superoxide dismutase activity: Comparison between study groups regarding serum SOD activity level was performed using anova test. Cases were compared with controls. There was a statistically significant difference in the serum mean SOD activity level for cases group (mean±S.E) (1.637±0.12) U/ml and control group (mean±S.E) (2.251± 0.14) U/ml, p value 0.002. Table (2).

Table (2): Serum SOD activity level comparison between term and preterm labor

Superoxide Dismutase (*U/mL*)						
Group	**N**	**Mean**	**S.E**	**Minimum**	**Maximum**	**P value**
Term	45	2.25	0.14	0.63	3.78	*0.002
Preterm	45	1.637	0.12	0.06	3.57	

*The result was highly significant.

Comparison of serum mean level of SOD between patient complain of contraction (mean±S.E) (1.655±0.23) U/ml and patient not complain of contraction (mean±S.E) (1.603±0.19) U/ml within preterm group show no statistical significant with p value 0.364.

Comparison of serum mean level of SOD between patient complain of pPROM (mean±S.E) (1.515±0.30) U/ml and patient not complain of pPROM (mean±S.E) (1.681±0.27) U/ml within preterm group show no statistically significant with p value 0.174.

Comparison of serum mean level of catalase between patient complain of contraction (mean±S.E) (572.039±67.11) pg/ml and patient not complain of contraction (mean±S.E) (446.705±24.67) pg/ml within preterm group show no statistically significant with p value 0.213.

Comparison of serum mean level of catalase between patient complain of pPROM (mean±S.E) (534.534±98.97) pg/ml and patient not complain of pPROM (mean±S.E) (527.243±87.23) pg/ml within preterm group show no statistically significant with p value 0.947.

The study show that the comparison of serum means level of catalase and SOD between prim and multigravida in preterm group no statistically significant. Table (3)

Table (3): Comparison Oxidative stress level between prime and multi gravida in preterm group

No. of previous pregnancy (in preterm group)		**Catalase (pg/ml)**	**SOD (U/ml)**
Prim gravida	Mean	542.56	1.38
	S.E	69.04	0.28
Multi gravida	Mean	522.19	1.76
	S.E	42.08	0.11
P value		*0.76	*0.16

*The result was non-significant.

The study show that the comparison of serum catalase and SOD mean level between prim and multigravida in term group no statistically significant Table (4).

Table (4): Comparison Oxidative stress level between prime and multi gravida in term group.

No. of previous pregnancy in term group		Catalase (pg/ml)	SOD (U/ml)
Prim gravida	Mean	773.94	2.24
	S.E	47.55	0.36
Multi Gravida	Mean	733.34	2.25
	S.E	19.04	0.15
P value		*0.42	*0.97

*The result was non-significant.

The study show that the comparison of serum means level of catalase and SOD between women with negative history of abortion and women with positive history of abortion in preterm group no statistically significant. Table (5).

Table (5): Comparison Oxidative stress level with history of abortion in preterm group

History of abortion (in preterm group)		Catalase (pg/ml)	SOD (U/ml)
Negative	Mean	532.62	1.58
	S.E	41.42	0.15
Positive	Mean	516.16	1.81
	S.E	67.85	0.12
P value		*0.85	*0.45

*The result was non-significant.

The study show that the comparison of serum means level of catalase and SOD between women with negative history of abortion and women with positive history of abortion in term group no statistically significant. Table (6).

Table (6): Comparison Oxidative stress level with history of abortion in term group

History of abortion (in term group)		Catalase (pg/ml)	SOD (U/ml)
Negative	Mean	739.54	2.15
	S.E	21.32	0.15
Positive	Mean	738.22	2.66
	S.E	16.14	0.24
P value		*0.97	*0.15

*The result was non-significant.

The study show that the comparison of serum mean level of catalase and SOD between women with positive history of preterm labor and women with negative history of preterm labor in preterm group no statistical significant. Table (7).

Discussion

Spontaneous preterm birth (sPTB) is a common major pregnancy complication leading to perinatal morbidity and mortality as well as short- and long-term sequelae [(6)]. The biologic mechanisms leading to PTB are incompletely understood. Oxidative stress may represent one possible mechanism. Oxidative stress biomarkers have been associated with adverse pregnancy outcomes [(7)] and several studies have shown that oxidative stress biomarkers are increased among women who go on to experience PTB or shortened gestation. However, the strength of association remains inconsistent across studies and discrepancies may in part be due to differences in biomarkers used to measure oxidative stress.[(8)]

Catalase enzyme is one of most abundant antioxidant enzymes which represent first line of antioxidant defense against free radical. [9]

In this study catalase activity was measured in both preterm and term groups and the results revealed that its serum levels is significantly low in preterm groups in comparison with term group. This inverse association of catalase enzyme and preterm labor was proved in other studies [10, 11]. This results may be due to increase in antioxidant defense as compensatory mechanism against increase oxidative stress in term group. Tiffany A. Moore, et al. found no differences in catalase levels in preterm maternal or cord blood samples between preterm and term pregnancies [12].Ramkumar Menon, et al measure catalase level in maternal plasma and found its levels was high in preterm labor in comparison with term labor [13]. These deferent results remain inconclusive, but indicate that a deferent finding in deferent studies may be caused by deferent studies circumstances, environmental factor, personal characteristics, diseases and other factors.

SODs are endogenous antioxidant enzymes that are catalyze the dismutation of O2• to H2O2 and O2.[14]

In this study the SOD activity was measured in maternal serum of preterm and term groups and the results show significant high level in term group in comparison with preterm. That's agree with the result of other studies [13, 11] This results may be due to increase in antioxidant defense as compensatory mechanism against increase oxidative stress in term group, Or due to normal increase in SOD level in this period of pregnancy [15] In other reviewed studies, researchers measured SOD in the preterm mothers. Herway et al. study EcSOD in maternal serum and did not showed significant differences between term and preterm birth [16]. Gunko, Pogorelova and Linde find minor expressions of Cu/Zn-SOD in women with preterm birth in comparison with women with term birth [17]. In the other studies, the researchers did not find significant differences in EcSOD levels between TB and PTB in cord blood [18] or in protein expression for Cu/ZnSOD in the myometrial tissue [19]. These inconclusive results indicate that SOD may be associated with race/ethnicity, personal factors, disease and environmental factors.

By comparing SOD level between pregnancies presented by pPROM and pregnancies presented by uterine contractions the results revealed decrease its level in pPROM pregnancies the same as Ramkumar Menon, et al study [13]. That due to increase oxidative stress which cause decrease antioxidant. In other study by Eryn H. Dutta et al. SOD1 measured in fetal membranes and show higher level in PTB than in pPROM whereas SOD2 showed no significant difference between the two groups [20].

Catalase level was compared between pregnancies presented by pPROM and pregnancies presented by uterine contractions the results revealed no significant deference between two groups, this results may be due to increase oxidative stress in two groups that cause decrease in antioxidant level. Ramkumar Menon measure serum catalase and found that its level in pPROM pregnancies was higher than spontaneous preterm labor which highlights the ambiguity associated with marker analysis of oxidative stress.[13] Eryn H. Dutta et al. found that catalase was not significantly different in fetal membranes of pPROM and PTB. [20]

Also, Soydinc *et al.,* did find lower catalase levels in the vaginal wash fluid in pPROM compared to a matched control group. [12]

Davut Sinan KAPLAN, et al. study total oxidative status and total antioxidant status in maternal colostrum of primiparous and multiparous mothers. and No significant differences were determined between two groups. [21]

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Conflict of Interest: None

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Comparative Clinical Study of Role of Saraswatchurna in Apasmar

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Abstract

Ayurveda explains disproportion in the three doshasvata, pitta and kapha singly or all of them together can cause Apasmar. Aggravated doshas get accumulated in hridaya and create the features based upon Doshic predominance, which cause illusion of the mind and visual hallucination and seizures (tonic spasms and clonic jerks). Often it is presented with outwarding of tongue, deviation of the eyes, dribbling of saliva with froth, tonic and clonic movements of limbs. Epilepsy is characterized by recurrent, episodic, paroxysmal, involuntary clinical events related with alternation of cerebral function. An epileptic attack is anrapid and unwarranted electric discharge of cerebral neurons. Saraswat Churna is a popular Compound drug which is successfully used in Psychological disorders. It is mentioned in "Unmada Prakaran" of well known Ayurvedic book of " Bhaishgya Ratnawali" that Saraswat Churna consists of different types of Medhya Rasayana herbs like Vacha, Shankhapushpi etc. and Brahmi Swarasbhawna is given to it for three times. Phenytoin (Diphenyl Hydantoin or Dilantin) is highly effective and widely prescribed anticonvulsant drug used in the treatment of grand mal and psychomotor epilepsy. Phenytoin has been used to treat thought, mood and behaviour disorders, cardiovascular disorders, neuro muscular disorders, gastrointestinal disorders, Endocrine disorders. As Dilantin showed adverse reactions like urticarial like skin rashes, gastric irritation, resulting in nausea, vomiting, hyperplasia and hypertrophy of the gums noted in 90% of patients was not noted in patients treated with Saraswat Churna.

Keywords: *Apasmar, Epilepsy, Saraswatchurna, Dilantin, Diseases, Treatment, Adverse Reactions.*

Introduction

Since time in memorable with existence of human being disease is associated. "Dis-ease" when a human being suffers from uncomfortableness or any alternation in normally; he is said to be diseased. Hippocrates is to modern system of medicine what Sushrut and Charak are to Ayurveda, But at least Hippocrates had bit of previous work to develop upon while Charak and Sushrut have done everything first hand and their work is pioneering, as well as astronomical. Transient loss of memory or loss of memory of convulsions is known as Apasmar[1,2]. In this disease patient gets convulsions, attacks of falling down and frothing from mouth. After convulsions patient is again normal. According to Ayurveda constituents of body are divided into two types 1st Sthula and 2nd Sukshma. Dosa, Dhatu and Mala are of sthula type and Atma, Indriya Mana are of Sukshma type. According to Ayurveda; Apasmar is a psychosomatic disorder. In more or less proportion Apasmar found in almost all countries. Being prevalent in both sexes, all age groups

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and any socio-economic condition, it has predilection for children. In all types of medical therapies we get description about disease which is similar to Apasmar and treatment of the disease. But still this disease remains the challenge for the therapists[3,4]. Through strong tranquilizers and sedatives of modern therapy are effective, they have adverse effect on mind. Hence an effective drug. Dilantin which is a drug of choice for all types of epilepsy is used by all Allopaths. In this study effects of Saraswat Churna in Apasmar are studied as against the allopathic drug Dilantin. In this study Saraswat Churna is used as a treated drug & Dilantin is used as a standard drug.This present thesis is an outcome of the above mentioned studies[5,6].

Material and Method

Selection of drug: Uptil now all the research work has been done on reinstate or reject whatever has been stated in the texts written thousands of years ago by the Acharyas.

There is no literature in which Saraswat Churna has been used up till now. According to Unmada Prakarma of Bhaishagyaratnawali daily intake of Saraswat Churna causes potentiating of Budhi, Dharanashakti, Dhairya and Smurti sequentially. Apasmar is defined as transient expression due to derangement of memory, intelligence and mind. Therefore I decided to work over use of Saraswat Churna in Apasmar.

Vacha the main ingredient of Saraswat Churna according to Ayuved has Medhya, Sangyasthapana, Akshepshamaka & Vedanasthapana action on central nervous system. According to Modern Science Vacha is Stimulant, antispasmodic & sedative in action.

Shankhapushpi according to Ayurved is Medhya, Balya for Brain & Nerves has Nidrajanana property.

Sunthi has stimulant & analgesic action.

Marich has antispasmodic & stimulant action on Nervous system.

Pippali is Stimulant & Medhya.

Ajamoda is balya for Brain & Central nervous system.

Kushtha is akshepshamak & avasadak.

Jeeraka is Vatanulomak & balya for brain.

Ashwagandhan is akshepshamak & analgesic.

After preparing churna of all twelve ingredients of 'Saraswatchurna' the mixture is given **Brahmi swarasbhavana** thrice. Brahmi is kaphavatshamak & has Apasmar & Unmadshamakprabhava. Brami is Kaphavatshamak & has Apasmar & Unmadshamakprabhava. Brami is also Medhya, Smaranshkti-wardhak (improve memory) Mastishka-Dharanashakti.

Thus according to Ayurved classics following are the qualities of the drugs used in 'Saraswat Churna' :- Antispasmodic, increases memory, tranquilises the over activity, sedative, hypnotic, brain tonic & Apasmarhara (antiepiteptic).

Therefore I have used Saraswat Churna for my research work.

Dilantin (Diphenylhydantoin): This is a major drug in the treatment of a variety of epileptic seizures with the exception of petit mal. Dilantin is a drug of choice, used by all Allopaths. In this study effects of Saraswat Churna in Apasmar are studied against the allopathic drug Dilantin.

Therapeutic Uses: This is important drug in the T/t of grand mal epilepsy, psychomotor seizures. Drug often controls focal cortical epilepsy but does not completely abolish the seizure activity.

Materials and Method:

A. Settings: A total 30 patients of Apasmar were randomly selected for the present study, from the Kayachikitsa OPD of Pakwasa Samnwaya Hospital and Bajaj Anusandhan Centre, Nagpur.

B. Research design: A Randomized Control Trial

1. Participants:

- Patients – Diagnosed patients of Apasmar(Epilepsy)
- Gender-Both Male and Female
- Age- From 15yrs-75yrs of age.

2]. Sampling procedure:

Random sampling

Grouping:

Groups	No. of patients	Age	Sex	Intervention	Dose/day	Duration
Group A Study Group	15	15 yrs to 75 yrs	Male and Female	Saraswat Churna	1 gm t.d.s. with Honey	6 months
Group B Control Group	15	15 yrs to 75 yrs	Male and Female	Dilantin	100mg t.d.s. with Plain water	6 months

4. Sample size: Sample size was taken 30

5. Population: Patients of Apasmar(Epilepsy) were selected from O.P.D. of Pakwasa Samnwaya Hospital and Bajaj Anusandhan Centre, Nagpur.

C. Data collection tools and process: Patients fulfilling inclusion criteria and willing to give written consent will be enrolled for study. The selected patient will be informed about possible outcome and side effects of interventional drug.

a. Inclusion criteria:

- Subject is between 15 and 75
- Ability to provide informed consent (or assent if under the age of 18)
- Ability to keep accurate seizure diaries
- Subject had prior neuroimaging with report available
- Diagnosed patients of Apasmar

b. Exclusion criteria:

- Subjects with significant progressive disorders or unstable medical conditions requiring acute intervention
- Change in AED regimen in the last 28 days
- Subject is currently taking > 3 concomitant AEDs
- Subject has had status epilepticus within the past 2 years

c. Specific Investigations:

- Manas Parikshan
- E. C. G.
- CBC
- C. S. F. Examination
- X- Ray Skull

d. Assessment Criteria:

1. Severity of attack

a. Grade 0: Myoclonic tremors

b. Grade 1: Multi focal clonic tremors

c. Grade 2: Generalized tonic tremors

d. Grade 3: Frothing + tongue biting

2. Frequency of convulsion

a. Grade 0: No convulsion

b. Grade 1: 1 episode/15 days

c. Grade 2: 1 episode/7 days

d. Grade 3: 1 or more episodes/day

3. Duration of convulsion attack

a. Grade 0: No convulsion

b. Grade 1: 5–15 s

c. Grade 2: 15–30 s

d. Grade 3: >30 s

4. Ictal features

a. Grade 0: No any features

b. Grade 1: Headache

c. Grade 2: Headache + drowsiness/delirium

d. Grade 3: Paresis + other complaints

D. Methodology: This is a Randomized Control Trial. The study was conducted at O.P.D. of Pakwasa Samnwaya Hospital and Bajaj Anusandhan Centre, Nagpur of Kayachikitsa department, as per inclusion criteria.

Written consent will be obtained from each patient. Total 30 patients were selected and they were divided

in two equal groups by random sampling method using lottery technique.

Preparation of Drug:

A. Contents of Saraswat Churna: Saraswat Churna contains mainly 12 types of herbal drugs in which eleven ingredients i. e. Kushtha, Patha, Ashwagandha, Shankhapushpi, Shunthi, Maricha, Pippali, Jeeraka, Krishna jeeraka, Ajmoda and Saindhav-lavana were taken in equal parts i.e. one part of each ingredient was taken and Vacha was taken in equal proportion of above drugs. Therefore eleven parts of Vacha & eleven parts of other drugs were taken. Fine powder of each ingredient was made by grinding method. Then the powder is mixed well after grinding. Then the mixture was given Brami Swarasbhavana thrice. And thus Saraswatchurna was ready for research work.

Comparative study of Rasa, Gun, Virya, Vipaka & Prabhava of contents of Saraswat Churna:

Table 1A

Name	Rasa	Guna	Virya	Vipaka	Prabhava	Doshaghnata
Kushtha	Katu	LaghuRuksha Tikshna	Ushna	Madhura	-	Kaphavatahara
Vacha	Katu	Laghu Tikshna Sara	Ushna	Katu	Medhya	Kaphavatahara
Shankhapushpi	Kashaya Katu Tikta	Snighdha Guru Sara	Sheeta	Madhura	Medhya	Vatapitthara
Patha	Tikta	Laghu Tikshna	Ushna	Katu	_	Tridoshaghna
Jeeraka	Katu	Ruksha Laghu	Ushna	Katu	_	Kaphavatahara
Krishna Jeeraka	Katu	Laghu Ruksha	Ushna	Katu	_	Kaphavatahara
Ashvagandha	Tikta Kashya	Laghu Snigdha	Ushna	Katu	_	Kaphavatahara
Sunthi	Katu	Laghu Snigdha	Ushna	Madhura	_	Kaphavatahara
Maricham	Katu	Tikshna RukshaLaghu	Ushna	Katu	_	Kaphavatahara
Pippali	Katu	Laghu Snighdha	Ushna	Madhura	_	Kaphavatahara
Ajamoda	Katu Tikta	Laghu Ruksha Tikshna	Ushna	Katu	_	Kaphavatahara
Saindhavalavana	Madhur	Swadu Laghu Anushna	_	Madhura	_	Tridoshnashak
Bramhi	Tikta Kashaya	Laghu Sara	Sheeta	Madhur	Unmada Shamaka Apasmar Shamka	Kaphavatahara

Table 1 B

Sr.No.	Gunas	Rasa	Vipaka	Virya
1	Laghu -12	Katu -7	Katu – 7	Ushna - 10
2	Ruksha - 5	Tikta - 5	Madhura - 6	Sheeta - 2
3	Tikshna – 5	Kashaya – 3		
4	Snigdha - 4	Madhura – 1		
5	Guru - 1			
6	Swadu - 1			

B. Dilantin: Dilantin which is a drug of choice for all types of epilepsy is used by all Allopaths. In this study effects of Saraswatchurna in Apasmar are studied as against the allopathic drug Dilantin.

Total no. of fifteen patients were treated by Saraswat Churna and fifteen patients were treated by Dilatin. The results obtained are represented graphically.

Statistical Data: In the present clinical study the response of Saraswatchurna and Dilantin on 15 patients each was observed. The data collected during the course of study is recorded below**.**

Table 2. Prakruti (According to Sharirik Dosha)

	Treated Group		Standard Group	
	No. of Patients	Percentage	No. of Patients	Percentage
Vata-pittaj	5	33.34	6	40
Vata-kaphaj	7	46.66	5	33.34
Pitta-kaphaj	2	13.33	2	13.33
Vata-pitta-kaphaj	1	6.67	2	13.33

Table 3. Prakruti (According to ManasikDosha)

	Treated Group		Standard Group	
	No. of Patients	Percentage	No. of Patients	Percentage
Satwik	1	6.67	2	13.33
Rajasik	12	80	10	66.67
Tamasik	2	13.33	3	20

Table 4. Duration of Illness

	Treated Group		Standard Group	
	No. of Patients	Percentage	No. of Patients	Percentage
1 to 5 years	2	13.33	1	6.67
5 to 10 years	5	33.34	7	46.66
10 to 15 years	6	40	6	40
15 to 20 years	2	13.33	1	6.67
Below 1 year	-	-	-	-

Table 5. Vataj Apasmar (12 Patients)

Symptoms/Signs	Treated Group		Standard Group	
	No. of Patients	Percentage	No. of Patients	Percentage
Kampa	4	44.44	3	33.33
Dantankhadan	7	77.77	8	88.88
Swasa	2	22.22	1	11.11
Visham Rudan	8	88.88	8	88.88
Udwamanthan Phenam	5	55.55	7	77.77
Abhikshanam Apasmaram	9	100	9	100
Ksheena Sanjanam Pratilabham	9	100	9	100
Anavasthitam Panipadam	8	88.88	7	77.77
Spurat Sakhthih Pratatam Ka Muhurmuhurh	6	66.66	7	77.77

Table 6. Kaphaj Apasmar(11 patients)

Symptoms/Signs	Treated Group		Standard Group	
	No. of Patients	Percentage	No. of Patients	Percentage
Chirat Apasmaram	6	100	6	100
Chirat Sangyam Pratilabham	6	100	6	100
Patantam	4	66.66	5	83.33
Nativikrita-chestanam	5	83.33	4	66.66
Guru	3	50	1	16.66
Hrillasa	2	33.33	2	33.33
Nidrarthah	6	100	5	83.33
Cheshtalpata	6	100	6	100
Shuklagurusnighdharupadarshanam	4	66.66	3	50

Observation and Results

It has been opined in A.P.I. that a final say regarding the relief to the patient can only be given after the period of five years follow up. Therefore in the present study the assessment of the results of the treatment in the terms of cured, markedly improved etc. is avoided.

An assessment of severity of symptoms recorded before and after treatment is used here to evaluate the efficacy of the treatment

Group 1:

Total no. of patients was 15.

Disturbance of intelligence, memory & orientation had shown better results compare to other clinical features

Symptomatic relief in percentage is as follows:

1. Disturbance of intelligence – 68.75% relief.
2. Disturbance of Orientation – 66.34 % relief.
3. Disturbance of memory – 65.81% relief.
4. Tongue bite – 52.33% relief.
5. Sphurat Sakthihprantam, muhurmuhurh – 35.29% relief.
6. Shukla guru snigdharupadarshanam – 19.78% relief.
7. Vishamrudan – 18.18% relief
8. Dantankhadan – 15% relief
9. AsamanVilapam – 15% relief
10. Chestalpata – 13.33% relief
11. Kampa – 12.05% relief

In Group 2:

The total no. of patients was 15.

In this Kampa, Dantan Khadan, Sphurat Sakthihprantammuhurmuhurh & seizure activity; drug had shown better results

Symptomatic relief in percentage is as follows:

1. Dantan Khadan– 78.28%
2. Kampa – 68.75%
3. Sphuratskhthihprantammuhurmuhurh -64.91%
4. Tongue bite-60%
5. Vishamrudan- 52.63%
6. Chestalpata – 44.44%
7. Asaman Vilapam-26.61%
8. Disturbance of intelligence- 24.44%
9. Shukla guru snighdharupadarshanam- 23.52%
10. Disturbance of Memory- 19.72%
11. Disturbance of orientation -19.04%

Therefore Saraswatchurna in following Clinical features is more effective than Dilantin:

1. **Disturbance of intelligence**-44.31% more effective than Dilantin.
2. **Disturbance of memory**-46.07% more effective than Dilatin.
3. **Disturbance of orientation**-47.30% more effective than Dilantin,

While in following Clinical features Dilantin; is more effective than Saraswatchurna:

1. **Dantan Khadan –** 58.28% more effective than Saraswatchurna.
2. **Khampa-** 56.25% more effective than Saraswatchurna.
3. **Visham rudan-**34.45% more effective than Saraswatchurna.
4. **Sphurat Sakthihprantammuhurmuhurh** – 29.72% more effective than Saraswatchurna.
5. **Cheshtalpata -** 21.11% more effective than Saraswatchurna.
6. **AsamanVilapam-** 11.61% more effective than Saraswatchurna.
7. **Shukla guru snighdharupadarshanam-** 03.74% more effective than Saraswatchurna.
8. **Tongue bite-** 7.67% more effective than Saraswatchurna.

Discussion

In this study entitled comparative clinical study of role of Saraswat Churna in Apasmar was conducted on 30 patients grouping them into two groups of 15 each (Treated group and Standard Group)[7,8.]

Group A administered by Saraswat Churna treated group and group b administered by Dilantin standard group and clinical investigation conducted before and after the starting of the treatment with the above drug for a duration of six months. And the findings were recorded monthly intervals and comparison made between before and after completion of treatment.Maximum patients were between 20 to 40 years. Maximum patients i.e. 36.66% were belonging to 31-40 years and 26.66% were belonging to 41-50 years.Maximum patients were Male i.e. 60%. 73.33% of patients were married and 26.66%of patients were unmarried. Majority of patients were having mixed vegetarian diet i.e. 70%. 50% of patients were Businessmen. 70 % were of middle class. Addiction of alcohol is one of the precipitating causes of epilepsy. From 30 patients 11 were male had addiction of alcohol[9,10,11]. 36.66% patients were of Vata-pittajprakruti, 40% patients were of vata-kaphajprakruti, 13.33% patients were of pitta-kaphajprakruti, 10% patients were of vata-pitta-kaphajprakruti. Prakruti indicated that Rajasik Prakrutiwere exhibited in majority of patients and disease is mainly causes by the predominance of Raja and Tama.80% patients were having duration of illness from 10-20 years[12-16]. As Dilantin showed adverse reactions like urticarial like skin rashes, gastric irritation, resulting in nausea, vomiting, hyperplasia and hypertrophy of the gums noted in 90% of patients andthese adverse reactions were not noted in patients treated with Saraswat Churna.

Conclusion

In Ayurveda system of medicine the diseases are classified according to Doshicpredominance as well as signs & symptoms. In the present Clinical study a detailed description of Apasmar by various Ayurvedic Acharyas

and the trial drug Saraswatchurna is selected from an authentic Classical text. Saraswatchurna is compared with standard drug Dilantin by clinical symptoms and signs as recorded by Ayurvedic system, investigations in Apasmar patients to find out whether is superior or not in comparison to standard drug therapy.

The result of Saraswat Churna is insignificant.

Ethical Clearance: Taken from institutional ethics committee.

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Critical Appraisal of Communicable Diseases in the Perspective of Ayurveda

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Abstract

Current situation of COVID -19 pandemic demands for search literature about in *Ayurveda* to generate various preveative & treatment modalities to over come over it. Considering this need, review regarding communicable disorders from available Ayurved literature was done extensively & elaborated under heads of concept of airborne communicable diseases in Ayurveda,its etiology, pathology & symptomatology with recommended various treatment measures given in Ayurvedascripctures. It is observed that COVID -19 can be corelated with various *Aoupasragic Rogas* or *Janapadodhwnasa Vyadhi* which has similar symptomatology similar with *Vata kapahaja Jwara*. Treatment modalieties of this *Jwara* & some preventives measures such as *Davuavayapasharaya* (spiritual practices like *Homa, Havana & Yoga*, *Dhoopana*), *Yuktivyapashara (Dinachrya, Rutucharya, Vyayam*, *Ayurvedic* drugs & herbs, *Upakrama* of detoxification, *Rasayana Chikitsa, Dhoopana*) *& Satvajaya Chikitsa (Sadvrutta & Achara Rasayana)* can be adopted in such condition.

Keywords: *COVID-19, Aoupasargic Roga, Pathophysiology, Davuavayapasharaya, Yuktivyapashara, Satvajaya Chikitsa.*

Introduction

COVID -19 is an entity among highly communicable disorder which generally spreads through air. There is no any successful pharmacological or non-pharmacological remedy is yet discovered till date. However, current dreadful outnreak of this dieases demands for such intervention & it is also necessary to check compatability of such modalities in the present era.

Persuing this aim, encouragement for researchs in vulnerable & sensitive area of COVID -19 with its prabable effective remedies in *Ayurveda* is really need of hour in current scenario. *Ayurveda* i.e. an ancient science of life & health, ever evolved throughout the globe & playsan imperative role in elaborating this concept of communicable diseases. As COVID -19 not directly elaborated by *Ayurveda* but considering it in a concept of *Anukata Vyadhi* given by *Acharya Charak*, explaration of COVID -19 can be possible with the help of different causative factors responsible for their generation & transmission, group of persons vulnerable for it, pattern & type of evolving symptoms, organs or structures involved in this disease, pathology involved, type of response given by patient on the basis of some fundamental principles of *Ayurveda*. As this exploration of these factors may help to establish some affirmative guidelines regarding preventative & curative aspect of COVID-19 in *Ayurveda* by breaking its basic pathology by doing root cause analysis. Through this paper, sincere efforts are done to compile the same to search effective & safe modalities in our ancient science.

A extensive literature search was done to review concept of communicable disorders, various clinical entities involved with *Pranavaha Srotas* (respiratory

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System) i.e. disorders of *Shwasanavaha Sansthana* with their causative factors, symptomatology by using available various *Ayurvedic* sculptures including *Samhita*, *Nighantu*, various research articles from different databases such as Google scholars, PubMed, *Shodhaganga.* Many P.G. & Ph.D. dissertations from different institutes till April 2020 are also reviewed. All these sources were screened & information related topic is identified & an attempt has done to compile this scattered information under different heads.

Observations and Results

As information collected from above different sources was observed in scattered form, therefore; it is narrated under heads of causative factors, various mode of transmission, organs or structures involved, *Samprapti* (probable mode of pathology), pattern, types symptoms regarding concept of airborne communicable diseases, treatment strategies in Ayurveda. All these heads will be explained one by one as follows:

Fundamental concept of airborne communicable diseases in *Ayurveda:* A many clinical conditions similar to COVID-19 are described in varius *Ayuvedic* manuscripts under the terms of *Janpadohwasna* or *Aupsargikarogas Roga* (diseases which are communicable in nature i.e, *Kushtha, Skin diseases, Jwara (Fever*) which are quite similar to epidemic disease which are caused due to vitiation of some factors such as air, water, location and seasons[1].

Causative factors & various mode of trasnmission: According to *Acharya Charaka, Adharma*[2] i.e. *Pradnyaparadh* which compromises of failure to follow *Dincharya & Ritucharya* (daily & seasonal regimen), *Vegavidharan (*suppression of urges*), Paapkarma (*sins*)* are the maincausative factors for such epidemic condition[3]. Apart from these, there are ample references of causative factors described in the form of *Dhatu Kshaya* (wasting), *Vega Sandharana (*suppuration of natural urges*), Ruksha Padartha Sevan* (intake of dry food), *Ati Vyayama* (excercise), *Rajasa* & *Dhooma Vatabhyam (*exposure to dust & smoke), *Atisheetambupana Sevana (*excessive intake of cold water*),* Vitiation of *Ama, Shuddhi Atiyoga* which are responsible for vitiation of *Pranavaha Strotas i*.e. respiratory system which are described in various *Ayurvedic* compendium in scattered form. Ultimately, all these factors play important role in the pathogenesis of various airborne communicable diseases[4].

Hetu Sevana

(contact with etiological factors i.e. *Rogotthan, Abhighata, Bhutabhisanga, Jantu-Kitta* transmission through *Mansahara* i.e. non-veg diet)

⇩

Strotovaigunya in *Vata* dominant *Kapha, Dushya i.e. Amashaya (Stomach) & Moolasthana of Pranvaha Strotas (*Lungs +Heart+Bronchi*)* & vitiation of *Vayu* + obstruction of *Vata* in the lower region

⇩

Upward movement of *Vata*

⇩

Vitiation of Pranavaha, Rasavaha Strotasa

⇩

Disturbed function of *Udana Vayu Dushti+ Agni Mandya*

⇩

Sama Rasa Dhatu+ Vitiation in in *Mukha, Kantha* & *Nasa* (throat, chest &oral cavity & nose which are main site of *Kapha* & *Udan Vayu* which results affected Mucosa of nose & mouth, conjunctiva of eyes exposed to potential respiratory droplets)

⇩

Pranavaha & *Rasavaha Strotasa Dushti Lakshana (*Symptoms of airborne diseases*)*

⇩

Dry or with phlegm/ *Vata Kaphaja Jwar* (Symptoms of corona) [8]

Fig. - 1: Pathophysiology of COVID-19.

While *Acharaya Sushruta* narrated different modes of transmission of such communicable disorders from person to person which consists of physical contact, expired air, eating with others in same plate, sharing bed (sexual contact also) using clothes, garlands and paste (*Anulepa* or cosmetics) [5] In addition to these, he further quoted that this transmission takes place by *Krumi*or *Jantu* which have appearance i.e. *Sukshma* (microscopic) & are *Ashukari* (rapid in action) in nature. This concept of *Sushruta* for causative factors of communicable diseases is highly matched with aetilogy of Covid -19 induced by Corona virus in contemporary science[6].

Pathology: *Pranavaha* & *Rasavaha Srotas*are mainly involved in pathogenesis of COVID-19. As *Hridaya*, *Mahasrotas* and *Murdha* are *Moolasthana* of *Pranavaha Srotas* and *Rasavaha Dhamani* are involved in transportation of *Pranvayu*[7] in the body which are also affected in this disease. Considering the various organs involved in this diseases & symptomolgy evolved, probable pathology of COVID -19 as per Ayurveda can be described in fig.no. 1 as follows:

Symptoms: The causataive organism of COVID-19 mainly attacks over respiratory & gastrointestinal system, so symptoms related with these two different systems are prominatly observed in this disease. Specific symptoms such as *Pratishyaya* (running nose), *Mookhpaka* (Sore throat), *Sakapha* or *Shushka Kasa* (wet or dry cough), *Angamarda* (Body ache), *Shirshoola* (headache) and Sneezing (*Kshavayu*) which are described by *Ayurveda* in various clinical eltities related with respiratory disorders, systemic disorders like *Jwara* (fever), *Shiroroga*, etc, All these symptoms generally reflects in COVID-19 also. There is a quite similarity between symptoms of communicable diseases which are airborne in nature with disorders of *Pranavaha Strotas*occurs due to its vitiation of this channel because symtoms such as shortness of breath, fever, sore throat, nasal congestion, rhinitis, chest pain, hemoptysis, diarrhea & fatigue described in viral communicable diseases are also observed as *Alpalpam* (short respiration) or *Shwasa, Jwara, Swarabheda, Pratishyaya, Uraha Shula, Rakta Stheevana*, *Atisara* & *Dourbalya* respectively.

Some symptoms mentioned under term of *Pranavaha Strotas Dushti Lakshana* e.g.*Atisrishtam* (Respiratory act is too long) *Atibaddham* (restricted respiration), *Kupitam* (agitated) *Alpalpam* (Shallow or short breaths) *Abhikshnan* (Frequent, or repeated, increased) *Sasabdam* (Stertorous) *Sashoola* (Painful respiration) & which are occurred due to vitiation of this*Strotas* may observed in severe complication of this COVID-19[8, 9].

In addition to these, *Vata-Kapahaja Jwara* can be correlated with COVID -19 due to similarity in symptoms such as *Pratishyaya (*cold or nasal congestion), *Kasa (*cough & sore throat), *Jvara (*Fever), *Shwvasa (*difficulty in breathing/breathlessness) & *Shirograha (*Headache) [10].Same opinion is given by Commentary *Chikitsa Tatwapradipika* also.*Shirshoola* & *Hrullas* which ocuured as a result of *Agnimandya* in *Vata-Kaphaja Jwara* are alsoexplained in modern science as headache & nausea.

Treatment strategies in *Ayurveda:* Till date; no any drug has specific claim to treat COVID 19, therefore, exploration of traditional remedies are highly needed in current scenario. In *Ayurveda*, all *Aacharya* insisted to take into consideration about nature of *Vyadhi* (disease) through *Dosha,* the *Sthathan Sanshraya* (site of manifestations), *Hetu* (etiological factor), *Adhisthan* (location), *Samsthana* (clinical feature), *Yoni* (site of origin), *Atmanam* (cardinal symptom) before adopting any treatment measures in any clinical conditions especially for *Anukta Vyadhi* i.e. diseases which are not mentioned directly with respect to its treatment or symptoms[11] & treatment should initiated only after assessment of these factors.

Generally due to close similarity in appearance of symptoms, treatment modalities of *Vata-Kaphaj Jwara* such as *Amapachaaka*, *Jwaraghna* & *Vatanulomana Chikitsa* can be applied here[12].Drugs having properties Drugs with properties such as *Vatakaphahar,* antioxidants, anti-inflammatory, immunomodulator, anitiviral, antimicrobialcan be used in this dieases. While concept of *Dhoopana* checks over the transmission of diseases from one person to another due to its antiseptic, antimicrobial & disinfectant properties[13][14][15].

According to *Chakrapani*, as *Vyadhibala Kshamatva* i..e immunity plays important role to prevent such diseases, its maintaince plays imperative role in prevention of this disease.immunity of body can better improved with the help of some hoilistic principles of *Ayurveda* such as *Dincharya* & *Rutucharya* through with matainanace of *Agni* can be done.As vitiation of *Agni* is main culprit for any disease, its maintaince imposes to avoid infection.Regular use of judicious use of *Panchakarma* therapies by healthy person is

also recommmded by *Achrayas* to main the health[16]. All these therapies have Antioxidants action which fight against cell damage & protects the body from oxidative stress. To maintain healthiness, physical as well mental health is equally important. To acuire mental helath, various remedies in the form of *Yoga, Achararasayana* & *Sadvrutta* are suggested[17].

In nutshell, three types of strategies i.e. *Daivayayapashraya, Yuktivayapashraya* & *Satavavajaya Chikitsa* are recommended by our ancient *Acharyas* for management of *Aoupasargic* & *Janpadodhwansa Vyadhi* such as COVID -19.

Discussion

The concept of communicable diseases along with theirvarious mode of spread has been very well elaborated by *Acharya Sushruta*.According to modern science, Bacteria, Virus, Fungi & Parasites are main causative factors for developing various communicable diseases like COVID-19.Corona virus (Jantu) is originated from the animal-like snake and bats.This fact is already quoted by *Ayurveda*before thousands years ago & there are such statements representing that many *AoupasargikRogas* (infectious diseases) have origin from different types of Worm (*Krumi*), Insect (*Kittak*), Virus (*Jantu, Raksha* or *Graha)* whichare *Sukshma* (microscopic), *Ashukari* (rapid in action) & they induces *Jvaraupanna* (Fever), *Snayudurbala* (muscle weakness or bodyache, fatigue ness, generalized weakness) . Basically all these organism generates such diseases due to vitiation of *Air (Vayu), Water (Jala), Time (Kala)* & *Region (Desha)* .This statement about the development of symptomatology by above causative factors in Ayurveda is also supported by modern science by stating that that most of diseases occurring due to Virus e.g.Viral fever have bodyche, general weakness & fever as chief symptoms. Along with that, such disorders are quick spreading in nature i..e highly contagious in nature.

All components of *Pradnaparadha*or *Adharma* are responsible for hampering immunity of an individual. Causative pathological factors described in *Samhita* in context of *Shwasa, Kasa, Hikka, Pratikshaya* can be taken into consideration while treatment of COVID-19 .*Ayurveda* has greatly appreciated for prevention of any disease rather than its treatment .Keeping this fact in mind, various preventative strategiesi.e avoidance of *Pradnaparadh* or *Adharma* or *Hetu* of some diseases such as *Shwas, Kasa, Pratishayay, Shroroga, Nasa Roga, Jwara (Abhishangaj)* through properadption of *Dincharya* & *Ritucharya*can be highlighted & planned for management of pandemic like COVID-19 because these strategy maintains sound health by boosting immunity. Through different modes of treatment & prevention, *Ayurveda* accepts the presence of micro - organism & most emphasis is given to maintain equilibrium of the internal environment & to avoid exposure to causative organism. For prevention, *NidanaParivarjana* in the form of*Dhoopana*, with *Bhootanashak Dravyas* (antimicrobials) has important role to remove causative factors by purifying the air.

While treating airborne infections such as COVID-19, etiopathological factors of *Vatashleshmak Jwar*, its *Chikitsa* in *Ayurveda* should be kept in mind. These causative factors are quite similar as told by contemporary science. There is an one major difference between treatment modalities of *Vatasleshmak Jwar* & COVID-19 that there is great emphasis is given to correct first *Agnimandya* (digestive as well as metabolic (fire) in Ayurveda.

Considering the season i.e. present environmental conditions, Physical and mental health, diet should be planned. Fruits, vegetables, cereals included in diet helps to maintain the health properly and they can feel light and further it help to proper digestion, it also leads to burn fats, calories, which will lead to healthy body and help to maintain to immunity .Various Ayurvedic herbs with antiviral, antioxidants, immunomodulatory, antitussive, antipyretics and bronchodilators properties & having capacity to strengthen the respiratory systemto improve the immunity in the form of *Rasayana* can be highly appreciated for prevention purpose in covid-19 outbreak[18][19][20].

Considering the basic pathology, causative & risk factors, specific clinical manifestations (sign & symptoms) for COVID -19 in terms of *Ayurveda*; rational use of *Davuavayapasharaya (*Spiritual practices like *Homa, Hvana & Yoga*, *Dhoopana*) [21], *Yuktivyapashara (Dinachrya, Rutucharya, Vyayam*, *Ayurvedic* drugs & herbs, *Upakrama* of detoxification, *Rasayana Chikitsa, Dhoopana) & Satvajaya Chikitsa (Sadvrutta & Achara Rasayana)* can be planned for prevention & management of communicable diseases like COVID-19.

Conclusion

As contemporary science has limitations in treatment modalities of COVID-19 & an extensive review of background of an essential and compulsory factors involved in pathogenesis of COVID-19 according to *Ayurveda*, it is possible to prevent & overcome over such similar clinical conditions with the rational application of the basic principles in this ancient science i.e. *Ayurveda* with its proper understanding.

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Study of Fresh Versus Frozen Embryo Transfer with Reference to Early Pregnancy Loss

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Abstract

Background: Etiology of Increased rate of early abortions associated with pregnancies happened after In Vitro Fertilisation are not understood fully till date. 'Freeze all embryos' is a new dictum of improving the overall success of In Vitro Fertilisation,although it's effect on early pregnancy loss is controversial in existing literature .So many studies stated that vitrification and thawing process although improves the positive pregnancy rate but cause early pregnancy losses after implantion, but other studies contradict it. Hence in present study we wish to find out the difference of early pregnancy demise in frozen thaw embryo transfer cycles in comparison to fresh embryo transfer cycle.

Trial Design: This is a cross sectional study which will be conducted in Wardha test tube baby centre, Acharya Vinoba Bhave Rural Hospital.

Method: 20 consecutive women conceived after In Vitro Fertilisation, either Fresh or Frozen embryo transfer and will be followed up further for rate of early abortions amongst them.

Expected Outcomes: Frozen embryo transfer cycles may have more chances of successful clinical pregnancies at 6-7 weeks of gestational age, but later on may result in early pregnancy losses.

Keywords: *Frozen, Embryo Transfer, Early Pregnancy Loss.*

Introduction

Presently frozen embryo transfer is one of the most recommended procedures in advanced treatment of infertily. As the success of In Vitro Fertilisation, is still limited in spite of so many advancements in all the fields of reproductive biology, frozen embryo transfer is a viable alternative to fresh embryo transfer considering that the endometrium and ovaries of fresh stimulation cycles are not properly prepared for implantation[1]. In frozen embryo transfer there could be better control of both endometrium and ovaries hence pregnancy rates are proposed to be increased and perinatal outcomes should be better than fresh embryo transfer, In few studies the rate of clinical abortion and biochemical pregnancy was reported around 20% after frozen embryo transfer [2].

Previously clinical indications for frozen embryo transfer (cryopreservation) were to help the cancer patient undergoing treatment, patients who have premature ovarian failure, to overall delay the fertility procedure for so many social factors[5].

Now days all freeze strategy is almost replaced the standard fresh assisted reproductive technique hence frozen embryo transfer is an important part of assisted reproductive technology. Previous studies suggested

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that procedure of frozen embryo transfer reduces the number of transferred embryos as well as also reduces the risk of multiple pregnancies, risk of Ovarian Hyper Stimualation Syndrome and ectopic pregnancies[1].

Elective frozen embryo transfer has been shown higher live birth rate than a fresh embryo transfer amongst the women with poor ovarian reserve or polycystic ovarian syndrome[4].

In spite of many advantages of vitrification and slow thawing process in frozen embryo transfer, there is a rising concern of increased number of early pregnancy losses and more chances of aneuploidy and abnormal genotype associated with it.

There is some controversy regarding the early positive pregnancy rates due to better implantation versus take home baby outcome of patient undergoing with healthy babies in frozen embryo transfer.

With this background, we would like to conduct the present study to compare the early pregnancy losses and their associated factors in fresh versus frozen embryo transfer.

Aim: This study aimed to find out the rate of early pregnancy losses in frozen and fresh embryo transfer cycles of in vitro fertilisation.

Objectives:

1. To compare serum β–Human Chorionic Gonadotrophins level on day 14th in post embryo transfer in fresh and frozen embryo transfer women.
2. To confirm presence or absence of sac in both the groups.
3. To compare the appearance of cardiac activity and other early foetal parameters in both groups
4. To compare early pregnancy losses in both the groups.
5. To compare late complications of pregnancy in both the groups.
6. To compare final outcome of pregnancy in both the groups.

Method

Study Design: Cross sectional study

Setting and Location: The study will be conducted in the rural area of Central India which caters up to 30 kms surrounding area.

Wardha Test Tube Baby Centre is the only centre providing advanced infertility care in whole of the district. This centre is associated with tertiary care fully equipped rural hospital

In Vitro Fertilisation lab, Acharya Vinoba Bhave Rural Hospital, Sawangi.

Period of recruitment and relevant dates will be decided before planning the stimulation protocol for these infertile women that is June 2020 onwards for 6 months and exposure will be for 10- 12 days.

The Follow up will be up to 12 weeks after conception

Analysis and Data Collection will be done after 9 months of the study. Subjects will be explained about the nature and purpose of the study after assuring confidentiality and taking informed consent the details of the patient will be entered in data forms the proforma of the subjects will be preserved comprising of demographic details, past medical, surgical and comorbid conditions. Personal and family history which might affect early pregnancy losses.

Participants:

Inclusion Criteria:

- Couples suffering from primary/secondary infertility fulfilling the indications of In Vitro Fertilisation .
- Patient with history of pregnancy loss with fresh embryo transfer.

Exclusion Criteria:

- Patient with abnormal semen parameter.
- Patients not giving consent for research.
- Patients having uterine agenesis.
- Patients not fit for In Vitro Fertilisation having viral infections like Human Immune deficiency Viral disease, Hepatitis B S Antigen etc.
- Patients with known history of early pregnancy losses.

Sources: Infertility patients at outpatient department of Acharya Vinoba Bhave Rural Hospital for seeking In Vitro Fertilisation treatment.

Method of Selection: All the women whose beta human corionic gonadotrophin, will come positive after 14 days of embryo transfer.

Outcome Parameters:

Primary Outcome:

1. To compare beta human corionic gonadotrophin level on day 14th in post embryo transfer in fresh and frozen Embryo Transfer women. [COMPARABLE]
2. To confirm presence or absence of sac in both the groups. [COMPARABLE]
3. To compare the appearance of cardiac activity and other early fetal parameters in both groups [EXPECTED TO BE LESS IN NUMBER IN Frozen Embryo Transfer]
4. To compare early pregnancy losses in both the groups.

Secondary Outcome:

1. To compare late complications of pregnancy in both the groups.
2. To compare final outcome of pregnancy in both the groups.

Predictors:

1. Abdominal pain after gestational sac visibility
2. Bleeding per vaginum after gestational sac visibility

Confounders:

1. Advanced Maternal Age
2. Ante Mullerian Hormone
3. Number of embryos formed

Diagnostic Criteria:

Signs and symptoms of inevitable abortion.

Bias:

1. Blinding is not possible because of cost and time duration.
2. Method bias.
3. Confounding bias.

Study Size: 8-15 cycles per month are carried out in Wardha Test Tube Baby Centre, Out of which 60 % are self stimulation cycles 70 % of In Vitro Fertilisation cycles will suit to eligibility criteria considering dropout rate So average comes out to be 4-5 patients per month

Sample size Calculation:

$N = \aleph^2 * N * p(1-p)/C^2(N-1)+\aleph^2 p(1-p)$

Total population = N=20 during 12 months

$\aleph^2$ = Chisquare value for 1 degrees at some desired probability level. This is 3.84 at 5% level of significance.

P = 50% proportion

Q = 100-p

= 50

C = Confidence interval of the one choice (95% CI)

= 0.05

$N = 3.84*20*0.5*0.5$

$(0.05)^2 *24+3.84*(0.5*0.5)$

= 23.52 = 19

Quantitative Variables:

Not applicable

Statistical Method: The statistical analysis will be carried out using the SPSS 22 software package (SPSS Inc., Chicago, IL).

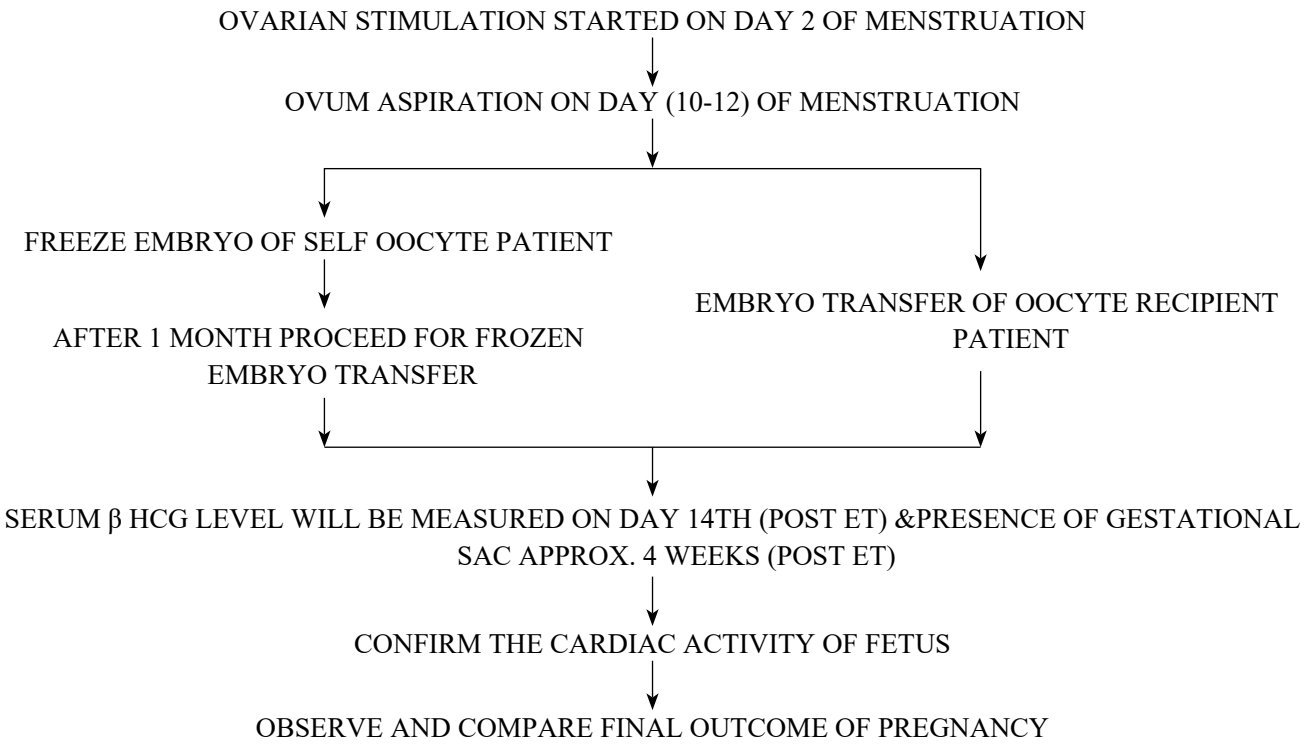
The method used is Chi Square test

Expected Results:

1. The β -HCG level on day 14th in post embryo transfer in fresh and frozen Embryo Transfer women would be COMPARABLE without stastical significance.
2. Presence or absence of gestational sac in both the groups would be COMPARABLE without stastical significance.
3. The appearance of cardiac activity and other early fetal parameters in both groups is EXPECTED TO BE LESS IN NUMBER IN Frozen Embryo Transfer cycles.
4. Early pregnancy losses may be more in Frozen Embryo Transfer groups.

Secondary Outcome:

1. The late complications of pregnancy would be COMPARABLE without stastical significance in both the groups.
2. The final outcome of pregnancy would be COMPARABLE without stastical significance in both the groups.



(DAIGRAMATIC REPRESENTATION OF RESEARCH METHODOLOGY)

Discussion

Hyper stimulation of ovaries in In Vitro Fertilisation is started to make gonadotropins level in the body at supraphysiological level which may influence the genetic makeup of oocytes and subsequently the embryos formed, so also reduce the endometrial receptivity for transferred embryos may be leading to failure of In Vitro Fertilisation treatment, hence by transferring the embryo in frozen cycle may lead body to come to normal endocrinal status and may improve the success of In Vitro Fertilisation by restoring the endometrial receptivity and selecting genetically normal embryos after thawing. Whether combined effect of both may increase or reduce the early pregnancy losses after successful In Vitro Fertilisation treatment will be studied .With this background we wish to conduct this study the pregnancy rate in frozen-thawed embryo transfer cycles is usually lower than that of fresh transferred embryos.[1]

There was an increased risk of loss after frozen Embryo Transfers versus fresh among women younger than 38 years in a study done by Hipp et all. [2]

In contrast to fresh cycle, women who received frozen cycle embryo had a significant increased risk of early miscarriage (P <0.001), while frozen cycle was linked with lower risk of late miscarriage (P =0.045) according to Lingminet all. [3]

According to study done by Zhang B et al. the risks of gestational diabetes mellitus, preterm birth, and small for gestational age were comparable between the frozen and fresh embryo transfer groups in both singleton and twin births. However, singleton infants born after frozen embryo transfer were more likely to be large for gestational age (25.2% vs. 17.5%; relative risk 1.44, 95% confidence interval 1.01-2.07, P=.044) than those born after fresh embryo transfer. [4]

In a study done by Basirat et all however,no significant differences between biochemical pregnancy rate (23% versus 18.8%, OR 1.301; 95% CI .95-1.774), gestational sac (95.6% versus 100% in Frozen Embryo Transfer, OR 0.60; 95% CI 0.54-0.67), and fetal heart activity (87.2% versus 93.6% OR .46; 95% CI .16-1.32) in fresh embryo transfe and Frozen Embryo Transfer cycles, respectively. P< 0.05 was considered statistically significant for all measures.[5]

Apart from fresh and frozen transfer, the study done by A Kaluarachchia stated that The success rate was analysed according to the duration of embryo freezing.

This showed a gradual decline in the implantation rate according to the duration of freezing.[6]

There was an increase in the risk of obstetric complications in pregnancies resulting from Frozen Embryo Transfer when compared to those emerging from fresh embryo transfers according to Roque et all.[7]

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Review on *Dhatryadi Churna*: An Ayurvedic Antipyretic Polyherbal Formulation

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Abstract

Introduction: In almost all Samhitas Jvara chikitsa is described with single or compound herbal drugs. Dhatryadi Churna is one such polyherbal churna formulation indicated in jvarachikitsa. But no data is available to know about its efficacy.

Aim and Objectives: To review *Dhatryadi Churna* w.s.r. to its antipyretic activity.

Material and Method: Relevant references were reviewed with respect to Dhatryadi Churna described in Samhitas and various Ayurveda texts such as Brihatrayee, Laghutrayee bhaishajyaratnavali etc. All the references were collected and reviewed thoroughly.

Observation and Results: Total 7 references with the name Dhatryadi Churna and Amalkyadi churna are recorded in various Samhitas and Ayurveda texts indicated in jvara chikitsa. Out of the total 5 ingredients, significant antipyretic activity in experimental animals in different dosages found in *Emblica officinalis, Terminalia chebula, Piper longum and Plumbago zeylenica.*

***Conclusion**:* Based onPharmacological properties and experimental studies conducted on individual drugs proved that Dhatryadi churna is a effective antipyretic formulation and can be used safely for treating Jvara.

Keywords: *Jvara, Dhatryadi Churna, Antipyretic activity.*

Introduction

In the Ayurvedic classics various forms of formulations are portrayed to achieve healthy and balanced life. *Panchavidha kashaya kalpana* represents the basic preparations upon which, in later period, many pharmaceutical method were developed for various medicine preparation to compete with need of all time availability, palatability, longer shelf life and efficacy. [1-2] There are more than 30 forms of formulations which are used by physicians in daily practice. Among them, herbal powder is one of the most accepted classical dosage form of Ayurvedic formulation advocated in various disease conditions. *Churna* in general is the fine powder of a completely dry drug, which is filtered through a clean cloth.[3]

In almost all Samhitas Jvara chikitsa is described with single or compound herbal drugs which includes various forms of formulations such as avaleha, arishta, vati etc. Dhatryadi Churna (DC) is one such compound powder formulation described in Ayurveda compendia. This formulation contains fine powders of *Amalaki*

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(*Emblica officinalis* Gaertn.), *Haritaki* (*Terminalia chebula* Retz.), Saindhav (Rock salt), *Pippali* (*Piper longum* Linn.) and *Chitraka* (*Plumbago zeylenica* Linn.) in equal proportion and indicated in the treatment of fever. There are many other references available where DC is described for treating fever and various other conditions such as loss of appetite,lack of digestive fire, eye diseases and constipation. Hence attempt is made in this paper to review DC described in various Samhitas and Ayurveda texts w.s.r. to its antipyretic activity.

Material and Method

Relevant references were reviewed with respect to DC described in Samhitas such as Brihatrayee and Laghutrayee andvarious Ayurveda texts such as Yoratnakar, Bhaishajya ratnavali etc. Published research papers related to pre clinical and clinical studies showing antipyretic activity of DC and individual drugs were searched. References were also searched for Amalkyadi churna and Amalkyadi gana as Amalki and Dhatri is synonymous.

Observation and Results

During the literary search total seven references were identified with the name DC and three were found as Amalkyadi churna (AC). The very first reference of Dhatryadi churna is found described in Sushruta Samhita in Dravyasangrahniya Adhyaya. Later it is described in other Samhitas and Ayurveda texts such as Sushruta samhita, Astanga Hridaya, Astanga Sangraha, Bhavprakash and. In Sushruta Samhita it is described with the name as *Amalakyadi gana*,in Sharangdhara Samhitait is described under the name *Amalakyadi churna* and in Bhaishajya Ratnavali as *Amalakyadi kwatha.* The ingredients and indications are compiled in Table 1.

Table 1: References of *Dhatryadi churna* in various Samhitas and Ayurveda Texts.

S.N.	Reference	Name	Ingredients	Indications
1.	Sushruta Samhita[4]	Amalkyadi Gana	Amalaki, Haritaki, Chitrak, Pippali	Jvara, Mandagni Netrya, Kaphanashak
2.	Ashtang Hriday[5]	Dhatryadi churna	Amalaki, Haritaki, Saindhav, Chitrak, Pippali	Jvara, Mandagni
3.	Ashtang samgraha[6]	Dhatryadi churna	Amalaki, Haritaki, Saindhav, Chitrak, Pippali	Jvara, Mandagni Vibandha
4.	Sharangdhar samhita[7]	Amalakyadi churna	Amalaki, Haritaki, Saindhav, Chitrak, Pippali	Jvara, Arochaka, Deepan, Pachana
5.	Yogartnakar[8]	Dhatryadi churna	Amalaki, Haritaki, Saindhav, Chitrak, Pippali	Jvara, Mandagni
6.	Bhavaprakash[9]	Dhatryadi churna	Amalaki, Haritaki, Saindhav, Chitrak, Pippali	Jvara, Mandagni Vibandha
7.	Bhaishajya ratnavali[10]	Amalakyadi kwatha	Amalaki, Haritaki, Chitraka, Pippali	Jvara, Deepan, Pachan, Kaphahar

Table 2: Pharmacological properties of Ingredients of *DC*

S.N	Ingredient	Rasa	Guna	Veerya	Vipak	Karma
1.	Amalaki [11] (Emblica officinalis.Burm.)	Pancharasatmak except lavan rasa	Laghu, Ruksha	Sheeta	Madhura	Vayahsthapana (C.S) Dahaprashaman Rasayan (B.P)[12]
2.	Haritaki[13] (Terminalia chebula. Retz)	Pancharasatmak except lavan rasa	Laghu, Ruksha	Ushna	Madhura	Shothahara, Kushthaghna Vishamjwaraghna (B.P)[14]
3.	Saindhav[15] (Sodii chloridum)	Lavan, Madhura	Snigdha, Sukshma Laghu	Sheeta	Madhura	Sukshma, Avidahi[16]
4.	Chitrak[17] (Plumbago zeylanica Linn.)	Katu	Laghu, Ruksha, Tikshna	Ushna	Katu	Deepaniya, Shulaprashaman Padushan[18]
5.	Pippali[19] (Piper longum Linn.)	Katu	Laghu, Snigdha, Tikshna	Anushna	Madhura	Deepaniya viryavardhak, pittashamak [20]

Researches on ingredients of DC.

Table 3: Experimental studies conducted on individual drugs of DC.

S.N	Drug Name	Part used	Type of Study	Reference
1.	*Emblica officinalis.*Burm.	Fruit powder	Experimental study- anti-pyretic and analgesic activities	Perianayagam, J.B et al[21]
2.	*Terminalia chebula.* Retz.	Fruit powder	Experimental study -*antipyretic activity*	Shukla P. et al [22]
3.	*Plumbago zeylanica* Linn.	Leaf extract	Experimental study *analgesicanti-pyretic activity*	Mittal V et al [23]
4.	*Piper longum* Linn.	Fruit powder	Experimental study -*anti-pyretic activity*	Evan Prince Sabina et al[24]

Pharmacologicalstudies:

Emblica officinalis. **Burm (Amalaki):** Study conducted by Perianayagam *et al*., on extracts of *Emblica officinalis* fruits observed potent anti-pyretic and analgesic activities. An ethanolic extract and aqueous extract (500 mg/kg) administered in a single dosedemonstrated considerable reduction in temperature in rats induced by brewer's yeast. Both the extracts brought outprominent inhibitory outcome on acetic acid-induced writhing response in mice in the analgesic test. The contents likephenolic compounds, tannins, alkaloids, carbohydrates and amino acids are established to be having antipyretic effect.[21]

Terminalia chebula. **Retz (Haritaki):** The flavonoids content of Terminalia chebula may also be responsible for its antipyretic activity by inhibiting prostaglandin synthesis in hypothalamus.

Shukla P*et al*., assessed the antipyretic activity of ethanolic extract of *Terminalia chebula* (EETC). The antipyretic activity was assessed by Brewer's yeast-induced pyrexia in Albino rats (120-130 g) of either sex. Single dose administration of 400 mg/kg and 600 mg/kg EETC showed substantial antipyretic activity, as compared to the standard paracetamol group. Thus this study proved significant antipyretic activity with ethanolic extract of *Terminalia chebula* Retz.[22]

Plumbago zeylanica **Linn*. (Chitrak):*** Mittal et al studied the antipyretic activity of ethanol extracted from dried callus and roots of *Plumbago zylenica*. Both the extracts were administered in mice at doses of 100,200 and 400 mg/kg to evaluate the peripheral and central analgesic activity by glacial acetic acid induced writhing and tailimmersion model respectively. Root extract significantly (P<0.01) reduced the writhing count at a dose 200 mg/kg whereas callus extract modified the pain threshold appreciably at 400 mg/kg. Root extract enhanced the reaction time significantly (P<0.01) in tail immersion model at 400 mg/kg. while callus extract failed to change the reaction time significantly during the observation period.[23]

Piper longum **Linn. (*Pippali*):** Extracts of *Piper longum* fruits possess potent anti-pyretic activities. The pyrexia was induced in experimental animal Mice by yeast injection. Piperine (20 and 30 mg/kg) and indomethacin (10mg/kg) were administered in single dose showed a significant (p<0.05) reduction in rectal pyrexia, similar to standard drug indomethacin without any adverse effects.[24]

Discussion

Total seven references were found with the name Dhatryadi churna or Amalakyadi churna. As Dhatri and Amalaki is synonymous. All the reviewed Samhitas described DC or AC with five ingredients as *Amalaki* (*Emblica officinalis* Gaertn.), *Haritaki* (*Terminalia chebula* Retz.), Saindhav (Rock salt), *Pippali* (*Piper longum* Linn.) and *Chitraka* (*Plumbago zeylenica* Linn.) in the treatment of fever and associated symptoms such as loss of appetite, lack of digestive fire and constipation.

Jvarahara drugs are known as antipyretics in medical science. Drugs which reduce the elvated body temperature by inhibition of prostaglandin synthesis are termed as antipyretic drugs[25]. In Ayurveda Samprapti (pathogenesis) of fever is described on the basis of state offactors responsible for digestion and metabolism/ transformation and dosas. According to Sushruta, Dosas aggravates due to consumption of causative factors and during respective time spreads to entire body and produces fever. Aggravated Dosas enters the Amasaya and combines with Agni, accompanying the Rasa, blocks the channels of Rasa and Sweda, impairs the function of Agni and expels out the Agni from the site of digestion and spread to all over body and gives rise to

Jvara. Dosas exhibit its exacerbated symptoms and signs and brings abnormality in the skin, nail, eyes, tongue, urine and faeces[26]. Acharya Charaka recommended *Langhana* (fasting therapy) as a first line of treatment for Jvara followed by digestive drugs[27].DC is such a polyherbal formulation with a perfect blending of *deepana, pachana* and *jvarahara* drugs. Antipyretic action of Dhatryadi Churna can be well understood by knowing the pharmacological properties and antipyretic experimental studies of its ingredients.(Table 2 and 3)

Probable mode of action of *Dhatryadi Churna* in *Jvara:* *Dhatryadi Churna*prominently consists of *Katu-Tikta-Lavan* rasa*, Madhura Vipaka*, *Sheeta Veerya* and *Laghu*, *Ruksha*, *Snigdha, Tikshnaguna*. Its *Katu-Tikta-Lavan*rasas digest and pacify Kaphapradhana doshas present in rasadhatu and correct aruchi (loss of appetite) and vishamagni (The state in which the action of Agni is rendered erratic either excessive or decreased, variable from time to time, due to the dominant influence of Vata). Due to its katu rasa and tikshna *Guna* it causes amapachana (substances having capacity to digest uncooked food material) and due to its laghu and tikshna property it causes srotoshodhana (purification of structural or functional channels meant for the transportation of Dhātus undergoing transformation) of rasagata doshas. Being ushna and tikshna, it acts as Svedajanana (substances that causes excessive sweating)[28]. Saidhava lavan commonly known as rock salt acts as a catalyst, help to enhance the action of other drugs in the formulation by deep penetration in the body after administration of drug. It also helps in developing the taste in the mouth[29]. Thus through all these properties of *DC*, Agni becomes normal leading to the subsidence of fever.

No clinical studies were found on DC regarding its antipyretic activity. Instead the individual ingredients of DC are reported to have antipyretic activities as depicted in table no 3. Hence combination of all these ingredients as DC may show synergistic effect and strong antipyretic activity. Various scientific studies conducted on individual ingredients of DC/AC showed that plants possess a range of phytochemicals such as alkaloids, flavonoids, glycosides, terpenoids, steroids, proteins and tannins which could lead to potential antipyretic activity[30]. Nabanita Roy et al conducted a study on phytochemical analysis of Amalkyadi churna revealed the presence of proteins, saponins, phenols, glycosides, tannins, alkaloids and fixed oil[31]. Mradu Gupta et al also confirmed in his research that flavonoids and its related compounds present in Jvarahar mahakashay preparation exhibit inhibition of archnoid acid Peroxidation which helps in decreasing prostaglandin levels thus reducing the fever. The results of the literary search of individual drugs of DC show antipyretic activity.However further studies are required on DC to elucidate the mechanism of inhibiting prostaglandin synthesis in hypothalamus which is responsible for reducing fever.

Conclusion

Literary review revealed that *Dhatryadichurna* is a compoundpolyherbal preparation containing *Katu tikta Lavan rasa, Madhura Vipaka*, *Sheeta Veerya* and *Laghu*, *Ruksha*, *Snigdha* and *Tikshnaguna*. Pharmacological properties and experimental studies conducted on individual drugs proved that *Dhatryadichurna* can be a potent antipyretic poly herbal formulation and useful for treating *jvara* safely.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Case Report on Oligohydramnios

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Abstract

Introduction: Oligohydramnios is an extremely rare condition where there is a deficiency of liquor amnii in the amount of less than 200Ml at the time of delivery. Sonographically, it is amaximum vertical pocket of liquor is < 2 cm or the amniotic fluid index is < 5 cm (less than 5 percentiles) is specified.

Patient History: A 23years old ANC & 8months mother was admitted on 11.9.2019 with the complaints of reduced fetal movements and abdominal discomfort.

Past History: Patient has history of blood transfusion 3months back and has history of cervical cerclage at 7months of pregnancy.

Clinical Findings: Reduced fetal movements, abdominal discomfort, leaking of the amniotic fluid, low amniotic fluid on ultrasound, low maternal weight gain, pre labor rupture of membranes.

Investigations: Ultrasonography (AFI=5cm), Hb% - 9.1gm%, MCV-74, MCH-23.5pico/gm, HCT-28.5%, Total WBC Count – 8000/cu.mm, Lymphocytes- 20%.

Surgical Management: Patient does not have any past surgical history.

Medical Management: Tab. AFI plus BD, L- Arginitrate sachet 5gm/BD/orally, Tab. iron OD, Tab. Calcium OD.

Nursing Management: Assess both maternal and fetal conditions carefully, fetal heart rate and vital signs should be assessed properly. During labor, an amnio-infusion should be given via intrauterine catheter. Fluid should be administered via amniocentesis before giving birth. The level of amniotic fluid may help by increasing the amount of oral fluid intake.

Conclusion: Patient was admitted to AVBRH and was diagnosed as oligohydramnios and got appropriate treatment and her condition has improved.

Keywords: *Oligohydramnios, amniotic fluid, cervical cerclage, amniocentesis.*

Introduction

Oligohydramnios is a disease characterized by an amniotic fluid deficit during pregnancy. Oligohydramnios is an extremely rare condition where there is a deficiency of liquor amnii in the amount of less than 200Ml at the time of delivery. Sonographically, it is a maximum vertical pocket of liquor is < 2 cm or the amniotic fluid index is < 5 cm (less than 5 percentiles) is specified[1].

Patient Identification: An ANC mother of 23 years old with 32.2 weeks gestational age from Hinganghat was admitted to ANC 12, AVBRH on 11th sept, 2019 with a complaints of reduced fetal movement, pain in abdomen and lower back area. After all the investigations, she was diagnosed as oligohydramnios.

Present History: Patient was apparently admitted in AVBRH on 11/9/2019 with a chief complaints of pain

in abdomen, pain in the lower back and reduced fetal movement.

Past History: Patient has history of mild anemia 3 months back where she has undergone the blood transfusion and she has history of cervical cerclage in 7 months of pregnancy for which the patient was hospitalized, she complaints pain in lower back and reduced fetal movement since 1 month back and came to AVBRH for further management.

Past interventions and outcome: My patient was diagnosed as oligohydramnios at 7 months of pregnancy where cervical cerclage has done at that time, from that time onward patient was admitted to hospital time to time for treatment. The treatment was found effective as the patient does not develop complications till then.

Obstetric History:

Menstrual History:

Age of menarche- 14 years

Duration of menstrual period- 3-4 days

Duration of cycle in days- 28 days

Regularity- regular

LMP – 28/6/19

EDD – 4/3/2020

POG – 32.2 wks

Antenatal assessment-

Weight – 45 kgs

Height – 15 cm

Edema – absent

Previous no. of antenatal visits – 2 visits

Treatment – patient has taken 2 doses of TT injection, and she's taking iron and calcium tablets.

Clinical Findings: Reduced fetal movements, abdominal discomfort, leaking of the amniotic fluid, low amniotic fluid on ultrasound, low maternal weight gain, pre labor rupture of membranes.

Etiology:

1. *Fetal conditions*- fetal chromosomal or structural anomalies, renal agencies, obstructed uropathy, spontaneous rupture of the membrane, intrauterine infection, post maturity, intrauterine growth retardation.
2. *Maternalconditions* - hypertensive disorders, uteroplacental insufficiency, dehydration, idiopathic.

Investigations:

1. CBC
   - HB% - 9.1 gm%
   - Total RBC Count – 3.87 mil/cu.mm
   - Total WBC Count – 8000/cu.mm
   - Total platelets count – 1.83 lacs/cu.mm
2. LFT
   - Total bilirubin – 0.6 mg/dL
   - SGPT – 20 U/L
   - SGOT – 40 U/L
3. USG
   - Single cell uterine fetus with 32.2 wks with oligohydramnios
   - AFI = 5CM

Therapeutic Intervention:

1. First trimester – counselling, serial USG
2. Second trimester – counselling, consider amnioinfusion, serial USG, termination of pregnancy SOS
3. Third trimester – deliver post term cases, serial USG & Doppler in IUGR, conservative management for protein pre-labor rupture of membranes till 34 weeks.

Medical Management:

- Tab. AFI plus BD
- L- Arginitrate sachet 5gm/BD/orally
- Tab. iron OD
- Tab. Calcium OD

Nursing Management:

- Assess both maternal and fetal conditions carefully, fetal heart rate and vital signs should be assessed properly. During labor, an amnio-infusion should be given via intrauterine catheter.

- Fluid should be administered via amniocentesis before giving birth.
- The level of amniotic fluid may help by increasing the amount of oral fluid intake.

Discussion

The patient 23 years old was apparently admitted in AVBRH on date 11/09/2019 . patient complaints of pain in the lower abdomen, lower back and decreased fetal movement, as she has complaints all this things patient was undergone several investigations like blood investigation, ultrasonography. After all this investigation patient was diagnosed as oligohydramnios where her amniotic fluid index(AFI) was 5cm. The condition of patient was improved after getting care by various multidisciplinary health care team. She was admitted in the ANC ward for further management[2,3].

Aclinical trial on oligohydramnios during the third trimester of pregnancy was done in 2012 at Moradabad city, U. P. The results shows that 24. 583. 99 SD was the patient mean age and out of these 46. 15% were the age between 21-25 years. 36%were primigravida and 64% patients were multigravida. Among 78 pregnant women 74% wasmild oligohydramnios and 25% was severe. Around 68% were preterm delivered which means who delivered before completing 37 weeks of gestational age. 72% cases were delivered by caesarean section, out of all these 51% were due to fetal distress, 33% were normal Cardiotocography (CTG) and 66% were abnormal at the time of admission. The normal colour of the amniotic fluid was found in 69% cases at the time of membrane rupture whereas 31% cases meconium stained was found. Mostly, in severe oligohydramnios the chances of caesarean section was high than the mild and moderate oligohydramnios group. Perinataloutcome like Apgar score, weight of the baby, meconium aspiration syndrome, respiratory distress syndrome and neonatal ward admission.65% cases were found to be low birth weight baby among 78 newborn babies. Among 78 babies, 21 babies APGAR score was less than 7 at 5 minutes. Respiratorydistress syndrome was suffered by 15% babies and 10% were suffered meconium aspiration syndrome. Due to all these complications 15 babies were admitted to NICU[4,5].

Oligohydramniosis well known to be associated with high adverse risk during the perinatal period. However, it is a poor indicator for the adverse effects during this period. But it is also used as a predictor for delivery[6,7]. So, closely monitoring of the volume of amniotic fluid during antenatal period is very helpful to find out the risk of the adverse effects during perinatal period.[8,9.]

Conclusion

Oligohydramnios is one of the most common complications during the pregnancy which can cause maternal mortality and morbidity. But if it is diagnose in early stage and if it is treated properly we can reduce the maternal and fetal complications. My patient has received proper care and her conditions also improved as evidenced by the patient conditions and the treatment is still going on till my last date of care.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Case Report on Full Term Normal Delivery

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Abstract

Background: Labor has been defined as that of the initiation of periodic painful contractions of progressive deletion as well as dilation of a cervix, followed by a decent part going to lead to the expulsion of its fetus & placenta from the uterus of mother. Post - natal treatment was its individualized treatment provided that meet the needs of the baby and mother after delivery/childbirth.

Case Report: Mrs. Nikita Matram 25-year-old female was admitted in AVBR Hospital with the complaint of pain in abdomen and increased the fetal movement on date 4/01/2020 and she delivered female baby on date 6/01/2020 and the baby weight was 2.8 kg at 1:40 am now her complaint was pain on suture side and weakness. but the patient has a previous history of abortion after marriage of four month two and half month of baby was abort. That time dilatation and curettage was done.

Patient delivered female baby throughout the vaginal delivery and baby and mother was normal after delivery baby was kept with mother on same bed and initiate breastfeeding within one hour after delivery.

Conclusion: Case report conclude that after normal delivery and post-natal care mother and baby was normal.

Keywords: *Full term normal delivery, post-natal care, newborn, delivery.*

Introduction

Giving birth, commonly defined of labor and birth, seems to be the conclusion of pregnancy once an or maybe more kids exit the mother via going through the womb or even the caesarean delivery and, throughout delivery, a baby is released through the womb. There are different forms of vaginal delivery. Supported vaginal and instrumental vaginal birth, accidental vaginal delivery, triggered vaginal birth, natural vaginal delivery.

Treatments that are consistent to protection. Given medical and scientific innovations through managing complex health problems, this same current setting with maternity services had also increased rates for healthy mothers and infants. There have been fears all around the community that evidence-based strategies and procedures across labour and childbirth are standard occurrence.[1] There is significant mis-use of advantageous techniques, over-use of detrimental or inefficient method, or lack of desire and over consequences of insufficiently measured method.[2] Enhance the efficiency with maternity services for both developed and emerging nations is an essential part with efforts to reduce maternal and infant mortality and morbidity.

Case Presentation: Mrs. Nikita Matram 25-year-old female diagnosed with G2,A1, with 38 .2 wks. of gestational age with full term normal delivery was admitted in AVBR Hospital with the complaint of pain in abdomen and increased the fetal movement on date 4/01/2020 and she delivered female baby on date

6/01/2020 and the baby weight was 2.8 kg at 1:40 am now her complaint was pain on suture side and weakness.. Before pregnancy the patient menstrual cycle was regular 28 days of cycle and duration was 3-4 days and Last menstrual period was 11/04/2019 and Expected date of delivery was 16/01/2020.

Mrs. Nikita Matram has a previous history of abortion after marriage of four month & two and half month of baby was aborted. That time dilatation and curettage was done.She has not used any contraception method & no any medical history like DM, Hypertension, TB, Asthma.she livesin joint family in their family five member are living together including newborn and she was belonging from middle class family and intheir houses all facilities are available like electricity, water supply from municipality etc. her monthly income was 25000/- per month. Mrs. Nikita and her family members were psychological stable. She maintains good interpersonal relationship with others. She is taking only vegetarian diet. She doesn't have any allergic reaction from any food and no any history of any bad habits like chewing tobacco, smoking etc.

Patient general examination was state of health was unhealthy, conscious, Body built thin, Posture erect, hygiene was good. general parameter height was 154 cm,weight 45 kg. Vital sign is Temperature 98˚c, Pulse 84 b/m, Respiration – 20 b/m, BP – 120/80 mmHg. In breast some changes occur because of pregnancy enlargement of breast, nipple was large erectile and discharge was present of milk secretion. In abdomen linea nigra, striae gravidarum present. Lochia rubra was present.

Investigations:

- CBC

  Hb 12.7 gm %

  Total RBC 3.92 million/cu mm

  Total WBC 13900 cu.mm

  Total platelet counts 2.69 lacs/cu.mm

- LFT

  SGPT 17

  SGOT 31

  Albumin 3.6 g/dl

- USG –

  Fetal no. – single

  Lie – variable

  Placenta – anterior, grade –II

  Presentation – variable

  Fetal movement – present

  Liquor – adequate

Impression of USG–single intrauterine live fetus of average gestational age of 27 weeks 1 day and corresponding to weight of 999GMS.

The drugs are used Inj. Metrogyl 100cc, I.V., TDS. Action–Metronidazole injection is also to prevent infection when used before, during, and after colorectal surgery. Metronidazole injection is in a class of medications called antibacterial. It works by killing bacteria and protozoa that cause infection, Inj. C-Tax 1 gm, I.V.,BD.,Action - C Tax 1gm Injection is an antibiotic medicine used to treat bacterial infections in your body. It is effective in infections of the brain, lungs, ear, urinary tract, skin and soft tissues, bones and joints, blood and heart. It is also used to prevent infections during surgery, Inj. Oxytocin 10 IU.Oxytocin is a uterine stimulant, prescribed for the initiation of uterine contractions and induction of labor in women as well as stimulation of contractions in cases where the uterus does not contract enough during labor.

If any complication occurs during normal delivery than patient refer for lower segment Cesarian section. Mother should take care of self and self-care mayinclude Rest and ambulance-early ambulation after delivery, hospital stay, diet, perineal care, care of bladder, care of bowel, sleep, care of breast, rooming in, aseptic and antiseptics, immunization post-partum exercise, follow up, daily observation and care of newborn. After normal delivery mother and baby should come for check up and follow up after discharge of six weeks, and explained the client about if any sign of infection and side effect of medication immediately informed to the doctors. Immunization of baby follow up is necessary.

Discussion

Present case reveals that the antenatal patient come in hospital with the complaint of pain in abdomen and increased the fetal movement on date 4/01/2020 and she delivered female baby on date 6/01/2020 and the baby weight was 2.8 kg at 1:40 am now her complaint was pain on suture side and weakness. After delivery mother and baby was normal Apgar score was ten and

baby was kept with mother in same bed it will help to initiate breast feeding, thermoregulation, create bonding between mother and child, mother learn how to provide the care to baby etc. after that pain was manage by painkiller. Condition of baby and mother was good.

Most broadly, the phrase 'natural life' in scientific research and healthcare policy refers to conception without and with minimal surgical intervention. The 2007 systematic review by both the prenatal care Planning Group, Making Natural Childbirth a Fact, provided for such a uniform approach of hospital birth to improve trust for auditors or tracking practice patterns A subsequent description defined natural delivery or non-assisted vaginal delivery without intervention of labour; epidural, spinal and general anaesthesia or episiotomy. Unlike many other meanings, an interpretation with Werkmeister has been restricted to a pregnancy and birth and it does not apply to birth outcomes like vertical presentation as well as intact perineum.[3]

This same delivering of a full-term baby originally referred with service only at gestational age of 37-42 weeks, even though defined by last menstruation cycle or through ultrasound dating as well as assessment. A Naegel rule is indeed a frequently was using formula besides predicting a due date focused mostly on date from the last menstruation cycle. Its legislation states a 28-day menstruation periods as well as a mid-stage ovulation. Ultrasound dating will be much more accurate, especially because once implemented early in the pregnancy and used to substantiate or adjust a due date focused on last menstruation cycle. About 11 per cent of singleton birth is pre-term and 10 per cent of all births are post-term. As a result, nearly 80 per cent of babies are born on even a full-term basis, but only 3-5 per cent of births arise mostly on expected delivery date.[4]

Healthcare offers patient care, encourages convenience, listens to emotional needs through a comprehensive wellbeing paradigm, and teaches nutrition or self-care. Even so, in today's popular health care setting, postnatal nurses are very often responsible to devices or for mothers and babies. It is critical that organizations develop trust and expertise in order to make a transition towards a humanizing birth feasible. This topic problem in such a major nursing review offers important resources to help caregivers encourage, endorse and defend regular birthsNursing staff have a special and significant role to play in deciding the treatment procedures encountered by women. Unfortunately, nursing staff may well be functionally removed from important method of data interchange as well as from making a contribution to a treatment plan. This can influence the ability of nursing to successfully encourage, help or secure regular births. Since medicalized conception presents a threat of iatrogenic damage to the both mothers and infants, nurses have a duty obligation to encourage natural delivery in order to improve patient health.[5] Studies on Prenatal diagnostic and treatment modalities were reviewed [6-7].

Conclusion

After delivery mother and baby was normal. Apgar score was ten at birth. Condition of baby and mother was good after pain management pain was reduced.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Case Report on Obsessive-Compulsive Disorder

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Abstract

Introduction: Obsessive-compulsive disorder is an anxiety disorder in which people have recurrent, undesirable thoughts, ideas or sensations (obsessions) that make them feel compelled to go and do something (compulsion) repeatedly. Repetitive habits, such as washing hands, inspecting items or bathing, can substantially interfere with the everyday routines and social experiences of a individual.

Symptoms: Behavioral changes like hyperactivity, impulsivity, repetitive movement, perfectionism and social isolation. Mood changes likes anxiety, anger and apprehension. Psychological changes like sleep disturbance, fear, repeatedly going over thoughts and narcissism.

Investigation: Hb% - 13 mg/dl; Glucose plasma random – 86 mg%; Serum Globulin– 3.2gm %; serum-Protein- 7gm %; HIV, HCV, HBsAg, VDRL, all of these tests were negative.

Surgical Management: Patient have no past surgical history.

Medical Management: Patient was treated with Ativan (Lorazepam) 3mg q6hr per oral and Therapies like Aversion therapy and Group Psychotherapy was also used.

Nursing Management: Assess for physical, psychological and social data. Aware for Impact of obsessions and compulsions on physical health, mood, self-esteem and natural capacity to cope. Remember that the defense mechanisms used, the nature or structure of the feeling, the suicidal risk, the capacity to work and the space accessible for social support services.

Conclusion: Patient was admitted to AVBRH and was diagnosed to have Obsessive-compulsive disorder. The got appropriate treatment and therapy and his condition has improved well.

Keywords: *Obsessive-compulsive disorder, anxiety, compulsion, obsession, lorazepam, narcissism.*

Introduction

Individuals with obsessive-compulsive personality disorder are very serious and Obsessive-compulsive personality disorder is very severe and formal, and has emotional difficulty. They are highly rigid, perfectionist, and keen on law. They are resistant to change about how tasks will be handled, and dedicate themselves to success to the exclusion of personal satisfaction. An extreme fear of making mistakes leads to a challenging decision-making process. The disease is relatively widespread and appears more often in men than in women. It appears to be most common in the oldest children within the family group.[1]

The obsessive-compulsive disorder is, according to ICD9, a state in which "the outstanding symptom is a feeling of subjective compulsion which must be resisted - to carry out some action, to dwell on an idea, to recall an experience, or ruminate on an abstract topic. Unwanted thoughts, which include the insistency of words or ideas are perceived by the patient to be inappropriate or

nonsensical. The obsessional urge or idea is recognized as alien to the personality, but as coming from within the self. Obsessional rituals are designed to relieve anxiety, e.g. washing the hands to deal with contamination. Attempts to dispel the unwelcome thoughts or urges may lead to a severe inner struggle, with intense anxiety."[2]

Patient Information: A male patient 47 years from Halwadi, Wardha was admitted to Psychiatric Ward, AVBRH on 10th January 2020 with acase of Obsessive-compulsive disorder.

Present Medical History: My patient was apparently asymptomatic 2 years back when he was working in a private company while he was firing from his job due to his lack of responsibility according to his employer. Since then, he started maintaining self-disciplined and directing himself to his work with fear of loosing the new job again. His behavior getting very excessive that leads to difficulty in coping with family and the society. He was brought to AVBRH by his wife on 10/01/2020, when he was diagnosed with Obsessive-compulsive disorder.

Past Medical History: My patient was apparently asymptomatic 2 years back when he was fired from his job due to lack of responsibility. He started devoting himself to his new job, maintaining new job self-disciplined that eventually leads to excessive behavior disorder like sleep disturbance, lack of interaction**,** perfectionist, difficulty in completing task due to perfectionism and strict standards, maintaining extreme hygiene of self and surroundings.

Family History: My patient belongs to a nuclear family, middle class living with his wife and two sons in their own house in Halwadi, Wardha. He is working as aa clerk in a private school and is a breadwinner of the family. His two sons, one is 11th standard and the other is 9th grade. His wife has a small grocery store in their house.

Clinical Findings: Behavioral changes like hyperactivity, impulsivity, repetitive movement, perfectionism and social isolation. Mood changes likes anxiety, anger and apprehension. Psychological changes like sleep disturbance, fear, repeatedly going over thoughts and narcissism.

Predisposing Factors: From a psychoanalytical point of view, one of overcontrol is the parental style in which the adult with obsessive-compulsive personality disorder was reared. Such parents want their children to live up to and reject their set standards of behaviour if they do not. Praise is bestowed on the child with far less frequency than punishment for unwanted behaviors. Individuals are specialists in this setting of knowing what they can not do to escape retribution and criticism, rather than what they should do to gain respect and praise. They learn to follow strict rules and regulations. Significant accomplishment are expected, taken for granted and acknowledged only occasionally by their parents, whose responses and decisions are restricted to pointing out transgressions and infringement of the rules.[1]

Pathophysiology: Anxiety, obsessive-compulsive,most likely and associated disorder arecaused by multiple factors. A graphic description of this multiple association hypothesis is provided in the Stress/Adaptation Transactional Model.

Mental Status Examination: A mental status examination was performed on my patient and it was found out that there was an impaired in the stream of thought and in the insight, the score was 2 i.e. the client denies of him having mental illness.

Diagnostic Assessment: Hb% - 13 mg/dl; Glucose plasma random – 86 mg%; Serum Globulin– 3.2gm %; serum-Protein- 7gm %; HIV, HCV, HBsAg, VDRL, all of these tests were negative.

Therapeutic Intervention: Tab Ativan (Lorazepam) 3mg PO x q6h. Therapies like Aversion therapy and Group Psychotherapy was also used.

Discussion

A male client of 47 years old from Halwadi was admitted to Psychiatric ward, AVBRH on 10th January 2020 with a complaint of strict personality and disciplined, which cause him to interact or cope with his family or society (according to the patient). According to the patient's wife, the patient was an extreme self-disciplined, perfectionist, and he also wanted to change the bedsheet, clothes, shoes and blankets every day and he was very violent when they disobey him and this behavior had started in the past 2 years ago. He was diagnosed as Obsessive-compulsive disorder. As soon as he was admitted to hospital investigations were done and appropriate treatment were started. After getting treatment, he shows great improvement and the treatment was still going on till my last date of care[2,3].

A study was done on, "An epidemiological study and severity assessment of obsessive-compulsive disorder in Warangal region, India". The study aimed at to clinically assess the prevalence and assess the severity of obsessive-compulsive disorder (OCD) in Warangal region, India. A prospective observational study was done for a duration of 6 months i.e. from July-December 2013 at various Neuropsychiatric Centres in Warangal region. A total of 113 patients (male: 65, female: 48) were screened using specially designed data collection form to collect and record demographic data. Structured interviews were conducted to obtain Yale Brown Obsessive- Compulsive Scale (YBOCS) scores. All the data were analysed by ANOVA and Chi Square test using Statistical Package for Social Sciences (SPSS) software version by dividing the patients into age groups of decades from 11 to 60. It was found out This obsessive-compulsive disorder was more common in subjects between the ages of 21 and 30 (46%) and literate in patients (98.2%). More patients were married (61.6%) than single patients (38%). The most prevalent obsession was fear of harming (81.25%) while of the most prevalent compulsions was repeating (76.1%). Based on YBOC scale, OCD severity was categorized into subclinical (2.7%), mild (23.9%), moderate (41.6%), severe (23%) and extreme (8.9%). The study concluded that the prevalence of OCD condition was more among the age group of 21 to 30 years and among literates. Sociodemographic factors like age, socioeconomic status and urban were risk factors in assessing the severity of OCD.[3] Fewarticles related to various aspects of prevalent psychological problems and associated factors were reviewed [4-11].

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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COVID 19–Laboratory Scenario

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Abstract

Background: Coronavirus is RNA virus, positive stranded, and is enveloped was first isolated in 1965, can cause symptoms like common cold, severe respiratory symptoms,gastrointestinal,liver and CNS symptoms. WHO announced the original name of coronavirus as COVID19. This virus has been named as Severe Acute Respiratory Syndrome Cov-2 currently and WHO has now declared this virus as emergency of public health concern. More than seventy thousand people in the China and 25 other countries have been infected.

Materials and Method: Various lab investigations like CBC, Real Time-PCR, d-dimer levels, cell culture, electron microscopy, Low platelet count, ESR, CRP levels, Chest CT, Xray, Liver and Kidney Function tests, SOFA (sequential organ failure assessment) score have been useful in diagnosing the causative agent.

Conclusion: Since no antiviral treatment has been effective till now so transmission can be slowed down by preventive measures like self-quarantine,isolation, maintaining distance from one another and use of personal equipment.

Keywords: *Coronavirus, COVID 19, SARS cov 2, Low platelet count, Real Time PCR.*

Introduction

Structure: Corona virus belong to family *Coronaviridae* is an RNA virus, positive stranded, and is enveloped of ca. 27000 - 31500 nucleotides present[(1)], For an RNA virus, this family is the largest known genome[(2)]. Corona means crown in Latin, so this virus when visualised under electron microscopy gives the appearance of coronavirus virions, membrane of virus gives projections in form of spikes, which gives the resemblance of a crown[(2)].

Coronavirus in humans was first isolated in 1965 from discharge of nasal cavity of patient having common cold and termed as B 814.[(2)]

This virus shares similarities regarding organisation and expression of the genome, in open reading frame encodes 16 non structural proteins. At the 5' end, 1 a/b is present, followed by/proteins mainly :Spike Protein, Envelope Protein (E), Membrane Protein (M) and Nucleocapsid (N) encoded by other open reading frames at the 3' end.[(2)]

Different strains known to infect humans are currently 6 including: Human coronavirus 229 E, OC 43, NL 63, HK U1, SARS coronavirus and MERS coronavirus[(2)].

SARS cov 2 shares similarities to some of virus generas, mainly beta subtypefound in bats.[(3)]

229 E and OC 43 are the viruses from alpha and beta lineage that cause fifteen to twenty nine percent of all common cold[(2).]

Coronavirus can cause symptoms like common cold, severe respiratory symptoms, gastrointestinal, liver and CNS symptoms.[(3)]

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History: WHO used a term novel coronavirus on December 29 2019, in reference to virus that was responsible for affecting the respiratory system of patient in Wuhansuffering from Pneumonia and officially, Novel coronavirus as Coronavirus disease (COVID 19) was announced by WHO and Severe Acute Respiratory Syndrome cov 2 (SARS- Cov-2) as the current name of virus[3].

It was confirmed by WHO that the local seafood market place in China, Wuhanwas associated with the outbreak of coronavirus epidemic but no specific animal was identified in association with this disease [3].

Epidemiology: During mid December 2019 in Wuhan, the outbreak of this disease started and up to February 18, 2020,more than seventy thousand people in the China and 25 other countries had been infected.[4].

2.2 to 3.3 is estimated basic reproductive number and 2.3 % as its mortality rate. Five thousand five hundred and forty four cases have been reported as of March 6, 2020, including 159 death[5].

The confirmed cases of Covid 19 Infection, up to 14th April, 2020 in India were 11,487 and out of which 393 have been deceased [6].

Most number of cases were found to be confirmed in Maharashtra (2,684). [6]

Animals like camels, cattles, cats, bats probably serve as hosts for coronavirus [3].

Mode of Transmission: Animal coronaviruses don't spread among humans generally with the exceptions of some like Severe Acute Respiratory Syndrome cov 2 and MERS which transmit mostly via respiratory droplets(via coughing or sneezing) through close contact with infected people[3].

Three main routes have been described as a route of transmission for Covid 19 according to new guidelines from health authorities in china as following :

1. Droplet transmission
2. Transmission through contact
3. Aerosol

One study indicated the potential transmission route for this virus infection as the digestive system in addition to these routes [3]

Laboratory Scenerio: Laboratory findings and investigations play a very important role in diagnosing the disease.

Molecular Profile :

Procedures suggested for suspected patientsare: Real Time PCR (RT-PCR)-for detection of the viral RNAin samples of lower respiratory tract like in sputum, throat swabsetc[3].

Specimen collection should be done and all specimens should be regarded as potentially infectious. Respiratory material should be collected at minimum .

Microbiological Profile:

Upper respiratory tract specimen:

1. Nasopharyngeal swab.
2. Oropharyngeal swabs and
3. Washes in patients who are able to walk about but not bedridden

Lower respiratory specimen:

1. Sputum for endotracheal aspirate.
2. BAL[3].

Blood, stool, urine specimens should also be collected[6]. Specimens should be transported to test centre immediately after sample collection with correct handling during transportation lab testing and confirmation with NAAT should be done followed by serological testing viral Sequencing and viral culture.[7].

Hematological Profile:

1. Complete Blood Count.
2. Platelet count
3. ESR levels

Biochemical Profile:

1. CRP
2. Lipid profile.
3. D- dimer level.
4. Procalcitonin.
5. Liver Function Test (LFTs)
6. Kidney Function Test (KFTs)
7. Potassium level.

Pan et al conducted a study, involving twenty one patients (71% women, from age ranging from 25 to 63 years) and frequent abnormalities reported as : Increased values of CRP, ESR, LDH and D dimers[8].

Wang et al studied the behavior of 6 laboratory parameters in 138 patients having Covid 19 infection and out of them, 33 were having a severe disease and noted significant differences between patients who needed ICU admissions and those who did not encompassing: High WBC count, Neutrophilia, lymphocytopenia, High LDH, Total Bilirubin, Creatinine, D-dimer and Procalcitonin[8].

Biomarker which is independently associated with severeity of the disease and mortality risk is Platelet count . It is also very simple as well as readily available[9].

In up to fifty five percent of patients in disease outbreak, low platelet count was found and it was considered as a risk factor regarding risk of mortality risk[9].

G Lippi et al conducted a metaanalysis including 1779 patients, 399 with severe disease and concluded that the patients who didn't survive had a significantly lower platelet count than the survivors[9].

Investigations like potassium levels can be a reliable, in time and sensitive biomarkers in patients with severe infections[10].

D Chen et al conducted a study and found that body temperature, CK,CK-MB, LDH and high levels of CRe active Proteins were significantly associated with a severity of low levels of potassium[10].

Some findings like high SOFA score (a score for assessment of sequential organ faliure), d-dimer levels > 1 microgram per mililitrecan tell about poor prognosis at an early age[11].

Fei Zhou et all conducted a study in which 191 cases were taken and divided them into two groups: survivors = 137, non survivors = 54 and concluded that old age, high SOFA score and d dimer levels more then 1 microgram per ml were associated with high risk of mortality[11].

Findings associated with death were like: Lymphopenia, lymphocytosis, Elevated LDH, cardiac markers like cardiac troponin 1, Creatinine Kinase, d dimer levels, Serum Iron, Interleukin 6, Prothrombin time and Procalcitonin[11].

An important investigation in suspicion of Covid 19 infection are the levels of CRP. The extent of lung lesion and disease severity are reflected by levels of C Reactive Proteins providing an important clinical evaluation index.[12].

L. Wang et al conducted a study in 27 positive patients and found that levels of C Reactive Proteins were correlated positively with lung lesions and reflected the severity of disease at early stages of the disease[12].

Radiological Profile:

1. Chest CT should be recommended in suspected cases of Covid 19 cases, for follow upas well as for initially revaluation, because respiratory system is primarily involved [12].
2. Chest X ray- Even before the onset of symptoms, CT findings may be present, so Chest X Ray have little diagnostic values at early stages.

Chest radiographs show features of ARDS, ranging from intermediate to advanced stages[12]. Lung and immune system damage are the main pathological changes.

Fibrin exudates of serous type, clear membrane formation in lung alveoli, with the congestion and edema[12].

In early stages, CT imaging reveals small, multiple patchy shadows, stromal changes with lung exudates, which develop as multiple ground glass and infiltrating shadows in both lungs[12].

Sana Salehi et all conducted a study in which 919 patients were taken for study and found characteristic pattern and distributed of CT findings as Ground Glass Opacities (88%), both lungs involvement (87.5%), and Multilobular involvement (78.8%).

Terlobularthickening of septa, Bronchiectasis, thickening of pleura with involvement of subpleura were Other CT findings. In setting of strong clinical suspicion for Covid 19 diagnosis, chest CT along with laboratory testing may be beneficial.

Sensitivity of CT Scan alone for the diagnosis was 76.4 %[12].

Cynthia S goldsmith et al gave a report which describe the events in which virus was isolated as well as identified by electron microscopy.

This study tells about various lab findings and investigations which have been used for diagnosing the etiological agent and also tells about various important investigations which should be done for screening for better outcome of the patients suffering from this disease.

There is no specific treatment confirmed to be effective against this virus till date, so symptomatic treatment with supportive care has been recommended regarding the patients which have been infected with Covid19 [3], along with some preventive measures to slow down the transmission of infection like isolation of cases,, disinfection of environment and use of personal equipments for safety[3].

Conclusion

This study give a brief idea about the disease including its epidemiology, mode of transmission, various lab investigations and findings, preventive measures etc.

Most studies have given their views on epidemiological and potential causes and many more studies are still trying to explore the prevention, safety and control measures.

However further research should be done to provide reliable and valid measures to face this type of problem in short term as well as long term.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Study of Lipid Profile and Estradiol for Evaluation of Cardiovascular Risk in Pre- and Post-Menopausal Women

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Abstract

Introduction: Following menopause, decreased oestrogen production from ovaries leads to lipoprotein profile derangement, drastic alterations in the metabolism of glucose and insulin, distribution of body fat, coagulation and fibrinolysis and vascular endothelium dysfunction. Oestrogens include many cardioprotective mechanisms that alter the vascular tone by enhancing the levels of nitrous oxide. Endothelial cells are stabilized by oestrogens, they augment antioxidant potential and alter fibrinolytic proteins. These are all cardio-protective mechanisms that are reduced with beginning of menopause.

Objectives: To evaluate the relation of oestradiol hormonal variation with plasma lipid concentrations in pre- and post- menopausal women.

Material and Method: 50 premenopausal women and 50 postmenopausal female were selected as subjects. Data were obtained via clinical assessment from laboratory investigations and questionnai.

Results: Serum levels of Total Cholesterol (TC), Triglycerides (TG), LDL-cholesterol, and VLDL-cholesterol in postmenopausal women were significantly elevated when matched with premenopausal women. For postmenopausal women, the level of HDL-cholesterol declined substantially. The concentration of estradiol in post-menopause women was significantly lower ($p<0.001$).

Conclusion: Menopause results in alterations in the lipid profile by reducing HDL and increasing total cholesterol (TC), triglycerides (TG), LDL-cholesterol and VLDL-cholesterol, thus raising the chances of cardiovascular disease. These shifts are due to decreased amounts of oestrogen that are observed in menopause.

Keywords: *Menopause, Oestrogen, Cardiovascular disease, Plasma lipids, Coronary heart disease.*

Introduction

Menopause refers to a state of complete menstruation cessation at the end of reproductive life because of loss of ovarian follicular function and menstrual cessation. There are numerous hormonal changes that occur after menopause in women and result in variations in lipid metabolism and increase the chances of coronary artery disease in women[1,2,3].

There are alterations in the metabolism of glucose and insulin, coagulation,distribution of body fat, fibrinolysis, and dysfunction of the vascular endothelium[3,4]. Coronary artery disease (CAD) is the major reason of death for women after the menopause. Post-menopausal women are 4 to 8 times more likely to die from CAD than from any other disease[5]. Framingham study results indicate

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that female CAD morbidity levels escalate faster than male morbidity rates after 45 years of age[6]. Numerous risk factors have been reported as responsible for CAD progression.

The incidence of CAD in women is lower up to the age of 50 years, but then the incidence in both men and women is similar[7]. As average lifespan in women is rising in terms of age and menopause remains relatively unchanged, so females are now spending more of their lives in post-menopause period. In India, 60 million women are over 55 years of age[8].

Oestrogen improves the permeability of the vessels by increasing production of nitrous oxide. Adequate Protein, Vitamins and Minerals intake is required for a healthy pregnancy[9]. It maintains a balanced profile of lipoprotein. It helps to maintain the endothelial cells, improves the antioxidant effect and changes the fibrinolysis protein. In menopause, all such cardioprotective functions are missing. Postmenopausal women face an elevated risk of developing cardiovascular disease and pre-eclampsia.[2,3,10]

A woman today, after the menopause, will live about a third of her life. After menopause the ovaries fail to produce significant quantities of oestrogen; thus, symptoms and diseases related with an oestrogen deficiency are of growing in significance for women's health[11].

Inadequacy of estrogen is a crucial factor that contributes in postmenopausal women's lipid metabolism derangement that is linked to increased cardiovascular risk[2,12]. Post-menopausal women currently account for over 30 per cent of India's female population at risk of CAD[13].

Low-density lipoprotein has been involved in coronary heart disease (CHD) progression. A predisposing factor for CHD is the accumulation of fatty plaques on the arterial walls (arteriosclerosis)[14].

Given comprehensive research on the effect of progestogens and oestrogens on lipoprotein and lipid metabolism[3,15,16]. It is not yet certain if changes in sex steroid levels are correlated with variations in lipid concentrations correlated with menopause period. Coronary artery disease (CAD) is evidently polyfactorial, and data of endogenous hormones could improve our assessment of CAD[17].

Our research aimed at finding variations in lipid status in menopause women and in regularly menstruating females and investigating the relationship between menopause status and associated hormonal variability with plasma lipid concentrations.

Material and Method

This cross-sectional study was carried out at Datta Meghe Medical College,Nagpur in collaboration with Jawaharlal Nehru Medical College Sawangi (Meghe) Wardha & Datta Meghe Institute of Medical Science (Deemed University) Maharashtra, India,between August 2019 to July 2020.

For this study, groups of 50 premenopausal women and 50 postmenopausal women were chosen randomly. The postmenopausal females who were studied were those with a history of natural menopause, who had menstrual cessation for at least one year. Those who had regular menstruation were premenopausal women who were studied.

Exclusion Criteria:

1. People with hypertension,
2. Cardiovascular disease,
3. Diabetes mellitus,
4. Hepatic,
5. Metabolic and renal disease
6. Those on exogenous hormones or hormone replacement therapy,
7. Lipid reduction medications.
8. Smokers,
9. Alcoholics,
10. Sedentary women
11. Skilled athletes or sports people

The research had been accepted by the Institutional ethical committee. Following a 12-14 hour overnight fasting, venous blood samples were obtained from subjects after obtaining their informed consents. But this was achieved for the premenopausal community on the 7th day of the last menstrual cycle. Samples were centrifuged, the plasma was separated, and analyzed.

The total cholesterol, triglyceride and HDL were estimated by an enzymatic method and serum LDL and

VLDL were calculated by using Friedewald's formula VLDL= TG/5.0 and LDL = TC - HDL - TG/5.0 (mg/dL)[18].

Oestradiol was estimated by using Chemiluminescence Immunoassay kit, which is a two step competitive binding immunoassay for the quantitative determination of 17-beta–oestradiol. Body Mass Index (BMI), as a measure of body fat was determined, based on height and weight of Pre-and Post-menopausal women It was calculated by following formula. Height was measured in Meter and weight was taken in Kgs.

Statistical Analysis: Data collected was entered into Microsoft Excel Worksheet and statistically analysed by using SPSS (Statistical Package for Social Sciences) version 23.0. For quantitative data, calculated values were processed by using: arithmetic mean, standard deviation and Student's 't' test. P value of< 0.05 was considered as statically significant.

Results

Table 1: Comparison of Plasma Lipids in Premenopausal and Postmenopausal Women

Plasma Lipids (mg/dl)	Pre-menopausal n=50	Post-menopausal n=50	P-Value
TC	151.42 ± 16.38	209.26 ± 27.48	< 0.001
TGL	126.3 ± 13.32	126.3 ± 13.32	< 0.001
HDL	47.56 ± 6.19	26.8 ± 5.4	< 0.001
VLDL	21.18 ±1.32	24.3 ± 2.40	< 0.001
LDL	84.76 ±20.32	159.38 ±29.42	< 0.001

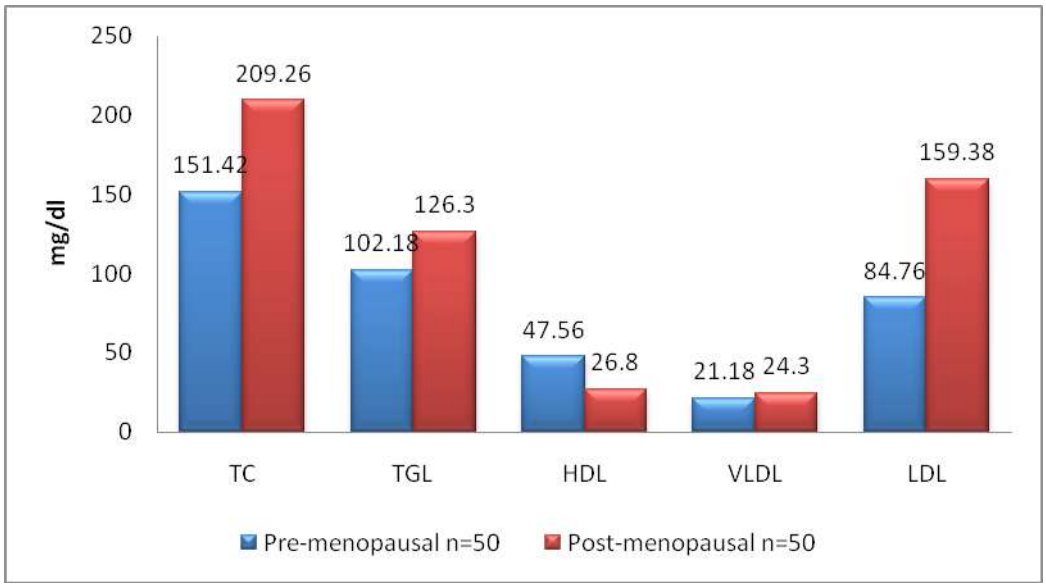


Fig-1: Comparison of lipid profile in pre and post menopausal women

In postmenopausal women, we found a significant rise in serum levels of Total Cholesterol (TC), Triglycerides (TG), LDL-cholesterol and VLDL-cholesterol compared with those in premenopausal women (p<0.001). The level of HDL-cholesterol in postmenopausal women was significantly reduced relative to that in premenopausal women (p<0.001) (Table 1 and fig 1).

[Table 2]: Comparison of Estradiol and BMI in Premenopausal and Postmenopausal Women

	Pre-menopausal n=50	Post-menopausal n=50	P-Value
Estradiol (Pg/ml)	168.48±41.28	42.32±11.20	< 0.001
BMI (kg/m^2)	20.7±4.3	23.9±4.12	>0.05

In our study, estradiol concentration in pre menopausal women (168.48 ± 41.28) was observed to be significantly higher (p<0.001) than post menopausal women (42.32 ±11.20) [Table-2].

According to the results of this study, there was no significant difference (p>0.05) in BMI between postmenopausal women (23.9±4.12) and pre-menopausal women (20.7±4.3) [Table 2].

Discussion

In postmenopausal women, we found a significant rise in serum levels of Total Cholesterol (TC), Triglycerides (TG), LDL-cholesterol and VLDL-cholesterol compared with those in premenopausal women (p<0.001). The amount of HDL-cholesterol in postmenopausal women was significantly lower than in premenopausal women (p<0.001).

Postmenopausal women in our cohort have demonstrated more significant dyslipidaemia than premenopausal women, in line with previous studies[19,20].

Our finding was comparable to the Ifueko (2013) analysis in which TC, TAG and LDL-C in postmenopausal women were significantly increased with "P" value < 0.001 than in premenopausal females[21]. Similarly, Shenoy and Vernekar (2015) observed a considerable increase in TC, TAG, and LDL-C but the HDL-C was not significantly increased in postmenopausal women compared to premenopausal females[22].

Our study results are consistent with other studies carried out by Kalavathi et al., where TC is shown to increase in postmenopausal females due to estrogen deficiency relative to premenopausal females and is statistically significant (P < 0.001) [12].

In our study, postmenopausal women had high TG and were statistically relevant (P < 0.001), relative to premenopausal women. Such results are consistent with other research carried out by Welty, Hallberg, and Svanborg[5,23]. For postmenopausal females, there is increased fat accumulation and increased release of free fatty acids into circulation, and excessive free fatty acids provide a substrate for hepatic TG synthesis[24].

In our study, in contrast with premenopausal women, postmenopausal women had elevated rates of LDL and were statistically important (P < 0.001). Those results are consistent with other studies[12]. Circulating estrogen regulates lipoprotein lipase (LPL). LPL catalyzes VLDL's hydrolysis to form intermediate-density lipoprotein and subsequently, LDL.

Regulation of various LDL receptors in Liver, is carried out by effect of Estrogen on lipid metabolism. Estrogen works on the hepatocytes on these LDL receptors and contributes to greater clearing of LDL-C particles[25]. By this process the serum LDL-C levels are regulated. Lack of Estrogen following menopause increaseshepatic TG and plasma LPL activity causing plasma LDL to concentrate and also contributes to LDL receptor down-regulation[26,27]. In our study, the VLDL was elevated and statistically significant (P < 0.001) in post-menopausal females relative to pre-menopausal females, and these results are consistent with studies by Welty [5].

Lack of Estrogen in postmenopausal women induces relative accumulation of small VLDL particles with cholesterol esters (CE) either due to elevated VLDL catabolism resulting in higher number of VLDL residual particles or increased cholesterol ester transfer protein activity[28]. The VLDL remnants are highly capable of interacting with smooth arterial muscle cells[29]. It is well known that VLDL alone constitutes a risk factor for cardiovascular diseases.

Anticipating the factors that impact the post-menopausal female's lipid profile will improve their cardiovascular risk profile by implementing strategies to control these mechanisms through modifying the relative risk factors during menopause transition.

The HDL was increased in premenopausal women relative to postmenopausal women in our study, and was statistically relevant (P < 0.001). These results

correspond to studies performed by Shenoyand Vernekar (2015)[22], and Sapkota et al. in 2015[23].

Estrogen raises HDL-C which is considered good cholesterol for CVS by raised hepatic synthesis of Apolipoprotein -A and decreased hepatic removal of HDL2 cholestrol by decreasing hepatic lipase enzyme activity. Because estrogen is reduced during menopause and this contributes to both of these functions being hampered[30].

The protective mechanism involving HDL could be due to its role in the transport of reverse cholesterol, resulting in the redistribution of cholesterol away from the artery wall and suppression of monocyte adhesion and antioxidant activity, which may avoid LDL oxidation[31]. In our analysis, we excluded the factors that could alter the lipid profile. BMI tests in pre- and postmenopausal women (P>0.05) do not indicate any important difference. So we concluded that those changes observed in these postmenopausal women's lipid profile are due to hormone estrogen deficiency and not linked to BMI. Similar findings have also been found in numerous other studies[12].

Conclusion

In the study,adverse changes in the lipid profile of postmenopausal women suggests that this group of people is at augmented risk of cardiovascular problems in the near future. Menopause contributes to increased lipid profile thus increasing the risk of cardiovascular disease. Postmenopausal females are at elevated risk of developing cardiovascular disease due to changes in lipid pattern and the loss of oestrogen's cardioprotective impact.

The decreased cardiovascular protective HDL is an indicator that menopause is an independent risk factor for the development of cardiovascular disease. Thus, it is necessary to encourage each and every postmenopausal woman to undergo screening for an abnormal lipid profile. Specific health education approaches are needed to prevent postmenopausal women from developing cardiovascular diseases.

In this high-risk population early and timely identification and primary prevention will reduce morbidity and mortality.

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Comparison of Quality Enhancing Method of Modern & Ayurvedic on Tulasi (Ocimum Sanctum)

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Abstract

Modern civilization, Industrialization and the population explosion has shrunken forests as well as the land available for cultivation in our country which has badly affected the availability of medicinal plants as the quantity of medicinal plants is reduced the quality also is compromised. The green revolution was brought about by the use of chemical treatments on the seeds, plants, used of the pesticides and chemical manures. It would be worthwhile to study whether similar pattern can be employed to increase the no. of plants and their quality in the field of cultivation of medicinal plants. Although, plant breeding appears to be the principal way of improvement, and can give good results in terms of resistance to pathogens, active component content and yield; the agronomical and the technological approaches are also very important. An agronomical method to improve the quality & quantity of the plants includes treatments of seeds & the growing plants with Bio-engineered herbal products along with the use of organic manures. The scientist in the field of botany and agriculture have developed some method of quality enhancing by treating seeds, seedlings or young shoots or buds in plant breeding with some suitable chemical treatments. These method have given satisfactory results in the form of increase in the yield. Hoping that the quality also improves it would be worthwhile to grow medicinal plants on the similar lines. The relevant literature reveals that Colchicine treatment on seeds or seedling or emerging buds for quality enhancing of medicinal plants will be worth studying.

Keywords: *Ocimum sanctum, Cholchicine, decoction.*

Introduction

Colchicine is a toxic natural product and secondary metabolite, alkaloid originally extracted from plants of the genus *Colchicum luteum i.e.* Colchicum luteum. Since chromosome segregation is driven by microtubules, Colchicine is also used for inducing polyploidy in plant cells during cellular division by inhibiting chromosome segregation during meiosis[1,2,3,4] half the resulting gametes therefore contain no chromosomes, while the other half contain double the usual number of chromosomes (i.e., diploid instead of haploid as gametes usually are), and lead to embryos with double the usual number of chromosomes (*i.e.* tetraploid instead of diploid)[5] While this would be fatal in animal cells, in plant cells it is not only usually well tolerated and properties of the plants can be enhanced. This necessitates to the plant with chemical treatments by suitable chemicals or organic materials on the seeds before sowing.

Ocimum sanctum (O. sanctum) is of family labiatae regarded in Ayurveda as a kind of 'elixir of life' and believed to promote longevity.

To generate the scientific evidences a comparative study of morphological and phytochemical of O. sanctumand O sanctum grown after treating it's seeds with O. sanctum decoction and colchicine and Colchicum luteum was studied.

Material and Method

Material:

1. *O. sanctum* seeds.
2. The materials used for treating the *O. sanctum* seeds
   i. Decoction of *O. sanctum*
   ii. Cholchicine
   iii. Colchicum luteum

Method

Sample A: *O. sanctum* plant grown after soaking *O. sanctum* seeds in distilled water for 24hrs.

Sample B: *O. sanctum* plant grown after soaking *O. sanctum* seeds in O. sanctum decoction for 24hrs.

Sample C: *O. sanctum* plant grown after soaking *O. sanctum* seeds in Colchicine solution for 24hrs.

Sample D: *O. sanctum* plant grown after soaking *O. sanctum* seeds in weak infusion of *Colchicum luteum* for 24hrs.

B. The **Decoction** was prepared as per the method mentioned in **Sharangadhar Samhita** which was used for treatment of seeds for 24hrs.

C. The Colchicine solution was prepared by dissolving 100mg of the powder of the chemical in 100ml of distilled water (1000ppm) and the 5ml of this solution was added to 5ml of distilled water to make it 500ppm. This solution was used to soak the seeds of O. sanctum for 24hrs.

D. The dried tuber of *Colchicum luteum* plant was crushed to obtain fine powder. 100mg of this powder was added to 100 ml of distilled water and mixture was churned for about 1hr. The mixture thus prepared was strained with fine cloth. The solid was discarded and the fluid was used in quantity of 5 ml to which 5ml of distilled water was added and the seeds of O. sanctum were soaked in this weak infusion for 24hrs.

The seeds thus germinated in the pattery dishes were transferred to the not manure soil. The plants of all the groups thus grown were sufficiently watered and were observed daily. After the appearance of inflorance i.e. after 2 months the plants were studied for following tests.

Morphological appearance, Phytochemical, HPTLC studies of all the groups.

1. **Morphological appearance:** Height of the plant, length of the root, charchaterstics of the stem and leaves were observed.

Solvent System: Toluene: Ethyl acetate: Formic acid: Methanol (3: 2: 0.4:0.4) was used as solvent system.

Chromatographic Development: Precoated TLC plates was used for Chromatography.

Observations and Results

Results of Morphological Charchacter of Tulasi: On the Comparison of Morphological features of the group A, B, C and D, the variation observed was significantly more than the control group A in all the three group of plants which were grown after treating the seeds with O. sanctum decoction (Group B), Colchicine solution (Group C) and weak infusion of *C. luteum* (Group D).

Table 1: Morphological Observation

Treatment	Observation Morphological Features
1. Control (A)	Height 11.2 cm shoot length 7.3 cm root length Secondary roots 16 no Stem color slightly purplish Stem clothed with soft silky hair but occurred frequently. Petiole length 2.1 cm Leaf Elliptical slightly acute, apex, margins slightly serrated. Leaf with 8-9 subveins.
2. T-1 Decoctions (B)	Height 22.2 cm Shoot length 12.1 cm root length Secondary roots length no33 Tertiary roots are also found. Petiole length 2.3-2.6 cm. Stems clothed with silky hair Leaves with elliptic ovate blade Apex acute.
T-2 Colchicine	Height 18.2 cm shoot length 19.1 cm root length Secondary root length no 24 Tertiary roots are also found. Stem colour dark purplish with abundant silky hairs. Secondary branched develop, petiole length 1.5 to 1.9 cm. Basal leaves with emarginated leaf apex, while upper ones are acute at obtuse leaf apex.
T- 3 Colchicum luteum (D)	Height 18.6 cm Shoot 9.2 cm root Secondary roots – 20 Leaf, petiole length 1.6-1.0 cm, margins Deeply surrounded with acute apex. Main vein with 8-9 subveins. Stem colour – slightly purplish Silky hair abundantly found

Table 2: Morphological character of all samples

Sample	Shoot	Root	Sec. Root	Petiole
A	11.2.	7.3	16	2.1
B	22.2	12.1	33	2.3-2.6
C	18.2	19.2	24	1.5-1.9
D	18.6	9.2	20	1.6-1.0

Table 3: Phytochemical Study of Samples

A, B, C and D of O. Sanctum

Sr.No.	Parameter↓/Samples→	A	B	C	D
1	Foreign matter %	0	0	0	0
2	Total Ash value %	7.87	6.34	7.35	8.59
3	Acid insoluble Ash %	0	0	0	0
4	Water soluble extractive %	68.75	69.88	68.98	69.45
5	Alcohol soluble extractive %	78.64	79.77	78.95	79.56
6	Total % of Alkaloids	24.65	26.82	26.45	26.55
7	pH	6.75	6.79	6.92	6.8

Table 4: HPTLC Analysis of Ocimum Sanctum of Different Treatment in Respect to Eugenol Content

No.	Start Rf	Start Ht	Max Rf	Max Ht	Max %	End Rf	End Ht.	Area	Area %	Eugenol %
1. Control	0.38	0.8	0.44	255.7	74.99	0.50	3.9	6593.5	83.47	0.24
2. Control	0.38	0.1	0.44	260.0	76.65	0.50	3.2	6725.2	84.94	0.24
3. Decoction	0.39	0.7	0.44	301.6	40.24	0.50	2.6	7877.4	47.41	0.29
4. Decoction	0.39	0.9	0.44	292.5	39.02	0.50	2.8	7850.3	47.24	0.29
5. Colchicine	0.39	0.4	0.44	309.3	41.66	0.50	3.0	8099.1	47.15	0.29
6. Colchicine	0.39	0.1	0.44	294.6	33.22	0.50	3.8	8000.1	46.41	0.29
7. Colchicum luteum	0.40	0.1	0.45	316.7	38.51	0.50	3.0	8287.9	42.80	0.30
8. Colchicum luteum	0.40	0.4	0.45	312.3	32.11	0.50	3.0	8161.2	37.14	0.30

Table 5

Samples	Eugenol Area	Eugenol %
Control (A)	6725.2	0.24
Decoction (B)	7877.4	0.29
Colchicine (C)	8099.1	0.29
Colchicum luteum (D)	8287.9	0.30

Discussion

These studies the plants were grown by treating the seeds of O. sanctum by O. sanctum decoction group B, colchicine and Colchicum luteum. The plants were grown for two months till inflorescence appeared. The morphological characters of all the groups were studied along with the chemical composition.

O. sanctum is having maximum medicinal and healing properties commonly occurred in near about all the regions of the India and mostly tropic & subtropics regions of the world. It is necessity to increase the quality and quantity of this valuable drug.

To provide a scientific basis for quality enhancement it decided to undertake four groups labeled as ‘A’ ‘B’ ‘C’ ‘D’. A - Control group, B – Decoction treated, C – Colchicine treated, D – Colchicum luteum treated. A

comparative study of all these samples was carried out. Some of them are mentioned Ayurvedic Pharmacopeia of India and Qualitative analysis of ICMR while the remaining are standard operative procedure explained by the experts.

Morphological character: It is observed that the height of plants group B is practically doubled (22.2cm) than that of control Group A (11.2 cm), where as that of group B grew 22.2cm Group C grew 18.2cm and Group D grew 18.6cm. It seems that the average growth in length of the shoot is maximum in group B followed by Group D & C in comparison with control Group A. The height of shoots is significantly more in the treated group.

The length of root of the control group A is 7.3 where as that of Group B is 12.1, Group C is 19.2 and Group D is 9.2 which indicates that the length of root of group C is largest than that of Group B, Group D and Group A but roots of group B and D are also longer than the control group. Roots are meant for giving the stability to the plant and providing nutrition this being one season plant stability is not much required and the roots are hardly used for medicinal purposes.

The numbers of secondary roots also highest in group B that is 33 and that of group C has 24, Group A has 16 and group D has 20 which suggest that the group B instead of growing in length grow in number suggesting that the nutrition provided to the plant is more.

The length of the petiole is also more in group B than that of group A group C and D are comparable with the control.

In comparison to control group A (7.87%) it was observed that the **Total Ash value%**content of sample C is 7.35 which is similar that of sample D is 8.59 which is significantly more than that of control group A. Ash value of group B is 6.34% which is significantly less than that of control group A and also of group C and D. Ash being an inert material lesser value of ash indicates that the organic material which is active is more in that sample implying that the plant which has lesser ash is more active hence it is concluded that the plants of group B are of better quality than control group A and other groups C and D.

The values of **acid insoluble ash** is zero % which if compared with the normal range i.e. not more than 3 % shows good quality of the samples. Acid insoluble ash denotes presence of siliceous matter in the drug which in lesser amount indicates better quality of the herbal drug. All the sample shows 0% acid insoluble ash indicating that the medicinal value of all the samples is good.

In comparison to control group A 68.75 % it was observed that the **water soluble extractive%**content of sample C is 68.98, sample D is 69.45 and that of sample B is 69.88 which is shows that the water soluble extractive is maximum in Group B which is significantly more than that of control group A and group C and D. the group C and control Group A are comparable where as group D it is significantly more.

It can be safely said that water soluble amino acids, carboxylic acid and vitamins are more in plant which has more water extractive values denoting greater activity therefore it can be concluded that in comparison with control and other groups, group B plant is more potent.

The presence of alkaloids, glycoside, steroids, anthraquinones and triterpenoids are estimated by alcohol soluble extractive values. It was observed that the **Alcohol soluble extractive%**content of sample C is 78.95, sample D is 79.56 and of sample B is 79.77 in comparison with control group A shows increase in the Alcohol soluble extractive% meaning there by the above said chemicals are comparable in the treatment groups but are significantly more than that of group A 78.64 indicating better availability of these chemicals.

In comparison to control group A 24.65 it was observed that the **alkaloids content** of sample C is 26.45, sample D is 26.55 and that of sample B is 26.82 which is showing improvement in the alkaloids in all th e treatment groups in comparison with group A treated groups are comparable with each other but are significantly more than that of control group A. Alkaloids are the major active ingredients of any plant. Their increase suggest better activity of the drug[6-16].

The drugs with low **pH** (acidic pH) have better absorption in intestine, as absorption of drug is dependent on pH of intracellular and extracellular fluid. So, the pH of the drug should be low and when the values were Compared to control group A it was observed that the pH Values of group C is slightly more where as that of Group B and D are similar to group A. It seems that there is no difference in pH of plant extracts in the treated groups.

It was noticed from the graph that **Eugenol %** in all the plants in the treated groups is significantly higher than that of control group A whereas there is negligible Difference among Eugenol % in B, C and D. eugenol being volatile oil is considered as active principle of the plant O. sanctum which is mostly used in respiratory tract disorder. Increase in eugenol after different indicates increase the potency of the plant.

So, considering this it can be said that decoction treatment & Colchicum luteum treatment to plants especially O. sanctum is equally effective as Colchicine used today. So, New Decoction & Colchicum luteum treatment can be considered as new options for quality enhancement in plants.

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OBE implies a paradigm shift toward supporting high levels of learning for all students. It focuses on what students can do and how do students relate to teaching (Biggs and Tang 2007).

That is, the instructor should provide guidance for students to achieve their outcomes by choreographing specific learning activities and assessment tasks (Van Schalkwyk 2007). Considering the above the most important aspect of OBE is to determine the learning outcomes[5].

"Pretorius (1998) has also laid down several characteristics of OBE:

- It is a learner-centered approach based on the philosophical assumption that all learners are achievers if allowed sufficient time to do so.
- There is freedom and flexibility for self-motivated learners to engage in enriching activities in order to attain the required outcome.
- Learners are focused on what is expected of them since they are exposed to the outcome right from the onset.
- Learners become accountable for their own learning when having to attain outcomes.
- An opportunity for flexible teaching strategies is offered to teachers since the emphasis is not only on whether the learner attains the selected outcome, but also on procedure.
- Learner success is established purely on whether the learner has attained the required outcome or not–and not in terms of the achievements of other learners.
- A variety of opportunities are offered to learners to demonstrate whether the outcome has been attained.
- Learner progression is based on demonstrated achievements.
- It is a long-term commitment based on the premise of continuous improvement.
- The notion of "great expectations for all to succeed", is emphasized.
- It focusses on the future and is able to address the changing needs of the community more readily.
- Community requirements tend to be addressed more directly because, in determining the required outcomes, there is the involvement of an extensive range of stakeholders such as parents, teachers and business leaders.
- The focus is on skills needed in everyday living and the requirements of the career environment, rather than on memorizing factual knowledge.

Rationale: There are very few studies related to OBE in Indian context with respect to analyzing the awareness, understanding,the reluctance and implementation of OBE practices in professional educational institutions.

Unemployment statistics of Indian youth especially in the days of reaping benefits of demographic dividend, has put forward a need to restructure the educational scenario.

This study makes a modest effort to give a visualization of the current scenario in educational sector by providing results about OBE quotient amongst faculty members,their perspective on application of the OBE and the effectiveness of OBE.

Literature Review: In a study conducted by Botha (2002), the research concluded that OBE is concerned with what students have learnt and how well they have learnt rather than what they were supposed to learn.

The shift in focus from teacher to student has led to constructivism in higher education (Ertmer and Newby 1993) where constructivism emerges from philosophy, psychology, sociology and education (Carlson 2003). It lays emphasis on knowledge as a social endeavor (Otting 2000).

In OBE teachers are stimulators of meaningful knowledge rather than mere transmitters of existing knowledge (Harris and Alexander 1998 in Kember 1997).

Black and Wiliam (2009) argue that every instruction is aimed at achieving some goals such as improving learning skills.

Transmission approach to knowledge delivery leads to superficial learning while an approach focused on students leads to in-depth learning. It is thus important to use appropriate method to transform superficial learning to in-depth learning (Trigwell et al. (1999).

Ebrahim (2010) and Allawneh and Mallah (2008) highlighted in their study that administrators incorporated the outcome based education concept in their system without adequate training to the members of faculty

on how to execute these concepts and render student centric learning. This leads to inability of the members of faculty to develop and enhance their teaching skills.

Further to this (Mc Alpine et al., 2006) stated that there was a lack of alignment between course goals, plans and classroom activities may be due to the lack of understanding of OBE by the administrators similar to the members of faculty.

Recent trends in higher education industry reflect that for assurance of quality in education focus should be on identification of outcomes of the course or program to be taught and alignment of these learning outcomes with the teaching pedagogy and assessment strategy in a manner to maximize the achievement of these outcomes. It's a process that begins with identification of outcomes i.e. what the students should be able to do at the end of the program, then organizing the curriculum accordingly, defining instruction and pedagogy and finally the assessment to ascertain that the intended learning has actually taken place. (Baron & Boschee, 1996; Deneen, 2009; Spady, 1994; Webb, 2009).

Laguador, J. M., & Dotong, C. I. (2014) in this study found out that faculty members may possess appropriate knowledge in certain area of the OBE implementation but not being practiced. Continuous participation of the faculty members in training and seminars is highly encouraged to provide them updates of the OBE process. Faculty members with high level of knowledge and understanding on the implementation of OBE have also higher possibility to contribute in the realization of the objectives of OBE through practice.

Research Methodology

Questionnaire Design: Structured close ended questionnaire has been used to collect data for the research. The questionnaire was developed based on literature review and objectives of the research. It takes into account the major components of OBE i.e. PO, CO, Pedagogy and Assessment.

The data is analyzed using both descriptive & inferential statistics

The study uses Convenience sampling method for data collection and data was collected based on the availability and willingness of the respondents.

Objectives:

Objective 1: To assess the knowledge of OBE amongst the faculties (Mgt & Engg)

Objective 2: To understand if OBE quotient differs with Gender and Age.

Objective 3: To understand the relationship between knowledge of OBE and acceptance/Reluctance of Teachers towards implementing/practice of OBE

Objective 4: To analyse the impact of OBE on teaching effectiveness of Faculty members.

Prominent Variables used in the research: OBE literacy quotient: OBE literacy quotient is the extent of knowledge pertaining to OBE concept and practices.

Items related to assess the OBE Quotient:

1. I have the conceptual knowledge of OBE.
2. I have gained the knowledge of OBE through the Seminars/Workshops/Self study
3. I understand how the Program Outcomes and Program Educational Outcomes of the College were formulated
4. I am aware of the process to formulate Course Outcomes using Bloom's Taxonomy
5. I understand the relationship between Course outcomes and Program outcomes.
6. I have clear understanding on how to do the OBE assessment
7. I understand the use of the direct assessment techniques in assessing the course outcomes.
8. I can assess the students by using Activities, Exercises, Projects, assignments to attain the course outcomes.
9. I understand the principle of making and using the rubrics for assessment.
10. I am ready to apply the OBE curriculum in the delivery of my instructions
11. I have used different techniques related to OBE in the delivery of my instructions
12. I was involved in implementing OBE at my college/ institute.

13. I understand the difference between PO, PSO, PE and GA
14. I can comfortably map COs with POs.
15. I can measure attainment of my course.

Teaching Effectiveness: Teaching Effectiveness is the result of the application of OBE practices and getting a positive evident learning outcomes.

Items related to Teaching Effectiveness: I find the students more engaged.

The students are able to think out of box.

The Teaching-Learning has become two way interactive process.

Higher order thinking skills of the students have improved

Clear teaching objectives and predefined expectations have inspired students to become creative and innovative thinkers.

Students understand WHY they are learning.

Increased student involvement allows students to feel responsible for their own learning

Students gained real world experience

Students are able to recall and implement/execute the concept.

Students are inspired and motivated about the subject

Hypothesis 1:

Context: The concept of OBE was first adopted in Engineering Colleges in India soon after India became a signatory member of Washington accord and later percolated to the various programs. So the researchers found it imperative to find if there was any difference in the OBE knowledge of the faculties in Management and Engineering colleges.

Ho1: There is no significant difference between the OBE Literacy Quotient of Various faculties of technical education (Engineering, Management)

Ha1: There is a significant difference between the OBE Literacy Quotient of Various faculties of technical education (Engineering, Management)

Hypothesis 2:

Context: It is well established from the Literature review that OBE knowledge is the need of the hour. But merely having knowledge will not suffice. So it was essential to explore the relationship between OBE quotient and acceptance of the faculties/teachers to implement the OBE practices in their classroom.

Ho2: There is no significant relationship between knowledge and acceptance of Teachers towards implementing/practice of OBE

Ha2: There is significant relationship between knowledge and acceptance of Teachers towards implementing/practice of OBE

Hypothesis 3:

Context: The employability of the students will enhance only if the teaching is effective especially if the students are able to apply the concepts taught by the teachers. Thus it was necessary to explore whether there exists any relationship between OBE literacy quotient and teaching effectiveness.

Ho3: Teaching effectiveness is independent of OBE literacy quotient.

Ha3: Teaching effectiveness is dependent on OBE literacy quotient.

Sample Size: 75 faculty members of Engineering & Management institutions (across Maharashtra).

Data Analysis & Interpretation

Descriptive and interferential statistics have been used, by applying SPSS 21 software package. Descriptive statistics have been used for summarizing the data to draw a meaning from the data.

Mean & Standard Deviation were computed to describe all the variables and Cronbach's Alpha test was performed to check the reliability of the data.

Reliability Statistics: Inferential statistics have been used for testing the hypothesis and drawing conclusions. T-Test was used to generalize the results of the sample for the complete population.

Analysis 1:

Test of normality

Shapiro-Wilk Test of Normality: To assess whether the data is normally distributed, Shapiro-Wilk Test of Normality is used for both data collected from Members of Faculty. The test reveals whether sample data are drawn from a normally distributed population or not.

Table 1: Applying Shapiro-Wilk test of normality to mean values determined and results obtained

Tests of Normality							
	Teaching in	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
OBE quotient	MBA	.150	47	.010	.947	47	.033
	ENGG.	.144	25	.195	.949	25	.235

The statistical value (p value) for the data is .235. Assumed level of significance is .05. Since the *p* value is greater than .05 (p>.05) the results are significant which means that the data is normally distributed.

Analysis 2:

OBE Quotient:

Table 2: The OBE quotient was calculated and the mean score was found as under:

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
OBE literacy Quotient	Equal variances assumed	.614	.436	.462	73	.646	.06276	.13596	-.20820	.33372
	Equal variances not assumed			.491	60.670	.625	.06276	.12772	-.19267	.31819

The significance value is greater than .05, which means that the null hypothesis is accepted. There is no significant difference between the OBE Literacy Quotient of Various faculties of technical education (Engineering, Management).

Independent Sample T-test was used to study the difference in the understanding of OBE by gender & Age of Members of Faculty (Teachers).

Analysis 3:

Table 3: Understanding of OBE differs by gender

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
OBE literacy Quotient	Equal variances assumed	.614	.436	.462	73	.646	.06276	.13596	-.20820	.33372
	Equal variances not assumed			.491	60.670	.625	.06276	.12772	-.19267	.31819

Independent sample test was conducted to generalize the results of the sample for the population. The results show that understanding of male members of faculty (3.88) did not show statistically significant difference to the understanding of female members of faculty (3.81), t (75) =.462, p = .646

Results are significant when calculated p value is less than assumed p value. Since $p > 0.05$, the null hypothesis is accepted & hence we conclude that understanding of OBE does not differ by gender of members of faculty.

Analysis 4:

Table 4: Different age groups shows different levels of OBE understanding.

Descriptives				
OBE quotient				
	N	Mean	Std. Deviation	Std. Error
20-30	8	3.55	.5574	.19710
30-40	31	3.711	.5857	.1052
40-50	27	4.068	.4878	.0938
50+	9	4.000	.4386	.14620
Total	**75**	**3.8578**	**.5573**	**.0643**

A Turkey Post hoc test revealed that the group with experience of 40-50 years showed higher level of understanding compared to 20-30 years & 30-40 & 50 above groups.

The graph clearly shows that members of faculty with experience between 40-50 years shows higher level of understanding of OBE compared to other members of faculty from the other two groups.

Analysis 5:

Table 5: Relationship between knowledge (OBE literacy quotient) and acceptance of Teachers towards implementing/practice of OBE

Correlations			
		OBE quotient	**Acceptance for practice of OBE**
OBE literacy quotient	Pearson Correlation	1	.293*
	Sig. (2-tailed)		.011
	N	75	75
Acceptance for practice of OBE	Pearson Correlation	.293*	1
	Sig. (2-tailed)	.011	
	N	75	75

*.Correlation is significant at the 0.05 level (2-tailed).

The p value is 0.011)which is less than 0.05, it means that we reject the null hypothesis and the correlation is significant and looking at the correlation quotient (r=0.293) the value of r is positive which means that there is a positive correlation between OBE Quotient and Acceptance of the Teachers to implement OBE practices.

Table 6: Interpretation

	Alternate Hypothesis	**Statistical Tool**	**Interpretation**
Ha1	There is a significant difference between the OBE Literacy Quotient of Various faculties of technical education (Engineering, Management)	T-test	The null hypothesis is accepted. There is no significant difference between the OBE Literacy Quotient of Various faculties of technical education (Engineering, Management)
Ha2	There is significant relationship between knowledge and acceptance of Teachers towards implementing/practice of OBE	Bi Variate Correlation	The alternate hypothesis is accepted
Ha3	Teaching effectiveness is dependent on OBE literacy quotient.	Bi Variate Correlation	The alternate hypothesis is accepted

Conclusion

The findings have demonstrated that some of the faculties lack the understanding of OBE which leads to monotonous teaching and less teaching effectiveness. It is a well-established fact that OBE is the need of the hour considering the fact that traditional education system is yielding unemployable youth due to a wide gap between the theory taught & practical knowledge required[6,7]. Members of faculty have also depicted low level of understanding of OBE which in turn leads to inadequate application of the concept in the education system and reluctance to implement OBE. The findings of the study also suggests that there is a clear linkage between Understanding the concept of OBE,Implementation of OBE in classroom teaching and Effectiveness in teaching. Proper training programs should be organized for the teachers in all technical programs.

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Impact of Outcome Based Education (OBE) on Teaching Effectiveness of Faculty Members of Professional Program

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Abstract

The growth and development of a country relies heavily on the Higher education which powers the supply of skilled and technologically advanced manpower.

Outcome based education is potentially successful curricular innovation for educational institutions as it not only overcomes the lacuna of traditional learning process but also it has the advantage of being future driven. OBE aims at assessing the learners on understanding, critical thinking, reasoning, reflection and action. It aims at ensuring that the students have an integration of knowledge and learning relevant and connected to real-life situations. Looking at the growing importance of Outcome based Education (OBE), This study is conducted to find the OBE literacy quotient of the faculty members in Management & Engineering colleges. A preview is generated with respect to the acceptance and/or reluctance of faculty members towards implementing OBE practices. The study also postulates the relationship between OBE knowledge and teaching effectiveness.

The data is analyzed through descriptive and inferential statistics.

The outcome of the research would be significant for the policy makers and administrators in determining the means to improve quality of education by proper implementation of OBE practices.

Keywords: *Outcome based Education (OBE), OBE literacy quotient, teaching effectiveness.*

Introduction

Looking at the fast changing global environment, it has become imperative to make changes accordingly in the teaching learning process. The regulatory bodies in India had proposed the outcome based education format earlier for the Engineering and Medical Institutions and now have percolated the policies in all the educational institutions[1].

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It is a task to change the mindset of the teachers who have been following the traditional classroom instructional pedagogy for years together. Thus there is a necessity to create awareness, train the faculty members, motivate them to design activities which targets towards the outcomes. The role of the teacher has to be changed from merely a instructor to a facilitator. Active learning rather than passive learning is the need of the hour for the students to sustain in the competitive world[2,3].

According to Pretorius (1998) and Genis (1997) OBE is a system that is relevant and appropriate to address future needs in a better manner compared to traditional approach. It also intend to implement the changes in technology and work environment more effectively[4].

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A to Z Activities for Engaging School Going Children During Lockdown

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Abstract

Pandemic of Novel Corona Virus disease is rampant and self-isolation or quarantine is one of the core strategies in flattening the curve of infection rates. It means that schools are going to be closed for relatively longer time. Such thought of staying at home with kids during the lockdown has built up a certain amount of stress and anxiety among parents. For them, it has become essential than ever to spend time with their kids and plan their day. Parents are suddenly getting confused for resources to keep their kids engaged in productive activities. With schools shut, children are also bound to feel more agitated and confused than ever, causing them to react in ways that may be new for parents. As such, it becomes important for parents to strike a balance between work and spending time with their kids, and understanding the ways in which the lockdown experience can be made simpler and healthier for the entire family.

Keywords: *Pandemic, quarantine, skill development, Covid-19.*

Introduction

The government of India Initially has declared a total lockdown, in the wake of Covid-19 pandemic, for 21 days which has extended further. Even the most tolerant people will have their limits tested as they are forced to stay indoors. Parents are wondering about lockdown activities to keep kids occupied[1,2,3]. Children are the most vulnerable section of society right now as schools across the country are closed and they are forced to be at home day in and day out. They dislike staying indoors too long and like to go out and play and interact with their mates. It is, therefore, the responsibility of the parents to keep children engaged during lockdown with productive quarantine activities. Zafirakou says it is important to limit screen time while children are learning at home[4,5.]

This can be done by engaging them in activities that are fun learning opportunities.

Ensure that the play area is hygienic and safe for your kid to plays alone there. Kids can be supervised through CCTV Camera.

Here are A to Z activities to keep kids busy during lockdown:

A. Art and Craft: By involving your kids in art and craft, you will not only be helping them pass the time easily but also be giving wings to their creativity. By making easy and simple craft projects helps the children to sharpen their artistic talents and whet their minds. Provide them lots of craft supplies such as paper sheets, glue, tapes, kid-friendly scissors, glitter, craft kits, etc. for setting up a creative space. Encourage your kid to create something new every day using those supplies.

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B. Build with Lego: Lego toys are so popular among children as it gives lot of fun but artfully, they also teach the fundamentals of physics. Start with simpler structures and as your kid begins to get hold of the concept and then move towards more advanced ones. This exercise alone will keep your kid occupied for hours and is perfect for beating boredom.

C. Chat with friends: It's really important for the children to communicate with their friends. Parents can set up online meetings with their children's friends. Not every day, but during the week, there should be some way they are connected with somebody else that's not you, and not another adult. They want their space and parents should allow them to talk with their friends to share their ideas.

D. Do It Yourself: Apart from the most obvious advantage of getting our children away from screens and gadgets, creative projects help children with inspiration, imagination and good motor skills. Getting messy sometimes and creating things is actually as important as outdoor play and reading. It helps to develop bilateral coordination, Fine motor skills,and Self regulation. It acts as a esteem booster and encourage self expression. 1

E. Exercise: A 2019 study published in the journal Proceedings of the National Academy of Sciences found that serotonin, a mood-regulating chemical the body produces, increases during exercise and can boost the energy and also associated with feelings of well-being, heightening of appetite, and lessening of mental depression. Combining regular physical activity with a healthy diet is the key to a healthy lifestyle. It should be variety of age-appropriate activities and with fun, so kids will come back for more. 2,3,4

F. Fancy dress celebration: Fancy dress also helps children develop emotionally, being a channel where children can freely express themselves. It also helps children communicate with others, and by playing the characters that they love they learn control their emotional responses. Fancy dress helps children develop their imagination. You can share these photographs to relatives and friends. 5

G. Gardening: A garden provides the opportunity to make a positive environmental impact. Planting a garden provides enjoyment. Watching your garden go from bare ground to ripe produce or beautiful plants offers a sense of satisfaction. It makes your child responsible and caring. Some gardeners find the activity relaxing and stress-relieving, for overall mental health benefits. Caring for the plants gives the entire family a chance to work together. Gardening also offers a form of moderate outdoor exercise.[6]

H. Helping in household chores: Children can learn a lot many things from doing household chores. Doing chores helps children to care for themselves, a home and a family. They **learn skills** they can use in their adult lives, like preparing meals, cleaning and organizing the home. It helps for easing family stress,and breaking away from gender stereotypes. Being involved in chores also gives children **experience of relationship skills** like communicating clearly, negotiating, cooperating and working as a team. When children contribute to family life, it **helps them feel competent and responsible**. Even if sometimes they don't enjoy it, when they keep on doing they get the feeling of satisfaction that comes with finishing a task[6,7.]

Here are some ideas for chores for children of different ages.

Toddlers (2-3 years):

- Pick up toys and books.
- Put clothes on clothes hooks.
- Set placemats on the dinner table.

Preschoolers (4-5 years)

- Set the table for meals.
- Help put clean clothes into piles for each family member, ready to fold.
- Help with grocery shopping and putting away groceries.
- Hand you wet clothes to be hung out to dry.

School-age children (6-8 years)

- Water the garden and indoor plants.
- Feed pets.
- Clean the bathroom sink, wipe down kitchen benches, mop floors or take out rubbish.
- Help hang out clothes and fold washing.
- Put away crockery and cutlery.
- Help with choosing meals and shopping.

- Help with meal preparation and serving, under supervision.

I. Indoor Games: By playing games, students become more motivated to learn, pay attention and participate in set tasks. Games help students to become a part of a team as well as take responsibility for their own learning. There are so many indoor games like chess, carrom, playing cards, UNO Snakeladder, Ludo and many more .Playing these games with family is really a great joy.[8]

J. Jigsaw puzzles: Jigsaw puzzles exercise the left and right sides of your brain at once. It improves your short-term memory and improves your visual-spatial reasoning. Jigsaw puzzles are a great meditation tool and stress reliever. Starting a jigsaw puzzle and keeping it on a table in your living room or kitchen is an invitation for the whole family to participate, whenever they have a few minutes to sit down and focus. It's a tactic that parents of teens can use for starting a conversation while working toward a shared goal.[9]

K. Kid's Recipes: This quarantine is a great time to teach your little chefs some simple dishes that they can make for themselves. Your child could be next master chef who knows?

If you want to get creative and need a little help, there are plenty of online sources that offer cooking classes and if you're confident in your skills, start your own online account and help others learn as well.

L. Learn a new language: Knowing a secondary or tertiary language can be really beneficial. It will open up new horizons for your kids. With the online world being just a touch away, learning a new language has never been this easy.There are plenty of apps online that teach new languages and are designed in the form of a game so that your children can have fun while learning.

M. Meditation: *Dr. Bryan Bruno, Founder and Medical Director at Mid City TMS explain, "*Meditation is one of the healthiest activities that a parent can do with their child. Meditation affects different parts of the brain, including the parietal lobe. Activity in the parietal lobe slows down during meditation, and children are able to process information more clearly."

Andrea Gurney, Licensed Clinical Psychologist, states "Kids who practice meditation and mindfulness reap a host of benefits, including but not limited to increased focus, self-control, coping strategies, emotional regulation, and better relationships."

These Changes Include:

- Kids feel calmer and can control their emotions better
- Kids have an increased emotional capacity to deal with stressful events
- Behaviour is improved
- Lower levels of depression and anxiety
- Improved memory

N. News Reading: There is a separate section for the kids at one page of newspaper where Sudoku, puzzles,riddles, tongue twister etc are published. These type of mind games helps in improving the vocabulary skills of children. Newspaper reading enhances the vocabulary of students. It is important for students, as early as Kindergarten, to start learning to read and gather information from newspaper. They help students develop daily reading habits that will last their entire lives. Studies have shown that students who learn using newspapers become more active citizens as adults.[10]

O. Online Activity Classes enrollment: Take time and try to understand the interest and hobbies of your kids. Accordingly, you can enroll them in different activity e-classes such as General Knowledge, Art and Craft, music classes, etc. Give them a specific fun task to do to base on what they have learnt in the activity class. This will help to channelize their energy while doing something creative and productive. You can also subscribe to an audio app for children like Audible, where children stories are shared in a fun and engaging way. They can even dance to their favourite songs or take up physical activity e-classes to keep their bodies active. Help your child pick a hobby in which he/she is genuinely interested.

P. Picture Stories: Orbis Pictus from 1658 by John Amos Comenius was the earliest illustrated book specifically for children. It is something of a children's encyclopedia and is illustrated by woodcuts. A Little Pretty Pocket-Book from 1744 by John Newbery was the earliest illustrated

storybook marketed as pleasure reading in English. For young readers, picture books are an important part of learning how to read. The rhythm and rhyme of picture books makes them easy to understand and fun to read aloud, allowing children to learn words quickly.[11]

Q. Quilling: Quilling is the art of rolled, shaped, and glued paper that results in creating a unified, decorative design. The name quilling is thought to come from the origin of the art; birds' feathers, or quills, were used to coil the strips of paper around. Quilling is also a great craft for kids as it teaches them fine motor skills and is a great craft to train their hand-eye coordination.[12]

R. Riddles: Young minds need to be fostered with something that gives them an opportunity to think over. Children are the buds in which we can instill our hopes for a great future. They are the ones that will make the world a better place to live in. Riddles for kids are the best form of solutions that helps to enhance their vocabulary. When you are sharing and solving riddles with children, it gives them an opportunity to bond with you and stay connected. Also, it helps them to break out of their comfort zone and mingle with others.

Riddles can help children form associations, link ideas and think, be creative and make conclusions; all of which are good ways of learning and remembering things quickly and with ease.[13]

S. Story Telling: Storytelling is used as a tool to teach children the importance of respect through the practice of listening. As well as connecting children with their environment, through the theme of the stories, and give them more autonomy by using repetitive statements, which improve their learning to learn competence. Vocabulary, comprehension, sequencing, memory and creative writing are all benefits from storytelling.[14,15]

T. Treasure Hunt: Hide a certain treasure in the house something that your kid will enjoy upon unearthing and lay out some clues to the hidden treasure. It would not only help you and your kid to pass the time during this period of quarantine but will also give you an opportunity to spend some quality moments together. Needless to say, the whole process of searching for clues will sharpen the mental faculties of the kids and help them grow.

Treasure Hunt play is essential to the healthy development of children; play helps them discover and explore, solve problems, test new ideas and experience friendship, cooperation and teamwork.[16]

U. Understanding of the situation: It is very important to explain the current situation to the children. They should understand the need of the lockdown. Parents should explain them what all precautions has to be taken by each of us during this situation. Try to solve the quarries arises in their mind.

V. Video call with relatives: Video conferencing helps to improve communication and re-establish relationships. During a video conference you get to see the body language and facial expressions of the participants. This leads to a more effective and faster collaboration. Modern technology provides new options to stay involved with your family. Video calling is now one of the most effective ways of staying in touch, and making these calls isn't a task that should be overlooked. In fact, it is absolutely vital that you find a way to stay connected.[17]

W. Writing the experience/letter: Online messengers and chat rooms may be the popular mode of communication these days, but nothing beats the charm of a letter. Besides serving as excellent memory archives and having great sentimental value, letters are great for teaching the art of penmanship to your young ones. Letters force the writer to be concise and clear and therefore, improve the thought process and help in developing clear thinking skills. Tell your kids to write their experiences or give them any theme to write.

X. X-mas tree making and decoration: Santaclaus is a favorite character of kids .Ask them to prepare the Christmas tree and decorate it. They would love it. Provide them all necessary things for decoration sometimes many things are available at home only.

Y. Yoga: One of the easiest and healthiest things you can do with your kids is to make them practice Yoga daily. The age-old discipline is great for improving overall health and keeps the energy levels high a necessity during these times!

It is also a great opportunity to teach your children the importance of having good health. If they learn to follow the discipline young, they will stay healthy all their lives.

Z. **Zumba dance:** Zumba Jr. is a great way for kids to have a good time while learning to exercise for an hour. They get a great workout in, meet some fun new friends and learn some hot dance moves while listening to various cultural music artists. Zumba is really fun way to spend quality time together as per the The National Association for Sport and Physical Education. There are so many benefits of zumba for kids.

- Improved Endurance
- Better Body Coordination, Gross Motor Skills & Control Over Their Body
- Teaches kids to incorporate fitness as a natural part of their lives
- Promotes an overall healthier lifestyle
- Develop teamwork respect confidence and cultural awareness

Parents can encourage creativity of children by

Teach them life skills: The most important thing is that now is a really good time to teach children things you want to be done. Not all lessons need to be academic – they can be life lessons we want our children to learn as well. Even how they should fold their clothes, mop up or vacuum. Take an hour out and show them how you want them to do it. Usually, life is very fast, and we have no time for this type of teaching. But they are invaluable skills.

Enjoy family time: In the evening, there should be family time. So everyone's devices go away, every household with children can now guarantee there is going to be family time.

Try and limit screen time: Research has linked screen time with an increased amount of sedentary behavior in children and teens. It is your job to encourage healthy behaviors and limit unhealthy ones sometimes this means making unpopular decisions like limiting your children's screen time. Let's get them reading books, drawing on toilet rolls, cooking, in the garden building things because they can't be the generation that's in front of screens learning, otherwise it will have a huge effect on them.

Proper and regular communication with kids can help them in understanding what is expected out of them during your work hours.

Encourage them to play independently: Train your children to be independent so that they are not always looking for you or someone else to accompany them at all times. This will make them confident and will also allow you to focus better on work. Focus on quality rather than quantity when it comes to spending time with our child. With clear communication and understanding, plan his/her activities for the day or the week.

Share the responsibility: We need to jointly plan and give them that sense of responsibility. It's quite powerful for them to take control of what they'd like to do. And if that's being in their room drawing, then give them that time, make them exhausted from doing that, because they will get bored very soon. And when they want to do other things, that are where, as a parent, you've already got all these resources and ideas ready to go.

Be Patient and Co-operative: There are going to be interesting, diverse scenarios taking place in homes at the moment. Don't get exhausted but calm and quite. Just remember that your children are transitioning as well. We need to use lots of conciliation and give and take. Every parent is concerned because we are taking up a new role here, which we have never had to do before. The positive thing is, we are going to get to know our children more[8,9.]

Quarantine doesn't have to mean boredom. There are plenty of activities that you can organize for your children which will not only keep them from getting bored but will also help them grow.

Stay positive, productive and proactive!

Ethical Clearance: Taken from institutional ethics committee.

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Yoga Helps to Improve Immunity & Fight Against Infection During Covid19

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Abstract

Corona is a pandemic disease but other than the corona also so many viruses and organisms effect on the respiratory system and harm on immune system of the body. Yoga plays a important role in the life. Yoga, perhaps, is one amongst the foremost effective and tried resistance boosters that we will adopt for a healthier life. Yoga could be a holistic follow that strengthens our body physically similarly as mentally. As a result, the body's defense conjointly improves in such a condition. A healthy life may be simply achieved if we have a tendency to learn to adopt a healthy modus vivendi, healthy food decisions and regular follow of yoga and meditation. Ayurveda, yoga, and meditation square measure the perfect factors to measure a healthy life. Surya Namaskar or Sun Salutation could be a sequence of twelve powerful yoga poses. Besides being an excellent vas physical exercise, Surya namaskar includes all 12 asanas and it will help to the growth of the physical body as well as mental peace. Surya Namaskar is additionally legendary to possess an associate vastly positive impact on the body and mind.

Keywords: *Corona, Yoga, Asanas, Pranayama, Surya Namaskar.*

Introduction

Corona is a Pandemic disease but other than corona also so many viruses that effects on the respiratory or the immune system of the body. A virus infects the body by getting into healthy cells. There, the encroacher makes copies of itself and multiplies throughout your body. The new coronavirus latches its high-pitched surface proteins to receptors on healthy cells, particularly those in your lungs. Specifically, the infective agent proteins bust into cells through ACE2 receptors. Once within, the coronavirus hijacks healthy cells and takes command. Eventually, it kills a number of healthy cells[1].

Coronaviruses square measure an oversized family of viruses that square measure common in folks and lots of totally different species of animals as well as camels, cattle, cats, and bats. Rarely, animal coronaviruses will infect folks and so unfold between folks like with MERS-COV, SARS-COV, and currently with this new virus (named SARS-COV-2).

The globe Health Organization declared an official name for this malady that's inflicting the 2019 novel coronavirus eruption, 1st known in the city, China. The new name of this malady is coronavirus malady 2019, abbreviated as COVID-19. In COVID-19, "CO" stands for "Corona", "VI" stands for "Viruses" and "D" for "Disease".There square measure many sorts of human coronaviruses as well as some that unremarkably cause delicate higher tract malady, like coronaviruses 229 E, NL 63, OC forty-three or HKU1 however SARS-COV-2 could be a new strain of coronaviruses that haven't been antecedently known in humans[2,3.]

The Clinical spectrum of COVID-19 ranges from delicate malady with non-specific signs and symptoms of acute respiratory disease to severe respiratory disorder with metabolic process failure and septic shock. There have conjointly been reports of symptomless infection with COVID-19. Current estimates of the time period vary from one to fourteen days, in step with the WHO and therefore the America Centers for malady

management and precautions. The median time period has been calculable to be five days. Transmission is also potential throughout the time period[4].

Virus Effects on Respiratory System: The virus moves down your tract. That's the airway that features your mouth, nose, throat, and lungs. Your lower airways have additional ACE2 receptors than the remainder of your tract therefore COVID-19 is additional doubtless to travel deeper than viruses just like the cold lungs may become inflamed, creating it robust for you to breathe this will result in respiratory disorder, associate infection of the small air sacs (called alveoli) within your lungs wherever your blood exchanges chemical element, and carbonic acid gas[5].

Yoga: Yoga plays an essential role in our life, word yoga was derived from the Sanskritic language word Yuj which suggests 'to join' or 'to unite'. This union isn't, merely, concerning your nose touching your knees as you bend to the touch your toes! The union spoken is that of your mind together with your body. You integration together with your surroundings and nature. And, finally, your individual consciousness with the universal consciousness.

The earliest recorded mention of the word 'yoga' is within the ancient Indian text, the Rig sacred writing - this body of data dates back to around 1500 BC! within the Atharva sacred writing, once more (dating to 1200-1000 BC), there's a mention of the importance of the management of breath. it's tough to pinpoint precise dates as a result of within the starting, the Vedas were, only, orally passed on from one generation to a different. Written records came to a lot of later.

Lord Mahavira and Lord Buddha's teachings shaped the first basis for Yoga Sadhana. whereas Lord Mahavira spoke of achieving salvation and freedom through meditation, Lord Buddha spoke of specific postures and meditation to realize enlightenment.

The most powerful Granth Bhagavad Gita conjointly came into existence during this amount. This text could be a dialogue between Lord avatar (universal consciousness) and aristocrat mythical being (human consciousness). Here, the Lord explains the ideas of Hindu deity, fate yoga (generous actions), devotion yoga (dedicated and caring actions), and Jnana yoga (knowledge)[6,7].

Maharshi Patanjali, thought-about the daddy of Yoga was the primary one to order the practices of yoga in, what's believed to be, the second century B.C.. Through his Yoga Sutras, he disseminated the means of yoga, and therefore the data it's to supply. This yoga was referred to as Raja yoga. He developed the Astanga yoga or the eight limbs of yoga, including yamas, niyamas, asanas, pranayama, pratyahara, Dharana, dhyana, and samadhi.

The idea of victimization yoga for treatment of diseases, fitness and to realize freedom from stress could be a fashionable, and rather, superficial perspective. In the past, yoga wasn't simply a method to get rid of diseases as an example, the stress on hygiene and cleanliness was a gift in the past similarly. But, this failed to talk over with solely brushing, laundry, and general morning ablutions. It enclosed keeping your mind, and by extension, your thought method conjointly, pure and clean. In different words, it junction rectifier to all-around well-being[8].

Yoga–The Resistance Booster: As the season's amendment, the possibilities of falling sick rise sharply. It's tough for our body to stay the pathogens unfree once the immunity levels square measure down and folks around America square measure sneeze and coughing. tho' we have a tendency to cannot shun from being connected with people who square measure sick, we will definitely work our thanks to strengthening our defense.

Antibiotics and different medicines do facilitate the body pass through the malady however they don't facilitate improve the body's immunity. Yoga, perhaps, is one amongst the foremost effective and tried resistance boosters that we will adopt for a healthier life it's an associate ancient art that strengthens the body and conjointly relaxes the mind. Let's have a glance at a number of easy yoga poses which will assist you to place up a robust resistance consequent time influenza season comes around:

Yoga could be a holistic follow that strengthens our body physically similarly as mentally. As a result, the body's defense conjointly improves in such a condition. A healthy life may be simply achieved if we have a tendency to learn to adopt a healthy modus vivendi, healthy food decisions and regular follow of yoga and meditation. Ayurveda, yoga, and meditation square measure the perfect factors to measure a healthy life[9].

Yoga isn't an alternative to medicines however rather a preventative live to stay diseases unfree. So,

let's not wait for this point for influenza to return knock at our door however rather beat it with ease.

Surya Namaskar: Surya Namaskar or Sun Salutation could be a sequence of twelve powerful yoga poses. Besides being an excellent vas physical exercise, Surya namaskar includes all 12 asanas and it will help to the growth of the physical body as well as mental peace. Surya Namaskar is additionally legendary to possess an associate vastly positive impact on the body and mind.

Surya Namaskar is best done early morning on an associate empty abdomen every spherical of Sun Salutation consists of 2 sets, and every set consists of twelve yoga poses you may realize many versions on the way to follow Sun Salutation. However, it's well to stay to 1 explicit version and follow it often for best results. Besides healthiness, Surya Namaskar conjointly provides a chance to precise feeling to the sun for sustaining life on this planet.

12 Steps of Surya Namaskar (Sun Salutation): Surya Namaskar (Sun Salutation) consists of twelve totally different postures. These 12 asanas are more beneficial for to improve the immunity and protect from infection and helpful for the physical growth.

Daily atleast 20 sryanamskar for beginner and more than 100 also performed by trained person.

1. **Pranamasana (Prayer Pose):** Pranamasana is that the 1st posture within the yoga sequence. To accomplish this create, stand upright on your mat and make sure that your feet square measure placed getting ready to one another. Next, take a deep breath, expand your chest, and relax your shoulder throughout inhalation, raise your arms from the facet and whereas eupnoeic be a part of each your palms along as if you're praying. The prayer posture or 1st salutation is complete.
2. **Hasta Uttanasana (Raised Arms Pose):** Ensure that your palms square measure joined along, a bit like within the previous prayer creates. Take a deep breath, elevate your arms, and slightly bent backward. Your striated muscle should keep getting ready for your ears.
3. **Hasta Padasana (Standing Forward Bend Pose):** Breathe out and bend forward from your waist. attempt to bit the ground together with your hands. However, make sure that your spine remains straight. whereas performing arts this creates, you must exhale slowly and completely.
4. **Ashwa Sanchalanasana (Lunge Pose):** Bend your knees slightly, in order that the palms will rest on the ground beside your feet. Take a deep breath, bring your right knee towards the proper facet of your chest, and stretch your left leg backward. Raise your head and appearance forward.
5. **Chaturanga Dandasana (Plank Pose):** Inhale and produce your right leg back similarly currently each your hands are right below your shoulders make sure that your body is parallel to the bottom.
6. **Ashtanga Namaskara (Eight boughed Pose):** This is conjointly spoken as salutations victimization eight points or elements. once performing arts Chaturanga Dandasana, exhale and slowly bring your knees down toward the ground. Rest your chin on the ground and keep your hips suspended within the air. If done properly, each of your hands, knees, chin, and chest can rest on the bottom whereas your hips can stay suspended within the air.
7. **Bhujangasana (Cobra Pose):** Rest your legs and midriff flat on the bottom. Position your palms beside your chest. Inhale and apply pressure on the hands to lift your higher body. At this time, your head and body part can gibe an elapid with a raised hood.
8. **Adho Mukha Svanasana (Downward Facing Dog Pose):** Keep your palms and feet wherever they're. Exhale and gently elevate your hips, in order that the body forms associate inverted 'V'. Straighten your elbows and knees. Look toward your navel.
9. **Ashwa Sanchalanasana (High Lunge Pose):** After performing arts Adho Mukha Svanasana, return to Ashwa Sanchalanasana by conveyance your right foot forward. Keep your left leg stretched behind you and appearance forward.
10. **Hasta Padasana (Standing Forward Bend):** Inhale and produce your left foot forward, specified it's next to your right foot. Keeping the position of your hands intact, exhale, and slowly bend your body part to enter into the Hasta Padasana create.
11. **Hasta Uttanasana (Raised Arms Pose):** Inhale and elevate your higher body. be a part of the palms and lift your arms on top of your head. Then bend backward as you probably did in step a pair of.
12. **Pranamasana (Prayer Pose):** This is the last step.

take a breath and stand straight during a relaxed manner. Lower the arms and hold your palms before your chest. This marks the top of the primary set of Surya Namaskar.

Benefits of Suryanamaskar: The benefits of Surya Namaskar square measure manifold. It helps in the higher functioning of the body and mental colleges. Here square measure a number of edges of Surya Namaskar:

- The postures square measure the right mix of warm-ups and asanas
- It helps to stay you disease-free and healthy
- Regular follow promotes balance within the body
- Improves blood circulation
- Strengthens the center
- Tones the gastrointestinal tract
- Stimulates abdominal muscles, Respiratory, vascular system, spinal nerves and different internal organs
- Tones the spine, neck, shoulder, arms, hands, wrist, back and leg muscles, thereby promoting overall flexibility
- Psychologically, it regulates the link of body, breath, and mind, so creating you calmer and boosting the energy levels with a sharpened awareness

Surya Namaskar is additionally vastly helpful for losing weight, tending, and hair care.

Benefits of Surya Namaskar for weight loss:

- It is an associate intensive workout that works on each a part of the body
- You will increase the number of rounds slowly and watch the pounds begin to disappear. The bends and stretches assist you to lose adipose tissue equally

Benefits of Surya Namaskar for hair:

- Surya Namaskar improves blood circulation to the scalp, thereby preventing hair loss
- The hyperbolic blood circulation nourishes the top and permits healthy hair growth
- Different poses facilitate stop graying of hairBenefits of Surya Namaskar for glowing skin
- Surya Namaskar improves blood circulation to any or all elements of the body, so keeping the skin young
- It will increase energy and vitality, thereby creating your face glow with radiance. This helps the skin retain its firmness
- Surya Namaskar prevents the onset of wrinkles by relieving the body and mind of stress

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Supplementation of Vitamin D in Diabetic Patients

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Abstract

Background: Nearly 30 percent to 50 percent of people are estimated to have deficiency of vitamin D, and insufficiency and vitamin D deficiency are recognized as global health issues in the world. Although the presence of hypo-vitamin D raises the risk of rickets and fractures, higher levels of vitamin D are also associated with hypertension (HTN), cardiovascular disease (CVD) and cancer. In addition, the vitamin D levels are also associated with diabetes mellitus (DM) and chronic kidney disease (CKD). Deficiency of vitamin D was linked to DM induction and progression. While the relationship between vitamin D and insulin secretion, insulin tolerance, and β-cell dysfunction is highlighted in patients with DM, evidence of vitamin D and DM levels is inconsistent, and well-controlled studies are required.

Aim: Supplementation of vitamin D in diabetic patients.

Material and Method: The study included 50 patients were given vita D supplement and 50 subjects were not given vita D supplement in diabetic people as the control group. Directly Observed Treatment in the Department of Clinical Biochemistry, Datta Meghe Medical College, SMHRC, Nagpur in collaboration with JNMC, ABVRH (Datta Meghe Institute of Medical Sciences Deemed To Be University), Sawangi, Wardha, Maharashtra.

Conclusion: This study evaluated that vitamin D supplementation can improve HbA1c, insulin resistance, and insulin in short-term intervention in T1DM & T2DM patients, suggesting that vitamin D can be considered as a therapeutic agent along with the other T1DM & T2DM treatments. The findings show that in non-additional diabetic patients, vit D concentration was substantially lower than the additional diabetic individuals.

Keywords: *Vitamin D,HbA1c,CKD, T1DM & T2DM.*

Introduction

Diabetes mellitus [DM] is one of the most common disease around the world, and their occurrence continues to increase[1]. Type 2 diabetes is portrayed by weakened pancreatic b-cell function, insulin resistance, and systemic inflammation [2] and there is proof that vitamin D regulates these mechanisms.[3] Vitamin D may directly affect β-cell function mediated by binding of the circling dynamic structure, 1,25-dihydroxyvitamin D [1,25(OH)2D], to the vitamin D receptor, which is expressed in pancreatic β-cells [4,5].

Low vitamin D levels have been recognized globally as a major health issue. Current literature provides evidence that hypovitaminosis D is associated with different health conditions, including bone health,

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cardiovascular disease, hypertension, cancer risk and diabetes mellitus. In particular, higher prevalence of low serum vitamin D [25-(OH)D] levels was observed in patients with type 2 diabetes whereas higher levels of vitamin D [25-(OH)D] were associated with lower risk of type 2 diabetes (T2DM)[6,7].

The International Diabetes Federation reports that there are about 285 million people with diabetes worldwide, or 7 per cent of the world's population. This figure is projected to reach 435 million by 2030.[7] An estimated 79 million people in the United States have pre-diabetes[7,8].

The following are recommendations for vitamin D in the DRIs developed by the Food and Nutrition Board at the Institute of Medicine. DRI is the general term for a set of reference values used to plan and assess the nutrient intake of healthy people.

Table 1: Current DRI Recommendations for Vitamin D[9]

DRIs for vitamin D	
Infants	
0–6 months	AI: 400 IU [10 µg/day]
7–12 months	AI: 400 IU [5 µg/day]
Children	
12-36 Months	600 IU [15 µg/day]
48 to 96 Months	600 IU [15 µg/day]
Older Children and Adults	
9 to 70 years	600 IU [15 µg/day]
Adults > 70 years	800 IU [20 µg/day]
Pregnancy and lactation:	600 IU [15 µg/day]

Material and Method

50 diabetic patients who referred to the clinics of Department of Clinical Biochemistry, Datta Meghe Medical College, SMHRC, Nagpur in collaboration with Jawaharlal Nehru Medical College, AVBR Hospital Sawangi, Wardha were selected as case group and 50 healthy subjects [as control group] were evaluated. Inclusion criteria were patients whose diabetes was confirmed and were between 30-60 years old and agreed to participate in this study. Exclusion criteria were: use any drugs consisting of vitamin D, any disorder except diabetes such as osteomalacia, osteoporosis, inflammatory rheumatism and patients treated with corticosteroids, using drugs which interfere with vitamin D metabolisms such as carbamazepine, phenobarbital, sodium valporate, gabapentin, isoniazid, mineral oil and calcitonin. The control group was selected as the normal population subjects.

According to season changes in Vitamin D level, all samples were collected in one season. Vitamin D level was measured with quantitative ELISA. Vitamin D deficiency was defined as serum 25(OH) D concentration of less than 20ng/ml, insufficiency as 20ng/ml <25(OH) D<30 ng/ml and sufficiency was defined as 25(OH) D higher than 30 ng/ml. The data were collected and analyzed. To determine the differences in vitamin D levels, in both groups we used t-test. A p-value less than 0.05 were considered significant.

Result

25 males and 25 females were enrolled in each group. The mean concentration of vit D in diabetic patients was 15.7±9.5 ng/dl and in the non supplementary diabetic patients was 12.2±6.5 ng/dl. The mean concentration of vit D in diabetic male patients was 10.04±6.4 and in non supplementary diabetic male patients was 8.23±2.6 ng/dl (p=0.219). The mean concentration of vit D in women with diabetes was 11.3±7.3 and in non supplementary diabetic women was 9.03±2.28 ng/dl.

Table 2: Vit-D levels in the case and in the control group

	Supplementary Diabetic Patients [study group]	Non Supplementary Diabetic Patients [control group]
Male	10.04±6.4 ng/dl	8.23±2.6 ng/dl
Female	11.3±7.3 ng/dl	9.03±2.28 ng/dl
Total	**15.7±9.5 ng/dl**	**12.2±6.5 ng/dl**

In the non supplementary diabetic group [control group], 34 patients had a deficiency of Vit-D, 10 patients with insufficiency of vit D and 6 patients had sufficiency of vit D.

Discussion

The results of this study showed that there was a statistical difference between vit D concentration in supplementary diabetic patients and the non supplementary subjects. Insufficiency of vit D in two groups also had higher prevalence[7,10,11].Need et al. showed that the patients who had higher levels of vit D concentration had lower FBS in comparison with

the other groups. These results were similar to our findings[11].

Other study that was performed by Daga et al. in the North of India demonstrated that 91.1% of diabetic patients had vit D insufficiency. In their study vit-D concentration in diabetic patients was 7.88±1.2, however, in non-diabetic individuals, it was 16.64±7.83. Different diets in two groups were considered this difference. [12]

Gagnon et al. found that the mean serum concentration of vit D in diabetic patients was lower than the non-diabetic individuals[12,13]. These results were almost similar to the findings of our study.

The weakness of this study is that we did this study only in one season and because of sunshine duration difference in each season level of vitamin D may undulate during the year, so this study cannot predict the condition of hypovitaminosis in this area.

Conclusion

Our findings demonstrated that vit D concentration in non supplementary diabetic patients was significantly lower in comparison to supplementary diabetic individuals. According to the high prevalence of vit D deficiency in this group, treating with vit D supplements maybe useful and seems to be necessary.

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Source of Funding: Self.

Conflict of Interest: Nil.

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Rasaushadhi (Herbomineral Formulations) Induced Nephrotoxicity in Children: Safety Concern Case Series

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Abstract

Background: Use of Rasaushadhi (Herbomineral formulations) in children is sensible topic. In last decade, some articles reported in various newspapers about nephrotoxicity of Ayurvedicherbomineral medicines. These issues create negative impression regarding the use of Ayurvedicherbomineral medicines. Ayurveda itself a time tested life science and provide guidelines to stay healthy and preserve the health. Such articles have done considerable damage to the reputation of ayurveda but at same time, it has raised many questions which need to be addressed. To make system more scientific and evidence based study was conducted at Paediatric department of Government institute in 2017. Paediatric department in this institute has used Rasaushadhi in children several times and not noted any problem regarding nephrotoxicity till date.

Aim: Question: Does Rasaushadhi (Herbomineral formulations) cause Nephrotoxicity in children? To determine Rasaushadhi (Herbomineral formulations) is "safe" study was conducted.

Materials and Method: Study was conducted for recurrent and reactive respiratory illness in children and herbomineral five drugs regimen was given to children of age 4 to 14 years for 45 days at 15 days interval. To rule out Nephrotoxicity after the use of Ayurvedicherbomineral formulations kidney function test was done. Patients were clinically observed in 4 follow ups, each follow up was conducted every 15 days. Last (Fourth) telephonic follow up was taken after six months of completion of treatment course to observe any renal obstructive pathology.

Results: Total 13 patients of case series were observed normal kidney function test reports. Last (Fourth) telephonic follow up was also reported normal, no any renal complications reported.

Conclusion: This clinical case series found that use of Ayurvedicherbomineral medicines in paediatric patients is safe and there is no relation with nephrotoxicity or any obstructive renal pathology when used properly.

Keywords: *Nephrotoxicity, Rasaushadhi, Herbomineral medicines, Kidney injury, Ayurvedic drug's safety, Paediatric nephrotoxicity, Kidney Function Test.*

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Introduction

Ayurveda is not a belief or faith based system. Charakasamhita defines a theory as a conclusion only once it has been established after several investigations in several ways and forms logic.[1] Rasashastra is a science of minerals aimed at intensively processing

and converting them into biomineral substances, to make them compatible for humans and to use them in an efficient and safe manner for medicinal purposes. Rasaushadhi's have 3 characteristics attributes-

1. Instant effective,
2. Requirement of very small dose,
3. Extensive therapeutic utility irrespective of constitutional variation.[2]

'Alpamatropayitwaat Arucera Apraangataha.

Kshipram Arogyadayitwat Ayushodyebhyo Adhiko Rasaha'.

(Vagbhata Rasaratnasamuchyaya.28/1, 1976a).

Rasashastra appeared as a big boon for humanity and a medicaments showed quick effectiveness even in very small doses.[3]But now a day there is big issue about the use of Rasaushadhi (Herbomineral medicine) in children regarding their safety and impurity. But there are lots of references in *Kashyapasamhita* about use of *Rasaushadihi* in children. *Acharya Kashyapa* has been mentioned herbomimeral preparations as internal use or external use in children e.g. *Lohitikagutika*in *akshiroga*a *anjana* (external application)[4], *Shilajatu* internal use in *raktagulma*. In Brihatrai and Laghutraisamhita of Ayurvedic classic has been mentioned Gold administration in newborn babies.

Nephrotoxicity is characterized as a rapid degradation in the function of the kidney due to toxic effects of medicines and chemicals.[5] Nephrotoxicity occurs when kidney-specific detoxification and excretion does not work properly due to damage or destruction of the function of the kidney by exogenous or endogenous substances. [6] In the past two years, this research study was aimed to make evidence based focus. The clinical case series of total 13 paediatric patients was conducted for 45 days exposure to rasaushadhi (Herbomineral medicines) and checked biomarkers of kidney functions to evaluate whether Rasaushadhi (Herbomineral medicines) cause any damage to kidney.

This study provides an evidence and built confidence in ayurvedic practitioners to use Rasaushadhi (Herbomineral medicines) without any hesitance.

Method

In this case series total 13 patients of 4 to 14 years age of recurrent and reactive airway disease were involved. All patients were diagnosed applying subjective and objective criteria. Patients involved in study received Rasaushadhi (Herbomineral medicines) for 45 days. Patients were clinically observed in 4 follow ups, each follow up was conducted every 15 days. Last (Fourth) telephonic follow up was taken after six months of completion of treatment course to observe any renal obstructive pathology.

Dose: As paediatric dosing system is different from adults. Appropriate dose of medicines given to the patients was calculated as per age according to Sharangdharsamhita[7] and the dose decided after consulting to experts. Five drugs regimen (Table 1) mixed well in powder form and given bid with honey after meal.

Anupana (vehicle): Honey

Consent: Well informed written consent was taken from parents.

Interventions:

Table 1: Rasaushadhi (Herbomineral medicines) {GMP certified pharma company}

Sr. No.	Medicines
1.	Abhrakabhasma
2.	Shwaskuthar rasa
3.	Laxmivilas rasa
4.	Shwasakaschintamani rasa
5.	Sitopaladichurna

Case Series:

Patient-1: 13 years male child; wt.-35.6kg, presented with the complaint of continuous dry cough; had history of Bronchial Asthma for 3yrs. Also had history of taking medications and nebulisation repeatedly and have had seasonal aggravation. Child was diagnosed Reactive Airway Disease and enrolled for study. Total 5 herbomineral Ayurvedic drug combination mentioned in table 1 was given for the period of 45 days with 15 days interval. Patient was clinically observed at follow up after every 15 days. After successfully completion of treatment course of 45 days, Kidney Function Test (KFT) was done at standard pathology lab. Last follow up was taken at 6 month of completion of treatment course to observe any renal complication or obstructive pathology.

Patient-2: 7years male child, wt. - 16.4 kg had complaint of rhinitis with h/o recurrent allergic rhinitis, specific food and seasonal aggravation, repeated medication and nebulisation for same illness. Child was enrolled for study after well informed consent. Herbomineral combination was given. After completion of treatment KFT was done. Last follow up was taken after 6 month on telephone.

Patient-3: 7 years female child; wt.-13.8kg, was presented with c/o breathlessness, cough and cold. Patient had h/o recurrent respiratory illness and allergic rhinitis and received treatment repeatedly for same illness, also had h/o bronchial asthma to grandparent. Patient was admitted in for Panchakarma (Vamana) procedure. Vamana karma was done on next day after that patient was enrolled for study. After successful treatment completion KFT was done, last f/u was taken on phone after 6 month.

Patient-4: 5years female child wt.- 13.1kg; had c/o cough and cold. Patient had h/o seasonal and specific food aggravation and h/o parental allergy. Patient had received medication repeatedly for recurrent respiratory infections (antibiotics and antihistamines). Patient was enrolled for study after well informed consent. 5 herbomineral drugs combination was given and f/u was conducted on time. after successful completion of treatment course LFT was performed at standard lab. Last f/u was taken on phone for any renal complication secondary to herbomineral drug consumption.

Patient-5: 7years female child wt.- 17.4kg had c/o cough and common cold. Patient had h/o allergic respiratory illness, seasonal aggravation and needed repeated medication for allergic respiratory illness. Patient was enrolled for study and given treatment for 45 days at 15 days interval. After course completion KFT was done. Last f/u was taken after 6 month.

Patient – 6: 5years female child wt.-14.6kg; had c/o cough and cold have had h/o specific food aggravation seasonal aggravation, allergy to parent (bronchial asthma to mother) child was diagnosed bronchial asthma by general practitioner. After well informed consent patient was enrolled for study and after completion of course KFT was done.

Patient –7: 13years male child wt. 27 kg; was diagnosed recurrent respiratory illness and enrolled for study after consent. Patient had completed treatment course after that KFT done at same institutional lab to check biomarkers.

Patient – 8: 11 years male child wt33.4kg had c/o dry cough and had h/o recurrent cough and allergic respiratory illness. Patient had h/o seasonal aggravation and parental asthma. Patient was enrolled for study and 5 herbomineral drugs combination was given for 45 days after 15 days interval. After course completion KFT was done. Last f/u was conducted after 6 month on phone for any urinary obstructive pathology.

Patient -9: 5years female child wt.-15.3 kg; had c/o cough with bouts. Patient had h/o recurrent respiratory illness and repeated medication and nebulisation for same illness. Patient was enrolled for study and 5 herbomineral drugs combination was given. After course completion KFT was done. Last f/u was taken after 6 month.

Patient -10: 4years female child wt.- 12.6kg; had c/o cough, cold, fever and nasal congestion. Patient had h/o recurrent respiratory infections and repeated medication, seasonal aggravation. After well informed consent from parent patient was enrolled for study. Total 5 herbomineral drugs combination was given to patient for 45 days at 15 days interval. After treatment course completion KFT was done at standard private pathology lab. Last f/u was taken on phone to observe any symptom of renal injury or any renal obstructive pathology.

Patient -11: 10years male child wt.-21.6kg; had c/o throat pain, cough and had h/o recurrent respiratory infections (thrice in a month).Also had h/o seasonal aggravation, parental allergy (father) and received repeated medication for same illness. On examination patient had diagnosed tonsil hypertrophy and adenoiditis. Patient was enrolled for study after informed consent. Patient had given 5 herbomineral drugs combination for 45 days. After successful completion of treatment course and on time f/u at 15 days interval KFT was done. Last f/u was done after 6 month.

Patient -12: 14 years male child wt.-50 kg; had c/o cough, common cold and fever. Patient had h/o parental allergy (Mother). Patient was enrolled for study after well informed written consent. 5 drug regimen of rasaushadhi was given to the patient for 45 days at 15 days interval. After successful completion of treatment course KFT was done. Last follow up was taken on phone after 6 months.

Patient -13: 4 year male child wt 13.5kg; had c/o

rhinitis. Patient had h/o recurrent respiratory illness and was taking antibiotics, anti allergic medicines for same illness. Patient had seasonal and specific food aggravation, parental allergy (Mother). Patient was diagnosed atopic rhinitis and enrolled for the study. 5 drug regimen of rasaushadhi was given to the patient for 45 days at 15 days interval. After successful completion of treatment course KFT was done. Last follow up was taken on phone after 6 months./

Results

Patients were observed in 4 follow ups, 3 follow ups from starting were taken at 15 days interval and at 3rd follow up KFT was done, last 4th follow up was taken on phone after 6 months to observe any obstructive pathology secondary to Ayurvedic medicines consumption. At last follow up on phone no any compliant related to renal injury was observed since at all.

Total 13 patients were involved in this case series. After completion of 45 days treatment course Kidney Function Test (KFT) was done and following value of biomarkers were observed.

Table -2 Kidney Function Test (KFT)

Sr.No.	Serum creatinine (mg/dl)	Blood Urea Nitrogen (mg/dl)	Blood urea(mg/dl)	Uric acid
Patient-1	0.9	11.29	24.17	-
Patient-2	0.46	-	40	5.01
Patient-3	0.78	-	21.16	-
Patient-4	0.9	7.94	17	-
Patient-5	0.7	9.81	21	-
Patient-6	0.68	-	18.50	-
Patient-7	0.8	14.49	31	-
Patient-8	0.8	12.15	26	-
Patient-9	0.45	-	26	2.83
Patient-10	0.7	10.75	23	-
Patient-11	0.8	8.64	18.5	-
Patient-12	1.22	-	40	2.59
Patient-13	0.8	9.81	21	-

Discussion

Use of metals and minerals in Ayurvedic drugs remains a most controversial issue for the followers of western medicine. These concerns not always true; mere presence of metal in a formulation will not develop adverse effects. Judicious administration considering all variants as explained in Ayurveda determines the benefit or otherwise. Pioneers of ayurveda have considered in detail about the possible ways by which untoward effects can occurred and provided all guidelines to avoid such occurrence.[8] Use of various Herbomineral medicines containing metals and minerals in therapeutics is an essential part of Ayurveda but safety and toxicity concern in the two decades creates debates in conventional community which attempted to damaging the glory of Ayurveda.[9,10]

Purpose of this study was to see the incidence of kidney injury due to Ayurvedic drug consumption. As Rasaushadhi (Herbomineral medicines) are used by Balroga department and by many of ayurvedic practitioners; and patient got relief from their illness as we expected. In spite of this adverse effect of Rasaushadhi (Herbomineral medicines) remains questionable. To answer such questions study was conducted. This study creates confidence to Ayurvedic practitioners to use herbomineral drugs in paediatric patients.

Conclusion

Proper use of Rasaushadhi (Herbomineral medicines) in paediatric patients is safe. It is clear from this case series use of herbomineral formulations that properly processed Ayurvedic medicines do not produce any toxic potential under judicious administration. Further to affirm the safety of rasaushadhi (herbomineral formulations) longitudinal study in large sample is required.

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Conflict of Interest: Nil.

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A Resistant Grave's Disease in Pregnancy: Case Report and Review of the Literature

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Abstract

Background: Thyroid hormones are crucial regulators of early development and play an important role in the maintenance of a normal pregnancy and in the development of the fetus, particularly the fetal brain. The effective management of Grave's Disease (GD) during pregnancy is challenging for the clinician. The most common causes of hyperthyroidism are autoimmune GD and multinodular goiter.

A 35 year old lady at 28 weeks of pregnancy, a case of Graves' disease on anti-thyroid drug with uncontrolled thyrotoxic symptoms despite regular medication didn't show clinical and biochemical normalization even with the maximum dose of antithyroid drug and antihypertensive drug permissible at pregnancy. At gestational week 28, T4 and T3 remained elevated with suppressed serum TSH and high levels of TSH receptor antibody levels. The patient had to be followed up medically with guarded prognosis. On follow up patient didn't show any clinical or biochemical remission. Despite high thyroid hormones levels and higher anti-TSH receptor antibody levels all throughout the gestation and with maximum dose of PTU the patient delivered a healthy baby with no clinical symptoms of thyrotoxicosis, goitre or any stigmata of PTU in the neonate. Mother's TFT was closed monitored and anti-thyroid medication was titred. We present a rare case of resistant GD in pregnancy, in different stages of pregnancy management encountered many challenges.

Keywords: *Graves' disease, GD, Pregnancy, Propylthiouracil (PTU), Carbimazole, PIH.*

Introduction

Hyperthyroidism in women who are of childbearing age is predominantly of autoimmune origin and caused by Graves' disease, affecting 0.2% of all pregnant women. During pregnancy due to physiological changes in the maternal immune system may influence the development other autoimmune diseases. Hyperthyroidism is defined by abnormally high levels of thyroid hormone caused by an increased synthesis and secretion of thyroid hormone from the thyroid gland.(1) 26.4% population of global adult affects due to hypertension, it was remaining the leading preventable risk factor for premature death and disability worldwide. In endocrine disorders including overt and subclinical hyperthyroidism and hypothyroidism Hypertension may be the initial clinical presentation. After thyroid dysfunction correction may normalize blood pressure (BP) in most cases, therefore checking thyroid function is essential during the workup for hypertension. Careful management and control of patients suffering from hyperthyroidism are important to prevent the possible complications related to the disease itself or to the treatment.(2,3)

Case Report: A 35-year-old pregnant woman Gravida 3 para1 live 1 abortion 1 came to the emergency department with complaints of absent foetal movements since 1 dayin her 28th weeks of pregnancy was admitted to Shalinitai Meghe Hospital and Research Centre, (DMIMS) Nagpur. The patient has never experienced bleeding or trauma before. A history of previous delivery

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underwent by LSCS was with a healthy male baby in view of foetal distress followed by after 2 year (2 month) spontaneous abortion. On admission she had raised blood pressure (BP-160/100 mm of Hg) with shortness of breath. She had no prior history of hypertension, diabetics, grave`s disease. Lab investigation showed normal hemogram with renal, liver, function test, blood glucose and serum electrolytes were normal Biochemical parameters. Thyroid profile was revealed biochemically hyperthyroid state, free T4 5.49 ng/dl (0.61-1.12), free T3 31.36 pmol/L (3.8-6) and TSH 0.002 µIU/ml (0.3-5.6). She had positive anti thyroid peroxidase antibodies (2107µ/ml; Normal ≤15 µ/ml) and negative TSH receptorantibody.

She had tachycardia (pulse132/min) and a regular, high volume pulse. However, there were no postural tremors, lid retraction, exophthalmos or other features of thyroid eye disease. Pulse pressure was wide (80mm Hg),blood pressure was raised at 180/100 mm Hg; there was significant bi-pedal oedema, patient was on tablet labetolol 200mg 4 hourly, tab. Nicardia20 mg 12 hourly, tab. Phenobarbiturate 60 mg at night, propylthiouracil (PTU) 150 mg 8 hourly. USG neck diffusely enlarged thyroid gland with increased vascularity. Sonographic evaluations of the fetus had been normal at time of intialpresentation (Fetal heart rate 154/mints, gestational age corresponded to chronological age and no anomalies). Cardiac status evaluated and was within in normal limits. Every four weeks patient continued to be thyrotoxic clinically, biochemically [free T4- 4.45 ng/dl, free T3- 28.3 pmol/L, TSH- 0.001 µIU/ml]. At 30 weeks gestation patient condition remained the same and fetus sonography was normal. At 34 week gestations we repeated TRab and the antibody titer continued to be high (TRab= 33 U/L).Normal fetus, placental unit and no advancement of bone age. During the follow up period patient had no symptoms or clinical signs of cardiac failure at any point of time despite being frank thyrotoxic. At 37 weeks gestation had underwent LSCS in view of raised BP and fetal distress, delivered female baby. No perinatal or post-natal complication was noted in baby and had normal anthropometric measurement at birth (birth weight 2.8 kg, length 50 cm and head circumference 34 cm) and no anomalies detected on clinical examination. There was no goitre, symptoms or signs of neonatal thyrotoxicosis. After 2 weeks of delivery the neonate though asymptomatic showed biochemical features of thyrotoxicosis [TSH: 0.021 mIU/ml (<20), FT4- 4.33 (1.0- 1.6ng/ml) and FT3 6.77 (2.6-4.4 pg/ml)]. The infant was monitored clinically and a repeat thyroid function test after 4 weeks showed normalization. PTU dose was titrated as required.

Discussion

The incidence of hyperthyroidism is 1 in 2000 pregnancies, with virtually all cases due to the autoimmune disease. Hyperthyroidism is hyperfunction of the thyroid gland characterized by a 15-20% increase in basal metabolism, sometimes accompanied by a mild enlargement of the thyroid gland.[4] About 90% of hyperthyroidism is caused by Grave's disease, both solitary and multiple toxic nodules and toxic adenomas. Generally Grave's disease is found that at young women that is between 20 to 40 years old and more in women than men with a ratio of 5: 1. Thyrotoxicosis is defined as excess of thyroid hormone", and this due to an increased synthesis of thyroid hormone in the thyroid gland (hyperthyroidism), but can be due to in the absence of hyperthyroidism. [5]

Pregnancy is a unique condition, in which the physiology of thyroid gland is mainly affected by 3changes, 1) due to increasedestrogen levels leads to increases levelof thyroxin binding globulin (TBG) from 12th weeks of pregnancy which reaches 2 times normallevels which will increase T4 and T3 levels in serum and change in thyroid size; 2) anincrease in the secretion of thyroid-stimulating factors(TSF) from the placenta especially human chorionicgonadotropin (HCG); and 3) pregnancy is accompanied byincrease in renal clearance of iodine and iodine loss through the photo-placental complex at the end of pregnancy leads to a decrease in iodine supply in the thyroid gland it will cause a relative iodine deficiency state. [6]

Clinical symptoms of Grave's disease are presence of tremors, non-infiltrative or infiltrative eye disorders, weight loss without knowing why, local myxedema, myopathy and onycholysis. All of these conditions never occur in a normal pregnancy. In pregnancy patients can experience hyperemesis gravidarum which can only be treated with anti-thyroid medications. An uncontrolled Graves' mother can have cardiac decompensation anytime. Infections, anemia, arrhthymias and PIH are known dreaded risk factors that can lead to cardiac failure.[7] In pregnancy, there will be immunosuppressant due to increased fetal suppressor T cell. These suppressor factors cross the placental barrier leads to supressed immune system of mother. Grave's disease often

becomes more severe in the first trimester of pregnancy, so that hyperthyroidism was diagnosed in first trimester of pregnancy. After delivery, these suppressor factors will disappear. This can explain why the exacerbation of hyperthyroidism occurs in the postpartumperiod.[8]

Antithyroid drugs that are used in the group of thioamides which work to inhibit thyroid hormonesynthesis through the blockade of the tyrosine molecule iodination process. Improvement can be seen in the first week and a new euthyroid state is reached after 4-6 weeks of treatment. The maximum permissible dose of antithyroid medication during pregnancy is 30 mg of methimazole or 40 mg of carbimazole or 600 mgof PTU. Propylthiouracil (PTU) and methimazole have been widely used in pregnant women with hyperthyroidism. In pregnancy PTU has many advantages as compared to methimazole, because PTU can inhibit the change of T4 to T3 besides inhibiting thyroid hormone synthesis and crossing the placenta less than methimazole because PTU has strong protein binds and is difficult to dissolve in water.[9] The baby was asymptomatic at birth and has elevated TSH receptor antibodies in cord blood, repeated TFT after 2 weeks of birth. At 2 weeks neonate had biochemical evidence of thyrotoxicosis with increased TSH receptor. After 2 weeks as the anti-thyroid drug clears from circulation and TSH receptor antibodies have a longer half -life (2 -3 weeks) and once the antibodies disappear the neonates achieve euthyroidism .[9,10]

Conclusion

Incidence of Grave's disease in pregnancy is not uncommon but resistant Grave's in pregnancy is very rare. Resistant Grave's disease is a difficult situation to manage and fatal complications are likely. Mother as well as neonates should frequently monitored as they are likely to have Grave's or hypothyroidism which is antibody mediated. There are difficult to manage this kind of patients due to no proper guidelines, endocrinologist find difficulty to maintain an optimal balance between maternal and fotal wellbeing.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Observational Study of Patients Presenting with Bleeding in First Trimester

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Abstract

Objective: First trimester is an important period during which there is risk of bleeding and may lead to spontaneous abortion. More than 80% spontaneous abortions occur in first trimester. The aim of this study was to determine the causes of first trimester bleeding.

Method: This was a cross sectional descriptive study conducted at Shalinitai Meghe hospital and Research centre Hingana, Nagpur during January 2020 to June 2020. The patients presenting to OPD with history of bleeding or spotting per vaginum, positive urine pregnancy test and less than 12 weeks of gestation were included in this study. Clinical examination and ultrasonography was done.

Result: Most of the patients presented with moderate bleeding and associated pain. The duration of bleeding was 2 to 5 days. Majority of cases were between 4-8 weeks (69.5%). History of prior miscarriage was present in 23.7%. 61% of cases were anemic. Threatened abortion was most common in patients presenting with bleeding per vaginum (in 47.5%). 18.6% patients were with incomplete abortion and 11.8% had complete abortion. There were 2 cases of unruptured ectopic pregnancy and 1 case of ruptured ectopic pregnancy. Salpingectomy was done in all the 3. Dilatation and evacuation was done in 12 cases. Gestational sac was present in 52 cases and the appearance was normal in 53.8% cases. Yolk sac was present in 65.4% cases. Fetal pole and cardiac activity was seen in 57.7%cases.

Conclusion: Bleeding in first trimester of pregnancy is the common complaint with which the pregnant women presents to the health care worker. The commonest cause is the threatened abortion. Other causes are incomplete, complete and missed abortions. Bleeding in early pregnancy may be early presentation of a serious situation like ectopic pregnancy. Ultrasonography, especially the Trans Vaginal is very important in concluding the causes of bleeding. Pregnant women should be educated in the first visit to antenatal OPD itself for the symptoms like bleeding per vaginum and to seek help from health care worker as soon as possible.

Keywords: *Vaginum, pregnant women, bleeding, first trimester.*

Introduction

First trimester is an important period during which there is risk of bleeding and may lead to spontaneous abortion. More than 80% spontaneous abortions occur in first trimester[1]. The causes for bleeding in first trimester can be spontaneous abortion, induced abortion, ectopic pregnancy, molar pregnancy and local factors. Terms that are used to describe bleeding during first trimester

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are threatened, inevitable, incomplete, complete and missed abortion[1].

The various possible causes of spontaneous miscarriages are abnormal conceptus, immunological factors, uterine abnormalities, endocrine factors, maternal diseases, infections[2]. The factors responsible for spontaneous abortion can be advancing maternal age, medical disorders, infections, medications, uncontrolled diabetes mellitus, thyroid disorders[3,4].

Pregnancies with first trimester bleeding and subchorionic hematoma were associated with similar risk of miscarriage and antepartum haemorrhage compared to patients with first trimester bleeding without subchorionic hematoma. Pregnancies with first trimester bleeding and subchorionic hematoma were associated with similar risk of miscarriage and antepartum haemorrhage although increased risk of preeclampsia, fetal growth limits, premature pregnancy, non-reassuring fetal heart rhythm, cäsarean delivery and low birth weight.[5-7]

Material and Method

This was a cross sectional descriptive study conducted at Datta Meghe Medical College, Shalinitai Meghe hospital and Research centre Hingana, Nagpur in collaboration with Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences, Sawangi (Meghe), Wardhaduring January 2020 to June 2020. The patients presenting to OPD with history of bleeding or spotting per vaginum, positive urine pregnancy test and less than 12 weeks of gestation were included in this study after prior informed consent. The pregnant women with history of intake of MTP pills were excluded from study. Clinical examination was done. Ultrasonography was done to note crown rump length, gestational sac, yolk sac, fetal pole and fetal cardiac activity.

Result

First trimester bleeding is a common problem during pregnancy and is a cause of major concern for the patient and family. The incidence of miscarriage is generally considered to be about 15% of all pregnancies[4]. Most of the patients presented with moderate bleeding and associated pain. The duration of bleeding was 2 to 5 days. This study included pregnant women from age 18 to 44 years. Maximum number was seen in the age group 26-30 years (59.4%)

Table 1: History of Prior Miscarriage

History of prior miscarriage	Number of cases (59)	Percentage
Present	14	23.7
Absent	45	76.3

In 23.7% women, history of previous spontaneous abortion was present. No such history was present in 76.3% cases.

Table 2: Gravida status of patients

Number of gravida	Number of cases (59)	Percentage
1	17	28.8
2	11	18.7
3	19	32.2
>/=4	12	20.3

Out of total cases, 28.8% cases were primigravida, 18.7% were second gravida, 52.5% cases were multigravida.

Table 3: Weeks since Last Menstrual period (LMP)

Weeks since LMP	Number of cases (59)	Percentage
<6	05	8.5
6-8	41	69.5
8-12	13	22

Majority of cases were between 6-8 weeks(69.5%). 8.5% cases were less than 6 weeks. 22% cases were between 8-12 weeks.

Table 4: Hemoglobin level

Hemoglobin level (gm/dl)	Number of cases (59)	Percentage
<7	08	13.5
7-11	28	47.5
>11	23	39

61% of cases were anemic. 13.5% cases were severely anemic and required blood transfusion.

Table 5: Clinical Diagnosis

Clinical Diagnosis	Number of cases (59)	Percentage
Threatened abortion	28	47.5
Incomplete abortion	11	18.6
Complete abortion	07	11.9
Missed abortion	10	16.9
Ectopic gestation	03	05.1

Threatened abortion was most common in patients presenting with bleeding per vaginum (in 47.5%). 18.6% patients were with incomplete abortion and 11.8% had complete abortion. There were 2 cases of unruptured ectopic pregnancy and 1 case of ruptured ectopic pregnancy. Salpingectomy was done in all the 3. Dilatation and evacuation was done in 12 cases.

Table 6: USG findings

Gestational sac	**Present**	**Absent**
	52	07
Gestational sac appearance (n=52)	**Normal**	**Abnormal**
	28(53.8%)	24(46.2%)
Yolk sac	**Present**	**Absent**
	34(65.4%)	18(34.6%)
Fetal pole	**Present**	**Absent**
	30(57.7%)	22(42.3%)
Cardiac activity	**Present**	**Absent**
	30(57.7%)	22(42.3%)

Gestational sac was present in 52 cases and not seen in 7 cases. The appearance of gestational sac was normal in 53.8% cases. Yolk sac was present in 65.4% cases. Fetal pole and cardiac activity was seen in 57.7%cases.

Discussion

Vaginal bleeding in early pregnancy requires prompt attention. A review of the menstrual history and prior ultrasonography can help establish gestational dating and determine whether the pregnancy location is known[8]. Bleeding equal to or heavier than a menstrual period and bleeding accompanied by pain are associated with an increased risk of early pregnancy loss[9].

A total of 59 of pregnant women who presented vaginal bleeding during the first trimester to OPD were enrolled into the study. The bleeding occurs at all gestational ages between the implantation of the embryo in the uterus and 12 weeks of gestation, mostly between 4-8 weeks in 69.5% in our study and 50.8% in that of Boco[10]. Threatened abortion (47.5%) was the commonest cause of first trimester vaginal bleeding. This similar trend was observed in other studies[11-13]. The clinical diagnosis of threatened abortion is presumed when bleeding occurs through closed cervical os before 20 weeks of gestation[1]. 18.6% women were diagnosed with incomplete abortions. This rate is comparable to that of Yang and al, who found a rate of 23.1%[12].There were 18.6% cases of stopped pregnancies (fetal demise). BelleyPriso and al found a rate of 20[13]. Bleeding from an ectopic pregnancy is the most dangerous cause of first trimester bleeding. It concerned 5.1% of the cases in our study. Belley and al found a rate of 5.2%[13]. Implantation of fertilized egg outside uterus is known as ectopic pregnancy. 11.9% were diagnosed as complete abortion[14]. This rate is similar to those of Coulibaly who found a rate of 9.1%[15]. Heavy bleeding occurred mainly in incomplete and complete abortions, while spotting and light bleeding occurred in threatened abortions, viable intra uterine pregnancy and missed abortion. Threatened abortions, incomplete abortion, missed abortion and ectopic pregnancy presented with bleeding, haemorrhage although increased risk of preeclampsia, fetal growth limits, premature pregnancy and lower abdominal pain[4,5,16]. The diagnosis was confirmed on ultrasound[17,18].

Conclusion

Bleeding in first trimester of pregnancy is the common complaint with which the pregnant women presents to the health care worker. The commonest cause is the threatened abortion. Other causes are incomplete, complete and missed abortions. Bleeding in early pregnancy may be early presentation of a serious situation like ectopic pregnancy. Ultrasonography, especially the Trans Vaginal is very important in concluding the causes of bleeding. Pregnant women should be educated in the first visit to antenatal OPD itself for the symptoms like bleeding per vaginum and to seek help from health care worker as soon as possible.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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The Effect of Eye-Hand Coordination Device on Subjects with Hemiparesis

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Abstract

"Coordination" the harmonious functioning of interrelated organs and parts, applied especially to the process of the motor apparatus of the brain which provides for the co-working of particular groups of muscles for the performance of definite adaptive useful response. In Current Clinical Practises there Less Devices Which can help in Gaining Eye-Hand Coordination. Objectives: To compare the effect of conventional training approach and eye-hand coordination along with conventional training. Materials and Method: The subjects in Datta Meghe College Of Physiotherapy were screened and 42 subjects were divided into 2 groups i.e Group A subjects were given Conventional Physiotherapy for coordination training and Group Eye-Hand Coordination Device along with given Conventional Physiotherapy for coordination training. The interpretation of the study was done on the basis of comparing pre-test and post-test assessment of NCT grading and FMA-UE.

Result: Intragroup comparison results showed that NCT grading and FMA-UE scale were statistically significant in both the groups ($p<0.0001$). Whereas the intergroup comparison results showed that Eye-Hand Coordination The device along with Conventional physiotherapy was statistically significant in improving NCT score ($p=0.0021$) and FMA-UE score ($p=0.0001$) than only Conventional Physiotherapy alone for Eye-hand In-coordination Conclusion: The Eye-Hand Coordination Device was Significantly effective when Given With Conventional Physiotherapy For treating In-coordination and improvement in coordination. Conventional Physiotherapy is also effective in treating in-coordination, but after a certain duration of treatment, the patient starts getting adapted and chances of achieving the plateau phase

Keywords: *Eye-Hand Coordination Device, Hemiparesis, NCT Grading, FMA-UE.*

Introduction

Coordinationis the even operating of interrelated vital organs and parts, applied especially to the process of the motor mechanism of the brain which provides for the co-working of particular groups of muscles for the performance of definite adaptive useful response. There are several types of coordination observed in humans namely intralimb, interlimb & visual-motor. Intralimb coordination refers to movements occurring within a single limb (eg: alternately flexing or extending the elbow). Inter-limb (bimanual coordination) refers to the integrated performance of two or more limbs working together (ex: between limb movements of the lower extremities and/or upper extremities during walking). Visual - motor coordination is the ability to integrate both visual and motor abilities with respect to the external environment in order to achieve a goal.[2]

Eye hand coordination which is a sub category of visual motor coordination hasits own merits with regards

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to activities of daily living. It is also named as eye-hand-head coordination because movement of the head is typically required for the eyes to fixate on a target or object[3,4].

Incoordination isobserved in patients following a vascular insult in brain. It is suggested that the lack of coordinated voluntary movement observed in hemiparetic patients may be due to impaired regulation of spinal neurons.

The structure getting involved in the incoordination pathology is the cerebellum it receives information from the sensory systems, the spinal- cord, and other parts of the brain and then regulates motor movements. voluntary movements like posture, balance, coordination and speech leading to smooth and balanced muscular activity are all impaired if there's involvement of this structure[1]. Direct damage while not causing paralysis or intellectual impairment, might lead to lack of balance, slower movements & tremors[5]. Complex physical tasks and fine motor functions including prehension would become unsteady and halting[6]. Coordination impairments associated with dorsal column medial leminiscal (dcml) lesions are somewhat less characteristic than those produced by either the cerebellum or the basal ganglia pathology.

Biofeedback a specialized form of feedback that provides information directly to a patient about internal biological mechanisms via a somewhat sophisticated electronic device.it provides a feedback to individuals about their internal physiological events in order to state whether they are normal or abnormal and in case of abnormal to correct them respectively[7]. Biofeedback may be in the form of auditory, visual, tactile, gustatory and olfactory signals. In this research biofeedback is used to inform patient about movement and whether his activity is taking place in a coordinated manner. The instrument used in biofeedback process here is non-invasive and can be easily operated. The main goal of biofeedback here is to improve motor performance along with coordination through auditory signals and motor learning. Motor learning leading to control over the motor activities requires signalling from the external world as well as his own proprioception. hence biofeedback works best as a substitute for proprioception. Here the patient must aim in fulfilling 3 things in a progressive manner. Initially the focus should be on adapting and understanding the relationship of the electronic signal to achieve a desired task. After accomplishing this stage the individual should progress to practice controlling the feedback signals and after achieving this the task should be performed for several times until he has gained full control and coordination over it.

The learning pyramid also known as the cone of learning, experience, learning cone, the cone of retention,the pyramid of learning, or the pyramid of retention is a group of popular learning models and representations relating different degrees of retention induced from various type of learning.this pyramid of learning is a layer wise presentation represented in percentages[8]. It originated in 1954 in a book called audio-visual method in teaching. It was developed by the national training laboratories institute in the early 1960s, on its main campus in bethel, main. This model generally displays the following representation mentioned as below: out of which our feedback method provide audio-visual method, demonstration method and practice method. Therefore, the individual retention rates for audio-visual method is 20%, for demonstration is 30% & for practice is 75%. In this all the three method are combined and used in the rehabilitation process of the patient making the treatment more effective and showing better results in the prognosis of the patient.

Materials and Method

Approval for the study was obtained from the institutional Ethical Committee of the University. The subjects in Datta Meghe College of Physiotherapy and shalinitai Meghe hospital were screened and those fulfilling the inclusion and exclusion criteria were involved. Participants were informed about the study and consent was taken. Pre-test assessment was taken by using Non-Equilibrium Coordination Test, Non-Equilibrium Coordination Test Grading and FMA-UE to assess the patient. 42 subjects were divided into 2 groups, Group A and Group B based on the inclusion and exclusion criteria. The treatment was started the first day when the subject came for consultation. Group A subjects were given Conventional Physiotherapy and Group B subjects were given Eye-Hand Coordination Device Along with conventional Physiotherapy. The post-test assessment was taken by using NCT Grading and FMA-UE to assess the patient. The interpretation of the study was done on the basis of comparing pre-test and post-test assessment of Non-Equilibrium Coordination Test Grading and FMA-UE

Group A: Conventional Physiotherapy For improving Coordination

- Frenkels Exercises for upper extremities
- Ball throwing to specific target.
- Ball Juggling
- Small ball tosing
- Target practise
- Wall ball bounce
- Aiming Practise

Group B: Eye-Hand Coordination Device - Patient should be in sitting position, patient will be asked to hold the hand piece which is of diameter 5cm, 4cm, 3cm, 2cm and 1cm accordingly.

- First the therapist will demonstrate the activity.
- The patient will be handed the hand piece and ask to move the hand piece from starting position to ending position and then again reach the starting point without much shaking of hand piece and with steadiness
- If the patient touches the wire then a buzzer will be on and light will blow and that will a biofeedback that means that the patient has to go to the starting point again for
- For starting we have asked the patient to perform the procedure on a wire which will be a straight line which is the basic level.
- The levels will be made difficult by making the wire into many different shapes.
- Once the patient gains confidence that he can perform the procedure easily he can go to the next level.

Statistical Analysis: Statistical analysis was done manually and by using the statistics software's INSTAT so as to verify the results derived. The statistical analysis of non-parametric data was done by Wilcoxon matched pairs test and Man-Whitney test. Wilcoxon matched pairs test was used for statistical analysis of pre and post intervention within the group. Man-Whitney test will be used for between groups statistical analysis of Group A and Group B (pre-pre and post-post intervention).

Table 1. Distribution According to Side Wise in Stroke

	Stroke			
	RT. Hemiparesis	**LT. Hemiparesis**	**TIA**	**Dropout**
Group A	8	13	0	
Group B	7	7	1	6
Total	**15**	**20**	**1**	**6**

Table 2: Data of NCT

	Non-Equilibrium Coordination Test Grading				p valve (Pre-Post)	Inference
	Pre-test		Post-test			
	Mean	**SD**	**Mean**	**SD**		
Group A	1.571	0.8701	2.619	0.7400	<0.0001	Extremely significant
Group B	1.533	0.5164	3.200	0.4140	<0.0001	Extremely significant

Table 3 : Data of FMA (Total Score)

	FMA-UE				p value (Pre-Post)	Inference
	Pre-test		Post-test			
	Mean	**SD**	**Mean**	**SD**		
Group A	33.000	4.764	43.095	5.665	<0.0001	Extremely significant
Group B	34.333	6.114	50.600	5.938	<0.0001	Extremely significant
p value (Pre-Pre) and (Post-Post)	0.5340		0.0001			
Inference	Not Significant		Extremely Significant			

Discussion

Eye-hand coordination is central to so many human activities–the tool used eating, sports and work, to name few as to be defining characteristics of typical human life, conversely, its disruption following the stroke, disease, injury, and developmental disorder leads to considerable degeneration in productivity and quality of life. Naturally eye-hand coordination involves the synergistic function of various systems including visual, balance system proprioception and the eye heads and arm control systems, plus aspects of awareness- like attention and, memory[9,10,11]. Eye hand coordination plays vital role in ADL activities Eg-Eating, Drinking, Doing Writing Work, Passing any object to the surrounding Cleaning, Typing in laptop or typing machine. In the patients with neurological impairment neurological impaired subjects during Rehabilitation usually, eye-hand coordination mostly gets untreated. The objectives of this study were to find the effect of eye-hand coordination device on coordination in subjects with hemiplegia. To find the effect of eye hand coordination device along with conventional physiotherapy in improving eye-hand coordination. To compare the effect of Eye-hand coordination device and conventional physiotherapy in improving eye-hand coordination[12,13]. The study was conducted with 42 subjects. Subjects were divided into two groups. Eye-Hand Coordination device along with Conventional physiotherapy (Group A) and Conventional Physiotherapy (Group B). Prior consent was taken. The treatment protocol was carried out for 6 days per week for 5 weeks. The outcome measures for this study were Non-equilibrium coordination test grading and Fugl-Meyer Assessment-Upper Extremity scoring system[14,15]. The results of this study showed that there was a significant improvement in Coordination, improving quality of life and reducing rehabilitation time after 5 weeks of intervention in both the group A and group B in Subjects with incoordination. Wilcoxon matched pairs test was used to analyzethe effect eye-hand coordination device and showed that there was extremely significant improving coordination in NCT Grading score (0.0097) and FMT-UE (p=0.0001). Researches have proved that there is improvement hand pointing accuracy. A Study Done by B. Biguer C. PrablancM. Jeannerod showed that the accuracy of pointing motion of the hand, directed at visual acuity 10° to 40° from the center, was measured in normal subjects. No visual feedback from the moving hand was available to the participants. The face could be either maintained fixed (head-fixed condition) or free to move (head-free condition) during the pointing movements. It was found that the flaw in marking was reduced for all targets in the head-free condition. This reduction was more important for the more eccentric spot. Enhancement in precision was observed without any remarkable change in either the latency or the duration of eye, head or hand movements[16]. Another Study done by Halil Ibrahim Ceylan and Ozcan Saygin conducted a study on Examining the Effects of Proprioceptive Training on Coincidence Anticipation Timing, Reaction Time and Hand-Eye Coordination The study stated that the effects of proprioceptive training on coinciding anticipation timing (CAT), reaction time and hand-eye coordination. 42 volunteer students participated in the paper. These students were randomly divided into two groups as control and experimental groups. An exercise program was applied to the experiment and control groups for approximately 3 days in 8 weeks, for about 45 to 60 minutes. Additionally, a 20-minute modified proprioceptive balance program was applied to the experimental group only. Paired sample tests were used. As a result, significant differences were found in the performance, reaction time and hand-eye coordination performances of the experiment group, pretest, and posttest ($p<0,05$). Significant differences were found in dominant hand visual reaction time and hand-eye coordination performances of the control group, pretest, and posttest ($p<0,05$). In conclusion, it is seen that proprioceptive training affect CAT performance and reaction time performances in a positive way[17,18].

Conclusion

The present study provided evidence to support the use of eye –hand coordination device along with conventional physiotherapy in improving NCT and FMA-UE scores in Subjects with Hemiparesis. In addition, results supported that among Eye-hand coordination device and conventional physiotherapy were significantly effective in improving eye-hand coordination and improving the quality of life in subjects having affected eye hand coordination in hemiparesis.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

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Evaluation of the Effect of Local Application of Kalpit Kasisadi Yogain the Managementof Ksharasutra Induced Hyper Granulation at External Opening Offistula-in-Ano: A Randomized Controlled Trial

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Abstract

Background: The *Ksharsutra* therapy is most fascinated para surgical process for fistula–in–ano. But during the procedure hyper granulations are typically seen near the external opening of fistula which may bleed or get infected if remain untreated. Moreover it may obliterate the pus drainage and thus complicate the wound. This consequence needs to be addressed. In *Sushrut Samhita*, raised soft tissues of wound is described as *Utsanna Mrudu Vrana Maansa* and specified to be treated by "*Avasadana Dravya*" with honey.

Aim: To evaluate the effect of LA of *Kalpit Kasisadi Yoga* with *Madhu*in management of hyper granulation, found at external opening of fistula-in-ano during its treatment with *Ksharsutra*.

Material and Method: 60 operated cases of fistula-in-ano with *Ksharsutra*, presenting with hyper granulation at the external opening of fistulous tract were randomly selected and divided into two groups.

Observation: The comparison between both the group indicates that effect of the therapy was very significant on thickness while insignificant result was obtained in area of hyper granulation. However the percentage of relief in both the criteria's in experimental group was above 90%.

Result: The LA of *Kalpit Kasisadi Yoga* in the management of *Ksharasutra* induced hyper granulation at external opening of fistula-in-ano is more effective than LA of *Madhu* alone.

Keywords: *Hyper granulation, Ksharsutra, Utsanna Mrudu Vrana Maansa, Avasadana Dravya.*

Introduction

Ayurveda classics at various places have emphasized on taking care of wounds which occur either as a result of vitiated *Doshas* or due to traumatic origin. In fact a detailed protocol to address various types of wound & their complications is illustrated in *Samhitas* for the management of wound. Being prime topic of the subject *Acharya Sushruta* has described 60 measures[1] in the management of *Vrana*in addition to its indication, contra indication and limitation while *Aacharya Charaka* has described 36measures.[2] These measures deal with each and every aspects of *Vrana* according to its type, condition and stages of healing.

In modern science wound healing has been

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described to be completed in 4 stages viz. inflammation, wound contraction, epithelialization and granulation tissue formation followed by scar formation which comprises various pathophysiological stages in itself. In normal circumstances wound heals through these stages, however hyper granulation tissue formation is an unusual event in wound healing.

Hyper granulation is said when granulation tissue grows above the level of wound edges. The presence of this tissue results in inhibition of fibroblast proliferation and prevents wound healing. The treatment consists of destruction of tissue by cautery, shave excision, aluminium chloride, copper sulphate or curettage. But the fact is that these hyper granulation tissue still remain there as a clinical problem.[3],[4]

Fistula-in-ano is itself a complicated wound, with muco-purulent discharge from its external opening. The incidence of a fistula–in–ano developing from an anal abscess ranges from 26% to 38%.[5]According to *Aacharya Sushruta,Vranathat* discharges pus, blood and mucous etc. are referred as *Kruchhraasadhya Vrana.*[6] That is why *Bhagandara*has been incorporated in *Ashtaumahagada* i.e. diseases with poor recovery rate.

Ksharsutra is a widely accepted line of treatment in fistula-in-ano which is designated as *Bhagandara* in ayurvedic texts. During the course of treatment it has been observed that many patients presented with hyper granulation tissue at the external opening of fistula- in-ano. This hyper granulation tissue delays the healing of fistulous tract.

In *Sushrut Samhita*, raised soft tissues of wound i.e. hyper granulation tissue is described as *Utsanna Mrudu Vrana Maansa* and advised to be treated by "*Avasadana Dravya*" with honey.[7]*Avasadana* denotes suppression of the soft tissue eruptions i.e. *Utsanna MruduMaansa*. '*Avasadaneitimaansasfotane'* - Dalhan

AvasadanaKarma is described by all the three acharyas *Sushrut* (Su.Chi. 1/83)*, Charak* (Ch. Chi. 25/39-43) and *Vagbhat*(A.H. Ut. 25/48) in management of *Vrana*. Various drugs are stated for *Avasadana Karma* in *Samhitas*[8] but on the basis of availability, following drugs were selected.

1) *Kasis* 2) *Saindhav* 3) *Kukkutand Twak Bhasma* 4) *Jatikalika* 5) *Karanj*

The above drugs were identified, obtained in purified form and were grinded to fine powder. Each drug of compound was mixed homogeneously in equal proportion. The prepared compound was stored in sterilized glass bottle. As the compound is not directly mentioned in the Ayurvedic classics and the first drug being *Kasis,*the drug of trial is designated as *Kalpit Kasisadi Yoga,* to be applied with *Madhu*. In Control Group only *Madhu*was used.

Hence the present research work is designed to assess the *Avasadana Karma* of the *Kalpit Kasisadi Yoga*in the management of *Ksharasutra* induced hyper granulation at external opening of fistula-in-ano in this trial.

Aims and Objectives:

- To study the effect of local application of *Kalpit Kasisadi Yoga* with *Madhu*in management of hyper granulation found at external opening of fistula-in-ano during its treatment with *Ksharsutra*.
- To compare the effect of local application of *Kalpit Kasisadi Yoga* and *Madhu* with local application of *Madhu*alonein management of hyper granulation found at external opening of fistula-in-ano during its treatment with *Ksharsutra*.

Material and Method

In this clinical trial, 60 operated cases of fistula-in-ano with Ksharsutra, presenting with hyper granulation at the external opening of fistulous tractwere randomly selected from OPD & IPD of Shalya Tantra Department of study center. The special research proforma was prepared for diagnosis and assessment of hypergranulation at external opening of fistula- in-ano.

Informed written consent was taken from all the patients included in this study. The patients were numbered from 1 to 60. The patients with odd numbers were selected in Group A. The patients with even numbers were selected in Group B.

Group A: Experimental Group–30 patients were treated with local application of *Kalpit Kasisadi Yoga* with *Madhu* OD for 10 days.

Group B: Control Group – 30 patients were treated with local application of only *Madhu* OD for 10 days.

Follow up of the patients was taken at 3rd, 7th and 10th day.

Criteria for Selection:

- Inclusion Criteria

1. Patient presenting with signs of hyper granulation at external opening of fistula-in-ano during the *Ksharsutra* therapy, irrespective of the length of the fistula tract.
2. Age of patients: 20 – 60 years.
3. Sex:- Both male and female of caste & religion.

- **Exclusion Criteria**

1. Patients suffering from uncontrolled diabetes, uncontrolled hypertension, tuberculosis, HIV, CA-Rectum, any malignancy in other parts of body.
2. Patients who had discontinued the treatment during follow up and patients who had developed any serious complications were dropped out from the study.

Criteria for Assessment: The assessment of the hyper granulation tissue was done on the basis of their dimensions. The dimensions were recorded before, during and after the treatment. The dimensions of hyper granulation were measured every time before applying the drug on subsequent follow-up day.

1. The length & width of the hyper granulation tissue was be measured by disposable millimeter ruler. Longest axis of tissue was recorded as length and width as the longest dimension perpendicular to length in the same plane. The area of hyper granulation tissue was calculated by Kundin's equation.[9]

This equation is to calculate the area of irregular surface.

$A_{kun} = L \times W \times 0.785\ mm^2$

2. The thickness of hyper granulation was measured by millimeter ruler. Thickness of hyper granulation tissue was its growth above the skin surface. The millimeter ruler was cut at its initial marking so to record thickness accurately. (As measured in mm, more than ½ mm was counted as 1mm and less than ½ mm was counted as 0.)

The area and thickness was measured in subsequent follow up rounds and after treatment.

Statistical Analysis:

- All the calculations were calculated through 'Graph Pad Instat' Software.
- **Paired't' test-**It was used for within the group assessment.
- **Unpaired "t" test-** It was used for comparison in Group 'A' and 'B'.

Observations:

1. Maximum patients of hyper granulation in the study were of age group 40 – 60 years in both the groups-

- Gr. A – 70%
- Gr. B – 60%.

2. In experimental group out of 30 patients 80 % patients were male and 20 % were female,In control group 56.67 % were male and 43.33 % patients were female.
3. Maximum patients in both the groups were Hindu as the trial was conducted in the Hindu dominated area.
4. Maximum patients in both the groups belonged to lower middle class category.
5. In both the groups, 70% patients were having mixed dietary habits.
6. In Group A patients 36.67% were having sedentary and 63.33% patients were active type of work. In control group 43.33% patients were of sedentary work and 56.67% patients were having active type of work.
7. In this study the data shows that in group A 10% patients were habitual alcoholic, 23.33% were smokers, 16.67% Tobacco chewing and 50% were of no habits, while in group B 13.33% patients were habitual alcoholic, 6.67% smokers, 16.67% of Tobacco chewing and 63.33% were not having any habits.

Table No. 1: Statistical Analysis of Area of Hyper Granulation Tissue In Group A & Group B (mm^2)

Area	N	Mean		% of relief	SD	SE	't' value	P value	Result
		BT	AT						
Group A	30	11.62	0.16	98.62	8.01	1.46	7.84	0.000	ES
Group B	30	12.48	3.24	74.04	6.26	1.14	8.08	0.000	ES

In Group A there was 98.62 % relief in Meanarea of hyper granulation tissue which was statistically ***extremely significant***. In Group B, there was 74.04 % relief which was also statistically ***extremely significant***.

Table No. 2. Statistical Analysis of Thickness of Hyper Granulation Tissue in Group A & Group B (mm)

Control group	N	Mean		% of relief	SD	SE	't' value	P value	Result
		BT	AT						
Group A	30	2.80	0.27	90.36	0.94	0.17	14.81	0.000	ES
Group B	30	3.30	1.40	57.57	0.62	0.11	16.26	0.000	ES

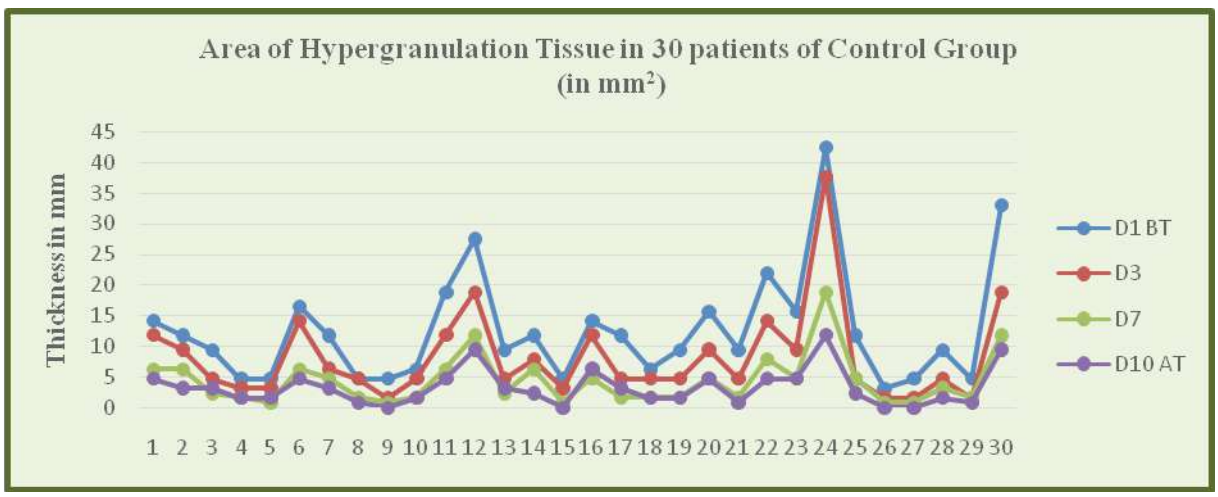
In Group A there was 90.36 % relief in Mean thickness of hyper granulation tissue which was statistically ***extremely significant***. In Group B, there was 57.57 % relief which was also statistically ***extremely significant***.

Table no. 3. Comparison between Group A and Group B

	Group	N	Mean Diff	SD	SE	't' Value	P Value	Result
Area	Group A	30	11.46	8.01	1.46	1.198	0.236	NS
	Group B	30	9.24	6.26	1.14			
Thickness	Group A	30	2.53	0.94	0.17	3.236	0.002	VS
	Group B	30	1.87	0.62	0.11			

Unpaired "t" test with Welch's correction was applied for comparison of Area and Thickness of hyper granulation tissue in Group A and Group B after treatment. It implies that

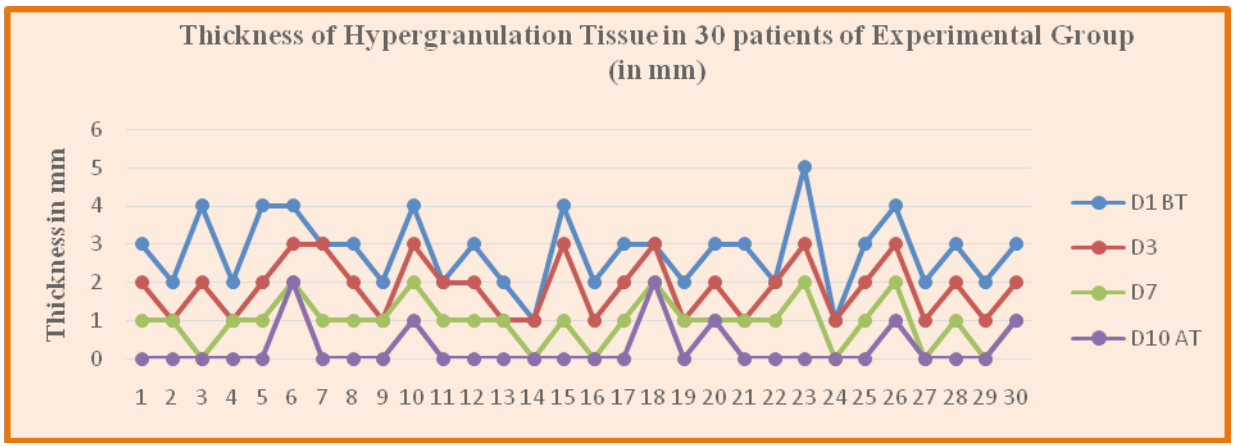
- Difference in Area of hyper granulation tissue after therapy between Group A & B is insignificant.
- Difference in Thickness of hyper granulation tissue after therapy between Group A & B is very significant.

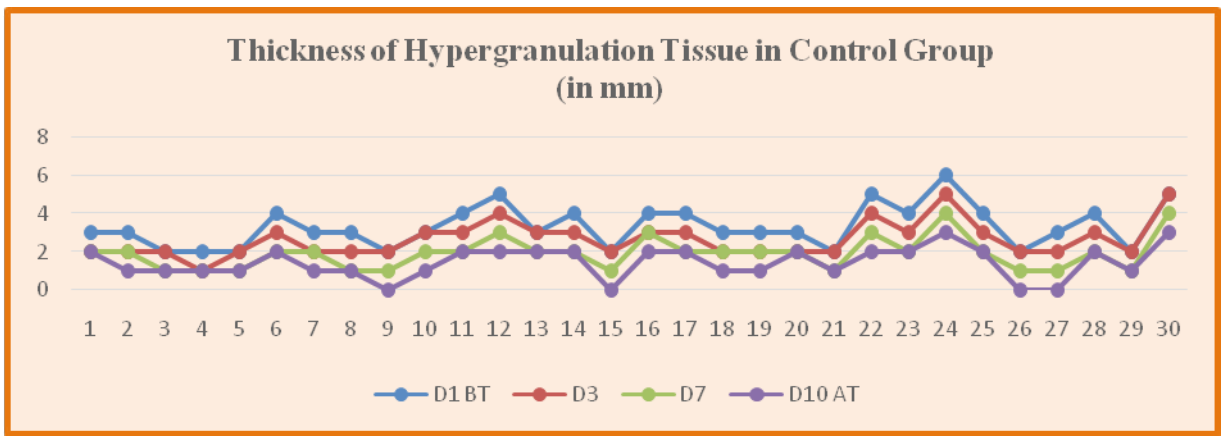


Effect on Area of Hyper Granulation Tissue: There was highly significant decrease ($p<0.001$) in area of hyper granulation tissue after treatment in both experimental and Control Group. In both groups highly significant decrease in area was observed after 3rd day of treatment onwards. On statistical analysis,mean difference in area of hyper granulation in both groups was found insignificant ($p>0.05$). However, the percentage relief was found to be 98.62% in Experimental Group and 74.04 % in Control Group.

The line diagrams shows that diminution of area of hyper granulation tissue in course of treatment is higher in number of patients in Experimental Group than that of Control Group.

Effect on thickness of hyper granulation tissue: There was highly significant decrease ($p<0.001$) in thickness of hyper granulation tissue after treatment in both experimental and Control Group. In both groups highly significant decrease in thickness was observed after 3rd day of treatment onwards. Mean difference in thickness of hyper granulation in both groups was found more significant ($p<0.01$) on statistical analysis. The percentage relief was found to be 90.36 % in Experimental Group and 57.57 % in Control Group.





The line diagrams shows that the reduction in thickness of hyper granulation tissue in course of treatmentis higher in number of patients in Experimental Group than that of Control Group.

Thus LA of *Kalpit Kasisadi oga* with *Madhu* is more effective in decreasing area and thickness of *Ksharasutra* induced hyper granulation at external opening of fistula-in-anothan *Madhu* alone.

Discussion

The *Kalpit Kasissadi Yoga* has dominance of *Kashaya-Tikta Rasa*, *Ushna Virya*, *Laghu Ruksha Guna*, and *Katu Vipaka*. In experimental group, *Kalpit Kasisadi Yoga* along with *Madhu*was used, hence there was an additive effect. *Avasadana Dravyas* have *Ushna Virya* so as *Kasis* is. *Amla, Kashaya, Tikta Ras* of *Kasis* adds to its *Vrana Ropana* and *Avasadana* properties.

In *Ayurvedic* literature, use of *Kasis* in chronic wounds with erupted wound edges and discharge has been postulated.[10] The ferrous sulphate acts as vaso-constrictor due its astringent property thus reducing the size of hyper granulation tissue.

Karanj with its *Tikta, Katu, Kashaya rasa* and *Ushna, Ruksha Guna* acts as *Kled-Shoshan, Stroro-Akunchan*. The seeds contain alkaloids, tannin and picric acids in addition to some reducing substance. The seeds yield a type of oil which has marked fungicidal, antiseptic, cleansing and healing properties when applied locally. *Karanj* is the active principle responsible for the curative effect of the oil in the skin diseases.[11]

Jatikalika is again of *Tikta-Kashaya Rasa* and *Ushna Virya*. The flowers contain pyridine and nicotinate derivatives, alkaloid jasminine and salicylic acid. Thus having anti inflammatory activity. Flowers are astringent and mild anaesthetic hence reduces oedema of hyper granulation tissue.[12]

Saindhav and *Kukkutand Twak Bhasma*possess hygroscopic activity thus dry and up the secretions of fistulous tract. *Kukkutandtwak Bhasma*contains Ca CO_3 which is recently recognized as an accelerator for healing of wounds.[13] *Saindhav* softens the hypertrophied fibrosed tissue.

In control group only *Madhu* was used, still highly significant results were obtained. This is because of multidimensional action of *Madhu* on wound which was established since ancient time. Due to *Kashaya Anurasa* (astringent) of *Madhu* there is constriction of the micro capillaries existing in the bed of hyper granulation tissue thus checking its growth. *Ruksha* and *Sukshma Guna* of *Madhu* causes *Kleda Shoshana* thus reducing the size of hyper granulation tissue.[14] According to modern science being hygroscopic, honey speeds up the healing of wound and dry up the secretions. It has antibacterial properties due to its acidic nature and enzymatically produced hydrogen peroxide. Thus with *Lekhana*, *Vrana Shodhana* and *Ropana* properties, *Avasadana karma* of *Madhu* is established.

Thus the *Kalpit Kasisadi Yoga* becomes very effective on hyper granulation tissue as compared to use of only *Madhu* in treatment of hyper granulation tissue of fistula-in–ano which is proved from the study results.

Conclusion

Hyper granulation, one of the rare consequences of wound is observed very frequently in patients of fistula-in-ano with *Ksharsutra* in it.Excessive secretions due persistent irritation, sensitivity, mal-preparation and faulty ligation technique, all associated with *Ksharsutra*, are the probable causes of hyper granulation tissue formation in fistula-in-ano.

Kalpit Kasisadi Yoga with *Madhu* shows a very effective *AvasadanaKarma*on*Utsanna Mrudu Vrana Maansa* (Hyper Granulation) in *Bhagandara* (fistula-in-ano), during its course of treatment with *Ksharsutra*, however local application of *Kalpit Kasisadi Yoga* is more effective than merely *Madhu*.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Anesthetic Management of Patients with Sickle Cell Disease Posted for Bipolar Prosthesis

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Abstract

In case of surgical procedures in sickle cell disease (SCD), patients are associated with high risk of perioperative complications like vaso-occlusive crisis, chest syndrome, post-operative infections, congestive heart failure, cerebrovascular accident and acute kidney injury. Preoperative assessment and stabilization like control of sepsis, blood transfusion, correction of hypoxia, hypothermia, dehydration and acidosis is needed to reduce peri-operative complications. Blood transfusion (Simple, manual exchange and automated exchange) remains an important therapeutic intervention in patients with SCD. The case study below shows the perioperative management of patients posted for bipolar prosthesis due to AVN (avascular necrosis). Adequate analgesia, incentive spirometry, early mobilisation and oxygen supplementation is the mainstay of post-operative management.

Keywords: *Sickle cell disease (SCD), Vaso-occlusive crisis (VOC), Avascular necrosis (AVN).*

Introduction

Sickle cell anemia (SCA) or sickle cell disease (SCD) is an autosomal recessive inherited hemoglobinopathy characterized by the presence of sickle hemoglobin (HbS), absent in healthy individuals. Homozygous individuals (HbSS) are characterized by chronic hemolytic anemia and repeated episodes of acute vascular occlusion leading to heart attacks, intense pain, and organ dysfunction, known as vaso-occlusive crisis (VOC).[1]

Different surgical procedures like hip replacement, obstetric surgery, gall bladder surgery, neuro surgery etc in SCD have been associated with relatively increased risks of peri-operative mortality, vaso-occlusive (painful) crisis, acute chest syndrome, post-operative infections, congestive heart failure, cerebrovascular accident and acute kidney injury.[2-3]

Hence preoperative assessment and stabilization like control of sepsis, blood transfusion, correction of hypoxia, hypothermia, dehydration and acidosis is needed to reduce peri-operative complications.

We are presenting a case of sickle cell disease with diagnosis of avascular nerosis (AVN) of femur posted for bipolar prosthesis.

Case Report: A 35 year old male, a known case of sickle cell disease HbSS pattern, was posted for bipolar prosthesis due to AVN. He was diagnosed to have sickle cell disease at the age of 12 year. He gave history of repeated hospitalization and blood transfusion since last 10 years. Last hospitalization was one month back and received 3 blood transfusions.

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On examination he was afebrile, pulse 100 beats per minute, blood pressure 130/90mmHg, SpO2 100%on room air. He had severe pallor. His respiratory, cardiac & neurological examination was within normal limits. Per abdomen exam revealed hepatomegaly. His lab investigation showed HB 5.6 gm %. Hematocrit was 16%, TLC- 17200/mm^3, platlet count, renal function, liver function, ECG and chest X-ray, were normal. USG abdomen showed mild hepatomegely and ABG showed mild metabolic acidosis. ESR and CRP were 112mm/hr and 78 mg/L. His urine culture was sterile while blood culture showed growth of methicillin senstive coagulase negative staphylococcus. 2Decho was normal and Hb electrophoresis showed HbS 65%. Airway and spine examination did not reveal any abnormality.

Patient was started on antibiotics according to culture sensitivity, warm intravenous fluid, tablets of sodium bicarbonate, folic acid, hydroxyurea. Oral paracetamol & tramadol were given for analgesia. Three blood transfusions were given. Patient was started on incentive spirometry. After one week his TLC count decreased to 10000, ESR & CRP normalized. Hemoglobin & hematocrit increased to 10.1 gm% & 31% respectively. ABG showed resolution of metabolic acidosis. So, patient was now posted for surgery.

Anaesthesia Management: Patient was kept NBM from midnight and allowed to take clear fluid up to 2 hours. Patients was given tablet alprazolam 0.5 mg and tablet pentaprozole 40 mg. Wide bore intravenous cannulation was done and warm IV fluid ringer lactate was started at the rate of 80 ml/hour to prevent dehydration in pre-operative period.

Spinal anaesthesia was planned for intraoperative management and femoral nerve block along with multimodal analgesia was planned for post operative period.

Pulse, blood pressure, oxygen saturation, temperature and ECG were monitored in intra-operative period.

Spinal anaesthesia was given with 25 G Quinkie needle at L4- L5 intervertebral space with 3 ml of 0.5% bupivacaine heavy with 75 mcg of buprenorphine.

Oxygen (5L/min) was given to the patient via facemask. Intravenous fluid 1.2 L of warm ringer lactate was infused along with one unit of blood transfusion. Urine output was maintained throughout the surgery at 40 to 50 ml/hour. Blood loss during surgery was approximately 400 ml. Patient's hemodynamic parameters remained stable in peri-operative period.

Femoral nerve block was given with 0.125% bupivacaine with 4 mg dexamethasone with the help of nerve stimulator in postoperative recovery room.

Patient was kept warm and oxygen supplementation was continued in postoperative period. Multimodal analgesia was supplemented in post-operative period with injection tramadol 100 mg and injection paracetamol 500mg thrice a day. IV fluid and antibiotics were continued in post-operative period. Early ambulation was encouraged.

Discussion

In patients with SCD undergoing surgery, peri-operative period is associated with increased risk of SCD related complications like increased risks of peri-operative mortality, vaso-occlusive (painful) crisis, acute chest syndrome (ACS), post-operative infections, congestive heart failure, cerebrovascular accident and acute kidney injury.[2-3] Careful preoperative assessment and management are important to reduce these risks. Peri-operative management requires a multidisciplinary approach, including surgeons, anesthesiologists, and hematologists.

Predictors of post-operative complications in patients with SCD include

1. Type of surgical procedure low, moderate or high risk.
2. Increased age- associated with disease progression
3. Frequency of recent complications-current activity of disease state
4. Hospitalization, marker of disease severity
5. Temporal clustering of ACS – progression of lung disease
6. Abnormal lung fields on radiograph – evidence of sickle chronic lung disease
7. Pregnancy –increased risk of maternal complications
8. Pre-existing infection – triggering agent for ACS
9. Haplotype – African haplotype have more severe disease than the Asian haplotype.[4]

Blood transfusion remains an important therapeutic intervention in patients with sickle cell disease (SCD), aiming to both increase the oxygen carrying capacity of blood and to reduce the complications of vaso-occlusion. Simple, manual exchange and automated exchange can be effective in reducing the acute and chronic complications of SCD.[5]

A randomized control trial compared conservative and aggressive preoperative blood transfusion in 604 operations in patients with sickle cell anemia. The conservative regimen (aiming for Hb 10 g/dL) was as effective as the aggressive regimen (aiming for Hb of 10 g/dL and HbS<30%) in preventing perioperative complications. Although alloimmunization was more common in the aggressive transfusion group. ACS was the most frequent clinical complication; seen in 10% of patients in both arms of the trial.[6] A subsequent RCT, the TAPS trial (Transfusion Alternatives PreOperatively in Sickle Cell Disease) compared preoperative transfusion with no preoperative transfusion in low- and moderate-risk surgical procedures in patients with HbSS or HbSb0 thalassemia. Patients randomized to transfusion were given a simple transfusion aiming for Hb>10 g/dL (if Hb<9 g/dL) or a partial exchange aiming for HbS<60% (if Hb>9 g/dL). Clinically important complications were significantly increased in the nontransfused group (39% vs 15% of patients, P = 0.023) and this difference was primarily explained by a marked increase in ACS in the un-transfused group (27% vs 3%).[7]

In our case patient was having initial hemoglobin 5.6gm% and HbS 65%. We did 3 simple blood transfusions to build hemoglobin to 10 gm% in preoperative period.

Urinary or respiratory tract infections are closely associated with the development of ACS.[8] Hence preoperative infection needs to be adequately controlledwith appropriate antibiotics. Chest radiograph, hemoglobin oxygen saturation, and lung function tests may delineate the degree of pulmonary pathology. Blood urea and serum creatinine concentrations and urine dipstick screening or proteinuria or occult urinary tract infection are useful and inexpensive screening tools. Other Investigations include screen - for antibodies, extended cross-match for E, C, K groups, arterial blood gas analysis, electrocardiogram, liver function tests, stool examination (mucosal ischaemia) and neurological imaging.[4]

During perioperative period, to avoid triggering of sickle cell crisis, factors like anxiety, emotional stress, infection, dehydration, acidosis, hypoxia, hypothermia, vascular stasis and increased blood viscosity should be addressed.[9]

Intracellular dehydration is a known trigger for HbS polymerization. Whenever possible, prolonged pre-operative fasting must be avoided.[9] The use of regional anaesthesia appears to be a useful means of pain control with effective blocking of nociceptive pathways and also prevent a vaso-occlusive crisis[10]. Use of tourniquet is safe to use provided optimum acid-base status and oxygenation are maintained throughout.[11-14]

Conclusion

SCD is a debilitating disease associated with increased risk of perioperative complications. Optimization of patient's laboratory parameters preoperatively is of utmost importance to prevent perioperative complications like VOC & ACS. Adequate control of infection, correction of anemia with simple or exchange transfusion can reduce the risk of complications. Adequate analgesia, incentive spirometry, early mobilisation and oxygen supplementation is the mainstay of post-operative management.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Triple Drug Therapy with Proton Pump Inhibitor a Better Option for Helicobacter Pylori Eradication

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Abstract

Introduction: H. Pylori is a gram-negative micro organism residing in the stomach of human. It is a important cause of acid peptic disorders. Bismuth based triple therapy,triple therapy regimens(Tripple PPI) and Quadruple therapy are the available treatment options to eradicate H. pylori. Controversy still presents regarding the superiority of treatment modality and results of available studies are variable. Hence this study was undertaken to evaluate and compare the efficacy of triple PPI, Quadruple therapy, and bismuth-based triple therapy in treating acid peptic disorders and Helicobacter pylori eradication. Method: This study was a prospective interventional. After the establishment of a clinical diagnosis of the acid peptic disorder, the patient was posted for endoscopy. H. pylori status was determined by the rapid urease test and culture examination. All patients divided into three groups and offered three different medical treatments the first group received bismuth bases triple therapy, the second group got Tripple PPI therapy and the third one received quadruple therapy. efficacy and comparison of all these treatment modalities were evaluated and assessed by symptoms, endoscopic findings, and results of the rapid urease test after 6 weeks. Results: In the present study, 150 patients with upper gastrointestinal symptoms were studied It was found that the maximum number of subjects belonged to the age group 31-40 yrs. Male outnumbers than females. Maximum patients had pain in epigastric region and were having gastritis as a commonest diagnosis., Symptomatic relief by Triple therapy with PPI was maximum than other therapies. Rapid urease test (RUT) was also suggest that treatment with it gives superior eradication rates. Discussion: In our study, we have found that the treatment regime with PPI Triple therapy has given maximum symptomatic relief, there is an association of H. Pylori infection in an acid peptic disorder like duodenal ulcer (86.66%), gastric ulcer (75%), and gastritis (82.85%). the efficacy of the Rapid urease test is 66% and that of culture is 38.66% in the diagnosis of H. pylori infection in acid peptic disorder. Conclusion: PPI Triple therapy is most effective in symptomatic relief and healing of the duodenal and peptic ulcer.

Keywords: *Gastritis, rapid urease test, dyspepsia, tetracycline.*

Introduction

The acid peptic disorder is a disorder in which due to hyperacidity, there is the damage of inner lining (mucosa) of the stomach and duodenum causing various manifestations like inflammation, ulcer, etc. A major symptom of the acid peptic disorder is a pain in the abdomen typically non radiating, burning, and located at in epigastrium, Other include nausea, vomiting, dyspepsia, haemtemesis, and melena, etc. Upper gastrointestinal tract disorders are common in surgical practice. A disease of the stomach and duodenum range from a benign disease like gastritis, peptic ulcer diseases which are usually having a self-limiting although a prolonged course with a potentially dangerous complication like perforation with its attendant's peritonitis both chemical and bacterial, to malignant diseases like carcinoma of the stomach. The upper gastrointestinal disease forms

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a major part of the total number of patients attending the outpatient clinic for gastrointestinal disorders. The treatment of peptic ulcers has been based on Schwartz dictum "No acid, no ulcer". Marshall and Warran in 1984 described microorganisms in the stomach of patients with gastritis and peptic ulceration first time in history. These investigators identified a spiral-shaped flagellated organism associated with peptic ulcer originally referred to as campylobacter pyloride. The organism is now known as Helicobacter Pylori[1].

H.Pylori is a gram-negative organism resides in the stomach of human and other animals,affecting half of the population of world[2].

H.Pylori is a common cause of peptic ulcer of stomach and duodenum leading to clinically present as recurrent abdominal pain. It is corelated with around 90% of duodenal ulcers and 80%of gastric ulcers, major cause of morbidity. Mortality associated however is low but can result in chronic illness leading to significant manpower loss it can also cause lymphoma and gastric carcinoma[3].

Present understanding about the aetiology of peptic ulcer disease, gastric cancer, and dyspepsia is under evolution. The invention of Helicobacter pylori as a infective aegent change our method to treat and diagnose it. Human beings are the only reservoir for Helicobacter Pylori. Direct transmission from person to person occurs via saliva and feces and infection also through contact with contaminated water[4].

The Nobel prize in physiology or medicine for the year 2005 was awarded to both J.Robin warren and Barry J Marshall. These Nobel winners made a astonishing and unbelievable invention that chronic inflammation in stomach and duodenum (Gastritis, gastic and duodenal ulcer), is due to an infection by bacterium H.Pylori. Many tried to disprove it [5]. As recent trend of decrease evidence of peptic ulcers was observed from last 40 years. The discovery of Helicobacter Pylori had a further major impact on the incidence of ulcer[6].

Bismuth based triple therapy comprising of colloidal bismuth subcitrate (120mg, q.i.d), tetracycline (500 mg, q.i.d) and metronidazole (400 mg, t.i.d) for 14 days is low cost, safe and have high success rates in metronidazole sensitive strains. Few investigators reveals upto 90 percent success rate. If tetracycline was replaced by amoxicillin then success rate decreased to 70 percent to eradicate H.pylori. Triple therapy (Tripple therapy with PPI) is a combination of a proton pump inhibitor and two antibiotics have now almost replaced the use of the classical bismuth-based triple therapy in all over world. This is due to less side effect and better eradication rate. Quadruple therapy **is** a try to get 100% removal of H. pylori by the addition of anti-secretory agents to the classic bismuth-based triple therapy. It has been noted that this quadruple treatment is more effective when a PPI is used as an anti-secretory agent rather than an H**2** receptor antagonist. Huang et al reported a promising eradication rates of 95% when omeprazole was added to the classical triple therapy.[7]

Controversy still presents regarding the superiority of treatment modality and results of available studies are variable[8]. Hence this study was undertaken to evaluate and compare the efficacy of triple PPI, Quadruple therapy, and bismuth-based triple therapy in treating acid peptic disorders and Helicobacter pylori eradication.

Method

The present study was undertaken in the department of surgery, Jawaharlal Nehru Medical College, Wardha in collaboration with Datta Meghe Medical College Hingana, Nagpur, Datta Meghe Institute of medical science (DMIMS), Sawangi, Meghe, Wardha, Maharashtra India. This study was a prospective interventional. The duration of this study was from September 2018 to September 2019. The numbers of patients were 150.

Sample size- 150

The sample size was calculated as per formula was around 134 Hence 150 sample size was sufficient ($N=2(Z\alpha+Z1-\beta)\ 2\ \sigma2/\Delta2$)

The present study was carried out on the patients attending surgical OPD as well as the surgical ward from September 2018 to September 2019 in a tertiary center. We selected 150 patients having gastrointestinal complaints. The complaints were a pain in the abdomen, usually, in the epigastric region, dyspepsia, nausea, vomiting, haemtemesis, melena, etc Detail history of patients was taken with regards to the symptom, their duration, and severity, other complaints, drug history. A thorough clinical examination and routine haematological examination of patients was done. After the establishment of a clinical diagnosis of the acid peptic disorder, the patient was posted for endoscopy. H. pylori status was determined by the rapid urease test and histopathological examination.

Inclusion Criteria: Patients between 15-60 years were having symptoms of the acid peptic disorder.

Exclusion Criteria:

1. Patients taking antibiotics and bismuth compounds or omeprazole 4 wks before endoscopy

2. Pregnancy.

Detail of Procedure Followed: Patients with clinical symptoms of APD were advised to attend the Gastroscopy clinic. The patients were kept nil orally at night and were advised to attend endoscopy clinic early in the morning Endoscopy was performed with Fujinon Gastroduodenoscope after obtaining informed consent. Esophagogastroduodenoscopy of each patient was done under surface local anesthesia with 4% Xylocaine viscous. Findings were noted and biopsies from the antrum and suspicious lesions were taken. Out of biopsies, one was subjected for a rapid urease test and the second biopsy specimen was used for histopathological examination. Endoscopic findings were recorded as gastritis, duodenal ulcer, gastric ulcer, gastric carcinoma, and endoscopically normal mucosa as per standard criteria.

All patients divided into three groups and offered three different medical treatments the first group received bismuth bases triple therapy, the second group got Tripple PPI therapy and the third one received quadruple therapy efficacy and comparison of all these treatment modalities were evaluated and assessed symptoms, endoscopic findings, and results of the rapid urease test after 6 weeks.

Data Collection: The data collected included the presenting complaints, clinical signs, endoscopic findings, results of rapid urease test, and histopathological examination.

Statistical Analysis: The presence of H pylori infection was correlated with symptoms, and endoscopy findings .this calculation was derived using SPSS 17.0 statistical software.

Ethical approval for the study was obtained from the ethics committee of DMIMS University.

Results

In the present study, 150 patients with upper gastrointestinal symptoms were studied It was found that the maximum number of subjects belonged to the age group 31-40 yrs. i.e. 26.66% followed by age group 21-30 yrs. ie 22.66%. It was found that of subjects male 112 and female 38.M: F 2.94:1. It was found that maximum no. subjects had pain in epigastric region 120 cases i. e. 80% followed by symptoms nausea 75 cases ie. 50%.

Table 1: endoscopic findings in patients with acid peptic disorder before treatment

Endoscopic findings	No. of Cases
Gastritis	70
Duodenitis	09
Duodenal ulcer	15
Gastric ulcer	O8
Gastric malignancy	02
Endosc. Normal mucosa but clinically symptoms of APD	46
Total	**150**

70 patients were having gastritis,15 had a duodenal ulcer,9 patients had Duodenitis, the gastric ulcer was found in 08 patients, gastric malignancy in 02 patients and 46 patients had endoscopic normal mucosa. The highest positivity of RUT was seen in patients of duodenal ulcers (86.66%), followed by gastritis (82.85%) and gastric ulcer (75%).

Table 02: Treatment regimen given in acid peptic disorder

Regimen	No. of Patients	Symptomatic relief	%
Bismuth Triple Therpy	38	24	60.52
Ppi.Triple Therpy	82	78	95.12
Quadruple Therpy	30	22	73.33

Symptomatic relief with PPI Triple therapy was 95.12%, followed by Quadruple Therapy in 73.33% and Bismuth triple therapy 60.52%.

Table 03: Gastroscopic findings after six weeks of treatment:

Endoscopic finding	No. of cases improved	%	No. of cases not improved	%
Gastritis	63	90	07	10
Duodenitis	08	88.88	01	11.11
DU	14	93.33	01	6.66
GU	06	75	02	1.25
GM	01	50	01	50
Endo. normal but clinically APD	42	91.30	04	8.69
Total	**134**	**89.34**	**16**	**10.66**

Out of a total of 150 patients who received treatment 134 patients (89.34%) improved and 16 (10.66 %) patients not improved.

Table no. 04: The result of rapid urease test and culture before treatment

Test	Positive	%	Negative	%
Rapid urease test	99	66	51	34
Culture	58	38.66	92	61.33

RUT test was positive in 66% of patients and negative in 34% of patients. Culture was positive in 38.66% patients and negative in 61.33%patients.

Table No. 05: the result of rapid urease test and culture after treatment

Test	Positive	%	Negative	%
Rapid urease test	15	10	135	90
Culture	12	8	138	92

RUT test was positive in 10% of patients and negative in 90% of patients. Culture was positive in 8% patients and negative in 92% patients 6 patients from bismuth-based treatment group 5 patients from quadruple and 4 from the Tripple PPI treatment group.

Discussion

In this study, 150 patients (112 men and 38 women), within the range of 15 to 72 years, with symptoms suggestive of acid peptic disorder, were included.

In our study, we found that acid peptic disorder was more common in the age group 31-40 years (26.66%cases). Followed by 21-30 years (22.66%cases) and 41-50 years (17.33% cases). In our study in patients with acid peptic disorder rapid urease test was positive in 99 patients (66%).

Name of the study	Year	RUT %	Culture %
Sivaprakash et al[8]	1994	38.7	43
Maimomma et al[5]	1994	65.8	47
Sharma B et al[7]	2006	59	-
In Our study	2008-09	66	38.66

In present study, the result with 3 different modalities of treatment was used as follows Bismuth triple therapy symptomatic relief in 34 patients (89.43%), PPI Triple therapy in 76 patients (92.68%) and Quadruple therapy in 28 patients (93.33%)

As per another study by Vikram kate (2005)[7]Bismuth therapy was used symptomatic relief in 30 – 90% patients, PPI triple therapy in 85-95% patients, and Quadruple therapy in 86-98% patients.

In present study, we found gastroscopic findings were improved 134 patients (88.93%) and not improved 16 patients (10.66%) after six weeks of treatment.

The cure rate of 80-85 % was achieved using combination therapy. Richard Guan et al, showed eradication of H. Pylori in 90% cases after a week with a triple regimen.

The present study was carried out on the patients attending surgical OPD as well as admitted patients in the ward who had clinical symptoms suggestive of acid peptic disease from September 2018 to September 2019 in the tertiary care center. A total of 150 patients were studied and advised to attend the Gastroscopy clinic.

1. The symptoms of acid peptic disorder were more in 31-40 years followed by 21-30 years.

2. In our study, we have found there was male predominance with male to female ratio of 2.94:1.
3. In our study, we found that there was an association of symptoms of acid peptic disease with H. Pylori infection. Pain in the epigastric region (80%) and nausea (50%) were the most frequent symptoms associated with H. pylori infection followed by dyspepsia (30.66%).
4. In our study, we have found that there is a strong association between H. Pylori in duodenal ulcer (86.66%), gastric ulcer (75%), and gastritis (82.85%).
5. In our study, we have found that the rapid urease test for H. Pylori was positive in 66% of patients and culture was positive in 38.66%.
6. In our study, we have found that the treatment regime with PPI Triple therapy has given maximum symptomatic relief (95.12%). The other treatment regimens and the symptomatic relief provided by them are as follows:
    a. Quadruple therapy 73.33%
    b. Bismuth triple therapy 60.52%.

Conclusion

From the study we could draw the following conclusion:

1. In our study, we have found that the incidence of Helicobacter infection in acid peptic disorder is 66%.
2. In our study, we have found that there is an association of H. Pylori infection in an acid peptic disorder like duodenal ulcer (86.66%), gastric ulcer (75%), and gastritis (82.85%).
3. In our study, we have found that the efficacy of the Rapid urease test is 66% and that of culture is 38.66% in the diagnosis of H. pylori infection in acid peptic disorder.
4. PPI Triple therapy is most effective in symptomatic relief and healing of the duodenal and peptic ulcer.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Correlation of Helicobacter Pylori Infection with Acid Peptic Disorders

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Abstract

Introduction: The acid peptic disorder is a disorder in which due to hyperacidity, there is the damage of inner lining (mucosa) of the stomach and duodenum causing various manifestations like inflammation, ulcer, etc. The acid peptic disorder is a disorder in which due to hyperacidity, there is the damage of inner lining (mucosa) of the stomach and duodenum causing various manifestations like inflammation, ulcer, etc. A major symptom of the acid peptic disorder is a pain in the abdomen typically non radiating, burning, and located at in epigastrium, Other include nausea, vomiting, dyspepsia, haemtemesis, and melena, etc. Upper gastrointestinal tract disorders are common in surgical practice. Helicobacter pylori is a common cause of acid peptic disorders. Still in literature there is a controversy still exist to question about role of Helicobacter in causing acid peptic disorders. So This study was undertaken to evaluate the correlation of H. Pylori infection with acid peptic disorders. Method: This study was a prospective observational. The duration of this study was from September 2017 to September 2019. The numbers of patients were 150. After the establishment of a clinical diagnosis of an acid peptic disorder, the patient was posted for endoscopy. H. pylori status was determined by the rapid urease test. The data collected included the presenting complaints, clinical signs, endoscopic findings, results of rapid urease test, and histopathological examination. The presence of H pylori infection was correlated with symptoms, and endoscopy findings. Results: In the present study, 150 patients with upper gastrointestinal symptoms were studied It was found that the maximum number of subjects belonged to the age group 31-40 yrs. It was found that of subjects male 112 and female. It was found that maximum number of subjects had pain in epigastric region 120 cases i. e. 80% followed by symptoms nausea 75 cases ie. 50%. about endoscopic findings .70 patients were having gastritis,15 had a duodenal ulcer,9 patients had Duodenitis, the gastric ulcer was found in 08 patients, gastric malignancy in 02 patients and 46 patients had endoscopic normal mucosa. The highest positivity of RUT was seen in patients of duodenal ulcers (86.66%), followed by gastritis (82.85%) and gastric ulcer (75%). Conclusions: Present study, we have found that there is an association of H. Pylori infection in an acid peptic disorder like duodenal ulcer, gastric ulcer, and gastritis.

Keywords: *Gastritis, duodenal ulcer, rapid urease test, dyspepsia.*

Introduction

The acid peptic disorder is a disorder in which due to hyperacidity, there is the damage of inner lining (mucosa) of the stomach and duodenum causing various manifestations like inflammation, ulcer, etc. A major symptom of the acid peptic disorder is a pain in the abdomen typically non radiating, burning, and located in epigastrium, Other include nausea, vomiting, dyspepsia, haemtemesis, and melena, etc. Upper gastrointestinal diseases are one of the common entities in surgical practice. A disease of the stomach and duodenum range from a benign disease like gastritis, peptic ulcer diseases which are usually having a self-limiting although a prolonged course with a potentially

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dangerous complication like perforation with its attendant's peritonitis both chemical and bacterial, to malignant diseases like carcinoma of the stomach. The upper gastrointestinal disease forms a major part of the total number of patients attending the outpatient clinic for gastrointestinal disorders. The treatment of peptic ulcers has been based on Schwartz dictum "No acid, no ulcer". The gastric acid has dominated the approach to the diagnosis and treatment of peptic ulcer disease. Marshall and Warran(1984) published a paper entitled unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration first time in history. These investigators identified a spiral-shaped flagellated organism associated with peptic ulcer originally referred to as campylobacter pyloride. The organism is now known as Helicobacter Pylori[1]. H.Pylori is a gram-negative organism resides in the stomach of human and other animals,affecting half of the population of world[2]. H. Pylori is a common cause of peptic ulcer of stomach and duodenum leading to clinically present as recurrent abdominal pain. It is corelated with around 90% of duodenal ulcers and 80%of gastric ulcers, major cause of morbidity. Mortality associated however is low but can result in chronic illness leading to significant manpower loss.it can also cause lymphoma and gastric carcinoma[3]. Present understanding about the aetiology of peptic ulcer disease, gastric cancer, and dyspepsia is under evolution. The invention of Helicobacter pylori as a infective aegent change our method to treat and diagnose it. Human beings are the only reservoir for Helicobacter Pylori. Direct transmission from person to person occurs via saliva and feces and infection also through contact with contaminated water[4]. The Nobel prize in physiology or medicine for the year 2005 was awarded to both J.Robin warren and Barry J Marshall. These Nobel winners made a astonishing and unbelievable invention that chronic inflammation in stomach and duodenum (Gastritis, gastic and duodenal ulcer), is due to an infection by bacterium H.Pylori. Many tried to disprove it [5]. As recent trend of decrease evidence of peptic ulcers was observed from last 40 years. The discovery of Helicobacter Pylori had a further major impact on the incidence of ulcer[6]. So This study was undertaken to evaluate the correlation of H.Pylori infection with acid peptic disorders.

Method

The present study was undertaken in the department of surgery, Jawaharlal Nehru Medical College, Wardha in collaboration with Datta Meghe Medical College Hingana, Nagpur, Datta Meghe Institute of medical science (DMIMS), Sawangi, Meghe, Wardha, Maharashtra India. This study was a prospective observational. The duration of this study was from September 2017 to September 2019. The numbers of patients were 150. The sample size was calculated as per formula was around 134 Hence 150 sample size was sufficient ($N=2(Z\alpha+Z1-\beta)\ 2\ \sigma2/\Delta2$). The present study was carried out on the patients attending surgical OPD as well as the surgical ward from September 2017 to September 2019 in the tertiary center. We selected 150 patients having gastrointestinal complaints. The complaints were a pain in the abdomen, usually in the epigastric region, dyspepsia, nausea, vomiting, haemtemesis, melena, etc. In personal history, the habit of tobacco chewing, cigarette smoking, alcohol consumption, and appetite were included. Detail history of patients was taken with regards to the symptom, their duration, and severity, other complaints, drug history. A thorough clinical examination and routine haematological examination of patients was done. After the establishment of a clinical diagnosis of an acid peptic disorder, the patient was posted for endoscopy. H. pylori status was determined by the rapid urease test and histopathological examination.

Inclusion Criteria: Patients between 15-60 years were having symptoms of the acid peptic disorder.

Exclusion Criteria: Patients taking antibiotics and bismuth compounds or omeprazole 4 wks before endoscopy. Pregnancy.

Detail of procedure followed. Patients with clinical symptoms of APD were advised to attend the Gastroscopy clinic. The patients were kept nil orally at night and were advised to attend endoscopy clinic early in the morning Endoscopy was performed with Fujinon Gastroduodenoscope after obtaining informed consent. Esophagogastroduodenoscopy of each patient was done under surface local anesthesia with 4% Xylocaine viscous. Findings were noted and biopsies from the antrum and suspicious lesions were taken. Out of biopsies, one was subjected for a rapid urease test and the second biopsy specimen was used for histopathological examination. Endoscopic findings were recorded as gastritis, duodenal ulcer, gastric ulcer, gastric carcinoma, and endoscopically normal mucosa as per standard criteria.

Data Collection: The data collected included

the presenting complaints, clinical signs, endoscopic findings, results of rapid urease test, and histopathological examination.

Statistical Analysis: The presence of H pylori infection was correlated with symptoms, and endoscopy findings .this calculation was derived using SPSS 17.0 statistical software.

Ethical approval for the study was obtained from the ethics committee of DMIMS University.

Results

In the present study, 150 patients with upper gastrointestinal symptoms were studied It was found that the maximum number of subjects belonged to the age group 31-40 yrs. i.e.26.66% followed by age group 21-30 yrs.ie 22.66%. It was found that of subjects male 112 and female 38.M: F 2.94:1

Table 01: Symptoms profile of patients

Symptom	No. of cases	Percentage
Pain in epigastric region	120	80
Nausea	75	50
Vomiting	42	28
Dyspepsia	46	30.66
Haemtemesis	15	10
Meleana	09	06

Table 3. shows the symptoms profile of study subjects. It was found that maximum no. subjects had pain in epigastric region 120 cases i. e. 80% followed by symptoms nausea 75 cases ie.50%.

Table 02: endoscopic findings in patients with acid peptic disorder

Endoscopic findings	No. of cases
Gastritis	70
Duodenitis	09
Duodenal ulcer	15
Gastric ulcer	O8
Gastric malignancy	02
Endosc. Normal mucosa but clinically symptoms of APD	46
Total	150

70 patients were having gastritis,15 had a duodenal ulcer,9 patients had Duodenitis, the gastric ulcer was found in 08 patients, gastric malignancy in 02 patients and 46 patients had endoscopic normal mucosa.

Table 3. Result of Rapid Urease Test in Acid Peptic Disease

Sr. No.	Diagnosis	No. of pts.	RUT positive	Percentage
1.	Gastritis	70	58	82.85
2.	Duodenitis	09	05	55.55
3.	DU	15	13	86.66
4.	GU	08	06	75
5.	GM	02	01	50
6.	Endo. Normal but clinically s/o APD	46	16	34.78

The highest positivity of RUT was seen in patients of duodenal ulcers (86.66%), followed by gastritis (82.85%) and gastric ulcer (75%).

Discussion

In this study, 150 patients (112 men and 38 women), within the range of 15 to 72 years, with symptoms suggestive of acid peptic disorder, were included. In our study, we found that acid peptic disorder was more common in the age group 31-40 years (26.66%cases). Followed by 21-30 years (22.66%cases) and 41-50 years (17.33% cases).

In similar studies following results were seen:

Table 4: comparison with previous studies:

Study	Year	Mean age of patients with APD
B Sharma[7]	2006	32.8
C. Rekha[8]	2007	40.89
GM Malik et al[9]	1999	34.80
J. Yakoob et al [10]	2005	40.89
Endale Tadesse et al[11]	2014	36
Present study	2019	38.48

Sex wise distribution: In our study, we found that Acid peptic disorder was more common in males as compared to females. M: F ratio was 2.94: 1

Table 5: Endoscopic Finding

Study	Gastritis	Duodenal ulcer	Gastric ulcer
In our study	82.85%	86.66%	75%
Yakoob et al (2005)[10]	65%	-	-
J M Pajures (2005)[12]	88%	90%	80-90%
Vandana Berry (2006)[13]	-	90%	80%
Richard G (2005)[14]	-	95%	80%

In our study, we found a maximum number of duodenal ulcer cases (86.66%) followed by gastric ulcer cases (75%) and gastritis (82.85%)

Symptom Analysis: In our study, we found epigastric pain, nausea, dyspepsia, and vomiting as the most common symptoms associated with the acid peptic disorder as detected on endoscopy (see table 3).

Vandana Berry[13] in their study found that most common symptoms are epigastric pain, nausea, vomiting, and dyspepsia

Tom Richard Okello (2006)[14,15] in their study found that the most common symptoms are a pain in the epigastric region, dyspepsia, haematemesis recurrent abdominal pain, vomiting, and miscellaneous. The findings in the present study are comparable to these studies

Table 6: comparison with previous studies for diagnosis

Name of the study	Year	RUT %
Sivaprakash et al [15]	1994	38.7
Maimomma et al [2]	1994	65.8
B Sharma [7]	2006	59
Our study	2019	66

In our study in patients with acid peptic disorder rapid urease test was positive in 99 patients (66%).

Conclusion

The present study was carried out on the patients attending surgical OPD as well as admitted patients inward who had clinical symptoms suggestive of acid peptic disease from September 20017 to September 2019 in the tertiary care center. A total of 150 patients were studied and advised to attend the Gastroscopy clinic.

1. The symptoms of acid peptic disorder were more in 31-40 years followed by 21-30 years.
2. In our study, we have found there was male predominance with male to female ratio of 2.94:1.
3. In our study, we found that there was an association of symptoms of acid peptic disease with H. Pylori infection. Pain in the epigastric region (80%) and nausea (50%) were the most frequent symptoms associated with H. pylori infection followed by dyspepsia (30.66%).
4. In our study, we have found that there is a strong association between H. Pylori in duodenal ulcer (86.66%), gastric ulcer (75%), and gastritis (82.85%).
5. In our study, we have found that the rapid urease test for H. Pylori was positive in 66% of patients.

From the study we could draw the following conclusion:

1. In our study, we have found that the incidence of Helicobacter infection in acid peptic disorder is 66%.
2. In our study, we have found that there is an association of H. Pylori infection in an acid peptic disorder like duodenal ulcer (86.66%), gastric ulcer (75%), and gastritis (82.85%).

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: self.

Conflict of Interest: Nil.

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Increased Paediatric Screen Time During Pandemic: A Cause of Concern to Child Health

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Abstract

COVID-19 has put the globe in an unforeseen and unexpected situation. COVID-19 has affected everyday life all over the world. Human population came face to face to this virus so gravely for the first time. During the initial days nearly all countries imposed lockdown to break the chain of viral spread. This created significant knock-on effects on the daily life of citizens. With the updates on COVID-19, the guidelines issued by medical authorities, categorized geriatric and paediatric age groups to be at high risk. In order to flatten the rising curve of exponential spread of the virus ban on gatherings resulted in enforced shut down of schools. Therefore children were bound to stay home. They were automatically exposed to the over use of electronic gadgets for recreation as well as education purpose .Hence, their screen time increased many fold. This paper describes the impact of COVID-19 and use of electronic screen on children.

Keywords: *COVID-19, Lockdown, screen time, children.*

Introduction

COVID -19 has taken a toll on every aspect of human life. Its rapid spread around the world, has posed enormous health, economic, environmental and social challenges to the global population. As a result, for the protection of their population mostly all the countries opted for lockdown. Nearly all nations are trying to slow down disease transmission by monitoring and treating patients, quarantining suspected individuals by touch surveillance, banning large gatherings, enforcing complete or partial lock-downs etc. The pandemic of corona virus is a serious disruption to the global economy. With the passage of time, to boost the sinking economy, gradually lockdowns are being lifted up. As,no records of any clinically approved antiviral drugs or vaccines that are effective against COVID-19 have been published so far. Therefore clinically vulnerable geriatric and paediatric age groups are forced to stay indoors, only. Due to schools shut all over still, over 1.*2 billion children* are out of the classroom globally. [1,2]As, the pandemic continues to exist globally, the education sector had to shift to the online method of teaching and learning.

During lockdown, children were already made to sit home. Due to lack of physical activity and social interaction, children engaged themselves with cell phones, computers, laptops, tablets and televisions. This added to many fold increase in the screen time of children. Parents also are struggling to maintain balance between work and childcare.[3] This is also one of the factors contributing to acceptance of kids engaging with gadgets.

Though, earlier there was high growth in education technology, it has been observed that there is a significant rise in usage of language apps, virtual tutoring, video

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conferencing tools, or online learning softwares, since COVID-19.[4]

The World Bank is working actively with education ministries in dozens of countries to support their efforts to use all kinds of educational technologies to provide distant learning opportunities to students while schools are closed, as a result of the COVID-19 pandemic.[5]

The Diksha, E-pathshala, Swayam, The National Repository of Open Educational Resources, are various free digital e-Learning platforms shared by the Ministry of HRD, India.

The decision of online schooling, is however, contributing to the increased screen time. During pandemic, the start of online education, urged parents to buy smart phones for schooling of kids. Not just schooling, many other online hobby classes also emerged on the web platform. Kids are being engaged in these activities, too. Thus, children are definitely spending much time on screen.

Aims: To assess the impact of pandemic on children education and health

Objectives:

1. To understand the effect of Covid-19 on education sector
2. To know the effect of online and offline screen activities on child health
3. To explore the possible solution to the problem

Materials and Method

Various Research articles, news bulletins, international reports, websites online available books, book chapters were reviewed thoroughly to understand and produce the contents.

Discussion

Many a number of researchers have done a comprehensive study on the evil effects of smart phones or cell phones on human health. Smartphones, Cordless phones emit radiofrequency radiation (RF). During the use of cell phones, brain is the easy target for radiations. Considering this fact, IARC (International Society for research on Cancer)evaluated the knowledge in case–control human studies by the Hardell group from Sweden and the IARC Inter-phone study .These studies provided supportive evidence of increased risk for brain tumors, like glioma and acoustic neuroma. (IARC Monograph, 2013). The scientific panel concluded that RF radiation in the frequency range 30 kHz–300 GHz is a Group 2B possible human carcinogen[6].

There are various possible health risks associated with overuse of mobile phones to mental and physical well-being, especially low IQ & inadequate behavioral growth in children, sleep deprivation, brain tumors and psychiatric disorders are major problems.[7]

During growing age, healthy spine is a prerequisite. When kids are slouching over a cell phone for many hours every day, their neck and back muscles are affected. So, they experience nagging pain in these parts. Pain, strain and aching neck muscles can cause a severe headache. Berolo, Wells, & Amick III in 2011 and Ning, Huang, Hu, & Nimbarte, in 2015, mentioned that hand held devices like smart phones and tablets have been associated with musculoskeletal pain and discomfort, particularly in children.[8][9]

Riadh et al., 2011 stated that as kids develop more quickly, their cell division is faster. Also their heads are more slender, hence they have bigger effect on their brain. Now, the prevalence of mobile phones use is increasing in children and possibility of children to be more sensitive than adults to potential adverse effects from exposure to RF-EMF, continuous monitoring of children's health in relation to mobile phones and tablets is needed.

Children spend 7-8 hours of time on smart phones/ computers alone, which is longer than they spend on any other activity. [10]

As children get used to personal space, it may further decrease children's social interaction. Face-to-face social interaction during childhood, is a major factor affecting the development of children's social competence [11] There is also the possibility of bidirectional associations between child social-emotional development and media use.[12]

Use of cell phones prior to sleep may affect the initial part of sleep by making changes in brain activity.[13]

Excessive screen time may lead to accelerated myopia excessive screen time.[14] Mobile phones are smaller devices that are generally held at a distance of 20–30 cm from the eyes, leading to digital eyestrain. It has been reported by Long et al that if the viewing

distances are closer, the resulting eyestrain symptoms are greater after reading for 60 min from a smartphone.[14]

Mostly digital screens are backlit and emit blue light or high-energy visible (HEV) light wavelengths. It has been found that eyes are sensitive to blue light exposure. Gradually, after a passage of time the overall damage may increase the chances and severity of eye disorders. (e.g. age-related macular degeneration and cataracts)[13]

Pediatric age group is growing up with touchscreen technology at their fingertips The speculations that the rising prevalence of asthenopia in the young may be due to over use of smart phones seems to be reasonable.[11]

An eye examination, reducing the amount of time that a child can continuously use the computer, the position of the computer, the lighting for glare on the computer screen, reduction of the amount of lighting in the room are the requirements for appropriate viewing habits in children.[14]

Similarly,the use of smart phones or tablets should also be under proper guidance by following sop of their usage.

Kesari *et al*. reported that mobile phone radiation may increase the reactive oxygen species, which plays an important role in the development of metabolic and neurodegenerative diseases. In an animal study,chronic exposure to Wi-Fi radiation appeared to cause behavioural alterations, liver enzyme impairment, pyknotic nucleus, and apoptosis in brain cortex.

With the advent of digital technology, entire population is exposed to some or the other effects of technology. Earlier generations have never been exposed during childhood and adolescence to this kind of radiation. Hence, it is going to take a long time to evaluate and conclude about the effects of radiation on younger population. Neuro-degenerative and carcinogenic changes take decades to manifest. But when earlier studies have already proved,use of smart phones as one of the possible causes of rise in cancer, education policy makers must keep in mind these established facts while designing education policy for younger population of the country.

In India, Swayam Prabha, a group of 32 Direct To Home (DTH) channels is devoted to telecasting of educational programs round the clock . The programmes are available across the country. The channels air courses for school education (class 9-12), higher education (undergraduate, postgraduate) as well as for out-of-school children, vocational education and teacher training.

A start up Think Zone" in Odisha, India has taken an initiative to reach out to children with no access to internet facility. It is broadcasting activity based learning modules with the help of a local radio channel for students aged 3 to 10.

Similarly, there is a need to explore other options of teaching and learning which are less harmful to paediatric age group. Many intellectuals assert that education system was already losing its relevance but the varying effectiveness of online learning cannot compete with the absence of classrooms.

Conclusion

To think of any long-term effects of the current online education scenario, will be a hasty step. But definitely a serious thought must be given to the future consequences of screen use by pediatric age group. Perhaps it is too necessary to educate children, but it still isn't that big problem at all. So, it is the need of the hour to think about affordability of imparting education at the cost of health. Thus, the other ways out for educating children must be explored. More the hours of use, more will be the absorption of radiations, so children must be taught to refrain from mobile phones. Landlines, Skype, and computer phone services, when connected to the internet with a cable, don't give off radiations, therefore the parents should take care that kids use those. The Wi-Fi routers in the home should be placed away from where people, particularly children, spend most of their time. To keep children healthy, overuse of cell phones and other wireless devices by kids must be restricted. We must not undermine children's mental and physical state day-by-day, caused by the excessive usage of cell phones for education as well as recreation.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Indirect Impact of COVID-19 Lockdown on Society and Environment

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Abstract

In a fairly short time, the worldwide spread of COVID-19 has brought a dramatic reduction in industrial activities, road traffic and tourism. During this time of crisis restrained human interaction with nature has appeared as a blessing for nature and the environment. Reports from around the world indicate that environmental conditions, including air quality and water quality in rivers, are improving after the COVID-19 outbreak, and wildlife is blooming. India has always been a pollution hub with enormous populations. But since COVID-19 announced the lockdown, air quality has begun to improve and all other environmental parameters such as water quality in rivers have begun to offer a positive sign of restoration.

Keywords: *COVID-19, Lockdown, Impact, society, Environment, Trade, Unemployment.*

Introduction

As of 21 August 2020, Covid-19, who emerged in Wuhan somewhere back eight months in December 19, has now taken shelter in host bodies in 213 countries across the globe infecting 22,589,017 people and 792,475 deaths. India has also reported more than 2,905,823 positive cases with 54,849 death tolls on the same day[1]. The disease remains uncontrolled, and the virus has no proven cure. Locking in homes and social distances is the only preventive step the country as a whole is taking. But as the human activities in most areas are limited, the country's natural environment has begun to heal itself. Factories, trade, aircraft and aviation are all at a standstill. Social media has immerged out as platform for people to stay connected with each other. Lockdown due to COVID-19 has also affected employment status of whole world. Worldwide import and export has been reduced substantially.

Aims and Objectives:

1. Discussion about the various effects of lockdown due to COVID-19 on environment, mental health, trade, unemployment etc.
2. Try to find solutions on adverse effects.
3. Try to find out solutions to maintain positive effects of lockdown.

Materials and Method

Material and data for this article is collected from authentic official websites and news channel reports from national and international platform.

Result

Due to lockdown, environment and society is influenced as follows:

Air Quality: Thanks to the effect on travel and industry of the coronavirus outbreak many regions

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and the world as a whole witnessed a decrease in air pollution.[2] The Energy and Clean Air Research Centre estimated that strategies for suppressing coronavirus spread, such as quarantines and travel bans, resulted in a 25% reduction in carbon emissions.[3] NASA uses an instrument for ozone monitoring (OMI) to measure and monitor the ozone layer and contaminants such as NO2, aerosols and others. According to scientists at NASA, the decline in NO2 emissions originated in Wuhan, China and spread gradually to the rest of the planet.[4]

Water Quality: Water in the canals in Venice cleared and saw greater flow of water[5]. The increase in water clarity was attributed to the settling of sediment which is disturbed by boat traffic and the decrease in air pollution along the waterways listed.[6]

Wildlife: Some animals have been seen in towns as people remain at home due to lockdown and travel restrictions. Sea turtles were seen laying eggs on the beaches they once avoided (such as the Bay of Bengal coast), due to lower human intrusion and light pollution rates.[7]

German scientist Rainer Froese has said that fish biomass will increase due to the sharp decline in fishing, and has projected that some fish such as herring could double their biomass in European waters.[8]

Carbon Emission: A study published in May 2020 found that the daily global carbon emissions declined by 17 percent during the lockdown steps in early April and could lead to an annual decrease in carbon emissions of up to 7 percent, which according to researchers would be the largest drop since World War II. They attribute these declines largely to reducing the usage of transport and manufacturing activities.[9]

Public Mobility: Public mobility has deteriorated dramatically due to constraints, including the imposition of a lockout in several countries, a Google Study reveals. Across India, shopping and leisure places saw the steepest decline across people's presence between February 16 and March 29 relative to traffic between January 3 and February 6. There was a significant rise in phone traffic from homes, meaning more people were staying at home. Visits to restaurants, shopping malls and movie theatres plummeted by 77 per cent between Feb. 16 and Mar. 29 compared to the period from Jan. 3 to Feb. 6. Visits to stores, markets and pharmacies have dropped by 65%. Visits to national parks, beaches and public gardens have been reduced by 57%. Compared to the period between Jan. 3 and Feb. 6, the use of public transport facilities such as buses and trains fell 71 per cent between Feb. 16 and Mar. 29. Workplace visits declined 47 per cent as many people were told to work from home. People stayed 22 per cent more at home than they did between January 3 and February 6.[10]

Impact on Trade: Trade in United States has been declined which shows impact on the trade in other countries of world.

Table: Effect of lockdown on Trade

Sr.No.	Trade	Export	Import
1	Passenger cars and light trucks	Decreased by 9.8 percent	Decreased by 6.2 percent
2	Engine and parts	Decreased 28.9 percent	Decreased by 23.0 percent.
3	Crude oil	Decreased 7.9 percent	-
4	Refined petroleum products	Decreased 9.8 percent	-
5	Medical equipment	Increased 24.2 percent	-
6	Medical diagnostic equipment	Increased 34.8 percent	-
7	Medical diagnostic products	-	Increased 44.4 percent
8	Disinfectants and sterilization products	Increased by 14.4 percent	-

Impact on Unemployment: The International Labour Organization (ILO) projected that the spread of corona virus would threaten more than 25 million jobs globally. Four out of five people (81 per cent) in the 3.3 billion global work forces are currently impacted by the complete or partial closure of the workplace. The United States, the United Kingdom, Canada and most European and Asian countries have already registered huge jobs

losses leading to significant increase in unemployment. The ILO in its report '', describes COVID-19 as 'worst global crisis since World War II'[11].

Low-paid and low-skilled informal workers are particularly concerned in low- and middle-income countries, where the industries and services have a high proportion of these informal employees, who account for 61 per cent of the global workforce or 2 billion men, and lack any social security. The sudden loss of livelihood will make them horrifying.

The CMIE (Centre for Monitoring Indian Economy) report showed a major job loss and corresponding substantial unemployment rate rise in March 2020. The unemployment rate in March 2020 stands at 8.7 per cent, which is far higher than the government's estimate of unemployment in 2017-18 at a 45-year high of 6.1 per cent. This is the highest jobless rate since September 2016. Over the same time, the unemployed have also risen from32 million to 38 million. The situation got worse when we went into the lockdown time last May week and the unemployment rate rose to 23.48%.[11]

Role of Social media during lockdown: During the lockout the social media plays a significant role. During the lockdown social media always update people's current affairs. The role of social media is very important in managing the anger, tension, and fear of the people and in helping they update the current scenario. Also Study indicates that active use of social media is better for you than passive use, so consider writing a blog or sharing status updates as a way to connect. And it will help you organize virtual encounters with family and friends. These should not limit themselves to chatting; they will engage in community activities such as watching films, playing drama and online conversation through video calls and share their views on academic aspects and other aspects of cooking together. Teachers are conducting online lectures for their students so they do not skip their academics by use of social media applications during the lockdown.

Only a couple of days of isolation may cause anxiety and depression to develop. Add to it the imminent possibility of constantly hammering a terrible disease through the media, and you have a prescription for several shades of mental and physical distress.

Impact on Mental health and behavior: Human beings have evolved into social beings, and are designed to live in collaborative communities. Feeling removed from families, friends and employers can be unbalanced and stressful for most people and can lead to psychological and physical health issues in the short or even long term. Psychological consequences of isolation may include a rise in levels of anxiety, aggression, depression, forgetfulness and hallucinations. In those with inherent pre-existing susceptibilities psychiatric disorders that are precipitated and even manifest without precondition in several others. Personal relationships help us cope with tension, and if we lack this outlet for steam, a massive emotional voice will result which, for an average person, is difficult to deal with.

Solitary inmates and patients in segregated medical facilities have also had negative psychological effects including elevated anxiety, panic attacks and elevated paranoia rates. This has been found that social isolation has a correlation with greater alcoholism. Researchers who had to live in remote areas such as deserts, the Antarctica, forests and outer space have confirmed that overcoming loneliness is a tough challenge. Yossi Ghinsberg, an Israeli explorer who spent several weeks alone in the Amazon, admitted making imaginary companions to fend off the loneliness. The shutdown of Covid has put forced isolation on many.

Young people live away from their families, trapped in small apartments with abysmal cooking skills. Many senior citizens living on their own seek companionship by meeting up in community parks with age mates. We find themselves marooned, robbed of social contact and even from their children's sporadic visits. Lack of playtime with peers causes children to become irritable and edgy.

Staying in a confined space with family is not a pink picture for everyone. It may be extremely oppressive and claustrophobic for large low-income families huddled together in small single-room houses. Children are not lucky enough to have a lot of board/electronic games or books to keep them busy. Add to this the profound uncertainty of running out of food funds and basic necessities. On the other hand, there are people with dysfunctional family dynamics, such as dominant, abusive or alcoholic partners, siblings or parents, who make a period of trial stay at home. There has been a worldwide increase in the incidence of suicide and physical abuse against women. Higher anxiety and depression often impair an individual's immune system and make them more vulnerable to disease.

Discussion

The issue today is not whether the Covid-19 passage will bring similar socio-political changes; experts predict it will be a long and damaging fight. This needs to be preparing for the long term. We need to control our population's physical and psychological harm by urgently diverting full resources to improve our health-care system. We have to maintain a fair distribution of food and essential needs among the less favoured groups. Before the lockout we tripped badly by misunderstanding their worries. Even more bungling will lead to messy rioting scenarios and food theft. The Government needs to move into action quickly to prevent losing legitimacy. A higher death toll also means that many families deprive members of their earnings and put greater burden on the welfare system. Throughout India, losses are the highest in the males of the 22-40 year age group, which is also the most active.

Import and export trade has decreased all over the world due to lack of transport; but environment is seen to be recovering up to great extent.

Today, social media like Twitter, Face book and Instagram have become the main information sources. They are also platforms for misinformation and false news.

Conclusion

Stigma and blame targeted at outbreak-affected communities may hinder international trade, finance, and relationships, prompting further unrest. Care needs to be taken to erase the stigma associated with disease, racism, religious propaganda and psychosocial impact and to be implemented through regular discussions with trained and specialist healthcare staff by setting up task forces and execution teams that are directly involved in healthcare delivery systems without creating any communication gaps between policy makers and ground level.

It is desperately necessary to set up mental health organizations specific to future pandemics with branches in many nations and individual healthcare institutions for research, delivery of mental healthcare and organization of awareness programs at both personal and community levels. To relieve psychological anxiety in the general population over this continuing pandemic, standardized websites and toll-free helpline numbers can be introduced. Social media should be used in good sense to educate people about dynamics of transmission, disease symptoms, and time when precise medical consultations are required. Strict government laws and legislation regarding fake news, social media rumours, disinformation and misinformation should be implemented to protect social media from devaluations.

The COVID-19 pandemic has clearly shown us how, even in the 21st century, a "virus" can adversely impact our lives and at the same time make us aware that the greatest assets of humanity are health, goodwill, compassion, unity, creativity and awareness.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

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Assessment of Age in Female Population by Radiographic Examination of Carpal Bones

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Abstract

Introduction: Assessment of the age of an individual from the appearance and the fusion of the ossification centers is a well-accepted fact in Medical and Legal professions. In the age estimation of juvenile offenders, radiological examination of bones gives valid idea of age needed in courts.

Objectives: Aim was to assess the age of the ossification of carpal bones at wrist joint in females. The objectives were to estimate the age specific difference in all subjects, also to study difference age of ossification in central part of India with other part of country and abroad.

Method: After obtaining the ethical clearance the subjects below the age of 20 years residing in central India were included in this study. As per inclusion criteria carpal bones forming wrist joint were examined radiologically with standard accepted procedures and guidelines to assess the age.

Result: The outcome of this study was very fruitful and knowledgeable as it revealed age specific difference and remarkable difference in the age of ossification as compared to the foreign countries and other parts of India.

Conclusion: Radiological examination plays very vital role in age estimation in judicial cases. It can be concluded that such studies should be conducted with good sample size and at regular intervals for academic as well as in judicial point of view.

Keywords: *Age, Asses, Ossification, Wrist joint, Radiological.*

Introduction

Age is helpful in identification of an individual which in turn is helpful in both civil and criminal cases according to Sangma William Bilkey ch. et al[1]. It has been also stated that the study of epiphyseal union of bones is considered a reasonable scientific and accepted method for age determination by the law courts all over the world[2]. **Forensic Osteology** is the branch of Forensic Medicine which deals with the study of the bones. Epiphysis of the bones unites during age periods which are remarkably constant for a particular epiphysis[3]. Epiphysis of the bones unites at the particular age and this is helpful in the age determination. In law the crime and punishment is entirely based on the criminal responsibility and this in turn depend on the age of a person[4].Reddy KSN stated that (2009) the bones of human skeleton develop from a number of ossification

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centers. At eleventh to twelth week of intrauterine life, there are 806 centers of ossification, at birth there are about 450. The adult human skeleton carries only 206 bones[5]. India is a vast country with diversity in social customs, multiple religions, dietary habits and variations in climatic conditions. In Modi's textbook it is quoted that owing to variation in climatic, dietetic, hereditary and other factors affecting the people of the different states of India, it cannot be reasonably expected to formulate a uniform standard for the determination of the age of the union of epiphyses for the whole of India[6]. Mehta Homi S (1963) observed that it has been approved by research in our country that the epiphysio-diaphysial union in Indian occurs about a year or two in advance of the age at which that occurs in Europeans[7]. According to Parikh CK Union of epiphysis in cartilaginous bones takes place earlier in the females by about 2 years than in males except in case of skull sutures where obliteration sets in little later and proceeds more slowly in females than in males and under tropical conditions ossification is observed earlier than in temperate areas[8]. According to Iscan M.Yasar and Loth Susan R(1984) estimation of age from the adult skeleton has been the most difficult part of Human identification studies. Human growth is continuous process which goes through first, a developmental stage and second, the maintenance of status quo. In the developmental stage, changes in skeletal and dental morphology occur in an age –age predictive sequence[9]. Jit and Balbir Singh revealed that the Precocity of epiphyseal union has been attributed to racial and climatic factors. Works in different regions of India-North (Punjab, Delhi and UP), East (Bengal) and South (Madras) have given different ages of fusion of the epiphysis. Further, workers in the same region have also given different ages of fusion of the epiphysis of the same bone and in the same sex. This difference could possibly be due to in adequate material or recording of incorrect ages of the subjects[10].

Hence, it was, decided to reestimate age radiologically in the central part of India, by including authentically examined subject, with valid age proof belonging to indigenous population.

Aims and Objectives: Radiological evaluation of carpal bones forming the Wrist joints (Scaphoid, Lunate, Triquetral, Pisiform, Trapezium, Trapezoid, Capitate, and Hamate)in female subjects was undertaken with the following aims and objectives:

1. To estimate the age from ossification of carpal bones of Wrist joint in relevant Subjects belonging to indigenous population of the central India.
2. To assess the age specific difference in ossification of bones in all the subjects.
3. To assess and evaluate the difference in the ossification in Central part of the India with other part of India on the basis of literature available.
4. To compare the deduced data with previously available data of other countries.

Material and Method

50 female subjects from age 0 to 20 years, were included in this study. The individual chosen for the study were evaluated and confirmed for the authentic proof of date of birth, physical fitness and their native place of central India. Individual involved in study were predominately right handed. A written informed consent and permission from Ethical Committee of the Institute was obtained.

Procedure of Radiography: After taking the written consent, the thorough physical examination and radiological evaluation was done. Training of Researcher was undertaken to get well acquainted with all the radiological procedures essential for X-Ray examination and developing X-Ray films. X-Rays were taken with the help of X-Ray machine in the Department. Minimum shots were taken to expose the joints involved in study and minimum and appropriate voltage settings of X-Ray machine were applied so as to avoid unnecessary radiation exposure of the subjects to get the desired qualities of X-Rays. The developed X-Ray films were studied and reporting was done with the help of by experienced Radiologist and Anatomist. All the radiological procedure was undertaken according to the present accepted standards. Skeletal maturity was evaluated according to the **Jits** and **Kulkarnis classification** as **Appeared or non appeared**[11].

The master chart was prepared and tabulated & was classified, analysed and compared with known standards. Data analysis was done in P4 computer using HPSS software. At the end conclusions were drawn which are compared with available results of various previous studies.

Table 1: Appearance of carpal bones in Female on right side.

Agein years	Scaphoid	Lunet	Triquetral	Pisiform	Trapezium	Trapezoid	Capitate	Hamet
<1-5	1(2%)	5(10%)	8(16%)	0(0%)	1(2%)	0(0%)	9(18%)	9(18%)
6-10	13(26%)	13(26%)	13(26%)	6(12%)	13(26%)	13(26%)	13(26%)	13(26%)
11-15	12(24%)	12(24%)	12(24%)	11(22%)	12(24%)	12(24%)	12(24%)	12(24%)
16-20	15(30%)	15(30%)	15(30%)	15(30%)	15(30%)	15(30%)	1(2%)	15(30%)
Total	41(82%)	45(90%)	48(96%)	32(64%)	41(82%)	40(80%)	49(98%)	49(98%)
ϰ2-value	26.96							
p-value	0.17, NS, p>0.05							

Scaphoid, Lunet, Triquetral, Trapezium, capitate, Hamate appeared in 1 (2%), 5 (10%), 8(16%), 1(2%), 9(18%), 9(18%) respectively in <1-5 age group. Pisiform and Trapezoid appeared in 6(12%), 13(26%) respectively in 6-10 age group.

Table 2: Comparison of the findings of the present study with the previous studies (in Yrs and months)

	Galstaun (Bengalis)	Nandy Apurba	Flecker (Australians)	Davies & Parsons (Englanders)	Present study
	Year	Year	Year	Year	Year
Triquetral					
Appearance	2-3	3	11/2	2-4	3
Lunate					
Appearance	2-3	4	4	3-4	4
Trapezium					
Appearance	4-7	4-5	5	6-7	5-6
Trapezoid					
Appearance	5-6	4-5	5	6-8	6-7
Scaphoid					
Appearance	6	6	5	6-8	5-6
Pisiform					
Appearance	9	9-12	9	12	9-10
Capitate					
Appearance	½	1	½	3-5	1
Hamate					
Appearance	1-14 mon.	1	6-10 months	4-5 months	1

Discussion

Scaphoid: In the female age groups of <1-5, 6-10, 11-15,16-20 years Scaphoid had appeared in 1(2%), 13(26%), 12(24%), 15(30%)%)subjects respectively .Scaphoid appeared earliest at 5-6 year in female subjects on right side.Galstaun G. (1937) in Bengali population found appearance of Scaphoid at 6 years in female[12]. According to present study Scaphoid appeared 1 years earlier in females than in Galstaun G study. Flecker (1942) in Australians population found that the appearance of Scaphoid at 5 years in female,but in present study Scaphoid has appeared 0-1 year later[13]. Davies & Parsons (1927) in his study on Englanders found that the Scaphoid appears at 6 - 8 years, which appeared 0 - 3 year earlier in current study[14]. According to Nandy A (2000) Scaphoid appears at 6 years, which is observed 0 - 1 year earlierpresent study[15].

Lunate: In the female age groups of <1-5, 6-10, 11-15, 16-20 years the Lunate had appeared in 5(10%), 13(26%), 12(24%), 15(30%) subjects respectively. In this study Lunate was appeared earliest at 4 years in females subjects.Galstaun G. (1937) in Bengali population found appearance of Lunate at 2-3 years, but in present study Lunate appeared 1-2 years later[12].

Flecker (1942) in Australians population found that the appearance of Lunate at 4 years in males, matching to this study[13].

Triquetral: In the Female age group of <1-5, 6-10, 11-15, 16-20 years Triquetral had appeared in 8(16%), 13(26%), 12(24%), 15(30%) subjects respectively. It can be concluded that, centre for Triquetral appeared at 3 year. Galstaun G. (1937) in Bengali population found appearance of Triquetral at 2-3 years. In comparison with this study in present study Lunate appeared nearly at similar age. Flecker (1942) in Australians population found that the appearance of Triquetral at 1 ½ year. On contrary to his findings in present study it is found that Triquetral has appeared 1 ½ year later.

Pisiform: In the female age groups of 6-10, 11-15, 16-20 years, Pisiform had appeared in 6(12%), 11(22%), 15(30%) subjects respectively. In this study Pisiform was appeared earliest at 9-10 years. Galstaun G. (1937) in Bengali population found appearance of Pisiform at 9 years in female, it is matching with present study. Flecker (1942) in Australians population found that the appearance of Pisiform at 9 year in females, in present study Pisiform has appeared 0 - 1 year later.

Trapezium: In the female age group of <1-5, 6-10, 11-15, 16-20 years, Trapezium had appeared in 1(2%), 13(26%), 12(24%), 15(30%) subjects respectively. In this study Trapezium was appeared earliest at 5-6 years. Galstaun G. (1937) in Bengali population found appearance of Trapezium at 5-6 years, in present study Trapeziumappeared nearly at similar age. Flecker (1942) in Australians population found that the appearance of Trapezium at 5 year in females, but here it has appeared 0 - 1 year later.

Trapezoid: In the female age group of 6-10, 11-15, 16-20 years, Trapezoid had appeared in 13(26%), 12(24%), 15(30%) subjects respectively. In this study Trapezoid was appeared earliest at 6-7 years. Galstaun G. (1937) in Bengali population found appearance of Trapezoid at 5-6 years which appeared 1-2 years later in present study. Flecker (1942) in Australians population found that the appearance of Trapezoid at 5 year, on contrary in present study it appeared at 1-2 years late.

Capitate: In the female age groups of 6-10, 11-15, 16-20 years, Capitate had appeared in 9(18%), 13(26%), 12(24%), 15(30%) subjects respectively. In this study Capitate was appeared earliest at 1 years. Galstaun G. (1937) in Bengali population & Flecker (1942) in Australians population found appearance of Capitate at 6 months, which appeared nearly 6 months later in present study.

Hamate: In the female age group of 6-10, 11-15, 16-20 years, Hamate had appeared in 9(18%), 13(26%), 12(24%), 15(30%) subjects respectively. In this study Hamate was appeared earliest at 1 years. Galstaun G. (1937) in Bengali population found appearance of Hamate at 1-14 months in present study Hamateappeared 2 months later[12]. Flecker(1942) in Australians population found that the appearance of Hamate at 6 – 10 months, on contrary to his findings in present study it is found that Hamate has appeared 2 – 6 months later[13].

Summary and Conclusions

1. This study was conducted exclusively on the young indigenous male population of Central India keeping in mind that very less literature about the age estimation from ossification of Wrist joint on both the right and left side is available involving this particular region of India.
2. The ossification of carpals at the Wrist joint in females on right is completed in all instances at the age 9-10 year respectively.
3. By comparing the available literature, the age of skeletal maturity in females in this region is nearly similar to those in state ofWest Bengal.
4. By comparing the available literature ossification center appeared one to two years earlier in this study with population of Central India than those in Australia and England.
5. As this study is done in Central India region the application of standards of this study can be considered ideal for application in the region of Central India.
6. As the sample size is limited further studies are necessary. Region wise studies should be conducted for better correlation and comparison.
7. Due to changing life style pattern, dietary, climatic,

behavioral factors age of ossification is changing as mentioned in the available literature. So as to evaluate these changes, studies are recommended in every region of India at regular time period for academic and judicial interest.

8. Along with the clinical and dental examination, radiological study plays an import role to arrive at the opinion about the age in medicolegal cases.

9. The opinion about age should be given always in the range. From this study range of 1-2 years of margin of error can be concluded.

Conflict of Interest: No conflict of interest was reported during conduction of study and preparation of this research article

Source of Funding: No fund was taken to carry out this study and preparing this article

Ethical Clearance: Due ethical clearance from institutional ethics committee was taken to undertake this study.

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Yash Scoring System in the Diagnosis of Acute Appendicitis

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Abstract

Background: Acute appendicitis is amongst the most encountered emergencies seen in casualties requiring intervention. The incidence of negative laparotomy is 15% to 25% and is associated with notable morbidity. Scoring systems are useful and logical for distinguishing acute appendicitis from non-specific abdominal pain. Presently many scoring systems exist that aids in the diagnosis of acute appendicitis but still fail to decrease the rates of wrong diagnosis and the negative appendicectomy rate. This study has been undertaken to evaluate the efficacy of the newer Yash scoring system which has been found more effective for the Indian population in diagnosing acute appendicitis.

Method and Material: A prospective study of the Yash scoring system was done on 50 patients. The decision of an appendicectomy was taken by the consultant surgeon. The outcomes of the Yash scoring system in terms of sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated.

Results: The Yash scoring system had sensitivity, specificity, PPV, NPV, and DA of 94.28%, 93.33%, 97.05%, 87.50%, and 94% respectively.

Conclusion: The Yash scoring system was a good tool to diagnose acute appendicitis.

Keywords: *Negative appendicectomy, C-reative protein, Hyperaesthesia in sherrens triangle, Blumberg sign.*

Introduction

Acute appendicitis is amongst the many encountered emergencies seen in casualties requiring intervention. There is a 6% chance of appendicitis in the general population[1]. Though the mortality has declined from 26% to 1% with the dawn of broad-spectrum antibiotics and timely surgery, it remains 5 to 15% in the elderly [2]. The incidence of negative laparotomy is 15% to 25% and is associated with notable morbidity, the frequency being more in women of reproductive age group (till 45%) due to the commonness of inflammatorytubo-ovarian diseases, ectopic pregnancy, and other gynecological pathology[3]. The delay in diagnosis may lead to rupture of the appendix in 17% to 40% of cases mostly in extremes of age. In the adolescents and elderly, it is linked with dramatic complications like an intra-abdominal abscess, wound infection, and ultimately death [4]. Thus, confirmation of acute appendicitis is very crucial to achieve lower morbidity and mortality rates.

The regular laboratory investigations of blood and urine are necessary. Leukocytosis is a convenient finding but is non-specific and may be missing in the elderly [5]. C – Reactive protein is elevated in almost all acute inflammatory conditions, hence is considered as a non-specific marker but its estimation guides a surgeon in reducing negative appendicectomies[6,7].

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Magnetic resonance imaging (MRI) as an investigation is only taken typically in the pregnant population where radiation exposure is not warranted. It provides outstanding resolution and is highly efficient in diagnosing acute appendicitis. MRI has high sensitivity and specificity of 100% and 98% respectively. The PPV and NPV of MRI are 98% and 100% respectively. It is also operator-independent. The only issue is its higher cost, motion artifact, and complexity in reading MRI by non-radiologists with limited experience [6].

Despite the use of all these imaging techniques, the incidence of negative appendicectomy is not decreasing. These unmerited operations have a complication rate of approximately 13%, which is nearby to that of an inflamed appendix. Removal of a healthy appendix has a mortality of 0.65%. Protracted clinical observations aiming to reduce undesired operations may mean a delay in operations in 28% of cases and considerable danger of perforation[7,8,9].

Scoring systems are useful and logical for distinguishing pain of acutely inflamed appendix from vague abdominal pain. Presently numerous scoring systems exist that aid in diagnosing acute appendicitis. However, these systems do not replace clinical acumen thereby just helping in the determination of acute appendicitis and aids in approaching a conclusion, whether a particular case should be operated or not, thus lowering the negative appendicectomy rate (NAR)[10,11].

The study done by Lamture YR et al[12] in India on the Yash scoring system reveals a sensitivity of 99.48%, a specificity of 92.86%, PPV of 99.48%, and NPV of 92.86%.Though the validity of the Yash scoring system is promising for the Indian population, its only disadvantage was in the mode of a single study.

Despite various scoring systems and developments in the diagnostic and imaging modalities, the diagnosis of appendicitis is in dilemma which fails to decrease the rates of wrong diagnosis and the negative appendicectomy rate. This sequentially increases the cost for diagnosis by the use of expensive radiological modalities like Computed Tomography and MRI thus causing a delay in the treatment leading to an increase in morbidity and mortality of the patients.[13]

This non-randomized prospective study has been undertaken to evaluate the effectiveness of the newer Yash score which has been found more effective for the Indian population in diagnosing acute appendicitis.

Method

The present study was undertaken in the department of surgery, Jawaharlal Nehru Medical College, Wardha in collaboration with Datta Meghe Medical College Hingana, Nagpur, Datta Meghe Institute of medical science (DMIMS), Sawangi, Meghe, Wardha, Maharashtra India.

Study Design: Prospective non-randomized study.

Study Population: All patients of acute appendicitis with age >15 years and <60 years.

Study Duration: July 2018-July 2020.

Sample Size: 50 patients

Inclusion Criteria: All patients with right lower quadrant pain and clinically diagnosed as acute appendicitis of age >15 years and <60 years.

Exclusion Criteria:

1. Patients with appendicular mass.
2. Patients of appendicitis with a known case of connective tissue disorder.
3. Patients with a past history of renal or ureteric stones and pelvic inflammatory disease.
4. Pregnant women.

Ethical clearance was obtained from the Ethics committee of Datta Meghe Institute of Medical Sciences (Deemed to be University) [Ref. No. DMIMS(DU)/ IEC/2018-19/7426].This prospective non-randomized study was conducted in Acharya Vinoba Bhave Rural Hospital, Sawangi. All the patients who fulfilled the eligibility criteria were subjected to routine hematological investigations, C-reactive protein, USG, and were scored based on the Yash scoring system.

The components of the Yash scoring system are as follows:

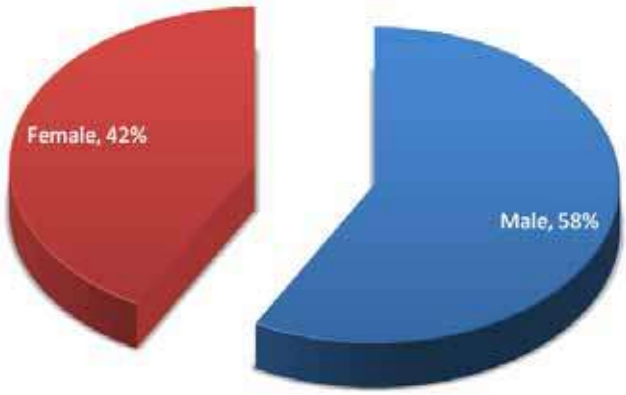
- Migration of pain to the right iliac fossa = 1 point
- Nausea and vomiting = 1 point
- Anorexia = 1 point
- Right iliac fossa tenderness = 2points
- Rebound tenderness = 1 point
- Hyperesthesia in Sherren's triangle = 1 point
- Fever = 1 point

- White blood cell count > 10,000 mg/dl =2 points
- C-reactive protein (> 15mg/dl) = 3 points
- Ultrasonography = 4 points

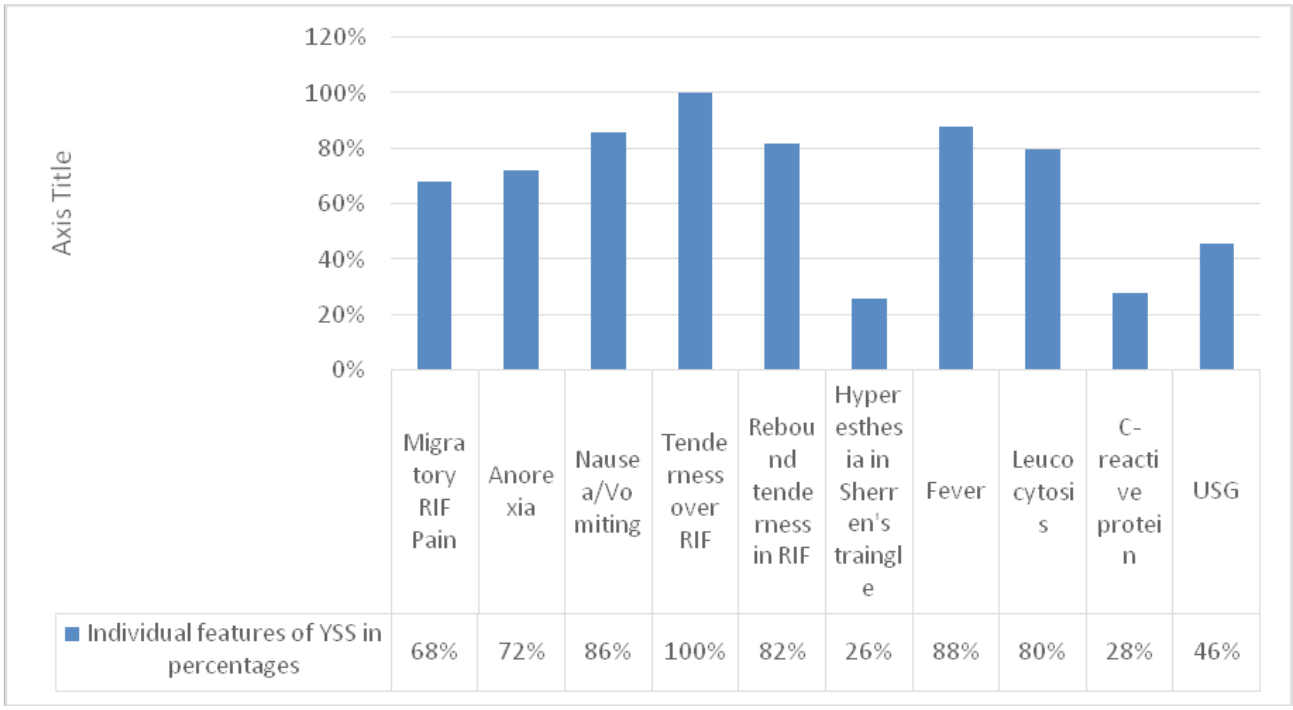
A score of 7 or more is suggestive of acute appendicitis requiring surgery[12].

The decision to operate was taken by a consultant surgeon by clinical diagnosis with help of other appropriate investigations in special circumstances. All patients underwent appendicectomy with prior consent and the specimen was subjected to histopathological examination for confirmation of diagnosis. The minimum criteria for acute appendicitis were the appearance of neutrophils in mucosa, submucosa, and lamina propria[1]. The result of the Tzanakis score and Yash score was reported independently. The result was correlated with the findings obtained on histopathological examination and the data was analyzed using the necessary statistical calculations using SPSS 24.0 version, the results were then presented.

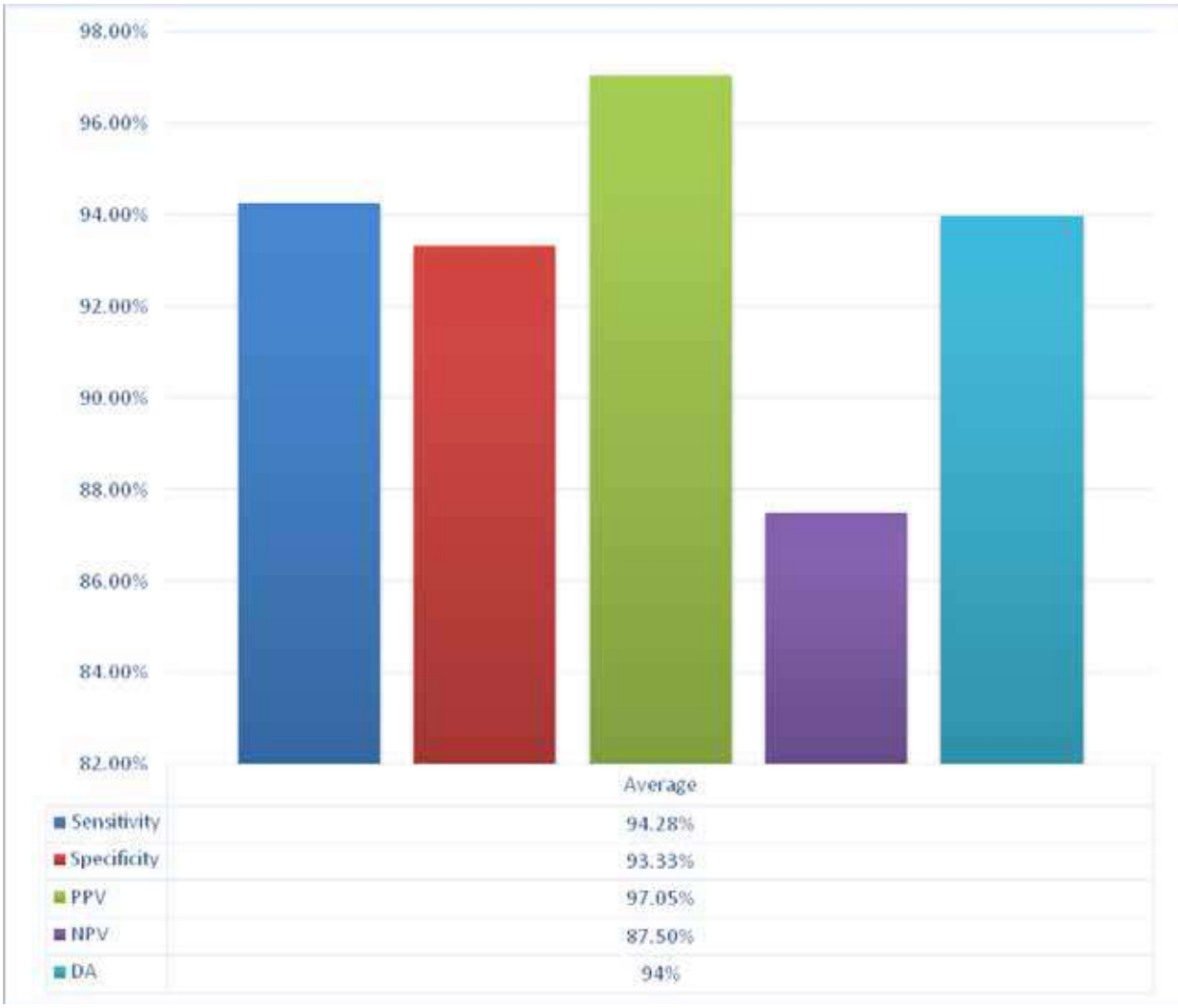
Observations and Results



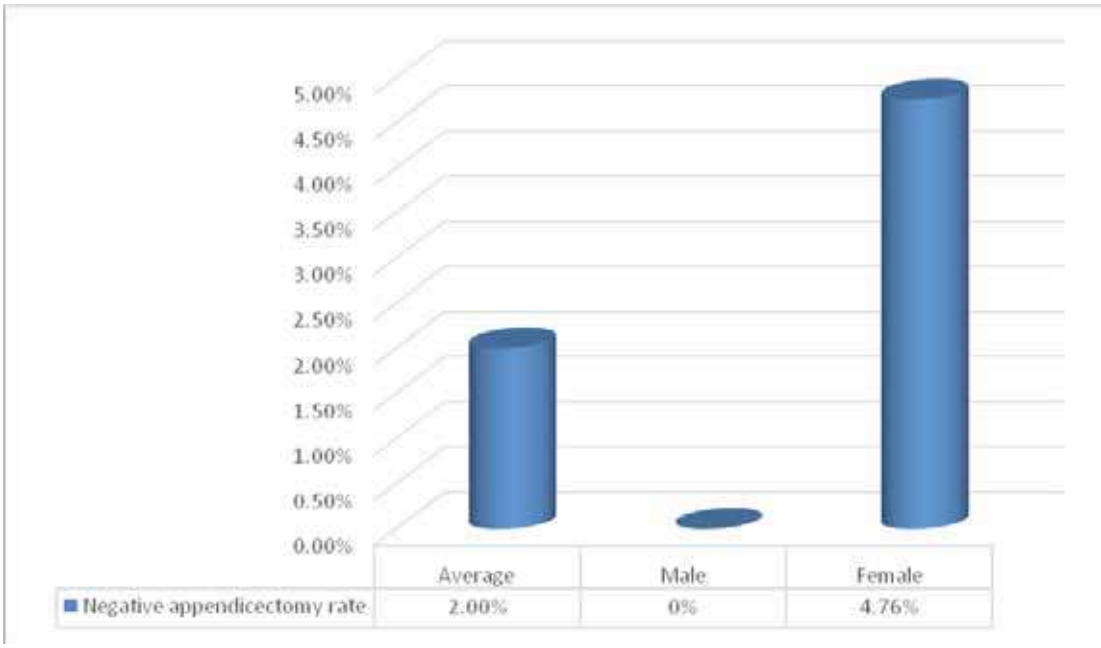
Graph No. 01: Gender distribution



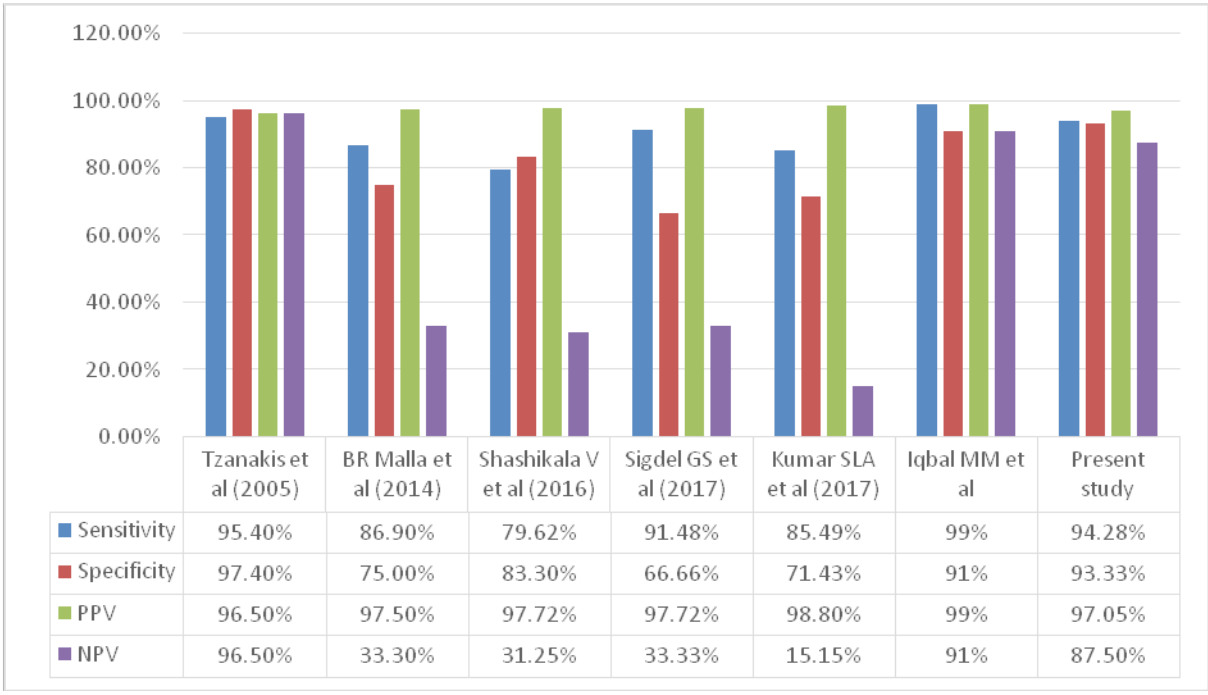
Graph 02: Characteristics of Yash score



Graph 03: Outcome of YASH scoring system



Graph 04: Negative Appendectomy rate of Yash scoring system:



	Tzanakis et al (2005)	BR Malla et al (2014)	Shashikala V et al (2016)	Sigdel GS et al (2017)	Kumar SLA et al (2017)	Iqbal MM et al	Present study
Sensitivity	95.40%	86.90%	79.62%	91.48%	85.49%	99%	94.28%
Specificity	97.40%	75.00%	83.30%	66.66%	71.43%	91%	93.33%
PPV	96.50%	97.50%	97.72%	97.72%	98.80%	99%	97.05%
NPV	96.50%	33.30%	31.25%	33.33%	15.15%	91%	87.50%

Graph 5: Comparison with various studies

Discussion

Acute appendicitis continues to exist as the most widespread emergency in the world. A delay in making a diagnosis is associated with various complications which increases the morbidity and mortality in patients therefore a prompt and reliable diagnosis of acute appendicitis is mandatory. Furthermore, negative appendicectomy also accounts for the loss of financial resources and is associated with morbidity in 10 to 15% of cases.

Despite big breakthroughs in the imaging field, there remains uncertainty in diagnosing acute appendicitis due to atypical presentations of the disease. It has been repeatedly shown that investigations like USG lack specificity due to its operator dependency whereas investigations like CT scan and MRI are highly-priced demand more advanced equipment and competency. This makes a detailed clinical examination with primary investigations such as leucocyte count as the backbone in diagnosing acute appendicitis. This has compelled many surgeons to use diverse scoring systems for diagnosing acute appendicitis. The clinical evaluation is reliable in 50% to 80% of cases. The evaluation is more complex in the extremes of age and women of reproductive age group due to atypical presentations.

The current study will deal with the evaluation of the Yash scoring system to diagnose acute appendicitis in a simple, reliable, and cost-effective way thereby reducing the negative appendectomy rate and thus morbidity associated with it.

In this study, the sample population consisted of 50 patients out of which 58% were males and 42% were females (see graph 01).

All the patients who accomplished the eligibility criteria were subjected to detailed clinical examination, routine hematological investigations, C-reactive protein, USG and were scored based on the Yash scoring system. The decision to operate the patient including patients with scores less than the cut-off value was based on the clinical assessment and judgment taken by the consultant surgeon. All the patients underwent appendectomy with prior consent and the specimen was subjected to histopathological examination to confirm the diagnosis.

In our study, 82% of the patients were operated by open method whereas 18% of patients were operated by laparoscopy. The study done by Malla BR et al[14] consisted of 200 patients in which 128 patients (64%) underwent appendectomy by an open method and 72 patients (36%) underwent appendectomy by laparoscopy.

The most common incision used in open appendectomy was Mc Burney's in about 83% of cases followed by the right Para median which was used in 17% of cases. Similarly, in the study done by Lamture YR et al[12] the most common incision used was Mc Burney's in 94.74% of patients followed by right Para median in 5.26% of cases. In our present study, 35 out of 50 patients (70%) had inflamed appendix intra-operatively. Out of the remaining 15 patients, five patients had enterocolitis, three patients had Meckel's diverticulitis, three patients had the pelvic inflammatory disease (salpingitis), two patients had a ruptured ovarian cyst and two patients had inflamed mesenteric lymph node (with or without pus) as the intra-operative findings. Shashikala V et al[9] in her study had five out of 50 patients with the alternative diagnosis; out of which one patient had enterocolitis and four patients had the pelvic inflammatory disease. Another study was done by Kumar SLA et al[13] his study also reported six patients with alternative diagnoses in which three patients had salpingitis, two patients had an ovarian cyst and one patient had Meckel's diverticulitis. The new Yash score described by Lamture YR et al [12] in 2017 has a significant role in identifying acute appendicitis. It differs from earlier scoring systems by including various parameters such as C-reactive protein, leukocyte counts, USG, and clinical data. A score of seven or more in patients was considered as acute appendicitis and such patients were subjected to operative intervention.

In our study, out of ten parameters, tenderness in the right iliac fossa was the commonest sign seen in 100% of patients. The other two signs i.e. rebound tenderness and hyperesthesia in Sherren's triangle was seen in 82% and 26% of patients respectively. The most common symptom was fever which was present in 88% of cases followed by nausea or vomiting which was found in 86% of the cases. Anorexia was seen in 72% of patients, whereas 68% of patients gave a history of migratory right iliac fossa pain. Out of the three investigations included in the scoring system, leucocytosis defined as WBC count more than 10000/mm^3 was present in 80% of cases. C-reactive protein with a value of more than 15mg/dl was present in 28% of cases with USG showing features of appendicitis in only 46% of cases (see graph 02).

In the present study, 34 patients had a score of seven or more, and16 patients who had a score of less than seven according to the Yash score. Out of the 34 patients who scored seven or more, there were 33 patients with features of appendicitis on histopathological examination whereas only one patient hada histologically normal appendix with no features of inflammation.Similarly, out of 16 patients who scored less than seven, there were 14 patients with a histologically normal appendix with no features of inflammation and only two patients with features of appendicitis on histopathology.

The sensitivity and specificity of the Yash scoring system in the present study were found to be 94.28% and 93.33% respectively. It had PPV and NPV of 97.05% and 88.50% respectively. The overall diagnostic accuracy of the Yash scoring system in our study was found to be 94% (see graph 03). The following results are comparable to the single original study done by Lamture YR et al in which the sensitivity, specificity, PPV, and NPV was 99.48%, 92.86%, 99.48%, and 92.85% respectively. The diagnostic accuracy of the Yash score reported by Lamture YR et al was 98.56%.

The negative appendicectomy rate (NAR) of the Yash scoring system in the present study was found to be zero percent in males and 4.76% in females. This discrepancy in NAR was due to the high probability of another possible diagnosis in females of reproductive age group such as pelvic inflammatory diseases and ovarian cyst. The overall NAR observed for the Yash scoring system was 2% (see graph 04) which is way lower than the accepted rate of 15 to 25%. In the study done by Lamture YR et al[12], the NAR was found to be 6.69% which is comparable to our study[14].

The negative appendectomy rate of the Yash scoring system was dramatically lower than other studiesand the diagnostic accuracy of this scoring system is better when compared with other studies (see graph no 5).

Conclusion

The study also shows that the Yash scoring system is a better tool to avoid unnecessary operations due to its exceptionally low negative appendectomy rate thus lowering morbidity in patients of acute appendicitis and thereby lowering the cost of treatment and prevention of misuse of valuable resources and manpower.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Tzanakis Scoring System in Acute Appendicitis Concern to Indian Population

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Abstract

Background: Acute appendicitis is amongst the most encountered emergencies seen in casualties requiring intervention. The incidence of negative laparotomy is 15% to 25% and is associated with notable morbidity. Scoring systems are useful and logical for distinguishing acute appendicitis from non-specific abdominal pain. Presently many scoring systems exist that aids in the diagnosis of acute appendicitis but still fail to decrease the rates of wrong diagnosis and the negative appendicectomy rate. This study has been undertaken to evaluate the efficacy of the Tzanakis scoring system for the Indian population in diagnosing acute appendicitis.

Method and Material: A prospective study to evaluate Tzanakis scoring system on 50 patients. The decision of an appendicectomy was taken by the consultant surgeon. The outcomes of the Tzanakis scoring system in terms of sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated.

Results: The Tzanakis scoring system had sensitivity, specificity, PPV, NPV, and DA of 71.42%, 66.67%, 83.33%, 50%, and 70% respectively.

Conclusion: Tzanakis scoring system in diagnosing acute appendicitis has a lower value in Indian populations.

Keywords: *Inflammation of the appendix,gangrene of appendix,perforation peritonitis.*

Introduction

Acute appendicitis is amongst the many encountered emergencies seen in casualties requiring intervention. There is a 6% chance of appendicitis in the general population[1]. Though the mortality has declined from 26% to 1% with the dawn of broad-spectrum antibiotics and timely surgery, it remains 5 to 15% in the elderly [2]. The incidence of negative laparotomy is 15% to 25% and is associated with notable morbidity, the frequency being more in women of reproductive age group (till 45%) due to the commonness of inflammatorytubo-ovarian diseases, ectopic pregnancy, and other gynecological pathology[3]. The delay in diagnosis may lead to rupture of the appendix in 17% to 40% of cases mostly in extremes of age. In the adolescents and elderly, it is linked with dramatic complications like an intra-abdominal abscess, wound infection, and ultimately death [4].Thus, confirmation of acute appendicitis is very crucial to achieve lower morbidity and mortality rates.

Ultrasonography (USG) with graded compressions has immense capacity in diagnosing appendicitis in its

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acute stage[5]. Various studies have been conducted on the utilization of these imaging systems. These studies reported a sensitivity of 75% to 94%, a specificity of 86% to 100%, and gross precision up to 96% [6]. Its superiority over computed tomography is the absence of exposure to ionizing radiation thus helpful in pregnant and pediatric patients. The major disadvantage is that it is operator dependent leading to inter-observer bias and failure to see the appendix during the scan does not rule out the possibility of having an inflamed appendix[5].

Despite the use of all these imaging techniques, the incidence of negative appendicectomy is not decreasing. These unmerited operations have a complication rate of approximately 13%, which is nearby to that of an inflamed appendix. Removal of a healthy appendix has a mortality of 0.65%. Protracted clinical observations aiming to reduce undesired operations may mean a delay in operations in 28% of cases and considerable danger of perforation [7].

Scoring systems are useful and logical for distinguishing pain of acutely inflamed appendix from vague abdominal pain. Presently numerous scoring systems exist that aid in diagnosing acute appendicitis. However, these systems do not replace clinical acumen thereby just helping in the determination of acute appendicitis and aids in approaching a conclusion, whether a particular case should be operated or not, thus lowering the negative appendicectomy rate (NAR).

The original study done by Tzanakis et al[8] in Greece reported sensitivity and specificity of 95.4% and 97.4% respectively. The diagnostic effectiveness of the score was 96.5% in his study. But in India, this scoring system was found inferior. For example, a comparative study between Tzanakis score and Alvarado score done by Shashikala V[9] in India revealed that Tzanakis scoring system had a sensitivity of 79.62%, a specificity of 83.3%, PPV of 97.72%, and NPV of 31.25% which was found better than Alvarado scoring system but still posing difficulties in diagnosis with a NAR of 12%. Another study was done by Iqbal MM et al[10] in Postgraduate medical center, Karachi, Pakistan revealed that the Tzanakis score had a sensitivity of 99%, the specificity of 91%, PPV of 99%, and NPV of 91% with a diagnostic accuracy of 95%. Similarly,A study done in Kathmandu Model Hospital, Nepal did by Sigdel GS et al [11] reveals that Tzanakis has a sensitivity of 91.48%, a specificity of 66.66%, and diagnostic accuracy of 90%.

Despite various scoring systems and developments in the diagnostic and imaging modalities, the diagnosis of appendicitis is in dilemma which fails to decrease the rates of wrong diagnosis and the negative appendicectomy rate. This sequentially increases the cost for diagnosis by the use of expensive radiological modalities like Computed Tomography and MRI thus causing a delay in the treatment leading to an increase in morbidity and mortality of the patients.

This non-randomized prospective study has been undertaken to evaluate the effectiveness of the Tzanakis score in diagnosing acute appendicitis.

Method

The present study was undertaken in the department of surgery, Jawaharlal Nehru Medical College, Wardha in collaboration with Datta Meghe Medical College Hingana, Nagpur, Datta Meghe Institute of medical science (DMIMS), Sawangi, Meghe, Wardha, Maharashtra India.

Study Design: Prospective non-randomized study.

Study Population: All patients of acute appendicitis with age >15 years and <60 years.

Study Duration: July 2019-July 2020.

Sample Size: 50 patients

Inclusion Criteria: All patients with right lower quadrant pain and clinically diagnosed as acute appendicitis of age >15 years and <60 years.

Exclusion Criteria:

1. Patients with appendicular mass.
2. Patients of appendicitis with a known case of connective tissue disorder.
3. Patients with a past history of renal or ureteric stones and pelvic inflammatory disease.
4. Pregnant women.

Ethical clearance was obtained from the Ethics committee of Datta Meghe Institute of Medical Sciences (Deemed to be University).This prospective non-randomized study was conducted in Acharya Vinoba Bhave Rural Hospital, Sawangi. All the patients who fulfilled the eligibility criteria were subjected to routine hematological investigations, USG, and were scored based on Tzanakis scoring system.

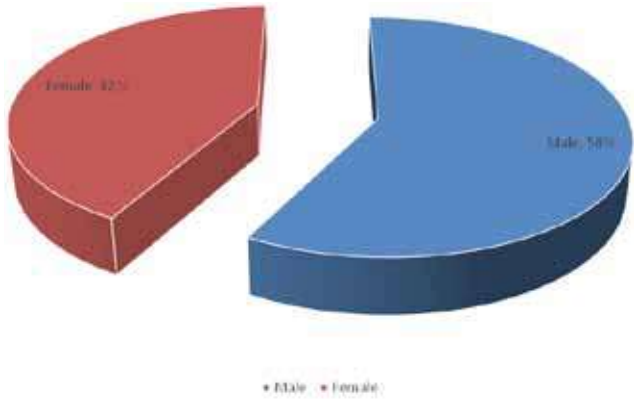
Tzanakis scoring system combines 4 variables as follows:

- Presence of right lower abdominal tenderness= 4 points
- Rebound tenderness(Bloomberg sign)= 3 points
- Laboratory findings: the presence of white blood cells greater than 12,000 in the blood= 2 points
- Ultrasound findings: the presence of positive ultrasound scan findings= 6 points

A score of 8 or more is suggestive of acute appendicitis requiring surgery [8].

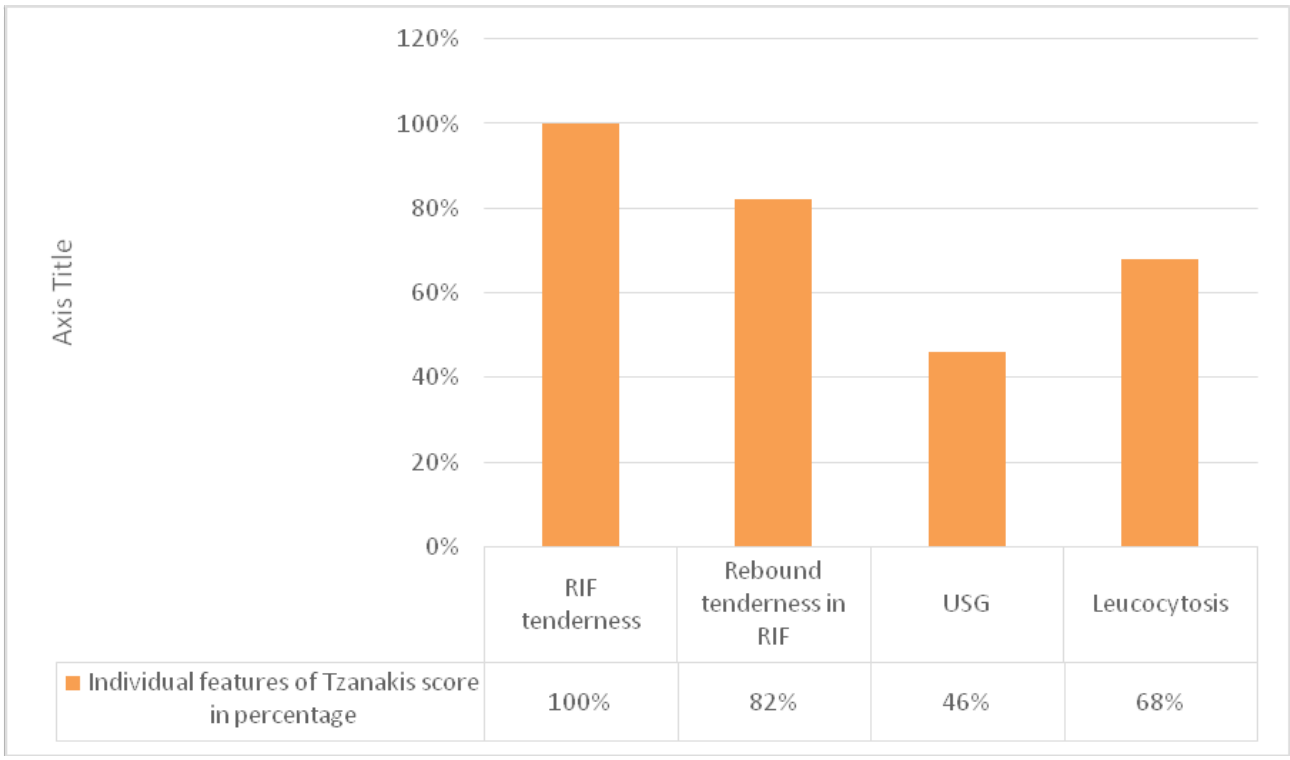
The decision to operate was taken by a consultant surgeon by clinical diagnosis with help of other appropriate investigations in special circumstances. All patients underwent appendicectomy with prior consent and the specimen was subjected to histopathological examinationfor confirmation of diagnosis. The minimum criteria for acute appendicitis were the appearance of neutrophils in mucosa, submucosa, and lamina propria[1]. The result of the Tzanakis score was calculated. The result was correlated with the findings obtained on histopathological examination and the data was analyzed using the necessary statistical calculations using SPSS 24.0 version, the results were then presented.

Observations and Results:

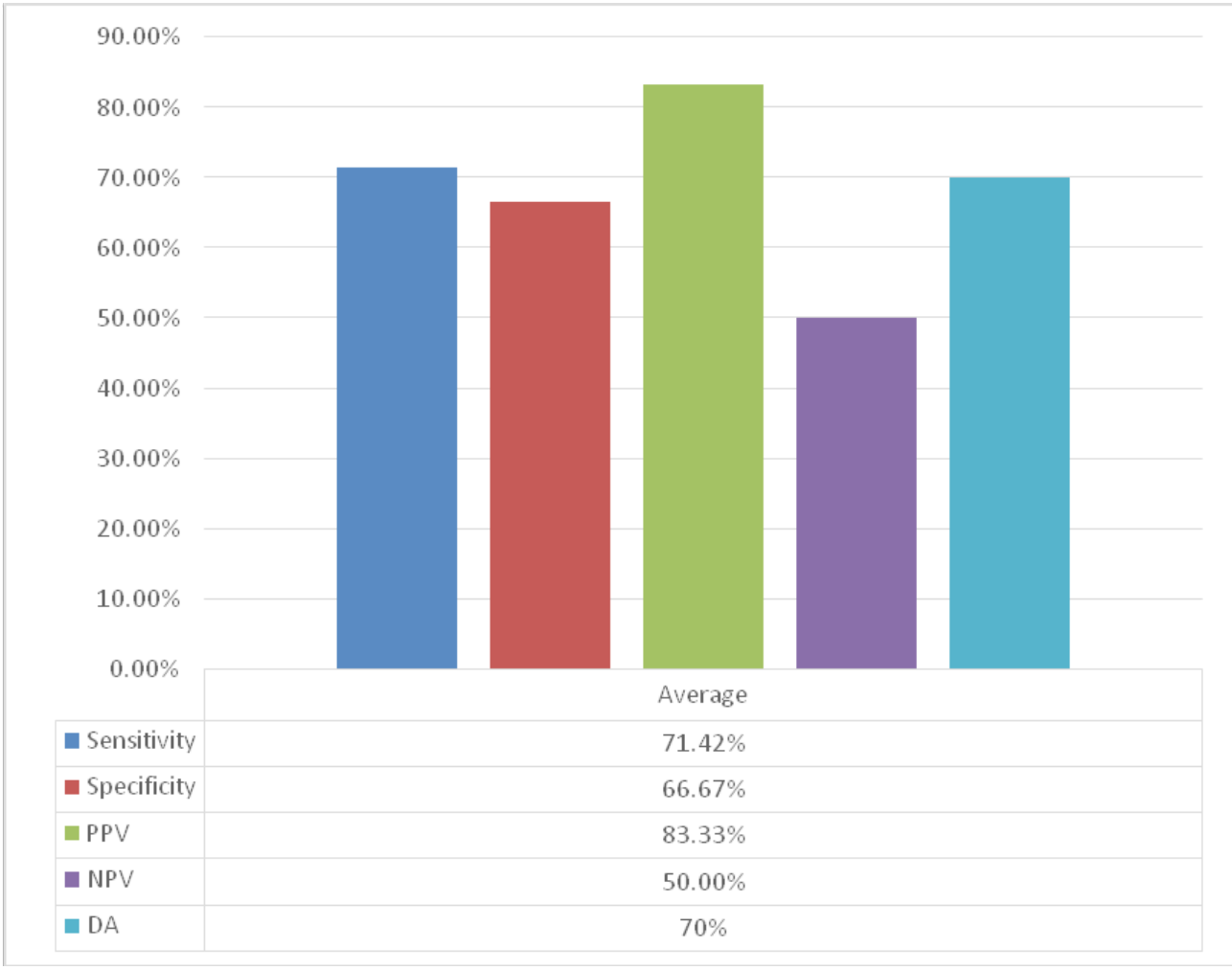


Graph No. 01: Gender

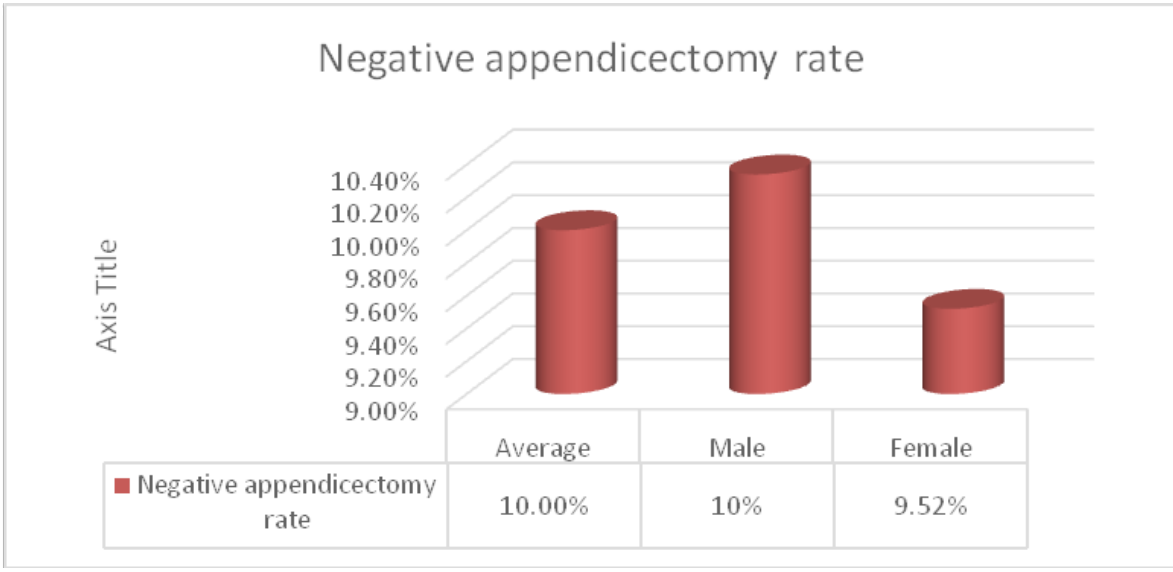
Graph 02: Individual characteristics of Tzanakis score :



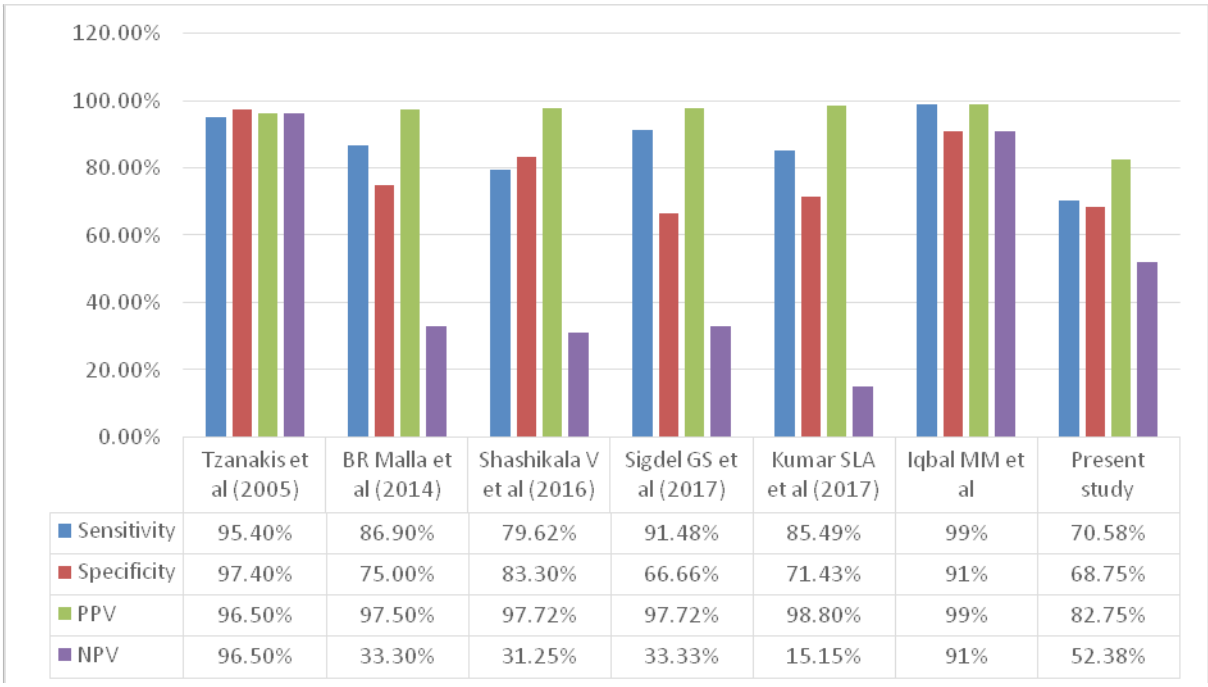
Graph 03: Outcome of Tzanakis Scoring system



Graph 04: Negative appendicectomy rate of Tzanakis scoring system:



Graph 5: Comparison of final results of Tzanakis scoring system of various studies with our present study:



	Tzanakis et al (2005)	BR Malla et al (2014)	Shashikala V et al (2016)	Sigdel GS et al (2017)	Kumar SLA et al (2017)	Iqbal MM et al	Present study
Sensitivity	95.40%	86.90%	79.62%	91.48%	85.49%	99%	70.58%
Specificity	97.40%	75.00%	83.30%	66.66%	71.43%	91%	68.75%
PPV	96.50%	97.50%	97.72%	97.72%	98.80%	99%	82.75%
NPV	96.50%	33.30%	31.25%	33.33%	15.15%	91%	52.38%

Discussion

Acute appendicitis continues to exist as the most widespread emergency in the world. A delay in making a diagnosis is associated with various complications which increases the morbidity and mortality in patients therefore a prompt and reliable diagnosis of acute appendicitis is mandatory. Furthermore, negative appendicectomy also accounts for the loss of financial resources and is associated with morbidity in 10 to 15% of cases.

Despite big breakthroughs in the imaging field, there remains uncertainty in diagnosing acute appendicitis due to atypical presentations of the disease. It has been repeatedly shown that investigations like USG lack specificity due to its operator dependency whereas investigations like CT scan and MRI are highly-priced demand more advanced equipment and competency. This makes a detailed clinical examination with primary investigations such as leucocyte count as the backbone in diagnosing acute appendicitis. This has compelled many surgeons to use diverse scoring systems for diagnosing acute appendicitis. The clinical evaluation is reliable in 50% to 80% of cases. The evaluation is more complex in the extremes of age and women of reproductive age group due to atypical presentations[12,13].

The current study will deal with the evaluation of the Tzanakis scoring system to diagnose acute appendicitis in a simple, reliable, and cost-effective way thereby reducing the negative appendectomy rate and thus morbidity associated with it.

In this study, the sample population consisted of 50 patients out of which 58% were males and 42% were females (see graph 01). The male to female ratio in our study was found to be 1.4:1 which is comparable to the study done by Kumar SLA et al[14] in which the male to female ratio was 1.6:1.

All the patients who accomplished the eligibility criteria were subjected to detailed clinical examination, routine hematological investigations, USG, and were scored based onthe Tzanakis scoring system. The decision to operate the patient including patients with a score less than the cut-off value was based on the clinical assessment and judgment taken by the consultant surgeon. All the patients underwent appendectomy with prior consent and the specimen was subjected to histopathological examination to confirm the diagnosis.

In our present study, 35 out of 50 patients (70%) had inflamed appendix intra-operatively. Out of the remaining 15 patients, five patients had enterocolitis, three patients had Meckel's diverticulitis, three patients had the pelvic inflammatory disease (salpingitis), two patients had a ruptured ovarian cyst and two patients had inflamed mesenteric lymph node (with or without pus) as the intra-operative findings. Shashikala V et al[9] in

her study had five out of 50 patients with an alternative diagnoses; out of which one patient had enterocolitis and four patients had a pelvic inflammatory disease. Another study was done by Kumar SLA et al[14]his study also reported six patients with alternative diagnoses in which three patients had salpingitis, two patients had an ovarian cyst and one patient had Meckel's diverticulitis.

Tzanakis et al [8], in 2005 revealed a more comprehensible system to help in making the diagnosis of appendicitis. It includes four parameters comprising of specific signs, laboratory and radiological investigations such as right iliac fossa tenderness, rebound tenderness over the right iliac fossa, leucocytosis (WBC count>12,000/mm^3), and USG. A score of 8 or more is considered as acute appendicitis.

In our study, out of the four parameters present in the scoring system, tenderness in the right iliac fossa was the commonest sign seen in 100% of patients whereas rebound tenderness and leucocytosis was found in 82% and 68% of the patients respectively. USG showed features of appendicitis in only 46% of cases (see graph 02).

In the present study, 30 patients had a score of eight or more, and 20 patients who had a score of less than eightaccording to the Tzanakis score.

The 30 patients who scored eight or more had 25 patients with features of appendicitis on histopathology whereas 5 patients had a histologically normal appendix with no features of inflammation.

Similarly,20 patients scored less than eight out of which there were 10 patients with a histologically normal appendix with no features of inflammation and 10 patients with features of appendicitis on histopathology.

The sensitivity, specificity, PPV, NPV, and diagnostic accuracy of the Tzanakis scoring system in our study was found to be 71.42%, 66.67%, 83.33%, 50.00%, and 70% respectively (see graph 03). The low sensitivity and specificity rate was due to the presence of high false-negative and false-positive results. The study done by Tzanakis et al [8] in 2005 had a sensitivity of 95.4%, the specificity of 97.4%, PPV of 96.5%, NPV of 96.5%, and diagnostic accuracy of 96.5% which is much higher as compared to our present study. This difference may occur due to low sample size, ethnic variation, and other demographic factors such as diet (see graph 5).

The study done by Shashikala V et al[9] in 2016 reported a sensitivity of 79.62%, a specificity of 83.3%, PPV of 97.72%, and NPV of 31.25%which were comparable to our present study. The study done by Iqbal MM et al[10] in 2018 had results similar to that of Tzanakis et al [8] with a sensitivity of 99%, a specificity of 91%, PPV of 99%, NPV of 91%, and diagnostic accuracy of 95% (see graph 5).

The study was done by Sigdel GS et al [11] and Kumar SLA et al [14] in 2017 had a specificity of 66.66% and 71.43% respectively which was comparable to our study whereas the NPV of both the studies was lower than our present study. Both studies had a sensitivity and PPV higher than our present study but the difference is not significant (see graph 5).

Similarly, the study done by BR Malla et al[15] in 2014 had sensitivity, specificity, PPV, and NPV of 86.9%, 75%, 97.5%, and 33.3% respectively. The outcome of the Tzanakis scoring system in the study conducted by Malla BR et al was comparable to our present study (see graph 5).

The negative appendicectomy rate (NAR) of the Tzanakis scoring system in the present study was found to be 10.34% percent in the males and 9.52% in females. The overall NAR observed for the Tzanakis scoring system was 10% (see graph no 04).

The negative appendicectomy rate in our present study for the Tzanakis Scoring system was comparable to NAR obtained in the studies done by Shashikala V et al [9](12%) and Iqbal MM et al [10](10.30%).

The disadvantage of the Tzanakis scoring system is that it does not include more clinical symptoms such as fever, nausea and vomiting, anorexia, migratory right iliac fossa pain, and supportive investigations such as CRP which augments the accuracy of the scoring system. Another factor for the low specificity of the Tzanakis score is that it gives the highest weightage to ultrasonography in its scoring system which has variable sensitivity and specificity due to inter-observer bias.

Conclusion

- Similarly, the Tzanakis scoring system had sensitivity, specificity, PPV, NPV, and DA 71.42%, 66.67%, 83.33%, 50.00%, and 70% respectively.
- The overall negative appendicectomy rate of the Tzanakis scoring system was 10%.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Comparison between the Tzanakis Scoring System and Yash Scoring System in the Diagnosis of Acute Appendicitis

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Abstract

Background: Acute appendicitis is amongst the most encountered emergencies seen in casualties requiring intervention. The incidence of negative laparotomy is 15% to 25% and is associated with notable morbidity. Scoring systems are useful and logical for distinguishing acute appendicitis from non-specific abdominal pain. Presently many scoring systems exist that aids in the diagnosis of acute appendicitis but still fail to decrease the rates of wrong diagnosis and the negative appendicectomy rate. This comparative study has been undertaken to evaluate and compare the efficacy of the Tzanakis scoring system with the relatively newer Yash scoring system which has been found more effective for the Indian population in diagnosing acute appendicitis.

Aim: To compare the efficacy of the Yash scoring system with the Tzanakis scoring system in diagnosing acute appendicitis.

Method and Material: A prospective comparison of the Tzanakis scoring system and Yash scoring system was done on 50 patients. The decision of an appendicectomy was taken by the consultant surgeon. The outcomes of the Tzanakis scoring system and Yash scoring system in terms of sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were calculated and compared.

Results: The Yash scoring system had sensitivity, specificity, PPV, NPV, and DA of 94.28%, 93.33%, 97.05%, 87.50%, and 94% respectively. The Tzanakis scoring system had sensitivity, specificity, PPV, NPV, and DA of 71.42%, 66.67%, 83.33%, 50%, and 70% respectively.

Conclusion: The Yash scoring system was significantly better than the Tzanakis scoring system in diagnosing acute appendicitis.

Keywords: *Appendix, laparotomy, score, diagnosis, inflammation.*

Introduction

Acute appendicitis is amongst the many encountered emergencies seen in casualties requiring intervention. There is a 6% chance of appendicitis in the general population[1]. Though the mortality has declined from 26% to 1% with the dawn of broad-spectrum antibiotics and timely surgery, it remains 5 to 15% in the elderly [2]. The incidence of negative laparotomy is 15% to 25% and is associated with notable morbidity, the frequency being more in women of reproductive age group (till 45%) due to the commonness of inflammatorytubo-ovarian diseases, ectopic pregnancy, and other gynecological pathology[3]. The delay in diagnosis may lead to rupture of the appendix in 17% to 40% of cases mostly in extremes of age. In the adolescents and elderly,

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it is linked with dramatic complications like an intra-abdominal abscess, wound infection, and ultimately death [4]. Thus, confirmation of acute appendicitis is very crucial to achieve lower morbidity and mortality rates.

Conventional history and clinical examination continue to exist as the potent and actual diagnostic modalities in pinpointing acute appendicitis[5]. The chronology is the commencement of periumbilical pain shifting to the right iliac fossa with subsequent development of anorexia and nausea or vomiting. The clinical examination may disclose signs resembling peritonitis such as local rebound tenderness (Blumberg's sign), guarding, rigidity, cutaneous hyperesthesia, and tenderness on rectal examination. Thirty-three percent of all cases with inflamed appendix present with unusual symptoms such as blunting of pain by the presence of overlying bowel in cases of retrocecal or retro iliac position of the appendix, increased urinary frequency, and tenesmus. Several conditions mimic appendicitis such as enterocolitis, terminal ileitis, pelvic inflammatory diseases, ureteric colic, peptic ulcer, diverticulitis, etc, and hence should be considered in the list of differential diagnosis[6].

The regular laboratory investigations of blood and urine are necessary. Leukocytosis is a convenient finding but is non-specific and may be missing in the elderly [2]. C – Reactive protein is elevated in almost all acute inflammatory conditions, hence is considered as a non-specific marker but its estimation guides a surgeon in reducing negative appendicectomies[7].

Plain x rays have validity in diagnosing appendicitis in only 8% of cases showing non-specific findings with low sensitivity and specificity [8]. The reliability of barium enema examination is 50% to 84%, but it has a major complication of caecal perforation when done in acute inflammatory conditions [9].Computed tomography (CT) is another imaging modality used to confirm appendicitis and is exceedingly efficacious and precise. It is operator independent and has shown a sensitivity of 90% to 100% and specificity of 91% to 99%.CT has a positive predictive value (PPV) of 92% to 98% and a negative predictive value (NPV) of 95% to 100%[10].

Ultrasonography (USG) with graded compressions has immense capacity in diagnosing appendicitis in its acute stage[10]. Various studies have been conducted on the utilization of these imaging systems. These studies reported sensitivity of 75% to 94%, a specificity of 86% to 100% and gross precision up to 96%[10,11]. Its superiority over computed tomography is the absence of exposure to ionizing radiation thus helpful in pregnant and paediatric patients. The major disadvantage is that it is operator dependent leading to inter-observer bias and failure to see the appendix during the scan does not rule out the possibility of having an inflamed appendix[10].

Magnetic resonance imaging (MRI) as an investigation is only taken typically in the pregnant population where radiation exposure is not warranted. It provides outstanding resolution and is highly efficient in diagnosing acute appendicitis. MRI has high sensitivity and specificity of 100% and 98% respectively. The PPV and NPV of MRI are 98% and 100% respectively. It is also operator-independent. The only issue is its higher cost, motion artifact, and complexity in reading MRI by non-radiologists with limited experience [11].

Despite the use of all these imaging techniques, the incidence of negative appendicectomy is not decreasing. These unmerited operations have a complication rate of approximately 13%, which is nearby to that of an inflamed appendix. Removal of a healthy appendix has a mortality of 0.65%. Protracted clinical observations aiming to reduce undesired operations may mean a delay in operations in 28% of cases and considerable danger of perforation [12].

Scoring systems are useful and logical for distinguishing pain of acutely inflamed appendix from vague abdominal pain. Presently numerous scoring systems exist that aid in diagnosing acute appendicitis. However, these systems do not replace clinical acumen thereby just helping in the determination of acute appendicitis and aids in approaching a conclusion, whether a particular case should be operated or not, thus lowering the negative appendicectomy rate (NAR).

The original study done by Tzanakis et al[13] in Greece reported sensitivity and specificity of 95.4% and 97.4% respectively. The diagnostic effectiveness of the score was 96.5% in his study. But in India, this scoring system was found inferior. For example, a comparative study between Tzanakis score and Alvarado score done by Shashikala V[14] in India revealed that Tzanakis scoring system had a sensitivity of 79.62%, a specificity of 83.3%, PPV of 97.72%, and NPV of 31.25% which was found better than Alvarado scoring system but still posing difficulties in diagnosis with a NAR of 12%. Another study was done by Iqbal MM et al[15] in

Postgraduate medical center, Karachi, Pakistan revealed that the Tzanakis score had a sensitivity of 99%, a specificity of 91%, PPV of 99%, and NPV of 91% with a diagnostic accuracy of 95%. Similarly,A study done in Kathmandu Model Hospital, Nepal did by Sigdel GS et al [16] reveals that Tzanakis has a sensitivity of 91.48%, a specificity of 66.66%, and diagnostic accuracy of 90%.

The study done by Lamture YR et al[17] in India on the Yash scoring system reveals a sensitivity of 99.48%, a specificity of 92.86%, PPV of 99.48%, and NPV of 92.86%.Though the validity of the Yash scoring system is promising for the Indian population, its only disadvantage was in the mode of a single study.

Despite various scoring systems and developments in the diagnostic and imaging modalities, the diagnosis of appendicitis is in dilemma which fails to decrease the rates of wrong diagnosis and the negative appendicectomy rate. This sequentially increases the cost for diagnosis by the use of expensive radiological modalities like Computed Tomography and MRI thus causing a delay in the treatment leading to an increase in morbidity and mortality of the patients.

This comparative non-randomized prospective study has been undertaken to evaluate and compare the effectiveness of the Tzanakis score with the relatively newer Yash score which has been found more effective for the Indian population in diagnosing acute appendicitis.

Method

The present study was undertaken in the department of surgery, Jawaharlal Nehru Medical College, Wardha in collaboration with Datta Meghe Medical College Hingana, Nagpur, Datta Meghe Institute of medical science (DMIMS), Sawangi, Meghe, Wardha, Maharashtra India.

Aim: To compare the efficacy of the Yash scoring system with the Tzanakis scoring system in the diagnosis of acute appendicitis.

Objectives:

1. To study the outcome of the Yash scoring system regarding its sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy.
2. To study the outcome of the Tzanakis scoring system about its sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy.
3. To compare the outcome of Yash and Tzanakis scoring system.

Study Design: Prospective non-randomized study.

Study Population: All patients of acute appendicitis with age >15 years and <60 years.

Study Duration: July 2018-July 2020.

Sample Size: 50 patients

The sample size was calculated by the modified Fischer's formula. The prevalence rate of appendicitis was taken as 6% from the study done by Yogesh Pralhad Chaudhari, Prasanna Gambhir Jawale[18] in 2015.

- Where $N \geq \{Z^2 1\text{-}\alpha/2 \times P \times (1\text{-}P)\}/d2$
- Z1-a/2 = is standard normal variate (at 5% type 1 error (P<0.05) it is 1.96 and at 1% type 1 error (P<0.01) it is 2.58). In most of the studies,P values below 0.05 are considered significant. Therefore, value of 1.96 is employed in formula**.**
- p = prevalence of appendicitis in community anticipated on the basis of past studies.
- d = Absolute error or precision – decided by researcher.
- p= 6%
- d= 0.07 (7% error of margin).

The minimum sample size would be 44.12 patients, thus 50 patients included in the study.

Inclusion Criteria: All patients with right lower quadrant pain and clinically diagnosed as acute appendicitis of age >15 years and <60 years.

Exclusion Criteria:

1. Patients with appendicular mass.
2. Patients of appendicitis with a known case of connective tissue disorder.
3. Patients with a past history of renal or ureteric stones and pelvic inflammatory disease.
4. Pregnant women.

Ethical clearance was obtained from the Ethics committee of Datta Meghe Institute of Medical Sciences (Deemed to be University) [Ref.No. DMIMS(DU)/

IEC/2018-19/7426].This prospective non-randomized study was conducted in Acharya Vinoba Bhave Rural Hospital, Sawangi. All the patients who fulfilled the eligibility criteria were subjected to routine hematological investigations, C-reactive protein, USG, and were scored based on Yash and Tzanakis scoring system.

Tzanakis scoring system combines 4 variables as follows:

- Presence of right lower abdominal tenderness= 4 points
- Rebound tenderness(Bloomberg sign)= 3 points
- Laboratory findings: the presence of white blood cells greater than 12,000 in the blood= 2 points
- Ultrasound findings: the presence of positive ultrasound scan findings= 6 points

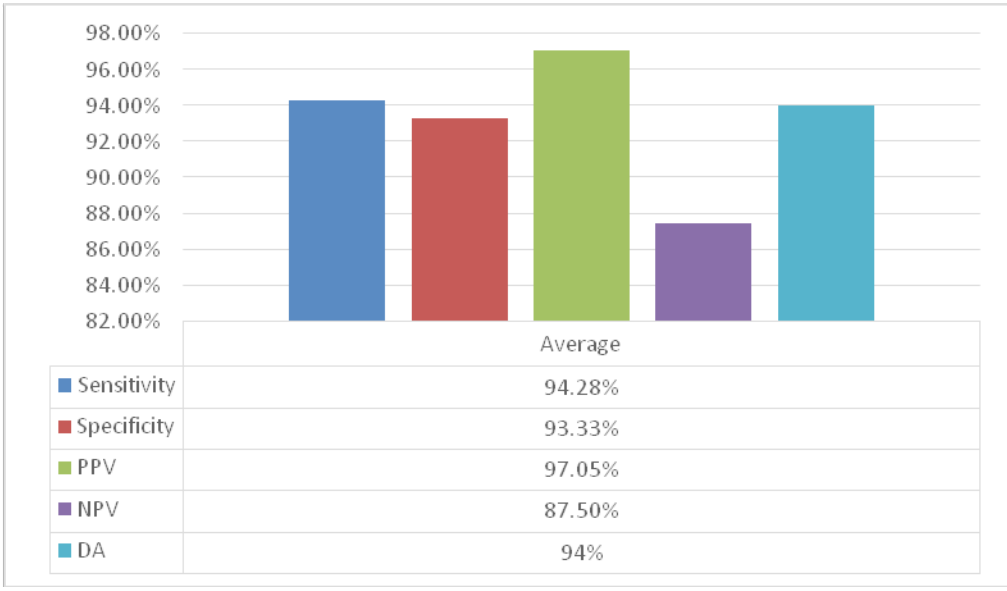
A score of 8 or more is suggestive of acute appendicitis requiring surgery [13]. Similarly, the components of the Yash scoring system are as follows:

- Migration of pain to the right iliac fossa = 1 point
- Nausea and vomiting = 1 point
- Anorexia = 1 point
- Right iliac fossa tenderness = 2points
- Rebound tenderness = 1 point
- Hyperesthesia in Sherren's triangle = 1 point
- Fever = 1 point
- White blood cell count > 10,000 mg/dl =2 points
- C-reactive protein (> 15mg/dl) = 3 points
- Ultrasonography = 4 points

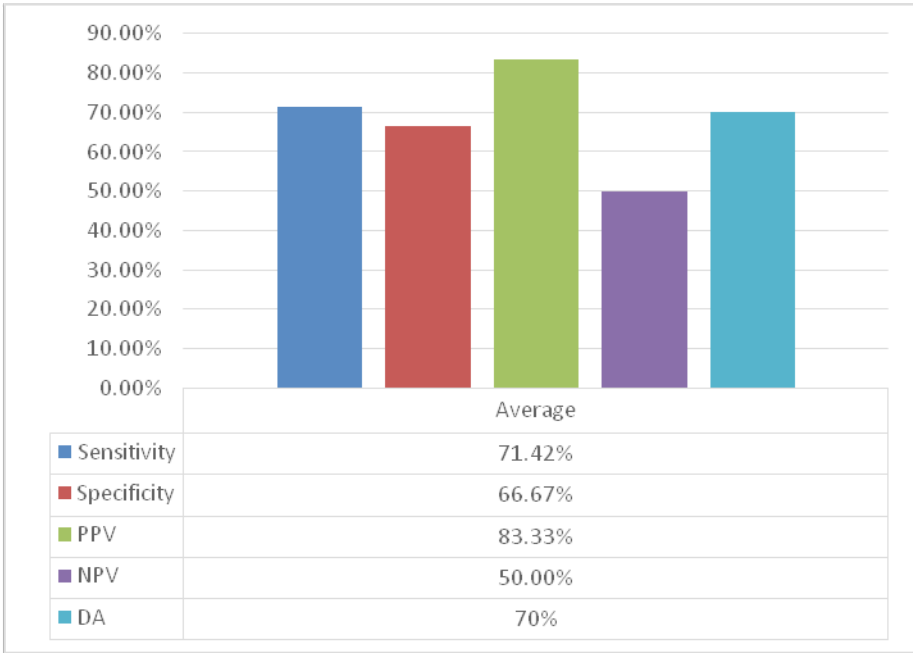
A score of 7 or more is suggestive of acute appendicitis requiring surgery[17].

The decision to operate was taken by a consultant surgeon by clinical diagnosis with help of other appropriate investigations in special circumstances. All patients underwent appendicectomy with prior consent and the specimen was subjected to histopathological examination for confirmation of diagnosis. The minimum criteria for acute appendicitis were the appearance of neutrophils in mucosa, submucosa, and lamina propria[1]. The result of the Tzanakis score and Yash score was reported independently. The result was correlated with the findings obtained on histopathological examination and the data was analyzed using the necessary statistical calculations using SPSS 24.0 version, the results were then presented.

Observations and Results

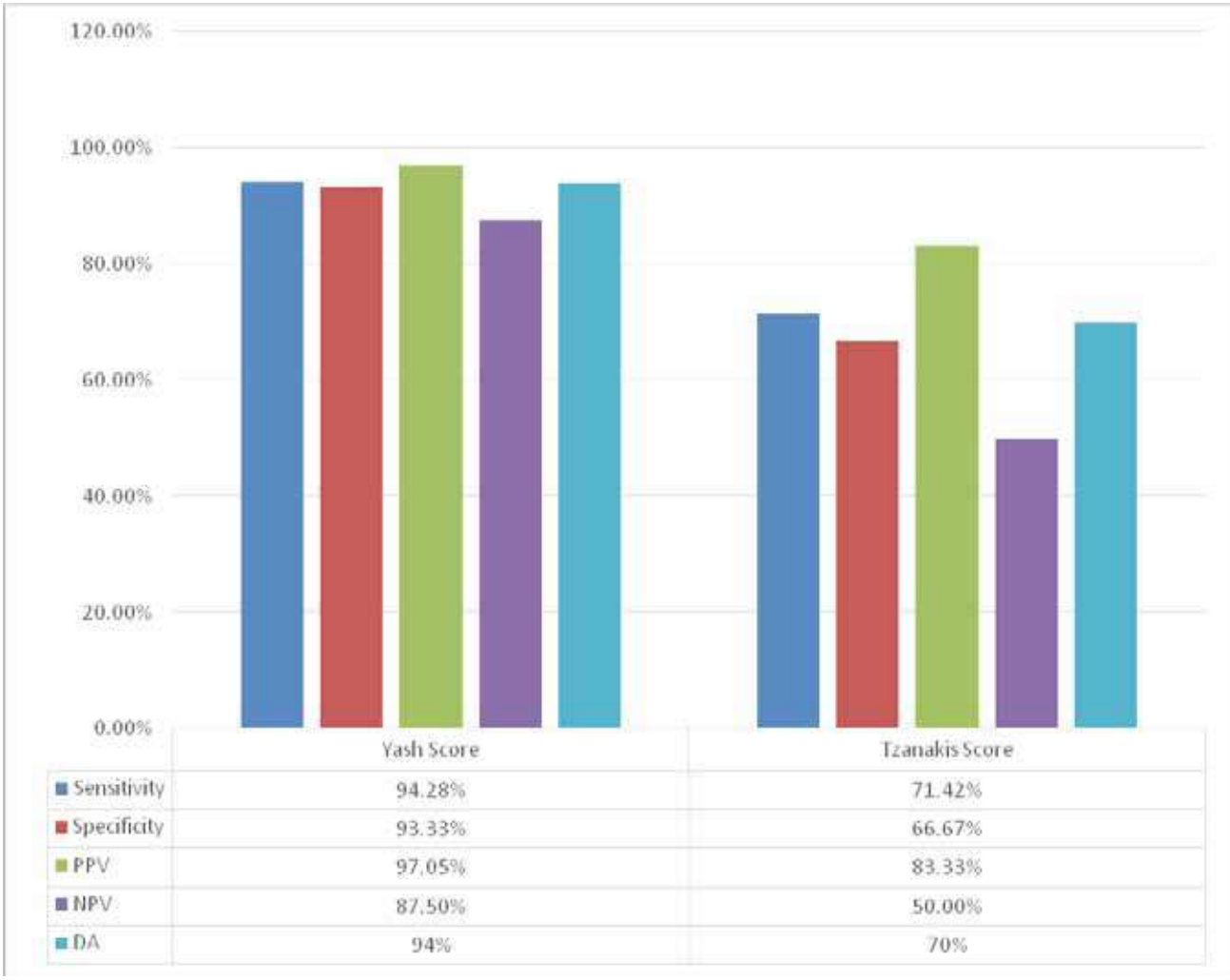


Graph 01: Outcome of YASH scoring system



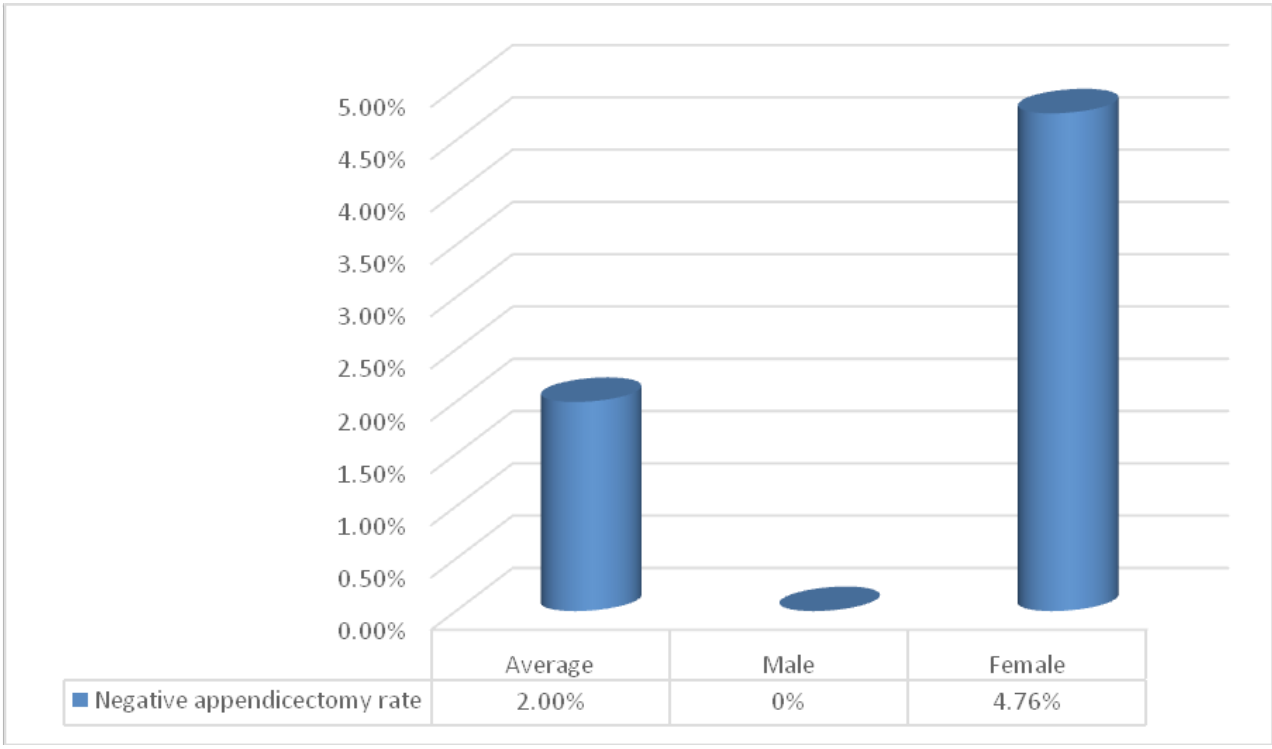
	Average
Sensitivity	71.42%
Specificity	66.67%
PPV	83.33%
NPV	50.00%
DA	70%

Graph 02: Outcome of Tzanakis Scoring system

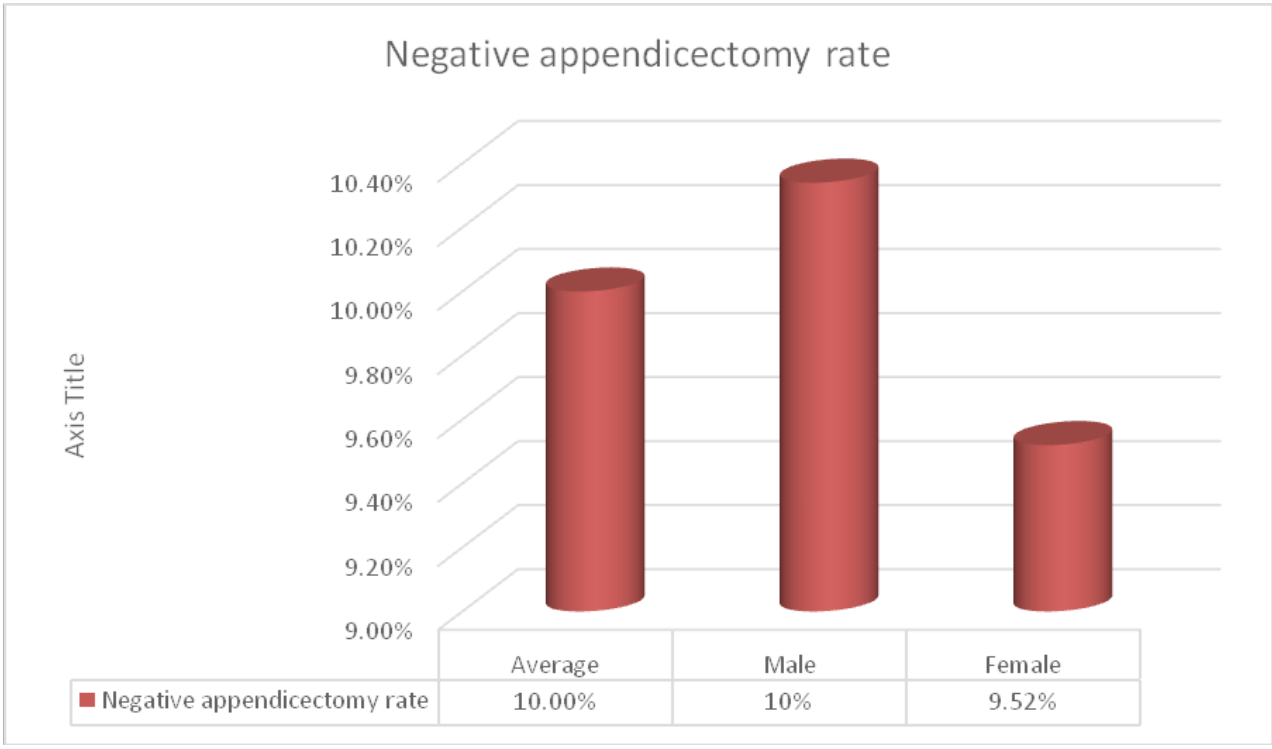


	Yash Score	Tzanakis Score
Sensitivity	94.28%	71.42%
Specificity	93.33%	66.67%
PPV	97.05%	83.33%
NPV	87.50%	50.00%
DA	94%	70%

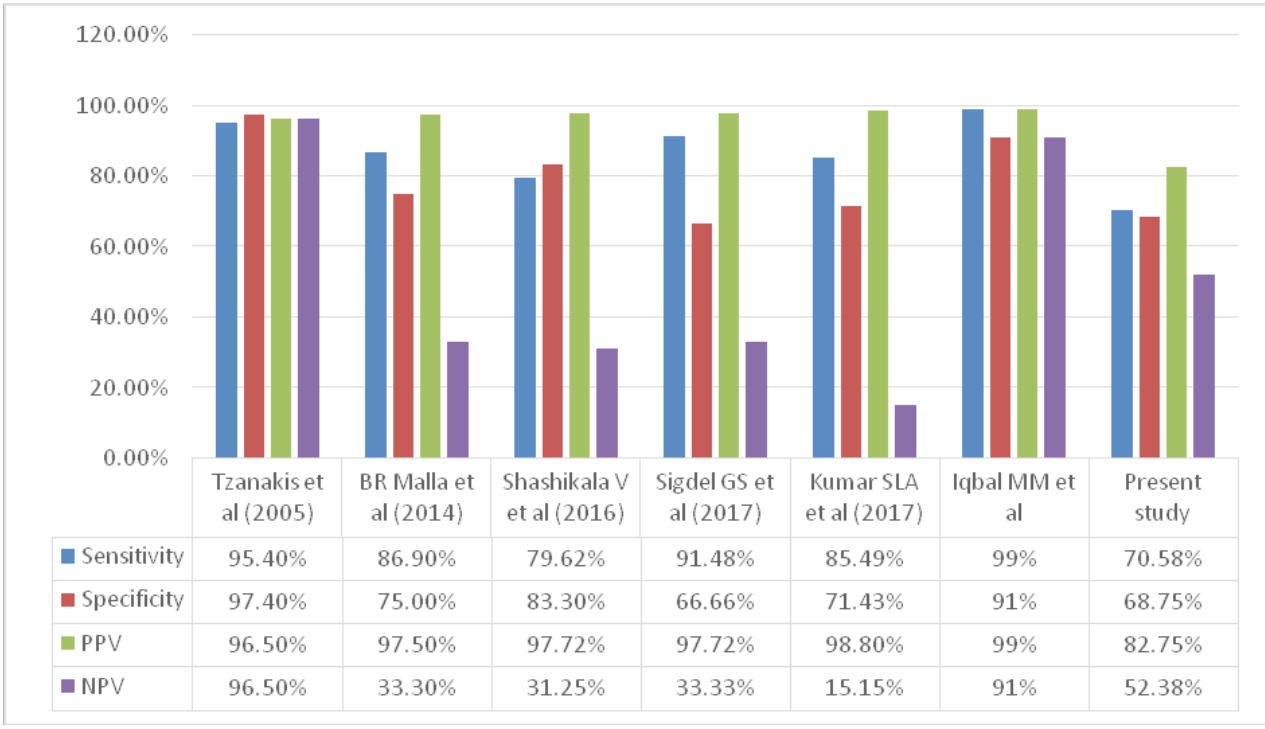
Graph 03: Comparison of the final outcome of Yash scoring system with Tzanakis scoring system:



Graph 04: Negative Appendectomy rate of Yash scoring system:



Graph 05: Negative appendicectomy rate of Tzanakis scoring system:



	Tzanakis et al (2005)	BR Malla et al (2014)	Shashikala V et al (2016)	Sigdel GS et al (2017)	Kumar SLA et al (2017)	Iqbal MM et al	Present study
Sensitivity	95.40%	86.90%	79.62%	91.48%	85.49%	99%	70.58%
Specificity	97.40%	75.00%	83.30%	66.66%	71.43%	91%	68.75%
PPV	96.50%	97.50%	97.72%	97.72%	98.80%	99%	82.75%
NPV	96.50%	33.30%	31.25%	33.33%	15.15%	91%	52.38%

Graph 6: Comparison of final results of Tzanakis scoring system of various studies with our present study:

Discussion

Acute appendicitis continues to exist as the most widespread emergency in the world. A delay in making a diagnosis is associated with various complications which increases the morbidity and mortality in patients therefore a prompt and reliable diagnosis of acute appendicitis is mandatory. Furthermore, negative appendicectomy also accounts for a loss of financial resources and is associated with morbidity in 10 to 15% of cases.

Despite big breakthroughs in the imaging field, there remains uncertainty in diagnosing acute appendicitis due to atypical presentations of the disease. It has been repeatedly shown that investigations like USG lack specificity due to its operator dependency whereas investigations like CT scan and MRI are highly-priced demand more advanced equipment and competency. This makes a detailed clinical examination with primary investigations such as leucocyte count as the backbone in diagnosing acute appendicitis. This has compelled many surgeons to use diverse scoring systems for diagnosing acute appendicitis. The clinical evaluation is reliable in 50% to 80% of cases. The evaluation is more complex in the extremes of age and women of reproductive age group due to atypical presentations.

The current study will deal with the evaluation of the Yash scoring system with the Tzanakis scoring system to diagnose acute appendicitis in a simple, reliable, and cost-effective way thereby reducing the negative appendectomy rate and thus morbidity associated with it.

In this study, the sample population consisted of 50 patients out of which 58% were males and 42% were females. The male to female ratio in our study was found to be 1.4:1 which is comparable to the study done by Kumar SLA et al[19] in which the male to female ratio was 1.6:1. A larger part of the study population (50%) was present in the age group of 15 to 29 years. The age group of 30 to 44 years had 26% of patients followed by 24% in the age group of 45 to 60 years. This is comparable to the study done by Kumar SLA et al[19] in which 40% of patients were in the 2nd to 3rd decade of life, 27% of patients in 3rd to 4th decade of life, and only 3% of patients in 5th to 6th decade of life.

All the patients who accomplished the eligibility criteria were subjected to detailed clinical examination, routine hematological investigations, C-reactive protein, USG and were scored based on Yash and Tzanakis scoring system. The decision to operate the patient including patients with scores less than the cut-off value

was based on the clinical assessment and judgment taken by the consultant surgeon. All the patients underwent appendectomy with prior consent and the specimen was subjected to histopathological examination to confirm the diagnosis.

In our study, 82% of the patients were operated by open method whereas 18% of patients were operated by laparoscopy. The study done by Malla BR et al[20] consisted of 200 patients in which 128 patients (64%) underwent appendectomy by an open method and 72 patients (36%) underwent appendectomy by laparoscopy. The most common incision used in open appendectomy was Mc Burney's in about 83% of cases followed by the right Para median which was used in 17% of cases. Similarly, in the study done by Lamture YR et al[17] the most common incision used was Mc Burney's in 94.74% of patients followed by right Para median in 5.26% of cases. In our present study, 35 out of 50 patients (70%) had inflamed appendix intra-operatively. Out of the remaining 15 patients, five patients had enterocolitis, three patients had Meckel's diverticulitis, three patients had the pelvic inflammatory disease (salpingitis), two patients had a ruptured ovarian cyst and two patients had inflamed mesenteric lymph node (with or without pus) as the intra-operative findings. Shashikala V et al[14] in her study had five out of 50 patients with an alternative diagnosis; out of which one patient had enterocolitis and four patients had a pelvic inflammatory disease. Another study was done by Kumar SLA et al[19] his study also reported six patients with alternative diagnoses in which three patients had salpingitis, two patients had an ovarian cyst and one patient had Meckel's diverticulitis.

The new Yash score described by Lamture YR et al [17] in 2017 has a significant role in identifying acute appendicitis. It differs from earlier scoring systems by including various parameters such as C-reactive protein, leukocyte counts, USG, and clinical data. A score of seven or more in patients was considered as acute appendicitis and such patients were subjected to operative intervention.

In our study, out of ten parameters, tenderness in the right iliac fossa was the commonest sign seen in 100% of patients. The other two signs i.e. rebound tenderness and hyperesthesia in Sherren's triangle was seen in 82% and 26% of patients respectively. The most common symptom was fever which was present in 88% of cases followed by nausea or vomiting which was found in 86% of the cases. Anorexia was seen in 72% of patients, whereas 68% of patients gave a history of migratory right iliac fossa pain. Out of the three investigations included in the scoring system, leucocytosis defined as WBC count more than 10000/mm^3 was present in 80% of cases. C-reactive protein with a value of more than 15mg/dl was present in 28% of cases with USG showing features of appendicitis in only 46% of cases.

In the present study, 34 patients had a score of seven or more, and16 patients who had a score of less than seven according to the Yash score.

Out of the 34 patients who scored seven or more, there were 33 patients with features of appendicitis on histopathological examination whereas only one patient hada histologically normal appendix with no features of inflammation.

Similarly, out of 16 patients who scored less than seven, there were 14 patients with a histologically normal appendix with no features of inflammation and only two patients with features of appendicitis on histopathology.

The sensitivity and specificity of the Yash scoring system in the present study were found to be 94.28% and 93.33% respectively. It had PPV and NPV of 97.05% and 88.50% respectively. The overall diagnostic accuracy of the Yash scoring system in our study was found to be 94%. The following results are comparable to the single original study done by Lamture YR et al[17] in which the sensitivity, specificity, PPV, and NPV was 99.48%, 92.86%, 99.48%, and 92.85% respectively. The diagnostic accuracy of the Yash score reported by Lamture YR et al was 98.56%.

The negative appendicectomy rate (NAR) of the Yash scoring system in the present study was found to be zero percent in males and 4.76% in females. This discrepancy in NAR was due to the high probability of other possible diagnoses in females of reproductive age group such as pelvic inflammatory diseases and ovarian cyst. The overall NAR observed for the Yash scoring system was 2% which is way lower than the accepted rate of 15 to 25%. In the study done by Lamture YR et al[17], the NAR was found to be 6.69% which is comparable to our study.

Tzanakis et al [13], in 2005 revealed a more comprehensible system to help in making the diagnosis of appendicitis. It includes four parameters comprising of specific signs, laboratory and radiological investigations such as right iliac fossa tenderness, rebound tenderness

over the right iliac fossa, leucocytosis (WBC count>12,000/mm^3), and USG. A score of 8 or more is considered as acute appendicitis.

In our study, out of the four parameters present in the scoring system, tenderness in the right iliac fossa was the commonest sign seen in 100% of patients whereas rebound tenderness and leucocytosis was found in 82% and 68% of the patients respectively. USG showed features of appendicitis in only 46% of cases.

In the present study, 30 patients had a score of eight or more, and 20 patients who had a score of less than eightaccording to the Tzanakis score.

The 30 patients who scored eight or more had 25 patients with features of appendicitis on histopathology whereas 5 patients had a histologically normal appendix with no features of inflammation.

Similarly,20 patients scored less than eight out of which there were 10 patients with a histologically normal appendix with no features of inflammation and 10 patients with features of appendicitis on histopathology.

The sensitivity, specificity, PPV, NPV, and diagnostic accuracy of the Tzanakis scoring system in our study was found to be 71.42%, 66.67%, 83.33%, 50.00%, and 70%respectively. The low sensitivity and specificity rate was due to the presence of high false-negative and false-positive results.

The study done by Tzanakis et al [13] in 2005 had a sensitivity of 95.4%, a specificity of 97.4%, PPV of 96.5%, NPV of 96.5%, and diagnostic accuracy of 96.5% which is much higher as compared to our present study. This difference may occur due to low sample size, ethnic variation, and other demographic factors such as diet.

The study done by Shashikala V et al[14] in 2016 reported a sensitivity of 79.62%, a specificity of 83.3%, PPV of 97.72%, and NPV of 31.25%which were comparable to our present study. The study done by Iqbal MM et al[15] in 2018 had results similar to that of Tzanakis et al [13] with the sensitivity of 99%, a specificity of 91%, PPV of 99%, NPV of 91%,and diagnostic accuracy of 95%.

The study was done by Sigdel GS et al [16] and Kumar SLA et al [19] in 2017 had a specificity of 66.66% and 71.43% respectively which was comparable to our study whereas the NPV of both the studies was lower than our present study. Both studies had a sensitivity and PPV higher than our present study but the difference is not significant.

Similarly, the study done by BR Malla et al[20] in 2014 had sensitivity, specificity, PPV, and NPV of 86.9%, 75%, 97.5%, and 33.3% respectively. The outcome of the Tzanakis scoring system in the study conducted by Malla BR et al was comparable to our present study.

The negative appendicectomy rate (NAR) of the Tzanakis scoring system in the present study was found to be 10.34% percent in the males and 9.52% in females. The overall NAR observed for the Tzanakis scoring system was 10%.

The negative appendicectomy rate in our present study for the Tzanakis Scoring system was comparable to NAR obtained in the studies done by Shashikala V et al [14](12%) and Iqbal MM et al [15](10.30%).

The result of the Yash scoring system as compared to the result of the Tzanakis scoring system in our present study reveals that the Yash scoring system had a better outcome than the Tzanakis scoring system and was more efficacious than Tzanakis scoring system in diagnosing acute appendicitis. The disadvantage of the Tzanakis scoring system is that it does not include clinical symptoms such as fever, nausea and vomiting, anorexia, migratory right iliac fossa pain, and supportive investigations such as CRP which augments the accuracy of the scoring system. Another factor for the low specificity of the Tzanakis score is that it gives the highest weightage to ultrasonography in its scoring system which has variable sensitivity and specificity due to inter-observer bias.

The negative appendectomy rate of the Yash scoring system was dramatically lower than that of the Tzanakis scoring system.

Conclusion

- The Yash scoring system had sensitivity, specificity, PPV, NPV, and DA of 94.28%, 93.33%, 97.05%, 88.50%, and 94% respectively.
- The overall negative appendicectomy rate of the Yash scoring system was 2%.
- Similarly, the Tzanakis scoring system had sensitivity, specificity, PPV, NPV, and DA 71.42%, 66.67%, 83.33%, 50.00%, and 70% respectively.

- The overall negative appendicectomy rate of the Tzanakis scoring system was 10%.
- Thus, our study reveals that the Yash scoring system is a valuable and plausible modality in detecting acute appendicitis and reducing NAR due to its outstanding outcome regarding its sensitivity, specificity, PPV, NPV, and diagnostic accuracy when compared to Tzanakis scoring system.
- The study also shows that the Yash scoring system is a better tool to avoid unnecessary operations due to its exceptionally low negative appendectomy rate thus lowering morbidity in patients of acute appendicitis and thereby lowering the cost of treatment and prevention of misuse of valuable resources and manpower.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Nerve Conduction Study in Early Diagnosed Cases of Hypothyroidism in Central India

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Abstract

Thyroid gland has multiple effects on the neuromuscular system and brain, its dysfunction is associated with characteristic symptoms and signs with functional alterations in many organs and systems. Thyroid disease may cause signs and symptoms of neuromuscular dysfunction. Hypothyroidism has been associated with the clinical features of proximal muscle weakness, mononeuropathy and sensorimotor polyneuropathy.Nerve conduction studies (NCS) are useful adjunct to the clinical examination of the peripheral nervous system as it is helpful to test speed of signals through a nerve. They help to delineate the extent and distribution of the neural lesion and distinguish two major categories of peripheral nerve disease demyelination and axonal degeneration. Present cross sectional studywas conducted at Central Neurophysiology Laboratory in tertiary care teaching hospital, J.N.M.C, Sawangi (M), Wardha catering to rural population of central India to assess the nerve conduction in early diagnosed hypothyroid patients. Total twenty seven patients; 20-50yrs of age were screened for nerve conduction.In our study we got mixed type of peripheral neuropathy. Out of 27 patients with age 18-30 yrs- 1(16.67%), 31-40 yrs- 13(61.90%) and 41-50 yrs -7 (33.3 %) had abnormal NCS. Occurrence of neuropathy was more common in female patients 80.95% as compared to male patients 19.05%.Sensory nerve action potential (SNAP) amplitudes and conduction velocity were significantly reduced ($p<0.05$) in cases diagnosed as hypothyroidism in median (77%), sural (70%) and ulnar nerves (33%)as compared to controls.

Keywords: *Nerve conduction study, hypothyroidism, central India.*

Introduction

Thyroid gland has modulatory effect on the neuromuscular system and brain through its secretions, thyroxin (T4) and triiodothyronine (T3) which are essential to maintain metabolism in the tissues that is optimal for their normal growth,function and maturation[1].

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Thyroid dysfunction is associated with characteristic symptoms and signs and functional alterations in many organs and systems. Central and peripheral nervous systems affection may provide the major presenting symptoms[2]. Prevalence of neuromuscular disorders related to thyroid dysfunction is 20-80 % [3].

Thyroid disease may cause signs and symptoms of neuromuscular dysfunction. Hypothyroidism has been associated with the clinical features of proximal muscle weakness; mononeuropathy and sensorimotor polyneuropathy [4]. Most of the patients of thyroid disorders with neuropathy are asymptomatic. The severity of the neuromuscular signs and symptoms are known to be related to the duration and degree of

hormonal deficiency[5]. Sometimes the nerve damage in untreated patients can lead to carpal tunnel syndrome and these patients end up getting hand surgery when they may have reversed that just by early screening with nerve conduction studies and getting treated for thyroid disorder[6].

The nerve conduction study (NCS) consists of Motor NCS, sensory NCS study. Motor nerve conduction studies assess motor axons by selectively recording muscle responses to nerve stimulation. In sensory nerve conduction studies SNAP is obtained by directly stimulating a sensory nerve and recording directly from it or its branches[7]. With a brief electrical pulse applied to the overlying skin, peripheral nerves can be easily stimulated and brought to an action potential [5]. Nerve conduction studies (NCV) are useful adjunct to the clinical examination of the peripheral nervous system. It is a test of the speed of signals through a nerve. They help to delineate the extent and distribution of the neural lesion and distinguish two major categories of peripheral nerve disease demyelination and axonal degeneration[6,8].

Complications occurring due to hypothyroidism are responsive to thyroid treatment or thyroid replacement therapy, but if are related to neuropathy then there could be a life long struggle with neuropathy pain if not treated promptly. Understanding neuropathy and hypothyroidism is crucial for all thyroid disease patients. Few studies are there who compare nerve conduction in early diagnosed hypothyroid disease with age and sex matched control group in rural population of central India. Electrophysiological studies are non invasive and helpful in early detection of neuropathy as they are most sensitive, specific and repeatable[9]. Neurological manifestations which may be noted in conjunction with systemic features of disease can be detected with nerve conduction studies[10,11]. Hence present study was conducted to assess the alterations of some selected variables of nerve conduction in early diagnosed hypothyroid patients and also to find out which type of neuropathy is more prevalent in those populations of rural area.

Aim: To assess the nerve conduction studies in the cases diagnosed as hypothyroidism.

Objectives:

1. To study the motor and sensory nerve conduction in the cases diagnosed as hypothyroidism.
2. To compare the motor and sensory nerve conduction of cases diagnosed as hypothyroidism with euthyroid subjects.
3. To evaluate type of neuropathy present in the cases diagnosed as hypothyroidism.

Method and Materials

Study Setting: Study was conducted at Central Neurophysiology laboratory in tertiary care teaching hospital, J.N.M.C, Sawangi (M), Wardha catering to rural population of central India.

Study Design: Cross-sectional comparative study.

Study Duration: Nine months

Sample Size:

Formula: $N = X^2.N.P\ (1-P)/C^2\ (N-1) + X^2.P\ (1-P)$

N = Total newly diagnosed patients

= 27

X^2 = Chi-square value for 1 degrees of freedom at some desired probability level is 3.84 at 5% level of significance.

P = 50% proportion

C = Confidence interval of one choice (95%)

= 0.05

$N = 3.84\times25\times0.5(0.5)/0.05^2\times49+3.84\times0.5\times0.5$

= 27

Study group -27 patient diagnosed as cases of hypothyroidism and

Control group -16 euthyroid subjects.

Inclusion Criteria:

- Study group included hypothyroid patients who were untreated of age 18-50 years[12] and both genders attending medicine outpatient department of AVBRH, Sawangi (M), Wardha.
- The diagnostic criteria for hypothyroidism were based on T_3, T_4 and TSH levels [4].
- Among the controls age and sex matched euthyroid subjects who had no past/present or family history of thyroid disorders and apparently healthy were included in the study.

Exclusion Criteria:

- Patients who were less than 18 years old to limit our study sample to the adult population.
- Patients suffering from secondary hypothyroidism or with other possible cause of neuropathy like diabetes mellitus, neuromuscular disorder, leprosy, drug induced neuropathy.
- Patients with family history of neuropathy, malignancy, HIV, liver and kidney diseases, myopathy, alcoholics, smokers and drug abuser were excluded from the study.

Methodology

After IEC clearance study was conducted in central physiology lab of AVBRH hospital, Sawangi (M) Wardha. Adult patients attending the outpatient department who were advised thyroid function tests were isolated and only those patients with biochemical evidence of hypothyroidism i.e. low tri – iodothyronine,thyroxine levels and thyroid stimulating hormone levels were enrolled for the study after getting their consent. Thyroid function tests were done in AVBRH hospital central lab by ADIVIA Centaur CP Immunoassay system of company Siemens (Photo-1). Normal values of lab were T3-0.60-1.81 ng/ml, T4-3.2-12.6 ug/ dl and TSH-0.25-5 µIU/ml. Patients were diagnosed cases of hypothyroidism who were not on any treatment, willing to participate in the study, were included in the study. Demographic parameters like height, weight and BMI were taken. Detailed history regarding neuromuscular symptoms with their duration based on questionnaire was asked. Age and gender matched healthy subjects with normal thyroid function tests were chosen as the control group for comparison.

Nerve conduction study was performed using the Neuron Spectrum 5 machine. The motor nerves tested were median, ulnar and peroneal. The sensory nerves studied were median,ulnar and sural. Parameters for motor nerve conduction study taken were latency (ms), amplitude (mv), conduction velocity (m/s) and for sensory nerve conduction study were amplitude (µv) and conduction velocity (m/s) [6]. Motor nerve conduction study involved stimulation of a motor nerve at two different sites with maximum stimulus, the distance was measured and automatically divided by conduction time between the two points (difference between proximal and distal motor latencies) which gave conduction velocity. Sensory conduction study (antidromic) involved stimulation of sensory nerves proximally and recording SNAPs with electrodes placed distally over the dermatomal distribution.

Result

Quantitative data analysis was done by using students unpaired' test and qualitative data by chi-square. All the values were expressed as mean and standard deviation (±SD) and p- value was considered statistically significant if found to be less than 0.05. GRAPHPAD PRISM 5.0 and SPSS 22.0 version was used.

Table 1: Distribution of patients according to demographic characteristics

Demographic Characteristics	Control (n = 16)	Hypothyroidism (n=27)	X^2 - Value	p-value
Mean Age	36.12±4.16	36.76±3.31	2.67	0.26,NS
18-30 yrs	0(0%)	2(7.41%)		
31-40 yrs	13(81.25%)	16(59.26%)		
41-50 yrs	3(18.75%)	9(33.33%)		
Gender				
Male	5(31.25%)	6(22.22%)	0.43	0.51,NS
Female	11(68.75%)	21(77.78%)		
M:F Ratio	1:2.2	1:2.5		
Wt (kg)	59.75±2.38	61.76±3.81	1.84	0.07,NS
Ht (cm)	155.50±5.54	154±5.75	0.79	0.43,NS

Demographic Characteristics	Control (n = 16)	Hypothyroidism (n=27)	X^2 - Value	p-value
BMI (kg/m2)	24.79±1.90	26.07±1.55	24.32	0.0001,S
Normal	16(100%)	6(22.22%)		
Overweight	0(0%)	9(33.33%)		
Obese	0(0%)	12(44.44%)		

Maximum 81.25% of the subjects in control group and 59.26% in hypothyroid group were in the age group of 31-40 years followed by 18.75% in control group and 33.33% in hypothyroid group were in the age group of 41-50 years which is statistically not significant using Chi-square test(א2-value=2.67,p-value=0.26).

Most of the subjects in control group (68.75%) and 77.78% in hypothyroid group were females which is statistically not significant (א2-value=0.43, p-value=0.51).

Mean weight of the subjects in control group was 59.75±2.38 and in hypothyroid group it was 61.76±3.81 and the difference was not significant using student's unpaired t test (t=1.84,p-value=0.07)

Mean height of the subjects in control group was 155.50±5.54 and in hypothyroid group it was 154±5.75and the difference was not significant using student's unpaired t test (t=0.79,p-value=0.43).

All(100%) of the subjects in control group had normal BMI, where as in hypothyroid group 44.44% of the patients were obese and 33.33% were overweight which is statistically significant א (א2-value=24.32, p-value=0.0001).

Table 2: Significant correlates of neuropathy

Demographic Characteristics	Abnormal Nerve Conduction(n=21)	Normal Nerve Conduction(n=6)	X^2- Value	p-value
18-30 yrs	1(4.76%)	1(16.67%)	1.00	0.60,NS
31-40 yrs	13(61.90%)	3(50%)		
41-50 yrs	7(33.33%)	2(33.33%)		
Gender				
Male	4(19.05%)	2(33.33%)	0.55	0.45,NS
Female	17(80.95%)	4(66.67%)		
Duration of disease				
<1 yr	6(28.57%)	3(50%)	0.96	0.61,NS
1-5 yrs	10(47.62%)	2(33.33%)		
>5 yrs	5(23.81%)	1(16.67%)		
BMI (kg/m^2)				
Normal	4(19.05%)	2(33.33%)	2.41	0.29,NS
Overweight	6(28.57%)	3(50%)		
Obese	11(52.38%)	1(16.67%)		
Signs and symptoms of neuropathy				
Absent	2(9.52%)	4(66.67%)	8.81	0.008,S
Present	19(90.48%)	2(33.33%)		

Maximum 61.90% of abnormal nerve conduction and 50% of normal nerve conduction was found in the age group of 31-40 years followed by 33.33% abnormal and 33.3% normal nerve conduction in the age group of 41-50 years,4.76% abnormal and 16.67 % normal nerve conduction in 18-20 years of age group in hypothyroid patients was observed which was statistically not significant (א2-value=1.00,p-value=0.60).

Most of the females 80.95% had abnormal nerve conduction while 66.67% had normal nerve conduction in hypothyroid group. Where as in males 19.05% had abnormal nerve conduction and 33.3% had normal nerve conduction which was statistically non significant (א2-value=0.55, p-value=0.45).

Maximum 47.62% of abnormal nerve conduction and 33.3% of normal nerve conduction was observed in 1-5 yrs duration, followed by 28.57% abnormal and 50% normal nerve conduction in less than one year,28.81% abnormal and 16.67 % normal nerve conduction in more than 5 years of duration of diseases in hypothyroid patients which was statistically not significant (א2-value=0.96,p-value=0.61).

Abnormal nerve conduction was observed maximum in obese hypothyroid patients 52.38% followed by 28.57% in overweight and 19.05% in normal weight hypothyroid group. Normal nerve conduction was found in 50% of overweight, 33.3% normal weight and 16.67% in obese hypothyroid patients which was not significant (א2-value=2.41, p-value=0.29).

Abnormal nerve conduction was present in 90.48% while 33.3% had normal nerve conduction in patients of hypothyroidism who showed signs and symptoms of neuropathy. Abnormal nerve conduction was present in 9.52% while 66.67% had normal nerve conduction in patients of hypothyroidism who didn't had signs and symptoms of neuropathy which was statistically significant (א2-value=8.81, p-value=0.008)

Discussion

Hypothyroidism is an endocrine disease and peripheral nervous system involvement is a well-documented. 20-80% of researchers have observed prevalence of neuromuscular disorders related to thyroid dysfunction, the severity of which correlates with the degree and duration of hormonal deficiency[3]. In fact neurological complications in hypothyroidism are a well-established finding. Peripheral nerve abnormalities known to be associated with hypothyroidism are entrapment neuropathy or sensorimotor polyneuropathy[13].

In hypothyroidism polyneuropathy is mild and rarely it could be a subclinical entity and hence most of these cases would have gone unnoticed if only clinical examination has been done as it alone may be insufficient to make a conclusive diagnosis specially to make a distinction between axonal and demyelinating disorders. At such times nerve conduction studies are particularly useful. They assess the functional integrity of sensorimotor conduction and give a reliable evidence of peripheral nerve dysfunction. In fact, the estimated prevalence of polyneuropathy diagnosed by electro physiologic tests is 718/1000 [(1).]

In our study on neurological examination two cases of hypothyroidism showed decreased reflexes. Out of 27 cases of diagnosed hypothyroidism 21 (77.78%) had at least one type of nerve conduction abnormality, most commonly in median and sural sensory nerves which was also observed in the studies done by Asia and Ettore et .al [(1,14)]. None of cases of hypothyroidism had signs of muscle atrophy or hypotonia. The commonly reported subjective complaints of tiredness, myalgias and weight gain were encountered in our study too. Tiredness, myalgias in 77.78%, weight gain in 59.26%, constipation in 37%, it correlates with other studies where it was 64% and 68% respectively [(14)]. Paresthesia were found in 33% and menstrual irregularities 22.2% was the next predominant symptoms. Similar results were also observed by Karne and Shetty et.al. [(8, 15)] The increased weight in cases of hypothyroidism would be due to accumulation of mucopolysaccharides, hyaluronic acid and chondroitin sulphate in the interstitial spaces. Those spaces being hydrophilic nature, retain water along with them resulting in weight gain. Also, hormonal imbalance leading to menstrual irregularities [(9)]. Neurological dysfunction associated with disorders of thyroid gland could be the result of hormonal imbalance or immune mechanism accompanying thyroid disease [(1)].

There are studies which reported primary axonal degeneration while some reported demyelination as the predominant feature of neuropathy in hypothyroidism[(8,15)]. In our study we got mixed type of peripheral neuropathy. SNAP amplitudes and Conduction velocity were significantly reduced($p<0.05$) in cases diagnosed as hypothyroidism as compared to controls in median (77%), sural (70%) and ulnar nerves (33%). Similar results were also observed by other studies [(14,15)].

Disturbed myelin synthesis during acute hypothyroidism may be the cause for demyelinating peripheral neuropathy in hypothyroid patients. Hormonal and metabolic changes associated with hypothyroidism are responsible for the nerve conduction changes in the form decreased nerve conduction velocity also thyroid hormones are involved in gene expression, neurotransmitter system and axonal transport , the plausible mechanism of axonal neuropathy is a hypothyroidism induced ATP deficiency with reduced activity of ATP ase enzyme may result in reduced SNAP amplitudes.

Similar to other researchers[9,13] distal latency and conduction velocity in our study was prolonged, amplitude was decreased in median motor nervesin early diagnosed hypothyroid patients as compared to that of control. This could be attributed to hypothyroidism leading to energy deficit due to decreased oxidation of nutrients also decreased degradation of glycogen leads to formation of glycogen deposits around the nerves. These metabolic alterations induced by hypothyroidism may initially damage the functions and later on induce structural changes in the nerves [16].The motor neural dysfunction seen in the present study may be linked to the various functional and structural changes in peripheral nerves associated with deficiency of thyroid hormones.

Presence of neuropathy correlated independently with advanced age and female gender. Out of 27 patients with age 18-30 years 1 patient (16.67%), 31-40 years 13 patients (61.90%) and 41-50 years 7 patients (33.3%) showed abnormal nerve conduction. Occurrence of neuropathy was more common in female patients 80.95% as compared to male patients 19.05%. Similar results were also seen in studies conducted by other researchers[1,8,15]. Neuropathy was more common with duration of disease 1-5 years (47.62%) and obesity (52.38%) but these findings were not statistically significant (P = 0.60,0.45,0.61 and 0.29) respectively. Signs and symptoms of neuropathy was present in 19 patients (90.48%) of early diagnosed hypothyroid patients which was statistically significant (P = 0.008) as shown in Table 2.

Conclusion

This study concluded that the polyneuropathy which is associated with early diagnosed hypothyroidism was largely of mixed type (axonal as well as demyelinating type). There was a predominant involvement of sensory nerves, especially the median nerve and sural nerve on nerve conduction study. In adults, as the hypothyroidism has insidious onset, diagnosis can be delayed by months or years and the neuropathic manifestations can go unrecognized for longer period. Clinical symptoms and laboratory assessment of thyroid function must be performed concurrently to determine the presence of thyroid dysfunction. Nerve conduction studies in cases of hypothyroidism are suggested, early in the course of the disease in order to detect nervous system involvement.

Limitations: Major shortcomings of this study are the small sample size. Furthermore, we could not perform EMG in patients.

Risk Factor: As it is non- invasive study there was no harm to the study subjects.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Work Related Musculoskeletal Disorders Assessment in Cab Drivers

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Abstract

Background: The cab drivers have to be in a particular posture for driving. They have to be in a prolonged sitting position till the time they are driving the car, this affects their back, neck, ankle &foot. it was found in the previous studies that these people develop work related musculoskeletal disorders & pain due to their wrong posture. This study focuses on assessment of musculoskeletal disorder in professional cab drivers.

Methodology: It is a one-time observational study,30 cab drivers were involved in the study of the age group 18-55 years. They were screened for the inclusion & exclusion criteria,then they were asked to fill the questionnaire for assessment, analysis of the result will be done finally.

Result: The result shows that the 70% of cab drivers were suffering from lower back pain

Conclusion: In this study we found that most of the car drivers suffers from back pain. They were advised for the posture correction.

Keywords: *WRMSD, posture, pain, musculoskeletal disorder.*

Introduction

Work-related musculoskeletal disorders (WMSD) are the category of musculoskeletal disorders that will include problem related to tendons, tendon sheaths, and synovial lubrication of tendon sheaths, and related to bones, muscles, and nerves of hands, wrists, elbows, shoulders, neck, and back.[1] Many factors make cab drivers different from other occupations in terms of exposure to the risk of work-related disorders. Previous studies said that cab drivers takes longer time in driving than other professions. The design of the seat can affect the posture of drivers and posture in turn also can effect both the comfort and physical conditions of a driver. Work-related musculoskeletal disorders (WRMD) and other postural harm may result in physiological illness that may grow over a long period due to prolonged mechanical stresses levied on the musculoskeletal system. Musculoskeletal pain includes a major health problem for the general population, distressing their quality of life, demanding increased need of health care and organization. A number of previous studies assessed the occurrence of musculoskeletal disorders amongst Iranian workers [2-4] and many other country. It has been suggested that people have changing perceptions about their musculoskeletal problem and insights about illness may influence health outcomes such as pain and disability directly or indirectly by their resultof coping. If a person reflects that musculoskeletal problem is a serious disease that medical care or health services can do little about, this belief may have an effect on the level

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of interference in daily life from that disease that the person reports and on their decision to consult or seek treatment for it. This discloses that perceptions may be an vitalissue to address as part of dropping the impact of disease and reassuring appropriate management.

After headaches and tiredness, back pain is the third most common health problem described by individuals. The exact cause of the augmented occurrence of low back pain (LBP) in populations of professional cab drivers is often uncertain. The most often reported risk factor for LBP is heavy physical workload such as lifting, awkward posture, and whole-body vibration. The prevalence and risk factors of WRMSDs among drivers would vary between countries due to differences in racial background, geographical location, ethnicity, and socio-demographics.. An earlier report found a 70.5% prevalence rate of WRMSDs among taxi drivers.

Bureau of Labour Statistics 2006 data on injury and illness show that the drivers of heavy trucks and tractor-trailers in the US are associated with the second-highest number of occupational illnesses and injuries for the past three years. It has been estimated that the indirect costs of one workday lost due to sickness absence amount to over than five hundred dollars [5]

A study in Japan inspected the risk factors of back pain reports that the prevalence of back pain among 285 taxi drivers was 45.8% which is consistent with other studies [6]. Some investigation showed risks of WMSDs in drivers are very high in Iran [7]. The evidence illustrated that people are driving at least half of working hours each day to endure three times more than other workers [8]. High prevalence of spinal disorders observed among the official worker, truck and bus drivers, particularly back and neck pain, frequently leads to invariable suffering and disease and possibly pre-retirement [9,10]. The aim of this study was to assess the musculoskeletal disorders among cab drivers of Delhi. This study will help us to determine the presence of WRMSD in drivers & to guide them for posture correction to help them in relieving their pain.

Methodology

In the methodology we use Nordic questionnaire. It is used to evaluate musculoskeletal symptoms, and the reliability has been checked out by test re-test system ,tells us that answers ,that are non-distinguishable fluctuate from zero to twenty-three percent, validity test tells us that answers that are non-distinguishable, changed between zero to twenty percent. The validity and reliability of the questionnaire were investigated and approved in different studies and several languages, including the Persian language[11,12] . This study design was one time observational.

Data Collection: The data collection procedures have been done recruiting the cab drivers of the age group 18-55 years & by using a "standardized general Nordic questionnaire". we collect data after taken an informed consent from subject they were explained about the study and they were asked to fill questionnaire. As this questionnaire is used to assess musculoskeletal symptoms& pain specifically back pain we have collected data from cab drivers of Delhi, India about musculoskeletal symptoms. The Inclusion criteria was drivers who drive cab only, drive cab, daily for at least 10 hours&cab drivers who have experience of at least one year of driving a cab&the Exclusion criteria was cab drivers, who were experiencing any kind of systemic or chronic musculoskeletal disease,Drivers who are involved in regular physical activity , Drivers with cardiovascular diseases

Result Analysis: Result was analysed by using SPSS 21 version. Study variables were measured using frequencies and discrete variables were measured by using mean and standard deviation, and as this is descriptive study, we use descriptive index test or frequency test or histogram test or box plot test.

On the analysis of the data we found 70% of cab drivers were suffering from lower back pain. Back pain was found to be most common problem for drivers in their daily activity. Back pain is affecting the daily works of the drivers so they are suggested exercise for their posture correction.

Data:

Age Distribution:

Table 1.1. Showing the Age Distrubution

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	18-25 Yr	4	13.3	13.3	13.3
	26-35 Yr	11	36.7	36.7	50.0
	35-45 Yr	11	36.7	36.7	86.7
	45-55 Yr	4	13.3	13.3	100.0
	Total	**30**	**100.0**	**100.0**	

Average Driving Time:

Table 1.2: Showing the Average time of driving

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	6-10 HR	12	40.0	40.0	40.0
	11-15	18	60.0	60.0	100.0
	Total	**30**	**100.0**	**100.0**	

Drivers Experience:

Table 1.3. Showing the Experience years of driving.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Less Than 5 Yr	8	26.7	26.7	26.7
	5-10 Yr	17	56.7	56.7	83.3
	11-15 Yr	3	10.0	10.0	93.3
	16-20 Yr	1	3.3	3.3	96.7
	20-25 Yr	1	3.3	3.3	100.0
	Total	**30**	**100.0**	**100.0**	

Table 1.4 Showing the percentage of lower back pain.

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	21	70.0	70.0	70.0
	No	9	30.0	30.0	100.0
	Total	**30**	**100.0**	**100.0**	

Discussion

In the current study found that most of the drivers were suffering from back pain. This affects their activity of daily living and stopping them from doing many works. This is a matter of serious concern because it affects their health & they must be guided for the posture correction & counselling will be done so that they must go for the regular visit to the physician & physiotherapist for the consultation & the treatment of their problem. In ROBB study on professional truck drivers, the most common reported disorders were low back, knee and neck pain.[13] The difference of spine pain between truck drivers and taxi drivers could be connected with the difference of vehicle vibration. Studies show that the vibration dose of taxi is lower than pain is more truck.[1415]

The vibration exposure level for taxi drivers is much lower than that for other professional drivers . Thirdly, direct back injury during motor vehicle accidents (MVAs) is another physical hazard associated with low back disorders of professional drivers.[16] The association between bending/twisting movements while behind the wheel and higher prevalence of LBP is also supported by recent reports from biomechanical experiments .[17,18] The observed association between long duration of car driving and LBP conforms to previous studies on other occupational groups often operating small automobiles.[11] Our cross-sectional analyses of the TDHS baseline data suggested that urban taxi drivers were a high-risk group for work-related LBP. The significantly higher prevalence of LBP (51%) in Taipei taxi drivers than other professional drivers (33%) in Taiwan is consistent with a previous study[18] High prevalence of musculoskeletal disease particular low back pain is possible due to prolong sitting position and absence of enough exercise among truck drivers[19]. Another point is a form of seats. Patterson et al., found that musculoskeletal disorders were the most prevalent health problem in bus drivers[20]. This study showed that low back prevalent in cab drivers.

In a study of Backman, about 70% of the drivers had suffered from musculoskeletal pain during the last month. In all, 40% of the drivers had often had back problem.[21]

Conclusion

The result of the current study showed the significance of frequent musculoskeletal disorders in cab drivers in different body regions, Among these, a lumbar area in drivers is more common .Present study concludes that there is high frequency of musculoskeletal disorders common in cab drivers in Delhi. Most commonly effected regions were upper back and lower back least common was wrist/hands region. This affects their activity of daily living & and stopping them from doing many works of day to day life as well this affects their job also.

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Immediate Effect of Buteyko Breathing and Bhramari Pranayama on Blood Pressure, Heart Rate and Oxygen Saturation in Hypertensive Patients: A Comparative Study

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Abstract

Aim: There are studies showing the use of Buteyko breathing technique & Bhramari Pranayama in reducing cardiorespiratory parameters in normal healthy individuals but to the best of our knowledge there is hardly any study that has reported comparative immediate effect of these two techniques. Thus the aim of our study was to evaluate the immediate effect of these two techniques on Blood pressure, heart rate and SpO2 in Hypertensive patients.

Method: The research design used was Comparative Experimental study design. Total 52 participants were screened and those meeting the inclusion criteria (n=42) were included in the study. The participants were randomly divided into two groups- Buteyko breathing and Bhramari pranayama Group of 21 each. Pre and immediate post intervention scores were measured in terms of systolic BP, Diastolic BP, heart rate and SpO2.

Findings: Inter group comparison (student's unpaired t test) showed that there was no statistically significant difference between the two groups in terms of Systolic and Diastolic BP, Heart rate and SpO2. The comparison of pre and post intervention valuesof the outcome measures in Buteyko group showed a significant difference in Systolic BP, Heart rate and SpO2 values whereas in Bhramari group there was statistically significant difference only the in Systolic BP values.

Conclusion: It can be concluded from the present study that Buteyko breathing and Bhramari pranayama are equally effective in immediately reducing the systolic blood pressure in hypertensive patients.

Keywords: *Buteyko breathing, Bhramari pranayama, Immediate effect, Hypertension.*

Introduction

Hypertension is defined as the persistent high blood pressure.[1] Systemic hypertension is one of the commonest non-communicable diseases in our country and also a prominent hazard to health[2] Around the world, hypertension is the foremost cause of premature mortality, with a rate of one in four men and one in five women.[3] Even though there is remarkable advancement in disease management, 30% of the global mortality are due to CVD.[2] It is considered as a silent invisible killer as the symptoms are usually invisible in early stagesupto occurrence of severe results. The international guidelines (2020) of Hypertension or elevated blood pressure diagnoses a patient with Hypertensionif systolic blood pressure (SBP) in the office or clinic is ≥140 mm Hg and/or diastolic bloodpressure (DBP) is ≥90 mm Hg. Further classification- Grade 1 Hypertension if SBP is

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140–159mmHg and/or DBP is 90–99 mmHg and Grade 2 Hypertension if SBP is≥160 mmHg and/or DBP is ≥100mmHg.[4]

Out of many treatment alternatives, the non-pharmacological interventions comprise a range of measures like reduction of weight, increasing physical activity, diet modification, tobacco and alcohol consumption restriction, yoga & meditationetc[6,7] Main aim of yoga is to makethe individual attain and maintain the "sukhasthanam" that is a dynamic sense of physical, mental and spiritual wellbeing. Pranayama - the science of well-ordered, conscious expansion of Prana (the life force) has many useful effects. A type of Pranayama, Bhramariis originated from the Sanskrit word 'Bhramar' meaning Wasp. The name Bhramari pranayama has come from the humming noise impersonating the flying wasp.[8] There are studies showing the immediate positive effect of Bhramari pranayama on resting cardiorespiratory parameters in normal healthy individuals.[10]

Yet another breathing technique was devised in 1950's by Dr. K P Buteyko.[9] It was basically developed to treat the asthmatics for hyperventilation. It is a unique breathing technique that utilises the breath holding and breath control exercises. Easy to understand and easy to perform are the merits of this technique. There are several studies claiming positive immediate effect of Buteyko breathing technique on cardiorespiratory para metes in healthy adults[12].

Aim: There is hardly any study that has reported comparative immediate effect of these two techniques. Thus the aim of our study was to evaluate the immediate effect of these two techniques on Blood pressure, heart rate and SpO2 in Hypertensive patients.

Materials and Methodology

Our study was a Comparative study. Total 52 Hypertensive (above 40 years of age) subjects residing in Nagpur region and outskirts were considered in the study. Study continued over a period of one month.

Inclusion Criteria:

1. Participants clinically diagnosed with hypertension in the past 20 years[13]
2. Age group -above 40 years
3. Participants with history of Coronary artery bypass grafting or angioplasty in the past 20 years.
4. Participants with addictions like alcohol, tobacco.
5. Participants having history of diabetes and thyroid.
6. Participants engaging in mild physical activity on daily basis.
7. Participants who have taken or are taking medications for hypertension.
8. Participants with a history of respiratory disorders but currently stable.

Exclusion Criteria:

1. Participants with active respiratory infection or kidney problems[21]
2. Cognitively unstable hypertensive patients.
3. Participants unwilling to cooperate in the study.
4. Participants with a history of organ transplant.
5. Participants undergoing any cardiac or respiratory rehabilitation program.

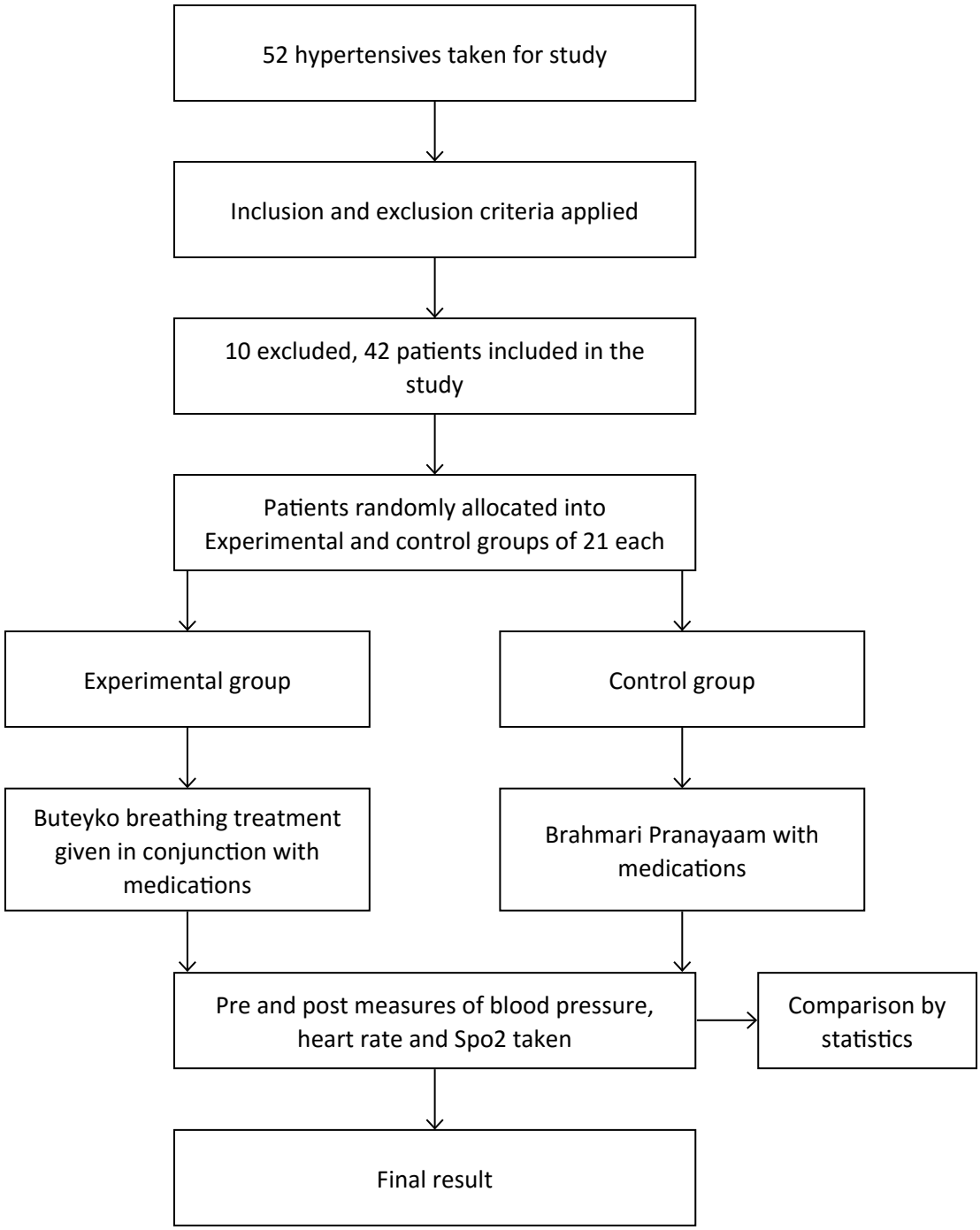
Materials: Digital B.P. apparatus, Pulse oximeter

Outcome Measures: Blood pressure, Pulse rate, Oxygen saturation

Methodology: 52 patients with hypertension were screened for the study. Ethical approval was obtained from Institutional Ethical Committee & an informed consent was obtained from the participants.Inclusion and exclusion criteria was applied and 10 patients were excluded from the study.

The study was conducted on 42 hypertensives fulfilling the inclusion criteria. The participants were randomly divided into two groups Buteyko and Bhramariof 21 each. One group performed the Buteyko breathing exercises and the other performed Bhramari pranayama.

The blood pressure, heart rate and Spo2 of the patients were taken pre Buteyko and Bhramari technique. The techniques were explained through video and audio to the patients and the patients were made to perform it. Immediately after the technique, the post readings of the blood pressure, heart rate and Spo2 were measured.



Buteyko technique:

Step 1: Control pause phase:

The patient was asked to sit in an upright chair, close the mouth and breath in normally through the nose for 30 seconds.

The patients were further instructed to:

- Breathe in a small breath and breathe out
- Then close both the nostrils with empty lungs, taking care lungs are not too void of air
- Keep a count for how much time one is comfortable before feeling urgency to breathe in again and hold on until then
- Breathe in byreleasing the nostrils
- Patients were asked to keep a watch on first breath after CP, to avoid taking a breath deeper than the one taken before CP. Patients were instructed to not

hold the breath for a longer duration as this may lead to deep breathing after measuring the CP.

Step 2: Shallow breathing

The patient was asked to:

- Sit up straight.
- Monitor the amount of air flowing through nostrils by placing the finger under the nose in a horizontal position.
- The patients' finger should lie close enough to nostrils so that the airflow can be felt, but not so close that the airflow is blocked[16]

Patients were asked to repeat Step 1 and 2 for 5 mins (or approximately 6 cycles)

Bhramari pranayama: In an ambient atmosphere patients were made to perform the Bhramari pranayama. The breathing technique was explained and demonstrated in the following manner

To start with, one has to be atease in a relaxed position (Sukhasana) with eyes closed, and maintainan erect and steady posture.

Placing the thumbs on the external auditory meatus, index and ring finger on the closed eyes and ring finger near the nostrils one should breath in slowly through both the nostrils for maximum of 5sec and exhale out completely again through both the nostrils for about 15sec. While exhaling one has to chant the word "AUMmmmmm" with a simultaneous nasal humming sound impersonating the sound of humming wasp. This leads to vibration of the inner nostril and laryngeal walls. With the completion of these steps one completes a cylcle of Bhramari pranayama. (RR 3/min).After 5 minutes of practicing Bhramari pranayama, the outcome measures were reassessed.

Once the pre and post measures of Blood pressure, heart rate and Spo2 were obtained, the statistics of the respective outcome parameters were calculated on the basis of MYSTAT 12 software.

Results

MYSTAT 12 software was used to calculate the results in this study. The outcome measures used were Systolic and diastolic blood pressure, heart rate and oxygen saturation.Mean and standard deviations were calculated to describe all the variables. The paired t-test and the unpaired t-test were used to verify differences between pre- and post-intervention

The gender ratio was 10:11 (10 females and 11 males) in Buteykogroup and 11:10 (11 females and 10 males) in Bhramari group. The difference in the mean age of both the groups was statistically not significant (Table 1).

Table 1. Baseline demographic data of both the groups

Group	Buteyko group	Bhramari group	t value	p value	Inference
Age (years)	60.57 ± 8.96	59.71 ± 8.95	2.17	0.86	Not significant
Gender ratio(M:F)	10:11	11:10			

Table 2: Mean and SD scores of Buteyko group and Bhramari groups, Pre intervention and Post intervention

	Buteyko Group				Bhramari Group				Between groups	
			Paired t test				Paired t test		Unpaired t test	
	Pre intervention Mean ± SD	Post intervention Mean ± SD	Mean Difference	p value	Pre intervention Mean ± SD	Post intervention Mean ± SD	Mean Difference	p value	p value	Inference
Systolic BP	140.75±21.27	125.55±26.42	15.2	0.003	139.61±6.04	129.95±8.25	9.66	0.004	0.56	Not Significant
Diastolic BP	83.15±10.75	84.56±6.56	1.41	0.56	81.28±5.22	82.77±4.98	1.49	0.31	0.31	Not Significant
Heart rate	78.31±12.55	75±8.21	3.31	0.001	75.06±9.07	74±9.65	1.06	0.86	0.62	Not Significant
SpO2	95.91 ± 2.23	97.89 ± 1.36	1.98	0.002	96.35 ± 2.65	97± 1.44	0.65	0.49	0.42	Not Significant

Inter group comparison (student's unpaired t test) showed that there was no statistically significant difference between the two groups in terms of Systolic and Diastolic BP, Heart rate and SpO2 (Table 2). The comparison of pre and post intervention values of the outcome measures in Buteyko group showed a significant difference in Systolic BP, Heart rate and SpO2 values whereas in Bhramari group there was statistically significant difference only the in Systolic BP values.

Discussion

The readings for pre and post values of Heart rate,SpO2 and blood pressure were taken for both Buteyko patients and Bhramari patients. Both the groups were compared for the effects using an unpaired t test. The results showed that there was no significant difference in systolic blood pressure, diastolic blood pressure, heart rate and SpO2 when the two groups were compared (p value - systolic B.P - 0.56,diastolic B.P - 0.31,heart rate - 0.62,SPO2 - 0.42)

However,individually Buteyko technique was helpful in improving systolic blood pressure (p value 0.003) and SpO_2 (p value 0.002), heart rate (p value -0.001)and bhramari was effective in improving systolic blood pressure. (p value 0.004)

The following could be the mechanism of action for Bhramari Pranayaam. Bhramari pranayama being a type of slow speed breathing exercise, it stimulates the parasympathetic nervous system. More they described that while exhaling, vibration effect on the nasal/ laryngeal mucous membrane together with the humming of "AUMmmm" lead to reflex apnoea by turning off inspiratory centre. It causes bradycardia through chemoreceptor sinu-aortic mechanism[14]

Further the neural respiratory elements may be responsible for a mechanism that clarifies how slow deep breathing affects the autonomic nervous system. During extended voluntary expiration, there is an increase in intrathoracic pressure causing more blood flow to the heart from lung and thus increasing the stroke volume. This leads to increased blood pressure causing stimulation of the carotid sinus baroreceptors. The increase in baroreceptor discharge leads to inhibitory action on the vasoconstrictor nerves and excites the vagus innervations of the heart. These may be the possible reasons for reduction in blood pressure in the present study. Another study by Jerath et al. explained that there is decreased oxygen consumption, heart rate and blood pressure resulting from slow deep breathing in pranayama, thus adding more strength to our study[15]

The Buteyko method is based on the concept that hyperventilation is the underlying cause of numerous medical conditions (Rosalba Courtney,2008)[16,17] One possible biochemical mechanism of Buteyko may be through its influence on nitric oxide (NO). NO is involved in a large number of physiological responses including bronchodilation and vasodilatation.[18] Ritu et al (2013) state that Buteyko breathing exercise is useful in management of respiratory rate and heart rate in chronic obstructive pulmonary disease patients.[19]Apart from this, it is also mentioned in studies that holding the breath can cause accumulation of carbon dioxide, that will lower blood pressure, as also breath holding will also improve the collateral ventilation which can be effective reasons for reduction of blood pressure, heart rate and improvement in SPO_2 in some patients. These can be the reasons that individually Buteyko and Bhramari have a significant effect on vital parameters.

The reason for no significant difference in both these two techniques can be that it was an immediate effect study. If the study was for a longer time, then maybe there would have been a significant difference noticed. There are several studies showing the effectiveness of Buteyko in cardiac and respiratory disorders over a long term.[9,10] There are also studies which show the effectiveness of Bhramari in long term on cardiac and respiratory disorders.[8] There are also studies that have shown that there is immediate effect on cardiorespiratory parameters by Buteyko technique.[12] So the study here proves thatButeyko and Bhramari techniques are both effective in hypertensive patients individually, but have no significant difference when compared for immediate effect.

Conclusion

It can be concluded from the present study that Buteyko breathing and Bhramari pranayama are equally effective in immediately reducing the systolic blood pressure in hypertensive patients.

Limitation: The present research was to study the immediate effect, thus the effect of long term treatment was not studied. Also the sample size was less.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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The Normal Response to Slump and Straight Leg Raising Test (SLR) in an Asymptomatic Subjects

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Abstract

Background: Neurodynamics is a test which check the mechanical movement of neurological tissue. It is used in an assessment to gain an impression of neural tissue mobility & sensitivity to mechanical stress. Slump and straight leg raising test are said to be the standard neurodynamics test for the lower limb.

Objectives: The aim of the study is to investigate the effect of neck and ankle position on knee and hip flexion movement during slump and straight leg raising (SLR) test and to know the neural tissue response to the neurodynamic test by addition of sensitizing manoeuver

Material and Method: It is an cross sectional study where 30 male & 30 female asymptomatic subjects in the age group of 18-39 years were taken

Results: It is concluded that there is a change in the range of movement in a normal asymptomatic subjection during Slump and SLR test. Therefore structural differentiating manouver has a significant effect on the body movement

Keywords: *Normal asymptomatic subjects, Neurodynamic test (slump & SLR test), Goniometer*

Introduction

Neurodynamic tests such as the straight leg raising (SLR) and slump test are frequently used for assessment of mechanosensitivity of neural tissue Sensitizing manoeuvres are limb or spinal movements added to neurodynamic tests, which aim to identify the origin of the symptoms by preferentially loading or unloading neural structures. A prerequisite for the use of sensitizing manoeuvres to identify neural involvement is that the addition of sensitizing manoeuvres has no impact on pain perception when the origin of the pain is non-neural.[1]

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Comprehensive evaluation of patients with low back pain must include a method of testing the integrity of related neural tissues. The straight leg raise test is recognized as the first neural tissue tension test to appear. During straight leg raise testing, the leg is elevated with the knee extended and the patient in a supine position. This places a tensile stress on the sciatic nerve and exerts a caudal traction on the lumbosacral nerve roots from L, to S,the straight leg raise test, nonneural structures, such as lumbar zygapophyseal joints, muscles, and connective tissue, can limit leg elevation and provoke patient discomfort during testing Flexing the cervical spine, dorsiflexing the ankle, and medially rotating the hip during the straight leg raise test increases tension exerted on the spinal cord, spinal dura, and lumbosacral nerve roots. In Maitland's slumptest, the patient is seated in full flexion of the thoracic and lumbar regions of the spine. Sensitizing manoeuvres are then systematically applied and released to the cervical

spine and lower extremities, while the tester maintains the patient's trunk position. The slump test evaluates the excursion of neural tissues within the vertebral canal and intervertebral foramen, detecting impairments to neural tissue mobility .They found that full spinal flexion, or flexion of the cervical, thoracic, and lumbar regions of the spine, produces lengthening of the vertebral canal. When the vertebral canal is elongated, the spinal dura is stretched, transmitting tension to the spinal cord, lumbosacral nerve root sleeves, and nerve roots. During full spinal flexion, the cauda equina becomes taut and the lumbosacral nerve roots and root sleeves are pulled into contact with the pedicle of the superior vertebra. When extension of the cervical spine is introduced, the dura and the nerve roots slacken as the vertebral canal begins to shorten. Extending the thoracic and lumbar spine increases the slack in the neural tissues as the vertebral canal continues to shorten.[2]

The straight leg raise (SLR) is a common neurodynamic test used to examine the mechanosensitivity of the lower extremity nervous system in individuals with low back or lower extremity pain. The SLR consisted of placing the knee in end range extension, determined by the examiner as end range resistance, followed by bringing the limb into hip flexion Pre-positioning in ankle dorsiflexion compared to plantar flexion is commonly utilized for purposes of structural differentiation during SLR testing. The movement was stopped at the first moment any sensory response was indicated, including but not limited to the sensation of stretch, pulling, tension, pain, numbness, or tingling.[3]

Methodology

1. **Study design:** Cross sectional study
2. **Study set up:** Physiotherapy department
3. **Selection criteria:**

Inclusion Criteria:

- Asymptomatic subjects were taken
- Age group 18-39 yrs

Exclusion Criteria:

- H/O of low back pain
- Joint injury
- Neurological or vascular impairment

Materials Used: Goniometer

Procedure: Permission to carry out the research were taken from the ethical committee of the Datta Meghe college of Physiotherapy, Nagpur. Subjects who were volunteered to participate in the study were selected keeping in view of inclusion and exclusion criteria. Structural differentiation or sensitizing manoeuvre have a significant effect on test response in terms of range of movement during neurodynamic tests.

The Slump & SLR tests are standard neurodynamics tests for the lower limb, it is demonstrated as follows:

Slump Test Procedure: The subject should be sat on table with the pelvis fixed and thigh fully supported with knees together. The patient is asked to slump the back into thoracic and lumbar flexion. A strap is positioned across the shoulder to maintain thoracic and lumbar flexion so that patient maintain the chin in neutral position. A universal goniometer was placed i.e stationary arm between lateral condyle of knee and greater trochanter and moving arm of goniometer should be placed between lateral condyle of knee and lateral malleolus of ankle. The zero was taken as the point of full extension.

For cervical flexion, the subject was asked to put their chin to chest & the examiner applied ankle dorsiflexion and passively extend the knee until the onset of resistance, then the range of knee flexion should be measured relative to zero. All time, the subject was monitored for the presence of adverse symptoms during testing.

Straight Leg Raising Test Procedure: The subject should be in a supine lying position with the trunk,shoulder and hip in a neutral position. A goniometer was positioned with the stationary arm aligned between the greater trochanter and midline of trunk and moving arm aligned between greater trochanter and lateral condyle of femur. The examined leg should be kept in full extension by placing the examined hand on thigh proximal to knee and other hand under the heel. Subjects were asked to dorsiflex the foot and elevates the leg in a sagittal plane about the hip joint until the onset of resistance. Then the range of hip flexion should be measured relative to zero.

All time, the subject was monitored for the adverse symptoms during testing.

Outcome Measures:

Knee flexion ROM during Slump test

Hip flexion ROM during straight leg raising test

Results

Table No. 1: Distribution of subjects according to age

Age Group	No of Subject	%
19-28	48	80
29-38	12	20
Total	**60**	**100**

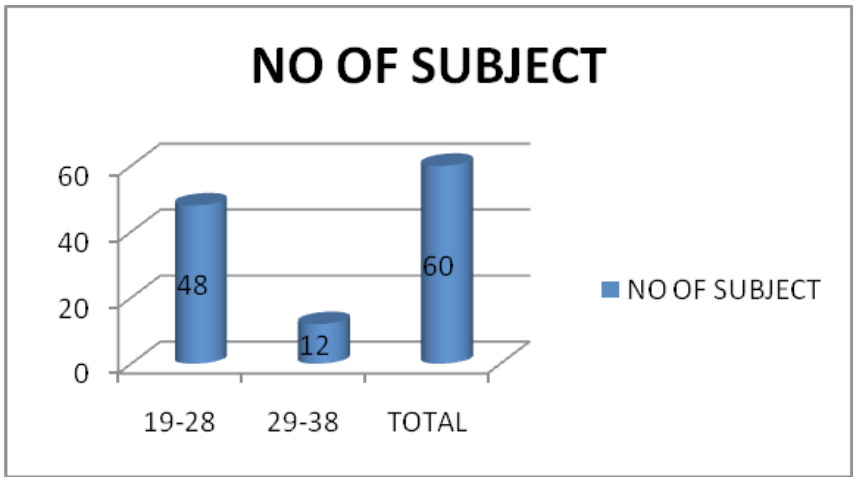


Figure 1

Table no 1 shows that, total 60 subjects were taken out of which 48 were participated in the age group of 19-28 years whereas 12 subjects in the age group of 29-38 years

Table No 2: Distribution of subjects according to Gender

Gender	No of Subject	%
Male	30	50
Female	30	50
Total	**60**	**100**

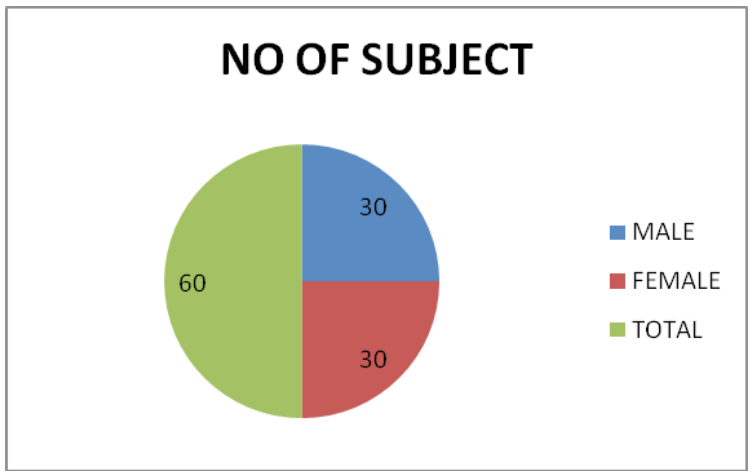


Figure 2

Table no 2 shows that total 60 subjects were taken out of which 30 male & 30 female who were volunteered to participate in the study in the age group of 19-38 years.

Table No 3: Mean and S.D of Flexion

	Male	Female
Knee flexion at slump neck flexed	42.7	37.77
Knee flexion at slump neck extended	48.6	47.6
SLR hip flexion at ankle dorsiflexion	73.9	73.3
SLR hip flexion at ankle neutral	82.23	81.23

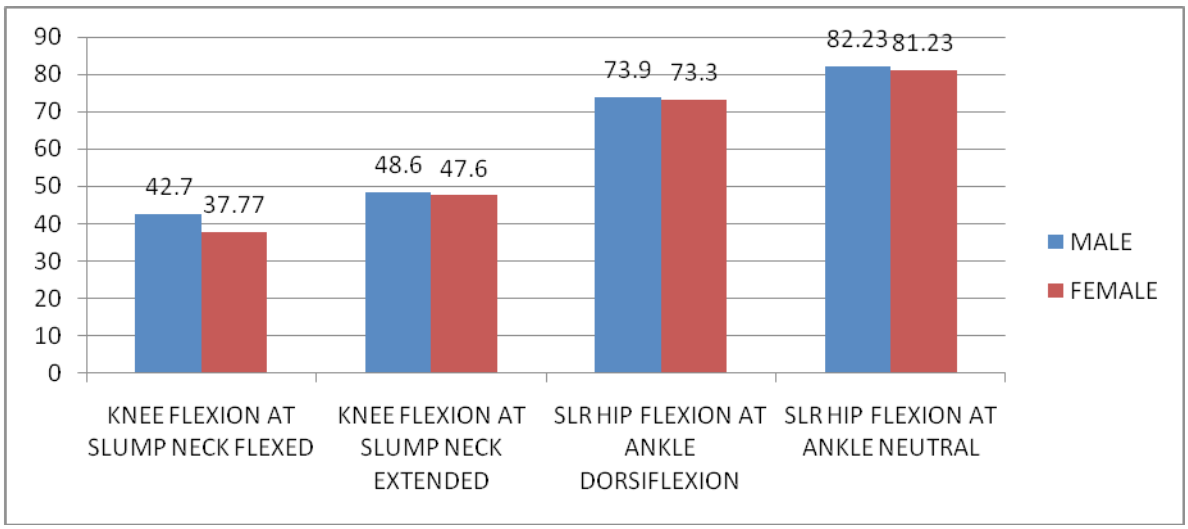


Figure 3

Table no 3 shows that range of knee and hip flexion angle during slump test and straight leg raising (SLR) test .It is found that there is an significant difference in knee & hip flexion angle in both male and female subjects.

Discussion

All data were analysed using statistical package, 60 subjects were taken (30 male and 30 female) who were volunteered to participate in the study between the age groups of 19-38 years. Structural differentiation or sensitizing manoeuvre have a significant effect on test response in terms of range of movement during neurodynamic tests. Cervical flexion & cervical extension during slump test whereas ankle dorsiflexion & ankle neutral during straight leg raising test act as a sensitizing manoeuvre. During slump test it is observed that knee flexion angle decreases when cervical flexion component wasadded, on the other hand knee flexion angle increases when cervical extension component was added both in male and female subjects.

This is supported by the study of P.C Lew, CA. briggs et al who conducted a study on asymptomatic patients between the age groups of 18-30 years he found that whether changing the cervical position during slump test altered posterior thigh pain/or tension in hamstring muscles,and there is an averaged over the group there was a 40% decrease in posterior thigh pain with cervical extension,it indicates that cervical movement did not change hamstring muscle tension the change in experimentally induced pain during cervical flexion was not due to change in hamstring muscle,it is concluded that posterior thigh pain caused by slump test and relieved by cervical extension arises from neural structures rather than hamstring muscle.[4]

This is also supported by Ketakijoshi, charu. Eapen et al who determine normal sensory and ROM responses during the movement components of thoracic slump test in an asymptomatic subjects,he conducted slump test in two sequences,proximal initiation which was proximal to distal and distal initiation which was distal to proximal its results shows the prevalence of sensory responses, its nature area and intensity,it concluded that the intensity of symptoms of proximal initiation

sequences was significant (p>0.05) when compared to distal initiation sequences and there is a change in ROM was significant for distal initiation when compared to proximal initiation,these normal responses is used as a reference when using thoracic slump test.[5]

During straight leg raising test it was found that there was a diminished range of hip flexion due to addition of ankle dorsiflexion, and increase in hip flexion due to addition of ankle in neutral position .Ankle dorsiflexion and neutral act as a sensitizing manoeuvre. The study was based on a sample of asymptomatic subjects in response to standard Neurodynamic test during slump and SLR test following the addition of structural differentiating manoeuvre.This study is supported by Benjamin s Boyd et al who conducted a cross sectional observational study on 20healthy subjects without low back pain,he measured hip flexion angle and surface electromyographic measures were taken and compared at the onset od symptoms P1 and at the point of maximally tolerated symptoms during SLR with ankle dorsiflexion and ankleplanter flexion, he concluded that addition of ankle dorsiflexion during SLR testing induces earlier distal muscle activation and reduces hip flexion motion.[6-8]

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Forensic Age Estimation from Proximal End of Femur: A Radiological Study in Living Individuals

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Abstract

Age determination is a very essential work in the field of forensic anthropology, which is a scientific study of human skeleton to determine age, sex and time of death to identify an individual. The identification is nothing but the recognition of an individual through various physical features or biological parameters. This study aims to examine the relationship between the stage of epiphyseal union of ossification centres at proximal end of femur & biological age in Chhattisgarh population. The study was carried out in 140 healthy subjects (70 girls and 70 boys) aging from 13 to 20 years. The obtained results revealed that the complete fusion of epiphysis of proximal end of femur is seen at 17-19 years. Females were consistently developing epiphysealunion at a younger age than their male counterparts with one year of difference. Results also suggest that the age of epiphyseal fusion at proximal end of femur is found to vary greatly all over the India, indicating the need for separate standards for separate regions.

Keywords: *Epiphyseal Union, Head of femur, Greater trochanter, Lesser trochanter.*

Introduction

Age determination by the epiphyseal fusion of bones is an intrinsic part of the biological characteristics by a forensic anthropologist to assist in achieving the identification of an individual. There are many well-known variables for identification of an individual like birthmarks, mal-formations, tattoo marks, scar marks etc. These types of identification are done by the forensic anthropologist. Several decomposed, burned, mutilated bodies are difficult to recognise & hence the identification of an individual becomes difficult[1,2]. Forensic anthropologist plays a crucial role in age determination & is one of the stringent problems in the medical justice system in both civil & criminal matters. As age advances, it becomes a wide problem in view of factors like nutrition, heredity, race, endocrinal etc. By taking into account the radiological analysis, this study will be of great help in further understanding the details of accurate assessment of age in Central Indian population[3,4,5].

Aims and Objectives:

1. To evaluate age specific difference in epiphyseal union at proximal end of femur.
2. To estimate age from epiphyseal union at proximal end of femur.
3. To compare bisexual difference in epiphyseal union at proximal end of femur.
4. To compare the findings of epiphyseal union at proximal end of femur in Central Indian population with other parts of India on the basis of previous studies.

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Material and Method

The present study was carried out in the Department of Forensic Medicine & Department of Radiology, SSIMS, Bhilai, Chhattisgarh. A total of 150 individuals participated in this study. The subjects included were students of 13-20 years of age from schools & colleges from Bhilai & Durg city. They are born to parents domicile from Central India & have lived here since birth. The subjects do not have any disease/deformity related to bones or chronic disease affecting the general health. An informed consent was taken from all subjects prior to each procedure. Skeletal maturity was evaluated according to the Jit & Kulkarni's classification[1] of four stages: Appearance, Non fusion, Partial fusion & Complete fusion. X-Rays showing clear gap between the epiphyseal and diaphysial ends & showing saw tooth like appearance end were designated as "Non-fusion" (NF) X-rays. X-Rays showing a line replacing the hiatus between the epiphyseal & diaphysial ends & not showing saw tooth like appearance were designated as "Partial Fusion" (PF) X-rays. X-Rays showing the same bony architecture in the diaphysis and epiphysis & showing scar of the previous stage were designated as "Complete Fusion" (CF). It was classified, analysed and compared with known standards. At the end conclusions were drawn, which were compared with available results of various previous studies.

Results

Table 1: Epiphyseal fusion in males:

Age (years)	Head of femur			Greater trochanter			Lesser trochanter		
	NF	PF	CF	NF	PF	CF	NF	PF	CF
13-14	3	7	0	4	6	0	3	7	0
14-15	1	9	0	2	8	0	1	9	0
15-16	0	10	0	1	9	0	1	9	0
16-17	0	8	2	0	8	2	0	8	2
17-18	0	4	6	0	3	7	0	4	6
18-19	0	0	10	0	0	10	0	0	10
19-20	0	0	10	0	0	10	0	0	10
Total	**70 (100%)**			**70 (100%)**			**70 (100%)**		

Table 2: Epiphyseal fusion in females

Age (years)	Head of femur			Greater trochanter			Lesser trochanter		
	NF	PF	CF	NF	PF	CF	NF	PF	CF
13-14	0	10	0	0	10	0	0	10	0
14-15	0	9	1	0	8	2	0	9	1
15-16	0	7	3	0	7	3	0	7	3
16-17	0	2	8	0	1	9	0	2	8
17-18	0	0	10	0	0	10	0	0	10
18-19	0	0	10	0	0	10	0	0	10
19-20	0	0	10	0	0	10	0	0	10
Total	**70 (100%)**			**70 (100%)**			**70 (100%)**		

In males, Head of femur shows: non fusion in 4 (5.71%) cases in age group of 13-15 years, partial fusion in 38(54.28%) cases in age group of 13-18 years & complete fusion in 28 (40%) cases in age group of 16-20 years. Complete fusion is seen in all the subjects in age group of 18-20 years. In females, Head of femur

shows: partial fusion in 28 (40%) cases in age group of 13-17 years & complete fusion in 42 (60%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects in age group of 17-20 years.

In males, Greater trochanter shows: non fusion in 7 (10%) cases in age group of 13-16 years, partial fusion in 34 (48.57%) cases in age group of 13-18 years & complete fusion in 29 (41.43%) cases in age group of 16-20 years. Complete fusion is seen in all the subjects in age group of 18-20 years. In females,Greater trochanter shows: partial fusion in 26 (37.14%) cases in age group of 13-17 years & complete fusion in 44(62.85%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects in age group of 17-20 years.

In males, Lesser trochanter shows: non fusion in 5 (7.14%) cases in age group of 13-16 years, partial fusion in 37 (52.86%) cases in age group of 13-18 years & complete fusion in 28 (40%) cases in age group of 16-20 years. Complete fusion is seen in all the subjects in age group of 18-20 years. In females, Lesser trochanter shows: partial fusion in 28 (40%) cases in age group of 13-17 years & complete fusion in 42 (60%) cases in age group of 14-20 years. Complete fusion is seen in all the subjects in age group of 17-20 years.

Discussion

Table 3: Comparison of Age of union (years)

Sr. No	Researcher	Region/population	Union of head, greater trochanter & lesser trochanter with shaft of femur (years)	
			Male	Female
1	Davies & Parson[2]	England	19-20	19-20
2	Hepworth[3]	Punjabi	15.5 - 17	15.5 - 17
3	Flecker[4]	Australians	17	14
4	Galstaun[5]	Bengalis	16-19	14-15
5	Chaurasia[6]	Indian	17-18	17-18
6	Parikh[7]	Indian	16 - 18	16 - 18
7	Nandy[8]	Indian	16 - 17	14 - 15
8	Vij[9]	Indian	17 - 18	17 - 18
9	Bhise S et al[10]	Mumbai	16 - 18	14 - 16
10	Sharma Y et al[11]	Udaipur	18-19	18-19
11	Devraj N et al[12]	Dhule	-	17-18
12	Present Study	Central India	18-19	17-18

When we compare our study with the previous studies for female population we found that our study is in agreement with studies conducted by Chaurasia[6], Parikh[7], Vij[9], Sharma Y et al[11] & Devraj N et al[12]. Whereas for male population we found that our study is in agreement with studies conducted by Galstaun[5], Chaurasia[6], Parikh[7], Vij[9], Bhise S et al[10] & Sharma Y et al[11]. If we carefully go through the above chart and analyse the overall age range for the Union of head, greater trochanter & lesser trochanter with shaft of femur, we found out that there is a lot of regional variation. So address this problem there is immense need of such studies in different regions of our country & these studies must include larger sample size.

Summary and Conclusion

1. This study was conducted exclusively on the young indigenous population of Central India.
2. The epiphyseal union at proximal end of femur in males is completed in all instances (100%)at the age of 18-19 years.
3. The epiphyseal union at proximal end of femur in females is completed in all instances (100%) at the age of 17-18 years.

4. Females were consistently developing epiphysealunion at a younger age than their male counterparts, with one year of difference.
5. Central Indian population is of mixed type comprising of various religions and castes. The opinion about age should always be given in the range.
6. From this study, range of 1-2 years of margin of error can be concluded.
7. For age estimation, relevant joints should be radiologically examined for different centres and opinion should be arrived considering the status of multiple centres.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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A Prospective Observational Case Series of Liver Injury in Paediatric Patients Secondary to Consumption of Ayurvedic Herbomineral Formulations

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Abstract

Background: Ayurvedic medicines especially herbomineral formulations are combinations of metals and herbs but not in a raw form. There are very good positive things in society about these herbomineral medicines and lots of misconceptions in modern world and modern scientist. They are much confused that how ayurvedic doctors can use metals in medicines; because they feel that metals are toxic to human body, metal can cause hepatotoxicity and nephrotoxicity. This is on scientific level totally confusion and misunderstanding in the mind of allopathy people. Why this confusion arises…? Actually they must little bit understand about ayurvedic science. This science never suggests to use these metals in the raw form. They are converting these metals totally nano particle level so original metal has been lost its molecular structure and it is completely new ayurvedic bhasmas are only oxides of metal. First purify the metal and after purification with lots of complicated biochemical processing, converting them into such molecule that molecule does not have a single raw metal molecule. This must be understood that final product is completely different than raw metal used. If try to study this things then nobody will challenge to ayurvedic science.

Aim: Observe the incidence of hepatic injury in paediatric patients due to herbomineral drug consumption.

Material and Method: In this prospective case series 5 drugs regimen of herbomineral medicine was given to 14 paediatric patients of age group 4 years to 14 years who were diagnosed as reactive and recurrent respiratory disease. The total duration of drug delivery was 90 days. After successful completion of treatment course Liver Function Test (LFT) of each patient was done and conclusion was drawn.

Outcomes: Total 14 patients, biomarkers of Liver Function Test were observed normal and at last follow up every patient was clinically fine.

Conclusion: Use of herbomineral medicines in paediatric patients is safe and it does not cause hepatotoxicity.

Keywords: *Hepatotoxicity, Liver injury, Herbomineral formulation, Paediatric, Drug Induced Liver Injury, Ayurvedic Medicines. Liver Function Test, Nephrotoxicity.*

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Introduction

Ayurveda believes in eradicating the disease right from its root and propound drugs that cause no complications. The metabolism of drugs depends upon the Bhautikagni's (enzymes) in liver and the Dhatvagni's in the cells or tissues.[1,2] In order to produce main desired

effect a drug must be present in appropriate concentration at the site of action. The optimum concentration of the drug depend upon the dose administered and the extent and rate of its digestion, absorption, localisation or bioinformation and excretion.[3] Despite rapid advancing theories in modern pharmacology; much a remains to be said about drug versus disease, pharmacokinetics, biotransformation, selective affinity of drugs to a particular cell or system.[4] Ayurveda believes in treatment without complications and therefore the Ayurvedic drugs as remain administered show s no encouraging pharmacodynamic or chemicotherapetic actions, they show only the pharmacotherapeutic actions well.[5]

The use of herbomineral Ayurvedic formulations is sensible and objectionable topic from the site of western science followers but at the same time the use of these medicines is interesting in Ayurvedic practitioners as they get desired effects among the patients. Various articles regarding the nephrotoxicity and hepatotoxicity secondary to consumption of Ayurvedic herbomineral and metallic medicines are found at internet. To address such issues the study was conducted to make evidence based knowledge and to claim the safe use of herbomineral medicines in paediatric population.

This prospective clinical trial was done with well informed consent in paediatric patients under the age group of 4 to 14 years which were diagnosed as Reactive and recurrent respiratory diseases and herbomineral five drug regimen was given for 3 months. Study was conducted at government Ayurvedic college osmanabad, Maharashtra in 2017-2018. Patients were observed for Hepatic pathological changes by applying biomarkers of Liver Functions.

This study provides evidence to use of Ayurvedic herbomineral medicines in children without causing harm to liver and also build confidence regarding the use of Ayurvedic herbomineral medicines among Ayurvedic practitioners and society also.

Material and Method

Study design

Study type: Observational, Prospective Clinical Trial.

0.- 14 participants of age group 4 years to 14 years.

Study duration: 1 year.

Group and cohorts: Single group/cohort diagnosed reactive and recurrent respiratory disease.

Sampling Method: Non-probability sample.

Study population- Children's in the age group of 4 to 14 years suffering from Reactive and recurrent respiratory diseases was involved in study and given 5 herbomineral drugs regimen for same illness.

Criteria: Inclusion criteria – children in the age group of 4 to 14 years presenting with complaints of recurrent respiratory illness episodes wheeze associated respiratory illness, reactive airway disease. Exclusion criteria - children suffering from tuberculosis, congenital heart disease and malformations, child suffering from any hepatic disease and any life threatening conditions.

Intervention: 5 herbomineral drugs regimen from standard and GMP certified Pharma Company.

1. Swaskaschintamani rasa[6]
2. Swaskuthar rasa[7]
3. Laxmivilas rasa[8]
4. Abraka bhasma (shataputi)[9]
5. Sitopaladi churna.[10]

Dose: Dose was decided as per age according to Sharangadhara samhita[11] and after discussed with higher authority of concerned subjects (Rasashastra department and paediatric department of institute). Above mentioned 5 drug regimens in powder form, 500 mg up to 7 years and 1 gm to above 7 years children BID after meal.

Anupana (vehicle): Honey

Duration of drug delivery: 5 drug combination was given for 3 months at one month interval.

Consent: Child was enrolled in study after taking well informed written consent from parents.

Follow up- total 4 follow ups were conducted at one month interval. At last follow up Liver Function test of each patient was done at authentic pathology laboratory.

Case Series:

Table 1: Case series/case details

Case	Age in years	Sex	Weight in Kg	Present complaints	History	Diagnosis	Treatment	Written Consent
Pt -1	14	M	35.6	Dry cough, cold, chest pain	Specific food and seasonal aggravation, repeated medication and nebulisation.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -2	7	M	16.4	Rhinitis	Repeatedly received medication and nebulisation.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt-3	7	M	13.8	Breathlessness, cough, cold	Asthama in grandparents, repeated medication and nebulisation.	Reactive airway disease	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt-4	5	F	13.1	Cough, cold	Seasonal and specific food aggravation, parental allergy, repeated medication and nebulization	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt-5	7	F	15	Rhinitis	Specific food aggravation, repeated use of nebulisation.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -6	5	F	14.6	Cough and cold	Parental allergy, specific food and .seasonal agrravation	Reactive airway disease	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt-7	13	M	27	Continuous rhinitis	Specific food and seasonal aggravation, repeated medication for same illness.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -8	11	M	33.4	Continuous dry cough	Grand Parental allergy, specific food and seasonal aggravation	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -9	5	F	15.3	Cough with bouts	Seasonal and specific food aggravation, parental allergy, repeated medication and nebulisation	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -10	4	F	12.6	Tonsillitis, fever, cough, cold.	Specific food and seasonal aggravation, repeated medications for same illness.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -11	10	M	21.6	Running nose	Seasonal and specific food aggravation, parental allergy, repeated medication and nebulisation.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -12	14	M	50.2	Cough, cold, fever	Seasonal aggravation, repeated medication, parental allergic rhinitis.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -13	4	M	13.5	Rhinitis	Seasonal and specific food aggravation, parental allerg.	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes
Pt -14	5	M	12.5	Cough, cold	Seasonal and specific food aggravation, parental allergy, repeated medication and nebulisation	Recurrent respiratory disease/illness	Swaskaschintamani rasa, swaskuthar rasa, lsxmivilas rasa, abhraka bhasma, sitopaladi churna.	Yes

Results

The present observational clinical trial was carried out to observe the liver injury after giving Ayurvedic herbomineral medicines to paediatric patients had same diagnosis. After successful completion of 3 months treatment course Liver Function Test was done and following results were obtained.

Table 2: Liver Function Test (LFT) Patient's Value

Sr.No.	Bilirubin Total (mg/dl)	Direct (mg/dl	Indirect (mg/dl	S.G.O.T. (U/L)	S.G.P.T. (U/L)	Total Proteins (g/dl)	Albumin	Globulin	Alkaline Phosphate (U/L)
Patient-1	1.00	0.6	0.4	23	12	6.7	4.4	2.3	214
Patient-2	0.35	o.35	0.00	31	18.3	-	-	-	-
Patient-3	0.86	0.19	0.67	41	25.6	6.7	3.6	3.2	101.3
Patient-4	1.0	0.6	0.4	37	28	6.7	3.6	3.1	-
Patient-5	1.2	0.7	0.5	38	20	5.8	4.2	1.6	-
Patient-6	0.87	0.34	0.53	40.3	39.2	-	-	-	115
Patient-7	0.9	0.3	0.6	28	19	6.4	3.5	2.9	-
Patient-8	1.0	0.4	0.6	41	33	7.0	3.5	3.50	-
Patient-9	1.96	1.37	0.59	31	23.3	-	-	-	-
Patient-10	1.0	0.6	0.4	38.9	22.1	6.7	4.2	2.5	-
Patient-11	0.54	0.24	0.30	39.7	20.6	7.27	4.25	3.02	-
Patient-12	0.48	0.46	0.02	19	11.3	-	-	-	-
Patient-13	0.9	0.5	0.4	30	22	7.9	4.5	3.40	-
Patient-14	0.5	0.2	0.3	35.1	16.5	6.24	3.64	2.6	-

Discussion

Generally speaking, Ayurvedic drugs possess no toxicity nor do they cause any complication and therefore, the Ayurvedic treatment never causes iatrogenic diseases (diseases caused by misguided treatment). But if the disease has not been properly diagnosed and only symptomatic treatment is given the duration of treatment is unnecessarily increased and obviously, this has socio-economic repercussions. Even though drugs have got active principles which with they work, they have some other fractions which counteract their bad effects, if any. Therefore Ayurveda advocates that drug should be used as a whole so that the desired effects may be had without any side effects.[12]

In Ayurvedic medicines the calculated proportion of ingredients in different formulations has a scientific meaning behind it. Disturbance in that proportion causes incompatibility. Once these explanations are accepted, it should be admitted that Ayurveda treats the patient while other systems of medicine treat the disease. To a lay and ignorant man, it is difficult to understand a disease without patient and a patient without disease.

There are some Ayurvedic metallic preparations, but they are fully and perfectly purified before administration. The finished product of Ayurvedic formularies does not cause any toxic or side effects. In some of the formulations antidotes are addedto avoid any possibility of toxic effects. Majority of Ayurvedic drugs show only the pharmacotherapeutic actions and do not cause any complication.[13]

Some articles published in journal regarding hepatic toxicity[14] and nephrotoxicity secondary to consumption of Ayurvedic medicines are observed and to address such articles the study was conducted. Paediatric department of this institute several times used Herbomineral medicines in children and does not noted any adverse effect of such medicines till date. Actual study was conducted over the paediatric patients suffered from recurrent respiratory illness and reactive airway disease[15], and herbomineral medicines (5 drug regimen)for 3 months. Along with this, to observe hepatotoxicity secondary to these combinations Liver Function Test was carried out after successful completion of treatment. And results was found no any pathological changes in the biomarkers

of liver function after given the herbomineral 5 drugs combination for 3 months in appropriate dose.

Conclusions

The present study,In this 14 patients observational clinical trial it was observed that use of Ayurvedic herbomineral medicines is safe and there is no relation to hepatotoxicity secondary to consumption of Ayurvedic herbomineral formulations.

Concluded as:

1. The aim of treatment is to cure the disease and to restore the health. Proper selection of drugs brings desired effects. Keeping all the pharmacological aspects in view, a drug should be chosen and employed to cure a disease.
2. Drug administration should be preceded by perfect diagnosis of disease and followed by expected results only.
3. the social implication of medicine is to see that man remains healthy and happy, and also useful to society. except in case where life itself is in danger, any drug causing iatrogenic disease should be socially and ethically contrabanded.

The Ayurvedic drugs fulfil all above requirements and therefore, satisfy the social obligation of medicine.

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Study of Non-Stress Test as a Screening Tool in Low Risk Pregnancies at Term Gestation

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Abstract

Introduction: Various antepartum surveillance method aim at early detection of fetal distress preventing intrauterine fetal death. Non Stress Test is one such tool used for screening antenatal patients. In this study non stress test is used to screen and monitor fetus of low risk mothers, to detect fetal distress take necessary intervention and correlate fetal outcome with test results.

Materials and Method: This was a prospective longitudinal study conducted in Shalini Tai Meghe hospital and Research Centre, Wanadongiri, Nagpur from august 2019 till August 2020. 180 Antenatal patients with 37 completed weeks in labour and with no high risk factors were included in the study. Non Stress Test was performed on the patients and according to NICE guidelines, the result was interpreted as normal or abnormal.

Results: Out of 180, 130 patients had normal Non-Stress Test and 46 patients had abnormal Non Stress Test. The percentage of normal delivery was 60.4% in normal non stress test group and 23.91% in abnormal non stress test group, where as the percentage of caesarean section was 33.58% in normal non stress test group and 63.04% in abnormal non stress test group. The most common indication for caesarean section in abnormal non stress test group was fetal distress with 68.96%. Meconium stained liquor was present in 73.91% patients with abnormal non stress test, with negative predictive value 72.34%, sensitivity 73.91% and specificity 72.34%. APGAR score < 7 at 5 minutes was present in 34.78% patients with abnormal non stress test group, with negative predictive value 81.36%, sensitivity 34.78% and specificity 97.76%. 18 babies from abnormal non stress test group were admitted in NICU with no perinatal mortality.

Conclusion: Non stress test is a reliable screening tool for detecting high risk fetus in low risk mothers. The probability of adverse outcome like meconium stained liquor, low APGAR score, NISU admission increases with abnormal non stress test.

Keywords: *APGAR score, NISU, stress test, liquor and risk fetus.*

Introduction

Maternal mortality has been drastically reduced in developing countries and now modern obstetricians are more focused on maternal as well as fetal wellbeing. Originally, fetus was still deemed a conception result, but today the fetus is deemed another patient, and it often needs as much supervision as mother's well being. Intra partum monitoring of fetal health is one of the main component of contemporary obstetrics education. Approximately 50 % of fetal mortality happens in low risk mothers without obvious cause.(1)

Prof E. H. Hon and Prof. Caldeyro Barcia invented cardiotocogram (CTG) for fetal monitoring (2). In the

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western countries,continuous fetal monitoring is being used extensively but due to economic constraints, itis not feasible in most of the developing countries like ours for practicing it. Ingemarsson et al.[3] has described analternative, in the form of Admission Test(AT) or Non stress test (NST) which is a short recording of FHR by cardiotocogramanduterine contractions of 15-20 minutes, at the time of admission in labour ward.

Non Stress Test is a non invasive, patient friendly, highly simple to perform and results are easily interpreted. The test detects transient FHR acceleration associated with fetal movements affecting the cerebral cortex and is caused by physiological and pathological effects on the brain of the fetus.

NST could be used as a screening technique to diagnose preexisting fetal hypoxia, and to schedule early intervention to avoid perinatal adverse outcome. The preset research was performed to forecast the perinatal result by conducting NSTas an admission test to minimize fetal morbidity and mortality by early diagnosis and intervention

Nice (2007) Classification of FHR trace feature[4]:

Feature	Baseline	Variability	Deceleration	Acceleration
Reassuring	**110-160**	**_>5**	**None**	**Present**
Non–reassuring	100-109 161-180	<5for 40-90min	Typical variable deceleration with over 50%of contraction, occurring for over 90min Single prolonged deceleration for up to 3min	The absence of acceleration with otherwise normal trace is of uncertain significance
Abnormal	<100 >180	<5 for 90min	Either atypical variable decelerations with over 50% of contractions or late decelerations, both for over 30 minutes Single prolonged deceleration for more than 3 minutes	The absence of acceleration with otherwise normal trace is of uncertain significance

Categorise cardiotocography traces as follows:

- **Normal:** All features are reassuring
- **Suspicious:** 1 non-reassuring feature and 2 reassuring features
- **Pathological/abnormal:** –1 abnormal feature or 2 non-reassuring features(4)

Aim of the Study: To study the role of Non Stress Test as a screening tool in management of low risk pregnancies in labor.

Materials and Method

Antenatal patients with 37 completed weeks in labour and with no high risk factors were included in the study. High risk pregnant patients like pre eclampsia, severe anaemia, heart disease, gestational diabetes mellitus, antepartum eclampsia, twins, previous lscs, intrauterine growth restriction were excluded from the study.

Informed written consent were taken from the patients. Detail history, general examination, systemic examination and obstetric examination was done. Routine blood investigations were done.

Non stress test was performed on the patient with patient in supine position. The Doppler transducer was strapped to the abdomen at the location where fetal heart sound was distinctly heard. The tocodynamometer was strapped at the fundus of the uterus. The fetal movement probe was given to the patient to press at the the time of fetal movement. Record was taken for full 20 minutes. Tracings were categorized according to NICE guidelines(4) as normal, suspicious and abnormal or pathological.

If NST was normal, monitoring was carried out with intermittent auscultation of fetal heart sounds. Repeat NST was done after 3 hours. If NST was suspicious, further extended NST was done for the patient for 40 minutes after following corrective measures like - patient

given left lateral position, oxygen was given, patient was hydrated with I.V fluids. If extended NST was found to be still suspicious continous monitoring with NST carried out till NST turns out to be normal. If NST turns abnormal, artificial rupture of membranes was attempted and decision for termination of pregnancy by LSCS, or instrumental delivery was taken dependingon amount and color of liquor and stage of labour.

Results

Total 180 antenatal patients in first stage of labour were included in the study. Maximum patients were in the age group of 21- 30 years i.e 82.22%. 11.11% patients were more than 35 years of age and 6.66 % patients were 18-20 years of age .51.66 % patients were primigravida and 48.33% were multigravida. Out of 180, 134 (74.4%) patients had normal NST recordings. 46 patients (25.55%) had abnormal NST out of which 26 had suspicious NST and 20 had pathological NST.

Table 1: Correlation of mode of delivery with admission NST

	Normal NST	**Abnormal NST**	
Ftnd	81(60.4%)	11 (23.91%)	92
Instrumental Delivery	8 (5.9%)	6 (13.04%)	14
Lscs	45 (33.58%)	29 (63.04%)	74
Total	134	46	180

As seen in the table, out of 134 patients with normal NST, 81 patients (60.4%) had full term normal delivery, 8 patients (5.9%) had instrumental delivery and 45 patients (33.58%) had caesarean section. Out of 46 patients with abnormal Non Stress Test, 11 patients (23.91%) had full term normal delivery, 6 patients (13.04%) had instrumental delivery and 29 patients (63.04%) had caesarean section.

Table 2: Indication of LSCS in all types of NST

Indication of LSCS	**Normal NST (n=134)**	**Abnormal NST (n=46)**
Fetal Distress	3(6.66%)	20 (68.96%)
Others	42 (93.33%)	9 (31.03%)
Total	**45**	**29**

In normal Non Stress Test Group, out of total 45 caesarean sections, 3 patients (6.66%) had caesrean section due to fetal distress. In patients with abnormal Non Stress Test, out of 29 caesarean sections, 20 patients (68.96%) had caesarean sections for fetal distress.

Table 3: Correlation of NST with Meconium stained liquor

Status of Liquor	**Normal NST (n= 134)**	**Abnormal NST (n=46)**	**Total (n= 180)**
Liquor Clear	121(90.29%)	12 (26.08%)	133(73.88%)
Meconium Stained Liquor	13 (9.70%)	34 (73.91%)	47(26.11%)

As seen from above table, liquor was clear in 121 patient (90.29%) with normal Non Stress Test, where as 13 patients (9.70%) with normal Non Stress Test had meconium stained liquor. Out of 46 patients with abnormal Non Stress Test, clear liquor was present in 13 patients (9.70%) while meconium stained liquor was present in 34 patients (73.91%). Out of total 180 patients 73 .88 % patients had clear liquor while 26.11% patients had meconium stained liquor.

Table 4: Correlation of NST with APGAR Score

APGAR Score	Normal NST	Abnormal NST
APGAR>7 AT 1 MIN	128(95.5%)	21(45.65%)
APGAR<7 AT 1 MIN	6(4.47%)	25(54.34%)
APGAR>7 AT 5 MIN	131(97.76%)	30(65.2%)
APGAR<7 AT 5 MIN	3(2.23%)	16(34.78%)

The table depicts the correlation between NST and APGAR score. At 1 minute, APGAR score was equal to or more than 7 in 128 babies (95.5%) and APGAR was less than 7 in 6 babies (4.47%) among patients with normal Non Stress Test. In babies of patients with abnormal Non Stress Test, 21 babies (45.65%) had APGAR at 1 minute,equal to or more than 7 and 25 babies (54.34%) had 1 minute APGAR less than 7. At 5 minutes, 3 babies showed improved APGAR more than 7, in normal NST group. So finally at end of 5 minutes, only 3 babies with low APGAR less than 7 were admitted in neonatal intensive care unit.

In abnormal Non Stress Group, 25 babies (54.34%) had APGAR score less than 7 at 1 minute. After 5 minutes, 9 babies showed improved APGAR of more than 7 . So at the end of 5 minutes 16 (34.78%) babies had APGAR less than 7 and were admitted to neonatal intensive care unit.

Table 5: Correlation of NST with fetal outcome

	Normal NST	Abnormal NST
Admission in NICU	4(2.98%)	18(39.13%)
Neonatal Death	0	0

4 babies (2.98%) were admiited to neonatal intensive care unit from normal Non Stress Test group, out of which 3 babies had low APGAR and 1 baby had good APGAR more than 7. 18 babies (39.13%) from abnormal Non Stress Test group were admitted to neonatal intensive care unit, out of which 16 babies had low APGAR, 2 babies had good APGAR with congenital anomalies. There was no neonatal death.

Performance Characteristics of Non Stress Test:

	Sensitiivity	Specificity	Positive Predictive Value	Negative Predictive Value
APGAR < 7 AT 1MIN	54.34%	95.52%	80.64%	85.90%
APGAR < 7 AT 5MIN	34.78%	97.76%	84.21%	81.36%
MSL	73.91%	90.29%	72.34%	90.97%
NICU Admission	39.13%	97.01%	81.81%	82.27%

Discussion

In our study,total 180 patients were studied. Out 180, 134 patients (74.44%) had normal Non Stress Test while 46 patients (25.55%) had abnormal Non Stress Test. In study conducted on similar subject, by Phelan et al [5], 85.4% had reactive Non Stress Test and 14% had nonreactive and 0.6% had unsatisfactory Non Stress Test. In Studies by Shrestha et al[6] and Panda et al[7] 10% and 14% had abnormal Non Stress Test respectively.

In this study, 60.4% patients had full term normal

delivery in normal NST group while 23. 91% patients had normal delivery in abnormal NST group. 33.58% patients had caesarean section in normal NST group and 63.04 % patients had caesarean section in abnormal NST group. This is comparable to study by Eden et al[8] where, caesarean section rate in reactive group was 23.2%, however in non reactive group caesarean section rate was lower as compared to our study i.e 37.7%.

Meconium stained liquor was found in 9.7% patients with normal Non Stress Test and 73.91% patients with abnormal Non Stress Test. In study done by Lohana et al[9], in reactive Non Stress Test Group, meconium stained liquor was present in 8.24% of reactive NST group which is comparable to our study while, in non reactive NST group the percentage of meconium stained liquor was 33.33% which is less compared to our study. The variation in gestational age of patients and number of post dated pregnancies can be accountable to the difference. The sensitivity and negative predictive value of the test for meconium stained liquor was 73.91% and 90.97 % respectively, which is comparable to study by Bano et al ([10]which showed negative predictive value to be 90.9%, and study by Khooshideh et al[11] which showed sensitivity of 62%.

APGAR score < 7 at 1 min was present in 4.47 % babies of normal Non Stress Group patients and 54.34% babies of abnormal Non Stress group patients. APGAR score < 7 at 5 min was present in 2.23 % babies of normal Non stress test group patients and 34.78% babies of abnormal Non Stress Test group patients. This was comparable to study done by Bano et al[10] which had APGAR<7 at 5min to be 3.4% in reactive group and 42.8 % in non reactive group.

For APGAR<7 at 5 minutes, the negative predictive value was 81.36% and specificity was 97.76% comparable to study done by Bano et al [10], with 98.8.% negative predictive value and 90.6% specificity respectively. The negative predictive value and specificity of APGAR<7 at 5 minutes is also comparable with the values of 95.23% and 83.33% respectively in study by Bhide et al[12]

4 babies (2.98%) were admitted to neonatal intensive care unit from normal Non Stress Test group and 18 babies (39.13%) from abnormal Non Stress test group. This is comparable to study by Bano et al[10], where, 3.6% babies were admitted from reactive nst group and 28.5% babies from non reactive Non Stress Group test.

Conclusion

Non stress test is a simple, noninvasive screening test which can be used to detect at risk babies in low pregnancies. It is easy to perform and the results are simple to interpret. It aids in early diagnosis of fetus with risk or hypoxia and helps in planning timely intervention. It is of great help in detecting meconium stained liquor with good precision. Non Stress Test is also a good predictor for babies with low APGAR score needing nicu facility so timely termination of pregnancies can be planned. Thus Non Stress Test is auseful tool to avoid obstetric litigation as parental expectation of a good outcome is extremely high.

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To Assess the Serum Vitamin D in Vitiligo Patients: SMHRC and AVBR Hospital of Vidarbha Region

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Abstract

Introduction: Vitiligo is a long-term skin condition that is characterized by skin patches which lose their pigment. The affected skin patches are becoming white and typically have narrow margins. The skin hair can also turn white. Mouth and nose inside might also be involved. Occasionally, the vitiligo "comes in families," implying a genetic origin. It ensures that the risk of other family members contracting vitiligo is not predictable. Approximately 25 to 50 per cent of people with vitiligo have a parent with vitiligo, and around 6 per cent have vitiligo siblings. Low serum levels of vitamin D have been associated with many autoimmune disorders and a number of other skin conditions. Vitiligo is an autoimmune disease which is characterized by immune processes that kill melanocytes. Melanocytes express vitamin D receptors, and vitamin D status can affect their function.

Objective: To assess serum Vit-D levels of vitiligo patients and contrast the outcomes and controls.

Material and Method: In all out, 50 vitiligo patients and 50 controls were taken on the examination. Vit-D levels were estimated from blood tests. Gathering correlations were performed utilizing fitting factual strategies.

Observation and Results: The patients had lower serum Vit-D levels than the controls, yet this distinction was not noteworthy ($p = 0.570$).

Conclusion: It stays obscure whether Vit-D inadequacy causes vitiligo. Bigger controlled investigations are required to demonstrate whether low flowing VIT-D is a causative factor in vitiligo.

Keywords: *Vitiligo, Vit-D, immune system illnesses, Etiopathogenesis.*

Introduction

Vitiligo affects approximately 2 percent of the world's population regardless of skin type, age and sex[1]. The malady may influence the two sexual orientations and all skin types[2] and may likewise be related with fundamental immune system sicknesses, for example, lupus erythematous, scleroderma, immune system thyroiditis and alopecia areata[3]. Diminished serum Vit-D levels are found in numerous immune system illnesses including foundational lupus erythematosus, diabetes mellitus, rheumatoid joint inflammation,

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different sclerosis and alopecia areata[4].Genetic factors play an important pathogenic factor. Multiple studies have included multiple genes in the pathogenesis of vitiligo, including studies on Jordanian population [5, 6].

Vitiligo has a significant psychological and social effect on individuals especially affected by women, or on exposed sites and more so in people of colour (POC) [7]. Vitamin D3 is a vitamin essential to humans. The majority of its active form is obtained by activating the pre-vitamin D3 formed in the skin following exposure to sunlight, in particular UVB (290–320 nm).Diet is only a minor vitamin source. This vitamin plays an important role in immunity (innate and adaptive), calcium control, and melanin synthesis; in addition, several diseases have been associated with decreased vitamin D levels. Melanocytes express vitamin D receptors which may indicate a possible role for vitamin D in melanocyte function regulation.[8].

Vit-D is a basic hormone that is orchestrated in the skin[9]. The dynamic type of Vit-D, 1, 25-dihydroxyvitamin D3, is a hormone that manages calcium and bone digestion, controls cell multiplication and separation and furthermore displays certain immunoregulatory capacities [10].Vit-D may influence both natural and versatile resistant reactions through receptors in T and B lymphocytes, macrophages and dendritic cells[11]. What's more, Vit-D3 increments tyrosinase action and melanogenesis by means of an atomic hormone receptor – the Vit-D receptor (VDR) in melanocytes[12]. Vit-D and its analogy are utilized to treat skin issue, including psoriasis and vitiligo[13]. Patients with vitiligo have been treated with topical calcipotriene [13]. Hardly any reports have examined the relationship among vitiligo and decreased Vit-D levels, yet these examinations give clashing outcomes [14,15]. Point: This examination expected to decide if patients with vitiligo have lower serum Vit-D levels contrasted with controls.

Material and Method

In all out, 50 patients determined to have vitiligo, in our outpatient division from 1 December 2013 to 31 March 2014, were taken a crack at the examination; 47 age-, sex and skin phototype-coordinated sound controls additionally took part. The patients were analyzed by a similar dermatologist and the analysis of vitiligo was made by clinical discoveries and a Wood's light assessment. Punch biopsy was performed on dubious cases, with the conclusion at that point checked. Point by point sickness and family ancestries were gotten from all patients. Different segment and way of life factors were recorded, including age, sexual orientation,skin model and sunscreen utilization. Members with liver or kidney issue, hyperparathyroidism, hypo-parathyroidism, any metabolic bone issue (for example osteoporosis or osteopenia) or fiery ailments were rejected from the investigation, similar to those taking Vit-D-or calcium-including drugs, or any fundamental or topical treatment for vitiligo inside the earlier month. Controls were selected from the accomplices or family members of patients, if not influenced by vitiligo, to limit contrasts because of dietary admission of Vit-D. Educated assent was acquired from all members and the neighbourhood Ethics Committee affirmed the examination, which was led as per the fundamentals of the Declaration of Helsinki. Blood tests were taken in the first part of the day after a base fasting time of 8 hour. Serum free T3, free T4, thyroid animating hormone, fasting glucose, against thyroid peroxidase antibodies, hostile to thyroglobulin antibodies, Vit-B12 and Vit-D levels were estimated.

Observation and Results

In complete, 26 (52%) guys and 24(48%) females were remembered for the investigation gathering. The benchmark group comprised of 30 (60%) guys and 20 (40%) females. The mean periods of the patient and control bunches were 31.96 ±10.68 and 32.45 ±9.52 years, separately. There was no huge distinction between the patient and controls as far as age ($p = 0.809$). All patients had summed up vitiligo as controlled by the nearness of reciprocal evenly circulated depigmented macules in trademark areas. The mean age at vitiligo macules beginning was 17.48 ±9.81 years. The term of the injuries extended up to 25 years. A family ancestry of vitiligo was accounted for in one patient. No patients had diabetes mellitus or Vit-B12 lack. Immune system thyroid ailments were accounted for in 14 (28%) patients. Vit-D levels were tried throughout months, from December 2019 to May 2020. The patients' serum Vit-D levels ran from 6 to 42 ng/ml (mean: 12.54 ±9.29 ng/ml); in the benchmark group they ran from 8 to 39 ng/ml (mean: 13.07 ±5.98 ng/ml). The patients had lower coursing Vit-D levels than controls, however this distinction was not critical ($p = 0.7352$).

Discussion

In the current investigation, we discovered lower

serum Vit-D levels in patients with vitiligo comparative with controls; be that as it may, this distinction was not huge. Both the patients and controls had exceptionally low circling Vit-D levels. This might be on the grounds that the blood tests were gathered during winter months. There are not very many examinations assessing serum Vit-D levels in vitiligo patients [14, 15]. Ustun et al. examined 25 vitiligo patients and 41 controls: deficient (< 30 ng/ml) or low (< 15 ng/ml) levels of Vit-D were seen in most of patients, yet the distinctions were not huge contrasted with controls [14]. These examiners expressed that countless examinations had announced low degrees of flowing Vit-D in immune system sicknesses; however it stays muddled whether this is a reason or consequence of immune system maladies [14]. Another examination researched 40 vitiligo patients and 40 age-and sexual orientation coordinated controls. Altogether lower serum Vit-D levels were found in the patient comparative with controls. The creators estimated on the chance of Vit-D supplementation for the treatment of vitiligo patients later on [15]. The pathogenesis of vitiligo remains to a great extent obscure. There are various hypotheses clarifying the pathogenesis of vitiligo, with all hereditary, immune system, autocytotoxic and neurogenic causes hypothesized. The immune system hypothesis is the best-bolstered one, since vitiligo might be related with other immune system sicknesses including malignant sickliness, hyperthyroidism, Hashimoto's thyroiditis, alopecia areata and adrenocortical disappointment. Besides, histological investigations have exhibited a high recurrence of cytotoxic T lymphocytes explicit to melanocytic antigens in vitiligo injuries, proposing an immediate, melanocyte-explicit T cell assault [16, 17]. Vit-D, which is a fat-dissolvable vitamin acquired by people through eating routine, is specifically compelling to dermatologists since it is combined in the skin by bright light. It has been utilized to treat psoriasis, vitiligo and other skin ailments for a long time [17]. The dynamic type of Vit-D, 1, 25-dihydroxyvitamin D3, directs calcium and bone digestion, yet in addition controls cell multiplication and separation and applies immunoregulatory exercises [13]. In a past report, it was accounted for those patients with comorbid immune system diseases are bound to have low serum Vit-D levels [16].Vit-D has anuclear receptor called Vit-D receptor (VDR). Vit-D receptors are available in the cells engaged with calcium and bone digestion, and furthermore in keratinocytes, melanocytes, fibroblasts and resistant framework cells of the skin. Polymorphisms in VDR are corresponded with expanded helplessness to numerous sclerosis, provocative entrail malady, rheumatoid joint pain and type 1 diabetes mellitus [19]. Vit-D applies a noteworthy impact on melanocytes and keratinocytes by means of different instruments. In vitro investigations have demonstrated that Vit-D3 is related with an expansion in tyrosinase action and melanogenesis [11], which may add to re-pigmentation in vitiligo macules. Vit-D analogs, including calcipotriol and tacalcitol, are known to improve re-pigmentation in vitiligo patients [20-22]. Another investigation announced that Vit-D applies immune-modulatory impacts by hindering the declaration of interleukin (IL)- 6, IL-8, tumor rot factor (TNF)- α, and TNF-γ [23]. Besides, it has likewise been indicated that the dynamic type of Vit-D lessens the apoptotic action prompted by UVB in melanocytes[13].

Conclusion

More investigation is required to outline the connection between VITAMIN D and vitiligo, to assess whether a low degree of serum Vit-D is a causative factor in vitiligo, and to learn whether Vit-D supplements are helpful for both the anticipation and treatment of vitiligo. Irreconcilable circumstance the creators proclaim no irreconcilable situation.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Immediate Effect of Buteyko Breathing in Hypertensive Patients: An Experimental Prospective Study

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Abstract

Background: There are ample studies showing effect of Buteyko breathing technique on cardiovascular parameters in asthmatics but very few studies have been done to see its immediate effect on hypertensive patients.

Aims: The aim of this study was to assess the immediate effectiveness of Buteyko breathing technique on hypertensive patients in terms of Systolic and Diastolic Blood pressure, Heart rate and Oxygen saturation (SpO2)

Method: The research design used was Experimental prospective study design. Total 50 participants were screened and those meeting the inclusion criteria (42) were included in the study. The participants were randomly divided into two groups-experimental and control Group, by computer generated randomised table into groups of 21 each. The experimental group performed the Buteyko breathing exercises. The control group did not receive any treatment. Pre and immediate post intervention scores were measured in terms of systolic BP, Diastolic BP, heart rate and SpO2.

Findings: On comparing the pre and post intervention values of the outcome measures, it was observed that there was statistically significant difference in Systolic BP, Heart rate and SpO2 values in Buteyko breathing group. No significant difference was seen in Pre and Post values of Systolic and Diastolic BP, Heart rate and SpO2 values in Control group. On comparing between the groups, it was observed that there was statistically significant difference between the two groups in terms of SpO2

Conclusion: Thus it can be concluded from the present study that Buteyko breathing technique has an immediate positive response on oxygen saturation level in hypertensives.

Keywords: *Hypertension, Buteyko breathing technique, SpO2, BP*

Introduction

Hypertension is defined as the persistent high blood pressure.[1] More than a billion people all over the world are affected with Hypertension[2] and it is anticipated that the number will increase to 1.56 billion in 2025[3,4] Globally, hypertension is the leading cause of premature deaths, with a rate of one in four men and one in five women.[5] For cardiovascular diseases, hypertension is one of the eminent risk factor. It is considered as a silent invisible killer as the symptoms are usually invisible in early stages until it takes a severe face later as of stroke, heart attack or chronic kidney disease.[2]

The Buteyko breathing technique named after its creator Dr. K P Buteyko was devised in 1950's.[6] It was basically developed to treat the asthmatics for hyperventilation. The beauty of Buteyko breathing technique is that it is easy to understand and patients can perform the exercise comfortably. It is a distinctive

breathing technique that utilises the breath holding and breath control exercises. This technique weighs more on shallow breathing pattern, correcting the hyperventilation. Thus basically it aims its attention on nasal breathing and holding the breath along with relaxation. The technique stresses on the optimum retention of CO2 which in succession has vasodialatory effect, resulting in the reduction in heart rate and blood pressure.[7]

There are several studies claiming successful effect of Buteyko breathing technique on Bronchial Asthma. A randomised control trial on Buteyko breathing technique as an addition to conventional treatment showed 39% increase in asthma control.[8] A similar study showing effects of Buteyko breathing technique on asthmatics revealed positive results.[9]Aslo there are a few studies showing immediate effect Buteyko breathing technique on Cardiorespiratory parameters in healthy adults[7], but to the best of our knowledge no study has been done to see the immediate effect of Buteyko breathing technique on cardiorespiratory parameters in Hypertensive patients. Thus the aim of our study was to evaluate the effect of Buteyko breathing technique on Blood pressure, heart rate and SpO2 in Hypertensive patients.

Methodology

The aim of the study was to assess the effect of Buteyko breathing technique on hypertensive patients.

Study Design:

Research Design: Experimental prospective study.

Sample Population: Hypertensive subjects residing in Nagpur region above 40 years of age.

Sample size: 50

Type of sample: Convenience sampling

Source of sampling: Nagpur district and outskirts

Place of study: Datta Meghe College of Physiotherapy

Duration of study: 1 month

Inclusion criteria:

1. Males and females clinically diagnosed with hypertension.
2. Age group -above 40 years
3. Patients with history of Coronary artery bypass grafting or angioplasty in the past 20 years.
4. Patients with addictions like alcohol, tobacco.
5. Patients having history of diabetes and thyroid.
6. Patients engaging in mild physical activity on daily basis.
7. Patients who have taken or are taking medications for hypertension.
8. Patients with a history of respiratory disorders but currently stable.

Exclusion Criteria:

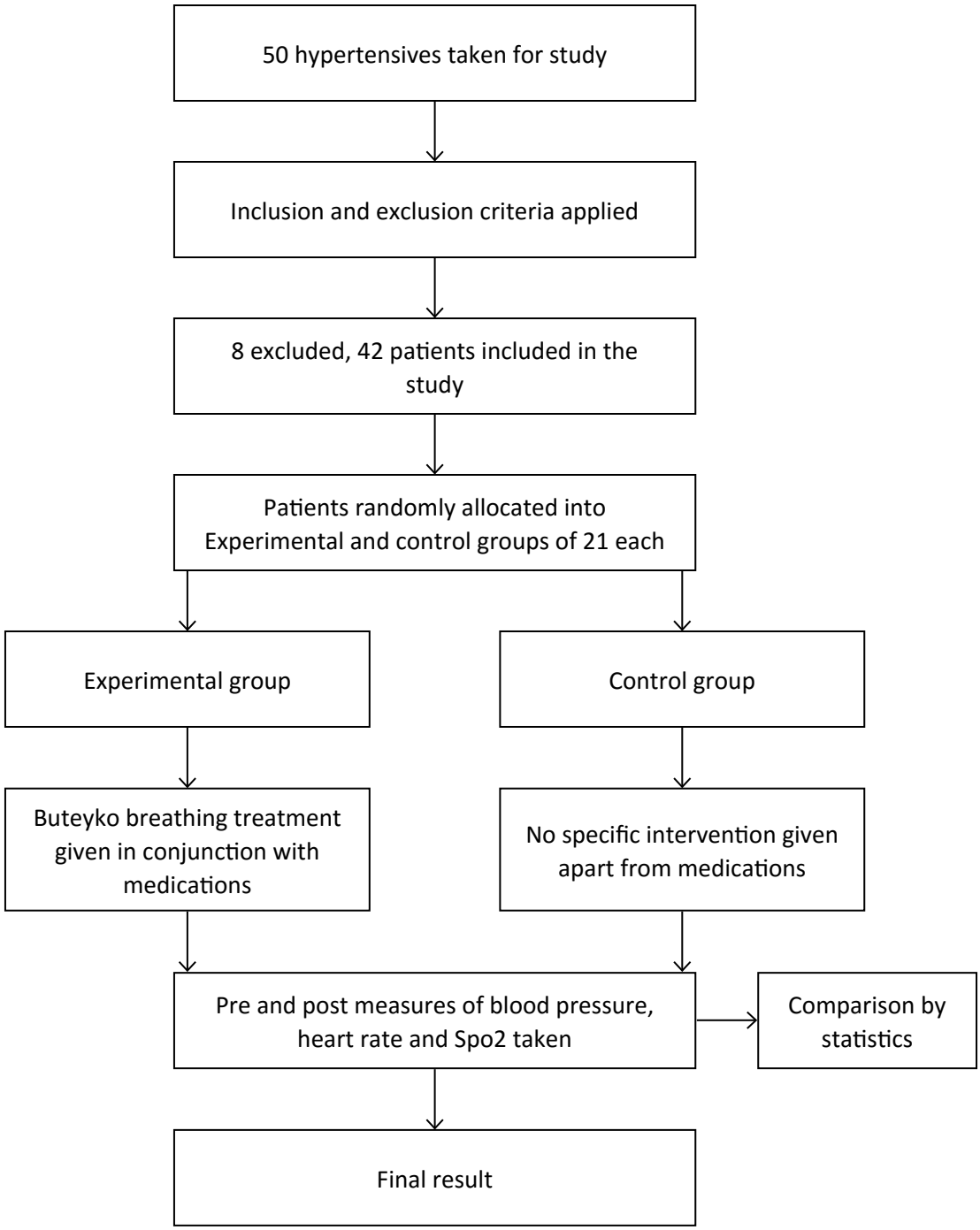
1. Patients with active respiratory infection or kidney problems.
2. Cognitively unstable hypertensive patients.
3. Patients unwilling to cooperate in the study.
4. Patients with a history of organ transplant.
5. Patients undergoing any cardiac or respiratory rehabilitation program.

Materials used: Digital B.P. apparatus, Pulse oximeter

Outcome measures: Blood pressure, Pulse rate, Oxygen saturation

The study was carried out at the Datta Meghe College of Physiotherapy, Wanadongri Nagpur. Ethical approval was obtained from Institutional Ethical Committee & an informed consent was obtained from the participants. 50 patients with hypertension residing in Nagpurregion were included in the study. The study was conductedfor a month. Inclusion and exclusion criteria were applied and 8 patients were excluded from the study. The study was conducted on 42 hypertensives fulfilling the inclusion criteria. The participants were randomly divided into two groups-experimental and control Group, by computer generated randomised table into groups of 21 each.

The Experimental group was given Buteyko Breathing technique.



A Digital B.P. apparatus and Pulse Oximeter were used in the study to measure Systolic and Diastolic Blood pressure, Heart rate and oxygen saturation. The blood pressure, heart rate and SpO2 of the patients were measured before the Buteyko technique. The technique was explained through video and audio to the patients and the patients were made to perform it. Immediately after the technique, the post reading were taken. The control group did not receive any treatment . The pre and the immediate post reading of the blood pressure, heart rate and Spo2 was taken in this group aswell after a 5 min interval.

Buteyko technique:

Step 1: Control pause phase:

The patient was asked to sit upright in a chair and breathe normally through the nose for 30 seconds (keeping mouth closed)

Subsequently instructions were given to the patient:

- Do not change your breathing pattern before taking control pause.
- Take a small breath in and a small breath out.
- Hold the nose on the "out" breath, with empty lungs but not too empty. (In order to prevent the air to enter the airways)
- Count for how many seconds you can last comfortably before need to breathe in again.
- Hold breath until feeling the first urgency to breathe in.
- Release nose and breathe in through it.
- First intake of breath after the CP should not be greater than the breath prior to taking measurement; patients were advised not to hold breath for too long as this may leadto taking a deep breath after measuring the CP.

Step 2: Shallow breathing

The patient were asked to:

- Sit up straight.
- Observe the air flow through the nostrils by placing a finger under the nose in a horizontally
- The patients' finger should lie just below the nostrils so that the airflow can be felt, but at the same time not blocking the airflow.[10]

Step 1 and 2 were repeated for 5 mins or 6 cycles approximately.

Once the pre and post measures of Blood pressure, heart rate and Spo2 were obtained, the statistics of the respective outcome parameters were calculated on the basis of SPSS software.

Result

The software MYSTAT 12 was used in this study. Outcome measures used were systolic and diastolic blood pressure, heart rate and oxygen saturation. Descriptive statistics such as mean and standard deviations were calculated to describe all the variables. The paired t-test and the unpaired t-test were used to verify differences between pre- and post-intervention. The statistical analysis was conducted at 95% confidence level, and $p<0.05$ was considered statistically significant.

The gender ratio was 12:9 (12 females and 9 males) in Group A and 11:10 (11 females and 10 males) in Group B. The difference in the mean age of both the groups was statistically not significant. (Table 1).

Table 1. Baseline demographic data of both the groups

Group	Group A Buteyko	Group B Conventional	t value	p value	Inference
Age (Years)	60.57 ± 8.50	60.71 ± 10.54	2.02	0.96	Not significant
Gender ratio (M:F)	12:9	11:10			

On comparing the pre and post intervention values of the outcome measures, it was observed that there was statistically significant difference in Systolic BP, Heart rate and SpO2 values in Buteyko breathing group. No significant difference was seen in Pre and Post values of Systolic and Diastolic BP, Heart rate and SpO2 values in Control group. On comparing between the groups, it was observed that there was statistically significant difference between the two groups in terms of SpO2 whereas no statistically significant difference was found between Systolic and Diastolic BP and Heart rate. (Table 2).

Table 2: Mean and SD scores of Buteyko and Control groups, Pre intervention and Post intervention

	Buteyko Group A				Conventional Group B				Between groups	
			Paired t test				Paired t test		Unpaired t test	
	Pre intervention Mean ± SD	Post intervention Mean ± SD	Mean Difference	p value	Pre intervention Mean ± SD	Post intervention Mean ± SD	Mean Difference	p value	p value	Inference
Systolic BP	139.57±23.48	126.00±28.44	13.57	0.002	139.57±22.82	19.42±20.83	0.14	0.79	0.08	Not Significant
Diastolic BP	83±12.51	84.42±6.86	-1.42	0.59	82.85±12.95	82.71±12.75	0.14	0.32	0.59	Not Significant
Heart rate	79.28±13.81	75±11.21	4.28	0.001	79.28±13.81	79.14±13.52	0.14	0.08	0.28	Not Significant
SpO2	97±1.22	98±1.44	-1	0.0001	97±0.94	97±1.09	0.00	1	0.01	Significant

Discussion

Table 2 shows the Pre-Post immediate effect comparison of Experimental and Control group. In data analysis, paired t test was applied for intra group comparison and unpaired t test was applied for intergroup comparison. On the basis of intra group comparison it can be said that there are positive changes of Buteyko breathing technique on Systolic blood pressure, heart rate and oxygen saturation in hypertensives. The results for the inter group comparison, showed a significant difference with Buteyko on SPO_2 (p value 0.01) as compared to the control group and thus it is seen that Buteyko technique has an immediate effect on increasing the SPO_2 level in hypertensive subjects.

Following could be the some of the reasons for the above results. According to the study by Yosreah et al(2019) titled "The effect of Buteyko breathing technique among patients with bronchial asthma: Comparative study" the major component of the Buteyko 'package' is to reduce hyperventilation through bouts of controlled reduction in breathing, known as 'slow breathing' and 'reduced breathing', combined with bouts of breath holding, known as 'control pauses' and 'extended pauses.'[10]

The Buteyko method is based on the concept that hyperventilation is the underlying cause of variety of medical conditions (Rosalba Courtney, 2008.) One possible biochemical mechanism of Buteyko may be through its influence on nitric oxide (NO). NO is involved in avarious of physiological responses including bronchodilation and vasodilatation[12]

According to Richa et al, Buteyko performed for 5 min at rest showed a significant increase in heart rate and reduction in systolic blood pressure, which is a normal physiological response. But however,they have notnoted any immediate changes in cardio respiratory parameters in the instances 'sub maximal exercise with Buteyko' and 'sub maximal exercise without Buteyko'.As also seen Review of the 'Australian government rebate on natural therapies' for private health insurance is is stated that there is no evidence about Buteyko Breathing Technique improving pulmonary function in adults. The reason for this can be that deep inspiration that is required to perform a lung function test might lead to bronchoconstriction and override any beneficial effect from the Buteyko breathing technique. This can explain the no significant change in diastolic Blood pressure and heart rate[7]Ritu et al(2013) stated that Buteyko breathing exercise is useful in management of respiratory rate and heart rate in chronic obstructive pulmonary disease patients[13,14]

Apart from this, it is also mentioned in studies that holding the breath can cause accumulation of carbon dioxide[15,16,17] that will lower blood pressure, as also breath holding will also improve the collateral ventilation which can be effective reasons for reduction of blood pressure, heart rate and improvement in SPO_2 in some patients. Thus these can be the reasons of the improvement in SPO_2 and reduction in systolic blood pressure in hypertensive patients in the study[18,19]. Also, since the immediate effect only was seen in the study, that can also justify why the diastolic blood pressure and heart rate was not significantly affected. The limitation of this study can be that it was a single intervention based study. Also it is recommended to use a larger sample size in further studies.

Conclusion

It can be concluded from the present study that Buteyko breathing technique has an immediate positive response on oxygen saturation level in hypertension.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Osteometric Evaluation of Human Skull for Sex Determination: A Comparative Study

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Abstract

Background: Sex determination from analysis of bony remains is one of the most important arduous job for medicolegal experts, especially in cases where identity of the deceased person is in question. Aims & objectives: Our study focuses on evaluation of different osteometric parameters of skull which will be helpful for sex determination.Material and Method: Current study was conducted on 60 human skull bones & they were studied by using calipers for various osteometric measurements. Results: In our study we observed that, skull length, interorbital breadth, biorbital breadth, maxillo-alveolar breadth, maxillo-alveolar length, bizygomatic breadth & nasion-prosthion length are the statistically significant variables & are good determinants for sex than rest of the parameters. As compared to them, cranial breadth & orbital height are poor parameters for sex determination. Conclusion: Human Skull bone as a whole & it's different measurements taken from anatomical landmarks & their osteometric analysis is a good determinant for sex of an individual.

Keywords: *Human skull, cranial osteometry, human identification, bony remains.*

Introduction

There are many challenges, a medicolegal expert faces during his work & sex determination from bony remains is one of such a crucial task to establish an identity of the individual. Such condition arises in many cases like homicidal investigation, archeological interest, mass disaster (both natural & manmade), cases where there is violation of human rights etc. Current global sex ratio is 1.018, which implies that if we are well versed with the aspects of sexual dimorphism then we can reduce our search result to nearly half of the population[1,2]. If whole skeleton is available for analysis then, sex determination is extremely authentic. But in most of the cases, part of the skeletal remains or some bones or even in extreme cases parts of the bony fragments are available for examination. Now in such cases, there arises a need of studies of various skeletal elements of individual bone for determination of sex of that individual. From the past studies it has been well established that sex determination from examination of skull bone & its components has precision rate of almost 85-95%[3]. There is a long list of factors that determine & influence the characteristics of bony prominences which are useful for sex determination, especially during the adolescence. Generally boys have exceptionally vigorous growth surge as compared to girls in their adolescent age group[4]. Roughly there are two techniques for determination of gender from bones & their characteristics. They are: visual evaluation & osteometric method. Visual method is comparatively very easy to asses, does not require any instrument to asses but is highly individual specific & depends on the skill of the forensic expert (which is variable). While the osteometric method is more objective, reliable, depends on indices & is less dependent of investigator's

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subjective evaluation. It in turn reduces the bias between two individual experts which means the results obtained from this method are highly reproducible. Our investigation is done to evaluate the dimorphic features of male and female crania of Central Indian origin based on osteometric analysis.

Material and Method

Current study was performed on dry skull bones at Datta Meghe Medical College, Nagpur which is a part of Datta Meghe Institute of Medical Sciences University. Institutional Ethics committee gave approval for the same. Intact skull bones which are showing well defined sexually dimorphic characteristics on visual examination were involved in our research. Skull bones were taken from departments of Anatomy & Forensic Medicine of Datta Meghe Medical College, Nagpur & Jawaharlal Nehru Medical College, Sawangi. Skull bones which are showing inconclusive dimorphic characteristics on direct visual examination/incomplete skull bones or skulls having any bony deformity were excluded from our study. On the basis of our inclusion & exclusion criteria, we finalized a total of 60adult skull bones out of which 35 were of males & 25 were of females.After reviewing the past researches regarding skull osteometry, we took ten measurements into our consideration. All the measurements were taken using Vernier calipers & sliding calipers. Following anatomical landmarks were included: ecto-conchion, dacryon, nasion, eurion, zygion, prosthion, opisthocranion, glabella, basion, alveolon, opisthion, ectomolare.

Table 1: Following measurements were taken into consideration for our study:

Variable	Abbreviation	Distance between
Maximum Cranial Length	MCL	Opisthocranion & glabella
Maximum Cranial Breadth	MCB	both the eurions
Inter-Orbital Breadth	IOB	both the dacryons
BiorbitalBreadth	BOB	outer & lateral margins of orbital rims
Orbital Breadth	OB	lateral & medial border of the orbit
Orbital Height	OH	superior & inferior border of the orbit
NasionProsthionLength	NPL	Nasion & prosthion
BizygomaticBreadth	BZB	two zygions
Maxillo Alveolar Breadth	MAB	two ectomolares
Maxillo Alveolar Length	MAL	Alveolon & prosthion

Our obtained data was analyzed using statistical package for social sciences (SPSS) software. Student's t- test was applied to study the mean differences in various cranial parameters. P- value< 0.05 was considered as significant.

$$\text{Index of sexual dimorphism} = \frac{\text{Meanvalueinmales}}{\text{Meanvalueinfemales}} \times 100$$

Results

Table 2: Descriptive statistics for cranial parameters

Variable	Male (N=35)		Female (N=25)		p – value
	Mean	SD	Mean	SD	
BZB	10.08	0.49	9.44	0.51	<0.0001
BOB	9.86	0.57	8.88	0.5	<0.0001
OB	3.87	0.25	3.55	0.33	0.0001
IOB	2.31	0.24	2.52	0.31	0.0477
OH	3.21	0.28	3.2	0.31	0.2174
NPL	4.81	0.42	4.19	0.57	<0.0001
MCL	13.46	0.6	12.41	0.65	<0.0001
MCB	10.39	0.63	10.21	0.28	0.3257
MAL	6.46	0.36	6.06	0.45	<0.0001
MAB	5.28	0.39	4.96	0.31	0.0012

In present study mean Bizygomatic Breadth was found to be significantly higher in males. Both Biorbital breadth and Orbital breadth were higher in males but no significant difference was observed between males and females in Orbital breadth whereas Biorbital breadth in males was significantly higher compared to females. Similarly inter-orbital breadth in males was observed to be significantly higher in this study. Orbital height was found have insignificant difference and was much similar in males and females. Nasion-Prosthion length and Maximum Cranial length were noted to be significantly higher in males while maximum cranial breadth was insignificant although it was higher in males compared to females. Both MaxillOo-Alveolar Length and Maxillo-Alveolar Breadth were found to be larger in males significantly.

Calculation of sexual dimorphism indices revealed that Nasion-Prosthion Length was strongest determinant of sex followed by Biorbital breadth, Orbital breadth, Maximum Cranial length. Inter-orbital breadth, Bizygomatic breadth, Maximum alveolar length and maximum alveolar breadth also showed good degree of sexual dimorphism. Maximum crania breadth and orbital height are observed to be poor determinants of sex in our study.

Table 3: Sexual dimorphism indices.

Variable	Index of sexual dimorphism
BZB	106.78%
BOB	111.04%
OB	109.01%
IOB	91.67%
OH	100.31%
NPL	114.80%
MCL	108.46%
MCB	101.76%
MAL	106.60%
MAB	106.45%

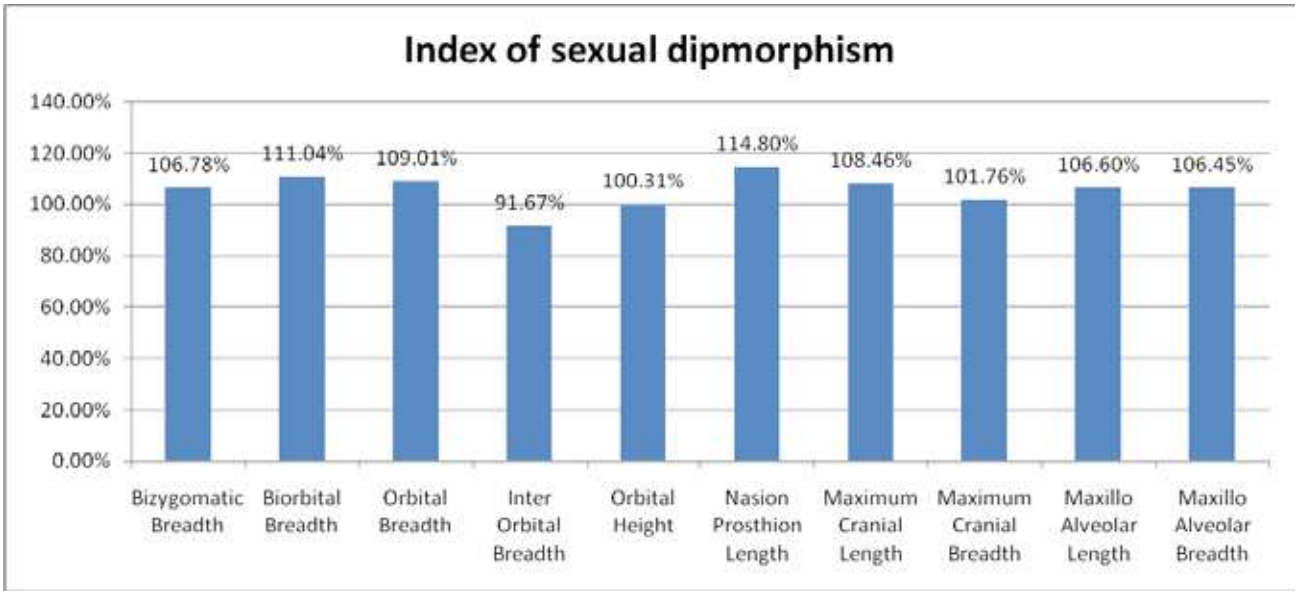


Figure 1: Graphical depiction of Sexual dimorphism indices

Discussion

In this study BB, BOB, IOB, OB, NPL, MCL, MAB and MAL were significantly higher in males compared to females. OB and MCB were found to be insignificant although they were comparative higher in males. The findings in our study are in agreement with study done by Gupta A et al.[5] The sexual dimorphism indices indicate that NPL is the strongest index followed by BOB, OB, IOB, and MCL. Also MAL, MAB, BZB are good at determination of sex while OH and MCB are poor determinants of sex from crania. These findings are similar reported to indices calculated by Gupta

A et al. When BZB, MCL, NPL were compared with study conducted bySteyn M et al[6] findings reported were similar and the parameters differed significantly among males and females. Steyn M et al. also reported that accuracy of prediction of sex from human skull was found to be 85.7%, which was highest among rest of the bony parameters which were analyzed in the same study. Thuangthong T et al[7] conducted a study on sex determination using computer aided software and compared those results with the findings obtained by conventional caliper method. In that study it was reported that OH and BZB differed significantly among both sexes. Also use of computer aided technique determined sex with greater accuracy as compared with use of conventional caliper method. A study done by Saini V et al[8]assessed various parameters for determination of sex among which BZB and BOB differed significantly similar to our study but in contradiction IOB was insignificant between both sexes. OH was insignificant in agreement to present study. Indices such as BZB(105.5), OB(103.295), BOB(103.251) in that study were not in agreement with indices found in our study BZB(106.78), OB(109.01) and BOB(113.04). Ramamoorthy B et al[9] conducted a similar study by using CT head scans of adult live subjects using various craniometric parameters (few of them are included in our study). They found out that accuracy for sex determination approximately ranged from 91.4% to 97.1% in multivariate analysis. Accuracy was highest when all variables were taken into consideration. The parameters which were similar in our study are in agreement with results obtained by Ramamoorthy B et al. Dayal M.R. et al[10] in their study reported that MCL, BZB, OB are significant while OH is not significant which matches to the results of present study. In the same study Dayal M. R. et al. reported that the accuracy of determination for sex is greater with mandible(85%) compared to that with cranium(80.8%). The findings in present study are in agreement with those in a study done by IscanM et al[11]in modern Japanese crania.

Conclusion: India is a diversified country with respect to climatic conditions, food habits, occupations, etc. With such a wide geographical variations, skeletal assortment to represent whole country is difficult. This study indicates the index for sexual dimorphism for various cranial parameters. In this study Nasion-Prosthion Length is observed to be strongest index for sexual dimorphism followed by Biorbital breadth, Orbital breadth, Interorbital Breadth and Maximum cranial length. These indices are useful for the population with similar groups. It is necessary to conduct more & more studies in geographically diversified population especially in different regions of our country on a larger scale so as to represent whole population as the results may not fully reveal true nature of sexual variation in parameters in a small sample size.

Ethical Clearance: Taken from institutional ethics committee.

Source of Funding: Self.

Conflict of Interest: Nil.

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Review on Utility of Antitoxic Preparations in Non-Poisonous Conditions w.s.r. to Agadtantra

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Abstract

There are hundreds of formulations in the market, which are mostly prescribed by the physicians depending upon their knowledge about the general context of reference. Analysis of the ingredients and the phalshruti of each formulation give a clear picture about them and also help to make a better application in clinical practice. Nowadays, most of the diseases are caused due to unconventional etiology. When a disease is caused by unconventional etiology, it can be managed effectively by using principles of Agadtantra. Currently most of the diseases require Nirvishikarana (Detoxification), for this reason Antitoxic formulations mentioned under Ayurveda which contains singleantitoxicherb, combinationofanti-toxicherbs, herbs-minerals combinations etc can be very effective insuch unknown etiology diseasedconditions. Antitoxic preparations with Vishaharatwa(Antitoxic) property, as one of the action have a better effect in general diseases than other formulation. This review article places of interest in the significant role of antitoxic formulations mentioned in Agadtantra (Ayurvedic perspective of toxicology) to overcome diseases which are produced due to unknown etiology.

Keywords: *Antitoxic preparations, Agadtantra, Non poisonous conditions, Vishaharatwa, Nirvishikarana.*

Introduction

Ayurveda an ancientIndianSystemofMedicine has principal scopethataretomaintain the good health and to cure the diseases or pathological conditions. In Ayurveda, Health is defined as the state of equilibrium of Doshas (Bio energetic humors), equilibrium in Agni (The governing processofdigestivesystem), equilibriuminDhatus(bod ytissues) and normal expulsion of Malas (wastes like feces, urine, sweat etc.)withstateofpeaceinAtma(soul), Indriya(senses)andMana (mind)[1] According to Modern Science, basically it is the phytochemical constituent in the herbs which leads to desired healing effect, such as saponins, tannins, alkaloids, alkenyl phenols, flavonoids, terpenoids, phorbol esters and sesquiterpenes lactones. In the management of general diseases or poisoning, Ayurveda focus on Equilibrium of Dhatu by maintaining equilibrium in Dosha and Agni with the help of various single herb or combination of herbs (Poly herbal formulation) or combination of herbs-minerals. The principle behind any formulation of Ayurveda in any disease or poisoning condition is to correct Agniandvitiation of Doshasviz. vata, pitta and kapha which may be seen in that specific disease or poisoning condition. In the management of visha, the primary focus is to remove visha before it has chance to harm Dhatu and ojus. In case of sthavara, we have an opportunity to eliminate by vamana or virechana. As jangamavisha is directly entered into rakta, it spreads quickly and damage the Dhatu much faster .Here we want to hurry to make sure quick paka of visha before this happens

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and for that ushna, teekshnadravyas are essential to be introduced though they are similar to visha in properties. In the detoxification of visha and containment of vata, ushnapradhan agada has a significant role. At this stage, we should remember the other side of the truth. As the properties of pitta and visha have common characteristics significantly, the pakaof ofvisha in pittapradhanvisha is enabled only with shita and tiktapradhandravya. Like sariva and chandana. Thus it is natural finding that some pittaharaaushadhis are also having vishahara property.

Many diseases are characterized by a component of something that refuses to undergo paka or digestion. This apaki component gives such diseases resemblance of vishajanyavyadhi . Normally a disease is managed with drugs that have qualities opposite to those that caused the disease. In diseased condition with higher visha component, we make use of another visha instead of employing the viparitguna. This exception becomes inevitable because the vyavayi and vikasigunas of the vyadhikarakvishdravya can be matched only by another vishadravya. The notable point here is that the vishadravyaemployed as a drug, even if ushna, doesn't aggrevate the potency of vishadravya that caused the disease. Even in the formulation of vishaharayogas. Teekshna and ushnagunas are dominant. With this understanding of the dynamics of visha and vishachikitsa, we can try to analyse the application of this management of diseases not caused by the visha.

The word Agada is derivative from Gada. Gada has two meanings one is Roga (Disease) and the other is Visha (poison). Agadaisadrug that conquers disease or poison. In Ayurvedic Samhitas various single herb, poly herbal formulation or herb-mineral formulation are mentionedasAntitoxic. Accordingly, single herbs or poly herbal formulation or herb-mineral formulations are used in that disease or poisoning condition. Physician can also change combinations, forms, vehicle of these formulations as per vitiation of Doshas in the patient ordisease. Charakacharya mentioned Vishghnamahakashaya which includes ten Vishghnadrugs[2] Drugs which act against these qualities of toxic substances are called as Vishghna (Antitoxic). Drugs mentioned under, work due to their Raspanchak antagonist to Visha (poison) and helps in the management of diseases.

Vishghna Mahakashaya contains:

1. Haridra (Curcuma longa)
2. Manjishtha (Rubiacordifolia
3. Suvahaa (Pluchealanceolata)
4. Sookshamaelaa (Elettariacardamomum)
5. Paalindee (Operculinaturpethum)
6. Chandan (Santalum album)
7. Kataka (Strychnospotatorum)
8. Shireesh (Albizzia lebbeck)
9. Sinduvaara (Vitex negundo)
10. Shleshmaataka (Cordia dichotoma).

Drugs which act against toxic substances are called as Vishaghna (Antitoxic). Some Vishaghna drugs act by Dravyaprabhava (virtue of their own nature), some drugs act by Gunaprabhava (by virtue of their properties) and some drugs act by Dravya Gunaprabhava (virtue of their nature as well as properties. These Vishaghna Dravya (Antitoxic herbs) and Vishaghna Yogas/Agadas (Antitoxic formulations) are used in various poisoning and diseased condition. Most of these antitoxic formulations acts as Raktashodhaka (Blood purifier), Tridoshaghna (causes equilibrium in body humors), Hridya (Cardio protective) and Ojavardhaka (Rejuvenate). Sushrutacharya has mentioned 95 antitoxic drugs in different Gana (Group) like Aaragwadhadi Gana (20), RodhradiGana (13), Arkadi Gana (14), Shyamadi Gana (19), Patoladigana (07), AnjanadiGana (8), Utpaladigana (7), Trapvadi Gana (7) in the chapter 38 Dravyasamgrahaniya of Sootrasthana[3]. Twenty antitoxic drugs are also mentioned in EkasaraGana in SushrutaSamhita[4]. AshtangHridaya describes 104 antitoxic drugs which are listed in Gana like AnjanadiGana (9), Patoladi Gana (6), Aaragwadhadi Gana (20), Rodhradi Gana (13), ArkadiGana (14), EladiGana (24) and ShamadiGana (18)[5]. These antitoxic drugs can be used singular or in combination with other drugs mentioned in that specific Gana. VishaghnaYogas (Antitoxic formulations) like Kshara agada[6], Dooshivishari Agada[7], BilwadiGulika[8], Maha agada[9], Ajita Agada[10], Sanjeevana Agada[11] etc. are described in Ayurvedainparticular poisoning as well as in diseased conditions with unknown etiology.

Some Antitoxic Formulations and their Mechanism of action:

1. **Bilwadi Gutika:** Most ingredients of Bilwadigutikahave ushna and teekshnaguna and pachana karma . The phalshruti includes almost all

jangamavisha, jwara, garavisha, ajeerna, visuchika .One safe interpretation is that this yoga corrects apakwa in rasa Dhatu and regulates vata. Clinically it has an extended efficacy in conditions like atisara, chardi and jwarawhrere ama is predominant.In psychiatric illness as well as somatic illness that affect the mind, with an appropriate anupana, this gutika stabilizes the mind.

2. **Kalyanaka Ghrita:** In the kalyanakaghrita described in Ashtanghridaya, Visha is an indication. Its specific utility is to produce hridayavarana in jangamavisha . Now most physicians use this yoga in manovyadhichikitsa. In comparision to Bilwadigutika, ushna and teekshnadravyas are less in Kalyanakaghrita and Tiktakaghrita. These two yogas execute their pachana karma more due to the vayu and aakash in the tikta rasa dravya and less due to the tejbhuta in the katu rasa dravyas.

3. **Aargwadhadikashay:** Aargwadadikashaya is generally employed kaphapittajanyavikaras. We all know its efficacy to reduce the dushtamedas . Its antitoxic property is one reason why it is particularly effective in dushtavrana and kaphajaprameha.

4. **Dhanwantaraghrita:** Even though this drug is mentioned in pramehaadhikara, the indications also include visha, gara, kushtha The majority of ingredients have ushna and teekshna property which makes it useful in pachana karma in the above mentioned diseases.

5. **Sanjeevani Vati:** The indication of this drug is mentioned according to its dose. It is indicated in minimum dose for ajeerna whereas an increased dosage for sanyasa and visha. This means the action of this drug is according to the pachana karma.

6. **Patolkaturohinyadikashayam:** This is mainly indicated inkaphapittapradhan diseases. It can be used in lutha Visha, Pittapradhan Visha and visarpa. It is used in this condition because of its rechana property.

Discussion

The current circumstances of world facing many health issues and occurrence of communicable diseases (Sankramaka Roga) are predominantly causing health problems. Acharya Charak mainly described the concept of Janpadodhwans as Sankramakroga, Aupasargikrogas which multiply from person to person, which resembles population demolition in an area. In current scenario, such types of diseases creating great health trouble since large number of global population affected with pathogens. The toxins; endotoxins or exotoxins releasedby microbial agents initiate pathogenesis of diseases.[12, 13, 14] The modern science has also mentioned some diseases which spread from person to person and occurs due to the microbial infections. These are huge challenges & creating a trouble over health care system, it is advised to include the use of Ayurvedatreatment.

Physical fitness of the people is deteriorating day by day duet ode forestation, globalwarming, indiscriminate use of pesticides-fertilizers in the farming, food adulteration, incompatible food, food additives-preservatives- sweeteners, increasing pollution levels, industrial emission, hormonal administrations in poultry-dairy, indiscriminate use of various synthetic drugs etc. In present circumstances, most of the diseases are caused due to unconventional etiology. For this unconventional etiology due to toxicants/chemicals Agadtantra focuses on ViruddhaAhar (Incompatible diet), GaraVisha (Artificial poison) and Dushivisha (polluting poison). All these toxicants/chemicals in the form of Viruddhaahara or Garavisha or Dushivisha enters into the human body by different routes and responsible for various. According to Ayurveda, diseases are produced due to imbalance of Dosha (Body Humors) and Dushya/Dhatu (Body tissues)[15]. Poison first vitiates the blood, then vitiates the humors Kapha, Pittaand Vatainsequencealong with their substrata, proceed to heart and then become fatal[16]. Vitiation of Doshas is very important factor in the production of both diseased and poisoning condition. Only difference is in the case of poisoning it vitiates blood first then body humors. Diseases caused by Viruddha Ahar, Garavisha and Dushivisha have same principle of management that is Shodhana Chikitsa (Eliminationoftoxins)by induction of emesis or purgation before using antitoxic formulations. As antitoxic formulation act as bloodpurifier, causes balance in body humors viz. vata-pitta- kapha, helps in rejuvenation, it reverses the action of poison and helps to become free from poison. These antitoxic formulations are useful in diseases caused by incompatible diet, artificial poison and pollutant poison. Therefore antitoxic preparations help to get better health status and longevity of the humanbeing. Ayurveda explains good health by using wholesome diet, lifestyle and natural remedies prepared from medicinal plants and minerals. Modern science generates the theory of balance between free radicals and antioxidants to maintain the goodhealth.

The diet which provokes bio energetic humors without eliminating them is considered as Viruddha Ahara[17]. Drugs and food materials incompatible with the normal body tissue elements are Viruddha[18]. Viruddhaahara generally do not have any effect on those who are habituated to it or consume it in a small quantity, their Agni (Digestive power) is strong and who are young and practicing exercise regularly[19]. Incompatible food like Garavisha (Artificial or synthetic poison) may beone of the reasons of several diseases anddeath[20]. For prophylaxis of these diseases, Ayurveda emphasizes on prohibition of incompatible food. Diseases caused by incompatible food may be treated with emesis, purgation, by using the drugs which act opposite to that particular disease and wholesome diet[21].

In current era, knowingly or unknowingly we the people are exposed to various types of poisons which may be of natural or artificial origin. Gara is the combination of substances, non poisonous or poisonous which exerts toxic effect after interval of sometime and as such does not kill the patient instantly. Low potency this poisons of artificial origin called as Garavisha after sometime it act as Dushivisha. Dushivisha becomes symptomatic when storage of chemicals/toxins get the rate of exposure is greater than rate of metabolism or excretion. Ayurveda explains this concept of accumulation of toxin in the body under the concept of Dushivisha. As Dushivisha is less dangerous it does not cause any immediate clinical features and lies inactive in the body for some years. . In current era diseases with unknown etiology are increased due to incompatible food, unhealthy lifestyle, exposure to toxic substances, pollutants, chemicals and drugs. In this condition, Dietetic foods and traditional herbal medicines work effectively. In the case of any formulation, it goes without saying that the clinical conditions indicated in the phalshruti will have atleast one common component of samprapti that threads through them. Apakwavastha is the key factor in all vishajanyavikaras. Similarly, the clinical success of the antitoxic preparations in jwara, pandu, ajeerna and visuchika points towards the common factor namely apakwavastha in rasa Dhatu.

Conclusion

Though Vishahraaushadhis (Antitoxic preparations) have many advantage to cure diseases, should not, need not and cannot be used in the management of common diseases. Only when the nidana, samprapti and lakshana is indicative of visha or vishasamancomponents, should we suggest the superior clinical contribution a vishaharaaushadhi is likely to make. In any common disease, we may identify a component or stage of the samprapti to be vishasaman. That would be the right time to employ Vishaharaaushadhis.

Nowadays, Agadtantra is the branch of Ayurveda is unnoticed due to less use in clinical practice by expert Ayurvedaphysicians . Most of the diseases caused in present scenario are due to unconventional etiology such as indiscriminate use of pesticides-fertilizers in the farming, food adulteration, food additives-preservatives-sweeteners, indiscriminate use of various synthetic drugs etc. For this unconventional etiology, In Ayurveda Agadtantra insist on Virudha Ahar (Incompatible diet/ food), GaraVisha (Artificial poison) and Dushivisha (Polluting poison). Oxidative stress can be correlated with the concept of Virudhaahar, Garavisha and Dushivisha. This unknown etiology causes diseases like cancer, cardiovascular disorders, neurological disorders, liver disorders, renal disorders, psychological disorders, skin diseases etc. For the management of such diseases of unknown etiology, routine drugs/treatment may not behelpful. Now it's time to explore and use the principles of Agadtantra with Antitoxic formulations not only to poisoning conditions but also in various diseases and pathological conditions to improve health status and longevity of human being. According tot he ideology of management in Agadtantra, in current era most of the disorders require, Detoxification, Elimination of Toxins, Blood purification, Equilibrium in body humors, Rejuvenation therapy. Anti- toxic formulations help in detoxifications. Thus, these anti toxic formulations mentioned in Agadtantra will be useful for society in the disorders of present scenario. Additional Preclinical and Clinical study have scope in this regard.

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Perception of Medical Professionals and Pharmacists towards Generic and Branded Drugs

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Abstract

Background: Doctors and pharmacist are in a state of dilemma at the time of prescribing and dispensing medicines. The Government of India is pushing the adoption of generic medicines to lower down the cost of treatment and the perception of the medical practitioners and the pharmacists can play a crucial role in the implementation of this initiative.

Aim and Objective: To understand the factors affecting the prescription of drugs among medical professionals. To understand the factors affecting the dispensing of drugs, To study determinants of prescription of generic drugs.

Methodology: A study is been conducted on doctors and pharmacist were the sample size was taken as 100, it is a purposive study where the data is been collected through questionnaires. Out of 100 participants, 50 were Doctors and 50 were pharmacist. The analysis of the data is performed in SPSS V20.

Result: Out of 100, About 59% of participants perceived that generic drugs are as effective as original drugs. However, interestingly, when they were asked if they ever experienced the difference in quality between the two, 23% participants believed that the generic drugs were not as efficacious Only 64% of the participants agreed that generic drugs are as safe as original drugs. About 46% of participants agreed that most of the generic drugs lack the quality of their branded counterparts. About 70% felt that more confidence should be built among patients about generic medicines. A majority of physicians and pharmacist 61% considered the socioeconomic status of the patient while prescribing medicines. About 24% of the doctors and pharmacist think that generic drug hasa high side effect compare to branded drug, whereas 64% says that the generic drug does not have much side effect. About 47% of the doctors and pharmacist think that they have high efficacy were as 47% thinks that the efficacy level of the drug is low compare to the branded drug."

Conclusion: This study found a gap between knowledge score and generic prescription practices. This can be improved by the combined approach of Information, Education and Communication (IEC).

Keywords: *Generic Medicine, Branded Medicine, Pharmacist, Doctor.*

Introduction

Prescription cost of medicines has increased drastically in the last decade and pharmacy outlets are looking for means for cost minimization by adopting generic fill rate[(1)]. Even in India also generic medicines have been considered as a tool to improve access to medicines for poor population[(2)]. A generic drug is a drug that is made and distributed without a patent. Formulation of the generic drug may be patented but its active ingredient is not patented. Generic medicines are not inferior in quality to any branded medicines and are as effective[(3)] as branded medicines. Even their

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dosage, side-effects, active ingredients etc. are similar to all branded medicines[4]. Generic medicines have to go through all strict procedures of quality standards before they are licensed to market.

For the treatment of a particular disease, it is recommended to give a chemical element/compound as a special medicine after all the research. Different companies sell different names to this element. The name of generic medicines is determined by an expert committee based on the name of the active ingredient present in that medicine. The generic name of the medicine is the same all over the world.

Benefits of generic medicines:

- Generic medicines are cheaper than branded medicines. With this, you can save a significant price every month.
- Generic medicines reach the buyer directly.
- Nothing is spent on the publicity of these drugs. So they are cheap.
- The government decides the price of these medicines themselves.

Generic medicines have the same effect, dosage and effects as branded drugs.

Difference between branded generic and generic drugs: The difference between the two is just the name. One is sold with a brand name and the other is sold with a generic name. But the reality is that both these are generic medicines, which are being made after the patent expires. Generic medicines are sold without any trademark or generic name instead of a brand name. The International Nonproprietary Name (INN) is a unique name given to identify pharmaceutical substances or active pharmaceutical ingredients.

National Drug Pricing Authority: The establishment of the National Drug Pricing Authority (NPPA) was conceived in 1994, based on the discussion in Parliament, in the order of the liberal procedure as prescribed in the amendments to the Drug Policy 1986. Approving the resources in the Drug Policy, 1986, the Cabinet-Committee on Economic Affairs approved the establishment of an independent body of subjects called the National Drug Pricing Authority in September 1994 and the NPPA in its light. Approved the establishment of the authority and the Government Resolution on the handing over of specialized works was notified on 29 August 1997. Thus the N.P.P.A. The date became fully functional from August 29, 1997.

Methodology

The study was undertaken to understand the perception of medical professionals and pharmacists about generic drug and branded drug. The study also aims at identifying determinants of prescription.

Study setting: The study was carried out at Nagpur city in Maharashtra State during the period November 2019 – February 2020.

Sampling: Purposive Sampling was performed to access a particular subset of people.

Inclusion criteria: Should have a minimum of 1 year of experience as a clinical practitioner or should be working in a pharmaceutical shop for more than 1 year.

Exclusion criteria: Students who are pursuing there a career for becoming Doctor or pharmacist, should not have any experience and not be an intern.

Sampling: A total of 100 samples were taken out of which 50 were Doctors and 50 were Pharmacist.

Doctors who are working in their clinic or are working in a government or private hospitals data is collected from them.

A pharmacist who is having their shops or are working in another shop and are registered under the pharmacy council of India, the questionnaire is circulated among them

The need for study:

1. To understand the factors affecting the prescription of drugs among medical professionals.
2. To understand the factors affecting the dispensing of drugs
3. To study determinants of prescription of generic drugs

Statistical Tools: The tools that were used in SPSS V20, MS Excel.

Study tool: The study tools that are been used are Questionnaires, literature review, observation and in-depth interview of the pharmacist as well as the doctors.

Primary data: Data has been collected through questionnaires. A Google form is prepared and is been circulated between doctors and pharmacist who are having experience minimum of 1 year.

Secondary: The secondary data was collected through journals and magazines which are verified.

Analysis: The analysis of the data is done by representations of charts and graphs.

Analysis and Interpretation:

Table 1: Ask for Generic

	Frequency	Percent	Valid Percent	Cumulative Percent
Often	13	13.0	13.0	13.0
Sometimes	36	36.0	36.0	49.0
Never	47	47.0	47.0	96.0
Always	4	4.0	4.0	100.0
Total	**100**	**100.0**	**100.0**	

Table 2: Ask for Branded

	Frequency	Percent	Valid Percent	Cumulative Percent
Often	12	12.0	12.0	12.0
Sometimes	40	40.0	40.0	52.0
Never	37	37.0	37.0	89.0
Always	11	11.0	11.0	100.0
Total	**100**	**100.0**	**100.0**	

Table 3: Confidence

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Agree	70	70.0	70.0	70.0
	Disagree	13	13.0	13.0	83.0
	Neutral	17	17.0	17.0	100.0
	Total	**100**	**100.0**	**100.0**	

Table 4: Explanation to Patient

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Often	11	11.0	11.0	11.0
	Sometimes	43	43.0	43.0	54.0
	Never	35	35.0	35.0	89.0
	Always	11	11.0	11.0	100.0
	Total	**100**	**100.0**	**100.0**	

Table 5: Education Level

	Frequency	Percent	Valid Percent	Cumulative Percent
Yes	64	64.0	64.0	64.0
No	25	25.0	25.0	89.0
Do Not Know	11	11.0	11.0	100.0
Total	**100**	**100.0**	**100.0**	

Table 6: Socioeconomical

	Frequency	Percent	Valid Percent	Cumulative Percent
Yes	61	61.0	61.0	61.0
No	25	25.0	25.0	86.0
Do Not Know	14	14.0	14.0	100.0
Total	**100**	**100.0**	**100.0**	

Discussion

This study was undertaken in 100 participants to evaluate the perception of prescribers and dispensers toward generic and original drugs. The study participants were the Doctors and pharmacist. At 100in number, Doctors and pharmacist were the highest participants with a clinical experience of 1–5 years. The reason for this could be that they were readily willing to participate in the study and present in the hospital premises at most times. About 59% of participants perceived that generic drugs are as effective as original drugs. However, interestingly, when they were asked if they ever experienced the difference in quality between the two, 23% of participants believed that the generic drugs were not as efficacious. We can, thus, conclude that there is a clear gap identified between the knowledge and perception of generic versus original drugs.

When asked about how many patients specifically ask for a generic drug it is been observed that only 4% of patient specifically ask for the generic drug whereas only 11% patient specifically asks for the branded drug. Whereas 40% never ask for a generic drug. Only 11% of the doctor and pharmacist explain the generic drug to the patient were like 35% of the doctors and pharmacist never giver explanation about the generic drug to the patient."

Only 64% of the participants agreed that generic drugs are as safe as original drugs. About 46% of participants agreed that most of the generic drugs lack the quality of their branded counterparts. About 70% felt that more confidence should be built among patients about generic medicines. A majority of physicians and pharmacist 61% considered the socioeconomic status of the patient while prescribing medicines. If the patient could afford, then the majority of participants preferred to prescribe original brand drug against those who referred to prescribe the generic drug. About 24% of the doctors and pharmacist think that the generic drug has a high side effect compare to branded drug, whereas 64% says that a generic drug does not have much side effect. About 47% of the doctors and pharmacist think that they have high efficacy were as 47% thinks that the efficacy level of the drug is low compare to the branded drug."

A majority of participants perceived that there was a significant difference between the price of generic and original brand drugs. Nearly similar findings were reported by studies done in different countries such as Australia, Iraq, and Malaysia. In one article, authors classify industry to innovating pharmaceutical companies and generics producers. It was interesting to note that a majority of healthcare providers were willing to substitute generic drugs but not for all the cases they treated, thus reinforcing the clear difference in the perception of the efficacy of two classes of the drugs. Physicians were doubtful about the efficacy of the generic drugs, which in turn shows the lack of confidence for the use of such drugs and lack of knowledge regarding generic drugs. This finding also corresponds to the earlier work where physicians were found to prescribe most of the medicines with their brand names[5]. "The overall results also corresponds to a study done in Bangladesh

where it was found that the medical practitioners [6] and pharmacy students were knowledge deficit about the various aspects of generic medicines [7]. Moreover the instances of patients switching back to branded drugs after opting for generic medicines are also noticed in the earlier studies [8].

We suggest that this could be overcome by training medical professionals at the undergraduate level by making curricular innovations. Basic information on health policy, pharmaceutical policy, essential drug list, innovators and generic medicines, and their availability and affordability should be provided. Health-care providers also doubted the efficacy of the quality of generic drugs although they thought it was cheaper. This could probably be owing to poor quality control of some generics. More number of medical representative visits resulting in more promotion of drugs by noncommercial sources could be the reason. We have also tried to understand the difference in the perspectives of various specialties toward prescription of generic and original drugs. As medical representatives visited doctors more frequently, they were the most common sources of drug information."

Conclusion

"Although a good percentage of doctors and pharmacist had good knowledge and attitude about generic medicines and the majority of them said that they prescribe/Dispense generic drugs but there was a meaningful proportion who expressed concerns about them. These beliefs could represent a significant hurdle to larger generic use and could lead to increased health-care expenses. Above all, it was observed in this study that the efficacy, safety, and quality profile of the medicine was the most important factor considered by physicians and pharmacist when they prescribe/ Dispense drugs. A sustainable generic pharmaceutical market requires active regulatory measures to build confidence among the physicians to prescribe them without any doubt. We would recommend conducting similar studies among health-care providers from private practice to understand their prescribing practices and find ways to improve them to reduce drug expenditures in a no medical reimbursement situation."

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Drivers of Home Health Nursing Profession

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Abstract

Introduction: It is important to determine the perceived factors of home health nursing professionals which are the drivers of home health nursing as a profession in developing countries.

Objectives: To identify the variables driving the growth of the home care nursing profession.

Methodology: Responses of 150 home health nursing professionals is processed with factor analysis.

Result: Analysis reveals that the three factors together explained 45.6 percent variation in the data.

Conclusion: The identified factors are "Comparative benefits over hospital care", "Exclusive benefits of home healthcare", and "Holistic view linking hospital care and home healthcare."

Keywords: *Home Healthcare, Holistic linkage between home healthcare and Hospital care, Home Health Nursing.*

Introduction

People tend to use healthcare services when it becomes urgent, as the behaviour of seeking healthcare is at the end of help-seeking behaviour[(1)]. These results in an increased number of chronic diseases and home healthcare are considered as one of the preferred modes of healthcare delivery for patients suffering from chronic diseases. Home Health Care is clinical medical care provided by Doctors, registered nurses, occupational therapists, physical therapists, and other healthcare and allied healthcare professionals at the patient's home. It is often prescribed as a part of a care plan following a hospitalization. The goal of home health care is to help an individual improve functions and lives with greater independence, promote the clients an optimal level of well-being, and assist the patient to remain at home, avoiding admission to a long term care institution. Home Healthcare is a relatively newer addition in the assortment of healthcare services in India[(2)]. As a matter of fact, in the United States alone home healthcare sector had employed around 1, 10, 000 registered nurses in the year 2008 and this sector was expected to grow by 66 per cent for the next ten years[(3).] However, home healthcare is characterised with many benefits of its own in comparison to institutional care such as lower cost, lower risk of healthcare-associated infection, and increased patient autonomy in terms of allowing the patients to stay with their family in times of sickness[(2)]. Considering the benefits of home healthcare, its acceptance in India is also on the rise. However, to meet the healthcare requirements of the home healthcare consumers, there should be an adequate supply of healthcare professionals and especially nurses who were willing to visit the patient's home to render medical care. Furthermore, there is a huge contrast between hospitals and patient's home in terms of work settings and this widens the range of risks and hazards which are peculiar to home settings of the patients. An earlier study has identified that hazards associated with slippery floors, and exposure to blood were rated by the Indian home health nurses as the high priority hazards, which required the management attention for risk reduction[(4).] Despite these challenges, the number of home health

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care providers are increasing, whereas the number of nurses and midwives ratio was 1.7 per 1000 populations, which is far less than the world average of 3.28 nurses per 1000 populations[5]. Moreover, in the year 2020, the world health organization has suggested that India alone requires 2.4 million nurses. However, with a new job avenue in terms of home healthcare nursing in India, it becomes pertinent to ponder upon what could be the driving factors for increasing adoption of the home health nursing role.

Objectives: The home healthcare sector has started to attract nursing professionals in India. However, the nursing professionals opting for home health nursing profession is still lesser in comparison to nurses opting for the hospitals. This put forwards a major challenge in front of the home health agencies in recruiting and retaining qualified, trained, and committed home health nurses in the organization. However, the numbers are slowly but steadily increasing and hence this raises the research question of what are the factors which drive the home health nursing profession as an option for the nursing graduates. Hence, this exploratory study is conceptualized to capture the perception of working home healthcare nurses towards the home health profession and to summarize these perceptions into the factors. The identified factors will be useful to the home health care providers and the recruitment agencies to attract and hire competent home health nurses.

Methodology

This cross-sectional study is carried out in the city of Bangalore of Southern India. The home care nurses working in three agencies were contacted. The nursing staff were selected based on systematic random sampling after getting the manpower list from the agency. The selection criteria were home healthcare nurses who have completed one month tenure in the home healthcare profession, as anyone lesser than that duration might not be getting an opportunity to experience the various facets of home health nursing profession. Furthermore, based on the analysis of related literature from the healthcare sector and the opinions of fifteen home health nurses, who were asked a two-part question, which was, what are the good things and the bad things you like in the home health profession and why home health is growing in India? A self-administered questionnaire was prepared, and the content validity of the questionnaire was checked by getting it reviewed from five subject experts with rich experience in the domain of home health nursing. The questionnaire consisted of ten demographic questions together with 15 items to identify the drivers associated with the growth of the home health nursing profession in India from the working home health nursing professional's perspective. Furthermore, and it was pilot tested on around 15 individuals, to assess the suitability of the questionnaire. However, the three items were removed due to low communality value, thus making the final questionnaire consisted of final 12 items. The internal consistency of the questionnaire was checked with the help of Cronbach alpha which came as 0.78, which is higher than the cut-off value of 0.73[6], See Table 1.

Table 1: Questionnaire

Sr.No.	Items	Cronbach Alpha
1	Facilitates access to preventive health services	0.73
2	Home care is a demanding job and only dedicated personnel must join this	
3	Allows terminal ill patient to pass away in the home environment	
4	Provides psychological support to the family	
5	It provides exclusive care that one will get anywhere else	
6	It is cost-effective than hospital care	
7	Offers a holistic assessment of the patient in the environment	
8	Risk of infection is less than hospital	
9	It should be preferred for a terminal ill patient	
10	Decreases rehospitalisation	
11	Home health care has new job avenues for nursing professionals	
12	It is important thus hospital and home care service providers must work closely	

The data were captured by a survey method where a total of 197 home health nursing professionals, working in the selected three home healthcare agencies of Bangalore City were contacted out of which only 165 nurses have responded and filled the questionnaire, thus making the response rate 79%. However, fifteen responses were discarded for incomplete information, thus at the end, 150 numbers of questionnaires were finally used for analysis and interpretation. The demographic details of the respondent are mentioned in Table 1, see below.

Table 2: Demographic details of the respondents

Gender (Percentage)	**Male**	**Female**		
	48.7	51.3		
Age (Percentage)	**20-30**	**31-40**	**41-50**	**51-60**
	28.8	41	29.5	.6
Years of experience in home healthcare (years)	**0-1**	**1-3**	**3-5**	**More than 5**
	24.4	37.2	34.6	3.8
Education (Percentage)	**Diploma**	**Bachelors**	**Masters**	**PhD and Above**
	29.5	67.9	2.6	Nil
Tenure of work with the current employer (Percentage)	**Less than 1**	**2-5**	**6-9**	**9 and above**
	45.5	51.9	2.6	Nil

The demographic data reveal that the females were slightly more than males with 51.3 % in the home health nursing profession. Additionally, the largest age group segment belonged to the age group 31-40 years with 41 % and very few were having experience in the home health nursing profession for more than 5 years i.e. 3.8 %. The largest section of the respondents was having a bachelor's degree in the educational qualification with 67.9 % and almost half of the respondents had an association with the current employer in 2-5 years of work experience.

Data reduction technique factor analysis[7] is used with extraction method principal axis factoring and rotation method Varimax with Kaiser Normalization. Kaiser Mayer Olkin (KMO) test is done for sampling adequacy and it was found to be .729, higher than the cutoff level 0.6[8], similarly, Bartlett's test of sphericity also found to be statistically significant $p<0.01$, implying that the correlation matrix is an identity matrix.

The factors were derived based on the criteria of having eigenvalues greater than 1, the identified three factors explained 45.6 percent of the variance. See Table.

Table 3: Total Variance Explained

Factor	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.883	32.356	32.356	3.329	27.744	27.744	2.698	22.486	22.486
2	1.641	13.675	46.031	1.155	9.626	37.371	1.697	14.138	36.624
3	1.516	12.636	58.668	.995	8.290	45.661	1.084	9.037	45.661
4	1.017	8.474	67.142						
5	.878	7.320	74.462						
6	.691	5.755	80.217						
7	.540	4.497	84.714						
8	.534	4.449	89.163						

Factor	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
9	.401	3.339	92.502						
10	.328	2.731	95.234						
11	.309	2.577	97.811						
12	.263	2.189	100.000						
Extraction Method: PAF.									

All the items loaded well on the factors with factor loadings higher than 0.5. Hence, it becomes evident that three factors are important contributors for the growth of home health nursing profession in India as perceived by working home health nurses, which are exclusive benefits of home healthcare, comparative benefits over the hospital, Holistic views linking hospitals and home health providers.

Table 4: Rotated Factor Matrix

	Factor		
	1	2	3
Facilitates access to preventive health services	.710		
Home care is a demanding job and only dedicated personnel must join this	.705		
Allows terminal ill patient to pass away in the home environment	.694		
Provides psychological support to the family	.527		
It provides exclusive care that one will get anywhere else	.525		
It is cost-effective than hospital care	.509		
Offers a holistic assessment of the patient in the environment	.458		
Risk of infection is less than hospital		.753	
It should be preferred for a terminal ill patient		.675	
Decreases rehospitalisation		.488	
Home health care has new job avenues for nursing professionals			.708
It is important thus hospital and home care service providers must work closely			.642
Extraction Method: Principal Axis Factoring. Rotation Method: Varimax with Kaiser Normalization.			

Based on the identified factors, the authors propose to depict the relationship between the three factors in figure 1.



Figure 1: Relationship between the identified Factors

Discussion

This study establishes that home health nursing profession in the organized sector in India is comparatively very new as ninety-six per cent of the home health nurses were having less than five years of work experience in home health nursing profession, this finding supports the postulation of an earlier study which has postulated that home health care is a new service segment in India[2]. Moreover, this finding also throws light on the attrition problem in the home health nursing profession as the work tenure of less than one-year duration with the current employer is around forty-five per cent, this is on the higher side, however, there is no earlier study to measure the attrition rate of home health nurses in India but this finding is relatable to another third-world country "Kenya" where the attrition rate for community health workers was found to be 33 per cent[9]. Another interesting finding of the study was that around 69% of the home health professionals were lesser than forty years of age. Moreover, only 3-4 % of the home health nurses had a master's degree in educational qualification, this finding was commensurate with an earlier study[4] also. Furthermore, the analysis of data reveals that there are majorly three factors which are promoting the growth of home health nursing services and the profession in India, these three factors are exclusive benefits of home healthcare, comparative benefits over the hospital, Holistic views linking hospitals and home health provider. The first-factor exclusive benefits of home healthcare highlight that some aspects of home healthcareare exclusively available with home health services only. This includes aspects such as enabling preventive healthcare in a better way than hospitals[10], home healthcare provides a unique opportunity which allows the terminally ill patients to spend their last times at home peacefully being around the family members, home healthcare also helps to reduce the caregiver burden by providing psychological support to the family members[11][12], home healthcare are more cost-effective than hospital care for chronic disease care[13] and lastly, home healthcare can provide holistic care to the patient i.e. all the levels of care such as preventive, promotive, restorative care can be provided with home health services. Similarly, nursing professionals also makes a direct comparison between the home health services and hospital care and finds that in some aspects home health is superior to hospital care for example with home healthcare services there is lesser risk of infection[14], it reduces the hospitalization[15] and it should be always preferred for terminally ill patients. Lastly, the third factor reveals that nursing professional also perceive that home health and hospital services are complementary to each other as this factor covers that home health service opens new job avenues for nursing professionals and both home health nursing professionals and hospital nursing professionals must work in close association in the interest of the patients. Which as a concept is also largely promoted in the earlier studies done in Germany, and these studies have insisted upon the home care integration with other healthcare services at all the levels (primary, secondary and tertiary) levels of healthcare delivery[16].

Conclusion

This study concludes that there is a positive perception of home health nursing professionals towards home health nursing profession and there are majorly three factors driving the growth of home health nursing profession in India, namely exclusive benefits of home healthcare, comparative benefits over the hospital, Holistic views linking hospitals and home health providers. Home healthcare is superior to hospital care in matters related to decreased infection, reduced res-hospitalization, and care for terminally ill patient care. Lastly, Home Health nursing professionals perceive that the home health nursing profession has started a new job avenue for them and it's important that hospital nursing professional and home health nursing professionals work in close collaboration to get the desired patient outcomes. In cognizance with the identified factors, it can be expected that in the comping years the numbers of nursing professionals opting for home health nursing profession is going to increase.

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Effectiveness of an Educational Program on Critical Care Nurses' Practices Regarding Endotracheal Suctioning of Adult Patients Who are Mechanically Ventilated in Hospitals at AL-Najaf, Iraq

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Abstract

Background: Nurses need to participate in many different educational activities that enhance and promote lifelong learning, professional and personal growth. Structured endotracheal educational program is developed to articulate new scientific approach by using researches in the clinical setting and apply research findings in clinical practice.

Objective: The study is aimed to evaluate the effectiveness of an educational program on critical care nurses' practices regarding endotracheal suctioning of adult patients who are mechanically ventilated in hospitals at AL-Najaf, Iraq.

Methodology: This study is carried out by using quasi-experimental of pre-test and post-test one group design. The study conducted over the period of time that started from December 10, 2019 and ended on February 20, 2020, by using observational schedule that concentrated on critical-care nurses' practices related to endotracheal suctioning. The study involved observation of critical-care nurses' practices prior to educational intervention and post-test evaluation of participants' practices after four weeks from implementing ETS educational program.

Results: The preliminary assessment of participants' practices related to endotracheal suctioning indicated that most of the nurses were not be committed to the best evidence-based recommended practice guidelines. Dramatically improvement in participants' practices occurred after implementing educational intervention with statistical highly significant indicators.

Conclusion: In-service educational program is a cornerstone in clinical care settings in terms of promoting healthcare providers' knowledge and practices that can lead to quality care and achieve desired outcomes of critically ill patient.

Recommendations: emphasizing on providing in-service educational program is an imperative aspect to enhance practicum skills for novice nurses. Incorporating most recent scientific approaches plays vitally important role in tracking recommended clinical evidence that reflects on embracing change to daily practice standards.

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Keywords: *Critical-care Nurses; Educational program; endotracheal suctioning; Evidence-based practice guidelines.*

Introduction

Patients who are critically ill generally hospitalized in critical care units. Most of these patients commonly having critical diseases such as respiratory failure and require mechanical ventilation (MV). Therefore, an artificial airway like endotracheal tube is commonly placed through the mouth to connect patients with MV in order to maintain breathing and aspirate airway secretions. Endotracheal suctioning (ETS) is one of the most frequent and important practiced procedures in critical care units. This procedure is often performed by critical care nurses in terms of removing accumulated secretions from the respiratory airway. The ETS includes threatening complications if it is performed incorrectly and may expose patient to risky deterioration.

Therefore, critical-care nurses need to be well trained, educated and competent in providing this procedure. In spite of published scientific practice modalities, many researchers addressed that nurses are having lack of commitment in terms of following evidence-based recommended practice guidelines[1-3]. A study carried out in Australia by Gilder, Parke, and Jull (2018) indicated that nurses were not adhering to follow international recommended guidelines related to ETS.[4] Moreover, a study in Turkey (2017) aimed to assess ICU nurses' knowledge and practices regarding ETS demonstrated that most of the nurses are relying solely on their individual experience rather than incorporating scientific approaches in performing suctioning.[5] Therefore, providing educational program based on clinical evidence is necessary to enhance nurses' practices and to guarantee excellent nursing care to patients who are mechanically ventilated.

Nurses should keep practicum skills, knowledge, and providing effective nursing care update. These aspects can be accomplished by attending continuous education. Schweitzer and Krassa (2010) asserted that "One way to keep current on nursing advances is to attend continuous opportunities." (p. 447).[6] Thus, developing structured educational program concerning ETS based on scientific clinical trials can prompt nurses' practice and achieve desired patient outcomes. Hence, the current study is aimed to evaluate the effectiveness of evidence-based practice educational program on critical-care nurses' clinical skills regarding endotracheal suctioning of adult patients who are mechanically ventilated in Hospitals at AL-Najaf AL-Ashraf City, Iraq. Accordingly, the evidence-based practice PICOT research question posed for this study is "Do critical-care nurses (P) who attend ETS educational program (I) improve their practice level (O) as compared to their pre-intervention practices (C) after four weeks from implementing educational program (T)". Moreover, the researchers hypothesized that there will be a statistical significant enhancement on the level of participants' practices about ETS after applying educational program as compared to their pre-test practices. General System Theory (GST) has been utilized to provide theoretical framework to guide this study.

Materials and Method

Research Design: This study is carried out by using quasi-experimental of pre-test and post-test one group design. The study involved evaluation of critical-care nurses' practices prior to educational intervention and post-test evaluation of participants' practices after four weeks from implementing ETS educational program.

Study Setting and Population: The population for the present study is nurses who are working in hospitals at AL-Najaf AL-Ashraf City, Iraq. The target population is nurses who are working in critical care units in selected hospitals. The accessible population is nurses who are met inclusion and exclusion criteria and who are working in AL-Sadder Medical City, AL-Hakeem General Hospital, and Middle Euphrates Hospital. The sampling technique was a non-probability, convenience sample of 87 out of 172 critical-care nurses who were interested in participating in this study.

The inclusion criteria for sample selection include:

1. Both male and female critical-care nurses at selected hospitals.
2. Critical-care nurses who were interested in participating in this study.
3. Critical-care nurses who were available at the period of data collection.

The exclusion criteria were as follow:

1. Nurses who were holding administrative position.
2. Nurses who were not available during the period of data collection.
3. Nurses who were not working in the critical care units.
4. Nurses who were not attending the ETS educational program.

5. Critical-care nurses who were selected for Pilot Study

Educational Program and Observational Checklist: The educational program has been developed based on extensive review of published studies from 2010 to 2020 and according to recent evidence-based recommended practice guidelines. The structured program is focused on improvement of participants' psychomotor skills by demonstrating and re-demonstrating essential steps in performing ETS. The objectives of the structured educational program are: to provide nurses the most recent scientific clinical evidence related to ETS, to articulate the significance of the suctioning procedure in terms of preventing ETS associated complications and enhancing patient outcomes, and to ameliorate collaboration between nurse educator (researcher) and critical-care nurses by identifying their areas where improvement can be made in order to meet the educational session goals. The researcher used various teaching-learning strategies to meet each individual nurse needs such as facilitate guided group discussion, role playing, concept map, problem solving approaches, and debriefing. However, the program concentrated on practices before suctioning, aseptic technique practices, practices during suctioning event, and post suctioning practices.

The observational checklist developed by Kelleher and Andrews (2008) was utilized and authors' permission was secured as well. The authors developed the clinical performance checklists based on comprehensive systematic reviews and recommended practice guidelines. The researchers took permission to adopt the final version of the tool. The observational schedule is peer reviewed to critical care nursing experts with experience in practice development. The authors did a pilot study to ensure validity and reliability.[7]

The tool contains 20 items that specified the most essential ETS practices: Prior to ETS practices includes nine items, and five items represents infection control practices, the suctioning episode incorporates five items, and post suctioning practices includes six items. These items can be found in the following tables. Moreover, after getting permission to use this observational checklist in the current study, we further requested opinion from ten experts from faculty members in adult nursing and anaesthesiologists with subspecialties in intensive care. The tool was valid and no major modifications were made. In addition, a pilot study was carried out for the reliability of the instrument and was analysed by using test retest method, which measured the coefficient of internal consistency. The obtained value for the instrument score was r = 0.81.

Method of Data Collection/Procedure: After getting the ethical approval from institutional review board, the researchers provided a brief description about the study purpose and informed consent was obtained to ensure confidentiality and privacy. We explained to nurses that observations would be occurred to investigate nursing-care practices related to infection control strategies in order to avoid bias during actual suctioning performances. The study began from December 10, 2019 and ended on February 20, 2020.

The participants were observed in their actual critical care units during morning and evening shifts by using observational checklist to document their actual practices against best evidence-based recommended practice. It took about 20 minutes for each observational checklist. The observation was done through three consecutive periods before implementing the educational program. Moreover, after providing an ETS educational intervention, the researcher used the same observational checklist three times in order to compare pre and post practices. The first observation checklist was done one day after presenting ETS educational program, while the second observation was carried out two weeks later and the third observation was done after one month from educational intervention to evaluate the effectiveness of educational program on nurses' practices.

Results

Eighty seven critical-care nurses are participated in this study. Demographic data for the study sample is shown in table 1. The results indicate that all nurses (100%) did not follow ETS guidelines. The vast majority (97.7) have not been attended previous educational or training program related to ETS. The majority of nurses (70.1%) had nursing diploma. Most of the participants (64.4%) had more than 5 years total experience in nursing, while only (12.6%) of them had more than 5 years experiences in critical care units (Table 1).

Table 1: Demographic Characteristics of the Critical-Care Nurses (N = 87).

Characteristics	Rating & Intervals	*n*(%)
Age/Years Mean 7.30 Standard Deviation 31.77	20–24	10(11.5)
	25–29	33(37.9)
	30–34	21(24.1)
	35–39	9(10.3)
	40+	14(16.1)
Gender	Male	64(73.6)
	Female	23(26.4)
Level of Education	Nursing Secondary School	5(5.7)
	Nursing Diploma	61(70.1)
	B.Sc. in Nursing	21(24.1)
Participation in Training/Educational Program	Yes	2(2.3)
	No	85(97.7)
If the answer is (Yes)	One Attendant	2(2.3)
Follow ETS Guideline	No	87(100)
Self-education	Yes	8(9.2)
	No	79(90.8)
If answer is (Yes)	Internet	6(6.9)
	Journal	2(2.3)
Total Experience as a Nurse	1–3 years	20(23.0)
	3–5 years	11(12.6)
	5 years and more	56(64.4)
Total Experience in Critical Care Units	Less than one year	29(33.3)
	1-3 years	24(27.6)
	3–5 years	23(26.4)
	5 years and more	11(12.6)

N = total study sample; *n* = sample number; (%) percentage.

Table 2: Evaluation of Nurses' Practices *Prior to Suctioning* at the Pre-test and Post-test.

Item	Observation	Pre-test	Post-test
		n(%)	*n*(%)
1. Patient Assessment: Did the nurse auscultate the patient's chest before ETS?	Done	29 (33.3)	73 (83.9)
	Not done	58 (66.6)	14 (16.1)
2. Patient preparation: Did the nurse explain to/communicate with the patient about the procedure?	Done	9 (10.3)	33 (37.9)
	Not done	78 (89.7)	54 (62.0)
3. Pre-suctioning hyper-oxygenation/hyperinflation	Done	71 (81.6)	73 (83.9)
	Not done	16 (18.3)	14 (16.1)
4. Sodium Chloride instillation	Done	79 (90.8)	7 (8.0)
	Not done	8 (9.2)	80 (92.0)

n = sample number; (%) = Percentage.

The results in table (2) indicate that the frequency and percentage of the correct performance in aspect "*Practices Prior to Suctioning*" is increased at the post-test as compared with pre-test. This gives an indication that there is an enhancement in the nurses' practices after the application of the ETS educational program.

Table 3: Evaluation of Nurses' Performances Regarding *Infection Control Practices*.

Item	Observation	Pre-test	Post-test
		n (%)	*n* (%)
5. Hands are washed prior to suctioning	Done	47 (54.0)	79 (90.8)
	Not done	40 (46.0)	8 (9.2)
6. Gloves are worn	Done	85 (97.7)	87 (100%)
	Not done	2 (2.3)	0 (0.0)
7. Apron is worn	Done	35 (40.2)	67 (77.0)
	Not done	52 (59.8)	20 (23.0)
8. Goggles/face mask worn	Done	30 (34.5)	70 (80.5)
	Not done	57 (65.5)	17 (19.5)
9. Sterility of suction catheter maintained until inserted into airway	Done	39 (44.8)	63 (72.4)
	Not done	48 (55.2)	24 (27.6)

n = sample number; (%) = Percentage.

Table (3) indicates that there is an improvement in frequency and percentage scores of participants' practices regarding infection control measures after implementing ETS educational program at post-test period.

Table 4: Evaluation of Nurses' Practices during "*Suctioning Event*"

Item	Observation	Pre-test	Post-test
		n (%)	*n* (%)
10. Size of suction catheter & Size of ET Tube: Done = Less than half internal diameter of ETS. Not done = More than half internal diameter of ETS.	Done	57 (65.5)	78 (89.7)
	Not done	30 (34.5)	9 (10.3)
11. Number of suction passes: *Done = two or less, Not done = more than two*	Done	60 (69.0)	82 (94.3)
	Not done	27 (31.0)	5 (5.7)
12. Length of time suction applied to airway: *Done = less than 15 seconds, Not done = more than 15 seconds*	Done	47 (54.0)	71 (81.6)
	Not done	40 (46.0)	16 (18.4)
13. Level of suction pressure: Done = 80 – 120 mmHg, Not done = more than 150 mmHg	Done	11 (12.6)	82 (94.3)
	Not done	76 (87.4)	5 (5.7)
14. Position of catheter when suction applied: *Done = withdrawal, Not done = insertion*	Done	49 (56.3)	82 (94.3)
	Not done	38 (43.7)	5 (5.7)

n = sample number; (%) = Percentage.

The results in the above table demonstrate that there is an enhancement in nurses' practices at post-test in regard to use suctioning catheter less than half internal diameter of ET tube. Similarly, items number 11, 12, and 14 indicate improvement in participants' practices at post-test. Concerning item number 13, the majority of nurses (87.4%) were exceeding the suctioning pressure limit in most of the time to more than 150 mmHg at the pre educational intervention. While after education, the participants adhered to recommended guidelines presented during the implanting ETS educational program.

Table 5: Evaluation of Nurses' Performance Regarding *Post Suctioning Practices*.

Item	Observation	Pre-test	Post-test
		***n* (%)**	***n* (%)**
15. Patient reconnected to Oxygen: *Done = less than 10 seconds, Not done = more than 10 seconds*	Done	56 (64.4)	65 (74.7)
	Not done	31 (35.6)	22 (25.3)
16. Post suctioning hyper-oxygenation/hyperinflation	Done	80 (92.0)	87 (100.0)
	Not done	7 (8.0)	0 (0.0)
17. Post ETS assessment: Did the nurse auscultate the patient's chest?	Done	32 (37.0)	65 (75.0)
	Not done	55 (63.0)	22 (25.0)
18. Patient reassured	Done	16 (18.4)	50 (57.5)
	Not done	71 (81.6)	37 (42.5)
19. Used catheter and gloves are disposed of in a manner that prevents contamination from secretions	Done	81 (93.1)	87 (100.0)
	Not done	6 (6.9)	0 (0.0)
20. Hands washed post suctioning	Done	41 (47.1)	72 (82.8)
	Not done	46 (52.9)	15 (17.2)

n = sample number; (%) = Percentage.

The finding in table (5) shows that prior to implementing ETS educational intervention, most of the critical-care nurses did not auscultate patient chest after suctioning procedure, whereas after education, most of nurses did auscultation examination. This table illustrates enhancement in the level of nurses' practices after providing the educational program.

Table 6: Mean Difference among Overall Nurses' Practices in Two Periods of Measurements (Pre-Test and Post-Test).

Phases of ETS	Pre-test	Post-test	Enhancement	Paired t-test	p- value S
	Mean (SD)	Mean (SD)	Mean (SD)		
Practices Prior to Suctioning	0.72 (0.43)	1.38 (0.42)	0.66 (0.11)	9.57	0.0001 HS
Infection Control Practices	0.88 (0.27)	1.19 (0.40)	0.31 (0.16)	6.01	0.0001 HS
The Suctioning Event	1.06 (0.33)	1.61 (0.28)	0.55 (0.9)	11.61	0.0001 HS
Post Suctioning Practices	1.11 (0.26)	1.43 (0.26)	0.32 (0.12)	8.14	0.0001 HS
Overall Practices	0.94 (21)	1.40 (0.22)	0.46 (0.7)	12.79	0.0001 HS

SD = Standard Deviation; S = Significance; HS = Highly Significance;

Paired t-test analysis is conducted to determine the difference in the mean of the nurses' practices at two points of measurements (pre-test and post-test). The study results presented in table (6) show that there are statistically highly significant differences in the nurses' practices at p-value less than 0.01. Based on the statistical mean, the results indicate that there is a highly significant improvement in the statistical mean in the post-test as compared with pre-test (i.e. there is an enhancement in the nurses practices after application the education program).

Discussion of the Study Finding

Regarding critical-care nurses' practices, the researchers observed participants during three times in each period of measurement (pre-test and post-test) in order to ensure the accuracy of recorded performances. The three recorded observations were statistically

analysed and calculated to compare overall mean scores for pre and post intervention. However, during pre-educational intervention period, the result of item no 1 regarding patient assessment shows that most of the participants did not perform auscultation technique before suctioning procedure (Table 2). This is consistent with what has been found in previous study in Finland that conducted by Jansson et al., (2013a) who stated that "the majority of suctions were carried out without clinical indicators (related to chest auscultation) being identified". (p.103).[3] A similar pattern of results was obtained from study in Iran that conducted by Haghighat and Yazdannik (2015) who reported that (90%) of nurses did not perform auscultation technique in terms of finding indications to ETS.[2]

On the other hand, after applying ETS educational program, the vast majority of participants performed auscultation in order to examine suctioning indications. This is evident that our educational intervention was an effective approach in terms of enhancing participants' practices. Our finding was directly in line with Azizian, Azadi, Veisani and Bastami (2020) who concluded that different educational method regarding ETS can be effective and significantly improving nurses' practices.[8] Özden and Görgülü, (2012) mentioned that nurses' practices about ETS improved after training program and reflected on enhancement of patient safety and excellent quality of nursing care.[9] The nurse must be aware that chest auscultation is a mandatory technique to determine clinical indicators for ETS. Performing chest auscultation is one of the most important assessment techniques that nurses should master to detect respiratory indicators to ETS.

In the same domain "*Practices Prior to Suctioning*" item number 4 "Sodium Chloride Instillation" the result indicates that most of participants had been doing normal saline instillation (NSI) before suctioning episode (Table 2). This was revealed in the study by Haghighat and Yazdannik (2015), where most of the nurses implemented NSI before suctioning event.[10] Similar study results that carried out in Baghdad Teaching hospital and Surgical Specialties Hospital/ Medical City Directorate by Majeed (2017) has also demonstrated that the majority of ICU nurses performed NSI.[11] AARC (2010) does not recommend using NSI because there is no enough evidence that articulated its benefits.[12] In spite of evidences that do not support using normal saline (NS), nurses still practicing this procedure. The reason for that could be lack of nurses' knowledge about recent evidence-based recommended practice guidelines. Oppositely, after implementing ETS educational program, the vast majority of participants did not use NSI prior suctioning (Table 2). Moreover, the statistical finding regarding participants' practices "*prior to suctioning"* revealed highly significant improvement in nurses' practices (p = 0.0001) after educational intervention as indicated in table (6). This is proved that our structured educational program has a positive impact on nurses' practices in terms of adhering to the best recommended practices.

In relation to the second aspect "*Infection Control Practices*" the result of pre-test shows that nurses were not fully adhere to infectious preventive measurements. For example, apron was not be worn in most of the participants (59.8%), many participants (65.5%) did not wear face mask and (55.2%) of nurses were not maintain sterility of suction catheter when inserted into airway (Table 3). Our finding is in line with Haghighat & Yazdannik (2015) who found that most of nurses in their study did not adhere to infection control practice guidelines such as (82.5%) of nurses did not wash their hands and goggles were not be worn in (90%).[2] Infection control practices are an essential aspect in terms of preventing hospital acquired infection specifically in ICU. The most common nosocomial infection in critical care units is ventilator-associated pneumonia (VAP) with the occurrence rates ranging up to 70%.[12] Jansson et al., (2013b) stated that despite current available guidelines regarding VAP prevention, nurses still have discrepancies between evidence-based best practices and their performances.[13] Another research study in Jordan conducted by Aloush (2017) indicated that nurses' commitment to infection control strategies regarding VAP were low. The researcher emphasized that poor adherence toward infection control principles could be due to the lack of in-service education for ICU nurses.[14]

Consequently, after providing in-service educational program, the result in table (3) indicates that most of the participants adhered to the best recommended practice guidelines related to infection control practices. Our results demonstrated that there is an improvement in nurses' practices occurred after applying ETS educational program. According to overall statistical analysis for the aspect "*Infection Control Practices*" the result appeared to be very highly significant (p = 0.0001) as indicated in table (6).

Concerning the next domain (table 4) "*The Suctioning Event*", our finding shows that there is a statistically highly significant improvement in participants' practices after implementing ETS educational intervention as illustrated in table (6). In addition, the last section (Table 5) namely "*Post Suctioning Practices"*, our finding revealed that the participants' performances enhanced significantly after educational intervention as delineated in table (6). These findings are in accordance with findings reported by Fernandes, Dasila, and Banerjee, (2018) who mentioned that "Regular in-service education programme at every hospital is required to keep the nurses updated with evidence-based guidelines to achieve positive patient outcomes" (p. 147).[15]

After application the ETS educational program, our finding shows that the overall evaluation of participants' practices in all domains was statistically very highly significant. This could be due to our structured educational program was focusing on the main aspects related to ETS. The second reason can be the study was guided by GST which provided a conceptual framework as a roadmap to delineate crucial steps in demonstrating and re-demonstrating practicum skills through input, output, and feedback process. Thus, our hypothesis is accepted at high level of significance ($p = 0.0001$) and null hypothesis is rejected as shown in table (6).

Conclusion and Recommendation

Directed by general system theory, evidence-based practice recommended guidelines, most recent studies, and standards of nursing care were used to develop an effective ETS educational program to improve practices of critical-care nurses in performing suctioning to patients who are mechanically ventilated. The structured educational intervention is provided in one session. For future researchers, different didactic approaches should be considered, for example using simulation training modalities and implementing several sessions over period of times to include more sample size are highly recommended. Studies recommended conducting continuous educational intervention to maximize the benefit in gaining knowledge and refining practical skills. Bridging the gap between knowledge and practices is the key to enhance the quality of nursing care and improve outcomes for patients who are under mechanical ventilation. In-service educational program based on evidence-based recommended practice guidelines is strongly advised to embrace change with updated scientific approach. Finally, ETS must be performed by well trained, educated and competent nurse to avoid potential adverse event of suctioning procedure.

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Nurse Educators Examine the Applicability of Health as Expanding Consciousness Theory: A Qualitative Review Study

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Abstract

Background: Health as expanding consciousness (HEC) is a grand theory by Margaret Newman, and it is one of those key tools to be used in order to understand a patient as a whole. The theory is based on identifying the pattern of an individual. The HEC focuses on all aspects of the individual as a social, cultural, physical, emotional, and spiritual. It works for understanding the disease as well as other factors pertaining to the pattern of the individual.

Objective: The present review study is aimed to provide inside comprehension on the applicability and feasibility of HEC from nurse educator perspectives based on review of qualitative studies.

Results: This study shows how successfully the theory was applied to coronary heart disease patients, women with multiple sclerosis or rheumatoid arthritis, and pain during perioperative period. Thus, the theory is effective in all scenarios and provides the nurse ways to understand patients from varied backgrounds. The authors of reviewed studies used the hermeneutic-dialectic approaches as a tool for the research study. The nurses played an important role by help the patients to recognize their pattern and move to a higher level of consciousness. The nurse-patient relationship built on mutual trust and respect, which helped both nurses and clients to interact effectively in order to identify their pattern.

Conclusion: Newman's theory can be applied in every nursing setting in both short and long-term interaction with the patient.

Keywords: *Health as Expanding Consciousness; Nursing Theory; Qualitative Review.*

Introduction

Nursing theory is one of the most important subjects that every nurse should know and gain knowledge about. Understanding the nursing theory is a crucial point, and it guides the nurses to apply their knowledge and experience in appropriate ways. Nursing theory is like a roadmap that helps the nurses to move forward and reach the desired outcome. However, nurses must know the main concepts about how to select a specific nursing theory to draw a broad framework that encompasses all nursing responsibilities. Additionally, it is essential that advance professional nurses use theoretical frameworks in nursing clinical settings, education, and research. In fact, nursing is a comprehensive field that deals with not only health and disease issues, but also with every single phenomenon that somewhat is related and incorporated with environment, individual, family, community, society, as well as global population.

As the patient seeks help and comes to the healthcare provider with not only health issues, but also with other problems. Hence, the Newman's theory focuses on

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health as expanding consciousness in which the person is viewed as a whole. Therefore, this approach is the best way to consider with the patient. This paper is focused on expanding consciousness of the patient and nurse through partnerships in order to find a new way of person-environment interaction in spite of the disease deterrent. Disease is not an enemy to fight against, but is a meaningful point that allows people to find new ways to interact with their environment.

Purpose: The purpose of this research article is to illustrate the importance of Newman's theory, which is supported by a research study done on patients with coronary heart disease, a woman with multiple sclerosis (MS) or rheumatoid arthritis (RA), and pain expression in the perioperative period of Somali Women. Newman's theory is based not only on the treatment of the disease, but also on the overall pattern of an individual in different cultures. This theory helps the nurse to expand the consciousness of the patient, simultaneously expanding the nurse's consciousness as well. This in turn supports nurses to improve their knowledge and experience in providing holistic care to an individual. Additionally, Newman's theory can be applied in multi-cultures as well.

Significance: People who live with acute or chronic diseases are facing so many difficulties, such as isolation, frustration, hopeless.[1] They feel unable to do something that they would like to do or have done before. Hence, they have lack in interest in life. However, Newman's theory is helping both nurse and client to expand their consciousness. Newman's theory helps patients to find new meaning of life and to interact with the environment by their remaining energy. Patients with multiple sclerosis or rheumatoid arthritis have restricted mobility and have difficulties to interact with their environment. The Newman's theory is a best approach to assist those patients in order to improve their interaction with the environment and implant the hope in their life.

Overview of the Theory: Newman's theory Health as Expanding Consciousness (HEC) is one of the most recent nursing theories. This theory focused on the health of all persons regardless of the presence or absence of disease. Health is a major concept of Newman's theory of health as expanding consciousness.[2] Newman (1994) provides a new perspective of health, which is opposite from the old definition of health as the absence of disease, and she explained health is a process encompasses that both disease and non-disease, and disease as a meaningful manifestation of the pattern of the whole, which is not the separate entity of the whole.[3] In addition, she believes that health and illness are the same entity and manifested in the pattern of the person. Disease and non-disease are each reflections of the larger whole, health is the pattern of the whole, and the wholeness is cannot be gained or lost.[4] Accordingly, when the person becomes ill, illness does not diminish wholeness, but wholeness takes on a different form. For example, when a person has certain disease, he or she can get to a healthy place even though that person has a particular disease, because disease is a reorganizing state and part of the wholeness, so the person can find a new situation (pattern) to live and interact with the environment. At the same time, that person is expanding his or her consciousness by changing his or her mind (consciousness) and becoming more aware about how to live and interact with new situation. According to Newman (2008), pattern is seen as information that depicts the whole, and understanding of the meaning of all of the relationships at once.[5]

Therefore, health can be identified by the pattern in which one interacts with the larger pattern of the environment and it is manifested in the way one moves, works, speaks, and relates with others. Moreover, when the person gains more information about his or her current situation, the person's pattern develops and the person begins to organize his or her life in high level, by which it becomes more congruent with environment interaction.

Based on Newman's theory, every person exhibits a unique pattern, which is constantly unfolding or open system that interacts with the environment.[6] In addition, Newman describes the nature of the pattern and she states that anything explicate (that which we can hear, see, taste, smell, touch), is a manifestation of the implicate (the unseen underlying pattern). In other words, there is information about the underlying pattern of each individual which we can sense about them, such as their movement, tone of voice, interactions with others, activity level, genetic pattern, vital signs. For example, we can recognize the patient's condition when he or she complains of pain by seeing the patient's facial expression or body posture. There is also information about the patient's underlying pattern in all that the patient tells us about his or her experiences and perceptions of pain. When the patient is providing us the necessary information by describing the pain such as aching, sharp, stabbing, throbbing, or on scale the pain from 1 to 10, or the patient tells us there are

contributing factors that increase pain, such as before or after eating, so all of these are subjective data. Hence, the pattern is just like objective and subjective data. The HEC perspective sees illness, disorder, disruption, and disequilibrium as an explication of the underlying implicate pattern of the person, family, and community.[6] These conditions need reorganization in order to keep balance between individual and environment interaction. Finding a new situation to adapt with is a part of the process of expanding consciousness.

Pattern recognition is one of the essence concepts of Newman's theory. Understanding the pattern is essential. The expanding consciousness is the pattern recognition. When persons face a disruptive situation such as during an illness, they are in a period of chaos. They may be unable to recognize their own pattern. A partnership with a nurse during this period can create an opportunity for reflection on a pattern and a shift in perspective. Nurse-client relationship often begins during a period of disruption, uncertainty, and unpredictability in patient's life. Becoming aware of the pattern of the other person is by becoming "in touch with one's own pattern" nurse should focus on the pattern of other person, acting as the reference beam in a hologram.[4] The nurse needs to be in touch with the patient and focusing on every single detail that interferes with the patient's condition in order to help the patient recognizes his or her own pattern and to clarify that the old rules of pattern are not working anymore, and need to shift into a new way of interaction with the environment. Dr. Newman (1999) referred to this period as a choice point in which the patient most needs nurses during this period, and by a partnership, the patient can recognize his or her pattern and make a decision to shift to a new pattern.[7] This transformation occurs by partnership with the nurse who provides all necessary information and helps the patient to adapt a new pattern, which more coincides with the patient's new condition. The client-nurse relationship becomes more in harmony during this period, both of them is growing by sharing information, experience, and perception, which reflects on expanding consciousness for both of them. We need to remind ourselves as nurses that when we get in touch with the patient it is to focus not only on treating symptoms, but also on identifying the patient's pattern and treating the patient as a whole rather than a part, as Dr. Newman mentioned and called a paradigm shift.[7]

Newman also mentioned the interrelatedness of time, space, and movement. Time and space are the temporal pattern of the patient, and they have a complementary relationship. People are constantly changing through time and space, and it shows a unique pattern of reality.[4] She also explained that time and space have a complementary relationship. Furthermore, Newman (1986) stated, "Individuals come into being from a state of consciousness, they were bound in time, found their identity in space, and through movement they learned the "law" of the way that things worked and then made choices that ultimately took them beyond space and time to a state of Absolut consciousness".[4] On the other hand, the person with limited mobility has limited space-time. However, transcendence occurs when the patient moves and finds a new meaning for life, which reflects that the patient reaches the higher level of awareness to interact with the environment.

Consciousness and expanding consciousness are also the major concepts of the theory of HEC. Consciousness is defined as both the informational capacity of the system (human being) and the ability of the system to interact with its environment.[4] Also, Dr. Newman (1986) asserts that consciousness not only includes the cognitive and affective awareness, but also the inter connectedness of the entire living system which includes physicochemical maintenance and growth process as well as the immune system. Each system in the human body has specific information and has an ability to interact with environmental system. The environment includes ourselves, our bodies, and the world around us. During the life span, people interact with environment, this interaction between complex information of systems reflecting by gaining experience and knowledge, which in turn these are contributing to expanding consciousness.[4] Life is a process of progression toward a higher level of consciousness. The process of expanding consciousness is defined by Newman (2008) as "a process of becoming more of oneself, of finding greater meaning in life, and reaching new heights of connectedness with other people and the world."[5] Nurses and their clients are gaining knowledge and experience from each other during interaction, and when there is a meaningful quality to their relationships. Nurses and their clients must be open and flexible in order to build mutual respectful and trustful relationships.

Moreover, relationships that are more open, loving, caring, connected, and peaceful are a manifestation of expanding consciousness. Unconditional acceptance is one of the most important issues for the nurse in order to build trustful and respectful relationship with clients.

Nurses must accept the client as he or she is, and whatever his or her health problem. Again, nurses must exhibit professional behavior such as paying attention to every single detail that may be very valuable to the patient. When the nurses are putting themselves in the patient's condition, they can understand and feel the patient's overall situation. Nurses must recognize what are the most important issues to the patient, and why the patient is concerning about these issues. Dealing with patient as a whole is a better approach to treat the patient. By doing so, the patient can realize that the nurse is caring about him or her and will be more open and cooperative to interact with the nurse.

Relevant Literature to the Theory: The following research article presented information on life patterns of persons with coronary heart disease by using Newman's theory HEC. The study included eleven clients (7 men and 4 women) in a cardiac rehabilitation program, a cardiovascular nursing specialist, and the investigators.[8] The most important part in this study is pattern, because the main concept of Newman's theory is that the disease is a manifestation of the individual's pattern, and the pattern can be seen in behavior and inner experience of the individual's pattern. The authors conducted this study by interviewing the clients and asked them to describe the most meaningful persons and events in their life. The interviews were audio-taped in order to organize them into chronological order. The nurses were considered as co-researchers and co-interviewers. The nature and purpose of the interview as collaboration between researchers and clients to understand the individual's overall life pattern was explained. The authors found similarities of patterns among these clients who have coronary heart disease (CHD) and classified them under three main themes, which are (a) the need to excel, (b) the need to please others, and (c) the feeling of being alone.[8] All of participants had these themes, but they expressed their thought in different manners. All clients tend to be successful people or they wish to be the best. These feelings were very important to them because their pleasure arose from recognizing of their accomplishments and from being the center of activity and in control. In addition, all of the clients had strong need to please others, but they were in difficult situations to express their feeling at that time. Moreover, they were experiencing loneliness. The feeling of being alone and lack of connectedness to others was the main cause for alcoholism and isolation. These are theoretical similarities of pattern, which reflect the pattern of the disease. However, the authors described these clients as having poor coping behaviors during the period of disruption (disease), and when the clients reached the choice point they realized that the old ways are not working with new situation (disease) and they need to change their life style.[8] They figured out that the disease crisis was the critical time of re-evaluation of the meaning and direction of their lives. Finally, most of them found things that made their life much easier and have meaningful aspects, such as closer relationships with their families and significant others, quit drinking, and find much more time for entertainment.[8] The role of the nurses was helping the clients to recognize their patterns, and then move forward to the state of absolute consciousness. Our opinion, this is a good research and precisely fits with Newman's theory HEC. The nurses cooperate or deal with the wholeness of the clients rather than treating the symptoms of the disease, and help patients to find new tracks to interact with their environments. The nurses assist the clients, as they are able to do interesting things and interact positively with their environment, rather than they are unable to interact with their environment because of their health problems. The nurses helped the patients to accept that the diseases are only parts of their health.

Neill in 2005 conducted research that supported Newman's theory about seven women living with multiple sclerosis or rheumatoid arthritis, both of which is considered as chronic and autoimmune diseases.[9] In this study, the author used hermeneutic-dialectic process of Newman's theory, which means the communication based on mutual respect and interpretation of content, focusing on meaningful experiences, surrendering control over responses provided, and sharing meanings and insights that evolved[9], in order to understand patterns of person-environment interaction. The author interviewed clients by using dialogue to pursue the communication with the patients' narratives. The study was conducted through partnerships over 2-year a period. The study allowed the clients to narrate their life-stories, in which the most important people and events they remembered were featured along with their patterns of person-environment interaction[9]. The synopsis of the seven women's stories that there was no movement or change in their patterns of person-environment interaction until they reached the choice point when the women developed new awareness of their situations and progressed to the transcendent stages of expanding consciousness. When the women reached the choice

point, they realized that the old ways of interaction with the environment were not working anymore, and during this time, they need new ways to interact with the environment by changing or re-modeling their life activities. However, the researcher assisted those women by helping them to recognizing their patterns. Then, they worked together and found meaning in living with MS or RA, even though their illnesses were not cured medically. Meaning was evident as new ways of living that restored harmony to their lives. All the seven women spoke of finding simple pleasure, being positive and gaining self-control.[9] New ways of living occurred during the transcendent stage of expanding consciousness, and represented a profound personal change and transformation. All the seven women started to think positively after reaching the choice point, and they began to reconnect with their families, practice simple hobbies, and manage their energy expenditure to avoid fatigue, identifying their limitations, and caring for themselves better than in the past.[9]

Neill (2005) declared that for nurses who interested to use HEC theory to guide their practice, these findings (finding simple pleasure etc.) suggest how best to interact with people who manifest health at different levels of expanding consciousness. Neill (2005) also asserted that nurses could become aware of identifying choice points in illness stories or life stories and might be able to move the patients toward higher level of awareness.[9]

The other research study that supported Newman's theory HEC was about pain expression in the perioperative period: insights from a focus group of Somali women. This study was conducted by Ness in 2009, about Somali women who live in Minnesota. Somali women have different pain expression than other cultures.[10] Nowadays, many nurses understand the importance of the variety of cultures and the impact of these cultures on nursing care process. Hence, caring of individuals from different culture needs more attention and it is a critical situation.[11] Ness (2009) used focus group design in the research study. The group was four Somali women who participated in this study. The researcher explained that pain expression is varying from culture to culture, and nurses need to understand those variations in order to recognize the patient needs. Delivering appropriate nursing care for patients from different culture needs a special attention on the way they express pain. The author used the Newman's theory as a theoretical framework and was focused on pattern recognition, interconnectedness, and wholeness, which offered an excellent integration for this project. The Newman's theory designates the major role of the nurse as helping others in identifying the power within them to progress to a healthy state.[12] The author used open-ended questions as a way to gain knowledge about their pain experience during perioperative surgery, and to identify patient patterns. Callister (2003) stated that the pain experience should be understood within the context of the beliefs, values, coping mechanisms, and life experiences of each patient.[13] However, the results of the study indicated that Somali women have unique perspectives on the pain experience. The references to Allah and the importance of expression in their native language during the pain experience are very helpful to know when caring for Somali women.[10] The results also showed that the Somali women have pain expressions and behaviors that are universal, which are the similar expressions of most people from different cultures. For example they expressed and described the pain as screaming, moaning, sighing, making a face, eye movements, guarding by holding the site of pain, holding the head in the hands, and movement of the head back and forth.[10] Additionally, those women spoke about their concern after surgery that they were worried about family because they were not able to do daily activities as they were before the surgery. Hence, the researcher provided some resources like social and community support that took care of their children, which were helping them to get better health and eliminate the hardship at that time. These finding will serve to strengthen culturally sensitive nursing practice and provide a positive impact on Somali women experiencing pain within the health care setting.[10] Finally, the women gained more awareness about pain experience while sharing their experience with each other as well as the researcher. The nurses can use the Newman's theory in multi-cultures in order to improve their interaction with patients from different cultures to reach the desired outcome.[10]

Synthesis of the Theory: The theory of health as expanding consciousness is a holistic nursing theory, and can be applied in both long and short-term nursing clinical settings. We believe that Newman's theory can be used in every interaction that nurses have with the patient. The following example exhibits how the Newman's theory can be applied in short-term setting in emergency room (ER). The female patient is 42 year old, comes to the ER for treatment of severe dehydration related to nausea and vomiting secondary to her AIDS infection. Based on this case, the nurse would apply the

Newman's theory, while the nurse is introducing her/ himself to the patient, the nurse will show the patient that she is highly respected, and she/he will do her/ his best to provide a good holistic health care. This is a good approach to show the patient in order to get a mutual respectful relationship. Then, the nurse would say according to the medical record it found that you are dehydrated, and we are going to help you for that, just in order to begin the conversation and discuss how the nurse is going to give her the IV fluid. Moreover, while the nurse is discussing the administration of treatment to her, the nurse would also ask her some other questions, such as how is everything going with you? Alternatively, do you have any other concerns or anything else I can do for you today?

Obviously, the questions will lead to open conversation, and then the nurse can identify or recognize the patient's pattern. During conversation, the nurse realized that the patient is a single mother for three kids and she feels isolated because there is no one around her who can offer a help. In addition, she has a full time job. She is worried about her kids and about her job as well. These are examples about this case, and the patient may demonstrate different situations. However, the key point is that the nurse must know what are the most important people or events to the patient and why. After knowing that, the nurse can work as partnership with the patient in order to assist the patient to recognize his or her pattern. Then, the nurse and patient can move forward to find a new way that would be much better and congruent with the patient's current situation. The nurse would give the patient some resources such as, social support or community support to help her by taking care of her kids and she can return to her job, and spend more time to take care of her health as well.

To sum up, the nurse focused not only on treating the symptoms of the patient, but also tried to recognize her pattern, and identify the most important issues that she was worried about. The nurse treated her as a whole. The nurse helped her to identify her pattern, and both of them are growing and gaining more awareness by finding new way of person-environment interaction.

Conclusion

In the final analysis, nursing theories have a great emphasis on nursing practice, education, and research. Nursing theories address the most valuable phenomena that improve nurses' knowledge, practice, and experience. Equally important, nursing theories are useful for individual, family, community, and society. This paper discussed one of the most important nursing theories, which is health as expanding consciousness by Margret A. Newman. This theory focuses on the health as a whole, and the disease as a part of the wholeness. Disease and non-disease manifests individual's pattern. Consciousness includes all the entire living systems in the human body. People during the life span are moving forward to the higher level of consciousness. The environment encompasses human beings and the world around them. Furthermore, the nurse-client relationship is very important part of the Newman's theory. Hence, both of them are gaining knowledge, expanding their consciousness in partnership, and exchanging their information by interacting with each other. Patients with acute or chronic diseases are seeking help and Newman's theory is an idealistic approach to assist these patients. This has been justified by the instances provided in this paper. It shows that this theory is dynamic in practice and can serve a variety of communities.

Recommendation: HEC is an excellent theory that can provide a roadmap and be applied in nursing research, clinical settings, and education. It is feasible and we strongly recommend applying this theory in short and long term clinical practice to achieve desired outcomes.

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Assessment of Satisfaction Levels of Health Insurance Policy Holders with Claims Settlement Process in Pune, Maharashtra

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Abstract

Introduction: Customer satisfaction is an important determinant of health insurance penetration. It is not only indicative of customer loyalty but is also helpful in identifying the needs of the customer and further necessity of product innovation. As compared to other verticals of insurance, health insurance is more complex as for the administration and implementation of the policy various stakeholders are involved. Theses take holders are namely hospitals, Third Party Administrators and health insurance agents and advisors. Equally important is the satisfaction with the claim settlement process, which significantly impact overall satisfaction with health insurance policies.

Aim and Objectives: To assess of customer satisfaction levels with the claim settlement process amongst health insurance policyholders in Pune, Maharashtra. Further, the study is aimed to identify the potential areas of dissatisfaction and weaknesses in the claim management system.

Materials and Method: The study design is and exploratory and descriptive. Sequential random sampling used to select participants from the data of health insurance policyholders who have availed the benefit of health insurance through hospitalisation. 5-point Likert scale was used to assess the responses on claim settlement process.

Results: Health insurance claims administration involves coordinated efforts of stakeholders viz insurance companies, TPA services, Hospitals and agents and advisors. The study shows scope of improvement in the claims settlement process. The policyholders experience depends on the coordination, timeliness and transparency in the process.

Keywords: *Health insurance policies, customer satisfaction, claim settlement.*

Introduction

India has conventionally been the country with low health insurance penetration. The out of pocket expenditure is still the most common source of healthcare expenditure.[1]

There has been considerable improvement the penetration health insurance in this decade. The sales of the insurance industry has almost doubled in the last 5 years. Oxford Economics projects revenue from health insurance premiums to reach US$3.5 by 2021, depicting growth of more than 12% annually.[2]

Effective functioning of the health insurance mechanism requires coordinated efforts amongst multiple stake holders. These are hospitalization, healthcare service, third party administration and agents. Equally important is the satisfaction with the claim administration process, which significantly impact overall satisfaction with health insurance policies. Likewise, it

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is important to focus on the existing customers along with new customers as it suggests satisfaction with the products offered. Also, it is seen that the efforts in retaining customers are less as compared to the targeting new customers as they are aware of the benefits of the policy. Further, with the increased competition among the insurance companies, the assessment of satisfaction levels is of utmost importance as policyholders have various choices available.

Satisfaction is the degree of fulfilment of the expectations from the product between perceived performance and expected performance for an individual. If the performance fails to meet the customers' needs they will become dissatisfied. Customer satisfaction has a lead role to play in renewal of health insurance policies as the policy are to be renewed annually. Researchers have considered health insurance as a most complex type of insurance as the service administration requires coordinated efforts from hospitals, TPAs and agents. Also, it is seen that there exists a constant struggle between the insured expectations and the insurers performance. Health insurance sector in India has much scope for improvement.[3]

The role of agents is also very influential in communication along with the personal and social demographic factors influencing the awareness about the health insurance policies. Awareness and opinion about health insurance and health insurance practice is strongly affected by communication and transparency[4]

According to a study, which examined the customer expectation and satisfaction of insurance policyholders towards the services provided by 11 life insurance companies in India, the companies were offering different products, though they were providing similar kinds of service. This shows the need of targeted approach. [5]

Studies focused on the factors influencing the customer satisfaction of policyholders in insurance industry have strongly advocated the need of evaluating service quality factors along with the socio economic factors.[6]

In the assessment of health insurance satisfaction three factors namely personal, marketing and social needs to be focused upon separately to understand the needs and preferences of the customers. It is also recommended that understanding the pattern of subscription of health insurance in different demographic groups and their awareness of health insurance plays a crucial role.[7]

Insurance companies need to focus on customer satisfaction strategies in insurance industry for a successful implementation of business. This is of strategic importance as customer satisfaction directly correlated to a firm's growth.[8]

Health insurance policies are usually for one year and are typically renewed every year. Customer satisfaction with the existing policies and the claims settlement transparency was found to be an important determinant of renewal of health insurance.[9]

Considering the recent innovations in health insurance sector, customer satisfaction is the one of the identified future areas for and associated challenges and enhancing reachability to complex issues involving health insurance. [10]

IRDAI report of 2019 states the claim settlement ratios of most companies. The report also states that there not all health insurance companies are showing positive claim settlement ratios. This means that there much scope of improvement and thus satisfaction.[11]

Studies also show variation in satisfaction levels of Policyholders who have availed policies from private sector and those purchased insurance from the public sector companies. Group policyholders are more satisfied with health insurance compared with family floater policies and individual policies.[13]

In such case the study of customer satisfaction becomes more important to identify potential areas of improvements which will ensure trust in insurance sector and improve the penetration of the same.

The evaluation of the satisfaction levels of these users will help in identifying whether the expectations are being met or not and it will highlight the problems faced and issues identified. This will also help to understand whether the present health insurance policy addresses the need of the respondents. It is important to know how satisfied the policyholders with the Hospitalization and claim settlement process. Health insurance policy administration involves stakeholders like insurance companies, TPA services, Hospitals stay, facilities and the overall treatment. Thus the experience depends on the whether the needs and preferences are being met or not.

Aim and Objectives: The objective of the study is to assess customer satisfaction with the claim settlement

process amongst health insurance policyholders in Pune, Maharashtra. Also, the study aims to identify the potential areas of dissatisfaction and weaknesses in the claim management system.

Research Methodology

The study is based on exploratory and descriptive research. Sequential random sampling was used to select participants from the data of health insurance policyholders. The study period was April – August 2019. The data was collected from 152 health insurance policyholders who had availed the benefit of hospitalisation through either cashless treatment or reimbursement from Pune, Maharashtra.

The respondents belonged to various demographic profiles. 5-point Likert scale was used to assess the responses of the policyholders. The parameters studied were communication transparency and timeliness, role of TPA and documentation and the overall coordination between various stakeholders.

Findings and Discussion

The findings of the study are presented below. Table 1 represents socio-economic characteristics of the respondents having availed benefits of health insurance. It shows that the sample taken adequately represents population in terms of age, gender occupation, education, marital status, income and healthcare expenditure.

Table 1 Demographic profiles of the respondents: The satisfaction levels of the respondents on various parameters of hospitalization and claim settlement have been discussed. The responses show significant scope for improvement. Most of the respondents were not satisfied with the communication and coordination between hospitals, TPA and insurance companies.

Table 1: Demographic profiles of the respondents

Characteristics	Pune N (%)
Area of residence	
Rural	1.3
Urban	98.7
Sex	
Male	56.5
Female	43.5
Age	
Below 25	5.9
25-40	52.7
40 - 60	35.9
Above 60	5.9
Occupation	
Business	14.8
Govt sector employee	12.2
Private sector employee	43.0
Retired and others	53.4
Education	
School Level	23.2
Graduate	33.8
Post graduate & Above	43.0
Marital status	
Married	89.0
Unmarried	11.0
Gross monthly income	
Less than Rs.20, 000	17.3
Rs.20, 001-Rs.30, 000	17.3
Rs.30, 001-Rs.50, 000	36.3
More than Rs.50, 000	29.1
Monthly percentage income spend on health	
Less than 5%	44.3
5-10%	37.6
10-20%	15.6
More than 20%	2.6

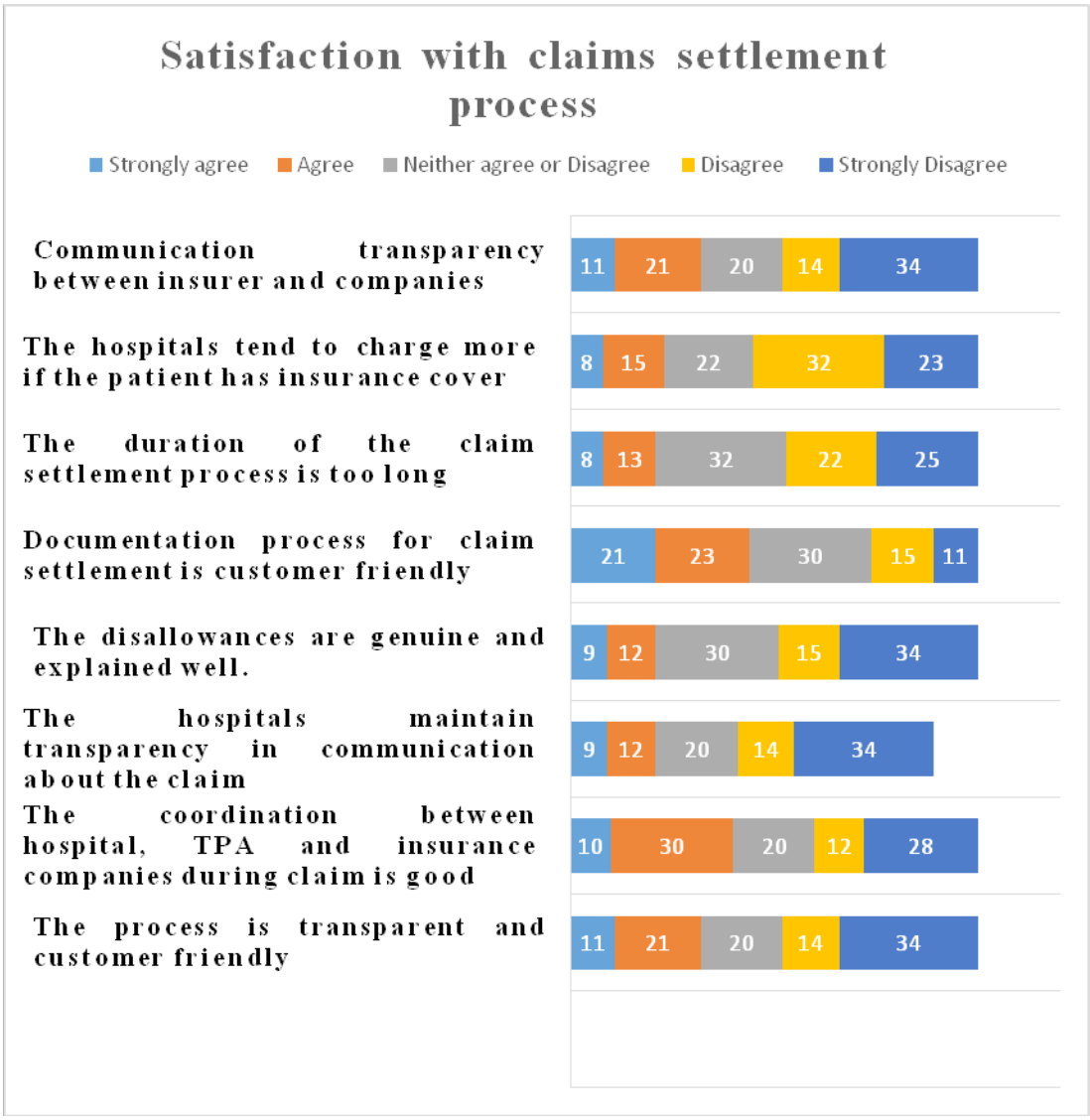


Fig 1 Satisfaction with claim settlement process

Fig 1 depicts the satisfaction levels of the respondents with respect to claim settlement process. Respondents stated that at the time of discharge the documentation process is very lengthy and the disallowances and other parameters are not well explained. This shows that process improvement is needed and communication and coordination needs to be improved to achieve maximum satisfaction level.

When enquired about the transparency and customer friendliness of process of hospitalization and claim settlement only 11 % respondents strongly agreed to the point. The results shows that there is much scope for improvement as the expectation of the policyholders is not being met. Efforts needs to be taken in this regard to make the process more customer friendly.

It is often seen that the communication transparency between insured and the company is not up to the mark. Only 21 % of the respondents agreed that communication was clear enough to understand the deductibles and co-payments.

There is a preconceived notion that the hospitals tend to charge more if the patient is having health insurance. The hospitalisation expenses exceeds than that of the self-paying patients. When enquired regarding the same. 22% respondents were neutral and 32% disagreed to the same.

It is see that the duration of claim settlement is long and exceeds up to 45 days. Often the disallowances in the health insurance policy are exceeding the expectation of

the policyholder and communication and understanding regarding the same is lacking. The respondents were questioned whether the disallowances were genuine or not to which 34% strongly disagreed and 15 % disagreed. This is of importance and ultimately affects satisfaction and further renewal of the policy in the subsequent years.

Hospitals are also one of the important stakeholder in the health insurance administration as the care providers. The role of hospitals in maintaining transparency in communication is important. 22% of the respondents agreed that the communication transparencies maintained.

It is often seen that the coordination between all stakeholders is important determinant of the satisfaction of the policyholders. In the study, it was found to be lacking. This shows that all the stakeholders be it hospitals, insurance companies or TPAs need to improve overall communication and coordination.

Conclusion

Health insurance claims administration involves stakeholders like insurance companies, TPA services, Hospitals stay, facilities and the overall treatment and involvement of agents and advisors. The study shows scope of improvement in the claims administration process. The policyholders experience depends on the coordination, timeliness and transparency in the process.

Recommendations: The claims settlement process needs to be made more customer centric and transparent. At the time of purchase of health insurance policy the policyholders should be explained about the disallowances, co-payments and deductibles. The hospitals and TPAs need to show more coordinated and targeted efforts towards documentation and communication in claims administration.

Relevance of the Study: Health insurance has become a dire necessity of the hour because of increase in the cost of medical care the medical expenses. The customers will purchase and renew policies if their needs are addressed. Results of the study will be helpful for insurance companies and TPAs operating in health insurance sector for improving their claims administration process and overall service quality.

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Ethical Clearance: IEC of SIU (Pune)

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A Study to Assess the Knowledge, Attitude and Practice about Management of Acute Exacerbation of Chronic Obstructive Pulmonary Disease amongst EMS Professionals in Pune, India

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Abstract

Background: Chronic Obstructive Pulmonary Disease (COPD) is one of the common, slow progressive disorder the respiratory system, considered an important cause of morbidity and mortality across the globe. Although being a chronic disability, COPD presents with acute exacerbations in the form of breathlessness and cough. Knowledge regarding about diagnosis of COPD, appropriate titration of Oxygen and effective management are essential to prevent mortality and morbidity due to COPD.

Objective: To assess the Knowledge, Attitude and Practice level about management of Acute Exacerbation of COPD among EMS Professionals.

Methodology: The study was conducted amongst 100 Emergency Medical Professionals in Pune, India. A previously tested and validated KAP questionnaire prepared by Sylvia Saade et al[2] was utilized for the study. The questionnaire was administered through online mode after obtaining informed consent from the respondents.

Discussion: The analysis of data shows EMS professionals are aware of the aetiology, pathophysiology and clinical features but require more training in management aspects of COPD. In terms of attitude towards COPD, broad agreement was found amongst respondents that COPD patients must quit smoking and passive smoking contributed to COPD. However, opinion amongst respondents with respect to recommendations regarding exercise was divided. In terms of Practices in COPD, broad consensus was found amongst the respondents on the usage and precautions regarding metered dose inhalers (MDI).

Conclusion: The study finding point towards a healthy degree of knowledge, attitude, and practice with regards to COPD amongst EMS professionals. Although the finding do red flag areas of improvement which can be focused upon in future training session. The study is limited by number of respondents yet attempts to provide a broad view on Knowledge, attitude and Practices in COPD.

Keywords: *COPD, Acute exacerbation, EMS professionals, Knowledge, Attitude and Practices.*

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Introduction

Chronic Obstructive Pulmonary Disease (COPD) is one of the common, slow progressive disorder of the respiratory system, considered an important cause of morbidity and mortality across the globe[1,2]. COPD is projected to be the third leading cause of death by the

year 2030 globally and the second leading cause of death after Road Traffic Accidents in India[3].

Although being a chronic disability, COPD presents with acute exacerbations in the form of breathlessness and cough. This can present as medical emergencies requiring hospitalizations and pose an increased risk of mortality[4].

In early phases of management of acute exacerbation of COPD, sufficient Oxygen must be administered to prevent hypoxemia, but if given in excess it can cause hypoventilation or dangerous hypercarbia. This can lead to fatality. A study by Michael. A. Austin *et al*[5] suggested that routine use of titrated Oxygen in prehospital setup for acute exacerbation of COPD significantly reduce mortality rate compared to high flow Oxygen therapy.

Emergency Medical Professionals are trained to administer Oxygen preemptively in Cardiovascular or Respiratory emergencies to ward off cardiacor respiratory failure, respectively. A study by Goktalay *T et al*[1] emphasized importance of training to the primary care providers for early detection, effective treatment and prevention of complications in case of COPD.

Hence, Knowledge regarding about diagnosis of COPD, appropriate titration of Oxygen and effective management are essential to prevent mortality and morbidity due to COPD.

Objective: To assess the Knowledge, Attitude and Practice about management of Acute Exacerbation of COPD amongst EMS Professionals.

Methodology

The study was conducted amongst 100 Emergency Medical Professionals in Pune, India. A previously tested and validated KAP questionnaire prepared by Sylvia Saade et al[2] was utilized for the study. The original 37 item questionnaire was meant for pharmacists. Only 23 items relevant to the Emergency Medical Professional were retained.

The twenty-three-item questionnaire comprised of three parameters –nine items testing Knowledge of which 4 items were open-ended questions and had to be answered within 25 to 30 words while the remaining 5 items were close ended requiring yes/no answers. Six items measuring Attitude and Eight items measuring Practice related to management of Acute Exacerbation of COPD required response in Likert scale method. All items were required to be mandatorily filled. The questionnaire was administered through online mode after obtaining informed consent from the respondents. The respondents were given two days to fill the questionnaire and revert. Any queries pertaining to questionnaire was clarified during data collection.

All 100 respondents reverted with completely filled questionnaire. The data was tabulated and statistically analyzed with the help of SPSS version 23.

Results

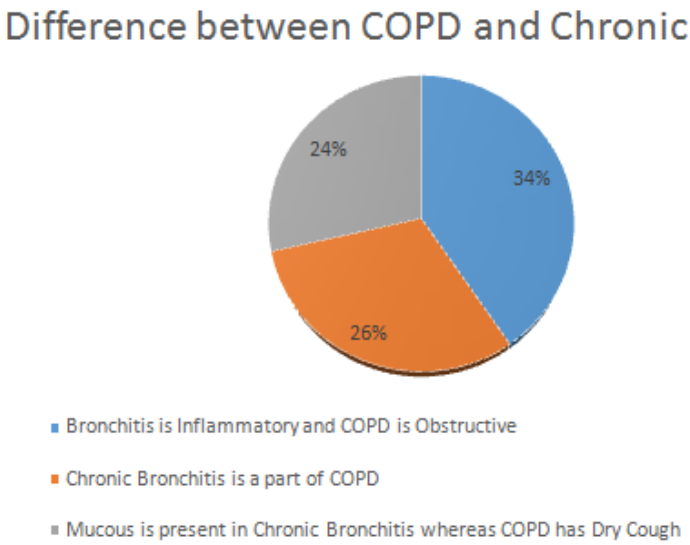


Figure 1

As shown in figure 1. and indicated about difference between COPD and Chronic Bronchitis, 34% of the respondents were of the opinion thatchronic bronchitis was inflammatory while COPD was of Obstructive Pathology.

26% respondents considered Chronic Bronchitis to be a subtype of COPD .

24% stated that COPD presented with dry cough while chronic bronchitis presented with mucoid cough.

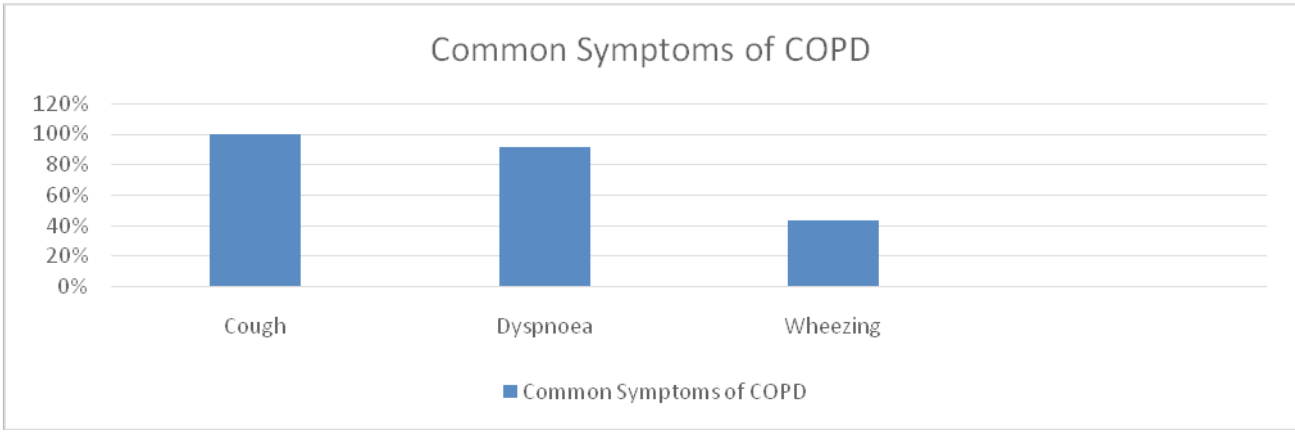


Figure 2.

As seen in figure 2. respondents stated cough, Dyspnea and Wheezing as common symptoms of COPD.

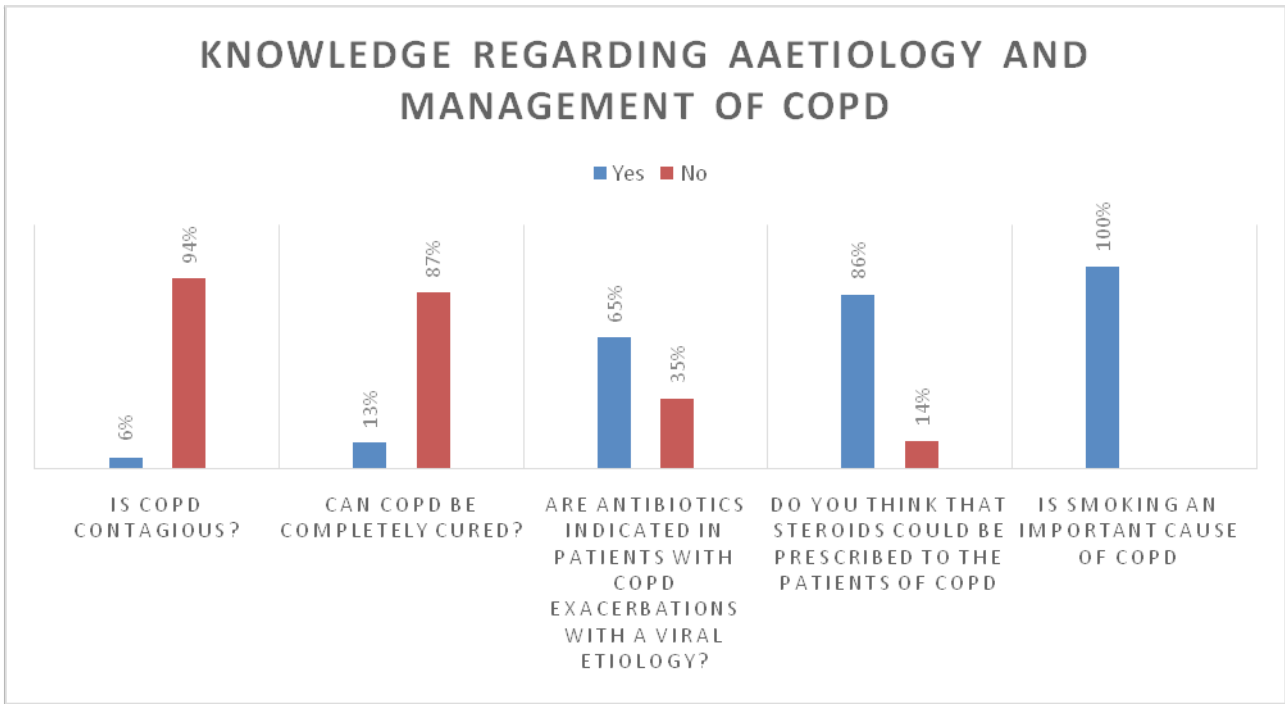


Figure 3.

As shown in figure 3. On the items pertaining to Knowledge regarding aaetiology and management of COPD, as many as 94% respondents felt COPD is not contagious while 87% respondents were of the opinion that COPD cannot be completely cured.

65% believed antibiotics are recommended in acute exacerbation of COPD due to viral aetiology while 86% felt that steroids could be prescribed to COPD patients.

All the respondents attributed smoking to be an important cause of COPD.

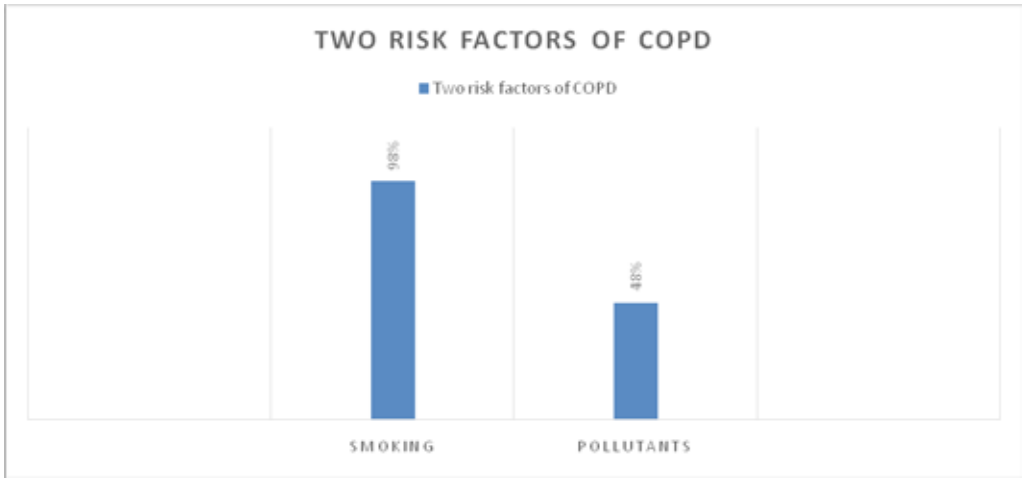


Figure 4.

As seen in figure 4, respondents stated smoking and pollutants as risk factors of COPD.

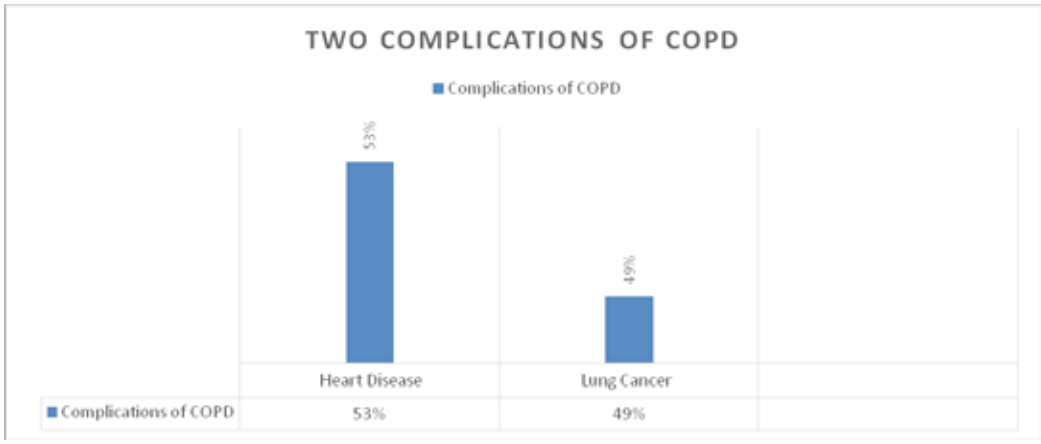


Figure 5.

As seen in figure 5, respondents stated the Heart disease and Lung cancer were common complications of COPD.

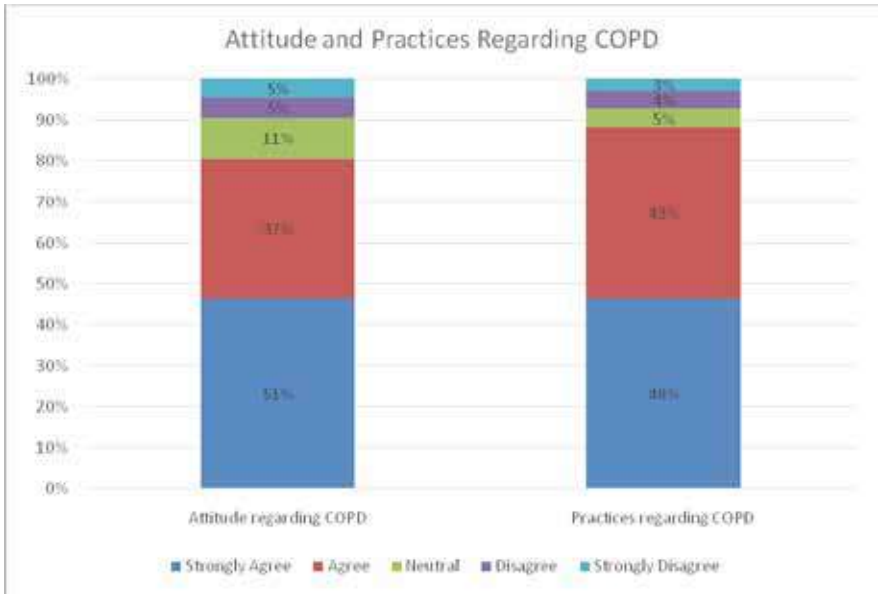


Figure 6.

As seen in figure 6. Broad agreement was found amongst respondents in parameters pertaining to attitude and practices regarding COPD.

Discussion

Most of the respondents were able to correctly differentiate between COPD and Chronic Bronchitis based on clinical picture. They were aware of the common symptoms of COPD. More than half of the respondents stated correctly that COPD is a non-communicable disease and has no permanent cure. All the respondents considered smoking to be an important cause and risk factor contributing to COPD. This shows that the respondents had a clear idea and in-depth knowledge regarding the aaetiology of COPD. In terms of Knowledge regarding management of COPD, most of the respondents correctly stated that steroids could be prescribed to COPD patients. However, more than half of the respondents incorrectly recommended antibiotics for acute exacerbation of COPD due to viral aaetiology. The analysis of data shows EMS professionals are aware of the aaetiology, pathophysiology and clinical features but require more training in management aspects of COPD.

In terms of attitude towards COPD, broad agreement was found amongst respondents that COPD patients must quit smoking and passive smoking contributed to COPD. Majority of the respondents agreed that inhalers and humidifiers are useful in acute exacerbation of COPD and there is requirement of long-term treatment in COPD patients. However, opinion amongst respondents with respect to recommendations regarding exercise was divided.

In terms of Practices in COPD, broad consensus was found amongst the respondents on the usage and precautions regarding metered dose inhalers (MDI). Majority of respondents believed that counselling the patient about worsening of situation and admission to tertiary care hospital during an exacerbation was essential.

Conclusion

The study finding point towards a healthy degree of knowledge, attitude, and practice with regards to COPD amongst EMS professionals. Although the finding do red flag areas of improvement which can be focused upon in future training session. The study is limited by number of respondents yet attempts to provide a broad view on Knowledge, Attitude and Practices in COPD.

Acknowledgement: We thank Sylvia Saade *etal* for granting permission to use the questionnaire for the research study.

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Ethical Clearance: Taken from IEC, SIU Committee.

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Effect of Facilitated Tucking on Physiological and Behavioural Responses to Pain among Infants Receiving Vaccination

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Abstract

Objective: To evaluate the effect of facilitated tucking on physiological and behavioural responses to pain among infants between experimental and control group before and after the IM vaccination

Method: A quantitative approach with true experimental design and consecutive sampling technique was used in this study. Researcher selected 100 infants up to the age of 6 month receiving IM vaccination in the immunization clinic, AIMS, Kochi, and they are randomly assigned to control and experimental group. Demographic and clinical data was collected using structured interview schedule. Heart rate and SPO2 was monitored 2 minutes prior to the vaccination and facilitated tucking was given to the experimental group before and during the immunization and routine position was given to the control group, after that behavioural response was assessed by using neonatal infant pain scale and physiological response such as heart rate and SPO2 was assessed after 5 minutes.

Result: The result showed that all the subjects in the control group had severe pain 100 %, whereas in the experimental group 50 % of the subjects had moderate pain and 50% of them had mild pain**.** Also there was a significant difference in the mean score of the physiological and behavioural response between the control and experimental group after the intervention

Conclusion: The study revealed that the Facilitated Tucking improves the emotional security and reduces the pain perception. The nurse or care givers can effectively implement facilitative tucking for reducing pain during vaccination in any setting as it is a very simple technique.

Keywords: *Facilitated tucking, behavioural response, physiological response.*

Introduction

Pain in newborns is a complicated, individualized, subjective, and universal finding[1]. The most common painful procedures performed during infancy are routine injections without pain management[2]. Vaccinations are first administered when babies are very young. An immunological product used to confer active immunity to a person against a particular disease is called vaccination. The process of producing artificial immunity in a subject is called immunisation[3]. Studies have shown that uncontrolled pain experienced during the early stages of life has negative and long-term side effects, such as distress, and that such pain negatively affects the development of the central nervous system[4].

The most important problem encountered while evaluating pain in newborns is the inability of babies to express pain verbally. Physiological parameters, behavioural method, and stress hormones have been evaluated to define the pain felt by newborns[5]. Pain experienced by newborns affects the heart rate, respiration rate, blood pressure, and tissue oxygenation,

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potentially causing these parameters to decrease or increase [6].

Pharmacological method used to relieve pain in newborns are reported to have side effects . Non-pharmacological and pharmacological method are considered for pain relief by health stuff [7]. One method of non-pharmacological method is the facilitated tucking; it is defined as "a sub-form of method of nesting the baby and the procedure of bringing the body to middle or even close position by holding the upper and lower extremities of the baby in flexion with hands." An infant can be held in the lateral, supine, or prone position while this method is performed [8]. It has been reported to prompt infants own regulatory systems, prevent painful stimulants coming from the outside world, and reduce the pain felt by the infant by enabling heat and touching stimuli [9]. In addition, this method stabilizes infants physiological parameters and helps them gain a feeling of safety based on the position, supports their motor development, and preserves their energy [10].

A semi–experimental study was conducted on the effect of Facilitated Tucking (FT) during Venipuncture on duration of crying in preterm infants. In this study, 70 preterm infants born at average 32 to 36 weeks' Gestational age (GA), who needed routine blood collection, were allocated to two intervention (35 neonates) and control (35 neonates) groups. In experimental group a nurse gave facilitated tucking to the infant. The results showed that the duration of crying after sampling in the two groups was statistically significant difference, this duration in control group was higher than the intervention group (P<0.05)The results of this study indicate that the facilitated tucking is effective in reducing the duration of crying infant.[11]

The primary responsibility of a nurse is to ensure that pain-relieving method are performed before and after a procedure during routine practices in addition to determining and relieving the pain. The purpose of this study was to evaluate the pain perceptions of infants during vaccinations performed in the facilitated tucking position and the classical holding position respectively.

Method

The present study used the quantitative approach with true experimental design and consecutive sampling technique. After obtaining the ethical committee clearance from the institution and informed consent by the mothers researcher selected 100 infants up to the age of 6 month receiving IM vaccination in the immunization clinic, AIMS, Kochi, and they are randomly assigned to control and experimental group . Demographic and clinical data was collected using structured interview schedule. Heart rate and SPO2 was monitored 2 minutes prior to the vaccination and facilitated tucking was given to the experimental group before and during the immunization and routine position was given to the control group, after that behavioural response was assessed by using neonatal infant pain scale and physiological response such as heart rate and SPO2 was assessed after 5 minutes.

Results

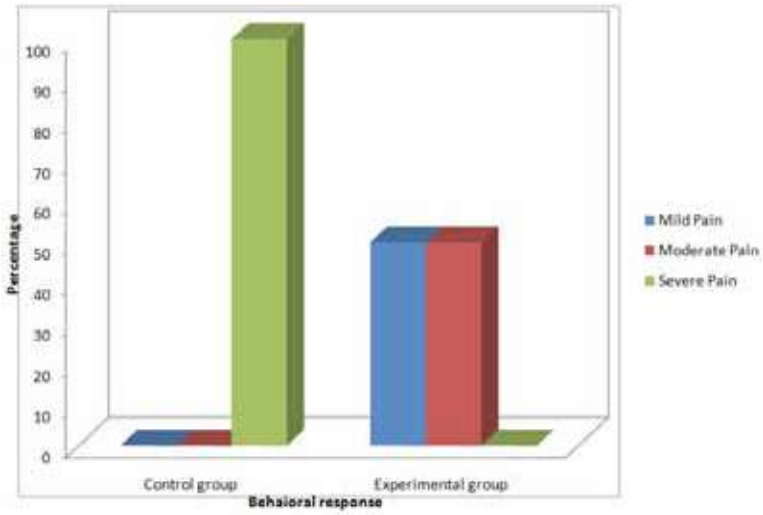


Fig 1: Distribution of subjects based on their behavioural response

The data depicts in the figure (1) shows that all the subjects in the control group had severe pain 100 %, whereas in the experimental group 50 % of the subjects had moderate pain and 50% of them had mild pain**.**

Table 1: Mean and standard deviation of physiological respons

	Control Group				Experimental Group			
	Min.	**Max.**	**Mean**	**Sd**	**Min.**	**Max.**	**Mean**	**Sd**
Heart rate before intervention	102	180	143.36	16.219	103	188	133.28	18.449
Heart rate after intervention	125	209	163.14	19.960	112	190	143.86	18.707
Oxygen saturation before intervention	97	100	99.82	.629	96	100	99.60	.857
Oxygen saturation after intervention	95	100	97.76	.938	97	100	99.16	.912

Table (2) shows that mean heart rate and SPO2 before and after the intervention in the control group were 143.36 +/-16.219, 163.14 +/-19.960, 99.82 +/--.629, 87.76 +/- .938, and the experimental group were 133.28 +/--18.449, 143.86 +/- 18.707, 99.60 +/- 0.857, 99.16 +/- 0.912 respectively .

Table 2 : Mean and standard deviation of behavioural response

	Control Group				Experimental Group			
	Min.	**Max.**	**Mean**	**Sd**	**Min.**	**Max.**	**Mean**	**Sd**
Behavioral response	6	7	6.92	0.274	6	7	6.92	0.274

The mean and standard deviation of behavioural response in the control and experimental group were 6.92 + 0.274, 6.92 + 0.274 respectively.

Table 3: Effectiveness of facilitated tucking on behavioural response

Behavioral response	**t –value**	**Df**	**p value**
Control group	-29.190	98	.0001**
Experimental group			

**: Highly significant

The above table (4) reveals that there was a significant difference in the mean score of the behavioural response between the control and experimental group after the intervention.

Table 4: Effectiveness of facilitated tucking on physiological response

Physiological response	**t -value**	**df**	**P value**
Heart rate	-4.983	98	.0001**
SPO2	7.568	98	.000**

** : Highly significant

The above table (5) reveals that there was a significant difference in the mean score of the physiological response between the control and experimental group after the intervention

Table 5 : Correlation between the behavioural and physiological response

Variable	**r value**	**p value**
Heart rate	0.489	0.001
Behavioral response		
SPO2	-0.630	0.001
Behavioral response		

Table (6) shows that there is a positive correlation between heart rate and behavioral response (r value is 0.489, p value is < 0.001 and there is negative correlation exits between SPO2 and behavioral response (r value is -0.630, p value is < 0.001)

Table 6: Association between physiological response with selected demographic variables

	Value	df	Level of significance
Age of the child	16.964	2	.0001**
Birth weight of the child	4.010	4	.405
Type of vaccine	18.721	2	.0001**
Order of vaccine	12.176	4	.016 *

*: Significant, **; Highly significant

The above table (7) reveals that there is a significant association between the physiological response and selected demographic and clinical variables such as age of the child, type of vaccine, order of vaccine.

Discussion

Facilitated Tucking is one of the simplest non pharmacological and cost effective techniques simulating the condition of being in uterus. This makes the infant comfortable, more secure with controlled response. It facilitates self-regulation by decreasing the physiologic response like prolonged heart rate elevation that contributes to the disequilibrium associated with pain and stress.

A study was conducted on a cohort of preterm infants (n = 42), divided into control (n = 21) and treatment (n = 21) groups, to determine the effect of facilitated tucking on pain relief during venipuncture on preterm infants in the neonatal intensive care unit. The severity of pain was measured using the Premature Infant Pain Profile score. The primary outcome measure was reduction in the Premature Infant Pain Profile scores. The Premature Infant Pain Profile score for the treatment group was significantly lower (M = 6·62, SD 2·598) than for the control group (6·62 ± 2·60, 8·52 ± 2·99 respectively, t = −2·202, p < 0·05).[12]

In the present study, the result shows that all the subjects in the control group had severe pain 100 %, whereas in the experimental group 50 % of the subjects had moderate pain and 50% of them had mild pain**.** Also there was a significant difference in the mean score of the physiological and behavioural response between the control and experimental group after the intervention

The above two studies shows that the facilitating tucking is very effective way of non –pharmacological method for reducing pain in infants. Therefore, the health care professionals must identify the distress and discomfort shown by babies to routine immunization, regarding pain perception, assessment and management in infants.

Conclusion

Facilitated Tucking improves the emotional security and reduces the pain perception. The nurse or care givers can effectively implement facilitative tucking for reducing pain during vaccination in any setting as it is a very simple technique. This motivated the investigator to take up an iniativeness to teach the health care professionals to practice facilitated tucking while giving vaccination. In this study the investigator aims to assess the effect of facilitated tucking on physiological and behavioural responses of neonates undergoing IM vaccination and it was concluded that the non-pharmacological method used was very effective

Financial Support and Sponsorship: Nil

Conflicts of Interest: There is no conflict of interest

Ethical Clearance: Approval was sought from the ethical review committee of AIMS, Kochi. Written informed consent was obtained from the entire participant prior to the study. Needed steps were taken up to maintain the confidentiality while doing data collection.

Authors' Contribution: Author has substantially contributed for the study from the conception of the problem, data collection, analysis and drafting the manuscript.

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A Cross-Sectional Study to Estimate the Presence of Anxiety in Injured Cricketers

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Abstract

Purpose: State anxiety is an unpleasant psychological state experienced by cricketers due to perceived performance stress. It is often an overlooked criterion for return to sports after an injury. There is a need to evaluate presence of anxiety as it leads to physical symptoms and hence injury. Mental training is a very important tool for handling anxiety but there is lack of training which further predisposes players to re-injury. This study aims to evaluate presence of anxiety in cricketers who have suffered sport-related injury and have returned to play. It also explores the method used by these cricketers to handle anxiety.

Method: 60 injured cricketers were selected based on inclusion and exclusion criteria. A baseline questionnaire was prepared to collect basic demographic data and Sports Anxiety Questionnaire (SCAT) which is a self-administered scale was included to determine presence of anxiety. Anxiety scores were calculated to determine the players level of anxiety.

Results: It was found that 65% players experience high anxiety, 32% had average anxiety and 3% had low anxiety levels. 45% of players reported re-injury.

Conclusion: Undiagnosed anxiety was found to exist in injured cricketers which may predispose them to re-injury. All the players were aware of mental training techniques but reported lack of guidance and professional help.

Keywords: *Anxiety, SCAT, cricket, injury, mental skills training, return to sport.*

Introduction

A sports injury has both physical and psychological consequences for the athlete. It can be viewed as part of a continuum where psychological factors are known to predispose an athlete to physical injury and physical injury can, in turn, lead to deteriorating psychological reactions. Psychological factors have been shown to be an important antecedent to the onset of athletic injuries and also play an important role in injury rehabilitation and ultimately successful return-to-play.[1] Evidence suggests that competitive athletes returning to sport following injury rehabilitation may experience a range of psychosocial concerns, like anxieties associated with re-injury, concerns about an inability to perform to pre-injury standards, feelings of isolation, a lack of athletic identity etc. These may adversely affect their return to sports performance.

Anxiety is an unpleasant psychological state experienced by cricketers due to perceived performance stress[1]. Cricket is a popular game in India wherein the demands of the game is more mental than physical. On one hand, it requires speed and precision and on the other hand, it requires strategic planning, focus and concentration. There is ample evidence suggesting the

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positive effects of anxiety on sporting performance in terms of physiological and physical readiness of the athlete. Smith and Smoll's proposed a conceptual model stating that when faced with a competitive sporting situation, an individual will make cognitive appraisals of the situation. These cognitive appraisals have a reciprocal relationship with physiological arousal. Hence anxiety, which causes a positive physiological arousal can turn into a negative outcome, if it remains unchecked.[2]

The concept of sports performance traditionally included participation in activities related to regular practice and competition; however, more recently, this has also included performance in activities that relate to sport injury prevention, rehabilitation, and the return to sport process[1,3]. To date, research has focused extensively on the role of anxiety in sports performance, to greater extent in injury occurrence and to some extent on injury rehabilitation. There is scarcity of studies on the presence of anxiety and its role in return to sports.

The deteriorating psychological responses are generally greatest immediately after sports injury and lessens during the rehabilitation process. However, they often rebound prior to return to sports, consistent with a U- pattern of recovery. If unaddressed and unresolved, these elevated psychological responses to the injury can slow rehabilitation progress and delay return to sports.[4]

After an injury, emphasis is often placed on the physical ability as a criterion to return to sport and not the psychological component. Psychosocial readiness is one criterion for return-to-play and it is essential the team physician understand that physical clearance to return-to-play may not correlate to psychological readiness. It is desirable that the team physician coordinate the athletic care network to monitor the psychological readiness of athletes who are preparing to return-to-play or have returned-to-play and coordinate psychological support services as needed.[3] Some of the most commonly used psychosocial interventions practiced by sportsmen include goal setting, imagery, relaxation strategies, and positive self-talk. Mental training is a very important tool for handling anxiety but there is lack of training for its correct application, which further predisposes players to re-injury.

With the above background in mind, there is a strong necessity to evaluate the presence of anxiety by means of easy clinical measures or pre-participation screening after an injury. There is a need to incorporate psychological readiness as a return to sport criteria. The need for our study was to find out presence and degree of anxiety in injured cricketers and the strategies implemented to handle their anxiety.

Methodology

The STROBE guidelines for reporting cross-sectional study was followed in writing this article. The study is a descriptive cross-sectional design. The objective of this study was to specifically identify anxiety in cricketers who have sustained an injury or injuries related to their sport. A minimum sample size of 60 was calculated. With regard to the primary aim of the study, sample size calculation indicated that at least 60 participants were needed to detect with a precision of 10% that 1 out of 20 cricketers suffer from a sports injury (power of 80%; confidence interval of 95%).

Independent Variables:

Injury in cricketers: Research has indicated that muscle injuries such as hamstring strains, groin strains and low back pain are the most common cricket injuries. On average, around 9% of cricketers have an injury at any given time, although in fast bowlers over 15% are injured at any given time. These injuries are due to the repetitive functional demands of the sport like sprinting and ball throwing

While injury incidence of these injuries has been largely studied in professional cricket, knowledge about the mental health among professional cricketers in general and specific to the impact of injury is still lacking.

Dependent variable:

Anxiety: Anxiety is one of the conditions seen as part of the common mental disorders seen in high performance athletes. In our study Sports Competitive Anxiety Test was used to measure levels of anxiety in the injured players. This scale was developed by Martens in 1977 and it aimed at measuring situation specific (competitive) trait anxiety. Trait anxiety predisposes one to perceive certain situations as threatening and to respond to these situations with varying level of state anxiety. Thereby implying that individuals with high trait anxiety tend to translate that anxiety into state anxiety. The SCAT is a self-administered Likert scale which contains 15 items, 10 of which measure symptoms associated with anxiety, with five others that

are not scored were included to reduce the likelihood of an internal response-set bias. The scores for the 10 items are summed to provide an overall measure, with a high score reflecting a greater tendency to experience competitive anxiety. A score of less than 17 indicates a low level of anxiety, 17 to 24 an average level of anxiety, and more than 24 a high level of anxiety.[9,10.]

Re-injury: It has been extensively suggested by researchers that anxiety leads to cognitive and somatic changes. These physiological changes hamper one's ability to focus and increases muscular tension.[1,12] This predisposes the cricketer to further injury. In our study, the cricketers were asked to self-report any injury or injuries experienced after the primary injury.

Procedure: Sixty participants were approached based on the selection criteria as semi-professional cricketers, having injury while playing cricket or during practice sessions, age group of 20-40 years and exclusion criteria of injury unrelated to the game. An electronic based form was administered to them which collected baseline information on demographic data, position in the game, injury related information, areas of injury, re-injury after the primary injury, mental training practices along with SCAT, which is a self-administered scale. It had instructions to answer how one feels when competing in sports. Out of 60, 12 participants did not respond even after verbal consent to participate in the study. We received 48 responses out of which 5 responses were above the age criteria as calculated by their date of birth and 3 responses had an injury about 8-9 years back. These responses were not taken into account as outliers in the study. Thesecricketers played 3 hours ± 1.8 on an average for 4 days a week ± 2, mean age of 28 years ±4.5, playing cricket since 10 years. They reported having injury since mean 4.5 years ±2.8. According to their position in the game, 45% were all-rounders, 22% batsman, 20% bowlers and 12% wicket-keeper. Descriptive statistics in the form of mean, median and standard deviation was used to calculate the results of this study.

Table 1: Baseline Characteristics

Parameter	Mean	SD
Age	28	4.5
Practice hours	3	1.8
Practice days/week	4	2
No. of years of playing	10	4

Results

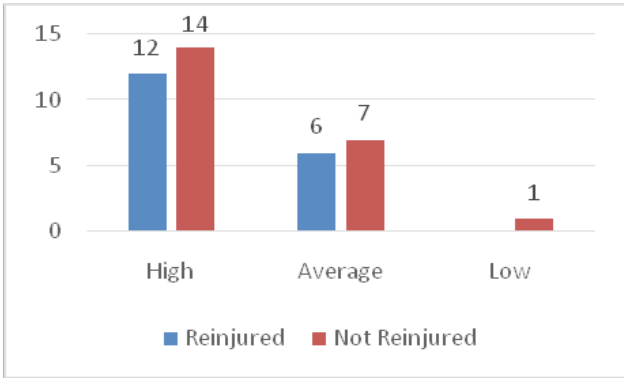
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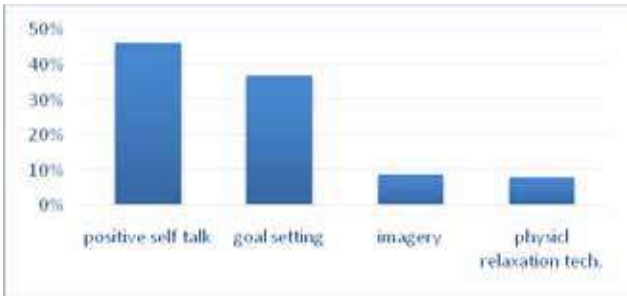
Graph 1 inference: 65% players-high anxiety, 32% players-average anxiety and 3%-low anxiety



Graph 2 inference: 45% of the population had re-injury and 55% did not suffer any re-injury



Graph 3 inference: Rate of re-injury incricketers suffering from high anxiety and moderate anxieties.



Inference: Commonly practiced mental training techniques.

Discussion

The findings of our study suggest that there was presence of anxiety in almost all players, with 65% (27±1.7) representing high level of anxiety and 32% representing average level of anxiety (22±2.36) Findings of similar study done in footballers suggests that the prevalence of mental health complaints ranged from 5% (burnout) to 26% (anxiety/depression) in 149 current players and from 16% (burnout) to 39% (anxiety/depression) in 104 former footballers.[13] Studies also suggest that injured players had higher scores for depression and anxiety than uninjured players, as previously described.[14] SCAT is a tool to measure trait anxiety which is an innate and relatively stable part of one's personality.[5,8,9] Individuals who have high level of trait anxiety have a tendency to perceive situations as potentially threatening and they consequently tend to experience more state anxiety. Evidence appears to affirm the role of not only trait anxiety but, other psychosocial factors such as stress, state anxiety, and daily hassles as potentially contributing to injury.[7] The SCAT scale has known to demonstrate a strong reliability (0.77–0.95).[5]

In our sample, we found out most common areas of injury were shoulder (28%), knee (21%), back (19%), wrist (16%), ankle (13%) and foot (2%). Since the sample size was very small, there was inconclusive results to link the of site of injury to the position of the players in the game of cricket. Maximum players were all-rounders.

Re-injury was reported in 45% of the players. This finding is supported by Podlog and Eklund who stated that re-injury anxiety during the return to sport phase can be detrimental for an athlete as it can lead to an increased risk of re-injury or secondary injury. Maximum players who had re-injury were found to have high anxiety levels. It is an established fact that high anxiety equates to high arousal. The spiraling effects of uncontrolled arousal are well documented as feelings of stress, unease, and worry that narrows the visual field, increase muscle tension, and increase distractibility, which in turn would subsequently disrupt the feed-forward and feed-back neuromuscular control required throughout athletic participation, leading to injury. it has been stated that reinjury anxiety, as compared to fear of reinjury, is more of a negative thought or worry of the consequences of injury (eg, additional surgery and more time in rehabilitation) while fear of reinjury is specific to fear of the injury itself. As there is no certainty about the extent of reinjury risk and situations, athletes possibly have more reinjury anxiety as opposed to fear of reinjury or these can occur together.[12] According to a study carried out at the IAAF World Championships in Athletics 2015, the anxiety experienced by elite athletes over illness symptoms is linked to the risk of being injured during competition and should be taken seriously.

An interesting finding of our study was the awareness and usage of mental training techniques. The cricketers reported using mental training to cope with anxiety, however it was not under guidance of a professional. The most used technique followed by our sample was positive self-talk and goal-setting. This highlights another lacuna in the domain of psychosocial rehabilitation of injured cricketers and as clinicians we should make efforts to work with sport psychology professionals in understanding and implementing techniques to improve athletes' coping mechanisms with the goal of decreasing anxiety.

Limitations and strengths of the study: There were drop-outs in our study leading to a smaller sample size. Additional samples could not be collected as this project was part of a time-bound internship completion for one of the authors.

Our sample consisted of club cricket/ semi-professional players; hence generalization of the results cannot be made, especially for elite cricketers. Elite cricketers would have better coping mechanisms and more awareness about the implementation of mental training strategies as compared to our sample.

The scale used to measure anxiety (SCAT) is a very old version. There are several new scales available but the author of this study has gone through the work of Martens (1977) and was convinced that this scale had the robustness to measure trait anxiety leading to state anxiety, thereby dealing with both components of anxiety. SCAT is also a Likert scale which accurately gives the level or degree of agreement on an item that is being measured. Hence more definitive responses are achieved. It justified the objective of our study which was the determine the presence and level of anxiety in the players.

The dependent variable of re-injury was self-reported by the players and we did not measure the intensity of either the primary injury nor the re-injury. This maybe a confounding variable in the interaction between anxiety and re-injury.

Our study implicit anxiety as one of the factors that may have contributed to re-injury. The confounding variable of physical strength, as criteria to return to sport, was not explored in our study. However, our baseline questions included use of physiotherapy rehabilitation services after injury to which the mean responses were positive.

Also, due to limitation of sample size, there was inconclusive findings on level of anxiety in different types of players in the game of cricket. Future research can be directed towards exploring the level of anxiety in bowlers, batsmen, fielders and wicket-keepers.

Conclusion

Undiagnosed anxiety was found to exist in injured semi-professional cricketers which may predispose them to re-injury. All the players were aware of mental training but reported lack of proper guidance and professional help.

Implications: The findings of this study can help physiotherapists to identify anxiety as a potential risk for re-injury. It also highlights the importance of mental/ psychological rehabilitation along with physical rehabilitation.

Conflict of Interest: There is no conflict of interest

Source of Funding: Self

Ethical Clearance: The study was undertaken after due clearance from the institutional ethics committee. Informed consents were taken from the subjects before data collection.

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Incomplete Kawasaki Disease: A Case Report

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Abstract

Vasculitis where underlying cause is not known is often related as Kawasaki Disease. It's important to know about such auto immune conditions as it directly affects the vessels of the heart and is responsible for death at an early age. The peculiarity of the disease is its characteristic oral manifestations which are often misdiagnosed as an oral disorder. Dental examination has a vital role in improving the diagnosis of auto immune conditions as auto immune conditions primarily manifest oral signs in their early stage. So, early diagnosis can drastically improve the treatment outcome of the disease and quality of life.

Keywords: *Kawasaki, vasculitis, Z score, auto immune.*

Introduction

Acute diffuse vasculitis without underlying etiology targeting mainly infants and young kids is known as Kawasaki Disease or Mucocutaneous Lymphnode Syndrome[1] and appearance of partial signs and symptoms is known as Incomplete Kawasaki Disease.[2]

The disease is not specific to any race but usually Asians are comparatively at a higher risk of getting affected.[3] By year 1990 only 3 published reports of Kawasaki disease existed in India but the number increased to 161 publications in span of twenty years.[3] First two case series in India were reported from Thiruvanthapuram and Chandigarh.[3] Incidence of 0.51 per 1,00,000 children under 15 years of age increased to 4.5 per 1,00,000 children under 15 in 2007, as reported by hospital based study in Chandigarh.[3] Till date an increased incidence rate of KD is seen in Northeast Asian and South Central Asian countries including India. As mentioned incidence has increased from 0.51 to 4.5 per 1,00,000 children under 15 by 2007. The uniqueness of the disease is that it is still often misdiagnosed and due to oral manifestations either the patient continues to receive treatment from a dental professional or due to persistent fever patient receives treatment for the commonly prevalent bacterial or viral infections. The most common reason for this has been identified is the lack of awareness about the disease.[2]

Kawasaki is often misdiagnosed with common febrile illness, viral or bacterial infections. The oral manifestations of the disease like bleeding gums, inflamed oral mucosa, coated tongue etc. Also often misleads the diagnosis. Lack of appropriate treatment can even lead to death.

Case History:

Patient's History: A 3 year old baby girl patient presented with the history of high grade fever recorded maximum of upto 104°F accompanied with chills. The oral acceptance of food was compromised and decreased urine output. There was a history of cold and cough and chronic recurrent tonsillitis, redness of oral mucosa and tongue with black coated appearance and inflammed papillas and Enlarged Tonsils **(Figure 1, 2 and 3).** Warm saline rinses, oral prophylaxis and multivitamins were started on consultation with a dental specialist and ENT. Patient was given Paracetamol, Augmentin syrup

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and cough syrup at home for 8 days but, there was no improvement.

Clinical Manifestations (Table 1): Tongue had a characteristic strawberry appearance. Throat was congested with conducted sounds from the chest and abdomen was soft on examination. Bilateral cervical lymphadenopathy of size 1.5 * 1.5 cm and Bilateral tonsillar enlargement was present.

Diagnosis and Treatment: Patient was diagnosed with fever of unknown origin. Immediate hospitalization of patient was done and I/V monocef, amikacin and rantac was given. Patient was unresponsive and no improvement was observed so 2-D Echo, X-Ray chest, Ultrasound of Abdomen and Blood Investigations comprising of Fever Panel 3 was advised for the patient **(Table 2 and 3)** Echo revealed the Z score of 2.18 and other blood investigations were suggestive of Incomplete Kawasaki Disease. There was no history of conjunctivitis, skin peeling, burning micturation and loose stools.

Immediately after diagnosis immunoglobulin G I/V, 2gms/kg,over 24 hours for a period of four days under hemodynamic monitoring was administered to the patient and tablet aspirin 30 mg/kg/day was given orally.

Treatment Outcome, Evaluation and Follow up: Child well tolerated the infusion and initial investigations post IVIG revealed raised ESR (102 mm in 1st hour), serum prolactin 1.96 ng/ml, Hb was 8.1g/dl, platelet was 6.38 lakhs and CRP was raised to 70mg/L. Liver and Renal functions were normal. Repeat ECHO was done which was normal. There were episodes of fever and 3 episodes of vomiting post I/V IG but it subsided with supportive management. Post IG therapy patient was kept on 75 mg Aspirin half tablet for 6 weeks OD. Patient was on constant follow up for the period of 6 months in which blood levels and an echo finding appeared to be normal and after that patient was advised for a review yearly with the basic blood test and 2-D echo.

Discussion

Kawasaki was described for the first time in the year 1967 by Tomisaku Kawasaki in the Japanese literature and in the year 1974 in the English literature.[4] He published a report of 50 patients and since then it has been one of the commonest factor for heart disease in Japan and North America.[5] In Asian continent the incidence of Japan, South Korea and Taiwan has been maximum.[6] In Japan the incidence of the disease was stated to be around 239 cases in 1,00,000 children under the age of 5 years.[2] Incidence of KD is also on rise in the Indian subcontinent as now more number of cases is reported in the literature.[7]

The underlying etiology of Kawasaki is still not clear but often individuals with compromised immunity become the target. Some of the most common organisms that are usually considered to originate the infection in such individuals are parvovirus, staphylococcus aureus, EB virus and mycobacteria.[1] Recent data correlates the severity of the disease with genetic predisposition. Superantigens like toxic shock syndrome is found to be the most suspected cause.[4]

The diagnosis of the disease is based on the characteristic history, physical findings followed by specific diagnosis kit. Raised inflammatory markers like ESR, CRP, neutrophil count are usually associated with the acute phase of the disease. Differential diagnosis of KD can be measles, scarlet fever, CMV, EBV etc.

Disease course is divided into three phases:[1]

1. Acute Febrile Phase: it exists for 7 to 14 days and ends with the resolution of fever. Conjunctivitis, erythematic changes in the oral cavity and extremities, rashes and cervical lymphadenopathy.
2. Subacute Phase: Time span from resolution of fever till end of 25 days. Desquamation of fingers and toes is observed, arthritis and thrombocytosis.
3. Convalescent Phase: clinical symptoms begin to disappear and ESR becomes normal.

In Classical case of KD fever persists for a minimum period of 5 days along with characteristic features of different phases. Cardiovascular manifestations mainly coronary aneurysms can be prominent in the acute phase and if untreated can be the leading cause of mortality. If the clinical criteria of the disease are not complete than Kawasaki is referred as Incomplete Kawasaki. The patient was categorised as the case of Incomplete Kawasaki as the changes in Z score were there but not that significant and the clinical features were also partially visible like strawberry tongue, cervical lymphadenopathy, rashes and fever. But incomplete Kawasaki should not be considered as mild form of KD.

Kawasaki has another important clinical sign usually seen in babies less than a year, reaction at BCG vaccination site but is not included in the classical

clinical criteria of KD.[8] Any redness or crust formation on site of inoculation should never be ignored as it can be suggestive of KD and should always be considered as the differential diagnosis of any febrile illness of unknown etiology or vasculitis.[8] American heart association suggests that children with fever not subsiding for 5 days or more, elevated CRP and ESR levels should go for Echocardiographic findings to rule out KD.[9]

The management of Kawasaki includes I/V immunoglobulin G and aspirin followed by periodic check up and repeated echocaridography. Standard dose of IVIG is 2g/kg of body weight in first 10 days along with high oral dose of aspirin around 80 to 100 mg/kg/day divided in different doses.[2]

Table 1: Physical Examination Findings

Signs	Status
General Examination	
Consciousness	Maintained
Orientation	Well Oriented
Genital Region	Rashes around the genital region were present.
Oral and Head & Neck Examination	
Oedema	Slight Oedema was observed in the lower extremities
Cervical Lymphadenopathy	Present, Bilateral, 1.5*1.5 cm
Tongue	Red Tongue with Black Centralised Coating and Inflammed Prominent Papillae.
Tonsills	Bilateral Enlargement
CVS	Heart Sounds Normal No Murmurs

Table 2: Laboratory parameters of Patient

	20.06.2018 (On day of Admission)	23.6.2018 (Post IVIG)	04/07/2018 (Follow Up)	7.08.2018 (Follow Up)
Hb (g/dl)	8.3	8.1	9.10	10.7
TLC (thous/ul)	14.86	10.65		11.61
DLC	62/25	33/57	28/58	40/50
PLATELET(thous/ul)	542	638	627	418
ESR(mm/1st hour)	80	102	60	08
CRP (mg/l)	89.2		0.70	0.52 (< 5)
CREATININE (mg/dl)	0.38	0.28	0.32	--
SGOT (IU/L)	19	27	31	30
SGPT (IU/L)	23	13	15	13
ALBUMIN(gm/dl)	2.6	2.88	3.64	4.63
GLOBALIN(gm/dl)	4.7	4.42	--	--
A:G RATIO	0.6	0.65	--	--
Bicarbonate	--	18.5	--	--
HLAb27 (PCR)	Negative	--	--	--
URINE Examination	10-15 WBCs Proteins Present	--	--	--
THROAT SWAB		--	--	--
Blood Culture	Negative	--	--	--
Typhi Igm	Negative	--	--	--
Urine Microscopic	WBCs 10-15, protein +	--	--	--
Throat Swab	Candida Kefyr+ve	--	--	--

Table 3: Z Score

Date	LMCA	LAD	LCX	RCA
20.06.2018 (Kobayashi Score)	3mm (Z score = + 2.18)	2.3mm (Z score = + 1.78)	1.4 mm (Z score = - 0.5)	2.1 mm (Z score = + 0.50)
25.06.2018 (Boston Score)	3mm (Z score = + 1.69)	2.45 mm (Z score = + 1.91)		2.5 mm (Z score = + 1.7)
06.07.2018 (Boston Score)	3.1mm (Z score = + 1.74)	2.3 mm (Z score = + 1.4)		2.3 mm (Z score = + 1.17)
23.11.2018 (Kobayashi Score)	2.3mm (Z score = + 0.26)	1.9 mm (Z score = + 0.23)	1.4 mm (Z score = -0.7)	2mm (Z score = + 0.25)



Figure 1: Erythematous changes observed on the tonsils.



Figure 2: Erythematous changes observed on the palate (Red Petechiae present on Soft Palate).



Figure 3: Blackish Discoloration of Tongue

Conclusion

The presentation of this case is with the motive to raise awareness about the fact that self medication can sometime prove to be fatal and conditions presenting with characteristic features should not be ignored. Oral health opens the door to systemic health so oral manifestations should be dealt carefully.

Ethical Clearance: Taken from IEC (Institutional Ethical Committee) of School of Dental Sciences, Sharda University.

Conflict of Interest: Nil

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The Prevalence and Structure of Arterial Hypertension and the Role of Apolipoproteins in its Development

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Abstract

The analysis of the inspection of the employees of Uzagrotehmash JSC was performed. A total of 1495 people aged 25-75 years have been examined (average age 46.3±0.3 years). The frequency of hypertension was 598 people. High blood pressure was recorded more often in women (25.9%) than in men (16%), hypertension is much more common at the age of 44-60 years and older, and hypertension has been found to be common in overweight people (31, 6%), a large proportion was found in the structure of hypertensive patients with hypodynamia (50.2%). Among the protesters, 26.9% of people know about high blood pressure, but do not treat it, and in 20.0%, high blood pressure was detected for the first time. The role of serum lipoproteins in the hypertension development was confirmed, which allows patients to be assigned to a high-risk group for the formation of cardiovascular diseases in subsequent life periods.

Keywords: *Blood pressure, risk factors, hypertension, dyslipidemia, prevention, risk, apolipoproteins A1 and B.*

Introduction

Arterial hypertension is a serious public health problem due to a further increase in its prevalence, as well as a lack of adequate control[1]. The prevalence of arterial hypertension in economically developed countries is 20-50% and is one of the main factors of disability and mortality in the population[2]. At the same time, the continuing increase in the incidence rate and the defeat of people of an increasingly young age makes cardiovascular diseases a major medical and social problem.[3]

Arterial hypertension leads to myocardial infarction and stroke, hear and renal failure[4]. Blood pressure at the preclinical stage in most cases is increased moderately, which is accidentally detected during preventive examinations[5].

Official statistics in assessing the disease prevalence are based specifically on the reversibility analysis, when the fact of the disease has already happened, and preventive measures are not so effective[6]. The registered incidence rate in the adult population is 2.3 times lower than the actual prevalence of this pathology. Thus, the appealability analysis does not allow us to evaluate the entire scope of the problem facing practical healthcare[7]. The most complete picture of the disease prevalence, its structure, severity can be given by epidemiological studies, and new preventive technologies development suitable for a specific age group can be realized only by analyzing a real existing epidemiological situation[8].

The situation is complicated by inadequate hypertension control in the population. At the same time, it is known that effective control of high blood

pressure reduces cardiovascular morbidity and mortality in patients with arterial hypertension. However, other factors influence the prognosis of the disease, which is reflected in the classification of arterial hypertension[6].

Epidemiological studies conducted in various countries showed the presence of ethnic characteristics in the prevalence of cardiovascular diseases[1]. The presence of these features, in addition to lifestyle, culture, environmental factors, etc., is determined, among other things, by genetics, whose role in the formation of cardiovascular pathology has been convincingly proved[4]. An important place in the development of cardiovascular diseases is occupied by overweight and metabolic disorders associated with it, smoking, stress, lack of exercise[2,5].

According to statistics, in Uzbekistan, among the causative factors of disability and mortality are diseases that occur with an increase in blood pressure[6].

Serum lipid values are the main tools for risk stratification in dyslipidemia. Serum cholesterol concentrations of low-density lipoproteins (LDL cholesterol) and high density (HDL cholesterol) are the most used lipid classes for this purpose[7]. Meanwhile, the quantitative indicators of LDL and HDL particles - apolipoproteins B (ApoV) and A1 (ApoA1), may be better risk markers than their cholesterol concentrations - LDL cholesterol and HDL cholesterol and the atherogenicity index (AI) traditionally used in domestic medicine[8].

Based on the foregoing, the study of the arterial hypertension prevalence and its risk factors, the creation of an adequate system for the prevention of cardiovascular diseases seems relevant for public health of the Republic of Uzbekistan.

The Aim of Research: To study the prevalence of arterial hypertension and risk factors for its development, using the example of Uzagrotehmash JSC employees examination, as well as assess the possibility of different classes of serum lipids in predicting its development.

Materials and Method

A total of 1,495 people aged 25-75 years have been examined (average age 46.3±0.3 years). The response was 63% (942 people) for men and 37% (553 people) for women. The quality control of the received information was carried out. Only representative data were selected for the analysis. Data is standardized by age structure.

The survey included: a survey on a standard questionnaire to identify passport data, level of education, profession, information about physical activity, the presence of bad habits, chronic noncommunicable diseases and their treatment, blood pressure measurements, anthropometry, it was found out whether a person knows about his blood pressure and if it is increased - is treated or not.

Blood pressure was measured twice on the right hand in a sitting position after 5 minutes of rest. The average of 2 measurements was analyzed as the level of systolic and diastolic blood pressure (SBP and DBP). The following criteria for arterial hypertension were used: SBP is equal to or greater than 140 mm Hg. Art. and/or DBP is equal to or greater than 90 mm Hg. Art., or the patient receives antihypertensive therapy.

The list of factors that were taken into account when assessing the risk of developing cardiovascular complications in patients with arterial hypertension included: age over 55 for men and 65 for women, smoking, obesity, low physical activity (NPA), lower-secondary education associated with low social status. Active smokers included people who smoked more than 100 cigarettes during their life and who currently smoke every day or sometimes, as well as people who smoke now less than once a week. Passive smoking was recorded in the presence of smoking relatives living with respondents. Overweight was recorded with a body mass index (BMI)> 25.0 kg/m2, including obesity - with a BMI> 30.0 kg/m2.

The level of total cholesterol (TC), high density lipoprotein cholesterol (HDL cholesterol) and triglycerides (TG) were determined in blood serum taken in the morning on an empty stomach after 12-14 hours of hunger on the EPOLL-20 semi-automatic analyzer (Austria). Reagents of the Human company (Germany) were used. The study included 111 people, 62 of them - the main group, 49 patients - the control group. The average age of the patients of the main group was 42.0±2.0 years, the age of the control group was 41.3±2.0 years. Criteria for inclusion of patients in the main group: the presence of arterial hypertension.

Statistical analysis of the results was performed using method of variation statistics. The significance of differences in the means was estimated based on Student's test at a 95% confidence interval ($p < 0.05$).

Results and Discussion

A comparative analysis of the arterial hypertension prevalence revealed a high prevalence of arterial hypertension among both men and women in all age periods.

The frequency of hypertension was 598 people, which corresponds to 67.7% for women (405 people) and 32.3% for men (193 people). Among them, 26.9% of people (161 people) are aware of high blood pressure, but are not treated, and 20.0% (120 people) were diagnosed with high blood pressure for the first time.

When studying the structure of arterial hypertension, it draws attention that increased blood pressure was recorded more often in women (25.9%) than in men (16%) ($p < 0.05$).

Age gradation was performed according to the classification of the World Health Organization (2012), the following age groups were identified: 18-44 years (youth); 44-60 years (average age) and 60-75 years (old age).

A comparative analysis of the frequency of hypertension occurrence depending on age is presented in table 1.

Table 1: The hypertension prevalence (in %) and the age of people in the studied groups

Age	Men		Women		Total	
	Abs.	%	Abs.	%	Abs.	%
Youth	32	16,6	53	13,1	85	14,2
Average age	68	35,2*	136	33,6*	204	34,1*
Elderly age	93	48,2*	216	53,3*	309	51,7*
Total	193	32,3	405	67,7	598	100,0

Note: *Reliability of data by a young age (P<0,05)

As can be seen from the data presented, most patients with hypertension are in advanced age (60-75 years). With increasing age, both among women and among men, the incidence of hypertension increases ($r = 0.569$ and $r = 0.609$, respectively).

The study revealed that arterial hypertension is often found in overweight people - 189 (31.6%) (Table 2). Hypodynamia was observed in almost half of the examined patients with hypertension (50.2%). Among patients with hypertension, the frequency of stressful situations occurrence is 54.5%, with women being more likely than men (62.5% and 37.8%, respectively). Dyslipidemia as one of the risk factors was noted in 41.5% of cases. Smoking was recorded in 37.5% of cases, in men in 95.3% of cases. A similar picture is observed in the analysis of alcohol abuse cases (9.4% and 29.5%, respectively).

Associations of cardiovascular risk factors with the prevalence of hypertension were evaluated using logistic regression. For this, the most significant risk factors were selected that have a reliable association with the prevalence of hypertension, regardless of age and gender, namely: body mass index ($p < 0.05$), alcohol abuse ($p < 0.05$), current smoking and past ($p < 0.05$), lack of exercise ($p < 0.05$), dyslipidemia ($p < 0.05$), stress ($P < 0.05$). In multiple logistic regression, risk factors affecting the prevalence of hypertension, regardless of age and gender: present and past smoking ($p > 0.05$), dyslipidemia ($p > 0.05$) did not reach statistical significance and were excluded from the analysis. The final model of risk factors affecting the prevalence of hypertension, regardless of age and gender, was body mass index ($p < 0.05$), stress ($p < 0.05$), and physical inactivity ($p < 0.05$).

Table 2: The prevalence of risk factors (in %) in the studied groups

Leading factors	The number of patients examined					
	M (n=193)		W (n=405)		Total (n=598)	
	n	%	n	%	n	%
Smoking	184	95,3	40	9,9	224	37,5
Hypodynamia	47	24,4	253	62,5	300	50,2
Overweight	25	13,0	164	40,5	189	31,6
Dyslipidemia	82	42,5	166	41,0	248	41,5
Stress	73	37,8	253	62,5	326	54,5
Alcohol abuse	57	29,5	38	9,4	95	15,9

In the study of blood lipid parameters revealed significant differences between the studied groups (table 3).

Table 3: Indicators of the lipid spectrum of blood plasma

Lipid Profile	Main Group (n = 62)	Control Group (n = 49)
Total cholesterol (mmol/l)	5,59±1,2	3,65±0,1*
Triglycerides (mmol/L)	1,9±1,1	0,71±0,03*
LDL cholesterol (mmol/L)	3,96±1,44	1,78±1,6*

Lipid Profile	Main Group (n = 62)	Control Group (n = 49)
Apolipoprotein B, g/l	1,07±0,02	0,73±0,01*
HDL cholesterol (mmol/L)	0,767±0,21	1,78±0,26*
Apolipoprotein A1, g/l	1,31±0,06	1,7±0,02*

Note: * P<0,01

The level of total cholesterol in the main group was significantly higher than in the control group (p <0.001), mainly due to an increase in atherogenic lipid fractions. At the same time, the average level of HDL cholesterol in the main group was below the normative values (p <0.001). Levels of lipoproteins A1 and B were significantly different in groups (<0.001).

Deviations in HDL cholesterol (36%) and TG (26%) were most often recorded, which is a marker violation of lipid metabolism in hypertension. Deviations in the concentration of ApoA1 were found in 24%, only 22% had deviations in the concentration of TC, this reflects the significance and necessity of studying the full lipid spectrum in patients with obesity. Increases in the concentration of LDL cholesterol and Apo B were recorded in 14% of patients in the main group. At the same time, 17.7% of patients (11 people) had normal lipid profile, thus, more than 80% of patients with hypertension had secondary lipid metabolism disorders.

The basis of atherogenesis is subendothelial deposition of particles containing LDL cholesterol[13]. Secondary dyslipidemia in hypertension is highly atherogenic for several reasons: small dense LDL particles are not effectively captured by the liver using LDL receptors, an increased number of circulating LDL particles increases the risk of their deposition in the subendothelial matrix, and a decrease in HDL content limits the reverse transport of cholesterol from tissue to liver.

The analysis showed that the ratio of ApoB/ApoA1 is a reliable predictor of atherogenesis in patients with hypertension and can be used to predict the disease development. The highest sensitivity and specificity corresponded to the value of the ratio ApoB/ApoA1 equal to 0.45.

Thus, with values of this lipid index greater than 0.45, it can be reliably assumed that this person is at risk for the hypertension development.

Conclusion

1. Arterial hypertension is a common disease - 40%. Among men, this disease occurred with a frequency of 32.3%, among women - 67.7%.
2. In the structure of arterial hypertension, both men and women in all studied age intervals revealed the presence of risk factors - overweight, physical inactivity and stress.
3. It is necessary to perform preventive examinations among the population, which will allow to identify the initial stages of arterial hypertension, and people with overweight, with physical inactivity and who have undergone stress should be considered a risk group for the hypertension development.
4. Among individuals at risk for the hypertension development, it is necessary to conduct a full lipid screening, including an assessment of the apolipoproteins A1 and B level.

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Hygienic Characteristics of Teaching Staff Labor Conditions in Different Universities of the Republic of Uzbekistan

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Abstract

The article is devoted to the study of the professional activities characteristics of university teachers, the possible risk factors that affect the state of teacher's health in the course of his work are considered, solutions for protecting the health of the teaching staff and the increase in the labor efficiency of teachers of higher educational institutions are proposed.

It was revealed that the actual time costs of teaching staff exceed the established standards of the teacher's working time; however, the teacher's work does not have clear regulation (except for scheduled classes); all types of work performed take up 95% of the working time and are integral elements of the teacher's activities. A general assessment of working conditions by the tension level, the harmfulness and danger degree, performed in accordance with SanPiN 0141-03, considering the most significant factors (the labor process intensity), allows to classify the labor of university professors in our republic as class 3 (harmful), 3 degrees of harmfulness.

Keywords: *Teacher, professional activity, working day timing, working conditions, fatigue, health.*

Introduction

Ensuring the safety of life and health of workers by improving working conditions in the labor process is one of the most important tasks of our State. The work of university teachers belongs to a field of activity that is not directly involved in the production process but continues to be one of the most widespread varieties of mental labor. It is well known that mental stress leads to fatigue much faster than physical ones[1]. Today in our country more than 22 thousand teachers share their knowledge with students.

What our country and the younger generation will become tomorrow, directly depends on those who teach us. The teacher in a higher educational institution (university) is a key figure in the educational process, the effectiveness of which is determined not only by the teacher's professional competence but also largely depends on his physical health.

The teaching profession deserves comprehensive and in-depth study, as it has several characteristic features. After analyzing a large number of literature sources, we can say that today the work on studying the working conditions of teachers of higher educational institutions, assessing their health status is extremely insufficient and they relate mainly to the period of 60-80s of the last century. In these works, adverse sanitary and hygienic working conditions of educational institutions' teachers were noted due to the mismatch of microclimate parameters in the classrooms in the winter, insufficient level of artificial lighting in the classrooms. A decrease in the education financing level in the 90s of the last century led to a reduction in the construction of new classrooms, laboratories and complexes, the impossibility of repairing and rehabilitating existing ones, replacing equipment, furniture, and ensuring the required levels of lighting and air-thermal conditions in lecture halls. In addition, in recent years there has been

a significant change in the labor process in educational institutions due to the introduction of some innovations in the educational process, and the teachers' working conditions remained the same as 20-30 years ago[2].

Now, a fairly limited number of studies have been conducted on the health of universities' teaching staff and the factors that shape the health[3], however, based on these works, we can conclude the significance of this kind of research.

The aim of research: Study and assessment of teaching staff's working conditions in various universities of the Republic of Uzbekistan.

Research Method

The objects of research were 3 largest universities of the Republic, located in Tashkent - the National University of the Republic of Uzbekistan named after Mirzo Ulugbek (NUUz), the Tashkent Medical Academy (TMA) and the Tashkent State Technical University named after Islam Karimov (TSTU), as well as the faculty of these educational institutions.

The assessment of social factors affecting teaching staff was performed according to the survey-questionnaire results of 430 teachers (15.9% of the average teachers' number among the three universities studied); the survey was based on a questionnaire recommended by the WHO regional office. To assess the content and intensity of teaching staff work, the working hours were timed (10-12 teachers in each university). Hygienic working conditions are studied in each university at the main workplaces; microclimate indicators (by seasons - 95 measurements), lighting of workplaces (88 measurements), noise level (90 measurements), electrostatic field (16 measurements), the number of aero ions in 1 m^3 of air (28 measurements), its anthropogenic pollution (content CO2 - 123 measurements), radiation environment (radon content, radiation dose rate - 30 measurements). Studies of hygiene factors were performed by generally accepted instrumental and laboratory method; all research results were subjected to statistical processing with the calculation of indicators M±m, and their assessment - according to the relevant SanPiNRUz.

Results

Work content and teaching staff employment level during the working day: During scientific research, we had the opportunity to compare the organization and working conditions of the teaching staff in our republic and in South Korea. During 2017-2019, in TMA, in the framework of international cooperation with the *State University of South Korea*, *3 business visits* of the teaching staff were organized (with our participation) to the above mentioned university in order to familiarize themselves with the organization and conduct of the educational process at the *State University of South Korea*.

During the working meetings, attention was paid to the peculiarities of the teaching staff and the organization of student tutoring. It was noted that the educational process is performed in well-equipped rooms with a favorable microclimate, good ventilation, proper lighting, and physiologically adapted furniture. With all types of work performed, there is widespread computer equipment use, and modern technical equipment and laboratory equipment are applied during the educational process. The teaching process is performed in accordance with very dynamic programs that consider both modern knowledge and requirements for specialists, and the wishes of students (master's program). Teachers perform only those tasks that require teaching, and the schedule of their work is not rigid, although in general the teaching load of teachers is quite large. The profession of a university teacher is considered very prestigious and, accordingly, the teacher's work is highly paid.

An important feature of the universities' teaching staff work of in our republic is that due to the versatility of the teacher's functions, he is forced to constantly change his workplace, so during the working day he can work in the classroom, in the office, in the laboratory, in the vivarium, in the hospital ward (medical university), in a hostel, etc.

To characterize the work content and the employment degree of the modern university teacher in Uzbekistan, we conducted a direct timing of the teacher's working day in each university under study. The study involved 9-12 teachers in each university, after which the averaging of the teaching staff in general was performed.

Timekeeping of the teacher's working day in all the universities studied has shown that the university teacher's work is currently characterized by a very wide range of work. In addition to classes with students in accordance with the schedule, the teacher performs many different types of work related not only to the

main areas of his activity. This includes preparation for lectures, workshops, seminars, advisory work, work with lagging students, a large amount of methodological work, scientific research, educational work, fulfilling public assignments, preparing reference and reporting materials, etc. All these activities are associated with the need of working with literature, applying modern information technologies and modern office equipment, working with a huge amount of information that requires analysis and relevant conclusions. It should be noted that, in addition to classes that are strictly regulated by the schedule, it is almost impossible to regulate other types of the teacher's work - they are performed either as necessary, or considering the temporary capabilities of the teacher.

The determination of the time spent on the performance of certain work types showed that for 80% of the teachers included in the study, the working day goes beyond the statutory 6-hour working day. The average statistical indicators of the teacher's time spent on various work types are characterized by Table 1.

Table 1: The results of the working timing day of a university teacher, M±m (n=20)

No	Work implemented	Actual elapsed time, hour (min- ′)	% of total time
1.	Lecturing	0,4±0,1 (24′)	4,6
2.	Lecture preparation, including presentation preparation	0,2±0,1 (12′)	2,3
3.	Conducting practical (seminar) classes	3,0±0,5 (180′)	34,5
4.	Preparation for practical exercises (laboratory base, handouts)	0,5±0,3 (42′)	8,0
5.	Preparation of training documentation, rating calculations	0,5±0,3 (48′)	9,2
6.	Consulting work	0,2±0,1 (12′)	2,3
7.	Verification of student work	0,3±0,1 (30′)	5,7
8.	Work on the Internet (reference materials, new data)	0,5±0,1(30′)	5,7
9.	Reception of missed classes	0,2±0,1 (12′)	2,3
10.	Preparation of materials at the request of deans, administration, scientific part, ministries, etc.	0,5±0,2 (30′)	5,7
11.	Preparation of teaching materials	0,5±0,2 (30′)	5,7
12.	Scientific work (Internet, library, collection of scientific data)	0,6±0,2 (36′)	6,9
13.	Educational work (curatorial, visiting dormitories, extracurricular activities)	0,2±0,1 (12′)	2,3
14.	Lunch break, personal distractions	0,4±0,2 (24′)	4,6
	Total	**8,0±0,2 час**	**100**

The data presented allow to determine that the teacher spends 95.0% of the total working time for all types of work, which is already 2 hours longer than the officially established working time of the teacher.

At the same time, it was revealed that the work of teachers is mainly accompanied by a static muscular load while in 30±4.6% of the working time in a free working position (standing or sitting), which allows us to characterize such work as a lightly stressed light physical work (except for physical teachers education, as well as teachers of certain medical disciplines (surgeons, traumatologists) requiring significant physical effort) - class 1b in SanPiN 0325-16.

When assessing the nervous load, it was determined that the teacher's work is characterized by high tension, due to both the mandatory fulfillment of the class schedule and the lack of time, increased responsibility for the work results, prolonged strain of visual functions, the need for large random access memory (a set of many changing elements that must be memorized during hours and a working day), high intellectual stress associated with performing creative work, frequent unrepeatable stressful situations when working with a large and diverse student population. The indicated labor characteristics of the teaching staff allow to determine the intensity of such work as hazardous (very hard) work of 3 degrees (SanPiN 0141-03).

It can be assumed that the teacher's actual work schedule in the universities under study, even with favorable hygienic factors of the working environment, can have an adverse effect on the body and, above all, on the central nervous system (CNS). It should be borne in mind that a high neuropsychic stress for a teacher is a factor affecting his body every day throughout the entire academic year and throughout his work at a university.

Hygiene factors' characteristics in workplaces: Based on the timing of the working day, we can assume that the main jobs of the teacher are classrooms and study rooms, and therefore, to characterize the main hygienic factors of the teaching staff environment, we studied these factors precisely in classrooms, laboratories and study rooms.

During a visual examination of these workrooms, it was noted that the rooms dimensions generally meet hygiene requirements. At the same time, despite the fact that the main part of the investigated premises is located and equipped in specially constructed buildings, violations of hygiene requirements were noted at many workplaces. Most often this relates to insufficient ventilation of the premises, the presence of idle lamps, non-compliance with the hygienic requirements of the furniture used, malfunctions of the water supply and sewage, i.e. the operating conditions of buildings and premises.

To characterize hygienic working conditions, we evaluated the microclimate, the workplaces illumination, the noise level, the electromagnetic field magnitude, the carbon dioxide concentration, measured the dose rate of external radiation and the content of radon in the working area air.

The microclimate parameters of the premises, which we studied in the cold and warm periods of the year, are presented in Table 2.

It was revealed that in the cold season the optimal parameters of air temperature as the main indicator of the microclimate take place only in the clinic, which is understandable, since for patients the requirements for the microclimate are more stringent. In all classrooms at the school day beginning, the air temperature was 4-5 degrees below the optimal values and approached those only at the end of the day. At the same time, in no case have we recorded air temperature below the established SanPiN 0324-16 border for the cold season (170 C). With regard to air humidity and its speed, the parameters of these indicators in all cases were within the established hygiene standards.

In the warm season, the air humidity at the measured points was within acceptable limits, but the air temperature was 1-4 degrees higher than the optimal parameters. The air movement speed in all rooms was 1.5-2 times lower than the recommended values. The indicated microclimate parameters in the warm season can be the cause of some tension of the thermoregulation function and increase the already high emotional stress level of teachers.

The work of most university teachers is associated with the need for long-term high voltage of the vision organs, with objects of discrimination 0.3-0.5 mm (fonts), high contrast on a light background, which allows to classify this work as a 3rd category of high-precision work, sub-category "G" (KMK 2.01.05- 98 "Natural and artificial lighting"), for which the normalized value of daylight factor with natural side lighting is 1.2, and the general level of illumination of workplaces is 200 lx (fluorescent lamps).

Table 2. The microclimate parameters of educational facilities in the universities studied in the cold and warm seasons

University	Place of measurements	Cold season, M±m			Warm season, M±m		
		Air temperature, °C	Air humidity, %	Air speed, m/s	Air temperature, °C	Air humidity, %	Air speed, m/s
TMA	Building 1	18,3±3,1	71,3±4,8	0,2±0,04	27,4±1,2	54,3±1,2	0,1±0,08
	Building 3	17,8±2,2	72,6±5,2	0,3±0,04	26,8±2,4	51,8±2,3	0,1±0,09
	Clinic (Department of Cardiology)	20,1±1,1	70,4±6,1	0,1±0,05	25,2±3,1	54,2±2.4	0,1±0,07

University	Place of measurements	Cold season, M±m			Warm season, M±m		
		Air temperature, °C	Air humidity, %	Air speed, m/s	Air temperature, °C	Air humidity, %	Air speed, m/s
NUUz	Building 1	17,2±2,2	71,6±5,1	0,2±0,06	26,4±2,2	60,0±0,8	0,2±0,04
	Pavilion 2	18,1±3,1	72,4±5,1	0,3±0,04	27,5±2,4	54,3±0,8	0,3±0,01
TSTU, main building	1st floor	17,1±2,2	73,0±1,5	0,3±0,09	26,2±0,8	55,6±0.9	0,3±0,08
	2nd floor	17,5±2,4	70,8±2,6	0,3±0.07	27,1±0,9	59,1±0,9	0,4±0,04
Hygienic norm (SanPiN 0324-16)		Optimal-21-23 Permissible-17-25	40-75	0,1-0,2	Optimal-24-26 Permissible-22-32	40-60	0,3-0,6

In the premises examined, the workplaces illumination parameters were determined both by the nature and purpose of the premises, their orientation, and the time of day and year (Table 3).

The best lighting parameters were identified in NUUz and TSTU, which are built according to individual projects developed for these universities. Both the daylight factor value and the combined illumination level of classrooms in these universities mainly correspond to hygienic standards, but the artificial lighting level in 32% of cases was 60-70 lux lower than hygienic requirements. This is mainly due to the fact that in some audiences there are faulty lamps.

As for TMA, we have studied the premises of buildings 1 and 3, which are the premises of former dormitories adapted for classrooms. In the studied rooms, the daylight factor value was 25% lower than the norm, and therefore artificial lighting is widely used in these buildings, which makes it possible to bring the general illumination level of classrooms to a hygienic norm. In the absence of artificial lighting, the level of illumination is 90-120 lux, which greatly complicates the work of both teachers and students.

Table 3: The illumination state of teachers' jobs in the universities studied, M±m

University	Place of measurements	Number of measurements	Daylight Indicators		The illumination level of working places	
			Room orientation	Daylight factor	Combined lighting, lux	Artificial lighting, lux
TMA	Building 1	14	East	0,8±0,1	170±50	150±60
	Building 3	14	Southwestern	0,9±0,1	170±48	154±50
	Clinic	10	Northeastern	1,1±0,1	134±60	132±60
NUUz	(Department of Gastroenterology)	10	Western	1,3±0,2	184±40	130±40
	Building 1	10	Northwestern southwestern	1,4±0,3	180±50	140±40
TSTU, main building	Building 2	10	Northeastern	1,4±0,2	180±49	140±38
	1st floor	10		1,5±0,2	188±50	138±42
	3rd floor	10	Northeastern	1,8±0,2	180±49	140±40
Hygienic norm (KMK 2.01.05-98)				1,2		200

No sources of intense noise were found in all the educational institutions studied - the noise level in the premises is mainly due to the presence of a large number of fairly active young people, whose communication, especially during breaks, creates a noise background in the rooms, which can be characterized as intermittent broadband noise. The measured total noise level in classrooms and classrooms (n = 12-14 in each university) during classes was 50±8 dBA, and during breaks - 60±7 dBA, which corresponds to hygiene requirements for highly skilled work requiring concentration (60 dBA according to SanPiN No. 0325-16). At the same time, attention is drawn to the fact that in the TMA buildings 1 and 3, in rooms whose windows face a street with heavy traffic, the noise level averages 72±4 dBA, reaching 85-87 dBA in separate time intervals (building 1), which is 12-27 dBA higher than the established hygiene regulations.

The widespread use of computer technology in various educational institutions suggests the possibility of forming electrostatic fields in the area of their location. We measured the electrostatic field value in the area where computers of both the old type (CRT screens) and new generations (LCD screens) are located. In accordance with SanPiN RUz No. 0303-12, when working on a computer for up to 8 hours, the permissible electrostatic field value should not exceed 10 V/m. Measurements made by us at the TMA Department of Communal Hygiene and Department of Informatics (16 measurements) showed that in the area where computers with CRT screens are located, the electrostatic field is 7.4±0.3 V/m, and with LCD screens - 2.05±0.5 V/m.

In most classrooms of the universities under research there are no man-made sources of intense chemical air pollution, but during some laboratory studies small amounts of various chemicals can be released into the air, and therefore, such laboratories must be provided with effective ventilation, including equipment for working fume hoods. During our research, we considered the fact that such equipment is practically absent in classrooms.

When conducting research, we did not reveal any classrooms of artificial chemicals in the air, which, in our opinion, is due to the fact that only demonstration chemical studies were conducted in classrooms during this period. At the same time, in the air of classrooms, we constantly revealed chemical components of anthropogenic origin - carbon dioxide, ammonia.

Table 4: Carbon dioxide concentration in classrooms, %, M±m

University	Time of measurements	Warm season		Cold season	
		Number of measurements	M±m	Number of measurements	M±m
TMA	Before class	15	0,05±0,01	12	0,08±0,03
	12^{00} - 13^{30}	15	0,12±0,08	12	0,09±0,03
	16^{00} -16^{30}	15	0,16±0,08	12	0,21±0,06
NUUz	Before class	12	0,04±0,01	10	0,06±0,09
	12^{00} - 13^{30}	12	0,08±0,02	10	0,09±0,03
	16^{00} -16^{30}	12	0,12±0,04	10	0,18±0,08
TSTU	Before class	14	0,04±0,01	11	0,06±0,02
	12^{00} - 13^{30}	14	0,11±0,06	11	0,14±0,05
	16^{00} -16^{30}	14	0,16±0,07	11	0,18±0,06

The determination of an indirect indicator of anthropogenic air pollution in classrooms - carbon dioxide - at various time intervals (Table 4) indicates that, both in the warm season and in the cold season, natural ventilation of the premises does not provide the necessary air purity. The CO2 concentration, indicating the absence of anthropogenic air pollution in residential and public buildings, is assumed to be 0.1% or lower. According to our data, such carbon dioxide concentration in classrooms is only before classes and, partually, in the classroom. By the end of the school day, an increased CO2 concentration (up to 0.24%) is detected in almost all classrooms.

The airborne content of the air ions indicates air pollution throughout the working day (Fig. 1.).

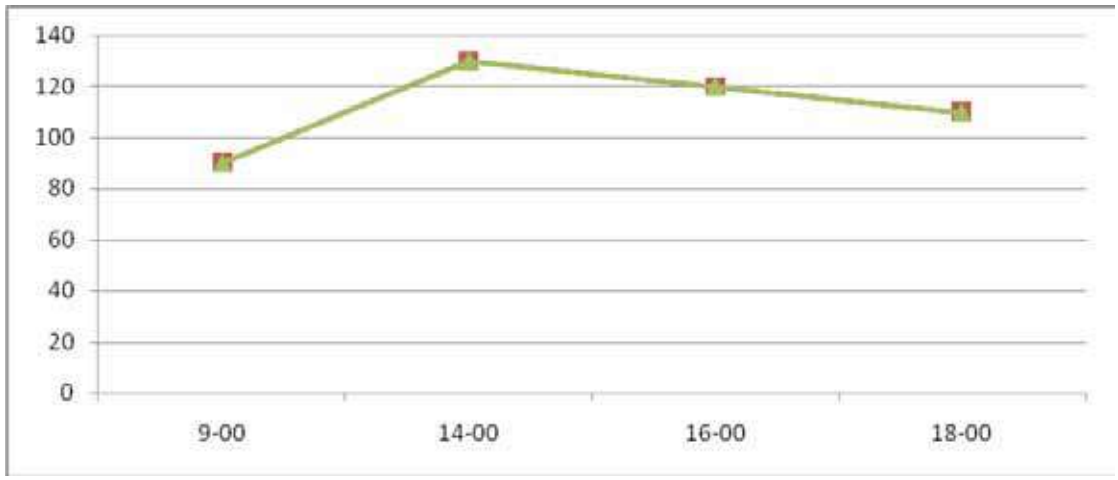


Fig. 1. The dynamics of the positive ions number in 1 m^3 of TMA classrooms air during the day.

Low-quality ventilation of classrooms is also evidenced by the results of measuring the concentration of radon in the air of these rooms (Table 5).

Table 5: The concentration of radon in the air of classrooms, Bq/m^3

University	Place of Measurements	Level	Number of Measurements	Concentration of Radon, M±m
TMA	1-Educational building	1	7	40,8 ± 4,5 Бк/м^3
		3	7	38,8 ± 3,8 Бк/м^3
		4	7	30,8 ± 2,5 Бк/м^3
	3- Educational building	1	7	39,8 ± 3,5 Бк/м^3
		3	7	34,6 ± 2,9Бк/м^3
		4	7	30,2 ± 5,1 Бк/м^3
	TMA hospital (Department of Cardiology)	2	7	36,7 ±,5 Бк/м^3
NUUz	Building 1	1	6	44,1 ± 4,5 Бк/м^3
	Building 2	2	6	35,8 ± 2,5 Бк/м^3
TSTU	Main building	1	6	40,6 ± 6,6 Бк/м^3
		3	6	31,8 ± 2,7 Бк/м^3
		5	6	30,5 ± 4,5 Бк/м^3

According to Zaredinov D.A. (2001), the average radon content in indoor air in the Republic of Uzbekistan is close to the national average - 30.8+3.5 Bq/m^3, but higher concentrations of radon are detected on the first floors of multi-storey buildings, where the underlying source of radon is : on the 1st floor up to 48.5 + 2.9, above - up to 25.7 + 1.6 Bq/m3.

The data obtained by us indicate that, despite the absence of high values of the measured indicators, their dynamics indicates a gradual deterioration in the quality of the air in the classrooms during the working day. These data reflect not only the quality of ventilation, but also the radiation exposure due to radon.

The level of general unprofessional radiation exposure on a person is determined not only by the incorporation of radon, but also by external background γ - radiation. The gamma background measured by us at the university premises was in TMA (n = 20) - 18-26 μR/hour, NUUz (n = 16) - 16-21 μR/hour, TSTU (n = 14) - 16-22 μR/hour.

We did not reveal higher indicators of external

exposure in computer labs in which the measured dose rate of external exposure was 23.5±2.8 μR/h. Only at one of the TMA departments (the former Department of General and Radiation Hygiene) during classes in radiation hygiene, during which a demonstration of exemplary radiation sources is conducted, a radiation dose rate of up to 42 μR/hour is recorded directly near the teacher's hands.

According to Zaredinov D.A. (2001), in our republic, the exposure dose rate of gamma radiation outdoors is 13 mcr/h-1, whereas in rooms it is higher (up to 23 mcr/h-1); our data confirm the results of D. Zaredinov's studies and indicate that the external gamma radiation level in the educational premises of universities is small and does not go beyond the background radiation, typical for the climatic and geographical conditions of the republic.

The above radiation factors characteristics, measured at the main teaching staff's workplaces in universities, allow to classify working conditions by this factor as optimal values that do not have a harmful effect on the body of workers.

Conclusions

Thus, the study of the universities' teaching staff content and the hygienic conditions of their work allows to draw the following conclusions:

1. The actual time costs of teaching staff exceed the established standards of the teacher's working time; however, the teacher's work does not have clear regulation (except for scheduled classes); all types of work performed take up 95% of the working time and are integral elements of the teacher's activities.
2. The general assessment of working conditions by the employment level, performed in accordance with SanPiN 0141-03, considering the most significant factors (the labor process intensity), allows to classify the university teachers' labor in our republic as class 3 (harmful), 3 degrees of harmfulness, whereas other hygiene factors, we have described working conditions as optimal or acceptable (table 6).

Table 6: Working conditions classes of universities' teaching staff in the Republic of Uzbekistan (according to SanPiN 0141-03).

Researched Factors	Working Conditions Classes					
	Optimal (1 Class)	Permissible (2 Class)	Harmful (3 Class)			
			1 Deg.	2 Deg.	3 Deg.	4 Deg.
Total noise	+	+				
Microclimate		+				
Lighting			+			
Ionizing radiation	+					
Labor intensity					+	

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Analysis of the Interrelation of Speed and High-Speed Endurance in 14-17 Years of Football Players

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Abstract

In the analysis of the optimal interrelation between the general and special high-speed physical qualities of young footballers of 14-17 years old, normative tests were used to measure speedy physical performance. It was determined the optimal age group in the test analysis processes of general and special speedy performance young group footballers.

Keywords: *Shuttle running 5*30m, standard "8" ("eight"), "high-speed equipment", complex coordination movement, sensitive period, "microgate" device, correlation coefficient, intensity.*

Introduction

From the first days of independence of Uzbekistan by the state, special attention, as well as other sports, was given to football. A clear confirmation of the above statements is that, according to the Decree of the Cabinet of Ministers of the Republic of Uzbekistan No. 144 of March 18, 1993 "On Measures for the Further Development of Football in the Republic of Uzbekistan", several measures have been outlined to restore the traditions of the Pakhtakor football club and determine the educational training complex "Kibray" the main training base of the national team. After one year after the adopted resolution, at the 1994 Asian Games held in Hiroshima and Nagasaki, the Uzbekistan national football team was successful in becoming champions. All this testifies to the increased attention of the state to football[1].

The further favorable condition of qualitative growth of football in republic became the Decision of the President of Republic Uzbekistan from March, 16th, 2018 for №ПП3610 "About measures on the further development of football", accepted with the purpose of formation of national team of Uzbekistan, and also perfection of preparation of gifted young shots and their inclusion in structure of a national team. The same decision provides specific instructions for the establishment of children's and youth football academies in the Republic of Karakalpakstan. These academies are created with the aim of training highly qualified football cadres from young talented players[2].

Relevance: In recent years, on the world stage, during international competitions, there has been an annual increase in the intensity of the pace of the game, i.e. the increase in the speed of the implementation of technical and tactical actions during the struggle for the possession of the ball. The growth trend of the game speed leads to the universality of the players' actions. At the same time, defenders of the flanks, who even perform the tasks of playmakers in the attack, take part in the attack (as a creative participant in organizing the attack and as a football player directly organizing the start of this attack). And this, over the course of many years of preparation, requires approaching the intensity of training loads to the pace of the speed requirements of the playing period, and this is especially necessary to consider when developing speed endurance for young players.

Such physical quality as speed must be developed from 13-14 years. The reason for this is that this age is considered a sensitive period in the development of speed[3].

The speed of young football players is developing at different rates depending on their abilities. In the growth of sportsmanship of young football players aged 12-13 years, the growth of physical performance, the integrated

development of speed and coordination qualities, and the implementation of special coordination of complex technical actions are of great importance.

14-15-year-old players are distinguished by their speed abilities, high activity of training and competitive actions, dribbling with complex coordination movements, and high-speed qualities.

And for 16-17-year-old players, physical performance, the integrated development of speed and coordination, the ability to perform complex coordination movements during the game are well developed.

- Due to the intensity of games during competitions, it can be very difficult to maintain a high level of speed qualities. In the process of training during the competition, it is necessary to develop their individual speed qualities taking into account the abilities of each player. In D. Sirenko's researches most suitable development of speed qualities was carried out by separate groups taking into account individual abilities and age of players, as a result of which speed qualities were developed optimally after application of the following exercises:

- Running from a high start on 15 meters: - 15 summer defenders, - 15-16 summer midfielders, 17 summer strikers;
- Running at 60 meters: - 16 summer defenders and midfielders, - 15-17 summer strikers;
- Run for 100 meters by 15 summer players;
- Driving the ball at 30 meters: - 14 and 15-year-old defenders, 13.15 and 17-year-old midfielders, 14.16, 17-year-old attackers [4].

Object and Subject of Research: As the object of the study, the study was chosen to study the development of general and special high-speed quality for 48 students of football players of the Republican boarding school of the Olympic reserve, aged 14 to 17 years, 12 people in each group. The subject of the study is the study of the correlation between general and special physical qualities by adopting standards: 30-meter running, 5 * 30m shuttle running, and the "8" («eight») test.

Aims and Purposes: The research aims to study the most suitable interrelation between general and special speed qualities of 14-17-year-old players, taking into account their age characteristics.

Based on the purpose of the research, the following tasks were selected:

- Application of norms of high-speed running without a ball for 30 meters and shuttle running 5*30m with a ball for studying the correlation between general and special high-speed qualities of 14-17-year-old football players;
- Using correlation analysis of features with general and special speed qualities of 14-17-year-old football players.

Research Practice: The research was conducted in July 2018 at the Republican School of Olympic Reserve with 12 football players in 4 age groups. Using a Microgate measuring instrument (Misrogate) during the study, 3 standard tests were applied (table 1):

The first standard test is "30 meters running." when starting a run from a high start, speed measuring instruments are installed at the "Start", "10 meters from the start" distances and on the "Finish" line. In the standard, 30 meters running, the speed of overcoming distances of 10 meters and 30 meters are determined simultaneously.

The second standard test is a shuttle running 5x30m. Microgate speed measuring instruments are installed at the intersection of the "Start" and "Finish" lines, where time is detected only when the football player has completely crossed these lines. This method determines the completeness of the intersection of the start and finish lines by football players.

The third standard test is the "8" ("eight") test. Four cones are placed on four sides in the form of a quadrangle and one cone is placed in the center. The distance between the cones is 10 meters. The soccer player must speed the ball from the cones in the corner to the cone in the center. The micro-gate speed meter shall be installed on the Start and Finish lines.

Methodologies

Tests have been used to check physical properties: Start speed "Running at 10 m", Longdistance speed - Running at 30 m", Shuttle running 5*30m, special coordination - Test "8".

Microgate Method:

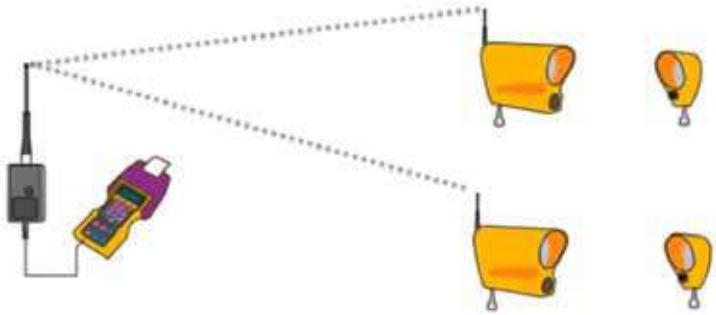


Fig. 1: The Device Microgate Racetimer 2

Source: http://www.microgate.it/Timing/Products/Kit-Racetime2-Light-Radio/Description



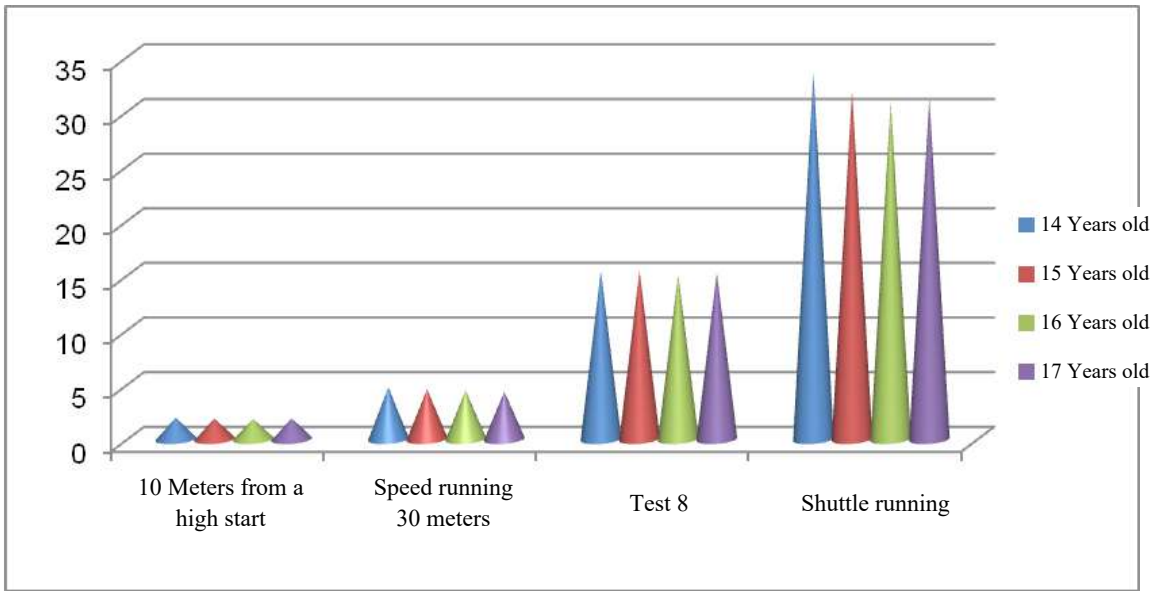
Fig. 1: Evaluation of Microgate Speed Measuring Device ("Microgate")

Table 1: The results of the time standards for running 10 meters from a high start, high-speed running 30 meters, shuttle 5x30m and test "8" for players aged 14-17 years old (2018)

№	Initials of a surname and a name				30metres per second running				10 metres per second running				5x30 metres per second shuttle running				Standard"8" («eight») (seconds)			
	14 years old	15 years old	16 years old	17 years old	14 years old/ sec	15 years old/ sec	16 years old/ sec	17 years old/ sec	14 years old/ sec	15 years old/ sec	16 years old/ sec	17 years old/ sec	14 years old/ sec	15 years old/ sec	16 years old/ sec	17 years old/ sec	14 years old/ sec	15 years old/ sec	16 years old/ sec	17 years old/ sec
1	T-K.	A-J.	T-M.	A-F.	4,62	4,42	4,39	4,42	2,06	1,84	1,92	1,81	30,61	31,94	31,76	35,81	14,08	15,56	15,1	14,92
2	G-F.	K-J.	J-M.	T-O.	4,87	4,88	4,52	4,37	1,95	2,02	1,09	1,9	34,48	32,7	32,04	29,62	16,25	15,31	16,4	15
3	B- A.	J-Sh.	T-Z.	X-M.	4,68	4,43	4,26	4,08	2,03	1,76	2,11	1,91	31,61	30,2	28,97	32,16	15,27	15,02	15,03	16,01
4	B-J.	A-N.	E- D.	Ch-A.	4,65	4,48	4,61	4,79	2,1	1,9	1,92	2,23	32,08	30	30,86	30,54	15,39	14,65	15,43	16,05
5	P-M.	F-O.	T-J.	A-B.	4,7	4,93	4,51	4,54	2,08	2,1	1,92	2,01	32,29	33,21	30,64	30,84	15,33	16,38	15,01	15,1
6	Ch- S.	E-A.	C- C.	B-O.	4,53	4,25	4,41	4,36	1,9	1,48	1,88	1,9	30,41	30,71	30,28	31,8	14,41	14,9	14,36	14,8
7	D-S.	R-M.	X-A.	M-Sh.	5,2	4,62	4,49	4,18	2,04	1,95	1,96	1,84	39	30,61	30,04	29,58	16,57	14,9	14,88	14,54
8	F-L.	O-D.	F-X.	I-Sh.	4,59	4,65	4,5	4,35	1,98	1,93	1,98	1,96	32,48	32,05	31,76	32,11	14,25	14,85	15,38	16,4
9	U-A.	P-I.	B- A.	S-B.	4,65	4,55	4,53	4,36	1,95	1,95	1,94	1,87	33,64	33,16	30,58	30,17	15,84	15,2	14,24	14,26
10	T- R.	F-A.	N-T.	R-M.	5,01	4,32	4,55	4,56	2,04	1,79	2	1,9	36,2	32	31,3	31,68	16,96	15,2	15,09	15,42
11	P-S.	J-J.	R-A.	M-M.	4,49	5,28	4,2	4,21	1,97	2,05	1,87	1,8	34,65	34,36	30,43	28,94	15,33	16,51	14,75	14,63
12	M-A.	S-N.	Ch-Sh.	R-M.	5	4,41	4,69	4,2	1,99	2	1,9	1,84	35,6	32,11	32,09	31,33	15,4	16,77	15,95	16
∑ Av.					**4,74**	**4,6**	**4,47**	**4,37**	**2,00**	**1,89**	**1,87**	**1,97**	**33,58**	**31,92**	**30,89**	**31,2**	**15,42**	**15,43**	**15,13**	**15,26**

The average result according to the standards is given in Table 1 which expressed in histogram 1 for each age group. Where marked: high scores of 17-year-old footballers in "30-meter speed"; it is noted that the highest results according to the standards are "10-meter speed", in "shuttle running 5x30m", "8" 16-year-old players.

Histogram 1: The average standard, running 10 meters from a high start, speed running 30 meters, shuttle running 5x30 m and test "8" for 14-17-year-old players (n = 12 in each age group)



- However, these indicators do not meet the standard level. In the book by Professor I. Koshbakhtiev "Department of Footballers' Training" the following standards are given in the race for 30 meters by age groups:
- Normative indexes in running for 30 meters for players of 15 years old: 4.4-4.45 seconds;
- Normative indicators in running for 30 meters of players of 16 years of age: 4.3-4.2 seconds;
- The standard rate of running for 30 meters for players of 17 years of age: 4.1-4.15 seconds.

From the above results (histogram 1) it can be seen that I. Koshbakhtiev's implementations do not meet regulatory requirements [5].

Research Results and their Comparative Analysis: For 14-year-old players in the "30-meter speed run" without the ball and the "5x30 meter shuttle race" with the ball, the correlation coefficient is r = 0.7. Compared with data of other ages, this is the best indicator r = 0.7, which indicates the presence of a high statistical relationship.

And for 15-year-old players, the result of the indicator is r = 0.6. It can be seen that the average correlation between the general and special speed qualities of 15-year-old football players is lower than that of athletes of 14 years of age.

The 16-year-old football players have r = 0.5. r = 0.5 indicates the existence of a weakly-average statistical relationship. It can be seen that the average correlation between the general and special speed qualities of 16-year-old football players is at a slightly-average level lower than that of athletes of 15 years of age.

The 17-year-old football players have r = 0.08. r = 0.08 indicates the absence of a correlation. However, although the results of a group of 17 summer athletes running 30 meters were higher than other age groups (table 1), we can observe a disproportionate relationship between overall speed and special endurance. And therefore, the correlation between general and special endurance shows a weak statistical result (table 2).

Table 2: In calculating the correlation between the general special and speed qualities of the players, the participants in the standard "high-speed running at 30 meters" -X and "shuttle running 5x30" - Y

	$\overline{X}$ (30m/s running)	$\overline{Y}$ (5x30 m/s shuttle running)	$X_i - \overline{X}$	$Y_i - \overline{Y}$	Xσ	Йσ	r
14 years old	4,74	33,58	0,11	0,09	0,220	2,525	0,7
15 years old	4,60	31,92	0,02	0,01	0,296	1,336	0,6
16 years old	4,47	30,89	0,02	0,07	0,138	0,934	0,5
17 years old	4,37	31,20	-0,02	0,18	0, 195	1,795	0,08

X_i – following the group of each age (n = 12) means one football player.

$\overline{X}$ – on the run for 30 meters on average n = 12.

Y_i – following the group of each age (n = 12) means one football player.

$\overline{Y}$ – in the shuttle run 5x30 meters on average n = 12.

average difference from n = 12 contingent results.

XσваYσ – sigma rate $\overline{X}$и$\overline{Y}$.

r - an indicator of the correlation coefficient.

Conclusions

At the current stage of intensive football - speed, one of the main conditions for organizing the effectiveness of the game quality. In professional football speed quality must be developed from childhood. At present, the world practice is developing more and more new tools and method used in the education of speed quality young football players. Performing technical movements (techniques) at a fast pace (in a high-speed mode) during training shows that the pace of training sessions adapted to the pace of the game. At the same time, modern football emphasizes the fact that a football player, in case of difficult situations during the game, can demonstrate high skill in performing technical moves at high speed.

From the accepted standards (high-speed running for 30 meters further running for 10 meters from a high start, shuttle running 5x30m, as well as the test "8") it is clear that 16-17-year-old players, with high performance, can be traced a weak proportional relationship between the overall high-speed (10 meters and 30 meters) and special endurance (shuttle running 5x30m).

Although, according to the adopted 4 standards, 14-year-old young football players had the lowest results in the group of players aged 14-17, their level of suitable interrelation between general and special endurance turned out to be the highest to other older age groups. From the literature studied during the study, it became known that with age, the process of neurohumoral regulation occurs in the athlete's body, in which, due to an increase in muscle mass, an increase in speed abilities occurs. However, their overall speed quality should be developed in proportion to (commensurate) special endurance. We can observe a similar development at a high growth rate for 14-year-old players (table 2). In measuring the standard of general speed quality "speed running 30 meters", to the standard for measuring special endurance "shuttle running 5x30 meters", they have an optimal correlation equal to r = 0.7.

If we take a group of football players of 16 years of age who, according to the four accepted standards, had the highest results in three, we can observe an average degree of the proportional statistical relationship between the general speed and special endurance (r = 0.5), although they had good results general speed qualities.

Athletes 16-17 years of age do not have enough time to develop special qualities.

Annotation: The article uses speed test physical criteria to analyze the degree of optimal correlation between the general and specific speed physical qualities of players between the ages of 14 and 17. As a result of the analysis of the tests, the age groups with the optimal correlation of general and specific speed quality were identified.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Role of Proinflamatory Cytokines in the Development of Clinical Picture of Myoma and Adenomyosis

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Abstract

The goal of research was to study the cytokine status in women with a combination of fibroids and adenomyosis to determine their role in the course of the disease. Method 165 women with uterine myoma and/or adenomyosis were examined. Patients were divided into 3 groups: 1-group - 75 women with a combined form of myoma and adenomyosis, 2-group - 47 women with diffuse adenomyosis, 3-group - 43 women with uterine myoma. The study was conducted in 2017-2018, on the basis of the Tashkent Medical Academy in the department of gynecology of the obstetric-gynecological complex No. 9 of Tashkent city.

The research method were a dynamic study of node blood flow by ultrasound with dopplerometry and an immunological blood test. For myoma 5 mg ulipristal acetate was prescribed for treatment for 3 months for at least 2 courses by 3 months with a 2 month break, and for adenomyosis - 2 mg dienogest for 6 months continuously. For the combined form of myoma and adenomyosis, treatment was started with dienogest for 6 months continuously, then UPA was prescribed for 3 months.

Results: The determination of the level of pro-inflammatory cytokines in patients before treatment showed the following results: the level of IL-1, IL-6 and TNF-α was higher in all three groups than in the control group, and in the group with combined pathology IL-6 was 1,5 times higher than in groups with isolated pathologies. After therapy, the decrease in the levels of IL-1, IL-6, and TNF-α was observed in all three groups. Also the level of anti-inflammatory cytokines IL-10 and VEGF exceeded significantly the control group, especially in the group with combined pathology, after the treatment, their level decreased significantly.

Discussion: An increase in the level of cytokines in the blood is most pronounced in combined forms of myoma and adenomyosis compared with isolated forms of myoma and adenomyosis.

Keywords: *Uterine myoma, adenomyosis, pro-inflammatory cytokines IL-1, IL-6, TNF, anti-inflammatory cytokines IL-10, VEGF.*

Introduction

This indicates the participation of the immune system in the development and progression of such hyperplastic processes of the uterus as myoma and adenomyosis[1].

Uterine fibroids and adenomyosis are ranked among the hyperplastic processes and diseases of the uterus[2]. However, each of these diseases has its own pathogenetic features, clinical implications and is characterized by various laboratory parameters. Uterine myoma have clinical symptoms more often with a proliferating node type. The most characteristic signs of it are menstrual irregularities, infertility and, in some cases, functional abnormality of adjacent organs[3]. Uterine adenomyosis is also manifested by disorder of menstrual and

reproductive function, pain syndrome[5]. At the same time, each of these pathologies has its own therapeutic approaches, including conservative prevention, which allows achieving positive results [6].

Modern ultrasonographs can not only distinguish myoma and adenomyosis, but also determine the degree of activity of the process[4]. However, over the past decades there has been an increase in the frequency of combined forms of myoma and adenomyosis among patients of reproductive and climacteric age. Often the presence of adenomyosis in the setting of myoma is detected only after a histopathological examination of the extirpated uterus. The relevance of the question lies in the fact that when identifying the combined form, the specialist raises the question: what diagnosis is a priority, what preparations the patient should be treated with, because not all preparations are uniquely effective for myoma and adenomyosis.

Recent studies have shown that the condition and function of various body systems, including the immune system is important in the development of myoma and adenomyosis,. The participation of the immune system has been studied well in endometriosis, but few studies have been conducted with its combination with uterine myoma, and their results are contradictory.

All this indicates the necessity for further research to improve the diagnosis of combined forms of diseases, to study the role of the immune system in the pathogenesis of these diseases.

The goal of this research was to study the cytokine status in women with a combination of myoma and adenomyosis to determine their role in the course of the disease.

Material and Research Method

The study was conducted in 2017-2018, on the basis of the Tashkent Medical Academy in the department of gynecology of the obstetric-gynecological complex No. 9 of Tashkent city. We examined 165 women with uterine myoma and/or adenomyosis. All the patients were divided into 3 groups: 1-group - 75 women with a combined form of myoma and adenomyosis, 2-group - 47 women with diffuse adenomyosis, 3-group - 43 women with uterine myoma. The study groups were formed according to the results of ultrasound, while the number of myomatous nodes and the degree of adenomyosis were payed attention to. The control group consisted of 29 healthy women. The age of the examined ranged from 20 to 45 years, the average age was 36.1±0.9 years.

The research method were a dynamic study of node blood flow by ultrasound with dopplerometry and an immunological blood test. An ultrasound study with dopplerometry made it possible to determine the presence of a simple or proliferating type of myoma node in patients and to establish the degree of adenomyosis, as well as to evaluate the effectiveness of the treatment by comparing the results of ultrasound before and after treatment. The effectiveness of treatment was also evaluated when questioning patients after treatment for the disappearance or decrease in the intensity of the pain syndrome.

An immunological study allowed us to determine the characteristics of the parameters of the immune system, including determining the level of pro-inflammatory cytokines IL-1, IL-6 and TNF and anti-inflammatory cytokines IL-10 and VEGF, to determine their role in the development of combined forms of uterine myoma, and in some cases infertility in women of reproductive age, compare indicators before and after treatment.

Interleukin-1 (IL-1) - a cytokine, a mediator of inflammation and immunity, is synthesized by many cells of the body, primarily activated macrophages, keratinocytes stimulated by B-cells and fibroblasts.

Interleukin-6 (IL-6) is a cytokine, an indicator of the immune response in diseases accompanied by inflammation, i.e. inflammatory mediator. IL-6 is a cytokine that coordinates immune and acute phase inflammatory responses, as well as oncogenesis and hemogenesis. Cytokines are specific proteins by which cells of the immune system can exchange information with each other and coordinate activities. Cytokines include some protein molecules - interferons, tumor necrosis factor (TNF), a number of interleukins, colonie-stimulating factor (CSF) and others.

Interleukin-10 anti-inflammatory cytokine is a human gene product. IL-10 has multiple pleiotropic effects on immunoregulation and inflammation. It reduces the expression of Th1 cytokines, increases the survival of B cells, their proliferation and antibody production.

Vascular endothelial growth factor (VEGF) is a signaling protein produced by cells to stimulate vasculogenesis and angiogenesis (the growth of new

vessels in an existing vascular system). The main functions of VEGF are the creation of new blood vessels, the enhancement of muscle growth after exercise, the maintenance of collateral circulation (the creation of new vessels by blocking existing ones). But, increased VEGF activity can lead to various diseases. Tumors (as well as uterine myoma and adenomyosis) cannot grow larger than a certain limited size without receiving adequate blood supply; and the tumors themselves are capable of expressing VEGF in order to grow.

Tumor necrosis factor (TNF - alpha, cachexin, cachectin) is an extracellular protein, a multifunctional pro-inflammatory cytokine, synthesized mainly by monocytes and macrophages. It affects lipid metabolism, coagulation, insulin resistance, endothelial function, stimulates the production of IL-1, IL-6, IL-8, interferon-gamma, activates leucocytes, one of the important factors of protection against viruses and intracellular parasites.

Excessive production of TNF causes hemodynamic disorders, diffusely increases the capillary permeability, the cytotoxic effect on body cells.

In our study, we performed a quantitative analysis of pro-inflammatory and anti-inflammatory cytokines in blood serum in 60 patients. 15 patients of each studied group, as well as in the control group donated blood for immunology research before treatment and after the course of therapy. The treatment was carried out depending on the revealed pathology: for myoma, ulipristal acetate 5 mg (UPA) was prescribed for 3 months at least 2 courses of 3 months with a two-month break, for adenomyosis - 2 mg dienogest for 6 months continuously. For the combined form of myoma and adenomyosis, treatment was started with dienogest for 6 months continuously, then UPA was prescribed for 3 months.

The Results of the Research

Ultrasound studies showed that the 1st group patients with the combined form of adenomyosis and myoma with clinical symptomatology (algodismenorrhea, infertility) revealed single (30 cases) or multiple (from 2x to 5 nodes - 45 cases) myomata.

Dopplerometry of the blood flow in nodes made it possible to determine a simple or proliferating myomatous node by the degree of quality of its blood supply. So in the 1st group with a combined form, about half of the patients (46.7%) had several myomatous nodes in combination with diffuse adenomyosis. A single myomatous node was combined often (25.3%) with a diffuse form of adenomyosis, and in some cases (14.7%)-combined with a diffuse focal form of adenomyosis. Several nodes of proliferating fibroids were also combined with a diffuse focal form of adenomyosis (13.3%).

Determination of pro-inflammatory cytokines level in patients before treatment showed the following results: the level of IL-1 in all three groups was almost equal (Table 1). In the 1st and 3rd groups, the level of IL-1 was 3.4 times, and in the 2nd group, IL-1 was 3.0 times higher than in the control group (P <0.001). A high level of IL-6 was observed more often in patients of the 1st group and was 3.3 times higher than in the control group (P <0.001).

Table 1: The level of pro-inflammatory cytokines in the blood serum of the examined patients before and after treatment (M±m)

№	Group	IL-1		IL-6		TNF-α	
		Before	After	Before	After	Before	After
1	The combination of myoma and adenomyosis n = 15	7,5±0,39* P<0,001	3,5±0,12[a] P<0,001	8,2±0,45* P<0,001	4,6±0,24[a] P>0,05	8,7±0,93* P<0,001	10,3±1,02 P<0,001
2	Adenomyosis n=15	6,7±0,38* P<0,001	3,2±0,22[a] P<0,01	6,0±0,79* P<0,01	4,3±0,26[a] P<0,01	12,7±1,12* P<0,001	14,6±0,95 P<0,01
3	Uterine myoma n=15	7,4±0,68* P<0,001	3,4±0,25* P<0,05	5,1±0,24 P<0,01	3,6±0,19 P<0,01	11,3±1,91* P<0,001	12,6±1,21 P<0,001
4	Control group n=15	2,2±0,15		2,5±0,21		1,5±0,07	

Note: *-acurate compared with the control group, [a] - acurate compared with the data before treatment

Also, compared with 2- and 3-groups, IL-6 exceeded 1.5 times. In the 2 group, this indicator exceeded the control group by 2.4 times (P <0.01), and in the 3 group 2 times (P <0.01).

In the 1-group with combined pathology TNF-α was 5.8 times, and in the second group for women with adenomyosis it was 8.5 times, in the 3-group it was 7.5 times higher than the control group, the accuracy was P <0.001.

After the therapy, decrease in the levels of IL-1 and IL-6 was observed in all three groups. The decrease in IL-1 was observed in all groups by an average of 2 times and the average values closed in on the control values. Also, a decrease in the cytokine IL-6 in the 1 group was observed by 1.8 times, and in the 2 and 3 groups by 1.4 times.

After treatment a decrease in the average of TNF-α, which is a pro-inflammatory cytokine was revealed in all groups. In the 1st group, the cytokine level was increased now only 2 times. The increase in its level was noted only 1.1 times in the 2nd and 3rd groups. accuracy P <0.001.

Determination of anti-inflammatory cytokines level showed that the level of IL-10 in patients with combined pathology was 3.2 times higher than in the control group (Table 2). In patients with isolated pathologies, the indicator was 1.9 times higher than in the control group. After treatment, the level of IL-10 decreased by 2 times in the 1-group. In the second group, a decrease was observed by 1.6 times and in the third group by 1.4 times.

A study of vascular endothelial growth factor (VEGF) indices showed that the highest indices were in patients of groups 1 and 2 and almost 4 times exceeded the control group (P <0.001). In the 3-group there was also a high indicator, but lower than in the 1- and 2-group, and 2.2 times higher than in the control group (P <0.001).

After the treatment, an accurate decrease in VEGF level was noted, compared with the indicators before treatment. In the 1st group the VEGF level decreased by 2 times, in the 2nd group by 1.9 times and in the 3rd group by 1.8 times.

Table 2: The level of anti-inflammatory cytokines in the blood serum of patients before and after treatment (M±m)

№	Group	IL-10		VEGF	
		Before	After	Before	After
1	The combination of myoma and adenomyosis n = 15	10,6±0,67*	5,0±0,3[a]	1282,6±119,8* P<0,001	628,7±27,4[a] P<0,05
2	Adenomyosis n=15	6,2±0,24*	3,8±0,21[a]	1243,5±127,4* P<0,001	653,2±30,36[a] P<0,01
3	Uterine myoma n=15	6,3±0,74* P<0,001	4,4±0,37[a] P<0,05	725,4±35,2* P<0,001	410,2±20,2[a] P<0,05
4	Control group n=15	3,3±0,14		325,0±22,0	

Note: * - the differences are accurate compared with the control group, [a] - the differences are accurate compared with the data before treatment

Discussion

Thus, the presence of diseases such as myoma, adenomyosis, or their combined forms in the examined patients contributes to the development of an imbalance of pro- and anti-inflammatory cytokines. The increase in their level in the blood is the most significant with combined forms of myoma and adenomyosis compared with isolated forms of myoma and adenomyosis. This indicates the participation of the immune system in the development and progression of such hyperplastic processes of the uterus as myoma and adenomyosis.

The choice of priority for prescribing preparations depended on the activity of myomatous nodules. With proliferating myomata, the preparation UPA was

prescribed first, then dienogest, and for simple myomata – vice versa. The treatment of these diseases contributed to the incremental recovery of pro- and anti-inflammatory cytokines level, which was close to the indicators of the control group.

Simultaneous ultrasound with dopplerometry after the treatment showed that stabilization of the growth of nodes in diameter of 5 and> cm and decrease in the size of small nodes were noted. The grade of blood flow in myoma nodes and in focal adenomyosis worsened, which was characterized by a decrease in the level of VEGF in the blood serum of patients, i.e. indicated the positive changes.

According to the patients survey, the clinical picture of the disease after treatment has also changed. Algomenorrhea disappeared in 24.2% of cases and its intensity decreased significantly in 61.8%, a slight improvement was noted by 14.0%. Reproductive function recovered in 67.9% within a year after the treatment.

Conclusions

1. The combined forms of myoma and adenomyosis in women of reproductive age are characterized by more significant imbalance of pro- and anti-inflammatory cytokines.
2. After the treatment, an accurate decrease in the level of pro-inflammatory cytokines is noted, which confirms the role of immunity in the development of myoma and adenomyosis.
3. The effectiveness of the treatment of combined forms of myoma and adenomyosis is characterized by the improvement in the clinical progression of the disease and restoration of fertility in 67.9% of cases.

Ethical Clearance: No ethical approval is needed.

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Pyeloplasty in Children: Advantages of External Trans-Anastomotic Drainage

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Abstract

Introduction: There are several forms of successful treatment and prevention for obstruction of the pyelourethral segment in pediatric patients. However, each method has its advantages and disadvantages. Namely, some those widespread surgical techniques frequently associate with different complications and subsequent financial deprivations. Therefore, the optimal method of external trans-anastomotic drainage is still disputable. In this study, we studied the results of pyeloplasty in children using a transanastomotic catheter.

Method: In this observational study, which was conducted from 2013 to 2018, we evaluated the outcomes of pyeloplasty in 132 childrenunder 3 years old. For all patients a trans-anastomotic catheter for urinary evacuation was used during the postoperative period. The observation period ranged from 6 months to 3 years (an average of 22±6,8 months). Antegrade pyelography was performed in 119 patients on the 6th day of postoperative period. The average hospital stay was 8.4 days (ranging from 6 to 16 days), most of the patients were discharged from hospital 7 days after surgery.

Results: 137 pyeloplasty was performed on 132 patients using a transanastomotic catheter. The observation lasted from 6 months to 3 years. Antegrade pyelography was performed in 119 patients on the 6th day after surgery. Of those 119 pyelograms taken, 108 (91 percent) showed adequate drainage after pyeloplasty. In 11 patients, partial obstruction was revealed, while in 8 (73 percent) children the possibility of the anastomosis returned to normal on its own about within 12 days. The transanastomotic catheter was removed on average 7-8 days after surgery. The average hospital stay was 8.4 days.

Conclusion: Urine evacuation with transanastomotic catheter after pyeloplasty in pediatric patients is highly effective and safe. Other comprehensive studies may be required to prove the further upsides of this technique.

Keywords: *Pyeloplasty, hydronephrosis, ureteral obstruction, children, drainage.*

Introduction

Dividing pyeloplasty is a common method of surgical treatment for obstruction of the pyelourethral segment in children with a success rate of 94% to 99% (Shahzad et al, 2014)[1]. As with all surgical interventions on the urinary tract, there are various method of urine diversion after pyeloplasty. Currently, many surgeons use an internal double pig tail stent (Elmalik et al, 2008 and Hsu et al, 2016)[2], or Salle's transanastomotic external stent, and some authors prefer non-drainage pyeloplasty (Kagantsov et al, 2012)[5]. According to some authors'

results on postoperative drainage of the upper urinary tract using a nephrostomy tube showed an increase in the incidence of urinary tract infections, the hospitalization period of patients, and the risk of secondary obstruction of the pyelourethral segment (Sibley et al, 1987)[8]. At the same time, there are ongoing discussions in the scientific literature regarding the optimal method for urine diversion after dissecting pyeloplasty in pediatric patients.

In this study, we studied the results of pyeloplasty in children under 3 years old using a transanastomotic catheter.

Materials and Method

This retrospective study was conducted from 2013 to 2018, included data from children under the age of 3 years with obstruction of the pyelourethral segment. After surgery, a transanastomotic catheter for postoperative urine evacuation was installed in all patients. In all patients, dividing pyeloplasty was performed through lateral access. After the first suture of the anastomosis placed between the pelvis and ureter, the distal end of the transanastomotic catheter (PVC catheter with a diameter of 8-10Fr) was inserted into the ureter, the other end of the catheter was taken out as a pyelostomy through the anterior wall of the pelvis. With further stitches the posterior semicircle of the anastomosis was formed, then the anterior one. When forming the anastomosis, a synthetic polyfilament suture material (6/0) was used. At the end of pyeloplasty, the intubating catheter was not fixed to the pelvis, since excessive fixation can significantly complicate its removal in the postoperative period. At the same time, a transanastomotic catheter adequately provided urine evacuation in two directions. Urine with a hemorrhagic shade, extending through the urinary catheter and the absence of urine discharge through the safety drainage, were indirect signs of the adequate function of the transanastomotic catheter. With adequate catheter function, ultrasound monitoring and antegrade pyelography were performed on the 6th dayafter operation in order to check the location of the catheter and the passabilityof the anastomosis. Then the catheter was clamped for 1 day. In the absence of parental complaints about the child's anxiety and negative dynamics during the control ultrasonography, the catheter was removed on the 7th day after the surgery.

Results

In 132 patients, 137 pyeloplasty was performed using a transanastomotic catheter (Table 1). The observation period ranged from 6 months to 3 years (average 22–6.8 months). Antegrade pyelography was performed in 119 patients on the 6th day after surgery. Of those 119 pyelograms taken, 108 (91 percent) showed adequate drainage after pyeloplasty. One patient experienced a slight extravasation of urine, which resolved independently after 1 week. In 11 patients, partial obstruction was revealed, while in 8 (73 percent) children the possibility of the anastomosis returned to normal on its own about within 12 days. The anastomosis impassibility lasted longer only in 3 patients, however, in two of them, normal urodynamics recovered after 16 days. Persistent obstruction of the pelvic ureteric segment was detected in one patient, which required installation of an internal stent. Ultimately, with intravenous urography 6 months after surgery, this patient had a good urine evacuation through the anastomotic zone. The transanastomotic catheter was removed on average 7-8 days after surgery. The average hospital stay was 8.4 days (ranging from 6 to 16), and discharge from the hospital occurred mainly on the 7th day after surgery.

Table 1: Clinical distribution of patients

Clinic course	Number of patients	
	n	%
Asymptomatic course	115	87
Palpable formation	4	3
Anxiety in the child (abdominal/lumbar pain)	1	0,8
Urinary tract infection	12	9,2
Total amount	**132**	**100**

Postoperative complications are provided in Table 2. None of the patients had progressive postoperative obstruction and re-pyeloplasty was required. Renal function did not deteriorate during postoperative examination, and no patient was noted soreness or anxiety associated with postoperative drainage with a transanastomotic catheter.

Table 2: Postoperative comlications

Complications	n	%
Urinary tract infection	2	1,5
Temporary obstruction of the ICP (internal stent)	1	0.7
Insignificant extravasation	1	0.7
Total	**4**	**2.9**

Discussion

Despite the presence of numerous method of urine derivation in the postoperative period, the optimal choice of the method of postoperative urinary evacuation after pyeloplasty remains under debates. While some authors argue that when using a nephrostomy, the risks of developing urinary tract infections increase and the length of hospitalization of patients elongates. other researchers, on the other hand, argue that using internal stents requires repeated anesthesia to remove the latter (Sibley et al, 1987)[8]. However, in many publications devoted to postoperative urinary diversion after pyeloplasty, the influence of the derivation method on the total number of long-term results of pyeloplasty is not well studied. They just comparedthe results with each other. So, Hsu et al (2016)[3], comparing the radiological results of pyeloplasty with nephrostomy and drainage pyeloplasty, did not note the significance of the differences between them. It should be emphasized that the improvement of surgical method, as well as the accessibility of modern suture materials, led to a significant improvement in postoperative results (Mendrek et al, 2019)[6]. The use of prophylactic antibiotic therapy also reduces the incidence of urinary tract infections, as evidenced by the results of our study. Thus, the incidence of urinary tract infections in our study was marely 1.5 percent. Therefore, in our opinion, preventive antibiotic therapy should be continued throughout the entire period of urine diversion. In this case, catheterization of the bladder in the early postoperative period is extremely important for adequate evacuation of urine from the bladder.

Wollin et al (1989)[9] believed that an empty bladder (low intravesical pressure) prevents the newly formed anastomosis from increased pressure, thereby reducing the risk of extravasation through the anastomosis. In addition, low intravesical pressure improves urodynamics in the anastomotic zone in the early postoperative period.

The literature describes the shortcomings of external method of urine evacuation, such as a longer period of hospitalization of patients after pyeloplasty (Hussain et al, 1994)[4]. The average hospital stay, in these earlier studies ranged from 9.8 to 24.8 days. However, in our study, the average hospital stay was 8.4 days. Meanwhile, removal of the transanastomotic catheter was usually performed without any difficulties, which allowed to avoid repeated anesthesia. In addition, external method of urine derivation have recently been criticized due to the increased frequency of various postoperative complications. However, the results of this study prove that using a transanastomotic catheter has several advantages. At the same time, the transanastomotic catheter provides urine derivation in two directions, allowing surgeons to adequately assess the state of the anastomosis. It also prevents the postoperative bending of the anastomosis zone, acting as an internal stent. In addition, it completely eliminates the need for repeated anesthesia during its removal, which is mandatory when removing the internal stent.

Conclusion

In our patients, the number of postoperative complications did not differ from those presented in the literature. The transanastomotic catheter serves as a reliable protective mechanism against postoperative edema and kink in the anastomotic zone, and prevents the extravasation of urine. And also makes it easy to perform x-ray examinations to assess the possibility of the pyelourethral segment. Furthermore, using a transanastomotic catheter completely eliminates the need for re-anesthesia for the removalof it.

Study Limitations: Further researchesare required to clarify the other advantages of transanastomoticcathetering in children. This was an observational study. Further comperative studies and secondary researches are needed to identify the most optimal therapy for children after pyeloplasty.

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Patient Consent: Written informed consent was obtained from allparticipants of the research for publication of this paper and any accompanying information related to this study. A copy of the written consent is available forreview by the authors.

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Pathogenesis Specifics of Allergic Reactivity in Hypertensive Conditions of Pregnants

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Abstract

Introduction: Significant allergic reactions and anaphylactic type cytotoxicity are registered in the pathogenesis of cell damage in pregnant women with hypertensive conditions. Determining role of indirect mast cell degranulation and allergic changes in neutrophils during pregnancy that complicated by preeclampsia, allows to identify the allergic status of the body, which is very importance for improving the quality of early diagnosis, treatment and prevention. The purpose of the study was to identify the allergic reactivity of pregnant women suffering from hypertensive disorders and to develop practical recommendations to improve the quality of early diagnosis.

Materials and Method: We observed 6 group of participants: pregnant women with clear allergy clinic, uncomplicated preeclampsia, pregnant women with light stage of preeclampsia, latent sensitization, pregnant women with preeclampsia combined with clear allergy clinic, conditionally healthy pregnant women – 1st control group, practically healthy non-pregnant women – 2nd control group, practically healthy men – 3rd control group. Using non-infection allergens, we called different types of allergic reactions and conducted clinical and allergic examinations, general test of bleed, urine, feces, indirect mast cell degranulation test and neutrophil alteration test.

Results: Within all control groups, the rate of positive reaction against the allergic agents made up less than 10 percent. In all pregnant women with clear clinical picture of allergies complicated or uncomplicated preeclampsia, as well as in pregnant women with preeclampsia with latent sensibilization, mast cell degranulation indexes were 4.5-8.6 times higher than that of control group. Among 90 examined pregnant women, positive reactions of indirect mast cell degranulation were observed in 204 cases.

Conclusion: In cell damage pathogenesis during preeclampsia, anaphylactic and cytotoxic type allergic reactions play important role. The positive reactions of indirect mast cell degranulation in pregnant women with an explicit allergy complicated by preeclampsia is significantly higher than in pregnant women with preeclampsia with hidden sensitization.

Keywords: *Hypertensive conditions in pregnancy, preeclampsia, allergy.*

Introduction

Preeclampsia of pregnant women has been continuing to be one of the urgent problems of modern medicine. This pathology, giving different complications during pregnancy and childbirth, remaining as one of the causes of maternal and perinatal morbidity and

mortality. Preeclampsia is especially lasts severely in pregnant women who permanently live in ecologically unfavorable regions (Sukhikh et al, 2015)[1]. According to world statistics, about half a million women die due to the different complications related to pregnancy and childbirth (Tumanova et al, 2017 and Mukhamedkhanova et al, 2019).[2]

According to results of researches conducted by Stokoz et al (2016)[3] and Chulkov et al (2015), about 1–4% of women aged 18–29 years and about 5–15% of women aged 30–39 years suffered from chronic hypertension between 1999 and 2004 in the United States. Along with this, in 2004, compared to 1998, the number of pregnancy complications caused by preexisting arterial hypertension increased by 50% and reached up to 1.7% of the total number of complications (Wang et al, 2019). Moreover, the number of preterm births and newborns with low weight for gestational age, as well as perinatal mortality rates in such pregnant women were 2-3 times higher compared to normotensive women (Perfilova et al, 2014; Paidas et al, 2016; and Wang et al, 2019)[4],[7]. The incidence of gestational hypertension increased from 10.7 to 30.6 per 1000 births, and 17% of pregnant women with gestational hypertension subsequently developed preeclampsia (Mosimann et al, 2019 and Wang et al, 2019).[5] However, it should also be mentioned that the maternal risks associated with gestational hypertension are usually less than 2 times lower than those of women suffering from chronic hypertension or moderate preeclampsia.

According to the data provided by WHO, the proportion of hypertensive syndrome in the structure of maternal mortality is about 20-30%, every year more than 50 thousand women die during pregnancy owing to complications associated with maternal arterial hypertension. Arterial hypertension increases the risk of detachment of a normally located placenta, massive coagulopathic bleeding. It can also cause eclampsia, impaired cerebral circulation, and retinal detachment as well (Marotti et al, 2017)[6].

The frequency of preeclampsia development is not same in different countries and depends to a large extent on the criteria used for its diagnosis. Although the problem of preeclampsia is studied from different perspectives, many of its aspects remain poorly understood (Stokoz et al, 2016)[4]. Among them, in our opinion, the allergy, more precisely, allergic reactivity of the body, plays very importance in the etiology, pathogenesis, clinic of preeclampsia and its complications. Chulkov et al, 2015; Perfilova et al, 2014; and Paidas et al, 2016)[8].

The prevalence of hypertensive conditions during pregnancy is ranges around 7–30 percent. This pathology worsens the prognosis in mother and in children, and is also causes perinatal mortality. Different complications like placental insufficiency, premature detachment of a normally located placenta, eclampsia, cerebral hemorrhage, acute heart failure, acute renal failure, syndrome of DIC develop with arterial hypertension (Perfilieva et al, 2015)[7].

Introduction of specific, clinical and allergological examinations of pregnant women with preeclampsia: collection of an allergic history, clinical and functional studies, in vitro allergy tests (indirect mast cell degranulation test, neutrophil damage index, determination of the functional activity of neutrophils by tetrazole recovery techniques dye and phagocytosis) into the complex of clinical and laboratory studieswill contribute to the early detection of allergic diseases in pregnant women, which has a great diagnostic, therapeutic and preventive value.

A study of the regional characteristics of the prevalence of pre-eclampsia of pregnant women in different regions of Uzbekistan using the methodology of epidemiological studies under the ISAAC international program allows us to clarify the true disease incidence the population (Mukhamedkhanova et al, 2019)[3].

Purpose of the study is to determine the allergic reactivity of the pregnant women body suffering from hypertensive disorders and to develop practical recommendations that improve the quality of early diagnosis of this serious illness based on the allergological concept. To determine the importance of combining allergies with hypertensive conditions in pregnant women. To determine the role of regional pollen factors in the development of an immediate type allergy in the pathogenesis of cell damage in pregnant women with pre-eclampsia using tests of indirect mast cell degranulation and allergic neutrophil alteration.

Materials and Method

168 individuals were under observation, including pregnant women with a clear clinic of allergy, uncomplicated preeclampsia (n=30), pregnant women with a slight degree of preeclampsia, latent sensitization (n=30), pregnant women with preeclampsia combined

with a clear allergy clinic (n=30), conditionally healthy pregnant women (n=30) - control group No. 1, practically healthy non-pregnant women (n=23) - control group No. 2, practically healthy men (n=25) - control No. 3. The age of pregnant women ranged from 17 to 39 years. The age of non-pregnant women and healthy men was 20-35 years old. We studied pregnant women with gestational age from 19 weeks to 40 weeks of their pregnancy. Among them, the first pregnancy - n=42 (35%); re-pregnants n=44 (36.7%); multiparous women n=34 (28.3%).

Among the allergic diseases in the examined pregnant women there were: allergic rhinitis, sinusitis, conjunctivitis-24 (20%); urticaria, Quincke's edema, neurodermatitis-15 (12.5%); drug allergy 8 (6.7%); food allergy 6 (5%); bronchial asthma 6 (5%); anaphylactic shock 1 (0.8%).

The results of studies in all pregnant women with allergic diseases were approximately the same. The numerical differences between the indicators were unreliable. Therefore, we combined them into one group: pregnant women with hypertension, combined with a clear clinic of allergic diseases.

The following standard non-infectious regional allergens were used: pollen (wormwood, quinoa, chinar, kenaf, aylanthus), household (house dust), epidermal (cat, dog hair, bird feather). Tissue allergens (placenta, kidneys) were prepared in a well-known way. The following method were used: clinical and allergic examination of patients, general blood tests, urine, feces, indirect mast cell degranulation test, neutrophil alteration test.

Allergy in the examined women was manifested in the form of allergic rhino conjunctivitis, allergic dermatoses, and even anaphylactic reactions[8]. In those cases, when pollen allergens were important in the etiology of allergies, a clear seasonality of the disease was observed (mainly in spring and summer seasons)[9]. With sensitization by household and epidermal allergens, the seasonality of the disease was not observed. When sensitizing to food allergens, patients indicated a connection between exacerbation of the symptoms of the disease and the intake of certain foods[10]. When eliminating causally significant foods, a remission of the disease was observed. Specific allergy diagnostics was carried out on the basis of a comprehensive clinical and allergological examination of patients, as well as in vitro allergic tests. The complex principle of specific diagnosis compares favorably with the fact that it allows you to identify specific allergic antibodies, establishes the specificity of the sensitization of an organism to a particular allergen, and conduct differential diagnosis[11]. An allergic history was collected on the basis of a specially compiled questionnaire. An allergic test for indirect mast cell degranulation (MCD) was performed according to generally accepted method. Anti-tissue antibodies were determined by the conventional photometric method. The phagocytic activity of neutrophils in the peripheral blood of patients was determined according to A. Shteltsner. An allergic neutrophil alteration test was performed according to V.A. Fradkin. To determine the functional activity of neutrophils, we used the Nitro Blue-Tetrazolium Test (NBT test)[12].

Results

In all control groups examined (conditionally healthy pregnant women, practically healthy non-pregnant women and practically healthy men), the frequency of positive reactions to tested pollen, domestic and epidermal allergens was below 10 percent and ranged from 4.4 + 0.2 - 3. 6 + 0.23. These indicators are non-specific and indicate the absence of sensitization of the body, since there are no examined specific IgE in the blood serum.

In all pregnant women with a clear clinical picture of allergies complicated or uncomplicated preeclampsia, as well as in pregnant women with preeclampsia with latent sensitization, mast cell degranulation values were significantly higher by 4.5-8.6 times compared to the control, and were within 30.2 + 0.92 - 38.0 + 0.44 ($p < 0.05$).

In general, among 90 examined pregnant women, positive reactions of indirect mast cell degranulation were observed in 204 cases, including pollen allergens in 115 (56.4±3.4%), household (house dust) in 50 (34.5±4.9%), epidermal - in 39 (19.1±2.7%).

The degree of mast cell degranulation in most pregnant women (92.1±3.8 - 94.5±10.1%) with anexplicit allergy clinic, regardless of the presence or absence of preeclampsia, was expressed in ++ and +++, while as in the majority of pregnant women with preeclampsia (91.7±7.9%) with latent sensitization, the degree of mast cell degranulation was weakly expressed (+).

In the vast majority of pregnant women with a clear allergy clinic, cases of combined polysensitization predominated, that is, simultaneous increased sensitivity to several unrelated allergens. For example, pollen+household, pollen+epidermal, etc. Only monosensitization to a particular allergen was observed in pregnant women with latent sensitization. Neutrophil damage in conditionally healthy pregnant women (control group No. 1) and practically healthy non-pregnant women (control group No. 2) were in the range of 0.019±0.001 - 0.002±0.001 (when using placental antigen) and 0.035±0.003 - 0.04±0.002 (when using renal antigen). The difference in the digital data of these two groups of controls is unreliable ($p > 0.05$). This fact indicates that the examined control groups of women have a minimal amount of anti-tissue antibodies in their blood serum. However, these antibodies do not cause serious tissue damage. Pregnant women with overt allergies, uncomplicated preeclampsia, showed an increase in neutrophil damage to 0.04±0.003 - 0.1±0.03, which is 2-2.5 times more than the controls ($p < 0.05$). In pregnant women with preeclampsia, neutrophil damage indicators are also greater than in pregnant women with latent sensitization 3-4 times (0.08±0.004 - 0.13±0.004), and in pregnant women with overt allergies - 4-5 times (0.1±0.002 - 0.19±0.004). These facts indicate the development of autosensitization in pregnant women, complicated by preeclampsia to the tissues of the placenta and kidneys.

First of all, we were interested in the issue of the presence of an immediate allergy. To clarify this issue, we used a methodological technique that allowed us to identify specific allergic antibodies belonging to the IgE class. In all pregnant women with allergies, regardless of the presence or absence of symptoms of hypertension in the blood serum, specific allergic antibodies to the tested allergens were detected. The frequency of positive reactions to the studied allergens in pregnant women with allergies without hypertension and in pregnant women with allergies combined with hypertension was approximately the same, respectively, 15.5-73.0 and 25.6-80.0 percent. The frequency of positive reactions in pregnant women with hidden sensitization combined with hypertension to pollen allergens was 8.6%, domestic - 13.3 percent, epidermal - 6.7 (average 0.6±2.0). The results of studies of this category of patients indicate that in pregnant women with latent (latent) sensitization, the titer of allergic antibodies, in comparison with pregnant women with an explicit allergy clinic, is significantly low ($p < 0$ 05).

However, they are all potential allergy sufferers, that is, at any moment the symptoms of allergies can occur. In addition, these data allow us to conclude that the presence of hypertension cannot serve as an obstacle to the synthesis of specific allergic antibodies in patients.

Discussion

In our opinion, in case of hypertensive conditions of pregnant women, there is no inhibition of the functional activity of immunocompetent cells responsible for the synthesis of specific allergic antibodies. This is evidenced by the absence of a difference in the frequency of positive reactions of indirect mast cell degranulation in two groups of pregnant women with an explicit allergy clinic: combined and not combined with hypertension, as well as positive reactions in pregnant women with latent sensitization combined with hypertension[11].

In the process of formation and development of allergic reactivity of the body, the inhibition of the functional activity of neutrophilic leukocytes is noted, which negatively affects the factors of cellular nonspecific protection of the body (i.e., the phagocytic activity of leukocytes decreases, the phagocytic number, phagocytic index, and the strength of phagocytosis).

Allergy in most cases is characterized by the development of polysensitization. In this regard, it was of some interest to analyze our data from these positions. In the literature, polysensitization means the presence of hypersensitivity in the same patient to two or more types of allergens. From our point of view, “the degree of development of an allergy clinic depends on the nature, nature and types of allergens. In this regard, depending on the nature of allergens, the following polysensitization options should be distinguished: 1) true - when one organism is sensitized to two or more related allergens at the same time: only pollen, or only domestic, or only epidermal, etc.; 2) combined - when one organism is sensitized to two or more non-infectious allergens of different nature: pollen and household, pollen and epidermal etc.; 3) mixed - when one organism is simultaneously sensitized to two or more allergens of non-infectious and infectious nature. According to our data, in pregnant women with a clear allergy clinic, cases of true and combined polysensitization predominated regardless of the presence or absence of hypertension: 93.3 and 76.7 percent respectively.A similar pattern was also observed in patients with latent sensitization - 90.0 percent.

At least two types of allergies in pregnant women with hypertensive conditions are noted: a clear clinical manifestation of allergic diseases and latent (latent) sensitization of the body without clinical manifestations of allergic diseases.

The clinical forms of allergy manifestation are diverse: pollinosis, allergic rhinitis and sinusitis, allergic dermatitis, bronchial asthma, anaphylactic shock. Hypertensive conditions associated with allergies are more likely to occur in repeatedly and multiparous women.

With a combination of hypertensive conditions with allergies, cases with a more severe clinical course of the underlying disease predominate, induced, premature and belated deliveries are more common, and surgical interventions are more often used.

In the etiology of allergies in pregnant women with gestosis, exogenous non-infectious allergens are essential. In the pathogenesis of cell damage during gestosis of pregnant women, a complex combination of allergy mechanisms of immediate (chimeric) and delayed (cellular) types is detected.Thus, the conducted studies allow us to come to the following generalizations - preeclampsia often occurs and develops against the background of an allergically altered body reactivity. It can be assumed that the severity of the clinical course of preeclampsia and its complications depends on the presence of a latent or overt allergy. The sensitization of the body in pregnant women is confirmed by a positive reaction of indirect mast cell degranulation, which indicates the presence of specific allergic antibodies (IgE) in the blood serum of the examined. With clinically pronounced forms of allergy, the sensitization of pregnant women, regardless of the presence or absence of preeclampsia in them, is clearly multivalent. The frequency of mixed forms of polysensitization is significantly higher. With latent forms of sensitization of the body of pregnant women, only monosensitization is noted.

The introduction of specific, clinical and allergological examinations of pregnant women with hypertensive disorders into the complex of clinical and laboratory studies: collecting an allergic history, clinical and functional studies, and setting up allergic tests in vitro will contribute to the early detection of allergic diseases in pregnant women, which has a great diagnostic, therapeutic and preventive value.

Conclusion

In the pathogenesis of cell damage during preeclampsia of pregnant women, allergic reactions of anaphylactic and cytotoxic type are of significant importance. The degree of positive and sharply positive reactions of indirect mast cell degranulation in pregnant women with an explicit allergy clinic complicated by preeclampsia is significantly higher than in pregnant women with preeclampsia who have hidden sensitization. Allergies in pregnant women are polyvalent in nature. Furthermore, determination of the parameters of the test for indirect mast cell degranulation and allergic neutrophil alteration in pregnant women complicated by preeclampsia, in combination with other clinical and laboratory studies, makes it possible to identify the allergic state of the body, which is of great importance in improving the quality of specific diagnostics, therapy and prevention.

Study Limitations: Further research is needed to clarify various aspects of the pathogenesis of preeclampsia from the perspective of modern allergology.

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Testing Techniques in the Evaluation of Severity of HIV-Associated Neuro-Cognitive Disorder in Children

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Abstract

Introduction: The number of HIV infected children exceeds 3 million worldwide. The most severe clinical manifestations and complications of the disease are HIV-associated neurocognitive disorder and HIV encephalopathy. These factors make the scientists search for method of optimizationin the diagnosis of neuro-HIV and identifying various pathological processes that determine the clinical course.

Materials and Method: 154 children were tested with HIV diagnosis. All patients were examined for neurological, psychological and cognitive disorders. Quality of life of sick children were examined by the questionnaire of PedsQL. In order to compare the results, study included 20 healthy adolescents (control group - CG), who also tested using the above method. Using the MS Excel software average values summarized and their standard deviations were calculated.

Results: All patients were diagnosed with 3rd and 4th stages of HIV. HIV-associated neurocognitive disorder: grade 2 in 100 patients – 64.94% and grade 3 - in 54 patients - 35.06%. Epilepsy was diagnosed in 31 patients, peripheral polyneuropathy in 7, vacuolar myelopathy in 8, and consequences of acute cerebrovascular accident in 9 with focal neurological deficit in 2. The Raven test: average test result was 18.68±4.51%. Aggressive behavior: negativism prevailed in 74 patients, and in 11 cases (7.14%) it was combined with physical aggression, and in 12 cases (7.79%) - with resentment. In addition, indirect aggression was detected in 18 patients (11.69%), resentment in 41 patients (26.62%), suspiciousness in 10 patients (6.49%), and 19 patients (12.34%) - irritation. Quality of life questionnaire revealed difficulties associated with physical activity (18.30±4.51 with maximum score of 32 points), with emotional states (15.50±2.23 with maximum of 20), social activity (14.10±2.93 with maximum of 20) and life in school (19.29±1.23 with maximum of 20).

Conclusion: HIV-associated neurocognitive disorder is manifested by a deterioration in intelligence, various forms of aggressive behavior and is associated with a decrease in the quality of life in terms of physical, emotional, social activity and learning at school.

Keywords: *HIV infection, HIV-associated neuro-cognitive impairment, children, quality of life.*

Introduction

About 3.4 million children worldwide are infected with the human immunodeficiency virus (HIV)/ AIDS. A significant part of them who have indications for antiretroviral therapy does not receive it today (UNAIDS, 2012)[1]. Neurological manifestations of HIV infection are common in both children and adults. A wide range of neurological conditions can be caused by the HIV, however, damage of the central nervous system

due to this virus, which affects the development of the brain in the embryo and baby, leads to the most severe complication of damage to the nervous system - HIV encephalopathy. In children not receiving treatment, the prevalence of the disease varies from 20% to 60% (Donaldet al, 2015; Foster et al, 2006 and Van Rie et al, 2007)[2]. The range of neurological complications that occur in children infected with HIV is quite wide and complex. One of the most common manifestations is HIV-associated neurocognitive disorder. In 18% of patients infected with HIV, it is the first clinical manifestation of AIDS (Saidkhodjaeva et al, 2019)[3];in 12 – 23 percent of them develop severe encephalopathy, usually during the first year of their life (Van Rieet al, 2008; Chiribogaet al, 2005; and Tardieu et al, 2000)[4]; in 35-50 percent of those children will develop encephalopathy in the future (Van Rie et al, 2007)[5]; in 50–90 percent of all HIV-infected infants and children develop anti-retroviral therapy deficiency (Kovacs, 2009).[6] Behavioral disorders are observed in 35 percent of all HIV-infected patients (Govenderet al, 2011), psychiatric manifestations - in 48-61 percent (Mellinset al, 2006; Wood et al, 2012; and Musisi et al, 2009).[7]

HIV affects the central nervous system shortly after the onset of systemic infection. Penetration into the central nervous system is largely mediated by infected macrophages-monocytes or $CD4^+$-T-lymphocytes (the effect of a Trojan horse), or both. Viral replicas isolated in the brain are predominantly CCR5-trophic, suggesting that virus invasion is driven by monocytes (Kramer-Hämmerleet al, 2005 and Banks et al, 2006). Once it penetrates the central nervous system, HIV basically infects perivascular macrophages and microglia, and blocks the proliferation of nerve progenitor cells. The expression of inflammatory neurotoxic neurotransmitters occurs in various types of CNS cells. Pathological changes include the formation of microglial nodules and large multinucleated cells, reactive astrogliosis, and the loss of a specific subpopulation of neurons. Damage and death of neurons are caused by several direct and indirect pathogenetic mechanisms. The consequences are the development of neurological and neurocognitive disorders.

These factors make us look for approaches to optimizing the diagnosis of neuro-HIV and identifying various pathological processes that determine the clinical picture (Mellinset al, 2006)[8]. Children with particularly weak cognitive and psychomotor functioning were most at risk of developing the disease. Prospective observational studies have found that significant neurocognitive impairment leads to a worse outcome of the disease and an increased risk of death, even against the background of combined etiological and pathogenetic treatment (Pearson et al, 2000).[9] Compared to healthy children, HIV-infected children have a much weaker development of early motor and cognitive skills. Although no clinical or clinical prognostic prediction scheme has yet been proposed, risk factors associated with HIV encephalopathy include maternal and child immune status, high plasma viral load, high levels of circulating monocytes, infection time, transmission route, and treatment options at an early stage (Le Doaréet al, 2012 and Abubakar et al, 2008).[10]

A study in the Democratic Republic of Congo examining the effect of HIV on the development of the nervous system in preschoolers found that HIV-infected children had a severe delay in cognitive function, motor skills, speech, and understanding[11]. Illustrating the complexity of the etiology of HIV-related cognitive and behavioral problems, the study also found that AIDS-infected orphans and HIV-negative children whose mothers had AIDS show a strong delay in their neurocognitive development, albeit to a lesser extent, in the area of development. than HIV-infected children (Van Rie et al, 2008)[12]. A review article of all studies in South Africa, evaluating the development, knowledge and behavior of HIV-infected children as their primary outcome, found evidence of a delay in cognitive function, speech and motor skills in children suffering from HIV[13]. However, this article also emphasizes the lack of information in this area, as well as the fact that almost all studies focus on a group of preschool children[14]. Also, according to the results of the study, it was shown that HIV affects all areas of the child's functioning, motor development is most obvious in terms of severity, early onset and resistance in all age groups. However, the development of motor skills was the most widely evaluated area, while the development of the language was less vigorously evaluated, therefore an accurate quantitative assessment of the effect cannot yet be made. (Abubakar et al, 2008).[15]

Purpose of the study is to evaluate the diagnostic significance of various testing method in assessing the severity of HIV-associated neuro-cognitive impairment in adolescent patients.[16]

Materials and Method

Study Population: The study included 154 children (92 boys - 59.74% and 62 girls - 40.6%) who are registered with HIV infection and are hospitalized in a Specialized Clinic of Infectious Diseases of the Republican AIDS Center (Tashkent, Uzbekistan) and receiving antiretroviral therapy (HAART). The mean age of the patients was 14.53±1.58 years (12-18 years), the duration of HIV diagnosis was 7.05±3.36 years (1-13 years), the duration of antiviral therapy was 6.41±3, 47 years old (1-13 years old). In 23 patients (14.94%), the vertical route of infection was diagnosed, and non the mother took HAART during pregnancy. In other children, the infection pathway is identified as parenteral. The average virus concentration was 345.85±181.45 copies/ml, while in 23 patients (14.94%) HIV RNA in the blood was not determined. The average number of $CD4^+$ cells was 461.91±230.32 in 1 mm^3 of blood.

Neurological Examination: A neurological study included a standard neurological examination, including an assessment of consciousness and some mental functions (speech, praxis, gnosis), functions of the cranial nerves, the state of the motor and sensory spheres, the autonomic nervous system, as well as neurophysiological and neuroimaging method of research (electroencephalography and magnetic resonance imaging).

Psychological and cognitive examination: All patients included in the study underwent the test aimed for studying the psychological and cognitive status, including test method: "The Raven progressive matrix scale" (state of intelligence), the Bass and Darki test modified by G.V. Rezapkina (diagnosis of various forms of aggressive behavior), and a pediatric quality of life questionnaire (PedsQL) for adolescents 13-18 years old.[17]

Intelligence Test: The Raven test, proposed for assessing the level of intelligence, is based on the use of the ability to learn based on the generalization of one's own experience and the creation of schemes to handle complex events, logical thinking. This test, convenient in application and simplicity in interpretation, repeatedly confirmed high indicators of validity and reliability.[18] The test allows it is not associated with linguistic abilities and skills and does not depend on the level of education. The Raven matrix consists of 60 images combined into 5 matrices, in each of which it is necessary to select the missing fragment, using the identified patterns.[19] In each matrix, tasks are progressively complicated. test tasks are performed without time limit, but it is noted how many tasks are completed correctly in the first 20 minutes. Thus, the Standard Progressive Raven Matrices test can be used both as a speed test (with time constraints on task execution) and a performance test (without time constraints).[20] The choice of the test application regimen should be made depending on the purpose and diagnostic conditions (first of all, the possibility of ensuring long-term continuous work of the test subject). The assessment is the percentage of correct answers, expressed as a percentage. Interpretation of the test allows us to distinguish 5 degrees of intellectual development: 1st degree - more than 95% - high intelligence; 2nd degree - 75-94% - intelligence is above average; 3rd degree - 25-74% - average intelligence; 4th degree - 5-24% - intelligence is below average; 5th degree is a defect.[21]

Test for behavior: Bass-Darki test, modified by G.V. Rezapkina, proposed in 2006, allows you to diagnose various types of aggressive behavior. The test includes 35 statement questions. The test subject is invited to apply the statement to patients.[22] If he reacts in a similar way, the answer is assigned 1 point. During the interpretation, all issues are divided into 7 forms of aggression. The form that received more than 3 points is recognized as dominant in the subject. According to the test results, the following forms of aggressive behavior are distinguished: physical aggression (statements 1,8,15,22,29), indirect aggression (2,9,16,23,30), irritation (3,10,17,24,31), negativism (4,11,18,25,32), resentment (5,12,19,26,33), suspiciousness (6,13,20,27,34), verbal aggression (7,14,21,28, 35).[23] The test is based on self-esteem. Its accuracy depends on the frankness of the subject. According to the results of the testing (increased level of aggression, inability to control emotions, etc.), it is not recommended to choose professions related to communication, maintenance, education, training - that is, all professions related to people. Low values for this test testify to your delicacy, pliability and non-conflict. However, these indicators may indicate a lack of perseverance in achieving their goals and defending their position.[24]

Quality of life Examination: The study of the quality of life of children is a new topical area of interdisciplinary research in domestic health care. The development of a methodology for studying the quality of life in pediatrics opens up the possibility of a comprehensive analysis of the physical, psychological

and social functioning of children. For this, our study used the pediatric quality of life questionnaire PedsQL, version 4 (for children aged 13-18), published in 1998 and translated into Russian, includes 23 situations that describe physical, emotional, social activity and school life. The questionnaire is recommended for studying the quality of life of healthy and sick children; using these tools, normative indicators of quality of life for the child population can be obtained. The use of the created tools in children with various diseases allows to expand knowledge about the attitude of children of a given age to their own health problems, treatment, degree of satisfaction with treatment; opens up new possibilities for assessing the impact of various diseases on the physical, psychological and social functioning of sick children. The test subject is asked to rate how each of these situations created difficulties in the past month. At the same time, 0 points are assigned to a situation that has never created difficulties, 5 points - in the case of constant difficulties with the described action. Thus, the maximum score for each situation is 5 points (almost impossible activity), the minimum score is 0 (no difficulty). The maximum score in terms of physical activity is 32 points, emotional social and complex activity is 20 points, the total maximum score is 92 point.

For comparison, the study included a group of 20 healthy adolescents of comparable age (control group - CG), who also tested using the above method.

Statistical Analysis: All data was entered into the software of MS Excel tables to calculate average values and their standard deviations. Intergroup difference was evaluated using Student's t-test for 2 comparisons. Frequency comparison was carried out using the chi-square table criterion.

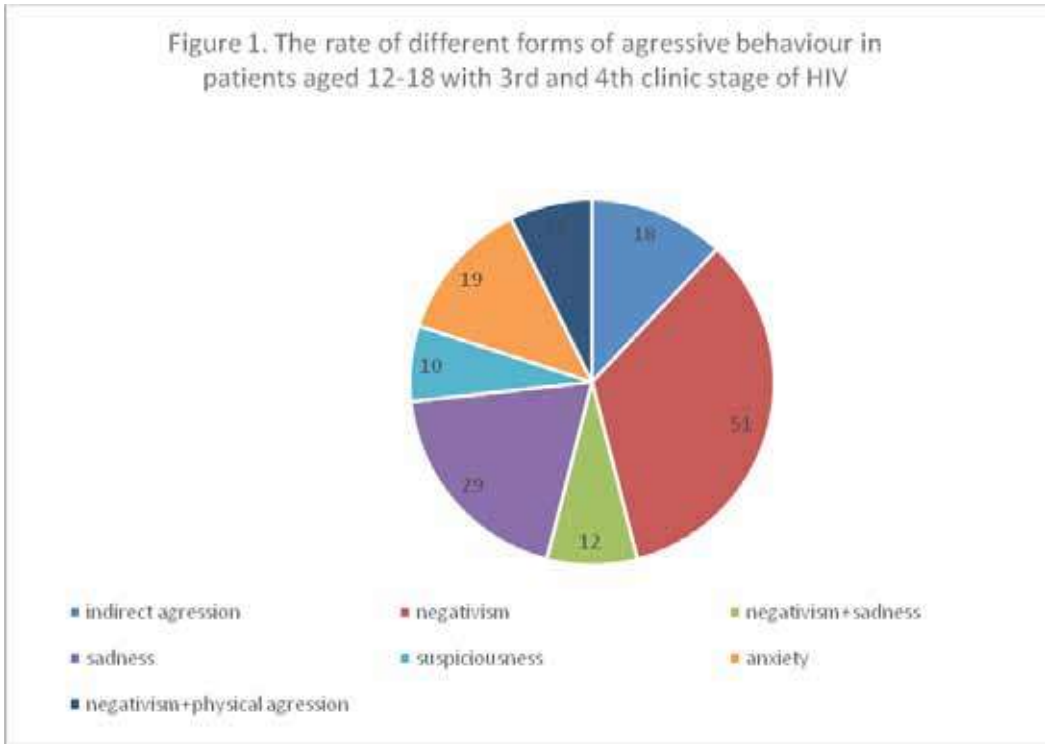
Results

During the study, all patients were diagnosed with 3rd and 4th (77 patients each — 50%) clinical stages of HIV infection involving the nervous system disorders in the form of an HIV-associated neurocognitive disorder (grade 2 in 100 patients — 64.94% and grade 3 - in 54 patients - 35.06%). In addition, epilepsy (20.13%) was diagnosed in 31 patients, peripheral polyneuropathy (4.55%) in 7 patients, vacuolar myelopathy (5.19%) in 8 patients, and consequences of acute cerebrovascular accident in 9 patients with focal neurological deficit (5.84%), in 2 patients - the consequences of herpetic meningoencephalitis (1.30%).

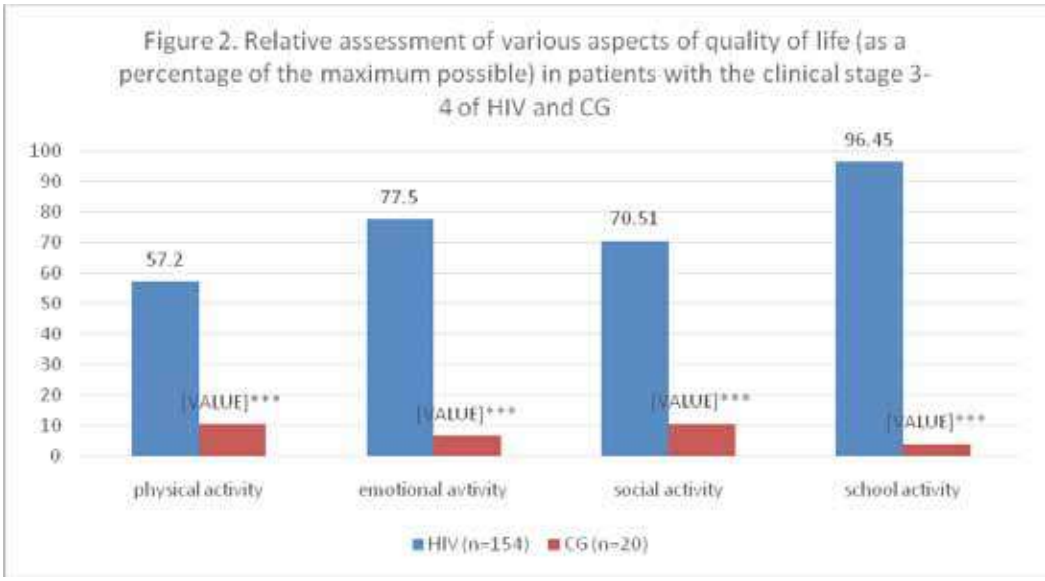
A neurological examination revealed a syndrome of autonomic dysfunction with impaired skin innervation and functional disorders of the gastrointestinal tractin 142 patients (92.21%), coordinating disordersin 96 patients (62.34%), in 52 patients (33.77%) focal motor disorders, including hemiparesis, in 22 patients (14.29%), in 48 patients (31.17%) speech disorders and elements of apraxia were noted, in 32 patients (20.78%) - insomnia and parasomnia syndromes. At the time of the examination, all patients were diagnosed with a lag in psycho-motor development, although in the period of 1-3 years these disorders were detected only in 26 patients (16.88%). Convulsive syndrome was diagnosed and confirmed by electroencephalography in 11 patients (7.14%).

The test using the Raven progressive matrix scale revealed a significant decrease in the intellectual level in the patients included in the study: the average test result was 18.68±4.51%, with a spread from 6 to 26%. At the same time, in 8 patients (5.19%) the indicator was within the boundaries of average intelligence (25-74%), in the remaining patients (146 patients - 94.81%) - below the average (5-24%). In the CG, the average score obtained in the Raven test was 52.31±4.38% ($p<0.001$, the significance of the difference with the index of the main group).

Some forms of aggressive behavior were observed in all patients included in the study (Fig. 1), while, according to the Bass and Darka test in the modification of Rezapkina, negativism prevailed (74 patients - 48.05%), and in 11 cases (7.14%) it was combined with physical aggression, and in 12 cases (7.79%) - with resentment. In addition, indirect aggression was detected in 18 patients (11.69%), resentment in 41 patients (26.62%), suspiciousness in 10 patients (6.49%), and 19 patients (12.34%) - irritation. Indirect aggression (5%) was found in CG in 1 teenager, negativism in 3 (15%) and resentment in 2 (10%) ($p <0.001$ significance of the frequency of aggressive behavior between the main and CG according to the tabular chi-square criterion).



The quality of life questionnaire, adapted for adolescents 13-18 years old, revealed difficulties associated with physical activity (18.30±4.51 points with a maximum score of 32 points, Fig. 2), with emotional states (15.50±2.23 points with a maximum of 20 points), social activity (14.10±2.93 points with a maximum of 20 points) and life in school (19.29±1.23 points with a maximum of 20 points). For an objective assessment of the quality of life in patients with HIV infection, CG was included in the study, in which the average quality of life scores were 3.27±0.26 (difficulties associated with physical activity), 1.28±0.07 points (difficulties associated with emotional state), 2.11±0.03 points (difficulties associated with social activities) and 0.74±0.02 points (difficulties associated with school activities, $p < 0.001$, significance of differences with the average value in the main group for all four parameters).



Note: *** - significance of differences between HIV and CG groups $p < 0.001$.

Discussion

There are several neuropsychological and neuroimaging studies of HIV-positive children with a safe CD4 count (more than 25%). A neuro-visual study found that such children showed far worse results than an HIV-negative control group using the Wechsles Abberviated Scales of Intelligence Verbal and Preformance IQ test and standardized testing of visual-spatial processing of information, visual memory, and executive functions [21], which found in this study in adolescents with 3-4 stages of HIV infection. Significant motor and cognitive impairments were also found in HIV-infected children[22]. HIV in older children is currently associated with more narrow and specific cognitive impairments, including difficulties in visual-spatial and motor integration, memory lapses, impaired attention, speech and socio-emotional deficit [23]. These features (in particular, violations of the executive function) can lead to profound functional impairment in the classroom.

Adult literature on HIV-related CNS damage supports a range of HIV-related neurocognitive disorders, including HIV-related dementia, mild neurocognitive disorder, and asymptomatic neurocognitive impairment [24]. The criterion is clinically useful, since it takes into account the conceptualization of a number of functional disorders and helps to formulate an individual treatment plan for adult patients with HIV-related problems. Similar spectrum criteria are needed in the case of HIV in children in order to better understand the effect and the social and educational management needed to support children infected with HIV. As a result of these improvements in HIV treatment, many children now live to a young age. Therefore, neurocognitive profiles appear in HIV-infected older children and adults. Given that HIV is a chronic disease, and given the limitations of antiretroviral therapy, a significant proportion of these patients are expected to develop neurocognitive deficits and symptoms of HIV-related dementia, which is reported in the adult HIV literature.

Conclusion

In adolescents with a 3-4 clinical stage of HIV infection, damage to the nervous system in the form of HIV-associated neurocognitive disorder is manifested by a decrease in intelligence, various forms of aggressive behavior and is associated with a decrease in the quality of life in terms of physical, emotional, social activity and learning at school. To assess the severity of these disorders, method of neuro-psychological testing using the Raven matrix scale, the Bass-Darki emotion test modified by Rezapkina and the PedsQL-4 questionnaire are accessible and diagnostically informative.

Study Limitations: The amount of participant in the control group was less than that of main group to compare. It might have affect some of our results. Thus, further researches can be conducted with same amount of participants in each arm.

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Ethical Approval: The ethical approval for the study was granted by the Committee of Ethical Approval for Researches under the Ministry of Health of the Republic of Uzbekistan.

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Conservative Treatment of Juvenile and Adolescent Idiopathic Scoliosis

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Abstract

The study relies on data received during treatment of 128 children and teenagers suffering idiopathic scoliosis. The subjects' median age is 10,2±0,39 years old. In the treatment group (90 juvenile subjects), treatment of scoliosis has been carried out according to an optimized conservative integrated algorithm with reliance on bone mineral density indicators and the Cobb's angle. The length of treatment averages 1,5±0,2 years (1 to 3 years).

The control group (30 subjects) has undergone traditional correction on an outpatient basis with the use of standard method: therapeutic exercise, massage, physiotherapy. The length of treatment averages 1.7±0,1 years (1 to 3 years).

Compared to traditional method, optimized conservative integrated treatment of idiopathic scoliosis demonstrates a 29% higher occurrence of positive results. Efficacy of brace treatment of moderate and severe progressive juvenile/adolescent scoliosis has been observed, as it has reduced spinal deformation and eased socio environmental adaptation.

Keywords: *Idiopathicscoliosis, conservative treatment, brace treatment, children.*

Introduction

Idiopathic scoliosis (IS) is a common and serious spinal disorder, which affects children and teenagers (T.N. Sadovaya, 2010; M.V. Mikhailovsky, et al., 2017, Brooks Smyrnis P. et al., 2009),[1] and causes cardiovascular and pulmonary disorders, severe postural abnormalities, earlydisabilityand, consequently, affects mental health (V.S. Mironov, 2010; H. Bouloussa. etal., 2019)[2]. Based on statistical data on idiopathic scoliosis, it can be noted that they have not changed much over the last decades: 1% to 12% of juvenile population are affected by scoliosis (Sadovaya T.N., 2010; Brooks Smyrnis P. et al., 2009)[3]. According to Sadovaya T.N. (2010),[4] idiopathic scoliosis accounts for 80% to 85% of all spinal deformities. It means that idiopathic scoliosis is one of the most frequently occurring juvenile musculoskeletal conditions. A more detailed statistical analysis reveals other features of idiopathic scoliosis as well: for the most part, it is diagnosed in children aged 10-15 years old, i. e. into adolescence. This is exactly when spinal deformation takes on the most formidable form – adolescent scoliosis (Grivas T.B. et al., 2009) affecting one out of every three or four patients.[5]

Based on the clinical picture and X-ray patterns, idiopathic scoliosis can be defined as typical and atypical. Typical scoliosis is diagnosed when there are curvatures of the vertebral column, mostly right thoracic and left lumbar curves with pronounced physiological

lordosed thoracic kyphosis and increased physiological lumbar lordosis. This type causes an abnormal convex side rotation of vertebrae. One out of every three casesis progressive, and it accounts for the majority of cases of structural heart defects, cardiac, respiratory, digestive and central nervous system disorders (Grivas T.B. et al., 2009).[6]

Fragmentation and disintegration of approaches explains the low efficacy of treatment of this spinal disorder.[7] The main problem affecting interdisciplinary interaction is limited use of fundamental knowledge within each healthcare field, which impedes development of fairly effective treatment method.[8] The huge number of existing symptomatic treatment method target separate stages of the pathological process sporadically and do not provide any ultimate solution.[9]

This study is aimed at optimizing the process of conservative treatment of juvenile and adolescent scoliosis.

Materials and Method

We have investigated 128 juvenile and adolescent subjects with IS. The patients were hospitalized to the Republican Center of Juvenile Orthopedics. Age groups have been formed according to the WHO classification, and the obtained data is shown in Table 1.

Table 1: Distribution of IS patients according to age

Age	Male subjects		Female subjects		Total	
	Abs.	%	Abs.	%	Abs.	%
0 to 3	5	45.5	6	54.5	11	8.6
4 to 6	5	25.0	15	75.0	20	15.6
7 to 9	11	40.7	16	59.3	27	21.1
10 to 13	15	41.7	21	58.3	36	28.1
14 to 17	16	47.1	18	52.9	34	26.6
Total	**52**	**40.6**	**76**	**59.4**	**128**	**100.0**

The majority of cases (28.1%) have been observed in children ages 10-13. The median age is 10.2±0.39 years old. According to some sources, this can be due to rapid skeletal growth during puberty and an increased risk of progressive scoliosis at this particular age. Sex distribution, as follows from the table, is biased towards the female sex (by a factor of 1.5), which corresponds to the data obtained from the sources.

Distribution according to the severity of deformities has been made according to the V.D. Chaklin's classification (1965). The study has involved children with spinal deformities with 31.3% (40/128) of them diagnosed with scoliosis Grade 1, 23.4% (30/128) - Grade 2, 17.2% (22/128) - Grade 3, and 28.1% (36/128) – Grade 4. Most juveniles have thoracic (35.2%, 45/128), thoracolumbar (27.3%, 35/128), combined(23.4%, 30/128), and lumbar(14.1%; 18/128) scoliosis.

In the control group, 66 children ages 5-17 have been examined. Based on the examination results, no spinal deformities have been observed in these children. Median age in the control group is 13.8±2.1 years old ($p<0.05$).

During the physical examination, doctors have checked patients for uneven shoulder height, shoulder-blade asymmetry, spinous process rotation,torsion in the form of a rib hump and muscular embankment in the lumbar area. It is important to check spinal mobility and stability through the pulling of the head. Pelvic tilts and lumbar lordosis are diagnosed in a standing position; abdominal muscles are checked in a lyingface-up position; back muscles are examined in a lying face-down position.

Scoliosis patients are examined in a standing position from back and front, in a side-lying position from back, in a standing position from a sagittal perspective, and in a bent-forward position from back and front (Fig. 1).

X-ray imaging has been conducted at the Department of Radiology of the JMDS-Service Clinic on a Silhouette radiographic system (General Electric), (128 images).

Of 128 children, 120 have been examined. A group of 8 children have failed to follow recommendation and regime and therefore have not completed the conservative treatment. The 120 juvenile subjects have been divided into two groups: the treatment group and the comparison group.

The *treatment group* has included 90 children: 33 (36.7%)boys and 57 (63.3%) girls. Treatment of the group's subjects has been administered with the use of the conservative integrated idiopathic scoliosis treatment method developed by us. The treatment has lasted 1 to 9 years (1.5 years on average), the follow-up period has lasted 1 to 13 years, averaging 2.2 years.



Fig. 1. Examination of patient A., 14 years old

The *control group* has included 30 children – 4 (13.3%) boys and 26 (86.7%) girls. The control group subjects have been treated on an outpatient basis according to standard method: therapeutic exercise, massage, physiotherapy. The treatment has lasted 1 to 6 years, on average – 2.1 years. The follow-up period has lasted 1 to 9 years, on average – 3.4 years.

Both control and treatment group subjects diagnosed with scoliosis, along with clinical symptoms, had ragiological signs indicating structural changes in vertebrae. Both groups have shown a 100% occurrence of spinal torsion. Wedge-shaped deformities of vertebrae and intervertebral cartilages have been observed in 43.3% and 63.3 control group subjects respectively and in 38.9% and 73.3% treatment group subjects respectively.

Therapeutic exercise is applied as essential therapy to patients with scoliosis Grade 1 and Grade 2 and as adjunct therapy to patients with scoliosis Grade 3 and Grade 4. Exercises are chosen individually depending on the severity of case, presence or absence of underlying diseases and contraindications. Exercises are intended for stimulation and strengthening of back muscles, which help straighten out the spine and other bones. No workouts, racing, jumping and spine-twisting exercises are allowed. Gym ball exercises and use of specific training devices have proved useful. The length of training courses varied between 2 and 6 months (3.5±0.01 months on average).[10]

Massaging scoliosis-affected areas helps muscles regain tone, improves blood circulation and metabolism. Therapy lasts 10 days, and it is repeated every two months over a 12-month period. Physiotherapy is prescribed for patients with a Cobb angle of 10 degrees or more. This kind of therapy improves blood flow and nerve conduction, eliminates pain and normalizes tissue metabolism. Treatment group patients have used Almagand Duna devices, as well as a Vitafon vibration massage device to stimulate core back muscles. Therapy lasts 10 days and it is repeated every two months over a 12-month period.

Brace therapy has been carried out with the use of a Cheneau type functional correcting brace. Measurements have been made individually on each patient. The use of an orthopedic brace is prescribed to patients with steadily progressing scoliosis and a Cobb's angle of 25-40 degrees (Grade 3 and Grade 4). Braces have been made on the basis of individual measurements for 18 subjects in such a way as to minimize discomfort. A brace should be worn several hours a day and removed only during

gymnastics and hydrotherapeutic procedures (Fig. 2) It should be used for a period of 12 months.



Рис. 2. Patient M., 13 years old. Scoliosis Grade 3. Brace therapy, view from the rear and front (a Cheneautype brace)

Late fate has been evaluated based on the results of clinical, X-ray and instrumental method of diagnosis, as well as based on patients' posture and appearance. We have included in the late fate the results of treatment achieved after 12 month of therapy.

The obtained data has been subject to statistical analysis on a Pentium-4 computer with the use of Excel package featuring statistical functions. Differences between median values have been deemed relevant with P-value *P<0.05.*

Results

Throughout the treatment, all patients have shown a quick improvement as early as by the fifth session, having less pain in the back, neck and legs. A clinical examination revealed a decrease in waist asymmetry in both groups (Table 1). In the comparison group, the asymmetry has decreased by 0.44±0.08 cm (the decrease ratio (DR)=41.0%, p<0.005), in the treatment group – by 0.33±0.07 cm (DR=56.3%, p<0.005). In the comparison group, the discrepancy between shoulder-blade and shoulder angles decreased by 1.0±0.5 cm (DR=30.1%, p<0.005), in the treatment group – by 1.4±0.6 cm (DR=54.3%, p<0.005).

Thus, the treatment group has shown a less severe spinous process rotation in the lumbar area, and the deterioration has been fairly rarer according to 3 out of 5 parameters. In the Grade 1 group, the clinical effect has been achieved both in the comparison and treatment group. In the treatment group, waist asymmetry has decreased by 58.4±1.4%, shoulder asymmetry – by 57.3±1.2%, while in the comparison group it has decreased by 23.5±1.3% and 24.4% respectively (p<0.005).

Table 1: Dynamics of clinical signs of scoliosis in the groups

Common signs	Treatment group (n=90)		P	Comparison group (n=30)		P
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
Deviation of the body	76 (84.4%)	56 (62.2%)	<0.05	25 (83.3%)	22 (75.9%)	>0.05
Thoracicspinous process rotation	82 (91.1%)	61 (72.2%)	<0.05	27 (90.0%)	25 (83.3%)	>0.05
Lumbar spinous process rotation	88 (97.8%)	75 (83.3%)	>0.05	29 (96.7%)	27 (90.0%)	>0.05
Shoulder-blade asymmetry	76 (84.8%)	38 (42.2%)	<0.05	28 (93.3%)	23 (76.7%)	>0.05
Breastbone-to-superior-iliac-spine asymmetry	90 (100%)	46 (51.1%)	<0.01	29 (96.7%)	21 (70.0%)	>0.01

Pisthecriterion of significance of the difference between pre-treatment and post-treatment indicators within a group.

The Grade 2 group has shown a somewhat lower effect than the Grade 1 group. In the comparison group, waist asymmetry has diminished by 34.3±1.2%, in the treatment group – by 42.4±1.4% (p<0.005). Shoulder-blade and shoulder asymmetry has decreased by 52.5±1.3% in the treatment group and by 45.4±1.1% in the comparison group. Clinical symptoms in juvenile and adolescent subjects with scoliosis Grade 3 have changed as follows: in the treatment group, waist asymmetry has decreased by 25.3±1.2%, in the comparison group - by 20.1±1.2%.

The treatment group has demonstrated a 6% increase in active spinal flexibility while performing forward bends and a 14.1% increase while performing backward leans. The asymmetry of left-side and right-side tilt depth values in patients with early stage and mild scoliosis has not exceeded 10%. The asymmetry has

been more pronounced in Grade 2 and Grade 3 groups (14.5%±6.2%).This category of patients has shown the best result in the treatment group, where the asymmetry ratio has decreased by 42%, while in the comparison group the ratio has decreased by 23%.

The conservative integrated treatment has boosted muscular endurance, raised tolerance to physical workload, as the subjects have done well in back therapy training and had no back pain after exercising. The investigation has revealed a potential for a longer abdominal muscle workout in the treatment group. At the lower end, the maximal increase – by 59% - has been observed in the treatment group (27.1±22.1 seconds before treatment vs. 86.0±29.3 seconds, p>0.05), while in the comparison group – by 20.2% (67.1±35.5 seconds before treatment and 86.0±29.3 seconds after treatment,p>0.05). The endurance of back muscles in a lying-straight position has increased in both groups by 27% to 30% (82.0±34.9 seconds before treatment and 103.0±34.3 seconds, p<0.05)

Discussion

A comparative analysis of the results of the therapy achieved by the treatment and control groups has shown that positive changes have been more pronounced in the treatment group. These changes have been observed both during a clinical and radiological examination. The biggest achievement is that the obtained data correlate and demonstrate mitigation of structural scoliosis symptoms. [11]

A review of the results achieved by the comparison group reveals mild positive clinical dynamics in juvenile and adolescent subjects. The Cobb's angle dynamics observed across the groups, which takes into account the degree and localization of curvatures, is presented in Table 2.

As follows from the table, the juvenile and adolescent subjects of the treatment group show a more pronounced decrease in the Cobb's angle regardless of grade, relative to the comparison group.[12]

The comparative analysis has revealed the most prominent positive results in the treatment group. The changes have been observed both during clinical and following radiological examinations.

A frontal radiograph made in a lying position (Fig. 2c) is used to evaluate frontal imbalance and determine the Kazmin's index of stability, which can be deemed as orthostatic interdependence of deformation parameters. The frontal images (Fig. 2d, e) made in maximal right- and left-side bending positions are functional *bending test* type radiographs.

It is advisable to plan orthopedic tests at this stage, depending on the clinical picture and bone maturity, at a two to six-month interval. During treatment, radiographic imaging is carried out as part of brace therapy; frontal and lateral images are made in a standing position (Fig. 3) The following radiographs reflect the result of the correction therapy, and their data is compared to initial curvature parameters, particularly with those of the bending test images, which are actually the target parameters for the correction therapy.

Table 2: Cobb's angle dynamics during the treatment

	Type of scoliosis	Severity of curvature (degrees)		Correction		P
		Pre-treatment indicators	Post-treatment indicators	Degrees	%	
Comparison group (n=30)	Grade 1 (n =7)	7.6±0.3	6.6±0.2	1.0±0.01	6.0	>0.05
	Grade 2 (n=8)	17.7±2.5	16.1±2.5	1.6±0.1	9.0	>0.05
	Grade 3 (n=9)	50.4±3.6	50±3.8	0.4±0.08	0.79	>0.05
	Grade 4 (n=9)	73.2±4.1	72.3±5.6	0.9±0.06	0.81	>0.05
Treatment group (n=90)	Grade 1 (n =29)	7.2±0.2	4.9±0.09	2.3±0.01	9.2	<0.05
	Grade 2 (n=20)	18.75±1.3	14.5±1.5	4.25±0.3	22.7	<0.05
	Grade 3 (n=11)	18.2±1.1	14.2±1.4	4±0.4	22	<0.05
	Grade 4 (n=30)	44±4.7	40.6±4.3	3.4±0.5*	7.7	>0.05



Fig. 2. X-ray images of an idiopathic scoliosis patient requiring brace treatment: frontal image made in a standing position (a); lateral image made in as tan ding position (b); frontal image made in a maximal right-side bending position (d); frontal image made in a maximal left-side bending position (e).



Fig. 3. Pre-treatment (a) and corrective brace therapy (b) radiographs of the spine: effect achieved following frontal major curve correction Th11-L3 on the left



Fig. 4. A 8-year-old girldiagnosed with moderatescoliosis: a –case record; b –a Cheneau type brace; c) radiograph received during initial medical examination; б) radiograph received following the brace therapy

In all 18 subjects deformities have improved. In 12 patients, the positive effect has lasted throughout the follow-up period (Fig. 4 a, b, c, d).

In five subject deformities continued to deteriorate throughout six months due to failure to follow brace treatment requirements. Correction has required two additional braces on average.

Two subjects needed a surgery, one due to refusal to undergo brace treatment; the other had a severe case of scoliosis without a possibility of curve correction.

Conclusion

Therefore, treatment of children suffering idiopathic scoliosis Grade 3 and Grade 4, with a Cheneau brace in combination with individual therapeutic exercises, in most cases helps eliminate scoliosis symptoms. However, in cases when a visibly good effect is achieved, which is purely cosmetic, without actually progressing in the correction of spinal deformities, surgical treatment is still admissible. Refusal to wear a brace is the most common cause of deteriorating deformities. It is necessary to carry out socio-educational work to provide early diagnostic of IS to avoid progressive spinal deformities and reduce the risk of having to undergo surgeries.

1. Juvenile and adolescent idiopathic scoliosis is characterized by a spinal dysfunction, as in 85.2% of scoliosis subjects deflection of spinous processes from the axis has been observed, 78.9% have shoulder-blade asymmetry, and 53.1% of subjects have shoulder asymmetry. The Nash and Moe method has helped reveal torsion in 13.3% of children.
2. Children and teenagers with IS have bone tissue pathologies resulting in a 2.8 times lower Z-score in mineral bone density tests, which signifies low osteosynthesis and structural changes in the spine.
3. Data obtained during mineral bone density tests on children and teenagers with IS can be used as prognostic criteria to evaluate the likelihood of deterioration.
4. The newly developed conservative integrated IP treatment method is more effective than traditional method, as it helps achieve a positive effect in 89.4% of cases compared to traditional method providing 56.9% of such cases.
5. The study has proved the efficacy of brace therapy in juvenile and adolescent IS subjects, as it has stopped the deterioration and helped correct axial spinal deformities.

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Comparative Clinical Signs of Herpetic Infection Coupled with HIV Infection

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Abstract

The article represents the results of the observation oft he comparative clinical examination of herpetic infection coupled with HIV infection in 57 children aged 1 to 15 years. There have been 41 boys (71,93%) and 16 girls(28,07%). The comparison group included 23 children oft he same age infected with HIV without herpetic infection.

The following laboratory research method have been used: a general analysis of blood, urine, feces, the study of the immune status, level of CD4 cells; Serological: by ELISA method antibodies to CMV and HSV (Herpes Simplex Virus) have been detected in blood. The results obtained indicate that coexisting diseases with the formation of complications and immunodeficiency development have been worsening the course of herpetic infection in HIV infected children and leading to a severe relapsing course.

Keywords: *Herpeticinfection, HIV infection, children, Herpes Simplex Virus.*

Introduction

The most significant group of opportunistic infections in HIV-infected children is the herpes group[3]. Infections caused by herpetic viruses draw attention by high prevalence in the human population. The most significant group of opportunistic infections in HIV-infected children is the herpes group[3]. Infections caused by herpetic viruses attract attention with a high prevalence in the human population. Among human viral diseases, herpetic infection (HI) occupies one of the leading places, which is explained by its ubiquitous transmission[4]. Herpeticinfectionis a group of diseases caused by herpes simplex viruses (HSV) of type 1 and 2. Atherpeticinfectionnotonlyepithelial, hematopoietic, nerve cells are affected, but also T-lymphocytes, which leads to the development of secondary immunodeficiency and generalized forms of the disease[5]. Herpes viruses are widespread in the human population, they are able to infect almost all organs and systems of the host organism, causing latent, acute and chronic forms of infection[3]. The cytopathogenic effect of herpes viruses is enhanced against the background of immunodeficiency condition. HIV infection is accompanied by the development of opportunistic diseases, among which herpes viruses take one of the leading places. The most common among them are lesions associated with herpes simplex viruses, zoster varicella, cytomegalovirus[1]. Herpes viruses can activate HIV, which is in the provirus stage, and is a cofactor in the progression of HIV infection and AIDS. In this regard, herpetic infection (HI) is one of the important AIDS-indicator disease. AIDS currently ranks third in the world in the group of deaths after diseases

of the cardiovascular system and neoplasms, and among children with HIV/AIDS it is the leading cause of death. The incidence of HI in the general population is 650-720 per 100 thousand population. Among HIV-positive, the same indicator is almost 10 times higher[2]. The viral etiology of the herpes group causes anabnormality of the functions of the immune system, resulting in the progression of immunopathological reactions, which reduces the control of virus replication, causing a manifestation or relapse of the disease[6].With herpes, immunodeficiency states develop due to the insufficiency of various parts of the immune system and its inability to eliminate the virus from the body. Persistent throughout life, sometimes in considerably high titres, virus-neutralizing antibodies if rather embarrass the spread but do not prevent the occurrence of relapses[6].

The urgency of the problem is determined by the high mortality rate of HIV-infected children with herpes infection, the absence of clear criteria for the diagnosis itself, as well as the natural difficulties in establishing this diagnosis.

The aim of the researchis to study the comparative clinical signs of herpes infection coupled with HIV infection.

Materials and Method

A clinical examination of herpetic infection in HIV-infected children has been conducted. 57 patients aged 1 year to 15 years registered at the Research Institute of Virology of the Ministry of Health of the Republic of Uzbekistan were under the supervision. Primary HIV diagnosis: a) Serological studies - the determination of antibodies to the human immunodeficiency virus has been carried out in the laboratory of the AIDS Center of the Ministry of Health of the Republic of Uzbekistan. The diagnosis of HIV infection has been verified in accordance with the National Protocol of the Republic of Uzbekistan, No. 117 as of March 17, 2005. The comparison group included 23 children of the same age infected with HIV withouth erpeticinfection. The results of repeated HIV tests were negative. Laboratory research method included a general analysis of blood, urine, feces, the study of the immune status, level of CD4 cells; Serological method: by ELISA, antibodies to CMV and herpes simplex virus (HSV) have been determined in the blood.

57 children aged 1 to 15 years have been under our examination: 41 boys(71,93%)and 16girls (28,07%).57 HIV-infected children with herpesvirus infection who have been HIV positive and herpesvirus infection has been serologically confirmed. The control group consisted of 23 children with HIV infection without herpes virus. Observations and general clinical studies have been conducted on the basis of the Research Institute of Virology of the Ministry of Health of the Republic of Uzbekistan. In terms of age, the children have been divided as follows: from 1 year to 6 years – 34children (59.65%), from 6 years to 10 years - 13 children(22.81%) and from 10 to 15 years - 10 children (17.55%). The diagnosis of HIV infection has been established on the basis of epidemiological, clinical and laboratory tests, confirmed by double detection of antibodies (AB) to HIV by enzyme-linked immunosorbent assay (ELISA). The diagnosis of HI has been established on the basis of the disclosed changes in the organs and systems taking into account the polytropy of the pathogen and the generalized nature of the lesions in the terminal stage of the disease. For each child, an examination card has been filled out, in which the passport part, the date of admission of the child, a detailed clinical diagnosis, medical history, infection routes, therapeutic measures, as well as the results of laboratory and instrumental research method throughout the observation period have been noted. Clinical and laboratory examination included: examination, thermometry, ultrasound of the abdominal organs, a general blood test, a biochemical blood test to determine the content of total protein, bilirubin and its fractions, AlAT, AsAT, sedimentary sample.

Statistical processing of the results has been carried out using the STATISTIKA V.5.5A program with the Student's t-criterion. The etiological interpretation of HI has been based on the results of polymerase chain reaction (PCR). A comparative analysis of the severity of the disease has been carried out with HIV-infected patients without HI[23], having similar HIV infection experience and similar sex and age characteristics. This group of patients (HIV + HI)[57] has undergone a comprehensive clinical and laboratory examination.

Results

The results of there search haves how nth at in the structure of coexisting diseases in young children frequent acute respiratory viral infections prevail with the formation of complications (18.7%), digestive tract infections (27.1%), fungal skin lesions (2.1%), in the group of children from 7 to 15 years there has been less

complications (p<0,05) rather than in younger children. An analysis of the results has disclosed that the most common in HIV-infected children without herpes infection are coexistingdiseases in the form of anemia in 19 (82.61%) cases, damage to the nervous system in 16 cases (69.57%), 14 cases of pneumonia(60.87%), 10 cases of tuberculosis (43.48%), 9 cases of intrauterine infection (39.13%), as well as 7 cases of chronic viral hepatitis B + C (30.44%),5 cases of thrombocytopenia (21.74%), 3 cases of sepsis (13.05%), 3 cases of chronic viral hepatitis C (13.05%), 2 cases of chronic viral hepatitis B (8.70%), 1 case of syphilis (4.35%). As HIV infection progresses, the severity of herpetic lesions worsens, which requires timely diagnosis[33] and therapeutic antiviral therapy[43]. From the epidemiological data, the identification of children at risk has been of great importance[75]. The characteristics of the children included in the researchhave been based on the classification of herpetic infection proposed by V.F. Uchaykin. The severity of the course of RHI has been determined by the frequency of relapses (Masyukova S.A. and others. 2007). The results of the research have shown that herpes simplex type 1 and type 2 occurred in 59.65–80.71% of HIV-infected people without herpes infection, causing mucocutaneous ulcerative lesions, diseases of the nervous system[14]. Herpes zoster is observed in 15.79–31.58% of patients with HIV infection and is one of the earliest markers of immunosuppression[12]. Coexisting diseases such as anemia (54 cases - 94.74%), nervous system damage (41 cases - 71.93%) pneumonia (38 cases - 66.67%), tuberculosis (33 cases - 57.90 %), toxoplasmosis (28 cases - 49.13%), intrauterine infection (26 cases - 45.62%), as well as chronic viral hepatitis B + C (24 cases - 42.11%), sepsis (20 cases - 35.09%), thrombocytopenia (15 cases - 26.32%), trichomoniasis (11 cases - 19.30%), chronic viral hepatitis C (10 cases - 17.55%), chlamydia (7 cases - 12.28%), syphilis (6 cases - 10.53%) are the most frequently met in HIV-infected children with herpesvirus infection[32]. The simultaneous action of such unfavorable factors as an early age at the time of infection, premorbid background, intensive parenteral loads exacerbated the course of herpes virus infection in HIV-infected children and also led to an early lethal outcome[50].

Discussion

An analysis of the clinical stages of HIV-infected patients with clinical signs of herpes virus infections shows that most of them 78.96% have been at stage 2 (stage 2B - 36.85%, stage 2C - 8.78%).

Studies conducted have shown that the prevailing form of relapse of herpetic infection in HIV-infected children is herpes of skin (52.64%).

The results of the researchhave shown that in children under 6 years of age complications have been significantly more likely to be detected than in the group of children from 7 to 15 years of age, where complications have been less common (p <0.05). Overcooling in 61.41% of cases, insolation in 8.78%, psychoemotional stress in 3.51%, acute respiratory viral infection in 57.90%, mechanical trauma to mucous membranes in 7.02%, and exacerbation of allergic dermatosis in 8.78% of cases have become provoking factors for the development of relapses of infection. Severe RHI course has been recorded more often in children under 6 years of age, the duration of relapse in children under 6 years of age has been significantly higher (p <0.05). The occurrence of relapse has been accompanied by intoxication signs, more pronounced in young children. The terms of epithelialization of defects have been delayed in children under 6 years of age by 7.4±1.7 days from the onset of the disease, and in the group of children from 7 to 15 years of age by6.4±2.8 days. The number of leukocytes in children under 6 years of age during the onset of relapse has been characterized by moderate leukopenia (5.0±1.76 *) (p <0.05) with relative lymphocytosis (64.9±1.40) (p <0.01) preserved in remission (61.4±1.82) (p <0.05). In the period of exacerbation of the disease in children over 7 years old, leukocytosis (6.4±2.15) (p <0.05) with relative lymphocytosis (40.1±1.22) (p <0.01) has been noted. The remission period has been also characterized by an increase in the percentage of lymphocytes (38.7±0.11) (p <0.05). The subpopulation composition in children from 1 year to 6 years of age with RHI during the period of exacerbation has been characterized by a pronounced decrease in the relative (22.6±1.35) and absolute (0.74±0.15) levels of CD4,% cells compared with the control group (37.7±1.47) (1.47±0.06) (p <0.001). During remission, their absolute number corresponded to (1.01±0.03) (p <0.05), the control group (1.47±0.06). In the group of children from 6 years to 15 years, there has also been a significant decrease in the relative content of SD4,% cells during periods of exacerbation (23.4±2.51) (p <0.001) and remission (27.3±3.09) (p <0.01). Their absolute number during exacerbation decreased moderately (0.63±0.12) (p <0.05), during remission it has corresponded to the norm (0.75±0.12). The indices of the absolute and relative content of T-cytotoxic lymphocytes during exacerbation

(40.1±1.22) and during remission (38.7±0.11) have not differed from the control values (34.2±1.35) in the group 6 years to 15 years. In children from 1 year to 6 years, there has been a significant increase in the relative content of SD8,% cells throughout the observation period (27.7±1.73) (p <0.01). Their absolute content has sharply increased during the exacerbation of the disease (1.14±0.17) (0.91±0.07) (p <0.001). In children from 1 year to 6 years, a decrease in the relative content of SD16,% cells during exacerbation (9.3±1.02) (p <0.01) and remission period (10.1±1.11) (p <0.05) of the disease has been noted. In children from 6 years to 15 years, the level of MK cells has decreased only during relapse (8.1±0.92) (p <0.05). The absolute number of CD16 cells in children from 1 year to 6 years and in the group from 6 years to 15 years has also decreased during the period of relapse (0.33±0.05) (0.26±0.06) (p <0, 05). In both groups, normal values of the relative number of B-cells have been recorded. Their absolute content has significantly decreased in children from 1 year to 6 years during remission (0.90±0.09) (0.51±0.03) (p <0.05).

The humoral immunity has been characterized by an increase in the values of all classes of immunoglobulins. The IgM level mg/μl in children from 1 year to 6 years has increased during the period of exacerbation of the disease (1.5±0.68) (p <0.05), and in remission period as well (1.5±0.19). In children from 6 years to 15 years, an increase in IgM mg/μl has been more pronounced during the exacerbation period (1.6±0.19) (p <0.01), normalizing during remission of the disease (1.3±0.15) (p <0.05). High values in children from 1 year to 6 years have been recorded throughout the course of the disease (p <0.01). In the group of children from 6 years to 15 years, an increase in the level has been observed only during exacerbation (p <0.01). The IgA content mg/μl in both groups has had a similar dynamics, increasing during an exacerbation of the disease (1.3±0.11) (1.8±0.22) (p <0.01). In remission, IgA hyperimmunoglobulinemia has persisted in both groups (1.2±0.26) (1.7±0.19) (p <0.05).

The results of the research have shown that in all HIV-infected children with herpes infection that we have observed, various forms of chronic HI have been diagnosed, the reactivation of which has occurred in 21 cases(36.85%), and the relapsecourse in 36 cases (63.16%). A comparative assessment of the severity of the disease shows that HI has been more often detected in HIV-infected patients at stages 2B and 3B, which means that the number of patients with a progressive course of HIV infection has prevailed. HI coupled with HIV with damage to skin and mucous membranes has been characterized not only by more abundant and widespread elements of the vesicular rash, but also by its slowed regression, lasting more than 1 month (from 6 to 8 weeks).

The results of the research have shown that with herpetic infection of HIV-infected children, severe immunosuppression is observed. The number of leukocytes in children under 6 years of age during the onset of relapse has been characterized by moderate leukopenia (5.0±1.76 *) (p <0.05) with relative lymphocytosis (64.9±1.40) (p <0.01) preserved in remission (61.4±1.82) (p <0.05). The subpopulation composition in children from 1 year to 6 years with RHI during the period of exacerbation has been characterized by a pronounced decrease in the relative (22.6±1.35) and absolute (0.74±0.15) levels of CD4,% cells compared with the control group (37.7±1.47) (1.47±0.06) (p <0.001). In the group of children from 6 years to 15 years, there has also been a significant decrease in the relative content of SD4,% cells during periods of exacerbation (23.4±2.51) (p <0.001) and remission (27.3±3.09) (p <0.01). The humoral immunity has been characterized by an increase in the values of all classes of immunoglobulins. The IgM level mg/μl in children from 1 year to 6 years has increased during the period of exacerbation of the disease (1.5±0.68) (p <0.05), and in remission (1.5±0.19).

Clinical case: Boy,GiyasovKomil, 3 years. Diagnosis at admission: Herpetic infection, HIV infection, stage 3 (persistent generalized lymphadenopathy, persistent hepatosplenomegaly, recurrent aphthous stomatitis, enlargement of the parotid salivary glands, recurrent upper respiratory tract infections, persistent fever, persistent diarrhea, ıpersistent oral candidiasis, severe recurrent bacterial pneumonia, HIV-associated encephalopathy). Coexistingdiagnosis: congenital heart disease (ventricular septal defect), Sturge-Weber phacomatosis, mild anemia (hemoglobin level - 98 g/l). A child has had multiple developmental stigmas. Immunological parameters: CD4 level - 23.6 cells/μl. Virological research method have not been available. ART initiated, scheme: lamivudine, zidovudine, nevirapine. In 2 months after the beginning of therapy, its clinical, immunological (CD4 - 27.7 cells/μl), virological (viral load has not been determined) effectiveness has been noted. The hemoglobin level has been 14 g/l, that caused the replacement of the medication.

Thus, the occurring immunological changes in herpes infection in HIV-infected children have disclosed signs of combined immune deficiency. The data presented in the research indicate that, with herpetic infection in HIV-infected children, the cellular and humoral immunity systems are suppressed and unbalanced. However, there is a functional inadequacy of the body's natural resistance system. The emerging immunological shifts of herpes infection in HIV-infected children indicate severe immunosuppression - leukopenia, lymphocytosis, a significant 1.3 times decrease in the content of CD3-, CD4-, cells, an increase in the levels of all classes of immunoglobulins in the period of exacerbation of the disease, signs of combined immune deficiency have been revealed. The data presented in the research indicate that, with a herpetic infection in HIV-infected children, the cellular and humoral immunity systems are suppressed and unbalanced. However, there is a functional inadequacy of the body's natural resistance system

Thus, the action of such unfavorable factors as an early age at the time of infection, coexisting diseases with the formation of complications, with the progression of immunodeficiency has worsened the course of the disease in infected children, and led to a severe recurrent course of herpes infection in HIV-infected people. Against the background of secondary immunodeficiency due to HIV, HI becomes generalized with multiple lesions of the internal organs and central nervous system.

Conclusion

It has been established that herpetic infection in HIV-infected children especially of the young age, has a course of severe and continuously recurring forms.

It has been found that the prevailing form of recurrence of herpes infection in HIV-infected children is herpes of skin (52.64%). Against the background of progression of immunodeficiency, a worsening of the course of the disease has been noted, the frequency, severity and complication of herpetic infections and their relapses increase, becoming generalized with multiple lesions of the internal organs and central nervous system.

It has been established that with herpes infection in HIV-infected children, severe immunosuppression is observed - leukopenia, lymphocytosis, a significant 1.3 times decrease in the content of CD3-, CD4-cells, an increase in the levels of all classes of immunoglobulins in the period of exacerbation of the disease.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Early Detection and Prevention of Suicidal Behavior in Adolescents

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Abstract

This article highlights the results of research, which help reveal high-risk groups to suicide among adolescents based on types of family relationships and provide timely medical, psychological and psycho-correction help for parents and children, predict suicidal behavior at early stages. An major role in prevention of adolescent suicides is played by the family and healthy family relationships, as they ensure harmonious development of a personality.

Keywords: *Adolescents; teenagers; personality specifics; emotional disorders; suicidal behavior; self-damage.*

Introduction

According to the World Health Organization (2018), over the past 30 years the number of suicide attempts committed by children and teenagers in the world continues to grow, especially in highly developed countries of Europe and America [12]. The necessity of studying suicidal behavior is explained by the growing number of suicides and suicide attempts among children and adolescents resulting from the negative influence of mass media and the virtual Internet environment, through which suicides are projected as role models and objects of worship[2]. Juvenile and adolescent suicide appears to be the most tragic manifestation of the weakness of existing anti-suicide barriers and young generation's inability to understand the value of life[8]. In this age group, suicide is the fourth (to injuries, infections and cancer) most common cause of death[3]. Along with the high rate of completed suicides, there are a growing number of those committed as a protest resulting from conflicts within a family[4]. Intrafamilial relationships and family climate are significant factors, which determine a child's family and social behavior and affect his/her future relationships with other people[1]. In puberty, symptoms of depression accompanied by inferiority complexes[10] interspersed with dysmorphophobic manifestations and proneness to suicidal behavior, tend to increase, in cases whereby they have not been diagnosed in time due to poor emotional climate in families and lack of harmony between children and parents[9]. In their research, Russian psychiatrists and psychologists highlight[3] a correlation between suicidal tendencies, deviant/delinquent behavior and socialized conduct disorders[6]. Suicidal behavior is triggered by depression, which is expressed in teenagers by somatic symptoms and behavioral incidents[11]. According to Zhukova N. Yu., suicidal tendencies result from progressing intrapersonal conflicts that develop due to the influence of external stress factors, as well as from psychopathological disorders, which trigger self-destructive behavior and are not related to any outer factors[7]. According to research conducted by Grigorenko E.A., adolescents at risk of suicide have specific traits of character: lack of confidence, timidity, social rejection, feeling useless and worthless, focusing on appearance imperfections, passive attitude, feeling helpless in difficult situations and conflicts, which occur in the rapidly changing society[5].

The goal of the research is to study family-related risk factors contributing to suicidal tendencies in teenagers to ensure early prevention and outline optimal psychocorrection tactics for this population category.

Materials and Method

The study has involved a group of 86 adolescent subjects ages 15 to 19 with depression-related behavioral disorders and suicidal intentions. The group has included patients with diagnostic criteria typical of ICD-10–F91.2 socialized conduct disorders. A structural personality query, developed by the Psychiatry Department in cooperation with clinical and social psychologists, has included a list of psychological test questions, which help identify suicidal behavior risk factors with reference to personality traits and severity of depression. To diagnose the parameters of a family system, the Family Adaptation and Cohesion Evaluation Scales (Olson and Portner) have been applied. The Columbia Suicide Severity Rating Scale (Posner K., Brent D., Lucas C., 2009) has been used to evaluate suicidal tendencies. The Zung Self-Rating Depression Scale has provided the most relevant measurements of depression symptoms in teenagers, and to evaluate anxiety levels, the Spielberger-Khanin State-Trait Anxiety Inventory has been used. To evaluate constitutional specifics of adolescents' personality, Lichko's Pathocharacterological Diagnostic Questionnaire has been applied.

Results

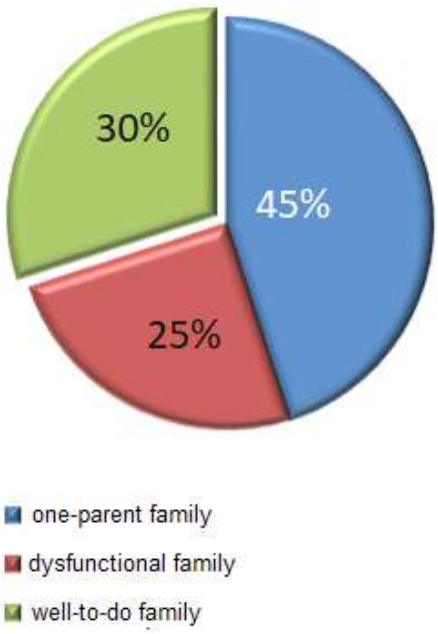


Fig. 1. Types of families, in which the subjects have been raised.

The psychological testing has shown that 45% of adolescents have grown up in one-parent families, 25% - in troubled family situations, 30% have been bred in well-to-do nuclear families. Chronic alcoholism and psychopathologies have been family affairs for 37% of the group. They have lived in conflict-ridden households, witnessed antisocial and amoral behavior and poor living conditions. The analysis of data obtained during the study has shown that 26% of teenagers, who veer toward antisocial behavior and minor felonies, have hedonistic inclinations and have been raised in dysfunctional families with destructive parenting habits, which have contributed to pathocharacterological protest reactions in the form of suicide attempts. Demonstrative, dramatic and manipulative suicidal behaviors have been observed in 28% of adolescents raised in well-to-do families n easy circumstances, yet emotionally detached and having poor interpersonal relationships with parents. The Family Adaptation and Cohesion Evaluation Scale has diagnosed the largest number of families practicing incorrect extreme parenting, which have upset family systems and caused a crisis in interpersonal relationships; 70% of subjects have been raised in emotional depravity and suffered lack of parental care and love (Fig. 1).

Discussion

Research of ambivalent family relationships has revealed a complete and utter detachment between family members, which has predetermined depression in teenagers, accentuation of personality traits and personality pathologies. Conflicts in destructive families have given rise to affective mental blocks with a negative perception of the world, which has exacerbated the disharmonic personality development and often caused negative emotions and involvement in so called virtual "death groups," which are controlled by online game managers. Comprehensive medical histories have confirmed the role of incorrect parenting and control in the formation of deviant behavior, including self-destructive behavior and its extreme manifestation–suicidal behavior. According to the Lichko A. E. classification, the following incorrect parenting and control practices have been detected: underprotection, dominant hyperprotection, promoting hyperrprotection, emotional parental rejection, increased moral responsibility. According to the study, most teenagers in the group have grown up in overprotective families (46%) and faced emotional rejection (25.5%). There have been a smaller number of cases of hyperprotection–dominant (14%), promoting (10%), and high moral

responsibility imposed on children (4.5%). Incorrect parenting has resulted in pronounced characterological personality changes. Underprotective parents have been indifferent to their children and failed to control their behavior, provide due attention, care and supervision, to demonstrate sincere interest in their businesses, emotions and hobbies. In extreme cases, this has been observed in utter disregard for a child's immediate needs like food, clothes, etc. There have been cases of concealed underprotection, when parents did actually control their children's behavior and lives, but formally. This style of parenting would result in a child being left completely unattended in the surrounding micro-social environment, where he/she would fall under seniors' influence and become a victim of early alcohol and drug abuse. Dominant hyperprotection has been caused by excessive protection by parents, their trying to control every little move, endless supervision and control, which have contributed to lack of confidence in children. These have been accompanied by excessive taboos, restrictions on children's scopes of activity and lack of independence. This type of hyprprotection goes hand in hand with authoritarian parenting with parents often perceived as iconic figures, whose word would be law. Families, where children have been treated as "golden children," would practice excessive patronage, overindulgence, and cater to his/her every desire and whim. Not only would these children be exempt from hardships and unpleasant duties – they would be praised for every little success and talent, and parents and relatives would always give a very high status to even minor abilities. From early childhood, these teenagers have believed that they are the best, most gifted and talented, and have been urged to strive for leadership and perfectionism. The study classifies this as promoting hyperprotection. Emotional rejection would result in a child feeling like he/she is a burden for his/her parents. The feeling tends to be more pronounced in families with younger brothers and sisters, who take the biggest part of attention and care. An extreme version of emotional rejection would be keeping a firm hand on children with disproportionately tough punishments and psychological abuse. Increased moral responsibility would take place in families with parents putting too much hope in their children, their performance and talents. Besides, some parents would attempt to get their children to realize their own uncompleted dreams, impose their opinion on them, limit their right of choice and force them to strive for perfection and success. The use of incorrect parenting tactics has resulted in the appearance of accentuated personality traits in adolescents, which are likely to evolve into personality pathologies (Fig. 2).

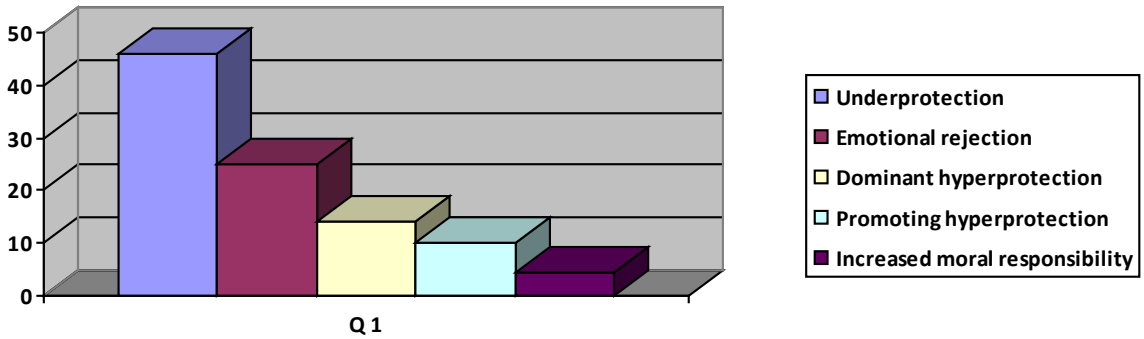


Figure 2. Parenting styles

The Lichko's pathocharacterological diagnostic questionnaire has helped identify the following types of accentuation: hysteroid accentuation has been observed in 9 subjects (10%), psychoasthenic accetuation – in 22 subjects (25.5%), labile accentuation – in 40 subjects (46%), epileptoid accentuation – in 12 subjects (14%), and in 4.5% of cases unstable accentuation has been observed. Labile personality accentuation has shown the highest percentage. These teenagers demonstrate mood swings, emotional instability, rapidly changing affective reactions, a long-standing focus on negative situations and memories from the past. Depression deemed as moderate on the Zung Self-Rating Depression Scale, has been observed in 6 subjects with hysteroid personality accentuation, who have striven to be recognized as the best among their peers, challenging for leadership and perfectionism. The hysteroid subjects have been characterized with conspicuous appearance, profanity,

falsehood, ostentatiousness and dramatic behavior. In terms of instability of personality, the most prominent signs of affective and emotional lability combined with pronounced impulsive outbreaks, antisocial activities, sometimes deviant inclination, and inability to build friendly relationships with peers have come to the picture. Of all subjects, 25.5% have been psychasthenic type, and common to these has been moderate depression accompanied by anxiophobic disorders. Psychasthenic adolescents are pursued by doubts, fears, obsessive memories, episodes of irrational anxiety, difficulty socializing, aloofness and hesitation.

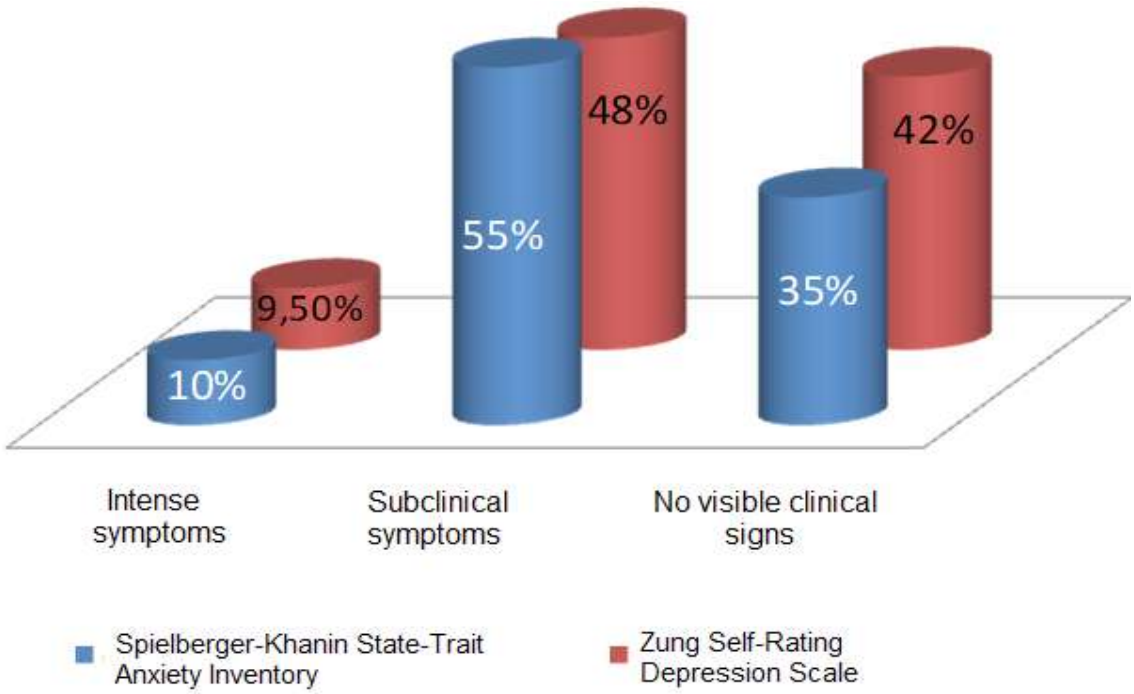


Figure 3. Structure of emotional disorders

The study of intensity of affective disorders on the Zung Self-Rating Depression Scale has revealed mild depression in 71% of the subjects and moderate depression in 29% of the subjects. The clinical picture of the depressive pathology would be manifested in low school performance, poor sleep and appetite, behavioral changes, unstable relationships with teachers and peers, detachment from parents and aloofness. The Spielberger-Khanin State-Trait Anxiety Inventory has revealed anxiety disorders of different intensity in 93% of subjects. Medium severe anxiety has been diagnosed in 56% of adolescent subjects, 27% have shown no clinically meaningful symptoms, and 16% of the subjects have demonstrated severe anxiety (Fig. 3). The most common factor contributing to self-destructive behavior consisting in suicidal thoughts and tendencies in adolescents, was incorrect parenting, which has resulted in interpersonal conflicts. The Columbia Suicide Severity Rating Scale (Posner K., Brent D., Lucas C., 2009), which has been used to evaluate suicidal tendencies, has determined 15% of the subjects as purely suicidal, 42% - as prone to suicidal thoughts and plans, 33% of the subjects are subjects with suicidal thoughts, who have made demonstrative, dramatic and manipulative suicide attempts. Most of them have been surface wounds near the bend of the arm or swallowing a large number of pills. Manipulative suicidal behavior has been triggered by the following factors: conflicts with parents (50%), with school and college teachers and mentors (25%), with peers (15%), and personal issues (10%). Most subjects would conceal suicidal intentions from parents and relatives, and they have been traced in social media dialogues, diaries and pictures. The study has revealed a correlation between teenagers' personality traits and type of suicidal attempts. Affective suicidal attempts have been observed in emotionally labile and psychasthenic personality types. Manipulative parasuicidal behavior has been diagnosed in hysteroid and emotive subjects. Impulsive suicide attempts have been made by epileptoid adolescents (Fig. 4)

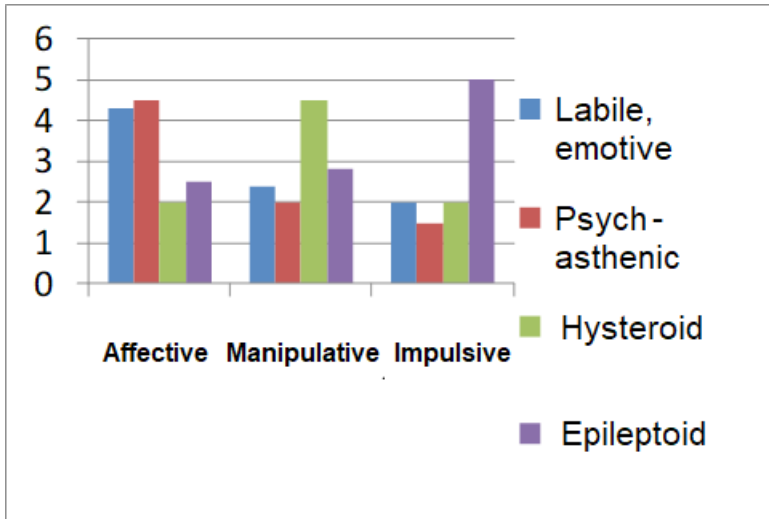


Figure 4. Correlation between types of personality and types of suicidal behavior

Clinical manifestations of self-destructive behavior mainly result from behavioral and stress-related disorders; they are associated with non-psychotic psychopathological conditions and are mostly demonstrative and manipulative by nature. Anxiodepressive and depressive neurotic disorders have been observed in emotionally labile teenagers. Mild depressive disorders have been diagnosed in hysteroid personality types. Moderate depressive disorders have been discovered in psychoasthenic adolescents. Dysphoric conditions have been found in epileptoid subjects.

Conclusion

The study of family-related risk factors of suicidal tendencies in teenagers has revealed that various manifestations of incorrect parenting in dysfunctional families contribute to development of labile, epileptoid and psychoasthenic personality accentuation in adolescents with intense personal and situational anxiety combined with proneness to depression-range suicidal behavior. Early detection of dysfunctional and ambivalent families and destructive parenting is paramount for adolescent suicide prevention and reduction of the suicide potential in this population group.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Certain Indicators of Diclofenac Sodium Pharmacokinetics in Patients with Rheumatoid Arthritis Considering Comorbide States

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Abstract

Introduction: Much attention is paid to pharmacokinetic (FK) researches for studying the processes of supply, distribution, biotransformation and excretion of medications at the present time, as well as identifying links between medical substances concentration and (or) its metabolites in biological fluids and tissues, and its pharmacological effect. In the treatment of rheumatoid arthritis (RA), non-steroidal anti-inflammatory drugs (NSAID) are widely used for a symptom therapy, where Diclofenac Sodium (DS) is most commonly prescribed. Duration of anti-inflammatory effect, effectiveness of NSAID in whole are directly dependent on the level of effective concentration of medications and circulation duration in blood in free form.

The Aim of the present research work is to study in a comparative aspect certain indicators of Diclofenac Sodium pharmacokinetics in patients with rheumatoid arthritis with gastric microbiocenosis disorders (gastric dysbiosis) and without disorders (without dysbiosis).

Materials and Method: 38 patients at the age from 18 to 60 years were examined, with I-II-III degree of disease activity. In addition to the general clinical examination, an immune-enzyme method and urease test were carried out to determine Helicobacter Pylori and highly effective liquid chromatography to determine Diclofenac pharmacokinetics.

Results: Conducted researches and analysis of their results indicate that in conditions of rheumatoid arthritis, particularly, in the presence of comorbid states, there is a decrease in the metabolism rate and an extension of NSAID half-excretion period, which increases the risk of side effects, especially to the gastrointestinal tract, and significantly affects the disease course and the results of the treatment.

Conclusion: In conditions of rheumatoid arthritis with comorbid states, there is more frequent occurrence of the NSAID side effects. Research conducted and analysis of their results indicate that in conditions of rheumatoid arthritis, particularly at comorbid states, there are reasonable changes in the NSAID pharmacokinetics. The decreasing of metabolic rate and the extension of the NSAID half-excretion increases the risk of side effects, especially on the side of the GIT, which significantly affects the disease course and results of the treatment. This circumstance dictates the need to take these results into account in the treatment of the studied pathology and in the development of a personalized approach to the treatment of rheumatoid arthritis.

Keywords: *Rheumatoid arthritis, Diclofenac Sodium, highly-effective liquid chromatography, pharmacokinetics.*

Introduction

Now a days a greatattention is paid to pharmacokinetic (FK) researches for studying the processes of supply, distribution, biotransformation and excretion of medications, as well as identifying links between medical substances concentration and (or) its metabolites in biological fluids and tissues, and its pharmacological effect. Clinical pharmacokinetics determines which dosage of medicines provides their necessary concentration in body media to achieve optimal therapeutic effect. For the present time several method for determining Diclofenac in biological fluids are known: potentiometric analysis[1], highly effective liquid chromatography (HELC)[2], capillary zone electrophoresis[3], spectrophluometry[4], thin layer chromatography[5], polar-graphic analysis[6].

It is generally known that in the treatment of rheumatoid arthritis (RA), non-steroidal anti-inflammatory drugs (NSAID) are widely used for a symptom therapy, where Diclofenac Sodium (DS) is most commonly prescribed. Duration of anti-inflammatory effect, effectiveness of NSAID in whole are directly dependent on the level of effective concentration of medications and circulation duration in blood in free form. These parameters in clinical practice determine a number of pharmacokinetics indicators, in particular, Cel (constant of elimination), Cl (medication clearance), T1/2 (half-excretion of medication), etc. In human body Diclofenac undergoes biotransformation under the influence of the cytochrome P_{450} enzyme system with formation of three primary metabolites: 3-hydroxydiclofenac, 4-hydroxydiclofenac, and 5-hydroxydiclofenac. Primary metabolites are conjugated to form two secondary ones - 4,5-dihydroxy-diclofenac and H-hydroxy-4-methoxydiclofenac (3,4-HMD)[9]. All metabolites significantly assign to the primarymedication in therapeutic activity[8]. The half-excretion of Diclofenac Sodium and four of its five major metabolites ranges on average from 1 to 3 hours, but for 3,4-HMDit reaches 80 hours. DS does not cumulate on long-term using, all its metabolites are excreted with urine and bile. 95.7% of the medication is bound with the serum proteins[7]. According to previous research works, the concentration of Diclofenac Sodium reached a maximum after intramuscular injection after 15 and 25 minutes[10]. Besides, it should be noted that most of NSAIDs belong to the category of medications metabolized in the liver, which makes the problem of metabolism studying in the body particularly relevant in practical terms.

Rheumatoid arthritis is often accompanied by visceral manifestations from other organs and systems, as well as concomitant diseases (comorbid state)[11], which can affect pharmacokinetic parameters of used NSAIDs.

The aim of the study is to research in a comparative aspect certain indicators of Diclofenac Sodium pharmacokinetics in patients with rheumatoid arthritis accompanied by gastric microbiocenosis disorder (gastric dysbiosis) and without gastric microbiocenosis disorder (without dysbiosis).

Materials and Method

There were 38 patients with reliably established RA at the age of 18-60 years, with disease duration of more than 5 years. RA patients were divided into 3 groups: RA patients without dysbiosis - 28.6%, RA patients with dysbiosis -42.8%, RA patients with dysbiosis and H.pylori- 28.6%.

For determination of Diclofenac in blood plasma a highly effective liquid chromatography (HELC) was used with mass spectrometry (MS). The Agilent 6420 Ultra Highly Effective Liquid Chromatograph (UHELC) device with triple quadrupole mass spectrometer was used (produced by Agilent Technologies, USA). HELC pure acetonitrile was supplied by Sigma-Aldrich Trading Co. (Schnelldorf, Germany). Ultra-pure water was obtained by a water purification system produced by Sartorius LabInstruments GmbHЄtCo.KG (Goettingen, Germany). All other chemical substances were of analytical purity and were used without further purification.

Preparation of Samples: Control blood samples were taken from the patients after Diclofenac tablets application (50 mg) (positive control) after 0.5, 6 and 12 hours. The blood samples were centrifuged at 2000 rpm during 6 minutes. After these measures the blood plasma was injected into HELC tube for the analysis.

For preparing standard Diclofenac samples, the substrate was dried, then the exact amount was weighed, and a basic water solution was prepared at a concentration of 1 mg/ml. Samples of different Diclofenac concentrations were prepared from this solution for both qualitative and quantitative analysis.

HELC-MS/MS screening: HELC analysis was performed with samples volume 1mcl,which was injected with the help of an automatic sampler. As a

mobile phase only water acidified with 0.01% formic acid or acetonitrile water added 0.01% formic acid for positive ion control at a flow rate of 0.2 ml/min was used.

Analysis were performed by Full Scan Mode (Fullscanmode) for determination of the chemical composition of the substance.

Qualitative and quantitative analysis of Diclofenac Sodium: Standard solutions of Diclofenac were newly prepared by diluting the base solution (1 mg/ml) in ultrafine water. Samples ranging from 0.01 to 1000 ng/µl were prepared for quantitative analysis for calibration, detection limit (DL) and quantitative limit (QL). These solutions were stored at 4ºC before the analysis. DL and QL were determined experimentally from the signal-to-noise ratio by diluting the basic concentration (1 mg/ml)

Results

The results of these studies are shown in Figures 1,2,3: As it can be seen from the Fig.1, in patients with rheumatoid arthritis without gastric dysbiosis, Cel is decreased for 34.4%. compared to the control index. At the same time in patients with rheumatoid arthritis with gastric dysbiosis this indicator is decreased to a greater extent (almost 1.5 times compared to the control). Considering that this indicator of pharmacokinetics reflects the rate of medication excretion from the body, it becomes obvious that in condition of rheumatoid arthritis the rate of medication excretion from the patient ‘s body is slowed down. And in accompaniment ofgastric dysbiosis this process is highly aggravated.

It is generally known that the rate of xenobiotics excretion, including medications metabolizing in the liver,first of all depends on the rate of their biotransformation (metabolism) in the body. In this regard, we studied the Cl indicator, which reflects the degree of body purification from the medication. Analysis of this indicator results also shows a significant inhibition of this body function in rheumatoid arthritis conditions, especially in the presence of comorbid states (Fig. 2).

Inhibition of medicine metabolism in the body and decrease of the rate of their excretion from the body is accompanied by accumulation of the applied medicine in the blood. Indeed, as it can be seen from the data presented in the figure, in patients with rheumatoid arthritis the indicator T1/2 is extended 1.5 times in comparison with the control. And in conditions of gastric dysbiosis, almost 2 times, respectively (Fig. 3).

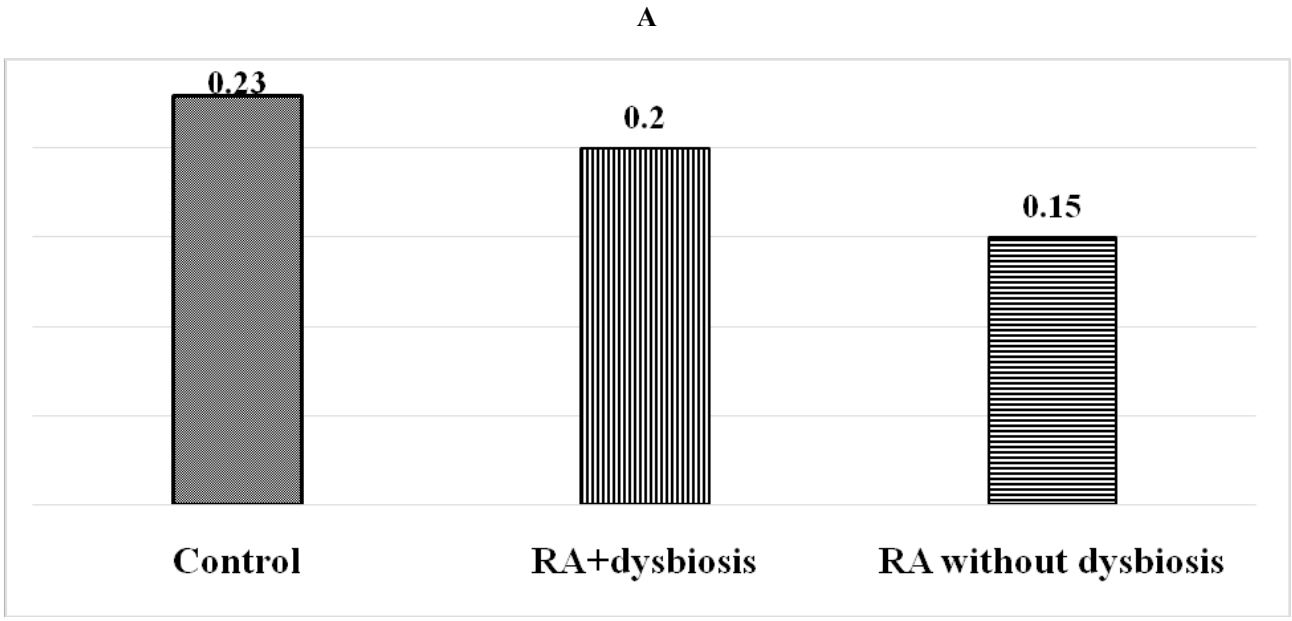


Fig. 1. Indicators of Diclofenac Sodium pharmacokinetics in patients with rheumatoid arthritis: A – indicator ofConstant Elimination (Cel)

B

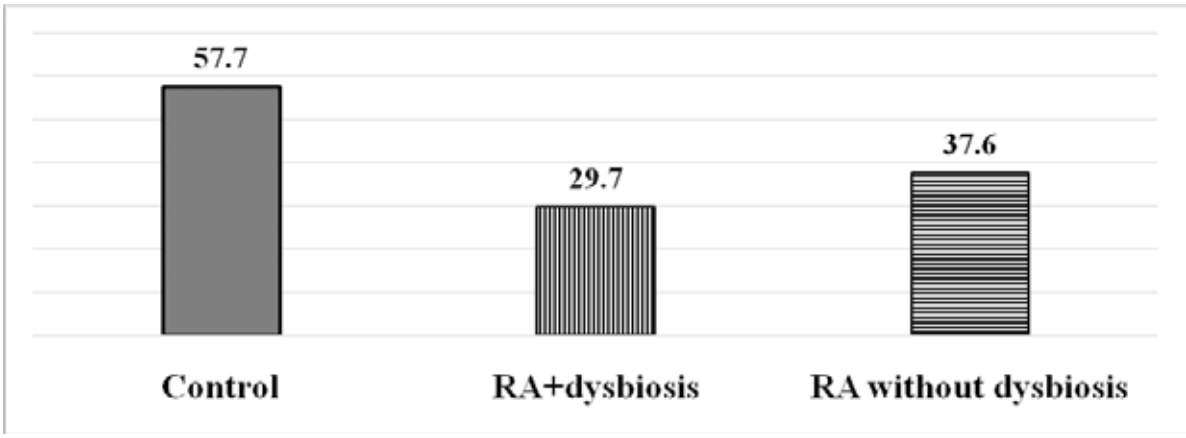


Fig. 2. Indicators of Diclofenac Sodium pharmacokinetics in patients with rheumatoid arthritis: B – clearance of the medication (Cl)

C

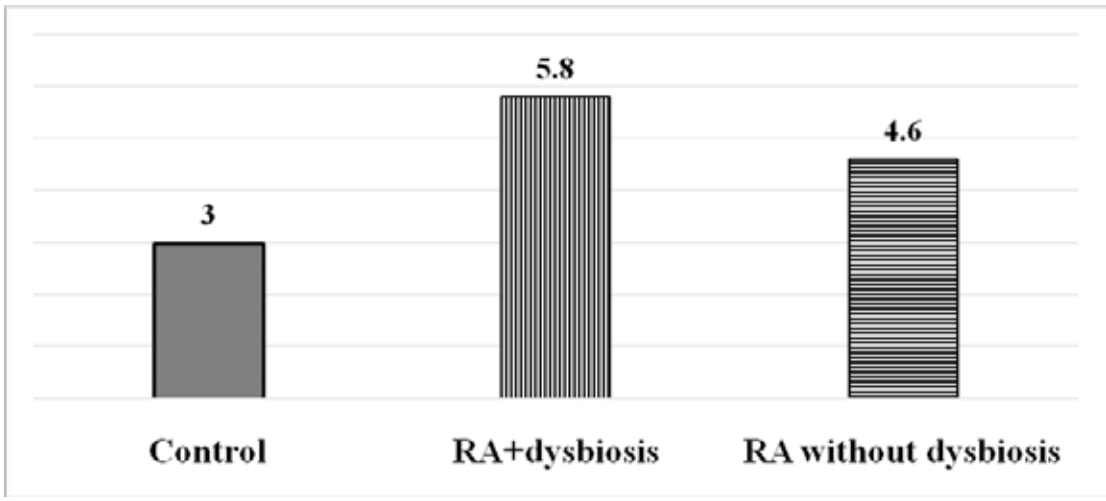
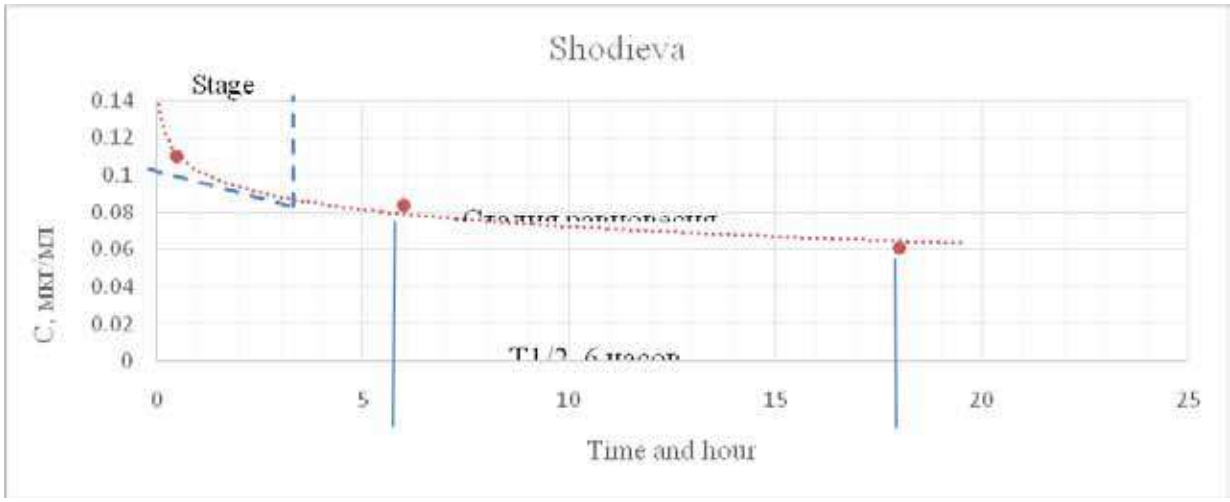


Fig. 3. Indicators of Diclofenac sodium pharmacokinetics in patients with rheumatoid arthritis: C –half-excretion period ($T_{1/2.}$).

The following is the example of calculating of the pharmacokinetic indicators of the patient with RA (Fig. 4)



V_p = Dose/C_0 = 25 mg/0,1 = 250 l

C_{el} = 0,693/$T_{1/2}$ = 0,693/6 hour = 0,1155h^{-1}

Cl = $V_p \times C_{el}$ = 250 l×0,1155h^{-1} = 28,875 l/h

Normally, Diclofenac is excreted in 2-3 hours. Let's assume, if this processtakes 2.5 hours, then

C_{el}(norm) = 0,693/$T_{1/2}$ = 0,693/2,5 hour = 0,2772 h^{-1}

$C_{el} < C_{el}$ (norm)

Cl(norm) = $V_p \times C_{el}$ = 250 l×0,2772h^{-1} = 69,3 l/h

Cl<Cl (norm)

Discussion

Therefore, in patients with rheumatoid arthritis there is a significant extension of the half-excretion of the researched medication, and in the condition of comorbid states this indicator grows even greater,that increases the risk of development of side effect.

Considering the results of pharmacokinetics, there are two variants for possible decrease of the NSAID side effects:

1. It is necessary to reduce the NSAID dose without changing the medicine taking intervals; or
2. Without changing the dose, to extend the intervals, i.e. reduce the multiplicity of medication appointment.

Besides, to protect gastric mucosa, it is preferable to add antacids, proton pump inhibitors, M-cholinoblocators and H2-histamine receptor blockers to the general treatment.

Thus, accordingto the above mentioned, we have also studied and analyzed the structure and frequency of side effects of the conducted treatment against the background of NSAID appointment in the examined groups of patients. The results of this analysis are given in the Table 1.

Table 1: Structure and frequency of manifestations of NSAID side effects in patients with rheumatoid arthritis

Lesion of GDZ	Group of patients		
	RA without dysbiosis	RA with dysbiosis	RA with dysbiosisand H. pylori
Heartburn (%)	50	58	80
Burp (%)	18	33	12
Heaviness in epigastrium (%)	12	42	50
Pains in epigastrium (%)	58	54	68
Constipation (%)	-	14	4
Poor appetite (%)	10	15	18
Esofagit (%)	77	50	88
Gastric ulcer and DPC (%)	10	25	28

Conclusion

As itis seen from the presented data above, in the group of patients with rheumatoid arthritis accompanied by gastric dysbiosis, the frequency of occurrence of the most characteristic signs of side effects becomes significantly higher than in the group of patients with rheumatoid arthritis without gastric dysbiosis. At the same time such manifestations as heartburn, heaviness in epigastria, pain in epigastria in the group of patients with rheumatoid arthritis and gastric dysbiosis increase for 38%, 19% and 26%, respectively. They also have a relatively high incidence of esophagitis and gastric and duodenal ulcers.

Therefore, in conditions of rheumatoid arthritis with comorbid states, there is a more frequent occurrence of the NSAID side effects.

Thus, researches conducted and analysis of their results indicate that in conditions of rheumatoid arthritis, particularlyat comorbid states, there are reasonablechanges in the NSAID pharmacokinetics. The decreasingof metabolic rate and the extension of the

NSAID half-excretion increases the risk of side effects, especially on the side of the GIT, which significantly affects the disease course and results of the treatment. This circumstance dictates the need to take these results into account in the treatment of the studied pathology and in the development of a personalized approach to the treatment of rheumatoid arthritis.

Consent: It is not applicable.

Ethical Approval: All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Conflict of Interest: Authors declare that there is no any conflict of interest for the results of the study.

Authors' Contributions: This work was carried out in collaboration between all authors. Author NKT designed the study, wrote the protocol, performed data collection, wrote the initial manuscript and revisions. Author MSK, BShA performed the literature review, data analysis and processing, manuscript writing, edition and revisions, author MVS helped with the writing of the manuscript. Author AMM helped with the statistical analysis. Author KLK edited the manuscript and revisions. All authors read and approved the final manuscript.

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Repeated Reconstructive Surgeries for Postoperative Complications of Anorectal Malformations in Children

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Abstract

The causes of postoperative complications in the correction of anorectal malformation were studied according to follow-up, general clinical and additional research method in 65 operated sick children. In the process of diagnosis and surgical correction, a number of diagnostics, tactical, and combined errors and shortcomings were identified that caused postoperative complications, which required a large number of repeated reconstructive operations. The authors recommend various options for tactical and operational - technical treatment approaches when solving the problems of repeated reconstructive operations. At the same time, it is proposed to study the causes, analyze errors, complications and failures, indications, terms, accesses, the volume of the operation and evaluate their effectiveness. The authors, thanks to the optimization of tactics, method of surgical and rehabilitation treatment, were able to improve the results of postoperative complications to 81.4%.

In the course of the study of catamnestic and examination data 65 of children with postoperative non-NAS in the process of diagnosis and surgical correction errors and deficiencies were revealed as: unreliable primary diagnosis, insufficiency of preoperative preparation, inadequate indication and choice of the method of operation, inadequate postoperative rehabilitation. All these had diagnostic, tactical, technical and combined character, which led to the formation of the postoperative HCA, which required a large number of repeated operations. Therefore, the authors suggest that to solve the problems of repeated operations is not limited to the improvement of surgical techniques, and to study the causes, analysis of errors, complications and failures that lead to repeated interventions, indications, timing, access, scope of surgery and their evaluation of effectiveness, medical and social rehabilitation of patients.

The authors, thanks to the optimisation of tactics, method of surgical and rehabilitation treatment, managed to correct the post-surgical NAC up to 80,4%.

Keywords: *Anorectoplasty, postoperative complications, anorectal malformation. Children, proctoplasty, postoperative failure of anal sphincter, anorectal malformations.*

Introduction

In the surgical treatment of anorectal malformations (ARM), reducing the number of postoperative complications (PC) remains a problem in pediatric surgery[18], which is far from being resolved[11] .According to the literature[1], after the initial surgical correction of the ARM, functional disorders of the sphincter apparatus of the rectum occur in 30-60% of patients, subsequently[9] accompanied by fecal incontinence and chronic constipation[2].

At the same time, the main cause of unsatisfactory results[10] in the treatment of ARM are diagnostic[6], tactical and technical errors[15], which often lead to anal incompetence (AI)[5] in children which requires repeated reconstructive surgery (RRS). Therefore, the optimization of surgical tactics, indications and the choice[18] of RRS method to reduce complications of ARM in children is an urgent problem in pediatric colorectal surgery[20].

The objective of the study was to improve the results

of repeated[4] reconstructive surgery for postoperative complications of ARM, based on[11] a study of the causes of complications, ways to optimize diagnosis, treatment tactics and develop preventive measures in children[17].

Materials and Method

For the period 2007-2020 In the departments of pediatric surgery 1st CCCH in Tashkent and MACH in Samarkand (clinical bases of the Department of Pediatric Surgery TashPMI and SamMI), 65 patients were hospitalized for re-correction of complications of ARM. The age of patients is: 3 - 12 months. - 6 (9.2%); 1-3 years - 27 (41.5%); 3 - 7 years - 20 (30.7%); 8-14 years old - 9 (13.8%); 14 - 18 years old - 3 (4.6%). A total of 139 RRS were conducted, of which: twice in 30 (46.1%); three times in 20 (30.7%); four times or more in 15 (23.9%) cases. At the same time, surgical approaches were: STD - 14; ASARP - 23; PSARP - 21; PP-81. The colostomy before RRS was imposed on 37 (57%) patients. Boys - 28 (43%) and girls - 37 (57%).

Concomitant pathologies and regional malformations had been noted in all re-operated, in 18 (40%) patients: agenesis and aplasia of the coccygeal and sacrococcygeal vertebrae, disorders of the innervation of the pelvic organs due to thickening of the terminal filament and spinal cord, etc.

Comprehensive examinations were carried out for all patients: general clinical examinations (medical history, follow-up, clinical examination with rectal examination and laboratory diagnostics); radiological (irrigography, fistulography); additional (ultrasound with doppler, MRI of the abdominal cavity and pelvis, electromyography of the sphincter apparatus of the rectum, rectal reflex and endoscopy). At the same time, we revealed defects, tears, relative thinning of the width, shortening of the length and asymmetry of the puborectal muscles and the external anal sphincter.

We carried out a clinical analysis of the causes of the occurrence of PC in the primary and repeated correction of ARM; The diagnostic tactical and technical errors that were made, the risk of complications during the operation were studied, and optimal method for their repeated correction were developed.

Patients had been divided into two groups. The first group consisted of 47 (72.3%) patients, the use of operations, against the background of mistakes and complications after the initial radical and repeated corrective surgery, leading to anal incompetence (AI). The second group - 18 (27.7%) of patients, against the background of combined regional malformations and congenital inferiority of the obturator apparatus of the rectum.

The study was carried out in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee of all participating institutions. Parents of the children had been informed about the studies and agreed to carry them out.

Results and Discussion

In the course of studying follow-up and clinical examination data of patients, the causes of complications in the correction of ARM, the clinically manifested AI (fecal incontinence) and stenosis of the anus and rectum (constipation), which needed to be carried out, were identified.

The identified causes of mistakes and complications were diagnostic, tactical, technical and combined.

I. Diagnostic: unreliable verification of diagnostics; insufficient assessment of malformations of neighboring organs; X-ray: improper laying of the patient or the absence of a mark on the anus when determining the height of atresia and the extent of stenosis, omission of deviation of the anorectal angle; clinically unrecognized congenital rectourethral fistula (RUF) and anus ectopia; inaccurate identification of anomalies in the initial diagnosis; inadequate identification of the causes and analysis of complications arising from the primary radical correction of the ARM; unreliable verification of the diagnosis during secondary correction.

II. Tactical: lack of preoperative preparation; wrong choice of indications; inadequate choice of operational access; non-optimal choice of the method of radical surgery or colostomy during the correction of the defect and in the event of complications; excessive attempt to search for perineal access to the high atresized end of the intestine; Inadequate postoperative rehabilitation and lack of surgeon qualifications.

III. Technical: defective implementation of the primary radical classical form of operation technique; damage to the muscles of the sphincters; underestimation

of the anomalies of the arcade vessels, significant intersections of the major vessels, too much tension or overextension of the mesenteric vessels during mobilization of the colon; extrafincter reduction of the distal part of the colon; accidental wound of the urethra with repeated mobilization of the colon; inferior fixation of the intestine during reduction; defective mobilization of the fistula, insufficient implementation of the plastic technique in rectovestibular (RVF) and RUF; technical mistakes during repeated operations.

IV. Combined, this type includes combinations of two or more types of groups of mistakes and complications at the same time. In some cases, the diagnostic mistakes made led to tactical ones, and they, in turn, to technical ones.

The results of the study showed that the causes of AI in the postoperative period were identified in the group of patients and are directly related to diagnostic, tactical and operational-technical errors (Fig. 1-2).



Fig. 1. Postoperative AI due to diagnostic, tactical, technical mistakes and complications in the correction of low forms of ARM



Fig. 2. Postoperative AI due to diagnostic, tactical, technical mistakes and complications in the correction of high forms of ARM

In the other group, not related to them: a combination of malformations of the nerve trunks, plexuses and fibers, defects in the distal part of the spine and chair, anomalies of neighboring organs.

The first group of causes of postoperative AI included intraoperative trauma, unsuccessful re-correcting operations and purulent-inflammatory complications due to diagnostic, tactical and technical errors that led to stenosis or dystopia of the anal canal, deformation of the perianal region, extinction of the mucosa of the reduced intestine, relapse of the fistula into the urinary tract or the reproductive system in children.

The second group of causes of postoperative AI was revealed in the background: disorders of the innervation of the pelvic organs due to thickening of the terminal filament and spinal cord in 4 cases; underdevelopment of the caudal vertebrae (aplasia or agenesis of the sacrum and coccyx) - in 10; underdevelopment of the muscular structures of the sphincter apparatus (aplasia or hypoplasia of the muscle complex) - in 4. These concomitant pathologies had been found among 18 operated patients in a separate and combined form (Fig. 3).

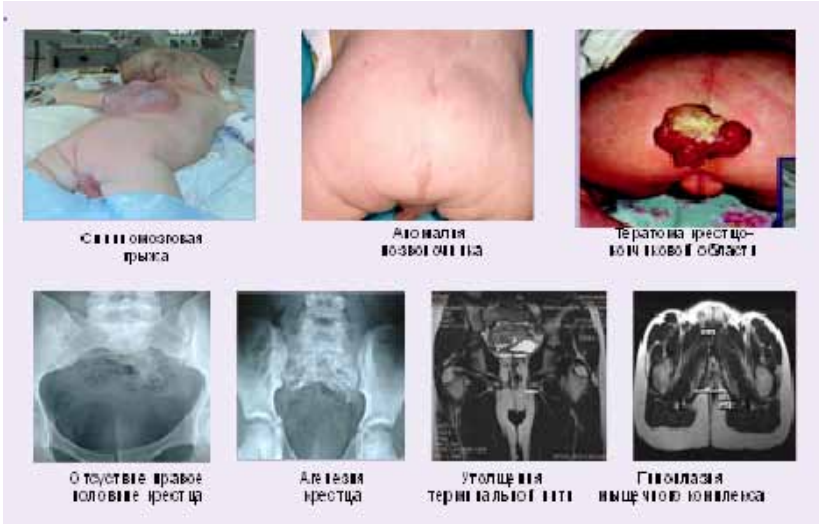


Fig. 3. Uncontrolled AI against the background of violation of the innervation of the pelvic organs, underdevelopment of the caudal spine and muscle structures of the sphincter apparatus.

In these groups of patients, despite an ideally performed primary radical anorectoplasty or RRS, AI was constantly left with the clinical course of fecal incontinence.

Thus, after the initial and repeated ARM correction, all PC for the period differs by: early and late.

Early Complications Include:

– *Suppuration with divergence of sutures of the perineal wound* - with poor-quality preoperative preparation, poor care and management of patients in the postoperative period.

– *Necrosis of the stump of the reduced intestine* - as a result of underestimation of the anomalies of the arcade vessels, significant intersection of the major vessels, too much tension or overextension of the mesenteric vessels in STDs, leading to impaired blood supply in the reduced intestine, its necrosis and sometimes pelvic peritonitis in the early postoperative period

Late Complications Include:

– *Stenosis* of the *anus and rectum*, *secondary megarectum* - arising in the late term, with a long inflammatory process in the area of the perineal wound, chronic ischemia of the wall of the reduced colon and after multiple perineal and peritoneal perineal proctoplasty, which clinical is manifested by chronic constipation.

– *Cicatricial deformity of the anus, cicatricial degeneration of the external sphincter and defects*

of the perineal septum in the form of an "artificial cloaca" ***- arising after a cicatricial process due to impaired blood supply to the wall of the reduced intestine and after multiple perineal proctoplasty due to relapse of the fistula, which clinical manifests by fecal and gas incontinence.***

– *Excess (standing) of the reduced intestinal mucosa* that arose after various types of proctoplasty and with technical errors in the formation of the neonus. The excess mucosa was represented by a small area or a large fragment of it, which clinically proceeds with fecal incontinence

– *A pararectal fistula* arising against the background of increased pressure by fecal masses in the rectum or clogging of it with "stones", with retraction of the reduced intestine, with an enema to create a false move. It usually opens far from the anus or gluteal region, which clinically occurs with fecal incontinence.

On the basis of complex method of examining patients and using ultrasound, MRI and EMG, we revealed anatomically: localization of defects, tears, relative thinning of the width, shortening of length, asymmetry and physiologically: impaired contractility of the puborectal muscles and external anal sphincter.

These data made it possible to assess the degree of damage to the sphincters in postoperative AI, to determine the tactics of choosing method for repeated surgical correction, intraoperative and postoperative measures for the prevention of complications.

In tactical terms, in all cases, in order to improve blood circulation and reduce the scar process, stiffness of perineal tissues, restore the tone of sphincter muscles of the 2-3-degree AI, a stage rehabilitation treatment was performed before each RRS. The rehabilitation treatment complex included: electrophoresis with lidase, electrical stimulation (ESM) of the anal sphincter and pelvic floor, LED laser in a separate and combination form. A sigmostomy, transversostomy and colostomy were applied in 57% of patients as a preoperative preparation. This allowed to improve the clinical results of surgical treatment by 2 times compared with the RRS

When conducting missile defense in the anorectal region, anterior and posterior sagittal access was often used, which allowed intraoperatively assessing the damage state, eliminating the defect of the sphincter of the anus and under visual control of plastic surgery. At the same time, muscle structures, blood vessels and nerves with minimal blood loss are minimally injured; local tissues are used to the maximum to restore the sphincter apparatus; significant mobilization of the rectum, which made it possible to carry out sphincteroplasty and sphincterolevoplasty and allowed to avoid excessive STDs.

The types and number of late complications leading to AI and the choice of RRS method in children are presented by us in table 1.

Table 1: Types and number of late postoperative complications and method of repeated surgical correction in children.

№	Types of postoperative complications	Amount-%	Method for repeated correction
1	Reduced stenosis of the anus and rectum	11 -16,9%	Elimination of stenosis with reconstruction of the anus and rectum
2	Extended stenosis of the anus and rectum	5 -7,6%	APR Elimination of extended stenosis with reconstruction of the anus and rectum.
3	Cicatricial deformity of the anus	7 -10,7%	Sigmostoma. Elimination of cicatricial deformity with reconstruction of the anus
4	Cicatricial deformity anus and vagina	4 -6,1%	Sigmostoma. Elimination of cicatricial deformity of the anus with reconstruction of the perineum
5	Defect of the rectovaginal septum	6 ()	Sigmostoma. PSARP. Sphincterolevatoroplasty
6	Acquired «Esthetic cloaca»	2 -3%	Sigmostoma. PSARP. Staged anorectoplasty

№	Types of postoperative complications	Amount-%	Method for repeated correction
7	Relapse of the RVF	8 -12,3%	Sigmostoma. PSARP. Stone's anorectoplasty.
8	Relapse of the RUF	3 -4,6%	Transversostomy. Epicystostomy. APR with the elimination of RUF
9	Acquired "Dystopia of the anus"	4 -6,1%	Displacement of the dystopic anus. Sphincteroleoplasty
10	Extrasphincterous lowering of the colon	4 -1%	APR With repeated lowering of the colon
11	Acquired pararectal fistula with anus deformity	2 -3%	Elimination of pararectal fistula Sphincteroplasty with reconstruction of the anus
12	Protrusion of the mucosa of the lowered intestine	7 -10,7%	Ablation of the protrusion of the mucosa of the lowered intestine
13	Deformation of the anus with cicatricial degeneration of the external sphincter	2 -3%	Transversostomy. APR with excision of cicatrix. Sphincteroplasty
	Total	65 -100%	100%

When conducting RRS in order to reduce postoperative AI, we drew attention to the following tactical aspects of a local nature: determining the degree of damage to the sphincter of the anus and rectum; the nature of cicatricial deformity of the anorectal region; the degree of protrusion of the mucosa, optimization of indications, choice of method, timing, ways of access to surgery. At the same time, ensure the preservation of the sphincter apparatus of the rectum; understand the intricacies of topographic and anatomical relationships of the perineum; to conduct adequate rehabilitation treatment and restoration of the physiological state of the newly created neo-anus and rectum.

In cases where there was no damage to the sphincter of the anus and direct and severe deformity, treatment was started with rehabilitation measures and only with inefficiency, RRS was performed.

In the presence of deformity (stenosis, ectopia, fistulas, defects) and mucosal standing, direct indications for RRS were determined regardless of the degree of damage to the sphincters.

When conducting RRS in children, we had based on the following techniques and intraoperative treatment tactics:

– If it possible, do correcting complications without abdominal access;
– Rehabilitation treatment was performed in the preoperative period to restore blood supply and innervation of the perineum;
– Strive for minimal tissue dissection when access to perineal;
– Exact removal of the rectum through the center of the retaining muscle complex;
– Strive to restore the anorectal angle with anorectoplasty, by maintaining the balance between puborectal and anococcygeal muscles;
– To ensure the elimination of tension of the reduced intestine in the area of the newly formed neo-anus.

It should be noted that RRS in children is performed in more difficult conditions than primary, and with less ability to perform proctoplasty in a classic form, with gross cicatricial changes in perineal tissue and chronic ischemia of the intestine. Moreover, with each repetition of RRS, the situation begins to deteriorate prognostically. Therefore, we recommend that in difficult situations (defects, cicatricial deformities) with pronounced AI for the effectiveness of RRS, it is imperative to apply a colostomy, conducts rehabilitation and comprehensive treatment of anemia and inflammatory diseases of the genitourinary, respiratory and cardiovascular systems.

Tactical moments of a general nature. This tactical approach significantly improved treatment outcomes with a decrease in the number of reoperations in children.

Thus, due to optimization of diagnostics, preoperative preparation tactics, method of intraoperative surgical correction, postoperative measures for the prevention of complications and rehabilitation, we obtained significant improvements in the results of treatment of postoperative AI. Long-term treatment results were rated as good, satisfactory and unsatisfactory.

The results of surgical treatment of postoperative NAS in children are presented in table 2.

Table 2: Results of surgical treatment of postoperative AI

Pathological conditions of complications by degrees of AI	Method of RRS	Results (Amount and %)			Total (%)
		Good	Satisfactorily	Unsatisfactorily	
1. AI of 1-degree, without significant damage to muscle structures and deformities of the anus.	Conservative (rehabilitation) treatment	16-23,2%	5-8,9%	-	21-32,2%
2. AI of 1-2 degrees, without significant damage to muscle structures, protrusion of the mucosa.	Removal of excess mucosa + sphincteroanoplasty + rehabilitation treatment	10-16%	8-10,7%	-	18-26,8%
3. AI of 2-3 degrees, with cicatricial deformity of the anorectal region.	Colostomy, ASARP or PSARP + sphincteroleo plasty + rehabili tation	4-5,4%	7-10,7%	6-7,3%	17-25%
4. AI of 3 degrees, with damage to all elements of the obturator apparatus.	Colostomy, APR, ASARP or PSARP + ARP with resto ration of the puborectal loop + sphincterolevato roanoplasty with reconstruction of ana tomical defects + multiple and phased rehabilitation treatment	-	3-5,4%	6-10,7%	9-16%
Total		**30-46,1%**	**23-35,3%**	**12-18,6%**	**65-100%**

As can be seen from the table 1., thanks to the optimization of tactics, surgical and complex rehabilitation treatment method, we had good results in 30 (46.1%) patients; satisfactory - at 23 (35.3%) and unsatisfactory - at 12 (18.6%).

Thus, we were able to adjust the AI in 53 cases out of 65 patients, which is 81.4% with good and satisfactory results. The optimal criteria were: optimization of the quality of preoperative diagnostics, preparation of the patient for surgery, timely detection of concomitant abnormalities of the neighboring organs, professional competence of the surgeon, adequacy of the choice of tactics, technique of performing corrective surgery, corrective surgery.

The second group - 18 (27.6%) patients, due to congenital inferiority of the obturator apparatus of the rectum against a background of a combination of regional malformations (aplasia, agenesis of the sacrum and coccyx, hypoplasia of the muscular complex) and violation of the innervation of the pelvic organs, an AI, despite an ideally performed primary radical anorectoplasty, RRS and prolonged stage rehabilitation treatment, unsatisfactory results were preserved in the form of fecal and urinary incontinence, to varying degrees and for long periods.

In these groups of patients, prolonged ESM of anal incontinence, rectal gymnastics, training enemas using the principles of biofeedback (in children older than 5 years), the development of an individual lifestyle up to 5 years and long courses of complex neurological treatment led to a satisfactory condition of patients, only in 5 (27.7%) cases.

Thus, in the first group of patients without concomitant pathologies against the background of purulent-inflammatory complications and diagnostic, tactical and technical errors as a result of which, they led to stenosis and dystopia of the anal canal, deformation of the perineum, extinction of the mucosa of the reduced intestine, relapse of the fistula into the urinary and reproductive system, RRS was provided in children. In the distant period, against the background of complex treatment, in these categories of patients, good results were obtained in 30 (46.1%) patients; satisfactory - in 23 (35.3%) and unsatisfactory - in 12 (18.6%).

In the second group of patients, in addition to

the correction of ARM, against the background of a concomitant disturbance of the innervation of the pelvic organs due to thickening of the terminal filament and spinal cord, underdevelopment of the caudal region of the vertebrae and the arm and levator muscle structures, good results were not noted, satisfactory - in 5 (27.7 %) and unsatisfactory - in 13 (72.3%) of the 18 cases.

Results

1. Indications for missile defense are determined taking into account 2-3 degrees of AI and deformation of the anorectal region.
2. By analyzing the causes, errors and complications, optimizing preoperative diagnostics, optimizing tactics, method of surgical and comprehensive rehabilitation treatment and postoperative preventive measures, it was possible to improve the results of RRS treatment by 81.4%.
3. For the prevention of postoperative complications of ARM during repeated correction in children, it is necessary to avoid possible diagnostic, tactical and technical errors by applying optimal tactics and techniques of repeated corrective surgery and ensuring high-quality rehabilitation treatment.

Ethical Clearance: No ethical approval is needed.

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Lower Extremity Deformities in Children with Vertebromedullary Anomalies

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Abstract

The article presents the literature data, results of diagnostics and treatment of 219 children with various morphologies of spinal herniation and neuroorthopedic disorders. It characterizes in detail motion disorders and deforming arthrosis of the lower extremities in 88.1% and 26% of patients. The authors state that the genesis of disorders can include the manifestations of spinal cord fixation syndrome in patients with spinal hernias with combined congenital and acquired forms of latent spinal dysraphism. The article confirms the feasibility of early preventive and therapeutic measures corresponding to the nature of the combined pathology, with the participation of specialists.

Keywords: *Spinal cord anomalies, spinal hernias, joint deformities, children.*

Introduction

Spinal cord (SC) anomalies are essential in the structure of the neural tube pathologies in children. They can occur in the form of isolated anomalies or combined with other spinal malformations[12]. Today, they are usually referred to as spinal dysraphism, spinal malformations, or vertebromedullary abnormalities (VMA). SC open anomalies are manifested in the form of a spina bifida with the formation of cystic SH [10](spina bifida cystica), and closed anomalies - with spina bifida and soft tissue cleavage with spinal cord extension along the entire length of the spine or in its segment (spina bifida occulta) [Voronov V. G., 2002; 2016; A.V. Rudakova, S.N., Larionov 2012]. VMA can also occur in any spinal segment, but more often in the lumbosacral region, mainly combined with other malformations that are located, in particular, in the brain. VMA is mainly caused by lipomyelomeningocele, terminal filament lipomas, medullary cone, diastomatomyelia[7], dorsal band fixation for patients with spinal hernia, or dermal sinus tracts. It is also followed by tethered spinal cord (TSP), which term had been proposed by H. J. Hoffmann et al., (1976)[11]. With normal intrauterine development of the fetus, in the third month, the spinal cord occupies the entire length of the spinal canal. In the next months, the spine grows faster than the spinal cord. As a result, in a newborn's body, the spinal cord ends at the level of the third lumbar vertebra[5], and by the age of 12-18 months it reaches only the lower edge of the LI or upper edge of the LII vertebra[8]. When the cone and epiconus of the spinal cord are located below the normal level and in the presence of corresponding clinical symptoms, the pathological component is considered tethered cord syndrome (TCS). In a series of 5.5 thousand pediatric patients, the frequency of TCS was equal to 0.1 % [Fariborz Samini et al., 2014]. The pathophysiological basis of this syndrome is mechanical traction of the caudal spinal cord, which is followed by a decrease in blood flow in the involved area, and causes ischemia and depression in the electrical activity of the spinal cord[2]. At the biochemical level, it causes inhibition of oxidative phosphorylation in the neuronal mitochondria[17]. Acquired forms of TCS occur after operations for SH, in children with post-traumatic or post-infectious conditions, that may include fixation and tension of the

caudal spinal cord ('spinal cord postfixation syndrome', or SCPS). [Kushel Yu. V., Zemlyansky M. Yu. 2010; Rudakova A.V. et al., 2011;]. This pathology is dynamic in its nature[3]. So, as a child grows, it prevents the age-related displacement of the spinal cord cone, and exacerbates neurological deficits[14]. The characteristic signs of this pathology include an increase in existing function disorders in a pelvic system or appearance of new ones, as well as sensory, motion and trophic manifestations with bone-articular deformities of the lower extremities[9], autonomic disorders and internal dysfunctions[15]. Up to date, there are no reliable data on the frequency and distribution of this syndrome in large localities. According to some authors, in 30-50% of pediatric patients with effects of spinal hernias, disturbances in the muscular equilibrium of paresis or paralysis of the muscles of the lower extremities lead to subluxation or dislocation in the hip joint during their whole life[1]. In children, during the load in extremities, the neutralization of deforming efforts decreases, and the joints of the corresponding spinal cord injury area become deformed[6]. With age, the type and severity of disorders, their deterioration or improvement depend on the severity and extent of the neurosegmental lesion[4]. For example, confounding static and dynamic factors without orthopedic prophylaxis aggravate deformations of the musculoskeletal system[13] (Ivanov S.V., Kenis V.M., 2013; Erol B, et all., 2005; Baindurashvili A.G. et al. 2013[16].

The aim of the study is to determine the frequency and nature of paralytic deformities of the lower extremities in children with spinal hernias.

Materials and Method

We have examined 219 pediatric patients (116 male, 103 female) with various forms of spinal hernias (SH), aged from 1 day to 18 years, and have determined the clinical and neurological manifestations of the disease. This pathology has been verified taking into account the data of functional and instrumental studies: ultrasound, MSCT, MRI of head, spinal cord and vertebral spine; electroneuromyography (ENMG) of the lower extremity muscles. The study was carried out in the Department of Elective Surgery of the Second Children's Surgical Clinical Hospital of the city of Tashkent and the Republican Specialized Scientific and Practical Medical Center of Traumatology And Orthopedics, Tashkent, Uzbekistan, in 2014-2019. The severity of the lower extremity motion disorders in children diagnosed with the peripheral link of the reflex arc has been evaluated using Modified Rankin Scale (mRS) ranging from 1 to 5. The nature and type of deformations of the body and limbs have been evaluated in accordance with generally accepted criteria.

Results

The diagnostic criterion for SH is a hernial protrusion of various sizes and shapes without neurological deficits, urological or proctological disorders with the musculoskeletal system deformations. Age distribution of hospitalized patients with SH is the following: up to 28 days - 24 (11%), up to 1 year – 113 (51.6%), from 1 to 3 years – 31 (14.2%) from 3 to 7 years - 17 (7.8%), from 7 to 12 years – 27 (12.3%), from 12 to 18 years - 7 (3.2%). Localization of pathology in the cervicothoracic region of the vertebrae has been observed in 5 (2.3%) patients; in the thoracic – 9 (4.1%), lumbar – in 76 (34.7%), lumbosacral - in 92 (42%), and sacral – 37 (16.9%). In 158 (72.0%) patients, diseases were manifested in cystic form, as a combination of hernia with teratoid and lipomatous formations (spina bifida complicate) in 32 (14.6%) patients, Chiari malformation (MK) – in 26 (11.9%), rachisis – in 3 (1.4%). Of 219 patients, the shell molds (meningocele) have been observed in 24 (10.9%) of patients, various types of meningocerebral forms - in 192 (87.7%), rachishisis - in 3 (1.4%). In the case of MK, the hernial sac contained meningoradiculocele in 13 (50%) patients and meningomyelocele in 13 (50%).

Up to date, spinal hernia or manifestations of latent spinal dysraphism are a marker for potential malformations of the central nervous and other body systems. Children should be examined in order to identify combined anomalies of the central nervous system and other organs and systems. Skin markers (lipomas, dermal sinus, hemangiomas, hypertrichoses and asymmetric gluteal folds) were signs of spinal dysraphism in 20 (6.3%) patients. In 10 (50%) patients, one or more skin stigmas were located in the zone of reticular protrusion, in 10 (50%) - in various areas along the spine. In 140 (63.9%) patients, diseases formed isolated malformations, in 79 (36.1%) mainly with meningoradiculocele - 53 (67.1%) and MK - 26 (32.9%). With the expanded applicability of special examination method, the detectability of combined abnormalities of the spinal cord, spine and other organs and systems increased. In 38 (48.1%) patients, signs of spinal cord fixation syndrome have been observed, in 41 (51.9%) - specific forms of myelodysplasia: hydromyelia - 9

(11.4%), diastematomy - 8 (10.1%), syringomyelia - 17 (21.5%), the combination of several forms - in 7 (8.8%). In some of these patients, the hidden myelodysplastic syndrome had been established long time after the surgery, at the rehabilitation therapy stage. This is due to the insufficient volume for preoperative examinations or surgery complications. Combined anomalies of other organs and systems have been revealed in 89 (40.6%) of 219 patients, of the musculoskeletal system – in 73 (82%), in the form of various vertebral disorders determined by radiation diagnostic method. In 219 patients, clinical and neurological manifestations of various forms of cystic spinal dysraphism depended on the location, size and anatomy of the defect. Their frequency was the following: cutaneous stigma - 4.5%, internal abnormalities - 14.6%, autonomic disorders - 19.2%, cranial nerve injuries - 22.4%, pelvic system dysfunctions - 76, 7%, sensitivity disorders - 53.9%, motion disorders - 88.1%, trophic-orthopedic disorders - 39.7%.

Discussion

According to MRS, in 18 pediatric patients (9.3%), motion impairment score was 4; in 119 (61.7%) – 3, in 18 (9.3%) – 1-2, in 38 (19.7%) - 0. In severe isolated hernias, abnormalities have been found in 94 (48.7%) children; in combination of SH with other spinal cord abnormalities - in 79 (40.9%); with spina bifida complicate - in 20 (10.4%). In 164 (85%) patients, abnormalities were bilateral in nature, in 29 (15%) - unilateral. Of 193 patients, 87 (45 %) had motion disorders followed by other abnormalities: 30 (34.5 %) - trophic disorders, 35 (40.2 %) - trophic disorders and joint deformities, 22 (25.3 %) - joint deformities and trophic ulcers. This is due to the fact, that in severe and combined osteoneural anomalies, spinal cord malformations increase with the corresponding increase in the frequency and severity of disorders. Motion disturbances - from the complete loss of functions or weakness of some muscle groups to an increased tone of other ones - cause deformations of different fixation and severity of the individual components. In 57 (26%) of 193 patients with motion disorders, various types of deforming arthrosis in the lower extremity joints have been observed, more often in the distal direction. Combined deformity of the hip, knee and foot joints has been observed in 4 (7%) patients; gluteal deformity - in 4 (7%) cases. In these patients, dislocation or subluxation in the hip joint has been also revealed. Knee joint pathologies, in the form of flexion contracture in the range of 150°-90°, have been observed in 2 (3.5%) patients. In 47 (82.5%) cases, polymorphic paralytic foot deformities have been revealed, in 28 (59.6%) cases - bilateral deformities with the prevailing equinus component of the heel-valgus or flat-valgus deformity. In 19 (40.4%) patients, unilateral pathology has been diagnosed. Musculoskeletal disorders combined with deformation in the lower extremity joints indicates a common pathogenetic mechanisms, aggravated by associated spine cord and vertebral anomalies. Early treatment and preventive measures can improve the patient quality of life in some way. The revealed disorders and their combinations depended on the localization and severity of myelodysplasia in the segmental innervation area of the spinal cord, to a certain extent. Of 57 patients with various deformities, 24 (42.1%) had movement disorders. In 6 (10.53%) children with a lumbar lesion due to the absence or weakness of the extensor and abductor muscles of the thigh, a subluxation or dislocation of the femur has been revealed, combined with the absence of arbitrary movements of the lower extremities and wheelchair ambulation. 18 (31.58%) patients with severe peripheral paresis (injuries to all lower extremity muscles with the knee jerk preserved and the Achilles and plantar reflexes absent, in some cases) were able to stand up with support for the upper limbs and bear weight on their knee joints, moving with crutches or other devices. 33 (57.89%) children with various feet deformations were able to move independently, even with limping.. Of 219 patients with severe and prolonged osteoneural anomalies in the thoracolumbar spine, 8 (3.6%) children with unproportioned body parts were bedridden (Fig. 4.16). The data presented indicate that static and dynamic disorders contribute to the formation of musculoskeletal system pathologies in the form of joints deformations. This occurs depending on the level and severity of the neurosegmental lesion, as a result of muscle traction and antagonist-synergist muscles imbalance. Electromyography performed in 98 (30.5%) patients have showed that the prevalence of neuromuscular disorders depended on the severity of spinal motion segment injuries. The topography of these disorders was determined by comparing the electromyography data and the scheme of segmental innervation of the lower extremity muscles, taking into account the proximal border of the spinal cord lesion. It was largely determined by the upper segment, which innervated the muscles with the complete function loss.

In such a way, we may conclude that a frequent combination of musculoskeletal and trophic disorders

combined with deformation in the lower extremity joints indicates a common pathogenetic mechanism, aggravated by associated vertebromedullary anomalies. Early treatment and preventive measures can improve the patient quality of life in some way. A comprehensive examination of patients is aimed at establishing the nature of spinal and combined pathology, revealing latent spinal dysraphism and related functional neuroorthopedic disorders. Early preventive and therapeutic measures should be carried out with the participation of specialists with the combined pathology competencies.

Conclusions

In 26% of patients diagnosed with meningeal forms of SH against the background of motion disorders, neuro-orthopedic manifestations have been observed. They are considered associated with the spinal cord fixation syndrome due to congenital or acquired latent spinal dysraphism.

Localization, nature of neuroorthopedic manifestations, and severity of disorders in dynamics depend on the level and extent of the neurosegmental lesion and myelodysplasia severity. For example, confounding static and dynamic factors without orthopedic prophylaxis aggravate deformations of the musculoskeletal system.

Paralytic deforming arthrosis with myelodysplasia has been mainly observed in the distal parts of the lower extremities, with the ankle joint affected in most of cases.



Fig. 1: Unproportioned body parts with contractures and deformities in the lower extremity joints in case of combined vertebromedullary anomaly: a) anterior view b) posterior view.



Fig. 2. Various types of lower extremity deformities with SH: a) bilateral talipes equinovarus; b) unilateral talipes equinovarus; c) lower extremity disproportion with the hip dysplasia.

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Diagnostic and Therapeutic Preventive Features of Sagittal Dentofacial Anomalies in Children

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Abstract

This article presents the specific features of clinical and radiological abnormalities in the mixed dentition. Individuality of such features is determined by the parameters of the maxillofacial region and the maxillary dimensions, in the form of late milk dentition abrasion, shape of the submaxillary retromolar area and the eruption level of the lower first and second multi-cuspid teeth according to the dental panoramic radiogram. All above was used as a basis for the assessment algorithm development for children with the dentofacial anomalies. Based on the results obtained, the recommendations has been proposed that allow to perform individual treatment for the children with this pathology.

Keywords: *Dentofacial anomalies, orthodontics, sagittal anomalies, anterior occlusion, distal occlusion.*

Introduction

According to WHO data, the prevalence of dentofacial anomalies is on average at least 50% (WHO, Geneva, 2016)[1]. According to the National Institute of Dentistry, USA, 40% of the total population have abnormalities in the teeth development and various dental formation disruptions[2]. Moreover, 15% of these individuals require surgical intervention for its elimination. The issues of timely elimination and removal of factors that adversely affect the normal formation of the dentofacial complex are rather relevant[10].

According to the world scientists, the occlusion anomalies affect the general and local diseases[5] of the human body and cause malfunctions of chewing, breathing, speech and swallowing[8]. The early diagnostics problem of latent forms of the sagittal dentofacial anomalies and its timely prevention and treatment is of undoubted practical interest[3]. It is due to the profound morphofunctional lesions that affect the proper development of chewing function, maxillary formation and occlusion[9].

This impairment is characterized by an unaesthetic appearance of the patient's face: a convex or concave profile, an unpretty smile, availability of a sagittal overjet between the jaws, speech impairment and open-mouth breathing, narrowing of the supramaxillary base, narrow Gothic palate[6] and malocclusion[4]. Obviously, this type of impairment has a skeletal and dentoalveolar nature that requires different treatment approaches to achieve a good result[7]. In order to achieve a satisfactory, aesthetic and functional result, the orthodontist needs to carefully analyze the diagnostic data, pay attention to all the morphological structures of this impairment and not to make mistakes that may deliver the inconvertible results when planning the treatment stages[11].

Most studies demonstrate that the incidence of sagittal occlusion abnormalities depends on the age of the patient[1], since some researchers believe that the incidence of occlusion abnormalities varies from 7 to 10 years at the early stages of mixed dentition[10]. It should be taken in into account that, most often, in addition to the functional maximum, adolescents and adult patients expect the maximum aesthetic result[6] from orthodontic treatment, which affects their psychosocial status in positive way[8].

In that respect, it seems promising to review the diagnostic and therapeutic measures aimed at improving the quality indicators of orthodontic care for children.

The development of the approach that is closest to the diagnostics and treatment of occlusion abnormalities to achieve an acceptable result, combined with the excessive growth of the upper or lower jaw and the anterior position of the supramaxillary dentoalveolar complex will help to achieve a stable aesthetic outcome during the treatment and avoid relapse. Therefore, the modern method of eliminating the factors affecting the normal dentofacial system formation are relevant.

The aim of this study is to determine the early latent forms of occlusion anomalies in children and to substantiate the effectiveness of treatment and preventive measures.

Materials and Method

The study is based on data from a survey of 90 patients during the period of mixed (6-12 years) and permanent dentitions (13-18 years), with the average age 13.4±0.3 years. 52 children (57.8%) had the posterior occlusion, and 38 - the anterior occlusion (42.2%). In order to compare the results obtained for the patients in the examined groups, we used the control group consisting of 19 children with the orthognathic occlusion.

The diagnosis was determined on the basis of data from the history of clinical, biometric, radiological examination of the patients, in accordance with the diagnostic criteria.

The clinical examination included a survey, facial and dental examination. The etiological and pathogenetic factors were identified that contributed to the malocclusion occurrence and development in the sagittal plane. During the facial examination, the attention was paid to the symmetry of its left and right halves, the intensity of the nasolabial and genial folds, and lip seal with the rest position of the mandible.

The particular attention was paid to the identification of morphological and functional disorders that led to the occurrence of a gap between the upper and lower dentitions in the frontal area, and a decrease in the height of its lower part.

In order to determine the structural features of the facial skull, and to determine the changes in the sagittal dimensions of the facial skull, the lateral teleroentgenograms of the patients' heads have been studied, as a result of orthodontic treatment.

On the basis of clinical examination, anthropometric measurement data of diagnostic maxillary models and analysis of the head teleroentgenograms performed in a lateral projection, the diagnosis and the treatment plan have been determined.

The orthodontic treatment consisted of the use of removable intraoral and extraoral devices (myobraces, cervical bandage, Delaire face mask, non-removable device for maxillary expansion), and edgewise techniques based on the brace systems of foreign manufacturers. The dentition correction was performed by the straight-wire technique of the Roth system. The results of instrumental correction controlled on the stone models determined the ability to complete the elimination process for the dentition occlusion abnormality and form the orthognathic occlusion.

Treatment stages for patients of the 1st study group with the distal occlusion:

1. **Early mixed dentition - 6-9 years:**
   - myobrace;
   - cervical bandage;
   - rapid maxillary expansion device.
2. **Late mixed dentition - 10-13 years:**
   - cervical bandage;
   - rapid maxillary expansion device;
   - braces with tooth extraction.
3. **Permanent dentition - 14-18 years:**
   - cervical bandage;
   - rapid maxillary expansion device;
   - braces with tooth extraction.

Treatment stages for patients of the 2nd study group with the distal occlusion:

1. Early mixed dentition - 8-12 years;
   - Frankel functional regulator;
   - high pull;
   - rapid maxillary expansion device.
2. **Late mixed dentition - 12-14 years:**
   - high pull;
   - rapid maxillary expansion device;
   - braces with removal of the upper first premolars.

3. Permanent dentition - 14-18 years:

- high pull;
- rapid maxillary expansion device;
- braces with removal of the upper first premolars.

Treatment stages for patients of the 1st study group with the anterior occlusion:

1. Early mixed dentition - 6-9 years:
   - myobrace;
   - Delaire face mask;
   - rapid maxillary expansion device.
2. Late mixed dentition - 10-13 years:
   - Delaire face mask;
   - rapid maxillary expansion device;
   - braces with the removal of the first premolars.
3. Permanent dentition - 14-18 years:
   - Delaire face mask;
   - rapid maxillary expansion device;
   - braces with removal of the lower first premolars.

Treatment stages for patients of the 2nd study group with the anterior occlusion:

1. Early mixed dentition - 8-12 years:
   - head-chin strap;
   - Frankel functional regulator;
   - plate with an inclined plane;
   - Delaire face mask.
2. Late mixed dentition - 12-14 years:
   - head-chin strap;
   - Frankel functional regulator;
   - Delaire face mask;
   - braces with removal of the lower first premolars.
3. Permanent dentition - 14-18 years:
   - head-chin strap;
   - Delaire face mask;
   - braces with removal of the lower first premolars.

The data obtained were subjected to statistical processing using the statistical analytic application software package with the calculation of arithmetic mean (M), mean square deviation (σ), standard error (m), and relative values (frequency, %). The confidence level of $P<0.05$ was accepted as the statistically significant changes.

Results

The clinical examination have revealed that 79 patients (87.8%; 79/90) were not previously consulted by an orthodontist and only 11 (12.2%; 11/90) performed regular medical check-ups. With that, the patients used the removable orthodontic appliances, but the treatment was not completed, for various reasons.

The prevalence of dentofacial anomalies is relatively more common with the mixed dentition than with the permanent dentition (42.2% versus 57.8%), however, the statistical analysis has showed that these differences are unreliable ($P>0.05$). When assessing the condition of the dentition and alveolar processes, the change in the dental arch (upper and lower) shape was determined. The maxillary V-shaped and trapezoidal, and the mandibular trapezoidal abnormal forms mainly predominated.

When analyzing the patient profiles, we found that in most cases, the children at the age of 6-12 years had a convex facial profile. Moreover, this effect occurs with almost the same frequency both in the case of the submaxillary underdevelopment and the maxillary overdevelopment. In the case of the submaxillary overdevelopment and the maxillary underdevelopment, the straight and concave profile was observed in children at the age of 6-12 years. In children at the age of 13-18 years with the distal occlusion, the convex profile was observed in all cases, while the children with the anterior occlusion had the concave profile.

During the analysis of the intraoral photographs, we identified the following specific features of the submaxillary development. 39 children at the age of 6-12 years had the specific features of the submaxillary retromolar area, among which 19 children had the alveolar crest narrowing, and 20 children showed the prominence of the retromolar area.

As a result, the data specifying the lack of space or dentition excess in the patients have been obtained. In the case of lack of space in the mandible, the alveolar crest shape of the retromolar surface of eruption of the submaxillary second molars is ill-defined and narrow. The reverse effect is observed in the children at the age

of up to 9 years: the more thickened the alveolar process crest, the closer the second molar germ is located to the eruption, thus, the clinical evidence demonstrates the excessive submaxillary growth and a wide submaxillary apical base (Fig. 1).



Fig 1. Premature eruption of the lower second molars resulted in excessive submaxillary growth (the patient is 11 years old).

Therefore, among the analyzed orthopantomograms, the largest number of patients with the delayed eruptions of the second molars is at the age of 12 to 14 years old, with the premature eruptions from 8 years to 11 years old. The severity of teeth overcrowding in the case of delayed eruptions of the second supramaxillary molars is increased with aging. The older the patient, the more difficult it is to prevent the anomaly development and occlusion deformation and vice versa.

In summing it up, it may safely be said that the computed tomography with 3D reconstruction forms the modern diagnostic method for anterior occlusion. The anterior occlusion treatment for the adolescents is possible without surgical intervention during early mixed dentition when using the extraoral devices (Delaire face mask), based on the growth modification, applied forces and with the false anterior occlusion.

The other reasons for the anterior occlusion development include the following: maternal illnesses during pregnancy, birth trauma, underdevelopment of the intermaxillary bone, maxillary adentia, retention and loss of the upper teeth, supernumerary teeth in the lower jaw, delayed second dentition, various children diseases, shortening of the lingual frenulum, hypertrophy of the palatine and lingual tonsils, macroglossia, bad sucking habits and some other bad habits, uneven abrasion of the temporary tooth cusps and uneven second dentition on the upper and lower jaws, irregular teeth position, infantile swallowing type, impaired tongue articulation during speech, acromegaly, impaired miodynamic balance (Fig. 2).

Fig 2. Occlusion contact disruption with the combined occlusion anomaly: anterior occlusion and cuspidate dystopia caused by excessive maxillary development and the submaxillary underdevelopment

Occlusively, the anterior molar contact in the sagittal plane (50% - 25 patients) prevailed with the neutral Angle's molar occlusion (50% - 25 patients). The palatine occlusion has been found in 20 patients (40%), and vestibulo-occlusion - in 11 patients (22%).

The teeth abnormalities in the transversal plane has been diagnosed in 62% of cases of the dentition anterior occlusion. In the frontal area, the incisor invert correlation has been detected in 23 patients (46%), sagittal mismatch has been diagnosed in 10 patients (20%), and direct occlusion - in 2 patients (4%).

In 50% of cases, there was a decrease in percent of the angle indication between the mandibular plane and the Frankfurter plane (25.43% - 25.27%). The mismatch in the relations between the upper and lower jaws was (79.7% - 80.13%). The indicators before and after the distal occlusion treatment in percent were equal to 112.7% - 116.4% (Table 1).

Table 1: Cephalometric study results among the examined patients

	Normal range	Before treatment	After treatment
FMIA	67±0,01	63,23±1,17	65,30±1,31
FMA	25±0,01	25,43±0,83	25,27±0,41
IMPA	88±0,02	89,27±1,26	88,37±0,73
AO-BO	2±0,0 мм	0,88±0,99	1,59±0,40
SNA	79±0,01	79,77±0,79	80,13±0,53
SNB	77±0,01	78,15±0,61	78,80±0,45
ANB	2±0,0	4,93±1,19	3,57±0,72
Effec. max. lengh	85±0,01	93,03±1,25	94,42±1,09
Effec. mand. lengh	105±0,01	112,69±1,61	116,37±0,90
Upper pharynx	8,58±0,65	8,78±0,61	8,58±0,65
Lower pharynx	11,23±0,50	12,25±0,65	11,23±0,50

An analysis of the parameters specifying the facial soft tissue profile has revealed an increase in the nasolabial angle of 15.8%, its decrease with the distal ratio of 25%, a decrease in the convexity angle of soft tissues and a decrease in the facial profile convexity of 33% with an anterior dentition relation. In summing it up, it is safe to say that the cephalometric data in lateral projection is the modern anterior occlusion diagnostic method. The anterior occlusion treatment for adolescents is possible without surgical intervention during the early mixed dentition using the extraoral devices (Delaire face mask) based on the growth modification, applied forces and with the false anterior occlusion.

Determination of the first maxillary premolar germ migration on a cephalometric image. According to Beamler's data, in the non-growing patients with the harmonious facial development and a normally formed permanent orthognathic occlusion, the 'stress axis' passes through the vertical axis of the first maxillary premolar. If the erupting first premolar was in front of the stress axis, it was indicated to remove the first premolars, and the cases of posterior position indicated the maxillary underdevelopment (Fig. 3).

Determination of the first maxillary premolar germ migration on the profile teleroentgenogram. 16 children at the age of 6-9 years old with the early clinical signs of sagittal abnormalities were subjected to cephalometric analysis of the upper premolar germ position. As a result, 7 children showed the anthroposition of the first premolar germs that was an early latent sign of rapid maxillary development or early migration of the upper posterior teeth (Fig. 4). 9 children demonstrated the retroposition of the first premolar germs being an early latelt sign of the delayed maxillary development, the distal position of the upper permanent posterior teeth germs or excessive supramaxillary development (Fig. 5).



Fig. 3. Determination of the first maxillary premolar germ migration on the profile teleroentgenogram



Fig. 4. Position of the upper premolar germs: a child at the age of 7 years



Fig. 5. Retroposition of the first premolar germs: a child at the age of 9 years

In some cases, the doctor may need a CT scan. This image only allows to estimate the spatial teeth arrangement relative to each other. This is necessary when planning the impaction movement, when it is extremely important to understand which tooth is located more superficially, and to select the right displacement vector so as not to touch the roots of other teeth. When using the OPTG, an assessment of the tooth position is possible only in one – frontal - plane.

In the course of the therapeutic measures performed for the children and adolescents, the neutral relations of the first molars according to Angle's class I was achieved. When evaluating the treatment outcomes, we noted the coincidence of the central line between the upper and lower teeth, formation of an interincisal angle within 118° and overlapping by the upper incisors of more than 1/3 of the height of the lower incisors.

The high level of satisfaction of the patient and his/her parents with the treatment results made it possible to positively evaluate its results. The formation of physiological dentition occlusion increased the chewing function efficiency. The results are presented in Table 2.

One year after the follow-up commencement, we recorded an increase in the number of people with physiological occlusion in the second group - in children who used a cervical bandage and non-removable orthodontic appliances. After two years, the treatment efficiency in children in the second group reached a maximum value that had a significant difference with the treatment results after one year. However, this indicator differs significantly from the similar indicators in the comparison group.

Table 2: Dentition condition dynamics during the treatment process

Treatment terms	Physiological occlusion				Anomaly at the formation stage				Formed abnormality			
	Control group		Comparison group		Control group		Comparison group		Control group		Comparison group	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Commencement of treatment	0	0	0	0	13	52	11	44	12	48	14	56
After 1 year	15	60	11	44	8	32	8	32	2	8	6	24
After 2 years	23	92	17	68	1	4	6	24	1	4	2	8
After 6 years	24		19		1		7		1		7	

After 24 months of active treatment, the malocclusion was corrected in the control group in 90% of cases, and in the comparison group - in 78% of cases. The patient profile was significantly improved, and the intermaxillary deviation was readjusted. The upper jaw was placed in its natural position. The cephalometric images showed the clear pattern of the correct maxillary growth direction in 23 patients (92%) of the control group and in 20 patients (80%) of the comparison group (Fig. 6).

The treatment results after two years show that in 92% of patients in the control group, occlusion stability was preserved, and the facial profile also remained aesthetically pleasing. In the comparison group, the stability was maintained only by 68% of children.

Therefore, the orthodontic treatment success in the growing patients with class II malocclusion depends on its growth direction and the appropriate selection of treatment time. That is why it is important to diagnose

an early accurate diagnosis, the degree of skeletal discrepancies, in order to prepare a treatment plan that is most suitable for the patient.

Class II or bite elastics can be used simultaneously and assist in changing the dentofacial growth direction. The combination of a cervical bandage is an effective approach to the early treatment of skeletal impairments. In addition, the achieved correct anterior teeth occlusion from the anterior position of the upper teeth and jaw has contributed to the mandibular growth.

Analysis of the study showed that in the control group of patients, in 94% of cases we achieved the good treatment results, in 6% - satisfactory, and in 0% - negative. In the comparison group, it was 68%, 24% and 8, respectively. The patients from the control group have also demonstrated a 3-fold increase in the treatment efficiency.



Fig. 6. Intraoral images of a patient with prognathia (a) before treatment, (b) after treatment, (c) 7 years after treatment

During the repeated studies, we found that in patients of the control group the relapses occurred in 6.7% of cases (3 patients), while in the experimental group - in 20.0% (9 patients).

Therefore, during the treatment process for the dentition distal occlusion, it is sustainable to use the proposed treatment with myofunctional devices such as myobrace, a non-removable maxillary expansion plate with a cervical bandage for the upper jaw and without certain teeth extraction. That allows to expand the upper dentition, while providing the lower jaw with the constructional occlusion.

Discussion

In most cases, the high occurrence of dentofacial abnormalities in children is characterized by the availability of morphologically immature structures. This opinion is supported by T.F. Vinogradova [1] and other researchers. According to T.F. Vinogradova, an eight-year-old child has a simultaneous growth of the coronal section of permanent molars, the premolar root system against the background of changes of the temporary molars that indicates the availability of various tissue maturity degrees. It is also noted that under the same influence of external and internal factors, the different forms and growth reactions are noted[3, 5].

According to our data obtained, the prevalence of dentofacial anomalies is relatively more common with the mixed dentition than with the permanent dentition (42.2% versus 57.8%). Gowever, the statistical analysis showed that these differences are unreliable (P>0.05).

According to the research performed by Listopadov M.A. et al., it has been found that the use of tomography allows to determine the anatomical and topographic features of the temporomandibular joints with the distal occlusion. This data must be considered during the treatment of patients with this impairment [4].

As a result, we have obtained data characterizing the lack of space or excess dentition in the patients. In the case of lack of space in the mandible, the alveolar crest shape of the retromolar surface of eruption of the submaxillary second molars is ill-defined and narrow. The reverse effect is shown by the children at the age of up to 9 years: the more thickened the alveolar process crest, the closer the second molar germ is located to the eruption. In such a way, the clinical evidence demonstrates the excessive submaxillary growth and a wide submaxillary apical base.

Voskanyan A.R. developed and implemented the express diagnostics of deviations in the teeth position, shapes and dimensions of the dental arch and its segments from the individual normal values. It is based on the use of template sets that provide complete information on monitoring the orthodontic treatment results [2]. Our data show that the severity of teeth overcrowding with the delayed eruption of the submaxillary second molar is increased with aging. The older the patient, the more difficult it is to prevent the development of occlusion abnormalities, and vice versa.

The delayed treatment of abnormal changes in the primary and mixed dentitions contributes to the formation of severe dentofacial abnormalities in the case of permanent dentition [3, 5], while the treatment initiated at the early stages reduces the implementation of comprehensive orthodontic treatment, including the permanent teeth extraction [7, 9]. In the course of our treatment of the dentition distal occlusion, it is sustainable to use the proposed treatment with the removable extraoral and intraoral devices with the cervical bandage for the upper jaw, with and without the certain dental extraction. All above allows to expand the upper dentition, while providing the lower jaw with the constructional occlusion.

Conclusions

1. The cephalometric indicators in the early diagnosis of sagittal occlusion in children helps to obtain the proper treatment planning and achieve the consistent long-term results. Due to the early diagnostics and treatment of sagittal occlusion anomalies, a significant improvement in aesthetics can be achieved.
2. Among the analyzed orthopantomograms, the largest number of patients with the delayed eruptions of the second molars is at the age of 12 to 14 years old, with the premature eruptions from 8 years to 11 years old. The severity of teeth overcrowding in the case of delayed eruptions of the second supramaxillary molars is increased with aging. The older the patient, the more difficult it is to prevent the anomaly development and occlusion deformation, and vice versa.
3. During the treatment process of the dentition distal occlusion, it is sustainable to use the proposed treatment with the removable extraoral and intraoral devices with the cervical bandage for the upper jaw, with and without the certain dental extraction. All above allows to expand the upper dentition, while providing the lower jaw with the constructional occlusion.
4. The economic efficiency of the proposed treatment method is aimed to reduce the number of relapses of this impairment by almost 3 times.

Ethical Clearance: No ethical approval is needed.

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Conflict of Interest: Nil

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A Study of Mental Health in Children with Vitiligo Using Projective Method

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Abstract

In the article, the authors present the results of their studies of mental health in children with vitiligo. The examination has been performed with 94 pediatric patients involved, using the 'House-tree-man', 'Kinetic drawing of the family' and 'Non-existent animal' projective method. According to the results of the study, it has been found that the mental health of children significantly affects the course of their disease.

Keywords: *Vitiligo, drawing test, mental health, severity of disease, aggression, low self-esteem.*

Introduction

Vitiligo occupies a special place among dermatological diseases that lead to severe cosmetic defects and mental suffering[2]. Vitiligo is a psychosocial problem that significantly affects the quality of life of patients[1]. Many researchers believe[4] that vitiligo is a serious cosmetic defect that occurs among people with a genetic predisposition to it and emotional disorders, in most cases[3]. Only in 4-7% of patients, vitiligo is combined with autoimmune thyroiditis. According to S. Mattoo, with vitiligo, adaptation and depression disorders are more common (75 and 18%, respectively)[6]. According to E. Colon, 39% of patients with localized foci on the skin face, are diagnosed with depressive disorders, and the same proportion of patients - with generalized anxiety disorder against the background of the manifestation of a skin disease. According to the data of the Research Institute of Dermatology and Venereology of the Ministry of Health of the Republic of Uzbekistan, in recent years, the number of vitiligo patients in our country has increased 3.4 times[8]. Within the territory of the Republic of Uzbekistan, dyschromia cases account for 21% of all skin diseases, and vitiligo cases account for 15.1%, while in other countries it does not exceed 3-4%. In 70% of cases, the disease primarily occurs at the age of 10 to 25 years. The number of children under 10 years, including newborns and infants, account for approximately 25% of all vitiligo patients[9]. In the midst stage, patients with vitiligo experience severe psycho-emotional imbalance, stress, irritation, socio-psychological discomfort, and inadequacy. Up to 5% of vitiligo patients suffer from severe depressive disorders, and in some cases, have suicidal thoughts. In children with vitiligo, emotional stress, low self-esteem and a deterioration in the quality of life are usually observed, which has negative impact on therapy and the course of their disease[5].

The purpose of the research has been to assess the mental health of children with various clinical manifestations of vitiligo.

Materials and Method

We have performed a mental health assessment for 94 children with vitiligo, aged 6 to 16 years, using

drawing tests. For many children, drawing is one of the ways to express their internality. This method makes it possible to assess the mental health and intelligence of children with vitiligo. The drawing tests were the following: 'Nonexistent animal' (NA), 'House-tree-human' (HTH) and 'Kinetic drawing of the family' (KDF). While studying the children' drawings, we took into account the whole drawing process, its details, structure, the features of graphic presentations, the positive or negative assessment of elements drawn by the patients themselves. We also paid attention to the verbal assessment of the drawing process by the children. The practical application of this method is effective due to its information value and the ability to track the dynamics of the children' mental state. During the study, behavioral characteristics such as self-doubt, insecurity, sensibilities, anxiety, fear, conflict, depression, low self-esteem, indecisiveness, problems in interpersonal relationships, lack of sociability, and social interaction difficulties have been revealed.

Results

During the drawing tests, 24 out of 90 children with vitiligo refused to perform the tasks, referring to a) lack of drawing skill b) discomfort due to the assessment of the drawing by the people present c) unclear purpose of the test proposed. These children were interviewed in confidence and were explained that the purpose of the study was not to evaluate their artistic or creative abilities. After that, they agreed to participate in research. Some children asked for a ruler to draw a straight lines. We explained to them that, according to the DTH test conditions, the drawing should be done by only manual means.

The ratio of the drawn picture in relation to a sheet means the psychological significance, value and importance of things, situations or relationships represented - both directly or symbolically - in the DTH drawing. 16 (17.02%) children drew a very small picture with weak lines, which signified a self-distrust and a feeling of inferiority. 21 (22.34%) children drew a picture at the bottom of the sheet, which was indicator of depressive state.

Analysis of the DTH test in the examined patients has showed the following results. 24 (25.53%) children drew a small house, standing far away, which characterized the feeling of loneliness or rejection of the patients. 13 (13.82%) of children drew a house without doors and windows, which indicated family relationship problems, and difficulties in being confident with their relatives. The chimney of a house with a puff of thick smoke, present in 20 (21.27%) cases, indicated the significant internal psychoemotional stress of children. Thin stream of smoke or the absence of a chimney in 26 (27.65%) cases indicated a feeling of lack of warmth and care at home. Closed windows of the house, drawn by 14 (14.89%) patients, mean alienation, hostility, difficulties in interpersonal relationships within the family. Clouds drawn by 25 (26.59%) children above the house indicated internal anxiety, fears and depression. Drawing details also reflect the level of emotions and intelligence of the patients. 14 (14.89%) children drew a human with a small amorphous head, which indicates their feeling of insecurity and concern about intellectual inadequacy. 8 (8.51%) of the children drew a human with small shoulders, which symbolized their shyness, a sense of their insignificance, and over diligence. An indinstinct and pale face in 17 (18.08%) drawings was indicative of fear and insecurity of children. Long arms in 10 (10.63%) cases were a sign of addiction, indecisiveness, the need for a family care and guardianship. As for the trees, we drew attention to such details as a shaded trunk, which meant an internal fear to be alone or hidden aggression. The lowered down tree branches meant the absence of aspirations, reluctance to make an effort, the difficulty in making decisions.

Discussion

When interpreting the results of the HTH drawing test, it is necessary to take into account the integrity of all the picture details. The presence of only one element does not indicate the presence of a certain psychological feature. According to the recommendations of R.F. Belyauskaite, all the qualitative indicators of the HTH drawing test have been combined into the following symptom complexes: 1) anxiety; 2) insecurity; 3) hostility; 4) depression; 5) self-distrust; 6) aggression; 7) communication difficulties; 8) sense of inferiority.

Table 1: Symptom complexes of HTH drawing in children with vitiligo

Indicators	Males (45)		Females (49)		Total (94)	
	Absolute.	%	Absolute.	%	Absolute.	%
Anxiety	13	13.82	16	17.02	29	30.08
Insecurity	9	9.57	16	17.02	23	24.46
Hostility	9	9.57	10	10.63	19	20.21
Depression	8	8.51	13	13.82	21	22.34
Self-distrust	8	8.51	11	11.70	19	20.21
Aggression	14	14.89	17	18.08	31	32.97
Difficulties in communication	9	9.57	10	10.631	19	20.21
Feeling of inferiority	12	12.76	15	15.95	27	28.72

After the analysis of the HTH drawing test, the following symptom complexes of different severity have been found: anxiety (30.08%), insecurity (24.46%), hostility (20.21%), depression (22.34%. The less pronounced symptoms were self-distrust (20.21%), a sense of inferiority (28.72%), aggression (32.97%), communication difficulties (20.21%) (Table 1).

'Kinetic drawing of a family' is a type of drawing test that illustrates the subjective situation of children with their family and friends. It helps to find out the children' relationship with their family members, and how significant they feel in their families, in their own opinion. The method of KDF drawing test makes it possible to identify difficulties in family relationships and emotional problems. 19 (20.21%) children did not draw faces of some their relatives, which means staying in an unhappy relationship and frequent conflicts with them. In 13 (13.82%) cases, the children did not draw themselves in the picture, which means notoriety, difficulties in self-expression in the family. When the children were asked why these or other relatives were absent in the drawing, some of them showed direct aggression towards their brothers, sisters or parents. Other children explained it by excuses: 'I forgot about him (her)', 'There wasn't enough space', 'I find him (her) difficult to draw'. In 19 (20.21%) patients, the drawing did not correspond to its verbal description, In this case, the family situation of the patients was considered as a conflict. If children drew all family members without exception, we paid attention to the size of the children themselves in the drawing, as well as their family members. The more attention and care is given to the children, the larger or proportional to other details they depicted themselves, and the less significant members of the family were painted small and distorted. We have also noted that in 20 (21.27%) cases, they drew themselves small, which means low self-esteem, insecurity among family members, and a sense of inferiority of the children. In 12 (12.76%) drawings, the children painted some family members fragmented, and the pictures were not integral. This indicates unfavorable family relationships, frequent conflicts, and sometimes hostility between some family members.

Aggressive attitude towards the family, fear towards certain family members has been determined in 29 (30.85%) cases. On the drawing, it was expressed by their isolated position from the family on a piece of paper, separation from the main picture by lines, frequent use of dashed or broken lines and dark colors. Quantitative assessment of KDF was carried out by the following system of symptom complexes: 1) family hostility 2) family conflicts; 3) anxiety; 4) a sense of inferiority in a family; 5) a favorable family situation.

After KDF drawing test, we identified the following main symptom complexes: a sense of inferiority in a family (37.23%), anxiety (25.53%). To a lesser extent, family hostility (26.59%) and family conflicts (30.85%) were present. A favorable family situation has been observed in families of 20 patients (21.27%) (Table 2).

Table 2: Symptoms of KDF drawing test in children with vitiligo

Indicators	Males (45)		Females (49)		Total (94)	
	Absolute	%	Absolute	%	Absolute	%
Family hostility	9	9.57	16	17.02	25	26.59
Family conflicts;	13	13.82	16	17.02	29	30.85
Anxiety	8	8.51	18	19.14	24	25.53
Feeling of inferiority in the family	19	20.21	16	17.02	35	37.23
A favourable family situation	9	9.57	11	11.7	20	21.27

The next drawing test is a projective research method - 'Non-existent animal'. This test is based on the theory of the psychomotor development of the children and expresses their psychoemotional state, anxiety and feelings. We invited children with vitiligo to draw a non-existent animal, which they had never seen before, and give it a name. Analyzes of NA drawing tests have been carried out with determining several parameters: position of the picture on the sheet, the central or supporting parts of the animal, the contours, lines of the figure, a positive or negative attitude, matching the animal's name with its image. 17 (18.08%) patients drew a non-existent animal in the lower part of the sheet, which indicates self-distrust and insecurities, depression or indecisiveness. In 13 (13.82%) children, the head was depicted with an open or crossed mouth with a rounded shape, which means that the children feel anxious and worried. In 23 (24.46%) cases, the head was depicted with horns, and there were claws on the extremities, which meant that the children are prone to self-defense and aggression. In 6 (6.38%) cases, uniformity of animal's legs was noted, which indicates conformity of judgments and a penchant for standards and commonplace. In a verbal survey and objective analysis, all animals were divided into three types: threatened, in need of protection - 26 (27.65%) cases, safe or neutral - 47 (50%) cases, and aggressive or threatening - 35 (37,23) cases. The mental health of patients was determined by the following symptom complexes: 1) irrationality 2) irrationality; 3) depression; 4) fear; 5) deliberation in decision making; 6)self-confidence; 7) feeling of insecurity from others; 8) asthenic feeling; 9) low self-esteem; 10) diffidence;

Table 3: Indicators of the 'Non-existent animal' drawing test in children with vitiligo

Indicators	Males (45)		Females (49)		Total (94)	
	Absolute	%	Absolute	%	Absolute	%
Irrationality.	16	17.02	21	22.34	37	39.36
Low self esteem	14	14.89	19	20.21	33	35.10
Oppression	9	9.57	11	11.70	20	21.27
Fear	14	14.89	12	12.76	26	27.65
Deliberation in decision-making	4	4.25	8	8.51	12	12.76
Self confidence	8	8.51	12	12.76	20	21.27
Feeling of insecurity from others	7	7.44	12	12.76	19	20.21
Asthenic feeling	12	12.76	18	19.14	30	31.91
Low self esteem	12	12.76	17	18.08	29	30.85
Diffidence	9	9.57	14	14.89	23	24.46

In NA drawing tests, such symptom complexes as diffidence (41.13%), low self-esteem (30.85%), asthenic feeling (31.91%), fear (27.65%), depression (21.27%), feeling of insecurity from others (20.21%) have been revealed to a lesser extent (Table 3).

An analysis of the results of drawing tests gives information about the mental health of children with vitiligo in a chronic somatic disease. The symptom complexes noted above form either the result of the vitiligo process, or a trigger factor leading to the manifestation or exacerbation of dermatosis.

Table 4: The severity of general - school, self-esteem, interpersonal - anxiety of children with vitiligo

Level	Males (45)		Females (49)		Total (94)	
	Absol	%	Absol	%	Absol	%
Extrinsic	4	4.25	4	4.25	8	8.51
Normal	9	9.57	15	15.95	24	25.53
Insignificantly increased	16	17.02	15	15.95	31	32.97
Increased	17	18.08	20	21.27	37	39.36
Very high	2	2.12	3	3.19	5	5.31

In order to determine the level of anxiety, we used the anxiety scale developed by A. M. Prikhozhan [11]. Types of anxiety included three types of situations: 1) school anxiety (incidents related to classmates, school, and communication with teachers); 2) self-assessment (situations actualizing self-identification); 3) interpersonal (situations that depend on the communication of children in the family, with their parents). After analyzing the general level of anxiety of the studied children of children with vitiligo in children, we have determined (Table 4) that anxiety was present in the majority of children - in 84 (89.36%) cases, as a relatively stable personality formation, with various degrees of severity. Patients with a high and very high level of anxiety are considered a risk group, and in our case, their number was 35 (37.23%). the cause of the occurrence in patients with such anxiety can be due to low self-esteem, traumatic for the child's psyche, real ill-being caused by the chronic course of the disease, problems in interpersonal situations, difficulties in school and family situations, an illness unnatural for healthy children, accompanied by great experiences. Children with increased timidity complain about the difficulty of adaptation in society, a decrease in the stability and effectiveness of education. Due to the long and frequent periods of treatment of children with vitiligo, their rhythm of life is violated, they lag behind their peers, and refuse to attend school for some time. All these factors increase emotional stress and create a kind of vicious circle. Some patients have a higher level of anxiety. These children can be attributed to a risk group related to the development of more pronounced forms, since negative situations associated with the disease, with its vivid clinical manifestations, relapses, will increase emotional distress and psychological discomfort. The number of such patients in our studies was 29 (28.72%).

Conclusions

Our studies prove the important role of the mental health of patients, which depends both on their personal qualities and the influence of the surrounding society, as well as the course, development, and prognosis of the disease. Timely identification and elimination of these psych emotional factors, correction of the psychological state of patients will undoubtedly contribute to improving the quality of life and will have a beneficial effect on the course of vitiligo.

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Methemoglobin Role in the Subdural and Epidural Hemorrhage Recentness Evaluation in Hot Climate

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Abstract

Methemoglobin (MetHb) is a physiological blood component that performs protective functions and neutralizes the endogenous hydrocyanic acid, hydrogen sulfide and other poisons. MetHb is slowly formed in the blood, its surplus is constantly restored while the content in blood remains low.

The spectrophotometric method has been used to measure the methemoglobin concentration in 72 traumatic subdural and epidural hematomas of various prescriptions. Based on the data obtained, the limitation of subdural and epidural hematomas has been determined by the methemoglobin concentration. The results are recommended for use in the forensic expert examinations.

Keywords: *Subdural and epidural hematomas, methemoglobin level, statute of limitations, forensic medicine, blood, hematoma, methemoglobin, intracranial.*

Introduction

Information on the methemoglobin concentration in the blood of healthy persons is very controversial. According to some researchers, MetHb is found in the blood of healthy adults in 23-25% of cases; however, its average concentration in the blood does not exceed 3 - 5%. Moreover, the concentration is higher in females than in males[1]. Many researchers believe that the MetHb content in human blood does not exceed 1%. According to others, MetHb formed in red blood cells during lifetime is reverted into hemoglobin by enzymes contained in red blood cells[2]. Moreover, the intracellular MetHb concentration is increased in corpses when the reducing enzyme substrates disappear from the red blood cells. 70% of red blood cells do not contain any methemoglobin.[3] With that, the more MetHB in cells, the less often they are found in the bloodstream.[4] Such distribution pattern indicates the active elimination of red blood cells with methemoglobin from the hematopoietic stream.

The MetHb content is increased depending on the erythrocyte aging. It is triggered by the direct changes in hemoglobin molecules. The electronic configuration of hemoglobin protein molecules is varying, therefore, the methemoglobin formation and reverse recovery are not uniform and depends on the medium pH.[5] In an acidic environment, oxidation is easier than in an alkaline one. Supposedly, it leads to the higher methemoglobin content in capillary blood than in venous blood. The MetHb increase in the blood is possible in various morbid conditions. However, under normal conditions, it is formed in the body in small quantities while quickly reverting into hemoglobin.[6]

Some researchers point out that assimilation and dissimilation in the body are not interrupted immediately and that the cadaveric red blood cells retain their gaseous metabolism for some time. As time passes after death, the MetHb content in the blood is increased both in the human cadavers and animal carcasses. An increase in ambient temperature accelerates the methemoglobin spontaneous generation in the blood of the dead, as well as the methemoglobin reduction and disintegration.

The processes of MetHb generation and reduction in the blood are directly dependent on the storage temperature requirements. Its most intense formation was recorded at a temperature of t18±24ºC.

As a result, the identification of posthumous biochemical patterns is of interest, both for determining the time of death and for assessing the recentness of brain injury in relation to the intracranial hematomas. Moreover, special emphasis was put on the temperature conditions in our Republic when studying the methemoglobin generation in the intracranial hemorrhages.[7]

In this regard, *the research objective* is to analyze the increase in MetHb concentration in the intracranial hematomas depending on the time of injury, as well as to reveal the trends in the process development based on the data obtained for determination of the injury time.[8]

The research materials and method: In order to determine the methemoglobin concentration in the blood, a spectrophotometric method was used. Spectrophotometry is based on measuring the monochromatic light flux attenuation degree as a result of selective light absorption by a dissolved substance.

The spectrophotometric method are based on the Beer-Lambert-Bouguer law of light absorption that determines the ratio between the impinging light intensity passed through the absorbed solution and monochromatic radiation. The log ratio between the light flux intensity entering the solution and the light flux intensity escaping from the solution is directly proportional to the substance concentration and the absorbing layer thickness: $D=lgIo/I=Cl\Sigma$. The lq value specifies the attenuation degree of the radiation intensity after its transmission through the solution. It is called the solution transmission density - D (A or Σ). The law indicates the linear dependence of D on the absorbing substance concentration (C), the absorbing layer thickness (I), the absorption coefficient or extinction coefficient (Σ).

The attenuation degree of the light intensity passing through the substance (solution) can describe the transmission: $T= Io/I= Io- \Sigma Cl.$ T (transmission) is the ratio of the radiation intensity transmitted through the absorbing solution to the initial radiation intensity.

Transmission can be ranged from 0 to 100%.

During the spectrophotometric analysis, the qualitative spectrophotometry is widely distributed in addition to the quantitative spectrophotometry, i.e. identification of dissolved matters by the light absorption curve shape or the so-called absorption spectrum.

In general, these curves are shown in a Cartesian coordinate system so that the optical density values (D) or extinction coefficients are plotted on the ordinate, and the length (in microns) is plotted on the abscissa. During the curve assessment, attention is brought to availability of the maximum and minimum light absorption values, its relative intensity, as well as the wavelengths that correspond to the spectrophotometric curve knees.

For this purpose, we took hematoma (subdural and epidural) and blood from the cerebral sinuses (to control the methemoglobin generation). The hematoma was ground in a glass homogenizer to a homogeneous liquid state for a hemolysate preparation. We used the cyanide method developed by M.S. Kushakovsky (19680 based on the indirect method by Evellyn and Malloy (1938).[9]

This method has a fairly high sensitivity and accuracy. It is notable for the following advantages:

1. It is simple and affordable.
2. It can also be used when, in addition to oxyhemoglobin (HbO^2) and methemoglobin (MetHb), other hemoglobin derivatives are available in the blood solution.

The repeatability of the results is very high. The single-measurement uncertainty did not exceed 0.2–0.3% methemoglobin of total hemoglobin.

The method is based on the well-known fact that the methemoglobin absorption band (acidic medium) disappears in the red region when cyanite is added. In this case, methemoglobin is turned into cyanmethogemoglobin.

If we take the total Hb (hemoglobin) concentration as 100%, then the MetHb concentration (x%) will be: $X= C\ MetHb\ x\ 100/C\ total\ Hb\%.$

In such a way, the calculation formula for the MetHb concentration is as follows: $X=(D_1-D_2)x11x100/3.6xD_3.$

The dependence of methemoglobin concentration on the time of injury was determined by the univariable linear regression analysis. According to this method, the constraint equation is as follows: $M=a_0+a_1t$, where M is the MetHb concentration, t is the time elapsed since the injury; a_0 and a_1 are the equation variables.

In order to perform quantitative methemoglobin determination in the substrates of intracranial hemorrhages, the spectrophotometric analysis for

methemoglobin concentration in the blood from the intracranial hematomas was performed in relation to 72 cadavers with the established terms of injury who were subject to a forensic medical study. The bodies were dissected according to the generally accepted method 24-36% after death. The blood from the superior sagittal cerebral sinus was used as a control.

The spectrophotometric study results, obtained as a difference in the MetHb concentration from the intracranial hemorrhages and the control blood group from the superior sagittal cerebral sinus at various times of brain injury, were subject to evaluation. The MetHb concentration between the study groups was compared in relation to 7 periods of post-traumatic hematoma development: up to 3 hours; up to 12 hours; up to 24 hours; up to 48 hours; up to 60 hours; up to 72 hours; more than 3 days.

Study Results

In the first group of the injured who suffered the brain injury and died within 3 hours after the injury (16 cases), it was found that the MetHb concentration in the studied hematomas ranged from 1.03 to 1.7, and the average value was 1.26±0.17.

The statistical processing of the results in 7 study groups revealed the statistically significant differences in concentrations at different times after the brain injury compared with the control group. Thus, in the 2nd group of injured with the period of up to 12 hours after the brain injury, the MetHb concentration was equal to 7.78±0.3% compared with the control values of 1.04±0.04% (P<0.01).

In the third group, where death after the brain injury occurred within one day, we observed the noticeable increase in the MetHb concentration up to 10.6±0.50% (P<0.01), in the fourth group, respectively, the MetHb concentration increased up to 15.21±0.7% (1.6±0.07% for the control group) (P<0.01).

The same trend is observed in other groups (V, VI, VII) where the methemoglobin concentration is gradually increased with increase in the body survivability period after the brain injury.

Table 1: Comparative values of methemoglobin concentration in the intracranial hematomas

Study groups according to the prescription of death coming	Methemoglobin concentration, %		
	Compared group	Control value	P<
1st group – death within up to 3 hours after the brain injury	1,7±0,06	0,82±0,02	0,01
2nd group – death within up to 12 hours after the brain injury	7,78±0,3	1,04±0,04	0,01
3rd group – death within up to 24 hours after the brain injury	10,6±0,50	1,5±0,06	0,01
4th group – death within up to 48 hours after the brain injury	15,21±0,7	1,6±0,07	0,01
5th group – death within up to 60 hours after the brain injury	16,7±0,63	1,8±0,12	0,01
6th group – death within up to 72 hours after the brain injury	18,2±0,74	1,8±0,14	0,01
7th group – death after 3 days	23,52±0,92	2,61±0,19	0,01

The 6th and 7th groups of post-traumatic intracranial hematoma development, indicated by a period of 3 or more days, demonstrated a slow increase in the methemoglobin concentration to an average value of 19.72%. When the injury survivability time is increased up to more than 10 days due to the organizational processes in hematomas (formed sacs and vascular neoplasms, fresh hemorrhages in the hematoma substrates), these MetHb concentrations have the quantitative variation and are not subject to adjustment.[10]

In such a way, the body survivability period after the brain injury and formation of intracranial hematomas leads to the increase in methemoglobin content in red blood cells. In part, the reasons for this is a decrease in methemoglobin reductive activity in the senescent cells. Contrarily, there are direct changes in the hemoglobin molecules. The spectrophotometric data obtained can undoubtedly serve as an additional criterion for recentness assessment of the resulting brain injury.[11]

At the next stage, we performed the recentness assessment of intracranial hematomas by the structural changes.[12]

Our observations were divided into seven periods, depending on the body survivability periods after the brain injury.[13]

The first period included 16 observations, the second period consisted of 12 observations, the third, fourth, fifth, sixth, seventh periods consisted of 8, 9, 10, 6 and 5 observations, respectively. During the first time period after the injury, the hemorrhages were represented by liquid blood with almost similar color regardless of localization and bleeding point. It should be noted that such a condition of hematomas remained unchanged during rather long-term storage of a corpse in the refrigerator.[14] The specific features of this study group were as follows: changes in the tinctorial collagen fiber properties in the form of pronounced basophilia, eosinophilia and insignificant cerebral edema, severity of vascular responses in the form of angioparesis and angiospasm. The hematoma substrates had the ratio of normal and abnormal red blood cells in favor of the former, moderate leukocytosis and lymphocytosis (Table 2).

The first post-traumatic period with the duration of up to 3 hours is represented by the pathomorphological changes typical for the inflammatory conditions: the changes in the tinctorial collagen fiber properties of the dura matter included basophilia (13.0±1.2%), eosinophilia (11.7±1.2%) and edema (6.5±0.7%) without any necrosis.

Table 2: Structural changes in the intracranial hematomas depending on the injury limitation

Morphological features of hematomas		Study groups						
		I – up to 3 hours	II – up to 12 hours	III – up to 24 hours	IV – up to 48 hours	V – up to 60 hours	VI - up to 72 hours	VII – after 3 days
Tinctorial properties of fibers (area, %)	Basophilia	13,0±1,2	12,7±1,4	8,7±1,3	0	0	0	0
	Eosinophilia	11,7±1,2	8,4±1,4	2,3±0,4	0	0	0	0
	Edema	6,5±0,7	7,6±1,5	10,2±1,3	10,4±0,6	11,1±1,2	1,5±0,4	1,6±0,3
	Necrosis	0	0	55,6±6,9	13,6±1,1	0	0	0
Vascular response, %	Tetanus	46,5±3,4	27,9±3,2	6,5±1,6	2,2±0,6	0	0	0
	Plasmorrhagia	0	30,4±3,2	78,7±4,2	82±5,6	0	0	0
	Fibrinoid necrosis	0	0	20,5±1,6	54,6±2,6	16,7±3,2	8,6±1,6	0
Normal red blood cells, %		95,6±6,4	92,5±5,2	87,0±3,8	56,8±1,8	32,6±2,6	27,8±1,2	25,6±1,2
Abnormal red blood cells, %		4,3±1,2	7,4±1,5	10,7±1,3	44,0±1,8	69,6±4,2	62,6±3,9	62,7±4,2
Normal white blood cells, %		28,1±2,8	30,4±1,2	24,0±2,6	55,8±3,2	16,7±1,8	14,0±0,8	18,6±0,6
Abnormal white blood cells, %		0	6,4±1,2	16,8±1,8	25,4±1,8	36,4±2,3	46,2±3,4	12,1±1,8
Macrophages, %		0	1,4±0,2	15,8±1,6	25,2±1,4	36,4±1,8	44,7±3,2	73,2±1,8
Hemosiderin, %		0	0	1,3±0,4	7,5±0,8	3,6±0,8	4,3±1,2	4,6±0,4
Fibrin strands, %		0	83,0±6,2	8,2±0,6	0	0	0	0
Fibrin mesh, %		0	0	76,4±4,1	14,2±1,6	0	0	0
Fibrin clot, %		0	0	0	15,7±0,5	76,6±4,4	8,6±0,8	0
Fibrin bodies, %		0	0	0	6,1±0,7	92,4±6,2	74,8±2,4	85,5±6,4
Lymphocytes, %		1,4±0,3	1,6±0,7	4,3±0,6	5,6±0,6	16,4±2,1	12,4±1,8	14,4±6,8
Fibroblasts, %		0	0	0	10,3±0,8	38,4±3,0	26,7±2,6	45,0±4,3
New vessels, %		0	0	0	0	35,4±3,2	44,6±4,0	82,4±4,6
Fascia, %		0	0	0	0	54,2±6,8	64,4±7,4	94,1±13,6

The vascular response was represented by spasms (46.5±3.4%). The hematoma substrates had the ratio of normal red blood cells (95.6±6.4%) with a small admixture of abnormal red blood cells (4.3±1.2%). The leukocytosis was noted mainly at the border with the dura mater due to the normal white blood cells (28.1±2.8%). This case is represented by the single lymphocytes (1.4±0.3%). According to the above specifications, there are statistically significant differences from the subsequent study group (Table 3).

Table 3: Changes in blood properties (in the intracranial hematoma substrates) during the first period after an injury (up to 3 hours)

Indicators of blood and hematoma properties	Degree of incidence in %
Basophilia	13,0±1,2
Eosinophilia	11,7±1,2
Edema	6,5±0,7
Spasm	46,5±3,4
Normal red blood cells	95,6±6,4
Abnormal red blood cells	4,3±1,2
Normal white blood cells	28,1±2,8
Abnormal white blood cells	0

The second injury survivability period (up to 12 hours) was described by an insignificant increase in cerebral edema (7.6±1.5%) and decrease in eosinophilia up to (8.4±1.4%) collagen fibers, decrease in the number of vessels with spasm (27.8±9.2%). The hematoma substrates adjacent to the dura mater showed an insignificant change in the ratio of normal (92.5±5.2%) and lysed red blood cells (7.4±1.5%), an increase in leukocytosis up to 30.4±1.2% cells with the leukocyte debris (6.40±1.2%).

The macrolesions (1.4±0.2%) and fibrin in the form of individual strands were detected for the first time in 83.0±6.2% of cases. The number of leukocytes is hardly varying. In the hematoma substrates on the periphery of the dura mater, the inflammatory response is increasing much more slowly (Table 4).

The survivability period after the injury (up to 24 hours) was described by the occurrence of necrosis in the collagen fibers (55.6±6.9%) in the setting of increasing cerebral edema (10.2±1.2%), decrease in basophilia (8,7±1.3%) and eosinophilia (2.3±0.4). The vascular response is represented by plasmorrhagia of the vascular walls (78.7±4.2%) and the first appeared fibrinoid necrosis. The normal red blood cells remain almost at the same level (87.0±3.8%), the number of both normal (55.8±2.6%) and destructed white blood cells (16.8±1.8%) is increased. The number of macrophages has been increased up to 15.8±1.6% of the cells, the lymphocytosis was accumulated up to 4.3±0.6% of the cells. Free hemosiderin was found with the value of up to 1.3±0.4% for the first time. Fibrin is available predominantly in the form of a mesh (76.4±4.1%).

Table 4: Changes in blood properties (in the intracranial hematoma substrates) during the death period after an injury (up to 12 hours)

Indicators	Degree of incidence in %
Basophilia	12,7±1,4
Eosinophilia	8,4±1,4
Edema	7,6±1,5
Spasm	27,8±9,2
Plasmorrhagia	30,4±3,2
Normal red blood cells	92,5±5,2
Normal white blood cells	30,4±1,2
Leukocyte debris	6,4±1,2
Macrophages	1,4±0,2
Lymphocytes	1,6±0,2
Fibrin strands	83,0±6,2
Abnormal red blood cells	7,4±1,5

The hematoma substrates located on the cerebral periphery demonstrate an insignificant increase in the number of leukocytes, as well as the first occurrence free hemosiderin (Table 5).

Table 5: Content of distinctive features in the intracranial hematoma substrates during the survivability period after injury (up to 24 hours)

Features	Degree of incidence in %
1	2
Basophilia	8,7±1,3
Eosinophilia	2,3±0,4
Edema	10,2±1,3
Necrosis	55,6±6,9
Spasm	6,5±1,6
Plasmorrhagia	78,7±4,2
Fibrinoid necrosis	20,5±1,6

Features	Degree of incidence in %
Normal red blood cells	87,0±3,8
Abnormal red blood cells	10,7±1,3
Normal white blood cells	24,0±2,6
Leukocyte debris	16,8±1,8
Macrophages	15,8±1,6
Hemosiderin	1,3±0,4
Lymphocytes	4,3±0,6
Fibrin mesh	76,4±4,1

The following survivability periods (24-72 hours) were described by a sharp increase in the collagen fiber necrosis (13.6±1.1%) on the setting of pronounced cerebral edema (11.1±1.2%). The basophilia and eosinophilia are not found. The vascular walls are subject to the fibrinoid necrosis (54.6±2.6%). The hematoma substrates adjacent to the dura mater showed a decrease in the number of normal red blood cells up to 32.6±2.6%, decrease in the number of white blood cells up to 16.7±1.8%, including 25.4±1.8% of destructed cells.

The level of macrophages (25.2±1.4%) and lymphocytes (16.4±2.1%) is increasing. Free hemosiderin reaches a maximum level of up to 7.5±0.8% of the lump. The fibrin clots are shown in 76.6±4.4% of cases. The fibroplasts are found for the first time (38.4±3.0%).

The injury survivability period of more than 3 days is described by the cerebral productive processes. The fascia formation was noted in 64.4±7.4% of cases. The number of fibroblasts, macrophages and lymphocytes reached the levels of 45.0±4.3%, 23.2±1.8%, and 14.4±6.8%, respectively.

The hematoma substrates demonstrate a sharp decrease in the normal red blood cells (up to 15.6±1.2%). The number of leukocytes is decreased up to 8.6±0.6%. Fibrins have the form of granular mass (85.5±6.4%). In the hematoma substrates on the cerebral periphery, the inflammatory response dynamics is slowed down. The macrophages (Table 6) in large numbers are found for the first time.

As a result, our studies using the spectrophotometric and pathomorphological data indicate specific changes in the methemoglobin concentration and structural parameters in the intracranial hematoma substrates, depending on the body survivability period after the severe brain injury.

Table 6: Morphological indicators in the intracranial hematoma substrates during the period after the injury (after 3 days)

Indicators	Degree of incidence in %
Edema	1,6±0,3
Normal red blood cells	25,6±1,2
Abnormal red blood cells	62,7±4,2
Normal white blood cells	15,6±0,6
Leukocyte debris	12,1±1,8
Macrophages	23,2±1,8
Hemosiderin	4,6±0,4
Lymphocytes	14,4±6,8
Fibroblasts	14,5±4,3
Fibrin grain bodies	85,5±6,4

While using the obtained data, we conducted the forensic medical studies based on the spectrophotometric and pathomorphological studies presented above.

When studying the brain injury cases with intracranial hemorrhage in the mortuaries of the Tashkent urban and Karakalpakstan branches of the Republican Scientific Center for Forensic Medical Examination, it was found that in 45.5% of studies, the injury terms were not determined. In this regard, we have attempted to determine the brain injury limitation period using the spectrophotometric and pathomorphological data obtained in relation to the people with the known injury terms.

Based on the data obtained as a result of structural and spectrophotometric studies of autopsy material of intracranial hemorrhages and the adjacent dura mater and the known brain injury terms, we have revealed the statistically significant differences between a number of time intervals that demonstrate specific manifestations of the inflammatory process.

Conclusions

1. The intracranial hematomas reach their maximum volume during the first few minutes after the injury, and one of the main factors determining the clinical implications is its generation rate.
2. The identification of post-mortem biochemical patterns is of concern, both for determining the death term and for assessing the brain injury limitation in the study of intracranial hematomas.

3. During the spectrophotometric study of the intracranial hematoma substrates, it is necessary to use the structural studies of intracranial hematomas and the adjacent dura mater according to our recommendations provided in this chapter in order to correctly interpret the results obtained and to avoid possible errors in determining the injury terms.

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Management of Nursing in Preschool Institutions of Ex-Soviet Countries

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Abstract

Recently,the activity of preschool institutions experiencing dramatic changes, which lead to the emergence of new requirements for medical care for children. The most important element of health care system is nursery. Current model of nursing is a science and practice aimed to improve the health status of each child. The main purpose of this review is to present different approaches of development of an integrated health care system in kindergartens used in ex-Soviet countries. Systematization of accumulated experience and knowledge will help to develop the system theoretical and practical provisions, when the activities of medical staff of preschool institutions are determined.

Keywords: *Nursing; preschool institutions; nurse's role; child.*

Introduction

Nowadays there is a sharp deterioration in the health status of children, which is one of the most significant problems facing society. Among various age groups, the most vulnerable are children attending preschool educational institutions. For this reason, improvement of health of children in preschool educational institutions is the primary task for pedagogical and medical staff. Kindergartens have been the site for health programming in many countries for long years. Since public education became compulsory in the mid-nineteenth century, the strategic role that pre-school institutions could play in promoting and protecting health became recognized. Nurseries and infant schools soon became the front line in the fight against infectious disease and the hub for providing a wide range of health and social services for children. As times changed, health programs in these institutions have changed to keep pace with the changing needs of children. Definition of pre-school institutions and their purposes**.** Nurseries/kindergartens are pre-school institutions designed to accommodate children under 6 years[1]. In most countries nursery is a socio-educational institution aimed for children aged between 3 months and 3 years. Nurseries provide children with appropriate conditions for harmonious development, while collaborating with their parents throughout the whole education process[2]. The kindergarten is a pre-school institution for children aged from 3 to 5 years. It aims at promoting children's development and providing educational activities[3]. Pre-school education is the first stage of basic education that focuses on the balanced development of children's potential, giving them the opportunity to develop their autonomy and socialization skills, preparing them for a successful education path, and supporting the families in children's education[4].

In recent years,the activity of preschool institutions experienced dramatic changes. These changes include introduction of specialists in different disciplines, such as sports, arts and dance. More attention from teachers and medical staff began to attend to organization of motor activity of children using modern medical equipment complexes. Also much more time is now attended to development of skills of independence, personal qualities and higher mental functions[5].

Discussion

The essence of nursing in preschool: Usually, medical care for children in preschool institutions on the territory of countries of former Soviet Union is the competence of medical staff serving this institution. It is also controlled by the authorities of sanitary supervision and public education[6]. Means and method of solving the problems of medical support for children in preschool educational institutions are diverse, dynamic in time and changeable. They are developed by various departments and specialists. Approaches to the same problem are often interpreted from opposite positions[7].

Diagnosis of pre-nosological conditions in children, which can no longer be rated as healthy and, at the same time, are not a disease plays the leading role in health care process of preschool institutions. Active preventive intervention at this stage of possible disease gives the greatest effect and the least material costs.

The greatest amount of preventive work falls on the shoulders of pedagogical staff of preschool educational institution, and doctor with nurse act as methodologists who plan, train and supervise the implementation of this work. Unfortunately, this is not always adequately perceived by educators and their assistants, who often refuse to perform some healthcare measures[8].

Nursing in a kindergarten as part of the healthcare system is a science aimed at solving existing and potential health problems in changing environment. The purpose of nursing is to carry out a nursing process. The nurse strives to carry out her work professionally, respecting and protecting children's rights and dignity, and helping to improve their relations with society. Recent model of nursing is a science and practice aimed to improve the health status of each child. Nurse is a person who shares the philosophy of nursing and carries out nursing practice professionally and creatively. Nursing is a job performed by a nurse in accordance with her functional responsibilities[9]. These functional responsibilitiescharacterized as maintaining and strengthening of health condition, patient's care and rehabilitation assistance[10].

Clinical training in nursing is also an essential period in medical students study. It allows students of medical colleges to learn as members of a team, in direct contact with healthy and ill children, and as part of a group in the planning, implementation, and evaluation of comprehensive nursing care based on the acquired knowledge and skills. Students are expected to develop necessary skills in order to deliver comprehensive health care to children. Students clinical training in this area often takes place in nurseries/kindergartens[11].

The health condition of children in preschool institutions. The Center for Disease Control and Prevention (CDC) noted that six categories of behavior are responsible for about 70% of children mortality and morbidity. These include unintentional and intentional injuries, diseases resulting from inadequate physical activity, and health problems due to inadequate dietary patterns. A significant part of our youth is at risk for dropping out of school as a consequence of a number of health and behavioral problems; further, many children do not have access to basic preventive and primary care[10].

According to statistics, almost every child attending kindergarten has suffered from flu or other acute infectious disease. Almost every fifth child is under observation with certain chronic disease[13]. Eye diseases lead among other illnesses with 32 cases per 1000 children. Approximately the same frequency (18-22 cases per 1000 children) observed in diseases of nervous, respiratory, digestive and musculoskeletal systems. It turns out that 20% health condition depends on the genotype, 20% on the impact of environment, 50% on the lifestyle, and only 10% on condition of medical care[12].

According to definition of World Health Organization, human health is the complete physical, mental and social well-being with absence of disease. Such well-being ensured by complex of external and internal factors[14]. Internal factors characterized by the features of morphological and psychological development of the child. One of the main criteria for health is the ability of human organism to adapt to conditions of environment and the ability to carry out activities in various settings.

According toinformation of Research Institute of Hygiene of children and adolescents of Russian Federation[15], there is four basic criteria of children's health:

1. Level of physical development and its harmony;
2. Level of physiological systems of organism;
3. The absence of disease during examination;

4. The degree of resistance to effects of pathological factors.

Development of the child's body is a process of qualitative and quantitative changes leading to a new, higher level of organization of all physiological systems of the body. This process includes three main interrelated factors: differentiation of organs and tissues, growth and formation.

The child's organism differs from the adult organism with a number of factors:

- Incomplete morphological and functional development of all physiological systems;
- Continuity of growth processes, development and differentiation of organs and tissues;
- High degree of reactivity to on external influences;
- Less resistance to environmental factors;
- Uneven growth and development at different age periods;
- Heterochronism (different periods) of maturation of various functional systems[16].

With age, the pattern of the most common diseases in children varies: in age under three yearsmost often children suffer from bronchitis, diathesis, rickets, anemia, malnutrition and dental pathology. Further children more affected by adenoids, tonsillitis and otitis. From 3 to 7 years most common diseases of childhoodare diseases of digestive system that closely associated with dental caries, diseases of pulmonary system, nervous system, sensory organs and allergic diseases[16].

The number of children with varying degrees of visual impairment and disease of musculoskeletal system is increasing dramatically. In children in age of 2-4 year functional disorders with the decrease in the body's resistance to diseases, are associated mainly with imperfection of the functions of thermoregulation and immune system are quite often.

Preschool children with abnormal physical development or neuropsychological condition, are usually more vulnerable to different diseases than others. These diseases further reduce their depressed immunity and increase their susceptibility to repeated disorders. Such condition have negative impactto study process of children and their ability to assimilate knowledge due to missed classes and poor performance[17]. Studies of children 's health at different levels of health in a preschool educational institution showed that in pupils of second health group health indicators from the beginning to the end of the year, as well as during the week, decreased more significantly then in children with relatively mild forms of health disorders and healthy children (for 30–35% and 12–15% in relation to the initial level)[18].

Access to Health Care in pre-school institutions: According to National Health/Education Consortium, millions of children in developed countries have inadequate insurance plans that fail to cover even such simple preventive services as immunization. About 12 million children do not get such basic preventive care as periodic physical examinations or immunizations at the proper intervals, and only half of all infant school children routinely receive health care. Moreover, 7.5 children require mental health services, fewer than one in eight actually receives them (GAO, 1994b)[20].

Access to children health care include concerns beyond mere financial issues. Transportation, convenience, and cultural sensitivity are also factors. Additionally, parental support and encouragement, as well as understanding the importance of health care and how to approach the result, influence the process. Even with access to health care, children may not be receiving the appropriate attention they need. Even children in those families with insurance and family doctors do not receive help from health care professionals for problems of greatest importance for their high-risk behaviors. Many studies have shown that an initial visit to doctor and physical examination for a new patient in pre-school institution should require not less than 30-45 minutes. Although 25% of doctor visits are first encounters, half of all visits last 10 minutes or less, 30% percent last 10-15 minutes, and only 4% are 30 minutes or longer[19].

The comprehensive pre-school health program that developed in ex-Soviet countries is seen as a new solution needed to deal with the problems of today's children in kindergartens[15]. It became clear to social committees that, although a variety of different models exist, comprehensive programs are still essentially an unrealized ideal in most institutions[21]. Many specialists came into the study with a range of backgrounds, and they determined that it needed to establish their own working definition of the term "comprehensive health program" which would guide further work with children[22].

The mainpreventive activities: The main priority of the activities carried out in the preschool educational institution is measures to prevent diseases. Currently, a number of infectious diseases are prevented by specificimmunization[22]. The incidence of viral infections of respiratory tract constitutesa high percentage of all diseasesamong preschool age children[23]. The vaccine against flu widely advertised during pre-epidemiological period does not always give expected results, its effectiveness decreases due to variety and variability of viruses[24]. Therefore, method aimed to increase protective mechanisms of the organism itself and to improve resistance to pathogenic, environmental factors play the leading rolein the system of health-improving of preschool institution[25].

The level of child's adaptation to changing environmental conditions have great importance in assessment of health. Leading pediatricians have repeatedly pointed out that non-specific prophylaxis of diseases is based on a set of general strengthening measures that include tempering and provision of full-fledged physical education that adequate to physiological capabilities of the child's body[21].

The protective reactions of the body enhanced by the systematic and regular use of small doses of the stimulus - low or high temperatures of water or air, and influence of natural factors. As a result, this leads to training of thermoregulation mechanisms and otherphysiological systems involved in strengthening of immunity.

One of the hallmarks of the Soviet recovery system is hardening. Hardening is an active process, representing a set of method for stimulating the hidden reserves of the body's defenses, improving it's flexibility and versatility. It is the development of a conditioned reflex to a particular irritation. Therefore, this is a training process, the construction of which in order to achieve the maximum final effect must be subject to certain physiological laws that underlie the mechanism for generating a conditioned reflex. The biological basis of any training process, including hardening, is adaptation to the acting loads, mainly cumulative adaptation. It is characterized by adaptive changes that occur under the influence of regularly repeated external influences. The properties acquired as a result of cumulative adaptation are stable and persist for some time after the cessation of external influences. Adaptive changes that occur in the child's body during the hardening process go through two stages: transitional and stationary. In the first stage, the adaptation process itself (hardening) takes place, in the second stage the adaptation state (hardening) is achieved, and if the body is not exposed to new influences or the load is insufficient, the process of readaptation will begin, a gradual return of all body systems to the initial level of functioning[1]. The hardening effect is achieved by a systematic, repeated exposure to a particular hardening factor and a gradual increase in its dosage[3]. Hardening is specific, as it is determined by a gradual decrease in the body's sensitivity only to the action of the hardening factor[5].

The body of preschool children characterized by insufficient functional condition of physiological systems, the purpose of which is to maintain thermal comfort. Therefore, the children's body responds with adverse reactions even to minor deviations in the weather. It is at an early and preschool age that hardening takes on special significance.

Hardening in a preschool institution is most advisable in the first half of the day before a sleep or immediately after a walk, depending on the season, local and climatic conditions. Also, good hardening agent is the sleep process, if it takes place in a well-ventilated room, under favorable hygienic conditions[8].

Anthropometric examination and determination of the degree of physical development according to its evaluation tables (standards) are the responsibility of nurse and their interpretation is the responsibility of the institution's doctor. Today there is a paradoxical situation when the standards of the 80-90s became unusable due to the changed biogenic situation. The acceleration of the young generation was replaced by stabilization and even a return to lower indicators of physical development of modern children[27]. On the other hand, the development of new regional age-gender standards requires the allocation of absolutely healthy children. Against the backdrop of Russia's economic difficulties, it is very difficult to implement this. Under these conditions, interest in genetic growth standards reappeared, the idea of which belongs to E.A. Shaposhnikov[26].

Studies conducted by this author, allowed him to establish a number of previously unknown statistical laws of the physical development of children. They are based on the law of repeatability of equal average values of mass and average parameters of proportions with equal average body length. Children of different nationalities, various social groups living in different

territories of country have average body weight and average parameters of the length of body, legs, arms, diameter of the shoulders, pelvis, chest and other somatometric signs, reduced to the same body lengths are approximately the same and remain virtually unchanged during generations.

The lag of growth processes, if it is not associated with the somatic pathology, means inhibition of the development and differentiation of internal organs. The greater lag in physical development of child means the more pronounced changes in the immune system. The change of an anthropometric indicator in the early stages allows doctor to suspect the development of pathology. It could be the emerging growth retardation; the development of dystrophies of various origins, obesity, narrow breasts, often accompanied by various anomalies in the development of the lung, broad chest, as manifestations of an increase in lung volume, micro- and macrocephaly, partially due to hydrocephalus. The physical development of the child depends on specific environmental factors and lifestyle. To assess physical development of group, it is necessary to conduct its individual assessment and give a final assessment of specific gravity of children with good physical development. The physical development of team is considered satisfactory if at least 68% of children have normal harmonious physical development.

In the early 90s of the last century, in the Soviet Union, with a total number of about 90-100 million children, 47–48 million cases of childhood infectious diseases and 36–39 million cases of flu were noted annually. But the incidence rate of diphtheria, whooping cough, measles, and other infection diseases is not determined by the state of science, but depends solely on the setting of preventive and health-improving measures in children's institutions. Preventive work in the preschool educational institution is defined by two main areas, the content of which is clearly delineated.

1. Scheduled preventive maintenance involves a set of measures to increase the specific and nonspecific resistance of the child's organism to adverse external influences and covers all healthy children in preschool education[11], that is, 1st and 2nd A health groups.
2. Wellness activities include the improvement of children's health from risk groups, primarily children with reduced immunoresistance (children of group 2nd B) and conducting a certain amount of medical and recreational activities based on the possibilities of preschool education[14]. Therefore, this service is best implemented in specialized health-improving preschool institutions for children with reduced resistance[15].

Conclusion

Thus, distinctive feature of the system of organization of medical care in preschool institutions of ex-Soviet countries is comprehensive pre-school health program, which is still the basis of successful development of children. Similar to United States and European countries, nursing plays a significant role in the implementation of preventive measures in preschool institutions. Along with this, the system of child health protection has a number of distinctive features expressed in the application of hardening method for prevention and a special approach to assessing the physical development of children.

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Development and Validation of UV-Spectrophotometric Method for Quantitative Assessment of Metronidazole in the "Metromed® Neo" Solution for Infusion

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Abstract

Introduction: Metronidazole is widely used in medicine and issues of quality control of its dosage forms are relevant. Objective - development and validation of a UV spectrophotometric method for monitoring the quality of metronidazole in the «Metromed® Neo» solution for infusion.

Materials and Method: The object of the study was a solution for infusion containing metronidazole. The method used is UV spectrophotometry.

Results: The relative error of the developed UV spectrophotometric method for the quantitative assessment of metronidazole in the solution for infusion was 1.9%. Validation characteristics of the method are within acceptable limits.

Conclusion: A UV spectrophotometric technique was developed and validated to assess the quality of the solution for infusion containing metronidazole. The results of the studies make it possible to use the developed and validated methodology in assessing the quality of metronidazole.

Keywords: *Metronidazole, UV spectrophotometry, solution for infusion, quality assurance, validation.*

Introduction

Antibacterial therapy is one of the most important components of intensive therapy of purulent-septic diseases, and its effectiveness has a significant impact on the course and outcome of the disease. Of particular interest is metronidazole. The mechanism of action of metronidazole is the biochemical reduction of the 5-nitro group of metronidazole by intracellular transport proteins of anaerobic microorganisms and protozoa [1].

In the US Pharmacopoeia, for the assessment of the authenticity and quantitative content of metronidazole in both substance and injectable dosage form[2], a method of high-performance liquid chromatography was proposed[3]. Existing method for assessing the quality of metronidazole do not always meet modern requirements. They have several disadvantages: the complexity, the duration of the implementation, the use of toxic organic solvents, expensive reagents and devices. This suggests that the problem of improving existing and developing new method for the analysis of metronidazole is relevant[4].

Spectrophotometry as a physicochemical method is characterized by high sensitivity and expressiveness. The development of simple, affordable, and highly accurate method for controlling the quality of drugs is essential for pharmaceutical manufacturers[5]. However, it should be noted that in order to confirm and ensure the quality of pharmaceutical products, the control method used must be validated. Currently, on the validation of analytical works, there are a number of regulatory documents, as well as scientific works, which describe in detail the conditions for the validation of analytical method [7].

Aim of the study was to develop and validate a UV spectrophotometric method for monitoring the quality of metronidazole in the Metromed® Neo infusion solution.

Materials and Method

Experimental Part

The infusion solution containing metronidazole has the following composition:

Composition:

Metronidazole 500 mg

(Brit. F., Heb. F,)

Sodium Chloride 800 mg

(Brit. F., Heb. F., FS 42 Uz-0129-2011)

Water for injection up to 100 ml

(FS 42 Uz-0512-2012)

Research on the development of metronidazole quality control method began with a study of the physicochemical properties of the substance.

Metronidazole or 2- (2-methyl-5-nitroimidazol-1-yl) ethanol is a white to white powder with a greenish-yellow tint, slightly soluble in water, acetone and alcohol 96%.

Results

Preparation of a working standard sample of metronidazole: 40 mg of metronidazole is placed in a 100 ml volumetric flask, a 0.1 M solution of hydrochloric acid is added, and the volume of the solution is adjusted with the same solvent. 5 ml of the resulting solution is transferred into a volumetric flask with a capacity of 100 ml, the volume of the solution is adjusted with the same solvent. The UV spectrum of the resulting solution was determined in the wavelength range from 230 nm to 350 nm in a cell with a layer thickness of 1 cm (Fig. 1).

The above spectrum indicates that metronidazole has a maximum absorption at a wavelength of 277 nm.

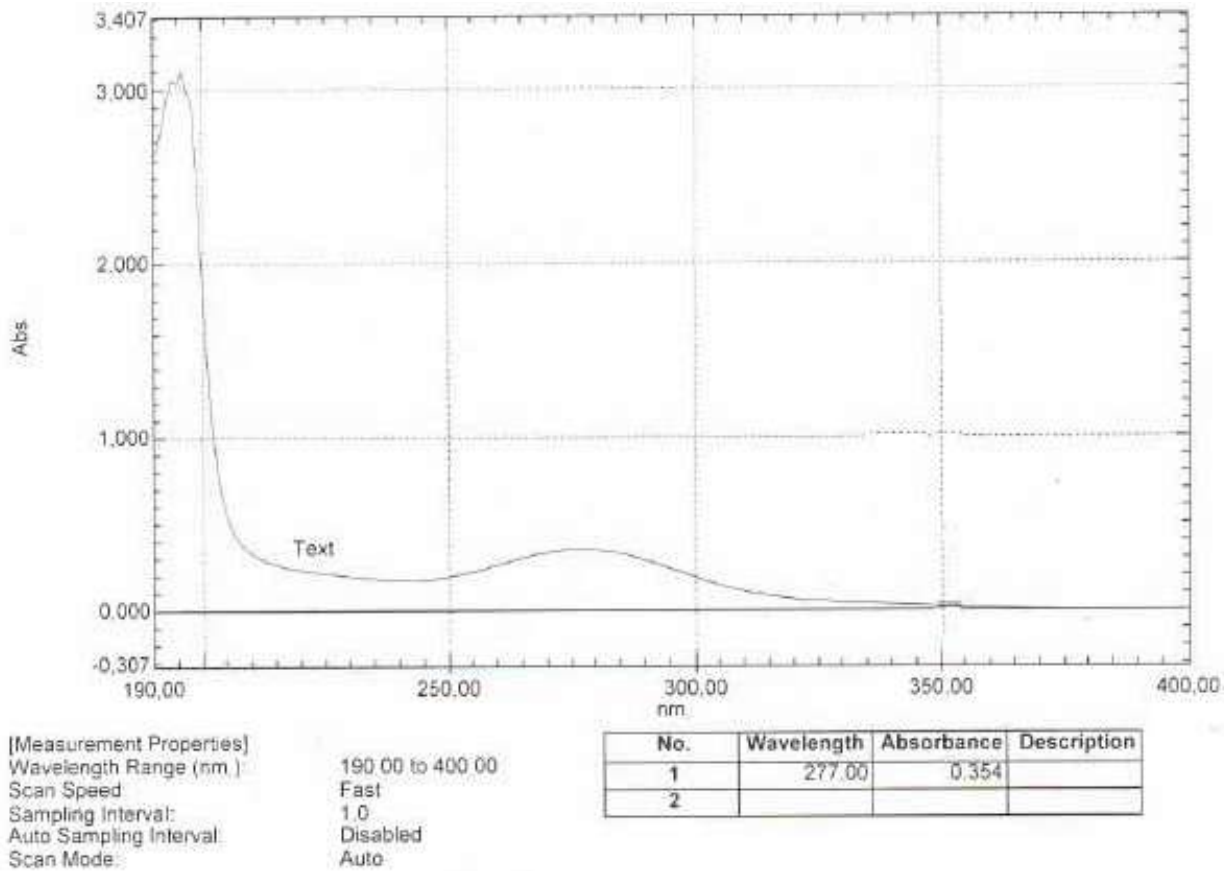


Fig. 1. UV spectrum of OCR metronidazole

To quantify metronidazole in the infusion solution, a test solution of metronidazole was previously prepared.

10 ml of the test solution of metronidazole was transferred into a volumetric flask with a capacity of 100 ml, the volume of the solution was brought to the mark with a 0.1 M hydrochloric acid solution, and stirred. 5 ml of the resulting solution was transferred into a volumetric flask with a capacity of 250 ml and the volume of the solution was adjusted to the mark with a 0.1 M hydrochloric acid solution and stirred. The optical density of the resulting solution was measured at a wavelength of 277 nm, using a 0.1 M hydrochloric acid solution as a reference solution.

The content of metronidazole in grams was calculated by the formula:

$$X = \frac{D \cdot 1 \cdot 100 \cdot 250}{375 \cdot 100 \cdot 0 \cdot 5} = \frac{D \cdot 5}{375},$$

where D is the optical density of the test solution.

375 - specific absorption rate of 1% solution of metronidazole.

The content of metronidazode (C6H9N3O3) in 1 ml of the drug should be from 0.0045 g to 0.0055 g.

Table 1: Metrological characteristics of the results of the quantitative assessment of metronidazole in the drug «Metromed® Neo», solution for infusion 500mg/100ml

The content of active substance, mg/100 ml	The limit of active substances in mg/ml	The average value of the quantity found, Xav	Sample Deviation, S	Relative Standard Deviation, RSD	Relative error, ε cf
Metronidazole					
500 mg	from 4.5 mg to 5.5 mg in 1 ml of the drug	4,720	0,1257	1,0%	1,9688
		4,759			
		4,773			

The results presented in table 1, indicate the reliability of the data.

Discussion

The next stage of the research was the validation of the developed UV spectrophotometry technique. Validation parameters were - correctness; precision (convergence, reproducibility), linearity, specificity.

1. The correctness of the analytical method characterizes the proximity of the test results obtained by this method to the true value.

Correctness was evaluated on the basis of at least 9 determination results at a minimum of 3 concentration levels in the limit of the analytical region (for example, 3 replicates of the determination for 3 analytical concentrations). Prepared solutions containing metronidazole in the amount of 80%, 100%, 120% of the declared value. The optical density of metronidazole in all mixtures was determined in three parallel determinations, and the quantitative determination of the active substance in each solution was calculated.

Acceptance criterion: the percentage of recovery when using concentrations of 80, 100 and 120%, adjusted to 100%, should be in the range from 98.0% to 102.0%.

Recovery percentage (R):

$$R = \frac{A}{B} * 100\%$$

where R (Recovery) - output, A – measured content, B-specified (actual) level.

The results of the analysis are presented in the table:

Table 2: Correctness of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug «Metromed® Neo», 500mg/100ml solution for infusion

The claimed amount of metronidazole in test solutions, mg/ml	Found amount of metronidazole in test solutions, mg	Regeneration, %	Metrological characteristic
3,3	3,31	100,3	X_{av} = 99,0; S = 0,96; S^2 = 0,92; Δx = 2,22; Δx av = 0,74; E_{av} = 0,74
3,3	3,29	99,9	
3,3	3,28	99,3	
5,0	4,95	99,0	
5,0	4,88	97,6	
5,0	4,99	99,8	
6,0	5,97	99,5	
6,0	5,88	98,0	
6,0	5,88	98,0	
The average percentage of regeneration		99,0%	

From the results of the table it is seen that the average percentage of metronidazole regeneration is 99.0%, which indicates satisfactory correctness of the method.

2. The precision of the analytical method characterizes the degree of closeness of the independent results of individual tests obtained in specific specified conditions. This characteristic depends only on random factors and is not related to the true value of the measured quantity. It is expressed by the standard deviation - the coefficient of variation. Extreme precision indicators - convergence and reproducibility.

2.1. Convergence characterizes the degree of consistency of the results of measurements (tests) obtained by the same method at identical test objects, in the same laboratory, by the same operator, using the same equipment, within a short period of time.

Acceptance criterion: the coefficient of variation of parallel determinations for 6 measurements should be no more than 2%.

Table 3: The convergence of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug "Metromed® Neo", solution for infusion 500mg/100ml

Solution No.	Found amount of metronidazole in test solutions, mg/ml	Arithmetic mean of all definitions, $\bar{X}$	Standard deviation, S	Coefficient of variation, CV
1	4,77	4,96	0,093	1,8
2	4,99			
3	5,00			
4	5,00			
5	5,01			
6	4,98			

The results presented in table 3 indicate a high convergence of the obtained data.

2.2. Reproducibility characterizes the measure of coincidence of the measurement results obtained by the same method on identical samples, in different laboratories, by different operators, using different equipment, a relatively long period of time between measurements, separate, presumably identical samples taken from the same batch of material.

Acceptance criterion: the coefficient of variation in each of the levels of accuracy is determined. The criterion of instrumental reproducibility is 1%, intralaboratory reproducibility is 2% for the quantitative determination of the main component.

The method for the quantitative determination of metronidazole in the analyzed medicinal product was tested in two laboratories:

1. Laboratory of expertise and standardization of medicines JV LLC Remedy Group.
2. Scientific laboratory LLC Medstandart.

The results of the analysis are presented in the table:

Table 4: Reproducibility of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug «Metromed® Neo», 500mg/100ml solution for infusion

Lab Name	Solution No.	Found amount of metronidazole in test solutions, mg	Arithmetic mean of all definitions, $\bar{X}$	Standard deviation, S	Coefficient of variation, CV
Laboratory of Expertise and Standardization of Medicines JV Remedy Group LLC.	1	4,77	4,96	0,0928	1,82
	2	5,00			
	3	4,99			
	4	5,00			
	5	4,98			
	6	5,01			
Scientific laboratory LLC Medstandart.	1	4,86	4,92	0,075	1,6
	2	4,88			
	3	4,85			
	4	4,9			
	5	5,02			
	6	5,01			

The calculated values of the coefficient of variation are within acceptable limits and indicate the reproducibility of the developed technique.

3. Linearity is established on the basis of test results that are proportional to the concentration of the analyte in the sample within the analytical method. The linearity of the results can be represented graphically in the form of a dependence of analytical signals on the concentration of a substance (at least 5). The following parameters are used to confirm the linearity of the analytical technique: the regression coefficient and the slope of the regression line.

The determination is usually carried out on solutions with concentrations of 80, 90, 100, 110 and 120%.

Acceptance criterion: the technique is considered linear if the correlation coefficient between a series of obtained values is not lower than 0.995.

The results of the study of linearity must be indicated in the graphic image.

Table 5: Linearity of the UV spectrophotometric method for the quantitative determination of metronidazole in the drug «Metromed® Neo», 500mg/100ml solution for infusion

№	The concentration of the solution,%	The concentration of the solution, mg/ml	Optical density value	Correlation coefficient, R
1	80	0,008	0,283	0,9993
2	90	0,009	0,315	
3	100	0,010	0,354	
4	110	0,011	0,389	
5	120	0,012	0,420	

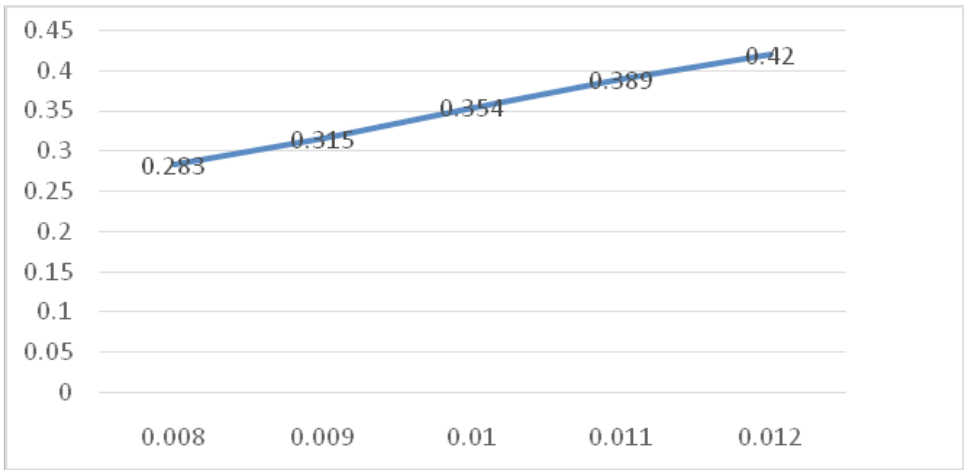


Fig. 2. The linear dependence of the optical density of metronidazole on its concentration in the test solution

From the data presented in table 5 and the graphic image, we can conclude that there is a linear dependence in a given concentration range. The value of corelation is within the acceptable range.

3. The specificity of the analytical method is determined by its ability to reliably determine the drug substance in the presence of impurity compounds, degradation products and excipients.

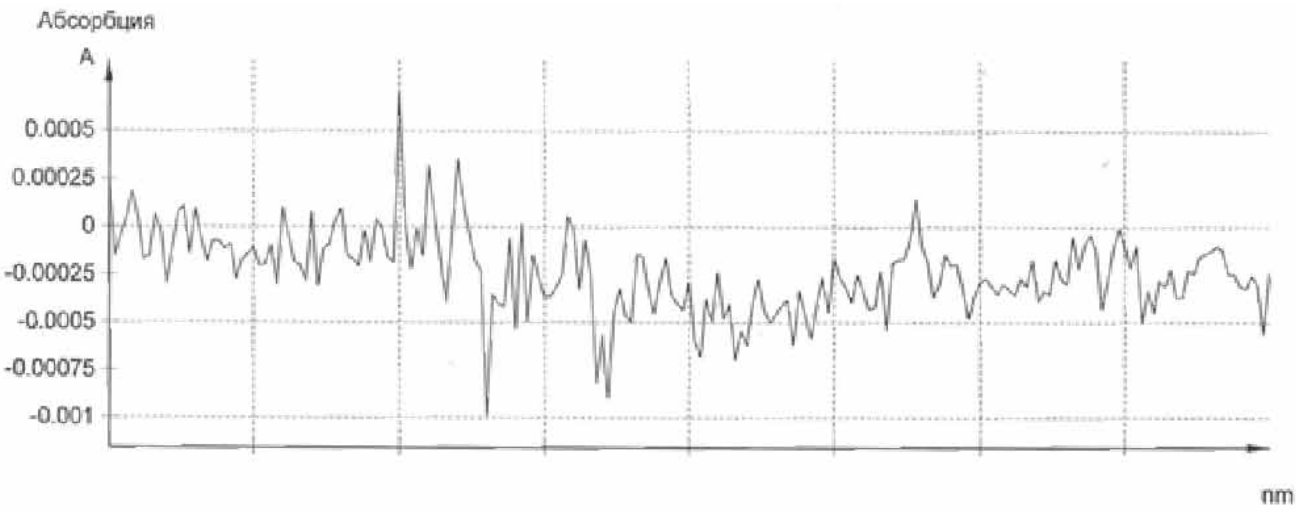


Fig. 2. The placebo absorption spectrum in the range from 250 nm to 320 nm

Preparation of a placebo solution: 800 mg of sodium chloride salt is dissolved in water for injection up to 100 ml. 10 ml of the resulting solution was transferred into a volumetric flask with a capacity of 100 ml, the volume of the solution was adjusted to the mark with a 0.1 M hydrochloric acid solution, and stirred. 5 ml of the resulting solution was transferred into a volumetric flask with a capacity of 250 ml and the volume of the solution was adjusted to the mark with a 0.1 M hydrochloric acid solution and stirred. The optical density of the resulting solution is measured at a wavelength of 277 nm, using a 0.1 M hydrochloric acid solution as a reference solution.

Acceptance criterion: impurities of the active substance, excipients and solvent for the test sample should not interfere with the quantitative determination of the basic substance.

From this spectrum it is seen that in the wavelength range from 250 nm to 320 nm, the placebo solution has no absorption in the UV light region. The maximum optical density obtained in a given wavelength range was 0.0005, which does not affect the quantification of the active substance.

Conclusion

Methodology was developed and validated for the quantitative assessment of metronidazole in the Metromed® Neo infusion solution. The obtained values of the validation characteristics are within acceptable limits, which allows using the developed technique in assessing the quality of the infusion solution.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Features of Manifestation of Optokinetic Nystagmus in Meniere'S Disease

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Abstract

The characteristics of optokinetic nystagmus (OKN) were studied in 20 healthy individuals and 30 individuals suffering from Meniere's disease in remission. The results recorded by the method of electronystagmography at frequency optokinetic stimulation 66,90 156 and field/min. found that in healthy people, the frequency of IPOs, the total amplitude and the speed of the slow phase(sp) nystagmus increases proportional to the increase in the frequency optokinetic stimulation; accordingly, consistently reduced the average amplitude window. In Meniere's disease, OKN indicators are generally characterized by lower values than normal, and an increase in the frequency of optokinetic stimulation is accompanied not only by a more pronounced decrease in the total amplitude indicators, but also by a decrease in the speed of the slow phase of OKN. After the glycerol load and with positive results of the glycerol test, the quantitative indicators of OKN increase in patients, approaching the physiological norm. It is concluded that it is appropriate to study the OKN in Meniere's disease to clarify the stage of the disease and predict the effectiveness of treatment.

Keywords: *Meniere,total amplitude, optokinetic nystagm, glicerol test.*

Introduction

According to the literature, the study of optokinetic nystagmus (OKN) is widely used for the differential diagnosis of hemianopsias[1], oculomotor disorders, and diseases of the Central nervous system[2].

In recent years, a number of papers have been published on the study of OKN features in disorders of both Central and peripheral parts of the vestibular analyzer [3]. It is experimentally proved that the peripheral labyrinth can give pathological optokinetic nystagmus[4]. Many authors first reported the neutralization of optokinetic nystagmus by supra-threshold post-caloric or post-dictatorial nystagmus in humans and indicated that sub-threshold vestibular stimuli increase the optokinetic response. Visual assessment of OKN disorders under the influence of stimulation in all directions in limes suffering from Meniere's disease and cochlear vestibulopathy caused by vegetative vascular dystonia[3], cervical osteochondrosis and other causes was not revealed[5]. However, when analyzing electronystagmogram was certain violations of the settings window. In this regard, it is advisable to further identify violations of the peripheral parts of the vestibular analyzer, in particular, in Meniere's disease, caused by the hydrops's of the labyrinth.

The aim of the study was to determine the features of the manifestation of optokinetic nystagmus in patients with Meniere's disease.

Materials and Method

A Total number of examined people in the clinic No. 1 of the Samarkand medical Institute were 50, including 20 healthy (control group) aged 17 to 41 years, who do not suffer from vestibular dysfunction, and 30 people with Meniere's disease aged 25 to 52 years, with a prescription of the disease from 5 months to 14 years. The examination of patients was carried out in the inter-access period.Method of work inspection, audiometric, vestibulometric study, as well as learning the OCN with the help of stat-tomographic recording. Audiometric examination was performed in a sound-attenuated chamber on an audiometer MA-30 (GDR). hearing tests included tonal threshold, supra-threshold and audiometry.

During vestibulometry, statokinetic stability was determined, and spontaneous and experimental

nystagmus obtained with caloric and rotational stimulation was recorded.

OKN was induced by observation of the examinee for optokinetic rotation of the drum, which caused the four black vertical stripes, a Rotating cylinder was carried out at three fixed angular velocities: 99, 135, 2340 in 1 second, which provides the frequency optokinetic stimulation, respectively, 66, 90 and 156 of the bands in the I min. In assessing the entry window takes into account the frequency nystagmoid movements, the amplitude and total average speed of nystagmus slow phase (sp) and efficiency bands. In addition, the severity of optosensory and opto vegetative reactions was recorded.

Based on the oral audiometry data, it was found that the examined patients had predominantly unilateral hearing loss on the side of the patient's labyrinth. The curves of the hearing thresholds were mostly horizontal, less often descending and ascending. In most cases, an interval of 10-30 dB was observed between the bone and air conduction curves. We noted low differential thresholds for the perception of sound power (0.4 0.8 dB at an over-threshold tone of 500 and 2000 Hz with an intensity of 20 dB), and a positive SISI test (80-100% of positive responses). According to vestibulometry, the predominant type of disorders was hyporeflexia, in rare cases hyperreflexia of vestibular excitability was observed.

Results

The frequency of OKN in healthy people increased linearly as the frequency of stimulation increased (from 1.2 at the frequency of stimulation of 66 bands/min to 2.64 at the frequency of 156 bands/min). There was an increase in the total amplitude (from 180 to 227.40) and SP (from 18.8 to 26.90/c). At the same time, the average amplitude tended to decrease (from 15 to 8.90) bands (the ratio of the number of nystagmal responses to the number of bands passing in the field of view per unit of time), regardless of the frequency of stimulation, was close to one. Optosensors and opto autonomic reactions were absent (table. 1).

Table 1: Characteristics of OKN in normal and Meniere's disease

Indicators	Main group			Persons suffering from Meniere's disease		
	Frequency of stimulation(number of bands per 1 minute)					
	66	90	156	66	90	156
Frequency OKN in Hz	1,2±0,1	1,6±0,1	2,64±0,02	1,18±0,01	1,55±0,015	2,43±0,06
The total amplitude (in degrees)	180±8,6	216,6±8	227,4±15,6	123,4±5,8	141,8±6,5	136,2±8,1
The average amplitude (in degrees)	15±0,72	13,7±0,65	8,9±0,55	9,9±0,48	8,3±0,48	5,2±0,29
SMF of optokinetic nystagmus (in degrees/s)	18,8±0,82	23,9±0,88	26,9±0,88	13,1±0,65	15,1±0,76	15,4±0,95
Band efficiency	1±0,1	1±0,1	0,957±0,12	0,952±0,022	0,928±0,016	0,831±0,028

When examining people suffering from Meniere's disease, the WINDOW parameters considered are of a different nature (see table. 1). The frequency of the window increases evenly (from 1.18 to 2.43 Hz). The average amplitude and performance indicators of the bands decrease linearly, and their value is significantly less than the "normal" one. The quantitative values of the total amplitude and CP of nystagmus increase to the frequency of stimulation of 90 bands/min, and with stimulation of 156 bands/min, their indicators decrease slightly, while in the control group, the characteristics under consideration increase linearly. In some patients, an increase in the speed of stimulation caused a feeling of vestibular "discomfort", manifested by dizziness and nausea.

To determine the stage of the process in the subjects, we performed a glycerol test, which is used in diagnostic practice to characterize the reversibility or irreversibility of pathological changes in the receptor formations of the labyrinth in Meniere's disease[5]. The patient was offered to take inside a mixture of pure medical glycerol in half

with sour fruit juice at the rate of 1.5 g of glycerol per 1 kg of body weight. Audiometry, vestibulometry, and optokinetic recording were performed up to h 3 hours after taking glycerol.

Discussion

According to the results of studies after taking glycerol, patients were divided into two groups. The first group included individuals who had a decrease in tone perception thresholds by 10 to 15 dB during audiometry, and with experimental vestibular samples, the nystagmal response indicators significantly increased. The second group included patients who did not have any significant changes in auditory and vestibular functions after the glycerol test.

Comparison of the results of optokinetic stimulation with the initial data showed that the subjects with a positive glycerol test showed an improvement in all the characteristics of OKN. So, for example, when stimulated 156 hollow/min, the total amplitude increased to 155.5" at the initial value of 136.2", and sp - to 17.8/s (before taking glycerol, this value was 15.49/s), in patients with a negative glycerol test, we did not note significant changes in the considered characteristics of the OKN.

Comparison of records allows us to conclude that with a positive glicerol test, there is a "revival" of the OKN in comparison with the original data.

Conclusions

Based on the results of the survey, the following conclusion can be made. In healthy people, there is a linear increase in the frequency, total amplitude, and speed of the slow phase of optokinetic nystagmus and a consistent decrease in the average amplitude with an increase in the frequency of optokinetic stimulation. In individuals suffering from Meniere's disease, OKN is characterized by lower parameters, and as the frequency of stimulation increases, the total amplitude of the CMF tends to decrease. Reduced endolymphatic labyrinth as a result of the action is accompanied by an increase in the quantitative characteristics of the WINDOW

Thus, the characteristics of OKN in Meniere's disease may be important for identifying additional criteria for diagnosing diseases of the labyrinth and predicting the effectiveness of treatment.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Some Features of the Bacteria of the Family Enterobacteriaceae Allocated from Small Cattle at Different Pathological Processes

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Abstract

Enterobacteria with a certain set of pathogenicity factors and capable of leading to the development of diseases of animals and humans are ubiquitous in nature, which can be recognized as the result of anthropogenic activity. Recently, they have been isolated with increasing frequency from soil, water, from fruits, vegetables, grains, plants, from representatives of various systematic groups of animals and humans. We have obtained bacteria from sheep with gastrointestinal pathologies. From sick animals, we have identified a large number of bacterial cultures belonging to species of different genera of the *Enterobacteriaceae* family. Among them, 190 cultures, species belonging to *Escherichia coli*, 124 cultures of *Enterobacter cloacae*, 74 cultures of *Morganella morganii*, 51 culture of *Citrobacter freundii*, 29 cultures of *Serratia marcescens*. For these microorganisms a large set of various pathogenicity factors was characteristic: antilysocyme, anti-complementary, anti-interferon activity, adhesive ability, hemolytic activity, multiple resistance to antibacterial drugs.

The aim of our work is to study the spread of enterobacteriosis, some features of the epizootic process in these diseases in the territory of the Chechen Republic

Keywords: *Epizoology, farm animals, enterobacteriosis, disease, small ruminants, pathogen, skin, wool.*

Introduction

Independence of the state is defined not only by the power of their military forces, but also by the capability to supply the population with food. According to the standards of UN FAO of the WHO, a person has to consume around 959.7 kg of plant and animal food products annually. In Russian Federation there are on average 701.6 kg of food per person annually[1]. Meat and milk consumption per person is 80% of the norm and fish consumption is 55% of the norm[2].

Only innovation-driven growth of APC can provide Russia with food supply security[3]. The issue of animal production intensification in Russian new economic conditions is one of the most acute, because it is directly connected with the quality of population nutrition and their life in general. Scientific approach to dealing with this acute issue is absolutely necessary, since food supply becomes a lever of economic and political pressure in international relations.[4]

At present, animal production industry is in crisis, it is considered unprofitable and has to cut the main qualitative parameters. This issue is especially acute in the present conditions of the imposed import substitution policy.

The popularity of import substitution policy in agricultural sector and on the food market is increasing. The problem of production intensification, based on innovation-driven technologies, in particular, on energy saving technologies, is very acute.

During the past years Russia has increased production rate, in particular, agricultural production rate, and, as a result, became one of the exporters. In 2014 export profit of agricultural production exceeded 20 billion dollars, which is higher by one forth than the profit from military equipment export and by one third than from gas resources export.

The concept of long-term social and economic

development of Russian Federation defined the main objective that population should be supplied with agricultural products and food by domestic industry. Thus, by 2020 Russia should reach the level of the recommended daily food norm of animal food products consumption. Meat production is expected to increase by 1.7 times and milk production is expected to increase by 27%. It is planned to provide the industry with breeding animals and increase their productivity[5] to the levels compatible with those of West European countries.

State support of establishment of the additional and modernization of the existing animal breeding genetic centers should provide the import substitution policy implementation by 80% and create conditions for parental and line breeding animals and their reproductive and productive characteristic improvement[4]. This is expected to develop animal breeding industry and increase its competitive advantages on Russian and world markets».[6]

«Successful animal production development is defined by veterinary policies that protect animal farms from infectious and parasitic diseases. Scientific research data[7] and practical experience in veterinary show that the majority of infectious diseases, as a rule, is diagnosed not as mono infection, but as associated parasite community[56]. It should be noted that parasite associations in farm animals are diverse in both taxonomic aspect (viruses, bacteria, protozoan, helminths)[8] and the pathologies caused».[9]

The diseases caused in animals and humans by different genera of the *Enterobacteriaceae* family, i.e. enterobacteriosis, are quite widespread. These diseases often lead to lethality in the infected individuals.

Enterobacteriosis pathogens are often isolated from soil, water, plant and animal products, domestic and wild animals, as well as humans. Among these microorganisms there are strains that differ significantly from each other by biological properties, ecological peculiarities, range of hosts, pathogenic factors and virulence. A number of species cause gastrointestinal problems. 12 out of 30 geniof the *Enterobacteriaceae* family–*Citrobacter*, *Escherichia*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, *Shigella*, *Yersinia*,cause different pathologies. In the Chechen Republic these diseases were registered annually in farm animals from 2002 to 2015. The annual season dependent dynamics of morbidity was identified in young small ruminants[9].

Materials and Method

For evaluation and analysis of enterobacteriosis epizootic situation (colibacillosis, salmonellosis, serratiosis, etc.) among small ruminants in the Chechen Republic, the authors used the data from annual reports from republic and regional vet laboratories. Epizoologic screening was performed from 2002 to 2015 according to the common methodical guidelines [10].

Isolation of opportunistic and pathogenic enterobacteria, their cultivation and evaluation of pathogenicity factors were conducted according to the common method.

Cultivation of microorganisms was done on 1.5% meat and peptone nutrient agar and nutrient broth. Bacteria culture was cultivated in thermostat at 37°C within 18-24 hours. The bacteria were isolated and identified according to the "Methodical guidelines to bacteriological diagnostics of associated intestinal infections caused by pathogenic enterobacteria in animal growing stock[11].

Morphologic, tinctorial and cultural properties evaluation was performed according to the method, outlined in the reference books on microbiology[12].

Disc diffuse method was used for identification of sensitivity range of the studied bacterial culture, isolated from sheep, to antibiotics[13].

Lysozymic activityof the studied cultures was assessed by the microorganism lysozyme capacity to split β-(1-4)-glycoside bonds of mucopolysaccharide complex in cell walls of the reference strain *Micrococcus luteus var. Lysodeikticus*[13].

Assessment of antilysozymic activity was done by the method of O.V. Bukharin et al.[13].

Adhesive activity of the studied bacteria was evaluated by the reaction of hemagglutination with 3% goat erythrocyte suspension in the presence of D-mannose and without it[13].

Hemolytic activity of the studied cultures was assessed on nutrient agar in the presence of 3-5% rabbit washed erythrocytes. Thiol dependent hemolysines were identified by cultivation on trypticase soy agar in the presence of 3-5% rabbit washed erythrocytes (washed three times in Hank's solution) according to the recommendations of Albesa I. et al[13].

Anti-interferon activity in the isolated cultures was studied by O.V. Bukharin and V.Y. Sokolov in association with antibacterial effect of leucocytal human interferon drug [14].

Anticomplementary activity was evaluated by the method of O.V. Brudastovet al[15].

Results

The results of the present study showed that 80% of enterobacteriosis cases in small ruminants were diagnosed as associated diseases and epizootically appeared on a wide territory in the Republic[9]. Their role and place in the formation of nosologic profile of the infectious pathology is quite significant[16].

During the years of screening, small ruminants, morbidity rate varied from 0.01% to 7.3% at serratiosis, from 0.05% to 11.5% at colibacillosis, from 0.01% to 3.7% at enterobacteriosis. Lamb mortality rate during these years varied from 0.02% to 2.2%, from 0.2% to 6.2%, from 0.01% to 1.5%, respectively. The present study allowed the authors to identify the peculiarities of serratiosis, colibacillosis and enterobacteriosis disease development in small ruminants on the territory of the Chechen Republic.

The study of this pathology annual dynamics allowed the authors to identify significant seasonal dependence in lamb morbidity rate. Thus, in February-March enterobacteriosis morbidity rate varied from 0.055 to 0.1%, decreased during the following months and increased again in June to 0.05%. Consequently, seasonal epizootological changes in these diseases are characterized by infection rate increase in the end of summer and in autumn and infection rate increase at the end of winter and in spring.

These bacterial pathologies in small ruminants have the following symptoms: appetite decrease, fatigue, low mobility, permanent diarrhea, decrease of life mass gain in comparison with healthy animals, hyperthermia, heart rate increase and tachycardia, unfavorable outcome rate during the first days of disease reached 60%.

Flashness of animal corpses was below the average, skin and wool around anal orifice, on the tail, hips and back abdominal part were covered with liquid feces, mucous membranes had signs of anemia. Pathological study of fallen animals showed peculiar changes in the intestine and parenchymatous organs, which were primarily observed in abdominal cavity: forth stomach mucous was irritated, tremellose infiltrated with hemorrhages. Mesenterium lymphatic vessels were enlarged, often hyperemic, fleshy on section cuts. Serosal abdominal layers often contained hemorrhages.

Splinter was slightly enlarged without significant alterations in most cases. Liver and kidneys had signs of anemia, often with hemorrhages under their coats, gall-bladder was filled with dark-green bile. Bladder mucous layer did not have significant changes in rare cases it was hyperemic.

Intestine content was rare, mixed with mucus and was red because of blood presence (catarrhal hemorrhagic colitis).Mucous membranes of large and small intestine were swollen, covered with mucus, hyperemic and often with hemorrhages. Mucous intestine layer had signs of necrotic colitis and proctitis localized in different parts of intestine in different animals. In most cases blindgut was filled with hydrous content and was inflated because of gases (meteorism)[17]. Thoracic cavity contained hemorrhages under serous membranes, significant deviations from the norm were not observed[18].

Enterobacteriosis was diagnosed based on bacteriologic tests performed in bacteriologic laboratory.

A lot bacterial cultures from the *Enterobacteriaceae* family were isolated from infected animals. Among them there were 190 cultures of *Escherichia coli* species, 124 cultures of *Enterobacter cloacae*, 74 cultures of *Morganella morganii*, 51 cultures of *Citrobacter freundii* and 29 cultures of *Serratia marcescens*. These microorganisms had a wide range of pathogenic factors: antilysozymic, anticompiementary, antiinterferon, adhesive, hemolytic activity and multiple resistance to antibiotics[19].

Thus, the studied microorganisms antilysozymic activity varied from 43.5% in representatives of genus Enterobacter to 86.2% in ygenus Serratia bacteria.

Antiinterferon activity was minimum 28.4% in genus Morganella and maximum in genus Escherichia representatives. Minimal number of cultures with anticomplementary activity was among representatives of genus Enterobacter and maximum number of strains with this feature was in genus Enterobacter.[17]

The study of goat erythrocytes agglutinative capability in the presence of D-mannose showed that

this feature was present in all the species of the studied enterobacteria. However, in different species this feature was present at different rate. Hemagglutinating capability was quite expressed in 94.7% of Escherichia strains. The identified hemagglutinins in the studied Escherichia strains had different sensitivity to mannose. Mannose resistant hemagglutinins to goat erythrocytes were identified in 73 Escherichia strains (39.2%). 24 out of 29 Serratia strains had hemagglutinating capability in the absence of D-mannose among Serratia, which is 82.75%. D-mannose resistant hemagglutinating activity was observed in 24 of the studied Serratia strains. The lowest number of strains with hemagglutinating activity (42 out of 124 (33.84%)) was identified among Enterobacter cloacae cultures. Among the studied Morganellastrains hemagglutinating activity was observed in 58.9% of cultures. The studied Morganella cultures produced mannose resistant hemagglutinins more often (63.64%) than mannose sensitive ones (36.4%). Goat erythrocyte agglutinating activity was identified in 180 out of 190 (94.7%) Escherichia strains. Hemagglutinins, identified in Escherichia strains, had different sensitivity to mannose. Thus, human erythrocyte agglutination was inhibited by mannose in 84 cultures (44.7%), and mannose resistant hemagglutinins were identified in 68 strains of the studied bacteria. Goat erythrocyte mannose resistant hemagglutinins were observed in 73 strains (39.2%).[18]

Among the studied cultures of microorganisms, the lowest amount of hemolytically active strains (16.1%) were found in genus Enterobacter and the highest amount of representatives with this feature was found in genus Citrobacter (58.8%).

All the studied cultures had sensitivity to 9 groups of antibiotics: group I – β-lactam antibiotics of penicillin group, group II – β-lactam antibiotics of cephalosporin group, group III – tetracyclines, group IV – aminoglycosides, group V –macrolides, group VI – rifamycins, group VII– glycopeptides, group VIII– nitrobenzenes (laevomycetin group), group IX – polypeptides (polymyxins).

The studied Escherichia strains showed higher resistance to β-lactams, tetracyclines, macrolides, rifamycinsandglycopeptides. The highest sensitivity in these cultures was to polymyxin and laevomycetin. Among Escherichia strains half of the studied cultures (47.7%) were the strains with multiply resistance to the used antibacterial drugs.[20]

Evaluation of Enterobacter cloacae cultures sensitivity to antibiotics showed that there was a number of strains with multiply resistance to drugs (45.16%). The studied Enterobacter cloacaestrains were mostly sensitive tomonomycin (81%), carbenicillin (29.0%), laevomycetin (17.8%) andneomycin (8.1%), to other antibiotics they were highly resistant.[21]

High ratio of the strains with multiple antibiotic resistance was identified among Serratia marcescens strains (68.96%).[22]

The studied Morganella cultures had absolute resistance to methicillin, streptomycin, ampicillin, benzylpenicillin, rifampicin, ristomycin, neomycin and erythromycin. Morganellas were sensitive togentamicin, kanamycin and laevomycetin. 82% of the studied Morganella cultures were multiple antibiotic resistance.

Among the studied *Citrobacter freundii*cultures, there were many strains with multiple antibiotic resistance(49.7%).

Discussion

The results of the present study allowed the authors to conclude that the disease outbreak and development in lambs was associated with both environmental factors (sharp temperature fluctuations in winter-spring seasons, traditionally high air humidity, high level of solar activity, etc. in the Republic) and uncontrolled indication of antibiotics to animals as food additives for growth stimulation, that led to appearance of strains with multiple resistance to antibiotics and high range of pathogenic features. Environmental factors are accompanied by the conditions of sheep barn housing in the Republic. This period coincides with high lamb birth rate, which leads to sheep concentration increase on the limited barn area. This, in its turn, leads to worsening of sanitary conditions in sheep farms. Feed supply and feed quality reduction (feed with insufficient amount of nutrients, vitamins, microelements, etc.) in the end of winter-spring season leads to animal immune system weakening.

Often, water and food, contaminated with pathogenic or opportunistic pathogenic bacteria, can become a source of infection because of sanitation and hygiene standards of animal keeping violation.

The obtained data indicates on widespread and unfavorable conditions of animal farm keeping in the

Republic, which makes sick and recovered animals the main source of pathologic infection.

During the analysis of epidemiologic situation in Chechen Republic, it was important not only to isolate bacteria cultures and identify their species, but also to confirm the fact that those particular cultures were the etiological agents that caused the diseases, because they had a number of pathogenic features defining their virulence. Development of labelling method, as well as identification of the isolated bacteria sensitivity to antibiotics, has great practical significance for establishing antibiotic therapy regimen for the sick animals on the territory of the Chechen republic.[13]

Conclusion

1. The results of the studies showed that enterobacteriosis of small cattle in 80% of cases occurs as associative diseases and have wide territorial boundaries of epizootic manifestations in the republic, and their role and place in the formation of the nosological profile of the infectious pathology of sheep is very significant.
2. From sick animals, we isolated a large number of bacterial cultures belonging to species of different genera of the *Enterobacteriaceae* family. Among them 190 cultures belonging to the species *Escherichia coli*, 124 cultures of *Enterobacter cloacae*, 74 cultures of *Morganella morganii*, 51 cultures of *Citrobacter freundii*, 29 cultures of *Serratia marcescens*. These microorganisms were characterized by a large set of various pathogenicity factors: antilysocyme, anticomplementary, anti-interferon activity, adhesive ability, hemolytic activity, multiple resistance to antibacterial drugs.

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The Communicative Skills Correction Technology of Children with Autistic Spectrum Disorder in the Crimea

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Abstract

The communicative skills correction technology in children with autism spectrum disorder is considered. In the Crimea centers and funds which provide support and assistance to families raising children with special developmental features, in particular autism, are being analyzed. The results of a communicative skills correction study in children with autism spectrum disorder are presented.

Keywords: *Technology, autism spectrum disorder (ASD), preschool age, communicative skills correction, health camp, Crimea.*

Introduction

In recent decades, the number of children with impaired mental development, particularly with autism spectrum disorder (ASD), has increased significantly at the national and regional levels.

«Several decades ago, there was one resident with autism spectrum disorder per 10,000 inhabitants. In 1995, there was already one person with autism per 5,000 people. In 2017, there was one person with autism spectrum disorder... the autistic spectrum is already prominent among 50 people» [1]. The earlier such people are assisted by specialists, the more likely their socialization will be. The growth dynamics of people with ASD and the need to correct their communication skills from early childhood predetermined the relevance of our study.

Among the scientists who studied the phenomenon of ASD, we can mention the works of E. S. Ivanov, E. R. Baenskaya, M. M. Liling, O. S. Nikolskaya, V. Bettelheim, U. Frith, L. Wing, who note that one of the "main violations preventing successful adaptation and socialization of an ASD child is its underdevelopment"[5]. Nevertheless, it is important to consider the technology of corrective actions and the creation of conditions for the development of communication abilities of children with ASD for their successful socialization. At the same time, the scientific literature reveals a lack of research on this problem, especially at the regional level. In this regard, the purpose of our study is to examine the correction of communication skills of children with ASD in the Crimean region and Sevastopol.

Research Methodology. Nowadays, according to children's neurologist of the Ministry of Health and Social Development, Professor A. Petrukhin, autism is among the most common disorders in Russia. The first works on the need for specially organized assistance to children with this type of dysontogenesis in Russian psychology can be considered the work by G.E. Sukhareva in 1955, which examined autism disorders in childhood schizoidia. In 2010, the main provisions of the "Special Federal State Standard for Primary Education and School System for Children with Developmental Disabilities of the Autistic Spectrum" were published. The document appeared due to the activity of O.S. Nikolskaya[9] and was prompted by the requirements of the reform of the education system in Russia.

According to Maureen Durkin, PhD in epidemiology, Professor of Public Health and Pediatrics (USA), «recent studies of the prevalence [in Russia] of eight-year-old children show that the number of cases of ASD can be 1.5% or even 2%» [4]. Among other facets of the problem in this area, apart from revealing the fact of autism in the Russian population, it should be noted that 92% of 100% of people with ASD in the US are adapted, while in Russia only 10% are adapted[3]. The third facet of the problem is that autism in adults is not recognized in Russia. Without knowing how many children in Russia

have autism spectrum disorders, it is impossible to build a literate system of state assistance to these children in health care, education, and social protection.

Modern scientists believe that ASD does not depend on a person's ethnopsychological peculiarities, social conditions of his life, his material well-being. At the heart of a child's adaptation in the process of socialization to the requirements of life in society is the degree of development of his or her communication abilities. «The main characteristic of the disorder is the permanent deficiency in social communication and social interaction. The main deficit in people with autism spectrum disorder is the skills of divided attention and reciprocity in contact»[2].

Due to the lack of social contacts in children with ASD, the emergence of difficulties in communicating with their loved ones and the world around them, an increase in primary lack of communication skills is formed due to the effect of «snowball». The speech function, the holistic picture of the world, and the sense of meaning are formed in a distorted way. «Echolalia, blurred speech, chanting pronunciation, speech with shorthand, a peculiar voice intonation with a predominance of high tonality, long naming of oneself in the second and third persons, absence of words in expressive speech that designate people close to the child, autostimulations realized in citing poems, songs, quotations, etc.».[6].

T. Grandin and M. Scariano note that such a child is limited by the fact that he can only build his relationship with the world around him through an adult. «Having lost contact with their emotional donor, translator, and arranger of meanings around them, such a child stops developing and can regress»[8]. By the period of enrollment in school, children with ASD show "a pronounced cognitive deficit, non-formation of arbitrary forms of activity, specificity of perception, thinking, and speech"[7], "in expressive speech, there is a predominance of a nominal vocabulary over the predicative one" [3].

The most competent assistance can be provided to the child in special centers for correction of communication skills disorders. In Russia, the First Federal Resource Center for Supporting Children with ASD and their Families was opened in 2016. Its main goal is to develop a system of comprehensive assistance to children with autism spectrum disorders.

On the other hand, there is the Regional Center for Autism, a unique school in St. Petersburg, where parents from all over Russia bring their children. The peculiarity of this Center is that it already works with speaking, adult children, who are prepared for the stage of self-determination. However, ASD children from preschool age who are not yet speaking cannot determine their interaction with the surrounding reality, and are not given due attention.

There are no specialized centers for developing communication skills for children with ASD in remote towns. All this creates a need to open new centers to improve the current situation. When searching for a location for a specialized center for developing communication skills for children with ASD, in our opinion, it is necessary to take into account whether there is a need for such a center in the region or whether the center already exists. On the other hand, it is important to take into account the climatic conditions, which should be aimed at creating favorable conditions that would enhance the possibilities of the corrective programs used.

Research results and Discussion. In this regard, let us consider the opportunities that the Crimean peninsula offers to solve the problem. Here, thanks to climatic conditions are enriched both physical and mental, and social health, which for a child with ASD, primarily manifested in the mastery of speech skills and basic communication. Crimea as a natural object provides the possibility of using complex therapy, parts of which are climatotherapy, landscape therapy, and aromatherapy. In a combination of mountain and sea air, phytoncides of medicinal plants and herbs: aromas of juniper, pine, arborvitae, lavender, sage, lemon wormwood, health improvement of spirit and body of the child, its hardening, which in turn leads to the strengthening of all components of health. Healing air, warm sea, the unique beauty of nature has a beneficial effect on the emerging body of the child: the child feels comfortable and has positive emotions; the result is a successful consolidation of skills learned by a specially designed program.

Now in Sevastopol, just over 100 people with ASD have been identified. But, according to the head of the organization «Special Children» A. Menanova, they «do not receive the quality assistance they need»[10].

Starting from 2017, Crimea began to open centers to work with children with various developmental features: the M. Lynskayacenter[11] and the centers of

"Territory of Speech" in Simferopol and Sevastopol. The latter's work is based on the method of T. Gruzinova and E. Gurina and is aimed at children with communicative disorders ranging from lack of speech to speech delays of various genesis (sensomotoralalia, autism spectrum disorders, dysarthric disorders), and includes three areas: ABA-therapy (Applied Behavioral Analysis), individual work with a neuropsychologist (neurogymnastics), game speech therapy. A distinctive feature of the centers is the use of author's innovative technologies contributing to the development of communication in children with ASD.

Our research was carried out on the basis of a speech therapy center «Territory of Speech» in the Crimea at the summer health camp «We're together». During the life of the student in the camp is communicative intensity (14 days). Group sessions are held in groups of up to 6 people with children 5-6 years old, the purpose of which is to acquire and develop skills to interact with peers in a safe environment. In individual sessions, a complex interaction with the following specialists is used: a speech therapist to practice speech skills; a neuropsychologist who performs neurogymnastics to restore normal brain function in a child; and an ABA-therapist.

During the two weeks of stay in the camp, the whole range of activities allowed to intensify mental processes, contributing to the development of communication skills of children of preschool age. Specially organized «parenting» events were held daily for parents.

Meetings» under the guidance of a psychologist consultant, as well as «Family Soft School» for parents and children by the method of E. Glushkova. The basis of the school's technology was the use of game exercises that develop flexibility, plasticity of the child, his or her ability to master his or her body and be active, perceptive, and adequate in any situation.

The peculiarities of the site where the psychocorrection was carried out required its creative improvement, as a result of which we developed and implemented the author's technological elements of the psychocorrection program for the development of communicative skills of preschool children with PAC. The first block of the program consisted of individual lessons with a game speech therapist according to the method of T. Gruzinova; the second block consisted of creative elements of the psychologically corrective program developed by us:

– Group lessons to develop communicative interaction;

It took two weeks, two hours a day. The following materials were used: pyramid, peg top, bubbles, balloons, chalk board, cards, napkins, grains, water. The sequence of corrective actions in the lessons is given below.

1. Children sit in a circle. Behind each child sits a tutor. The whole process is managed by the leader of the communication group.
2. The leader of the group gives the following instructions: "Who should I give the bubbles to?" (or any other kind of material).
3. The leader looks carefully at which of the children took the initiative in the form of a gesture request, a vocal request. If the children cannot ask for it themselves, the tutor does it for the child with a physical prompt.
4. The child who has asked the presenter is given an object for manipulation.
5. All children command the child by name (for example: the presenter gave N bubbles): N, "blow". Execution of the command is based on the capabilities of the child: if the child can not address the N by name and does not look at him, the tutor helps the child by turning his head and pointing to the N.

If the child does not speak or imitate a gesture, the tutor helps him with a physical clue. This happens in the following way: the tutor takes the child's hand, puts it on his lips and makes a verbal gesture "blow". As time goes by, the hint becomes weakened and, as a result, the child can give instructions to the participant on how to communicate. Each child has the opportunity to manipulate any presented leading object.

At the end of the communication group training, each participant is trained to give instructions to the other and perform them independently;

- Yard games - bodily, visual, audio communication with the purpose of

"See me, notice me." Children in individual games learned to see each other, notice each other and interact with each other through different channels of perception. This is the direction of psychocorrective work combined and developed all aspects of communication. An example of author's yard games is presented in Table 1.

Table 1: Examples of copyright pickup games

Game Title	Accompaniment	Game participants	Actions of the game participants
Playhouses and cards	The active melody in major tone is switched on.	1. Group Leader 2. Tutors 3. Kids.	The presenter places the playhouses of four basic colors (green, yellow, and red, blue) in any part of the room. While the music is playing, the children run in a chaotic direction. When the music stops, the presenter raises a card of any color and gives instructions: find a playhouse like this. Each participant independently or with the help of a tutor finds a playhouse of the corresponding color.
Flower Bees	The active melody in major tone is switched on.	1. Group Leader 2. Tutors 3. Kids.	The presenter distributes hats with the image of bees to all children, and then spreads flowers sewn from fabric on the lawn (fabric of green color). Flowers have the same appearance and are equal to the number of children. While the music is playing, children run in a chaotic direction. When the music stops, each child finds a free flower for himself or herself or with the help of a tutor and becomes on it. Then the game can become more complicated depending on the possibilities of the group as a whole. The instruction is added: Remember, where your flower is, and when the music is over, find it.

- Creative workshop.

The creative workshop implied the development of small and large motors, the development of skills for independent creative activity. Creative workshops based on the elements of ART-therapy were conducted as follows. Every day the theme was chosen from what the children saw with their own eyes in the correctional camp: mountains, rocks, sea, plants. Then the trainees took seats at the table next to the tutor and tried to display what they saw with a brush on canvas or a plastic on a board.

Each learner gave out materials to others. For example, if he gave out child N, he would go with a piece of paper, and all the other students, according to their abilities, would ask for a piece of paper for themselves. Someone would do it vocally, someone would gesture, bending their palm. If the child did not have speech skills at all and could not ask with a gesture, the tutor gave a physical clue. Thus, the trainees gave out all the materials to each other, and they communicated based on their communication skills: vocally or by gestures, independently or with a physical clue. An important element of this communication was eye to eye contact. «Eyes» followed by the presenter and tutor. After all the materials had been distributed to the student, the presenter gave the instruction: «cut» and showed the verb with a gesture (he collected his index and middle finger, depicting scissors). During the instruction, the tutors from time to time reminded the child to 'cut' vocally and with a gesture to reinforce the skill and automate it.

Every day the creative workshop ended with a finished work, which was hung in a special room for creativity. All children had an opportunity to come to the hall, find their drawing and show their parents the element of their creativity. An opportunity was provided to consolidate the learning process with positive emotions: a manifestation of joy from the results of their creative activity and the creative work done.

In general, children from families that participated in the work of the «We are together» remedial camp received positive results, as shown in table 2.

Table 2: Results of children with ASD who underwent «intensive» summer camp in Crimea

Name	Age	Diagnosis	Pre-intensive course performance	After-intensive course performance (new elements)
1	5 years 2 months	ASD	Requests:0 Indicative gesture:- Sounds arbitrary:- Sounds involuntary: P,B. Words:-	Requests:1 (give - gesture) Indicative gesture:+
2	5 years 9 months	ASD	Requests:0 Indicative gesture:- Sounds arbitrary:- Sounds involuntary: P,B. Words:-	Requests:1 (give, open - gesture) Indicative gesture: +
3	6 years	ASD	Requests: 1 (give – vocally) Indicative gesture:- Sounds arbitrary: “Papa” (Dad) Sounds involuntary: B,D. Words:-	Requests: 3 (open, close, blow - with gestures).
4	6 years 2 months	ASD	Requests:0 Indicative gesture:- Sounds arbitrary:- Sounds involuntary: P,B. Words:-	Requests:2 (give, water - in gestures). Indicative gesture:+
5	5 years 7 months	ASD	Requests: 1 (give – gesture) Indicative gesture: + Sounds arbitrary:- Sounds involuntary: H,P. Words:-	Requests: 1 (give - vocally)
6	5 years 10 months	ASD	Requests:0 Indicative gesture:- Sounds arbitrary: Ba Sounds involuntary: P. Words:-	Requests:1 (give - gesture) Indicative gesture:+ Sounds arbitrary: P
7	5 years 6 months	ASD	Requests:0 Indicative gesture:- Sounds arbitrary: Vi Sounds involuntary: Di. Words:-	Requests:1 (give - gesture + vocal) Indicative gesture: + Sounds arbitrary: D.
8	5 years 4 months	ASD	Requests: 2 (give, open – gesture) Indicative gesture: + Sounds arbitrary: - Sounds involuntary: Pa. Words:-	Requests: 2 (give, open -vocally)
9	5 years 8 months	ASD	Requests: 0 Indicative gesture: + Sounds arbitrary: Ma Sounds involuntary: P,D. Words: Mama (Mother)	Requests:1 (give - gesture + vocal) Sounds arbitrary: P, D

Name	Age	Diagnosis	Pre-intensive course performance	After-intensive course performance (new elements)
10	6 years 2 months	ASD	Requests: 0 Indicative gesture:- Sounds arbitrary: P Sounds involuntary: B. Words:-	Requests:1 (give – gesture) Indicative gesture: +
11	5 years 9 months	ASD	Requests: 1 (open – vocally "aka") Indicative gesture: + Sounds arbitrary: M Sounds involuntary: D. Words:-	Requests: 1 (open vocally "aka + gesture")
12	6 years	ASD	Requests: 0 Indicative gesture: + Sounds arbitrary: Mi Sounds involuntary: Ti. Words:-	Requests: 2 (open, give - gesture)
13	5 years 11 months	ASD	Requests: 1 (give – vocally) Indicative gesture: + Sounds arbitrary: Give Sounds involuntary: P,B. Words:-	Requests:1 (give - vocal + gesture) Sounds arbitrary: P, B
14	5 years 10 months	ASD	Requests: 0 Indicative gesture:- Sounds arbitrary:- Sounds involuntary: M,P. Words:-	Requests:2 (give, water - gesture) Sounds arbitrary: M, P
15	5 years 6 months	ASD	Requests: 1 Vkluchi (Turn on) Indicative gesture: + Sounds arbitrary: Ku, Chi, Chu Sounds involuntary: P,B. Words:-	Requests:2 (give - vocal, blow - gesture) Sounds arbitrary: P,B

The data in Table 2 are the result of a comprehensive impact on communication skills development for children with ASD.

Conclusion

Due to a properly organized and implemented program, each child has received positive results, which will later be consolidated to automatism and applied in life. So, as a result of the educational development program in the summer camp all its participants, children with ASD who came with their families from different regions of Russia, achieved results. Let us cite the parents' opinion about the summer camp program: "We are happy: the impossible has turned out to be real". Of course, it is necessary to have more such centers so that all families with children with ASD could, with the help of the correctional programs of these centers, develop their children and improve their worldview, peace and harmony of families.

It is envisaged to continue further research in order to improve the technology considered and to create conditions for children with ASD to achieve results for everyone.

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Indices Changes of Central Hemodynamics at Carry Out Anesthesia with Keeping Independent Breathing at Children

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Abstract

Introduction: The aim of study was to research hemodynamic shifts in conditions of carry out combined anesthesia on the base of isoflurane with preservation of spontaneous breathing at small surgical operations in children.

Materials and Method: The research was carried out at 44 sick children at the age from 3 to 14 years with inguinal and inguinal hernias (41,7 %), hydrocele was (19,4 %) section of abscesses and suppurations were (38,9%). The duration of anesthesia benefit at 58,3 % patients was from 30 to 40 minutes, at 40,66 % it was about 60 minutes.

Results: The clinical monitoring was performed with study AP, HR and oxygen. For objective evaluation of cardiovascular functional condition, the echocardiography way was use. The first group patients were introduced intravenously the ketamine solution in doze 3 mgr/kg of body weight, propofole in dose 2 mgr/kg of body weight and izoflurane up to 3 ob % in 30-40 minutes after premedication. The second group patients were introduced intravenously the ketamine solution in dose 4-5 mgr/body weight and sibazone in dose 0,4 mgr/kg.

Conclusion: Complex clinical study and taken results of hemodynamic parameters showed the efficiency of carried out anesthesia with safety of independent breathing at small surgical operations at children.

Keywords: *Combined ànesthesia, small surgical operations, propofole, ketamine, sibazone, izoflurane.*

Introduction

Any operation, even small surgical intervention[2] or diagnostic manipulations are multicomponent stress which may be incompatible[3] with the life of many people[5].

The small surgical operations are more often performed in conditions of general anesthesia at children. The given necessity was proved by physiological peculiarities of child's organism, keeping safety of psychoemotial sphere, age features and severity of trauma. But these drugs in recommended doses often give the series negative effects including: suppression of independent breathing, and, the reducing amount of dose leads to non-adequate anesthesia[6]. The choice of anesthetics or their combinations depended on patient's

state, presence severe accompanied diseases, mainly, from the side of cardiovascular system, and also from pharmacodynamics and pharmacokinetic properties of drugs[7].

For solving the given problems some authors[1] offer to use several drugs of different action direction which allow to reduce their doses, and, at the same time to save the demanded level[4] of anesthesia[6].

Nowadays at carry out series small surgical operations in children the special place takes anesthetics, they are propofole and ketamine, which in combination with inhalation anesthesia isoflurane are matching with their pharmacological actions. These combinations of drugs have definite advantages of safe independent breathing[2].

In child's anesthesiology the increase dose and concentration of using drugs lead to depression contractive ability of myocardium[3], considerable changes in hemodynamic indices, which may lead to severe, and, in some cases to irreversible complications at patients with sharply reduced reserves of adaptation[8]. All the above mentioned have the definite actuality of searches more secure ways of anesthesia with safe independent breathing at children.

The aim of study is to research hemodynamic shifts in conditions of taking combined anesthesia on the base of izoflurane with keeping spontaneous breathing at small surgical operations at children.

Materials and Method

The study was carried out in 44 sick children at the age from 3 to 14 years with inguinal and scrotum hernias (41,7 %), hydrocele (19,4%) and at section of abscesses and suppurations.

The premedication was directed to remove vagal reflexes, anxiety, prevention of nausea and vomiting. The duration of anesthetic benefit at 58,3 % patients was to 30 minutes, at 40,66% persons to 60 minutes. Complex clinical study and taken physiological indices such as statistic parameters of cardiac rhythm allowed to evaluate objectively the level of functional shifts in child's organism at presence pain syndrome, which was necessary condition for choice adequate and effective anesthesia.

The patients' examinations were carried out at the following stages: before operation (outcome), after premedication, at introducing narcosis, at maintain period, at waking up time.

For objective evaluation of functional state for cardiovascular system the complex of bloodless electrophysiological ways of blood flow study were used. With this purpose the echocardiography way with analysis of final-diastolic size(FDS), final-systolic size(FSS) of left ventricle, R-R interval and expulsion time were used. The study was carried out on echocardiograph "Interscan 250" (Germany) with sensor 3,5 MHz. The systolic, diastolic and middle arterial pressures were measured by oscillographic method. With the purpose of more detailed and exact estimation the functions of left parts in heart with the help of computer analysis EchoCG the next indices of myocardium contraction ability and diastolic function of left ventricle: cardiac index (CI), beat index (BI), fraction of expulsion (FE), fraction of shortening (FS), specific peripheral resistance (SPR) were carried out.

The control after HR (heart rate) and AP (arterial pressure) were constantly carried out with monitor "Life Scope" (Japan). The index of oxygen saturation was determined with the method of pulse oxymetry.

During premedication the atropine sulfate 0,1 % - 0,01 mgr/kg body weight, dimedrol 1 % solution 0,1 mgr/kg body weight were prescribed, at open repositions for premedication enhancement: 0,5% solution of sibazone in dose 0,25 mgr/kg body weight and 5% ketamine solution in dose 2,5 mgr/kg body weight were additionally administered.

The patients of the first (main) group in 30 – 40 minutes after premedication ketamine solution in dose 3 mgr/kg body weight and propofole in dose 2 mgr/kg body weight and iziflurane inhalation to 3 ob% were introduced. The patients of the second group the ketamine solution in dose 4 – 5 mgr/kg body weight and sibazone in dose 0,4 mgr/kg were intravenously introduced. The maintain doses of drugs composed ½ part from the main ones.

Statistic processing of quantity indices, being taken at the result of special electrophysiological researches was carried out with the help of mathematical statistics. For statistically true results differences were taken only those meanings of "P" which were found in Student table and were less than 0,05. The factual material was given in relative meanings for leveling of age differences for homeostasis on the stage of study (initial data were

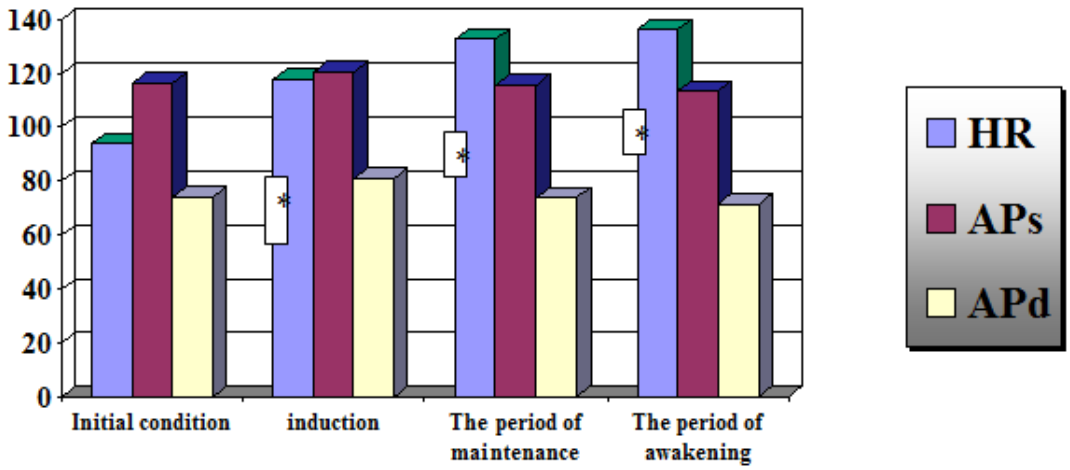
taken for 100%). The authenticity was calculated on the ratio to initial and previous stage of study.

Results

On the stage of introduction narcosis, the loss of consciousness became on the background of intravenous propofole introduction in dose 3 mgr/kg intravenously. After introduction ketamine the insignificant AP (arterial pressure) increase was marked on 5,5% from the level of initial one. RRM (respiration rate movement) was 22 – 36 times per minute. At performing operation more than 30 minutes the patients were intravenously introduced the second dose of ketamine and propofole composing 2/3 part of the main dose. Inhalation with izoflurane was to 1,5 ob%. In 40-50 minutes after carry out operation the patients answered questions in adequate way, orientated themselves in time and space, they recover consciousness in 1,5 hours after performing operation.

The indices of HR (heart rate), AP (arterial pressure) and oxygen saturation ($sP0_2$) at introduction period of anesthesia with use ketamine and propofole were characterized with stability. Smooth falling asleep was marked during 30-40 seconds after bolus introduction propofole, moreover, the disorder of breathing wasn't marked, skin coverings were warm and had usual color. The cornea was moist and shining, cornea reflex was moderately reduced, the eyeballs made swimming movements and they were fixed centrally. The pupils were moderately constricted with safe the reaction to light. The HR (heart rate) increased on 6,36%, there was tendency to increase APc on 3,2% and APd on 2,9% in comparison with their initial data. At introduction propopole in dose 3 mgr/kg the hypotension was observed to 5% from initial index only in 10% cases of observations. In other cases, AP wasn't changed, may be, in connection with ketamine action to increase AP. Thus, ketamine corrected propofole action and reduced hypotensive effect of propofole.

The waking up period was not long and continued 30-40 minutes it was depended on general dose, time of introduction the last sodium oxibutirate dose and individual features of patients. The extubation of trachea was performed at appearance adequate spontaneous breathing. The skin coverings were rose and warm, the vision, larynx, pharynx, coughing reflexes and moving activity were fast restored. APc reduced to 2,86%, APd to 3,49% in comparison with initial data.



* Authenticity of indices differences in comparison with primary ones (P<0,05)

Figure 1. Changes of indices APc, APd и HR at carry out combined anesthesia with ketamine and propofole

At use ketamine in combination with sibazone at children the fast and smooth induction was observed, moreover, depending on dose in that or another level the cardio-respiratory effect as decrease of arterial pressure and reducing breathing, sometimes apnea at fast introduction of drugs, were present. Together with it the sibazone doses for induction anesthesia and its maintain are various depending on age and way of induction (induction dose is from 3 to 5 mgr/kg weight).

Discussion

At combined anesthesia with use ketamine and sibazone the smooth falling asleep of patients was marked during 30 – 60 seconds after bolus introduction of ketamine in dose 5 mgr/kg and sibazone in dose from 3 – 5 mgr/kg body weight, moreover, the disorder of breathing wasn't marked, the skin coverings were warm and had usual color. It was marked the increasing HR on 8,24%, decreasing APc on 6,68% и APd on 4,76% in comparison with initial data (tabl.2).

After introduction calculated dose of ketamine and sibazone the sedative effect was observed. Children began falling asleep and didn't react upon external sounds. The eyeballs made slow swimming movements, the pupils were constricted, their alive reaction to light was saved, the cornea was moist and shining, corneal reflexes were saved. The relaxation of muscular tonus was marked. The respiration rate decreased to 2-3 cycles per minute, moreover, its depth wasn't changed. The tendon and skin reflexes, warm, tactile and pain sensitivity were decreased. In 2-3 minutes was the loss of consciousness. The expressed changes of AP weren't marked either. After introduction ketamine in dose 4-5 mgr/kg body weight, the pupils were sharply constricted with preservation weak reaction to light. The reflexes were depressed. The breathing was smooth and deep. At introduction period of anesthesia, the increase APc index was marked on 7,98% and HR on 32,68% (P<0,05) in comparison with their initial data. At the period of maintain anesthesia the clinical displays were characterized with stability of general symptoms, being marked at the period of introducing narcosis. At the most traumatic moments of operation the researching indices were unauthentic measured.

The results of hemodynamic researches at children at carry out combined ahesthesia with ketamine and propofole are given in table 1. In comparison with initial data on premedication background the increase HR was on 25,27% and specific peripheral resistance (SPR) was on 24,52%. Other indices were little changed. At introduction period of anesthesia, the decrease of CI was on 10,64%, increase of HR was on 15,46% and SPR was on 25,58%. The indices BI, SAP, CI were changed a little in comparison with previous period. During the anesthesia maintain the definite indices changes of central hemodynamics were also observed. Thus, at the most traumatic operation stages in comparison with previous period the tendency to decrease indices CI on 2,38%, BI on 2,97% and SPR on 7,55%, moreover, HR increased on 12,52%.

Table 1. Indices of central hemodynamics in children at carry out combined anesthesia with ketamine, propofole and izoflurane

Indices	Outcome	Premedication	Introduction period	Traumatic stage of operation	Final stage of operation
BI ml/m^2	41,54±1,22	40,49±3,15	41,74±2,01	40,5±3,68	39,63±4,19
SAP mm.m.c.	88,85±1,21	91,2±2,93	94,22±3,75	87,81±4,0	85,44±2,56
HR min^{-1}	94,0±2,38	117,75±2,87*	117,93±3,93*	132,7±8,41*	136,01±8,45*
CI l/min x m^2	4,7±0,4	5,0±0,5	4,2±0,2	4,1±0,4	4,0±0,4
SPR con.un.	40,42±5,31	50,33±7,74	50,76±5,85	46,93±5,17	48,16±7,56

Note: * Authenticity of different indices in comparison with initial meaning (P<0,05).

The data on analogical researches of central hemodynamics at carry out combined anesthesia with ketamine and sibazone are given in table 2.

Table 2. Indices of central hemodynamics in children at carry out anesthesia with ketamine and sibazone

Indices	Outcome	Premedication	Introduction period	Traumatic stage of operation	Final stage of operation
BI ml/m^2	44,86±0,79	51,12±2,14*	49,08±1,91	50,34±2,15*	53,54±6,91
SAP mm. m.c	72,42±2,27	77,03±2,29	77,03±2,4	75,97±2,78	73,03±2,06

HR min^{-1}	92,14±2,09	125,26±3,07*	122,25±2,13*	130,52±5,23*	143,75±7,93*
CI l/min. x m^2	4,83±0,35	5,11±0,21	4,91±0,19	6,63±0,48***	8,03±1,39*
SPR con.un.	71,18±4,74	88,1±10,6	82,24±8,69	83,72±10,6	72,88±4,87

Note: *Authenticity of different indices in comparison with initial meaning (P<0,05). ** authenticity of different indices in comparison with previous stage of study (P<0,05). *** authenticity of different indices in comparison with initial and previous stages of study (P<0,05)

At introduction period of anesthesia, the increase of HR on 32,68% (P<0,05), SPR on 23,77%, BI on 9,41% in relation to their initial meanings were observed. At the most traumatic stages of operation the increase of CI, HR, SI accordingly to 37,27%, 41,65% and 12,22% (P<0,05), and, in comparison with previous stage of study these indices were changed unauthentically. At the end of operation in comparison with the stage of traumatic moment for operation the indices of central hemodynamics were changed a little., which had unauthentical character.

At all main stages in two groups of combined anesthesia the increase SpO_2 index was marked.

Thus, the study clinical symptomatology and noninvasive hemodynamic indices allowed to state that the course of given kinds of anesthesia was accompanied minimum hemodynamic shifts that witnessed on adequacy of anesthesia course.

Conclusion

1. Combined anesthesia with use little doses of ketamine, propofole in combination with inhalation izoflurane at small surgical operations at children was characterized with smooth clinical course, fast waking up from narcosis.
2. In condition of carry out anesthetic benefit with use ketamine, propofole and isoflurane the moderate changes of main indices in central hemodynamics without decrease of the heart performance were marked in children.
3. Use combined anesthesia with ketamine, propofole in combination with izoflurane is optimum method of anesthesia and safe independent breathing at carry out small surgical operation in children.

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Consent: Written informed consent was obtained from all participants' parents of the research for publication of this paper and any accompanying information related to this study. A copy of the written consent is available for review by the authors.

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Current View of the Problem: A New Approach to Covid-19 Treatment

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Abstract

Coronavirus infection COVID-19 (acronym for *Corona Virus Disease 2019*), formerly Coronavirus infection 2019-nCoV is a potentially severe acute respiratory infection caused by SARS-CoV-2. It is a dangerous disease that can take the form of an acute respiratory viral infection of the lung or a severe form, the specific complications of which may include viral pneumonia leading to acute respiratory distress syndrome or respiratory failure with a risk of death.

Keywords: *Melatonin, cell, production, infection, treatment*

Introduction

The disease is caused by a new virus, people have no acquired immunity to it, people of all age categories are susceptible to infection[1]. The virus is spread by airborne droplets through inhalation of sprayed droplets with the virus by coughing or sneezing, as well as through the virus getting on the surface and then getting into the eyes, nose or mouth. Effective preventive measures include frequent hand washing and observance of respiratory hygiene rules[2].

In about 15% of cases, the disease is severe with the need for oxygen therapy, and in 5% more patients are critical. Globally, as of 01 April, the mortality rate is estimated at around 5%. According to the analysis of data for 1099 patients as of February 28, 2020, 91.1% of patients with COVID-19 were diagnosed with pneumonia[3]. Figures may change over time.

On 11 March, the spread of the virus was declared a pandemic[4]. The epidemic is the first ever verifiable pandemic in human history[5]. It makes sense for Governments to prepare lists of trained personnel who can take control of the situation, as well as lists of medicines[6], personal protective equipment, supplies and equipment needed for treatment[7]. World Health Organization (WHO) encourages countries to train hospitals, protect health workers and decide whether some form of social distancing is necessary[8].

In response to the epidemic, the World Health Organization (WHO) has declared a public health emergency of international concern, and risks at the global level are assessed as very high[9]. The situation is developing rapidly, with a daily increase in the number of cases and deaths. Various scientific and clinical studies are being conducted[10].

The 2019-nCoV strain was first detected in December 2019 as a result of nucleic acid analysis in a patient with pneumonia[11].

Since the nucleic acid content is highest in the cell nucleus, it was named after the Latin word “nucleus” (“cell nucleus”, Latin).[12] However, nucleic acids are not only found in the nucleus, where they are definitely the most, but also in chloroplastics and mitochondria[13]. DNA and RNA nucleic acids are present in cells of all living organisms and perform the most important functions for storage, transfer and realization of hereditary information[14].

The coronavirus infection COVID-19 enters deeply into the cells to the level of the nucleus[15]. Once the virus (or its nucleic acid) penetrates the cell, the cell resources are switched to the synthesis of viral nucleic acid and proteins[16]. The genes are expressed. Gene expression is the process by which genetic information from a gene (DNA nucleotide sequence) is transformed into a functional product - RNA or protein.[17]

Some stages of gene expression can be regulated: transcription, translation, RNA splicing and the stage of posttranslational modification of proteins.[18] The process of activating gene expression in short double-stranded RNA is called RNA activation.[19]

When the cellular resources are exhausted, its shell is torn and ready viral particles are poured out of it.[20] This way, other cells are affected. Fibrous tissue is formed in the place of the bloated cell.[21]

Expression of genes is a substrate for evolutionary changes, as control over time; place and quantitative characteristics of the expression of one gene may have an impact on the functions of other genes in the body as a whole.[22] This is called a gene mutation. [22,23]

There are no specific antiviral treatments or prophylaxis against the disease yet. Since, to the level of the nucleus is not yet reached any antiviral drug.[24]

In most cases (about 80%), no specific treatment is required and recovery takes place on its own.[25] Severe forms of disease are more likely to develop in older people and in people with certain diseases, including asthma, diabetes and heart disease. In severe cases, means are used to maintain the functions of vital organs[26].

Today we want to tell you about melatonin, the "sleep hormone".

Melatonin is a hormone that is naturally secreted by our body and whose functions are crucial for our physical and mental well-being.[27] It is closely linked to the control of circadian rhythms, our biological clock, and thus helps us sleep better.[28]

Among other things, this substance is involved in neuroendocrine, neurophysiological and immune processes, which are necessary for many vital tasks of the body.[29] However, its production takes place only at night and, if something prevents it, a person has trouble sleeping and a good rest.[30]

Melatonin hormone was discovered in 1958 by A.B.Lerner. It is the main hormone of epiphysis - the organ that transmits information about the light regime of the environment into the internal environment of the body.[31] Changes in melatonin concentration have a noticeable daily rhythm - usually a high level of the hormone during the night and low levels during the day.[32] Maximum concentrations of melatonin in human blood are observed between midnight and 5 am local sunshine time. Melatonin is produced by the main secretory cells of epiphysis - pinealocytes.[33]

The biological half-life time of melatonin is 45 minutes. This means that for research purposes, blood samples must be collected at short intervals in order to determine the period of melatonin production.[34] In addition, a patient's sleep disturbance during the night in order to collect samples may affect the blood melatonin levels. These problems can be avoided by determining melatonin metabolite levels: melatonin sulfate (6-sulfatoxymelatonin) and 6-hydroxyglucuronide in urine. 80-90% of melatonin is secreted to the urine as melatonin sulfate. The concentration of melatonin sulfate in the urine correlates well with the total level of melatonin in the blood during the collection period. [35]

Melatonin synthesized in epiphysis enters the blood and cerebrospinal fluid, the liquor, through which it accumulates in the hypothalamus. In addition to blood and cerebrospinal fluid, melatonin is found in urine, saliva and amniotic fluid[36].

Melatonin is a rare example of a hormone that has both membrane and nuclear receptors[4].

In the human body melatonin is synthesized from the amino acid tryptophan, which is involved in the synthesis of neurotransmitter (neurotransmitter) serotonin, and it in turn turns into melatonin under the influence of the enzyme N-acetyltransferase.[38] Melatonin has been shown to be an indole derivative of serotonin and to be synthesized at night by the enzymes N-acetyltransferase and hydroxyindol-O-methyltransferase. About 30 μg of melatonin is synthesized in an adult during the day, its concentration in serum at night is 30 times higher than during the day, and the maximum concentration is about 2 hours of the night local sun time, on average for many observations.[39] Melatonin is transported by serum albumin, after liberation from albumin it binds to specific receptors on the membrane of target cells penetrates into the nucleus and there performs its action. Melatonin is quickly hydrolyzed in the liver and excreted with urine; the main metabolite is 6-hydroxymelatonin sulfate, the content of which allows to indirectly judging the production of melatonin epiphysis.[40]

The body also contains melatonin, which is formed outside the epiphysis. This discovery was made in 1974 by Russian scientists N.T. Reichlin and I.M. Kvetnoy, who discovered that melatonin is synthesized in cells of the worm-like intestinal process.[6] Then it turned

out that melatonin is also formed in other parts of the gastrointestinal tract, as well as in many other organs.

The secretion of melatonin is subject to the daily rhythm, which in turn determines the rhythmicity of gonadotropic effects and sexual function. Synthesis and secretion of melatonin depend on the illumination - excess light reduces its formation, and a decrease in illumination increases the synthesis and secretion of the hormone. In a person with a normal daily routine (sleep at night), melatonin accounts for approximately 70% of the daily production of melatonin. In clinical conditions, it has been established that sleep deprivation during night hours leads to a disturbance in the daily rhythm of melatonin production - production at night is reduced and approaches the daily level.[2,13]

Melatonin secretion rhythm shift also occurs when flying to other time zones.

Melatonin is also known as N-acetyl-5-methoxytryptamine and N-[2-(5-Methoxy-1H-indol-3-yl) ethyl] acetamide.

Melatonin production depends entirely on the biological clock of the human body and the change of the natural day-night cycle. Since melatonin production depends on the length of the daylight, many animals use it as a «seasonal clock». People, like animals, have *less melatonin in summer than in winter*.

So, melatonin can regulate the functions that depend on the photoperiod - reproduction, migration behavior, seasonal molting. In bird and mammal species that breed at long bottoms, melatonin suppresses gonadotropin secretion and reduces sexual activity. In animals that breed at a short light day, melatonin stimulates sexual activity. The effect of melatonin on reproductive function in humans has not been sufficiently studied. During puberty the peak (night) concentration of melatonin decreases sharply. In winter the number of menstrual cycles not ending in ovulation is on average higher than in summer. In women with pituitary amenorrhea the concentration of melatonin is reliably higher than in healthy women. These data suggest that melatonin suppresses reproductive functions in women.

Melatonin is a solid, absorbing or dissolving fat, a water-soluble substance that is a powerful *natural antioxidant*. By synthesizing it, the body is protected from the excessive formation of free radicals - metabolic products that cause rapid aging and cancer.

Melatonin has a unique ability, unlike other antioxidants, to penetrate into any cell in any part of the body and have a specific protective effect on the nucleus - the central structure of the cell containing DNA, i.e. the structure that *allows the damaged cell to recover*. Melatonin is known to have a stimulating effect on the enzyme glutathionoperoxidase, which has antioxidant properties. Melatonin may also exist in a synthetic form, which is used as an additive with sleeping pills and antioxidant properties. This becomes relevant with age when the activity of the body's internal secretion glands dies out.

Antioxidant Effect: Melatonin neutralizes the damaging effects of oxidation processes, which are the main cause of skin aging and ageing. The most important function of melatonin is its antioxidant activity, which manifests itself everywhere in the body, as melatonin penetrates all organs and tissues. The mechanism of antioxidant action is manifested in the fact that melatonin has a pronounced ability to bind free radicals, including those formed by the lipid peroxidation of hydroxyl radicals, and exogenous carcinogens, and it activates glutathione peroxidase - a factor in protecting the body from free-radical damage. The main functions of melatonin's antioxidant action are aimed at *protecting DNA*. To a lesser extent, it protects proteins and lipids.

Melatonin is the strongest known endogenous free radical absorbers. In recent years, there has been evidence that melatonin can be found not only in plasma, but also in the nuclei of cells and protect the nucleus macromolecules from oxidative damage in all subcellular structures.

Immune stimulating effect: An important consequence of long-term stress is stress immunodeficiency. Melatonin helps to normalize immunological indicators.

Melatonin and other epiphyseal hormones may be classified as geroprotective. A connection has been established between the degree of age-related epiphysis involution and tissue shriveling. It is known that in aging the degree of immunological protection decreases, and melatonin, as has been repeatedly stated, has immunomodulatory activity. With age, the concentration of melatonin in human blood decreases. When its level decreases below 20% of the norm, the leukocytes stop recognizing parasites in the blood and viruses, the infection of the organism starts.

Melatonin stimulates the immune system (immunostimulant) as it participates in the regulation of thymus and thyroid function, increasing the activity of T-cells and phagocytes, which is a warning for a number of diseases and, as laboratory studies show, slows down the growth of seven types of cancer cells, including breast and prostate cancer cells.

Lack of melatonin in the body: Experiments on laboratory animals showed that with melatonin deficiency caused by the removal of receptors, animals began to age faster: earlier menopause began, free radical cell damage accumulated, insulin sensitivity decreased, obesity and cancer developed.

Melatonin extrapine synthesis: The amount of hormone that is produced in the pineal gland is not enough to provide so many biological effects of melatonin. Studies have shown that significant amounts of melatonin are found in the blood of experimental animals after pineal gland removal. It is now firmly established that the pineal gland is not a monopolistic organ of melatonin production. Extrapineal sources of melatonin synthesis are enterochromaffin cells of gastrointestinal tract (EC-cells), main serotonin depot cells (contain up to 95% of all endogenous serotonin) - melatonin precursor. This hormone has been synthesized in a large number of neuroendocrine cells of airways, lungs, in the cortical layer of kidneys and along the border between the cortical and cerebral adrenal layer, under the hepatic capsule, in paraganglia, ovaries, endometrium, prostate, placenta, gallbladder and inner ear. Melatonin synthesis has also been found in non-endocrine cells such as fat cells, lymphocytes, platelets, eosinophilic leukocytes, in the thymus, pancreas, cerebellum, eye retina and in some endothelial cells.

Functionally, many cells producing melatonin belong to the so-called diffuse neuroendocrine system - a universal system of adaptation and maintenance of homeostasis in the body.

Within this system there are two melatonin links in the producing cells:

- Central (includes the pineal gland and visual system cells), where the rhythm of melatonin secretion coincides with the rhythm of "light-dark";
- Peripheral - all other cells where the secretion of the hormone does not depend on light.

With age, the activity of epiphysis decreases, so the amount of melatonin decreases, sleep becomes superficial and restless, sleeplessness is possible.

Symptoms of melatonin deficiency

- Insomnia and other sleep disorders
- Violation of sleep and waking cycles
- Weakening the immune system
- Fluctuations in blood pressure
- Disorders of mental adjustment
- Anxiety and depression conditions

The main causes of disturbance of natural melatonin production:

- Disturbances in sleep and waking,
- Regular consumption of alcohol and nicotine
- Unbalanced nutrition
- Natural decrease in melatonin production with age
- Shift schedule, night shifts
- Sleeping in an illuminated room

Aspirin, beta-blockers can reduce melatonin levels in the body. Sedative antidepressants, tranquilizers and other sedatives may reduce the expected effect.

Returning to the beginning, almost all bats (as well as winged bats in general) lead nightlife and sleep during the day. In addition, bats often take shelter in cracks in trees, rocks or cracks in buildings. Sanctuaries can be caverns in trees, caves, grottoes and various artificial structures, both aboveground and underground. Bats are able to fall into a daze, accompanied by reduced metabolic rate, breathing intensity and heart rate; many can fall into prolonged seasonal hibernation (up to 8 months). Retinol and tocopherol levels in bats have been found to be high enough to withstand the absence of bonded antioxidants for a long time while maintaining the reserves required for reproduction.

Bats are a natural reservoir of rabies and, according to the WHO, Marburg, Ebola and coronavirus viruses are among the most dangerous infections, a list of the most dangerous known pathogens for humans that cause fatal diseases. There is evidence of the involvement of bats in transmission.

Conclusions

1. Melatonin production decreases with age, which may adversely affect the clinical course of coronavirus infection COVID-19.
2. Unhealthy lifestyle reduces melatonin production.
3. Medicines reduce melatonin levels.
4. Melatonin makes bats not die, but become carriers of infection.
5. The number of coronavirus infected during the summer months may increase by lengthening the daylight hours. Excess light reduces melatonin formation.

Recommendations: The usage of Melatonin in the treatment of patients with coronavirus COVID19 may have a beneficial effect on the outcome and outcome of treatment. Thus, Exogenous Melatonin activates killer cells and monocytes in both bone marrow and spleen with a latency period of 7-14 days. These are components of nonspecific immune protection; some authors believe that melatonin can block tumor growth and destroy infected cells. Melatonin has been found to stimulate the synthesis and release of opioid peptides and thus participate in the regulation of cell hemopoiesis and humoral immunity. The introduction of melatonin in vaccination potentiates the production of specific antibodies. It is known that under stressful and significant physical activity there is an activation of pro-inflammatory factors (cytokines and eicosanoids), the level of alpha tumor necrosis factor (TNF-α) increases, and the level of lymphocytes in blood increases. Lipopolysaccharides (LPS) potentiate these effects - a situation that can occur under inadequate, draining the body's defenses physical activity. At the same time, there are increasing manifestations of oxidative stress, which leads to the violation of the integrity of the hemato-intestinal barrier and LPS in the blood. The fact that melatonin suppresses the inflammatory reaction in response to physical activity should be taken into account in the preparation of athletes and recovery activities in people engaged in hard physical work. Due to its antioxidant and anti-inflammatory properties, melatonin can reduce stomach mucosa damage caused by many damaging factors: stress, alcohol, bile and non-steroidal anti-inflammatory drugs.

Activation of macrophages in pathological conditions leads to an increase in nitric oxide (NO) production. This process is not indifferent to the body and can lead to the development of degenerative diseases. Melatonin inhibits these changes by inhibiting the expression of inducible macrophage NO-synthesis. The introduction of pharmacological doses of melatonin prevented the development of damage in vivo, and inhibited peroxidation and induction of NO in cell culture of the large intestine.

Thanks to intensive research in recent years, there have been reports of the successful use of melatonin in bacterial meningitis as a protection for brain neurons from damage, to normalize sleep and general condition in attention and hyperactivity disorder, in sleep phase disorders syndrome, migraine and headache attacks, in complex therapy of multiple sclerosis, chronic fatigue syndrome, bronchial asthma night attacks, fibromyalgia, irritable colon syndrome, respiratory distress of newborn syndrome.

Nevertheless, the research of the chemical and biological properties of melatonin allows its wide application in medical practice, as experimental data confirm its positive effect on the course of both physiological processes and leveling of many pathological conditions.

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Morphological Characteristics of Intestinal Vessels of Animals with an Experimental Model of Diabetes Mellitus Type 2 Complicated by Microangiopathy

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Abstract

Diabetes mellitus and its complications are considered far from unresolved problems of modern medicine. An important aspect in solving fundamental problems is experimental research aimed at assessing the state of change of pathogenetic important key positions in the development of such a formidable complication of diabetes as angiopathy. On the other hand, surgeons' great interest in bariatric and metabolic surgery, as method of surgical treatment of type 2 diabetes mellitus, requires in-depth studies to assess the state of angiogenic changes in the organs of the gastrointestinal tract, namely in the intestine. The article describes the morphological changes in the vascular capillary bed in the dynamics of the development of a new experimental model of diabetes mellitus, namely the chemical method of diabetic angiopathy. The development of an experimental model of diabetic angiopathy showed the staged nature of the changes in the form of the initial signs of angiopathy, with perivascular infiltration and edema in the form of congestive plethora in the flesh until the development of vascular hyalinosis, which indicates the irreversibility of the processes of pathological transformation. Thus, the results of morphological studies in the dynamics of modeling of diabetic angiopathy indicate the development of diabetes mellitus with its complication in the form of microangiopathy.

Keywords: *Diabetes mellitustype 2, microangiopathy, vascular morphology, diabetic angiopathy, bariatric surgery, obesity.*

Introduction

One of the important world problems of drug therapy for type 2 diabetes, along with the prevalence of this disease, is its lack of effectiveness. It is known, that compensation of type 2 diabetes mellitus, through its traditional treatment, reaches only 30% of patients, and remission in a minimal proportion of patients [1]. Nowadays, it has been proven, that a clinically significant alternative in the treatment of type 2 diabetes is bariatric surgery, which is used in patients with obesity[2], i.e. with a BMI (body mass index) above 35 kg/m2 and providing attainment of diabetes compensation in 76.8% of cases[14].

The great clinical interest are the possibilities of surgical treatment[3] of type 2 diabetes mellitus in patients with normal or reduced body mass index[15]. However, the effectiveness of such operations[4] requires fundamental research in the experiment, proving the safety and effectiveness of this procedure, including taking into account the existing pathomorphological transformations as a form of diabetic gastropathies and enteropathies[5]. The basis of their pathogenesis[7], of course, is the development of diabetic angiopathy[8].

The literature presents the results of experimental studies of the effect of bariatric and metabolic operations on the condition of the mucous membrane of the stomach and intestines, in particular, changes in its cellular balance[9]. Nevertheless, there is no information regarding the characteristics of angiogenic changes in the intestinal walls and stomach in the background of progression of type 2 diabetes. In turn, this would make possible to evaluate objectively the positive and negative aspects of the effect of these operations in the treatment of type 2 diabetes with a normal body mass index, but with the presence of diabetic angiopathy.

Materials and Method

In the experiment were used laboratory Wistar rats weighing 150-250 g on a normal laboratory diet. By the beginning of the experiment, each group consisted of 10-12 rats. 112 animals were used overall.

Diabetic microangiopathy in animals was modeled according to original technique developed by us ("A method of modeling diabetic angiopathy" Patent No. IAP 03642 PV RUz of 2006). The simulation was carried out as follows: laboratory rats weighing 150-250 grams on an empty stomach under ether anesthesia were injected 100-110 mg/kg of doxorubicin with 0.9% sodium chloride solution intraperitoneally, and 48 hours later, they were once daily injected intraperitoneally for 3 days 0.2-0.4 ml of a 70% sorbitol solution. In dynamics, starting from the first day after the injection of the last dose of sorbitol, we observed the development of diabetes mellitus and the manifestations of its angiogenic complications. The main criterion in the reproducibility of the disease was the presence of a stable level of glycemia (at least 9 mmol/l on an empty stomach) starting from the 3rd day after doxorubicin injection. Over the next 30 days, the animals developed diabetic microangiopathy. Animals with normal glycemia level were removed from further researches, and the results obtained during the studies were canceled. Carbohydrate metabolism: blood glucose (mmol/l) was determined on a VitrosDT-60 automatic biochemical analyzer (Austria) using reagents express medical chemistry.

For histological examination, Pieces of the studied intestinal zone were fixed in neutral formalin, Carnoy fluids and embedded in paraffin. Cross-sections were stained with hemotoxylin-eosin according to Van Gieson. Light microscopy and morphometry were carried out on a XSZ-20 trinocular microscope (China) with an optical resolution of 4x to 400x with a direct electronic nozzle in digital format.

Results

With a 5-day period of the experimental model, there were noted mild manifestations of diabetic microangiopathy.

They were manifested a certain increase in proliferation and swelling of arteriole endothelial cells, and areas of plasma impregnation of their walls, mainly in subendothelial zones with plasma proteins, detected in the form of small foci.

Swelling of endotheliocytes in some places was accompanied by destructive changes in some of them. Sometimes perivascular edema and weak lymphohistiocytic infiltration around individual arterioles were detected.

On the 10th day of observation in the capillaries and venules, were noted along with the phenomena of stagnant flushing, with a tendency to hemolysis, thinning of the walls of vessels containing, in addition to red blood cells, a lot of neutrophils.

On the 20th day of the reproduction of diabetic microangiopathy morphologically, more significant disorders of most capillaries and arterioles were observed, compared with those of the previous observation period. They manifested themselves in a marked swelling of endotheliocytes, flattening of the nuclei with a thickening of their basement membrane, and in some cases, their desquamation into the lumen of the vessels. Plasma impregnation with fixation of plasma components in the subendothelium was observed in the vessel wall. In addition to thickening of the basement membranes in arterioles, thickening of their layers and the presence of pericytes in small vessels were observed. Around the blood vessels revealed edema and perivascular infiltration.

On the 30th day of the reproduction of diabetic microangiopathy, the lumen of the capillaries looked wide with the phenomena of stagnant flushing. Some of them were in a state of thrombosis (Fig. 3). Arterial capillaries were characterized by changes in blood cells in the form of adhesion of red blood cells to the surface of endotheliocytes, and in some places, to the basement membrane. In addition to narrowing the lumen of some arterioles, due to thickening of the walls, as a result of plasma soaking, swelling and desquamation of endothelial cells in their lumen, sections of thinning of the walls were found (Fig. 4) with the release of red blood cells.

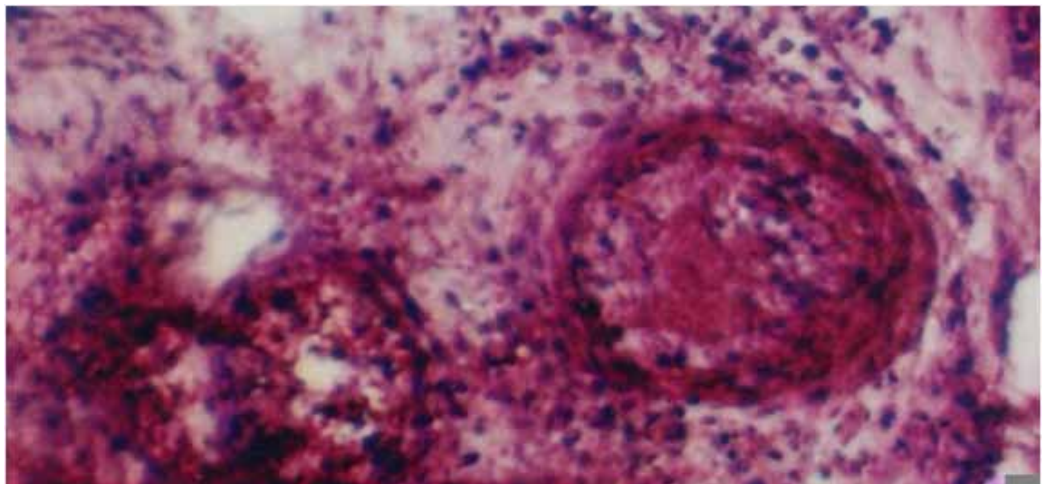


Figure 1. 30 days of modeling diabetic microangiopathy. Flushing of capillaries with signs of thrombosis and adhesion of red blood cells to the basement membrane. Hemotoxylin and eosin. Enlarged 400x.

Venules were characterized by widened lumen and severe perivascular infiltration.

40 days of modeling diabetic microangiopathy were manifested pronounced swelling of endothelial cells, its hypertrophy, destruction, detachment from the basement membrane, and their rejection into the lumen of the vessel.

Discussion

Sometimes, processes of pericytes or smooth muscle cells were immersed in the formed cracks. The marked thickening of the walls of most arterioles was accompanied by hyalinosis of their walls, which was both segmental and circulatory nature. A more significant, compared with the early observation period, plasma impregnation of the vessel walls was revealed, moreover, it was observed in all layers. A significant thickening of the walls of the arterioles occurred, mainly due to an increase in the thickness of the endotheliocytes' basement membrane. In some cases, it's profusion into the lumen of the vessel was determined, leading, together with the above changes, to a significant narrowing of it or complete obliteration (Fig. 6). In separate observations, on the 50th day of modeling, re-calibration was detected in arterioles, leading to occurence of "vasa vasorum". (fig 3).

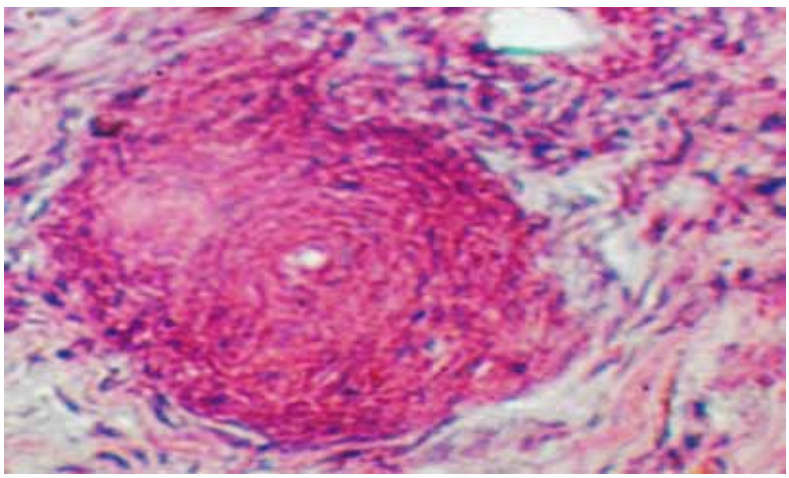


Figure 2. 40th day of modeling diabetic microangiopathy. Hyalinosis, thickening of the arteriole wall with a sharp narrowing of the lumen (obliteration). Hemotoxylin and eosin. Enlarged 400x.

In the later stages of the experiment (60-80th day), maintaining all of the above morphological signs of microangiopathy, the phenomena of complete loss of the endothelial lining by the hyalinized vessels are noted. In view of this, they were represented by acellular tubules which lumen was filled with nests of red blood cells, with the occurrence of thrombosis. These terms were characterized by significant problems of blood cells, in particular, the phenomena of hemolysis, and areas of thrombosis. As a result of this, erythrocytes redistributed to the surface of endotheliocytes (Fig. 4).In addition to the accumulation of erythrocyte aggregates resembling the shape of mulberries, swollen and torn away from the basement membrane endotheliocytes clogged the lumen of the vessels. Moreover, in some cases, perivascular pronounced proliferation of connective tissue, forming a kind of connective tissue "couplings", was observed.

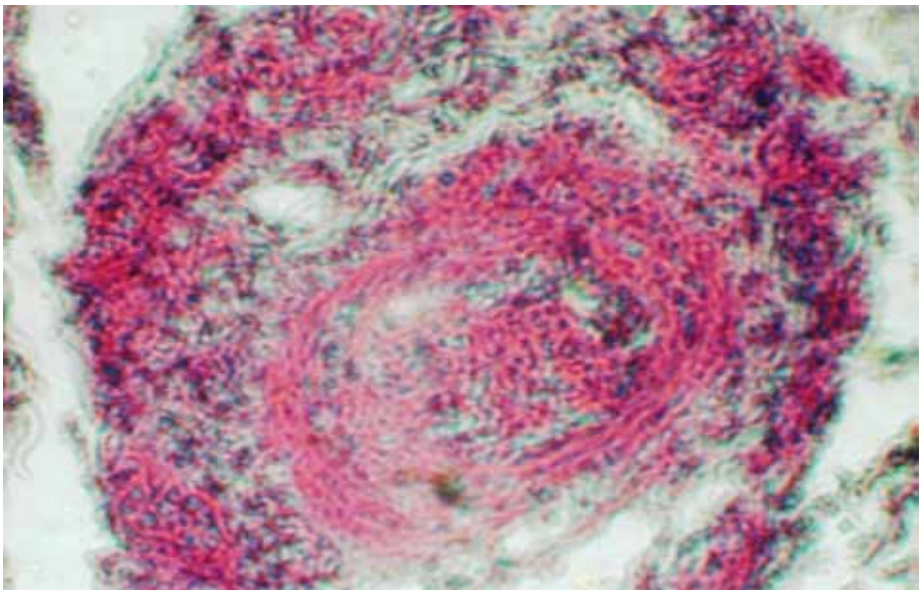


Figure 3. 50th day of diabetic microangiopathy. The phenomenon of percolibration with the advent of "vasa vasorum". Hemotoxylin and eosin. Enlarged 400x

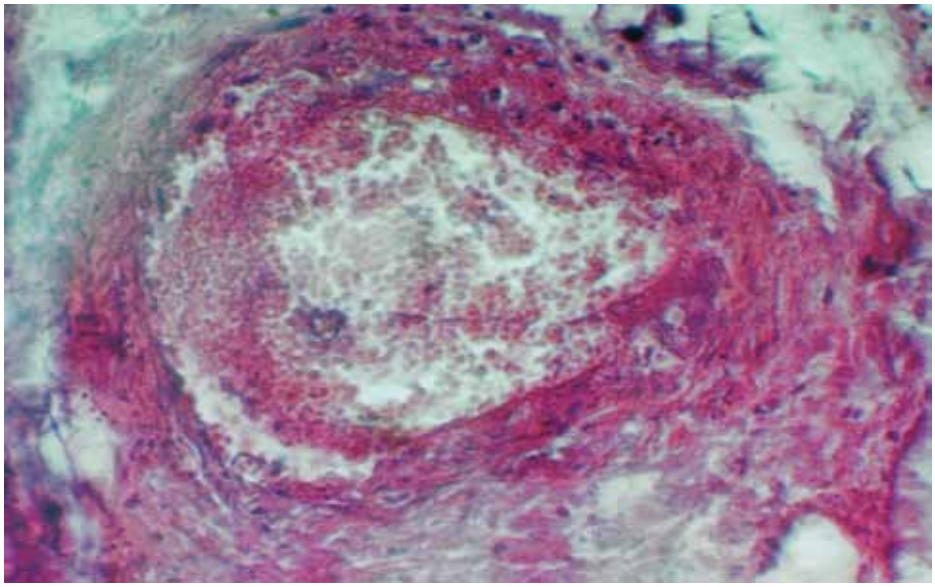


Figure 4. 60th day of modeling diabetic microangiopathy. Hyalinosis of the wall of the arterioles with a complete absence of the endothelial layer, the phenomenon of hemolysis of red blood cells with their location at the basement membrane. Hemotoxylin and eosin. Enlarged 400x

Conclusion

Thus, the results of our morphological studies in dynamics from 5 to 80 hours show that diabetic microangiopathy leads to the diabetes mellitus with its complications, which indicates the presence of damaged vessels when a distinct degree of microangiopathy is seen. This is due to pathomorphological changes in blood vessels. It is characteristically, that changes in experimental diabetes mellitus were exposed to all structural components of the vascular wall.

According to a number of authors, diabetes mellitus is a metabolic disease, called diabetic microangiopathy [10]. For this, the most characteristic morphological sign is the presence of a pronounced degree of severity of thickening of the basement membranes, plasma impregnation[11], hyalinosis of the walls of capillaries and arterioles, narrowing of lumen of blood vessels, proliferation of endothelial cells, perithelium, their destruction and rejection, changes in the rheological characteristics of blood [12].

You know, that diabetes mellitus with diabetic microangiopathy for 80 years is the result of all pathogenetic changes:

I period up to 10 hours: the allocation of mild signs of angiopathy with swelling of the endothelium, arterioles, soaking their walls, plasma protein, perivascular infiltration and edema in the form of stagnant congestion. Thinning of the walls of the capillaries was observed.

In the II period of experimental modeling (days 20-30), progressive processes are observed, which manifest themselves in deeper structural changes in the main vessels. In addition to plasma impregnation of the subendothelial departments of arterioles, proliferation of endothelial and perithelial cells, as well as their desquamation, enlightenment due to leakage of the walls was observed. It was also noted the expansion of the lumen of small vessels, their stagnant congestion, infiltration of adventitia layers.

III period (40-80th day) was characterized by a more significant development of morphological signs of diabetic microangiopathy. Along with morphological changes identified in the early stages, the phenomenon of hyalinosis of the majority of the walls of the arterioles, as well as their marked plasma soaking, were noted. Swelling and detachment of endotheliocytes with intercalation of smooth muscle cells into the vessel wall with their perical calibration and the formation of vasa vasorum, as well as a significant narrowing of the lumen of arterioles. At the same time, perivascular edema, infiltration and proliferation of connective tissue were observed. Changes in blood cells were manifested in their tendency to hemolysis, adhesion on the lumenal surface, the appearance of red blood cell aggregates with the development of thrombosis. Moreover, a pronounced lesion of endotheliocytes, their detachment from the basement membrane led to an increase in the clearance of arterioles, and the reaction of smooth muscle cells and pericytes contributed to the thickening of the basement membranes. Prolonged plasma impregnation causes the development of hyalinosis in microvessels.

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Clinical and Biological Importance of Micro RNA in the Formation of Women Reproductive Losses

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Abstract

Habitual miscarriage is currently one of the most frequent complications of the gestational process, which is diagnosed in 10-25% of pregnant women. (IuA, 2017) Habitual abortion (miscarriage) - spontaneous interruption of pregnancy two or more times in a row.

So, high expression of miR133a may mediate a decrease in HLA-G expression at the protein level and may be implicated in the pathogenesis of habitual miscarriage, since HLA-G provides immune tolerance in pregnancy[49]. So, the literature data suggest that microRNA dysregulation may be related to the pathogenesis of various obstetric complications, but accurate methodological approaches to the diagnosis and prediction of pregnancy complications using microRNA require further study.

Keywords: *Microrna, pregnancy, placenta, gene.*

Introduction

In some countries, three or more abortions are considered habitual, but examination for the cause of the abortion is recommended after two abortions[2]. The frequency of Habitual miscarriage in the population is up to 5% of pregnancies. In the structure of miscarriage, the frequency of miscarriage is up to 20%. Habitual miscarriage is a polyethological complication of pregnancy based on reproductive disorders[1] The causes of miscarriage, both episodic (single or repeated) and recurrent (loss of three or more consecutive pregnancies), are similar. They can be combined into the following groups: genetic, infectious, endocrine, anatomical, immunological, and idiopathic[3] Biomarkers, which are present in the mother's serum, will be the most valuable, as obtaining the mother's blood is a safe and affordable method, and blood can be obtained in sufficient quantities.[4] The exchange of information between the functional systems of the mother and fetus is important for normal pregnancy, fetal development and growth. This connection reduces the risk of rhesus conflict and coordinates biological resources for the benefit of both organisms. Previously, the mother-fetal link was thought to provide hormones and growth factors that are soluble in the blood or bound protein carriers and serve as paracrine or endocrine signals to control healthy pregnancy. It is now clear that nucleic acids, and especially small RNA molecules, circulate between tissues and affect them at the cellular level.

The main class of non-coding RNA and one of the most well studied is the family of small regulatory RNA called micro-RNA. The term micro-RNA was first introduced in the early 2000s. Micro-RNA were originally described in the Caenorhabditis Elegans nematode in 1993 and later were found in the genome of many organisms, including humans. The human genome encodes more than 1000 micro-RNA species and about 60% of the human and other mammalian genes are targeted[5]

Micro-RNA's (miRNA's) are endogenous RNA's ranging in size from 20-22 nucliotides that can play

an important regulatory role in animals and plants by targeting mRNA's for cleavage or translational repression. Oligonucleotide micromatrixes have shown that microRNAs are highly enriched in the placenta. (Wu et al., 2012) RNA-induced jamming complex to the RNA target, with subsequent weakening of gene expression by inhibiting matrix RNA (mRNA) translation and/or RNA degradation. This is accomplished by complementary binding their «seed sequence» to the target in the 3'-nontransmitted mRNA region either by detaching the Cap 5'-structure (decapitation) or deadenylating the poly(A)tail. Consequently, they participate in transcription and posttranscriptional regulation of gene expression and play an important role in various biological processes[6].

Cells transcribe microRNA precursors (primary transcripts) (PRI-microRNA), which undergoes multistage biogenesis with the formation of mature microRNA 18-25 nucleotides in length. The synthesis of microRNA begins in the nucleus, with RNA polymerase II-mediated transcription, using genomic DNA as a matrix. A long primary microRNA (known as PRI-microRNA) is then formed, which forms a complex secondary structure by folding loops and studs.) The dual-chain structure of RNA can be easily recognized by the nuclear protein DGCR8, which functions in combination with Drosha, the protein that cuts through RNA, due to the catalytic domain of RNAase III, forming a precursor microRNA molecule in the form of a stud. PRE-microRNA can bind to the nuclear transport factor, the exporter-5, and is exported to the cytoplasm by the energy of guanosine triphosphate. In cytoplasm, PRE-microRNA is broken down by endonucleases specific to double-stranded RNA, resulting in the formation of microRNA: duplex of ~22 nucleotides in length. As a rule, only a few can bind to Argonaute proteins in the RNA-induced Gene Jamming Complex (RISC) and participate in the RNA interference process. The remainder is destroyed by the gene jamming complex. miRNA's specifically expressed in human placenta were found in the sera of pregnant women and were found to be significantly elevated in comparison with those of non-pregnant women; their levels increased with gestational age and decreased after delivery, providing a new group of molecular markers for monitoring pregnancy[7].It should be noted that the expression profile of tissue-specific and disease-associated microRNA's is more selective and stable than the general mRNA profile. The biological efficacy of specific mRNA's is in their subsequent conversion into specific proteins as compared to microRNA's, which act as key regulators of polymer genes. So, microRNA more accurately reflects physiological changes in the body. After long researches it was proved that microRNA is everywhere present in body fluids and the mechanism of genetic exchange between cells in horizontal order takes place. Circulating or extracellular microRNA's are more stable and protected against RNAase degradation. This protection is achieved either by complexes with different proteins or by inclusion in different types of extracellular vesicles. MicroRNA's have been shown to be ten times more stable than mRNA's, which have a half-life expectancy of ~10 hours. The average half-life of microRNA, according to a mathematical model, is 119 h and some microRNA's, such as 125b microRNA (half-life, 225 h), are more stable than others. This is probably due to their inherent structural features[8]. The use of single-primer real-time PCR or real-time PCR using high-capacity platforms ensures that quantitative analysis of microRNA from tissue or plasma/serum will be easier and more accurate. This highlights the ease of using microRNA as a biomarker for the development of practical method to detect obstetric pathology. MicroRNA analysis shows that tissue diversity is reflected in the microRNA expression profile. In addition, the expression profile of pathological tissue differs significantly from that of normal tissue, which can be useful for clinical diagnostics. So, aberrant microRNA expression is associated with cellular dysfunction and a number of pathological conditions such as cancer, inflammatory and immune diseases, cardiovascular disorders[9]. Abnormal expression of miRNA's has been observed in multiple diseases of the human reproductive tract, including preeclampsia, endometrial endometrial adenocarcinoma, leiomyoma, and ovarian cancer, endometriosis, and pregnancy recurrence. The following review provides an update on the role of microRNA and gynaecological diseases, covering not only the impact of microRNA regulatory violations on the origin of each disease, but also shows the potential useful diagnostic and therapeutic tool that miRNA can play in these gynecological pathologies[10]

The increased expression of specific microRNA's causes a repressive transmission from the mRNA target, while the decreased expression of these microRNAs has the opposite effect. These changes in mRNA translation define a distinct protein expression profile, affecting multiple molecular pathways, and induce many different

cell and tissue changes. Thus, the cumulative effect of multiple microRNAs can affect human health as well as determine the outcome of a particular disease. These data may allow for a better understanding of changes associated with genital tract diseases and may be useful for developing newer therapeutic approaches as well as less invasive and more effective diagnostic tools[11]. MicroRNAs play an important role in regulating many events, such as inflammatory and immune responses, cellular cycle, differentiation, apoptosis and tissue remodeling. The precise regulation of the expression of these genes is fundamental to the normal functioning of the endometrium. Thus, dysregulation of microRNA expression is associated with certain gynecological pathological processes such as endometriosis, leiomyoma and habitual miscarriage[12]. The microRNA expression profile has been shown to be more important than the mRNA expression profile for discriminating normal and pathological tissues. As a result, microRNA may have a significant impact on the development of new and more accurate diagnostic method as well as more effective therapeutic approaches. A number of microRNAs specific to the human placenta have been detected using the Real Time Polymerase Chain Reaction (PCR-RT) method, each of which has an individual expression level[12]. Some specific microRNA (microRNA516-5p, 517*, 518b, 520a*, 520h, 525 and 526a) are considered pregnancy-specific microRNAs. The importance of mRNA in early embryo development has been identified in a number of species, from Caenorhabditis elegans to mammals.[13-18]It has been shown that in these organisms miRNA-supplied gene regulation is necessary for normal embryogenesis, as evidenced by the reduced survival of embryos in mutant embryos.

The importance of microRNA is confirmed by the fact that deletion of Dicer1 gene (Dcr-1) in mice leads to stopping embryo development on 7.5 days due to differentiation defects[19]. Jan et al.[20] reported similar effects between 12.5 and 14.5 days of pregnancy due to altered embryonic angiogenesis, which also appears to be regulated by microRNA. These researchers have demonstrated that, since miRNAs act as posttranscriptional suppressors of target genes, a defect in Dicer leads to defects in miRNAs. This, in turn, causes the over-expression of target genes such as Ang-1 and PTEN in embryos. In the same way, Medeiros et al [21]. have demonstrated that miRNA Mir-290-295 deficiency leads to partial embryo death and germ cell defects. Micro-RNAs are also highly expressed in human embryonic stem cells. [23]. Rehn and Co. found that miR-520 may be closely involved in human embryonic cell function. Other miRNAs, such as miR-302 and miR-200 families, were similarly observed in human [30] and mouse embryonic stem cells[31]. Rosenbluth et al.[34] studied 754 microRNAs, were expressed in 14 blastocysts (five females, four males and five aneuploid embryos) with matrix screening. They confirmed using real-time quantitative PCR (RTR) the most expressed microRNAs including miR-372, miR-720 and miR-302c in 27 blastocysts (seven males, eleven females and nine aneuploids). These researchers observed that human blastocysts express a large number of microRNAs, which are necessary for the survival and development of embryos, as well as to preserve pluripotency of stem cells. Most of them have already been registered in human embryonic stem cells[29] mammalian embryos[35] or primate placenta[37]. Moreover, when comparing male and female embryos, these researchers found that one miRNA, miR-518d-5p, was expressed 5.6 times in male embryos, indicating some degree of sexual differentiation even at the blastocyst stage. Interestingly, Mir-518d-5p mRNA belongs to C19MC cluster, the largest human cluster where miRNA genes are organized. In these clusters, miRNA genes are expressed only in the placenta and can participate in the development and implantation of the placenta. Similarly, miRNA expression was found to be higher in mouse embryos than in mature tissues, confirming their role in embryo development [38]. More recently, Rosenbluth et al.[39] have demonstrated that miRNA can be detected in In Vitro Fertilization (IVF) culture media where they are differentially expressed according to the fertilization method, chromosome status and pregnancy outcome. These results show that miRNAs are potential markers for predicting success after IVF[40].

Concentrations of some other microRNAs are significantly higher in plasma in pregnant women than in non-pregnant women. Moreover, the concentration of some microRNAs (e.g. microRNA 141) increases with increasing gestation period[41]. The highest intensity of expression in the placenta is typical for microRNA 517a, 517b, 516b, 525 -5p, 512-3p, 515-3p[42]. It is also noted that microRNA 517a-3r, 519 a-3r, 520 s-3r are determined in mesenchymal stromal cells of the placenta, being an indicator that the specific functions of the placenta are not limited only to the trophblast. At the same time, the intensity of microRNA expression of 125b5p in the trofooblast is much higher in the third trimester compared to I. The different expression of microRNA patterns in the nap tree of the placenta in

the first and third trimesters of pregnancy highlights the individual profiling of microRNA and ensures normal development of the placenta. Therefore, the level of microRNA expression can be an indicator of placenta changes during pregnancy depending on the period of gestation and development of the choroid branches[8]. The search for microRNAs that could serve as biomarkers of complications during pregnancy was initiated by Chim and colleagues in 2008. At the time, about 100 microRNAs were known to be expressed primarily in the placenta, but it was not known whether microRNAs obtained from the placenta would be detected in the mother's blood and useful in diagnosis. In a landmark study, Chim and his colleagues suggested that most plasma microRNAs, which are not pregnancy-related, originate in the hematopoietic compartments, and therefore they compared 157 microRNA profiles isolated from the placental tissue in the third trimester of pregnancy with microRNA isolated from the mother's blood cells. They identified 34 microRNAs that were present in the placenta at concentrations tens of times greater than those in the maternal blood cells. Importantly, the researchers additionally showed that the four most common of these placental microRNAs (microRNA-141, microRNA-149, microRNA-299-5p, and microRNA-135b) were found in the mother's plasma during pregnancy but were not found in postpartum plasma, another confirmation that they were of placental origin. Placental specific microRNAs were subsequently detected in plasma and serum and in a number of other studies. These data clearly show that placental microRNAs are present in the maternal bloodstream[8]. It was originally thought that nucleic acids (DNA and RNA) of placental origin enter the maternal bloodstream as apoptosis cells. Lo et al. have shown that microRNAs are exported from the human placental syncytial trophoblast to the maternal bloodstream through exosomes, and suggested that circulating microRNAs derived from the trophoblast reflect the physiological state of pregnancy and can be used for diagnostic purposes. Because their concentrations probably reflect tissue-specific physiological or pathological conditions, many recent studies have focused on identifying microRNAs that correlate with them and thus allow predicting complications of pregnancy. As for the normal human placenta, the expression of microRNA belonging to the C19MC, C14MC and miR-371-3 clusters depends on the gestational period. C14MC, the largest microRNA cluster containing 52 microRNA genes, is preserved without significant structural changes and is unequivocally determined in placental mammals. This suggests the important role of C14MC microRNA in controlling neurogenesis, embryonic development, transcription regulation and RNA metabolism. The C19MC cluster of 46 microRNA genes plays an important role in human embryonic development in the same way as the insulin-like growth factor-2 (IGF-2). It should be noted that some of the C19MC cluster microRNAs were found in the maternal bloodstream during pregnancy, including microRNA-515-3p, microRNA-516-5p, microRNA-517A, microRNA-517c, microRNA-518B, microRNA-520a *, microRNA-520h, microRNA-526a and microRNA-526b. Thus, maternal, circulating microRNAs may have the potential to become a new diagnostic tool for pregnancy pathology[47]. The cluster of microRNA-371-3 consists mainly of three microRNAs, HSA-Mir-371- 5p, HSA-microRNA-372 and HSA-Mir-373-5p and is located on chromosome 19. The cluster mainly expresses itself in the placenta, and the amount of its microRNA decreases in the process of development, therefore it seems to be important for the maintenance and regulation of proliferation and apoptosis. In addition, it was found that microRNA's from the cluster microRNA-17-92 are expressed in the normal motensive placenta. These microRNA's are important for angiogenesis and affect the expression of many genes, namely HIF1A, interleukin-8, tissue inhibitor metalloproteinase-2, metallopeptidase-2 matrix, VEGFA, efrin-B, and efrin receptor B4. The abundant expression of microRNA in amniotic fluid has been confirmed; it contains mainly those microRNA's that are involved in supporting embryonic development and immunity, thus protecting the fetus from external pathogens and the mother's immune response[44]. Some microRNAs with oncogenic and immunologic suppressor characteristics, such as cluster microRNA-17-92, microRNA-371, C14MC, and C19MC, mentioned above, have been found to express themselves more strongly in the first trimester of pregnancy, a microRNA of oncosupressors and those microRNAs that promote differentiation and have hematopoietic activity, including LET-7 family microRNA and cluster microRNA-29 and microRNA-34, are more strongly expressed in the third trimester of pregnancy. In addition, aberrant expression of some microRNA's has been recorded in placenta or lint in women with pathological pregnancy. Expression of HSA-MIR-184, HSA-MIR-187 and HSA-MIR-125b-2 in the lint of patients with normal pregnancy loss has been found to be significantly increased, while expression of HSA-MIR-520F, HSA-MIR-3175 and

HSA-Mir-4672 has been significantly reduced compared to that of women with normal pregnancy.

Studies in the laboratory have shown that microRNA is involved in the regulation of proliferation, migration, invasion, apoptosis and angiogenesis of trophoblast cells. Thus, it is quite probable that microRNA is necessary for normal development of the placenta and that pathological expression of microRNA may lead to defective placenta and abnormal pregnancy, resulting in miscarriage[46]. It has been shown that seven specific placental microRNAs present in maternal plasma (microRNA-516-5p, microRNA-517, microRNA-518b, microRNA-520a, microRNA-520H, microRNA-525 and microRNA-526a) are associated with pregnancy and may have diagnostic potential. There is a direct correlation between the level in maternal plasma of extracellular pregnancy-associated placenta-specific microRNA-515-3p, MIR-517A, MIR-517c and microRNA-518B and placental mass. The relationship between placental preposition and extracellular pregnancy-associated placental-specific microRNAs in the mother's plasma has been identified. They found significantly higher concentrations in plasma of extracellular MIR-517A and significantly lower concentrations in plasma of extracellular MIR-518B in the placenta predestination group in comparison with the control group and suggested that the level of circulating extracellular MIR-517A can be a prognostic marker of the risk of bleeding at late stages of pregnancy and massive bleeding at delivery. Other studies have provided evidence that functional oligonucleotide polymorphisms (OP) of microRNA-125 may increase susceptibility to habitual miscarriage. Two variants of the allele in primal-microRNA-125 have been identified, namely rs41275794 and rs12976445, which lead to changes in microRNA-125 production. Genes of inhibitory factor leukemia receptor (LIFR) and ERBB2 are targets of microRNA-125. LIF is a gene involved in the growth of the trophoblast and differentiation of the human placenta, which, like ERBB2, regulates the proliferation of stroma cells and differentiation of the trophoblast cells in the placenta in the period after implantation. In addition, ERBB2 can stimulate angiogenesis and invasion by increasing the concentration of vascular endothelial growth factors (VEGF). All these data suggest that these genes may play an important role at early gestational ages and their disruption may lead to PPB in some patients.

Expression of microRNA-125 A-T haplotype decreases in microRNA-125, which leads to increased expression of LIFR and ERBB2. Apparently, ectopic expression of LIFR may partially block LIF activity and cause disturbances of trophoblast growth and differentiation during the post implantation period, which leads to the usual loss of pregnancy. Finally, further studies have investigated associations of microRNA polymorphisms including MIR-146aC> G, MIR-149T> C, MIR-196a2T> C and MIR-499A> G in women with habitual miscarriage. Patients suffering from miscarriage showed a higher frequency of microRNA-196a2CC and microRNA-499AGþG genotypes compared to the control group of healthy patients. The combination of both mutations showed synergistic effects; all these polymorphisms are largely related to idiopathic loss of pregnancy.

Functional genomics has been widely studied, and recently endometrial gene expression signature has been used for implant window screening. Endometrial microRNA has been identified in recent years, during the middle secretory phase, and it is assumed that it regulates cell proliferation and differentiation, which may affect embryo implantation, but their exact role is not yet understood.

In order to study microRNA (miRNA), a prospective cohort study including 12 good female infertility predictions and endometrial gene expression tests during natural cycles before IVF treatment, ERA analysis revealed 5 receptive and 7 post-receptive endometrial samples. No depressed miRNA's were found comparing receptive and post-receptive endometrium. However, eight miRNA's were found to be activated with a multiple of more than 3.0 changes in susceptible samples. Among them, two positively regulated miRNA's were statistically significant: has-miR-374a-5p (multiple: 8.9, $p = 0.03$) and has-miR-17-5p (multiple: 8.9, $p = 0.03$). Despite their insignificance, the other two miRNAs had high multiples of has-miR-30a-5p (multiples of 9.7, $p = 0.116$) and has-miR-103a-3p (multiples of 10.5, $p = 0.125$). Eight microRNAs have been identified as being differently compressed in the receptive endometria by ERA analysis and may be considered as new potential candidate biomarkers for endometrial susceptibility.

Researches show that miR-374a and miR-103 family members are miRNA's associated with cell proliferation, mainly in cancer, while miR-17 and miR-30 family members were identified in the uterine cavity during the implantation window and may be associated with endometrial susceptibility[48].

Recent studies have evaluated the effect of microRNA on the regulation of the HLA-G gene in habitual miscarriage. HLA-G is a non-classical antigen of the main Histocompatibility Complex (MNS) and is expressed almost exclusively on the surface of trophoblast lint in the transplacental barrier during pregnancy, playing a crucial role in the immune tolerance of the mother to the fetus. After microRNA microchipping in habitual miscarriage patients and a control group of patients undergoing planned abortions, it was found that excess expression of microRNA-133a was found in abortions with abnormal karyotype. Further analysis showed that HLA-G is the target of miR133a. It is regulated by binding to a 3'' untranslatable area of HLA-G.

So, high expression of miR133a may mediate a decrease in HLA-G expression at the protein level and may be implicated in the pathogenesis of habitual miscarriage, since HLA-G provides immune tolerance in pregnancy[49]. So, the literature data suggest that microRNA dysregulation may be related to the pathogenesis of various obstetric complications, but accurate methodological approaches to the diagnosis and prediction of pregnancy complications using microRNA require further study.

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The Value of Neurospecific Protein S-100 and Lipoproteins in the Development of Multiple Sclerosis

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Abstract

This article describes the clinical picture of multiple sclerosis (MS) depending on indices of the neurospecific protein s-100β and low-density lipoproteins. In patients with MS in the phase of activity, high levels of s-100β protein and LDL were found. It means that in patients with MS, demyelination and degeneration process continues, and as a result, patients are more likely to develop new symptoms. Thus, the study results of s-100β protein and LDL can be used to predict possible development of adverse symptoms in multiple sclerosis.

Keywords: *Multiple sclerosisneurospecific protein lipoproteins central nervous systemdemyelinating diseases.*

Introduction

Multiple sclerosis (MS) is a fairly widespread dysimmune neurodegenerative disease of the central nervous system, affecting mainly young people and almost inevitably causes disability at a certain stage of its development. Early diagnosis of multiple sclerosis is considered one of the most significant tasks of modern neurology. The etiology, pathogenesis and factors, affecting demyelination in multiple sclerosis, are still not clearly understood.

Demyelinating diseases belong to the group of autoimmune diseases, develop with destruction of the myelin sheaths in the central and peripheral nervous system. According to scientific literature, this process is caused by imbalance of 1,2,3-group cytokines of T-lymphocytes. An increase in the activity of T-1 lymphocytes leads to an increase in pro-inflammatory cytokines: IL-2, TNF-alpha, IFN-gamma, IL-12, causing a loss of tolerance to autopeptides and enhancement of autoimmune processes[5]. Synthesized “anti-myelinic” “antibodies” leading to central nervous system demyelination, dramatically reduce impulse conduction along nerve fibres. In patients’ blood, in relation to antigens of brain structural elements, that is, oligodendrocytes - to myelin basic protein (MBP), S-100 glial protein, antibodies are formed and detected[3]. The detected S-100 glial protein is considered to be an inducer of glial cell destruction. Sensitivity to this immunocyte protein in 70% of cases is determined in the blood; it is explained by destruction of glia and neurons. Also, in 50% of cases, the integrity of neuronal membranes is broken. The following types of s-100 protein are present: s-100 άά (specific to skeletal muscles, heart muscles, kidneys, liver); s-100 άβ (specific to glial cells, melanocytes); s-100 ββ (specific to Schwann cells). An increase in the concentration of s-100 άβ and s-100 ββ in cerebrospinal fluid and plasma is a brain damage marker. The results of s-100 study can be used to predict possible development of adverse symptoms in multiple sclerosis. In humans, elevated S100B levels have been detected in various clinical conditions. Brain injury and ischemia are associated with an increase in S100B concentration, probably, due to destruction of astrocytes. In neurodegenerative, inflammatory and mental diseases, elevated S100B levels may be caused by secreted S100Bs or release from damaged astrocytes. This review summarizes published S100B data regarding human brain damage and neurodegeneration[6]

An increase in the level of this protein is detected in the cerebrospinal fluid and blood plasma of patients with MS with exacerbation[4].

In MS pathogenesis, lipid storage disease is also

present. Lipids comprise 70% of the myelin sheath dry weight. They are structurally and functionally significant, their composition under demyelinating diseases undergoes great changes. The lipids involvement in all 4 development stages of the demyelinating process is demonstrated. In animal models, encephalithogenicity of myelin glycolipids was shown[7]. Thus, lipids may act as ligands of innate immunity receptors or pro-inflammatory agents that potentiate activity of innate immunity receptors and affect the inflammatory process with demyelinating diseases.

Despite great successes in documenting cellular and molecular mechanisms, underlying multiple sclerosis pathophysiology, the autoimmunity structure also cannot provide an exhaustive explanation of the disease etiology. I propose a new framework for MS as a lipid metabolism dysfunction. In particular, lipid metabolism homeostasis is destructed during the acute phase of inflammatory response, induced by a pathogen, trauma or stress, starting a feedback loop of increased oxidative stress, inflammatory response and proliferation of cytotoxic foam cells, crossing the blood-brain barrier, catalysing myelin and preventing remyelination. Understanding MS as a chronic metabolic disorder, makes it possible to single out four aspects of the disease onset and progression: 1) its pathophysiology; 2) genetic susceptibility; 3) environmental and pathogenic triggers; and 4) asymmetric sex ratio in patients. It also provides new treatment options.[2].

Cholesterol is an important component of mammalian cell and myelin membranes. In this systematic review we studied the relationship between cholesterol levels and cholesterol turnover markers in blood circulation and/or cerebrospinal fluid (CSF) and disease outcomes in adults with clinically isolated syndrome (CIS) or confirmed MS. Studies show that elevated levels of circulating cholesterol of low density lipoproteins (LDL), total cholesterol, and,in particular, apolipoprotein B and oxidized LDL, are associated with adverse clinical and MRI outcomes in MS. These relationships were observed back in the CIS. Studies also show that oxysterols, cholesterol precursors and apolipoprotein E may be a marker of specific disease processes in MS, but further research is required to clarify these processes and relationships. When analysed together, these data mean that cholesterol and cholesterol turnover markers are potentially suitable for clinical use as biomarkers of disease activity and may even be involved in MS pathogenesis. [1]

Purpose of our Study: to study the role and importance of neurospecific protein s-100β and lipid profile indices in the pathogenesis of multiple sclerosis development.

Material and Research Method

40 patients with multiple sclerosis (MS), aged from 21 to 45 years (mean age 33±1.5) were examined. Of these, 14 (35%) were men and 26 (65%) were women. All patients underwent clinical and neurological examinations and head MRI. Patients were divided according to MS course: with remitting MS - 18 (45%), primary progressive MS - 7 (10%) and secondary progressive MS - (15) 37.5%. The study of MS duration from the disease onset showed that patients with duration of "1-5 years" were 58%, "5-10 years" - 22%, "over 10 years" - 20%. There were 26 patients with MS in the active phase (65%), and 14 (35%) in the remission phase. A neurospecific s-100β protein (s-100β protein) (normal < 0.11 μg/L) and low-density lipoproteins (LDL) (normal < 2.5 mmol/L) were studied in blood plasma.

Study Results

The study results showed that in patients with MS, s-100β protein values vary from 0.06 to 0.22 μg/L (mean 0.17±0.09 μg/L). In 70% of cases, this index is higher than normal (28 patients). A study of neurospecific protein indices depending on the disease course showed, that in patients with a remitting course, s-100β protein was 0.14±0.1 μg/L, and, accordingly, in a primary progressive course - 0.21±0.0 μg/L, secondary progressive course - 0.18±0.06 μg/L, that is, in all types of course, s-100β index is increased, but the highest index is registered in the primary progressive course. In MS active phase, s-100β protein index was increased 0.13±0.01 μg/L, and during remission, within the normal range, - 0.08±0.2 μg/L.

The s-100β protein index did not differ by sex. Thus, in men it was 0.12±0.01 μg/L and in women - 0.14±0.02 μg/L.

It was determined that the longer the disease lasts, the more s-100β protein index is increased, regardless of the activity phase. With duration of the disease for "1-5 years" s-100β was 0.16±0.1 μg/L, "5-10 years" - 0.21±0.05 μg/L and "more than 10 years" - 0.2±0.1 μg/L.

Studies showed that in patients with MS, LDL levels ranged from 2.2 mmol/L to 4.65 mmol/L, mean of 3.6±0.1 mmol/L.

In all MS forms, mean LDL value is increased, especially with a secondary progressive course (3.6±0.1 mmol/L), remitting course (3.4±0 mmol/L) and primary progressive course (3.1±0.02 mmol/L).LDL in the active phase (3.5±1.3 mmol/L) differs sharply from that during remission phase (3.0±1.1 mmol/L). There were no gender differences in LDL indices (male - 3.4±0.03 mmol/L and female - 3.4±0.15 mmol/L).

The longer MS duration is, the higher LDL index is. So, with duration of "1-5 years", this index was 3.4±0.01 mmol/L, "5-10 years" - 3.6±0.1 mmol/L, "more than 10 years" - 3.7±0.2 mmol/L.

When protein indices s-100β and LDL were compared, it showed a negative correlation (r = - 0.12).

Conclusions

Evaluation of study results showed, that in all MS forms, the mean index of neurospecific protein s-100β and LDL was increased. It means, that our patients have a demyelinating and neurodegenerative process. A study of the disease duration showed, that the longer the disease lasts, the more intensive demyelination (degeneration) process is. A comparative study, depending on the disease course showed, that in a primary progressive course, the protein index s-100β is permanently high, LDL index is high in a secondary progressive course. Both analysed parameters are higher in the active than in the remission phase. Based on study results, in MS patients, high s-100β protein and LDL indices demonstrate, that in patients with MS the demyelination (degeneration) process continues; as a result, patients are more likely to develop new symptoms.Based on the analysis of studied parameters, depending on MS activation and remission, it can be observed that s-100β protein index in the active phase is increased, whereas in the remission phase it is within the normal range; LDL index is increased in all phases. Logically, patients in active phase of the disease re treated for demyelination (degeneration) processes (hormone therapy, interferons, immune adjuvants). As a result, s-100β protein index in the remission phase is within normal range. However, as a rule, patients with MS do not receive lipid-improving drugs. On the other hand, it takes a long time, on average, 3-6 months to normalize the lipid profile. But, by this time, either the disease exacerbation or the disease progression occurs.

Thus, in MS treatment, the disease phase shall be transferred from the active to remission phase, patients shall also be treated against risk factors, contributing to emergence of demyelination (degeneration) processes. This is a very important tactic for secondary prevention of the disease.

Thus, the study results of s-100β protein and LDL can be used to predict possible development of adverse symptoms in multiple sclerosis.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

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Epidemic Process of Salmonellosis in Tashkent

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Abstract

In the latest decade, epidemiological features of salmonellosis morbidity have changed, in particular, the fraction of humans and animals, caused by S. enteritidis, has increased. It was caused by social changes in the population life: widespread development of private property, increased population migration, rapid development of the public catering system, etc. The features of salmonellosis epidemic process were analysed in Tashkent. S. enteritidis has also dominated in the causal structure of salmonellosis, registered in food products and other environmental objects over the past twenty years in Tashkent.

In the long-term dynamics of salmonellosis morbidity in Tashkent, there is a downward trend: in 1991 - 92.85 cases per 100 thousand people, in 2017 - 13.2, that is, over a 26-year period, the morbidity decreased by 6.7 times.

Keywords: *Salmonellosis, epidemiology, prevention.*

Introduction

Salmonellosis, called the “disease of civilization” is so widespread that at present it cannot be eliminated in any country, the morbidity rate can only be decreased[7]. Salmonellosis occupies a special place among acute intestinal infections. Unlike dysentery, typhoid fever, colibacillosis and cholera, they are caused by microorganisms being pathogenic for animals and humans in natural conditions[9]. There are two salmonella reservoirs in nature - animals and humans. There are two salmonella reservoirs in nature - animals and humans[10]. The expansion of international trade in animals and animal products, feed, a high concentration of livestock in a limited area, environmental changes, leading to the emergence of new Salmonella properties[3], introduction of modern mass technologies for slaughter and processing of animals, expansion of the catering network, are the main factors, contributing to salmonellosis spread[5].

In the latest decade, epidemiological characteristics of salmonella morbidity in Uzbekistan have changed, in particular, the fraction of people, suffering from S. enteritidis, the incidence of Salmonella infection in farm animals, birds, and the environment has increased[2]. It was caused by social changes in the population life: widespread development of private property, increased population migration, rapid development of the public catering system, etc[1].

In the 90-ies, in Uzbekistan, as a result of political and economic reforms, significant changes took place in the system of food production and sale, including poultry products. Most catering and trade enterprises became privately owned. At the same time, a sanitary-epidemiological service reform was introduced, accompanied by a sharp limitation of the frequency of inspections of controlled objects and the volume of microbiological studies of environmental samples. These social changes had a serious impact on salmonellosis epidemic and epizootic processes[6].

In this situation, there is a need for a theoretical justification of measures to control and prevent salmonella infection, considering current features of not only epidemiology, but also epizootology of salmonellosis enteritis. Meanwhile, the epizootic process of salmonellosis enteritis among farm animals and birds and its effect on intensity, dynamics and structure of the population morbidity in Uzbekistan have not been studied. Currently, the prevalence of bacterial diseases in animal husbandry worldwide is 16.2%, of them 10.9% are mixed bacterial diseases. In the development and increase of livestock yield, being the agricultural

main sector, it is important to increase livestock on state farms, farms and private farms, increase their yield, get healthy livestock, provide good care and protect it from various diseases[10]. The increasing epidemiological role of livestock and poultry, industrial poultry products, relationship of epizootic and epidemic processes call for restructuring of the existing system of salmonellosis epidemiological surveillance according to epizootological and epidemiological surveillance principles[7].

The purpose of this study was to research the prevalence of salmonellosis morbidity in Tashkent and determine epidemic process features of this infection.

Materials and Method

The study was based on official data of the State Sanitary and Epidemiological Surveillance Department on salmonellosis causal structure and retrospective epidemiological analysis results of salmonellosis morbidity in 1992-2017 in Tashkent. The epidemiological survey results of 114 salmonellosis foci, registered in Tashkent, were studied. The method of epidemiological survey of foci and epidemiological analysis were applied: descriptive research method (observation technique, study method of spatial spread of diseases, method for temporal distribution cases in time, method for cases distribution among different population groups, statistical method), analytical research method (formal logic, statistical, cohort and retrospective study method).

Results and Discussion

The spatial distribution of salmonellosis diseases was contrasting, with Tashkent being extremely unfavourable in terms of salmonellosis, high morbidity was also registered in Tashkent, Ferghana and Andijan regions (Fig. 1).

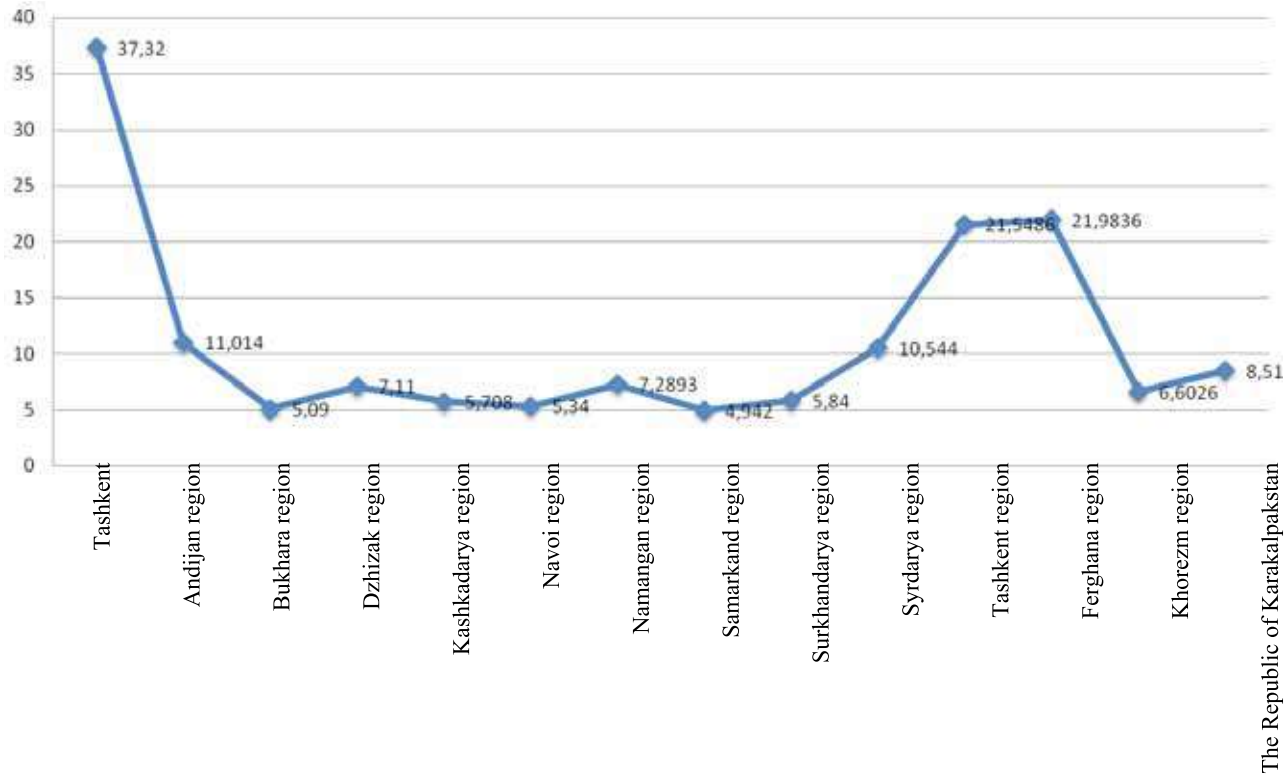


Fig. 1. Spatial distribution of salmonellosis morbidity in the Republic of Uzbekistan over 26 years (1991-2017)

One of the most important epidemiological determinants, defining the nature of salmonellosis epidemic process is attributed to serovar, prevailing in the causal profile. S. enteritidis has dominated in the causal structure of salmonellosis, registered in food products and other environmental objects over the past twenty years in Tashkent. The proportion of diseases caused by S. enteritidis in the general structure of salmonellosis

was s follows (1992 - 17.1*%,* 2008 - 35*%).* Another serovar, S. infantis, rarely registered in humans until the 1990s, subsequently occupied sufficient proportion in the causal structure of salmonellosis (1992 -6 6% 2008 r.-14.6%).

The causal structure ofsalmonellosis in humans and the serotypic profile of salmonella, registered in food products and environmental objects, often were the same. Common serovars, dominating in humans and food products were as follows: S, typhimurium, S. enteritidis, and S. infantis. Moreover, these salmonella were polyresistant to antibiotics.

The study results of animal salmonellosis showed significant infection of birds, pigs and cattle to a lesser extent. Salmonella, registered in animals, were distributed in 13 serological variants. Adapted Salmonella serovars were mostly registered. In the long-term dynamics of salmonellosis morbidity in Tashkent, there is a downward trend: in 1991 - 92.85 cases per 100 thousand people, in 2017 - 13.2, that is, over a 26-year period, the morbidity decreased by 6.7 times (Fig. 2).

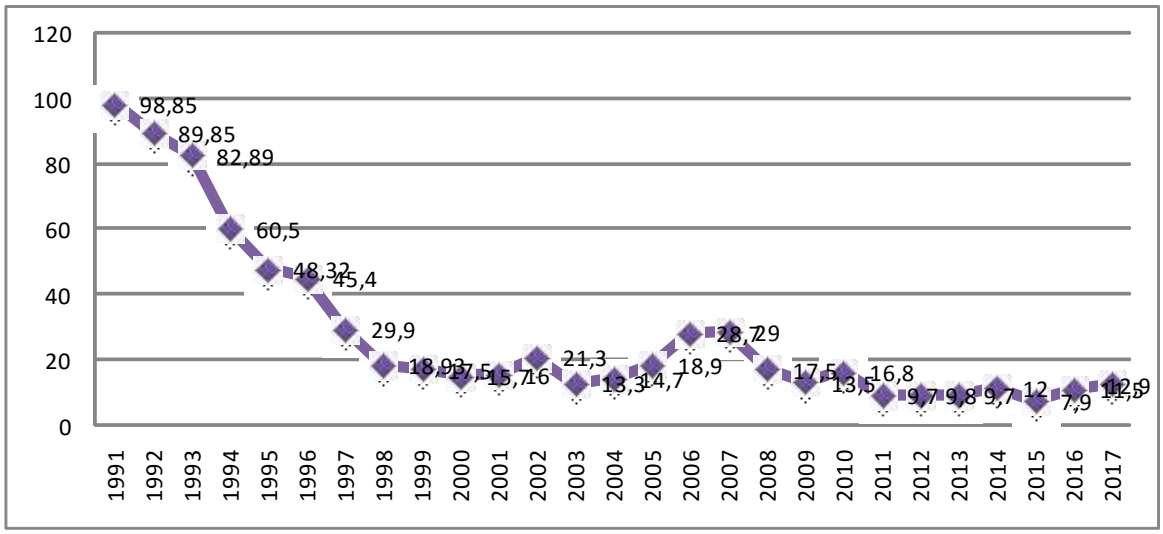


Fig. 2. The dynamics of salmonellosis morbidity in Tashkent during 26 years (1991-2017) in int. indices

The salmonellosis incidence is registered in all age groups. Social factors had the greatest effect on morbidity formation in children under the age of 14. We determined that children under the age of 14 are more likely to suffer from salmonellosis, and a significant number of patients are observed among adolescents of 15 years of age and older. The salmonella mostly enters the human body orally by consumption of meat and dairy products that do not meet hygiene requirements. Considering a great number of private catering facilities are organized, they often sell food, very popular among children and adolescents (fast food, pizza, grilled chicken, etc.), the control over food preparation and storage is not always proper, which is so it may be the cause of salmonellosis. All these factors present mass transmission pathogens in the period of a seasonal increase in the morbidity among older people[4].

The monthly distribution of salmonellosis morbidity was quite regular, with the highest rates in Tashkent recorded during hot months: up to 60% of cases were diagnosed during June-September. Such seasonal distribution is related to activation of nutritional transmission factor (meat, dairy products, vegetables, fruit). The role of open water as an additional factor in the transmission of infection is not excluded, since salmonella of the same serovars are detected in water as in humans. According to Kh.B. Magzumov's data (1975), in the conditions of

Uzbekistan, developed horticulture, vegetable growing, and picking vegetables and fruit in summer also contribute to salmonella infection, the special experimental studies have shown significant salmonella contamination of vegetables and fruit.

The epidemiological survey of the foci showed sporadic morbidity (92%). Multiple foci amounted to 8%. Most of the multiple foci were registered in children's hospitals, child care facilities and apartments. The foci were different by nature: food or household episodes; the causative agent was transmitted among children, especially in hospitals, in households and among adults with food products

Conclusions

1. In recent years, in the causal structure of salmonellosis, a decrease in proportion of S. typhimurium has been observed, S. enteritidis and S. infantis became more significant.
2. A long-term study (26 years) of the dynamics of of salmonellosis morbidity in Tashkent showed a downward trend.
3. In regions of the republic, cases of morbidity are distributed unevenly due to social changes: widespread development of private property, increased population migration, rapid development of the public catering system, etc.
4. The salmonellosis morbidity is registered in all age groups.
5. The morbidity is predominantly sporadic by nature, there are minor multiple foci related to food and domestic transmission.

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Source of Funding: Self

Conflict of Interest: Nil

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Effectiveness of Integrated Treatment Strategy for Diabetic Retinopathy in Patients with Diabetes Mellitus: A Case Report

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Abstract

Vascular pathologies are considered as severe complications of diabetes mellitus, which occur very frequently in patients. Namely, diabetic retinopathy, being one clinical form of diabetic angiopathy, may lead to distressing socioeconomic burden like decreasing visual acuity and blindness. In the literature, many treatment method have been described and used in many centres.

We present a case of 60-year-old diabetes sufferer, who underwent combined treatment strategy including panretinal laser coagulation, enzyme therapy, vitreoretinal surgery and intravitreal administration of an inhibitor of angiogenesis, which was highly effective in terms of restoring visual acuity with minimal periprocedural complications.

Keywords: *Diabetes mellitus, diabetic retinopathy, panretinal laser coagulation, vitreoretinal surgery, intravitreal administration of inhibitor of angiogenesis.*

Introduction

Diabetes mellitus is associated with vascular complications very frequently, which clinically presents with micro- and macrocircular disturbances. The basis of these disorders are hyperglycemia, metabolic disorders and insulin resistance due to the insulin deficiency and/or dysfunctions of its working mechanisms (Gurieva et al, 2019)[1].

Diabetic retinopathy (DR) is considered to be the fifth leading cause of visual impairment worldwide, and is a huge social and economic burden to the healthcare system[2]. Taking in account the escalating prevalence of DR in the world, WHO identified a variety of approaches to screening for the blindness prophylaxis, early diagnosis and treatment of the population in different conditions[4].

In the treatment of DR and diabetic maculopathy (DM), laser coagulation of the retina is remaining as the gold standard of the therapy due to its accessibility and long-lasting propitious effects after the treatment.[5]

Today, there are three main strategies in the treatment of patients with proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME): pharmacological (compensation of glycemia, control of blood pressure, metabolism correction)[6], laser and surgical (intravitreal administration (IVA) of anti-VEGF medicines and vitrectomy) (Fayzrakhmanov et al, 2017; Bhat et al, 2017; Figueira et al, 2018; Rong et al 2017)[7]. The combination of these treatment strategies often provides a more pronounced positive effect (Mitchell et al, 2011 and Nguyen et al, 2012)[8].

According to some researchers, anti-VEGF drugs are an important addition to the arsenal of treatment for PDR (Zhao et al, 2018)[9]. Efficiency and safety of local anti-VEGF therapy in DME was proved by a largeinternational multicenter studies undertaken in accordance with all rules of evidence based medicine (EBM) (Cheung et al, 2014 and Do et al, 2016)[10].

The complexity of the treatment of the PDR, in many cases, is defined by the absence of a unique opinion on the pathogenetic mechanisms of development of pathological processes in the posterior part of the eyeball, although for this issue is devoted a large number of studies (Yuldasheva, 2014).[11]

The purpose of the studywas to present a clinical case, demonstrating the efficiency of complex treatment of PDR and DME in a patient with second type diabetes mellitus.

Case Presentation: Patient S., was born in 1959, suffering from second type diabetes mellitus during 18 years. He admitted to the Clinical Ophthalmic Hospital of the Ministry of Health of the Republic of Uzbekistan in January 2015 with complaints of decreased vision, fog in front of both eyes. Vis OD = 0.04 sph - 3.0 D = 0.6-0.7. Vis OS = 0.05 sph - 4.0 D = Vis 0.8. The intraocular pressure of both eyes was normal. The front section is not changed. Local opacities of the lens. There are separate floating opacities, which is not fixed to the retina in the vitreous body.

In the fundus of eye: fibrovascular tissue along the temporal vascular arcades, neovascularization of the optic disc, cystic macular edema (up to 480 μm in height in the right eye, 420 μm in the left one). The level of glycemia ranges from 6.8 to 8.9 mmol/L. Microalbuminuria up to 0.086 g/l. Blood pressure was 130/80 mm.Hg. Diagnosis: Proliferative diabetic retinopathy, cystic macular edema, complicated with catarrh bilateral cataract. Second type diabetes mellitus, severe course, subcompensated stage.

In February 2015, to patient was performed panretinal laser photocoagulation in full volume (OD - 2350 coagulates, OS - 1693 coagulates). All stages of laser interventions went without complications. Visual acuity with correction OD - 0.2, OS - 0.7. On the left eye,we could manage to stabilize the pathologic process. In November of 2015, the patient admitted to our clinic with the presence of hemophthalmus in OD and a decrease in vision up to 0.06. Considering the freshness of the pathological process, the patient underwent resorption therapy with 0.5 ml Gemaza enzyme in combination with a solution of dexamethasone 0.5 in the same syringe. 5 parabulbar injection were performed. Wobenzym tablets were administrated orally (3 tablets three times per day), as systemic enzyme therapy. This drug is proved as absorbable agent in hemophthalmia. In the dynamics of observation, visual acuity improved to 0.2 with correction and it became possible to examine the eye fundus, where the growth of fibroglial tissue on the retina was observed ophthalmoscopically. At the control examination after 1 month in the right eye, the growth of the phenomenon of cystic macular edema and proliferation intensified with the development of traction retinal detachment. According to the results of OCT, the height of detachment of neuroepithelium in the macula increased to 759 microns on the right eye, epiretinal fibrosis proceeded in the central region. In February of 2016, patient was admitting with recurrent hemophthalmus and tractional retinaldetachment detected on the “B” scan, which were an indication for two-step vitreoretinal surgery on the right eye. The patient performed a subtotal vitrectomy 25 G with a temporary tamponade vitreal cavity perfluoroorganic compound substituting the silicon oil, removing epiretinal fibrosis and endolasercoagulation. During the postoperative period arterial hypertension was observed in the patient up to 180/100 mm Hg, which was cut off with medicines. Arterial blood pressure was steadily normalized by the use of ACE inhibitors at the level of 110/70 - 130/80 mm Hg. The glycemic range was between 5.7 and 7.2 mmol/L. Proteinuria up to 0.072 g/l. At the end of the surgical treatment, Vis OD = 0.1 n/corr. Vis OS = 0.05 sph - 4.0 D = 0.6. We detected a partial regression in fibrovascular proliferation. Three months later, on the right eye silicone oil was replaced with saline.

However, in May 2016, relapsed partial gemoftalms in the right eye first, and from July 2016 - in the left eye as well with decreased visual acuity to hand movement of a person, and 0.1, respectively. In the “B” scan,opacification was noted with the presence of cell suspension in the eye vitreous haze. Taking in account the previous experience that conservative therapy may not be effective due to the larger volume of hemorrhage in the vitreous body and the presence of neovascularization of disc of optic nerve, it was decided to intravitreal administration of an inhibitor of angiogenesis (IVAIA). From May to November 2016, patient had 4 injections of IVAIA on avitreal right eye, then from July to December 2016 4 injections were performed in the left eye. Injections were performed in the operating room under local anesthesia through the flat part of the ciliary body 27 G needle through tunnel access with dose of 1.25 mg. Postoperative examinations were carried out on the next day and after 7 days. During and after intravitreal injection of IVAIA we did not note any ophthalmic and somatic complications. During the treatment, active resorption of hemophthalmus, regression and obliteration of neovascularization, especially on the optic disc and in the vascular arcades, a decrease in fibrovascular proliferation, and a decrease in neuroepithelial edema in the macula were observed. As of March 2017, Vis OD = 0.1 sph - 3.5 D=0.3-0.4. Vis OS=0.08 sph - 3.75 D=0.5-0.6. Thickness of neuroepitheliumaccording to OCT OD

- 360 microns on OS - 257 microns. In October 2017, during the next subsequent examination noted the rapid progression of cataract in the right eye. Therefore, to the patient was performed facoemulsification of complicated cataract with IOL of Acrysof implantation on the right eye. The operation went without any complications. As of February 21, 2018. Vis OD = 0.3 sph - 1.5 D = 0.4-0.5. Vis OS = 0.1 sph - 4.5 D = 0.7-0.8. Objectively: OD - IOL in a capsule bag. In the lower parts of the vitreous cavity, fibrotic changes. Fundus of the eye: all parts of the retina adjoined, no fresh bleedings. Remains of glial tissue on the optic nerve disc and preretinal fibrosis in the lower segments are detected. Dystrophic changes in the macula, focal atrophy of the pigment epithelium. The thickness of the neuroepithelium on the OCT is 255 microns. OS - initial clouding of the lens. In ophthalmoscopy: optic disc is pale pink, borders are clear. In the course of the temporal vascular arcade, fibrovascular proliferation was reduced, there were no fresh hemorrhages. Architectonics of central part of macula is saved. The thickness of the neuroepithelium is 247 microns. The patient was under observation of an ophthalmologist at the place of residence, follow-up examinations are every three months, with the additional anti-angiogenic therapy if needed.

Discussion

In our opinion, this case report is very interesting for several reasons. During the entire period of observation, we used all method of treatment, ranging from conservative therapy with enzymes and hormones, further laser-coagulation of retina, vitrectomy with silicone oil tamponade, followed by its removal and intravitreal administration of an inhibitor of angiogenesis.

According to the standards of treatment of diabetic retinopathy, with complicated hemorrhagic changes, conservative enzyme therapy was proved and is effective in early treatment of the patient with partial hemophthalmia with relative visualization eye fundus.

The rest of the treatment method we used affect the level of VEGF-factor in the eye cavity: panretinal laser coagulation by blocking the production of neovascular factor in zones of poor perfusion, subtotal vitrectomy - by mechanically removed the VEGF from the cavity vitreous body, the introduction of an angiogenesis inhibitor by binding its molecules to a specific antigen. However, combined use of several anti-VEGF techniques demonstrates high efficacy in cases of advanced PDR.

In this clinical case, our IVA anti- VEGF technique once in every first 6 weeks (1 time in 1.5 months) 4 times, and then 1 time in 2-3 months for 2 years was enough effective. Intravitreal administering of VEGF inhibitors in standard dosage, not only inhibit the progression of neovascularization, epiretinal gliosis and cystoid macular edema development, but also ensured the preservation of the productive central vision. Our studies prove that stopping the processes of proliferation and neovascularization on timely prolonged, massive intravitreal administration of angiogenesis inhibitors is fully proved. Taking into account that with increasing duration of diabetes mellitus, pathophysiological processes in the retina cannot be improved independently, refractory edema of the macular zone does not spontaneously resolved, especially patients with the background of increasing arterial blood pressure are not guaranteed from relapse of hemophthalmus. So, we recommend to conduct IVAIA throughout the whole life for patients with indications.

Our 5-year observation period for patients with PDR who received intravitreal angiogenesis inhibitors showed that most of them managed to achieve improved functional results during the entire observation period and with a minimum number of IA injections after 3 years. During the observation period, no systemic adverse events were observed in the patient. Exceptions group consisted of patients who had undergone acute cerebrovascular accident or myocardial infaction during the last 6 months.

In the literature the potential risk of systemic side effects of intravitreal administration of VEGF-factor inhibitors are clearly described, namely - arterial hypertension, proteinuria, gastrointestinal bleeding, impaired wound healing process, which is particularly unfavorable in diabetic patients.

Conclusion

The case study shows that combined use of several anti-VEGF techniques guaranteesthe effectiveness of treatment for advanced proliferative diabetic retinopathy, complicated with DME and recurrent hemophthalmus. In planning vitrectomy after applying the anti- VEGF drugs, it is reachable to decrease fibrovascular tissue hemorrhages, therefore there is less intraoperative bleedingand, in many cases, it is fully avoidable this kind of complications.

Patient Consent: Written informed consent was obtained from the patientfor publication of this case report and any accompanying information related to this study. A copy of the written consent is available for review by the authors of the paper.

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Organ-saving Operations in Comprehensive Treatment of Lower-Ampullary Rectal Cancer

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Abstract

In recent decades, in many countries of the world there has been an increase in the incidence of colorectal cancer (CRC). The annual occurrence of 50 new cases of colorectal cancer per 100,000 population determines a 5% population risk of developing the disease throughout life 1. An alarming fact is that for the 100 newly diagnosed patients with CRC, more than 70 deaths occur, of which, in the 1st year from the moment of diagnosis, about 40%. This circumstance is due to the fact that when patients first go to the doctor, advanced forms of CRC (stage III-IV) are diagnosed in 62.4% of patients. In 25-30% of patients, distant metastases are detected at the time of surgery. In recent years, there has been a tendency to expand indications for organ-preserving operations in the CRC.At the same time, it is necessary to formulate clear criteria for their implementation on the basis of a comparative analysis of long-term treatment results, the frequency of local regional relapses, the development and implementation of techniques using staplers, as well as the justification of indications for the use of combined and complex treatment programs that increase the radicalism and ablasticity of surgical interventions.

Keywords: *Colorectal cancer, radiation therapy, ampullar colorectal cancer, perineal extirpation of the rectum.*

Introduction

The main and most effective method of treating CRC is the surgical method, in compliance with the principles of oncological radicalism. However, the use of only the surgical method of treating CRC demonstrates low efficiency - over the past 20-25 years, 5-year survival rates, which average 52%, have remained stably low. In this case, the cause of death of approximately 68% of patients is relapse of cancer after surgery, and the incidence of loco-regional metastases ranges from 20-40% 3. These failures determine the feasibility of finding ways to increase an acceptable and effective method of treatment through the use of combined and complex treatment method for CRC.

Recent decades have been characterized by the search for optimal regimens for combined and complex treatment of CRC by combining the surgical method with radiatiotherapy or chemotherapy in order to improve the treatment outcomes of a severe category of patients with a locally advanced process and the presence of metastases [4].

In modern clinical oncology, the search for the most effective method for combining surgical intervention with various options for radiation therapy (preoperative, intra- or postoperative), as well as large-fraction ones 5. At the same time, to date, there are no clear indications on the timing, doses and method of exposure to radiation therapy in PKK, which indicates the need to find ways to improve and increase the effectiveness of this method of exposure [6].

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Practical significance of the research results: Developed indications for organ-preserving surgical interventions for lower ampullar colorectal cancer, the most effective variants of chemoradiation method have been proposed in the practical health care system of Uzbekistan.

The developed variants of the schemes and method of combined and complex therapy, improvement of the surgical technique, method of correction and prevention of complications help to reduce the incidence of early postoperative complications, mortality rate, as well as the occurrence of relapses and metastases, which will increase the effectiveness of treatment, improve the quality of life of patients and reduce terms of their rehabilitation.

Based on the studies, the basic scientific provisions that are made are substantiated and formulated:

1. An analysis of the dependence of the number of postoperative complications in patients with colorectal cancer associated with performing surgical intervention showed that after organ-sparing surgeries, complications were observed in 14 (11.2%) patients, and after performing peritoneal perineal extirpation of the rectum (PPER) - in 14 (9.5%) patients. Analysis of postoperative complications of a general nature showed that their number did not depend on the type of surgical intervention.
2. On the example of 120 patients with rectal cancer with T3-4N0-1M0 stages of the disease, the possibility of performing organ-saving operations in this category of patients is shown. The presence of severe concomitant pathology along with the individual characteristics of tumor growth may serve as a contraindication to performing such operations.
3. New method have been developed for the complex treatment of patients with colorectal cancer, including various schemes of preoperative high-dose radiation therapy, as well as method of preoperative lymphatic polychemotherapy with subsequent organ-preserving operations.
4. The five-year survival rate for patients with colorectal cancer after complex treatment with organ-preserving surgeries was 75.2%, and in patients in the control group after complex treatment with PPER, 74.8%. Moreover, in the group of patients with organ-sparing surgeries after carrying out the method of lymphatic polychemotherapy, this indicator amounted to 76.1%, and after various schemes of high-dose radiation therapy - 73.0%.
5. Organ-saving operations in the complex treatment of patients with rectal cancer with T3-4N0-1M0 stages of the disease significantly increase the quality of life of this category of patients, their social and labor rehabilitation, without compromising overall and relapse-free survival rates, which makes it possible to recommend the use of these techniques in complex treatment of this category of patients.

Diagnosis and treatment of locally advanced colorectal cancer: The five-year survival of patients with CRC in the T1N0M0 stage after surgical treatment is 90%; T2N0M0 after combined treatment - 70-80%; T3N0M0 after combined treatment - 60-65%, and after only surgical treatment 50-55%. T1-3N1-3M0 after combined treatment is 50-55%, and after only surgical treatment - 25-30%. In the T4N0-1M0 stage, 10-15% .[8].

The situation when the neoplasm has exceeded not only the intestinal wall, but also the periintestinal tissue and has spread to nearby organs, or to the pelvic peritoneum, perivesical tissue, etc. commonly called locally distributed RC.9. More broadly, the concept of locally distributed RC may include the following definition: an immobile or limitedly mobile tumor, circularly covering all the walls of the intestine, 5 cm long, narrowing the lumen of the intestine with the involvement of neighboring organs and tissues and possible formation of fistulas or purulent cavities, and not having distant metastases [10].

The most adverse forms are mucous adenocarcinoma, cricoid-cell, undifferentiated cancer, and unclassified tumors. With a relatively small damage to the intestinal mucosa, they metastasize faster and more often spread not only to the entire intestinal wall, but also to surrounding tissues and organs. PKK can germinate in any adjacent organs or tissues. The bladder, small intestine, pelvic bones, ovaries, uterus, posterior wall of the vagina, prostate and seminal vesicles are most commonly affected. However, such germination is not always accompanied by distant metastasis, therefore, in such cases, it is advisable to conduct combined resections.

Local tumor growth and lymphogenous metastasis in RC are interrelated, but not always parallel. So, 15% of tumors limited by the muscle layer of the intestinal

wall are accompanied by lymphogenous metastasis. When germinating in all layers of the intestinal wall, the lymph nodes are affected in more than 50% of cases. At the same time, even when the tumor invades the neighboring organs (locally advanced forms of cancer), lymphogenous metastasis is often (30%) absent.

According to many authors, 70-80% of patients with RC enter clinics with stage III-IV disease. Among the reasons for the late diagnosis of RC, it should be noted the absence of a mandatory standard examination scheme for a patient who suspects this disease, the inadequate use of previously developed diagnostic method, and the limited use of new examination method [11].

The primary RC is characterized by the fact that the tumor remains localized for a long time and grows relatively slowly compared to other tumors of the digestive tract. Localized and slow growth is also characteristic of metastases to the lymph nodes. Given this biological feature of RC, it was suggested that by increasing the number of lymphadenectomies, a reduction in the number of relapses can be achieved. At the same time, according to many researchers, the average five-year survival of patients, even when the operation is expanded to total pelvioabdominal lymphadenectomy, ranges from 45 to 60% .[12].

In this regard, increasing the effectiveness of treatment for RC, today remains an urgent problem of modern oncology. In recent decades, there has been a tendency to expand indications for organ-preserving operations in the RC. Many authors note that even with a pronounced spread of the cancer process, distant metastases may be absent, and it is possible to perform extended surgical interventions 13. At the same time, it is necessary to formulate clear criteria for their implementation on the basis of a comparative analysis of the long-term results of treatment, the frequency of local regional relapses; the introduction of combined and comprehensive treatment programs that increase the flexibility of surgical interventions [14].

Modern directions of organ-preserving surgical treatment of colorectal cancer: Modern method of surgery for colorectal cancer are numerous. To choose one of them is directly dependent on the location of the tumor, its type of growth, degree of spread, its anatomical features, the patient's condition and the surgeon's qualifications. When choosing surgical tactics in patients with colorectal cancer with sprouting into neighboring organs, one should be guided not only by such criteria as the size of the tumor, its location, growth form and histological structure, which always determine the technical capabilities of radical surgery, but such the most important prognostic factor, as the depth of tumor invasion [15]. An operation can be considered radical only if, along with a sufficiently wide resection of the area of the intestine affected by the tumor, the zone of possible regional metastasis is also removed.

The main and most effective method of treating RC is the surgical method, in compliance with the principles of oncological radicalism. However, the use of only the surgical method of treating RC demonstrates low efficiency - for 20–25 years, 5-year survival rates after radical surgeries remain at 46–62%, which is an average of 52%. Many authors attribute the main causes of failures in the surgical method of treatment to the occurrence of relapses and distant metastases that occur at different times after surgery. The cause of death in approximately 68% of patients is cancer recurrence after surgery, and the incidence of locoregional metastases ranges from 20-40% .16. These failures determine the feasibility of finding ways to increase an acceptable and effective method of treatment through the use of combined and complex treatment method for RC.

Among the many factors that determine the nature and extent of surgical interventions, and first of all organ-preserving ones, the fundamental role belongs to the degree of local spread of the tumor process and the level of tumor localization in the rectum. In RPK, typical operations depending on the height of the primary tumor are: anterior resection, abdominal-anal resection of the rectum with sigmoid reduction, obstructive resection (Hartmann's surgery) and abdominal-perineal extirpation (BPE) of the rectum [17].

Common forms of RC are often the main reason for abandoning active surgical tactics. Often, patients in this group perform symptomatic palliative surgery, in which there are a large number of postoperative complications and high rates of postoperative mortality. At the same time, the quality of life of these patients is significantly deteriorating, which is due to the increasing effect on their body of the not removed primary tumor and the progression of metastatic growth. The average life expectancy of these patients does not exceed 3–7 months.[18]

The main argument of opponents of superradical

interventions is the high technical complexity and invasiveness of the latter. However, many surgeons do not agree with this judgment, since long-term world practice of performing combined and advanced operations not only allows us to hope for good long-term results, but also opens up great prospects for improving the quality of life of patients by performing simultaneous or delayed plastic surgeries [19].

The current trend in locally advanced RC can be attributed to the tendency to perform saving interventions in the form of trans-abdominal resection and abdominal-anal resection of the rectum, naturally, in the absence of oncologicalcontraindications. The postoperative mortality in these interventions is not much higher than that in conventional operations - for example, 30-40% of patients undergoing combined operations survive a 5-year period. One of the important contraindications for performing combined operations is the presence of concomitant diseases that cannot be corrected in the preoperative period, especially in elderly patients .[20].

Materials and Method

In our work, a retrospective analysis of the clinical data of 120 patients with colorectal cancer (CRC) with a T3-4N0-1M0 stage of the disease, who were treated in the Department of Oncoproctology, Republican Cancer Research Centre, Ministry of Health, Republic of Uzbekistan in the period from 2005 to 2015, was carried out.

Patients were divided into the main and control clinical groups. The main group included 60 patients with RC who underwent various types of operations during complex treatment: abdominal-anal resection of the rectum (AARR), anterior resection of the rectum (ARR), Hartmann's operation. The control group included 60 patients with RC who underwent abdominal-perineal extirpation of the rectum (APER).

In this case, the main and control groups of patients with RC were additionally divided into 3 subgroups depending on the nature of chemo- and radiatiotherapy (tables 1,2). Thus, patients in 1 subgroup received surgical treatment with endolymphaticpolychemotherapy (ELPCH) and regional lymphatic polychemotherapy (RLPCH) and hypoxiradiotherapy (HRT), in 2 subgroups received surgical treatment in combination with radiatiotherapy (RT), in 3 subgroups received surgical treatment in combined with ELPCH and RLPCH.

We did not include in the observation of patients with acute intestinal obstruction, requiring emergency surgery, with a generalization of the process in the abdominal organs, ascites, lymphostasis, the presence of distant metastases, as well as with severe concomitant diseases requiring specialized treatment.

Table 1: Distribution of patients with colorectal cancer depending on the type of treatment in the main group, n=60

Sub-Group	Type of operation	Combinationtherapy	Number of patients	
			Abs.	%
1	PRR, Hartmann's operation	ELPCHT	21	35,0
2	PARR	ELPCHT	23	38,3
3		LT	16	26,7
Total			**60**	**100**

Table 2: Distribution of patients with colorectal cancer depending on the type of treatment in the control group, n=60

Subgroup	Type of operation	Combination therapy	Number of patients	
			Abs.	%
1	PPER	ELPCHT+RLPCHT+HRT	19	31,7
2		ELPCHT+RLPCHT	24	40,0
3		LT	17	28,3
Total			**60**	**100**

A study of the distribution of patients by gender and age showed that in the main group of patients with RC there were 34 men (56.7%) and 26 women (43.3%), in the control group these figures were 36 (60%) and 24 (40%) respectively. The average age of patients in the main group was 49.1±0.8 years, in the control group - 48.9±0.7 years.

Table 3: Distribution of patients with colorectal cancer by gender and age in the control group, n=60

Age	Gender				Total	
	Men		Women			
	Abs.	%	Abs.	%	Abs.	%
18-45	18	30,0	9	15,0	27	45,0
46-60	11	18,3	8	13,3	19	31,6
60 and upper	7	11,7	7	11,7	14	23,4
Total	**36**	**60**	**24**	**40**	**60**	**100**

A study of the anamnesis of patients with PKK showed that in 17 (14.2%) of them, the duration of the disease was up to 3 months, in 42 (35%) - up to 6 months, in 36 (30%) - up to 1 year, in 13 (10.8%) - up to 2 years and 12 (10.0%) - up to 3 years (table 5).

Table 4: Description of anamnesis of patients with colorectal cancer, n=120

Anamnesis of disease	Patients groups				Total	
	Main		Control			
	Abs.	%	Abs.	%	Abs.	%
Up to 3 months	3	5,0	6	10,0	17	14,2
Up to 6 months	14	23,3	12	20,0	42	35,0
Up to 1 year	19	31,7	26	43,3	36	30,0
Up to 2 year	17	28,3	14	23,4	13	10,8
Up to 3 year	7	11,7	2	3,3	12	10,0
Total	**60**	**100**	**60**	**100**	**120**	**100**

This subjective indicator shows the presence of an erased RC clinic, as evidenced by a three- and six-month history in less than half of patients (44.1%).

When conducting a rectoscopic examination, the exact localization of the tumor process and its extent was established (table 6). As follows from the table, in the patients examined by us, the distal location of the tumor was mainly determined. So, during proctoscopy in 58 (48.3%) patients with CRC, the tumor was limited to localization in the anal canal. In rectoscopy in 50 (41.7%) patients, the tumor process was determined at a distance of 5–9 cm from the anus and in 12 (10%) patients at a distance of 10 cm or more from the anus level.

Table 5: Localization of the tumor process in relation to its location from the anus, n=120

Tumor localization	Patients groups				Total	
	Main		Control			
	Abs.	%	Abs.	%	Abs.	%
In the anal canal (up to 3-4 cm)	28	46,7	30	50,0	58	48,3
At a distance of 5-9 cm from the anus	26	43,3	24	40,0	50	41,7
At a distance of 10cm from the anus	6	10,0	6	10,0	12	10,0
Total:	**60**	**100**	**60**	**100**	**120**	**100**

In assessing the prevalence of the disease, the TNM system was used (8th edition, 2008) (table 7). As follows from the table, stage IIa of the disease occurred in 18 (30.9%) patients, IIb in 32 (19.1%) and stage IIIb in 70 (50.0%) patients. Moreover, in 32 (26.7%) patients there was a local spread of the tumor process (T4N0-1M0).

The study of the histological structure of tumors showed that adenocarcinoma was predominantly found in 106 (88.3%) patients. At the same time, moderately differentiated adenocarcinoma was found in 39 (32.5%) patients, low-differentiated adenocarcinoma in 31 (25.8%) and highly differentiated adenocarcinomas in 36 (30.0%) patients with RC. Cricoid cell adenocarcinoma was diagnosed in 5 (4.2%) patients. Other histological forms of tumors were found in 9 (7.5%) patients.

Endolymphatic and regional lymphatic administration of chemotherapydrugs.

Preoperative endolymphatic polychemotherapy (ELPCH) and regional lymphatic chemotherapy (RLPCH) were performed in 47 (39.2%) patients with CRC, of which 23 (19.2%) were patients in the main group and 24 (20.07%) were in the control group.

At the same time, 21 (17.5%) patients of the main group underwent ELPCHT and RLPT in combination with hypoxiradiotherapy (HRT); in the control group, this type of treatment was used in 19 (15.8%) patients. Other 23 (19.2%) patients of the main group and 24 (20.0%) patients of the control group received only ELPCH and RLPCH.

Thin polyethylene catheters (PE10) were used for cannulation of the lymphatic vessels (Fig. 1).

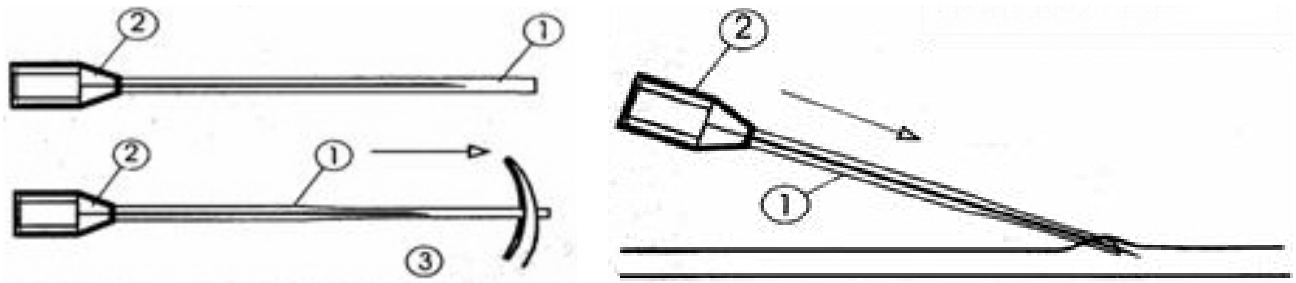


Fig. 1 Method of catheterization of the peripheral lymphatic vessel of the lower limb. 1 - mandrin of an epidural catheter; 2 - polyethylene catheter PE 10; 3 - clamp.

Direct introduction of the cytostatic agent into the lymphatic channel provides a direct supply of the drug to the tumor and to metastases in the regional lymph nodes. This leads to an increase in the antitumor effect of chemotherapy with a decrease in the general and local toxicity of chemotherapy.

The duration of ELCHT was 96 hours continuously:

1. Methotrexate (Mtx) - 50 mg/m2 for 24 hours in 24 hours, 1 day;
2. 5-fluorouracil (5-Fu) - 1000 mg/m2 for 24 hours, 2, 3 days - 48 hours.

RLPCH (with the preliminary introduction of a cocktail of lymphostimulants, consisting of: novocaine - 0.25% - 4 ml, heparin - 2500 U, furosemide - 0.5 ml):

1. Mtx - 50 mg/m2 1 time, 4 days;
2. 5-Fu - 1000 mg/m2 1 time, 5.6 days.

The catheter is cut 2-3 mm longer than the mandrel. The protruding end of the catheter is fixed with a clamp and stretched until the catheter becomes thin (0.25-0.45 mm), while it should fit snugly against the mandrel. Then the catheter is cut 1-2 mm shorter than the mandrel.

Lymphatic catheterization for long-term administration of drugs was carried out as follows. The site of the procedure was chosen large interdigital gap on the rear foot. After treating the skin with 5% iodine solution and 96% alcohol, 1 ml of 1% methylene blue or 0.4% indigo carmine diluted in 0.25% 1.0 ml novocaine solution is introduced into the first interdigital space to stain the lymphatic vessel; retreating 5-6 cm upwards, a longitudinal skin incision of 2-3 cm was performed, a stained lymphatic vessel was found, it was isolated from adipose tissue and a dental spatula was placed under it. Previously, two ligatures were performed under the vessel, which were fixed with clamps directed to the proximal part of the wound.

To enlarge the vessel in diameter, it was pinched, pressing it above the incision with fingers. A cotton swab produced massage below the incision. After filling the vessel and increasing its size in diameter, a dental spatula was brought to the distal part of the wound. The lymphatic vessel became inactive. The vessel was punctured in its lower part by a specially prepared catheter, which was fixed with ligatures in two places above the entry into the lymphatic vessel. Then the mandrinwas removed, the lymphatic vessel was checked for leaks by introducing a solution of novocaine through a catheter. 2-3 nodal sutures were applied to the skin. The free edge of the catheter was fixed with a band-aid to the skin. A needle was inserted into the free end and a chemotherapeutic system was connected. The drugs were injected with an electronic dispenser (ДЛВ-1) at a rate of 1.0 ml/h. within 72 hours. In this case, Mtx was administered continuously - 50 mg/day x m2 for 24 hours, on days 2 and 3 of 5-Fu - 1000 mg/day x m2 for 48 hours. Then the lymphatic catheter was removed, and after treating the wound with 5% iodine, an aseptic dressing was applied.

After 6 days, a peripheral lymphatic vessel was catheterized on the back of the other foot, and the remaining half of the course dose of chemotherapy was slowly introduced into the catheter.

On the 4th day, RLPCHT was performed by injecting drugs into pararectal fiber, taking into account the localization of the tumor (Fig.).

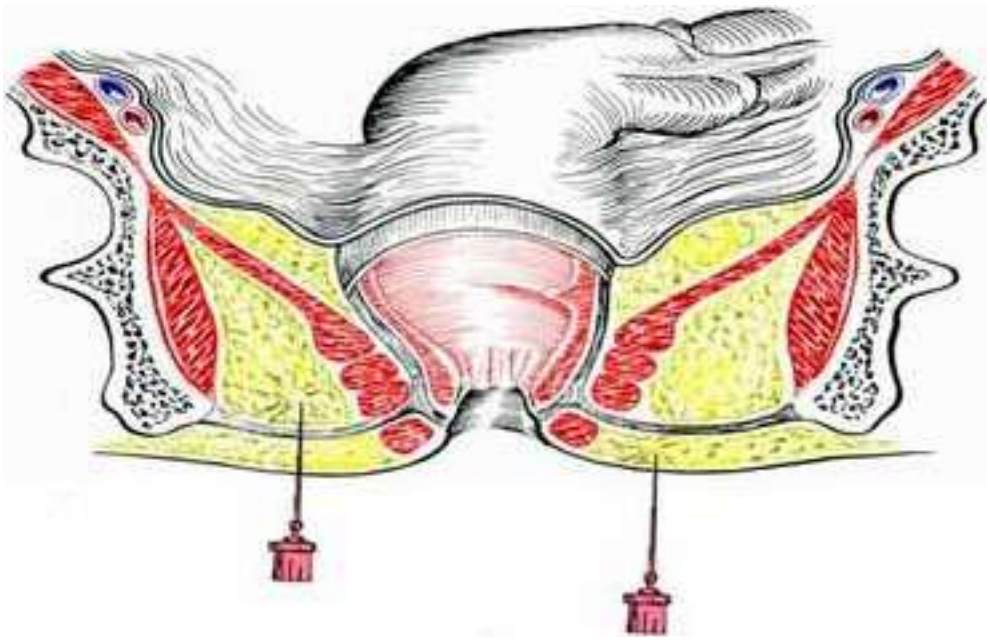


Fig. 2. Conducting regional lymphatic chemotherapy for colorectal cancer.

So, in case of a lesion of the anal canal by a tumor, the preparations were injected into the perianal tissue, and in case of a tumor of the middle and upper ampullar parts of the rectum, it was injected into the pararectal tissue closer to the tumor (i.e., to a depth of 8-10 cm from the skin).

To perform RLPCHT after thoroughly treating the perianal skin with 5% iodine solution and 76% alcohol, departing from the transition line 2 cm laterally, under the control of the finger inserted into the rectum, a needle was inserted into the pararectal tissue, leading its end towards the tumor.

For lymphostimulation, a cocktail of 0.25% 4.0 ml novocaine solution, 0.5 ml furosemide solution and 2500 IU heparin solution was administered. Then, without removing the needle, 3-5 minutes after complete lymphostimulation, the chemotherapy was administered through the same needle. Having removed the needle, a warming 40% alcohol compress was applied to this area for 5-6 hours. RLPCHT was performed on days 4 and 5, on day 4, Mtx was administered - 50 mg, then on day 5 5-Fu - 1000 mg of chemotherapy.

Along with the lymphotropic administration of water-soluble chemotherapy drugs, detralex was used 2 tablets 3 times a day during the course of chemotherapy in order to stimulate lymph drainage and lymphosorption in the lower extremities, to improve the penetration of chemotherapy drugs into the peripheral lymphatic channel and increase the concentration of these drugs in the lymph.

To prevent thrombosis in the lymphatic and venous bloodstream, a low molecular weight anticoagulant, fraxiparin, was administered at a dose of 0.3 ml subcutaneously 1 time per day during the administration of chemotherapy, under the control of PTI and VSK. In order to reduce the local cytotoxic effect by additional dilution of the residual amount of chemotherapy drugs in the tissues and at the same time to re-stimulate their lymphosorption, a 0.5% novocaine solution up to 50 ml was injected subcutaneously at the site of the previous chemotherapy injection, immediately after removing the cuff from the hip. At the end of the procedure for the lymphotropic administration of water-soluble chemotherapy drugs to improve venous outflow and prevent venous thrombosis of the lower extremities, elastic bandaging of the lower extremities was performed after subcutaneous administration of novocaine.

Surgery was performed 7-14 days after the last lymphatic administration of drugs, during the period of the most pronounced decrease in the biological activity of the tumor due to PCHT.

Effectiveness of treatment of patients with colorectal cancer depending from prognostic factors: The results of organ-preserving operations in the complex treatment of CRC were tracked for three and five years. Cases of relapses and metastases were analyzed, and the survival of patients over a three- and five-year period was assessed, depending on prognostic factors.

During the three-year follow-up, local relapses in the main group occurred in 10 (16.6%) patients with RC and in the control group in 13 (21.7%) patients. Relapses developed at follow-up from 6 to 27 months. The average relapse rate was 11,8±2,4 months.

Table 6: The frequency of relapse over a three-year period in patients with colorectal cancer, depending on the type of surgical treatment

Type of operation	The number of patients in groups	Patients groups			
		Main, n=60		Control, n=60	
		Abs.	%	Abs.	%
PRR with the anastomosis	Main, n=12	1	8,3	–	–
PARR with the relegation proximal colon in the anal canal	Main, n=14	2	14,2	–	–
PARR with there legation Sigma in anal canal	Main, n=25	5	20,0	–	–
PARR with colostomy	Main, n=6	1	16,6	–	–
Hartmann's operation	Main, n=3	1	33,3	–	–
PPER with sigmostomy	Control, n=60	–	–	13	21,7
Total		**10**	**16,6**	**13**	**21,7**

The results of a univariate analysis showed that the frequency of relapse over a three-year period in patients with RR was higher after the PPER in 13 (21.7%) patients than in organ-preserving operations in 10 (16.6%) patients.

The frequency of relapse over a three-year period in patients with colorectal cancer, depending on the type of preoperative lymphatic chemotherapy:

An analysis of the dependence of the recurrence rate on the lymphatic chemotherapy regimen revealed that, in the ELPCHT + RLPCHT + HRT regimen, a smaller number of tumor relapses was observed compared to the regimen without HRT. In the main group of patients, in the first case, relapses were observed in 3 (14.2%) patients, while in the case of PCHT without HRT, in 4 (17.3%) patients.

The number of relapses in the case of a single GRT of 13 Gray turned out to be slightly lower compared with the ROD of 5 Gy to SOD 25 Gy. So, in the main group of patients with RC, with a 13 GyGRT regimen, relapses were detected in 3 (14.2%) patients, and with a 5 Gy ROD regimen up to 25 Gy SOD, in 3 (18.7%) patients.

In stage IIa of the disease, the number of relapses was minimal and was observed in the main group in 1 (10.0%) patients with PKK, in stage IIb in 2 (12.5%), and in stage IIIb, the maximum number of relapses was found in 7 (20.6%) of patients.

The histological structure of the tumor also influenced the development of relapses in the distant postoperative period. The maximum number of tumor relapses in patients of the main group was found in undifferentiated form of cancer in 1 (33.3%) patients, in moderately differentiated adenocarcinoma in 5 (20.0%), and in low-grade adenocarcinoma in 3 (20.0%)) The least relapses were observed with highly differentiated adenocarcinoma - in 1 (7.1%) patients with CRC.

The macroscopic form of tumor growth also significantly affected the number of recurrences of RC. In the endophytic form of growth in the main group, we observed the highest number of relapses - in 4 (25.0%) patients, in the exophytic form - the smallest number of relapses - in 1 (7.1%), and in the mixed form of tumor growth, relapses were found in 5 (16.7%) patients.

Over a three-year period, tumor metastases to distant organs (liver and lungs) occurred in the main group in 5 (8.3%) patients with RC and in the control group in 4 (6.7%) patients. Metastases developed in the period from 6 to 18 months, which averaged 11,3±2,5months.

Moreover, the type of surgical intervention significantly influenced the development of metastases in the distant postoperative period, which is associated with various morbidity and volume of resected tissues of various surgical techniques. So, after Hartmann's operation, distant metastases occurred in 2 (66.7%) patients, after PARR with the relegation of the colon into the anal canal - in 1 (7.1%), after PPER with sigmostomy - in 4 (6.7%) and after PARR with the relegation of tyesygma into the anal canal - in 2 (8.0%) patients.

As a result of ELPCHT + RLPCHT in combination with HRT, distant metastases developed in 1 (4.8%) patients with major group RC, while metastases were detected in 2 (8.7%) patients with the same chemotherapeutic effect, but without HRT.

At the same time, we did not observe significant differences in the incidence of metastases over a three-year period in patients with colorectal cancer depending on the type of preoperative radiation therapy. So, after a single GDT at a dose of 13 Gy in the main group, metastases developed in 1 (4.8%) patients, and after an irradiation regimen of ROD of 5 Gy to SOD of 25 Gy - in 1 (6.2%) patients.

The incidence of metastases in the distant postoperative period also depended on the stage of the tumor process. So, in stage III b, metastases were detected in 4 (11.7%) patients of the main group, in stage II b - in 1 (6.2%) and metastases were not observed in stage II a.

The histological structure of the tumor also influenced the development of distant metastases. In the main group of patients with cricoid-cell form of cancer, metastases were found in 1 (100%) patients, with low differentiated adenocarcinoma - in 1 (6.7%), and with moderately differentiated adenocarcinoma - in 3 (12.0%). With highly differentiated adenocarcinoma, metastases were not observed.

In the endophytic form of tumor growth, metastases in the long-term postoperative period were determined in 2 (12.5%) patients of the main group, in the exophytic form of growth in 1 (7.1%), and in the mixed form of tumor growth in 3 (10.0 %) patients with RC.

An analysis of the three-year survival of patients with RC, depending on the type of surgical treatment performed, showed that the highest rate was observed after RC with anastomosis, where it was 91.7%. 3-year survival of patients after PARR with relegation of the colon was 85.7%, after PARR with relegation of sigma into the anal canal - 84.0%, after PPER with sigmostomy - 81.6%, after PARR with colostomy - 83.3%, and after Hartmann's operation - 66.7%.

A study of the three-year survival of patients with RC depending on the type of preoperative lymphatic chemotherapy showed that after the treatment regimen ELPCHT + RLPCHT + HRT, this indicator in the main group was 86.4%, and after ELPCHT + RLPCHT without HRT - 83.7%.

At the same time, the three-year survival rates after preoperative radiation therapy were almost identical and amounted to 83.8% after a single HRT at a dose of 13 Gy in the main group, and 80.6% after an ROD of 5 Gy to an SOD of 25 Gy.

The stage of the disease significantly affected the indicators of three-year survival of patients with RC. So, in the main group of patients, this indicator in stage IIa was 90.0%, in stage IIb - 87.5%, in stage IIIb - 79.4%.

The histological form of the tumor also significantly affected the three-year survival of patients with RC. In the main group of patients with cricoid adenocarcinoma and with undifferentiated cancer, mortality of patients for this period of time was not observed. With highly differentiated adenocarcinoma, this indicator was 92.8%, with moderately differentiated adenocarcinoma - 88.0%, with low-grade adenocarcinoma - 80.0%. Three-year survival rates for low-grade cancer were 33.3%.

Three-year survival with the exophytic form of tumor growth in the main group of patients with RC was 92.8%, with the endophytic form - 75.0% and with the mixed form of tumor growth - 83.3%.

An analysis of the five-year survival of patients with PKK, depending on the type of surgical treatment performed, showed that the highest rate was observed after PRR with anastomosis, where it was 83.3%. the five-year survival rate of patients after PARR with relegation of the colon was 71.4%, after PPER with sigmostomy - 81.6%, after PARR with sigma relegation in the anal canal - 68.0%, after PARR with colostomy - 50.0%, and after Hartmann's operation - 66.7%.

A study of the five-year survival of patients with RC depending on the type of preoperative lymphatic chemotherapy showed that after the treatment regimen ELPCHT + RLPCHT + HRT, this indicator in the main group was 76.1%, and after ELPCHT + RLPCHT without HRT - 69.6%.

At the same time, the five-year survival rates in the main group of patients after preoperative radiation therapy were slightly lower and amounted to 76.2% after a single HDT at a dose of 13 Gy in the main group, and 62.5% after the radiation treatment regimen of 5 Gy to SOD 25 Gy.

The stage of the disease significantly affected the five-year survival of patients with RC. So, in the main group of patients, this indicator in stage IIa was 80.0%, in stage IIb - 81.2%, in stage IIIb - 61.8%. The histological form of the tumor also significantly affected the five-year survival rate of RC patients.

In the main group of patients with undifferentiated cancer, with cricoid adenocarcinoma, with mucous cancer, with squamous cancer, five-year survival was not observed. With highly differentiated adenocarcinoma, this indicator was 78.6%, - 80.0%, with moderately differentiated adenocarcinoma - 88.0%, with low-grade adenocarcinoma - 60.0%.

Five-year survival with an exophytic form of tumor growth in the main group of patients with RC was 78.6%, with an endophytic form - 56.2% and with a mixed form of tumor growth - 73.3%.

Conclusions

1. An analysis of postoperative complications of a general nature showed that their number did not depend on the type of surgical intervention. The rather large clinical material shows the possibility of performing organ-preserving operations in patients with lower ampullar cancer of the rectum.
2. A new method has been developed for the complex treatment of patients with colorectal cancer, including preoperative high-dose hypoxiradiotherapy according to schemes with a single exposure at a dose of 13 Gy (in 37 patients) and irradiation with medium fractions of 5 Gy up to SOD 25 Gy (in 36 patients), followed by organ-conserving operations. The five-year survival rate for patients with colorectal cancer after endolymphaticpolychemotherapy and regional lymphatic chemotherapy with subsequent

organ-preserving surgeries was 69.6%, with the addition of preoperative hypoxiradiotherapy to 76.1%. The five-year survival rate after a single hypoxiradiotherapy at a dose of 13 Gy with subsequent organ-preserving surgeries was 76.1%, and after an irradiation regimen of ROD of 5 Gy to SOD of 25 Gy - 62.5%.

3. A new approach has been developed for the complex treatment of patients with colorectal cancer, including preoperative endolymphatic polychemotherapy and regional lymphatic polychemotherapy (in 47 patients), as well as in combination with hypoxiradiotherapy (in 40 patients), followed by organ-preserving operations.

4. An analysis of the five-year survival of patients with RC, depending on the type of surgical treatment, showed that the highest rate was observed after PRR with anastomosis, where it was 83.3%. the five-year survival rate of patients after PARR with relegation of the colon was 71.4%, after PPER with sigmostomy - 81.6%, after PARR with sigma relegation in the anal canal - 68.0%, after PARR with colostomy - 50.0%, and after Hartmann's operation - 66.7%. In patients of the control group, the five-year survival rate after performing PPER with sigmostomy was 68.3%.

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Microhemodynamic Parameters of Cortical Substance of Kidneys at Experimental Hydronephrosis

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Abstract

Under conditions of unilateral occlusion of the ureter, the development of microhemodynamic disorders of the cortical substance of the kidneys was studied both on the damage side and on the contralateral (opposite) side. It has been established that disorders on the side of occlusion depend on the duration of obstruction of the lumen of the ureter, which determines the development of tissue hypoxia and violates trophic renal parenchyma. Dynamically functional disorders of the microvasculature occur at the early (on the 1st, 3rd, and 5th day), and irreversible changes in the renal parenchyma at the later (on the 14th and 30th day) stages of the experiment. The contralateral one responds to turning off the function of one kidney by adaptive restructuring of the microhemocirculatory system, which is manifested by dilatation of microvessels and an increase in the linear velocity of blood flow in them, which ensures its hyperfunction.

Keywords: *Microcirculation, rheology, hydronephrosis.*

Introduction

Hydronephrotic transformation of the kidneys as a result of impaired passage of urine is one of the most common forms of pathology, leading, as a rule, to the destruction of the affected kidney, a complete loss of its function[5]. Most studies on the activity of the kidneys in obstruction of the urinary tract reveal various aspects of the pathogenesis of hydronephrosis[7], structural changes in the renal parenchyma[6], and mechanisms of sanogenesis during restoration of urinary tract patency[9]. Recently, researchers have been paying close attention to the pathogenesis of microhemodynamic tissue disorders[1]. Changes in microhemodynamics lead to a decrease in adequate tissue perfusion, which naturally causes deep metabolic disturbances[4]. Disorders in the microcirculation system are the central link in the chain of disorders leading to the development of dysfunction and irreversible structural changes[7]. In the kidney, which performs the function of fluid filtration and absorption and is a well-vascularized organ with two capillary networks, pathological changes in microcirculation become extremely important. However, until now, insufficient attention has been paid to the study of the role of microhemodynamic disorders in the dynamics of the development of hydro-nephrotic transformation, which is largely due to the unsatisfactory state of the methodological base for obtaining reliable results. The current level of development of television technology allows you to plan and conduct intravital studies of internal organs in the dynamics of the development of various pathological conditions. The purpose of the study was to study the dynamics of microhemodynamic disorders of the cortical section of the renal parenchyma with unilateral full ureteral occlusion.

Materials and Method

Under the conditions of the experiment, we studied the state of microhemodynamics of the cortical region of both kidneys during the development of unilateral hydronephrosis. The experiments were conducted on 90 (48 experimental, 36 false-operated, 6 intact) outbred adult males. Occlusion of the right ureter was carried out by its complete ligation. Microcirculation of the renal cortex was examined on the 1st, 3rd, 5th, 7th, 14th and 30th days after ureter ligation. Kidney bio microscopy was performed under general anesthesia with etaminal at a dose of 8 mg/100 g of body weight of the animal.

The control was animals that underwent a laparotomy with revision of the retroperitoneal space without ureter ligation. In order to optimize research and improve the quality of the results obtained, we used a system of television analogs of the digital conversion of microstructures with morphometry of the microcirculation parameters. The results of the study were recorded on digital media.

Results

Normally, under the microscope, single efferent arterioles and peritubular capillaries are visible. The diameter of arterioles varies between 18.7–25.8 μm. The bloodstream in them is jet, in a continuous flow. The linear velocity of blood flow in arterioles was (0.557±0.073) m. Peritubular capillaries extending from the efferent arteriole, widely anastomosing each other, form a network with polygonal cells, extend along the tubules. The boundary between the tubules and capillaries differed by: due to whitish crimped stripes that appear when light is refracted by the epithelium of the tubules. It is noteworthy that almost all capillaries visible in the field of vision function. The diameter of the capillaries is - (9.70±0.38) μm of blood flow in them in a continuous flow, jet, blood flow speed –(0.446±0.044) ms.

Discussion

During studying the microvasculature, after the first day, the angioarchitectonics of the vessels were preserved on the obturated side, the individual capillaries were turned off and filled with blood plasma. They alternated with dilated capillaries in which blood flow was slowed down by 61.2% compared with the values of intact animals (table). The contours of the capillaries are even, clear. During this period, on the contralateral side, all the vessels visible in the field of view functioned, which were somewhat dilated. In individual PICs, the colloidal contents of the blood stream are fine-grained. In the control group of animals, the microvasculature was characterized by a distinct contour of the vascular bed, an insignificant (by 19.1%) decrease in the linear velocity of blood flow.

Table 1: Morphometric parameters of microhemocirculatory channel of animal kidneys with occlusion of the right ureter

Term of the experiment, day	Diameter of the capillaries of PICs, μm		Blood flow speed, ms	
	Right kidney	Left kidney	Right kidney	Left kidney
Intact animals	9.70±0.38		0.446±0.044	
1st	$\frac{10.60 \pm 0.84}{9.20 \pm 0.66}$	$\frac{9.60 \pm 0.51}{9.10 \pm 0.72}$	$\frac{0.173 \pm 0.034*}{0.381 \pm 0.043}$	$\frac{0.361 \pm 0.023}{0.389 \pm 0.057}$
3rd	$\frac{10.12 \pm 1.09}{9.50 \pm 0.71}$	$\frac{9.80 \pm 0.34}{9.70 \pm 0.82}$	$\frac{0.195 \pm 0.045*}{0.397 \pm 0.093}$	$\frac{0.339 \pm 0.022}{0.410 \pm 0.062}$
5th	$\frac{11.20 \pm 0.33*}{9.60 \pm 0.59}$	$\frac{10.20 \pm 0.48}{9.90 \pm 0.76}$	$\frac{0.142 \pm 0.036*}{0.431 \pm 0.063}$	$\frac{0.427 \pm 0.039}{0.410 \pm 0.055}$
7th	$\frac{12.30 \pm 0.68*}{9.80 \pm 0.55}$	$\frac{10.70 \pm 1.37}{9.50 \pm 0.63}$	$\frac{0.126 \pm 0.031*}{0.428 \pm 0.026}$	$\frac{0.461 \pm 0.067}{0.425 \pm 0.055}$
14th	$\frac{13.70 \pm 0.92*}{9.30 \pm 0.72}$	$\frac{11.20 \pm 0.64}{9.40 \pm 0.66}$	$\frac{0.090 \pm 0.013*}{0.415 \pm 0.041}$	$\frac{0.526 \pm 0.028*}{0.427 \pm 0.034}$
30th	$\frac{}{9.80 \pm 0.65}$	$\frac{12.60 \pm 0.71*}{9.6 \pm 0.8}$	$\frac{}{0.435 \pm 0.059}$	$\frac{0.533 \pm 0.032*}{0.423 \pm 0.021}$

Note: Numerator - values of experimental group, denominator - false-operated.
*The results are reliable in relation to control (P<0.05)

Three days later the ligation of the ureter on the obturated side, the areas of dilated capillaries alternate with the areas that function normally: without signs of stagnation. The blood flow velocity in the capillaries is - (0.195±0.045) mm/s continuous, even flow. The contours of the vessels are clear, correct, without pathological changes. Observed separate sections of blood vessels, clogged stagnant blood, as well as the foci of the capillary network that are emptied, are erased in these sections of the boundary between the individual PIC loops. On the 3rd day of the experiment, in the opposite kidney, there was an expansion of blood vessels with streaming continuous blood flow and some homogenization and the disappearance of the characteristic glow of the PIC epithelium. On the 5th day of the experiment, the angioarchitectonics were disrupted on the obturated side, only a few sharply expanded capillaries function, the blood flow in them is grainy, the boundaries are somewhat blurred. Most of the capillaries are turned off from the bloodstream. The boundary between the capillaries and the PIC is blurred. The renal parenchyma in these areas is homogenized. From the side of the opposite kidney, total abrasion of the vessels was observed. The blood flow velocity in them exceeded the corresponding indicator at the previous study period by 25.9% (see table). Plots of diapedesis are marked in places, which indicates o increased permeability of capillaries PIC. In these areas, the PIC is filled with dense contents.

On the 7th day of the experiment, the angioarchitectonics of the kidneys was disrupted due to the disappearance of the microvessels and PIC circuits, which are homogenized in most areas. Along with this, there are areas of functioning capillaries, which are sharply expanded, the blood flow in them is slow, granular, sometimes segmented. Identified areas of clogging of capillaries of stagnant bloodstream. The boundaries between the PIC loops are erased. In contrast to the obstructed, contralateral night was characterized by areas of vasodilation.

The borders of vessels are clear, without turning off. The blood flow is-(0.461±0.067) mm/s, which is slightly higher values of the intact group of animals.

On the 14th day, the renal antioarchitectonics was preserved, the contours of the vessels were smooth and clear, alternation of half-blood capillaries with plasmatized, collapsed, non-functioning ones was noted. blood flow continuous, trickle, fast. Individual PIC capillaries are sharply expanded, overflows with stagnant blood. The contours of the non-functioning vessels are blurred. There are separate sections of perivascular hemorrhages. On the opposite side, the angio-architectonics of the kidney is preserved, all vessels visible in the field of vision are functional. The contours of the vessels are even, clear. Perivascular space without inclusions. The bloodstream is fast, stream, continuous its speed is 11.8% lower than the values of the intact group of animals.

On the 30th day of the experiment, almost complete destruction of the kidney parenchyma was observed at the occlusion side. The vascular bed of the cortical crown of the affected kidney was practically undetectable. A lot of scraps of starting plasmatized and hyalinized capillaries appeared in separate thrombus capillaries. In the opposite kidney, previously detected compensatory manifestations in the microhemocirculatory system were noted: the vessels were dilated, their circuits were clear continuous; a small tortuosity of the vessels due to an increase in pressure in them.

Thus, in animals experiment a progressive decrease in renal blood flow on the side of the obstruction was established, due to an increase in the resistance of the renal microvessels associated with thrombosis and the removal of most peritubular from the bloodstream, their destruction due to a sharp increase in hydrostatic pressure. These changes, in turn, lead initially to focal, and later dates (on the 14th and 30th days of the experiment) - to diffuse necrosis and atrophy of the kidney parenchyma. No disturbances were detected in the false-operated animals. During the entire study period, the vascular architectonics were preserved, the boundary between them and the loops of the proximal convoluted tubules was clear. The blood flow in the components of the microcirculatory system accessible for microscopy was jet, in a continuous flow. The mental blood flow indices in microvessels practically did not differ from those of intact animals.

Thus, a comparative analysis of the results of the study in the experimental group and the group of false-operated animals allows us to conclude that the characteristic dynamics of unidirectional changes in the early stages is really associated with the activation of the sympathetic-adrenal system. The absence of any changes in the microcirculatory system of the kidneys in the later stages of the experiment, when the influence of stress factors associated with operating trauma is completely

excluded, once again confirms the significance of stress factors in the occurrence of microcirculatory disorders.

Conclusion

Disorders of the microvasculature of the kidneys on the side of occlusion depend on the duration of obstruction, which contributes to impaired trophism of the renal parenchyma, the development of tissue hypoxia and the emergence of dynamic functional disorders in the early days (on days 1, 3, 5) and irreversible changes in the renal parenchyma at a later date (on the 14th and 30th day) of the experiment. 2. To turn off the function of one kidney, the other responds by adaptive restructuring of the microhemocirculatory system, manifested by dilatation of microvessels and an increase in the linear velocity of blood flow in them, which ensures its hyperfunction.

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Health Quality Assessment of Patients with Toxic-Diffuse Goiter

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Abstract

In recent decades, there has been a significant increase in the number of patients with thyroid disorders[1,4]. The widespread introduction of highly informative diagnostics method (ultrasound, fine-needle aspiration biopsy, tests on hormones and levels of antibody) and screening studies into medical practice led to an increase in detection rate of thyroid disorders[4,5,8]. Among patients with thyroid pathology, the thyrotoxicosis syndrome significantly complicates the course of thyroid disorders, which makes it difficult to choose a rational treatment strategy, affects the long-term result of treatment, and significantly worsens the disease prognosis[8,10].

Keywords: *Thyroid disorders, Graves' disease, goiter, patient, grave.*

Introduction

In clinical practice, it is appropriate to distinguish 3 main diseases that occur with thyrotoxicosis syndrome: toxic diffuse goiter (Graves' disease), multinodular toxic goiter (MTG), and toxic adenoma (TA) of the thyroid gland. The surgeons most often face such disorders. In more rare cases, the cause of thyrotoxicosis development is iodine-induced thyrotoxicosis and inflammatory thyroid disorders - thyroiditis[8]. The thyroid disorders with thyrotoxicosis syndrome have characteristics of serious thyrotoxicosis complications that significantly worsen the course of the disease and its prognosis and influence the quality of life, such as endocrine ophthalmopathy, cardiomyopathy, arrhythmia, heart failure, weight loss, heat intolerance, insomnia, muscle weakness, pretibial myxedema, women often have menstrual disorders, men – gynecomastia, decreased libido, erectile dysfunction [1]. In case of Graves' disease, the endocrine ophthalmopathy (EOP) develops in 25-70% of cases[3], pretibial myxedema – in 2%[1]. In 5-22% of all cases the thyrotoxicosis syndrome occurs with cardiomyopathy, especially among elderly people that suffer from MTG[8]. The toxic diffuse goiter is a disease with such characteristics as increased production of thyroid hormones and diffuse growth of the thyroid gland (TG) of various degrees. The Graves' disease incidence varies from 30 to 200 per 100 thousand people within the course of one year. In regions with normal iodine supply, Graves' disease is the most common cause of persistent thyrotoxic condition, and in iodine-deficient regions, Graves' disease competes with the functional autonomy of the thyroid gland (nodular and multinodular toxic goiter) within the etiological structure of Graves' disease toxic goiter [4]. Graves' disease is one of the main causes of the endocrinological help request among population, it includes 80% of all thyroid hyperfunction cases and is most common in the age of 20 to 40 years; the disorders rate among women is in 10-20 times larger. The presence of symptoms that complicate the course of the disease undoubtedly negatively influence the health, performance and quality of life of patients [11]. In accordance with the evidence-based medicine, the study of the quality of life (QOL) among patients is mandatory in order to determine the efficiency of the treatment method [2]. The key treatment method of thyroid disorders with thyrotoxicosis syndrome ca n be combined into three groups: complex medicamentous therapy, radiotherapy (RT) and surgical treatment, used

separately and in various combinations. Each method has both advantages and disadvantages, which is a wide topic for discussion in the literature[12]. The standards of patients management with thyrotoxicosis (in accordance with the Russian, American, European, Chinese, Korean, Japanese, Australian, Latin American thyroid associations) differ significantly [13]. QOL (quality of life) is an indicator of a patient's physical, psychological, emotional and social functioning on the basis of their subjective condition perception[14].

The purpose of the research: To study the degree of toxic diffuse goiter incidence among thyroid pathologies and assess the quality of life among urban population of Urgench (Khorezm region, Uzbekistan) on the basis of the received therapy.

Materials and Method

The study included patients with thyroid pathology who sought medical service of endocrinological dispensary in Urgench from 2014 to 2019. We analyzed the medical history of 697 patients with thyroid pathology retrospectively. All patients passed the complete physical examination: General Clinical researches; hormones level detection (total T3, SV. T4, TSH); immunological indicators examination (Ab-r TSH,Ab-TPO). In addition to hormonal research, there was an ultrasound examination of the thyroid gland. There were scintigraphy and fine-needle biopsy of the thyroid gland among patients with nodules (by indications). We examined the timely visits of patients to endocrinologists, assigned standard therapy of toxic diffuse goiter. In order to study the quality of life among patients with Graves' disease, we used the SF-36 quality of life questionnaire, which reflects patients 'satisfaction with their physical and mental condition, social functioning, and self-assessment of the pain syndrom severity. We determined the sum of total score by 8 parameters: physical functioning, role functioning, pain intensity, general medical condition, vitality, social functioning, emotional functioning, and psychological health. The questionnaire has such design that a higher score reflects a higher level of quality of life. In addition, 20 healthy volunteers also completed a questionnaire to obtain benchmark indicators specific to healthy individuals. This questionnaire has Uzbek translation, and the patients managed to answer all the questions by themselves.

We used the quantitative assessment in regard to the following indicators: PF – physical functioning which reflects the extent to which the health state limits the performance of physical loads such as self-care, walking, stair climbing, heavy loads handling, body bending, and significant effort tolerance; RP is the influence of physical state on the role functioning (work, daily activities); BP – bodily pain and its impact on the ability to engage in daily activities like household duties inside and outside of the house; GH – general medical condition – health condition assessment by the patient at the moment and the prospects of a cure, resistance to disease; VT – vitality, a rise of vitality means feeling full of energy or, on the contrary, weakened; SF – social functioning, determined by the degree to which physical or emotional condition restricts social activities (communication); RE – influence of emotional state on role functioning, seeks to assess the extent to which emotional state interfere with work or other regular daily activities, as well as a heavy timetable, descrease of workload and its quality; MH – characteristics of mental health (depression, anxiety, general indicator of positive emotions).

The research materials were subject to statistical processing by means of statistics software packages Excel 2003, STATISTICA 6.0 (Stat-Soft, 2001).

Research Results and their Discussion

Table 1: The number of patients with thyroid cancer who applied to the endocrinological dispensary in the city of Urgench.

№	Nosology	Quantity
1.	Toxic diffuse goiter	109
2.	Diffuse-nodular goiter	24
3.	Toxic adenoma	45
4.	Diffuse goiter with euthyroidism	136
5.	Nodular goiter with euthyroidism	98
6.	Diffuse-nodular goiter with euthyroidism	72
7.	Autoimmune tireodit of thyrotoxic phase	23
8.	Autoimmune thyroidit with euthyroidism	79
9.	Condition after strumectomy	66
10.	Cysts of the thyroid gland	44
11.	Thyroid cancer	1

We examined the medical history of 697 patients with thyroid pathology. Among them there were 453 women and 244 men within the age range from 18 and

65. The duration of the disease with thyrotoxicosis was on the average of 8.3±2.1 years. The analysis of the patient's medical history revealed 109 (15.63%)patients with (Graves' disease), diffuse-nodular goiter among 24 (3.4%), toxic adenoma among 45 (6.48%), diffuse goiter with euthyroidism among 136 (19.51%), nodular goiter with euthyroidism among 98 (14.1%), diffuse-nodular goiter with euthyroidism among 72 (10.3%), autoimmune thyroiditis (AT) thyrotoxic phase among 23 (3.3%), AT euthyroidism among 79 (11.33%), condition after strumectomy among 66 (9.5%), thyroid cysts among 44 (6.31%), thyroid cancer in case of 1 (0.14%) patient (table 1).

The examination of the patient's medical history revealed that 178 (25.5%) patients within the group of the examined patients had thyrotoxicosis syndrome. We verified the thyrotoxicosis syndrome by the determination of thyroid hormones. The patients with Graves' disease had the average level of TSH around 0.1±0.02 MED/l, SV. T3-7,8±1.1 PG/ml, SV. T4-22.6±2.4 PG/ml, antibodies in relation to TSH receptors 2.3±1.2 IU/l, antibodies in relation to thyroperoxidase (TPO) - 58.7±3.1 IU/l (table.2.) Whereby, the reference values for TSH were 0.3-4.5 MED/l,for SV. T3 2.0-4.2 PG/ml, for ESV.T4-8,9-17,2 PG/ml, antibodies in relation to TPO-0-30ME/l, antibodies in relation to TSH receptors 0-1,49 IU/l.

Table 2: Changes in thyroid hormones in accordance with the pathology

Nosology of TG pathology	Control group, n=20	Graves ' disease, n= 109	Diffuse-nodular goiter, n= 24	Toxic adenoma, n=45
TSH, mU/l	2,2±0,7	0,1±0,02*	0,1±0,05*	0,1±0,02*
SV. T3, pg/ml	0,8±0,3	7,8±1,1*	6,2±1,3*	6,5±1,6*
Sv. T4, pg/ml	14,2±0,9	26,7±0,3*	29,4±0,4*	23,4±0,3*
Antibodies to TSH receptors, IU/l	0,3±0,02	3,4±0,5*	2,5±0,1*	2,9±0,5*
Antibodies to thyreoperoxidase, IU/l	20,6±3,4	58,7±3,1*	24,3±2,1*	27,7±2,7*

Note: Statistical significance to CG: * P<0.05 in relation to CG.

In case of endocrine profile interpretation, we found indications among 109 patients with Graves' disease, among them there were 72 (66 %) of female patients, and 37 (34 %) of male patients.

We diagnosed diffuse enlargement of the thyroid gland (TG) with palpation among each patient with Graves' disease in accordance with O. V. Nikolayev. We used the ultrasound examination in order to determine the volume and structure of the thyroid gland. We observed an TG increase of the 1st degree among 23 (21.1%) patients, of the 2 degree among 54 (49.5%), of the 3 degrees among 27 (24.8 %), of the 4 and 5 degrees among 5 (4.6%) patients. The ultrasound examination determined the volume of TG, with an average volume of 24.8±7.3 cm^3 among women and 32.3±9.3 cm^3among men.

By utilisation of services, we divided all patients with Graves' disease into 4 groups in accordance with the duration of the disease. The group 1 included patients with duration of the disease of up to 6 months, the group 2 up to 12 months and more than a year, the group 3 –3-5 years and the group 4 more than 5 years.

Table. 3: The complications development among patients with Graves' disease in accordance with the disease duration

Groups	Goiter heart	Endocrine ophthalmopathy	Toxic hepatitis	TSH, mU/l
1 group, n= 62	2 (3,2%)	24 (38,7%)	-	0,3±0,01
2 group, n=74	5 (6,8%)	32 (43,2%)	1(1,4%)	0,2±0,02
3 group, n=41	6 (14,6%)	26(63,4%)	2 (4,8%)	0,3±0,01
4 group, n=28	5 (18%)	19 (67,8%)	2 (7,1%)	0,4±0,01

In group 1, there were 62 patients who received thyrostatics with a daily dose of 25.4±2.1 mg/day (average TSH level of 0.3±0.01 Med/l), in group 2, 74 patients (thyrostatics with a dose of 20.3±2.4 mg/day and an average TSH level of 0.2±0.01 Med/l), in group 3, 41 patients that use of thyrostatics with a daily dose of 15.1±1.6 mg/day (average TSH level of 0.3±0.01 Med/l), in group 4, 28 patients that used thyrostatics with a daily dose of 12.2±1.1 mg on average (average TSH level 0.4±0.01 MED/l).As you can see, with the duration of the disease, there is a decrease in the dose of thyrostatics, but whereby there is no clinical improvement. The patients with Graves' disease with a duration of up to 6 months: we observed clinical and biochemical remission among 23 (37%) patients. The patients with Graves' disease with a duration of up to the 1st year: we observed clinical and biochemical remission among 34 (46 %) patients, in group 3 among 8 (19.5 %) patients and in group 4 - among 9 (32.1%) patients. The average TSH level was 1.2±0.05 med/l. we observed such complications as goiter heart among 2 (3.2%) patients, endocrine ophthalmopathy among 24 (38.7%) patients in the group 1, in group 2 among 5 (6.8%) patients, among 32 (43.2 %); in group 3 among 6 (14.6%), among 26 (63.4%);in group 4 among 5 (18%) and 19 (67.8%) patients, respectively. (table 3.). We did not detect any toxic hepatitis within the first group, there was 1.4% in group 2, 4.8% in group 3, and 7.1% in group 4 (table 3.) Within the examination of patients in these groups in relation to the treatment of Graves' disease, we observed that in group 1, 28 (45.1%) of patients showed improvement in their condition, 34 (54.9%) patients continued treatment with thyrostatics, despite the fact that there were indications for surgical treatment. In group 2, 24 (32.4%) patients recovered, 37 (50.0%) continued medication, and 13(17.6%) patients passed surgical treatment - total thyroidectomy. Among 24 recovered patients, the indicator of antibodies in relation to TSH receptors was on the average of 0.76±0.03 IU/l, respectively, these patients did not have clinical implications of thyrotoxicosis. In case of group 3 analysis, we observed recovery among 2 (4.9%) patients, respectively, the average level of antibody in relation to TSH receptors was 0.96±0.07 IU/l, 29 (70.7%) then the patients continued medication,since they had a high indicator of 2.3±0.05 IU/l that refer to the disorder persistence, and 12 (24.4%) passed operations. In group 4, 2 of patients (7.2 %) there was a health improvement (the average TSH level of 1.6±0.02 Med/l and TSH receptor in relation to antibodies by 0.72±0.02 IU/l), and 22 (78.6%) continued treatment with thyrostatics (average TSH level of 0.2±0.04 Med/l and TSH receptor in relation to antibodies by 2.5±0.09 IU/l) and 4 (14.3%) of patients passed medical operations(table.4.) The patients who had complaints with characteristics of the clinical thyrotoxicosis and the value of antibodies in relation to TSH receptors higher than the reference values, continued to take antithyroid medicines.

We should note that not all patients within the dynamics of treatment passed the examination on antibodies in relation to TSH receptors, since we indicated the start of disorder remission by the absence of thyrotoxicosis symptoms and normalization of antibodies in relation to TSH receptors and hormone indicators within the scope of thyroostatic therapy.

Table. 4: Dynamic observation of patients with toxic diffuse goiter

Group of patients	Recovered, n (%)	Prolonged medical treatment, n (%)	Surgical management, n(%)
1 group, n= 62	28 (45,1%)	34 (50%)	-
2 group, n= 74	24 (32,4%)	37 (50,0%)	13 (17,6%)
3 group, n= 41	2 (4,9%)	29 (70,7%)	12 (24,4%)
4 group, n= 28	2 (7,2 %)	22 (78,6 %)	4 (14,3 %)

The diffuse-nodular goiter within the TG pathology constituted 24 (3.4%) patients, and toxic adenoma 45 (6.45%) patients. During palpation and ultrasound examination, we found nodes up to 1 cm among 26 (37.7%) patients, and more than 1 cm among 43 (62.3%) patients. We made the diagnosis by means of hormone testing and TG scintigraphy. We performed the scintigraphy in the RSPC of the endocrinology. We found inflammation spots by scintigraphy. All patients received the medicamentous therapy with thyrostatics

until subclinical hypothyroidism progress within 3-6 months and passed surgery operations.

In case of the conducted thyroostatic therapy analysis among patients with Graves' disease, we found that among them 28 (25.6%) patients received long-term medication for up to 10 years with inappropriate medicamentous therapy within the latest medicine doses in regard to the severity of thyrotoxicosis and it caused various complications of Graves' disease. Subsequently, in case of ineffective medicamentous therapy for more than 5 years, 20 (71.4%) patients had a thyroidectomy, among them 9 (45.0%) patients had subtotal thyroidectomy and 11(55.0%) had total thyroidectomy. We performed the thyroidectomy in the department of surgery in the city endocrinological dispensary of Urgench. Within the treatment period from 3 to 5 years, 6 patients with cardiovascular complications did not referred for surgical treatment service and continued thyrostatics treatment. In case of operated patients observation within 3 and 6 months, we observed a decrease in the functional activity of the TG among 8 (27.6%) patients, since there was no prescription for timely hormone replacement therapy (HRT) with thyroid medications. Among the operated patients, only 5 patients (17.2%) in the early postoperative period received HRT with levothyroxine at a dose of 1.2±0.5 mcg/kg per day without full compensation. In the study of TSH and SV. T4 patients we diagnosed subclinical hypothyroidism among these, with an average TSH level of 5.4±0.7 MED/l. In order to correct these disorders, we increased the dose of levothyroxine to 1.6 mcg/kg. Whereby, the average TSH level was 5.4±0.7 MED/l. 15 patients after thyroidectomy did not come for reexaminations. In accordance with the indications, if the patient refuses surgical treatment, there were recommendations in regard to 2 patients to pass treatment with radioactive iodine in the radiological Department in the RSPC of the endocrinology.

In addition, we diagnosed T3 toxicosis among 2 women, whereby both had a common fraction. In the medical history analysis of these women, we found that they used HRT for a long time together with sex hormones. On ultrasound examination of the thyroid gland, we diagnosed a diffuse goiter of 1-2 degrees without any structural changes of TG. It is known that the medication intake leads to an increase in total T3, whereby the content of total and SV. T4, as well as TSH stay within the norm[7]. Clinically, these women did not have any complaints. These women stopped HRT by sex hormones and they got a prescription to use other non-hormonal method of pregnancy prevention.

We used a QOL questionnaire SF-36 in order to study the quality of life among patients with Graves' disease on the basis of the received therapy. We divided all patients with Graves' disease into 2 groups. The first group included 30 patients with Graves' disease who had received medicamentous therapy for more than three years and the second group 24 patients after thyroidectomy in case of Graves' disease. Among 29 patients under surgery, 3 (10.3%) had hypoparathyroidism as postoperative complication, and 2 (6.9%) had recurrent nerve paresis. These patients visited an endocrinologist and an otorhinolaryngologist in order to pass rehabilitation. We did not include these patients into this group. Among the patients of both groups, all parameters of quality of life present with examination were significantly lower than among healthy individuals ($P<0.001$) by all scales of vitality within group 1: $P<0.01$ for the vitality scale in group 2 and $P<0.001$ for all other parameters in group 2. (table 5.). Between the patients of two groups there were the following differences: the parameters physical functioning, vitality, social, emotional functioning and mental health were significantly higher in the group of patients after total and subtotal thyroidectomy ($P<0.001$),the role functioning was not significantly different in both groups of patients, and indicators of social functioning were significantly higher (30.7%) in the group of patients under medicamentous therapy by thyreostatics($P<0.001$), as endocrine ophthalmopathy was more large in this group. Within the comparison of the QOL among patients with Graves' disease within both groups, we found that the average values of physical functioning, role-based physical functioning, social functioning , role- based emotional functioning and mental health after surgery significantly exceed similar indicators among patients who received medications with thyrostatics, although the average level of TSH in both groups did not significantly differ. In the first group, the average TSH level was 2.3±0.02 Med/l, in the second group 3.1±0.05 MED/l. The emotional functioning and psychological health in the 2 group of patients are higher by 40.7% and 22.5%, respectively.

Table 5: Parameters of QOL assesment among patients with Graves' disease in accordance with the treatment

Parameters	CG, n=20	1 group, n=30	2 group, n=24
Physical functioning	60,35±2,21	17,43±3,08***	22,33±5,38***^
Role functioning	64,52±3,28	24,10±3,24***	28,14±3,62***
Pain	12,16±3,74	20,13±1,41***	15,32±6,72***
General medical condition	58,17±3,26	45,23±2,33***	47,02±3,14
Vitality	78,06±2,76	30,50±2,11***	46,25±4,22**^
Social functioning	52,13±1,84	27,33±2,59***	39,95±3,02***
Emotional functioning	81,57±2,41	29,53±1,70***	49,81±2,97***^
Psychological health	74,65±2,57	48,27±1,95***	61,28±2,64^

Note: Statistical significance with CG: * * P<0.01, ***P<0.001; statistical significance between groups 1 and 2: ^P<0.05 CG-control group

Thus, the timely appointment for thyroidectomy in cases of Graves' disease in real time leads to patients health improvement. After thyroidectomy, all patients should pass a hormone examination in order to fulfill timely hormonal replacement therapy. Timely appointment for hormonal replacement therapy with thyroid medications improves the quality of life and prevents the development of complications within various organs and systems.

Conclusions

1. Among the examined patients of the Urgench endocrinological dispensary that suffered the thyroid pathology in 2014 - 2019,we observed:15.6% of toxic diffuse goiter, 3.4% of diffuse-nodular goiter, toxic adenoma in 6.4% cases, diffuse goiter with euthyroidism in 19.5% cases, nodular goiter with euthyroidism in 14% cases, mixed goiter with euthyroidism in 10.3% cases, autoimmune thyroiditis, thyrotoxic phase in 3.3% cases, autoimmune thyroiditis with hypothyroidism in 11.3% cases, condition after strumectomy in 9.5% cases, thyroid cysts in 6.31% cases, thyroid cancer in 0.14% cases.
2. The quantity of patients with toxic diffuse goiter and goiter heart was 3.2%, endocrine ophthalmopathy 38.7% of patients within the first group, within group 2 6.8% and 43.2 %; within group 3 in 4.6% and 63.4%; within group 4 in 18% and 67.8%, respectively. There was no diagnosis of toxic hepatitis within the first group, 1.4% within group 2, 4.8% within group 3, and 7.1% within group 4, respectively. We should note that with an increase in the duration of inadequate thyrostatic therapy, the percentage of these complications increases, which confirms the need for correct medicine management and a timely transition to radical method of treatment.
3. In case of ineffective medication, only 26.6% of patients had a thyroidectomy. After surgery, only 17.2% received HRT with levothyroxine at a dose of 1.2±0.5 mcg/wt per day without full compensation. The remaining 55.1% of patients did not come to reexaminations. In order to improve the diagnosis and treatment of thyrotoxicosis syndrome in the Khorezm region, we recommend to conduct a number of seminars for doctors.
4. In comparison between the QOL among patients with Graves' disease after timely thyroidectomy, the average values of physical functioning exceed by 23% in relation to medicamentous therapy, the indicators of emotional functioning and mental health, as well as social functioning significantly exceed by 40.7%, 22.5% and 30.7%, respectively, similar indicators of patients under medicamentous therapy with thyrostatics.
5. We observed hypoparathyroidism among 0.3% of patients with toxic diffuse goiter after total thyroidectomy and among 6.9% of patients with recurrent nerve paresis, which indicates the need for training of operating surgeons in Urgench at the workplace of experienced surgeons.

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Prognosis Innovative Approaches in Relation to Results and Complications of Coronary Remedial Surgical Procedure in Case of Acute Coronary Syndrome

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Abstract

In modern cardio surgery, it has become possible to predict not only the outcome of surgical interference in order to save life, but also its effectiveness in terms of the quality of life improvement. It is proved that coronaro correcting technologies can not only soften the clinical picture of chronic coronary insufficiency, but also increase the contractility of the ischemic myocardium, as well as significantly improve the patient's quality of life, if the myocardial ischemia has the character of hibernation, and not stanning. The ability to determine which variant of the ischemic syndrome occurs in the patient before surgery helps to predict the interference effectiveness. The method used for this purpose (radioisotope scintigraphy, positron emission tomography, MRI, pharmacological stress echotests) are technically or economically inaccessible, difficult to apply in the perioperative period, and sometimes dangerous.

The purpose of the research: to offer an informative, accessible and safe method of preoperative identification of the hibernating myocardium among patients with IHD for successful prediction of the interference effectiveness.

Materials and Method: 83 patients with acute and chronic forms of IHD were under study. In the first group (49 people), there was a coronary stenting (CS). In the second group (34 patients), there was a heart bypass surgery (HB). There was the comparative assessment analysis in regard to variant of ischemic syndrome and the effectiveness of both operations by means of precardial ECG mapping and stress echotest with dipyridamole.

Results: In case of IHD acute forms (acute coronary syndrome, acute myocardial infarction) CS and HB operations had significant positive dynamics in regard to indicators of coronary blood flow within the course of ischemia and myocardial contractility in accordance with precordial mapping (area reduction of ischemia - AST and the decrease of the ischemia degree - ΣST) and stress echotest with dipyridamole (increased contractility of EF indicator and a reduction of ESV, increase in the number of eukinetic segments of the myocardium and decrease of hypokinetic segments). We observed the same dynamics, but much less pronounced, in accordance with ECG mapping and stress echotest data after both operations within the group with chronic forms of ischemia.

Conclusion: The precardial ECG mapping is informative and available for use in regional cardiosurgical clinics as a method of the hibernating myocardium identification, which is a more perspective variant of ischemia in case of IHD in terms of left ventricular contractility increase.

Keywords: *Coronary stenting, coronary bypass, prognosis, stress echocardiography, ECG mapping.*

Introduction

Currently, the prognosis of outcomes and complications in surgical treatment has gone far from the primitive principle of the patient's chances comparison in regard to a favorable outcome within conservative and surgical treatment. We consider currently performed high-tech operations as the most complex and effective (but at the same time with risk possibility) among all known coronarocorrecting operations. The new data on the pathogenesis of coronary insufficiency and variants of ischemic syndrome have created a demand for such prognosis method that allow us to calculate not only the patient's chances of survival after surgery, but also the improvement possibility degree in regard to the pumping heart function. Whereby it makes available not only as "prognosis quo ad vitam ", that is, the prognosis for survival after surgery, but also as" prognosis quo ad valetudinem", that is, the prognosis in regard to the patient's quality of life after surgery. And one can determine it by which of the ischemic syndrome variants occurs in case of this patient.

Thus, in order to calculate the patient's chances to pass coronarocorrecting interferences with high positive result, it is important that the preoperative examination helps to detect the presence of a hibernating myocardium. Whereby in this variant of ischemic syndrome (unlike stanning) the improvement of coronary blood supply restores (or at least significantly improves) their contractile function. In case of coronary syndrome promising variant includes hibernating myocardium, and effective revascularization as a result coronarocorrecting operation, one can expect a significant increment of the left ventricle contractile ability with reduction of heart failure signs, a positive clinical dynamics and improvement in quality of life.

Currently, the most widely used in coronary surgery, that is, relatively accessible, simple and informative is the indirect method of the hibernating myocardium identification – the method of stress echocardiography with dobutamine (dipyridamole or nitroglycerin). However, this method is technically quite complex, in addition, during the perioperative period, it is dangerous for patients with acute forms of IHD. We have proposed a method of precardial ECG mapping for this purpose. The origins of this method refer to the 70s of the last century in order to estimate the size of the necrosis focus in case of myocardial infarction, the area of the so-called "peri-infarction zone", or the ischemic myocardial necrosis focus around it. We consider this zone as a zone of "critical blood supply" and, as practice confirms, when the coronary blood flow improves, it completely recovers and returns to the normally functioning myocardium. If the treatment was not quite effective, this zone increases the area of the necrosis focus with complete elimination from the heartbeat[1].

Materials and Method

In order to assess the prognosis reliability in relation to complications, outcome and effectiveness of surgery operation, we examined the status of the ischemic myocardium (a form of ischaemic syndrome) and its dynamics as a result of coronary correction by CS method, and also CABG method, in two groups of patients – with acute (acute coronary syndrome, acute myocardial infarction) and chronic (angina FC II-IV) forms of IHD with initial signs of heart failure. There was an integral assessment of the heart pumping function recovery based on the results of stress echotest with dipyridamole.

The study included 83 patients of both sexes (62 men and 21 women) in the age from 38 to 65 years (average age 54.3+1.21) with acute and chronic forms of IHD. There was randomization, in which the criteria of non-inclusion were: age≥65 years; acute myocardial infarction or acute stroke, a medical history of HB or CS operations; multifocal (more than 3 vessels) demages of the coronary bed; the presence of resistant arterial hypertension, chronic bronchopulmonary conditions, thrombophilic and anaemic syndromes, heart failure above grade II in accordance with NYHA. We established such criteria because the assessment did not refered to the effectiveness of any medical or surgical interference, but to the reliability of prognosis technology.

We divided the patients under study into 2 groups. The patients of the first group (49 people) passed coronary stenting. This group included 17 patients with acute and 32 patients with chronic forms of IHD.

The second group consisted of 34 patients with acute (11 people) and chronic (23 people) forms of IHD, who passed HB. A retrospective analysis showed that the age-sex structure of all subgroups differed slightly from each other.

We performed the precardial ECG mapping five times for all patients of both groups: before the interference, 30 minutes after the operation, and 1, 3, and 6 days after the operation.

We performed the pharmacological EchoCG test with dipyridamole twice-before the interference (for one day) and before the discharge.

Results and Discussion

The research results are in tables and diagrams.

Table 1: The dynamics of area (AST) and degree of myocardial ischemia (ΣST) among patients with acute and chronic forms of IHD during treatment with the CS method

№	Time of study	ACS, AMI		CCI		P_{1-3}	P_{2-4}
		AST (M±m)	ΣST (M±m)	AST (M±m)	ΣST (M±m)		
1	Before surgery	27,84±1,66	88,42±6,67	8,13±2,81	10,81±3,11	p<0,02	p<0,001
2	After 30 ‘ p/operation	27,17±1,04***	63,19±4,48***	6,47±1,12	7,46±2,88	p<0,01	p<0,001
3	1 day	25,34±2,11***	52,68±6,64***	6,15±0,92	6,93±1,27	p<0,02	p<0,001
4	3 day	23,76±0,86***	48,46±6,40***	5,44±0,86	5,12±1,88	p<0,01	p<0,001
5	6 day	17,32±1,08***	54,17±6,18***	4,78±0,46	4,64±1,26	p<0,01	p<0,001

***High degree of statistical significance (p<0.02) between 1-2, 1-3, 1-4, 1-5.

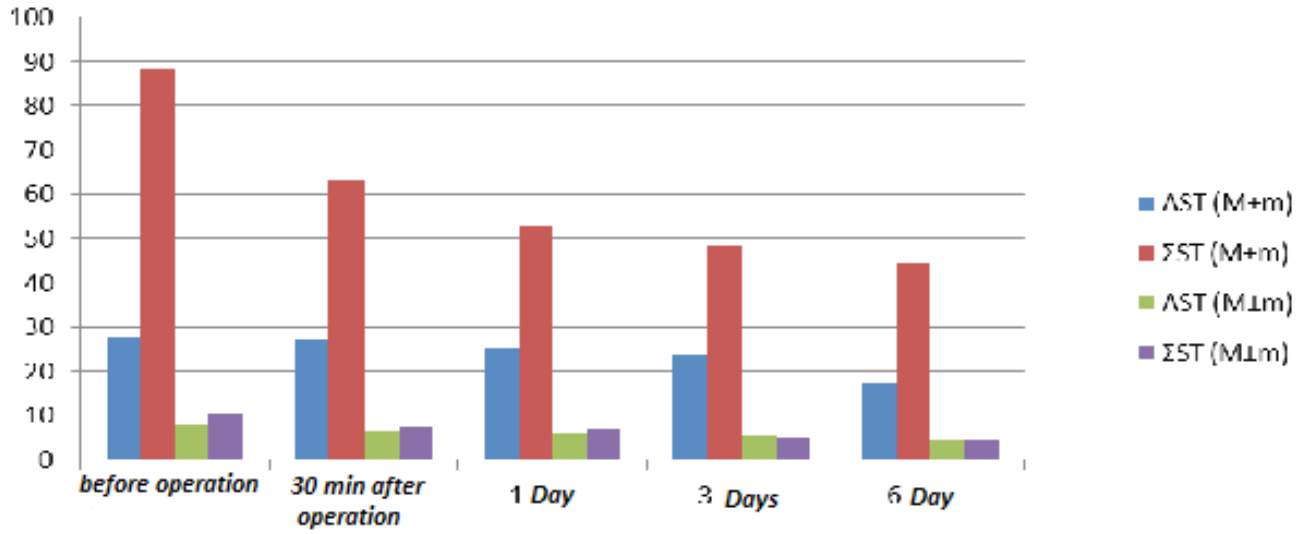


Diagram 1: The dynamics of area (AST) and degree of myocardial ischemia (ΣST) among patients with acute and chronic forms of IHD during treatment with the CS method

Table 2: The dynamics of area (AST) and degree of myocardial ischemia (ΣST)among patients with acute and chronic forms of IHD during treatment with the HB method

Time of study	ACS, AMI		CCI		P_{1-3}	P_{2-4}
	AST (M±m)	ΣST (M±m)	AST (M±m)	ΣST (M±m)		
Before surgery	27,50±1,93	92,34±4,93	9,25±1,67	8,31±1,29	p<0,02	p<0,005
After 30 ‘ p/operation	24,67±1,20	67,58±8,04	10,27±2,53	11,69±2,17	p<0,02	p<0,005
1 day	20,83±1,42	57,17±8,26	8,77±3,14	9,25±1,75	p<0,02	p<0,005
3 day	19,50±1,02	50,34±7,21	6,17±2,35	7,62±1,14	p<0,02	p<0,005
6 day	18,83±1,30	36,67±8,89	5,81±1,62	5,64±1,28	p<0,02	p<0,005

***High degree of statistical significance (p<0.02) between 1-2, 1-3, 1-4, 1-5.

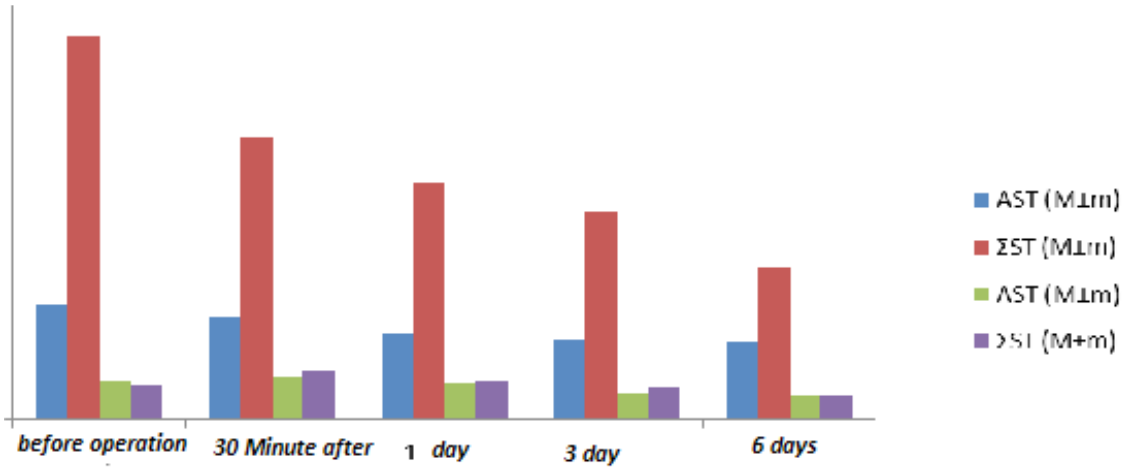


Diagram 2: The dynamics of area (AST) and degree of myocardial ischemia (ΣST) among patients with acute and chronic forms of IHD during treatment with the CABG method

As can be seen from the data provided in tables 1 and 2, we selected patients with clinically pronounced forms of the disease, with demonstrative laboratory and instrumental shifts, for both CS and CABG surgery operations. It is evident from the high initial indicators of affected area filed (AST) and degree of ischemization (ΣST) in both groups (conservative and surgical treatment). Almost all of them are active, working, highly educated people with a fairly high social status, "advanced" in the achievements of modern medicine, who value this status and their work. That is why, in contrast to conservative patients, within the choice between the proposed medication and surgical method of treatment, they preferred surgical interference. The main motivator of this choice was that the operation, even if there is a certain risk, provides a higher quality of life, gives more prospects to save the ability to work.

Both among patients treated with the CS method and in the group who passed HB, from the 1st day to the end of observation (day 6), there was a rapid and statistically significant ($p<0.02$) positive dynamics both in terms of area reduction (AST c 27.84±1.66 in the CS group, as well as from 27.50±1.93 in the HB group before surgery to 17.32±1.08 and 18.83±1.30 by day 6, respectively), and in aspect of the myocardial ischemia degree (ΣST c C 88.42±6.67 and 92.34±4.93 before treatment, up to 54.17±6.18 and 36.67±8.89 by day 6 in both groups, respectively).

In the groups with chronic IHD, treated by method of CS and HB, the results of ECG-mapping were less clear, however, quite convincing. Here, however, the elevation is not typical, but depression of the ST segment (which is specific for CCI) and this shift is less obvious than in acute forms of IHD – usually within 1-2 mm of the isoelectric line.

As can be seen from tables 1 and 2, the initial (pre-operation) indicators of the area and degree of myocardial ischemia in both groups were almost identical – AST, both in the CS group, and CABG, respectively, 8.13±2.81 and 9.25±1.67, and ΣST, respectively, 10.81±3.11 and 11.27+2.64. The dynamic control within the specified time period also demonstrated positive, but significantly slower changes of the ST segment than among patients with acute forms of IHD: by day 6, the AST indicator decreased within both groups to 5.81±1.12 and 6.19+2.03 (P0,05), respectively.

In our opinion, this difference in the extent and severity of myocardial ischemia among patients with acute and chronic forms of IHD reflects the difference in the state of the myocardium in two different variants of ischemic syndrome. It means that in acute forms, the hibernating state prevails, and in chronic forms – stanning. In order to confirm (or exclude) our assumption, we performed a pharmacological EchoCG test with dipyridamole.

We performed this functional test in accordance with the recommendations of the European Echocardiographic Association[2]. We performed the test twice-before the operation and on the 6th day after operation.

we performed the pharmacological stress echotest with dipyridamole among 28 patients from a sample in which there was ECG mapping in order to assess the effect of HB or CS surgery. In each of 4 groups, we

selected 7 patients by method of random sampling (group 1-ACS, who passed HB; group 2-with chronic IHD, who passed HB; group 3 – ACS, who passed CS; group 4 – with chronic IHD, CS). The criteria of non-inclusion: 1) severe conduction abnormality; 2) patients with chronic bronchopulmonary diseases with bronchoobstructive syndrome (bronchoconstrictor effect of dipyridamole); 3) hypotension with systolic A/D<100 mmHg.

The data on changes in cardiodynamic parameters (ejection fraction-EF and final systolic volume - ESV) among patients with ACS before and after CS and CABG operations in accordance with the echo test with dipyridamole are in table 3 and diagram 3.

Table 3: The changes in cardiodynamic parameters and local contractility among patients with acute forms of IHD during CS and HB surgery operations in accordance with the EchoCG test with dipyridamole

The indicators of cardiodynamics and local contractility	CS		HB	
	Before the CS surgery operation	6th day after CS	Before the HB surgery operation	6th day after HB
ESV, ml	114,6±4,3	77,3±3,1	109,6±4,3	74,3±3,4
EF, %	45,8±6,4	55,4±8,3	47,5±7,1	56,3±6,6
The LV segments:				
Eukinetic	168,4±7,0	232,8±6,2	192,6±5,7	236,7±8,4
Hypokinetic	62,2±4,1	24,5±2,2	62,3±4,4	32,5±2,4
Akinetic	58,3±5,6	32,4±3,5	34,5±3,1	20,1±1,7

As can be seen from table 3, before surgical interference of both types, patients showed a slight increase in ESV, which we associate with the area of the ischemic zone and indicates the latent heart failure. The degree of ESV increase in both groups was approximately the same, and the difference between them was statistically unreliable (p>0,1), which indicates the comparability of patients in both groups.

We confirmed the presence of left ventricular contractility disorders among patients of both groups by a decrease in the EF index. Moreover, these indicators among the patients of both groups did not differ significantly (p>0,2), which indicates a qualitative randomization.

By the 6th day after surgery (both in the HB group and in the CS group), myocardial contractility increased statistically in a valid way (p<0,02).

Moreover, there was a solid confirmation of such dynamics by both EchoCG indicators – both a decrease in ESV and an increase in EF (p <0.02).

Such character of changes in cardiodynamic is quite consistent with the dynamics of precordial ECG mapping indicators that showed in both groups statistically significant (p>0,02) improvement of coronary circulation within the ischemic area, both in terms of its vastness (by AST, p<0.05), and in terms of reducing the extent of ischemia (by ΣST, p<0,02).

The pronounced and rapid (in less than a week after surgery, recovery of coronary blood flow) improvement of the ischemic myocardium state and its contractility in accordance with the EchoCG test with dipyridamole helps us to explain the achieved positive effect of both surgery types by the fact that in ACS there is a hibernating version of the ischemic syndrome. While in the case of stanning, it would be impossible to count on such a rapid and pronounced positive effect of surgery. To confirm this assumption, we conducted a pharmacological EchoCG test with dipyridamole within a group of patients with chronic IHD.

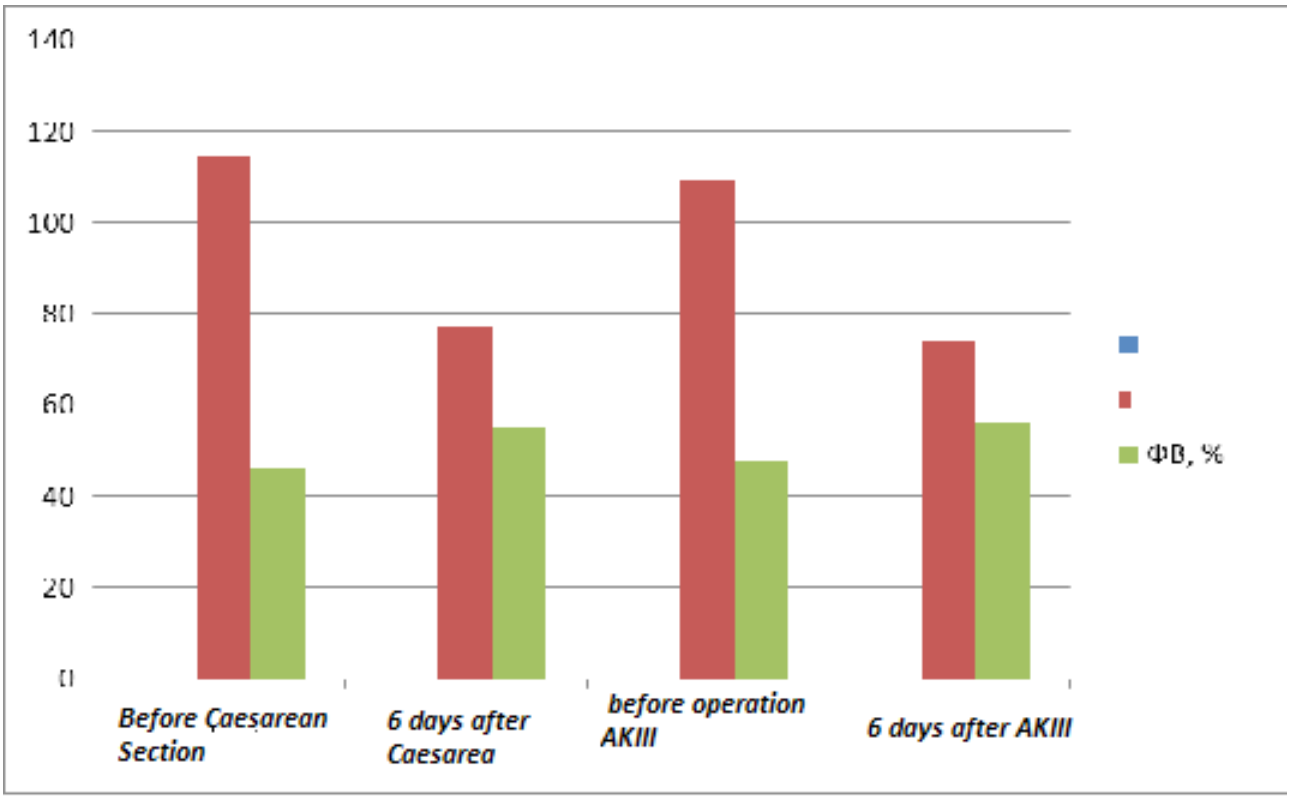


Diagram 3: The dynamics of ECHOCG parameters (ESV, EF) during CS and HB operations before and after the interference

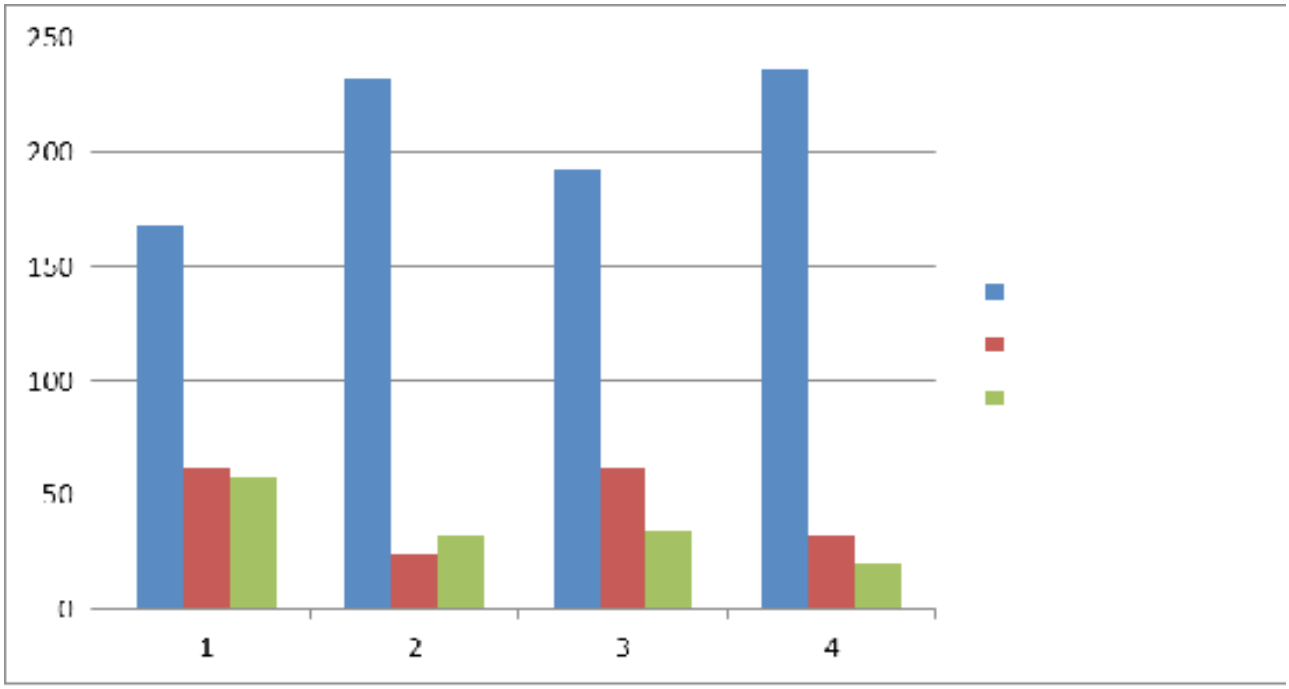


Diagram 4: The dynamics of the myocardial segments kinetics during CS and HB operations before and after the interference

Table 4: The changes in cardiodynamic parameters and local contractility among patients with chronic forms of IHD during CS and HB surgery operations in accordance with the EchoCG test with dipyridamole

The indicators of cardiodynamics and local contractility	CS		p	HB	
	Before the CS surgery operation	6th day after CS		Before the HB surgery operation	6th day after HB
ESV, ml	101,4±2,6	93,4±6.3	>0,05	113,1±3,7	69,3±6,1
EF, %	49,9±6,8	55,4±5,6	>0,05	42,2±5,1	53,3±4,8
The LV segments:					
Eukinetic	196,3±0,1	212,6±1,5	<0.05	188,4±1,1	202,9±0,6
Hypokinetic	43,0±3,3	28,3±4,1	>0,05	58,3±2,9	43,1±2,2
Akinetic	36.6±1,7.	29,5±5,0	>0,05	36,7±2,2	28,9±1,4

As can be seen from the table, among patients of both groups, in accordance with EchoCG data, there was initially a significant decrease in myocardial contractility, manifested by an increase in left ventricular of ESV and decrease of LV. It is natural, since it is known that the main markers of blood supply deterioration are chest pain (optional symptom) and segmental decrease in left ventricular pumping function (obligate symptom), usually without clinical indication and with identification by instrumental method.

The analysis of changes in cardiodynamic parameters (in accordance with the EchoCG test with dipyridamole) within two groups of patients with chronic IHD (treated with CS and HB) revealed the absence of significant improvement in left ventricular contractility after surgery to improve coronary blood flow, both by this and another method. Both indicators under study, both ESV and EF, showed some improvement, which became statistically unreliable (p>0,05). It would be groundless by definition to expect more significant changes – as is known, the term "stoned myocardium" refers to an ischemic area of the heart muscle, in which the maximum possible recovery of coronary blood supply has already occurred within these conditions (+at the expense of a decrease in coronary spasm, neoangiogenesis, recanalization of the thrombus; - at the expense of akinesia and hypokinesia). Such conclusion bases on the EchoCG research results of local myocardial contractility-in both groups of patients within the postoperative period,there was a statistically significant p<0,05 increase in the number of eukinetically shrinking segments,while the reduction in the number of hypo – and akinetic segments was statistically insignificant (p>0,05). Consequently, this suggests that in acute variants of IHD, there is hibernation of myocardium (reduction of heart function to a level adequate to reduce coronary perfusion in order to save persistent cardiomyocytes), when the restoration of blood flow leads to the restoration of full ischemic cardiomyocytes functioning. In contrast to stanning, in which chronic coronary hypoperfusion within chronic variants of IHD leads to an irreversible decrease in the contractile function of the LV myocardium (complete absence of persistent cardiomyocytes in the presence of Hypo - and akinetic sections of the myocardium).

As an illustration, we present extracts from the medical histories of operated patients.

Conclusion

The precardial ECG mapping is informative and available for use in regional cardiosurgical clinics as a method of the hibernating myocardium identification, which is a more perspective variant of ischemia among patients with IHD in aspect of left ventricular contractility increase.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Characteristics of Cerebral Hemodynamics and Vascular Responsiveness of Brain Arteries among HIV-Infected Patients

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Abstract

The damage features of the cerebral hemodynamics disorders and decreased responsiveness of brain vessels among the patients with HIV are the pathogenetic basis for the formation of HIV encephalopathy development. We observed patterns of changes in cerebral blood flow and immunological reactivity among patients at preclinical stages of HIV. It indicates that the vascular system of the brain is highly sensitive to the action of treponemous toxin and is responsive to damages at the early stages of the disease.

Keywords: *HIV, diagnostics, cerebral hemodynamics.*

Introduction

The HIV infection under course of slow progression influences not only the body's immune system[6]. The virus spreads throughout all the vital organs of the human body. In nine cases out of ten, the virus damages the patient's nervous system, and the HIV encephalopathy develops[4].

Although the pathomorphological studies show that one can detect the damage to nervous system in 90% of AIDS patients, clinically neurological complications in 50-70% of patients, and in 10% of cases they are the first clinical implications of the disease. Neurological complications are one of the main causes of disability and death in case of HIV infection[11].

HIV enters the CNS at early stage (macrophages and monocytes act as a" Trojan horse", within which HIV penetrates through GERD, but apparently does not directly infect neurons, and numerous neurological complications connect with the neurotoxic action of substances produced by HIV, or factors released by infected macrophages or microglial cells [8](for example, cytokines that release amino acids, etc.).[10] One can assume that some viral proteins, within the connection with neurons, can disrupt the functioning of calcium or other ion channels or affect neurotransmitter processes, which can eventually cause the death of neurons[5].

The most common neurological complication of HIV infection is HIV encephalopathy (HIV-associated dementia). One can detect it among the half of patients within the end-stage. The HIV encephalopathy develops within the scope of severe immunosuppression and systsystemic symptoms of the disease (weight loss, lymphadenopathy, alopecia), but in 3-5% of patients with HIV encephalopathy is the initial or even the only symptom of AIDS12. Pathomorphologically, we found the diffuse changes in the periventricular and deep parts of the white matter, which refer to demyelination or extravasation of plasma proteins as a result of damage to the hematoencephalic barrier, multinucleated giant cells in the cerebral cortex and white matter, astrocytic gliosis, microglial nodules,[7] perivascular lymphocytic infiltration, and a decrease in the number of neurons [9].

The first symptoms are of non-specific character: headache, rapid fatigue, decreased libido, loss of interest to work, inattention, memory loss.[12] The HIV-associated dementia combines with emotional and behavioral disorders, and may include depression[2], dysphoria, apathy, limited motivation, and lethargy. Gradually there is a development of subcortical-frontal type dementia. There often are indications of sleep disorders. About half of the patients suffer motor disorders developments: akinetic-rigid syndrome, postural instability and gait disorders, pyramidal insufficiency, generalized

hyperreflexia, cerebellar ataxia[6]. The condition progressively worsens from month to month [9]. As there is a progression of disorder, loss of orientation, confusion, epileptic attacks, and akinetic mutism develop. After the development of dementia, average death occurs after 6 months[11].

However, there are only a small number of studies in the Russian literature which refers to the early symptoms of HIV encephalopathy. There is no clear information on the issue of cerebral hemodynamics and vascular responsiveness of the brain arteries, which allows a practical doctor to get an idea of the current state within the scope of the actual problem.

The purpose of the research: To evaluate cerebral hemodynamics and vascular responsiveness of brain arteries among HIV-infected patients.

Materials and Method

We examined 56 HIV-infected patients with a verified serological and clinical diagnosis, whose average age was 35.7±1.3 years, among them there were 31 (55.4%) men and 25 (44.6%) women.

Under a detailed survey of patients, we found that the majority of patients (58.9%; 33/56) had a history of injectable narcotic substances usage, 12.5% of patients reported sexual HIV infection (7/56), and 28.6% did not know the route of HIV infection.

We divided all the patients into 4 groups. The 1st and 2nd groups included 12 and 14 people, respectively, who differ by the level of CD4 lymphocytes in the blood plasma (by the threshold level of 500 CL/ml). The patients of these groups did not have clinical implications of HIV infection in accordance with experts at the time of the study, and did not receive antiretroviral therapy.

Group 3 included 12 HIV-infected patients who had no clinical complaints or symptoms at the time of the study, but under antiretroviral therapy.

A special place belongs to the 4th group, which included 16 HIV-infected patients who were not active recipients of narcotic substances for at least 6 months before the study, as well as those infected with hepatitis C. All patients received highly active antiretroviral therapy (HAART).

The clinical observations underline that within the early stages of HIV infection, the most frequent are reactive neurotic states and symptoms of as the no vegetative syndrome.

The physical examination of patients revealed a disorientation by place, time and personality from 26.8% (15/56) to 43.9% (24/56), we noted the disorders of higher integrative functions in the form of memory and attention loss - in 19.6% of cases (11/56).

In case of the neurologic examination assessment, the majority of patients in the first three groups did not complain about difficulties in memorizing, focusing, although some had headaches, dizziness and light coordination disorders.

At the same time, the psychological examination of patients, as well as those within the first two groups, i.e. at the subclinical stage of the HIV-infection course, showed process disorders of the memorizing and information reproduction. In the fourth group, we noted focal neurological symptoms, while motor disorders, hypertension syndrome, and pronounced memory disorders prevailed. All the patients with an infection period of more than 120 months complained about difficulties in memorizing the current events, memory impairment for long-ago events, poor concentration within their daily duties at the workplace, which interfered with routine work and required active concentration and great effort.

We put the state of cerebral hemodynamics and vascular reactivity of brain arteries undr study by standard method with sensors under frequency of 2, 4, 8 MHz on the Spectromed-300 device (Russia). The research algorithm in regard to the state of cerebral hemodynamics and vasomotor reactivity included:

– Study of the main arteries within the head and intracranial arteries by method of extra - and intracranial dopplerography in order to determine the speed characteristics of blood flow, pulsation indices and circulatory resistance;

– Study of vasomotor reactivity on the basis of the compression test results. It is known that short-term finger compression of the common carotid artery (CCA) on the neck leads to a decrease in perfusion pressure and the development of a transient hyperemic response after compression stops, which allows us to calculate a number of indicators that characterize the autoregulation reserves. The patients (without constrictive lesion of arteria carotis) passed 5-6-second CCA compression with the termination

of compression in the diastole phase. We recorder the average linear blood flow rate (LBFR) within the middle cerebral artery (MCA) before compression of the ipsilateral CCA-V1, during compression-V2, after termination of compression-V3, as well as the recovery time of the original LBFR-T. With the obtained data, we calculated the coefficient of overshoot (CO) by the formula: CO = V3/V1[15].

The obtained data were subject to statistical processing by the means of statistical analysis applications package with the calculation of the arithmetic mean (M), mean square deviation (σ), standard error (m), and relative values (frequency, %). We used the confidence level P<0.05 in relation to statistically significant changes.

Results and Discussion

We characterized the cerebral hemodynamics disorder among the patients in accordance with the dopplerographic test output by a decrease in blood flow velocity within the ICA, 24.5 and 33.9 % in the MCA - 25.4 and 34.5 % in VA - 24.3 and 44.7% in LCX - 21.7-32.6% (1 and 4 groups, respectively) relative to values of the control group (table 1).

There were also signs of increased vascular tone among all the vessels under study in accordance with the increase in the pulsation index (Pi) and circulatory resistance (Ri) by an average of 1.5 and 2.3 times within group 1 and 1.8 and 2.75 times within group 4 (table 2).

There was no any hemodynamic significant stenosis of the head main arteries among the examined patients in any of cases (their presence was a criterion for exclusion from the study due to the compression tests risk).

Table 1: Hemodynamic indicators of blood flow within the main arteries of the head and intracranial arteries at rest

Vessels	LBFR (cm/s)		
	1 Group	4 Group	Control
ICA, right	40,2±5,8*	25,1±3,8*^	53,2±6,4
ICA, left	41,4±5,9*	29,9±4,1*^	51,9±5,9
MCA, right	48,7±6,2*	30,2±4,5*	62,4±11,3
MCA, left.	47,1±6,8*	32,1±3,1*^	65,2±10,7
VA, right	27,2±4,7*	20,4±3,6*	37,6±7,8
VA, left	29,1±3,7*	19,8±2,6*^	38,0±8,7
LCX	36,6±4,8*	21,3±4,3*^	46,0±5,6

Note: * - the output reliability by control indicators (P<0.05); ^ - the output reliability by group 1 indicators (P<0.05)

There was confirmation of the possibilities decrease for collateral blood flow (anatomical link of the cerebral vascular reserve) among the patients under examination by the depression relative to the control parameters of the residual blood flow rate in the MCA(V2) at the time of compression of the ipsilateral CCA by 19.3 and 28.1%, respectively, in groups 1 and 4 (table 3).

Table 2: Hemodynamic indicators of vascular tone within the main arteries of the head and intracranial arteries at rest

Vessels	Pi, s.u.			Ri, s.u.		
	1 Group	4 Group	CG	1 Group	4 Group	CG
ICA						
Right.	1,30±0,12*	2,6±0,12*^	0,85±0,21	0,77±0,2*	1,54±0,15*^	0,55±0,16
Left.	1,30±0,11*	2,45±0,10*^	0,83±0,19	0,79±0,1*	1,58±0,18*^	0,53±0,15
MCA						
Right.	0,98±0,12*	1,96±0,01*^	0,56±0,14	0,7±0,1*	1,40±0,16*^	0,50±0,10
Left.	0,94±0,09*	1,88±0,12*^	0,57±0,14	0,6±0,1*	1,20±0,12*^	0,51±0,09
VA						
Right.	0,89±0,13*	1,78±0,13*^	0,78±0,11	0,63±0,1*	1,26±0,15*^	0,52±0,08
Left	0,91±0,11*	1,82±0,12*^	0,74±0,10	0,7±0,1*	1,41±0,15*^	0,52±0,07
LCX	0,79±0,20*	1,58±0,18*^	0,54±0,19	0,76±0,1*	1,9±0,18*^	0,56±0,09

Note: *The output reliability by control indicators (P<0.05); ^ - the output reliability by group 1 indicators (P<0.05).

Table 3: Hemodynamic parameters of blood flow by the intracranial arteries during a compression test

Hemodynamic parameters	1 Group	4 Group	Control
V_1MCA (cm/s)	47,7±6,2*	33,9±4,1*^	63,8±9,7
V_2MCA (cm/s)	26,4±1,4*	19,5±1,1*^	31,8±3,4
V_3MCA (cm/s)	57,6±5,7*	36,1±2,9*^	86,8±7,1
CO	1,21±0,07*	0,98±0,01*^	1,36±0,09
T (s)	10±2*	19±0,3*^	6±2

Note: * - the output reliability by control indicators (P<0.05); ^ - the output reliability by group 1 indicators (P<0.05)

A decrease in the coefficient of overshoot among patients with respect to control by 11.6 and 16.9%, respectively, indicated a pressure within the functional link of cerebrovascular reactivity, in particular, its myogenic component due to a structure disorder of the vascular wall and its tone. The revealed 1.7-and 2.3-fold increase in the recovery time of the blood flow rate to the initial one reflected a disorder of the vascular reactivity metabolic circuit as a symptom of general dysmetabolic processes which develop within the body of HIV-infected patient.

Thus, the hypersthenia of vessels suggests a lack of dilatatory mechanisms functioning at the level of the capillary bed, which indicates a pressure within the mechanisms of brain vessels adaptation. Such condition iwithout correction can lead to the vessels failure and the development of dyscirculatory processes in the CNS among the HIV-infected patients already at the preclinical stages of the disease.

We conducted analysis of dopplerographic test output within groups 2 and 3 patients (Fig. 1) in order to determine the effect of HAART on cerebral hemodynamics and vascular reactivity of brain arteries among HIV-infected patients.

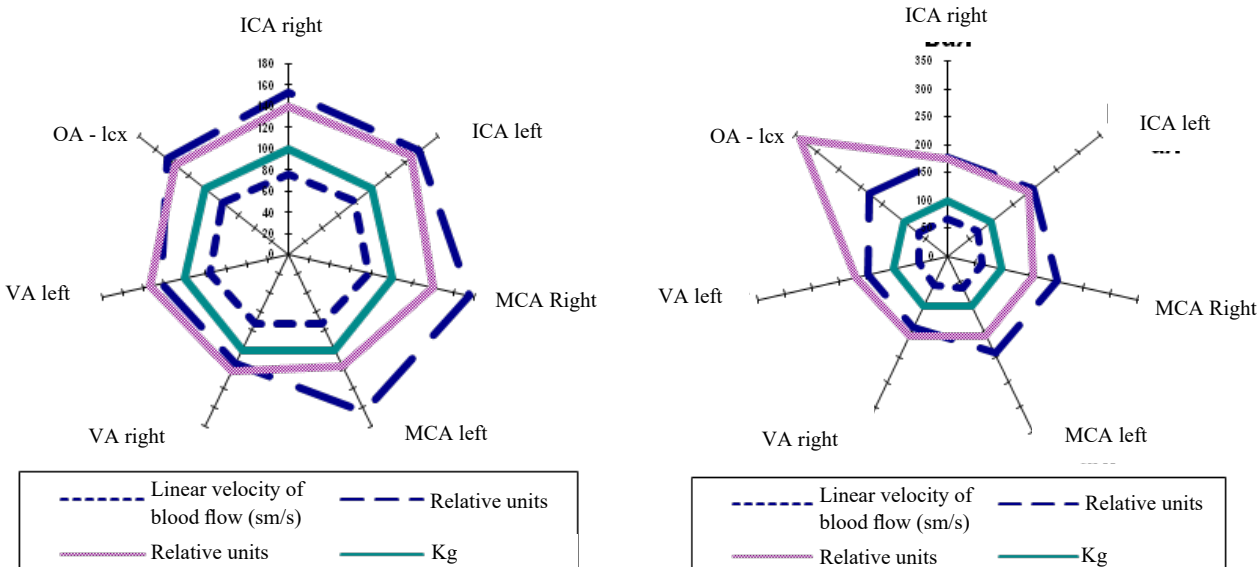


Fig. 1. Hemodynamic indicators of blood flow within the main arteries of the head and intracranial arteries at rest

There has been no proof in regard to the HAART effect on the state of the brain vessels, but the patients of group 3 had a tendency to the blood flow rate disorder in relation to the indicators of patients from group 2.

The observed patterns of changes in cerebral blood flow and immunological reactivity among patients of the 1 and group indicate that the vascular system of the brain is highly responsive to the action of treponemous toxin and is responsive to damages at the early stages of the disease (tables 4, 5).

Table 4: Hemodynamic indicators of blood flow within the main arteries of the head and intracranial arteries at rest

Vessels	LBFR (cm/s)		
	1 Group	2 Group	Control
ICA, right	40,2±5,8*	35,1±5,8*^	53,2±6,4
ICA, left	41,4±5,9*	35,9±6,1*	51,9±5,9
MCA, right	48,7±6,2*	40,1±5,7*^	62,4±11,3
MCA, left.	47,1±6,8*	42,4±7,1*	65,2±10,7
VA, right	27,2±4,7*	21,7±5,6*	37,6±7,8
VA, left	29,1±3,7*	20,6±4,6*^	38,0±8,7
LCX	36,6±4,8*	31,2±5,3*	46,0±5,6

Note: * - the output reliability by control indicators (P<0.05); ^ - the output reliability by group 1 indicators (P<0.05)

There was a significant increase in ESR (40.03±4.74 mm/h) and a reduced hemoglobin content (99.1±4.03 g/l) among the patients with focal changes by CT and MRI.

The average content of CD4 + lymphocytes among the patients with changes in cerebral hemodynamics and vascular responsiveness was 343±41.5/mcl, with diffuse lesions 152.2±16.6/mcl, and significantly lower with focal lesions (112.8±28.7/mcl, p<0.05).

Table 5: Hemodynamic indicators of vascular tone within the main arteries of the head and intracranial arteries at rest

Vessels	Pi, s.u.			Ri, s.u.		
	1 Group	4 Group	CG	1 Group	4 Group	CG
ICA						
Right.	1,30±0,12*	1,50±0,14*	0,85±0,21*	0,77±0,2*	0,96±0,2*^	0,55±0,16
Left.	1,30±0,11*	1,60±0,13	0,83±0,19*	0,79±0,1*	0,99±0,21*^	0,53±0,15
MCA						
Right.	0,98±0,12*	1,12±0,11*^	0,56±0,14*	0,7±0,1*	0,78±0,15*	0,50±0,10
Left.	0,94±0,09*	1,10±0,12*^	0,57±0,14*	0,6±0,1*	0,81±0,11*^	0,51±0,09
VA						
Right.	0,89±0,13*	1,12±0,13*^	0,78±0,11*	0,63±0,1*	0,81±0,90*^	0,52±0,08
Left	0,91±0,11*	1,09±0,14*	0,74±0,10*	0,7±0,1*	0,87±0,15*^	0,52±0,07
LCX	0,79±0,20*	0,97±0,18*^	0,54±0,19*	0,76±0,1*	1,9±0,15*^	0,56±0,09

Note: * - the output reliability by control indicators (P<0.05); ^ - the output reliability by group 1 indicators (P<0.05)

The patients without pathological changes in hemodynamics and vascular responsiveness of the brain arteries, had the average duration of infection around 5.8 years, and the average content of CD4 + lymphocytes was higher than 500/mcl.

Conclusion

Thus, the cerebral hemodynamics disorders and decreased responsiveness of brain vessels among the patients with HIV are the pathogenetic basis for the formation of HIV encephalopathy development. With concern to the hemodynamic and metabolic disorders in case of AIDS, as well as their complex role in the pathogenesis of cerebrovascular and neurological complications, it is neccessary to include a combined medication in therapy design among the patients at preclinical stages of HIV in order to improve the state of cerebrovascular responsiveness, reduce the phenomenon of vasospasm in the cerebral vessels and normalize metabolic processes within the body, which will improve the condition of patients with HIV and their quality of life.

All the above-mentioned statements highlight the need for further study in regard to the complex of hemato-encephalic disorders among HIV patients. The authors recommend the method of transcranial stress-dopplerography to evaluate the parameters of cerebral hemodynamics among these patients.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Cardiovascular Development Abnormalities in Young Athletes

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Abstract

The article discusses the issues of small anomalies of heart development in young athletes. The question of the admission of young athletes with small anomalies of heart to sports and their impact on the state of the cardiovascular system during exercise is still an open question. To avoid subjectivity and the subsequent over diagnosis of small anomalies of heart, their objectification is necessary, through a quantitative assessment.

Keywords: *Small abnormalities of the development of the heart, additional chord, mitral valve prolapse, young athletes, sports, ventricular fibrillation.*

Introduction

Physical education and sports require increased functional capabilities of all body systems, including CVS. In this regard, any deviations, especially from the CVS, can negatively affect both the health and success rate of athletes. The term CDMA is usually used to denote inherited or congenital structural changes in the valvular apparatus and/or its connective tissue framework, including the main vessels, in the form of various anatomical abnormalities that are not accompanied by hemodynamically 'gross' disorders [1]. False ('supplemental', 'abnormally located') chords (tendons) are manifested by the presence in the ventricular cavity of additional formations in the form of connective tissue cords. Unlike normally located chords, these formations have an ectopic attachment not to the valves of the atrioventricular valves, but to the free walls of the ventricles and interventricular septum [3]. The term 'tendon' reflects the position of the abnormal cord as a geometric body which crosses the cardiac chamber. True chords are fibrous strands consisting of collagen fibers. They receive blood and innervation from the heads of papillary muscles, supply and innervation of which provided by muscle trabeculae located at their base [4]. Some clinical data and numerous morphological studies suggest that transverse, diagonal and longitudinal false tendons of basal and median localization, as well as multiple left ventricular false tendons (LVFTs) have an independent clinical significance, as it often acts as an independent cause of ventricular extrasystole. With that, single apical LVFTs are usually considered as normal variant [2]. In recent years, an active study of intracardiac structures has clearly demonstrated not only the independent clinical significance of CDMA, but also their close relationship with heritable connective tissue disorders (HCTD)[3]. The development of HCTD is based on disruption of the genes responsible for the synthesis or decay of the extracellular matrix components. A large group of HCTD is caused by mutations in the genes of extracellular matrix proteins (collagens, fibrillin, tenascin, etc.). These genes mutations lead to the development of many mendelating HCTD, the number of which exceeds 250, and most of them are very rare. Connective tissue dysplasia (CTD) is a pathological condition of organs and tissues. It is manifested in a decrease in the content of certain types of collagen or a violation of their ratio, and leads to a decrease in the strength of connective tissue of various organs and systems. In the development of CTD, gene mutations are of primary importance, since they encode the synthesis and spatial organization of collagen. They are also responsible for the formation of the structural components of the matrix and enzymes involved in fibrillogenesis. In addition, there is a complex of exogenous factors that cause a violation of

collagen synthesis. Dysplastic changes can be due to the impact of adverse environmental conditions, inadequate nutrition or stress. The severity of these changes can be affected by polymorphic variants of the genes of the renin-angiotensin system associated with the activity of antioxidant enzymes [5].

As a result, connective tissue dysplasia syndrome (CTDS) forms a pathology of a multifactorial nature with a wide range of clinical signs and a fairly high prevalence. Its further study will contribute to the development of method for identifying patients who respond positively to additional physical exertion, and those for whom such exertion may be undesirable or even harmful [7, 1].

Single false tendons don not form a contraindication to sports. With multiple false tendons, exercise is also not contraindicated, in the absence of cardiac arrhythmias. Located in the pathways of the inflow or outflow of the left ventricle, the false tendons can contribute to the emergence of a local phenomenon of ultrahigh speeds (aliasing phenomenon) and, as a result, lead to injury or the fibrosis development. Being an abnormal way of conducting an exciting impulse, false tendons can cause ventricular arrhythmias, including ventricular fibrillation. Patients with supplementary chords of the heterogeneous structure ('beam' type) are recommended for more frequent and thorough medical supervision, in order to identify early signs of aCVS arrhythmic overstrain [9].

The aim of this study is to do research of the frequency of CDMA occurrence in young athletes.

Materials and Method

The research was conducted in the Department of Sports Medicine of the City Adolescent Dispensary (Tashkent). The main group consisted of 305 athletes involved in various sports in children and youth sports schools (CYSS), aged 6-18 years - 113 females and 182 males. The control group consisted of 305 children of the same age who were not involved in sports. All children were permanent residents in Tashkent. All athletes underwent an echocardiographic examination. Statistical processing of the obtained data has been performed using the Student's t-test for the significance level $P<0.05$.

Results and Discussion

As a result of the analysis of echocardiographic examination of children, a number of CDMA have been identified. The frequency of their occurrence in comparative groups is presented in Table 1.

Table 1: Frequency of occurrence of CDMA in examined children

CDMA types	Frequency of occurrence of CDMA (%)			
	Main group (n=305)		Control group (n=305)	
	Abs.	M±m	Abs.	M±m
False tendons of the left ventricle (FTLVs)	72	23,6±2,4*	52	17,0±2,15*
Mitral valve prolapse (MVP)	3	0,98±0,56	4	1,31±0,65
Open foramen ovale (OFO)	2	0,65±0,46	1	0,33±0,33
Tricuspid valve prolapse (TVP)	2	0,65±0,46	2	0,65±0,46
Left ventricular abnormal trabeculae (LVAT)	1	0,33±0,33	0	

* - $P<0.05$ relative to the main group of the control group

As Table 1 shows, the most common CDMA (23%) in both groups - especially in athletes - is asupplementary chord. The second most frequent mitral valve prolapse is 0.98% in the main group, and the dependence of CDMA frequency on physical activity has not been observed.

Table 2: The frequency of occurrence of CDMA in the examined children, depending on gender

CDMA	Number of children with this CDMA (frequency of occurrence (%)							
	Main group-72				Control group-52			
	Females		Males		Females		Males	
	Abs, %		Abs, %		Abs, %		Abs, %	
False tendons of the left ventricle (FTLVs)	31	43±5,8*	41	56,9±5,8*	29	55,7±6,8	23	44,3±6,8
Mitral valve prolapse (MVP)	2	66,6±6,1	1	33,4±5,5	1	25±6,0	3	75±6,0
Open foramen ovale (OFO)	1	50±5,9	1	50±5,9	-		1	100
Tricuspid valve prolapse (TVP)	1	50±5,9	1	50±5,9	-		2	100
Left ventricular abnormal trabeculae (LVAT)	1	100	-		-		-	

Note: * - P<0.05 confidence of differences compared to the main and control groups

According to Table 2, it is clear that FTLVs in the main group is 1.3 times more common in males than in females (56.9% and 43%; respectively, P <0.05). By contrast, mitral valve prolapses (MVP) in the main group in females are 2 times more often than males (66.6% and 33.4%, respectively). In other cases, the frequency of CDMA occurrence depending on gender in the main group has not been detected. The following table presents data on the quantitative ratio of FTLVs in the main and control groups (Table 3)**:**

Table 3: The number and frequency of LVFTs in the experimental and control groups

FTLVs	Number of children from this CDMA (Frequency of occurrence%) n=305			
	Main group n=72		Control group n=52	
	Abs, %		Abs, %	
Single	69	95,8±2,3*	50	96,1±2,6*
Double	2	2,7±1,91	1	1,92±1,9
Multiple	1	1,38±1,35	1	1,92±1,9

Note: * - P<0.05 confidence of differences compared to the main and control groups

As Table 3 shows, the frequency of occurrence of single FTLVs is the same in children involved and not involved in sports activities. Double FTLVs are more common in children involved in sports.

In such a way, single FTLVs predominate in both groups. Duplicate FTLVs have been found to a lesser extent, and the rarest in frequency are multiple FTLVs, that are 1-2% of all FTLVs cases.

Conclusions

As a result of the research performed, it can be concluded that the most common CDMA is a supplementary chord. False tendons can cause ventricular arrhythmias, including ventricular fibrillation, which should be taken into account when admitting children to sports and planning their training regime. No gender differences in the studies have beennoted. However, it has been found that the frequency of occurrence of single chords in athletes is higher than double and multiple ones, which affects CVS in a positive way. Although a single supplementary chord does not affect the cardiac hemodynamics as often as other CDMA do, it is necessary to carry out mandatory regular supervision of transverse chords through electrocardiographic examination.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Form of Erythrocytes and Laser Doppler Flowmetry of Skin with ACNE and Use of Medication FarGALS in its Comprehensive Treatment

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Abstract

Using the express method of a thick smear and morphometry, the state of erythrocytes obtained from the acne and finger region has been studied. Using laser Doppler flowmetry, the state of microcirculation of the skin of the finger and the acne location area has been studied.

It has been found out that comprehensive treatment with the use of FarGALS promotes the reduction in blood of pathological forms of erythrocytes and the restoration of microcirculation parameters.

Keywords: *Erythrocyte, blood, acne, pathological, microcirculation.*

Introduction

Dermatoses and infectious skin lesions are accompanied not only by significant structural changes, but also by pronounced abnormalities in the ratio of discocytes - normal erythrocytes and their pathological forms. These abnormalities are noted both in peripheral blood obtained from the finger, and, especially, in blood obtained from areas of pathologically altered skin [2].

Shifts in the ratio of discocytes and pathological[3] forms of erythrocytes cause pronounced microcirculation abnormalities[6].

However, studies of changes in the shape of erythrocytes and microcirculation with acne[7], which is one of the most common skin pathologies, especially in adolescents and young adults [4]. have not been conducted.

The medication FarGALS is registered by the Pharmaceutical Committee of the Ministry of Health of the Republic of Uzbekistan and is approved for clinical use.

It is included in the pharmacotherapeutic group - “antiseptic and wound healing agent”, has a pronounced antioxidant effect, has the property to enhance metabolism in tissues, increases oxygen consumption by them, accelerates the regeneration of damaged tissues, promotes neovasculogenesis [1]. These properties of FarGALS make in possible to suggest that it has a positive effect on erythrocyte membranes, normalization of the ratio of discocytes and their pathological forms, and contribute to the activation of microcirculation.

In this regard, the purpose of this study is defined: to study the effect of FarGALS with its complex use in the treatment of acne on erythrocytes and the state of microcirculation.

Materials and Research Method

Before and after the course of treatment, all patients’ blood obtained from the finger and acne area has been studied. Blood for light microscopy has been studied with the use of the express method of a thick smear (EMTS) - Patent Office of Uzbekistan - “Method for the Determination of RBC Forms” No. MKI 6 A 61 B 10/00, Program “Express Diagnosis of RBC Forms” No. ED-5-05.

For this, 2 drops of blood have been fixed for 3-4 minutes in 1 ml of a 2.5% solution of glutaraldehyde. Then a drop of fixed blood has been placed on a glass slide. A cover glass has been horizontally lowered onto it. A layer formed underneath is a “thick smear” - suitable for studying using a light microscope equipped with a digital camera.

Erythrocyte form ratio has been calculated at a magnification of 10x40 with a sample of at least 1000 erythrocytes for each stage and series of studies. Erythrocyte studies have been performed using Biolam-I2 and Axioscop 40 - ZEISS microscopes. Light-optical micrographs have been obtained on an Axioscop 40 - ZEISS microscope coupled to a digital camera with a data storage in a computer using the Microsoft-Windows XP-Professional application programs.

Laser Doppler flowmetry has been performed using LAKK-01 devices with a single-channel analyzer (LAZMA, Russia) coupled to a Pentium-Sh personal computer and ALT - Vostok LDF-01 device developed by domestic manufacturers ("Naf" company). A comparative assessment of the data obtained using LAKK-01 and ALT - Vostok LDF-01 has been carried out. These studies have shown the full authenticity of the devices.

LDF has been used to assess microcirculation of the skin of the finger (control) and of the face of 8 patients with normal skin (control) and in patients aged 14-24 with acne on the face. The standard conditions for registering LDF have been observed [2].

Conventional treatment included standard therapy. FarGALS has been used as a local application.

Results of the Research and Discussion

With acne, the proportion of pathological forms of erythrocytes is slightly less than half of all red blood cells (pic. 1 tab. 1).

Morphometry of blood erythrocytes obtained from the gums and from the finger (in presence of acne) has shown that pathological forms dominate among erythrocytes. This is especially pronounced in blood obtained from the area of acne.

The conventional treatment of this pathology leads to a significant reduction in the pathological forms of erythrocytes, both in the blood from the acne area and in the peripheral blood obtained from the finger (pic. 1 tab. 1).

The use of FarGALS in the comprehensive treatment of acne leads to a significant increase in the proportion of discocytes and a decrease in the proportion of pathological forms of erythrocytes. This tendency appears both in the blood from the acne area and in the peripheral blood from the finger.

A characteristic feature of blood from the finger, and especially from the acne area, is a sharp increase in the proportion of erythrocytes with a crest (pic. 1 tab. 1). Comprehensive treatment and, especially, treatment using FarGALS significantly reduces the number of pathological forms of erythrocytes, but their share remains much higher than the control level (pic. 1 table. 1) Laser Doppler flowmetry has shown that acne causes microcirculation abnormalities as in the skin of finger, and especially in the area of localization of acne. AmaxCF/AmaxLF, which reflects the measurements of arterial blood flow, decreases in the acne area by more than 3 times. AmaxHF/AmaxLF, which reflects the measurements of venous blood flow, decreases by more than 2 times. MEI - the microcirculation efficiency index reduces by 2 times.

Medication therapy leads to a tendency to normalize the studied parameters. When using FarGALS, all the studied parameters are close to the control level (tab.2).

Pathological forms of erythrocytes such as stomatocytes, echinocytes and erythrocytes with a crest are found in peripheral blood and are in the norm. However, their total share does not exceed 11-12%. Erythrocyte is the most differentiated cell in the body of mammalian animals and humans and does not have either organelles or nuclei. This determines their sensitivity to various changes in blood parameters in various pathological conditions. The shifts in the ratio of discocytes (normal red blood cells in the form of a biconcave disc and their pathological forms) are determined, first of all, by changes in the properties of their plasma membrane [2]. An increase in the proportion of pathological forms of erythrocytes leads to the abnormality of microcirculation, since discocytes have the ability to change their shape when passing through narrow capillaries with its subsequent restoration[2].

The shifts we have found in the ratio of discocytes and pathological forms of erythrocytes regarding the presence of acne are especially pronounced in the blood obtained from the skin of the acne location area. This is accompanied by shifts in all studied microcirculation parameters. Changes in blood from the finger accompanied by impaired microcirculation parameters confirm the systemic nature of microcirculation disorders in acne, where erythrocytes play a leading role.

FarGALS contributes to the restoration of the ratio of discocytes and pathological forms of erythrocytes and improves microcirculation of the skin.

Conclusions

1. With acne, there is an abnormality of the ratio of discocytes and pathological forms of erythrocytes in blood obtained from the finger and especially from the area where acne is located.
2. FarGALS contributes to the restoration of the ratio of discocytes and pathological forms of erythrocytes in blood obtained from the finger and from the area where acne is located.
3. The use of FarGALS in the comprehensive therapy of acne, along with a reduction in the proportion of pathological forms of erythrocytes, leads to an improvement in microcirculation.

Table 1: The ratio of discocytes and pathological forms of erythrocytes regarding the presence of acne and after treatment with FarGALS

Erythrocytes form	Control 1gr.	Blood from finger before treatment 2 gr.	Blood from acne area before treatment 2 gr.	Blood from acne area, treatment without FarGALS,.	Blood from finger, treatment with FarGALS,
Discocytes	89±1,7%.	65± 1,4%*	54± 1,6%*	70± 1,6%**	81±1,2%**
Echinocytes	4±0,6%.	6± 1%*	7± 1%*	5± 1%**	4±0,2%**
Stomatocytes	3±0,04%.	14 ±0,2%*	19 ±0,9%*	12 ±0,2%**	6±0,1%*
Eryth. with crest	3± 0,01%	13±0,3%*	18±0,4%*	12±0,2%**	7±0,2%**
Irreversible forms	1±0,07 %	2±0,2%*	2±0,2%*	1±0,2%**	2±0,1%**

* Statistically significant (P <0.05) compared with control 1

The devices used make it possible to obtain over 20 parameters characterizing the state of microcirculation. An indicator of microcirculation - MI, characterizes the total microvascular blood flow. It can be represented by the following expression: MI (t) = M + .δMI (t). "M" reflects the level of perfusion (in relative units) and its value is the total reflection of the average speed of movement of erythrocytes.

In the control, M indicators are higher than in patients with periodontitis and are respectively 16.6 and 11.5 in the alveolar ridge. The earlobe is 8.5 and 5.7 respectively. In the crest δ is 4, in the earlobe is 2, while the microcirculation efficiency index (MEI), which reflects the ratio of passive and active processes in the microcirculation system, is more than 2 times higher in control compared with that in patients with periodontitis. The latter have shown a more than 1.5 times decrease in the coefficient of variation (CV), which reflects the vasomotor activity of microvessels (tab.1).

A pronounced decrease in the parameter of the amplitude coefficient (arteries/vasomotors) reflecting the relative parameter of the arterial (arteriolar) component of microcirculation has been noted. With periodontitis, the amplitude coefficient of the venous (venular) component (veins/vasomotors) reflecting the venous component of microcirculation has been especially sharply reduced (tab.1).

Conventional treatment leads to a pronounced tendency to normalize the studied parameters.

With a comprehensive treatment using ILBI, the normalization of all the studied microcirculation parameters has been observed (tab. 1).

The study of erythrocytes of the blood from the gums and from the finger has shown that with periodontitis there is a pronounced shift in the ratio of discocytes and pathological forms of erythrocytes. This is especially true for echinocytes and erythrocytes with a crest (tab. 2).

Conventional treatment leads to a pronounced tendency to normalize the ratio of discocytes and pathlogical forms of erythrocytes (tab. 2).

When using ILBI in combination with conventional treatment, there is a restoration of the proportion of discocytes to almost normal levels, especially in the blood from the finger. The content of stomatocytes and erythrocytes with a crest remains quite high (tab. 2).

Table 2: Indicators of laser Doppler flowmetry in periodontitis and its treatment using ILBI

	Control (n=8)		Acne (n=12)		Conventional treatment (n=12)		Conventional treatment + FarGALS (n=10)	
	Finger skin	Face skin	Finger skin	Acne area skin	Finger skin	Acne area skin	Finger skin	Acne area skin
	M±m	M±m	M±m	M±m	M±m	M±m	M±m	M±m
AmaxCF/AmaxB (LF)	0,25±0,1	0,24±0,2	0,2±0,01*	0,08±0,001*	0,22±0,02**	0,16±0,02**	0,23**	0,14**
AmaxHF/AmaxB (LF)	0,4±0,01	0,4±0,01	0,3±0,07*	0,2±0,04*	0,3±0,08**	0,24±0,06	0,38±0.01	0,36±0,01
MEI = $A_{LF}/(A_{CF} + A_{HF})$	2,9±0,1	2,8±0,01	2,5±0,03*	1,5±0,02*	2,6±0,04**	2,1±0,01	2,6±-,02	2,2±0,02
M	16±0,2	14,5±0,3	12,4±0,3*	9,8±0,3*	14±0,2**	12,4±0,2	15,2±0,3	12,9±-4
δ	4,0±0,3	3,8±0,2	2,8±0,1*	1,9±0,03*	3,2±0,1	1,5±0,1	3,6±0,2	3,8±0,1
CV	18,6±0,5	19±05	14,±0,4*	10,0±0,4*	16,±1,0	12±0,5	16,±0,4	14,0±0,3

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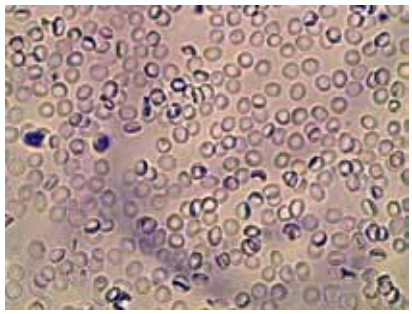
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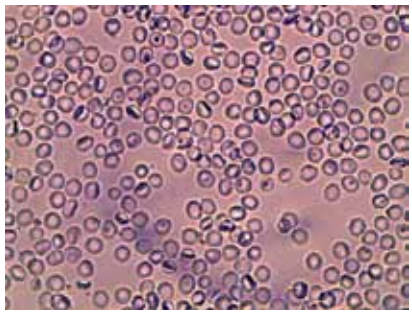
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Blood from the finger. Norm. (control). EMTS 10x40. pic a) Prevalence of pathological forms of erythrocytes in blood: b) from acne area, c) from the finger. EMTS 10x40. Reduction of pathological forms of erythrocytes in blood post conventional treatment d) from acne area, e) from the finger. EMTS 10x40. Significant reduction of pathological forms of erythrocytes in blood: e) from acne area, б) from the finger. Conventional treatment with FarGALS. EMTS 10x40.

Ethical Clearance: No ethical approval is needed.

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Conflict of Interest: Nil

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Social and Demographic Characteristics of Elderly and their Lifestyle in Developing Countries: On the Example of Uzbekistan

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Abstract

Background: Elderly people have a variety of medical problems and functional limitations. Most older people experience multiple chronic illnesses as they age, which is one of the reasons for reducing of their physical activity. Undoubtedly, one of the ways to prevent the occurrence and exacerbation of chronicdiseasesis to maintain a healthy lifestyle, which increases the period of active life and helps support physical activity.

Method: We used a random selection method to obtain reliable and representative data: cluster and stratified selection. In Tashkent city, we selected 2 clusters out of 11 clusters, where the proportion of elderly and senile people was the highest and 674 elderly were included into the research; in the Tashkent region 3 clusters were selected, and 473 elderly were included to our sample. Our sample was divided into 3 social categories: the first category included lonely elderly people living alone in their apartments in the amount of 310 people, the second one included lonely elderly people living in nursing homes for the elderly, in the amount of 240, and the 3rd category included elderly people living with their families, in the amount of 597 people. In order to assess the respondents' lifestyle, we studied their daily routine, dietary regimen and nature, leisure, bad habits and physical activity.

Results: Married respondents were 2.1 times more among elderly living with their families, and 5.7 times more amongelderly living alone in their apartments compared to elderly living in nursing homes. Lonely elderly of "middle-old" age are in more difficult state due to significant proportion of this age-category suffer with various disabilities. It is find out that most respondents of the research do not pay proper attention to a balanced diet. Every second elderly person in our study suffered with obesity and had an increased body mass index (BMI: 25.1<). More than half of the respondents (54.6 + 3.2%) from nursing homes smoked tobacco products 3 times more than those living with their families. Alcohol consumption among older people living in nursing homes was 6 times higher compared to older people living with their families. With an increase of the age, the number of elderly engaged in any kind of physical exercise decreased sharply.

Conclusions: The study and analysis of the socio-demographic characteristics and lifestyle of elderly in Uzbekistan revealed that divorce and loss of spouse are negative factors, whereas the presence of a family and the residence of elderlywith relatives have a positive impact on their lifestyle and, accordingly, on their health status.

Keywords: *Loneliness, elderly people, nursing homes, lifestyle, living conditions, bad habits.*

Introduction

The group of elderly patients with multiple chronic diseases is growing around the world[13]. On average, there are 2 or more chronic diseases for one elderly patient[7], while elderly patients experiencing the mostsense of loneliness might have 5-6 chronicdiseases[12]. Polymorbidity, defined as the presence of two or more long-term health conditions, tends to increase with age[1]. These health conditions are currently not treatable, but can be controlled with medicines or other treatments[3].

Numerous studies indicate that smoking increases the risk of coronary disease and death from cardiovascular diseases in people over 60 [9]. In another hand, the alcoholism of the elderly is frequently observed phenomenon that causes significant harm to the health of this category of population. According to research, factors associated with excessive drinking in older people are male gender, social isolation, loneliness, and divorce[10]. Some studies have shown a correlation between alcohol abuse and serious somatic diseases, a decrease in self-esteem of one's health, a large number of visits to the doctor[2], a greater frequency of depressive symptoms, a decrease in social contacts and, as a consequence, a decrease in the quality of life [11].

Malnutrition and dehydration in older people leads to an increase the number of falls, the vulnerability to infections, the loss of energy and mobility, the poorhealing of wounds, and the confusion. In nursing homes, the low body mass index (BMI) of dwellers is associated with a lower quality of life[4].

This study helps to understand the social and demographic characteristics of older people in Uzbekistan and their lifestyle, and could be useful in designing measures to protect and strengthen health of elderly in the other low- and middle-income countries.

Method

The objects of the study were people of retired age living in the Tashkent city and the Tashkent region: elder people living alone in their apartments (310), were considered as a 1st social category, elder people living in nursing homes (240), were considered as a 2nd social category, and elderly people living with their families (597), were considered as a 3rd social category. Atotalof 1147 elderly people composed ours ample.

The subjects of the study were the social and demographic characteristics of elderly people and their lifestyle.

The lifestyle of elderly was studied by using a sociological research method through the conduction of a survey-interview. All data was entered in the "Elderly Lifestyle Study Questionnaire".

According to the WHO age classification, people aged 60-74 years old are elderly, 75-89 years old are senile, and over 90 years old are centenarians. We took these age periods into account when dividing our research object into the age groups.

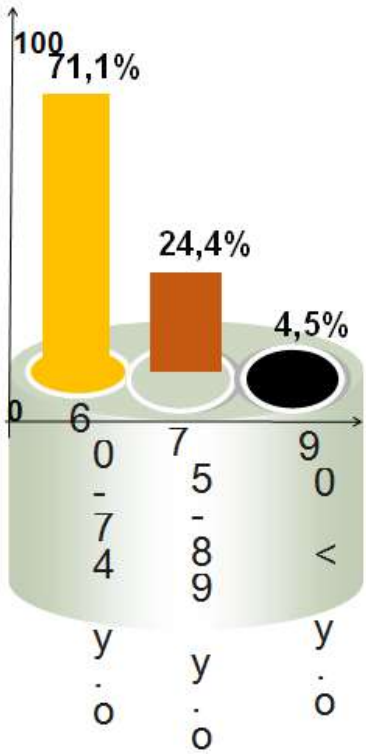
We studied living conditions, daily routine, dietary regimen and nature, leisure, bad habits and physical education along with assessment lifestyle of respondents. Elderly people were asked also about the amount of hot food consumption per day and its character to assess the regimen and nature of nutrition. A special place was given to determining the BMI of the elderly, the amount of consumed meat per week, the number of raw vegetables and fruits servedon average per week. During the study the smoking status of respondent (smoker, former smoker, did not smoke at all), the frequency and types of smoked tobaccowas clarified. Moreover, the alcohol consumption, its frequency and the amount of a standard dose of consumed alcohol over the past month was also specified to assesstheir bad habits. All respondents were asked about the performing physical training, its nature (high-intensity, medium-intensity, and low-intensity), frequency and duration of each leisure activity.

The interview data and medical records were transformed into an electronic database - MS Office Access 2018, which was then exported to the IBM SPSS Statistics version 20 statistical package. Relative and average values were calculated with the calculation of mean error (m), confidence coefficient (t) and error probability (R). To conduct the study, the approval from the National Ethics Committee of the Ministry of Health of the Republic of Uzbekistanwas obtained. After familiarizing the respondents with the goals and objectives of our study, they filledout the consent to participate in this scientific work.

Results

Analysis of the social and demographic data of our sample showed that the average age of the examined individuals was 72.5 +1.6 years. The distribution of our sample by age groups was carried out according to WHO criteria, the group of elderly, from 60 to 74 years old, the group of senile, from 75-89 years old and the group of centenarians, from 90 years and above. A large

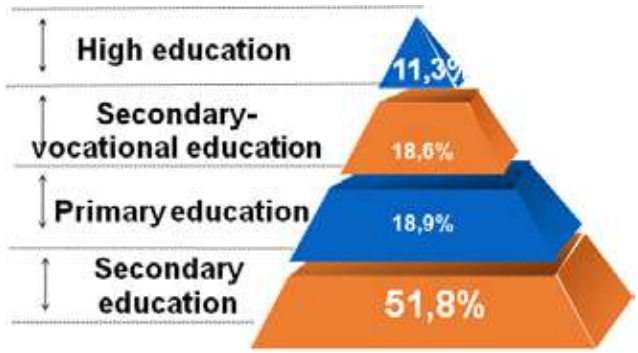
proportion of the respondent was in the age group of the elderly (71.1%) and a smaller proportion was in the age group of centenarians (4.5%) (Fig. 1)



Picture 1. Age structure of respondents

The interesting data was observed in general distribution of the sample according to their social categories; in the category of lonely elderly people living alone in their apartments, 3.4 women corresponded to one man, and in the category of elderly people, living with their families, 2.4 women, whereas in the category of elderly living in nursing homes, this ratio was reversedwith almost 2 times more men corresponding to one woman.

Most of the respondents had a secondary education, however, it should be noted that the largest proportion of elderly people living in nursing homes were people with primary education (31.7%). (Fig. 2)



Picture 2. Distribution of respondent's education level

In the structure of marital status elderly people who interpreted their condition as "widowhood" (40.5%) were dominated. Married respondents, among elderly living with their families, were 2.1 times more than elderly living in nursing homes and 5.7 times more than lonely elderly living alone in their apartments (table 1)

Table 1: Distribution of family status of respondents according to their social categories (in %)

Marital status	Social categories			Total
	1st Category	2nd Category	3rd Category	
Married	9,7±1,6	25,8±2,8	55,1±2,0	35,7±1,4
Divorced	10,6±1,7	31,2±2,9	11,1±1,2	15,2±1,06
Never married	23,2±2,3	7,9±1,7	1,2±0,3	8,6±0,8
Widows/widowers	56,5±2,8	35,1±3,0	32,6±1,9	40,5±1,4
Total	100,0	100,0	100,0	100,0

It is noteworthy that there were almost 3 times more divorced elderly in nursing homes than among the 3rd social category, i.e. elderly living with their families[11]. This, which may be due to two reasons: firstly, in older generations, divorce and widowhood are an unpleasant and negative factor that lead to loneliness; secondly, according to various studies, the presence of a family is one of the factors that have a positive effect on the residence of the elderly in around of relatives and, naturally, on life expectancy[10].

It should be noted that lonely elderly are in the most

difficult situation, because a significant large proportion of these people have various disabilities. Unfortunately, the largest proportion of elderly is in the senile age group, which amounted to 63.8%.

The study of the composition of the social status of the participants in our sociological survey showed that 2/3 of them (65.0% + 1.4%) were non-working pensioners, disabled people of the groups I, II and III, composed respectively, 0.53%, 13.5% and 3.8%. Working retirees accounted for only 3.7%.

The social position in the context of social categories is significantly varies. It is important to note that in the 1st social category, people with disabilities of the groups I and II were mostly observed, whereas, in the 3rd social category elderly people had II and III disability groups. There were no persons with disabilities of the group III in the 1st and 2nd social categories (table 2).

Table 2: Distribution of social status of respondents according to their social categories (in %)

Social status	Social categories			Total
	1st Category	2nd Category	3rd Category	
Disabled of the I group	12,9±1,9	0,84±0,5	-	0,53±0,2
Disabled of the II group	20,0±2,2	14,2±2,2	9,9±1,2	13,5±1,0
Disabled of the III group	-	-	7,4±1,1	3,8±0,5
Working retirees	2,3±0,7	5,8±1,5	3,7±0,7	3,7±0,5
Non-working retirees	67,2±2,6	51,7±3,0	69,0±1,8	65,1±1,9
Others	9,0±1,6	27,5±2,8	10,0±1,2	13,4±1,0

Every second elderly person in our study was suffered with obesity and had an increased BMI (25.1<). Respondents with a BMI of 18.1-25.0 accounted for almost 1/3 of all social categories. The data shows the presence of obesity in the studied individuals, which may be associated with the peculiar nutrition and physical activity of the respondents (table 3).

Table 3: Comparative analysis of BMI among elderly people according to their social categories (in %)

Social categories	Average age	BMI		
		<18,5	18,6-25,0	25,1<
1st category	72,4±1,5	10,6±1,8	28,4±2,6	61,0±2,8
2nd category	73,4±1,6	20,0±2,6	29,2±2,9	50,8±3,2
3rd category	71,8±1,6	13,9±1,0	28,5±1,3	57,6±1,5

Note: (p≤0,05) in comparison with the respective groups

With the increasing of age, performing physical exercises is reducing. A small amount of elderly people (24.9%) is engaged only in low-intensity physical exercises.

The needs of elderly people for medical and social assistance significantly depend not only on their health status and disability degree, but also on such important factors as living conditions, organization of leisure, nutrition regimen, bad habits, and on physical activity.

Considering the human health as a harmonious development of the bodily and spiritual abilities of a person, it is important to bear in mind that human health is formed in connection with environmental conditions, in general, and in particular with his lifestyle.

One of the important indicators characterizing the living standard of the family and having a significant impact on the state of human health is living conditions, i.e. the conditions where a person lives and spends most

of his time; this applies, first of all, to lonely elderly people. An analysis and a comprehensive assessment of the living conditions of the elderly people indicate that 36.5+2.7% of lonely elderly living in their apartments lived in a one-room apartment, 36.8+2.7% in a two-room apartment, 16.1+2.9% in a three-room apartment and 10.6+1.8% in a four or more room apartment. These indicators among elderly people living with their family, respectively, amounted to 11.2+1.3%, 23.3+1.7%, 22.1+1.7%, 43.4+2.0%. Among elderly people living in nursing homes, 92.9+1.7% of them live in one-room ward, and 7.1+1.7% in two-room ward. The study showed that in all the studied social groups in Tashkent, almost 100% lonely elderly had more than 9 m^2 area per person, and had a centralized provision of hot and cold water, heating, sewage. Elderly from the 1st social category who live in the Tashkent region, 67.1+2.7% did not have central heating system, half (50.0+2.8%) were not provided with centralized hot water and 21.0+2.3% were not provided with centralized sewage system. Almost the same situation is observed in elderly people living with their family. In general, 23.7+2.3% of the 1stsocial category, 38.3+2.1% of the 2ndsocial category and 51.2+2.7% of the 3rdsocial category are living in good living conditions. The rest have quite satisfactory living conditions.

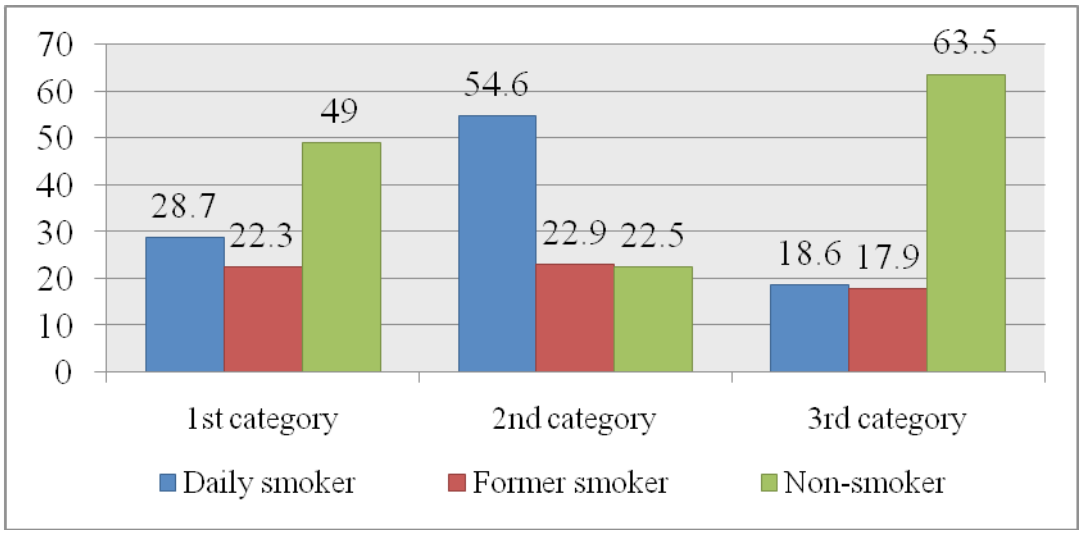
Almost all lonely elderly have a TV, newspapers and magazines in their house. However, it should be noted that 31.6+2.6% of elderly people living alone in their apartment do not have a TV, 51.3+2.8% did not have a radio and telephone, and 52.3+2.8% are not provided with newspapers and magazines, 85.5+2.0% do not have a computer. Whereas, people living in nursing homes much better provided with the necessary conditions for their leisure and cultural recreation. So, of those living in nursing homes, 97.9+0.9% responded that they have access to a TV, more than half (64.2+3.1%) replied that they have access to a radio, 49.2+3.2 % indicated the availability of updated newspapers, 60.0+3.0% indicated the presence of magazines in the place where they live.

As a factor affecting human health also can be included factors such as daily routine, nutrition, recreation, and others.

During interview of the studied groups, a total of 4.6+0.6% noted that they have a monotonous food, 66.4+1.4% - a varied food, 8.3+0.8% - diet food, and 11.8+1.0% prefer to eat fatty food.

Most respondents do not pay proper attention to a balanced diet. Most of the respondents from the 1st social category noted that they consuming insufficient amount of meat, dairy products, vegetables and fruits.

The problem of smoking and alcohol consumption among the elderly deserves a separate attention. More than half of the respondents (54.6+3.2%) from nursing homes smoked tobacco products 2.0 times frequently compared to lonely elderly people living alone in their apartments (28.7+2.6%) and 3 times more than elderly people living with their families (18.6+1.6%) (Fig. 3).



Picture 3. Distribution of respondents according to their smoking status by social categories (in %)

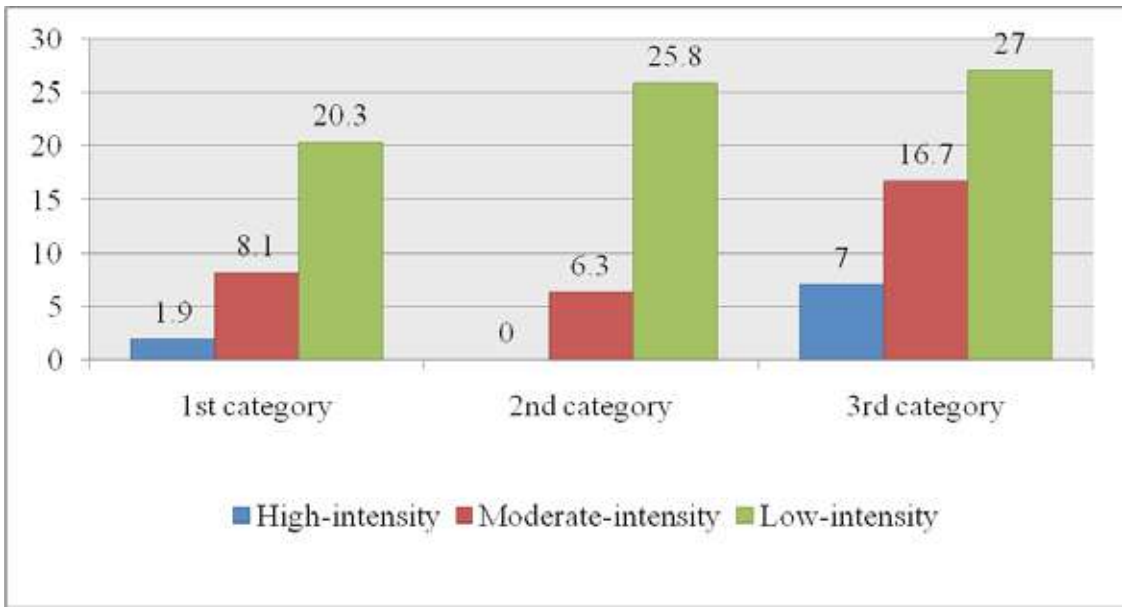
The same trend was observed in assessing alcohol consumption. The data presented in Table 4 show that alcohol consumption is 6 times frequent among elderly people living in nursing homes compared with elderly people living with their families, and 3 times more than elderly people living alone in their flats.

Table 4: Alcohol consumption of elderly people according to their social categories (in %)

Frequency of alcohol consumption	Social categories		
	I	II	III
Daily	4,5+1,2	14,2+2,3	2,0+0,6
Weekly	6,5+1,4	25,0+2,8	2,7+0,7
Monthly	5,8+1,3	12,7+2,1	4,0+0,8
Non-drinking	83,2+2,1	48,1+3,2	91,3+1,2

It has been established that a small number of lonely elderly people regularly engaged in moderate-intensity physical exercises. So, in the first social category, their specific weight was 8.1+1.5%, in the third social category - 16.7+1.5%, and among people living in nursing homes, the second social category, their amount was significantly low (6.3+1.5%), compared with social categories I and III. Lonely elderly engaged in low-intensity physical exercises in all comparative groups were close to each other, but nevertheless their amount was much lower (Fig. 4).

With an increase the age, the number of elderly people who are engaged in any physical exercise began to decline sharply. So, if for lonely elderly people aged 60-74 years, the proportion of persons regularly engaged in physical exercises was equal to 25.4+2.7%, while in the age group of centenarians(90 years and above) its specific weight was equal only to 3.2+0.5%. Apparently, this is due to the fact that with the increase in the age, the number of chronic diseases increases as well as anatomical and physiological characteristics of the body changes which in turn influence to frequency of performance physical activity.



Picture 4. Assessment of performance physical activity among elderly people according to their social categories (in %)

Discussions

Conducted comprehensive studies of the elderly people revealed specific socio-demographic characteristics and lifestyle features for this category of population.

The average age of all examined individuals was 72.5+1.6 years. 65.1% of the surveyed individuals were non-working pensioners, 17.9% were disabled and 3.7% were working retirees. Every second elderly person living in nursing homes, or separately, in their

apartments, was widows and never married. At the same time, the proportion of people who have never been married, among elderly people living in nursing homes and lonely elderly living alone in their apartments, was 19.3 times (23.2%) and 6.6 times (7.9%) more than elderly people living with their families (1.2%). Most of all, nursing homes in Uzbekistan were crowded with men (64.6%) than women (35.4%), as well as with elderly people with primary education level (31.7%).

Anthropometric indicators of individuals did not practically differ among elderly from different social categories ($p > 0.05$).

Among elderly persons living alone in their apartments, more than 1/3 did not have access to the media compared with elderly people living in nursing homes. A small proportion of elderly people (24.9%) are engaged in low-intensive physical exercises. Elderly people engaged in physical exercises are significantly higher among those who live with their families.

The problem of loneliness in old age gives rise to a whole series of medical and social problems. It was found that 48.0% of the respondents, at the time of survey, lived outside of their families. In older age groups, divorce and widows (widowers) are negative factors that lead to loneliness. The presence of a family, the residence of the elderly in around of relatives has a positive effect on their health status and on their life expectancy. The needs of elderly people for medical and social assistance largely depend not only on their state of health, but also on the degree of limitation of their life activity.

Conclusion

It can be concluded that lonely elderly men are highly dependent on the help of others, and widowers and people with primary education are most likely to be in nursing homes. On a person who is distant from his family, the factor of loneliness affects sharply negatively.

Most of the elderly people live in good and quite satisfactory living conditions and have the necessary leisure facilities for recreation, which should be considered as a favorable factor in the socio-hygienic characteristics of the studied groups.

Along with the development of physiological and biochemical foundations for rationing the nutrition of different population groups, it should be paid special attention on the necessity of consuming vegetables, fruits, dairy and meat products for the people above the working age to maintain and strengthen their health status.

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Efficient Means of Development of Speed Abilities Based on Analysis between Morphofunctional Indicators with Speed Qualities for Footballers on the Stage of Advanced Specialization

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Abstract

The article revised analyzes of indicators of morphofunctional parameters in the interconnections between speed indicators of football players at the stage of in-depth specialization. The experiment was conducted during the competitive period, using the method of Bioimpedansometry and the computer mathematical-statistical program SPSS for parametric non-parametric correlation.

Keywords: *Pearson-Brave correlation, Kendal and Spearman correlation, SPSS computer program, Bioimpedancemetry method, standard "8" ("eight"), physique, metabolic rate, bone mass density index.*

Introduction

Optimal change of the functional state of players by the intensity of the game is a requirement of modern football[2]. The increased functional state of football players over 15 km per match is evidenced by the rate of qualitative growth of players physical endurance in overcoming long distances during a single game, as well as the players performance of technical tricks at high speed and for a long time[1].

Relevance: The research of dependencies by method of mathematical statistics and the theory of probability, in particular by correlation analysis, helps researchers establish regularities that previously unknown to them[3]. Obtaining accurate and operational information in the analysis of sports parameters that are complex and difficult[4]. At the same time, for optimal planning of training loads, it is necessary to process the measured data during the training sessions, to assess the state of a football player after stress training is an invaluable tool in the researcher's arsenal, and especially at the stage of in-depth specialization.[5].

Object and Subject of Research

Eight football students of the RSHIOR (The Republican Olympic Reserve Boarding School) at the aged of 14 to 15 years were selected as **the object** of research. **The subject** of the research is to study of the correlation between morphofunctional and speed qualities, such as the percentage of total fat, the percentage of water in body weight, muscle mass in kg., physique, metabolic rate, bone mass density index, high start running at 10 and 30 meters, shuttle run 5 * 30m and the test "eight".

The Purpose of the Research: The purpose of this work was to research the interconnections of four speed characteristics such as the run of footballers at 10 m, 30 m, shuttle running 5*30 m and the test "eight" with physiological indicators and as a result to obtain quantitative measures of these interconnections in the form of coefficients of paired correlations to reveal effective means of developing speed qualities [6].

Method of Research

The SPSS package of computer programs for mathematical statistics, including a software procedure that implements parametric and nonparametric method of correlation analysis were used to achieve this purpose.

The physiological indicators of athletes were eight signs: body length, body weight, percent of total fat and water in the body, muscle mass, physique, metabolic

rate and bone density index. The indicators were taken using Tanita MC-980 equipment (Japanese - made) and the Bioimpedansometry method (Fig. 1).



Fig. 1. Tanita Bioimpedance Meter MS-980.

Bioimpedansometry method. The study of the component composition of the body by the method.

Assessment of body composition of athletes using bioimpedansometry method. Bioimpedansometry (BIR) or bioimpedance analysis of the diagnostics of an athlete's body composition by measuring impedance - the electrical resistance of body parts. An apparatus designed for bioimpedance measurement is called a bioimpedance meter (Tanita company, Japan). The method is based on measuring the resistance of the bioelectric resistance of body tissues (in particular, the following parameters were recorded: body length (cm), body weight (kg), relative fat mass (%), relative water mass (%), TBW (%), type of addition, level of metabolism, bone density index. (Fig. 2.2.3.)



Fig. 2.2.3. Tanita Bioimpedance Meter MS-980.

Information on the effect of physical exercises on length of body weight in young athletes is scarce. This is due to the difficulty of organizing lengthy experiments with subjects who regularly engage in physical exercises for several years. In addition, it is extremely difficult to take into account the effects of changing factors such as diet and heredity that significantly affect body size in a long study on a young man.

There is a bioimpedance method for determining body weight and percentage of fat in an athlete's body. With regular measurement of body weight and the percentage of body fat, one can accurately determine how the structural composition of the body is measured in favor of active muscle tissue. The normal fat content in young men for 18 year olds is 14-20%.

It is important to note that the composition of the body in young athletes varies, and as in adults, adipose tissue increases (Table No. 2.2.3.)

Bioimpedanceometry (BIR) or bioimpedance analysis is a method of diagnosing human body composition by measuring impedance - the electrical resistance of body parts - in different parts of the body. The device used to perform bioimpedance measurement is called a bioimpedance meter (Tanita). The method is based on measuring the bioelectric resistance of body tissues ("impedance" - resistance, "bioimpedance" - resistance of biological tissues). On the basis of the received data the conclusion about normative broken hydration of tissues, lipid and water-salt exchange is made and the results are monitored during the whole period of training work. According to the data in Table 2 translated, it should be noted that the average result of boys' body weight was 59.9 kg, their physique is large. Since the normal percentage of water $\bar{X} = 67.5\%$. The physique of trained, but observed lack of fat body weight (6.4%). Table 2.2.3.

Table 1: Body composition data of young soccer players in the Bioimpedansometric method.

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№	**Surname and first names of students**	**Hight (cm)**	**Weight (kg)**	**% total fat**	**% water**	**Muscle mass (кg)**	**Phusique**	**Metabolic rate**	**Physiological age**	**Bone mass density index**	**Internal fat**
	Norm										
1	Begmatov Azizbek	176	65,8	3,0	69,6	59,4	8	1847	12	3,1	1
2	Platov Izzat	170	63,9	-7,5	65,8	56,1	8	1755	12	3	1
3	Ortiқboyev Dierbek	182	66,4	-5,0	66,5	59,9	8	1861	12	3,1	1
4	Tўkhtasinov Zainiddin	169	61,7	-5,5	68,2	55,3	8	1726	12	2,9	1
5	Khabibjonov Shohzhokhon	168	64,4	10,2	63,6	55,0	5	1727	12	2,9	1
6	Rakhmatullaev Shazod	166	56,9	-6,2	67,3	50,7	8	1592	12	2,7	1
7	Ergashev Davron	168	59,0	-5,9	67,5	52,8	8	1651	12	2,8	1
8	Tursunov Jahongir	174	64,3	-6,7	65,9	57,0	8	1779	12	3,0	1
9	Sarimsaқov Abror	180	62.2	-5.0	70.9	56,2	8	1750	12	3,0	1
10	Shokirov Ramzan	171	63,1	-6,3	66,8	56,1	8	1751	12	3,0	1
11	Ganizhanov Husan	180	58.6	-5.0	67.1	52.9	7	1653	12	2.8	1
12	Akbarov Jahongir	157	46.4	-5.0	71.8	41.8	8	1342	12	2.3	1
13	Zhumabaev Suhrob	172	66.8	-5.7	68.0	59.8	8	1861	12	3.1	1
14	Yodgarov Ahmad	185	79.9	12.4	60.3	66.3	5	2091	12	3.5	1
15	Toshpulatov Asadbek	180	72.4	-6.6	65.9	64.3	8	2002	12	3.4	1

Table 2: Form for measuring body composition using the bioimpedance method

	Body Fat Percentage			**The percentage of internal fat (%)**			**Bone mass content (kg)**			**The percentage of water in the body (%)**
	Low level	**Optimal**	**High level**	**Standart**	**Elevated level**	**High level**	**Low level**	**Optimal level**	**High level**	**Standart level**
Standart	11-13%	14-20%	21-23%	1-6	7-12	13-30	до 65 кg	65-95 кg	от 95 кg	60-70%
							2,66кg	3,29 кg	3,69 кg	

Antropometric body type

«-» - low level

«0» - moderate level

«+» - high level

Code	Type	Fat	Muscles
1	Hidden fullness	++	--
2	Fullness	+	-
3	Heavyweight	+	+
4	Not trained	0	-
5	Standart	0	0
6	Trained	0	+
7	Thin	-	-
8	Muscular	-	+
9	Very muscular	-	++

№	Surname and first names of students	Hight (cm)	Weight (kg)	% total fat	% water	Muscle mass (кg)	Phusique	Metabolic rate (kcal)	Physiological age	Bone mass density index	Internal fat
Standart		-	-		60%	-	8	-	12	-	1-2
1											
2											
n											
∑ average											

Discussion

Correlation analysis is used to determine the degree of closeness of the interconnection between two random variables X and Y. The coefficient/correlation is used as a measure of bonding. The correlation coefficient is estimated by sampling the volume of n related observation pairs (x, y) from the joint general population X and Y. There are several types of correlation coefficients, the application of which depends on assumptions about the joint distribution of X and Y values.

Based on the above-mentioned speed and physiological indices of the athletes, we have created a sample in Excel file format consisting of eight observations (rows) and twelve indices (columns), a copy of which was then used as the source data for the correlation analysis program. Because the processed file has fewer observations, in addition to the parametric method (calculation of correlation coefficient by Pearson-Brave method) non-parametric method (method of rank correlations of Kendal and Spearman) of calculation of correlation coefficients are used.

The Results of the Research

Before starting the calculations, descriptive statistics and exploratory analysis programs were performed to determine the basic statistical characteristics and check the distribution of the initial data for normality (Table 1).

The table shows the name, average, standard deviations, and the number of all the studied indicators.

Table 3: Descriptive Statistics Table

№	Indexes	Average	Std. dev.	N
1	2	3	4	5
1	10m running	1,8825	,17990	8
2	30m running	4,5375	,20070	8
3	Shuttle running 5*30m	31,8638	1,09520	8
4	Test«8» «eight»	15,4075	,55633	8
5	Hight (cm)	170,7500	4,43203	8
6	Body mass (кg)	61,2125	5,62810	8
7	% total fat	2,0875	9,72220	8
8	% water	65,8750	3,00464	8
9	Muscle mass (кg)	52,9625	3,51281	8
10	Physique	6,6250	1,40789	8
11	Metabolic rate	1665,3750	107,59705	8
12	Bone mass density index	2,8250	,17525	8

Table 2 presents the correlation matrix containing paired correlation coefficients obtained as a result of the work of the program for calculating correlation coefficients by the Pearson-Brave method.

Table 4: Pearson pair correlation table

Correlation by Kendal	10m running	30m running	Shuttle running	Test «8» «eight» рка»	Hight (cm)	Body mass (кg) (кг)	% total fat	% water	Muscle mass (кg)	Physiqueия	Metabolic rate
30m running	,805*										
Shuttle running5*30m	,586	,652									
Test «8» «eight»	,548	,824*	,737*								
Hight (cm)	,454	,091	,245	,228							
Body mass (кg)	,100	-,015	,538	,320	,123						
% total fat	-,015	-,183	-,259	-,072	-,131	,526					
% water	,035	,098	-,407	-,312	-,102	-,973**	-,596				
Muscle mass (кg)	,092	-,034	,615	,313	,252	,951**	,302	-,910**			
Physique	-,069	,082	,049	-,145	-,063	-,685	-,940**	,761*	-,515		
Metabolic rate	,095	-,033	,603	,313	,229	,968**	,350	-,929**	,998**	-,551	
Bone mass density index	,147	-,035	,628	,300	,414	,879**	,180	-,829*	,979**	-,420	,967**

An analysis of the data in the table showed that, out of the speed characteristics of athletes, the 10-meter run correlates not with all physiological indicators, except for the growth indicator, has no correlation, and growth correlates with an average degree of tightness with a correlation coefficient equal to r = 0.454.

The indicator of running 30 m also does not correlate with all, even with growth, physiological indicators, except for the "eight" test r = 0.8.

The shuttle running indicator 5 * 30 m showed a completely different picture concerning the physiological characteristics of athletes. This indicator correlates with physiological indicators of body length and the percentage of total fat in a weak degree, and with the latter in the opposite direction, with coefficients equal to r = 0.245 and r = -0.259, respectively. And with indicators, body weight, percentage of water, muscle mass, metabolic rate and bone density index, with an average degree of bond strength with the corresponding correlation coefficients r = 0.538, r = -0.407, r = 0.615, r = 0.603, r = 0.628. Only the type addition indicator did not correlate with the shuttle run indicator.

The "eight" test indicator with three physiological indicators (growth, percentage of total fat, and type of addition) had no correlation and correlated with other physiological indicators to an average degree of tightness with a correlation coefficient in the side-chapel r = 0,300.

Due to the smaller number of observations, in addition to the parametric method, we had to use nonparametric method for calculating pair correlation coefficients based on the Kendal rank method of correlation (Table 3) and Spearman (Table 4).

According to the Kendal ranking method (Table 3), the 10-meter running correlates on average with the growth indicator (r = 0.556) and with the percentage of total fat and water in the body, to a weak degree, respectively r = - 0.327 and r = 0.266.

Running on 30 m by this method had a correlation connection only with the index of the percentage of fat of the total in the opposite direction with the value of correlation coefficient, equal to r = - 0,327, and with other physiological indexes of running on 30 m did not correlate.

The shuttle running 5 * 30 m according to the Kendal rank correlation method had the same weak tightness of connections with two physiological indicators: muscle mass and metabolic rate (r = 0.286). This indicator also weakly correlated with bone mass index (r = 0.355), and shuttle running did not correlate with other physiological indicators.

The «eight» test only correlated with three indicators, but weakly. These indicators are growth, body weight, and the percentage of water in the body with the corresponding correlation coefficients r = 0.327, r = 0.367, and r = -0.286.

The correlation of the «eight» test with other physiological indicators was very weak.

Table 5: Kendal pair correlation table

Correlation by Kendal	10m running	30m running	Shuttle running 5*30м	Test «8» «eight»	Hight (cm)	Body mass	% total fat	% water	Muscle mass (кg) (кг)	Physiqueия	Metabolic rate
30m running	,667*										
Shuttle running 5*30m	,473	,618*									
Test «8» «eight»	,327	,473	,571*								
Hight (cm)	,556	,222	,182	,327							
Body mass (кg)	-,182	-,036	,214	,357	,255						
% total fat	-,327	-,327	-,214	-,071	-,036	,429					
% water	,255	,109	-,143	-,286	-,182	-,929**	-,500				
Muscle mass (кg)	-,109	-,109	,286	,143	,327	,786**	,357	-,857**			
Physique	,252	,168	,041	-,206	-,084	-,536	-,866**	,536	-,371		
Metabolic rate	-,109	-,109	,286	,143	,327	,786**	,357	-,857**	1,000**	-,371	
Bone mass density index	,000	,000	,355	,197	,401	,749*	,197	-,749*	,906**	-,273	,906**

According to the Spearman ranking method (Table 4), the 10-meter run correlates on average with the growth indicator (r = 0.590) and with the percentage of total fat in the body in the opposite direction and with the type of addition, lower than the average degree, respectively r = - 0.443 and r = 0.285.

Table 6: Spearman pair correlation table

Correlation by Sperman	10m running	30m running	Shuttle running 5*30м	Test «8» «eight»	Hight (cm)	Body mass	% total fat	% water	Muscle mass (кg)	Physique	Metabolic rate
30m running	,777*										
Shuttle running 5*30m	,587	,766*									
Test «8» «eight»	,407	,611	,690								
Hight (cm)	,590	,271	,299	,503							
Body mass (кg)	-,060	,000	,405	,476	,395						
% total fat	-,443	-,419	-,310	-,024	-,060	,619					
% water	,228	,168	-,262	-,333	-,275	-,976**	-,667				
Muscle mass (кg)	-,144	-,132	,333	,214	,359	,905**	,429	-,929**			
Physique	,285	,158	,126	-,252	-,095	-,693	-,945**	,693	-,441		
Metabolic rate	-,144	-,132	,333	,214	,359	,905**	,429	-,929**	1,000**	-,441	
Bone mass density index	,037	,037	,408	,284	,510	,840**	,259	-,840**	,964**	-,327	,964**

Conclusions

Running 30 m above the mentioned method very weakly correlates with the physical index of growth (r = 0.271) but with the index of the percentage of total fat in the body in the average degree and the opposite direction with the value of the correlation coefficient, equal to r = - 0.419, and with other physiological indicators correlation was not observed.

The shuttle running 5 * 30 m according to Spearman's rank correlation method had lower average connections

with the following physiological indicators: height, body weight, total fat percentage, muscle mass, metabolic rate and bone density index with corresponding correlation coefficients 0.299, 0.405, -0.310, 0.333, 0.333, 0.408.

Test «eight» to an average degree correlated with two physiological indicators. These indicators - growth (r = 0.503) and body weight (r = 0.476), and with two indicators - the percentage of water in the body and bone density index, weakly correlated with the corresponding correlation coefficients r = -0.333 and r = 0.284.

To develop the speed abilities of the players at the stage of advanced training to use the means of special physical training using a situational method. It is necessary to assess the data during the preparation.

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Modern Trends in the Prevention of Liver Echinococcosis

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Abstract

The results of diagnostics and treatment of patients with liver echinococcosis were analyzed for the period from 2010 to 2018. The follow-up amounted to 59 patients with recurrent liver echinococcosis. The acid-forming function of the stomach was analyzed in 34 patients who were on inpatient treatment for liver echinococcosis. Indicators of secretion were reliably biased towards hypoacidity. We examined 31 families of patients. The total number of examined was 93. In 66,7% of family members who had previously been operated on for echinococcosis, reduced acidity was found. This indicates that the risk of infection with echinococcosis is much higher in a hypoacid state than in normoacidic or hyperacidic gastric juice. A method of using drugs based on albendazole in the preoperative period, as well as to prevent relapses in the postoperative period, has been developed and proposed. The analysis of the incidence of liver echinococcosis in all districts of the Khorezm region was carried out. In all areas, especially in the most endemic ones, sanitary and educational activities are carried out both among the population and among the medical staff of regional clinics. Conducted interconnection with the veterinary service of the region, as well as with the sanitary and epidemiological bodies. The proposed method for applying Decasan residual cavity treatment and ultrasonic cavitation has shown impressive efficiency due to the lower number of relapses.

Keywords: *Liver echinococcosis; hypoacidity; prevention of echinococcosis with hypoacidity; perioperative prevention of recurrence of liver echinococcosis; method of echinococcectomy.*

Introduction

The relevance of the problem. In the Republic of Uzbekistan, more than 4.5 thousand operations per year are performed for liver echinococcosis[5], and more than a quarter of them suffer from complications[13].

In recent years, there has been a significant increase in the incidence of echinococcosis, with the number of patients with complicated forms reaching 5–40% [15]. Most often the liver is affected, in about 31 - 92% of cases, less often - the lungs, rarely - other organs. Mortality reaches 1.7 - 4.4%, the number of postoperative complications - 5.4 - 57.8% [14].

The difference between echinococcosis and other diseases is the difficulty of preventive measures, difficulties in early diagnosis, the use of only surgical method of treatment[5].

The increasing number of cases of echinococcosis, and many medical and social problems associated with it[7], make it necessary to further search for the causes of infection and method of prevention of this common and severe disease.

Analysis of the literature has shown that many issues of intraoperative and postoperative prevention of echinococcosis remain unresolved and contradictory. Most of the research, including fundamental monographs, scientific articles, as well as PhD and doctoral dissertations, is devoted to the technical features of performing echinococcectomies, using the latest

developments in the field of medical equipment, tools, including "Ultracision", "CUZA Exel+", "LigaSure", cystpericystectomy using an ultrasonic dissector, cystpericystectomy using an ultrasonic destructor-aspirator [8]. Of course, these method are progressive and should be implemented. However, the vast majority of clinics do not have such equipment[9].

Recurrent echinococcosis of the liver, strictly associated with the area of primary localization of the parasitic cyst, accounted for 26.6% of all recurrent forms. A large proportion of recurrent echinococcal cysts in segments remote from the primary focus (43.4%) and even lesions of another share (20%) call into question the role of the fibrous capsule of the primary cyst as the main factor of disease recurrence, which means that it is expedient to improve the method of its heat treatment, pericystectomy and wide use of resection technologies[10].

In 1985–1986, in vitro experimental studies on the effect of low-frequency ultrasound (LFU) on the germinal elements of echinococcus (GEOE)[4] were conducted in the Republican Center for Surgery named after Academician V. Vakhidov[3].

Studies have shown that, LFU has a powerful parasitocidal effect on GEOE. Even exposure of LFU to the GEOE for 1 to 2 minutes, causes a marked alteration of cells and other structures of the germinative envelope. Changes in brood capsules and protoscolexes, accompanied by the destruction of their membrane components, are irreversible.

The chemotherapeutic effect on GEOE is associated mainly with the use of various albendazole derivatives. The number of courses is unpredictable and depends on the success of chemotherapy and ranges from 8 weeks to 18 months.

However, a meager amount of literature has been devoted to the prevention of recurrence of echinococcosis in the postoperative period and, in general, to the prevention of infection by this pathology in a global, organizational direction. This explains the very disappointing situation regarding the prevalence of echinococcosis in the Republic.

Materials and Method

The results of diagnostics and treatment of patients with liver echinococcosis were analyzed at the Department of General surgery, traumatology and orthopedics of the Urgench branch of TMA and the Khorezm regional multidisciplinary medical center for the period from 2010 to 2018. Out of the total number of patients – 261 – a catamnestic analysis was performed in 225 patients. The follow-up volume was 59 patients with recurrent liver echinococcosis.

Relapse of the disease was detected in 52 cases out of 225 follow-up groups, and recurrence of echinococcosis was observed in 7 cases out of 36 patients in the prospective group. The largest number of relapses occurs in the following areas: Khiva, Khanka, Khazorasp. In addition, relapses were found in patients operated on in other regions of the Republic. On average, relapse occurred within 5±0.63 years. Repeated relapses occurred in 28 patients within 4±0.53 years.

In the existing literature there are only single and scattered reports of a direct relationship between the incidence of echinococcosis and the degree of inhibition of gastric acid production[1]. Found literary information about the role of hypoacidity in the development of echinococcosis creates the prerequisites for an in-depth information search in this direction.

Scientific studies of past years have shown that infection with echinococcosis is much more likely in patients with low acidity of gastric juice [12]. Based on the results of these studies, we purposefully conducted a clinical examination of family members of patients who underwent echinococcectomy from the liver. It was necessary to give an answer to the question of what dependence is the probability of infection with liver echinococcosis on the acid-forming function of the stomach in adults, as well as what frequency of infection with this disease in persons with normal gastric acidity among family members operated for liver echinococcosis under the same living conditions. We analyzed the acid-forming function of the stomach in 34 patients who were on inpatient treatment for liver echinococcosis by pH-metry. The indicators of secretion were significantly shifted towards hypoacidicity, which indicates a significant inhibition of the acid-forming function of the stomach in patients with liver echinococcosis.

Since we have provided district-based statistics on the incidence of liver echinococcosis in the Khorezm region, it was also advisable to conduct a similar quantitative study of dispensary data on the examination of family members operated on for liver echinococcosis.

A total of 31 families of our patients were examined.

The total number of examined was 93. The age of the examined ranged from 21 to 57 years, on average - 38±0.8 years.

Results

In general, the examined did not present complaints during the initial examination. Of the 93 people at the initial examination, 3 were diagnosed with gallstone disease, however, none of this group presented any complaints.

Initially, the examination began with ultrasound sonography (US) control. With ultrasound scan, echinococcosis was not found in 84 people (90.3%). In 6 (6.5%) cases, a single echinococcosis of the liver was found, and in 3 people (3.2%), a comprehensive examination using ultrasound scan and X-ray examination revealed multiple and combined echinococcosis of the liver and lungs. In case of a single liver echinococcosis, the average diameter of the cysts was 4.7 cm, and in patients with multiple lesions, the size of the liver cysts was on average 3.9 cm and for the lungs 4.1 cm. Detection of echinococcosis in a relatively initial stage, therefore, is, in our opinion, is extremely important, since the small size of the cysts can be subjected to chemotherapy without resorting to unnecessary surgical interventions.

In 9 patients with echinococcosis detected during clinical examination, the pH value of the gastric juice was checked.

In the main part of patients, members of families previously operated on for echinococcosis, reduced acidity was found (66.7%). This suggests that with a hypoacid state, the risk of infection with echinococcosis is much higher than with normoacid or hyperacidity of gastric juice. Those 3 patients with normal and high acidity, in our opinion, were infected with echinococcosis due to ingestion of a large number of oncospheres along with poorly washed vegetables. This circumstance requires a systematic sanitary-educational work among the population along with medical examination of family members of patients who have previously undergone echinococcectomy.

Discussion

Since 1983, the world practice has been using the anthelmintic drug albendazole and its derivatives for the treatment of echinococcal cysts less than 5 cm in size and anti-relapse prevention after surgical treatment. This provision is also enshrined in a resolution of the congress of the Association of Hepatologist Surgeons of Russia and the CIS countries (2014). Retrospective analysis of medical records revealed that 5% iodine solution and 96% ethyl alcohol were used to treat residual cavities. Subsequently, we used the following scheme for the treatment of residual cavities: treatment with 5% iodine solution, 70% ethyl alcohol, and 0.02% Decasan solution. In addition, with medium and large sizes of the residual cavity and the intrahepatic location, ultrasonic cavitation was used with the equipment SIGMA -01, 1990 (France) with frequency of 26.4-26.6 KHz.

We have developed and proposed a methodology for the use of drugs based on albendazole in the preoperative period, as well as to prevent relapse in the postoperative period. Three days before the operation, we use Zentel in a dose of 10-12 mg/kg of body weight. We assume that the period Of zentel's presence in the blood, according to the data of manufacturers' research, is 8-12 hours [11]. By circulating in the bloodstream, Zentel interferes with the implantation of living protoscolexes in the tissue, and also eliminates the risk of dissemination.

In the postoperative period, a week after discharge from the hospital, we prescribe taking Zentel at a dose of 10-12 mg/kg for 28 days together with simultaneous administration of hepatotropic drugs (Carsil, Essentiale, etc.). After this, a break is required for 14 days. We offer 3 to 4 courses of preventive chemotherapy. For multiple lesions, we use 4 to 5 courses.

If a hypoacid condition is detected, we offer a diet that stimulates the secretion of gastric juice, including broths with spices, mashed vegetables and fruits, boiled low-fat meats, fish and poultry, rye stale bread, cabbage and cabbage juice, peppermint, decoctions of St. John's wort, wormwood, calendula, lemons, butter and vegetable oils, cottage cheese, sour milk, buttermilk, juices. In addition, we recommend multivitamins, drugs containing hydrochloric acid, such as acidin - pepsin, enzyme drugs, biostimulants - aloe, pantocrine and others.

In order to prevent liver echinococcosis, as well as for early detection of the disease, we have proposed a method according to which relatives of the operated patients are invited to the clinic, they are given an ultrasound scan. If echinococcosis is detected, in addition to other studies necessary for the upcoming

operation, an analysis of the acidity of gastric juice is carried out. In addition, we identify the most endemic areas of the region where cases of liver echinococcosis are more common. In all areas of the region, especially in the most endemic, sanitary and educational events are held both among the population and among the medical personnel of district clinics. For this purpose, the media are also used - regional newspapers, radio messages. The interconnection with the veterinary service of the region, as well as with the sanitary and epidemiological bodies.

Thus, a retrospective analysis showed the ambiguity of the results of various method of echinococectectomy from the liver. A rather significant number of recurrences of echinococcosis of the liver is detected. There are several reasons for this. Firstly, the level of acidity of gastric juice matters, low acidity facilitates infection with echinococcosis. Secondly, the method of processing the residual cavity is important, since protoscolexes can remain alive and penetrate deep into the surrounding tissues (metastasis). Analysis of long-term results shows that the detection of recurrent liver echinococcosis most often occurs at 4-5 years after surgery. At the same time, there is a need for a control inspection every six months using ultrasound as a screening method.

The proposed method of surgery, including preoperative chemoprophylaxis, postoperative chemoprophylaxis for three years, a method of treating the residual cavity using LFU with Decasan should, in our opinion, significantly reduce the likelihood of recurrence of liver echinococcosis. Preventive and sanitary-educational work among the relatives of the operated patients, as well as close relationship with the veterinary and sanitary-epidemiological services, in our opinion, can significantly reduce the incidence of liver echinococcosis among the population of the Republic.

Conclusion

A specific study of the incidence of liver echinococcosis in the regions of Uzbekistan is essential to reduce the incidence of this pathology, which is of great social and economic importance. The analysis of the incidence of echinococcosis of the liver in all areas of the Khorezm region. The greatest number of morbidity and relapses is observed in Bagat (17.4%), Khiva (16.2%), Khazarasp (13.0%), and Khanka (11.0%) districts.

In a comparative assessment of various method for treating the residual cavity using devastation and technological method, the proposed method for applying the treatment of the residual cavity to Decasan and ultrasonic cavitation showed impressive efficiency due to the smaller number of relapses.

A complex of preventive measures has been developed to reduce the incidence of liver echinococcosis in the Khorezm region, including postoperative preventive devastation therapy, systematization of sanitary and educational work among the population and medical personnel, a clinical examination of family members of patients operated on for echinococcosis, including a study of gastric acid acidity, use of the media.

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Disturbance of Coagulative Hemostasis among Patients with Henoch-Schonlein Purpura

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Abstract

The Purpose: To examine the state of thrombocytic and coagulation hemostasis among patients with immune microthrombovasculitis**.**

Materials and Method: The study included 75 patients with immune microthrombovasculitis. Among them, rmen - 24 (32%), women - 51 (68%), the average age is 42.4±1.3 years. The control group consisted of 15 practically healthy persons.

Results and Discussion: A study of coagulation hemostasis among patients with immune microthrombovasculitis showed the presence of hypercoagulation shift with the increase of fibrinogen concentration, prothrombin index, shortening of the active partial thromboplastin time and blood clotting time, the appearance of hybrid monolith complexes - level increase of D-dimer positive ethanol test.

Conclusions: The most common clinical form of IMTV is the skin-joint form of the disease (62.2%), 89.3% of patients were admitted with an acute form of the disease. Women get sick twice as often (67.3%) than men (32.7%), the highest incidence is in the young age group from 18 to 49 years (87.3%). There is hypercoagulability in case of immune microthrombovasculitis.

Keywords: *Immune microthrombovasculitis, coagulation hemostasis.*

Introduction

Immune microthrombovasculitis (IMTV) - is an autoimmune disease characterized by generalized vasculitis which involves small skin vessels, the gastrointestinal tract, kidneys, joints, and, rarely, the lungs and central nervous system (CNS). The causes of the disease can include infections of various aetiology, parasitic invasions, various medical products, the introduction of serums, vaccines, etc. IMTV mainly affects microcirculatory vessels, such as arterioles, capillaries, and post-capillary venules[3].

IMTV is a widespread disease from the systemic vasculitis group of immunocomplex nature[11] and in accordance with epidemiological studies[16], IMTV constitutes 59% of allnosologically forms [19]. The annual incidence of IMTV reaches 20-25 per 100,000 of population, and there is an increase in the incidence [14]. Children get sick more often than adults[5] and in accordance with some data, the ratio of IMTV between children and adults is 8:1 – 9:1[17].

The studies of the hemostasis system have led to the conclusion that the leading link in the process of blood

clotting disorders among IMTV patients is the tension of thrombolytic-vascular hemostasis and the acquisition of procoagulant properties by endothelial cells under the influence of immune aggression. In case of hemorrhagic vasculitis, "aseptic inflammation" occurs in small vessels, as well as the formation of immune complexes that circulate within the bloodstream[9]. Under the influence of soluble immune complexes and cytokines activated by them, components of the complement system, aseptic inflammation of micro vessels develops with more or less deep focal endothelial dystrophy and vascular wall disruption[12]. As a result, phlebarteriectasia occurs with divergence of endothelial cells, violation of vascular permeability and its integrity[7]. Then, the secondary hemorrhages of skin blood vessels and internal organs develop.

There was a evident activation of coagulation hemostasis, i.e. a state of thrombophilia with a tendency to hypercoagulation and thrombosis, as well as the development of disseminated intravascular coagulation syndrome in acute situations [13].

In 90 % of cases, the disease debuts with arthritis or arthralgia [15] and joint syndrome is the diagnostic criteria of IMTV [20]. In general, joint damage (usually knee, ankle, wrist) exists among 60-80 % of patients with IMTV, more often develops among adults and combines with myalgia [8]. As an adult, joint pathology takes the form of chronic or migrating intermittent polyarthritis[22].

The high prevalence and increase in the number of patients with IMTV, the severity of the clinical course, determine the important socio-economic significance of this problem.

The Purpose of the Research: To examine the state of thrombocytic and coagulation hemostasis among patients with immune microthrombovasculitis**.**

Materials and Method of the research. The study included 75 patients with IMTV who were under treatment in the Department of Hematology in the multidisciplinary clinic of the Tashkent Medical Academy. Among them, men - 24 (32%), women – 51 (68%), the age range of patients was from 19 to 70 years, the average age was 42.4±1.3 years. The control group consisted of 15 practically healthy persons in comparable age, as well as 6 men (53.4 %) and 9 women (46.6%), whose average age was 39.4±1.2 years.

We divided all the patients under study into 4 groups in accordance with the clinical implications of the disease. The first group included 14 (18.7%) patients with cutaneous form, the second group consisted of 47 (62.6%) patients with cutaneous-articular form of IMTV, the third group included 8 (10.7%) patients with mixed abdominal form and the fourth group included 6 (8%) patients with mixed renal form of the disease.

Research Method

the research method included patient examination, general blood analysis by the hematological analyzer Mindray BC 5000 (China),), blood clot retraction, platelet adhesion and aggregation by the aggregometer Biola Alat-2 (Russia), fibrinogen, prothrombin index (PTI), fibrinolytic activity, activated partial thromboplastin time (APTT) by the coagulometer Humaclot Junior (Germany), antithrombin III, D-dimers by the Roche Cobas C 311 biochemical analyzer (Switzerland).

Results and Discussion

An analysis of the patients distribution by age and gender showed that among patients there was a predominance of women, the number of men was almost half the number of women.

The age composition study of the examined patients showed that the majority of patients were young; 24% (18) were in the age from 18 to 29 years and 60% (45) were patients in the age from 30 to 49 years.

The diagnosis based on clinical symptoms and laboratory data. Patients with a cutaneous form of IMTV were hospitalized with a severe form of the disease. 13 (92.8%) patients of the first group suffered the acute start of the disease. All patients had multiple small-point hemorrhagic rashes on the skin of the lower and upper limbs and within the abdominal area, sometimes of a coalesced character. We observed the maculopapular rash with necrotic skin among 5 (35.7%) patients. 47 patients had the skin-joint form of IMTV, we observed it within the scope of skin lesions in the form of a symmetrical hemorrhagic rash, joint damage. The most characteristic were swelling, soreness, restriction of joint function, often marked symmetrical involvement of the joints: knee, ankle, elbow and wrist. 8 patients constituted the third group with diagnosis of abdominal IMTV on the basis of characteristic spastic pains in various parts of the abdomen, as well as conditions of nausea and vomiting. Abdominal syndrome developed simultaneously with skin rashes. 2 patients had the

enterorrhagia. We found the most severe complications among patients with IMTV refered to 6 patients with kidney damage. The main clinical signs of kidney damage were micro and macrohematuria, proteinuria, cylindruria, edema, and increased blood pressure.

Thus, the analysis of the research results showed that the main part (62.6%) constituted the patients with skin and joint disease, women had the disorder twice as often (68%) as men (32%), the highest incidence is within the young age group in the age range from 18 to 49 years (84%).

Among 75 patients with IMTV who passed treatment in 2019, the state of hemostasis was under study within comparison with the control group (tables 1, 2). In accordance with the table 1, the number of thrombocytes in IMTV group was more high than in the control group, although it remained within normal values. At the same time, there was no significant difference between the thrombocyte values of both groups. The study of thrombocytes functional characteristics showed that the patients of the main group have a distinct increase in the adhesive and aggregation properties of thrombocytes. Thus, in the group of patients with IMTV, the aggregation time increased and constituted 119.3±2.7%, while in the control group it was 66.7±4.1%. this is significantly more high than the initial data. The observed significant activation of thrombocytes aggregation was among 38 patients (88.3%), while 5 (11.7%) patients had the thrombocytes aggregation time within the normal values. The adhesion of platelets in the main group significantly differed from the control group and increased to 62.4±1.2%, and within the control group it was 41.2±0.97%.

Table 1: Thrombocytic indicators among patients with IMTV, M±m.

Indicators of hemostasis	Control group n=20	IMTV, n=43
Thrombocytes (10^9/l)	232,8±9,75	258,8±6,65*
Retraction (of 0.3-0.5 s)	0,32±0,02	0,25±0,02
Aggregation, %	66,7±4,1	119,3±2,7*
Adhesion, 20-40%	41,2±0,97	62,4±1,2* *

Note: * - accuracy in relation to the control group *- p< 0,05; * *-p< 0,01.

The parameters that characterize the platelet link of hemostasis also include the identification of blood clot retraction. The study of the retraction time among IMTV patients showed that the initial indicators were more short than in the control group, which indicates an increase in thrombocyte activity, but without any reliable difference.

In order to assess the state of plasma coagulation hemostasis, we studied blood clotting factors, physiological anticoagulants and products of fibrin degradation.

The studies have shown that among the patients with IMTV, the initial values of coagulation hemostasis indicators demonstrated a state of hypercoagulation. (table 2).

In the direction of hypercoagulation, the indicator of blood clotting time (BCT) changed. In the group of patients with IMTV, the BST was shortened to 2.1±0.13 minutes, which is more than half in comparison to the control group (4.8±0.21 minutes). The research results of activated partial thromboplastin time (APTT) showed that a shortening of this indicator occurred among 55 (73.3%) patients. As can be seen, the APTT in the control group was 35.2±0.54 seconds, and in the group of patients, it decreased to 25.2±0.48 seconds. (p<0.001).

Table 2: Indicators of coagulation hemostasis among patients with IMTV, M±m

Indicators of hemostasis	Control group, n= 15	IMTV, n=75
BCT (5 min)	4,8±0,13	2,1±0,12* *
aPPT, sec (45 sec)	35,2±0,54	25,2±0,48* **
PTI, % (90-105%)	86,4±1,24	104,0±1,33* *
INR	1,25±0,04	0,82±0,03* *
Fibrinogen (2.0-3.5 g/l)	2,4±0,19	4,9±0,17* *
Hematocrit, % (40-50%)	41,2±0,9	48,8±0,82*
Von Willebrand factor	96,2±1,2	118,6±1,8* *
Antithrombin III %	100,3±5,2	87,8±3,1 *
FA, sec(140-240 s)	142,2±6,14	256,2±5,18 ** *
D-dimers µg/ml	0,58±0,03	2,1±1,6*
Ethanol gelation test	negative	positive

Note: * - accuracy in relation to the control group, * - p< 0.05; * * - p<0.01; * * *-p< 0.001

The analysis of coagulation hemostasis state among patients within the IMTV group in accordance with indicators of prothrombin, INR and fibrinogen indicates clearly expressed hypercoagulation. The evidence of

it is a significant increase in the prothrombin index by 104.0±1.33% in the group of IMTV patients, while in the control group it is 86.4±1.24% ($p<0.01$), the average INR values in the main group were significantly shortened in comparison with the control group (1.25±0.04 and 0.82±0.03, respectively, $p<0.01$).

An increase in plasma fibrinogen concentration also characterized the state of hemostasis with a tendency to hypercoagulation, so among patients of the main group it was 4.9±0.17 g/l, and within the control group it was 2.4±0.19 g/l. ($p<0.01$). The hematocrit index in the groups under study did not deviate from the norm and was within the range from 41.2 to 48.8%.

The indicators of coagulation hemostasis also include fibrinolytic activity of blood. In our study, this indicator within the group of patients was significantly more high than in control group. In case of hypercoagulation syndrome, it is important to determine markers of inopexia. Our ethanol gelation test and determination of D-dimer content showed that there is an activation of fibrinolysis processes, which is indicated by an increase in the main group of the fibrinolytic activity indicator almost twice in comparison with the control group. The ethanol gelation test was positive among 61 (81.4%) patients, while within all control group it was negative. The content of D-dimers in blood plasma of patients with IMTV significantly increased, this indicator was almost four times more high than in the control group. It is very important to assess the state of hemostasis in order to study the anticoagulant hemic system, which is to balance shifts in the clotting system. Normally, these important systems of the body are in a state of dynamic equilibrium. For this purpose, the activity of a physiological anticoagulant, antithrombin III (AT III), was under study. In our study, this indicator was more low than the values of the control group and constituted 87.8±3.1% and 100.3±5.2%, respectively.

Thus, our study of the hemostasis coagulation link among patients with IMTV showed the presence of significant deviations in regard to hypercoagulation shift. We indicated it by an increase in the concentration of fibrinogen, PTI, shortening of APTT and BCT, low INR, and the appearance of degradation markers in relation to fibrin-fibrinmonomer complexes (an increase in the D-dimers level, a positive ethanol gelation test).

Conclusions

1. One found that the most common clinical form of IMTV is the skin and joint form of the disease (62.2%), with the acute form of the disease refered to 89.3% of patients.
2. Women get sick twice as often (67.3%) than men (32.7%), the most high incidence is in the group of the young age within the range from 18 to 49 years (87.3%).
3. The study of hemostatic parameters among the patients with IMTV indicates a state of hypercoagulation. The evidence of hypercoagulation state was an increase in the aggregation properties of thrombocytes, an increase in the quantity concentration of plasma clotting factors and fibrin degradation products in the blood, and a decrease in the anticoagulant properties of plasma.

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Some Features of Hemorrhagic Syndrome in Chronic Diffuse Liver Diseases

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Abstract

Objective of the Study: To characterize the hemorrhagic syndrome in patients with chronic hepatitis and liver cirrhosis of viral cause.

Materials and Method: 80 patients with liver cirrhosis of viral cause have been studied, 20 patients with liver cirrhosis of non-viral cause and 62 patients with chronic viral hepatitis.

Results of the Study: Hemorrhagic syndrome was pronounced in patients with liver cirrhosis of viral cause, and much less pronounced in the group of patients with liver cirrhosis of non-viral cause. In patients with chronic viral hepatitis B and C, bleeding symptoms were much less common.

Conclusions: Severe hemorrhagic syndrome with liver cirrhosis of viral cause requires a detailed study of coagulation and vascular-platelet hemostasis for the prevention and treatment of bleeding.

Keywords: Hemorrhagic syndrome, liver cirrhosis, chronic hepatitis, hemostasis.

Introduction

Hemostasis is an evolutionarily developed, multicomponent protective function of the body. Its physiological significance is mainly to ensure the formation of a fibrin clot and maintain blood in the liquid state[1].

The liver plays an important role in hemostasis; therefore, diffuse lesions of the liver parenchyma are the cause of complex disorders of hemostasis [7]. In liver cirrhosis, changes in the hemostasis system are complex and multidirectional. In the occurrence of hemostasis disorders in liver diseases, complex mechanisms of interaction of platelets, coagulation factors, and the fibrinolysis system are involved[14]. Therefore, chronic or acute liver diseases, including viral hepatitis, alcoholic hepatitis, chronic hepatitis and cirrhosis, often have a profound effect on the hemostatic system[12].

Many liver diseases lead to thrombocytopenia, since protein coagulation factors and hematopoietic platelet growth factor thrombopoietin are produced in the liver[8]. According to many researchers, one of the reasons for the development of thrombocytopenia is an increase in antiplatelet antibodies in liver diseases[10]. Thrombocytopenia in liver diseases leads to an increased risk of bleeding, which may be a contraindication for antiviral therapy, liver biopsy and surgery [5].

Decreased synthesis of coagulation factors by liver cells plays a key role in changes in hemostasis. It also leads to an altered fragile[6] balance of blood coagulation factors, which is the reason for the development of

coagulopathy and the occurrence of hemorrhagic syndrome in liver diseases [13].

Bleeding is the most common clinical manifestation, due to thrombocytopenia and thrombocytopathy, to impaired synthesis of clotting factors, and activation of fibrinolysis. Other actual clinical problems are bleeding in patients with liver cirrhosis[3], bleeding esophageal varices, hematomas, hemorrhagic purpura, nasal hemorrhage, gingival hemorrhage and menorrhagia [2].

Timely diagnosis of hemostatic disorders in patients with chronic viral hepatitis and liver cirrhosis and appropriate hemostatic therapy will reduce the risk of hemorrhagic complications. It is noteworthy that previous researches concerned with the bleeding in chronic hepatitis and liver cirrhosis are few, and their results are contradictory. This explains interest in further research in this area.

Objective of the study: To characterize the hemorrhagic syndrome in patients with chronic hepatitis and liver cirrhosis of viral cause.

Materials and Method

Clinical studies were performed in the departments of Hematology and Hepatobiliary Pathology of the multidisciplinary clinic of the Tashkent Medical Academy, since 2016 to 2019. The study included 162 patients with chronic diffuse liver diseases, including 80 patients with decompensated liver cirrhosis of viral cause (Child-Pugh Class B), 20 patients with decompensated liver cirrhosis of non-viral cause(Child-Pugh Class B), and 62 patients with chronic viral hepatitis of moderate activity.

When establishing a diagnosis of liver cirrhosis and chronic hepatitis of viral cause, past medical history of the patients was taken into account (transfusion of blood components, dental treatment, etc.), as well as characteristic clinical syndromes (hemorrhagic, anemic, astheno-neurotic, icteric, etc.) and laboratory and instrumental studies data. The detection of hepatitis virus markers by ELISA and PCR, blood analysis, the determination of hepatitis B virus DNA (HBV) and hepatitis C virus RNA (HCV) and D (HDV) with the determination of genotypes was mandatory. In patients with chronic hepatitis and liver cirrhosis of viral cause, the viral load was more than 1,000,000 IU/ml.

The diagnosis of liver cirrhosis and the degree of hepatic cell failure has been established with the consideration of WHO recommendations (2008), according to the Child-Pugh classification,and based on diagnostic criteria. In order to establish the degree of fibrosis in patients with liver cirrhosis, an ultrasound scan (US), MSCT, and liver fibroscanhavebeen performed. The survey included patients with chronic hepatitis and liver cirrhosis who did not undergo antiviral therapy.

All patients examined by us were divided into 6 groups: group 1 included 30 patients with HBV liver cirrhosis, group 2 - patients with HBV+HDV liver cirrhosis, group 3 - 30 patients with HCV liver cirrhosis, group 4 - 20 patients with liver cirrhosis with negative results for markers of viral hepatitis. Group 5 included 32 patients with chronic viral hepatitis B of moderate activity, and group 6 - 30 patients with chronic viral hepatitis C of moderate activity.

Among 162 patients included in the study, there were 92 males (56.79%) and 70 females (43.21%). The age of patients ranged from 21 to 69 years, the average age was 48.2±12.1 years. Number of patients of working age accounted for 43.97%.

In order to determine the type of hemorrhagic syndrome, we studied patients' complaints, their past medical history and objective data: gingival hemorrhage, nasal hemorrhage, menorrhagia, bleeding esophageal varices and hematomas.

Research Result

The study included patients with liver cirrhosis of viral and non-viral cause, and chronic viral hepatitis B and C of medium activity. Liver cirrhosis in these patients was classified as decompensated (Child-Pugh Class B).

In order to determine the type of hemorrhagic syndrome, we studied the patients' complaints, their past medical history and objective data. Patient complaints were divided into the following types: anemic syndrome, sideropenia, symptoms of funicular myelosis, icteric syndrome, hemorrhagic syndrome and symptoms of liver failure. In order to identify hemorrhagic syndrome, in addition to patients' complaints, we paid special attention to objective data and a medical history.

The patients' main complaints were divided by the following hemorrhagic symptoms: nasal bleeding, gingival bleeding, skin petechiae, hemorrhoidal bleeding, bleeding esophageal varices, menorrhagia andgastrointestinal bleeding.

Analysis of the clinical manifestations of bleeding according to complaints, medical history and objective data of the patients showed that the hemorrhagic symptoms were very diverse during the examination. For example, epistaxis occurred in 59 (34.3%), gingival hemorrhages in 52 (30.2%), skin petechiae in 46 (26.7%), hemorrhoidal bleeding in 36 (20.9%), bleeding esophageal varices in 35 (20.4%), gastrointestinal bleedingin 29 (16.9%) and menorrhagia in 20 (11.6%) patients (Fig. 1).

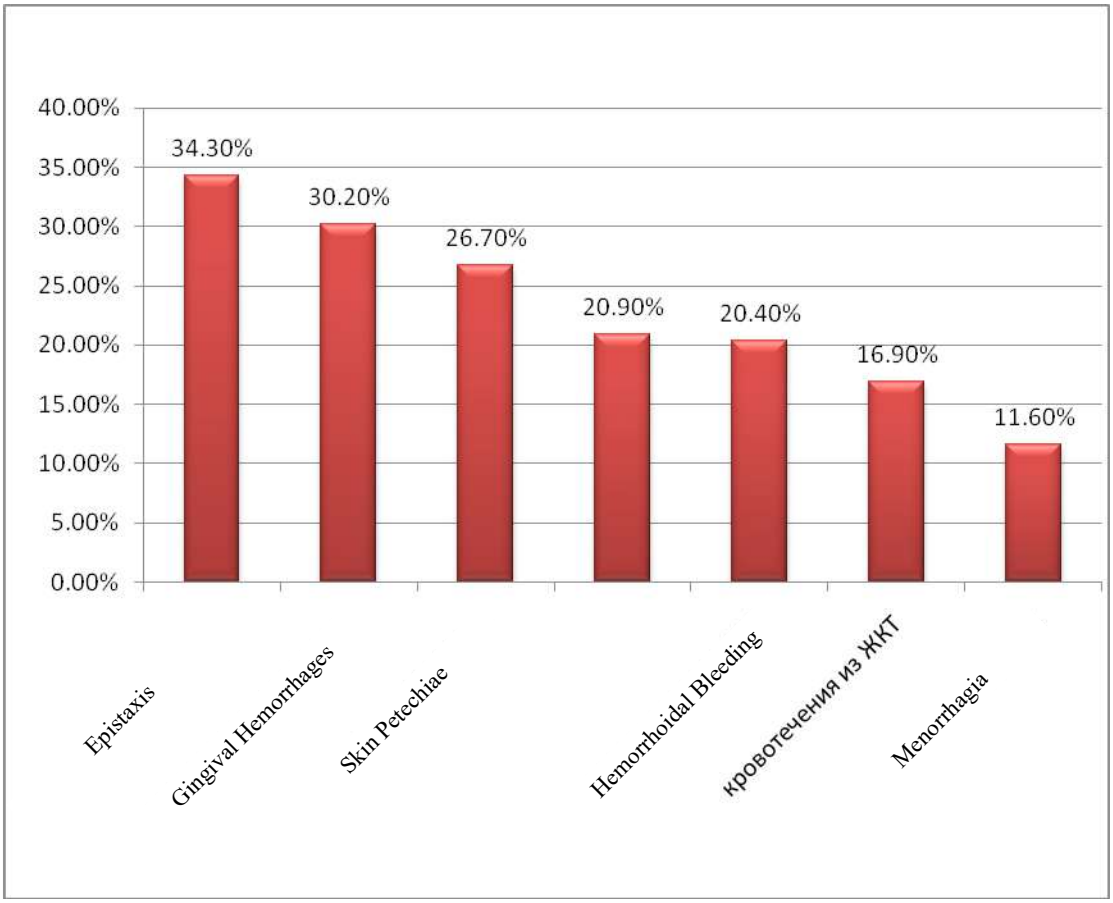


Fig. 1. Degree of incidence of hemorrhagic syndrome in patients with liver cirrhosis and chronic viral hepatitis

Hemorrhagic syndrome with large fluctuations has been found in different patient groups. For example, it was more pronounced in groups 1 and 2. In group 1, symptoms of bleeding occurred in 19 (63.3%) patients, including nasal hemorrhage in 17 (56.7%), gingival bleeding in 17 (56.7%), skin petechiae in 13 (43.3%), hemorrhoidal bleeding in 12(40.0%), bleeding esophageal varices in 12 (40.0%), gastrointestinal bleeding in 10 (33.3%)and menorrhagia in 8 (26.7%) patients.

In group 2, the incidence of hemorrhagic syndrome was highest and was observed in 14 (70.0%) patients. Nasal hemorrhage occurred in 12 (60.0%), gingival bleeding in 13 (65.0%), skin petechiae in 11 (55.0%), hemorrhoidal bleeding in 8 (40.0%), bleeding esophageal varices in 9 (45.0%), gastrointestinal bleedingin 7 (35.0%), and menorrhagia in 5 (25.0%) patients.

In group 3, symptoms of bleeding occurred in 16 (53.3%) patients. Nasal hemorrhage were observed in 16 (53.3%), gingival bleeding in 13 (43.3%), skin petechiae in 12 (40.0%), hemorrhoidal bleeding in 10 (33.3%), bleeding esophageal varices in 9 (30.0%), gastrointestinal bleeding in 7 (23.3%)and menorrhagia in 4 (13.3%) patients.

Group 4 consisted of patients with liver cirrhosis with negative results for markers of viral hepatitis. In this group, hemorrhagic syndrome was much less pronounced

than that with liver cirrhosis of viral cause. A detailed examination of patients' complaints and medical history followed by physical examination has showed that of hemorrhagic symptomsoccurred in 5 (25.0%) patients. Epistaxis occurred in 5 (25.0%), gingival bleeding in 5 (25.0%), skin petechiae in 4 (20.0%), hemorrhoidal bleeding in 3 (15.0%), bleeding esophageal varices in 3 (15.0%), menorrhagia in 4 (20.0%), and gastrointestinal bleeding in 3 (15.0%) patients.

The list of the main complaints of bleeding from patients, and their frequency in different clinical groups are shown in Table 1.

Table 1: Structure of hemorrhagic syndrome in patients with liver cirrhosis and chronic viral hepatitis

Hemorrhagic syndrome	Patient groups					
	Group 1 n=30	Group 2 n=20	Group 3 n=30	Group 4 n=20	Group 5 n=32	Group 6 n=30
Nasal hemorrhage	17 (56,7%)	12 (60,0%)	16 (53,3%)	5 (25,0%)	2 (6,3%)	2 (6,7%)
Gingival bleeding	17(56,7%)	13 (65,0%)	13 (43,3%)	5 (25,0%)	1 (3,1%)	1 (3,3%)
Skin petechiae	13 (43,3%)	11 (55,0%)	12 (40,0%)	4 (20,0%)	2 (6,3%)	1 (3,3%)
Hemorrhoidal bleeding	12 (40,0%)	8 (40,0%)	10 (33,3%)	3 (15,0%)	0	0
Bleeding esophageal varices	12 (40,0%)	9 (45,0%)	9 (30,0%)	3 (15,0%)	0	0
Gastrointestinal bleeding	10 (33,3%)	7 (35,0%)	7 (23,3%)	3 (15,0%)	0	0
Menorrhagias	8 (26,7%)	5 (25,0%)	4 (13,3%)	4 (20,0%)	0	0

Group 5 and 6 consisted of patients with chronic viral hepatitis B and C of moderate activity with no hemorrhagic syndrome. In group 5 of patients with chronic viral hepatitis B, hemorrhagic syndrome occurred in 5 (15.6%) cases: nasal hemorrhage occurred in 2 (12.5%), skin petechiae in 2 (12.5%), gingival bleeding in 1 (3.1%) patients. In group 6 of patients with chronic viral hepatitis C, hemorrhagic syndrome occurred in 4 (13.3%), with nasal hemorrhage in 2 (6.7%), gingival and skin bleeding in 1 (3.3%) patients.

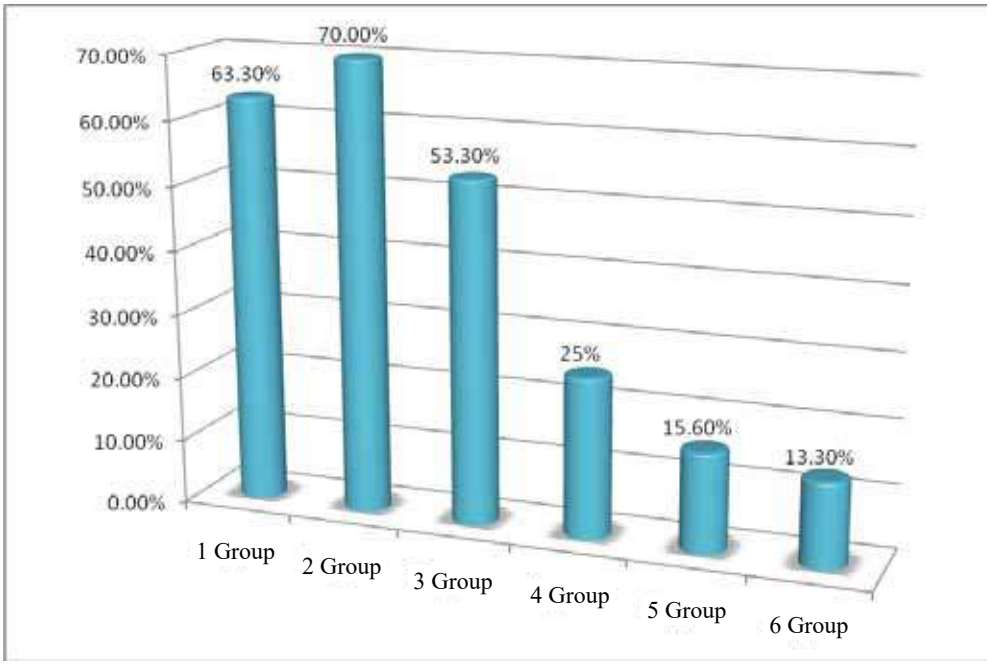


Fig. 2. Degree of incidence of hemorrhagic syndrome in patients with liver cirrhosis and chronic viral hepatitis.

As the above data shows, hemorrhagic syndrome was pronounced in the group of patients with HBV and HBV+HDV liver cirrhosis, in 63.3% and 70.0%, respectively. In the group of patients with HCV liver cirrhosis, bleeding symptoms occurred in 53.3% cases, and in the group with liver cirrhosis of non-viral cause, it was found twice less – in 25% of cases. For example, in chronic viral hepatitis B and C of moderate activity, hemorrhagic syndrome occurred in 15.6% and 13.3% cases, respectively (Fig. 2).

The study of the hemostatic system in the examined patients allowed us to determine the following structure of the incidence of hemorrhagic diathesis in patients with chronic hepatitis and liver cirrhosis of viral cause (Table 2).

Table 2: Nosological structure of acquired hemorrhagic diathesis in patients with chronic diffuse liver diseases

Groups	Acquired coagulopathies		Acquired thrombocytopenias		Acquired thrombocytopathies	
	abs.	%	abs.	%	abs.	%
Group 1 (n=30)	19	63.3	11	36.7	26	86.7
Group 2 (n=20)	13	65.0	9	45.0	16	80.0
Group 3 (n=30)	15	50.0	16	53.3	21	70.0
Group 4 (n=20)	6	30.0	5	25.0	10	50.0
Group 5 (n=32)	2	6.3	1	3.1	15	46.9
Group 6 (n=30)	1	3.3	2	6.7	13	43.3
Total (n=162)	**56**	**36.3**	**44**	**28.3**	**101**	**62.9**

As the Table shows, a study of the vascular-platelet and coagulation hemostasis links demonstrated that the majority of hemorrhagic diathesis in all groups is represented by acquired thrombocyopathies, while thrombocytopenias and coagulopathiesare much less common. Of 30 patients with HBV liver cirrhosis, 26 (86.7%) were diagnosed with acquired thrombocytopathy, while thrombocytopenia in this group was diagnosed in only 11 (36.7%) patients, and acquired coagulopathy - in 19 (63.3%). The same trend was also observed in patients with HBV+HDV and HCVliver cirrhosis. In the group 5 of patients with liver cirrhosis with negative results for markers of viral hepatitis, the incidence of hemostatic pathology was almost 2 times less than that in patients with liver cirrhosis of viral cause. For example, acquired thrombocytopathy occurred in 10 (50%), acquired thrombocytopenia - in 6 (30%), and acquired coagulopathy - in 5 (25%) patients.

Acquired thrombocytopathy was diagnosed in patients with chronic viral hepatitis B in 15 (46.9%), acquired coagulopathy - in 1 (3.1%) and thrombocytopenia and 2 (6.3%) patients. In patients with chronic viral hepatitis C, acquired thrombocytopathy was diagnosed in 13 (43.3%), acquired coagulopathy - in 1 (3.3%) and thrombocytopenia - in 2 (6.7%) patients.

Conclusions

1. The main hemorrhagic symptoms in patients with chronic hepatitis and liver cirrhosis of viral causehave been divided as follows: nasal hemorrhage in 59 (34.3%) cases, gingival bleeding in 52 (30.2%), skin petechiae in 46 (26.7%), hemorrhoidal bleeding in 36 (20.9%), bleeding esophageal varices in 35 (20.4%), gastrointestinal bleeding in 29 (16.9%) and menorrhagia in 20 (11, 6%).
2. Hemorrhagic syndrome has been found in a group of patients with liver cirrhosis of viral cause: with HBV liver cirrhosis – in 63.3%, with HBV+HDV liver cirrhosis – in 70.0%, with HCV liver cirrhosis - in 53.3% cases. In patients with liver cirrhosis of non-viral cause (group 4), hemorrhagic syndrome has been found more than 2 times less often – in 25% of cases.
3. In chronic viral hepatitis B and C, hemorrhagic syndrome was much less common –in 15.6% and

13.3% cases, respectively. This indicated a lesser violation of the hemostatic system than with liver cirrhosis of viral cause.

4. Since theliver cirrhosis of viral cause is followed by pronounced hemorrhagic syndrome, a detailed study of coagulation and vascular-platelet hemostasis is required for the prevention and treatment of bleeding.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Possibilities of Ultrasound in the Diagnosis of Congenital Dysplasia of the Hip Joint

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Abstract

Hip dysplasia is still the most common pathology in the structure of orthopedic pathology in children, and its diagnosis is difficult, and even with timely diagnosis, treatment does not always bring success, we were faced with the task of optimizing radiation diagnosis of this pathology based on assumptions about the multiple etiological nature of dysplasia.

To solve this problem, we selected 75 children from our ultrasound clinic. There were 52 girls and 23 boys with an average age of 14 weeks.. Based on the ultrasound study, a technique has been developed to assess the degree of maturity of the skeleton. Ultrasonic standards for the structure of the hip joints for children living in the iodine-deficient region have been developed. Ultrasound signs of somatic, orthopedic and neurogenic forms of hip dysplasia were revealed.

Keywords: *Young children, multiple etiological dysplasia, hip joints.*

Introduction

Congenital hip dislocation remains a topical issue in pediatric orthopedics. Hip joint dysplasia and congenital dislocation of the hip are the most common pathology among all the malformations of the musculoskeletal system that are expected and ranges from 5 to 16 cases per 1000 newborns[1]. The frequency of this pathology according to various authors varies from 3 to 20% (in endemic areas) [2]. In ecologically unfavorable areas, there is an increase in the frequency of this disease compared with the average European level [2].

The late diagnosis of dysplasia and dislocation is associated with poverty and nonspecific clinical symptoms in newborns and children of the first months of life, and untimely treatment, in turn, leads to a significant number of unsatisfactory results [3]. The traditional diagnostic method remains X-ray, recording changes only in bone structures, the number of which in children in the first months of life is relatively small[4]. According to various domestic and foreign authors, an X-ray examination of the hip joints becomes informative at the age of no earlier than 3-5 months of life[5].

Among the many alleged causes of dysplasia are the elderly parents, orthopedic diseases, heredity, bacterial, protozoal and viral diseases of the mother during pregnancy, endocrinopathy, toxicosis of pregnant women, vitamin D deficiency, hormonal changes in the mother, and pelvic delivery of the fetus and some others[6]. In addition, there are few reports in the literature about the effect of perinatal damage to the central nervous system on the formation of the pathology of the hip joint. Neurogenic dysplasia obtained in the experiments of Ratner AY et al. [7] and his staff described, however, the study of radiation signs of neurogenic dysplasia of the hip joint in children was not conducted.

For a long time, the diagnosis of dysplasia and

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dislocation of the hip joints was made on the basis of an examination by an orthopedist and the results of an X-ray examination[8]. However, the use of radiography up to 3 months of age is associated with an unjustified risk of exposure to the child, and the interpretation of radiographs is difficult due to the predominance of cartilage in the elements of the child's hip joint[9]. Therefore, indications for x-ray examination are limited. In recent years, due to the widespread introduction of ultrasound in pediatrics, the situation has changed. This method allows you to visualize soft tissue and cartilage components, there is no radiation exposure and the need for special preparation of the patient. The possibility of using the method as a screening and for dynamic control during therapy is also important[10].

The fundamental work of R. Graf in 1978, which served as the basis for the development of orthopedic ultrasound diagnostics in pediatrics, contains a number of diagnostic method and schemes that can reliably diagnose various degrees of dysplasia and dislocation[11]. However, this work and research by a number of late authors are devoted exclusively to the orthopedic problem and do not contain data on the possibility of a multi-etiological lesion of the joint and their radiation diagnosis[12].

The above arguments determined the purpose of our study, which was to develop ultrasound criteria for diagnosing the results of etiology-related disorders in the formation of hip joints - dysplasia - in children of the first year of life[13].

The aim of the study was to determine the significance of early ultrasonographic diagnosis of congenital hip dysplasia in children.

Materials and Method

We selected 75 children from our ultrasound clinic. There were 52 girls and 23 boys with an average age of 14 weeks (2 to 40). Everyone had a standard assessment with a history and physical examination. Ultrasonography of both hips using a 5 MHz linear sensor of the Siemens Sonoline 1 apparatus (Siemens, Erlangen, Germany) was performed by a pediatric orthopedic surgeon, who then classified the hips according to the Graf method and made recommendations for treatment. After obtaining parental consent, the babies then underwent a second ultrasound from another examiner who did not have a history and physical examination and did not see the first record. Thus, a total of 150 hips were examined, each of which had two ultrasound scans (300 strips in hard copy).

For ultrasound examination of the hip and hip joints, we used the method of R. Graf. In total, including control, 1640 ultrasound examinations were performed. The child was laid on its side, the leg was bent at an angle of 20-30° in the hip joint, which made it possible to obtain a better oblique section.

Since there is no lumbar lordosis in newborns, the optimal scan was when the scan plane (linear sensor) was parallel to the lumbar spine in the projection of the greater trochanter of the femur. Moving the sensor posteriorly allowed us to obtain an image of a mid-section of the femoral head, which has the form of a spherical formation of reduced echogenicity and fine-grained texture.

Normally, the femoral head was centered on a Y-shaped cartilage. The bony part of the roof was visualized at a slight angle to the horizontal line, the cartilaginous part (limbus) was determined as a hyperechoic strip covering the femoral head. The main center line was drawn along the horizontally located edge of the ilium, parallel to it, which corresponded to the center of the acetabulum. The bone bottom line was drawn through the bone protrusion and the Y-shaped cartilage, thus obtaining the angle alpha - the angle of inclination of the bone roof of the acetabulum. The cartilaginous roof line was drawn through the bony protrusion along the base of the limbus and received a beta angle - the angle of inclination of the cartilaginous roof of the acetabulum. The magnitude of these angles and the degree of ossification of the femoral head judged the maturity and anatomical usefulness of the joint.

Results

Indications for ultrasound examination are: symptoms of "click" and "slip" in the first week of life; hip abduction restriction; asymmetry of the gluteal folds; shortening of the leg; violation of hip rotation; birth in the buttock; increased muscle tone in the lower extremities; the presence of pathology of the hip joints in close relatives.

Normally, the joint head is centered in the acetabular cavity. The bony part of the roof is visualized almost horizontally, the cartilaginous part (limbus) is defined as a hyperechoic strip covering the head of the joint. The main line is drawn along the edge of the ilium, parallel to

it, through the center of the acetabulum. The bony roof line runs through the bony protrusion and the U-shaped cartilage and forms an angle alpha. The cartilaginous roof line is drawn through the bony protrusion at the base of the limbus and forms the angle of the betta.

Discussions

According to the results of ultrasound, 72.41% of children were diagnosed with types 1a and 1b of the hip joints (according to G. Reingard). Clinically and sonographically, types 1a and 1b correspond to the age of the child - these are healthy joints. The bony part of the acetabulum is well defined, the bone bay window is slightly flattened or rectangular, the cartilaginous part of the roof covers the head of the femur, the bone-cartilage ratio is greater than or equal to 2/3. The angle α is greater than or equal to 60°. Angle β less than 55° - type 1a; angle β is greater than 55° - type 1b.

Type 2a of the hip joints was diagnosed in 11.6% of children. This is a variant of the physiological delay in the development of hip joints in children under 12 weeks of age, at which the angle α is less than 59°, but more than 50°, respectively, the angle β is more than 60°.

Type 2b of hip joints was detected in 6.33% of children - hip dysplasia in children older than 3 months. The bone acetabulum is underdeveloped, the bone bay window is rounded, the bone-cartilage ratio is less than 2/3, the cartilaginous part of the roof covers the head of the femur. Angle α is less than 59°, but greater than 50°, angle β is greater than 60°.

Type 2c of the hip joints was found in 0.51% of children. This is a variant of severe dysplasia at any age. All components of the joint are underdeveloped. The bony part of the acetabulum is flattened, the bone bay window is rounded or flat, the cartilaginous part of the acetabulum is expanded, but still covers the femoral head. Angle α is less than 49°, but greater than 43°, angle β is greater than 65°, but less than 72°. This type of joint, without appropriate treatment, entails a progressive decentration of the femoral head.

In 0.25% of the child, type 3a of the hip joint was revealed - congenital dislocation of the hip. The bony part of the acetabulum and the bay window are flat, the cartilaginous part of the acetabulum is displaced cranially, since the femoral head cannot be fixed in the acetabulum, its decentration occurs. The structure of the cartilaginous part of the roof is not changed. The angle α is less than 43°.

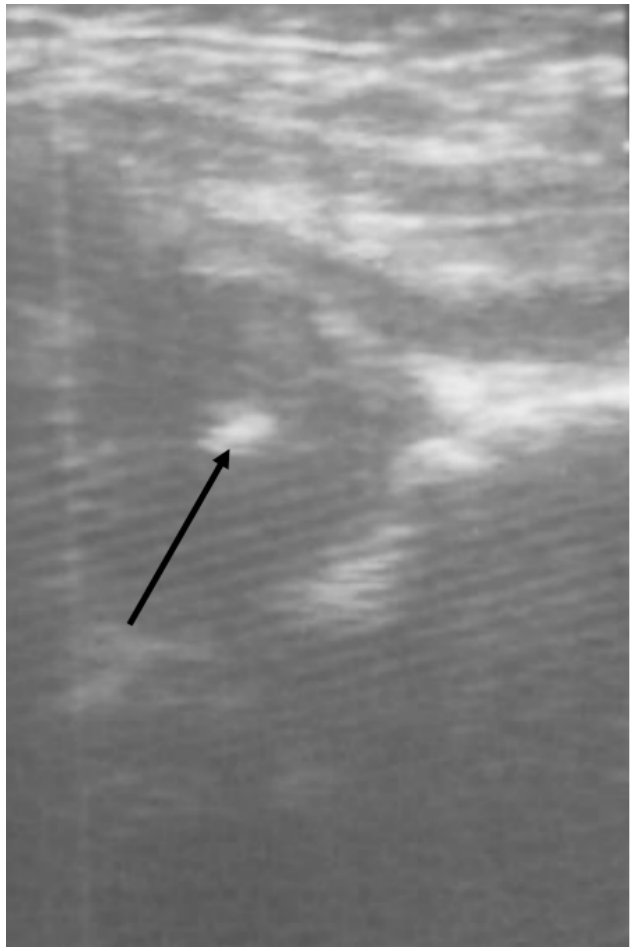


Fig. 1. Ultrasound image of the unchanged hip joint of a child of three months of life.

Note: The angle of inclination of the bone roof of the acetabulum α=68°; the angle of inclination of the cartilaginous roof to the trochanter of the female cavity is β=54°. The arrow shows the core of the ossification of the femoral head.

All children with identified pathology were referred for consultation with an orthopedic surgeon.

As a result, 70% of children with timely diagnosed pathology after the treatment have healthy joints of the first type, 30% of children with preserved pathology were sent for a second course of therapy.

Clinical screening for congenital hip dysplasia during the first year of life is considered a reliable strategy for reducing the incidence of dysplasia with a late diagnosis or hip dislocation, but only clinical screening sometimes leads to late diagnosis. Thus, ultrasound screening was used to further reduce the frequency of late diagnosis. Most ultrasound screening method for congenital hip dysplasia are based on a combination of static morphological assessment and dynamic stability assessment. The combination of universal clinical

screening and selective ultrasound screening is widely used throughout the world. Although it is logical to assume that selective ultrasound screening of high-risk patients should reduce the late diagnosis of congenital hip dysplasia, available studies have not confirmed this assumption. Further studies are needed to elucidate the role of ultrasound in screening for congenital dysplasia of the hip joint.

We have developed and patented an ultrasonic technique for determining the degree of maturity of the bone skeleton by means of the ossification of nuclei of ossification of the capitate and hooked bones of the wrist, which should be visible in a child aged 2 months. If a child over two months of age displays the indicated ossification nuclei and nuclei of the femoral heads, then this condition, with all other normal parameters, is estimated as the age norm (Fig. 1)

If the nuclei of ossification of the bones of the wrists were visualized, and the nuclei of the heads of the femurs were not detected, this condition was defined as a local violation of ossification in the area of the hip joints and a sign of somatic dysplasia of the hip-joint. If the nuclei of ossification were absent either in the carpal bones or in the heads of the femur, then a conclusion was drawn about a systemic slowdown in the ossification of the skeleton. It is these children, that is, those who suffered from local and systemic disorders of ossification and who had clinical signs of hip joint pathology, made up the II (1) group with somatic dysplasia.

Children suffering from somatic dysplasia of the hip joints, in 80.88% of cases had minor changes in the angles α and β, but without going into a state of subluxation and dislocation. This, in our opinion, is of great importance for the following treatment, since in such children, vitamin-D prophylaxis and treatment of rickets or rickets-like disease are primarily needed. The close attention of the pediatrician and the correction of somatic pathology leads to a complete restoration of the joint structure within 2 months after the start of treatment. Subsequently, during follow-up observation, these children were assigned to a group that did not have an uropathic pathology. Consequently, infants suffering from somatic dysplasia do not need to use fixing orthopedic devices, which in this case bring unconditional harm!

Conclusions

The study confirms the need for ultrasound of the hip joints for children under 6 months of age on an outpatient basis, avoiding unjustified radiation exposure. Using the information obtained makes it possible to conduct corrective therapy in a timely manner for all types of hip joints in the early period, followed by their correct formation.

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Patient Consent: Written informed consent was obtained from all participants of the research for publication of this paper and any accompanying information related to this study. A copy of the written consent is available for review by the authors.

Conflict of Interest: The authors declare that they have no competing interests.

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Clinical and Instrumental Evaluation of the Effectiveness of Surgical Treatment of Chronic Generalized Periodontitis Using RANK-RANKL-OPG Biomarkers

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Abstract

The problem of clinical and instrumental evaluation of the surgical treatment effectiveness of chronic generalized periodontitis using RANK-RANKL-OPG biomarkers is discussed in the article. Currently, the important role of Pro - and anti-inflammatory cytokines in the regulation of the immune-inflammatory response has been established [2]. Progressive destruction of bone tissue in patients with periodontal diseases is associated with a combination of cytokines IL-1β, IL-6, TNF and prostaglandins E2. The use of Alhadaya in complex therapy with surgical treatment will allow to control the level of biomarkers of bone inflammation, which will allow to use this method for the diagnosis of periodontal diseases and as the outcome of treatment, in addition to traditional research method (clinical, functional, x-ray, densitometric).

Keywords: *Dentistry, periodontitis, biomarker, RANK-RANKL-OPG.*

Introduction

According to WHO (World Health Organization), periodontal tissue pathology ranks 2nd in the structure of dental morbidity and is the main cause of premature tooth loss[1]. Chronic generalized periodontitis (CGP) develops under the influence of local and general factors, the leading of which is considered bacterial. The impact of periodontal pathogens on the tissues of the periodontal complex triggers a number of responses from the body, leading to the development of inflammation. Currently, the important role of Pro - and anti-inflammatory cytokines in the regulation of the immune-inflammatory response has been established[2]. Progressive destruction of bone tissue in patients with periodontal diseases is associated with a combination of cytokines IL-1β, IL-6, TNF and prostaglandins E2 [3]. These cytokines can activate osteoclastogenesis and bone resorption by osteoclasts. The increased migration of macrophages under the influence of cytokines and their constant presence in tissues exacerbates the destructive processes in the periodontium. It is known that there is a close relationship between the immune and bone systems. The study of the mechanisms of bone resorption associated with the development of periodontitis, Paget disease, osteoporosis, rheumatoid arthritis, multiple myeloma and metastatic bone tumors highlighted the importance of deeper research in the field of osteoimmunology [4].

A breakthrough in understanding the process of bone remodeling was the discovery of a signaling system consisting of the nuclear factor kß activator receptor (RANK), its ligand (RANKL), and osteoprotegerin (OPG) [5]. RANKL is a transmembrane protein – a representative of the family of tumor necrosis factors that plays a key role in osteoclastogenesis, differentiation, activation, and apoptosis of osteoclasts [6]. An important preparatory stage of osteoclast formation is the activation of RANKL, which occurs by binding to RANK located on osteoclast precursors. OPG, being a natural receptor trap for RANKL, competes with RANK for binding to

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RANKL [7]. By blocking this process, OPG suppresses bone resorption, giving priority to bone formation. Disorders of the balance of the RANK/RANKL/OPG system were also detected in various osteopathies, as well as in periodontitis. A significant increase in the content of RANKL in the periodontal pocket fluid is registered in the early stages of the disease [8]. In moderate to severe chronic generalized periodontitis, mixed saliva contains a high content of specific markers of bone remodeling-osteoclast activating factor (RANKL) and osteoprotegerin (OPG), which are absent in saliva in healthy individuals[9]. However, until now there are no clear correlations between the success of surgical treatment and the presence or absence of biomarkers of RANK-RANKL-OPG inflammation, so it seems relevant to conduct such a study.

The aim of the study is to increase the effectiveness of surgical treatment of chronic generalized periodontitis according to the RANK-RANKL-OPG biomarkers.

Materials and Method

To achieve this goal, 65 patients with severe chronic generalized periodontitis have been examined. The average age of patients was 35.43±0.68 years. To determine the quality of treatment, biomarkers of inflammation such as RANK-RANKL-OPG have been used.

The state of bone metabolism has also been examineded by determining the content of local regulators in blood plasma - sRANKL, osteoprotegerin (OPG) and sclerostin by enzyme immunoassay, according to the attached instructions.

In the formation, differentiation and activity of osteoclasts, the main role is played by the cytokine system RANKL-RANK-OPG. RANKL is a ligand of the Kappa nuclear factor activator receptor (antigen 254, TNF-1L). RANKL is produced by osteoblastic cells and interacts with the Ranktransmembrane receptor of osteoclasts and their precursors. When RANKL interacts with RANK, the signal activates intracellular cascade mechanisms and affects the NF-kB-nuclear factor Kappa b, which, with the help of TRAF receptors, goes from the cytoplasm to the nucleus and increases the expression of the nfatc1 protein, which is a specific trigger that triggers the transcription of intracellular genes that form the processes of osteoclastogenesis. OPG (osteoprotegerin) is a soluble (false) receptor for RANKL, synthesized by vascular stroma and endothelial cells. OPG is a blocker of RANKL-RANK interaction and, as a result, inhibits osteoclast formation and bone resorption. Sclerostin is a regulator of the canonical Wnt/β-catein signaling pathway of osteoblastogenesis. It is produced by osteocytes and mineralized hypertrophied chondrocytes, binds to LPR-5 (lipoprotein receptor 5), which is a Wnt-coreceptor, which leads to the destruction of the ubiquitin-proteosomal mechanism of β-catenan in the cytoplasm and a decrease in Wnt signaling with a decrease in osteogenesis.

The soluble receptor-activator of factor b was determined by a set of reagents "sRANKL" of Biomedica Medizinprodukte Gmb H and Co K G "(Germany), the osteoclast inhibitor factor – osteoprotegerin (OPG) by a set of reagents" Osteoprotegerin", sclerostin – " Sclerostin "of The same company on a semi-automatic analyzer"Uniplan". All patients gave their voluntary written consent to participate in the experiment. Statistical data processing has been performed using the program STATISTICA 6.0.

Results

Before the surgical treatment of chronic generalized periodontitis, all the patients underwent a study of biomarkers in the blood (the RANK-RANKL-OPG system). So, as an inhibitor of RANKL, we recommend the drug Alhada (black cumin oil). Alhada was appointed on 1 capsule 2 times a day for 6 months. No serious side effects found during patient follow-up. Thus, during the collection of anamnesis and catamnesis of patients, it was found that in 2 patients during the collection of apampesis vitae, chronic kidney disease of stage 2 was detected. Since patients with reduced renal function are particularly susceptible to hypocalcemia, which is accompanied by an increase in the level of parathyroid hormone, such patients were prescribed in addition to Alhadaya and vitamins of group D (calcium D3 nicomed) 1 tablet 3 times a day.

Discussion

Immediately after the use of Alhadaya and surgical treatment of chronic generalized periodontitis, the gums are pale pink, moderately moistened. The depth of the gingival furrow is 0.28±0.003 mm after treatment, there is no mobility of the teeth. The CPR index before treatment was 24.1±1.41, while after surgical treatment and the influence of Alkhaday it was 11.3±0.56 ($p \leq 0.05$). The hygiene index (HI) before treatment was 73.2±2.08, while after complex surgical treatment with Alhadaya

was 45.1±2.12 (p≤0.05). The bleeding index (PBI) before treatment was 2.1±0.05, while after treatment it was 0.8±0.33 (p≤0.01). The gingival index (GI) is 1.9±0.06 before treatment and 0.7±0.15 (p≤0.01) (after complex application of Alhadaya and hirrugic treatment).

After a General analysis of blood mineral metabolism, the total calcium level before treatment was 2.31±0.01 mmol/l, while after treatment it was 2.12±0.01 mmol/l (p≤0.05). Ionized calcium was 1.24±0.041 mmol/l before treatment, and after 1.12±0.007 mmol/l (p≤0.05). Parathyroid hormone before treatment was 56.7±2.14 PG/l, and after treatment 41.5±1.26 PG/l (p≤0.01). Calcitonin before treatment was 2.7±0.24 ng/l, and after treatment-4.3±0.23 ng/l (p≤0.001). Osteocalcin 5.5±0.57 ng/l before treatment, and after treatment 12.5±0.39 ng/l (p≤0.001).

The RANKL content before treatment was 0.23±0.005 pmol/l (p≤0.001) after treatment, the RANKL content was 0.14±0.003 pmol/l (p≤0.001). OPG before treatment was 4.35±0.004 pmol/l (p≤0.001), after treatment was 3.51±0.003 pmol/l (p≤0.001). Sclerostin before treatment was 297±0.4 pmol/l, after treatment was 210±1.2 (p≤0.05).

Osteoprotegerin (OPG) is a soluble receptor for RANKL. It is synthesized by osteoblastic type cells, as well as β-lymphocytes, vascular stroma and endothelial cells. OPG is a competitor to the interaction of RANKL with RANK and therefore, as a consequence, inhibits the formation of osteoclasts and bone resorption.

Sclerostin is produced by osteocytes and mineralized hypertrophic chondrocytes. It binds to the LRP-5/6 receptor, which is a coreceptor of the Wnt/β-catenin signaling system necessary for the stimulation of osteoblastogenesis and osteoblast functions, and has an inhibitory effect

Conclusions

Thus, osteoblasts express a high level of the nuclear factor activator receptor ligand Kappa beta and osteoprotegerin in the patients with chronic generalized periodontitis with reduced bone mineral density . The use of Alhadaya in complex therapy with surgical treatment will allow controlling the level of biomarkers of bone inflammation, which will allow to use this method for the diagnosis of periodontal diseases and as the outcome of treatment, in addition to traditional research method (clinical, functional, x-ray, densitometric).

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

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Comparative Analysis of Clinical and Laboratory Parameters and Assessment of Antiretroviral Therapy Effectiveness in HIV-Associated Cryptococcal Meningoencephalitis in the Case of Immune Inflammation Syndrome

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Abstract

Cryptococcal meningoencephalitis is an opportunistic mycosis. Earlier in medical practice single cases of the disease were encountered, now the problem becomes more and more urgent due to the increase in the HIV infection spread, as persons with impaired immune systems are affected. In most cases, cryptococcosis refers to HIV-associated diseases. However, today there are unexplored clinical, virologic immunological aspects of HIV-associated cryptococcosis. The comparative rarity of the multi-organ nature of the lesion, the lack of objective clinical and laboratory data that allow to timely suspect and confirm a cryptococcal infection, lead to errors in the diagnosis and treatment of this pathology. The main goal of our work is to study the prevalence of HIV-associated cryptococcosis, the clinical and laboratory features of HIV-associated cryptococcosis in patients associated with inflammatory immune restoration syndrome to increase the diagnosis and treatment efficiency in Uzbekistan.

Keywords: *HIV infection, cryptococcal meningoencephalitis, immune inflammation syndrome, antiretroviral therapy.*

Introduction

The development of deep mycoses in HIV-infected people is one of the most pending problems of the last decade. The combination of these two infections has increased over the past 10-12 years, which is associated with both an increase in the incidence of HIV infection and an increase in recorded cases. One of the most important risk factors for the occurrence of HIV-associated deep mycoses is the immunodeficiency state and the likelihood of developing severe clinical forms of the disease is determined by the severity of immunodeficiency. Among HIV-associated deep mycoses in patients with deep immunodeficiency, the infection caused by Cryptococcus neoformans often began to be recorded [1]. Cryptococcosis is recognized as the second most important mycotic infection. HIV-associated cryptococcal infection is detected with unequal frequency in different regions of the world: in Europe - 2-4%, in America - 30%. According to F. Odds (2009), in the world they annually sick from 700 thousand to 1 million people. And according to the Center for Disease Control (CDC, USA), about 1 million cases of cryptococcosis in HIV-infected patients are registered annually in the world [1]. According to large retrospective studies of HIV-infected patients, cryptococcosis occurs from 1.85% in Russia to 9.1% in the United States[8]. In economically developed countries, the cryptococcosis incidence is 30–66 cases per 1 million people per year [2]. Cryptococcal infection occurs mainly in patients with T-cell immunodeficiency and is characterized by frequent damage to the central nervous system. Quite often, cryptococcosis is the first AIDS stage manifestation. This figure varies from 39% in the United States (1992-2000) and 41.7% in Tunisia (1991-2000) to 58.8% in France (after the mid-90s) and 67.2% in Italy (1985-1999).

One of the most common forms is a cryptococcal infection caused by Cryptococcus neoformans (Sanfelice) Vuillemin (1901), especially C. neoformans var. neoformans, less commonly C. neoformans var. Gatii. [3]. The main causative agent of cryptococcosis is Cryptococcus neoformans, less commonly the disease is caused by C. Laurentii and C. Albidus. Pathogenic C. neoformans strains have a pronounced neurotropism, so the clinical cryptococcosis forms in most cases end in damage to the brain and its membranes [9]. A disease feature is the clinical manifestations severity and a very high mortality rate. During the initial therapy period, 10–25% of patients die, another 30–60% die within the next 12 months [6]. Mortality from cryptococcosis in the United States is 12%, and in Africa - 75–90%. The highest frequency of deaths from cryptococcal infection in HIV-infected patients was observed before the introduction of highly active antiretroviral therapy. With cryptococcal meningoencephalitis in HIV-infected untreated patients, it reaches 100%. Without HIV infection specific treatment, with a decrease in CD-4 lymphocytes of less than 100 cells/mm^3, the cryptococcosis incidence is from 4 to 30% and significantly decreases with the early ART prescription [2]. The main diagnostic method for cryptococcal infection is the cryptococci detection with microscopy and/or culture in the study of CSF, blood, and other substrates. Cultural diagnosis is considered the "gold standard" in diagnosis. In this case, the growth of colonies appears on the 3rd - 15th day, and it is difficult to isolate a pure culture of C. neoformans: it is known that pathogens of fungal infections are present in various mycoassociations in samples of clinical material, especially in HIV-infected patients. However, in terms of growth quality, yeast-like fungi are often ahead of mold fungi and it is much more difficult to isolate micromycetes of the genus Cryptococcus in culture compared to Candida sp. Determination of Cryptococcus neoformans antigen in cerebrospinal fluid, blood by latex agglutination test, which according to published data is found in 90% of patients, is still problematic due to the lack of available test systems[3]. Antibodies to the pathogen in blood serum are detected only in one third of patients with cryptococcal meningitis and are sometimes present in the serum of healthy people. Therefore, at present, the microscopic examination of cerebrospinal fluid remains the simplest and most informative laboratory method in the diagnosis of cryptococcosis [4]. According to the literature, the most severe course of disease generalized cryptococcosis forms have[13]. During the initial period of therapy, 10–25% of patients die, another 30–60% over the next 12 months. [14]. In this regard, there is an urgent need for early diagnosis and intensive care of cryptococcosis. The clinical manifestations of cryptococcosis are nonspecific and depend on the localization of the process. The most common clinical signs are febrile body temperature, persistent headache, dizziness, impaired consciousness, less commonly coughing, shortness of breath, rashes on the skin. In many clinical trials with cryptococcal meningitis or meningoencephalitis, about 75% of researchers have noted cases of high intracranial pressure caused by a rapid increase in cerebrospinal fluid [14]. The most characteristic development of subacute meningitis. In some patients, the disease is asymptomatic. Often with a cryptococcal lesion, a disorder of consciousness develops, visual acuity decreases, focal symptoms appear in the form of hemiparesis, ataxia, damage to the cranial nerves. The main feature of the cryptococcal meningitis clinical manifestations, according to a literature review, is negative meningeal symptoms, it is extremely rare for a patient to be clearly positive[11]. Mental disorders were noted in the form of decreased memory, uncriticality, euphoria, depression, changes in personality characteristics and even signs of deep encephalopathy[4]. The disseminated clinical forms of cryptococcal mycosis in HIV-infected people include damage to many organs and systems, especially the membranes of the brain (cryptococcal meningoencephalitis occurs in 80% of cases), as well as the heart, bones, kidneys and adrenal glands, eyes, prostate gland and lymph nodes[3]. Of particular scientific and practical interest are cases of generalized cryptococcosis forms with disseminated damage to almost all organs and tissues. In these situations, deep multiple organ dysfunction often leads to death until the moment when the attending physician can identify unfavorable background mycosis conditions in the form of various variants of immunological insufficiency [1].

Rare non-meningeal forms of infection include damage to the lungs, skin, joints, eyes, adrenal glands, gastrointestinal tract, liver, pancreas, prostate and urinary tract. Skin lesions with disseminated infection are found in 10-15% of HIV-infected patients with cryptococcosis [7].

Clinical guidelines for the treatment of cryptococcal meningitis currently need to be reviewed. The standard of combination of amphotericin B deoxycholate (amphotericin B deoxycholate, amphoto-B) and flucytosine (flucytosine) adopted in developed countries

is not acceptable in low- and middle-income countries, due to the high cost of flucytosine. For this reason, the combination of amphotericin-B and fluconazole (fluconazole) is of interest. According to the literature, antagonism is possible theoretically and in vitro between these antifungal drugs [10]. Such a combination was used for the early treatment of cryptococcal meningitis by an international team of scientists led by Dr. Peter G. Pappas from the University of Alabama, USA. The authors convincingly showed that the early combination treatment of cryptococcal meningitis with amphotericin B and fluconazole is well tolerated, and when using high-dose (800 mg per day) fluconazole also improves the results. In an accompanying editorial, Dr. Harrison T.S. from St.George University in London notes that the worst results in the low-dose fluconazole group could be due to the antagonism between amphotericin B and fluconazole [10]. Using the currently recommended treatment method based on Amphotericin[15], the clinical course of the disease in those who survive is often complicated by a prolonged or recurrent disease. There are unexplored clinical, virologic, immunological aspects of HIV-associated cryptococcosis[4]. The comparative rarity of the lesion multiple nature, the lack of objective clinical and laboratory data allowing to suspect and confirm cryptococcal infection with HIV in a timely manner, lead to errors in making a timely diagnosis, which entails errors in the initial pathogenetic treatment[6].

The question remains open whether prescribing antiretroviral therapy (ART) is possible and necessary shortly after diagnosis of cryptococcal etiology. In the literature, there is only evidence that an antiretroviral drug from the group of protease inhibitors indinavir restrains capsule formation in vitro[14]. The higher thickness of the capsule in patients receiving ART than in patients who have not received therapy or received only antimycotics, is probably associated either with the therapy ineffectiveness or with the fact that the capsule is not only a virulence factor but also protects cryptococcus from the macroorganism[16]. Another major concern for doctors is the of Immune Reconstitution Syndrome (IRIS) development. Quite often, cryptococcosis develops as a primary opportunistic infection against the background of an inflammatory immune system restoration syndrome. IRIS occurs in 15–20% of individuals who develop cryptococcosis initially and only then start ART, which leads to relapse and a more severe clinical course of the disease[15]. The development of more effective prevention/treatment method, as well as ensuring a safe and quick ART start, and its effectiveness in the treatment of antimycotic drugs, is important and is one of the main components of improving the HIV-associated cryptococcosis outcomes in patients. By the present date, the timing of antiretroviral therapy start remains uncertain[17].

Aim of Research: The main objective of this research is to study the prevalence of HIV-associated, identifying the clinical and laboratory cryptococcosis manifestations associated with inflammatory immune restoration syndrome in Uzbekistan.

Materials and Method

To achieve the aim and solve the tasks in the work, a general scientific methodology was used, based on an integrated systematic approach using epidemiological, clinical, microbiological, molecular genetic and statistical method. The prevalence of cryptococcosis in HIV-infected people in Uzbekistan was studied based on statistics from the Republican AIDS Center from 2012 to 2018. A screening method was performed by rapid tests for the presence of C. neoformans antigen in 347 naive adults living with HIV with a CD4 level of ≤100 lymphocytes in quick tests. The immune system state was studied by the total number of T-lymphocytes and their subpopulations in accordance with the differentiation clusters CD3 +, CD4 + and CD8 + lymphocytes using a Partec Cy FLOW Counter cytofluorimeter. HIV virus nucleic acids were detected by the Polymerase Chain Reaction method on a Rotor Gene 6000 TM. The diagnosis was made according to the EORTC/MSG (Europen Organization for Research and Treatment of Cancer/Mucoses Stady Group) criteria based on positive results by the rapid testing method for the presence of CrAg, as well as the determination of the pathogen C. neoformans in a microspy smear stained with Indian ink and culture growth on the Saburo environment. From instrumental research method, a computer tomogram of the brain, lungs, and X-ray examination of the upper and lower respiratory tract were also performed. A retrospective and prospective analysis of the clinical manifestations of 347 outpatient records and medical records was performed. The results of antifungal and antiretroviral therapy were evaluated in 37 patients with HIV-associated cryptococcal meningitis and meningoencephalitis who were hospitalized at the clinic of the Research Institute of Virology and the Specialized Infectious Diseases Hospital and at the Ust'-Izhora

Clinic, St. Petersburg, Russian Federation; then they were observed on an outpatient basis in dispensary departments of the RC AIDS, 14 regional AIDS centers. The research period is from 2012 to 2018. The criteria for selecting patients for the 1st (main) group were: cryptococcal meningoencephalitis and meningitis; adherence to ART and its beginning (no more than 6 months); positive immunological and virologic response to ART with the development of a clinical worsening of the condition with meningeal symptoms. The control group included 17 patients with HIV-associated cryptococcal meningoencephalitis and 20 patients in the main group with HIV-associated cryptococcal meningoencephalitis and meningitis taking ART antiretroviral therapy for a period of 1 to 12 months. The age index of the 1st and 2nd study groups ranged from 19 to 53 years (average 26.3±2.3 years, 83% of men).

Results

According to the research results, the cryptococcosis frequency in HIV-infected patients according to the long-term statistics of the Republican AIDS Center (from 2012 to 2018) amounted to 3.9% of HIV-infected patients with opportunistic diseases. Potential factors for the cryptococcosis development have been studied. Conducted in the case-control study. As hypothetical risk factors for the development of cryptococcosis in HIV-infected people, we included medical factors (HIV infection and its duration, the presence of chronic diseases of the upper respiratory tract, chronic hepatitis C, tuberculosis, diabetes mellitus, frequent hospital treatment, severe immunodeficiency of the patient, the development and presence of the clinical stages of HIV infection), as well as "social and behavioral" (employment, living conditions, being in a penitentiary institution, dependence on psychoactive substances, as well as socio-demographic risk factors for the development of the disease. In the group "case" 37 patients were included with a confirmed cryptococcosis diagnosis and the control group consisted of 23 patients without cryptococcosis who were observed in the same AIDS center or clinic at the same time. The groups were comparable by sex, age, territory of residence, stage of HIV infection, the number of CD4 < 100 cells/μl of lymphocytes, absent or taking ART.

The study revealed that cryptococcosis is significantly more likely to occur in HIV-infected patients living in private uncomfortable homes, where access to clean drinking water was difficult and there was no sewage. A direct relationship was found between the cryptococcosis occurrence and the active use of narcotic substances. The likelihood of developing cryptococcosis was also determined by the severity of immunodeficiency. The duration of HIV infection, a low level of limocytes (CD4≤100), previous hospitalizations created favorable conditions and created a high level of cryptococcal infection risk. The results obtained showed a direct relationship between these signs and the likelihood of developing cryptococcosis (OS 2.659 and 3.711; CI = 1.335-9.517). When examining 347 HIV-infected patients, a low level of limocytes (CD4≤100), serum antigenemia was detected in 11.2%. In 4.3% of patients, high titers were determined (≥1: 1024). High titers of the pathogen subsequently were a marker of poor prognosis. The research determined the time from the onset of symptoms to diagnosis on average 24.5 days passed (from 14-80 days). Cryptococcosis is rarely the only opportunistic infection in HIV-infected patients. Often combined with candidiasis (68% and 59%), cytomegalovirus infection (1.7% and 1.2%), and tuberculosis (17.2% and 15.3%). Severe intoxication and cerebral symptoms in cryptococcal meningoencephalitis were noted in 63% of patients of the first and second groups. Tachycardia (heart rate 88-104 per minute), hypotension (from 100/60 to 80/50 mm Hg), dyspnea (BH 22-38 per minute) were recorded in 48% of the 1st group and 52% of cases in 2nd group of patients. In our study, only 3 patients of the 2nd group and one patient of the 1st group were diagnosed within the first 14-18 days from the start of the disease. Upon admission, all patients complained of severe headache in the frontal and temporal areas, the average duration of which was 14-16 days in both groups. Irritability, nausea, vomiting, dizziness, unstable gait were observed in 47% of patients of the 1st group and 54% of the 2nd group of patients. Visual impairment (double vision) was noted in 5.4% of patients of the 2nd group. In the 1st group of patients, body temperature rose to 38–40°C and remained with the wrong type of temperature curve (88.4±3.21 cases) from 18 to 32 days, weakness (84±3.83), persistent generalized lymphadenopathy (44±4.23), sleep disturbances (87.5±4.11). Arthralgia disturbed 19.7±4.32 patients. Respiratory tract lesions with oropharyngeal mycoses were predominant among group 1 patients, and showed a productive cough in 77.5±3.97 cases (versus 48±3.87 in group 2 patients) and shortness of breath in 67.5±3, 7 cases (versus 24.3±3.45 in patients of the 2nd group). 45% of patients of the 1st group and 27% of patients of the 2nd group showed meningeal symptoms. Focal

symptoms (hemiplegia, nystagmus, anisocoria) were observed in 12.5±3.1 patients of the 1st group and in 7.3±3.7 patients of the 2nd group. Patients were depleted, the skin was pale, marked peeling of the skin in both groups was noted. Peripheral lymph nodes are enlarged to 1 cm, dense, painless. Tachycardia (heart rate 88–104 per min), hypotension (from 90/60 to 80/50 mm Hg), shortness of breath (BH 22–38 per min) were noted. Hypochromic anemia with erythrocyte counts from 2.3 to 3.3×10/12/L, leukopenia from 1.5 to 3.9×10/9/L, shift of the formula to the left (relative neutrophilia), lymphopenia were found in the hemogram in patients with hypochromic anemia from 3 to 7% and high ESR (39–66 mm/h). The number of CD-4 lymphocytes in the blood ranged from 7 to 86 cells/μl. The viral load was 131,000–634,000 copies of HIV RNA in 1ml of blood in the 1st group and 723,000–1,232,000 copies of HIV RNA in 1ml of blood in the 2nd group. Magnetic resonance imaging (MRI) revealed hydrocephalus, arachnoid changes in the cerebrospinal fluid. In patients of the 1st group revealed multiple nodes in the parenchyma of the brain, meninges, basal ganglia and midbrain. In patients of the 1st and 2nd group, cortical atrophy was observed. With repeated MRI performed in 3 patients of the 2nd group after 9-14 days, structural disorders of the basal nuclei of the white matter of the cerebral hemispheres, signs of focal encephalitis appeared.

For diagnostic purposes, all patients underwent puncture of the cerebrospinal fluid (CSF). The results of CSF tests showed a sharp increase in cerebrospinal fluid pressure (190-450 mm water column). Liquor is transparent. Microscopically in the cerebrospinal fluid, mainly lymphocytic cytosis was observed (from 12 to 1824 cells/μl), the protein content was increased (450-1400 mg/l), and glucose was reduced (0.1-2.5 mmol/l). The number of leukocytes in the CSF in 4 patients of the 2nd group was normal. The causative agent was detected by CSF microscopy in 89.1% of patients in both groups. Repeated adequate correction of intracranial hypertension using spinal puncture was performed in 53% of cases of the 1st group and 33% of cases of the 2nd group. Considering that in 5 patients with a three-time study of CSF with a different time interval (from 1 to 3 months), the pathogen was determined, resistance studies were performed in connection with long-term fluconazole therapy. An analysis was made of the IRIS effect on the following factors development: quantitative characteristics of CSF culture, initial CD4 lymphocyte count and viral load before ART, pressure during spinal puncture, leukocytes levels, protein, glucose in CSF.

Cultural diagnosis was performed using standard Saburo medium. Colonies of Cryptococcus neoformans were usually seeded after 48–72 hours. At the same time, cryptococcus was determined microscopically in 70%, culture was isolated in 77%, which made it difficult to evaluate the antimycotic therapy effectiveness in 23% of patients. Obtaining a cryptococcus culture made it possible to determine the sensitivity of the isolated strain to antifungal drugs. The sensitivity of the pathogen during the initial CSF study was 93±3.9 in the 1st group, and 91.2±3.7 in the 2ndgroup to amphotericin B; 89±3.9 in the 1stgroup, and 73±3. 9 in the 2ndgroup to fluconazole. The combination of these drugs was used as an etiotropic treatment in accordance with international recommendations. We used doses of amphotericin B at 0.7–1 mg/kg/day and fluconazole at doses of 800 mg/day, as recommended in modern guidelines. Fluconazole inhibits the synthesis of ergosterol in fungi, has excellent absorption and penetration into the CSF, and is a less toxic drug compared to amphotericin B. This treatment regimen allowed us to more quickly get the initial antifungal effect and the possibility of avoiding the toxicity of amphotericin B considering the therapy duration. Headache disappearance by the 7th day of antifungal therapy was observed in 12% against 18% of the 1st and 2nd groups of patients, and by the 14th day in 69% and 48% of the 1st and 2nd groups of patients. Treatment was considered successful if cerebrospinal fluid cultures were negative after 2 and 10 weeks, or if positive disease dynamics were observed in patients after 2 weeks and no symptoms after 10 weeks. Cryptococcus in CSF was determined by PCR in 31 cases. Before treatment, the microbial load ranged from 17,700 to 6,456,700 copies in 1 μl. During treatment, there was a decrease in load from undetectable values to 2,900 in the 1st group of patients and up to 12.348 copies in the 2nd group. Despite the ongoing therapy, 3 patients (1 from the 1st group and 2 from the 2nd group) did not observe treatment efficacy and the disease continued to progress. These patients in the consolidated phase of treatment switched to introconazole for 8-10 weeks of treatment. After 5–9 days of the consolidated treatment phase, 2 patients experienced deterioration of health, signs of respiratory and cardiovascular failure, cerebral coma developed, which resulted in death on the 42–48th day of the disease. The overall mortality rate was 5%-5.2% of cases, with no significant difference between the groups. The average number of CD4 lymphocyte cells

among patients of the 1st group at the time of diagnosis was 13.7±3.27 cells/μl, while in the 2nd group it was two times or higher (28.1±3, 23 cells/μl). By the 3rd month of specific treatment of cryptococcus, the number of CD4 increased to 56.3±3.7 cells/μl in the 1st group and to 78±3.2 cells/μl in the 2nd group. Patients of the 1st group during the prevention of relapse of the disease for 2-3 months, was prescribed antiretroviral therapy. In the 2nd group of patients, the average number of CD4 cells at 6 months after initiation of ART was 73±3.24 cells/μl, in patients of the 1st group for this period the number of CD4 cells was 103±3.78 cells/μl. HIV RNA in plasma was not detected (i.e., <400 copies/ml) in 8 (44%) patients of the 2nd group and in 15 (83%) of the 1st group at 6 months of ART. The data showed that patients in whom IRIS manifested with cryptococcal meningoencephalitis, had a significantly faster start and more progressive severe course. But the response to treatment with antifungal drugs on the background of ART was faster in the 2nd group.

Discussion

Thus, the cryptococcosis frequency in HIV-infected patients in Uzbekistan was first determined. The low rates of cryptococcosis among HIV-infected people in Uzbekistan are noteworthy. It amounted to 3.9% of HIV-infected patients with opportunistic diseases. This may be due to insufficient quality of diagnosis. For the first time, "medical" and socio-demographic risk factors for the cryptococcosis development in HIV-infected patients were identified. The most significant combinations of risk factors were the combination of the no employment with the fact of frequent hospitalization in hospitals, the lack of supportive prevention of the disease (in 32.6±5.99%), poor social conditions (in 13.7±4.27%) examined in the group observations. The results of the study confirm the direct relationship between the degree of immunodeficiency in HIV infection and the occurrence of cryptococcosis. The disease significantly more often develops with a CD4 T-cell count of ≤100 cells/μl. Also, the importance of the absolute number of leukocytes and lymphocytes in the peripheral blood was revealed, with their reduction, the risk of cryptococcosis in HIV-infected patients significantly increases. The results of the study also confirm the need for rapid testing for CrAg and fluconazole prophylaxis for cryptococcal disease in patients with CD4≤100 lymphocytes. The results of the study are consistent with the literature that cryptococcosis occurs against the background of severe insufficiency of cellular immunity. Therefore, screening with rapid tests for detecting CrAg in all HIV-infected patients with a CD4 level of ≤100 is necessary. The low information content of the specific clinical picture of the disease requires doctors when neurological symptoms appear, and requires microscopic examination of CSF in patients with prolonged persistent headache, even in the absence of meningeal symptoms. Cryptococcal meningitis should always be included in the differential diagnosis of chronic or subacute meningoencephalitis since the clinical features are not specific. In our studies, the typical clinical cryptococcosis manifestations in HIV-infected patients were an acute and chronic process development with high fever, a manifest clinical picture of CNS damage in the form of meningoencephalitis. The specific features of the course of cryptococcal meningoencephalitis in HIV-infected patients is the development of a manifest CNS lesion in the absence of clinical signs of disturbance on the part of the internal organs. The disease is characterized by a severe course in 92.6±5.99% of cases. It should be noted that most patients observed by us (83.0±3.72) had meningeal symptoms, although according to the literature, they are found only in 30.0% of patients suffering from cryptococcosis. None of the patients observed by us in vivo revealed clinical symptoms of lesions of the skin and other internal organs characteristic of a generalized pathological process. However, when conducting mycological studies revealed the presence of C. neoformans in cerebrospinal fluid in all patients of both observation groups. Uncharacteristic changes were CSF. The most demonstrative was an increase in protein from 0.47 to 4.84 g/l in 88% of patients, which indicated an increase in the permeability of the blood-brain barrier. There was also a decrease in glucose from 0.47 to 2.1 mmol/l in 36% of patients. 57% had lymphocytic or mixed pleocytosis ranging from 12 to 293 cells/μl, which indicated a weak inflammatory reaction. Liquor hypertension did not play a significant role in the severity of the course of the disease in both study groups. Increased attention is required not only to the patient's condition, but also to microbiological CSF monitoring with determination of the pathogen sensitivity during treatment. This will allow timely determination of the possible resistance formation to antifungal drugs. Adequate antimycotic therapy performed in the early days determined the positive dynamics of the pathological process.

An early assessment of treatment results (on days 10-14) is possible based on mycological CSF examination and a decrease in the microbial load determined by PCR (decrease after 2 weeks by 10 thousand times).

Maintenance therapy based on fluconazole (200 mg/day) with poor adherence or early cessation of treatment can lead to relapse of cryptococcal meningitis, which was noted in patients of the 1st group (84±4.7). Itraconazole was used as rescue therapy in 3 cases of fluconazole resistance.

Another aggravating factor in the disease development was the significantly expressed degree of immunosuppression (CD4≤100 cells/μl) in the observed patients. An increase in the content of CD4 + cells to a greater degree in patients with HIV-associated cryptococcal meningoencephalitis during ART contributed to faster and more successful treatment, and vice versa, the late ART start in patients with low CD4 counts increases the risk of cryptococcosis and other opportunistic diseases. But despite the increase in the absolute amounts of CD4 + lymphocytes in the main group, the fact is obvious that an increase in their number does not yet prove the normalization of the immune response lost in the early stages of HIV infection. The presentresearch once again shows that antifungal treatment with antiretroviral drugs helps to restore CD4 cell levels more quickly, as well as preventing the inflammatory immunity syndrome development, which can lead to severe cases of generalized forms.

Conclusions

1. It was found that the cryptococcosis frequency in HIV-infected patients was 3.9% of HIV-infected patients with opportunistic diseases.
2. Among the risk factors for the development of cryptococcosis in HIV-infected patients of the main group were socio-demographic (no employment, unsatisfactory housing and utilities) and medical (frequent hospitalization, lack of maintenance prophylaxis with fluconazole). The combination of these factors was the most significant.
3. The presence of a direct relationship between the degree of immunodeficiency in HIV infection and the cryptococcosis occurrence has been determined. The disease significantly more often develops with a CD4 T-cell count of ≤100 cells/μl.
4. The low information content of the specific clinical picture of the disease dictates the need for routine rapid testing for CrAg and fluconazole prophylaxis for cryptococcal disease in patients with CD4≤100 lymphocytes.
5. Cryptococcal meningitis should always be included in the differential diagnosis of chronic or subacute meningoencephalitis since the clinical features are not specific.
6. Characteristic changes in the CSF in cryptococcal meningoencephalitis were an increase in protein, a decrease in glucose levels, without much difference in the compared groups.
7. Microbiological CSF control and monitoring with determining the pathogen sensitivity during treatment allowed to timely determine the resistance formation to antifungal drugs.
8. Antifungal treatment on the background of antiretroviral drugs contributes to a more rapid increase in the level of CD4 cells.

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Treatment of Acute Lung Abscesses Considering their Non-Respiratory Function in Patients with Diabetes

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Abstract

During the examination and treatment of 465 patients with acute lung abscesses on the background of diabetes mellitus, a preoperative preparation program was developed that takes into account the phase of the process (group 1 - septic course of the process, group 2 - stabilization, group 3 - remission), the severity of endotoxemia and non-respiratory lung function. Patients of the 1st group against the background of basic long-term intra-arterial catheter therapy used the scheme of correction of non-respiratory function of the lungs that we developed, sanitized purulent foci with an electrolyzed solution of sodium hypochlorite. In 202 (79.9%) of 254 patients of the 1st group, it was effective that allowed to refuse the operation, while only 52 (40.6%) were not operated on in the 2nd, and 26 in the 3rd (31.3%) patients. In parallel, a pronounced normalizing effect was achieved in correcting the degree of violation of non-respiratory lung function against the background of delimitation and stabilization of the process. This allowed in 109 (60.9%) of 179 operated patients to confine themselves to lung resection with 8.7% of postoperative complications in the 1st group versus 18.4% in the 2nd and 24.6% in the 3rd, which emphasizes the effectiveness of our preoperative preparation.

Keywords: *Lung abscess, diabetes mellitus, non-respiratory lung function, diagnosis, conservative and surgical treatment.*

Introduction

Dissatisfaction of surgeons with the results of treatment of acute lung abscesses in patients with diabetes mellitus, due to the continued high mortality (up to 70%)[1] in gangrenous forms, contributes to the emergence of a large number of studies on various issues of the pathogenesis and treatment of this disease [12].

Against the background of the progress of resuscitation and intensive care in recent years in the treatment of acute lung abscesses in patients with diabetes mellitus[3], a combination of signs of disturbances in the activity of various organs and systems, defined by clinicians as multiple organ failure syndrome, began to be noted [9].

In our opinion, among the mechanisms of development of this syndrome[2], insufficient attention is paid to impaired non-respiratory function of the lungs, representing the main protective barrier that prevents the [18]generalization of infection and intoxication with subsequent disorders of almost all vital systems of the organism [17].

The treatment of acute lung abscesses in patients with diabetes mellitus is complex, is long and is accompanied by a large number of complications[19]. There is still no unity in the treatment of this disease. As a rule, they begin with intensive, complex therapy, including antibacterial drugs, non-specific anti-inflammatory drugs, detoxification therapy[13], sanitation of the tracheobronchial tree, and correction of metabolic disorders[7].

The indication for surgery is the absence of the effect of this treatment, carried out within 3 weeks, the chronization of the process that occurs[5], coming, according to various authors, in the period from 6 to 14 weeks from the time of the disease, the presence of life-threatening complications [6]. The weight of these criteria is estimated by various authors is far from unambiguous.

The foregoing determined the purpose and

objectives of this study: to clarify the indications for conservative, preoperative treatment of patients with acute lung abscess with diabetes mellitus, considering violations in non-respiratory lung function to improve immediate and long-term results of surgical treatment.

Material and Research Method

465 patients with acute lung abscesses were observed against diabetes mellitus at the age from 28 to 60 years old, divided into clinical groups[10]. The 1st group consisted of 254 (54.6%) patients with a pronounced clinical picture of inflammatory destructive process in the lung, which against in the background of prolonged selective intra-arterial catheter therapy, carried out targeted correction of non-respiratory lung function according to the method developed by us[12]. The 2nd group consisted of 128 (27.5%) patients with a stable course of the disease who used long-term intra-arterial catheter therapy according to the traditional scheme with generally accepted correction of metabolic disorders [14].

The 3rd group consisted of 83 (17.9%) patients who received purely traditional conservative treatment in other hospitals and transferred to our clinic to continue treatment with remission of the inflammatory process. This division of patients with acute lung abscesses in the presence of diabetes reflects different stages in the development and implementation of method for diagnosing and treating these diseases in our clinic.

The Results Obtained and their Discussion

The majority of patients - 341 (73.3%) were admitted within 2.5 months from the moment of illness, 119 (25.6%) patients were ill for no more than 3 months and only 5 (1.1%) were longer. We have developed a quantitative method for assessing the degree of impairment of non-respiratory lung function (compensated, sub compensated and decompensated) in patients with chronic non-specific inflammatory lung diseases on the basis of integral blood levels of albumin, globulins, total phospholipids and lipids, phosphatidylcholine and sphingomyelin.We used this method, with small additions and changes reflecting the degree of the inflammatory, mainly bacterial, process in the broncho pulmonary system, in the treatment of patients with acute lung abscesses with diabetes mellitus. A comprehensive clinical and biochemical study in patients with acute lung abscesses in diabetes mellitus during the stages both conservative and surgical treatment, led to the conclusion that they have a varying degree of non respiratory lung function impairment, deepening in the early postoperative period due to the presence of a lung abscess in the parenchymaa factor in surgery and general anesthesia.

The main rule in the treatment of patients with acute lung abscesses in the presence of diabetes mellitus was early and adequate endobronchial or transthoracic drainage and sanitation of the purulent-necrotic lesion in the lung. In 54.3% of cases, these rehabilitation method were used simultaneously.

Patients of the 1st clinical group, taking into account the degree of impairment of non-respiratory function of the lungs, used intravenous infusion of fat emulsion preparations (nirpid) and protein-synthetic enhancing agents by daily intraarterial drip of 100ml of a 10% solution of albumin or alvezin with intramuscular injection against the background of basic therapy. retabolil. Conservatively treated with the achievement of a certain positive result were 286 (61.5%) patients with acute lung abscesses associated with diabetes mellitus, 179 (38.5%) patients were operated on. In the first clinical group of 208 people, complete recovery was achieved in 163 (78.4%), and clinical remission was achieved in 39 (18.7%) patients with acute lung abscesses with diabetes mellitus. Disease progression was observed in 6 (2.9%) patients. In the 3rd clinical group, complete cure without surgery was achieved only in 4 (15.4%) of 26 patients, and clinical remission in 8 (30.8%) patients. It should be noted that a positive effect was achieved to a greater extent in the most severe patients.

So, in the 1st group, 202 (97.1%) patients achieved cure and persistent clinical remission. Only 6 (2.9%) patients who were admitted to the hospital with a running process, expressed symptoms of endotoxemia and respiratory failure were found to be ineffective; their disease progressed and they died of septic shock.

In the 2nd clinical group, the results of conservative treatment were more modest: only 16 (30.8%) patients achieved complete cure. Clinical improvement was noted in 20 (38.5%), treatment had no effect in 14 (26.9%) and the progression of the process, resulting in death, in 2 (3.8%) patients.

In the 3rd clinical group, complete cure and clinical remission were achieved in 12 (46.1%) treated, 14 patients (53.8%) were found to be ineffective. In general, conservative treatment was effective in 250 (87.4%) patients. Of these, 183 (64%) achieved a

complete cure, 67 (23.4%) patients refused surgery to achieve clinical remission. In 28 (2.8%) patients, the effect of treatment was not observed, and in 8 (2.8%) the pathological process progressed, therapeutic measures were ineffective, and patients died from various causes.

179 (38.5%) patients with acute lung abscesses were operated on the background of diabetes mellitus. Of these, 46 (18.1%) of the 1st clinical group, 76 (59.4%) of the 2nd and 57 (68.7%) of the 3rd. The main operation was a lobectomy performed in 109 (60.9%) patients. Advanced operations (pulmonectomy, bilobectomy, lobectomy with resection of segments) were performed in 70 patients[18]. The prevalence in the structure of organ-preserving interventions, we explain the preoperative use of long-term intra-arterial catheter therapy, which ensures the delimitation and stabilization of the destructive process in the lung[11].

It should be noted that the performed interventions were rather complicated and traumatic, which caused a number of complications after operations in 32 patients. The main group of complications is associated with impaired bronchial obstruction resulting in atelectasis and pneumonia in the remaining part of the lung (13 and 5, respectively).Empyema on the background of fistula of the bronchus stump occurred in 2 patients. The residual cavity in the early postoperative period, as a result of incomplete expansion of the remaining part of the lung, appeared in 4 (2.23%) patients. Postoperative bleeding was noted in 1 (0.56%) patient. Pulmonary embolism was the cause of death in 3 (1.7%) operated patients. Least of all were complications from the postoperative wound that occurred in 2 (1.12%) patients. Fixing the number and type of postoperative complications, it should be noted that in the 3rd clinical group they were noted in 14 (24.6%) patients, in the 2nd - in 14 (18.4%) patients and in the 1st clinical group - in 4 (8.7%) examined. Analyzing and explaining the results of conservative and surgical treatment of patients with acute lung abscesses against diabetes mellitus, we studied the dynamics of the clinical manifestations of the inflammatory process at the main stages of treatment. 46 (25.7%) of the 179 patients operated on in the clinic received a pronounced clinical presentation of the inflammatory process in the lung, which required intensive preoperative treatment, taking into account the degree of impairment of non-respiratory lung function.

Using method of conservative correction, it was possible to stabilize the process and achieve remission before surgery in 171 (95.5%) patients. Of these, the process was stabilized in 42 (23.46%) patients of the 1st group, remission was observed in 3 (1.68%) of the 1st and 14 (7.82%) patients of the 2nd clinical group, there was no effect from preoperative treatment only in 1 patient of the 1st group, and in 7 patients with a stable course of the disease deterioration occurred. These patients were operated on for vital indications.

Thus, by the time of the operation, the number of patients with acute lung abscesses in the presence of diabetes mellitus with symptoms of severe inflammatory process, decreased under the influence of conservative treatment to 4.47%, with stabilization of the process increased to 54.19%, and remission was achieved in 41.3% the patient, which confirms the effectiveness of the proposed conservative measures, but it is not clear why the results of preoperative treatment in patients with acute lung abscesses of the 2nd and 3rd clinical groups are worse than in patients with more pronounced clinic of the disease..

As indicated above, patients with acute lung abscesses in the presence of diabetes mellitus have a certain degree of impairment of non-respiratory lung function, manifested in various combinations of disorders of both systemic homeostatic[6] and local protective reactions. Considering the fact that the compensated and balanced degree of impairment of non-respiratory lung function is usually a dynamic and reversible process [19], and impairment of non-respiratory function of the lungs of patients with acute lung abscesses during diabetes mellitus can vary, we analyzed the degree of impairment of non-respiratory lung function within the clinical groups [15].It was found that 179 (38.5%) patients of the 1st clinical group had severe and moderate impairment of non-respiratory lung function characteristic of compensated changes, and 75 (16.1%) revealed subcompensated disorders.

Decompensated disturbances of non-respiratory lung function in patients of the 1st clinical group were not recorded. In 98 (21.1%) patients of the 2nd clinical group, compensated violations of the non-respiratory function of the lungs were revealed, disorders of the type of subcompensated recorded in 18 (3.9%) of the examined and less often decompensated were observed in 12 (2.58) %) surveyed.

For patients with acute lung abscesses in the presence of diabetes mellitus in the 3rd clinical group,

a decompensated form was found in 56 (12%) and subcompensated in 27 (5.8%) of them.

The data presented indicate that the stabilization and remission of the process in patients with an acute lung abscesses during diabetes mellitus, which are clinically determined, in most cases is not accompanied by normalization of non-respiratory lung function[1], which requires adequate[10] treatment of patients with acute lung abscesses, considering their degree of disturbances non-respiratory function, which meets the requirements of rehabilitation[14], which provides an increase in the proportion of patients specific weight with clinical recovery and remissions, and for staged surgical treatment reduces the number of inflammatory postoperative complications, improves immediate and long-term outcomes.

Conclusions

1. Conservative treatment was effective in 250 (53.76%) patients with acute lung abscesses in the presence of diabetes mellitus. Of these, 183 (64%) patients achieved complete recovery.
2. The proposed conservative, corrective preoperative treatment made it possible to stabilize the process and achieve remission before surgery in 171 (95.6%) operated patients with acute lung abscesses with diabetes mellitus;
3. Effective preoperative preparation, carried out considering the degree of violation of non-respiratory function of the lungs, limits and stabilizes the inflammatory process, which allowed 109 (60.9%) patients with acute lung abscesses to limit themselves to economical lung resection with 17.9% of complications in the postoperative period.
4. Clinical stabilization and remission of the process, is not always accompanied by normalization of the non-respiratory function of the lungs, which requires rehabilitation of patients taking into account their degree of violation of this activity.

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Increasing the Effectiveness of Public Information on Risk Factors and Self-Control of Hypertensive Disease in Primary Link

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Abstract

The past 5-year data from 222 randomiy selected outpatient cards of hypertensive patients who were followed up at a family polyclinic (n=54) and in a number of rural ambulances (n=168) of the Republik of Uzbekistan were retrospectively analyzed. The physicians from the Tashkent family polyclinic and rural ambulances of a number of the Republic's regions, who had taken 10-month retraining courses for general practitioners, were questioned. Questionnaires were used to study 156 and 119 physicians before and after retraining, respectively. Two hundred and fifty-six hypertensive patients followed up at the family hospital and rural ambulances were interviewed using questionnaires and examined. Then some of them (a study group) took a course of training at a school for the hypertensive patient and the others (a comparison group) did not participate in the education program. All the patients were followed up for 2 years with a subsequent reexamination and study. Most outpatient cards give recommendations for non-drug treatment incompletely. In a number of cases, the physicians who attach importance to the detection and correction of risk factors has substantially increased after education. The patients who had taken training courses were found to be significantly ($p<0,001$) more aware of their having hypertension and the major risk factors of hypertensive disease, to be more adherent to treatment, and to know its adequacy.

Keywords: *Arterial hypertension, hypertensive disease, school for the hypertensive patient, patient education.*

Introduction

AH in the world affects about 1 billion people and more than 7.1 million deaths per year are associated with high blood pressure (BP)[1]. According to WHO, about 20% of the world's adult population have problems associated with high blood pressure. Alarming forecasts of WHO experts have been published, according to which, by 2025, a peak in the growth of arterial hypertension (AH) is expected, the incidence of which will reach 29%[2]. adult population of the planet. As a result of a targeted health policy in the second half of the last century, a significant reduction in mortality from CVD was achieved in the countries of North America, Western Europe, Japan and Australia[3]. So, in

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1976-1980. in the American population, the number of patients receiving treatment was 31% of all patients with hypertension, and in 2004-2009 already 59%. Control of blood pressure (at a level below 140/90 mm Hg) for the same period was 10% and 34%, respectively [4]. In the CIS countries, the epidemiological situation correlates with global data. Studies conducted in different regions of Russia over the past 20 years indicate that hypertension remains one of the most common diseases. About 30 million people suffer from it, which makes up 20% of the total population[5]. Given the main role of hypertension in the development of coronary heart disease (CHD) and stroke, control of blood pressure becomes an important task for healthcare systems in the vast majority of countries[6]. The widespread prevalence of CVD risk factors is due to the modern pace and lifestyle, characterized by stress, physical inactivity, smoking, excessive salt, calorie intake, insufficient consumption of fruits, vegetables and potassium [7].

The importance of the study of hypertension is directed by the efforts of many clinical studies in Europe and the USA [8]. According to N. Engl (2015), only 5–10% of hypertensive patients in Europe and 25.6% in the United States achieved BP control[9]. Global urbanization and social insecurity lead to the launch of one of the diverse pathogenetic mechanisms for increasing blood pressure - psychoemotional stress[10]. As the Framingham study showed, tachycardia accompanying stress not only contributes to the progression of atherosclerosis, but also increases the incidence of significant cardiovascular complications (CVC) of myocardial infarction (MI), cerebral stroke (CS), and sudden coronary death [11]. According to Russian scientists, psycho-emotional stress activates the sympathoadrenal system and the hypothalamic-pituitary-adrenal axis, creating conditions for chronic inflammation (C-reactive protein, tumor necrosis factor, interleukin-1B, interleukin-6), leading to oxidative stress followed by dissonance between vasoconstriction. and vasodilators, which is manifested by hypertension[13].

The purpose of the study is to increase the effectiveness of prevention and treatment of AH by educating patients in hypertensive schools at the level of the primary health care (PHC). To achieve the goal, the following tasks were formulated: to study patient management tactics, the knowledge of general practitioners about the principles of prevention and treatment, as well as the commitment of patients with hypertension to compliance with non-pharmacological and medical recommendations at the PHC level; to develop a structured program for the education of patients with hypertension with information interventions in the conditions of city family clinic/rural medical center (CFC/RMC); compare the results of patient adherence to treatment, analyze the results of therapeutic and preventive interventions before and after 24 months. observations; the creation of an electronic program of dispensary monitoring of patients with AH in primary care settings.

Materials and Method

A retrospective analysis of data (over the past five years) of 222 randomly selected outpatient records of patients with hypertension, registered in the joint venture (n=54) and in some Polyclinics of the Republic of Uzbekistan (n=168) was performed. Data from a retrospective analysis of outpatient maps are compared with the results of examination of patients (interrogation, examination, anthropometry, blood pressure measurement, determination of cholesterol, etc.). Interviewed by doctors of the Tashkent polyclinic and medical centers in rural areas of the republic, who took 10-month retraining courses for general practitioners. A survey of 156 doctors before training and 119 doctors after training was conducted, as well as a survey and examination of 236 patients with hypertension, consisting of a follow-up in polyclinics and medical centers. In the future, part of them (the main group; n=122) took a course at the school of hypertensive patients, and part (comparison group; n=114) did not participate in the educational program. All patients were under our supervision for 2 years with subsequent re-examination and examination. In the main group (age of patients from 45 years to 71 years, average - 51.75±1.85 years; 29.5% of men, 70.4% of women), the distribution of patients by stages was as follows: stage I AH - 45 (36, 8%) people; Stage II - 62 (50.8%); Stage III - 15 (12.2%). The crisis course of the disease was observed in 40 (32.7%) patients. The comparison group consisted of 114 patients (80 (70.1%) women and 34 (29.8%) men, aged 44-75 years, average - 52.6±1.62 years), treated by the traditional method and not participating in the educational program. In this group, stage I GB was detected in 41 (35.9%) people; Stage II - 61 (53.5%); Stage III - 12 (10.5%) people. The crisis course of the disease was observed in 37 (32.4%) patients. The groups were comparable in terms of the main clinical and laboratory instrumental indicators.

In the main group of patients, 46 (37.7%) patients had an average risk of cardiovascular complications, 63 (51.6%) were at high risk, and 14 (11.4%, $p < 0.05$) were very high. In the comparison group - 41 (35.9%), 62 (54.3%) and 11 (9.6%, $p < 0.05$) patients, respectively.

Schools of hypertension are organized on the basis of the Training and Medical Center at the Tashkent Medical Academy, located in SP 32 of Tashkent and RMC of two regions of the republic; teaching materials are developed taking into account the specifics of the work of family doctors. When compiling the training technology, the level of patient education was taken into account. In our opinion, taking into account the mentality of our population, 10 classes can be considered optimal (1 time per week, preferably on Saturday). Training material should be submitted in accordance with a structured program in a logical sequence according to the sections specified in the training program.

When organizing schools of hypertension, it is necessary to adhere to the following method of conducting classes: a set of patients in groups of 10-12-14 people; Venue - specially prepared thematic room; necessary resources for training: banners, a multimedia projector, phantoms, scales, a height meter, a measuring tape, an apparatus for measuring blood pressure, etc.; presentation of material - interactive conversation-lecture; duration of classes 90-120 minutes with a 15-20 minute break;

Students must be persuaded to keep a diary of patient self-control with the assessment and monitoring of controlled risk factors, the results of blood pressure indicators and a schedule for taking antihypertensive drugs. In order to acquire practical skills for self-monitoring and self-help with an acute increase in blood pressure, pay special attention to classes where patients learn the principles of non-drug and drug treatment of hypertension. To teach the methodology of independent outpatient daytime measurement of blood pressure, pulse counting and other practical skills (determining BMI, staffing an emergency first-aid kit and providing self-help with an acute increase in blood pressure), in order to adequately control blood pressure indicators. This method allows you to assess changes in blood pressure and heart rate in the early morning and during the day several times before going to bed, is easy for the patient, informative and increases patient adherence to treatment, allows you to control the effectiveness of antihypertensive therapy.

Self-ambulatory daytime monitoring of blood pressure is recommended as a mandatory method for the study of hemodynamics on an outpatient basis in assessing the effectiveness of antihypertensive therapy, in the diagnosis of hypertension, especially in hypersensitive patients with high levels of anxiety and depression, as well as in patients without any symptoms.

Results and Discussion

A retrospective analysis of risk factors recorded in the outpatient records of the clinic (n=54) and medical stations (n =168) yielded the following results: burdened heredity was established in the clinic in 34 (62.9%) cases and in medical centers in 88 (52.3%), smoking - in 16 (29.6%) and 47 (27.9%) cases, overweight - in 18 (33.3%) and 35 (20.8%), alcohol abuse - in 9 (16.6%) and 37 (22.0%), hypercholesterolemia - in 21 (38.8%), stress - in 38 (70.3%) and 115 (68.4%), respectively. The most common risk factors such as burdened heredity, stress, overweight, and lack of exercise.

In most outpatient cards, recommendations for non-drug treatment are not given in full. Table salt restriction is recommended by 35 (64.8%) polyclinic doctors and 53 (31.5%) rural doctors; auto-training - 24 (44.4%) and 64 (38.0%); restriction of fat intake - 31 (57.4%) and 71 (42.2%) and 54 (32.1%); weight loss in obesity - 37 (68.5%) and 71 (42.2%); regular physical education - 37 (68.5%) and 67 (39.8%), respectively. A comparative analysis of retrospective indicators of outpatient charts with data from an oral survey of doctors and examination of the same patients revealed a significant discrepancy ($p < 0.001$) of these risk factors. These data allow us to conclude that in most patients the timely detection and correction of risk factors for hypertension were not performed.

Studying outpatient cards, we drew attention to the rules for the appointment of antihypertensive drugs with proven effectiveness. β-Adrenergic blockers in the clinic are prescribed in 44.4% of cases, in medical centers - in 8.9%; diuretics - in 35.1 and 10.7%, respectively; calcium antagonists - in 24 and 5.9%; ACE inhibitors - in 51.8 and 42.2%, respectively. Despite the appointment of first-line antihypertensive drugs, dibazole, Adelfan, Raunatin, etc. were also prescribed, in courses, without a specific regimen and duration of administration. Antihypertensive drugs are regularly prescribed by 35 (64.8%) doctors of the polyclinic and 30 (17.8%) by medical points, by short courses - 19 (35.1%) and 138

(82%) doctors, respectively, the differences in both cases are highly significant (p <0.001).

Anonymous questionnaire of doctors at the polyclinic and medical centers that underwent 10-month retraining courses revealed an underestimation of risk factors and their correction by doctors in patients with hypertension. After training, the number of doctors who attach importance to the identification and correction of risk factors significantly increased (p<0.001). Thus, 46.0% of polyclinic doctors and 30.1% of medical centers recommended a decrease in salt intake before training, 92.3 and 76.1% respectively, after training; weight loss before training - 26.9 and 15.0% of doctors, after - 96.1 and 85.0%, respectively; restriction of fat intake was recommended before training by 20.6 and 16.1% of doctors, after - 94.2 and 43.2%, respectively.

Most doctors do not fully understand the method of modern prevention of hypertension. Reducing the negative factors associated with lifestyle is the main goal of preventing hypertension, an effective way to reduce blood pressure and prevent complications. The role of the general practitioner in convincing patients to follow simple recommendations is extremely large.

236 patients were enrolled in a questionnaire survey of patients under clinical observation for AH. When analyzing questionnaires, the following groups of signs were evaluated: patient awareness of hypertension, blood pressure levels, its complications; the ability to provide self-help with a sharp increase in blood pressure; knowledge of non-drug method of treating AH; possession of practical skills (independent measurement of blood pressure, determination of heart rate, etc.); attitude to taking antihypertensive drugs.

Patients participating in the educational program significantly (p <0.001) improved their knowledge of risk factors and practical skills to improve the quality of AH control (Table 1). In the group of patients who did not undergo training, a special difference in the provision of self-help before being taken under observation and after 2 years of observation was not detected. All 122 patients who completed the training mastered the skills of independent measurement of blood pressure and pulse counting. In the comparison group, these indicators remained almost unchanged. Repeated questionnaires after training showed a significant (p <0.001) increase in the number of patients taking antihypertensive drugs, 82.7% of those trained began to "constantly" and "from case to case" with an excessive increase in blood pressure - 9.0% of trained patients.

The majority of patients in the comparison group do not possess self-help skills with a sudden increase in blood pressure and self-monitoring of blood pressure. In addition, one of the principles of drug antihypertensive therapy (taking drugs with regularity and duration) is not supported. After 2 years of observation, the rules for taking antihypertensive drugs almost did not change. The results of a survey of patients prove the need for training AH patients in a number of skills that allow them to provide self-help in a timely manner with an acute increase in blood pressure and maintain its target level while observing non-pharmacological treatment method and rules for taking antihypertensive drugs.

One of the main links of the ongoing measures for the prevention of hypertension on an outpatient basis should be the increase in the educational level of patients. If a general practitioner takes the time and develops an individual program for non-drug and drug treatment for each patient, then it will be very difficult to implement it, since untrained patients lack motivation for treatment. Only after informing about the principles of prevention of AH and its complications does the patient increase adherence to treatment, they become responsible for their health. The school of hypertensive patients at the PHC level is an ideal and suitable method for increasing the knowledge, abilities and skills of patients for quality control and monitoring of their condition.

Among the factors affecting the effectiveness of treatment of patients with hypertension, along with the clinical features of the course of the disease and the correctness of medical prescriptions (choice of drug, dose, dosage regimen, etc.), the adherence of patients to treatment is of great importance, i.e. completeness and correct implementation of medical recommendations.

Patients who underwent training experienced positive dynamics in the lipid spectrum: the number of patients with hypertriglyceridemia (p <0.001) and hyper-LDL cholesterol (p <0.001) decreased (Table 2). All patients stopped drinking alcohol (p <0.001), quit smoking; physical inactivity was observed only in 30.3% (p <0.001). The number of patients with impaired glucose tolerance decreased (p <0.001).

Table 1: Comparative results of a questionnaire survey of patients, before and after 2 years of observation

Relevant Question	Core group before training (n=122)		The main group after training (n=122)		Comparison group source data (n=114)		After 2 years of follow-up (n=112)	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Can you help with an acute increase in blood pressure?								
• Yes	21	17,2	120	98,3	23	20,1	28	25,0
• No	101	82,7	2	1,6	91	79,8	84	75,0
Do you know how to measure blood pressure on your own?								
• Yes	45	36,8	122	100,0	34	29,8	41	36,6
• No	77	63,1	-	-	80	70,1	71	63,3
Do you know how to independently determine your heart rate?								
• Yes	8	6,5	122	100	6	5,2	6	5,3
• No	114	93,4	-	-	108	94,7	106	94,6
Do you think that overweight affects blood pressure?								
• Yes	28	22,9	122	100	29	25,4	34	30,3
• No	67	54,9	-	-	44	38,5	40	35,7
• I do not know	27	22,1	-	-	41	35,9	38	33,9
Do BP indicators change with age?								
• Yes	24	19,6	122	100,0	21	18,4	29	25,8
• No	29	23,7	-	-	32	28,0	24	21,4
• I do not know	69	56,5	-	-	67	58,7	59	52,6
Do you know about acute and chronic complications of AH?								
• Yes	87	71,3	122	100,0	79	69,2	80	71,4
• No	35	28,6	-	-	35	30,7	32	28,5
How do you take medicine for pressure?								
• Constantly	14	11,4	101	82,7	12	10,5	29	25,8
• From case to case (with an increase in blood pressure)	89	72,9	11	9,0	79	69,2	67	59,8
• Short courses (10-14 days)	19	15,5	-	-	23	20,1	16	14,2

Table 2. Dynamics of controlled risk factors for cardiovascular diseases in patients trained at the school of hypertension

Risk factors	Data before training (n=122)		2 years after training (n=122)		ρ
	Abs	%	Abs	%	
Excessive intake of salt more than 5-6 g/day	10	8,1	3	2,4	<0,001
Smoking	13	10,6	5	4,0	<0,001
Alcohol abuse	11	9,0	-	-	
TG> 2.0 mmol/L	24	19,6	14	11,4	<0,001
LDL cholesterol> 3.0 mmol/L	69	56,5	33	27,0	<0,001
HDL cholesterol <1.0 mmol/L	42	34,4	24	19,6	<0,001
Impaired glucose tolerance	12	9,8	2	1,6	<0,001
Sedentary lifestyle	85	69,6	37	30,3	<0,001

Significant changes in the clinical picture of the disease were also noted: dizziness and headaches began to occur less frequently ($p < 0.001$), there was a tendency to decrease dyspnea during exercise ($p < 0.001$) (Table 3). The number of patients suffering from angina pectoris, as well as those with rhythm disturbances, has not increased.

Table 3. Change in symptoms of hypertension in patients trained at the school of hypertension

Clinical symptoms	Before training (n=122)		2 years after training (n=122)		ρ
	Abs	%	Abs	%	
Dizziness	42	34,4	5	4,0	<0,001
Headache	91	74,5	5	4,0	<0,001
Exertional dyspnea	16	13,1	6	4,9	<0,001
No angina pectoris	71	58,1	71	58,1	>0,05
FC I	30	24,5	33	27,0	>0,05
FCII	21	17,3	18	14,7	<0,05
Heart rhythm disturbances, incl. ventricular and supraventricular extrasystole, atrial fibrillation	14	11,4	14	11,4	>0,05

Trained patients significantly (p <0.001) increased the use of long-acting antihypertensive drugs; ACE inhibitors - from 19.6 to 91.8%; β-blockers - from 29.5 to 39.3%; calcium antagonists - from 6.5 to 59.0%; diuretics - from 13.1 to 66.3%, respectively. Blockers of AT-1 receptors before training were taken in 5.7% of cases; after 2 years of observation, the frequency of administration of these drugs significantly (p <0.001) increased and amounted to 13.1%.

Patients trained in the school of hypertension have a significant (p <0.001) improvement in knowledge of AH risk factors and their knowledge of hypertension, increased adherence to treatment and drug treatment to achieve the target blood pressure level (Table 4). The course of AH was accompanied by a significant decrease in the frequency of hypertensive crises and hospitalization, side effects of drug therapy. In the comparison group, against the background of the prescribed therapy, positive reliable dynamics were noted; patients began to control blood pressure more often, the selection of adequate drug therapy improved, although patient awareness of the presence of hypertension, patient knowledge of the main risk factors for AH, treatment adherence remained low, and the decrease in the number of hypertensive crises was not significant.

Table 4. The main results of the observation of patients who have passed and have not been trained at the school of hypertension

Index	Main group				p	Comparison group				p
	Before training (n=122)		After training (n=122)			Initial data (n=114)		After 2 years of follow-up (n=112)		
	Abs	%	abs	%		abs	%	Abs	%	
AH awareness	66	54,0	121	99,1	<0,001	71	62,2	75	66,9	>0,05
Patient knowledge of the main risk factors for GB	56	45,9	121	99,1	<0,001	36	31,5	38	33,9	>0,05
The incidence of hypertensive crisis	40	32,7	4	3,2	<0,001	42	36,8	37	33,0	>0,05
Hospitalized for emergency	40	32,7	4	3,2	<0,001	42	36,8	37	33,0	>0,05
Permanent independent monitoring of blood pressure	63	51,6	118	96,7	<0,001	25	21,9	32	28,5	<0,001
Drug treatment of hypertension with achieving the target level of blood pressure	14	11,4	101	82,7	<0,001	12	10,5	29	25,8	<0,001
Adherence to treatment	87	71,3	120	98,3	<0,001	24	21,0	25	22,3	>0,05
Side effects of drug treatment of AH	38	31,1	5	4,0	<0,001	40	35,0	38	33,9	>0,05

It should be noted that 118 (96.7%) patients began to independently control blood pressure according to the SADMAD technique and keep a self-monitoring diary, which contributed to increasing adherence to drug therapy, and improved the relationship between the doctor and the patient.

The most difficult thing for patients was the conscious implementation in practice of such measures to normalize a lifestyle as reducing body weight, increasing physical activity, observing the principles of a balanced diet, restricting the use of salt, quitting smoking, and alcohol abuse. After 2 years, 48 (39.3%) patients increased physical activity, 49 (40.1%) - limited the consumption of table salt. Of the dietary recommendations for 98 (80.3%) patients, limiting the intake of animal fats and an increase in the diet of unsaturated fats were easily feasible. In general, these measures were accompanied by a decrease in blood cholesterol, a decrease in the number of patients with impaired glucose tolerance.

The role of independent ambulatory daytime monitoring of blood pressure (SADMAD) in the formation of adherence to treatment was revealed. In the main group of SADMAD in the amount of 10 measurements per day 51 (41.8%) patients performed; 6 measurements per day - 35 (28.6%); 4 measurements per day - 22 (18.0%); 2 measurements per day - 10 (8.1%). SADMAD was carried out before prescribing drugs and against the background of antihypertensive treatment.

After reaching the target level of blood pressure during therapy, subsequent examinations for patients with medium and low risk who regularly measure blood pressure at home should be planned at intervals of 6 months. For patients with a high and very high risk, as well as for patients receiving only non-drug treatment, and for people with low adherence to treatment, the intervals between visits should not exceed 3 months. The regularity of medical examinations that ensure continuous monitoring can be ensured by involving patients in activities related to training and the acquisition of self-control skills.

Treatment of a patient with hypertension is carried out continuously, in most patients - for life, since its cancellation is accompanied by an increase in blood pressure. With persistent normalization of blood pressure for 1 year and compliance with measures to change the lifestyle of patients with low and medium risk, a gradual decrease in the number and/or reduction of doses of antihypertensive drugs is possible. Reducing the dose and/or reducing the number of medicines used requires an increase in the frequency of visits to the doctor and the administration of SADMAD at home in order to ensure that there are no repeated increases in blood pressure.

It should be noted that in a number of scientific papers it has been noted that the effectiveness of educational programs has recently been declining[14]. They only temporarily improve the situation [15], help control blood pressure, but do not affect other risk factors, for example, body weight [17].

Conclusion

1. At the primary care level in most patients with hypertension, risk factors are not fully identified; some of them do not correct the identified modifiable risk factors; the vast majority of patients use antihypertensive drugs in short courses, not observing the regularity and duration of admission.
2. Patients with hypertension are not sufficiently aware of the risk factors (33.9%) that affect the course and prognosis of the disease: do not have the skills of self-control (63.3%) and self-help (75%) with an acute increase in blood pressure; there is a low commitment (24%) of patients to the implementation of medical recommendations.
3. Antihypertensive therapy in patients with hypertension not participating in the educational program does not significantly affect controlled CVD risk factors and does not lead to the achievement of target blood pressure levels (29%).
4. The school of hypertensive patients is an effective system for organizing patient education and conducting complex therapy in the clinic and medical centers; significantly contributes to the prevention of CVD, adequate control of systolic and diastolic BP (96.7%).
5. Adequate pharmacotherapy is optimally possible in patients participating in the educational program. In our study, this training allowed us to achieve the target blood pressure level in 82.7% of patients, reduce the pharmacological burden on the body, and thereby reduce the number of adverse reactions to drug therapy.
6. Due to the insufficient development of personal programs for the follow-up of patients with AH, the

effectiveness of treatment is reduced, the course and prognosis of the disease are aggravated, leading to a deterioration in the quality of life and an increase in the cost of treatment.

7. The introduction of an electronic dispensary monitoring program facilitates the implementation of treatment and prophylactic measures, allows to identify the leading CVD risk factors, and conduct their timely correction and monitoring. With the help of such programs, patients with lesions of target organs and ACS are detected at the early stages of their development, which allows for adequate treatment and prevention of severe complications of hypertension.

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Peculiarities of the Pregnancy in Women with Hepatobiliary System Pathology

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Abstract

During pregnancy, there is a significant restructuring of various organs and systems, including liver functions. Due to the increase in circulating blood volume, most indicators of liver function may differ from their level in non-pregnant women. In the liver of pregnant women, the synthesis of a large class of proteins (excluding immunoglobulins), fibrinogen, prothrombin, blood coagulation factors (V, VII, X, XI, XII, XIII), fibrinolytic factors (antithrombin III, proteins C and S) increases. Hepatic enzymes (serum transaminases, γ-glutamyltranspeptidase), as well as the bilirubin content do not change during the physiological course of pregnancy[1].

Keywords: *Pregnancy, cholestasis, syndrome, acid, intrahepatic, preeclampsia.*

Introduction

Alpha-fetoprotein (AFP) is produced by the fetal liver and usually its level in the blood increases[2]. Alkaline phosphatase can be increased in the third trimester, due to its production by the placenta and the development of bone tissue of the fetus[3].

At the end of II and III trimesters, 50% of pregnant women may experience clinically insignificant varicose veins of the esophagus, resulting from compression of the inferior vena cava by the enlarging uterus and a decrease in venous return[4].

Altered hepatic tests are found in 3-8% of all pregnancies [5]. The main causes of liver pathology are very diverse. Traditionally, they are divided into related and not related to pregnancy [6]. The liver pathology that developed during pregnancy includes indomitable (excessive) vomiting of pregnant women, acute fatty hepatosis, pregnant HELLP syndrome, and intrahepatic cholestasis of pregnant women.

Non-pregnancy related liver pathology is represented by acute viral hepatitis, cirrhosis and portal hypertension, liver tumors, bile duct diseases, etc.

A mortality rate of 0 to 25% has been reported among mothers with liver diseases associated with pregnancy. According to Carmen S. G. et al., Liver diseases that occur during pregnancy were observed in 11.24% of pregnant women attending the National Institute of Perinatology in Mexico City over a three-year period. Concomitant liver diseases were found in 10.8% of patients, mainly associated with preeclampsia (9.9%). Only in 0.56%, preeclampsia was caused by a liver disease that existed earlier: acute or chronic HCV detected in this group (0.12%) [7].

Indomitable pregnant vomiting (hyperemesis gravidarum) is the most severe form of nausea and vomiting in pregnant women, leading to dehydration, ketosis and weight loss of over 5%. indomitable pregnant vomiting complicates 0.3–2% of pregnancies[8, 9].

Indomitable pregnant vomiting is not considered a true liver disease, but it is associated with an abnormal functional liver test (FPP) in approximately half of patients, while the increase in serum aminotransferases reaches 200 U/L. Other biochemical abnormalities may also be observed, such as an increase in serum amylase and lipase [10]. The standard of definition is the occurrence of more than three episodes of vomiting per day with ketonuria and weight loss of more than 3 kg or 5% of body weight, the diagnosis is usually made clinically after excluding other causes. Clinical studies of indomitable pregnant vomiting reveal dehydration, acidosis due to malnutrition, alkalosis due to loss of hydrochloride and hypokalemia [11].

Risk factors for developing indomitable pregnant vomiting include hyperthyroidism, psychiatric illness, cystic skidding, pre-existing diabetes, pregnancy in a pregnant woman, multiple pregnancy, increased body mass index[12], and high daily intake of saturated fat before pregnancy[15]. One study also identified female fetus as a risk factor[14]. According to Saad A., Jaquelyn F (2019) in the history of gastrointestinal disorders and asthma play a significant role in the development of indomitable pregnant vomiting[16].

According to other authors, the exact etiology of indomitable pregnant vomiting is unknown. Apparently, there is a direct correlation between the severity and the level of human chorionic gonadotropin (hCG), which reaches a maximum at 10 weeks of gestation. indomitable pregnant vomiting is more often observed in trophoblastic tumors and multiple pregnancy, i.e. with an abnormally high level of hCG. The latter activates the thyroid stimulating hormone receptor (TSR), which leads to the suppression of TSR and an increase in the level of thyroxine (T4). A positive correlation was found between the level of hCG, T4 and severity of indomitable pregnant vomiting[17]

Pregnant Intrahepatic Cholestasis (ChB) is the most common liver related pregnancy disorder. This is a reversible form of cholestasis, which is characterized by itching and an increase in the level of bile acids in serum on an empty stomach or after eating. Intrahepatic cholestasis of pregnant women is spontaneously resolved in the first 6 weeks. after childbirth, but often recurs during subsequent pregnancies [18]. The frequency is 0.2–2%. The highest incidence was recorded in South America and Europe. The incidence of intrahepatic cholestasis in pregnant women is more common among pregnant women infected with hepatitis C virus [19].

The incidence of intrahepatic cholestasis in pregnant women varies depending on geographic location and ethnicity: 3–5% of pregnant women in Chile, 1% in Europe, 0.7% in the UK, 0.3–1.0% in the United States and are rare in African countries. Seasonality indicates a higher incidence in the winter months in some countries. If jaundice is the initial symptom, then a further examination of the pregnant woman is necessary. It has been found that serum autotoxin, lysophospholipase D necessary for angiogenesis and neuronal development during embryogenesis, is a highly sensitive and specific diagnostic marker that distinguishes pregnant intrahepatic cholestasis from other itchy pregnancy disorders and liver diseases associated with pregnancy. Ultrasonography of the liver is necessary to exclude gallstone disease [20].

Kremer et al. A 2010 animal study found that autotoxin, a serum enzyme that converts lysophosphatidylcholine to LPA (lysophosphatidic acid), which causes itching, increased markedly in patients with spoils compared to pregnant women and patients with cholestasis compared to pregnant women without itching. Kremer et al. In another 2015 study in which 145 women participated, it was also reported that increased serum autotoxin activity is a highly sensitive, specific and reliable diagnostic marker for pregnant intrahepatic cholestasis, distinguishing pregnant intrahepatic cholestasis from other itchy pregnancy disorders and liver diseases associated with pregnancy. According to the authors, pregnancy and oral contraception increase serum autotoxin to a much lesser extent than intrahepatic cholestasis in pregnant women. [21].

Intrahepatic cholestasis of pregnancy affects 0.1–2% of pregnant women; in 1–4 women, gestation can be complicated by premature birth, fetal asphyxia, meconium staining of amniotic fluid, and stillbirth. The results of a large Swedish cohort study showed that pregnancy, in which the concentration of bile acid in maternal serum is 40 μmol/L or more, can be complicated by spontaneous preterm delivery[23], the presence of meconium in the amniotic fluid and fetal asphyxia. Another UK cohort study confirms these findings and showed that pregnancy outcomes[22] in women with intrahepatic cholestasis in pregnant women and a serum bile acid concentration of 40 μmol/L or more often lead to fetal death compared to 2205 women with uncomplicated pregnancy [25].

A differential diagnosis of intrahepatic cholestasis in pregnant women is carried out with viral hepatitis, autoimmune hepatitis, primary biliary cholangitis and gallstone disease [26]. pregnant intrahepatic cholestasis first appears at 28-30 weeks of pregnancy in the form of itching, especially pronounced at night. Almost half of patients develop jaundice[27].

Itching of the palms and soles is a key symptom. Skin rash is absent. Jaundice is rarely observed in patients with intrahepatic cholestasis in pregnant women. Serious adverse effects on the fetus are fetal distress, premature birth, prematurity, and fetal death. An increase in serum bile acids to 100 μmol/L or higher is associated with an increased risk of fetal death. Most societies recommend delivering a pregnant woman at 37 weeks because there is an increased risk of fetal death in the last 4 weeks of pregnancy. The risk of relapse in subsequent pregnancy is high and can reach 90% [28].

Intrahepatic cholestasis of pregnant women is more often observed with multiple pregnancy and in women who have previously been treated for infertility. In the etiology of intrahepatic cholestasis of pregnant women, genetic, endocrine and environmental factors[29] play a role. Apparently, an increase in estrogen and progesterone levels due to pregnancy can contribute to the development of this pathology [30].

Additional risk factors include maternal old age, multiple pregnancies, a personal or family history of the disease, preexisting liver disease, and a history of cholestasis when taking oral contraceptives.

Exogenous progesterone also plays a role in pregnant intrahepatic cholestasis. The study showed that 64% of patients who developed intrahepatic cholestasis in pregnant women took orally micronized progesterone to reduce the risk of premature birth. 70% of patients after progesterone withdrawal and 10% of patients after progesterone dose reduction experienced relief of pruritus before childbirth[31].

Many studies have shown that high estrogen levels are associated with the development of intrahepatic cholestasis in pregnant women, such as multiple pregnancies, the effect of ovarian hyperstimulation, and second trimester gestation[32]. pregnant intrahepatic cholestasis shows similar characteristics as in women taking high estrogen birth control pills. High levels of circulating estrogen can cause cholestasis in genetically predisposed women with intrahepatic cholestasis in pregnant women. Several animal studies have shown that sulfate metabolites of progesterone are partial agonists of the farnesoid X FXR receptor (bile acid receptor). Progesterone sulfate metabolites alter the hepatobiliary transport system, disrupting the functioning of the main bile acid receptor in the liver[33].

Multidrug Resistance Protein 3 (MDRP3) is a key carrier of phospholipids across the tubular membrane. A mutation of this gene leads to a loss of function and, consequently, an increase in serum bile acids. The MDRP -3 mutation is located on chromosome 7q 21.1 and was identified in 15% of cases of intrahepatic cholestasis in pregnant women. Altogether, ten different mutations were identified. Heterozygous mutations cause transport dysfunction, while a complete lack of transport function is associated with severe liver disease. The genetic tendency to intrahepatic cholestasis of pregnant women has been proved and there is information about pedigrees in which inheritance is of a limited type by gender of the dominant system.

Some environmental factors also play a role in the etiology of intrahepatic cholestasis in pregnant women, including the level of selenium in the region [34].

Immunological dysfunction also plays an important role in the pathogenesis of intrahepatic cholestasis in pregnant women. Thus, an increase in serum bile acid level causes a change in the immune system from a TH2-mediated response to TH1. As pregnancy proceeds to TH1, the immune response has more adverse outcomes, many risks of pregnant intrahepatic cholestasis are associated with the immune system.

Ovadia et al. conducted a comprehensive meta-analysis including 23 studies that compared perinatal outcomes in women with intrahepatic cholestasis of pregnancy (n = 5557) and healthy control group (n = 165136). The results of meta-analysis demonstrate the relationship of intrahepatic cholestasis of pregnancy with an increase in fetal complications, as described below[35]. Sudden intrauterine death of a fetus, meconium-stained amniotic fluid, spontaneous preterm birth, iatrogenic preterm birth, fetal distress. Maternal bile acids are transported through the placenta to the fetus and accumulate in the amniotic fluid, causing complications. The cause of preterm birth is unknown, but is possibly related to the accumulation of bile acid in the uterine myometrium, which causes increased uterine activity. Sudden death of the fetus is the most

serious complication of IVB; the cause of fetal death is not entirely understood; possibly associated with the toxic effect of bile acids on the fetal heart, causing arrhythmias and chorionic spasm, causing a decrease in the supply of maternal oxygenated blood to the fetus, causing asphyxiation [36].

Acute fatty hepatosis of pregnant women. Acute fatty hepatosis of pregnant women (AHFS), first described by Shihan, is a rare but serious disease, with a frequency of 1 to 7,000 per 16,000 pregnant women [37].

The accumulation of fatty acids in hepatocytes caused by various pathophysiological mechanisms is known as fatty liver disease. For example, metabolic syndrome or obesity, in which lipolysis is suppressed as a result of hyperinsulinemia, therefore, can provoke non-alcoholic fatty liver disease (NAFLD) or more severe non-alcoholic steatohepatitis (NASH). With a prolonged course of the disease, this condition can lead to cirrhosis of the liver. However, when this occurs during the gestational period, it is called acute pregnant fatty hepatosis (AHFS). AHFS is a devastating condition that can rapidly progress to fulminant liver failure with associated coagulopathy or hepatic encephalopathy[38].

The prevalence of acute fatty liver disease is 1 in 10,000-15,000 pregnancies. This life-threatening condition often develops in the second half of pregnancy (range: 27–40 weeks of pregnancy), usually close to delivery. The differential diagnosis is carried out with fulminant viral hepatitis, drug-induced hepatitis, idiopathic pregnancy cholestasis, with Reye syndrome in adults, as well as with hemolysis syndrome and an increase in liver enzymes and a decrease in platelets (HELLP syndrome)[39]. In the UK, it occurs at a rate of 1 in 20,000 pregnancies [40].

The etiology of AHL is completely unknown, and the connection with geographical or ethnic characteristics is also unknown[41].

Risk factors for the development of AHFS include congenital LCHAD deficiency, 1st pregnancy, multiple pregnancy (in patients with AHFS up to 25%), preeclampsia (in patients with AHFS up to 50%) and male fetus (3 times more often), underweight newborns, history of AHLH [42].

Chen et al. Indicated that twin pregnancy can be a protective factor for maternal outcome. Conversely, Knight et al. Came to the conclusion that double pregnancy is at a higher risk of AHL, similar to Davidson et al., Who have demonstrated this condition may increase the risk of AHL [43].

Maternal mortality reached 70%, but more recent estimates range from 7% to 18%, which is secondary to advances in the supportive care of these patients. Maternal complications include postpartum hemorrhage, renal failure, hypoglycemia, disseminated intravascular coagulation (DIC), pancreatitis, and pulmonary edema.

Perinatal mortality reaches 85%, and in more recent data, rates range from 9% to 23%. Due to urgent need, approximately 75% of the births are premature, and the average gestational age at birth is 34 weeks. All infants of mothers with AHFS are tested for defects in the FAO (fatty acid oxidation), because rapid detection and treatment can reduce mortality and morbidity. Relapse of AHB during subsequent pregnancies is rare, but often occurs in carriers of LCHAD mutations[40].

Fat accumulates in the liver due to the excess intake of free fatty acids (FFAs) in the liver, a decrease in the rate of β-oxidation of FFAs in the mitochondria of hepatocytes, excessive formation and absorption of FFAs in the intestines, a decrease in the synthesis of lipoproteins of different densities in the liver itself and a functional liver test caused by liver disease . The normal fat content in the liver does not exceed 5%, and with AGB, it rises to 13-19%. Of great importance for the development of steatohepatosis is insulin resistance [44].

Prevention by prenatal diagnosis after mutations in the family are possible by selecting chorionic villi at 11 weeks of gestation.

Maternal and antenatal fetal death in pregnant women with AHFS are 10% and 45%, respectively [45].

Biochemical signs of LCHAD deficiency are the accumulation of long chain 3-hydroxy fatty acids, such as 3-hydroxylauric acid, 3-hydroxymyristic acid, 3-hydroxypalmitic acid and 3-hydroxy dicarboxylic acid in the systemic circulation and increased excretion of 3-hydroxy dicarboxylic acids in the urine.

It is reported that children with LCHAD deficiency develop sudden death with hypoglycemia, cardio-respiratory failure, acute heart failure, severe neonatal cardiomyopathy, hepatic dysfunction and acute liver failure, skeleton myopathy with rhabdomyolysis. Next, 34% of children with disabilities die between four days

and 10 years after birth. In addition, mental disorders, retardation, developmental disorders, eye abnormalities, and sudden infant death may develop. It is believed that such damage to many organs is associated with the lipotoxicity of the intermediate accumulation of toxic 3-hydroxy fatty acid.

In the medical history of pregnant women with severe AHFS, placental abruption, premature birth, and fetal death were noted.

For the diagnosis of AHFS, the Swansea Criteria have been proposed and validated in large cohort studies in the United Kingdom. These criteria include symptoms and laboratory findings. Six or more of the following criteria are needed to establish a diagnosis of AHFS according to Swansea criteria[46].

1. Vomiting;
2. Encephalopathy;
3. Polydipsia and polyuria;
4. Abdominal pain;
5. An increase in the content of bilirubin (more than 0.8 mg/dl or 14 μmol/l);
6. Hypoglycemia (less than 72 mg/dl or 4 mmol/l);
7. Leukocytosis (more than 11 · 109/l; often 20-30 · 10 * 9/l);
8. Increased levels of transaminases (often 3-10 times higher than normal - more than 42 IU/l);
9. Renal dysfunction (creatinine content of more than 150 μmol/l);
10. An increase in the level of ammonia (more than 47 μmol/l);
11. An increase in the level of uric acid salts (more than 340 μmol/l);
12. Coagulopathy (prothrombin time more than 14 s; APTT more than 34 s);
13. Ascites or hyperechoic structure of the liver during ultrasound examination (ultrasound);
14. Microvesicular steatosis with liver biopsy and histological examination[31]. In the future, jaundice (more than 70% of cases), ascites (40%), fever (45%), headache (10%) and such severe complications as gastrointestinal bleeding (20-60%), PP (50%) develop), DIC (55%), hepatic encephalopathy (60-80%) and acute pancreatitis[5].

Symptoms of liver damage include epigastric pain or pain in the upper right quadrant, probably from hepatomegaly as a result of a stretching of the Glisson capsule. An increase in hepatic enzymes to double normal values classifies severe preeclampsia. Typically, coagulation rates remain normal until the progression of liver failure or placental abruption. Liver damage is a consequence of vasoconstriction, a decrease in hepatic blood flow, leading to hepatic ischemia, periportal hemorrhage, endothelial damage and fibrin deposition in the liver[47].

Recommendations of the American College of Gastroenterology (ACG)[33]:

- Women with AHFS must be delivered promptly; observation tactics are not suitable
- all women with AHFS and their children must undergo molecular testing for LCHAD,
- Children of women affected by AHL should be carefully monitored for the presence of LCHAD, including hypokinetic hypoglycemia and liver enlargement[8].

In a study by Meng et al. involving 43 patients[26] at the center of tertiary levels, prothrombin time, plasma fibrinogen, and platelet counts correlated with recovery time, while white blood cell count, blood glucose, and liver aminotransferase showed no significance in predicting. Transfusion of fresh frozen plasma (FFP), cryoprecipitate, or platelet concentrates in the case of abnormal standard tests such as prothrombin time (PT), activated partial thromboplastin time (APTT), or fibrinogen can prevent further bleeding complications. However, relative fluid overload can lead to acute lung damage and infection resulting from the use of these drugs. In addition, current standard coagulation tests are considered only weak predictors of bleeding in critically ill patients; Therefore, additional method such as thromboelastometry can be used. Thromboelastometry was used in the early prediction of bleeding complications in seriously ill patients. In a report by Crochemore et al., It was found that this method effectively detects a hypocoagulable condition and reduced quality of fibrinogen function at the beginning of an operation. Fibrinogen and prothrombin complex concentrates were administered to the patient and a cesarean section was performed without any major bleeding. Recombinant

factor 7 injection is also recommended along with supportive care in the intensive care unit [26].

Another important point concerns the deficiency of vitamin K, which is observed in newborns born to women with AGB. Cases of severe intracranial hemorrhage due to an early deficiency of vitamin K in a newborn whose mother has been diagnosed with GBV have been reported .

A recent multicenter retrospective study in China shows that male fetal sex, postpartum diagnosis of AHF, intrauterine fetal death, DIC, altered prothrombin time (PT), and activated partial thromboplastin time (APTT) can be potential factors that affect maternal complications [34].

Currently, artificial liver support therapy (ALST), which includes plasma exchange (PE) and molecular adsorbent of the recirculation system (MARS), is relevant. It is believed that the effect of ALST occurs due to the removal of circulating endotoxins, replacement of normal coagulation factors and proteins, stopping coagulopathy and, finally, improving liver function [25].

N-acetylcysteine (NAC), a precursor of glutathione, has some success in patients with Non Acetaminophen-induced acute liver failure, due to its anti-inflammatory, antioxidant, inotropic and vasodilator effects, which contribute to the improvement of vital microcirculation and oxygenation survival, as reported by Mumtaz K. Et al.[34].

A prospective study of 47 patients with this disease showed that new treatments such as plasmapheresis and the use of activated protein C were practiced in specialized centers, with variable results. Plasmapheresis, apparently, can be a promising treatment option for patients with AHFS, since it partially replaces liver function by removing ammonia, endotoxins, bilirubin, and inflammatory cytokines from maternal circulation .

Preeclampsia Preeclampsia is a multisystem disorder that affects 5 to 10% of all pregnant women, in some cases it develops multiple organ dysfunction syndrome, causing impaired renal and central nervous system function, hematological and liver dysfunctions. The disease can progress, causing microangiopathy, which mainly affects the kidneys, liver and brain. Thrombocytopenia, liver dysfunction, microangiopathic hemolytic anemia, acute renal failure, placental abruption, visual impairment, stroke, convulsions and maternal mortality are serious consequences of preeclampsia[7].

Preeclampsia is a complication of pregnancy, characterized by a deep violation of the functions of vital organs and systems, including the liver, which, depleting its reserve capacity as pregnancy progresses, becomes more vulnerable. The liver is a target organ in severe forms of preeclampsia. Preeclampsia is accompanied by severe dyslipidemia: the content of triglycerides of unesterified fatty acids (NEFA), total cholesterol, very low density lipoproteins (VLDL), low density lipoproteins (LDL) is much higher, and the level of high density lipoproteins (HDL) is lower than in uncomplicated pregnancy. Preeclampsia is characterized by a more pronounced than with a normally developing pregnancy, the appearance of unique, different from VLDL, lipoproteins loaded with triglycerides. Perhaps such an overload is associated with an increase in insulin resistance, a decrease in the intensity of β-oxidation in the liver, or a decrease in the activity of triglyceride catabolism due to inhibition of LP-lipase by cytokines.

Dyslipidemia, against the background of a decrease in the content of antioxidants in the blood, predisposes to the development of endothelial dysfunction. NEZHK, having high toxicity, contribute to the formation of free oxygen radicals. A possible mechanism explaining a significant increase in the level of NEFA during preeclampsia is an increase in the content of lipophospholipase in maternal blood flow. Its source in violation of trophoblast invasion is microvillosis membrane. [4].

Angiogenic markers have been identified and can confirm the diagnosis of PE in women without hypertension or proteinuria. These include a decrease in placental growth factor, an increase in serum soluble endoglin, and an increase in the soluble VEGF receptor[35].

However, the identified biomarkers and risk factors for the development of PE are only modestly predictive.

In PE, a generalized spasm of the vessels is observed, which leads to an increase in systemic vascular resistance and a pressor reaction to endogenous vasoconstrictors. Damage to vascular endothelium leads to the deposition of platelets and fibrin in the sinusoids, causing hepatocellular necrosis and hemorrhage in zone 1. Detection of necrosis and hemorrhage in zone 3 is associated with shock in severe preeclampsia. In mild cases of preeclampsia, a moderate increase in serum

AST/ALT/alkaline phosphatase is observed, as well as minor signs of DIC with thrombocytopenia. Jaundice is rare, but in its presence it manifests itself as a terminal and hemolytic reaction in its etiology, and the total serum bilirubin often does not exceed 6 mg/dl[33,37,38].

Conjugated bilirubin, albumin, and PT tend to remain normal. Liver damage, consisting of spasm of capillaries of liver tissue and fibrin deposition within the portal and periportal regions of the liver lobules, can lead to lobular ischemia and necrosis of hepatocytes.[6].

In earlier studies, it was shown that LCHAD mRNA, protein expression, and enzyme activity were reduced in the placenta of pre-eclampic patients. In addition, a decrease was found in mitochondrial oxidation of long chain fatty acids in the placenta obtained in patients with preeclampsia compared with the control group, which indicates that a decrease in the oxidation or defect of placental fatty acid in LCHAD may contribute to the pathogenesis of preeclampsia. Maternal AFLP is highly associated with fetal transfer and LCHAD enzyme deficiency [30].

Since one of the leading values in the pathogenesis of preeclampsia is given to microcirculatory disorders, which, in turn, lead to multiple organ failure, the liver, as an organ with a developed capillary system, is always more or less involved in the process of tissue hypoxia and microcirculatory disorders, the degree of which Depends on the severity of preeclampsia. Currently, impedance hepatography is one of the most sensitive functional method for examining the liver, revealing a violation of microcirculation, which occurs even before the increase of biochemical parameters. This method gives information about blood supply and microcirculation in the liver, about sinusoidal blood flow, the indicators of which correlate with data on morphological changes in liver tissue and its functional disorders. Of course, laparoscopy and puncture biopsy of the liver are invasive and unsafe diagnostic method for pregnant women with preeclampsia. In addition to impedance hepatography, there are also other non-invasive, safe method - such as ultrasound, dynamic hepatobiloscintigraphy and the study of the reticuloendothelial system (RES). The ultrasonic method allows you to evaluate the size of the liver, gall bladder, the presence of obvious pathological conditions - subcapsular hematoma of the liver, and also using echodsitometry it is possible to determine different sections of the density in the liver tissue, which is a criterion for fatty hepatosis. Dynamic hepatobyl scintigraphy is a functional morphological radionuclide study of the liver using a 99mTc-HIDA radionuclide. A feature of the drug is a low radiation load on the patient (0.2 m), which does not limit its use even in pediatric practice. This method allows a comprehensive assessment of both the functional and morphological state of the hepatobiliary system. The most diagnostically difficult cases are severe liver pathology during pregnancy, which are manifested by hepatocellular insufficiency syndrome and in which there is no correlation with the severity of preeclampsia. So, in 40-60% of cases of severe forms of liver pathology associated with pregnancy, there was no classical triad of symptoms characteristic of preeclampsia. So, with HELLP syndrome, an increase in blood pressure up to 160/100 mm. Hg. Art. met from 26-56% of cases, edema was recorded in 55% of patients, pronounced proteinuria - 85% of cases, in some cases, manifestation of preeclampsia occurs in childbirth, followed by rapid progression of clinical symptoms, the development of DIC and severe liver pathology. The manifestation of the complete HELLP syndrome against the background of severe preeclampsia and eclampsia was established in 53.5% of cases [39].

Finally, PE has been recognized as a marker for future cardiovascular disease, given the common risk factors, persistent endothelial dysfunction, and damage to the target organ. In the examined cohort, women with liver dysfunction due to PE and HELLP had a previous history of hypertension and diabetes mellitus, and were more prone to the subsequent development of metabolic disturbances [18].

Obstetric complications in PE include fetal growth restriction syndrome (10–25%), placental abruption, premature birth (15–65%), and fetal death. The condition of the maternal liver associated with preeclampsia/ eclampsia does not require special treatment, and the medical approach is based on the regulation of blood pressure, the reduction of concomitant symptoms and the timely treatment of seizures. After 37 weeks, women with preeclampsia should give birth. If in severe cases a deterioration of the fetus or mother is observed, childbirth should be taken in a period of 24–34 weeks [6].

HELLP syndrome: The term HELLP syndrome was first proposed in 1982 by L. Weinstein. This acronym includes: Hemolysis - the appearance of free hemoglobin in serum and urine; Elevated Liver enzimes - increased levels of AST, ALT; Low Platelets - Thrombocytopenia.

HELLP syndrome occurs in less than 1% of all pregnancies, but in 20% of pregnancies complicated by severe symptoms of PE. HELLP syndrome can occur during urgent delivery (18%), premature birth (53%) or after childbirth (30%). Risk factors include a family history of HELLP syndrome and preeclampsia or HELLP in previous pregnancies, occurring in 10–20% of patients.

Laboratory data are important for determining intravascular hemolysis, peripheral smear schistocytes, elevated FPP (usually ALT), and low platelet counts. All three HELLP elements must be observed in a pregnant patient before they are diagnosed. The level of serum aminotransferases can be increased by more than ten times. At the same time, unconjugated hyperbilirubinemia due to hemolysis may be present.

The pathogenesis of the HELLP syndrome is not entirely clear, but there is probably an abnormal development of the vascular system of the placenta and new defects in the endothelial cells of the mother's blood vessels, which leads to poor perfusion in various organs. Less commonly, LCHAD deficiency in the fetus is involved in its pathogenesis .

Insufficient immune tolerance leading to fetal trophoblast damage in early pregnancy is probably the initial sign of HELLP. Endothelial trauma and fibrin deposition may cause the HELLP microangiopathic process. As a result, hemorrhage and liver necrosis may develop. Maternal risk factors include a high body mass index, treatment for infertility, a history of preeclampsia or HELLP. HELLP syndrome may be the initial manifestation of antiphospholipid syndrome [9]. The main pathophysiological changes in HELLP syndrome occur mainly in the liver. The main link in the development of the syndrome are disorders in the hemostatic system due to damage to the endothelium and intravascular activation of the coagulation system. Deposition of fibrin in the sinusoidal capillaries of the liver leads to central necrosis with the formation of stasis and tension of the glisson capsule. Further progression of the process can lead to rupture of the liver. Subcapsular hematomas and liver ruptures more often occur with prenatal manifestations of the HELLP syndrome. Spontaneous liver ruptures are characterized by high maternal mortality (over 50%). If this vicious circle is not interrupted in time, then within a few hours, DIC develops with fatal bleeding.

Immunohistochemical analysis of the liver with HELLP syndrome showed the presence of large amounts of leukocyte elastase and TNF-α in the area of hepatocyte necrosis, which indicates the role of these factors in liver damage[4].

In approximately 65% of cases, pain is observed in the right upper quadrant of the abdomen or epigastrium, in 35% - nausea and vomiting, in 30% - headache, less often - bleeding and jaundice. In a significant number of cases of HELLP syndrome, pregnant women have no complaints. 85% of patients have an increase in blood pressure. Proteinuria is often found [3]. Excessive weight gain and edema precede the development of HELLP syndrome in 50% of cases.

The pathogenesis of HELLP syndrome has much in common with the pathogenesis of preeclampsia, DIC, and AHFS, which is reflected both in clinical manifestations and laboratory signs, as well as in the morphological picture of liver damage. Such mechanisms include impaired tone and vascular permeability (vasospasm); neutrophil activation, cytokine imbalance (increased levels of interleukin IL-10, IL-6 receptor and transforming growth factor β3 (TGF β3), and the concentration of CCL18, CXCL5 and IL-16 is significantly reduced); fibrin deposition and microthrombosis in microcirculation vessels; increased activity of plasminogen activation inhibitors (PAI-1); violation of the metabolism of fatty acids (long-chain deficiency of 3-hydroxyacylCoAdehydrogenase long chain 3-hydroxyacyl dehydrogenase) (LCHAD), characteristic for fatty hepatosis. Antiphospholipid syndrome and other variants of thrombophilia, various genetic abnormalities that play a role in the development of preeclampsia are of great importance in the development of HELLP syndrome. These anomalies are associated with the presence of polymorphism of genes encoding components of the lipid metabolism system (LPL, ApoE, LCHAD), antioxidant protection (EPHX, LPL, CYP1A1, SOD), immunological protection (HLA-G, TNFα (TNFα), IL-1, IL-10, CD14 receptor, CTLA-4), hemostatic systems (FVL, MTHFR, prothrombin, CBS, PAI-1, GP-IIIA, FXIII, FXVII, fibrinogen), placental factors (STOX1, SERPINA3, ACVR2, IGF- I, IGF-II), and factors involved in the regulation of vascular tone (AGT, ACE, AT1R, REN, PRCP, eNOS, ET-1, ER, Flt-1, ENG, VEGF, PIGF) .

HELLP syndrome usually develops in the third trimester or after childbirth and is associated with increased complications: as maternal (placental abruption, disseminated intravascular coagulation, hematoma and rupture, and acute kidney damage) and newborns (prematurity and low birth weight).

The clinical picture of HELLP syndrome varies greatly. The clinic may be asymptomatic or may appear with symptoms such as pain in the upper right quadrant of the abdominal cavity, enlarged liver, nausea, vomiting, jaundice, or confusion.[41].

Although the etiopathogenesis of this condition remains unclear, histopathological findings in the liver show intravascular deposition of fibrin, which is likely to result in hepatic sinusoidal obstruction, increased intrahepatic pressure followed by liver necrosis, intraparenchymal and subcapsular hemorrhage, and, ultimately, capsule rupture.

In a large retrospective cohort study involving 442 pregnancies complicated by HELLP syndrome, maternal mortality was 1.1%. The clinic is similar to VCB (intrahepatic cholestasis of pregnant women), prematurity remains the most common risk associated with complications from the fetus. According to Gul et al., 72 cases of perinatal mortality accounted for 34% before the 32-week gestation period and 8% after 32 weeks [29].

Cholelithiasis: Gallstone disease (cholelithiasis) is the second most common indication for surgery during pregnancy. Acute cholecystitis can occur in 20% of women under 40 years of age [43].

Risk factors for gallstone disease include an increased body mass index (BMI) (> 25 kg/m2), it is confirmed that 2.7% of women with normal BMI developed gallbladder disease before pregnancy, compared with 11% in women with obesity.

Gallstones are more common during pregnancy because the secretion of cholesterol increases compared with bile acid and phospholipids, thereby causing supersaturated bile. In addition, the volume of the gallbladder increases and emptying decreases, thus leaving an oversaturated bile in the gallbladder with the possible formation of gallstone [8].

Another mechanism suggests that while estrogens increase cholesterol secretion, progesterone decreases the secretion of soluble bile acids, and this contributes to the accumulation of insoluble bile acids predisposing to stone formation [44].

Biliary sludge and gallstones are diagnosed using ultrasound. Biliary sludge, also known as microlithiasis, is described as low-level echoes that shift when position changes and do not have an acoustic shadow, while gallstones have high-level echoes larger than 2 mm in size after postacousticshading[8, 45].

Symptomatic cholelithiasis may have typical signs of biliary colic in the upper right quadrant or epigastrium, abdominal discomfort, bloating, nausea.

Asymptomatic gallstones are found in 3.5% of pregnant women, and in 90% of cases they caused cholecystitis during pregnancy. Despite the predisposition to the formation of bile sludge and stones during pregnancy, cholecystitis occurs in only 1% of pregnant women [46]. Adverse obstetric outcomes include spontaneous abortion and premature birth.

Serious complications are cholangitis, sepsis, jaundice, cholelithiasis, pancreatitis, perforation and the formation of an abscess. In the absence of serious complications, conservative treatment is the initial option with normalization of bowel function, analgesics (opioids or non-steroidal anti-inflammatory drugs) and broad-spectrum antibiotics.

Nonsteroidal anti-inflammatory drugs should be used with caution after 32 weeks because of the risk of developing oligohydramnios and narrowing of the ductus arteriosus. Preferred antibiotics include cephalosporins and clindamycin. However, some researchers believe that a conservative approach is associated with higher relapse rates from 40% to 70%.

A study performed by Jelin shows a higher risk of fetal death (7%) was observed among patients who underwent conservative treatment, compared with patients who underwent laparoscopic cholecystectomy (2.2%).

In case of complications due to common bile duct stones, such as cholangitis or pancreatitis, endoscopic retrograde cholangiopancreatography with sphincterotomy and stone removal is a safe method with minimal risk of exposure to fetal ionizing radiation. Optional cholecystectomy can be performed after childbirth.

A review comparing conservative and surgical treatment for gallstone disease showed multiple remissions in the group that received non-surgical treatment, with 27% of them eventually having surgery, 19% in the first, 60% in the second and 10% in the third trimester. The frequency of preterm birth (3.5% and 6%) and fetal death were 2.2% and 1.2%, respectively. Cholecystectomy is recommended in the second trimester, due to the risk of termination of pregnancy in the first trimester and the risk of premature birth in the third trimester [48].

Cholecystitis: Diseases of the biliary system occupy one of the leading places among diseases of the digestive system and are found mainly in young people[3]. Chronic cholecystitis is one of the most common human diseases, takes third place after cardiovascular disease and diabetes. According to the VI World Congress of Gastroenterologists, 10% of the world's population suffers from chronic cholecystitis. In developed countries, the frequency of the disease is 10-15% and doubles for every decade [49]. During pregnancy, conditions arise that impair the function of the liver and biliary tract, which is associated with an increase in the load on the liver due to the neutralization of the vital products of the fetus, the mobilization of energy resources, and the need to strengthen metabolic processes. Pregnancy becomes a provoking factor contributing to the development of the disease[3].

The main role in the development of chronic cholecystitis is played by two factors: infection and stagnation of bile due to dyskinetic disorders. The latter, as a rule, determines the clinical manifestations of the disease. Hypomotor dyskinesia is characterized by a flaccid contractile ability of the gallbladder and ducts, as a result of which an insufficient, very small amount of bile enters the duodenum. Pregnant women are characterized by hypomotor disorders, since the main hormone prevailing in the second half of pregnancy is progesterone, which relaxes all smooth muscle organs (the main physiological meaning of its action in the pregnant woman is relaxation of the uterus, obstruction of premature birth, miscarriage). The basis of hypomotor dyskinesia is insufficient, weak emptying of the gallbladder. It is motor disorders that determine the clinical manifestations of chronic cholecystitis in pregnant women, and not inflammation.

Chronic cholecystitis (XX) worsens during pregnancy in 30-35% of women, and in most cases in the third trimester of pregnancy. In 88% of cases, the disease manifests itself in pain. Depending on the gallbladder dysfunction, pregnant women complain of dull, aching, bursting or sharp pains in the right hypochondrium, radiating to the right shoulder blade, shoulder or collarbone. Along with the pain of patients, feelings of nausea, bitterness in the mouth, vomiting, heartburn, bloating, and unstable stools are disturbing. In the second half of pregnancy, 25% of women associate the appearance of pain with fetal movement[3].

Clinical and biochemical blood tests should be evaluated in pregnant women with caution, since neutrophilic leukocytosis can only be a leukemoid reaction to pregnancy[50]. Peripheral blood tests usually do not show abnormalities. In some cases, moderate leukocytosis with a shift in the leukocyte formula to the left and an increase in ESR are noted. An increase in the activity of transaminases (ALT and AST), alkaline phosphatase and g-glutamyl transpeptidase (GGT), hyperbilirubinemia, and hypercholesterolemia is sometimes found in a biochemical blood test [61].

Buddy Chiari Syndrome. Baddi-Chiari syndrome (BCS) is defined as obstruction of the outflow of hepatic veins and is sometimes associated with myeloproliferative disorders. Up to 20% of cases of BCS occur in women who took oral contraceptives, were pregnant or gave birth in the previous two months. Because pregnancy alone is a prothrombotic condition; a physiological decrease in protein S concentration may explain the increased incidence of BCS during pregnancy. These patients have a risk of exacerbation during pregnancy due to an increased concentration of female sex hormones. Clinical signs include pain in the upper right quadrant, jaundice, and ascites. Dopplerography is very important for diagnosis. Treatment includes anticoagulation at the beginning, identifying the causes of the procoagulant, and bypass or liver transplantation in extreme cases[8].

Risk factors for developing BCS include injuries, hypercoagulant conditions, myeloproliferative disorders, oral contraceptives, and pregnancy. With BCS, abdominal pain, hepatomegaly and ascites are usually observed. Doppler sonography in most cases is enough to establish a diagnosis[2].

Thus, a review of recent literature in recent years on the pathology of the hepatobiliary system in pregnant women has shown that this problem is relevant and requires further study. The development of medical

science and practice today allows you to correctly establish the type of liver pathology by applying modern research method, although some of them are invasive. However, very often the pathology of the hepatobiliary system can first manifest itself during pregnancy, which limits both diagnostic and therapeutic options. Given the importance of this problem, the influence of the pathology of the hepatobiliary system on pregnancy and perinatal outcomes, and further studies to better understand the mechanism of development of these complications are relevant.

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Achilles Tendinopathy and Associated Factors among Recreational Ballet Dancers: A Cross Sectional Study

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Abstract

Background: Achilles tendinopathy (AT) describes an overuse condition which common experienced by dancers. The reason is because dance is a physical activity involving short explosive runs, turns, jumps, leaps and weight bear on metatarsal heads which forcefully contract the calf muscles and this causes more pressure on Achilles tendon. However, lack of studies on AT in dancers has been reported.

Objectives: To find out the prevalence and risk factors of Achilles tendinopathy among recreational ballet dancers.

Method: Quantitative approach, non- experimental and descriptive research design was conduct on a sample of 53 female and male recreational ballet dancers. Ages varied from 15- 30 and at least with 2 years of ballet dancing experiences were selected by purposive sampling from the ballet dance company or studios. Screening tools and prevalence sheet priority given to the subjects to fill in to select appropriate population who passed the study's inclusion and exclusion criteria. Then researcher required to palpate the Achilles tendon area to confirm the problem before distribute the questionnaire form to ballet dancers. All the data were using percentage and mode to analyze. Results: Our findings show 28.3% of the recreational ballet dancers had AT and footwear is the highest average risk factors among training duration, limited warm up and cool down, and muscle strength. Besides that, our findings also show BMI, unfamiliar choreography, training surface, medication, flexibility and lower limb abnormalities are not the risk factors that cause them to have AT.

Conclusion: AT is common among recreational ballet dancers and footwear is the highest average of risk factors among the four risk factors. These findings provide clearly

Information for dancers, teachers, sport therapists, health professionals and parents to prevent or treat the injuries more effectively and help to develop strategies aimed at reducing the prevalence of ballet injuries.

Keywords: *Achilles Tendinopathy, Ballet dancers, Calf muscle, Heel pain, Physical therapy, Questionnaire, Training surface.*

Introduction

Achilles tendinopathy (AT) describes an overuse condition[1] that causes pain, swelling and stiffness of the Achilles tendon[2] Achilles tendon is the strongest tendon in the human body connecting the heel with the calf muscles[3] . "It is used in locomotion by controlling the lowering of the forefoot to heel strikes and heel off the ground during walking and jumping"[4]. "AT results when the demand placed on the Achilles tendon is greater than its ability to function"[5] and it is common in runners, track and field and racquet athletes as well as soccer and volleyball players[6]. Besides that, Achilles tendon has been suggested for dancer one of the most sensitive parts of the body [7] because ballet dancers

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force their turnout, leading to increased pronation in the mid foot and hind foot, dancing on pointe, on demi-pointe, or in plié are places force on the Achilles tendon. Therefore, pain and injury involving Achilles tendon are also common among ballet dancers[8]. "AT which is not limited to the athletic populations is the most frequently reported injury related to the ankle and foot in different sports" [9]. The reason is because "recreational sports activities are getting more and more popular and athletes are being pushed to enhance their performance; hence injuries in sports are increasing"[6]. Around 35% of highland dancers had tendonitis injury, 18% of ballet dancers will suffer an Achilles injury at some stage in their dance life and around 30-40% may suffer an Achilles or arch tendon damage in Irish Dance[10].Besides that, a systematic review done by Hincapie CA, Morton EJ and Cassidy JD (2008)[11] showed that the foot and ankle sustained the majority of injuries within the lower extremity and reported incidence rates are between 17% and 67%. Another study claimed that AT is considered an injury common to the dance community, although the prevalence and incidence of AT has not been specifically identified in the dance literature[12]. This condition will cause pain, swelling, weakness and stiffness of the Achilles tendon in the morning[13], impaired ability to perform strenuous activities[14] and will affect almost all the foot movements of the dancer. This condition will also result in financial outlay by the dance company[15], owing to dancers unable to dance or time loss from dance class, rehearsal, or performance[16]. Current treads on assessment are Ultrasonography, MRI, palpation, passive and active range of motion, Thompson's test and single leg calf raise[17]. Treatment for Achilles tendon injury in ballet dancers are conservative with rest, refraining from dancing, and local treatment such as ice and adhesive strapping, anti-inflammatory drugs, local injections, thermotherapy, laser therapy and stretching exercises[18]. Previous research had described several risk factors for Achilles tendon injury in Football, Cricket and Badminton Players and runners[19]. There is no evidence about whether recreational ballet dancers have the same risk factors of AT as studies on AT in recreational dancers are lacking. Achilles tendon injury will affect the dancers' ability to perform at an optimum level and will cause pain to be felt when walking or running. Therefore, it is importance to identify the risk factors in order to help develop strategies aimed at reducing the prevalence of ballet injuries and makes it clear information for dancers, teachers, sport therapists, health professionals and parents to prevent or treat injuries in recreational ballet dancers[20]. To fill the gap in the literature, population will be on recreational ballet dancers of both genders, will use purposive sampling because to minimize bias and this sampling is commonly used, moderate cost, and the sample will meet a specific objective. In addition, the questionnaire to be established by review of researcher will add in those questions which previous research had recommended getting more clear information about the risk factors.

Method

This study will utilize a Quantitative approach, non-experimental, descriptive research design to find out the prevalence and risk factors of AT among recreational ballet dancers. This study was conducted for a period of four months. The first month was allotted for proposal revisions, and acquisition of necessary permissions and approvals. The second month was the implementation of the study. The third month includes data collection, preparation and analysis and the final month covers the writing of the results and final paper presentation. Before starting the procedure, the researcher sends the informed letter to the Dance Company to get the permission to conduct the study. An informed consent form (IFC) will be provided to each subject stating their willingness to participate in the study. During the study, researcher give priority to the screening tools and prevalence sheet to all the dancers who are willing to give consent to select the appropriate population who passed the study's inclusion and exclusion criteria. After selecting the dancers who are suitable to conduct this study, Achilles tendon area was palpated to confirm the problem. The questionnaire will be given to the dancers at that movement. On the questionnaire dancers will indicate the BMI, training hours in a week, unfamiliar choreography, duration of warm up, stretching and cool down exercises, type of floor surfaces, shoes type during practice, medication history, flexibility, foot posture, and calf muscle strength. The instrument utilized in this study is purely questionnaire-based. A self-developed questionnaire was adopted from researchers whom carried out similar studies. To enhance content validity, experts in Kinesiology, Growth and Development and Physical Therapy reviewed the questionnaires and then the questionnaires were adjusted based on their recommendations. The measures used to determine the factors influencing the likelihood of sustaining an injury were one questionnaire. The one questionnaire administered by the researcher were factors of AT. To develop content reliability, a pilot test will be

conducted on 6 female or male dancers aged 15- 30. Upon completing the questionnaire, the dancers were asked to identify any question they found unclear or confusing. The questionnaire was then adjusted thereby making it easier to understand for the participants in the study. After 2 weeks, the questionnaire was conducted again on the same dancers to prevent the recall bias and enhance the reliability. The screening tools and prevalence sheet also provided to obtain the subject's name, age, gender, ethnic, occupation, company/studio name, ballet grade/level, and number of years in ballet dance, any Achilles tendon injuries/pain, and symptoms of AT, time of injury, doctor diagnosis, surgery history on lower extremity, history of joint instability, details of treatment received, and any other musculoskeletal disorder. All descriptive information was analyzed using measures of central tendency such percentage and mode as measure of dispersion. The statistical treatment of data used Statistical Package for Social Sciences (SPSS) v Version 24.

Results

A total of 108 recreational ballet dancers were recruited and 53 subjects met the inclusion criteria. A total of 53 (100%) female subjects with age range: 15-19 years (n=42, 79.2%), 20-24 years (n=9, 17%), 25-30 years (n=2, 3.8%) participated in the study. Figure 3.1.1 shows the prevalence of Achilles tendinopathy was 28.3% (15/53) among recreational ballet dancers. A number of extrinsic and intrinsic risk factors have been identified in Table 3.1.1 for ballet dance related AT, including BMI, training duration, unfamiliar choreography, limited warm up and cool down, training surface, footwear, medication, flexibility, lower limb abnormalities and muscle strength. Not only that, Figure 3.1.2 shows footwear is the highest average of risk factors (57.77%) among training duration, limited warm up and cool down, and muscle strength. The BMI of 53.3% (n=8) recreational ballet dancers were under normal weight status (18.5- 24.9) which clearly shows that most of the ballet dancers are under normal weight status. For the training duration of recreational ballet dancers, 40% (n=6) dancers usually practiced 0-3 hours of ballet dance per week. However, before they were injured 46.7% (n=7) of dancers practice more than 9 hours of ballet dance per week. During the period to cope with exam or competition or performance, 46.7% (n=7) of ballet dancers practiced more than 9 hours per week. This result shows that there was a sudden increase of training duration., For the unfamiliar choreography, 60% of recreational ballet dancers do not feel difficulty in moves in the choreography will cause them to get injured. The result of limited warm up and cool down shows that, before ballet dance there are 7 (46.7%) of recreational ballet dancers use 0-5 minutes to warm up and 7 (46.7%) of dancers use 5-10 minutes to do stretching exercise. However, after the ballet dance 7 (46.7%) of dancers do not perform any cool down or stretching exercises. These results clearly indicated that there is a limited duration of warm up and stretch before ballet dance and lack of cool down exercises after ballet dancing. Next, the result of training surface showed that 13 (86.7%) of recreational ballet dancers are using sprung wood as a floor surface during practice time in studio and there are 13 (86.7%) dancers get injured on ballet studio instead of examination hall, competition area or home practice. For the footwear, 53.3% (n=8) dancers usually wear soft shoes for practice whereas for the shoes that causes pain on Achilles tendon, there are 60% (n=9) dancers having pain when wearing pointe shoe to dance. About the tightness of the shoe ribbon, 60% (n=9) dancers proper fit to tie the shoe ribbon. The result of medication shows that 15 (100%) of recreational ballet dancers do not take any medication like OCP, HRT, Fluor quinolone antibiotics, high blood pressure medication, and DM medication. The result of lower limb abnormalities and flexibility shows that 53.3% of recreational ballet dancers have normal foot posture in standing upright position. 66.7% of dancers having normal hamstring flexibility and 53.3% of dancers having normal calf flexibility. Therefore, most of the recreational ballet dancers are having normal foot posture and flexibility. The muscle strength of 33.3% (n=5) recreational ballet dancers able to do 20-24 single heel rises on right side, which clearly indicate the weakness of the right lower limb muscles, although 33.3% (n=5) recreational ballet dancers are able to do more than 25 single heel rises on both sides.

Discussion

In the present study, 53 recreational ballet dancers with age range from 15-30 have completed the questionnaire. The aim of this study was to investigate the prevalence and risk factors of AT among recreational ballet dancers. In this descriptive study, the prevalence rate of 28.3% was observed which suggests that AT is common among the recreational ballet dancers because this result is higher than the prevalence in ballet dancers done by Hullachan (2008) where a prevalence of 18% was reported. Regarding the BMI of the respondents in

this study, most frequently recreational ballet dancers (53.3%) are under normal weight status and none of the dancers are overweight or obese as this indicate that BMI is not the risk factors of AT among recreational ballet dancers. This result agrees with a prior study by Longo, U.G., et al. 9) which reported that weight and height did not have influence in the development of AT. Another study by Franceschi, F., et al. (2014) also stated that, significant associations found for AT in people who were overweight or obese compared to normal weight. Furthermore, previous research stated that unfamiliar choreography is one of the contributing factors to AT because they have "insufficient time to become accustomed to the movements and fine-tune their technique accordingly". Although difficulty choreography will cause AT, but according to our findings, most of the recreational ballet dancers (60%) do not feel difficulty in the moves. Therefore, unfamiliar choreography is not one of the risk factors of AT among recreational ballet dancers. Based on the result of this study, training surface is not one of the risk factors of AT among recreational ballet dancers because most of the dance floor they commonly used for practice is sprung wood. According to Motta-Valencia, K. (2006), sprung wood as a floor surface during practice are advantageous for dancing because they are built with layers of different materials and a specific resiliency. Hence, the most important properties of ballet dance surfaces are resiliency, surface friction and shock absorption. The reason that causes them more prone to overuse injuries is the floor material which lack of spring. According to the result of current study, most of the dancers are having normal flexibility therefore flexibility is not one of the risk factors of AT. The reason is because normal flexibility in the hamstring and calf muscles will not cause tension in the Achilles tension and impact the functioning of ankle joint. A study done by Rabin et al shown that limited flexibility of the gastrocnemius and soleus increases absorption work by the plantar flexors during walking and running, which may lead to increased strain on the Achilles tendon. Another study by Miller and Royal Surrey County Hospital (2017) stated that having poor flexibility like tight calf muscles and/or hamstring are the causes of AT. In this report, most of the dancers are having normal foot posture instead of flat arch in standing upright position. This result indicates that lower limb abnormities is not one of the risk factors of AT among recreational ballet dancers because normal foot posture does not lead to an increase torsional stress on Achilles, whereas flat arch foot posture prone to Achilles tendonitis. According to Carey, Achilles tendon has been stressed due to the flattened arch pulls on calf muscles and it will cause the tendon to become inflamed and painful if continuous stress applied on the heel and tendon. Another studies by Footbionics also found that there was a correlation between excessive pronation and increased strain on the medial portion of the tendon. "People who taking medicines from a group called fluoroquinolones will have an increased risk of developing AT. It is also more common in people who have high blood pressure, high cholesterol or diabetes". The reason is because fluoroquinolone antibiotics have toxic effect that may cause tendonitis or spontaneous rupture of the Achilles tendon. However, current study find out that 100% (n=15) of recreational ballet dancers do not take any medication like OCP, HRT, Fluoroquinolone antibiotics, high blood pressure medication, and DM medication. Therefore, medication is not one of the risk factors that caused them to have AT. In current study, most of the recreational ballet dancers are having limited duration of warm up and stretch before ballet dance and lack of cool down exercises after ballet dancing. This result clearly indicates that limited warm up and cool down are one of the risk factors of AT among recreational ballet dancers because each of the dancers do not take about 15 minutes for warm up before the class and cool down after the class and also stretching exercises. According to a study done by Malliou, P., et al. (2007), there is an important co-relation between the rate of injuries and the duration of the warm up and cool down because the result shows that, the number of injured instructors appears significantly smaller when they take part in warm up and cool down about 15 minutes. Another study by Woods also stated that, before beginning the activity, a warm up and stretching protocol should be applied within 15 minutes in order to increase blood flow to the tissues, reduce injury and increased flexibility. Furthermore, training duration is also considered one of the risk factors of AT among recreational ballet dancers because in present study result shows that there was a sudden increase of training duration from about 3 hours ballet dance per week to more than 9 hours per week during the season to cope with exam or competition or performance. This result agrees with prior study that dancers are having risk of sustaining an injury when dance more than 8 hours per week due to overwork and fatigue. Martin, S. also claimed that pre-professional dancers are especially easily to get Achilles tendonitis when they increase their training, like starting a summer intensive.

In another study which done on runners by Hein, T., et al. (2014), overtraining is described as a major risk factor for AT, including excessive training distance, changes in training routines, increases in training intensity and faster training pace. Besides that, footwear is also one of the risk factors of AT because current study result shows that most of the dancers are feeling pain on Achilles tendon when wearing pointe shoe for practice. Pain is noticeable when wearing pointe shoe because "Achilles tendon is stressed when on pointe or demi pointe due to the forceful contraction of the gastrocnemius and soleus muscles". However, for the tightness of the shoe ribbon, current study result shows that most of the dancers are proper fit therefore shoe ribbon is not excessively tightened around the ankle which do not cause further compression on Achilles tendon . Muscle strength is also one of the risk factors of AT among recreational ballet dancers because 33.3% dancers are unable to perform more than 25 single heel rises on right sides although another 33.3% of dancers are able to do more than 25 single heel rises on both sides. This result clearly indicates that there was reduced muscle strength over the right lower limb. There are many reviewed articles have reported 25 raises for a general healthy population but as a recreational ballet dancer more raises should be performed for both lower limb. The reason is because the dancer spends a good deal of time with releve (ankle plantar flexed) movement, therefore more raises to perform is important for dancers to improve the calf muscle strength and prevent more stress on Achilles tendon and lead to AT. Mahieu, N.N., et al. (2006) showed increased risk of AT with reduced plantar flexion strength in the calf muscles due to decreases of the ability of the Achilles tendon to absorb high forces during intense stretch-shortening cycles. Our findings clearly demonstrated that training duration, limited warm up and cool down, footwear and muscle strength are the risk factors of AT among recreational ballet dancers whereas BMI, unfamiliar choreography, training surface, medication, flexibility and lower limb abnormalities are not the risk factors that cause them to have AT. Furthermore, results also have shown that footwear is the highest average risk factors (57.55%) among the four risk factors. The weakness of the current study is unable to recruit male recreational ballet dancers and small number of samples collected in the study because most of the male dancers are participating in other dance forms besides ballet dance. On the contrary, major strength of current study is the fact that information was collected by questionnaires form which has validity and reliability. The questionnaires form also been established by review of researcher with reference to recommendation of previous study. Besides that, subjects also able to answer the questionnaires themselves without any incomplete data or claimed that they do not understand the questionnaire. Secondly, our study clearly provided a preliminary data on the prevalence and risk factors of AT among recreational ballet dancers where previous study was mentioned there was a lack of studies on AT among ballet dancers.

Conclusion

In a nut shell, 53 recreational ballet dancers in age range from 15 to 30 are participated in this study with completed the questionnaire which establish by researcher. We found that AT is an overuse condition which commonly reported among recreational ballet dancers with the prevalence rate of 28.3%. A number of extrinsic and intrinsic risk factors have been identified in this study such as BMI, training duration, unfamiliar choreography, limited warm up and cool down, training surface, footwear, medication, flexibility, lower limb abnormalities and muscle strength but our findings clearly demonstrated that training duration, limited warm up and cool down, footwear and muscle strength are the risk factors of AT among recreational ballet dancers. Besides that, among this four risk factors, footwear is the highest average of risk factors (57.55%) for AT. According to this findings, it indicates the risk factors of AT among recreational ballet dancers and shows the highest average of the risk factors among the four risk factors of AT which provide clearly information for dancers, teachers, sport therapists, health professionals and parents to prevent or treat the injuries more effectively and help to develop strategies aimed at reducing the prevalence of ballet injuries.

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Multidisciplinary Approaches in Surgical Treatment and Rehabilitation of Patients with Defects of Trachea Anterior Wall

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Abstract

Introduction: Elimination of persistent and extensive defects of the anterior wall of the trachea is one of the urgent problems and continues to be the subjects of researches and discussions of the thoracic surgeons, plastic surgeons and otolaryngologists. The authors presented the results of treatment of 118 patients with extensive defects of trachea in subglottic division of the larynx and soft tissues of the neck after laryngo-tracheostomy or tracheostomy.

Materials and Method: The authors consider that the choice of the method of plastic surgery or reconstruction of the tracheal defect is determined individually in each case. If the size of the defect of the trachea is up to 2x4 cm, the sufficient depth of the trachea lumen corresponds to the lumen above and below the stoma, the common axis of the trachea and the distal part of the larynx are without displacement and deformation of the lumen, then a skin-muscle plasty of the defect with the substitution of local tissues is recommended.

Results: Local three-layer or four-layer skin-muscle plastic operations were performed in patients with defect sizes from 1.0 sm to 2.0 sm wide and from 2.0 sm to 4.0 sm long, with a defect depth of more than 8 mm, i.e. anterior-posterior size of the tracheal lumen and a stable formed lumen of the respiratory tract in the zone of plastic contraction without the presence of a protector (T-shaped stent or tracheostomy cannula). Eleven patients with extensive defects of the anterior-lateral walls of the trachea and distal larynx underwent complex reconstructive operations using microsurgical techniques.

Conclusion: The use of displaced flaps allows: to form a mucous membrane in the defect area without tension of the lateral walls of the trachea (the first layer); to close the defect (or to cover the cartilaginous implant) with a well blood-supplied second flap. A complex-compound prefabricated flap (Delta-pectoral skin-fascial-cartilaginous flap) can be used in the presence of a persistent defect and formed tracheal lumen at this level, without signs of restenosis of the respiratory tract, in the absence of inflammation and infection of tissues around the defect. The skin-muscle-bone revascularized complexes of auto-tissues correspond all the requirements of tracheal surgery, allowing to reduce the risk of infectious and trophic complications.

Keywords: *Trachea, extensive defects, tracheostomy, T-shaped stent, skin-muscle plasty, auto-rib cartilage, reconstructive surgery, delta-pectoral flaps.*

Introduction

At the present time there is a tendency for increasing of the long-term ALV (artificial lung ventilation) cases, which is associated with the development of the level of resuscitation life support[1]. Concerning this fact there is an increase in the number of patients with CST (cicatricial stenosis of the trachea), so the relevance of this theme causes no doubt[2]. On the third day of the ALV in the cartilaginous half-rings of the trachea pathological changes are observed[10], which are specific for chondrolysis, sequestration and necrosis[3]. These pathological processes cause the cartilage to loss its support function, which further leads to the narrowing of the tracheal lumen[13]. The occurrence frequency of cicatricial stenosis of trachea after conducting resuscitation actions, accompanied by prolonged lung ventilation, and later tracheostomy, according to different authors' data, ranges from 1 to 25%[13]. Endoscopic examination is the main in the detection of stenotic diseases of the respiratory tract[7].

In specific CST, the main method of treatment is a circular resection of the trachea with the formation of an anastomosis for restoration of the respiratory tract integrity. If the patient has tracheomalacia, the volume of surgery intervention expands. In some cases, resection should be avoided for staged reconstructive operations with the formation of respiratory tract lumen on the T-shaped stent. Distinct respiratory disorders may require tracheostomy or endostenting. Also the implantation of auto-tissues and fixing of biocompatible ceramic rings is possible. Hybrid and biodegradable stents have been developed, but the assessment of their clinical effectiveness is not sufficient, so they are not widely used. Thus, the diagnosis of CST should be aimed at identifying not only the stenosis of the respiratory tract lumen, but also intramural changes in the tracheal wall, including tracheomalacia.

Plasty of extensive defects of the cervical trachea involves the restoration of not only soft tissue defect[12], but also mucous membrane and tracheal skeleton function[7]. The latest requires sufficient supply of wholesome skin, free of hairs, in the reconstruction zone[6]. The absence of such areas near the tracheal defect (sharp dystrophic or cicatricial changes)[2] requires the use of tissues taken from other areas of the patient's body (the formation of the Filatov's stem, the displacing of skin-fascial flaps on the vascular pedicle, autotransplantation of complex flaps on microvascular anastomoses)[4].

A large number of possible tissues and materials, as well as method for surgeries aimed at eliminating the defect[5] of the cervical trachea are proposed[11]. The main requirements for them are clearly formed: they must have sufficient size, provide a frame function and the possibility of evacuation of the discharges, as well as be resistant to infection [9]. Despite of this, the problem is relevant and continues to be the subject of researches and discussions of thoracic surgeons, plastic surgeons and otolaryngologists[14]. The choice of a method for eliminating the tracheal defect depends on its size, the depth of the respiratory tract lumen and the state of the surrounding tissue defect[8]. At a small size of the defect, with the depth of the respiratory tract more than 1.5 cm, and non-specific cicatricial process around the defect, it is possible to use local plasty[7].

Materials and Method

In the Department of Lungs Surgery and Mediastinum at SI "RSSPMCS named after acad. V. Vakhidov" in the period from 2008 to 2019, 118 patients with defects of the anterior wall of the cervical trachea were hospitalized. Previously all patients underwent the stages of endoscopic coagulation, bougienage of tracheal stenosis with the subsequent plasty of the trachea with the formation of the lumen by the T-shaped stent.

In patients with persistent extensive defects of the anterior wall of the trachea and soft tissues of the neck, with a defect size not exceeding 2 sm in width and up to 4 sm in length, a three-layer skin-muscle plasty was carried out with a simultaneous elimination of the defect by local tissues. At the presence of large defects, we use prefabricated scin-fascial and scin-muscle flaps.

Results

Local three-layer or four-layer skin-muscle plastic operations were performed in patients (n=91) with posttraheostomy defects of the anterior wall of the trachea and soft tissues, with defect sizes from 1.0 sm to 2.0 sm wide and from 2.0 sm to 4.0 sm long (Fig. 1), with a defect depth of more than 8 mm, i.e. anterior-posterior size of the tracheal lumen and a stable formed lumen of the respiratory tract in the zone of plastic contraction without the presence of a protector (T-shaped stent or tracheostomy cannula).

After plasty of the defect we performed an intraoperative fiber-optic bronchoscopy through the endotracheal tube by pulling it in subglottic part of the

larynx for visual inspection of the sutured defect. At the same time, the tightness of the created anterior wall in the form of a skin insert, the mapping of layers, the presence or absence of deformation and/or contraction of the trachea in the plastic zone were investigated.

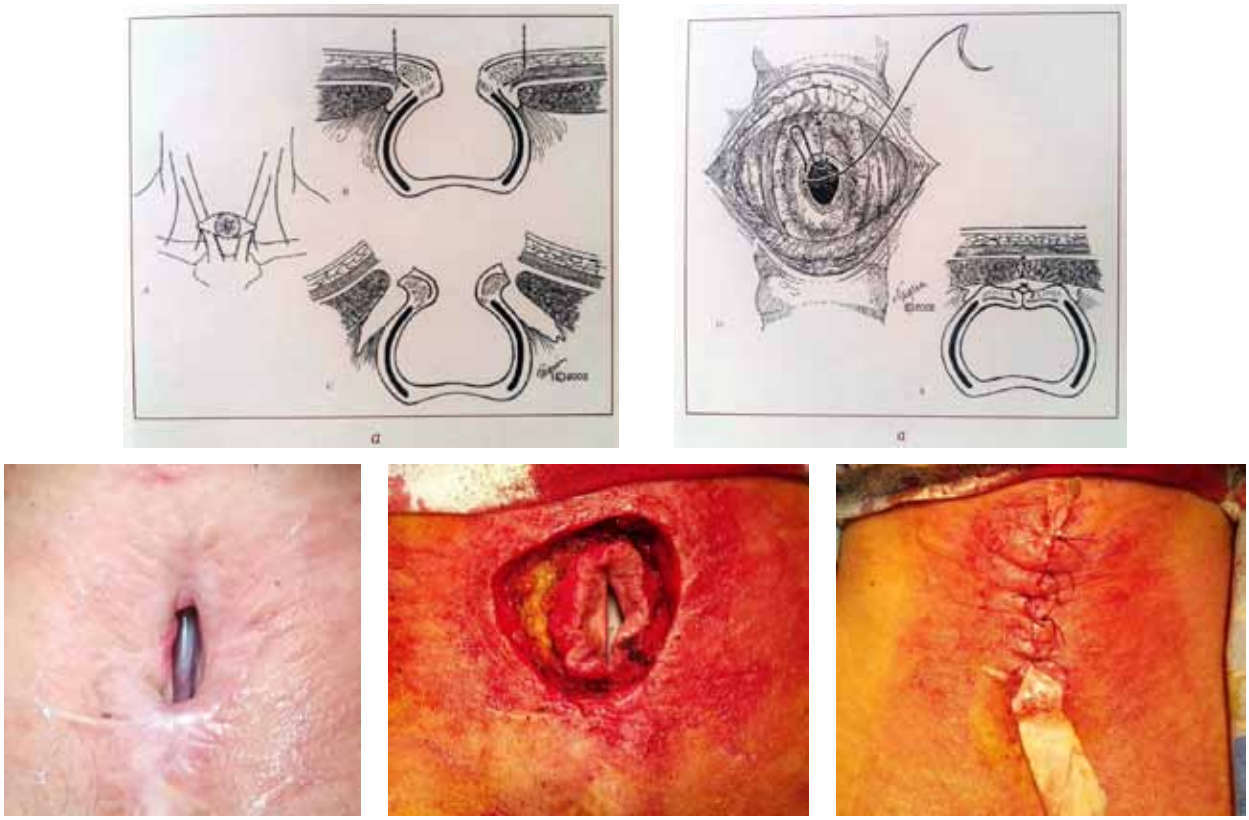


Fig. 1 Scheme and intraoperative photos of four-layer skin-muscle plastic.

Discussion

In the case of insufficiency of the sutures in local plasty, restenosis and large size of the defect of the anterior wall of the trachea, we performed plastic surgery with displaced flaps, including implantation of the autorib cartilaginous graft.

At three-layer skin-muscle and skin-muscle-cartilaginous plasty, the suturing of the muscles as a second layer often leads to a stenosis of the trachea lumen and reduces the depth of the respiratory tract in the defect area. The significant cicatricial process of surrounding soft tissues prevents the approach of the wound edges. In this case, the solution of the problem is the use of displaced flaps from the surrounding areas. The most acceptable are: lateral, upper-lateral and supraclavicular flaps.

In 11 patients with an extensive defect of the anterior-lateral walls of the trachea and the distal part of the larynx, the plasty with the use of displaced flaps on the vascular pedicle were performed. In 2 cases with defect sizes of more than 5x3 cm, the plasty was performed with the use of a pre-fabricated skin-fascial-cartilaginous delta-pectoral flap on the vascular pedicle (Table 1).

The use of the displaced skin-fascial flaps on a vascular pedicle for closure of the defect of the trachea can allow to restore the mucous membrane area of the trachea defect without the use of surrounding cicatricial tissue and cover the defect with a second flap with no tension on the wound edges.

Table 1: Plasty of tracheal defect by displaced flaps

Type of plasty	Used flaps in plasty of:		Number
	Mucosa of the trachea (first layer)	The wound defect (second layer)	
Plasty with two displaced flaps	Upper-lateral flap	Lateral flap	3
Displaced flap + free skin graft	The supraclavicular flap	Free skin graft	3
Closure of the defect + displaced flap	Skin flap from the edges of the defect	The supraclavicular flap	3
Delto-pectoral flap (DPF) + autorib cartilage	DPF skin+cartilage	Free skin graft	2

Clinical Example: Patient A., 42 years old. Diagnosis: Posttracheostomy cicatricial stenosis of the upper third of the trachea. Defect of the anterior wall of the trachea.

Patient with persistent open defect of the anterior wall of the trachea and soft tissues of the neck. The patient has previously undergone several operations: 1) plastic surgery of tracheal lumen on the T-shaped stent (14/10/2015); 2) closure of the lower 2/3 of the defect with skin-muscle plasty (03.02.2017); 3) re-stenting with a T-stent due to restenosis (29.08.2018).

This time the defect is eliminated by two stages with the use of rotational skin-fascial neck flaps. Initially, a skin-fascial supraclavicular flaps on the left and on the right were formed (12.11.2019). We raised the skin-fascial flaps and sewn back with the abandonment of rubber outlets ("biological straightening of the flap"). (fig. 2).



Figure 2. Stage 1- formation of flaps.



Figure 3. Stage II – plasty of the defect of the trachea

The next stage – the plasty of the anterior wall of the trachea by previously formed skin-fascial flaps (20.11.19).

The edges of the previously formed skin-fascial flap on the left were isolated and rotated to form the neo wall of the trachea. The sutures of the previously formed supraclavicular skin-fascial flap on the right were excised, a flap with good blood supply, was moved to the defect area without tension to close to the tracheal defect the second layer (fig. 3).

The control TBFS in the dynamics - the skin insertion is determined on the anterior wall of the trachea 2 cm below the vocal cords, hermetically. The lumen of the trachea in the defected area is wide (fig. 4).



Fig. 4 Tracheobronchoscopy 8 days after plastic closure of tracheal defect.

Clinical example of plasty of extensive defects of trachea with prefabricated delto-pectoral flap on a vascular pedicle.

Patient P., 28 years old, with a diagnosis: Cicatricial stenosis of the upper and middle third of the trachea. Extensive cicatricial defect and deformation of the anterior wall of the trachea and soft tissues of the neck. Cicatricial stenosis of the left main bronchus. Bronchiectasis of both lungs. Anamnesis: in 1992, at the age of 2 years, she was hospitalized to the regional hospital in Fergana with inflammatory process of lungs. Had an intensive care for 7 days on ALV, tracheostomy was done. In a subsequent, since 1992 up to the present time the patient has undergone multiple surgeries for cicatricial stenosis of the trachea in Moscow, Khabarovsk, at the Otolaryngology Department of the Tashkent State Medical Institute. MSCT of the thorax dated 09.06.2016: an irregular thickening of the trachea walls to 8-10 mm with stenosis of the lumen throughout a minimum diameter of 9 mm. Stenosis of the left main bronchus to 5.8 mm. Half-rings of the trachea are calcified. Due to the fact that the patient had multilevel tracheal stenosis, the defect was large and in the middle of the defect there was a connective tissue septum on the posterior wall. First, it was necessary to fix a T-shaped stent to expand the middle part and align the tracheal axis (Fig. 5). It took about 1.5 months.



a **b** **c**

Figure 5. Multifocal cicatricial stenosis of the trachea. Extensive cicatricial defect of the anterior-lateral walls of the trachea and soft tissues of the neck (a). A week after the fixing of the T-stent (b). After 6 weeks (c).

Due to the presence of extensive defect of the anterior wall of the trachea and soft tissues of the anterior surface of the neck, the plastic surgery was performed by a prefabricated Delta-pectoral flap. The size of the defect was 3 sm wide and 8 sm high, the depth of the lumen was 1.2 sm. The axis of the respiratory tract was broken, the distal division of the larynx and upper third of the trachea in the projection of the defect had the direction of the axis from behind to forward in the sagittal direction, and the middle third of the trachea in a retrosternal area had the axis direction from front to back in the sagittal direction.

We have improved the method of forming a pre-fabricated flap. As a supporting tissue in this case we had used an autorib-cartilaginous skeleton, implanted into the composition of the flap. Depending on the parameters of the tracheal defect, a part of the cartilage was taken from the area of connection of 6th and 7th ribs, according to the generally accepted technique in the plastic surgery. Subsequently the formed cartilage framework was implanted subfascially into the distal part of the delto-pectoral flap.

Stages of surgery:

Stage I. The formation of prefabricating delto-pectoral flap with implantation of autorib-cartilaginous skeleton into the distal part of the flap (Fig. 6).



a **b** **c**

Figure 6. Delto-pectoral flap marking (a). Preparation of rib cartilage (b). Condition after the implantation of rib-cartilaginous graft (c).



Figure. 7. A month after the pre-fabrication of a delto-pectoral flap with implanted cartilage before lifting and rotating the flap.

No indications of restenosis were observed for a long period during the observation. So it was decided to perform the 2nd stage of the surgery: the displacing of the previously prepared skin-cartilage flap with the closure of the lower 2/3 of the tracheal defect with the formation of the mucous membrane.

Stage II. Raising the pre-fabricated flap with the formation of a round stem. The substitution of the defect of the trachea with the restoration of the mucosal membrane (on account of the flap skin) and cartilage skeleton (on account of implanted autorib cartilage). Closure of the donor area and the fascial part of the flap with split skin (Fig. 8).

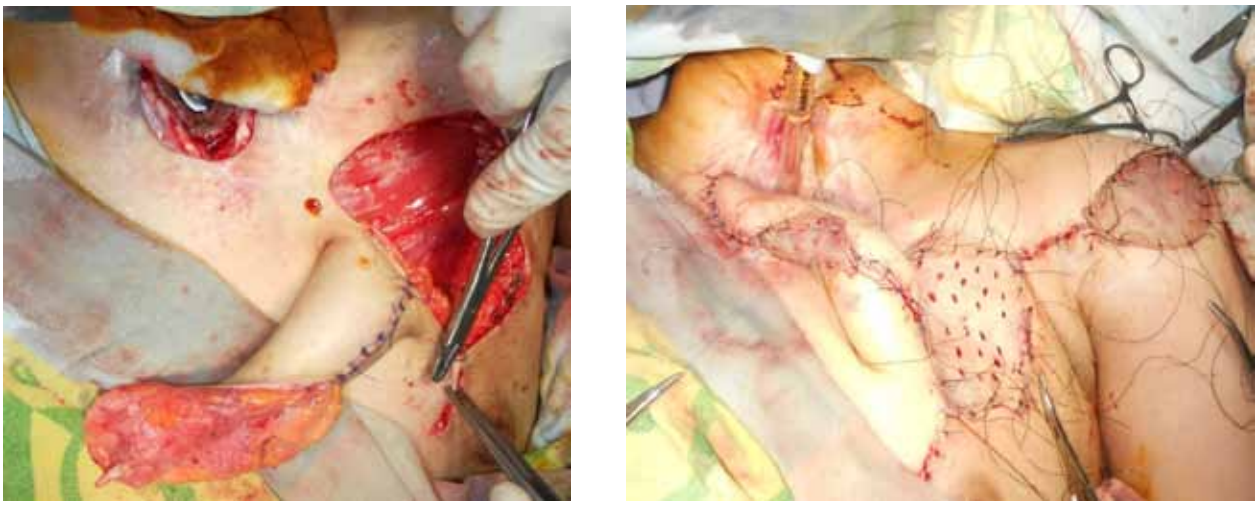


Figure 8. Raising of previously formed pre-fabricated flap. Suturing of the tracheal defect.

Stage III. The final plasty of the tracheal defect (Fig 9, 10).

Biological preparation of the flap is followed by cutting off its pedicle with suturing along the axis of the trachea from the left side and strengthening the frame by autocartilage.

On 22.12.2018 partial plasty of the defect of the trachea by pre-prepared skin-fascial cartilage flap was performed. The postoperative period proceeded without complications. Primary healing of wound was noted. Sutures were removed on the 14th day after the surgery. Diagnostic TBFS was conducted: the trachea is wide, stable. The patient was observed for a long period, indications for restenosis were not observed.



a **b** **c**

Figure 9. Biological preparation of the flap (a). Amputation of the flap (b). Suturing of the flap along the axis of the trachea from one edge (c). The arrow shows the implanted autoccartilage.

On 11.01.2009 the plasty of the residual defect of the cervical trachea under the local anesthesia was performed by suturing the fabricated delta-pectoral flap on the right side with full closure (Fig. 10).



a **b** **c**

Figure 10. Complete closure of the tracheal and soft tissue defect in 2 weeks. Before surgery (a). Suturing the flap on the right side (b). Extensive defect is completely closed by the flap.

MSCT shows that the wide lumen of the trachea is visualized with a deformation in the middle third, which does not significantly affect the passability. Inside of the displaced flap, the autorib cartilage is traced, running diagonally from left to right, crossing the longitudinal axis of the formed anterior wall of the trachea (Fig. 11).



Figure 11. MSCT of the trachea after the 3-rd stage of the surgery.

At bronchoscopic control the tracheal lumen is also wide with a deformation in the middle third, up to 11 mm in diameter in the narrowest part.

In future, it is planned to cut off excess flap tissue. Thanks to the multi-stage plastic surgery, we managed to eliminate the extensive defect of the trachea and soft tissues, because of which the patient had been suffering for 26 years and was limited both physically and mentally, and the result of it undoubtedly is the main basis for improving the quality of life.

Conclusion

Thus, the choice of the method of plasty or reconstruction of the tracheal defect is determined individually in each case. If the size of the defect of the trachea is up to 2x4 cm, sufficient depth of the trachea lumen corresponds to the lumen above and below of the defect, and the single axis of the trachea and the distal part of the larynx is without displacement and deformation of the lumen, then a skin-muscle plasty of the defect with the substitution by local tissues is used. Rotational skin-fascial flaps also can be used, including pre-implantation of the cartilage frame. The indication for the use of displaced flaps for plasty of the tracheal defect is a satisfactory depth (more than 1.5 sm) and defect size not more than 4x2 sm. With a significant cicatricial process around the defect (as a result of repeated surgical interventions) and inability to use the Bokstein method, the using of skin-fascial displaced flaps on the vascular pedicle is known to be effective.

The use of displaced flaps allows: to form a mucous membrane in the defect area without tension of the lateral walls of the trachea (the first layer); to close the defect (or to cover the cartilaginous implant) with a well blood-supplied second flap. A complex-compound prefabricated flap (Delta-pectoral skin-fascial-cartilaginous flap) can be used in the presence of a persistent defect and formed tracheal lumen at this level, without signs of restenosis of the respiratory tract, in the absence of inflammation and infection of tissues around the defect. When using displaced flap to form a mucous membrane in the area of the tracheal defect, it is advisable to use the first stage of biological preparation of this flap, which consists in its separation and re-fixation by sutures to the mother bed. The task of this stage is to strengthen the axial circulation and increase the survival of the flap. The main stage is performed on the 12-14th day after the first stage.

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Barrier Factors In Maintaining Breastmilk Volume

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Abstract

Objective: Breast milk (ASI) is a nutrient that is produced from cellular secretion and transportation activities in the breast organs. The development cycle of the organ of ASI secretion begins to form from the neonate, puberty to pregnancy [1]. Until now, several assumptions that state that the production of breastfeeding of a nursing mother are influenced by various external and internal factors. External factors involve the environment, sociodemography, and even sociocultural[2].

Method: The volume of milk assessed on days 7th, 8th, 9th, postpartum. Milk volume measured through pumping using a Medela Swing Double Pump electric breast pump for 15 minutes, the weighted milk is then returned for drinking to the baby using new media (spoon or cup feeding). Sociodemographic variables, obstetric history, and lactation history were measured using an observation form, postpartum is then grouped into two groups; breastfeeding mothers with optimal and sub-optimal milk production. The relationship between variables was tested by the chi-square test and logistic regression test.

Results: Occupational variables ($p < 0.049$; OR = 2.61), initiation of early breastfeeding ($p < 0.022$; OR = 2.73), frequency of breastfeeding ($p < 0.003$; OR = 4.62), duration of breastfeeding ($p < 0.018$; OR = 2.76) demonstrates relationship with milk volume.

Conclusion: This research succeeded in proving several external factors such frequent and timing that supported the mechanical mechanism of breast milk production.

Keywords: *Early Breastfeeding Initiation; Breastfeeding Barrier; Breastfeeding Duration; Milk Volume.*

Introduction

Breast milk is a nutrient produced from the activity of cellular secretions and transportation of the breast organs. The development cycle of the organ of ASI secretion begins to form from the neonate, puberty to pregnancy.[1] Several assumptions state that various external and internal factors influence the production of breast milk for a nursing mother. External factors involve the environment, sociodemography, and even sociocultural.[2] Internal factors involve psychosocial, cognitive, affective, obstetric, and biological history.[3]

Extensive work to establish factors that affect timing and initiation of breastfeeding. Various studies have shown that breastfeeding predictors differ around

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the world. Notice in Australia, Iceland, Ireland, Scotland, and the US that breastfeeding rates are higher among older women with higher education.[4] Besides, higher-income is also associated with high rates of breastfeeding in countries such as Sweden[5], and rural Jamaica.[6] By comparison, a study in Zhejiang China that examined breastfeeding initiation and prevalence found that younger mothers with lower education and family income were more likely to breastfeed.[7] According to Kogan, breastfeeding rates depend on multiple factors, including socio-economic and demographic factors. Mother's age, employment, income and not smoking contribute to higher breastfeeding rates.[8]

There are many reasons mothers chose not to breastfeed their children. Fear of pain, physical distress, lifestyle constraints, and embarrassment have all described as reasons a woman prefers not to breastfeed.[9] The most common reason that is also a factor for mothers not giving breast milk is the lack of milk production. Mother's causes before age 6 could be more linked to maternal factors. Community factors constitute 20% of mothers' reasons to avoid exclusive breastfeeding. Community help is one reason mothers avoid early breastfeeding. Some studies also shown that mothers consider social support as a more important factor than health care.[10] Often, insufficient support from family, friends, and health providers may be a cause for women to choose not to breastfeed or quit breastfeeding.[11] Therefore, this study aims to see whether there is a difference in milk production between all the risk factors mentioned, namely, sociodemographics, obstetrics, and lactation history.

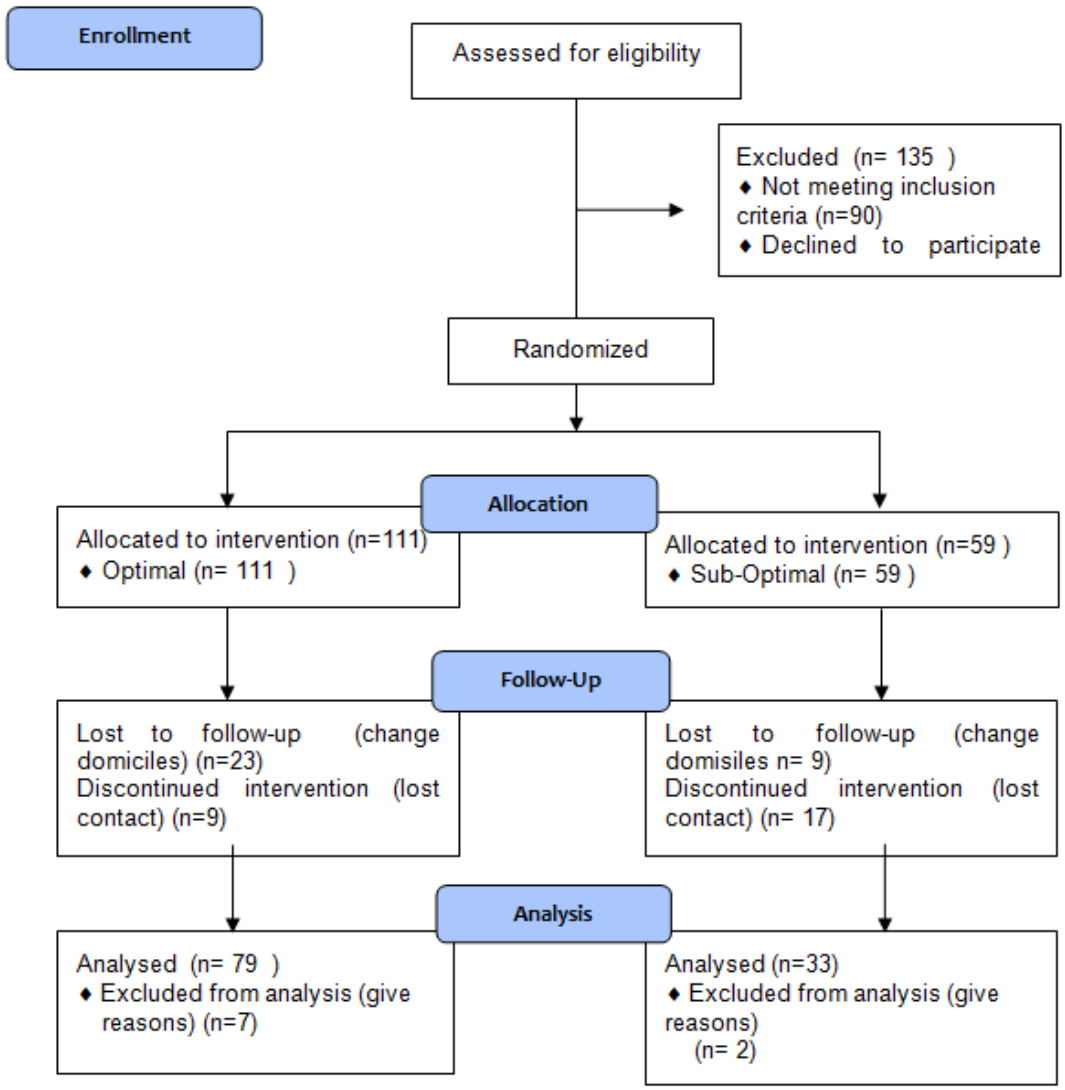


Figure 1: Flow Diagram of the medical research ethics

Methodology

Samples: Mothers who met the inclusion criteria, namely third-trimester vaginal delivery, had no history of pregnancy complications or risky, delivered at the study site, willing to be observed until the study period ended. Mothers who are willing to participate sign an informed consent accompanied by their respective families. Collection of variables, including sociodemographic characteristics, obstetric history, and lactation history, were measured using an observation form at the beginning of recruitment at the final antenatal care examination. The volume of milk assessed on days 7, 8, 9, postpartum.

Statistical Analysis: This study uses a cross-sectional study design. A total of 113 research samples were selected purposively at two primary healthcare clinics (Kassi-Kassi and Jongaya Clinic) with a large number of visits of pregnant women with relatively low coverage of exclusive breastfeeding in the city of Makassar, South Sulawesi, Indonesia in the month of April-December 2019.

Milk volume measured through pumping using a Medela Swing Double Pump electric breast pump for 15 minutes, the weighted milk is then returned for drinking to the baby using new media (spoon or cup feeding). The volume of milk is calculated and multiplied by the frequency of breastfeeding for three days and then averaged. The measurement results classified into two; optimal breast milk production ≥ 440 ml/day and sub-optimal <440 ml/day[12].

Sociodemographic variables (age, education, occupation), obstetric history (parity, baby birth weight, sex), and history of lactation (initiation of early breastfeeding, frequency of breastfeeding, and duration of breastfeeding) were analyzed using the chi-square test. Multivariate logistic regression analysis used to assess which risk factors were correlated with the amount of milk. The medical research ethics committee approved all research procedures of the Medical Faculty of Hasanuddin University number 171/UN4.6.4.5.31/ PP36/2019.

Results

A total of 113 participants who were successfully analyzed depicted in table 1. The sociodemographic variables referred to in this study include age, education, and occupation. Education transformed into two categories; higher education equivalent to DIII and S1, low equivalent to elementary, junior high, high school. Work also transformed into two categories into work and non-work. Bivariate analysis demonstrates insignificant relation on several sociodemographic variables, except for job ($p<0.049$).

Working is one of the common barriers to breastfeeding for many breastfeeding mothers. Parting with the baby will reduce the frequency and duration of breastfeeding. Although alternative milk pumps exist, not all biological reflexes replaced with mechanical stimulation. The odd ratio shows that mothers who do not work have a volume of milk production 2.61 times more than mothers who work.

Table 1. Corelations between sociodemographic, obstetric, and lactation with milk volume

Variable	Breastmilk Volume				p*	OR
	Sub-optimum (31)		Optimum (72)			
	n	%	n	%		
Age						
< 20 and >35	7	38.9	11	61.1	1.000	1.14
20-35	34	35.8	61	64.2		
Work						
Working mother	15	53.6	13	46.4	0.049	2.61
Household mother	26	30.6	59	69.4		
Education						
Low	29	41.0	59	59.0	1.461	0.53
High	12	48.0	13	52.0		

Variable	Breastmilk Volume				p*	OR
	Sub-optimum (31)		Optimum (72)			
	n	%	n	%		
Parity						
Primipara	14	42.4	19	57.6	0.511	1.44
Multipara	27	33.8	53	62.2		
Birth Weight (gr)						
<2.500/>4.000	3	50.0	3	50.0	0.666	1.81
2.500-4.000	38	35.5	69	64.5		
Baby sex						
Male	18	37.5	30	62.5	0.973	1.09
Female	23	35.4	42	64.6		
Early Breastfeeding Initiation						
No	21	51.2	20	48.8	0.022	2.73
Yes	20	27.8	52	72.2		
Breastfeeding Frequency						
< 8 times/day	15	65.2	8	34.8	0.003	4.64
≥8 times/day	26	28.9	64	71.1		
Breastfeeding Duration						
< 15 minute/session	25	49.0	26	51.0	0.018	2.76
≥15 minute/session	16	25.8	46	74.2		

*Chi Square Test alternatif Fisher's Exact Test

Obstetric variables in this study include parity, baby sex, baby birth weight. These three variables are not significant for milk production. Lactation variables include the history of Early Breastfeeding Initiation, frequency of breastfeeding and duration of breastfeeding. All three showed a positive significance with milk production. Mothers who give birth and get Early Breastfeeding Initiation are at least 2.73 times higher potential to produce more milk volume than those who do not get Early Breastfeeding Initiation. Mothers who breastfeed more often (more or equal to eight times per day) have a potential of 4.62 times to produce more milk volume than mothers who breastfeed less than eight times per day, as well as the duration of each breastfeeding process, has an Odd ratio of 2.76 times higher. The duration reported in this study is an estimate based on the subjective assumptions of the mother and the average results of data collection for seven days analyzed in the transformation of categorical data more/ equal to and less than 15 minutes for one breastfeeding session.

Table 2. Logistic regression risk factor for milk production

Factors	p	Odds Ratio	95%CI
Working mother	0.034	2.969	1.086-8.114
No Early Breastfeeding Initiation	0.018	0.345	0.143-0.830
Breastfeeding frequency < 8 times per day	0.025	3.390	1.164-9.876
Breastfeeding duration < 15 minute per session	0.041	2.559	1.039-6.303

*Variable(s) all entered on step 1(based on table 1), after performing 6 step of backward likelihood ratio method, tested correlation and controlling confounder, the last fit model explained milk production among breastfeeding mothers.

After conducting a simultaneous analysis using multiple logistic regression, by entering all the variables at the beginning of the test step to consider the possibility of confounders and intercorrelations between variables,

the results shown in Table 2, which most determine the volume of milk production is the frequency of breastfeeding (OR: 3.390), working mother (OR: 2,969), duration of each breastfeeding session (OR: 2.559), and initiation of early breastfeeding (OR: 0.345).

Even though a working mother gives birth without early breastfeeding initiation, she still able to maintain the volume by breastfeeding more frequent and extended duration of each session. Two mechanical factors frequency of sucking and duration of sucking will ensure emptying of the breast and increase production supplies. Both activity as if perfect rhythm between the need and availability of milk with infants.

Discussion

The ratio of mothers who produce optimal milk volume is 2.3 to sub-optimal; this means that more than 113 research subjects can breastfeed optimally. Sociodemographic factors occupy an obvious path within the conceptual framework of milk production, such as age, education, and employment. This study shows that more than half of mothers in the age range of 20-30 years have optimal milk production, but mothers aged over 30 years also record similar facts. Research conducted at the Department of Nutrition at the University of California shows that "older" primiparous mothers (24 years or more) produce less milk in the first week of postpartum than younger primiparas (16-23 years). This research assumes that breast milk produced "depends on the amount of functional tissue in the breast," which may decrease with age. In Sweden, reporting that breastfeeding rates are declining, one of the factors that inhibit exclusively breastfeeding mothers for more than two months is primipara under the age of 25 years.[13]

In another study also revealed that young age and marriage at less than 20 years not only have an impact on the maturity of the mother's soul in babysitting but affect the mother's performance starting initiation of breastfeeding faster.[2]

Education, in many studies, is a favorable variable to study. Education assumed as the basis for everyone to form normative behavior. This research shows that mothers are generally highly educated (equivalent to D3, S1-2), meaning that mothers have sufficient intellectual power to support their skills in breastfeeding and caring for babies. Although statistically, there is no difference between mothers with high and low education in terms of milk production, this is because traditions and habits heavily influence the context of breastfeeding in a particular family or culture. The element of milk production is in the body and breast tissue, which logically has nothing to do with the external aspects of education.

One study showed that the context of breastfeeding practice is influenced by traditional practices even though they (mothers) lack education or lack understanding of the ins and outs of milk production. Breastfeeding is a derivative of habits passed down from generation to generation, views on breast function and the quality of milk produced and the standards of behavior that exist in a community or tribe to be determinants of breastfeeding can be well accepted and implemented or not.[14] However, in other studies, data found that mothers with high status have higher production than those with low education.[15] In various searches, it is rather difficult to find studies that specifically study the relationship between education and milk production. However, studies examining the relationship between a mother's knowledge and the success of breastfeeding for up to six months are numerous. Some of them prove that educated mothers (72% secondary and 90.9% graduated) are more likely to give colostrum to their babies than those who are illiterate (38.9%). Although the duration of exclusive breastfeeding and breastfeeding is not related to mother's education, knowledge about breastfeeding is very important for parents with higher education (63.6% and 52.8%) compared to parents who are illiterate or have low education. Other studies in developed countries also show the fact that highly educated mothers (62.0%) more exclusively breastfeed for up to two months than low education (38.0%) (Cato et al., 2017). In Europe, a comparison of cross-sectional studies conducted between 2006-2016 revealed that early breastfeeding initiation and shorter breastfeeding duration found in mothers with less education than those with higher education based on the ISCED (International Standard Classification of Education) education classification.[16]

The provision of breast milk for six months requires high commitment. One of the most challenging commitments fulfilled for mothers is the attachment of time between themselves and their babies. Everyone will naturally experience boredom in the same activities every day, especially for six months. The most significant barrier is working mothers. Work reported as the most frequent reason to stop breastfeeding.[17]Breastfeeding and working require more effort and commitment.

Many workplace policies that are not in line with the extended maternity leave policy make this situation more difficult.[18] In this study, significant work related to milk production. The Odd ratio shows that working mothers are 2.62 times lower risk of producing milk compared to mothers who do not work. This pathway can be explained through separate time-feeding scenarios starting when the mother returns to work. Maintenance of milk production can be done by breastfeeding on demand, breastfeeding periodically, and pumping periodically. Leaving baby with grandmothers appears in a different situation in feeding style. Grandma does not have superior soothing ability compared to their mother with her breasts. Choices that can be taken by working mothers are pumping milk. Dairy milk requires a storage medium, care that is more troublesome than direct breastfeeding. Grandma also certainly is not always painstaking in preparing dairy milk. If the workplace situation does not allow mothers to leave the office more often for breastfeeding purposes, then direct breastfeeding will be replaced by alternative dairy milk.

Giving expressed breastmilk has different dimensions for each person. It started with the availability of breast pumps, how to pump, the storing method, the defrosting process, the feeding process after pumped, and many other dimensions that must overcome. Not to mention dealing with the composition of expressed breast milk, which has decreased the quality of macronutrients during storage. Degradation of some macro components of fat and calories observed in frozen milk stored for three months under -20 degrees Celsius.[19] Even, the mechanical obstacles in the process of pumping milk. Some natural reflexes will disappear, such as mother-baby bonding that reinforces neuro-response to hormonal secretions.

The pumping mechanism is different from the suction mechanism in infants; pumping tends to have a constant rhythm. Some milk pumps have developed pumping technology with different rhythms following the baby's suction pattern, suction strength, and adjustable flow. This type of pump is usually much more expensive than pumps in general, and not all mothers can afford to buy or rent. Although the suction technology in a milk pump resembles a baby's suction rhythm, it still leaves several natural scenarios such as skin to skin touch and lactogenesis, which only develop optimally through direct breastfeeding. However, no studies have yet found that review differences in the speed of milk secretion indirect breastfeeding and pumping; the loss of intimacy between mother-baby interaction during breastfeeding is clearly of different quality.

The phase of lactogenesis is progressive in pregnancy. Lactogenesis in the first is different from the second pregnancy. The process of repetitive Lactogenesis characterized by an increase in the quality of lactation. Primipara is one of the risk factors for inhibition of Lactogenesis II and reported that primipara has a potential 3.16 times more likely to experience delayed lactogenesis II compared to multipara.[20] Hampering the phase of Lactogenesis II, i.e., no discharge of milk beyond 72 hours postpartum, can result in the cessation of breastfeeding before six months.

This study did not show differences between primipara and multipara in the two milk production groups. The number of mothers who had optimal milk production was higher in both groups because the proportion of research subjects belonging to optimal milk was 2.3 times the sub-optimal group. Specific findings in Thailand are specific to other areas of rural northern Thailand.[21]Transferred milk volume and breastfeeding duration did not vary significantly between multipara and primipara. Babies in the primipara community took much longer to get the same amount of milk on day 15 and day 45, but the period of subsequent breastfeeding was shorter and more like that of other partners' babies. Perhaps they need more time before breastfeeding is known. Babies' weights and lengths were identical at each time between classes. Research in Nigeria also found that maternal age, parity or social status did not impact breastfeeding patterns.[22]

Research with different results shows that women who have previously breastfed had significantly different breastfeeding experiences than those who were first. They have a shorter duration of breastfeeding, have a more considerable delay in labour until the first attempt at breastfeeding, are less likely to breastfeed at least eight times in the first 24 hours, and have more breastfeeding problems during their breastfeeding period. These variables might contribute to the finding that first time mother who had received formula milk while still in the hospital, delayed Lactogenesis, and reduced the likelihood of achieving their intended breastfeeding goals, as well as shorter breastfeeding duration.[23]

The percentage of sexes of female babies is higher than male babies. Gender data in this analysis helps to identify the baby's own birth prevalence. Scientifically

found no difference in the amount of milk between the sexes of female and male, because the need for infant nutrition is directly proportional to the increase and growth of baby's weight in the first year. Many myths have also developed among the community that baby boys are stronger at breastfeeding than baby girls; this may connote the nature of the male identic with the power. To some extent, this myth will lead to the natural perception that a baby girl is breastfeeding less than a baby boy. Although empirical evidence illustrates that male babies breastfeed more than female babies is still rarely found, so it is necessary to emphasize the focus of the problem is on the mother and their behaviour.[24]

Infant birth weight is also not related to milk production because birth weight influenced by many things including; maternal factors such as maternal age, parity, teenage pregnancy, smoking history [25]; nutritional factors such as haemoglobin levels and folic acid deficiency, history of HIV infection, abortion and unwanted pregnancy.[26]

Variable early breastfeeding initiation is a decisive contributory factor that affects the amount of milk production. The initiation process carried out as early as possible to provide the broadest possibility for babies after birth to get the best of the skin-to-skin breast crawl process, utilizing all of their instinctive abilities within a period of 30 minutes to 1 hour after delivery. Before the breastfeeding instincts fade after 8 hours, new-borns tend to fall asleep more. Early Breastfeeding Initiation is the right start for mothers and babies to start learning to suckle and breastfeed. Along with the process and time goes to know each other until breastfeeding formed. Early Breastfeeding Initiation ensures that every baby gets colostrum rich in vitamins and protective factors.[27] Neonatal abstinence in early breastfeeding initiation has a higher frequency of NOD2 gene mutation, which causes a decrease in immune function compared to opposite.[28]

The suction on the nipple stimulates the secretion of prolactin and oxytocin so that the initial initiation is a precursor of milk secretion, the faster and more prolonged the process of Early Breastfeeding Initiation takes place, the more consistent the milk produced. In this study, Early Breastfeeding Initiation was significantly related (p-value <0.02) to milk production. The Odd Ratio reveals that mothers who initiate early breastfeeding 2.73 times more produce milk than mothers who do not initiate early breastfeeding. In the Early Breastfeeding Initiation intervention study with a combination of direct breastfeeding and regular breast milk pumps, the average volume of milk was higher on the third and seventh days (73.9mL and 225.2mL) compared to the control group that only did direct breastfeeding (25.4mL and 69, 2mL) (Fok et al., 2019). This research will reinforce the previous statement that barriers to working mothers eliminated by regular milk pumping. Early Breastfeeding Initiation also prevents the occurrence of "delayed lactogenesis II" on the third day of postpartum, and significantly precursors of increasing milk volume.[30]

The frequency of breastfeeding a baby is different in each phase of time. At the beginning of the first-third month, babies can suckle 7-9 times per day or more than 12 times. This tight connection dismissed the mother chance to leave her baby even if an several hour. Usually, hungry babies will cry at intervals every 1.5 to 3 hours. However, babies who are getting bigger will have a longer breastfeeding interval. The duration also varies depending on the adaptation of each baby. In this study, the frequency of breastfeeding significant related to milk production, as well as the duration of breastfeeding. Milk production increases 4.62 times more for women who breastfeed more often, and 2.76 times for women who breastfeed longer. Meaning the frequency of breastfeeding and duration of breastfeeding (every one breastfeeding session) is a critical point in increasing milk production, it related to the process of breast drainage. The longer the breastfeeding duration, the more it will ensure each breast is empty, thus increasing the supply of milk volume.[31]

Nevertheless, the nature of breast milk production is quite adaptive to baby. Research reveals that breast adaptability to baby suction compare to no other. Babies who sucking fast rhythms but short time, are having the same quality to suckle slowly and long time. Both of these adaptation models ensure the baby gets the same quality milk composition between the foremilk hindmilk.[31],[12]

In addition to drainage and frequency, lactation techniques will support mother for prolonged breastfeeding. The most important corrected technique regarding to positioning and attachment.[32] These two crucial points, especially in new-borns, need to assist by the midwife. Incorrect position resulted in many problems, such as blister nipples, hard nipples, swollen, and painful sensation when suckled.[33] Each mother's pain adaptation differs depending on prior

knowledge and perception of physiological pain during lactation. Inadequate physiological response during the breastfeeding process adds to the anxiety that will ultimately make the mother vulnerable to falling in postpartum depression cases. The performance of breastfeeding will decrease in women who experience postpartum depression, several factors that contribute include age, working mother, socioeconomic status, number of children, and family support[34].

Inappropriate lactation management, giving them the opportunity for early breastfeeding cessation. Most women who breastfeed and experience constant nipple pain can achieve normal milk production. Breastfeeding length and frequency are the same as pain-free mothers. The longer period of breastfeeding in pain groups, however, is correlated with lower milk production rates.[35]This study emphasizes that emptying the breast when expressing milk or direct feeding will keep the baby's milk required.[31] Although the sociodemographic barriers addressed in this study are not statistically related to milk production, working mothers, early initiation, frequency and length of breastfeeding are findings that preserved the milk production pathway through external mechanisms.

Conclusion

This research succeeded in proving several external factors such frequent and timing that supported the mechanical mechanism of breast milk production. Therefore the midwife and all other breastfeeding support program must provide a better assisted education in breastfeeding management, and other feeding alternative among them who still face unavoidable barriers such as working mothers.

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Effectiveness of Septic Tanks Floating in Reducing COD, TSS, Temperature and pH of Waste Water Black

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Abstract

Sanitation is the provision of facilities and services for disposal of human waste such as urine and feces. To reduce diseases arising from poor environmental sanitation, efficient technology that can be utilized by communities in coastal areas and small islands is one of the facilities what is needed for access to sanitation is a septic tank. This study aims to determine the sanitary conditions of communities in coastal areas and small islands and to determine the effectiveness of appropriate technology for floating septic tanks in coastal areas and small islands. The quality of wastewater that is important to know is Total Suspended Solid (TSS), Chemical Oxygen Demand (COD), Temperature and Hydrogen Power (pH).

Keywords: *Effectiveness; Septic Tanks; Floating; Coastal; Small Islands.*

Introduction

Sanitation is a public health effort that focuses on supervision of various environmental factors that affect the degree of human health, so it prioritizes efforts to prevent various environmental factors so that the emergence of disease can be avoided.[1] Sanitation is also a process to maintain a clean and hygienic place, especially the availability of clean water, solid and liquid sewage systems.[2]

People living in coastal areas and small islands everyday life they are exposed to health risks, among others, the lack of availability of clean water.[3] Human behavior in using latrines and adapting to the environment is one of the factors in shaping the culture of society.[2] Healthy water is a necessity for people on this planet. This includes people who live on the coast and on small islands. People on the coast and small islands, however, find healthy water very difficult to find because of limited water resources. In addition, available surface water also appears to have been polluted by domestic wastewater.[4] Water quality has a direct effect on health, given the nature of water which is easily contaminated by various microorganisms and easily dissolves various materials.[5] The nature of such conditions is very easy to function as a medium for distribution or spread of disease. There are several diseases that fall into the category of water borne disease, or diseases carried by water. While the types of microbes that can spread through water are quite a lot including bacteria, protozoa and metazoan.[6]

Based on the results of laboratory tests conducted on cutlery samples, it was found that all samples contained bacteria. This is caused by several factors that cause the presence of germs (bacteria) in eating utensils, namely the quality of washing water.[7] Well water on these small islands is vulnerable to contamination, especially on small islands and densely populated areas where the limited availability of land prevents people from building standard septic tanks.[4]

The impact of waste disposal is not only for humans but also for biota in marine waters, aquatic ecosystems are being degraded by anthropogenic pollution on a global scale.[8] Such as Hg concentration in mackerel. Hg concentration in mackerel is 1.346 mg/kg while blood clams range from 0.772 to 3.111 mg/kg.[9]

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It is important to evaluate the level of groundwater threats posed by the on-site system and review the strategies available to limit further groundwater degradation.[10] To minimize the occurrence of contamination of water in the area of small islands and coastal areas, it is necessary to treat domestic wastewater before being discharged into water bodies, one of which is the re-engineering of septic tanks with an appropriate technology approach. Septic Tank Engineering with the appropriate technology approach is an urgent need to address the problem.[11] A septic tank that is commonly used in the community is a conventional septic tank. However, the use of septic tanks is still not optimal because the processing efficiency has only reached 65%, causing only 22.5% of the total organic pollutants that can be processed.[12]

The quality of wastewater that is important to know is Total Suspended Solid (TSS), Chemical Oxygen Demand (COD) and Hydrogen Power (pH). The level of treatment carried out before the waste is discharged into water can contain organic and inorganic materials which are generally measured by parameters, COD, TSS and others.[13] COD describes the total amount of oxygen needed to chemically oxidize organic material, both biodegradable and biodegradable (non-biodegradable) biodegradable to CO2 and H2O. The results of COD research in waters in the upstream, middle and downstream successively value is 62.176 mg/l; 69.176 mg/l and 64.48 mg/l. COD values in uncontaminated waters are usually less than 20 mg/l, whereas in polluted waters can be more than 200 mg/l. pH indicates the need for pretreatment to prevent interference with conventional wastewater treatment processes.[14]

Method and Materials

This research was carried out on the coast and small islands. This type of research is descriptive research based on the quality standards set in PUPR PERMEN No. 4 of 2017 which refers to the planning of the building under the septic tank according to the Indonesian National Standard (SNI).

Floating septic tank prototype planning includes, the criteria for planning a black water effluent treatment tank consisting of two parts. Based on the source of waste, the first tub is a reservoir of waste water from the toilet. Solid and liquid waste that enters through a small pipe that enters tub 1 and is station 1 (inlet). Station 1 is connected to the goose neck closet from the household toilet will be decomposed in tub 1 which is station 2 at the top. This section is where the aerobic process takes place and the middle is the path and the bottom part is where the anaerobic process occurs. During crossing at station 2, the waste will decompose into gas, water and mineral mud with a breakdown time of at least 3 days. Station 2 is like two connected by a tub 1 by using pipe model of T.

The top part of the tub is where the aerobic process takes place, the middle part is the path and the bottom part is where the anaerobic process takes place. During crossing the tub, waste will decompose into gas, water and mineral mud.

At outlet or station 3 is part of the outermost pipe or large pipe is a pipe overflow. A waste quality inspection sample will be conducted at the outlet. One of the factors that become a balance in choosing the type of floating septic tank is the limited land.

Septic tank design is made by utilizing a drum with a volume of around 200 liters, which is equipped with pipes and pipe accessories for complementary components and design improvements (Figure 1).

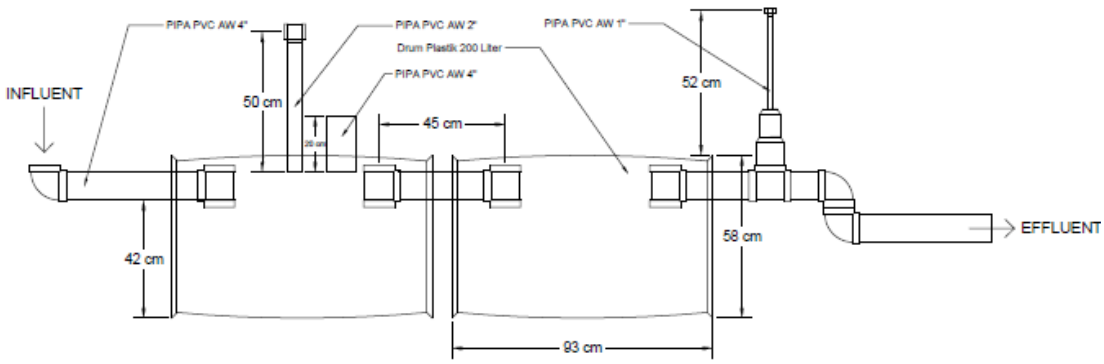


Figure 1. Floating Tank Septic Tool Design

Characterization in waste management from biological, physical, chemical and fluorescence types is combined with information about the system, design, tank type, tank management and number of users. From various studies that have been conducted several biological characterizations show that the total concentrations of coliform and Escherichia coli (E. coli) are: 103-108 and 103-107 MPN/100 mL.[15]

Other research results also provide new quantitative evidence that septic tanks are a real problem for water quality in coastal areas during ecologically sensitive periods, especially where waste is discharged directly into the flow network.[16]

Results and Discussion

From the results of interviews with people in the research area, the level of public knowledge about waste treatment, especially liquid waste, is still very low (Table 1). The survey found that 85% of the residents directly disposed of domestic liquid waste directly into water bodies and 15% were channeled into drainage. Based on the level of knowledge and habits of the community, using the instrument ask respondents about the need for fulfilling clean water that is used daily.

Table 1. Survey Results Based on Clean Water Sources.

Indicator	PDAM/ Gallon	Groundwater (Well)
Clean Water Source	44%	64%
Drinking/cooking water sources	88%	12%
Water Source for Washing/Bathing	25%	75%

The condition of community houses still does not meet these health requirements because of land limitations and the economic conditions of the community. Most people work as fishermen and scavengers used goods. The lack of sanitation in the community greatly influences the emergence of various diseases caused by the environment such as diarrhea and skin diseases.

For defecation, most residents make facilities without septic or directly discharged into water bodies. At low tide the dirt will stay and become a place to fly flies, dirt will disappear at high tide.

To reduce the risk of diseases arising from community sanitation conditions that are still lacking in need of good handling include the procurement of integrated waste treatment facilities that can be used by all local communities (Table 2).

Table 2. Types of Diseases Often Affected in the Community

Diseases Often Experienced by Communities	Diarrhea	Skin disease	Fever
Percentage of respondents	45%	36%	19%

Table 3. Survey Results of Domestic Liquid Waste Management in the Study Area

Type of Liquid Waste	Disposed of directly into water bodies	Streamed Through Drainage/Septic Tanks
Gray Water	75%	25%
Black Water	70%	30%

The results of waste management are still very lacking, this is due to lack of public awareness, land conditions and inadequate facilities of the waste management system (Table 3).

One of the main threats to groundwater and surface water quality is the impact of poorly designed, constructed or maintained wastewater treatment systems.[5] Sources of pollutants originating from septic tanks are recognized as significant contributors to pathogen flow and nutrient loads, but there is little research data in the UK that assesses the potential risks posed by septic tank effluents (STE) on water, human and health quality.[15] In recent years, the potential for groundwater pollution from septic systems on site has emerged as a serious problem in the United States.[10]

Conclusions

From the results of research on obesity that has been carried out the sanitary conditions of communities in coastal areas and small islands are still very lacking so that diseases arising from the environment are still very high, waste management with floating septic tanks in the management of domestic black water waste is effectively used, the levels of the parameters COD, TSS, temperature and pH are safe to dispose of into water bodies. Environmental management and appropriate technology is needed to minimize the occurrence of environmental pollution due to direct disposal of waste into water bodies.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

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Maternal and Perinatal Outcome on Pregnancy with Covid-19 Infection at Dr. Wahidin Sudirohusodo Hospital Makassar During the Period of April-July 2020

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Abstract

Objective: The aims of this research is to notice maternal and perinatal outcomeon pregnant women with covid-19 infection at Dr. Wahidin Sudirohusodo Hospital During the Period of April-July 2020.

Method: This research is using descriptive method which is by taking data from the pregnant women with covid-19 infection at Dr. Wahidin Sudirohusodo Hospital during the period of April 1st – July 12th 2020. The data covers the age, gestational age, amount of parity, symptoms, pregnancy complication, supportive examination results such asneutrofil score, limfosit, CT-scan Thorax, method of delivery, baby's APGAR score, covid baby status, maternal death, perinatal death.

Results: There are 26 pregnant patients characteristic subjects with covid-19 infection, mostly 20-35 years old, aterm gestational (>37 weeks), multiparity, low educational background, unemployed, mostly with no symptoms, increasing limfositneutrofil ratio rate >3,13. Good maternal outcome, there are no maternal death in this research. Perinatal outcome from 24 babies, 5 babies are confirmed covid-19 positive and all are in good condition.

Conclusion: This research concludes good maternal and perinatal outcome.

Keywords: *Maternal Outcome, Perinatal Outcome.*

Introduction

Global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or Coronavirus Diesease 2019 (COVID-19) has developed by the increased death rate, which also assures the identified vulnerable population in the society. From the data of Covid-19 Acceleration Handling Task Force in Indonesia by July 12th 2020, there are 75.699 people confirmed to be positive, 36.638 people confirmed to be recovered, and 3.606 people confirmed to be departed. In the first research, which described the clinical characteristic and investigate the possibility of SARS-CoV-2 vertical transmission on nine pregnant women with Covid-19 that have been confirmed by laboratory examination, showed that the effect of COVID-19 on pregnant women are similar to adult women who are nor pregnant and there are no evidence of vertical transmission because SARS-CoV-2 is not detected by amniotic fluid, umbilical cord blood and neonatal throat swab from the six cases.[1]

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To date, the largest series reporting pregnancy and neonatal outcomes, with 99 COVID-19 infected pregnant women, shows that having COVID-19 during pregnancy are not related to the increase of damaging risks such as spontaneous premature birth. Among the 100 neonatusdelivered by these women, not even one is infected by SARS-CoV-2.[2]Based on very rare data until today, conflicting evidence from nucleic acid based assays and anti body test on neonatus delivered by mother with Covid-19 has appeared to cause further controversy for the vertical transmission risk during pregnancy.[1,2]Due to the foetus and the newborns, innate and adaptive immune system immaturity make them susceptible to infection.[3]Factors dysregulationsuch as cytokines and cascades May have damaging consequences on the developing and brain function. Therefore, not being able to notify whether the infection agent is able to infect nowborns with vertical transmission is interesting.[4]

Pregnant women and their babies need to be evaluate as potentially risky group in today's COVID-19pandemic. From gathered limited information about this novel coronavirus and drastically increasing disease burden, it is necessary for scientific information about this disease to be Sharedbriefly and practically. Result data of infected women and pregnant women perinatal with SARS-CoV-2 are limited to some reports and case series. The size of small samples and the findings are vary. The changes of policies in countries hit by pandemic, developing clinical management guidance, and uncertainty about the reliability of the result make the reports difficult to be interpreted. Therefore, this research is conducted so it is possible to notice maternal and perinatal external result on pregnancies with COVID-19.

Method

This research is using descriptive method by taking the data on pregnant women infected by Covid-19 at Dr. Wahidin Sudirohusodo General Hospital during the period of April 1st – July 12th 2020. The data covers the age, gestational age, amount of parity, symptoms, pregnancy complication, supportive examination result such as neutrofil ratio, limfossit, CT-scan Thorax, method of delivery, baby's APGAR score, baby's covid status, maternal death, perinatal death.

Result

During the period of April 1st – July 12th 2020, there were 26 covid-19 infected pregnancy cases obtained at Dr.Wahidin Sudirohusodo Hospital. Table 1 showsthat the characteristic of pregnant patients with covid-19 are to those with the age of 20-35 years old, which are 20 cases (76.9%). On the age <20 years old and >35 years old, 6 cases were found (23.1%).In terms of the gestational age, the most pregnancy cases with covid-19 are at the aterm gestational age of (>37 weeks) are 22 cases (84.6%). There are 4 cases (15.4%) in cases with Preterm pregnancy (<37 weeks). From the amount of parity, the most cases happen to be on the multipara, which are 17 people (65.4%). And on the nullipara there are 9 cases (34.6%). Most of the subject in this research have low educational background, which are 15 cases (57.7%) and unemployed subjects, which are 17 cases (65.4%).

Table 1. Research Subject Characteristic

Characteristic	Pregnancy with Covid-19	
	N	%
Age		
20-35 years old	20	76.9
<20/> 35 years old	6	23.1
Gestational Age		
< 37 weeks	4	15.4
> 37 weeks	22	84.6
Parity	9	34.6
Nulliparity	17	65.4
Multiparity		
Working Woman		
Well Educated	11	42.3
Lower Educational Background	15	57.7
Occupation		
Employed	9	34.6
Unemployed	17	65.4
Covid-19 Symptom		
Yes	8	30.8
No	18	69.2
Pregnancy Complication		
Chronic Hypertension	1	3.84
PEB	4	15.4
Gestasional DM	1	3.84
SLE	1	3.84
Hyperthyroid	1	3.84
Hepatitis B	1	3.84
No complication	17	65.4
Total	26	100

Table 2 shows pregnancy case with covid-19 taken from supportive examination. Rated parameter is the limfositneutrofil ratio and Thorax CT-scan. The cut off

point of neutrofil and limfosit ratio used in this resaerch is 3.13. In the research subject it is known that limfosit and neutrofil ratio generally increased >3.13 which are 7 cases (34.6%). From the Thorax CT-scan done by the research subjects, there are 6 cases (23.1%) with pneumonia, but caused no death on the mothers and there are 20 cases (76.9%) with no radiological disorder or also know as normal.

Table 2. Laboratory and Radiologic Findingson Pregnancy with Covid-19 at Dr. Wahidin Sudirohusodo Hospital during the period of April-July 2020

Laboratory and Radiologic Findings	Pregnancy with Covid-19	
	N	%
Neutrofil Limfosit Ratio (NLR)		
< 3,13	7	34.6
> 3,13	19	65.4
CT chest		
Pneumonia	6	23.1
Normal	20	76.9
Total	**26**	**100**

From Table 3, it is known that maternal external result is taken from the delivery method, most of the method are by cesarean section which are 17 cases (70.8%). From those with cesarean section, 11 of them come with Obstetric Medical indication, which are previous post cesarean section (8; 47.1%), severe preeclampsia with eclampsia impending sign (2; 11.8%), and macrosomia (1; 5.9%). There are also other cases like 1 case of abortus at the gestational age of 12 weeks and 1 conservative treatment case for dypepsia. There are 6 cases (35.3%) of cesarean section for covid-19 infection.

Table 3. Maternal Outcome on Pregnancy with Covid-19 at Dr. Wahidin Sudirohusodo Hospital during the period of April-July 2020

Maternal Outcome	Pregnancy with Covid-19	
	N	%
Mode of delivery		
Vaginal delivery	7	29.2
Caesarean delivery	17	70.8
Outcome		
Life	26	100
Died	0	0
Total	**26**	**100**

Table 4 shows that perinatal external result of pregnancy with covid-19. From 24 baby external result, there are 5 babies (20.8%) confirmed to have positive covid-19 and normal APGAR score. 1 baby (4.25%) was born with severe asfixia from mother with severe preeclampsia pregannacy complication But with negative status of Covid-19. And there were 16 babies (66.7%) born with negative covid status.

Table 4. Perinatal Outcome of Pregnancy with Covid-19 at Dr. Wahidin Sudirohusodo Hospital during the period of April-July 2020

Perinatal Outcome	Covid-19 Status of the Baby	
	Positive	Negative
APGAR Score		
Normal	5 (20.8%)	15 (62.5%)
Asphyxia	0 (0%)	1 (4.2%)
IUFD	0 (0%)	3 (12.5%)
Outcome		
Life	5 (20,8%)	16 (66.7%)
Died	0 (0%)	3 (12.5%)
Total	5	19

Discussion

Pregnancy cases with covid-19 during the period of April 1st– July 12th 2020 at Dr. Wahidin Sudirohusodo General Hospital are generally patients with no symptoms (OTG), 18 people (69.2%). This findings suit to the research in China that there were 23.3% of pregnant women with Covid-19 that showed no symptoms during the peak of the pandemic in the Province of Hubei, China.[2]There are also 8 patients with symptoms, the experienced symptoms of coughing (2; 7.72%), coughing followed with the experience of Hard breathing (4; 15.4%), anosmia (1; 3.84%), diarrhea(1; 3.84%). This goes along with the research in China that indicates that infected patients' clinical characteristic during their pregnancies show similarity with other infected adult who are not pregnant.[2] From the total of 26 cases, there were 9 patients (34.6%) that came with complication complaints during their pregnancies. 4 cases (15.4%) of severe preeclampsia, 1 case (3.84%) of chronic hypertension, 1 case (3.84%) of hypertiroid, and 1 case (3.84%) of B hepatitis. All pregnancy complications are not related to covid-19. The increasing of the NLR in this research is in line with the research conducted in Spain, where the NLR is increasing on pregnant women infected by Covid-19 and decreasing after clinical condition treatment.[5] The research in China

showed that patients with covid-19 have increased value of neutrofil and decreased value of limfosit on severe phase.[6]The Thorax CT-scan examination did not show radiological disorder or also know as normal. On the contrary, in every research in China, they found radiological disorders on the Thorax CT-scan, which is 96.3 & of Ground glass opacity.[2]

Maternaloutcome pregnancy with covid-19 showed all subjects in the research were in good condition and discharged after two swab test and confirmed to be negative. This suits the research that shows majority of pregnant women with Covid-19 on their third trimester and deliver the babies by cesarean section, showed no maternal death. On a contrary, pregnant women by respiratory infection virus like H1N1 reported to have 4-5 bigger possibilities to develop into severe disease compare to non pregnant women on common population.[7]In this research, there are 3 babies (12.5%) who passed away from mothers who experienced fetal death in the womb (KJDR). External perinatal of 5 babies that confirmed to be positive are in good condition. This also suits the research in Cinawhich perinatal outcomeis good with mothers infected by Covid-19.[2]

Conclusion

There are characteristics of 26 pregnant patients infected by Covid-19, where most of them are 20-35 years old, aterm gestational age of (>37 weeks), multiparity, low educational background, unempleyed, most of them show no symptoms, increasing rate of neutrofillimfosit>3.13. Good maternal external result, no maternal death in this research. Parinatal external result from 24 babies, 5 of them are confirmed to be covid-19 positive and all of them are in good condition.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Effect of Pravastatin Provision on Endotelin-1 Levels in Preeclapmsia's High Risk Patients

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Abstract

Objective: To determine the effect of pravastatin administration on endothelin-1 levels in patients at high risk of preeclampsia.

Method: There are 70 subjects in this research that consist of 34 subjects with the risk of high preeclampsia who get aspirin and 36 subjects of research to get Aspirin and Pravastatin. Subjects are given therapy started the gestational age of 12-19 weeks in 6 days until 36 weeks of pregnancy. Endhotelin-1 levels prior to administration of therapy compared with the levels of endothelin-1 after the administration of treatment.

Results: The test results show the administration of aspirin or aspirin and Pravastatin is shown to provide the changes were significant at levels of endothelin-1 after the administration of treatment. It is characterized by the decrease in the levels of endothelin-1 which were significantly ($p < 0.005$). But by assessing percentage decrease in the provision of aspirin with pravastatin give change levels of endothelin-1 is large (45.74%) compared with administration of aspirin alone (28.8%).

Conclusion: From this study it can be concluded that endothelin-1 levels before administration of aspirin and pravastatin were 86.18 ng/L and after administration of 46.76 ng/L. Giving aspirin and pravastatin 20 mg in patients with high risk factors of preeclampsia provide reduced levels of endothelin-1 is greater than the prevention of preeclampsia that is used when it is only aspirin.

Keywords: *Patient risk of high preeclampsia, Aspirin, Pravastatin, Levels of Endothelin-1.*

Introduction

Preeclampsia is a multisystem disorder in pregnancy that is characterized by hypertension and proteinuria after 20 weeks period of gestation. Abnormalities that occur in 5 stars - 7 % pregnancies, with figures incidence of 23.6 cases per 1,000 births in the United States the United t.[1] Preeclampsia is a specific pregnancy disease and is still the leading cause of maternal-neonatal mortality and morbidity worldwide, accounting for more than 70,000 - 80,000 maternal and 500,000 perinatal deaths.[2]

The imbalance of pro and antiangiogenic factors as well as the activation of mediators of immunity that contribute to excessively inflamation is a major cause. The etiology of preeclampsia is unclear, but several recent studies have shown that an imbalance between angiogenic factors (*Vascular Endothelial Growth Factor* (VEGF) and *Placental Growth Factor* (PLGF)) and anti-angiogenic (*Soluble fms-like Tyrosine kinase 1* (sFlt-1) and *Placental Growth Factor* (PLGF)) and anti-

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angiogenic (*Soluble fms-like Tyrosine kinase 1* (sFlt-1) and *Placental Growth Factor* (PLGF)) *soluble endoglin* (s-eng)) plays an important role in the pathogenesis of this disease.[2]

Endothelin 1 (ET-1) is an isoform primary that produced by endothelial humans and peptide 21-amino-acid is a vasoconstrictor potent. Levels of ET-1 in plasma increased in pregnant women normotensive, but women with preeclampsia had levels of ET are much higher. Endothelin-1 has proven be the holder of the key to the activation signal molecular that caused stress oxidative in patients with preeclampsia that inhibits the release of ET-1 is one of the alternative in preventing the occurrence of preeclampsia.[3]

The International Study Collaboration conducted a meta- analysis of the effectiveness of antiplatelet agents (especially aspirin) for the prevention of preeclampsia. Low- dose aspirin (60-150 mg per day) can reduce the risk of preeclampsia by 24%.[4]On the other, Ahmed et al propose a statin as a new therapeutic strategies in which case it is based on studies of experimental animals showed that pravastatin (inhibitor coenzyme-A reductase 3-hydroxy-3-methilglutaril) has a protective role in the uteroplacental circulation and blood vessel cells.[5]

When this, pravastatin has demonstrated the effect of protection against endothelial cells vaskular induces the expression of H eme O ksigenase (H O -1) and inhibits cytokine release from anti-angiogenic factor sFlt-1 and zinc. Increased levels of sFlt-1 and zinc causes a decrease in VEGF and PlGF free on placental cells and endothelial vessels of blood which ultimately resulted in systemic vasoconstriction and endothelial dysfunction vessels of the blood of the mother.

Therefore, the purpose of the research is to determine the effect of administration of aspirin and pravastatin on the levels of endothelin - 1 in patients Be risk of high preeclampsia .

Material and Method

Design of study: This study was a quasi-clinical trial with randomization, which carried out clinical trials on control groups and cases during the 1- year study period.

Population of study: Samples of research are taken from a woman pregnant at high risk of preeclampsia with age pregnancy 12 weeks - 19 weeks 6 days of the visit to the clinic of the General Hospital of Makassar and Jumpandang Baru health care center. Sampling was carried out by block randomization in the administration of therapy. The provision of interventions carried out *single blinding* that participants can determine the allocation of the group based on the amount of drug that is given.

Study Interventions: This study was conducted to assess the effect of giving pravastatin to pregnant women who are at high risk of preeclampsia. Subject tests are given aspirin 1 x 80 mg orally until the age of term pregnancies as a treatment standard (group A), while the group of samples are given intervention in the form of aspirin 1 x 80 mg orally plus pravastatin who consumed 2 times a day until the age of term pregnancies in the same time (group B).

Randomization: If the patient agrees to be a participant in this study, it is mandatory to sign the research consent sheet. Participants will then be randomized into two groups: those who receive a combination of pravastatin and aspirin, and those who receive aspirin only. Participants will take the drug until the end of pregnancy Endothelin-1 levels will be checked before and after drug administration.

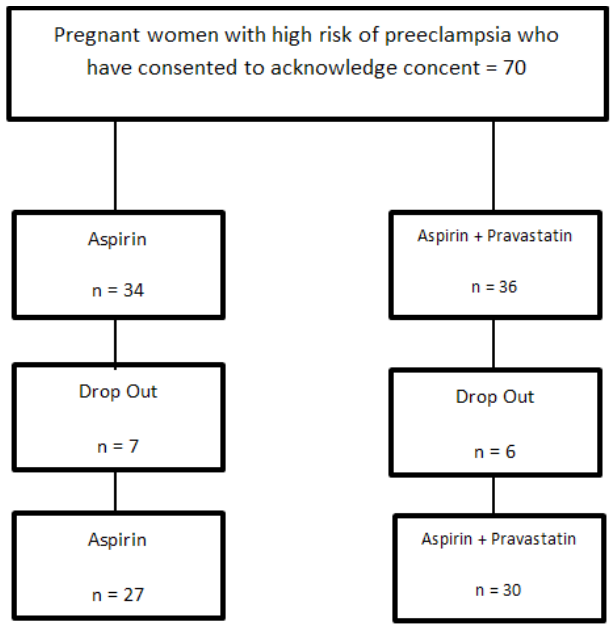


Figure 1. Flowchart of screening of participans

Study Outcomes: Results of the study is expected to provide information about the profile levels of endothelin - 1 on a mother pregnant as well as the effect of giving aspirin and pravastatin on the levels of endothelin - 1 in patients Be risk of high preeclampsia .

Sample Size: In the study of this, a large sample in each group of the intervention and the control is determined by a formula as follows

$$n1 = n2 = \left[\frac{(Z\alpha + Z\beta)S}{X1 - X2}\right]^2$$

N is total samples required for each intervention and control groups . Zα is a type I error, which is 5% with a one-way hypothesis with a constant value of 1.64 . Zβ is k esalahan type II set at 10% by value of the constant 1.28 . S is s impang raw (Verdonk et al., 2015) with a value of 0.42 . X1 - X2 is s elisih minimum considered significant at 0.21 . So the value of n in this study are:

$$n1 = n2 = \left[\frac{(1.64+1.28)0.42}{0.21}\right]^2 = 34$$

Statistical Analysis: Data were collected and have been analyzed and then processed by computer using SPSS. Normality test was performed with Kolmogorov-Smirnov (samples> 50). If a normal distribution is obtained (p> 0.05) a paired T test is used and if an abnormal distribution is obtained (p <0.05) the Wilcoxon test is used to compare the average serum Endothelin - 1 level before and after drug administration in each each sample and control group . Then if a normal distribution is obtained (p> 0.05) an unpaired T hypothesis test is used and if an abnormal distribution is obtained (p <0.05) the Mann-Whitney test is used to compare the average difference in serum Endothelium - 1 levels between sample groups with a control group.

Ethical Approval: Before the study is conducted, the researcher asks the feasibility of conduct of the Commission of Ethics Research Biomedical on Human Faculty of Medicine, University of Hasanuddin Makassar and has been approved by the number of register 601/ H4.8.4.5.31/PP36- KOMETIK/2018 by chairman Prof. Dr. dr. Suryani As'ad, M.Sc., Sp.GK and secretary Dr. Agussalim Bukhari, M.Med ., Ph.D., Sp.GK .

Results

Samples Characteristics:

Table 1. Demographic Characteristics of Research Subjects.

Variable	Aspirin (n = 34)		Aspirin + Pravastatin (n = 36)		P value
	n	%	n	%	
Age					
<20 years old	0	0	0	0	
20 - 35 years old	17	50	23	62.9	0.469 [a]
> 35 years old	17	50	13	37.1	
Parity					
• 0 children	3	8.8	7	19.4	0.734 [a]
• ≥ 1 child	31	91.2	29	80.6	
Education					
• Basic education	15	44.1	22	61.1	0.15 [b]
• Further education	19	55.9	13	36.1	
Body Mass Index (BMI)					
• Normal	7	20.6	8	22.7	
• Overweight	9	26.5	6	16.2	0.643 [a]
• Obesity	18	52.9	22	61.1	

a: *chi-square* test, b: *mann-whitney* test

Table 2. Risk Factors Research subjects.

Variable	Aspirin (n = 34)		Aspirin + Pravastatin (n = 36)		P value
	n	%	n	%	
Diabetes					
• Yes	0	0	0	0	-
• Not	34	100	36	100	
Kidney failure					
Yes	0	0	0	0	-
• Not	34	100	35	97.2	
Hypertension					
• Yes	11	32.4	7	19.5	0.243 [a]
• Not	23	67.6	29	80.5	
Gestational Diabetes					
• Yes	0	0	0	0	-
• Not	34	100	36	100	
History of Preeclampsia					
Yes	13	38.2	14	38.8	0.881 [a]
• Not	21	61.8	25	61.2	

By follow up, there were 1 or 3 subjects who *dropped out* for various reasons.

Differences Endothelin-1 se is not the provision of treatment

Table 3. Differences in Endothelin-1 before and after administration of aspirin alone with aspirin and pravastatin.

Variable	Aspirin + Pravastatin (Mean±SD)	Aspirin (Mean±SD)	P value
Endothelin-1 Pre- intervention	86.18±129.46	109.61±199.33	0.948 [b]
Endothelin-1 Post Intervention	46.76±95.1	78.04±166.97	0.655 [b]

b: *mannwhitney* test

Changes Endothelin-1 prior to and throughout has been the provision of treatment:

Table 4. Changes in Endothelin-1 levels before and after administration of aspirin alone with aspirin and pravastatin

Variable	Endothelin-1 Pre-intervention (Mean±SD)	Endothelin-1 Post Intervention (Mean±SD)	Percentage of Decrease (%)	P value
Aspirin + Pravastatin	86.18±129.46	46.76±95.1	45.74	0.008 [c]
Aspirin	109.61±199.33	78.04±166.97	28.8	0.046 [c]

C: *Wilcoxon* Test

Based on *mannwhitney* test, table 3 shows the p-value have no differences s of endothelin-1 level before and after therapy in both groups of treatment ($p > 0.05$). The intervention that is given to the subject of research at the given on samples with endothelin pre-intervention were homogenous. So the intervention is given in the sample with endothelin start of the homogeneous.

Based on Wilcoxon test, the administration of aspirin or aspirin and pravastatin shown to provide the changes were significant at levels of endothelin-1 after the administration of treatment . It is characterized characterized by a decrease in the levels of endothelin-1 were significantly ($p < 0.005$). But by assessing the percentage decrease in the provision of aspirin with

pravastatin give change levels of endothelin-1 is large compared with the administration of aspirin alone.

Discussion

Preeclampsia is a specific hypertensive disease in pregnancy which involving multisystem. Usually, after gestation age of 20 weeks, most often in the near term, can be superimposed with other hypertensive disorders. Preeclampsia, the most common form of high blood pressure (*BP*) which could complicates pregnancy, is mainly determined by the incidence of new onset hypertension and the presence of new onset proteinuria. However, although both of these criteria are regarded as classical definitions of preeclampsia, some women come with hypertension and multi-systemic signs that usually indicate the severity of the disease without the presence of proteinuria.[6]

If proteinuria is not present, the diagnosis of preeclampsia is enforced when there is hypertension with thrombocytopenia (plateletcount less than 100,000 per microliter), the presence of impairedliver function (increased levels of liver transaminases in the blood totwo times from the normal concentration), the new development of renal insufficiency (increasecreatinineserum greater than 1.1 mg/dl or doubling thecreatinine serum with outany other kidney disease), the presence of a lung edema,or a brain disorder or new onset visual impairment.[6]

Table 2 showed that both the sample and the control Group have the most age in the 20 – 35 years old, in which the sample Group consist of 23 Samples (62.9%) and 17 sample for the control Group (50%). In the Sample group, the most parity count is ≥ 1 as many as 29 Samples (80.6%) and similarly to the control group as many as 31 Samples (91.2%). Further, most samples's education in sample group were elementary which is 22 Samples (61.1%) and the control group found that most of the samples at the advanced education were 19 Samples (55.9%). Then, based on body mass index (BMI) both the sample and the control was found mostly in obese condition which are 22 Samples (61.1%) and 18 Samples (52.9%) respectively.

In particular in table 3 only obtained risk factors with a history of hypertension and previous preeclampsia history in sample groups as much as 7 Samples (19.5%) and 14 samples (38.8%) as well as in a control group of 11 samples (32.4%) and 13 samples (38.2%).

Based from table 1 and table 2, the age, the number of parity, the education status, the body mass index and the previous history of the disease such as hypertension and preeclampsia was not found a meaningful differences in both Research groups, where the value of p > 0,005 on each research variable. This finding indicating that both groups have the characteristics of respondents who are homogeny.

In this study, endothelin-1 levels were the aim. Thus, the study does not found meaningful differences on the levels of endothelin-1 before and after therapy in both groups of treatment. Even if theendothelin levelon the control group(aspirin) much higher than the treat group (aspirin + pravastatin), however, the levels are not so much distinct. This is due to a spread of data that may be too broad due to the range in the sample rate because of the difficulties to control several factors due to the research limitations namely race, ethnic, dietary and previous history of illness. This is evidenced by the unusual data spread from the beginning in both groups in statistical tests. Based on a study by Rahman Shah (2007) endothelin-1appears to have a diverse role as a vascular and growthmodulator and as a mediator in many cardiovascular and non-cardiovascular diseases. ET-1 is the primarily a paracrine substance that acts locally which appears to contribute to the maintenance of basal vascular tone. Moreover, it is also activated in some diseases, including congestive heart failure, arterial hypertension, atherosclerosis, endothelial dysfunction, coronary artery disease, renal failure, cerebrovascular disease, pulmonary arterial hypertension, and sepsis. The increased concentration of *ET-1* can be attributed to increased activity of endotheline-modifier enzymesand to indicate endothelial damage in the circulation of pregnant women with preeclampsia.

After the intervention was found a decreased level in endothelin-1 for both groups with aspririn or pravastatinintervention which there was decreased levels of endothelin-1 by 28.8% in the Control Group (aspirin) pre- intervention from 109.61±199.33 to 78.04±166.97. However, the decrease in endothelin-1 in the sample group (aspirin + pravastatin) is greater than 45.74% from the rate of 86.18±129.46 to 46.76±95.1 after intervention. Based on the findings of this study was observed that both the administration of aspirin and aspirin and pravastatinom could reduce the levels of endothelin-1. Nonetherless, due to the absence of normal value from Endothelin-1, it is not yet clear whether the decrease in the rate is up to normal value or only decrease

its level from the initial rate. This is one of the limitation of this study. This is in line with the research conducted by Raditya Ery Pratama, dkk(2018) in Surabaya which proves that the combination of pravastatinom and standard therapy lowers Endothelin-1 levels.

This research is able to provide data on Endothelin-1 levels after aspirin therapy in high-risk preeclampsia groups. Further, this data shows that there is a decrease in the level of endothelin-1in the group of aspirin but the level of endothelin-1 after therapy is quite relatively high. The data can be an initial input, that perhaps the aspirin is not good enough to suppress endothelin-1 in order to prevent preeclampsia, as it is known that the preeclamicpatomecanism is multifaktorial, not only caused by the increasing in endothelin-1.

The results of this research are as well as the study conducted by Costantine*et al* for *the Eunice Kennedyshriver National Institute of Child Health and Human Development Obstetric-Fetal pharmacology Research Units Network (OPRU),* where it publishes a pilot of a multicenter, *double-blind*, placebo-controlled test, random of the woman with a single pregnancy with gestation age 12 to 16 weeks which randomly receives either 10 mg of pravastatindaily (n = 10) or placebo (n = 10).[7] Four women in the group given the placebo had high preeclampsia with a concentration of *sFlt-1 (soluble fmslike tyrosine kinase-1);* Three of them showed severe conditions compared to the pravastatin given group. Thus, pravastatinshows beneficial benefits in preventing the occurrence of preeclampsia.[7] In addition, there are also numbers of preeclampsia incidence and lower premature births, concentration of sFlt-1 and sENG (Endotelin) decreased in subjects who administered pravastatin. Although the difference for these results does not achieve statistical significance.[8]

Another study by Hassanain*et al* (2018) reported 4 patients having significant hypertension (90-105/155-200 mm Hg) with proteinuria ranging from 840 to 2990 mg/24 hours later administered daily pravastatin(40 mg) since the first day of admission Hospital.[6] Then in line with the effects of proangiogenic pravastatin, little but not significantly lowered the anti-angiogenic factor *sFlt-1 (soluble fmslike tyrosine kinase-1)* and $_{S}$*ENG (soluble Endoglin*) which was observed in the group given pravastatincompared to placebo group.[6]

For Evidence-Based Preeclampsia Prevention found that therapy with 150 mg of aspirin in high-risk women subjected to preeclampsia (based on algorithms that utilize maternal factors and biophysical and biochemical measurements) that began at gestation age 11 to 14 weeks lowered preeclamsia incidents compared to women who received placebo.[9]

Apart from the data limitation of aspirin, in 2013, the *American College of Obstetricians and Gynecologists (ACOG)* recommends administering daily aspirin (80-100mg) for women with preeclampsia history in previous pregnancies before gestation 34 weeks age or preeclampsia history in the previous two pregnancies.

Low-dose Aspirin given to pregnant women with high risk of preeclampsia may decrease preeclampsia incidence of up to 10-24%, lowering the incidence rate of IUGR by 20%, and premature delivery by 14%. However studies have shown that low dose aspirin administration does not lower the preeclampsia incidence at gestation age more than 37 weeks.[10] Similarly, other studies, stating that low-dose aspirin is useless to prevent preeclampsia in patients with chronic hypertension.[7] Therefore, it is preferred to give pravastatinom to decrease the incidence of preeclampsia.

This study shows the administration of aspirin and Pravastatinand aspirin proved to provide significant changes in endothelin-1 levels after administration of therapy is in the Group ofPravastatinom ($P < 0.05$, $p = 0.008$) and on the control group (Aspirin) ($P < 0.05$, $p = 0046$). However, a greater reduction in endothelin-1 levels was given to the group of pravastatin. Therefore, pravastatinis more preferred as a therapy to lower endothelin-1 levels in prevention of preeclampsia cases.

Some recent studies have also added pravastatinas an agent other than low dose aspirin to prevent preeclampsia. Pravastatin is chosen due to the basis of preeclampsia pathogenesis having similarities with cardiovascular disease i.e. systemic endothelial dysfunction.

Pravastatin is chosen from various other statins drugs due to its hydrophilic properties. Its protein binding is the lowest therefore drug interactions are also minimal (Lecarpentier*et al*, 2012). Oral absorption is about 40-75%, passing the first metabolic rate in the liver. Excretion of pravastatinin the form of intact through the liver and kidneys (secretion in tubule). The average absorption time is 2.4 hours with a bioavailability of 18%. The time to reach the maximum concentration in plasma averages one hour after oral administration.[11]

Statins act pleiotropic on various mechanisms: reverse angiogenic imbalance by increasing vascular endothelial growth factor (*VEGF*) and placental Growth Factor (PLGF), and reduce antiangiogenic factors such as *soluble fmslike tyrosine Kinase-1 (sFlt-1)* and *soluble Endoglin (sENG)*.[6]

Pravastatin is a competitive inhibitor of a *3 hydroxy-3-methyl-glutaryl-coenzymeA*reductase which is an enzyme catalyzing step limiting levels in the synthesis of cholesterol. Pravastatin is the most hydrophilic polar compound among current statins and is therefore considered to have limited ability to traverse the placenta. It is also the most powerful inhibitor of sterol synthesis.[6]

All of the biological effects of pravastatin can restore maternal endothelial dysfunction so that maternal vascular reactivity can be reduced. It can prevent maternal systemic vasoconstriction so that blood pressure will not increase.[11]

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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A Model of Cigarette Advertisement Policy in Preventing Children Smoking Habits in Palu City, Indonesia: A Systematic Review

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Abstract

Objective: The high prevalence of smokers in Indonesia has led the government to issue various policies. One of the policies issued by the government to reduce or limit the prevalence is the cigarette advertisement policy. The purpose of this systematic review is to develop a model of cigarette advertisement policy in preventing children smoking habits in Palu City, Indonesia.

Method: This research used a systematic literature review. Various references were collected from online database including reports, journals, and books in the last 10 years. The journals were mostly from the scholarly journals. The articles were screened according to the research objectives. The keywords used are cigarette advertisement policy and children smoking habits.

Results: This study showed that the use of a model of cigarette advertisement policy is very effective in preventing children smoking habits. Cigarette advertisement regulation policy is the government's commitment to protect children, as an effort to protect the younger generation from cigarette advertisements that are intense and have a very large influence on smoking behavior. Cigarette advertisements are proven to encourage children to try cigarettes, repeating the behavior so that it eventually becomes a habit.

Conclusions: This paper concludes that a model using cigarette advertisement policy is effective for prevent children smoking habits. Further recommendation will be provided.

Keywords: *Cigarette Advertisement Policy; Children; Smoking.*

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Introduction

Some of the motivations behind smoking habits are to get recognition (anticipatory beliefs), to eliminate relief (reliefing beliefs) and to think that their actions do not violate the norm (permission beliefs or positive)[1]. The higher death rates caused by smoking and the high social costs incurred due to the effects of smoking encourage the need to optimize the implementation of government policies in changing the behavior of cigarette consumers. On the other hand the cigarette industry contributes significant revenue to the government as well as providing employment for the community as well as tobacco farmers. An alternative way out of the phenomenon as mentioned above, basically is not an easy and simple problem, because it will require a large amount of data and information regarding the attitude of the smokers towards government warnings. In addition, data and information on opportunity costs arising from the reduction of cigarette industry output are also needed, as well as public opinion about the government's social responsibility in tackling the tendency to consume cigarettes[2].

Demarketing policies of the government and the cigarette industry aim to reduce the level of cigarette consumption in the community. In accordance with Government Regulation No. 81 of 1999 concerning the Arrangement of Cigarette Billboards, and even permanent warnings are contained in every cigarette package. Government protection as a decision maker (dicision maker) in the form of regulations that affect the public interest, both the cigarette industry and the public. This policy product can meet social expectations and demands. Public policy is real action, not just what you want to do. Advertising arrangements aim to persuade people not to consume harmful products[3].

WHO (World Health Organization) data in 2015 shows that the prevalence of active smokers in the world currently reaches 17.4%. Indonesia ranks first in Asia based on the number of smokers over the age of 15 years with the prevalence of 38.5% of Indonesia's population smoking, namely 73.3% of the male population and 3.8% of the female population. The National Basic Health Research (Riskesdas) data in 2018 shows that the prevalence of Indonesian smokers in the population aged 10-18 years continues to increase, namely in the Riskesdas data in 2013 of 7.2%, 2016 National Circular Survey data of 8.8% and in the data Riskesdas in 2018 increased to 9.1%. The Riskesdas data in 2018 explained that the prevalence of smokers every day and sometimes age ≥ 10 years in Central Sulawesi was 31.2%. The number of smokers in Central Sulawesi exceeds the National Prevalence of 28.8%.

Data from the Palu City Health Office revealed that smokers under the age of 20 (<20) in the last three years continued to increase, namely in 2015 as many as 153 children smoked, in 2016 there were 315 children smoked, and in 2017 there were 1,073 children smoking. Data from the Palu City Health Office further explained that of the 13 Puskesmas in Palu City, the highest number of smokers under the age of 20 (<20) was found in Talise Health Center with a total of 748 smokers smoking, then at the PuskesmasBirobuli with the number of smokers as many as 101 children smoked, as well as Mamboro Health Center with the number of smokers as many as 49 children smoked.

Seeing the problem above, the researcher was interested in conducting research on how a model of cigarette advertisement policy in preventing children smoking habits in Palu City, Indonesia?. The purpose of this systematic review is to develop a model of cigarette advertisement policy in preventing children smoking habits in Palu City, Indonesia.

Method

This research uses literature review. Literature sources of this review are generally obtained by searching through the internet in various national and international journals, and e-books. Generally the journal is indexed by Google Scholar, Scopus, Elsevier, Science Direct, and others. Generally the literature collected is in accordance with the themes raised and published in the last 10 years and some longer journals and textbooks for additional literary needs. About 100 articles and using English are reviewed. The cigarette advertisement policy is the main guide, and children smoking habits are in addition. The articles were filtered and found as many as 50 articles in accordance with the purpose of the study. The keywords used are cigarette advertisement policy and children smoking habits.

Results

This study showed that the use of a model of cigarette advertisement policy is very effective in preventing children smoking habits. Cigarette advertisement regulation policy is the government's commitment to protect children, as an effort to protect the younger

generation from cigarette advertisements that are intense and have a very large influence on smoking behavior. Cigarette advertisements are proven to encourage children to try cigarettes, repeating the behavior so that it eventually becomes a habit.

Cigarette advertisements as a promotional medium for cigarettes and various types are very potential to shape the attitudes and behavior of teenage smoking. Knowledge about cigarettes is obtained through cigarette advertisements, both the latest types of cigarettes and the dangers of smoking itself.

Most children like cigarette advertisements on TV and when they see advertisements on TV, there is a feeling to try the cigarette brand in the ad. Sometimes cigarette advertisements do not encourage enthusiasm to smoke and smoke not because they see advertisements on TV or in print media, and the artists they idolize never star in. Sometimes smoking is not because seeing an ad on TV or in print and cigarette advertising does not make the spirit to smoke. And sometimes like cigarette advertisements on TV and when seeing advertisements on TV, there is a feeling to try the cigarette brand in the ad, and the idol artist has starred in cigarette advertisements.

Children are affected by cigarette advertisements on TV, such as buying interest to try the cigarette brands displayed on these advertisements and affect the child's mindset of smoking because cigarette advertisements give a positive impression that makes children more confident, so this shows that children are exposed to advertisements cigarettes will have the opportunity to behave more smoking than respondents who are not exposed to cigarette advertisements.

Cigarette advertisements are packaged as attractive as possible by raising the theme of friendship, friendship and togetherness. Cigarette advertisements are made very attractively and creatively touching the psychological side that shows the image of brave, macho, trendy, cool, togetherness, relaxed, optimistic, male, full of adventure, creative, critical and various other things that boast and represent the conscience of young people and adolescents . This shows that it effectively affects children to behave in smoking.

Advertising is a very effective promotional media in shaping public opinion in the field of cigarettes, therefore almost all cigarette entrepreneurs can spend large amounts of funds for advertising purposes each year, experts at WHO (World Health Organization) state cigarette advertising can stimulate someone to start smoking, can inhibit smokers who want to quit smoking or reduce their cigarettes, can stimulate smokers to smoke more, and motivate smokers to choose certain brands of cigarettes.

The onslaught of cigarette advertisements in electronic media (audiovisual) has a big influence, especially for novice smokers who are generally children. Cigarette advertising and sponsorship is a comprehensive strategy undertaken by the cigarette industry to influence children and adolescents.

Seeing advertisements in the mass media and electronics that show that smokers are a symbol of virility or glamor make children often triggered to follow the behavior of such advertisements. The cigarette industry can enter people's lives by becoming the main sponsor of various sports shows on television, organizing music events on various campuses and schools that attract the attention of many children and adolescents who are one of the objects of cigarette industry advertising targets, offering scholarships for high achieving students. Cigarette advertisements usually contain scenes that present natural beauty, fitness, success. Though smoking itself can cause pollution that pollutes the environment and damage health.

Discussions

There are very few studies that assess the equity of tobacco control interventions or policies affecting young people. The most consistent price or tax increases have a positive equity impact. There is a need to strengthen the basic evidence for the impact of tobacco equity on intervened youth groups[4].

The impact assessment of one of the tobacco control policies recommended by WHO is contained in the MPOWER term (Monitor tobacco use and prevention policies; Protect people from tobacco smoke; Offer help to stop using cigarettes; warn about the dangers of tobacco; enforce advertising bans, promote tobacco and sponsor sponsorship), at least one interesting result in children aged 0-12 years. The main outcomes were: perinatal death, premature birth, hospital asthma and respiratory infections in the hospital[5].

Weekly smoker experiments, plain packaging increases visual attention to health warnings from cigarette branding. Daily smokers, even relatively early

smokers, seem to avoid health warnings on cigarette packs. Adolescents who have never smoked are aware of special health warnings on the type of cigarette pack, a finding that might reflect their decision not to smoke[6].

Exposure to electronic cigarette advertising reduces children's perception of the dangers of smoking. An updated meta-analysis of three studies with 1935 children found that exposure to various types of e-cigarette advertisements (glamorous, healthy, flavorful, not scented) had a lower danger than smoking one or two tobacco cigarettes[7].

The high school environment, targeted advertising makes black people more promotions and lower prices for leading menthol cigarette brands. This evidence contradicts the manufacturer's claim that the availability of promotions is not based on race or ethnicity. It also highlights the need for tobacco control policies that will limit gaps in retail marketing exposure to cigarettes[8].

Higher cigarette prices will reduce the smoking prevalence rate of black, Hispanic, female subpopulations faster than the overall youth population and other youth subpopulations. In addition, this study concludes that a smoke-free air law will reduce the prevalence of smoking for the youth population as a whole with the greatest reduction in the male subpopulation[9].

Awareness of health risks and support for a smoke-free spiritual policy is very high in Ghana. Exposure to tobacco advertisements or promotions is limited and most smokers try to quit. Whether this finding is the cause or effect of the current low prevalence of smoking is still uncertain[10]. The most comprehensive review of motivational interviews to stop smoking is done to date. These findings indicate that the motivational interview approach to stop smoking now can be effective for adolescents and adults. However, useful comparative efficacy tests need to be carried out[11].

Implementation of important steps to reduce WHO FCTC demand is significantly associated with lower smoking prevalence, with a reduction in tobacco-related morbidity and mortality in the future. These findings validate the call for strong implementation of the WHO FCTC in the WHO Global Action Plan for Prevention and Control of Non-Communicable Diseases 2013-2020, and in advancing United Nations Sustainability to Development Goal 3, setting global targets for reducing tobacco use and premature death from non-communicable diseases to one-third in 2030[12].

About one in ten high school students are smokers, one in three smokers smoke more than once per day, and a quarter of nonsmokers ever smoke in China. Smoking rates are higher among students from the Tujia nation than Han. This study provides some important information for future tobacco control programs between secondary school students in ethnic minority autonomous regions and minority settlements in multi-ethnic countries[13].

Very low levels of educational attainment by fathers, smoking more and also consuming drugs, low academic achievement, having permission from parents to smoke, and attending public schools are all related to increasing the higher risk of smoking in the school environment[14]. Siblings and friends who smoke can have an important influence on children's cognitive vulnerability at the age of 9-10 years to smoke. While some differential findings based on gender are observed, this may not be enough to guarantee separate preventive interventions. However, further research is needed[15].

Having a smoker in the family affects not only the children's intentions and attitudes towards smoking, but also the effectiveness of smoking prevention education. Especially it is difficult to increase "intention not to smoke as children", "intention to refuse cigarettes when offered", "attitude away from smokers", and "desire that their families not smoke". Smoking prevention education programs become less effective, in situations that mentioned in raising awareness and attitudes to smoking among elementary and junior high school students[16].

Productivity losses due to premature death and illness caused by smoking are estimated at $ 2.84 billion and $ 941 million respectively. The findings support a strong need for tobacco control and prevention programs to reduce the health and economic burden caused by smoking in Alabama. This result is used by state health workers to describe the true costs of smoking in Alabama and to advocate for improved tobacco control policies[17].

Conclusions

Based on the results of this study, it can be concluded that a model using cigarette advertisement policy is effective for prevent children smoking habits. Further recommendation will be provided.

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A Transtheoretical Model in Controlling Smoking Behavior in Junior High School Students in Palu City, Indonesia: A Systematic Review

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Abstract

Objective: The average child first tries and is accustomed to smoking when sitting in seventh and eighth grade junior high school (SMP) or around the age of 12-13 years. In TTM Model (Transtheoretical Model), there are 5 stages of TTM. The purpose of this systematic review is to develop a model of transtheoretical in controlling smoking behavior in junior high school students in Palu City, Indonesia.

Method: This research used a systematic literature review. Various references were collected from online database including reports, journals, and books in the last 10 years. The journals were mostly from the scholarly journals. The articles were screened according to the research objectives. The keywords used are transtheoretical model and smoking behavior in junior high school students.

Results: This study showed that the use of a transtheoretical model is very effective in controlling smoking behavior in junior high school students. There are 5 stages of TTM, namely pre-contemplation (contemplation to stop smoking), contemplation (contemplation to stop smoking in the next 6 months), preparation (contemplation to stop smoking in the next 30 days), action (already stopped smoking in 6 months), and maintenance.

Conclusions: This paper concludes that a model using transtheoretical is effective for control smoking behavior in junior high school students. Further recommendation will be provided.

Keywords: *Transtheoretical Model; Smoking; Students.*

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Introduction

Smoking behavior has become a culture in the Indonesian nation. Teenagers, adults, even children are familiar with these deadly objects.Very massive exposure to cigarettes can negatively affect the quality of life of 83.4 million Indonesian children. There is already plenty of evidence that shows that children are targeted

by the cigarette industry to make them loyal customers in the future. In addition to influencing health, the impact of cigarette consumption can also erode the economy and cause social impacts.

The school environment is considered important to be intervened because the school setting is considered more efficient in carrying out actions, very good for developing emotional, academic, cognitive, and other behaviors that support health. The average child first tries and is accustomed to smoking when sitting in seventh and eighth grade junior high school (SMP) or around the age of 12-13 years. Due to including early adolescents, they try smoking because of high curiosity, as well as environmental influences and peers who are very strong in their age. This shows that child smokers are increasingly caused by regulations or policies that are still obtuse.

In TTM Model (Transtheoretical Model), there are 5 stages of TTM, namely pre-contemplation (not thinking to stop smoking), contemplation (thinking to stop smoking in the next 6 months), preparation (thinking to stop smoking within the next 30 days), action (has stopped smoking in 6 months), and maintenance (has stopped smoking for more than 6 months)[1].

WHO (World Health Organization) data in 2015 shows that the prevalence of active smokers in the world currently reaches 17.4%. The National Basic Health Research (Riskesdas) data in 2018 shows that the prevalence of Indonesian smokers in the population aged 10-18 years continues to increase, namely in the Riskesdas data in 2013 of 7.2%, 2016 National Circular Survey data of 8.8% and in the data Riskesdas in 2018 increased to 9.1%. The data presented shows that there is an increase in the prevalence of smokers aged ≥ 10 years in Central Sulawesi, by 30.7% with the number of smokers every day at 26.2% and smokers sometimes at 4.5%.

Data related to age starting smoking every day for ages 10-14 years, the city of Palu ranks third largest in Central Sulawesi at 13.5%. Many factors cause the interest of junior high school students to buy and consume cigarettes in Palu City.

Seeing the problem above, the researcher was interested in conducting research on how a transtheoretical model in controlling smoking behavior in junior high school students in Palu City, Indonesia?. The purpose of this systematic review is to develop a model of transtheoreticalin controlling smoking behavior in junior high school students in Palu City, Indonesia.

Method

This research uses literature review. Literature sources of this review are generally obtained by searching through the internet in various national and international journals, and e-books. Generally the journal is indexed by Google Scholar, Scopus, Elsevier, Science Direct, and others. Generally the literature collected is in accordance with the themes raised and published in the last 10 years and some longer journals and textbooks for additional literary needs. About 150 articles and using English are reviewed. The transtheoretical model is the main guide, and smoking behavior in junior high school students are in addition. The articles were filtered and found as many as 100 articles in accordance with the purpose of the study. The keywords used are transtheoretical model and smoking behavior in junior high school students.

Results

This study showed that the use of a transtheoretical model is very effective in controlling smoking behavior in junior high school students. The research on motivation and method for stopping smoking is based on the concept of the Transtheoretical Model (TTM) developed by Prochaska & Diclemente. There are 5 stages of TTM, namely pre-contemplation (contemplation to stop smoking), contemplation (contemplation to stop smoking in the next 6 months), preparation (contemplation to stop smoking in the next 30 days), action (already stopped smoking in 6 months), and maintenance.

The process of changing smoking cessation can be adapted through six stages of behavior changethrough a transteoritic model, which is a theory that ratesindividual readiness to act or behave healthily, and make a strategy or process of changeto help individuals through the stages of changeto the action and maintenance stage. According to this theory,individuals who are most likely to succeed in changing behavior are individuals who conduct business based on strategies that are appropriate to the readiness stage to replace. The basic theory of the transteoritic model isbehavior change is a process andeveryone is at a different level related to motivation and readiness to change. With regard to smoking behavior, this model identifies five stages of preparedness can be applied to all types of behavior change.

First, precontemplation can be defined as a state of individuals who do not have the desire to change behavior. Most individuals are onthis stage is not even aware that they have behavior problem. Thus, the individual is in stages this is very difficult to be motivated to change behavior. For example, a smoker refuses to put an end to smoking because he feels he does not have the smoking behavior problem that he maintainsso they don't have thoughts or considerations to stop smoking.

Second, contemplation is the condition of a person conscious or start thinking about the existence of a problem of behavior that is maintained, but not yet made commitment to action. At this stage, a smoker starts to think of a time when he has to end smoking behavior due to various negative effects of cigarettes began to be felt because they have not been able to make a commitmenttowards smoking behavior, they will look for the moment and the right conditions to motivate to stop smoke. The process of change that occurs at this stage includes consciousness raising, which is the condition of the individual who discover and learn new facts, ideas and tips that support change towards healthy behavior; dramatic relief, which is feeling negative feelings, such as fear or anxiety about the risk of implementationunhealthy behavior; environmental reevaluation, namely being aware of the negative influence of that behavior unhealthy or positive influence of healthy behavior in the environment around individuals; and self-reevaluation, namely realizing that behavior change is important as part of self identity.

Third, preparation, which is the stage when individuals intend to change behavior in the near future. At the stage, this smoker is ready to quit. Process of change what happens at this stage is self-liberation, the individual make a strong commitment to change. In some cases, smokers start to make plans to stop such as determining when to stop or reducing the amount of cigarette use. Some individuals begin to think of strategies that were carried out on her day must stop.

Fourth, action, as a stage when individuals begin change his behavior to resolve the problem. The act of changing behavior and the factors that support it requires a commitment totime and energy. Most individuals will prove themselves that he is able to put an end to smoking because they have prepared themselves with strategies to deal with the urge to smoke again. Smokers have taken action to quit and are still within the first six months of the smoke-free period. This stage involves several behavioral change processes, namely contingency management, namely increasing appreciation for new healthy behaviors and reduce appreciation towards unhealthy behavior; helping relationships, that is, seeking and using social support for healthy behavior change; counterconditioning, i.e. replace unhealthy thoughts and behavior with alternative behaviors that support change behavior; and stimulus control, which is to throw reminders that can lead individuals to get involved in unhealthy behavior and added a reminder that leads to healthy behavior.

Fifth, maintenance, which is the stage when individuals keep changing behavior from possible relapse (returning to behavior that has been abandoned). Smokers compare the benefits they have gained from stopping smoking with a desire to return to smoking. The process of behavior change at this stage is the same with the action stage. If someone is able to remain free of addictive behavior for more than six months, people it is assumed to have been in the maintenance phase of stopping smoking. Similarly, someone who has stopped smoking but is still on the first year of his smoking-free period.

The sixth, termination, is the last stage which can be applied to addictive behavior. On at this stage, unhealthy behavior will never be back and the individual has no fear relapse. Even though the individual feels depressed, anxious, bored, lonely, angry, or stressed, they are sure that they will not return to behavior old unhealthy as a solution problem. Relapse or recurrence is more a rule of addictive behavior, so the theoretical model describes the stages of change behavior.

Discussions

The study results will provide valuable information regarding how each predictor variable is connected and path through adoption of smoking cessation behavior. By understanding mechanisms of behavior change, health professionals could develop effective smoking cessation interventions with consideration of roles of self-efficacy in the smoking cessation behavior change[2].

The students in the experimental group were given Transtheoretical Model-based planned education, and students in the control group were not given any education. As a result of last tests of the experimental and the control groups, it was observed that there was a statistically significant difference between measurements of "Social interaction and habit strength" (p=0.003),

which is a subscale of it and "Temptation scale total score" (p=0.004), "Being able to cope with the social environments and negative sense (p=0.03), "Being able to cope with the habit strength" (p=0.001), which are subscales of "Self-efficacy scale", "Processes of change scale" total score, and its subscales: "Conscious raising" (p=0.006), "Dramatic relief" (p=0.001), "Environmental reevaluation" (p=0.035), "Self-reevaluation" (p=0.007)[3].

As the change stages progressed, the scores of Decisional Balance sub scale Coping Pros and mean scores of the subscales Habit Strength and Weight Control of Self-Efficacy Scale scores increased in a statistically significant (p<.01)[4].This program has the potential to be applied as stand-alone practice or as part of more intensive interventions to promote substance use cessation[5].

Evidence for the validity of the TTM as it applies to tobacco use is strong and growing; however, it is not conclusive. Eight different staging mechanisms were identified, raising the question of which are most valid and reliable. Interventions tailored to a smoker's stage were successful more often than nontailored interventions in promoting forward stage movement. Stage distribution is well-documented for U.S. populations; however, more research is needed for non-U.S. populations, for special populations, and on other TTM constructs[6].

A TTM-based intervention programme was potentially effective in passive smoking prevention by improving knowledge, experiential and behavioural processes and self-efficacy among pregnant women and women with young children. A higher percentage of mothers with young children had progressed in stages of change post-intervention compared with pregnant women[7].

Given the relative ease of dissemination, these programs could be applied as stand-alone practices or as part of more intensive interventions[8].In Chinese high school students suggest tailored health education and practical interventions should be adopted to reduce sedentary time and thereby improve the efficacy of health promotion[9].

The results provide support to the advantages of increased self-efficacy and the role of decisional balance for the intervention program development for smoking cessation[10].This smoking cessation program increased self-esteem, attitude toward smoking, perceived control over smoking and decreased smoking per day among male high school students. Therefore, schools and parents should focus on developing these factors to encourage students to quit smoking[11].

Nearlytwo-thirdsof smokers in Turkey and more thantwo-thirds in Thailand were in the precontemplation stage during the last survey wave assessed. The proportion of smokers in the preparation stage increased in Turkey but declined in Thailand. Identifying stages of cessation helps guidepopulation-basedtargeted interventions to support smokers at varying stages of change toward quitting[12].

The program was effective in smoking cessation and influencing stages of change but did not change psychosocial factors such as self-efficacy and stress. It is suggested a program should be developed to change psychosocial variables on a long-term basis. It is also desirable to involve peers and families of adolescents who smoke when planning programs to enhance social support[13].

Furthermore, the results showed that measures of self-efficacy decreased, while temptation increased from precontemplation to action stages. The pros of smoking behavior increased toward action stage, while the cons decreased[14].A higher overall prevalence of adolescents in precontemplation stage for smoking cessation was observed. Such group displayed more unfavorable smoking-related behaviors and characteristics than adolescents in other stages. The evidence is limited due to the lack of studies in adolescents living in low-income countries[15].

Transtheoretic model is a significant tool for smoking cessation with its ability to use different models of behavior changes. This flexibilitaly of Transtheoretic model makes the model treatment of choice in different addictions. In this review we focus on the features of Transtheoretic model[16].

Conclusions

Based on the results of this study, it can be concluded that a model using transtheoreticalis effective for control smoking behavior in junior high school students. Further recommendation will be provided.

Ethical Clearance: No ethical approval is needed.

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Conflict of Interest: Nil

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Development and Standardization of Capsular Pharmaceutical Dosage form from Dry Extract of Ferula Tenuisecta

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Abstract

The tradition of herbal medication by plants and products of natural origin in the treatment of various diseases exists thousands of years. Currently, despite the achievements of chemistry and biotechnology, the relevance and popularity of the herbal medicine is quite high. The therapeutic effect peculiarity of the medications from herbal medicinal raw materials is that the therapeutic effect does not occur immediately and is not always obvious, as in case of medications producted by chemical synthesis. However, medications which contain biologically active substances (BAS) of plant origin, unlike synthetic ones, do not cause allergies, are low-toxic, have a favorable effect on the body, and do not have side effects during the continuous use.

Keywords: *Capsule, average, herbal medicine, extract, ferula.*

Introduction

Ferulatenuisecta (lat. *Fērulatenuisēcta*) is popular in folk medicine of Central Asia, the medicinal raw material of which is the milky juice of roots, gums and resin[1]. It acts as a antifatigue, antibacterial, anti-inflammatory, antifatigue and antitumor medication. One can use ferula both internally and externally. One takes an infusion for internal use. Ferula can be used for internal use in order to debride a wound, and it is also can be used to lubricate wounds, tumors, boils, and trophic ulcers. It is possible to make compresses by a warmed bandage on the sore spot.

The Institute of the Chemistry of Plant Substances named acad. S. Yu. Yunusov of the Academy of Sciences of the Republic of Uzbekistan has developed a technology for the sum determination of terpene compounds and then the production of a dry extract. We found the estrogenic activity within the screening study of extracts, sum and separate compounds of Ferulatenuisecta.

Materials and Method

In order to make Summary in respect to the composition and technology of capsules of Ferulatenuisecta dry extract, we put under study the main physical, chemical and technological properties of the substance samples . These properties are interrelated and can influence the process of high-quality capsules production with the necessary therapeutic effect in a certain way. The substance-powder of Ferulatenuisecta dry extract and the encapsulated mass were subject to physical, chemical and technological research. From the technological properties of the dry extract, the fractional composition, porosity, flowability, tap density, angle of natural slope, compressibility, compression ratio and residual moisture were under study.

The research findings of dry extract technological properties showed unsatisfactory values of flowability and angle of natural slope, which will obviously have a negative impact on the filling process of capsules, so we had the task to improve these indicators by the optimal selection of the excipients and technological process.

Table 1: The results of the technological properties study in relation to dry extract of Ferulatenuisecta

№	The indicators under study	UOM	Results
1.	**Fractional Composition:** +1000 -1000 + 500 - 500 + 250 - 250 + 150 - 150 +125 - 125	µm, %	 2.2 20.1 45.3 12.4 11.6 8.4
3.	Tap density	kg/m^3	452,00±2,07
4.	Flowability	10^{-3} kg/s	0,55±0,83
5.	Angle of natural slope	degree	72.00
6.	Compressibility	H	70,0±5,0
7.	Residual Moisture (70^0C)	%	5.00

In case of capsules development[2], where dry extract is the main substance (which, as a rule, does not have a satisfactory value of the flowability index), one can use the method of pre-wet granulation in order to select the optimal technology, as well as to improve the technological indicators. As for the completeness and objectivity of the paper, we decided to select the optimal composition and technology of the capsular pharmaceutical dosage form.

As the principal vehicle, we selected microcrystalline cellulose (MCC)[3] of the following brands, "MCC-101" and "MCC-102" these MCC brands are most popular in the production of medicines among domestic pharmaceutical enterprises. MCC-102 is superior to MCC-101 by its technological parameters, which makes it possible to use it for tableting by direct compression, but from an economic perspective, the price of MCC-102 is 50% higher than MCC-101. Also, potato and corn starches were used as vehicle, both of these excipients also have leavening property, which in turn can improve the flowability index[4]. As excipients we also used: lactose monohydrate (as a moisture-absorbing and adsorbing agent), aerosil[5] (as a fluidity-enhancing and adsorbing agent), talc, magnesium stearate, calcium stearate (as antifriction substances that improve the flowability).

In addition, we used the following excipients as for the pre-wet granulation method: ethyl alcohol (various concentrations), starch paste (various concentrations) and purified water.

After that, we made a number of experiments on the composition selection with the subsequent preparation of various masses in order to fill the capsules. The following table shows the most optimal compositions of encapsulated masses in terms of both technological indicators and the highest economic profitability.

Table 2: The compositions of capsules selected with the use of various excipients

Name of medications and excipients	Quantity of medications and excipients, mg				
	1	**2**	**3**	**4**	**5**
Dry extract	40	40	40	40	40
MCC-102	295				
MCC-101		295	200	195	198
Corn starch			155		155
Potato starch	60	60			
Lactose				155	
Talc				5	2
Aerosil	2	2	2	2	2
Magnesium stearate	3	3	3	3	3
Average weight	400	400	400	400	400

In the selection of the encapsulated mass various compositions and their subsequent preparation, we have consistently studied the technological properties obtained by both dry blending and pre-granulation method. The research results of the encapsulated mass technological properties you can find in table 3.

We used purified water; ethyl alcohol of 40, 70, 96% concentrations; 3, 5, 10% starch paste as moistening agent. We obtained granulated masses with each binding agent. The results of the experiments showed that the most optimal binding agent was 5% starch paste.

Table 3: The research results of the encapsulated masses technological properties obtained with various excipients

№	Verifiable indicators	Units of Measure	Compositions				
			1	2	3	4	5
1	Fractional composition, µm						
	+1000	%	-	-	-	-	-
	-1000 +500		0.60	0.49	8.20	6.20	6.40
	-500 +250		3.40	3.51	12.10	9.55	9.55
	-250 +150		10.15	9.45	12.75	18.46	18.7
	-150 +125		65.45	72.14	54.25	47.24	47.0
	-125		20.4	14.41	12.7	18.55	18.35
2	Flow ability	10^{-3} kg/s	6,4±1,1	3,8±0,2	2,6±0,1	2,8±0,2	6,7±0,2
3	Angle of natural slope	degree.	40	58	60	65	45
4	Tap density	kg/m^3	560±0,8	520±0,8	720±1,8	638±3,1	670±2,1
5	Residual moisture (70°C)	%	4	3.5	4-5	4-5	3

The production of granules by pre-granulation worked in accordance with the following method: the substance and excipients separately passed through a sieve with a hole diameter of 150 µm. We weigh the desired amount, after we mix the substance and excipients for 40 minutes, after we granulate them by wet granulation in the desired amount of starch paste as a binding agent. We line the resulted wet mass by a thin layer on a baking tray with parchment paper and place it in exsiccator, we dry it at a temperature of 60-70°C up to the optimal relative humidity of the mass (3.6-3.0%). We pass the dried mass through a sieve with a hole diameter of 1000 µm by pressing, thus we form granules. We powder the resulted prepared granules with magnesium stearate, talc and aerosil pre-sifted through a sieve with a hole size of 125 µm. The resulted mass goes to the capsule filling process.

On the basis of technological properties research results, we have proposed the most optimal composition of capsules with Ferulatenuisecta dry extract:

Dry extract	– 40 mg
MCC-101	– 198 mg
Corn starch	– 155 mg
Talc	– 2 mg
Aerosil	– 2 mg
Magnesium stearate	– 3 mg
Average weight	**– 400 mg**

The next stage of our research was to study the quality indicators of capsules with Ferulatenuisecta dry extract and encapsulated mass by the method given in the literature.

Description: Hard gelatine capsules of bright red color, with volume №1. The encapsulated mass in the form of granules with adelomorphous shape, of brown color.

Identification: The ultraviolet spectrum of the solution obtained for quantitation within the range from 230 nm to 290 nm has a maximum absorption at (260±2) nm.

Shake 0.4 g of the powder poured from the capsules with 8 ml of glacial acetic acid for 5 minutes and filter it through a paper filter (GOST 12026-76). Add 0.2 ml of vanillin solution to sulfuric acid to the filtrate, and a blue-green staining appears (a sesquiterpene ferutinin residue).

Weighing of the average mass. In order to weigh the average mass, we weigh 20 unopened capsules together and determine the average weight of the capsule. Then weigh each capsule separately and compare it with the average weight. The deviation of the each capsule mass should not exceed±10% of the average mass. Then carefully open the same 20 capsules, remove the contents

as completely as possible and weigh each shell. For soft capsules with liquid or pasty contents, we wash the shell with ether or other suitable solvent before weighing with the following air removal of the solvent. Determine the average mass of the capsule contents. Unless there are no any specified instructions in the individual monographs, the mass deviation of each capsule contents from the average mass should not exceed±10%, with the exception of two capsules, in which a deviation is possible of up to±25. If more than 2 capsules, but not more than 6 have deviations from the average weight within the range of 10 to 25%, then we determine the contents of each capsule and the average weight of the contents of 60 capsules with 40 capsules in addition. No more than six capsules out of 60 can have deviations from the average weight of more than±10% and there should not be any capsules that have a deviation in the mass of the contents of more than±25%. We use the contents of 20 or 60 capsules for quantitation of medicinal substances and other indicators under individual monographs.

Disintegration: One can determine it by a special laboratory identifier.[6] The device design consists of a "basket", a vial with a volume of 1 l (for liquid), a thermal device that maintains the temperature of the liquid within (37±2)°C, an Electromechanical device that supplies the basket with a reciprocating motion by a frequency of 28 to 32 cycles per 1 min.

The main part of the device consists of a rigid basket with a mesh bottom which supports six cylindrical glass tubes, whose length is (75.5 - 79.5) mm, internal diameter is (20.7-23) mm and wall thickness is (1.0 - 2.8) mm. The glass tubes locate vertically and two plastic plates hold them (at the top and bottom) The parameters of plates are: a diameter (88-92) mm, thickness (5-8.5) mm, it has six holes with diameter (22-26) mm. The holes are equidistant from the center of the plate and from each other. A square grid with cells of size (1,8 - 2,2) mm and a stainless steel wire of diameter (0,57 - 0,66) mm fixes to the lower plane of the plate. The rods hold the plates in vertical position along the entire circumference. Another rod fixes in the center of the plate upper plane, which allows you to attach the basket to a mechanical device. You can raise and lower the basket by it. The time of movement up and down should be the same.

We select 16-18 capsules for the test. One sample is in each tube. We lower the basket into a container with liquid and turn on the device. At the end of the specified time, we withdraw the basket. Examine the state of the samples. All the tested samples must completely disintegrate. If 1-2 capsules do not fall to pieces, we repeat the test with the remaining samples. At least 16 of the 18 selected samples must completely disintegrate.

One can consider that sample have passed the test if it leaves no residue, or a residue is in the form of a soft mass that falls in parts at the slightest touch of a glass stick.

The findings: the tested capsules completely disintegrate within 14 minutes.

Quantitation: We place about 0.4 g (precisely weighed amount) the powder poured from the capsules in a measuring flask with a capacity of 100 ml, add 80 ml of 96% alcohol and shake for 10 min., then bring the volume of the solution with the same solvent to the mark, filter it through a paper filter (blue tape), discarding the first 10 ml of filtrate. We transfer 1 ml of filtrate to a measuring flask with a capacity of 25 ml, bring the volume of the solution with 96% ethyl alcohol up to the mark and mix. We measure the optical density of the resulting solution by a spectrophotometer at a wavelength of 260 nm in a cuvette with a layer thickness of 10 mm. We use 96% ethyl alcohol as a reference solution.

At the same time, we measure the optical density of the ferulen work standard solution. We calculate the content of ferulen in one capsule, in grams (X) by the formula:

$$X = \frac{D_1 a_0 * 100 * 1 * 2 * b}{D_0 * a_1 * 100 * 1 * 50} = \frac{D_1 * a_0 * b}{D_0 * a_1 * 2}$$

Where:

D_0 is the optical density of the ferulene work standard solution;

D_1-optical density of the test solution;

a_0 - dose weight of the ferulen work standard, in grams;

a_1 - dose weight of the powder poured from the capsules, in grams;

b-the average weight of one capsule in grams.

The content of ferulen in one capsule should be from 0.036 g to 0.0044 g (in terms of feerutinin from 0.009 g to 0.011 g) with count of one capsule average weight.

Table 4: The quantitation metrological description of capsules with dry extract of Ferulatenuisecta

Series	Dose weight, g	Findings		Metrological description
		By dose weight, g	In one capsule, g	
011117	0.400	0.160	0.040	X_{avg} = 0,159 t(95%,4)=2,78 S^2 = 0,00000525 S = 0,00229±DX = 0,00636±DE = 4,006 P = 95
021117	0.405	0.158	0.039	
031117	0.410	0.156	0.038	
041117	0.41	0.162	0.039	
051117	0.40	0.160	0.040	

Note: Preparation of the solution with the work standard of ferulen. We place about 0.04 g (precisely weighed amount) of ferulene (in terms of ferutinin 0.01 g) in a 100 ml volumetric flask, add 80 ml of 96% alcohol and dissolve with continuous stirring. Then we bring the solution volume with the same solvent to the mark and mix. We transfer 3 ml of the resulted solution to a measuring flask with a capacity of 25 ml, bring the volume of the solution with 96% alcohol to the mark and mix. The solution must be freshly prepared.

Packaging: 10 capsules in a contour – cell package in accordance with GOST 64-074-91 (All-Union standard) from polyvinyl chloride film in accordance with GOST 25250-88 or imported and printed lacquered aluminum foil in accordance with TU 48-21-270-78 or imported or 10, 20, 30, 50 and 100 in polymer vials (banks) in accordance with GOST 64-15390981-02:2003 from low-pressure polyethylene in accordance with GOST 16338-85E or high-pressure polyethylene in accordance with GOST 16337-77, the vials must have labels made of label paper by GOST 7625-86e or writing paper by GOST 18510-87e.[7]

We place packs, contour cell packages by 1 [no. 10(1x10)], by 2 [no. 20(2x10)], by 3 [no. 30(3x10)] pieces together with the instructions for use in a pack (pencil case) of box cardboard in accordance with GOST 7933-89E or other imported and with packing lists on accordance with OST 64-7-382-84 placed in a box of box cardboard in accordance with GOST 8273-75 or bagged by GOST 2228-81E. The boxes or stops are pasted with a parcel made of wrapping paper by GOST 8273-75 or tied with cotton threads in accordance with GOST 6309-87 or twine made of bast fibers by GOST 17308-88, the ends of which are pasted with a label made of label paper in accordance with GOST 7625-86E or writing paper in accordance with GOST 18510-87E.

Table 5: The research results of technological properties in relation to encapsulated mass and quality indicators of capsules

№	Verifiable indicators	Units of Measure	Results
1	**Fractional composition, µm** +1000 -1000 +500 -500 +250 -250 +150 -150 +125 -125	%	- 6.40 9.55 18.7 47.0 18.35
2	Flowability	10^{-3}kg/s	6,7±0,2
3	Angle of natural slope	degree.	45
4	Tap density	kg/m^3	670±2,1
5	Residual Moisture (70°C)	%	3
6 7 8 9 10	Appearance Authenticity Deviation from the average weight of capsules Disintegration Quantitation (in terms of ferutinin)	g, % min. g	Red capsules with volume № 1 respectively 0,394±0,13 14 ± 2,32 0,009-0,011

The identification of stability and capsules shelf life. The purpose of stability investigation is to obtain data on changes over time in the quality of the medication under the influence of various environmental factors, such as temperature, humidity and light, as well as to establish the frequency of repeated studies of the active substance or the shelf life of the medication and recommended storage conditions[8].

The stability tests of the Pharmaceutical Dosage Form must be in the intended packaging for presentation on the market (including, if necessary, any secondary packaging and container labels).

The use of rational packaging[9] is the main way to prevent a decrease in the quality of encapsulated medications in storage. Therefore, the type selection of packaging and packaging materials is individual in each case in accordance with the physical and chemical properties of the substances within the capsules.

One of the most important requirements for packaging materials is the protection of capsules from light, atmospheric moisture, air oxygen, and microbial contamination. Today, we use the traditional packaging materials such as paper, cardboard, metal, and glass for capsule packaging. Along with the traditional materials, film packages made of cellophane, polyethylene, polystyrene, polypropylene, polyvinyl chloride and various combined films on the basis of them are popular. The most promising film contour packages, which are produced on the basis of combined materials by the method of thermal welding, are strip pack (tape) and cellular pack (blister).

In order to make tape packaging it is popular to use laminated cellophane tape in various combinations, aluminum foil, laminated paper, laminated polyester or nylon polymer film. One can make packaging by heat welding of two-placed materials. The special machines make such packs. As a thermoforming film, hard (non-plasticized) or weakly plasticized polyvinyl chloride (PVC) with a thickness of 0.2-0.35 mm or more is most popular for usage. PVC film is well formed and heat-bonded with various materials (foil, paper, cardboard, covered with a thermolak layer). The film covering by polyvinyl chloride or ethylene halogenation reduces gas and vapor permeability. For hygroscopic medicines, we recommend to use polypropylene, but it is more difficult to form. In addition, it is more tough than PVC. Polystyrene is also well formed, but is unpopular due to its high water permeability. In order to close the cells, aluminum foil is quite popular. On the inside, it is covered with glue or thermal adhesive film, on the outside-with varnish. Aluminum foil is impervious to water vapor and gases, well protects the medications from the fragrance permeation. The packaging, one layer of which is aluminum foil, provides high tightness.

The main function of containers, packaging and capping is to isolate a separate dosage (portion) of a medication not only from other dosages of the same medical product, but also from the effects of so-called environmental factors: light, atmospheric air pressure, atmospheric natural gases and vapors, as well as from the evaporation into the surrounding atmosphere of other medical products, chemicals and products that one stores in the same or in neighboring rooms. In other words, one determines the suitability of a package by four properties: protection, safety, compatibility, and performance (functioning and/or delivery of medicines). In case of the packaging/capping system assessment, we must consider the following factors: design materials of the packaging/capping device; surface treatment and/or technological additives; active ingredients and vehicles that are part of the Pharmaceutical Dosage Form; sterilization and/or other similar processes; finally, storage conditions.

Storage conditions have a great influence on the stability of medicinal substances within capsules and on their physical and chemical parameters.

An increase in air temperature and the effect of direct sunlight have a negative impact on the quality of capsules. Therefore, one stores the capsules at room temperature in a dry, protected from light place.

Stability tests should include studies of such characteristics in respect to the finished medicinal product that are subject to changes during storage and may reasonably affect quality, safety and/or efficacy. One should study physical, chemical, biological and microbiological properties (in accordance with the specific situation). Thus, the study of medical product stability is an additional source for development and improvement of the requirements that determine the quality of medical products.

One stores the hard gelatine capsules with a dry extract of the Ferulatenuisecta at room temperature (20±5)°C, humidity (60±5)%. These conditions correspond to the natural method of storage, the research duration is1 year with intermediate quality checks every 6 months the research continues. The findings show the value of the verifiable indicators within the normal range, and one can also note that the various containers and packaging materials selected for capsules storage ensure safe storage and stability of the medical product. Thus, we can recommend the packaging materials used in the experiments for further use.

Also, one of the most popular research method within the stability identification of medical products is the method of “accelerated ageing”. The essence of the method is to increase the storage temperature at the expense of a thermostat usage, and in the presence

of more advanced equipment that can also control humidity indicators and automate the process, one can achieve more accurate research results. In accordance with the experiments results, the optimal temperature for the capsules was 40°C, as at the storage temperature of 50–60°C, the quantitative indicators of the capsules showed some changes. The packaged capsules were put in a thermostat (40°C) and every 46 days (corresponding to 6 months of storage at room temperature) passed the quality indicators examination. As a result of the experiments, the shelf life of capsules with dry extract of Ferulatenuisecta was 184 days (on the basis of 2 year storage at room temperature), the results are in the table.

Table 6: The capsules shelf life identification by natural storage

Types of packaging GOST, OST, TU	Verifiable indicators	Primary results	Shelf life, months	
			6	12
1	2	3	4	5
Plastic bottles with screw cap (64-20-8780) brown glass vials (OST 64-2-71-80)	Appearance Average weight, g Disintegration, min. Authenticity Amount of ferutinin, g	Respectively 0.4010 14 Respectively 0.010	Respectively 0.4011 14.30 Respectively 0.010	Respectively 0.4012 15 Respectively 0.0098
Brown glass vials with plastic screw caps (64-287-81)	Appearance Average weight, g Disintegration, min. Authenticity Amount of ferutinin, g	Respectively 0.4012 14 Respectively 0.009	Respectively 0.4013 14.25 Respectively 0.009	Respectively 0.4015 14.50 Respectively 0.009
Contour-cell packaging materials (OST 64-744-6-81)	Appearance Average weight, g Disintegration, min. Authenticity Amount of ferutinin, g	Respectively 0.4011 14 Respectively 0.009	Respectively 0.4017 14.15 Respectively 0.009	Respectively 0.4019 14.30 Respectively 0.0087

Conclusions

One selected the optimal composition of the encapsulated mass and the subsequent study of the technological properties, the findings showed an improvement in the flowability and angle of natural slope after the addition of excipients and pre-wet granulation. Also, on the basis of the obtained optimal composition, the average weight of capsules was 0.4 g and the size of the most optimal gelatin capsule in accordance with the volume № 1.

There were complex experimental studies in respect to the main quality indicators of developed capsules in accordance with the State Pharmacopoeia of XI edition, notably: organoleptic properties, average mass, mass uniformity, disintegration, authenticity, quantitation. One determined the shelf life and stability of capsules by the method of "accelerated aging" and natural storage[10].

We selected the packaging and storage conditions for the received capsules, and the shelf life of the capsules was 2 years.

Ethical Clearance: No ethical approval is needed.

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Comparative Analysis of Human Kidney Venous Vessels at Various Method of Radiation Research

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Abstract

The aim of the study was to conduct a comparative assessment of the spatial characteristics of the kidney veins in a planar projection, in stereo projection, and according to the results of computed tomography after 3D modeling. To compare and identify the truth of the morphometric parameters of the renal veins, as well as the options for their formation during x-ray and computed tomography, 136 corrosive preparations of the kidney veins were made, which were taken as a control group. To identify the degree of similarity of the renal veins parameters on corrosion preparations, X-ray diffraction patterns (2D) and on computed tomograms (CT) with a helix pitch of 1.0 and 2.5 mm after 3D modeling, a comparative assessment of the obtained parameters was performed (degree of the angle similarity - simPhi; the degree of length similarity is simLen; and the general degree of similarity is sim). It was revealed that the greatest degree of parameters similarity was found between corrosive preparations and computed tomograms of kidney veins with a spiral pitch of 1.0 mm, which was 98.0%. Studies have shown that the informational content of x-ray examination of the kidneys' veins depends on the options for the formation and location of blood vessels in the gates of the kidney relative to the frontal, horizontal and sagittal planes. The informativeness of computed tomography studies does not depend on the options for the formation of renal veins, it determines the step of the spiral.

Keywords: *Kidney, renal veins, 3D - modeling.*

Introduction

Today in the literature there are single and conflicting data[2] on the morphometric indicators of human renal venous vessels[13]. The ambiguity of this information[9] depends on various methodological approaches used[6] in the study of these vessels[4]. Thus, morphometric indicators[3] obtained in the study of autopsy material do not always correspond to the data of radiation diagnostics[14]. There are frequent discrepancies in the data of X-ray diffraction patterns[5] with anatomical preparations[15]; in addition, there have been cases of the absence of certain sections of the intraorgan kidney veins[11] on the X-ray diffraction patterns[16].

Today, the clinic uses computed tomography[21] to study the vascular bed of the kidney[20]. However, this method does not always allow obtaining the exact parameters of blood vessels, since after 3D reconstruction[19] their true sizes may be lost[18], and sometimes some anatomical details important for diagnosis are missing or artificially formed structures[14] sometimes appear[17].

Materials and Method

We have made 136 corrosive preparations of the kidneys' venous channel of people in the age range from 22 to 90 years who died from diseases not related to kidney pathology. As the mass for pouring, Styracryl, Protacryl + Kraplak was used to simulate a radiopaque substance. To compare and identify the truth of the morphometric parameters of the renal veins, as well as the options for their formation during x-ray and computed tomography, the corrosion preparations of the kidney veins were taken as a control group[4].

Research Algorithm:

1. On corrosion preparations, 5 of the most common renal vein formation variants were identified.
2. Corrosive preparations of the kidneys veins were

subjected to digital radiography on an apparatus Clinodigit Compact X - FRAME, (Italy). The current strength was 200 mA, the voltage was 65 kV, the exposure was 300 seconds, and the focal length was 120 cm.

3. Corrosion preparations of the kidneys veins were subjected to computed tomography scanning using the Light Speed VCT apparatus (Germany). The current strength was 132 mAs, the voltage was 140 kV, the spiral pitch was 1.0 and 2.5 mm, followed by 3D modeling.

4. Corrosion preparations of the venous vessels of the kidneys, their lengths and diameters were measured on corrosion preparations of kidney veins, X-ray angiograms and computed tomograms.

5. To identify the degree of similarity of the renal veins parameters on corrosion preparations, X-ray angiograms (2D) and on computed tomograms (CT) with a helix pitch of 1.0 and 2.5 mm after 3D modeling, a comparative assessment of the obtained parameters was performed (degree of similarity of the angle - simPhi; degree of similarity of length - simLen; and general degree of similarity - sim).

The degree of similarity of corresponding vectors' pairs was revealed. Each category of the studied parameters was associated with a characteristic vector. We used the cosine measure of vectors proximity[1]. For this, they began to determine the function $simPhi(V_i, V_j)$ according to the formula 1.

$$simPhi(V_i, V_j) = \frac{\arccos(cos\theta)}{\frac{\pi}{2}} * 100\% \quad (1)$$

Further, the function $simPhi(V_i, V_j)$ between corresponding vectors V_i и V_j uses the cosine of an angle.

The cosine of the angle, knowing the corresponding coordinates of the vectors, was revealed through the scalar product according to the formula 2.

$$cos\theta = \frac{\vec{V_i} \cdot \vec{V_j}}{|\vec{V_i}| \cdot |\vec{V_j}|} \quad (2)$$

the scalar product was calculated by the formula 3:

$\vec{V_i} \cdot \vec{V_j} = \sum_{k=0}^{N} V_i^k * V_j^k$, где N – was the rank of the corresponding vector (3)

$|\vec{V_i}|$ и $|\vec{V_j}|$- the lengths of the corresponding vectors V_i и V_j.

We determined them according to the formula 4:

$$|\vec{V_i}| = \sqrt{\sum_{k=0}^{N} V_i^k * V_i^k} \quad (4)$$

As a result, simPhi was revealed - this is the degree of similarity of the angles of venous vessels. Identification of the correspondence and similarity degree of the venous vessels parameters by angle was insufficient. For this, an additional measure of correspondence and similarity of vectors along the length of venous vessels was introduced, i.e., a function $simLen(V_i, V_j)$ was revealed according to the formula 5:

$$simLen(V_i, V_j) = \frac{\min(|\vec{V_i}|, |\vec{V_j}|)}{\max(|\vec{V_i}|, |\vec{V_j}|)} * 100\% \quad (5)$$

Further, to reveal a comprehensive measure of correspondence and similarity of the two studied vectors, the formula 6 was used.

$$sim(V_i, V_j) = simPhi(V_i, V_j) * simLen(V_i, V_j) \quad (6)$$

6. All digital material obtained, and the data of instrumental research method were processed using variation statistics method using a workstation with an Intel Core2Duo T5250 processor 1.5 GHz, RAM up to 2 GB on a Windows 7 platform. In the course of work, the Excel application package from *Microsoft Office 2007*.

Results

It was revealed that in 32.4% of cases, the renal vein is formed from the upper and lower pole veins. The average diameter of the renal vein was 26.2±1.3 mm. The fusion angle of the upper and lower pole veins was on average 21.2±1°. The length of the superior pole vein was on average 18.2±0.9 mm, and that of the inferior pole vein was 27.2±1.3 mm. According to the analysis of X-ray angiograms from the same corrosion preparations of the kidney veins, the average diameter of the renal vein did not differ from the parameters obtained on the corrosion preparations of the kidney and amounted to 26.4±1.3 mm. The average angle of confluence of the upper and lower pole veins was less than on corrosive preparations and amounted to 20.2±1°. The length of the superior pole vein averaged 17.2±0.8 mm, and the length of the inferior pole vein 26.4±1.3 mm. According to the analysis of computer tomograms (spiral pitch 1.0 mm with 3D modeling), the average diameter of the renal vein was 26.3±1.3 mm, not significantly different from the data on corrosion preparations. The average fusion angle of the upper and lower pole veins also did not differ

from the data obtained by morphometry of corrosive preparations and amounted to 20.1±1°. The average length of the superior pole vein did not differ from the data on corrosive preparations and amounted to 18.3±0.9 mm. However, analysis of computed tomograms (2.5 mm pitch) showed significant differences in average values. So, the average diameter of the renal vein was significantly different and was 28.2±1.4 mm. The fusion angle of the upper and lower pole veins on average significantly differed from the parameters obtained on corrosion preparations and amounted to 16.7±1°. The average length of the superior pole vein was 16.4±0.8 mm, and that of the inferior pole vein was 24.3±1.2 mm, which was less than with corrosive preparations.

A comparative analysis of renal vein morphometry data on radiographs and computed tomograms with data on corrosion preparations showed that the similarity degree of the parameters was: simPhi - (3D, 2D) - 98,2%, simPhi - (3D, KT1) - 98,5%, simPhi - (3D, KT2,5) - 89,9%, simLen - (3D, 2D) - 97,8%, simLen - (3D, KT1) - 99,3%, simLen - (3D, KT2,5) - 96,5%, sim - (3D, 2D) - 96,0%, sim - (3D, KT1) - 97,8%, sim - (3D, KT2,5) - 86,8%.

In 25.4% of cases, renal veins are formed from the ventral and dorsal veins. It was revealed that the average diameter of the renal vein is 10.8±0.5 mm. The average fusion angle of the ventral and dorsal veins was 27.2±1.3°. The length of the ventral vein averaged 33.2±1.6 mm, and the dorsal vein 39.1±1.9 mm. Analysis of x-ray diffraction patterns showed that in 14% of cases, the shadow of the ventral vein in the frontal projection was visualized as an upper pole, and the dorsal as a lower pole (orthogonal principle of the depth of location). In 26% of cases, the shadows of both veins were projected onto each other, giving out the projection of a single trunk (orthogonal principle of competing lines). Morphometric analysis of computed tomograms (spiral pitch 1.0 mm) did not reveal significant differences from indicators obtained on corrosive preparations of kidney veins. However, computer tomograms (spiral pitch 2.5 mm) revealed that the average diameter of the renal vein did not differ significantly from the data of corrosive preparations and was 13.2±0.6 mm. The fusion angle of the ventral and dorsal veins differed and averaged 26.5±1.3°. The average length of the ventral vein was 26.2±1.3 mm, and that of the dorsal vein was 23.4±1.1 mm, which was significantly less than on corrosion preparations.

When comparing morphometry data, the degree of similarity was: simPhi - (3D, 2D) - 84.2%, simPhi - (3D, CT1) - 99.7%, simPhi - (3D, CT2.5) - 84.9%, simLen - (3D, 2D) - 84.0%, simLen - (3D, KT1) - 99.9%, simLen - (3D, KT2.5) - 79.7%, sim - (3D, 2D) - 70, 8%, sim - (3D, KT1.5) - 99.7%, sim - (3D, KT2.5) - 67.7%.

In 8.4% of cases, the renal vein is formed from the superior pole, inferior pole and dorsal central veins. The value of the diameter of the renal vein averaged 9.1±1.0 mm. The fusion angle of the upper and lower pole veins averaged 18.1±0.9°. The confluence angle of the upper pole and dorsal central veins was 29.3±1.4°. The fusion angle of the dorsal central and lower pole veins averaged 28.1±1.4°. The length of the superior pole vein was on average 21.1±0.1 mm, while the diameter was 8.4±0.4 mm. The length of the dorsal central vein averaged 12.8±0.6 mm, and the average value of its diameter was 5.5±0.2 mm. The length of the inferior pole vein averaged 14.8±0.7 mm, while its diameter averaged 8.1±0.4 mm.

Analysis of x-ray diffraction patterns showed that in 18% of cases, the shadow of the dorsal central vein was visualized as the central one (orthogonal principle of the depth of location), located in the same plane with the rest of the veins. The average diameter of the renal vein was 9.4±0.4 mm. The fusion angle of the upper and lower pole veins averaged 17.2±0.8°. The fusion angle of the upper pole and dorsal central veins was on average 13.2±0.6°, and the dorsal central and lower pole veins 14.1±0.6°. The length of the superior pole vein was, on average, 20.2±1.0 mm, and its diameter 8.7±0.4 mm, which did not significantly differ from that on corrosion preparations. The length of the dorsal central vein averaged 9.3±0.4 mm, and its average diameter was 5.8±0.2 mm. The length of the inferior pole vein was on average 13.1±0.6 mm, and its average diameter was 8.5±0.4 mm.

According to the analysis of computed tomograms (spiral pitch 1.0 mm), it was revealed that the average values of their morphometry data did not differ significantly from those on corrosive preparations. However, analysis of computed tomograms from the same corrosive preparations (2.5 mm pitch) showed significant differences in morphometric parameters and the appearance of non-existent parts. So, in 6.2% of cases, a central vein and insufficient visualization of interlobar and arc veins were visualized on computed tomograms. The diameter of the renal vein was on average 11.3±0.5

mm. The fusion angle of the upper and lower pole veins was on average 17.1±0.8°. The confluence angle of the upper pole and dorsal central veins was 27.3±1.3°. The fusion angle of the dorsal central and lower pole veins averaged 26.4±1.3°, which was significantly different from morphometry data on corrosion preparations. On average, the length of the superior pole vein was less than on corrosive preparations and was 19.4±0.9 mm, and its diameter was 10.2±0.5 mm. The length of the dorsal central vein was also less and amounted to 10.4±0.5 mm, while its diameter was on average equal to 7.1±0.3 mm. The length of the inferior pole vein was on average 12.3±0.6 mm, while its average diameter was 10.3±0.5 mm. A comparative analysis of morphometry data showed that the degree of similarity was: simPhi - (3D, 2D) - 79.1%, simPhi - (3D, CT1) - 99.8%, simPhi - (3D, CT2.5) - 95.5 %, simLen - (3D, 2D) - 70.2%, simLen - (3D, KT1) - 99.7%, simLen - (3D, KT2.5) - 93.0%, sim - (3D, 2D) - 55.5%, sim - (3D, KT1) - 99.6%, sim - (3D, KT2.5) - 88.8%.

In 4.2% of cases, renal veins are formed from the ventral, dorsal and lower pole veins. In 13.0% of cases on X-ray diffraction patterns from the same corrosive preparations, the ventral veins were visualized as upper pole, dorsal veins as central (the principle of location depth). On x-ray angiograms, these veins were falsely projected in the frontal plane in the form of an upper pole, central and lower pole vein. In 16.0% of cases, when the veins are located in the same projection, the shadows are subtracted, and the ventral and dorsal veins overlap each other, which creates the false impression of a single venous trunk (the principle of competing lines).

Discussion

Based on the researches, it was established that certain options appear on the radiograph[10], which is consistent with the opinion of a authors' number[16]. According to the results of our research, variants were observed, as a rule, where the shadows of the kidneys veins were projected in the same geometric plane[11]. In axonometry, they are called competing lines, giving a single shadow in one plane[16]. If a separate image element (a section of a kidney vein) was an object of perception, then options in a planar projection were visualized relative to the depth of the venous vessels[7]. On radiographs of corrosive preparations of the veins of the kidney, the true parameters (angles of fusion of the veins, lengths of the kidneys veins and their diameters) were distorted. Analysis of computed tomograms with a helix pitch of 1.0 and 2.5 mm, followed by 3D modeling, revealed possible distortions of the spatial organization of the venous vessels of the kidneys, which depend on the helix pitch (changes in diameters, angles of vascular fusion). According to the study, after 3D - modeling, computed tomograms do not contain some important anatomical structures for diagnosis, and sometimes artificially formed parts appear in the form of additional (non-existent) venous vessels[4], which we met in 28.6% of cases and is consistent with data from several authors[10].

Conclusion

A comparative assessment of the spatial characteristics of the kidney veins in planar projection, in stereo projection, and according to the results of computed tomography with increments of 1.0 and 2.5 mm after 3D modeling showed that the highest degree of the parameters similarity was found between corrosion preparations and computed tomograms of kidney veins with a spiral pitch 1.0 mm, which was equal to 98.0%.

Studies have shown that the informational content of x-ray examination of the kidneys veins depends on the options for the formation and location of blood vessels in the gates of the kidney relative to the frontal, horizontal and sagittal planes. The informativeness of computed tomography studies does not depend on the options for the formation of renal veins, it determines the step of the spiral.

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Specifics of Left Ventricular Remodeling and Daily Blood Pressure Profiles in Young and Middle-Aged Servicemembers Dealing with Arterial Hypertension

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Abstract

Objective: Investigating structural and geometrical parameters, specifics of LV remodeling, as well as daily BP profiles in young and middle-aged service members dealing with hypertension Stage 1 and Stage 2. A group of 86 male subjects has been researched, including 52 young (26-44 y/o) and 34 middle-aged (45-55 y/o) subjects with AH. The cardiac function has been evaluated through echo cardiography, and daily BP monitoring has been carried out. The ***results*** of the study have shown that young and middle-aged servicemen with AH have a normal LV geometry (59.6% of young subjects (31), 55.9% of middle-aged subjects (19)), and concentric LV remodeling has been observed in 40.4% of young subjects (21) and in 22.1% of middle-aged (15) subjects with AH. According to data obtained during 24-hr BPM,as compared to the normal LV geometry group, in the concentric LV remodeling groups the occurrence of the dipper AH profile has decreased both in young (28.5% vs. 39%) and middle-aged (40% vs. 47.7%) subjects.

Keywords: *Arterial hypertension; echo cardiography, left ventricular remodeling, 24-hr arterial blood pressure monitoring.*

Introduction

Arterial hypertension (AH) is a leading mortality and disability risk factor among CVDs, being far ahead of other risk factors. It has been confirmed by many authors, as they note that in 2015, AH was the most common cause of premature death, killing 10 million and disabling more than 200 million people (5).

An important morphological and functional change, which characterizes thetarget organ's state, is left ventricular (LV) myocardial remodeling. This and other changes consist in LVH (left ventricular hypertrophy), which results from structural and functional modification of the heart, which adapts to increased hemodynamic load and neurohumoral activation.

Echo cardiography of the heart and vessels is still the most advanced non-invasive methodof diagnosing left ventricular remodeling. The diagnostics plays an important role in evaluation of left ventricular myocardium mass, which is an important life expectancy prognostic factor for AH and other patients [Mancia, G.]. The most common method used to classify types of LV remodeling in AH patients is the A[1]. Ganau classification (1992), according to the 2013 ESC/ESH Guidelines, which relies on LVMMI and left ventricular wall thickness[6]. A gradual increase in the LV wall thickness and progressing concentric LV hypertrophy lead to an increased risk of cardiovascular complications[12].

AH in service members attracts closer attention, because high blood pressure (BP) can affect contract servicemen's ability to properly carry out their professional duties and therefore undermine national security and defense capability[8].

Materials and Method

A group of 86 male AH patients, who are service members, have been examined. Group 1 comprises 52 young servicemen aged 26 to 44 y/o (median age – 36.25±5.1 y/o); Group 2 consists of 34 middle-aged servicemen with AH aged 45 to 55 y/o. median age of the group is 47.5±2.6 y/o. Age distribution was made

according to the WHO classification pattern, which deems the age of 18 through 44 as young and the age of 45 through 59 - as middle age. Also, the subjects were divided into subgroups according to the type of LV remodeling.

Male subjects aged 20 through 60 years old and suffering arterial hypertension Stage 1 and Stage 2 have had all inclusion criteria[2]. Non-inclusion criteria are: female gender, old and senile age, non-sinusoid heartbeat, symptomatic AH, diabetes type 1 and/or type 2, IHD, malignant neoplasms, cardiac insufficiency, acquired or inborn cardiac defects, hepatic, renal and/or respiratory insufficiency.

The structural/functional state of the heart has been evaluated through echocardiography with the use of a Neusoft ultrasound system (made in China) in the B- and M modes while relying on the standard method. Left ventricular mass (LVM) has been calculated according to a cubic formula recommended by ASE experts (2015): LVM (g) = 0.8 × (1.04 × ((LV EDD + 3CLV + IVS)^3 – (LV EDD)^3)) + 0.6. [11]. The LVM (LVMMI) index has been calculated as a ratio between LVMMI and TBSA (total body surface area)[4]. LV geometry has been evaluated as recommended by experts from the American Society of Echocardiography Association and from the European Association of Cardiovascular Imaging, as the method help calculate LVMMI and relative left ventricular myocardial thickness (RLVMT, unit = (2x3C LV)/(LV EDD)).

Based on LVMMI and RLVMTIvalues, the following LV remodeling types have been specified: normal geometry LV remodeling (NGLVR): LVMMI is within the normal range with TBSA less than 0.42; concentric LV remodeling (CLVR): LVMMI is within the normal range with TBSA more than 0.42; concentric LV hypertrophy (CLVH): LVMMI exceeding the normal value with TBSA more than 0,42; eccentric LV hypertrophy (ELVH): LVMMI exceeding the normal value with TBSA less than 0.42 [9].

The 24-hr blood pressure monitoring procedure has been conducted with the help of a Shiller BP-102 system and according to the generally accepted method, and it has continued for 24±1.5 hours on average. Based on nighttime BP decreases, four daily BP profiles have been specified: *dippers* (DI>10%<20%), *non-dippers* (DI<10%), *over-dippers* (DI>20%) and *night-peakers* (DI<0).

Data obtained during research has been processed for statistical purposes on a Pentium-4 computer with the help of the Microsoft office Excel-2012 package and integrated statistical processing functions. Parametric and non-parametric variation statistics method have been used with calculation of the mean indicator being studied (M), standard deviation, relative values (frequency, %), statistical significance of measured values, with mean values compared, has been measuredthrough the use of Student's-t test with calculation of error probability (P).

Results and Discussion

The analysis of the study has shown that young and middle-aged servicemen with AH had normal LV geometry and concentric LV remodeling. Particularly, 59.6% of young subjects (31) and 55.9% of middle-aged subjects (19) with AH have had normal LV geometry, while 40.4% of young subjects (21) and 44.1% of middle-aged subjects (15) with AH have had concentric LV remodeling. No cases of eccentric or concentric LV hypertrophy have been revealed.

Meanwhile, LVM in young subjects with AH and normal LV geometry is 151.2±10.1g, in young subjects with concentric LV remodeling – 165.7g±11.4g ($P<0.01$), and LVMMI is 73.5±5.6g/$m^2$and 80.2±6.1g/m^2respectively ($P<0.05$).

Middle-aged AH patients with normal LV geometry have shown a LVM of 154.7±9.9 g, those with concentric LV remodeling – 166.6g±11.3g ($P<0.01$).

Middle-aged subjects with concentric LV remodeling have demonstrated the highest left ventricular posterior wall thickness (LVPWT) – 1.18±0.06cm, in young subjects, LVPET has been 1.11cm±0.03cm ($P<0.05$). Young and middle-aged subjects with normal LV geometry have shown a LVPWT of 1.08±0.05cmand 1.09cm±0.04cm respectively.

As to inter ventricular septum thickness (IVST), geometry changes have not caused any increase; on the contrary, a slight increase has been observed in patients with normal LV geometry: respectively, the normal LV geometry and concentric LV geometry young-age groups have had an IVST of 1.04±0.06cm and 1.02±0,07cm. In middle-aged subjects, it has been 1.07±0.0cm and 1.05±0.07cm respectively. The insignificant increase in these characteristics in subjects with normal LV geometry may be due to the septum's anatomic features,

as it has a convex shape, and depends on what part of the septum these indicators refer to.

Thus, LVM, LVMMI, and LVPWT tend to increase in patients with LV remodeling. There is a direct correlation between left ventricular mass and occurrence of cardiovascular events[15].

Concurrently, ejection fraction (EF), which reflects cardiac contractility, has been similar in all study groups (P>0.05) (Table 1).

Table 1: Echo cardiography test results in young and middle-aged service members diagnosed with AH

Indicators	Young		Middle-aged	
	Normal LVG(31)	Concentric LVR (21)	Normal LVG(19)	Concentric LVR(15)
EDD, cm	4.69±0.22	4.6±0.16	4.7±0.19	4.67±0.21
ESD, cm	2.89±0.24	4.03±5.3	3.03±0.23	2.9±0.2
EDV, ml	100.8±20.89	97.6±8.35	108.4±10.1	104.1±24.4
ESV, ml	33.45±6.47	32±5.2	37.2±6.5	32.6±5.4
SV, ml	69.4±7.5	65.5±7.8	70.0±6.08	71.5±23.7
EF%	67.5±4.4	66.6±4.9	65.4±4.08	66.1±4.2
IVST, cm	1.03±0.06	1.02±0.07	1.07±0.05	1.05±0.07
LVPWT, cm	1.08±0.05	1.11±0.03	1.09±0.04	1.18±0.06**•
LVM, g	151.2±10.1	165.7±11.4**	154.7±11.9	166.6±10.3**
LVMMI, g/m^2	73.5±5.6	80.2±6.1	76.03±7.4	84.42±10.5
RWT, units	0.39±0.022	0.44±0.01	0.39±0.021	0.44±0.021

Note: * P<0.05, ** P<.01 differences between the subgroups, • P<0.05, •• P<0.01 differences between Group 1 and Group 2.

Concentric remodeling (normal LVM with an increased relative wall thickness) is characterized by peripheral vascular resistance, low cardiac index and high arterial stiffness[14]. Concentric LV remodeling is believed to be the earliest stage of the pathologic process that precedes left ventricular hypertension[13].

The results justify the necessity of early AH diagnostics and administration of strict and effective anti-hypertension therapy to servicemen. In turn, these measures help stabilize the left ventricle's state and stop the progress of the disease in patients dealing with the process of LV remodeling.

Table 2: 24-hr blood pressure monitoring indicators in young and middle-aged servicemen with AH

BP (mmHg)	Young		Middle-aged	
	Normal LVG (31)	Concentric LVR (21)	Normal LVG(19)	Concentric LVR(15)
SBP, daytime	152.4±6.1	160.9±5.95**	153.3±5.4	165.9±5.4**
DBP,daytime	85.1±5.12	92.8±5.9**	86.0±5.58	93.2±4.47**
SBP, nighttime	132.9±6.2	147.8±8.5**	139.3±3.04	149.4±8.3***
DBP,nighttime	77.12±5.2	85.7±6.4**	79.8±4.5	88.5±6.4**
SBP, 24-hr	142.5±8.3	155.1±8.8**	148.5±7.9	157.7±4.4***
DBP, 24-hr	83.4±8.4	89.5±8.1	84.3±4.5	87.4±5.9
Mean pulse pressure	66.4±3.5	78.4±4.9**	66.6±6.9	77.87±5.33**

Note: * P<0.05, ** P<0.01 are differences between the groups

Hemodynamics indicators (SBP, DBP, MAP, HR) have been studied in all the groups, and the results are shown in Table 2. BP checks in service members dealing with AH, based on the type of LV remodeling, have helped reveal the following specific features: all groups of AH subjects with concentric LV remodeling have shown an increase in SBP, DBP, MAP, and HR (P<0.01) (except 24-hr HR in the middle-age group relative to AH with normal LV geometry). These indicators were relevant in the young age group (P<0.01).

Also, the analysis of SBP, DBP and mean hemodynamic pressure in the middle-age group of servicemen has revealed a transition from normal LV geometry to concentric LV geometry, but the results have not shown any relevant differences. Based on the above, it is possible to track the relationship between the indicators and the LV geometry; i. e., as these indicators grow, the LV geometry shifts toward remodeling, which is a primary precursor to hypertrophy in itself.

24-hr blood pressure monitoring of servicemen with AHis necessary not only for analysis of prognostically important indicators and daily BP profiles, but also for adequate AH control, because it gravely affects military capacity.

Table 3: Distribution of AH patients according to daily BP profile types

24-hr BP monitoring	Young		Middle-aged	
	Normal LVG (31)	Concentric LVR (21)	Normal LVG(19)	Concentric LVR (15)
Dipper	12 Patients – 39 %	6 Patients – 28.5%	9 Patients – 47.4%	6 Patients – 40%
Non-dipper	18 Patients – 58%	13 Patients – 62%	9 Patients – 47.4%	9 Patients – 60%
OVer-dipper	1 Patient – 3 %	0	1 Patient – 5.2%	0
Night-peaker	0	2 Patients – 9.5%		0

It has been observed that compared to subjects with normal LV geometry, concentric LV remodeling groups, both young (28.5% vs. 39%) and middle-aged (40% vs. 47.7%), have shown a lower occurrence of the *dipper* profile. And vice versa, the occurrence of the *non-dipper* profile has increased in subjects with concentric LV remodeling in both the middle-age (62% vs. 58%) and young (60% vs. 47.7%) groups. Finally, 9.5% of young subjects with concentric LV remodeling have had the *night-peaker* profile (see Table 3).

The results of the study show that a shift from the normal LV geometry changes the daily AH profile, increasing in the number of *non-dippers* and generating *night-peaker* profile cases[7]. In turn, these indicators lay ground for unfavorable prognoses, because they are associated with cardiovascular complications (myocardial infarction, CVA, cardiac insufficiency)[3].

Thus, early diagnostics of AH and effective treatment of servicemen can help keep the LV in a healthy state. However, absence of concentric and eccentric hypertrophy signifies an appropriate pharmacological control of AH indicators in service members. It is known that most scientists consider insufficient lowering of BP during sleep and its dramatic increase in the morning to be an independent risk factor of left ventricular hypertrophy and consequently, cardiovascular complications.

That is exactly why it is important to evaluate nighttime BP levels. Daily BP profile changes accompanied by increased *non-dipper* profile occurrence should be regarded as unfavorable factors, which should also be taken into account when administering a drug therapy. The goal of the treatment, which is part of arterial pressure control therapy, is reversal of the remodeled left ventricular myocardium in this group of patients to reduce the risk of cardiovascular complications.

Conclusion

1. Ithasbeenfoundthatsomeyoungandmiddle-agedservicemenwith AHhaveanormalLVgeometry (59.6% (31) ofyoungand 55.9% (19) ofmiddle-agedsubjects), andconcentricLVremodelinghasbeenobservedin 40.4% (21) of youngand 44.1% (15) of middle-aged subjects.

2.ComparedtothenormalLVgeometrygroup, inc on

centric LV remodeling groups the occurrence of the *dipper* AH profile has decreased both in young (28.5% vs. 39%) and middle-aged (40% vs. 47.7%) subjects.

3. The occurrence of the *non-dipper* AH profile has increased in middle-aged subjects with concentric LV remodeling (62% vs. 58%), as well as in young subjects (60% vs. 47.7%). In 9.5% of the subjects with concentric LV remodeling, the *night-peaker* profile has been observed.

4. 24-hr blood pressure monitoring has revealed at rend of increasing BP along with a change in LV geometry from normal tore modeling.

Ethical Clearance: No ethical approval is needed.

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Features of Pain Syndrome of Patients with Brucellosis if Damaged Nervous System

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Abstract

The article presents the features of the clinical course of chronic brucellosis at the present stage, the issues of diagnostics and treatment, taking into account the results of our own observations of patients with damage of the nervous system and musculoskeletal system. There is characteristic of clinical and epidemiological, laboratory and instrumental diagnostics of chronic brucellosis, the issues of etiopathogenetic therapy are considered.

Keywords: *Brucellosis, diagnostics, treatment, Milgamma.*

Introduction

The steady increase of the brucellosis incidence is becoming a global problem in the world[1]. Brucellosis is a particularly dangerous and socially significant infection, that causes significant economic damage and a high level of disability of patients[3]. Disorders from the nervous system in chronic brucellosis (CB) are one of the most common, and lesions of both the central nervous system are detected, the clinic of which fits into the syndromes of arachnoiditis, meningoencephalitis, myelitis, and the peripheral department in the form of polyneuropathies, radiculopathies, plexitis, neuritis[6].

At the present stage of treatment of patients, three directions are distinguished: specific treatment with brucellin, pathogenetic and symptomatic therapy[4]. Currently, the most attention is paid to the issues of rehabilitation[7] treatment, which is the most promising direction in the treatment of patients with peripheral form of chronic brucellosis[8].

The aim of the study is to improve the pain treatment with damage of the nervous system of patients with brucellosis[4].

Materials and Research Method

Under our supervision, there were 32 patients complaining of pain, which are: women - 85%, men - 15%. The age division is: from 24 to 30 years - 11.5%; from 30 to 40 years old - 19.2%; from 40 to 50 years old - 15.4%; from 50 to 60 years old - 38.5%; older than 60 years - 15.4%, the age of patients - from 39 to 84 years (53.7±2.3). The duration of the disease ranged from 0 to 52 years: 51.6%, patients were ill for up to 5 years, 19.4% of patients - 5-10 years, 10-15 years - 9.6%, 19.4%.

All patients were divided into two groups: the main - 24 patients who received traditional treatment with the addition of Milgamma and the control group - who received only traditional treatment.

Clinical and epidemiological examination with specific laboratory diagnostics (enzyme-linked immunosorbent assay with antibodies to brucellosis pathogens (Br. Abortus, Br. Melitensis), Haddlson plate agglutination test, Rites agglutination test in test tubes, indirect hemagglutination reaction with brucellosis diagnostic, Coombs antiglobulin reaction, Burne skin-allergic test). From additional method, clinical, biochemical and instrumental (radiography of the joints and spine) studies were carried out. The dynamics of pain perception was evaluated on a 10-point visual analogue scale (ВАШ) before and after drug administration.

Results and Discussion

In the primary epidemiological diagnosis of brucellosis in most patients (61.5%), the professional nature of the disease with a contact path of infection was

established: 38.5% were workers at the meat processing plant who received specific prophylaxis irregularly, with long interruptions, and also did not always use personal protective equipment (gloves), often noted scratches, cuts, bruises in the processing of raw meat. Veterinarians accounted for 7.6%, who are infected by contact with sick animals (cattle, pigs). 11.4% were milkmaids, possibly infected while caring for cattle, and were also in contact with sick animals, their excreta. In 3.7% of the patients, a presumably mixed (aerogenic and alimentary) route of infection was observed (they were engaged in shearing sheep, where there were cases of brucellosis among animals, as well as raw milk and dairy products were consumed). In 37.5% of patients, an alimentary route of infection was observed: eating unboiled milk and raw dairy products: feta cheese, cream, sour cream, etc.

As a result of a clinical examination, taking into account the timing of infection and the epidemiological history, we found chronic brucellosis with a combined lesion of the osteoarticular and nervous systems in 65.3% of patients, and a primary lesion of the osteoarticular system in 35.6%. The changes are identified during radiography of the joints, which indicating a deeply advanced process: deforming arthrosis of the knee joints - in 46.1%; deforming arthrosis of the hip joints - in 33.6%; humeroscapular periarthrosis - in 27.7%; deforming arthrosis of the wrist joints and joints of the hands - in 30.5%; deforming arthrosis of the interphalangeal and metatarsophalangeal joints of the feet - in 11.5%; degenerative-dystrophic changes in the spine (osteochondrosis, subchondral osteosclerosis, deforming spondylosis, uncovertebral arthrosis) - in 80.2% of patients (Fig. 1).

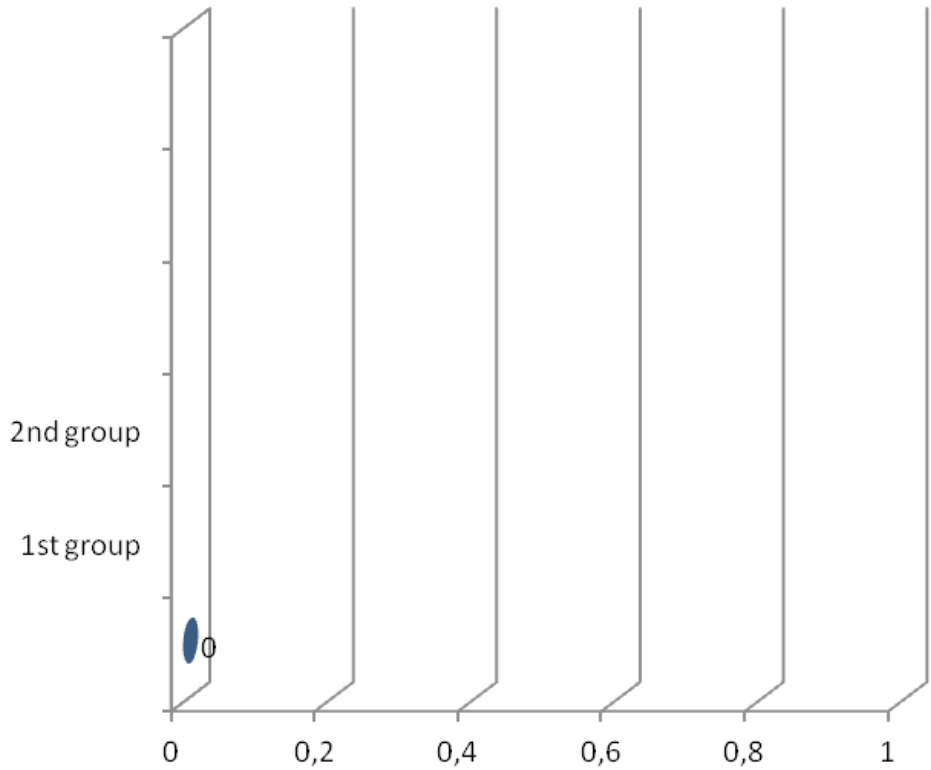


Fig. 1. Changes identified during joint radiography

The most common and characteristic for brucellosis is the defeat of the sacroiliac joints - sacroileitis, which is rare in rheumatism and other infectious diseases, which allows the patient to suspect a brucellosis process[7]. Changes in the sacroiliac joints rarely attract the attention of practitioners in connection with the presence of concomitant symptoms from the muscles, sacral and sciatic nerves, therefore, sciatic nerve neuritis or osteochondrosis of the lumbosacral spine with radicular syndrome is more often diagnosed.

Lesions of the soft skeleton in most cases manifest as inflammatory nodules[9] in the connective tissue, the so-called fibrositis, pathognomonic for brucellosis, especially for primary chronic forms[4]. They can form in the form of seals of various sizes and soreness everywhere, where there is connective tissue: in subcutaneous fat, fascia, aponeurosis, periosteum, surrounded by joints, nerve trunks and roots, they may later dissolve or remain in the form of scars[9].

The development of the clinic of modern brucellosis was reflected in the features of damage of the nervous system[1]. Lesions of the peripheral nervous system became softer, and the central nervous system became rarer or not observed at all[10]. So, in chronic brucellosis (8.7%), neurological syndromes often occur with a variety of diffuse symptoms, complex types of disorders of sensitivity and motor sphere (for example, the type of brain tumor, multiple sclerosis, stem encephalitis, etc.); the most common symptoms of a functional nature in the form of neuralgia (35.6%)[4]. In patients with chronic brucellosis observed by us, lumbar ischalgia, neurodystrophic form, cervicalgia, sensorimotor polyneuropathies and other disorders were recorded in 30.5% of cases.

The clinical and epidemiological diagnosis was confirmed by the results of specific laboratory diagnostics: by IFA in 46.2% of patients, antibodies to the causative agents of brucellosis (Br. Abortus, Br. Melitensis) of the IgG and IgM classes were determined, in 57.7% - by the positive Hedlson agglutination test. In 46.1% of patients, a positive Rite agglutination reaction was determined in the titer from 1/200 to 1/800; as well as in 34.6% of patients, positive results were simultaneously observed both in IFA and in the agglutination reaction of Rite and Hedlson. It is important that the Byrne allergic skin test in all patients (100%) with chronic brucellosis was positive.

The revealed "spread" of the frequency of positive serological reactions in chronic brucellosis is natural and indicates the need to use at least two method[4]. The coincidence of positive IFA results with Rite agglutination reaction was found in 46.0% of cases, with indirect hemagglutination reaction - in 37.0%; with the Coombs antiglobulin reaction - in 100%[11]. The Burne test refers to tests of the third level of diagnosis to detect the sensitization of the body to brucella, its degree reflects the final stage of the interaction of the antigen and the immune system[9].

The tactics of treating patients with chronic brucellosis is determined by the phase and form of the disease and the state of the reactivity of the body, which confirmed by the results of clinical, laboratory and instrumental examination. The issue of prescribing antibacterial therapy, which is not indicated for chronic inactive and residual brucellosis, as well as for the prevention of relapse, remains controversial in real practice. Indications for the appointment of antibiotic therapy are not sufficient only positive serological tests and allergy tests, and most importantly - a clinical exacerbation of chronic brucellosis infection. We used etiotropic therapy for the treatment of patients with chronic brucellosis during exacerbations: tetracycline antibiotics (iv videoccin or doxycycline per os) - in 77.9%; aminoglycosides (gentamicin) - in 56.7%; III generation cephalosporins (ceftriaxone) - in 23.7% of cases.

But still, the leading principle in the treatment of chronic brucellosis is pathogenetic using non-steroidal anti-inflammatory drugs[4] (diclofenac, ortofen, etc.), given the persistence of the inflammatory process, desensitizing agents (mainly groups of mast cell membrane stabilizers - ketotifen, azelastine, lodoxamide, etc.) as well as metabolic correction preparations (B vitamins, almiba, nicotinic acid, pentoxifylline, cytoflavin, reamberin, remaxol, etc.)[2], taking into account oxidation-reduction disorders that correlate with the degree of adjustment allergic organism [11].

During a recurring course of the disease, as well as with a chronic process, immunotropic therapy is indicated, taking into account the degree of immunological rearrangement of the patient's body with brucellosis. The appointment of immunocorrective therapy is considered necessary 1-3 weeks after taking antibiotics[11]. It is recommended to use immunocorrectors of various groups: immunomodulators of endogenous origin (immunoregulatory peptides obtained from central organs of the immune system (thymus and bone marrow), cytokines, interferons, immunomodulators of exogenous origin (pyrogenal, zymosan, prodiogisan, lycopid, bacterial lysates), synthetic immunomodulators (levamisole, pentoxyl, methyluracil, immunofan), cycloferon, an interferon inducer[12].

Patients were divided into 2 groups: in the main group (22 patients), milgamma was prescribed in combination with the above traditional treatment, in the control group (10 patients) only traditional therapy

was performed. The effectiveness of treatment was evaluated on the 10th day and 24th day on the visual analogue scale, as well as after 3 and 6 months (Fig. 2). Throughout the study, according to the visual analogue scale, there was a tendency to a higher efficiency of the milgamma combination in relation to traditional treatment, however, this difference reached the level of statistical significance only by the 24th day. Using the neuropathic pain scale, it was shown that only against the background of combination therapy, such characteristics of pain as intensity and severity significantly decreased, pain tolerance improved. A significant and moderate effect against the background of the introduction of milgamma was observed in 66% of patients, in the control group - in 34% ($p < 0.05$). 3 months later pain was absent or was minimal in 63% of patients in the main group and in 50% of patients in the control group ($p < 0.05$). The results obtained indicate that the complex of B vitamins can potentiate the analgesic effect of non-steroidal anti-inflammatory drugs and contribute to a more rapid and persistent regression of pain.

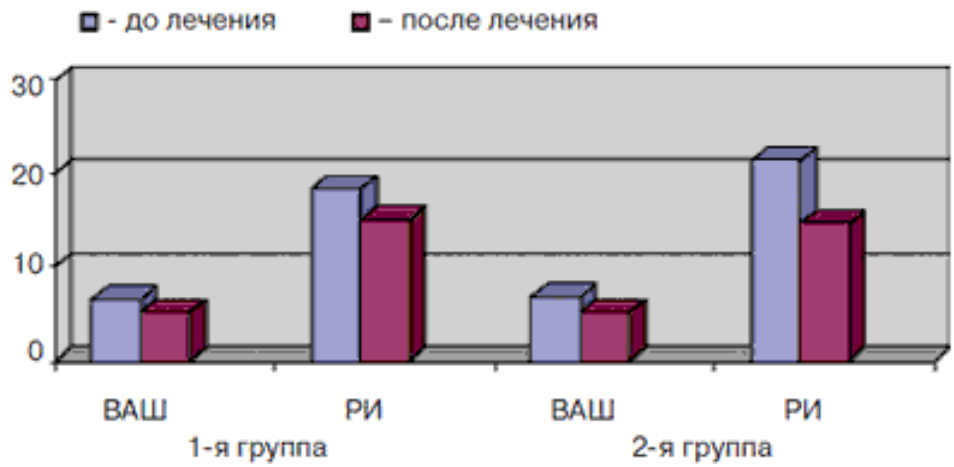


Fig. 2. Dynamics of pain according to VAS before and after treatment

According to the general clinical impression scale against the background of the use of combination therapy, including milgamum, on the 10th day a significant effect was noted in 6 patients (25% of the patients who entered the study), satisfactory (moderate - in 19 (41%), minimal improvement - 8 (17%), no improvement - 7 (17%). In the control group, a significant effect was observed in 2 patients (10%), satisfactory - in 4 (24%), minimal improvement - in 9 (41%), lack of effect - in 5 (25%). The effectiveness of therapy, initial intensity and length flaccidity, the presence of symptoms of loss, the severity of vertebral syndrome were not affected by the age.

Conclusions. Thus, the diagnosis and treatment of chronic brucellosis in the practice of a neurologist is a difficult task and is determined by the incidence rate in the region, both among animals and among people, the professional readiness of practical doctors and the availability of diagnostic technologies. At the same time, it should be taken into account the epidemiological history, the predominance of latent and primary chronic forms of low activity with damage to the joints and nervous system (with characteristic polyofocal), autonomic reactions, the presence of fibrositis, prolonged subfebrile condition, which determine the clinical differential diagnosis. Specific diagnostics are determined by various methodological levels - from the isolation of the pathogen to the determination of its antigens and antibodies, as well as the degree of sensitization to brucella. Hence, the treatment of chronic brucellosis depends on the activity of the infectious process, which requires the appointment of antibacterial agents and reasonable immunotropic therapy.

The received results confirm the efficacy and safety of milgamma preparations in the treatment of pain in patients with damage to the nervous system in brucellosis. They suggest that high doses of neurotropic vitamins as part of milgamma preparations can potentiate the analgesic effect and contribute to a faster

regression of the pain syndrome. The peculiarity of our study was that, firstly, we showed the effectiveness of a complex of B vitamins (using the example of a milgamma preparation) with such a severe form of back pain as vertebrogenic radiculopathy. Secondly, we found that milgamma effectively acts on the neuropathic component of brucellosis pain. Thirdly, it is shown that the addition of a 14-day course of oral administration of the drug milgamma compositum to the 10-day course of intramuscular administration of milgamma promotes a more complete manifestation of the therapeutic potential of the drugs. Finally, the data obtained indicate that the effectiveness of the combined course of treatment with milgamma can be observed not only in the short, but also in the medium term (3 months), which was also facilitated by its prolongation by oral administration of the milgam compositum tablets.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Adaptation Mechanisms of Shift Workers in Areas of the Far North

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Abstract

One uses the labor migration in various sectors of the economy. A high degree of stress on the health of shift workers leads to rapid fatigue and the development of irreversible pathology. Within the observation of two groups during the 52-day shift, the 1st group-before the start of shift work, the 2nd group - after the completion of shift labor organization, one put the labor activity under study, and there was time study of key functions performed by workers. The body of shift workers functions with a constant level of stress in regard to regulatory adaptation mechanisms to the circumstances of the industrial and climatic environment. In the study of employees ' health, there was a decrease in performance at the end of the 1st month of shift work with confirmed Vascular and autonomic dysfunctions within the cardiointervalography. In accordance with the cardiointervalography and measured physical strength by the method of dynamometry, there were dynamic indicators in groups at the stage of warming-up and during the completion of works. The study of cardiointervalography revealed a negative impact of labour conditions on the cardiovascular system. In accordance with the results of the study, the proposals may increase the workforce productivity.

Keywords: *Functional reserves, shift method, compensatory adaptation, labor productivity, cardiointervalography.*

Introduction

At the current rate of economic growth[1], there is a need for industrial development within the Far North regions by the means of labor reserves[2] from all over the country and abroad. The shift workers suffer a high level of stress in respect to the regulatory mechanisms.[3] In order to ensure stable workforce productivity, it is necessary to set vital indicators in regard to a level that depends on the organization of labor. The body functions that mainly depend on the organization of shifts are the cardiovascular, respiratory and other life support systems.

Materials and Method of Research

One used a comprehensive approach in order to study the body reserves of shift workers. In addition, one applied to method in order to reduce the level of stress at the commencement and end of works.

One managed the work in accordance with the research plan of Industrial University of Tyumen and Tyumen state medical University and it is part of the program «Adaptation recovery mechanisms to the conditions of Arctic». Within the research, one examined the parameters of functional indicators within the cardiovascular and respiratory systems of the workers' bodies, as well as the stock of physical health reserves of workers who operate on oil-field equipment by shifts [3,4]. In accordance with the results of the study, it is possible to develop measures that contribute to the improvement of job performance during the various periods of adaptation. The research method of heart rate variability was cardiointervalography, since it is highly informative, both under familiar environmental conditions and under extreme conditions. During the study, one performed the analysis of the R and P waves in their standard lead, and we measured the morphofunctional reserve by the dynamometry. The key base was the research results

in the summer period of shift works, which constitutes 52 days, the average daily air temperature is +12 ^{0}C. In order to study the indicators of the body fatigue and adaptation, we formed 2 groups, the 1st group refered to the time before the start of work, the 2nd group refered to the time at the end of works, one conducted the study by the method of dynamic observation in respect to the state of workers during the adaptation period and the entire shift time.

Research Results

Currently, the national economy has a rapid growth due to the increase in demand for oil and gas production, thereby it stimulates the increase of pipeline and transport flow. Within the pipeline operation at the Sporyshevskoye oil field, Gazpromneft-Noyabrskneftegaz (JSC) developed a plan to increase the amount of oil product transportation and oil production in order to meet the required volumes. The shift set-up workers of oilfield equipment ensure the continuity of oil production within the infrastructure of PJSC «Gazprom». One uses the oilfield equipment for unconstrained production profile, transportation and storage of oil. For this purpose, more than 100 oil wells located on the territory of the Sporyshevskoye oil field participate in the process of maintenance. This equipment is under continuous service of at least 56 shift workers, their shift change happens every 52 days.

In this mode, the oil production has a full year time span, the productivity directly depends on changes in the climatic conditions of labor migration, all this affects the functional adaptation reserves, as well as the phases of work capacity within the scope of professional activities. At the beginning of the shift period, the workers pass the adaptation stage, and during this time period there is a high stress level of compensatory and adaptive reactions[5]. The increase in work capacity occurs during the next work cycle, which constitutes the period of two weeks[6]. Under the dynamics changes analysis in accordance with the indicators the increase is up to 14%, but by the end of the first working month, there is a decline in labor activity due to occurrence of vascular and autonomic dysfunctions[7]. Under the cardiointervalography output (hereinafter- Cardiointerval recording), one found that when the period of adaptation starts, the body of workers passes through the phases of warming-up and fatigue. In order to analyze the variability of heart rate under the usual environmental conditions, in order to assess the adaptation of the cardiovascular system, and in case of functional loads during shift works, it was advisable to use the method of cardiointervalography[2]. The cardiointervalography group's indicators are in table 1.

Table 1. Cardiointerval recording indicators for heart rate variability

Indicators of Cardiointerval recording	1 Group (n=56) start of the shift period	2 Group (n=56) end of shift period
In prone position		
M (modal value)	15,97±0,25	17,1±0,06*
Mo (mathematical expectation)	0,93±0,01	1,57±0,01*
Amo (mode amplitude)	33,3±1,42	31,34±0,39
ΔX (range)	0,58±0,04	0,83±0,01
IN1 (stress index)	93,84±4,32	70,2±1,79*
VBI1 (vegetative balance index)	118,16±2,60	93,89±2,09*
VICP1 (validity indicator of the control process)	52,68±1,3	46,05±0,70*
VRI1 (vegetative rhythm indicator)	5,97±1,10	5,02±0,08*
In the upright position		
M (modal value)	14,29±0,28	15,54±0,10
Mo (mathematical expectation)	0,86±0,01	1,1±0,01*
Amo (mode amplitude)	44,03±2,1	39,28±0,61
ΔX (range)	0,53±0,05	0,77±0,01
IN2 (stress index)	171,11±8,20	120,19±5,53*

Indicators of Cardiointerval recording	**1 Group (n=56) start of the shift period**	**2 Group (n=56) end of shift period**
IN2/IN1	2,13±0,08	2,22±0,05
VBI2 (vegetative balance index)	191,6±6,36	144,13±5,73*
VICP2 (validity indicator of the control process)	78,87±3,18	65,13±1,41*
VRI2 (vegetative rhythm indicator)	8,06±0,49	6,57±0,19*
In prone position		
M (modal value)	16,13±0,22	17,19±0,03*
Mo (mathematical expectation)	0,93±0,01	1,17±0,01*
Amo (mode amplitude)	33,74±0,74	30,48±0,33*
ΔX (range)	0,59±0,01	0,83±0,01*
IN3 (stress index)	91,82±1,55	68,09±1,48*
IN2/IN3	2,17±0,07	2,27±0,07
VBI3 (vegetative balance index)	115,61±1,85	91,08±1,83*
VICP3 (validity indicator of the control process)	53,38±1,85	45,11±0,56*
VRI3 (vegetative rhythm indicator)	5,77±0,28	5,02±0,07*

Under the study of indicators in relation to workers, one found that the shift workers of the 1st group have:

- Increased heart rate at rest 87.3±1.52;
- Increase in A_{MO} (mode amplitude) 33.3±1.42; p<0.05;
- Range (0.58±0.04; p<0.05);
- Maximum stress index (93.84±4.32; P0, 05).

In accordance with the observations results, it became possible to establish moderate sympathicotonia, the tonicity predominance of sympathetic segment Fig. 1.

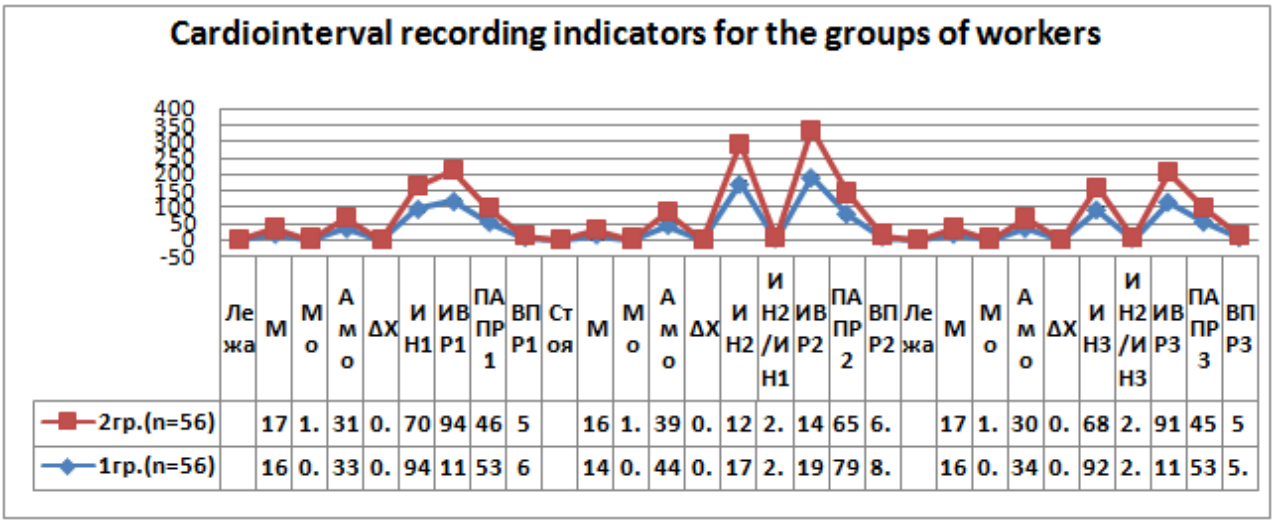


Figure 1. Cardiointerval recording indicators for the groups of workers

In the study of groups by the method of cardiointervalography, one determined the influence of vegetative reactivity on the indicators of the lower margin of the sympathetic type. One observed the vegetative activity within the range (1.83±0.08; P0, 05).

One observed the increase in VBI among the subjects of the 1 group under study at the stage of adaptation (vegetative balance index) – which is a direct indicator of the control loop activity (118.16±2.60; P0, 05). The high values in figure 2 confirm this statement:

- vegetative rhythm indicator - VRI (5.97±0.10; P0, 05);
- validity indicator of the control process (52.68±1.3; P0, 05).

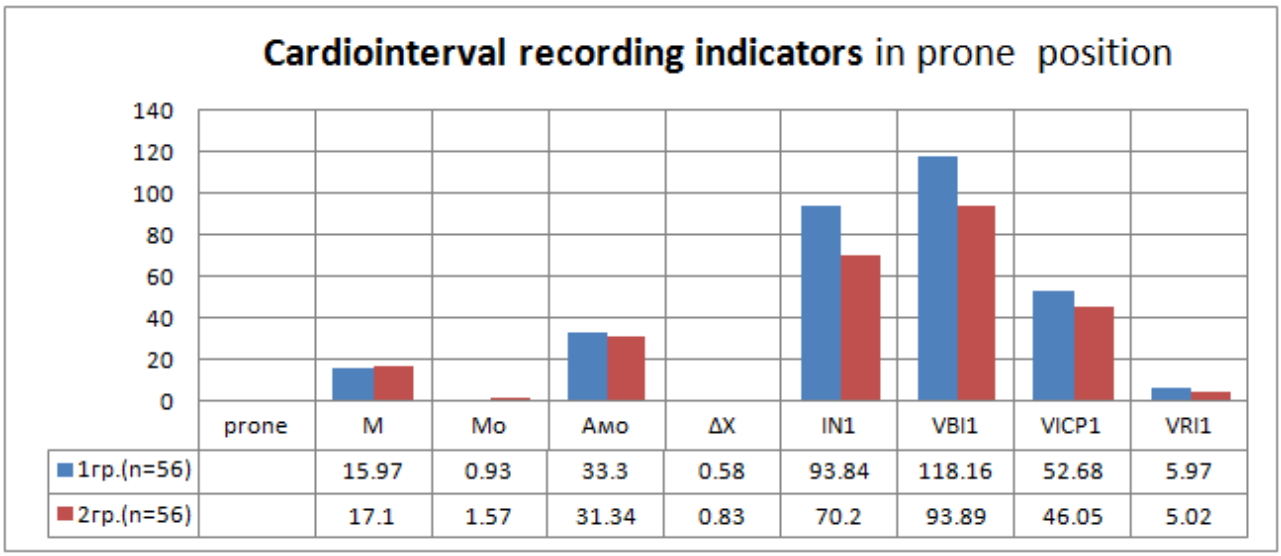


Figure 2. Analysis of vegetative reactivity in prone position during the shift period.

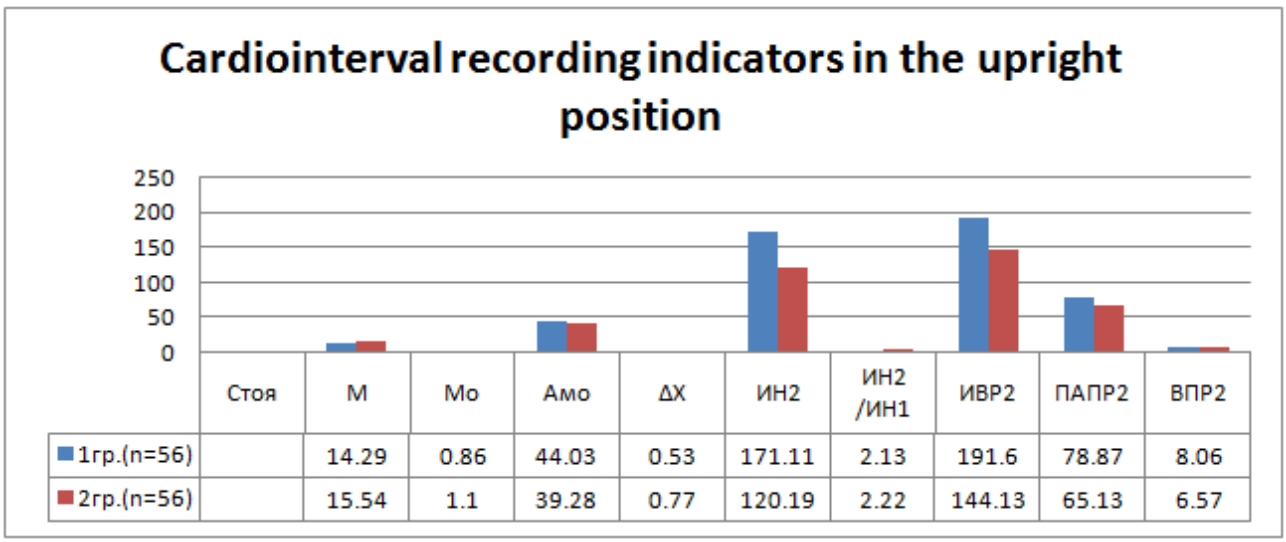


Figure 3. Analysis of vegetative reactivity in the upright position during the shift period.

The analysis output for test in the upright position differs by groups under study.

At the adaptation stage in group 1 Fig. 3:

- heart rate indicators - (94.17±1.9; p<0,05);
- the maximum level of VBI - (191,6±0,05; p<0,05);
- maximum values of the stress index (IN) 171,11±2,4; p<0,05;
- the mode amplitude increased (44,03±2,11; p<0,05).

In accordance with the results of the study, in accordance with the data on 2 group of workers at the stage of constant work, one observed the decrease in heart rate (97,1±1,5; p<0,05), with minimal stress index and large values of the variation range.

In prone position, the characteristics data for adaptation within group 2 belongs to Fig. 4:

- decrease in heart rate (74,2±1,6; p<0,05);
- minimum VBI - (91,08±0,04; p<0,05);
- minimum values of the stress index (68,09±1,6; p <0,05);
- decrease in the mode amplitude (30,48±1,91 p<0,05).

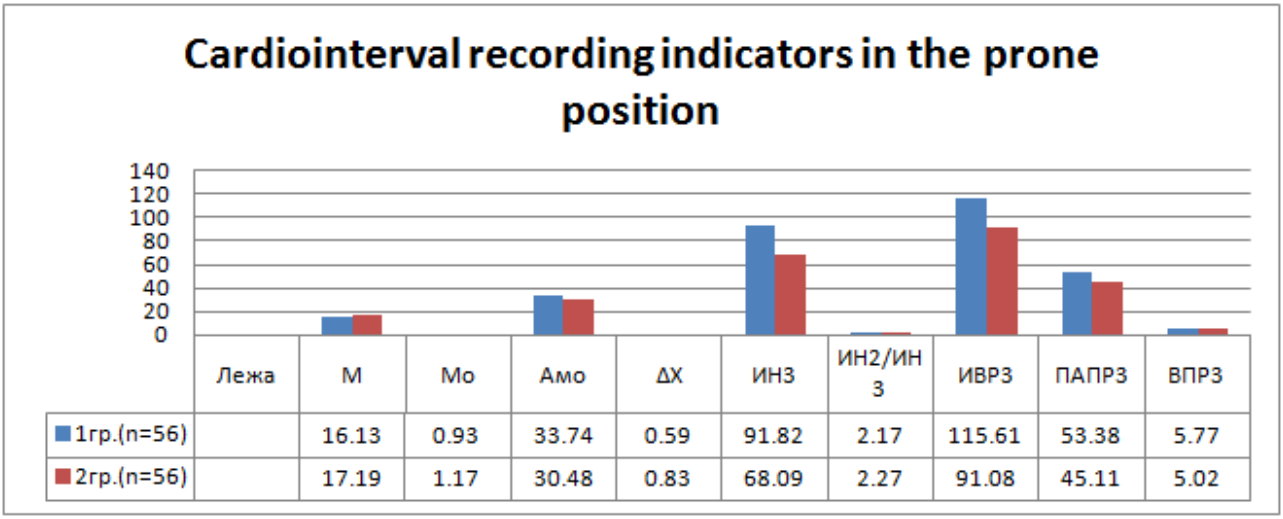


Figure 4. Analysis of vegetative reactivity in relation to the orthostasis of workers in prone position

The research results of orthostasis showed the same type of reaction in respect to the cardiovascular system within all groups, at the beginning of shift work in group 1, and at the end of shift work in group 2, one has observed:

1. Heart rate explicitness;
2. Decrease in range;
3. Increase in modal value;
4. Decrease in stress index.

In accordance with the method of cardiointervalography and the method of dynamometry in relation to physical strength determination[8], the set-up workers of oilfield equipment had satisfactory results at the stages of the shift period start and end[9].

One registered the condition of eitonia as the general state of the workers body regulatory systems and the vegetative reactivity in relation to orthostasis, see Tab. 2.

Table 2. Vegetative and morpho-functional criteria within groups of workers.

№	Criteria	1 Group, n=56	2 Group, n=56
1	Vital capacity (l)	3,95±0,02	3,89±0,02
2	Heart rate (b/min)	74±0,62	78±0,34
3	SBP (Systolic blood pressure) (mm Hg)	125±0,42	130±0,62
4	DBP (АДД) (mm Hg)	80±0,82	86±0,73
5	PP (mm Hg)	45±0,52	44±0,4
6	IN1	93,84±4,32	70,2±1,79*
7	VBI1	118,19±2,60	96,89±2,09*
8	VICP1	52,68±1,3	46,5±0,70*
9	VRI1	5,97±0,10	5,02±0,08*
10	IN2	171,11±8,20	120,19±5,53*
11	VBI1	191,6±6,36	144,13±5,73*
12	VICP2	78,67±3,18	65,13±1,41*

№	Criteria	1 Group, n=56	2 Group, n=56
13	VRI2	8,06±0,49	6,67±0,19*
14	IN3	91,82±1,55	68,09±1,48*
15	VBI3	115,61±1,85	91,08±1,83*
16	VICP3	53,38±1,85	45,11±0,56*
17	VRI3	5,77±0,28	5,02±0,07*
18	Right hand strength (kg/body mass)	47±0,12	50±0,41
19	Left hand strength (kg/ body mass)	45±0,21	48±0,28

Under the analysis of all indicators one revealed a change in the correlation coefficient:

- in the first group from 0.27 to 0.691;
- in the second group from 0.3 to 0.71.

The indicators highlight the dynamics of shift workers adaptive capabilities, which requires a high level of body functional stamina[9]. As a result of shift works the vital health indicators of shift workers will decrease[10]. Thus, within the comparative analysis of functional values, one underlines the criteria of work capacity dynamics and develops the suggestions for workforce productivity optimization.

Analysis of the functional indicators disorders among the shift workers indicates their acclimatization nature, the contrast of climatic factors, the severity of the labor process and the functional reserves of the body as an instability of physiological functions. A significant factor is the state of shift workers psychophysiological status[8].

Conclusions

1. The shift work method influences the health, causes the deterioration of functional activity. One observes the greatest responsiveness of the cardiovascular system both at the beginning and at the end of adaptation in relation to the work process within both groups.
2. Due to insufficient compensatory and adaptive responses, there is no complete adaptation to environmental factors and in accordance to the calculations under study, one observed such conditions among 78% of set-up workers of specialized equipment, and at the end of the shift work period, there is an increase in 22% of workers, as the fact of adaptation.
3. The level of energy consumption during respiration of shift workers increases, due to a decrease in effective external respiration.
4. At the beginning of the work process, the workers had a higher heart rate at rest by the Cardiointerval recording (91,1±1,51; $p<0.05$ and an increased mode amplitude (33.3±1.42; $p<0.05$). It indicates a high level of stress in respect to the regulatory systems.

One recommends the work safety service to prevent the onset of fatigue by control of labor modes within the production process in order to optimize the workforce productivity. Restoration of the body functional constants serve as the basis for the improvement of work capacity indicators. In accordance with these patterns, one can manage the processes of fatigue and recovery and thus increase the level of efficiency among the shift workers.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Assessment of Survivability of Vitrified Embryos Depending on the Stage of Development

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Abstract

Freezing embryos of excellent and good quality allows you to maintain reproductive potential for patients in the future and achieve pregnancy using one stimulation cycle. Cryopreservation of embryos can be carried out at any stage of development. Vitrification as a method of cryopreservation combines the use of highly concentrated solutions of cryoprotectants and rapid (almost instantaneous) cooling by immersing samples directly in liquid nitrogen. Due to this, the main goal of vitrification is achieved - the absence of the formation of ice crystals, which can damage the cell structure. The advantages of vitrification are as follows: a high concentration of cryoprotectants and a high cooling/thawing rate eliminate the damage caused by the formation of intracellular ice, as well as the undesirable osmotic effects of dehydration and rehydration due to extracellular crystal formation.

Keywords: *Embryo, blastocyst, cell, pregnancy, icm1, te1.*

Introduction

The main criteria for the effectiveness of the vitrification system is the survivability of the embryos. According to the data of the Vienna Consensus (The Viennaconsensus: report fan expert meeting on the development of ART laboratory performance indicators, 2017), the survival rate of blastocysts (embryos of 5-6 days of preimplantation development) is 90% or more[1].

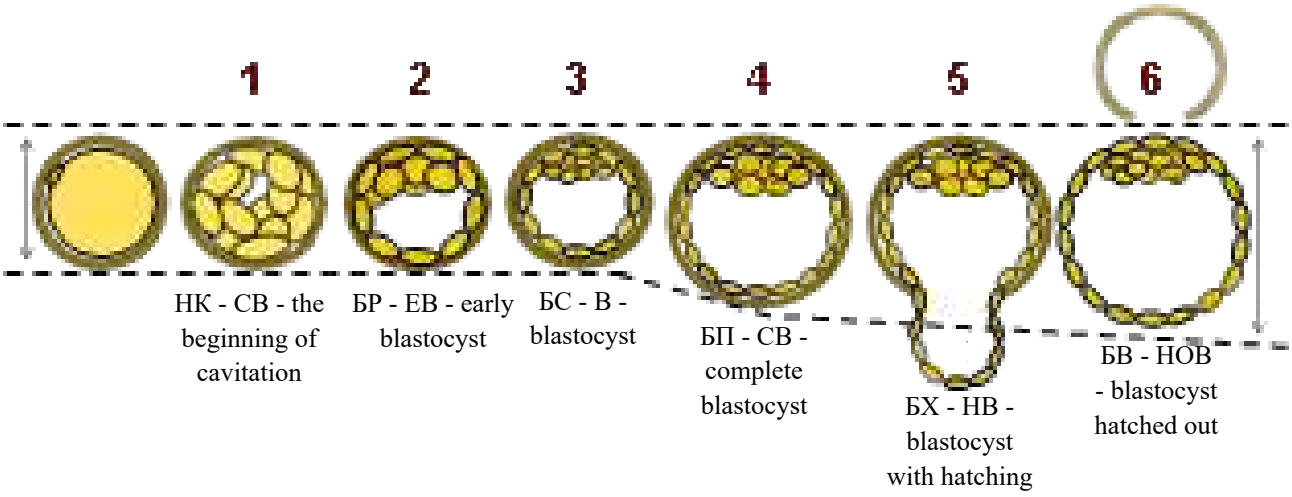
Objective of the Research: Efficiency of various types of carriers for embryo freezing and embryo survivability after thawing under the ART programs.

Materials and Method

The work has used human embryos, the study of which has been carried out in compliance with international ethical and legal standards for the treatment of human embryos [Art. 18 of the Council of Europe Convention on the Protection of Human Rights and the Dignity of the Human Being while Using the Advances in Biology and Medicine, 1997][2]. The study of embryos has been carried out as part of in vitro fertilization cycles at the embryological laboratories of "IDK Medical Company" CJSC. The use of germ cells and human embryos in scientific research has been authorized by the ethics committee of the Samara State Medical University of the Russian Ministry of Health. Embryos have been identified under the control of a stereo microscope (Nikon, Japan). For incubation under conditions of 5% O2, SOOC incubators (Australia) have been used. Vitrolife (Sweden) have been used to cultivate embryos up to 5-6 days of embryonic development. Irvine Scientific environments (USA) and environments of open CryoTop (Japan) and closed CryoTip (USA) types have been used for vitrification of embryos. The fundamental difference between open and closed carriers is the presence/absence of direct contact of the vitrified object with liquid nitrogen. Before freezing, blastocysts of 5-6 days of cultivation have been assessed according to the international classification[6]. Blastocyst assessment takes into account three main parameters: the percentage

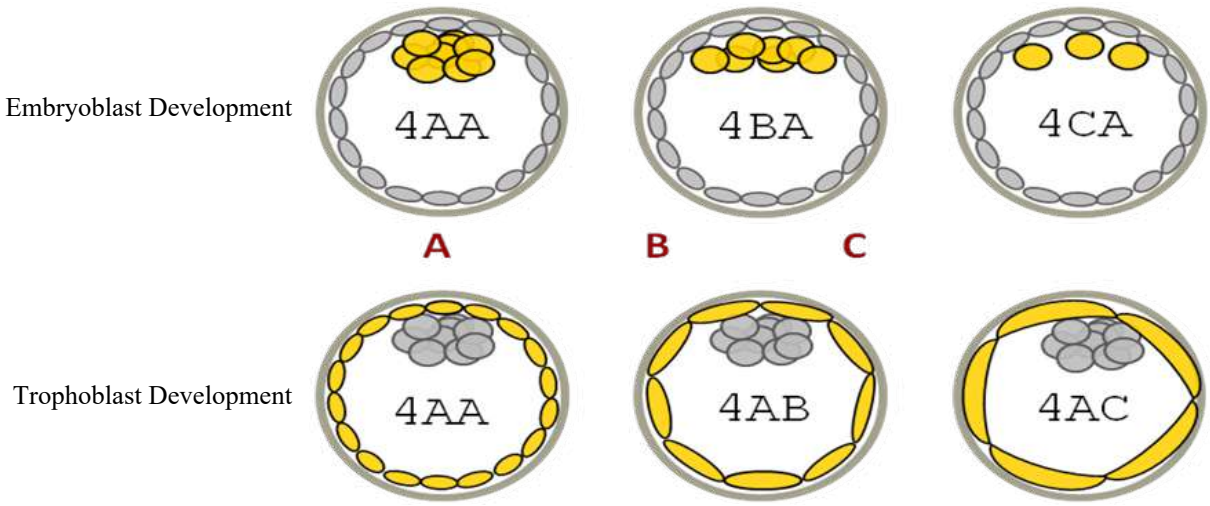
of expansion (increase) of the embryo (blastocyst), the development of internal cell mass and trophectoderm.

Assessment of intracellular mass has been performed under an inverted microscope: (A) densely filled with many cells; (B) weakly grouped with several cells; and (c) very few cells. The following classification has been used for trophectoderm: (A) many cells forming a cohesive epithelium; (B) few cells forming a loose epithelium; and (C) very few large cells. After thawing, the blastocysts have been subjectively assessed according to the degree of repeated expansion of the blastocoel, the degree of cell reorganization, and the degree of cell survivability by two embryologists.



Pic. 1. Blastocyst Assessment by D. Gardner (1999)

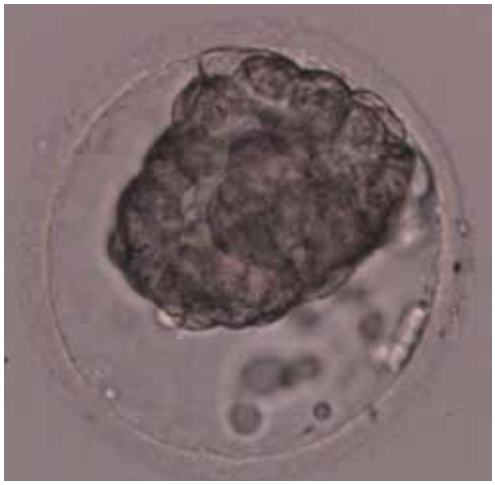
Comment: CB-the beginning of cavitation, EB-early blastocyst, B-blastocyst, CB-complete blastocyst, HB-blastocyst with hatching, HOB-blastocyst hatched out.



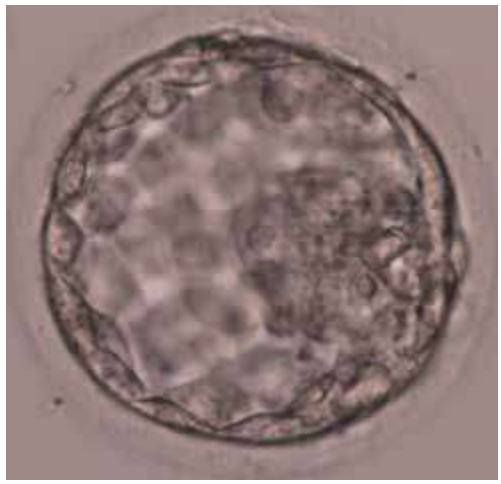
Pic. 1. Blastocyst Assessment by D. Gardner (1999)Illustartions: R.Shafei, 2008

Statistical processing of the results has been performed on a computer in the environment of statistical calculations R (R v.3.5.3, R Studio v.1.1.463), the primary data input has been performed using MS Excel spreadsheets. Descriptive statistics method, tests for comparing proportions, including an exact binomial for small samples and a single-sample proportional test with continuity correction for large samples have been used.

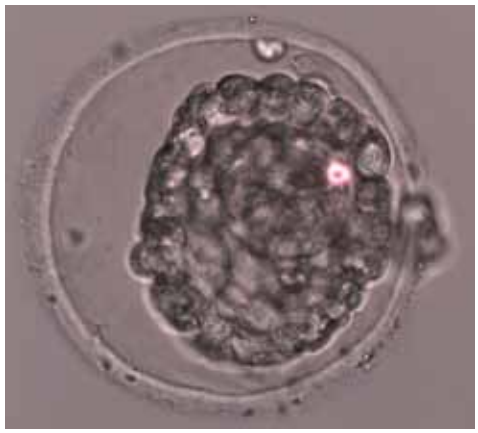
Research Results and Discussion



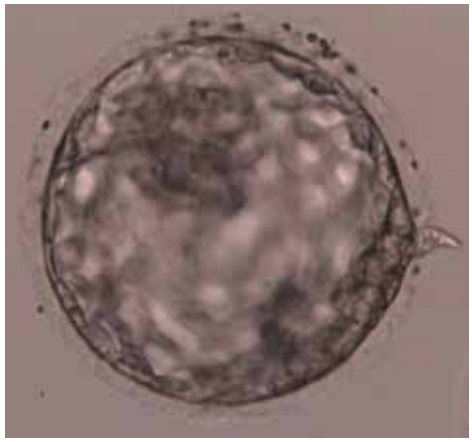
Picture 3. 5-day embryo, stage of collapsed human blastocyst, 10X eyepiece, 20X lens (immediately after thawing)



Picture 4. Blastocyst embryo, 4AA, 10X eyepiece, 20X lens (2 hours after thawing)



Picture 5: 5-day embryo, stage of collapsed blastocyst, 10X eyepiece, 20X lens (immediately after freezing)



Picture 6: Blastocyst embryo, 4AA, 10X eyepiece, 20X lens (2 hours after thawing)

The pictures show embryos immediately after freezing and after 2 hours (Pic. 3-6). 1-2 hours are needed to restore the blastocyst and to morphologically assess its viability. If the embryo has more than 50% intact cells, then it is considered viable.

Table №1. Embryo survivability depending on embryo developmental stage

No.	Embryo development stage	ICMdevelopment stage	Trophectoderma development stage	Number of embryos	Survivability, %
1	0.morula	<NA>	<NA>	5	100.00
2	1.beg.of cav.	<NA>	<NA>	156	90.33
3	2.earl.blast	A.icm1	A.te1	1	100.00
4	2. earl.blast	<NA>	<NA>	187	90.00
5	3. blast	A.icm1	A.te1	41	100.00
6	3. blast	A.icm1	B.te1	109	95.51

No.	Embryo development stage	ICMdevelopment stage	Trophectoderma development stage	Number of embryos	Survivability, %
7	3. blast	A.icm1	C.te1	3	100.00
8	3. blast	B.icm1	A.te1	8	100.00
9	3. blast	B.icm1	B.te1	141	89.33
10	3. blast	B.icm1	C.te1	27	92.16
11	3. blast	C.icm1	C.te1	2	100.00
12	3. blast	<NA>	<NA>	1	NA
13	4. blast	A.icm1	A.te1	193	96.96
14	4. blast	A.icm1	B.te1	168	97.90
15	4. blast	A.icm1	C.te1	1	100.00
16	4. blast	A.icm1	<NA>	1	100.00
17	4. blast	B.icm1	A.te1	21	100.00
18	4. blast	B.icm1	B.te1	107	97.21
19	4. blast	B.icm1	C.te1	13	100.00
20	4. blast	C.icm1	B.te1	1	100.00
21	4. blast	C.icm1	C.te1	1	100.00
22	4. blast	<NA>	<NA>	1	100.00
23	5. blast	A.icm1	A.te1	43	96.43
24	5. blast	A.icm1	B.te1	15	100.00
25	5. blast	B.icm1	A.te1	7	100.00
26	5. blast	B.icm1	B.te1	8	81.82
27	6. blast	A.icm1	A.te1	5	100.00
28	6. blast	A.icm1	B.te1	4	75.00
29	6. blast	B.icm1	B.te1	3	100.00
30	<NA>	<NA>	<NA>	133	37.68
	Total			**1406**	**94.49**

From table 1 it follows that a total number of 1406 embryos have been analyzed at different stages of development. The embryo survival range is quite wide and ranges from 75 to 100%. It should be noted that embryos of the 6th degree of expansion, namely, hatched blastocysts, demonstrate the lowest survival rate. Probably this is due to the lack of a fertilization membrane and the cytotoxic effect of cryoprotectors on trophectoderm cells. The number of embryos in this group is extremely small to draw any unambiguous conclusions on this subject.

In more numerous groups (more than 100 cases), the survival rate has been more than 95%, which indicates a high degree of cryotolerance of excellent and good quality embryos. Nevertheless, we considered it necessary to conduct a further, more detailed analysis of the dependence of the frequency of pregnancy on the stage of the frozen blastocyst (degree of expansion, the severity of ICM and TE). On the basis of the degree of expansion, the indicators of the pregnancy rate (PR, positive ultrasound) increase with an increase in the degree of expansion from the stage of onset of cavitation of the blastocyst to increased blastocyst (degree of expansion 4), reaching a maximum in hatched blastocysts lacking a fertilization membrane. This trend is quite natural: an embryo with a higher level of organization and cell differentiation gives higher PR indices. However, the decline in the blastocyst group with the 5^{th} expansion degreeis noteworthy; these are embryos that have been frozen during hatching. Apparently, embryos of this developmental stage are the

most vulnerable to the effects of the freezing procedure. The difference in the proportion of positive ultrasound has significantly differed between the stages ($p < 0.01$). That is, the percentage of PR-positive ultrasound scan has been growing from morula (20.0%) to 6-blast (66.7%) with a slight "failure" at the stage of expanded [5] blastocysts (37.5%). Carrying out a comparative analysis according to the following criterion — the degree of development of intracellular mass (ICM), we have obtained the expected results: the higher the degree of compaction of cells in the ICM, the higher the PR indices. In blastocysts with ICM A (581 embryos), the PR rate has been 51.3%, in blastocysts with ICM B (334 embryos) - 39.8%. The differences are significant at $p = 0.004$. It should be noted that in the group of blastocysts with intracellular mass C (there are extremely few or no cells), we have received 50% of PR. This indicator can hardly be considered objective due to the extremely small group (4 embryos), since embryos with a similar morphology, as a rule, are not supposed to be frozen. The pronouncement of trophectoderm, the degree of development of which is most significant in the process of implantation, directly correlates with the PR data. When analyzing 918 embryos, we have found that blastocysts with class A trophoctoderm (318) give the most indicators - 57.5% of PR, blastocysts with class B trophoctoderm (553) - 42.9% of PR, with class C trophoctoderm - 27.7% of PR. These differences are significant at a level of $p < 0.01$.

To assess the effectiveness of different carriers, the following criteria have been taken into account: survival rate, average transfer embryo/s score, average number of transfer embryos, pregnancy incidence rate, birth rate and loss (table 1).

Table 1. The main criteria for the effectiveness of the cryoprogram of embryos between open and closed types of carriers

	Closed carrier (CryoTip)	Open carrier (CryoTop)	p
Average age of the patient, years	34,1	33,5	
Number of thawed embryos	611	678	
Survivability, %	84,8%	95,1%	<0,0001*
Average score of embryos/transfer	3,9	4,1	
Average number of embryos/transfer	1,2	1,3	
PR, % (per transfer)	39,5	44,2	0,001*
Birth rate, % (per clinical pregnancy)	72,7	67,3	0,003*
Loss, % (per clinical pregnancy)	27,3%	24,3%	0,044*

In 2015-2016, 1289 embryos were thawed on the types of carriers under consideration. Embryo survivability on closed carriers has been 84.8%, on open - 95.1%, and this difference is statistically significant ($p < 0.0001$). The average transfer embryo score in group I has been 3.9, in group II - 4.1. The average number of embryos per transfer is 1.2 and 1.3, respectively. The pregnancy rate has been statistically significantly different ($p < 0.0001$) and has amounted to 39.5% (group I) and 44.2% (group II). The birth rate for clinical pregnancy, taking into account the known outcomes, has been 72.7% in group I, 67.3% in group II, and losses have been 27.3% and 24.3%, respectively. Birth rates and losses have a statistically significant difference.

Conclusion

Thus, conducting a comparative statistical analysis, the influence of all components of the blastocyst assessment—the stage, the degree of ICM development, and the pronouncement of trophectoderm—on the degree of survival and the level of pregnancy rate, has been disclosed. Embryos with the highest level of development of intracellular mass A and trophectoderm A give the highest results[3]. Unexpected results have been obtained on the PR in the group of embryos with intracellular mass C (50%), they can hardly be considered natural given the small number of cases (4).[4] The degree of expansion of blastocysts also shows a

direct relationship with PR, with the exception of stage 5 preimplantation development blastocysts (blastocysts with hatching). Apparently, this energetically active process for the embryo (when the embryo expands at regular intervals) is extremely important. The embryo is trying to find the most favorable place for rupture of the membrane of fertilization and exit from it. In fact, this stage of development is a kind of critical period of development for the embryo[5]. Accordingly, at this moment, it seems to be the most vulnerable to the impact of internal and external factors. Vitrification in a certain way at this moment is the very external factor that determines lower PR indices in embryos of this developmental stage[6].

According to the results of the analysis, the use of open carriers in cryopreservation allows us to conclude about a higher level of embryo survivability. Most likely this is due to direct contact of the object with liquid nitrogen and a high freezing rate, which can be a decisive factor in determining the success of vitrification on an open carrier. Clinical indicators (incidence of clinical pregnancy, childbirth and pregnancy loss), which directly depend on the level of survivability, also demonstrate a statistically significant difference in favor of using open carriers in embryological practice[7].

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Changes in Blood Counts of Patients with Chronic Atrophic Rhinitis

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Abstract

Chronic atrophic rhinitis among all ENT organ diseases happens in 10% of cases. This process is very rarely diffuse and mainly from an ailment, the nasal cavity and nasopharynx are sometimes tracheal membranes. The aim of the study was to study the indicators of a general analysis of blood, iron and ferritin in the blood. A study was conducted of 20 people with a diagnosis of atrophic rhinitis. After the initial reaction, expressed in the form of increased bone marrow production, a significant predominance of lymphocytes sets in again.

Based on this, it can be concluded that a degenerative shift with an increase in the number of lymphocytes should apparently indicate a chronic infectious irritant.

Keywords: *Subatrophy, crusts, ferritin, iron, neutropenia.*

Introduction

It is probably impossible to find a person in the whole world who would not know what a runny nose is, although [1]this is a fairly common occurrence.[3] Very few treat the common cold, and in vain, because one of the complications of this disease can be atrophic rhinitis[6], which implies an inflammatory disease of the nasal cavity and nasopharynx [9]. The occurring changes in the nasal mucosa are dangerous, and can lead to further changes in bone tissue[2]. The causative agents of atrophic rhinitis are infections, which indicates a negative effect on the body[3]. Subatrophic rhinitis occurs in 10% of patients with chronic diseases of the nasal cavity, and is characterized by a prolonged course, unexpressed symptoms of dryness and itching in the nose, and difficulty breathing[11]. Chronic diseases can provoke this condition. Chronic subatrophic rhinitis occurs due to endogenous and exogenous factors[15]. Diseases of the gastrointestinal tract, genitourinary and nervous systems are common causes[13]. They contribute to the development of pathogenic (pathogenic) microflora, the accumulation of cell breakdown products in the body. In this case, the regulation of restoration of the epithelium of the nasal cavity is disrupted, the cells begin to be replaced by connective tissue[16]. The atrophic process is very rarely diffuse.

The nasal cavity and nasopharynx sometimes suffers from an condition is inherent in the lake, when all layers of the wall of the nasal cavity are deeply affected. With subatrophic rhinitis, epithelial cells undergo mainly dystrophic changes, their number sharply decreases, they are unable to secrete a sufficient amount of protective mucus. Atrial epithelium loses its excretory function[9], and its pH (acidity) rises, which contributes to the development of pathogenic microbes [14]. Clinical signs of the disease develop gradually and at the same time, a bacterial runny nose worsens.[4] Mucous secretions acquire a purulent appearance (green color). Thickening occurs, the formation of crusts and the blood supply to the mucous layer is disrupted, degenerative changes in the tissues begin[7]. One of the leading causes of chronic atrophic rhinitis is long-term exposure to various environmental factors[8], which is especially important for residents of ecologically unfavorable territories, as well as for workers of harmful industries[14].

The main clinical manifestations of the disease occur with prolonged inhalation exposure to industrial factors, such as wood, coal dust, gaseous products formed during the cooking of metals, processing of petroleum products, etc. It is known that after 2-3 years of working in harmful production, workers note the appearance of typical symptoms of chronic atrophic rhinitis. Subjective

complaints are confirmed by objective research data: a decrease in the speed of mucociliary transport (saccharin test), a decrease in the temperature of the mucous membrane of the anterior divisions of the nasal septum, a violation of secretory function (a test with weighing) is determined. The main clinical manifestations of chronic atrophic rhinitis include a feeling of dryness in the nasal cavity, itching and abundant formation of dry crusts, which are found not only in the anterior, but also in the posterior part of the nasal cavity (differential diagnosis with anterior dry rhinitis). The fetid smell that bothers some patients is probably due to the presence of specific bacteria.

The sense of smell is impaired, weakness, anemia, and panic attacks occur periodically due to repeated episodes of nosebleeds[15]. Prolonged constant irrigation and adverse microclimate can lead to further damage to the structures of the nasal cavity, up to perforation of the nasal septum [17]. The disease is often associated with similar atrophic processes in the pharynx. Diagnosis of chronic rhinitis is based on the patient's subjective complaints and objective examination data (including endoscopy of the nasal cavity). For the purpose of differential diagnosis, it is necessary to perform allergological studies (test panel), make a seeding of the separated nasal cavity to determine the flora and sensitivity[1], as well as conduct computer tomography of the paranasal sinuses (to exclude pathological foci in the paranasal sinuses, the initial manifestations of some autoimmune diseases)[6]. Equally important is a violation of the blood supply to the mucous membrane of the nasal cavity, often associated with age-related changes. Diseases such as heart disease, pulmonary emphysema, tumors of the chest cavity, which make it difficult to drain blood from large veins and develop congestion in the mucous membrane of the upper respiratory tract, also play a large role in the development of atrophic rhinitis. The development of this disease can provoke prolonged use of vasoconstrictor nasal drops.

An important role is assigned to the hereditary factor. Separately, it should be said about the development of atrophic rhinitis in children. Teenagers are at risk during puberty, especially girls. Most likely, this is due to sharp hormonal bursts in the body, as well as vitamin deficiency and a decrease in immune resistance. As international medical experience shows, all atrophic processes in the body are interconnected. Therefore, together with damage to the nasal mucosa, they can detect similar processes in the esophagus or stomach.

The aim of the study was to study blood counts in patients with chronic atrophic rhinitis.

Materials and Research Method

In the clinic of the Samarkand Medical Institute from 2017 to 2019, 20 people were examined, of which 11 men and 9 women with a diagnosis of chronic atrophic rhinitis aged 15-70 years. Patients were divided into 2 groups. The first group of patients was 9 patients, after surgical interventions on the septum and nasal cavity. Complained about the formation of crusts in the nose, atrophy, dry mucous membrane, weakness, respiratory failure. The second group of patients was 11 patients who had not previously been operated on on the nasal cavity and nasal septum. Complained of dryness and crusting of the nose. All of them had signs of atrophic lesions of the nasal cavity, but had not previously been on an outpatient appointment with an otorhinolaryngologist.

The patient underwent a study of a general analysis of blood, iron, and ferritin in the blood. As a rule, a study of plasma for iron and ferritin is carried out from 8 to 10 in the morning. The analysis is given on an empty stomach. The day before the blood sampling, you should refrain from eating fatty foods and drinking alcohol. It is performed in conjunction with studies on hemoglobin, transferrin, total iron binding capacity of serum (OGSS) and ferritin. The results are needed to detect iron deficiency, diagnose and monitor the treatment of anemia, hereditary hemochromatosis, infections, systemic inflammatory diseases and malabsorption of substances in the intestine. Blood sampling is performed from a vein. Within 30 minutes before the fence, you need to refrain from smoking and physical activity, to avoid feelings and emotional stress. To get the correct results 7-10 days before the blood donation, it is necessary to stop taking food supplements and medicines containing iron.[6] The study is carried out by the colorimetric photometric method (with ferrozine). Normally, in men, the iron content in serum is 11.6–31.3 μmol/L, in women - 9.0–30.4 μmol/L [7].

The term of the analysis does not exceed 1 business day. The determination of iron is carried out by the colorimetric method, often using ferrosin[2]**.** Diagnosis of chronic rhinitis is based on individual patient complaints and objective examination data, including endoscopy of the nasal cavity [3].Taking into account one of the main links in the pathogenesis of atrophic rhinitis the principles of treatment are formulated: 1) stimulation of

local and General blood circulation, i.e. increased supply of the mucous membrane with nutrients; 2) moistening of the nasal mucosa and preventing the formation of crusts; 3) combating local pathological microflora.

One of the main points is the systemic effect on the trophism of the nasal mucosa is considered mandatory, but this is a ineffective method of treating atrophic processes. Some authors recommend irrigation with infusions of medicinal herbs (sage, calendula flowers, walnut leaves, tree aloe), often recommend peach, olive oil, retinol acetate and other oil drops.

Results of the Study

Summarizing and summarizing the data of the blood picture, we can reduce them to the following: the total number of leukocytes in 57.5% of cases was normal, in 25% there was little expressed leukocytosis and in 17.5% some leukopenia. The neutrophilic group of leukocytes was characterized by a decrease in the percentage and was characterized by degenerative forms of both segmented cells and stab cells. The number of rod-shaped in most hemograms was reduced and only in 12 cases, slightly increased, although degeneration was quite pronounced. The number of eosinophils in the overwhelming majority of patients 64% was normal in others, but it gave some fluctuations in one direction or another. The number of monocytes, with few exceptions, did not have any particular deviations, but it is difficult to note a certain regularity in the changes in their percentage content on our material.

Analyzing the hemograms of patients with atrophic rhinitis shown here, we tried to find any relationship between the clinical picture of the disease and blood changes. The most interesting in our material is the fact that the number of neutrophils appeared to be significantly lower than the average norm. Any irritation produces a functional change in the leukocyte formula. The longer and more intense the irritation, the more it acts on the leukopoietic organs**.**

Table 1: Revealed altered hemogram parameters in patients with atrophic rhinitis

	Leukocytosis	Lymphocytosis	Ferritin reduced less than 10 ng/ml	Fe in the blood is reduced less than 5.83 µmol/l
1 group (n= 9)	7	6	8	9
2 group (n =11)	8	7	11	11
Total 20	**15**	**13**	**19**	**20**

As can be seen from table 1 in the first group of patients, characterized by more vivid clinical phenomena and the duration of suffering, the decrease in the number of segmented and the presence of a shift is presented more sharply. Obviously, the irritation that caused these changes had a depressing effect on the production of the neutrophilic moiety. In the second group with less pronounced clinical symptoms, the irritating moment probably did not cause this oppression, and we have some neutropenia with a slight shift or normal blood counts.

In the study of serum iron in the blood in all examined patients, we revealed low rates. Normally, in patients, normal values of iron for an adult patient are indicators from 5.83 to 34.5 µmol/L. In 11 patients, the indicators were in the range of 4.35 µmol/L, in 9 patients 6.35 µmol/L. A certain degenerative shift in these sufferings indicates the constancy of the cause acting on the body. Under physiological conditions, ferritin reflects the iron content in the blood. So 1 µg/l of blood ferritin corresponds to 8 mg of deposited iron. In 2 groups of patients, a decrease in blood ferritin below 10 ng/ml was also noted.

Two large systems, the opposite in nature of the lymphocytes and ferritin in the blood, are closely related to each other. With a decrease in the total number of leukocytes with neutropenia and a degenerative shift, lymphocytosis occurs, and against this background, a decrease in iron and ferritin in the blood is detected. A certain degenerative shift indicates the constancy of the cause acting on the body. The absence of a neutrophil core shift always speaks against infection.

In the aforementioned disease, after the initial reaction, expressed in the form of increased bone marrow production, a significant predominance of lymphocytes sets in again.

Conclusion

Based on this, it can be concluded that a degenerative shift with an increase in the number of lymphocytes should apparently indicate a chronic infectious irritant. But less pronounced changes not only do not exclude infection, but confirm our conclusion by the fact that the infection, which is an irritant, has not yet acquired the significance of a chronic factor in these patients due to certain conditions. Although in patients with chronic atrophic rhinitis, a decrease in iron and ferritin in the blood is also observed, which basically can and is the main factor causing the formation of dryness and crusts in the nose.

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Work-Related Musculoskeletal Symptoms among Academicians in Malaysian Universities

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Abstract

Work-related musculoskeletal disorder is (WMSD) defined as injuries or disorders of the musculoskeletal system, which includes muscles, nerves, tendons, joints, cartilages, and spinal discs that may be associated with exposure to risk factors in the workplace. This study is to determine the prevalence of work-related musculoskeletal symptoms and analysis of risk factors among the academicians of university in Malaysia. Index Terms–Academician, Work-related Musculoskeletal Symptom Work-related musculoskeletal symptoms (WMS) has been identified as "occupational cramps" or "occupational myalgia", in relation to an occupation or work-related activities.

Keywords: *Musculoskeletal Symptoms, muscles, nerves, tendons, joints, cartilages.*

Introduction

This study is to determine the prevalence of work-related musculoskeletal symptoms and analysis of risk factors among the academicians of university in Malaysia. Index Terms–Academician, Work-related Musculoskeletal Symptom Work-related musculoskeletal symptoms (WMS) has been identified as "occupational cramps" or "occupational myalgia", in relation to an occupation or work-related activities[1]. Work-related musculoskeletal symptoms that can be presented in an occupation are pain, ache, numbness, tingling or any discomfort[2]. The symptoms are arising from repetitive, continuous, and unnatural movements in relation to work[3]. The prevalence of work-related musculoskeletal symptoms present in different anatomical areas are differs from each field of profession. Those musculoskeletal symptoms could be a sign of WMS. Those symptoms often occur as a consequence of their interaction with the working environment in fulfilling the demands of task in physical, psychosocial, environmental, and cognitive demands[4]. It has been suggested that a linkage exists between work-related musculoskeletal symptoms and risk factors involving one's occupational conditions and physicality[5] suggest these symptoms causing difficulties in performing job-related tasks and activities of daily living (ADL). According to the statistics from Ministry of Education Malaysia (2019)[6], it shown that the academicians in higher learning institutions throughout Malaysia in 2010 are 64,882 and it has increased to 90,483 in 2017[7]. The roles of academicians are significant in the higher learning academic system of Malaysia to deliver knowledge and to produce qualified students. Since the population is getting larger and the various studies shown that prevalence of work-related musculoskeletal symptoms of other teaching profession is also high, the academicians is needed to be studied on this issue[8]. As Conclusion, the academicians in Malaysia might at risk of having work-related musculoskeletal symptoms as other teaching professional. It cause various disadvantage towards the academicians, university and healthcare system[9] .However, there is paucity of the prevalence of work-related musculoskeletal symptoms among academicians of universities in Malaysia. Thus, the prevalence of musculoskeletal symptoms among academicians in university is conducted[10].

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Methodology

This study utilized a quantitative approach, non-experimental, Cross-sectional research design7. This research design use to describe the result in quantitative or numeric data, which is prevalence of work-related musculoskeletal symptoms by study the population of academicians in university.It is the most suitable to use for the aim to measure the prevalence of a problem which is work-related musculoskeletal symptoms in this study, by taking a cross-section of the population of the academicians in a university [11]. The survey is designed by using Microsoft Word 2010 for printed form and also prepared in online form through Google Forms Website at https://docs.google.com/forms/u/0/. The questionnaire is distributed via email given the internet link of online form or in printed form to the academicians. The targeted population is full time academicians of both genders, from 23 to 60 years old, with minimum one year of teaching experience. Teaching experience is required for at least a year as this study is going to investigate whether they have any trouble from the past 12 months in any region of the body[9]. Exclusion criteria Subjects who reported any musculoskeletal injuries such as fracture and soft tissues injuries and recent surgery in any of the regions of the body within 12 months were excluded. Any neurological condition, systemic disorder and cardiovascular condition are also excluded from the study[12]. This is to minimize the individual cause that might be affecting the result of the study rather than the occupational factor. The subject is academicians recruited from a university from six faculties by using non-probability sampling method convenience sampling that whoever passed the study's inclusion and exclusion criteria[13]. The six faculties are Business, Dentistry, Engineering, Medicine and Biomedical Science, Pharmacy and Physiotherapy. The sample size is determined by using the sample size calculator powered by Survey Monkey Website [14]. The final sample size is able to reach as much as 84 subjects. This study reviewed by the Faculty of Health & Sport Sciences Research Review Committee, MAHSA University (FRRC)[15]. All procedures, assessment, tools that used in this study have been approved by the FRRC. Informed Consent Form is provided to each subject stating their willingness to participate in the study[16]. The main tool of this survey study is the general type of Nordic Musculoskeletal Questionnaire (NMQ)[17]. The questionnaire was prepared in two forms which are printed and uploaded in Google From. The subject was identified as the academicians in a university based on the targeted population needed with a sufficient amount of sample size, the convenient of the location and time for researcher to travel to the research site. After filling of the questionnaire was carried out into two ways, either through Google Form Website or through a questionnaire filling session in the academicians' office. For faculty of physiotherapy, medicine and biomedical science and business, the responses are collected through Google Form Website, while faculty of medicine and biomedical science, pharmacy and dentistry are having a questionnaire filling session in the office. For those faculties that were having a questionnaire filling session in the office of the academicians the researcher would brief the academicians about the study aims, study process, questionnaires and to obtained informed consent before the questionnaire begin. The subject was also allowed to ask question to the researcher if having doubts. The variables under the descriptive data are age, gender, faculty, years of teaching, hours of working per week, preferred teaching instrument, and the prevalence of work-related musculoskeletal symptoms were collected. All the data, statistics is analyzed by using statistic software IBM SPSS Version 21.

Results

The main result of this study shows that the general prevalence of work-related musculoskeletal symptoms among academicians in university is as high as (n=62) 73.8%. This result involves symptoms in any nine region of the body as stated in the general Nordic Musculoskeletal Questionnaire; nearly three quarters of the academicians are having the work-related musculoskeletal symptoms. Only (n=22) 26.2% academicians are free from any WRSS from all the nine body regions.

Table No. 1: Demographical Data Collected

Gender	**Frequency**	**Percentage (%)**	**Mode**	
Female	39	46.4	Male	
Male	45	53.6		
Total	**84**	**100.0**		
Age (years)	**Frequency**	**Percentage (%)**	**Mode**	**Median**
23-30	13	15.5	31-40	31-40
31-40	45	53.6		
41-50	12	14.3		
51-60	14	16.7		
Total	**84**	**100.0**		

The table shows the total number of subjects are 84 academicians .Table 4.1 shows that Age range of the academicians is from 23 to 60 years; there are 13 academicians (15.5%) at 23-30 years, (53.6%) 45 academicians at 31-40 years,(14.3%) 12 academicians at 41-50 years, and (16.7%)14 academicians 51-60 years. The mode and median of age is at 31-40 years. These show that most of the subjects are in 31-40 age groups (53.6%), while age 41-50 years is the least (14.3%). The table illustrate about the prevalence of the work-related musculoskeletal symptoms at any time during the last 12 months from the highest to the lowest. It is followed by shoulder (42.9%), neck (40.5%), lower back (27.4%), knees (26.2%), ankle/feet (22.6%), wrists/hand (15.5%), hip/thigh (11.9%) and elbow (6.0%). The highest prevalence of work-related musculoskeletal symptoms for the last 12 months is at the shoulder (40.5%), while the lowest is at elbow (6.0%). The top three regions highest in prevalence is shoulder (42.9%), neck(40.5%), lower back (27.4%). The least three region in prevalence is elbow (6.0%), hip/thigh (11.9%) and wrists/hand (15.5%). The body region that has the highest symptoms at any time during the last 7 days is Neck (23.8%) while the lowest is also elbow again (2.4%) among all the regions. The top three regions highest in prevalence is neck (23.8%), shoulder (22.6%) and lower back (21.4%). The least three region in prevalence is elbow (1.2%), hip/thigh (6.0%) and wrists/hand (6.0%).By comparing this result with studies on school teachers, this result (73.8%) is within the range of school teachers which is 40% to 95%. This results suggest that most of the academicians in university is at high risk of develop WMSD according to the relative body regions. The results investigating the body region that have work-related musculoskeletal symptoms at any time during the last 12 months, whether that symptom affects normal activity and whether it presents during the last seven days. Shoulder, neck and lower back had commonly become the top three highest regions. The three common body regions of top three highest ranking are similar with the systemic review of WMSD among school teachers12. Both the academicians and the primary and secondary school teachers are having the same three common work-related musculoskeletal symptoms on shoulder, neck and lower back with slight difference in the ranking. The contributed factors are prolonged sustained sitting during reading, marking assignment, office meeting; prolonged standing up during teaching sitting13. The second result is the prevalence of the work-related musculoskeletal symptoms at any time during the last 12 months. From the 73.8% of academicians, the top three regions that having the highest ranking is shoulder (42.9%), neck (40.5%) and lower back (27.4%). Next, the body region that has the highest symptoms at any time during the last 7 days is neck (23.8%), shoulder (22.6%), and lower back (21.4%). Erick and Smith (2011) stated that the neck region is the first most common region to have symptoms . The ranking result of the body region that has the highest symptoms at any time during the last 7 days which is neck (23.8%) is parallel to the finding of Erick and Smith12. The ranking for the result during the last 12 months is slightly different due to different methodology used in various articles. Some articles investigate whole body region, some just specifically check

Table 2

Have you at any time during the last 12 months have trouble in :			
Parts of body	**Frequency**	**Percentage (%)**	**Ranking**
Neck	34	40.5	2
Shoulder	36	42.9	1
Upper Back	23	27.4	4
Elbow	5	6.0	9
Wrists/Hand	13	15.5	7
Lower Back	32	38.1	3
Hips/Thigh	10	11.9	8
Knees	22	26.2	5
Ankle/Feet	19	22.6	6

During the last 12 months,have you been prevented from carrying out normal activity because of this trouble in:			
Parts of body	**Frequency**	**Percentage (%)**	**Ranking**
Neck	10	11.9	2
Shoulder	8	9.5	3
Upper Back	7	8.3	4
Elbow	1	1.2	7
Wrists/Hand	5	6.0	6
Lower Back	17	20.2	1
Hips/Thigh	5	6.0	6
Knees	7	8.3	4
Ankle/Feet	6	7.1	5
Have you at any time during the last 7 days have trouble in:			
Parts of body	**Frequency**	**Percentage (%)**	**Ranking**
Neck	20	23.8	1
Shoulder	19	22.6	2
Upper Back	12	14.3	4
Elbow	2	2.4	8
Wrists/Hand	5	6.0	7
Lower Back	18	21.4	3
Hips/Thigh	5	6.0	7
Knees	6	7.1	6
Ankle/Feet	10	11.9	5

Discussion

There is also a lot of study which presented similar prevalence. Shoulder and neck pain among the academicians also can contribute by writing on the whiteboard, sometimes overhead and using computer[14]. This study found most of the academicians preferred using both projector and whiteboard (63.1%). Besides, there are more academicians choose to used projector (29.8%) than whiteboard (7.1%). The use of projector compare to writing on whiteboard might be able to reduce the incidence of shoulder and neck pain during teaching. The teaching instrument among the academicians and teacher in Malaysia most likely is different. The academician writing on the board is comparatively lesser but more on using the projector while teaching. The usage of computer might be more for the academicians such as typing in repetitive movement and reading assignments compare to manual marking on paper. The prevalence of neck pain among school teachers is significant positive associated with improper and static postures, sustained sitting and standing[15]. Other risk factors that were significant associated with neck pain are age, female, years of teaching, regular exercise and job satisfaction. Besides that, chronic neck pain was associated with multiple joint pain and daily living activity limitation. Incidence of neck pain increased with the amount of neck flexion. Regarding the low back pain, academicians found to have lower prevalence (27.4%) compare with primary school teachers of Klang valley, Malaysia (40.4%) in a similar cross-sectional study by using Nordic Questionnaire[16]. The main factor that contribute to low back pain among the primary school teachers is lifting load (28.0%);second is prolonged sitting (25.2%).Besides, poor mental health (OR 1.11, 95% CI 1.06-1.15) was also is the risk factor to low back pain. This might indicate that the academicians have a lesser or different risk factor of low back pain .Next, the academicians probably have lesser lifting load factor compare to primary school teachers. The used of teaching and assignment material of university are mostly in electronic form while the school in Malaysia mostly used books. The needs of carrying

books and students assignments probably are higher compare to academicians in university. The student age group for academicians and primary school teachers is much different as the university students are relatively independent compare to young children. The risk lifting load might be reduce for academicians as it could be assisted by the students in university during teaching or co-curriculum activities. The lifting work most likely need to be done by the teachers themselves in school as the students are still young children . The availability of the office also found to be significantly associated with low back pain[13]. Teacher who does not have their own office in the school are more likely to develop low back pain compare to those who have (AOR=0.52, 95%CI: 0.33, 0.81). This explained other reason for the lower prevalence of lower back pain among academicians compare to teachers. The working environment of the academicians in private university most likely is better compare to usual government school in Malaysia. Design and type of the chair and table size could affect the sitting posture increase risk of low back pain. Better ergonomic design of chair would provide better rest for the academicians in sitting and after teaching in standing. In terms of psychological factor, teachers who were unhappy with their working culture and environment were 1.82 times more likely to experience LBP (AOR=0.55, 95%CI: 0.36, 0.86)[13]. According to the previous symptoms presented, the next finding is the body region that has been prevented from carrying out normal activity at any time during the last 12 months. Lower back (20.2%), neck (11.9%), shoulder (9.5%) is the top three body region that are disabling among the academicians in university. These three regions again ranked as the top three as the first result. However, lower back is the most disabling region among all. Low back pain disability was significant associated with female gender (OR: 2.47, 95% CI: 1.52-3.99, $p<0.001$) and history of low back injury (OR: 3.01, 95% CI: 1.92-4.74, $p<0.001$) among teachers in Botswana. Besides, low back pain disability was also associated with psychosocial job demands, job insecurity and supervisor support. However, not all factors remained statistically significant when evaluated in the logistic regression model[12]. In terms of activity limitation, standing and ADLs are found to be more disabling compare to other activities among the teachers[16]. The work performance and activity limitation related to low back pain among the academicians should be studied in future. Next, elbow, wrist/hand and thigh/hips are commonly rank as three lowest prevalence among all nine regions. The elbow is the least among all nine regions at any time during the last 12 months (6.0%), last seven days (2.4%) and affecting normal activity during the last 12 months (1.2%)17. also found that elbow and wrist have lower prevalence. For thigh/hips, the pain of this region and the lower extremities are least to be concern even among the teachers as there is no study specifically study about the work-related musculoskeletal symptoms in thigh/ hips. The might explained by the prevalence of thigh/ hips is relatively low compare to other regions.

Conclusion

The main result shows the prevalence of work-related musculoskeletal symptoms among academicians in university is as high as 73.8%. This findings suggest that most of the academicians in university is at high risk of develop work-related musculoskeletal disorder especially on shoulder, neck and lower back region with slight variance in ranking. Shoulder is the highest at any time during the last 12 months .Neck has the highest prevalence at any time during the last seven days. Lower back is found to be affecting normal activity at any time during the last 12 months. Next, elbow, wrist/ hand and thigh/hips are three common region ranked as the lowest prevalence. Elbow is the least among all nine regions at any time during the last 12 months, last seven days and affecting normal activity .As conclusion, the academicians in university Malaysia is at risk of having work-related musculoskeletal symptoms as other teaching professional. This study presented the prevalence of the work-related musculoskeletal symptoms among academicians in university .It might use to apply to other academicians and other educators in Malaysia as well. According to the result in this study, we will be able to identify that shoulder, neck and lower back region that academicians suffer from work-related musculoskeletal symptoms . The information analyzed under this study would be useful in understanding the prevalence and given a direction to investigate the possible risk factor among academicians in develop evidence based practice for education on preventive measures in workplace and ergonomic guideline for academicians to prevent work-related musculoskeletal symptoms.

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Pre-Clinical Study of General Toxicity of the Medication "Fitin-S"

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Abstract

A pre-clinical study of the general toxicity of the medication "Fitin-S" has shown that it is referred to class IV of low toxic compounds. The medication "Fitin-S" does not have aaccumulative and local irritant action. At multiple intramuscular administration to rats and rabbits it does not influence the behavior and the dynamics of animals growth, does not cause a toxic effect on the composition of peripheral blood, kidneys' and liver's function as well as on the pathomorphology of animals organs and tissues. There is a slowdown in the process of blood coagulation in rabbits at doses of 16 and 32 mg/kg during the whole study period. In 1 month of a recovery period all the indicators have been changing within physiological norm. All the aforementioned data allows us to make a conclusion that the medication does not cause a toxic effect on the organism of animals.

Keywords: *General toxicity, medication "Fitin-S", acute toxicity, cumulation, chronic toxicity, local irritant action, pathomorphological study.*

Introduction

The purpose of studying the medication "Fitin-S" is to establish the nature and severity of its damaging effects on the body of experimental animals and assess its safety.

Materials and Method

The study of acute toxicity of the medicationshas been carried out according to the method of Litchfield and Wilcoxon on white mice, both sexes, weighing 20±2.0 g of 6 animals in each group, 150 mice has been used in total[2]. All pharmacological studies have been performed on healthy sexually mature animals (mice) exposed to the quarantine of at least 10-14 days[1].

The medication tested has been injected intravenously, subcutaneously and intramuscularly as a single dose of 100 to 10000 mg/kg. The animals were monitored hourly during the first day of the experiment in the laboratory, while the survival rate during the experiment, general condition, possible convulsions and death were used as indicators of the functional state of the animals. At the end of the experiment, the average lethal dose (LD50) has been calculated and the toxicity class has been determined[3]. The study of accumulation of "Fitin-S" has been carried out according to the Lim's method, which allows to evaluate not only accumulation, but also addiction. The objective of the study was to identify a possible accumulative property in the solution of the medication tested. The trials have been performed on 10 mice of both sexes weighing 20±2.0 g. The medications have been administered intramuscularly according to the scheme for 24 days from 0.1 to 1.15 from LD_{50}[4].

The general toxic action of "Fitin-S" at multiple intramuscular administration has been studied in 40

non-linear white rats (males and females) with the initial weight of 110±10 grams and in 16 gray-colored rabbits of both sexes with the initial weight 2,0±0,2 kilograms. Animals were in vivarium on a normal diet. The trial animals have been divided into 4 groups: 10 rats (5 males and 5 females) and 4 rabbits (2 females and 2 males) in each. The medication has been administered intramuscularly daily (7 days a week) for 30 days to rats at doses of 20, 40 and 100 mg/kg and to rabbits at 8.0, 16.0 and 40 mg/kg. The control animals have been injected with distilled water in a bioequivalent volume. All animals have been kept in standard vivarium conditions on a full-fledged food and water diet.

The condition of animals during chronic intramuscular administration has been evaluated by the following parameters of peripheral blood: haemoglobin contents, gr/dl; the number of red blood cells,10^{12}/l; reticulocytes,%$_0$; thrombocytes, 10^9/l; leucocytes,10^9/ land leukogram; liver function evaluation - by glucose content,mmol/l; total protein,gr/dl; alanine - and aspartate - aminotransferases (AlAT, AsAT) in blood serum. Biochemical tests have been performed using test kits from CypressDiagnostica, Belgium. Renal function has beenevaluated by diuresis with a water load (5% of body weight) for 4 hours, blood urea, urine acidity (pH), the presence of nitrites, glucose, leukocytes etc. in the urine (qualitative reactions) with the Combina kit, Germany, and by urinary sediment.

Hematological and biochemical studies were performed after 10 and 30 days post the day of the experiment. At that time animals were weighed. At the end of the experiment some animals have been decapitated and the material has been taken for pathomorphological studies according to the list approved by Pharmacological Committee of the Republic of Uzbekistan[5]. The rest of animals has been left to study the recovery process.

The general direction of changes in the coagulation process under the influence of medication has been assessed by the records of thromboelastograms performed on the thromboelastograph Tromb-2.

The "open field" test is a study of behavior due to the animal being in an unfamiliar open space, which it cannot leave. The animal is put in the central square, and the latent period of exit from it is recorder. The number of squares into which the animal entered is registered (horizontal motion activity - HMA), as well as the number of rises on the hind limbs ("upright posture" – VMA), the number of holes the animal sniffed(exploratory activity - EA), the number of washes (number of grooming acts -NGA) and defecations (number of bolus - NB). Based on these parameters, motion activity, orientative-motion activity, anxiety and autonomic reactions are evaluated. The above parameters are fixed for 4 minutes from the moment of placing the animal[7].

The conjunctival sample is a very sensitive test and, in some cases, even discloses the reaction of animals to an allergen at mild allergization and negative skin tests. The trials have been performed on 12 rabbits, weighing 2,0-2,5 kg, which have been instilled with 0,1 ml of 0,5 and 5% medication solution in the left eye, and with 0,1 ml of distilled water in the right eye (a controlled one). The reaction has been recorded in 15 minutes (quick reaction) and in 24-48 hours (delayed hypersensitivity) and evaluated according to the following scale (in points). Besides, the level of hyperemia, swelling, and lacrimation have beenregistered[8].

The local-irritant action of "Fitin-S" has been studied on 12 rats weighed 150±10 grams. The animals have had their hair clipped on both sides of the spinal column (4 areas) 2x2 cm in size.0,5 ml of Fitin-S in 0,5 and 5% solutions have been applied on two clipped areas on the left side of the back. The medication has been applied for 10 days. The controlled areas were the right clipped areas to which distilled water has been applied in the same volume. The monitoring has been carried out for 14 days. The skin reaction has been recorded daily according to the skin sample scale in points[8].

Statistical processing of the obtained data has been carried out with the definition of the Student's criterion using statistical programs WindowsExcel 2010.

All experimental animals have been kept in the same conditions and on a common diet with free access to water and food.

Results

The general action and "acute" toxicity of "Fitin-S" have been determined in mice at a single intravenous, intramuscular and subcutaneous administration. Each dose of the substance has been studied in 6 animals. The monitoring has been conducted for 14 days.

The medication has been administered to mice intravenously at doses of 200, 300, 400, 500 and 600 mg/kg in the form of a 0.5% solution, intramuscularly and

subcutaneously at doses of 100-500-1000-2500-2700-2800-2900-3000-3300-3600 mg/kg as a 5% solution.

When studying the acute toxicity of the medication "Fitin-S" with intravenous administration, a 5% solution of the medication has been diluted with 0.9% sodium chloride in a ratio of 1: 1; 1: 5 and 1:10. The obtained solutions of the "Fitin-S" medication have been administered intravenously at doses from 100 to 400 mg/kg in a volume not exceeding 0.5 ml per animal weight[3]. As can be seen from the data in table 1, after administration of the medication at doses causing toxic phenomena, convulsions and death of some mice have been observed within 72 hours. The average lethal dose (LD50) of intravenous administration of the "Fitin-S" medication to mice has amounted to 347 (320 ÷ 460) mg/kg.

The results of studies with subcutaneous and intramuscular administration have shown that "Fitin-S" at doses of 100-1500 mg/kg does not cause changes in the general condition of the animals. When the medication is administered at doses of 1800-2500 mg/kg, after 30-60 minutes there has been a decrease in the motion activity of animals, while the response of mice to external stimuli has remained unchanged, and no lethal outcome has been observed. With the introduction of the medication at doses of 2800-3600 mg/kg after 60-120 minutes, anxiety of mice, shortness of breath, short-term convulsions and the death of some animals during the day has been registered.

Table 1: The results of indicators of "acute" toxicity during parenteral administration of the medication "Fitin-S" to mice

Animal species, type of administration	Sex	Dose mg/kg	Number of animals in a group/number of animals died	LD_{10} -m+m mg/kg	LD_{16} -m+m mg/kg	LD_{50} -m+m mg/kg	LD_{84} -m+m mg/kg
Mice, intravenous	Males	150	6/0	233 -4,8 +6,0	294 -6,0 +7,6	347 -9,3 +13,1	460 -9,7 +12,3
		200	6/2				
		250	6/4				
		300	6/5				
		400	6/6				
Mice, intramuscular	Males	1800	6/0	2000 -380 +450	2266 -300 +330	2560 -350 +380	2893 -500 +550
		2000	6/0				
		2200	6/2				
		2500	6/3				
		2800	6/5				
		3000	6/6				
Mice, subcutaneous		2200	6/0	2240 -300 +330	2566 -320 +420	2900 -340 +380	3277 -440 +480
		2500	6/1				
		2900	6/3				
		3000	6/4				
		3300	6/6				
		3600	6/6				

Discussion

Thus, the study of the acute toxicity of "Fitin-S" has shown that the medication is referred to class IV of low toxic compounds. The LD_{50} during intravenous, intramuscular and subcutaneous administration to mice has amounted to 347(320÷460) mg/kg respectively, 2560 (2266÷2893) mg/kg and 2900(2566÷3277) mg/kg.

When choosing the doses for the study of chronic toxicity of pharmacological substances their accumulative effect should be taken into account. That's why before conducting experiments on chronic toxicity we have been studying the accumulation via sub-chronic toxicity according to the method of Lim and co-authors which allows to evaluate not only accumulative properties but also addiction, and we have determined the accumulation index, the ratio of LD50 with a single administration to LD50 with a multiple administration.

The studies conducted have shown that the accumulation index $K_к$ has amounted to$K_к=LD_{50n}/LD_{50,}$ 2944/2560=1,15. In other words, the medication "Fitin-S" does not have the accumulative effect, the accumulation rate is 1,15.

The local-irritant effect of the medication has been studied on rats and rabbits. The results of monitoring have shown that "Fitin-S" in 5 and 10% concentrations does not cause even the slight reddening of the conjunctiva of the rabbit eye in either 15 minutes or 24 or 48 hours. Application of the medication for 10 days on the skin of rats also does not cause irritation, redness, swelling or other visible changes on the skin and the effect of "Fitin-S" is estimated at 0 points.

According to this a conclusion that the medication "Fitin-S" in 5 and 10% concentrations does not have the irritant effect on the conjunctiva of rabbits and on the skin of rats can be made.

The results obtained at daily intramuscular administration of "Fitin-S" to rats and rabbits are represented in Tables 3-4. As can be seen from the data given in tables 3-4, in 10 and 30 administration at all the studied doses there has been no significant deviations in the composition of the peripheral blood of rats and rabbits in the hemoglobin content of erythrocytes, in the number of blood cells themselves, their precursors (reticulocytes), and leucocytes. Leukocyte counts in rats and rabbits are within the physiological norm.

Based on this, it can be concluded that "Fitin-S" at the studied doses with intramuscular repeated administration does not have a toxic effect on the quantitative composition and morphology of the peripheral blood of experimental animals.

Table 2: Indicators of peripheral blood of rats at daily intramuscular administration of the medication "Fitin-S" (M±m; n=10)

Indicators	Control	10 days, dosemg/kg			30 days, dosemg/kg		
		20	40	100	20	40	100
Hemoglobin, gr/dl	14,7±0,3	15,8±0,7	15,9±1,0	15,0±1,0	14,8±0,2	14,0±0,1	13,3±0,2
Erythrocytes, 10^{12}/l	5,2±0,5	5,8±0,5	5,0±0,6	5,8±0,4	5,1±0,3	4,8±0,2	5,0±0,6
Reticulocytes, %	4,2±0,2	5,4±0,3	5,8±0,3	3,9±0,3	6,5±0,4	6,5±0,5	4,3±0,4
Thrombocytes,10^9/l	675±11,5	830±12,5	600±11,0	550±10,5	675±12	725±14	725±14
Leucocytes,10^9/l	10,2±0,8	11,6±0,8	12,2±0,9	13,3±0,7	8,4±0,6	9,1±1,2	11,9±0,7
Neutrophils:							
Bands, %	1,8±0,2	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2
Segmentonuclear, %	25,0±1,0	27,4±0,2	27,2±0,8	27,5±1,0	28,5±1,5	28,7±1,3	28,5±1,5
Eosinophils, %	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,3	2,0±0,2	2,0±0,2
Monocytes, %	3,0±0,3	2,6±0,2	2,9±0,3	3,0±0,3	3,0±0,3	3,0±0,3	3,0±0,3
Lymphocytes, %	64,2±1,8	66,0±3,4	66,8±3,2	65,5±3,5	65,0±1,6	64,3±2,7	64,5±1,5

*P < 0,05 to control

Table 3: Indicators of peripheral blood of rabbits at daily intramuscular administration of the medication "Fitin-S" (M±m; n=4)

Indicators	Control	10 days, dosemg/kg			30 days, dosemg/kg		
		8	16	40	8	16	40
Hemoglobin, r/dl	9,6±0,3	9,9±0,7	12,0±0,5	11,2±0,1	9,2±0,7	8,4±0,5	8,4±0,1
Erythrocytes, 10 ^{12}/l	4,5±0,5	4,1±0,5	5,0±0,6	4,7±0,8	4,3±0,5	4,4±0,6	4,0±0,8
Reticulocytes, %	3,5±0,4	4,0±0,6	3,2±0,5	5,5±0,5	4,4±0,6	3,2±0,2	4,0±0,3
Thrombocytes,10^9/l	350±11,5	250±6,5	225±13,0	400±8,5	250±6,5	325±13,0	400±8,5
Leucocytes,10^9/l	8,0±0,8	8,0±0,6	8,25±0,9	5,4±0,4*	9,4±0,6	9,0±0,9	8,4±0,4
Neutrophils:							
Bands, %	1,8±0,2	2,2±0,2	1,9±0,2	2,0±0,2	1,8±0,2	2,0±0,2	1,9±0,2
segmentonuclear, %	25,0±1,0	27,4±0,2	27,2±0,8	27,5±1,0	27,0±0,2	27,2±0,8	27,5±1,0
eosinophils, %	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2	2,0±0,2
basophils, %	1,0±0,1	0,8±0,6	1,1±0,1	1,0±0,1	1,2±0,1	1,1±0,1	1,0±0,1
monocytes, %	3,0±0,3	2,6±0,2	2,9±0,3	2,8±0,3	2,6±0,2	2,8±0,3	2,9±0,3
lymphocytes, %	63,2±1,8	65,0±3,4	65,8±3,2	64,7±3,5	65,0±3,4	65,8±3,2	64,5±3,5

*P < 0,05 to control

Diuresis after 10 days and 30 days of the experiment under conditions of water load did not differ significantly in the experimental and control rats. In rats, the state of nitrogen metabolism, the criterion of which is the urea content in the blood serum, did not deviate from the control after 10 days and 1 month of the experiment. In the urine during the entire experiment, both in the experimental groups and in the control group, no changes in the studied urine parameters have been detected. The acidity of urine in all the studied groups has not been changing during the entire experiment and its pH was 6.0-7.0. No pathological changes in urine sediment have been detected.

The results obtained have led to the conclusion that the medication "Fitin-S" with chronic administration does not have a toxic effect on renal function in the studied doses.

The liver function in experiments on rabbits and rats has been studied with the use of the most sensitive tests. The levels of total protein, glucose, and enzyme activity (AsAT, AlAT) in the blood serum of all experimental rats after 10 and 30 days of the experiment have not differed from the control (Table 4.5).

Thus, the medication "Fitin-S" at intramuscular daily administration at the studied doses does not have a toxic effect on liver function.

Table 4: Some indicators of liver function of rats at intramuscular administration of the medication "Fitin-S" in a chronic trial (M±m; n=5)

Study timeline	Dose mg/kg	Total protein, gr/l	AlAt, mmol/l	AsAt, mmol/l	Glucosemmol/l	Urea mmol/l
10 days	Control	84±5,3	0,26±0,03	0,35±0,04	5,1±0,4	4,4±0,42
	"Fitin-S" 20	78±5,2	0,28±0,02	0,35±0,03	5,62±0,4	4,6±0,4
	40	82±5,5	0,22±0,02	0,27±0,02	5,9±0,4	4,4±0,42
	100	82±5,2	0,21±0,02	0,39±0,04	5,9±0,4	4,6±0,4
30 days	Control	72±5,6	0,35±0,03	0,40±0,03	5,6±0,4	4,2±0,4
	"Fitin-S" 20	86±5,6	0,3±0,02	0,36±0,03	5,5±0,4	4,3±0,4
	40	76±6,0	0,28±0,02	0,36±0,02	5,6±0,4	4,5±0,42
	100	76±6,0	0,28±0,02	0,4±0,03	5,5±0,4	4,6±0,4

P > 0,05 to control

Table 5: Some indicators of liver function of rabbits at intramuscular administration of the medication "Fitin-S" in a chronic trial (M±m; n=5)

Study timeline	Dose mg/kg	Total protein, gr/l	AlAt, mmol/l	AsAt, mmol/l	Glucosemmol/l	Urea mmol/l
10 days	Control	84±5,3	0,16±0,03	0,25±0,04	2,1±0,4	4,4±0,42
	"Fitin-S"-8	68±5,2	0,18±0,02	0,22±0,03	0,62±0,4	4,6±0,4
	16	82±5,5	0,18±0,02	0,27±0,02	1,9±0,4	4,4±0,42
	40	82±5,2	0,21±0,02	0,3±0,04	2,9±0,4	4,6±0,4
30 days	Control	72±5,6	0,25±0,03	0,32±0,03	5,6±0,4	4,2±0,4
	"Fitin-S"-8	86±5,6	0,3±0,02	042±0,03	5,5±0,4	4,3±0,4
	16	76±6,0	0,26±0,02	0,36±0,02	5,6±0,4	4,5±0,42
	40	76±6,0	0,28±0,02	0,34±0,03	5,5±0,4	4,6±0,4

P > 0,05 to control

As the medication "Fitin-S" is supposed to be applied parenterally, it was important to find out how the process of blood coagulation changes at multiple application. The experiments have been held on 12 chinchilla rabbits, males and females weighing 2,0±0,2 kg. The blood has been studied in 10 and 30 days post the administration of "Fitin-S" at doses of 8,0, 16,0 and 40,0 mg/kg.

The results of the impact of the medication "Fitin-S" on the process of blood coagulation are represented in table 6. Thromboelastogram data indicate that after 10 and 30 administrations, "Fitin-S" does not affect the blood coagulation process at a therapeutic dose of 8 mg/kg. With an increase in the therapeutic dose by 2 and 5 times (16 and 40 mg/kg), the blood coagulation process changes, which is expressed in an increase in the indicator K, the time of clot formation, which depends on the concentration of thrombin formed and the amount of fibrinogen; and a decrease in MA, a decrease in the elasticity of the clot (E) and the index of hypercoagulation Ci, which indicates a slight decrease in the process of blood coagulation.

Table 6: The dynamics of changes in blood coagulation of rabbits with chronic administration of the medication "Fitin-S" M±m; n=4)

TEG Indicators	Control	In 10 days/dosemg/kg			In 30 days/dose mg/kg		
		8,0	16,0	40,0	8,0	16,0	40,0
R, mm	50±2,1	40±2,0	45±1,0	50±1,0	40±2,0	45±1,0	50±1,0
K, mm	15±1,0	15±1,1	25±1,0	30±0,6*	15±1,1	25±1,0*	30±0,6*
R+K, mm	65±3,5	55±3,0	70±3,0	80±6,6	55±3,0	70±3,0	80±6,6
R/K	3,3±0,2	2,8±0,1	1,8±0,1	1,7±0,1*	2,8±0,1	1,8±0,1*	1,7±0,1*
MA, mm	69±1,5	66±1,0	64±1,0	62±1,4	66±1,0	64±1,0	62±1,4
t, mm	100±10	100±10	100±10	100±10	100±10	100±10	100±10
S, mm	115±11	115±10	125±11	130±12	115±10	125±11	130±12
T, mm	165±14	155±14	170±15	180±16	155±14	170±15	180±16
Ci (MA/R+K)	1,1±0,1	1,2±0,1	0,9±0,1	0,8±0,06	1,2±0,1	0,91±0,1	0,8±0,06
E, MAx100/100-MA	222±19	194±16	178±15	163±14	194±16	178±15,0	163±14
ITC (E/S)	1,9±0,1	1,7±0,1	1,4±0,1	1,26±0, *	1,7±0,1	1,4±0,1	1,26±0,1*

*P ≤ 0,05 to control

Thus, the intramuscular administration of the medication "Fitin-S" to rabbits at a therapeutic dose of 8 mg/kg does not cause anyeffect, andat doses of 16.0 and 40.0 mg/kg leads to a slight slowdown in blood coagulation process throughout the entire period of administration (30 days).

The study of the effect of the medication "Fitin-S" on the central nervous system and peripheral nervous system in the open field test has shown that after 10 days of administration of "Fitin-S" for 4 minutes, it has not changed the horizontal and vertical motion activity of rats at doses of 20, 40 and 100 mg/kg. After 30 days of the experiment, a sedative effect has been observed, which was expressed in a reduction in the number of intersections (horizontal activity) and the number of postures (vertical activity) and the number of "holes" (exploratory activity). Throughout the entire experiment, "Fitin-S"has not significantly affected the vegetative reactions of the body (the number of "boluses", the number of washings).

Table 7: Impact of "Fitin-S" on behavioral reactions of animals in the "open field" test (M±m; n=6)

Medication, dose, mg/kg	Latentperiod, sec	Motion activity		Quantity		
		Horizontal, number ofintersections	Vertical, number ofpostures	Holes	Washes	Bolus
In 10 days						
Control	1,0±0,1	46,3±3,4	5,3±0,4	9,7±1,0	1,0±0	1,0±0,1
"Fitin" C-20	0,5±002	38,0±2,6	5,5±0,4	8,0±0,4	1,3±0,1	1,8±0,15
40	0±0	43,0±2,4	5,5±0,4	10,8±0,8	1,0±0,1	1,3±0,11
100	0,75±002	49,0±3,6	7,8±0,6	6,0±0,4*	0,3±0,01	0,4±0,04
In 30 days						
"Fitin" C-20	2,2±0,2	14,0±1,0*	2,5±0,2*	2,5±0,2*	0,45±00,2	1,0±0,3
40	3,8±0,2	32,3±3,0	3,6±0,2*	4,8±0,4*	0,6±0,04	1,4±0,1
100	1,0±0,1	26,0±2,3*	2,0±0,2*	5,0±0,4	1,3±0,1	0,5±0,02

*P ≤ 0,05 to control

The study of integral indicators has shown that the dynamics of the body weight of the experimental animals at the studied doses after 10 and 30 days of the experiment has not differed from the control. Throughout the experiment animals were active, neat, they ate food normally, drank water, their hair was smooth, shiny. The behavior of experimental rats has not differed from the behavior of control groups of animals.

A macroscopic examination of sacrificed animals has disclosed the correct location of the internal organs. There is no free fluid in the pleural and abdominal cavity. Tissues of the lungs, stomach and intestines are also of a normal color, without signs of edema, hemorrhages and ulcerations. Pancreas, kidneys and adrenal glands are unchanged.

At the end of the experiment, 1 cm^3 of organs has been taken from experimental animals for histological examination. This site has been fixed in 10% normal formalin. Then the tissue has been embedded in paraffin and sections of histological preparations 3-5 microns thick have been prepared and stained with a mixture of hematoxylin + eosin. The obtained preparations have been analyzed under a Leica-1000 DM microscope (Germany), I0x eyepiece, 10x and 40xlens [6]

The results of histological studies have shown that basically their histostructure is comparable with the morphology of control groups of animals. In the stomach, the mucous membrane is well preserved, glandular cells are without any changes. In the small intestine its main elementsare villi and crypts. They are well prominent, villi are finger-shaped, the border of enterocytes is distinguished, the stroma of the villi and the epithelial layer are moderately saturated with lymphocytes, are distinctly distinguished in the enterocytes. In the colon, the crypts are lined with mucous cells, the lumens of the crypts are enlarged and filled with mucoid secretion.

In the liver of experimental animals treated with the medication, no significant changes have been found. In experimental animals at the studied doses, the majority of hepatocytes are in a state of hydropic edema with granular inclusions, and moderately medium-droplet fat inclusions on the periphery. In addition, small foci of cellular infiltration have beendisclosed in the liver parenchyma in some areas. Basically, as usual, the boundaries of the lobules are not distinct in the liver, the hepatocytes are polygonal in shape, their cytoplasm is pink and contains a large, roundish one or two nuclei. In stellate macrophages, small grains of hemosiderin have also been found, as well as in the control. The structure of the various departments of the nephron, renal corpuscles, proximal and distal parts are without any changes. The structure of the capsule and cellular elements of all departments of the nephron indicates their functional viability.

White and red pulp are seen in the spleen; the structure of these elements does not differ significantly from that of control animals. The study of the structural organization of the myocardium is indistinguishable from indicators in the control group, consists of rectangular cardiomyocytes, with a weakly pronounced transverse striation.

The lungs have a characteristic pattern and are represented by numerous dilated alveolar vesicles and atelectasis sites. The interalveolar walls contain numerous blood capillaries, the walls of the bronchi and blood vessels are without any significant changes.

The histology of the thymus is represented by the cortical and brain zones, with signs of age-related involution. The cortical zone is represented by lymphoid cells and trabecular interlayers. Also, the brain area is represented by lymphoid follicles, without clear centers of reproduction.

In this regard, it should be noted that the differences found are a manifestation of the species or age characteristics of animals.

Thus, the results of histological studies allow us to conclude that “Fitin-S” at the studied doses is the optimal medication, its long-term use does not cause noticeable structural destruction or damage and can be recommended for further clinical trials.

Conclusion

Pre-clinical study of the general toxicity of the medication “Fitin-S” has shown that it is referred to class IV of low toxic compounds. The medication “Fitin-S” does not have anaccumulative and local-irritant effect. With repeated intramuscular administration to rats and rabbits, it does not affect the behavior and dynamics of animal weight, does not have a toxic effect on the composition of peripheral blood, kidney and liver function, and also on the pathomorphology of animal organs and tissues. With repeated use, there is a slight slowdown in the blood coagulation process in rabbits at doses of 16 and 32 mg/kg throughout the study period. After 1 month of the recovery period, all these indicators have been changing within the physiological norm. All of the above data allow us to conclude that the medication does not have a toxic effect on the animal organism.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

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Hemodynamic Shifts in Children During the Induction into Narcosis Period

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Abstract

The article focuses on the study of hemodynamics of children during the period of induction into anesthesia. The analysis of the study results has been carried out – 80 children aged 3 to 14 years undergone medical procedures on diseases of abdominal cavity and anorectal area. The study results have shown that the combination of sevoflurane and ketamine in small doses has caused an effective analgesic effect with a significant vegetative stabilizing effect providing a stable course of the following stages of anesthesia and postanesthetic period.

Keywords: *Combined anesthesia, induction period, measurements of central hemodynamic, inhalation anesthetics, fentanyl, ketamine.*

Introduction

Induction narcosis is the most essential stage of anesthesia. Mistakes and inaccuracies during the induction narcosis[2] and its complications may become the very reason for the chain of abnormities that entail a negative outcome in the future[7]. It is known that medicines applied during the induction period are not neutral for the organism and might become the reason for serious complications due to hard-hitting medicamental effect of the components[9] of anesthesia on cardiovascular system[6]. Induction period might be accompanied by significant hemodynamic shifts[5]. The most frequent complications of the anesthesia period of children are the disorders of cardiac rhythm and central hemodynamics[11].

The purpose of the work is to study the impact of various types of induction on the condition of central hemodynamics during the surgery on abdominal cavity and anorectal area of children[4].

Materials and Method

The work is based on the analysis of the study results – 80 children aged 3 to 14 years 80. The study has been carried out with children who have undergone surgery because of the diseases of abdominal cavity – 42 children (52,5%) and of anorectal area – 38 children (47,5%).

A complete clinical examination has been held. For the purpose of the objective evaluation of the condition of cardiovascular system a method of echocardiography with the analysis of end-diastolic (EDD) and end-systolic (ESD) dimensions of the left ventricle, R-R interval and ejection time was applied. The study has been carried out using the echocardiograph “Sonoscape”

(China, regnumber cfda 20123231386) of patients on the following stages: prior to surgery (initial), post premedication and post-induction.

Premedication was aimed at eliminating anxiety, preventing nausea and vomiting. Atropine sulfate at a dose of 0.01 mg/kg, diphenhydramine - 0.1 mg/kg, sibazon - 0.25 mg/kg and ketamine - 2.0 mg/kg body weight were prescribed as a part of premedication.

Small doses of ketamine (1 mg/kg) and propofol (1 mg/kg) were administered intravenously to patients of the first group in 30-40 minutes post premedication as well as inhalation of sevoflurane at a dose 2,0 – 3,0 vol%.

Before a skin incision, a fentanyl solution of 3 μg/kg was injected intravenously. The maintenance dose of sevoflurane was 1.5 vol%, the fentanyl dose was 2 μg/kg.

Patients of the second group were exposed to inhalation of isoflurane at a dose 2,0 vol% and intravenous doses of fentanyl of 5 μg/kg and droperidol of 0,4 mg/kg body weight 30-40 minutes post-premedication.

The obtained data has been processed via the set of auxiliary programs Statistica for Windows, Release 5,5 Stat Soft, Inc, with the calculation of arithmetic mean value (M), the average error (m), all digital data has been presented as M±m. The significance of differences was assessed by Student's criterion (t) with a known number of observations (n). The differences were considered significant at $P < 0.05$.

Results

After the premedication patients of the first group has shown an increase in the measurement of the heart rate frequency (HRF) by 6% ($P<0,05$), the tendency to an increase in the measurement of the arterial pressure: systolic arterial pressure (SAP) by 2,3%, diastolic arterial pressure (DAP) by 2,9% (Table.1). During the induction period, propofol and sevoflurane have caused fast and smooth induction, a smooth sleep onset within 30-60 seconds of patients has been registered. While the study period, an insignificant decrease of the heart rate frequency measurement by 4,08% has been noticed compared with similar measurements of the premedication stage. The systolic and diastolic arterial pressure measurements were characterized by the downward tendency respectively by 0,7% and 0,9%.

Table 1. Measurements of the HRF, SAP and DAP at the induction anesthesia period with the use of ketamine, propofol and sevoflurane

Measurements	Study Stages		
	Initial	Premedication	Induction
HRF	108,25±7,46	114,75±8,40*	110,06±6,80
SAP	89±8,20	91,06±9,30	90,43±9,15
DAP	45±7,79	46,31±8,56	45,87±7,50
Sp O2	95,31±0,60	97,56±0,51*	98,25±0,68*

Note: * Reliability of differences in measurements compared to the original ($P < 0,05$).

Discussion

On a premedication stage patient of the second group have shown an increase in the measurement of HRF by 5,5%. SAP measurements - by 3,0%, DAP measurements - by 1,7%. Isoflurane inhalation has caused a slow induction and the solutions of fentanyle and droperidol have been injected intravenously in this connection. After the fentanyle injection, a respiratory depression has been observed, an auxiliary ventilation has been applied and a tracheal intubation has been implemented after the administration of muscle relaxants. Skin cover was warm and had a regular staining whilst the oxygen saturation indicator increased by 3,9%. HRF measurement increased by 2,0%, an insignificant increase in the SAP by 1,2% and DAP by 0,2% compared with similar measurements of the original period has been observed (Table 2).

On the background of the administration of medicines for induction – ketamine, propofol and sevoflurane after the trachea intubation an insignificant change in hemodynamics not negatively affecting a hemodynamic system has been observed. Introduction period of general combined anesthesia with the use of small doses of has been characterized by smooth clinical course.

Table 2. Measurements of the HRF, SAP and DAP at the induction anesthesia period of children with the use of isoflurane, fentanyle and droperidol

Measurements	Study stages		
	Initial	Premedication	Induction
HRF	115±2,38	121,33+ 2,87*	118,93+ 3,93*
SAP	88,40±2,69	91,7±2,87	89,5±4,21
DAP	45,16±2,69	45,92±3,51	45,07±4,32
SpO2	95,38±0,58	97,44±0,6*	99,07±0,73

Note: * Reliability of differences in measurements compared to the original (P<0,05).

When conducting a study of the central hemodynamics measurements of patients of the first group, in the introduction period compared with the similar measurements of the initial period, after the trachea intubation the increase in HRF measurements by 7,6%, CI by 18,5% (P<0,05), some tendency to the increase of SV measurement by 9,3% have been observed. At the same time, a downward tendency to the decrease in the measurements of EF by 5,6% and SPVR by 8,2% has been noted (Table 3).

Table 3. Measurements of central hemodynamics during the anesthesia induction period of children with the use of isoflurane, fentanyle and droperidol (n=40)

Measurements	Initial	Premedication	Induction
EF	70.49±0.85	68.28±1.03	66.55±0.96*
SV	24.59±1.61	22.9±1.31	26.88±1.63
HRF	97.8±2.21	107±2.11*	105.18±1.91*
SPVR	30.29±1.54	32.16±1.77	27.81±1.04**
CI	2.87±0.09	3.02±0.11	3.4±0.1***

Note: * Reliability of differences in measurements compared to the original (P < 0,05).

Table 4 represents the data on the study of central hemodynamics during general anesthesia of children with the use of isoflurane, fentanyle and droperidol. The increase in the measurements of HRF and Specific peripheral vascular resistance.

Table 4. Measurements of central hemodynamics during the anesthesia induction period of children with the use of isoflurane, fentanyle and droperidol (n = 40)

Measurements	Initial	Premedication	Induction
EF	71.96±1.48	71.51±1.17	72.05±1.4
SV	24.87±1.54	23.56±1.31	23.67±1.35
HRF	96.48±2.07	109.15±2.2*	109.9±2.33*
SPVR	29.7±1.41	25.52±1.25*	26.59±1.42
CI	2.81±0.14	3.08±0.15	3.12±0.17

Note: *Reliability of differences in measurements compared to the original (P < 0,05).

In the introduction period of anesthesia after the trachea intubation compared with the premedication stage only a slight tendency to the measurement change has been noted. In comparison with the initial period, only HRF was subjected to a significant change and increased by 13,9%. Only the SV and SPVR measurements had a downward tendency by 4,8% and 10,5% respectively. Subsequent laryngoscopy and intubation do not cause such a pronounced hyperdynamic response as with induction with ketamine and barbiturates. Hypotension was prevented and eliminated by infusion loading.

The results of the existing study show that the combination of sevoflurane with propofol and ketamine in small doses has caused the effective analgesia with a significant vegetative stabilizing effect associated with central suppression of sympatic activity, and has ensured a stable course of subsequent stages of anesthesia and post-anesthesia period. Patients experienced a rapid restoration of the preoperative level of neuropsychic status after surgery.

Conclusions

1. Insignificant changes of the main measurements of central hemodynamics are contingent to the condition of patients during the introduction period, the trachea intubation and the manipulations taken. The vasodilating properties of propofol and sevoflurane suring the introduction narcosis determine a switch hypodynamic reaction of the circulatory system.
2. The evaluation of the clinical course of the introduction period and of the changes of the main measurements of central hemodynamics at conducting the two variants of the combined anesthesia has shown smooth introduction into narcosis and therefore an auxiliary stable course of the subsequent anesthesia stages.
3. Applying small doses of ketamine and propofol coupled with sevoflurane is an optimal method of induction during traumatic and extensive surgery in the abdominal cavity and anorectal area in children.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Comparative Analysis of the Results of Traditional and New Distance Learning Method Among the Master's Degree Students and Physicians in Uzbekistan

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Abstract

The rapid changes in medical theory and practice require physicians to undertake continuing medical education. The low level of qualifications of physicians can eventually lead to medical errors. There is a special currency to measure and evaluate the obsolescence of knowledge, the so-called half-life of knowledge.

Materials and Method: Analysis of results and evaluations using various forms of training method on one topic between 2nd year master's degree students (masters) of Tashkent pediatric medical institute and physicians. Training was conducted traditionally (face-to-face form for periods of each time 1 hundred 10 minutes) and distance-based (internet point of-care learning and journal-based continual medical education). The results of tests among 72 masters and 84 physicians were retrospectively analyzed.

Results: According to the study, it was found that the average rating in the group of Masters via traditional method with an "unsatisfactory" evaluation was 2 (4%) while in the group of physicians with the same test the number with an unsatisfactory evaluation was 9 (21%), (which is four times more). The number of Masters who received a satisfactory evaluation was 12 (23%) (18 physicians received the same evaluation, representing 43%). The number of Masters who got a good evaluation was 23 (43%) and only 10 or 24 % of the physicians got the same score (which is twice less than the result of masters) and 16 (30%) Masters received an excellent rating (5 physicians received the same mark or this was 3 times less).

Discussion: More than half of the physicians (being 27 or 64%) responded to the test with an evaluation of "satisfactory" and "unsatisfactory", while only 14 (26% or 2.5 times less) Masters received such marks. Only 15 (35%) physicians received "good" or "excellent" rating, whereas among the masters, this result was 39 (60% or 2 times higher than the responses of physicians). However, among the participant soft the online program unsatisfactory response was not observed. 8 (42%) participants answered satisfactorily, 11 (58%) of the participants responded with good or excellent marks.

Conclusions: Following the results of the analysis, the study showed that the increase in theoretical knowledge has been identified in a larger number of masters. The knowledge gained through traditional teaching or distance (online and journal form of training) learning, seems virtually identical, and the study seems to indicate that they even surpass the traditional method of training.

Keywords: *Medical error, obsolescence, chronic diseases.*

Introduction

The rapid changes in medical theory and practice require physicians to undertake continuing medical education both on the workplaceas well as at home, with additional financial costs associated with accommodation and meals[1]. There are more than 72000 (10000 population-24) physicians in Uzbekistan and Each physician should improve his/her qualification by earning 288 credits within every 5 years. Initially, physicians have to earn 144 credits at the Tashkent Institute of Postgraduate Medical Education (TIPME),

and the other credits can be earned in other short trainings[2]. In the American literature, there is a special currency to measure and evaluate the obsolescence of knowledge, the so-called half-life of knowledge[4]. As a result of obsolescence of knowledge as new information appears, the competence of physicians is reduced by half in a 3-4 years time period[3]. This rapid obsolescence of knowledge dynamics may be relevant to setting educational standards and planning training program accordingly[5].

The purpose of the study: Analysis of results and evaluations using various forms of training method on one topic between 2nd year master's degree students(masters)of Tashkent Pediatric Medical Institute and physicians.

Materials and Method

Materials are the results of the tests obtained among 136 respondents who have undergone training on "Improving method and cost-effectiveness of prevention of several chronic diseases", consisting of a series of 15 questions, and research method are both mathematical as well as statistical.

To achieve the objective, we, together with the Tashkent Pediatric Medical Institute and the Tashkent Institute of Postgraduate Medical Education (TIPME) have developed a training programme on "Improving approaches and cost-effectiveness of prevention of several chronic diseases. Training was conducted traditionally (face to face form for periods of each time 1 hour and 10 minutes) and distance-based (internet point-of-care learning and journal-based CME).

Results

The results of tests among 72 masters and 84 physicians were retrospectively analyzed (Tab. №1).

Traditional teaching method for masters and physicians. Second year Masters have been trained at the department of "Public Health" in 2014. For physicians,the training was conducted at the Institute of Health and Medical Statistics. After training, each participant was given 20 minutes to answer the questions with the following results. A comparative evaluation of results was carried out among the masters of the Russian group before and after training. Before the training, the average number of correct answers was 10,9 (73%), whereas after the training this figure increased to 11,3 (78%). The masters of the Russian group were informed in advance that they would be tested, whereas the masters of the national groups (Uzbeks) were tested immediately after the presentation without warning. According to test results among national groups, the average number of correct answers was 11,3 (or 75%). The average number of correct answers among the practitioner-physicians after the training was significantly lower than those Masters of Russian and national groups and was 8,7 (58%).

Internet point-of-care learning. To explore and introduce new forms of learning we had put our training program "Improving approaches and cost-effectiveness of prevention of several chronic diseases" on to the website of the Medical Association of Uzbekistan (www.avuz.uz). A web application was developed to save the data. Participation in the test tasks,training and testing weredone on a voluntary basis. After registering, participants received access to training program. Thus, 19 masters and physicians were registered and participated on this topic. All data were saved in the database of the website with the consent of the participants, which included the participation date, the amount of time to answer the tests and the percentage of correct answers. At the initial level, the average number of correct answers was 10,6 (70,6%) spending 7,4 minutes on average, while after the training, the average number of correct answers was 11,7 (73%). However, online learning allows reading, printing of educational material and learning in a convenient location for the physician.

Journal-based training. In 2010-2015, more than 1200 physicians participated in journal-based trainings in Uzbekistan, 1021 of whom have been awarded a certificate. Training theme: "Improving method and cost-effectiveness of prevention of several chronic diseases",was published in the "Bulletin of the Medical Association of Uzbekistan." In the journal form of training 42 physicians participated from various regions of Uzbekistan, including (Tab. №2)

Tables 1: Participants of journal-based training.

№	Region	Number of participants
1	Republic of Kazakhstan	1
2	Bukhara region	8
3	Andijan region	7
4	Surkhandarya region	2
5	Tashkent city	3
6	Tashkent Region	3

№	Region	Number of participants
7	Jizzakh region	5
8	Namangan region	2
9	Kashkadarya region	1
10	Samarkand region	7
11	Navoi region	2
12	Syrdarya Region	1
	Total	42

Journal-based learning is not free of charge: and every physician was required to pay 10 euros. All the successful physicians(65% of the test) were given certificates that could be used in the delivery of documents for obtaining a certificate of qualification.

Discussion

Comparative analysis of the results and assessment (Tab. №3). According to the study, it was found that the average rating in the group of Masters via traditional method with an"unsatisfactory" evaluation was2 (4%) while in the group of physicians with the same test the number with an unsatisfactory evaluation was 9 (21%), (which is four times more). The number of Masters who received a satisfactory evaluation was12 (23%) (18 physicians received the same evaluation, representing 43%). The number of Masters who got a good evaluation was 23 (43%) and only 10 or 24 % of the physicians got the same score (which is twice less than the result of masters) and 16(30%) Masters received an excellent rating (5 physicians received the same mark orthis was 3 times less).

More than half of the physicians (being 27or 64%) responded to the test with an evaluation of "satisfactory" and "unsatisfactory", while only 14(26% or 2,5 times less) asters received such marks. Only 15(35%) physicians received "good" or "excellent" rating, whereas among the masters, this result was 39 (60% or 2 times higher than the responses of physicians).

However, among the participants of the online program unsatisfactory response was not observed. 8 (42%) participants answered satisfactorily, 11 (58%) of the participants responded with good or excellent marks.

There were no candidates with unsatisfactory ratings among the participants of the journal-based training, 6 participants (14%) got a satisfactory rating, 36 (86%) of the participants got good or excellent response. Because of the payment for this type of training, we assume that physicians are more motivated to read carefully the training program and can consult with colleagues before completing and submitting test items for review by experts of Tashkent Institute of Postgraduate Medical Education - this may explain the best result answers.

Conclusions

Following the results of the analysis, the study showed that the increase in theoretical knowledge has been identified in a larger number of masters in contrast to the practicing-physicians, although there is no doubt that the physicians are more experienced. The knowledge gained through traditional teaching or distance (online and journal form of training) learning, seems virtually identical, and the study seems to indicate that they even surpass the traditional method of training. These data show that how physicians are trained and their requirements need to be reviewed with an emphasis on the development of distance learning method of training(online and journal-based form) and also experience can be learned from developed countries.

Ethical Clearance: No ethical approval is needed.

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Conflict of Interest: Nil

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The Role of Robotic Mechanotherapy in the Recovery of Mobility in Patients after an Acute Cerebrovascular Accident

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Abstract

Hemiparesis of various severity is a common type of motor disorder resulting from an experience of a stroke. It is motor disorders that lead to the disruption of such categories of activity as the ability to move and self-care, which entails a limited patient mobility. The study has included 35 patients with motor disorders resulting from acute cerebrovascular accidents (CA), and the patients have undergone medical rehabilitation.

Clinical research method (Ashworth and Rivermead scales) and mathematical statistics have helped assess the positive effects of robotic mechanotherapy, which was used in the rehabilitation of stroke patients.

As a result of rehabilitation measures with the use of robotic mechanotherapy, 68.2% of the examined subjects have developed significant strength in the hand, and 45.5% - in the leg ($p<0.005$). In the remaining subjects, no significant improvement in the motor function has been observed, although mobility has improved in all examined patients and the average Rivermead mobility index has been 11.4±2.1 out of 15 ($p <0.001$).

Keywords: *Acute cerebrovascular accident, stroke, paresis, validated scales, Ashworth scale, Rivermead Mobility Index, robotic mechanotherapy, Armeo Power device, Lokomat device.*

Introduction

Although the issue is very evident, as is the need in finding solutions, data provided by the World Health Organization (WHO) suggests that cerebrovascular disorders of various genesis are still a leading mortality and disablement factor. It should be noted that a number of international authors (Sarti C., Rastenyte D., Cepaitis Z., et al., 2000; Howard G., Howard VJ., Katholi C., et al., 2001; National Center for Health Statistics. Health, United States, 2005: With Chartbook on Trends in the Health of Americans[1]. Hyattsville, 2005; Claire L. Allen, 2008; Shinichiro Uchiyama, Noriaki Nakaya, Kyoichi Mizuno, 2009; Benamer H.TS., Grosset D., 2009) consider the stroke issue a global epidemic, which claims lives and threatens the world. The annual 0.2% (2000 new cases per 1,000,000 of people) increase in stroke incidence, as well as the growing mortality during each following year (4,400,000 deaths), high disability rate, low complete recovery rate, impact nations' socioeconomic indicators[2].

According to statistics, in 2017, cardiovascular disorders were the number one cause of death (59.7%), and the most common causes of these were ischemic heart disease, arterial hypertension and their

complications (myocardial infarction, cerebrovascular accidents, etc.) Men aged 30 to 59 are three times more likely to die of infarction and twice more likely to die of a stroke than women[3]. According to some authors, the incidence of strokes in Uzbekistan ranges between 0.9 and 1.4 cases per 1000 people, while mortality in the acute period can reach 35 to 40% (Asadullaev M.M. et al, 2002; Akhmedov O.T. et al., 2002; Gaphurov B.G., Alikulov A.N., 2000)[4].

According to published data, in Belarus the total number of disabilities resulting from cerebrovascular accidents is 6500, and only 10% to 15% of patients get back to work[5].

It is important to provide proper diagnostic of the state of the motor function with a mandatory quantitative assessment of the severity of dysfunctions and disabilities, which can be completed with the help of validated scales and tests[6].

It is motor functions that disrupt such abilities as movement and self-care and limit patients' movement[7].

Recovery of mobility is a paramount goal of the complex rehabilitation system. In medical rehabilitation, the choice of recovery tactics implies an early start, use of individual approaches and integrated method, which, in turn, can help improve patients' mobility[8]. Normalization of pathologically impaired functions is associated with broad incorporation of robotic mechanotherapy into the recovery process. Regaining joint movement with the use of specially designed devices does provide for optimal physical activity even in the most severe cases, when a patient is unable to move independently[9]. An advanced robotic device uses computer programs that regulate workload on an individual basis[10]. A specific thing about the tactics is proportionate exercise load, abduction, adduction, flexion, extension, and rotation [12].

The goal of the study is to evaluate the efficacy of robotic mechanotherapy in the treatment of mobility dysfunctions as part of a rehabilitation program for apoplectic patients.

Materials and Research Method

The study has involved 35 patients with stroke-related motor dysfunction, who underwent medical rehabilitation in the Republican Clinical Hospital of Medical Rehabilitation (the Republic of Belarus, Minsk District).

A group of 23 subjects (66%) have had ischemic CA, 12 subjects (34%) have had hemorrhagic CA. The patients were hospitalized on the 11th to 15th day from the beginning of the condition. All subjects have undergone a rehabilitation program, which includes medicinal therapy, walking training, coordination and equilibrium exercises, physiotherapy (electric stimulation, magnetic and laser therapy, heat therapy, cryo-therapy, massage), and social rehabilitation.

The treatment group (Group 1) has consisted of 20 subjects, including 9 female subjects (45%) and 11 male subjects (55%). Median age has been 56.6±9.6 (Table 1). Apart from the general exercises, robotic mechanotherapy exercises were added to the treatment group's program.

The control group (Group 2) has consisted of 15 patients (8 men and 7 women) aged 53.8±7.8 with similar demographic characteristics, type of stroke and severity of neurologic impairment, and their rehabilitation program has not included robotic mechanotherapy.

Table 1: Sex and age Distribution

Group	Number of subjects			Median age
	Male	Female	Total	
Treatment	11	9	20	56,6±9,6
Control	8	7	15	53,8±7,8
Total	**19**	**16**	**35**	**55,2±8,7**

The Department of Medical Rehabilitation has developed an individual medical rehabilitation program for both treatment and control group based on clinical symptoms and specifics of the main disorder, age and underlying pathologies.

Robotic monotherapy has been carried out with the use of devices intended for functional upper limb therapy (Armeo Power) and lower limb (Lokomat) therapy, manufactured by Hocoma AG, Switzerland. In the groups, the treatment has lasted for 21 to 28 days. Robotic mechanotherapy has been carried out on a daily basis before the afternoon, sessions have lasted 30 to 40 minutes. The procedure has been applied to the lower and upper limbs at a time with an interval of 15 to 20 minutes.

To recover the function of the affected upper limb, an Armeo Power training device has been used for bilateral forearm pronation/supination and wrist flexion/extension[13]. The training has included exercise

for the upper limbs with affected elbow and shoulder joints, which uses the biofeedback principle with the involvement of the wrist's prehensile function and compensation of the forearm's and shoulder's weight[10]. The training would start with minimal possible workload and amplitude of movements with a gradual increase in the intensity and complexity of exercises[12]. For the lower limbs, the Lokomat system has been used, as it consists of robotic walking orthoses, a body mass support system and a running machine [14]. The training would start from a 100% body-weight unloading with a full and complete control of the walking pattern by the Lokomat device.

The training has implied a gradual lowering of the body-weight unloading along with increasing exercise duration, walking speed, hip elevation, and development of an independent walking function. During a session, a patient is suspended on a belt harness. It is possible to adjust the load on the lower limbs depending on the patient's ability to stand, and enables patients to walk with or without assistance. At the early stage, the robot assists in making passive movements with the lower limbs and, as the patient 's motor function recovers, the amount of active movement increases.

Clinical research method (with the use of a modified Ashworth spasticity scale to determine the intensity of paresis and evaluate muscle strength, the Rivermead Mobility Index to evaluate mobility) have been used to evaluate the subjects' neurological status, and they have served as criteria of efficacy of the rehabilitation procedures. The newly obtained results have undergone statistical processing with the use of the applied Statistica 7.0 package (StatSoft Inc., the USA), and the Student's t-test.

The Ashworth Scale has been used to evaluate muscular resistance while performing passive joint movements. The starting position is lying face up, fully relaxed – for measurement of elbow and knee flexion and extension. Next, five consecutive passive elbow and knee joint movements are made over a length of time needed to pronounce the figure 1969 (nine hundred sixty-nine). The exercise helps evaluate the average muscle tone change index. The movement should be made within the range/until pain.

The muscle tone test parameters are:

0 = No increase in muscle tone;

1 = a slight increase minimal tension at the ends of the flexion/extension range of motion;

2 = a slight increase with minimal muscular resistance (tension) less than halfway into the whole range of motion;

3 = a moderate increase throughout the range of motion with no resistance to passive movements;

4 = a significant increase with high resistance to passive movements;

5 = rigid flexion/extension (flexion/extension contracture).

The Rivermead Mobility Index (RMI) is one of the simplest and easiest-to-perform measures of not only a patient's walking, but general mobility as well. The RMI value is the number of points assigned by a question, based on which a physician can answer positively. The RMI value can range from 0 (unable to make any voluntary movements without assistance) to 15 (capable of running a distance of 10 m). The test is usually done for 10 minutes.

To evaluate mobility, different parameters have been used, such as rolling over in bed, moving from lying to sitting, maintaining balance in the sitting position, moving from sitting to standing, standing without support, moving and walking around a room with (if necessary) or without support tools, walking upstairs, walking outside one's living space (on even surfaces), walking around a room without using support tools, picking up objects (see Table 2).

Table 2: RMI criteria

№	Activity	Question
0	Immobile	What kind of movements does a patient make during a day?
1	Rolling over in bed	Can you roll from back to side without help?
2	Moving from lying to sitting	Can you move from lying to sitting on the edge of your bed?
3	Maintaining balance in the sitting position	Can you sit on the edge of your bed without support for 10 seconds?

№	Activity	Question
4	Moving from sitting to standing	Can you get up (from various types of chairs) over less than 15 seconds and stand for 15 seconds (holding on to something with your hands or, if necessary, using a support tool)?
5	Standing without support	Monitoring a patient standing for 10 seconds without support.
6	Moving	Can you get up from your bed and sit down on a chair without assistance?
7	Walking around a room with support (if necessary)	Can you walk 10 m using support tools, but without using someone else's help?
8	Walking upstairs	Can you climb up a stair flight without help?
9	Walking outside one's living space (on even surfaces)	Can you walk outside your home on a sidewalk without help?
10	Walking around a room without using support tools	Can you walk 10 m around your apartment without using a crutch, orthosis or someone else's help?
11	Picking up objects	If you have spilled an object, can you walk 5 m, pick it up and get back?
12	Walking outside one's living space (on uneven surfaces)	Can you walk outside your house without someone else's help on uneven surfaces (grass, gravel, snowdrifts, etc.)?
13	Taking a bath	Can you get into and out of your bathroom (shower room) without supervision and take a bath/shower alone?
14	Walking four steps up and down	Can you walk four steps up and down without holding onto the railing, but using support tools?
15	Running	Can you run 10 m without limping over 4 seconds (brisk walking is allowed)?

Results and Discussion

Before the rehabilitation procedures started, the majority of subjects had moderate and severe mobility deficits. For instance, according to the Modified Ashworth Spasticity scale, in the treatment group (n=20), 31.8% of subjects had moderate or severe hand paresis; 27.3% of the subjects had very severe hand paresis, 9.1% had mild hand paresis. More than half of the group (59.1%) had severe leg paresis, 31.8% had moderate leg paresis, 9.1% were dealing with mild cases. In the control group (n=15), 28.7% of the subjects had moderate hand paresis, 33.4% - severe had paresis, 29.4% - very severe hand paresis; 98.5% of the subjects had mild hand paresis, more than half of the group (62.3%) had severe leg paresis, 29.6% - moderate leg paresis, and 8.1% of the subjects had mild leg paresis (Table 3).

Thanks to the rehabilitation procedures, which have involved robotic mechanotherapy, 68.2% of subjects have gained hand strength ($p<00.5$), 45.5% of the treatment group subjects have gained leg strength; likewise, the control group subjects have shown a 52.7% increase in the hand strength, and 38.6% - in the leg strength (see Table 4).

Table 3: Clinical characteristics of subjects with mobility deficits

Affected area	Type of paresis	Treatment group	Control group
		Incidence (%)	
Hand paresis	Very severe	27.3	29.4
	Severe	31.8	33.4
	Moderate	31.8	28.7
	Mild	9.1	8.5
Leg paresis	Severe	59.1	62.3
	Moderate	31.8	29.6
	Mild	9.1	8.1

Table 4: Follow-up muscular strength and spasticity dynamics according to the Ashworth scale in the treatment and control groups.

Affected area	Treatment group		Control group	
	Points			
	Before treatment	After treatment	Before treatment	After treatment
Hand paresis	3.65±1.01	2.95±1.16*	3.93±0.92	3.4±1.08
Leg paresis	3.4±0.73	2.95±1.07*	3.46±0.72	3.07±0.93

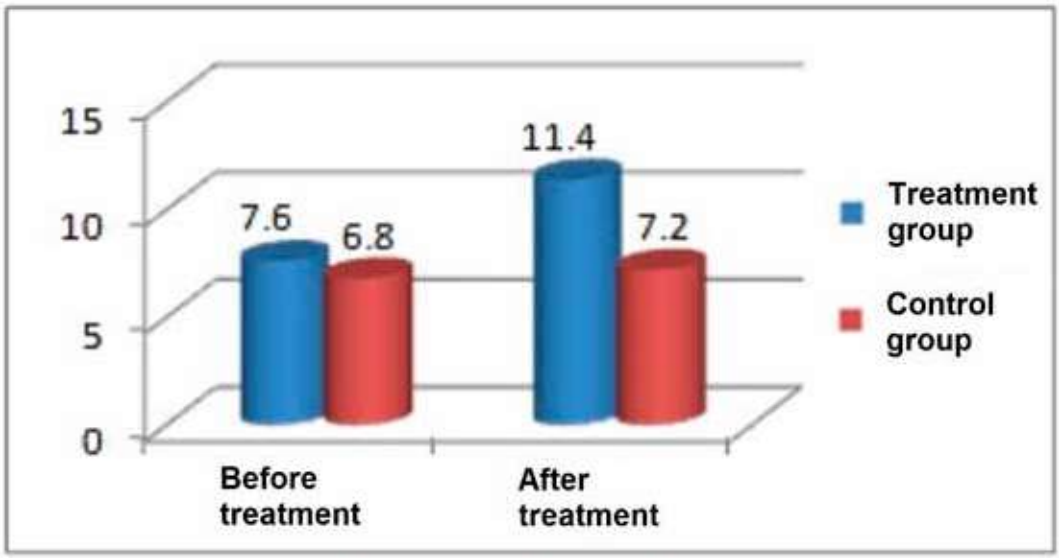


Figure 1: Post-rehabilitation mobility dynamics

The average admission RMI value was 7.6±3.5 in Group 1 and 6.8±3.7 in Group 2 out of 15.

After the rehabilitation procedures, all subjects showed a better mobility, and the improvement was more pronounced in the treatment group: the Rivermead Mobility Index averaged 11.4±2.1 in the treatment group and 7.2±3.2 in the control group out of 15 ($p<0.001$) (Fig.1.). This has been achieved through patients' adaptation to a mobility impairment and development of compensatory and substitutive functions.

The locomotion training with partial body weight unloading with the use of a suspension harness enables free hand movement and more natural walking movement pattern. These devices feature motors, which provide for continuous symmetrical and timeless training sessions.

The dynamics shows a significant spasticity decrease in the treatment group, where subjects have undergone robotic mechanotherapy. A continuous sensor feedback from extensor load receptors, which is observed during this type of walking and hand movement, help to stimulate neuronal networks, which activate muscle locomotion activity patterns and provide for neural plasticity.

The integrated therapy with the use of robots has helped speed up the patients' walking, improved biochemical walking characteristics, reduced muscle tone in the upper and lower limbs in the group of subjects under control dealing with post-stroke hemiparesis during rehabilitation.

Conclusion

The research has made it possible to conclude that the program, which has included robotic mechanotherapy, has been more effective, as it is seen from the higher Ashworth muscle strength indexes in affected limbs, increased Modified Rivermead Mobility Indexes, as well as more a pronounced improvement of impaired functions. Subjects, who have not gone for additional training, have shown minimal improvement according to these characteristics.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Development and Standardization of Dental Plant-Based Preparation in the Republic of Uzbekistan

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Abstract

Nowadays herbal medicines are widely used in official medical practice in Uzbekistan. At the same time, the most common among both imported and domestic plant-based preparations are liquid dosage forms. The modern stage of application of these medicines is based on the rational use not only of the accumulated experience, but also on the achievements of scientific medicine in the study of the composition and features of biological activity of medicinal plants and isolated from them active compounds. Studies have shown that these drugs contain unique in composition combinations of biologically active substances, providing a wide range of therapeutic and preventive action. To solve the problem developed the composition and obtained on its basis a liquid extract for the treatment of diseases of the oral cavity of the mixture of plants such as chamomile drug, sage drug, St. John's wort, calendula drug in the ratio of raw materials - extraction fluid 1:1, established the most optimal extraction fluid - 70% ethyl alcohol and obtained alcohol extraction.

The resulting liquid extract was conditionally called "Parodonfit" and analyzed according to the requirements of regulatory documentation. Each of the plants has its own quality standard. Qualitative and quantitative analyses of biologically active substances of liquid extract "Parodonfit" with the use of physical and chemical method, such as thin layer chromatography, chromatography spectrophotometry, gas-liquid chromatography was carried out. Biologically active substances in the liquid extract - flavonoids and tannins - were determined during the research. The sum of flavonoids was determined by TLC, where compounds of routine, quercetin, cinarozid were isolated. Qualitative analysis of tannins and determination of heavy metals were conducted by pharmacopoeia method. Quantitative determination of active substances of cinarozid by chromatospectrophotometry was carried out. The tannins in recalculation for tannin - 3,2 % and in recalculation for proanthocyanides - 6,38 % were found out by chemical reactions according to the GD (governing documents). Quality indicators for liquid extract "Parodonfit" have been developed according to the requirements of the normative document: physical and chemical indicators, ethyl alcohol concentration, heavy metals, microbiological purity. The received results have shown perspective and expediency of development of highly effective multicomponent medical products on the basis of medicinal vegetative raw materials.

Keywords: *Plant-based preparation, dentistry, thin layer chromatography, chromatospectrophotometry, wild chamomile, St. John's Wort, calendula and garden sage.*

Introduction

Periodontal disease is one of the most difficult problems of modern dentistry. According to WHO, its level varies from 55% to 98%. In 15-19 years, it's about 55-89 %, in 35-44 years - 65-98 % [1,2]. Wide prevalence and significant «rejuvenation» of this pathology, adverse effect of periodontal infection foci on the body, as well as high level of complications up to complete loss of teeth - all this determines both medical and social significance of this problem. In addition to performing therapeutic and prophylactic dental manipulations, the protocols for the treatment of dental diseases include the prescription of various pharmaceutical preparations, such as antibacterial preparations, non-steroidal anti-

inflammatory preparations, calcium preparations, and preparations for the local therapy of periodontal diseases. Taking into consideration the continuous development of the pharmaceutical industry, the introduction of new drugs, the identification of new aspects of contraindications, side effects of certain drugs, all this requires a dentist to constantly update his knowledge in the field of pharmacotherapy [3].

In connection with this, recently in medicine herbal medicines (plant-based preparation - PBP) have been used more often for treatment of this disease[4], which is primarily due to good tolerability and absence of side effects in the vast majority of cases, as well as the presence of various biologically active substances and trace elements in their composition[5].

Many medications are used to treat diseases of the oral mucous membrane. Their choice depends on the nature of the course of inflammatory process (acute, exacerbation, chronic), the presence of pain, the type of elements of lesion, including soft tissue defect, oral hygiene. In diseases of the mucous membrane of the mouth used various dosage forms: decoction, infusion, infusion, ointment, plant-based paste, plant-based cocktail and their combinations.[6]

The results of the study showed that the range of Drug Products for the prevention and treatment of diseases of the oral cavity there are several types of DF (Dosage Forms) (solid, liquid, soft and gaseous), but the majority of drug products are produced in the form of solid DF. A relatively even distribution of liquid medications by quantity in Uzbekistan and abroad has been revealed. Soft dosage forms are mainly represented among drug products produced abroad.

At the analysis of Dosage Forms in solid aggregate state it has been revealed that among foreign there are three drug products produced in the form of tablets for resorption, while domestic medicinal products are represented by medicinal herbal substances (MHS) and produced in packets and filter - packets.

The analysis of the assortment revealed the presence of only two foreign drug products in the form of sprays.

At the next stage the analysis was carried out with a breakdown of the total assortment of dosage forms of sprays, the results are shown in Fig. 1.

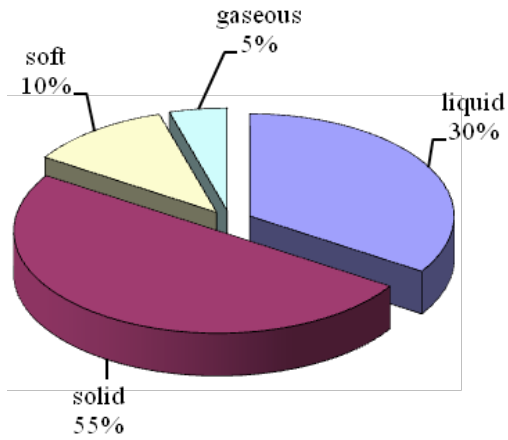


Figure 1: Specific weight of Dosage Forms in the range of drug products registered in the Republic of Uzbekistan, used for the treatment of stomatosis

Carried out marketing analysis of herbal drug products used for prevention and treatment of stomatosis in the pharmaceutical market [7]of the Republic of Uzbekistan, showed the prospects and feasibility of developing highly effective drugs based on local medicinal herbal substances (MHS).[8]

Standardization of multi-component preparations of plant origin is one of the most urgent tasks in modern pharmacy[9]. Determining the authenticity and analysis of the quality of these drugs is a complex procedure that must meet all modern requirements for the standardization[10] of multicomponent drugs. In addition, the growing demand for multicomponent preparations of plant origin used for prevention and treatment of stomatosis makes them potential targets for falsification [12].

Research Objective: Nowadays, studies on the conversion of multi-component mixtures of medicinal plants, which are used in the form of infusions and broths, in the total drugs - extracts are relevant. In obtaining the extracts provides maximum yield of biologically active substances, which increases the pharmacotherapeutic effect. The advantages of liquid extracts and dosage forms on its basis include convenience for use and resistance to storage. In addition, the standardization of active substances allows for control at the stage of production and the accuracy of dosing the finished product, so standardization of liquid extracts and dosage form is no less important.

In order to create medicinal products based on plant raw materials with standard active ingredients and

predictable therapeutic effects, it is necessary to study the relationship between the chemical composition and biological activity. A wide range of biological activity - anti-inflammatory, astringent - has flavonoids and tannins of medicinal plants. The qualitative and quantitative composition of flavonoids and tannins depends on many factors, such as differences in chemotypes and growth conditions of medicinal plants, production and storage technology, etc. In this regard, the need to develop rapid and effective method to determine the qualitative and quantitative composition of flavonoids and tannins, both in plant raw materials, and in phytotherapy based on it is an urgent task.

The article is based on the task of creating a composition for oral cavity treatment by means of such a selection of components that would provide a complex effect of the composition on the affected tissues of the oral cavity, resulting in a high therapeutic effect with simultaneous reduction of negative side effects.

Moreover, the composition of the components is selected in such a way that it represents different groups of biologically active compounds, which, on the one hand, provide a wide range of biological activity (antimicrobial, anti-inflammatory, astringent). Development of new approaches for the standardization of herbal medicines using thin layer chromatography, GLC chromatograph and chromatography spectrophotometry method.

Materials and Method

It is offered a dental product, which includes – wild camomile flowers of the pharmacy, sage leaves, calendula flowers, herb St. John's wort pierced in a ratio of 1:1:1:1.

To develop the technology of obtaining a phytopreparation, initially conducted pharmacognostic studies of raw materials. Wild camomile flowers of the pharmacy, sage leaves, calendula flowers of the medicinal product and the herb of St. John's wort pierced for research were prepared in autumn within the natural range.

We selected the optimal extraction fluid in the following way. We studied 4 extraction fluids: ethyl alcohol with concentrations of 40, 50, 60 and 70%.

Alcohol extraction from plants - liquid extract "Parodonfit" was obtained by repercalation in a battery consisting of three percolators. Repercolation, i.e. repeated (multiple) percolation, allowing the maximum use of the solvent capacity of the extraction fluid, to obtain concentrated extractions with complete exhaustion of the raw materials. The amount of raw material to be loaded into each of the three percolators is calculated. The same amount of raw material is loaded into each percolator. The method of Repercolation is considered to be economically justified for long-term production.

Medicinal plant raw materials are shredded to particles not exceeding 3 mm in size. The shredded raw materials are separated from the dust through a sieve with a hole diameter of 0.25 mm and 30 g are removed. Place the raw material in 3 percolators of 10 g. The first percolator is filled with extraction fluid (70% ethyl alcohol) with an open crane percolator to form a "mirror". The faucet is closed after the extractant displaces the air and left for 2 hours. The displaced liquid is poured into a second percolator. The first percolator is filled with a clean extraction fluid until a "mirror" is formed. After air displacement the first and second percolators are left for 2 hours. From the second percolator poured the amount of extraction in the third percolator, the extraction of the first percolator poured into the second percolator, the first percolator filled with pure extractant until the formation of the "mirror". Three percolators are left for 3 days. From the third percolator is pushed out extraction with a volume of 10 ml. From the second percolator extraction is poured into the third percolator, from the first to the second percolator. The second and third percolators are left for 2 hours. Extraction with the volume of 10 ml is displaced from the third percolator. Pour the extraction from the second percolator into the third percolator and leave for 2 hours. From the third percolator the last extraction of 10 ml is obtained. All portions of the finished product obtained from each percolator are combined, stored and filtered.

The resulting complex product refers to liquid alcohol-containing plant-based preparation. The current regulatory documentation contains general articles on such pharmaceutical forms as "Tincture" and "Extracts", for which the following indicators are mandatory[13]:

- Description
- Authenticity
- Heavy metals
- Alcohol concentration
- Quantitative determination

- Microbiological purity.

Authentication was determined by the following method of analysis: *for qualitative analysis of tannins* we add 1 g of sodium acetate crystals and 10 drops of 1% iron ammonium alum solution to 5 ml of filtrate.

Thequalitative composition of the extract for flavonoids was studied by the TLC test method on "Merck" plates with silica gel COF_{254} for aluminum with the size of 15x10 cm in the system of solvents n-buthanol: acetic acid: water (in a hundred 4:1:2). To detect phenolic compounds of the resulting extract chromatographed ascending method. To identify adsorption zones, 0.05 ml of water-alcohol extract and 0.05 ml of state standard reference sample alcohol solutions of rutin, quercetin and cinaroside were applied to the starting line. Detection of adsorption zones was performed in UV-light at 254 nm length. Three adsorption zones were detected: quercetin (Rf = 0.85); rutin (Rf = 0.55); and cinaroside (Rf = 0.75).

Determining heavy metals to 1 ml of liquid extract add 1 ml of concentrated sulfuric acid, gently burn and calcinate. The resulting residue is processed by heating 5 ml of saturated ammonium acetate solution. Filter through an ashfree filter, wash 5 ml of water and bring the filtrate volume to 200 ml. 10 ml of the obtained solution should pass the test for heavy metals (no more than 0.01% in the preparation). To 10 ml of the resulting solution add 1 ml of diluted acetic acid, 2 drops of sodium sulfide solution and after 1-minute compare with 1 ml of reference solution B, and the same amount of reagents as added to the test solution.

The ethyl alcohol concentration was determined by the developed method with the use of Agilent Technologies "GC 6850 Network GC System" gas chromatograph. Ethyl alcohol concentration in the obtained extract was determined by the developed method using the method of GLC on Agilent Technologies "GC 6850 Network GC System" gas chromatograph.

Chromatography was performed under the following conditions: DB-624 Capillary 30.0m x 250µm x 1.40µm; furnace temperature from 40˚C - 120˚C, duration of analysis-8.0 min, injection 1µl, split 50: 1, injector temperature 120°C; detector temperature 260°C; mobile phase 0.8 ml/min helium (He); flame-ionization detector (FID); air and hydrogen speeds 450 ml/min and 40.0 ml/min.

Preparation of the test solution: Place 10 ml of the test extract with a pipette in a measuring flask with a capacity of 100 ml and bring the volume to the mark with distilled water and stir. The solution is filtered through a 0.45 µm membrane filter.

Preparation of 70.0% RDF ethanol work standard sample solution. To do this, take 66.5 g (precision hanging) of 99% ethyl alcohol and placed in a measuring flask with a capacity of 100 ml, bring the volume to the mark with distilled water and stir. (Solution 1).

10 ml Solution 1 is placed in a measuring flask with a capacity of 100 ml, bring the volume to the mark with distilled water and stir.

Identification of ethyl alcohol on chromatograms of the tested sample is carried out by comparison of retention times of the standard sample. Chromatographs of 1.0 µl of the test solution and ethanol PSO solution shall be alternately taken, obtaining at least 3 chromatograms for each solution.

The reliability of the results of the analysis was checked by determining the suitability of the chromatographic system by: -resolution (R) of ethyl alcohol peaks (not less than 2.0); -association coefficient (T) of ethyl alcohol peaks (not exceeding 2.0); -relative standard deviation (RSD) (not exceeding 2.0%).

The main groups of active substances of the medicinal product "Periodonfit" were quantified. The quantitative content of the sum of flavonoids in terms of cinaroside was determined by chromatospectrophotometry. For this purpose, chromatographic plate "Merck" UF 254 (10 x 15) was divided into 3 strips. On the starting line was applied as a 3 cm strip of 0.05 ml of the extract, on the second strip 0.05 ml of work standardcinaroside solution, the third strip was left free - control. The plate with the applied samples is air-dried for 1 hour and chromatographed upward in a chamber with a pre-saturated mixture of solvents chloroform-methanol-acetic acid (4:2:2) for 30 minutes. When the solvent front passes 7 cm, the plate is removed from the chamber, dried in the air, marked in the UV light (254 nm) zones containing cinaroside at the level of the witness spot. The zone of sorbent containing cinaroside and the zone of control experience, transferred into flasks with lapped corks with a capacity of 50 ml, eat 10 ml of 80% ethyl alcohol and heated in a water bath (60°C) for 30 minutes. With constant stirring the eluate is filtered through the filter. paper and determine the optical density on the SF at a wavelength of 352 nm in the cuvette with a layer thickness of 10 mm.

Preparation of work standart Cinarozid: 0.2 g (so-called) dissolve 80% alcohol in a measuring flask with a capacity of 100 ml (when heated).

Determination of the quantitative content of tannins in the drug: take a pipette 25 ml of the resulting extraction in a conical flask with a capacity of 750 ml, add 500 ml of water, 25 ml of solution of indigosulphonic acid and titrate when continuously stirred with a solution of potassium permanganate (0.02 mol/l) to golden yellow color.

In parallel, a control experiment is conducted.

1 ml of potassium permanganate solution (0.02 mol/l) corresponds to 0.004157 g of tannins in terms of tannins.

Microbiological purity. For the manufacture of high quality and effective plant-based preparation (EPBP) is a prerequisite for quality control (QC) procedure at various stages of the technological process. A special place is occupied by the control of microbiological purity (MBC) of the resulting EPBP.

In accordance with the requirements of the regulatory document definition was carried out in Petri dishes with sterile nutrients: meat - peptone agar - for the quantitative determination of growing colonies, blood agar - to determine hemolytic bacterial strains, yolk and salt agar - to detect pathogenic staphylococcus, Endo - to detect intestinal bacterial groups, Saburo - to excrete fungi. In nutrient media were brought the test drug in different concentrations (1: 2: 4), at least three cups of Petri. All cups with crops were incubated in a thermostat at 37°C. Initial recording of the results was made in 24 hours and 48 hours, the final one - in 5 days. After incubation, the growing colonies were counted and identified.

Results and Discussion

The quality and efficiency of therapeutic action of plant-based preparation directly depends on the method of isolation and receipt of biologically active substances from medicinal plants in the form of extracts. For example, it is known that such traditional method of extraction as decoction, infusion and tincture can allocate about 15-25% of biologically active substances in the medicinal plant. In this case, labile (unstable) biologically active substances are destroyed and the extract significantly loses its medicinal properties. At the same time with these method of allocation of biologically active substances in extracts together with them gets a large enough amount of ballast substances (resinous substances, hydrocarbons, fiber, hairs of plants). These substances, on the one hand, may have a negative effect on the body itself. And it should also be noted that the more used plants in the collection of the extraction, the more pronounced these negative effects, the harder to obtain a quality extract.

Camomile flowers, sage leaves, calendula flowers and St. John's wort herb are pharmacopoeia plants and are of great interest in dentistry as a source of BAS (biologically active substance). Preparations based on these plants are widely used in medicine.

The technical result obtained in the implementation of the invention is expressed in the increase of therapeutic effect with the simultaneous elimination of negative side effects, which ultimately leads to a reduction in the recovery time of patients. Moreover, the composition of the components was chosen in such a way that it represented different groups of biologically active compounds, which, on the one hand, provide a wide range of biological activity (antimicrobial, anti-inflammatory, regenerating, astringent, styptic, capillary strengthening, adaptogenic, immunocorrective action). In this regard, 4 extractants were studied - ethanol with concentrations of 40, 50, 60 and 70% for the liquid extract which includes - chamomile flowers, sage leaves, calendula flowers, St. John's wort herb pierced in the ratio 1:1:1.

The sum of flavonoids was quantified by spectrophotometry. Extraction properties of each object were studied three times; the results were statistically processed and are presented in Table 1.

Table 1. Quantitative determination of the sum of flavonoids in each extract by spectrophotometry.

Extraction fluid	Flavonoid content of extraction, in %
Ethyl alcohol 40%	0.37±0.05
Ethyl alcohol 50%	0.93±0.05
Ethyl alcohol 60%	1.22±0.05
Ethyl alcohol 70%	1.55±0.05

So, as a result of the conducted studies, the most optimal extraction fluid was found - 70% ethyl alcohol and alcohol extraction from a mixture of plants such as chamomile medicinal, sage medicinal, St. John's

wort pierced, calendula medicinal in the ratio of raw materials - extraction fluid 1:1.

The usage of the method of recolonisation instead of maceration allows for more complete extraction of the whole complex of BAS due to a higher concentration gradient, which is created by passing a pure extraction fluid through the raw material. This composition is a biologically active complex compound obtained by mixing components with known therapeutic properties, taken in the ratio providing a given therapeutic effect without negative side effects.

Further research was conducted on qualitative and quantitative indicators of liquid extract in accordance with the requirements of the regulatory document.

The resulting liquid extract is a transparent liquid of brown color, with a pleasant aromatic smell of medicinal herbs and a bitter spicy taste.

At the qualitative analysis of tannins in the liquid extract on the surface of the crystals in the neutral zone of the filtrate black-green coloring appears, which meets the requirements of the ND.

The qualitative composition of the extract for flavonoids was studied by the TLC (thin layer chromatography) method in the system of solvents n-buthanol: acetic acid: water (in a hundred 4:1:2). As a result of the conducted studies 3 adsorption zones were found: quercetin (Rf = 0.85); rutin (Rf = 0.55); cinaroside (Rf = 0.75). The results of TLC analysis of the flavonoid sum are shown in Fig. 2.

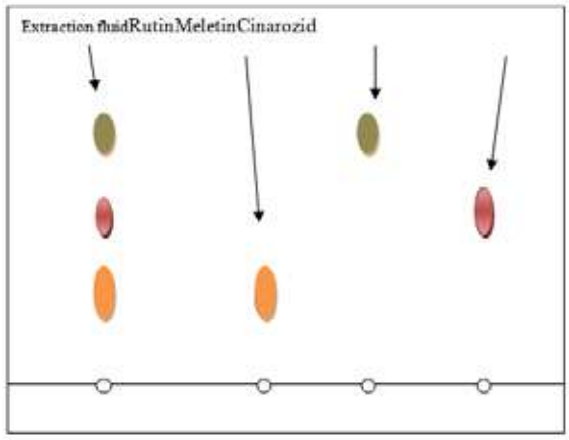


Fig. 2. Chromatogram of liquid extract and alcohol solutions of rutin, meletin and cinarozid as witnesses

When determining the qualitative indices, it was found out that there are no heavy metals in the extract (the color of the test solution is less than that of solution B). Low opalescence is observed.

Ethyl alcohol concentration was determined by the developed method with the use of GC (gas chromatography) method. Identification of ethyl alcohol on chromatograms of the investigated samples was carried out by comparison of retention times of a standard sample. The results are presented in Fig.3-4.

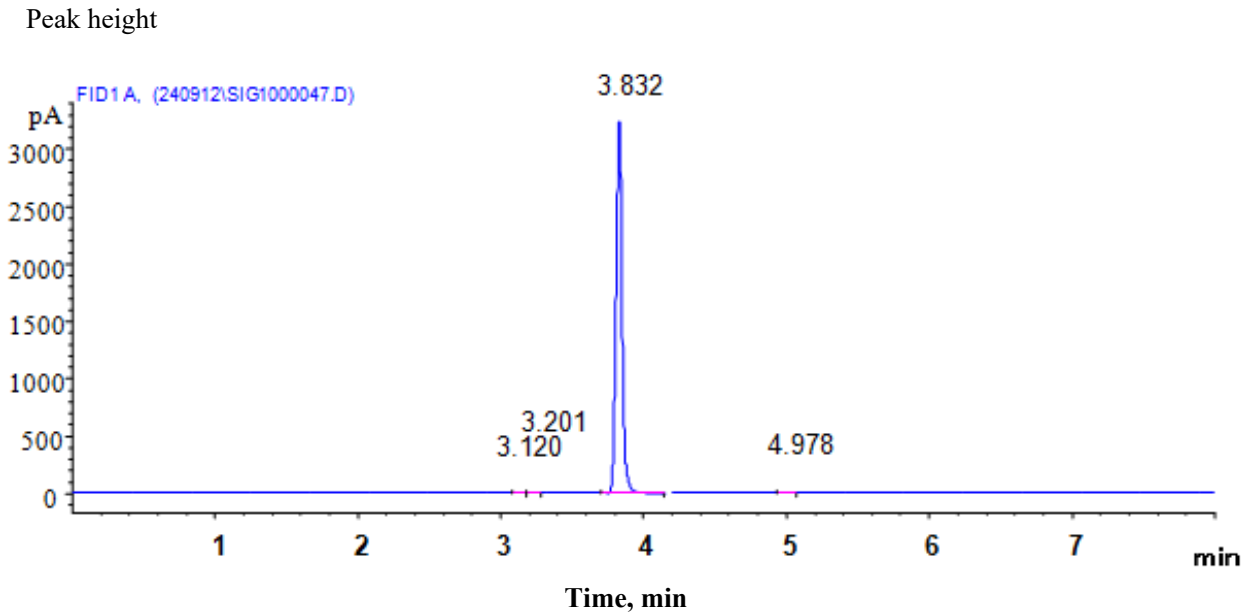


Fig. 3. Chromatogram of standard sample of ethyl alcohol

Peak height

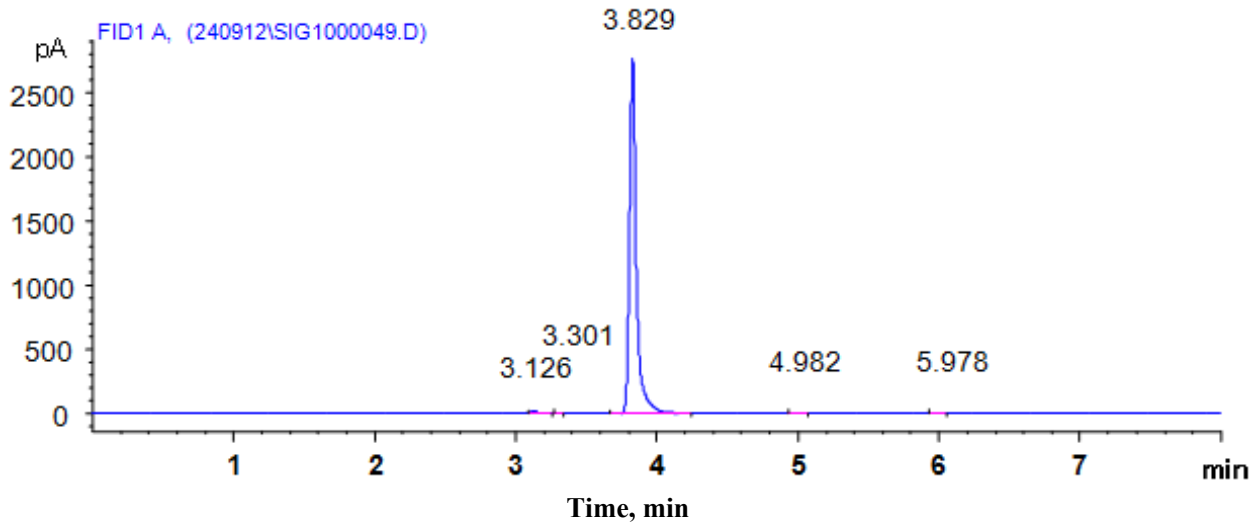


Fig. 4. Chromatogram of "Parodonfit" extract

On the chromatogram the peak with retention time of 3,829 min corresponds to work standard of ethyl alcohol with retention time of 3,832. The concentration of ethyl alcohol in "Parodonfit" extract was calculated by formula

$$X = \frac{H_1 \bullet m_0 \bullet 0 \bullet 100 \bullet P}{H_0 \bullet 100 \bullet 100 \bullet V}$$

H_1-peak height of ethyl alcohol from chromatograms of tested solution; H_0-peak height of ethyl alcohol from chromatograms of ethanol work standard solution; m0 - mass of 96% of ethyl alcohol in grams; V - volume of tested solution used for analyses, in milliliters; P - volume of 70% of ethyl alcohol, for preparation of ethanol work standard solution, in percent.

As a result of the tests carried out it was found out that the quantitative concentration of ethyl alcohol in "Parodonfit" extract was not less than 62.0%.

The quantitative determination of the main groups of active substances in the Parodonfit drug was performed. The quantitative content of the sum of flavonoids in terms of cinarozid was determined by chromatography spectrophotometry to determine the optical density in NF at 352 nm wavelength. The cinarozid concentration of the extract was 0.12%.

Determination of the quantitative content of tannins in the drug was conducted by chemical reactions given in the regulatory document. The concentration of tannins (X) in percent converted to absolute dry raw materials is calculated by the formula:

$$x = \frac{(V - V1) * 0{,}004157 * 250 * 100 * 100}{m * 25 * (100 - W)}$$

Where V - the volume of potassium permanganate solution (0.02 mol/l) spent on titration extraction, in ml;

V1- Volume of potassium permanganate solution (0.02 mol/l) used for titration in control trial, in ml;

0.004157 - amount of tannins corresponding to 1 ml of potassium permanganate solution (0.02 mol/l) (in terms of tannin), in grams;

m - Mass of raw materials in grams;

W - Loss in mass when drying the raw material in percent;

250 - Total extraction volume in ml;

25 - Extraction volume taken for titration, in ml.

The amount of tannins was 3.2 % in terms of tannins and 6.38 % in terms of proanthocyanides.

The research of microbiological purity was conducted in accordance with the requirements of the regulatory document «Method of microbiological control of drugs». A methodology for determining microbial quality was developed.

The results obtained are presented in Table 2.

Table 2: Microbiological control results

Target drug	Selected microorganisms				
	Aerobic Gram-positive bacilli	Staphylococcus	Micrococcus	Escherichia bacteria	Mushrooms
Liquid extract	0	0	0	0	0

Under studying "Microbiological purity" of liquid extract "Periodonfit" the obtained liquid drug corresponds to this index, according to which such drugs should contain no more than $5x10^3$ aerobic bacteria, 10^2 yeast mold fungi in the absence of *Pseudomonas aureginosa, Staphylococcus aureus, Escherichia coli, Salmonella* and no more than 102 other intestinal bacteria. The assessment of quantitative and qualitative composition of the contamination flora did not reveal any deviations from the requirements of SP (State Pharmacopoeia) XI. Quality indicators have been established and will be included in the draft regulatory documents.

Conclusions

1. Based on the data on application of medicinal plants in folk, traditional and scientific medicine, the review of the literature on chemical structure, taking into account a raw-material base it is proved and the optimum composition from four kinds of medicinal vegetative raw materials which is a basis for reception of anti-inflammatory stomatological means is developed.
2. The resource-saving technology was developed by the method of recolation, which consists in the extraction of phytocomposition with alcohol - 70%.
3. Method of standardization of phytocomposition - liquid extract - with the use of chromatographic and spectrophotometric method - TLC and chemical reactions have been developed, the presence of: flavonoids, tannins in liquid extract "Parodonfit" has been established. The phenolic composition of the liquid extract was studied and the following were identified: rutin, quercetin, cinaroside.
4. Tanning materials were studied by quantitative method of analysis calculated on tannin and proanthocyanides.
5. Quality indexes for liquid extract "Parodonfit" according to requirements of normative document are developed: qualitative and physical indexes, ethyl alcohol concentration, heavy metals, microbiological purity.
6. The results obtained can be further used to obtain, standardize and develop a regulatory document for a new combined herbal remedy "Periodonfit" used in dental practice.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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A Complex of Risk Factors for Developing Dependence on the Combined Abuse of Hashish and Tramadol

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Abstract

Introduction: The study of a set of risk factors for a social-premorbid narcological personality dependent on the combined abuse of hash and tramadol.

Materials and Method: 129 male patients were examined, which were divided into three groups. Group I (main) -41% of patients (n = 53) with a combined abuse of hashish and tramadol, Group II (1 control) -34% of patients (n = 44) with addiction from opiates abusing tramadol without a doctor's prescription, group III (2 control) - 24.8% of patients (n = 32) with a cannabis addiction F12.2. The influence of biological, socio-psychological and personality risk factors on the development of the formation of premorbid-social narcological personality was analyzed. To identify the strength of the relationship between exposure to the factor and the disease, the relative risk (RR) was calculated.

Results: An analysis showed a combination of factors of involvement in psychoactive substances: the presence of a family history of hereditary alcoholism and drug addiction in relatives of -62.3% ($P < 0.001$ and $P < 0.001$), transferred craniocerebral loss of consciousness injuries-39.6% ($P < 0.05$), inferior family-73.6% ($P < 0.01$; $P < 0.01$), single child-50.9% ($P < 0, 05$; $P < 0.05$) or senior - 41.5% ($P < 0.05$), unstable personality type - 50.9% ($p < 0.05$, $p < 0.001$).

Conclusion: The analysis of premorbid-social indicators revealed a relative risk of the formation of a narcological personality and thereby determined a new set of factors for which an algorithm of primary prevention measures was developed.

Keywords: *Concomitant abuse, cannabis, tramadol, risk factors.*

Introduction

According to the latest World Drug Report, in 2017 year approximately 271 million people, or 5.5% of the world's 15–64-year-old population, used drugs, and 35 million people suffer from drug-related disorders. Cannabis continues to be the most widely used drug in the world. A drug use survey showed that 4.7% of the population aged 15–64 years old used prescription opioids for non-medical purposes, with tramadol undoubtedly the most common in terms of opioid abuse. The proportion of patients with codependence is growing.[1] Mental and behavioral disorders caused by the simultaneous use of several psychoactive substances become an extremely relevant and rapidly growing problem in adolescence. Adolescents who use psychoactive substances are a high-risk group for the simultaneous administration of several psychoactive substances, in which use becomes abuse and often addiction. [2]

According to the narcological service of the Ministry of Health of the Republic of Uzbekistan over the past 10 years in the republic there has been a positive trend in the development of the narcological situation by its main indicators. However, the use of pharmaceutical opioids has increased. The Government Decree broadened the lists of prohibited and restricted in circulation narcotic drugs, psychotropic substances and their precursors

(substances used for the manufacture of drugs). In particular, Tramadol was included in list No. 2. [3] Today in Uzbekistan there is a tendency to a combination of abuse of cannabis and tramadol, tramadol and lyrics, tramadol and sedatives. Polydrug abuse is a debatable issue in narcology. Various types of interaction of psychoactive substances with their joint use are described. In foreign and domestic scientific reports, dissertations, the problem of epidemiology, the clinical features of intoxication and withdrawal syndrome, the medical and social consequences of the combined use of psychoactive substances, the aspects related to the study of risk factors, the features of the formation and patterns of development of multidrug are not covered enough.

The risk of formation of addictive behavior is the result of multifactorial interaction, where each of the factors or combination forms a dependence. [4,5] Prerogative risk factors include perinatal pathology, hereditary burden of substance abuse and mental illness, history of craniocerebral trauma, premorbid personality deviations, incomplete family composition, types of education, asocial microenvironment, early onset of use of psychoactive substances.[6,7,8] Separately distinguished as an effective factor is the composition and quality of the social environment.[9] Personality traits are an intermediate phenotype that mediates the development of various psychopathologies, including the development of dependence on psychoactive substances. Psychological trauma, stress at an early age increases the risk of developing a dependence on psychoactive substances.[10,11] Some authors say that for each type of psychoactive substance there are dominant risk factors for the development of addiction.[12] Although most studies have shown a high degree of association between cannabis use and the use of other illicit drugs, the predictors of the transition from cannabis to other illegal drugs remain largely unknown.[13] In the framework of the biopsychosocial model, the formation of a disease requires a combination of three groups of individual factors (domains): biological, personal and social. All domains are important for the onset, development and maintenance of the disease, are closely interrelated and cannot be considered separately.[14]

The study of risk factors for the development of addiction allows for primary prevention, already at the primary link. Knowledge of biopsychosocial factors allows you to choose the right prevention strategy for both primary and secondary.[15]

The aim of our study was to study a set of risk factors for a socially premorbid narcological personality, which is dependent on the combined abuse of hash and tramadol.

Materials and Method

A clinical study was conducted among 129 patients who were treated for drug addiction in the City Narcological Dispensary and the Republican Narcological Center from 2016 to 2018. All male patients, aged 21 to 45 years. The examination was carried out using clinical, psychopathological and anamnestic method with filling in a specially developed patient map, indicating the clinical and dynamic characteristics of the disease, biological, social and individual psychological risk factors. All patients were divided into 3 groups. Group I (the main group) included 41% of patients (n = 53) with a diagnosis of F19.2 - combined abuse of hash and tramadol, group II (control group) -34% of patients (n = 44) with a diagnosis of F11.2, patients with opiate dependence, abusing tramadol without a doctor's prescription in order to achieve euphoria, who did not consume other opiates before taking tramadol. Patients with addiction to opiates who abused tramadol for economic reasons (high cost of psychoactive substances) or other reasons (difficulty in access to psychoactive substances, conflict with the law, and fear of being detained) were excluded. Group III (control) included 24.8% of patients (n = 32) with a diagnosis of hash addiction F12.2. In all three groups, the second stage of the disease was diagnosed. The duration of the disease in the examined ranged from two to five years. Criteria for exclusion from the study were comorbid mental pathology, dependence on other types of psychoactive substances.

The results obtained in the study were evaluated using the statistical program R-studio. In order to establish the statistical significance of differences between groups of qualitative characteristics, we used the contingency table using the Pearson $\chi 2$ criterion. To identify the strength of the relationship between exposure to the factor and the disease, the relative risk (RR) was calculated. If RR = 1, then there is no connection between the factor and the disease. For RR> 1, it shows a direct connection, and for RR <1, reverse connection. Differences were considered statistically significant at $P < 0.05$.

Results

The average age in the three studied groups at the time of admission to the medical institution corresponded to the first period of adulthood - 21-35 years. Patients with a combined dependence on hashish and tramadol occupied a median position of 31.2±1.89 years. A younger age is the prerogative of tramadol dependence - 28.1±1.61 years. Hashish addiction amounted to - 32.0±2.59 years.

When studying the narcological history of patients, it was found that the formation of the syndrome of dependence on combined abuse of hashish and tramadol corresponds to the general principles of the formation of dependence (Table 1).

Table 1: The main clinical and dynamic indicators of the formation of the syndrome of dependence on the combined abuse of hash and tramadol.

Indicator	I group (n=53)		II group (n=44)		III group (n=32)	
	Abs	%	Abs	%	Abs	%
Age of the first intake of hashish (years)	18,2±0,59		15,5±0,59**		16,09±0,60*	
Duration of episodic use (month)	3,69±0,28		2,5±0,19***		3,7±0,31^^^	
Duration of systematic use before withdrawal syndrome (years)	2,47±0,19		2,2±0,18		2,7±0,26	
Average dose of psychoactive substances consumed per day	1-2 cigarettes - 5-6 tramadol tablets		20-25 Tablets of tramadol		2 cigarettes	

Note: * - Differences regarding the data of group I are significant (* - P <0.05, *** - P <0.001); ^ - differences relative to the data of group II are significant (^^^ - P <0.001).

Discussion

The formation of dependence on the combined abuse of hash and tramadol began with the consumption of cannabinoids and the subsequent accession of opioids, in particular tramadol. They began to use them earlier by about 18-22 months compared with the start of the use of tramadol. The duration of episodic administration depended on the prevailing effect in the state of intoxication of the first hash samples. If the first samples were characterized by a subjectively pleasant experience of euphoria, then the duration of episodic administration did not exceed 3 months. With the development of unpleasant sensations associated with general intoxication disorders, the episodic stage increased on average to 4 months. The episodic stage was of a group nature with the transition to a systematic reception in solitude. The prevailing motives for the transition to polinarcotism were ataractic - 39.6% and submissive - 22.6%. Not worn in character.

Biological Factors: When comparing biological factors in the main group and control groups, a number of statistically significant differences were revealed. The presence of alcoholism and drug addiction among relatives was significantly more common in the main group - 62.3% (P <0.001 and P <0.001), which is an important risk factor for the development of dependence on psychoactive substances. The quantitative characteristic of hereditary burden-degree, [16] which was analyzed by the number of relatives with addiction, determined a "high" degree of hereditary burden in the group with combined abuse of 35.8% (P <0.01 and P <0.01), for no indicator of an "average" degree of hereditary burden in the studied groups revealed significant differences (P> 0.05). The prevalence of traumatic brain injury with loss of consciousness in the main group (39.6%) than in the control groups (P <0.05), which can play the role of a pathoplastic factor, is noteworthy. In pathologies from other systems, no differences were found (P> 0.05).

Socio-psychological factors: It all starts with the family, a small social group that performs a set of functions, where such functions as emotional, educational and spiritual and moral often fall out. The vast majority of the studied in group I - 73.6% (P <0.001; P <0.001) were brought up in single-parent families. Guardianship was not a significant factor among the subjects (P> 0.05).

Table 2: The frequency of occurrence of clinical and biological risk factors for the formation of a narcological personality in the studied groups

Clinical and biological risk factors		I Group(n=53)		II group (n=44)		III group (n=32)		R_1	R_2
		Abs.	%	Abs.	%	Abs.	%		
One parent's alcoholism		17	32,1	16	36,4	12	37,5	0,88	0,86
Alcoholism in 2 parents		7	13,2	8	18,2	5	15,6	0,73	0,85
Alcoholism and drug addiction in relatives		33	62,3***^^^	12	27,3	7	21,9	2,28	2,85
Mental illness in relatives		3	5,7	5	11,4	3	9,4	0,50	0,60
The degree of hereditary burden in the family	Addiction in father	8	15,1	4	9,1	4	12,5	1,66	1,21
	Addiction in 2 x-3 relatives	19	35,8**^^	4	9,1	3	9,4	3,94	3,82
Incidence of upper respiratory tract diseases		8	15,1^	7	15,9	11	34,4	0,95	0,44
Traumatic brain injury with loss of consciousness		21	39,6*^	9	20,4	5	15,6	1,94	2,54
Hepatitis		5	9,4	6	13,6	4	12,5	0,69	0,75
Neurotic disorders		12	22,6	11	25,0	9	28,1	0,91	0,81
Chronic gastritis		7	13,2	12	27,3	4	12,5	0,48	1,06
HIV infection		2	3,8	3	6,8	0	0	0,55	

Note: * - Differences relative to group II data are significant (* - P <0.05, ** - P <0.01, *** - P <0.001); * - differences regarding group III data are significant (^ - P <0.05, ^^ - P <0.01, ^^^ - P <0.001)

Intra-family relations give an important role in the development of a person who is prone to drug addiction in the future. Intra-family relations are the first instance of interaction with society. When studying the role of the family (Table 3), it was found that the predominant type of upbringing in the first group was the dominant hyperprotection - 60.3% (P <0.001; P <0.001), in the second group, the concurrent hyperprotection was 38.6% (P <0, 05), and in group III, hypoprotection is 43.7% (P <0.01). The vast majority of all studied (52.7%) noted an unfavorable psychological climate in the family, with a lack of understanding of each other. Family relationships could be affected by the birth order of the child in the family. So, those examined in the main group were more often the only sons - 50.9% (P <0.05; P <0.05) or the eldest in the family - 41.5% (P <0.05), and for groups I and II it was common to be the middle or youngest child - 52.3% (P <0.001) and 53.1% (P <0.001).

Table 3: The role of the family in the development of the pre-narcotic personality of the studied groups

Family Relationship Factors		I group (n=53)		II group (n=44)		III group (n=32)		R_1	R_2
		Abs	%	Abs.	%	Abs.	%		
Type of parenting	Hypoprotection	7	13,2^^	10	22,7	14	43,7	0,58	0,30
	Dominant Hyperprotection	32	60,3***^^^	11	25,0	7	21,9	2,42	2,76
	Indulgent hyperprotection	9	16,9*	17	38,6	5	15,6	0,44	1,09
	Emotional rejection	2	3,8	4	9,1	4	12,5	0,42	0,30
	Cruel relationship	2	3,8	2	4,5	0	0	0,83	
	Increased moral responsibility	1	1,9	0	0	2	6,2		0,30
Family Birth Order	The only son	27	50,9*^	12	27,3	8	25	1,87	2,04
	Older child	22	41,5*	9	20,4	7	21,9	2,03	1,90
	Middle or junior	4	7,5***^^^	23	52,3	17	53,1	0,14	0,14

Note: * - Differences regarding the data of control group 11.2 are significant (* - P <0.05, *** - P <0.001); ^ - differences regarding the data of the control group 12.2 are significant (^ - P <0.05, ^^ - P <0.01, ^^^ - P <0.001)

The data suggest that the microsocial conditions considered could influence the formation of the pre-narotic personality.

The result of the study of the level of education among patients did not significantly differ (p> 0.05) (Table 4). A characteristic field of activity was revealed for the main group, which is represented by trade and sales of 43.4% (P <0.05) and for group II, marketing and advertising in 36.4% (P <0.01), and characteristic for group III labor activity is not identified.

Table 4: Distribution of subjects by level of education and labor activities in the study groups

Figures		I group (n=53)		II group (n=44)		III group (n=32)		R_1	R_2
		Abs	%	Abs.	%	Abs.	%		
Level of education	Secondary education	20	37,7	21	47,7	12	37,5	0,79	0,91
	Specialized secondary education	25	47,2	15	34,1	13	40,6	1,38	1,07
	Incomplete higher and higher	8	15,1	8	18,2	7	21,9	0,83	0,60
Type of activity	Transport and logistics	18	34	11	25,0	13	40,6	1,36	0,84
	Trade and sale	23	43,4*	9	20,4	9	28,1	2,12	1,54
	Marketing and advertising	7	13,2**	16	36,4	7	21,9	0,36	0,60
	State service	3	5,7	1	2,3	3	9,4	2,49	0,60
	Education	2	3,8^	7	16	0	0	0,24	
Total		**53**	**100**	**44**	**100**	**32**	**100**		

Note: * - Differences relative to group II data are significant (* - P <0.05, ** - P <0.01); ^ - differences relative to group III data are insignificant (P> 0.05)

When studying family status, it was determined that patients with cannabis addiction were significantly more likely to be divorced - 37.5% (P <0.05).

When studying personality factors, the personality type was considered (Table 5). Patients with a combined dependence of 50.9% (p <0.05, p <0.001) were characterized by an unstable type, with a pronounced craving for entertainment and with the desire to remain without mother's control, with a tramadol dependence - hysterical 22.7% (P <0, 05), and for hash-epileptoid personality type - 31.5% (p <0.01).

Table 5: The distribution of subjects by type of personality

Personality type	I group (n=53)		II group (n=44)		III group (n=32)		R_1	R_2
	abs	%	abs	%	abs	%		
Asthenic	1	1,9	0	0	3	9,4	1,66	0,20
Psychasthenic	6	11,3	3	6,8	7	21,8	0,00	0,52
Schizoid	0	0	1	2,3	0	0	1,25	
Cycloid	3	5,7	2	4,5	1	3,1	0,33	1,81
Epileptoid	2	3,8^^^	5	11,4	10	31,5	0,33	0,12
Hysterical	4	7,5*	10	22,7	3	9,4	1,72	0,81
Unstable	27	50,9*^^^	13	29,5	4	12,5	0,00	4,08
Paranoid	0	0	1	2,3	0	0	0,92	
Insensitive	10	18,9	9	20,4	4	12,5	1,66	1,51

Note: * Differences regarding the data of control group 11.2 are significant (* - P <0.05); ^ - differences relative to the data of the control group 12.2 are insignificant (^^^ - P <0.001)

Conclusion

A correlation analysis of the influence of risk factors for initiation of combined abuse of hashish and tramadol, which lead to the formation of a socially premorbid narcological personality among the studied groups, allows us to conclude that patients with a combined pathology of the cannabinoid group and tramadol have a large history of hereditary burden of alcoholism and drug addiction in relatives with a "high" degree, the presence of hereditary burden in parents does not affect the formation of drug addiction oh personality. Craniocerebral trauma with loss of consciousness is directly related to poly-narcotism. Addiction syndrome was consistent with the general principles of addiction, but they started using cannabis later than other patients. The analysis showed that excessive control of parents over their attempts to do something, limitation of activity, independence, abuse of fault, and not an explanation lead to psychological strangulation. Moreover, the children were the eldest or only child. The dominant hyperprotection was explained by the family climate and family inferiority, which led to the development of an unstable personality type in patients with combined dependence. All these factors are interrelated and thereby create a complex of risk factors predisposing to the formation of a combined dependence on the cannabinoid group and tramadol.

The selected complex of risk factors allows us to develop an algorithm for primary prevention of identifying and deactivating risk factors, on the principle of the relationship between teachers of an educational institution, general practitioners and a narcological dispensary, law enforcement agencies, neighborhood gatherings and youth movements.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Obtaining and Studying Technology of Dry Substance Silver Nanoparticles Obtained by "Green Synthesis" Method Using Scutellaria Iscandaria L.

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Abstract

Silver nanoparticles were synthesized by the method of "Green Synthesis" using extracts of Scutellaria Iscandaria L. The "Green" pathway for the synthesis of silver nanoparticles has been described using an aqueous extract of the plant Common Skullcap as a reducing and stabilizing agent. A dry powder of silver nanoparticles was obtained from the Synthesized Suspension. The role of Luteolin flavonoid contained in Scutellaria Iscandaria L. extract in the synthesis of silver nanoparticles as a reducing and stabilizing agent is shown, the possible mechanism of nanoparticle formation is given. The synthesized silver nanoparticles have been studied by infrared spectroscopy, mass spectroscopy (ICP-MS), and high-performance liquid chromatography (HPLC). The size and shape of the obtained nanoparticles were characterized by the Atomic Force Microscopy (AFM) method. The antimicrobial activity of dry powder and aqueous suspension with silver nanoparticles was also studied at the Strains Test: St.aureusATCC 25923, B.subtilis ATCC 6633, Candida alb. 885-653.

Keywords: *Silver nanoparticles, extract of Scutellaria Iscandaria L., green synthesis, dry powder, infrared spectroscopy, Atomic Force Microscope (AFM), HPLC, Luteolin, antimicrobial activity, St. aureus ATCC 25923, B.subtilisATCC 6633, Candida alb. 885-653.*

Introduction

Over the last two decades, there has been a significant growth in research on the properties of nanoscale particles and various nanomaterials, which is associated with a wide range of opportunities for their practical application[1]. Applied developments in this direction abroad are being successfully introduced into production by enterprises in the nanoindustry; there is reason to believe [2] that further progress in this direction will make it possible to successfully solve many problems (energy supply, ecology, raising living standards, fighting epidemics and reducing mortality from various diseases, etc.) that arise at the present stage of development of human civilization.

Silver nanoparticles destroy the cell walls of bacteria[4], causing their immediate death from physical destruction rather than from toxin poisoning. Bacteria break down and die, and nanoparticles are able to function further.[5] This way of destroying bacteria leaves them unable to adapt, develop a protective mechanism and pass it on to future generations[3]. The undoubted advantage of nanoparticles over silver ions is that the surface coating of nanoparticles is protective on the one hand and can act as a platform for the transfer of various active substances on the other[6].

Nowadays, with the development of nanotechnologies, interest in silver as an antibacterial and bactericidal agent has increased greatly[7]. Nanotechnology has made it possible to reduce the cost of silver-based drugs and make them more affordable in the treatment of many infectious diseases. Among more than 800 items of consumer nano-goods, the greatest demand in the world is for products containing silver nanoparticles[8].

To date, a new method for obtaining nanoparticles of metals is considered the method of "green synthesis"[9].

The approach based on the method of "Green Synthesis" is non-toxic, environmentally friendly and economically efficient in comparison with other method, as in the method vegetable extracts are used as reducing agents and stabilizers of nanoparticles[10] .

The main advantage of plant-based biological systems[11] for the production of nanoparticles is the biological safety of the process and the possibility of obtaining[12] the necessary volume of production without additional costs[13].

The most frequently used method for obtaining metal nanoparticles in aqueous solutions are those based on the reduction of metal ions in the solution under conditions conducive to the formation of nanoparticles. The method differ mainly in the type of reducer and stabilizer (a substance that helps to preserve nanoparticles in solution in nanosize state). Nanoparticles can be synthesised by chemical reduction - with the use of chemical reducers and stabilisers (pure chemical synthesis) or with the use of current or radiation sources providing reduction by hydrated electron and other reducing particles (photochemical, radiation-chemical and electrochemical synthesis) and biological reduction - with the use of water media containing biological reducers and stabilisers (biological or "green" synthesis). In the latter case, water extracts from various biological objects (green plants, fungi, bacteria, etc.) are used.

To create safe medicines or materials containing silver nanoparticles, it is necessary to obtain aqueous solutions of such nanoparticles, which have high antimicrobial activity and at the same time low enough toxicity for the human body. To solve this problem, over the past 10-15 years, studies have been conducted on the action of the same solution of nanoparticles on bacterial cultures and normal culture cells. Unfortunately, as far as we know, such parallel research is very few, which does not allow us to draw an unambiguous conclusion about the possibility of creating appropriate drugs.

From the literature it is known that the search for new effective means to combat cancer has shown, in particular, that, in addition to antimicrobial activity, silver nanoparticles also have cytotoxicity for various types of malignant cells. Such data have been obtained for aqueous solutions of nanoparticles in studies of their action on the viability and functional activity of various cell cultures [14]. This suggests that solutions of these nanoparticles can be used to create drugs for oncology that are sufficiently effective and at the same time do not cause such serious side effects as those currently used in chemotherapy. But even in this case, it is necessary to obtain solutions of nanoparticles, which show, in the same concentration, high toxicity for malignant cells of this type and low toxicity for normal cells of the organ or tissue concerned.

To date, it has been established that the action of silver nanoparticles on cultured cells of both bacteria and mammals is significantly dependent on the way the nanoparticles are produced. So, for the development of both antimicrobial and antitumor drugs based on silver nanoparticles, the actual task is to create a method for obtaining aqueous solutions of these nanoparticles, ensuring their high efficiency as an active component in drugs and at the same time low toxicity to the human body.

It is known that formation of silver nanoparticles occurs at the expense of various biologically active substances that are in medicinal plant raw materials. In particular, plant flavonoids may include metal ions in the chelating complex and restore them. Flavonoids contain various functional groups that can cause the formation of nanoparticles. On the territory of Uzbekistan there is a sufficient number of medicinal plants rich in flavonoids, in particular Scutellaria IscandariaL. The -OH groups inherent in flavonoids may be responsible both for the reduction of silver ions to nanoparticles and for their role as stabilizers[15].

In this regard, today it is relevant to study the possibility of biosynthesis of metal nanoparticles, including silver, using domestic plant raw materials and justification of the possibility of formation of nanometals by modern physical and chemical method.

Some flavonoids have the ability to chelate metal ions through their carbonyl groups or π-electrons, the presence of such mechanisms may explain the ability of flavonoids to adsorb on the surface of the emerging nanoparticle. This means that they take part in the initiation stages of nanoparticle formation (nucleation) and further aggregation in addition to the bioremediation stage. In addition, isolated flavonoids and flavonoid glycosides have the ability to induce formation of metallic nanoparticles.

Among the great diversity of flavonoids containing plants growing on the territory of Uzbekistan, special attention is drawn to Scutellaria Iscandaria L. from

Labiatae (ScutellariaLamiaceae), which along with various flavonoid compounds is rich in other biological active substances such as glycosides, essential oils, organic acids, macro and micro elements, tannins, etc.[16].

"Green synthesis" method, as opposed to chemical synthesis, are cheaper. They allow nanoparticles to be produced[19] in unlimited quantities without significant energy costs[17], as they are considered cost-effective, and are also non-toxic. Biological method are environmentally safe as they do not use toxic compounds[18]. According to many authors[21], a variety of plant metabolites including terpenoids, flavonoids, polyphenols, sugars, alkaloids, phenolic acids and proteins are transferred to water extracts. These biologically active substances contain the hydroxo and aldehyde groups responsible for the reduction of silver ions, and the carboxylic group responsible for stabilizing the resulting nanoparticles.

A number of silver nanoparticles obtained in this way exhibit antibacterial and fungicidal properties, which makes them promising for use in new medical and cosmetological preparations. Antibacterial properties of silver nanoparticles depend on their size: the smaller the particle, the stronger is the bactericidal effect, as smaller nanoparticles have a larger surface area, which provides better interaction with the bacteria.

Silver nanosuspensions are in demand in various industries. Taking into account that the production of silver nanosuspension is mass-produced, it becomes clear that their production must be rational, efficient and these products must meet high quality and safety requirements. For example, in the production of pharmaceuticals or cosmetics with silver nanoparticles, it is more efficient to produce a dry powder of silver nanoparticles, where the exact quantity of silver nanoparticles can be calculated. Thus, dry silver nano-silver powder is more efficient than a suspension of silver nanoparticles.

In connection with the studied data, we find it interesting to study the formation of silver by the method of "green synthesis" using Scutellaria Iscandaria L. and study their antibacterial properties.

Research objective is to obtain an aqueous suspension and dry substance with silver nanoparticles by the method of "Green Synthesis" using a plant of the genus Common Skullcap, as well as the study of their physical, chemical and antimicrobial properties.

Materials and Method

The above-ground part of Scutellaria Iscandaria L. collected in July in the Pontifical district of Namangan region of the Republic of Uzbekistan was used as plant raw material, while the silver ion source was 0.01 M nitrate.

Production of silver nanoparticles was carried out by "green synthesis" method.

Extract Obtaining: Water extraction of dried grass was used as a regenerating agent. For this purpose, 1 kg of shredded raw material was placed in the extractor, 10l of purified water was poured, and then left to swell for 24 hours and received the first drain. Then we added 10 liters of purified water and got the first drain. Both drains were combined and filtered. The received suspension was stored in a refrigerator at temperature 20°C[22].

Silver Nitrate Preparation: To produce 0.01 m of silver nitrate, 80 l of distilled water was added to 136 g of silver nitrate powder. Thoroughly stirred until completely dissolved.

Phytosynthesis of Silver Nanoparticles: For the phytosynthesis of silver nanoparticles, a solution of silver nitrate ($1*10^{-3}$ mol/l) in a ratio of 1:4 was added to the resulting extract. Nanoparticles were synthesized at room temperature until light changes. The pH value of the solution was 5.5.

Production of dry substance with silver nanoparticles: To obtain a dry powder of silver nanoparticles, the liquid extract was first converted into a dense form using a rotary evaporator, thus obtaining 5 liters of the dense extract. Dry extract powder with silver nanoparticle from a dense suspension was obtained by means of Lyophilic drying.

Quantitative determination of the content of micro- and macro-elements in the dry substance by the method of ICP-MS.

By results of studying the elemental composition of dry substance of silver nanoparticles by method ICP-MC it is possible to make calculation of silver for carrying out physico-chemical, microbiological analyses.

The method of impurity(Li, Be, Sc, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Nb, Mo, Rh, Ag, Pd, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, W, Re, Ir, Pt, Au, Tl, Pb, Bi,

Th и U) elements in soils, Soils and bottom sediments are based on a highly sensitive multi-element analysis method - mass spectra with inductively coupled plasma (ICP-MS).

IR-spectroscopy research: IR spectra of the initial extract of Scutellaria Iscandaria L. and the slurry after phytosynthesis were recorded on an IR spectrophotometer (Cary 630 Ftir Agilent Technologies USA) in the frequency range of 4000-400 cm^{-1} (resolution 4 cm^{-1}, number of scans of the sample 50). A certain amount of solution was applied to the KRS substrate, dried for about 5 minutes and the spectrum was recorded.

Study of identification and quantitative determination of flavonoid content: The identification and quantification of flavonoids in Scutellaria Iscandaria L. was studied by HPLC with photometric detection on Agilent 1100. Luteoline, Apigenin, Rutin, Quercetin (ICC lnc, USA Indofine) standards were used. For the preparation of a standard sample with a concentration of 0.05 mg/cm^3, 5 mg standard is placed in a measuring flask with a capacity of 100 cm^3, bring the volume to the mark with methanol. To prepare the sample for the suspension with silver nanoparticles add 2 ml of hydrochloric acid and heated in a water bath with a reverse refrigerator for 1 hour 30 minutes. The solution is quantitatively transferred into a measuring flask for 100 cm^3 and brought to the mark 70% ethanol. Then the flask is placed for 5 minutes in an ultrasonic bath.

Further, the sample for HPLC analysis is filtered through a membrane filter and used for analysis. Conditions for chromatographic analysis: column: 5 µm, 250×4.6 (e.g. Agilent 5 µm C18(2)); mobile phase: acetonitrile - 0.1% trifluoroacetic acid solution pH 2.6 (40:60); mobile phase rate: 1.0 cm^3/min; Column Temperature: 30^0C; Detection: UV, λ= 254 nm. Volume of Introduced Sample: 10 mm^3.

The content of the indicator components is calculated according to a graduation chart or a formula:

$X = C \times S1 \times V\ S2 \times m$,

C — concentration of the corresponding standard solution, mg/cm^3;

S1 – the area of the peak determined by the component in the analysed sample;

S2 — area of the peak determined by the component in the standard sample;

V — is the total dilution volume of the sample, cm^3;

m — hinge mass, gr.

AFM (Atomic force microscopy) study of silver nanoparticles: Method - atomic force microscopy (AFM) to visualize the resulting nanoparticles. The procedure for preparing samples for atomic force microscopy consists in their immobilization on an even substrate. The substrate material can be varied in a wide range depending on the tasks at hand. Traditionally atomic-smooth substrates made of mica, graphite and other layered materials as well as various glasses, polymeric materials and metal surfaces are used as substrates.

Study of antimicrobial activity by microbiological method: The antimicrobial activity of silver nanoparticles was determined[23] by the Diffusion method in Agar and serial dilutions on solid nutrient media[24].

Diffusion in agar on a dense medium (nutrient medium) by comparing the size of the growth depression zones of test microbes.

The following strains were selected: St. aureus ATCC 25923, B.subtilisATCC 6633, Candida alb. 885-653.

Nutrient media: Wednesday No.1, Wednesday No.2.

Nutrient media were prepared in accordance with the manufacturer's instructions.

Autoclaving - 1/atm (121°C) for 15 minutes.

Inoculum Preparation: Neglected daily culture St. Aureus, B. subtilis, Candida alb. grown in a densely nutritious environment. Microbial suspension was prepared by suspending isolated colonies from a 24-hour culture to a standard turbidity of 0.5 McFarland.

Solution Preparation:

Working Solutions:

Tested solution #1: Dry powder with silver nanoparticles obtained by green synthesis method using aqueous extract of common skullcap.

Distilled water was used as a solvent and diluent.

All solutions were applied 100 µl each in wells with one cup of Petri. Then they were kept at room temperature for 1-2 hours.

The cups were incubated in a thermostat at 36 (±1) C^0 for 18-24 hours. After incubation the growth delay zones were measured to the accuracy of 1 mm with the HiAntibiotic Zone Scale CPW 297. The results were taken into account by changing the growth delay zones of microbes around the well (including the diameter of the well itself).

Results

During our Green Synthesis studies, we observed that the color of the observed suspensions was changing. During 1 hour to 4 hours the color of the solution changed from yellow - brown to dark brown light. The change in color indicates an increase in the concentration of silver nanoparticles, as well as an increase in particle size. The liquid extract was then thickened to a thicker form using a rotary evaporator. A 5-liter thick suspension was obtained. Then it was dried by sublimation. From 1 kg of raw material 75 g of dry substance with silver nanoparticles was obtained. Dry fine amorphous powder is dark brown, hygroscopic, slightly crumbling, the smell is weak and peculiar.

The elemental composition of dry substance was studied quantitatively. In particular, the amount of silver in the studied samples was 2500 ppm (2500 mg/kg), which is - 0.25% .

It is known that silver deposition in concentrations of 10-20 µg/ml causes reduction of tumor necrosis factor, interleukin-1, interleukin-6, and at concentration of nanoparticles at the level of 100 µg/ml 50% survival rate of A549 lung cancer cells is achieved. According to EPA (USA) recommendations, the daily permissible dose of silver (RfD) that does not cause cell necrosis is 5 µg per 1 kg of body weight per day.

IR spectroscopy study of Scutellaria Iscandaria L. extracts and extract suspensions with formed silver nanoparticles are presented in Fig.1 and 2.

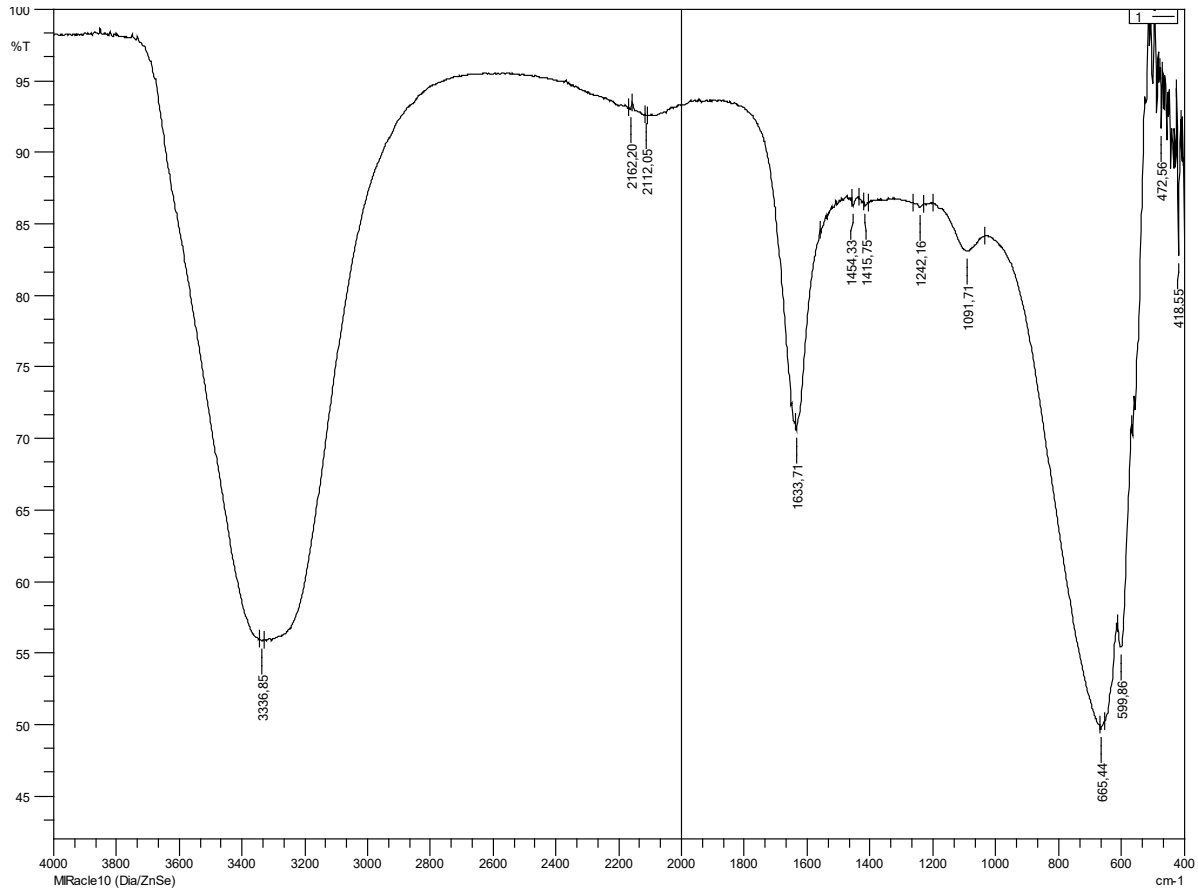


Fig. 1. IR absorption spectrum of Scutellaria Iscandaria L. extract.

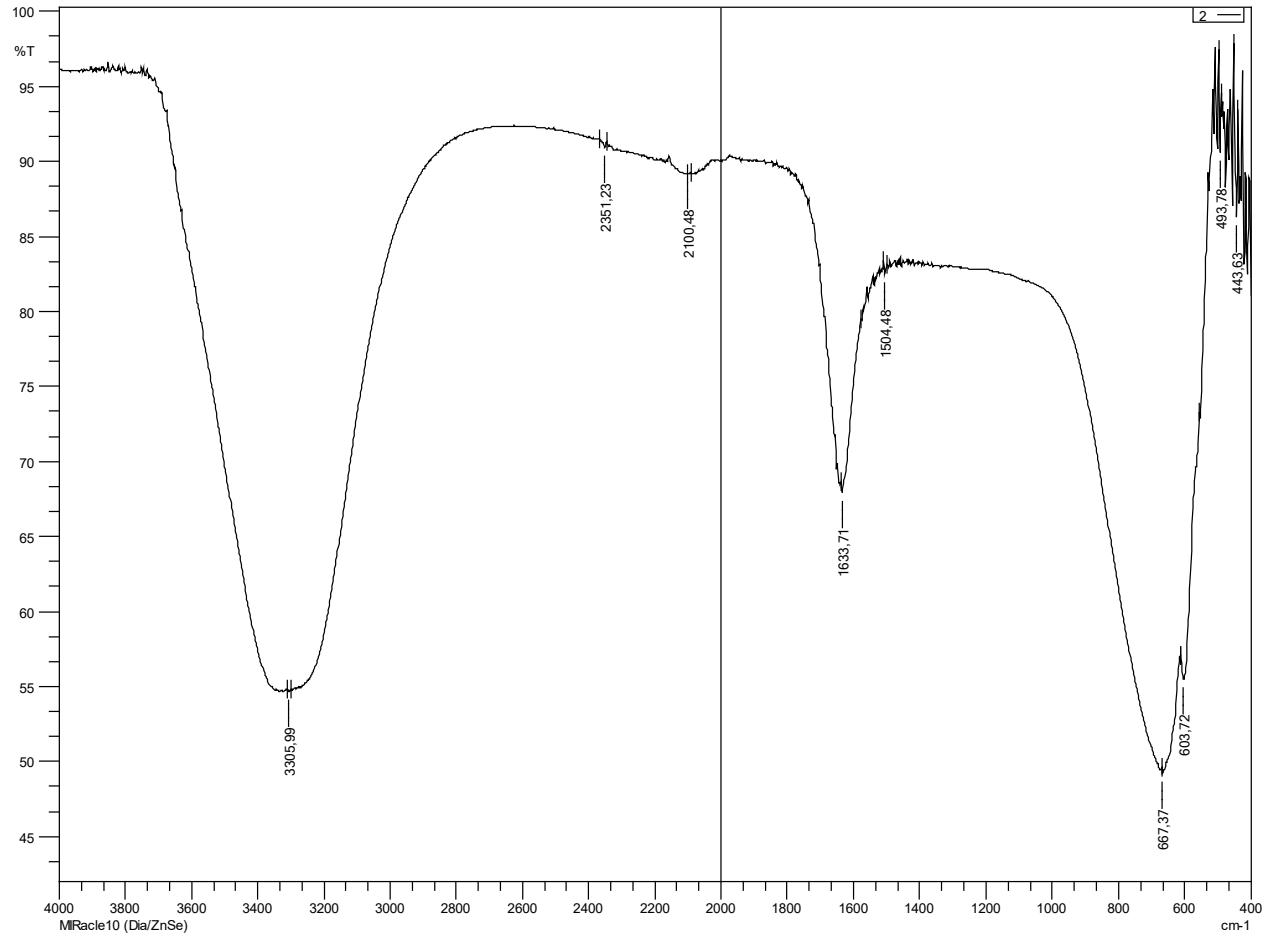


Fig 2. IR spectrum of silver nanoparticles absorption using Scutellaria Iscandaria L. extract.

In the presented infrared spectra, the following characteristic peaks in the absorption area can be distinguished: 3336-3305 cm^{-1}, 2112-2100 cm^{-1}, 1633 cm^{-1}, 1454-1504 cm^{-1}, 1091 cm^{-1}, 400-667 cm^{-1}. It is known that these peaks are connected with valence oscillations of hydroxyl groups in alcohols or phenolic compounds, functional groups C-O of phenol, C-H of aromatic ring.

Removed IR spectra (Fig.1, 2) showed the fluctuations of the temple ring at 1633 cm^{-1}. In the spectrum of the extracts under study there is a wide absorption band in the region of 3336 cm-1, which refers to the valence oscillations of OH-groups in alcohols and phenolic compounds.

After the interaction of $AgNO_3$ solution with Scutellaria Iscandaria L. extract, instead of the band at 3336 cm^{-1}, there is a band at 3305 cm^{-1}, which suggests that hydroxyl groups of phenolic compounds that are part of the extract take part in the restoration of silver ions and formation of nanoparticles.

Absorption bands at 1415 and 1454 cm^{-1} refer to the valence vibrations of the benzene ring at low intensity. Absorption bands in the area of 1242 cm^{-1} belong to the valence oscillations of C-O phenol, while absorption bands in the area of 665 cm^{-1} characterize the deformation oscillations of C-H of the aromatic ring. Taking into account that Scutellaria Iscandaria L., contains flavonoid compounds, which participate in formation of silver nanoparticles, we have carried out a comparative chromatographic study of water extraction of Scutellaria Iscandaria L. and suspensions with silver nanoparticles.

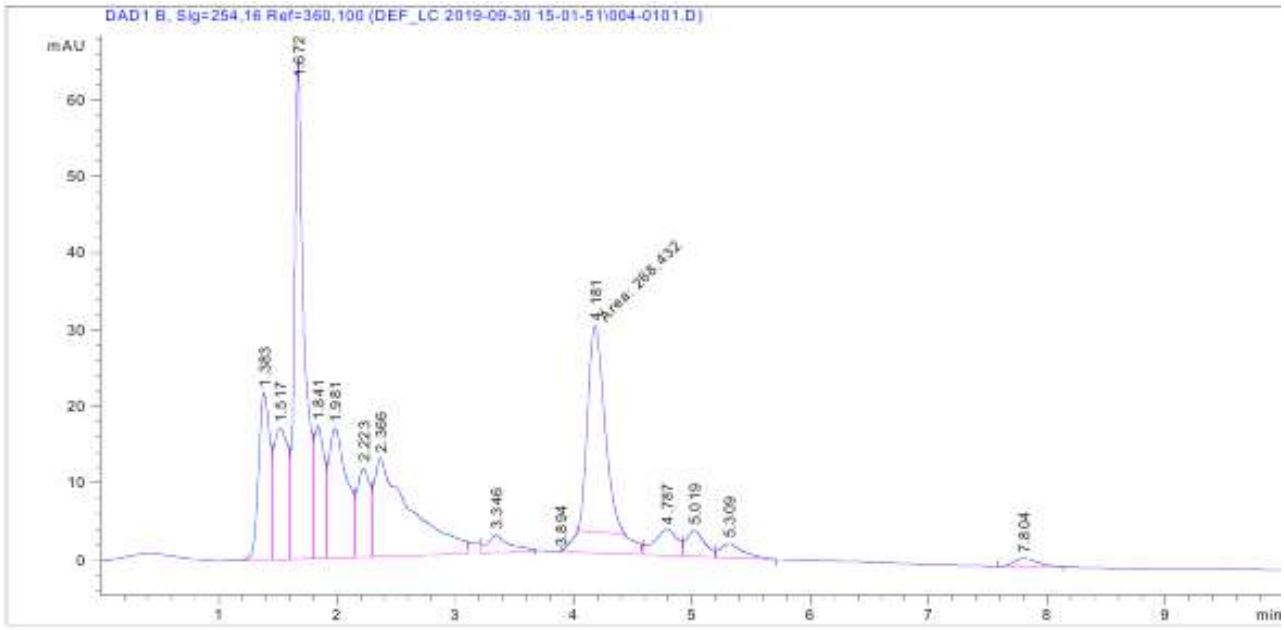


Fig. 3. Luteolin chromatogram.

To begin with, chromatograms of Lutheonine standards were taken (Fig. 3).

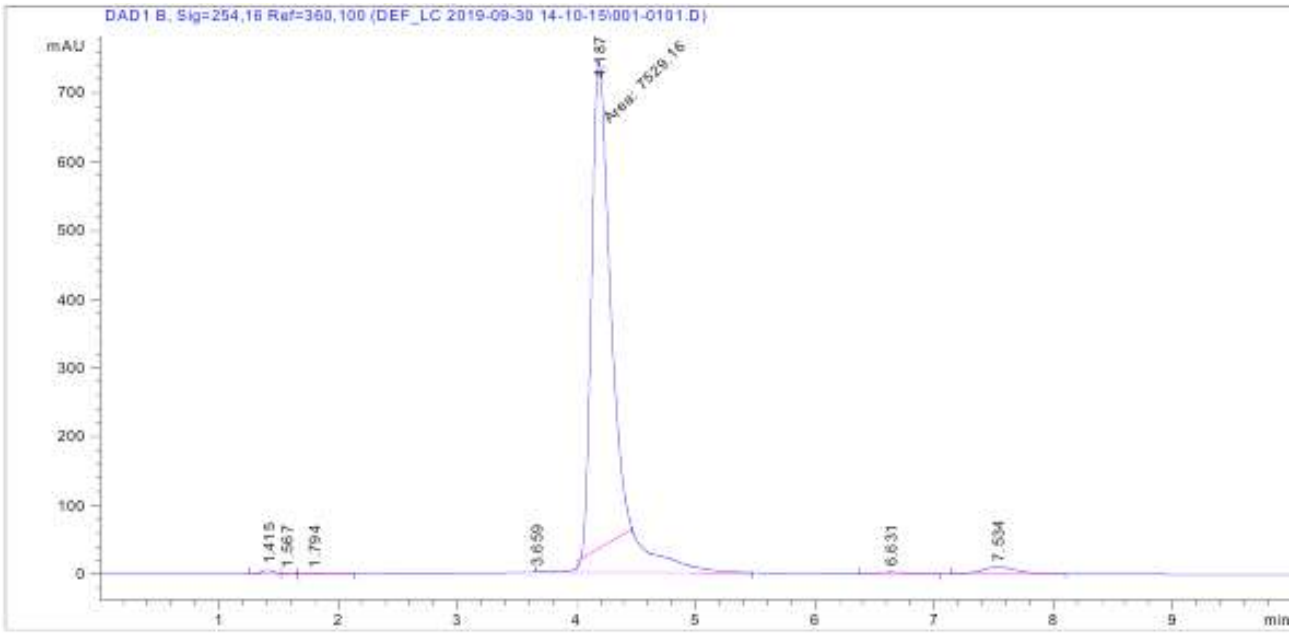


Fig. 4. Water extraction chromatogram of Scutellaria Iscandaria L. (Determination of flavonoids: Luteolina).

The chromatogram shows that the peak holding time for the Luteolin standard is 4.1 minutes.

Further, the water chromatogram of Scutellaria Iscandaria L. was taken. As can be seen from the picture below, the chromatogram also shows peaks in the area of 254 nm, whose retention time (min) characterizes the presence of only Luteolin - 4.1 minutes.

According to the results of the analysis, the content of Luteolin in the samples of Scutellaria Iscandaria L. extract is - 0.0062 mg/g (fig.), and in the suspension with formation of silver nanoparticles - 0.0039 mg/g (fig.).

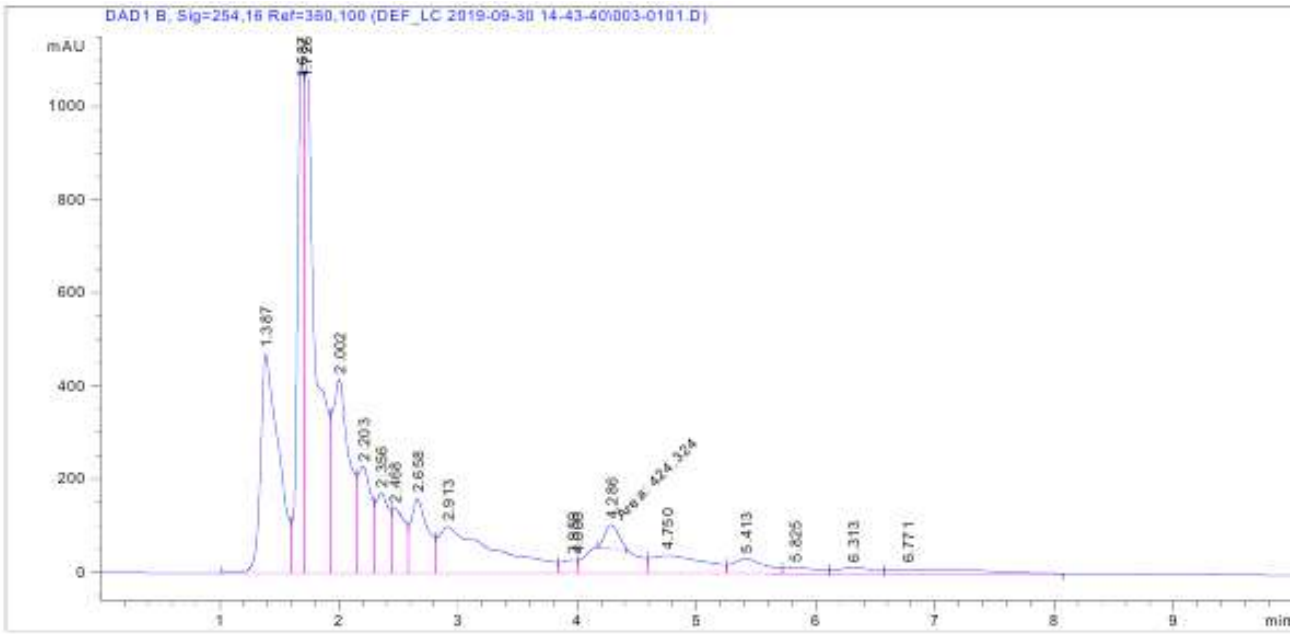


Fig. 5: HPLC - water extraction chromatogram of Scutellaria Iscandaria L. with silver nanoparticles.

Table 1: The generalized results of chromatographic analysis are presented.

Standard Names	Contents of Scutellaria Iscandaria L. water recovery standards.	Contents of Scutellaria Iscandaria L. water extraction standards with silver nanoparticles.
Concentration of mg/g		
Luteolin	0,0062	0,0039

As can be seen from the data presented, the flavonoids contained in Scutellaria Iscandaria L are actively involved in the formation of silver nanoparticles[25].

As is known from literature data[26], the rich phytochemical composition of the extracts used suggests its complex action, for example, as a reducing, stabilizing agent. The mechanism of nanoparticles formation consists mainly of three stages: ion recovery, clustering, and further growth of nanoparticles. Features of each stage depend on the nature of the reducing agent, its concentration, pH, $AgNO_3$: concentration of the reducing agent. The -ON groups present in flavonoids such as luteolin are responsible for the reduction and play the role of stabilizers, therefore, the resulting suspension with nanoparticles can be stored as colloids.

As is known from the literature, the rich phytochemical composition of the extracts used suggests its complex action, for example, as a restorative and stabilizing agent. There is no corresponding literature explaining the mechanism of flavonoid recovery and stabilization of silver nanoparticles. The mechanism of formation of nanoparticles consists mainly of three stages: restoration of ions, clusterization and further growth of nanoparticles. Features of each stage depend on the nature of the reducing agent, its concentration, pH and concentration of the reducing agent. According to some researchers, the -OH groups present in flavonoids, such as Luteolina, may be responsible for the recovery and play the role of stabilizers, so the resulting suspension with nanoparticles can be stored in the form of colloids.

Luteoline reacts with Ag+ as acid through the most reactive hydroxyl groups attached to the carbon atoms of the aromatic ring, which can restore silver ions to nanoparticles and provide stability against agglomeration (Fig. 6).

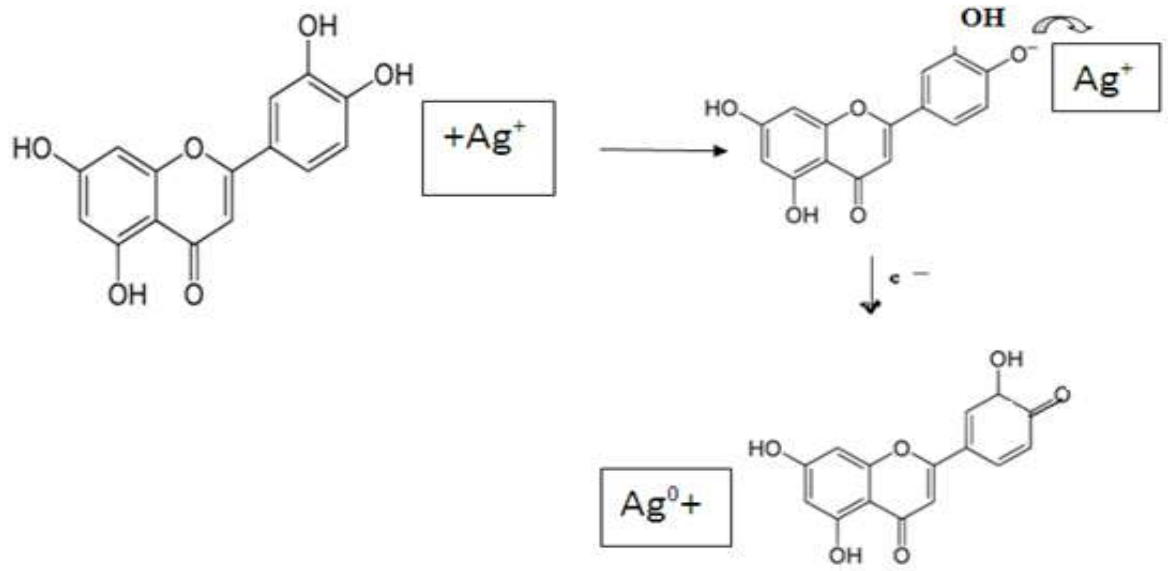


Fig. 6. Approximate mechanism of silver ions reduction to silver nanoparticles by the Luteolin molecule.

From this we can conclude that the formation of silver nanoparticles was due to all flavonoids inherent in the extract of Scutellaria Iscandaria L.

Further, we studied the size and shape of the formed silver nanoparticles by Atomic Force Microscopy.

According to microscopic studies, the morphological characteristics (shape, size, structures) of silver nanoparticles were studied. In particular, the size of silver nanoparticles derived from extracts of medicinal plants Scutellaria Iscandaria L. was 45.7 nm (25% of the total number of particles) with a basic fraction (81%) ranging from 36.6 nm to 64 nm.

Determination of the antimicrobial activity of the preparation by serial dilution makes it possible to determine more precisely the minimum concentration of the preparation that inhibits bacterial growth[27].

The bacteriostatic dose was considered to be the concentration of the preparation, which inhibited the growth of the culture of the test strain, and the bactericidal amount of the preparation, which completely inhibited the growth of microbes. Sensitivity of microorganisms was estimated by a unified method: group 1 was considered highly sensitive, group 2 - sensitive, and group 3 - stable.

Stable - the strain is not suppressed at the concentrations of the drug created in organs and tissues at recommended dosing modes.

Absence of the zone of microbial growth delay around the well indicates that the experimental strain is not sensitive to this experimental drug [28], at a zone with diameter up to 10 mm the strain is considered to be insensitive. In standard conditions of experience by the size of zones formed by preparations, it is possible to judge about the degree of sensitivity of the investigated microorganism.

Based on the results of ICP-MS, the amount of silver was determined to be 0.25%, from these data for analysis of antimicrobial activity in terms of dosage was taken dry powder of silver nanoparticles in 3 different concentrations. 1-0,5%; 2-0,25%; 3-0,125%.

The results of the evaluation of sensitivity and inhibition diameters of growth zones of silver nanoparticles were carried out in Table 2.

Table 2

No	Test strains	Test Results Diameters of microbial growth inhibition zones
1	Staphylococcus aureus ATCC 25923	25-32 mm
2	Bacillus subtilis ATCC 6633	36-40 mm
3	Candida albicans ATCC 885-653	30-38 mm

The results of the conducted studies (Table 2) showed that the greatest efficiency of the studied sample

was observed in relation to Bacillus subtilis ATCC 6633- the diameter of the inhibition zone was 36-40 mm and Candida albicans ATCC 885-653 - the diameter of the inhibition zone was 30-38 mm, also Staphylococcus aureus ATCC 25923 - the diameter of the inhibition zone was 25-32 mm.

Silver has a wide range of antibacterial activity, including antibiotic-resistant gram-positive and gram-negative strains. There is a direct correlation between silver's concentration, particle size and its antimicrobial action. The higher the concentration and smaller the particle size, the more effective silver is at inhibiting bacterial growth. The treatment of infection caused by microorganisms in this category is usually effective when tested preparations are used in recommended doses. The sensitive ones were Staphylococcus aureus, Candida albicans with a growth delay zone around wells, which was 30-40 mm. Category-induced infection treatment may be effective when the test subject is administered at elevated doses or when the focus of infection is localized in those organs or tissues in which elevated drug concentrations are created due to physiological features. In addition, after observing the results, we kept the cups in the thermostat for another 1 week and observed microbial growth. During 1 week no microorganism growth was found in the culture.

Wasteless Technology: On the basis of the above data, water was vaporized in the process of obtaining a thick extract. As silver nanoparticles are considered to be small metal particles, they were not only in the dense suspension but also in the water part. On this basis, we found it interesting to study the antimicrobial activity of the evaporated water part.

First, we studied the amount of silver in water using the ICP-MS method. The amount of silver in the tested samples was 0.023 mg/l.

Table 3

No	Test strains	Test results Diameters of microbial growth inhibition zones
1	Staphylococcus aureus ATCC 25923	-
2	Bacillus subtilis ATCC 6633	36-38-40 mm
3	Candida albicans ATCC 885-653	-

During the antimicrobial activity study, water with nano-silver was also taken at 3 x concentrations.

The results of the sensitivity and inhibition diameters of microbial growth zones of water with silver nanoparticles are given in Table -3.

No activity was detected on Staphylococcus aureus and Candida albicans during the studies. The absence of a microbial growth delay zone around the well indicates that the test strain is not sensitive to this test drug. Only Bacillus subtilis ATCC 6633 was considered sensitive, with a growth inhibition zone diameter of 36-38-40 mm.

Conclusions

For the first time, a dry substance of silver nanoparticles was obtained by the "Green Synthesis" method using an extract from a plant of the Common Skullcap. The role of Luteolin flavonoid contained in Scutellaria Iscandaria L. extract in the synthesis of silver nanoparticles as a reducing and stabilizing agent is shown, the possible mechanism of nanoparticle formation is given. Synthesized silver nanoparticles have been studied by IR-spectroscopy, Mass Spectrometry (ICP-MS), High Performance Liquid Chromatography (HPLC). The size and shape of the obtained nanoparticles were characterized by the Atomic Force Microscopy (AFM) method. Their antimicrobial activity was also studied on the test strains: St.aureusATCC 25923, B.subtilis ATCC 6633, Candida alb. 885-653.

IR spectroscopy data suggest that hydroxyl groups of phenolic compounds in the extract take part in the reduction of silver ions and the formation of nanoparticles.

It follows that the Scutellaria Iscandaria L. extract contains flavonoids that participate in the formation of nanoparticles. We have investigated the role of flavonoids, in particular, Luteolin method of HPLC, contained in the extract of Scutellaria Iscandaria L. in the reduction of silver ions in the solution with the formation of silver nanoparticles.

It was found that Luteolin is also actively involved in the restoration of silver nanoparticles, so its content in the biosynthesis process is reduced by half. The formation of silver nanoparticles is confirmed by the appearance of the color of the resulting ash, as well as the results of atomic force microscopy, which allowed to obtain an image of silver nanoparticles. The AFM image allowed us to establish that silver nanoparticles obtained using Scutellaria Iscandaria L. extract have a size of 45.7 nm (25% of the total number of particles)

with a basic fraction spread (81%) of 36.6 nm to 64 nm. Consequently, Scutellaria Iscandaria L. may be a promising raw material for the synthesis of silver nanoparticles.

Silver nanoparticles obtained with the use of Common Skullcap extracts have been shown to exhibit strong antimicrobial activity; they have bactericidal effect on Bacillus subtilis, Staphylococcus aureus, Candida alb.

Based on previous data, it can be concluded that the silver nanoparticles obtained with the use of Common Skullcap extract can be used to treat inflammatory processes. The results of the study confirmed that the bactericidal dose of the drug was higher than the bacteriostatic dose.

Comparative data on the antimicrobial activity of silver nanoparticles powder and water with silver nanoparticles allow concluding that not only the concentration of nanoparticles, but also the biological active substances contained in the dry powder with silver nanoparticles play a major role.

Later, when planning the production of dry substance with silver nanoparticles, we can use the water obtained in the process of evaporation to obtain a thick extract in various areas, such as in cosmetology, meal grass for addition to feed, because in the process of extraction was not used organic substances. On this basis, we can conclude that the selected technology for the phytosynthesis of nanoparticles will relate to the wasteless technology.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Assessment of Informativity of Dopplerographic Determination of the Ankle-Brachial Index

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Abstract

The modern concept of the diagnosis of peripheral artery disease is one of the urgent problems of modern medicine. Peripheral Artery Disease (PAD) is a pathology characterized by the formation of plaques in the peripheral arteries. As reported at the Scientific Session soft the American Heart Association in 2007, the prevalence of asymptomatic PAD in adults in the United States was constantly increasing, and now it is found in almost 5% of adults aged 40 years and older. In addition, PAD is an indicator of the condition of the arteries and its presence is associated with an increased risk of heart attack and stroke.

Keywords: *Vascular ultrasonography, dopplerography, peripheral artery disease, ultrasound duplex studies, ABI.*

Introduction

Prevention issues as a strategic direction of healthcare development in Uzbekistan are contained in all state legislative acts of recent years[1]. However, many of the necessary projects have not been properly implemented in everyday practice, including a strategy aimed at preventing chronic lower limb ischemia[2]. One of the reasons for the high mortality rate is the lack of effective primary prevention measures that ensure the timely identification and correction of risk factors for the development of pathology[4].

Unfortunately, today there is very little accurate data on the epidemiology of obliterating diseases of the arteries of the lower extremities. Information on the results of published studies on this issue is fragmentary and contradictory, which does not give a complete picture of the prevalence of this process in different regions, among different age and ethnic groups, the dynamics and outcomes of these diseases. According to published data, the prevalence of obliterating diseases of the arteries of the lower extremities in the countries of Europe and America is 2.1-20.7%, and this pathology is extremely rare in the indigenous peoples of Asia. Great importance is attached to the study of the prevalence, risk factors, and clinical features of these diseases among various ethnic groups. There are only a few short reports on the prevalence of obliterating diseases of the lower limb arteries among indigenous peoples[3].

The aim of the study. The study of the prevalence of peripheral arterial disease among residents of the Khorezm region aged 45-90 years and the development of an optimal model for its ultrasound diagnosis for general practitioners.

The degree of influence of unmodifiable and modifiable risk factors, as well as their combinations, in the occurrence of arterial pathology of the lower

extremities in this contingent is studied, taking into account the identified regional characteristics.

An objective picture of the prevalence of stenosing atherosclerosis of the arteries and hemodynamic disorders of the lower extremities in this population is obtained.

For the first time, an analysis of the information content of non-invasive diagnostic method in patients with PAD was carried out. The criteria for stenotic lesions of the arteries according to the values of the ankle-brachial index

Materials and Method

Among the examined 487 (87.0%) patients, no PAD was detected, and 73 (13.0%) patients were diagnosed with PAD. Arterial events were noted in the lower extremities in 38 (6.8%) patients. The proportion of patients with average SBP at the ankle level of 300 mm in different areas was generally the same (22 (30.1%) patients in the city of Urgench, 6 (8.2%) of them in the Urgench district, 3 (4.1%) patients in Hankha, 4 (5, 5%) patients in Bagat, 4 (5.5%) patients in Khazarasp, 7 (9.6%) patients in Yangarik, 6 (8.2%) patients in Khiva, 10 (13.7%) patients in Kushkupir, 2 (2.8%) patients in Shavat, 3 (4.1%) patients in Yangibazar, 6 (8.2%) patients in Gurlen).

The average ABI was 0.92+ 0.23 and was less than 0.9 in 13.0% of patients. The average ABI decreased with age; in elderly patients, the ABI was 0.96 + 0.18 versus 0.86 + 0.25 in senile patients. In addition, approximately twice as many elderly and senile patients of 63 (14.7%) patients had an ABI of less than 0.9 compared with middle-aged patients 10 (7.6%). When analyzing the ABI taking into account the type of patients, the ABI was less than 0.9 in 16 (22.0%) patients with coronary heart disease (mean value of the ABI 0.91+ 0.24), in 15 (21.0%) patients with cerebrovascular disease (the average value of the ABI is 0.91+ 0.23), in 6 (8.0%) patients with multiple vascular pathology (the average value of the ABI is 0.87+ 0.25) and in 36 (49.0%) patients with only cardiovascular risk factors (average LPI 0.94+ 0.22). When taking into account the living area of the Khorezm region, the ABI is less than 0.9 in: 22 (30.1%) patients in the city of Urgench, 6 (8.2%) of them in the Urgench district, 3 (4.1%) in Hankha, 4 (5.5%) in Bagat, 4 (5.5%) in Khazarasp, 7 (9.6%) in Yangarik, 6 (8.2%) in Khiva, 10 (13.7%) in Kushkupir, 2 (2.8%) in Shavat, 3 (4.1%) in Yangibazar, 6 (8.2%) in Gurlen.

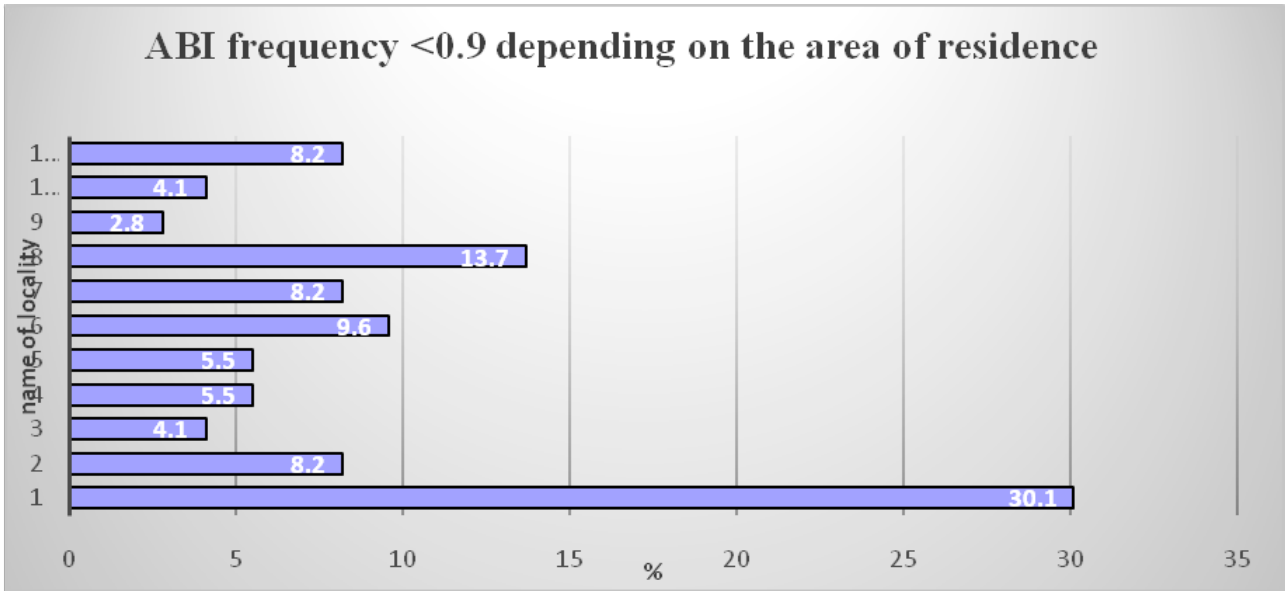


Figure 1. ABI frequency <0.9 depending on the area of residence

The prevalence of ABI values of 1.3 among patients with and without diabetes did not differ significantly and amounted to 3.0 versus 2.2% between the two groups.

Table 1: The indicators of the ankle-brachial index in groups

ABI values	Total (n-560)	Coronary (n-149)	Cerebrovascular (n-111)	Multivascular (n-39)	Only RF(n-261)
Average (M±SD)	0,92 ±0,23	0,91±0,24	0,91±0,23	0,87±0,25	0,94± 0,22
0,5	6 (1,1%)	1 (0,7%)	1 (0,9%)	1 (2,6%)	3(1,2%)
0,5-0,7	26 (4,6%)	5 (3,4%)	6 (5,4%)	3 (7,7%)	12 (4,6%)
0,7-0,9	41(7,3%)	10(6,7%)	8(7,2%)	2 (5,1%)	21 (8,0%)
0,9-1,1	300(53,6%)	78(52,3%)	57(51,4%)	20(51,3%)	145(55,6%)
1,1-1,3	128(22,9%)	35(23,5%)	25(22,5%)	9 (23,0%)	59 (22,6%)
More 1,3	59 (10,5%)	20(13,4%)	14(12,6%)	4 (10,3%)	21 (8,0%)

The determination of the ankle-brachial index led to a noticeable change in the distribution of patient types.

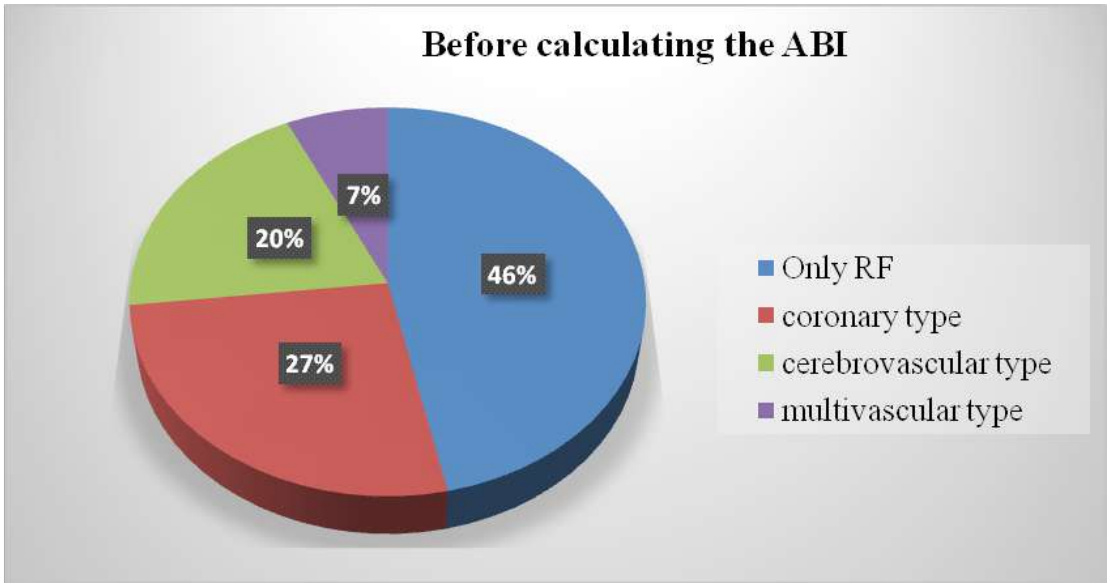


Figure 2. Patient distribution before calculating the ankle-brachial index in peripheral artery disease

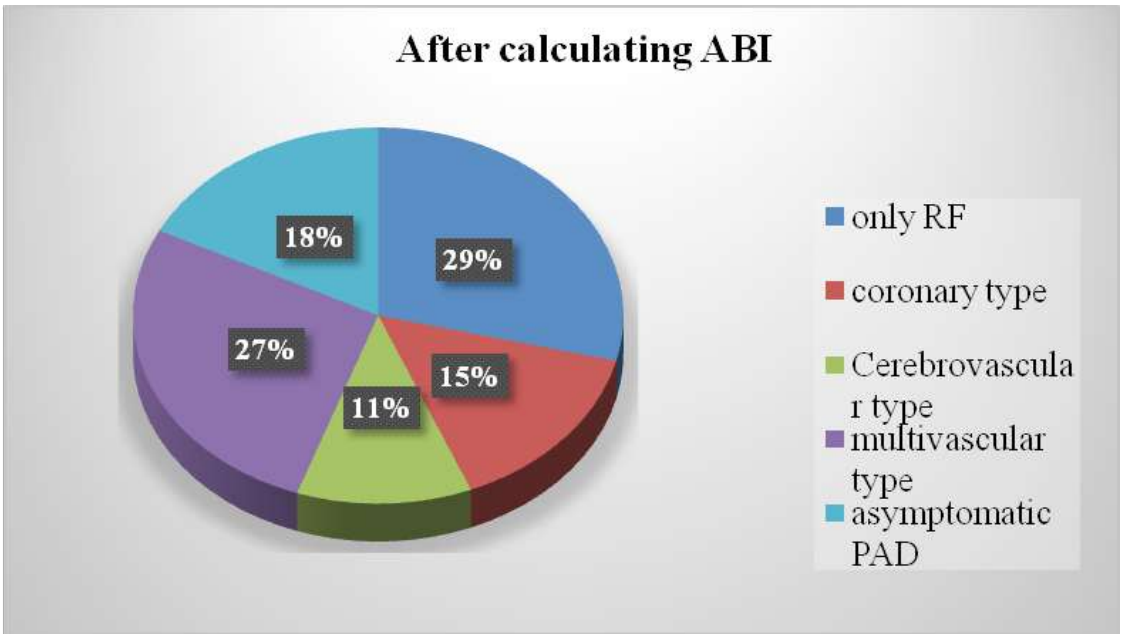


Figure 3. Patient distribution after calculating the ankle-brachial index for peripheral artery disease

Results

When enrolled in the study, patients with high risk and asymptomatic PAD were divided into four groups: patients with [1] documented coronary artery disease, [2] documented cerebrovascular disease, [3] documented multivascular disease (both coronary and cerebrovascular disease) and [4] the presence of at least one cardiovascular risk factor without ischemic or cerebrovascular disease. After evaluating the ankle-brachial index, coronary patients with PAD and cerebrovascular patients with PAD were counted as patients with multiple vascular lesions. Almost (38%) of the examined individuals, initially rated as having only RF, had PAD (36 (49.3%) of the total). The percentage of patients with multiple vascular lesions, which initially was 19 (3.4%), increased to 39 (7%) after measuring the ABI.

Widespread RF PADs include obesity, dyslipidemia, arterial hypertension, diabetes mellitus, smoking, and increased age.

BMI of patients ranged from 17.1 to 48.6 with an average of 26.9 kg / m² + 4.5% kg / m². 1.4% of the subjects were overweight, 1.5%[8] were of normal weight, 40.7% were obese, 19.6% were obese 1 st, 5.1% were obese 2 st, and 0.8% had obesity 3 tbsp. Only 32.6% of participants had normal BMI. and 66.2% were at least overweight. 33.5% of patients with PAD suffered from obesity, which is less than the proportion of people with obesity in patients with irreplaceable LPI (44.4%). Patients with a BMI of at least 30 (class I obesity) accounted for 40% of patients with PAD and 21.0% in patients without PAD (which roughly corresponds to the proportion in the average population). A significant relationship between BMI and PAD was revealed.

Discussion

Overall, 25.4% of participants suffered from diabetes (25.0% of women and 25.6% of men). The average disease duration was 12.1 + 10.1 years. 45% of all diabetics had PAD, compared with 26.4% of non-diabetic patients, and significantly more diabetics than non-diabetics with PAD. Overall, 33.5% of patients with PAD had diabetes mellitus. Of all diabetics, 44.4% had a history of myocardial infarction and 86.4% had arterial hypertension.

In addition to history, dyslipidemia was determined by laboratory determination of LDL (low density lipids), HDL (high density lipids), triglycerides and total cholesterol (HDL <1 mmol / L, LDL> 4.2 mmol / dL), triglycerides> 2 3 mmol / L (150 mg / dl) and total cholesterol> 6.2 mmol / L (240 mg / dl). 21 (37.8%) patients with high cholesterol had PAD, 20.3% of those without high cholesterol suffered from PAD. 5.3% of patients had high LDL levels. Of all patients with hyperlipidemia, 35.4% had PAD, 46.0% had myocardial infarction, and 88.0% had arterial hypertension.

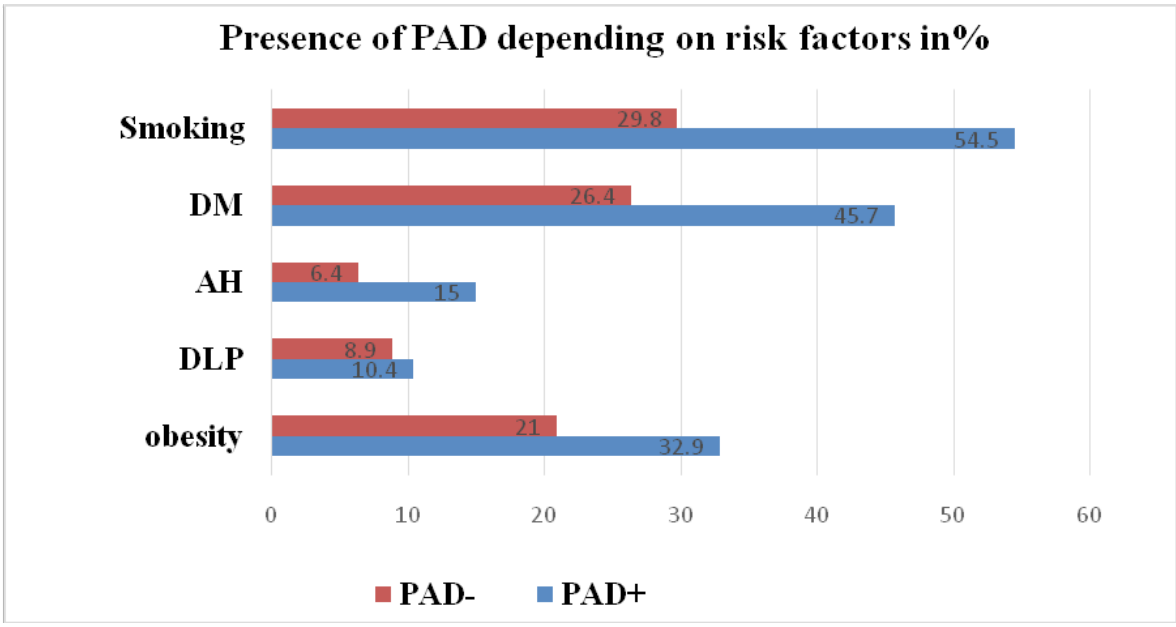


Figure 4. The presence of PAD for various cardiovascular risk factors: obesity, dyslipidemia, arterial hypertension, diabetes mellitus, smoking

Overall, 83.9% of all patients had hypertension. 34.0% of patients with arterial hypertension also had pathological ABI. 82.9% of patients with pathological ABI had arterial hypertension. 4.3% of the subjects were smokers (2.6% of women and 5.3% of men). Significant relationships have been identified between PAD and smoking. 29.8% of patients who never smoked had PAD, compared with 37.7% of former smokers and 54.5% of smokers (43.1%) ($p < 0.05$).

10 (7.6%) of patients under the age of 60 suffered from PAD. 44 (13.7%) patients out of 320 elderly patients had PAD. Over 75 years old, 19 (17.6%) of 108 patients suffered from PAD. Thus, there is a correlation of age with the presence of PAD.

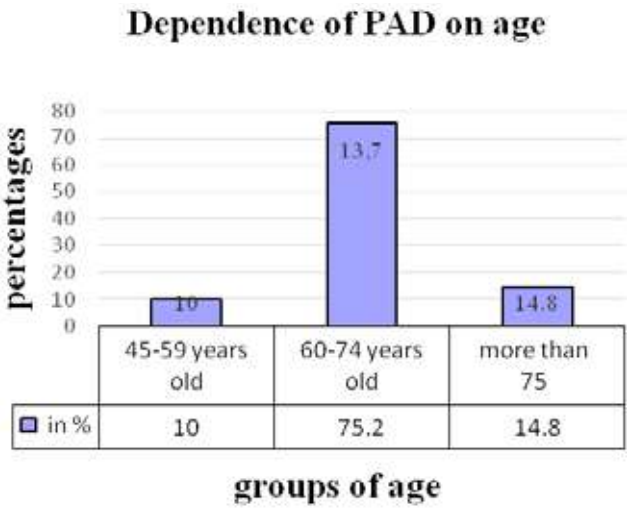


Figure 5. Frequency of PAD depending on age

Table 2: Distribution of the number of risk factors

The number of risk factors	Absolute number	%
0+1	128 (29 + 99)	25,20
2	299	58,86
3	132	25,98
4	18	3,54

Traditional risk factors were analyzed: hyperlipidemia, elderly and senile age, arterial hypertension, smoking and diabetes mellitus. The number of RFs for each patient was calculated. The distribution is shown in table.

Since only 26 patients had one risk factor. 117 patients had 2 RFs, 85 patients had 3 RFs, and 6 patients had 4 RFs. A correlation was found between the number of RFs and the presence of PAD. 11.1% of patients with 1 risk factor or without it had PAD. 50% of patients with two risk factors suffered from PAD. Patients with 3 risk factors had a PAD probability of 36.3%. 2.6% of patients with four risk factors had PAD.

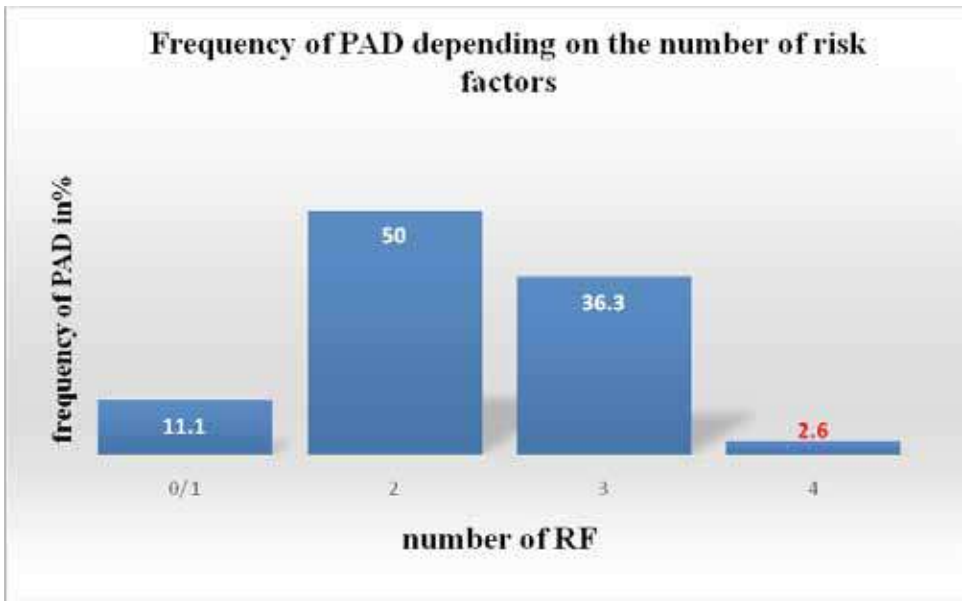


Figure 6. The frequency of PAD depending on the number of RF

Other possible RFs included the presence of first-line relatives with PAD. 13.4% of patients had a hereditary burden.

Among the concomitant diseases of PAD, other diseases of the cardiovascular and cerebral system (MI, IS, TIA) were analyzed. Among patients who had myocardial infarction, 39% had PAD or asymptomatic elevated ABI, compared with 31% of patients who did not have myocardial infarction. 45.1% of patients with PAD had a history of myocardial infarction. 36.4% had MI, in the absence of PAD. In 42.9% of patients with the condition after IS / TIA, PAD was diagnosed, compared with 32.6% of patients without cerebrovascular disease. In patients who complained of angina pectoris, 36.8% of cases had PAD. Significant correlation of selected concomitant diseases with PAD was not observed.

Conclusion

The results can be used in the work of medical expert commissions. The baseline examination should include a medical history, examination, palpation and auscultation, as well as a measurement of the ankle-brachial index.

The necessity of compulsory use of the ankle-brachial index (ABI) for the diagnosis of asymptomatic lesions of the arteries of the lower extremities in a screening examination of the elderly and senile, as well as people with high cardiovascular risk, has been proved.

The developed criteria for the values of the ankle-brachial index make it possible to diagnose lesions in the arteries of the lower extremities and to predict violations in the main arteries of the head and heart

Given the high diagnostic accuracy in assessing arterial disease of the lower extremities, color Doppler mapping of arteries is sufficient for decision-making in the treatment of these patients and may reduce the number of diagnostic angiographies. The method allows to assess the degree of damage, to clarify the topical diagnosis of PAD and to give an objective assessment of the functional state of the limbs, which can be effectively used in planning and monitoring treatment.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Significance, Effectiveness and Prospects of Development of Enzyme Preparations in Modern Pharmaceutical Practice

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Abstract

This article presents topical issues devoted to the study and prospects for the development of enzyme preparations in modern pharmaceutical practice in the Republic of Uzbekistan. In the context of existing resources, the rational use of high-quality, effective, safe medicines and the creation of new ones by combining local raw materials with an innovative approach is a priority. The expediency of combinations of drugs useful for medical practice for effort or combination of effects is noted.

In medicine, nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat rheumatoid arthritis and other inflammatory diseases. NSAIDs are the basic drugs in the pharmacotherapy of many chronic, acute diseases, normalizing biochemical parameters and the functional state of blood cells. These drugs are taken over-the-counter and for a long time, and therefore resolving issues, related to the safety of their use, the search for a combination is relevant. Currently, domestic developments of NSAIDs (fensulkal, blizketazin) are being carried out by combining enzymes (to enhance the effect) and in justified rectal and transfermal medicinal forms. Many diseases are associated with impaired secondary enzyme deficiency. Clinical studies confirm the high effectiveness of the combination of NSAIDs with enzyme preparations. The classification of proteolytic enzymes, the importance, scale and resources of the prevalence of plant enzymes in the Republic are presented. Of particular interest is the papaya (melon tree). Papain and chymopapain are latex enzymes of the fruits of the melon tree.

Keywords: *NSAID's, enzymes, enzyme preparations, pharmacy.*

Introduction

Currently, the most topical issues of rational use of drugs is the ability to assess the effectiveness, safety and quality, in circulation of medicines throughout their "life cycle": from the development, quality criterion before registration studies, the procedure of examination before going into wide clinical practice and the period of stay in the pharmaceutical market. In the XXI century medicine came with a powerful arsenal of drugs. Global pharmaceutical industry generates tens of thousands of medical products.[1]

The problem of drug provision, rational and safe pharmacotherapy has now become one of the most urgent tasks of the clinicians and pharmacists[2,]. This is due, on the one hand, with the ever increasing market of medicines (drugs) and the volume of scientific information[3], on the other - with the need to determine the most popular groups and classes of drugs.[4]

In the context of existing health care resources rational use of high-quality, efficient, safe medicines and the creation of new combination of using local raw materials with an innovative approach is a priority.

This requires the introduction of optimal evidence-based approaches that receive the greatest benefit and cost-effective to carry out, rational, effective and safe therapy combination drugs. [5]

Quality drugs - the basis of the safe treatment of patients. It is known that safety of drugs characterized by the absence of adverse reactions when it is used, and the efficiency – by the level of pharmacotherapeutic effect achieved by using the minimum dose of the active ingredients.

Adverse drug reactions (ADR) are a major problem of pharmacotherapy. The problem of safety conducted drug therapy involves the collection of information about their occurrence. Information about all the side effects of drugs used for more in-depth study of the causes of severe drug complications and their prevention measures. However, in recent years, along with the improvement of the work on timely notification of any cases ADR, remains unresolved the question of creating the conditions for a more complete account of all of these reactions. According to the International Center for the monitoring of adverse reactions to the WHO (Uppsala, September 2006, the Republic of Uzbekistan entered), it is considered that 2-3% of the general population of people suffering from the side reactions of drugs. [5, 6.]

The development of modern scientific and practical directions allows individualized drug selection, dosing regimens taking into account features.

The possibilities of modern clinical pharmacology study of drugs opens promising directions and have a great future.

These features and method should be actively used in the expert evaluation of the efficacy and safety of drugs. Questions of the rational, and hence effective, quality and safe pharmacotherapy are gone far beyond the medical community; they rightly belong to the most important strategic tasks of the day, a full program of import substitution drugs.

This is the basis of creating and entering the pharmaceutical market in the broad appeal of highly effective drugs.

In medical practice, often use multiple drugs simultaneously. Thus, they can interact with each other by changing the severity and nature of the main effect, its duration (prolongation), and the effort or weaken side and toxic effects.

Combined differences drugs are often used to enhance or combination of effects, i.e., appropriate combinations useful for medical practice.

The pharmacological interaction related by the fact that one substance alters the pharmacokinetics or pharmacodynamics of other component of the mixture. Furthermore, it is possible chemical and physical-chemical interaction of the substances in their joint application. [7]

In medicine, for the treatment of rheumatic, arthritic and other inflammatory diseases are widely used non-steroidal anti-inflammatory drugs (NSAID). NSAID are the basic drugs in the pharmacotherapy of many chronic, acute illnesses, normalize the biochemical parameters and functional status of the blood cells and connective tissue. More than thirty million people worldwide take NSAID on a daily basis, and 40% of patients older than 60 years and about 20% of hospitalized patients. [8]

However, their long-term use in effective doses is often difficult because of the frequent complications. Strategically important question remains to use the well-known and affordable drugs, adjusting the therapeutic dose, dosage form, or by combining with the possibility of obtaining synergy among them, reducing their toxicity, increasing safety and to introduce in the domestic pharmaceutical practice.

In a variety of publications of domestic and foreign scientists pay attention to the fact that 30% of men and 50% of women taking the recipe less form of NSAID and 60% of patients taking the drug without consulting a doctor.

Among NSAID ibuprofen, due to the relatively low toxicity, has been widely used in pediatric and geriatric medicine. Low toxicity of ibuprofen is proportional to its therapeutic efficacy. Relevance of research on the combination of a rectal and transdermal dosage forms is the need to increase the therapeutic efficacy of ibuprofen and its security application. [9]

The feasibility of using enzymes is associated with their ability to enhance, accelerate the therapeutic effect in various diseases in clinical practice. They act as a catalyst for biochemical processes, stimulate metabolism. Many scientists, as well as Uzbeks, devoted their work to the study and introduction of papaya into domestic practice.

Another important representative of proteases (animal or microbial) is serratiopeptidase, which is called the "silkworm enzyme", because it is produced from Serration bacteria found in the intestines of silkworms. Serratiopeptidaseis a proteolytic enzyme for the treatment of inflammation, and reduces the intensity of inflammation due to the hydrolysis of biologically active substances. Widely used in Japan, USA, Germany, Italy.

Thus, it is shown that drugs, which are natural physiologically active protein compounds (enzymes) have gained a worthy place among the means of practical medicine. Actual aspects of marketing research of drugs containing enzymes and presented on the pharmaceutical market of the Republic of Uzbekistan (RUz) have been carried out. The data for 2017, 2018 and 2019 were studied on the market of the Republic of Uzbekistan dominated by drugs containing enzymes of imported production (73%), and especially of animal origin (80%). The data presented in the State Register of Medicines by country (foreign, CIS, domestic) as well as by drug companies and pharmacotherapeutic groups are presented. The main types of dosage forms are coated tablets (53.13%). It is noted that the use of local raw materials containing enzymes with an innovative approach by combining drugs in rational dosage forms of quality control is a government priority in the development of the domestic pharmaceutical industry.

Materials and Method

The search for new classes of chemical compounds that are promising for targeted synthesis of anti-inflammatory, antimicrobial agents will reduce the financial costs of the development of new antimicrobial agents.

In the Uzbek scientific research chemical and pharmaceutical Institute named after A. Sultanov synthesized new biologically active drug-fensulkal, which is a bisulfite derivative of phenylglyoxylic acid, and benzoketazone.

Fensulkal characterized moderate toxicity and has pronounced anti-inflammatory and anti-microbial properties, which in total is very important for pathogenetically oriented treatment of many gynecological diseases. Results patent studies showed that the potassium salt of sodium bisulfite phenylglyoxylic acid derivative is a novel, previously non-synthesized and non-described compound (patent RUz IAP №02245/ 04.10.02). [10]

Today fensulkal used in the form of tablets (0.1g active substance TPA42 Uz- 0663-2003 and ointments (0.5% and 3% PAE 42 Uz-0187-2007).

Also benzketozon (thiosemicarbazone) NSAID recommended for rheumatic diseases (3%) ointment and combined gel.[11]

Currently under operation of the development combining with enzymes and antiallergic means in reasonable rational medicinal forms.

Many diseases (if not most) are associated with a violation of the qualitative and quantitative composition of enzymes in the body, the occurrence of secondary enzyme deficiency. When conducting pharmacotherapy, special attention must be paid to those drugs that have active metabolites formed during biotransformation. There are drugs that are initially inactive, and only as a result of biotransformation from them is active metabolites formed that determine the pharmacodynamic effects (Fig. 1)[12].

Prodrugs and their Active Metabolites: The Role of Cytochrome P-450 Isoenzymes:

Spironolactone	CYP 3A4	Canrenone
Clopidogrel	CYP 2C19	2-oxo clopidogrel
Enalapril	Carboxyesterase	Enalaprilate
Azathioprine	Xanthine oxidase	Mercaptopurine
Primidone	CYP 3A4	Phenobarbital
Tamoxifen	CYP 2D6	Endoxifene
Losartan	CYP 2C9	E-3174

Fig. 1. Examples of prodrugs, active metabolites, which are formed with the participation of biotransformation enzymes.

Results

The most common reason that inflammation does not proceed as necessary for a full and quick recovery is a violation of catabolic processes (destruction) and the release of the focus of inflammation from destroyed tissues, which again is caused by an imbalance in the work of enzymes. If these processes are improved, the damage zone will decrease and will be better prepared for the beginning of the recovery phase of inflammation with high chances of a more favorable outcome.

This fact has led us to the idea of the need to introduce enzymes into the body from the outside by combining drugs.

A lot of importance is the fact that with NSAIDs and antibacterial agents and enzymes (serratiopeptidase) working senergically helps to enhance their positive effects.[12]

Clinical studies confirm the high effectiveness of the combination of antibacterial agents and enzyme preparations in most acute and chronic inflammatory infections. With the simultaneous administration of antibacterial drugs in combination with proteolytic enzymes, the effectiveness of treatment increases, while the side effects of antibacterial agents, as well as the risk of complications, including intestinal dysbiosis, are reduced. Examples are diseases such as sinusitis, bronchitis, cystopyelitis, adnexitis, prostatitis, etc.

In the same way that serratiopeptidase potentiates the action of antibiotics; it potentiates the action of non-steroidal and steroidal anti-inflammatory drugs and therefore is often prescribed in combination with them or as a continuation of treatment after a short course of non-steroidal anti-inflammatory drugs according to all their indications.[13]

The use of enzymes (proteolytic enzymes) in the treatment of various diseases has its own history. Since ancient times, in the treatment of purulent wounds, abscesses and other purulent diseases of soft tissues, they used pumpkin juice, applied the pulp of papaya or green pineapple. Since all these plants contain a large number of enzymes, they contributed to more rapid cleansing and healing of wounds.

Enzymes are substances that are present in the tissues and cells of all living organisms and are able to accelerate the chemical reactions that occur in them many times. Enzymes can be simple proteins, built entirely from polypeptide chains and decomposed by hydrolysis only into amino acids. Simple proteins are hydrolytic enzymes (e.g., proteases, lipases, and ribonuclease) that perform their function in the absence of coenzyme. In most cases, enzymes are complex proteins. Complex proteins (holoenzymes) contain, along with the protein part (apoenzyme), a non-protein component (coenzyme or prosthetic group) (Fig. 2).

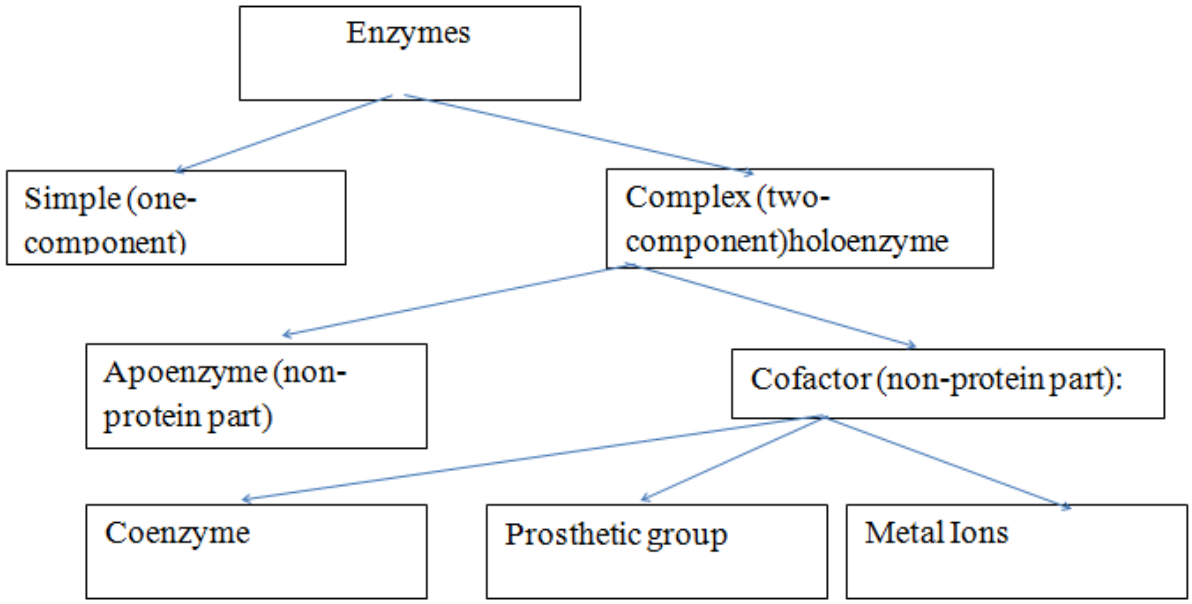


Fig. 2 Nomenclature of enzymes.

A substance whose chemical transformation catalyzes an enzyme is called a substrate.

Classification and nomenclature of enzymes: In 1961, the International Union of Biochemistry and Molecular Biology developed a systematic nomenclature, according to which all enzymes are divided into 6 main classes, depending on the type of catalyzed chemical reaction.

Each enzyme has a common working name, and a systematic one, used to uniquely identify the enzyme.

Working names are formed from combining the name of the substrate, the type of reaction and the end of "-ase". For example: lactate + dehydrogenation + ase = lactate dehydrogenase. The systematic name of the enzyme is formed as follows: name of substrates: name of the type of chemical transformation + ase. The same lactate dehydrogenase will have the systematic name "l-lactate: over + oxidoreductase".

Each of the six classes of enzymes has its own serial number, strictly assigned to it. [14]

In 2009, global sales of enzymes and enzyme preparations amounted to 440 million euros, including enzymes produced using traditional (classic) producer strains of 180 million euros (41% of total sales) homologous to GMM 1-2 safety classes - 132 million euros (30% of total sales). The sales of enzymes from heterologous GMMs of safety class 3 amounted to 128 million euros (29% of total sales in the global food market).[15]

Discussion

The production of enzyme preparations is one of the most large-scale and dynamically developing branches of biotechnology. Large volumes of production and a wide range of enzyme preparations are due to their demand in various industries, medicine, and scientific research.

According to the current classification, about 2,000 enzymes have been identified. The industry produces about 250 types of enzyme preparations. At the same time, about 99% are accounted for by 18 enzyme preparations.[16,17]

Sources of enzyme preparations.

Of all the existing natural sources of enzymes, microorganisms, some plants or individual organs of plants and animals that can accumulate significant amounts of enzymes are of practical interest for large-scale production of enzyme preparations.

Plant raw materials. The source of enzymes can be sprouted cereal grain, which can be used directly as a technical enzyme preparation or feedstock to obtain purified preparations. As a raw material for proteinases use the latex of melon tree and ficus, green juice of pineapple, papain, bromelain, ficin papaya, pineapple, figs.

The technology for the production of enzyme preparations from plant and animal raw materials includes two main stages: collecting enzyme-containing raw materials; isolation and purification of the target product.[18]

Proteolytic enzyme. These enzymes catalyze the hydrolysis of the peptide bond in the molecules of proteins and peptides.

Previously, proteolytic enzymes were classified into proteinases that break down proteins into polypeptides, and peptidases that hydrolyze polypeptides to amino acids. According to modern classification, proteases are divided into endo- and exopeptidases. The former can hydrolyze deep peptide bonds and break down proteins. In turn, endopeptidases, depending on the structure of the active center, are divided into serine, thiol, acid (carboxyl) and metalloproteinases. Exopeptidases can cleave terminal amino acids. In this regard, exopeptidases are divided into:[18,19]

- Aminopeptidases catalyzing the cleavage of N-terminal amino acids;
- Carboxypeptidases catalyzing the cleavage of C-terminal amino acids;
- Dipeptidases exhibiting specificity for dipeptide substrates.

Proteases are widely used in agriculture, food industry, and medicine.

Proteases of technological importance are divided into plant, animal and microbial.

Plant proteases. Papain and chymopapain are latex enzymes of the fruits of the melon tree. They belong to the group of thiol proteinases, activated by reduced glutathione and cysteine.

Ficin and bromelain. The first is obtained from figs, the second - from pineapple juice. These enzymes belong to thiol proteases. Both enzymes have similarities in properties and use with papain. They are also used to remove protein turbidity in beer and to soften meat.

Proteases of animal origin. They have a huge role in digestion processes.

Trypsin is a serine proteinase secreted by the pancreas, which is used as a crude pancreatin in the food industry for the production of hydrolysates.

It hydrolyzes peptide bonds formed by arginine and lysine.Trypsin is secreted by the pancreas as an inactive trypsinogen precursor and is activated by another enzyme.

Chymotrypsin - is secreted by the pancreas into the small intestine in the form of inactive chymotrypsinogen. It is activated by trypsin; wherein two dipeptides are cleaved off.

Hydrolyzes peptide bonds formed by tyrosine, tryptophan, phenylalanine. [20]

Pepsin - is produced by the gastric mucosa in the form of pepsinogen, which turns into active pepsin under the influence of HCl or by cleavage of one peptide bond.

Pepsin is part of the drug, tonic agents, chewing gum.

Microbial proteases. Fungal and bacterial proteases exhibit trypsin-like, pepsin-like, thiol-like, and other actions. There are enzymes with optimum in acidic, neutral and alkaline environments.

The feasibility of using proteolytic enzymes is systematically associated with their ability in clinical practice to enhance, accelerate the therapeutic effect in various diseases, affecting their introduction to the general patterns of their development, inflammation activity in patients with rheumatoid arthritis, ulcerative colitis, etc. Currently, proteolytic enzymes are effectively used to reduce the inflammatory process. [20]

Proteolytic enzymes do not belong to anti-inflammatory drugs of direct action. They act as a catalyst for biochemical processes, triggering or accelerating biochemical reactions in the inflammation zone, where there is a local deficiency of their own proteolytic enzymes, in which the duration of inflammation increases and recovery is delayed. Visher T.L et. al., 1976

Proteolytic enzymes (PE - catalyze the hydrolysis of the peptide bond in the molecules of proteins and peptides) provide reliable relief of inflammation as a result of pronounced fibrinolytic activity and exert an effect through anti-inflammatory, immunomodulating, antiaggregant, fibrinolytic, decongestant, and analgesic effects (Klein G. et al. 1999). .

Depending on the purpose of the application, certain requirements are imposed on the enzyme preparations with respect to the composition of the enzymes, the optimal conditions for their action, the degree of their purification, which is especially important for microbial preparations that require chemical, microbiological and toxicological control, the use of excipients, cost, etc. [14]

The search and implementation in medical practice of new highly effective enzyme preparations of plant origin is of certain interest. [21]

One of the properties of enzyme preparations is the ability to potentiate and facilitate the action of other drugs, as well as reduce their side effects.

The ability of enzymes to increase the concentration of antibacterial agents in the blood, to facilitate their penetration into the tissues and thereby increase the effectiveness of therapy is well known (Koyama A. et al., 1986; Selan L. et al., 1993).

World medicine limits the use of antibiotics. Scientists believe that in the future, antibiotics can be replaced by super-antibodies, for which the cell wall will not be an obstacle, will be able to penetrate into the cells and destroy pathogenic bacteria, viruses and toxins. They are testing antibody modification technology that allows them to freely enter cells and leave them [22, 23.]

In the modern world, much attention is paid to the use of biologically active herbal preparations in medical practice.

A typical representative of plant proteases is papain and chymopapain, and belongs to the group of thiol proteases.

Papain is a monothiol cysteine endoprotease.[16] By the nature of the enzymatic action, it is called “plant pepsin.” But, unlike pepsin, papain is active not only in acidic, but also in neutral and alkaline media (pH range 3–12, optimum pH 5). It retains activity over a wide temperature range. [22]

The papain catalytic center contains a dithioacyl group. [15,]

Papain also has anti-inflammatory properties. Without acting directly on the focus of inflammation, papain stimulates metabolism, which affects the acceleration of the regeneration of inflamed tissues[18]. Papain increases blood flow and destroys toxic substances in the focus of inflammation[24,]. In addition, papain destroys toxins released by many pathogens, participates in the synthesis of arginine[25], an amino acid

that stimulates growth hormone, which in turn is very important for the regeneration of liver cells[26,]. Papain also accelerates the healing of wounds, trophic ulcers and pressure sores. [27]

Exopeptidases having the ability to cleave terminal amino acids have already been indicated that proteolytic enzymes catalyze the hydrolysis of the peptide bond of proteins and peptides.

Papaya breaks down proteins into polypeptides and amino acids, and hydrolyzes any peptide bonds.

The homeland of the melon tree is Central and South America. It is cultivated in all tropical countries of the world as a fruit tree.

Uzbekistan has all the climatic conditions to cultivate a melon tree (papaya), the fruits of which can be used as raw materials for enzyme preparations, and the aerial part for obtaining effective medicines. A lot of works have been devoted to the phytochemical and proteolytic study of papaya growing in Uzbekistan (Aziizov I.K., Rakhimov M.R., Musaeva N.A. 2000) [27]

As a result, the substance and injection drug were approved for medical use in Uzbekistan. (2000)As a result of the study, useful amino acids were identified and the anti-inflammatory effect of the papaya substance was revealed, as well as the anti-inflammatory and immunostimulating activity of the tincture of the aboveground part of the domestic papaya.

The leaves contain free and bound phenolic compounds, tannins, organic acids, steroid and triterpene saponins, flavonoids, lipids, coumarins, glucose, alkaloids used in the treatment of tuberculosis and having choleretic and diuretic properties.

In Peru, papaya leaves are famous as an indispensable tool for healing wounds. Recently, papaya made a sensation in the medical world: Indian scientists have discovered that the cortex of a melon tree contains a substance that is 250 times more effective in inhibiting the growth of cancer cells than the most modern and advanced drugs. Research is underway (bark has never been used in medicine before), if contraindications are not identified, papaya will give the world an effective cure for a terrible disease.[23,24]

Papain is used in medicine: ophthalmology, neurosurgery, neuropathology, gastroenterology, urology.

Properties: It is capable of breaking down proteins of polypeptides and amino acids, acting on any peptide bonds (except for bonds of preline, glutamic acid of a dislocated carboxyl group), breaking down proteins deeper than most enzymes of animal and bacterial origin, and dissolving dead cells.

It has: proteolytic, anti-inflammatory, anticoagulant, dehydration, analgesic, bactericidal, hemolytic properties. Another important representative of proteases (animal or microbial) is serratiopeptidase. Serratiopeptidase is an enzyme for the treatment of inflammation. Serratiopeptidase is a proteolytic enzyme (that is, it is used by the body to break down proteins to amino acids), which is sometimes called the “silkworm enzyme” because it is produced from Serration bacteria found in the intestines of the silkworm. This enzyme is responsible for the splitting of the silkworm cocoon. [28]

One of the most widely used proteolytic enzymes in the treatment of inflammatory diseases in Japan, Germany, Italy, the USA for more than 40 years is serratiopeptidase. It is present in all living systems, but was first discovered in the body of a silkworm, which uses it to dissolve a cocoon when a butterfly appears[20]. Doctors have long appreciated the anti-inflammatory and analgesic properties of this natural substance and have successfully used it as an alternative to anti-inflammatory drugs[28]. Serratiopeptidase has proven itself in the treatment of post-traumatic and postoperative edema, as well as the relief of pain and inflammation in rheumatic diseases (Esch P.M. et al., 1989; Panagariya A. et al., 1999).[29]

Over the years, serratiopeptidase (an enzyme of animal origin) has been shown to be effective in sports injuries, sprains and ruptures of the ligaments, fractures and dislocations, postoperative edema, the risk of transplant rejection, and cavity surgery, not only to improve healing processes, but also to prevent postoperative adhesions, for the treatment of ENT pathology and inflammatory lung diseases - not only with the aim of resolving inflammation as soon as possible, but also giving dynamic properties to the mucociliary system of transport thanks to the action thins mucus. Also, it increases the effectiveness of the treatment of acute inflammatory dermatoses. The drug reduces breast edema and is indispensable for mastitis in breast-feeding thanks to not only high efficiency, but also safety.

Serrata has a direct effect on the pathogenetic mechanisms of these diseases and contributes to their cure due to the fact that:

- Reduces the intensity of inflammation due to the hydrolysis of biologically active substances - inflammatory mediators (bradykinin, histamine, serotonin, etc.), a decrease in the dilatation of capillaries and the regulation of their permeability;
- Working synergistically with non-steroidal anti-inflammatory drugs and antibacterial agents, enhances their positive effect. [15, 29]

The authors of studied the interaction of local and general reactions of the body in patients with traumatic osteomyelitis of the lower jaw of the fermentative drug "Serrata[17]".

It should also be noted that many take with chronic inflammatory pathologies, which means for a long period (key enzyme No. 41 of October 20, 2008. Weekly "Drugstore").[20-23]

However, many studies of this substance were poorly structured and did not include control groups. Further research will shed light on the ability of serratiopeptidases to treat these and other conditions.

A prospective study of the effects of serratiopeptidase (Aniflazim) on postoperative edema and pain was carried out with the participation of three randomized groups of patients. Sixty-six patients participating in this study received surgical treatment due to rupture of the lateral ligament of the knee joint.

In addition to reducing swelling, subjects also noted a decrease in pain.

According to BioMed Research International, for 2017, serratiopeptidase reduces swelling by reducing the amount of fluid in the tissues, thinning it and facilitating outflow. In addition, the enzymatic activity of this drug dissolves the dead tissue surrounding the damaged area, which helps to speed up the healing process.

Serratiopeptidase was noted to be superior to placebo in reducing pain, swelling, and chest tightening. A moderate to noticeable improvement was observed in 85.7% of patients receiving the supplement, while among the placebo group; only 60% of the subjects experienced a similar improvement. A "noticeable" improvement was found in 22.9% of patients receiving treatment, compared with 2.9% of the placebo group.[30]

It helps relieve symptoms of superficial vein thrombophlebitis. [21]

Italian researchers have compared the efficacy of serratiopeptidase and seaprose S in the treatment of inflammatory vein disease. Both treatments have been found to effectively reduce pain and alleviate the symptoms of thrombophlebitis.

In most studies, serratiopeptidase was used in a dosage of 10 to 60 mg. However, additional studies are still required to determine the optimal dose.

Also, judging by the available research data, this enzyme is an effective tool to reduce swelling after surgery and can be an alternative to conservative measures, such as the use of ice.[31, 32]

One of the properties of enzyme preparations is the ability to potentiate and facilitate the action of other drugs, as well as reduce their side effects. The ability of enzymes to increase the concentration of antibacterial agents in the blood, to facilitate their penetration into tissues and thereby increase the effectiveness of therapy. (Koyama A. ct al, 1986, Senan L.et al., 1993)

Most enzyme preparations are available in the form of dragees or tablets in enteric coatings, which protects the enzymes from being released in the stomach and destroyed by hydrochloric acid of gastric juice. The size of most tablets or dragees is 10 mm or more. Nevertheless, it is known that solid particles with a diameter of not more than 2 mm with an optimal size of 1.4 mm can be evacuated from the stomach at the same time as food[21.]

If the tablet or dragee is in the stomach for a long time, the enteric coating is destroyed, and the enzymes inside are inactivated. However, proteoastic enzymes are used in suppositories in gynecology. (Longidase).

Suppositories are also used for the registration of the Ruhr University G. Bohuli, Germany.

Thus, we see that drugs, which are natural physiologically active protein compounds (enzymes, their inhibitors and activators, hormones), have found their rightful place among the means of practical medicine. Unfortunately, the daily clinical use of enzymes is limited by both economic factors - their high cost and low availability, and their rapid inactivation under the conditions of the body, and various adverse reactions caused by or as foreign proteins (antigenicity,

allergenicity, toxicity, etc.). Significant number of these obstacles can be eliminated through the use of enzymes in a stable, immobilized form, especially since the efforts of engineering enzymes today operated by a significant number of method of covalent and non-covalent fixation of enzymes on insoluble and soluble carriers of diverse nature.

Modern medicine is increasingly using highly purified preparations of physiological active substances (PAS) of a protein nature in various fields of clinical medicine as promising drugs for medical treatment due to their extremely high activity and specificity. [33, 34]

We have given relevant aspects of the marketing research of drugs containing enzymes on the pharmaceutical market of the Republic of Uzbekistan. The results obtained indicate the feasibility of further marketing research of the nomenclature of drugs to assess the prospects of creating and introducing new domestic drugs containing enzymes.

A content analysis of drugs containing enzymes presented on the pharmaceutical market of the Republic of Uzbekistan and included in the State Register of Medicines of the Republic of Uzbekistan was carried out.

In the process of conducting a content analysis, as an object of study, we studied the data on the registration of drugs of enzyme origin based on the materials of the "State Register of Medicines and Medical Devices" for the period 2017, 2018 through the first half of 2019, and a list of essential drugs.[36, 37]

Content analysis is a formalized method for studying textual graphic information, which consists in translating the studied information into quantitative indicators and its static processing. In our studies, we developed and used a scheme for conducting content analysis.[35]

Currently, 63 trade names of pharmaceutical preparations containing enzymes are registered in the Republic of Uzbekistan, taking into account various forms, dosages and packaging. The pharmaceutical market of the Republic of Uzbekistan is mainly dominated by drugs containing imported enzymes (73%).

In accordance with the State Register of Medicines, we conducted an analysis of the nomenclature of drugs containing enzymes of origin. Currently, enzymes of animal origin prevail (80%), (tab. 1).

Also, all drugs containing enzymes can be divided into pharmacotherapeutic groups: proteolytic agents (for external and internal use), digestive enzyme preparations, immunomodulating agents, enzyme preparations (systemic effects), multivitamin preparations and other drugs. The largest number of trade names of drugs containing enzymes is noted in the pharmacotherapeutic group "enzyme preparations" (54.5%), Fig. 3.

Table 1. Analysis nomenclature medicaments containing enzymes origin.

№	By origin	2017 y.		2018 y.		2019 y.	
		Amount	%	Amount	%	Amount	%
1.	Plant	5	7.94%	7	10.94%	8	12.12%
3.	Microbial synthesis	5	7.94%	6	9.37%	6	9.09%
2.	Animal	53	84.12%	51	79.69%	52	78.78%

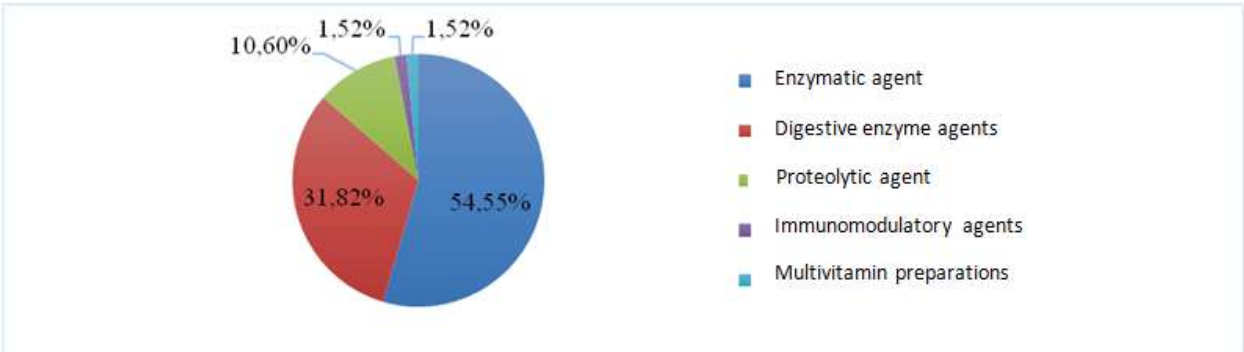


Figure 3. Distribution of trade names of drugs containing enzymes by pharmacotherapeutic groups.

A content analysis of enzyme drugs by type of dosage form was also carried out.

In the course of the analysis of the market for drugs containing enzymes, it was found that the main form of the dosage form for this group are coated tablets (53.13%).It can be concluded that a structured content analysis of enzyme drugs was carried out by comparing quantitative and qualitative characteristics according to the criteria: pharmacotherapeutic group, origin of the assortment of non-CIS countries, CIS countries and the Republic of Uzbekistan.

A relatively large number of enzyme preparations of foreign production were identified (73%), of which drugs imported from the CIS countries (24%), from foreign countries (49%), and domestic (25%) and (27%) for the period 2017- 2019, which suggests the relevance of replacing imported drugs with drugs of local origin obtained on the basis of substances of local raw materials. Moreover, Uzbekistan is rich in raw materials for the production of enzymes of plant and animal origin. Also relevant is the issue of obtaining drugs using local raw materials with an innovative approach, combining PAS to improve absorption and obtain a synergistic effect and reasonable selection of a rational dosage form.

Conclusion

The issue of strategic importance remains the use of well-known and affordable drugs in various combinations in various dosage forms, taking into account the possibility of obtaining the synergistic effect of their action, thereby reducing toxicity, increasing safety and introducing them into domestic pharmaceutical practice.

Moreover, the main emphasis is on those types of products, the introduction of which into production does not require large expenditures, time and money, and will reduce future consumption in their imports. The use of local raw materials with an innovative approach by combining drugs in rational dosage forms of quality control, standardization is an urgent and priority task of the government in the development of the domestic pharmaceutical industry.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Comparative Analysis of the Spectrophotometric and HPLC Method for Quantitative Determination of Fensulkal in a Substance

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Abstract

According to the World Health Organization (WHO), non-steroidal anti-inflammatory drugs (NSAIDs) are effective in treating pathologies accompanied by inflammatory and painful processes of various etiologies. More than thirty million people in the world receive NSAIDs daily, with 40% of these patients over 60 years of age, and about 20% of inpatients.

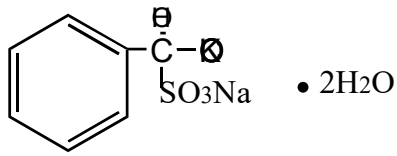
The development vector of pharmaceutical industry in the years of independence is aimed at developing and introducing import substitution drugs produced in our country.

Keywords: *Fensulkal, method, determination, chromatography.*

Introduction

In the Republic of Uzbekistan, special attention is paid to the development of pharmaceutical industry and providing the population with domestically produced products. In this regard, expanding the range of antimicrobial and anti-inflammatory drugs, using domestic resources, is a priority in the development of science and technology in the direction of modernization of production and technology to introduce domestic developments of drugs and medical products. A number of government resolutions are aimed at supporting domestic manufacturers producing pharmaceuticals, so in the production of domestic medicines equally, preference is given to drugs developed from raw materials of plant and synthetic origin. As well as standardization and quality control of medicinal products plays a crucial role in providing the population with effective and safe medicines[1].

The Uzbek Scientific Research Institute of Chemistry and Pharmaceutics named after A. Sultanov synthesized a new biologically active drug - fensulkal, which is a bisulphite derivative of phenyl glyoxylic acid. The results of patent studies have shown that potassium salt of sodium bisulphite derivative of phenyl glyoxylic acid (fensulkal) is a new, previously not synthesized and not described compound [7].



The substance is characterized by moderate toxicity and has strong anti-inflammatory and antimicrobial properties, which together is very important for pathogenetic treatment of many gynecological diseases[2]. Scientists of Tashkent Pharmaceutical Institute together with the above mentioned institute are working on creation of pharmaceutical forms and development of effective method of analysis[3].

Standardization and quality control of medicinal products plays an important role in providing the population with effective and safe medicines[4]. Nevertheless, along with the search for new medicinal products it is necessary to solve the issues of

development of method for their analysis[5],. Fensulkal in substances is quantitatively determined by several method such as chromatography spectrophotometry, spectrophotometry, HPLC[6]. Comparative analysis of the method for quantitative analysis of fensulkal in a substance is relevant as a result of the selection of a more valid one for the purpose of introducing into the quantitative analysis method the first time combined preparations containing this PAI (pharmaceutical active ingredient) are obtained[7].

Research Objective: Comparative analysis of the spectrophotometric, chromato-spectrophotometric and high-performance liquid chromatography (HPLC) method for quantitative determination of fensulkal in PAI.

The research subjects were samples of production batches of fensulkal (PS 42 Uz-0185 - 2017).

"Silufol UV-254" (Czech Republic) plates were used for thin layer chromatography (TCH) and chromatography spectrophotometric method. Various organic solvents were used as a mobile phase (MP): chloroform, acetone, ethyl acetate, hexane, etc. in different variations and ratios [5,6,7]. In work they used liquid chromatograph "Agilent Technologies 1100 series" with software "Chemstation A.09.0" with four-channel gradient pump, degasser and spectrophotometric detector. Acetonitrile, methanol as well as 1% acetic acid solution and buffer solutions with different pH values were used as organic solvents. The optical density of the investigated solution (0.001% fensulkal solution) was measured at a layer thickness of 10 mm in the range of wavelengths from 200 to 400 nm relative to the working standard (WS) of fensulkal. Purified water was used as a comparison solution. The quantification of the active substance was calculated using a formula:

$$X(\%) = \frac{D_1 * C * V_p^1 * V_p^2 * 100}{D_0 * a * V_{ali}^1} \quad (1)$$

Where, D_1 - the value of optical density of fensulkal in the test solution; D_0 - the value of optical density of working sample (WS) of fensulkal; C- the concentration of the preparation in 1 ml of standard solution g/ml; a- the quantity of the test substance; $V_p^1 * V_p^2$ – the volumes of dilution of the quantity; V^l_{ali} - the volume of aliquot part.

The aqueous solution of fensulkal has a maximum absorption at a wavelength of 252±2nm. The spectrum of absorption of fensulkal is shown in Fig. 1,2.

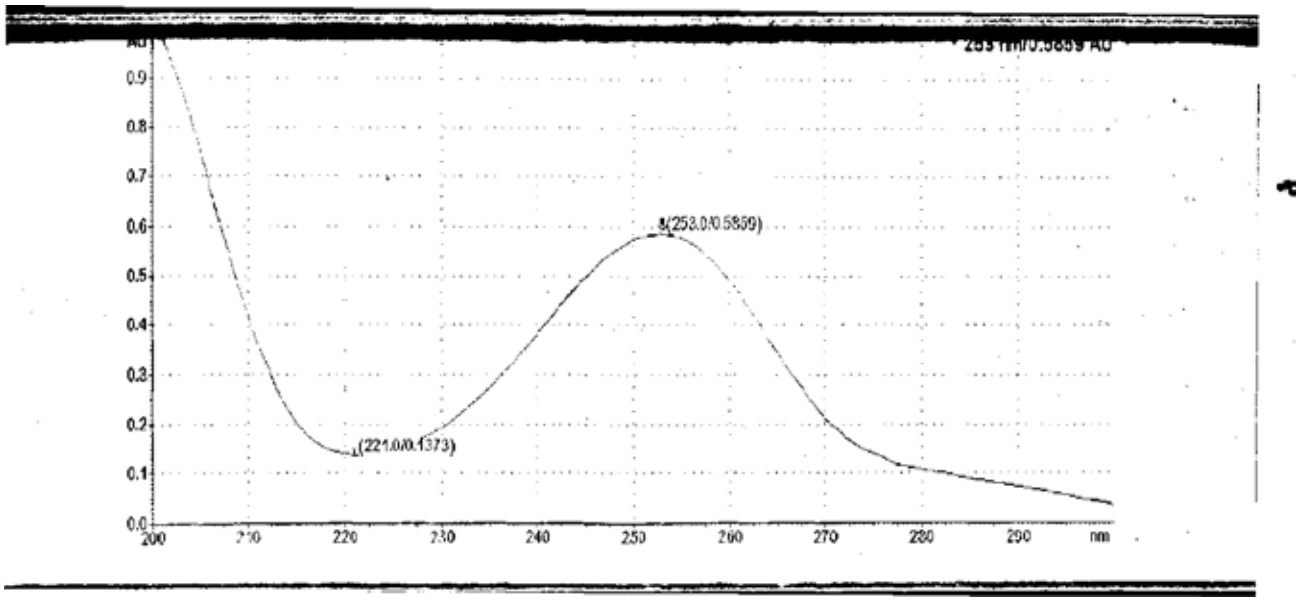


Fig. 1. UV spectrum of WS fensulkal

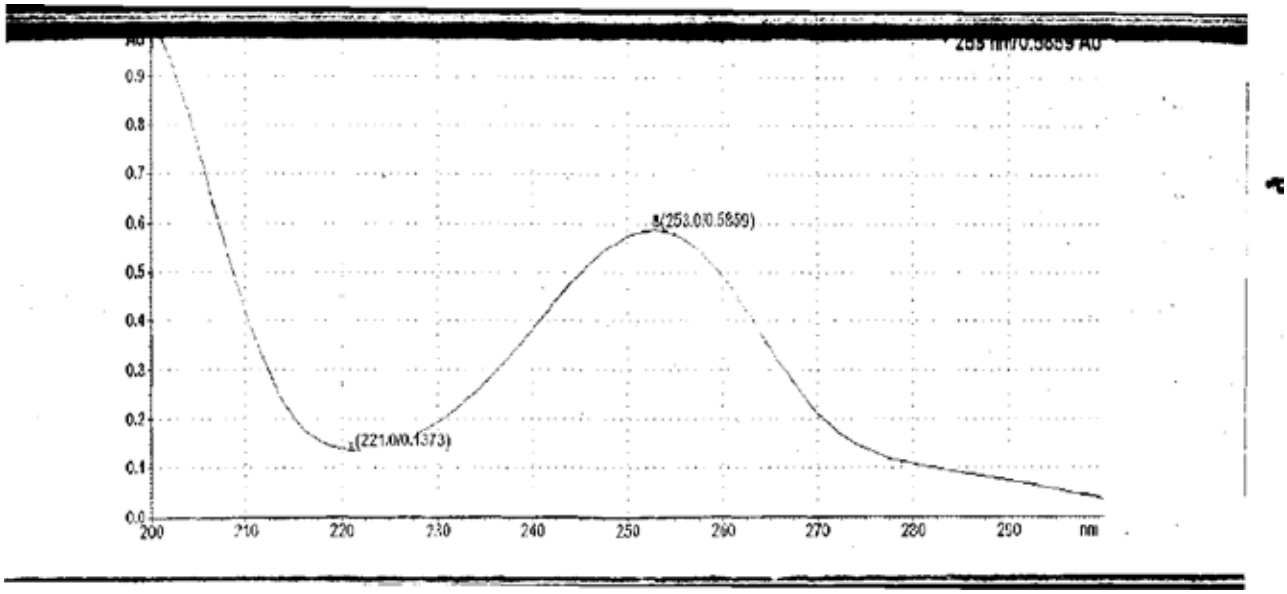


Fig. 2. UV spectrum of the test sample of fensulkal

The received data of quantitative determination of fensulkal in PAI by spectrophotometric method are given in Table 1.

Table 1: Results of the analysis of the quantitative determination of fensulkal in PAI by UV spectrophotometry

No.	Quantity of fensulkal, gr	X, %	Metrological properties
Fensulkal			
1	0,0250	98,90	X_{av}= 99,0 f =4; t(P;f)=2,78; S^2=0,078; S=0,837; ΔX_{cp}= 0,99; ε_{av}= 0,9%
2	0,0249	98,95	
3	0,0249	98,95	
4	0,0250	99,10	
5	0,0251	99,10	

Table 1 shows that the relative error of the method was 0.9%, which also confirms the accuracy of the results obtained within the method.

For the chromatography spectrophotometric determination of 0.2 g of fensulkal was placed in a measuring flask with a capacity of 100 ml and diluted with water to the mark. Then a 0.05 ml water solution of the test sample was applied to the starting line of the "Silufol-UV_{254}"chromatographic plate using a calibration capillary. At the same time, a standard sample of 0.01 ml of witness substance containing 1.0 mg of fensulkal was chromatographed. The plate was chromatographed and after the mobile phase reached the front line, the plate was dried at room temperature. The adsorption zones were detected in UV-light at λ = 254 nm. For quantitative determination of the localization zone, the adsorption zones were eluated, transferred to a paper filter and 100 ml of fensulkal was washed with purified water. Optical density of the obtained eluates was measured on spectrophotometer "Agilent 8453E" at 252 nm. The quantitative content of fensulkal was calculated using the following formula 1.

Preparation of WS solution of fensulkal: About 0.025 g (precise quantity) of fensulkal (VFS 42 Uz-0185-2007) is placed in a measuring flask with a capacity of 50 ml, add 20 ml of a mixture of purified water with methanol, shake to dissolution, bring the volume of the solution to the mark and stir. Place 1 ml of the resulting solution in a measuring flask with a capacity of 50 ml and bring the volume of solution with purified water to the mark. The solution is used freshly prepared. The resulting solutions are filtered through

a filter "Millipor" with a pore size of 0.45 µm. 20 µl of the obtained solutions are injected into the injector of the chromatograph and analyzed under the above conditions, receiving at least 5 chromatograms. The content of fensulkal, in % is calculated by formula (2):

$$\tilde{O}(\%) = \frac{S_1 * a_0 * 2 * P}{S_0 * \grave{a}_1} \quad (2)$$

Where, S_1 - the area of peak fensulkal on a chromatogram of a test solution; S_0- the area of peak fensulkal on a chromatogram WS of fensulkal; a_0– quantity of WS Fensulkal, gr; P – Fensulkal content in WS, %.Content of $C_8H_{10}O_8SNaK$ (fensulkal) should be not less than 98,5 %. The obtained results are presented in Table 2.

Table 2: Results of quantitative determination of fensulkal by chromatography spectrophotometric method

No.	Quantity of Fensulkal, gr	$X_{\%}$	Metrological properties
1	0,2001	98,7	X_{av} = 99,16%; f = 4; t(P;f) = 2,78; S^2 = 0,31; S = 0,51; ΔX_{av} = 0,69; ε_{av} = 1,9%
2	0,2000	99,0	
3	0,2004	99,2	
4	0,2000	99,3	
5	0,2001	99,6	

The data presented in Table 2 confirm the accuracy ($\varepsilon_{average}$=1.9%) of the results obtained and the reproducibility of the developed method for determination of the quantitative content of fensulkal.

HPLC analysis was performed on a liquid chromatograph by "Agilent Technologies" with a column 150 mm long, 3.0 mm in diameter, filled with Zorbax Eclipse XDB C-18 sorbent with a particle size of 5 µm, equipped with a UV detector with a variable wavelength (254 nm) and an isocratic pump. The data were processed using the "ChemStation" software. The relative standard deviation of the peak areas of the received chromatograms should not exceed 2.0% (Fig.3).

As a mobile phase several systems of organic solvents in different ratios were used.

In terms of retention time and symmetry of peaks we have chosen the following conditions for chromatography:

- 150x3.0 mm analytical column filled with Zorbax Eslipse XDBC18 sorbent with 3.5 µm particle size;
- Column temperature - indoor;
- Detection - 254 nm;
- Mobile phase degassed and filtered mixture of methanol and water in ratio (25:75);
- Injector loop volume - 20 µl;
- Flow rate - 0.6 ml/min.

The Fensulkal separation factor (K`) was 0.86, the number of theoretical plates was more than 2250 and the asymmetry factor did not exceed 1.1. The results of the quantitative determination are presented in Table 3.

Table 3: Results of the quantitative determination of fensulkal by HPLC method

No.	Quantity of Fensulkal, gr	$X_{\%}$	Metrological properties
1	0,0510	98,1	X_{av} = 99,08%; f = 4; t(P;f) = 2,78; S^2 = 0,42; S = 0,64; ΔX_{av} = 0,8; ε_{av} = 1,5%
2	0,0509	99,2	
3	0,0520	99,0	
4	0,0510	99,8	
5	0,0501	99,3	

The data presented in Table 3 confirm the accuracy (ε_{av}=1.5%) of the results obtained and the reproducibility of the developed method for determination of the quantitative content of fensulkal.

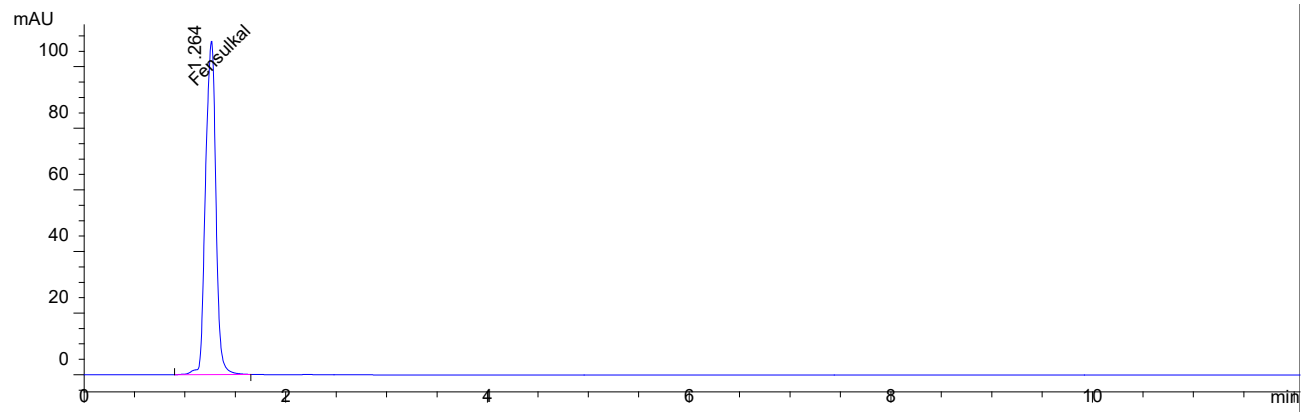


Fig. 3. Chromatogram of WS fensulkal

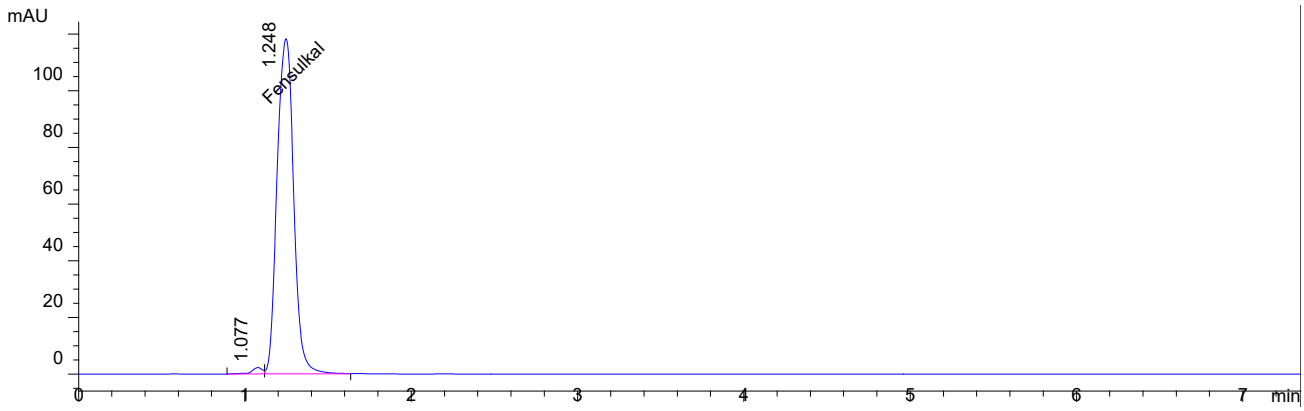


Fig. 4. Chromatogram of the test sample of fensulkal

From the above chromatograms (Fig. 3,4) we can see that at column temperature 20^0C the retention time of fensulkal is 1.248 min. The results of quantitative determination are presented in Table 3.

Since the HPLC method of determining the quantitative content of fensulkal is accurate enough to validate this method on several indicators.

Linearity was assessed by the response of the analytical instrumentation, and model solutions of different concentrations were prepared, corresponding to a range from 25% to 100% of the range specified in the respective technique. These solutions were analyzed on the "Agilent Technologies" liquid chromatograph under the conditions specified above. The results obtained indicate that the best range of linearity is observed in the range of concentrations of the preparation from 0.001 mg/ml to 0.3 mg/ml (Table 4, Fig. 5).

Table 4: Dependence of areas of chromatographic peaks on solution concentration (Linearity)

Solution concentration, mg/ml	Chromatographic peaks area
0,001	51,27
0,10	3649,44
0,15	7350,09
0,20	11039,68
0,30	14765,40
Correlation coefficient	0.999422
Standard deviation	1315.841881
Linear Angular Coefficient	7.01688E-06

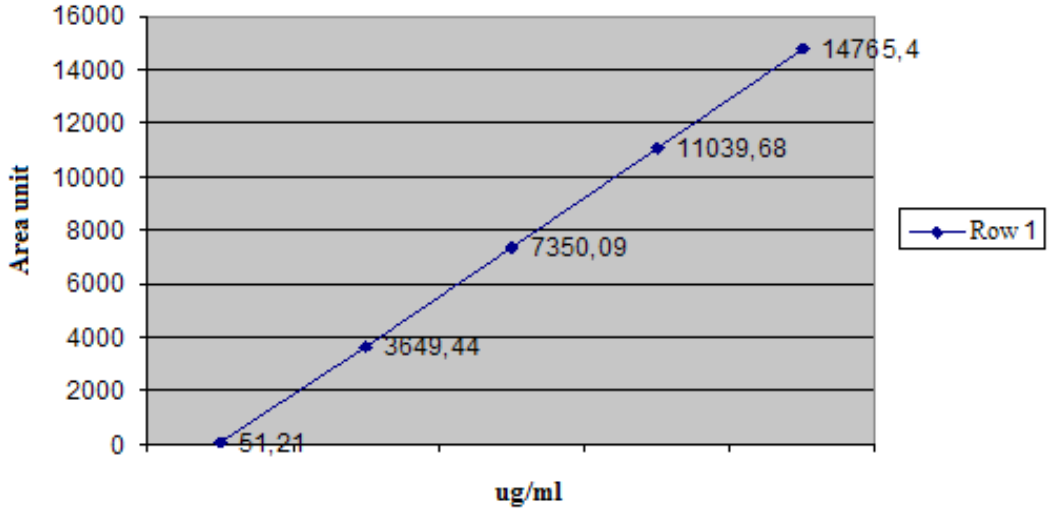


Fig. 5. Fensulkal Linearity Graph

Correctness of the technique was determined by three times determination of 7 analytical concentrations of the substance under study (Table 5). The quantitative content of fensulkal was calculated by the above formula.

Table 5: Evaluation of the correctness of the developed HPLC methodology of the fensulkal

Number of active component from declared, %	Quantity of fensulkal, gr	Found, gr	Regeneration, %
70	0,0350	0,0348	99,4
80	0,0401	0,0398	99,2
90	0,0451	0,0446	98,8
100	0,0502	0,0497	99,0
110	0,0551	0,0547	99,2
120	0,0601	0,0595	99,0
130	0,0650	0,0647	99,5
Average regeneration rate			99,15

From the results in the table we can see that the average regeneration rate is 99.15%, which indicates the correctness of the technique. So, we have developed a new method of separation, detection and determination of the quantitative content of the substance fensulkal by HPLC. The validation of the technique has been carried out; such parameters as sensitivity, linearity, accuracy, reproducibility, selectivity and correctness have been established.

Similarity (e.g. the same spectrophotometer, the same laboratory assistant, the same day, identical reagents) was shown by analyzing 6 prepared samples at 100% concentration of the pharmaceutical substance in the solution under study. The results were evaluated and calculated by calculating the mean value, standard deviation, coefficient of variation and confidence interval. The number of tests required for routine control of each sample was established in the specification depending on a certain coefficient of variation and boundaries of the norm. With the help of intermediate precision it was shown that the analytical technique ensures the obtaining of consistent results regardless of changes in external circumstances provided the homogeneity of the sample under study. All conditions for the laboratory have been taken into account in the validation process. Typical variables were variability, staff, day of analysis and instrument.

Conclusion

Method have been developed to control the quality of fensulkal in substances using UV-, chromatography spectrophotometric, and HPLC method of analysis, the

relative error of which was 0.9 percent for the UV-, chromatography spectrophotometric method; 1.9 percent for the chromatography method; and 1.5 percent for the HPLC method. The developed method are included in the pharmacopoeia article of fensulkal and can be used for quality control not only of fensulkal in PAI, but also in medicinal forms with its content.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Technologies for Producing Capsules "Yuyuzin"

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Abstract

Hypertension is a disease whose main symptom is an increase in blood pressure due to neuro-functional disorders of vascular tone. Hypertension affects men and women equally. The disease usually begins after the age of 40. This is a very common pathology. It is called the disease of the fall of life, although in recent decades hypertension has become much younger.

Capsule,substance, extract, calcium Hypertension is one of the causes of disability and mortality in patients with disorders of the cardiovascular system.

One of the causes of hypertension is prolonged and frequent neuropsychological stress, prolonged stress. Hypertension very often occurs in people whose work is associated with constant emotional stress. People who have suffered a concussion often suffer from it.

The paper presents the results of the development of the optimal composition and hard gelatin capsules based on the dry extract of Jujube fruit - Ziziphusjujuba (jujube, jilanjija). The formulation and technology of the single - source pharmaceutical product in the form of capsules "Yuyuzin" with antihypotensive activity was developed. As excipients used MCC, Aerosil, calcium stearate.

Keywords: *Technology, excipients, substance, capsule, antihypotensive activity.*

Introduction

More than 1 billion people on earth suffer from arterial hypertension (AH). AH remains the most common disease of the cardiovascular system in many countries. It has been diagnosed in 30% of adult population in developed countries, and it has reached 44% among men of working age, and it has reached 55-60% among people over 60 years of age. AH is the most common reason for medical appointment and administration of medications [1].

AH is also a leading cause of cardiovascular morbidity and mortality. It has been established that the risk of Myocardial Infarction increases with an increase in blood pressure (BP). Acute cerebrovascular accident In 95% of cases, 94% of Myocardial Infarction in women and 75% in men occur against the background of hypertension. Studies show that about 75% of elderly patients with heart failure have a background of AH. It is also the most common cause of atrial fibrillation, which occurs among other permanent forms of arrhythmias. In general, the incidence of hypertension in patients with cardiovascular pathology is 96%, and only 4% of patients do not have this background disease[2].

One of the most important tasks of modern pharmaceutical technology is the development of dosage forms boosting to enhance the bioavailability of drugs and reduce side effects. This is achieved in various ways, among which the usage of special excipients

and technological method can be distinguished. Of the greatest interest from oral dosage forms are capsules, due to a number of advantages and positive characteristics: accuracy and uniformity of dosage, corrective ability, high bioavailability, stability, aesthetics.

Currently, much attention is paid to the search for new antihypotensive medications based on local drug raw materials. One of such unique plants growing in Uzbekistan is Jujube - *Ziziphinsjujube* (jujube, jilanjija). Jujube fruits have been used as a hypotensive agent in traditional medicine [3]. The technology for obtaining a dry extract of Jujube fruits, based on Jujube fruits with a hypotensive effect, was developed at UzKFITI[4].

Objective: Formulation development and technology of the single - source pharmaceutical product in the form of capsules "Yuyuzin"

As you know, the technology of solid dosage forms of herbal preparations has its own characteristics, associated with the physicochemical properties of the starting substances, which must be taken into account when choosing excipients when establishing the regime of production operations.

The initial stage in the development of the technology for producing solid dosage forms of drugs is the study of the structural, mechanical and technological properties of active substances, depending on which the pressed material can behave differently. According to the indicators of the technological properties of the substance, it is possible to predict the types and quantities of necessary auxiliary substances, as well as the progress of the process [5].

The Jujube fruits substance we have been studying is a product of plant origin, which has a variety of physical and mechanical properties and absorbability. Absorbability plays an important role in the technology of solid dosage forms (TDLF) containing plant extract It affects the technological properties of the substance, such as fluidity, bulk, compressibility, elasticity, viscosity, ductility, which determine a number of technological parameters, such as the rate of granules formation, pressure, speed of pressing.

Our studies showed that the substance has non-positive technological parameters (Table 1).

In order to scientifically substantiate the type and amount of excipients introduced into the capsule composition and the optimal encapsulation method, the physicochemical and technological properties of the dry extract of Jujube fruits have been studied according to the method described in the literature. The results of the experiments are presented in table 1.

Table 1: The results of the study of the technological properties of dry extract of Jujube fruits

№	Test characteristic	UM	Results
1	**Grain particle size**	μm,%	
	+500		0.4
	-500 +300		5.8
	-300 +200		37.4
	-200 +150		49.3
	-150 +100		6.3
	-100		0.8
2	Friability	μm,%	no
3	Angle of repose	degree	42
4	Bulk volume	g / ml	0.66
5	Residual humidity	%	5

As can be seen from table 1, the examined dry extract as a whole does not have satisfactory technological characteristics for the production of encapsulated dosage forms, which requires the introduction of excipients into the capsule composition.

It is necessary to choose an optimal particle size distribution for the development of a dosage form of the drug in the form of capsules.

Analysis of the dry extract showed that it contains about 5% moisture, i.e. dry Jujube fruit extract is a hygroscopic substance.

Preliminary pharmacological studies to determine the specific hypotensive activity of the dry extract of Jujube fruits showed that the optimal single dose is 0.35 g.

As a result of studying the properties of the dry extract, it was found that it is a brown powder with sweet astringent taste and distinct smell.

The following are the results of the development of the technology for producing an encapsulated dosage form of the dry extract of Jujube fruits. In addition to pharmacologically active substance, auxiliary ingredients that must be biologically indifferent are introduced into the composition of the mass for filling capsules in order to give it necessary technological characteristics, as well as to set, if necessary, biopharmaceutical properties[6].

Considering the above factors, various excipients has been used to obtain the capsules of the Yuyuzin preparation. Fillers or diluents are most often used. They give the mixture for filling capsules the necessary optimal volume. For these purposes, in the manufacture of medicine in the form of hard capsules, milk sugar, microcrystalline cellulose (MCC), dibasic calcium phosphate, crospovidone and other substances are used that allow to adjust bulk density and give the filler necessary flowability, and MCC, in addition, allows to slow down the absorption process, which is important for the prolonged action of the drug. Tamped powders break up 2 times longer than freely filled ones, but the difference becomes insignificant with the introduction of disintegrants. Aerosil, talc, calcium carbonate are used In this quality. Calcium or magnesium stearate, etc. are commonly used to impart friability. MCC, aerosil-200, potato starch, lactose anhydride, crospovidone, calcium stearate in various proportions were tested as auxiliary substances.

Subsequently, each batch was individually moistened with 96% ethanol, 2%, 5%, starch paste, MC gel, Na-CMC, MCC, Aerosil, starch, lactose, crospovidone, calcium stearate to form the optimal wet substance. The data are shown in table 2.[7]

As can be seen from table 2, we tested MCC, aerosil, lactose anhydride, crospovidone, calcium stearate in various ratios as auxiliary substances. Based on the results, the capsule mass had been prepared in 8 different formulations and the influence of excipients on the technological properties of the substance was determined (table 3).

Table 2: The composition of the components of the capsules Yuyuzin

Composition, g	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dry Peony Extract Evasion	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
MCC	0.095	0.08	0.092	0.095	0.085	0.07	-	0.02
Aerosil	-	0.01	-	0.002	0.01	-	-	-
Lactose	-	0.01	-	-	-	-	0.01	0.01
Crospovidone	-	-	-	-	-	0.03	0.09	0.03
Calcium Stearate	0.005	-	0.008	0.003	0.005	-	-	-
Total weight (g)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45

Table 3: The results of studies to determine the technological properties of the substance and masses obtained on the basis of the composition selected for encapsulation Yuyuzin

Studied properties	**Compositions**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Form	**Brown powder**							
Fractional composition, µm								
+ 500	42	50	45	40.7	50.4	48.4	44.4	50.2
- 500 + 300	35.8	35.8	35	25.8	39.2	36.8	38.8	36.8
- 300 + 200	7	7.4	6.8	6.4	7.3	7.0	6.4	7.4
- 200 + 150	1.5	1.9	1.3	3.3	1.9	2.3	2.0	2.3
- 150 + 100	1.1	1.3	1	2.3	1.2	1.3	1.2	1
- 100	0.2	0.0	0.8	1.5	0.0	0.0	0.8	0.0
Friability µm,%	no	22.1	no	19.8	24.9	20.2	no	19
Angle of repose degree	47	31	48	43	30	44	41	44
Bulk volume (g / ml)	0.26	0.39	0.32	0.28	0.43	0.40	0.24	0.28
Residual humidity %	5	3.5	4.8	5.5	3.1	4.5	5	4.1

The analysis of the physicochemical properties of the capsules was carried out in accordance with the requirements of National Pharmacopeia, vol. XI. The results of the analysis showed that of the 8 investigated compounds, the most satisfactory results were obtained for compounds 2 and 5. However, taking into account the presence of the production of excipients in the Republic of Uzbekistan, composition 5 has been selected as the most suitable in the future for organizing the production of Yuyuzin capsules.

To obtain Yuyuzin capsules of 0.35 g each, to 37.2 g of the crushed dry extract of Jujube fruit, 9.0 g of MCC, 0.53 g of calcium stearate, 1.06 g of Aerosil are added and the mixture passes through a sieve with a hole size of 0.25 mm. Then the resulting mass in the amount of 47.7 g is moistened with 10 ml of 96% ethyl alcohol and repassed through a sieve with a hole size of 0.63 mm. The obtained granules are dried at a temperature of 45 + 5 ° C for 30-45 min to the optimal residual humidity.

The dried mass in the amount of 45.1 g is sieved through a sieve and the finished granules are transferred to the stage of filling capsules. 0.45 g "0" gelatin capsules are filled on a capsule filling machine from Pharma-ChemIndustris (India). 100 pcs capsules received.

The resulting Yuyuzin capsules of 0.45 g are packed in 30 or 60 pcs in bottle.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Adaptive Capabilities of Underweight Newborns and Development Features in their First Year of Life

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Abstract

Introduction: The issue of caring underweight infants is vital today because of the relatively high mortality rate. The perinatal death in full-term babies born with low birth weight is 3–8 times higher than in children with normal weight and takes the second place in the structure of perinatal mortality after prematurity. Moreover, the lack of a well-established concept for the management of such children in the first year of life in the literature, make us to conduct additional studies in this sphere. In order to evaluate the adaptive capabilities of children and monitor their physical development, growth curves and development of children checked according to WHO standards.

Method: 281 newborns and 272 mothers were examined. Newborns were divided into 2 groups: group 1-83 premature infants with low body weight; group 2-103 full-term newborns with low body weight. The control group consisted of 95 full-term newborns. Quantitative determination of human placental growth factor (PlGF) was performed based on the enzyme-linked immunosorbent assay. Correlation analysis and statistical processing of the gained data were calculated on MS Excel - XP software and statistical analysis of the results was carried out using method of variation statistics.

Results. 83 mothers had babies born prematurely with low body weight relative to gestational age (group 1); in 102 mothers - full-term, but with low body weight (group 2). The control group consisted of 87 women whose newborns were born on time (38.4 ± 6.3 weeks) and body weight corresponded to gestational age. A high risk of birth with low body weight in pregnant women was observed with signs of fetal growth retardation by ultrasound examination (OR = 11.58), with anemia (OR = 8.17), other malnutrition (OR = 5.75), placental insufficiency (OR = 5.46). Signs of perinatal cerebral hypoxic-ischemia of the 2nd degree were noted in groups 1 and 2, respectively, in 73.5% and 66.0% of children.

Conclusion: Pregnant women who have proteinuria and preeclampsia are 3 times more likely to have babies with low body weight compared to pregnant women with normal renal function and blood pressure. Underweight preterm infants are born in severe asphyxia, with a severe degree of RDS, severe disorders of the central nervous system; they require primary resuscitation measures more often, longer antibacterial therapy.

Keywords: *Premature infants, intrauterine growth retardation, adaptation, neurological disorders, placental growth factor, neurospecific protein S100b.*

Introduction

According to the World Health Organization (WHO), the problem of nursing for underweight babies is still actual due to the relatively high mortality rate. Out of 2.7 million dead newborns, 60-80% are premature and/or low birth weight infants (United Nations, 2015; and UNICEF, 2014)[11]. The perinatal mortality of full-term babies who born with low birth weight is 3–8

times higher than in children with normal weight and takes the second place in the structure of perinatal losses after prematurity (Galinsky et al, 2018; and Plomgaard et al, 2017)[7,10]. Premature babies die 30-35 times more often than babies born on time, and perinatal mortality in premature pregnancies is 30-40 times higher than in urgent births. Among children born with low body weight, the proportion of healthy ones do not exceed 10-25%, and the percentage of severe neurological abnormalities (childhood disabilities - cerebral palsy, blindness, deafness, mental retardation) varies from 12 to 32%. In 32.5% of children born with low weight, deviations in physical development persist even by the age of 1 year (Barkun et al, 2013;)[2].

Conflicting thoughts are available in the literature for infants born with low birth weight. Some authors claim a decrease in the number of children with disabilities, while others say that the number of children in this category is not decreasing today (Kometova et al, 2017; Pisani & Spagnoli, 2016; and Wojtczak-Soska & Lelonek, 2010)[9]. In this regard, the problem of studying the clinical and biochemical characteristics of the adaptation of underweight newborns and the optimization of the tactics of medical care for this category of children is extremely important and represents both scientific and practical significance (Lebedeva et al, 2015; and Wojtczak-Soska & Lelonek, 2010)[4,14].

The current approaches to assessing the severity of a condition are based on a diagnosis, risk factors for the therapy used, and changes in the physiological functions of the newborn's organism[1]. However, the true condition of the patient is determined only by the last of the listed factors. This is the reason for the active search for new markers that have diagnostic and prognostic value in the development of the pathological process (Akramova,; and Variane et al, 2017)[2,13].

In this regard, recently special attention has been paid to the determination of neurospecific proteins, neurotrophic and growth factors in the blood serum of newborns from the first days of life[5]. Significant changes in the levels of the above factors in blood serum can be detected much earlier than structural disorders that can be detected by modern method of neuroimaging (Breatnach et al, 2016; Laptook, 2016; and UNICEF, 2014)[6,12].

The lack of a unified concept for the management of such children in the first year of life, necessitate additional studies in this area aimed at optimizing the care of children born with low body weight and reducing the incidence and mortality of children in this category (Akhmedova, 2016).[3]

The aim of our study was to evaluate the prognostic significance of anamnestic factors and biochemical markers in the birth of underweight newborns, as well as to establish the features of their development in the first year of life (Laptook, 2016)[8].

Material and Method

Study Population: Taking into account the goals and objectives of the study, the research was carried out in the City Perinatal Center of Tashkent, Republican Specialized Scientific-Practical Medical Centre of Pediatrics, and the family polyclinics of 44, 45, 46, 47 in Tashkent. Overall, 281 newborns and 272 mothers were examined (18 of the examined newborns were twins). Newborns were divided into 2 groups: group 1 - 83 premature infants with low body weight relative to gestational age; group 2 - 103 full-term newborns with low body weight relative to gestational age. The control group consisted of 95 full-term newborns (38.4 ± 6.3 weeks), whose body weight corresponded to gestational age. Follow-up observation was carried out on 72 lightweight newborns regarding gestational age in the first year of life.

Laboratory Tests: Quantitative determination of human placental growth factor (PlGF) was carried out based on the enzyme-linked immunosorbent assay "Quantikine Human" (USA) of the solid-phase method in the blood serum of mothers and their newborns. Using ELISA, S100b protein was determined in the blood serum of 89 newborns, among them 40 full-term newborns were with normal body weight and 49 newborns with low body weight. S100b protein content was determined by enzyme-linked immunosorbent assay using serum reagents "CanAg" (Sweden).

Anthropometric assessments: To assess the adaptive capabilities of the body in children and monitor their physical development, growth curves and development of children were observed according to WHO standards. Anthropometric data: indicators of body weight, body length, BMI (body mass index - kg/m^2), as well as age-related achievements of the stages of motor development were plotted on curves and evaluated according to the recommended WHO growth and development standards (2006).

Medical care for underweight newborns and its evaluation: Evaluation of the effectiveness of the proposed medical care for children with low birth weight was carried out according to the results of observation and research in 72 children up to the age of one. Everyone was recommended the rules and stages of conducting underweight children in the first year of their life. During the study, the examined children were divided into 2 groups: the first group consisted of 49 children who underwent medical care in accordance with the proposed recommendations in full; the second group consisted of 23 children who received the recommendations in part because of non-compliance with the recommendations on the part of their parents (moving to another place of residence, mother's studies at a university, etc.).

Recommendations for caring for underweight children in a maternity complex were as follows: joint stay with the mother; prevention of hypothermia; ensuring the proper organization of breastfeeding; if necessary, resuscitation and treatment of newborns according to the approved clinical guidelines.

Recommendations for the care of underweight children in a clinic with our additions were as follows: exclusively breastfeeding in the first 6 months of life, the longest possible preservation of breastfeeding until reaching 2 years of age; monitoring the implementation of the daily regimen and nutrition; increase immunity through regular hardening (walks, water procedures, general massage); prevention of rickets, hypovitaminosis A and iron deficiency anemia; carrying out preventive vaccinations according to an individual schedule, monthly monitoring and evaluation of the growth and physical development of children, and, if necessary, more often against the background of correction of revealed violations. The first patronage to underweight children the day after discharge from the maternity hospital or hospital (wards of the first and second stages of nursing), since such children give higher rates of morbidity and infant mortality. Then, in the first month, an examination by a general practitioner (GP) or pediatrician once a week, from 1 to 6 months - 1 time in 2 weeks, 6-12 months - 1 time per month, according to indications - more often; examination of the child at home - in the first 3-4 months during periods of epidemic of infectious diseases. At each examination, it is necessary to conduct anthropometric measurements with an assessment of the dynamics of body weight, body length and BMI. Examination of narrow specialists: an orthopedist - every month for the first 3 months, then once a quarter; ophthalmologist and otorhinolaryngologist - at the 1st month of life, repeatedly at least 2-3 times a year (1 time per quarter); neuropathologist - 1 time in 3 months in the first year of life.

Statistical Analysis: To carry out correlation analysis and statistical processing of the obtained results, the MS Excel - XP programs were used. Statistical analysis of the results was carried out using method of variation statistics.

Results

The results of the anamnesis and clinical observation of 272 mothers in a hospital are presented, of which 83 mothers had babies born prematurely with low body weight relative to gestational age (group 1); in 102 mothers - full-term, but with low body weight (group 2). The control group consisted of 87 women whose newborns were born on time (38.4 ± 6.3 weeks) and body weight corresponded to gestational age.

It was found that a burdened obstetric and gynecological anamnesis and a complicated course of pregnancy and childbirth (pathological preliminary period, primary and secondary weakness, premature detachment of a normally located placenta) in mothers of children with low body weight of two groups relative to the control ($p < 0.05$).

Table 1: Assessment of risk factors for birth of low birth weight infants depending on the course of pregnancy

Risk factors	Attribute risk	Coefficient of relevant risk
USI data (chorion thickness)	0,91	11,58
Anemia	0,88	8,17
Malnutrition	0,84	5,75
Multiple pregnancy	0,82	5,58
Placental failure	0,81	5,46
Proteinuria	0,79	4,86
Arterial hypertension	0,77	4,41
Infection	0,76	4,23
The risk of abortion	0,76	4,23
Preeclampsia	0,64	3,28

As can be seen from table 1, a high relative risk of giving birth to children with low body weight in pregnant women was observed with signs of fetal growth retardation by ultrasound examination (ultrasound)

(chorion thickness) (OR = 11.58), with anemia (OR = 8.17), other malnutrition (OR = 5.75), placental insufficiency (OR = 5.46).

The risk ratio for manifestations of proteinuria and preeclampsia ranged from 4.86 to 3.28, indicating a direct relationship between these conditions and the severity of their influence. In pregnant women who had the above complications, children were born 3 times more likely to be born with low body weight relative to gestational age compared to pregnant women with normal functional state of the kidneys and blood pressure.

The analysis of risk factors for the birth of children with low body weight confirmed the multifactorial nature of their occurrence. Thus, the risk of having a baby with a small body weight varies widely and largely depends on the risk factors that caused them, the criteria that form the basis of its calculation.

A comparative analysis of the peculiarities of adaptation of the body of newborns showed that when children were admitted to the pathology department, the age of the children in groups 1 and 2 did not differ and amounted to 4.9 ± 0.6 and 4.6 ± 0.6 days, respectively. Indicators of physical development of the studied children at birth are presented.

There is no doubt that in the neonatal period, the physical development of newborns with low body weight significantly differs from full-term infants at a high level of statistical significance ($p < 0.001$). When assessing the condition at birth, it was found that all children of the 1st group were born in serious condition, the 2nd group - in most cases (66.0%) of moderate severity, and in the control group - in satisfactory.

The Apgar score at 1 and 5 minutes of life (Table 3) was significantly lower in infants born with low birth weight compared with full-term infants ($p < 0.001$). in the second group, the Apgar score was higher, but unreliable.

Table 2: Apgar score for 1st and 5th minutes of life in low birth weight infants

Apgar score	Group 1 (n=83)	Group 2 (n=103)	Control group (n=95)
At the 1st minute	3,6±0,2 ***^^	5,7±0,2**	7,8 ± 0,1
At the 2nd minute	5,1±0,2 ***^	6,3±0,2*	8,9 ± 0,1

Note: * Reliability of the data for the control group (* -p <0.05; ** - p <0.01; *** - p <0.001); ^ - data reliability between the 1st and 2nd group (^ -p <0.05; ^^ - p <0.001)

Table 3: Diseases of newborns with low body weight

Disease	Group 1 (n=83)		Group 2 (n=103)	
	Abs.	%	Abs.	%
Respiratory distress syndrome (RDS)	78	94,0±2,6	69	67,0±4,6
Bronchopulmonary dysplasia	23	27,7±,9	12	11,7±3,2*
Pneumonia	49	59,0±5,4	27	26,2±4,3**
Enterocolitis	36	43,4±5,4	28	27,2±4,4
Cerebral ischemia 2 stage	61	73,5±4,8	68	66,0±4,7
Cerebral ischemia 3stage	8	9,6±3,2	5	4,9±2,1
Intraventricular hemorrhage 1st stage	5	6,0±2,6	8	7,8±2,6
Intraventricular hemorrhage 2nd stage	3	3,6±2,1	2	1,9±1,4
Intraventricular hemorrhage 3rd stage	6	7,2±2,8	3	2,9±1,7
Periventricular leukomalacia	10	12,0±3,6	7	6,8±2,5
Anemia	72	86,8±3,7	52	50,5±4,9*
Jaundice	70	84,3±4,0	85	82,5±3,7

Note: The reliability of the data between group 1 and 2 (* - p <0.05; ** - p <0.001)

Signs of perinatal hypoxic-ischemic brain damage of the 2nd degree were recorded in groups 1 and 2, respectively, in 73.5% and 66.0% of children, degree 3 - 2.2 times more in premature infants. Grade 3 intraventricular hemorrhage (IVH) was diagnosed 2.5 times more often in premature infants than in full-term infants with low body weight (7.2% and 2.9%, respectively). Periventricular leukomalacia (PVL) was also significantly more often observed in premature infants than in full-term babies with low body weight ($p < 0.05$) (Table 4).

In all newborns, jaundice was conjugative in nature. Newborn children of groups 1 and 2 had a longer icteric period than newborns of the control group. There were no significant differences in the characteristics of icteric syndrome in newborns with low body weight. Jaundice in preterm infants was observed in 84.3% of cases and in 82.5% of cases in newborns of group 2. Severe anemia at birth prevailed in group 1 infants ($p < 0.01$). The high frequency of diseases of the nervous and respiratory systems (RDS, cerebral ischemia) in underweight children is due to the more pronounced immaturity of these systems at the time of birth. RDS was registered in 94.0% of children of the 1st group and in 67.0% of newborns of the 2nd group.

In the assessment of the length of infants with low body weight in the hospital, it was found that statistically significantly longer children from group 1 spent both in intensive care (19.3 ± 2.4 days, $p<0.05$) and in the neonatal pathology department (41,4 ± 1.9 days, $p<0.001$).

Among the syndromes of central nervous system damage in underweight preterm infants, the syndrome of oppression associated with exposure to hypoxic factors and muscle hypotension predominated, and in children of the 2nd group, vegeto-visceral disorders and muscle hypertonicity (Table 5).

Table 4: Syndromes of posthypoxic damage to the central nervous system (CNS) in newborns with low body weight

Diseases	Group 1 (n=83)		Group 2 (n=103)		Control group (n=95)	
	Abs.	%	Abs.	%	Abs.	%
Increased neuro-reflex irritability	21	25,3**	25	24,3**	6	6,3
Oppression Syndrome	46	55,4***	32	31,1^***	1	1,1
Vegeto-visceral disorders	33	39,8***	38	36,9***	7	7,4
Muscular dystonia: - hypotension - hypertension	 56 27	 67,5*** 32,5***	 36 45	 35,0^*** 43,7***	 1 4	 1,0 4,2
No disorders in CNS	-	-	-	-	76	80,0

Note: * The reliability of the data between the groups of underweight newborns and the control (** - $p < 0.01$; *** - $p < 0.001$); ^ - reliability between groups 1 and 2 (<0.05)

Table 5: S100b in preterm low birth weight infants depending on the severity of asphyxiation

Duration of Observation	1st Subgroup	2nd Subgroup	Control Group
At the birth	2571,8±55,5*	1428,7±56,1*	571,5±56,9
On the 2-3 days of birth	3886,4±51,2*^	2714,5±49,5*^	574,2±46,3

Note: * Data reliability in relation to the control group (* - $p < 0.001$); ^ - reliability of data in relation to indicators at birth and on the 2nd-3rd day of life (^ - $p < 0.001$)

Motor activity was reduced in 1 group in 56 newborns (67.5%), and in the second - in 36 (35.0%) newborns. Muscle tone was changed in all children with low body weight, with muscle hypotension occurring in most children of the 1st group (67.5%), and in the 2nd group - hypertension (43.7%). All newborns with low

body weight showed hyporeflexia and rapid exhaustion of unconditioned reflexes.

In newborns of the control group, disorders of the central nervous system were observed in 20.0% of newborns, with increased prevailing neuro-reflex excitability (6.3%) and muscular dystonia in the form of hypertonicity (4.2%).

In 33 (39.8%) premature infants with low body weight relative to gestational age, a syndrome of vegetovisceral disorders was revealed, in full-term infants with low body weight this percentage was 36.9%. It was manifested by a violation of microcirculation, thermoregulation and motility of the gastrointestinal tract. Equally often, microcirculation disturbances (marbling of the skin, pallor, acrocyanosis) and thermoregulation disorders were observed, which required the need for newborns to be in the couveuse. Violations of the motility of the gastrointestinal tract in the form of regurgitation and constipation were 2 times more often observed in premature infants than in children of the control group. In preterm infants with low body weight, inhibition syndrome was 2.2 times more common than increased neuro-reflex irritability syndrome, and in full-term infants with low birth weight - 1.3 times. The revealed syndromes of posthypoxic damage to the central nervous system during the observation process were characterized by a periodic change in the phases of cerebral activity.

The level of S100b depended on the gestational age - the highest rates at birth were recorded in newborns born at 28-30 weeks of gestation (811.2 ± 46.9, $p < 0.05$). In this group of newborns, an increase in the level of S100b was also observed for 2-3 days (1075.6 ± 31.8 ng / l, $p < 0.01$), while in full-term infants the level of S100b did not significantly differ from the indicator at birth (Figure. 1).

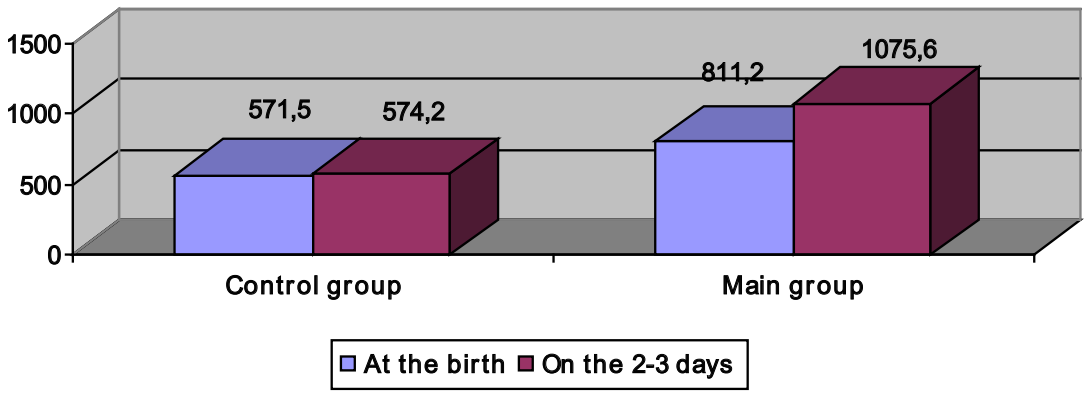


Figure 1: S100b in newborns at the 2nd-3rd days of life

In children born in severe asphyxia (37 newborns), on the first day, the serum concentration of S100b protein was significantly higher than standard values (4-5 times) and averaged 2571.8 ± 55.5 ng/l, whereas Compared with newborns with an Apgar score of 5–7 points, an increase in S100b level was observed by a factor of 2–3.

Among the newborns, 2nd subgroups with an Apgar score of 5-7 points (38 newborns) showed an increase in S100b content by a factor of 2–3 compared with the control group (25 full-term infants) and averaged 1428.7 ± 56.1 ng/l (tab. 6).

Correlation analysis in the compared groups of newborns revealed a pronounced inverse relationship between the level of S100b in the blood serum of newborns and the severity of asphyxiation at birth, characterized by Apgar score at 1 minute: the lower Apgar score, the higher the serum S100b protein level ($r = -0.756$; $P < 0.01$). It should be borne in mind that on the first day of life, an increase in serum concentration of S100b in newborns with brain hypoxia is probably due to the neuroprotective effect of this protein, however, an increase in concentration on the first day should not exceed a 3-fold increase compared with the upper limit of normal. Exceeding the concentration of S100b in blood serum by 4 or more times on the first day is usually an unfavorable prognostic sign, indicating destructive changes in brain cells, and indicates the

possible formation of severe structural brain disorders within 10-14 days.

An analysis of the level of human PlGF in the blood serum of mothers of the main and control groups revealed that in the main group the level of PLGF was significantly lower ($p < 0.01$) than in the control group (84.3 ± 13.4 and 275.0 ± 26, respectively 5 pg / ml) (Figure. 2).

This fact may indicate a disorder of the processes of invasion of chorionic villi and the formation of insufficiency of the second wave of trophoblast invasion in conditions of a low level of PlGF.

In the study of PlGF in umbilical cord blood in premature infants with low body weight (28 children), we found that this indicator was significantly reduced compared to the control group (20 children) 2.5 times and 1.6 times compared with the group full-term infants with low body weight relative to gestational age (27 children) (Figure. 3).

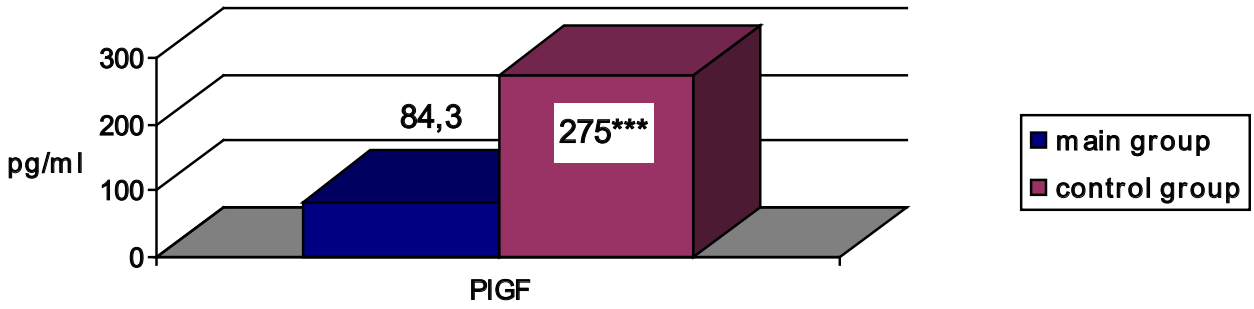


Figure. 2. Indicators of PlGF in a comparative aspect in mothers

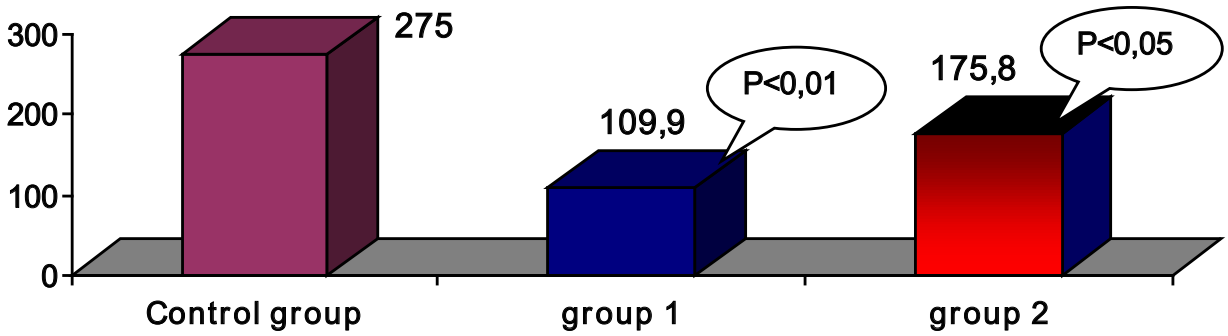


Figure 3: PlGF in newborns

The decrease in PlGF indicators correlates with the formation of low body weight ($r = + 0.49$, $p < 0.05$), and also has a direct correlation with the state of health of premature infants with low body weight relative to gestational age ($r = + 0.51$, $p < 0.05$).

On the 4th day of life, in order to clarify the state of the central nervous system and identify distant neurological disorders, neurosonography was performed. It was revealed that newborns without neurological impairment, by the age of 6 days, are safe in relation to the formation and development of the central nervous system, they can conditionally be considered healthy children. Cerebral ischemia of the first degree, obviously, was due to a hypoxic lesion of the central nervous system transferred in the prenatal period. She was characterized by minor neurological symptoms and the absence of significant changes in the parameters of neurosonography.

When studying the relationship between the level of PlGF in mothers and the level of S100b in their newborns in the formation of low fetal body mass, a strong inverse correlation relationship was established ($r = -0.875$, $p < 0.001$), with a decrease in the level of PlGF, a significant increase in S100b was noted ($p < 0.05$)

During the study, children were divided into 2 groups: the first group consisted of 49 children who received full medical care in accordance with the proposed recommendations; the second group consisted of 23 children who received the recommendations in part because of non-compliance with the recommendations on the part of their parents (moving to another place of residence, mother's studies at a university, etc.).

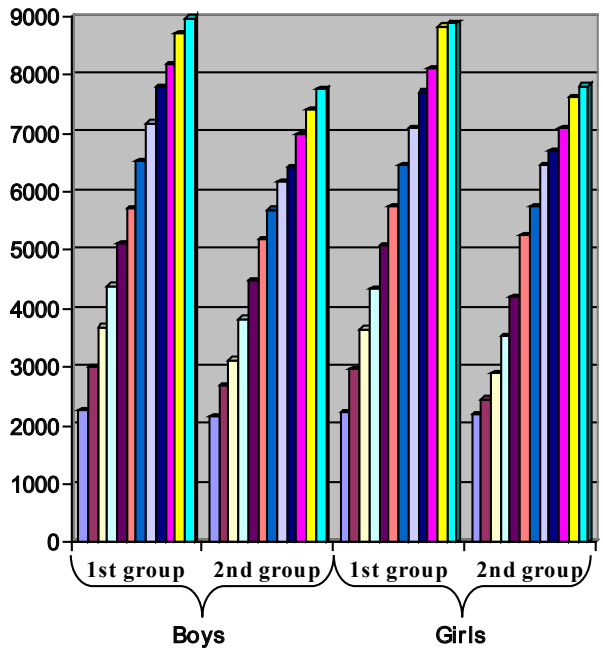
The analysis showed that the proposed methodology for the management of this category of children contributes to the leveling of disorders in neurological status.

As can be seen from the data presented in table 7, the recovery of tendon and unconditioned reflexes occurred in a larger percentage of children of group 1 ($p < 0.05$) compared with children of group 2. The proposed method gave a more pronounced effect in the restoration of motor and sensory functions, accompanied by an improvement in muscle tone, which is associated with the stimulation of reparative processes of the central nervous system in children who received full medical care.

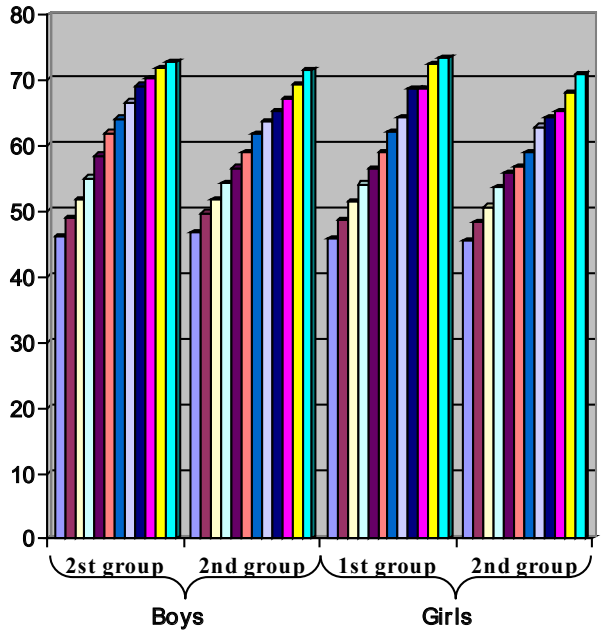
Table 6: The comparative effectiveness of the medical care for children in their first year of life

Values	Group 1, n=49		Group 2, n=23		P
	Abs.	%	Abs.	%	
Normalization of tendon reflexes	29	59,3	8	48,4	<0,05
Normalization of unconditioned reflexes	47	96,3	13	74,2	<0,05
The level of motor activity	33	66,7	9	54,8	<0,05
Muscle tone improvement	47	96,3	13	64,5	<0,01
Improving turgor tissues	45	92,6	12	54,8	<0,01
Body weight gain	47	96,3	13	71	<0,01

When assessing the physical development of the examined children every month during the first year of life, a statistically significant difference was revealed between the groups of underweight children ($p < 0.05$-0.01) (Figure 4).



Body weight to age



Body length to age

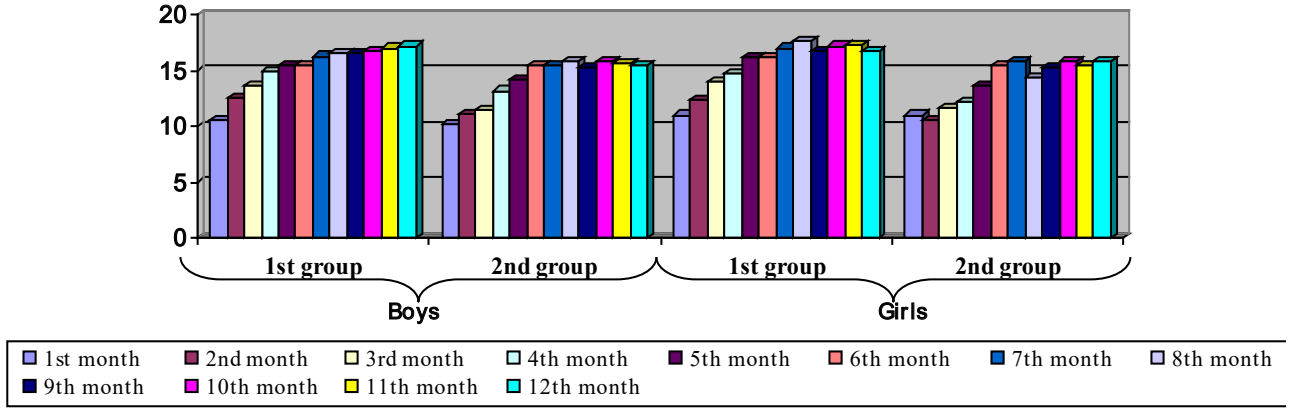


Figure 4. Dynamics of anthropometric data in underweight children in the studied groups

Children of group 2 at all periods of observation, body weight and BMI were less than children who fully complied with the proposed recommendations ($p < 0.05$). Particularly pronounced lags in physical development were noted in children in group 2 at the age of 6 to 12 months.

One of the indicators indicating the nutritional status of a child is BMI. As can be seen, in children of the 2nd group in all age periods there is a significant decrease in BMI, more pronounced after 6 months of life. Children with indicators below two standard deviations are regarded as having reduced weight and a moderate degree of protein-energy malnutrition (PEM). In children of the 2nd group at the age of 12 months, the indicators of weight, body length, and BMI were in the interval "(-1CO) - (-2CO)". These mass indicators do not correspond to the norm, but tend to normalize.

Monitoring of anthropometric measurements in children of group 1 in the first 6 months showed that at birth, the average body mass and body length correspond to -3CO, BMI - within -3CO and -2CO; from 2 months, the dynamics of increase in body weight increases and is in the range of -3CO - -2CO, while the rate of increase in body length lags behind over the next months and corresponds to values below the -2CO curve, due to this, BMI increases. So, in 2 months, BMI corresponds to values lying in the range -2CO - -1CO; at 3 months - -1CO, at 4 and 5 months - from -1CO to the median, i.e. according to this indicator, they correspond to standard values, at 12 months - the median. This dynamics is typical for boys and girls. These results indicate that low birth weight in the first 3-6 months causes a violation of the harmonious development of the baby with normalization by 12 months (Figure. 4). It should be noted that in the 2nd group, 56.3% of children showed a lag in physical development, while in the 1st group this percentage was 22.2%.

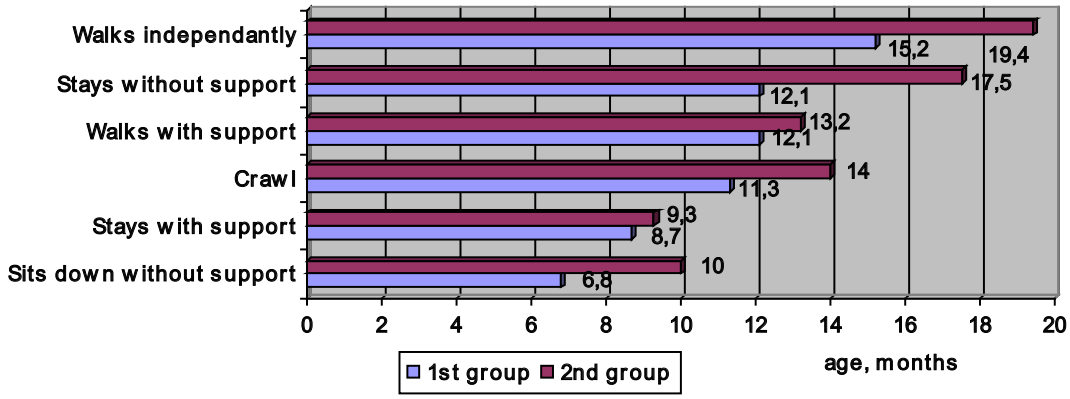


Figure 5: Indicators of motor development of children born with low weight

The characteristic features of the motor development of underweight children who have not received medical recommendations in full and the lag in motor development are presented in Figure. 5. So, all skills that are an indicator of motor development of children were later developed in children of this contingent, especially skills such as "sitting without support" (10.0 ± 0.85, p <0.05), "standing alone" (17, 5 ± 0.8, p <0.01) and "walks independently" (19.4 ± 0.9, p <0.01)[4].

As it is all-known factor that, children born with low body weight are the maximum risk group for the formation of severe pathology leading to disability, and they are prescribed a huge number of drugs and manipulations, which often lead not to an improvement in condition, but to a breakdown of natural compensation. When studying the incidence of children differentially depending on management method, it was found that children who received the introduced care (group 1) were much less likely to have acute respiratory infections - 8.4 ± 1.3 times versus 14.6 ± 1.3% times (p<0,01). The duration of the disease in the first year of life was significantly less, almost twice, in the 1st group of children: respectively, 51.6 ± 2.4 versus 91.3 ± 2.4 days in the 2nd group (p <0.01). The duration of treatment was also shorter when using the introduced management of underweight children. The frequency of pneumonia and bronchitis in the 1st group was 1.6 ± 0.19 times, in the 2nd group - 2.3 ± 0.1 times; the duration of the disease in group 1 was 13.7 ± 1.6 days, in group 2 - 21.9 ± 2.3 days. The duration of pneumonia treatment in group 1 was less (13.3 ± 1.7 days) than in group 2 (19.8 ± 2.8 days).

Significantly less frequently diarrhea was observed in children of the 1st group (1.6 ± 0.2 times), in children of the 2nd group the frequency was 2.7 ± 0.3 times (p <0.05). The duration of the disease and the need for prolonged treatment was less in group 1. The frequency of rickets in children of the 1st group was more than 2 times less and amounted to 5.3 ± 1.2, in the 2nd group - 11.0 ± 0.8 (p <0.001). The duration of signs of rickets was also 2 times less in group 1 - 160.0 ± 3.2 versus 324.6 ± 2.9 days (p <0.001), which indicates a significant proportion of the influence of the principles of management of underweight children during the first year of life. The frequency of anemia in underweight children was also different depending on the principles of their management. It was revealed that in the 1st group the incidence rate of anemia was almost twice lower than in the 2nd group and amounted to 4.2 ± 1.3% versus 7.6 ± 1.6%.

Discussion

In general, a significant decrease in the frequency of diseases among children of group 1 was established compared with children of group 2 (18.5% versus 34.4%, respectively, p <0.01). According to the dynamic observation, the frequency of visits to SP and SVP for diseases (diarrhea, bronchopneumonia, acute respiratory viral infections, otitis media) decreased by 1.8 times, the frequency of hospitalizations decreased. At the same time, in the 2nd group, the frequency of hospital admissions to the hospital ranged from 5 to 6 times a year, while in the 1st group - on average 2 times a year.

Thus, in a differentiated study of the effectiveness of the proposed method for caring for underweight children, it was found that children who received incomplete recommendations lagged behind physical and motor development, and were also more likely to suffer from diseases such as acute respiratory infections, pneumonia, diarrhea, rickets, and anemia.

Based on the data obtained, it can be concluded that for the prevention of birth of low birthweight infants, it is necessary to improve the quality of antenatal care for pregnant women with the correction and treatment of identified disorders and diseases, taking into account the identified prognostic factors; in order to ensure normal development and reduce the incidence of diseases in children born with low birth weight relative to the gestational age, it is necessary to provide care and follow up for them during the first year of life in accordance with additional recommendations on tactics of managing underweight children with adherence to modern WHO/ UNICEF technologies.

A high relative risk of giving birth to underweight infants in pregnant women is a delay in fetal development according to ultrasound (chorion thickness) (OR = 11.58), with anemia (OR = 8.17), and placental insufficiency (OR = 5.46). For low birth weight infants, especially premature infants, low adaptive capabilities of the body are characteristic. Underweight preterm infants are born in severe asphyxia (3-4 points on the Apgar scale), with a severe degree of RDS, severe disorders of the central nervous system; they require primary resuscitation measures 1.7 times more often, longer antibacterial measures (3-4 courses - 1.4 times more often, 5 or more - 2 times more often) and respiratory therapy.

The excess on the first day of the concentration of S100b in blood serum by 4 or more times is an

unfavorable prognostic sign for the formation of severe structural disorders of the brain, as evidenced by neurosonography indicators - signs of intraventricular hemorrhage, PVL and ischemic manifestations.

A low level of PlGF (below 100 pg/ml) in the blood serum of mothers indicates a high risk of intrauterine growth retardation and the birth of underweight newborns, which is confirmed by the correlation between the mother's PlGF level and newborn body weight. The adaptation and development of diseases of premature infants with low body weight relative to gestational age is also directly affected by the low level of PlGF in the blood serum of the child himself.

Neurospecific protein S100b in newborns and placental growth factor PlGF in mothers and newborns are biochemical markers of the birth of low birth weight infants, their adaptation disorders with the development of diseases of the central nervous system and other systems

Conclusion

The risk ratio for pregnant women with proteinuria and preeclampsia varies from 4.86 to 3.28, which indicates a direct relationship between these conditions and the severity of their effect. Pregnant women who have the above complications are 3 times more likely to have babies with low body weight relative to gestational age compared to pregnant women with normal renal function and blood pressure. Care and observation during the 1st year of life, according to additional recommendations on tactics of conducting underweight children with observance of modern technologies. WHO/UNICEF helps to improve central nervous system disorders (normalization of tendon and unconditioned reflexes, physical activity), physical and motor development, as well as reduction 1.9 times the incidence rate among children of this contingent.

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Ethical Approval: The ethical approval for the study was granted by the Committee of Ethical Approval for Researches under the Ministry of Health of the Republic of Uzbekistan.

Consent: Written informed consent was obtained from all participants' parents of the research for publication of this paper and any accompanying information related to this study. A copy of the written consent is available for review by the authors.

Conflict of Interest: The authors declare that they have no competing interests.

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The First 5 Years Characteristics of Growth and Development of Children Residing in the Regions of Uzbekistan

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Abstract

Introduction: Nutritional issues in children are associated with a complex of factors. Early assistance to the child is feasible with the on time detection of developing deviations in the health, growth and development. The actual tasks were to determine the level of growth and development of children in the regions of the Republic of Uzbekistan, assess the effectiveness of programs implemented in the country to improve nutrition and prevent micronutrient deficiency based on the application of new standards for the growth and development of children, as well as develop relevant proposals and recommendations.

Materials and Method: 3969 children under 5 years old living in 6 regions of Uzbekistan were observed. In order to monitor the growth and development of children, anthropometric measurements were carried out in the following periods: the first measurements - at birth, the second and third - at the age of 15 days and 1 month, then monthly until the expiration of 12 months, 1 time in 3 months - between 12 and 36 months, every 6 months - between 36 and 60 months and then annually. An immunological study was conducted as well for children with malnutrition.

Results: The percentage of BF in the city of Tashkent was 78.6%, Tashkent region - 66.4%, Khorezm region - 67.3%, Fergana region - 85.7%, Bukhara region - 72.2% and the Republic of Karakalpakstan - 89.0 % Based on those results, it can be stated that in the region where the percentage of IHV was lower (Tashkent and Khorezm regions), the percentage of children with low body weight is greater. By the end of 2012 and the beginning of 2013, the percentage of children of the first 6 months of life with reduced weight decreased in all regions. The dynamics of reduced weight by the end of 2012 among children aged 6-12 months was positive

Conclusion: Anthropometric indicators in children of the first 5 years of life, regardless of gender, in almost all studied regions in terms of body weight and body length/height in most cases were same level. In children under 5 years old, low body weight relative to age prevailed among the forms of impaired physical development and nutrition in all regions.

Keywords: *Malnutrition, growth and development impairment, children, socio-economic regions, Uzbekistan.*

Introduction

Taking into account the importance of normal growth as an overall indicator of health status, the World Health Organization (WHO) has developed standards for the growth and development of children[1]. These standards allow us to show how children should grow, to monitor

their growth and development with the adoption and conduct of timely preventive measures (Bogomolova, 2015; Khodzhieva et al, 2016 and *Growth reference 5-19 years. BMI-for-age*, 2007)[17].

Children's health and development are highly dependent on nutrition and the environment. The nutritional problems of children, in turn, are determined by a complex of factors, among which the well-being of the region where the child lives and the formation of eating behavior in the family are leading ones (Gelashvili et al, 2018 and Namazova-Baranova et al, 2018)[5,8,11]. For some regions of the world, there is a problem of chronic malnutrition, which leads to the development of chronic nutrient deficiency in children and continues to be the cause of infant mortality, developmental disorders, including intellectual lagging, and increases the risk of various infectious diseases (Akhmedova et al, 2016; Izotova, 2015; Khodzhieva et al, 2016 and Lobstein et al, 2015)[1,7,12]. Rapid and excessive growth leads to a higher prevalence of cardiovascular disease and obesity. According to the WHO, in 2010, about 115 million children had experienced a reducein body weight, 55 million had low body weight in relation to growth, and 171 million children under the age of five had stunted growth (L.D. I. 2015. *Health in the European Union*, 2011)[7]. In addition, in 2010, 43 million preschool children in developing and developed countries were overweight or obese. The prevalence of childhood obesity in low- and middle-income countries has accelerated over the past 10 years; according to WHO estimates, in 2015 this coefficient should have reached 11%, which is close to prevalence in countries with incomes above the average (12%) (*WHO child growth standards*, 2016)[17].

In the world, great focus is given to the scientific and organizational-methodological studies in determining the characteristics of the physical development of children and improving rational nutrition, taking into account the regional factors in the system of medical prevention (D.T. A.Bogomolova, 2015)[2]and Tanagaet al, 2010)[9],[10]. In this regard, special attention is paid to conducting targeted research: assessment, taking into account regional characteristics of the physical development of children by introducing new standards for the growth and development of children recommended by WHO; determining the frequency of various forms of malnutrition and their relationship with major diseases and immunological reactivity of children; improving the system for assessing factors affecting the health, physical and intellectual development of children (*Health in the European Union*, 2011 and *WHO child growth standards*, 2016)[13,16].

Analysis of the literature showed that early assistance to the child is possible with the timely detection of developing deviations in the health, growth and development (Ashurova, 2015 and Namazova-Baranova et al, 2018)[8,11].

The WHO multifocal study of growth standards (WHO, 2006) was designed to provide data on "how children should grow up," including criteria for selecting children for the study: favorable socio-economic conditions for the growth of the child, breastfeeding (in the first 6 months - BF), compliance with the recommended method of feeding infants, lack of medical or environmental problems, mothers quitting smoking, giving birth to one full-term baby. In order to obtain useful information about the physical development of the child, it is necessary to have several successive measurements of the indicators of the physical development of the child according to the recommended dates of their implementation and apply them to the physical development curves derived from the corresponding reference groups of the population (Geht, 2015 and Lobstein et al, 2015)[14,15].

The aim of the study was to determine the characteristics of the physical development of children in their first five years of life and evaluate the effectiveness of programs to improve their nutrition based on modern organizational and methodological approaches.

Materials and Method

Study Population. A survey (monitoring the growth and development of children) of 3969 children of the first 5 years of their life in 6 regions of the Republic of Uzbekistan was conducted. Representatives of 5 geo-economic regions of Uzbekistan were included in the study: the Republic of Karakalpakstan and the Khorezm region - the West (1592 children), Bukhara region - Central (442 children), Tashkent region - Central-East (707 children), Ferghana region - East region (500 children) and Tashkent city (728 children).

Data Collection: At the first stage, for monitoring and evaluation of growth and development of children in the regions using growth curves according to WHO standards, a monitoring questionnaire was developed and approved by Order of the Ministry of Health of the Republic of Uzbekistan (No.145 dated March 30,

2007). In order to monitor the growth and development of children, anthropometric measurements were carried out in the following periods: the first measurements - at birth, the second and third - at the age of 15 days and 1 month, then monthly until the expiration of 12 months, 1 time in 3 months - between 12 and 36 months, every 6 months - between 36 and 60 months and then annually. If necessary, more frequent weight measurements were carried out, especially to check the adequacy of breastfeeding in the presence of deviations in the growth and development of the child and to evaluate their correction.

An immunological study was conducted in children with malnutrition. Determination of the absolute and relative content of lymphocytes, populations and subpopulations of lymphocytes (total pool of T-lymphocytes (CD3) and B-lymphocytes (CD20), subpopulations of T-helper cells (CD4), T-suppressors (CD8) and natural killers (CD16) in the blood was carried out according to the method of Zalyalyeva (2003) using monoclonal anti-lymphocyte antibodies produced by the Research Institute of Immunology of the Russian Federation (Moscow). Concentrations of serum immunoglobulins (IgG, IgM, IgA) were determined by the standard sets "IgA total - IFA-BEST", "General IgM - IFA-BEST", "General IgG - IFA-BEST" produced by VECTOR-BEST CJSC, Novosibirsk (Russia) by enzyme-linked immunosorbent assay, phagocytic activity of neutrophils - FAN was studied using melamine formaldehyde latexes. Immunological studies were carried out jointly with the Institute of Immunology of the Academy of Sciences of the Republic of Uzbekistan.

Statistical Analysis: To carry out correlation analysis and technological processing of the obtained results, the MS Excel - XP software was used.

Results

The assessment and monitoring of the growth and development of 1,592 children living in the Western region are given: 879 children in the Republic of Karakalpakstan and 713 children in the Khorezm region. It was found that children at birth corresponded to median indicators. In the Republic of Karakalpakstan, the average birth weight for girls was 3.32 ± 0.02 g, body length 50.3 ± 0.1 cm, body mass index (BMI) 13.06 ± 0.06 kg/m^2; in boys, respectively, 3.44 ± 0.03 kg, 51.2 ± 0.1 cm and 13.1 ± 0.08 kg/m^2. These indicators indicate that at birth, indicators of weight, body length and BMI relative to age corresponded to standard indicators according to the WHO growth curves. The weight, body length and BMI of children aged 0-6 months, regardless of gender, corresponded to the level of indicators in the field of -1CO + 1CO, which indicates the harmonious development of children. At the age of 12 months, the girls' body weight corresponded to 8.98 ± 0.05 kg, body length 73.9 ± 0.2 cm, BMI 16.4 ± 0.07 kg/m^2; in boys, respectively, 9.88 ± 0.06 kg, 75.6 ± 0.2 cm and 17.3 ± 0.1 kg/m^2. In girls, all indicators of physical development were in the range of -2CO - + 2CO, which corresponds to the standard. A similar dynamics of physical development by 12 months of life was also observed in boys in this region. However, starting from 7-8 months of life, the pace and development of 87.8% of children slowed down to a greater extent as a result of late (37.0%) and irrational feeding (39.4%). In dynamics from 12 months in girls and boys, body length / height indicators on average corresponded to -1CO. BMI on average in children from 12 months to 5 years old corresponded to normative indicators in accordance with WHO standards.

Among the examined children in the Khorezm region, 51.6% were boys (368) and 48.4% were girls (345). The average birth weight for girls was 3.43 ± 0.04 kg, body length 50.3 ± 0.1 cm, BMI 13.6 ± 0.2 kg/m^2, i.e. all indicators corresponded to normative indicators - median (O) according to the WHO growth curves. The weight, body length and BMI of children aged 0-6 months also corresponded to the median level. In children 9-12 months old, body weight was in the range + 1CO + 2CO, body length in the range - 0 + 1CO, in accordance with this BMI - in the range - 0 + 1CO. These data show that in this age period, physical development corresponds to the standard, but an increase in body weight is noted. It should be noted that most children (87.9%) were exclusively breastfed and breastfed. Indicators of weight, body length / height and BMI for children under the age of 5 years inclusive by region are presented in Figs. 1, 2 and 3.

Bukhara region located in the central geo-economic region. Anthropometric measurements were carried out in this region among 442 children, of which 46.6% were girls (206 children) and 53.4% were boys (236 children). At birth, the average weight, body length, and BMI were in the -1CO and median areas. After 9 months of life, deviations from the median are observed towards a decrease in body weight.

The study of growth and physical development indicators in the Central-Eastern region of Uzbekistan was carried out among 707 children in the Tashkent region, of which 443 were boys (48.5%) and 364 girls (51.5%). In children of the Tashkent region from 12 months to 5 years, the values of body length/height and body weight relative to age were in the region of -2CO and + 1CO.

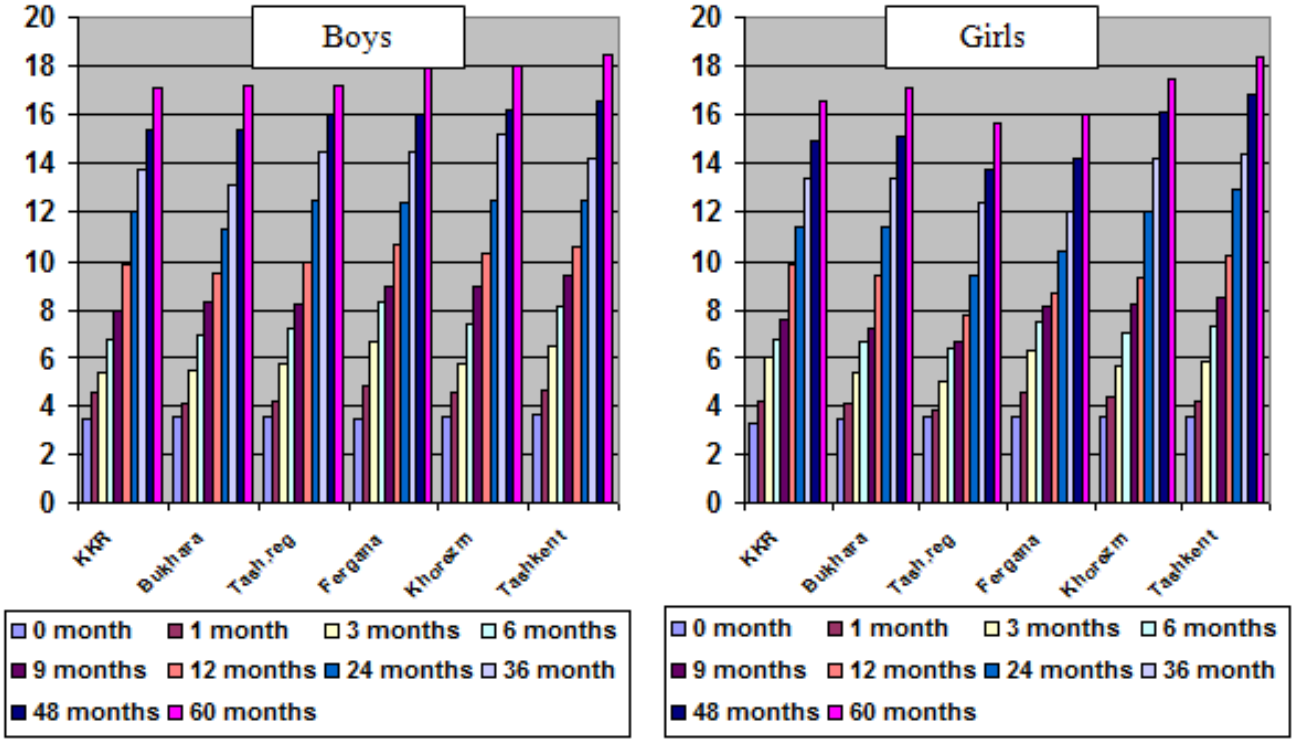


Fig. 1. Body mass index (g) of children under 5 years old inclusive

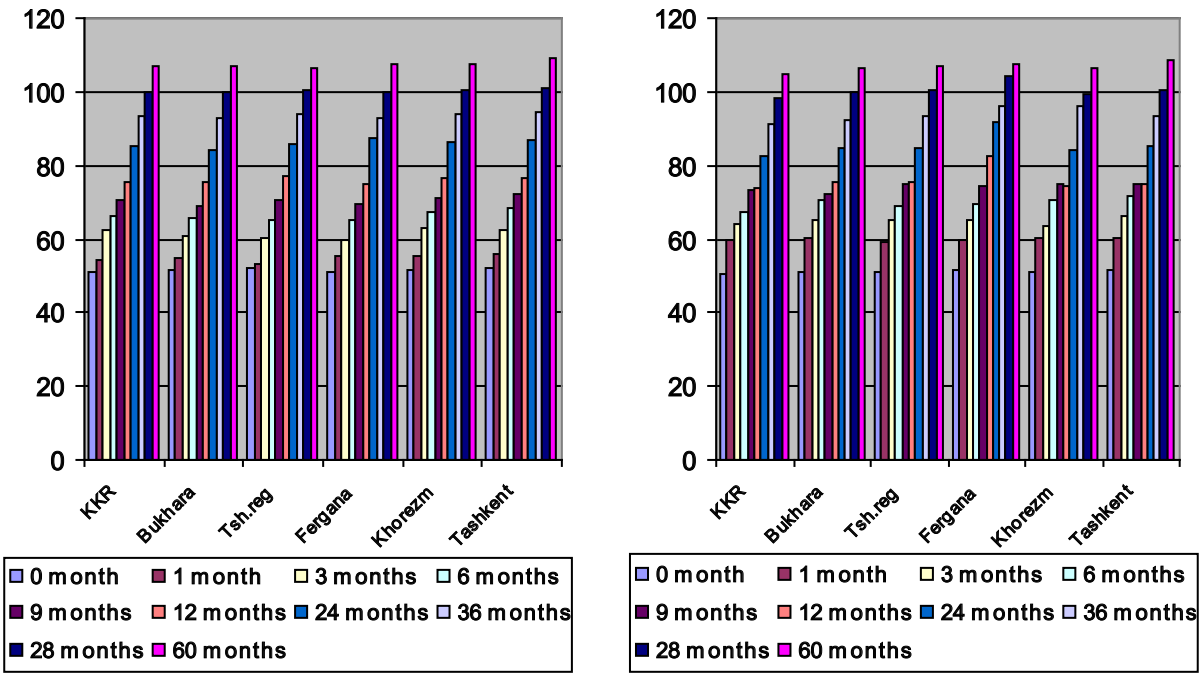


Fig. 2. Indicators of body length/height (cm) for children under 5 years old

Anthropometric measurements and monitoring of the growth and development of 500 children living in the Fergana region were carried out. Of the children examined, boys accounted for 49.2% (346 children), and girls 50.8% (354 children). An analysis of the studies showed that in children living in the Fergana region, up to 10 months of life, indicators of physical development corresponded to the range of -1CO and median (0). Subsequently, a decrease in body weight was observed in the dynamics, the indicators of which were on average in the range of -1CO. Body length/growth indicators were in the same range, BMI in the range -1CO + 2CO.

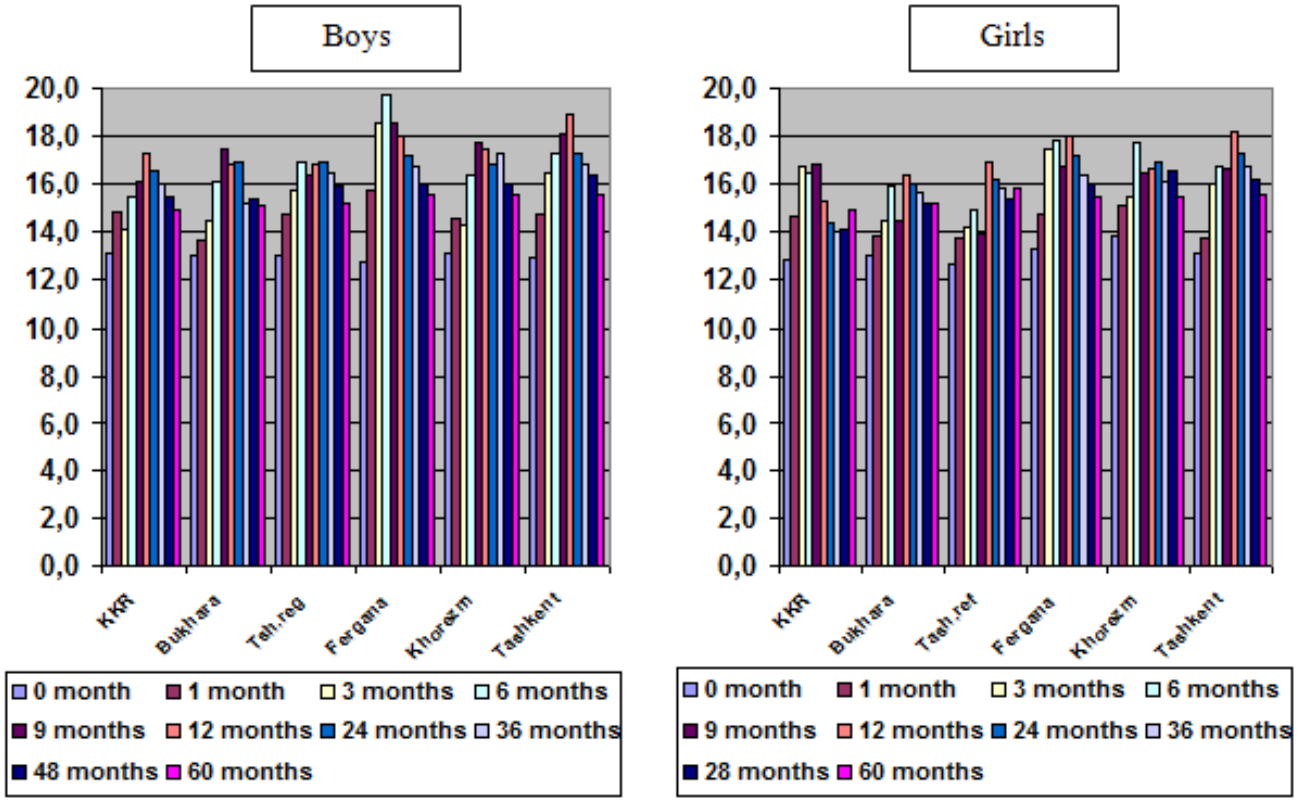


Fig. 3. BMI indexes for children (kg/m^2) up to and including 5 years old

The analysis of growth and physical development indicators was carried out among 728 children of the first 5 years living in Tashkent. The study included 364 girls and boys. Children living in Tashkent showed normative indicators, which in most cases corresponded to the range of -1 CO + median (O) according to the WHO growth curves for all age periods of life.

Anthropometric indicators of children living in the city of Tashkent correspond to the median values for all anthropometric data of the median level, the girls of the Fergana region in terms of body weight and, accordingly, BMI in the first 7-8 months of life slightly exceed their peers from other regions. From 9 months of age, there has been a decrease in the rate of increase in body weight in girls of the Fergana region, and starting from this age, the body mass index is higher for girls in Tashkent and the Khorezm region.

We examined children aged 1 to 2 years with moderate PEM living in the Khorezm region (43 children) and the Republic of Karakalpakstan (44 children), the control group consisted of 34 children in Tashkent. An analysis of the results of an immunological examination of children revealed that against the background of leukopenia relative to the control group, T-immunodeficiency was observed, expressed in a decrease in the level of CD3 + lymphocytes in 76.7% of the examined children of the Khorezm region and 84% in children of Karakalpakstan. A reduced number of immunoregulatory CD4 + subpopulations relative to the control group ($p < 0.01$) was observed in 34 of 43

examined children (79.0%) living in the Khorezm region, and 36 of 44 children (81.8%) living in Karakalpakstan. The content of the relative number of CD8 + lymphocytes in children of the Khorezm region did not significantly differ from the data of the control group, however, in 8 out of 43 children (18.6%) there was a reduced level of T-suppressors / cytotoxic lymphocytes. In children with PEM, the level of CD8 + lymphocytes was reduced ($p < 0.05$). Possibly, insufficient protein content in the diet causes a decrease in the expression of CD8 + lymphocytes in these children compared with children without PEM. There was a significant increase in the relative content of natural killer cells (CD16 +) against the background of the relative and absolute deficit of the total pool of T-lymphocytes (CD3 +). The increase in the relative CD16 + content in children of Karakalpakstan was more pronounced than in children of the Khorezm region ($p < 0.01$). Note that the absolute content of CD16 + in this case did not differ from the control (Table 2). Severe functional deficiency of phagocytes was also noted (the content of active phagocytes was less than 50%).

Table 2: Nonspecific protection factors and parameters of humoral immunity in children with PEM in the Aral Sea regions (M ± m)

Immunological index	Control group (Tashkent), n=34	Regions	
		Khorezm region, n=43	Karakalpakstan Republic, n=44
CD3+	44,5±0,4	39,6±0,2	38,5±0,2
CD4+	27,8± 0,3	25,6±0,2	23,1±0,2
CD8+	19,7± 0,2	19,2± 0,2	16,1± 0,1
CD4/CD8	1,42±0,02	1,33±0,02	1,44±0,02
CD16+,%	10,8 ± 0,1	15,7 ± 0,2*	17,4 ± 0,2*
CD16, 10^9/l	0,46 ± 0,01	0,43 ± 0,01	0,41 ± 0,01
ФАН - Neutrophil phagocytic activity NPA	57,5±0,3	36,4 ± 0,4*	34,2 ± 0,3*
CD20+	20,4±0,2	29,2 ± 0,3*	27,3 ± 0,3*
CD20, 10^9/l	0,93±0,004	0,8±0,01*	0,64 ±0,02*
IgG,g/l	6,9±0,05	4,3±0,03*	3,9±0,03*
IgA,g/l	0,81±0,005	0,52±0,006*	0,65±0,005*
IgM,g/l	0,87±0,005	0,39±0,004*	0,38±0,004*

Note: * The values are significant relative to the control group ($p < 0.05$-0.001).

In general, expressed deviations by average in all regions of the examined children compared with new WHO standards are not observed. The indicators of physical development of children of the first 5 years of life who were breast-fed up to 2 years of age are practically at all age stages in all studied regions at the median level or in the range -1CO - + 2CO in terms of body weight and body length/height, from -1CO to + 1CO according to BMI.

The first stage of malnutrition is reduced weight, which can quickly occur, quickly stop and indicates the acute and/or chronic disease that caused this malnutrition (malnutrition, diarrhea, ARI, etc.). Monitoring results in 2007 showed that in Tashkent, the percentage of children with reduced weight relative to age was 2.7% in children aged 0-6 months, 4.3% in children of the first year of life; in the Tashkent region, respectively 4.5% and 8.4%; in the Fergana region - 4.1% and 6.2%; in the Republic of Karakalpakstan - 4.6% and 6.0%, in the Khorezm region - 5.9% and 7.1%; in Bukhara region - 5.7% and 6.5%.

As it is well known that the most effective intervention for this age group is exclusively breastfeeding (IHV). The percentage of BF in the city of Tashkent was 78.6%, Tashkent region - 66.4%, Khorezm region - 67.3%, Fergana region - 85.7%, Bukhara region - 72.2% and the Republic of Karakalpakstan - 89.0 % Based on those results, it can be stated that in the region

where the percentage of IHV was lower (Tashkent and Khorezm regions), the percentage of children with low body weight is greater. By the end of 2012 and the beginning of 2013, the percentage of children of the first 6 months of life with reduced weight decreased in all regions. The dynamics of reduced weight by the end of 2012 among children aged 6-12 months was positive, i.e. the percentage of children of this age with this form of malnutrition in all regions decreased. This percentage ranged from 2.2% in Tashkent to 3.9-3.5% in Tashkent and Khorezm regions.

The monitoring results showed that in 2007 in the group of children over the age of one year and under 5 years old, lower weight was more marked in Khorezm (8.4%) and Tashkent (7.0%) regions. In dynamics, by 2012 this indicator decreased in all regions and ranged from 2.0% in Tashkent to 5.3 and 5.9%, respectively, in Tashkent and Khorezm regions.

One of the main indicators of the physical development and nutritional status of young children is a growth indicator. The largest percentage of children over the age of 5 and up to 5 years old with low growth for a given age was found in Tashkent and Khorezm regions (6.7 and 7.0%), in Tashkent this figure was 3.6%, in Karakalpakstan - 6.0%, in the Ferghana region - 5.1%. In general, in all regions, there has been a positive trend in reducing this form of malnutrition by the end of 2012, and in early 2013 the indicator of low growth among children aged 5 years old in virtually all regions decreased by 1.2-1.5%.

Discussion

The nutritional status of young children is also assessed by the prevalence of low body weight for this growth, which is regarded as protein-energy malnutrition (PEM). The monitoring results showed that in 2007 the percentage of children with moderate PEM in Tashkent amounted to 2.7% in children aged 0-6 months, 2.8% in children of the first year of life, i.e. in age dynamics there is no deterioration of this indicator; in the Tashkent region, these indicators amounted to 6.2 and 3.0%, respectively. 2012 studies showed that PEM, regardless of the age and region of residence of children, decreased and ranged from 1.8% in Tashkent to 3.2% in the Tashkent region among children aged 5 years.

Today, according to WHO, obesity is an increasing problem in the growth and development of children. Monitoring showed that the percentage of overweight and obesity in Tashkent amounted to 3.9% in children of the first 6 months, 9.3% in children of the first year of life; in Bukhara region - 2.2% and 4.5%, respectively; in the Tashkent region - respectively 1.8% and 3.6%; in the Fergana region - 6.2% and 3.0%; in the Khorezm region - 2.2% and 4.2%, respectively; in the Republic of Karakalpakstan - 3.8% and 2.8% of children, respectively. As you can see, in the dynamics in the regions and the Republic of Karakalpakstan there is a decrease in the number of overweight and obese children. The opposite picture is noted in the capital - this indicator by the end of the year actually increased by 3 times.

In the Fergana region, overweight and obesity prevailed in the first 11 months of boys, on average, BMI at this age stage corresponded to the range of overweight relative to the body length of the corresponding age (+ 1CO + 2CO) with a fluctuation from median (0) and above + With. The lowest indicator of overweight and obesity in 2007 was observed among children of the first 6 months in the Tashkent and Bukhara regions, the first year - in the Republic of Karakalpakstan. In dynamics, by the end of 2012, in all age stages, the leading position in the frequency of overweight and obesity continues to be held in Tashkent (E.S B 2015)[4].

Thus, among the forms of malnutrition in all regions low body weight prevails relative to age, this form is more pronounced in regions where the rate of BF coverage is less. This fact also contributes to a more pronounced growth retardation. Malnutrition in children after 6 months is exacerbated by the untimely and inadequate introduction of complementary foods. For the capital of our republic, especially among children by the end of the first year of life, overweight and obesity are more characteristic.

In-depth analysis of the results of the survey of 356 children with malnutrition and various diseases. Among the surveyed, the most prosperous is the situation in Tashkent, where the highest percentage of children with functional impairments and transient deviations in health status is noted (2nd health group) - 88.6% of the examined. Among the children of the first 5 years of life, children with the 2nd health group made up 74.4% in the Republic of Karakalpakstan, 67.9% in the Bukhara region, 65.4% in the Tashkent region, 78% in the Fergana region, 78 1%. Among all examined children with chronic diseases and the risk of transition to chronic diseases in Tashkent city made up 11.4%. Respiratory diseases and anemia predominate in the structure of

functional disorders and transient diseases in a state of health. The leading positions in the structure of chronic pathology are occupied by diseases of the circulatory system (22.0% of children), pathology of the nervous system and behavior disorders (20.7% of children), and pathology of the digestive system (13.7% of children).

It is known that a diet affects the functioning of the immune system. Based on the results of monitoring and evaluating growth and physical development, we selected 32 children aged 3 to 5 years with PEM. With protein-energy malnutrition, a metabolic imbalance is noted, which, of course, should affect the child's immune system. The research results showed that already in the initial period of malnutrition in the immune system of children, changes take place. In particular, a decrease in the percentage of CD3 + (42.5 ± 0.2% versus 54.7 ± 0.2% in the control, $p < 0.05$) was recorded, mainly due to a decrease in CD4 + (26.6 ± 0.1% against 34.6 ± 0.2% in the control, $p < 0.05$). However, the absolute values of the content of T- and B-lymphocytes did not differ from the indicators of the control group, possibly due to the fact that in children with PEM, relative lymphocytosis was observed (60.4 ± 2.3%). The relative number of T-suppressors / cytotoxic lymphocytes (CD8 +) among children was reduced to 18.1 ± 0.1% versus 22.0 ± 0.2% in the control. The relative content of total B-lymphocytes (CD20 +) and natural killer cells (CD16 +) was reduced compared to the control group to the level of 21.5 ± 0.1% (at 28.1 ± 0.2% in the control, $p < 0.05$) and 11.4 ± 0.2% (at 17.5 ± 0.3% in the control, $p < 0.05$), respectively. The phagocytic activity of neutrophils was significantly lower than the control values - 40.1 ± 0.1% versus 46.4 ± 0.4% in the control ($p < 0.05$). Analysis of the functional activity of B-lymphocytes showed a significantly reduced level of immunoglobulins of the main classes by 2-2.5 times compared with the control. Thus, the level of IgG in children with malnutrition decreased to 4.1 ± 0.04 g/l, IgA to 0.68 ± 0.01 g/l and IgM to 0.38 ± 0.004 g/l compared with the data in the control group, where the level of IgG, IgA, IgM was respectively 8.2 ± 0.08 g/l, 1.05 ± 0.01 g/l and 0.83 ± 0.01 g/l.

Deficiency of phagocytic protection was detected in 43.7 ± 5.3% of the examined children. In the examined children of the Khorezm region, the percentage of active phagocytes was reduced by 1.3 times, and in children of the Republic of Karakalpakstan by 1.4 times relative to the control group.

The relative number of B-lymphocytes (CD20) exceeds the control group by 1.36 times ($p < 0.05$) in children in the Khorezm region and 1.27 times ($p < 0.05$) in children in Karakalpakstan, and the absolute content total B-lymphocytes significantly reduced relative to control values in both groups ($p < 0.05$).

Immunological disorders in children with respiratory and nutritional diseases were exacerbated in the presence of malnutrition. A feature of immunological reactivity in the examined children with BENP is a deficiency of phagocytic defense ($p < 0.01$), low blood levels of immunoglobulins IgG, IgA, IgM ($p < 0.05$).

Conclusion

Anthropometric indicators in children of the first 5 years of life, regardless of gender, in almost all studied regions in terms of body weight and body length/height in most cases were at the median level (0) at birth, in the range -1CO - + 2CO - at the age 0-6 months, in the range of -2CO - + 1CO - at the age of 6 months to 5 years; according to body mass index in the range -1CO - + 1CO - in children of all ages, which corresponds to normal indicators according to the standards of growth and development recommended by WHO. Furthermore, in children of the first year of life, low body weight relative to age prevailed among the forms of impaired physical development and nutrition in all regions. This form is more pronounced in regions where the coverage rate for exclusively breastfeeding in the first 6 months and breastfeeding up to 2 years of age is less (Tashkent and Khorezm regions). The same trend was observed in subsequent age stages. Violation of the physical development and nutrition of children after 6 months was exacerbated by the untimely and irrational introduction of complementary foods. In the dynamics of the 5-year monitoring, by the end of 2012 - the beginning of 2013, a decrease in the frequency of all forms of developmental and nutritional disorders, regardless of the age and gender of children, was noted in all regions. The leading position in the frequency of overweight and obesity was held by children in Tashkent and the Fergana region. In the Fergana region, overweight and obesity prevailed in the first 12 months in boys. In dynamics, by the end of 2012, in all age stages, the leading position in the frequency of overweight and obesity continues to be held in Tashkent. Immunological abnormalities in moderate early BENP were mainly manifested in defects of the T-cell immunity, which were characterized by a low content of CD3 + and CD4 + lymphocytes, which indicates that

T-cell proliferative response to antigens weakens. A decrease in the production of immunoglobulins, due to a violation of the T-cell regulation of their synthesis, is also characteristic. These immunological disorders have important prognostic value in determining the body's resistance to diseases.

Study Limitations: We could not include the prevalence of somatic diseases among children under 5 years old. If any research included such data, it might show more clear picture of influence of malnutrition on the general health condition certain regions. Thus, further researches may be required to describe the prevalence of malnutrition and its impact of the health condition of local children.

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Age-Related Features of Mast Cell Reactivity in Allergies

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Abstract

Introduction: During last few years, a novel scientific sphere has developed and is successfully growing, associated with the study of age-related changes in immune and allergic reactions at the cellular level. Although there is a fact that mast cells are the subject of many researches, many aspects of their physiology and pathology remain unsolved. Moreover, clarification in the age-related features of mast cell reactivity makes a great contribution to the expansion of our view of point on the characteristics of the clinic, etiology and pathogenesis of allergies in ontogenesis.

Materials and Method: We used 443 guinea pigs of both sexes of different ages, previously experienced 10-14 day-quarantine control under conditions of vivariums. Animals were constantly monitored, body weight was periodically determined, and rectal temperature was measured. Sensitization and anaphylactic reactions were reproduced by conventional method. Normal horse serum was used as an allergen. The resolving dose of a specific allergen was administered once intravenously or intraperitoneally 14-16 days after the start of the first sensitizing dose of the allergen. Collected digital data processed on electronic microcalculator Rokwell international Model 44 (USA).

Results: With age, the rate of filling mast cells with granules increases. In guinea pigs of an older age, as compared to newborns, the rates of filling mast cells with granules are 1.3-2.2 times greater ($p < 0.05$), which is consistent with the literature. Spontaneous mast cell degranulation was observed in intact animals. Their frequency ranged from 5.4 to 8.6% and, in principle, did not depend on age. A comparative analysis of the frequency of degranulation, depending on age, showed that the frequency of degranulation in sensitized newborns is slightly lower than in older animals. However, with age in sensitized guinea pigs, the frequency of degranulated mast cell forms increased and the mast cell reactivity in one month aged pigs became approximately the same as in mature animals.

Conclusion: In general, the functional activity of mast cells increases with age, and by the age of one month reaches the level of functional activity. In the development of specific therapy, a decrease in the titer of homocytotropic antibodies and the percentage of mast cell degranulation is vital.

Keywords: *Allergy,mast cells, sensitization, degranulation, allergens, age-related features.*

Introduction

During the life of organ systems of human body, namely, homeostasis, protective and adaptive reactions, metabolic processes, blood circulation, respiration and others, an important role is played by connective tissue and blood. Among various connective tissue cells, mast cells are particular and learnt with high interest by scientists (Nasonov, 2017)[1]. They accomplish

extremely important functions: participate in the regulation of homeostasis, secrete biologically active substances (heparin, histamine, serotonin, dopamine, etc.), affect the entire system of connective tissue, and are actively involved in allergic reactions. In this regard, during the recent years, a new scientific field has arisen and is successfully developing, associated with the study of age-related changes in immune and allergic reactions at the cellular level (Baglay et al, 2015)[2]. For a long time, the prevailing opinion in medical science was that cells are always damaged as a result of the formation of an immune complex on the surface of their membranes (Tsibulkina et al, 2017)[3]. It has now been clearly established that the formation of immune complexes on the surface of target cells is not always accompanied by cell destruction (Gushchin, 2019)[4]. In most cases, it is not destruction, but excitation, that is, an increase in their functional activity. This information opens up a new promising area that allows us to develop pharmacological method for the management of allergic reactions and not it is possible to use drugs that reduce and inhibit excitatory processes in mast cells (Kryshen et al, 2018)[5].

Despite the fact that mast cells are the subject of many researches, many aspects of their physiology and pathology remain unsolved[6]. So, for example, the question of age-related features of mast cell reactivity in normal and allergic reactions is of great scientific interest (Artishevsky et al, 2016). The fact is that mast cells are among the most reactive cells in the body. They quickly respond to various exogenous and endogenous effects (Namazova-Baranova et al, 2017)[7]. The study of mast cell reactivity in the dynamics of allergy development allows us to determine the pathological process in the early stages of their development, which is of great diagnostic, therapeutic and preventive value. It is believed that the clarification of the reactivity of mast cells allows one to indirectly judge the general condition of the organism as a whole. In addition, mast cell reactivity can be used to objectively evaluate the effectiveness of therapy (Abaturov et al, 2017)[8].

Thus, elucidation of the age-related features of mast cell reactivity contributes to the expansion of our ideas about the features of the clinic, etiology and pathogenesis of allergies in ontogenesis[9]. Above mentioned points make us to conduct this research. The identification of age-related features of mast cell reactivity in allergic reactions, in our opinion, will contribute to the successful solution of certain fundamental aspects of the National Program of the Ministry of Health of the Republic of Uzbekistan for the treatment and prevention of allergies[10].

The aim of the study was to identify age-related features of mast cell reactivity in allergic conditions and to develop criteria for therapeutic effectiveness based on an experimental model of specific hyposensitizing immunotherapy[11].

The age-related features of reactivity, quantitative content, filling of cells with granules, functional activity of tissue (mesentery) and peritoneal mast cells in intact guinea pigs have been established[12]. It was revealed that the body of guinea pigs in early postnatal ontogenesis (newborns, two weeks) is reactive to exogenous allergenic effects[13]. It was determined that the reactivity of mast cells of guinea pigs increases with age and reaches a level of reactivity in mature adults. It was established that mast cells of young guinea pigs are actively involved in the development of allergic reactions of an immediate (anaphylactic) type[14].

Criteria have been developed for evaluating therapeutic efficacy based on an experimental model of specific hyposensitizing immunotherapy in guinea pigs of different ages[8]. It has been established that the most important objective indicators of experimental specific therapy of anaphylactic allergy are the determination of the level of HCA titer in blood serum, the frequency of mast cell degranulation and the anaphylactic index[3].

Materials and Method

Study Population: The experiments were conducted on 443 guinea pigs of both sexes of different ages, which previously passed 10-14 day-quarantine control under conditions of vivariums: experimental - 274, control - 169. By age, guinea pigs were distributed as follows: newborns - 108 (24.4%), two-weeks - 117 (26.4%), one-month - 120 (27.1%), mature - 98 (22.1%).We paid attention to the general condition, feed intake, mobility, shine of the coat, absence of outflows from visible mucous membranes (eyes, nose, anal and genital openings). Animals were constantly monitored, body weight was periodically determined, and rectal temperature was measured. Animals were fed with normal (traditional) food. Before the experiments animals were not fed during one day. In general, we provided with all the necessary requirements for experimental research. Sensitization and anaphylactic reactions were reproduced by conventional method[1].

Normal horse serum was used as an allergen. The resolving dose of a specific allergen was administered once intravenously (hind paw) or intraperitoneally 14-16 days after the start of the first sensitizing dose of the allergen. The severity of general anaphylactic shock and the calculation of the anaphylactic index (AI) were evaluated by Weigle et al. The histamine shock is reproducible in two versions: 1) the deadly histamine shock was reproduced by intravenous administration of 0.1% histamine at the rate of 4-6 mg/kg of animal body weight; 2) non-fatal histamine shock was reproduced by subcutaneous administration of histamine at the rate of 2 mg/kg of animal body weight[2].

Homocytotropic antibodies (HCA) in the blood serum of sensitized guinea pigs are determined by the generally accepted method of reproducing a cutaneous passive anaphylactic reaction. The reaction was considered positive if the diameter of the stained spot on the skin was at least 6 mm. Tissue (mesentery of the small intestine) and peritoneal mast cells in guinea pigs were obtained by a well-known method in compliance with sterility.

Mast cells were fixed in absolute alcohol and stained with 1% solution of toluidine blue in 60° alcohol. The frequency of granular mast cell forms showed in percentage by viewing different fields of view of smears under a microscope with a magnification of 5x95. Specific therapy was carried out according to the scheme developed by us. To control animals, sterile saline was injected subcutaneously at the same time instead of a specific allergen.

Statistical Data Analysis: Digital data processed on electronic micro calculator Rokwell international Model 44 (USA). The following indicators were calculated: 1) arithmetic and geometric mean values; 2) the negative logarithm of the geometric mean titer of antibodies when the base is equal to two; 3) the correlation coefficient; 4) the significance of differences between the compared average values. Information was considered reliable provided that t22, and $p < 0.05$.

Results

An analysis of our own experimental studies revealed the age-related features of mast cell reactivity in normal and in allergic reactions, anaphylactic type. In intact guinea pigs, mast cells are scattered in all organs and systems of the body. However, their quantitative content in tissues compared with other animal species (rats, mice, hamsters) is much less.

Nevertheless, in the development of allergic reactions of guinea pigs, they are crucial (Eliseeva et al, 2016). In this regard, it was of some interest to clarify the age-related characteristics of the number of mast cells in guinea pigs. In the mesentery of the small intestine of intact newborn guinea pigs (with an increase of 100 times), the number of mast cells, in comparison with guinea pigs, is more than that of older age (two-weeks, one-month, sexually mature) for 1.9-3.7 times (Table 1), which is consistent with the literature.

It is believed that this phenomenon is associated with a deep change in the environment after birth, mechanical influences during childbirth, etc. According to some researchers, the decrease in the number of mast cells after birth is explained by the blockade of the color of the protein-polysaccharide interaction, which is more important for the proliferation of mast cells. The analysis of mast cell fullness by granules was observed with great interest; where biological active substances are concentrated (histamine, serotonin, etc.).

Guineapigsage	The number of mast cells in the field of view (M±m)	Mast cell degranulation rate (M ± m%)
Newborns (n=10)	268,9±11,2	5,4±1,6
Twoweeks (n=10)	136,0±6,2*	6,2±1,3
One month (n=10)	84,1+1,1*	7,6±0,6
Sexuallymature (n=10)	72,7+2,2*	8,6+0,7
Total: 40	---	---

Note: * The difference in comparison with the performance of newborns is significant ($p < 0.05$).

They determine the pathochemical phase of the pathogenesis of an allergy of an immediate type. The indicator of filling mast cells with granules is given great importance because a smaller filling with granules does not indicate that they are poorly developed, but, on the contrary, increase their functional activity. According to our data, age-related features of filling mast cells with granules are observed in intact guinea pigs. With age, the rate of filling mast cells with granules increases. In guinea pigs of an older age, as compared to newborns, the rates of filling mast cells with granules are 1.3-2.2 times greater ($p < 0.05$), which is consistent with the literature.

Thus, on the whole, the following regularity was revealed — in guinea pigs of different ages: in newborns, in comparison with older animals, the total number of mast cells is greater, and the nature of filling the cells with granules is less.

According to our data, spontaneous mast cell degranulation was observed in intact animals. Their frequency ranged from 5.4 to 8.6% and, in principle, did not depend on age (table. 1). However, the age-related features of mast cell degranulation in allergic reactions were clarified. Sensitization showed a significant increase in the mast cell degranulation rate, in comparison with similar indicators in intact guinea pigs, 1.2-3.0 times ($p < 0.05$). A comparative analysis of the frequency of degranulation, depending on age, showed that the frequency of degranulation in sensitized newborns is slightly lower than in older animals. However, with age in sensitized guinea pigs, the frequency of degranulated mast cell forms increased and the mast cell reactivity in one month aged pigs became approximately the same as in mature animals (Table 2).

Table 2: Age-related features of the frequency of degranulation of mast cells of the mesentery of the small intestine in guinea pigs with sensitization and anaphylactic shock (n = 145).

Age of animals	Sensitization (M± м%)	Anaphylactic shock (M± m%)	Control (M± м%)
Newborn	10(6,5+0,8)	21(15,2+0,8)*	6(5,3+1,3)
Two weeks	10(12,3+0,9)**	19(27,2+1,3)*	6(6,0+1,1)
One month	10(25,6±1,3)**	20(61,0±1,6)*	6(8,0+0,7)
Sexually mature	10(21,9+2,3)**	21(65,0+2,1)*	6(7,3+0,7)
Total	**40**	**81**	**24**

Notes: Between indicators of anaphylactic shock and control * (*). and also between the sensitization and control indicators (**) reliability ($p < 0.05$).

The study of the reactivity of guinea pigs of different ages with experimentally reproduced anaphylactic shock was also of some interest. In the literature, there is conflicting information on this issue. Some researchers believe that in the early postnatal age, the body does not respond to allergenic effects, asimmunocompetent system is poorly developed. Other researchers believe that newborns are actively sensitized. It turned out that guinea pigs in early postnatal ontogenesis are reactive to exogenous allergenic effects, as evidenced by the development of general anaphylactic shock with parenteral administration of a resolution dose of a specific allergen. Along with this, age-related features of anaphylactic shock were revealed: in young animals (newborns, two weeks old), cases of mild and moderate severity of anaphylactic shock (AI = 1.7-2.6) prevailed, and in one-month-old and mature ones, severe and fatal (AI = 2 9-3.7). In the mechanism of development of allergic reactions in general and anaphylactic shock in particular, mast cells and their biological active substances are crucial. In anaphylactic shock, the frequency of mast cell degranulation in comparison with similar indicators in sensitization was significantly ($p < 0.05$) higher in newborns - 2.3 times, two-week 2.2 times, one-month 2.3 times, and mature - 2.9 times. Similar results were noted in the study of reactivity of peritoneal mast cells sensitization and anaphylactic shock (table. 3). With sensitization, compared with intact guinea pigs, the mast cell degranulation rate was 1.6 times higher in newborns in two weeks, 3.4 times higher one-month - 5.7 times, sexually mature - 7.2 times (p-0, 5). With anaphylactic shock, the frequency of mast cell degranulation was even greater than with sensitization.

Table 3: Age-related features of the frequency of peritoneal mast degranulation in guinea pigs during sensitization and anaphylactic shock (n = 81).

Age of the animals	Sensitization (M± м%)	Anaphylactic shock (M± m%)	Control (M± м%)
Newborn	12(10,7+0,97)**	12(13,5+0,88)*	6(6,5:0,96)
Two weeks	15(22,2+0,37)**	15(26,4+1,11)*	6(6,5.0,96)
One month	16(39,8:0,70)**	16(50,8+0,70)*	6(6,8.0,98)
Sexually mature	14(45,8+1,4)**	14(73,9+2,43)*	6(6,3+0,7)
Total	57	57	24

Note: the differences between anaphylactic shock and control (*), as well as between sensitization and control, are significant ($p < 0.05$).

Discussion

In the mechanism of development of anaphylactic shock in guinea pigs, the decisivehistamine is important (Soldatov et al, 2016). According to our data, with histamine shock, the frequency of mast cell degranulation increased sharply, moreover in older animals, which indicates a sharp increase in the functional activity of these cells. In histamine shock, the mast cell degranulation rate in sexually mature guinea pigs was 4 times higher than the mast cell degranulation rate in newborn guinea pigs.

The question arises: why in rats, mice and hamsters, where there are many times more mast cells than guinea pigs, the clinical symptoms of anaphylactic shock are weakly expressed? Apparently, this is due to the fact that their mast cells are scattered in all tissues and organs more or less evenly. In addition, immunocompetent cells of these animals weakly synthesize humoral antibodies (Johansson et al, 2001). In the body of guinea pigs, the spread of mast, cells around the smooth muscles of the bronchioles, bladder and intestines is much greater than in other animals. In this regard, during allergic reactions on the cytoplasm of mast cells of guinea pigs, a large amount of histamine is released, which causes spasm of bronchioles, contraction of the smooth muscles of the gastrointestinal tract and bladder, and expansion of peripheral small arteries and arterioles (Reese et al, 2002). As a result of this, all the main symptoms of anaphylactic shock acutely develop: asphyxia, hypoxia, involuntary defecation and urination. One of our tasks was to study the importance of mast cells in the mechanism of development of specific therapy. Although specific therapy has been used in practical medicine for more than 90 years, nevertheless there are many unresolved issues, among which age-related features of indications and contraindications, the importance of mast cells in the mechanism of development of specific therapy, are important.

Studies have shown that specific HCA are detected in the blood serum of sensitized animals. However, the HCA titer depended on the age of the experimental animals: fewer (3.5 ± 1.2) y newborns and more (7.5 ± 0.6) in older guinea pigs. After specific treatment, the HCA titer in animals of all age groups, compared with the HCA titer before treatment, decreased 1.7-2.3 times ($p < 0.05$). The study of mast cell reactivity with specific therapy showed the following results. The frequency of mast cell degranulation compared with indicators before treatment significantly decreased ($p < 0.05$). Specific therapy also effectively prevented the development of general anaphylactic shock. All animals remained alive. Excellent results were observed in the sexually mature (AI = 0), good results were observed in the one-month (AI = 0.1), two-week (AI = 0.3) and newborns (AI = 0.6). Analysis of the actual material indicates that mast cells also take an active part in the mechanism of development of specific therapy, along with cells of the immunocompetent system.

In general, currently, there are various theories regarding the pathogenesis of specific therapy: increasing the synthesis of blocking antibodies, neutralizing specific allergic antibodies, switching the synthesis of allergic antibodies to the synthesis of blocking antibodies, strengthening the factors of nonspecific defense of the body, strengthening the synthesis of anti-inflammatory factors, etc.

All these theories are based on actual clinical and experimental facts (Nadein et al, 2012). Our data support the theory of neutralizing specific allergic antibodies.

Indeed, according to our data, after specific therapy, the titer of specific HCA significantly decreased in all age groups of animals, including newborns, two-week and one-month-old ones. However, the mechanism of this phenomenon is not clear. There is an opinion that when conducting specific therapy, the process of multifaceted "microshocks", that is, the interaction of HCA with allergens, occurs. Thus, allergic antibodies are gradually eliminated. However, not all scientists agree with this treatment. It is believed that inhibition of HCA synthesis is crucial in the mechanism of specific therapy. Our data are also consistent with the theory of inhibition of the synthesis of mediators by mast cells. According to our data, there is a significant decrease in the frequency of mast cell degranulation in all age groups of animals. Thus, using a complex of allergological and immunological method, it was possible to identify age-related features of the reactivity of tissue and peritoneal mast cells, both in norm and in allergic reactions of anaphylactic type.

Conclusion

The following age-related features of tissue (mesentery of the small intestine) mast cells in intact guinea pigs are noted: their quantitative content in newborns is greater in 1.9-3.2 times, and the filling rate of cells with granules is 1.3-2.2 times less than that of two-weeks, one-month and sexually mature ones; functional activity of some (minimum) partmast cells of all age groups of guinea pigs is increased, as evidenced by the presence de granulated cell forms; in general, the functional activity of mast cells increases with age, and by the age of one month reaches the level of functional activity, the characteristics of the reaction are fat and mature.

Furthermore, the organism of guinea pigs in early postnatal ontogenesis (newborns, two weeks) is reactive to exogenous allergic effects, as evidenced byidentification of specific homocytotropic antibodies in the blood serum of sensitized animals and the development of anaphylactic shock with repeated parenteral administration of a specific allergen, with mild and moderate cases predominate in young people (AI = 1.7 and 2.6),and in one-month-old and sexually mature - heavy and fatal (AI = 2.9 and 3.7). The functional activity of tissue and peritoneal mast cells significantly increases in all age groups of guinea pigs, and with active sensitization in1.2-3.0 times, anaphylactic shock - 2.8-8.9 times, histamine shock - 3.0-10.6 times.An experimental model of specific therapy with a positive therapeutic effect can be successfully reproduced on young guinea pigs(newborns, two weeks, one month). In the mechanism of development of specific therapy, a decrease in the titer of homocytotropic antibodies and the percentage of mast cell degranulation is essential.

Recommendations: It is fact that the indicators of the frequency of mast cell degranulation, in combination with other clinical and laboratory data, can be used to objectively assess the initial state of an allergic reactive organism, which will improve the quality of specific diagnosis of allergies in the early stages of its development, and also when clinical symptoms are absent, that is, with latent sensitization. An experimental model of a specific hyposensitizing immunoterium FDI in guinea pigs of different ages can be used to evaluate the therapeutic efficacy of different allergens non-infectious origin. The discovery of new scientific facts about the active participation of mast cell mast cells in early postnatal ontogenesis during sensitization and allergic reactions broadens our understanding of the age-related features of the pathogenesis of immediate (anaphylactic) allergy in developing organisms.

1. The age-related features of reactivity, quantitative content, filling the cells with granules and reactive tissue (mesentery of the small intestine) and peritoneal mast cells in intact guinea pigs are noted.
2. The reactivity of mast cells of guinea pigs increases with age and reaches a level of reactivity in mature adults.
3. The organism of guinea pigs in early postnatal ontogenesis (newborns, two weeks) is reactive to exogenous allergenic effects. As evidenced by the successful reproduction of sensitization and anaphylactic reactions.
4. The mast cells of young guinea pigs (newborns, two weeks) take an active part in the development of allergic reactions of the immediate type, as evidenced by a significant increase in their functional activity during sensitization, anaphylactic and histamine shock.
5. In young guinea pigs, an experimental model of specific hyposensitizing immunotherapy is successfully reproduced, in the mechanism of development of which a decrease in the titer of homocytotropic antibodies and the percentage of mast cell degranulation is essential.

Study Limitations: The study is animal based research. Expected outcomes and results of the interventions might be different on humans. Thus, in case of implementation the achievements of the study on patients, this factor has to be taken into account.

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Ethical Approval: During the study respected the principles of a human attitude towards laboratory animals. The ethical approval for the study was granted by the Committee of Ethical Approval for Researches under the Ministry of Health of the Republic of Uzbekistan.

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Correlation of the Main Parametres of Immun Systeminchildren with Localized Scleroderma by the Stages of the Disease

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Abstract

Introduction: In spite of significant development in modern medicine, the tendency of increase in the number of patients with localized scleroderma (LS) remaining actual with more severe clinical courses. Therefore, LS remains as one of the urgent problems of healthcare system. The etiology and pathogenesis of the disease is still unclear. Yet, LS frequently associates with deep and wide immune disorders and subsequent complications.

Materials and Method: We studied 45 children with LS, of whom 25 patients had a linear form, 16 had plaque form, Pazini-Pieriniatrophodermia – in 2 participants, scleroatrophic lichen in 2. The control group included 30 conditionally healthy children. We conducted monitoring of the cellular immunity system with immunophenotyping using specific monoclonal antibodies to surface molecules CD3, CD4, CD8, CD16, CD20, CD25, CD54, CD95. Determination of the main classes of immunoglobulins in serum - IgG, IgA, IgM was also carried out.

Results: There was a significant decrease in the number of T-helpers - CD4: at the stage of erythema and edema by 1.3 times, with sclerotic and atrophic - by 1.2 times. The content of cytotoxic CD8-lymphocytes significantly decreased at the stages of erythema, edema and sclerotic on average by 19.4%. As a result of more significant inhibition of CD8, the CD4/CD8 ratio became larger (by 24%, 25%, and 11%, respectively, at the stage of erythema, sclerosis, and atrophy) than in the group of conditionally healthy patients. Patients with LS, a statistically significant decrease in the serum percentage of total T-lymphocytes - CD3, and at the stage of erythema and atrophic edema it was 11.4%, and at the stage compaction - 6.3% compared with the parameters of control group.

Conclusion: According to the level of inflammatory process with LS, immune dysfunctions are associated with a decline in the total amount of blood lymphocytes, namely CD4 T-helper cells and CD8 T-suppressors, CD54 endotheliocyte adhesion molecule, especially in the stages of erythema and edema or sclerosis. Learning these parameters may help to assess the immunological changes, and can be informative for the determination of the course, prognosis and further treatment.

Keywords: *Morphea, localized scleroderma, children, cellular immunity, humoral immunity, interleukin.*

Introduction

Localized scleroderma (LS) or “morphea” is a progressive lesion of the connective tissue of the dermis and hypodermis, without involvement of internal organs in the process with a predominance of fibro-sclerotic and vascular disorders, such as obliterating endarteriolitis with widespread vasospastic changes. The disease develops on the background of genetically determined

imperfection of immunoregulatory processes, resulting in the formation of antibodies to own cells and their components, the occurrence of immunocomplex inflammation, leading to tissue damage (Mannanov & Moon, 2018; and Moon, 2019)[1].

Despite intensive studies during the last few decades, there is a tendency to increase the number of patients with LS with a more severe clinical course, thus, LS remains as one of the urgent problems of modern medicine[2]. The etiology has not yet been identified well, and the pathogenesis of the disease is also unclear[3]. The issues of clarifying the links in the pathogenesis of LS, determining diagnostic markers of the severity of the process, and substantiating new treatment method based on certain disorders remain as actual problem today (Moon, 2019; and Shostak et al, 2015)[4]. However, the main role in the development of scleroderma is played by 3 theories of pathogenesis, immune disorders, metabolic pathology and vascular disorders in the connective tissue. One of the main links in the pathogenesis of scleroderma is immune disorder (O'Brien et al, 2017; and Torok et al, 2015)[5]. However, the data on this issue are still disputable and require some improvements. It is important to evaluate the immune disorders in the blood, leading to morphological changes in the lesions by considering the course of dermatosis.

With localized scleroderma, pathological autoimmune processes develop with signs of activation of the T-cell link of the immune system, associated with ineffective suppressor mechanisms in the immune system, which are represented by cytotoxic T-lymphocytes and some other subpopulations of T- and B-lymphocytes.An increase in IL-2, IL-4, IL-6, and soluble IL-2 receptors in patients with limited scleroderma indicates activation of cellular immunity (Suzanne, 2018)[6].

Dysregulatory disorders are accompanied by increased production of pro-inflammatory cytokines IL-2, TNFα and INF-γ, contributing to the development of early autoimmune inflammation[7].There is no doubt that the pathogenesis of scleroderma is associated with a dysfunction of human immunity[8]. Evaluation of the immune status with diagnostic, therapeutic, prognostic and preventive purposes in patients with limited scleroderma remains an extremely urgent issue[9].

The aim of our work was to study the characteristics of the immune status of children with LS at different stages of the disease[10].

Materials and Method

Study Population: We studied 45 children with LS from January 2013 to December 2019, of whom 25 patients (55.5%) had a linear form, 16 (35.5%) had plaque form, Pazini-Pieriniatrophodermia–in 2 participants (4.4%), scleroatrophic lichen in 2 (4.4%). The degree of severity, characterized by a mild course, was observed in 12 children, II degree, moderate in severity - 22, III degree, which was characterized by a severe course - in 11.Of the total number of patients with LS in the abdominal and linear form, 16 (35.6%) patients were diagnosed with the stage of erythema and edema.In 18 patients (40%), the stage of sclerosis was diagnosed (I degree of severity - 4, II degree - 9, III degree - 5), 11 - stage of atrophy (I degree of severity - 4 people, II degree - 5, III degree - 2).

All patients with Pasini-Pieriniatrophodermia were in the stage of sclerosis (I severity). Patients with scleroatrophic lichen were in the acute stage of II severity and II severity. Thus, we studied children with LS at the stage of erythema and edema - 16 (35.6%), at the stage of sclerosis -18 (40%), atrophy - 11 (24.4%). The number of boys was 12 (26.6%), and girls - 33 (73.3%). The mean age of the examined was 6.8 ± 0.8 years.

Clinical and laboratory tools: The diagnosis of LS was verified and established according to clinical and laboratory data, and the severity according to the dermatological indices of the LoSCAT (Localized Scleroderma Assessment Tool) including mLoSSI (Modified Localized Skin Severity Index), LoSDI (Localized Scleroderma Damaged Index), based on an assessment of 3 separate processes - erythema, skin tightening, the appearance of new elements or an increase in existing ones during the previous month, the degree of atrophy of the skin and subcutaneous fat, as well as the severity of pigmentation (Zulian, 2017; and Teske & Jacobe, 2018)[11].

The control group included 30 conditionally healthy children who permanently reside in Tashkent city and the Tashkent region (18 girls and 12 boys, average age 7.4 ± 0.9 years).

Monitoring the state of the cellular immunity system was carried out by immunophenotyping using specific monoclonal antibodies to surface molecules CD3, CD4, CD8, CD16, CD20, CD25, CD54, CD95 of the "Scientific-Production Center" LLC (Moscow). The determination of the main classes of immunoglobulins in

serum - IgG, IgA, IgM, is the main method for assessing humoral immunity and their determination is of great diagnostic value in the diagnosis and monitoring of primary immunodeficiencies and autoimmune diseases. The content of immunoglobulins A, M, G in serum was determined by the enzyme-linked immunosorbent method "Immunoglobulins A, M, G - IFA". The concentration of circulating immune complexes was determined according to the method of Grinevich and Alferov (Mannanov & Moon, 2018; and Shostak et al, 2015)[12].

Immunocompetent cells were verified using "Chemicon" monoclonal antibodies (MCAs) (product of USA) in mature T-lymphocytes (CD3), T-helpers (CD4), T-suppressors (CD8), B-lymphocytes (CD22), macrophages (CD16), IL-1 and IL-2. Cells expressing receptors for HLA-Drantigen were determined using the MCA HLA-Dr antigen of the same company. The relative volumes of immunocompetent cells were determined by the aqueous field of view of the microscope in terms of 100 cells.

Results

Immunomorphological studies were carried out in 45 children with LS, of which 16 patients (35.6%) of the abdominal and linear forms were diagnosed with erythema and edema (I severity -5 people, II degree - 8, III degree - 3), in 18 patients (40%) - the stage of sclerosis (I degree of severity -4 people, II degree - 9, III degree - 5), in 11 patients (24.4%) - stage of atrophy (I degree of severity -4 people, II degree - 5, III degree - 2). The age of patients ranged from 2.5 to 17 years (average age was 6.8 ± 0.8 years), among whom there were 12 boys (26.6%), and girls - 33 (73.3%), the duration of the disease from 1 month up to 6 years.

When analyzing the obtained data of the immune parameters of the blood of children with LS, inhibition of the T-system of immunity was revealed - a statistically significant decrease in the serum percentage of total T-lymphocytes - CD3, and at the stage of erythema and atrophic edema it was 11.4%, and at the stage compaction - 6.3% compared with the parameters of control group.

There was also a significant decrease in the number of T-helpers - CD4: at the stage of erythema and edema by 1.3 times, with sclerotic and atrophic - by 1.2 times. The content of cytotoxic CD8-lymphocytes significantly decreased at the stages of erythema, edema and sclerotic on average by 19.4%. As a result of more significant inhibition of CD8, the CD4/CD8 ratio became larger (by 24%, 25%, and 11%, respectively, at the stage of erythema, sclerosis, and atrophy) than in the group of conditionally healthy patients.

The content of CD8-lymphocytes at the atrophic stage increased up to the level of control group. Despite the revealed disorders of the immune system, no statistically significant differences were found in patients with different numbers of lesions.

An increase in the immunoregulatory index indicated an activation of the immune system in the stage of edema and erythema, as well as in the stage of early sclerosis, however, a decrease in this indicator in the stage of atrophy and sclerotherapy in the later stages showed inhibition of immunity (Table 1).

Table 1: The content of lymphocyte subpopulations in the blood of patients with LS depending on the stage of the disease in children

Indexes	Control n = 30	Stage of the disease		
		Edema n = 16	Sclerosis n = 18	Atrophy, n = 11
CD3+,% (Overall pool of T-lymphocytes)	56,5 ± 1,3	42,0 ± 1,3	47,8 ± 2,2	41,4 ± 2,9
CD4+,% (T-helpers)	33,8 ± 1,4	22,3 ± 1,2	26,2± 1,1	27,1 ± 1,8
CD8+,% (T-citotoxic lymphocytes)	22,6 ± 0,6	15,2 ± 1,8	16,3 ± 1,4	25,0 ± 1,7
CD4/CD8 (immunoregulation index)	1,8 ± 0,04	2,47 ± 0,22	2,27 ± 0,27	1,37 ± 0,18
CD16+,% (natural killers)	14,5 ± 0,8	18,5 ± 1,7	19,3 ± 2,6	19,9 ± 1,5
CD20+,% (B-lymphocytes)	20 ± 0,7	24,5 ± 0,5	25,3 ± 2,5	26,0 ± 1,0
CD25+,%	7,4 ± 0,5	15,4 ± 3,0	18,1 ± 0,5	16,5 ± 1,0
CD95+,% (factor of apoptosis)	17,2 ± 0,5	22,3 ± 1,3	26,8 ± 1,0	24,1 ± 1,5

Indexes	Control n = 30	Stage of the disease		
		Edema n = 16	Sclerosis n = 18	Atrophy, n = 11
CD54+,% (factor of adhesion)	23,4 ± 1,1	14,2 ± 1,2	11,8 ± 1,0	17,1 ± 1,8
Leucocytes, $x10^9$/l	5,98 ± 0,27	6,45 ± 0,42	6,71 ± 0,38	6,64 ± 0,33
Lymphocytes, $x10^9$/l	2,26 ± 0,19	2,05 ± 0,16	2,16 ± 0,15	2,63 ± 0,11

It was important that significant decrease in the blood serum of patients with respect to the content of intercellular adhesion molecules, which are surface antigens of activated B-lymphocytes-CD54, namely, 36% at the stage of erythema and edema, 38% at sclerotic, 28% at atrophic stages of the disease compared with the control group. The content of adhesion molecules of endothelial cells CD54 at the atrophic stage of the disease remained significantly increased compared with the sclerotic stage by 41.7%.

It should be noted that the number of CD95 + cells responsible for apoptosis in sick children at the stage of atrophy increased by 27.2%.Especially statistically significant differences in the number of lymphocytes of different phenotypes were found for CD16, CD25 and CD95, the content of which was increased compared with conditionally healthy patients.

The stages and nature of the immunological process reflect the expression data of activation markers that depend on the functional activity of immunocompetent cells. Interpretation of the expression of active markers on the surface of lymphocytes showed a significant increase in apoptosis processes, in patients with LS as evidenced by an increase in cells carrying apoptosis receptors - CD95, the most pronounced increase was at the sclerotic stage - by 57.6% compared with the control group.

The relative content of CD95 in the stages of edema and atrophy was 31.2% and 41.7%, respectively. At the same time, an increase in the expression of receptors for CD95 testified to a significant destruction of lymphocytes, which led to immunodeficiency, a decrease in the number of lymphocytes, inhibition of T-suppressors and an increase in circulating immune components (CIC)in the blood.

In the examined patients, the content of markers of the early stage of cell activation, IL-2 CD25 activators increased by 2.1 and 2.4 times in the cases of erythema and edema and atrophic, respectively, and 2.2 times in the sclerotic stage compared with the control. As you know, IL-2 is a pro-inflammatory cytokine, which leads to inflammation, the appearance of severe vasculitis. Its level remained high throughout the entire disease, which led to a chronic process, activation, and the appearance of a significant pool of proliferating lymphocytes in LS. The amount of natural killers - CD16 increased on average by 35.2%, a change in the content was especially pronounced during the sclerotic and atrophic stages. A significant increase in the percentage of activation markers of the B-cell chain of the CD20 immune system was 22.5%, 26.5%, 30%, respectively, at the stages of erythema and edema, sclerotic and atrophic, compared with the control group.

The dynamics of changes in the lymphocyte phenotype was observed in the sclerotic and atrophic stages of the OS against the background of an increase in the total number of leukocytes and a relative decrease in lymphocytes. A decrease in the number of T-lymphocytes with an imbalance of subpopulations against the background of the development of activation processes and their increased tendency to apoptosis probably leads to a loss of control of T-lymphocytes in relation to B-cells, with their subsequent activation.

Our results indicate a significant increase in blood levels of IgM and IgG in the examined LS patients compared with the control against the background of insignificant changes in the content of IgA. The most pronounced was an increase in IgG, the content of which increased by 24.3%, 15.1% and 39.8%, respectively, at the stages of edema, compaction and atrophy.

In children with LS in the blood serum, an increase in the level of large and small circulating immune complexes of the CIC by 47.4% and 26.9% in the sclerotic and atrophic stages of the disease, by 63.9% in the stage of erythema and edema compared with the control (table 2). The production of antibodies, including autoantibodies, is controlled by the corresponding cytokines.

Table 2: The main indicators of the humoral immunity in the blood serum of children with LS

Indexes	Control, n = 30	Stage of the disease		
		Edema n = 16	Sclerosis, n = 18	Atrophy, n = 11
Ig A, g/l	1,82 ± 0,11	1,93 ± 0,14	1,71 ± 0,09	1,74 ± 0,11
Ig M, g/l	1,4 ± 0,07	2,01 ± 0,05	2,1 ± 0,04	1,93 ± 0,07
Ig G, g/l	9,73 ± 0,32	12,1 ± 1,77	11,2 ± 1,48	13,6 ± 1,29
CIC, y.e.	38,2 ± 3,3	62,6 ± 8,4	56,3 ± 9,2	48,5 ± 4,6

In the blood serum of patients with LS in the stage of edema, a significant increase in the content of IL-1β was detected on average 4.9 times, IL-4 2.05 times and IL-6 8 times compared with the control. In the sclerotic stage, these indicators increased by 3.7 respectively; 1.93 and 5.7 times; at the atrophic stage - 5.7; 1.7 and 3 times (table 3).

Table 3: The main indicators of the content of cytokines in the blood serum of children with OS, depending on the stage of the disease

Indexes	Control, n = 30	Stage of the disease		
		Erythema and edema, n = 71	Sclerotic n = 47	Atrophic, n = 37
IL-1β	5,68±2,12	27,92±3,12	21,1±2,38	32,41±2,83
IL-4	5,75±2,05	11,81±1,23	11,07±1,24	9,93±0,9
IL-6	5,71±1,7	45,53±6,09	32,32±5,23	17,42±1,93
TNFα	2,85±0,63	11,36±1,2	9,74±1,61	8,18±1,52
IFN-γ	273,3±28,9	95,8±9,1	78,5±8,2	168,3±12,1

Discussion

IL-1β, which is a pro-inflammatory cytokine, has numerous common effects and can contribute to the development of the systemic nature of the pathological process by forming autoantibodies and increasing the concentration of CRP in the blood. It should be noted that IL-4 and IL-6 are one of the frequently determined cytokines in the blood serum of patients with scleroderma. (O'Brien et al, 2016; and O'Brien et al, 2017).

In addition to B-lymphocytes, monocytes, macrophages and fibroblasts are also sources of IL-6 in scleroderma (Toroket al, 2015; and Suzanne, 2018). IL-6 is involved in the induction of almost the entire complex of local manifestations of inflammation. It affects the migration of phagocytes, enhancing the production of chemokines that attract monocytes and lymphocytes, and weakening the production of chemokines that attract neutrophils. The pro-inflammatory effects of IL-6 are less pronounced than that of IL-1 and TNFα, in contrast to which it does not enhance, but inhibits the production of pro-inflammatory cytokines (IL-1, TNFα and IL-6) and chemokines by cells involved in the inflammatory process (Toroket al, 2015). Thus, IL-6 combines the properties of pro- and anti-inflammatory cytokines and is involved not only in the development, but also in limiting the inflammatory response (Speeckaert et al, 2016).

Cytokines play an important role in the regulation of all aspects of the functioning of the immune system, including the differentiation of lymphoid cells, inflammation, the development of the adaptive function of the immune response, and others. One of the most important regulators of reactions of both non-specific and adaptive immune responses is TNFα and IFN-γ. TNF is produced both by cells of the immune system (B and T lymphocytes, basophils, eosinophils, dendritic cells, nK-cells, neutrophils) and other types of cells (astrocytes, fibroblasts, glial cells, keratinocytes), however, the main producers are monocytes and tissue macrophages (Toroket al, 2015; and Florez-Pollack et al, 2018).

IFN-γ is synthesized by activated antigen-specific T-lymphocytes (CD4 + th1 type). Interferon-γ has a strong immunoregulatory effect and occupies one of the central places in the regulation of adaptive immune response (Florez-Pollack et al, 2018; and Suzanne, 2018). In the blood serum of patients with LS, an increase in TNFα content was observed compared with the norm, and it was most pronounced at the stage of erythema and edema of the disease by 3.9 times, its content was slightly lower at the sclerotic and atrophic stages - at 3.4 and 2.9 times, respectively, compared with the control.

Another dynamics was observed for the content of IFN-γ, in the form of a significant decrease of 2.9 times at the stage of erythema and edema, 3.5 times at the sclerotic stage and 1.6 times atrophic compared with the parameters of the group of conditionally healthy patients. Perhaps a decrease in the synthesis of IFN-γ is associated with a low content in patients with LS of their main producers of CD4 lymphocytes.

Thus, our results indicate that the leading link in the pathogenesis of LS is a significant change in the indicators of humoral and cellular immunity. In the studied stages of LS, against the background of an increased tendency of blood lymphocytes to apoptosis, there is a loss of control of T-lymphocytes in relation to B-cells, with their subsequent activation and a significant change in the quantitative and qualitative composition of the lymphocyte subpopulation. These changes are accompanied by immune disorders in the skin.Histological examination of the skin in the erythematous stage as a manifestation of acute vasculitis revealed volumetric intradermal lymphocytic infiltrates.A significant increase in the number of lymphocytes indicates the activation of the function of the mononuclear phagocyte system in response to the destruction of all elements of the dermis (Walker et al, 2017).

In the sclerotic stage of the disease, the total number of mature lymphocytes remained at the levels detected in the erythematous stage, while the number of macrophages tended to decrease.Such dynamics testified to a decrease in antigenic stimulation in the body, which was combined with the attenuation of alternative exudative processes, the beginning of the development of sclerotic processes in the papillary and reticular layers of the dermis, and partial atrophy of the skin appendages, especially hair follicles and sebaceous glands (Walker et al, 2017; and Florez-Pollack et al, 2018).A decrease in the number of IL-1 producing cells with a decrease in the number of lymphocytes indicates a subsidence of the inflammatory process in the skin and a decrease in the secretion of neutral proteases - collagenase and elastase, which stabilizes the structure of connective tissue and inhibits further collagen destruction.

Together with a decrease in the total number of T-lymphocytes and their varieties in the sclerotic stage, a significant change in the number of macrophages was detected, which was associated with the disposal and elimination of skin tissue destruction products. The decrease in the number of cells of the T-system of immunity compared with their content in the previous stage was consistent with the data of a histological study of biopsy samples of the skin of patients with LS in the sclerotic stage, when, against the background of pronounced sclerosis of the dermis, hypodermis and atrophy of the epidermis, signs of immune inflammation are either absent or weak with the exception of individual cases requiring a correct assessment of the nature of the process and the correction of conventional therapy.

In a histological examination of the atrophic stage of LS against severe sclerosis, developing hyalinosis, reduction of blood vessels and appendages of the skin, the extinction of alternative-destructive and inflammatory processes, the number of total T-lymphocytes reached low values, but did not decrease to normal values.Such dynamics of the indicators of immune homeostasis in the skin indicates that the final completion of immune inflammation in the body and skin does not occur, and therefore the patient has a chronic and relapsed disease. Thus, the results of histological analysis of the skin at the atrophic stage of OS reflect the extinction of the immune process as sclerotic and atrophic changes in the skin develop, but none of the indicators tend to normalize, and this is due to the fact that in the sclerotic, and even more so in the atrophic the stage of LS is no longer the decay products in the amount that was at the erythematous stage. The general state of histological changes in LS can be characterized as activation of the T-system of immunity, inhibition of the B-system of immunity. High rates of CD3, CD4 and CD8 from the very beginning of the disease and their weak dynamics indicate that a high activity of immune inflammation damages the walls of blood vessels with the development of plasma leakage, fibrinoid swelling and fibrinoid necrosis surrounding tissue with collagen destruction.

A significant decrease in the levels of CD4, IL-1 and IL-2 producing cells, the number of CD16 macrophages and a decrease in the immunoregulatory index are a prognostic sign of the transition to the next stage.

It should also be noted that in patients with LS in the atrophic stage, there is no normalization of all immunological parameters characterizing the absence of remission, but only clinical improvement against the background of persistent disease.

Conclusion

Depending on the stage of the inflammatory process of LS, immune disorders are accompanied by a decrease in the total amount of blood lymphocytes due to subpopulations, namely CD4 T-helper cells and CD8 T-suppressors, CD54 endotheliocyte adhesion molecule, especially in the stages of erythema and edema or sclerosis. Due to the destruction of cellular contacts of endotheliocytes, the vesselwalls thickened, develop plasma impregnation and migration of immune cells into the perivascular space and dermis, which is confirmed by histological studies at different stages of the pathological process.

The study confirmed the important role of the activation of the T-cell link of the immune system in the pathogenesis of OS development, characterized by the appearance of immune inflammation, mainly from CD4 and CD8 lymphocytes, as well as producing cells of IL-1 and IL-2, which are inflammatory mediators. Interpretation of the results of immunograms in patients with LS revealed a significant increase in the blood of pro-inflammatory - IL-1β, IL-6, TNFα, anti-inflammatory (IL-4) cytokines and macrophages CD16, B-lymphocytes CD20, activator IL-2 CD25 with a simultaneous decrease in IFN-γ, which is evidence of the activation of both T and B-systems of immunity.

LS at all its stages, accompanied by an increase in blood levels of class G and M immunoglobulins, CIC. A decrease in the level of CD8 lymphocytes contributes to an increase in the level of CICs, which damage the basement membrane, and contribute to the acquisition of damaged antigenic characteristics and production of autoantibodies and new immune complexes. The blood level of markers of the early stage of cell activation, CD25, IL-1β, IL-4, IL-6, TNFα, is a diagnostic sign of inflammatory activity in children with LS. These indicators represent highly sensitive markers in assessing immunological changes, and are also informative for the determination of the course, prognosis and further treatment.

Study Limitations: The amount of participant in the control group was less than that of main group to compare. It might have affect some of our results. Thus, further researches can be conducted with same amount of participants in each arm.

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Forensic-Medical Criteria for Evaluation of Poisoning by Alcohol Surrogates

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Abstract

Introduction: According to statistical data, fatal poisoning by alcohol and its surrogates account for more than 55% of all poisonings. Fatal poisoning by alcohol surrogates equals for about 3-5%. The number of poisonings by organic solvents and technical fluids taken orally as alcohol surrogates has risen sharply. The widespread availability of these compounds and poor public awareness of their toxic properties lead to poisoning by these substances, and the frequency of such poisoning has a strong tendency to increase.

Materials and Method: 20 archival forensic medical reports on the corpses of people who received chemical intoxication due to poisoning by alcohol surrogates. The subject of the study was the effect of poisoning by alcohol surrogates on human systems and organs. Forensic medical examination of the corpses was carried out according to the generally accepted method in the first 10-18 hours, but no later than 24 hours after death. Quantitative determination of alcohol surrogates in biological material was carried out by gas chromatographic and spectrophotometric method.

Results: The most common signs of external examination of corpses in all cases studied during the study were clearly defined bluish-violet cadaveric spots (in 100% of cases), pale pink conjunctiva (in 86% of cases), and cyanosis puffiness of the skin of the face (in 74% of cases). Meanwhile, the most significant of the microscopic signs characteristic of most of the studied cases during forensic histological examination were: plethora of vessels in the lung tissue (in 100% of cases), edema of the pia mater and brain tissue (in 86% of cases), edema and focal hemorrhages in the lung tissue (in 84% of cases).

Conclusion: Respiratory, cardiovascular systems and pancreas are most vulnerable target organs for alcohol surrogates and leads to early morbidities in people who consume chronically. The changes can be found macroscopically as well as microscopically in corpses during forensic medical examinations.

Keywords: *Alcoholism, mortality, myocarditis, gas chromatography, cadaveric spots, alcohol substitute.*

Introduction

The term “surrogate alcohol” refers to a diverse array of products. Some researchers have defined surrogate alcohol as a subcategory of “unrecorded alcohol.” In addition to the definitions above for surrogate alcohol, unrecorded alcohol includes alcoholic beverages that are illicitly obtained or made legally for home use. Nonbeverage alcohol is another term used in the literature. Acute alcohol intoxication presents with a range of clinical challenges including behavioral disinhibition, metabolic derangements, and organ compromise. All acute alcohol intoxication may present with inebriation, gastroenteritis, and central nervous system depression. General management of ethanol-based alcohol intoxication consists of thorough history taking, supportive care, airway protection, trauma assessment, and correction of dehydration and electrolyte abnormalities. (Green et al, 2018)[1].

According to statistical reports, fatal poisoning by alcohol and its surrogates account for more than 55% of all poisonings (Ruziev & Yadgarova, 2018)[2]. Fatal poisoning by alcohol surrogates accounts for about 3-5%. The number of poisonings by organic solvents and technical fluids taken orally as alcohol surrogates has risen sharply (Iskandarov & Najmitdinov, 2018).[3] Poisonings with household and industrial fluids, which, apart from ethyl alcohol, include propyl, isopropyl, isobutyl and isoamyl alcohols (primus liquid, methylated spirits, brake fluid, technical glass washing liquid, various varnishes) are observed quiet often. Propyl alcohol poisoning accounts for 29% of all surrogate poisoning, 65% for isobutyl alcohol and 6.5% for isoamyl alcohol. Among these poisonings, the percentage of deaths is especially high (Shamsiev et al, 2017)[5].

The toxic effect of methanol is mainly associated with its metabolic products (formaldehyde, and especially formic acid), which suppress the cytochrome system and oxidative phosphorylation, thereby causing ATP deficiency, especially in the brain tissue and in the retina. Butyl alcohol causes a narcotic effect, acts mainly on the central nervous system (Ruziev, 2017)[4].Many surrogatesof alcohol, for example, ethylene glycol (EG) and its esters (cellosolves; CZ), at present, widespread use both in industry, in transport and in everyday life, and in the maintenance of various technical systems (Neufeld et al, 2016; Razvodovsky, 2017; and Rehm et al, 2015)[6]. The widespread availability of these compounds and poor public awareness of their toxic properties lead to poisoning by these substances, and the frequency of such poisoning has a strong tendency to increase.

In the Republic of Uzbekistan, this topic has not been studied enough; there is lack of information on exact definitions of which organs and how these organs are affected by poisoning with alcohol surrogates.

The aim of the research was to develop modern criteria for assessing acute poisoning by alcohol surrogates.

Materials and Method

Study Population: The objects of the study were 20 archival forensic medical reports on the corpses of people who received chemical intoxication due to poisoning by alcohol surrogates. The subject of the study was the effect of poisoning by alcohol surrogates on human systems and organs. The study was conducted at the Republican Scientific and Practical Center for Forensic Medical Examination of the Ministry of Health of the Republic of Uzbekistan.

Forensic Medical Examination: Forensic medical examination of the corpses was carried out according to the generally accepted method in the first 10-18 hours, but no later than 24 hours after death. Quantitative determination of alcohol surrogates in biological material was carried out by gas chromatographic and spectrophotometric method. For forensic histological examination, pieces of internal organs were fixed in 10-12% formalin solution and enclosed in paraffin blocks. Histological sections were stained with hematoxylin-eosin. In all expert cases, alcohol substitution poisoning was confirmed by case materials, the clinical picture of poisoning, the results of a forensic medical examination of a corpse, data from a toxicological laboratory, forensic chemical analysis and histological examination. The objects of toxicological analysis were the internal organs of the corpse, biological fluids, gastric lavage, vomit, as well as fluid residues in various containers found at the scene.

Results

Out of 20 materials, 12 men and 8 women aged from 30 to 50 years. Analysis of the distribution of victims by gender yielded the following results (Figure 1):

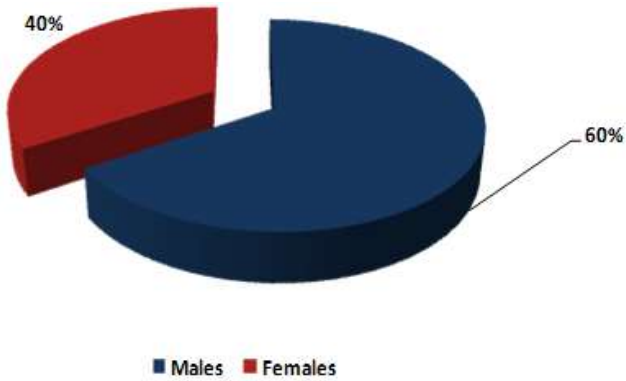


Fig. 1. Distribution of victims poisoned by alcohol surrogates by gender

The study of the results of an external study of corpses based on the materials of forensic medical examinations in cases of acute poisoning by alcohol surrogates allowed us to highlight some signs characteristic of a number of cases studied during the research. The frequency of occurrence of these signs is presented in table 1.

Table 1: The frequency of occurrence of signs characteristic for a number of cases of acute poisoning by alcohol surrogateslearned in an external study of a corpse

Signs	Absolute number of cases	Proportion of cases (%)
Cadaverous spots of cyanotic purple, well defined	20	100,0
Pale pink conjunctiva	17	86,0
Cyanosis and puffiness of the skin of the face	19	74,0
Mucus in the nasal passages and oral cavity	2	8,0
Exophthalmos	1	6,0

As it is shown in the table above, the most common signs of external examination of corpses in all cases studied during the study were clearly defined bluish-violet cadaveric spots (in 100% of cases), pale pink conjunctiva (in 86% of cases), and cyanosis puffiness of the skin of the face (in 74% of cases). Data on the occurrence of such signs as mucus in the nasal passages and oral cavity (in 8% of cases) and exophthalmos (in 6% of cases) in the materials of the study are not so significant.

An analysis of the results of forensic histological studies showed that in the microscopic picture of changes in acute poisoning with alcohol surrogates, some indicators characteristic of a number of cases studied can also be distinguished. The frequency of occurrence of these indicators is shown in table 2.

Table 2: The frequency of occurrence of microscopic changes characteristic of a number of cases of acute poisoning by alcohol surrogates studied in a forensic histological study

Signs	Absolute number of cases	Proportion of cases (%)
Edema of the pia mater and brain tissue	17	86,0
Vascular congestion in lung tissue	20	100,0
Perivascular hemorrhages in the tissues of the pia mater and brain	7	36,0
Edema and focal hemorrhage in lung tissue	17	84,0
Cardiosclerosis	15	76,0
Sclerosis of the blood vessels of the heart	14	68,0
Unbalanced pulp plethora in spleen tissues	8	42,0
Sclerosis of trabeculae and trabecular vessels in the tissues of the spleen	8	42,0
The process of pancreatic autolysis	14	68,0
Pancreatic hemorrhage	5	26,0
Fatty hepatosis	10	50,0
Dystrophic changes in hepatocytes	7	34,0
Dystrophic changes in the renal tubules	14	68,0
Uneven blood vessels in the kidney tissue	12	60,0

The data presented in the table shows that the most significant of the microscopic signs characteristic of most of the studied cases during forensic histological examination were: plethora of vessels in the lung tissue (in 100% of cases), edema of the pia mater and brain tissue (in 86% of cases), edema and focal hemorrhages in the lung tissue (in 84% of cases), cardiosclerosis (in 76% of cases), sclerosis of the blood vessels of the heart (in

68% of cases), the process of autolysis in the pancreas (in 68% of cases), dystrophic changes in the kidneys tubules (in 68% of cases) and uneven blood supply vessels in kidney tissues (60% of cases). Less significant were fatty hepatosis (in 50% of cases), uneven pulmonary congestion in spleen tissues (in 42% of cases), and sclerosis of trabeculae and trabecular vessels in spleen tissues (in 26% of cases). The least significant signs were signs of perivascular hemorrhage in the tissues of the pia mater and brain (in 36% of cases), dystrophic changes in hepatocytes (in 36% of cases), hemorrhage in the tissues of the pancreas (in 36% of cases). The figures given in these tables are characteristic for changes caused by chronic alcohol dependence, which indicates that the victims, in all studied cases of poisoning by alcohol surrogates, suffered from chronic alcoholism[10].

However, during the analysis of archival data, specific indicators were also found that were found in cases of poisoning with certain alcohol surrogates. In 6 cases of ethylene glycol poisoning, an external examination of the bodies of victims of acute ethylene glycol poisoning showed cyanosis of the skin and mucous membranes, as well as pinpoint hemorrhages on hyperemic conjunctiva of the eyes[11].

In an internal study of corpses, vascular endothelial damage, venous congestion of organs, hemorrhages in the pleura, epicardium, endocardium and gastric mucosa were observed[12]. The cyanosis of the membranes of the brain was detected. Expansion of the right heart regions and their overflow with blood were revealed, uneven blood filling and myocardial edema were noted. Puffiness and hyperemia of the mucous membranes of the upper respiratory tract, single subpleural and intrapulmonary hemorrhages, plethora, emphysema and pulmonary edema, swelling and plethora of the gastric mucosa with single small hemorrhages were observed[14]. An increase and swelling of the kidneys, tension of the capsule were detected. The tissue of the kidneys in the incision was cyanotic.

A study of the results of clinical and laboratory studies in cases of poisoning with alcohol surrogates showed that the most effective method for their determination are toxicological analysis and gas-liquid chromatography. The analysis of the results of chemical-toxicological studies allowed to distribute the victims of poisoning with alcohol surrogates as follows (Table 3.):

Table 3: Distribution of victims by identified alcohol surrogates

Alcohol surrogates	Number of cases	
	Abs	%
Ethylene glycol	2	10%
Methyl alcohol	3	15%
Amyl alcohol	1	5%
Chloral hydrate	1	5%
Dichloroethane	2	10%
Tetraethyl lead	1	5%
Propyl alcohol	7	14%
Butyl alcohol	1	5%
Acetone	2	10%

As the data in this table show, 4 detoxification method were used in victims of acute poisoning by alcohol surrogates. Moreover, hemodialysis was carried out in all cases of poisoning, hemosorption in 58% of cases, enterosorption in 36% of cases, and forced diuresis in only 14% of cases. This choice of detoxification method is explained, first of all, by the features of these method.

Among the microscopic signs, the most significant ones were: vascular congestion in the lung tissue, edema of the pia mater and brain tissue, edema and focal hemorrhage in the lung tissue, cardiosclerosis, sclerosis of the blood vessels of the heart, autolysis process in the pancreas, dystrophic changes in the renal tubules and uneven blood vessels in the tissues of the kidneys. In addition, specific signs and morphological features of poisoning by each of the alcohol surrogates were revealed.

Discussion

According to data we obtained during our study, cadaverous spots of cyanotic purple, pale pink conjunctiva and cyanosis and puffiness of the skin of the face are the most common macroscopic signs in corpuses died from unrecorded alcohol surrogates. Furthermore, the most significant of the microscopic signs characteristic of most of the studied cases during forensic histological examination were: plethora of vessels in the lung tissue, edema of the pia mater and brain tissue, edema and focal hemorrhages in the lung tissue, cardiosclerosis, sclerosis of the blood vessels of the heart, the process of autolysis in the pancreas, dystrophic changes in the kidneys tubules and uneven blood supply vessels in kidney tissues.

One clear example is that the index of lethal poisoning with ethyl alcohol and its surrogates in Russia remains one of the highest in the world. It is explained by high alcohol consumption in Russia (about 15 liters of pure alcohol per capita annually), consumption of mainly strong beverages (about 80%), extensive sales of adulterated strong alcoholic beverages (more than 25%), virtually no preventive work in the population, and other causes. The index of lethal poisoning with alcohol and its surrogates in the country and its regions is one of the most important parameters in evaluation of the socioeconomic well-being of the population (Tomilin et al, 1999)[7].

Unrecorded alcohol includes illegally distributed alcohol as well as homemade or surrogate alcohol which is unintended for consumption by humans (e.g., cosmetics containing alcohol). The highest unrecorded alcohol consumption occurs in Eastern Europe and some of these countries have an over proportional liver cirrhosis mortality. Compounds besides ethanol have been hypothesized as being responsible for this observation. On the other hand, chemical investigations were unable to prove that unrecorded alcohol regularly contains contaminants above toxicological thresholds (Lachenmeier et al, 2014)[8].

However, illegally produced spirits regularly contain higher percentages of alcohol (above 45% by volume), but for considerably less costs compared with licit beverages, potentially causing more problematic patterns of drinking. In this review, it is investigated whether patterns of drinking rather than product composition can explain the liver cirrhosis mortality rates.

Statistical examination of World Health Organization country data shows that the originally detected correlation of the percentage of unrecorded alcohol consumption and liver cirrhosis mortality rates disappears when the data is adjusted for the prevalence of heavy episodic drinking. It may be concluded that there is currently a lack of data to demonstrate causality between the composition of illicit spirits (e.g., higher levels of certain contaminants in home-produced products) and liver toxicity on a population scale. Exceptions may be cases of poisoning with antiseptic liquids containing compounds such as polyhexamethyleneguanidine, which were reported to be consumed as surrogate alcohol in Russia, leading to an outbreak of acute cholestatic liver injury, histologically different from conventional alcoholic liver disease (Lachenmeier et al, 2014)[9].

Conclusion

Among the microscopic signs, the most significant were: vascular congestion in the lung tissue, edema of the pia mater and brain tissue, edema and focal hemorrhage in the lung tissue, cardiosclerosis, sclerosis of the blood vessels of the heart, autolysis process in the pancreas, dystrophic changes in the renal tubules and uneven blood vessels in the tissues of the kidneys.

If morphological or histological changes characteristic of poisoning by surrogates of alcohol are detected, it is necessary to conduct comprehensive forensic toxicological studies of corpses.

Study Limitations: The amount of participant in both arms was less to prove the hypothesis. It might have affect some of our results. Furthermore, the type of surrogate was not clear in all cases. Some corpses did not have any relatives who might be asked for elucidation of the cases. Thus, further researches can be conducted with more amount of participants in each arm.

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Ethical Approval: The ethical approval for the study was granted by the Committee of Ethical Approval for Researches under the Ministry of Health of the Republic of Uzbekistan.

Consent: Written informed consent was obtained from the relatives of corpses of the research for publication of this paper and any accompanying information related to this study.

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Nephroprotective Effects of 2nd Type Sodium-Glucose Co-Transporter Inhibitors: A Literature Review

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Abstract

Introduction: A wide range of oral hypoglycemic drugs with various mechanisms of effect are available for the treatment of patients with 2nd type diabetes mellitus today. Despite the high effectiveness of these agents in the normalization of glycemia, they cause different chronic complications in many patients with 2nd type diabetes mellitus. However, during the last years, a range of principal new data has been accumulated on the pleiotropic effects of the last classes of oral hypoglycemic agents — inhibitors of the 2nd type sodium-glucose cotransporter (SGLT2).

Materials and Method: Three electronic databases were checked to find out studies reporting the data on the mechanisms and clinical significance of the nephroprotective effect of inhibitors of the sodium-glucose cotransporter of 2nd type (glyphlozines) in patients with 2nd type diabetes mellitus. The primary outcome was to learn the worldwide tendency on this matter and to compare the results of different researchers.

Results: We found 25 studies as eligible for our review. The review presents data on the mechanisms and clinical significance of the nephroprotective effect of inhibitors of the sodium-glucose cotransporter of 2nd type (glyphlozines). The effects associated with the influence of representatives of this pharmacological group on the glomerular filtration rate, glycemia, diuresis, ketogenesis and other factors are discussed. The results of recent experimental and clinical studies aimed at studying certain aspects of the nephroprotective effect of SGLT2 inhibitors in 2nd type diabetes mellitus and other pathological conditions were analyzed.

Conclusion. SGLT2 inhibitors associate with a multicomponent nephroprotective effect, which confirmed by the results of both experimental and clinical studies. The nephroprotective potential of SGLT2 inhibitors is maximally realized in the presence of diabetes and remains not entirely clear in the case of another kidney pathology.

Keywords: *Diabetes mellitus, oral hypoglycemic drugs, 2nd type sodium-glucose cotransporter inhibitors, chronic kidney disease, diabetic nephropathy, nephroprotection.*

Introduction

Currently, a large number of oral hypoglycemic agents (OHAs) with various mechanisms of action are available for the treatment of patients with 2nd type diabetes mellitus (Thrasher, 2017)[1]. In spite of high effectiveness of OHA for normalizing glycemia,

in a significant proportion of patients with 2nd type diabetes chronic complications may develop[2]. Namely, the most unfavorable prognostic value associate with diabetic nephropathy and cardiovascular complications (Demidova, 2016)[1]. Thus, all modern drugs for the treatment of 2nd type diabetes are examined for their nephron- and cardioprotective properties (Muskiet et al, 2015; Bandanna et al, 2016)[2,3]. In recent years, a lot of principal novel data has been collected on the pleiotropic effects of representatives of the last classes of OHA — inhibitors of the 2nd type sodium-glucose cotransporter (SGLT2) (Vallianou et al, 2016; and Satoh, 2018)[3].

The main contribution of the kidneys to the regulation of carbohydrate metabolism is due to the processes of renal reabsorption of glucose[4]. Normally, in a one day, about 180 g of glucose is filtered through the kidneys (Maltese et al, 2015)[5].Reabsorption of glucose from the lumen of the renal tubule is provided by the carrier proteins (sodium glucose cotransporters) family of SGLT, which carry out active glucose transport[6], coupled with sodium, into the tubule cell[7]. Further, carrier proteins of the GLUT family located on the basolateral membrane, passively transport glucose from the cell to the interstitial space according to its concentration gradient (Prie, 2014)[8]. Almost 90% of glucose is reabsorbed in the S1 segment of the proximal convoluted renal tubule with the participation of SGLT2transporters[9], which have low affinity but very high capacity[10]. About 10% of glucose is absorbed more distally - in the S3 tubule segment using SGLT1 transporters, which, on the contrary, are characterized by high affinity, but low capacity.[11]

The combination of sequential effects of these transporters ensures the achievement of aglucosuria in healthy people.[12] The kidneys are the main location for SGLT2transporters.[13] The presence of a mutation in the SLC5A2 gene encoding these transporters leads to the development of a hereditary disease that occurs with a frequency of up to 0.3% in the family - renal glucosuria population (Vallon, 2015)[6],[14]. In familial renal glucosuria[15], in the absence of carbohydrate metabolism disorders and signs of tubular dysfunction[16], constant glucosuria is observed, which may exceed 100 mg/day.[17] In most patients, the disease proceeds without severe clinical symptoms, and renal function does not change[18].

Purpose of the studyis to learn the current literature data on the functional mechanisms and clinical significance of the nephroprotective effect of inhibitors of the sodium-glucose cotransporter of 2nd type[19], to investigate the clinical results and outcomes of treatment with hypoglycemic drugs in patients with 2nd type diabetes mellitus[20].

Materials and Method

Protocol and registration: The review was conducted following the guidelines set by the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Moher et al, 2009)[21].

Eligibility and exclusion criteria: Both primary and secondary studies were included for this review. We applied 10-year time filter (from 2010 to 2020)[22] for eligible studies. Only English papers were included. The review covered both prospective as well as retrospective data analyses. We did not used any restrictions regarding the place of publication[23].

Search Strategy: Three medical electronic databases (PubMed, EMBASE and Cochrane) were searched to find the studies reporting the conservative (influence of medicaments) effects on the renal function in patients with diabetes mellitus[24]. We applied 10-year time filter for eligible studies[25]. In the search strategy techniques on the web sites mentioned above, we used the following key words:

Diabetes mellitus, oral hypoglycemic drugs, 2nd type sodium-glucose cotransporter inhibitors, chronic kidney disease, diabetic nephropathy, nephroprotection.

Study Selection: Titles and abstracts of the papers screened by the authors of the review using the search method mentioned above in order to identify the studies that match the inclusion criteria[26]. Found items were searched and compared again for duplicate papers, deleted one were found any similar studies.[27]Eligible studies were checked to find any more interesting sources for our review. As soon as we found any interesting paper that suitable for our criteria of eligibility, full texts the study were reached and real till the end. Any disagreements between reviewers, resolved by discussion.

RESULTS

Study Selection: Overall, 1224 items found searching through electronic databases outlined above. Double checked the records obtained reviewing the titles and abstracts.[28] Next, we identified 450 identical studies that were deleted after wards.[29] Then, remaining 382

records were screened excluding 296 findings owing to different reasons. Further, the titles of 114 studies were read, 72 articles found as potentially eligible, which obtained in full text.[30] All full text articles were learnt by the authors of the review. Finally, we included 25 studies to our review. Owing to the colorful style, method and outcomes of the studies (heterogeneity), we did not perform meta-analysis.[31]

Study Characteristics: It was established that one of the mechanisms of regulation of SGLT2 expression is autocrine: pro-inflammatory cytokines (interleukin-6 and alpha tumor necrosis factor) produced by renal epithelial cells are capable of increasing SGLT2 expression by the same type of cells (Fig. 1.) (Maldonado-Cervantes et al, 2012).[32]

In another study, conducted using proximal tubules of human kidney cells, it was shown that up-regulation of SGLT2 does not occur directly under the influence of elevated glucose concentrations, but as a result of exposure to these cells transforming growth factor beta, overproduction of which is characteristic for diabetic kidney damage (Panchapakesan et al, 2013)[33].

A large number of studies have proved that inhibition of SGLT2 leads to a wide range of physiological changes that are potentially favorable for 2nd type diabetes[11]. The main direct and indirect renal effects of SGLT2 inhibitors in 2nd type diabetes are summarized in Fig. 2. Below, based on an analysis of the results of key experimental and clinical studies of recent years, certain aspects of the nephroprotective effect of SGLT2 inhibitors are discussed[34].

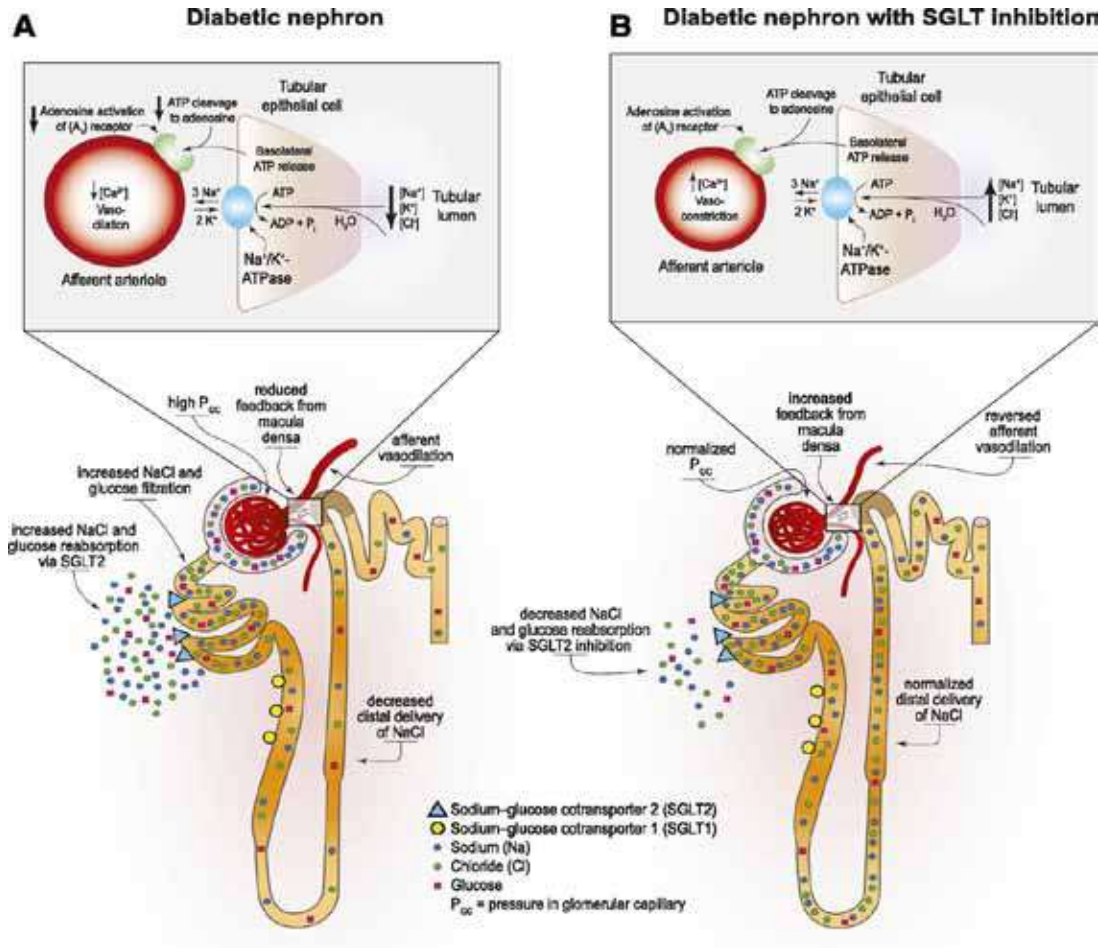


Fig. 1. The effect of diabetes mellitus and inhibition of SGLT2 on the hemodynamics of nephron. A: Increased glucose reabsorption with SGLT2 in the proximal tortuous tubule reduces the delivery of solutes to the dense spot. As a result, the release of ATP from the basolateral membrane of the tubular epithelial cells is reduced, the production of adenosine is reduced and the vessels of the afferent arteriole dilate. B. SGLT2 inhibitors restore the delivery of solute to a tight spot, followed by activation of adenosine and prevention of vasodilation of the afferent arteriole (Maldonado-Cervantes et al, 2012).

In the first experimental studies aimed at studying the nonprotective properties of the inhibition of the sodium-glucose cotransporter, florizin, a competitive inhibitor of SGLT1 and SGLT2 of natural origin, was not suitable for use in clinical practice owing to the presence of pronounced side effects (Vallon & Thomson, 2017)[35].

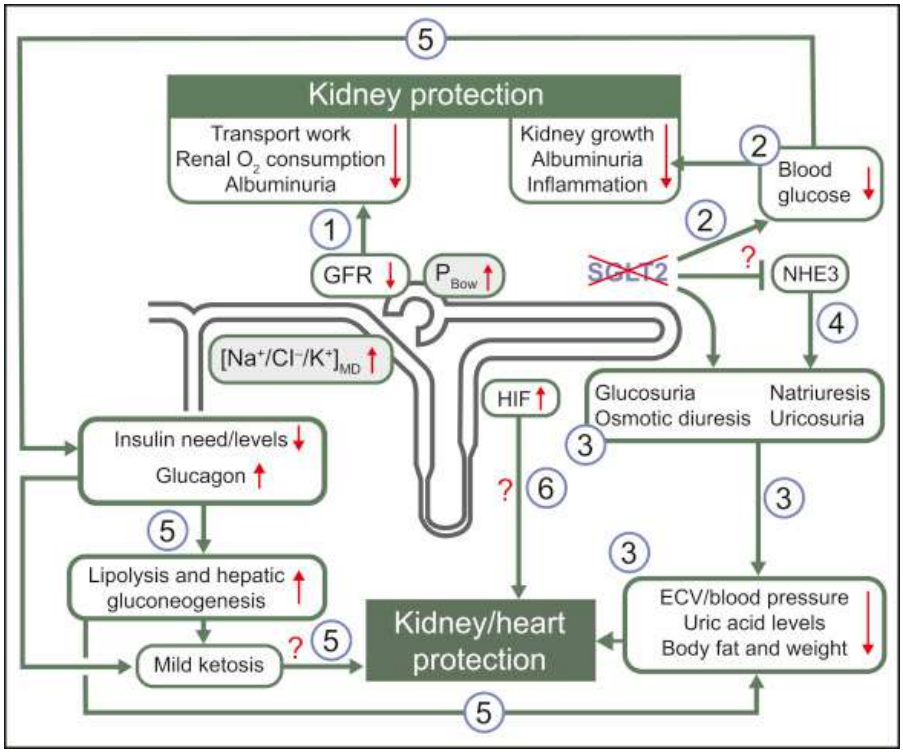


Fig. 2. The main mechanisms of the nephroprotective action of SGLT2 inhibitors (Vallon & Thompson, 2017) (1) A decrease in glucose and sodium reabsorption in the prochymal tubules leads to an increase in pressure in the Bowman space (BPM), and a decrease in glomerular filtration rate (GFR), and also affects the function of dense spot cells of the juxtaglomerular apparatus. [2] By normalizing blood glucose levels, SGLT2 inhibitors indirectly block the increase in the level of glycation end products, the overproduction of pro-inflammatory cytokines and other negative effects of hyperglycemia.[3] SGLT2 inhibitors have a diuretic, natriuretic and uricosuric effect, which helps to reduce extracellular fluid volume, blood pressure, uric acid level in the blood and body weight.[4] It is suggested that there is a relationship between the activity of the 2nd type sodium glucose cotransporter and the sodium hydrogen ion exchange protein (NHE3), which may have an additional beneficial effect on blood pressure.[5] A decrease in insulin demand and an increase in glucagon levels lead to the activation of lipolysis and gluconeogenesis and, as a result, to a slight increase in the level of ketones, which can have cardio- and nephroprotective effects.[6] Inhibition of SGLT2 may increase the production of renal hypoxia-induced factor (HIF), which has nephroprotective properties.

In a study by Malatiali et al. (2015)[14,36], florizin has been shown to reduce the severity of glomerular hyperfiltration in rats with streptozotocin-induced diabetes. The research conducted by Osorio et al. (2012)[16]proved a decrease in oxidative stress using the same model of diabetes[37]. In a study conducted on rat mesangial cells, the addition of Florizin blocked the resizing of these cells when they are incubated in a solution with a high glucose content (Wakisaka et al, 2016)[38].

Most studies conducted on various genetic and non-genetic models of diabetic kidney damage using modern synthetic SGLT2 inhibitors (empagliflosin, dapagliflosin, luseogliflosin, tofogliflosin) also confirmed the presence of pronounced nephroprotective properties in this pharmacologicalgroup.[39] It was shown in BTBRob/ob mice that the use of empagliflozin was associated with a decrease in the level of albuminuria and expression of inflammatory markers, a decrease in glomerular hypertrophy, and expansion of the mesangial

matrix, however, when these laboratory animals were administered angiotensin II, only the antialbuminuric effect was preserved (Gembardt et al, 2014)[40],[17]. In a study on db/db mice, the administration of empagliflozin reduced the expression of fibronectin and transforming growth factor beta genes[18], but did not affect the level of albuminuria and urinary excretion of markers of tubular damage KIM-1 and neutrophilic gelatinase-associated lipocalin (NGAL) (Gallo et al, 2016)[19,41]. In a study where authors used of empagliflozin (unlike telmisartan) did not have a nephroprotective effect on mice with knockout of the endothelial nitric oxide synthase (eNOS) gene, in which diabetes was induced by the administration of low doses of streptozotocin (GangadharanKomala et al, 2014)[42].

According to Ojima et al. (2015)[43], the use of empagliflozin in rats with streptozotocin-induced diabetes reduced the expression of the receptor for glycation end products, as well as decreased urinary excretion of the oxidative stress marker 8-OH-deoxyguanosine and the tubular lesion marker L-FABP (hepatic protein that bindsfatty acid)[22]. Moreover, in this study of relatively short duration (4 weeks), the antialbuminuric effect of SGLT2 inhibition was not observed[44].

Another SGLT2 inhibitor, dapagliflozin, also showed pronounced nephroprotective properties in a study on db/db mice. In particular, there was a decrease in kidney infiltration by macrophages, suppression of mesangial expansion and interstitial fibrosis, and a decrease in albuminuria. At the same time, creatinine clearance and blood pressure did not undergo significant changes (Terami et al, 2014)[45].

In a study on Akita mice (type 1 diabetes model), the effects of dapagliflozin and insulin therapy were compared[25]. After 12 weeks of treatment, no significant intergroup differences were observed in glycemia, HbA1c, body weight and blood pressure, but diuresis and fluid intake were higher in the group treated with the SGLT2 inhibitor. In mice treated with dapagliflozin, morphological changes in the kidneys (macrophage infiltration, interstitial fibrosis) were less pronounced than in animals that were on insulin therapy. When studying the proximal tubule cell culture, a decrease in the severity of oxidative stress under the influence of dapagliflozin was also demonstrated (Hatanaka et al, 2016)[26].

Another comparative study that compared the nephroprotective effects of tofogliflosin and losartan was conducted on db/dbline mice. It was shown that both tofogliflozin and losartan can reduce the level of albuminuria by 50-70%, however, only the SGLT2 inhibitor reduced the severity of glomerular hypertrophy (Nagata et al, 2013)[27].Most researchers agree that a decrease in the level of albuminuria in experimental studies is observed when using high doses of SGLT2 inhibitors, while other effects (antifibrotic, anti-inflammatory, inhibition of oxidative stress) are manifested in a wider range of doses (Kawanami et al, 2017)[28].

In a comparative study conducted using a non-genetic model of rats with insulin resistance against a high-fat diet, it was shown that the SGLT2 inhibitor dapagliflozin has a more pronounced nephroprotective potential than metformin (Jaikumkao et al, 2018)[29].

A study of combination nephroprotective therapy on a model of streptozotocin-induced diabetes in salt-sensitive rats (Dahl S line) showed that the use of the SGLT2 inhibitor luseoglyphlozin in combination with the ACE inhibitor lisinopril is associated with more pronounced beneficial effects (hypotensive effect, decrease in renal hyperfiltration) than the appointment of any of these drugs separately (Kojima et al, 2015)[30].

Gallo et al. (2016)[31]showed that the use of empagliflozin in conjunction with metformin has the advantage of influencing the synthesis of type IV collagen and connective tissue growth factor in the kidneys[32].In recent years, the effectiveness and safety of SGLT2 inhibitors in chronic kidney disease with a significant reduction in glomerular filtration has been actively studied.[33]

In a study completed by L. Li et al. (2018)[34], it was demonstrated that inhibition of SGLT2 in rats undergoing subtotal nephrectomy (without diabetes) does not lead to intrarenal and systemic activation of the renin-angiotensin-aldosterone system, which, according to several researchers, may be a side effect of this pharmacological effect in case of development of hyponatremia against the background of severe renal pathology[35].

Use of dapagliflozin in rats undergoing subtotal nephrectomy is not associated with a decrease in the expression of profibrotic cytokines in the kidneys and a slower progression of nephrosclerosis (Zang et al, 2016)[36]. With a less significant decrease in the volume of functioning renal tissue against the background of

diabetes, the nephroprotective properties of SGLT2 inhibitors are preserved, which was demonstrated in mice with 2nd type diabetes KK/Ay line undergoing unilateral nephroectomy(Tahara & Takasu, 2018)[37].

In a study of the effects of SGLT2 inhibition in animals with chronic kidney disease induced by a diet containing excessive amounts of oxalates, dapagliflozin did not show beneficial renal effects[38].

Thus, the presence of nephroprotective properties in this class of drugs with non-diabetic kidney damage and their practical significance are currently in doubt (Ma et al, 2017)[39].

Nevertheless, experimentally confirmed the presence of nephroprotective effects in SGLT2 inhibitors mediated by their effect on the expression of hypoxia inducible factor (HIF-1). Corresponding data were obtained on a model of ischemia-reperfusion of kidneys in mice (Chang et al, 2016).[40] Other authors have shown that under hypoxic conditions, the expression of SGLT1 and SGLT2 by tubule epithelial cells decreases (Zapata-Morales et al, 2014).[41]

Less studied is the question of possible organoprotective and, in particular, nephroprotective effects associated with the accumulation of ketogenesis products, which is activated against the background of SGLT2 inhibition.In an experimental study conducted by T[42]. Shimazu et al., It was shown that the administration of one of these metabolites, beta-hydroxybutyrate, to mice is associated with a decrease in the expression of oxidative stress and lipid peroxidation markers in the kidney after intravenous injection of paraquat (Shimazu eta al, 2013)[43]. In addition, there are experimentalstrong evidence of the use of ketone bodies by the kidneys as an energy substrate. Under conditions of ketonemia, their utilization increases sharply, which inhibits the uptake of other substrates by the kidneys and reduces oxygen consumption (Guder et al, 1986).[44]

One of the largest clinical studies that examined the nephroprotective effects of SGLT2 inhibitors is the EMPA-REG OUTCOME study. In this study, the nephroprotective effect of empagliflozin was manifested not only when it was prescribed at a maximum dose of 25 mg per day, but also when using a dose of 10 mg per day. Adding empagliflozin to standard therapy (about 80% of patients received ACE inhibitors or angiotensin receptor blockers) was associated with a 38% reduction in the risk of nephropathy progression with macroalbuminuria, a 44% increase in serum creatinine, and a 55% increase in renal replacement therapy. At the same time, SGLT2 inhibitor therapy did not reduce the likelihood of microalbuminuria in patients, which was a common event both in the main group and in the placebo group (51.5% and 51.2%, respectively).[45]

At the initial stage of empagliflozin therapy, predictable decrease was observedin GFR, however, in the future the GFR value in the main group remained relatively stable (decrease by 0.19 ± 0.11 ml/min/1.73 m^2 per year), while in the placebo group, a more pronounced decrease in GFR (by 1.67 ± 0.13 ml/min/1.73 m^2 per year).

In addition, after the cancellation of empagliflozin, an increase in GFR was observed, which further increased the intergroup differences in this indicator (Wanner et al, 2016)[46]. In addition to the obvious mechanisms of cardio- and nephroprotective effects of empagliflozin, clearly demonstrated in EMPA-REG OUTCOME, the possible clinical significance of enhancing ketogenesis and the role of the corresponding energy substrates are discussed in the scientific literature (Fig. 3) (Mudaliar et al, 2016).[47]

The CANVAS study examined the effect of the canagliflozin SGLT2 inhibitor on cardiovascular and renal outcomes in 2nd type diabetes. The results of the study showed that the addition of canagliflozin to standard therapy was associated with a 47% reduction in the risk of reaching a combined endpoint, which included a steady 2-fold increase in serum creatinine concentration, the development of the terminal stage of chronic kidney disease, and death from renal causes. In the placebo group, the average annual decrease in GFR was 1.2 ml/min/1.73 m^2 more. The mean urine albumin / creatinine ratio in the group of patients receiving canagliflozin was 18% lower (Perkovic et al, 2018).[48]

In the DECLARE-TIMI study, the administration of dapagliflozin to 58 patients with 2nd type diabetes in addition to standard therapy was associated with a 24% reduction in the risk of reaching a combined endpoint that included a sustained decrease in GFR of at least 40% to below 60 ml/min/1,73 m^2, the development of the terminal stage of chronic kidney disease and death from renal or cardiovascular causes (Wiviott et al, 2018).[49]

The nephroprotective effect of SGLT2 inhibitors in 2nd type diabetes is considered a class effect, which is indirectly confirmed by the results of the above studies and some secondary analyzes (Clegg et al, 2019).[50]

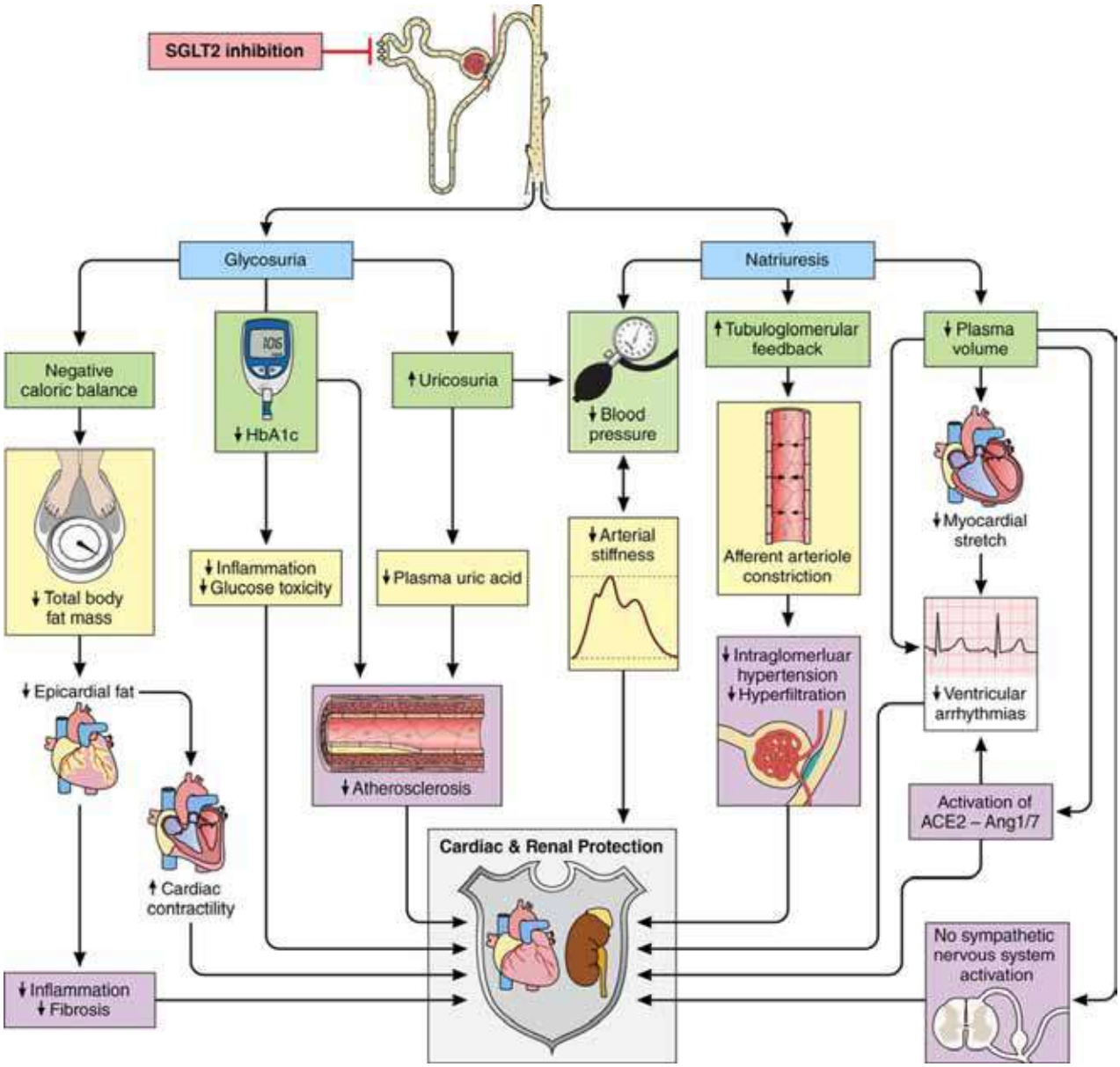


Fig. 3. Physiological mechanisms involved in cardio and nephroprotection during inhibition of SGLT2. (Mudaliar et al, 2016)

There is little data on the ratio of the nephroprotective effects of various representatives of this pharmacological group, and they were obtained mainly using indirect comparisons. In a large meta-analysis, summarizing the treatment results of more than 39 000 patients from 58 studies, the superiority of empagliflozin was indirectly confirmed. In patients on dapagliflozin therapy, according to the results of this work, the relative risk of adverse renal outcomes was higher than in patients taking placebo. In addition, empagliflozin was the only inhibitorSGLT2, which can reduce the risk of acute kidney damage compared with placebo (Tang et al, 2017).According to the results of the research conducted by Cherney et al. (2014), prescribed empagliflozin to patients with type 1 diabetes with renal hyperfiltration, lead to a decrease in serum nitric oxide concentration, an increase in renal vascular resistance, and a decrease in effective renal plasma flow and GFR. These changes were recorded both in hyper- and in normoglycemia. At the same time, these parameters did not significantly change with the use of empagliflozin in patients with type 1 diabetes and normal GFR values.

A secondary analysis of the results of this study with the calculation of the indicators of intrarenal hemodynamics according to the Gomez (1951) method showed that in patients with hyperfiltration even with normal systemic blood pressure and normoalbuminuria there was observed intrapulmonary hypertension, which decreased during treatment with empagliflozin due to a change in the resistance of afferent renal (Skrtic et al, 2014)[42].

When analyzing the effect of the dopagliflozin SGLT2 inhibitor on the excretion of markers of inflammation and damage to the tubules of the kidneys, the ability of this drug to reduce the excretion of the kidney damage molecule KIM-1 and interleukin-6 with urine was established. At the same time, the excretion of other markers (monocytic chemoattractant protein 1, neutrophilic gelatinase-associated lipocalin, and the hepatic form of the protein binding fatty acids) did not change (Dekkers et al, 2018)[43].

Researchers pay much attention to the study of the efficacy and safety of SGLT2 inhibitors in patients with CKD with a decrease in GFR. The EMPA-REG OUTCOME study demonstrated the beneficial renal effects of empagliflozin in 2nd type diabetes, both in patients with GFR ≥60 ml/min/1.73 m^2 and in patients with GFR 30-59 ml/min/1.73 m^2(Wanner et al, 2016)[34]. The DERIVE study evaluated the efficacy and safety of dapagliflozin in patients with 2nd type diabetes and stage 3 CKD. The use of dapagliflozin in this group of patients was associated with a statistically significant decrease in glycated hemoglobin, body mass index and systolic blood pressure without an increased risk of adverse events (Fioretto et al, 2014)[44].

In a meta-analysis that included 11 clinical trials, it was shown that the effect of dapagliflozin on hematocrit, body weight, and systolic blood pressure does not depend on the initial values of GFR. In addition, a more pronounced decrease in albuminuria occurs in patients with the lowest GFR (Petrykiv et al, 2017).[49]

Discussion

The review of the current literature on diabetic nephropathy showed that SGLT2 inhibitors were characterized with a multicomponent nephroprotective effect and it was confirmed by the results of both experimental and clinical researches. The renal influence of this pharmacological group is associated with many pathogenetic changes in diabetic nephropathy. Thus, the nephroprotective effect of SGLT2 inhibitors is maximally realized in the presence of diabetes and remains not full clear in the case of other kidney pathologies.

Of considerable interest is the study of the nephroprotective effects of combination therapy, including SGLT2 inhibitors. A RACELINES study is currently underway to study the renal effects of a combination of empagliflozin and a dipeptidyl peptidase-4 linagliptin inhibitor. The design of the study involves the addition of metformin in the first group - empagliflozin for 8 weeks, followed by the transition to the combination of empagliflozin + linagliptin, in the second group - linagliptin for 8 weeks, followed by the transition to the combination of empagliflozin + linagliptin, in the control group - followed by an increase in its glycoslid doses after 8 weeks. Based on the results, it is planned to evaluate the effect of empagliflozin and linagliptin on GFR, the excretion of albumin and electrolytes, urine pH, body mass index, pancreatic beta cell function, and biochemical parameters (US National Library of Medicine Clinical Trials Database). The safety of the combination of empagliflozin and linagliptin was demonstrated in a previous study (Lewin et al, 2015).[47]

Experience in studying the renal effects of SGLT2 inhibitors in non-diabetic patients is currently extremely limited. A study is currently being conducted in which, using the method of functional magnetic resonance imaging, the effect of empagliflozin on the oxygenation of the kidney tissue of healthy volunteers with different body mass index is evaluated (Muller et al, 2018).[48]studied the effects of dapagliflozin administration in patients with focal segmental glomerulosclerosis for 8 weeks and found no significant effect on renal hemodynamics and proteinuria.

The publication of the results of a placebo-controlled study RECEDE-CHF, dedicated to the study of the effects of adding empagliflozin to therapy with loop diuretics in a group of patients withheart failure. In addition to the effect on diuresis, the study plans to evaluate changes in the level of cystatin C, GFR, sodium and albumin excretion in the urine during combination therapy (Mordi et al, 2017).[50]

Conclusion

Thus, SGLT2 inhibitors are characterized by the presence of a multicomponent nephroprotective effect, which is confirmed by the results of both experimental and clinical studies. The renal effects of representatives

of this pharmacological group have points of contact with a large number of links in the pathogenesis of diabetic nephropathy, and therefore the nephroprotective potential of SGLT2 inhibitors is maximally realized in the presence of diabetes and remains not entirely clear in the case of another kidney pathology. Currently, the greatest scientific and practical studies are devoted to the comparative effectiveness of various SGLT2 inhibitors in nephroprotection, as well as the possibilities of personalizing treatment and combination pharmacotherapy.

Study Limitations: The review did not include the quality assessment and risk of bias within included studies. This might affect the general outcomes as well as conclusion of this review. Thus, further literature review and meta-analyses on this topic can be conducted with the quality assessment.

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Assessment of Hearing in Children with Cerebral Palsy

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Abstract

Introduction: The objective of this study is to identify the prevalence and nature of auditory analyzer pathology in children with different forms of infant cerebral palsy by recording otoacoustic emission and recording short latency brainstem auditory evoked potentials.

Materials and Method: The subject group consisted of 75 children, 40 (53%) males and 35 (47%) were females, ranging from 3 to 14 years of age with a confirmed diagnosis of cerebral palsy on the basis of a psycho-neurological hospital in Tashkent. (Uzbekistan). The examination of children was carried out by the following method: clinical examination of patients (general and otorhinolaryngological) and functional research method of the auditory analyzer.

Results: All children underwent acoustic impedancemetry, including tympanometry with determination of the type of tympanogram and acoustic reflex. The data obtained revealed a bilateral lesion of the peripheral part of the auditory analyzer. Beilateral sensorineural hearing loss was detected in 21-I-II degree, in 12-III degree and in 15-IV degree. Marked changes in the main parameters of BAEP were detected in the study of the stem structures of the auditory analyzer in children with cerebral palsy. Thus, disorganization, configuration disturbances of the component composition (I, III, V) were noted.

Conclusion: Thus, the study revealed a disorder of auditory function by sound-detecting type and justified the need for hearing correction in these children. Evaluation of auditory function made it possible to determine the level of auditory analyzer injury and localization of the process from the results of a complex, objective examination.

Keywords: *Cerebral palsy, hearing impairment, otoacoustic emission, brainstem auditory evoked potentials.*

Introduction

Cerebral palsy is a complex disease of the central nervous system, affecting not only body movement and muscle coordination[1], but leading also to complications such as hearing impairment[2], which in turn affects the delay in speech and mental development[7].

In children with cerebral palsy, due to the delay of brain development 4 or its pathology of development[3], there may be a later formation of speech function, these children understand the speech addressed to them,[8] but their own speech is formed at a later date3. At the same time in pathogenesis of speech pathology the leading disorder can be damage of auditory analyzer, and disorder of hearing-motor conditional connections leads to insufficiency[9] of motor component of speech in children with cerebral palsy, which further forms delay and underdevelopment of phonematic hearing[10].

According to various sources, cerebral palsy develops in 2-3.6 cases per 1000 live births and is the

main cause of childhood neurological disability in the world[11]. The prevalence of cerebral palsy in Uzbekistan is 3.7 cases per 1000 newborns (Mutalova Z.D. The structure of diseases of children with disabilities in Uzbekistan[12] under 16 years old (2012-2014) Institute of Health and Medical Statistics).

Among premature babies, the incidence of cerebral palsy is 1%. In newborns [13]with a body weight of less than 1500 g, the prevalence of cerebral palsy increases to 5-15%, and with extremely low body weight - up to 25-30%[14].

Thus, the leading and main ones in cerebral palsy are motor disorders, in case of change of muscle tone, coordination disorders, presence of pathological synsikinesis and senergia, involuntary movements[14], as well as disorders of pose. Motor disorders have an adverse effect on the formation of mental, speech and auditory functions (Alieva Z.S., 2001,2005)[16].

A frequent disorder in children with infantile cerebral palsy is speech disorder, the frequency of which according to some authors is between 65 and 85% (Aidinov M.B., Pravodina-Vinarskaya, 1959; Bohme, 1966), others from 70 to 80% of children (Semenova K.A., 1984, Badalyan L.O., 1988; Aliyeva Z.S., 2001, 2005)[17].

Speech disorders can be associated with both deep mental retardation and the deafness bordering deafness. Often these speech disorders are combined [8].

In addition to damage to certain brain systems, secondary underdevelopment or later formation of those parts of the cerebral cortex that are extremely important for speech function and most strongly develop already in postnatal ontogenesis (premotor-frontal, dark-temporal regions of the cerebral cortex) plays an essential role in the genesis of speech disorders in these children (Klossovsky B.N., 1949; Megun G., 1960, Anokhin ILK., 1961). Since speech is a complex[19] multi-level functional system[21], correction of speech disorders in children with cerebral palsy is important,22 along with the study of auditory function[23]. Due to the fact that24 speech impairment may be due to hearing loss [25].

Four main forms of cerebral palsy are distinguished depending on muscle tone disorders:

- Spastic,
- Ataxic
- Dyskinetic (subdivided into athetoid and dystonic forms)
- Mixed forms of cerebral palsy.

Cerebral palsy is also classified regarding the affected parts of the body:

- Hemiplegia (damage to one half of the body - right or left),
- Diplegia (paralysis that affects both halves of the body, but more of the legs),
- Tetraplegia (damage to the arms and legs).

According to (Semenova K.A., Makhmudova N.A., 1979) hearing impairment is noted in 10-40% of children with cerebral palsy, more often with a hyperkinetic form[26].

When a diagnosis is correct and made in a timely manner[27], it gives an opportunity, as early as possible, to begin correction of auditory disorders and integration of the child[28] into the speech environment, thus improving the quality of life of the baby[29].

The aim of this study was to determine the prevalence and nature of auditory analyzer pathology in children with different forms of infant cerebral palsy by recording otoacoustic emission and recording short latency brainstem auditory evoked potentials.

Materials and Method

The subject group consisted of 75 children, 40 (53%) males and 35 (47%) were females, ranging from 3 to 14 years of age. Clinical examination of children began with a thorough history of the child, starting from the moment of birth to the present period. The largest number of children 48, which amounted to 64% (Table 1), was with frequent forms of cerebral palsy (spastic diplegia).

Table 1: The distribution of the examined children in the form of cerebral palsy

Forms of cerebral palsy	Amount of children		Total
	M	D	
Spastic diplegia	27	21	48
Hemiparetic	9	3	12
Atonic-astatic	4	4	8
Hyperkinetic	1	4	5
Bilateral hemiplegia	2	-	2
Total	**40**	**35**	**75**

A significant part of the children's history had a combination of several risk factors that inevitably entail the addition of other factors and thereby sharply increase the likelihood of hearing loss.

Such a factor, for example, is birth weight less than 1500 g, which entails hypoxia, the need for prolonged mechanical ventilation and the use of ototoxic drugs. Thus, it was found that most of the examined children 53 (70.7%) were born prematurely (from 24 to 36 weeks of gestation), 48 (64%) of children with low body weight.

At 64.5% (31 people) had burdened obstetric history: a history of abortion had a 15 (31%) women, the share of abortions and dead born had by 10.4% and 12.5%, respectively. Analysis and statistical processing of 23 (48%) labor histories of pregnant women with various gestoses.

The examination of children was carried out by the following method: clinical examination of patients (general and otorhinolaryngological) and functional research method of the auditory analyzer. All children underwent acoustic impedancemetry, including tympanometry with determination of the type of tympanogram and acoustic reflex.

When registering tympanograms, 48 % of children received type «A», 31% - type «B» and 21% - type «C».

In order to differential diagnosis of conductive and sensorineural hearing loss, as well as to study the correlation of data obtained during acoustic impedancemetry depending on the condition of the middle ear, childrenwere recorded caused by otoacoustic emission at the frequency of the distortion product. The stimulus intensity was used in the range from 50 to 70 dB, while the level of two tones was the same or varied by 5-10 dB. Based on measurements of the DPOAE amplitude obtained by presenting tones of different frequencies, a DP gram was automatically constructed — a graph of the dependence of the DPOAE amplitude on the tone frequency. For each point of the curve, the background noise level was also calculated. The amplitude of DPOAE exceeded the noise level by at least 3 dB.

One of the main objectives of this study was the differential diagnosis of auditory analyzer disorders. Currently, the use of acoustic auditory evoked potentials is of great importance toclarify the extent and location of lesions of the auditory analyzer. For this purpose, children underwent an electrophysiological study of hearing using the method of recording short latency brainstem auditory evoked potentials- an assessment of the state of the stem structures of the auditory analyzer. When registering the BAEP, broadband clicks, tonal pulses with a rise frequency and a duration of 2 ms were used as sound stimuli; with a presentation frequency of 11/sec, 2,000 responses accumulated, analysis time - 20 ms; bandwidth - 30-1500 (12 dB per octave).

Results

The data obtained revealed a bilateral lesion of the peripheral part of the auditory analyzer. Beilateral sensorineural hearing loss was detected in 21 - I - II degree, in 12 - III degree and in 15 - IV degree.

Marked changes in the main parameters ofBAEP were detected in the study of the stem structures of the auditory analyzer in children with cerebral palsy. Thus, disorganization, configuration disturbances of the component composition (I, III, V) were noted. The smoothness of the components was noted due to a decrease in the amplitude of the response by 2-2.5 times. The analysis of time parameters showed that, with a similar dependence of latency on changes in the intensity of the stimulus in children with cerebral palsy, in contrast to the norm, the time parameters of the main components of the BAEP (I, II,V) are significantly delayed: I - by 0.1±0, 6; III - by 0.2±0.06 and by V - by 0.37±0.07 ms. Peak intervals of the main components I - III; I - V; III - V are increased in comparison with the norm in the whole range of reactivity of BAEP. Studies have shown that the most pronounced peak-to-peak I - V intervals are increased by 0.18 ms compared to normal. In children with cerebral palsy with deep hearing impairment, a narrow dynamic range is observed, on average not exceeding 40-45 ms. An L - shaped dependence of latency on the increase in stimulus intensity was revealed; a significantly higher percentage of impaired volume function is noted.

Thus, the study allowed us to identifyimpaired auditory function in the sound-absorbing type in children with cerebral palsy. Assessment of auditory function allowed the results of a comprehensive, objective examination to determine the level of damage to the auditory analyzer and the localization of the process, as well as conduct electro-acoustic rehabilitation of this category of children.

As you know, the leading and main in cerebral palsy are motor disorders that have an adverse effect on the formation of mental, speech and auditory functions [26]. A frequent violation in children with cerebral palsy is speech disorder, the frequency of which, according to some authors, ranges from 65 to 85% [1,3], while others from 70 to 80% of children [8,12,34]. Speech disorder may be delayed speech development (late formation of the motor component of speech, especially its sound-producing side), dysarthria (pseudobulbar, extrapyramidal, cerebellar, cortical). Speech disorders can be associated with both deep mental retardation and hearing loss bordering deafness. Often, these speech disorders are combined.

The study of the stem structures of the auditory analyzer in children with cerebral palsy, having normal thresholds of auditory sensitivity, revealed pronounced changes in the main parameters of the BAEP: disorganization, disturbances in the configuration of the component composition (I, II, V) were noted; smoothing of components was noted due to a decrease in the amplitude of theresponse by a factor of 2-2.5. Analysis of time parameters showed that, with asimilar dependence of latency on changes in stimulus intensity in children with cerebral palsy, in contrast to the norm, the time parameters of the maincomponents of short avokedbreain (I, II, V) significantly tightened: I - by 0.1±0.7; III - by 0.1 8±0.05 and by V - by 0.39±0.08 ms. Peak intervals of the main components I - III; I - V; III - V are increased in comparison with the norm in the whole range of reactivity of SVP. Studies have shown that the most pronounced peak-to-peak I - V intervals are increased by 0.18 ms compared to normal. In children with cerebral palsy with deep hearing impairment, a narrow dynamic range is observed, on average not exceeding 40-45 ms. An L - shaped dependence of latency on the increase in stimulus intensity was revealed, a significantly higher percentage of impaired volume function (FCFN) was noted.

In a comparative aspect, the signal response in children with cerebral palsy was less mature. The amplitudes of the waves of auditory evoked potentials, as a rule, were smaller in comparison with normal values. In contrast, the peak-to-peak intervals in most cases corresponded to normal values. Table 2. presents the values of BAEP depending on the etiological factor of cerebral palsy.

Table 2: The values of BAEP depending on the etiological factor of cerebral palsy

Etiological factor	Number of patients	Peak Interval I-V	Amplitude V waves	% To I wave ratio
Very low weight	9	3.93 ± 0.25	0.35 ±0.10	1.25 ±0.66
Asphyxia	26	4.01 ±0.24	0.28 ±0.09	1.02 ±0.67
Unknown etiology	12	4.07 ±0.27	0.32 ±0.12	1.14 ±0.72
Control group	25	3.95 ±0.18	0.37 ±0.10	1.25 ±0.61

In the history of the mother, we paid great attention to the course of pregnancy and childbirth. The presence of toxicosis in the mother, its course and duration, the use of ototoxic drugs during the pregnancy (aminoglycoside series, “loop” diuretics).

Discussion

Of the 9 children we examined with cerebral palsy, 4 children were born using cesarean section before the 30th week of gestational age, of which three children had birth weight less than 1500g. 2 children born on time had hyperbilirubinemia (bilirubin content of more than 20 mmol/ l). With hemolytic disease of full-term newborns, there were 3 children.

A significant portion of the children have a history of a combination of several risk factors. Some of them inevitably entail the addition of other factors and thereby sharply increase the likelihood of hearing loss. Such a factor, for example, is birth weight less than 1500 g, which entails hypoxia, the need for prolonged mechanical ventilation and the use of ototoxic drugs.

In most cases, in children with cerebral palsy, the amplitude ofI and V waves and the ratio of V waves to I were less than the average values characteristic of this age. Thus, the obtained values showed a significant difference in the amplitude of the V wave during cerebral palsy, due to the birth of very low body weight,

asphyxiation, and unknown etiology. The amplitude of the V wave in children born with a very low birth was slightly reduced, but did not significantly differ from the control group.

Some children showed poor identification of waves 2 and 4, sometimes even I waves. Basically, this observation was made in children with cerebral palsy of unknown etiology, who had deep damage to the central nervous system.

The results showed that there are no significant differences from the control group in the values of the peak intervals I-V, I-III and III-V in children with cerebral palsy, regardless of its etiology. There were also no significant differences in the interaural difference in the latencies of the V peaks on both sides of the interaural difference for the interval I-V, I-III and III-V. Compared with the control group, the interval I-V was slightly longer in children with cerebral palsy of unknown etiology. In children with cerebral palsy, against the background of very low body weight, the interval I-V was shortened.

As the obtained data show, a significant decrease in the amplitude of the V wave and, accordingly, the V / I ratio is observed in children who survived asphyxia at the beginning of life. There is also a tendency to shortening of the 1st wave, which leads to a relative increase in the V / I ratio. Of the 78 children with cerebral palsy, 21 (26.1%) had a significant decrease in the amplitude of the V wave, and 10 (12.5%) had a significant decrease V / I ratio. Absence of waves was noted in 14 cases (17.5%).

Only 10 children with cerebral palsy (12.5%) had a significant lengthening of the interval I-V, which was often accompanied by a significant decrease in the amplitude of wave V. Deviations in intervals I-III and III-V and ratios III-V / I-III were rare. An increase in intraural difference (6.3%) was noted in 5 (6.3%) cases. This increase was mainly due to the unilateral shortening of intervals III-V and IV (2 children with very low birth weight) or the unilateral extension of 2 intervals (2 children with unknown etiology of cerebral palsy).

In total, 48 out of 75 children with cerebral palsy had deviations in auditory evoked potentials, which manifested itself in a significant decrease in the amplitude of the V wave, a decrease in the V/I ratio, an increase in the IV interval, and the absence of I or more response waves. The most often seen deviations were manifested during the registration of the V wave, which appeared by a decrease in its amplitude. In some children with cerebral palsy, more than one deviation from normative indicators was recorded. These children often, although not always, had profound disorders of the central nervous system.

The results showed a significant difference between children with cerebral palsy and the control group. The present study showed that the main changes in cerebral palsy are manifested in the form of depression of the signal and a decrease in the amplitude of the V wave, as a rule . These changes mainly reflect a decrease in the size, or synchronization of neural activity in the auditory part of the brain. These changes may be due to neurological abnormalities or be directly a manifestation of cerebral palsy. Summarizing, we can say that there is a depression of waves, including wave I. A decrease in the amplitude of the I wave is observed, accordingly, the V / I ratio increases. As a result, even with a significant decrease in the amplitude of the 5 wave, the V / I ratio changes little and remains within the normal range. This can largely explain why a significant decrease in the V / I ratio is less often detected compared to a decrease in the amplitude of the 5th wave. This result shows that, compared with the V / I ratio, the 5-wave amplitude is more sensitive when registering auditory evoked potentials with a reliable reflection of auditory function in clinical practice.

When assessing auditory evoked potentials, it should be taken into account that waves 2 and 4 are characterized by great variability and are less reliable than waves I, III, and V. In assessing the configuration of the curve, in some cases precipitation of individual components was noted. The curves with the loss of the first, third, and fifth peaks were not encountered in the analysis. The maximum loss occurred in the second, fourth and sixth components. According to the established opinion and literature data, it is these peaks that are distinguished with the least frequency. However, according to its own regulatory data, the secrecy of these peaks does not differ much from the secrecy of the rest, based on which we can conclude that configurational changes are quite common with spastic diplegia. The technique for recording auditory evoked potential includes mandatory monotonous audiometry, which allows you to get an idea of the state of auditory function. An increase in the auditory threshold above the laboratory norm was noted in 18 (30%) cases in 34 (28.3%) studies. That is, almost every third patient had a one-sided increase in the auditory threshold

The main trends in the parameters of the short-latency auditory evoked potential were a decrease in the amplitude and an increase in the latency of the components. A significant ($p < 0.05$) increase in latency was noted for the I, II, III, IV and VI components of the potential. An increase in absolute latencies per se cannot be a sign of conduction disturbance at the stem level, especially given how often disturbances in the function of the peripheral part of the analyzer were encountered. However, a significant increase in the inter-peak interval III – V indicates dysfunction of stem afferents at the bridge-mesencephalic level. The detected decrease in the amplitude of the I, II, V components of the potential ($p < 0.05$) is most likely a consequence of the dysfunction of the peripheral unit of the analyzer.

A fall in the amplitude V / I ratio below 0.75 was observed in 20%, below 0.5 - in 6.7% of patients in 5 (4.2%) studies. The analysis of peak-to-peak intervals indicates the truth of the revealed changes in the conductive ability of stem afferents. The analysis of amplitude relationships allows us to confirm the presence of a nuclear-dysfunctional component in the pathophysiological picture of spastic diplegia, however, the frequency of occurrence does not allow us to see in it the key mechanism of the pathogenesis of stem disorders present in the clinical picture of the disease. The revealed changes in the conductive properties of stem afferents contribute to the picture of the dysfunction of stem structures during spastic diplegia and can have residual demyelination nature.

Thus, the study revealed a disorder of auditory function by sound-detecting type and justified the need for hearing aids in these children. Evaluation of auditory function made it possible to determine the level of auditory analyzer injury and localization of the process from the results of a complex, objective examination.

Conclusion

1. The use of objective method for the study of hearing in children with cerebral palsy allows you to identify the nature and extent of hearing impairment.
2. The pathogenesis of cerebral palsy combines dysfunction of the nuclear apparatus of the brain stem and impaired conductive properties of stem afferents.
3. The study of hearing at an early age with the help of objective method helps to timely detect hearing impairment and delineation of deafness and hearing loss from a number of diseases that delay speech development, which is an important link in the chain of rehabilitation measures aimed at correcting difficult - the structure of the defect in children with Cerebral palsy.
4. Our study indicated the need for an early (from the first days of life) and comprehensive objective examination of auditory function in children with cerebral palsy. Early diagnosis of the pathology of the auditory analyzer provides timely correction of auditory-speech disorders in children with cerebral palsy.

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Clinical Features of Early Diagnosis and a Choice of Method of Surgery for Hirschsprung Disease in Infants

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Abstract

The aim of this study is to improve the results of surgical treatment of Hirschsprung's disease in infants by choosing the most effective method.

This article presents the results of the treatment of Hirschsprung's disease in 61 patients of infancy. However, immediate and long-term results of the children's treatment with HD, operated on in infancy, using the traditional Soave-Boley method and modern minimally invasive technologies according to TENTC and the Georgeson method, which show different results, have been studied. Among 26 patients who had been operated on by the Soave - Boley method, the following results were obtained : excellent - in 10 (38.4%); good - in 8 (30.7%); satisfactory - in 6 (23%) and unsatisfactory - in 2 (7.6%). Among 29 patients operated on by the TEPT method, except for one patient, excellent and good results were obtained in 28 (96.6%) patients. What is more, among 6 patients who had been operated on by the Georgeson method, excellent and good results were obtained in all cases; there were no satisfactory or unsatisfactory results. It should be noted that among the operated HD patients according to the Soave-Boley method, there were severe patients, of which 7 patients had a colostomy in the neonatal period.

At the same time, radical one-stage surgical treatment for distal forms of HD using minimally invasive TEPT method is a priority in infancy, which can reduce complications to a minimum with improved functional outcomes of treatment, relative to two-stage treatment.

Keywords: *Hirschsprung's disease, treatment, surgical, diagnosis, pediatric.*

Introduction

Recently, the authors have recommended effective early diagnosis and surgical correction, the priority of which is a one-stage radical correction of Hirschsprung disease (HD) and the use of minimally invasive method. In this regard, the choice of optimal method for surgical treatment of HD in infancy is of particular relevance.

Regarding the reliability of the diagnosis of children with HD, taking a biopsy from the rectal mucosa is the «Gold Standard» for morphological diagnosis[1]. However, there is evidence of controversy and debatable questions about the validity of biopsies in young children with HD in literature[3]. Many authors claim that by the time of birth, intramural, ganglion cells still do not reach full morphological maturity[6]. They are especially immature in the caudal part of the colon, in the submucosal layer in particular[8]. This immaturity is physiological; final maturation occurs gradually. Consequently, it is believed that at an early age, a rectal biopsy is contraindicated and unreliable[22]. If this concept is not taken into account and take a biopsy in cases of suspected GB, then this diagnosis will be made often and unreasonably, especially in premature infants[23].

Other authors believe that the lack of preliminary morphological diagnosis and the preservation of the proximal dysganglionic segment of the colon during radical surgical treatment leads to unsatisfactory results[5].

At the present stage, the cases when there were no clear signs of HD according to irrigography became the indication[4] for carrying out a laparoscopic biopsy of the colon[21].

There is information that in some cases the histological structure of the colon also had a deviation from the norm. In 50% of children[9], a histochemical reaction suggested a diagnosis of intestinal agangliosis, in other cases, it only complicated the further diagnostic search[10]. So, low information content of histochemical diagnosis is explained by the absence of not only a nerve ganglia and nerve cells in the intestinal wall[12], but also parasympathetic fibers with their total lesion[13].

The available histochemical and morphohistochemical research method for determining the activity of tissue AChE in HD have been carried out for a long time with high reliability[2], however, its radical applications are not always available[11]. The modern neurohistochemical express method for a short research time provides information with high reliability (97-99%), however, it requires highly qualified specialists, expensive equipment and reagents that are not always available[18].

As well as the inclusion in the examination program of infants suffering from chronic constipation to identify HD, a morphohistochemical method[11], was not always reliable, which was not always reliable14, moreover, the applied clinical, radiological and functional method did not always make it possible to make a correct diagnosis[16]. Thus, many-sided and debatable questions on determining the localization of taking biopsy material from the rectal mucosa or laparoscopically from all the muscular - submucous layers of the colon and the low reliability of the biopsy in the diagnosis of HD remain open. Moreover, in an inaccessible situation, pediatric surgeons need to optimize and accurately solve the issues of intraoperative diagnosis, measures for the prevention of complications and relapses of the disease in infants.

Moreover, in surgical treatment of HD in infants, against the background of many concomitant diseases, malformations of other organs and morphofunctional immaturity of a child's body[7], preoperative preparation and postoperative management of patients causes particular difficulties[2]. On the other hand, the use of existing modified abdominoperineal proctoplastics (APR) using Soave technology (Boley, Lenyushkin) is more traumatic[17]. Surgeries of lowering the colon through the serous-muscular case of the rectum according to the Soave method with the creation of a "seamless anastomosis" in 2 stages are the safest for a patient, and their effectiveness reaches 90% in children older than infancy[12]. Fundamentally, the operation of laparoscopically assisted intestinal reduction is not different from the operation of Soave, however, it allows treatment in one stage and has all the advantages of minimally invasive interventions[13].

Regarding the results of surgical treatment of HD in children, according to the authors, in the postoperative period, having bowel movements is disturbed in 37% of sick children. At the same time, astriction occurs in about 8-20% of children, enuresis occurs in 5-26% of cases, which is unsatisfactory and leaves much to be desired[18]. The authors note that the introduction of minimally invasive method for the correction of Hirschsprung disease has led to a change in the spectrum of complications, the main of which were : colorectal anastomosis failure (3.4%) and its stenosis (8.5%)[22].

Therefore, in order to prevent postoperative complications and relapses of the disease in infancy, each method of operation according to Soave - Boley, Georgeson and Transanal endorectal pull-through (TEPT) according to Delatorre - Mandragon and Ortego - Salgado, in solving tactical and operational-technical issues requires optimization of indications, a differentiated choice of method depending on the anatomical form, degree of compensation and the course of the disease, which is an urgent problem in pediatric surgery.

Objective: Improve the results of surgical treatment of Hirschsprung disease in infants by choosing the most effective method.

Materials and Method

The immediate and long-term results of surgical treatment of HD in 61 infants were studied by us for the period 2010-2019. Patients' age: up to 3 months. - 8 (13.1%); 3-6 months - 17 (27.8%); 6-9 months - 20 (32.7%); 9-12 months - 16 (26.2%). By the forms of the disease were: rectal - 13 (21.3%); rectosigmoidal - 43 (70.4%); subtotal - 5 (8.1%). According to the clinical course: acute - 9 (14.7%); subacute - 39 (63.9%); chronic - 13 (21.4%). Congenital concomitant pathologies occurred: Down's disease - 1, aplasia and hypoplasia of kidneys - 3, coccyx agenesis - 3. There were 53 boys, 8 girls.

All children underwent general physical, laboratory, radiation (plan radiography, polypositionalirrigography, U/S) and morphological (macro preparation of colon agangliosis zones resected during radical surgery) studies.

Patients were divided into 2 groups: 1 - group (control) - 29 patients operated on by the Soave - Boley, Lenyushkin method; 2 - group (main) 32 operated on patients, among them: 26 cases by the TEPT method (Delatorre-Mandragon) and in 6 cases by the Georgeson method.

The effectiveness of each method has been studied by evaluating on a point scale according to the questionnaire data of operated patients in a remote period of 1-4 years. Clinical results were evaluated as: excellent, good, satisfactory and unsatisfactory.

Results and Discussion

Early diagnosis of HD in infants has its own specific features, and at the same time, the degree of maturity of the child, the age category, associated pathologies, the length of the aganglinal and the degree of (compensatory) expansion of the suprastenotic zone are of no small importance.

Enterocolitis is known to be a serious complication of HD in pre- and postoperative periods. Enterocolitis can develop at any age, from the neonatal period to the older age, regardless of medication or surgery. Recurrent enterocolitis can occur even in the presence of an externalized colostomy [].

According to our observations, during the neonatal period and early infancy (up to 3 months) there were 7 patients who received a clinical picture of acute low intestinal obstruction, a history of which noted: diarrhea, bloating, fever, anxiety due to spastic pain in abdomen, intoxication, hypovolemia and loose bowel movement with an admixture of blood. At the same time, the first thing was to exclude enterocolitis associated with Hirschsprung disease. After a plan radiography of the abdominal cavity, these patients underwent irrigography (Fig. 1a, b).



(A) **(B)**

Fig. 1. A - plan radiography of the abdominal cavity; B - Irrigography

In which the narrowed (aganglionic) zone in the distal colon was clearly contrasted, the transition zone in the form of a funnel-shaped expansion over the affected area in 5 patients with subtotal and 2 patients with a long zone of rectosigmoid form of the disease and manifestation of enterocolitis.

Having concomitant enterocolitis against the background of irritation of the intestine with spasmodic areas that was created with a false appearance of pathological narrowing. It means that during the period of newborn and early infancy, the risk of false-positive, and false-negative results of irrigography increases by

almost 3 times. Therefore, the x-ray area of narrowing does not always coincide with the area of the taken biopsy and agangliosis.

And also in 6 hypotrophic, functionally immature infants with enterocolitis in the initial early diagnosis of HD, the implementation of a layer-by-layer rectal biopsy on the inflamed mucosa and thinned intestinal wall was impossible.

That is why, in almost all cases, due to inaccuracy, inaccessibility and sometimes the lack of possibility of biopsy immunohistochemically, morphohistochemically and neurohistochemically, we did not carry out express method [11,14,15,19].

In patients (in 19 cases) older than 3 months with HD with a long aganglionic zone, a more pronounced picture of obstruction with high pressure had been observed with increasing suprastenotic expansion of the colon - left subtotal and long rectosigmoid form (Fig. 5a., b.).



(A) **(B)**

Fig. 2. Hirschsprung disease in infants: (A) left-side subtotal; (B) rectosigmoid form with a long zone of aganglionosis.

In a short zone with compensatory courses of the disease (in 40 cases), it is not always shown as in the classical form: narrowed, transitional zone with suprastenotic funnel-shaped extensions of the colon. At the same time, the sensitivity and specificity of irrigography in newborns and infants was significantly lower due to only breastfeeding.

With supplementary feeding and the transition to mixed, artificial and natural feeding, the clinical picture of chronic bowel movement retention began to increase in patients, and against this background, the use of polypositionalirrigography in these patients has greatly expanded the range of diagnostic capabilities of HD in infants.

Despite the limitations or sometimes difficulties of using morphological and other research method in the early diagnosis of HD in infants, we consider polypositionalirrigography and preoperative diagnostic laparoscopy to be more optimal and reliable method. We carried out preoperative diagnostic laparoscopy with dubious diagnosis of HD and the length of the zone of aganliosis, taking into account the choice of the method of operation in 6 patients.

In the surgical treatment of HD, until recently, though STI according to Soave - Boley and Lenyushkina, which we performed in the first group of patients in 25 cases, were considered optimal and more physiologically substantiated method in children. These operations are performed in 2 stages, the abdominal stage is almost identical, but they are not free from shortcomings and complications. At the abdominal stage, in the depths of the pelvis against the background of technical difficulty during demucosis of the rectal wall, as close as possible to the anus, internal anal sphincters are often damaged and perforation occurs, which often causes pelvic peritonitis, interfacial abscess, and stenosis.

Moreover, with excessive blind demucosis of the intestine, blunt trauma (with instruments) and impaired blood and lymph formation in the adjacent pelvic organs (sphincter muscles, ovaries, prostate gland, urethra) in the long term, the consequences often lead to fecal and urinary incontinence, as well as are the background state in the development of diseases of the reproductive organs in boys and girls.

However, at the modern level, with the improvement of new minimally invasive and high-tech TEPT operations with video-assisting at the abdominal stage - with Georgeson's method and without it, we had the opportunity to operate sick children with HD during the neonatal and infancy years.

With the gain in experience, we have developed various tactical approaches for choosing the method of operation and optimizing the indications depending on the anatomical shape, degree of compensation, and the course of HD.

In case of subtotal and proximal left-sided long forms of HD disease in 6 cases (agangliosis zone length over 30 cm), in the main group of patients, in order to reduce intra-abdominal complications, the Soave-Boley method was replaced by laparoscopic diagnostics and colon mobilization. The perineal stage is an oncoming transanaldemucosis "from the bottom up" with the reduction of the mobilized intestine. After resection of the agangliosis zone, a primary colorectal anastomosis (Georgeson operation) was created, the results of which were successful outcomes.

In rectal and rectosigmoid forms of HD in infancy, a long zone of agangliosis in the rectum and sigmoid colon (up to 30 cm) was performed by TEPT (Delatorre - Mandragon and Ortego - Malgado), in which demucosis was performed by transanal access with lowering of the colon endorectally. After resection of the agangliosis zone, the primary colorectal anastomosis is transanally superimposed (Fig. 3.). The operation by using TEPT method was performed in 26 children with HD in infancy, a rectal and rectasigmoid form of the disease. Moreover, all operations were performed without prior colostomy.



Fig. 3. TEPT - Delatorre - Mandragon and Ortego - Malgado

When performing TEPT, it should be noted that it is possible to determine the level with wide gut resection in order to prevent relapse of the disease, since the younger the age of patients, the less clearly and contrast the narrowed area and suprastenotic enlarged part of the intestine are manifested during irrigography. This is most characteristic of subacute (rectal and rectasigmoidal form) and atypical (enterocolitis associated form) clinical course of the disease, which complicates early diagnosis and intraoperative determination of the level of resection. For that purpose, to determine intraoperatively the location of the level of wide resection and ensure the optimal radicality of TEPT method, we studied morphologically the state of the histostructure of the nerve plexuses, nodes, cells and fibers in the wall of the transitional and enlarged parts of the removed intestine near the gross specimen. At the same time, in 9 patients with HD, the gross specimen obtained by wide resection of the colon was subjected to a morphological study, the reason for which was the additional loops with dolichosigmoid.

The initial, middle and upper parts of the suprastenotic enlarged zone of the colon (Fig. 4). has been studied morphologically.



Fig. 4. A gross specimen obtained by a wide resection of the colon.

At the same time, in the initial section of the enlarged part of the intestine, at a distance of 3 cm from the transition zone, areas of dysgangliosis were noted, characterized by places of absence, atrophy, destruction, hypertrophy and hyperplasia of the preserved nerve plexus nodes.

Further, when studying the mucous membrane of the middle suprastenotic enlarged part of the intestine, at a distance of up to 5 cm, from the transition zone, their atrophicity was noted, expressed in wide gaps in the crypts, diffuse nature of the edema, loosening of its own connective tissue plate with foci of inflammatory infiltrate and sclerosis. There are relatively hypertrophic and hyperplastic nerve nodes due to swelling of nerve cells, fibers, and Schwann's cells hyperplasia (Fig. 5a).

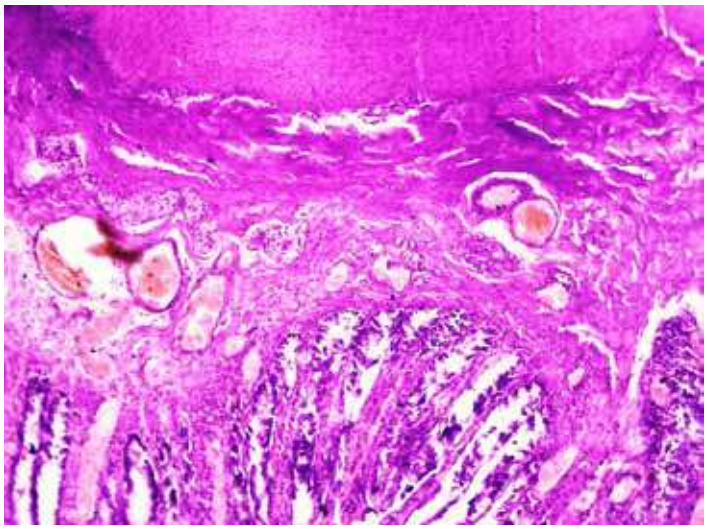


Fig. 5a. The submucous layer of the suprasthenic part of the intestine with HD in an infant is up to 3-5 cm long . Hypertrophy and hyperplasia of the nerve plexuses against the background of chronic inflammation. H & E stain Magn: 10x20.

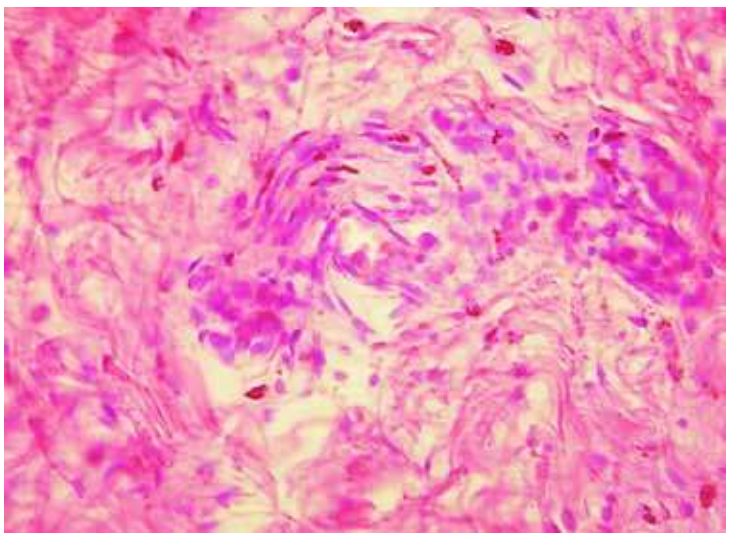


Fig. 5b. The submucous layer of the suprasthenic part of the intestine with HD in an infant is up to 3-5 cm long . Hypertrophy and hyperchromasia of Meissner nerve nodes with hyperplasia of Schwann's cells. H & E stain Magn: 10x40.

Nerve cells are located in one pole of the node with signs of hypertrophy and hyperchromasia, in the other pole of the node there are nerve fibers from hyperplastic Schwann's cells (Fig. 5b). In the submucosal layer of the middle expanded part of the intestine, at a distance of up to 5 cm from the transition zone, the presence of nerve nodes of various size and composition is noted. In these nerve nodes there are few nerve fibers and glial cells and they are located on the periphery.

Meissner nerve nodes of round shape with a small number of nerve cells (Fig. 6a), where, on the contrary, there are more nerve fibers circles of Schwann's cells form a thin membrane (Fig. 6b), which covers, depending on the degree of decompensation. These secondary morphological changes at the level of the less pronounced dilated "normal" part of the intestine lead to normalization of the structure of nerve nodes in the mucous and submucosal layer up to 5 cm from the transition zone, depending on the degree of compensation of the suprastenotic expansion of the colon.

Fig. 6a. The submucous layer of the suprasthenic part of the intestine with HD in an infant is up to 3-5 cm long . Meissner nerve nodes with a large number of nerve cells and a thin myelin sheath. Magn: 10x40.

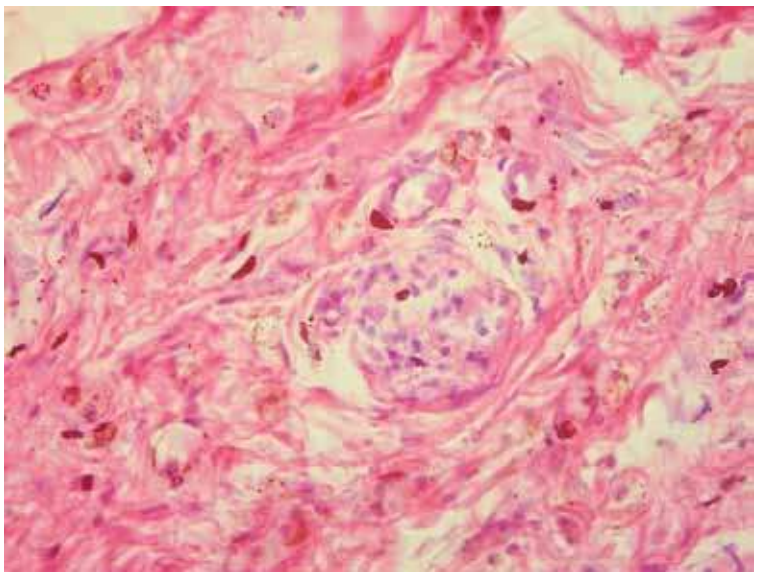


Fig. 6b. The submucous layer of the suprasthenic part of the intestine with HD in an infant is up to 3-5 cm long. . Meissner nerve nodes with a small number of nerve cells and a large amount of nerve fibers. H & E stain Magn: 10x40.

In the intramuscular layer of the enlarged part of the intestinal wall, separate Auerbach nerve nodes with dystrophic, atrophic, and destructive changes up to 5 cm long are noted (Fig. 7a). In this case, mainly nerve cells, glial cells and nerve fibers underwent dystrophic changes, and Schwann's membranes underwent atrophic changes. Microscopic examination of the wall of the intermediate part of the expansion of the colon shows a picture of the proliferatively inflammatory form of chronic colitis. The muscle layer is thickened due to hypertrophy of smooth muscle cells and the presence of interstitial lymphohistiocytic infiltrate with atrophy and destruction of the nerve ganglia. Auerbach's nerve nodes are compressed by inflammatory infiltrate, atrophic nerve cells are compressed and small, hyperchromatic. Pathomorphological changes of the nervous system of the colon correspond to hypogangliosis (Fig. 7b).

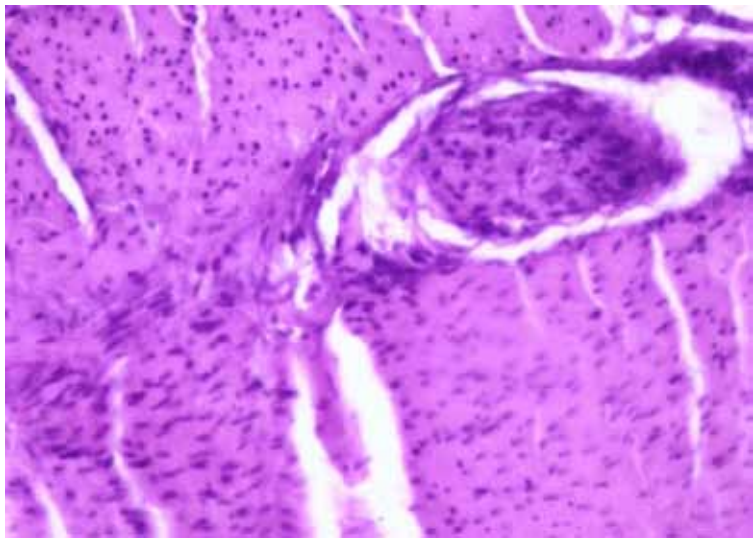


Fig. 7a. The muscle layer of the suprasthenic part of the intestine is up to 3-5 cm long. with HD in an infant. Separate Auerbach nerve nodes with dystrophic, atrophic and destructive changes. H & E stain Magn: 10x40.

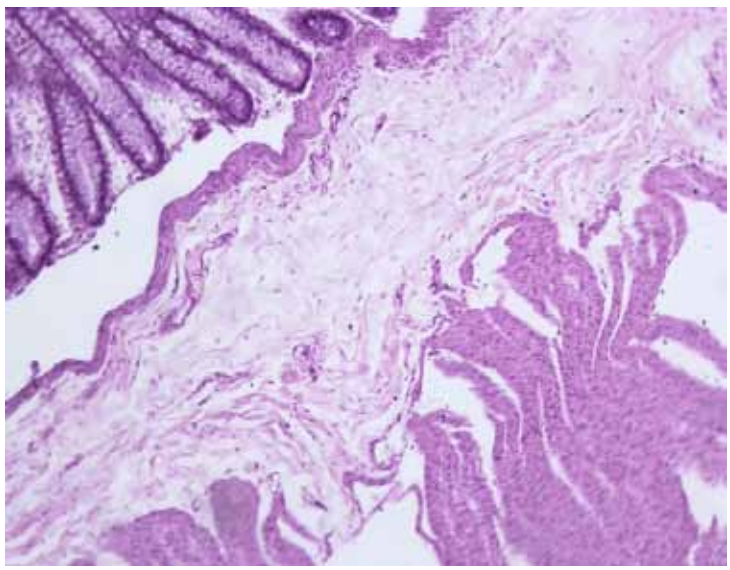


Fig. 7b. The muscle layer of the suprasthenic enlarged part of the intestine up to 5 cm long. Auerbach's nerve nodes are compressed by inflammatory infiltrate, atrophic nerve cells are compressed and small, hyperchromatic. The Auerbach nerve plexuses correspond to colon hypogangliosis. H & E stain Magn: 10x40.

Moreover, in different topographic sections of the wall of the enlarged part of the intestine, the Auerbach nerve plexuses differ in histoarchitectonics and sizes from each other. In the first section, where the mesentery is attached there are neurovascular bundles and nerve nodes are larger, there are many cells located rarely in one microscope objective, there is only one node. The farther the section of the intestinal wall from the zone of attachment of the mesentery, the denser the Auerbach nerve plexuses are located, in one microscope objective there are 2-3 nodes. They have few nerve cells, the nerve fibers are loosened, and the circumferences of the myelin sheaths are thick due to hyperplasia of Schwann's cells.

Thus, at the level of the suprastenotic enlarged zone at a distance of up to 5 cm, thesigns of irreversible sclerosis and fibrous degeneration of the muscle layer, degenerative -inflammatory changes in the mucous membrane,dysganglionic "inferior" sections of the nerve plexuses, which can subsequently serve as a source of development of hypoperistaltics, the cause of chronic colostasis in the long term after surgery have been diagnosed. Based on the data of histomorphology, we can conclude that to determine the optimal level of the upper border of the resection for rectal and rectosigmodal forms of HD, even with minor dilatation of the suprastenic zone, there are varying degrees of dysganglionic and "inferior" sections of the nerve plexuses. This is why, a wide resection at a distance of at least 5 cm from the enlarged zone is recommended and it is considered to be an optimal site for the resection zone and prevent recurrence of the disease.

Such intraoperative tactics for determining the level of wide resection in the mobilized part of the intestine was used in 28 patients with TEPT surgery, and in 8 patients it was established with diagnostic laparatomy. In the remote period, all patients with no signs of chronic constipation obtained excellent and good results.

At the same time, we have proved that the place and level of wide resection of the suprastenotic zone in infants depending on its compensatory expansion for the prevention of postoperative chronic constipation and relapse of the disease in a more distant period after surgery is of natural significance.

The advantages of TEPT were: radicalism; low injuries; one-stage; the absence of postoperative complications from the abdominal cavity and pelvic organs; smooth postoperative course. Contraindications were: severe vital malformations, acute severe enterocolitis, severe dilatation of the proximal intestine, severe general somatic state of the child, Hirschsprung associated enterocolitis (HAE), multiple, deep erosive ulcers in the mucosa of the rectum ampule and purulent inflammation of the parianal region.

In solving the tactical issues of diagnosis and treatment of HD in infancy, enterocolitis is the main and prognostically key condition for postoperative complications and mortality. Upon admission to the intensive care unit, newborns with HAE undergo intensive therapy, which includes correction of water-electrolyte and protein balance, cleansing and therapeutic enemas. If the length and localization of the agangliosis zone with an acute course of the disease is inaccurate, a colostomy is first applied above the site of obstruction and a floor biopsy from the distal sections is simultaneously performed. Further, when agangliosis is detected histologically, the zone in the lower part of the ostomy, within 3-6 months after the complete elimination of enterocolitis, the second stage is the operation of Soave-Boley with the simultaneous elimination of colostomy. We used this tactical approach in 3 patients with enterocolitis associated with HD, in whom, after surgical treatment, a successful fate was obtained in the late late period.

Also, we conducted 6 newborns and young children who had been accepted from the region with a severe course of HAE, where against the background of diarrhea syndrome, severe dilatation of the proximal intestine and with a general somatic status, a colostomy was imposed on an emergency basis. In the future, after removing the patient from the septic state and treating enterocolitis at the age of 7-9 months, a radical Soave-Boley operation was performed in the second stage with the simultaneous elimination of colostomy.

In other cases, with an uncomplicated form of HD and with the ex ception of total agangliosis, a one-stage correction of HD was performed. With incomplete primary diagnosis and with the presence of a very long zone of agangliosis, before TEPT, we used laparoscopic diagnostics in 10 patients to clarify the areas of elongation and expansion, localization and the true length of the zone of narrowing and suprastenotic expansion. After clarification of the above, the optimal location of the resection level was determined, the marks were left on the suprastenotic enlarged area, 5 cm from the transition zone, the proximal part of the colon agangliosis was

mobilized by separation of adhesions and the fixative ligament, where conditions for the possibility of TEPT were created.

With subtotal, total and left-sided disease with a long zone of agangliosis, clipping and resection of the blood supplying large mesenteric vessels of the second and third order. Then, a pull-through operation of the large intestine (Georgeson's operation) through the proctal access was performed . Moreover, the crucial points are: a meticulous execution technique, adequate hemostasis, maintaining good blood supply for the prevention of ischemia and preventing torsion and tension of the reduced intestine. These points prevent possible com plications in the postoperative period of HD in young children.

In acute type of HD, in the presence of intestinal obstruction, HAE with toxic dilatation of the colon, perforation of the blind and other parts of the colon, was carried out by colostrum with biopsy at different levels of the colon and rectal biopsy. With the subtotal and total form of HD in 3 cases, a Soave - Boley operation was performed with the simultaneous elimination of the colostomy. With the subtotal and total form of HD in 3 cases, the Soave-Boley operation was performed with the simultaneous elimination of colostomy.

According to our clinical observations, in all cases, complications in the intra- and postoperative period associated with the technique, the operation was not observed. In the postoperative period, in all cases, a Foley catheter No. 28-32 was inserted into the rectum, and turunda soaked in «Bakstims» balsam was left between the coloanal anastomosis and periodically changed after 2 days. The course of the postoperative period in all patients proceeded satisfactorily. Patients were usually discharged from the hospital 7 days after surgery.

Enterocolitis occurred in one patient in the early postoperative period. Stenosis in the area of the anastomosis often caused partial low intestinal obstruction against the background of circular and segmental-circular anastomosis, and was not a high risk factor. At the same time, the passage of a smaller Foley catheter through the anastomosis, the picture of mechanical intestinal obstruction was quickly eliminated. Two weeks later, during the control finger test, part of the stenosis was eliminated conservatively by dilating the narrowed area and rupturing the absorbable sutures of the colorectal anastomosis, which was carried out easily.

In the postoperative period, the failure of the coloanal anastomosis in our observations has never occurred. We didn't come across violations of bowel movement, including constipation, incontinence and enuresis. Functional constipation can be treated with conservative method, such as cleansing enemas and diet.

In addition to the use of Georgeson's surgery and TEPT, a 3-fold decrease in the number of postoperative complications was obtained in comparison with the control group.

During the monthly follow-up examination, complaints of constipation of varying intensity were observed only in 35% of patients. In these patients, with a long period, there were clinical signs of constipation until the filament was completely absorbed (up to 3 months) at the site of the anastomosis, due to its partial narrowing. At the same time, cleansing enemas and bougienage of stenosis to the age norm were performed. In order to restore the neuro-reflex connection in the anorectal zone, 1-3 courses of electrical stimulation were performed. In this case, the signs of functional disorders of the stool gradually disappeared and the normal act of defecation was restored.

To study long-term results, questionnaires were conducted 3 months after the radical operation, in 45 sick children with HD at the age of 1-3 years, by scoring on a point scale. The list of questionnaire questions is presented in table No. 1, where clinical violations of the act of defecation were evaluated as: good, satisfactory, unsatisfactory.

Table 1: Questionnaires to assess the long-term results of surgical treatment of Hirschsprung disease.

Symptoms	Long-term results					
	Good	Point	Satisfactory	Point	Unsatisfactory	Point
1. Abdominal distention	No	10	Often	5	Constantly	0
2. Difficulty in the act of defecation	No	10	After 3 days with difficulty, but independently	5	After 4-6 days, not on their own, after an enema	0
3. Incomplete bowel movements	No	10	Partially	5	Yes	0
4. Reducing bowel movement diameter	No	10	Partially reduced	5	Sharply reduced	0
5. Lack of a bowel movement and the urge to defecate	No	10	Partially	5	Yes	0
6. Fecal incontinence without the urge to defecate	No	10	Partially	5	Yes	0

Moreover, if the result was 45-60 points, we rated it good; 30-45 points - satisfactory; below 30 points - unsatisfactory.

Long-term results of surgical treatment of Hirschsprung disease in infants by choosing a method are presented in Table 2.

Table 2: Long-term results of surgical treatment of Hirschsprung disease in infants by method choices

Anatomical forms	Method operations	Amount of operated Patients (%)	Long-term results (n- 61)			
			Unsatisfactorily	Satisfactorily	Good	Excellent
Rectal 13(21.3%)	TEPT	13(21.3%)	-	-	34.9%)	10(16.4%)
Rectosigmoid 22 (36.1%)	TEPT	10(16.4%)	-	-	2(3.2%)	8(13.1%)
	Soave - Boley	12(19,7%)	-	2(3.2%)	5(8,1%)	5(8,1%)
Rectosigmoid with a longzone19 (31,1%)	TEPT	6(9.8%)	-	1(1.6%)	2(3.2%)	3(4.9%)
	Soave - Boley	7(11.5%)	-	1(1.6%)	1(1.6%)	5(8,1%)
	Georgeson	6(9.8%)	-	-	2(3.2%)	4(6.5%)
Rectosigmoid with a long zone + HAE2(3,2%)	Soave - Boley	2(3.2%)	-	1(1.6%)	1(1.6%)	-
Subtotal form + HAE + colostomy 5 (8.1%)	Soave - Boley	5(8,1%)	2(3.2%)	2(3.2%)	1(1.6%)	-
Total		**61(100%)**	**2(3,2%)**	**7(11,5%)**	**17(27,9%)**	**35(57,3%)**

At the same time, we obtained long-term results in general for surgical treatment of HD in infancy: excellent - 57.3%, good - 27.9%, satisfactory - 11.5%, unsatisfactory - 3.2%.

Among 26 patients who had been operated on by the Soave - Boley method, the following results were obtained : excellent - in 10 (38.4%); good - in 8 (30.7%); satisfactory - in 6 (23%) and unsatisfactory - in 2 (7.6%). Among 29 patients operated on by the TEPT method, except for one patient, excellent and good results were obtained in 28 (96.6%) patients. And also among 6 patients operated according to the Georgeson method, excellent and good results were obtained in 100% of cases, there were no satisfactory and unsatisfactory results. It should be noted that among the operated HD patients according to the Soave-Boley method, there were severe patients, of which 7 patients had a colostomy in the neonatal period.

Thus, according to the study of the long-term results of the first group of patients operated on by the Soave-

Boley method: in the late period there was stenosis in the anastomotic zone with a violation of the bowel movement as a constipation with complete and partial absence of a feeling of urge. For children with satisfactory and unsatisfactory results, a stationary examination was proposed with irrigography, ultrasound of the abdomen and perineum, if necessary, examination of feces for coprology and dysbacteriosis, rectal ulceration, ESM and the complex rehabilitation treatment post-mortem until the symptoms disappear for 1-3 years.

In the second group of patients operated on by the TEPT method in the remote period after constipation surgery, they were episodic in nature, and urinary incontinence was not observed. Fecal incontinence was noted only in the form of a temporary nature by the type of increased stool and fecal incontinence were in the presence of loose stool. After receiving the ESM and bougieurage and the complex rehabilitation treatment, they gradually disappeared within 6–9 months.

Thus, the study of immediate and long-term results of the children's treatment with HD, operated on in infancy, using the traditional Soave-Boley method and modern minimally invasive technologies according to TENTC and the Georgeson method, shows different results.

At the same time, radical one-stage surgical treatment for distal forms of HD using minimally invasive TEPT method is a priority in infancy, which can reduce complications to a minimum with improved functional outcomes of treatment, relative to two-stage treatment.

Results

1. In the surgical treatment of distal forms of HD in infants, the TEPT method is the method that is preferred for selection and the colostomy is not placed on.
2. With proximal and decompensated forms of HD with the presence of a colostomy, the optimal method of radical surgery is abdominoperineal proctoplastics according to the Soave (Boley and Lenyushkin) technology with the simultaneous elimination of the colostomy.
3. In the complicated, acute course of the subtotal form of HD during the neonatal period, primary emergency colostomy application saves the life of a child and contributes to the treatment of bacterial and HAE and the subsequent use of abdominal-perineal proctoplasty according to the Soave technology (Boley and Lenyushkin) with the simultaneous elimination of the colostomy.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Especially the Development of Myocarditis in Hypothyroidism in Postnatal Ontogenesis

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Abstract

The morphological changes in the myocardium of the offspring were studied when the mother had hypothyroidism in the rat to the female mother during the feeding of the rats.

The aim of our work was to identify structural and morphological changes in the walls of the heart of baby rats born to mothers in a state of hypothyroidism and the use of the antioxidant Mexidol.

The material for the experiment, served microscopic myocardial preparations of 50 rats of different periods of early postnatal ontogenesis.

Results: The morphological structure of the myocardium of the heart of baby rats born to mothers, dystrophic changes were observed in a state of hypothyroidism. The observed changes reached a maximum by the 14th day of studies, manifested by perivascular and interstitial lymphohistiocytic infiltrate. Changes should also occur in the ultrastructure of the heart, which leads to changes in myofibrils, damage to the mitochondria, which affect the microvessels with a violation of the components of the vascular walls, which are developing intercellular edema.

Keywords: *Heart, myocardium, hypothyroidism, myocytolysis, mexidol.*

Introduction

One of the most common forms of functional disorders of the thyroid gland is hypothyroidism, which develops as a result of a long-term persistent deficiency of thyroid hormones or decreasing their biological effect on the cellular level. Hypothyroidism may not be detected for a long time. This is due to the gradual, inconspicuous start of the process, satisfactory well-being of patients in mild to moderate degrees of illness, erased symptoms, regarded as overwork, depression, pregnancy.

Increasing of thyroid hormones causes systemic changes in the body[8]. Thyroid hormones regulate energy metabolism in the cells of organs, and their deficiency is manifested in a decrease in tissue oxygen consumption, a decrease in energy expenditure and the processing of energy substrates7. With hypothyroidism, the synthesis of various energy-dependent cellular enzymes necessary for the normal functioning of the cell is disrupted[12]. In the case of advanced hypothyroidism, mucinous (mucous) edema occurs - myxedema, most pronounced in connective tissue. Myxedema develops as a result of excessive accumulation of glycosaminoglycans in the tissues, which, having increased hydrophilicity, retain water.

The most frequent and serious complication of both thyrotoxicosis and hypothyroidism is damage to the cardiovascular system[6], so this problem is not only endocrinological, but also cardiological[5]. Heart pathology with thyroid dysfunction is often the leading disease in the clinic and often leads to disability, and in severe cases, to death in this category of patients[10]. The clinical picture shows a variety of complex cardiac arrhythmias[1], arterial hypertension, dishormonal cardiomyopathy with the development of chronic heart failure[2]. Meanwhile[3], data on the pathogenetic mechanisms of the development of "thyrotoxic" and "hypothyroid" hearts are scarce and contradictory[4]. An analysis of recent data shows that cardiovascular pathology remains the main cause of death in all developed worlds[14]. The heart is most often exposed to the pathological effects of endogenous and exogenous stimuli[13], as a result of which not only a violation of its function occurs, but also various pathological processes develop throughout the body[11]. It is known that heart damage is a very complex, poorly predictable and difficult to recognize based on clinical symptoms, medical and physiological process[9]. The defeat of any organ, in particular also the heart, naturally begins with the defeat of blood vessels and connective tissue stroma of the organ. In the subsequent lesions, the actual muscle tissue of the heart is exposed. Therefore, with heart lesions, the main quantitative and qualitative changes occur both in the stroma - vascular, and in the muscle structures of the heart. Structural changes are necessarily accompanied by clinical symptoms in the form of impaired cardiac activity.

The aim of our work was to identify structural and morphological changes in the walls of various parts of the heart of baby rats born to mothers in a state of hypothyroidism and the use of the antioxidant Mexidol.

Material and Method

The object of the study was the hearts of 50 white outbred rats of the following age groups: 3, 7, 14, 21, 30 days. Animals were divided into 3 groups. The first group of animals consisted of female rats receiving mercazolil at a dose of 5 mg per 100 g of body weight for 30 days, then for the month before pregnancy, a maintenance dose of mercazolil was used at a rate of 2.5 mg per 100 g. After pregnancy and during feeding, rats were injected with mercazolil in a maintenance dose of 2.5 mg per 100 g through a probe. As a solvent for mercazolyl used 1% starch paste. The second group of the experiment consisted of female rats who were injected with mercazolilum on the same days of the experiment as in the first group, and after pregnancy they received Mexidol at a dose of 0.5 mg per 100 g of weight together with mercazolilum. The third group included female rats of the mother of the control group, which, after birth, rat pups were daily fed on an empty stomach, depending on the term, for water. After each experimental week, the hormone level from the rat caudate vein was determined.

Results and Discussion

The control and experimental groups of animals were kept in the same vivarium conditions. At the end of the experiment, the rat pups of the experimental and control groups were killed under ether anesthesia. After that, the heart was isolated from animals, fixed in 10 per cent neutral formalin, followed by piping in alcohols, pouring in paraffin and preparing histological sections. Sections of 8-10 microns thick were prepared from paraffin blocks. Microsections were stained with hematoxylin and eosin, van Gieson.

The ventricular endocardium of the control group of animals consisted of longitudinally directed bundles of collagen fibers. A histological section reveals places where longitudinally lying bundles of collagen fibers interwoven with each other. Bundles of collagen fibers located closer to the ventricular myocardium are intertwined with bundles of collagen fibers from connective tissue layers located between bundles of cardiomyocytes of the myocardial inner layer (See App. A). The bundles of elastic fibers of the ventricular endocardium lie loosely compared to bundles of collagen fibers. In bundles of fibers adjacent to the myocardium of the ventricles, the density of arrangement increases, the direction of which changes from longitudinal to oblique, and are interwoven with bundles of connective tissue located between bundles of cardiomyocytes of the inner layer of the myocardium. Reticular fibers in the endocardium of the ventricles are located close to each other.

Upon further study of the slice, the cardiomyocytes of the outer layer of the myocardium are directed longitudinally, in the middle layer circular directed beams are detected; the inner layer contains weakly obliquely oriented bundles of cardiomyocytes. The inner bundles of fibers as they approach the endocardium acquire a more oblique direction and pass into the papillary muscles. A study of the direction of the bundles of ventricular myocardial fibers showed that the

circularly directed layer does not always have a clear orientation. The bundles of fibers of the middle layer on the lower wall of the ventricles are directed obliquely and deviate towards the endocardium. Between the layers of ventricular myocardial cardiomyocytes, the border is weakly expressed. They fit snugly together. The inner layer of the myocardium consists of parallel located bundles of cardiomyocytes running in parallel with the endocardium. The outer layer of the myocardium has a loose structure in which cardiomyocytes are located in different directions (See App. B).

In the middle layer of the myocardium of the left ventricle of the heart, bundles of cardiomyocytes are located perpendicular to the inner layer. In the center of cardiomyocytes, 1-2 oval-shaped nuclei are determined. The nucleus of cardiomyocytes is located in the center of the cell, while myofibrils are located on the periphery. In the right ventricle, bundles of cardiomyocytes in the layers of the myocardium are similar to the left ventricular myocardium. But unlike the left ventricle in the myocardium of the right ventricle, the thickness of the circular layer of cardiomyocytes is 2-3 times thinner than the thickness of the longitudinal layers of cardiomyocytes.

In the myocardium of the ventricles of the heart, depending on the site, bundles of collagen fibers have different directions. At the top of the heart, the bundles of collagen fibers are directed obliquely, while part of the bundles of collagen fibers changes direction from oblique to longitudinal. In the inner layer of the myocardium, bundles of collagen fibers lie in the longitudinal direction, separating bundles of cardiomyocytes from each other. In the middle layer of the myocardium, collagen fibers form bundles having a circular direction. When studying the outer layer of the myocardium, bundles of collagen fibers lie obliquely between bundles of cardiomyocytes. In the inner layer, the reticular fibers lie longitudinally and in the region of the apex of the heart they are intertwined with the reticular fibers from the outer layer of the myocardium of the heart. In the middle layer of the myocardium, the reticular fibers are located in a circular direction between the bundles of cardiomyocytes. Reticular fibers around bundles of cardiomyocytes form a network of various sizes and shapes (See App. C). The direction of the bundles of connective fibers depends on the direction of the cardiomyocytes.

The interventricular septum consists of two longitudinal and one circular layer. The longitudinal layers on the left and on the right are from the corresponding longitudinal layers of both ventricles, and the middle circular layer is formed due to the circular layer of the left ventricle of the heart. The internal diameter of the arteriole ranges from 9.5 to 15.2 microns and an average of 11.7 ± 0.6 microns. The inner diameter of the venules ranges from 15.7 to 20.5 microns. Myocardial sinusoids are elongated, oval or irregular in shape. The thickness of the inner diameter of the capillaries is from 5.7 to 11.4 microns, an average of 9.3 ± 0.6. Myocardial blood vessels are direct along the bundles of cardiomyocytes (See App. D). Around the cardiomyocytes and blood vessels are bundles of collagen and elastic fibers. The fibrous structure of the connective tissue of cardiomyocytes connecting with the blood vessels captures the capillaries around the muscle fibers.

In the epicardium of the ventricles of the heart, bundles of collagen and elastic fibers lie longitudinally, and have a higher density than bundles of collagen and elastic fibers of the endocardium. In the epicardium of the ventricles, the reticular fibers are located longitudinally.

The histological picture of 3-day-old rats undergoing hypothyroidism did not reveal significant differences compared with the control group. Cardiomyocytes had an oblong shape, formed muscle fibers, in the center of the fiber an oval-shaped nucleus was determined and myofibrils were clearly differentiated. In the subepicardial zone of the myocardium, dilated and full-blooded veins were detected with signs of redistribution of blood by the presence of clotted red blood cells, and in the myocardial stroma, beginning edema was observed. The endocardium is also somewhat thickened and uneven, in some places foci of depression and deepening in the form of cracks and vessels of Tebesia are visible. Endothelial cells are somewhat hypertrophied and hyperchromic, sometimes with foci of enlightenment in the basement membrane.

A morphological study of the heart of 7-day-old pups born to mothers in a state of hypothyroidism, with minor changes in the form of expansion of visible vessels (See App. E). An increase in the permeability of microvessels and vessels of the venous link was accompanied by the release of the liquid part of the blood through the vessel wall to the surrounding connective tissue (See App. F). Due to inflammatory infiltrate, the vascular wall thickens and the lumen narrows. These discirculatory

disorders lead to perivascular edema, loosening of the intramuscular space. At the same time, cardiomyocytes are loosened with the development of dystrophy and vacuolization in their cytoplasm. Myofibrils have a granular appearance, of different thickness and density. During this period, the epicardium of the heart is significantly thickened due to the proliferation of young connective tissue cells and swelling of fibrous structures. The endocardium is also swollen, in places forms uneven thickenings from proliferative endothelial cells.

At the age of 14 days, destructive and inflammatory phenomena join the above-described discirculatory and dystrophic changes. In the myocardial stroma, increased swelling was noted mainly in the perivenular and pericapillary spaces. Collagen fibers are swollen, loosened, in some places there is a separation of collagen bundles, swelling of the main substance of the connective tissue with the initial signs of surface disorganization. Connective tissue cells are also swollen, their nuclei slightly increase in size. Vascular disorders are common, endothelial cells in the vessels, acquire a rounded shape. In the cytoplasm of cardiomyocytes, small vacuoles were found filled with a clear cytoplasmic fluid, i.e. hydropic dystrophy develops. Intracellular edema is focal in nature, along with dystrophically altered cardiomyocytes, unaffected cells are found. In the myocardium of the left ventricle of the heart, around the vessels and in places in the stroma, inflammatory foci consisting of lymphohistiocytic cells appear (See App. G). In this case, cardiomyocytes are loosened with the development of dystrophy and vacuolization in their cytoplasm. Myofibrils have a granular appearance, different thickness and density. The endocardium is also swollen, in places forms an uneven thickening of proliferative endothelial cells. At this time, the epicardium of the heart is significantly thickened due to the proliferation of young connective tissue cells and swelling of fibrous structures. A histochemical study reveals a significant decrease in the number of collagen structures in the composition of the epicardium, instead of them, the content of SHIK-positive substance increases, especially in the walls of blood vessels. Endothelial cells are somewhat hypertrophied, hyperchromic, sometimes with foci of enlightenment in the basement membrane.

The study process of growth dynamics of the wall thickness of both the left and right ventricles of the heart of rat pups born by mothers in hypothyroidism state, depending on different parts of the heart, showed that the wall thickness of all departments is less than the by control indices. Comparing the thicknesses of wall of the right and left ventricles of the animals of the experimental group with the control group, a significant lag in myocardial indices is revealed. These changes are being most pronounced in pups of 14 days of age. The thickness of the left ventricular myocardium is less than that of the control group by 29 per cent. The thickness of the right ventricle compared with the control group is less by 17 per cent (Table).

On the 21st day, vascular disorders persist in the myocardium: pronounced plethora of veins, stasis, numerous perivascular hemorrhages of a diapedetic nature and an increase in the intensity of edema is noted. Edematous fluid is located between the muscle fibers, as if pushing them apart. Swelling of not only connective tissue cells, but also their processes was revealed. The swollen processes of neighboring fibroblasts are in contact with each other and, as it were, form syncytium. In the myocardial stroma, numerous small infiltrates from lymphocytes, histiocytes and fibroblasts. In cardiomyocytes, signs of protein hydropic dystrophy with the development of intracellular edema are observed, numerous foci of plasmolysis throughout the myocardium are determined. Plots of plasmolysis (intracellular myocytolysis) look like optically empty spaces. With intracellular myocytolysis, dissolution of myofibrils in certain sections of the fiber along its length is noted. In adjacent areas, myofibrils are preserved. With silvering according to Foote, the formation of reticular fibers in the form of coarse clumps and intermittent thick dark brown fibrous structures is determined. A histochemical study using the Van Gieson method reveals loosening and a decrease in the number of collagen structures, especially in the epicardium, and an increase in the content of SHIK-positive substance. Elastic fibers of the endocardium and in the intramuscular stroma in a state of loosening and tearing due to edema and disorganization of the connective tissue.

The study process of the dynamics of growth of wall thickness of both the left and right ventricles of the heart of rat pups born by mothers in a state of hypothyroidism with the use of Mexidol depending on different parts of the heart showed that the wall thickness of all departments is less than by the control indices. Comparing the wall thicknesses of the right and left ventricles of the animals of the experimental group with the control, a significant lag in myocardial indices is revealed. These changes are being most pronounced in pups of 21 days of age. The thickness of the left ventricular myocardium is less than

that of the control group by 29 per cent. The thickness of the right ventricle compared with the control group is less by 17 per cent (Table).

After 30 days, interstitial edema intensifies and spreads to the entire myocardium, reaching the greatest value in the perivenular spaces. Due to edema, swelling of collagen fibers, their delamination and shedding are observed. The main substance swells and collapses, signs of disorganization of the connective tissue appear (See App. H). When stained with toluidine blue, the phenomenon of metachromasia is observed. Dystrophic changes in the myocardium acquire a diffuse character, increased resorption of the cytoplasm, intracellular edema is noted. The foci of plasmolysis are numerous and larger than on the 21st day. Intracellular myocytolysis extends to the entire myocardium; total damage to cardiomyocytes is noted. In the focus of myocytolysis, most myofibrils are absent, single fibrils are visible. At the same time, marked loosening of the subendocardial and intramural layers of the heart wall due to edema and dissociation of cardiomyocytes is noted.

The study process of the growth dynamics of thickness of wall of both left and right ventricles of a heart of baby of intact rats at different stages of postnatal ontogenesis depending on the parts of the heart walls has showed that by the 30th day of the current study, comparing to 3 days of age, thickening occurs on average in all parts of the heart 1.7 times, only in the interventricular septum - 1.5 times. These morphometric data are confirmed by microscopic rearrangements occurring in the dynamics of postnatal ontogenesis (Table).

The subepicardial layer of the myocardium remains dense, where cardiomyocytes form large muscle bundles. Inflammatory infiltrate and sclerosis are defined around the vessels. When staining according to the Weigert method, it is noted that in the walls of the arteries the inner elastic membrane is thickened, winding, sometimes homogenized. In the muscle layer and in the surrounding connective tissue, elastic fibers are fibrous and lysed.

In cardiomyocytes with small foci of myocytolysis, the nuclei are preserved. In cardiomyocytes with large foci of myocytolysis, the nuclei are destroyed with the development of collicational necrosis. Remains of fibers in the form of tubules surrounded by a sarcolemma are found in foci of collication necrosis; the contents are not stained. Sarcolemma is thickened and well stained with picrofuxin. The described tubules are collapsed, around them there are excessive deposits of glucosaminglycans.

In some areas, counter-abnormalities and small foci of coagulation are determined, fragmentation of muscle fibers is observed in some places. However, collision processes dominate in the myocardium.

Since metabolic imbalance primarily affects the energy supply system of cells, the therapy should be aimed at increasing energy generation and increasing myocardial resistance to hypoxia. The second experimental group included 26 rats, which were daily injected with mercazolyl and antioxidant Mexidol.

In rat pups 3 days old born from mothers to hypothyroidism and receiving Mexidol, the normal histological picture is determined in the rat myocardium, pathohistological changes in the myocardium are not detected.

After 7 days of birth, vascular disorders in the form of plethora and expansion of veins, stasis, plasmorrhagia are noted (See App. E). Vascular disorders persist, there is an increase in plasmorrhagia, plasma impregnation and fibrinoid swelling of the walls of the arteries.

After 14 days from the start of the experiment, against the background of the use of Mexidol in the myocardial stroma, a small edema was found mainly around the veins. In individual cardiomyocytes, intracellular edema was detected in the form of accumulations in the cytoplasm of vacuoles filled with tissue fluid.

Intracellular edema is focal in nature. In the myocardial stroma, vascular disorders persist in the form of plethora, stasis and venous stasis. When staining with toluidine blue, metachromasia was not detected; fibrinoid swelling of the walls of the artery is observed.

After 21 days, edema was found in some places in the myocardial stroma, which is focal in nature and has a lower intensity than in the first experimental group. In areas of edema, collagen fibers swell and homogenize. There is a superficial disorganization of connective tissue with the accumulation of glycosaminoglycans. On histological preparations, mucoid swelling is observed (See App. I).

In places in the cytoplasm of cardiomyocytes, hydropic dystrophy is observed, which is focal in nature. The transverse striation of muscle fibers is preserved, in some areas myofibrils swell. Vascular disorders become less intense.

After 30 days, against the background of daily use of the antioxidant Mexidol, partial relief of vascular disorders is noted. In the myocardial stroma, edema develops, which extends to almost the entire myocardium, however, the intensity of the edema is less pronounced compared with the experimental group I. In the connective tissue of the myocardial stroma, swelling and decomposition of the main substance, accumulation of glucosaminglycans, swelling, homogenization and partial decomposition of collagen fibers with the formation of a fibrinoid are observed. At this time, for the first time in the stroma, small focal infiltrates from lymphocytes, histiocytes, and fibroblasts were detected (See App. J).

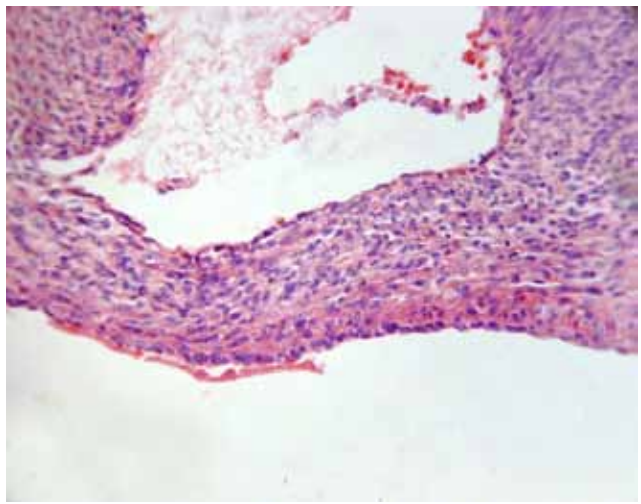
At the indicated times, a picture of intracellular edema is observed in the myocardium and for the first time against the background of Mexidol use, small foci of plasmolysis (myocytolysis) appear. These foci are optically empty portions of the cytoplasm with complete preservation of the plasmolemma. Compared with the experimental group I, the processes of myocytolysis are focal in nature.

Thus, with the daily use of the antioxidant Mexidol in rats of the experimental group II, no histological changes in the myocardium were detected in the first 7 days. The first signs of myocardial damage (beginning periventricular edema of single cardiomyocytes) appear on the 14th day, that is, 7 days later than in the first experimental group. An expanded picture of a change in the myocardium such as diffuse intracellular edema, myocytolysis was detected on the 28th day, then the network was later compared by comparison in the first experimental group. Destructive processes with total damage to the myocardial fibers in the experimental group II of rats were not detected. Regenerative processes with the replacement of damaged myocardial cells with connective tissue proceeded more intensively in comparison with the experimental group I. The above suggests that Mexidol has a protective effect on the myocardium under conditions of hypothyroidism.

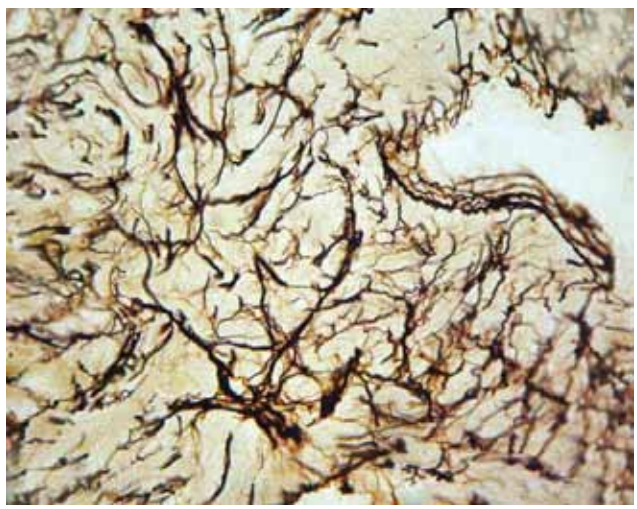
Appendix A: The wall of the left ventricle of the heart of a rat of 14 days of age in the control group. Collagenic fibers of the endocardium. Coloring: according to van Gieson. Vis.: approx. 10, vol. 40.



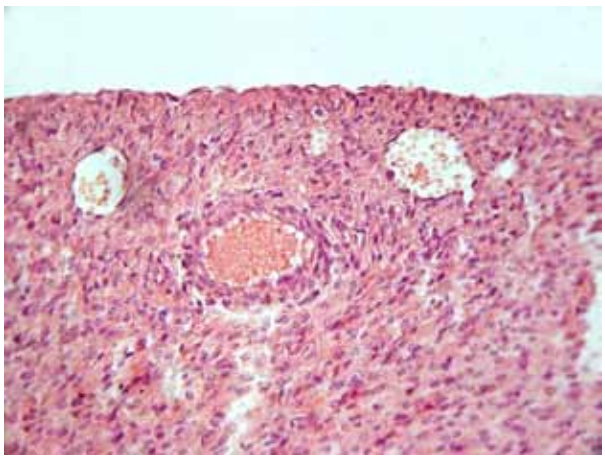
Appendix B: The side wall of the right ventricle of 21 days of age of the control group. Coloring: hematoxylin and eosin. Vis.: approx. 10, vol. 20.



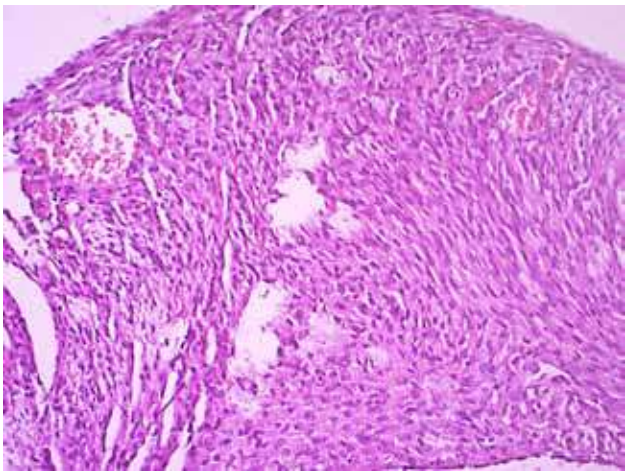
Appendix C: The front wall of the left ventricle of the heart of rats of 14 days of age in the control group. 1. Increased content of endocardial reticular fibers. 2. Reticular myocardial fibers. Coloring: imputation according to Foot in the modification of Yurina. Vis.: approx. 10, vol. 40.



Appendix D: Vessels of the posterior wall of the left ventricle of 21 days of age in the control group of animals of the subepicardial layer. Coloring: hematoxylinandeosin. Vis.: approx. 10, vol. 40.



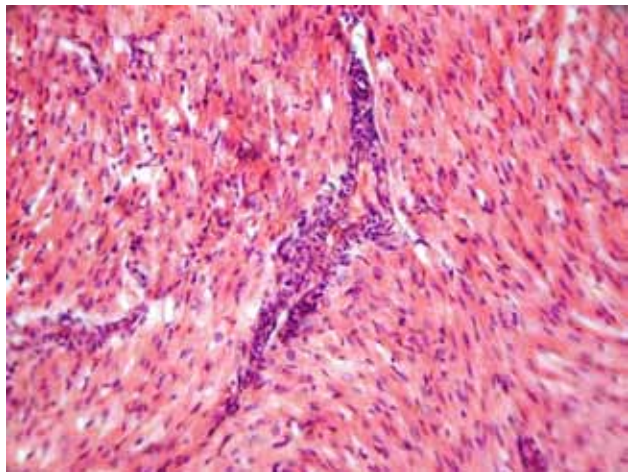
Appendix E: Anemia of the pancreatic vessels of the heart and blood supply. Hematoxylinandeosinstain. Vis.: vol. 20, approx. 10.



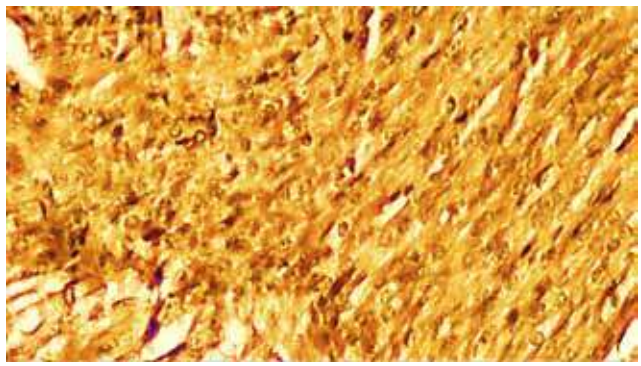
Appendix F: Pancreatic tissue of a 7 day old rat with blood and vasodilatation. Painting according to Van Gieson, Vis.: vol. 10, approx. 10.



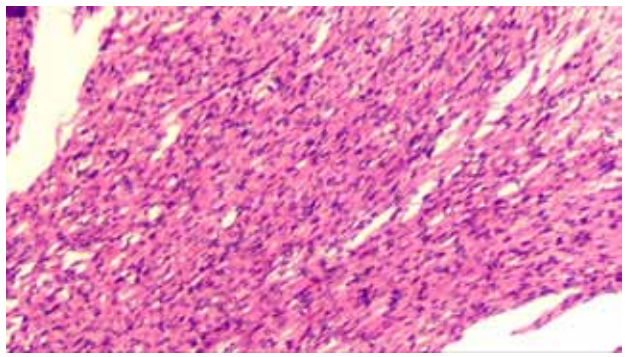
Appendix G: LV wall of a 14-day-old experimental group rat. Lymphohistiocytic infiltration around the vessels. Hematoxylin and eosin stain. Vis.: vol.20, approx. 10



Appendix H: Myocardial tissue with slight sclerosis of the capillaries. The staining of the vessel wall is densely red. Coloring according to Van Gieson, Vis.: vol. 40, approx. 10



Appendix I: The cytoplasm of some cardiomyocytes is unevenly colored, sarcolemma is clearly defined. In the sarcoplasm, transverse bands are visible. Hematoxylin and eosin stain. Vis.: vol. 20, approx. 10



Appendix J: Wall of the right heart core of a 30-day-old rat born to mothers with hypothyroidism. Myocardial hypercellularity is preserved. Hematoxylin and eosin stain. Vis.: vol. 20, approx. 10

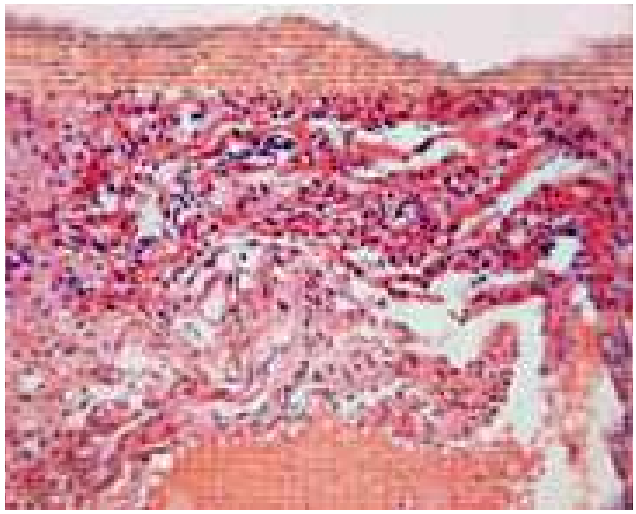


Table: Indicators of the thickness of the layers of the lateral wall of the ventricles of the rat heart, in microns

Days	Ventricles	Control	1st Group of experiment	2nd Group of experiment
3	L, n=9	1149±33	960±22,1	958±22,0
	R, n=9	381±12	295±7,4	328,5±8,2
7	L, n=7	1327±33,2**	976±28,3	1045±30*
	R, n=7	442±11,9**	328±6,6**	342±6,84
14	L, n=9	1544±35***	1105±28**	1272±32***
	R, n=9	500±14,0**	360±9,7*	412±11***
21	L, n=8	1738±46,9**	1419±33***	1513±35**
	R, n=8	571±14,3**	470±13***	507±14***
30	L, n=8	1935±21,3***	1565±35,5*	1758±22,3***
	R, n=8	610±18,1**	402±51,1**	540±14,9**

Findings:

1. In transient experimental hypothyroidism, the use of mercazolil in animals in the ventricular myocardium results in dystrophic, destructive and atrophic changes in cardiomyocytes, diffuse edema and stromal fibrosis. The first signs of hypothyroidism were detected on days 7-14, a detailed picture develops on the 21st day.
2. When using an antioxidant in the myocardium of experimental animals, signs of hypothyroidism occur on the 21st day, and a detailed picture on the 30th day.
3. The intensity and prevalence of morphological changes are less pronounced, destructive changes in the myocardium are not detected.

The use of antioxidants in experimental hypothyroidism in laboratory animals has a protective effect and prevents the development of severe destructive changes in the myocardium.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Neuroimmunological Characteristics of Idiopathic and Symptomatic Epilepsy in Accordance with the Clinical Course

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Abstract

The authors have scientifically determined an immunoreactivity enhancement, which one can indicate by a change in the level of neurotropic autoantibodies in relation to proteins and neurotransmitter receptors in epileptogenesis and the formation of aberrant plasticity among patients with symptomatic and idiopathic epilepsy. Epilepsy is a variant of autoimmune dysregulation, in which an increase in the content of autoantibodies in relation to neuro-specific proteins and neurotransmitters causes the genesis, progression and preservation of the pathological process in accordance with the etiological factor.

A new impulse to study the problem of epilepsy immunopathogenesis should be the ideas of in the last decade about the function inseparable integration of the two main integrative body systems: the central and the immune nervous systems[2]. On the basis of information about neuroimmune interaction, new scientific disciplines — Neuroimmunology and neuroimmunopathology formed[5].

Keywords: *Epilepsy, pathogenesis, immunoreactivity, neurotransmitter, serum, protein.*

Introduction

Currently, thanks to the researchers of immunoneuroendocrine regulation, we know that in the regulation of homeostasis involves plenty of natural autoantibodies (Ab) that reversibly interact with different antigens (AG) of its own body[3]. Natural autoantibodies pass through the synthesis within the body of healthy persons since the fetal life and throughout life [8].

As a result, healthy persons of different genders and ages have only minimal differences (immune fingerprints) in the serum content of various AT. We found that the AT production of different specificity regulates (by the principle of feedback) by the content of the appropriate target antigens4. It indicates the presence of powerful mechanisms for maintenance of the physiological (normal) level of production and secretion of various AT variants[6]. The part of Ab does not have a specific organ orientation and interacts with the ubiquitous AG (collagen, DNA, cytochromes, etc.). The other part interacts with organ-specific antigens (neuro-specific proteins, organ-specific isoenzymes, insulin, etc.). This also applies to Ab which interacts with nerve cell proteins or neurotropic Ab (NAb).

Among the patients with diseases of the nervous system, there are often changes in the serum content of NAb. Whereby, the researchers mainly pay attention to situations characterized by an increase in the neurotropic antibodies content of a certain specificity, which stands as pathological autoimmune aggression[7]. However, in accordance with the regulatory functions of Ab, the disorders of serum ratios in refard to different types of NAb can also be important, and the growth in the production of certain Ab can have not only a pathological, but also an adaptive-compensatory value[6].

Recently, there is more and more attention in regard to the participation of a single neuroimmunological network within the genesis of many diseases[3]. There is a wide discussion of epilepsy immunological aspects in modern literature8.

The pathogenic role and the diagnostic value of Ab to the brain proteins and the receptors of neurotransmitters require further study[2].

The Purpose of the Research: To study the immunoreactivity of neurotropic autoantibodies and neurotransmitters in case of symptomatic and idiopathic epilepsy.

Materials and Method

The study bases on a survey in relation to 86 patients with epilepsy in the age of 16 to 70 years, among them 23 patients had idiopathic epilepsy and 64 patients had symptomatic focal epilepsy. Each patient passed thorough preliminary anamnestic and clinical selection under method of stratified randomization through inclusion and exclusion criteria. The criteria for patients inclusion within the study: adults, epileptic attacks at the time of hospitalization or in history, idiopathic and symptomatic epilepsy. The exclusion criteria: children, cryptogenic epilepsy, pseudepileptic seizures, psychogenic reactions, conversion seizures (hysteria). The scope of the study included: clinical neurological examination, physical status research, neuropsychological testing of higher cortical functions, neurophysiological examination (EEG), laboratory method (clinical blood analysis, blood biochemistry, immunity status test), functional research method (ECG, EchoCG), neuroradiological research method (MRI or CT scan of the brain).

The seizures type determination is in accordance with the International League Against Epilepsy (1981). The diagnosis of epilepsy correspond to the International classification of epilepsy (ILAE 1989, 2001)[12].

Quantitation of serum immunoreactivity of antibodies in relation to neurotransmitter receptors (glutamate, GABA, dopamine, serotonin, and holinoreceptors) and neuro-specific proteins (NF200, Gfap, S100, and OBM) performed by means of solid-phase immunoenzyme method ELI-N-Test and test kits of the same name, manufactured by international research center "Immunculus" (Russia). We determined the values of AAb immunoreactivity within the range from 80 to 140 Units, and the Ab1/AIAb2 immunoreactivity index within the range from 0.8 to 1.2[16] as the norm.

We compared the results of the study with data from a control group output that included 32 clinically healthy individuals (in the age from 24 to 65).

We performed the analysis of the obtained indicators by the software package "SPSS for Windows" and "STATISTICA" Microsoft Excel with material processing with the method of variation statistics. We maintained the reliability assessment of the obtained results by the paired method with the Student's t-test . We considered the differences significant at $p<0.05$.

Research Results

We determined the level of AAb in relation to neurotropic proteins and neurotransmitters in the blood serum of 59 patients with epilepsy. We formed the following clinical groups to conduct immunology research : the first group - 17 patients with idiopathic epilepsy; the second group – 42 patients with symptomatic epilepsy. The control group consisted of 16 clinically healthy individuals.

The results of the conducted studies showed that among clinically healthy individuals (control group), natural AAb in relation to the studied neurotropic proteins within certain limited titers are normal components of the blood serum among practically healthy people, and therefore, in accordance with the recommendations of the test system manufacturers, they determined the design as "internal standard serum" (table 1).

In case of immunology research results assessment, we found that both groups of patients differed from the control group, both in terms of the level and degree of the immunological indicators dispersion under research. In order to identify possible pathogenetic features, we performed the analysis of neuroimmune relationships in both groups in comparison with the control group.

Table 1: The level of serum autoantibodies in relation to neurotropic proteins among patients with symptomatic and idiopathic epilepsy, ie.

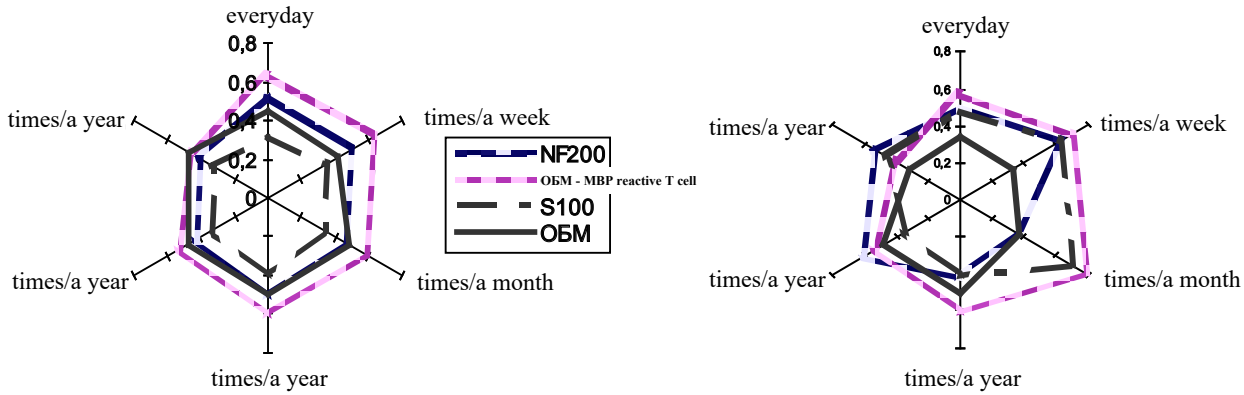
Indicators of	SE (n=42)	IE (n=17)	Control (n=16)	SE and IE, P<	SE and KG, P<	IE and KG, P<
NF200	121,8±8,2	97,6±8,9	72,9±7,1	0.05	0.05	0.05
GFAP	102,5±8,3	80,4±7,2	57,9±5,7	0.05	0.01	0.05
S100	124,1±4,6	150,3±11,8	77,5±7,6	0.05	0.01	0.05
MBP	102,4±8,0	78,8±7,7	58,8±5,5	0.05	0.01	0.05

We found that in the group of patients with SE, the levels of the studied AAb in relation to all neuro-specific proteins significantly exceed the values of not only the «internal standard serum», but also similar indicators of patients with IE. Thus, we found that the highest level of AAb in both groups was the level of AAb in relation to S100, whereby among patients with SE this indicator exceeded the standard values by an average of 1.6 times (P<0.01), and among patients with IE – of 1.9 times (P<0.05). At the same time, there was a significant increase in the AAb output within the group with IE in comparison with SE (150.3±11.8 cu vs 124.1±4.6 cu, P<0.05). Such a significant increase in the level of AAb in relation to the S100 protein, which is a calcium-binding protein, may be a confirmation of the hypothesis that one of the links in the pathogenesis of epileptic attacks is a growth in the permeability of neural membranes for Ca2+ ions with an increase in their intercellular space concentration.

The examination of the AAb level in relation to the NF200 protein also revealed a significant increase in their titers among patients of both groups (on average of 1.7 times for SE and 1.3 times for IE, respectively, P<0.05). Whereby, there was also a significant predominance of the AAb level in relation to NF200 among the patients with SE (121.8±8.2 units vs. 97.6±8.9 units, P<0.05), which indicates excessive plasticity, which apparently contributes to the preservation of stronger pathological connections of the epileptic system among patients with SE (Johnston M. V., 2004).

The level of serum AAb to the neuro-specific protein MBPa,ong patients of both groups also significantly exceeded the values of the "internal standard serum" (on average of 1.7 times among patients with SE; p<0.01 and 1.3 times among patients with IE; p<0.05), while on average of 1.3 times higher than these indicators among patients with SE in relation to patients with IE, (102.4±8.0 cu vs 78.8±7.7 cu, P<0.05).

The level of serum AAb in relation to GFAP was approximately at the same level as AAb to MBP and was 102.5±8.3 cu among patients with SE, and 80.4±7.2 cu among patients with IE, significantly exceeding the indicators of the control group by an average of 1.8 (p<0.01) and 1.4 times(p<0.05), respectively. The comparative analysis between the groups also revealed a statistically significant increase in AAb in relation to MBP among patients with SE in comparison to IE by 1.3 times (P<0.05). As a result of this analysis, we found a positive correlation between the duration of epilepsy and AAb levels in regard to all neurotropic proteins under study, which mostly referred to patients with SE. We found a similar direct relationship between the level of AAb output and the frequency of seizures among the patients under examination. Moreover, this relationship did not depend on the etiological factor of epilepsy (Fig. 1).



Symptomatic epilepsy **Idiopathic epilepsy**

Fig. 1. The correlation relationship between the frequency of seizures and the level of autoantibodies in regard to neurotropic proteins in accordance with the type of epilepsy

Thus, clinical and immunological analysis revealed a clear pattern relation between the content of antibodies and the neurotropic proteins in conformity with the aetiology, duration and course of SE and IE. The regularity of the detected increase of neurotropic AAb in close relationship both with each other and with the

frequency of seizures is evidence of the neuroimmune dysregulation aggravation with the increase of the clinical course severity among the patients with epilepsy. The disruption of the brain's immune barriers permeability leads to the AAb formation in regard to neurotropic proteins, which increases the lack of the brain trophic support and the progression of damage processes. In case of differentiation of the obtained results in accordance with the form of epilepsy,we found that the greatest sensitization of immune cells to the neuro-specific antigens NF200, GFAP and MBP is with SE, which, in turn, is a reliable reflection of the destructive process and pathological penetrance of the blood-brain barrier.

The table 2 contains the research results of the AAb level in relation to neurotransmitters. Within the comparative analysis of the indicators which refer to patients with both IE and SE against the data of the "internal standard serum", we found a significant unidirectional increase in the individual level of serum AAb immunoreactivity in relation to the receptors of all neurotransmitters under study. We obtained an interesting data in regard to patients with concern to the level of AAb in relation to choline receptors. In particular, the patients with IE had the level of AAb in relation to this neurotransmitter 162.9±5.3 cu, with above-limit value of the "internal standard serum" by 4.8 times ($p<0.01$), and the indicators of patients with SE by 3.5 times (46.7±4.9 cu, $p<0.05$).

Table 2: The level of serum autoantibodies in relation to neurotransmitters among patients with symptomatic and idiopathic epilepsy, cu.

Indicators of	SE (n=42)	IE (n=17)	Control (n=16)	SE and IE, P<	SE and KG, P<	IE and KG, P<
CHL	46,7±4,9	162,9±5,3	33,9±3,3	0.05	0.05	0.01
GLU	73,8±5,3	98,1±8,8	57,2±4,9	0.05	0.05	0.01
GABA	53,7±5,4	71,8±4,7	45,4±4,9	0.05	-	0.05
DA	62,6±5,0	74,4±4,2	46,9±4,9	0.05	0.05	0.01
SER	62,9±5,3	76,9±3,4	56,2±4,6	0.05	-	0.05
V-zav.(B-зав.) Ca-channel	98,5±7,0	78,6±6,4	45,9±4,2	0.05	0.05	0.01

The further analysis of neuroimmune relationships among patients with epilepsy showed that the level of AAb in regard to glutamate and voltage-dependent Ca-channels was also significantly high among the AAb under study within both groups.

Thus, the indicators of patients with SE constituted 73.8±5.3 and 98.5±7.0 cu, respectively, which exceeded the indicators of the "internal standard serum" by an average of 1.3-2 times ($p<0.05$), and in regard to patients with IE – 98.1±8.8 and 78.6±6.4 cu, respectively with increase of the standard indicators by an average of 1.7-2 times ($p<0.01$).

Among patients with IE, the AAb in relation to glutamate significantly exceeded not only the control values, but also the indicators of patients with SE (respectively 98.1±8.8 and 73.8±5.3 cu, $p<0.05$). Such a significant increase in the level of AAb in regard to glutamate among the patients of both groups indicates the evident disorder of the excitative processes as activation result of membrane neurotransmitter receptors and mechanisms of glutamate excitotoxicity. Whereby, we note more significant disorders of the glutamateergic system within the course of IE.

One a priori can determine the obtained output as evidence of a gross imbalance within the glutamatergic system among patients with IE, which is the starting point for the neuronal sprouting processes.

The levels of AAb to GABA, dopamine and serotonin within the examined groups also exceeded the standard indicators and were approximately at the same level. However, we found the significant differences from the "internal standard serum" only among patients with IE (AAT GABA 71.8±4.7 cu, $p<0.05$; AAT DA 74.4±4.2, $p<0.01$; AAb SER 76.9±3.4 cu, $p<0.05$).

We found the significant differences among the

patients with SE only within the ratio of AAb in regard to dopamine (62.6±5.0 cu, P<0.05).

The high level of autoantibodies in regard to GABA is evidence of disorders within the GABA-ergic system, which increase the neurotoxic effects of glutamate on the one hand, and inhibit the structure of the antiepileptic system on the other hand (Gusev E. I., Geht A. B., 2009).

In particular, AAb significant increase in relation to this neurotransmitter among patients with idiopathic epilepsy may indicate a gross imbalance in the glutamatergic system and a marked depletion of the GABA-ergic system within this group of patients, which is the starting point for the processes of neuronal sprouting.

The high levels of AAb in relation to dopamine and serotonin among patients with IE and their significant difference from the indicators of the "internal standard serum" confirm the close relationship of the glutamateergic system with the system of biogenic amines, whose dysregulation leads to a detrimental effect on neurons and has a proepileptic effect. One can interpret such ratio of AAb as evidence of a significant autoimmune response from the nervous tissue, which, in turn, contributes to the maintenance of the pathological epileptic system among patients with IE.

Thus, the AAb increase in relation to the ligand-binding site of neurotransmitter receptors (Glu-P, GABA-P, DOF-P, Ser-P, and Hol-P) indicates changes within the corresponding neuron systems. More high serum level of AAb in regard to neurotransmitter receptors may indicate the presence of different mechanisms in order to implement the neurotransmission and neuroplasticity among patients with IE and SE.

As a result of correlation analysis, we found a positive relationship between the duration of epilepsy and the levels of AAb in regard to the receptors of all neurotransmitters, most closely expressed among patients with IE in comparison with SE. The study of correlation relationships between neurotransmitters and the frequency of paroxysms has established a direct average and strong dependence of the number of paroxysms on the level of neurotransmitters. The strength of correlation relationships did not depend on the form of epilepsy.

The obtained output confirms the assumption in regard to multi-factorial causes of the development and progression within the epileptic process (Fig. 2).

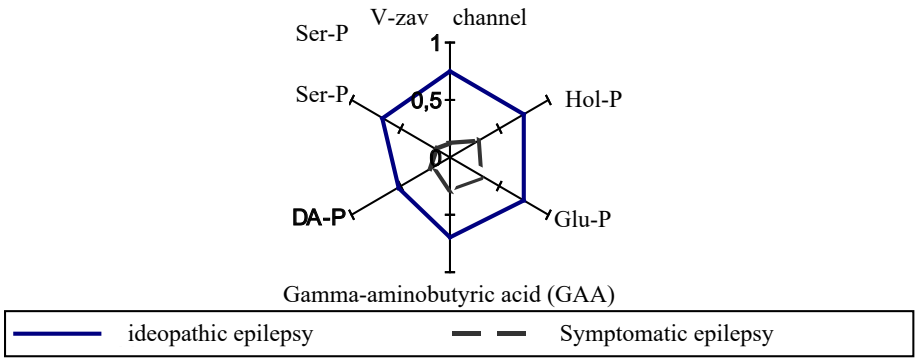


Fig. 2. The correlation indicators between the duration of epilepsy and levels of AAb in regard to neurotransmitter receptors

Thus, the regularity of the autoantibodies detected increase in regard to all neurotransmitters under study within close relationship with each other, as well as with the frequency of seizures and duration of the disease is evidence of neuroimmune dysregulation aggravation as the clinical course severity in cases of epilepsy .

The correlation analysis of the cognitive disorders relationships and the content of antigens in relation to the main neurotransmitters, in particular GFAP, among the patients with SE found a strong relationship between the indicators of clock drawing test (r=-0.753 and -0.755) and MMSE (r=-0.806 and -0.892), as well as with tests for speech activity (r=-0.736, -0.540 and r=-0.562, -0.642), words memory (R=-0.679 and R=-0.753, respectively) and repeating numbers in reverse order (R=-0.568 and R=-0.695, respectively).

With the level of AAb in relation to the rest of the neurotropic proteins, there was a weak relationship, and in some cases it was absent at all. The relationships of AAb level in regard to neurotransmitter receptors and cognitive disorders among patients with SE were weak.

The influence of the AAb level in relation to GABA by the indicators of clock drawing test (r=-0.274), MMSE (r=-0.272), phonetically mediated associations (r=0.268), word memorization (r=-0.204) and repetition of numbers in reverse order (r=-0.265), which expressed by the indicators of inverse and direct weak correlation relationships.

Thus, we found the only significant inverse correlation among the patients with SE between the level of AAb in relation to GFAP and MBP and the indicators of neuropsychological tests. The obtained data once again convincingly confirm both the fact of pathological BBB permeability among patients of this group, and the significance of this fact in the occurrence and maintenance of cognitive disorders in case of SE.

The study of correlation relationships between similar neuroimmune indicators and the results of neuropsychological tests among patients with IE showed a different nature of the relationships within this group in comparison with SE. Thus, in case of correlation analysis among patients with IE, we found weak relationships between the indicators of AAb in regard to GFAP and MBP and the parameters of the cognitive sphere. In particular, we registered the most significant indicators of reverse correlation within the clock drawing test assessment (R=-0.361 and r=-0.355, respectively) and the speech activity test (R=-0.322 and r=-0.298, respectively).

There was a high, medium, and weak correlation relationship between the indicators of neuromediators and neuropsychological tests. We obtained the particularly evident output within the relationship of cognitive disorders with the level of AAb in relation to choline and GABA receptors. Thus, one can establish the following relationships with the indicators of clock drawing test (R=-0.852 and r=-0.869, respectively) and MMSE (R=-0.795 and r=-0.790, respectively), as well as with the indicators of the speech activity test (r=-0.697 and r=-0.872 with semantically mediated associations and r=-0.872 and r=-0.635, respectively), word memory (R=-0.697, r=-0,826) and the numbers repetition in reverse order (R=-0,725 and R=-0,822).

The obtained data presents a great scientific interest and a priori helps us to determine the identified cognitive disorders among patients with IE, since it is known that GABA receptors mediate “long-term” GABA - dependent reactions within the central nervous system by complex processes trigger in relation to various neurotransmitter systems interactions (in particular, GABA and cholinergic). The identified neuroimmune dysregulation causes the neurotransmitter processes disorders at all levels of synaptic regulation, and as a result, there is a neurotransmitter imbalance formation, a priori in the cortex and hippocampus – the leading ares of the cognitive functions implementation.

In case of neuroimmunological indicators study inregard to patients with epilepsy, we found that circulating AAb in relation to neurotransmitter receptors, in particular to glutamate, GABA, dopamine, serotonin and holinoreceptors, in the blood serum of patients with epilepsy indicate changes within the corresponding neuron systems. A higher serum level of AAb in relation to neurotransmitter receptors among patients with SE may indicate the presence of different mechanisms for the implementation of neurotransmission and brain plasticity in cases of IE and SE.

Thus, we indicate the heterogeneity of patients with epilepsy in terms of molecular development mechanisms, the causes of epileptic attacks. In particular, one can use the circulating AAT in relation to neurotropic proteins and neurotransmitter receptors within the blood serum of patients with epilepsy as additional prognostic “immunobiochemical” criteria for the course of the disease and the efficiency of antiepileptic treatment.

Conclusions

1. One of the leading mechanisms within the epilepsy pathogenesis is a complex change of neuroimmune relationships under a unidirectional increase in the level of autoantibodies in relation to the neuro-specific proteins S100, GFAP, NF-200, MBP and the neurotransmitters glutamate, GABA, dopamine, serotonin, serotonin and voltage-dependent Ca-channel. Whereby, the key link within the pathogenesis of idiopathic epilepsy is a neurotransmitter imbalance, and in the pathogenesis of symptomtomatic epilepsy – an increase in the level of AAb in relation to GFAP and MBP.
2. The changes in the levels of antibodies in relation to the neuro-specific proteins S100, GFAP, NF-

200, MBP and the neurotransmitters glutamate, GABA, dopamine, serotonin, serotonin and voltage-dependent Ca-channel depend on the form and duration of epilepsy, the nature and frequency of seizures. We noted a high specificity of increasing antibody levels in regard to patients with increase of disease severity.

3. The rise of antibodies level in relation to neurospecific proteins S100, GFAP, NF-200, MBP and the neurotransmitters glutamate, GABA, dopamine, serotonin, serotonin and voltage-dependent Ca-channel within epilepsy can serve as a prognostic criterion of severe disease and used at an early stage in the selection of adequate antiepileptic therapy and the choice of further tactics patients management.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Our Experience in Treatment Acute Surgical Diseases of the Abdominal Cavity in Patients with Situs Inversus Viscerum (Transposition of Internal Organs)

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Abstract

Situs viscerumtotalis is a fairly uncommon anomaly of the mirror-imaged arrangement of the peritoneal cavity organs with a frequency of distribution in the range from 1: 5000 to 1: 20 000. Such an arrangement of internal organs is found in one case per 10 million[2]. In the early embryonic stage, the internal organs are located along the midline of the body. Normally, in the course of their subsequent development, they grow and rotate to the right, and – exceptionally rarely – to the left, which leads to the reverse arrangement of internal organs, i.e., to their transposition. In the case of complete (total) transposition of internal organs, all of them are inverted. In the case of partial transposition, it involves inversion of all or some organs of one of the body cavities (heart, stomach, duodenum and caecum, spleen). Dextrocardia, a condition in which the cardiac apex is pointed to the right, was first described by Marco Severino, in 1643. Matthew Baillie first described situs inversus more than a century later.

Keywords: *Patient, abdominal, organ, internal, surgical.*

Introduction

In 1600, Fabricius reported the first known case of liver and spleen reversal in humans. Kuchenmeister in 1824 recognized situs inversus in a living person for the first time. In 1897, Wehsemeyer was recognized for being the first to demonstrate the transposition of internal organs with an x-ray. This unusual anatomy of internal organs causes difficulties in diagnosing and treating diseases.

Situs viscerum inversus is an autosomal recessive genetic condition. Although itdoes not form a pathology in itself, it may be associated with certain abnormalities, mainly cardiac, which can be potentially life-threatening[2]. In case of complete or partial inversion of internal organs (viscerum inversus totalis or partialis), the abdominal organs are located on the opposite side. That means that the liver is placed on the left, the heart on the right, the spleen on the right, etc. According to Russian and foreign researchers, there are too few data on surgical interventions, in particular laparoscopic ones, in such patients[1]. The cases of acute surgical pathologiesin patients with complete transposition of internal organs are extremely rare in the world science literature.

Acute appendicitis is the most common surgical disease that surgeons, therapists, gynecologists, and other medical specialists deal with on a daily basis[3].

For the first time, the vermiform appendix of the cecum was described and drawn by Leonardo da Vinci in 1472. The term 'appendicitis' was first proposed by American surgeon Reginald H. Fitz, in 1886, at the Convention of the American Medical Association. In 1725, the first appendectomy was performed by Claudius Amyand, in England. The first fully laparoscopic appendectomy was carried out by K. Semm in 1983. Acute appendicitis is the reason for about 7% of abdominal surgeries. The incidence of acute appendicitis in patients with situs inversus totalis is about 0.016-0.024%[4].

Among urgent surgical pathologies, perforation of gastric and duodenal ulcers is about 1.5%, and, according to various researchers, among patients with peptic ulcer, it occurs in 5-30% of cases.

The main diagnostic criteria for ulcer perforation are free gas in the abdominal cavity which is found during the plain radiography and an ulcer with signs of perforation detected during fibrogastroduodenoscopy (FGDs). However, the removed clinical picture of perforation (covered and atypical) occurs in almost 36% of cases. In this regard, diagnostic laparoscopy is of particular diagnostic significance, since it allows to verify the diagnosis, assess the prevalence of peritonitis and determine further treatment tactics. As it is known, the main method of treatment of patients with ulcer perforation is operative[4]. With that, this pathology combined with situs inversus totalis is much less common than other urgent surgical pathologies[5]. The first case of perforation of duodenal ulcers with dextroposition was described in 1986.

Based on data from foreign literature, we can conclude that the occurrence of surgical pathologies in patients with situs inversus totalis is extremely rare. According to many researchers, to date, about 60 cases of laparoscopic cholecystectomy, 7 cases of colorectal cancer, isolated cases of pancreatoduodenal resection, hemihepatectomy and angiographic interventions in patients with situs inversus totalis have been described in the world scientific literature[3].

In clinical practice, when examining patients with a mirror-imaged arrangement of internal organs, there is a high probability of anatomical disorientation, which can lead to incorrect diagnosing or treating the disease. In this regard, we decided to share our experience of diagnosis and treatment of acute surgical diseases of the abdominal cavity with complete transposition of internal organs.

Below are 3 clinical cases of acute surgical pathologies with complete transposition of internal organs.

Case 1 and 2. In September and October 2019, patient A (23 years old) and patient B (21 years old), sought medical attention in the Department of Surgery of the TMA Multidisciplinary Clinic. Both patients complained of pain in the left iliac region, nausea, and fatigue. According to anamnesis, patient A. had been ill for the last three days, and experiencedpain of this nature for the first time. The patient A. also complained of aching pain in the right iliac region, followed by spreading to the left iliac region and area around the navel, nausea, expulsion of gastric contents, dry mouth and fatigue. At home, patient A took Mezyme and No-shpa medicines, which had a short-term positive effect. Subsequently, there was an increasing pain in the left iliac region. According to anamnesis, patient B had been ill for one day, and experienced pain of this nature for the first time. The patient B. also complained of aching pain in the epigastric region with subsequent spread to the left iliac region and around the navel. Among other symptoms, there was nausea, a single expulsion of gastric contents, dry mouth and fatigue. The patient B. did not seek medical attention in any medical institutions, andtook painkillers which had a temporary positive effect. Subsequently, there was an increasing pain in the left iliac region and around the navel.

According examination data, in both patients, abdomen participates in respiration, it is symmetrical, soft during palpation; in the left iliac region there is pain and local tension of the muscles of the anterior abdominal wall. The Kocher-Volkovich, Rovsing, Sitkovsky, Razdolsky, Obraztsov, Voskresensky, Bartomier-Michelson's signs results are positive. The Shchetkin-Blumberg sign's result is negative in all abdomen areas. Intestinal peristalsis noises are heard.

In the course of laboratory and instrumental research method, both patients underwent chest x-rays (Fig. 1).

Description of x-ray images: the chest is symmetrical. The edge shadows are not deformed, with a uniform structure. Soft tissue shadows without features. The pulmonary fields are fully pneumatized, without focal and infiltrative shadows. The roots of the lungs are poorly structured. The pulmonary pattern is not reinforced. Pleural sinuses are free. Heart: dextraposition is not enlarged, aorta without features.

On an abdominal MSCT scan, it was found: stomach, spleen, tail and body of the pancreas are located on the right side; liver, gall bladder, head of the pancreas, inferior vena cava – on the left. The shapes, sizes, and structure of organs have not been changed. The lymph nodes of the abdominal cavity and retroperitoneal space are not enlarged.

Both patients were diagnosed with acute appendicitis based on their anamnesis, physical examination, and instrumental data. Both patients underwent Laparoscopic appendectomy, using the RZ Medizintechnik, Germaniya device (Fig. 3).

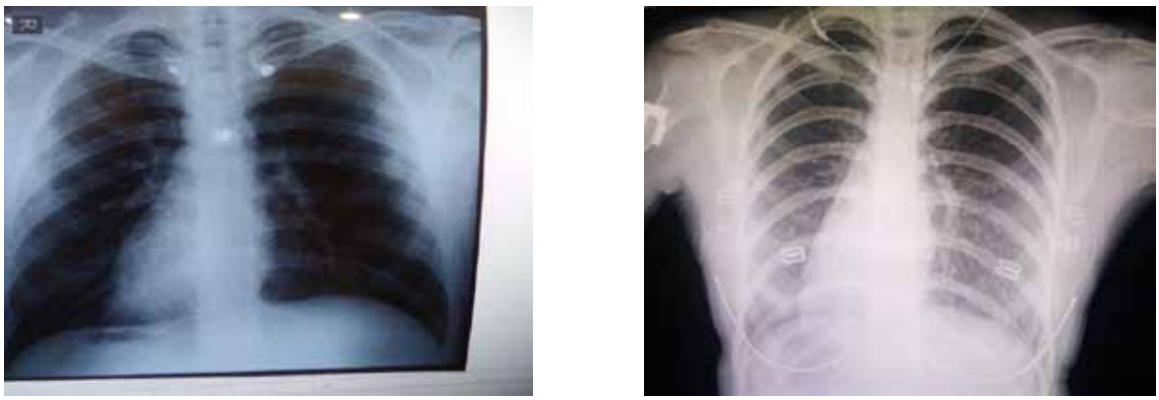


Fig. 1. Chest x-ray (patient A and B)

In order to clarify the diagnosis, an abdominal MSCT scan was performed, using a Siemens device (Fig. 2).

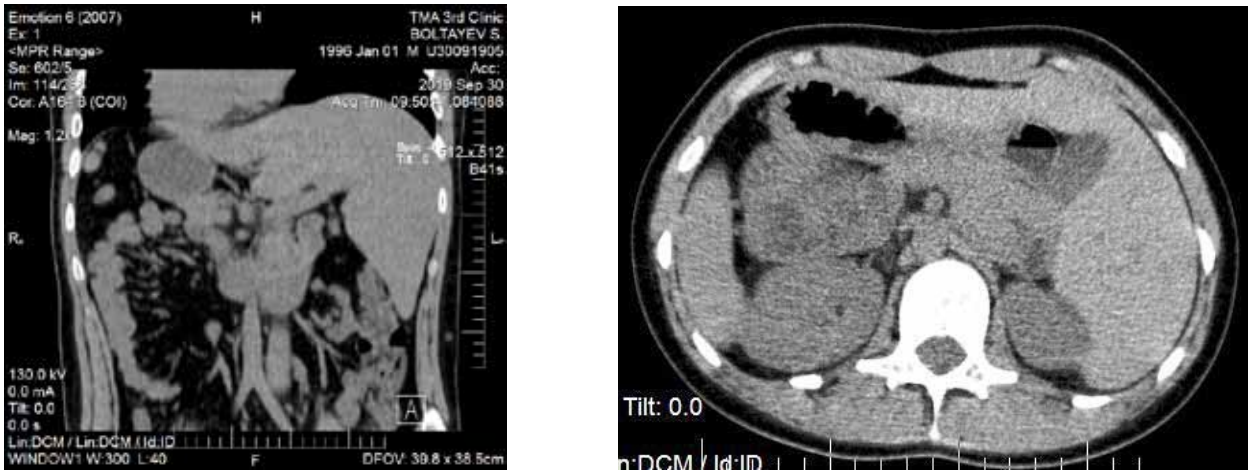


Fig. 2. Abdominal MSCT scan (patient A and B)

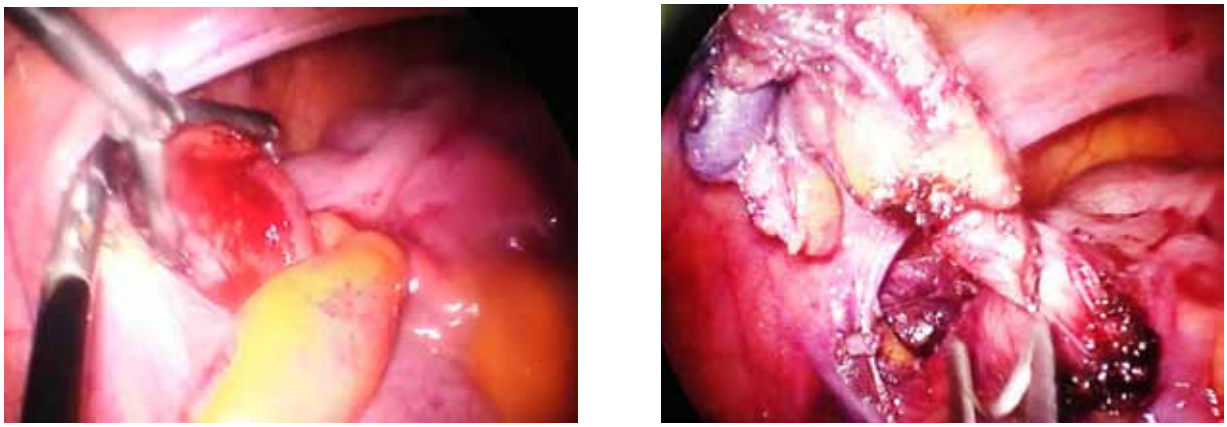


Fig. 3. Laparoscopic appendectomy (patient A and B)

The position of the surgeon and assistant, as well as the location of the trocars was opposite to the standard location for laparoscopic appendectomy (Fig. 4).

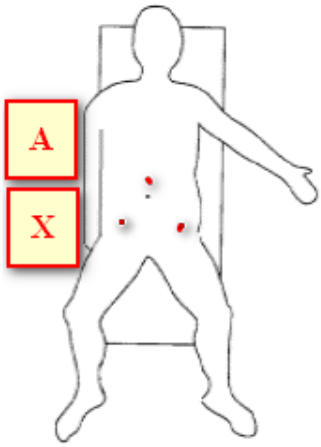


Fig. 4. Position of surgical team (X – surgeon; A-assistant) and location of trocars in laparosocpic appendectomy for patients with situs inversus viscerum

In both patients, surgical intervention proceeded without technical difficulties. For control purposes, a drainage was installed in a small pelvis. Postoperative diagnosis for both patients: *Main diagnosis:* acute phlegmonous appendicitis. *Related diagnosis:* aitus inversusviscerum. (histological study No. 3717-20 and No. 3717-35). The postoperative period proceeded without complications. On the 2nd day after the operation, after aabdominal ultrasonography (no free fluid was detected in the abdominal cavity and pelvis), the drainage tube was removed. Both patients were discharged on the 3rd day after the operation.

It can be concluded that performing laparoscopic appendectomy for patients with situs inversus totalis is not convenient in terms of ergonomics. It is also technically difficult.

Case 3. In September 2019, patient S applied to the Department of Surgery with complaints of pain in the epigastric region (in the left half of the abdomen), nausea, vomiting that did not bring relief, and fatigue. According to anamnesis, the patient denied ulcerative anamnesis. The aspect of the disease corresponded to a perforation of a stomach ulcer or duodenum: the 'knife-like' pain began in the epigastric region. During a local examination, the following data were found: the tongue is dry, with a white coating, the abdomen is not involved inrespiration, symmetrical, there is a surgical scar 10x0.5 cm in the right iliac region, consistent; during palpation, sharp pain in the epigastric region and in the left half, throughout the abdomen, the tension of the muscles of the anterior abdominal wall is determined, the Shchetkin-Blumberg sign result is positive in all abdomen parts.

Taking into consideration the previous appendectomy and identified situs inversus totalis in the patient, wehave performed the following instrumental research method:

Ultrasound impression: Total inversion of internal organs, free fluid in the abdominal cavity (Fig. 5).



Fig. 5. Abdominal ultrasound (patient S)

Chest and abdominal x-ray: Cardiac dextroposition!!!Abdominal radiography shows a free gas under both domes of the diaphragm (Fig. 6).



Fig. 6. X-ray of the chest and abdomen (patient S)

The patient underwent an emergency Laparoscopic repair of duodenal ulcer perforation and abdominal drainage (Fig. 7). Performing laparoscopic surgery was technically difficult, given the non-standard position of surgical team and location of trocars.

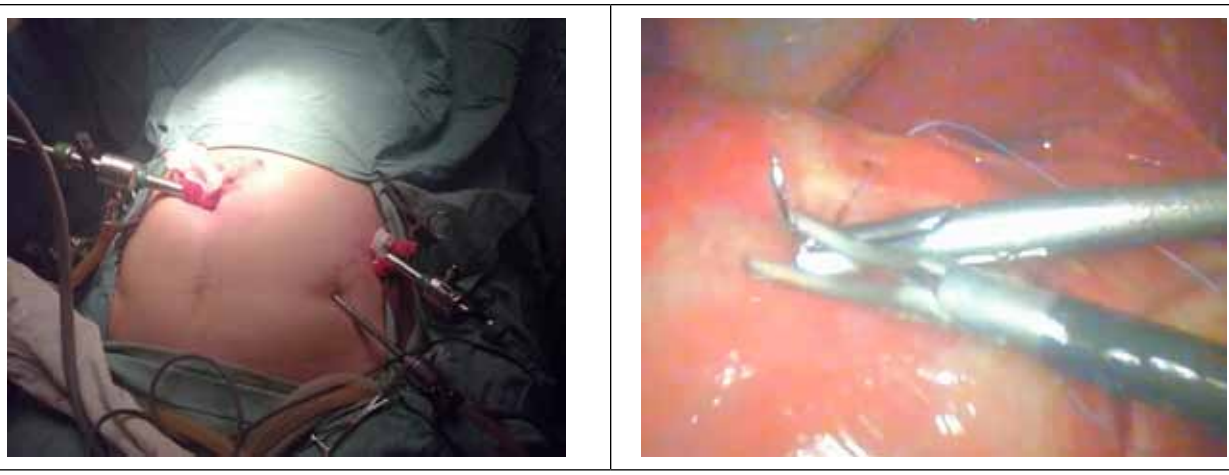


Fig. 7. Laparoscopic repair of duodenal ulcer perforation (patient S.)

The surgery lasted 1 hour and 20 minutes, and was completed with no complications. During the revision in the subhepatic region, the presence of dried serous-fibrinous fluid is found. With the further revision, no free fluid was detected in the suprahepatic region, in the right and left lateral channels, in the pelvis and in the interstitial spaces. On the anterior wall of the duodenum there is a perforating hole measuring 0.5x0.5 cm, with the infiltrative torus around it, up to 1 cm. The perforation is repaired with two-row interrupted sutures. Postoperative diagnosis: Primary diagnosis: duodenal peptic ulcer. Complication: duodenal ulcer perforation. Local serous-fibrinous peritonitis. The postoperative period proceeded without complications. On the 3rd day after the surgery, nasogastric tube was removed, on the 4th day after the surgery, drainage of the abdominal cavity was removed, after the control abdominal ultrasound. On the 5th day after the operation, the patient was discharged from the hospital in satisfactory condition.

Careful collection of anamnesis and performing all necessary diagnostic method (x-ray, ultrasound) makes it possible to timely and correct diagnosis and choice of treatment tactics.

Conclusion

The presence of a complete inversion of internal organs in patients with acute surgical diseases of the abdominal cavity does not form a contraindication to laparoscopic surgery. However, such arrangement of organs can create some inconvenience for surgeons. Due to this, correct installation of laparoscopic ports and rational positions of the operating team members seem to be necessary. The high probability of iatrogenic injuries during manipulations in the abdominal cavity requires increased attention from the surgeon. The operation should be performed by a team with significant experience in endovideosurgery.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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State Rubricator of Scientific and Myoma, Senior Reproductive Age and IVF/IVF+ICSI is there Any Connection?

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Abstract

In women of older reproductive age, due to the physiological decrease in the ovarian reserve and more frequent concomitant gynecological disorders, the effectiveness of the IVF/IVF-ICSI protocols is significantly reduced, which is associated with a smaller number of obtained oocytes and low quality of embryos 5.

Keywords: *Myomectomy, myoma, numb, embryo.*

Introduction

According to World Health Organization report in 2010, 20–25% of women suffer from myoma, and it is estimated that around 235 million women are affected worldwide, representing 6.6% of the global female population[1]. The location of myoma is crucial in the results of ART. In particular, submucous myoma reduces significantly the implantation and pregnancy by ART. It was found that the submucous myoma, which deforms the uterine cavity, compromises a risk ratio of 0.3 for pregnancy and 0.28 for implantation after ART compared with infertile and women without myoma[3].

Myomectomy is an alternative to hysterectomy for women who want to save their uterus, regardless of their reproductive plans. A number of studies have been conducted to determine the effect of myoma and myomectomy on fertility; the research data were widely miscellaneous [3].

We studied 124 women aged 35-43 years who were treated in the Department of Assisted Reproductive Technologies, which used in vitro fertilization and/or intracytoplasmic sperm injection.

The goal of the research was to study the effect of the presence of intramural or subserous myoma and myomectomy (laparoscopically or laparotomically intramural-subserous or subserous) in the anamnesis on the number of oocytes derived, the dose of gonadotropins expended, the quality of the embryos, and the effectiveness of IVF/IVF-ICSI.

Patients with submucous or intramural uterine myoma (according to the FIGO III-VII classification) up to 4 cm. Patients with submucous uterine myoma (FIGO 0-II) were not included in the study group.

The Method of Research

Clinical examination of patients included a medical history, primary inspection, pelvic examination, ultrasound investigation of the pelvic organs using a vaginal probe.

The following indicators of ovarian stimulation in IVF cycles were analyzed in the study:

- Course dose of gonadotropins;
- Number of growing follicles;
- Frequency of paracentesis of ovary;
- The average number of oocytes obtained by paracentesis;
- The frequency of paracentesis of ovary in which no oocytes were obtained;

The following indicators were used in the study to describe the character of the early development of the embryo:

Morphological characteristics of embryos (on the 3rd day of development)

In addition, the analysis of the dependence of the effect of ovarian stimulation on the presence of

uterine myoma and myomectomies (laparoscopic and laparotomic) in the history.

Results

During stimulation and paracentesis of patients of older reproductive age, an accurate difference in the number of oocytes was obtained in the IVF/IVF-ICSI protocol with uterine myoma. So, 22 oocytes (75.86%) were obtained in patients with uterine myoma, 87 oocytes (92.55%) (p * <0.05) were obtained in patients without uterine myoma.

The connection of uterine myoma with the number of oocytes:

	Patients without uterine myoma	Patients with uterine myoma	Total	p
Oocytes not obtained	7	7	14	
	7,45%	24,14%	11,38%	
1 or more oocytes obtained	87	22	109	*<0,05
	92,55%	75,86%	88,62%	

The same connection was observed in the group of patients with myomectomy. During paracentesis of patients with myomectomy, 11 oocytes were obtained, without myomectomy, 98 oocytes.

Table: The connection of the number of oocytes obtained with the presence of myomectomy in the history

	Patients without myomectomy	Patients with myomectomy	Total	p
Oocytes not obtained	10	4	14	
	9,26%	26,67%	11,38%	
1 or more oocytes obtained	98	11	109	0.05
	90,74%	73,33%	88,62%	

The following table shows the accurate difference in the higher dose of gonadotropins expended in myomectomy, in contrast to non-operated patients. So, for myomectomy, an average of 2437.67 ± 241.15 MU of gonadotropins is expended during stimulation. And with stimulation of patients not operated on for fibroids 1953.57 ± 80.35 MU.

The number of follicles and subsequently oocytes is greater in patients without uterine myomectomy. And also, the number of correctly fertilized zygotes with two pronuclei is almost 2 times higher in patients without uterine myomectomy, which is associated with the initially large number of oocytes obtained. The total number of embryos is also greater in patients without myomectomy in a history, in particular class B embryos.

Table: Character of the cycle in patients with myomectomy

Indicant	Myomectomy	M±m, ME	Number	ANOVA	p
The total dose of gonadotropins expended	Yes	2437,67 ±241,15	15	0.040	0.013
	No	1953,57 ±80,35	107		
Number of follicles	Yes	5,866667 ± 0,735926	15	0.256	0.362
	No	7.17	107		
Number of oocytes	Yes	4,33 ± 0,89	15	0.116	0.167
	No	6,40 ± 0,47	107		
2 polar bodies (fertilization +)	Yes	2,75 ± 0,60	12	0.260	0.308
	No	3,78 ± 0,29	107		
Class B embryos	Yes	0,25 ± 0,18	12	0.071	0.085
	No	1 ± 0,134	107		

When analyzing the effectiveness of pregnancy with and without uterine myoma in the fresh transfer cycle, differences in pregnancy were not obtained.

	Without myoma	With myoma	Total
Biochemical pregnancy	23	4	27
	28,05%	18,18%	25,96%
The presence of an ovum on an ultrasound scan (21 day after embryo transfer)	20	4	24
	24,69%	18,18%	23,30%

Conclusions

The number of oocytes obtained is reduced due to the physiological decrease in ovarian reserve in women of older reproductive age, when using ART. A history of surgery on the ovaries reduces undoubtedly the ovarian reserve, and in the future the number of oocytes obtained during paracentesis. It would seem, surgical measures on the uterus should not have affected the ovarian reserve. In this study it was found that surgical measures on the uterus, in particular, myomectomy, reduce ovarian reserve. So, accurately more oocytes were obtained in the absence of myomectomy in a history in patients of older reproductive age (?). It can be assumed that after myomectomy due to the formation of adnations, impairment of blood flow in the ovaries, the ovarian reserve decreases.

The presence of myomectomy in a history does not affect the quality of oocytes, embryos

The presence of myomectomy in the patient's history can lead to an increase in the dose of gonadotropins for stimulation, which increases the financial costs of the stimulation cycle.

In a fresh transfer cycle, the presence of uterine myoma in women of older reproductive age does not affect pregnancy.

Perhaps if uterine myoma is not symptomatic, in women of older reproductive age who have not carried out their reproductive plans, it is advisable to conduct a myomectomy after receiving oocytes, before embryos transfer. which, in turn, will not increase the dose of gonadotropins expended, and will allow to obtain a larger number of oocytes.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Inconsistency of Seams on the Uterus after Cesara Section Problems and Ways of their Solution

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Abstract

Cesarean section is currently the most common delivery surgery, which leads to the occurrence of diverse in nature changes from the internal genital organs. This primarily concerns the uterus, where a postoperative scar forms in the area of the former incision. This article presents histomorphological and immunohistochemical signs of a uterine scar after cesarean section in the lower uterine segment. The features of tissue regeneration in the area of the incision made on the uterus after cesarean section were revealed. Some method for diagnosing and treating uterine scar failure are also presented.

Keywords: *Cesarean section, uterine scar, ultrasound, hysteroscopy.*

Introduction

In all countries of the world there is a steady increase in the frequency of Caesarean section (CS). Over the past few decades, the frequency of this operation in the world has more than doubled and in Uzbekistan is on average about 24%. According to data published in world literature, one in four women has a cesarean section and a fifth has complications in the early postoperative period and in the long term.

The increase in abdominal delivery is due to the expansion of indications to it from the fetus, an increase in the number of older primiparas[4], with existing comorbidity, and the emergence of new reproductive technologies[25]. At the same time, according to the literature, a small number of criteria or features of the course of the postoperative period in patients with inconsistency of sutures on the uterus after cesarean section were isolated[34].

Currently, issues of repair of the suture on the uterus after CS, which largely determines the favorable course of subsequent pregnancies and childbirths[5], are becoming increasingly urgent[25]. A study of literature data showed a significant variability of information about the causes of the formation of an insolvent scar on the uterus.

Numerous studies have found that the increase in infectious[1] complications in obstetrics is largely due to an increase in the frequency[20] of cesarean section[24]. The increase in abdominal delivery is explained by an increase in severe somatic and obstetric pathology, frequent repeated operations, an increase in the age of primiparas in combination with other factors of obstetric and gynecological history[6]. A little justified tendency to an increase in the frequency of abdominal delivery during pregnancy and childbirth complications or severe somatic pathology poses a risk of secondary or late uterine suture failure[7].

Purulent-inflammatory complications after cesarean section are 8-10 times more common than after spontaneous[24] delivery and occupy one of the first places in the structure of maternal morbidity and mortality[32]. The frequency of peritonitis after CS ranges from 0.2 to 1-1.5% [1, 20, 35].

A large number of factors influence the formation of a suture on the uterus after CS: the presence of somatic and gynecological diseases (especially chronic inflammatory diseases), the course of pregnancy, the duration of labor, the technique of performing CS, the duration of surgery, the amount of blood loss and the adequacy[44] of its replenishment[2], and the course of the postoperative period[8]. It is believed that the main reasons leading to the development of an insolvent uterine scar are postoperative purulent-septic complications[17] in the early postoperative period[17], termination of pregnancy or the presence of any intrauterine interventions during the first year after CS [19]. Many authors consider intrauterine interventions after CS as a possible cause of direct trauma to the area of the postoperative[22] suture and violation of the formation of a scar on the uterus[8],

It is known that the postpartum period, even in the physiological course, and especially in the presence of risk factors, is favorable for the development of infectious complications, with surgical delivery, the postoperative period and associated additional significant risk factors (edema, ischemia, tissue destruction in the seam area, the presence of microhematomas, foreign suture material). Therefore, if the frequency of endometritis after spontaneous labor on average is 5%, then after surgical labor it varies significantly (from 2 to 54.3%), averaging up to 15% [34]. The consequences of severe endometritis are devastating for women's reproductive health: these are chronic endometritis, chronic pelvic pain syndrome, menstrual dysfunction, miscarriage, and secondary infertility [23].

According to some authors, the structure of complicated forms of purulent inflammation was dominated by complications caused by prolonged wearing of intrauterine contraceptives (44%), and postpartum diseases took the second place (16.9%) [50], while the risk factors for the development of purulent-septic puerperal complications were : "Inflammatory" obstetric and gynecological history (endometritis after childbirth - 35.3%; mastitis - 8.2%; wound infection - 23.5%; endometritis after abortion - 18.2%; cervical erosion - 23.6% acute and chronic salpingoophoritis - 12.7 and 29.1%, respectively); a previous history of infertility (21.8%); wearing an intrauterine contraceptive prior to a real pregnancy (4.1%); previous or existing chronic diseases of the bronchopulmonary system (29%); placenta previa in incision (38.6%); surgery in conditions of sharp thinning of the lower segment (22.7%); the presence of technical errors during the operation (using the Gusakov method - 72.7%; the use of rough manual method for removing the head - 13.6%; the use of a continuous suture for suturing the uterus - 38.6%; the use of reactogenic suture material - 9%; inadequate hemostasis - 9%); the duration of the operation is more than 2 hours (4.5%); the presence of pathological blood loss (9%).[51]

As you know, the optimal technique for conducting COP is still debatable. Most obstetrician-gynecologists are supporters of a "blunt" uterine dissection after a small incision is made with a scalpel according to the Gusakov[47]– Zanchenko method and less commonly, the uterus is dissected using scissors according to Derfler[49]. V.A. Ananyev et al. Found that biopsy specimens during surgery according to Derfler do not affect the most intracellular systems that are most sensitive to damage. Moreover, maintaining the integrity of the cell membranes and the respiratory apparatus of the cell ensures the maintenance of organelles homeostasis and oxidative phosphorylation[16]. At the same time, in the Gusakov – Zanchenko section, deep, mostly irreversible, ultrastructural changes prevail, reflecting the destruction of not only the trophic, but also the contractile apparatus of the myometrium cells [41].

At the same time, there is evidence that the determining factor in the formation of a wealthy scar is the nature of the healing of the wound surface with the predominant formation of muscle or scar tissue. So, if healing proceeds according to the type of primary (with the predominant formation of muscle tissue), then such additional risk factors as placenta localization, the period of time after CS, the production of medical abortions after CS in the final outcome are of unprincipled importance[46]. Gabidullina R.I. et al., based on histochemical studies, noted the complexity of the healing process of the uterus after CS surgery [13]. In later works, it was shown that during the healing of the uterus, two types of regeneration can be observed: substitution, which is defective regeneration, and restitution[45], in which the scar is not clinically detected. Naturally, the replacement of a myometrial defect not with an inert connective[117], but with smooth muscle tissue exhibiting contractile activity is more adequate[146].

Undoubtedly, the main etiological factor in the formation of an inferior uterine scar and generalization of infection is postpartum endomyometritis[15]. Features of the clinical picture of postpartum endometritis are: primary chronic course or short acute phase of

the manifestation of the disease, often hidden behind various "masks" (lochio and hematometer); further erased clinical course, caused, as a rule, by the use of massive antibacterial therapy (on average, two courses - 4–5 antibiotics) and various method of local uterine sanitation: vacuum aspiration, curettage of the uterus, lavage (in 75.5% of puerperas); latent or late (after discharge) manifestation of the inflammatory process with a undulating course of the disease[2] (remission - exacerbation); the predominance of infiltrative and necrotic forms of inflammation in the absence of typical clinical and laboratory signs of infection[3].

All diseases, including postpartum and postoperative, have specific symptoms, reflected in subjective complaints and data from an objective study.

The development of inflammatory and postoperative complications always "goes through" successive stages and is clearly identified when collecting information about the history of the disease, provided that the doctor knows the possible course of the disease and poses directed questions (the presence of risk factors, the initiation of the disease, its development, duration and frequency of hospitalizations, the nature and duration of treatment). Moreover, almost always, even after a certain time, you can identify the cause of the formation of an insolvent scar after CS (more often - endometritis, less often - obvious technical errors of the operation).

It should be noted that information on technical difficulties in performing the operation (difficulties in removing the fetal head, tears in the lower segment, difficulties in hemostasis, additional suturing, etc.) is almost impossible to obtain from extracts from the maternity hospital, while at 40 % of patients with an unsuccessful suture on the uterus after CS, if the extract contains a standard description of the operation, hematomas of parametria and/or hematomas of the postbubble tissue were revealed clinically and by ultrasound, and during the repeated operation[25], multiple unsystematic ad mass ligatures outside the suture line on the uterus (more often in the area of vascular bundles with ligation of one or both uterine arteries) [26].

Risk factors for the formation of an insolvent uterine scar after CS[48]:

- The patient has an "inflammatory"[22] obstetric and gynecological history (endometritis, mastitis, wound infection after previous birth, postabortion endometritis; endocervicitis, acute and chronic salpingo-oophoritis, infertility)[47];
- The presence and exacerbation during pregnancy of chronic inflammatory extragenital diseases (especially diseases of the bronchopulmonary system), the presence of anemia;
- Long-term operation, the presence of pathological blood loss;
- Placenta previa in the incision, the operation in conditions of sharp thinning of the lower segment in patients with clinically narrow pelvis ("low" or cervical CS);
- Urgency of the operation, especially the need for rapid delivery due to the development of acute fetal hypoxia, including in patients with premature detachment of the normally located placenta (in this group, the highest perinatal losses are 154 ‰);
- The use of a continuous suture for suturing a wound on the uterus;
- The use of Gusakov's technique, rude manual techniques for removing the head.

Clinical manifestations of a failed seam (scar) on the uterus after CS: Since the main reason for the formation of an unsuitable suture (scar) on the uterus after CS is endometritis, these patients are characterized by a complicated postoperative period and a later discharge from the obstetric hospital (on the 10-11th day).[2] Unfortunately, at present, postpartum endometritis in general and postpartum endometritis after CS[3], in particular, in most patients has a "erased"[9], subclinical course and is characterized by the following,[49]:

- At best, a short "acute" phase of the manifestation of the disease with a further "erased" clinical course of endometritis, often "hiding" under the masks of uterine subinvolution and lochio-hematometers (with a mild or mild inflammatory-intoxication syndrome);
- Late manifestation of the disease, often after discharge from the maternity hospital;
- The appointment in the postoperative period of long or repeated courses of antibiotic therapy;
- Insufficient effect of conservative treatment, which is often supplemented by the use of various instrumental method of sanitation of the uterus: vacuum aspiration, diagnostic curettage, hysteroscopy, lavage (47%);
- The wave-like course of the disease during its progression and episodes of deterioration of the state

of puerperas after the end of antibiotic treatment (short-term effect).

– The predominance of infiltrative, necrotic forms of inflammation in the absence of both typical clinical and laboratory signs of acute inflammatory process. Due to the absence of typical signs of purulent inflammation, necrotic forms of panmetritis were especially difficult for clinical and ultrasound diagnosis and were detected only with hysteroscopy;

– The rapid progression of the disease, despite its "erased" course.

Complaints upon admission of patients do not reflect the actual severity of their condition, therefore, a thorough history and examination are crucial for making a diagnosis. With a vaginal examination, the size of the uterus exceeds the value corresponding to certain days of the postpartum period, its consistency is soft. In most patients, the main pathological changes are observed in the area of the operation zone, i.e., the area of the anterior wall of the uterus, isthmus and neck, while the severity of inflammatory-necrotic changes in the anterior wall of the uterus and isthmus reflects a lag in the formation of the cervix (the neck is free in the form of " sails "hangs in the vagina, freely passes 1-2 fingers). The presence of infiltrates (hematomas) behind the gallbladder is a characteristic unfavorable clinical sign, indicating the development of secondary failure of sutures on the uterus. Even in cases where infiltration is not determined, the uterus is fixed to the tissues of the anterior abdominal wall in the region of its lower segment.

At present, also the features of the course of postpartum infectious diseases are the "worn out" laboratory picture. It is inappropriate to focus on leukocytosis as the main marker of the inflammatory process (it is observed only in 11% of postpartum patients, in the rest the indicators do not exceed the normative). More typical for puerperas with endometritis are an increase in ESR, the presence of moderate anemia (on average, 97.8 g/l), hypo- and dysproteinemia, which is expressed in a decrease in the concentration of albumin and an increase in globulins, as well as an increase in the concentration of C-reactive protein (72, 3%). Leukopenia is a poor prognostic sign indicating a septic course of the process. In severe forms of postpartum infection, systemic disorders prevail, for example, severe hemostasiological disorders (with the presence of uterine and pelvic vein thrombosis), immune disorders with damage to the receptor link (an excessive pathological concentration of antibodies to γ-interferon receptors appears, blocking receptors). Sepsis is a manifestation of a systemic inflammatory response, which is based on a generalized inflammatory reaction[9] - a kind of "malignant intravascular inflammation", leading to endothelial damage and the development of multiple organ failure [42].

Ultrasound diagnosis of insolvent uterine scar: The leading ultrasound method for identifying postpartum endometritis and especially a failed uterine scar[15]. To date, the most informative criteria for the condition of the scar are: the structure of the myometrium, the presence of areas of high acoustic density, vascularization of the uterine segment[38]. The results of echoscopy coincided with intraoperative data in 80.3% of cases. False positive results, when with unsatisfactory echographic parameters, the scar on the uterus was without features, amounted to 12.1%. False negative, when an insolvent uterine scar was an accidental find during surgery, in 7.6% of cases [40].

Analysis of echograms in postpartum patients with complications after CS revealed a number of common characteristic signs indicating the presence of endomyometritis and impaired repair processes in the suture or scar on the uterus:

– Subinvolution of the uterus, an increase and expansion of its cavity;

– The presence in the uterine cavity of inclusions of various sizes and echogenicity (intracavitary serous fluid, pus); the presence on the walls of the uterus of linear echopositive structures (in the form of an intermittent or continuous contour), reflecting the imposition of fibrin in all patients;

– Heterogeneity of the myometrium in the region of the scar, anterior and posterior walls of the uterus.

The main ultrasound signs of a failed scar on the uterus after CS are:

– of the cavity - the presence of a "niche" conical shape with a depth of 0.5 to 1.0 cm or more, when the top of the niche reaches the outer contour of the anterior wall of the uterus;

– A local change in the structure of the myometrium in the region of the scar or a diffuse change in the myometrium of the anterior wall of the uterus in the form of multiple inclusions of reduced echogenicity with fuzzy contours (microabsection sections);

– Local circulatory disturbance in the scar area - the absence of a diastolic component of blood flow (focal necrosis) or a sharp decrease in blood supply to the entire front wall of the uterus, when it was not possible to visualize the blood flow velocity curves;
– A sharp decrease in blood supply to the anterior wall and an increase in blood flow in the region of the posterior wall.

The following echographic signs are prognostically unfavorable, indicating complete failure of the suture (scar) on the uterus after CS:

– Expansion of the uterine cavity over the entire length of more than 1.5 cm;
– Sharp deformation of the cavity in the scar area: the presence of a cone-shaped niche, the apex of which reaches the outer contour of the anterior wall of the uterus (complete divergence of sutures);
– Diffuse change in the structure of the myometrium of the anterior wall of the uterus in the form of multiple inclusions of reduced echogenicity with fuzzy contours (areas of microabsection);
– A sharp decrease in blood supply to the anterior wall of the uterus (it is not possible to visualize blood flow velocity curves) with an increase in blood flow in the region of the posterior wall (systolodiastolic ratio <2.2 and resistance index> 0.5);
– The presence of echographic signs of hematomas, abscesses or infiltrates in the parameters, pelvis and abdominal cavity.

The development of criteria for the viability of the uterine scar after cesarean section using ultrasound has been the subject of many works by domestic and foreign authors [25, 38,40]. In the study, pay attention to the thickness of the lower uterine segment, its uniformity, the presence or absence of acoustic seals, their heterogeneity, fragmentation. According to the researchers, on the basis of the obtained echograms, scars with a total thickness of less than 4 mm, individual thinning sites (less than 3 mm), heterogeneous scars with a large number of acoustic seals, indicating the growth of connective tissue in the thickness of the anterior wall of the lower uterine segment, are considered insolvent. Almost all authors note the high information content of this method for the study of the uterine scar[2].

Some authors noted that hysteroscopy is indicated for all obstetric patients with endometritis, and the sooner this intervention is undertaken (with a diagnostic or therapeutic diagnostic purpose)[3], the better the outcome of the disease. The only exceptions are patients who have indications for surgical treatment (panmetritis, purulent lesions of the appendages and fiber, the presence of complete failure of the suture (scar) [38]on the uterus according to ultrasound), and hysteroscopy in this case does not make sense[40].

Hysteroscopy allows not only to identify postpartum endometritis and determine its shape (fibrinous, purulent, due to residues of placental tissue, necrotic), determine the condition of the suture on the uterus after CS, but also sanitize the uterine cavity and conduct differential treatment [50].

Morphological and histochemical changes in the uterine scar after CS: The presence of morphofunctional "solvency" of the uterine scar is one of the main criteria that determine[28] the outcome of pregnancy and childbirth[30] in women with a history of cesarean section[32], however, due to the complexity of the object of study, data on factors and mechanisms to date underlying the formation of a full scar on the uterus is not enough. Uterus scar formation should be considered as part of the modern concept of wound healing.

The wound and reparative processes are a complex of successive[30] local changes and the numerous common reactions[19] associated with them, which can be combined in three stages regardless of the location of the wound: the stage of inflammation[29], the stage of repair and the stage of remodeling [40]

By remodeling, the authors understand not only the reorganization of the scar, but also (to varying degrees)[28] the morphofunctional[29] restoration of lost tissue[32] (smooth muscle, epithelial, mesothelium, etc.). The wound process, which results in the formation of scar tissue, is accompanied by complex multicomponent interactions of cellular elements[30]: it is carried out mainly with the participation of mast cells, lymphocytes, macrophages and fibroblasts[29]. The interconnection of cells is carried out through cytokines[32] and growth factors: platelet growth factor, transforming β-growth factor, main fibroblast growth factor, epidermal growth factor, vasculoendothelial growth factor, etc.[32] . Studies show that macrophage-fibroblastic and collagen-fibroblastic interactions, through which the feedback between the breakdown[28] and production of collagen is realized, play a key role in the regulation of reparative

growth of connective tissue and sclerogenesis[29]. The optimal course of intercellular interactions underlies physiological neoangiogenesis, since it is the formation of small vessels that is necessary for the full regeneration of the connective tissue and muscle components in the initial stages of wound healing[2]. Hemomicro circulation determines[36] the level of tissue metabolism and oxygenation: the more severe the defeat of the individual components of the microcirculation[46], the more pronounced are violations of the transcapillary exchange and rheological properties of the blood [29].

In a number of works devoted to the study of reparative processes after various operations on the uterus, it has been shown that during the healing of the dissected uterine wall, two types of regeneration can be observed: both substitution (inferior regeneration) and restitution, in which the scar is not clinically detected [36]. Replacing a defect not with an inert connective, but with smooth muscle tissue exhibiting contractile activity is more advantageous. The morphofunctional usefulness of the uterine scar after cesarean section surgery largely depends on the course of the processes of its formation.[5] The various clinical manifestations of anatomical and functional inferiority of the uterus due to inferior wound healing after cesarean section are combined [29]with the concept of "operated uterus disease", the most severe manifestation of which is uterine lacerations in the scar during subsequent pregnancies and childbirths[32].

Features of the repair of uterine tissue determine the formation of the solvency of the scar after CS. Many factors influence the course of repair in the uterus, for example, anemia, preeclampsia, inflammatory processes, immune status, technique of surgical intervention and suture material used, duration of surgery and blood loss, postoperative period, and the time until the next pregnancy [11]. A number of complications (intrauterine interventions, inflammatory diseases of the genitals, intra- and postoperative complications) 1.5 times increase the risk of scar failure [14]. Many researchers have found that the formation of morphofunctional usefulness of the myometrium in patients with CS by a transverse section in the lower segment of the uterus occurs within 1–2 years after surgery [15].

An analysis of the literature on wound repair issues showed that individual, genetically determined features of the course of the wound process, as well as the nature of abdominal delivery, can be considered promising areas in this area. Today, the field of molecular genetic research continues to develop: numerous studies have shown the role of genetic factors, as well as gene polymorphism in the healing process [9]. A certain effect on the nature of wound healing on the uterus can be exerted by the time of the operation of cesarean section - in a planned or emergency manner. The effect of this indicator on the outcome of labor for the fetus is shown.

According to the results of the analysis of significant clinical and statistical material, the most favorable outcome for the fetus is observed with the so-called planned abdominal delivery, when the operation is performed with the beginning of regular labor. This is explained by the influence on the fetus of mechanical and metabolic factors from the mother's body, which lead to a powerful release of stress hormones that provide more optimal conditions for the adaptation of a newborn to extrauterine life [16]. The results of modern and, unfortunately, few studies indicate that the nature of the operation of cesarean section can play an important role in the process of scar formation on the uterus. The potential impact of cesarean section time on wound repair on the uterus can be assumed on the basis of modern ideas about the reparative process described above, taking into account the specific changes occurring in childbirth both at the body level and at the level of the uteroplacental-fetal complex[43]. A change in the reactivity of the immune system during childbirth has been shown to predominate over proinflammatory factors, which leads to structural changes in the cervix, rupture of the membranes and initiation of labor [34].

With the development of regular labor in the myometrium of the lower uterine segment, an increase in the content of leukocytes that produce interleukin (IL) -1β, tumor necrosis factor alpha (TNF-α) and IL-6 is observed [33]. The migration of neutrophils and macrophages into the myometrium during childbirth is due to increased production of chemoattractants for monocytes (MCP-1) and IL-8 [31]. IL-1β and TNF-α stimulate the formation of endogenous prostaglandins due to activation of phospholipid metabolism [43]. IL-6 increases the expression of oxytocin receptors in the myometrium [13]. In addition, IL-1β and TNF-α increase the production of matrix metalloproteinase-9 by myometrial cells, which can play an important role in the process of placenta separation[13]. A study of the concentration of endothelial cell adhesion molecules in the lower uterine segment showed that during urgent delivery there is a significant increase in the level of the intercellular adhesion-1 molecule (ICAM-1),

which provides adhesion of neutrophils, monocytes and lymphocytes to activated vascular endothelium with subsequent extravasation and migration to the lesion inflammation. ICAM-1 is also involved in cell contact interactions in immune responses: a T-lymphocyte with a monocyte, a cytotoxic T-lymphocyte with a target cell [13]. In a study on the mechanical properties of the lower uterine segment during full-term pregnancy and urgent delivery, it was shown that the viscoelastic properties of the myometrium change during childbirth, while the lower uterine segment becomes denser [27]. The time taken to perform a caesarean section affects the thickness of the lower uterine segment in subsequent pregnancies: with abdominal delivery during pregnancy, the thickness of the lower uterine segment, according to ultrasound, is 2.4 mm (interquartile range from 2 to 3.2 mm), while as with the operation in the active phase of labor, 3.1 mm (interquartile range from 2.5 to 3.9 mm)[37]. It has been suggested that the migration of leukocytes into the uterine wall during childbirth and their production of proinflammatory[26] cytokines play an important role not only in initiating and maintaining normal labor[14], but also in the process of repairing the wound surface in the placental area in the postpartum period, however, detailed studies in this direction[2] have not been carried out[9].

Therefore, it is necessary to further study the process of wound repair on the uterus using modern molecular genetic[9] and immunological method[15], which will expand the existing understanding of the course of the wound process with the subsequent development[29] of new diagnostic method, as well as optimizing the prevention of "insolvency" of the uterine scar after cesarean section[14] .

In their observations, the authors noted the expression of vascular endothelial growth factor VEGF and epidermal growth factor receptor EGFR, which is also common for transforming growth factor[29]. It is these growth factors that provide mitogenic activity of endothelial cells and fibroblasts, cell migration and differentiation, stimulate angiogenesis and the development of granulation tissue, which occupied a significant amount of the forming scar[31]. It should be noted that the presence of extensive areas of granulation tissue in the period of 6 months[14] or more after cesarean section indicates a slow healing process[27]. This fact may be associated with massive areas of necrosis[19]. A large volume of necrotic tissue slows down the process of granulation formation and their maturation into connective tissue, the organization of the extracellular matrix, scar formation and, moreover, its remodeling. The formation of foci of disorganization in fibrous and smooth muscle tissue lacking cellular components, collagen, fibrin, blood vessels, acid mucopolysaccharides and glycoproteins, proteinases, with perifocal expression of the macrophage marker CD68 may be associated with the outcome of persistent granulomatous inflammation[39].

Hence, the main reason for the formation of an insolvent uterine scar after CS is the primary severe inflammatory or traumatic necrotic damage to the endo- and myometrium with delayed and inferior maturation of connective tissue[29].

Given the high frequency of postpartum complications in women after cesarean section, as well as the absence of a tendency to reduce it[31], there is a need to determine preclinical markers of complications[19], in our case, uterine suture failure, to develop an algorithm for managing them[39], depending on the degree of risk of complications and new highly effective method of surgical treatment that can preserve the reproductive function of women.

The significance of immunological and biochemical changes in the development of uterine suture failure after cesarean section in their functional relationship, as markers of preclinical diagnosis, remains virtually unexplored, which determines the urgency of the problem and the need for targeted research in this direction.

The diagnosis of uterine scar (suture) on the uterus and the possibility of reconstructive surgery remain poorly understood.

Discussion

Diagnosis of a failed suture (scar) on the uterus after CS is always difficult, therefore, the diagnosis should be verified during a consultative examination after a sequential comprehensive examination including a clinical examination (special attention is paid to a thorough medical history, study of the features of the course of a previous pregnancy, surgery and postoperative period, analysis of discharge from obstetric and other hospitals), ultrasound and endoscopy. Even if it is possible to use the most modern research method, the main diagnostic method that determines the professional qualifications, the level of clinical thinking of a doctor and the subsequent direction of diagnostic measures is clinical.

Currently, laparoscopic metroplasty is known only at the stage of pregnancy planning with localization of the postoperative scar within the lower segment of the uterine[27] body, while hysteroresection with "smoothing" of the edges of the niche and ablation of the endometrium in the defect zone in patients not planning pregnancy[16], which allows to effectively save woman from complaints and significantly improve the quality of her life [40].

Conclusion

In view of the increase in the frequency of CS and its complications, it becomes important to search for prognostic criteria for the usefulness of the uterine scar in the postpartum period. However, the currently accepted[38] method for assessing the usefulness of the scar are not sufficiently informative and are often invasive, which limits their application. Currently, the issues of diagnosis and reconstructive surgery for an inconsistent uterine scar after a cesarean section in the postpartum period, as well as the volume and advisability of surgical intervention in patients with this pathology, have not been fully studied.

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Stability Research of "Biomayrin" Capsules

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Abstract

Research objective is to study the stability and shelf life of Biomayrin capsules, which were obtained by chemical modification of high-molecular weight compounds by incorporation into polymers.

Materials and Method: "Biomayrin" capsules weighing 600±6 mg containing isoniazid, rifampicin, ethambutol, polygalacturonic acid and calcium stearate, obtained by disk dosing into capsules No.0. The shelf life was set by the method of "accelerated aging" when the samples were stored in PVCA1 blister packs (20 capsules each) at the temperature of 60^0C in the thermostat TV-80-1. The tests "Decomposability" of capsules and "Average mass and homogeneity by mass" were conducted in accordance with the requirements of the State Pharmacopoeia of the XIV edition. Experiments were conducted in at least three repetitions. Decompressibility of capsules was determined on PJ-3 Tablet Four-usage Tester (China). Isoniazid, rifampicin and ethambutol were quantitatively determined in solution by spectrophotometry (SF). The analysis was performed on SHIMADZU UV-1601, UV-VISIBLE spectrophotometer in 1 cm cuvette against the background of the solvent. The pH value was controlled with the EV-74 universal ionometer. Mathematical processing of the results was carried out according to General monograph. 1.1.0013.15 "Statistical processing of chemical experiment results" using standard computer programs MsExcel.

Results: The results on properties of capsules at storage of 60^0C and 25^0C are shown, after dissolution the content of active substances (isoniazid, rifampicin and ethambutol) in preparations was within the limits of regulated norms (60-120 mg), at 60^0C it is for isoniazid 101, 7-106.7 mg, at 25^0C 100.1-106.0 mg for rifampicin the regulated norm was 72-132 mg and at 60^0C it was 94.2-92.2 mg, and at 25^0C it was 94.7-91.2 mg, as well as ethambutol hydrochloride content was 60-120 mg and at 60^0C it was 62.5-60.5 mg, and at 25^0C it was 62.0-60.2 mg.

Conclusion: "Biomainrin" capsules with different expiration dates were investigated, and also the change of their quality according to the indicators "Active substance content" and "Dissolution" was found. It was shown that after the expiration of the shelf life there is a gradual decrease in the active ingredient content.

Keywords: *Anti-tuberculosis drug, biomayrin, isoniazid, rifampicin, ethambutol hydrochloride, spectrophotometry, shelf life, stability study, dissolution, capsules.*

Introduction

During the production of solid formulations[1] the corresponding required indicators of finished product quality should be provided[2], including appearance, decomposition[3], average mass and homogeneity by mass, dissolution, foreign impurities and stability during storage[4].

Nowadays, in medical practice, the problem of treatment of lung tuberculosis patients occupies an important place in the structure of phthisiologicalpathology[5]. Lung tuberculosis is an infectious disease which occupies the second place among infectious diseases by mortality. Approximately 9 million people in the world get TB every year and about 2 million die from it[6]. This figure is increasing due to unfavorable living conditions, frequent stressful situations, contact with tuberculosis patients, poor and unhealthy diets, and the presence of diseases and bad habits that weaken immunity[7]. Low-molecular synthetic drugs currently used for tuberculosis treatment are highly toxic and have no ability to penetrate selectively into phagocytes (eaters of cells) containing the tuberculosis pathogen[8]; in addition, they are rapidly excreted from the body without providing a prolonged action, since they only have an effect on extracellular tuberculosis[10] pathogens [9]. WHO has recommended a tuberculosis control strategy known as the Directly Observed Treatment Short course[11]. An integral part of the program is the creation of a reliable system for supplying high quality, effective TB medicines to health care institutions[12]. First of all, it is relevant for standard "first-line" medicines used for treatment of common (sensitive) tuberculosis[13]. Poor quality of one of the medicines taken by patients at this stage violates the whole system and may lead to transition of easily treatable sensitive form of the disease to multidrug-resistant tuberculosis[14]. Improving the efficacy as well as reducing the side effects of anti-TB drugs remains a topical issue [15].

The combined anti-tuberculosis drug "Biomayrin" was developed at the Institute of Bioorganic Chemistry in the Academy of Sciences of the Republic of Uzbekistan by chemical binding of isonicotinic acid hydrazide with modified dialdehyde of polygalacturonic acid, with subsequent inclusion of ethambutol and rifampicin in the macromolecule by means of ionic bond [16].

The combination of isoniazid, ethambutol, and rifampicin along the chain of modified polysaccharides allowed creating a drug[17], which not only combines the properties of these drugs, but also manifests new properties due to its macromolecular nature[18] - prolonged action, membranotropicity, change of activity, possible synergetic action, and others[19]. Creation of combined drug of macromolecular nature "Biomayrin" due to activity increase (at lower content of individual drugs in macromolecule) and latitude of pharmacological action leads to significant reduction of side effects[20].

The research purpose is to develop method of standardization, study stability and establishment of shelf life of "Biomayrin" 600 mg capsules.

This article presents the results of the study of stability of the capsule form of the anti-tuberculosis drug "Biomayrin".

Subject of Research: The subject of research is capsules "Biomayrin" with the mass of 600±6 mg containing isoniazid, rifampicin, ethambutol, polygalacturonic acid and calcium stearate obtained by disk dosing into capsules No.0.

Materials and Method

The research on development of standardization method, stability studies and determination of shelf life for "Biomayrin" capsules was carried out using materials and equipment on the basis of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan[16]. Besides, we considered it expedient to study the dissolution profiles of "Biomayrin" capsules, since their change in time provides additional information when studying the stability of drugs and to justify the shelf life.

Shelf life time of capsule "Biomayrin" was set by the method of "accelerated aging" at storage of samples in PVCA1 blister packages (20 capsules each) at 60^0C in the thermostat TV-80-1.

Testing "Decompossibility" of capsules and "Average mass and homogeneity by mass" were conducted in accordance with the requirements of the State Pharmacopoeia of the XIII edition. The experiments were conducted in at least three repetitions. Decompressibility of capsules was determined on PJ-3 Tablet Four-usageTester (China). Isoniazid, rifampicin and ethambutol were quantitatively determined in solution by spectrophotometry (SF). The analysis was performed on SHIMADZU UV-1601, UV-VISIBLE spectrophotometer in 1 cm cuvette against the background of the solvent. The pH value was controlled with the EV-74 universal ionometer. OFS.1.1.0013.15 "Statistical processing of chemical experiment results" using standard computer programs MsExcel. The "Dissolution" of the combined drug was investigated according to [20] using the RCZ-6C3 device of the "Rotating Basket" type. At the first stage, purified water (pH 7.0) in the volume of 900 ml at a temperature of 36.5±0.50 C was used as a medium for dissolution at a basket rotation speed of 150 rpm and the duration of

the analysis was 60 min. At the second and third stage, when determining the dissolution profile of the capsules, two dilution media similar to pH of human stomach (pH 1.2) and pH of duodenum (pH 8.0) were consistently used. Sampling for analysis was carried out every 15 min during 1 h. Release of isoniazid, rifampicin and ethambutol as one of the main groups of biologically active substance of the combined drug was determined by direct spectrophotometry on the device "UV-7504" in a cuvette with thickness of 1 cm at wavelength 262±2; 336±2 and 450±2 nm.

Results and Discussion

To determine the quantitative content of isoniazid, the content of 10 capsules was thoroughly stirred and selected 100 mg (precise attachment) of powder, placed in a measuring flask of 100 ml, 50 ml 0 was added, 1 M of hydrochloric acid solution dissolved in an ultrasonic bath for 15 minutes, brought the volume of the solution with the same solvent to the mark, 5 ml of the resulting solution was placed in a measuring flask with a capacity of 100 ml and brought the volume of the solution to the mark 0.1 M with hydrochloric acid solution. The obtained solution was filtered through a "White Ribbon" filter, discarding the first 10-15 ml of filtrate, optical density was measured at 262 nm wavelength. At the same time the optical density ofisoniazid solution was measured.

Calculation of isoniazide (mg) content in Biomayrin capsule was performed by the formula:

$$\frac{D \times 100 \times 100 \times m_{st} \times 1 \times m_{caps}}{D_0 \times 5 \times m_{test} \times 100 \times 100},$$

Where: D1 – optical density of the test solution.

D0 – optical density of SSS (Solution standard samples) isoniazid solution.

m_{caps}– average mass of content of one capsule, in mg.

m_{test}– precise hinge of the test specimen, in mg.

m_{st}- precise attachment of standard sample, in mg.

The quantitative content of rifampicin was determined using the above method and optical density was measured at a wavelength of 472 nm. The optical density of rifampicin SS solution was measured in parallel. The calculation of rifampicin (mg) content in Biomayrin capsule was carried out by the formula:

$$\frac{D \times 25 \times 100 \times 2 \times m_{st} \times m_{caps}}{D_0 \times 5 \times m_{test} \times 100 \times 100},$$

Where: D_1 – optical density of the test solution.

D_0 – optical density of SS rifampicin solution.

m_{caps}– average mass of content of one capsule, mg.

m_{test}– precise hinge of the test sample, in mg.

m_{st}- accurate attachment of standard sample, in mg.

Determination of the quantitative content of ethambutol by the above method, with a difference after filtration to 10 ml of leachate was applied 1 ml solution of iron ammonium alum, thoroughly stirred and left for 5 minutes. Then 0.2 ml of solution of mercury rhodiumized was added and stirred again. In parallel, a similar treatment with 10 ml of SS solution of ethambutol dihydrochloride was performed. After 10 min the optical density of the analyzed solutions was measured at a wavelength of 457 nm.

The calculation of the content of ethambutol (mg) in the Biomayrin capsule is done by the formula:

$$\frac{D \times 100 \times m_{st} \times m_{caps}}{D_0 \times m_{test} \times 100}$$

Where: D_1 – optical density of the test solution.

D_0 – optical density of ethambutol SS solution.

$m_{капс}$– average mass of one capsule content, mg.

$M_{исп}$– precise hinge of the test sample, in mg.

$m_{ст}$- accurate attachment of standard sample, in mg.

According to SF (State Pharmacopoeia) XIV, a formula based on the Vant-Goff law is used to determine whether the experimental storage period at elevated temperature (C) corresponds to the storage period (C) at 25^0C:

$$SP=K*P_{ex},$$

Where the coefficient of conformity $K=A(t_e–t_{xp})/10$, the temperature coefficient of chemical reaction speed (A) is 2.5. At a temperature difference $t_e–t_{xp=}60\text{-}25=35^0$C the conformance coefficient K=9.0. Calculated terms of experimental storage at 60^0C are given in Table 1, the results of studies are shown in Table 2.

Table 1: Consistency of experimental storage terms at 60^0C and 25^0C

At 60^0C	At 25^0C
6 days	3 months
12 days	6 months
18 days	9 months
24 days	12 months
36 days	18 months
48 days	24 months

Table 2 Properties of experimental capsules during storage at 60^0C

Term of experimental storage at 60^0C, a day.	Decompossibility, no more than 20 min	Average weight and homogeneity in weight, deviations ±10%.	Dissolution, at least 75%.		
			Isoniazid from 60.0 to 120.0 mg	Rifampicin from 72.0 to 132.0 mg	Etambutol from 60.0 to 120.0 mg
0 days (Start)	15 min	587,92mg. +8,17% -5,94%	93,6 %		
			106,7 mg	94,2 mg	64,5 mg
6 days	15 min	585,62mg. +6,70% -3,14%	91,1%		
			105,6 mg	94,7 mg	64,2 mg
12 days	15 min	580,45mg. +4,17% -3,25%	95,6 %		
			104,7 mg	94,2 mg	62,7 mg
18 days	16 min	583, 22mg. +5,45% -2,67%	92,4%		
			106,0 mg	93,6 mg	62,1 mg
24 days	16 min	586,34mg. +7,23% -4,34%	94,6 %		
			105,7 mg	92,9 mg	62,0mg
36 days	16 min	585,56mg. +6,79% -3,35%	90,2 %		
			106,2 mg	91, 7 mg	61,6 mg
48 days	16 min	584,18mg. +6,20% -4,62%	89,5%		
			101,7 mg	92,2 mg	60,5 mg

Table 3: Properties of experimental capsules during storage at25 ^{0}C

Term of experimental storage at 60^0C, a day.	Decompossibility, no more than 20 min	Average weight and homogeneity in weight, deviations ±10%.	Dissolution, at least 75%.		
			Isoniazid from 60.0 to 120.0 mg	Rifampicin from 72.0 to 132.0 mg	Etambutol from 60.0 to 120.0 mg
Start	15 min	585,0 mg.+6,6%-3,54%	93,1%		
			106,0 mg	94,7 mg	62,5 mg
3 months	15 min	585,62 mg.+6,70%-3,14%	91,0%		
			105,0 mg	93,2 mg	62,2 mg
6 months	16 min	580,40 mg.+4,17%-3,25%	95,6 %		
			104,7 mg	93,0 mg	61,7 mg
9 months	16 min	583, 22 mg.+5,45%-2,67%	92,4%		
			106,0mg	92,6 mg	60,1 mg

Term of experimental storage at 60^0C, a day.	Decompossibility, no more than 20 min	Average weight and homogeneity in weight, deviations ±10%.	Dissolution, at least 75%.		
			Isoniazid from 60.0 to 120.0 mg	Rifampicin from 72.0 to 132.0 mg	Etambutol from 60.0 to 120.0 mg
1 year	16 min	586,27 mg.+7,23%-4,34%	94,6 %		
			105,5 mg	92,0 mg	60,1 mg
18 months	16 min	585,56 mg.+6,79%-3,35%	90,2 %		
			104,2 mg	91,7 mg	61,1 mg
2 years	16 min	584,18 mg.+6,20%-4,62%	89,5%		
			100,1 mg	91,2 mg	60,2 mg

The results of determining the content of active substances in dissolution in the studied preparation are presented in Tables 2 and 3.

As follows from the above data on the properties of the capsules during storage of 60^0C and 25^0C, after dissolution the content of active substances (isoniazid, rifampicin and ethambutol) in preparations with the shelf life up to 2018. is within the limits of regulated norms (60-120 mg) at 60^0C is for isoniazid 101.7-106.7 mg, at 25^0C 100.1-106.0 mg for rifampicin the regulated norm should be 72-132 mg and at 600C is 94, 2-92.2 mg, and at 25^0C 94.7-91.2 mg, and also the content of ethambutol hydrochloride should be 60-120 mg and at 60^0C of storage its content was 62.5-60.5 mg, at 25^0C 62.0-60.2 mg. In the study of samples with expired shelf life (2018) a moderate decrease in the level of active substances was noted.

In order to obtain the most complete picture of Biomayrin quality changes during storage, we considered it expedient to study its release from the investigated samples along with the tests of "Active substance content" [22].

The results of the experiments to study the release of the active ingredients of the combined preparation from the capsules in the device "Rotating basket" are presented in Fig. 1 - 3.

The graphs show that the amount of substances transferred to different solvents in 60 min varies within the limits for:

- Isoniazid in water purified from 74.0 to 92.0%; in acidic medium from 56.0 to 84.6%, and in buffer solution from 65.5 to 83.0%;
- Rifampicin in the first medium from 58.3 to 84.0%; in the second medium from 44.0 to 77.0%; in the third medium from 62.0 to 85.0%;
- Ethambutol on Wednesday from 65.0% to 85.0%; on Wednesday 2 from 67.0% to 85.0%; on Wednesday 3 from 64.0% to 77.0%.

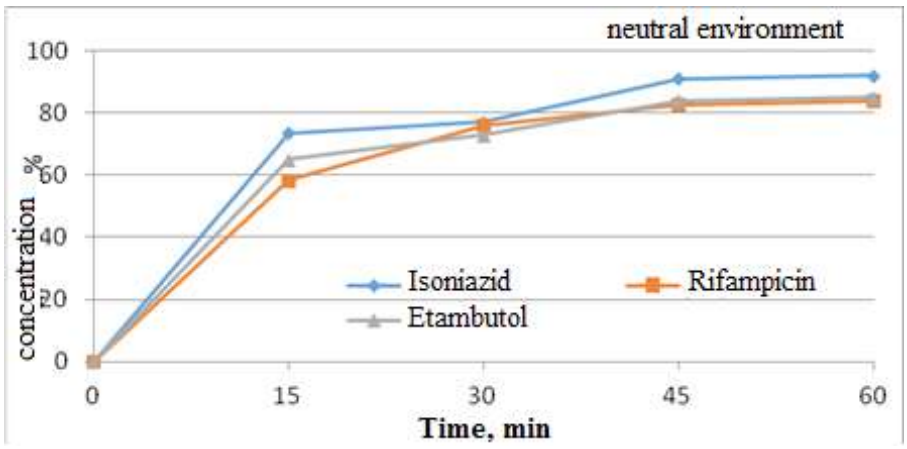


Figure 1: Kinetic release profile of DS (drug substance) from capsule at 36.5±0.5^{0}C and V = 900 ml, pH = 7.0 (dissolution test method)

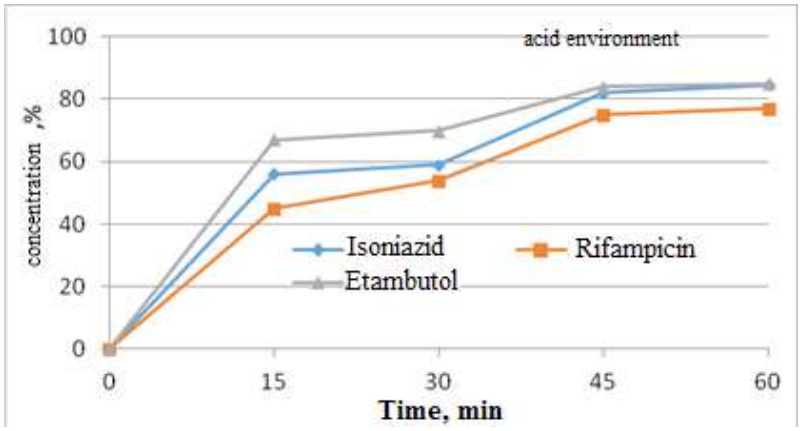


Figure 2: Kinetic release profile of DS (drug substance) from capsule at 36.5±0.5^{0}C and V = 900 ml, pH = 1.2, 0.1 m hydrochloric acid (dissolution test method)

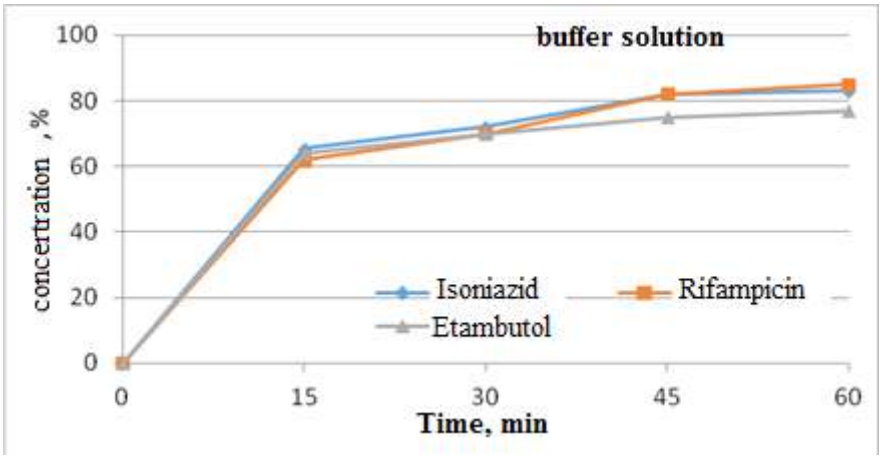


Figure 3: Kinetic release profile of DS (drug substance) from capsule at 36.5±0.5^{0}C and V = 900 ml, pH = 8.0 (buffer solution) (dissolution test method)

Based on the results of the first set of experiments in the dissolution test, we can conclude that isoniazid, rifampicin and ethambutol dissolve well within 60 minutes in all the solutions we used.

Based on the above studies, a table was made on the results of the accelerated stability test for Biomayrin capsules. The results are given in Table 4.

Table 4: Results of the accelerated stability test of "Biomayrin" capsules

Test	Specifications	0 Days (Start)	6 Days (3 Months)	12 Days (6 Months)	18 Days (9 Months)	24 Days (12 Months)	36 Days (18 Months)	48 Days (24 Months)
Description	Hard gelatin capsules No.0, light green, containing brown powder with a slight peculiar smell	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate
Originality	Spectrum of approx. 233 nm, 262 nm, 269 nm, 336 nm and 473 nm	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate
	Add 10 ml of water to the powder of Biomairin capsule contents shake for 10 minutes and filter. To the resulting filtrate add 0.2 ml of copper (II) sulfate solution and 0.5 ml of diluted sodium hydroxide solution; blue staining appears.	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate	Appropriate

Test	**Specifications**		**0 Days (Start)**	**6 Days (3 Months)**	**12 Days (6 Months)**	**18 Days (9 Months)**	**24 Days (12 Months)**	**36 Days (18 Months)**	**48 Days (24 Months)**
Average mass and homogeneity by mass	From 553.0 mg to 636.0 mg Deviations from average weight ±10%.		587,92 mg. +8,17% -5,94%	585,62 mg. +6,70% -3,14%	580,45 mg. +4,17% -3,25%	583, 22 mg. +5,45% -2,67%	586,34 mg. +7,23% -4,34%	585,56 mg. +6,79% -3,35%	584,18 mg. +6,20% -4,62%
Decompressibility	Not more than 20 min		15 min	15 min	16 min	16 min	16 min	16 min	16 min
Dissolution	Minimum 75%		93,6 %	91,1 %	95,6 %	92,4 %	94,6 %	90,2 %	89,5 %
Quantitative content	Isoniazid	From 60,0 to 120,0 mg	106,7 mg	105,6 mg	104,7 mg	106,0 mg	105,7 mg	106,2 mg	101,7 mg
	Rifampicin	From 72,0 to 132,0 mg	94,2 mg	94,7 mg	94,2 mg	93,6 mg	92,9 mg	91,7 mg	92,2 mg
	Etambutol	From 60,0 to 120,0 mg	62,5 mg	62,0 mg	61,7 mg	62,1 mg	62,6 mg	61,6 mg	60,5 mg

Conclusion

In the research of samples with expired shelf life (2018) was found out that capsules “Biomayrin” at storage of 60^0C and 25^0C, after dissolution the content of active substances (isoniazid, rifampicin and ethambutol) in preparations is within the limits of regulated norms (60-120 mg) at 60^0C is for isoniazid 101, 7-106.7 mg, at 25^0C 100.1-106.0 mg, for rifampicin the regulated norm 60^0C was 94.2-92.2 mg, and at 25^0C 94.7-91.2 mg, and also the content of ethambutol hydrochloride at 60^0C was 62.5-60.5 mg, at 25^0C 62.0-60.2 mg. This indicates that, when stored at different temperatures the content of active substances is within the limits of regulatory standards, as well as a moderate decrease in the level of active substances.

As well as, according to the results of the test “Dissolution”, it was found that the number of active ingredients transferred to water purified from “Biomayrin” capsules is 87% on average, in acidic environment - 82.2% on average, in buffer medium - 81.6%. Comparison of the data allows us to conclude that the release of active substances in the buffer solution is slower than in a neutral environment (purified water). The results show that the method of “dissolution”: the degree of release of biologically active substances in 1 hour is at least 81%.

It was found that at the end of the shelf life (2 years) gradually reduces the rate of “dissolution” from 93.6% to 89.5% and changes the profile of release of active substances from the capsule forms of drugs.

Ethical Clearance: No ethical approval is needed.

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Conflict of Interest: Nil

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Protection of Human Rights in the Context of Combating of Counterfeiting Medicines: Theoretical and Legal Issues of International Cooperations

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Abstract

The article highlights the problematic issues of implementing of the best international practices in protection of a human and human rights, and counterfeiting medicines in the context of globalization. Every state exists for man, for the protection of universally recognized rights and freedoms. Counterfeiting of medical products and other similar crimes require attention in any country in the world.

Counterfeiting is not an exception in developed countries in Europe, such as the Netherlands, Belgium, Sweden, Switzerland, Finland, Norway, Denmark, and Austria. This problem is global for, both, legal and public administration science. It is widespread throughout the world, and the negative effects of this crime have severe consequences that affect people's lives and health.

Keywords: *Counteraction management, counterfeiting of medicines, globalization, digitization, protection of a human and human rights.*

Introduction

The development of Europe, Asia, North and South America, Africa, Australia and Oceania is currently linked with the implementation of a number of urgent reforms, including the modern transformation of crime-fighting method. The global problem of counterfeiting in medicines is in need of immediate solution worldwide.

When a person dies because of adulteration of medicines or loses health, the consequences are deplorable. Buying medicines from pharmacies, a patient does not always pay attention to their quality. The regulation of this issue at the international level makes it possible to issue responsibility for the above-mentioned crimes in the states and stop it, or at least significantly reduce their number normatively. In the case of purchase of counterfeit medicines, a person may file a lawsuit with the administrative court to protect their rights in the field of public-legal relations in the event of inaction or unlawful decisions by the relevant authorities.

Literature Review: Counterfeit products reach the highest levels in countries with imperfect legal frameworks in the regulation of drug trafficking and lack of efficiency in regulatory oversight of counterfeit drugs. In most countries with developed regulatory framework and market control, such drugs are rarely found[1,2], [p. 29]. Ukrainian scientist Khromykh A.G. conducted an expert survey to determine the main factors affecting the adulteration of medicines by developing a questionnaire with more than 150 experts. Therefore, according to experts, the most important factors influencing the circulation of counterfeit medicines are identified as follows: a developed system of selling medicines

through the global Internet information network and mail that does not allow the control bodies to check their quality; high cost of imported medicines, which makes the sale profitable; etc. These practices and the review of scientific sources have made it possible to identify also the most promising at the present stage of the technology of protection of pharmaceutical products from adulteration[3], namely: their origin through electronic registration.

The World Health Organization estimates that counterfeit drug sales are about $ 200 billion annually, equivalent to 10-15% of the global pharmaceutical market[4]. In Russia, the problem of counterfeiting of medicines, as in most countries of the world, remains urgent and needs to be addressed by amending the legislation.

A number of international acts have been adopted in order to address the problem of counterfeiting of medicines. For example, in Ukraine, the Council of Europe Convention on Counterfeiting of Medical Products and Similar Health-Related Crimes[5] entered into force on 1 January 2016. The Universal Declaration of Human Rights[6], promulgated by the General Assembly of the United Nations on 10 December in 1948; and the Convention for the Protection of Human Rights and Fundamental Freedoms, issued in1950 and its number ETS No. 5[7]; the European Social Charter, issued in 1961, ETS No. 35[8]; the Convention on the Development of the European Pharmacopoeia (1964, ETS No. 50)[9]; and Protocol thereto 1989, ETS No. 134); the Convention on the Protection of Rights and Dignity on the application of biology and medicine; Convention on Human Rights and Biomedicine, issued in 1997, ETS No. 164)[10]; Additional Protocols, issued in 1998 ETS number 168; issued in 2002; ETS number 186, issued in 2005; CETS number 195, issued in 2008; CETS number 203; the Convention on Cybercrime issued in 2001, ETS number 185 are International instruments for governing these issues[11]. Rule makers have duly drawn attention to other relevant international legal instruments and programs. They were paid to those which were conducted by the World Health Organization and included the work of the IMPACT Group, the European Union, and the G8 (since 2014 - G7). Determined to effectively achieve the overall goal of combating crime, which includes counterfeiting of medical products and similar health-threatening crimes, in particular, by fixing new crimes and criminal penalties relating to these crimes[12].

Aims: Studying and generalizing the best international experience in counterfeiting medicines, as well as exploring the problematic issues of its implementation in the context of globalization.

Method

The research material is a modern international regulatory framework governing the fight against counterfeiting of medicinal products, foreign and domestic scientific works of specialists. The system method, structural and functional method, techniques of logical method (analysis, synthesis, deduction and induction), method of legal statistics, legal modeling were used in writing the article.

Results

For example, it is worth noting that Article 165 of the Criminal Code of Poland establishes responsibility for actions such as the manufacture and putting into circulation of harmful substances, foodstuffs or other public goods, as well as pharmaceuticals that do not meet the mandatory requirements, which do not meet the requirement of conditions of quality[13]. Denmark's criminal law, for example, establishes liability for actions such as the sale of medicines that do not meet these goals and may cause harm to health[14]. In the United States, the Food and Drug Administration is the primary body responsible for monitoring compliance with pharmaceutical law. Along with the Food and Drug Administration, as a federal body, each state has its own supervisory authority[15].

For example, in October 2011, pharmaceutical giant subsidiary Glaxo Smith Kline was found guilty of four counts of counterfeit medicines. The lawsuit is also considered a civil suit filed by the Office of Criminal Investigations alleging that Glaxo Smith Kline has accused the Food and Drug Administration Medicaid program of thinking information about low-quality drugs of its own manufacture. The case, in which the Office of Criminal Investigations employees represented the State, resulted in the fine of Glaxo Smith Kline. It was fined $ 140 million in criminal proceedings and $ 600 million in civil proceedings[15;16]. During the unscheduled audit, 18 violations were detected. One of them was critical. Nine were significant. There were eight non-essentials. In view of the presence of a critical disorder, which may lead to the production of substandard medicines and which may harm the health or life of a person, a decision was made to issue an order to remedy the violations

of the License terms. The enterprise is obliged to stop the production of medicines until the critical breach of the License Terms. However, considering the available material and the facts of the case, examining and evaluating the evidence presented in their totality, the court decided to dismiss the claim in its entirety. The court considers that on the day of the court hearing there are no grounds for taking the measures mentioned in the claim. The violation, which were classified as critical ones after an unplanned measure of state supervision (control) with respect to compliance by the entity with the requirements of the Licensed Conditions of Business Activity in the Production of Medicinal Products "Zaporizhzhia Autogeneous Plant" and the Company", was independently eliminated by the defendant. The company[17].

Discussion

Counterfeit drugs cause both social and economic harm to society according to V. Pashkov, A. Soloviov, A. Olefir. Counterfeit and substandard medicines should be distinguished. Although there are recommendations for patients to identify counterfeit drugs, the law is essential in counteracting their circulation. Opposite actions can be carried out at the level of general rules, as well as through specific regulatory requirements. The latter can be divided into economic (setting rules for market participants, how to detect and remove counterfeit medicines) and those, imposing penalties for offenses. In all European Union countries studied, the use of counterfeit medicines is strictly prohibited and administrative liability is envisaged for such actions. At the same time, there is no single approach to criminal liability as defined by national governments[18],[p. 848].

Being studied the regulatory framework and the work of international scientists; it is fair to point out that the leading countries in the development of the digital economy are Great Britain, Japan, Singapore, New Zealand, Estonia, United Arab Emirates, Israel and more.

It is important to give a theoretical characterization of the term "digitization", which comes from the English "digitalization" and in translation means "digitization", "digitization", or "digitization". At the same time, nowadays domestic scientists are increasingly using this term, based on the requirements of practical transcription, and therefore the terms "digitization" or "digitization" have become widespread. This approach has several advantages, since the term "digitization", by the right remark of K. Lapina-Kratasyuk, covers a wider range of meanings than its synonym "digitization". This is due to the fact that it is in European and American research traditions that the effects of the transition from analogue to digital type of information encoding have been studied not only as technological but also as social, cultural, and anthropological processes[19]. According to K.O. Kuprina and D.L. Khazanova, digitization is theway of bringing any kind of information into digital form[19; 20]; with. [259]. The Polish Government[21] adopted the Digital Government for 2014-2020. Digitization in the country is developing quite actively. Special Ministry of Digitization or Digitization - Ministerstwo Cyfryzacji is engaged in the introduction of new technologies.

Having conducted research of scientific sources, it is right to emphasize that scientists Vasylieva T. A., Lieonov S. V., Rubanov P. M., Vasilyeva TA, note that the digitization of the economy is considered to be a fundamental factor of economic growth, innovation and competitive environment, job creation and social progress in general[22; 23]. In Russia, there is a law on the circulation of medicines, in which the terminology has been successfully developed, for example, the concept of pharmaceutical activity, etc.[24], which, for example, is absent in the Law of Ukraine on Medicines, is enshrined. Pursuant to Article 9 of the Law, medicinal products are allowed to be used in Ukraine after their state registration, except in cases provided for by the Law. Registered by the competent authority of the United States of America, Switzerland, Japan, Australia, Canada, a medicinal product registered by a competent authority of the European Union under a centralized procedure for use in the territories of those countries or Member States of the European Union, only materials on method of quality control of medicinal product are added. Materials of the registration dossier and samples of the packaging of a medicinal product, labeled in a language for the labeling of medicinal products, instructions for the use of a medicinal product in a language must be in accordance with the requirements of this Law; document confirming payment of the registration fee are added too[12]. This makes it possible to regulate this issue legally and avoid crimes in Ukraine, since the person, his life and health, according to Article 3 of the Constitution of Ukraine, is of the highest legal value[25].

The problem of counterfeiting of medical products and other similar crimes require attention in every state, because the negative consequences of this crime have

serious consequences that affect the lives and health of people. The introduction of positive foreign experience makes it possible to legally secure the responsibility for the aforementioned crime in international countries and stop it.

Conclusion

Because of the conducted research, we propose strengthening of legal responsibility for falsification of medicines in the countries of the world, the legislation of which provides insufficiently severe criminal penalties or even administrative liability. We believe that the manufacture and trade (both domestic and export) of counterfeit medicines should be a particularly serious crime, for which criminal liability in the form of imprisonment for 15-20 years with confiscation of property should be provided. The rights of individuals should be protected by an administrative court in the sphere of public-legal relations when appealing against decisions, actions (inaction) of state bodies.

However, in the first place, it is necessary to create the appropriate conditions for the effective fight against counterfeit medicines at the international level. This requires the use of innovative digital technologies already in use in the leading countries of the world, including the European Union. It is necessary to create in each country a unified electronic system for the identification of goods, including medicines that would be publicly available on the Internet for everyone, including every consumer of medicines. When buying these products from their smartphone, they will be able to open the program, take an electronic ID photo, and verify the authenticity of the medicinal product, obtain information about the manufacturer, date and place of manufacture, etc. Such programs should also be used by business entities to prevent counterfeiting. At the same time, both the persons who manufactured the product and the guilty officials of the economic entities that sell them must be prosecuted. Legal entities and entrepreneurs must be subject to considerable administrative liability - € 100,000, such as in Spain.

A significant aspect of the problem of drug identification is its global, international level. The worldwide locomotive of digitization in counterfeiting of counterfeit medicines should be the World Health Organization, which is already taking care of these problems. To counteract the counterfeiting of medicines globally, it is necessary to develop an international program coordinated by WHO. Authoritative intergovernmental associations, such as the European Union, the Eurasian Economic Union and other trans-state entities, should be involved in the development and implementation of this program. At the national level, such work should be undertaken by relevant health ministries through the creation of appropriate structures of their own subordination or through interaction with national digitization bodies. In the positive experience of Poland, it is appropriate to create special ministries (or other authorities) that would develop modern technologies.

In the future, a global database of certified medicinal products should be created for use of any citizen of any country in the world in the age of the pharmaceutical market globalization. And also give access to such a court system.

Thus, the implementation of the proposed approach will significantly reduce the number of counterfeit drugs in the world, as the inevitability of criminal punishment for such crimes will become evident.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Indonesia.

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Cytology and Genotyping of High-Risk Human Papilloma Virus from Female Sex Workers in Makassar

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Abstract

Objective: This study aims to determine the results of cytological examination and genotype of high risk HPV in women work as femalesex workers (FSW)at Makassar, South Sulawesi.

Method: This study designed as analytical study with a cross sectional analysis approach and sample came from female sex workers in the Makassar city. Research was conducted in the localization area of sex worker, and conducted from March 2019 - September 2019. The research sample consist of women with reproductive age who sex workers, and meet the inclusion criteria. The cytological examination was performed using the Thin Prep Pap Test and the DNA HPV detection (genotyping) using the reverse dot blot "flow through" hybridization method. Data were analyzed with SPSS.

Results: Our study found that most of the FSWs who were sampled are > 30 years old (61.3%), had <9 years of education (66.3%), were married (77.5%), nulliparous (62.5%), being FSW> 1 year (86.3%), age at FSW ≥ 15 years (73%), age at first coitus ≥15 years (82.3%), number of sex partners within a month ≥5 (61.3%), the last number of sexual partners in the last 1 month<5 (58.8%), did not have an Sexual Transmitted Infection (STI) (96.3%). The number of FSWs who using and not using condoms was comparable (50% each), and all samples had never vaccinated with HPV vaccine. It was found that based on the type of lesion, most FSW samples had non-specific and non-bacterial cervicitis (60%) and the remaining 40% had no lesions. Based on the distribution of HPV genotypes in commercial sex workers, most of the samples had a high risk HPV of 25%. Duration to be FSW connected with high risk HPV ($p < 0.05$).

Conclusion: There is a significant relationship between duration of being FSW and high risk HPV genotype. Our study highlited that FSWs who work for >1 year are prone to having high risk HPV.

Keywords: *Cytology of FSW, HPV genotype of FSW, Makassar.*

Introduction

Cervical cancer is the third most common type of cancer in the world, and is the only cancer that can be detected early therefore prevented its development. It is estimated that in 2008 worldwide there were 530,232 women diagnosed with cancer and 275,008 of them died.[1] Most cervical cancers (85%) occur in developing countries such as Indonesia.[2] The growth rate of cervical cancer has a drastic difference in developed countries compared to developing country. Globally, the burden of cervical cancer in developing countries is around 85% -88% of deaths from cervical cancer. The growth rate of cervical cancer in developing countries is still increasing, by adding the incidence of both of cervical

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and breast cancer will be the main cause of death in women of reproductive age by 2025.[3]

Cervical cancer often found in women aged> 50 years, but in developing countries the incidence frequently found in women with reproductive age. In countries that do not have good screening programs, cervical cancer sufferers are mostly diagnosed at an advanced stage with incurable stage.[4] In addition, the prevalence and genotype of HPV infection differ in each population. There are currently two types of HPV vaccines, namely for types 16 and 18, both of which significantly reduce the prevalence of HPV infection caused by these two types of HPV.[5] However, the effect of HPV vaccination on the prevention of HPV infection and cervical cancer also differs based on the distribution of HV genotypes in each region.

Cervical disease is highly contagious and the risk of infection increases with increased sexual activity.[6,7] Female sex workers are among the high-risk groups who most likely infected and transmit HPV because having more than one sexual partner.[7] Studies on female sex workers in Cambodia show the highest prevalence of HPV types 51 and 70 dominant compared to types 16, 71 and 81.[8] However, other research in Turkey shows that female sex workers are mostly infected with HPV types 18, 16 and 50.[9] A meta-analysis of HPV infections in female sex workers in Asia shows that there are differences in the prevalence of HPV types among southeast and eastern Asia. The predominant types of HPV that infect in eastern Asia consist of types 16, 18, 58, 56 and 52 while HPV infections in southeast Asia are mainly caused by types 52, 16, 58, 18 and 66.[10]

Cytological examination has become a standard examination of cervical cancer screening over the last 50 years. Cervical cancer screening examination with cytology has reduced the incidence and mortality rates due to cervical cancer. Screening of cervical cancer is effective step because the nature of this cancer has a long preinvasive phase so that minimal changes can be detected before it turns into a malignancy or metastasis. This examination evaluates the existence of cell abnormalities resulting from cervical epithelial specimens. Conventional cytological examination has a sensitivity level (70% -80%) and specificity (94-97%) to detect high-grade precancerous lesions.[1]

The high level sensitivity of HPV DNA testing has the advantage of a very high negative predictive value even against adenocarcinoma precursors, which are often not detected by cytological examination.[11]

Research on the cytology and genotype of HPV in commercial sex workers has never been conducted in South Sulawesi, especially Makassar. Therefore, it is necessary to conduct research on the cytology and genotype of HPV in women commercial sex workers in Makassar to stabilized data of this area and for women commercial sex workers regarding the risk of HPV infection and transmission and the importance of educational programs for to prevent the transmission of cervical cancer.

Method and Sample

Our study conducted as an analytical study with cross sectional analysis approach which was carried out in a localization area of Makassar City for 6 months, starting from March 2019 to September 2019. Sampling was done by consecutive sampling i.e subjects in the study sites that meet the requirements inclusions were taken as research samples. The sample of this study consist of women with reproductive age who work as commercial sex workers, who meet the inclusion criteria that is female subjects who work as commercial sex workers, aged 15 - 50 years, actively engaging in sexual relations with more than 1 sexual partner in the past 1 month, the patient is not menstruating, the patient is not using tampons, vaginal medicines, vaginal contraception, vaginal douche for 48 hours before the examination, the patient is not allowed to have sexual course within 24 hours before the examination, and the patient is not pregnant. Our sample including 80 commercial sex workers who were willing to be involved in the study.

Subjects were asked to fill out a questionnaire accompanied by a research assistant. Furthermore, subjects were given an oral explanation of the examination and taking swabs in the endocervix. After the subject understands, it can be asked for willingness to participate in research. Informed consent sheet are provided for patient thatwilling to participate in this study.

The process of collecting specimens is carried out by the clinician using a broom like brush or endocervical brush and spatula to the ecto-endocervical transformation area. Then the endocervical brush is inserted into a container containing the PreservCyt solution (Cytyc, Boxborough, MA) by turning/rinsing about 10 times. By using Thin Prep Processor homogenisation of specimens

with a certain centrifuge speed, and separating materials such as mucus, debris and washing blood from cells without damaging the grouping of cells. After going through the dispersion process, the cells are transferred to specific glass objects in a representative and accurate way through the filtration process and form a thin layer of one layer of cells (thin layer - monolayer cell). The next process of closing and closing the slide is in accordance with the routine process of pap's smear inspection.

Specimens from HPV (cervical swab) examination were put into a sterile 1ml Phosphate Buffered Saline (PBS) solution and then cortized for 3x15 seconds. Furthermore, 0.5 ml of each sample was used for the extraction of nucleic acids and the remaining preparations were frozen at -20 ° C. Extraction, detection and determination of HPV type genotypes were performed using the PCR method. Data were analyzed with SPSS.

Result

During the study period, 80 female sex workers were involved in the study. Data distribution were normal by Kolmogorov Smirnov test when the p value is> 0.05. In this study, the value of p> 0.05 was obtained indicating all data were normally distributed therefore chi-square statistical tests would be carried out.

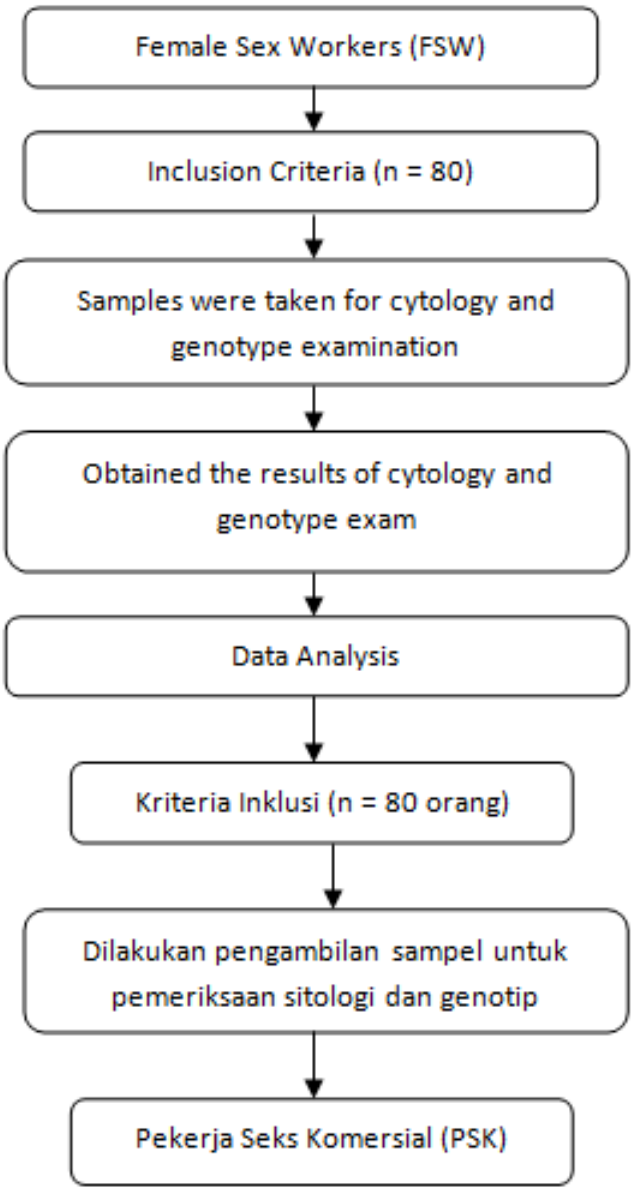


Figure 1 Flow chart

Tabel 1: Frequency Distribution of Variables

Variabel	N	%
Age		
<30 years	31	38,8
>30 years	49	61,3
Education		
<9 years	53	66,3
≥9 years	27	33,8
Marital Status		
Not married	18	22,5
Married	62	77,5
Parity		
Multiparous	16	20,0
Primiparous	14	17,5
Nuliparous	50	62,5
Duration being FSW		
<1 years	11	13,8
≥1 years	69	86,3
Age become FSW		
<15 years	7	8,8
≥15 years	73	91,3
Age ar first coitus		
<15 years	14	17,5
≥15 year	66	82,5
Sex partners in one month		
≥5	49	61,3
<5	31	38,8
Sex partners in last month		
≥5	33	41,3
<5	47	58,8
Condome usage		
Never/occasionally	40	50
Always	40	50
Sexual transmitted disease (STD)		
Yes	3	3,8
No	77	96,3

Table 1 showed that the majority of CSWs who became our sample were> 30 years old (61.3%), had received education in <9 years (66.3%), were married (77.5%), nulliparous (62, 5%), became CSW >1 year (86.3%), age at CSW ≥15 years (91,3%), age at first coitus ≥15 years (82.5%), number of sex partners in a month ≥ 5 (61.3%), the number of last sexual partners in the last 1 month <5 (58.8%), did not experience sexually transmitted infections (96.3%). The number of CSWs using and not using condoms was comparable (50% each), and all samples has never vaccinated.

Table 2: Pre-Cancer LesionsCategoriesAccordingto CervicalCytology Examination Based on Fluid (SSBC) in Female Sex Workers

Tipe Lesi	n	%
Non-specific bacterial cervicitis	48	60,0
Negatif lesion	32	40,0

Our result in table 2 showed that most of samples have Non-specific bacterial cervicitis (60%) and the remaining 40% do not have lesions.

Table 3: Distribution Genotipe HPV in Female Sex Workers

Lesion Type	n	%
High-risk HPV	20	25
Low-risk HPV	6	7,5
High-Low risk HPV	24	30
No HPV and other types	30	37,5

Table 3 showed the distribution of HPV genotypes in female sex workers, most of the samples had a 25% high risk HPV, a 7.5% low risk, a 30% high and low risk and 37.5% did not have an HPV.

Based on Figure 2 our study showed that 5 people infected with type 16 HPV, people infected with type 18 HPV were not found, 44 people infected with other types of High Risk HPV, 43 people infected with Low Risk HPV .

In Figure 3 it can be seen that of the 49 HPV types of high risk women of FSWs, HPV type 45 were detected in 6 people, type 51; 6 people, type 16; 5 people, type 33; 5 people, type 35; 5, type 52; 3 people, type 39; 2 people, type 66; 2 people, type 56; 2 people, type 68; 2 persons. And types 5,42,53,58,73 and 82 each in one person, respectively. The other types obtained in 5 people.

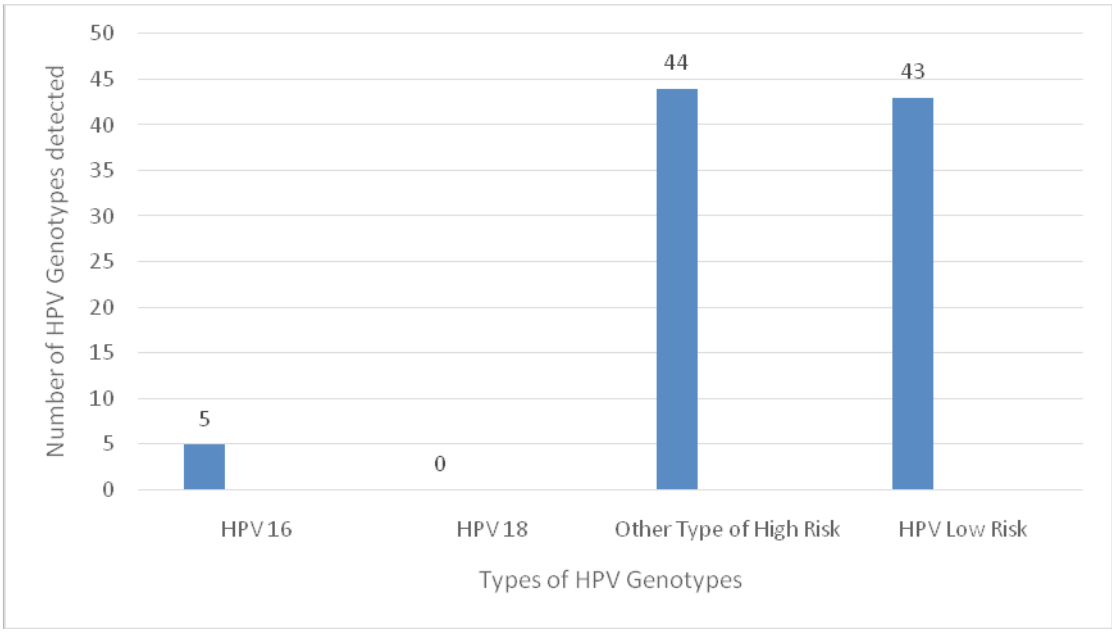


Figure 2 Distribution of HPV type 16, HPV type 18, high risk HPV, low risk HPV in female FSWs in Makassar

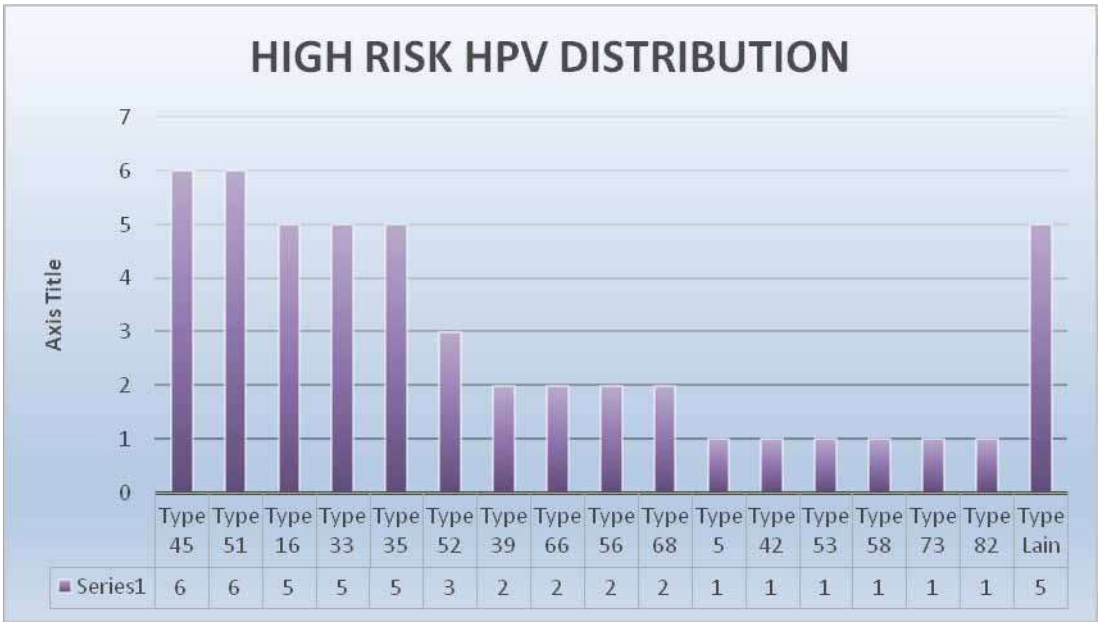


Figure 3 Distribution of High Risk HPV types in women of CSW in Makassar

Table 4: The relationship between the type of cytology of pre-cancerous lesions with the characteristics of female sex workers

Variable	Non-specific cervicitis and bacteria		Negative		p-value	OR (95% CI)
	n	%	n	%		
Age						
<30 years	20	41,7	11	34,4	0,640	1,364 (0,538–3,450)
>30 years	28	58,3	21	65,6		

Variable	Non-specific cervicitis and bacteria		Negative		p-value	OR (95% CI)
	n	%	n	%		
Education						
<9 years	34	70,8	19	59,4	0,412	1,662 (0,649–4,275)
≥9 years	14	29,2	13	40,6		
Marital Status						
Not married	9	18,8	9	28,1	0,477	0,590 (0,205–1,699)
Married	39	81,2	23	71,9		
Parity						
Multiparous	10	20,8	6	18,8	0,232	-
Primiparous	9	18,8	5	15,6		
Nuliparous	29	60,4	21	65,6		
Duration being CSW						
<1 year	9	18,8	2	6,2	0,185	0,289 (0,058–1,437)
≥1 years	39	81,2	30	93,8		
Age became CSW						
<15 years	3	6,2	4	12,5	0,429	0,467 (0,097–2,242)
≥15 years	45	93,8	28	87,5		
First coitus age						
<15 years	9	18,8	5	15,6	0,952	1,246 (0,376–4,130)
≥15 years	39	81,2	27	84,4		
Sexual partner within a month						
≥5	30	62,5	19	59,4	0,963	1,140 (0,456–2,850)
<5	18	37,5	13	40,6		
Sexual partner for last month						
≥5	21	43,8	12	37,5	0,746	1,296 (0,519–3,237)
<5	27	56,2	20	62,5		
Condom usage						
Never/Occasionally	20	41,7	20	62,5	0,110	0,429 (0,171–1,073)
Always	28	58,3	12	37,5		
STD						
Yes	3	6,2	0	0,0	0,271	0,938 (0,871–1,009)
No	45	93,8	32	100		

Uji Chi Square p<0.05

Table 4 illustrates the relationship between the type of cytology of pre-cancerous lesions and the characteristics of commercial sex workers. No association was found between age, education, marital status, parity, length of time a prostitute, age at prostitution, age of first coitus, number of sex partners in the past 1 month, use of condoms and STIs with the type of cytology of pre-cancerous lesions (p <0.05). While the relationship to vaccine use cannot be analyzed because all samples have never received an HPV vaccine.

Tabel 5: Relationship between High Risk HPV genotypes and characteristics of commercial sex workers

Variable	High Risk HPV		Other type-HPV		p-value	OR (95% CI)
	n	%	n	%		
Age						
<30 years	7	8,75	24	30	0,691	0,658 (0,230–1,881)
>30 years	13	16,25	36	45		
Education						
<9 years	15	18,75	38	47,5	0,339	1,737 (0,555–5,432)
≥9 years	5	6,25	22	27,5		
Marital Status						
Not married	5	6,25	13	16,25	0,757	1,205 (0,369–3,937)
Married	15	18,75	47	58,75		
Parity						
Multiparous	3	3,75	13	16,25	0,716	-
Primiparous	3	3,75	11	13,75		
Nuliparous	14	17,5	36	45		
Duration being FSW						
<1 year	6	7,5	5	6,25	0,015	4,714 (1,254–17,717)
≥1 years	14	17,5	55	68,75		
Age became FSW						
<15 years	3	3,75	4	5	0,253	1,941 (0,420–8,976)
≥15 years	17	21,25	56	70		
First coitus age						
<15 years	6	7,5	8	10	0,089	1,408 (0,456–4,351)
≥15 years	14	17,5	52	65		
Sexual partner within a month						
≥5	11	13,75	38	47,5	0,508	1,069 (0,387–2,955)
<5	9	11,25	22	27,5		
Sexual partner for last month						
≥5	8	10	25	31,25	0,895	1,439 (0,505–4,099)
<5	12	15	35	43,75		
Condom usage						
Never/Occasionally	10	12,5	30	37,5	1,000	1,069 (0,388–2,942)
Always	10	12,5	30	37,5		
STD						
Yes	0	0	3	3,75	0,308	1,351 (1,183–1,542)
No	20	25	57	71,25		

Chi Square test $p<0.05$

Table 5 illustrates the relationship between the High Risk HPV genotype and the characteristics of female sex workers. No relationship was found between age, education, marital status, parity, age at becoming a sex worker, number of sex partners in the past 1 month, use of condoms and STIs with the HPV genotype ($p <0.05$).

There was a significant correlation between duration of FSW and High Risk HPV genotype (p = 0.015) with an OR value of 4.714. That is, FSWs who have peddled sex for >1 year 4,714 times are more at risk of having high risk HPV.

Discussion

Cervical HPV infection is very contagious and the risk of infection increases with increasing sexual activity. Female sex workers are in a high-risk group infected and transmit HPV because having more than one sexual partner. In this study we tried to analyze the risk factors for pre-cancerous lesions and HPV genotype in female sex workers in Makassar City.

Based on the type of lesion, the majority of samples from FSW had non-specific and non-bacterial cervicitis (60%) and (40%) the rest had no lesions. The results of this study are also not much different from previous studies which showed that femalesex workers had more positive cervicitis (80.4%) results compared with negative results (19.6%). [12] Cervicitis is a frequent case of sexually transmitted infections occur in female sex workers where cervical inflammation occurs as a result of infection by sexual organisms, most often caused by Chlamydia trachomatis or Neisseria gonorrhoeae.[13]

Based on the distribution of HPV genotypes in female sex workers, most of the samples had high and low risk HPV (62.5%) and 37.5% did not have HPV. A meta-analysis of HPV infection in female sex workers in Asia found that the prevalence of HPV among female sex workers was 12.8 - 84.8%. Female sex workers are 10 times more likely to have HPV infection compared to the general population. The analysis also showed that the prevalence of HPV in the Southeast Asian region was dominated by HPV types 52 (12.9%), 16 (8.5%), 58 (5.2%), 18 (5.0%), and 66 (4 9%). [10]

Age is one of the factors causing precancerous lesions and HPV infection. In this study the majority of FSWs with non-specific cervicitis lesions and bacteria aged> 30 years. High-risk HPV infections are also more common in women> 30 years old (16,25%). However, in the bivariate analysis there was no association between age with lesion type (p = 0.640) or HPV genotype (p = 0.691). The results of this study are supported by previous studies which showed that women aged 31 - 40 years and 41 - 50 years were most at risk of having pre-cancerous lesions (65% and 63.5%, respectively) 14 However, contrary to the results of studies in women workers sexually reported in the Philippines that age 25 years or younger is more at risk of having an HPV infection.[15]

In this study, the majority of samples were low-educated. In samples with non-specific cervicitis and bacterial lesions 70.8% received education for <9 years, while based on genotype type 18.75% samples with education <9 years were infected with high-risk HPV. But in the bivariate analysis, education was not significantly related to the type of lesion and HPV genotype (p = 0.412 and 0.339, respectively). The results of this study are supported by research in Nepal which found that HPV infection was lower in educated women compared to uneducated one.[16]

Most of the samples in this study were married. The prevalence of nonspecific and bacterial cervicitis lesions is higher in married women (81.2%). High-risk HPV infection is also more common in married women (18.75%). However, in the bivariate analysis no relationship was found between marital status and lesion type (p = 0.447) and high-risk HPV genotype (0.757). The results of this study are supported by previous studies which reported that the prevalence of HPV infection was higher in married women, especially married more than once. [16]

In this study the majority of samples were nulliparous women. Nonspecific and bacterial cervicitis lesions are higher in nulliparous women (60.4%). Low and high risk HPV infections are also more common in nulliparous women (17.5%). But in the bivariate analysis parity was not related to the type of lesion and HPV genotype (p = 0.232 and 0.716, respectively). Most groups with positive pre-cancerous lesions had children <4 (34.2%) and they also did not find a significant relationship between parity number and risk of developing cervical pre-cancerous lesions (p = 0.280) .[17]

In this study non-specific and bacterial cervicitis lesion were higher in women who became FSWs for >1 year (81.2%) but in contrast to high-risk HPV infections were more common in women who had FSWs> 1 year (17.5%). In the old bivariate analysis, FSW was not related to the type of lesion (p = 0.185) but significantly correlated with high-risk HPV genotype (p = 0.015) with OR 4,714 (95% CI 1,254-17,717) meaning that being FSW >1 year was at risk. against high-risk HPV infection. This is consistent with the results of previous studies that reported that women who worked as FSWs for 6 months

- 2 years were more at risk for HPV infection. But after 2 years, the risk of HPV is proven to decrease. This was hypothesized due to immunity to HPV in women who have worked as sex workers> 2 years.[15]

Most of the samples aged ≥15 years when they became FSWs, and the age at first coitus was mostly at the age of ≥15 years. No significant relationship was found between age at FSW and age at first coitus with nonspecific and bacterial cervicitis lesions as well as with high-risk HPV infection (p = 0.429 and p = 0.952); (p = 0.253 and p = 0.089). Women with non-specific bacterial and bacterial lesions and high and low risk HPV genotypes have more sexual partners ≥5. However, no significant relationship was found between the number of sexual partners with the type of lesion and high-risk HPV genotype (p = 0.963 and 0.508, respectively). The benefits of condoms in preventing HPV in women are still a matter of debate. There has been a reported decrease in HPV infection in female sex workers who use condoms, but it is not fully effective in protecting against HPV infection. In this study, no relationship was found. There is an argument that condoms cannot completely cover the infected area

This study has several limitations including the small sample size and cross-sectional design. And in this study the sample collected is still under the institution where the results obtained may be different from the free sample or freelance. Further research is needed with larger samples and better designs to confirm the results of this study.

CONCLUSION

Based on cytological examination of precancerous lesion types in female sex workers in Makassar, most of the FSW samples had non-specific and bacterial cervicitis (60%) and the remaining 40% had no lesions. No relationship was found between age, education, marital status, parity, age at becoming a sex worker, number of sex partners in the past 1 month, use of condoms and STIs with high-risk HPV genotype (p <0.05). There was a significant correlation between duration becameFSW and high risk HPV genotype (p = 0.015) with an OR value of 4.714. That is,FSWs who have work as sexual worker for >1 year 4,714 times are more at risk of having high risk HPV.

Ethical Clearance: No ethical approval is needed.

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Comparing the Effect of Combined Therapy of Extracorporeal Shock Wave Therapy and Ice Massage with Combined Therapy of Therapeutic Ultrasound with Paraffin Wax Bath in Treating Trigger Finger

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Abstract

Introduction: Trigger finger is a common hand disorder characterized by locking, catching, or clicking with range of motion of the affected finger. Purpose: The aim of this study was to compare the effectiveness of combined therapy of extracorporeal shockwave therapy (ESWT) and ice massage to the combined therapy of therapeutic ultrasound (US) and paraffin wax bath in treating trigger finger. Method: 19 patients were assigned to the US + wax bath group while the remaining 10 were assigned to ESWT + ice massage group. The effectiveness of the treatment was assessed using visual analog scale (VAS) to measure pain score and Quick-Disabilities of the Arm, Shoulder, and Hand (Quick DASH) questionnaire to assess the functionality of the affected limb at 1st, 2nd, 3rd and 4th week after the initial treatment. Results: The US + wax bath group had reduced more pain and improved more hand function than the ESWT + ice massage group for all visits. Conclusion: This study was not able to prove the hypothesis that ESWT + ice massage group was better than US + wax bath in treating trigger finger. However, a similar study like this but with larger sample size, individualized protocols for ESWT participants, longer study durations and conducted by experts in ESWT is able to show which treatment is better in treating trigger finger.

Keywords: *Trigger finger, shockwave therapy, ultrasound, paraffin wax bath, ice massage.*

Introduction

Trigger finger also known as Stenosing tenosynovitis is one of the most common hand disorders. It occurs when there is difference in size between the tendon and the pulley which the tendon passes through[1]. This difference may due to Narrowing or thickening of the A1 pulley or enlargement of tendon (flexor digitorum profundus and flexor digitorum superficialis) secondary to Degeneration or Tendinosis.[2] This abnormality results in characteristic locking, catching, or Clicking with range of motion. Women are six-times more likely to develop trigger finger than men, with a higher Incidence in patients with Diabetes Mellitus (DM) and Rheumatoid Arthritis (RA) (Adams & Habbu, 2015)[3]. Trigger Finger has no known cause. Female gender, DM, RA, gout and occupations that requiring repetitive finger motions are known risk factors for developing trigger finger (DynaMed, 2017)[4]. Patient may complaint of pain at the level of the A1 Pulley (around the metacarpophalangeal joint)[5], reduced grip strength, and clicking, triggering or locking of digi[6]t. He/she also having difficulty in the extension of digit, a full range of Motion of the affected digit can only achieved by using the opposing hand to assist. In advanced disease, flexion contracture may occur (digit becomes fixed in flexion)

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(DynaMed, 2017)[7]. Initial conservative management in adults with trigger finger is recommended by the European HANDGUIDE Group (Huisstede et.al)[8]. Conservative treatments that can be used include oral nonsteriodal anti-inflammatory drugs (NSAIDs) for temporary pain relief, corticosteroid injection[9] (first line treatment for trigger finger), splinting and physical therapy[10]. Surgical intervention is only recommended if the symptoms persists and are significant in nature after the start of conservative management more than one month (Huisstede et.al)[11]. Ultrasound (US) has been used in clinical practice since the 1950s and still remains as a popular intervention for a various clinical problems (Watson, 2017). US waves can be delivered continuously or in pulses. Continuous US generate heat which increases the tissue temperature[12]. This thermal effect is often used in chronic sprains and strains, and for pain relief. Pulsed US tend to generate non-thermal effects such as cavitation and micro streaming. The non-thermal effects are used in stimulation of tissue regeneration, protein synthesis in fibroblasts and tendon repair (Tsai, Tang & Liang, 2011).Since the early 1990s, extracorporeal shock wave therapy (ESWT) has been used in the management of tendon pathologies to ease pain and improve function (Malliaropoulos et.al, 2016)[13]. The physiological and therapeutic mechanism of ESWT is not fully identified but there are some effects that have been confirmed (Watson, 2015). One of the reasons of using ESWT in therapy is that it can bring a tissue from a more chronic to a more acute stage and stimulates repair sequence that has been stalled [14]. There are several treatment effects of ESWT that have been confirmed: mechanical stimulation, increased local blood flow, increased cellular activity, analgesic effect on afferent nerves and breaking down of calcific deposits (Watson, 2015).Therapeutic ultrasound (US) and extracorporeal shockwave therapy (ESWT) have been compared in various conditions in other studies but there are no studies comparing these two modalities in treating trigger finger [15]. So this study is able to compare the 2 modalities and see which one is better in treating trigger finger. Yildirim et.al (2015) had conducted a randomized clinical trial that compared the efficacies of ESWT and corticosteroid injection for the management of trigger finger. In his study, 40 patients with trigger fingers were randomly assigned to ESWT or corticosteroid injection group (20 patients for each group). Visual analogue scale (VAS) was used to measure pain. A 0- to -10 point trigger finger assessment was used to measure the frequency of triggering (FT), the severity of triggering (ST) and functional impact of triggering (FIT)[16]. The Quick-Disabilities of the Arm, Shoulder, and Hand (QuickDASH) self-administered questionnaire was used to measure the symptom severity and functional status of the patient. All these clinical measures were assessment before treatment and after one, two and six months. At the end of study, 36 patients out of the original 40 patients completed the study (19 from ESWT group; 17 from injection group). Both groups showed statistically significant improvements in all outcome measures after treatment. In conclusion, ESWT was suggested as an alternative treatment for patients who didn't prefer corticosteroid injection. Malliaropoulos et.al (2016) had conducted a retrospective cohort study to evaluate the effectiveness of radial extracorporeal shockwave therapy (rESWT) for the treatment of trigger finger. 44 patients with 49 affected fingers or thumb who took part in the study were each given an individually adapted rESWT protocol. The Roles and Maudsley score was used to assess functional outcome after rESWT treatment. VAS was used to measure pain severity. Outcome measures were assessed at baseline (pretreatment) and after one, three and twelve month follow-up appointments (post-treatment). At the end of study, significant reductions in pain score and functional improvement were seen between baselines and all follow up assessments. The study was able to prove the effectiveness of rESWT in treating trigger finger but requiring randomized controlled trials to provide further evidence of the effect [17]. Dogru et.al (2017) had conducted a study that investigated the effect of rESWT in treating trigger finger. 16 patients (18 affected fingers) diagnosed with trigger finger grade 1, 2 and 3 according to Quinnel classification took part in the study. They were subjected to 10 sessions, twice a week of rESWT. Pain scores, general functional, range of motion, grip strength and pinch strength were assessed at every week for five weeks during treatment and 3 months after treatment. It was observed that the patients shown decrease in pain levels and increase in general functional capacity, range of motion, grip strength and pinch strength[18].

Methodology

This was a prospective randomized controlled clinical trial with follow-up at 1st, 2nd, 3rd and 4th week after the initial treatment. Patients who signed informed consent were selected after they were given a brief but detailed explanation of the study aims, benefits and the study procedures. The research protocol was reviewed and approved by the AIMST University

Human Ethics Committee (AUHEC), and it was carried out in accordance with the principles of the Declaration of Helsinki. A formula for a two sided-test of 5%, is $m(size\ per\ group) = \frac{2c}{\delta 2} + 1$ where $\delta = \frac{[\mu 2 - \mu 1]}{\sigma}$ is the standardized size and μ1 and μ2 are the means of the two treatment group, σ is the common standard deviation . The study was started at 17th December 2018 and ended at 13th February 2019. The treatment duration for each visit was about one hour including examination of the patient's condition, and retaking the measurement of pain score and QuickDASH score, and providing the necessary treatments to treat the patient. The details of the treatments are in 3.8 Techniques. There are 2 groups in this study: the US + wax bath group (Group A) and the ESWT + ice massage group. (Group B) These 2 groups will be using different techniques: Group A will be using ultrasound and wax bath; Group B will be using ESWT and ice massage. However, both groups will be given identical exercises that are beneficial in treating trigger finger.

	Intervention
Group A: US, paraffin wax bath and therapeutic exercise	US: Sonopuls 490 that was available in the hospital was used to administer US. The dosage was 3MHz, intensity of 0.5 W/cm^2 and duty cycle 50%. Duration: 5 minutes. The ultrasound gel was applied at the top of the skin of the palm. An electrode was then placed on the area covered by the gel. The electrode was then moved in uniform speed and in circular motion at the affected area for 5 minutes. Paraffin wax bath: The subject was asked to wash his hand before dipping his hand into the wax bath. The subject dipped his hand inside the wax bath for 1 second and then lifted up his hand from the wax bath. He needed to dip his hand 7 times to form a coat of wax around his hand. Subject's hand was wrapped by a plastic before further wrapped by an insulator grove to prevent the heat from dissipating. Treatment duration: 15 minutes. Stretching. The subject's affected finger was stretched by hyper-extending his affected finger and held the position for 10 seconds. The stretch was then released. This was repeated for 15 times. Resisted finger spread using rubber band. Begin by pinching the tips of fingers and thumbs. Put an elastic band around the fingers. The fingers were moved away from the thumb so that the band became tight The position was held for 10 seconds and the stretch was released. This was repeated for 15 times.
Group B: ESWT, ice massage and therapeutic exercise	ESWT: It was administered using Shock Master 500. The dosage is 1000 shocks, 2.1 bars and 15 Hz. Ultrasound gel was placed on the skin of the affected area. The d-actor electrode was placed on subject's affected area. Ice massage for 5 minutes. It was being performed on the area which ESWT was given by using cyrocup. Stretching. The subject's affected finger was stretched by hyper-extending his affected finger and held the position for 10 seconds. The stretch was then released. This was repeated for 15 times Resisted finger spread using rubber band. Begin by pinching the tips of fingers and thumbs. Put an elastic band around the fingers. The fingers were moved away from the thumb so that the band became tight The position was held for 10 seconds and the stretch was released. This was repeated for 15 times.

Data analysis was done by using Statistical Package for the Social Sciences (SPSS) verison 21 (Armonk, NY, IBM Corp). Analysis of varience (ANOVA) with repeated measure was used to test differences in the pain score and QuickDASH scores between 2 groups at the baseline (initial visit) and subsequent visit. Independent t-test was used to compare the difference between US + wax bath group and ESWT + ice massage group on the 1st visit and subsequent visit.

Result

Table 1: Demographics And Clinical Data Of The Patients

	Frequency	Percentage (%)
Study Group		
US+ paraffin wax bath	19	65.5
ESWT+ ice massage	10	34.5
Gender		
Male	6	20.7
Female	23	79.3

	Frequency	Percentage (%)
Ethnicity		
Malay	12	41.4
Chinese	17	58.6
Age range		
40-49	8	27.6
50-59	13	44.8
60-69	6	20.7
70-79	2	6.9
Affected digits		
Thumb	5	17.2
Index	11	37.9
Middle	2	6.9
Ring	10	34.5
Little	1	3.4

Table 1 showed that there are 29 patients taking part in this study. There were no dropouts. 19 patients in US + wax bath group (65.5%) while 10 patients in ESWT + ice massage group (34.5%).

Table 2 Mean Pain Scores of Both Study Groups and Tests of Within-Subjects Effects

Quick DASH	Mean	SD	df	F	P	Partial Eta Square
1st visit						
Ultra sound	36.8316	18.20406				
ESWT	31.8000	11.13613				
2nd visit						
US	34.1000	17.09662				
ESWT	31.6200	9.39537				
3rd visit						
US	30.5474	15.41988	1.991, 53.754	8.067	0.000	0.230
ESWT	33.1600	10.56800				
4th visit						
US	24.8737	15.86778				
ESWT	31.3400	10.42595				
5th visit						
US	22.4895	12.88836				
ESWT	31.3400	10.42595				

Note: SD: standard deviation, df: degree of freedom

Table 2 showed the mean pain scores of both study groups: US + wax bath group and ESWT + ice massage group in each visit and Tests of Within-Subjects Effects.

Table 3 Mean Quick DASH Scores of Both Study Groups and Tests of Within-Subjects Effects

Pain score	Mean	SD	df	F	P	Partial Eta Square
1st visit						
Ultra sound	5.2632	1.62761				
ESWT	2.6000	1.57762				
2nd visit						
US	4.8241	1.53707				
ESWT	2.6000	1.57762				
3rd visit						
US	3.9474	1.47097	2.031,	13.739	0.000	0.337
ESWT	2.6000	1.57762	54.842			
4th visit						
US	3.2632	1.40800				
ESWT	2.8000	1.22927				
5th visit						
US	3.0000	1.15470				
ESWT	2.8000	0.42164				

Note: SD: standard deviation, df: degree of freedom

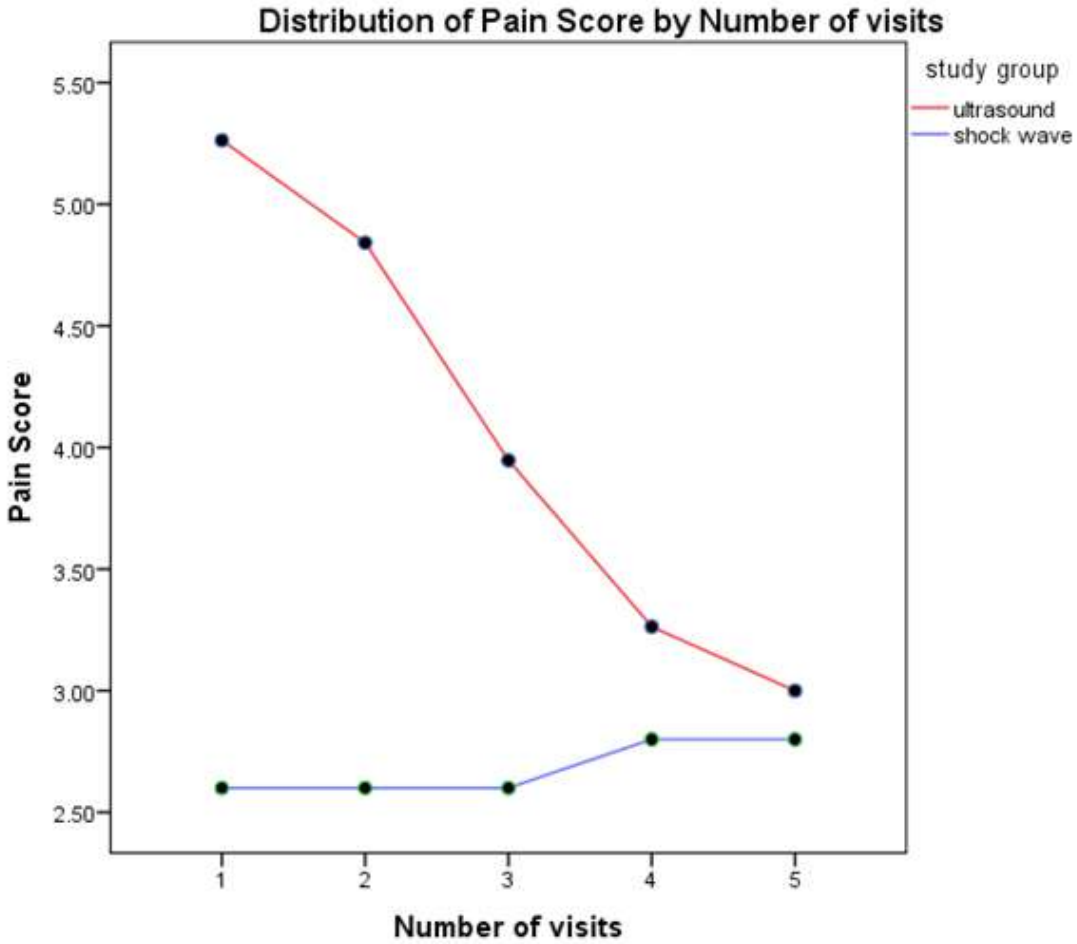


Figure 1 Graph of Distribution of Pain Score by Number of Visits

Figure 1 showed that the mean pain score recorded in the US + wax bath group declined steadily from the 1st visit until the 5th visit.

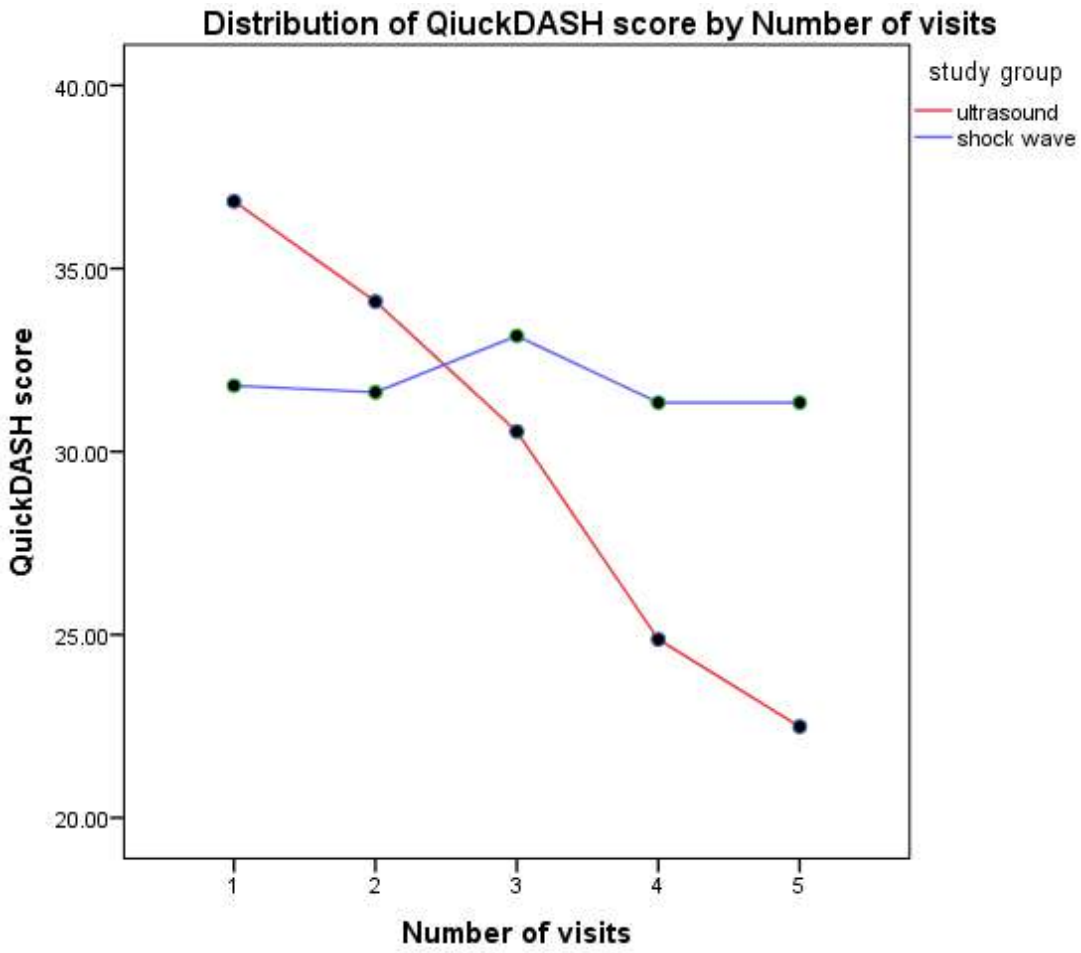


Figure 2 Graph of Distribution of QuickDASH Score by Number of Visits

Figure 2 showed that mean QuickDASH score recorded in the US + wax bath group declined steadily from the 1st visit until the 5th visit.

Table 4. Mean difference in pain score between visits of both groups

	Mean	SD
Mean difference of pain score between 1st and 2nd visit		
US + wax bath	-.4211	.76853
ESWT + ice massage	.0000	.00000
Mean Difference of pain score between 2nd and 3rd visit		
US + wax bath	-.8947	.45883
ESWT + ice massage	.0000	.00000
Mean Difference of pain score between 3rd and 4th visit		
US + wax bath	-.6842	.74927
ESWT + ice massage	.2000	.42164
Mean Difference of pain score between 4th and 5th visit		
US + wax bath	-.2632	.56195
ESWT + ice massage	.0000	1.15470

Note: SD: standard deviation

Table 4 showed the mean difference of pain scores between visits of both study groups: US + wax bath group and ESWT + ice massage group.

Table 5 Result of mean difference in Pain Score between US + wax bath group and ESWT + ice massage group in First and Subsequent Visits

	t	df	P	Mean difference	Std. error Difference	95% Confidence Interval of Difference	
						Lower	Upper
Pain score difference between 1st and 2nd visit	-2.388	18.000	0.28	-.42105	.17631	-.79147	-.05063
Pain score difference between 2nd and 3rd visit	-8.500	18.000	.000	-.89474	.10526	-1.11589	-.67359
Pain score difference between 3rd and 4th visit	-4.065	26.784	.000	-.88421	.21754	-1.33074	-.43768
Pain score difference between 4th and 5th visit	-.832	27	.413	-.26316	.31618	-.91190	.38559

Note = df: degree of freedom

Table 5 showed the comparison of the mean difference of pain scores between visits in the US + wax bath group with the mean difference of pain scores between visits in the ESWT + ice massage group.

Discussion

The findings of the study show that the combined therapy of therapeutic US and wax bath is more effective than the combined therapy of ESWT and ice massage in reducing pain scores and improving upper limb functionality of trigger finger patients in all visits. The effectiveness of both groups in treating trigger finger can be seen clearly in figure 4.1 and figure 4.2 as both groups show that the US + wax bath group is able to decrease pain score and QuickDASH score steadily throughout the visits but there are no improvement in the ESWT + ice massage group

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Correlation between Duration of the Second Stage and Pelvic Floor Muscle Strength in Primiparous Women Following Vaginal Delivery and Caesarean Section

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Abstract

Introduction: Women underwent caesarean section tend to have higher pelvic floor muscle strength;however, pelvic floor muscle strength in women underwent caesarean section in the second stage of labor have not been widely studied. This study aimed to assess correlation between duration of the second stage of labor with pelvic floor muscle strength in primiparous women underwent vaginal delivery and caesarean section.

Materials and Method: In this prospective study, 58 primiparous women underwent vaginal delivery and second–stage caesarean section were recruited from hospitals affiliated with Hasanuddin University in Makassar, Indonesia. Pelvic floor muscle strength was measured at 6 weeks following delivery. Correlation analysis was performed to estimate the correlation between pelvic floor muscle strength and duration of the second stage.

Results: Demographical characteristics were similar between groups. Primiparous women in caesarean section group had longer duration of second stage than women in vaginal delivery group(206,38 + 107,66 minutes versus 61,89 + 36,67, $P < 0.001$). Mean pelvic muscle floor strength in primiparous women underwent second stage caesarean section were similar to women underwent vaginal delivery(41,99 + 8,59 cm H_2O versus. 41,83 + 12,44 cm H_2O, $P = 0.954$). Duration of the second stage had no correlation with pelvic floor muscle strength in both vaginal delivery (r= 0,248; $P = 0.195$) and caesarean section (r = -0,083; $P = 0.669$).

Conclusion: Duration of the second stage may not alter pelvic floor muscle strength regardless of mode of delivery.

Keywords: *Pelvic floor muscle strength, duration of second stage, vaginal delivery, caesarean section.*

Introduction

Pelvic floor is an important structure that serves as mechanical and functional support of pelvic organs[1,2]. Damage to pelvic floor muscles may lead to increased morbidity associated with pelvic organ dysfunction such as urinary incontinence, anal incontinence, and pelvic organ prolapse[3]. Delivery and aging are both known as significant risk factors that may contribute to pelvic floor dysfunction[3].

Pelvic floor integrity is provided by coordinated mechanism from pelvic muscles, nerves, and connective tissues. Pelvic connective tissue may become principal support system when damage occur to pelvic floor muscles and eventually lead to pelvic organ dysfunction[4].

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In many studies, pregnancy and childbirth were the main risk factors for decreased pelvic floor muscle strength and function through stretch and denervation mechanisms[5–7]. Numerous studies have demonstrated that vaginal delivery was considered as the main risk factor for developing pelvic floor dysfunction compared with caesarean delivery[8–10]; however, the studies did not specifically compare the second–stage caesarean section and vaginal delivery in primiparous women.

Prolonged second stage of labor is still widely found in our regions especially at primary health center. Complicated geographic and administrative system may delay referral process and management, thus lead to extremely prolonged second stage of labor.

The current study was undertaken to find correlation between duration of second stage of labor and pelvic floor muscle strength in vaginal delivery and second – stage caesarean section. Besides, we also would like to find if there had been any difference in pelvic floor muscle strength between two groups.

Materials and Method

This study was an observational prospective study conducted in several regional hospitals affiliated with Hasanuddin Univeristy in Makassar, Indoensia between February 2019 – February 2020. Primiparous women underwent vaginal delivery and second – stage caesarean section were recruited by consecutive sampling. Inclusion criteria of this study were primiparous woman with a history of term delivery, 20–35 years old, birthweight from 2500 g–4000 g, no prior gynecologic surgery and troublesome systemic illnesses, and without third to fourth degree perineal tear.

Ethical and research approval for this study were obtained from Hasanuddin University Ethical Committee. All participants were provided by written informed consent. Eligible participants were then evaluated at 6 weeks following delivery. Pelvic floor muscle strength was measured by Peritron perineometer 9300 V. The device consists of a 28 mm diameter – compressible vaginal probe connected to microprocessor. Pelvic floor muscle strength was measured in cm H_2O following insertion into vagina. The measurement are reliable and reproducible as demonstrated by previous studies[11,12].

Before performing measurement, pelvic muscle contraction was ascertained by digital palpation into vagina. Participants were then instructed to contract their muscles as they were trying to hold in gas. Contraction of the abdominal and gluteal muscles had to be excluded in this maneuver. Participants were requested to contract their pelvic muscles as strong as possible, and maintain their contraction as long as possible. Pelvic muscles were than allowed to relax after contraction. After confirming correct pelvic contraction, the probe, covered with condom, was inserted into vagina and three contractions were measured, with a 10 second rest interval. Pelvic floor muscle strength was averaged over the three recorded contractions.

Demographical and clinical data were mainly acquired from medical record. Pelvic floor dysfunctions such as urinary incontinence, anal incontinence, and pelvic organ prolapse were assessed concomittanly with pelvic floor strength measurement. We used Questionnaire for Urinary Incontinence Diagnosis (QUID) to identify any postpartum urinary incontinence following delivery[13]. Anal incontinence was assessed by Wexner scoring system[14]. Pelvic organ prolpase was assesed by Pelvic Organ Prolaps Quantification system (POP-Q)[15].

Demographical and clinical characteristics, such as age, occupation, antenatal care frequency, education, maternal body mass index, active phase progression, and birthweight were analyzed by Pearson chi square or Fisher's exact test. Duration of second stage and pelvic muscle floor muscle strength in both groups were tested for normality by Shapiro Wilk normality test. Independent *t* test or Mann Whitney test were used to compare pelvic floor muscle strength and duration of the second stage between groups. Spearman rank test was used to estimate the correlation between duration of the second stage and pelvic floor muscle strength in both groups. Data regarding urinary incontinence between groups which assessed by Questionnaire for Urinary Incontinence Diagnosis was analysed by Mann–Whitney test after performing Shapiro Wilk normality test. Fisher's exact test was used to analyse Wexner score and POP-Q system between groups.

All data analyses were performed using SPSS 24 statistical software. Statistical significance for all analysis was defined at 5% significance level.

Results

This study focused on primiparous women underwent vaginal delivery and second – stage caesarean section. Secod–stage caesarean section is not as common

as other caesarean sections performed before entering second – stage caesarean section; however, we were able to evaluate 29 participants in this group. Moreover, in our region the duration of the second stage of labor may be extremely long as demonstrated in our study which revelaed the longest duration of second stage of labor was 450 minutes.

Of 58 participants fulfiled eligibility criteria of our study, demographical and clinical characteristics such asmaternal age, occupation, educational level, number of antenatal care visits, neonatal birthweight, and active phase progression were analysed as summarized in Table 1. Primiparous women in vaginal delivery group tended to be younger (P = 0.061). Both groups were likely to have a role as housewife at evaluation (P = 0.256). Educational level was not different between groups (P = 0.083). Almost all women in both second – stage caesarean section and vaginal delivery group had more than 4 times antenatal care visits during pregnancy (89.7% versus 96.8%). All demographical characteristics did not show any significant association with mode of delivery (P> 0.05).

Twenyty–one women (72.4%) women in both groups had normal body mass index (P = 0.771). Abnormal body mass index was not likely to be found in our study population. Twenty–one women (72.4%) in second – stage caesarean section group had neonatal birthweight > 3000 g compared with only 14 women (48.3%) in vaginal delivery group. Women underwent vaginal delivery were more likely to have lower neonatal birth weight even if it was not statitically significant (P = 0.060). Of 29 women in second–stage caesarean group, 21 women (72.4%) had slower progression of active phase of labor, whereas only 13 (44.8%) women in vaginal delivery had this progression abnormality (P = 0.033).

Mean duration of the second stage of labor in women underwent caesarean section was 206.38 + 107.66 minutes. This finding was extremely different with mean duration of the second stage of labor in vaginal delivery group, which was 61.89 + 36.67 minutes (P< 0.001) as shown in table 2. The longest duration of the second stage of labor in caesarean section and vaginal delivery were 450 minutes versus 180 minutes. Mean pelvic floor muscle strength in second – stage caesarean section and vaginal delivery group were similar (41.83 + 12.44 cm H_2O versus 41.99 + 8.59 cm H_2O, P = 0.954). One women in second – stage caesarean section had lowest mean pelvic floor muscle strength among all participants, which was 14.9 cm H_2O. The comparison is shown in figure 1.

Our main goal in this study was to determine if there was any correlation between duration of the second stage of labor and mean pelvic floor muscle strength in both groups (Table 3). We found there was no correlation between duration of the second stage and pelvic floor muscle strength in (r = – 0.083, P = 0.669). Moreover, duration of the second stage and pelvic floor muscle strength in vaginal delivery group also did not show any correlation (r = 0.248, P = 0.195). These correlations are depicted in figure 2a and 2b.

Postpartum urinary incontinence was assessed by Questionnaire for Urinary Incontinence Diagnosis (QUID) as shown in table 4. Stress urinary incontinence was more prone to occur in second – stage caesarean group than vaginal delivery (0.34 + 0.18 versus 0.24 + 0.51 P = 0.044). Maximum QUID stress score in second – stage caesarean was 2.0. QUID urge scores were similar between second – stage caesarean section and vaginal delivery (0.17 + 0.47 versus 0.10 + 0.40, P = 0.410).

Anal incontinence was assessed by Wexner score. Score of zero means perfect anal continence, whereas score equal to or more than 1 indicates any anal incontinence. There was no woman in vaginal delivery group that had experienced any episodes of anal incontinence (Table 5). Three women (10.3%) in second – stage caesarean group had experienced some episodes of anal incontinence, all of which were flatal incontinence. However, they did not have persistent symptom at 6 weeks following delivery. Ultimately, anal incontinence had no associations with mode of delivery (P = 0,237).

Anterior pelvic organ prolpase was found in 2 women (6.9%) in both groups. Uterine prolapse occured in 2 women in second – stage caesarean group and 1 women in vaginal group. In contrast to 2 women (6.9%) with posteior pelvic organ prolapse in second – stage caesarean section, there was no posterior pelvic organ prolapse found in vaginal delivery group. Pelvic organ prolapse and mode of delivery did not show any significant association (p > 0.05).

Discussion

We determined all primiparous women in the main

inclusion criteria as to avoid any confounding variables that may be found in multiparous women. In addition, nulliparous women are considered to have higher mean pelvic floor muscle strength[16]. There were no significant differences in sociodemographic characteristics between groups in our study ($P > 0.05$)

Women lived in rural areas were at increased risk to have prolonged second stage of labor due to referral delays. This may be caused by geographical and administrative obstacles. In addition, women underwent second – stage caesarean section were more likely to have prolonged active phase of labor as reflected by previous study[17]. In our study, we may not be able to identify the exact cause of prolonged second stage as we did not rule out cephalopelvic disproportion as our exclusion criteria.

Our findings suggest that there is no correlation between duration of the second stage of labor and pelvic floor muscle strength in both groups when measured at 6 weeks following delivery. Previous study demonstrated that prolonged second stage may confer greater risk to have decreased pelvic floor muscle strength but the study did not specify delivery method and duration of the prolonged second stage[18]. Fetal head compression at second – stage of labor may incite high pressure to birth canal[19]. This process may severely injure pelvic floor muscles especially in prolonged second stage of labor. This effect may not be accurately determined because most women with prolonged second stage would eventually end up in caesarean section, as demonstrated in our study.

Duration of the second stage in our study was extremely different between groups. Mean duration of the second stage in second – stage caesarean was 206.38 + 107.66 minutes, meanwhile in vaginal delivery group the second stage duration was 61.89 + 36.67 minutes. Difference between groups may result from prolonged length of time to find referral facilities, differences in response time between hospitals, and the ability of health care provider to assess labor progression.

Prolonged second stage of labor which dominantly managed by caesarean section is not likely to decrease pelvic floor muscle strength. There was no difference in pelvic floor muscle strength between groups in our study. We were not able to find primiparous women underwent vaginal delivery with remarkable prolonged second stage of labor as in caesaren section. This limitation did hinder us to find the exact relationship between prolonged second stage of labor and vaginal delivery. Nonetheless, we are still able to infer that prolonged second stage may not have detrimental effect on pelvic floor muscles when managed by caesarean section.

Mean pelvic floor muslce strength in primiparous women underwent vaginal delivery did not differ significantly with second – stage caesarean group (41.99 + 8.59 cm H_2O vs 41.83 + 12.44 cm H_2O). This findings differ from previous study demonstrated that mean pelvic floor muscle strength in women underwent vaginal delivery with episiotomy was 32.71 + 14 cm H_2O and without episiotomy was 53.88 + 20.9 cm H_2O [16]. In addition, the study also found that mean pelvic floor muscle strength in women underwent elective caesarean section was 52.9 + 21.29 cm H_2O and in emergency caesarean section was 48 + 21.04 cm H_2O. Another study found that mean pelvic floor muscle strength in women underwent vaginal delivery and second – stage caesarean section were 27 cm H_2O versus 36.5 cm H_2O, though the study did not performed exclusively for primiparous women[18]. Different results between studies may result from distinct subject characteristics, as well as time interval between delivery and pelvic floor evaluation in which we performed at relatively shorter duration following delivery.

Our study demonstrated that there was no significant difference in pelvic floor muscle strength between groups. This may result from full recovery of pelvic floor muscle that may take place at 6 weeks following delivery. However, a study has stated that restoration of pelvic floor muscle strength may be achieved at 8 weeks following vaginal delivery. Moreover, pelvic floor damage exclusively occurs in a woman with a history of vaginal delivery[5]. This may confirm similarities of pelvic floor muscle strength between groups in our study in which no extensive pelvic damage occured in second – stage caesarean section and full restoration of pelvic muscle did take place in vaginal delivery.

Women in second – stage caesarean section group were more likely to experieced stress urinary incontinence as evaluated by *Questionnaire for Urinary Incontinence Diagnosis* (QUID). In contrast to our study, some studies described that women underwent vaginal delivery were more prone to have stress urinary incontinence compared with women underwent caesarean section[19,20]. The difference with our study may result from special population enrolled in our study, which limited to second

– stage caesarean section. In addition, our study suggests that stress urinary incontinence was more likely to occur in older women and women with a history of prolonged second stage. Another study demonstrated that there was no relationship between duration of the second stage of labor and urinary incontinence occurence[21]. However, the study did not specify the mode of delivery. All women with episodes of stress urinary incontinence had neonatal birthweight more than 3000 g. This finding is different with previous study demonstrated that there was no association between birth weight and stress urinary incontinence; nevertheless we did not estimate this association as the number of subjects with stress urinary incontinence were ultimately low[22].

In contrat to stress urinary incontinence, urge urinary incontinence tend to occur in younger women with normal body mass index. Many studies suggest that urge urinary incontinence is not associated with mode of delivery and may occur sporadically following delivery[23]. Although urge urinary incontinence was more likely to occur in women with second – stage caesarean section, we were not able to determine if it was a true urge urinary incontinence as we did not perform urinalysis examination to rule out genitourinary tract infection. Our study suggests that both stress and urge urinary incontinence may occur transiently following delivery as none of our participants had persistent symptoms.

Perfect anal continence was found in all women underwent vaginal delivery, whereas 3 women in second – stage caesarean group had experienced some episodes of anal incontinence following delivery. This findings may indicated that second – stage caesarean section may confer greater risk to have anal incontinence especially in prolonged second stage of labor. Prevalence of anal incontinence following delivery is extremely small and flatal incontinence is the most frequent type occur following delivery[24,25]. Moreover, postpartutm anal incontinence tends to occur sporadically without identifiable risk factors as shown in our study[26].

Pelvic organ prolapse occurences were low regardless of mode of delivery. A previous study described that about 18% primiparous women underwent vaginal delivery and 7% women underwent caesarean section would eventually have second stage pelvic organ prolapse when evaluated at 6 months following delivery[9]. The study may indicate mode of delivery as a risk factor for pelvic organ prolapse; however, the study did not specifically conducted in second – stage caesarean section[27]. Another study found that only 11% women would eventually have pelvic organ prolapse regardless of the mode of delivery[18].

In our study, prolonged second stage and vaginal delivery may not be a risk factor for developing pelvic organ prolapse; however, we were not able to estimate the effect of extremely prolonged second stage in vaginal delivery. Previous study suggested that prolonged second stage of labor may increase the risk of levator anii muscle damage about 5.32 times, but he did not specify the duration of the second stage[28]. However, even if damage occurs in levator ani muscle, it may eventually recovered following delivery.

Limitations to our study were that there was siginificant difference of the duration of the second stage of labor between groups. This would impede our ability to predict the correlation between groups accurately. Nevertheless, we are able to infer that even extremely prolonged second stage would not cause extreme detrimental effect on pelvic floor muscle strength when managed by caesarean section. Relatively low sample size in our study may serve as a model for further study to be conducted in larger sample size and longer duration for evalution following delivery.

Table 1. Demographical and clinical characteristics

Variable	Caesarean section (n = 29)	Vaginal delivery (n = 29)	P
Age			
20 - < 25 years	10 (34.5%)	19 (65.5%)	0.061*
25 - < 30 years	11 (37.9%)	6 (20.7%)	
30 – 35 years	8 (27.6%)	4 (13.8%)	

Variable	Caesarean section (n = 29)	Vaginal delivery (n = 29)	P
Occupation			
Housewife	19 (65.5%)	24 (82.8%)	0.256*
Low workload	9 (31.0%)	5 (17.2%)	
High workload	1 (3.5%)	0 (0%)	
Educational level			
Primary school	4 (13.8%)	2 (6.9%)	0.083*
Juniro high school	8 (27.6%)	3 (10.3%)	
Senior high school	7 (24.1%)	16 (55.2%)	
Undergraduate or higher	10 (34.5%)	8 (27.6%)	
Antenatal care visits			
< 4x	3 (10.3%)	1 (3.4%)	0.611**
≥ 4x	26 (89.7%)	28 (96.6%)	
BMI (Kg/m^2)			
<18,5	2 (6.9%)	4 (13.8%)	0.771*
18,5 – 24,9	21 (72.4%)	21 (72.4%)	
25 – 29,9	4 (13.8%)	3 (10.3%)	
30 – 34,9	1 (3.4%)	1 (3.4%)	
35 – 39,9	1 (3.4%)	0 (0%)	
Neoantal Birthweight (g)			
< 3000 g	8 (27.6%)	15 (51.7%)	0.060*
≥ 3000 g	21 (72.4%)	14 (48.3%)	
Active phase progression			
Normal	8 (27.6%)	16 (55.2%)	0.033*
Prolonged	21 (72.4%)	13 (44.8%)	

* Chi Square test, ** Fisher's Exact test

Table 2. Comparison of duration of the second stage of labor and mean pelvic floor muscle strength between caesarean section and vaginal delivery

Group	n	Duration of second stage (min)			P	*P****
		Min	Max	Mean ± SD		
Second – stage Caesarean section	29	60	450	206,38 ± 107,66	<0.001*	0, 007
Vaginal delivery	29	10	180	61,89 ± 36,67		0,007
Mean pelvic floor muscle strength (cm H_2O)						
Second – stage Caesarean section	29	14,9	68,3	41,83 ± 12,44	0.954**	0,568
Vaginal delivery	29	28,5	63,9	41,99 ± 8,59		0,550

*Mann – Whitney test, ** Independent t test, ***Shapiro – Wilk normality test

Table 3. Correlation between duration of second stage and pelvic floor muscle strength in second stage Caesarean section and vaginal delivery.

	Group	Pelvic floor muscle strength (cm H_2O)	
		r	*P**
Duration of second stage (min)	Caesarean section	-0,083	0,669
	Vaginal delivery	0,248	0,195

*Spearman's rank correlation

Table 4 . Comparison of Questionnaire for urinary incontince (QUID) in second stage Caesarean section and vaginal delivery

Group	n	QUID (stress)			*P**
		Min	Max	Mean ± SD	
Second stage Caesarean section	29	0,0	2,0	0,24 ± 0,51	0.044
Vaginal delivery	29	0,0	1,0	0,03 ± 0,18	
		QUID (urge)			
		Min	Max	Mean ± SD	
Second stage Caesarean section	29	0,0	2,0	0,17 ± 0,47	0,410
Vaginal delivery	29	0,0	2,0	0,10 ± 0,40	

*Mann – Whitney test

Table 5. Comparison of Wexner score and POP-Q with mode of delivery

Variables		Caesarean section (n = 29)	Vaginal delivery (n = 29)	*P**
Wexner score 0		26 (89,7%)	29 (100,0%)	0,237
Wexner score≥1		3 (10,3%)	0 (0,0%)	
Prolapse	**Stage**			
Anterior	0	27 (93,1%)	27 (93,1%)	1,000
	1	2 (6,9%)	2 (6,9%)	
Uterine	0	27 (93,1%)	28 (96,6%)	1,000
	1	2 (6,9%)	1 (3,4%)	
Posterior	0	27 (93,1%)	29 (100%)	0,491
	1	2 (6,9%)	0 (0%)	

*Fisher's exact test

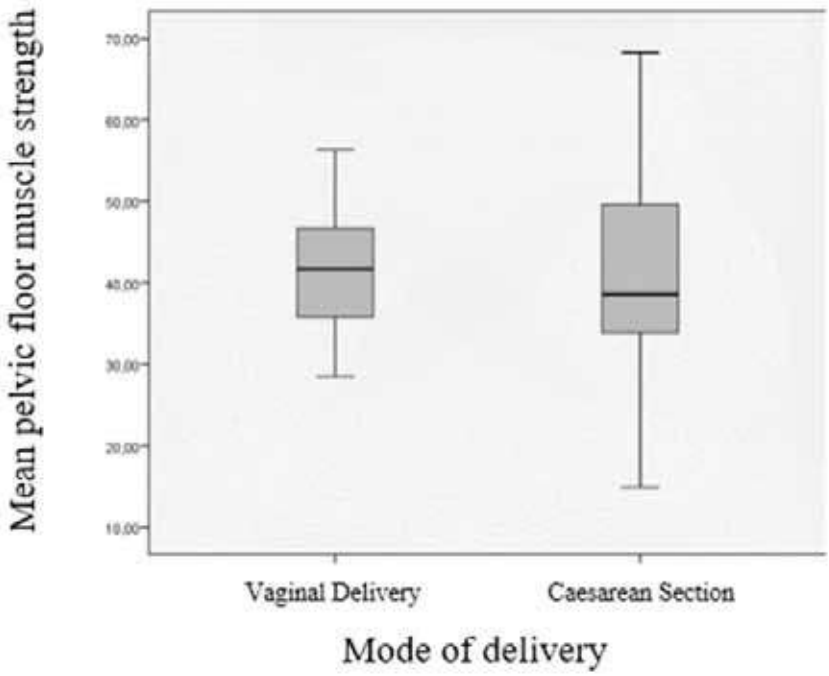
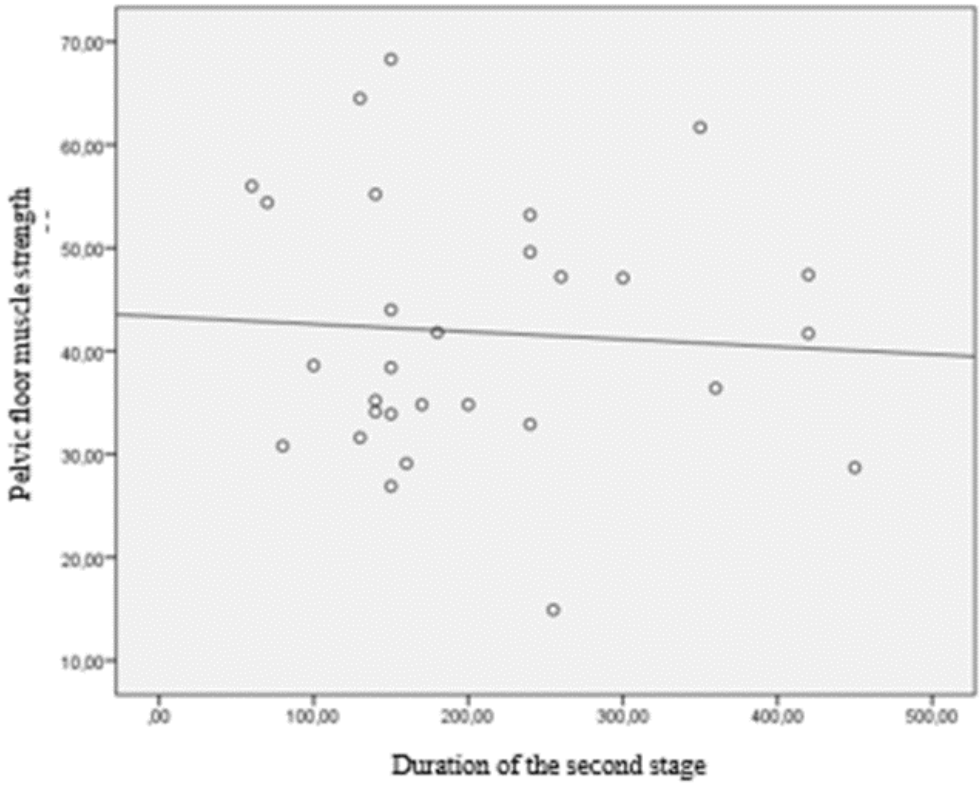
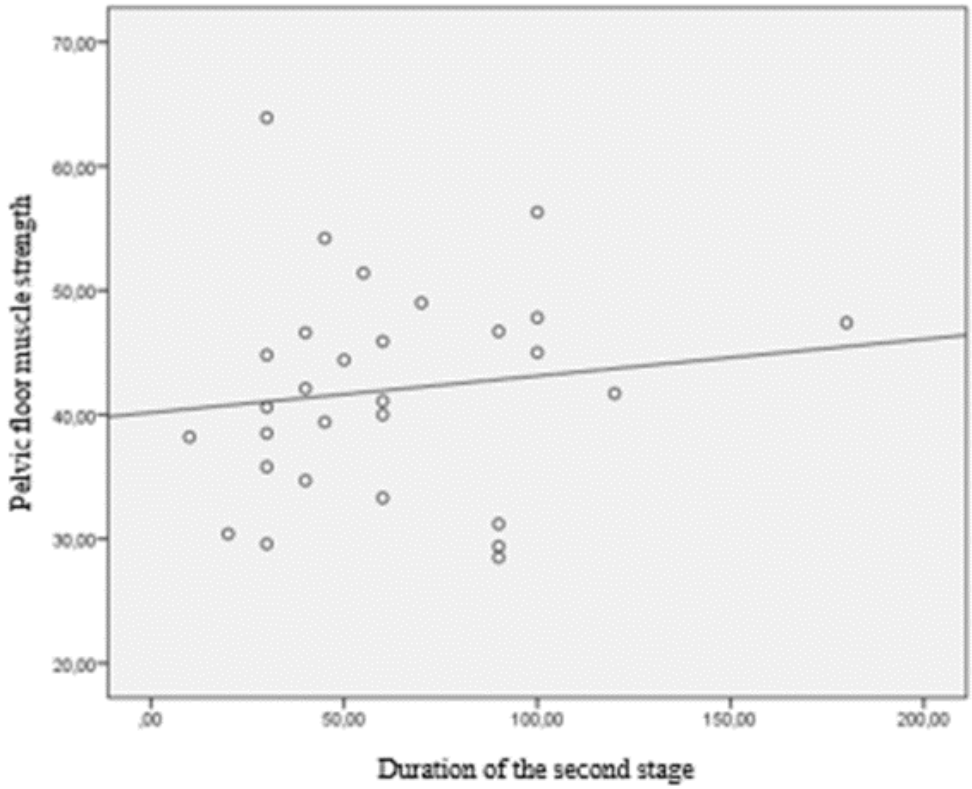


Figure 1. Comparison of mean pelvic floor muscle strength between caesarean section and vaginal delivery.



(a)



(b)

Figure 2. Correlation between duration of second stage and pelvic floor muscle strength in caesarean section (a) and vaginal delivery (b).

Conclusions

Pelvic floor muscle strength in primiparous women underwent second stage Caesarean section and vaginal delivery did not differ significantly after evaluation in 6 weeks following delivery. Duration of the second stage between groups was extremely different. Moreover, there were no correlation between duration of second stage and pelvic floor muscle strength on both groups. There were no differences in pelvic organ dysfunction between groups.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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User Centered Portable Suspension Therapy Unit Design & Testing on Healthy Volunteers

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Abstract

Background: Suspension is a therapeutic skill that suspends a body and eliminates effects of friction to ease the movements. It is very beneficial in both musculoskeletal and neurological field.

Objective: To make suspension therapy more accessible for a physiotherapist domestically which provide an opportunity for patient to receive suspension therapy in any occasion.

Study Design: Pilot study.

Sample Size: 30 healthy subjects from Hospital or AIMST University (with various body sizes).

Discussion: The overall satisfaction level for the Portable Suspension Kit is pleasant. The subjects acknowledge that suspension therapy helps to improve range of motion and is suitable for home rehabilitation and both statements score the highest which is 3.9667±0.66868 out of 5. On the contrary, the subjects agree less on the stability of the frame which results show 3.7±0.98786 out of 5. Whereas, for R.A. tracker, the outcome of accuracy level turned out to be 65.2% which is of moderate range and the result is acceptable for a new prototype. $P > 0.652$ is not statistically significant due to the sensor sensitivity of calibration. Thus, we strongly agree that the deficiency needed to be improved to allow accurate visual feedback.

Conclusion: The making of 'Portable Suspension Kit' is successful and indeed is convenient to carry around. Suspension therapy can now be widely used in any occasion on the patients. Nevertheless, it still has more to improve especially on the stability and the material used to make it safer, lighter and easier to carry.

Introduction

Suspension therapy is very beneficial in both musculoskeletal and neurological field; it has some disadvantages which are yet to be overcome. It is big, heavy and immobile causes it impossible to Transport and limit the suspension exercise only to a specific location. In medical search engines such As Pub Med, NCBI, and Medscape is lack of information and article for sling suspension Therapy[1]. This may be due to suspension therapy is not widely used in the medical field as it requires

More time to set up the slings and cuffs. Besides, this topic is overlooked[2]. Although had some research did the research or study, most of them were focused on low back pain or urinary incontinence, which was lack of information focus on musculoskeletal and neuromuscular[3]. Suspension therapy also known as sling therapy is one of the therapeutic technique where a part of the body is supported by a sling and suspended by ropes to the fixed point on suspension frame[4]. It frees the body from friction and helps to move the body easier with less resistance. (John George B, 2002)[5]. Hence,

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this helps the patient in reducing the axial load on the joint while the patient is performing the exercises. Suspension therapy can be either used as an assistance exercise or resistance training[6]. There are 3 main principles used in suspension therapy which is friction, pendulum and eliminating gravity movements. Firstly, fiction is produced when a particular surface moves on another. In suspension, friction is less, therefore causes smoother and easier movement. Secondly, a pendulum is a heavy material suspended by a weightless thread. When the force is applied to the pendulum it results in movement[7]. In the human body, pendular motion occurs mainly at the shoulder and hip joints, forward leg movement and arm swing while walking[8]. This helps to maintain muscle properties, increase range of motion and strengthen the muscle. Lastly, it comes to eliminating gravity movement, the patient should at least have muscle power grade 2 for suspension exercise[9]. The therapist is able to alter the patient's position while doing the exercise[10]. The accessories used in suspension therapy, e.g. suspension frame, connecting rope, wooden cleat, dog clip or S-hook and spring. Connecting ropes provide attachment for a sling to the steel and able for adjustment in length[11]. Though its effect such as strengthening muscle, increase range of motion, and improve coordination are similar to those of other forms of exercise[12], additional characteristics of suspension therapy make it[13] particularly useful and unique. Other than those mentioned[14], the suspension also helps to improve stability of the joint, stimulate the sensorimotor system and improve patient confidence to undergo exercise or rehabilitation programme[15].

Method and Subjects

A qualitative research model in the form of a prospective type quasi experimental study design is carried out in this study. The study is conducted in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy & USA, Institute of Paramedical Sciences, Kannur, India and Mekelle University Ethiopia. In this study all Academic and non-academic professionals are the population for this study. Conveniently 800 participants among the academic professionals are selected in collaboration with AIMST University Malaysia, University of Indonesia, NMT Institute Italy, USA, and Mekelle University Ethiopia & Institute of Paramedical Sciences, Kannur, India.

Standard Application procedure: Patients diagnosed by Low back and neck pain for at least 6 months,

Features: Width Cm 5 and Length 30 cm.

Reference points: Intergluteal line - Vertebral column.

Patient standing with hands resting on a table, anterior lumbar flexion of 45°. Apply the base of the tape on the interglutea line and laterally 2 cm from the vertebral column. Remove the paper leaving only a small piece to hold the strip. Ask the patient to round the lumbar section (ask to "pull in the belly", the skin over the lumbar area must be stretched). The tape is applied with absolutely no tension[16]. The tape is applied parallel to the column and must end at exactly the same level. The treatment was given twice per week for 6 weeks (8-10 sittings). The cure tape is approved by the FDA and the product name is Tape and bandage, adhesive. Before the primary neuromuscular taping application VAS (visual analogue scale for pain scaling) and back pain index (Oswestry Low Back Pain Disability Questionnaire) were collected and this procedure was continuing till the end treatment sessions. This portable suspension kit is invented to help the physiotherapist to make use of the suspension therapy domestically. Because we found out that there is difficulty in applying suspension therapy in a ward, home-based rehabilitation and nursing house[17]. The difficulties are due to the large size and heavy weight of the suspension frame which make it not portable and mobile. The new invention portable suspension kit is foldable and light in weight which can be carried by the physiotherapist. It can follow the patient wherever he is and can be easily kept. It offered a portable and mobile frame to perform the suspension therapy in need of the patient which is very beneficial in the rehabilitation progression. The reason why we are inventing a R.A tracker is to calculate the repetition and accuracy of the patient performing the movement instructed which is very important for the outcome of the rehabilitation. Setting a target for a patient to complete can be a motivation in rehab. Sometimes, they may get bored, sleepy and during exercise and forget in counting the repetition. Hardship may be felt during rehab, so patient sometimes will find excuses for not completing the exercise that is given[18]. This tracker is now useful in this case by helping them calculate the repetition when they are performing the exercise and cheating is prevented because a repetition can only be counted as one when u reach a targeted angle and during back to starting position

it should be less than 5 degrees. Repetition is important during rehabilitation because the neuronal circuits can be changed by the neuroplasticity in the brain's ability. For example, increasing the amounts of the task repetitions proved that it can help in cortical changes and functional improvement in a post-stroke patient. The main point to focus on rehabilitation professionals should be the number of repetitions and the type of activity performed per session. According to the current research shows that most of the physiotherapy session does not contain the proper and sufficient amount of repetitions in each session to cause actual changes in the brain. Thus, one item of focus for rehabilitation professionals should be the number of reps and type of activity performed in each session. Current research, however, shows that most therapy sessions do not contain the proper amount of repetitions per session to cause actual changes in the brain. Typical therapy sessions, whether physical, occupational, speech, or behavioral, last about one hour per session with the therapist employing various tasks and exercises throughout the session[19].

Methodology

The study duration is about one year and treatment duration is one treatment twice a week with 30 minutes per session which held at AIMST University Physiotherapy Clinic and private clinic with the size of 30 samples from AIMST University Physiotherapy clinic and private clinic. The sample was contacted twice a week and one treatment session is 30 minutes.

Results

Table 1: Sample Characteristic

	Frequency	Percentage
Gender		
Male	15	50%
Female	15	50%
Age Category		
21 - 30	13	43.3%
31 - 40	9	30.0%
41 - 50	4	13.3%
51 - 60	2	6.7%
61 - 70	1	3.3%
70 - 80	1	3.3%
Weight Category		
Less than 50 kg	4	13.3%
51kg – 70kg	12	40.0%
71kg – 90kg	9	30.0%
91kg – 110kg	5	16.7%
More than 110kg	0	0%
Height Category		
Less than 150cm	2	6.7%
151cm – 160cm	4	13.3%
161cm – 170cm	8	26.7%
171cm – 180cm	12	40.0%
More than 180cm	4	13.3%

Table 1 shows this demographic characteristic of samples participated in this study. In this study, consists of 15 male and 15 female as a sample with a percentage of 50% respectively. The sample was divided into 6 groups in the age category. Between the age of 21 to 30 consists of 13 samples (43.3%); 9 samples (30.0%) in the age between 31 to 40; 4 samples (13.3%) in the age group within 41 to 50; 2 samples (6.7%) with age range from 51 to 60; the age group with the range of 61 to 70 and age within 71 – 80 only have 1 sample each (3.3%). We divided the weight category into 5 groups. We have 4 persons (13.3%) in the group with a weight of less than 50kg. weight range from 51kg to 70kg is 12 persons (40.0%) from the weight range from 51kg to 70kg; 9 samples (30.0%) from the weight group of 71kg to 90kg and 5 samples (16.7%) from the last weight group with the range of 91kg to 110kg. In this study, we did not include any sample with the weight that exceeds 110kg. There were 5 different groups divided in the height category. For the height less than 150cm consists of 2 samples (6.7%); 4 samples (13.3%) with the height range of 151cm to 160cm; 8 samples (26.7%) with the height range of 161cm to 170cm; 12samples (40.0%) with the height range from 171cm to 180cm and 4 samples (13.3%) with the height more than 180 cm. Table 5.1 shows the weight, height and after 4 sessions of 2 weeks treatment samples satisfaction level on pain relief, range of motion, muscle strength, home rehab, and stability. The mean weight value of the samples is 69.0667±18.69710 and the mean height of the samples is 169.3000±11.16383. The average satisfaction level on pain relief is 3.8333± 0.59209; improving in range of motion is 3.9667±0.61495; improving in muscle strength 3.8000±0.66436; for home rehabilitation purpose is 3.9667±0.66868 and stability of the frame is 3.7000±0.98786. Table 5.2 shows the correlation

between weight, height, and stability. The correlation test shows that body weight is significantly related to height (r= 0.851, P< 0.01). In comparison between body weight and stability, r= -0.692 which demonstrates that body weight is significant to stability (P< 0.01). In regards to the correlation between height and stability, the result depicts the coefficient -0.652 which clearly shows that height is significant to stability (P< 0.01) in figure 1.0 which is the receiver operating characteristic (ROC) graph with the angle of movement against repetitions. In table 5.3 the result demonstrates the area under the curve is 0.652. The percentage of the sensitivity is 65.2% and the result of the area under the curve is not statistically significant (P> 0.05). Even though the result is not significant but it is still considered moderate and acceptable. The coordinates of the curve in table 5.4 shows that if the test results can be improved to 27.5 % than the sensitivity can be enhanced to 96 %.

Table 2: Descriptive state of weight, height and satisfaction level on pain relief, range of motion, muscle strength, home rehab, and stability.

Variable	Mean	Std. Deviation
Weight	69.0667	18.69710
Height	169.3000	11.16383
Pain relief	3.8333	0.59209
Range of motion	3.9667	0.61495
Muscle strength	3.8000	0.66436
Home rehab	3.9667	0.66868
Stability	3.7000	0.98786

Table 3: Correlation between weight, height and stability.

	Mean	Std. deviation	1 Body Weight	2 Height	3 Stability
Body Weight	69.0667	18.69710	1	0.851**	- 0.699**
Height	169.3000	11.16383	0.851**	1	- 0.692**
Stability	3.7000	0.98786	- 0.699**	- 0.692**	1

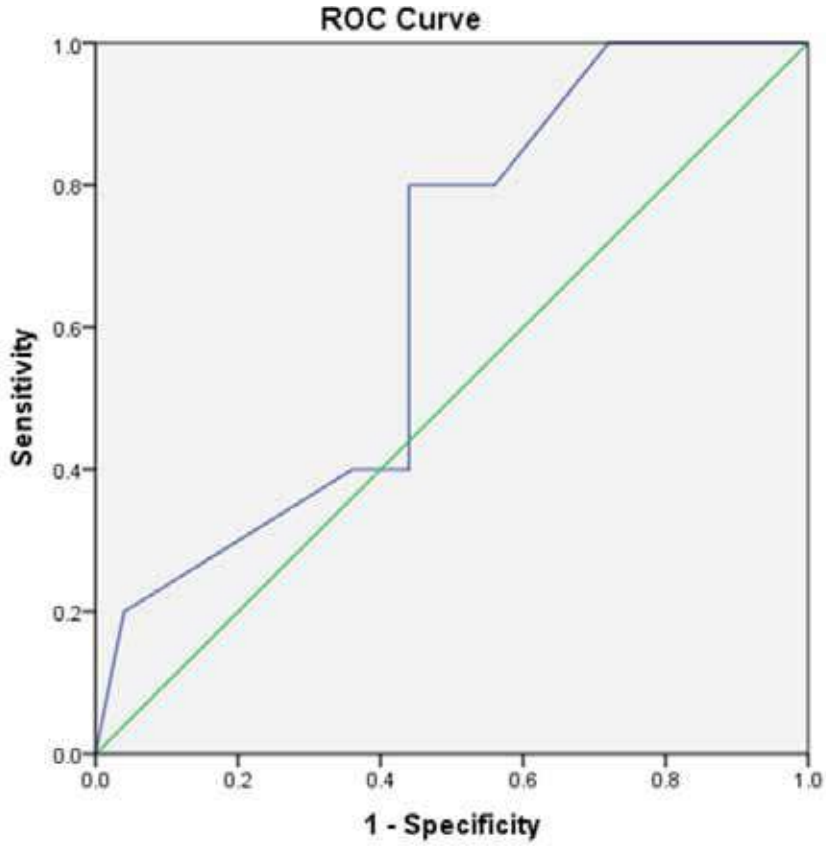


Figure 1: Receiver operating characteristic (ROC) curve of the angle of the movement against repetitions.

Table 4: Shows the area under the curve with the angle of movement as the test variable.

Test Result Variable(s): angle of movement		
Positive if greater than or equal to	**Sensitivity**	**1-Specificity**
24.0000	1.000	1.000
27.5000	.960	.800
32.5000	.640	.600
37.5000	.560	.600
42.5000	.560	.200
47.5000	.440	.200
52.5000	.280	.000
57.5000	.200	.000
62.5000	.120	.000
67.5000	.080	.000
72.5000	.040	.000
76.0000	.000	.000

Table 5: Coordinates of the curve

Area	Std. Error	95% Confidence Interval		P
		Lower Bound	Upper Bound	
0.652	0.120	.416	.888	0.290

Discussion

The 'R.A. Tracker' is advantageous for physiotherapists in decreasing the workloads, the mission of counting the repetition and observing the angle can now be done by the tracker. It alleviates the burden on the physiotherapists so that they can pay attention to observing any secondary movement or the appropriate muscle action. The tracker uses battery-powered liquid-crystal display (LCD) as display of the device. The repetitions and angle can be pre-set which makes the treatment effective by providing biofeedback to the patient. This allows the patient as well as the therapist to be aware of the quantity and quality of the movements, at the same time enables the therapist to set a target to the patient. In general, the 'Portable Suspension Unit' performs a more professional appearance in contrast to the traditional suspension therapy. During the trial of the R.A. tracker, 15 repetitions of specific movements are prescribed to the subjects. The examiner was beside the subject comparing the exact movement and angle while comparing it to the tracker. The outcome turned out to be 65.2% which is in mid-range. The accuracy is acceptable for a new prototype. There are many reasons that caused it to be less precise and further enhancement is needed to make it more perfect. $P > 0.652$ is not statistically significant due to the sensor sensitivity of calibration. Sensitivity is significant as it refers to the input signal required to project the output of the device and it shows the true positives in some situations. Therefore, its deficiency needed to be improved to allow accurate visual feedback. The calibration process takes time due to the Auto-Calibration process in the Digital Motion Processor (DMP) itself. The DMP uses an algorithm to Auto Calibrate the values over time.

This time varies across each MPU6050 sensor as there is a slight variation and offset in the sensor due to the manufacturing process. This would affect the speed of the auto-calibration process. One way to improve the auto-calibration speed is to supply an offset for the sensors prior to the auto-calibration. The offsets are unique to every MPU6050 sensors due to the manufacturing process and slight variations in each sensor. (V. Christ, 2017) the sensor becomes less accurate over time due to gyro drift in the gyroscope within the sensor. The gyroscope has an internal bias or noise within the sensor. The bias or noise changes over time due to various factors such as temperature or just an inherent noise in the sensor. Hence when the raw values are fed into the DMP it would auto-calibrate based on the current bias or noise.

Conclusion

In conclusion, we found out that suspension therapy still very high demanding for rehabilitation treatment in our country no matter in hospital, private clinic, etc. through our survey, the heavy, big and immobile suspension frame brings a lots of inconvenience for the physiotherapist who wish to do home rehabilitation, ward and for patient with difficulty in ambulation and more. The survey result shows that the majority agreed that suspension therapy brings tons of benefits to the patient and our invention The Portable Suspension Unit will help in fulfilling their needs for domestic purpose. We also gained some bits of advice from the minority which not satisfied with our invention. From here we'll have more discussion and enhancement regarding the limitation of our portable suspension kit and take into consideration to make changes such as the design, materials, and weight to make it more convenient and functional.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Comparison of Gestational Diabetes Mellitus Screening between the One Step Method and the Two Step Method

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Abstract

This research aimed to compare the results of gestational diabetes mellitus (DMG) screening based on the One Step and Two Step method. This research was a cross-sectional study conducted in pregnant women 24-28 weeks of gestation who meet the research criteria. The diagnosis of gestational diabetes using the One Step and Two Step method was each carried out on 53 pregnant women so that the total sample of the research was 106 pregnant women. The results showed that 28.3% of pregnant women (15/53) were diagnosed with DMG with the One Step method compared with 3.8% (2/53) of pregnant women diagnosed by the Two Step method. There was a significant difference ($p < 0.05$) between the diagnosis results of the two DMG diagnosis method. Further analysis of DMG risk factors showed that the highest positive likelihood ratio (LR +) was found in the history of macrosomia with LR + = 4.45 with the One Step method while the highest LR + for the Two Step method was found in obesity factors for pregnant women with LR + = 6.57. The highest positive predictive value (PPV) for the One Step method of 46.70% was found in pregnant women with risk factors for obesity while the Two Step method was found in pregnant women with a history of DM (PPV = 100%). Pregnant women diagnosed with DMG were significantly greater in the One Step method than the Two Step method. History of macrosomia (is found) as a risk factor for pregnant women diagnosed with DMG with the One Step method, while a history of DM for the Two Step method.

Keywords: *Diabetes mellitus, pregnancy, screening, risk factors.*

Introduction

Gestational diabetes mellitus (DMG) is a carbohydrate intolerance of varying degrees that occurs or is first known during pregnancy. Diabetes mellitus is a medical complication that often occurs during pregnancy. According to the American Diabetes Association (ADA) in 2004 gestational diabetes mellitus occurred in about 4% of all pregnancies in the United States, and 3-5% in the United Kingdom. The prevalence of gestational diabetes mellitus in Europe is 2-6%[1].

Increased perinatal morbidity and mortality in pregnancy with DMG is directly correlated with maternal hyperglycemia. The effect shown by mothers with gestational diabetes mellitus is that they have high risk of excess weight gain, the occurrence of preeclampsia, eclampsia, cesarean section, and cardiovascular complications to maternal death. After delivery occurs, the mothers are at risk of developing type 2 diabetes mellitus or recurring gestational diabetes in subsequent pregnancies while babies born to mothers who have gestational diabetes are at high risk of experiencing macrosomia and birth trauma. In addition, babies are also at risk of developing hypoglycemia, hypocalcemia, hyperbilirubinemia, respiratory distress syndrome, polycythaemia, obesity and type 2 diabetes mellitus[2].

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Screening is done to identify asymptomatic individuals who are at high risk for having or are in the development of certain diseases. Screening can be done as a two-step or one-step process. In the two-step technique, the first step is carried out to identify individuals who are at risk of disease, so that the second step (a more definitive diagnostic test, but usually more expensive and complex than screening) is only done in groups that are truly at risk and not carried out in groups low risk. While the One Step technique is done by filtering all individuals. Over the past three decades, the procedure and criteria which are most widely used to detect early DMGs is the two-step procedure (wherein): oral glucose tolerance test 1 hour 50 g and is followed by 3 hours 100 g for positive cases at initial screening. The One Step procedure is carried out with a simple step of performing an oral glucose tolerance test of 2 hours 75 g but (it) requires fasting for 8-14 hours before the glucose check[3].

Until now, there is still no agreement on the most appropriate screening method or DMG screening for pregnant women in Indonesia. For this reason, an approach is needed about screening for DMG so that it can improve pregnancy outcomes for both mother and baby.

This research aimed to compare the results of gestational diabetes mellitus (DMG) screening based on the One Step and Two Step method.

Methodology

This research was an analytic study with cross-sectional approach. Sampling was done by simple random sampling with the ordinal method until the required number of samples was met. The minimum sample required for this research is 53 samples for each of the One Step and Two Step method so that the total sample of this study was 106 samples.

Sample: The sample in this research were pregnant women who came to the Obstetrics and Gynecology clinic in Wahidin Sudirohusodo Hospital, Jumpandang Public Health Center, Tamalanrea Jaya Public Health Center, to undergo a pregnancy check during the research period that met the inclusion and exclusion criteria. Inclusion criteria in this research were pregnant women — who are 18 years old or older, gestational age are between 24 and 28 weeks, who have single pregnancies, and who are willing to be involved in the research. The exclusion criteria in this research were pregnant women who are having medical or obstetric complications during pregnancy, being treated for diabetes mellitus, and not being able to undergo an Oral Glucose Tolerance Test (TTGO) due to nausea or vomiting.

Procedure: The way this research worked was by explaining the purpose and objectives of the research to pregnant women, if they are willing to take part in the research, pregnant women sign an agreement. Anamnesis was conducted on the research participants and other data were recorded in the research sheet, then DMG diagnosis test with the One Step method and Two Step method were carried out.

GDM diagnosis using the One Step method: Patients were asked to fast for at least 8 hours before the test. OGTT was performed in the morning after fasting — with 75 grams of glucose in 250 mL of water. Glucose level examination was done when the patient was fasting, 1 hour and 2 hours after OGTT. Analysis of blood glucose levels was carried out using the GOD-PAP method in serum at Prodia Laboratory Makassar. The diagnosis of GDM is positive if one or more of the following criteria has an abnormal (greater) value:

Glucose Examination	Glucose level	
	mg/dL	mmol/L
Fasting blood sugar	92	5.1
Glucose at 1 hour	180	10
Glucose at 2 hour	153	8.5

GDM diagnosis using the Two Step method:

Step 1. 50 gram glucose loading test (without fasting) was carried out, plasma glucose levels were measured 1 hour after glucose loading. If the plasma glucose level on 1 hour after glucose loading> 140 mg/dL (7.8 mmol/L) then (the test)would be followed by OGTT 100 grams of glucose.

Step 2. OGTT 100 grams of glucose was performed on patients in a state of fasting. Glucose level examination was performed when the patient was fasting, 1 hour, 2 hours and 3 hours after OGTT. Analysis of blood glucose levels was carried out using the GOD-PAP method in serum at Prodia Laboratory Makassar. The diagnosis of GDM is positive if two of the four plasma glucose measurements exceed the following values:

Glucose Examination	Glucose level	
	mg/dL	mmol/L
Glucose 50 mg/dL	105	5.8
Glucose 100 mg/dL at 1 hour	190	10.6
Glucose 100 mg/dL at 2 hour	165	9.2
Glucose 100 mg/dL at 3hour	145	7,9

Data analysis: Differences in the characteristics of the research sample between the two groups were tested by the independent t test, the Mann Whitney test and the Chi square test. Differences in diagnosis results between the One Step and Two Step method and differences in the characteristics of the research sample based on the diagnosis of DMG were tested by Fischer's exact test. Data were analyzed with SPSS with significance level (p) <0.05.

Results

This research involved 106 pregnant women consisting of 53 pregnant women getting DMG diagnosis test with the One Step method and 53 pregnant women with the Two Step method. This research was carried out on pregnant women with 24-28 weeks gestation and had passed the screening process for DMG as a research sample.

Table 1. The characteristics of research samples

Characteristics	One Step Method (n = 53)	The Two Step Method (n = 53)	
Mother's age (mean ± SD year)	27.3 ± 5.1	29.4 ± 6.1	0.056 *
Parity			
Primipara	26 (49.1%)	22 (41.5%)	0.558 ***
Multipara	27 (50.9%)	31 (58.5%)	
Gestational age (mean ± SD week)	25.8 ± 1.2	25.9 ± 1.3	0.487 **
Family history of DM			
Yes	5 (9.4%)	4 (7.5%)	1,000 ***
Not	48 (90.6%)	49 (92.5%)	
Macrosomia History			
Yes	4 (7.5%)	2 (3.8%)	0.678 ***
Not	49 (92.5%)	51 (96.2%)	
BMI (kg/m^2)			
≥ 30	44 (83%)	46 (86.8%)	0.786 ***
<30	9 (17%)	7 (13.2%)	

*Independent t test, **Mann Whitney Test, ***Chi Square Test

Table 1 shows (that) the proportions for the age of pregnant women, parity, gestational age, risk factors for family history of DM, history of childbirth, macrosomia and BMI ≥30 kg/m^2 for both DMG diagnosis method is almost the same. The results of further statistical tests on the research sample in the two diagnosis groups showed no significant difference in characteristics (p> 0.05) between the two research groups so that the two groups were comparable.

Table 2. The diagnosis of DMG based on the One Step and Two Step method

DGM diagnosis	One Step Method (n = 53)	Two Steps Method (n = 53)	p*
Positive	15 (28.3%)	2 (3.8%)	.001
Negative	38 (71.7)	51 (96.2%)	

* Fischer Test

Table 2 shows (that) the general prevalence of DMG in this research was 16.04% (17/106). DMG was diagnosed more in the One Step method than the Two Step method. DMG diagnosis results of this research consisted of 15 pregnant women (28.3%) diagnosed by the One Step method and 2 pregnant women (3.8%) diagnosed by the Two Step method and there were significant differences ($p < 0.05$) between the diagnosis results from both method of diagnosis of DMG.

Table 3. The comparison of DMG screening diagnosis results based on risk factors

Risk Factors	One Step				Two Step			
	PPV (%)	NPV (%)	LR +	LR -	PPV (%)	NPV (%)	LR +	LR -
DM history	26.70	97.40	3.49	0.26	100.00	96.10	-	0.50
Macrosomia History	26.70	100.00	4.45	0.00	0.00	96.10	0.00	1.04
BMI	46.70	94.70	4.28	0.27	50.00	88.20	6.57	0.88

PPV = positive predictive value, NPV = negative predictive value, LR + = positive likelihood ratio, LR- = negative likelihood ratio

The data in table 3 shows (that) the highest positive likelihood ratio (LR +) was found in the history of macrosomia with LR + = 4.45 with the One Step method while the highest LR + for the Two Step method was found in the obesity factor of pregnant women with LR + = 6.57. The highest positive predictive value (PPV) for the One Step method of 46.70% was found in pregnant women with risk factors for obesity while the Two Step method was found in pregnant women with a history of DM (PPV = 100%).

Discussion

This research aimed to compare the results of gestational diabetes mellitus (DMG) screening based on the One Step and Two Step method. In this research, the prevalence of DMG was 16.04%. Research by Melchior et al (2017), reports that regardless of the screening method used, the prevalence of DMG has tended to increase in the past decade[4]. Another study by Ferrara (2007), reports that in the last 20 years there is an increase in the prevalence of DMG between 10-100% in the world[5]. This increase is in line with the increasing epidemic of type 2 diabetes mellitus and obesity. Globally, the prevalence of hyperglycemia in pregnancy (DMG and type 2 diabetes in pregnancy) is estimated to reach 15%. A retrospective cohort study of 567,191 pregnant women found a prevalence of 13.2% [4]. The prevalence of DMG varies between 1-28% depending on the characteristics of the population.

The true prevalence of DMG is difficult to estimate because of differences in risk factors in the study population, such as maternal age, BMI, diabetes and ethnic prevalence. In addition, the screening strategy, the testing method used, and the glycemic threshold used also differ. The use of data in reporting also greatly influences the prevalence of reported DMG.

The research also showed that pregnant women diagnosed with DMG were higher in the One Step method compared to the Two Step method. Some researches show the same results as this research. Research in Turkey in pregnant women with gestation of 24-28 weeks and using universal screening of 50 grams of TTGO reports a prevalence of DMG of 14.5% with the One Step method and 6% with the Two Step method[6]. Another research in Thailand also shows a greater diagnosis of DMG done by the One Step method (32%) compared to the Two Step method (10.3%)[7]. Research in Iran also shows the incidence of DMG is also higher with the One Step method (9.3%) compared to the Two Step method[8]. Conversely, a meta-analysis in 2. 333 pregnant women reports no significant difference in the incidence of DMG between the One Step and Two Step method [9]. However, this meta-analysis also shows that the One Step method is associated with better maternal and perinatal outcomes compared to the Two Steps method in terms of lower incidence of preterm labor, NICU care and neonatal hypoglycemia. The Two Step Method also deals with reducing the number of DMGs that cannot be followed up (lost to follow up) by about 5% thereby reducing the incidence of DMG [10]. lower NICU care and neonatal hypoglycemia. The Two Step Method also deals with reducing the number of DMGs

that cannot be followed up (lost to follow up) by about 5% thereby reducing the incidence of DMG [10]. The One Step Method increases the prevalence of DMG from 1.03 to 3.78 times compared to Two Step [11]. The results of this research are comparable to Brown's research that found a positive diagnosis of DMG with the One Step method increased about 2-fold from the positive diagnosis result by the Two Step method. Another research based on differences in the risk of DMG in pregnant women which are low risk and high risk shows DMG increased from 7% to 11.7% in high risk pregnant women using the One Step method [12]. Other researches have even reported a higher incidence of DMG (between 16% to 27%) with the One Step method compared to Two Step [13,14].

The difference in the prevalence of DMG between the One Step and Two Step method between researches can be caused by differences in population characteristics such as sample size, ethnicity/race, and DMG risk factors. Several studies have shown that ethnic/racial factors have implications for the prevalence of DMG worldwide. The prevalence of DMG increases significantly in European populations in the One Step method compared to Two Step.

The DMG diagnosis approach is still being debated to this day. Based on the results of this research it was found that the diagnosis of DMG was significantly higher by the One Step method compared to Two Step method. There are several arguments related to the benefits and risks of using the One Step method as a DMG diagnostic approach. The One Step method is superior to the Two Step method because the women examined and showed positive results with the One Step method but negative with the Two Step method (meaning, having symptoms of lighter glucose intolerance) still have a higher risk for experiencing pregnancy complications such as gestational hypertension, preeclampsia and cesarean section, moreover, the babies born are also at risk of complications. Thus women who tested positive for the One Step method are eligible to be diagnosed with DMG and given treatment to reduce the risk of complications. Another advantage of the One Step method is that the level of patient compliance is higher compared to the Two Step method which is reported to be around 15% of patients not taking the second test. The use of the Two Step method can be a burden on patients. In addition to these two reasons, the One Step method is also more cost effective and results in maternal and neonatal outcomes have been proven to be better. A meta-analysis of 4 randomized controlled researches comparing these two approaches shows that the diagnosis and treatment of DMG with the One Step approach is associated with better maternal and neonatal outcomes. Compared to the One Step method, the second step with the Two Step approach will delay diagnosis and treatment. The One Step Method improves patient compliance and diagnosis can be established earlier. In high risk cases such as history of DMG, history of macrosomia, older maternal age, multiple pregnancy and others, screening with the One Step method can be done at the first antenatal visit.

Meanwhile, a counter argument was put forward by Hosseini et al (2018), who reports that the One Step approach tended to identify low-risk women as DMG positive[8]. This will increase anxiety and health costs because more antenatal visits and more ultrasound examinations will be needed, including an increase in the need for additional laboratory tests without clear benefits. ACOG does not recommend the use of the One Step method because there is not enough data available that the use of these criteria will increase maternal and neonatal outcomes and may even increase health costs.

Based on this research the DMG diagnosis results are higher when it is diagnosed with the One Step approach compared to Two Step. The One Step Method is recommended by IADPSG and ADA because it is more sensitive for identifying DMG. DMG diagnosis using the One Step method is used to uniform DMG diagnosis procedures for all pregnant women so as to increase the sensitivity of the screening method and the prevalence of DMG, pregnant women diagnosed with DMG earlier are expected to improve pregnancy outcomes for both mother and baby [15]. Increased sensitivity is associated with a low threshold for positive results (only one abnormal value is needed) and a cut-off value is also low.

Research conducted in Thailand states that the One Step method is not suitable for routine screening in clinics with as many ANC patients as in developing countries, because screening tends to be complicated for it requires prior fasting, with no real evidence of clinical improvement in pregnancy outcomes [7].

The One Step Method provides benefits for patients and health care providers because it can provide diagnostic results in just one ANC visit. But the increased prevalence of pregnant women with DMG also increases health costs, without any real evidence of the benefits of

treatment. Women diagnosed with DMG are also likely to face unintended consequences, such as increasing the risk of labor by cesarean section, requiring intensive newborn assessment, increasing the cost of care, and possibly experiencing psychosocial burdens.

This research also found that the possibility of pregnant women diagnosed with DMG was greater if they had a history of macrosomia and obesity in the One Step method while a history of DM and obesity for the Two Step method. This means that pregnant women who have a high risk of DMG will be more likely to experience DM during pregnancy compared to pregnant women without risk factors or low risk. Hosseini's research shows that pregnant women who are older than 35 years old and having a family history of DM significantly increased DMG development for both in the One Step and Two Step method. It further shows that a high BMI before pregnancy and lack of exercise during pregnancy and a history of previous cesarean section supports the development of DMG especially for the Two Step method [8]. Therefore, the presence of high risk factors for DMG supports the formation of DMG diagnoses for both DMG diagnosis method.

Some weaknesses of this study are (1) the sample used is different for the two diagnostic method so that diagnostic tests cannot be performed to see the sensitivity and specificity of both method; (2) it does not compare the results of pregnancy outcomes (from) both maternal and neonatal between the two examination method so that further research is needed with larger samples to conclude the most effective screening method.

Conclusions

Based on the results and discussions, it can be concluded that pregnant women diagnosed with diabetes mellitus by the One Step method were 28.3% and by the Two Step method were 3.8%. The prevalence of gestational diabetes mellitus is significantly greater with the One Step method compared to the Two Step method. History of macrosomia and obesity were found to be risk factors for pregnant women diagnosed with DMG by the One Step method while a history of DM and obesity (were found to be risk factors) for the Two Step method.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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To Compare the Effectiveness of Taping and Arch Support on the Flexible Flat Foot on a Random Population

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Abstract

Background: Flat foot is also known as Pes planus caused by injury, prolonged stress on the foot, obesity, and faulty biomechanics. This will cause the posterior tibial tendon dysfunction, collapse of the foot arch and developed flat foot (Pes planus).

Objective: Many of the research studies show the prevalence of flexible flat foot is on raise in recent times due to any number of factors. The majority of researchers focused independently on the effectiveness of Kinesio taping and arch support. So we aim to compare the effectiveness of Kinesio taping and medial arch support.

Method: The study design is an experimental study. The study will be conducted at Aimst University, Kedah, Malaysia. Total of 30 participants of both the gender, age between 18 to 30 years, who have a limitation of ROM and pain while performing dorsiflexion and plantar flexion, will be recruited. Each participant will undergo pre-test and post-test screening for an assessment of Body Mass Index (BMI), ROM, pain, physical examination, and Staheli Arch Index. Later the participants will be divided into two groups by a convenient sampling method. Group A participants will undergo exercises related to strengthening of foot muscles along with non-allergic taping technique while Group B participants will undergo exercises related to the strengthening of foot muscles along with medial arch support.

Data Analysis: Data collected is using IBM SPSS Statistics 23 for analyzing the data. For statistical analysis of the range of motion, VAS scale and Staheli Arch Index, Wilcoxon Signed Ranks Test was used. To compare the outcome of Kinesio Taping and Medial Arch Support, Mann-Whitney U test was used.

Result: After statistical analysis, a significant improvement was found in the range of motion and VAS Scale. The level of improvement ($p<0.05$) was significantly high with Kinesio Taping in flat foot.

Conclusion: The present study concluded that the application of Kinesio tape to flat feet was an effective intervention method for immediately reducing abnormally increased foot pressure and tone and stiffness in the lower extremities muscles and considered to be the most effective intervention for subjects with flat foot.

Keywords: *Flat foot, Kinesio tape, Medial Arch Support, Pes planus.*

Introduction

Background: Flat foot defines as a postural deformity in the combination of the medial longitudinal arch collapse. According to the International Journal of Physiotherapy and Research (2015), the flat foot is also known as Pes Planus caused by injury, prolonged

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stress on the foot, obesity, and faulty biomechanics[1]. This will cause the posterior tibial tendon dysfunction, collapse of the foot arch and developed flat foot (Pes planus). Flat foot can sometimes contribute problems in knees because this condition can alter the optimal alignment of legs. Clinical assessment will determine the characteristics and deformity of the joint[2]. Along with assessment, range of motion (ROM) would place a major roll on the assessment. The ankle is the part of the lower limb, the distal portion of the leg and proximal portions of the foot [3]. The foot contained 26 small bones for weight-bearing, and force distribution. The bony alignment creates three arches to provide efficient weight distribution while avoiding compression of plantar neurovascular structures. There are, lateral and medial longitudinal arch and the transverse arch together create an architectural vault which is one of the strongest load-bearing structures known to mankind[4]. The ankle joint is formed by talus, articular surface of the lower tibia and medial malleolus and lateral malleolus of the fibula. The movements that allow by ankle joint are plantar flexion, dorsiflexion, inversion, and eversion[5]. The foot is anatomically and biomechanically, subdivided into three parts, hindfoot, midfoot and forefoot[6]. The Rearfoot and the hindfoot is the talus and calcaneus bone. The midfoot consists of navicular, cuboid and three cuneiforms and the forefoot consist of fourteen bones of toes and five metatarsals[7]. Flat foot defines as a postural deformity in the combination of the medial longitudinal arch collapse[8]. According to the International Journal of Physiotherapy and Research (2015), the flat foot is also known as Pes planus caused by injury, prolonged stress on the foot, obesity, and faulty biomechanics[9]. This will cause the posterior tibial tendon dysfunction, collapse of the foot arch and developed flat foot (Pes planus). Galen is the most famous physician and he is the first author of the flat foot (Pes planus). Galen didn't use the term (flat foot), but he described it and characterize the patients as liopothes or smooth feet people. There are two types of flat foot[10]. Flexible flat foot is a common type of flat foot. The foot arch will be normal at rest (not during standing or walking), and it will disappear when contact with the ground[11-13]. A rigid type was fixed deformation in the flat position on ankle joint, with or without weight-bearing. Normally the pain will appear in the medial part of the hindfoot and along into posterior tibial tendon. Flat foot can sometimes contribute problems in knees because this condition can alter the optimal alignment of legs. Clinical assessment will determine the characteristics and deformity of the joint. Along with assessment, range of motion (ROM) would place a major roll on the assessment [14].

Methodology

The study design is an experimental study. The study will be conducted at Aimst University, Kedah, Malaysia. Total of 30 participants of both the gender, age between 18 to 30 years, who have a limitation of ROM and pain while performing dorsiflexion and plantar flexion, will be recruited. Each participant will undergo pre-test and post-test screening for an assessment of Body Mass Index (BMI), ROM, pain, physical examination, and Staheli Arch Index. Later the participants will be divided into two groups by a convenient sampling method. Group A participants will undergo exercises related to strengthening of foot muscles along with non-allergic taping technique while Group B participants will undergo exercises related to the strengthening of foot muscles along with medial arch support.

Result

Total of 30 participants of both the gender, age between 18 to 30 years, who have a limitation of ROM and pain while performing dorsiflexion and plantar flexion, will be recruited. Each participant will undergo pre-test and post-test screening for an assessment of Body Mass Index (BMI), ROM, pain, physical examination, and Staheli Arch Index. Later the participants will be divided into two groups by a convenient sampling method. Group A participants will undergo exercises related to strengthening of foot muscles along with non-allergic taping technique while Group B participants will undergo exercises related to the strengthening of foot muscles along with medial arch support.

Group A: Group A participants will undergo to perform a 30 min exercises related to the strengthening of foot muscles that consisted of a 5 min warm-up exercise, a 20 min main exercise, and a 5 min concluding exercise along with a non-allergic taping technique.

Group B: Group B participants will undergo to perform a 30 min exercises related to the strengthening of foot muscles that consisted of a 5 min warm-up exercise, a 20 min main exercise, and a 5 min concluding Body Mass Index (BMI). The height of each subject was measured using sewing tailor tape and the weight was measured using a digital weight scale. The BMIs of the subject was calculated by divided the body weight by the square of height (kg/m^2). BMI was taken during

assessment because there is a relationship between Body Mass Index and flat foot. Obesity is one factor that causes flat foot. It increases static and dynamic plantar pressure. As a result, the appearance of flattened medial longitudinal arch4. BMI categories – Underweight: below 18.5. Normal: 18.5 – 24.9. Overweight: 25 – 29.9 and Obesity: 30 – 39.95. Range Of Motion (ROM): Ankle joint range of motion was measured by using goniometer as a measurement tool for ankle joint to find out any limitations in the range. For ankle dorsiflexion, the subject is supine with legs off the table. The fulcrum is aligned with the lateral malleolus. The stationary arm is in line with the midline of the lower leg; use the head of the fibula for reference. The moving arm is parallel to the fifth metatarsal. Normal ROM of dorsiflexion is between 0 and 20 degrees. Ankle plantarflexion, the subject is supine with legs off the table. Goniometer alignment is the same as for dorsiflexion. Normal ROM is 45 degrees. For subtalar inversion, the subject is prone to lying with the foot hanging off the table. The fulcrum is placed in the two malleoli. The stationary arm is aligned with the midline of the leg and moving the arm with the midline of the calcaneus. The ROM is between 0 and 30 degrees and subtalar eversion, method are the same as subtalar inversion. The ROM is between 0 and 25 degrees. Pain Scale: Pain evaluated by using the Visual Analog Scale (VAS Scale). A briefly explained to the subjects and showed the scale; that they rated their pain using numbers, faces and words. Toe Raising Test: A toe raising test was conducted under a physical examination of all the subjects. The purpose of this test to check the flexibility of Pes planus. The test was performed with the patient weight-bearing, while the therapist dorsiflexes the hallux. Positive result: (arch formation) from the flat foot being flexible. Negative result: (lack of arch) from the flat foot being rigid. This special test was described by Jack and explained by Hicks due to the "windlass action" of the plantar fascia6. After done with the assessment, the participants equally divided into two groups. Each group 15 participants. Group A was provided exercise and taping technique and Group B was provided exercise and arch support. Exercise along with medial arch support.



Figure 1: Toe raising test

The collected data were recorded and tabulated. The data was analyzed using statistical package for social science (IBM SPSS Statistics 23) to present the finding of the study - compare the effectiveness of Kinesio taping and medial arch support on random population. Range of motion, VAS scale, and Staheli Arch Index, Wilcoxon Signed Ranks Test was used for statistical analysis. The Wilcoxon Signed Ranks Test used to determine whether there were changes in between pre-test and post-test. To compare the outcome of Kinesio Taping and Medial Arch Support, Mann-Whitney U test was used. After statistical analysis, a significant improvement was found in the range of motion and VAS Scale. Results were considered significant at $p<0.05$.

Table 1: Demographic Characteristics of Study Samples

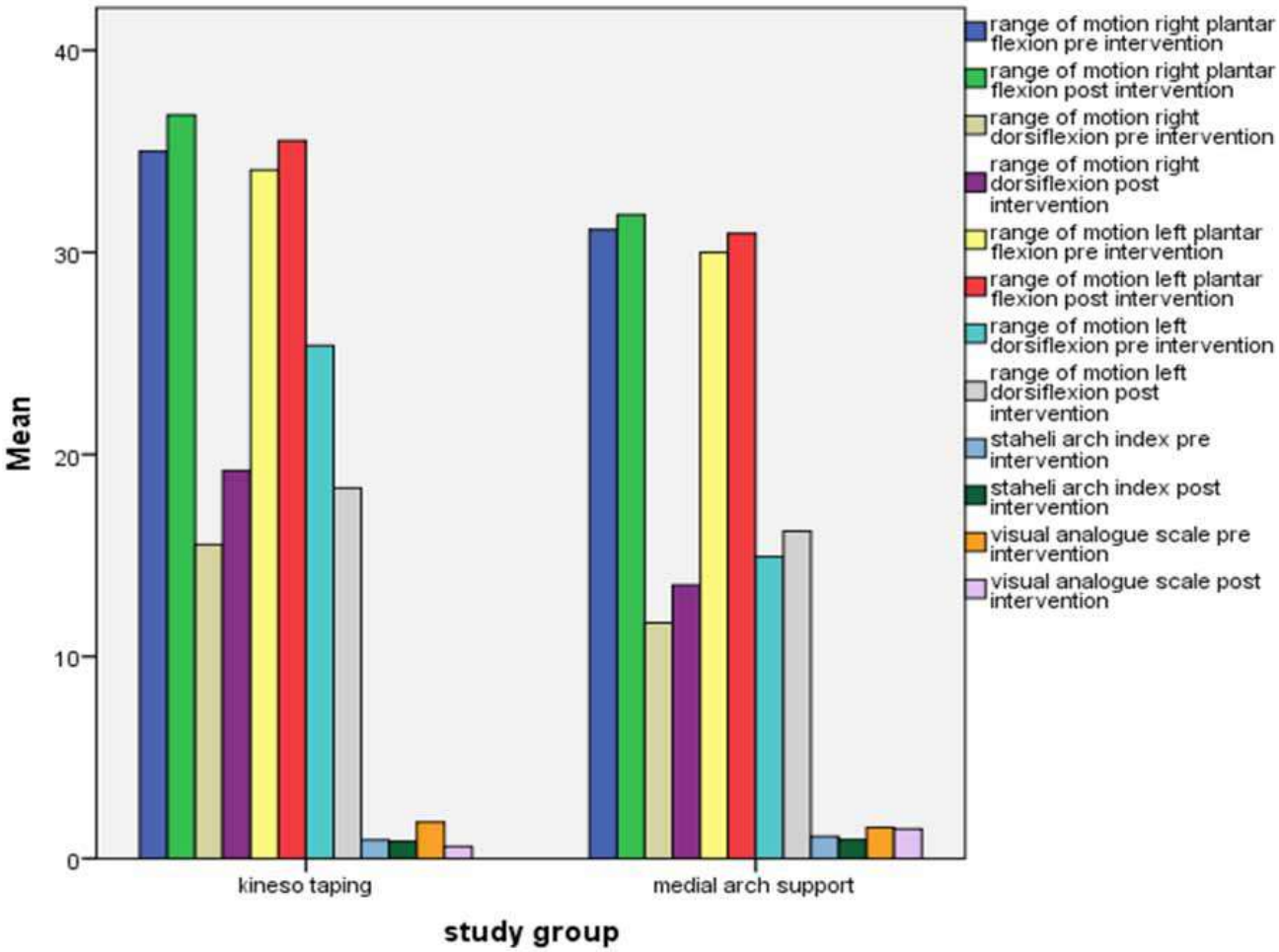
Gender	Mean (SD)	Frequency	Percentage (%)
Male		12	40.0
Female		18	60.0
Race			
Malay		3	10.0
Chinese		10	33.3
Indian		17	56.7
Study group			
Kinesio taping		15	50.0
Medial arch support		15	50.0
Age	21.6 (2.5)		
Weight	64.1 (18.3)		
Height	162.7 (9.4)		
BMI	24.0 (5.5)		

Table 2: Results of ROMPFR, ROMPFL, ROMDFR, ROMDFL, SAI and VAS Measures in Kinesio Taping and Medial Arch Support

	Median	Range	Percentile		Z	pa
			25th	75th		
Kineso taping						
ROMPFR-pre intervention	35.0000	30.0000	25.0000	45.0000		
ROMPFR-post intervention	40.0000	25.0000	50.0000	40.0000		
ROMPFL-pre intervention	35.0000	25.0000	30.0000	40.0000		
ROMPFL-post intervention	35.0000	26.0000	30.0000	40.0000	-2.226	0.026
ROMDFR-pre intervention	15.0000	15.0000	12.0000	20.0000	-2.536	0.011
ROMDFR-post intervention	18.0000	13.0000	17.0000	22.0000	-3.316	0.001
ROMDFL-pre intervention	15.0000	145.0000	15.0000	20.0000	-1.791	0.073
ROMDFL-post intervention	16.0000	22.0000	15.0000	22.0000	-1.633	0.102
SAI-pre intervention	0.9000	0.5000	0.8000	0.9211	-3.491	0.000
SAI-post intervention	0.8000	0.5000	0.8000	0.9000		
VAS-pre intervention	2.0000	3.0000	1.0000	2.0000		
VAS-post intervention	0.0000	4.0000	0.0000	1.0000		
Medial arch support						
ROMPFR-pre intervention	30.0000	15.0000	25.0000	35.0000		
ROMPFR-post intervention	30.0000	25.0000	26.0000	36.0000		
ROMPFL-pre intervention	30.0000	35.0000	20.0000	40.0000		
ROMPFL-post intervention	30.0000	35.000	21.0000	40.0000	-2.041	0.041
ROMDFR-pre intervention	10.0000	10.0000	10.0000	15.0000	-1.841	0.066
ROMDFR-post intervention	12.0000	15.0000	10.0000	17.0000	-2.724	0.006
ROMDFL-pre intervention	15.0000	20.0000	12.0000	20.0000	-2.388	0.017
ROMDFL-post intervention	15.0000	20.0000	15.0000	20.0000	-1.633	0.102
SAI-pre intervention	0.9000	2.3000	0.8000	1.2000	-0.333	0.739
SAI-post intervention	0.8000	0.5000	0.8000	1.2000		
VAS-pre intervention	1.0000	2.0000	1.0000	2.0000		
VAS-post intervention	1.0000	3.0000	1.0000	2.0000		

Table 3: Comparison Of Outcome Results Between Kinesio Taping And Medial Arch Support

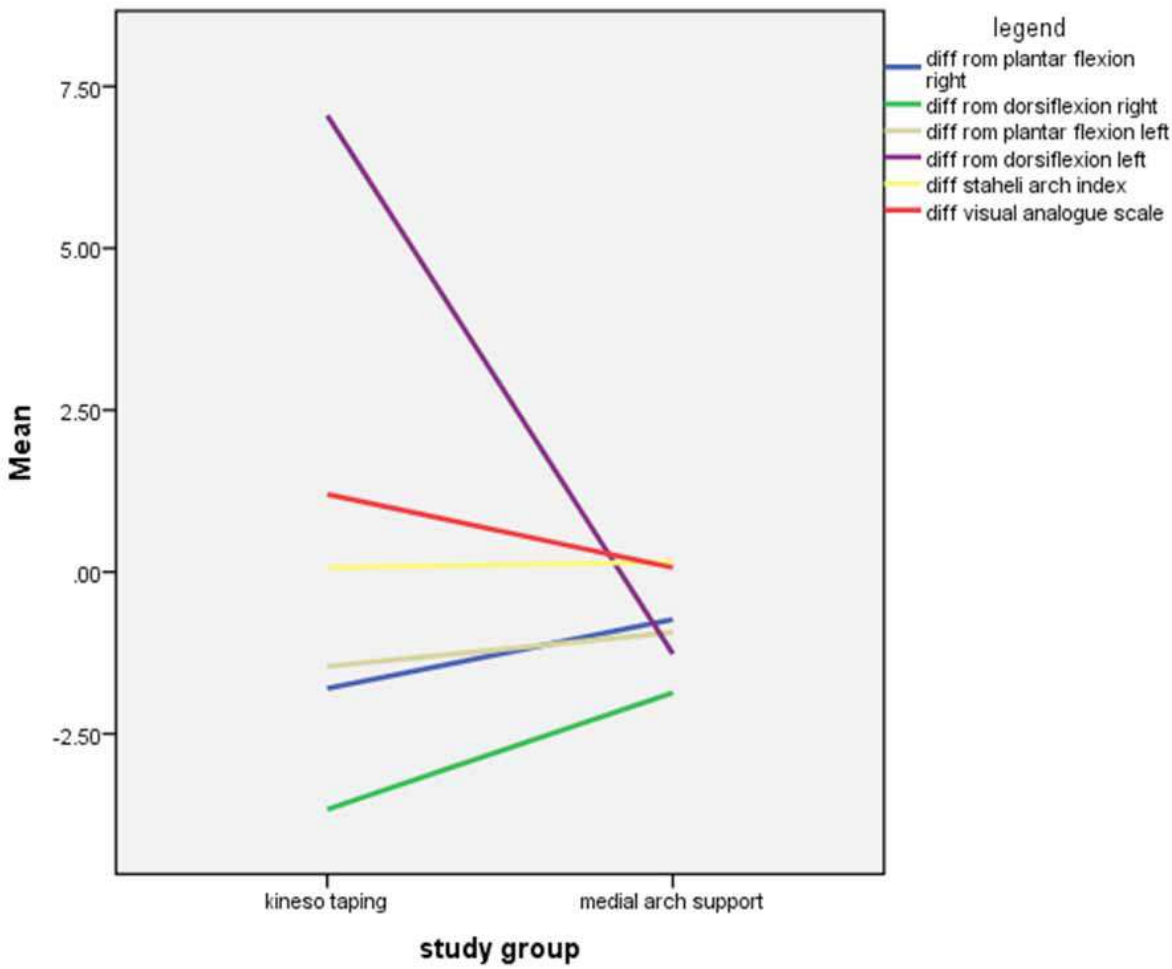
Study group	Outcome	Median	Range	Percentile		U[a]	P
				25th	75th		
Kineso taping Medial arch support	ROMPFR	0.0000	7.0000	-2.0000	0.0000	94.500	0.386
	ROMPFL	0.0000	9.0000	-2.0000	0.0000	81.500	0.146
	ROMDFR	-2.0000	7.0000	-5.0000	-0.7500	58.000	0.020
	ROMDFL	-1.0000	144.8000	-3.0000	0.0000	101.500	0.633
	SAI	0.0000	2.5000	0.0000	0.1000	85.000	0.220
	VAS	1.0000	1.0000	0.0000	1.0000	30.500	0.000



Graph 1: The distribution measures of pre and post-intervention for ROMPFR, ROMPFL, ROMDFR, ROMDFL, SAI and VAS between Kinesio Taping and Medial Arch Support

Table 1 shows the demographic characteristics of 30 subjects included gender, race, study group, age, weight, height, and body mass index category. According to Table 2, for the subjects treated with Kinesio tape, the ankle range of motion, showed significant differences in pre-test and post-test of plantar flexion on the right side with 'Z' value -2.226 at $p<0.05$. There was a significant difference between pre-test and post-test of Medial Arch Support in plantar flexion on the right side with 'Z' value -2.041 at $p<0.05$. There was a significant difference in pre-test and post-test of Kinesio tape in plantar flexion on the left side with 'Z' value -2.536 at $p<0.05$. Comparison of pre-test and post-test of Medial Arch Support in plantar flexion on the left side with 'Z' value -1.841 at $p>0.05$, thus stating that there is no significant difference. There was a significant difference

in dorsiflexion of the right sides between the Kinesio tape group and the Medial Arch Support group ($p<0.05$). Pre-test and post-test of Kinesio tape in dorsiflexion on the left side with 'Z' value -1.791 at $p>0.05$, thus stating that there is no significant difference. For the subjects treated with Medial Arch Support, the pre-test and post-test of dorsiflexion on the left side show a significant difference with 'Z' value -2.724 at $p<0.05$. There is no significant difference in Staheli Arch Index on pre-test and post-test between the Kinesio tape group and the Medial Arch Support group ($p>0.05$). There was a significant difference in pre-test and post-test of Kinesio tape in the Visual Analogue Scale with 'Z' value -3.491 at $p<0.05$. Comparison of pre-test and post-test of Medial Arch Support in Visual Analogue Scale with 'Z' value -0.333 at $p>0.05$, thus stating that there is no significant difference. Table 3 shows the comparison of outcome values for Range of motion, Staheli arch index and Visual analogue scale test within the group and between the groups by Mann-Whitney U test. For the range of motion of right plantar flexion on both sides, the 'U' value is 94.500 on right and 81.500 on the left side at $p>0.05$, thus stating that there is no significant difference between the groups. Hence there is a significant difference of dorsiflexion in both groups at the right side with 'U' value is 58.000 at $p<0.05$. Nevertheless, the 'U' value of left side dorsiflexion is 101.500 at $p>0.05$, thus stating that there is no significant difference between the groups. For Staheli Arch Index, the 'U' is 85.000 at $p>0.05$. So, stating that there is no significant difference between the groups. There is a significant difference in the Visual Analogue Scale in both groups with the 'U' value is 30.500 at $p<0.05$. Graph 1 and 2shows the distribution on measures of pre-test and post-test for Range of motion, Staheli arch index and Visual analogue scale test within the group and between Kinesio Taping and Medial Arch Support. The result significantly shows that an improvement in Kinesio Tape than Medial Arch Support.



Graph 2: Improvement in Rompfr, Rompfl, Romdfr, Romdfl, Sai and Vas Measures Between Kinesio Taping and Medial Arch Support

Discussion

This study was done to compare to the effectiveness of Kinesio taping and medial arch support on the flexible flat foot on a random population in AIMST University. The outcome measure used in this study was Range of Motion, Staheli Arch Index, and VAS Scale. Subjects are selected after confirming with the parameter of the arch which measured by Staheli Index Arch.

Plantar Arch Index = A/B

A = Measurement of the support width of the central region to the foot.

B = Measurement of the support width of the heel region to the foot

All 45 participants selected and agreed to take part in this study. However, there were 15 dropouts and a total of 30 participants completed the course of the research study. 12 male and 18 female subjects participated in this research. The reason of subjects for dropouts was declined to participate, due to semester breaks, and a packed schedule. Consents were gained from all participants before the assessment. Each participant undergo pre-test and post-test screening for an assessment of Body Mass Index (BMI), ROM, pain, physical examination, and Staheli Arch Index. The participants divided into two groups by a convenient sampling method. Group A participants will undergo exercises related to strengthening of foot muscles along with non-allergic taping technique while Group B participants will undergo exercises related to the strengthening of foot muscles along with medial arch support. Data of a total of 30 subjects were taken for analysis. The findings of this study were support the hypothesis. This result reflects in agreement with, after applying Kinesio tape, foot pressure, pain and foot arch on the bilateral leg significantly reduced and improved in a range of motion. According to the subject's feedback at the end of the research session, those who applied Kinesio Tape claimed that they feels comfort and better after applied Kinesio Tape, pain was reduced and improvement seen in the ankle movement. Therefore, Kinesio tape was a more effective method than Medial Arch Support to improve muscle tone, range of motion and the foot pronation decreased. Some of these studies are similar to the current study was observed, especially the increase in peroneus longus activity. This study was conducted to compare the effectiveness of taping and arch support on the flexible flat foot on a random population. Weng-Sam Siu et al, stated that using Kinesio tapes that reinforce the transverse arch for static stability and facilitate tibialis posterior for dynamic stability immediately after its application and increase the muscle activity of tibialis anterior during treadmill running in individuals with functional flatfoot, which was similar results of the present study. Joong-San Wang et al stated that the application of Kinesio tape to flexible flat feet was an effective intervention method for immediately reducing abnormally increased foot pressure and tone and stiffness in the lower extremities muscles. Agnieszka Straczynskaa et al stated that the application of Kinesio taping was a statistically significant decrease in pain and improvement in the deviation angle of the calcaneus was identified. Sivachandrian et al stated that the exercise programs significantly improved the angle of the arch foot, navicular height, and medial longitudinal arch. In additional, Smith et al. (Smith, Coates, Leung, & Creaby (2015) compared rigid and elastic anti-pronation taping effect on exercise related leg pain in females. The elastic tape was ranked as the most comfortable condition by a higher percentage of the participants. This implies that the elastic tape, like Kinesio tape, which was intended to provide therapeutic effects, may also provide greater comfort during exercise. Robert Yolo et at stated that taping acts as controlling pronation of the foot by pulling the calcaneus anteriorly and medially, thus limiting hindfoot eversion and restricting the associated talar adduction and plantarflexion. When the subtalar motion is limited, the arch is elevated subsequently reducing the stretch on the plantar aponeurosis. Similarly, the medial arch height is raised as the tape pulls the lateral aspect of the foot medially. According to Park et al. (2015); Tsai, Chang, & Lee (2010) have reported a reduction of pain in patients with plantar fasciitis and hallux valgus after taping. Our flatfoot participants did not have such pain level. Pain scores were not more than 4 (out of 10) due to the acute stage of their condition. These grading was taken before the treatment. Range of motion is defined as the movable range of a joint's potential movement. As for the ankle range of motion, the subject is asked to perform ankle plantar flexion and dorsi flexion. The measurements were taken with the help of goniometer. Quackenbush et al (2008) reported that ankle taping in female athlete's involked significant differences in pre- and post- exercise active range of motion for plantarflexion and Dorsiflexors. In the present study, the taping group showed significant differences in dorsiflexion and plantarflexion at $p<0.05$. This is consistent with prior studies suggesting that,

with taping, the ankle range of motion is greater after exercise than before. Because of the loosened taping during exercise, ankle range of motion increases after exercise. After the treatment session, it was shown that there was a statistically significant reduction in pain and improved in a range of motion in the post-test in Group an after applied Kinesio Tape.

Conclusion

This study was done to compare to the effectiveness of Kinesio taping and medial arch support on the flexible flat foot on a random population aged 18 to 29 years. This conclusion was drawn from the statistics shown in the SPSS after the data analysis was done. There was a statistically significant reduction in pain and improved in a range of motion in the post-test in Group an after applied Kinesio Tape. Thus, the results of the present study concluded that the application of Kinesio tape to flexible flat feet was an effective intervention method for immediately reducing abnormally increased foot pressure and tone and stiffness in the lower extremities muscles and it improved medial longitudinal arch. The study also concluded that taping is effective for 24 hours in the management of flexible flat feet. Having said that, it was statistically found in this study. These findings may have important implications for clinical practice as they will help guide treatment choices for flat foot.

Ethical Clearance: No ethical approval is needed.

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The Relationship between Prediabetes and Body Mass Index of Outpatients at Dr. Dody Harjoto Hospital at Maros Regency, South Sulawesi, Indonesia

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Abstract

Objective: The prevalence of prediabetes is increasing worldwide. According to the International Diabetes Federation in 2016,6.7% of the world's population lived with this condition and estimated to rise to 7.8% in 2040. In general, prediabetes is accompanied by excess body mass index. Therefore, the purpose of this research was to determine the relationship between prediabetes and body mass index of outpatients in the internal medicine polyclinic at Dr. Dody Sarjoto Hospital, Maros Regency, South Sulawesi.

Method: This was an analytical research design with cross sectional approach, involving 143 prediabetes patients. Secondary data were obtained from the record books of the hospital and subjected to Chi Square test.

Result: The highest number of prediabetes cases were within the age range of 40-50 years (58.7%), among female (73.4%), patients with high school education (58.7%), and in 81.1% of patients with family history of DM. Furthermore, the result of the Chi Square test was 0.041, greater than <0.05, indicating a relationship between prediabetes and BMI.

Conclusion: There was a relationship between the cases of prediabetes and BMI of outpatients at dr. Dody Sarjoto Hospital, Maros Regency, South Sulawesi

Keywords: *Prediabetes; Overweight; Obesity; Body Mass Index.*

Introduction

Prediabetes is a health challenge with serious impact and emerging as epidemic in the world, especially in Indonesia. It is a condition characterized with increase in blood glucose known as hyperglycemia, but not high enough to be regarded as diabetes mellitus. The prevalence of prediabetes is increasing every year worldwide, and in 2016, the International Diabetes Federation estimated the adult population with prediabetes to be 6.7%, which was equivalent to 318 million people. It was also estimated that this figure would reach 7.8% in 2040, equivalent to 480 million population of the world.[1] Based on a study, the population of prediabetes in America of people over 20 years old between 1999 and 2010 was 35.5% while the prevalence in UK was 35.5%, 15.5% in China, 14.8% in Spain and 22.4% in Bangladesh.[2] Its prevalence at Indonesia in 2009 was around 10.2% and increased to 36.6% in 2013.[3] According to IDF (2017), Indonesia is the 3rd country with the highest number of prediabetes between the ages of 20 to 79 years. A research showed

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that 25% of prediabetes cases within a period of 3-5 years developed into type 2 Diabetes Mellitus (T2DM), about 50% remained as prediabetes, and while 25% reversed to normal blood glucose.[4]

Generally, prediabetes has a great chance of reversing to normal condition, therefore, it could be managed in order to prevent or delay its development into T2DM. One of the best ways of managing it is by avoiding some of the modifiable risk factors such as excess body weight, obesity, lack of physical activity, smoking, alcoholic, hyperlipidemia/dyslipidemia, and high blood pressure. However, the non-modifiable risk factors cannot be avoided by humans and these include age, genetic, and history of diabetes etc.[5] Physiologically, blood glucose are stored in muscle, liver, or fat cells as an energy source, but in insulin resistance condition, glucose are unable to enter these cells, consequently, blood glucose tend to rise but not sufficient to cause diabetes clinically. This condition eventually makes the pancreas unable to secrete insulin as needed, thereby leading to excessive and uncontrolled sugar production in liver, a condition which makes the blood sugar to rise and eventually leads to T2DM.[6]

Diabetes is a metabolic disease characterized by hyperglycemia caused as a result of abnormalities in insulin secretion or action or both.[7]The International Diabetes Federation reported about 382 million DM cases worldwide in 2013 and it was predicted it would rise to 592 million in 2035. Similarly, the World Health Organization (WHO, 2014), reported that 1 million people died as a result of diabetes in year 2000, with a prevalence of around 2%, which then increase to a death of 1.5 million people in 2012 with a prevalence of about 2.7%. Also, 70% of these worldwide deaths due to DM happened in developing countries including Indonesia. According to the results of Basic Health Research (*Riskesdas*) in 2013, the prevalence of diabetes from ages ≥15 years was 6.9% and increased to 10.9% in 2018.[8]

Body mass index (BMI) is an indicator of the level of adiposity in the body, calculated from the body weight (BW) in kilograms divided by height (TB) in meters squared (m^2). It does not directly measure body fat, but shows the body fat measurements such as underweight, normal, overweight and obesity.[9] Underweight increases the risk of infectious diseases while excessive weight i.e, obesity, is an important factor in insulin resistance pathogenesis. BMI is often recommended for evaluating obesity in adults due to the fact it is easier to conduct and inexpensive. BMI level is associated with body fat, which is a risk factor for prediabetes.[10] Obesity is characterized by excessive accumulation of body fat, which increases the risk of hyperglycemia. This condition results in increased secretion of free fatty acids, hormones, and pro-inflammatory substances such as interleukin-6 (IL-6), tumor necrosis factor α (TNFα) which triggers insulin resistance and damage to insulin pancreatic beta cells, which could develop into prediabetes characterized by hyperglycemia.[11] According to a research in South Korea, obesity is 1.5 times at risk of developing into prediabetes.[12] Similarly, the *Riskesdas* data in 2007 showed that obesity significantly increased the possibility of prediabetes by 1.5 times compared with people with normal BMI. The prevalence of obesity according to *Riskesdas* in 2013 was 18.8%, which then increased to 21.8% in 2018.[8] There are various risk factors for obesity, which include genetic, psychological environment, lifestyle, age, sex, economic status, and use of contraception especially the hormonal type. Also, a research showed that obese individuals have 1.63 to 11.58 times greater risk of developing DM compared with non-obese, and the more severe the obesity, the greater the risk of developing DM.[13] Hyperglycemia in overweight and obese people is caused by increase in the free fatty acids which reduce glucose transporter translocation into plasma membrane. This eventually results in insulin resistance in muscle and adipose tissue, initially forming proinsulin with molecules larger than insulin. Usually, proinsulin is stored in the pancreas until it is needed by the body. When secreted into the bloodstream, proinsulin is broken down into 2 parts namely; connecting peptides and active insulin hormones. The main function of the hormones is to reduce glucose levels in the blood. According to Guyton's theory, obesity is a predisposing factor for increased blood sugar. This is because the Langerhans island beta cells are less sensitive to stimulation (insulin resistance) or due to the rising sugar levels and obesity suppresses the number of receptors insulin in cells throughout the body. Therefore, the purpose of this research was to determine the relationship between prediabetes and body mass index (BMI) of outpatients at dr. Dodysarjoto Hospital, Maros Regency, South Sulawesi, Indonesia.

Research Method

This is an analytical descriptive cross sectional study. The research subjects were selected through purposive sampling method, involving all outpatients

treated at Internal Medicine Polyclinic of Dr. Dody Sarjoto Hospital from April 2018 to April 2019. Out of the 2,150 patients sampled during this period, only 143 met both the inclusion and exclusion criteria, hence, were the ones involved in this research. The inclusion criteria include; male and female aged between 18-70 years, fasting blood glucose levels 100-125mg/dl or/and blood glucose levels after 2 hours (post prandial) 140-199mg/dl.

The exclusion criteria are patients with diabetes history and incomplete general data. Secondary data were collected from Dr. Dody Sarjoto Hospital medical record book and the general data were analyzed using SPSS 22 program. The univariate analysis was used to test the normality of the data, as well as the frequency distribution, while bivariate analysis involved the use of Chi-Square Test.

Results

The results of this study are presented in the following table.

Table 1: Characteristics of research respondents.

Characteristics	Frequency	Percentage (%)
Age (Years)		
<28	5	3.5
29 - 39	13	9.1
40 - 50	84	58.7
>51	41	28.7
Gender		
Male	38	26.6
Female	105	73.4
Work		
Yes	62	43.4
No	81	56.6
Education		
D1-D3	21	14.7
S1-S3	28	19.6
Junior High School	10	7.0
Senior High School	84	58.7
DM Family History		
Yes	116	81.1
No	27	18.9
Total	**143**	**100.0**

The highest number of respondents with prediabetes was 84 (58.7%), found between the ages of 40-50 years, while the lowest number of 5 (3.5%) was found at ages <28 years, as shown in Table 1. Also, 81 of the respondents, representing 56.6%, do not work, while the highest number of prediabetes had senior high school as the highest level of education, 84 (58.7%). In addition, there were more respondents, 116 (81.1%), with family history of DM.

Table 2 The data of body mass index.

Body Mass Index	f	%
Normal	6	4.2
Overweight	128	89.5
Obese	9	6.3
Total	143	100.0

Based on Table 2, there were only 6 respondents (4.2%) with normal BMI, majority, 128 people (89.5%) were overweight while 9 people (6.3%), were found to be obese.

Table 3 Chi-Square test.

	Value	Df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	9.800a	4	.044	.041
Likelihood Ratio	13.460	4	.009	.014
Fisher's Exact Test	9.962			.019
N of Valid Cases	143			

Based on Table 3, the Chi-Square results was 0.041, since this figure <0.05, it means that there is a relationship between prediabetes and body mass index.

Discussion

Prediabetes is a condition characterized by elevated blood sugar above the normal but not sufficient to be recognized as diabetes mellitus. The age range with the highest number of prediabetes was 40-50 years, with 84 (58.7%) people. The high prevalence of prediabetes in this age range was due to the aging process taking place at that point. The production of enzymes associated with insulin is disrupted at this point. Also, there are changes in cell permeability, as well as the response of cell nucleus to insulin, a condition which allows an increase in the blood glucose. There were more cases of prediabetes among women because they generally have less muscle mass, consequently, it is easy to experience insulin resistance within the muscles in moderate to severe levels. Additionally, there were prediabetes cases among patients with no permanent job due to the fact that

they do not perform routine physical activity. Majority of these people just sit at home and watch television, a behavior which increases the risk for prediabetes. The highest number of cases was found among in the high school graduates.

This is possibly due to the fact the largest range of graduated in Indonesia are from the public high schools, hence, more respondents were at this education level. People with family history of DM have a higher risk of coming up with prediabetes. The results of this study support this fact with Table 1 showing 116 (81.1%) prediabetes cases with family history of DM. This is in line with the the results of a multicenter research conducted by Wagner in Germany which showed that family history of DM was significantly associated with the emergence of prediabetes (OR = 1.4 with 95% CI = 1.27: 1.54 p <0.001). It further stated that family history of DM increased the risk of prediabetes by around 40%.[14] In general, there are more obese people with prediabetes compared with individuals with normal body weight. In addition, BMIs above the normal limits have a higher tendencies of coming up with various kinds of complications such as prediabetes, diabetes, hypertension, etc. According to a study, BMI mechanism plays a major role in insulin resistance, as BMI above normal causes peripheral resistance to glucose uptake, thereby decreasing the sensitivity of pancreatic beta cells.[15] Moreover, a research showed that 80% of obese or overweight people experience hyperglycemia[16], while another research conducted in Iraq showed that BMI <25 kg/m^2 had 2.7 times risk of causing hyperglycemia.[17]

Insulin resistance in prediabetes happens in the muscle, liver, and fat tissues. Additionally, the result obtained from the Chi-Square test was 0.041, meaning that there was a relationship between prediabetes and the BMI, as there were more cases among obese patients compared with people with normal body weight. This could be due to the accumulation of excess visceral fat in prediabetes patients which causes insulin resistance in the liver and muscles, and consequently glucose uptake leading to hyperglycemia. The adipose tissue is recognized as an endocrine organ producing several peptides which are both proinflammatory and anti-inflammatory. This inflammatory process causes dysfunction in pancreas causing insulin resistance, and if this condition continues, it leads to increase in glucose levels and impaired lipid metabolism.[1] Under normal conditions of sufficient and sensitive insulin levels, the insulin works on its receptors found on the cell surface, enters the cells and eventually allows glucose to enter the cells, which are used as energy or as energy reserve, with normal blood glucose level the end result.[17] This is not the situation in obese people, as increase in lipopolysaccharides (LPS) mRNA induces TNF-α factors and protein levels, a process leading to inflammatory processes in leptin-1 and PAI-1 obese patients. In general, an increase in TNF-α in fat tissue of obese patients is an indication of insulin resistance.

Furthermore, obesity is a dominant cause of insulin resistance associated with decrease in the number of receptors and the failure of post-receptor to activate the tyrosine kinase, which is a subunit B in the insulin receptor. Activating this complex activates beta-phosphorylase and insulin-mediated action which control blood sugar levels. Consequently, hyperinsulinemia arises due to the failure in the delivery of signals to regulate blood sugar levels. Generally, the risk factors of diabetes include hereditary factors, physical activity, dietary intake, metabolism and hormonal metabolism. Increased glucose and fat usually result in increased transport of fatty acids into adipose and lipogenesis, which is under the influence of insulin. Also, partial oxidation of fatty acids produces ketones which are alternative fuel source for the brain and various organs. Moreover, fat tissues are known not only as storage points for energy reserves but as dynamic network with various functions. This condition of excess fat tissue common in the modern society is also associated with insulin resistance.[18]

Conclusion

Based on the *Chi square* result, 0.041 (<0.05), there is relationship between prediabetes and BMI of outpatient at Dr. Dody Sarjoto Hospital, Maros Regency, South Sulawesi, Indonesia.

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Conflict of Interest: Nil

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Pandemic Corona (Covid-19) and Health Law Protection

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Abstract

Pandemic Corona (Covid-19), which struck all countries in the world, not only caused anxiety but also the number of victims that were not insignificant. This has caused the state to make efforts to manage health that is categorized as a disaster. Standards for health services also follow the rhythm of the victims that are not small every day. For this reason, it is necessary to examine whether the risk of spreading a pandemic in health is part of legal protection and whether a pandemic is part of disaster mitigation that receives legal protection. The linkage to health protection during the pandemic and law as the basis for policies to be able to implement health decisions and policies, which certainly requires effective and fast treatment.

Keywords: *Pandemic, Corona (Covid-19), disaster, health law.*

Introduction

At the beginning of 2020, which is usually the spirit of countries in starting the economic movement and various other development programs began, suddenly faced with the harsh reality of the emergence of malignant diseases that attack humans not only occur in one continent but simultaneously in all corners of the world.

It was reported from Wuhan that on December 29, 2019 in a hospital in Wuhan, Hubei Province, China the emergence of an acute pneumonia was unknown. The PRC government then informed WHO about the emergence of this disease after going through a verification process.

WHO describes the initial conditions[1]: On 30 December 2019, three bronchoalveolar lavage samples were collected from a patient with pneumonia of unknown etiology – a surveillance definition established following the SARS outbreak of 2002-2003–in Wuhan Jinyintan Hospital. Real-time PCR (RT-PCR) assays on these samples were positive for pan-Betacoronavirus. Using Illumina and nanopore sequencing, the whole genome sequences of the virus were acquired. Bioinformatic analyses indicated that the virus had features typical of the coronavirus family and belonged to the Betacoronavirus2B lineage. Alignment of the full-length genome sequence of the COVID-19 virus and other available genomes of Betacoronavirus showed the closest relationship was with the bat SARS-like coronavirus strain BatCovRaTG13, identity 96%.

The PRC National Health Commission said that: On January 8, 2020 the pathogen from this incident could be identified as the 2019 coronavirus novel (nCoV-2019), and its gene structure was immediately sent to WHO.

Due to the serious condition, WHO then announced[2]: The second meeting of the Emergency Committee convened by the WHO Director-General under the International Health Regulations (IHR) (2005) regarding the outbreak of novel coronavirus 2019 in the People's Republic of China, with exportations to other countries, took place on Thursday, 30 January 2020, from 13:30 to 18:35 Geneva time (CEST).

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WHO subsequently established a committee, the Public Health Emergency of International Concern (PHEIC) tasked for:[3]

The Committee's role is to give advice to the Director-General, also provides public health advice or suggests formal Temporary Recommendations as appropriate.

Then on 12 February 2020 the International Committee on Taxonomy of Viruses (ICTV) declared that nCoV-2019 became the official name of severe acute respiratory coronavirus 2 syndrome ((SARS-CoV-2), and on the same day WHO declared SARS-CoV- The 2 official names are corona virus disease 2019 (COVID-19).

With the outbreak of the disease which by WHO is categorized as a pandemic, lead the author to analyze whether the risk of pandemic spreading in the health becomes part of legal protection and whether the pandemic is part of disaster mitigation that gets legal protection.

Discussion

Scope of Pandemic: Definition of Pandemics in the Big Indonesian Dictionary (KBBI) means that it is widespread (about disease) in a region, continent or throughout the world. WHO defines this term as a new pathogen epidemic that spreads easily from person to person throughout the world. A pandemic is when it spreads in several countries or continents and usually affects large numbers of people. Global distribution.

In the current health conditions the world community is experiencing a period of concern with the corona virus pandemic issue. WHO also mentioned: WHO has been assessing this outbreak around the clock and we are deeply concerned both by the alarming levels of spread and severity, and by the alarming levels of inaction. We have therefore made the assessment that COVID-19 can be characterized as a pandemic.[4]

Calling from WHO: Prevention, Preparedness, Public health, Political leadership, And most of all, people. We're in this together, to do the right things with calm and protect the citizens of the world. It's doable.[5]

For this reason, it is hoped that the need of collaborative not only from within the national of country but also unite movement from countries with strategic-preventive-repressive in togetherness, where countries unite in fighting the pandemic. Especially for Indonesia the question arises whether the risk of a pandemic spreading is part of legal protection and whether pandemics have become part of disaster mitigation?

Health Regulations and Epidemic Measures: In Indonesia, indeed, several legal rules for health care have been regulated and more specifically regarding the pandemic contained in the following rules:

Law No. 4 of 1984 concerning Infectious Diseases: This is the legal basis for handling disease outbreaks[6] that have been in force for decades. UU no. 4 of 1984 interpreted outbreaks of infectious diseases as an outbreak of an infectious disease in a society where the number of sufferers increased significantly more than was normal in certain times and regions and could cause havoc. This law not only regulates how to overcome it, but also regulates criminal sanctions for perpetrators of criminal acts. Threatened parties are those who prevent the prevention of communicable diseases.[7]

Government Regulation No. 40 of 1991 concerning Management of Communicable Diseases: This Government Regulation is the implementing regulation of Law No. 4 of 1984, which in essence regulates how prevention efforts[8] are carried out. Valid from July 3, 1991, PP No. 40 of 1991 regulates what the central and regional governments must do if there is an infectious disease outbreak.[9]

Law No. 24 of 2007 concerning Disaster Management: In this Law alluded to non-natural disasters[10] namely disasters caused by events or a series of non-natural events, among others in the form of technological failure, modernization failure, epidemic, and disease outbreaks. UU no. 24 of 2007 is more a regulation that regulates post-event, which is to overcome risks arising from a disaster. The spread of infectious diseases can be categorized as a non-natural disaster. Responsibility for disaster management basically rests with the government, central and regional. This law also regulates the rights and obligations of citizens, as well as the role of businesses.[11]

Law No. 36 of 2009 concerning Health: Entered into force October 13, 2009, Law No. 36 of 2009 specifically regulates infectious diseases in one chapter (Chapter X). Activities that can be carried out are promotive, preventive, curative, and rehabilitative for affected individuals or communities.

Minister of Health Regulation No. 82 of 2014 concerning the Control of Communicable Diseases: Is the implementing regulation of Law No. 36 of 2009 concerning Health. Diseases transmitted to humans based on Minister of Health Regulation can be caused by biological agents, including viruses, bacteria, fungi, and parasites.[12] Steps[13] that can be done are reduction, elimination, and eradication.

Law Number 6 Year 2018 concerning Health Quarantine (State Gazette of the Republic of Indonesia 2018 Number 128, Supplement to the State Gazette of the Republic of Indonesia Number 6236): Public health emergencies[14] themselves are extraordinary public health events marked by the spread of infectious diseases and/or events caused by nuclear radiation, biological pollution, chemical contamination, bioterrorism, and food that pose health hazards and potentially spread across regions or across countries.

Furthermore, Law 6/2018 mandates that the central government and regional governments are responsible for protecting public health from diseases and/or risk factors for public health that have the potential to cause health emergencies through the implementation of public outrage.[15]

Before determining public health emergencies, the central government first determines the types of diseases and risk factors that can cause public health emergencies. In this regulation it is stated that the central government determines and revokes public health emergencies.[16]

Government Regulation in Lieu of Law Number 1 Year 2020 Regarding State Financial Policy and Financial System Stability for Handling Corona Pandemic: Government Regulation in Lieu of Law (Perppu)[17] concerning state financial policies and financial system stability.[18]

There is an additional budget for spending and financing the 2020 state budget for handling Covid-19, the total additional expenditure and financing for the 2020 state budget for handling Covid-19 is Rp405.1 trillion. With regard to the Transfer to Regional and Village Fund Policies, adjustments to Transfer allocations are made Regions and Village Funds include, among others.

1. Adjusting the allocation of Revenue Sharing Funds based on economic development and/or state revenue;
2. Adjusting the allocation of General Allocation Funds per region based on certain criteria determined by the Minister
3. Finance;
4. Increase/decrease the allocation of Special Transfer Funds, transfer of allocations between Physical budget fields or adjustments to the use of Special Transfer Funds due to certain conditions that require an urgent budget; and/or
5. Adjusting the Village Fund budget ceiling.
6. In addition, there are arrangements regarding the prioritization of the use of the Village Fund, which can be used, among others, for direct cash assistance to the poor population in the village and activities to handle the 2019 Corona Virus Disease pandemic (covid-19).

Government Regulation Number 21 Year 2020 concerning Large-Scale Social Restrictions in the Context of Accelerating Handling of Corona Virus Disease 2019 (COVID-19): The PP 21 of 2020 concerning Large-Scale Social Restrictions in the framework of Accelerating Handling of Corona Virus Disease (COVID-19)[19] is certainly decided with much consideration according to the culture of the Indonesian state. Lockdown policy as applied in many countries, India for example causes chaos and surprising social problems. But the key to this is self-limitation and the movement of individual citizens to stay at home, limiting the frequency and number of mass gatherings to later conquer this pandemic coronavirus.

Presidential Decree No. 11 of 2020 concerning Determination of Corona Virus Disease 2019 (COVID-19) Public Health Emergency: Presidential Decree No. 11 of 2020 concerning Determination of Corona Virus Disease 2019 (COVID-19) Public Health Emergency is taken by considering[20]

Article 4 paragraph (1) of the 1945 Constitution of the Republic of Indonesia; Law Number 6 of 2018 concerning Health Quarantine (State Gazette of the Republic of Indonesia of 2018 Number 128, Supplement to the State Gazette of the Republic of Indonesia Number 6236);

Government policy

The role of the government in terms of medic and non-medic:

1. Mass rapid test with large coverage of residents

suspected of being exposed to the Covid-19 corona virus. Rapid testing involves all parties, from government hospitals, SOEs, the TNI-Polri, to the private sector, research institutions and universities that can also be involved.

2. Providing availability of medical devices and stopping exports of medical devices. The government ensures the availability of medical devices to prevent the transmission of the Covid-19 corona virus, such as masks and hand sanitizers. One of the ways taken is to stop the export of masks, hand sanitizers, or other medical devices needed to prevent corona from being stopped for a while.
3. Food stock

   Ensuring the availability of Indonesian food stock (logistical affairs agency (Bulog) for the community during the Covid-19 pandemic.
4. Work, study and worship policies from home
5. Involvement of religious leaders and appeals for not going home on Muslim Celebration Day (Eid Al-Fitr).

   President Jokowi requested that religious institutions and figures be involved in helping the government prevent the spread of the corona virus which caused Covid-19 disease.[21]
8. Incentives for medical personnel

   The President instructed the Minister of Finance to formulate incentives for health workers who handle Covid-19 patients.
7. Incentives for small businesses

   President Jokowi is aware that social distancing policies to avoid the spread of the Covid-19 corona virus can have an impact on the business world.

Therefore, Jokowi asked the Coordinating Minister for the Economy AirlanggaHartarto to formulate incentives for the business world, particularly micro, small and medium enterprises (MSMEs).

Strategies and mechanisms of the Ministry of Health of the

Republic of Indonesia: In dealing with the pandemic covid-19, it is necessary to study how the mechanism of health protection in Indonesia and its implementation strategy.

Document	Date	Keterangan
Tentang Coronavirus Coronavirus Disease (COVID-19)	March 2020	Ministry of Health
Circular letter of P2P director general Number: HK.02.02/II/753/2020 About the 3rd Revision Preparedness Guidelines for Novel Infection of Corona Virus (COVID-19)	March 16, 2020	P2P director general
Guidelines for the Prevention and Control of Coronavirus Disease (COVID-19)- Rev 4	March 27, 2020	Ministry of Health
Circular letter Number HK.02.01/MENKES/202/2020 concerning the Self-Isolation Protocol in Handling Coronavirus Disease (COVID-19)	March 16, 2020	Ministry of Health
Decree of the Indonesia Minister of Health Number KH.01.07/MENKES/169/2020 concerning the Designation of a Reference Hospital for Prevention of Certain Emerging Infection Diseases	March 10, 2020	Ministry of Health
Advice on the use of masks the community, during home care and in health care settings in the context of the novel coronavirus (2019-nCoV) outbreak	March 10, 2020	Ministry of Health
Risk communication and community engagement (RCCE) readiness and response to the 2019 novel coronavirus (2019-nCoV)	March 10, 2020	Ministry of Health
Decree of the Indonesia Minister of Health Number HK.01.07/MENKES/182/2020 about the Inspection Laboratory Network for Coronavirus Disease 2019 (COVID-19)	March 16, 2020	Ministry of Health
Decree of the Indonesia Minister of Health Number HK.02.01/MENKES/199/2020 about Handling Communication for Coronavirus Disease 2019 (COVID-19)	March 16, 2020	Ministry of Health
Decree of the Head of the National Disaster Management Agency Number 13.A Year 2020 concerning the Extension of the Status of Specific Emergency Disasters of the Corona Virus in Indonesia	March 16, 2020	Head of the National Disaster Management Agency
Circular of Head of Hajj Center Number: HJ.01.01/2/946/2020 about Spread Prevention Efforts Coronavirus Disease (COVID-19) To All Indonesian Hajj Pilgrims	March 18, 2020	Head of Hajj Center

Establishment of the Covid-19 Task Force by the Government of Indonesia:

In Indonesia, the response to disasters has been anticipated by forming ad hoc bodies such as:

1. The National Committee for Avian Flu Control and Pandemic Preparedness in Indonesia
2. BRR (Aceh-Nias Rehabilitation and Reconstruction Agency) after the 2004 Tsunami
3. Mitigation plans for example in DKI Jakarta Flood Disaster Mitigation Plan.

In its implementation, disaster management is generally included in the provisions of Law No. 24 of 2007 concerning Disaster Management with technical implementation through the National Disaster Management Agency in regulation No. 4 of 2008 concerning Guidelines for the Preparation of Disaster Management Plans, in the introduction stated that Indonesia's broad demographic and natural conditions tend to be affected by very high disasters and varying in types:

Indonesia, which consists of a group of islands, has a very high potential for disasters and also varies greatly in terms of types of disasters. These natural conditions and the diversity of population and culture in Indonesia create the risk of natural disasters, human-made disasters and complex emergencies, although on the other hand they are also rich in natural resources

The types of disasters that can arise are: In general, the risks of natural disasters include disasters caused by geological factors (earthquakes, tsunamis and volcanic eruptions), disasters caused by hydrometeorology (floods, landslides, drought, typhoon angina), disasters due to biological factors (outbreaks of human diseases, plant/livestock diseases, pests, pests plants) as well as technological failures (industrial accidents, transportation accidents, nuclear radiation, chemical pollution). Disasters caused by human activities are related to conflicts between people due to the struggle for limited resources, ideological, religious and political reasons. While complex emergencies are a combination of disaster situations in a conflict area.

In dealing with such disaster conditions, integrated and directed preparation is needed: The complexity of the disaster problem requires a careful arrangement or planning in its handling, so that it can be carried out in a directed and integrated manner. The countermeasures that have been carried out so far have not been based on systematic and planned steps, so that overlapping often occurs and there are even important measures that are not addressed.

In the implementation of disaster management, so that each activity in each stage can run in a directed direction, then a specific plan is prepared at each stage of disaster management.

At the stage of a disaster in a situation where a disaster does not occur, a Disaster Management Plan (Disaster Management Plan) is prepared, which is a general and comprehensive plan that covers all stages/ areas of disaster work. Specifically for certain disaster prevention and mitigation efforts there are plans called mitigation plans,

At the stage of a disaster in a situation where there is a potential for disaster, a preparedness plan is prepared to deal with an emergency based on a single hazard scenario (single hazard) then a plan called a Contingency Plan is prepared.

When an Emergency Response is carried out an Operational Plan which is the operationalization/ activation of the Emergency Plan or Contingency Plan that has been prepared previously.

During the Recovery Stage, a Recovery Plan is prepared, which includes rehabilitation and reconstruction plans carried out in the aftermath of a disaster. Meanwhile, if a disaster has not yet occurred, then in anticipation of future disasters the preparation of instructions/guidelines for the post-disaster management mechanism will be carried out.

The pandemic disaster management policy that was declared as a non-natural disaster was then followed up with a special team namely the Task Force created because certain conditions had occurred with the presence of COVID-19 transmission in Indonesia, which needs to be anticipated. In order to accelerate the handling of COVID-19, quick, precise, focused, integrated, and synergic steps are needed between ministries/agencies and local governments.

The legal standing of this team is Presidential Decree (Keppres) No. 7 of 2020 concerning the Task Force for the Acceleration of Corona Virus Handling. The Task Force for the Acceleration of Handling

COVID-19 is under and is responsible to the president. The Head of the National Disaster Management Agency Lieutenant General Doni Monardo was appointed as the Chairperson.

In accordance with the provisions of the Presidential Decree, the Task Force also aims to accelerate the handling of the corona virus through synergy between ministries/institutions and local governments.

The contents of Presidential Decree (Keppres) No. 7 of 2020 contains the Coordinating Minister for Law and Legal Affairs and the Minister of Health designated as Deputy Director, Minister of Finance as secretary with a number of Ministries/Institutions as directors. However, this Presidential Decree was later amended by the presence of KEPPRES Presidential Decree (Keppres) No. 9 of 2020 with the following composition:

KEPPRES Number 7 Year 2020	KEPPRES Number 9 of 2020
The steering committee consists of 27 elements which include: 19 ministers and institutional elements such as the Head of the National Intelligence Agency (BIN), the Head of BPOM, the Chief of Staff of the President, the Head of LKPP, the Commander of the TNI, the National Police Chief, the Head of the BPKP, and the Governor of Indonesia	**Steering member becomes 33 elements:** 33 ministers and institutional elements such as the Head of the National Intelligence Agency (BIN), the Head of BPOM, the Chief of Staff of the President, the Head of LKPP, the Commander of the TNI, the National Police Chief, the Head of the BPKP, and the Governor of Indonesia

Preparation of hospital mechanisms: The Ministry of Health's government regulations have indeed revised the guidelines for the prevention and control of the Corona or Covid-19 virus, including the addition of categories of asymptomatic people (OTG) categories. This term is certainly not many people know about it.

The term OTG is used to categorize those who are asymptomatic and who have a risk of contracting from people who are confirmed positive for Covid-19, but who have close contact.[20]

"Close contact is someone who has physical contact or is in a room/visit, within a radius of 1 meter with PDP (Patient under Supervision) or Covid-19 confirmed case, within 2 days before a symptom case arises and up to 14 days after a symptom case arises, "wrote the Ministry of Health in the document.

During this time the public only knows a variety of medical terms that are used to categorize patient status, such as people in monitoring (ODP) and patients under surveillance (PDP).

The three criteria for someone who is potentially an OTG due to close contact, namely:[21]

Health workers, who inspect, treat, deliver and clean the room in the case treatment area (Corona) without using personal protective equipment (PPE) according to the standard.

People who are in the same room with the case within 2 days before the case symptoms arise and up to 14 days after the cases symptoms occur.

People traveling together (radius of 1 meter) with any type of conveyance/vehicle in the 2 days before the case of symptoms develops and up to 14 days after cases of symptoms appear.

In the Regulation of the Minister of Defense of the Republic of Indonesia Number 39 Year 2014 Regarding Disaster Management at the Hospital of the Ministry of Defense and the Indonesian National Army it is stated that:[21]

The implementation of Disaster Management is a series of efforts that include the establishment of disaster risk policies, prevention activities, emergency response, and rehabilitation.

It is also regulated in Article 1 point 10 regarding:

Hospital Disaster Management Plan is a planning activity from the Hospital to deal with disaster events, both planning for disasters that occur inside the Hospital (Internal Hospital Disaster Plan) and Hospital planning in dealing with disasters that occur outside the Hospital (External Hospital Disaster

All accredited hospitals in Indonesia must have a disaster management plan for a hospital or Hospital

Disaster Plan (HDP). However, because the value for HDP is only 20 percent and can pass without making plans, it is not uncommon for hospitals to only make documents and not socialize them to all staff. Furthermore, hospitals often do not make disaster threats a culture in hospitals.[20] Therefore, in the event of an internal disaster or the arrival of an external victim, or sending a team to the disaster area or experiencing a natural disaster, the hospital experiences difficulties in HDP application.[20]

Said by Lucky Tjahjono[20]: Health crises arising from disasters require coordinated handling from various parties both cross-program and cross-sectoral related in handling health crises in hospitals due to disasters. By integrating and coordinating the handling of the health crisis starting from the efforts of prevention, mitigation, preparedness, emergency response to the recovery of the health crisis in the Hospital due to the disaster by the various parties involved, it is expected that the impacts arising from the disaster can be minimized.

In addition, hospitals must be able to apply the concept of planned disaster management. Where hospitals should follow the procedure mentioned in Minister of Health Regulation 75 of 2019 on Health Crisis Management, the aim[20] is to provide guidance to Health Crisis Management actors at the regional and national levels, so that a coordinated, planned, integrated, and comprehensive health crisis management system is established to provide protection to the community from threats, risks and impacts of health problems.

Minister of Health Regulation No. 75 of 2019 concerning Health Crisis Management in it regulates the implementation of Health Crisis Management at each stage of the Health Crisis namely pre-crisis health, emergency response and post-crisis health. Health Crisis is an event or series of events that result in fatalities, injuries/illnesses, displacement, and/or the presence of potential hazards that have an impact on public health that requires quick responses beyond normal habits and inadequate health capacity.[21]

Disaster situations make vulnerable groups such as pregnant women, infants, children and the elderly vulnerable to disease. In these circumstances the risk and transmission of the disease increases. The hospital has a key role in handling health especially with pandemic conditions. WHO states:[1]

Hospitals and other healthcare facilities play a critical role in national and local responses to emergencies, such as communicable disease epidemics

Hospital readiness is needed which leads to networking in the health sector[1]: To ensure that the hospital is at all times in a state of preparedness to participate fully, efficiently and effectively in the coordinated health-sector response to an emergency, such as a communicable disease epidemic

In the global disaster by Covid-19, it was stated that[21]: By observing regional outbreaks in the context of Italy's case growth, we can start to model and anticipate both what the ultimate capacity to provide care will be and when that capacity will be exceeded.

That in handling cases, observation and planning are needed in dealing with the problem in our respective countries. The problem often faced by hospitals is that they already have SK COVID-19 task handling units and their duties and functions, but do not yet have an internal communication channel and an external communication channel. For example, the logistics sector is already included in the ICS and has a clear function assignment, but when there is a problem of lack of PPE, the logistics sector does not know where to coordinate, whether to directly ask external parties or through the internal commander first. This communication flow is important to be understood by the ICS team to facilitate systematic coordination between fields (internal) and outside the team (external). Thus all information needs and reporting activities can be met properly.

Management in handling a pandemic should be[1]: To ensure that the hospital has established the mechanisms and procedures including those for more strategic all-hazards emergency risk assessment and specific epidemic event risk assessment, prevention, preparedness, response and recovery that are needed for overall coordination of the hospital's epidemic risk management activities.

In following management it will be able to take steps in handling more leverage. The concept of handling the COVID-19 pandemic is basically the same as the concept of disaster management. The difference is only in the basic principle of handling because of differences in the nature of the causative agent. It is time for the hospital to activate ICS which has been formed to handle the COVID-19 pandemic. ICS that is formed can be said to be running if it has fulfilled 3 functions, namely

what doing what (division of tasks), communication (communication channel), and what if (backup plan).

Wang CJ stated[21]: Taiwan have been successful at combating the virus through aggressive testing and containment measures. South Korea, while initially inundated with patients from a rapidly spreading cluster, has successfully mitigated spread (at least temporarily) with minimal associated mortality. These examples provide encouragement that a well-executed public health response can minimize the potential for operational crises. The common themes in success have been massive testing, adaptive policy recommendations for different regions, and communication by public officials that is constant, transparent, and honest. The public needs to trust public officials and see the good and bad news every day.

Joseph J. Cavallo emphasize that: Actions taken now by society and health care systems will determine whether history regards 2020 as a great public health achievement or an epic failure of our public health and health care infrastructure.

Eric Toner[22]: Because it will take considerable time to fully understand the epidemiology of COVID-19, it is reasonable to begin preparations using a model we have studied extensively for decades and that seems similar to COVID-19—pandemic influenza. The threat of a novel influenza pandemic has stimulated international, national, and local planning and preparedness efforts for years. In the event of a 1918-scale flu pandemic, hospitals would be flooded with sick patients seeking care.

Conclusion

In the study of data relating to regulations and policies on health and regarding outbreaks of disease (pandemic) there are legal instruments and their applications.

In the real risk of pandemic spread if legal interpretation is used and also see the legal regulation has become part of the legal protection itself and applies everywhere. As a country that upholds the welfare state, Indonesia in particular is inseparable from the protective ties to its people. By the way the law has stated that *Salus populi suprema lex*, which means that people's salvation is the highest law, then in a legitimate manner, society must be saved. In the study of normative-positive legal rules, there are many rules that regulate and mention disaster mitigation. Even though in the regulations and legal basis, it is not specifically mentioned for its type but has been included in the general classification that disasters include non-natural disasters that include plague and/or pandemic. Concerning the issue of pandemics being part of disaster mitigation has been decomposed from the highest regulations to the implementing regulations, which contain definitions to the operational and technical implementation. Indonesia has also included these rules in existing health policies (health stakeholders including hospitals as the spearhead of medicinal treatment). We realize this condition is not perfect, there are still shortcomings, especially for hospitals that must include later in the Hospital Emergency Management Plan During the COVID-19 Epidemic, for pandemic outbreaks other than dengue fever and malaria. Cross-sectorial cooperation in a pandemic will be very helpful and provide hope that the storm from this pandemic will end soon.

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The Analysis of Antenatal Care During Covid-19 Pandemics in The Working Area of Public Health Center of Tangerang City

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Abstract

Covid-19 spreads from one person to another person through close contact in the form of droplets to respiration track. The physiological changes that happen to the body system is primarily on respiration and immune system. Such changes give higher possibility on pregnant mothers to be infected by covid-19. In order to decrease the infection risk, national policy suggests to omit hospital visit which is not important and changes it into distance care or virtual visit using phone or video call. Quality Antenatal Care (ANC) service is a service provided to pregnant mothers according to the service standard. Quality service is formed from service quality dimension. This research was carried out using analytical survey through cross sectional approach. The research population was all pregnant mothers in the working area of Public Health Center of Tangerang City chosen through total sampling. The data were collected using questionnaire (google form). Research finding resulted that during Covid-19 pandemics, there was 72.2% of the subjects who obtained suitable Antenatal Care. The research concluded that service quality of ANC during Covid-19 pandemics was in accordance with the service guidelines established by the government.

Keywords: *Service Quality, Antenatal Care Scope, Covid 19, public health care center.*

Introduction

Data regarding the effect of Covid-19 on pregnant mothers is still limited. However, information regarding diseases related to Covid-19 indicates that children are rarely exposed to it (Chavez, Long, Koyfman, & Liang, 2020)[3]. In 2 reports issued, there were 18 assessments done on Covid-19 disease, in which all of them occurred during the third trimester. Fetal distress and premature birth also seen in several cases, the two assessments were cesarean delivery and there was no proof indicating infection on womb. (Meliala, 2020)[10].

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Physiology changes on immunity and respiratory system can cause pregnant mothers to be more susceptible to Covid-19 infection. Up to now, there is no effective vaccine found yet. Thus, it is suggested that pregnant mothers have to refrain from unnecessary travel, avoid crowd, public transportation and contact with sick people as well as always maintain personal and social cleanliness (Liang & Acharya, 2020)[8].

In order to decrease the risk of infection, pregnant mother who does not have any particular worries regarding her pregnancy does not need to seek for pregnancy care, instead they can find online material to support the pregnancy. As an example, a midwife has started a series of YouTube video to guide pregnant mothers in passing weeks before and after giving births (Furuta, M., & Furuta, M. 2020)[5]. It also includes maintaining social distancing as well as often washing hands using soap and running water for 20 seconds (Poon et al., 2020)[12].

Social distance is the best protection mechanism thus the visit for the second trimester through tele health is recommended to protect the patients and service provider health (Laupacis, 2020)[10].

Midwife needs to be contacted before visiting the clinic so that they will come without any guide, in this case the health provider needs to be informed to apply the right self-protection procedure (Boelig, Saccone, Bellussi, & Berghella, 2020)[2,5]. The planning of individual labor and team-based approach through multispecialty consultation is used (Rasmussen, Smulian, Lednicky, Wen, & Jamieson, 2020)[15].

The quality of ANC care can be seen based on the factors affecting its effectiveness, compliance and sustainability of the care (Nwaeze, Enabor, Oluwasola, & Aimakhu, 2013)[11,14]. Quality service is formed from the service quality dimensions including reliability, responsiveness, assurance, empathy and tangible (physical appearance of the service) (Yuwono, Hartati, & Winarko, 2019)[18].

During the covid-19 pandemics in Indonesia, the Antenatal Care provision in either hospital, public health center,or independent midwife practice must follow the guidelines established by the Ministry of Health of the Republic of Indonesia. The guideline explains that the pregnant mothers need to participate in pregnant mothers online class, pregnancy examination in the second trimester need to be done through tele communication or postponed expect there is dangerous symptoms, pregnancy examination is done through appointment so that they do not need to wait for long, health worker needs to wear protective equipment level 1 which are head cover, medical mask, face shield, handscoon, working clothes and shoes (Enfagrow & Care)[4].

Method

The current research was done through analytical survey of cross sectional approach. The population involved in this research was all pregnant mothers in the working area of Public Health Center of Tangerang City. The total of the samples was 115 pregnant mothers chosen through total sampling. The inclusion criteria proposed were pregnant mothers who were involved in the WhatsApp group during the data collection. This research was carried out in March 2020.

Result

Table 1. The Scope of Antenatal Care Provision

The Scope of ANC Provision	Frequency	Percentage %
Inappropriate	32	27.8
Appropriate	83	72/2
Total	**115**	**100**

Based on Table 1, it is indicated that among the 115 respondents, those who obtained inappropriate antenatal care was 32 respondents (27.8%), while the remaining 83 respondents (72.2%) obtained appropriate antenatal care.

Table 2. Quality of Antenatal Care Service

Quality of ANC Service	Frequency	Percentage %
Good	113	98.3
Poor	2	1.7
Total	**115**	**100**

Table 2 informs that among 115 respondents, those who considered the antenatal care service as good were 113 respondents (98.3%), while the other 2 respondents (1.7%) stated that the antenatal care service provided was poor.

Table 3. Service Quality on the Scope of Antenatal Care Provision

Service Quality	Scope of ANC Provision						p-value	OR
	Inappropriate		Appropriate		Total			
	f	%	f	%	f	%		
Tangible								
Inappropriate	3	9.4	7	8.4	10	100	0.872	1.123
Appropriate	29	90.6	76	91.6	105	100		

Service Quality	Scope of ANC Provision						p-value	OR
	Inappropriate		Appropriate		Total			
	f	%	f	%	f	%		
Reliability								
Inappropriate	1	3.1	6	7.2	7	100	0.409	0.414
Appropriate	31	96.9	77	92.8	108	100		
Responsiveness								
Inappropriate	8	25.0	2	2.4	10	100	0.000	13.500
Appropriate	24	75.0	81	97.6	105	100		
Assurance								
Inappropriate	4	12.5	1	1.2	5	100	0.008	11.713
Appropriate	28	87.5	82	98.8	110	100		
Empathy								
Inappropriate	3	9.4	1	1.2	4	100	0.32	8.483
Appropriate	29	94.6	82	98.8	111	100		

Table 4. The Provision of Antenatal Care (ANC) on Service Quality

The Scope of ANC Provision	Service Quality				Total		P
	Good		Poor				
	F	%	F	%	F	%	
Inappropriate	30	93.8	2	6.3	60	100	0.022
Appropriate	83	0.0	0	100	4	100	
Total	**113**	**98.3**	**2**	**1.7**	**115**	**100**	

Discussion

Based on the research result presented in Table 3, it indicates that among 115 respondents, those who obtained inappropriate antenatal care service were 32 respondents (27.8%).

Antenatal care is health service performed by professional worker for pregnant mothers during their pregnancy period, according to the minimum standard of antenatal service (Singh et al., 2019)[17].

According to the researchers' analysis, there were more respondents who considered the Antenatal Care service as appropriate, compared to those who considered as inappropriate. This is because the midwives have performed the service according to the standard established by the government during Covid-19 pandemics in which standard examination is still done (examination of body weight and blood pressure, measurement of upper arm circumference, high fundal examination of the uterus, examination of fetal heart rate and provision of vitamin of blood booster). However, there were also midwives who did not perform the examination in accordance with the scope of Antenatal Care service.

Based on the research result presented in Table 4

1. **Relationship between Tangible and Service Quality on the Scope of Antenatal Care Provision:** This research was in line with the research conducted by Firdausi in the working area of Kabila Public Health Center of Bone Balango District obtaining p value of 0.00< 0.05(Ramadhani, 2014)[14]. Consumers' satisfaction regarding a product depends on the direct proof obtained from the availability of equipment completeness, capacity of product quality and comfortable facility (Razak, 2019)[16].

The researchers assumed that pregnant mothers' good satisfaction on tangible dimension is related to the service provided by midwives that has been in

accordance with the standard of Antenatal Care service and Operational Standard of Service during Covid-19 pandemics. Midwives have provided service by giving distance in the waiting room and providing hand sanitizer to wash hands before entering the waiting room.

2. **The Relationship of Reliability and Service Quality on the Scope of Antenatal Care Provision:** Reliability is one of the dimensions which consist of uncomplicated procedure in accepting patients quickly, accurately and on time starting from the service to the treatment (Berliana & Amelia, 2018)[1].

The researchers analyzed that pregnant mothers considered the service given by the midwives as fast and accurate in responding to the pregnant mothers' need and complaint. Compliance and reliability of the midwives in meeting the service standard is one of the factors affecting the pregnant mothers' satisfaction on antenatal care service such as using the examination equipment as usual (weighting the body weight, measuring the blood pressure, examining the fetal heart rate and providing counselling). However, there were still many midwives who did not wear complete Personal Protective Equipment (PPE) during the pandemics due to the equipment which was difficult and expensive to obtain in the market.

3. **Relationship between Responsiveness and Service Quality on the Scope of Antenatal Care Provision:** Nurmawati (2010) stated that the pregnant mothers who considered the responsiveness dimention as in accordance with the scope of service werw 69 (86%) people, starting that good relationship between human will create good relationship (Indrayani & Sari, 2020)[6]. Fast service can be distinguished between the patients' service who where provided by special room in order to obtain fast service (Berliana & Amelia, 2018)[1].

According to the researchers' analysis, the high appropriateness of this dimension was affected by the fast response given, especially on the complaint experienced by the pregnant mothers and provision of information regarding Covid-19 prevention that must be done by pregnant mothers.

4. **Relationship between Assurance and Service Quality on the Scope of Antenatal Care Provision:** The current research result is in accordance with the result of research done by Firdausi (2014) in which as many as 71 respondents (89%) felt the availability of assurance, friendly environment, in this case is friendly service as the stage to improve the quality of public health facilities (Nair & Panda, 2011)[10,17]

The research also assumed that assurance is factor determining the service quality, because through the assurance, patients can feel safe during the examination and satisfied by the information provided as well as assured regarding the privacy by the polite service given, so that worries on Covid-19 infection can be overcome.

Relationship between Empathy and Service Quality on the Scope of Antenatal Care Provision

Current research is in line with the research performed by Yuniarti (2015) stating that there was relationship between empathy and patients' quality by p value of 0.000. This research also in accordance with research carried out by Nurani (2012) saying that by giving health counselling, screening and follow-up to pregnant mothers adjusts the visit time which is one of the actions to prevent Covid-19 (Qiao, 2020)[13].

Empathy given by the midwives during Covid-19 pandemics was analysed as good in which the midwives have given easiness for the patients in examining their pregnancy. Although the control schedule was made based on appointment, all of the examination can be implemented well.

Conclusion

Based on the research that has been done, it can be concluded that:

1. Based on the exposure of Antenatal Care, it indicated that 115 respondents have obtained appropriate Antenatal Care during Covid-19 pandemics by 72.2% of respondents.
2. Based on the service quality, among 115 respondents, it was stated that the five indicators have relationship with the service quality.
3. There was relationship between tangible, responsiveness, assurance and empathyon ANC service provision which is according to Covid-19 guidelines for pregnant mothers in Public Health Center of Tangerang City.

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The Effectiveness and Safety of Convalescent Plasma as a Therapy for COVID-19 Patients: A Literature Review

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Abstract

COVID-19 is a new disease caused by SARS-CoV-2 with an increasing number of cases. until now, no specific vaccine or antiviral therapy regimen has yet been found. A therapy that has been known for approximately a century ago called convalescent plasma therapy has been discussed again as an effective and safe therapy for COVID-19 patients. Conduct a literature review of scientific studies related to the effectiveness and safety of convalescent plasma as a therapy for COVID-19 patients. Convalescent plasma has proven to be effective because it can improve the clinical condition felt by patient, prevent the entry of viruses into cells and inhibit the virus replication. This therapy is also proven to have no harmful effects, so it is safe to be performed by paying attention to certain things such as conditions that must be fulfilled by the donors.

Keywords: *COVID-19, SARS-CoV-2, Convalescent Plasma, plasma, virus, therapy*

Introduction

In Wuhan (China), at the end of 2019, there was an outbreak of the corona virus that had never been identified in humans(1). WHO officially labeled this kind of virus as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the resulting disease as coronavirus disease (COVID-19)(2). Infected by COVID-19, a person will show a number of symptoms such as fever, cough, rapid breathing and difficulty breathing. In severe cases, it causes pneumonia, severe acute respiratory syndrome and the worst possibility, death(1).

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WHO affirmed that on May 10, 2020 there were 3,925,815 people worldwide infected by COVID-19 and 274,488 people reported dead(2). COVID-19 is currently a major concern throughout the world(3). The number of infected cases keeps increasing, but specific vaccines or antiviral therapy regimens have not been found yet (4–6). Treatment only focuses on supportive care in the form of oxygen administration, ventilation, fluid therapy, low-dose corticosteroid combination therapy, antivirals and atomized inhalation of interferon (4).

Convalescent plasma therapy has been utilized since a century ago and is now re-discussed as an effective therapy for COVID-19 patients(7,8). The convalescent plasma is a blood plasma obtained from a person who has been declared cured of the infection process and has antibodies against certain pathogens. This plasma can be transfused to patients with varying degrees of severity of infection as a relative treatment(8).

Convalescent plasma has been used in a variety of previous infectious diseases, such as influenza,

cytomegalovirus infections, Middle East Respiratory Syndrome (MERS-CoV), Severe Acute Respiratory Syndrome (SARS-Cov), H1N1 - H5N1 avian flu and Ebola[9–11]. The United States Food and Drug Administration (FDA) has now approved the use of convalescent plasma as a therapy for critical COVID-19 patients. However, the use of this therapy follows the usual system of new drug investigations[12].

Based on the aforementioned issues, the main focus of this paper is to conduct a review of previous studies related to the effectiveness and safety of convalescent plasma as a therapy in COVID-19 patients.

Method

Some international journal databases such as Pubmed, Proquest and the Indonesian Scientific Journal are sources of literature search conducted through Google Scholar. The literature search aims to find a lot of information related to convalescent plasma therapy and COVID-19. It is done using the keywords "convalescent plasma" and "COVID-19".

Results and Discussion

Efforts to create the SARS-CoV-2 vaccine and COVID-19 treatment have now been carried out by many scientific laboratories. However, referring to previous therapeutic experience of infectious diseases, a convalescent plasma therapy might be an effective therapeutic option in the treatment of COVID-19[6].

A good result of convalescent plasma therapy has been demonstrated by a study conducted at Wuhan Huoshenshan Hospital on 6 patients who were confirmed to be positive for COVID-19 with different clinical, laboratory and radiological symptoms. The study was carried out based on the results of a Reverse Transcription-Polymerase Chain Reaction (RT-PCR) throat swab in male and female patients with an age range of 28 - 75 years who had confirmed COVID-19. They had a variety of main conditions ranging from fever, shortness of breath, recurrent infections of COVID-19, to those with comorbidities with Sjogren's Syndrome. Convalescent therapy was given to each patient after more than 4 weeks from the initial complaint that appeared. The therapy was given 1-3 times with a dose of 200 ml for 30 minutes each time of transfusion. Evaluation results showed that this therapy could reduce the clinical symptoms felt by the patient, improve lung conditions evidenced by a gradual reduction in consolidation and ground glass opacity occured in the lungs, clear SARS-CoV-2 which could lead to a double increase in titers of IgM and IgG antibodies against SARS-CoV-2 in patients receiving the therapy[13].

Another study was conducted on 4 male and female patients who were critically ill due to SARS-Cov-2 infection with an age range of 31-73 years. Each patient had comorbidities including hypertension, chronic obstructive pulmonary disease (COPD), chronic kidney disease and pregnancy. Patients obtained treatment in the ICU, experienced acute respiratory distress syndrome (ARDS), respiratory failure, septic shock and dysfunction and even multiple organ failure. Each patient was given 1-8 times convalescent plasma therapy with a volume of 200-400 ml each. The results indicated that the convalescent plasma therapy had a good effect on the patients' condition. This therapy improved their clinical condition, radiological picture and laboratory picture by which they no longer needed therapy for COVID-19 management. The duration required between administration of convalescent plasma therapy with a negative RT-PCR throat swab was in the range of 3 to 22 days[14].

There was also a research conducted at Shenzhen Third People's Hospital in China on the use of convalescent plasma therapy for COVID-19 patients. The study was conducted in 5 critical patients who were characterized by the following conditions: respiratory failure requiring mechanical ventilation, shock identified by vasopressor therapy and elevated lactate levels (> 2 mmol/L) despite adequate fluid resuscitation, or organ failure. The amount of plasma given to each patient was two consecutive transfusions of 200 - 250 ml with a total plasma of 400 ml. After conducting the convalescent plasma therapy, the patients' clinical condition improved, which was indicated by a decrease in body temperature, an increase in PAO_2/FIO_2 and chest radiology results showed a gradual improvement. Laboratory results showed a decrease in the number of viruses; some viruses become negative and there was a decrease in the number of inflammatory biomarkers (C-reactive protein, procalcitonin and IL-6). Four patients who had received mechanical ventilation and ECMO no longer needed 9-day respiratory assistance after the plasma transfusion [15].

Similar study was also carried out in three different hospitals in Wuhan on 10 patients with severe COVID-19. Each patient received a 200 ml convalescent plasma

therapy which was transfused within 4 hours. This therapy had a good effect on COVID-19. All symptoms in these 10 patients, especially fever, cough, shortness of breath and chest pain disappeared or, in other words, most of their condition improved within 1 to 3 days after the transfusion was performed. Antibody titer against SARS-CoV-2 increased in 5 patients and had a constant level in 4 other patients. The amount of SARS-CoV-2 RNA in each patient decreased and was not detected after 2-6 days of convalescent plasma therapy[16].

In Korea, a study on the effectiveness of convalescent plasma therapy was also conducted in two COVID-19 patients with acute respiratory distress syndrome (ARDS). The study was conducted on days 7 and 22 after the initial appearance of symptoms in each patient. Positive effects were shown after the administration of the therapy including improvement of clinical conditions, chest X-ray results accompanied by an increase in PaO_2/FiO_2, reduction in the number of inflammatory biomarkers and SARS-CoV-2 RNA showing negative results on days 20 and 26 after administration of therapy[17].

In addition, a study conducted on 21 COVID-19 patients obtaining intensive care in Henan Province of China showed that 6 patients given 300 ml convalescent plasma therapy had viral clearance and a longer survival rate than patients without plasma therapy[18].

Based on the aforementioned results of studies, the convalescent plasma therapy was effective for COVID-19 patients. It has been mentioned in the literature that antibodies mediated by the humoral response are very important for preventing or treating viral infections. Some parts of the antibody reduce the amount of virus by binding the epitope to the external surface of the virus particle, thus it prevents the entry of the virus into the cell and also prevents the replication of the virus[19].

The research stated that no harmful effects appeared after the plasma therapy was carried out[16,20]. However, one of the patients showed reddish spots on the face that easily disappeared after getting the therapy[20]. To avoid the harmful effects of therapy, it must be ensured that the plasma donor has completely healed. Here are some conditions that have to be fulfilled by the donors: 1) Free of fever for at least three days; 2) Reduction of respiratory symptoms; 3) Negative SARS-CoV-2 RT-PCR in two consecutive tests (one day interval between tests); 4) Anti-SARS-CoV-2 is positive, while HIV, HBV, HCV and other respiratory viruses are negative; and 5) Plasma should be taken after three weeks of disease onset[13,15,20].

Conclusion

This study established a conclusion based on the results of the research that had been carried out in various places with clinical symptoms, the results of radiological examinations and the results of various laboratory examinations. It concluded that therapy using convalescent plasma was proven effective since it could minimize the clinical symptoms felt by patients, prevent the entry of viruses into cells and inhibit viral replication. This therapy was also proven to have no harmful effects by which it was safe to do by paying attention to certain things such as conditions that needed to be fulfilled by donors.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Indonesia.

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Clinical Characteristic of Covid-19 Infection in Pediatric: A Systematic Review

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Abstract

SARS-CoV-2 is a new type of corona virus that has been identified as a cause of COVID-19 cases. This disease is transmitted quickly, causes a global pandemic and can affect all ages, including children. In children, cases are still rare and hard to be found. Because the prevalence of events in children tends to be smaller than in adults, information about the clinical characteristics of COVID-19 infection in children is still lacking. The aim of this research is to find out the clinical characteristics of COVID-19 infections in children. Conducting a literature review of various scientific studies related to the clinical characteristics of COVID-19 infection in children. The most common clinical symptoms found in pediatric patients with COVID-19 infection are coughing, fever and gastrointestinal symptoms where these symptoms usually tend to be mild to moderate and often the symptoms are not specific or asymptomatic. Laboratory results are generally normal but the CT-Scan results show suspicion towards pneumonia in the majority cases of children.

Keywords: *Clinical characteristics, COVID-19, Children.*

Introduction

A new type of corona virus has been identified as the cause of a group of pneumonia cases detected in Wuhan City in early 2020. WHO officially named this disease as coronavirus 2019 (COVID-19) and the International Coronavirus Study Group (CSG) decided to name the virus SARS-CoV-2.[1]

At 26 April 2020, there were 2,804,796 total confirmed positive cases worldwide with 193,710 fatal cases (6.90%). Based on this, there are studies that use data based on a certain time span. The study took data from 149,760 laboratory confirmed cases in the United States from 12 February to 02 April 2020. The cases were analyzed based on known age. The results obtained were among 149,082 (99.6%) cases, 2,572 (1.7%) were children aged <18 years.[2,3]

Based on the most recent case reports, 90% of the 2,143 children diagnosed through laboratory examinations or clinical symptoms of COVID-19 infection are reported to be asymptomatic, mild or moderate. The remaining 5.2% are heavily infected and 0.6% are in critical condition. Children and adolescents infected with SARS-CoV-2 generally only experience mild symptoms and radiological manifestations. Children rarely experience severe cases of infection or are in a critical condition.[4,5]

Therefore, based on these data it can be said that cases of COVID-19 infection among children are still quite rare and symptoms tend to be milder or absent,

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so this article review needs to be done to increase anticipation regarding the clinical characteristics of COVID-19 infection in children.

Method

The method used in compiling this article review is use research journals related to COVID-19 infection in children. The journals used in the study of literature are obtained through a database of international journal providers Elsevier and the Scientific Journal of Indonesia through Google Scholar. Journal searches are performed using the keywords "*COVID-19*" and "*child*".

Result and Discussion

Coronavirus (CoVs) is a group of RNA viruses with a single, encapsulated strain. This virus can mutate quickly and zoonoses (risk of spreading from animals to humans). One example of this virus is SARS-CoV which appeared in 2002 and causes severe acute respiratory syndrome. SARS-CoV-2 which currently appears is a new type of corona virus causing COVID-19 outbreaks in China and various other countries, causing a global pandemic.[6]

This virus spreads through droplets when infected patients cough, talk loudly or sneeze. Contact with the mouth, nose or conjunctiva of the eye through contaminated hands is also a source of transmission. Close contact with patients infected with SARS-CoV-2 is one of the ways in which infections occur in children. In addition, it has been reported that SARS-CoV-2 can also be transmitted via aerosols, the fecal-oral route and from mother to baby. This virus can still live in feces after a few weeks leading to transmission of oral-stool. Thus, because live virus strains can be isolated from feces, this indicates the potential for infection from bodily waste.[7,8,9,10]

Although this virus spreads very quickly, the cases in children are much lower compared to adults. One reason that may cause cases of COVID-19 in children tends to be lower because children have less outdoor activity and do less international travel so that the chance of contacting the virus is smaller.[11,(12)]

According to national guidelines, the diagnosis of COVID-19 is confirmed using RT-PCR with specimens taken from nasopharyngeal swabs and detection of lesions by chest CT-Scan. Intake of nasopharyngeal swabs are performed on all patients, including children.[13]

The severity of COVID-19 is classified based on clinical symptoms, laboratory testing and chest X-ray imaging, including asymptomatic infections, mild, moderate, severe and critical cases. The diagnostic criteria are as follows:

1. **Asymptomatic:** Without clinical symptoms and signs, chest imaging is normal while the 2019-nCoV nucleic acid test shows positive results.
2. **Mild:** Symptoms of acute upper respiratory tract infections including fever, fatigue, myalgia, cough, sore throat, runny nose and sneezing. In some cases there is no fever, but often there are gastrointestinal symptoms
3. **Medium:** With pneumonia, fever and cough are common, mostly dry cough followed by productive cough. Some may have wheezing and wet or dry crackle. Some cases may not have clinical signs and symptoms, but chest CT shows subclinical lung lesions.
4. **Severe:** Early respiratory symptoms such as fever and cough, can be accompanied by gastrointestinal symptoms such as diarrhea. Dyspnea occurs by central cyanosis and decrease in oxygen saturation is followed by other manifestations of hypoxia
5. **Critical:** Children can quickly develop into acute respiratory distress syndrome (ARDS) or respiratory failure and may also experience shock, myocardial injury or heart failure, acute kidney injury, encephalopathy and coagulation dysfunction. Organ dysfunction can be life threatening.[14,(15)]

Based on the results of the Chang TH et al study, analyzes conducted in all age groups of children (neonates-adolescents) showed the majority of patients had mild to moderate infections (98%) with more than half (59%) patients who had symptoms of fever and 46% had symptoms cough. Only 12% of patients have gastrointestinal symptoms. About 26% of patients have no specific symptoms. On the results of laboratory tests, the Chang TH et al study showed lymphopenia in 32% of cases. Some children (9%) even experience severe lymphopenia. Radiological examination results on 3 children showed consolidation and ground glass opacity. In 27% of cases there were no lesions in the lungs.

Chen J et al results showed that among the 12 pediatric patients infected with COVID-19, the main symptom most commonly experienced was coughing (75%) followed by fever (58.3%) and then 4 patients

reported having diarrhea (33.3 %). On laboratory examination, most patients (11/12) had normal white blood cell levels and 2 patients had lymphopenia. Dynamic changes from the laboratory that included WBC, the results of leucosyte and platelet counts occurred in 3 patients. Data collected from days 8-14 of the 3 patients showed normal laboratory results.

In the study of Xiaoxia L et al, the most common complaint experienced by pediatric patients at the Children's Hospital of Wuhan was coughing (48.5%). After that followed by pharyngeal erythema (46.2%) then fever with a range of 37.5oC (41.5%). From the results obtained, 32.1% of pediatric patients suffer from fever above 38oC with the majority temperature ranges ranging from 38.1 oC - 39 oC. Other symptoms were diarrhea (8.8%), fatigue (7.6%), rhinorrhea (7.6%) and vomiting (6.4%). In addition, 4 out of 171 children (2.3%) are known to have decreased oxygen saturation to below 92% and 26.7% of children have tachypnea. 42.1% of children also reported experiencing tachycardia while 27 other children (15.8%) were reported asymptomatic. Investigations showed that the CT scan results of 56 patients (32.7%) showed ground glass opacity and local spot images in 32 people (18.7%). Bilateral shading was also seen in 21 children (12.3%) and interstitial abnormalities occurred in two children (1.2%).

In the study of Su et al found that among 9 pediatric patients, 2 children had a fever in the range of 37.4-38.5 oC (22.2%) and 1 child had a cough (11.2%) and 6 others did not symptomatic (66.7%). In addition, based on the results of laboratory tests, 8/9 (88.9%) children have normal levels of white blood cells or decreased levels of white blood cells that are consistent with the characteristics of viral infections. Six children (66.7%) showed an increase in CKMB. ALT, AST and liver and kidney tests are within normal limits. The results of radiological examination found bronchitis in two children (22.2%) and one person with bronchial pneumonia (11.1%). One boy who was older than his twin showed pulmonary consolidation and ground glass opacity on the first day of hospitalization but the lesions disappeared after five days of treatment. In five other children (55.6%) no abnormal radiological features were found.

In the study of Sun et al at the Children's Hospital of Wuhan in pediatric patients with severe COVID-19 infection, the most common symptom was polypneu (8/8), followed by fever (6/8), cough (6/8)), phlegm (4/8), diarrhea (3/8), muscle fatigue/pain (1/8), headache (1/8) and constipation (1/8). The results of pulmonary auscultation examination showed the presence of rales in the left or right lobe in 5 patients (5/8) while in 3 other people there were rales (3/8). For the CT scan results showed there were six patients with bilateral pneumonia and two patients with unilateral pneumonia. CT scan images also include many features such as spotting (7/8), *ground glass opacity* (6/8), pleural effusion (1/8) and *white-lung like image*s (1/8). Patients with severe COVID-19 infection, septic shock and multiple organ dysfunction syndrome are the most common complications in critically ill patients.

In the study of Du et al several differences were found in clinical features between adult patients and pediatric patients. The findings show that the most common clinical symptoms in children are fever and dry cough but when compared with adults, these symptoms tend to be less common in children. In addition, other reports show that a decrease in lymphocyte counts is one of the more common clinical features. But in pediatric patients, there is no significant reduction in lymphocyte levels. On the other hand, the number of neutrophils in pediatric patients actually decreases so that it can be stated that lymphocyte levels cannot be an indicator of diagnosis of pediatric cases.

In the study of Du et al, also found that an increase in LDH is more common in pediatric patients than adult patients so that children are more likely to suffer heart muscle damage. In contrast, elevated levels of CRP and IL-6 are seen in adults, but not in children. For the results of chest X-ray examination, the majority of children and adult patients found abnormalities in the form of pneumonia and often bilateral. However, pneumonia in adult patients is more severe than in children. Even among patients who are not asymptomatic, 62.5% of cases have pneumonia. Therefore, lung damage caused by SARS-CoV-2 remains relatively clear.

In this study it was suggested that the pathogenesis of SARS-CoV-2 is closely related to the interaction of viruses with angiotensin-converting enzyme 2 (ACE2) cell surface receptors. The number of ACE2 receptors in children is lower than in adults so this is likely the main reason why children experience fewer symptoms and organ dysfunction. In short it can be said that most cases of COVID-19 infection in children are classified as mild.

Management for adult patients and children is

generally not much different. However, in severe but non-emergency cases, administering oxygen to younger pediatric patients is recommended using nasal prong (nasal prong) or nasal cannula to make it more acceptable. In addition, in adult patients with a critical condition (septic shock), norpinephrine is considered a first-line treatment. Epinephrine or vasopressin can be given afterwards to reach the MAP target. Whereas in pediatric patients, epinephrine is considered as a first-line treatment while norepinephrine is only additional if shock still occurs even though the dose of epinephrine given is optimal.[22,23]

Conclusion

The conclusions of the many journals that have been reviewed as intended are to find out the clinical characteristics of COVID-19 infection in pediatrics. The authors conclude that the clinical characteristics of pediatric patients with COVID-19 infection include clinical symptoms and examination results. The most common clinical symptoms found in pediatric patients with COVID-19 infection are coughing, fever and gastrointestinal symptoms where these symptoms usually tend to be mild to moderate and often the symptoms are not specific or asymptomatic. For the investigation itself, it is generally obtained the results of a normal leukocyte level, but the results of the CT-scan of the majority of pediatric patients show spots, consolidation, ground glass opacity which indicates suspicion toward pneumonia.

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Tobacco Smoke and Pregnancy Outcome: Literature Review

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Abstract

Pregnant women have a risk for tobacco smoke both actively and passively. Various effects of tobacco smoke on pregnancy have been investigated. Pregnant women are usually exposed to enviromental tobacco smoke (ETS) in various places with different duration of time. Cigarette smoke has a severe effect not only on pregnant women but also on the fetus. This is a literature review. Various references were collected from online database including reports, journals, mostly in the last 10 years. The journals were mostly from the scholarly journals. The articles were screened according to the research objectives. The keywords used are tobacco smoke, environmental tobacco smoke and pregnancy outcome. Tobacco smoke can effect the immunity, hormonal and metabolic system of the pregnant woman, therefore disturbing the growth of the fetus. Tobacco smoke also known to increase the risk of premature birth and reduce gestational age. Tobacco smoke cause several problems in newborn such as respiration distress, low birth weight (LBW), neural disorder, sudden infant death syndrome (SIDS) and congenital anomaly.

The effect of tobacco smoke, actively of passively, have worse outcome for the pregnant women and their babies.

Keyword: *Pregnancy Outcome, tobacco smoke, ETS, LBW, preterm birth.*

Introduction

Smoking pregnant women have an increased risk of having an ectopic pregnancy, spontaneous abortion and other complications leading to pregnancy complications as well as the placenta. The fetus also has the risk of exposure to tobacco smoke, more than thousands of types of hazardous chemicals, especially nicotine, tar and carbon monoxide can have an effect on the fetus and cause unwanted disorders or abnormalities[1].

Environmental tobacco smoke (ETS) is a complex mixture consisting of most of the smoke emitted from the smoker's body, the smoke produced by burning cigarettes and the surrounding air[2].Exposure of ETS in pregnant women causes increased levels of carbon monoxyde(CO), nicotine and cotinin in maternal serum or urine, in the fetus and in amniotic fluid. The effect of ETS on pregnant women can occur from the first semester to the third semester. Pregnant women are usually exposed to ETS in various places with different duration of time. Places that have the potential to become ETS exposure locations include at home, at work and the outside environment [3.]

The effect of exposure to cigarette smoke on secondary smokers has not been widely studied in developing countries even though the prevalence of smokers is higher in developing countries than in developed countries. The increasing number of smokers in the world causes ETS exposure to secondary smokers become a concern for the global health. Environmental conditions such as population density make the ETS effect on health increased. Research in developing countries has found that ETS exposure in pregnant women is associated with poor pregnancy outcomes[3].

Tobacco smoke and pregnancy: One effect of the cigarette smoke exposure on pregnant women is its effect on the mother's immune system. In pregnancy changes occur in the mother's immune system to prevent rejection of the fetus. Exposure to ETS can cause changes in the immune system of pregnant women. Changes include an increase in activated leukocytes and a decrease in the percentage of regulator T lymphocyte cells (Treg cells). Smoking during pregnancy also affects the function balance between Th1 cells (T helper lymphocytes) and Th2 cells, causing an increase in the production of cytokines, proinflammatory chemokines and Th1 growth factors. In addition, the percentage of macrophages and NK cell residues is higher in smokers in the first semester [4]

Smoking is also associated with physiological changes in hormones during pregnancy. In smoking mothers there are a decrease of estrogen, globulin bindingsex hormone and human chorionic gonadotropin (hCG) compared to non-smokers. Nicotine as the main component of cigarettes has a substantial impact on the hormones of pregnant women, nicotine causes excessive stimulation of the acetylcholine receptors, releasing vasoactive catecholamines. The activation of nicotine receptors causes the release of acetylcholine, dopamine, serotonin, growth hormones and adrenocorticotropic hormones and glutamate, these hormones significantly influence fetal growth. Smoking is also associated with changes in metabolism during pregnancy. Some reports say that there is a strong relationship between smoking and the incidence of diabetes mellitus and disruption of insulin sensitivity, which can also have an effect on fetal metabolism[4].

Tobacco smoke exposure can also effect the fetus. Exposure to nicotine in pregnant women can cause vascular placental vasoconstriction, decrease placental blood flow and reduce trophoblast invasion which results in inhibition of good placental circularization leading to placental hypoxia causes disruption of placental invasion. Placenta previa is a form of placental invasion disorder[4]. Nicotine can cause a significant decrease in the mitotic potential of cytotrophoblast tissue in vitro. This effect is also found in smoker women. This effect can explain the mechanism of impaired placental development during the early phases of pregnancy due to placental ischemia which can cause fetal death[5.] Low placental weight strongly related with LBW [6].

Beside nicotine and cotinin, CO levels are also the found highest in smokers. The presence of CO in the body causes oxygen binding to hemoglobin decrease due to the higher affinity of hemoglobin for CO. CO Exposure tothe fetus prevents the release of oxygen and then converted to carboxyhemoglobin. The result is a decrease in tissue oxygenation through competitive inhibition with oxyhemoglobin. Prolonged CO exposure in pregnant women can cause significant permanent damage to the brain of a fetus which sensitive to hypoxia. Nicotine is also considered to have a bad effect due to the stimulation of nicotine cholinergic receptors and their neuroteratogenicity effects.[4,7,8]Kadmiun, the other components found in cigarettes are embryotoxic and tetarogenic in animals. Increased cadmium levels in maternal and placental blood are inversely correlate to zinc levels in placental cord blood, suggesting that cadmium interferes with zinc transfer to the fetus and causes impaired fetal growth.[4]Nicotine also interferes with the micro RNA which is important for the maturation of fetal stem cells.[9]

The effects of tobacco smok on the fetus are considered to be multifactorial, including indirect effects such as poor nutritional status associated with anorexiagenic effects of nicotine exposure and CO. Placental blood flow decrease is associated with the vasoconstrictive effect of catecholamines released from adrenals and nerve cells after nicotine exposure. The direct effects of nicotine on the nicotinic acetylcholine receptors that occur during the formation of the fetal brain are also factors that may contribute.[10]

Tobacco Smoke and Preterm Birth: The rate of preterm birth is quite high around the world.In Europe rates of preterm births are reported between 5 - 9%, while in developing countries and the United States the rate of preterm births reaches 12%. The cause of preterm birth is often difficult to determine. Some risk factors associated with preterm birth include excessive distension of the uterus, low economic status and smoking[11].

Smoking is known as a risk of spontaneous and elective preterm birth, but has a stronger relationship with spontaneous preterm birth. Passive smoking in pregnancy also has a risk of preterm birth both spontaneous and elective 5. Elective preterm birth may be associated with cigarette-related obstetric complications such as placenta previa, placental abruption and impaired fetal growth. Research shows that smoking during pregnancy

increases the risk of preterm birth by 25% [11]. The exposure of ETS also specifically has an influence on preterm birth[12,13].

Active smoker mothers have been accepted as a risk for preterm birth, for around 14% of preterm births. One mechanism that links cigarette exposure with preterm birth is the CYP1A1 genotype and GST (glutathione S-transferase). Abnormalities in these genes make mothers more vulnerable to exposure to hazardous substances such as cigarette smoke.[14] Cotinin levels as anindicator of cigarette exposure are also strongly related to preterm birth.[4] The danger of non-smoking tobacco is also seen in the research of Munmun et al. Which shows that non-smoking tobacco (chewed and swallowed) increases the risk of preterm birth.[15]

There are four mechanisms proposed by Goldstein et al in 1964 to explain the relationship between smoking and preterm birth: (1) Decreased maternal appetite caused by smoking leading to a decrease in nutrition for the fetus, (2) vasoconstriction caused by smoking results in decreased blood supply to the fetus, reduced fetal nutritional supply and slowing the release of catabolism results, 3) cigarettes may have a direct effect of toxins on the fetus and 4) an increase in fetal CO levels causes reduced oxygen transport capacity and teratogenic properties.[11,16]

The mechanism of smoking effect on preterm birth is currently believed to be likely caused by various effects that play a simultaneous role. CO levels in cigarettes are about 4% of the volume, after dilution of air in elveoli CO levels in smokers are 400-500 ppm. A room filled with cigarette smoke, CO levels can reach 100 ppm, causing the risk of CO exposure to non-smokers in the room. In blood CO is bound to hemoglobin and forms carboxyhemoglobin, because its affinity is about 200 times of oxygen, hemoglobin binds stronger with CO than oxygen. The binding of CO to hemoglobin causes the oxygen affinity of hemoglobin to increase, so that the remaining oxygen bound to hemoglobin is difficult to release. Carbon monoxide can cross the placental blood barrier. Carboxyhemoglobin levels in the fetus are usually higher than the levels in the mother. Increased levels of carboxyhemoglobin in the fetus cause fetal hypoxia. With decreased oxygen delivery to cells, CO also has an effect on the intercellular process by interfering with the work of cytochrome enzymes.[11]

Research on rhesus monkeys shows that nicotine administration does not cause changes in oxygen partial pressure so that the fetus also does not experience changes in oxygen pressure. Fetal hypoxia in this study was caused by decreased placental blood flow. Other studies on sheep have also shown that nicotine causes an increase in catecholamines and decreases uterine blood flow. Although data from studies in monkeys and sheep show that nicotine decreases uterine blood flow, research data in humans are very limited.[11]

Tobacco smoke can also increase the risk of preterm rupture of placental membranes through several mechanisms, 1) tobacco smoking decreases immunity and becomes a predisposing factor for infection, 2) tobacco smoking decreases copper levels and ascorbic acid in the blood,leading to a decrease in the elasticity of the placental membranes and increase the risk of rupture. Both of these mechanisms have the potential to cause preterm birth[11]

Prostagladin has been known to trigger labor. Prostaglandin levels such as F2-isoprostane as a marker of oxidative stress are found in the amniotic membrane and amniotic fluid in smokers. F2-isoprostane levels increased 3 times compared to non-smokers. Increased F2-isoprostane levels is considered asa mechanism that associated smoking and preterm birth. Smoking is also considered to increase the sensitivity of the uterus to the contractile hormone. Research by Egawa et al in mice shows that inhalation of cigarette smoke increases the contractile activity and sensitivity of myometrium to oxytosis.[11,17]Cadmium found in tobacco smoke interacts with calcium and effects myometrial activity. Cadmium may modulate the function of the oxytocin receptors in the myometrium. Increased levels of cadmium in pregnant women are found to be associated with an increased risk of preterm birth.[11,18]Another condition associated with preterm birth is necrotizing-enterocolitis (NEC). The study of Ding et al in smoking pregnant women showed an increased risk of infant death by NEC in the preterm newborn. Death in neonates by NEC is difficult to prevent with a mortality rate of 15-20%.[19]

Spontanious Abortion: Spontaneous abortion or miscarriage is one of the most common pregnancy complications, about 12-26% of known pregnancies. Generally the literature on the relationship between smoking and abortion does not show any consistency although some studies have concluded that smoking can cause abortion. A meta-analysis study by Pineles et al. Showed the relative risk of abortion during pregnancy

is 1.32. The risk of abortion increases with the number of cigarettes. One cigarette per day increases the risk of abortion by 1%. Secondary smokers have an 11% increased risk of abortion.[20]

Tobacco Smoke Effect on Newborn: Various studies provide an overview of the effects of cigarette smoke on fetal growth and development during pregnancy and infant growth and development after birth

Respiratory Disorder: Based on several studies children who are exposed to cigarette smoke can experience lung growth disorders and are more prone to lung infections and asthma. Exposure to cigarette smoke in pregnant women can also affect the newborn.[1]Smoking during pregnancy can cause the newborn's lungs failed to reach maximum function and continues with decreased lung function. Preterm births in most smokers also cause disruption of lung maturation. Diseases caused by these infants include wheezing, bronchitis, hospitalization due to lung infections and asthma in children.[21]

Generally, children who are exposed to nicotine during pregnancy also experience exposure to cigarette smoke in childhood. This raises the question whether the increased risk of asthma is caused by exposure to prenatal or post natal cigarette smoke. Research by Pattenden et al showed that exposure to cigarette smoke during pregnancy without post natal exposure still shows an association with an increased risk of asthma.[22,23]

Immune factors are also considered to play a role in impaired lung development in fetuses exposed to cigarette smoke. In animal models, there is a change in immune cell phenotype after exposure to cigarette smoke/nicotine. Wang's study et al showed a decrease in Th1 cytokine production after pre-natal and post-natal cigarette smoke exposure associated with increased Th1 immune cell recruitment to the lung parenchyma. By adjusting for risk factors for infection, secondary smokers have an immune system which tends to Th2 leading to an allergic phenotype, small airway inflammation and asthma-like symptoms. Exposure to cigarette smoke pre natal and post natal also shows an imbalance of Th1/Th2 causes susceptibility of airway reactivation.[24]

The mechanism of exposure to nicotine causes various pathological conditions in the lungs is not yet fully understood, but based on some data it was found that nicotine causes various anomalies in the lungs. Histological studies in animals show goblet cell hypertrophy after nicotine exposure and it is associated with asthma in the community.[22,25,26]

Low Birth Weight: Low birth weight babies are the variables most widely studied the effect of smoking mothers during pregnancy or exposure to cigarette smoke in pregnant women on pregnancy outcomes. Data shows that LBW is more common in smokers (12.4%) compared to nonsmokers (7.7%). Women who smoke have a 1.5 to 3.5 times greater risk of giving birth to LBW and the risk increases along with the cigarette consumption.[1]

The physical environment has an important role in determining the weight of babies born and their health in the future. Research in Jordan shows that exposure to ETS in non-smokers pregnant women causes an increase in LBW. Increased ETS exposure increases the risk of LBW infants. All ETS exposure in the home, office and outside environment has the potential to reduce the weight of newborn. It was also found that second and third semester exposures were the most vulnerable time of exposure causing LBW. LBW infants tend to be more at risk for neurological problems including cerebral palsy, seizures, severe mental retardation, respiratory diseases and other morbidity.[3]

It has been ascertained which component of cigarettes affects the flow of uterine blood and primarily affects the birth weight of the baby.[4] Lee et al study showed that exposure to ETS in pregnant women also has the potential to cause LBW and the effect of smoking on LBW from active smokers has the same mechanism as the effect of smoking on LBW in secondary smokers. The risk of small infants according to pregnancy is also increased in the group of mothers with high ETS exposure compared with mothers with low ETS exposure[27]

Behaviour and Neural Disorder: Nicotine exposure to the fetus can affect fetal brain development [10]. A study in Finland shows that heavy nicotine exposure causedthe infant born with attention deficit/ hyperactivity disorder (AHDH5 or attention deficit/ hyperactive disorders.[28]Fetuses exposed to tobacco smoke during pregnancy ware found to have a risk of cardiac autonomic abnormalities during sleep especially in preterm babies. Changes in autonomic activity are at risk of causing neurological and cardiological complications[29]

Research on nicotine exposure to lung development in the fetus is mostly done in animals. Perinatal nicotine exposure in mice causes an increase of airway reactivity.

Nicotine also causes an increase in smooth muscle volume in the distal bronchi and hyperreactive airway in sheep.[21]

Sudden infant death syndrome (SIDS): One of the most severe complications of nicotine exposure in pregnancy is sudden infant death syndrome or SIDS. The cause of SIDS is not clearly known in infants less than one year old suddenly die without definite explanation even after a thorough investigation. Exposure to nicotine is known to be a risk factor for SIDS, but the mechanism of nicotine exposure that causes infant death is unclear and is still being debated. But some animal studies provide some clues.[22,30,31]

Research by Cohen et al in rats shows that nicotine exposure causes disruption of catecholamine synthesis in newborn mice. The study also showed that the mice exposed to prenatal nicotine had impaired response to induced hypoxia. Other studies have shown that mice exposed to nicotine during pregnancy have a risk of death after exposed to hypoxic conditions and have disruption of catecholamine release from adrenal medulla. Human and mouse responses to hypoxia include the release of catecholamines from the adrenal medulla to maintain cardiovascular function. Disorders of catecholamine release in neonates exposed to nicotine during pregnancy may cause an increased risk of SIDS.[22,32]

Congenital Anomaly: The effects of tobacco smoke on infanstcongenital anomalies have been investigated. Exposure to tobacco smoke is known to be associated with fetal heart defects including atrial septal defects, atrioventricular septal defects, transposition of large arteries, craniosynostotic cleft palate and gastroschisis.[4,33,34] Exposure to ETS in pregnancy could also affects the abnormalities of the fetus. Research by Hoyt et al. shows that exposure to mothers as secondary smokers is associated with anencephaly and craniorachischisis, spina bifida, palatoschisis (cleft palate), labioschisis (cleft lip) with and without palatoschisis, agenesis or bilateral renal hypoplasia.[35]

Other studies have also shown an association of ETS exposure in pregnancy with anorectal atresia,[36]neural tube defect,[37]and limb abnormalities [38]Meta-analysis study by Salmasi et al on ETS exposed mothers showed a slight increase risk to have a smaller babies, have a congenital anomaly and tends to have smaller head[39]

Conclusion

Tobacco smoke effect the immunity, hormonal and metabolic system in pregnant women. The substance found in tobacco smoke were found to be the cause of some pregnancy complication leading to poor pregnancy outcome such as preterm birth, spontaneous abortion and adverse effect on tha infants. The infants could suffer respiratory disorder, behavior and neural disorder, SIDS and another congenital anomaly. The effect of tobacco smoke, actively of passively, associated with poor outcome for the pregnant women and their babies.

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Correlation Between Oxygen Saturation, Uric Acid and Creatinine Level to Maternal Outcome of a Woman with Severe Preeclampsia

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Abstract

The aims of the research is to measurecorrelation between oxygen saturation, uric acid and creatinin level to maternal outcomes of a woman with severe preeclampsia. The research is observational with cross sectional cross design. The amount of samples is counted by using categorical correlative analysis uncoupled and the sample taking is using total sampling technique which collected 135 women sample with severe preeclampsia in Wahidin Sudirohusodo General Hospital Makassar. The data is analyzed by using SPSS 21. The result of this research shows oxygen saturation (p=0.021) the uric acid has (p=0.006) association with complication on severe preeclampsia. Oxygen saturation (p=0.000) and uric acid (p=0.028) also has the association with the mortality on severe preeclampsia. Creatinine has no relation with the complication (p= 0.56) and mortality (p=0.358) on severe preeclampsia. The logistic regression analysis shows that oxygen saturation (OR=5.443; p=0.016) and uric acid (OR=4.333; p=0.004)has the relation with the complication on severe saturation. This research concludes that oxygen saturation and uric acid has relation with the complication and mortality on severe preeclampsia.

Keywords: *Severe Preeclampsia, Oxygen Saturation, Creatinine, Uric Acid.*

Introduction

The number of maternal death in Indonesia is still very high, which is 305 per 100,000 life birth in 2015 based on the data showed by Indonesia Republic Minister of Health. This findings is still far from the target planned in the 5th Millenium Development Goals (MDGs) for 2015 which is 102 per 100.000 life birth (Wibowo dkk, 2016)[1].

Until today, preeclampsia and eclampsia still become Obstetric service issue and the mother and baby mortality beside bleeding and infection. The number of preeclampsia case is around 5-10% of the whole pregnancy cases with each country gaining different number of it. In 2015 the World Health Organization (WHO) estimated 303,000 maternal death in the whole wide world. According to the MDGs, the number of maternal death is decreasing 43% since 1990 (Primadi dkk, 2015). World Health Organization estimates that preeclampsia cases are seven times higher in developing countries compare to ones in developer countries. The preeclampsia prevalence in developer countries is 1.3%-6%, while in developing countries is 1.8%-18%. There are 128.273 and around 5,3% preeclampsia incidents in Indonesia and there has not been any indication of the incidents decreasing in the past two decades (Wibowo dkk, 2016; Primadi dkk, 2015). The data is compiled by fetomaternal Division of Wahidin Sudirohusodo General Hospital Makassar, which shows there were 175 severe preeclampsia cases in 2016.

There are several indicators to notify maternal outcomes complication on preeclampsia, which are from oxygen saturation, uric acid and creatinine. <93% oxygen saturation is one of the high risk factors of maternal complication from respiration and non respiration system (Alexandra et al., 2011).

The purpose of this research is to rate the association

of oxygen saturation, uric acid and creatinine level in maternal outcomes of patients with severe preeclampsia.

Methodology

The research is and observational research and cross sectional design. The amount of samples is counted by using categorical correlative analysis uncoupled and the sample taking is using total sampling technique which collected 135 women sample. The samples are pregnant women with severe preeclampsia diagnosis admitted at Dr. Wahidin Sudirohusodo General Hospital Makassar during the period of the research with included and excluded criteria. The included criteria in this research are pregnant women diagnosed with severe preeclampsia who are willing to take part in the research. The excluded criteria are subject with complication before oxygen saturation, uric acid and creatinine test and have history of kidney disorders, cardiopulmonary and metabolic disease. The procedure in the this research is by taking blood samples in order to examine the uric acid level and the creatinine in the laboratory and also to check the oxygen saturation by using pulse oxymetry attaching informed consent signed by the subjects. The data analysis consists of descriptive analysis and hypothesis test. In descriptive analysis, nominal and ordinal data is stated in frequency and percentage distribution. The hypothesis test is using Chi square for free and bound categorical scale. To identify the confounding variable on the outcomes, Chi square test is performed to indicates the rate of the risk. The amount of influence is stated based on the magnitude of risk which is know as Relative Risk (RR) for bivariate analysis. The rate of RR > 1 and the range of trust is not 1, which means the variable is considered to be risk factor. The boundary limits is when $p < 0.5$ with the interval of trust as 95%. The data analysis is conducted by using SPSS program for Windows.

Results

From the research, there are 135 women samples with severe preeclampsia in Dr. Wahidin Sudirohusodo General Hospital Makassar. Some common characteristic are researched suc as gravidity, educational background, occupation, age, gestational age and maternal outcomes. Table 1 shows there no significant differences between the groups of primigravida and multigravida and their relation to mortality and morbidity on severe preeclampsia with percentage 42,2% and 57,8%.

Most of the research correspondents have low educational background (65,2%). Most respondents of the research have steady job, 75 people (55,6%). This reasearch shows that no correlation between occupation incidents on severe preeclampsia.

Most of the respondents of the research are diagnosed with severe preeclampsia at the gestational age of <34 weeks, 85 people (63%). The severe preeclampsia incidents in this research tend to happens to the high risk age group, with the percentage of 59,3%, 80 people.

Table 1. Characteristics of Research Subjects

Characteristics	n	%
Gravidity		
Primigravida	57	42.2
Multigravida	78	57.8
Working Women		
Well Educated	47	34.8
Lower Educational Background	88	65.2
Occupation		
Employed	75	55.6
Unemployed	60	44.4
Gestational Age		
<34 weeks	85	63.0
>34 weeks	50	37.0
Age		
Low risk	55	40.7
High risk	80	59.3
Total	135	100

Table 2. Correlation between oxygen saturation, uric acid and creatinine towards complication incident of severe preeclampsia

Variable	Complication	Without Complication	p
SpO_2			
Normal	18 (78.3%)	106 (94.6%)	0.021*
Abnormal	5 (21.7%)	6 (5.4%)	

Variable	Complication	Without Complication	p
Creatinine			
Normal	10 (43.5%)	59 (52.7%)	0.565**
Abnormal	13 (56.5%)	53 (47.3%)	
Uric Acid			
Normal	7 (30.4%)	72 (64.3%)	0.006**
Abnormal	16 (69.6%)	40 (35.7%)	

*Fisher's Exact Test, **Continuity Correction

Table 2 shows correlation between SpO2, creatinine, uric acid and the existence of any complication what so ever to patients with severe preeclampsia. The level of SpO2 indicates significant association of complication on severe preeclampsia with the rate of p=0.021. The creatinine level does not show any significant association on the complication incident to severe preeclampsia with the rate of p=0.565. On the other hand, uric acid shows significant association with the complication on severe preeclampsia. Severe preeclampsia complication tends to happens to respondents with abnormal level of uric acid (69,6%). This indicates that uric acid has significant association to severe preeclampsia complication incident (p=0.006).

Table 3. Correlation between oxygen saturation level, creatinine, uric acid, to severe preeclampsia mortality

Variable	Life	Died	p
SpO_2			
Normal	124 (94.7%)	0 (0%)	0.000*
Abnormal	7 (5.3%)	4 (100%)	
Creatinine			
Normal	68 (51.9%)	1 (25,0%)	0.358*
Abnormal	63 (48.1%)	3 (75,0%)	
Uric Acid			
Normal	79 (60.3%)	0 (0%)	0.028*
Abnormal	52 (39.7%)	4 (100%)	

*Fisher's Exact Test

The data in table 3 shows correlation between the level of SpO2, creatinine and uric acid towards the incident of mortality to severe preeclampsia patients. The severe preeclampsia patients who finally died had abnormal SpO2 level. On a contrary, only 7 respondents (5%) with severe preeclampsia who survive have abnormal SpO2 level. Correlation between SpO2 level with mortality incident on severe preeclampsia shows significant relation with the rate of p>0.05 (p=0.000).

Creatinine shows no significant association on severe preeclampsia mortality (p=0.358). There are 68 People (51,9%) who survive from the group of severe preeclampsia with normal creatinine level. That number does not show much difference from the group severe preeclampsia who survive with abnormal creatinine level, which is 48,1%. The uric acid has significant relation with mortality incident on severe preeclampsia with the rate of p=0.028. All the death case over severe preeclampsia have abnormal uric acid level. The surviving severe preeclampsia patients dominantly have abnormal uric acid level (60,3%).

Table 4. The effect of oxygen saturation and uric acid towards severe preeclampsia morbidity

Variable	p*	OR
SpO_2	0.016	5.443
Uric Acid	0.004	4.333

*Logistic regression analysis

Table 4 show the logistic regression analysis from the oxygen saturation and uric acid level variable to predict the complication on severe preeclampsia cases. The data in the Table shows the level of SpO2 (OR=5.443; p=0.016) and uric acid (OR=4.333; p=0.004) has significant predictive rate over severe preeclampsia case.

Table 5. The effect of oxygen saturation and uric acid towards severe preeclampsia mortality

Variable	p	OR
SpO_2	0.995	0.000
Asam Urat	0.996	0.000

*Logistic regression analysis

The data in table 5 shows the effect between oxygen saturation and uric acid towards the incident of mortality to severe preeclampsia case. The data also shows that oxygen saturation and uric acid have no significant effects towards severe preeclampsia mortality (p=0.995).

The aims of this research is to identify the association of SpO2, creatinine and uric acid towards the incident of complication on severe preeclampsia patients.

Primigravida is one of the risk factors on preeclampsia cases. It suspected that the case may show because the incompetency of the immune system during first pregnancy between maternal and fetoplacenta tissue (Grum et al., 2017). Our research has shown that there is no significant difference between the groups of primigravida and multigravida with the incidents of morbidity and mortality on severe preeclampsia cases.

Luo et al (2007), in his systematic study shows that the primipara has 2,4 times tendency to experience severe preeclampsia compare to the ones in multipara group. This is different from the study that stated severe preeclampsia cases are more to be found in multipara. Immune system maldaptation suspected as a factor taht may contribute to the high incidence of preeclampsia in primigarvida (Luo,Z. C., *et. al*, 2007)

This research also shows that from 135 severe preeclampsia samples, only 4 people (3,0%) of them died. Curiel-Belsara et al (2011) in the research shows that the rate of mortality on severe preeclampsia reaches 1,5% and tend to happens to multipara. This also indicates that severe preeclampsia has lover mortality number but has more complication cases (17%) (Curiel-Belsara et al., 2011).

Early onset preeclampsia is the condition where the preeclampsia incident happens on the 34th week of gestational age. Late onset preeclampsia is one of the terminologies used when the preeclampsia develops after the 34th week of gestational age (Raymond & Peterson, 2011). Wojtowicz et al (2019), in their research show that early onset preeclampsia tend to experience more severe complication compare to late onset preeclampsia. In our research, we have come across different statement, where are no significant differences between early onset preeclampsia and late onset preeclampsia towards severe morbidity and mortality incident.

Sari et al (2019), in their research shows that severe preeclampsia tend to happens more on the high risk age group. Grum et al (2017), shows that there are no significant differences between age and severe preeclampsia incidents. It is similar to the research we have conducted where most of the severe preeclampsia patients come from high risk group, which is < 20 years of age or > 35 years of age. Von Dadelszen et al (2011), in their research show that there no relation between age with complication incidents on preeclampsia cases. The result is similar to our research, where there are no significant differences found between the age group and mortality and morbidity happens to severe preeclampsia cases.

The oxygen saturation in the research shows that there is significant association that triggers the complication on severe preeclampsia (p=0.021). This suits to the research conducted by Millman et al (2011), that stated that oxygen saturation is able to act as main preeclampsia predictor if it is combined with the accompaniment complaints such as difficult to breathe or chest pain (AUC ROC 0.73; 95% CI 0.67-0.78). Von Dadelszen et al (2011), shows that level of SpO2 <90% also acts as predictor on severe preeclampsia complication.

Creatinine level is one of the parameters used to measure the renal function on patients with severe preeclampsia. Vyakaranam et al (2015), in the research shows that creatinine with 80% sensitivity and 77,4% to measure the renal damage that happens to preeclampsia patients by using 0,7mg/dl limitation. The creatinine level on patients with severe preeclampsia have higher average rate, which is 0.94 mg/dl ± 0.26 SD. Our research has failed to indicates the significant relation between the level of creatinine and the complication incident on severe preeclampsia cases. This is probably caused by the Method od study.

Manjareeka & Nanda (2012), in their research shows that the level pf uric acid on patients with severe preeclampsia have higher rate compare to the normal group. The research shows that the level of uric acid has no relation with the increase blood pressure of preeclampsia patients. On a contrary, the level of uric acid can work as the reflection of kidney function and as free radical scavenging which can be more protective on preeclampsia patients. The research that we have conducted shows higher level of uric acid found on severe preeclampsia patients with complications. This may also support the previous researches which explains that uric acid may has protective role in damaging the organ on severe preeclampsia.

At first, uric acid might decrease at the first trimester with the rate of 3 mg/dl, this happens for the uricosuria effect from the estrogen and the increase of renal blood stream. Uric acid level will be higher related to worse maternal and fetal outcomes. The average uric acid rate on severe preeclampsia patients is 7.88 ± 3.11 mg/dl higher compare to normal group (Kumar & Singh, 2019). The research suits the research we have done where most of the severe preeclampsia patients have complications (69,6%) and die (100%) have abnormal uric acid level.

Conclusion

Based on the result and the discussion of the research, it can be concludes that oxygen saturation and uric acid level have significant correlation with severe preeclampsia mortality and morbidity, while creatinine level has no significant correlation on severe preeclampsia mortality and morbidity incidents. Oxygen saturation and level of uric acid are together have significant effect on the morbidity incident. But it shows no significant effect on severe preeclampsia mortality incidents.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Indonesia.

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Marriage Tradition of Indigenious Community of Baduy and The Covid-19 Prevention

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Abstract

Baduy is a group of people who are still primitive, because they still survive with the principles of a long life full of local wisdom, including in conducting marriage rituals. However, the problem with marital procedures is contrary to positive law in Indonesia. In addition, the marriage ceremony conducted by the Baduy indigenous people involves many people which is also contrary to government policies regarding social distancing in handling the Covid-19 outbreak. The purpose of this study is (1) to analyze the marital status carried out by the Baduy community in positive law in Indonesia, (2) to analyze the efforts of the Baduy community to prevent the spread of covid-19 in the midst of procuring a marriage ritual that involves many people. The method in this paper uses normative juridical research and uses three sources of legal material, namely primary, secondary and tertiary. Data analysis was performed descriptively qualitative. The results showed that (1) the legality of the marriage of the Baduy community certainly had no legality and this marriage was not recognized by the state, (2) the marriage ceremony of the Baduy community could still be carried out as usual, bearing in mind that this community was very closed with the outside world long before the Covid-19 pandemic, but this clearly cannot be applied in outside Baduy communities which incidentally still open access for outsiders to visit

Keywords: *Baduy, Covid-19,Indegenious People, Marriage, Tradition.*

Introduction

Baduy indigenous people are a group of people who adhere to their customs, rituals and religion. Baduy community name is actually urang kenekes. They are an Indonesian people who live in the Kendeng Mountains in Lebak Regency, Banten, West Java. In their belief, Baduy people first worshiped supernatural beings, that is, spirits, supernatural spirits that were considered to be the ancestors of the givers of life and death. That spirit is what animates everything. As the sole holder of power called Batara Tunggal. The place where the supernatural creature lives is near the springs of the Ciujung River and the Cisemet River. The shrine is worshiped and is called the Arca Domas. This place of worship is now strictly forbidden to outsiders.

Baduy community trust which is very thick towards spirits makes them have their own traditional ceremonies. The traditional ceremony is used in a variety of memorials and activities, including in marital activities. Usually in the traditional ceremony, the Palawari on the women's side by Ambu, the Baduy tribe calls for the mothers to wear black Kemben traditional clothes and bring rice cone. Then, gather in one of the houses of the people to do rituals and prayers led by Pu'un or the king of Baduy Dalam. Pu'un is the supreme leader of adat as well as adat and religious government. Not only that, after the ritual, the customary leaders or leaders of the Baduy tribe gather in a bale or hall to perform the ratification of the surrogate pair and are led by Pu'un (https://cnnbanten.id : 1). The traditional marriage ritual carried out by the Baduy community is sacred and involves many people.

The ritual has become a natural thing and a normal routine conducted by the Baduy people. However, the problem now is that Indonesia is facing a Covid-19 pandemic outbreak, which in order to overcome the outbreak the government issued a policy on social distancing that must be obeyed by all people to avoid the spread of Covid-19. This regulation clearly contradicts the traditional rituals performed by the Baduy tribe, which involve many people and cause crowds.

Marriage is basically legal, if done according to each religion or belief. Legitimate marriage if done according to State law according to Article 2 (1) of Law no. 1/1974. The intended religious and religious law is not only the law found in the book, but also all the legal provisions (which are still valid for each group). Both of which have preceded this National marriage law. (see Article 66).

The marriage is generally based on mutual liking, but there are marriages that although the bride and groom already like each other, but the marriage is carried out with no outright or underhand marriage or marriages siri or marriage siri, namely marriages that are conducted based on religious or customary rules customs and are not recorded at the marriage registrar's office, not recorded at the KUA for those who are Muslim or in the Civil Registry Office (KCS) for those who are not Muslim. The term siri comes from the Arabic sirran, israr which means secret. Siri marriages, according to the meaning of the word, marriages that are carried out in secret or secret (Yunus, 2009 : 34).

Likewise what happens to the customary law of Baduy people, where a marriage is valid if an orderly procedure has been carried out according to the law of belief, the marriage has been considered valid according to customary law and in the marriage there are traditional rituals that must be carried out by both partners. Customary marriage rituals carried out by Baduy people are based on the beliefs they profess, that is to spirits or supernatural spirits, which beliefs have not been recognized in Indonesian law because they conflict with the provisions of the Almighty God.

Hazairin argues that customary law is not statutory law although as a law it has received temporary recognition in the transitional rules of Article II of the 1945 Constitution. Legislation is always in written form (written law), whereas customary law is not written law. So what is the fate of marriage law according to customary law if it has to do with religious or religious law. Provisions of customary law that conflict with the provisions of religious law or with statutory provisions in the field of religious law. The religion in question is any religion or belief in the belief in the Almighty, if he has legal provisions in the field of marriage. The beliefs that are still held in small groups here and there lie outside the scope of national marriage laws should enter into religion before they do marriages according to the 1974 national marriage law (Zarin, 2014 : 24).

The registration of marriage is affirmed by the state in legislation (Article 2 paragraph 2 of the UUP), which aims to benefit citizens. Because with a siri marriage for men who are not responsible, it will be easy not to do what is their duty to his wife and children, easily getting divorced at will.

As one of the consequences of a legal marriage will result in legal consequences, such as the rights and obligations of husband and wife, marital property, mutual relations between parents and children (nasab), child care obligations (hadhanah) and inheritance. One result of a legal marriage, a child born from such marriage is a legitimate child, has a perfect civil relationship with both parents, as stipulated in Article 42 of the UUP Article 99 of the Presidential Instruction. Number 1 of 1991 concerning Compilation of Islamic Law. Civil relations in question include the right of nasab (lineage) of children connected to the father (in Islam), the right of fulfillment of the livelihood of parents to children, the right of care and education (hadhanah), the right to inherit each other, the right of marriage guardianship for fathers over daughters and other civil rights.

Then the problem in this paper is related to the legal status of marriages committed by the Baduy community. This is because in conducting marriages, Baduy people use their own customary law and are not familiar with the registration of marriage in the office of the marriage registrar, both KUA and KCS. For this reason, the first problem statement raised was how is marriage in the Baduy indigenous community when viewed in positive law in Indonesia? In addition, in conducting marriages, Baduy indigenous people perform traditional rituals or ceremonies that involve large numbers of people and cause crowds, which is against the social distancing policy that is being intensified by the government in its efforts to deal with the spread of the Covid-19 virus. So what about the efforts of the Baduy indigenous people in preventing the spread of Covid-19 in the midst of procuring a marriage ritual that involves many people

Research Method

The type of research used is normative juridical research (legal research) or library law research, namely research conducted by examining mere library materials or secondary data (Soekanto & Mamudji, 2011 : 3).This research was conducted by reviewing cases based on applicable laws and regulations and based on other secondary data obtained.

Result and Discussion

Baduy Indigenous Marriage Rituals: Marriage is a natural law that must occur and be carried out by every human being without exception. This is a perception of the people in the Baduy. A remote tribe located in the foothills of the Kendeng mountains, Kanekes village, Leuwidamar District, Rangkasbitung Regency, Banten has its own unique wedding culture. The Baduy marriage system is the Monogamy marriage. Which means that a Baduy man cannot have more than one wife and a polygamy marriage is a taboo subject. Marriage of the first son (sister) of one lineage to the last daughter (sister) of another lineage. A younger sibling cannot have a marriage before his brother has a marriage (ngarunghal). Furthermore, in practice, there is no difference between Bedouin cousins and cousins between cousins so that there is a tendency for marriages to occur in the closest family, which can occur up to fourth level cousins. In Baduy,they called it, baraya (Badruzaman, 2020 : 35)[2].

In a marriage in Baduy is a process that is not a joke among Baduy residents, because after marriage the married couple inevitably have to be able to meet their own needs. Therefore, before the customary marriage takes place there is a series of traditional processes that must be undertaken by the bridegroom. The process before the marriage consists of bobogohan (the introduction of a mate), marriage proposal and marriage contract.

Bobogohan is a time of introduction between men and women who will get married or married. Men visit women, their future wife. However, the arrival of this man must not be alone. He must come with his male friends. Because in Baduy, unmarried men and women cannot be seen together. In addition, men must help their future in-laws work in the fields. The woman's parents will assess the work of her future daughter-in-law, if it is appropriate to accompany her daughter later. In Baduy, new families must support themselves by working in the fields. However, not all prospective brides undergo bobogohan. Children who are arranged for marriage are often notified and brought together on the day of the wedding ceremony. In the belief of Baduy indigenous people, children cannot refuse arranged marriages by their parents, because they will accept the law of karma. The atmosphere of the Bobogohan program is usually accompanied by a musical instrument playing that is carried by the men. The Baduy call marriage as a harmonious pillar, meaning that marriage must be done, because if not then he will violate his nature as a human being. After the agreement of the two parties to get married, then the application stage was conducted.

The next stage is applications that go through three processes, namely applications submitted from male families to female families. The first application was submitted to express the desire to propose a daughter (expressing the seriousness of the man to the woman). After eight months, the second application was submitted. The second proposal is proof of the seriousness of the family of a man marrying a daughter of the family.After five months, a third application is submitted and if marriage is approved, it can be held immediately (Halwany, 2020 : 3)[4].

All three applications must be passed by every Baduy resident who will hold a marriage, especially in Inner Baduy. For Outer Baduy, the number of applications can be less than three times. During this application period, the male proposal may still be rejected.

After all the processes are gone through, a wedding ceremony is held that can only be held in the fifth month, kanem, katujuh. This calendar is based on pikukuh. Pikukuh which means rules and teachings that must be carried out by the Baduy community, these rules regulate what is permissible and what is prohibited in the Baduy tribe which has been outlined by the ancestors of the Baduy community.

In general, the bride and groom's wedding procession will say the shahada (like the consent of the Kabul), witnessed by Naib as the leader. The registration of marriage by KUA did not apply in Baduy, it was hindered by the belief they believed in. In Baduy, a marriage ceremony and a reception for the bride and groom were held at the Balai Adat, led by Pu'un to legalize the marriage. In the provisions of the marriage system the Baduy people do not recognize polygamy and divorce. They are only allowed to remarry if one of them has died (Halwany, 2020 : 4).

The Baduy people have always held fast to all the provisions and rules set by Pu'un (Head of Custom). Adherence to these provisions is an absolute guide to living life together. In addition, driven by strong conviction, almost the entire Baduy Outer and Inner Baduy people have never opposed or rejected the rules applied by the Pu'un. By living life according to the customs and rules set by the Head of Indigenous people

there, a community with a very peaceful and prosperous society is created. Their lives are essentially the same as other people's lives. The only difference is that there are so many traditional rules that they must obey.

- **Marriage of Baduy Indigenous Peoples Judging from Positive Law:** The Baduy community is a tribe that has a very strong principle. We can see this in the marriage of the Baduy community. Baduy people are very strong in regulating marriages. For the Baduy community, marriage is a natural law that must be implemented, which is usually referred to by Baduy Marriage as a "harmonious pillar", namely that marriage must be carried out so that they do not violate their nature as humans.

Marriage as mentioned in Law No. 1 of 1974 in Article 1 Paragraph 2 which reads "marriage is a bond of birth and heart between a man and a woman as husband and wife with the aim of forming a happy and eternal family based on a Godhead. In carrying out a marriage, the most important thing that must be fulfilled is the harmony and marriage conditions. Pillars of marriage are part of the nature of sustainability. If one of the Pillars is not fulfilled, then the marriage is invalid alias canceled. The condition of marriage is something that must exist when marriage takes place (Asnawi, 2004 : 50)[1].

Aside from the Pillars and Marriage Terms, another more important thing is related to marriage administration. Because the administration of this marriage has an impact on the recording of the marriage. Recorded marriage is a form of written evidence of marriage. Regarding the provisions on marriage registration have been regulated in Article 2 of Law no. 1/1974 which states:

1. Marriage is legal, if it is done according to the law of each religion and its beliefs.
2. Each marriage is recorded according to applicable laws and regulations

Marriage registration is an administration activity of marriages conducted by Marriage Registration Employees (PPN) domiciled in the Office of Religious Affairs (KUA) within the scope of the territory of the two brides' candidates to hold marriages that are Muslim and in the Civil Registry Office (KCS) for non-Islamrecorded according to applicable laws and regulations (Mardani, 2016)[5,3].

The importance of marriage registration is very influential for family life, community life and state life. So that marriages that are not registered can have a negative impact, namely the marriage does not have any legal force in protecting the rights and fulfillment of the obligations of each party, both husband and wife (Mardani, 2016)[5]

If in the future a violation is committed by one of the parties, the injured party cannot claim any legal rights. Actors who are absent from their obligations, legally do not have an obligation to account for what has been done to their partners. Because the bond established in the marriage is not in accordance with the provisions of the marriage law in force in Indonesia and the marriage is considered illegal before the law. Therefore, marriages that are carried out without being registered and registered by the Registrar of Marriage, then the marriage has the potential to cause harm and neglect of obligations in the marriage bond.

As we know, the Baduy people adhere to the Sundanese wiwitan religion. This Sundanese wiwitan belief is a belief in the worship of the forces of nature and ancestral spirits (animism and dynamism) held by traditional societies. Every Baduy must have a Sundanese religion. However, as for those who are Muslim, it is a Baduy community that converted to Islam.

Regarding the legality of the marriage of the Baduy people, of course there is no legality and this marriage is not recognized by the state, because this marriage is only carried out in front of the headman which is carried out based on adat. This marriage was not registered at the civil registry office in accordance with the provisions on marriage registration as regulated in Article 2 of Law 1/1974 and Law Number.22 year 1946 jo Law Number 32 year 1954.

- **Baduy Community Efforts in Preventing the Spread of Covid-19 in the Middle of Marriage Procurement that Involves Many People:** The Baduy Dalam tribe in Lebak Regency, Banten, has their own way to ward off the spread of the corona virus or Covid-19. Even with this method, it has been proven that no Baduy people have been infected by the deadly virus. Baduy Traditional Elder who is also the Head of Kanekes Village, Leuwidamar District, Lebak Regency, Jaro Saija, stated that in order to ward off the corona virus, his party carried out the Kawalu ritual for approximately 3 months(Puji & Setyo : 2020)[6].

To prevent transmission, during the ritual the residents are prohibited from going outside the area, especially Jakarta, Tangerang, Bekasi, Depok and Bogor which have now been designated as red zones. While those who are overseas are asked to go home immediately. Those who return to their hometowns are first required to check their health at the local health center. In addition, the promotion of clean living in order to avoid the Corona virus in accordance with instructions from the Health Office and traditional rules in Baduy.

In addition, efforts are being made to temporarily close traditional tourism objects in Baduy, Kanekes Village, Lewidamar District. The decision was the result of deliberations with traditional institutions, the Village Consultative Body (BPD) and representatives of handicraft entrepreneurs. This is expected to make the Covid-19 virus not spread to baduy tribal communities, both inside and outside (Soekanto, Soerjono & Sri Mamudji. 2011)[7].

Related to the traditional Baduy tribal wedding ceremony that involved many people in the midst of the pandemic of the 19th plague, this should have received attention. Previously we separated first between the Baduy tribe and the outside Baduy tribe. According to the opinion of the writer, the marriage ceremony in the Baduy tribe is no problem if it is still held as usual. This is because, the indigenous Baduy tribe is indeed very closed and only government visits are allowed to enter the area. In addition, people are also not permitted to go outside the area. In addition, the ritual ritual of kawula has also been held.(Yunus, M. 2009)[8]

However, the condition is different from the baduy tribe community outside, where this area can still be visited by outsiders even though they have to go through a series of health procedures first. In baduy society outside it would be nice if the marriage ceremony for the time being did not need to involve many people, as would be done in the tradition of the Baduy. This was done to break the spread of the Covid-19 virus in the Baduy community itself.**(**Zarin. 2014**)**[10]

Conclusion

Baduy indigenous marriages are conducted traditionally and based on the belief they believe makes the marriage is not recorded at the office of the registrar of marriage, both KUA and KCS. This is indeed legally customary, but for the legality issue of the marriage of the Baduy people there is certainly no legality and this marriage is not recognized by the state.

Furthermore, for the problem of the marriage ceremony of the Baduy indigenous people which involves many people, it should be addressed wisely. In dealing with this problem, it is first separated between the outer Baduy tribe and the inner Baduy tribe. In the Baduy tribe in the marriage ceremony in the Baduy tribe there is no problem if it is still carried out as usual. This is because, the Baduy indigenous tribe very closed so there is no circulation of people in and out. On the other hand, outside Baduy tribal communities cannot be applied as such because these communities still open outside community visits. Customary marriage ceremonies to outside Baduy tribal communities should be limited in terms of the number of people as will be applied in the Seba Baduy tradition.

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Overcoming of COVID-19 Transmission: The Fulfillment of Right to Health and Education

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Abstract

The spread and transmission of the virus is very fast and widespread has become a concern of all people in the world to prevent the transmission and overcoming of COVID-19. This paper aims to analyze the legal aspects in overcoming COVID-19 transmission through the application of learning from home, both for schools and colleges. The research is a normative-legal research with statute, case and conceptual approaches. The results show that the attention of the government is more focused on economic and health issues and does not provide a balanced portion of education. Implementation of schooling or lectures from home is currently only regulated in a Ministerial Circular so that it is only a suggestion. Therefore, in order to be able to realize the protection and fulfillment of the right to education during Covid-19 pandemic, the government should issue legislation that becomes a reference for education providers and for the community so that their right to education is still granted under any circumstances.

Keywords: *COVID-19; Constitutional Rights; Right to Health; Right to Education.*

Introduction

Protection of the citizen's rights in a rule of law is a must. The fulfillment of this right is an obligation and responsibility of the State to protect and fulfill rights in all situations and conditions.[1] Looking to case law regarding civil commitment, many scholars and some lower courts have concluded that isolation and quarantine are constitutional only when the government can show by clear and compelling evidence that they are the least restrictive means of protecting the public's health.[2] But one this condition that faced by the world including Indonesia is pandemic Corona Virus Disease 2019 or known as Covid-19. The spread and transmission of the virus is very fast and widespread has become a concern of all people in the world and every country taking rapid steps in order to prevent the transmission and overcoming of Covid-19.

Until now, after 4 months since the first-transmission was announced by the President of the Republic of Indonesia, the curve of increasing the number of positive or reactive viruses continues to grow even in some areas experiencing a very significant increase. Then, prevention of covid-19 transmission was addressed by the government with the issuance of various regulations on all aspects of community life even in some areas that have imposed large-scale social restrictions (hereinafter abbreviated as PSBB – *Pembatasan Sosial Skala Besar*) although the effectiveness of this step cannot yet be said to be effective in the prevention and overcoming of Covid-19.

The aspect of community life that is strongly affected by the spread of Covid-19 is education.[2] Since March, schools and campuses have closed so that the entire learning process is done online or online-learning. Certainly, this is a new thing for most Indonesian people, especially among people who have not or rarely come into contact with technology and informatics. Various complaints from the public regarding the implementation of education from home (school/online-lectures) were not uncommon, ranging from difficulties in understanding learning method to the difficulties of funding related to this system.

The government policy to implement learning from home is carried out in the presence of a circular from the Ministry of Education and followed up by each local government. Online learning solutions is one of the government efforts to provide protection and fulfillment of the community right to health, but on the other hand, various difficulties

faced by the community related to the complexity and additional costs because online learning is the duty of the State to take solutions and attitudes that can provide a balance between the protection and fulfillment of the right to health and education.[3]

Beginning in June 2020, when it began to spread the discourse to re-open education and stopping online-learning system, protests from the community also arose because of fears if schools and campuses became new clusters in the transmission of Covid-19. However, on the other hand there were also circles people who have started to not feel at home with online-learning because it causes various complications and difficulties.

The government's policy to implement learning from home is carried out in the presence of a circular from the Ministry of Education and followed up by each local government. Online learning solutions is one of the government efforts to provide protection and fulfillment of the community' right to health, but on the other hand, various difficulties faced by the community related to the complexity and additional costs because online learning is the duty of the state to take solutions and attitudes that can provide a balance between the protection and fulfillment of the right to health with the right to education. This is interesting for the author to do a study related to legal aspects in overcoming Covid-19 transmission through the application of learning from home both for schools and colleges.

Method

The research is a normative-legal research conducted by examining library materials or secondary data consisting of primary, secondary and tertiary legal materials. The materials are arranged systematically, reviewed then drawn a conclusion in relation to the problem under study. It is carried out by examining library materials or secondary data.

- **Guarding Right to Health and Education: The Constitutional and Human Rights Dimensions:** The discourse on the rule of law began to develop as ideas emerged about the theory of natural law, which grew in Europe in the seventeenth to eighteenth centuries. In general, in the theory of the rule of law, there are 2 (two)types of conceptions about the rule of law, which consist of the concept of the rule of law in the sense of *rechtsstaat* and in the sense of the rule of law. The term *rechtsstaat* is known in Continental-European countries, this understanding was developed among others by Immanuel Kant, Paul Laband, Julius Stahl and Fichte. Whereas, the rule of law was developed in Anglo-Saxon countries, adherents of the common law as pioneered by A.V. Dicey in England. However, basically the two conceptions have one similar purpose, namely the existence of protection of human rights and respect for the dignity of man.[5]

The protection of human rights in a rule of law is carried out among others by establishing a constitution which is a binding basic law based on the highest authority or the principle of sovereignty adopted in a country and in the environment of democratic countries, the people are the holder of sovereignty that determines the enactment of a constitution.[6] Constitutionalism today is considered a necessary understanding for every modern country. This was stated among others by C.J. Friedrich, in essence, that the general agreement or consensus among the majority of the people regarding the idealized building pertains to the State. The organization of the state is needed by members of the political community so that their shared interests can be protected or promoted through the establishment and use of mechanisms called the State.[7] The consensus that guarantees the establishment of constitutionalism in modern times is generally understood to rely on 3 (three) consensus, as follows:[8]

1. Consensus on general goals or ideals (the general goals of society or general acceptance of the same philosophy of government).
2. Consensus on the rule of law as the basis of government or State administration (the basis of government).
3. Consensus on the form of institutions and constitutional procedures (the form of institutions and procedures).

In the view of Frans Magnis Suseno, the rule of law is State authority that must be exercised on the basis of good and fair law which is the basis of all State actions and the law itself must be good because it is in accordance with what is expected by society from the law and is fair because of the intent the basis of all law is justice characterized by (1) legal certainty, (2) demands for equal treatment (3) democratic legitimacy and (4) demands for reason.[9] The ideals of the Indonesian rule of law can be clearly seen from the goals of the Indonesian state contained in the Fourth Paragraph of the 1945 Constitution of the Republic of Indonesia, which is to

protect all Indonesians and all over Indonesia, promote public welfare, educate the nation' life and participate in carrying out world order. These four objectives are affirmed without prioritizing their fulfillment so that the duty of the state in realizing protection is equal and equal to the State's duty in realizing prosperity and intellectual life of the nation.

The protection and fulfillment of the right to health and education is one of the tasks of the State which cannot be ignored under any circumstances. The right to healthas stipulated in Article 28H paragraph (1) which reads "*every person has the right to live in prosperity physically and mentally, to live and to have a good and healthy living environment and the right to obtain health services*." Normatively, the 1945 Constitution does not state that the right to health is a human right or part of human rights. However, because Article 28H is in a chapter or articles regulating human rights, the material is considered human rights or part of human rights.[10] The Indonesian Constitution has affirmed that the right to health is a fundamental right for humans. The basic philosophy of guaranteeing the right to health as a human right is *theraison d'etre* of human dignity. Health is a fundamental right of every human being. Therefore, every individual, family and community have the right to obtain protection for their health and the government is responsible for regulating and protecting so that the community is fulfilled the right to live healthy.[11]

The right to health as a fundamental right that demands state responsibility in its fulfillment is reinforced in the declaration made by WHO and UNICEF known as *Almaata* declaration, namely the important WHO and UNICEF Declaration of Almaata adopted at the International Conference on Primary Health Care in 1978. The conference reaffirms the right to health which is part of human rights, where the fulfillment of the right to health is the responsibility of the State and the goals of the whole world which must also be supported by various sectors.[13]

In the literature, there are several terms used in referring to the right to health, namely: *human right to health*, *right to health*, or *the right to attainable standard to health*. In addition, there are also other terms such as *right to health*, *rights to health care*, *right to health protection* and in broad terms *health rights*. In other words, the mention of the right to health still varies between one author and another, so the term has not been generally agreed upon.[12]

Right to health does not always mean the right for everyone to be healthy and not sick, or the obligation of the government to provide expensive health care facilities that are beyond the government' capacity. But this right is more demanding that the government and public officials can make policies and work plans that lead to the availability and affordability of health service facilities for all in all possibilities and as much as possible in a relatively short time. Article 12 Paragraph (1) of *the International Covenant on Economic, Social and Cultural Right* (ICESCR), the right to health is explained as "the right of everyone to enjoy the highest standards that can be achieved for physical and mental health" does not cover the area of health services.

The regulation of human rights in the constitution has shown that human rights are constitutional content that has the power to bind all parties, including state and regional government officials, executive, legislative and judiciary in the fulfillment of human rights and this is not only the responsibility of state administrators and government, however, is the responsibility of all people and all citizens even though responsibility for the implementation of human rights rests with the government, including the main responsibility in fulfilling the right to education.[14]

- **Reality of Right Protection to Health and Education in COVID-19 Pandemic:** The right to health and education are rights that must be protected and fulfilled by the State under any conditions including during the Covid-19 pandemic. For the fulfillment of the right to health, during the Covid-19 pandemic, the government has issued several regulations, namely:

1. Perppu No. 1 of 2020 concerning State Financial Policy and Financial System Stability for Handling CoronaVirus Disease 2019 (Covid-19) Pandemic.
2. Presidential Instruction No. 4 of 2020 concerning re-focusing activities, budget reallocation and procurement of goods and services in the context of accelerating the handling of Corona Virus Disease 2019 (Covid-19).
3. Presidential Decree No. 11 of 2020 concerning Stipulation of Community Health Emergency of Covid-19.
4. Presidential Decree No. 12 of 2020 concerning the Stipulation of non-natural disasters the Corona Virus Disease 2019 (Covid-19) as a national disaster.

5. Government Regulation No. 21 of 2020 concerning Large-Scale Social Restrictions in the Acceleration of Handling Corona Virus Disease (Covid-19) was established in Jakarta on March 31, 2020 by President Joko Widodo.
6. Presidential Decree No. 7 of 2020 concerning the Task Force for the Acceleration of Corona Virus Disease 2019.
7. Presidential Decree No. 9 of 2020 concerning Amendment to Presidential Decree No. 7 of 2020 concerning Task Force for the Acceleration of Handling Corona Virus Disease 2019 (Covid-19).

In addition to the rules above, the relevant ministries also issued regulations and adopted policies in the context of overcoming Covid-19 transmission, including:

1. Regulation of the Minister of Trade No. 23 of 2020 concerning Provisional Prohibition of Antiseptic Exports, Mask Raw Materials, Personal Protective Equipment and Masks.
2. Regulation of the Minister of Law and Human Rights No. 11 of 2020 concerning Provisional Prohibition of Foreigners Enter the Unitary Territory of the Republic of Indonesia.
3. Regulation of the Minister of Finance No. 23/ PMK.03/2020 concerning Income Taxpayers Affected by Corona Virus Outbreak.
4. Regulation of Financial Service Authority No. 11/ POJK.03/2020 concerning the National Economic Stimulus as a Countercyclical Policy on the Impact of Covid-19 Pandemic.
5. Regulation of the Minister of Health No. 9 of 2020 concerning Large-Scale Social Restriction Guidelines in the Accelerating of Handling Corona Virus Diseases 2019 (Covid-19)

The State is a central actor who holds the main responsibility in implementing policies based on international law or international treaties where the right to health is protected.[15] In carrying out these efforts the state must take strategic steps in the fulfillment of the right to health, by involving all stakeholders in the health sector both from the government and from the private sector. In this case the right to health as a human right legally gives birth to a relationship between an individual and the state, where state obligations relating to human rights must refer to three principles namely respect, protect and fulfill rights for health which is part of human rights.[16] This became the main reason so that in the Covid-19 pandemic, the government issued several regulations, namely:

(a) PerppuNo. 1 of 2020 concerning State Financial Policy and Financial System Stability for Handling the Corona Virus Disease 2019 (Covid-19) Pandemic
(b) Presidential Instruction No. 4 of 2020 concerning re-focusing activities, budget reallocation and procurement of goods and services in the context of accelerating the handling of Corona Virus Disease 2019 (Covid-19).
(c) Presidential Decree No. 11 of 2020 concerning Determination of the Public Health Emergency Covid-19.
(d) Presidential Decree No. 12 of 2020 concerning the Stipulation of non-natural disasters the Corona Virus Disease 2019 (Covid-19) as a national disaster.
(e) Government Regulation No. 21 of 2020 concerning Large-Scale Social Restrictions in the Acceleration of Handling Corona Virus Disease (Covid-19) was established in Jakarta on March 31, 2020 by President Joko Widodo.
(f) Presidential Decree No. 7 of 2020 concerning the Task Force for the Acceleration of Corona Virus Disease 2019.
(g) Presidential Decree No. 9 of 2020 concerning Amendment to Presidential Decree No. 7 of 2020 concerning Task Force for the Acceleration of Handling Corona Virus Disease 2019 (Covid-19).

As described above it can be seen that a number of regulations related to health have been issued but for the right to education it is only regulated in a circular of the Minister of Education so that it appears that the government' priority is on regulations related to health. UNICEF, WHO and IFRC in COVID-19 Prevention and Control in Schools in March 2020 states that in a situation of faster virus transmission, schools must be closed but the education process must continue to run through online-learning activities using various media. This is based on UNESCO data that 1.5 billion students and 63 million teachers at the elementary to secondary level in 191 countries affected by the Covid-19 pandemic and become extraordinary because it has never happened before.

The education world experienced a very significant change because there was a change in the way of learning from face-to-face learning to online learning and digital transformation which is considered the safest way in deciding the spread of the Covid-19 outbreak.

The government' policies related to online or online learning in general provide new experience for all stakeholders related to education and with this policy the government expects that the right to education remains protected even though it is under the Covid-19 pandemic. However, the realization of these government policies cannot be avoided from various barriers that require an effort to arrange the ideal method and mechanisms in overcoming various problems in learning this online method. The reality of Indonesian society shows that information technology does not touch all levels of society. Online learning cannot be done if the school or parents do not have sufficient capital to access the tools. This learning will not occur when teachers and students alike do not have computers, cellphones, or adequate quotas and internet networks. Many complaints both from educators, students and parents related to the implementation of learning from home. Many educators have complained about the limited availability of technological facilities, operational capabilities and internet network limitations in some areas.

To conduct a good learning process, there are basic elements that must be fulfilled, namely curriculum, human resources, teachers, lecturers and staff of teaching facilities and infrastructure. In the atmosphere of the Covid-19 pandemic, there is a need for regulations that can provide regulation and become guidelines for the world of education in conducting teaching and learning processes so that they do not lose the right to education. This regulation must be able to answer all obstacles faced by the community in obtaining their right to education during the Covid-19 pandemic. This regulation not only regulates the procedures but also regulates funding problems in the implementation of education from home because it cannot be denied that the implementation of online learning method requires large costs so that the rules related to the budget and funding must be set forth in the legislation that guides and guides the implementation of certain tasks.

Conclusion

Education is a right that must be protected and fulfilled by the State including during Covid-19 pandemic. Today, the attention of the government is more focused on economic and health issues and does not provide a balanced portion of education. Implementation of schooling or lectures from home is currently only regulated in a Ministerial Circular so that it is only a suggestion. Therefore, in order to be able to realize the protection and fulfillment of the right to education during Covid-19 pandemic, the government should issue legislation that becomes a reference for education providers and for the community so that their right to education is still granted under any circumstances.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Indonesia.

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Medical Tourism Awareness of Health Workers

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Abstract

The aim of this study is to determine the medical tourism awareness of health workers in public hospitals in South Eastern Anatolian Region. The study was carried out between January-March 2020 and 392 health workers participated in the study. The reliability value of scale, used in the study, was counted as very high (0.921). The medical tourism awareness of health workers in the study was found as low in general (2.89). In the sub-dimensions of scale, the awareness of health workers was found as low. When the sub-dimensions of medical tourism awareness scale used in the study analysed, it was found out that health workers are generally unsure at sub-dimensions of institutional competence (2.83), the effects of medical tourism (2.73), the problems of medical tourism (3.11), medical tourism potential of city (2.83) and medical tourism practices of public hospitals (2.84). According to the results of correlation analysis between sub-dimensions, a positive relationship between all sub-dimensions was determined.

Keywords: *Health, Medical tourism, Health tourism.*

Introduction

Health tourism, which began to be frequently spoken in recent years and regarded as an export opportunity by several countries, is referred as an important foreign exchange inflow for Turkey that attains the high quality in the delivery of health care. Thermal tourism services, which were previously tried to be provided only in thermal facilities, started to spread in health care services later and was referred as medical tourism (MT). Especially, private hospitals and intermediary institutions, had the opportunity to both earn higher income and develop themselves in terms of institutional and labour force and export in health care services by seeing the opportunity in the health tourism and using available health resources.By making health tourism in Turkey a government policy since 2006, a department within Ministry of Health was established and tourism policies regarding the Ministry was started to be developed. New steps have been taken towards especially MT and private sector has been encouraged in this regard.

Today, people make great efforts to stay healthy and increase their life quality. With this effort, individuals who want to get cheaper, more quality and out-of-turn health service travel to abroad. Travels of those people have led a new and different field as a MT to arise in the tourism sector (Connel, 2006, p. 1093)[6]. MT is defined as "trips which are made to abroad by a tourist for treatment and rehabilitative health services" (Pollard, 2010)[9].MT means "benefitting from cross border health opportunities to get affordable services which are not available or do not have long waits in one's home country" (Carrera and Bridges, 2006)[5]. According to (Yun Yu and Gyou Ko2012:80), MT is to travel to overseas countries for treatment and care. (Lee and Spisto 2007:1)[8]defined MT as "travelling to another country in order for tourist to feel better or get treatment". According to (Khan and Alam 2014:258)[7], it istravelling of someone to another country which is made to get healthy again in cases like dental treatment and organ transplantation.

Factors that motivate tourists to made MT to receive treatment in another country are; shorter waiting periods than those of their countries, decrease in cost, private treatments, more quality care, the charm of new and

different, non-covered treatment, high-level technology, desire to be treated in the countries having scientifically medical activity all around the world (Woodman 2009, Ministry of Health 2012:13)[13]

In terms of medical tourism, our country has important touristic destinations, critical labour force potential with young population and trainability and several advantages by providing appropriate conditions to treat patients more quality and economically in a positive environment and most of the health institutions are at a level that can compete with the West (Eriş, 2019)[3]. In addition, insufficient publicity, inadequacy in terms of human rights, negative image, the lack of coordination with foreign insurance companies, not giving adequately weight to marketing strategies and marketing researches, the lack of coordination between Ministries and sectors are among the disadvantages (Tengilimoğlu, 2005:92)[11].

The aim of this study is to determine the medical tourism awareness of health workers, in public hospitals in South Eastern Anatolian Region.

Material and Method

Population and Sample: The population of study consists of approximately 15.000 workers in the public and university hospitals in the city centre of Şanlıurfa, Gaziantep, Batman, Diyarbakır, Adıyaman, Mardin and Siirt. With simple random sampling method, the size of sample was determined as 377.

Data Collection Method: Two method as face to face interview and drop off/pick up method were used to collect data. The study was carried out in January, February and March 2020. During the conducting of questionnaire, 392 questionnaires, filled out by voluntary participants, were included in the assessment and analysed was made. The questionnaire, created in the study named "A Research on Health Care Worker's Awareness of Health Tourism: Example of Ahi Evran University Education and Research Hospital Workers" carried out by Acar and Turan (2016)[1], was taken as reference. In the questionnaire, created to be used for data collection, there are 27 statements and participants are asked to give answers according to 5-Likert scale.

The Analysis of Study Data and The Assessment of Study Findings: Data collected in the study was analysed with SPSS 20 package programme. In order to determine the demographic and personal features of hospital workers, which constitutes of sample group, the analysis of frequency, percentage distributions were made; correlation analysis was made to determine the arithmetic means of answers given by health workers to the statements in the scale and their standard deviation and relationships between sub-dimensions of the scale.

Findings: Finding obtained are presented in this chapter of the study.

Table 1: The Reliability Analysis Findings for Scale and Sub-dimensions

Scale and Sub-dimensions	The number of statements	Cronbach's Alpha
The Scale of MT	27	0.921
Institutional Competence	11	0.914
The Effects of MT	5	0.687
The Problems of MT	3	0.720
MT Potential of City	4	0.657
MT Practices of Public Hospitals	4	0.715

The findings of reliability analysis of scale and its sub-dimensions in the study were shown in Table 1. According to the findings, the reliability of scale and sub-dimensions are found as sufficient or very high.

Table 2 The Frequency Distribution of Demographic Information of Hospital Workers in the Study

		Number	Percentage
Sex	Male	200	51.0
	Female	192	49.0
Age	18-25	67	17.1
	26-40	174	44.4
	41-65	151	38.5

		Number	Percentage
Education	High School	65	16.6
	Two-year Degree	99	25.3
	Undergraduate	166	42.3
	Graduate/Postgraduate	62	15.8
Job	Doctor	57	14.5
	Nurse	143	36.5
	Other Health Workers	145	37.0
Department	Administrative Personnel	49	12
	Medical Department	318	81.1
	Administrative Department	74	18.9
City	Diyarbakır	65	16.6
	Urfa	75	19.1
	Antep	58	14.8
	Batman	46	11.7
	Siirt	56	14.3
	Adıyaman	45	11.5
	Mardin	47	12.0
	Total	392	100

The distribution of frequency for the demographic information of hospital workers that participated in the study are shown in Table 2. It was find out that hospital workers work in 7 cities, Şanlıurfa, Diyarbakır, Gaziantep, Batman, Siirt, Adıyaman and Mardin, located in South Eastern Anatolian Region, 51% of them are male, 44.4% of them are at 26-40 age group, 42,3% of them have bachelor's degree, 37% of them are in the other health workers group and 81.1% of them work in a medical department.

When Table 3 analysed, hospital workers state that they are unsure regarding the medical tourism awareness in general. When the sub-dimensions of medical tourism awareness scale, used in the study, analysed, they state that hospital workers are generally unsure at dimensions of institutional competence (2.83), the effects of medical tourism (2.73), the problems of medical tourism (3.11), medical tourism potential of city (2.83) and medical tourism practices of public hospitals (2.84). According to the results of correlation analysis between sub-dimensions, a positive relationship between all sub-dimensions was determined.

Table 3. The Means of Answers given to MT Awareness

	Mean	Std. D.
Institutional Competence	2.83	0.909
The Effects of MT	2.73	0.928
The Problems of MT	3.11	1.089
MT Potential of City	2.83	0.961
MT Practices of Public Hospitals	2.82	0.936
The Scale of Medical Tourism Awareness Total	2.84	0.741

Table 4 Correlations of Sub-dimensions of Medical Tourism Scale

		Institutional Competence	The Effects of MT	The Problems of MT	MT Potential of City	MT Practices of Public Hospitals
Institutional Competence	Cor. coeff.	1	,478**	,265**	,541**	,673**
	p		,000	,000	,000	,000
The Effects of MT	Cor. coeff.		1	,371**	,501**	,525**
	p			,000	,000	,000
The Problems of MT	Cor. coeff.			1,000	,432**	,394**
	p				,000	,000
MT Potential of City	Cor. coeff.				1,000	,618**
	p					,000
MT Practices of Public Hospitals	Cor. coeff.					1,000
	p					

**Shows the significant relationship for p<0,01

The correlation relationships between the sub-dimensions of medical tourism scale are shown in Table 4. When the table analysed, it is seen that there are positive relationships between all sub-dimensions of medical tourism scale. When the sub-dimensions, in which the positive relationship is highest, analysed, it seen that it is between “MT practices of public hospitals” and “institutional competence” (.673). The lowest positive relationship was found between “the problems of MT” and “institutional competence” (.265).

Conclusion and Suggestions

MT has become a field that comes to prominence within the health tourism in recent year. MT which especially draws the attention of private hospitals, is a field on which public and university hospitals should put emphasis.

In this study, it is aimed to determine the MT awareness of hospital workers in the public hospitals in South Eastern Anatolian Region. According to the data obtained from the study, it was found out that 392 hospital workers that participated in the study are unsure (2.84) regarding the scale, used in the study, in general. Hospital workers did not present a positive or negative opinion because they could not be sure regarding the statements in the scale in general and they stated that they are unsure generally. In addition, it is predicted that they did not present a certain opinion regarding the scales in the studybecause hospital workers do not have adequate information on medical tourism.

When the sub-dimensions of scale in the study analysed respectively, hospital workers are unsure regarding all sub-dimensions. At the first sub-dimension of scale, institutional competence, hospital workers stated that they are unsure regarding both general dimension (2.83) and the means of answers given to statements. Hospital workers stated that they disagree with the statements, “This Hospital follows the scientific publications and practices” (2.60) and “This Hospital is proper for health tourism in terms of technological equipment” (2.60), the means of answers give to the statement, “This Hospital is appropriate for medical tourism practices in general” (2.70), was found as low in general and they stated they are unsure regarding the statement. Generally, it was found out that hospital workers are unsure about whether the hospital they work for is institutionally adequate for medical tourism.

At the effects of medical tourism sub-dimension, the second sub-dimension of scale, it is seen that hospital workers are unsure in general (2.83) and according to the answers given to the statements. When the statements of the effects of medical tourism sub-dimension analysed one by one, hospital workers stated that they are generally unsure regarding the statements, “Foreign patients are the source of income for our hospital” (2.65) and “Foreign patients enable our city

to develop economically" (2.69). There is not a certain opinion regarding the other statements of the effects of medical tourism sub-dimension. In general, they have doubts regarding the effects because they do not know the medical tourism.

At the problems of medical tourism sub-dimension, another sub-dimension of the scale, it was found out that hospital workers are generally unsure (3.11). When the means of answers given to the 3 statements of the problems of medical tourism sub-dimension analysed, it is seen that they are unsure regarding all three statements.

At the sub-dimension of medical tourism potential of city, another sub-dimension of scale, it was found out that hospital workers are generally unsure (2.83). Although hospital workers stated that they disagree with the statement, "This city has adequate reserves in terms of thermal springs" (2.58), they stated that they are unsure regarding other three statements.

At the sub-dimension of medical tourism practices of public hospitals, another sub-dimension of scale, it was found out that hospital workers are generally unsure (2.823). Although hospital workers stated that they disagree with the statement, "Foreign patients can prevent us from giving adequate attention and care to native patients" (2.52), they stated that they are unsure regarding other three statements.

When the correlation relationships between all sub-dimensions of medical tourism scale analysed, it is seen that there is a positive relationship between all sub-dimensions of medical tourism scale. It can be said that sub-dimensions of scale have positive effects on each other. It is seen thata positive increase in one of the sub-dimensions would cause a positive increase in other sub-dimensions.

When the literature review analysed, it is seen that there are limited studies regarding the medical tourism awareness of health workers. As a result of the study, carried out by (Acar and Turan 2016)[1], they found out that health workers are unsure regarding the medical tourism potential of city in which they work and the medical tourism practices of hospital for which they work. They obtained results which are similarasour study. In a study regarding the medical tourism perceptions of doctors that work in a university hospital, carried out by (Erdoğan 2018)[2], it was found out that doctors are unsure regarding the medical tourism potential of both their hospital and Isparta. In a study regarding the medical tourism awareness of health workers, carried out by (Dökme et al. 2018)[4], it was found out that health workers are highly anxious about the providing of health services in their hospitals.

In conclusion, it is the seen that the medical tourism perceptions of health workers in their city and hospital are low. That the managers of public hospitals, especially Ministry of Health, primarily give trainings to health workers about medical tourism and make hospitals ready for medical tourism would be helpful.

Two authors collaborated at all stages of this research. Ethical approval was obtained from Harran University Social and Human Sciences Ethics Committee.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Indonesia.

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Evaluation of the Results to Digestive Endoscopy Patients in Azadi Teaching Hospitalin Kirkukcity

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Abstract

Background: Esophagogastroduodenoscopy (EGD)endoscopic findings, such as esophagitis, bleeding, ulcer and malignancy, represent a public health problem.

Aims: To evaluate patients results after doing digestive endoscopy and to determine the, characteristics and results of endoscopy among patients in Kirkuk city hospitals.

Methodology: The samples purposively collected from the patients who referred for Esophagogastroduod endoscopy unit in Azadi teaching hospital in Kirkuk city for period from one September 2016 to the 12th September 2018. The study sampled consist of (70) patients with digestive diseases were selected (male & female). A questionnaire was developed for purposive of study & included 2 parts were demographic data, endoscopy characteristics. The data were arranged for, age,gender, residence and the results of the procedure.

Results: The age group (20-29) years was represent (31.4%) of the sample, the female represent (64.3%) from the sample, the residence (70.0 %) were living in urban and the frequency of endoscopy is majority of the sample (72.9%) were scheduled for two time.

Conclusion: The study showed highest percentage endoscopic finding gastritis and cancer for indication and repeated vomiting for complication.

Recommendation: The study recommended promote best health practices and early nursing management optimized for the endoscopy of G.I.T disease to reduce damage and prevent complications.

Keyward: *Evaluation, Digestive Endoscopy.*

Introduction

Philipp Bozzini was the first one who developed endoscope technology in 1806 in German Mainz utilizing "for the examinations of the canals and cavities of the human body"[(1)]. At the beginning of the eighteenth century a flexible fibrotic type of endoscope and since that became as part of standard practice in medicine and Gastrointestinal tract endoscope which is characterized by easiness and safety with a highly valuable assistance method in diagnostic and therapeutic. Further to the that low in cost and mortality[(2)]. Aforementioned technology can be used to examine gastrointestinal (GI) symptoms such as difficulty swallowing, abdominal pain, nausea, vomiting and hemorrhage. Another utilizing is diagnosed by taking a biopsy investigating GI system for inflammation, bleeding, anemia, tumors and bleeding. The third use can be used for treatment expanding narrowed esophageal, bleeding vessel cauterization and cutting off an extra or a foreign bodies removing[(3)]. Peptic ulcer (duodenal and viscus ulcer) is common in men and prevalence is distended with age with a top prevalence of twenty eight point eight% in five the last decade of life[(4)]. The examination of endoscopic is vital, evaluating and managing higher and lower canal issues. Organic process disorders are very frequent inside the normal population [(5)]. The function of endoscopy in bleeding peptic ulcer disorder is critical. It is a positive device in diagnosis, prognostication and medical aid of harm ulceration. The desires of excellent endoscopy – receipt of an accurately indicated procedure, a right diagnosis and excellent care have to be done with the minimal threat to the patient

and take place in a properly – geared up facility staffed through true education and in a position endoscopists[6]. Bleeding is the 1st danger of issues after an endoscopy is expanded if the technique involves casting off a piece of tissue for trying out (biopsy) or treating a digestive device problem. The second danger is the infection in most endoscopies that consist of an examination and biopsy and risk of contamination is low and in the end danger is tearing of the gastrointestinal tract. The risk will increase if the extra procedures such as dilation to widen esophagus, are carried out[5].

Methodology

Design: To descriptive design was applied in the present study to achieve the objectives was from one September 2016 to the 12th September 2018.

Sampling: A Purposive sampling of (70)sample selected from the patients were attended hospital according to following criteria:Patient who done the endoscopy in any part of GIT system, where, male and female with ages ranged between (10- 60 and over).

Setting: The present study was conducted at azadi teaching hospital which receiving large number of patient who have GIT endoscopy done for them for diagnosis or treatment.

Tools: A questionnaire was adopted and developed for the purpose of the study, it comprises of (2) parts, part (I) included demographic characteristic of patients which consists of (9) items. It includes (age, gender, residence, marital status, educational Level, occupation, smoking, NSAID, number of endoscopy). Part (II) endoscopy characteristics. This part was concerned with the patients characteristics about endoscopy. It comprised of(36)items . That's classified as the following: Endoscopic organ site use, Indication of endoscopy, Reason of endoscopy, Result of endoscopic and Complication after GIT endoscopy. All items were measured by using 3-likert scale option were used in the rating scale as(Always, Sometime, Never) . The data were collected through the utilization of constructed questionnaire, interview technique with the patients in hospital. The data Was collected between (21thNovember in 2016 to 29th March in 2017).

Data Analysis: Data were analyzed by using descriptive statistics, which include Descriptive statistical method (Frequency, percentages and Mean of score) and Inferential statistical method (ANOVA), by using; Statistical Package for Social Science (SPSS) version (23) is used for data analysis at (P.value ≤ 0.05).

Results

Table (1): Distribution of the Socio-Demographic Characteristic of the Study Sample (No=70).

Variable	Frequency	Percentage
Age		
20-29	22	31.4
30-39	10	14.3
40-49	10	14.3
50-59	16	22.9
60 and above	12	17.1
Total	**70**	**100.0**
Gender		
Male	25	35.7
Female	45	64.3
Total	**70**	**100.0**
Residence		
Urban	49	70.0
Rural	21	30.0
Total	**70**	**100.0**
Marital status		
Single	20	28.6
Married	46	65.7
Widow	4	5.7
Total	**70**	**100.0**
Level of education		
Illiterate	11	15.7
Read and write	9	12.9
Primary	17	24.3
Secondary	10	14.3
Institute	9	12.9
College	14	20.0
Total	**70**	**100.0**
Occupation		
Employee	17	24.3
House Wife	29	41.4
Jobless	11	15.7
Free Work	6	8.6
Student	7	10.0
Total	**70**	**100.0**
Smoking		
Yes	45	64.3
No	25	35.7
Total	**70**	**100.0**

Variable	Frequency	Percentage
NSAID		
Aspirin	12	17.1
Ibuprofen	30	42.9
Induced	10	14.3
More than one	18	25.7
Total	**70**	**100.0**
Number of endoscopy		
1Time	14	20.0
2Time	51	72.9
3 Time	5	7.1
Total	**70**	**100.0**

Table (1) indicates the socio-demographic characteristics of the whole study sample. The it shows that the highest percentage of age groups is between (20-29 years) and constituted (31.4 %) and according to gender, the high percent was of females and constituted (64.3%). With regard to residence, (70.0%) of patients were living in urban areas, most of the patients were married and constituted (65.7%). (24.3 %) of the sample were primary school,As regard to occupation, (41.4 %) patients were housewives. With relation to smoking, the majority of the subjects (64.3%) were smokers. The high percent in ibuprofen and constituted of (42.9%), were NSAID. Finally, in respect to the frequency of endoscopy, the majority of the sample (72.9%) were scheduled for two time.

Table (2) Mean of scores for objects of patients' endoscopy concerning the organ site use of endoscopy.

Endoscopic Organ site Use	Always		Sometime		Never		MS
	f	%	f	%	f	%	**1.7286**
Esophagus	15	21.4	21	30.0	34	48.6	2.8429
Stomach	63	90.0	3	4.3	4	5.7	1.8571
Duodenum	19	27.1	22	31.4	29	41.4	1.3429
Colon	8	11.4	8	11.4	54	77.1	

Table (2) indicates that the mean of score was highly significant inobjects (2) and moderately significant inobjects (3) and low significant in objects(1-4) .

Table (3) Mean of scores for items of patients' endoscopy regarding the indication of endoscopy.

Indication For Endoscopy	Always		Sometime		Never		MS
	f	%	f	%	f	%	
Infection	35	50.0	13	18.6	22	31.4	2.1857
Paralytic illus	6	8.6	20	28.6	44	62.9	1.4571
A dhescion or rupture of bowel	5	7.1	13	18.6	52	74.3	1.3285
Hemorrhage	16	22.9	15	21.4	39	55.7	1.6714
Spasm and obstruction of the bowel	24	34.3	22	31.4	24	34.3	2.0000
Stress and life style	28	41.4	9	12.9	32	45.7	1.9571

Table (3) indicates that the mean of score was moderately significant in items (1-5-6) and low significant in items (2-3-4) .

Table (4) Mean of scores for matter of patients' endoscopy as regards the reason of endoscopy.

Reason For Endoscopic	Always		Sometime		Never		MS
	f	%	f	%	f	%	
Haemtemesis-Millennia	15	21.4	16	22.8	39	55.7	1.6571
Acute–Chronic Epigastria	50	71.4	2	2.9	18	25.7	2.4571
Chronic constipation	10	14.3	19	27.1	41	58.6	1.5571
Chronic Diarrhea	10	14.3	12	17.1	48	68.6	1.4571
Alternating Bowel motion	13	18.6	18	25.7	39	55.7	1.6286
Abdominal mass	2	2.9	14	20.0	54	77.1	1.2571

Table (4) indicates that the mean of score was highly significant inmatter (2) and low significant in matter(1-3-4-5-6).

Table (5) Mean of scores for substance of patients' endoscopy about the result past endoscopy.

Result After Endoscopic	Always		Sometime		Never		MS
	f	%	f	%	f	%	
Previous bleeding	9	12.9	17	24.3	44	62.9	1.5000
Gastritis	31	44.3	6	8.6	33	47.1	1.9714
Ulcer	14	20.0	17	24.3	39	55.7	1.6429
Esophagitis	12	17.1	15	21.4	43	61.4	1.5571
Bleeding Duodenal and Ulcer	9	12.9	7	10.0	54	77.1	1.3571
Mass in G.I.T	3	4.3	12	17.1	55	78.6	1.2571
Celiac Disease	5	7.1	24	34.3	41	58.6	1.4857
Esophageal	5	7.1	19	27.1	46	65.7	1.4143
Esophageal Cancer	55	78.6	11	15.7	4	5.7	1.2714
Gastro Duodenititis	34	48.6	20	28.6	16	22.9	1.7429
Colon Cancer	55	78.6	11	15.7	4	5.7	1.2714
Rectal Cancer	55	78.6	12	17.1	3	4.3	1.2571

Table (5) indicates that the mean of score was moderately significant insubstance (2-10) and low significant in substance(1-3-4-5-6-7-8-9-11-12) .

Table (6) Mean of scores for items of patients' endoscopy regarding the Complication After Endoscopy.

Complication After (G.T.I) Endoscopy	Always		Sometime		Never		MS
	f	%	f	%	f	%	
Poor Appetite	31	44.3	16	22.9	23	32.9	2.1143
Repeated Vomiting	43	61.4	14	20.0	13	18.6	2.4286
Bleeding	10	14.3	18	25.7	42	60.0	1.5429
Chest Pain	24	34.3	4	5.7	42	60.0	1.7429
Perforation	6	8.6	25	35.7	39	55.7	1.5286
Hypertension	4	6.7	22	36.7	34	56.7	1.4857
Emphysema	5	7.1	19	27.1	46	65.7	1.4143
Allergy	1	1.4	18	25.7	51	72.9	1.2857

Table (6) indicates that the mean of score was highly significant in items(1-2) and moderately significant in items (4) and low significant in items (3-5-6-7-8).

Table (7) statistical Differences between Gender, Smoking, NSAID, Number of endoscopy and Associated with Results to Digestive Endoscopy.

Categories	S.O.V	Sum of Squares	df	Mean Square	F	Sig.
Gender	Between Groups	11.048	31	.356	2.696	S.
	Within Groups	5.024	38	.132		
	Total	**16.071**	**69**			
Smoking	Between Groups	7.810	31	.252	1.159	N.S
	Within Groups	8.262	38	.217		
	Total	**16.071**	**69**			
NSAID	Between Groups	55.152	31	1.779	.776	S.
	Within Groups	87.148	38	2.293		
	Total	**142.300**	**69**			
Number of endoscopy	Between Groups	14.471	31	.467	1.570	S.
	Within Groups	11.300	38	.297		
	Total	**25.771**	**69**			

Table(7)demonstrates that there is a significant difference in gender, NSAIDs, number of endoscopy and no significant of smoking in respect to the patient's digestive endoscopy at $P < 0.05$.

Discussion

Table (1) indicates the socio-demographic characteristics of the whole study sample. The it shows that the highest percentage of age groups is between (20-29 years) and constituted (25.7%)

The explanation of this result the young age in our country face several issues like security situation and no gift of utilized there for the young age became additional nerve-racking and suffer from completely different issues like Gastro enteric issues. Mentioned that over seventieth of all ulceration cases occur in people between the ages of (25 and 64) years(7).

According to gender, the high percent was of women and constituted (64.3%). Interpret of this end result related to pregnant may reasongastro esophageal reflexes disorder,also can also related to use of contraceptive medication. Also the women additionally appear to have slower emptying of meals from the belly than men. This can also be necessary in explaining why women have a tendencyto trip nausea and bloating more regularly than men. Also supported this result when they noted that the incidence of duodenal ulcer in male to female is 2 – 3: 1 whilst in gastric ulcer are 1:1.(8)

With regard to residence, (70.0%) of patients were living in urban areas.Because the clinics place in center of governoratein this manner we obtain most of them beginning urban area. Also the results appears most the patients were married and constituted (65.7%). (24.3 %) of the sample were primary school, As regard to occupation, (41.4%) patients were housewife. Mentioned that occupation has been related to the progress of peptic ulcer (PU) (7). With relation to smoking, the majority of the subjects (64.3%) were smokers. The high percent in Ibuprofenand constituted of (42.9 %), were NSAID. Finally, in respect to the frequency of endoscopy, the majority of the sample (72.9%) were scheduled for two time.

Table (2) Mean of scores for items of patients' endoscopy regarding the organ site use of endoscopy. Table (2) indicates that the mean of score was highly significant in items(2-stomach) and moderately significant in items (3 Duodenum) and low significant in items (1- esophagus and 4-colon), Declarethe Peptic ulcer extra common happens with endoscopy and come out in 20% - 40% of people who live in industrialized countries and merely about 15%-25% of these people in fact have an ulcer (9).

Table (3) Mean of scores for items of patients' endoscopy regarding the indication of endoscopyand shows the mean of score was moderately significant in items (1-infection, 5-spasm and obstruction, 6 life style

events). Mention to the majority patients alluded for endoscopy complain of symptoms that come below the general heading of indigestion (10)

Table (4) indicates that the mean of score was highly significant in items (2-chronic constipation). Interpret of this result related to the constipation consider a big problems there for when patients suffer from this problems will try to find the main cause for this problems by endoscopy. Is disagreement with our learn and reveal that every the patients with a finding of esophageal tumor were properly referred but 10 absent of 70 patients with a diagnosis of gastric cancer, all elderly more than 50 years, had unfortunate indication for EGD (research of primitive cancer in patients by way of adenocarcinoma metastases) (11)

Table (5) about results of endoscopic and shows that the mean of score was moderately significant in items (2-gastritis and 10-gastro dudenitis) . Mention that thegrades of endoscopic analyze as oesophagitis, metaplastic epithelium in the gullet, hiatal hernia or imperfect sphincter closure, soreillness, erosive or nodular gastritis and cancer. In expansion, the worked abdomen was scored (12)

Table (6) indicates of complications of endoscopy and shows that the mean of score was highly significant in items (1-poor appetite and 2-vomiting). Account so as to complications of esophagogastroduodenoscopy (EGD) is connected by a 0.1% occurrence of complications. The largelyimortant of these complications are perforation thenhemor- rhage. Desire, Mallory-Weiss tears and former “minor” complications such since retroesophageal swelling have too beendepicted (13).

Table(7) illustrate that around is a significant difference in sexual category, NSAIDs, number of endoscopy and no significant of smoking in accept to the patient’s digestive endoscopy on $P < 0.05$. Recover that the main pathology (tumour, ulcer, or stricture) was found by endoscopy in 787/3815 (21%) patients through digestive disorder. Age, male sex, hemorrhage and anemia were found to be significant but vulnerable independent indicators of endoscopic result. A multivariate prediction regulation based on these factors had bad predictive accuracy (c statistic=0.62). Using a simplified prediction rule of age >45 years before the attendance of some “alarm” symptom, affectability was 87% and specificity was 26%. Rising or declining the age reduce bad did not significantly advance the prognostic truth (14).

Conclusion

Further than one quarter of the study test their age ranging beginning (20 – 29) years. Although height percentage in married status who had investigative and therapeutic endoscopes among too much voltage, food preparation and anxiety at home. One third of studies patient’s smoker followed over half of studies sample taking NSAIDs. The more I have done them Endoscopy they are two-off due to the early diagnosis of the sickness. The study showed elevated percentage of endoscopic finding site in stomach and duodenum.

Recommendation:

1. Promote best health practices and early nursing management optimized for the endoscopy of G.I.T disease to reduce damage and prevent complications.
2. Availability of endoscopic facilities at all hospitals in city and make it accessible to all population and thus enables earlier diagnosis of digestive tract diseases.
3. Expansion of the service with endoscopic, more relevant accessories and diagnosis and facilitate therapeutic measures.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Indonesia.

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Non Genetic Study for Autism Child in Related to Age And Sex of Autism Child, Parental and Maternal age And Degree of Relative and Family History with the Disease or Any Other Psychological Disorder in Anbar Governorate

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Abstract

Aims of this study were find the relation of the non-genetic factors related to the parents of the autism child, the child himself and the family history in specific points because there is no perfect causes for the autism., the autism child including the age of mother during delivery of these child, the degree of kinds hip between the parents, the sex of the child. The information taking from 80 children present in institute of the Virgin Mary for Autism & Balsam institute for Autistic patients, from both sex, the age of them between 3-16 years who suffer from Autism. Data collected we distributed forms to autistic families for the purpose of taking the information we needed. There is no significance relation between parental or maternal age and autism, male greater than female, family history not effects on the presence or not of autistic disorder.

Keywords: *Autism, non genetic factors, Anbar governorate.*

Introduction

The autism is defined as a severe social problem which marked by difficult communicated with environment or the surrounding people, in the speaking, the repetitive and the behavioral limitations and an strong and over response to any events in the surrounding [(1)]. The autism is considered as a chronic disorder that could be occurred at early childhood, that divided into three types of disturbances in behaviorwhich are: the social irritability, a speakingproblems and a repetitive form of the behavior [(2)]. It is also explained as a clinical problems which appear as delayed in development or deviation in the way of development.[(3)]. In 1990s the reported cases of autism were increased until in 2000s.this result of increasing in number change The numbers of the reported cases of the autism increased dramatically in the 1990s and in the early 2000s.these increasing in number belong to change in the practices of diagnosis,the patterns referral,the time of the diagnosis and the social awareness[(4)].

The outward appearances of the autistic children may not indicate as a disorder. The diagnosis of the state occur by taking all the information of the patient t, any neurological or physical disturbances. There are many possible causes for autism belong to before the delivery period which are include disturbance of respiratory system, anemia, the mother taking large amount of drug during pregnancy of autistic children, or the occurrence of bleeding after the 1 st trimester and the amniotic fluid defect[(5)].autism also have a genetic change in spite of there is no one know the number of gene that affected[(6)].

The common cause of the autisimanomalies of the chromosome 15 and the chromosome 17q21,numerical and formal abnormalities of the sex chromosomes, are the common cause of the autisim.[(7)] for ASDs the environment also play a role in the development of the disorders . the possible causes for autism spectrum disorder as an environmental factor are radiation and mercury[(8)]. The studies which talked about consider the MMR vaccination as one causes for autism,another study provide a strong refuse for these hypothesis [(9)] the mixing of DPT and MMR vaccines may affect the immune system of children that enhance the autistic biomedical cascade [(10)]. The are many studies suggest the family factors such as the education level, age of child and parents while the age of parents related to many

disorder or health problems such as cancer of childhood period, schizophrenia, death of fetus, however,the relation of parental age and autim still not clear [11].

Another articles study that congenital malformations have appositive relation with low birth weight(<2, 500 g), fetus birth less than 37 weeks the low birth boys according to gender related study say that it is increased risk of autism greater than low birth weight girls (<2,500 g) [12]. When there are many differences between The monozygotic twins this will enhance the non-geneticeffects of the autism [13]. Because of there are many limitations in the studies made before or after the birth for the risk factor in autism this proved the relation between them still unknown [14].

The aim of the study: To find the relation between the non-genetic factors related to the parents of autism child, the child himself and the family history in specific points because there is no perfect causes for the autism.

Research Methodology

The information taking from 80 children present in institute of the Virgin Mary for Autism & Balsam institute for Autistic patients, from both sex, the age of them between 3_16 years who suffer from Autism. Data collected we distributed forms to autistic families for the purpose of taking the information we needed. The sample was divided firstly on the base of age of the autistic child into three groups which are under the age of 5 years, between 5-9 years and above 10 years. In addition the sample divided on the base of sex into two groups which are male and female. In this study the age of parents(father and mother) was taken at the day of the child birth. In addition to that the relative degree was taken for the parents of the autism children (relative or not). Family history with autism or any other psychological disorder was taken.

Results

Statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25) was used to do analysis for the data.Mean, percentage,frequency,percentage,standard deviation and range are used in presenting the data . Pearson Chi-square test (□2-test) with application of Yate's correction or Fisher Exact test were measure the significance of difference of different percentages (qualitative data). Statistical significance was considered whenever the P value was equal or less than 0.05.

The male was 67 (83.8%) and the female was 13 (16.3). the age was 7 (8.8%) under the age of 5 years and 50 (62.5%) between 5 and 9 years and 23 (28.7) above 10 years old.

		No	**%**
Age of autistic child (years)	<5y	7	8.8
	5---9y	50	62.5
	=>10y	23	28.7
	Mean±SD (Range)	8.0±2.7 (3-15)	
Sex of autistic child	Male	67	83.8
	Female	13	16.3

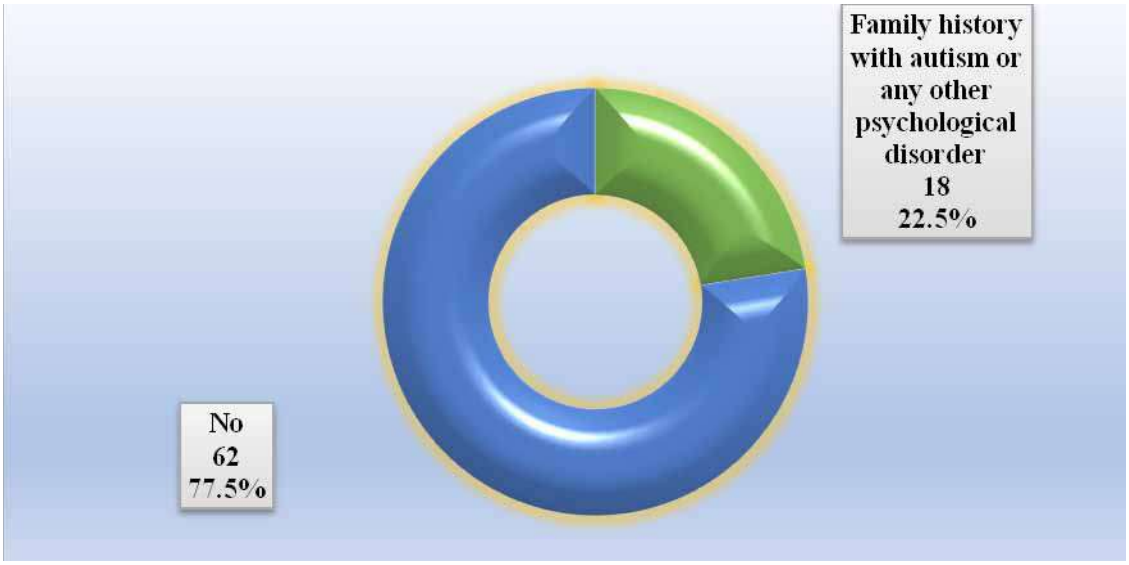
In this study the age of mother and father was taken at the day of the child birth and it found that the mother age was less than 20 year for 7 child (8.8%), 20-29 years for 46 child (57.5%), 30-39 years for 25 child (31.3%) and more than 40 years for 2 child (2.5%). The fathers were 20-29 years for 31 children (38.8%), 30-39 years for 42 children (52.5%), more than 40 years for 7 children (8.8%).

		No	**%**
Mother age at birth (years)	<20y	7	8.8
	20---29	46	57.5
	30---39	25	31.3
	=>40y	2	2.5
	Mean±SD (Range)	26.8±5.7 (18-45)	

		No	%
Father age at birth (years)	<20y	-	-
	20---29	31	38.8
	30---39	42	52.5
	=>40y	7	8.8
	Mean±SD (Range)	31.3±5.8 (20-48)	

The relative degree was taken for the parents of the autism children (relative or not) and it was found that 26 (32.5%) relative and 54 (67.5%) was not relative. Family history with autism or any other psychological disorder was taken and it was found that 18 (22.5%) with positive history and 62 (77.5%) was with negative history.

		No	%
Parental degree (relative or not)	Yes	26	32.5
	No	54	67.5
Family history with autism or any other psychological disorder	Yes	18	22.5
	No	62	77.5



		Family history with autism or any other psychological disorder				P value
		Yes		No		
		No	%	No	%	
Age of autistic child (years)	<5y	2	28.6	5	71.4	0.780
	5---9y	10	20.0	40	80.0	
	=>10y	6	26.1	17	73.9	
	Mean±SD	8.0±3.2		8.0±2.6		
Sex of autistic child	Male	13	19.4	54	80.6	0.132
	Female	5	38.5	8	61.5	

		Family history with autism or any other psychological disorder				P value
		Yes		No		
		No	%	No	%	
Mother age at birth (years)	<20y	1	14.3	6	85.7	0.734
	20---29	12	26.1	34	73.9	
	30---39	5	20.0	20	80.0	
	=>40y	-	-	2	100.0	
	Mean±SD	26.6±4.5		26.9±6.1		
Father age at birth (years)	<20y	-	-	-	-	0.855
	20---29	7	22.6	24	77.4	
	30---39	10	23.8	32	76.2	
	=>40y	1	14.3	6	85.7	
	Mean±SD	30.7±5.2		31.5±6.0		
Parental degree (relative or not)	Yes	5	19.2	21	80.8	0.627
	No	13	24.1	41	75.9	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level						

		Parental degree (Relative)				P value
		Yes		No		
		No	%	No	%	
Age of autistic child (years)	<5y	3	42.9	4	57.1	0.764
	5---9y	15	30.0	35	70.0	
	=>10y	8	34.8	15	65.2	
	Mean±SD	8.4±3.0		7.7±2.6		
Sex of autistic child	Male	22	32.8	45	67.2	0.884
	Female	4	30.8	9	69.2	
Mother age at birth (years)	<20y	4	57.1	3	42.9	0.458
	20---29	13	28.3	33	71.7	
	30---39	8	32.0	17	68.0	
	=>40y	1	50.0	1	50.0	
	Mean±SD	26.5±5.9		27.0±5.7		
Father age at birth (years)	<20y	-	-	-	-	0.048*
	20---29	15	48.4	16	51.6	
	30---39	10	23.8	32	76.2	
	=>40y	1	14.3	6	85.7	
	Mean±SD	29.6±5.7		32.1±5.7		
Family history with autism or any other psychological disorder	Yes	5	27.8	13	72.2	0.627
	No	21	33.9	41	66.1	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level						

Discussions

Among all the professionals there is agreement that the autism is one of the most difficult and puzzling diseases. It is considered as a neuro developmental disorder and characterized by the impaired of social interaction; its prevalence has increased in recent years[15].

In our study the high percentage of patients was between the ages 5-9 years which was 62.5%, while under the age 5 years the percentage was 8.8% and above the age 10 years it was 28.7% and this findings are in disagreement with the findings of Farida El-Baz et al [16] who found 46% of the cases was at the age of 1.5 years and 4% at the age of 4 years. and also in reverse to the findings of Gray and Tonge[17] who found high percent of autism children at the age of 1-2.5 years. In contrast our findings were in agreement with the findings of Paul et al. [18] who reported that the average age of identifications is 5.7 years. This study showed that the percentage of the males was more than the females (males 83.8%, females 16.3) and this was in agreement with the study of Farida El-Baz et al [16] who found that the percentage of the males was 71.3% and the females was 28.7% and this was also consistent with the study of Itzchak et al [19] in which the males was 81% and the females was 19%. Regarding the maternal and paternal age of the autism children at the birth our study showed the high percentage was the age of 20-29 years of the mothers (57.5%) followed by the age of 30-39 years (31.3%) while for the fathers the high percentage was at the age 30-39 years (52.5%) followed by the age of 20-29 years (38.8%) and this was in disagreement with the study of Kolevzon et al [8] who found that the risk of the autism increased with the advanced age of both father and mother. While the study of Marissa et al [20] showed that increased maternal age, regardless the paternal age, may associated with greater risk of autism. Our study dealt with the consanguinity of the father and mother of the autistic children and showed that 67.5% of them are not relative and 32.5% of them are relative and this findings was in agreement with the findings of Farida El-Baz et al [16] in their study who found that 87% of the parents of the autistic children was non consanguineous while Muhle et al [21] showed that the families of the autistic individuals tend to show a set of cognitive disorders which are not seen in the other family groups. This study showed the percentage of 77.5% of the autistic children were with no family history with autism or any other psychological disorder in contrast 22.5% of them were with a history of family autism or psychological disorder and this was in reverse to the findings of SherllyXie et al[22] in their study who suggested that the family history mental or neurological disorders was associated with the increased risk of autism and also Bilder D et al. [23] showed in their study the family history of autism was found in 16% of the cases versus 1%of the controlled cases.

Conclusions

There is no significance relation between parental or maternal age and autism, male greater than female, family history not effects on the presence or not of autistic disorder.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Indonesia.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Parent's Knowledge upon Nutritional Status of Primary School Children toward Low-Income Households in Al-Hilla City, Iraq

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Abstract

Background: Nutrition has a main impact on the health promotion of school-age children, it is crucial that the rising energy demands that come with this age are met daily with high nutritional value foods. School-age children get a well-balanced diet, an average of 2400 calories per day required to meet growth needs, as the child's size grows, as well as the amount of food required

Method: Descriptive design study was used and get the stated objectives the period of one month (from the first of January, 2020 to the first of February, 2020).

Results: The data analysis was indicated that the largest percentage of gender is female (97%); all of them are in the fifth class and 32% of mothers and 31% of fathers are graduated from primary school, most of them from urban area 86%.

Conclusion: All of the children were in the 5th class—most children with normal weight**.** Most children have breakfast daily. Most of the children have three meals daily. Most mothers choose easy to prepare food for her child. There is no a relationship between education of the mother and eating breakfast. However, there are a relationship between monthly income and eating fruits .

Keywords: *Parent's Knowledge, Nutritional Status, low-income households, Primary School Children.*

Introduction

Anthropometrics may be responsive measures of infant and child health, growth and development which it one most widely accessible, cheap and non-invasive approach used to measure human body size, proportion and composition[(1)]. The ultimate goal of the nutritional assessment, according to WHO in 2013 to improve human health[(2)].

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To ensure optimum work capacity, normal reproductive performance, adequate immune reactions and resistance to infections, sufficient food and nutrition are important for proper growth and physical development. An inadequate diet can cause severe forms of malnutrition in children, deficiencies in vitamin A and disorders of iodine deficiency, also World Health Organization, in 2013 has proposed various anthropometry-based indices to determine the school-aged children's nutritional status. Body mass index (BMI) has now been well developed as the most important predictor for adolescent nutritional status[(3)]. Several research has looked at adolescent nutritional status from different parts of the world[(4)].

Children's nutritional status plays a crucial role in their wellbeing, with malnutrition accounting for (45%) of all deaths among children under five years of age and

stunting development among another 165 million[5]. With the positive success of various programs for child protection, more children reach school age (ages 6-12)[6]. Unfortunately, minimal attention was paid to school-age children's health and nutritional status, especially in developed countries with a greater focus on enhancing their access to education[7].

However, undernourished and obese school children may struggle to meet their maximum cognitive potential with an eventual decline in school academic achievement and later in adulthood, low job ability[8],[9],[10]. Hundreds of millions of children in urban areas of the world are growing up in the midst of poverty and deprivation, with the rising incidence and complications of childhood malnutrition [11]. Some socio-demographic features such as age, gender and order of birth have also been found to affect their health and nutritional status[12].

Methodology

This part will present the methodology of the current study and all numerous stages that passed through, from the beginning of its approval and ending with the analysis of its collected data.

Design of the study: A descriptive design study was used to get the stated objectives the period of one month (from the first of January, 2020 to the first of February, 2020).

Administrative and Ethical Arrangements: Before the gathering of the data and formal administrative permissions, the researcher was obtained to conduct the study.

1. The researcher obtains ethical approval from the Ethical Researcher Committee, University of Babylon/Faculty of Nursing.
2. The Central Statistical Organization has approved the study tool.

Ethical considerations: This study was accepted by the Faculty Nursing/University of Babylon. Several strategies were utilized to protect the responder's rights, who agreed to participate in this study. Next, verbal, oral consent of samples was obtained before the questionnaire was administered. The samples were told of the study intent and that they were entitled to refuse to participate. It has also been told the samples that they should refrain from answering any questions and can terminate at any time.

The setting of the study: The study was conducted in Altatbiqat primary school and 14 tmmuz primary school at Hilla city center.

Population and sampling plan: This quantitative study using a descriptive correlational design to assess Parent's Knowledge upon Nutritional Status of Primary School Children toward low-income households in Al-Hilla city. A convenience sample of (n=100) consenting parents. Data were selected through the period of one month (from the first of January, 2020 to the first of February, 2020).

The Instrument of the study: The questionnaire that is used in this study contains two parts as the following:

Part 1/A. Includes the Child's demographic information, which consists of (4) items that include age, class, Gender, number of brothers.

Part 1/B. Knowledge of Parent demographic information, which is consisted (educational level of mother and educational level of a father, monthly income, number of family members and Residence).

Part 2: Feeding Aspect According B.M.I consist of (6 Items).

Part 3: Food Choosing for Parents consist of (17 Items).

Rating and scoring:

The study instrument is rated on: A three-level Likert scale was used for knowledge items to measure the responses (Important, Kind of important and Not Important) and all statements are scored as 3= Important, 2= Kind of important and 1= Not Important, 3 represents the high -value scale.

The validity of the questionnaire: A preliminary copy of the questionnaire was sent to (10) experts. Responses from the expert suggested that slight changes needed to be made to some items. This adjustment was made according to their recommendations, few items needed to be omitted and others were added according to expert notice.

Statistical Data Analysis: Various statistical method were used to interpret and test the study findings using the Statistical Package of Social Sciences (SPSS) version 25 and Microsoft Excel (2016). All continuous

quantitative variables and the generated overall knowledge scores were tasted for normality distribution and all were followed by the statistical normal distribution.

Results

Table (1): Nutritional Status, according to B.M.I.

Variables	Group	Frequency	Percentage
Weight	Less than normal	15	15
	Normal	83	83
	Obese	2	2
	Excessive obesity	0	0
	Total	100	100
High	Normal	84	84
	Tall	10	10
	Short	6	6
	Total	100	100
Is he/she have breakfast?	Yes	93	93
	No	7	7
	Total	100	100
Is breakfast at home or at school?	Home	91	91
	School	9	9
	Total	100	100
Is he/she have any fruits daily?	Yes	87	87
	No	13	13
	Total	100	100
Number of meals daily	Less than three	5	5
	Three	82	82
	More than three	13	13
	Total	100	100

Table (3) indicated that most of the sample with normal weight of 83% and tall 84%.

Table (2): Point of view about food choices by parents(total score)

	Group	F.	%
Point of view about food choices by parents	Not Important	16	16
	Kind of important	21	21
	Important	63	63

F :Frequency % :Percentage

Table (3) shows that most families about 63% point of view about food choices that Important.

Table (3): Relationship between some variables in present study

Demographic data	Rating	F	%	P-Value
Relationship between Education of mother and eating breakfast for child	Do not read and write	8	8	X^2 =5.895 N.S=.435 Df=6
	Write read and	17	17	
	Primary school	31	31	
	Middle school	16	16	
	High school	10	10	
	Institute	8	8	
	Bachelor and more	10	10	
Monthly income and eating fruits	Enough	26	26	X^2 =8.954 Sig=.01 Df=2
	Not enough	34	34	
	Kind of enough	40	40	

F : Frequency % :Percentage X^2: chi-square-test. sig: significant df :degree of freedom, N.S: not significant

The table (4) shows the relationship between some variables in the present study. Regarding education of mother and eating breakfast for child this table reveals that is no relationship them. (P-value = 0.435) at the ($p \leq 0.05$) level of significance. In respect, monthly income and eating fruits the result shows that relationship between them. (P-value = 0.01) at the ($p \leq 0.05$) level of significance.

Discussion

School-age children are unable to meet their needs with the necessary nutrients that lead to building a fully developed body because unhealthy lifestyle neglects low-income households to their needs. Nevertheless, our study children's nutritional status was especially optimal, in line with the other researchers[13],[14].

Many studies were done in Islamabad and Abbottabad showed gross malnutrition in school children[15],[16]. In the present study, most of the children, 83% were in optimal nutritional status and had sound skeletal growth despite their society and economic status. These results are in disagreement with the results of Fisher and Birch[17]. They indicated that parents influence the development of children's eating habits, not only through the foods that they make available to children, but also through their eating patterns, mealtime activity and child-feeding activities. Child-feeding habits of parents are correlated with eating patterns of children, including different eating styles, food intake and preferences and energy intake control.

In this study, there was no a relationship between having breakfast for children and the educational level of mothers. This result disagrees with Al-Shookri in Oman, who revealed that there was a positive correlation between children's dietary food intake scores and the mothers' level of education which nutritional knowledge was due to dietary intake, which the importance of the fact that nutrition-related education and information for mothers can improve their off spring's dietary intake[18]. Finally, the data analysis was showed that relationship between monthly income of father and eating fruits.

Conclusions

1. All of the children were in the 5th class.
2. Most of children with normal weight**.**
3. Most of children have breakfast daily.
4. Most of the children have three meals daily
5. Most of mother choose easy to prepare food for her child.
6. Most of mother choose rich protein food for her child.
7. Families' ability to provide and choose suitable and healthy food for their child its good.
8. Recommendations
9. The study recommends the following:
10. Nutritional programs training for teachers and parent

11. Create a nutritional assessment database based on school policy at all primary schools in Hilla City.
12. Supply of material for calculating height and weight in schools.
13. Group Calculator by using a measurement system to control on BMI.
14. Making a health education pyramidal food guide.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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Right to Health Protection in the Former-Soviet Constitutions: Constitutional and Legal Analysis

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Abstract

The constitutional framework for legal aid to available health care therefore determines one of the strategic areas of policy of democratic, social and law-governing states (former Soviet states). This determines the relevance of studies of resistance to constitutional and legal regulation of the health system formed as a result of changes in the skin and radical structure of social structure in previous USSR states. The article contains the constitutional and legal analysis of the right to health protection in the former-Soviet constitutions by reviewing the constitutional healthcare provisions and specifying the groups of qualified bearers of right to health protection and variety of rights as the healthcare system elements. The studies are to be continued, as well as the review and summary of information about constitutional and legal regulation of the right to health protection in the former-Soviet states are needed.

Keywords: *Health, healthcare, right to health protection, Constitution, former-Soviet states.*

Introduction

The public welfare directly depends on the health status of the population that is conditioned by availability of the medical assistance, medical supplies, as a whole and its services, in particular. That is why the constitutional framework of the legal support of the available healthcare set forth one of the strategic areas in the state policies of the democratic, social and law-governed states (the former-Soviet states constitute themselves as such). This determines the relevance of study of contradictory features of the constitutional and legal regulation of the healthcare systems in the former USSR states formed as a result of the accelerated and radical formation changes in the social structure.

Method

To prepare the article, the following general scientific and special study method were used: analysis, synthesis, information search, structured system analysis, dogmatic, comparative legal.

Results and Discussion

The similar topics were studied by S.V. Agievets, K. Bendukidze, A.O. Dichko, L.V. Zhylskaya, O. A. Kovalenko, N. P. Kryzina, V.S. Lysenko, V.M. Oleynik, V. Petrovsky, V.V. Puchkova, F. S. Reder, S.A. Rudy, S.G. Stetsenko, M. Taner, A. Urushadze, M. A. Shevchenko, L.Z. Shupa, et al. The studies are to be continued, as well as the review and summary of information about constitutional and legal regulation of the right to health protection in the former-Soviet states are needed.

There are broad and narrow definitions of the term "right to health protection" in the scientific literature. Broadly, this is a collection of legal norms regulating the relations of the population health protection in socio-economic, healthcare and other spheres of the state life. Narrowly, this is an intersectoral legal institution directly aimed at health protection (Agievets SV 2012)[1].

The constitutional lawmaking of the states-the union republics of the former USSR in the past - is distinguished by inert continuity of the lawmaking aimed at sectoral

regulation of the social sphere, including the right to health protection. The statements on the guaranteed free medical aid are contained in the most of the new Constitutions of the CIS members and others.

That is why, to guarantee the constitutionally objectified right to health protection, its international legal recognition, documentation, as well as provision and preservation of the implementation mechanisms are of critical importance and have been realized since H2 XX century in view of the lawmaking process of the UNO and WHO and other subjects of the international legal relations.

The principle of priority and rule of the international law embodied in the Constitutions of the former-Soviet states provides for the required state obligation on the guaranteed safeguards for the human and civil rights to the available healthcare and excludes any avoidance of its fulfilment that is motivated by explanations regarding insufficient capacities of the national economy.

The mandatory legislative regulation of the available right to healthcare by all the former-Soviet states is determined by their UN membership as the parties to the Covenant on Economic, Social and Cultural Rights of 1966. The Covenant Preamble sets forth the regulation of the UN charter binding the states "to promote universal respect for and observance of, human rights and freedoms" (Agievets, S. 2002)[2]

Substantiating the above provisions, L.V. Zhilskaya fairly points out the unlawful interpretation of the principle of "the gradual implementation of obligations" embodied in the Covenant as ensuring these rights only when the state achieves a certain economic development level, whereas according to the above obligation, all the member states, irrespective of the degree of their national welfare, must undertake the immediate steps to exercise economic, social and cultural rights not postponing them for an indefinite period (Zhilskaya LV. 2005)[3].

Clause 1, Article 12, of the Covenant contains a mandatory provision for the member states regarding recognition of the right of everyone to the highest achievable level of physical and mental health specified in cl. 2 of this Article: «*a*) the provision for the reduction of the stillbirth-rate and of infant mortality and for the healthy development of the child; *b*) the improvement of all aspects of environmental and industrial hygiene; *c*) the prevention, treatment and control of epidemic, endemic, occupational and other diseases; *d*) the creation of conditions which would assure to all medical service and medical attention in the event of sickness" (**Agievets, S. 2002)** [2.]

The mentioned provision in brief or in detailed wording is reflected in the Constitutions of all the former-Soviet states, at this, the detailed wording assumes the presence of a system of the state guarantees for ensuring the maximum possible level of mental and physical health.

The healthcare issues are highlighted in Articles 37, 41, 42 of the Constitution of the Russian Federation. The basic scope of the right to health protection, including the medical aid, is included in cl. 1, Art. 41, providing the relevant right to everybody, that is not only to the citizens. At the same time, the medical aid guarantees consisting in the free medical aid provision by the state and municipal healthcare institutions out of the relevant budget, insurance contributions, other revenues, are addressed to the citizens only. Cl. 2 of Art. 41 provides for financing the federal programs on health protection and promotion, undertaking the measures to develop the state, municipal and private healthcare systems. But at the same time, the activity facilitating the health promotion by developing the physical training and sports, establishing the ecological and sanitary and epidemiological welfare is only "encouraged". Consequently, ensuring the natural human right to health and medical aid is not guaranteed by Art. 41 of the Russian Constitution in contrast to the legal rights of a citizen. This contradicts the provision of cl. 1 of Art. 17 concerning recognition and guarantee of the human and civil rights and freedoms in the Russian Federation pursuant to the general principles and norms of the international law and this Constitution. Nevertheless, cl. 3 of Art. 41 establishes the responsibility of the state, in the persons of officials of the state system, for life and health of people. The sanction for suppression by such officials of the life and health threatening facts and circumstances attracts a legal responsibility as provided by the Federal Law. At the same time, cl. 3 of Art. 37 grants to everyone the right to work in the conditions meeting the safety and hygiene requirements and Art. 42 - the right to favourable environment and reliable information on its state that is substantiated by the restorative justice norm on compensation of damage caused to health and property by an environmental offence (The Constitution of the Russian Federation 1993)[4].

Notwithstanding that cl. 3 of the Constitution of Ukraine of June 28, 1996 recognizes the human life and health as the highest social value, such contradiction is also present in the wording of the human right to health protection in cl. 49 of the Constitution declaring the right of everyone to health protection, medical aid and medical insurance to be provided by the state funding of the relevant socio-economic, medical-sanitary and health improvement and prevention programs. At the same time, the assessment criteria of the proper level of the medical services - their efficiency and availability, embodied in para. 3 of this Article refer not to everyone but only "all citizens". The free medical aid must be provided by the state and municipal healthcare institutions and the state shall "promote" the development of such institutions of all legal forms of organization. At this, the state guarantee is contained in the norm prohibiting the reduction of the network of the state and municipal healthcare institutions, although the current implementation of the medical reform in Ukraine actually transforms this guarantee into the declaration. According to para. 4 of Art. 49, the state shall take care of developing the physical training and sports, ensuring the sanitary and epidemiological welfare. Art. 50 contains a natural human right to the life and health safe environment that simultaneously provides for compensation of damage caused by infringement and a guarantee for free and available information with the right of its distribution and prohibition of its classification (The Constitution of Ukraine. 1996)[5].

The Constitution of the Republic of Belarus of 1994 (as amended and supplemented, adopted at the National Referendums on November 24, 1996 and October 17, 2004) clearly restricts a range of subjects having the guaranteed right to health protection with its citizens. The guarantees embodied in Art. 45 include free treatment in the state healthcare institutions, available medical services, development of the physical training and sports, environmental improvement activities, opportunity to use the recreational institutions, labour protection improvement; and those in Art. 47 include the right to social security in old-age, in case of disease, disability, loss of earning capacity. and only the norm stated in Art. 46 grants the right to favourable environment and compensation of damage caused by breach of this right of everyone (The Constitution of the Republic of Belarus 1994)[6].

The specific feature of Art. 37 of the Constitution of Georgia of August 24, 1995 as amended on October 15, 2010 is the interpretation of health insurance as the available means of the medical aid being a right of everyone. At the same time, the free medical aid is limited by the legally established "specific conditions". The state control covers all healthcare organizations, pharmaceutical production and trade. All people have the right to harmful environment, use the natural and cultural environment. The environmental conservation and protection and sustainable use pursuant to the ecological and economic interests of the society constitute the common obligation of "all" and the state (The Constitution of Georgia. 2005 Aug 24., Kryzina NP,KOO. 2016)[7, 8].

The Constitution of the Republic of Armenia of July 05, 1995 as amended on December 06, 2015, Art. 85, very briefly declares the right of everyone to health protection according to the law, thus restricting the list and procedure of the basic free medical services not mentioning the medical aid and guarantees (The Constitution of the Republic of Armenia. 1995) [9].

The Constitution of the Republic of Azerbaijan of November 12, 1995 (as amended and supplemented on 25/07/2016) and vice versa, Art. 41 more meaningfully sets forth the right to health protection and medical aid covering everyone, binding the state to undertake necessary measures to develop all kinds of healthcare on the basis of various kinds of ownership, guarantee the sanitary and epidemiological welfare, create the opportunities for various forms of the medical insurance. In the given wording, the right to health protection and right to medical aid are considered as individual interconnected varieties of healthcare rights. The state provides for development of all kinds of healthcare in various forms of organization, guarantees the sanitary and epidemiological welfare, creates the opportunities for various forms of medical insurance. Similarly to the Russian Constitution, the officials concealing the facts and events that create danger for health and life shall be brought to the responsibility as provided by the law(The Constitution of the Republic of Azerbaijan. 1995 Nov 12.) [10.]

The Constitution of the Republic of Moldova of July 29, 1994, Art. 36, briefly and without details, guarantees the right to health protection, declares the minimum level of the free state medical supplies and refers to the organic act establishing the national healthcare system structure and facilities intended for physical and mental health protection (The Constitution of Georgia 2005

Aug 24, The Constitution of the Republic of Azerbaijan. 1995 Nov 12)[8],[11].

The Constitution of the Republic of Kazakhstan (adopted at the National Referendum on August 30, 1995) (as amended and supplemented as of 23/03/2019 г.), Art. 29, grants the right to health protection and statutory guaranteed scope of the free medical aid to the citizens only. The medical aid can be paid not only at the private health care centers and when provided by the persons engaged in the private medical practice, but also at the state medical institutions on the basis and in a manner provided by the law (The Constitution of the Republic of Moldova. 1994 July 29)[12].

According to Art. 47 of the Constitution of the Kyrgyz Republic of June 27, 2010, the right to health protection, the state-created conditions for medical services and measures to develop the state, municipal and private healthcare sectors shall cover all people. But it does not specify the scope of subjects of the right to free medical services, as well as medical services on favourable conditions in the scope of state guarantees provided by the law. Similarly, to Russia and Azerbaijan, there exists the legal responsibility of the officials for concealing the health and life-threatening facts and events (The Constitution of the Kyrgyz Republic. 2010 June 27.)[13].

The Constitution of the Republic of Uzbekistan of December 08, 1992, is limited by the right of everyone to the qualified medical services declared in Art. 40 (The Constitution of the Republic of Uzbekistan1992 Dec 8.)[14].

The Constitution of Turkmenistan of May 18, 1992, as amended and supplemented on December 27, 1995, Art. 52, stipulates the right of "each person" to health protection, including free use of services of the state healthcare institutions. Along with the paid medical services, it also embodies the alternative medicine on the basis and in a manner provided by the law. Art. 53 grant to every person the right to life and health favourable environment, reliable information on its state and compensation of damage caused to health and property by an environmental offence or natural disasters (The Constitution of Turkmenistan. amended and supplemented. 1992 May 18) [15].

According to the Constitution of the Republic of Tajikistan (as revised by Referendums on 26/09/1999, 22/06/2003, 22/05/2016), Art. 38, the right to health protection cover everyone and the free medical aid shall be provided by the state institutions as legally provided. This also refers to the other forms of the medical aid. The measures to improve the environment, create and develop the mass sports, physical training and tourism are the responsibility of the state (The Constitution of Turkmenistan. 1992 May 18) [16].

The Constitution of the Republic of Lithuania of October 25, 1992, Art. 53, binds the state to take care of the people's health and guarantee the medical aid and services to a person in case of disease. But the law establishes the procedure of free medical aid provided at the state healthcare institutions for the citizens only. The state encourages the physical training and promotes or the sports. The environmental protection against harmful impacts is a common responsibility of "each person" and the state (The Constitution of the Republic of Lithuania. 1992 Oct 25) [17].

The Constitution of the Republic of Latvia adopted at the general meeting of the Constitutional Assembly of Latvia on February 15, 1922, revised on 22/07/2014, cl. 111 of Chapter VIII, is limited by the statement of the state health protection and guarantee of minimum medical aid to everyone and cl. 115 – by the statement of right of everyone to live in the favourable environment by providing the information on the state of the environment and taking care of its protection and improvement (Kryzina NP,KOO. 2016 No.2. 2018, The Constitution of the Republic of Estonia. 1992 June 28.)[18, 19].

The Constitution of the Republic of Estonia of June 28, 1992, is distinguished by the major social right to health protection as provided by cl. 28 stating that everyone has the right to health protection. A citizen of Estonia has a priority right to the public support in old age, in case of disability, loss of breadwinner, poverty. Its kinds, size, provisions and procedure are established by the law. Simultaneously, if not contradicting to the law, such support can be provided to foreign citizens and stateless persons coming to Estonia (Kryzina NP,KOO 2018, Dichko GO. 1993 July 16)[8,18]

The considered constitutional and legal provisions of the former-Soviet states concerning the right to health protection make it possible to conclude on the following:

1. The guaranteed free medical aid (maintenance, services) is present in the most of the post-Soviet constitutions as a result of inertia of the Soviet

constitutional system.

2. The priority of the international right is one of the principles of the state of law and the most important factor for the required fulfilment by the former-Soviet states of their constitutionally acceptable international liabilities on guaranteeing the right to health protection and implementing the complex measures to ensure availability and efficiency of the healthcare system.

3. The constitutional provisions on free medical aid (maintenance, services) is embodied as the relation with the paid aid in various ratios, but a common feature of the post-Soviet constitutions is a statutory restriction of the free-of-charge basis and a trend to its minimization.

4. The scope of subjects - the qualified bearers of the right to health protection - is represented in all constitutions by two main groups: all individuals having the natural human rights and citizens having the legal rights. At this, the absolute majority of the constitutions provide the free medical aid (maintenance, services) for the citizen only.

5. In all post-Soviet constitutions, the right to health protection is related to the right to health living environment that is ensured by common responsibilities on its conservation and protection by a human, citizen and state. Simultaneously, there is the human and civil right to compensation of damage caused by violations in the sphere of natural management. At this, there is the constitutionally accepted legal responsibility of officials for concealing the information concerning any danger to life and health.

6. A number of constitutions include the rights to improvement of occupational hygiene and labour protection, physical training and sports, use of recreational facilities demonstrating these states striving for achievement of the ecological and sanitary and epidemiological welfare, maximum possible level of mental and physical health of population.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in turkey.

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The Influence of Labor Environment Settings on Labor Progression

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Abstract

The environment in labor can have a great effect on the fears and anxieties experienced by the mother. Anxiety can inhibit cervical dilatation, so that the perception of pain increases. Cortisol is secreted in response to tension and anxiety, it affects the progress of cervical dilatation. The increasing concentration of cortisol during pregnancy and continue to rise until labor. Anxiety is usually associated with increased pain during labor and modification of labor pain can be done through psychological and physiological mechanisms. One way to modify psychological labor pain is by setting the delivery environment. The purpose of this study was to analyze the effect of the setting of the labor environment on the progress of labor.

The results showed the average delivery progress (cm/hour) in the treatment group 1.38 (0.43) and the control group 1.23 (0.52), obtained p = 0.058, this indicates there was no difference in labor progress in both study groups. Based on the statistical test results of the effect of the setting of the delivery environment on the progress of labor, the p value = 0.015 shows that there is an influence of the delivery environment setting on the progress of labor and the RR value of 3.67 (IK = 1.14-11.84), which means that the mother gives birth in a Independent Midwifery Practice (IMP) who did not manage the delivery environment had a risk of having a cervical opening < 1 cm/hour by 3.67 times compared to mothers giving birth at the IMP who did the setting of the delivery environment.

The conclusion of this research is that there is a positive influence of the delivery environment to the progress of labor.

Keywords: *Labor progress, labor environment, childbirt, cortisol, cervical dilatation.*

Introduction

Childbirth is an amazing event that can be experienced and remembered in every woman's life. Birth will change a woman's life and the care given has the potential to affect both physically and emotionally in the short and long term. The goal of caring for women during childbirth is to create positive experiences for a woman and her family while maintaining physical and psychological health (Susilowati E. 2014, Iravani M, Zarean E, Bahrami M. 2015).[1,2]

The environment in labor room can have a great effect on the fears and anxieties experienced by the mother (Munro J, Jokinen M. 2012).[3] Anxiety is usually associated with pain that increases during labor and can be in either modify labor pain through psychological and mechanisms of physiological.(Lowe NK 2002)[4] One way to modify labor pain psychologically is by regulating the labor environment.

Fear and pain cause stress which results in adrenaline expenditure. This results in vasoconstriction and reduced vascularization to the uterus, resulting in a decrease in uterine contractions which will cause prolonged labor. This is less beneficial for the mother and fetus in the mother's uterus (Reck C, Zimmer K, Dubber S, Zipser

B, Schlehe B, Gawlik S. 2013)[5] Stress during labor can also reduce the amplitude and frequency of uterine contractions and thus, labor will last long and allow for *caesarean section* (SC).(Namazi M, Akbari SAA, Mojab F, Talebi A, Majd HA, Jannesari S. 2014).[6]

It also can increase adrenaline so that blood vessel constriction occurs so that oxygen supply to the fetus decreases. Decreased blood flow also causes weakening of uterine contractions and results in prolonged labor.[5] hormones, such as catecholamines, cortisol, epinephrine and beta-endorphins, whichare secreted in response to tension and anxiety, are involved inthe progression of cervical dilatation. It is affecting the muscles of the uterus and reduce the strength of the contract of the of the uterus and efficiency in the delivery so eventually will prolonged labor, increasing pain and trigger anxiety. (Masoudi Z, Akbarzadeh M, Vaziri F, Zare N, Ramzi M. 2014).[7]

Method

Population and Sample: Subjects in this study were all women in the Independent Midwifery Practice (IMP)who met the inclusion criteria, were not included in the exclusion criteria and were willing to participate in the study by signing the informed consent sheet after explanation.

Inclusion and Exclusion Criteria: The inclusion criteria in this study were primigravida, latent parturient phase with gestational age 37-4 0 weeks, normal pelvic size, estimated fetal weight of 2500-4000 grams, single fetus, head presentation, desired and or planned pregnancy, mother accompanied during pregnancy, mother who has an anxiety score ≤ 59 . Exclusion criteria were premature rupture of membranes, blind and deaf, mothers who had attended pregnancy exercises and yoga.

Research Design: This study is a quasi-experimental study with a non equivalent control group design. The research subjects were divided into two groups namely the treatment group and the control group. In the treatment group of the Midwifery Practice (IMP), maternity interventions were given in the form of labor environment settings which included lighting arrangements, wall color arrangement, arrangement of medical equipment, use of natural scenery, use of music, furniture arrangement, temperature regulation and using plants to neutralize fishy odors the labor environment, while the control group using environmentally deliveries already exist.

This research was carried out simultaneously with members of the research team in developing the delivery environment. Data collection was performed in the treatment group and the control group. The treatment group was given an intervention in the form of childbirth at the Independent Midwifery Practice (IMP) that had done the delivery environment. The maternity control group at IMP is in accordance with the existing delivery environment.

Measurement Result

The main outcome to be measured in this study is the difference in labor progress (cervical opening) in the intervention and control groups. Other results that will be measured in this study are anxiety scores, pain scores and cortisol levels, gestational age and birth weight .

Research Flow: In the Independent Midwifery Practice (IMP) which is made as an intervention group, the delivery environment will be arranged in accordance with the draft delivery arrangements that have been made and the Independent Midwifery Practice (IMP) which is used as a control group using the existing delivery environment. This study requires examination of cortisol levels in maternal, so the researcher collaborates with the Laboratory of the Faculty of Medicine Unpad to examine cortisol levels. Examination of cortisol levels is done once, namely in the latent phase, blood samples are taken for examination of cortisol levels carried out by midwives. Then to obtain the inclusion criteria anxiety score ≤ 59 performed an anxiety score assessment by other researchers. Furthermore, the measurement of labor progress (cervical dilatation) starts from the opening of 3 cm to the opening of 10 cm, is checked every 4 hours and the average opening (cm/hour) is assessed .

Research Instrument: The instruments used in this study were partographs and observation sheets to assess the progress of labor.

Location and time of research: This research was conducted at the Independent Midwifery Practice (BPM) of West Bandung Regency and Cimahi in July-September 2016.

Ethical Aspect: The ethical aspects considered include (Wirakusumah FF, Satari MH. 2011)[8]

1. The principle of respecting human dignity and respect (*Respect for person)*

In this principle, research subjects are given information about the research to be carried out including: procedures, benefits, volunteerism, confidentiality of data, inconvenience and compensation as well as officers who can be contacted if there is anything that needs to be discussed in connection with research. The research subject is free to determine its participation in following the course of the study, it must be stated in writing by signing the consent form (*informed concent)* as respondents.

2. The principle of benefits and no harm *(Beneficence)*

The advantage of this research that can be felt directly by respondents is obtaining the comfort of giving birth in a room that has been arranged so that labor is expected to run smoothly.

3. The principle of *justice*

Researchers always be honest, careful, professional, humane. Researchers also always pay attention to the factors of accuracy, equality, accuracy, intimacy, psychological and religious feelings of respondents.

Results

In the study of the effect of the setting of the delivery environment on the progress of labor the characteristics of the subjects can be seen in the following table:

Table 1 Characteristics of research subjects

Characteristics	Group		Value of p
	Treatment	Control	
	(n = 30)	(n = 30)	
Mother's age (years)			0.812 *
Average	22.90	22.70	
Median	23.00	22.50	
Range	17-29	17-32	
Gestational age (weeks)			0.317 **
Average	38.33	38.03	
Median	38	38	
Range	37-40	37-40	
Birth weight (gram)			0.215 **
Average	2,925	3,040	
Median	3,000	3,000	
Range	2,500-3,400	2,500-3,500	

Note: * T test is not paired, ** Mann-Whitney Test

Based on table 1 it can be seen that the statistical test results obtained on maternal age characteristics p value = 0.812, gestational age values p= 0.317 and birth weight (BW) obtained p value = 0.215 it indicates that there is no significant difference between the characteristics mothers in the treatment group and in the control group, so that both of these research groups were considered homogeneous.

Differences in cortisol levels (µg/dl) and pain in the two study groups can be seen in the table below:

Table 2 Differences in Cortisol and Pain Levels in the Treatment and Control Groups

Cortisol (µg/dl)	Group		Value of p
	Treatment	Control	
	n = 30	n = 30	
Cortisol			0.988 *
Average	371,201	429,125	
Median	361,211	334,185	
Range	140,000-579,856	160,050-895,831	
Pain			0.109 *
Average	4.97	5.67	
Median	5	6	
Range	3-7	2-10	

Explanation: * Mann-Whitney

From table 2 it can be seen that the statistical test results obtained cortisol values (µg/dl) p = 0.988 and pain values obtained p = 0.109 it shows that there is no significant difference between the treatment group and the control group, so both of these research groups are considered homogeneous.

To analyze the comparison of the progress of labor in the treatment group and the control group can be seen in the following table:

Table 3 Comparison of Progress in Labor in the Treatment and Control Groups

Variable	Group		Value of p
	Treatment	Control	
	(n = 30)	(n = 30)	
Labor Progress (cm/hour)			0.05 8 *
Average	1.38	1.23	
Median	1,2	1.08	
Range	0.8-2, 3	0.5-2.3	

Note: * Mann-Whitney test (one- sided test)

From table 3 it can be seen that the results of statistical tests for the progress of labor (cm/hour) in the treatment group and the control group obtained p = 0.058, so this shows that there was no difference in labor progress in the two study groups.

To analyze the effect of the setting of the delivery environment on the progress of labor the following calculation results are obtained:

Table 4 The influenced of Labor Environment Settings on the Progress of Labor

Group	Cervical Opening				RR (95% IK)	Value of p
	< 1 cm/hour		≥ 1 cm/hour			
	n = 30	%	n = 30	%		
Control	11	36.7	19	63.3	3.67(1.14-11.84)	0.015 *
Treatment	3	1 0	27	90		

Note: *Test two proportions (one party)

From table 4.4 the statistical test results show that in the control group subjects who had cervical opening < 1 cm/hour were 11 (36.7%) and in the treatment group were 3 (10%), whereas in the control group subjects who had cervical opening ≥ 1 cm/hour was 19 (63.3%) and in the treatment group was 27 (90%). The difference in the proportion of labor progress in the two groups was found to be p = 0.015, so this shows that there is an influence of the delivery environment on the progress of labor. Furthermore, by calculating the relative risk of the relationship between treatment with the progress of labor obtained RR = 3.67 (IK = 1.14-11.84), meaning that mothers who give birth at Independent Midwifery Practice (IMP) who do not do labor settings have a risk to have cervical opening < 1 cm/hour 3.67 times compared to mothers who gave birth at Independent Midwifery Practice (IMP) who carried out the delivery environment.

Discussion

The environment in the process of childbirth can have a great effect on the fears and anxieties experienced by the mother, such as an unfamiliar environment, then the existence of medical interventions and the existence of negative thoughts such as childbirth which is always followed by pain and then the mother is afraid of the condition of the baby being born. Anxiety is usually associated with pain that increases during labor and childbirth pain can be modified through psychological and physiological mechanisms. (Lowe NK.2002).[4]

The environment is the biggest factor influencing the healing process in medical facilities that is equal to 40%. The physical environment affects 94% of the ease or difficulty of giving birth.[9,10] A physical environment has an influence on human thoughts, feelings and behavior. As a built environment, space is a stimulus (stimulation from outside) that is able to be responded by the human sensory system (vision, hearing, taste, smell and touch), which psychologically has the potential to form perceptions that indirectly affect emotional and human behavior. (Sari SM. 20030)[11]

Pain during labor can be caused by uterine contractions and cervical opening (stretching down the uterus). According to control theory, there is a relationship between pain and psychological problems such as anxiety.[12] The environment in labor can have a great effect on the fears and anxieties experienced by the mother. (Munro J, Jokinen M. 2012)[3]

Simultaneously, the hypothalamus-pituitary-adrenal (HPA) axis and *the sympathetic nervous system* (SNS) organize various circumstances psychological (eg: emotional processing) and physiological (eg: hormonal endocrine activation and cardiovascular) process to ensure that the maintenance of homeostasis of the organism that is being challenged to stress. (Thoma MV, Marca RL, Brönnimann R, Finkel L, Ehlert U, Nater UM. 2013)[13] At the same time, the perception of the injury will enable (HPA) axis, where corticotropin releasinghormone (CRH) produced in the hypothalamus into the bloodstream, causing the release of adrenocorticotropic hormone (ACTH) and other substances. ACTH then activates the adrenal cortex to release cortisol.(Melzack R. 1999)[14] During labor an increase in CRH levels stimulates an increase in myometrial prostaglandin receptors, prostaglandin release, oxytocin, fetal cortisol and beta-endorphins. (Dixon L, Skinner J, Foureur M. 2013)[15]

The speed of the opening of the cervix itself is influenced by various factors, namely the strength and weakness of uterine contractions will affect the process of cervical opening. To be able to perform contractions oxytocin hormone secretion is needed. Oxytocin is a hormone produced in the hypothalamus and is transported through the axoplasmic flow to the posterior pituitary which, if given the right stimulation,

will be released into the blood. This hormone is named oxytocin based on its physiological effects, namely the acceleration of labor by stimulating contraction of the uterine smooth muscle.(Lestari I, Abadi A, Purnomo W. 2012)[9]

A major proposition from the gate control theory is that to reduce pain affects the center of the brain that serves attention, cognition and emotion which activates nerve impulses to close the " gate" located in the dorsal horn of the spinal column. A second proposition from gate control theory is that pain is a sensory interaction, motivation and a component of middle control.[17] The presence of pain interferes with the brain's homeostatic regulatory system, resulting in stress and requires a complex program to restore homeostasis.(Newburn M, Singh D 2003)[10]

The pain causes stress which results in adrenaline expenditure. This results in vasoconstriction and reduced vascularisation to the uterus, resulting in a decrease in uterine contractions which will cause prolonged labor.[5] Oxytocin also reduces stress by way of centralized activate the parasympathetic nervous system, which gives the effect of calm and healing; and by reducing activity in the sympathetic nervous system which reduces fear, stress and stress hormones and increases socialization.(Buckley SJ. 2015)[18]

Increased anxiety and pain increase catecholamine release and alpha receptor stimulation by the sympathetic nervous system, which in turn causes vasoconstriction, increased muscle tension and decreased uterine blood flow, increased blood pressure, loss of uterine contractions, slowed labor progress and increased metabolism and oxygen consumption to mother. [4, 19] Stress during labor can also reduce the amplitude and frequency of uterine contractions and thus, labor will last for a long time and allow for *caesarean section* (SC).(Namazi M, Akbari SAA, Mojab F, Talebi A, Majd HA, Jannesari S. 2014)[6]

Lighting can be used creatively and seriously to create a birth space that is calming and relaxing. [1] At the beginning of labor, catecholamines (stress hormones) have the potential to stop labor. When a woman feels very afraid of pain, is in the hospital, labor can fail to progress.(Lothian JA. 2004)[20] Darkness stimulates the body to produce melatonin, which then increases oxytocin production. Found a link between lighting arrangements with oxytocin production. The presence of oxytocin affects the production of endorphins.(Gedey S. 2014)[21] Expending endorphins can make mothers feel relaxed.

A causal relationship between an increase in nocturnal endogenous melatonin and a decrease in core temperature. On the other hand, melatonin secretion is usually suppressed by bright light and thus, endogenous melatonin levels are almost zero during the day.(Kondo N, Taylor NA, Shibasaki M, Aoki K, Muhamed AMC. 2009)[22]

Color can affect the human soul strongly or can affect human emotions. Color can also affect one's mood.(Darmaprawira S. 2002).[23] The use of color in the delivery room must be considered because it can stimulate the neocortex to release endorphins. Bright colors must be avoided because it can stimulate the neocortex. (Stenglin M, Foureur MJ. 2013)[24] The neocortex provokes the release of adrenaline and inhibits the physiology of birth.(Jenkinson B, Josey N, Kruske S. 2014) [25] Penyimpanan equipment deliveries in wardrobes can create peace for the mother and reducing the clinical atmosphere.[25] Labor equipment should be stored and kept away from the view of maternity so that the mother does not experience anxiety.(NTC Policy Briefing 2011)[26] The use of natural elements can also help reduce stress and pain felt during labor, for example by exposure to natural scenes.[27]

In addition to lighting arrangements, the color/use of wall paint, the arrangement of medical equipment and the use of natural elements, it is also important to note the importance of the arrangement of furniture. Furniture influences the atmosphere of the delivery room. Minimalist furniture and arrangement is very important to note, given the importance of sufficient space for mobility in the delivery room.(Jenkinson B, Josey N, Kruske S.)[25] The existence of restrictions on mobility contributes to increased stress and tension. (Manizheh P, Leila P. 2009)[28]

The use of music in the labor room can also help mothers to deal with pain that is good to increase stimulation and pain threshold. (Gedey S. 2014)[21] Music provides a pleasant sensory stimulus, causing endorphin release. In addition, music therapy is also a cognitive process that can stimulate the control system through the production mechanism of endorphins, so that with the stimulation of the descending control, the "gate" area will close the transmission of pain to the brain.

Music therapy has vibration and harmony that can be used according to the listener so it will feel comfortable. In addition, because the vibrations of music produce vibrations or the delivery of air to the ear, the vestibular organ (balance tool) also gets the impact of music so that it relaxes.(Malehere NS. 2013, Dwirahayu Y. 2014)[29, 30]

Adding new odors in the labor environment to change odorous substances into substances that are less stimulating. (Suma'mur. 1996)[31] Like adding plants in the labor environment that can neutralize fishy odors in the labor environment.

Improve the physical environment, increase women's expectations and confidence. Women believe that the birth environment can affect the ease or difficulty of labor and the facilities provided can affect opportunities for normal birth or risks that require emergency surgery. (Newburn M, Singh D. 2003).[10]

Conclusion

The conclusion of this research is that there is a positive influence of the delivery environment to the progress of labor.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both

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Development of Spectrophotometric Method for Analysis of Framicetine Sulphate in a Medicinal Product and its Validation

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Abstract

A procedure for the quantitative determination of framycetin sulfate in the drug 'Framex' (nasal spray) 8000 IU/ml has been developed, using spectrophotometry (SF) method. The developed method has been validated using the following indicators: specificity, correctness, linearity, repeatability and intralaboratory accuracy. It has been found that the developed methodology is valid for all characteristics and can be used for the quantitative determination of framycetin sulfate in the substances and composition of medicinal products.

Keywords: *Framycetin sulfate, validation, spectrophotometry, framex, antibiotic, aminoglycosides.*

Introduction

On the current pharmaceutical market, there are very few drugs with antibacterial activity, that can be used for children under 1 year old. These include the drug 'Framex' (produced by Aseptica, Uzbekistan)[1]. The nasal spray composition is based on the antibiotic of the aminoglycoside series of the topical bactericidal action of framycetin. 1 ml of the drug contains 8000 units of framycetin[2].

It is a common fact that aminoglycosides are highly active against most gram-negative and gram-positive microorganisms - pathogens of upper respiratory tract infections. However, systemic antibacterial drugs of these groups have a side ototoxic effect, which makes it impossible to use their antimicrobial potential in bacterial infections of the nasal cavity, nasopharynx and paranasal sinuses in children. The indicated side effects can not be expected with the local administration of these drugs, since, according to the results of clinical studies, the level of their systemic absorption during topical use is extremely low[1].

One of the urgent problems of pharmaceutical chemistry is the development and validation of theroutine analysis method for the quantitative determination of the active pharmaceutical ingredient in substances and finished medicinal products. The developed method are designed to minimize the consumption of solvents and reagents, the duration of the analysis, and to obtain the most accurate result. Validation of an analytical method is an experimental proof that the method is suitable for solving the proposed problems[2].

In our work, we used the drug 'Framex' (nasal spray) containing 8000 IU/ml of framycetin sulfate. The pharmacologically active ingredient of the drug is framycetin sulfate[3]. In its chemical structure, it is 2-deoxy-4-O-(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-D-streptamine sulfate. The chemical structure of framycetin sulfate allows its staining using basic conditions (pH=9-10)[4].

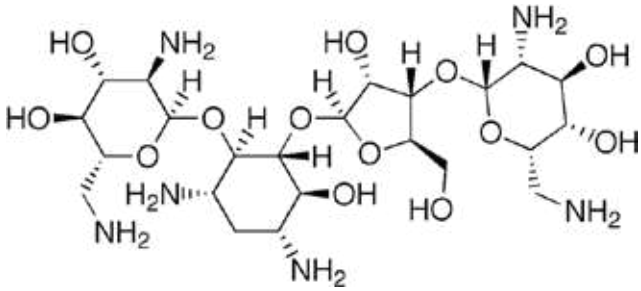


Fig. 1. Structural formula of framycetin

The aim of this work is to develop and validate the SF method for the quantitative determination of framycetin sulfate in substances and the drug 'Framex' (nasal spray).

Materials and Method. In our study, we used class A measuring glassware, an CY 224 CITIZEN analytical balance (India), a 'UV-1800' UV spectrophotometer (manufactured by Shimadzu Corporation, Japan) (wavelength range from 190 to 1100 nm, relative accuracy less than 0.1%)

The specificity of the method is determined by comparing the values of the active ingredient in the analysis of the solvent (form), the active ingredient and the pharmaceutical product.

The linearity of the method is determined by at least 5 different dilutions of the test solution in the range of application of at least 80-120% of the concentration of the analyte in the test solution[5].

Repeatability is carried out using tests of at least 6 prepared samples at 100% concentration of the active ingredient in the test solution.

The correctness of the method determines the response of the active ingredient in placebo[6]. The placebo concentration is 100% of the weigh indicated in the control method. The active ingredient is added to the model mixture in accordance with the required concentration level (lower limit - max. 80, 100%; upper limit - min. 120%)[7].

The object of the study was the drug 'Framex' (the content of framycetin sulfate - 12.5 mg/ml), the corresponding MPhM (manufacturer's pharmacopoeial monograph)is 42 Уз-26491354-2019. Solutions of 80% (0.40 mg/ml), 90% (0.45 mg/ml), 100% (0.50 mg/ml), 110% (0.55 mg/ml), 120% (0.60 mg/ml) were prepared. The substance of framycetin sulfate 'Suzhou Pharmaceutical Technology CO., LTD' (China) (content of active ingredientis 654 IU/mg) was used as a working standard sample (WSS). A WSS solution of framycetin sulfate with concentrations of 0.5 mg/ml was prepared. We prepared WSS, test and blank solutions. All three flasks were simultaneously placed in a water bath for 15 minutes at a temperature of 70-80 ^{0}C. Then, the flasks were removed, cooled to room temperature and water was added, in order to bring the total volume of solution to the mark. The optical density of the obtained solutions was measured on a spectrophotometer at a wavelength of 564 nm in a cuvette with a layer thickness of 10 mm, using a blank solution as a compensation solution[8].

Results and Discussion

The obtained solutions were measured using a spectrophotometer under the optimized conditions given in the article. The obtained spectra confirm that the measurement conditions are optimized and the absorption spectrum of the test solution prepared for quantitative determination in the region from 200 to 700 nm corresponds to the spectrum of the WSS solution of framycetin sulfate and has maxima at a wavelength of 252 nm (3), 401 nm (2) and 564 nm (1).

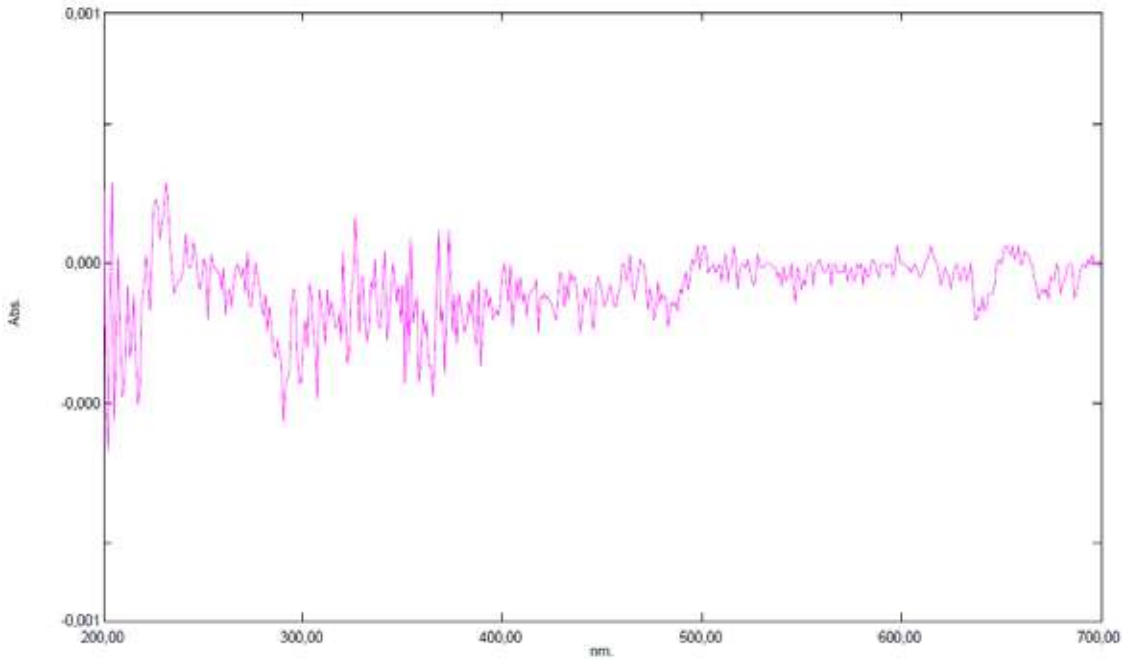


Fig. 2. Absorption spectrum of the blank solution

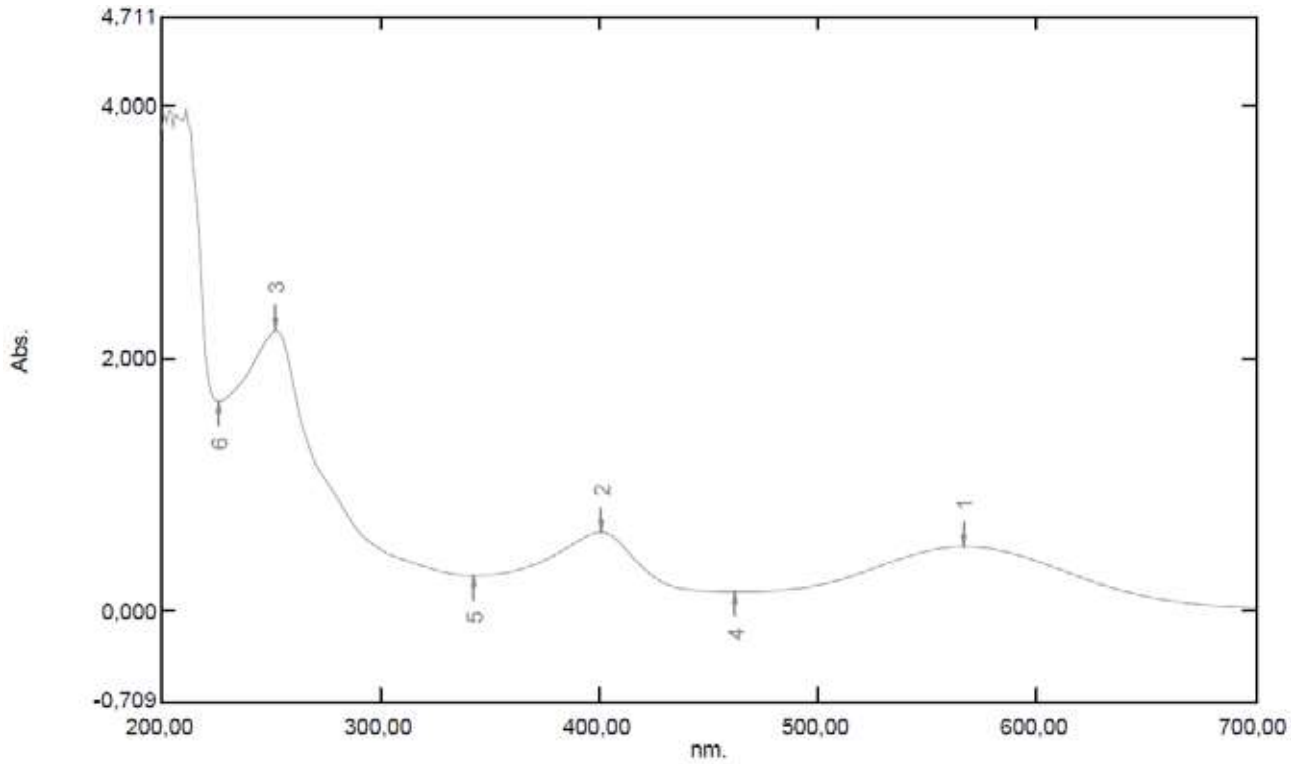


Fig. 3. WSS absorption spectrum of framycetin sulfate (C=0.50 mg/ml).

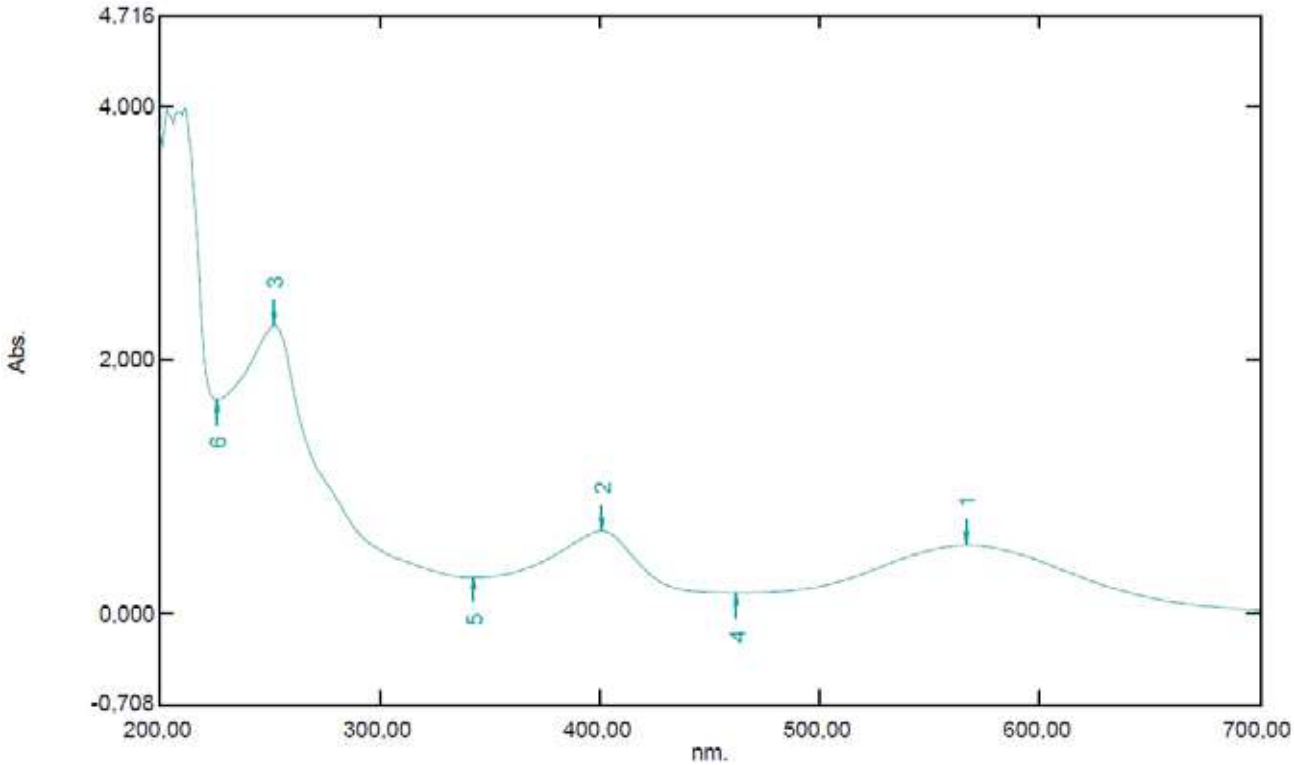


Fig. 4. Absorption spectrum of the test solution (C=0.50 mg/ml).

Fig. 1-3 shows that, under optimized conditions, the maximum values of the WSS and test solutions are identical. The developed technique indicates that the conditions for preparing samples and measuring spectra for determining the quantitative content of framycetin sulfate are specific.

Specificity. The blank solution, standard solution and pharmaceutical product were measured. The optical density of the samples was measured at a wavelength of 564 nm.

Assessment criteria: neither solvents nor reagents used for sample preparation should distort the result.

Linearity. We prepared and measured solutions of the drug with an active ingredient concentration in the range from 80 to 120%: 2 solutions with an active ingredient concentration of 80%, 2 solutions with an active ingredient concentration of 90%, 2 solutions with an active ingredient concentration of 100%, 2 solutions with a concentration active ingredient of 110% and 2 solutions with an active ingredient concentration of 120%. The linearity results are shown in Table 2.

Based on the obtained data, a dependence of optical density on the concentration of framycetin sulfate in solution can be found. The program also draws a trend line and determines the regression equation.

Table 1: Results of linear dependence of optical density on the concentration of framycetin sulfate

No.	Concentration level, %	X (Weigh of drug in mg/ml)	Y (Optical density, A.U.)
1	80	0.4012	0.283
2	80	0.4007	0.281
3	90	0.4501	0.315
4	90	0.4509	0.316
5	100	0.5006	0.352
6	100	0.5000	0.350
7	110	0.5499	0.386
8	110	0.5504	0.388
9	120	0.5993	0.421
10	120	0.6001	0.423
Average		0.5003	0.352

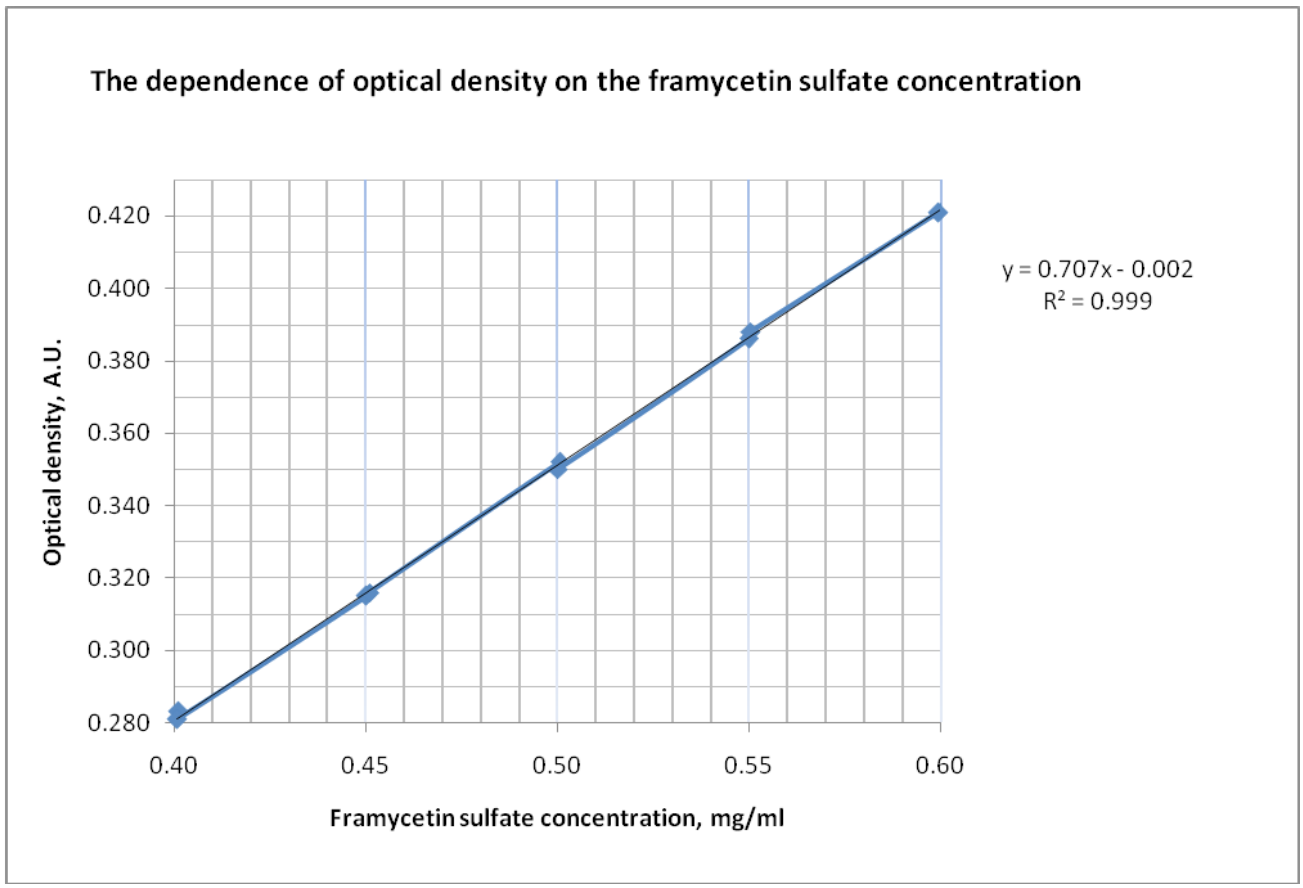


Fig. 5. Graph of the linear dependence of the optical density on the concentration of framycetin sulfate.

Criteria for assessing the linearity: correlation coefficient ≥ 0.99.

Correctness. We prepared and measured drug solutions with an active ingredient concentration in the range from 80 to 120%: 3 solutions with an active ingredient concentration of 80%, 3 solutions with an active ingredient concentration of 90%, 3 solutions with an active ingredient concentration of 100%, 3 solutions with a concentration active ingredient of 110% and 3 solutions with an active ingredient concentration of 120%. The correctness results are shown in Table 2.

Table 2: Results of determining correctness for the developed methodology

Concentration level, %	Optical density, A.U.	Specific content of framycetin sulfate, IU/ml	Specified content of framycetin sulfate, IU/ml	Response, %
80%	0.283	6493.4	6400.0	101.46
80%	0.281	6447.5	6400.0	100.74
80%	0.280	6424.6	6400.0	100.38
90%	0.315	7227.7	7200.0	100.38
90%	0.316	7250.6	7200.0	100.70
90%	0.314	7204.7	7200.0	100.07
100%	0.350	8030.7	8000.0	100.38
100%	0.352	8076.6	8000.0	100.96
100%	0.353	8099.6	8000.0	101.24
110%	0.386	8856.8	8800.0	100.64
110%	0.388	8902.6	8800.0	101.17
110%	0.386	8856.8	8800.0	100.64
120%	0.421	9659.8	9600.0	100.62
120%	0.423	9705.7	9600.0	101.10
120%	0.422	9682.8	9600.0	100.86
Average				100.76

We performed statistical processing of the obtained t_{kp} data, calculated the average value, the common standard deviation, the coefficient of variation, and the relative confidence interval. The results are shown in Table 3.

Table 3. Results of static processing of correctness determination

Statistical characteristic, %	Results
Average value	100.76
Standard deviation	0.3789
Coefficient of variation	0.3761
Lower limit of the confidence interval (P=95%)	100.55
Upper limit of the confidence interval (P=95%)	100.97

Criteria foraccessing correctness: average value 97.5-102.5%, coefficient of variation ≤ 2.0 %, confidence interval should include 100%.

Repeatability. For the study, 6 solutions of the drug at 100% concentration of the active ingredient were prepared. The analysis was carried out on an interday basis for each sample, by two different analysts, using different measuring glassware. The repeatability results are shown in Tables 5 and 6:

Table 4. The results of the determination of repeatability obtained by the chemist-1 (n≥6; P=0,95)

Sample No.	Optical density, A.U.	Specific content of framycetin sulfate, IU/ml	X_i-X_{cp}	$(X_i-X_{cp})^2$
1	0.352	8076.6	7.6483	58.4968
2	0.352	8076.6	7.6483	58.4968
3	0.351	8053.7	-15.2966	233.9872
4	0.352	8076.6	7.6483	58.4968
5	0.351	8053.7	-15.2966	233.9872
6	0.352	8076.6	7.6483	58.4968
Average		8069.0		

Table 5. The results of the determination of repeatability obtained by the chemist-2 (n≥6; P=0,95)

Sample No.	Optical density, A.U.	Specific content of framycetin sulfate, IU/ml	X_i-X_{cp}	$(X_i-X_{cp})^2$
1	0.350	8030.7	-15.2966	233.9872
2	0.351	8053.7	7.6483	58.4968
3	0.351	8053.7	7.6483	58.4968
4	0.350	8030.7	-15.2966	233.9872
5	0.351	8053.7	7.6483	58.4968
6	0.351	8053.7	7.6483	58.4968
Average		8046.0		

We performed statistical processing of the obtained t_{kp} data, calculated a single standard deviation relative confidence interval. The results are shown in Table 6.

Table 6. The results of static processing of the definition of repeatability

Statistical characteristic, %	Results of Chemist-1	Results of Chemist-2
Lowest value, IU/ml	8053.7	8030.7
Highest value, IU/ml	8076.6	8053.7
Average value	8069.0	8046.0
Standard deviation	90.6228	90.6228
Coefficient of variation	1.1231	1.1263
Lower limit of the confidence interval (P=95%)	99.67	100.58
Upper limit of the confidence interval (P=95%)	102.05	100.58
t_{kp} (95% and N1+N2-k=10) 2.228	1.43	
F(95% f1=N1-1;f2=N2-1) 5.05	1.00	

Criteria for accessing repeatability and ruggedness: coefficient of variation ≤1.5% (n≥6), confidence interval F(5%,5,5): ≤ 5,05, t(5%,10): ≤ 2,228.

Conclusions

The method of quantitative determination of framycetin sulfate in the drug 'Framex' has been developed and validated using SF method. Based on the results of the intra-laboratory experiment, it has been found that the metrological characteristics of validation parameters as correctness, specificity, repeatability, linearity and ruggedness do not exceed the validation criteria. The stability of the method can be reproduced in the laboratory. The deviation of a single value is 100.7% with a confidence probability of 95%.

Ethical Clearance: No ethical approval is needed.

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Comprehensive Integrated Assessment of Health, Lifestyle and Medical Services for Children at the Age of their First Year

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Abstract

Introduction: Children are the main value of any society, which plays a huge role in its development and prosperity. Meanwhile, they are the most vulnerable sector that have to be protected from negative physical and mental factors as well as diseases. A wide range of factors can affect the health of children, especially in their first year of life. Deep knowledge on those triggers may help to reduce the risk of developing different pathological conditions among them.

Materials and Method: An integrated analysis and risk assessment of different pathologies in 767 outpatient children under 1 year were cunducted. A survey of the parents of those children was also conducted. The objectivity of the information obtained is guaranteed by the directed application of the method and techniques used in public health and healthcare, namely, medical-statistical analysis, sociological, psychological and expert analysis.

Results: Many childrenhad a low risk of developing chronic diseases, including those with a hereditary predisposition and had a IIB health group, these are children with background of conditions and risk factors that can cause the formation of a chronic pathology. The third group of healthy children was represented by children with chronic pathology in the stage of compensation, the number of such babies was small (5.9±0.85%), as well as healthy babies with I (6.0±0.86%) and II A (4.2±0.72%) by health groups. Furthermore, more than half of the children of the first year of life had a normal functional state of the body. However, in 43% of cases, the functional state was impaired, which is manifested by the presence of various pathological conditions.

Conclusion: Comprehensive integrated approach and using evidence-based medicine principles for studying the medical conditions in children under 1 year-old can be valuable and help us to obtain reliable data on the health conditions and affecting risk factors.

Keywords: *Children of the first year of life, functional state, health group, medical care, evidence-based medicine.*

Introduction

Maintaining the health of a child is an essential part of the task of ensuring the health of a nation, which reduces the creation of necessary set of conditions and an overall improvement in the quality of life, politically and legally constituting one of the foundations of national security (De Jong et al, 2017)[8]. In general, we are talking about a set of special measures that protect the health of children, their development, starting from birth. Children are the main value of society, contributing to its development and prosperity, but at the same time they are the most vulnerable part, which needs protection from diseases, physical and mental factors.

Recent decades have determined the need for a transition to more modern, cost-effective and effective preventive technologies for maintaining and optimizing the health of the child population, especially children from birth to 1 year. Among all age groups, special attention should be paid to young children, since it is during this period that risk factors can be realized and

various chronic pathologies can form (Da Cunha et al, 2016)[7]. A variety of factors can influence the health of a child in its first year of life, the knowing those triggers may help to reduce the risk of developing different pathological conditions.

During the last few years, many studies have been published in the literature that reflect this topic of issues. Perinatal damage to the central nervous system (CNS) is the most frequently detected pathology in children of the first year of their life (Sovetkhan et al, 2019)[3].There is evidence of a relationship between central nervous system prenatal damage and somatic pathologies, as well as the negative impact of prenatal injuries of central nervous system on neuropsychic development and mental health of children (Dvorkina et al, 2015)[2].

Rickets is the most common background disease in young children (TT C, 2016)[6]. However, due to several reasons, the diagnosis of rickets is not always established by local pediatricians. Yerenkov et al. (2016) suggests using ultrasonic densitometry in order to increase the efficiency of diagnosis of rickets in young children.

Another common background disease is iron deficiency anemia (IDA) among children (Dvorkina et al, 2015)[2]. Ivanova et al. proved that there was a link of between iron deficiency anemia with a slowdown of psychomotor development in children of the first year of life. It has been proven that anemia with its untimely and inadequate treatment can irreversibly affect cognitive functions (Peterkova et al, 2016).

An electrocardiographic study is widely implemented in diagnostic algorithms for assessing the health status of young children. When studying the state of the cardiovascular system in children of the first year of life, Kharlamova et al. detected posthypoxic cardiopathy in 76.4% of children, however, in half of them, it's the symptoms extinguished by the age of one month (Bezler & Galitsa, 2015)[1]. Congenital heart defects are also diagnosed frequently in the early neonatal period (Peterkova et al, 2016)[3]. An echocardiographic examination of 1012 children in the first half of life indicates a high incidence of minor cardiac abnormalities, in particular, additional trabeculae in the cavity of the left ventricle. In the study of Kuleshova et al (2014), a high frequency of functional disorders of the digestive tract, especially in children in the first half of life was proved. The health of children in their first year of life largely depends on the type of feeding. Kashin et al. (2005), analyzing the physical development of children of the first year of life depending on the type of feeding, showed that children who were on early artificial feeding had higher weight growth rates by the end of the first year of life. However, in the group of babies who were breast-fed for a long time, a mesosomatic body type (85.3%) with harmonious development (84.1%) was more often observed. Similar data was presented by other authors (Puzankova et al, 2015; Savchenko et al, 2015). (De Jong M CATJvWM. 2017;Atyasova et al 2005)[8] showed that infants who were breast-fed are 1.8 times more likely to be diagnosed with dystrophy, 2 times more likely to have deficiency anemia, and 1.7 times more purulent-septic diseases compared to breastfed babies[1].

Legonkova et al. (2011)[10] conducted a multicenter open comparative prospective study, which aimed to study the health status of children who were on different types of feeding. As a result of long-term observation, it was shown that children receiving breast milk had higher rates of physical and neuropsychic development, a lower incidence of acute respiratory diseases and atopic dermatitis.

Clinical examination is a dynamic monitoring of the level of development and the state of health (of children) with the aim of early diagnosis of initial deviations, timely correction as well as providing the conditions necessary for an optimal state of health and development (Bezler & Galitsa, 2015)[1].

The main methodological principles of dispensary observation in the first year of life are as follows: 1) continuous coverage of children living in the polyclinic service area, the unity of the preventive and medical work of the district doctor; 2) differentiated monitoring of children and preventive, improving, curative measures, depending on the individual characteristics of the child; 3) the leading role of the district children's clinic (or district central hospitals) in the follow-up with the wide involvement of advisory centers; continuity between institutions of various profiles in the examination and management of children; 4) a prognostic approach in assessing the capabilities of the immediate and distant level of health and development of the child; 5) the introduction of new non-traumatic method of preclinical diagnosis and computer monitoring (integrated automated systems) of medical examinations and the dynamics of children's health indicators.Thus, the only way to improve the health of young children, reduce their morbidity and disability is to fully strengthen the

state pediatric service and its central link responsible for medical examination - children's district polyclinics - with the widest participation of specialists from research institutes and departments of pediatrics in medical examinations of the pediatric population, pediatric centers.

Purpose of the study is to develop recommendations for improving the treatment and preventive care for children in their first year of life based on a comprehensive study of their health status and lifestyle.

Materials and Method

To achieve this goal, it is necessary to identify the risk factors for morbidity and mortality in children of the first year of life. It is also need to be identified the combinations of risk factors that are most unfavorable from the point of view of the development of morbidity and the occurrence of a "death case" in children of the first year of life. As for the interaction of risk factors with each other, as well as with factors that favorably affect the child's body, these issues have been studied extremely insufficiently. An increase in the incidence of the disease, an increase in the proportion of children often ill in the structure of the incidence of children in many countries, are attributed by most authors to changes in the conditions and lifestyle of families. The study of the interaction of health and lifestyle with the use of multivariate analysis can make a significant contribution to the development and improvement of the primary and secondary prevention of children. The approaches to choosing an object of study for studying the lifestyle on the health of children in their first year of life can be different, and each approach, in our opinion, has a number of advantages and disadvantages. The elimination of these shortcomings can be promoted by a combination of separate approaches, each of which involves solving a separate problem. An integrated approach to the selection of the object of study, in our opinion, makes it possible to obtain more informative data. In this article we will consider some approaches to the selection of the object of socio-hygienic research, the influence of the lifestyle of the development of diseases and the occurrence of a "death case" of children of the first year of life. First of all, it is necessary to decide which group of children should be examined to identify risk factors for children's health. Risk factors for disease, death act on the body until the moment when an accurate diagnosis is made or the causes of death are identified.

From our point of view, their identification can be approached in two ways: by conducting a retrospective study using the analytical method and by prospectively studying risk groups selected taking into account known factors. The first of these approaches is used more often and is technically simple. In this case, the examined group of children (patients - often ill) is formed with a confirmed, specified and final diagnosis or cause of death. A control group may be - health of children (who have never been ill for a year) of the same age and gender.

To characterize the choice of research base, from our point of view, is essential. The volume (n) and sampling method (cluster, stratified, random mechanical, randomized) were determined. The second approach to the selection of the object of study is based on known risk factors for morbidity and causes of death in children of the first year of life. Since the number of these factors is very significant, it is necessary to consider which of them should be relied upon in the selection of the studied group of children to study the influence of lifestyle, diagnosis on morbidity, or causes of death of children. For example: rest and nutrition of pregnant women, body weight at birth, the nature of feeding, may themselves be the result of a particular lifestyle and, therefore, are not suitable for the selection of the studied group.

Study Population: Based on the foregoing, 767 outpatient records of children under 1 year were analyzed. A survey of the parents of those children was also conducted. The objectivity of the information obtained is guaranteed by the directed application of the method and techniques used in public health and healthcare, namely, medical-statistical analysis, sociological, psychological and expert analysis.

In our opinion, for the selection of the examined group in order to prospectively study the impact of lifestyle on children's health, it is possible to use factors such as the mother's age (up to 20 years), the body weight of the child at birth (up to 1,500 grams), the sex of the child, the order of birth a child.

In this case, control groups are selected in accordance with which of the factors is taken as the basis for the choice of the examined group. So in the control group can include healthy children born to mothers whose age was 21-24 years old, born from 2-3 pregnancies, with a body weight at birth of 3000-3499 gr. So, the control group should correspond to the subject - by age, gender,

place of residence, examination of children in family clinics. In the study, it should be kept in mind that often male children born from mothers up to 20 years old, from the first birth, with low birth weight, who were breastfed, are ill. One of the criteria for children's health is the assessment of ontogenesis features.

In the study, the risk group selected by such factors as the age of the matter, gender, the child's birth order, and body weight of the child at birth should be used to compare the lifestyle of children who are often ill and who have never been ill during the study period, and observation should be carried out from their birth and during the first year of life.

Statistical Analysis: For statistical processing and analysis of the obtained data, personal computers of the Intel Centrino type (Core2 Duo) and Microsoft Office 2007 packages were used.

Results

In order to evaluate ontogenesis, genealogical, biological, and social anamnesis were collected, and the burden coefficient of the genealogical anamnesis in children of the first year of life was calculated and gained the results as follow (Table 1).

Table 1: The structure of the genealogical history in children of the first year of life by burden ratio (M±t, %)

Burden coefficient	Children under the study n = 767
Low	58,03±1,80***
Moderate	41,97±2,73
Significant	-
High	-

Note: *** - p <0.001

The table shows that most children had a low risk of developing chronic diseases, including those with a hereditary predisposition.

As a result of a comprehensive assessment of the health status of children in the first year of life, a health group was determined (Table 2).

Most of the children under one year old had a IIB health group, these are children with background of conditions and risk factors that can cause the formation of a chronic pathology. The third group of healthy children was represented by children with chronic pathology in the stage of compensation, the number of such babies was small (5.9 ± 0.85%), as well as healthy babies with I (6.0 ± 0.86%) and II A (4.2 ± 0.72%) by health groups.

Table 2: The health group of children aged 1 year (M±t, %)

Healthy group	Children under the study n = 767
I (1)	6,0±0,86
IIA (2)	4,2±0,72
IIБ (3)	83,9±1,32***
III (4)	5,9±0,85
IV (5)	-
V (6)	-

Note: *** - p1-3, 2-3, 4-3 <0.001

The level of the functional state of the body of children is reflected in the table. 3.

Table 3: The functional level of children in their first year of life (M±t, %)

Functional condition level	Children under the study n = 767
Normal	56,9±1,80***
Poor	43,1±1,80
Worse	-

Note: *** - p <0.001

The table shows that more than half of the children of the first year of life had a normal functional state of the body. However, in 43% of cases, the functional state was impaired, which is manifested by the presence of various pathological conditions. So, small abnormalities of the development of the heart (false chord, open oval window) and pyeloectasia were found in 63.1 ± 1.76% of infants. Perinatal damage to the central nervous system was diagnosed in 65.1 ± 1.72% of children, possibly due to a deterioration in the health status of expectant mothers and the pathological course of pregnancy. Almost every fifth child of the first year of life has atopic dermatitis (19.6 ± 1.43%) and iron deficiency anemia (21.3 ± 1.48%). Rickets was diagnosed in 10.3 ± 1.10% of infants, dystrophy by type of malnutrition - in 7.6 ± 0.95%. 5.5 ± 0.82% of children in the first year of life were observed by an infectious disease specialist for intrauterine infection. Only 7.2 ± 0.93% of children under one year-old did not have background diseases. All this raises questions about the additional search for risk factors for the development of these diseases and the improvement of preventive measures.

Thus, as a result of a comprehensive study of the health status of children of the first year of life living in Tashkent, it was found that the IIB health group prevails (83.9 ± 1.32%), most children have a low risk of developing chronic diseases, including with a hereditary predisposition (58.3%), a good level of resistance (87.3%) and the normal functional state of the body (56.9%).

However, the presence of babies with a retardation in neuropsychic development (36.0%), congenital malformations (63.1%) and weight loss (8.0%) pose new challenges for the outpatient service in terms of strengthening pedagogical and psychological support for families with small children, more effective interaction with obstetric care services and improvement of medical check-ups for children in their first year of life.

The number of children with the first health group during the year ranged from 0 to 3%. Upon discharge from the maternity hospital 2A, a sufficiently large number of newborns had a health group ($p < 0.01$). Subsequently, in the process of neonatal screening and clarification of the anamnesis, the number of children with a 2A group of health significantly decreased, without significant differences (Fig. 1).

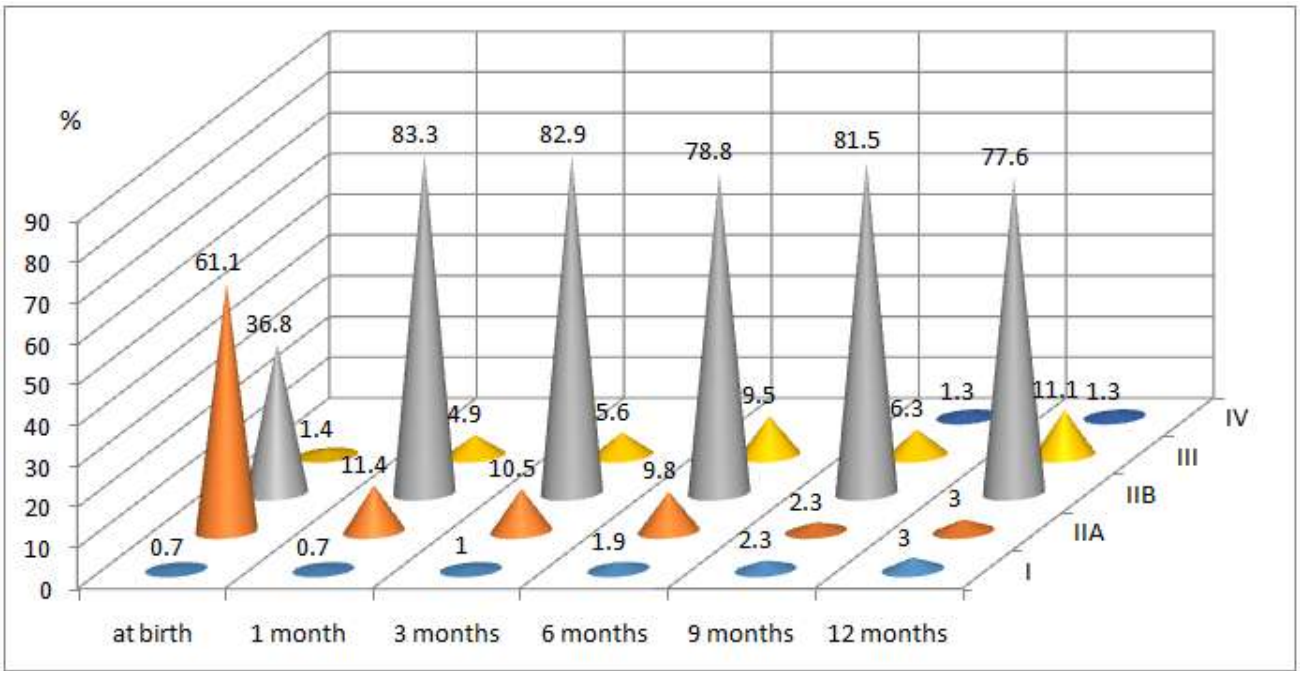


Fig. 1. Dynamics of monitoring children depending on the health group during the first year of life

The number of children with the third group of health did not exceed 13%. At the age of 12 months, children with chronic pathology in the compensation stage were found in 11.1% of cases. The fourth group of health was observed in one baby (1.3%).

Discussion

Analysis of scientific and medical publications on the topic of the study indicates that children in their first year of life, their state of health, taking into account the importance of socio-hygienic factors, are a fairly common object of scientific research. However, to date, a comprehensive study of the health status of children of the first year of life has not been carried out taking into account indicators of the physical development of morbidity and mortality, health levels of children have not been developed and determined, taking into account complex factors, assessment tables of physical development have not been studied and developed, and children's health has not been established taking into account the leading factors of lifestyle and the quality of medical care. Treatment and prophylactic and organizational and methodological measures have not been developed to improve the health status and differentiated medical care of children in their first year of life, taking into account a set of factors affecting their health.

The literature mentions a significant number of risk factors for children's health, the identification of which is carried out mainly by comparing the frequency of the studied symptoms in the group of patients and healthy children (Bezler & Galitsa, 2015; Sovetkhan et al, 2019)[1,3]. In this case, the question arises about the specificity of individual factors, which to date cannot be considered resolved (Dvorkina et al, 2015; and Peterkova et al, 2016)[2,3].

On the basis of the data obtained, it was established that the family history had a low coefficient of burden. The adaptation period in the maternity ward in newborns proceeded, as a rule, without pathology ($p < 0.05$). Most of the newborns discharged from the hospital had II A health group ($p < 0.01$).The number of children with the first group of health during the year was insignificant (0.7%), group II A in the process of neonatal screening and clarification of the anamnesis significantly decreased from 61.1% upon discharge to 7% at 12 months of a child's life. Number children from group IIB by the end of 12 months of life increased almost 2 times ($P < 0.05$) from indicators at birth. A similar picture was noted for the III group of health ($P < 0.05$).

However, in this case, the influence of lifestyle cannot be ruled out, therefore it is advisable to conduct a comparative study of the influence of several complex lifestyle factors in children of the first year of life, to eliminate the influence of previously known risk factors for morbidity and mortality in children. A comparison of the risk factors for morbidity and mortality of children and their interaction as a cause and effect for various approaches to the selection of an object, the study will allow us to determine with sufficient accuracy the most significant factors and their unfavorable combinations, and to develop recommendations on this basis for the primary prevention of children's morbidity and secondary prevention of causes death of children. Prevention is carried out by the family in which the child lives and primary health care institutions (SP, SVP, Central District Hospital, Central City Hospital, etc.), each of which contributes to this great business in accordance with its capabilities.

In order to develop specific recommendations for the family and medical institutions for the formation and upbringing of a healthy child, it is necessary not only to identify risk factors for children's health, but also to carefully study the contingent of sick children and the features of the work of outpatient and inpatient facilities, the relationship, and the continuity in their conduct primary and secondary prevention of morbidity in children.

Conclusion

Thus, a comprehensive integrated approach and using evidence-based medicine principles for studying the medical condition of children under 1 year-old in conjunction with their lifestyle factors will make it feasible to obtain the most reliable data on the state of health and affecting risk factors. Moreover, this method helps us to develop evidence-based recommendations for primary and secondary prevention of diseases that cause of postnatal death.

Study Limitations: The study was conducted on children living in Uzbekistan. Therefore, some of the results and outcomes of the study are specific for locals only. Thus, if needed, further researches can be conducted worldwide or in certain areas.

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Ethical Approval: The ethical approval for the study was granted by the Committee of Ethical Approval for Researches under the Ministry of Health of the Republic of Uzbekistan.

Consent: Written informed consent was obtained from all participants' parents of the research for publication of this paper and any accompanying information related to this study. A copy of the written consent is available for review by the authors.

Conflict of Interest: The authors declare that they have no competing interests.

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Walkability Index Based on Pedestrian Needs in the Losari Beach area of Makassar City

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Abstract

Walkability Indexis the result of measuring the comfort level of pedestrian paths which will be a benchmark for developing pedestrian paths that are feasible for everyone. Jpedestrian paths (sidewalks) can be said to be feasible if they can meet pedestrian needs based on several criteria such as accessibility, circulation, safety, security, beauty, cleanliness, noise, shade, and connectivity. The purpose of this study is to obtain a pedestrian comfort index based on pedestrian needs in the Losari Beach Area and Fort Rotterdam. To produce this comfort index, it is necessary to identify the factors and measure the level of comfort based on pedestrian needs, and to determine the priority of pedestrian needs..The pedestrian path available in this area is known as "Bundaratta", located in the recreation/natural tourism area (Losari Beach) and cultural tourism (Fort Rotterdam) people of Makassar City. With the function of the land as a recreation area, a comfortable pedestrian path is urgently needed by local and foreign tourists to enjoy and reach the existing facilities around the area. This research is a quantitative descriptive study, using a Likert scale to obtain a level of comfort and the Analytical Hierarchy Process (AHP) to obtain priority for the comfort factor. The results showed that the pedestrian comfort index along Losari Beach to Fort Rotterdam is 60.20 and it is in the comfortable category, but it still needs some repairs and improvements in several location segments so that this pedestrian lane falls into the category that is truly comfortable and even very comfortable. for its users.

Keywords: *Comfort index, pedestrian path, pedestrian need.*

Introduction

The term pedestrian or pedestrian comes from the Latin word pedesterpedestris, namely people who walk or pedestrians. Pedestrian also comes from the Greek word pedos which means foot so that pedestrian can be interpreted as a pedestrian or person walking, Pedestrian is also defined as the movement or circulation or movement of people or humans from one place to the origin to another as a destination (destination) by foot(Rubenstein, 1992)[1]. Another definition of a pedestrian is a person who dominantly uses feet (walking) and/or one part of the body (for example with hands for people who do not have legs) to move from one place to another and not in a state of using a vehicle. (Pattisinai, 2013)[2].

Broadly speaking, the motivation of a person in walking, like pedestrians using the sidewalk, divided into 2 (two) types of pedestrians, namely functional pedestrians and optional pedestrians (Gatoet Wardianto, 2017)[3]. Functional pedestrian activities are routine every day or periodically and occur throughout the year, such as walking to school, to work, shopping for daily necessities which are classified as necessary activities, namely compulsory activities. Meanwhile, functional pedestrian activities are optional (optional), such as taking walks, recreation/excursions in historical areas, shopping tours, culinary tours[4], walking for health purposes and even taking walks without a specific purpose or other social activities[5].

Losari Beach area is a natural recreation/tourism area, as well as Fort Rotterdam which is a recreation/ cultural tourism area in Makassar. Pedestrian activity in the area is optional (optional). Very busy at certain times, especially on weekends or holidays. Local and foreign tourists will enjoy the natural beauty of the Losari beach and see the historical buildings of Fort Rotterdam. These

places can be reached by foot along the pedestrian path "Bundaratta"[6].

A. Pedestrian Need Concept: Alfonso (2005)[7], states that pedestrian activity is based on 5 (five) factors of human needs, namely the feasibility factor, the accessibility factor, the safety factor, the comfort factor and the pleasurability factor. The feasibility of a pedestrian path (sidewalk) can be measured from the level of fulfillment of pedestrian needs based on 8 (eight) criteria, namely mobility, accessibility, continuity, connectivity, safety, comfort, security and beauty(Gatoet Wardianto, 2017)[8]. On the other hand, Fruin (1979)[9], states that facility planning for pedestrians (sidewalks)[10] must pay attention to 7 (seven) main targets, namely safety, security, security, convenience, continuity, comfort, system integration (system coherence)[11], and the level of congestion (level of service/LOS). The seven factors are interrelated and influence each other[12].

Proper pedestrian paths or sidewalks must be able to meet pedestrians' needs, not just for walking, but also for leisure, walking, (strolling), and interacting with other pedestrians. (Gatoet Wardianto, 2017)[13]. A well-designed built environment without the presence of pedestrians is not an active social place with a high level of community. This will cause loss of vitality and livability in the area(Rafiemanzelat, Emadi, & Kamali, 2017)[14].

B. Pedestrian Comfort Concept: Sander and Mc Cormick (1993)[15] describes the concept of comfort as a condition of one's feelings towards something and is very dependent on the person experiencing the situation[16]. In other words, comfort is the fulfillment of conditions or conditions that a person wants to stimulate which is received and caused by environmental conditions around him[17]. The desired condition or condition depends on the perception of each individual. The environmental quality of the pedestrian path will affect a person's level of comfort when walking so that in planning it is necessary to consider the human scale, good design, materials, space for walking and a good surface for walking are determinants of pedestrian comfort. (Zakaria & Ujang, 2015)[18].

The word "index" itself according to the Big Indonesian Dictionary (KBBI) from linguistics means the ratio between two certain linguistic elements that may be a measure of a certain characteristic; pointer[19]; If it is related to comfort, it can be defined that the Walkability Index is a list of measures based on the standards referred to to assess a person's comfort level[20], in this case pedestrians, to environmental conditions around the pedestrian path[21]. One of the indicators can be referenced from several studies related to pedestrian comfort on pedestrian paths that produce standards for measuring comfort levels, such as the Global Walkability Index (GWI)[22].(Habibian & Hosseinzadeh, 2018)[23]

Comfort is the main requirement in planning pedestrian or sidewalk facilities by considering pedestrian path conditions related to pavement quality, size, cleanliness, weather, availability of city facilities, circulation, even though the surrounding climatic conditions will affect them on foot.(Corazza, Mascio, & Moretti, 2016).[24]

Here are some of the factors on pedestrian needs that will affect the comfort of walking based on the opinions of several experts and researchers:

(a) Mobility, is a pedestrian requirement related to the space for pedestrians to easily carry out their activities. Pedestrian mobility is determined by the following factors: the track surface, the track width and the absence of obstructions along the sidewalk(Gatoet Wardianto, 2017)[24]. Dimensions (width) of pedestrian paths that are not optimal, uneven surfaces are a major barrier for elderly pedestrians (elderly people) because they are related to where and their safety (Strohmeier, 2016)[16]. Likewise halya with aand permanent obstructions in the form of trees and the placement of city facilities, as well as temporary barriers in the form of several peddlers, motorbike parking, pedicabs and others that will affect pedestrian comfort(Erna & Leksono, 2016)[25]

(b) Accessibility, is the need for pedestrians to access/ reach the desired destination points without obstacles and barriers. Accessibility is the key in making pedestrian paths walkable or pedestrian friendlyfeet, (Rafiemanzelat et al., 2017). Thus, the pedestrian path is easily accessible to everyone, including those with special needs, such as wheelchair users, blind people or the elderly (Zakaria & Ujang, 2015).

(c) Continuity, is the need for pedestrians to be able to carry out walking activities in a sustainable manner, not cut into pieces or stop. The available sidewalk network must ensure the continuity of movement for pedestrians(Gatoet Wardianto, 2017).

(d) Connectivity, is the complete integration of pedestrian paths with transportation modes so that a person can navigate the entire system easily (Bhattacharyya & Mitra, 2013). The unavailability of access to public transportation and the lack of connection of pedestrians and pedestrians is an obstacle to pedestrian mobility (Strohmeier, 2016)

(e) Safety, is the need for pedestrians to avoid traffic accidents. The use of traffic control signals or by pedestrian traffic light signals will create a safe zone between cars and pedestrians, thereby reducing the risk of conflicts and accidents. (Dorohin, Zelikov, & Denisov, 2018). Especially at crossings and road junctions, these control signals are needed to ensure pedestrian safety Elderly(Kim, 2019). The existence of information signs is very useful for pedestrians to provide an overview of the availability/direction of facilities in the area being traversed (Koh Puay Ping & Wong, 2013)

(f) Convenience is a pedestrian need related to climate and weather conditions (heat, sun, wind and rain) as well as the physical conditions of the pedestrian path. Protection can be done by placing trees, shelters, benches or other objects in certain locations that can be used to sit if pedestrians feel tired or just to enjoy the view(Gatoet Wardianto, 2017).

(g) Security is a need for pedestrians to avoid fear of crime that can threaten their lives. The CPTED (Crime Prevention Through Environmental Design) concept is a concept that was built in a certain way to reduce or prevent crime or crime.(Gatoet Wardianto, 2017). Adequate lighting arrangements are also an important factor for attracting visual interest and preventing nighttime crime.(Bhattacharyya & Mitra, 2013)

(h) Beauty is the need for pedestrians to get a pleasant physical atmosphere or environment when doing their activities on foot on the pedestrian path.Trees and parks along the pedestrian path, as well as the scenery around it is what attracts pedestrians. (Koh Puay Ping & Wong, 2013). In this case the role of architects and urban designers in designing streetscapes will be able to refresh and rejuvenate tired pedestrians.(Bhattacharyya & Mitra, 2013).

(i) Circulation is rotation or circulation. This is closely related to the dimensions of the pedestrian path. Comfort can be reduced due to poor circulation, such as the absence of a clear division of space for the circulation of humans and motorized vehicles. Pedestrian circulation is closely related to the dimensions of roads and pedestrian paths, the place of origin of circulation and the exact destination of pedestrian circulation, the purpose of travel, the time of day and the volume of pedestrians.(Ashadi., Rifka H., 2012).

(j) Nature or Climate Style, is a natural condition and climate that occurs at one time. Condition Weather such as rain, wind and temperature have a significant impact on pedestrian behavior as this will influence people's interest in walking(Arana et al., 2014).Modification of city infrastructure, in this case pedestrian paths in accordance with local climatic conditions, will attract pedestrians to continue walking in all seasons and throughout the day.(Shaaban & Muley, 2016). The existence of a shelter such as a gazebo or sheter and vegetation in addition to having aesthetic qualities, plays an important role in urban sustainability, because it has a positive influence on climate and pollution prevention.(Lamour, Morelli, & Marins, 2019).

(k) Fragrance or smell,Bad smells or odors are generated by untreated trash cans, open drainage (gutters) around and odors from motor vehicle exhausts. This unpleasant odor will be smelled by pedestrian path users so that it will reduce pedestrian comfortHygiene and maintenance factors are related to the presence of garbage, or maybe the pungent odor it causes will affect a person walkingi(Erna & Leksono, 2016)

(l) Noise, Noise is unwanted noise in a certain level and time and can cause disturbances to human health and environmental comfort. The permissible noise level for urban areas, especially for trade and service areas and for recreation, ranges from 65 - 70 dB.To minimize, plants are needed as a barrier around the pedestrian path.The arrangement of plants on the sidewalk (on the side of the road) is adjusted to the width of the land, starting from the shoulder of the road to the channel threshold or the Rumija limit. This can reduce the amount of air pollution or noise(Natalia Tanan & Gede Budi Suprayoga, 2015).

(m) Shape,The shape of the pedestrian path design must be adjusted to human standards in order to create a sense of comfort. Wide sidewalks, free from obstacles, freedom of walking, with good quality

coating, green and leafy to provide safety and comfort to users(Lamour et al., 2019). Research results from R. Projadi, Sangkertadi and RC Tarore (2014) revealed that there are 4 (four) physical characteristics of the pesedrian lane that will affect pedestrian comfort, namely shape, dimensions, color and texture.

(n) Cleanliness,is a state free from dirt, including something clean in addition to adding to the attractiveness of the location, it also adds to the feeling of comfort because it is free from garbage and eliminates the unpleasant odors it causes. among them, dust, trash, and odors. Something clean that will add attractiveness as well as convenience for pedestrians (Feybe G. Kaliongga., Veronica A. Kumurur., 2014). It is necessary to arrange and place trash bins on the pedestrian path, the number and distance of every 20 meters at meeting points such as intersections with the size adjusted to the needs. The materials used are materials with high durability such as metal and printed concrete(Ministry of PUPR, 2018)

(o) Lighting, Sufficient lighting for pedestrian paths is a means to increase security against criminal threats in order to create comfort on pedestrian paths (Feybe G. Kaliongga., Veronica A. Kumurur., 2014). Lighting is needed for safety, comfort and aesthetics(Muhammad Muslihun, 2013). The location of the lighting on the pedestrian path is placed every 10 meters with a maximum height of 4 meters, and the materials used are materials with high durability such as metal & printed concrete. (Ministry of PUPR, 2018).

(p) Supporting Elements,Pedestrian path design is no longer oriented to the aspect of beauty alone, but rather focuses on the comfort felt by pedestrians. For this reason, complementary elements of pedestrian paths are needed, including paving, lights, signs, sculptures (vocal points), bollards, benches, shade plants, telephones, kiosks, shelters and canopies, clocks, trash cans, stops and utilities. (Rubenstein, 1992).

This study will use the following variables:

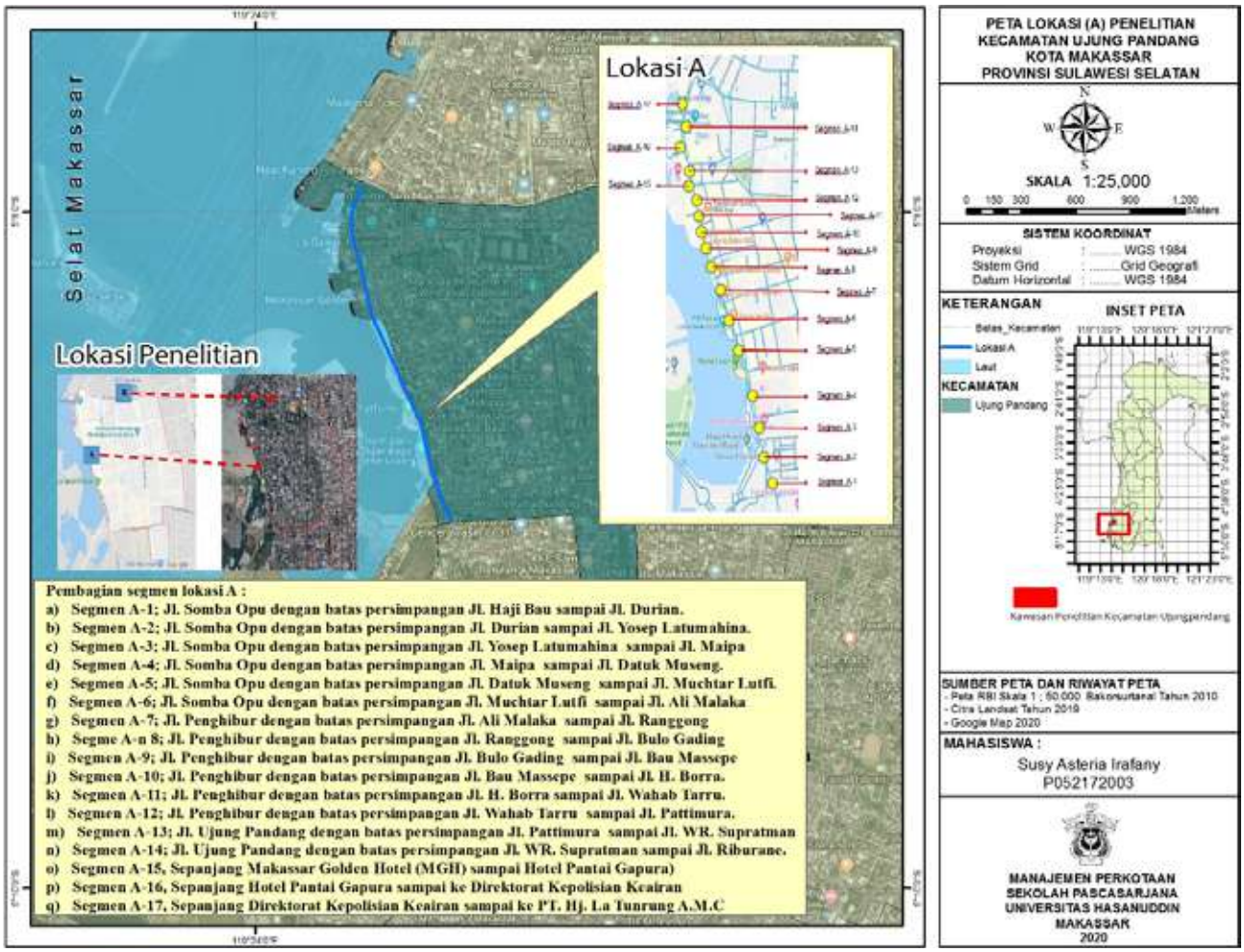
Table 1. Variables and sub-variables for determining the pedestrian lane comfort index

Variable	Sub Variable	Source
Accessibility	• Facilities for pedestrians with special needs • Ramp and Marka	Untermann (1984), R. Judge (2002), Alfonso (2005), Rubenstein (1992) and G. Wardianto (2017)
Beauty	• Pedestrian path material • Garden/Flower Pot • Seat	
Circulation	• Pedestrian path dimensions • The existence of obstructions on the pedestrian path	Untermann (1984) and R. Judge (2002)
Shade	• Shelter/shelter • Vegetation/shade plants	
Cleanliness	• The quantity and quality of bins • Cleanliness level	
Security	• Security systems (CCTV, security posts) • Pedestrian path lighting	Untermann (1984) R. Judge (2002), Alfonso (2005) and Krambeck (2006)
Safety	• Differences in the level of the pedestrian path and the road body • Availability of markers and signs/signals for pedestrian paths • Pedestrian path conditions • Material surface texture • The crossing	Krambeck (2006), Rubenstein (1992) and G. Wardianto (2017)
Noise	Noise canceling facilities	R. Hakim, 2002
Connectivity	Continuity of pedestrian paths Facilities move between modes of transportation	G. Wardianto (2017)

Source: Author's Analysis, 2020

A. Research Method

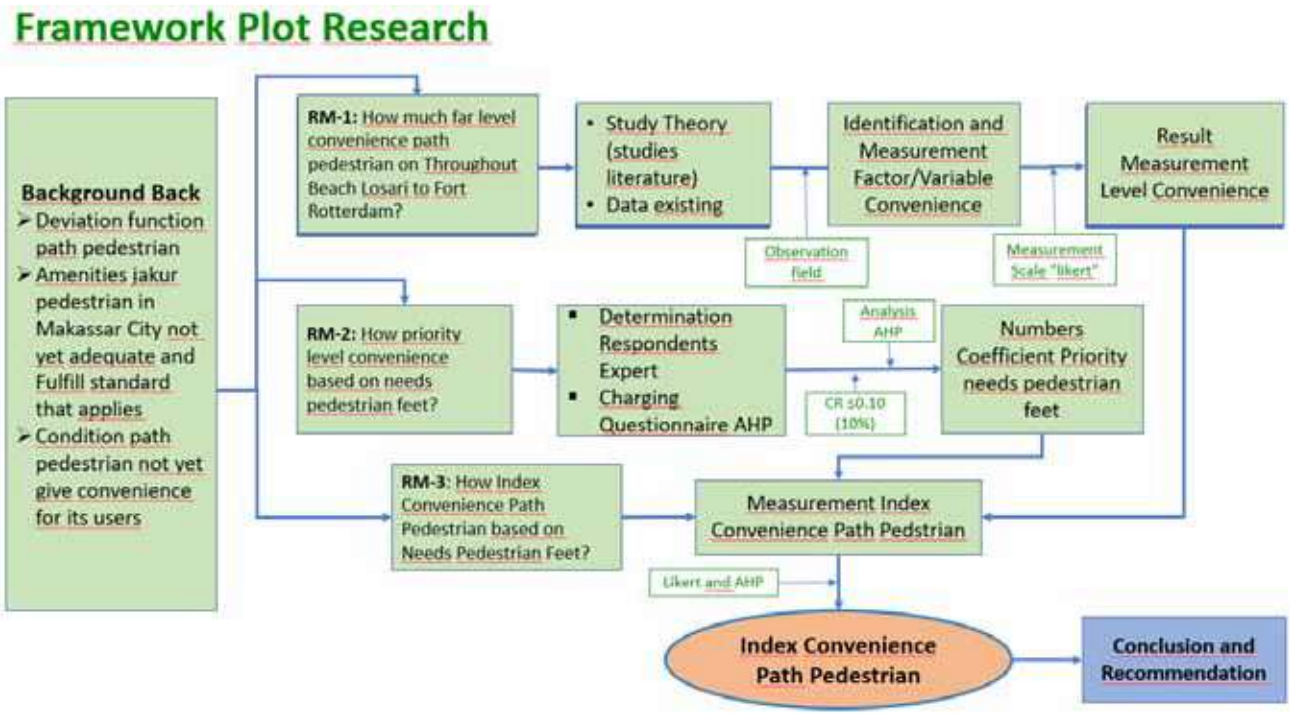
Research Sites: The object of this research is the pedestrian path along Losari Beach to Fort Rotterdam, Makassar City. As for asite selection welds. This is based on the consideration that natural and cultural recreation areas are quite dense with pedestrian activities. This pedestrian path is long enough so that to obtain more valid data, the pedestrian path is divided into 17 (seventeen) pedestrian lane segments.



Source: Author, 2020

Figure 1. Segment Division of Research Sites

Data Collection and Analysis Techniques: This type of research is a quantitative descriptive study with the Analytical Hierarchy Process (AHP) analysis technique. Primarydata collection techniques are carried out through direct observation in the field, structured interviews using a questionnaire and documentation in the form of recorded images/photographs related to the research location. All data related to variables/sub variables will be assessed based on a Likert scale (0 - 5) to obtain a level of comfort. Whereas for priority determination, the variable assessment was assessed by 7 (seven) expert respondents and the data obtained would be processedusing the Analytical Hierarchy Process (AHP) method and the results will be tested for the level of consistency with limitsThe maximum value of Consistency Ratio ≤ 0.1 or 10%. Walkability Index is obtained by multiplying the AHP weighting coefficient with the results of measuring the comfort level of each variable.



Source: Researcher Analysis, 2020

Figure 2. Research Framework

B. Research Results and Discussion:

Measurement of Pedestrian Path Comfort Level: The comfort level of the pedestrian path along Losari Beach to the Fort Rotterdam is measured using variables/sub variables that are measured/assessed as referred to from several applicable regulations (Ministerial Regulations, Ministerial Circular, Decree, Guidebook, etc.). Variables/sub variables are measured using a Likert scale with a score of 1 to 5, where a score of 1 indicates a very bad/very uncomfortable condition, a score of 2 indicates a bad/uncomfortable condition, a score of 3 indicates a fairly good/comfortable condition, a score of 4 indicates a good condition./comfortable and a score of 5 indicates very good condition/very comfortable. The Likert scale obtained is converted into a value at intervals of 0 to 100 to show the results of the index calculation.

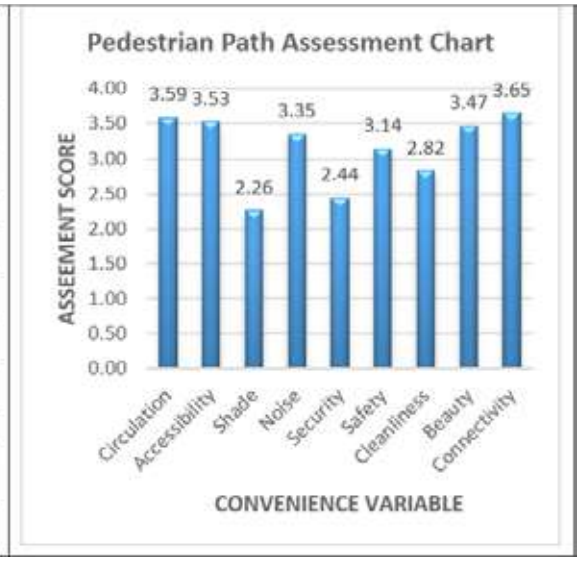
Table 2. Likert Scale and Value Conversion

Likert scale (0-5)	Value conversion (0-100)	Information
1	0 to <20	Very bad/very uncomfortable
2	20 to <40	Bad/uncomfortable
3	40 to <60	Good enough/comfortable enough
4	60 to <80	Good/comfortable
5	80 to 100	Very Good/very comfortable

Source: Author's analysis, 2020

Table 3. Average score of pedestrian comfort level assessment

No.	Variable	Average Score (Score 1 - 5)
1	Circulation	3.59
2	Accessibility	3.53
3	Shade	2.26
4	Noise	3.35
5	Security	2.44
6	Safety	3.14
7	Cleanliness	2.82
8	Beauty	3.47
9	Connectivity	3.65
Average		**3.14**



Source: Author's Analysis, 2020

The table and graph above shows that the average value of the pedestrian paths on the pedestrian path along Losari Beach to Fort Rotterdam is 3.14 and is included in the quite good/quite comfortable category.

The lowest score can be seen in the shade variable: 2.26 (bad/uncomfortable). The sub variable that is very influential on the assessment of this variable is the availability of shelter facilities such as shelters (covered seats) which are not available in every segment of the location. Roofed shelters are only found at bus stops on segments A-5 and A-17 and they are not utilized optimally. This shelter can also be used by pedestrians as a place to change the mode of transportation. The lowest average score below 3.00 is also found in the safety and cleanliness variables. For security factors, security facilities are needed in the form of CCTV facilities that can reach the pedestrian paths at this location. Likewise, the provision of special lighting facilities for pedestrian paths. What is currently available is a street lamp whose illumination level cannot provide optimal lighting for the pedestrian path. Pedestrian lane special lights also function as aesthetic ornaments that beautify pedestrian paths. The existence of security officers can also be empowered to help control the security of pedestrian paths. For the hygiene variable, almost all pedestrian path segments in this location do not provide special trash cans to accommodate pedestrian trash. Based on the prevailing standards, these bins are placed at a distance of about 20 meters and at meeting points (eg intersections). This is necessary to ensure the cleanliness of the pedestrian path. Pedestrian lane special lights also function as aesthetic ornaments that beautify pedestrian paths. The existence of security officers can also be empowered to help control the security of pedestrian paths. For the hygiene variable, almost all pedestrian path segments in this location do not provide special trash cans to accommodate pedestrian trash. Based on the prevailing standards, these bins are placed at a distance of about 20 meters and at meeting points (eg intersections). This is necessary to ensure the cleanliness of the pedestrian path. The pedestrian lane special lights also function as aesthetic ornaments that beautify the pedestrian path. The existence of security officers can also be empowered to help control the security of pedestrian paths. For the hygiene variable, almost all pedestrian path segments in this location do not provide special trash cans to accommodate pedestrian trash. Based on the prevailing standards, these bins are placed at a distance of about 20 meters and at meeting points (eg intersections). This is necessary to ensure the cleanliness of the pedestrian path. Almost all pedestrian lane segments in this location do not provide special trash cans to accommodate the garbage originating from pedestrians. Based on the prevailing standards, these bins are placed at a distance of about 20 meters and at meeting points (eg intersections). This is necessary to ensure the cleanliness of the pedestrian path. Almost all pedestrian lane segments in this location do not provide special trash cans to accommodate the garbage

originating from pedestrians. Based on the prevailing standards, these bins are placed at a distance of about 20 meters and at meeting points (eg intersections). This is necessary to ensure the cleanliness of the pedestrian path.



Source: Author, 2020

Figure 2. Pedestrian Path Condition

The variable average score (3.14) with an interval of 3.00 - <4.00 is in the quite comfortable category, but that does not mean that it does not require improvement but needs to be improved; For safety variables, the surface of the pedestrian paths, especially in segment A-17, needs to be physically repaired because around 50% of the pedestrian paths are damaged (holes) so that it can endanger pedestrian safety. Pedestrian signs (prohibited signs, warning signs, command signs and directional signs) are also not available at this location. Likewise, the pedestrian lane is very minimal, making it difficult for pedestrians to move places because they have to cross a fairly dense vehicle lane. This crossing route is not visible in the Losari beach area, even though there are many pedestrian activities only in the Fort Rotterdam area. For noise variables, plants that are able to reduce noise from motorized vehicles are needed on the road around the pedestrian path. The variables of accessibility and circulation are closely related to the ease of access to reach the pedestrian path and the freedom to move pedestrians on the pedestrian path without any obstacles. Arrangement of supporting facilities such as placing lighting lamps, aesthetic accessories (seats, flower pots, etc.) need to be rearranged so that they do not interfere with pedestrian access and circulation. Connectivity between pedestrian lanes also needs to be considered, especially the connectivity of pedestrians and the available pedestrian paths.

Prioritizing Pedestrian Needs: The determination of priority scale uses the AHP (Analytical Hierarchy Process) method based on 9 factors of pedestrian comfort. To obtain the priority scale, an AHP questionnaire was compiled which will be filled in by experts, namely people do not have to be experts in the field of pedestrian paths, but understand the problems to be studied. Resource persons or informants who become expertists and meet the consistency test requirements come from government elements as many as 3 people, 2 academics, city observers and 1 person each using the pedestrian path.

To obtain an assessment matrix from several respondents, the mean measurement was carried out based on 9 (nine) variables using the Geometric Mean formula.

The formula for the Geometric Mean is as follows:

$$GM = \sqrt[n]{X1 \times X2 \times X3 \times \ldots xXn}$$

Where: GM = Geometric Mean

X1, X2, X3,…, Xn = Weight of the assessment of variables 1, 2, .., n

n = Number n (order)

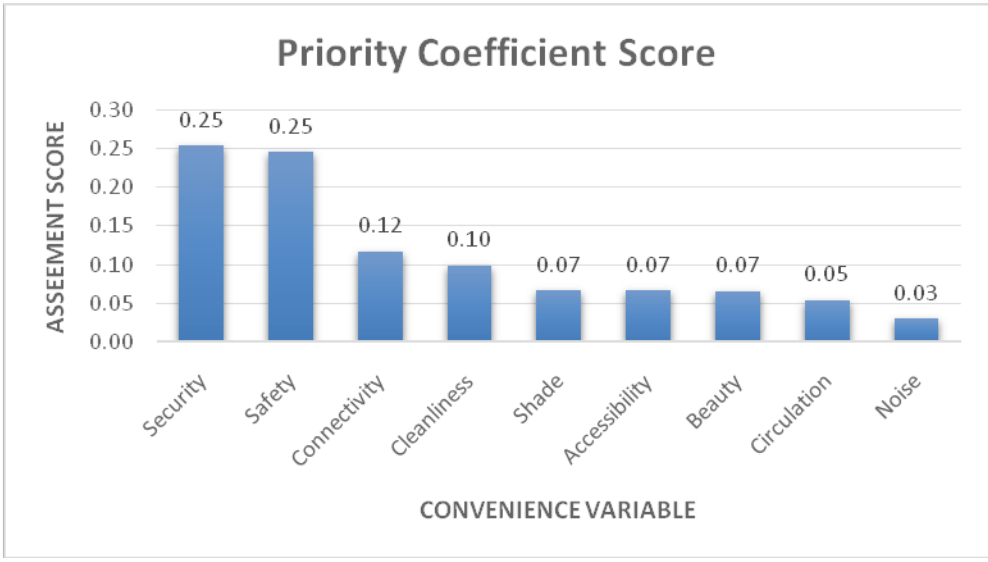
Based on the Geometric Mean formula, the average measurement results are obtained and then the eigen values are calculated to obtain the Priority Vector which is then re-tested for the validity of the data (level of consistency) using the Consistency Ratio (CR) formula as follows:

$$CR = CI/IR$$

Where: CR = Consistency Ratio (requirement CR ≤ 0.1)
CI = Consistency Index ((λ max-n)/(n-1))
IR = Index Random Consistency

Based on the formula above, the calculation of the Consistency Ratio (CR) is 0.015 (consistent, CR ≤ 0.1)

Based on the Analytical Hierarchy Process (AHP) data, which is derived from geometric mean data, the data on the priority order of the pedestrian comfort factors are obtained as follows:



Source: Author's analysis, 2020

Figure 5. Priority sequence graph based on AHP at location A

According to respondents from several experts, the priority needs of pedestrians that really need to be considered in the development of pedestrian paths in this location are the safety and security factors. As a recreation/cultural tourism area, there will be many pedestrians using this pedestrian path, so that the safety and security factors for pedestrians are things that need to be the priority of development so as to provide a sense of comfort for pedestrian lane users. Provision of security facilities (CCTV/security posts/security guards) and lighting needs special attention. Meanwhile, to ensure the safety of pedestrians on pedestrian paths, it is necessary to pay attention to the existence of ramps that are according to standards, availability and placement of markers and signs/signals for pedestrian paths as well as textures and materials used by pedestrian paths. The connectivity factor between pedestrian lanes and transportation mode facilities also needs to be considered in maintaining the continuity of the use of pedestrian paths in Makassar City. This is related to the physical ability of pedestrians and the mileage that pedestrians can travel so that facilities are needed to change modes of transportation to vehicles or public transportation.

Measurement of Pedestrian Track Comfort Index in Makassar City: Measurement of the pedestrian comfort index at each research location uses the results of the assessment of the comfort level of the pedestrian paths obtained from field data by taking into account the priority coefficient from the results of data processing Analytical Hierarchy Process (AHP). To calculate the pedestrian comfort index, the researcher will add up

the multiplication of the comfort level value (column 4) with the priority coefficient (column 3) of each variable. The index numbers obtained for each location will be converted to values 0–100.

Table 4. Walkability Index

Influence Variable	Priority Coefficient	Priority Level (%)	Comfort Level (Scale 1 - 5)
(1)	(2)	(3)	(4)
Circulation	0.0545	5.45	3.59
Accessibility	0.0665	6.65	3.53
Shade	0.0673	6.73	2.26
Noise	0.0306	3.06	3.35
Security	0.2534	25.34	2.44
Safety	0.2454	24.54	3.14
Cleanliness	0.0990	9.90	2.82
Beauty	0.0659	6.59	3.47
Connectivity	0.1174	11.74	3.65
Location Convenience Index A (conversion values 0-100)		**60.20 (Convenient)**	

Source: Author's analysis, 2020

Table above shows that the Walkability Index at location A is 60.20 and is included in the comfortable category (interval 60 to <80). Based on columns (3) and (4), it shows that the security variable has a major priority in improving pedestrian paths at this location, which is 25.34%. Field conditions indicate that the level of comfort is included in the uncomfortable category (2.44) due to the lack of availability of security systems (CCTV, security posts and security guards) which are able to reach all areas of the pedestrian path and the unavailability of lighting specifically intended for pedestrian paths.

Likewise, the safety factor is the second priority (24.54%) in the development of pedestrian paths at this location. Based on the results of the assessment of the level of comfort for each location segment, it shows that the safety sub-variables, namely the availability of markers and pedestrian paths/signs are not available. Likewise, the availability of zebra crossing facilities is inadequate, only available around SombaOpu Fortress, even though these facilities are urgently needed by pedestrians to ensure the safety and security of pedestrians.

The connectivity factor occupies the third priority in improving pedestrian pathways (11.74%), even though it is in the quite comfortable category (3.65). Connectivity between pedestrian lanes and transportation modes is important in improving pedestrian pathways at location A. The existence of 2 (two) bus stops as a place to change the mode of transportation at this location needs to be optimized for use so as to be able to increase pedestrian comfort. Cleanliness and shade factors also need special attention because they are included in the uncomfortable category with the fourth and fifth level of development priority. In this case, the availability of trash and shelter facilities needs to be a concern in providing a sense of comfort to pedestrians.

The pedestrian path accessibility factor at location A is quite comfortable because it is easy to access by normal pedestrians and those with special needs, only need to be equipped with road markings such as a zebra crossover. Other factors such as beauty, circulation and noise which are categorized as comfortable enough, need to be maintained even if the comfort needs to be increased by completing facilities that are not yet available.

C. Conclusions and Recommendations

The conclusions from the results of this study are as follows:

1. The results showed that the pedestrian path along Losari Beach to Fort Rotterdam was in the quite good/quite comfortable category (score 3.14). Improvement needs to be done, starting from providing several facilities to physical development in several location segments

2. The priority of pedestrian needs on pedestrian paths in this location places security, safety and connectivity as the main priority in the development of pedestrian paths in Makassar City. The improvement and development of these three factors need serious handling in order to create a pedestrian path that is comfortable for everyone without exception.

The recommendations of this study are addressed to the Makassar City Government or to further researchers in the following:

1. In developing pedestrian nets in Makassar City, factors such as security, safety and connectivity have become top priorities in addressing one of the increasingly complex challenges of urban development while still paying attention to other supporting factors.
2. Further researchers are advised to carry out more in-depth and detailed research on the priority needs of pedestrians that have been obtained in this study.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Features of Neuroimaging Diagnostics of Transient Ischemic Attacks

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Abstract

Transient ischemic attacks (TIA), as precursors of cerebral strokes, occupy an important place among all forms of cerebrovascular insufficiency. Regarding epidemiology of transient ischemic attacks (TIA), there are no accurate data in most countries. In the United States, up to 5 million adult citizens per year suffer from these episodes, many TIA remain undiagnosed. These episodes of a sudden and short-term neurological deficit have long been considered as benign and harmless. Most general practitioners and patients misunderstand or insufficiently understand TIA's nature and significance, perhaps, this is the reason of insufficient medical interest, lack of statistical data on this nosological unit. Transient ischemic attacks (TIA) are clinically defined as rapidly developing focal and sometimes diffuse (cerebral) dysfunctions of the brain that are caused by local ischemia and last no longer than a day[1]. Over the past two decades, many views on TIA have changed significantly, approaches to the diagnosis and treatment of patients have become much more intense and aggressive. Modern knowledge about TIA is of paramount importance both for proper organization of patient care and educational programs among the population; their importance can hardly be overestimated.

Keywords: *Transient ischemic attack, Cardiomagnyl, ischemic stroke, prevention.*

Introduction

Transient ischemic attacks (TIA), as precursors of cerebral strokes, occupy an important place among all forms of cerebrovascular insufficiency. Regarding epidemiology of transient ischemic attacks (TIA), there are no accurate data in most countries. In the United States, up to 5 million adult citizens per year suffer from these episodes, many TIA remain undiagnosed. These episodes of a sudden and short-term neurological deficit have long been considered as benign and harmless. Most general practitioners and patients misunderstand or insufficiently understand TIA's nature and significance, perhaps, this is the reason of insufficient medical interest, lack of statistical data on this nosological unit. Transient ischemic attacks (TIA) are clinically defined as rapidly developing focal and sometimes diffuse (cerebral) dysfunctions of the brain that are caused by local ischemia and last no longer than a day [1]. Over the past two decades, many views on TIA have changed significantly, approaches to the diagnosis and treatment of patients have become much more intense and aggressive. Modern knowledge about TIA is of paramount importance both for proper organization of patient care and educational programs among the population; their importance can hardly be overestimated. TIA shall be considered an urgent situation for the following reasons: • first - the risk of ischemic stroke development after TIA is rather high; • second - effective secondary prevention is available. In this respect, early and reliable diagnosis of TIA is utterly important, allowing correct treatment of this pathology. In TIA diagnostics, ultrasonic Doppler examination and computer tomography are used in most

cases. Usually, TIA is an acute episode in the general clinical picture of chronic progressive cerebral ischemia, neuroimaging method also make it possible to detect dyscirculatory encephalopathy signs. Atherosclerosis is defined as the most common cause of TIA in Western Europe and America in 80-90% of cases. Atherosclerotic multiple lesions are more common than isolated ones. According to Spiridonov A.A. (1996), pathology of only one carotid artery occurs in 51.7% of cases. The lesion of one vertebral artery was observed in 38% of cases. The incidence of multiple BCA lesions ranges from 50 to 93.8%. BCA lesion in 33% of cases is located intracranially, or in the zone, inaccessible for direct surgical revision. Other 67% were extracranial, 52% of these were located in CCA bifurcation area, 20% in the area of vertebral artery mouths and 9% in the area of the main branches of the aortic arch.Pathological deformations of carotid and vertebral arteries takes the second place in the structure of TIA causes. The reasons for development of pathological deformations have not yet been determined. Most authors believe that they result from congenital (embryogenesis disorder) or acquired factors (weakening of the artery wall elastic structure, atherosclerotic artery lesions, age-related anatomical changes in the cervical spine), or functional adaptive mechanisms aimed at pulse wave reduction (with arterial hypertension) and blood flow uniformity.

The aortic arch syndrome (AAS) is another cause of BCA lesion. It is observed in approximately 10-15% of patients and is a polyetiological autoimmune vasculitis with a primary lesion of the aorta and main arteries of the elastic type. AAS affects people aged from 10 to 40 years, more often women[7,9,15]. AAS is characterized by multiple lesions of the aortic arch branches, often symmetrical, for example, two carotid and two subclavian arteries. Most often, the subclavian artery is occluded and almost twice as often - the left subclavian artery. Most often, a subclavian artery is occluded and almost twice as often - a left subclavian artery.

In 5-10% of cases, TIA is caused by relatively rare diseases, causing lesion (blockage or narrowing) of the BCA and resulting local brain ischemia. Acute and blunt vascular injuries with arterial thrombosis, BCA dissection, BCA fibromuscular dysplasia, as well as extravasal vascular compression.

Dissection of pre-cerebral and, less commonly, cerebral arteries is the cause of about 2% of TIA cases, it is more typical for young women. BCA and VA dissection can occur due to trauma, unsuccessful manipulation of the cervical spine or developmental abnormalities. A hematoma, formed under the intimal artery, causes its lumen narrowing and can cause thrombosis or embolism.

The clinical picture of TIA is very diverse, but with this pathology there are no clear pathognomonic symptoms, their presence would help clinicians in diagnositics nd timely selection of treatment tactics for detected lesions, including surgical revision of pathology. If TIA is located in the carotid pool, then, as a rule, transient paresis or paralysis is observed. With TIA in the vertebral-basilar pool, the main clinical symptom is dizziness.

The purpose of our work was to study method of ultrasonic Doppler examination and computer tomography for TIA diagnosis. The study included 76 patients (47 men and 29 women, aged from 45 to 80 years (mean age 62.3 ± 5.1 years) Control group (15 patients) included practically healthy individuals of the same age, with no signs of cerebrovascular insufficiency.

TIA etiological factors in our observations were as follows: cerebral atherosclerosis 68.42% (52 patients) and a combination of cerebral atherosclerosis and hypertension 31.57% (24 patients). The course of hypertension in our observations was malignant with average indices of blood pressure of 170 ± 6.4 mm Hg and frequent increases in systolic blood pressure over 220 mm Hg, worsening the picture of cerebrovascular insufficiency.

In the clinical picture, outside TIA, cerebral complaints to headaches, dizziness, which intensified at head turn and change of the body position, sleep disturbances, performance impairment and memory problems prevailed. In the presence of diffuse focal symptoms in the form of tendon anisoreflexia, oral automatism symptoms, pathological stop signs, most of our patients (80.2%) were diagnosed with dyscirculatory encephalopathy. It should be noted,that in most patients, clinical symptoms spoke for dyscirculatory encephalopathy, primarily, in the vertebral-basilar pool. In the latter case, there was vertebral-basilar insufficiency of II or III degree, which was also determined by the method of ultrasonic Doppler examination and computer tomography. The study of cerebral hemodynamics and main artery of the head was performed using ultrasonic Doppler examination on VAZOSKAN device, manufactured by Sonicaid (England) using sensors with

a frequency of 2.4.8 MHz and diameters of 14, 10 and 6 mm. The studies were performed in the city diagnostic centre.

According to Spiridonov A.A. (1996), pathology of only one carotid artery occurs in 51.7% of cases causes TIA development. The lesion of one vertebral artery was observed in 38% of cases. The incidence of multiple BCA lesions ranges from 50 to 93.8%. To determine the role of occlusive lesions in TIA development, ultrasonic Doppler examination was performed. The study of cerebral hemodynamics and main artery of the head was performed using ultrasonic Doppler examination on VAZOSKAN device, manufactured by Sonicaid (England) using sensors with a frequency of 2.4.8 MHz and diameters of 14, 10 and 6 mm.

Magnetic resonance imaging studies were performed in Radiology Department of the 3rd clinic of Tashkent Medical Academy using Magnetom Open Viva (Siemens) device with a magnetic field strength of 0.2 Tesla in coronary and transverse projections using a common flexible RF coil for the patient in a neutral position on his back.

Results

The patients' condition upon admission in most cases 80% (32 patients) was regarded by us as moderate. It is important to note that in most cases, the severity of patient's condition was due to a number of neurological symptoms, but not impairment of consciousness. For description of transient disorders, the following aspects were evaluated: TIA duration and incidence, probable vascular pool, morbidity, prevalence of focal or cerebral symptoms. Moreover, in most of our observations, TIA were predominantly focal over cerebral. Anamnestic data of patients with TIA are shown in Fig. 1.

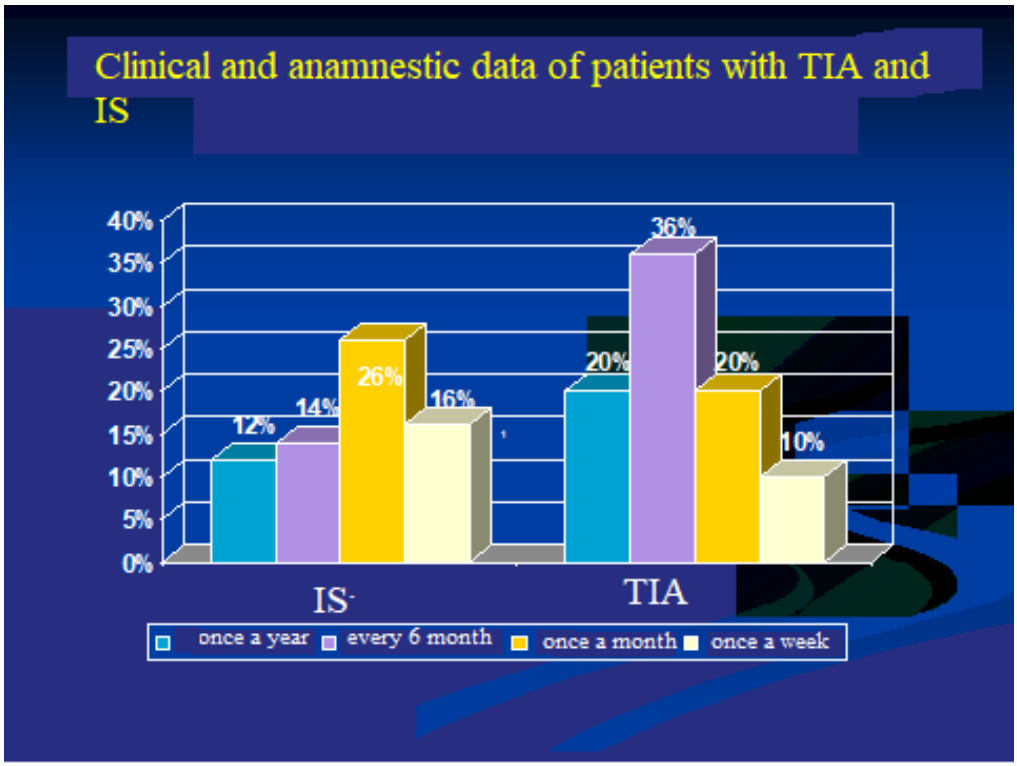


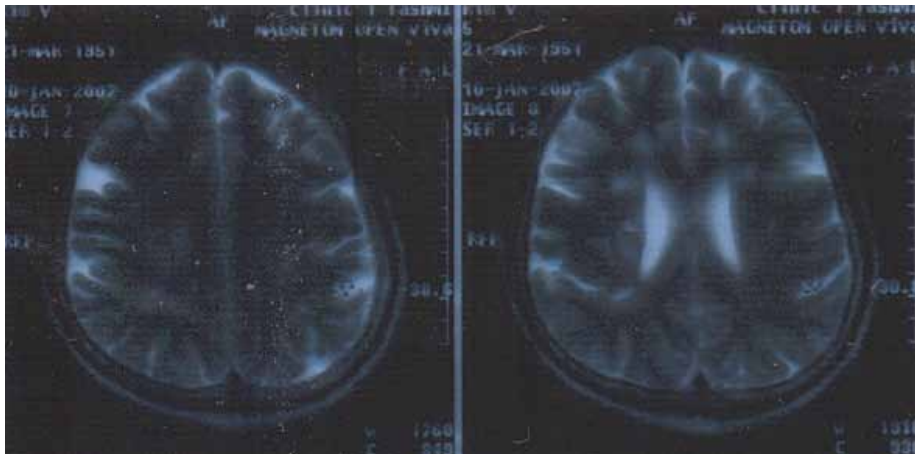
Figure 1. Clinical and anamnestic data of patients with TIA and ishemic stroke (IS).

As can be seen in Fig. 1, IS most often developed in patients with TIA incidence once a week or once a month. The highest risk of IS development was observed at a TIA incidence of once a week and reached 33.3%. The duration of TIA in this group of patients was up to 10-16 hours (according to anamnestic data). Based on clinical and anamnestic studies, we analysed the dependence of the risk of stroke development on the incidence of previous TIA in 18 patients who had a stroke against TIA (Fig. 2). In all cases, the stroke vascular pool coincided

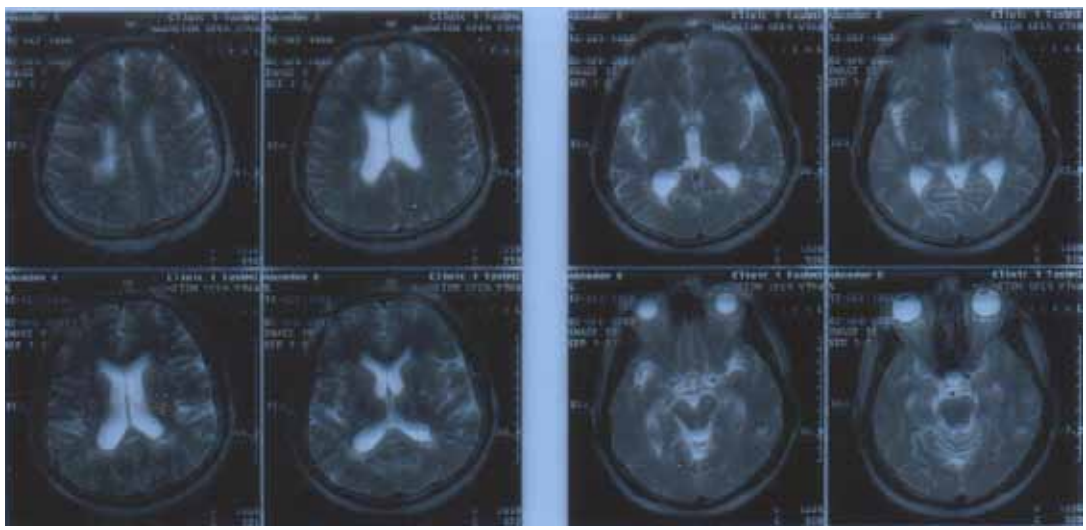
with the TIA localization. Besides, as presented data show, the incidence of stroke and TIA coincided when they were localized in the right carotid system (31% and 37%, respectively). At the same time, TIA in the left carotid system were observed 3 times less often (18%) than strokes in the same pool (55%). In contrast, TIA in vertebral-basilar pool were observed 3 times more often than strokes in the same vascular pool. The obtained results can be explained by anatomical features of the vascular pool structure, as well as by compensatory characteristics of these vascular pools.

The MRI studies revealed the dependence of TIA clinical picture the severity of brain atrophy. To determine the latter, based on MR images, we calculated indices of the lateral and third ventricles. Our studies showed that in the group of patients with frequent TIA (more than 2-3 times per month), indices of lateral ventricles were 1.3 and were much higher than in patients with TIA 1 time in 6 months or 1 year (0.8). Besides, dependence of MRI indices on the degree of dyscirculatory encephalopathy and etiological factors, caused cerebrovascular insufficiency.

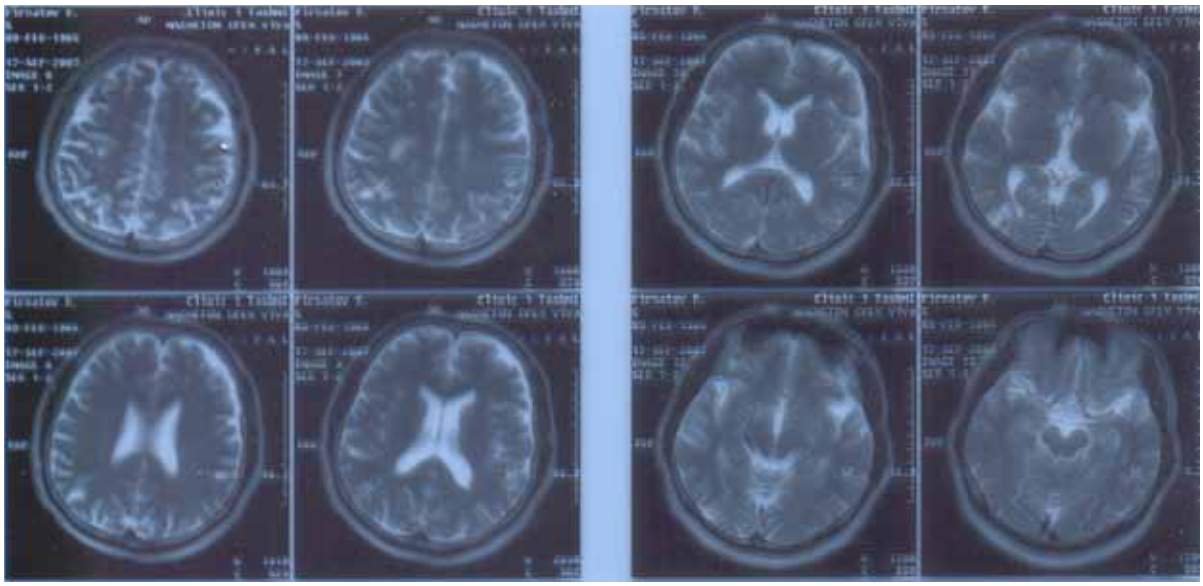
Multiple foci of reduced density no larger than 1 cm were found on MR images in the periventricular zone.



The number of the above foci also depended on the TIA incidence. Besides, in the group of patients with TIA incidence of more than 2-3 times a week, an expansion of subarachnoid space and sylvian fissures was observed, showing probable external brain atrophy and the state of cerebral hemodynamics. Typically, the cause of TIA in the latter case was a combination of arterial hypertension and cerebral atherosclerosis.



In patients with TIA on the background of cerebral atherosclerosis, we revealed only the expansion of sylvian fissures and subarachnoid space, with indices of lateral ventricles not significantly different from those of the control group.



Thus, as our studies showed, the clinical picture of TIA is characterized by various symptoms and diagnosis complexity, considering its retrospective character. The incidence, duration and vascular pool of TIA are correlated with the risk of cerebral stroke. The clinical picture of TIA is also largely determined by the brain condition, in particular, the severity of its atrophy. The latter, depending on the etiological factor, manifests itself in the form of predominantly external or internal forms. The number of foci of reduced density in the periventricular zone is also directly dependent on TIA incidence.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Results of Transurethral Correction of Stenotic Developmental Abnormalities of Ureterovesical Junction in Children

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Abstract

Purpose: Improving the results of treatment of stenotic developmental abnormalities of the ureterovesical junction (SDA UVJ) in children.

Materials and Method: Treatment analyzed included 88 pediatric patients (125 ureters) with SDA UVJ, aged 3 months - 15 years.

Results: Endoscopic stenting has been performed using antireflux stents, with subsequent bladder drainage using a urinary catheter. We have also developed an inverted V-shaped ureterocele incision. The UVJ control calibration has been performed using calibration catheters. If the UVJ diameter did not correspond to the age norm, the second stage of treatment was carried out by dilating with conical bouges or by UVJ balloon dilating. Transient complications developed in the immediate postoperative period have been successfully eliminated using conservative measures, in all cases.

Conclusions: The study results imply the effectiveness of endoscopic method in correcting SDA UVJ in children.

Keywords: *Obstructive megaureter, stenotic developmental abnormalities of ureterovesicular junction, endoscopic treatment, children.*

Introduction

The endosurgical treatment of patients with UVJ abnormalities have not almost been used in pediatrics, due to the lack of differential diagnostic criteria for various forms[8] of this disease or indications for such an intervention [14].

Studying the urodynamic disturbance mechanisms led us to the fact that the existing open surgical procedure of patients with stenotic anomalies had to be reconcepted, and the list of indications for endosurgical interventions[7] - expanded. The technique of transurethral ureteroplasty for UVJ stenotic anomalies, using bouginage or balloon dilatation of UVJ with ureteral stenting and ureterocele incision[13], had been previously recognized as effective. For the first time in pediatrics, this approach to the treatment of obstructive megaureter was described by N.V. Dorasvely in his work (1990), which presented two cases of successful endoscopic ureteroplasty using a Fogerty balloon dilator[10]. However, the discrepancies in the results of these interventions performed in younger children still remain[12].

Endosurgical method of treating children with UVJ developmental abnormalities have an increasing number of its supporters[1]. However, insufficiently studied mechanisms of urodynamic disturbance reduce the positive results of such interventions[2]. Forms of UVJ developmental anomalies are diverse, while in children, functional anomalies predominate[6]. All this explains why the differentiated treatment strategy has been chosen for pediatric patients[8].

The aim of this study is to improve the results of treatment of SDA UVJ in children.

Materials and Method

The treatment analyzed included 88 pediatric patients (125 ureters) with SDA UVJ, aged 3 months - 15 years. The research was carried out in the Department

of Intermediate Level Pediatric Surgery (Head of the Department - prof. M.M. Aliev) of TashPMI (Rector - prof. B.T. Daminov), and in the Department of Pediatric Surgery (2014-2019) of the Republican Specialized Scientific and Practical Medical Center of Pediatrics (Director - prof. D.I. Ahmedova).

Indications for endoscopic transurethral correction of organic stenotic anomalies (SA) of UVJ have been determined based on the nature of functional disorders - mild, moderate or severe - according to our diagnostic research algorithm.

3 types of endoscopic correction of UVJ have been performed in 88 patients (125 ureters) with SA (Table 1).

Table 1: The nature of endoscopic interventions performed in children with SA UVJ

Types of UVJ anomalies	Endoscopic method of treatment			Total
	UVJ bouginage**	UVJ balloon dilatation**	Ureterocele incision	
OSA*	8 (11)	21 (29)	16 (18)	53 (66)
FSA*	21 (35)	22 (32)	-	35 (59)
Total	29 (46)	43 (61)	16 (18)	88 (125)

Note: The number of ureters is provided within brackets. * - SA - stenotic anomalies; O - organic; F - functional. ** - UVJ bouginage and balloon dilatation have been performed with mandatory stenting of the lower third of the ureter.

As shown in Table 1, in our practice, we preferred UVJ balloon dilatation with stenting of the lower third of the ureter. In 16 children (18 ureters), the ureterocele incision was performed only with its intravesical form.

During examination cystoscopy, the ureterocele opening was catheterized with a flexible conductor to a depth of 8-10 cm. The ureterocele opening bouginage was performed consecutively using conical bouges Biorad Medisys PVT (India) 6-8-10-12 Fr, along the entire UVJ, under triple video+Carm+ultrasound control. Bouginage up to 6 Fr was carried out for children under the age of 3 years, up to 8 Fr - for children from 4 to 7 years, and up to 12 Fr - for children over 7 years.

Balloon dilatation was performed using a balloon catheter manufactured by Biorad Medisys PVT (India). Balloon catheter was passed over using a flexible conductor, UVJ dilatation up to 6Fr with a pressure of up to 2 Atm/bar was performed for children under the age of 3 years, up to 8Fr with a pressure of up to 3 Atm/bar - for children from 4 to 7 years, and up to 10Fr with a pressure of up to 4 Atm/bar - for children over 7 years. The abundant flow of turbid urine with the failure of a bouge or balloon was considered as an efficacy criterion for dilation (P. Puri et al. 1991).

Results

After bouginage or dilatation, a polyurethane stent with a diameter of 4-10 Fr of the same manufacturer was installed in the lower third of the ureter at one end and into the bladder cavity at the other end. Then, it was passed through the conductor using a 'pusher' (Fig. 1). Stents were placed in the ureter for a period of 20-30 days.

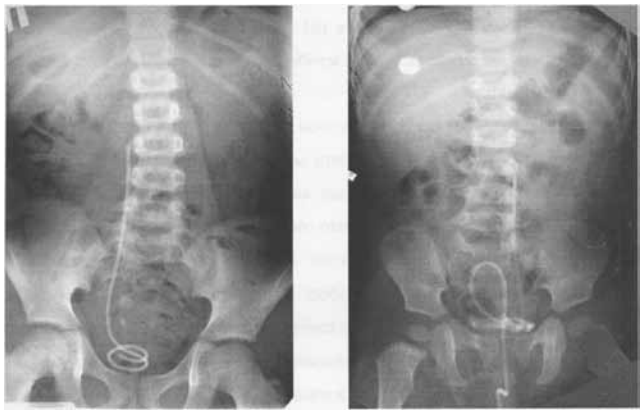


Figure 1. Plain urography. Normal position of the 'low' stent.

During primary stenting of the ureter, the diameter of the stent did not largely exceed 5-6 Fr. With repeated stenting (7 cases), its diameter was 8-10 Fr. After 20-30 days, when the stent was removed, it was determined by cystoscopy whether it is necessary to replace the stent. With that, UVJ control air-blast calibration was performed using calibration catheters. If the UVJ diameter did not meet the age norm, the second stage of treatment included dilating using conical bouges or UVJ

balloon dilating. Then, the larger stent was installed for a period of 20-30 days.

Outcome of treatment in children using double J (JJ) stenting has been more successful than that through high derivative urine using percutaneous puncture nephrostomy. Indications for percutaneous puncture nephrostomy have been determined by pronounced deviation of the ureter and a sharp decrease in its contractility in case of insufficient derivation of urine from the lower ureter. Based on the foregoing, a modification of stents has been developed, that promoted improved drainage of the upper urinary tract. For this purpose, so-called 'low stents' were used. After endosurgical intervention, they were inserted up to the middle third of the ureter or the first knee-shaped bend. In this way, appropriate urinary drainage from all parts of the collecting system was ensured. A thin line from the distal end of the 'low' stent was withdrawn through the external urethral opening, which made it possible to remove the stent without additional instrumental interventions. Endoscopic stenting was performed with antireflux stents Biorad Medisys PVT (India) with the subsequent drainage of the bladder - on the first or the second day - with a Foley 8-12 Fr urethral catheter. The drainage time was determined depending on the age of the child and the urethra diameter.

Discussion

For ureterocele incision, we used endoscopic equipment manufactured by KARL STORZ (Germany): an electrochemical resectoscope 10 CH with loop-shaped electrodes and a hook-shaped cold knife. During ureterocele incision - transverse or semi-lunar - the ureterocele upper wall contracted between the two fixation points (Waldeyer's ring and the proximal urethra), and the free edge of the artifical opening was moved in the proximal direction, as a result of which the opening became U-shaped. The submucous ureteral segment was shortened inversely in proportion to the length of the discission, which could not ensure a tight closure of its walls in the area of the opening.

Taking into account this disadvantage of the conventional method above, we have developed an inverted V-shaped ureterocele incision. In this case, the contracting upper wall of the ureterocele, while retaining both fixation points, does not displace the proximal border of the articular opening, since it does not have free edges oriented perpendicular to the axis of the ureter. Due to this, the length of the submucosal part of the ureter remains unchanged and depends only on the length of the incision performed. After contraction, the upper and lower walls of the ureterocele close tightly, which improves antireflux function. The incision was short, its length did not exceed the length of the working part of the electrode and the hook of the cold knife (3 mm). The intervention was completed by draining the bladder with a Foley 8-12 Fr urethral catheter, at the first or the second day.

UVJ endosurgical intervention was always carried out against the background of antibacterial prophylaxis, 1-2 days before the intervention, depending on the results of common urine and bacterial urine culture tests, determining the sensitivity of the flora to antibiotics.

After surgery, patients stayed in the hospital within 2.06±0.49 days, on average (1-3 days). Upon discharge, patients were advised to maintain an increased fluid intake and take uroseptics and antihistamines, in order to prevent sensitization to stent material. Recommendations had to be observed for the entire period of 20-30 days before the removal of the stent and the subsequent transition from the inpatient to outpatient setting. The dynamics of emptying the lower third of the ureter and the pyelocaliceal system was evaluated through ultrasound, which was performed every 10 days for 1 month. The tendency to a decrease in the diameter of the lower third of the ureter within 2 weeks largely indicated the reversibility of changes in the enlarged ureter. On the 20th-30rd day after ureteral stenting, patients were re-hospitalized, in order to remove or replace the stent. A control excretory urography was performed with 1 or 2 images, depending on the initial state of the upper urinary tract (UUT). After ureterocele incision, the mycystic cystography was performed, in order to extend the study materials.

Transient complications (ureter atony, hemorrhagic cystitis, exacerbation of pyelonephritis, dysuria) developed in the immediate postoperative period have been eliminated by conservative measures, in all patients. Such measures consisted of antibacterial, anti-inflammatory therapy and physiotherapeutic procedures that stimulated ureter contractibility (Table 2).

Table 2: Complications of the immediate period after endoscopic correction for UVJ stenotic abnormalities in children

Method of endoscopic correction	Types of complications			
	Leukocyturia (FOV)	Proteinuria (g/l)	Atony of the ureter (urine discharge/min)	Dysuria
UVJ bouginage n=17	19,3±1,25	0,18±0,9	1,83±0,06	7 (33,3%)
Balloon dilatation UVJn=27	17,1±0,69	0,05±0,1	1,55±0,07	8 (33,3%)
Ureterocele incision n=11	28,3±0,52	0,07±0,07	1,9±0,05	4 (50%)

Note: $P < 0.05$.

As can be seen from Table 2, the most frequently observed complications of the immediate postoperative period were exacerbation of pyelonephritis and transient urodynamic abnormalities. Infusion and antibacterial therapy led to normalization of body temperature, urine tests and elimination of dysuria.

Excretory urography has been performed in 29 patients (46 ureters), in order to evaluate the results of UVJ endoscopic augmentation with low stenting within 1-3 months after the treatment. Of the 46 cases of examination of kidneys and ureters, in 35 cases (76.1%) a decrease in the degree of enlargement of UUT was recorded, by an average of 47.5±1.1% (Fig. 2, 3, 4), in 9 cases (19.6%) - no changes, and in 2 cases (4.3%) - an increase in the UUT size.



Figure 2. Patient A., 7 years. Excretory urography. III grade megaureter on the left. Before the UVJ bouginage with low stenting on the left.

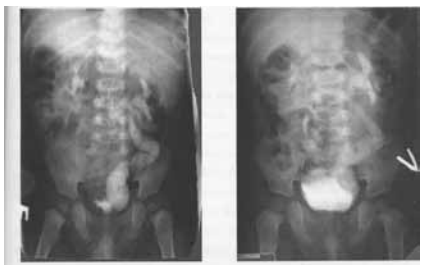


Figure 3. The same patient Excretory urography 1 month after UVJ bouginage with low stenting on the left.



Figure 4. The same patient Excretory urography 3 months after repeated UVJ bouginage with replacement of the low stent on the left after 30 days.

In order to evaluate the results of UVJ endoscopic balloon dilatation with low ureter stenting, within 1-3 months after treatment, excretory urography results have been studied in 43 patients (61 ureters). Of the 61 cases of examination of kidneys and ureters, in 54 cases (88.5%) a decrease in the degree of UUT enlargement was recorded, by an average of 58.4±1.2% (Fig. 5, 6), in 5 cases (8.2%) - no changes, and in 2 cases (3.3%) - an increase in UUT size.

Excretory urography has showed the following results of endoscopic surgery using the modified method of inverted V-shaped ureterocele incision. In 16 patients (18 ureters) a decrease in ureterocele lumen was observed by an average of 68.3%, in 14 ureters (77.8%) - by 57.8% ($p<0.05$) (Fig. 8), in 1 (5.5%) - without reducing UUT size. In 3 cases (16.6%), the vesicoureteral reflux has been observed (Fig. 6, 7), and in 1 case - urethenosis (Fig. 8).



Fig. 5. Patient V., 5 years. Excretory urography. II grade megaureter on the right, III grade megaureter on the left. Before UVJ balloon dilatation



Fig. 6. The same patient Excretory urography after 3 months. Positive result.



Fig. 6. Patient S., 6 years Excretory urography. Intrauterine ureterocele on the right.



Fig. 7. The same patient Cystography. Unsatisfactory result. Vesicoureteral reflux on the right.



Fig. 8. Patient F., 4 years. Excretory urography. Ureter and ureterocele lumen reduction.

After analyzing the results of the UVJ dilatation with low stenting, we have identified the following reasons for unsatisfactory indicators: 1) stent prolapse into the bladder lumen (Fig. 9.) 2) stent migration into the ureter lumen (1 case) 3) accumulation of salts on the stent surface and its obstruction, which contributed to the exacerbation of pyelonephritis (1 case). When dissecting the ureterocele in a modified way, the fusion of the edges of the incision and the excessive ureterocele tissue dissected have been revealed, which caused vesicoureteral reflux.

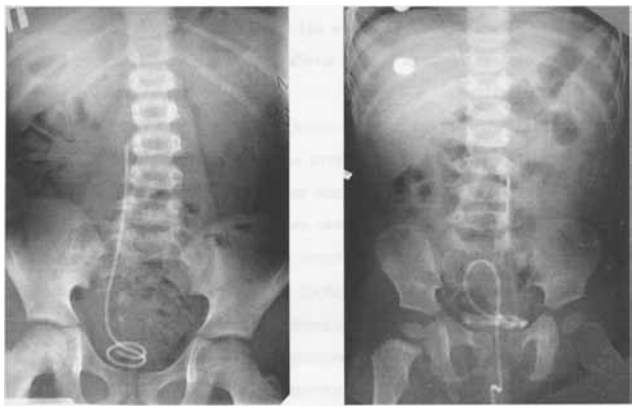


Figure 9. Patient P., 3 years. Plain urography. Stent prolapses into the bladder.

In order to study positive urodynamics after endoscopic correction in SA UVJ within 1-3 months, we used the following special research method: ultrasound of the ureter-cystic urinary discharge (UCUD) using pulse-wave dopplerometry and transformational echopieloscopy (TEC) with diuretic load (Table 3). In patients with positive urodynamics, with pulse-wave dopplerometry of the ureter-bladder discharge of urine, the frequency, duration and maximum speed of UCUD were close to normal values. Analysis of transformational echopieloscopy has showed that the more pronounced the initial dilatation of the ureter, the less its expansion at the time of examination.

The maximum expansion of the distal ureter (35-40% of the initial size) was observed at 5-8 minutes of the examination, and the restoration of the original size usually occurred at 35-45 minutes. During the study period, the expansion of the ureter in this group of patients did not exceed 1.5 cm in diameter, which indicated that its wall tone is preserved.

Table 3: Positive urodynamics in the immediate period after endoscopic correction in children with SA UVJ.

Method of endoscopic correction	The number of ureters	UCUD Doppler ultrasound			TEP		
		Frequency of discharge per 1 min.	T_c (sec)	V_{max} (m/s)	T_{max} (min)	P_{max} (%)	T_i (min)
UVJ bouginage	46	1,93±0,56	1,56±0,18	0,31±0,04	7,3±0,78	39,2±0,46	44,2±0,89
UVJ balloon dilatation	61	2,9±0,2	1,8±0,4	0,34±0,03	5,8±1,1	36,5±3,6	41,1±0,78
Ureterocele incision	18	2,4±0,36	1,2±0,1	0,33±0,02	6,4±0,44	35,8±0,37	37,6±0,69

Note: T_c - duration of one urine discharge; V_{max} - maximum speed of urine discharge; T_{max} – time of maximum expansion of renal collecting system (min); P_{max} – maximum expansion of renal collecting system (%); T_i - the restoration (reduction) time of the size of renal collecting system to its initial values (min.);- $P<0.01$

Conclusions

The obtained results have showed the effectiveness of endoscopic method for the correction of UVJ stenotic anomalies in children. The revealed causes of conservatively reversed complications and unsatisfactory results have mainly depended on the severity of urinary infection and have made it possible to improve the conservative treatment of children with AS UVJ.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Epidemiological Characteristic of Aphthous Stomatitis and Real Clinical Practice of Management of Patients in Uzbekistan

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Abstract

Introduction: The article provides an epidemiological description for patients with chronic recurrent aphthous stomatitis (RAS) and the frequency of their reversal in the general structure of diseases of the oral mucosa from 2014 to 2018. The structure of clinical forms of RAS in the Republic of Uzbekistan (ICD-10) was taken. The study of the dynamics of frequency and debut of various clinical forms of RAS, the gender characteristics of RAS has led to the need for the introduction of modern standards for examination, treatment, and prevention of the disease. The goal of the research is to study the frequency of occurrence of RAS, as well as the real clinical practice of managing patients in the Republic of Uzbekistan and its compliance with existing recommendations.

Materials and Method: Patients' medical records and records of the patients' consultation journal for five years of work (from 2014 to 2018) were studied.

Results: Taking into account the gender and age characteristics of patients with RAS will determine the real clinical picture and prevalence of this disease, increase the ability of monitoring, medical examination and providing timely specialized care, allowing to prevent the progression and maintain the working capacity of patients with this pathology of OCR.

Conclusion: The frequency of RAS at the general reception for diseases of OCR is 6.22 ± 0.42%; in the general structure of the disease, the fibrinous form prevails 3.46%; the incidence of necrotic periadenitis is 3.15%; herpetiform aphthous stomatitis is recorded in 2.25% of patients and 1.25% - a symptom in Behcet's disease. The debut of RAS falls on the age period of 18-34 years (62.50%), which indicates a negative trend of "rejuvenation" of this pathology. For effective treatment and rehabilitation of patients suffering from RAS, it is necessary to conduct a comprehensive examination attracting internists, an appointment of means of immunomodulating therapy and means effectively correcting the most important homeostatic mechanisms in humans.

Keywords: *The fibrinous form of chronic recurrent aphthous stomatitis, Setton's aphthae, herpetiform aphthous stomatitis, symptom in Behcet's disease.*

Introduction

Chronic recurrent aphthous stomatitis is one of the most common diseases of the oral mucosa, characterized by recurrent rash of aphthae and a prolonged course with periodic manifestations[12]. Recurrent aphthous stomatitis (RAS) is a disorder characterized by recurrent ulcers on the oral mucosa in the absence of other symptoms. RAS presents several pathological conditions with similar clinical manifestations. According to the international classification of diseases (ICD-10), RAS refers to ICD-10: K12.0 ICD-10/K00-K93 CLASS XI Diseases of the digestive system/K00-K14 Diseases of the oral cavity, salivary glands and jaw/K12 Stomatitis and related lesions[13].

Six clinical forms (fibrinous, necrotic, glandular, scarring, deforming and lichenoid) of ASD have been

distinguished since 1978, based on the analysis of clinical and morphological manifestations. According to foreign authors, ASD is characterized by three main manifestations: small aphthae (Mikulich aphthosis), located mainly on the mucous membrane of the lips, cheeks, the bottom of the oral cavity, large aphthae (necrotic periadenitis of oral mucosa, or Sutton's disease) and herpetiform ulcers – multiple aphthae of round forms[14].

According to various authors, the prevalence of RAS in the overall structure of the pathology of OCR ranges from 1.27 per 1000 population to 8.68 ± 0.62%. Recurrent aphthous stomatitis (RAS) occurred among the population of Sverdlovsk region, in 6.9% cases. Large aphthae, up to 1 cm in diameter, very painful, epithelizing for a long time (up to 5 weeks), the so-called Setton`s aphthae, were diagnosed in 12% of patients with RAS[7]. According to the WHO, RAS occurs in 20% of the population, and adults at the age from 20 to 40 suffer from recurrent chronic aphthous stomatitis[4].

In general, it was determined that the prevalence of RAS had a tendency to increase, which is due to the global aging of the population, an increase in the pathology of OCR, an increase in somatic pathology, and uncontrolled use of immunomodulators and medications with antibacterial properties[10].

In everyday clinical practice, patients, applying for dental care with mucosal diseases present one of the most difficult problems due to difficulties in diagnosis and treatment. The official reports of the dental service of the Republic of Uzbekistan do not include indicators of the incidence of chronic aphthous stomatitis[4]. However, there is an urgent need for studying the prevalence of gender-related morbidity indicators, the age limits for the debut of pathologies belonging to this group of diseases. Information on the epidemiology of aphthous stomatitis in the country should stimulate the development of modern method of diagnosis, therapy and rehabilitation of patients with a similar pathology[10].

Information about the prevalence and characteristics of the clinical course of the disease is necessary for planning the activity of a medical organization and its management; planning of therapeutic and preventive measures; establishing health indicators and the prognosis of morbidity[12]. Knowledge of the prevalence and intensity of dental diseases is the basis for developing the need for medical personnel and resources.

The goal of the research is to study the frequency of occurrence of RAS, as well as the real clinical practice of managing patients in the Republic of Uzbekistan and its compliance with existing recommendations.

Materials and Method

We studied the medical records of patients who applied for dental care for the treatment of diseases of the oral mucosa at the dental clinic of Tashkent State Dental Institute TSDI for five years of activity (from 2014 to 2018).

We used the classification of RAS proposed by WHO for identifying the disease: 1. The fibrinous form of RAS (Mikulich aphthosis); 2. Necrotic periadenitis (Setton's aphthae) (recurring scarring deep aphthae, deforming aphthae, creeping aphthae); 3. Herpetiform aphthous stomatitis; 4. Symptom in Behcet's disease.

When studying the history of development of the disease, the emphasis was on identifying the preclinical stage of the disease, the onset of the disease, the duration of the disease, the stage of the disease at the time of diagnosis, the first symptoms, the dynamics of the signs of the disease, previously conducted treatment, its effectiveness, drugs prescribed for treatment, their tolerability, the presence of allergic reactions.

Data about patients were recorded in the Unified Card, designed for this purpose.

The control sample in this series of activity amounted to 40 healthy individuals of the corresponding gender, age and ethnic composition who underwent a complete dental examination.

Results

208 patients applied for treatment of the aphthous stomatitis from 2014 to 2018, thus, the frequency of RAS in the total frequency of RAS treatment complaints was 6.22 ± 0.42%. It should be noted that over the entire sampling period, the frequency of RAS in the overall structure of the RAS diseases did not undergo significant differences and ranged from 5.32 ± 0.42% (2014) to 7.62 ± 1.05% (2016) (table 1). Thus, for the diseases that make up the group of aphthous mouth ulcers over the past 5 years, the peak of visits was in 2016-2017, which in general can be described as a negative trend in morbidity increase.

Table 1. Dynamics of the appealing frequency regarding RAS in the general structure of OCR diseases

Diseases	Years					
	2014	2015	2016	2017	2018	Total
RAS	32 / 5,22 ± 0,90	38 / 5,32 ± 0,87	47 / 7,52 ± 1,05	49 / 7,11 ± 0,97	42 / 5,97 ± 0,89	208 / 6,22 ± 0,42
Total with OCR disease	612 / 100,0	714 / 100,0	625 / 100,0	689 / 100,0	703 / 100,0	3343 / 100,0

Note: In the numerator – the number of patients; In the denominator, in % of the total number of patients.

The unit weight of the registered clinical forms (within the framework of ICD-10) within the group of aphthous lesions of the oral cavity is presented in Table 2.

Table 2. The structure of clinical forms of RAS in the Republic of Uzbekistan (ICD-10)

No.	Clinical Form	Abs	%
1.	Fibrinous Form, Mikulich Aphthosis	115	55,29±3,45
2.	Necrotic Periadenitis (Setton's Aphthae) (Recurrent, Deep Aphthae, Deforming Aphthae, Creeping Aphthae)	61	29,33±3,15
3.	Herpetiform Aphthous Stomatitis	25	12,02±2,25
4.	Symptom in Behcet's Disease	7	3,36±1,25
5.	**Total**	**208/100,0**	

Table 2 shows that the mainly fibrinous form of the disease (Mikulich aphthosis) is recorded, the proportion of which in the general structure of the diseases was 55.29 ± 3.45%; a significant proportion – 29.33 ± 3.15% was occupied by severe periadenitis (Setton's aphthae, recurrent, deep aphthae, deforming aphthae, creeping aphthae); 12.02 ± 2.25% in the total structure of RAS accounted for herpetiform lesions and the proportion of Behcet's syndrome was the smallest – 3.36 ± 1.25% (table 2).

It should be noted that the established trend towards a higher incidence of fibrinous disease was recorded throughout the study period. Throughout the entire period of research, a traditionally high unit weight of severe periadenitis was noted (table 3).

Table 3. Dynamics of frequency of various clinical forms of RAS

Clinical course of RAS	Years				
	2014	2015	2016	2017	2018
Fibrinous form	20 / 62,5±8,56	26 / 68,21 ± 7,56	20 / 42,55 ± 7,21	23 / 46,94 ± 7,13	26 / 61,90 ± 7,49
Setton's aphthae	6 / 18,75±6,90	10 / 26,32 ± 7,14	17 / 36,17 ± 7,13	18 / 36,73 ± 6,88	10 / 23,81 ± 6,579
Herpetiform aphthous stomatitis	5 / 15,62±6,42	1 / 2,63 ± 2,60	8 / 17,02 ± 5,48	6 / 12,24 ± 4,64	5 / 11,90 ± 5,01
Symptom in Behcet's disease	1 / 39,25±3,08	1 / 2,63 ± 2,60	2 / 4,26 ± 2,71	2 / 4,08 ± 2,80	1 / 2,38 ± 2,5

Clinical course of RAS	Years				
	2014	2015	2016	2017	2018
Total	32 / 100,0	38 / 100,0	47 / 100,0	49 / 100,0	42 / 100,0

Note: In the numerator – the number of patients; In the denominator, in % of the total number of patients.

Clinic development debut among patients with various clinical forms was registered at age from 18 to 24 – 23.08 ± 2.92%; from 25 to 34 – 39.42 ± 3.39; from 34 to 44 – 23.56 ± 2.94%; from 45 to 54 – 9.61 ± 2.04%. According to archival data, in 4.33% ± 1.41 (9) patients, the disease manifested at the age of more than 55 years, which corresponds to global data. The results obtained indicate a negative trend of "rejuvenation" of this pathology. At the same time, in 62.50 ± 2.34% of patients, clinical manifestations of the disease formed in the age period of 18 – 34 years (table 4).

Table 4. Debut of various clinical forms of RAS

Age groups, years	Clinical course				Total
	Fibrinous	Setton's aphthae	Herpetiform aphthous stomatitis	Symptom in Behcet's disease	
18 -24	20 / 17,39±3,53	15 / 24,59 ± 5,51	10 / 40,0 ± 9,80	3 / 42,86 ± 18,70	48 / 23,80 ± 2,92
25-34	45 / 39,13±4,54	21 / 34,42 ± 6,08	12 / 48,0 ± 9,99	4 / 57,14 ± 18,70	82 / 39,42 ± 3,39
35-44	32 / 27,82±	15 / 24,59 ± 5,51	2 / 8,0 ± 5,43	— / — / —	49 / 23,56 ± 2,94
45-54	12 / 10,43±4,8	7 / 11,47 ± 4,08	1 / 4,0 ± 3,92	— / — / —	20 / 9,61 ± 2,04
≥55	6 / 5,22±2,03	3 / 4,92 ± 2,80	— / — / —	— / — / —	9 / 4,33 ± 1,41
Total:	115 / 100,0	61 / 100,0	25 / 100,0	7 / 100,0	208 / 100,0

Note: In the numerator – the number of patients; In the denominator, in % of the total number of patients.

In the analysis of gender differences, it was determined that chronic recurrent aphthous stomatitis is mainly registered among women: over the past 5 years, the frequency of women reporting about RAS was 68.75 ± 3.21% against the corresponding frequency among men – 31.21 ± 3.21%. This trend continued throughout the studied period (Table 5).

Table 5. Gender characteristic of RAS

Gender	Clinical course				Total
	Fibrinous	Setton's aphthae	Herpetiform aphthous stomatitis	Symptom in Behcet's disease	
Men	33 28,70±4,22	21 34,43 ± 6,08	10 40,0 ± 9,80	1 14,28 ± 13,30	65 35,25 ± 3,21
Women	82 71,30±4,22	40 65,57 ± 6,08	15 60,0 ± 9,80	4 57,14 ± 18,70	143 68,75 ± 3,21
Total	115 100,0	61 100,0	25 100,0	7 100,0	208 100,0

Seasonal occurrence of exacerbations was noted, chronic recurrent aphthous stomatitis, as a rule, manifested in spring-autumn period (table 6). Moreover, in 23.08 ± 2.92% of patients, the onset of the disease occurred in autumn period of the year; in 36.54 ± 3.34% in winter; in 25.96 ± 3.03% in spring, and only in 14.42 ± 2.43% the onset of the disease occurred in summer season (figure 1).

Table 6. Distribution of patients with RAS by seasons of the year

Clinical course of RAS	Seasons			
	Autumn	Winter	Spring	Summer
Fibrinous form	26 22,61 ± 6,03	42 36,52 ± 5,52	31 26,96 ± 6,04	16 13,91 ± 6,32
Setton's aphthae	14 22,95 ± 6,07	20 32,78 ± 5,38	17 27,87 ± 6,10	10 16,39 ± 6,76
Herpetiform aphthous stomatitis	6 24,0 ± 6,16	11 44,0 ± 5,69	5 20,0 ± 5,49	3 12,0 ± 5,93
Symptom in Behcet's disease	2 28,57 ± 6,52	3 42,86 ± 5,68	1 14,28 ± 4,76	6,39
Total	48 100,0	76 100,0	54 100,0	30 100,0

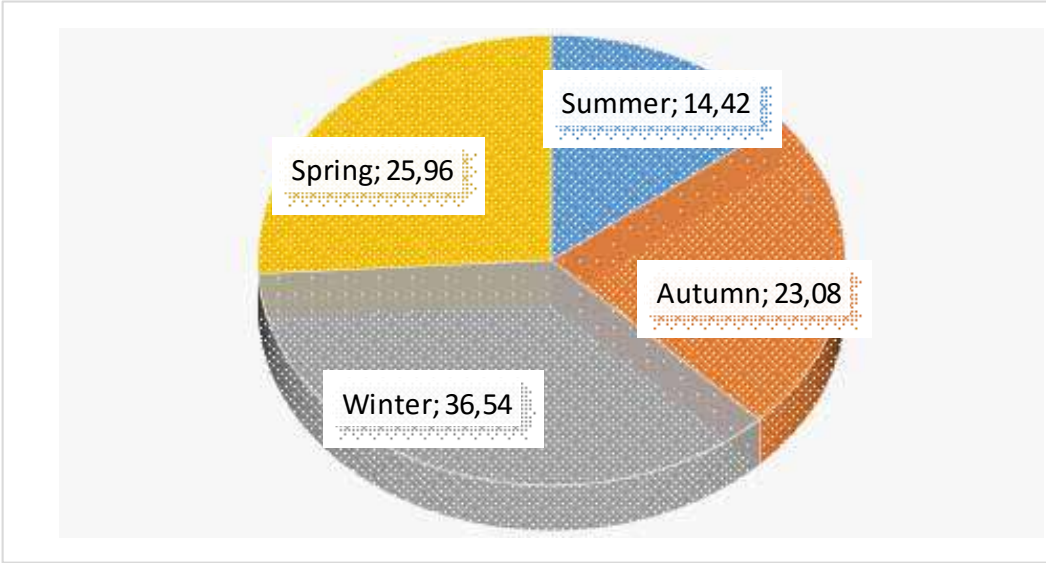


Fig. 1. Distribution of patients with RAS by seasons (in % of the total number of the diseased)

Discussion

Analysis of the duration of the disease at the time of treatment showed that in most patients the disease lasted 3-5 years, the maximum duration at the time of treatment was registered in a more severe form (Setton's aphthae, necrotic periadenitis, deforming aphthae), the average disease duration was 6.88 ± 0.28 year; with a fibrinous form, the disease lasted 3.44 ± 0.17 year and with herpetiform aphthous stomatitis and a symptom in Behcet's disease, 2.84 ± 0.11 and 4.46 ± 0.21 year, respectively.

Analysis of the completeness of the diagnosis, quality of treatment and prevention of RAS revealed the following:

When collecting an anamnesis, as a rule, data on the patient's profession and the presence of occupational hazards and bad habits (smoking, consuming nasvai (a type of non-smoking tobacco product), alcoholism, etc.) were ignored; the nature of nutrition and the use of products that can cause allergic reactions was not taken into account); the history of allergy was not taken into account, the hereditary burden and the presence of RAS among relatives of the first and second lines of kinship were not found out, information about the transferred and concomitant diseases was ignored.

When collecting the anamnesis of only 140 (67.31%) patients, the time of the onset of the first symptoms, the presence of pathology in the past, the nature of the previous treatment and its results were specified. Data on intolerance to drugs and materials, stress that can trigger this exacerbation, acute condition/disease or exacerbation of a chronic disease that developed less than 6 months before seeking dental care, as well as work involving contact with occupational hazards, were obtained from 122 (58.65%) patients.

Incomplete implementation of diagnostic procedures is noted at the stage of diagnosing the disease. A visual examination with a full description of the elements of the lesion was performed in 108 (51.92%) patients, palpation of the organs of the oral cavity, external examinations of the maxillofacial part, and palpation of the maxillofacial part were not performed in any patient, as well as the degree of opening of the mouth and restriction of mobility of the lower jaw is also not determined.

The oral cavity using additional tools is not examined. Carious cavities are not investigated using a dental probe and periodontal pockets using a periodontal probe. Occlusion and degree of pathological tooth mobility are not determined, as well as the diagnosis of the state of the dentofacial system using method and means of beam imaging. Histological examination of the oral tissue preparation and a biopsy of the oral mucosa was not implemented. Determination of oral hygiene and periodontal indices was carried out only in 54 (25.96%) patients.

At present, the importance of the immune system and disturbances in the microbiocenosis of the most important biotopes of the body in the severity of the clinical course of diseases of OCR is not questioned. In this connection, special attention was paid to the study and prescription of drugs for immunity and intestinal microbiocenosis.

When diagnosing the disease, only 45 (21.63%) patients underwent consultative examination and were prescribed examination and treatment by a general practitioner (gastroenterologist, dermatologist, endocrinologist, occupational therapist, neuropathologist), somewhat more often, consultation with an orthopedic dentist was assigned in 63 (30.29%) patients.

It is obvious that the prognosis of RAS and the relapse rate largely depend on the timeliness and accuracy of the diagnosis. The selected sample based on the results of archival data, the diagnosis of chronic recurrent aphthous stomatitis was carried out on the basis of:

– only anamnesis and objective examination data – in 108 (51.92%) patients;
– anamnesis, objective examination, assessment of dental status data – in 54 (25.96%) patients;
– data of anamnesis, objective examination, assessment of dental status, consultative examination by a physician (gastroenterologist, dermatologist, endocrinologist, occupational therapist, neuropathologist) – in 45 (21.63%) patients;
– data of anamnesis, objective examination, assessment of dental status, assessment of the immune status, study of intestinal microbiocenosis, consultative examination by a physician (gastroenterologist, dermatologist, endocrinologist, occupational therapist, neurologist) – in 30 (14.42%) patients.

In the study of the non-pharmacological assistance provided, it was found that oral sanitation with the implementation of professional oral hygiene was carried out only in 59 (28.36) patients; teaching patients the oral hygiene with carrying out controlled toothbrushing – in 81 (38.94%) patients; removal of odontolith, grinding of sharp edges of teeth, replacement of metal fillings and prostheses from dissimilar metals, orthopedic correction, polishing of dentures and elimination of galvanosis – in 45 (21.63%) patients.

In the overwhelming majority of cases, drug therapy in 200 (96.15%) patients consisted of applying painkillers – 1-2% lidocaine solution, 1-2% trimecaine, cleaning the surface of aphthae from fibrinous or necrotic plaque using proteolytic enzymes: trypsin and chymotrypsin, as well as antiseptic treatment with antiseptic solutions: 0.05% chlorhexidine solution, 1% hydrogen peroxide solution, 0.01% miramistin solution, etc. Subsequently, epithelizing therapy with ointments carotoline, solcoseryl, actovegin and cholisal gel was prescribed.

Prescription of dietary therapy was established in 153 (73.56%) patients. It should be noted that there were no prescriptions in the treatment regimens, including sedative and antihistamines, nonspecific hypersensitizing therapy, metabolic correction drugs (calcium preparations, vitamin therapy), drugs that correct microcirculatory disorders and metabolic trophic processes that normalize the lipoperoxidation process, as well as specific hyposensitizing therapy.

Quite rarely, immunomodulating therapy was registered in 102 (49.04%) patients.

Taking into account the gender and age characteristics of patients with RAS at a dental appointment for OCR diseases allowed to determine the real clinical picture and prevalence of this disease, to increase the possibilities of monitoring, clinical examination and provision of timely specialized care to prevent the progression and preserve the working capacity of patients with this OCR disease.

Conclusion

1. The frequency of RAS at the general reception for diseases of OCR is 6.22 ± 0.42%; in the general structure of the disease, the fibrinous form prevails 55,29 ± 3,46%; the incidence of necrotic periadenitis – 29,33 ± 3,15%; herpetiform aphthous stomatitis is registered among 12,02 ± 2,25% patients and symptom in Behcet's disease among 3.36 ± 1.25%.
2. The debut of RAS falls on the age period of 18-34 years (62.50%), which indicates a negative trend of "rejuvenation" of this pathology. Women morbidity prevails in the general structure of the disease – 68.75 ± 3.21%.
3. For adequate treatment of RAS, it is necessary to introduce modern standards for the examination, treatment and prevention of the disease.

The results of the study can be used in practical work for predicting the risk of the onset and development of OCR diseases among the population, as well as for improving therapeutic and preventive measures. For the effective treatment and rehabilitation of patients suffering from RAS, it is necessary to conduct a comprehensive examination with participation of internists, the appointment of the immunomodulating therapy and agents that effectively correct the most important homeostatic mechanisms of the body.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Approach to Managing the Patients with Idiopathic Thrombocytopenic Purpura: A Case-Control Study

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Abstract

In this study, we present the results of treatment of 224 patients with idiopathic thrombocytopenic purpura (ITP). In this study, we present the comparative results of treatment with inhalation and traditional method by glucocorticoid hormones and splenectomy (SE). It was established that the usage of glucocorticosteroid hormones in the manner of cold doses inhalation was more effective in 87, (2%) of patients in comparison with traditional method of treatment – 75,(7%) and in this way the remission of disease approaches on 6,3 days earlier. For the treatment of these patients we used splenectomyand created new approach to the operation. This method, which was executed in the early post operational period, was more effective in 96, (9 %) of cases compared with traditional approach.

Keywords: *Thrombocytopenia, glucocorticoids, splenectomy, complications, prophylaxis, hormone inhalation.*

Introduction

Idiopathic thrombocytopenic purpura (ITP) is one of the common forms of hemorrhagic diathesis[1]. It is known that the basis of the pathogenesis of ITP is an autoimmune process from any cause which result in the synthesis of antiplatelet autoantibodies with an antimegakaryocytic orientation occuring in the human body[2].

ITP is not a rare disease in the structure of hematological pathology. So, for example, in Uzbekistan ITP is registered among children with a frequency of 1.5 - 2.0 cases per 100 000people[2,3].

The pathogenetic treatment of ITP is the use of glucocorticoid (GCS) hormones, however, taking per os hormones often gives gastrointestinal complications[4].

Given the pronounced side effects with prolonged use of corticosteroids, especially when taken per os[5], other method of corticosteroid administration are being sought to reduce or prevent complications of corticosteroids[6]. In this regard, the inhalation method of administering GCS hormones to patients with ITP is noteworthy[7], although in the available literature we could not find works devoted to the study of the use of GCS hormones in the form of inhalations in patients with ITP[8].

When performing splenectomy (SE), various method of mobilizing the spleen and processing the elements of its gates areused[9]. Bleeding during SE and after surgery is from 300 ml to 1 litre, postoperative pancreatitis occurs up to 6% and has no tendency to decrease[10]. Complications associated with the technique of surgery adversely affect the results of treatment[11].

Therefore, the improvement of both conservative and surgical treatment of ITP is undoubtedly of great scientific and practical interest for modern hematology and surgery[12].

Materials and Method

Patients Data: This case-control study retrospectively recruited 224 consecutive patients with a diagnosis of «ITP» in the surgical and pediatric wards of the clinic Scientific-research institute ofHematology and blood transfusion, Ministry of Health, Republic of Uzbekistan Among patients with ITP with a chronic form was 187, with an acute form was 37. Men were 88 and women were 136. Only 95 patients (42.4%) received the hormonal treatment: - including traditional

treatment - 48 patients (men –18 (37.5%), women –30 (62.5%), inhaled hormonal treatment of 47 patients, 22 (46.8%) of them were men, and 25 women (53.2%). SE was performed in 129 (57.6%) of patients with chronic ITP (of these, 40(31.1%) of men and 89 (68.9%) of women). The traditional method of SE was performed in 65 (50.4%) patients; we proposed this method in 64 (49, 6%).

To verify ITP, the following criteria were used below (except for complaints, medical history and objective examination):

1. Thrombocytopenia (platelets $<150x10^9$/L) in the absence of other abnormalities in the calculation of blood cells.
2. The absence of clinical and laboratory signs of the disease in blood relatives.
3. Normal - or increased number of megakaryocytes in the bone marrow.
4. The absence of clinical manifestations of other diseases or factors that can cause thrombocytopenia in patients.
5. The effect of corticosteroid therapy.

During SE, the amount of blood loss was determined by weighing the operating dressings - balls, before and after the operation, and blood loss after the operation from the drainage tube entering the dishes with a mark. In addition, patients with ITP underwent ultrasound for examination of internal organs, electrocardiography, and, according to indications, an endoscopic examination of the gastrointestinal tract.

Patients complained of hemorrhagic syndrome: petechiae and ecchymoses on the skin, nasal, uterine bleeding, bleeding from the gums and the gastrointestinal tract, which often appeared with platelet counts <20.5 - 30.0 x 109/L. The duration of the disease at the time of the first visit to the hospital ranged from 3 days to 5 months in acute ITP. In the anamnesis, the duration of the disease ranged from 6 months to 20 years with a chronic form of ITP and during this time patients received hormones in tablets from 2 to 10 times.

GCS was prescribed in the traditional treatment of 2.0-2.5 mg/kg in the acute form, 1.0-1.5 mg/kg in the chronic form of ITP per os and parenterally. Inhalation (solution of prednisolone and dexamethasone) was carried out on a nebulizer inhalation device “Boreal” (manufactured in Italy) at a dose of 1.0-2.0 mg/kg. In addition, patients received fibrinolysis inhibitors, vascular wall protectors, biological membrane stabilizers and topical treatment for nasal and gingival bleeding. Red blood cell mass was transfused for patients with severe anemia.

In 50 (52.6%) patients with ITP, gastrointestinal complications were revealed; gastroduodenitis in 21, gastric ulcer in 6, duodenal ulcer in 5, colitis in 2, gastrointestinal discomfort in 11, stomach pain in 5.

SE was performed in 129 patients with ITP. For 64 patients of the experimental group, splenectomy was performed by the method we proposed - during revision of the spleen, its mobility and connection with the circumference were assessed, its posterior surface was separated from the parenteral peritoneum by hemostasis in the direction of the pedicle and posterior surface of the stomach.

Then the spleen was easily dislocated into the wound, starting from the lower pole, it was freed from the ligaments and previously bandaged; two clamps were applied over the ligature, then dissected between the clamps and the stump was stitched. Next, a window of 0.5 x 0.5 cm in size was opened in the anterior sheet of the peritoneum from the upper pole of the spleen and the gastro-splenic ligament was ligated in the aforementioned manner.

If the width of the leg is large, more tissue is taken when the poles are highlighted. The front sheet of the peritoneum in the leg area does not open, this preserves the integrity of the tissue and hemostasis. The next stage of the operation is the preliminary ligation of the spleen leg with catgut over the capsule of the pancreas, under the control of the back surface, so as not to damage the tail of the pancreas. At the same time, all the elements of the spleen leg are assembled into a single stem with a straightened axis, which greatly improves subsequent clamping over the ligature.

This, in turn, is the prevention of bleeding. Subsequently, 2-3 clamps are applied over the ligature, and the spleen is removed. The stump is bandaged and stitched. Performing SE in this way prevents damage to the tail of the pancreas, creates reliable hemostasis, maintains the integrity of adjacent tissues

Results

Inhalation was carried out at a dose of 1.0 to 2.0 mg/kg per day. Of 18 patients with ICU, a clinical and hematological effect (CGE) was obtained in 13 (72.2%) patients, an average of 6.5 days, a clinical effect (CE) in 2 (11.1%) patients, on average for 5.5 days. Clinical and hematological effect (K and KGE) were obtained in 15 (83.3%) patients, on average for 6 bed days. In 3 (16.7%) patients, the effect was not obtained. In patients with CITPwho received inhaled therapy of corticosteroid hormones, the hemorrhagic syndrome began to disappear by days 3 and 4, the platelet count began to rise from days 5 and 6 of treatment. 19 patients (adults - 9, children - 10) with ICU, starting from the first or second day of admission, received hormones (prednisone and dexamethasone) in tablets and injections at a dose of 1.5 - 2.0 mg/kg with other HA drugs per day. The results of traditional hormone therapy in adults and children with ICU have shown that CGE was obtained in 13 (68.4%) patients, on average for 9.8 bed days. CE was obtained in 2 (10.5%) patients, an average of 16.5 hospital days. K and CGE was obtained in 15 (78.9%) patients, on average, for approximately 11 bed days. In 4 (21.1%) patients, the effect of hormone therapy was not obtained even after carrying out hormonal treatment for more than 3-4 weeks they were ineffective.

The results of inhalation therapy of sick children and adults with a chronic form of ITP (CITP),inhalation was performed at a dose of up to 2 mg/kg per day. Of the 29 patients who received inhalation therapy, CGE - in 13 (44.8%) patients, an average of 6 bed days, CE in 13 (44.8%) patients received an average of 7 days, 3 (10.4%) - without effect. Twenty-six (89.7%) patients received K and KGE, on average for 7 bed days. Starting from days 2 and 4, in all patients whose hemorrhagic syndrome has subsided.

29 patients with CITP(children - 9, adults - 20) received traditional treatment, including hormones at a dose of 1.0 - 1.5 mg/kg per day as a pathogenetic therapy of HK. From the traditional treatment of HK with hormones, sick children and adults with HITP received CGE of 6 (20.7%) patients, on average, for 9 bed days. Clinical remission (CR) in 15 (51.7%) patients, an average of 11 bed days. K and RAG were obtained in 21 (72.4%) patients, an average of 11 bed days, 8 (27.6%) patients were without effect with the preservation of hemorrhagic syndrome. Hemorrhagic syndromes began to disappear from 3-5 days in patients with remission.

Thus 47 patients withAITP and HITP received dosed cold inhalation of GCS hormones, while in patients with AITP K and RAG were obtained in 83.3% of cases on average in 6 days, on the contrary to traditional treatment 78.9% and 11 days. In patients with HITP K and RAG received in 89.7% of patients, in 6.5 days, on the contrary 72.4% and 10.7 days Of the 47 patients who received inhalation therapy 26 (55.3%) patients with average of 6 bed days admission received a CGE, in 15 (31.9%) with average of 7.0 bed days received a CR and in 41 (87.2%) patients with average of 6 bed days received K and CGE. In 6 (12.8%) patients, the effect was not obtained and they still had skin hemorrhagic diseases, in the form of ecchymoses[14].

ITP splenectomy results for years 2000-2002 in the generally accepted manner, 65 (50.4%) patients with ITP were operated among them: children - 37 (56.9%), adults - 28 (43.1%). Moreover, the total blood loss amounted to between 122.8 - 7980 ml per patient. During the operation, a total of 5590 ml of FFP and 9715 ml of erythrocyte mass were transfused. In total, 235.5-15305 ml were transferred per patient[15].

A complication such as damage to the pancreas during surgery was observed in 12 (18.5%) patients. Days 1 and 2 after surgery, 4 (6.2%) patients had an increase in temperature to 38^0C and 3 (4.6%) patients had complaints on the 2nd day after surgery for pain in the left hypochondrium and lumbar region. From the drainage tube, hemorrhagic discharge amounted to 100.0 ml or more on the 1st and 2nd day. In 50% of patients, the drainage tube was removed on the 1st day after surgery[16].

Relaparotomy for internal bleeding after SE was performed in 2 (3.1%) patients. The immediate postoperative results in patients with ITP with a chronic course of K and RAG amounted to 91.6% of patients. We proposed and performed SE on 64 patients with ITP with a chronic course. Moreover, the total blood loss is estimated to be between 76.8 - 4915 ml per patient. During the operation in 3 (4.7%) patients, the tail of the pancreas on the catgut ligature was damaged, but in the postoperative period, a pancreatitis clinic was not observed[17].

After surgery, hemorrhagic discharge from the drainage tube was up to 30 ml. The immediate postoperative results in patients with ITP with a chronic course of K and RAG were in 96.9% of patients. In 90%

of cases, drainage tubes were removed on the 1st day after surgery. There were no complications during and after the operation[18].

Discussion

Thus, the inhaled method of administering GCS hormones in dosed cold form on the Boreal nebulizer apparatus to patients with acute and chronic ITP is not inferior compared with traditional treatment with oral and parenteral administration of HA and has such positive aspects as;lack of complications from inhalation, good tolerance of the procedure, especially by children; prevention of neurosis, hysteria, pain associated with the manipulation of corticosteroids with hormones in children; lack of withdrawal syndrome; prevention of parenteral infection;economic efficiency by reducing the amount of hormones, blood components, and other drugs introduced by HA; and reducing the number of days. In a comparative aspect, with traditional treatment in acute ITP, 4.4, in chronic ITP 4.2 days earlier, remission was received.

The method we proposed allows us to preserve the integrity of adjacent tissues, prevents damage to the tail of the pancreas, and prevents bleeding during and after surgery. At the same time, the duration of the operation decreased by 10-15 minutes, bleeding during the operation decreased by 45.7 ml in one patient, and after the operation by 2-3 times. Postoperative pancreatitis, relaparotomy, and death were not observed. Erythrocyte mass, blood components and other drugs are saved.

Conclusion

In summary, the inhalation administration of GCS hormones in dosed cold form on the Boreal nebulizer apparatus to patients with acute and chronic forms of ITP is an alternative to the existing traditional method of conservative treatment. From inhalation administration of HA, in a comparative aspect, with traditional treatment for acute ITP, 4.4, in chronic ITP 4.2 days earlier, a remission was received. It prevents a number of complications of corticosteroids therapy and the transmission of parenteral blood-borne infection. Inhalation administration of corticosteroids hormones in patients with ITP disease is indicated, especially in childhood and in patients with diseases of the gastrointestinal tract. Contraindicated in patients with severe chronic condition against the background of the underlying disease and intolerance to inhaled corticosteroids.The use of an advanced method of splenectomy - this operation is less traumatic, has a milder course of the postoperative period, less blood loss and a minimal number of complications.

The use of an advanced method of splenectomy reduces blood flow during splenectomy by 41.4 ml, furthermore, it reduces the need for blood transfusions by 2-3 times. The proposed method reduces risk of damage to the pancreas by 4 times and the development of postoperative pancreatitis. It also reduces the duration of the operation by 10-15 minutes. A good result in the immediate postoperative period is achieved after splenectomy in the proposed.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Study of Hematological Changes in Local Goats (Capran Aegagrus) at Al-Najaf Province, Iraq

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Abstract

Goat is among widespread small ruminants in the Iraq and important animal provides nutrient resources for as well as economical source. The blood values and hematologicalparameters can be greatly affected by environmental factors such as stress, low grassland pasture, parturition and climate. Thus, it can be reflected by blood analysis and reliable medium for health and illness status of individual animals.

200 of normal and healthy goats (90 males and 110 Females) were selected depending on clinical examination in Al-Najaf slaughterhouse (Al-Najaf, Iraq). The study was conducted during the summer season. The gouts were divided into subgroups according to sex and age. Then, Blood samples were collected from the jugular vein for the determination of hemoglobin, packed cell volume, red blood cells count, and white blood cells count and sub determination of white blood cell was included neutrophils, small and large lymphocytes, monocyte, eosinophil, basophil and band-cell. Result showed there are nonsignificant different between male and female hematological parameters in all age period except small lymphocytes in gout larger than 1 year old and Neutrophils for less than 6-month age. In Conclusion: This study includes values that are considered to be the first to be published for the difference in local goats' breeds (Capran Aegagrus) At Al-Najaf Province, Iraq. Such data is considered as preliminary data which can be used as a reference for further studies.

Aim of Study: The aim of the study was to measure hematological parameters in order to monitor the health status of goat breeding with different age in males and female goat.

Keywords: *Hematological, hematological parameters, Capran Aegagrus.*

Introduction

Goat is among widespread small ruminants in the middle east, as well as is considered one of the domestic animals which an important animal provides nutrient resources for humans (Addass *et al.* 2010)[1]. Additionally, is an important economic source in several countries such as Iraq, Jourdan, Oman and other contraries (Kozuh, 2010; Al-Bulushi *et al.* 2017)[2]. Goat meat is well known to be unique meat. In addition, goat meat is not interchangeable with meat from other species that have the same –approximately- maturity and fatness levels (Ivanović *et al.* 2016)[3], as well as goats can be reared under traditional husbandry system in the open atmosphere and breeding under modern husbandry (Gebreyowhens *et al.* 2018)[4]; which are believed as perfect animals to be reared, firstly because they are able to survive severe conditions and secondly because they are able to produce high-quality and milk and milk derivatives (Darcan *et al.* 2018)[5]. The blood values and hematological parameters can greatly be affected by factors like low nutritional grassland pasture, parturition, climatic factors and stress (Mohammed *et al.* 2016; Mahmoud *et al.* 2016)[6]. Besides being one of the useful and reliable parameters to use in order to assess health and illness status of individual animals (Al-Bulushi *et al.* 2017)[7], blood is a guide for several metabolic processes and represents the healthy state of the organ functions and productions of the animal and a realistic measure to evaluate the management practices, nutrition progress and diagnosis in farms (Farooq *et al.* 2011; Momenah, 2014; Panadi *et al.* 2018)[8].

Thus, the aim of the study was to investigate and monitor the health status of goat breeding through the measurement hematological parameters of different age in males and female goat and knowledge different between male and female blood parameter value.

Material and Method

Animals: Two hundred (90 males and 110 Female) of goats which were selected normally healthygoat depending on clinical examination in Al-Najaf slaughterhouse (Al-Najaf, Iraq). The gouts were divided into subgroups according to sex and age. However, four age group of two sex goats were study, its included 0 - 6 months, 6 - 12 months, 1–2 years, and above the 2 year, as showed in table-1.

Table 1: The numbers and ages of goat according to sex

Sex	Number	Age
Males (90)	15	0 – 6 months
	25	6 – 12 months
	19	1 – 2 year
	30	Above 2 year
Female (110)	14	0 – 6 months
	31	6 – 12 months
	35	1 – 2 year
	31	Above 2 year

Blood Sampling: Blood collection was performed from the jugular vein followed by placing blood into a tube containing ethylenediaminetetraacetic acid, placed on ice and transferred immediately to the laboratory to be used for the assessment of blood hematological parameters.

Hematology Analyzer: It is an automated hematology analyzer that is used for analyzing feline blood including the determination of hemoglobin (Hb), packed cell volume (PCV), red blood cells count (RBC), and white blood cells count (WBC) within 2 hours after sample collection. Sub determination of white blood cell was included neutrophils (N), small and large lymphocytes (L), monocyte (M), eosinophil (E), basophil (B) and band-cell (BC).

Statistical Analysis: All data were presented as mean ± standard error of mean (SEM). Graph Pad PRISM8 software was used to analyze data. Shapiro–Wilk normality test was used to test whether our data are normally distributed followed by 2-way ANOVA to determine any significant difference. Those data with p value($p<0.05$) was considered significantly different.

Result

The result in table-2 showed compared between blood hematology in goat in age 0-6 months, however showed no significant different between parameters except in neutrophils since female neutrophils higher than male neutrophil significantly at (P=0.0003).While in table-3 result showed no significant different between hematological parameters of 55 goats (24 males and 31 female)at age 6-12 months**.**

Additionally, result in table-4 explained compare between hematological parameters of 54 goats (19 males and 35 female) at age between 1-2 years which fond no significant different between male and female except in term of large lymphocyte which higher significantly at (P= 0.0046)in female.

Result showed comparehematological parameters between male and female at age above 2 year in table-5 showed only significant different at (P=0.0025) in smalllymphocyte which higher in males' goat than females, while no significant different in others parameters.

In general, from tables-6 observed in compared hematological parameters between all age of 200 goat (90 males and 110 females) showed no significant different in parameters except in small lymphocyte parameter which was higher significantly at (P= 0.0161) in males' goats than females as showed in table-6.

Table 2: 0–6 months

Parameters	Male	Female	P Value
Age	3.8±0.5	2.76±0.45	0.9960
Hb	6.70±0.1	6.76±0.11	>0.9999
PCV	27.13±0.2	26.6±0.26	>0.9999
RBC	12.42±0.3	12.6±0.14	>0.9999
WBC	7.73±0.1	6.75±0.32	0.9975
S.lymph	41.66±1.3	42.84±1.97	0.9870
L.lymph	26±1.3	24.07±1.78	0.6936
Neutrophils	26.4±1.1[a]	31.30±1.45[b]	0.0003
Band-cell	3.26±0.5	2.76±0.28	>0.9999
Eosinophils	0.8±0.2	0.69±0.17	>0.9999
Basophil	0.13±0.09	0.07±0.07	>0.9999
Monocyte	1.46±0.3	1.92±0.30	>0.9999

Values as means ± stander error of 28 Goats (15 males and 13 females)

- [ab]refer to significant differences between groups ($P < 0.05$)

Table 3: - 6 – 12 months

Parameters	Male	Female	P Value
Age	8.19 ± 0.35	8.82 ± 0.34	0.8593
Hb	8.35 ± 0.17	8.17 ± 0.12	>0.9999
PCV	27.75 ± 0.30	27.10 ± 0.35	0.8351
RBC	12.00 ± 0.13	11.91 ± 0.12	>0.9999
WBC	8.46 ± 0.16	8.51 ± 0.16	>0.9999
S.lymph	29.92 ± 0.35	29.84 ± 0.23	>0.9999
L.lymph	29.96 ± 0.49	29.65 ± 0.27	0.9996
Neutrophils	34.46 ± 0.66	35.13 ± 0.61	0.8098
Band-cell	0.21 ± 0.12	0.58 ± 0.23	0.9978
Eosinophils	1.92 ± 0.25	1.58 ± 0.30	0.9992
Basophil	1.33 ± 0.24	1.10 ± 0.25	>0.9999
Monocyte	2.25 ± 0.29	1.90 ± 0.20	0.9989

Values as means ± stander error of55 Goats (24 males and 31 females)

- [ab]refer to significant differences between groups ($P < 0.05$)

Table 4: 1 – 2 years

Parameters	Male	Female	P Value
Age	1.53 ± 0.08	1.44 ± 0.07	>0.9999
Hp	8.57 ± 0.11	8.13 ± 0.08	0.9999
PCV	25.65 ± 0.28	24.94 ± 0.21	0.9893
RBC	9.15 ± 0.17	9.27 ± 0.16	>0.9999
WBC	8.99 ± 0.17	9.15 ± 0.13	>0.9999
S.lymph	22.32 ± 0.71	20.66 ± 0.78	0.2027

Parameters	Male	Female	P Value
L.lymph	25.32 ± 0.82 a	27.83 ± 1.23 b	0.0046
Neutrophils	47.74 ± 0.57	48.06 ± 0.46	>0.9999
Band-cell	0.00 ± 0.00	0.00 ± 0.00	>0.9999
Eosinophils	1.63 ± 0.22	1.80 ± 0.22	>0.9999
Basophil	0.53 ± 0.16	0.54 ± 0.13	>0.9999
Monocyte	2.26 ± 0.56	2.46 ± 0.32	>0.9999

Values as means ± stander error of 28 Goats (19 males and 35 females)
- [ab] Means values between groups with different superscripts are significantly different at ($P < 0.05$)

Table 5: Above 2 years

Parameters	Male	Female	P Value
Age	2.65 ± 0.07	3.29 ± 0.12	0.9607
Hp	10.43 ± 0.13	10.08 ± 0.17	0.9998
PCV	26.65 ± 0.35	26.03 ± 0.42	0.9725
RBC	10.62 ± 0.14	9.85 ± 0.14	0.8721
WBC	9.71 ± 0.19	9.61 ± 0.16	>0.9999
S.lymph	22.39 ± 1.06a	20.37 ± 0.72b	0.0025
L.lymph	26.77 ± 0.81	27.90 ± 0.60	0.3712
Neutrophils	47.74 ± 0.28	47.30 ± 0.24	0.9984
Band-cell	0.00 ± 0.00	0.00 ± 0.00	>0.9999
Eosinophils	1.29 ± 0.21	1.47 ± 0.25	>0.9999
Basophil	0.65 ± 0.15	0.67 ± 0.15	>0.9999
Monocyte	2.03 ± 0.20	2.57 ± 0.22	0.9909

Values as means ± stander error of 28 Goats (15 males and 13 females)
- [ab] Means values between groups with different superscripts are significantly different at ($P < 0.05$)

Table 6: Between male and female

Parameters	Male	Female	P Value
Age	4.10 ± 0.31	4.21 ± 0.31	>0.9999
Hp	8.84 ± 0.16	8.52 ± 0.12	>0.9999
PCV	26.81 ± 0.18	26.06 ± 0.19	0.9154
RBC	10.98 ± 0.15	10.59 ± 0.15	0.9997
WBC	8.84 ± 0.12	8.81 ± 0.12	>0.9999
S.lymph	27.65 ± 0.89a	25.83 ± 0.82b	0.0161
L.lymph	27.19 ± 0.46	27.92 ± 0.50	0.9309
Neutrophils	40.56 ± 0.96	42.17 ± 0.72	0.0520
Band-cell	0.61 ± 0.16	0.50 ± 0.11	>0.9999
Eosinophils	1.45 ± 0.12	1.51 ± 0.13	>0.9999
Basophil	0.72 ± 0.10	0.68 ± 0.10	>0.9999
Monocyte	2.04 ± 0.17	2.27 ± 0.14	>0.9999

Values as means ± stander error of 28 Goats (15 males and 13 females)
- [ab]refer to significant differences between groups ($P < 0.05$)

Discussion

This study reported a wide range of hematological values such as RBC, PCV, Hb, WBC in addition to white blood differential count in Local Goats (*Capran Aegagrus*) at Al-Najaf Province, Iraq. Findings reported in this study are generally consistent with previous studies which discussed healthy goats. As shown in tables (2, 3, 4, 5), the levels of hemoglobin were within the normal range for male and female and at different age which results are agreed with (Al-Rukibat and Ismail, 2019) which detection of hematologic variables in Shami goats and (Khan *et al.* 2016) since analyzed the blood sample of Maraz male and female goats. On other hand, Polatet al.(2018)[9] found changes in levels of Hb during month and seasons, which result is contrary to that of Polat et al.(2018)[10] them found significant increase in Hb levels of male than female in all age of Angoragout.

Comparison of the findings with those of other studies table (2, 3, 4, 5) confirms theno significant different in the levels of RBC are generally found higher in males than female in the all age but not significantly as reported that values of hematological parameters in most animal species (Yaqub et al. 2013)[11], while Polatet al.(2018)[20] found significant higher in values RBC of male than female[12].

The levels of white blood cells count for the examined does were within the normal range as showing in tables (2, 3, 4, 5,6) for goats in all age as reported in varied studies values of WBCin different sex goat and age (Fadare et al. 2012; Pehlivan et al. 2017)[13] but not agreed the Polatet al.(2018)[14] which found WBC higher in values of female goats than males. In addition, similar values of WBC were reported in this study to those reported in other studies (Jarić et al. 2015, Nayak and Acharya, 2020)[15] which there are no significant different between gander in WBC value. In organisms[16], WBC are cells of the immune system that represent the integrity of bone morrow to produce cells important for peripheral circulation and storage in different organs or pools (Yaqub et al. 2013)[17]. In this result showed small lymph are higher significantly in male than female as showed in result of (Mohammed etal. 2016)[18].

In general, the tables showed there are not significant different in PCV levels between genders in all age period as showed in other studies (Jarić et al. 2015, Nayak and Acharya, 2020)[19].

Conclusion

Findings obtained in this study are the first to be published regarding the difference in local goats' breeds (Capran Aegagrus) At Al-Najaf Province, Iraq. In addition, it can be concluded that such data is considered as preliminary values that can be used as a reference for further. Furthermore, it can help veterinarians in terms interpretation of laboratory data, selection of an appropriate treatment and diagnosis in capra aegagrus in zoological studies.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Game Method of Rehabilitation of Children with Infantile Cerebral Paralysis

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Abstract

Nowadays, the problem of the vital functions of patients with infantile cerebral paralysisis in the combination of various aspects of rehabilitation: rehabilitation and recreation, physical and psychological, social, emotional and educational. In a comprehensive rehabilitation system, more attention has been paid recently to new organizational forms, such as the game method.

The aim of research: To develop a new rehabilitation system for children with infantile cerebral paralysis.

Materials and Method: We examined 52 children aged 2 to 12 years with various forms of infantile cerebral paralysis. The control group, we were engaged in physical therapy in a game form along with mothers.

Results: Children who played in a game way with their mothers improved movement in the limbs, improved mood, enjoyed doing exercises and had a positive result more than the previous group.

Keywords: *Game method, disability, speech disorders, rehabilitation.*

Introduction

Relevance: Disability of children with cerebral pathology takes the first place in the structure of childhood disability according to the neurological profile and its severity is due to both motor, mental and speech disorders. The rehabilitation success depends not only on the severity of the damage to the central nervous system (CNS) of the child, but also on timely diagnosis, correct organization of the treatment process, starting from the first years of the child's life[4]

The Aim of Research: To develop a new form and system of outdoor games in the rehabilitation of children with the consequences of infantile cerebral paralysis, by unconventional means.

Tasks of Research:

1. To develop new game method for children - with the consequences of infantile cerebral paralysis in order to correct motor abilities.
2. To substantiate the effectiveness of the game technique developed by us on the physical condition of children with the consequences of infantile cerebral paralysis.

The Object of Research: Examined children aged 2 to 12 years with the consequences of infantile cerebral paralysis.

Table 1: Forms of infantile cerebral paralysis in the studied patients.

Clinical forms of infantile cerebral paralysis	Number of patients with infantile cerebral paralysis	Boys	Girls	Age (Average)
Spastic diplegia	4-7.69%	1-1.9%	3-5.8%	2.8
Hemiplegic form	21-40.38%	9-17.3%	12-23%	5.4
Double hemiplegia	7-13.46%	4-7.7%	3-5.8%	3.7
Hyperkinetic form	6-11.5%	3-5.8%	3-5.8%	8.2
Atonic-astatic form	2-3.85%	-	2-3.85%	1.9
Mixed form	12-23%	4-7.7%	8-15.4%	2.2

Table 2: Number of main group patients

Clinical forms of infantile cerebral paralysis	Number of patients with infantile cerebral paralysis	Boys	Girls	Age (Average)
Spastic diplegia	2-3.85%	1-1.9%	1-1.9%	2.9
Hemiplegic form	11-21%	6-11.5%	5-9.6%	4.9
Double hemiplegia	4-7.7%	2-3.85%	2-3.85%	4.2
Hyperkinetic form	4-7.7%	1-1.9%	3-5.8%	7.3
Atonic-astatic form	1-1.9%	-	1-1.9%	1.3
Mixed form	8-15.4%	4-7.7%	4-7.7%	2.6

Table 3: Number of control group patients

Clinical forms of infantile cerebral paralysis	Number of patients with infantile cerebral paralysis	Boys	Girls	Age (Average)
Spastic diplegia	2-3.85%	1-1.9%	1-1.9%	2.9
Hemiplegic form	10-19.2%	6-11.5%	4-7.7%	4.7
Double hemiplegia	3-5.8%	1-1.9%	2-3.85%	3.8
Hyperkinetic form	2-3.85%	-	2-3.85%	6.6
Atonic-astatic form	1-1.9%	-	1-1.9%	1.6
Mixed form	4-7.7%	1-1.9%	3-5.8%	2.9

All children are divided into 2 groups (30 main and 22 control). All children after 10 days of treatment in the hospital were discharged home. All parents had lessons so that they could independently deal with the child with infantile cerebral paralysis at home. The main group all these activities should be performed in a game way, without forcing a child. Particular attention was paid to the fact that the child himself wanted to be engaged.

All used integrated systems in a game way together with mothers should be affected by the ability to feel the child's self-confidence. Since the rehabilitation of children with infantile cerebral paralysis is complex, it includes psychological, social, medical, pedagogical and other aspects. At the same time, physical education is the most important part of the process[6]. Classes were held 6 times a week mainly in the morning. The lesson duration was 20-30 minutes depending on the condition and mood of the child. All children in dispensary settings received heat therapy for 1 month. Thermal procedures were applied in the form of paraffin-ozocerite applications with a temperature of 45-50°C on the hypertonic and hypotonic muscles of the limbs. A general massage was applied for 15 minutes daily. Massage is used with stroking, rubbing, kneading and vibration techniques depending on the age, severity and degree of the disease for 10 minutes №10. Dispensary conditions in the gym of physiotherapy exercises were performed physiotherapy

4-5 times a week for 15-20 minutes for a course of 30 procedures. To assess the psycho-speech development of the child, the clinical psychological assessment Gnome was used. The results of the study were transferred into points. The maximum score was 10 points.

With the functional classification of infantile cerebral paralysis - GMFCS (Gross Motor Function Classification System) proposed by R. Palisano et al. (1997), the degree of motor skills development and movement restrictions in everyday life was taken into account for 5 age groups of patients with infantile cerebral paralysis.

Table 4: Studied Population Level according to GMFCS

	Level 1	Level 2	Level 3	Level 4	Level 5
Main group	2	4	8	4	2
Control group	2	6	6	5	1

The motor characteristics were investigated on a 100-point scale, which after the study was again measured and compared with the previous result. Since the performance of physical exercises ensures the participation of higher mental functions in the process of mastering motor skills and has a great influence on the nervous system development, we contributed to the mental development of the child with conscious movements[1]. They explained to their mothers that rehabilitation is timeliness, interconnectedness, continuity[5]. The treatment of this disease has long been unpromising [5]. Of great importance for the further rehabilitation of children with infantile cerebral paralysis is the early diagnosis of the disease. The sooner specialists begin to work with the child, the greater the chance to prevent, and not subsequently correct, the delayed reaction of parents, deviations in speech and psyche.[3] Another important criterion for successful rehabilitation work with children with disabilities with infantile cerebral paralysis is to participate in a rehabilitation event for the mothers of a sick child. In this regard, the application. For the restorative effect on the body of a child with infantile cerebral paralysis, exercise therapy with the game method e is a very urgent problem. The technique is a game way and even more so with mothers as a means of physical rehabilitation of children with infantile cerebral paralysis has a very good result[1]. Movement disorders that limit or impede normal physical activity adversely affect all aspects of personality development [6]. The nature of the rehabilitative effects on the child's body with the consequences of infantile cerebral paralysis was complex, supported the motivation of disabled children to permanent classes, conducted classes in a mode corresponding to the child's capabilities, created a favorable psychological background. [1] The comprehensive system of rehabilitation impacts that we used in our work consisted of:

- Special physical exercises that do not bother the child.
- Physical exercises with light gymnastics.
- Game classes with mothers.
- Sports and lightweight outdoor games[4].

The fundamental direction of our thesis is the game method without fail along with mothers.

Results

Physical rehabilitation expressing the improvement of motor abilities by the game method together with the mothers of children with disabilities showed high efficiency. Under the influence of prolonged physical exercises in the muscles of a child with infantile cerebral paralysis, adequate motor sensations arose. The postures and positions of the limbs in the process of medical gymnastics returned to normal, muscle tone and involuntary movements decreased. The child began to properly feel the postures and movements, which is the most important powerful incentive in the process of medical gymnastics [7]. As a result of an integrated rehabilitation system introduction, the game method with mothers and children suffering from the consequences of infantile cerebral paralysis gave a very positive effect.

Table 5: Comparative evaluation of psychocognitive parameters in children with infantile cerebral paralysis before and after the study

Field of study	Field parameters	Main group		Control group	
		Before treatment	After treatment	Before treatment	After treatment
Emotional Functions	Total emotions	3.0±0,2	4.2±0.3	3.1±0.1	3.4±0.1
	Activity	4.0±0.1	5.5±0.6	3. ±0.1	3.4±0.6
	Activity in response	3.0±0.3	4.0±0.3	2.8±0.5	2.9±0.8
Cognitive functions	Attention	4.8±0.6	5.5±0.8	3.8±0.3	4.2±0.5
	Thinking	2.4±0.2	3.4±0.5	2.5±0.1	2.6±0.3
	Game features	5.5±0.1	7.5±0.3	5.2±0.8	5.5±0.8
Child behavior	Food	5.4±0.3	6.5±0.6	5.0±0.8	5.2±0.8
	Life skills	5.2±0.8	6.6±0.3	5.0±0.1	5.4±0.2
Touch functions	Vision	6.5±0.6	7.2±0.8	5.8±0.8	6.2±0.3
	Hearing	6.4±0.8	6.8±0.1	5.8±0.6	6.0±0.2
	Tactile sense	4.5±0.1	5.2±0.3	4.4±0.3	4.4±0.6
Motor skills	Facial expression	3.5±0.3	4.2±0.6	3.6±0.1	3.8±0.3
	Statics Kinetics	3.6±0.3	4.0±0.1	3.2±0.3	3.4±0.6

Table 6: After the study, the obtained results of large motor functions

	Level 1	Level 2	Level 3	Level 4	Level 5
Main group	2	1	9	2	1
Control group	2	7	6	4	1

After the study, the level of large motor function moved in a positive direction.

Table 7: Motor characteristics before and after the study

Parameters	Main group		Control group	
	Before study	After study	Before study	After study
Support development	34.2±23.2	42.3±12.4	24.5±11.2	25.5±10.3
Head holding	42.3±14.5	54.4±14.4	36.6±15.4	37.6±38.2
Hand balance	51.2±13.4	57.6±15.6	48.7±5.6	51.3±12.3
Torso control	54.3±12.3	59.5±14.5	51.3±11.2	51.7±12.4
Walking Verticalization	44.3±15.4	49.4±12.4	38.4±12.4	41.3±42.3
Item Hold	7.5±3.4	8.4±2.2	6.7±1.5	6.8±1.4
Hold posture	42.2±11.3	48.4±14.5	44.5±11.3	43.7±14
Walking speed	18±3.4	22±1.5	15±2.2	15.5±1.4

After completing all the tasks, the child's position was evaluated using a 100-point value. The results above 80 points meant the children without infantile cerebral paralysis without significant motor impairment. 74-80 points - borderline motor disturbance, 64-73 - average degree and below 60 - severe degree.

All these activities, classes with mothers, especially game method, had a beneficial effect on both the physical and mental state of children with the consequences of infantile cerebral paralysis.

Wellness activities using long-term rehabilitation

with a set of exercises for the movement amplitude development, led to positive dynamics in the main groups, regardless of the pathology severity. The proposed rehabilitation program allowed children with infantile cerebral paralysis to increase the range of motion in the joints and muscles.

Thus, a comprehensive system of rehabilitation measures in the game method along with mothers has shown its effectiveness. It gives an improvement in the functional state of the musculoskeletal system, a change in the independence of the child and a positive result when used in practical work with people with disabilities suffering from the consequences of infantile cerebral paralysis.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Psychoemotional State of the Universities' Teaching Staff in Uzbekistan

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Abstract

The results of assessing the psychophysiological state of modern teachers of Uzbekistan leading universities as the basis for the preventive measures development to maintain their health are presented. It is shown that the teachers' cardiovascular system (CVS) functional state during the working day basically does not go beyond physiological fluctuations, and the functional state of the central nervous system is characterized by a decrease in the quality of the work performed. Revealed a high frequency of emotional burnout (EB) signs in teaching staff in comparison with the administrative and technical employees of universities; the average statistical indicators of the signs of the teaching staff of different universities do not have statistically significant differences. The average level frequency of emotional exhaustion among the teaching staff members is 52-60%, a high level of personal achievement reduction - 44-50%; the average level of depersonalization is 22-40%. For the EB signs frequency, the work experience at the university matters; at the beginning of the academic year, emotional burnout indicators are less pronounced than at the end.

Keywords: *University teachers, psychophysiological state.*

Introduction

In modern conditions of widespread information technology development, a change in the requirements for higher professional education and, consequently, increasing the effectiveness of university teachers as leading subjects of higher education is inevitable[1]. Moreover, the quality of training depends not only on the degree of teacher's professionalism, but also on his internal state - physical and mental health, which, in turn, largely depends on the teacher's working conditions[4].

The main factors of the teacher's work are: a busy schedule with an increased psychoemotional state, significant voice load, prolonged static load with a slight general muscle load, a large amount of visual work[4]. The busy schedule of the teacher, even with favorable hygienic factors of the working environment, can have an adverse effect on the body and, above all, on the central nervous system[1]. It should be borne in mind that a high neuropsychic stress for a teacher is a factor affecting his body every day throughout the entire academic year and throughout his work at a university[3].

The aim of the present research was to assess the psychophysiological state of modern teachers of universities in Uzbekistan as the basis for the development of preventive measures to preserve the health of the universities' teaching staff (faculty).

Research Object and Method

The research object is the teaching staff of three leading universities of the Republic of Uzbekistan: National State University of Uzbekistan (UzNU), Tashkent State Polytechnic University (TSTU), Tashkent Medical Academy (TMA). As comparison groups, we used the results of the psychophysiological state research of the universities' administrative and technical personnel[5].

The research was performed in two stages. At the first stage, the functional state of the cardiovascular (CVS) and central nervous systems (CNS) was studied during the working day[6]. At the second stage, the frequency and nature of the emotional state symptoms of the teachers' body was studied, taking into account gender, age and work experience[7].

We assessed the CVS and central nervous system functional status in the spring (May 2018) in two universities with a significant difference in the areas of study - humanitarian (TMA) and technical (TSTU). In each of these universities from 30 to 60 people of each gender were studied at the beginning and at the end of the academic day. The CVS condition was assessed by the pulse rate and blood pressure, the central nervous system condition - by Anfimov's correction test (Komarova I.A., Melnikov I.Yu., 2014)[8]. In accordance with the recommendations of these authors, in assessing the functional state of the central nervous system, two indicators were considered - the accuracy factor of task performance (A) and the mental productivity coefficient (P).

The emotional burnout (EB) state was revealed by setting up a test for emotional burnout (Boyko V.V., 2002), which contains 22 statements about the feelings and emotions associated with the teaching staff work.

Testing contains the selection of various answers to 22 questions characterizing three indicators of EB: emotional exhaustion, depersonalization and personal achievement reduction.

Emotional exhaustion (EE) is characterized by a decrease in mood and emotional background, fatigue, depression, apathy, a feeling of frustration, and indifference.

Depersonalization is characterized by a violation of relationships with colleagues at work, indifference to team members, and a lack of positive aspects vision in their activities.

The personal achievement reduction implies self-doubt, the possibility of further development and new achievements.

Results and Discussion

Evaluation of some indicators of the functional state of the body of teachers during the working day. Features of the teaching staff of universities are of the greatest importance for the functional state of the cardiovascular and central nervous system, which was taken into account when organizing functional studies.

Functional state assessment of the teaching staff body showed that during the working day in the teacher's body, functional changes are formed, the nature of which depend on specific functions, as well as on gender and work experience.

The functional state of the tested teachers' CVS system is characterized by the data of Table 1. It is revealed that, on average, the indicators studied are within the physiological norm, both before and after work. At the end of the working day, TMA teachers showed a slight increase in systolic and diastolic pressure and heart rate, while TSTU teachers showed only an increase in heart rate. At the same time, a large scatter of individual indicators was noted, as a result of which the average statistical values did not have significant differences either during the working day or when comparing the faculty indicators of two universities.

Table 1. Functional state indicators of the teachers' body at the beginning and at the end of the working day*

University	Time of research	Number of tested	Indicators			
			Arterial pressure		Heart rate	Breathing rate
			Systolic	Diastolic		
Women						
TMA	Before work	30	113 ±5	73± 2	76± 2	20± 1
	After work	30	122± 4	79± 4	79± 3	20± 2
TSTU	Before work	31	116± 8	74± 3	73± 4	23 ± 2
	After work	60	116±7	74± 2	75± 4	23± 2

University	Time of research	Number of tested	Indicators			
			Arterial pressure		Heart rate	Breathing rate
			Systolic	Diastolic		
Men						
TMA	Before work	29	119± 3	74± 2	76± 2	19± 1
	After work	29	124±4	79± 3	80± 4	20 ± 1
TSTU	Before work	61	124±4	82± 4	74 ± 5	22± 2
	After work	60	123±6	81±4	76± 3	22± 2

In all cases, for indicators before and after work, P> 0.05.

At the same time, it was revealed that for such an indicator as systolic pressure, work experience is important (Fig. 1, 2). Moreover, the differences in the indicator before and after work with work experience of 21 years or more were statistically significant in both women and men.

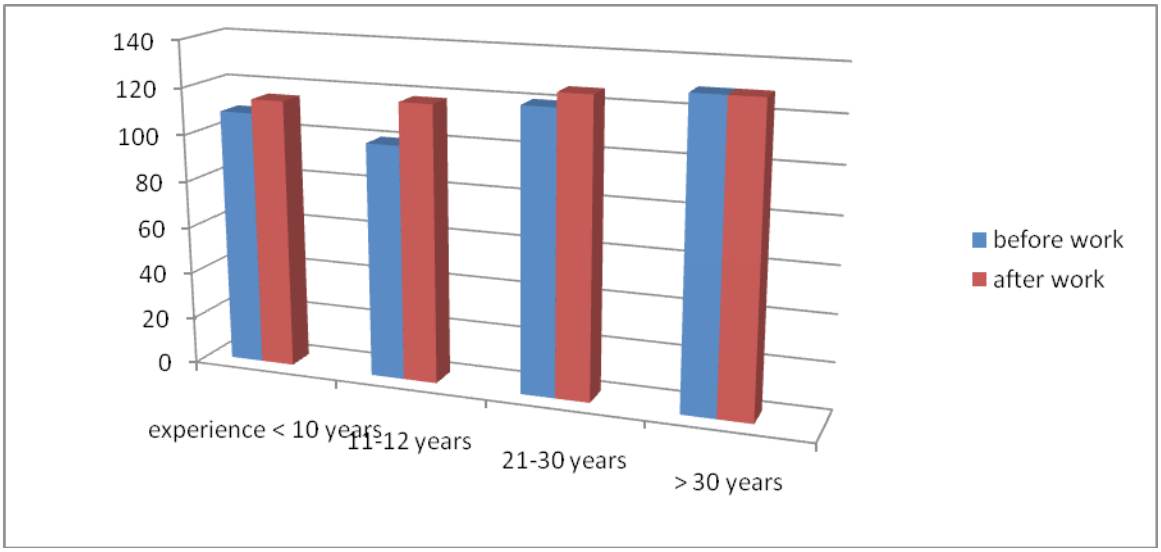


Fig. 1. Change in systolic pressure of TMA female teachers depending on the work experience, mmHg

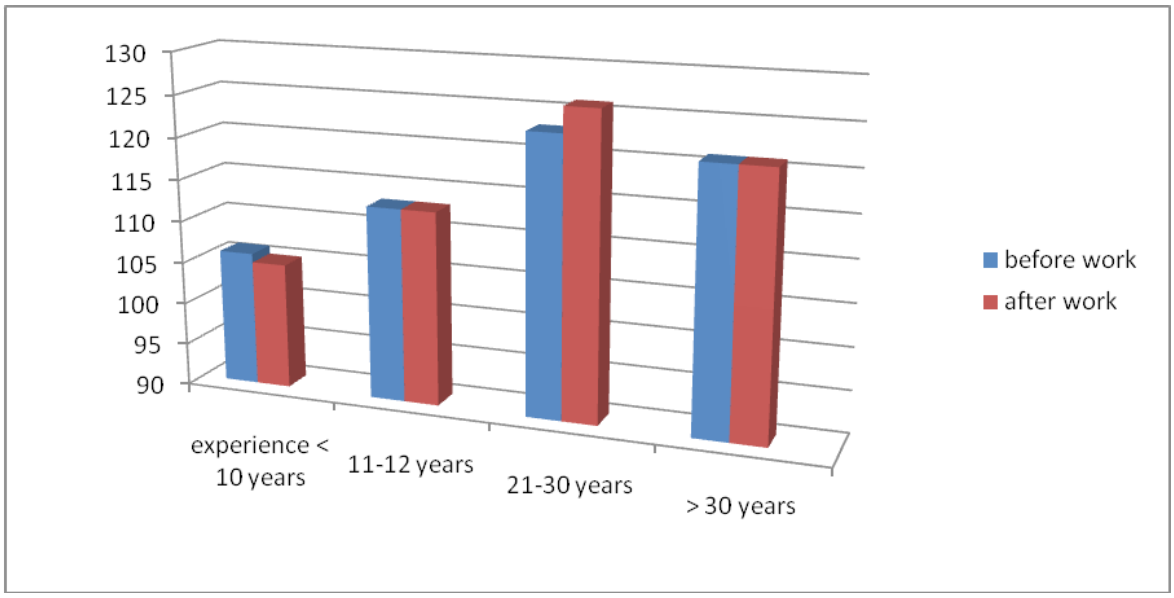


Fig. 2. Change in systolic pressure ofTSTU female teachers depending on the work experience, mmHg

More significant changes were characteristic of the central nervous system's functional state. The amount of work performed by teachers characterizes the mental productivity coefficient, the value of which, for men and women of different universities with rare exceptions, did not have significant differences according to the data of Table 2. At the same time, a number of features are revealed that are characteristic all universities' teaching staff under research. Thus, in all universities the productivity coefficient at the end of work is significantly lower than at the beginning, and the decrease in this indicator in all universities is more noticeable in women: in men, the coefficient decreases by 8.8-12.2%, and in women - by 11.6-24.6%.

Table 2. Comparative characteristics of changes in the productivity coefficient of universities' teaching staff under research at the beginning and end of the day, M±m

University	Men			Women		
	Before work	After work	P	Before work	After work	P
TGTU	659±6	594±4	<0,01	679±8	512±4	<0,01
NUUz	589±8	517±6	<0,01	659±7	558±3	<0,01
TMA	637±5	581±3	<0,01	593±10	524±9	<0,01

The same regularities were revealed with regard to the accuracy of the work performed: the accuracy coefficient of all teachers by the end of the day significantly decreased in all the universities studied (Table 3), and for women this decrease was more pronounced than for men - by 9-17% and 11-19%, respectively.

Table 3. Comparative characteristics of changes in the accuracy coefficient of the task performed by teachers of the studied universities at the beginning and at the end of the day, M±m

University	Men			Women		
	Before work	After work	P	Before work	After work	P
TGTU	0,85±0,04	0,77±0,03	<0,05	0,78±0,04	0,63±0,03	<0,05
NUUz	0,78±0,04	0,67±0,04	=0,05	0,79±0,03	0,67±0,04	<0,05
TMA	0,82±0,05	0,68 ±0,05	=0,05	0,80±0,05	0,71±0,03	<0,05

It was also noted that in all universities the degree of change depends not only on the gender of teachers, but also on their age and work experience. As an example, Table 4 shows the characteristics of TSTU indicators dynamics considering age, gender and work experience.

Table 4. The mental productivity coefficient (P) depending on the age and work experience of TSTU teaching staff

Age or work experience	Men n = 52		Women n = 48	
	Before work	After work	Before work	After work
With regard to age				
Up to 30 years	660±39	611±34 P>0,05	668±38	464±40 P<0,05
31-40 years old	662±28	654±27 P>0,05	840±16	590±95 P<0,05
41-50 years old	752±60	569±41 P<0,05	590±57	464±51 P>0,05
51 years or more	562±40	542±52 P>0,05	619±47 P	533±35 P>0,05

Age or work experience	Men n = 52		Women n = 48	
	Before work	After work	Before work	After work
With regard to work experience				
Up to 10 years	681±31	638±25 P>0,05	680±36	470±38 p<0,05
11-20 years	665±27	616±32 P>0,05	643±57	507±48 P<0,05
21-30 years	655±50	590±53 P>0,05	593±64	515±56 P>0,05
31 years or more	532±50	502±66 P>0,05	633±55	508±50 p>0,05

It was revealed that the central nervous system of men is more resistant to the effects of neuropsychic stress, both considering age and work experience: only for teachers aged 41-50 years, at the end of the working day, a significant decrease in the number of performed mental work. In women, the central nervous system is more labile at a young age - a significant decrease in work productivity was found only in women under the age of 40 years and in people with work experience of up to 20 years (apparently younger than people with more work experience), and the younger women, the changes are all the more pronounced: for people under 30 years of age and with work experience of up to 10 years, productivity by the end of the day decreases by 30.9-38.9%, and at the age of 31-40 years and with work experience of 10-20 years - 21.1-29.8%.

A more accurate indicator turned out to be the accuracy factor of the task, which characterizes the quality of the work performed: a significant deterioration in the indicator was noted in most of the studied age and senior groups of teaching staff in both men and women (table 3); only in the age groups of 31-40 years (for men - and at the age of 51 years or more) the deterioration in the quality of work performed by the end of the day was unreliable. In the experience groups,there was no deterioration in the quality of work only for men with an experience of more than 31 years; in other observations, it was found that the less work experience, the more the quality of its work decreases by the end of the day.

In TMA and NUUz, the dynamics of the functional state indicators of the central nervous system, considering the age and work experience, was similar (Table 5).

Table 5. The accuracy factor of the task, depending on the age and work experience of the TSTU teaching staff

Age or work experience	Men n = 52		Women n = 48	
	Before work	After work	Before work	After work
With regards to age				
Up to 30 years	0,84±0,03	0,80±0,04 P>0,05	0,80±0,03	0,58±0,05 P<0,05
31-40 years old	0,92±0,02	0,88±0,02 P>0,05	0,80±0,07	0,70±0,03 P>0,05
41-50 years old	0,94±0,03	0,70±007 P<0,05	0,76±0,04	0,60±0,04 P<0,05
51 years or more	0,70±0,05	0,70±0,03 P>0,05	0,75 ±0,03	0,66±0,44 P>0,05

Age or work experience	Men n = 52		Women n = 48	
	Before work	After work	Before work	After work
With regards to work experience				
Up to 10 years	0,87±0,02	0,78±0,03 P<0,05	0,80±0,03	0,58±0,04 p<0,01
11-20 years	0,90±0,02	0,76±0,04 P<0,05	0,80±0,04	0,69±0,001 P<0,05
21-30 years	0,88 ±0,02	0,70±0,05 P<0,05	0,80±0,05	0,68±0,04 P=0,05
31 years or more	0,65±0,06	0,70±0,08 P>0,05	0,76±0,04	0,64±0,05 P=0,05

Identified similar shifts in the central nervous system's functional state in all tested individuals, in our opinion, are caused by constant neuro-emotional stress, which is the main harmful factor in the activities of the universities' teaching staff and which, ultimately, lead to the emotional burnout development.

The identification and assessment results of the psychoemotional burnout state in the studied population. Emotional burnout indicators in accordance with the methodology used (emotional exhaustion, depersonalization, personal achievement reduction) can be detected in a variety of people, but their nature and severity depend on many factors, including on the characteristics of work.

It can be assumed that one or another EB level is characteristic of all people, in connection with which we tested a group of university employees (50 persons) not belonging to the teaching staff - laboratory assistants, technical workers, accounting workers; this group of researchers served as a control in relation to the teaching staff of universities. It was revealed that emotional burnout signs do occur in this group of patients, but their level is very variable. Thus, low level emotional exhaustion is typical for most people in this group (64.0 ± 6.8%), while a high level of this indicator is noted only in 2 out of 50 cases (4.0 ± 2.8%). A more characteristic EB sign in this group is depersonalization, the average level of which is observed in 52.0 ± 7.1% of the examined, and the personal achievement reduction is characteristic of most of these individuals - its high level was detected in 84% of cases. It is obvious that the claims of this group of people were quite high, but they were practically not realized, which nevertheless did not oppress them, without causing despondency, without depriving the feeling of joy in life.

The data obtained also allow us to conclude that EB indicators can be detected in any person; therefore, the significance of control in such studies is less than considering the level and nature of changes in emotional burnout indicators recommended by the testing methodology.

The emotional burnout possibility and degree assessment of the universities' teaching staff in Uzbekistan, conducted by us in the three universities under research, allowed us to establish a number of important patterns.

General characteristics of emotional burnout among teachers of the studied universities are presented in Table 6.

Table 6. The level of emotional burnout signs at the studied universities' teaching staff, %, M±m

University	Number of tests	Changes level, M±m					
		Low		Average		High	
		abs	%	abs	%	abs	%
1. Emotional burnout							
Control	50	32	64±6,8	16	32,0±6,6	2	4,0±2,8
TMA	M-30	23	21,9±4,0**	59	56,2±4,8*	23	21,9±4,0*
	W-71						
UzNU	M-50	33	33,0±4,7**	39	39±4,9	28	28,0±4,5**
	W-50						
TSTU	M-50	16	16,0±3,7***	56	56,0±5,0*	28	28,0±4,5**
	W-50						
2. Depersonalization							
Control	50	20	40,0±6,9	26	52,0±7,1	4	8±3,8
TMA	M-30	38	36,2±4,7	45	42,8±4,8	22	20,9±4,0*
	W-71						
UzNU	M-50	42	42,0±4,9	44	44,0±5,0	14	14,0±3,5
	W-50						
TSTU	M-50	38	38,0±4,8	24	24,0±4,3**	38	38,0±4,8**
	W-50						
3. Reduction of personal achievement							
Control	50	3	6±3,3	5	10±4,2	4,2	84,0±5,2
TMA	M-30	20	19,0±3,9*	33	31,4±4,5*	52	49,5±5,1**
	W-71						
UzNU	M-50	26	26,0±4,4*	30	30,0±4,6*	44	44,0±5,0**
	W-50						
TSTU	M-50	20	20,0±4,0*	28	28,0±4,5*	52	52,0±5,0*
	W-50						

Pcomparing to the control: * p<0,05, ** p<0,01, ***p<0,001

It is necessary to note a fairly wide individual scatter of indicators, however, the data presented in the table indicate that we practically did not reveal any reliable differences in the average statistics in different universities, with the exception of 3 of 27 indicators. This suggests that the dynamics and level of emotional burnout indicators in all universities are approximately the same, although the percentage distribution of indicators among universities is slightly different (in 88.9% of cases, p> 0.05).

In all 3 universities, the most typical is the average level of change in the EB indicators (from 28.0% to 56.0% of the tested); less often, a low level of changes (from 16.0% to 42.0%) is noted and even less often, a high level (from 14.0% to 52.0%). Of the 3 EB indicators, the highest indicators of emotional exhaustion are characteristic of the average level (39.0-56.2%), for depersonalization - also the average level (24-44.0%), and for the personal achievement reduction - the high level (44-52.0%).

When comparing the EB indicators of the teaching staff with the control group, it was found that the teaching staff is characterized by significantly higher indicators of high and medium levels of emotional

exhaustion and depersonalization, while the personal achievement reduction of medium and high level is more characteristic of individuals in the control group. This allows to conclude that if dissatisfaction with the achieved is typical for 75-80% of the teaching staff, then in the control group, claims of 94% of the tested people were not implemented, which confirms the relative importance of control in this research.

At the same time, comparison with control shows that the teaching staff is characterized by a significantly higher frequency of unfavorable EBindicators. This makes it necessary to identify factors contributing to the formation of the EB of the faculty. It is completely obvious that typical hygienic working conditions (microclimate, lighting, ventilation, etc.), which are not particularly rigid, cannot be the EB cause, although a prolonged deviation of these conditions from hygiene requirements can play the role of contributing factors in the EB development.

We hypothesized that risk factors for EB can be gender, age, and work experience at the university. To assess the significance of sex for EB, the test results were distributed in each university by the frequency and EB indicators level depending on gender. The data presented indicate that both men and women in all universities have unfavorable EB indicators (medium + high level), amounting to 52-85%.

Despite the fact that the natural values of indicators in men and women have some differences, a statistical analysis of the data showed that in no case have we revealed any significant differences in the indicators in these groups. At the same time, the comparative characteristics of the men and women indicators are almost the same in all universities (Table 6). This is also clearly seen in Fig. 3, where the frequency of various levels of EB indicators in men and women at TSTU is presented.

Of all the EB indicators in both men and women, the highest frequency - at the level of average indicators (52-60%) - is emotional exhaustion characteristic. A high frequency of a high level indicator (44-50%) is characteristic for the personal achievement reduction. Given that in the control this indicator is also the highest, it can be assumed that dissatisfaction with the achieved is characteristic of any person and should be considered as a factor in stimulating human activity in the event that this indicator is not predominant in relation to other EB indicatorsю

EEDPAR

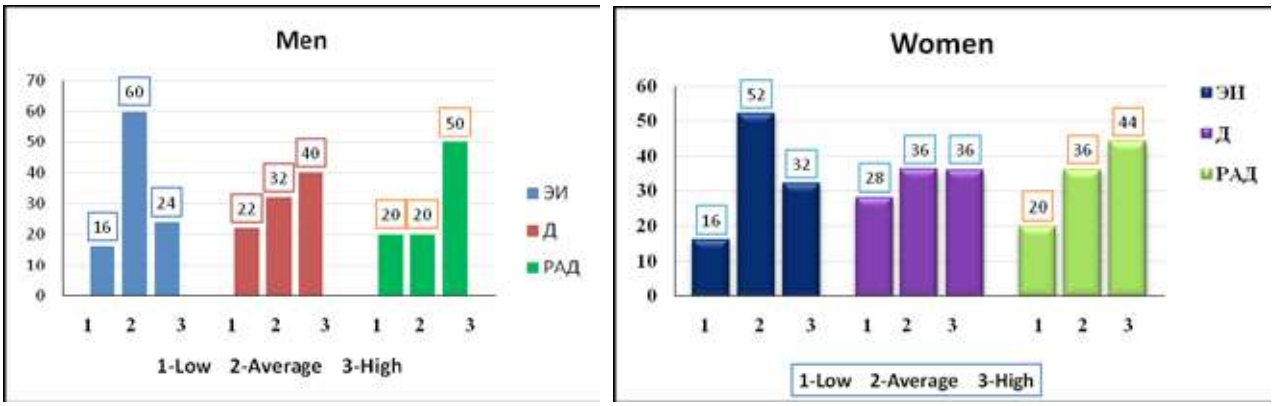


Fig. 3. The frequency of TSTU teaching staff's emotional burnout indicators depending on gender,%

When assessing the significance of the age for EB, we tested all persons under research into age groups: up to 30 years, 31-40 years, 41-50 years and older than 50 years. Given the absence of significant differences between men and women, when assessing the significance of age, the division of subjects in age groups by gender was not carried out.

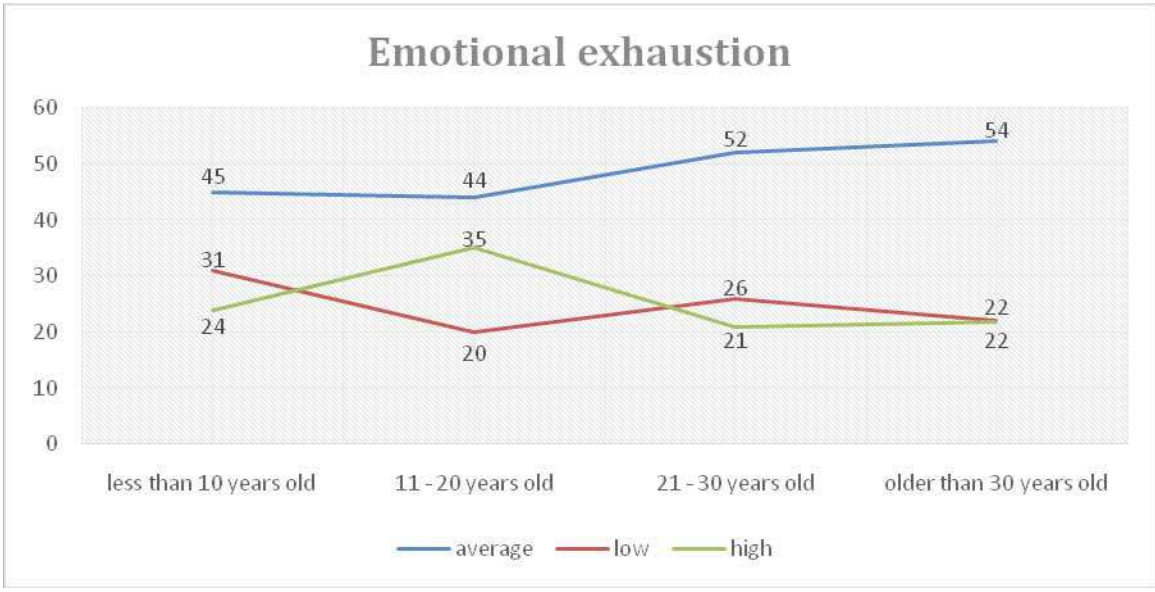
First of all, a comparative analysis of the frequency of the most significant indicator, emotional exhaustion, was carried out in the age-gender groups. The results of this analysis are presented in Tables 7 and 8, from which it can be seen that the distribution of the frequency and level of this indicator in all 3 universities studied is similar, i.e. differences in indicators between universities are not significant.

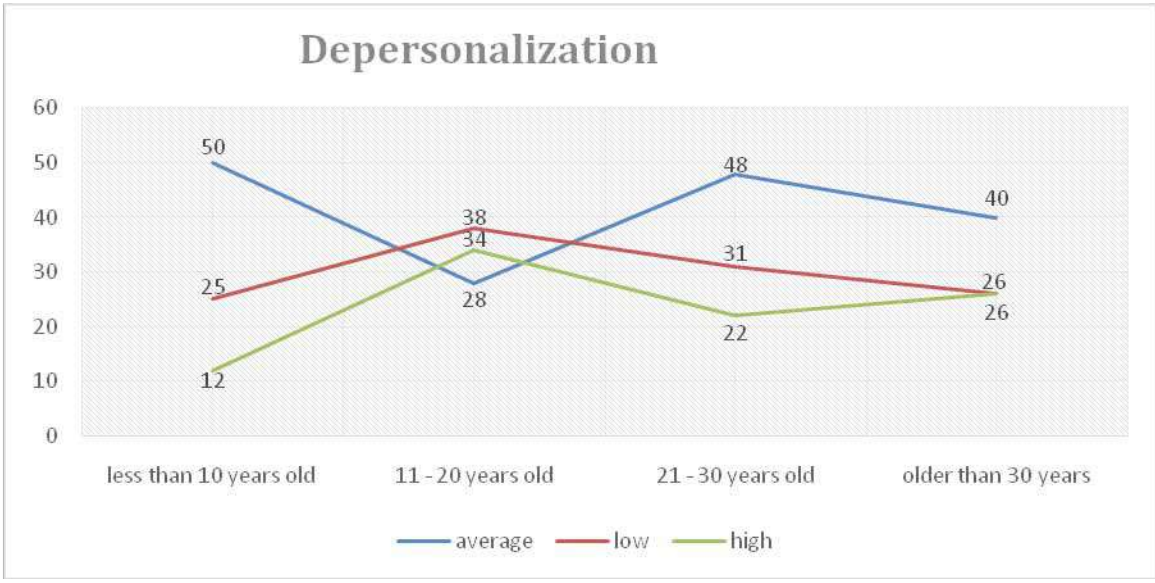
In general, the following patterns can be traced: less often in all age groups there is a low level of emotional exhaustion (3.6-8.1%), somewhat more often (7.8-10.7%) - a high level, most often (7, 2-18.2%) - the average level of EI. At the age of 31-40 years, the frequency of unfavorable types of EE (medium + high) significantly increases (2-3 times in comparison with people under 30), and in 41-50 years - 2.5-7 times compared with the group under the age of 30 years.

After 50 years, the frequency of unfavorable EE levels decreases, but still remains significantly higher in relation to the first age group. Consequently, the most vulnerable group in relation to emotional exhaustion are people aged 41-50 years, i.e. the time of the most active teachers.

The dynamics of depersonalization and personal achievement reduction frequency is similar to the dynamics of emotional exhaustion frequency, but the indicators values are significantly lower than EE; in addition, a significant change in indicators in older age groups compared with the age of up to 30 years was revealed in the group of 41-50 year old teachers and in isolated cases at the age of 31-40 years (high level of "D" and "PAR") and 50 years or more (increase in the number of people with low levels of PAR).

An important factor for the formation of negative trends of emotional burnout turned out to be seniority at the university. Its influence on the level of the studied indicators was mixed. This is evidenced by the data of and graphically shown on Fig. 4.





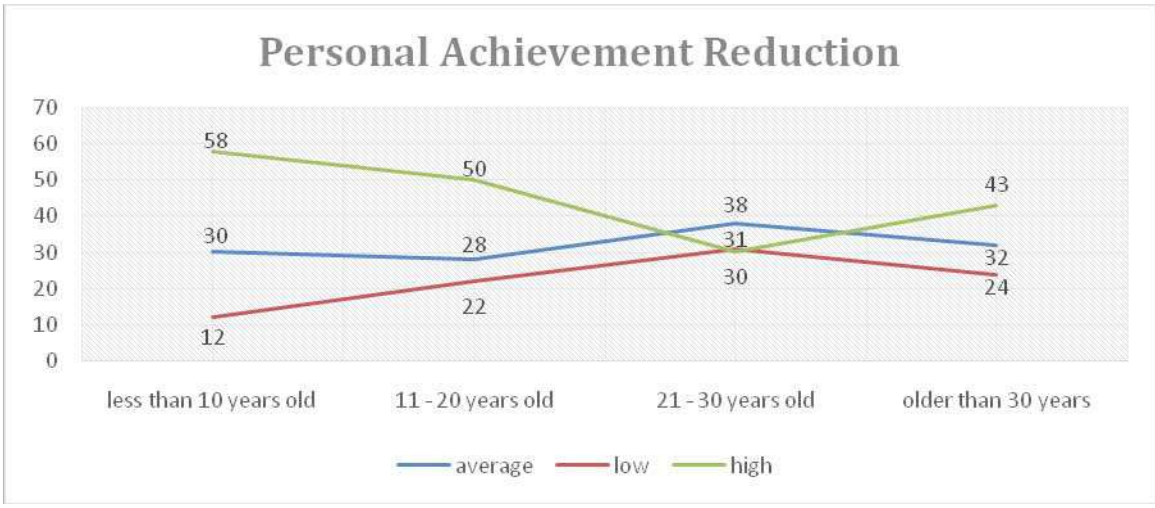


Fig. 4. Dynamics of the emotional burnout indicators level depending on the work experience, %

The indicator of a low EE level changes insignificantly with an increase in the work experience, being detected in 20-30% of the examined persons. Almost at the same level is a high level of changes in EE. The average EE level naturally grows with an increase in the work experience: in individuals with an experience of 21-30 years, the average EE level is recorded 7% more often, and with the experience of more than 30 years - 11% more than with the experience of up to 10 years.

Low and high level of depersonalization occurs in the range of 22-34% and is little dependent on experience. The average level of depersonalization is more characteristic for people with a short work experience (up to 50%), then it decreases almost 2 times (up to 28%), and for people with a work experience of more than 20 years it increases 1.5-1.7 times.

The dynamics of a high PAR level is interesting. Persons with an experience of up to 10 years have a high level of doubt in their own abilities in 58% of cases, then the level of self-esteem increases - with the experience of 21-30 years, only 30% of the tested do not believe in their own strength, but with the experience of more than 30 years, self-confidence is again decreases - 43% of the respondents showed a high level of doubt in their capabilities.

We also studied the psychoemotional state of a TMA teachers group (50 people) at the beginning and at the end of the academic year. The results of these studies showed that by the end of the academic year the number of unfavorable indicators of the psychoemotional state increases significantly. So, by the end of the academic year, the number of teachers with a high level of emotional exhaustion increases 2.5 times, depersonalization - 4 times, with a personal achievement reduction - 2.4 times. This suggests the great importance of a properly organized summer vacation to restore the psychoemotional state of the teacher.

The study of the work content, the functional and psychoemotional state of the universities' teaching staff allows us to draw the following conclusions:

1. Assessment of the functional state of the teachers' CVS throughout the working day indicates that the average statistical indicators of the CVS functional state throughout the working day do not go beyond physiological fluctuations, although the systolic pressure level, especially in people with a long history of work, increases significantly.
2. When assessing the functional state of the central nervous system, a decrease in the quality of the work performed was revealed: a significant deterioration in the indicator was noted in most studied age and senior groups of faculty members in both men and women; it was revealed that the less work experience, the more its quality decreases by the end of the day.
3. Signs of emotional burnout are detected in people who are not involved in pedagogical activities, but the main indicator level of emotional exhaustion in such individuals is low; these individuals are most characterized by a high level of personal

achievement reduction. The revealed facts indicate the relative importance of control in such studies; more informative is the account of the level and nature of the signs of EB.

4. The studied group of teachers is characterized by a wide individual scatter of EB indicators, but the average statistical indicators of EУ, D, and PAR in different universities do not have statistically significant differences:

– The studied group of teachers is not characterized by a low level of changes in the EB indicators, while the prevalence of adverse parameters of changes is noted, i.e. medium and high level changes.

– For EE and D, the most characteristic is the average level of changes, significantly higher than in the control group: for PAR, a high level of changes was detected less often than in the control group, which once again confirms the relative importance of control.

– The average level of emotional depletion of the teaching staff environment is 52-60%, a high level of PAR - 44-50%; average level of D - 22-40%; therefore, the main sign of EB is emotional exhaustion.

5. There were no statistically significant differences in the EB indicators depending on gender - the indicators are almost the same for both men and women in all universities. Adverse parameters of changes in EB (medium + high level) were identified in 52-85% of teachers

6. The most vulnerable age group with a high and medium level of EE are people aged 41-50 years, ie age of the most active teachers.

7. Work experience in a university does not practically change the low and high level of EE and D indicators, while the average level of change in these indicators increases with a work experience growth; a high level of PAR, characterizing the self-assessment of the teaching staff members, was noted among those with the longest work experience, the highest frequency of positive self-esteem was revealed with work experience of 21-30 years, and a sharp decrease in self-esteem of their capabilities was revealed in those with experience of more than 30 years; at the beginning of the academic year, emotional burnout indicators are less pronounced than at the end; this indicates the great importance of rational relaxation for the teachers' psychoemotional state.

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Efficacy of Gastric Bypass in the Treatment of Obesity-Associated Carbohydrate Metabolism Disorders

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Abstract

We have operated on 42 patients ages 22 to62 suffering different forms of obesity and obesity-related metabolic disorders. Body mass indexes have ranged from 28 to 62 kg/m^2. All patients have had symptoms of metabolic disorders of different intensity. Our goal has been exerting maximal influence on carbohydrate metabolism processes rather than mere body weight reduction. The patients have undergone sleeve gastrectomy, laparoscopic mini-gastric bypass, Roux en Y gastric bypass, as well as modified gastric bypass with Rouxen Y gastrectomy with a 180-cm bypass and 70-cm alimentary loop as an independent way of surgical treatment of diabetes type 2 associated with obesity. Sleeve gastrectomy in terms of weight control has demonstrated a 73% efficacy, and gastric bypass –a 89% efficacy. The efficacy of surgeries in patients with carbohydrate metabolism disorders through Roux en Y gastric bypass has totaled 96%,classic Roux en Y gastric bypass - 92%, sleeve gastrectomy – 60%. Roux en Y gastric bypass andmini-gastric bypass are more effective in the treatment of obesity and associated metabolic disorders compared to sleeve gastrectomy. Gastric bypass has proved effective even in diabetes patients with body mass indexes up to 30 kg/m^2.

Keywords: *Obesity, metabolic syndrome, diabetes type 2, bariatric and metabolic surgery, gastric bypasss, mini-gastric bypass.*

Abstract

Today, the incidence of obesity has grown to proportions of an epidemic, especially in the civilized world. In 2016, more than 1.9 billion mature people above 18 were deemed overweight[1]. More than 650 million of those were diagnosed with obesity[2]. According to the World health organization (WHO), as of 2016, 39% of adults (men - 39%, women – 40%) were overweight[3]. In 2016, about 13% of mature population of the planet (men – 11%, women – 15%) were diagnosed with obesity[4]. Within a period from 1975 through 2016, the number of obese people tripled[5]. In Europe, the incidence of obesity ranges from 5% to 23% in men and from 7% to 36% in women[6]. In Uzbekistan, the percentage of overweight or obese people is 24.9%. According to WHO, obesity is a non-infectious epidemic of the 21st century[7].

In 2000, WHO came up with a proposal to lower the overweight threshold for the mongoloid race from 25kg/m^2 down to 23.5kg/m^2, and the obesity threshold – from 30kg/m^{2}down to 27.5kg/m^2[8]. The initiative was put forth because epidemiological research data had shown that mongoloids began to deal with overweight-related issues at a lower body mass index [4][8].

In cases when the body mass index reaches 40 kg/m^2 or higher, even when no complications are observed[9], morbid (pathological) obesity is diagnosed[10]. With complications, such as diabetes type 2, arterial hypertension, dyslipidemia, and lower limb joint pathologies, obesity is classified as morbid at a body mass index of 35 kg/m^2 or higher[11].

The body mass index is subject to criticism for not reflecting the fat/muscle ratio and distribution of fat around the body[12]. For example, it can classify an elderly person with low muscle mass as one with ideal weight, while a heavy-muscled athlete can be termed as overweight or obese. However, so far, body mass index is the only internationally recognized body weight evaluation criterion[13].

Over the past decades, scientists and clinicians have

switched to a complex approach to studying different metabolic disorders and obesity-related conditions[14]. They have concluded that these pathologies share symptoms and generalized them as "metabolic syndrome"[15].

Metabolic syndrome (X syndrome) is a kind of price we pay for urbanization, as it entails physical inactivity, improper nutrition, and sedentary life mode, which can cause serious health problems in future[16]. There is even a symbolic pattern of these consequences: metabolic syndrome – 10 to 20 years old - atherosclerosis – 10 to 20 years old – myocardial infarction, stroke[17].

A person can be diagnosed with metabolic syndrome if he or she has at least three of the following symptoms:

- Overweight;
- Arterial hypertension;
- High blood sugar;
- Dyslipidemia (abnormal lipid profile);
- Early atherosclerosis;
- Ischemic cardiac disease;
- Gout;
- Increased male hormone levels in women.

Today, the most effective and reliable method of morbid obesity treatment is bariatric surgery[18]. Operations that limit food intake and food absorption result in pronounced and continuous weight loss and mitigate the majority of associated conditions[19]. The effect lasts for lifetime, frees patients from having to follow strict diets[20], significantly improves and prolongs life[8,26,46]. Anti-obesity surgeries have been in practice since the 1950s, when the first jejunoileal bypass surgery was conducted in the USA[21]. Over the past 25 years or so, other types of bariatric surgeries have been developed and are now broadly applied (intragastric balloon placement, biliopancreatic bypass, lap band surgery, sleeve gastrectomy)[22].

Today, the number of anti-obesity surgeries per year in the world exceeds 340 000 (as of 2018) [18]. There are lots of factsproving the positive effect of bariatric operations, which lasts for long periods[23].

First of all, the effect of an operation depends on its type. Patients lose 45% to 95% of unhealthy weight, and the effect continues for 1 to 1.5 years[24]. If there are serious underlying conditions, simple bariatric surgeries may be ineffective, so more complex operations – gastric or biliopancreatic bypass – may be required[25].

The initial goal of bariatric surgeries is body weight control[26]. Later they proved effective against diabetes type 2, as it was observed in most patients, who had lost weight after an operation[27]. With different underlying signs of metabolic syndrome, most effects appear during the first month after the surgery[28]. In other words, diabetes, hypocholesterolemia and other symptoms go away before the patient loses weight[29]. This has produced the term "metabolic surgery", which means selective surgical correction of metabolic disorders [54].

In 2009, the American Diabetes Association included these operations in the standards that are applied in the treatment of diabetes type 2 and underlying obesity, and in the USA these operations are covered by insurance companies[30]. In some European (Italy, France, Great Britain) and other countries (Israel), surgeries required for treatment of morbid obesity are covered by medical insurance too[31].

Although there are tons of research and scientific publications concerning bariatric and metabolic surgery, there are lots of issues that still need to be resolved[32]. So far, there are no unified or universal standards that would regulate the use of bariatric surgeries based on indications and surgeon's choice[33]. Given the high spread and incidence of morbid obesity and related metabolic disorders in Uzbekistan[34], we actively use bariatric and metabolic surgery to treat this category of patients while relying on our own observations and studies[35]. Based on these studies, we have presented some results and conclusions concerning the choice of effective surgical tactics in the treatment of patients suffering carbohydrate metabolism disorders with underlying obesity and dyslipidemia[36].

Materials and Method

We operated on 42 patients at Clinic 1 of the Tashkent City Clinical Hospital over the period from 2015 through 2019, ages 22 to 62, dealing with various types of obesity and related metabolic disorders[37]. The body mass index ranged from 28 to 62 kg/m^2. All patients had symptoms of metabolic disorders of different intensity[38]. It should be noted that 20 subjects had applied for a surgery for lack or short duration of effects of conservative treatment (diet, physical exercise, use of dietary supplements)[39]; 22 patients had applied

for treatment due to diabetes type 2 (disease duration – 3 to 15 years)[40]; therefore (see a description below) our goal was to exert maximal influence on carbohydrate metabolism rather than mere reduction of body mass index[41], as we realized the inseparability of bariatric and metabolic procedures[42].

Prior to a surgery, the patients had undergone the following tests:

- Complete blood count;
- Biochemical blood count (ALAT, AST, uric acid, creatinine);
- Coagulogram;
- Blood clotting time;
- Carbohydrate metabolism indexes (blood sugar level; HbA1Cglycosolated hemoglobin; fasting and after-food insulin, if necessary; C-reactive protein);
- Lipid spectrum;
- Imaging: ECG, ultrasound, EGD;
- Other consultations (endocrinologist, diabetologist, hepatologist, cardiologist, therapist, emergency physician and anesthesiologist)[43].

A group of 8 patients underwent sleeve gastrectomy, 23–gastric bypass with Rous en Y (7 patients were subject to gastric bypass with Rous en Y as independent treatment of diabetes type 2 associated with obesity)[44], 11 patients were subject to mini-gastric bypass (MGB/OAGB) with the use of bypass and alimentary loops of different length (Table 1)[45]. Also, a group of 11 patients underwent laparoscopic mini-gastric bypass devised by R. Rutledge with the formation of a small stomach with a volume of 150 ml to 200 ml, and a 4cm to 5cm thick gastroenteroanastamosis was fixed over a length of 150cm to 200cm from the ligament of Treitz[46].

Table 1. Types of surgeries required to treat obesity and obesity-related diabetes type 2

Type of surgery	Sleeve gastrectomy	Roux en Y gastric bypassmodification	Roux en Y gastric bypass	Mini-gastric bypass
Obesity	8	-	7	5
Obesity combined with diabetes	-	7	9	6

Results and Discussion

During the early postoperative period, disease management required special care (control and correction of blood sugar and other indicators) during the first several days; later, all patients were managed on the Fast Track Recovery basis. All patients were discharged after 5 to 7 days with their state defined as satisfactory. Later, all patients were subject to regular monitoring for all related parameters (blood sugar, fasting and after-food insulin, weight loss dynamics, etc.)

Patients, who had sleeve gastrectomy, lost 25 kg of weight on average; it should be noted that after a year weight loss would slow down dramatically or stop completely (Fig. 1)

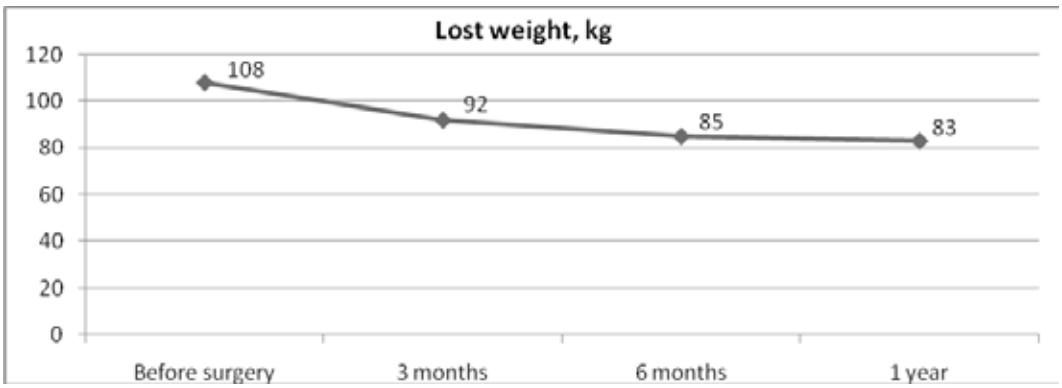


Figure 1.

On the average, patients lost 54 kg during the year following a gastric bypass surgery (Fig. 2).

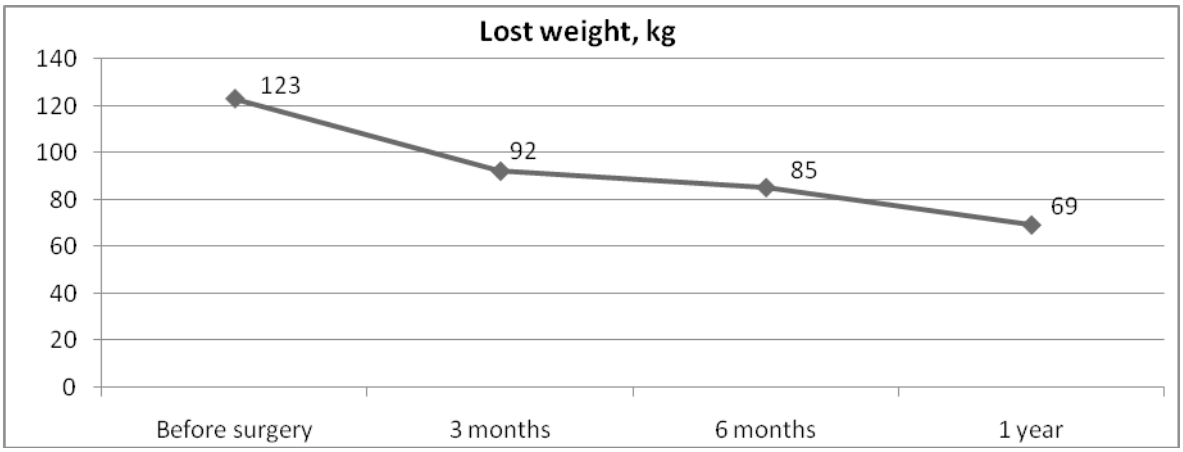


Figure 2.

The percentage of lost weight has been calculated according to the formula:

$$\text{EWL\%} = \frac{\text{Excess weight (kg) in the end}}{\text{Lost weight (kg)}} \times 100\%$$

and totaled 70%on average for sleeve gastrectomy cases and 85% for gastric bypass cases.

As mentioned above, 22 patients had diabetes type 2; therefore, the type of surgery was chosen with special consideration to the duration of the disease, clinical symptoms and their intensity, fasting and post-meal blood sugar and other metabolic syndrome indicators; however, special attention was paid to fasting and after-meal C-peptide, insulin and C-reactive protein blood levels, HOMA-IP, atherogenic and abdominal obesity indexes, which has enabled us to achieve an up to 98% accuracy in prediction of the efficacy of surgical diabetes treatment (Fig. 3). Also, a differential approach was applied to each patient with reference to carbohydrate and lipid metabolism indicators when choosing an optimal length of bypass and alimentary loops and stomach size. This has helped us achieve full and complete remission of diabetes in patients with a body mass indexof up to 30 without losing much of excess weight.

Test results of patients with carbohydrate metabolism disorders and their dynamics during the postoperative year demonstrating a complete remission of both clinical and laboratory diabetes indicators (Fig. 3 and 4)

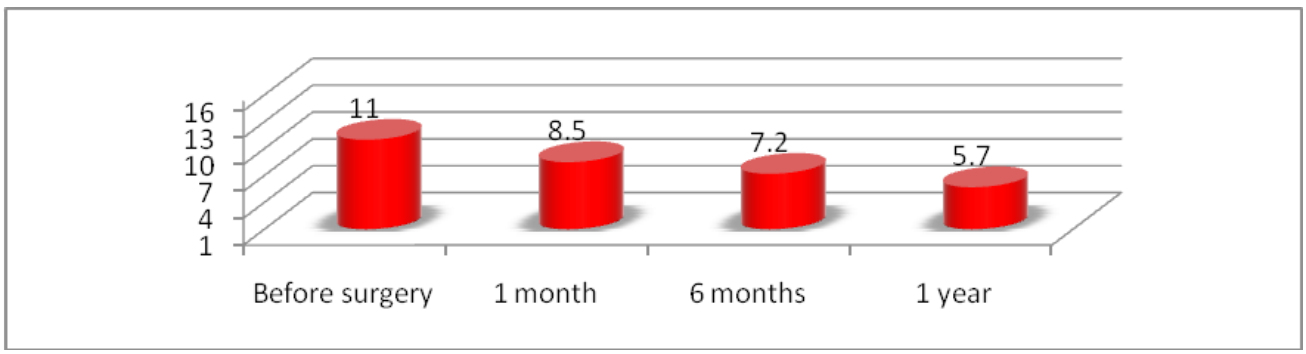


Fig 3. Average blood sugar level**

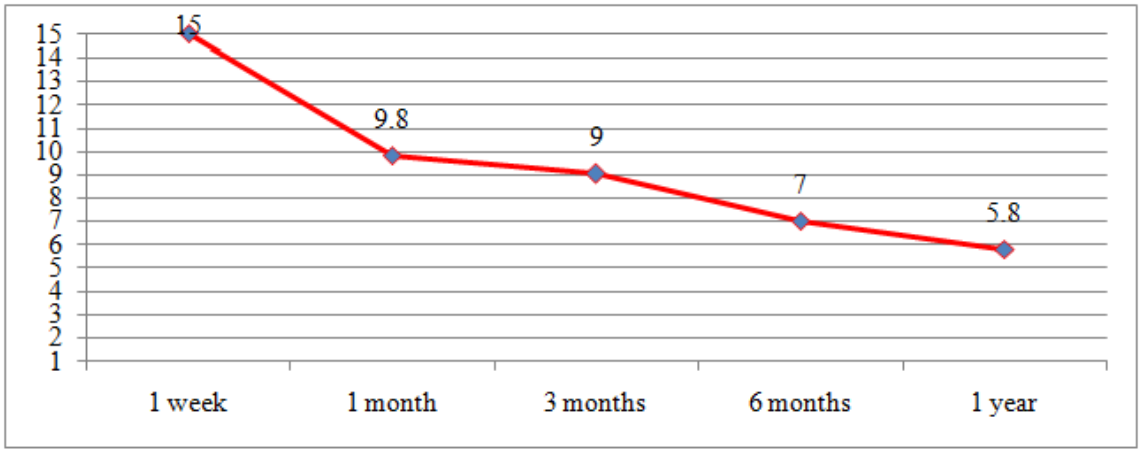


Figure 4. Blood sugar dynamics**

We detected normalization of insulin secretion in response to eating, which indicates a decrease in or disappearance of insulin resistance (Fig. 5).

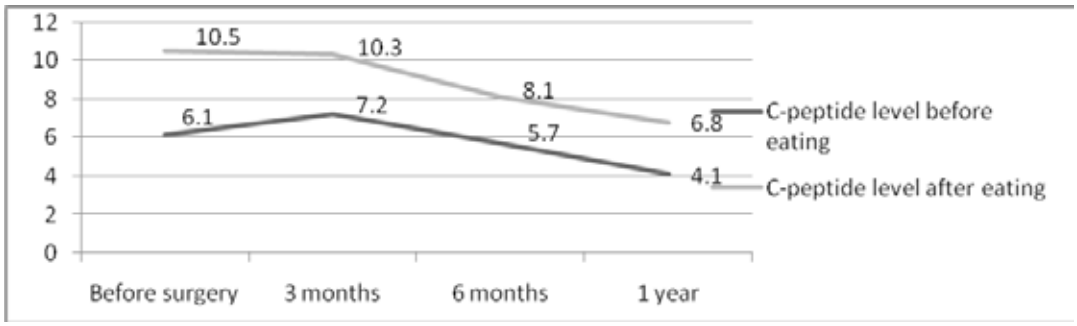


Figure 5. Postoperative C-peptide level

Also, it should be noted that six patients had been receiving 32 units of insulin on average before a surgery with blood sugar exceeding 10 mmol/l. Three patients canceled insulin injections within a month following a surgery due to a complete remission of diabetes; two patients cut down on insulin within three months following a surgery. Only one patient had to reduce insulin injections to a minimum of 10 units of simple (Actrapid) insulin per day.

All obesity patients demonstrated an increased C-reactive protein, which is a sign of metabolic syndrome; after an operation, C-protein levels would decrease as shown in Figure 6.

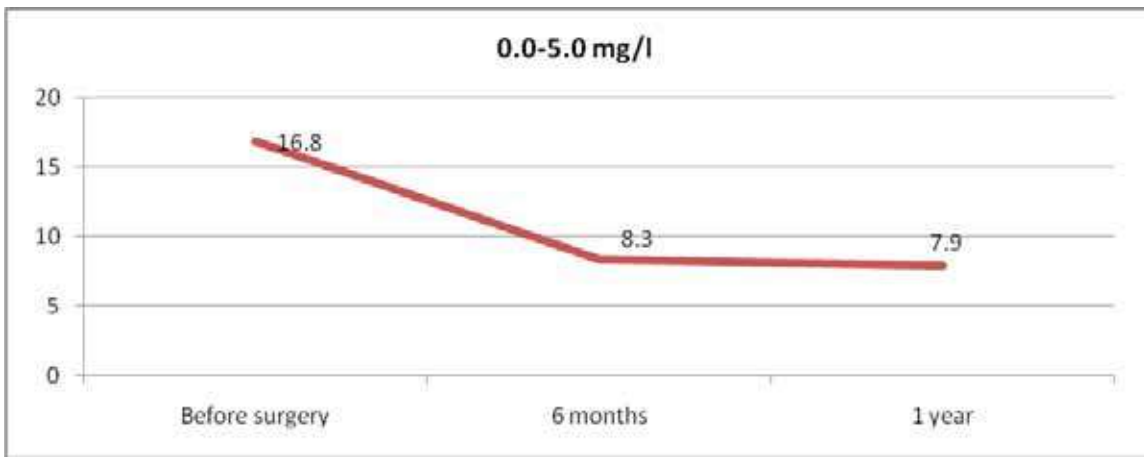


Figure 6. C-reactive protein level dynamics

Table 2. Pre-* and postoperative metabolic syndrome indicators**

	Before surgery*	After surgery (1 year)**	Norm
Blood sugar	9.81	5.8	4-7 mmol\l
Blood sugar after eating	12.9	6.7	4-7 mmol\l
Glycosylated hemoglobin	8.10	6.6	4-7%
Fasting insulin	20.4	0.2	0.7-9.0 mU/l
Insulin level after eating	32.1	6.6	0.7-9.0mU/l
Blood pressure	150/90	130/80	
Atherogenic index	5.0	4.8	Optimal up to 4

* All preoperative tests were made as the patients were taking medications and following strict diets (sugar-lowering, antihypertensive,etc.)

** All postoperative tests were made without the patients' aking of any medicines or following diets(sugar-lowering, antihypertensive, etc.)

Evaluation of the efficacy of bypass surgeries in the treatment of metabolic syndrome revealed a more pronounced weight and metabolic symptom reduction compared to restrictive surgeries (Table 3).

Table 3. Efficacy of bypass and restrictive surgeries

	Weight loss	Diabetes	Arterial hypertension	Dyslipidemia
Modified Roux en Y gastric bypass	98%	95%	92%	98%
Roux en Y gastric bypass	90%	90%	95%	90%
Mini-gastric bypass	85%	92%	85%	85%
Sleeve gastrectomy	73%	No data available	50%	40%

Table 4. Postoperative metabolic improvement in 42 (100%) patients

	Average BMI	Diabetes type 2	AH	Dyslipidemia	Other diseases (apnea, joint and spinal diseases)
Before surgery	38	52%	87%	98%	60%
After 3 months	35	4,6%	36%	40%	42%
After 6 months	32	2,3%	17%	21%	33%
After a year	29	2,3%	8%	9%	18%

We specified general and specific postoperative complications. One of the patients, who had undergone a modified Roux en Y bypass surgery, developed Potassium, Magnesium and Calcium microelement deficiency resulting in dysmetabolic polyneuropathy manifesting itself in different forms of paresthesia. General postoperative complications were observed in three patients; two of them had postoperative hernias, and one developed postoperative wound festering(Table 5); there were complications after the use of the traditional median approach only. No complications occurred after laparoscopic surgeries.

Table 5. Postoperative complications

	Lethal	Specific		General	
		Micro- and microelement deficiency	Dysmetabolic polyneuropathy	Hernia	Wound festering
Modified Roux en Y gastric bypass	0	1(2.3%)	1(2.3%)	1(2.3%)	1(2.3%)
Roux en Y gastric bypass	0	0	0	1(2.3%)	0
Mini-gastric bypass	0	0	0	0	0
Sleeve gastrectomy	0	0	0	0	0

Thus, the conclusion is that the most effective method of treatment of morbid obesity and associated diseases is gastric bypass surgery[47].

However, this type of surgery is technically challenging and may cause unfavorable postoperative reactions[48]; gastric bypass remains effective with the use of an individual approach to each patient and when determining the length of intestinal loops[49].

Sleeve gastrectomy is purely restrictive and therefore not very effective in the treatment of metabolic syndrome[50], and it has a high obesity recurrence rate, as patients may regain weight over several years following the surgery[51]. Gastric bypass is a kind of gold standard of surgical treatment of obesity and related disorders[52]. Non-invasive gastric bypass surgeries significantly reduce the occurrence of general surgical and specific complications[53].

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Efficiency of Patient-Controlled Epidural Anesthesia after Lower Limb Joint Replacement Surgery

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Abstract

At present, there are many drugs and method of anesthesia, each has its advantages and disadvantages. The aim of anesthesia is not only to eliminate suffering and create psychological comfort, but also to improve the quality of life, accelerate postoperative functional rehabilitation, reduce the incidence of complications. The problem of postoperative pain treatment is relevant both in our country and abroad. According to literary sources, 30–75% of patients suffer from severe pain in the postoperative period. Today there is a great number of research, recommendations and guidelines for postoperative anesthesia. *(*Volchkov V.A., Kovalev S.V., Kubynin A.N. Modern aspects of postoperative anesthesia (literature review)//Bulletin of St. Petersburg University. Medicine. 2018. V. 13. Issue 3. p. 245-270).

Keywords: *Analgesia, pain, postoperative, analgesic.*

Introduction

The study results of postoperative pain level after various surgical procedures are of special interest. (Ovechkin A. M. Postoperative pain: state of the problem and current trends in postoperative pain management// Regional anesthesia and treatment of acute pain. 2015. V. IX, No. 2. p. 29-39.)[1] In particular, after orthopedic interventions. An effective method of postoperative analgesia is preemptive analgesia (PEA) combined with use of local anesthetics and various adjuvants. The main drugs for PEA are represented by solutions of local long-acting anesthetics in low concentration, providing analgesia without motor blockade. Narcotic analgesics and adrenergic drugs are most often used as adjuvants. (Yousef A. A., Atef A. M., Awais W. M. Comparison of fentanyl versus meperidine as supplements to epidural clonidine-bupivacaine in patients with lower limb orthopedic surgery under combined spinal epidural anesthesia//BMC Anesthesiol. 2015. Oct. 14. No. 15. p. 146-155.) (Bell R. F., Dahl J., Moore R. et al. Perioperative ketamine for acute postoperative pain// Cochrane Database Syst Rev. 2015. Jul. 2(7). p. 344–348.)[2]

High-tech analgesia method include regional analgesia/anesthesia and patient-controlled analgesia. Patient-controlled analgesia (PCA) or on-demand analgesia is an alternative to the traditionally used analgesic “for cause”or planned analgesic[2].

According to this procedure, the patient himself, by pre-prescribed doses, administers the analgesic using a programmable syringe perfusor[3]. Anesthesia is achieved as a result of repeated injections of small analgesic doses[4]. When in pain, the patient titrates analgesic administration, evaluates its effect and determines the most acceptable level of pain[5]. The working principle of PCA is easily understood by patients. As the pain increases, the patient, by pressing the control unit button, titrates a bolus dose of analgesic until the desired effect is achieved[6].

PCA has several advantages over traditional administration of analgesics. It ensures effective analgesia according to patient's individual needs; quick desired effect; reduction of time of the patient without analgesia; stable analgesic concentration in blood plasma . Besides, when PCA is applied, it is possible to save medical personnel time, decrease the incidence of side effects and shorten their treatment in hospital.[2]

Therefore, the purpose of our study was to evaluate PCA efficiency after orthopedic surgery for prosthetics of lower limb joints.

Material and Research Method

The study was performed in 34 patients aged from 65 to 90 years (average age was 78 ± 8 years) with a physical status of ASA 3 or higher. Patients were hospitalized in the traumatology department of TMA clinic with a diagnosis of: Coxarthrosis; Gonarthrosis. All patients had a concomitant pathology: coronary heart disease - 25, hypertension - 27, atherosclerosis - 28, chronic bronchitis - 19, peptic ulcer - 10. Patients underwent orthopedic surgery: 20 patients - hip replacement, 14 patients - knee replacement.

All examined patients were divided into 2 groups. In the 1st group (n = 15), postoperative analgesia was performed traditionally by administration of opioid analgesics (morphine or promedol) by the patient's request. In the 2nd group of patients (n = 19), postoperative analgesia was performed using "Accumate -1100. Electronic PCEA. Electronic PCA" Device. 0.25% solution of longocain, 5 ml, was fractionally injected into the epidural catheter by a patient every 4 hours. During 24 hours after surgery. The study and control groups of patients were comparable in age, volume and surgery duration.

P.R. Bromage scale was used to evaluate the motor blockade. Postoperative analgesia was evaluated using a visual analogue scale (VAS), hemodynamic parameters (heart rate, SBP, BR,) SatO2, stress hormone cortisol and glucose in blood plasma. Hemodynamic parameters were measured at the following stages: 1 - initially, before analgesia, 2 - in 20 minutes after analgesic administration, 3 - in 24 hours after surgery.

The obtained results were processed using parametric statistics method using Statistica 6 program. Data were presented as mean (M) with standard deviation and evaluated using Student's t-test. Differences were considered significant at $p < 0.05$.

Results and Discussion

Before analgesia in patients of both groups, respiratory and hemodynamic parameters were almost identical. As can be seen in the following table 1, all patients experienced severe pain (more than 7 points according to VAS) before analgesia, which was clinically accompanied by tachycardia, moderate hypertension and rapid breathing.

In the 1st group, introduction of standard analgesia decreased in pain severity. Parenteral administration of narcotic analgesics has often proved to be insufficiently effective for pain relief, although pain intensity was higher than 4 points according to VAS, which is an indication for administration of opiates. However, use of drugs in this group was associated with quite a large number of complications and side effects (hypotension - 2, decreased saturation - 3, bradypnea - 1), which are more often observed in elderly patients (over 80 years old). At the same time, postoperative nausea and vomiting were more often recorded in the group (n = 6). Nearly 30% of patients in the group suffered from adverse reactions.

In the 2nd group, where PCA was used, not a single case of severe pain was recorded, the lowest levels of pain syndrome were observed. Mild motor blockade (1-2 Bromage points) was observed in 3 patients of the group and (p = 0.5). Motor blockade occurred in the first 12 hours after surgery and resolved after the patient increased intervals of using the patient's control unit. Epidural anesthesia, in terms of risk/benefit ratio is not less efficient. The main disadvantages of neuraxial analgesia are represented by arterial hypotension (n = 2), acute urinary retention (n = 1), post lumbar puncture syndrome (n = 2). No other adverse events, typical of neuraxial blockade, including infectious ones, were recorded.

As a result of the postoperative analgesia study with use of PCEA, no statistically significant changes in hemodynamic system indicators and a decrease in systolic and diastolic pressure were observed (see Table 1). Also, in contrast to the control group, in the study group of patients, cardiac depression with a decrease was not recorded. It may be due to the patient's selection of the optimal analgesic dose, when, upon reaching the comfort state in the first hours after the surgery, patients maintained this state at an individual level.

In our studies, all patients did not suffer from severe pain. In study groups, there was not a single case of complications associated with side effects of drugs or anaesthesia during the hospitalization period.

Table 1: Indicators of hemodynamics, respiration and pain syndrome severity

Indicators	Group 1			Group 2		
	Stage 1	Stage 2	Stage 3	Stage 1	Stage 2	Stage 3
	128.5±5.7	119.5±5.4	120.5±5.2	126.7±5.2	123.5±5.7	121.5±5.3
ABP mm Hg	85.6±5.3	81.6±5.1	81.4±5.3	84.5±5.1	84.1±5.3	83.1±5.1
Heart rate, bpm	86.8±7.4	78.8±7.4	73.8±3.4	85.8±8.0	74.8±7.4	71.8±4.4
BR/min	18.6±2.4	14.6±2.4	14.6±2.4	18.5±2.6	16.6±2.5	15.6±2.5
Sat O_2, %	98.3±1.5	96.3±1.5	97.3±1.5	98.1±1.7	98.3±1.8	99.3±0.6
VAS, points	7.4±1.3	3.6±1.8	3.5±1.5	7.2±1.9	1.5±0.5	1.2±0.2
Cortisol, n/mol/l	186.1±51.1	184.5±49.1	178.1±43.1	186.2±52.1	149 ± 43.1	145.3±32.1
Glucose, mmol/l	6.1±1.3	4.26±0.4	4.3±0.3	6.2±1.5	4.09±0.3	4.04±0.1

Analysis of the analgesic effect study results showed, that in the postoperative period, using patient-controlled epidural analgesia (PCEA), a clear tendency to a decrease in cortisol and glycemia levels was observed, which was not the case with the control group. The decrease in cortisol is due to nociception interruption in the lesion, because there is no activation of hypothalamo-pituitary-adrenal axis (HPAA). The humoral pathway is a direct entry of inflammatory mediators into the blood and HPAA activation. The blood glucose level is almost the same, with a slight fluctuation, indicating HPAA deactivation.

The results of studies, performed to evaluate PCA efficiency, showed that analgesia shall be started as early as possible. In patients, who started PCA for mild pain (at rest), good results were observed more often by 30 %, than in patients with severe pain. Inadequate analgesia is observed in 15% of patients, when PCA was used for severe or unbearable pain. At the same time, if PCA started with absent or slight pain at rest, inefficient analgesia was observed in only 3% (p is less than 0.05) of patients. Analgesic action starts 20-30 minutes earlier, as no time is spent for a doctor's permission and a nurse's injection.

The patient-controlled analgesia is a method that makes it possible, depending on the analgesic to be administered, in 82-95% of patients to select an efficient individual dose and control pain.

Thus, PCA method provides adequate analgesia according to the patient's individual needs.

Conclusions

1. PCEA is a reliable way to provide analgesia in the postoperative period with total arthroplasty of lower limb joints.
2. The use of prolonged analgesia in low-concentrated solutions of local anesthetics provides sufficient hemodynamic stability and preservation of motor activity of lower limbs. Advantages of the method are as follows: rapid analgesia, bypasses gastrointestinal tract is not involved, patient-controlled, programmable.
3. Epidural analgesia with extended infusion provides reliable nociceptive protection, use of low concentrations of local anesthetics prevents motor disorders. As a result, early activation of patients is possible even with use of epidural analgesia.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Our Experience of Vitiligo Therapy Through Transplantation of Skin Autografts

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Abstract

Vitiligo is a common disease with a characteristic depigmentation of the skin and not fully understood pathogenesis. Epidermocytes obtained from donor sites of their own skin have been transplanted into depigmented skin sites in 28 patients with 48 foci at the age of 19–35 years with disease duration from 4 to 15 years; the duration of patient monitoring is 12 months. Dermabrasion of the focus has been performed by a dermabrider. Epidermal grafts for transplantation have been obtained by cutting off the covers of the blisters extracted at a negative pressure of 200-300 mm Hg. All patients have tolerated the collection of skin fragments and transplantation of epidermocytes well. After 10-12 PUVA sessions applied to 19 patients, complete repigmentation of 19 foci was observed; repigmentation in 75–80% of the area — in 15 foci; on 50% of the area - in 10 outbreaks, less than 50% - in 4 outbreaks. The greatest effect has been noted in patients with localization of foci of depigmentation on the trunk and proximal limbs, and with skin phototype III. The achieved result was maintained throughout the observation period (12 months).

Keywords: *Vitiligo, transplantation, PUVA.*

Introduction

Vitiligo is one of the most common types of hypomelanosis, and it holds a unique position among various types of juvenile dyschromia [2]. Today, experts admit vitiligo's multifactorial nature and discuss the role of endocrine, nervous, autoimmune and genetic factors, as well as malignancies and other diseases in the condition's pathogenesis[3].

Followers of the oxidation-reduction homeostasis theory suggest that the death of pigmentation cells is caused by intensive free-radical oxidation [4]. The diversity of hypotheses makes it difficult to develop[1] a uniform approach and relevant treatment guidelines. Therefore, treatment of vitiligo has always posed serious problems, and it is still not effective enough[2].

There are two diametrical method of treatment, which are aimed at creation of one-type pigmentation. The first method lies in depigmentation of skin that surrounds the affected area; the second one – in stimulating the pigmentation of a discolored focus until it takes on the normal skin color.

The second method is more common. The most feasible solution is using various cosmetics that mask discolored areas.

However, not all cosmetics can satisfy patients due to their short life, high incidence of allergies and high price.

Another opportunity consists in normalization of the functioning of pigment cells within depigmentation foci. Interestingly, researchers have tried to employ almost every newly discovered scientific fact of a chemical's influence on chromogenesis to boost the pigmentation process in vitiligo patients. Therefore, an abundance of medicineshave been tested in vitiligo therapy, including copper-based drugs, hormonal drugs and vitamins, methyldopa, flurouracil, dinitrochlorobenzene, sampren, chingaminum, oxyferriscirbone[7].

Today, exciplex laser is an increasingly practiced technique. However, every case is followed by reports about complications and side effects, such as persistent erythema and hyperpigmentation at the edge of a depigmentation focus[6].

Over the past few years, autograph transplantation of both skin and melanocytes has been resumed [9]. These works are regarded with particular interest thanks to a rapid growth of cell technologies, which have paved the way for melanocyte culture from donor sites and implantation of these into depigmentation foci[5]. In vitiligo therapy, transplantology has passed the following stages:

1. Skin grafting;
2. Skin autografting;
3. Non-cultured epidermal cell suspension melanocyte transfer;
4. Cultured epidermal graft transplantation;
5. Cultured autologous melanocyte transplantation;
6. Non-cultured extracted hair follicle outer root sheathe transplantation.

An advantage of cell transplantation is an opportunity to extract a population of melanocytes from a tiny fragment of skin, which can be quite enough to repigment a large area.

The method' weak points are the complexity of procedures, having to purchase specific equipment and set up a laboratory, a time-taking and challenging cell extraction process (15 to 30 days), high cost of treatment, a risk of mutagenic and carcinogenic effects of some growth factors and nutritional mediums applied during cell culture.

As of now, there are no fully standardized cell transplantation method, and long-term results of their use are not clear.

Objective: clinical assessment of the effectiveness of epidermocyte culture and their transplantation onto depigmented areas.

Materials and Method

We have monitored 28 patients aged 19 to 35 years old with 48 vitiligo foci and disease duration of 4 to 15 years; the length of monitoring is 12 months (19 female and 9 male subjects) with different vitiligo forms. The following vitiligo triggers have been observed: acute emotional suffering, hepatobiliary and gastrointestinal diseases, family history of vitiligo (3 subjects have relatives with vitiligo).

Distribution of subjects by Fitzptrick skin phototype: 22 subjects have had skin phototype III; 6 subjects have had skin phototype II.

We have transplanted non-culture epidermal cells into 48 foci in 28 vitiligo patients.

Stage 1: Dermabrasion of depigmented area

(Dermabrader SAESHIN, KOREA)



Figure 1: Cutting off blister covers

Stage 2: Epidermal grafts, which were to be transplanted into abraded vitiligo-affected areas, have been obtained by cutting off blister covers.

Obtaining epidermal grafts with the help of a blister (at a negative pressure of 200 to 300 mm Hg during 2 to 3 hours).

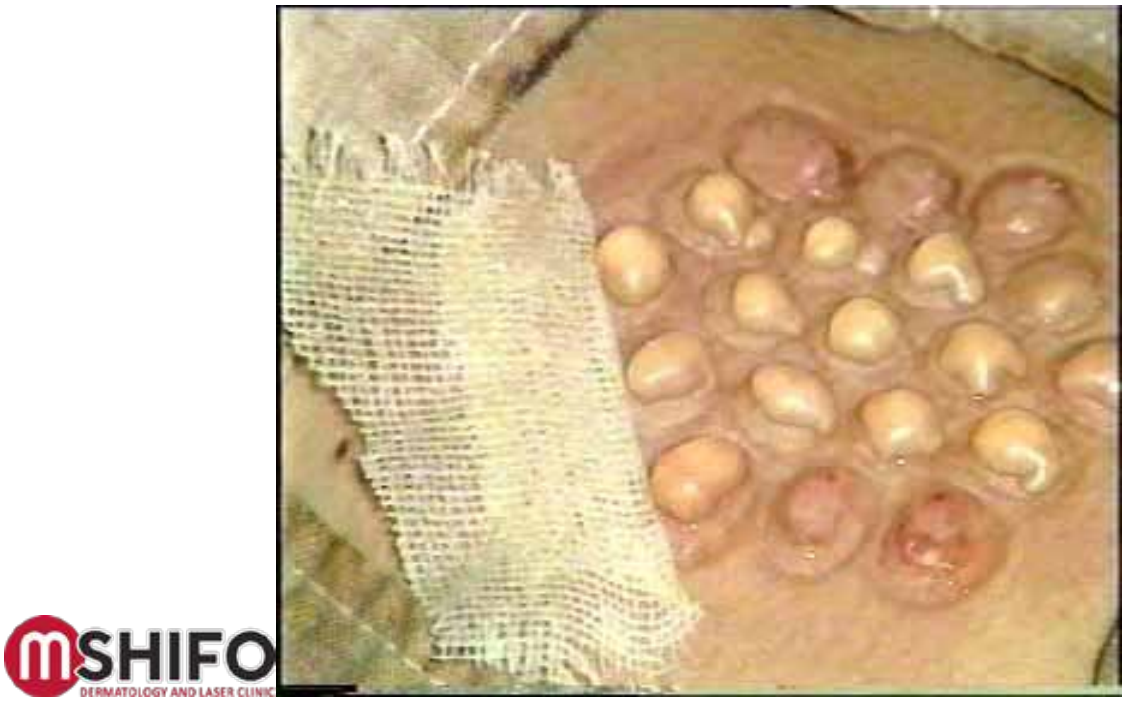


Figure 2: Pan Dulce

Stage 3. The operation ended in the fixation of the grafts and prevention of secondary contamination of the donor site and transplantation site.

Results of vitiligo treatment through epidermal cell transplantation: All subjects previously received various types of traditional and non-traditional treatment without reaching any visible effect. Three patients have had foci with cicatrical changes resulting from folk healers' burning manipulations.

All patients have undergone autologous epidermal cell transplantation. Skin samples have been obtained with the use of aseptic techniques, anesthesia with a 1% lidocaine solution and disinfection with a 70% ethanol solution. All the patients have tolerated extraction of skin fragments and epidermal cells transplantation well.

The skin fragments – 5 to 8 mm blister covers – have been extracted from a symmetrical part of the body or from the buttocks. The samples have been placed into dermabraded depigmented foci. Hemostatic compression bandages have been applied to donor and transplantation sites. We would remove them after 10 to 14 days, ensure that the graft was healing, then we would apply long-wave ultraviolet radiation (with the use of a PUVA device) starting with a 10 to 15 seconds long sessions and gradually increasing the length.

The efficacy of the therapy has been evaluated based on the speed of epithelialization and pigmentation, and the size of repigmentation areas. The follow-up monitoring has lasted 12 months.

After 10 to 12 PUVA sessions, 19 patients showed a full and complete repigmentation in 19 foci, a 75% to 80% repigmentation in 15 foci, a 50% repigmentation in 10 foci, and a smaller than 50% repigmentation in 4 foci. Patients with depigmentation foci on the trunk and proximal limbs and Fitzpatrick skin phototype III showed a more pronounced effect. The effect has lasted throughout the follow-up period (12 months).

Female patient Sh.; diagnosis: vitiligo; 20 days following an epidermal cell transplantation (pre-treatment and post-treatment)

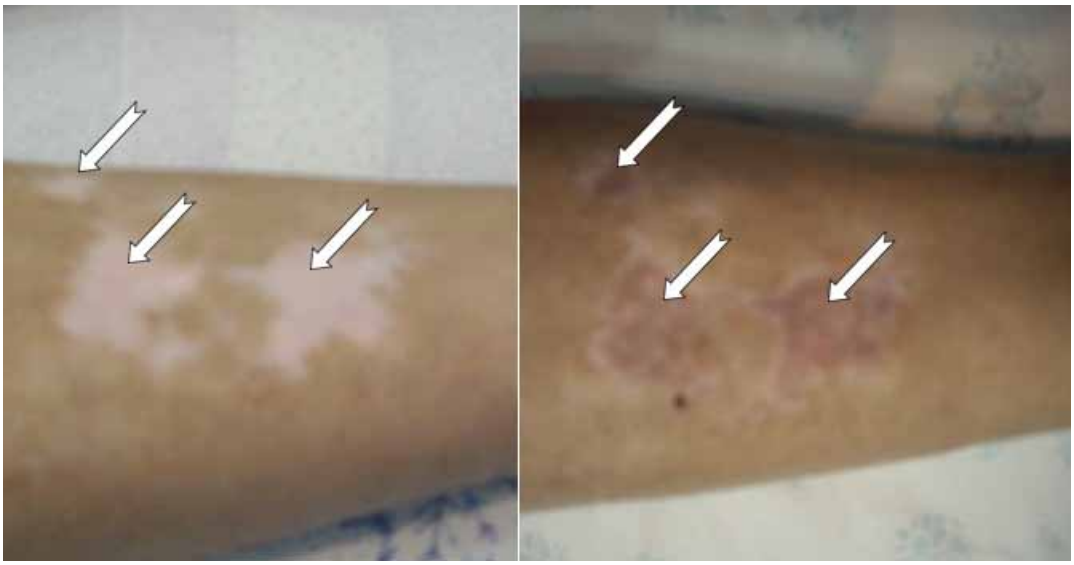


Figure 3: Vegetable

Figure 3: Female patient K. Diagnosis: vitiligo; three months following epidermal grafting



Figure 4: Scar

Results and Discussion

Lately, given the development of regenerative medicine, researchers demonstrate an increasing interest in cell auto grafting method, which consist in transplanting skin grafts into depigmented areas; the grafts contain mature and precursor melanocytes. Today, both culture and non-culture cell transplants are used. However, no comparative analysis of any of these method' effectiveness or long-term results has been carried out so far. Transplantation of blister covers is more preferred because the procedure is simple, inexpensive, quick, and it does not cause serious complications.

The choice of a transplantation method depends on the size of affected areas and localization. Treatment of vitiligo-affected areas like back wrist and foot, knee and elbow, has turned out to be ineffective.

Conclusions

Autologous epidermal cell transplantation in the treatment of different types of vitiligo is a feasible and very promising method, which ensures a pronounced and lasting effect without complications.

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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Circulating and Renal Endothelin-1 Expression Lead to Glomerular Impairment in High Fat-Induced Diet Wistar Rats

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Abstract

Objective: This study aimed to determinelevels of expression ET-1 in serum and renal tissue associated with changes in kidney structure.

Method: The rats were divided into two groups. The Obese group was given a high-fat-induced diet chow and a standard diet chow for the control group. ET-1 protein expression and localization in the glomerulus of the kidney were examined. The serum of blood checked for ET-Iusing ELISA method and assessed the ET-1 expression of the glomeruliby Immunohistochemistry (IHC). Mesangial expansion is an indicator to evaluate the degree of glomerular damage by Periodic Acid–Schiff(PAS staining). Associations between the immunoreactivity for ET-1 in glomeruli and the degree of kidney injury were examined.

Results: Lee Index was significantly higher in the obese group than in the control group ($p<0.05$). ET-1 immunoreactivity was considerably higher in the obese group than the control rat. It was a strong correlation with enhancingmesangial expansion glomeruli using PAS staining of the renal tissue ($rs=0.765$; $p= 0.001$). Contrast to ET-1 levels in the serum revealed no significant in obese rat groups ($p>0.05$).

Conclusion: These data confirm the role of ET-1 will provide changes in the levels of glomeruli impairment, but serum levels may well fail to reflect the local expression at the tissue level.

Keywords: *Endothelin-1; Obesity; Glomerular impairment; Chronic kidney disease.*

Introduction

Obesity is a complex multifactorial disease involving an excessive amount of body fat. Kelly et all projects that the prevalence of overweight and obesity will increase to 58% of the adult population by 2030.[1–3] Obesity is not just a cosmetic concern;it is responsible for the growing prevalence of chronic diseases. A meta-analysis was conducted to investigate the association of obesity with more mild kidney disease, including proteinuria (CKD stages 1–2) and low kidney function estimated glomerular filtration rate (eGFR) <60 ml/mnt / 1.73 m2). This study supports the concept that obesity is a direct cause of CKD.[4] This effecthas also been thought to have a strong relationship with the role of ET-1 as a vasoconstrictor agent in obese rats effects on kidney functions.[5,6] Another study,Ferri C et al., revealed a significant increase in plasma ET-1 levels in obese patients.[7] Long term incubation fat tissue increased effect of ET-1 could be a mechanism that contributes to increased basal lipolysis in human obesity.[8] It is suggested that ET-1 plays a vital role in regulating kidney function.

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Endothelins (ET) are a family of peptides that act as potent vasoconstrictors and pro-fibrotic growth factors. ET-1 is integral to renal and cardiovascular pathophysiology and exerts effects via autocrine, paracrine, and endocrine signaling pathways tied to the regulation of aldosterone, catecholamines, and angiotensin. In the kidney, ET-1 is critical to maintaining renal perfusion and controls glomerular arteriole tone and hemodynamics. It is assumed that ET-1 influences the progression of chronic kidney disease (CKD).[9] A correlationbetween ET-1 systemic levels and increased endothelin receptors in the kidney have been reported. Local expression of ET-1, especially in the obese kidney itself, is still unclear, so this is what underlies to see the relationship between levels and distribution ET-1 in kidneys in Wistar obese rats compared with Control Wistar rats.[6] The aims of this study provide differences in the expression/levels of ET-1 in the kidneys. It can be used as a reference to see the involvement of ET-1 in kidney pathophysiology in obese patients to develop new strategies in the prevention or treatment of obese patients suffering from kidney disease to reduce patient mortality and improve quality of life.

Material and Method

This study is an experimental study with observing high fat-induced rats reach a Lee Index> 300 for the obese group and maintained obesity status 3-4 month and non-obese rats as Control were then terminated and measured the planned variables. We hoped that the design of this time could show the effect of abnormalities on kidneys due to obesity.

Animal Experiment: This study used sixteen male Wistar rats (Rattus norvegicus), aged 12-16 weeks with an average weight of 185 g (Laboratory of Pharmacy, Faculty of Pharmacy, Hasanuddin University). Rats were housed in cages at 23-25 °C temperature and under a 12-hour day-night cycle. All of the rats were adapted for seven days.On day8th, rats were randomly divided into two groups. The Control Groups (n = 8) was fed a standard laboratory diet containing 4.7% crude fat (CE-2; CLEA, Japan), and the other (Obese groups; n = 8) was fed a High-Fat induced diet containing 14.13% crude fat (Quick Fat; CLEA, Japan),and were allowed to eat and drink water at any time (ad libitum). All of the rats were adapted for seven days in a cage made from plastic material 25 x 35 cm in size. Each cage contained a rat and was reposed by chaff and given cover by woven wire. The protocol of this study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia (number: 1141/UN4.6.4.5.31/PP36/2019). Nutritional details of the two diets are provided in Table 1. Bodyweight and food intake were measured every three days throughout the experimental period.

Table 1. The nutritional content of two experimental diets

Ingredients	CE-2	Quick Fat
Moisture (%)	8.65	6.47
Crude protein (%)	25.1	24.7
Crude fat (%)	4.51	14.13
Crude fiber (%)	5.05	3.33
Crude ash (%)	6.97	5.6
NFE (%)	49.72	45.77
Energy (kcal)*	339.9	409.1
Hardness (kg/cm^2)	27.6	12.4
Fat energy (%)	11.94	31.46

*Calculated by multiplying crude protein (4), crude fat (9), and NFE (4)

Lee index was measured the naso-anal lengths, the body weight, and calculated the Lee index between the study groups. This index was calculated by dividing the cube root of body weight (g) by naso-anal length (cm) and multiplying the result by 1000. Lee Index>300 indicated obese status. Obesity groups and the control groups were maintained for 3-4 months (175 days). Both groups of mice were terminated and measured the planned variables. For termination, rats were anesthetized with intraperitoneal injection using a ketamine cocktail. Xylazine and acepromazine at a dose of 0.1 mL / 10g BW, then we obtained the right kidney to 10% formalin buffer for IHC ET-1 analysis of paraffin block tissue.

ET-1 Serum Analysis: The blood has drawn from the tail, and retro-orbital vessel, then centrifuged at 3000rpm to extract serum. A commercial Rat Endothelin 1 ELISA Kit PicoKine ™ (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK0952) was used to assess for circulatory ET-1 level, which followed the manufacturer's manual. Data wereanalyzed by SPSS with all data presented by the mean and standard deviation.

Immunohistochemical Staining Procedures: We used 4 μm paraffin sections of the right kidney sample on the warming block in a 60ºC oven for 30 minutes.

The next step to deparaffinization and rehydration procedure begins with successive submersion in Xylene: twice each for 5 minutes 100%, 90%, 80%, and 70% ethanol concentration for 5 minutes. The rehydration antigen is given for 10 minutes using citrate buffer at 103°C, then incubatedto block endogenous peroxidase activity with 3% H2O2 for 10 minutes at room temperature, rinsed for three times each time for three minutes with Tris-buffered saline (TBS), and then incubatedfor 30 minutesin 10% bovine serum albumin (BSA) at room temperature in a humidified room. The tissue area that has been circledby the PAP penon glass objectsand dripped with primary antibodies, monoclonal anti-ET-1 (GeneTexCat No. GTX22786) that has been diluted using a 1:250 dilution and incubated at room temperature for 2 hours. The sections wererinse three times for three minutes each with PBS and incubated for 30 minutes with anHRP conjugated secondary antibody at room temperature. Then, they are rinsed and dropped with diaminobenzidine (DAB) substrate, and then balanced with hematoxylin. The final step is dehydrated and mounted with Entellan medium.

Right kidney samples prepared with ET-1 immunostaining that were analyzed and mounted, then assessed. ET-1 expression was reported in glomeruli.In each specimen, the staining intensity of ET-1 recorded by two independent observers in 10 adjacent high power fields (±100 glomeruli) and graded as 0 (staining not detectable), 1 (minimal immunostaining in some cells), 2 (weak immunostaining intensity in all cells) and 3 (strong staining in all cells). The mean grade was calculated by an averaging grade assigned by the two authors and approximating the arithmetical mean to the nearest unity.[10]

Glomerular Impairment: The glomerular injury was scored based on PAS staining. Scoring was done by grading mesangial expansion by looking at the pink color of the mesangial matrix and glomerular basal membrane. The lesions were graded on a scale from 0 to 4: 0: normal; 1: the injury involves less than 25% of the glomerulus; 2: the injury involve 25 to 50% of the glomerulus; 3: the injury involves 50 to 75% of the glomerulus; and 4: the extensive damage affecting more than 75% of the glomerulus.[11]

Statistical Analysis: Data were analyzed using SPSS version 18. Quantitative variables were expressed in means ± SDs. Shapiro–Wilk test was used to analyze the normality of distributions. The comparison of quantitative data was compared using a t-test to analyze variables with normal distribution and equal variances. Mann–Whitney U test was used to analyze the differences when data did not correspond to a normal distribution andthe graphics using Prism 6; GraphPad Software.A p-value of < 0.05 was considered statistically significant. Correlation analysis was measured by *spearmen's* rho.

Results and Discussion

Obesity Status: Table 2 shows that the obesity status of each group. It offers significant differences (p= 0.000) between the obese and the control groups after being fed for 63 days and maintenance until the day before terminated (175 days) (p=0.002). There was a significant increase in the Lee index of the obese group compared to the control group.

Excess weight gain and obesity may cause renal dysfunction, including glomerular hyperfiltration, sodium retention, enlargement of Bowman's space, increased glomerular cell proliferation, mesangial matrix expansion, inflammatory cell infiltration, and tubulointerstitial lesions. These early renal alterations can progress to more intense and diffuse lesions in the kidneys, such as focal segmental glomerulosclerosis, proteinuria, and tubulointerstitial lesions, observed in prolonged obesity.[12]

Table 2. Comparison Lee index between control and obese rats group.

	63 day		175 days	
Variable	**Control (Mean±std dev)**	**Obese (Mean±std dev)**	**Control (Mean±std dev)**	**Obese (Mean±std dev)**
Weight (gr)	283.25±44.98	325.50±27.33	341.50±50.07	417.125±37.68
Length (cm)	21.94±0.73	22.00±0.65	23.00±0.65	23.56±0.68
Lee index	297.96±8.38	311.88±1.84**	303.97±7.66	316.8786±5.81*

Calculated with an independent sample t-test. *p<0.05; ** p<0.001

Systemic level of ET-1: Table 3 shows that the level of serum ET-1 is not increased significantly (p>0.05). This is relevant to earlier studie[13], has been demonstrated that circulatory levels of ET-1 might not directly reflect the full physiological. Normally circumstances, ET-1 produced by endothelial cells is released abluminal and, therefore, the circulating level of ET-1 is thought to be the result of spillover,the half-life of ET-1 plasma is less than 2 min, owing to its efficient harvesting in the pulmonary or renal vascular beds.[14] ET-1 appears to be foremost a locally acting paracrine substance, rather than a circulating endocrine hormone. Besides, it is vital to bear in mind that ET-1 plasma concentration is dependent not only on generation but also on renal and receptor-mediated clearance and enzyme-mediated metabolism of the peptide.[13] Nevertheless, ET-1 plasma concentration could be useful as an index of ET-1 synthetic activity and venous plasma. ET-1 concentrations have been used as markers for the endothelial synthesis of the peptide.[7,13]

Table 3. Level of serum ET-1 (pg/ml)

Group	n	Mean±std dev	Median (Range)	*p*-value*
Control	8	9.5±1.60	9 (8-12)	
Obese	8	11.37±1.85	10.5 (10-15)*	0.062

*Calculated with a Mann Whitney U test *p*>0,05

Interpretation of ET-1 immunohistochemistry: The glomerulus is the functional filtration unit of the kidney. Glomerular injury is frequently associated with progressive CKD. ET-1 expression is positive staining of the glomeruli by Immunohistochemistry (IHC) shows a significant difference between the obese group and the Control group, *p* value=0.03 (*p*<0.05)(Figure 1). William et al. investigated the immunolocalization of ET-1 in the vasculature and glomerulus of the normal kidney. It is abundantly expressed in the endothelium of glomeruli, arteries, veins, and capillaries. Although ET-1 was rarely detected in the mesangium of the normal kidney[15], By contrast, in stress-induced condition shows mesangial localization of ET-1 peptide consistent in cultured human mesangial cells.[15,16] ET-1 activation can escalate the progression of chronic kidney disease (CKD) via ET-1 and ETA mediated pro-fibrotic pathways, playing a role in conditions such as diabetic nephropathy (DN), hypertensive nephropathy and focal segmental glomerular sclerosis (FSGS).[12]

The local effect expression of ET-1 in the kidney, especially in the glomerulus, was significantly different between the obese group and the control group (p> 0.05). Glomerular injury and scarring is the hallmark of CKD progression regardless of the underlying disease. Increased production of ET-1 has been found in multiple conditions associated with CKD, including diabetes and insulin resistance, obesity, and dyslipidemia. ET-1 binding of ETA stimulates renal fibroblasts, driving increased extracellular matrix synthesis, inducing collagen production, and mesangial cell proliferation with the secretion of fibronectin and type IV collagen furthering scar formation.[12]

Figure 1 Light microscopy of kidney sections immunostained for endothelin-1 (ET-1, 400x). Immunostaining scores were graded as follows; (1a) score 0 =No staining, (1b).score 1= minimal immunostaining in some cells; (1c). Score 2 = weak immunostaining intensity in all cells; (1d). Score 3= strong staining in all cells. 1e. Semiquantitative analyses of kidney sections stained for ET-1 scores are reported as means±SE. The significance level for the obese group compared to the control group **Mann Whitney u test *p*=0,003 (p<0.05)

Glomerular Impairment: The effect of increasing ET-1 expression in the glomerulus correlates with the increase in mesangial expansion in the glomerulus can be seen in figure 2. Grossly, endothelial cells comprise 45%, mesangial cells 25%, and epithelial cells 30% of all cellular elements of the glomerulus. The mesangial cell is PAS-positive (pink color). An increase in extracellular matrix implies an increase in mesangial matrix or basement membrane material. In the former instance, this may be in a uniform and diffuse pattern in all lobules or cause a nodular appearance to the mesangial healed proliferative lesions are PAS-positive. It leads to the obliteration of capillaries and solidification of all or

part of the tufts. Sclerosis may be associated with the destruction of the urinary space by collagen and an increased extracellular matrix in the capillary tufts. An older and less precise term is glomerular hyalinization. Complete sclerosis is known when the entire glomerulus is involved. When the entire glomerulus is involved, this is known as complete sclerosis[17], but no data reaches score 4 (diffuse sclerosis) in this study. Figure 2B revealed discrete and focal morphological changes in glomeruli indicate early glomerular impairment in the obesity group than in the control group ($p<0.05$).

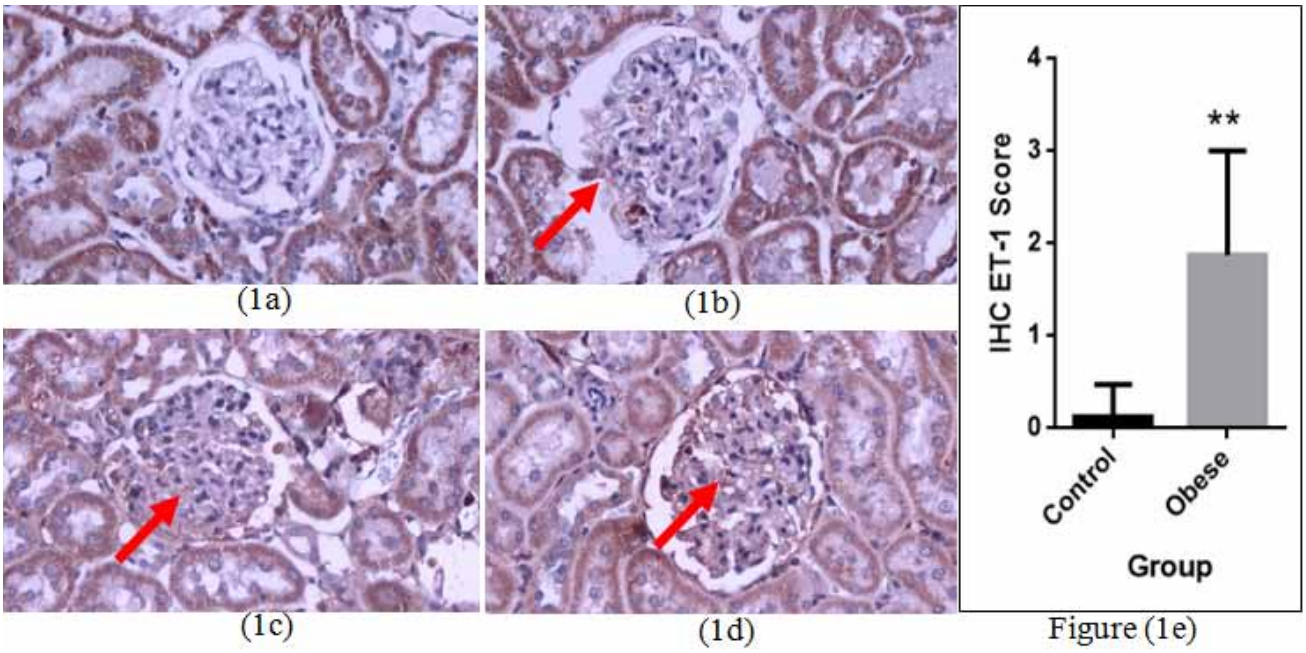


Figure 2A. Light microscopy of PAS staining of the glomerulus from Control dan Obese (400x). The mesangial expansion score of glomeruli was graded as follows: 0 represents no lesion (2a), 1 represents the mesangial expansion of 25–50%=2; (2b), >50–75%=3 (2c), (2e). Semiquantitative analyses of kidney sections stained for the PAS staining score are reported as means±SE. The significance level for the obese group compared to the control group. *Mann Whitney u test *p*=0,03 (p<0.05).

The Correlation Glomerular ET-1 glomerular expression and glomerular impairment: A significant correlation between ET-1 expression and glomerular impairment is based on PAS staining. The spearman's rho correlation value is 0.765, indicates that the direction of correlation is positive with strong correlation strength at the p= 0.001. The mesangial matrix is a contractile function important in changing the filtration coefficient. Accumulation extracellular matrix to be responsible for progressive CKD involves glomerular sclerosis and interstitial fibrosis.Although glomerular endothelial cells are probably the principal source of ET-1 in normal conditions, glomerular ET-1 can increase in the presence of proteinuric renal disease. ET-1, probably secreted abluminal from glomerular endothelial cell modulates podocyte and mesangial cell structure and function. The mesangial cell itselfmakes it possible to produce a significant amount of ET-1.[17] We conclude that the effects of ET-1 can cause changes in glomerular filtration area and intraglomerular hemodynamics.

Conclusion

In summary, our results revealed the local effect expression of ET-1 in the kidney, especially in the glomerulus, leads to glomerular injury as a hallmark of CKD progression regardless of the underlying disease.However, systemic or circulating ET-1 level is detected from serum might not directly reflect the local expression at kidney injury in obesity. Further studyis needed to assess the relationship between GFR, metabolic disturbances in the blood to determine the degree of kidney function, and what about the receptors (ETA and ETB receptor).

Ethical Clearance: No ethical approval is needed.

Source of Funding: Self

Conflict of Interest: Nil

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The Study of Antiallergic Activity of the Phytocollection of "Phytoallergoderm"

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Abstract

The antiallergic activity of "Phytoallergoderm" phytocollection was studied, which was recommended for topical treatment of a topic dermatitis, on the model of allergic contact dermatitis, caused by skin application of turpentine. The following drugs were used as comparison: antihistamine "Psilo-Balsam" gel, manufacturer: OJSC "Nizhpharm" Russia; glucocorticoid (topical steroid) "Dermoveit" cream 0.05%, manufacturer: "Glaxo Smith Kline Pharmaceuticals SA" Poland; zinc product "Zinc" ointment, manufacturer: NPP "Radiks" Uzbekistan.

During the experiment, it was found that the test drug has a reliable anti-allergic activity, which is clearly caused of decrease in total allergic reactions, in the reduction of recovery time and in a decrease in the dynamics of the severity of allergic reactions. Also, the study of antiallergic activity showed that the test drug has a comparable antiallergic activity with the etalon synthetic drug.

The foregoing indicates the presence of a reliable anti-allergic activity of the phytocollection "Phytoallergoderm", which is comparable to reference synthetic preparations.

Keywords: *Atopic dermatitis, experimental allergic contact dermatitis, turpentine, phytocollection.*

Introduction

Atopic dermatitis (ATD) is a chronic allergic skin disease with a genetic predisposition and complex development mechanisms that lead to impaired immune response, the development of allergic inflammation and the appearance of a specific symptom complex, which is characterized by age-related features of the localization and morphology of the inflammation hearth, as well as excruciating skin itching[1]. ATD is a socially significant problem due to its wide prevalence, as well as a significant impact on the quality of life, limitation of working capacity up to disability (Elisyutina, 2014).[2]

Today, glucocorticosteroids are widely used in the external treatment of atopic dermatitis, which, despite their effectiveness, have a number of side effects and often cause complications in the form of pyoderma (Elisyutina, 2014 and Skripkin, 2000).[3] However, the treatment of skin diseases with medicinal plants remains a promising direction, since medicinal plants act in a complex manner, and being non-toxic rarely cause adverse reactions (Skripkin, 2000 and Imamaliev, 2015)[4].

Based on the foregoing, we have developed a phytocomplex (phytocollection) "Phytoallergoderm", recommended for external treatment of a topic dermatitis, and having the following composition: tricolor violet grass; hop cones; licorice roots and; flowers of chamomile pharmacy, the leaves of nettles; flowers of marigolds medicinal; the grass of horsetail (Kochueva, 2014)[5].

The aim of the study was to investigate the antiallergic activity of phytocollection "Phytoallergoderm", recommended for the local treatment of atopic dermatitis[6].

Materials and Method

The study was carried out in accordance with the requirements of the current GLP regional standard- "good laboratory practice", as well as in compliance with all the rules and regulations of the "European

Convention for the Protection of Vertebrate Animals used for experimental and scientific purposes". In the study, were used healthy animals, that have passed their quarantine for at least 10-14 days.

A study of the anti-allergic activity of the drug was carried out on a model of allergic contact dermatitis caused by skin application of turpentine (turpentine oil) (Kochueva, 2014). Turpentine is known to be an industrial allergen, and external exposure to turpentine causes a strong allergic reaction, including dermatitis. It is known that when an allergen is exposed, histamine and a number of other inflammatory mediators are released, which contribute to the formation of an allergic reaction.

As comparison drugs (control drugs) were used:

– Antihistamine "Psilo-Balsam" gel for external use 1% in 20 g (tube), manufacturer: OJSC Nizhpharm, Russia, active ingredient: Diphenhydramine 1%;

– Glucocorticoid product (topical steroid) "Dermoveit" cream 0.05%, 25 g each (tubes), manufacturer: "Glaxo Smith Kline Pharmaceuticals SA" Poland, active ingredient: Clobetasol 0.05%;

– Zinc product "Zinc" ointment, manufacturer: "Radiks" Company, Uzbekistan, active ingredient: Zinc oxide 10%.

To study the antiallergic activity, the tincture was prepared the collection by GF-XI, in the ratios of 1:5, 1:10, 1:15 (the water was added taking into account the water absorption coefficient CV = 2.5 ml/g). The solids content of the infusion made up 2.97% (1: 5), 1.96% (1:10) and 1.47% (1:15) respectively.

The experiments were carried out on 60 white mice (both sexes) with a body weight of 18-22 g, which were divided into groups of 6 animals in each. In the experiment were used only healthy animals that have passed quarantine for at least 10-14 days.

To do this, turpentine was applied to the animals daily and once for six days on pre-depilated skin of the right femoral part (hair was cut out a day before the experiment), the depilation area was 2.25 cm^2 (1.5 x 1.5 cm). Turpentine was applied in the form of a moistened cotton swab, while the exposure diameter was in the form of a circle, with a diameter of about 1 cm and an area about 0.785 cm^2. As a result, pronounced morphological changes in the skin were observed in the animals, accompanied by skin itching, anxiety, lichenization, and hyperkeratosis.

Then, on the next day after the last application of turpentine, the animals of the test groups and comparison groups were injected once and daily with drugs until complete recovery:

1. Control group (control) - animals without treatment;
2. Test group No. 1 – the affected skin of the animals was smeared with 2.97% infusion of phytocollection (through a cotton swab), at a dose of 445.5 mg/kg (0.3 ml/20 g);
3. Test group No. 2 – the affected skin of the animals was smeared with 1.96% infusion of phytocollection (through a cotton swab), at a dose of 94 mg/kg (0.3 ml/20 g);
4. Test group No. 3 - the affected skin of the animals was smeared with 1.47% infusion of phytocollection (through a cotton swab), at a dose of 220.5 mg/kg (0.3 ml/20 g);
5. Comparison group (reference group) No. 1 - 1% Psilo-Balm gel was applied to the affected skin at a dose of 35 mg/kg (0.07 g / 20 g);
6. Comparison group (reference group) No. 2 - on the affected skin was smeared with 0.5% Psilo-Balm gel, at a dose of 18 mg/kg (0.07 g/20 g);
7. Comparison group (reference group) No. 3 - 0.05% Dermoveit cream was applied to the affected skin at a dose of 1.75 mg/kg (0.07 g/20 g);
8. Comparison group (reference group) No. 4 - 0.025% Dermoveit cream was applied to the affected skin at a dose of 0,88 mg/kg (0.07 g/20 g);
9. Comparison group (reference group) No. 5 - 10% Zinc ointment was applied to the affected skin at a dose of 350 mg/kg (0.07 g/20 g);
10. Comparison group (reference group) No. 6 - 5% Zinc ointment was applied to the affected skin at a dose of 175 mg/kg (0.07 g/20 g);

During the experiment, a skin condition was assessed daily, according to the severity of inflammation and skin reaction. The severity of signs of inflammation (hyperemia, edema, pain) was evaluated in arbitrary units:

0 – absence of visible reactions;

1 – pale pink erythema throughout the site or along its periphery;

2 – bright pink erythema throughout the site or along its periphery;

3 – red erythema throughout the site;

4 – skin infiltration and edema (thickening of the skin fold) in the presence or absence of erythema;

5 – erythema, severe infiltration, about local ulceration (necrosis), hemorrhages, about the formation of crusts are possible.

The criterion for assessing pharmacological activity was:

– Reduction in the amount of an allergic reaction (inflammation), compared with the control;

– Reduction in the recovery time of animals, compared with control;

– A decrease in the dynamics of an allergic reaction (inflammation), as compared with control.

To assess the amount of an allergic reaction, after the experiment was completed, the skin reaction scores for each animal were summarized, observed throughout the entire period of the experiment (during the treatment period), and the antiallergic activity of the drug was judged by the amount received.

When conducting the experiment, the room temperature is in the range of 18-25^0 C and the relative humidity in the range of 40-70%.

The results were processed by the method of variation statistics according to the Student criterion at p= 0.05 (Khabrieva, 2005 and Mirobov, 2012). The tables show the arithmetic mean values (M), the corresponding standard error of the mean value (m), student criterion (t), the number of samples (n), and confidence limits (lower confidence limit/upper confidence limit).

Results

When assessing the changes in total allergic reaction, it was found (Table 1) that the test drug in doses of 445.5 mg/kg (54.1%), 294 mg/kg (62.8%) and 220.5 mg/kg (56, 4%) had a reliable antiallergic effect. In this case, the highest antiallergic effect in the test drug was observed at a dose of 294 mg/kg.

In the case of preparation “Psilo Balm” at doses of 35 mg/kg (47, 6 %) and 18 mg/kg (51.2%) also observed a significant anti-allergic effect. At the same time, the highest anti-allergic effect of the drug “Psilo-Balm” was observed at a dose of 18 mg/kg.

In the case of the drug “Dermoveit” at a dose of 1.75 mg/kg (50.0%), a significant anti-allergic effect is observed. However, at a dose of 0.88 mg/kg (12.6 %), although an antiallergic effect was observed, it was not significant. Moreover, the highest antiallergic effect of the drug “Dermoveit” was observed at a dose of 1.75 mg/kg.

Table 1: Results study of antiallergic activity (according to the total allergic reaction) (M±tm; p = 0.05; n = 6)

Group	Result	% effect
The control	41.00 (34.07÷ 47.93)	-
“Fitosbor” (at a dose of 445.5 mg/kg)	19.17 (14.84÷ 23.49)	54.1 %
“Fitosbor” (at a dose of 294 mg/kg)	18.67 (15.24÷ 22.09)	62.8%
“Fitosbor” (at a dose of 220.5 mg/kg)	21.33 (13.21÷ 29.45)	56.4 %
“Psilo-Balm” (at a dose of 35 mg/kg)	21.50 (16.63÷ 26.37)	47, 6 %
Psilo-Balm (at a dose of 18 mg/kg)	20.00 (14.29÷ 25.71)	51.2 %
Dermoveit (at a dose of 1.75 mg/kg)	20.50 (14.88÷ 26.12)	5 0, 0 %
Dermoveit (at a dose of 0, 88 mg/kg)	35, 83 (27.69÷ 4 3, 97)	12.6 %
“Zinc ointment “ (at a dose of 35 0 mg/kg)	17.17 (14.02÷ 20.31)	58.1%
“Zinc ointment “ (at a dose of 175 mg/kg)	17.17 (13.11÷ 21.23)	58.1%

In the case of "Zinc Ointment" at doses of 350 mg/kg (58.1%) and 175 mg/kg (58.1%) also observed a significant anti-allergic effect. Meanwhile, the highest anti-allergic effect of the drug "Zinc Ointment" was observed in both doses of 350 mg/kg and 175 mg/kg.

When the experimental data on the decrease in the total allergic reaction of the most effective doses of the test drug compared with the most effective doses of the comparison drugs, it turned out that the difference between them was not statistically significant.

When assessing the change in the timing of recovery, it was found (Table 2) that the test drug in doses of 445.5 mg/kg (40.6%), 294 mg/kg (48.7%) and 220.5 mg/kg (45.9 %) significantly reduces the recovery time. In this case, the greatest decrease in the recovery time for the test drug was observed at a dose of 294 mg/kg.

In the case of the Psilo-Balsam at doses of 35 mg/kg (48.6 %) and 18 mg/kg (44.3 %), a significant decrease in the recovery time is also observed. In this case, the greatest decrease in the recovery time for the drug «Psilo-Balm» was observed at a dose of 35 mg/kg.

In the case of the drug "Dermoveit" at a dose of 1.75 mg/kg (44.3 %), a significant decrease in the recovery time was observed. However, at a dose of 0.88 mg/kg (5.7 %), although a decrease in recovery time was observed, it was not significant. At the same time, the largest reduction in the recovery time for the Dermoveit drug was observed at a dose of 1.75 mg/kg.

In the case of preparation «Zinc Ointment « at doses of 350 mg/kg (51.4 %) and 175 mg/kg (51.4 %) also observed a significant decrease in recovery terms. The greatest reduction in drug recovery terms «Zinc Ointment « is observed in both dosages of 350 mg/kg and 175 mg/kg.

If we compare the experimental breakdown by the reduction of the time of recovery the most effective doses of comparator drugs, the difference between them was not statistically reliable.

Table 2: The results of the study of antiallergic activity (by the timing of recovery) (M±tm; p = 0.05; n = 6)

Group	Result	% effect
The control	11.67 (9.95÷ 13.38)	-
"Fitosbor" (at a dose of 445.5 mg/kg)	6.50 (5.62÷ 7.38)	40.6 %
"Fitosbor" (at a dose of 294 mg/kg)	6.33 (5.25÷ 7.42)	48.7%
"Fitosbor" (at a dose of 220.5 mg/kg)	6.67 (4.71÷ 8.62)	45.9 %
"Psilo-Balm" (at a dose of 35 mg/kg)	6.00 (5.06÷ 6.94)	48.6 %
Psilo-Balm (at a dose of 18 mg/kg)	6.50 (5.40÷ 7.60)	44.3 %
Dermoveit (at a dose of 1.75 mg/kg)	6.50 (5.21÷ 7.79)	4 4.3 %
Dermoveit (at a dose of 0, 88 mg/kg)	11.00 (7.75÷ 14.25)	5.7 %
"Zinc ointment " (at a dose of 35 0 mg/kg)	5.67 (5.12÷ 6.21)	51.4%
"Zinc ointment " (at a dose of 175 mg/kg)	5.67 (5.12÷ 6.21)	51.4%

When assessing changes in the dynamics of an allergic reaction (inflammation), it was found (Table 3) that the test drug at doses of 445.5 mg/kg and 294 mg/kg already on the 3rd day of treatment, and at a dose of 220.5 mg/kg by 4 the day of treatment there was a significant decrease in the severity of the allergic reaction, which was traced to the end of treatment. In that case, the most pronounced decrease in the severity of the allergic reaction in the test drug was observed at a dose of 294 mg/kg.

In the case of the Psilo-Balsam preparation at doses of 35 mg/kg and 18 mg/kg, a significant decrease in the severity of the allergic reaction was observed already on the 4th and 3rd day of treatment, which can be traced to the end of treatment. Meanwhile, its most pronounced decrease in the severity of the allergic reaction in the Psilo-Balsam, which was observed at a dose of 18 mg/kg.

In the case of the drug « Dermoveit « at a dose of 1.75 mg/kg already on the 3rd day of treatment, there

was a significant decrease in the severity of the allergic reaction, which can be traced to the end of treatment. However, at a dose of 0.88 mg/kg, a significant decrease in the severity of an allergic reaction was observed only on the fifth day. In that case most of its marked reduction in severity of an allergic reaction in preparation « Dermoveyt « was observed at a dose of 1.75 mg/kg.

In the case of the drug "Zinc ointment" in doses of 350 mg/kg and 175 mg/kg already on the 4th and 3rd day of treatment, there was a significant decrease in the severity of the allergic reaction, which can be traced to the end of treatment. At the same time, its most pronounced decrease in the severity of the allergic reaction in the drug « Zinc Ointment « was observed at a dose of 175 mg/kg.

If we compare the experimental data by the decrease of the dynamics of an allergic reaction (inflammation) of the most effective dose of the drugs under experiment and the most effective doses of comparator drugs, it reveals that the experimental data of the test drug and «Psili Balm» and « Dermoveyt « are comparable, and in the case the «Zinc ointment», there was no significant difference.

Table 3: The results of a study of changes in the dynamics of an allergic reaction (inflammation) (M±tm; p = 0.05; n = 6)

Days Treatment	The control	"Fitosbor" (at a dose of 445.5 mg/kg)	"Fitosbor" (at a dose of 294 mg/kg)	"Fitosbor" (at a dose of 220.5 mg/kg)
1 day (baseline)	5.00	4.67(4.12÷5.21)	5.00	5.00
2 day	5.00	4.67(4.12÷5.21)	5.00	5.00
3 day	5.00	4.33(3.79÷4.88)	4.33(3.79÷4.88)	4.50(3.93÷5.07)
4 day	5.00	2.67(1.81÷3.52)	2.33(1.25÷3.42)	2.83(1.03÷4.64)
5 day	5.00	1.83(0.80÷2.87)	1.33(0.25÷2.42)	1.67(0÷3.73)
6 day	4.67(4.12÷5.21)	0.67(0÷1.94)	0.50(0÷1.38)	1.17(0÷3.09)
7 day	3.83(2.80÷4.87)	0.33(0÷1.19)	0,17(0÷0.60	0.67(0÷1.94)
8 day	2.83(1.44÷4.23)	0	0	0.50(0÷1.38)
9 day	2.00(0.67÷3.33)	-	-	0
10 day	1.33(0.06÷2.60)	-	-	-
11 day	0.83(0÷1.87)	-	-	-
12 day	0.33(0÷0.88)	-	-	-
13 day	0.17(0÷0.60)	-	-	-
14 day	0	-	-	-

Table 3: The results of a study of changes in the dynamics of an allergic reaction (inflammation) (M±tm; p=0.05; n = 6)

Days Treatment	"Psilo-Balsam" (dose of 35 mg/kg)	Psilo-Balm dose of 18 mg/kg)	Dermoveit (dose of 1.75 mg/kg)	Dermoveit (dose of 0.88 mg/kg)	"Zinc ointment" (dose of 350 mg/kg)	"Zinc ointment" (dose of 175 mg/kg)
1 day (ex. Comp.)	5.00	4.83(4.40÷5.26)	4.83(4.40÷5.26)	5.00	5.00	5.00
2 day	4.83(4.40÷5.26)	4.83(4.40÷5.26)	4.83(4.40÷5.26)	5.00	5.00	4.67(4.12÷5.21)
3 day	3.83(2.44÷5.26)	3.83(3.04÷4.62)	3.83(3.04÷4.62)	5.00	4.17(3.13÷5.20)	3.83(2.80÷4.87)
4 day	3,50(2,05÷4.95)	3.17(2.13÷4.20)	3.17(2.13÷4.20)	4.83(4.40÷5.26)	2.33(0.50÷4.17)	2,33(0.90÷3.77)
5 day	2.17(0.13÷4.20)	2.33(0.90÷3.77)	2.33(0.90÷3.77)	4.33(3.79÷4.88)	0.67(0.12÷1.21)	1.33(0÷2.91)
6 day	0.50(0÷1.38)	1.00(0÷2.76)	1.00(0÷2.76)	3.83(2.61÷5.06)	0	0

Days Treatment	"Psilo-Balsam" (dose of 35 mg/kg)	Psilo-Balm dose of 18 mg/kg)	Dermoveit (dose of 1.75 mg/kg)	Dermoveit (dose of 0.88 mg/kg)	"Zinc ointment" (dose of 350 mg/kg)	"Zinc ointment" (dose of 175 mg/kg)
7 day	0.17(0÷0.60)	0.50(0÷1.38)	0.50(0÷1.38)	2.83(0.80÷4.87)	-	-
8 day	0	0	0	2.67(0.40÷4.93)	-	-
9 day	-	-	-	2.33(0.27÷4.40)	-	-
10 day	-	-	-	1.67(0÷3.62)	-	-
11 day	-	-	-	1,50(0÷3,22)	-	-
12 day	-	-	-	1.17 (0÷2.56)	-	-
13 day	-	-	-	0.50 (0÷1.38)	-	-
14 day	-	-	-	0	-	-

Discussion

There are some data in the literature analyzing the effectiveness of antiallergic drugs. One interesting study analyzed the results of the treatment in forty-one patients[7]. Authors observed a significant difference between the acute radiation dermatitis scores on the test and the control arms[8]. Mometasone furoate cream reduced the stages of acute radiation dermatitis when the radiotherapy used over 6000 cGY[9]. Compared to the control arm, local application of mometasone furoate significantly reduced the singes of dermatitis like itching and pain regardless of the radiotherapy dose (Liao, 2019)[10].

Bao et al conducted a trial treating one hundred and sixty-four cases of allergic rhinitis with the Chinese medicine Biminkang mixture. That mixture was mainly included the Kazak folk herb Artemisia rupestis. They compared the results of 52 cases of allergic rhinitis were treated with the patent medicine Biyankang. The results showed that Biminkang mixture was an effective drug in treating allergic rhinitis (Bao et al, 1997)[11].

However, another study concluded investigating the overall effects of herbal therapy against allergic pathologies concluded that there is an existence of a number of clinically important interactions, although cause and effect relationships have not always been established. Herbs and drugs may interact either pharmacokinetically or pharmacodynamically. Yet, for many of the interactions discussed here, the understanding of the mechanisms involved is incomplete. Taking herbal agents may represent a potential risk to patients under conventional pharmacotherapy (Izzo, 2005)[3].

Based on the data obtained, we can conclude that the test drug has a reliable antiallergic activity, which is reliably manifested in the decrease of total allergic reaction, in a reduction in the recovery time and in a decrease of the dynamics of the severity of allergic reactions[4]. Also, the study of antiallergic activity showed that the tested drug has a comparable antiallergic activity with the reference and synthetic drugs, since the experimental pharmacological activity of the most effective doses of the drug, which we tested and the reference synthetic drugs have no statistically significant difference[9].

Conclusion

The antiallergic activity of the phytocollection for the treatment of eczema in comparison with the reference drug was studied. According to the results of the study of antiallergic activity, the testing drug has a reliable antiallergic activity, which is comparable to reference synthetic drugs.

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The Effect of Social Support on Pregnant Women Health in Minangkabau Matrilineal Communities

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Abstract

Objective: This research aims to explore the effect of social support on the health of pregnant women in the Minangkabau matrilineal community, Indonesia, from the perspective of the husbands, community leaders, health cadres and workers.

Method: This study employs qualitative research, while purposive sampling techniques were used to select 51 informants comprising 18 husbands, 14 community leaders, eight medical workers, and 11 health cadres. In addition, the data collection method involved focus group discussions and in-depth interviews and was analyzed using content analysis.

Results: The findings showed the social support of the Minangkabau matrilineal community towards pregnant women was non-optimal and was in the form of emotional, informative, and instrumental assistance.

Conclusion: The improvement in the health status of pregnant women is achievable with an intervention to increase social support, particularly in the Minangkabau matrilineal community, West Sumatra.

Keywords: *Matrilineal; Pregnant Women; Social support.*

Introduction

According to the 2015 Inter-Census Population Survey (SUPAS), Maternal Motherly Rate (MMR) in Indonesia is 305 per 100,000 live births.[1] Furthermore, interventions to reduce this are majorly focused on increasing access to trained health workers, and obstetric emergencies management.[1] The factors causing non-access comprise subordinate societal status, culture, and religion[2], and results in the inability of women to refer to pregnancy-related complaints, difficulty in taking initiatives, and medical treatment delays.[3] Therefore, to enhance women activities in health services, empowerment is a necessary component of the healthcare system.[4]

Several myths, stereotypes, and labels about women's roles are inseparable from the prevailing social

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construction because the origin is the socialization process in the family, education system, and other communal environments. In addition, the kinship system is a significant aspect of social structure and, in Indonesia, comprises patrilineal and matrilineal. While patrilineal refers to a community custom where the descent line originates from the father's side, matrilineal relates to the mother's side.

The Minangkabau are the only Indonesian ethnic practicing the matrilineal system and the largest adherents globally. Moreover, the women there hold a more important position because of the presence of a child signifies the next generation.

However, the maternal health and antenatal care overview in this region shows an appalling condition. Hence, K4 coverage in West Sumatra province is at 73% while the lowest is Tanah Datar District, with a 3-year decline comprising 72.4% (2015); 70.6% (2016) and 64.06% (2017).[5]

There is little research focuses on the social support of pregnant women in matrilineal societies. Therefore, this study contributes information about the subject matter, particularly in the Minangkabau ethnic group, from the perspective of the husbands, community leaders, health cadres, and workers.

Research Method

Research Design: A qualitative design was employed to determine the social support of the Minangkabau matrilineal community for the health of pregnant women, and the study showed emotional, informative, and instrumental assistance.

Research Informant: Fifty-one native Minangkabau informants were selected by purposive sampling techniques and comprised husbands, community leaders, health cadres, and medical workers. Furthermore, this was done in collaboration with the head of Subdistrict, the head of the Public health center, *Wali Nagari, Wali Jorong*, public figures, and other community groups, including the PKK.

Research Sites: The study was conducted in Sungayang and Pariangan Subdistrict, Tanah Datar District, and West Sumatra Province in Indonesia.

Data Collection Technique: The data collection method involved detailed informant interviews and focus group discussions.

Data Analysis Technique: The data were analyzed using content analysis and information triangulation techniques.

Research Result

Emotional Support: The focus group discussions and in-depth interviews of the husbands, community leaders, and health workers showed emotional assistance from the community as a form of social support towards pregnant women's health. However, this was insufficient because the attitude was not widespread. Also, emotional support entails attention, empathy, and concern.

" .....In our area, there are still a small number of people who provide support to pregnant women"(Sytoma03)

Local health workers affirmed the absence of special support for pregnant women in the region.

" …special support for pregnant women "(Sytnks 01)

Furthermore, certain husbands expressed emotional support by giving attention and encouragement to their wives.

*"……*giving attention to the wives)*....(*sysm02; sysm04; sysm06)

"……cheering the wives*(*sysm02; sysm04; sysm06)

The cadres confirmed provision of emotional support by husbands in the form of attention giving.

"……*was given attention by the husband*…. (sykdr01, sykdr02, sykdr04, sykdr05)

Also, the cadre disclosed good emotional support from the local community.

"…*several social support given to pregnant women by the* community…."*(Kpr01)*

According to certain community leaders, communal emotional support involves inquiry on the condition of pregnant women.

"...the community asks about the condition of pregnant women…."(tomapr01, tomapr02, tomapr03, tomapr04, tomapr05, tomapr06, tomapr07)

The health workers reinforced an additional communal effort of communication and sharing of

pregnancy experiences with the women during available periods.

"…the community talks to pregnant women about prenatal experiences…"(Prnks02)

Also, health professionals revealed a custom of the local community culture, particularly for first-time pregnant women, called the 7-month tradition.

"…the community carries out a festive seven-month-of-birth ceremony, involving preparation of a pumpkin meal, particularly for the first child " (prnks03)

Therefore, there is a consensus by cadres, health workers, and community leaders on observable daily communal empathy for pregnant women through inquiry on the pre-natal stage and problems faced.

Informative Support: Informative support entails the provision of advice, direction, or feedback, to positively influence the recipient. Furthermore, the interviews with health workers revealed this form of assistance through the supply of information, advice, and suggestions to conduct pregnancy checks with health workers.

"the community reminds pregnant women to check with midwives or at the health centers …."(Prnks02 dan Prnks04).

The cadre understands the duties and functions of providing wellbeing information to pregnant women. Hence, women call on health workers.

"….*the cadres provide information to pregnant women….." (kpr02).*

The husbands, community leaders, health workers, and cadres affirm the participation of the community through encouragement to check with health workers rather than traditional healers. Also, pregnant women are actively reminded of the examination schedule.

"people inquire about the condition of pregnant women, share food restrictions and give reminders to check up "(*Tomapr 01-07)"*

Similarly, certain husbands provide informative support by constantly prompting the wives about the nutritional needs, check-up schedule, and consumption of Fe tablets.

"…..*there are husbands who do not care about the pregnancy* …"(sykdr02)

"….a small number of husbands remind their wives to attend pregnancy checkup.." (prkdr05)

The health workers claim the information offered to pregnant women by the public is usually based on related personal experiences or habits. This information is conveyed informally at spare times.

"…the community socializes with pregnant women to share antenatal experiences….. "(Prnks02)

"….occasionally, the community reminds pregnant women to check with the midwife or at the public health center.…" (Prnks03).

Instrumental Support: The interviews with health cadres showed instrumental support by the Minangkabau matrilineal community through the availability of privately owned public transportation to serve as an ambulance to transport pregnant women to more adequate facilities.

"…the cars of people in the village are borrowed during an emergency " (sykdr01).

"….*several residents believe providing personal cars to transport pregnant women to the hospital brings fortune, and hence, give permission*…."(sytoma05)

Discussion

Pregnant women require adjustments to both physical and psychological changes. Furthermore, emotional support is needed due to the inability of some to properly acclimatize. These women share the comfort and discomfort felt, pregnancy development and birth concerns to trusted persons.[6]

Edmons et al. (2011) posit four support types usually obtained by pregnant women, comprised of practical assistance with routine activities, information or advice, emotional support and guarantees, and resource or material provision. Furthermore, the most frequent support includes good mothers, mother-in-law, sister-in-law, and husband.[6]

Socio-cultural influences affect pregnant women and families during the welcoming period. In addition, several ceremonies are carried out during the3rd and seventh month, birth and postpartum periods based on the customs of each region.[7]

Pregnancy is a crisis phase in the life cycle of a woman, and people in various cultures pay attention to this. Also, the several rituals required indicates the community views pregnancy as an extraordinary event. Therefore, public attention towards pregnant women is a form of social support.[8] The traditional philosophy of "badunsanak" (a strong solidarity culture with fellow kin group members) identified among the Minangkabau people. Furthermore, this relationship is apparent in the social support practiced by family members. Informative social support is easier to provide due to efficiency, effectiveness, ability to share through any media, without the requirement of personal information.[7]

Bagalkot et al. (2018) found informal peer-to-peer female networks to play a major role in Indian pregnant women care, through the sharing of stories, experiences and general knowledge.[9]

Therefore, a mindset of women support is necessary to foster the ability to solve related and family problems, including facility choice, means of transport, alternative house and children care, costs, fulfillment, among others. Moreover, the habit of living in "a gadang house" (large house) consisting of several families in the matrilineal Minangkabau community enables the provision of this assistance. However, this living together habit has begun to fade, but the principles of cooperation and collaboration remain strongly maintained in their daily lives.

Therefore, the basic instrumental support encompasses direct assistance, comprising finances, facilities, personnel, and other related assistance. This is aimed at facilitating pregnancy care and monitoring. Furthermore, concern and encouragement from the family makes pregnant women feel cared for and motivated to be careful.[6,10]

Also, a feature of the Minangkabau community is the deliberation among the tribes where there are problems faced.

Conclusion

The research shows the social support of the Minangkabau community towards the health of pregnant women is not optimal, although a unique culture and potential exists. The assistance provided is limited to emotional, informative, and instrumental support. Therefore, further studies on the factors influencing social support are needed for use as a basis for making health promotion interventions models to the community based on local culture. Through this, improving the health status of pregnant women in Minangkabau Indonesia is achieved.

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Understanding Patient Compliance During Orthodontic Treatment

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Abstract

The practice of orthodontics has sometimes been described as involving two distinct but related processes, the physiological-mechanical and the psychological-educational. Proper application of appliances to the physiological-mechanical is essential to successful treatment, but no amount of attention by the orthodontist to sophisticated techniques is likely to have much effect, if those efforts are met with resistance or indifference on the part of the patient. But how does one counter that indifference or overcome that resistance? How does one even understand it? This is a most serious and baffling problem, because it is very difficult for those in the health professions to distinguish which patients will be compliant and which will not, and to know what to do about it.

Keywords: *Patient Compliance, Orthodontic Treatment, Behavioral Pattern.*

Introduction

For centuries, medical practitioners have been concerned with unsuccessful outcomes of treatment. Physicians have sometimes rationalized their failures as resulting from an incurable illness, ineffective medication, or divine interference. Some research conducted in the past twenty years suggests that unsuccessful outcomes are not so much a function of patient biology, drug potency, or heavenly interference as they are a matter of noncompliance. The medication may not have been taken in the prescribed way, or may not have been taken at all[1].

Some orthodontists believe that their specialty and its attendant problems are so unique, that what applies to physicians does not apply to them. No doubt, some aspects of the practice of orthodontics are unique. However, orthodontists have their share of noncompliant patients, who affect both the quality of care and cost-effectiveness. A noncompliant patient is less likely to achieve quality results, and more likely to become a "special case", requiring more time, effort, and staff attention than more compliant patients. This time and energy loss might indeed be charged for, but this usually penalizes the parent, not the uncompliant patient. There is very little reason to believe that charging additional fees brings about a cooperative attitude in an adolescent patient, who does not pay them. Moreover, charging additional fees shifts the emphasis to the payer of the penalty and away from the noncompliant patient.

Compliant/Noncompliant Research: It makes sense for the orthodontic specialty to pay attention to what general medicine has learned about noncompliance, by reviewing the relevant literature. Most of the research has consisted of comparisons of compliant and noncompliant patient groups drawn from communication studies and general medicine. However, the psychological principles involved apply generally throughout health care delivery. From such research factors that are not believed to influence compliance are:

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1. The severity of the illness.
2. Patients' demographic characteristics, although there may be some sex differences.
3. Patients' knowledge about the illness and an understanding of how to follow the regimen, which are necessary for compliance, but not sufficient by themselves to guarantee it[2].

Factors which are believed to influence compliance are:

1. Some psychological characteristics of the patient (although these tend to be complicated and confounding).
2. Beliefs that patients have about their illness and treatment.
3. Some features of the interaction process between doctor and patient.[1,2]
4. Some features of the treatment regimen.

A summary of the research on compliance, based on hundreds of investigations, is provided on the accompanying chart.

Psychological Characteristics: Compliance is not a simple matter of "kinds of people"— those who will comply and those who will not. Compliance is behavior, just as noncompliance is. Since behavior is observable, one can know eventually who the compilers and noncompliers are; but, other than in behavior, how do compliers and noncompliers differ from one another? Do they have traits or characteristics or personalities, which would distinguish them from one another? Even though the behavior may appear to be a simple matter of cooperating or resisting, the human motives behind the behaviors may be highly complex and confusing. Nevertheless, there are a few guidelines for understanding the person behind the behavior. These have been called general response tendencies.

Yeasaying/Naysaying: The first of the general response tendencies has been termed "overall agreement" or the "yeasaying-naysaying tendency"[3] It describes people who have a typically favorable or typically unfavorable response to whatever they encounter. Some people, as a matter of personality, would be agreeable to a treatment regimen and accept it, while others are predisposed to be negative and resist it. In either case, the response has nothing to do with what the doctor says or does. These people are prone to respond to most situations that way.

The yeasayer's general attitude is described as stimulus acceptance. He is ready to respond affirmatively, impulsively, and quickly. He is attracted by enthusiastic presentations, while naysayers are repelled by the same thing. Naysayers are extremely careful and critical, trusting objective data, rather than feelings or impulses. Alert clinicians may be able to distinguish between yeasayers and naysayers, and treat them differently.

Persuasibility: The second guideline for understanding the person behind the behavior is called "general persuasibility" Women were found to be more susceptible to persuasion than men. During childhood, girls tend to be more obedient to commands and directions from adults, but this diminishes with maturity. As children, they are more easily influenced by young boys than by young girls, but they are not as easily influenced by adults, once they reach puberty. Young boys are especially concerned with maintaining their status in their peer group, and are probably more vulnerable to peer group pressure than girls.

It is important to remember that, although there are average numbers, there are no average people. Even though women may be generally more persuasible than men, it is difficult to predict just which women they will be; whereas men do tend to respond more consistently to persuasive messages. Janis6 has hypothesized the following characteristics associated with persuasibility, but applying more to men than to women:

1. Individuals who are hostile toward the people they encounter in daily life are predisposed to remain uninfluenced by any form of persuasion[4].
2. People who are socially withdrawn are relatively uninfluenced by any form of persuasion.
3. People with rich fantasy lives — with strong responses to rich imagery and words and pictures — tend to be more persuasible than those whose fantasy responses are more constricted.
4. People with low self-esteem— as manifested in feelings of personal inadequacy, social inhibition, and depressive affect— are predisposed to be more readily influenced than people with high self-esteem.
5. People with an "other-directed" orientation are predisposed to be more persuasible than those with an "inner-directed" orientation. It seems reasonable that a person who relies more on his own opinions

than those of others would be less influenced by the opinion of others. While those with basic feelings of insecurity and those who rely on external authority may be more easily influenced by persuasive messages and more willing to comply, this is not the case with dogmatic individuals. For them, the messages which oppose their beliefs are more likely to be rejected. Dogmatic persons dismiss evidence and logic with which they disagree. They may be influenced in their peripheral or inconsequential beliefs, but will resist information which they cannot incorporate into their existing central beliefs. There is some evidence that levels of dogmatism can be changed[5].

There are some psychological characteristics which help to determine compliance-noncompliance. Among these are agreeing tendencies, general persuasibility, the sex of the patient, dogmatism and centrality of the belief in question. Even though they have been insufficiently researched, these appear to be potentially crucial concepts for understanding what is involved when a patient cooperates or does not cooperate with a treatment regimen[6].

Patient Beliefs: While the seriousness of the illness has very little to do with patient compliance, what a person understands and believes about the illness and the treatment might. For example, a person who believes that the treatment extends over "too long" a period, or a person who believes that the treatment is "too complicated' will typically not comply with treatment. The combination of the "too long" and "too complicated" beliefs almost ensures noncompliance. "Too long" and "too complicated" are subjective and relative. Too long or too complicated compared to what? They are a "judgment call" based on belief, rather than something as factual as the severity of the illness or the difficulty of the procedures involved in treatment.

Patient beliefs about the illness and treatment and doctor-patient interaction seem to be tied together, although not completely. Some of the beliefs may come from the doctor, but a good many may not. Some may be no more than myths or "old wives' tales" Recent studies on the "Health Belief Model" conclude by recommending that the health worker systematically explore the patient's beliefs related to illness and treatment. Examples related to orthodontics might include:

"Is there anything that worries you about getting orthodontic treatment?"

"Can you think of any problems you might have in wearing headgear?"

"Have you known anyone else who has had orthodontic treatment?"

"Did they have any problems with it?"

"How did things turn out for that person?"

The "Health Belief Model" suggests that patients' beliefs can change from visit to visit, and that these types of questions should be asked at each visit to monitor those changes. However, in terms of cost and efficiency, this is a very expensive approach to improving patient compliance. There is good reason to believe that the "beliefs factor" is closely related to the "doctor-patient interaction factor", and that it is not the Health Beliefs Questionnaire which facilitates compliance, although it fosters a better understanding. It is rather the fact that the health worker must spend a considerable amount of time with each patient, asking questions and demonstrating a sincere interest in the patient, which increases his compliance[7].

Doctor/Patient Interaction: In an attempt to create a questionnaire to predict which orthodontic patients would comply and which would not, the authors put together a variety of items related to self-concept, attitudes toward dental hygiene, attitudes toward authority, and the interaction between the doctor and the patient. Using what is known as the method of "known groups", the questionnaires were given to hundreds of patients who were identifiable as extremely compliant or noncompliant in orthodontic offices in Texas, Kansas, and Colorado. Since the groups were selected as the extremes of compliance, it was felt that they might answer some of the questions differently, and that those items might be the best predictors of compliant-noncompliant behavior. The only items that produced opposite responses between the two groups were:

"My orthodontist praises me when I do well."

"My orthodontist cares about my feelings."

"My orthodontist is fair in judging my progress."

These items are not so much about the patient or even about the orthodontist as they are about the relationship between the patient and the orthodontist. Interaction influences that relationship, which in turn influences compliance. Several factors have been found

to interfere with doctor/patient interaction and, thus, reduce compliance. These factors include:

1. The doctor seeking information from the patient without providing the reasons why the information is being gathered, as if the information were only important for the doctor.
2. Tension emerging during the interaction, which is not addressed or resolved.
3. Patients feeling that their expectations are not being met, or feeling that the doctor is treating them impersonally.

It would appear that, if a health worker intentionally set about establishing such a relationship with patients, compliance would significantly increase. Carolyn Hudak-Murphy tried that at the University of Denver with arthritic patients and found it didn't work. Perhaps it was because the patients suspected that they were being manipulated and that the relationship was not as genuine as it was presented to be. Another explanation is that compliance may flourish in an authoritarian environment. It suggests that one person knows what is best for another, and that the other person should accept that. There is the possibility that equality in a relationship interferes with that authoritarian format and interferes with compliance. It suggests that, while the doctor can encourage compliance by being personal and authentic, he may very well defeat his compliance aim by being too friendly and making the patient a peer[8].

Rachelle Kaye, at the University of Denver, was interested in finding out what mode of communication was most successful in securing compliance from a doctor who was not living up to the hospital standards of quality of care. What Kaye discovered was that it was not oral versus written communication or individual versus group communication that made the difference in achieving change. It was pointing out to the physician that there was a discrepancy between the standard previously agreed upon and his failure to maintain that standard[9].

If these findings might apply to noncompliant orthodontic patients, an orthodontist would avoid a personal attack on the patient, and concentrate instead on calling attention to the fact that the patient had earlier agreed to a certain treatment regimen, but was now falling short of what he had agreed to. Some health practitioners have found it useful to tape-record the interviews at which the regimen was agreed to, and play them back to the patient. They are like an oral contract that can be reviewed, when necessary. They place the responsibility exactly where it should be— on the shoulders of the person who is not living up to the agreement.

The question of compliance/noncompliance is involved in virtually every case a doctor treats. At the same time, it is a baffling problem, not easy to understand because the causes are complex, and not easy to deal with because what works with one patient may not work with another. In some instances, the noncompliance may be a feature of the psychological makeup of the patient, and the beliefs that the patient has about the condition and treatment, and can in a way be "blamed" on the patient. Although the doctor may not have much effect on the psychological characteristics of the patient, he might take those into account in attempting to influence the beliefs of the patient to secure compliance[10].

It is also important for the doctor to know that the actual treatment regimen influences compliance and that a doctor who reduces negative aspects and optimizes positive aspects of the treatment regimen will increase his or her chances of treating cooperative patients. Some features of the treatment regimen, such as length of treatment, maybe "givens" and, as such, are unavoidable conditions of the treatment. Others can be altered in a positive way.

Enhancing patient compliance: It is not uncommon that there needs to be any learning of psychology in the field of orthodontics, yet, in the approach to the patient there will always exist an element of psychology that will enhance the progression of treatment.

Usually, patients do not co-operate in treatment in one/more of the following ways:

- Frequent absence from scheduled appointments
- Frequent damage to orthodontic appliances
- Unpleasant or negative office behavior
- Failure to attend to proper hygiene
- Failure to wear headgear and/or elastics
- Failure to wear retaining devices

The question to ask here is **Why do they exhibit such a behavioral pattern?** To this there may be several reasons.

Firstly, if we were to analyze the age at which most

kids approach for treatment they would fall in pre-pubescence or early adolescence. Classically these are the ages when repressed expressions exist between the parent and child. The struggle is between the child's ownership of his/her body and the parents' demands that the child does as he is told. Taken in this perspective, the parent might be saying "we will fix your body the way we like it." The child in turn may be revolting in a passive-aggressive way by saying "it is my body and I shall resist any efforts you might make to change it.

Secondly, every human being reacts in different ways to pain. The mouth per se is an organ for the ingestion of food, a means for the expression of thoughts and feelings and an orifice of erogenous significance. For kids intrusion by means of placing foreign objects may not produce dramatic pain but they tend to place things in a figure of overestimation thereby introducing psychological factor[6].

Thirdly, appliances in the mouth in addition to improving smile and appearance do invite ridicule by others in one way or the other. Whether the response is positive or negative is to be perceived by the individual who is undergoing the treatment. For instance, individuals with braces may be greeted with remarks such as 'tinsel teeth', 'metal mouth', 'tracky smile' and so on. These remarks may end up in a response that ranges from complete withdrawal to a blow in the mouth. However, there is the other section of individuals who perceive wearing braces as a badge of pride. These individuals look upon themselves as being different from others and flashing their braces earns them the reputation of being a subculture of an 'elite' background[11].

But still there does exist that small group for who before any orthodontic appliance had been applied were considered as unacceptable or unwilling among their peers. When they decide to finally go ahead with the treatment the appearance of the braces draws remarks of jeer. They thus become fearful of it and withdraw from any situation that singles them out. So they tend to miss appointments and not co-operate in the hope that the inattentiveness will ward away[12, 13]

Conclusion

Fourthly, there is a belief that by orthodontic treatment the result should be more esthetically pleasing. It is intended to make one more attractive, pleasant and socially more pleasing. This is generally the idea with which most people opt for treatment. Yet there are many whose self-image is essentially negative. They could not be made attractive because they do not feel so from the very start. It is better for such people that they do not invest in orthodontic treatment. They do not want to be more attractive because they feel themselves unattractive. What these youngsters are saying is that any external cosmetic appearance will not make me look better and is an unwelcome assault on my self-assumption which is not only negative but over the years have become so.

The way out with such patients would be to:

1. Conceptualize, describe and define the various attitudes towards orthodontics in young patients.
2. Develop instruments, which will help to assess the attitude of the patient.
3. Once the patient has been identified, the next plan is to develop plans and processes to modify or counteract the results of a negative attitude pattern.

It would appear that the Orthodontist is placed in direct conflict with the unrestrained and/or indifferent adolescent. It would seem advantageous to develop programs like the **O**rthodontic **A**ttitudinal **T**est **S**urvey (**OATS**) which is a questionnaire designed to tap patient attitudes about appearance, authority and invasion, comprehend the attitude of the individual and address the patient's needs and deal specifically with hidden feelings that can often become a hindrance in patient compliance. Booklets, programs and personal relationships based on this approach actually serve to control the battles that are likely to exist between the orthodontist and the patient.

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Plasma Cell Gingivitis Due to an Antitartar Toothpaste: An Unusual Case Report

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Abstract

Plasma cell gingivitis is a rareform of atypical gingival inflammation which is characterized by erythematous marginal gingival hypertrophy which may involve the adjacent attached gingiva. Plasma cell gingivitis (PCG) is an atypical form of gingivitis and is characterized by massive infiltration of plasma cells into the subepithelial connective tissue. It is a rare condition; the cause of which is still not fully understood. It is often attributed to an allergic reaction to the components of chewing gums, dentifrices or various diet components. A case of PCG is presented here in a 52 -year old male who switched to an anti-tartar toothpaste on the advice of a dentist which manifested in a generalized form. This report is the first, as far as we know, that relates PCG to the use of Glister toothpaste manufactured by Amway. The histological examination revealed the infiltration of polyclonal plasma cells. The PCG disappeared within two weeks of stopping the use of the dentifrice.

Keywords: *Plasma cell gingivitis; Anti-tartar toothpaste; Glister toothpaste.*

Introduction

The increase in the size of gingiva is a common feature of gingival inflammation. Several causes for gingival hyperplasia have been incorporated in the dental literature like an inflammatory, drug-induced, neoplastic enlargements, systemic diseases like leukemia[1,2] chronic kidney disease patients on immunosuppressants,[3] to name a few. Among them, few are uncommon and come under the category of conditioned enlargement. Plasma Cell Gingivitis (PCG), also known as plasma cell gingivostomatitis is an abnormal type of gingival inflammation which involves marginal as well as attached gingiva. The exact cause of its appearance is not known, but it is believed to be an allergic or exaggerated response to the ingredients of chewing gum, toothpaste/ or maybe to some dietary elements.

In the early 1940s and 1950s, many reports of mucosal hypersensitivity to edible gum had come out. The cinnamon compounds in them, as well as, toothpaste were identified as the etiological agents in causing PCG. The histopathology revealed a dense penetration of plasma cells separated by collagenous stroma seen predominantly in the keratinized gingiva. These findings can mimic some fatal diseases like multiple myeloma and solitary plasmacytoma, and hence a thorough case history to rule out these conditions is warranted.

Similarly, desquamative gingivitis has also been reported with the use of anti-tartar dentifrices. Pyrophosphates and flavouring agents have been identified as the offending agents. Oral reactions to cinnamon compounds (cinnamon oil, cinnamic acid, cinnamic aldehyde)[4-6] used to mask the taste of

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pyrophosphate produce intense erythema of the gingiva characteristic of PCG as seen in this particular case.

Case Report: A 52-year-old man reported to the outpatient department of Periodontics and Oral Implantology with the chief complaint of bleeding and swollen gums for one month. The medical and dental history was non-contributory. There was a particular emphasis on diabetic status as diabetes significantly increases the chances of periodontal disease as compared to non-diabetic patients.[7] The patient did not report of any deleterious habits like smoking, alcohol intake or any form of tobacco usage. He was well built and nourished and was cooperative. He had undergone scaling six months back. Extraoral examination showed no gross facial asymmetry with a straight profile and competent lips. Upon further asking, he told that he had started using Glister toothpaste around two months back and had developed swollen gums 2-3 weeks after that. Intraoral examination revealed strawberry red coloured enlargement of gingiva present throughout with rolled out marginal gingiva and blunt interdental papilla. Gingiva appeared friable, granular and bled easily, and desquamative gingivitis was also present (Figure 1).He was advised to change the toothpaste in his daily regimen for two weeks. There were minimum plaque and calculus, which was not consistent with the inflammation seen in the oral cavity. No cutaneous lesions were present elsewhere. Complete hemogram and blood sugar levels revealed no abnormalities and were well within normal limits.

A provisional diagnosis of Severe chronic generalized gingivitis was made. A conservative approach was planned initially with patient education, motivation followed by phase one therapy. The patient was also instructed to rinse with 0.2% chlorhexidine twice daily. Since the patient was using anti-tartar toothpaste, we raised our suspicion towards PCG and hence a biopsy was taken from the representative site. On the persistence of the symptoms after two weeks of phase one therapy, the patient was scheduled for a biopsy of the lesion. The procedure was carried out under strict asepsis, and all the protocols were followed. The patient was administered local anesthesia, and after subjective symptoms were positive, we used a Bard parker handle and a No 15 BP blade, gingival tissue was removed from the attached gingiva of the maxillary anterior region and was subsequently sent for histopathological examination. (Figure 2) The patient was advised routine post-operative instructions and necessary medications were prescribed. While the histopathological reports were awaited scaling and root planing was continued. In the subsequent visits, the patient reported improvement in the condition. Histopathology revealed an abundant congregation of plasma cells in the connective tissue without the presence of any atypical cells, and hence a final diagnosis of Plasma Cell Gingivitis was made. (Figure 3) Within one month of the stoppage of the anti-tartar toothpaste, the lesion completely subsided without any treatment. (Figure 4).



Figure 1. Initial diagnosis for PCG



Figure 2: Gingival tissue for histopathological examination

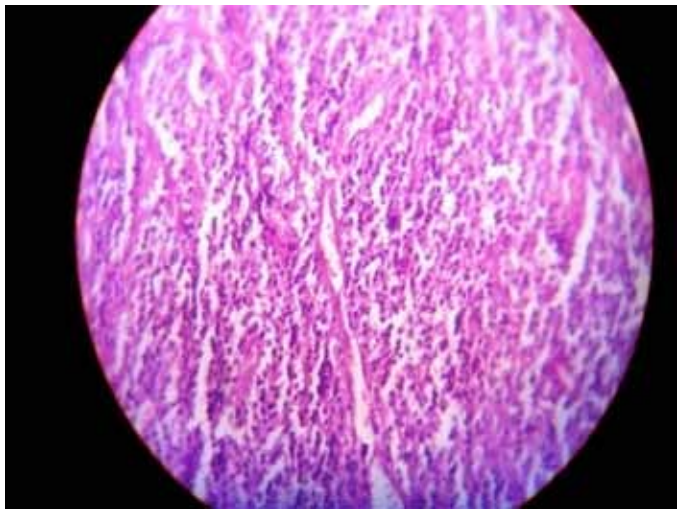


Figure 3: Histopathological examination



Figure 4

Discussion

Plasma cell gingivitis is an uncommon and rare condition that manifests clinically as a diffuse erythematous gingival lesion which may or may not extend to the mucogingival junction.[8] The number and infiltration of plasma cells vary in variouscases and are said to result from an allergic hypersensitivity reaction to various flavouring agents used in chewing gums and dentifrices. Lamey et al. 1990[6] and Miller RL 2002[9] reported that Cinnamonaldehyde, which is usually added to dentifrices to mask the unpleasant taste of pyrophosphate, has been associated with the development of PCG. The etiology is primarily unknown but is believed to be associated with the cinnamon compounds used to mask the taste of pyrophosphates in anti-tartar toothpaste. Macleod and Ellis in 1989 reported a case related to the use of herbal toothpaste. Cinnamon, when used as a flavoring agent in toothpaste, was found to be anetiological factor in gingivitis.[8] Miller et al. had reported 14 cases of cinnamon induced stomatitis.[9] The present case also had desquamative gingivitis which further stresses the role of cinnamon compounds for the same as the patient was using anti-tartar toothpaste. The differential diagnosis of the condition is critical because of its similarity with other mucosal lesions. Most cutaneous disorders were eliminated for consideration by the absence of skin lesions, a negative Nikolsky sign and no abnormalities in the hemogram in this case. Differential diagnosis needs to be done to exclude it from other aggressive lesions such as pemphigus, and once the diagnosis of PCG is confirmed, we should scout for the antigenic substance. In this particular case, the patient had switched to anti-tartar toothpaste (Glister from Amway), and that was the only contributing history.

Conclusion

This case again stresses the role of history taking in arriving at a correct diagnosis. Irrational use of herbal or anti-tartar toothpaste should be avoided, and as the use of herbal products are on the rise. Early diagnosis of plasma cell gingivitis is essential because it mimics other aggressive diseases like leukaemia and multiple myeloma.

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Rethink Retraining: A Review

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Abstract

As we advance with time, there is a change in the attitude of the kids and their parents which in turn demands a change in the pediatric dentist's approach to them. When we treat young children, there is an added responsibility to gain their co-operation for rendering the best treatment. Sometimes, the added challenge will be to gain the confidence and co-operation of the child as well as the parents with a previous unpleasant dental experience. Hence, there is a need to re-evaluate the retraining of a patient by considering advanced and innovative behavioral guidance and management techniques.

Keywords: *Retraining, Reframing, Dental Anxiety, Behavioral management.*

Introduction

Establishing a connection with the child, the child's family and the dental team is an energetic process. This can be in the form of an exchange of pleasantries, voice tone, body language, facial expressions and other ways of non-verbal communication. The dentist needs to have at their dispersal a variety of behavior management and communication techniques to deal with children of different mindsets.[1,2]

The complexity of growing up involves a lot of things that can be pleasant as well as frightening to the children and they learn to deal with new experiences as they develop their cognitive skills. In this regard a very important but, often overlooked is the consideration of the cognitive stage of child patients before we approach them. So, when the patient with a previous unpleasant dental experience ends up on your dental chair, it is better to start retraining the patient by first assessing the cognitive stage of child development she/he is in.Retraining involves usage of a combined approach of different behavior management and modification techniques to assist the child to show favorable behavior.

Retraining Techniques in Sequence:

1. **Pre-requirements of a dental set up:** Before even thinking of treatment for any patient it is important to have a clean dental operatory set up. Dental clinics should not have a cluttered environment as it leads to a cluttered mind. The dental set up should attract and instigate some sort of inquisitiveness in a child patient.
2. **Attire of the dentist:** When dealing with children with 'White coat anxiety' it is better to avoid wearing the usual doctors coat or replace it with a less threatening attire.
3. **Appointment timings and duration:** Morning appointments with brief waiting time and short treatment time of not more than 30 minutes is appropriate to avoid appointments in their nap or playtime which might make them more restless and irritable.
4. **Team Effort:** Pleasant and warm welcome to the child and the parents by the receptionist and other staff members will put them at ease and calm them.[3]
5. **Positive pre-visit imagery:** Before taking the child to the operatory chamber, showing them images that are agreeable and compliant to the child puts them in the path of acceptable behavior. This can be used in any patient. The objectives of positive pre-visit imagery are to:
   - Provide children and parents with visual information on what to expect during the dental visit.
   - Provide children with a context to be able to ask providers relevant questions before dental procedures are initiated.[4]

6. **Functional questionnaire:** Set of questions can be asked to gather information regarding the child's temperament and their previous dental visit such as:
   - How did the child behave during the past dental/ medical visits?
   - Age of the child in the last dental visit?
   - What is the anxiety level of the parent? (anxiety in parents is reflected in the child's behavior)
   - How does the parent think the child would behave in the operatory?[2]
   - What caused the unpleasant dental experience in the past? (Any particular treatment/incident)
   - Visual scaling of the patient's perception regarding dental treatment if the patient is old enough to interpret it.[5]
7. **Recognizing dental anxiety:** Feeling of restlessness about the dental treatment that is not essentially connected to a particular external stimulus is defined as Dental Anxiety. In the opinion of Chadwick and Hosey (2003), children are familiar with anxiety and the symptoms depend on the age of the child. Crying is a common expression of anxiousness in children. Phase of development plays an important role in the way a child deals with anxiety. Sometimes, children cannot be supported as they may be very young or have special health care needs with whom cooperation may not be accomplished. Anxiety of a child can also have their roots in parent anxiety levels or previous dental experience Recognition of this paves the way for a different path to be taken for a child with unpleasant dental experience.[6]
8. **Communication:** Communication has a twofold purpose as a preventive measure in behavior management (setting the tone of the environment into which the child enters) and a part of the behavior management strategy later.

Verbal communication: The choice of words is extremely important to establish the environment. Words of affirmation and expectations of behavior can be communicated to the patient by the doctor, assistant, or receptionist. Multiple targets may also be used to convey information. For example, affirmation can be given to the child for good behavior by telling the assistant or the parents how well the child is doing. Similarly, explanations about potential behavior management strategies can be given to the parent in case of an uncooperative child. When a child and his or her family do not speak a language known to the practitioner then an interpreter may be necessary. Employing auxiliaries or staff with the language skills represented by the surrounding community is favorable. Training the auxiliaries to communicate in a way consistent with the dentist's is important in such cases. In any event, nonverbal communication becomes more significant.

Non-verbal communication: It involves communicating with a child through physical contact, posture, facial expression and eye movements.[7] Either way, communication must be appropriate to the developmental age of the patient and the cultural context of the family.[4]

Reframing: Reframing is defined as, "taking a situation outside the frame that up to that moment contained the individual in different conditions and visualize (reframe) it in a way acceptable to the person involved and with this reframing, both the original threat and the threatened"solution" can be safely abandoned." It is a part of neuro-linguistic programming that helps to strengthen the relationship between the dentist and the child. The content is perceived depending on how it is framed. So, to change the behavior of the patient we need to change the content in the frame by changing the meaning or sense of the situation or by changing the context. [8,9]

Direct observation: Patients are shown a video or are permitted to directly observe a young cooperative patient undergoing dental treatment to familiarize the patient with the dental setting and dental procedure. This also allows the patient and parent to enquire about the dental procedure in a safe environment. It is based on the "Observational learning theory" by Bandura given in 1969. It aids in removing the fear of dental treatment and it can also be used to make the child aware of the expected co-operative behavior like the model(live or video) while undergoing treatment.[10, 11]

Ask tell ask: This method involves enquiring the child about their current emotions and anxiety level (ask) and then offer reassurance by explaining the procedures through demonstrations and non-threatening language appropriate to the cognitive level of the patient (tell). After comforting the child is again enquired how they are feeling (ask).[2] If the concerns continue, we need to address them and assess the situation, and modify the procedures or behaviorguidance techniques

if necessary. This will assess the anxiety that may lead to non-compliance during treatment and also teach how the procedures are going to be accomplished.[12]

Teach Back: A similar strategy called teach-back is similar to the dentist or dental assistant asking the patient to **teach back** what they have learned. It may be useful for patients with low literacy who cannot rely on written reminders. It is important to inculcate this process as a part of the routine. The patient is asked to tell/demonstrate what they have understood and if they express it incorrectly or doubtfully, we need to reteach/ demonstrate them.[4]

Successive approximation/Tell show do: "Tell show do" technique was given by Addleson in 1959. This is one of the most effective techniques of behavior modification. The child is told about the procedure in a language and manner understandable to the child after which the child is shown the equipment and the performing method(approximation). When the child becomes assured and relaxed, the treatment can begin.[3] This is most beneficial for a child who is progressing beyond the pre-cooperative stage. This also effective in an uncooperative child with 'fear' as a core issue. This is used to even demonstrate patience and concern in compensatory and overprotective parents and to retrain their vocabulary in front of kids. Successive approximation can be used to gradually expose the child to invasive procedures.[7]

Being tolerant and truthful: It is important, to tell the truth to children as that is what lets us gain their confidence. In case if they are about to receive an injection, they need to be told the same and shown how little pain would be caused like that of a prick or a mosquito bite. This way they would be prepared to receive what is told to them. Lying that there won't be any pain and then inflicting pain will only shatter their confidence. At the same time, it is of utmost importance for the dentist to be patient and maintain their tolerance levels even in case of an uncooperative child.[5]

Systemic desensitization: Introduced by Joseph Wolpe in 1952, this method introduces dental treatment to children progressively from a competing response such as relaxation to progressively increasing the threatening stimuli.[6] The first sitting involves only examination and diagnostic x-ray procedures if required followed by less invasive procedures like scaling, restorations, sealants,etc and then invasive procedures like extractions or endodontic treatments in the consecutive visits. This will tune the child to the dental setting and treatment procedures.[5]

Memory restructuring: It is a behavioral approach in which memories associated with a negative or difficult dental event are restructured into positive memories using information suggested after the event has taken place. Restructuring involves four components: visual reminders; positive reinforcement through verbalization; concrete examples to encode sensory details; and a sense of accomplishment. A visual reminder could be a photograph of the child smiling at the initial visit (i.e., before the difficult experience). Positive reinforcement through verbalization could be asking if the child had told her parent what a good job she/he had done at the last appointment. Concrete examples to encode sensory details include praising the child for specific positive behavior. The child then is asked to demonstrate these behaviors, which leads to a sense of accomplishment. The aim is to restructure difficult or negative past dental experiences and improve patient behaviors at subsequent dental visits.[13, 14]

Flexibility: In the current times, it is not uncommon to come across patients who show uncooperative behavior despite trying the common behavior management and retraining techniques. In such cases treatment shouldn't be done by force or immediately resort to protective stabilization. It is better to give time to the child and schedule another appointment for treatment after gaining their trust in the initial visit.[5]

The Current Needs of Parents from a Dentist: The parents of today seem to be moving toward a more laissez-faire attitude in parenting by providing the child with lavish freedom and choices leading to them having no self-control and discipline in some cases. Perhaps this is the reason we have newer categorization of children.[15] Children of today often present with a variety of behavioral problems such as attention- deficit hyperactivity disorder, conduct disorder, oppositional defiant disorders, and other disruptive behavior disorders. This calls for a change in the professional guidelines for behavioral guidance as well as retraining procedures.[16] Verbal reprimands and voice control are far less favored when compared to time outs. Interestingly, in a 2002 survey of 577 diplomates of the American Board of Pediatric Dentistry88 percent of respondents felt that parenting styles had changed in their practice lifetime, with 92 percent believing that these changes were

"probably or bad." Most respondents (85 percent) also believed that these changes resulted in "somewhat or much worse" patient behavior. In concert with changing parental attitudes, the diplomates reported less use of assertive behavior management techniques.[17] Younger practitioners, who may have grown up with modern values of parenting bear the influence of the modernist generation.[7]

Using classic behavior management techniques with assertive method is not favorable in the current times. Usage of physical restraints is not acceptable to most parents and Hand over-mouth exercise has fallen out of favor even with dental practitioners, possibly as a result of the implications of abuse. Voice control is questionable to overindulgent parents who consider it against the self-esteem of the child.

Conclusion

So, right now it is important to understand every child's parenting method of disciplining their children and incorporate it in our approach to the child along with behavior management science and it should be custom made for every child as they are all different from one another in one way or the other. Our goal should be to maximize their comfort levels on our dental chair especially in cases requiring retraining.

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Tuberculosis: The Interrelationship with Oral Health

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Abstract

One-fourth of the population globally is infected utilizing a bacterial infection-tuberculosis (TB). TB remains the most contagious killer disease globally having 10 million people falling ill with TB. The transmission route of TB is through the air and when symptoms occur, Therefore, as a dentist we need to be aware of oral tuberculosis such that proper protocol can be followed in our daily practice. This article highlights the important oral manifestation of tuberculosis and its confirmed diagnostic criteria so that a dentist can follow all the guidelines for proper management of a tuberculosis-infected dental patient.

Keywords: *Dental protocols, Oral manifestations, Tuberculosis.*

Introduction

Tuberculosis (TB) kills five thousand people every day. About 25% of the population is infected with tuberculosis globally whereas, in India, the TB incidence is 199 recent cases per 100000 population per year, with the global average being around 130. However, TB remains the most contagious killer disease worldwide having 10 million persons falling ill with TB as per report till 2018.[1] Maximum counts of current TB cases have been found in Southeast Asia, Western Pacific regions and Africa. Recently 8 countries were reported which were meant for 2/3rd of the new Tuberculosis cases globally.[2]

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The bacteria usually attack the lungs, but they can also damage other parts of the body. Oral cavity provides a variety of surfaces for bacterial colonization, and each environment supports a characteristic bacterial population. The composition and quantity of the bacterial population on mucosal and tooth surfaces are influenced by many factors, such as the integrity of the soft-tissues, oral hygiene, saliva, diet, and the host's immune and inflammatory responses. Transmission of TB occurs very frequently in the dental clinics either from doctor to patient or from patient to dental staff. Therefore, as a dentist we need to be aware of oral tuberculosis such that proper protocol can be followed in our daily practice.

Microorganisms responsible for this disease is Mycobacterium Tuberculosis and the transmission route of TB is through the air and when symptoms occur, they generally consist of cough (sometimes blood-tinged), weight loss, night sweats, and fever. The majority of people infected with the bacteria results in tuberculosis don't have symptoms and, in such cases, it is known as latent tuberculosis.[3] Secondary tuberculous lesions of the mouth are more commonly found. An oral manifestation is observed as a painless ulcerated lesion in the palate, lips, or tongue, along with persistent

cervical lymphadenopathy. Although most cases of oral TB manifests with a chronic ulcerative mucosal lesion, it may also present as a granuloma, osteomyelitis, or salivary gland disease.[3]

The social and economic impacts are devastating, including poverty, stigma and discrimination. The world has committed to end the TB epidemic by 2030[4] so we need a dynamic, global and multisectoral approach to reach this goal. Hence, this review article focuses on the oral manifestations of tuberculosis and guidelines for management by the dentist.

Oral Manifestations of Tuberculosis:

Tuberculous ulcer presents as single or multiple, superficial, or deep, painful or painless ulcers with an irregular border which tends to increase slowly in size. Usually develop as a small tubercle which then softens to form a shallow, ovoid ulcer with undermined margins and is lined with pale granulation tissue.[5] Tiny single or multiple nodules called "sentinel tubercles" may be seen surrounding the ulcer. Tongue is most commonly affected. However, lesions also occur on the floor of the mouth, gingiva, palate, and lips.[6] Provisional analysis of an ulcer in the mouth because of tuberculosis can be traumatic ulcers, syphilitic ulcers, aphthous ulcers as well as various carcinomas like squamous cell carcinoma, lymphoma etc.[7]

Tuberculous gingivitis may appear as nodular or it can be seen as the papillary proliferation of gingival tissues which is diffuse along with hyperaemic.[8] There may be an absence of any clinical attachment loss, alveolar bone loss or significant cervical lymphadenopathy. Sometimes tuberculous gingivitis can be seen simultaneously with marginal periodontitis and enlarged cervical lymph nodes or may present as periodontal loss of tooth shore up leading to loose teeth and gingival overgrowth.[4] A biopsy of the lesion is mandatory for arriving at the diagnosis of TB.

Tuberculous dental periapical granuloma: Three routes can be perceived to be entry portals for the tubercle bacillus. Incursion into the dental pulp throughout a very deep carious lesion by the acid-fast bacilli in the saliva is one route. The second route is hematogenous and the third is a deep periodontal pocket. The lesions are usually painless and sometimes involve a considerable amount of bone by relatively rapid extension.[9]

Tuberculous involvement of extraction sockets of teeth: Healing of the tooth extraction sockets is delayed and the socket gets filled with "tuberculous granulation tissue" consisting of many pink to red elevations.[10]

Tuberculous osteomyelitis of maxilla and mandible: Rate of occurrence of tuberculous osteomyelitis is less than 2% of skeletal TB.[11] The mandibular association is more frequent than maxilla and alveolar and angle regions have maximum affinity.[12] Tuberculous osteomyelitis commonly affects the adults; however, in some cases children are also affected. The spread of infection may be by direct transmission from infected sputum, throughout a tooth socket because of extraction or mucosal opening coupled with a completely erupted tooth or by regional extensions of soft tissue lesions and also it can be through hematogenous spread. Diagnosis of tuberculous osteomyelitis is a significant challenge as the smears for acid-fast bacilli usually do not yield positive results.[11-13]

Tuberculosis of maxillary sinus – It is usually a disease of adults and remains an underdiagnosed entity. It is usually secondary to pulmonary or extrapulmonary TB resulting from the bloodstream or by direct extension.[14] It is found as nasal discharge, stuffed nose and formation of crust along with epistaxis. It resembles as fluctuant swelling, i.e. Pott's puffy tumor as well as a malignant lesion. 3 varieties of sinonasal TB : (i) There can be involvement of mucous which can result in the formation of poly using minimal pus discharge, this type is more common; (ii) There can be involvement of bone with the occurrence of fistula and rich discharge of acid-fast bacilli (AFB); this type can lead to midfacial defect; (iii) the third type is hyperplastic that has presence of granuloma and mimics a malignancy.[15] The confirmation of Tuberculosis sinusitis is generally decided on the nonexistence of reaction to regular common antibiotics as well as by bacteriological culture or polymerase chain reaction assay. Sinus surgery may be required for sinus drainage and specimen collection. CT or magnetic resonance imaging can be helpful to figure out the extent of disease. Appearance of calcification in sinuses on CT scans can be indicative of sinonasal TB, and imaging findings are mostly nonspecific.[16]

Tuberculosis of temporomandibular joint: Tuberculosis of the TMJ is very rare in occurrence; therefore, only a few cases have been reported.[16,17] The clinical manifestation of TB infection of TMJ is unspecific and can resemble osteomyelitis, arthritis, cancer or any kind of chronic joint disease. Therefore,

TMJ TB should be taken as a confirmed diagnosis when patients present with pain and stiffness of the joint or with chronic joint diseases.[10,11] The presence of signs and symptoms is menacing like Nocturnal type of muscular spasm can lead to soft and elastic joint tumefaction, typically without erythema and with edema that will be resulting in early to severe and localized periarticular muscle atrophy.[12] Treatment of TMJ TB consists of conventional drug therapy.

Tubercular sialadenitis: TB of the salivary gland is a rare condition even in countries like India, where the disease is rampant.[18] The most common salivary glands involved in primary TB are parotid glands, whereas in systemic TB submandibular glands are most commonly involved.[19] There are two clinical forms of tuberculous parotitis. First is the localized form which is common and involves intraglandular/periglandular lymph nodes. It may be secondary to nodal infection. It presents as slow-growing, non-tender localized swelling in front and below the ear.[15] Diagnosis of tubercular parotitis is very difficult because of the absence of symptoms and may often be misdiagnosed as a benign parotid tumor.[16] A detailed history, examination and FNAC have been advocated for diagnosis of tubercular parotitis.[14]

Tuberculous lymphadenitis: Tuberculous lymphadenitis in the cervical region, also known as scrofula, is considered as the common site of extrapulmonary TB which accounts for up to 5% of the cervical lymphadenopathy. It often affects children and young adults in the age range of 30–40 years and shows female predilection.[20] It can be manifested as multiple neck mass which can affect deep lymph nodes and may relate with supraclavicular and axillary node involvement. Patients present with slowly enlarging asymptomatic lymph nodes in the persistent neck. Fine - needle aspiration cytology (FNAC) and direct microscopic screening for acid-fast bacilli (AFB) are recommended intended for the usual daily diagnosis of tuberculous lymphadenopathy along with the culture that remains the gold standard for diagnosis.[21] Diagnosis often requires biopsy. Therapy includes various types of anti-TB chemotherapy, surgical excision, or a combination of surgery and chemotherapy.[21]

Lupus vulgaris: It is the most common form of cutaneous TB found in individuals with moderate immunity and a high degree of tuberculin sensitivity that spreads through a hematogenous or lymphatic route.[22] Eighty percent of the lesions are on the head and neck and most often on the face around the nose, eyelids, lips, cheeks, ears. Females are affected two to three times more often than males.[22] Lupus vulgaris skin lesions are of five types – (a) plaque, (b) ulcerative or mutilating, (c) vegetating, (d) tumor-like and (e) papulonodular. Commonly found LV form is the plaque form that accounts for 32% of cases, whereas the destructive and deforming of all lesions is the ulcerative form.[20] The distinctive lesion is a reddish-brown plaque, containing nodules which show an "apple-jelly" color when pressed with the help of a glass spatula (diascopy).[23]

Apart from the above-mentioned oral manifestations, droplets from the contaminated sputum may result in tuberculous tracheitis and laryngitis resulting in hoarseness, coughing, and pain along with tuberculous ulcers on the tonsils resulting in dysphagia.[11]

Diagnosis Confirmation:

1. Recording detailed medical history including general and local signs and symptoms of the patient.
2. Symptoms that might help to distinguish oral tuberculosis from other varieties are fever along with lymph nodes enlargement and gastrointestinal disorders. Also, chronic cough and excessive fatigue can be seen.
3. Incisional biopsy is required to be done for each oral ulcer that lasts for more than 3 weeks .[11,17,18,22]
4. The existence of granulomatous seditious access with Langhans giant cells along with lymphocytes can be found along with foci of caseous necrosis of the tissue during histopathological assessment.
5. For confirmed diagnosis microbiological culture of sputum should be done[7,11,12,21] and also Ziehl-Nielsen stain of sputum swab to be done for acid-fast bacilli which can be positive.[11-13,22]
6. Its mandatory for are all individuals suspected of TB to do posterior-anterior (PA) and lateral view chest radiograph. The positive findings suggestive of TB are disseminated infiltrations, nodules, fibrotic scars and cavities in the lungs and also pleural effusion.[12,19]
7. One of the common Tuberculosis diagnostic tests is the Mantoux tuberculin skin test however its positive result cannot always mean active TB. There might be an indication of previous contamination or intake of the BCG vaccine.[3,5]

8. Currently interferon-g release assays (IGRAs) are now in use as a substitute of the Mantoux test because of its higher sensitivity and specificity.[9] But in suspicious patients, molecular tests (PCR) may be helpful.[1,7,13]

Protocols for management of tuberculosis patients in dental settings: A detailed medical history and physical examination of patients, specifically high-risk people should permit the dentist to make out Tuberculosis patients who can then refer for medical conduct. Sputum culture should be done for the patients who are already on anti-tuberculous treatment (ATT) to make certain that disease is not in active state.[11]

Budding routes of transmission of contamination include:

- Contact transmission through blood, oral fluids or other body fluids.
- Contaminated objects like instruments or equipment or any environmental surfaces by indirect transmission.
- Transmission route through eyes, nose, mouth and/or mucous membranes with droplets/splatter containing microorganisms.
- Breathing of microorganisms present in the air which can float for a longer period.

Recommended protocols for tuberculosis patients:

1. Limiting the utilization of ultrasonic scalers as well as very good speed handpieces in actively infected patients. Aerosolized M. tuberculosis can also survive more than nine hours. High volume suction should be mandatory for carrying out any procedure to minimize aerosol generation.
2. Rubber dam isolation should be used to have high vacuum suction but to be avoided in case the patient has a productive cough.
3. Proper hand hygiene should be maintained and individual protective things like eye shields, facemasks, head caps, gloves as well as surgical gowns needs to be followed.
4. Properly constructed, soft pleated, high filtration face masks have to be used and face masks should have at least 95% Bacterial Filtration Efficiency (BPE) for particles more than 3µm diameter.
5. Dilution of contaminated operating air/aerosols should be done for dental operatories. TB rooms should have effective air evacuation with either exhausted or HEPA-filtered if recirculation is necessary.
6. Standard fumigation of dental operatories should be done along with cleaning and disinfecting significant and contact surfaces like Dental chair and accessories. Anti-bacterial sprays can be utilized.
7. Barrier method and high valued filters otherwise UV light in the exhaust air ducts.
8. All hospitals and clinics definitely should conduct an annual report on risk assessment for Tuberculosis infection.

Conclusions

Tuberculosis is highly contagious and infectious transmitted disease which have created a rank in the list of deadliest disease globally. Since the route of transmission and all its causative factors are common especially in the hospital and dental settings, therefore detailed information and its oral manifestation need to be aware of among the health care professionals for its prevention. As per the motto of this review, the dental health professionals should follow all the protocols for the management of any tuberculosis patient. Diagnosing the disease at the early period will increase the morbidity and mortality of tuberculosis-infected patients. Hence, it becomes the responsibility of the dentist to consider tuberculosis in the provisional diagnosis of suspicious oral lesions to avoid unwanted delay in the treatment of this disease.

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Tunnel Technique for Anterior Interdental Papillary Reconstruction with Amniotic Membrane and PRF Membrane: A Case Report

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Abstract

Aesthetics play a crucial role in enhancing the outlook and self-assurance of a person.Loss of interdental papilla which occurs in the anterior maxillary region, usually known as the "black triangle" is one of the demanding missions to take in hand as it requires precise and meticulous handling of tissues in the interdental area thereby posing a great challenge inperiodontal plastic surgical procedures. Various non-surgical and surgical techniques have been recommended with varied success rates for its management. The present case report presents a tunnel technique for interdental papillary reconstruction procedure using platelet-rich fibrin and amniotic membrane.

Keywords: *Papillary recession, black triangle, amniotic membrane, PRF.*

Introduction

The space between two adjacent teeth is occupied by interdental papilla which protects the underlying periodontal tissue thereby acting as a physical barrier against microbial invasion and plaque accumulation as well as improving the aesthetics and self-maintenance by the patient. Improper tooth brushing habits, abnormal position of the tooth,extension of inflammation from gingiva to periodontal tissues, parafunctional habit, faulty orthodontic and restorative procedures are factors leading to interdental papillary loss. The interdental papillary loss have been classified by Nordland and Tarnow classification in 1982[1], taking interdental contact point,facial and interproximal cemento-enamel junction into consideration.

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Papillary reconstruction has been one of the most difficult periodontal plastic surgical procedures to master and has a very low success rate. Forced eruption, use of gingival and porcelain veneers are some of the non-surgical techniques which have been used with a successful outcome. However, managing the lost interdental papilla surgically is critical as the only source of blood supply in the narrow space at the base of the papilla. Various surgical techniques such as Beagle's technique[2], Modified Beagle's technique[3], Robert Azzi technique[4], Han and Takei technique[5] and surgical reconstruction using connective tissue graft and also regenerative materials have been carried out so far.

In recent years, platelet-rich fibrin (PRF) has gained a lot of popularity as a predictable regenerative material in the treatment of osseous and gingival recession defect because of the abundance of growth factors which are slowly released from the fibrin matrix and hasten wound healing process[6,7]. Few studies have also been reported the use of Amniotic Membrane (AM)in the reconstruction of anterior papillary recession. However, the adaptation of such membranes is quite difficult during surgery. Therefore, the present case report presents a novel tunnel technique for anterior interdental papillary reconstruction.

Case Report: A 25-year-old healthy female patient reported to the Department of Periodontology and Oral Implantology, Institute of Dental Sciences, Bhubaneswar with the chief complaint of receding gums between the upper anterior teeth. She was concerned about her look since the increased space was visible during smiling. She reported that the unaesthetic gap persisted since last one year and had slightly increased recently. Clinical examination revealed class I papillary loss between maxillary central incisors (Figure 1). There was no evidence of periodontal pocket and no bone loss was revealed radiographically. A plan for surgical reconstruction of the lost papilla was made and explained to the patient. A written patient consent was taken for the same. Routine blood investigations did not reveal any deviation from normal health.

Surgical Procedure: After performing intraoral asepsis with 0.2% chlorhexidine digluconate rinse for 30 seconds,local anesthesia (2% lignocaine) was administered in the region of #11 and #21. The distance from the contact point to the bone crest was evaluated by transgingival probing, using the UNC15 periodontal probe which was found to be 5 mm. A small horizontal slit incision was made apical to the mucogingival junction in the interdental region of #11 and #21. This was followed by a crevicular incision with the central incisors without splitting the interdental papilla.A pouch in the interdental area was created with the help of the tunneling instrument to relievethe gingivopapillary from its periosteal attachment (Figure 2a). A similar slit incision was made on the palatal aspect and using an Orban's knife the entire papillary complex was relieved from its base(Figure 2b).

Platelet-rich fibrin[8] membrane was prepared by following the standard procedure of withdrawing 10 ml of patient whole blood intravenously and centrifuging for 10 min at 3000 rpm. The amniotic membrane was procured from the tissue bank of Tata Memorial Hospital, Mumbai. Amniotic membrane was then inserted from the facial slit incision (Figure 3a) and pushed horizontally so that a portion could be pulled from the palatal aspect and manipulated to remove any folds (Figure 3b). The prepared PRF membrane was then inserted underneath the Amniotic membrane so that it lay interdentally pushing the entire papillary complex coronally. The amniotic membrane was then tucked internally, adapted and sutured on the facial (Figure 3c) and palatal side (Figure 3d). The coronally advanced papillary complex was stabilized with 4-0 vicryl sutures, secured on the labial surface of the tooth followed by placement of periodontal dressing (Barricaid)at surgical site. Suitable post-operative medications were prescribed and instructions were explained. Suture removal was done after 10 days and followed till 6 months (Figure 4). Fig 5 (a-d) shows the schematic diagram of the proposed technique.



Figure 1: Preoperative view showing loss of interdental papillae in 11 and 21



Figure 2a: Papillary complex was relieved from its base using a tunneling instrument; Figure 2b: Horizontal slit incision given on the palatal side and relieved

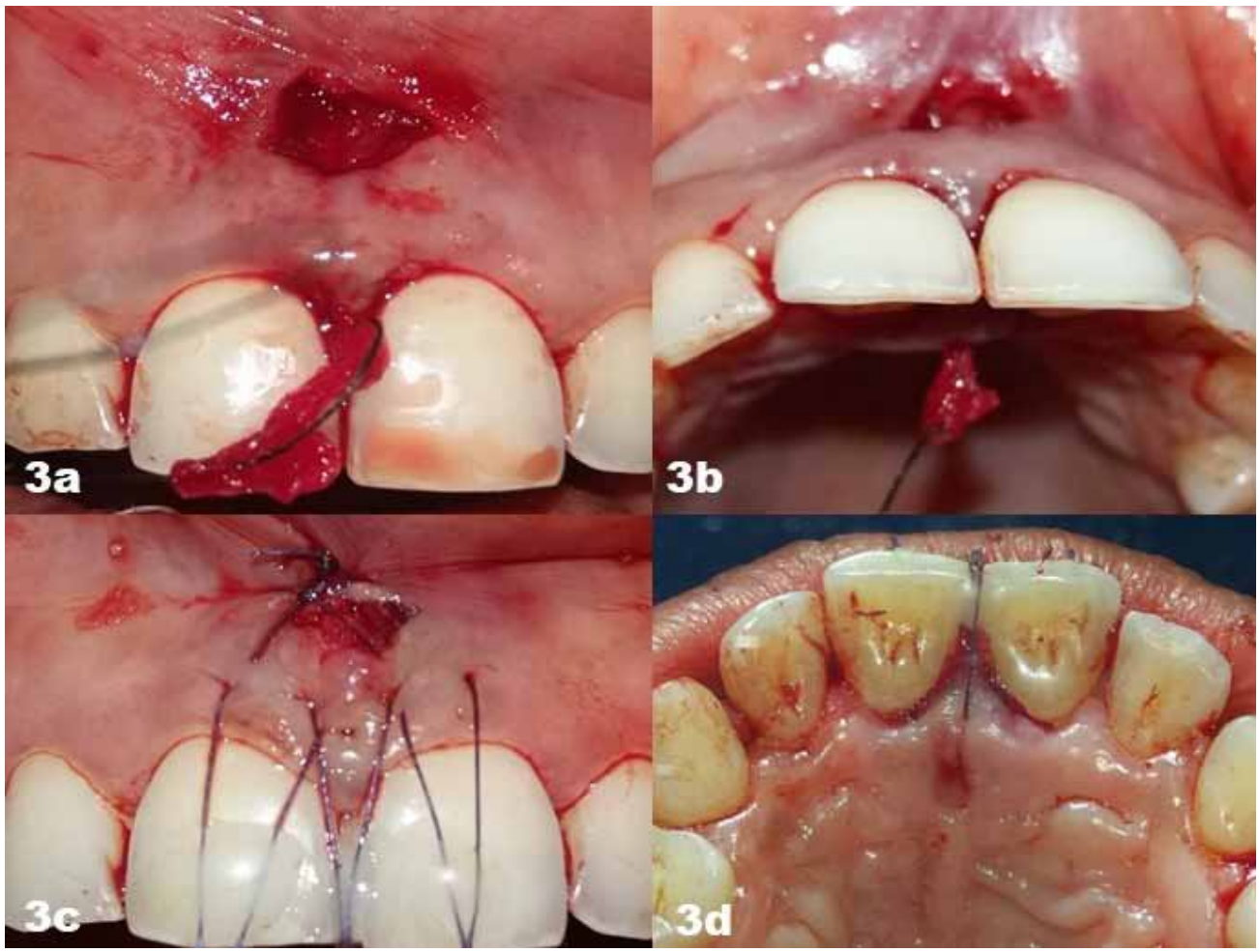


Figure 3a: AM being tucked into the pouch created and passed through the interdental area; Figure 3b: AM emerging from the palatal aspect; Figure 3c: Incisions closed with 4-0 vicryl sutures on the buccal aspect after placement of PRF; Figure 3d: Incisions closed with 4-0 vicryl sutures on the palatal aspect



Figure 4: Postoperative view after 6 months

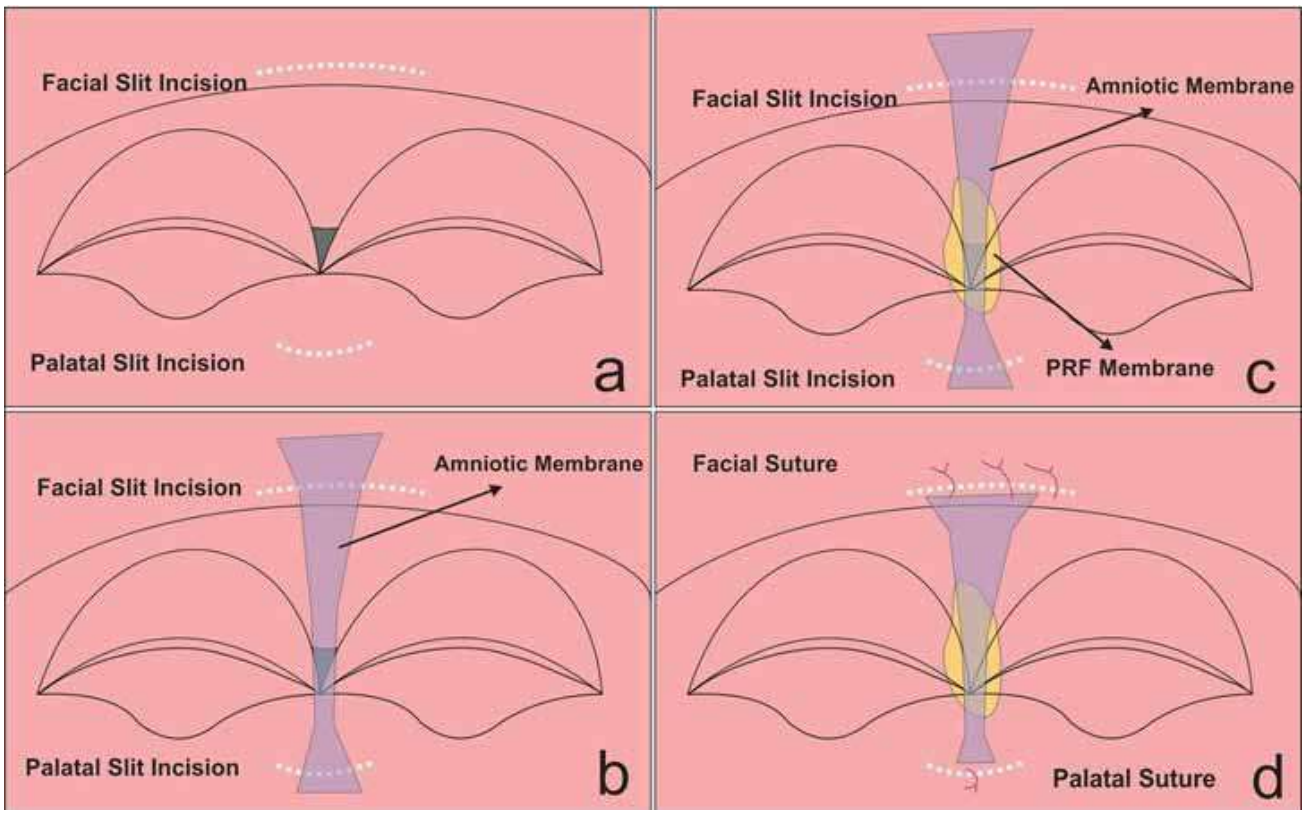


Figure 5. Schematic diagram of the technique (a-d)

Discussion

The enhancement of lost interdental papillae is however one of the most difficult periodontal plastic surgical procedures as the interdental papilla is an end-organ that is deprived of its blood supply. Many techniques have been employed for its reconstruction but no predictable absolute surgical procedures advocated for its reconstruction are present currently. The shape of the interdental papillae depends on a variety of factors such as contact point between two adjacent teeth, presence or absence of degree of recession, and width of proximal tooth surfaces.

The tunneling approach used by us is a step further which has been used earlier where the approach was restricted to the facial side only, in that there was an addition of palatal window. This technique is particularly

beneficial in cases where a barrier membrane is used. The palatal window makes the manipulation of the membrane very easy especially when an additional regenerative component such as PRF is used in conjunction with the membrane.

As suggested by Tarnow et al[9],if the distance from the contact point to the bone crest is 5 mm, there will be complete fill of the interproximal embrasures of the various techniques put forth for the amplification of the lost interdental papillae, placement of connective tissue graft under the flap showed statistically significant results inthe improvement of both papilla presence index score and papillary height when observed for a period of 12 months[10]. Due to the small, restricted space interdentally, any form of free grafting cannot be utilized since the surface area for blood supply to the donor tissue is minimal. Patient morbidity,post-operative discomfort and involvement of the second surgical site are the demerits associated with soft tissue grafting[11]. To overcome this, Arunachalam et al [12], used PRF in the reconstruction of papillae and stated that papilla regenerated was structurally stable in its new positionwhen reviewed at 6 months postoperatively.

Amnion tissue is a potent source of stem cells that helps in the stimulation of growth of the fibroblast,formation of granulation tissue and neovascularization. In addition, the stem cells found within tissue enhance clinical outcomes[13]. It also possessesan ability to form an early physiologic "seal" with the host tissue precluding bacterial contamination[14] and various studies also support its potential to reduce the host immunologic response [15].Sharma et al in 2015[16] in a study reported that amniotic membrane provides an effective alternative to autograft tissue as the self-adherent nature of the amniotic membrane significantly reduced surgical time and it is also easier to perform thus making it membrane of choice. Shetty etal[17] studied bilateral multiple recession coverage with platelet-rich fibrin (PRF) in comparison with amniotic membrane and found that the clinical outcome of the surgical procedure accounted for 100% root coverage, an enhanced gingival biotype, with both the membranes.

However, in this study we obtained a partial coverage of the papillary recession which could be attributed to various factors such as lack of following a standardized protocol, use of microsurgical instruments and evaluation of biologic width.

Conclusion

Thus, at the end,it could be concluded that the surgical technique evaluated for reconstruction of interdental papilla using PRF and AM was fairly successful. Although many refined approaches showing good clinical results, have been proposed to restore interdental papilla, use of PRF and amniotic membrane may be the solution for interdental papilla augmentation. To confirm the use of amniotic membrane as a good alternative for the treatment of papillary recession, more longitudinal studies are required in this field of periodontal plastic surgery.

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Ameloglyphics: A Biometric Analysis

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Abstract

Forensic Odontology is a budding science that utilizes dental knowledge in the court of law. It has now proved itself as an important science in medico-legal issues and personal or individual identification, gender & age determination. Ameloglyphics stands for imprints of the tooth and the enamel rod end patterns on the surfaces of the tooth. Teeth are resistant to environmental alterations like fire, desiccation, or decomposition. Enamel rod patterns are unique and are different for every individual. This review covers the basics of using enamel rod end patterns that are employed in personal identification in natural calamities.

Keywords: *Forensic odontology; Ameloglyphics; Tooth prints.*

Introduction

Forensic odontology is a branch of dentistry that involves the application & identification of individuals comparing the ante- and post-mortem records. Like fingerprints, ameloglyphics are analogue to identification and is unique with a record that remains for lifetime.[1] There should be working establishments & centers with facilities at the dental laboratories. Even it should be mandatory to maintain records for all dental clinic & hospitals either government or private. [2]

Amelo stands for enamel whereas glyphic suggests that carving thus Ameloglyphics stands for enamel rod patterns and tooth prints. Enamel could be a spin-off of germ layer cells. [3] Enamel rod is the structural unit which is synthesized by specialized cells the ameloblasts. Ameloblasts form enamel rods in associate undulating and inter-twining with the enamel rods.[1] Enamel descends from the ectoderm. It is a complex organized procedure derived from specialized cells the ameloblasts. They are laid in an undulating and inter-twining path that provides a high tensile strength that traverse in different directions.[4]

Biometrics-play a vital role in determining the tooth prints. They have constraints when decomposed, burned, fragments of calcified tissues are the remnants. As enamel is highly mineralized that forms enamel prism or rods (Hunter Schreger bands)so it withstands the degradation procedures during post mortem.[5] Biometric analysis of enamel conceals each intra- and inter-individual variation that is distinctive and specific to either gender. The formation of the enamel prisms may be categorized into 3 main patterns with Prisms being circular, aligned in parallel rows and keyhole look types.[6]

Orientation of enamel rods: The orientation of enamel rods is perpendicular to the dentin surfaces they are slightly different in deciduous and the permanent dentition. In deciduous dentition, enamel rods lie in a horizontal plane in the cervical and middle third that becomes oblique in the incisal and occlusal third with vertical in the incisal edge or the cusp tips.[3]

In permanent dentition arrangement is similar to deciduous teeth in the occlusal and middle thirds but

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show a rootward inclination or outward orientation in cervical third. The enamel rods also known as enamel prisms are described as circular, parallel or key whole appearance type patterns. In another classification they classified these enamel rods into Wavy branched & un-branched, Linear branched & un-branched, Whorl open, closed and Loop varieties[7.]

Recording Enamel rod end patterns: There are several techniques for recording the patterns of enamel rods that are useful in mass disasters. In Ameloglyphics, recording of enamel rod is done by using acid etchant, acetate peel technique, and automated biometrics for producing accurate enamel rod end patterns.[4] Macroscopically pattern of enamel rods appears incrementally on the tooth surface as perikymata but microscopically, they have a unique direction, which is different from adjoining group of enamel rods unique to ever person.[8,9]

1. **Acid Etching:** It removes the surface mineral element within the rod and rod sheath. But as the rods and rod sheaths have different mineral density, the etching leads to uneven dissolution of the surface enamel with the removal of the smear layer.

   Procedure: Initially the tooth is scaled and polished acid etched using 37% orthophosphoric acid for 30 s the preferred site being the buccal/lingual surface of tooth. Thereafter the surfaces are rinsed with water, dried & conditioned using a drop of acetone and covered using cellulose acetate film and left undisturbed for 20 minutes which is pulled gently and transferred to a clean glass slide for microscopy. Slides are visualized under a light microscope under different magnifications along with a digital camera that is sent for biometric conversion using fingerprint analysis software.[10]

   Uses: It can be used as an accurate personal identification tool. Ameloglyphics is an easy, cheap and fast procedure. It can be readily used as an adjunct antemortem dental record of firefighters, soldiers, jet pilots, etc.

2. **Peel technique:** It enables the study of the tooth structures in a 3-dimensional form that is completely mineralized as enamel. This process doesn't require the routine decalcification, sawing, and mounting.[11]

   Procedure: It is a modification of the cellulose acetate peel technique Here the enamel rod end patterns are duplicated on cellulose ester paper, rubber base impression materials, plastic wrap tape. It completely reproduces the distinctive patterns, sub-patterns of surface enamel rod end. Verifinger software readily matches the enamel rod end patterns with specific identification.[6]

   Advantages: This process is simple & inexpensive with a rapid method in making replicas of dental hard tissue surfaces.[12]

3. **Automated Biometric analysis:** Biometric-based identification is primarily used for fingerprint recognition, facial recognition, and iris scanning using automated systems and software tools. Similarly, amyleglyphics have certain desirable prerequisite characteristics, like uniqueness, easy transmit ability, to acquire as uninvasive as and distinguishable to humans. Software applications like M2SYS fingerprint SDK software system, Sig ID biometric fingerprint software system, Verifinger standard SDK etc.[7,13]

Minutiae points are the broken lines that appear as endings of lines, dots, small lines, ponds, bifurcations. Multiple bifurcations are collectively termed minutiae. The minutiae sets can be coordinated using manytechniques. In the case of fingerprints, matching a print poses a big computational problem due to the following three factors: Poor quality, very large fingerprint database, structural alteration of the fingerprint images.[14] These become irrelevant in tooth prints because being a hard surface enamel rod ending produces a good quality digital image and does not show distortion as in the fingerprints.[8] Thus forensic investigation, the team should compulsorily have dental personnel who specializes in forensic odontology. [15]

Need for Further Research: Dental caries being a complex process involving microbiologic action resulting in destruction as well as loss of tooth structure. Ameloglyphics can be utilized in determining tooth surfaces affected by caries than on relatively unaffected surfaces. Until now it doesn't illustrate any predisposing structural variations in dental caries or any variation in the shape or orientation of the rods.[9,16]. Even as we are aging, changes occur in the enamel structure due to continuous micro and macro wearing like attrition, abrasion & erosion of the tooth surfaces. Thus inducing a possible change in the structure of the rods. So the pattern is to be recorded and checked for any specific pattern. Thus requiring a need for further research.[17]

Conclusion

Enamel is the hardest structure in the body, it is exposed to continuous wear & tear. Processes like routine tooth brushing, abrasion, erosion, exposure to acidic occupational hazards, wear the enamel to expose the underneath layer. Since teeth can withstand extreme temperatures, dental findings should be a technique of choice for disasters. Thus, the biometrics reveals that the enamel rod-end patterns are unique for individuals. The reproducibility and reliability of the pattern suggests its utility as a reliable tool for identification in mass disasters.

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Prognosis of Periodontal Disease-A Dilemma

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Abstract

Prognosis is a statistical measure of the likely outcome for an individual who undergoes periodontal treatment. Determining prognosis at an early stage before treatment planning would help the clinician to render the periodontal treatment in confidence. The prognosis is determined by assessing the factors which could make the outcome of rendered treatment favorable as well as the factors which can negatively affect the outcome. Therefore, it is crucial for the clinician to judge the prognosis with his knowledge, expertise and patient's compliance. This review outlines all the factors which could possibly affect the prognosis and the clinical application of the same.

Keywords: *Periodontal Disease; Prognosis; Periodontal Treatment; Risk factors.*

Introduction

Periodontics is one of the areas of dentistry that is especially directed to the total-mouth concept, which means that, success or failure is not measured on a tooth-by-tooth basis, such as is the case in operative dentistry. The prime objective of therapy is to maintain the mouth in health throughout the life of the individual. Prognosis is defined as a prediction of the probable course, duration and outcome of a disease based on a general knowledge of the pathogenesis of the disease and the presence of risk factors for the disease.(1) It is a statistical measure of the likely outcome for an individual who undergoes treatment. Prognosis is determined before the treatment plan after the diagnosis of the condition is made. The determinants of prognosis include the nature of disease/ condition, severity of the disease, the type of treatment it requires (influenced by clinician's expertise and itPrognosis can be classified in to: Overall prognosis (dentition as a whole) and Individual tooth prognosis (more specific, and determined after the overall prognosis and is affected by it.(2)

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Factors Affecting Overall Prognosis: The factors affecting overall prognosis include Type of periodontitis, Age, Systemic background, Malocclusion, Assessment of periodontal status and prosthetic possibilities, Plaque control, Smoking, Cooperation of the patient, Individual tooth prognosis, Genetic factors and Stress

1. **Type of Periodontitis:**
   - i. **Slowly progressing periodontitis:** Onset is in the 3rd and 4th decade and progresses slowly. It responds to conventional treatment if not severe. Prognosis is directly related to severity of inflammation and height of remaining bone.
   - ii. **Rapidly progressing periodontitis:** It occurs in second decade and exhibit rapid bone loss. Either slight inflammation not suggestive of underlying osseous defect or extensive inflammatory reaction may be present. They have a hopeless prognosis, however some cases respond to conventional therapy and antibiotics.
   - iii. **Refractory periodontitis:** Patients are resistant to treatment due to impaired defense

mechanism, resistant bacteria, untreatable morphologic problems or combination of these factors.

iv. **Prepubertal periodontitis:** This type of periodontitis is associated with systemic disease in children, most cases prognosis is poor.

v. **Juvenile periodontitis:** It starts at puberty, limited initially to 1st molars and incisors. It is associated with specific flora; patient may have leukocyte defect. Prognosis is poor, but administration of systemic tetracycline in conjugation with surgical pocket therapy has good results.(3)

2. **Patient's age:** Prognosis is better in older patients with comparable levels of remaining bone. Prognosis is not good in younger patients because of rapid periodontal destruction in a short period of time and they may suffer from aggressive periodontitis.

3. **Systemic background:** Prevalence and severity of periodontitis is higher in patient with systemic disorders like diabetes, hyper parathyroidism, and nutritional deficiency. Incapacitating conditions like Parkinson's disease adversely affects the prognosis. Newer automated oral hygiene devices are useful for these patients to improve prognosis.

4. **Malocclusion:** Irregularly aligned teeth, malformation of jaws, abnormal occlusal relationships interfere with plaque control. Orthodontic or Prosthetic correction is essential for success of periodontal treatment. Overall prognosis is poor for the patients with occlusal deformities that cannot be corrected.

5. **Smoking:** Smoking plays an important element and considered to be one of the environmental risk factor in pathogenesis of periodontal disease. Smokers do not respond well to conventional method of treatment. Patients (smokers) with slight to moderate periodontitis have fair to poor prognosis. Patients (smokers) with severe periodontitis have poor to hopeless prognosis.(4)

6. **Patient cooperation and compliance:** Prognosis critically depends on patient's behaviour, perception, desire to retain his natural teeth, attitude and oral hygiene maintenance. The patient's cooperation in following the prescribed treatment protocols and post treatment instructions also affect the prognosis.

7. **Plaque control:** Bacterial plaque plays an important role in initiation and progression of periodontal disease and is considered as the primary etiologic factor. Effective removal of plaque either during treatment procedure or patient's approach to follow plaque control on a daily basis is critical for successful periodontal therapy and for prognosis.(5)

8. **Genetic factors:** IL-1 gene polymorphisms leads to production of increased IL-1β which is believed to associated with pathogenesis of chronic periodontitis.(6) Prior knowledge and diagnosis of patient's IL-1 genotype and smoking status would help the clinician to judge the prognosis and comment on the survival rate.

9. **Stress:** Physical, mental, emotional and environmental stress may affect the patient's response rate to the periodontal therapy. Proper identification of these factors on the patient's behalf would give a clear picture about the overall prognosis of the patient.

Factors Affecting Individual Tooth Prognosis: Factors affecting individual tooth prognosis include percentage of bone loss, probing pocket depth, Bone loss patterns and its location, presence of furcation involvement, tooth mobility, tooth morphology, crown to root ratio, root concavities and its forms, caries, non vital teeth, occlusal relationship, developmental grooves, enamel projections, teeth adjacent to edentulous area, relation to adjacent teeth.

1. **Percentage of bone loss:** Greater the bone loss, poorer the prognosis. As bone loss exceeds 50% the prognosis worsens rapidly. More irregular the bone loss, the poorer is the prognosis.

2. **Distribution and type of bone loss:** Horizontal bone loss, pocket elimination is easier. When vertical/trough like bone defects are present, the feasibility of osteoplastic, new attachment attempts or bone grafting must be considered. (7)Vertical bone loss with 1 or 2 walled infrabony defects or interproximal bony craters have worse prognosis than horizontal type. However, considering vertical defects, three walled infrabony defects and two walled defects have better prognosis than One walled defects for bone regeneration.(8)

3. **Periodontal pocket:** Pocket depths, clinical attachment, degree of bone loss, and nature of pocket are important in determining prognosis.(9) The extent, location, depth and complexity should

be taken into consideration.

4. **Mobility:** Teeth with 4–5mm remaining bone or grade 3 mobility has doubtful prognosis. Teeth that can be rotated or depressed is much more seriously involved than a tooth with horizontal mobility. Mobility due to occlusal trauma has favorable prognosis.

5. **Furcation involvement:** The problems associated with furcation involvement are, difficulty of access to surgical area, inaccessibility of the area to plaque removal by the patient.[10] The divergence of the root, the accessibility to the clinician for better instrumentation, the trunk of the tooth are to be considered when determining prognosis.

6. **Relation to adjacent teeth:** In dealing with teeth of questionable prognosis, the chances of successful treatment should be weighed against any benefits that would serve to adjacent teeth if the tooth under consideration was extracted. Heroic attempts to retain a hopelessly involved tooth may jeopardize the adjacent teeth.

7. **Caries, Non-vital teeth, Tooth resorption:** In teeth with extensive caries, feasibility of adequate endodontic therapy should be considered before undertaking periodontal therapy. The resorption of root (internal or external) leads to mobility of the tooth and this can affect the treatment outcomes therefore jeopardizing the instrumentation, maintenance of oral hygiene and success of any periodontal therapy rendered to the tooth n question. Vitality of the tooth also plays an important role in deciding the prognosis of the periodontal therapy. The non-vital tooth behaves differently than vital ones. It is believed that new attachment can occur in cementum of both non-vital and vital teeth, however the nature of attachment is not even close to regeneration.

10. **Tooth morphology:** Root form and morphology, Crown root ratio**,** Root concavities**,** Developmental grooves**,** Enamel projection are to be considered. Prognosis poor for teeth with short, tapered roots and relatively large crowns. Developmental grooves which sometimes appear in the maxillary lateral incisor (palate-gingival groove) or in the lower incisors, create an accessibility problem. (5.6% maxillary lateral incisors and 3.4% maxillary central incisor). Cervical enamel projections on the root surface interfere with the attachment apparatus and may prevent regenerative procedures from achieving their maximum potential. Therefore, there presence may have a negative effect on the prognosis for an individual tooth.

Clinical Applications: Careful analysis of the factors determining individual and overall prognosis, will allow the clinician to assess the type of prognosis.[1] The below mentioned criteria are used to determine the type of prognosis in a patient suffering from periodontal disease:

1. **Excellent prognosis:** The prognosis is considered excellent when the patient presents with no evident bone loss**,** however, the gingival condition is maintained at an excellent condition. The patient is cooperative and complaint with no history of systemic or any environmental risk factors.

2. **Good prognosis:** The prognosis is considered to be good when there is adequate amount of remaining bone support along with the periodontium, and the patient as well as the clinician has adequate possibilities to control etiologic factors and establish a maintainable dentition. The patient is cooperative with no history systemic or any environmental risk factors. And systemic factors if present, it should be well to moderately controlled.

3. **Fair prognosis:** The prognosis is considered fair in case of patient presents with less than adequate remaining bone support along with weak periodontal support and tooth mobility. There may be presence of Grade I furcation involvements. The patient cooperation may be acceptable and can take adequate maintenance and plaque control measures. There also may be presence of limited systemic or any environmental risk factors.

4. **Poor prognosis:** The prognosis is considered fair in case of patient presents with moderate-to-advanced bone loss, weakened periodontal support, tooth mobility along with Grade I and II furcation involvements. There may be presence of difficult-to-maintain areas. The patient's cooperation and compliance may be doubtful and there is always presence of systemic or any environmental risk factors.

5. **Questionable prognosis:** The prognosis is considered fair in case of patient presents with advanced bone loss, weakened periodontal support, tooth mobility along with Grade II and III furcation

involvements. There may be presence of inaccessible areas. The patient's cooperation and compliance may be doubtful and there is always presence of systemic or any environmental risk factors.

6. **Hopeless prognosis:** The prognosis is considered fair in case of patient presents with advanced bone loss, weakened periodontal support, Grade III tooth mobility. There may be presence of non maintainable areas. The patient's cooperation and compliance may be doubtful and there is always presence of uncontrolled systemic or any environmental risk factors. Extraction is indicated in such a scenario.

Excellent, good and hopeless prognoses are the only prognoses that can be interpreted with a reasonable degree of accuracy. Fair, poor and even questionable prognosis depends on a large number of factors that can interact in an unpredictable number of ways. In many of these cases, it may be advisable to establish a provisional prognosis until phase I therapy is completed and evaluated.

Prognostic Judgement and Treatment Planning: Treatment can alter prognosis. The severity and distribution of the lesions, the ADA classification type and complicating factors of the diseases must be assessed to determine the effect of the therapy. Severity distribution of inflammatory and traumatic factors, severity depends on pocket depth, degree of bone loss, tooth mobility, crown root ratio. Distribution of disease may be generalized or localized. Inflammatory and traumatic factors must be considered and may produce variation in prognosis of individual teeth.

Conclusion

Periodontal prognosis, a fascinating aspect of periodontal therapy, requires experience and judgment as well as a keen attention to meticulous detail. The fate of the dentition sometimes rests on the decision of the therapist. Patients almost always ask for reassurance on the chances of retaining natural teeth and usually express their questions in the form of an expressed doubt on the advisability of proceeding with therapy. Hence, the determination of prognosis is a vital and essential step in periodontal treatment planning.

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Chronic Inflammatory Gingival Enlargement Managed by Scaling and Root Planing with Curettage: A Case Report

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Abstract

Gingival enlargement (GE) is defined as an increase in the size of the gingiva. It is also known as gingival overgrowth. A wide variety of etiological factors play an important role in the development of GE. GE may be acute or chronic. However chronic inflammatory GE is most commonly found. Prolonged exposure of dental plaque is the most common etiological factor of chronic GE. A 23-year-old girl came to the Department of Periodontics, with a chief complaint of swelling of gums in lower front teeth regions. On clinical examination, there was grade II GE in 33 to 43 regions along with the presence of calculus in the lingual side, and poor oral hygiene too. It was planned for phase I therapy followed by phase II therapy. So scaling and root planing was performed and after 4 weeks curettage was done under infiltration local anesthesia. The subject was instructed to maintain oral hygiene. After 2 months, it was found complete resolution of GE. She was very satisfied with the result.

Keywords: *Curettage, gingival enlargement, root planing,scaling.*

Introduction

Gingival enlargement (GE) is the most common characteristic of gingival disease.[1] Increase in size of gingiva is known as GE.[2] These GE may represent as mild enlargement of the isolated interdental papilla to segmental. It may sometimes be uniform and marked enlargements affecting one or both jaws. These GE may be due to various etiopathogenesis. It may be due to multifactorial origins such as from interaction between host and environment, or to various stimuli.[3] But it generally develops from plaque-induced or any associated with systemic hormonal disturbances. It is also associated with the manifestation of leukemia, thrombocytopenia.[4] These GE may sometimes lead to functional disturbances such as altered speech, difficulty in mastication, and esthetic and psychological problems.[2]

Inflammatory GE may be divided into acute or chronic. But chronic GE is much more common. This chronic inflammatory GE is due to prolonged exposure to plaque accumulation.[1,4] This plaque-induced inflammatory GE is easily treated with debridement of plaque and calculus and encouragement of oral hygiene.[5] Sometimes it is subsided without curettage. But in case of fibrotic GE gingivectomy is needed.[6] This case report is presenting chronic inflammatory GE and its treatment.

Case report: A 23-year-old girl came to our Department of Periodontics, with a chief complaint of agum swelling in front lower region, gum bleeding and bad breath. The patent had marked the swelling and told that the swelling gingiva had increased in size since then. Onclinical examination, it was found that there

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was grade 2 GE which involved marginal and papillary gingiva, slight erythematous color, loss of stippling, obliterated contour, bleeding on probing in the complaint area (figure 1).[7] The gingiva appeared as soft and friable with a shiny and smooth surface. There was the presence of false periodontal pockets.[1]



Figure 1: Preoperative view showing enlarged gingiva of mandibular teeth

She was almost systemic healthy. She had no tobacco chewing habits. However there was the presence of sub gingival calculus, especially in the lower anterior region, which was the main culprit of enlarged gingiva. On further investigation, she maintained poor oral hygiene and poor plaque control.

Treatment: Scaling and root planing was done. After 4 weeks, there was some amount resolution of GE with no bleeding on probing as shown in figure 2. So the next plan was decided to curettage. Under local anesthesia (2% lignocaine hydrochloride with 1:80,000 epinephrine), curettage was performed in the anterior mandibulararch (33 to 43) with the help of Gracey curettes # 1, 2, 3, 4. The patient was advised to perform proper oral hygiene maintenance and trained the proper brushing technique (Modified Bass Technique).



Figure 2: After 1 month of Scaling and root planing



Figure 3: After 2 month of curettage

Results

The present case was againre called after 2 months of curettage. It was found that there was a complete resolution of GE as shown in figure 3. There was no bleeding on probing, presence of stippling and scalloped gingiva, melanin pigmented pink health gingiva. She was satisfied with the final result.

Discussion

Chronic inflammatory GE is the most common occurrence of GE which is mainly originated as a result of prolonged exposure to dental plaque.[1] So factors that favor the plaque accumulation and retention include poor oral hygiene, maligned teeth, loss of tooth function, any defective restoration, previous history of orthodontics treatment, mouth breathing habits, etc. Treatment of chronic inflammatory GE depend upon the understanding the underlying the causes and pathological changes.[2]

In the present case, there is a case of chronic inflammatory GE. Generally, these type GE is associated with prolonged exposure of dental plaque and later accumulation dental plaque. This type of GE can be managed by regular professional scaling and root planing along with good patient compliances. If the GE is not resolved, then curettageis done after 1 month. If still the GE is not resolved and gingiva becomes fibrotic, then gingivectomy or flap surgery should be performed.[2,3,6]

Conclusion

Chronic inflammatory GE in our case was due to mainly poor hygiene maintenance. There was more inflammatory component in our case. So there was a drastic reduction of GE after scaling and root planing and followed by curettage. To prevent relapse of GE, it is very important to maintain oral hygiene, as well

as it needs oral hygiene education supplemented with positive motivation at the initial stage of the treatment.

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Post Orthodontic Cosmetic Detailing

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Abstract

Post orthodontic cosmetic detailing is perhaps one of the most deceiving and difficult phases of orthodontic treatment. The cosmetic detailing procedures are considered as a part of the total scheme of treatment to enhance the stability and esthetics of the cases. In recent times, a great deal of emphasis is placed on achieving perfect finishing and detailing of the orthodontic treatment, so that the results are pleasing to the eye. This article aims to provide comprehensive knowledge about post orthodontic cosmetic detailing.

Keywords: *Esthetics, finishing and detailing, cosmetic detailing, orthodontic treatment.*

Introduction

Enhancement of facial beauty is one of the primary elective goals of patients seeking dental care. The goal of orthodontic treatment should be the attainment of the best possible esthetic result. Orthodontists enjoy a high level of patient appreciation, partly because their results are almost always an esthetic improvement. Still, many orthodontists finish treatment short of the finest possible esthetic result[1].

The finishing of orthodontic cases can usually be enhanced through restorative techniques such as cosmetic contouring, bleaching, bonding, porcelain laminating, or crowning. Each patient should be educated in advance about these techniques and told that the orthodontist will evaluate the treatment after the appliance therapy and decide what, if any, additional treatment would be beneficial. This should be a joint consultation with the patient's general dentist to ensure that all choices are considered[2,3].

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The following are certain procedures that are considered under the cosmetic finishing:

1. Gingivectomy
2. Gingivoplasty
3. Bleaching
4. Veneers.
5. Porcelain jackets
6. Selective contouring.
7. Bridges and
8. Cosmetic build-ups

Gingivectomy: It is done to eliminate gingival enlargements – aesthetic considerations particularly in the anterior maxilla either by conventional surgery or by electrosurgery. Pockets on each surface of the tooth are explored and marked with pocket markers. Kirkland knives are used for labial and lingual incisions and Orban's knife for interdental incisions. The incision is given apical to points marking the course of the pockets and directed coronally to a point between the base of the pocket and the crest of the bone. The excised pocket wall is removed along with the granulation tissue and any remaining calculus or necrotic cementum. The area is covered the area with surgical pack[4,5].

Gingivoplasty: It is Similar to the gingivectomy technique. Gingivoplasty is reshaping of the gingiva to create physiological gingival contours, with the sole

purpose of recontouring the gingiva in the absence of pockets[6].

Conservative alteration of tooth contours and contacts:

Reshaping of teeth: Rounding of sharp angles–prophylactic measure to stress and to help prevent chipping and fracture of incisal edges is done to achieve youthful feminine characteristics; Rounding the incisal edges, reducing facial line angles, opening incisal embrasures. Opposite characters are considered masculine.

The patient must understand what is involved and just want to have the alteration made. The patient should be explained about the expected postoperative result. Areas to be reshaped should be marked with a pencil. Diamond instruments, abrasive points are used contouring, finishing and polishing[7].

Many orthodontic results can be improved by a slight reshaping, or cosmetic contouring, of the teeth. This can help achieve an illusion of straightness, especially in cases where patients demand early removal of appliances or where particular teeth are impossible to position ideally.

Contouring is useful in compromise treatment. When the occlusion is good but anterior crowding is present, minor tooth repositioning followed by cosmetic contouring will often improve the appearance[8].

Irregularly fractured incisal surface: In these cases, **the** esthetic result can be achieved by slightly shortening incisal edges and reshaping both teeth to symmetrical form. Similar procedures are done to recontour the tooth[9].

Alteration of embrasures: Anterior teeth may have embrasures that are too open, e.g. when laterals are congenitally missing. Facial surface and cusp angle of some canines reshaped to appear like lateral. But in many instances, mesio incisal embrasure remains too open. Composite restorations can be done to enhance the esthetic contour and correct open embrasure. Evaluation of occlusion before restoration determines if the addition will be compatible with functional movements.

Coarse flame-shaped diamond is used to remove overly convex enamel surfaces and to roughen area to be augmented. Wedge with abrasive strips is used in difficult proximal surfaces. Polyester strip is inserted to protect the adjacent tooth during acid etching. After etching, rinsing, and drying the contoured strip is positioned. Either self-cure or light cure material is inserted and the strip closed for polymerisation[10].

Closure of Diastema: The most frequent site of diastema is between the maxillary central incisors. Prominent labial frenum with nonelastic fibers extending proximally is often the cause. Other factors are congenitally missing tooth, undersized or malformed teeth, Bolton discrepancy, supernumerary and heredity. Diastema should not be closed without first recognizing and treating the underlying cause. Occasionally diastema is too large to esthetically close with comp augmentation alone. In such cases, spaces are best redistributed orthodontically among anterior teeth so that symmetrical and equal composite additions can be made[11].

Discolored teeth:

Discolorations are of two types; Extrinsic and intrinsic: Extrinsic discoloration is due to the remnants of Nasmyth's membrane, poor oral hygiene, existing restorations, eating habits and presence of chromogenic bacteria. Most of the surface stains can be removed by routine prophylactic procedures. Some require micro abrasion or macro abrasion[12,13].

Intrinsic discolorations are generally caused by deeper internal stains or enamel defects and are more difficult to treat than external types. It is due to hereditary disorders, medications, excess fluoride, high fever etc. Tetracyclines create the most distracting, generalized, intrinsic stains. Severity will depend on several factors such as–dosage, duration and the type of tetracycline analog. Yellow-orange to dark blue-gray (more difficult to treat) type of staining is the most difficult to treat[14].

White spots: Poor oral hygiene during orthodontic treatment creates decalcified areas. White spots with intact enamel surface–evidence of remineralization–no invasive procedures. Mild discolorations are best left untreated, bleached or treated conservatively with micro or macro abrasion, because no restorative material is as good as the healthy, natural tooth structure. The patient should be told that the gingival tissue never would be as healthy when adjacent to a restorative material as when next to normal tooth structure.

Bleaching treatments: The lightening of the color of the tooth through the application of a chemical agent to oxidize the organic pigmentation in the tooth

is referred to as bleaching. Most bleaching techniques required some form of hydrogen peroxide in different concentrations.

Mechanism of action–Bleaching agents creates oxidation of organic pigments. Generally has a life span of 1-3 yrs, although the change may be permanent in some cases. With all bleaching techniques there is a transitory decrease in the bond strength of the composite is generally expected. Residual oxygen or peroxide residue in the tooth which inhibits the set of bonding resin. No loss in bond strength is noted if the composite treatment is delayed at least 1 week after cessation of any bleaching[15].

Micro/Macro abrasion: It is called Microabrasion as it involved the surface dissolution of enamel by acid along with the abrasiveness of the pumice. Croll modified the procedure by reducing the concentration of acid to 11% and adding silicon carbide particles –'Prema' for improved and safer means of removal of superficial stains. They represent conservative alternatives for the removal of superficial discolorations. As the term implies, the stained areas or defects are abraded away only when stains or defects do not extend beyond a few tenths of a millimeter (0.2-0.3mm) in depth. If the defect persists a restorative alternative is indicated[16].

When should they be used?: The nature and extent of the defect should be evaluated. Non-hereditary developmental demineralization defects (white or light brown fluoretic enamel and the idiopathic white or light brown spots) versus the incipient carious lesion should be properly differentiated. *Incipient caries* usually near gingival margin where abrasion contraindicated (thin enamel). Smooth surface, opaque or chalky white when dried but invisible when hydrated. It is generally reversible by improved oral hygiene.

Developmental discolored spots (opaque white or light brown) are due to unknown local traumatic events during amelogenesis and are Idiopathic. They occur on Intact, smooth and hard surfaces usually in the incisal half. The patient should be informed that an accurate prognosis for microabrasion cannot be given. It will be tried first and if unsuccessful due to the depth of defect exceeding 0.2-0.3mm, the tooth will be restored with tooth-colored restoration. Surface discolorations due to fluorosis can be removed by microabrasion if the depth is within 0.2-0.3mm[17].

Technique–Rubberdam to isolate and protect the gingiva from acid. Prema paste is applied to a defective area with a special rubber cup with fluted edges. Low-speed handpiece, to avoid excessive removal of tooth structure. Treated areas are polished with fluoride-containing prophy paste to restore surface luster. Immediately after treatment topical fluoride to enhance re-mineralisation[18].

Microabrasion: Simply utilizes 12-fluted composite finishing bur or a micron finishing diamond in high-speed handpiece to remove defects.

Care should be taken to monitor the removal of tooth structure.

Air-water spray is recommended – facilitates assessment of defect removal.

It should be emphasized that these techniques involve the physical removal of tooth structure and does not remove stains or defects through any bleaching phenomena. *Microabrasion have b*etter control over tooth removal

Microabrasion*: It is done using* high-speed instrumentation. It is technique sensitive and can have catastrophic results. The procedure is faster and does not require a rubber dam or special instruments. It is easier to remove the defect with Macroabrasion[19].

Veneers: It is a layer of tooth-colored material that is applied to the tooth for esthetically restoring localized or generalized defects or intrinsic discolorations. It is made of chairside composite, processed comp, porcelain, or cast ceramic materials. Veneers are indicated on the facial surfaces that are malformed, discolored, abraded, and/or eroded. *Partial veneers* are indicated for the restoration of localized defects or areas of intrinsic discoloration. *Full veneers are indicated for the r*estoration of generalized defects or areas of intrinsic discoloration. Discolored mandibular anterior are rarely indicated for veneers, because the facio-incisal portions are thin and usually subject to biting forces and attrition.

Conservative bridges: In selected cases conservative bridges can be made by acid etching and bonding a pontic to the adjacent teeth. According to the type of pontic are of four types; Natural tooth pontic. Denture tooth pontic. PFM or All Metal with metal retainers and All-porcelain pontic. They are used for the conservation of natural tooth structure. Because of

the bonded nature of these bridges retention is never as strong as conventional bridges. Patients should be told of potential risks of dislodgement and must be cautioned not to bite hard food[20].

Periodontal Plastic Surgery and Orthodontics: For many years, periodontal mucogingival surgery was employed only to cover exposed roots with soft-tissue grafting. Today, periodontal plastic surgery is used not only to enhance esthetics in a variety of ways, but also to aid in orthodontic treatment.

Periodontal plastic surgery is the term for a procedure that corrects or eliminates an anatomic, developmental, or traumatic deformity of the gingiva or alveolar mucosa. This would include treatment of excessive gingival display, marginal tissue recession, asymmetrical or unesthetic gingival margins, and localized alveolar-ridge deficiencies, as well as exposure of unerupted teeth for orthodontic movement.

If excessive gingival display, commonly called a "gummy smile," or high lip line is caused by insufficient clinical crown length, this can be corrected with gingival contouring.

Root-coverage techniques can be used to correct inadequate attached gingiva, root sensitivity, or an unesthetic appearance. Although the success of root coverage for treatment of canine marginal tissue recession was once unpredictable, Miller has recently developed a reliable technique using a free gingival graft after citric-acid root conditioning. Langer and Langer have described a subepithelial connective tissue graft that improves the esthetics of root-coverage grafting; this technique is particularly useful in treating the recession of canine marginal tissues[21].

Esthetic finishing of an orthodontic case often involves periodontal plastic surgery. The art of periodontal procedures can be used to improve facial esthetics and complement the results of orthodontic treatment.

Conclusion

Post orthodontic cosmetic detailing is probably the most challenging and difficult stage of treatment. In certain cases, it takes much effort for planning the detailing to provide better esthetics. The time spent by individual clinicians in detailed finishing is an important factor in the variation of treatment duration. Orthodontists must keep them in mind and take care of the beginning of treatment to minimize errors in the different stages so that he has to do the minimum amount of work during the actual stage of finishing and detailing thereby providing efficient treatment to his patient.

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Theories of Disease Causation: An Overview

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Abstract

Disease is an important entity in healthcare. In-depth understanding of disease causation aids in planning prevention and steps to tackle the disease in a population. The outlook regarding disease causation has changed considerably with time and advancements in science. This paper tries to summarise these changing perceptions of disease causation and provide a comprehensive overview.

Keywords: *Disease Causation; Theories; Models.*

Introduction

Disease is usually considered the opposite of health, a deviation from the normal function. Webster defines disease as "a condition in which body health is impaired, a departure from a state of health, and alteration of the human body interrupting the performance of vital functions". However, from an ecological view, disease is defined as "a maladjustment of the human organism to the environment".[1]

The key to finding remedies for the cure of diseases, necessitates an understanding of the cause of the disease. But our perception of disease causation has undergone a vast alteration with the ever changing advancement of science and its contribution to health. Our explanation of disease causation is based on these:

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Theories of disease causation:

i. Theories of the pre-modern era
ii. Germ theory of disease
iii. Biomedical model
iv. Epidemiological triad
v. Dever's epidemiological model
vi. Theory of multifactorial causation
vii. Web of Causation
viii. Wheel of disease causation
ix. Other theories
    - Lazaru's theories of stress response
    - Wolf's theory of stress, organ maladaptation and disease
    - Holmes and Rahe's theory of life change and the onset of illness

i. Theories of the pre-modern era: These theories were in play before the existence of microorganisms was established, till the end of the 18th century.

a. The demonic theory: According to this theory, the disease is a result of being possessed by demons, or evil spirits. Hence the sick person

was subjected to sorcery to rid the body of the evil occupant.

b. **The punitive theory:** For a long time,the disease has been thought to be caused as a punishment for any bad deed done, as an outcome of the outrage of Gods. Hence the diseased individual is to appease the deity to rid himself of the disease.

c. **The miasmatic theory:** It is the notion of bad air. Breathing in certain areas was proposed to cause disease, such as air around swamps, marshes and air at night times was thought to cause an array of diseases.

d. **Theory of four humors:** It proposes that the body is constituent of four humor; blood, phlegm, yellow bile and black bile. Any imbalance in these resulted in disease. Blood-letting was considered the most common method of treating disease.[2]

The Ayurveda believes that the imbalance of the three Doshas (tridosha) is the reason behind disease causation. While the Chinese medicine considers a discrepancy between the male (yang) and female (yin) principle resulting in disease.[1]

ii. **Germ theory of disease:** The demonstration of bacterial presence in the air by Louis Pasteur and Anthrax is caused by bacteria by the turn of the 18th century caused a drastic shift in the understanding of disease causation. Thus, focus from empirical causes such as bad air and wrath of God was altered to scientifically plausible causes like the presence of specific microorganisms.

The germ theory implies the causal effect is one to one, i.e. a single microorganism is the culprit behind a specific disease. Eg. Mycobacterium tuberculosis bacteria and the occurrence of tuberculosis. But that seldom is the case, as many diseases cannot be explained by this one to one causal relation but in reality, an interaction of various other contributory factors.[3]

iii. **Biomedical Model:** This envisions the healthy human body as an efficiently functioning machinery, and any resulting aberration as malfunction of the component organs. Eg. Hypothyroidism caused by improper functioning of the thyroid gland.

But the human body seldom functions independently of its surrounding environment. This theory overlooks the complex and summative role of psychological and social attributes.[4]

iv. **Epidemiological triad:** It proposes the disease is an outcome of imbalance of interactions between three essential components, Host, Agent, and Environment. Disease is caused when a susceptible host is exposed to the causative agent, in a compatible environment.

It's a broader concept and overcomes the limitations of germ theory. Intercepting any of the three links provides a means of halting the disease process, thereby highlighting areas for focus preventive efforts.[5,6]

v. **Dever's epidemiological model:** This model highlights the interplay of four factors namely: Human biology, Lifestyle, Environment and Health system. All of which can have a positive or a negative effect.

Human biology includes genetics along with complex physiological systems, factors that are related to maturation and aging. While, lifestyle factors include daily habits, customs, and traditions; environmental factors are all the living and non-living factors that surround us. Health care system is comprised of accessibility and availability of health services.[7]

This model is used to explain those diseases where, rather than a causative organism, harmful living conditions and lifestyle are to be blamed.

vi. **Multifactorial causation:** Pettenkofer proposed that disease is a result of many factors as opposed to germ theory where the idea of a single cause was used. Improvements in public health and medicine brought about a decline in communicable diseases. But other noncommunicable ailments were on a rise which could not be explained based on the germ theory of disease. Hence single cause was deemed to be oversimplifying etiology of a disease where factors such as social, cultural, genetics, and economic factors were overlooked. Also having multiple causes for a disease meant numerous method of preventing that disease. But it was essential to prioritize the sequence of modification of the causal factors to tackle the disease causation.[1,8]

vi. **Web of causation:** Was proposed by MacMahon, Pugh, and Ipsen (1960), who argued that multiple factors causing a disease cannot be explained using a linear causal relation as, there are complex

precursors to each causal component in the chain that have their respective complex interactions that overlap each other. Hence giving the metaphorical appearance of a complicated ***web of interactions*** rather than a linear causal relation.

This web of causation gives each component equal prominence in recognizing determinants and helps in planning interventions. It integrates social factors to biomedical etiological factors.[9]

vi. **Wheel of disease causation:** This was proposed by Mausner and Kramer in 1985. It eliminates the agent as a sole cause of disease, but emphasizes the complex interaction of physical, biological, and social environments. It also introduces genetics into the mix. The outer circumference is divided between environmental factors comprising of social, biological, and physical factors. The core depicts the genetic component.[6,10,11,12] (Figure 1).

Rothman's Component Causes and Causal Pies Model: It proposes that the causation of disease is by a collection of factors, which are represented by a pie.

This model discriminates the causal factors into ***Sufficient and Necessary causal factors.***

Sufficient cause: is a set of minimum conditions, events, or factors that are required to produce a given outcome. These factors that form the ***sufficient cause*** are called **component causes**. A disease can have more than one sufficient cause.[6]

Necessary cause: is a causal factor whose presence is imperative for the emergence of the effect. If the disease cannot occur without the factor being present then the causative is termed as ***"Necessary".***A component that appears in every pie or pathway is a necessary cause.

So, in figure 2, component cause"A" is present in all pies making it a necessary cause.

The disease can be controlled by removing one component from the pies or the removing the factor that is common to all pies.

Eg: Mycobacterium Tuberculosis bacteria is ***necessary*** to cause tuberculosis but cannot cause the disease in isolation, i.e. is not ***sufficient*** to cause the disease. A host of other factors are required such as impaired nutrition or immunity which can be deemed ***sufficient cause***.

Postulates of Disease Causation: Postulates explaining the process of disease causation have been given by Henle and Koch in 1877 and then by Hill and Evans in 1965. Though our perception about disease causation has changed greatly due to advancements in the field of science, these postulates still hold with some modifications.[13]

a. **Henle-Koch's Postulates:** Postulates were formulated by Robert Koch and Friedrich Loeffler. These postulates helped the germ theory of disease gain popularity over other theories in the 1800s. Certain sets of criteria are to be met before a particular infectious disease agent can be accepted as a cause for a disease.

- The microorganism must be found in abundance in the subject suffering from the disease, but should not be found in healthy subjects.
- The causal microorganism should be isolated from the diseased subject and be grown in pure culture.
- The cultured microorganism thus grown should cause disease when introduced into a healthy organism.
- The microorganism must then be re-isolated from the inoculated, diseased experimental host subjects and cultured and identified as the original causative agent.[14]

However, Koch's postulates can't be applied in all scenarios.[15] As in instances of asymptomatic carriers of diseases such as typhoid and cholera or where two or more microorganisms cause disease. Additionally, some symptoms can be brought about by multiple microorganisms.

b. **Hill-Evans Postulates:** Austin Bradford Hill in 1965 proposed criteria for assessing evidence of causation. These supersede Henle-Koch postulates and considered extensions of Mill's Method of inductive inference (mentioned later). Each criterion contributes to the strength of the causal relationship between a factor and disease.[16]

- **Strength:** Stronger the association between the factor and the disease, more likely it is the causative agent.
- **Consistency:** Consistent observations seen in different scenarios and across different populations strengthens the likelihood of the

causal effect.

- **Specificity:** More specific an association between a factor and an effect, greater the probability of the effect.
- **Temporality:** Exposure to the cause should precede the effect.
- **Biological gradient:** Greater exposure should cause a greater incidence of disease. The strength of causality is reinforced when lesser exposure decreases incidence.
- **Plausibility:** A valid scientific explanation of the causal relationship, though limited by current knowledge is essential.
- **Coherence:** The findings should be coherent with the causal argument.
- **Experimental evidence:** When a feasible causal relationship should be demonstrated.
- **Analogy:** Similar effects should be caused by similar factors.[17]

Another method of inferring causal relation is based on Mill's Eliminative Method of Induction. John Stuart Mill put forth five method of experimental reasoning in his paper System of Logic in 1843. If a specific effect (E) is to be investigated to be caused by the presence of certain factors then the following method are used to reason.[18]

1. Method of agreement: looks for factors present in all circumstances where (E) occurs.
2. Method of difference: advises to look out factors that are present when (E) occurs and the factors that are absent when (E) doesn't occur.
3. Joint method: combining the method of agreement and difference, reinforces the causal relationship.
4. Method of residues: if a range of factors are believed to cause a range of effects, with exception of one factor let's say 'C', and there is an effect that cannot be explained by all the other factors, then we could infer that Factor 'C' is the cause for that effect.
5. Method of concomitant variations: If reducing of a factor causes a reduction in the effect and increasing a factor increases the effect, then a causal relationship between the factor and the effect can be established.[19]

Hence, having a clear picture about the causal mechanism of disease, aids in understanding the disease process, its course and treatment planning. Proper knowledge of disease causation also helps in planning epidemiological studies, adjusting for confounding factors, and ensuring a competent method of research.

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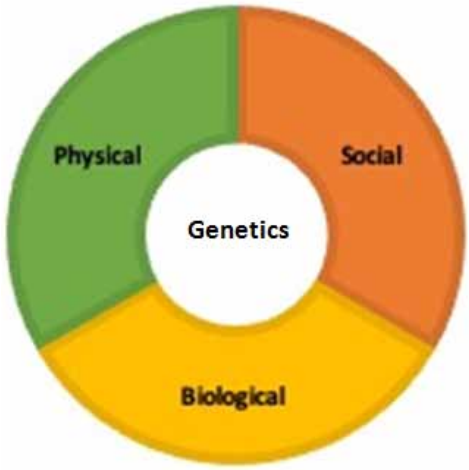


Figure 1. Epidemiological Wheel

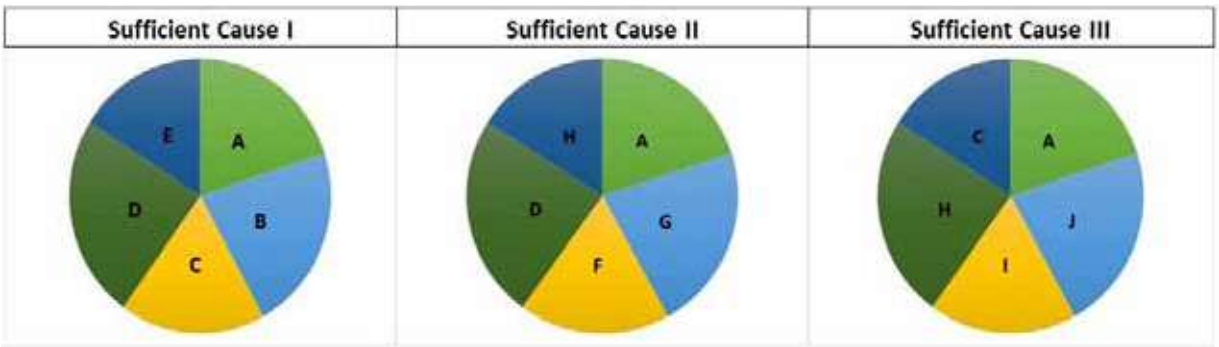


Figure 2. Rothman's Causal Pies

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Dental Negligence During Prosthetic Treatment

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Abstract

Dentists treat patients intending to restore the patient's health. However adverse effects may occur despite all their efforts, some of which could have otherwise been prevented, hence amounting to malpractice. Any patient coming to the dental office needs to be diagnosed accurately first to formulate the correct treatment plan. Its the sole responsibility of the clinician to make the patient aware of the treatment needs and difficulties during and post the procedures. A thorough diagnosis with a proper record of the case history & consent should be obtained. As the dental malpractice lawsuits are extremely, expensive and emotionally draining for the clinician.

Keywords: *Dental Negligence, prosthetic treatment, diagnosis.*

Introduction

Dental malpractice is a form of medical malpractice focussing on injuries suffered during dental visits which range from obvious trauma to dental or oral tissues, negligence by the treating dentist, or misconduct like abuse or molestation of sedated patients. Often dental patients have an underlying systemic disease and are on multiple drugs; lack of proper history taking by the dentist or ignorance regarding the drug interactions or medical complications, even administration of correct dental treatment may still lead to medical emergencies and can be designated as dental malpractice. With advancements in oral and dental treatment modalities, many issues regarding the wrong diagnosis, procedural delays in treatment, improper management and failure in follow-ups have cropped up, comprising dental practice.[1,2] Fixed oartial dentures or crown and bridge are responsible for one of the greatest proportions of negligence claims, damages & legal costs against dentists.

Impression Making: All prosthetic procedures start with impression making and judicious selection of the impression material is the key. Depending upon the accuracy of the structures needed to be recorded the impression material is selected. Here an in-depth knowledge regarding the manipulation and setting times of the various impression materials used is essential. Hypersensitivity to impression materials also needs to be noted in the history taking and then substituted. Any mistake in impression making leads to significant problems in the prosthesis thereby amounting to negligence and malpractice which can be easily prevented by using correct techniques and expertise.[3,4,5]

Diagnosis & Treatment Planning: Prosthodontic treatment requires to thoroughly evaluate every detail from initial patient interaction to the various treatment phases to follow-up & post-treatment care. Problems during treatment could be due to errors during history taking and overlooking of the initial examination.[6]

Articulated casts during the planning of fixed prosthodontic treatment are essential as they provide

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static and dynamic relationships of the teeth. Unmounted casts can give information as to the alignment of the individual arches without providing information about the functional relationships. The diagnostic casts, when attached to an articulator,simulates the jaw movement.[7,8]

Diagnostic Cast Modification: One advantage of having accurately articulated diagnostic casts is that proposed treatment procedures can be rehearsed on the stone cast irreversible changes in the diagnostic procedures are essential when attempting to solve complicated problems.[9] Even the most experienced clinician may have difficulty deciding between different treatment plans.

Diagnostic cast modifications include the following:

1. Changing arch relationship, preparatory to orthognathic procedures when surgical correction of skeletal jaw discrepancy is to be performed.
2. Changing the tooth position before orthodontic procedures.
3. Modifying the occlusal scheme before attempting any selective occlusal adjustment.
4. Trial tooth preparation and waxing before fixed restorative procedures.[10]

On many occasions, it will be necessary to combine two or more of these options. Most treatment planning decisions–whether preparation design, choice of abutment teeth, selection of an optimum path of withdrawal of a fixed partial denture, or deciding to treat a patient with a fixed partial denture or a removable partial denture can be simplified by adhering to these diagnostic techniques.[11]

A logical and systematic approach to diagnosis helps the operator avoid mistakes. Often a completely edentulous patient presents with a resorbed residual alveolar ridge incapable of providing retention for complete denture prosthesis and pre-prosthetic surgery is required.[12] A good treatment plan keeps the patient informed about the conditions, extent, time and treatment costs along with the home care and professional follow-up to achieve success.

Correction of existing disease & Prevention of future disease: Determining and reduction of the initiating factors with the improvement of the resistive factors are crucial to arrest the disease process. Like oral hygiene, instruction tends to improve gingival & periodontal health. Treatment should be directed to correct impaired function primary of mastication, speech and esthetics.[13] Treatment should be planned in such a manner that it shouldn't compromise unwise attempts to improve appearance. They should be advised about the possible adverse consequences of treatment.

Selection of Abutment Teeth: Replacement of missing tooth is done by fixed partial dentures with the use of abutment tooth unless bone support is weakened by periodontal disease.[14]

Cantilever fixed partial dentures have the unilateral side of pontic attached to a retainer. Here forces are tolerated when periodontal structures are directed in the long axes of the teeth. Cantilevers induces lateral forces on the supporting structures leading to tipping rotation or drifting of the abutment.

Assessment of abutment teeth is important before the tooth preparation. Radiographs along with thermal and electrical stimulation of tooth should be done to access the pulpal health. Teeth, when pulpal health is compromised, should be treated endodontically before the denture.[12]

Endodontically treated abutments with post and core have better retention and strength. Failures do occur, mostly on teeth which have shorter roots or less coronal tooth structure.[13]

Unrestored abutments: are the one ideal for the abutment. It is to be conservatively made to obtain good retention & aesthetics. The margins can be made which should contain restorations.

Post- insertion problems: The clinical procedures involved in fabrication and insertion can cause irreversible pulpal damage. Correct techniques should be followed for reduction of tooth, temporary fabrication of restorations, with proper cementation of the final restoration along with the post-insertion care.[14]

Table 1. Symptoms dental negligence during prosthetic treatment

Thermal Sensitivity	Pulpal discomfort from temperature change is frequently encountered following cementation of fixed partial dentures (1) The temporary restoration which don't cover all prepared tooth surfaces, (2) A loose temporary restoration that allows seepage, of oral fluids over the prepared surfaces, (3) A temporary restoration that places excessive occlusal forces on the prepared teeth.[15]
Discomfort during Function	Pains during chewing due to premature centric or excessive contact during eccentric mandibular movement
Gingival Inflammation	Occurs post cementation of prosthesis. This can occur as a result of faulty cervical contour, marginal fit, or embrasure form. Even inadequate oral hygiene aids could cause food retention & inflammation.[16]
Retention of Food	This occurs usually around pontics and connectors. This cannot be completely avoided but proper oral hygiene measures help in removing the food.
Trauma to the cheek or Tongue	Cheek and tongue are the common areas of trauma due to poorly polished portions of the prosthesis. Pontics when placed in areas in which the tongue and cheek were previously not restricted or when flanges of dentures are sharp or cusp-to-cusp or ends -lo-end occlusal relationship without normal horizontal overlap is present. [17]
Sensitivity of Sweets	This occurs when the prepared tooth is not completely covered by the final prosthesis. Sweet sensitivity occurs when the luting agent has undergone dissolution or if the abutment retainer is loose.
Tooth Mobility	Occurs due to poor occlusal relationships, overloading of prosthesis affecting the periodontal ligament and supporting bone causing excessive movement. [18]
Neuromuscular Discomfort	Pain in the temporomandibular joint & associated muscles occurs due to improper occlusion created by a prosthesis. The discomfort caused by occlusal contact on the prosthesis, causing the patient to habitually brings other teeth into a different position. The new mandibular position can create neuromuscular pain in such patients as a result of positional changes in the ligaments and muscles associated with the temporomandibular joint. It must be recognized that other factors may cause pain.[19]
Nonspecific Complaints	Like feels different or slightly uncomfortable this is due to an additional force applied to the abutment teeth, a slight occlusal discrepancy, or simply the presence of an artificial tooth.[20] Esthetics of the prosthesis, Not Cost-effective, Candida infection due to denture stomatitis,improper vertical dimensions causing discomfort with clicking noise, or an unaesthetic appearance. Use of a suction cup in the maxillary denture bearing surface leads to unequal pressure on the hard palate leading to ulceration or even perforation of the palate.[21]

Conclusion

No denture can adequately replace the natural teeth in totality. It can serve in restoring some function and aesthetics but prosthesis however good it maybe does not always satisfy the patient. Moreover, a dentist is not always liable to be sued for malpractice as dental prosthesis involves a lot of lab work which is done by dental technicians and the dentist cannot be only held responsible for the result. Thus, adhering to the most conservative procedure that is in the patient's best interest.

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The Mysterious Chemo-Preventive Player in Oral Cavity: Retinoids

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Abstract

Vitamin A is considered as one of the essential micronutrients which are extensively associated with chemo-preventive and therapeutic modalities. Carcinogenesis is a complex disease where environmental and genetic factors act as a predisposing cause bringing about alterations in the overall cell microstructure. Possible role of Retinoid, which is a form of Vitamin A, has been recognized in suppressing breast cancer, lung carcinoma, cancer of the colon, prostate cancer, blood cancers and to some extent the cancers of the oral cavity. Retinoid is known to act through RAR/RXR receptors to form ligands and thereby affect the transcription process and controls angiogenesis, abnormal proliferation, and metastasis. Some varieties of leukemia have shown better results with Retinoid therapy. However its role in oral premalignant and malignant lesions are not much known. This review discusses the physiologic, epigenetic and molecular role of Retinoids in lesions of the oral cavity.

Keywords: *Retinoids, Oral Carcinoma, Chemopreventives.*

Introduction

Oral carcinoma is a major area of concern that has increased incidence and associated with a 5 years survival rate in 50% of patients.[1] Chemoprevention refers to the term that includes repairing and reversal of the cellular defects thereby preventing the initiation of disease and its further progression into a tumour. The rich source of Vitamin A is vegetables and animal products such as milk, eggs, cod liver oil, spinach, carrot, butter, etc. These are a precursor to retinol that consists of a retinoid molecule family. The extracted forms are retinol, retinal, pro-vitamin A like carotenoids and retinoic acid. Esterification of retinol takes place in the Liver and then it is distributed to the body parts through the bloodstream. Chemopreventive role of Retinoids was first identified by Sporn MB et al 1976.[2]

RXR or rexinoid receptoris a cell membrane receptor and its isoforms are α,β,γ. These isoforms allow swift movement of retinol in the cell as Retinoic Acid (RA). Similarly 9 cis RA, 13 cis RA and all TransRA(ATRA) are derivatives of retinoic acid. RA and Retinoic Acid Receptor (RAR) with α, β, γ isoformsbind together with hormone binding receptors causing gene transcription. Retinoid Acid Receptor β (RAR β) expression has shown alterations in leukemia,squamous cell carcinoma, breast carcinoma, etc.[3]

Immunomodulatory role of Retinoids: Vitamin A levels are maintained at a physiological level to protect the host from daily harmful stimuli thereby providing immunity. In autoimmune diseases the harmony between the T Helper cell (Th cells) and Regulatory T cell (Treg) population is disrupted.

(a) Th17 acts as a mediator for initiating autoimmune responses that help to protect the host from undesirable bacterial and fungal stimuli.

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(b) Treg, a member of the FOXP3+ family suppresses massive effector T cell activation by producing interleukins.[4,5] The role of RA is to directly influence FOXP3+ Treg cells in regulating the ratio between Treg and Th17 thereby preventing any untoward body's reaction to inflammatory responses. These changes cause improved resistance of the host towards the autoimmune disease.

(c) ATRA reduces anti-DNA antibodies. According to *Kinoshita K and Wasserfall C* et al, ATRA is used to treat autoimmune diseases like SLE and type 1 diabetes.[6,7] ATRA is also known to inhibit virus-induced T cell death in HIV positive patients. It down regulates the fasL production and alters the ratio of CD8+ and CD4+ T cells. [8,9]

(d) According to Gupta and Sansoni, et al. age-related changes can be due to a progressive increase in effector/cytotoxic CD8+ T cell population producing IL-2, IFN-γ and TNF α.[4,5]

However,a study by *Mecocci P*et al has shown that elevated levels of RA provide resistance to antigenic load in elderly patients.[10]

(e) T Lymphocytes that originate from thymus cells generate T Cell Receptors (TCRs) that carry an important signal for the maturation of T cell.[11] The survival of T lymphocyte depends upon antigen expression in acute diseases which increases IL-2 and BCL-XL expression and prevents apoptosis in the cell.[12,13]

Retinoids regulate healthy reserve of T lymphocyte by allowing maturation of normal cells and selective apoptosis of defective cells.

Retinoids act synergistically enhancing glucocorticoid-induced death and decreasing TCR–mediated death of thymocytes and T cell hybridomas.[14,15]

Tumor suppressionrole of Retinoids: Interaction of retinoid and its receptors downstream the proliferating tumor cells. They either undergo apoptosis or allow the abnormally proliferative cells to differentiate.

It has been observed that expression of RAR-β is either lost or masked hence resulting in reduced intracellular binding of RA.[16,17]

Similarly, specific members of cytochrome (CYP 450) maintains the concentration of retinoic acids in the cell, for a specific period.

A link between RA signal and carcinogenesis may be established via the expression of enzymes of the CYP 26 superfamily. The anti-proliferative property of retinoids is diminished by the enhanced expression of CYP 26.Oxidative stress on the cell is prevented by extending the half-life of RA intra-cellularly, this is done by CYP 26 enzymes which help in preventing oncogenesis.[18]

Role of Retinoids in Apoptosis: Angiogenesis and neo-vascularization promote proliferation and metastasis of a tumor. Angiogenesis is a known predisposing factor to support the survival of neoplastic cells. It is present in all types of head and neck cancers with increased expression of VEGF. Anti-apoptotic activity of tumors can be associated with disrupted expression of NF-Kβ in carcinomas. Increased NF-Kβ expression is seen in bacterial or viral stimuli that lead to inflammation. Increase in NF-kβ expression means there is increasing resistance of the tumor to treatment. Retinoids act through the mitochondrial pathway to release cytochrome c into the cytosol which allows the caspase pathway to activate and cause apoptosis. According to Chen Z and Di Donato, et al, expression of NF-Kβ is seen due to phosphorylation of IKB (inhibitor of kappa beta) by IKB Kinase (IKK) which allows polyubiquitination and proteasome-dependent degradation of its inhibitors.[19] Then the entry of NF-Kβ into the nucleus permits various gene transcriptions to prevent apoptosis.

Anti-proliferative role of retinoids

Size, stage, size and type of tumor influences the fate and prognosis of a disease. Similarly Preferentially Expressed Antigen in Melanoma (PRAME),that is present in tumors is also expressed.PRAME can trigger cytotoxic T cell-mediated immune response in melanoma and other solid tumors like HNSCC. Usually PRAME is expressed weekly or negatively but in tumours its presence can be exponential. PRAME act as a co-repressor of the retinoid signaling pathway. Epping MT et al concluded that the direct mechanism of action of PRAME is unknown but it might prevent ligand-receptor activation of retinoids by acting as an antagonist that allows the growth of tumor cells and differentiation of malignant stem cells.[20] It also inhibits the action of retinoic acid thereby explains the failure of RA treatment in malignant and premalignant lesions in the literature.

Role of RA in the treatment of various conditions:

Oral Lichen Planus: Oral lichen planus is an autoimmune disease that affects the oral mucosa, nails, skin, and genital mucosa. There is a tendency for malignant transformation to SCC in about 1-2 % of affected patients.[21] Often characterized by the presence of inflammatory mediators like cytokines that interact between keratinocytes and T lymphocytes.[22] It is postulated that gene dysregulation is caused by aberrant productions of the cytokines and causes autoimmune reactions with basal cell apoptosis. Expression of (MHC) class II antigen, human leukocyte antigen DR and variable degree of epithelial thickness are features of lichen planus.[23] Vitamin A deficiency might favour the reprogramming of Treg cells into IL-17. RA treatment regulates Th 17 synthesis by inhibiting IL that further influences Treg cells to regulate peripheral tolerance.[24] The plasticity and phenotype of Treg cell production are maintained by RA which helps in controlling a disease. Cytokines production can also be influenced by local microenvironmental which may be controlled by introducing Vitamin A at early stages to reverse the pathogenesis.

Squamous cell carcinoma: Initiation, progression and metastasis of a complex multistep disease is influenced by genetics and environmental factors. Proliferation of stratified squamous epithelium is limited to the basal and parabasal layer and the maturation of the cell is the sequential process of terminal differentiation. In neoplastic lesions IL-8 and TGF β maintain angiogenesis. Retinoic acid treatment suppresses angiogenesis by neutralizing the IL 8 concentration in cancer cells.Retinoids inhibit the cell growth and GJIC- connected cells in HNSCC.[25]Retinoids exert their pleiotropic effects by binding to nuclear receptors in cells and causing inhibition of cell proliferation, modulation of cell differentiation and enhancement of apoptosis.[26,27]

Leukoplakia/Epithelial Dysplasia: The proliferation of basal and suprabasal cells of epithelium associated with a malignant transformation due to extrinsic factors such as tobacco products and with a high rate of proliferation as 43% are features of Leukoplakia. Ramaswamy G, et al. studied the association of intrinsic factors with leucoplakia, decreased plasma levels of vitamin A, B12, C, β carotene, and folic acid were found in patients with oral leukoplakia.[28]

Use of antioxidants like vitamin A block the generation of reactive oxygen species which further prevent normal cell to undergo genetic aberration thereby may revert the tissue to normal state.[29] Vitamin A supplementation and habit can control the variable degree of epithelial dysplasia. Meir, et al. observed that loss of RAR β mRNA as a result of hypermethylation in premalignant lesions may be restored by application of 13 cis RA. Vitamin A can exert its effects when initial signs and symptoms are noted, but once the disease process is advanced, no satisfactory results could be obtained solely. At present, further studies are still under research to estimate the effect of Vitamin A at the molecular level following treatment.

The molecular modification of vitamin A, aids a role in the expression of growth factors, differentiation of cells, immunity, reproduction and repair of epithelial tissue. In oral tissue, deficiency of endogenous vitamin A may cause defective tooth formation and hypo/ dyskeratosis of oral mucosa due to dysregulation caused during the development of dental lamina and oral ectoderm respectively.

Future Perspectives & Conclusions: This article attempts to review and present the chemotherapeutic effects of vitamin A and its analog as a preventive measure in oral premalignant lesions and oral cancer. Extensive research is being conducted to evaluate the usefulness of the molecule as a chemotherapeutic drug, though promising results are seen in leukemia and high-risk breast cancerous lesion but its usefulness in treating epithelial premalignant and malignant lesion is still under question. The effective use of chemoprevention may decrease the potential malignant transformation but it is yet to be authenticated by long term studies to analyze the effectiveness of retinoids following treatment. Moreover, we still stand deficient to identify the targeted receptor which maintains inter and intracellular homeostasis. Invent of targeted markers has given a promising scope to understand the molecular bases of Vitamin A therapy. Research should, therefore, focus on retinoid molecules that can delay the metabolism of RA inside the cell like RAMBA (Retinoic Acid Metabolism Blocking Agents). Validation of treatment to therapy must be viewed using the latest biomarkers to overcome undue exposure in resistant cases. More clinical studies are needed in this direction for treating epithelial malignancies using retinoids as a targeted epigenetic approach.

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Pemphigus Vulgaris: Oral Pathologist as Sentinal

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Abstract

The word 'Pemphigus' has been derived from a Greek term meaning "blister". Pemphigus vulgaris is a chronic autoimmune vesiculobullous lesion primarily manifesting as intraoral blisters, gradually involving other parts of the mucous membrane and skin. Though the exact etiology is unknown the lesion is characterized by the production of autoantibodies directed against the intercellular substances thus categorizing it as an autoimmune disorder. Early diagnosis and management determine the course of the lesion hence dental practitioners should be well aware of the clinical features of pemphigus vulgaris as early diagnosis is of considerable value since therapy is simplified when the lesion is confined to the mouth. Here we are reporting a case of a 40 year old female presenting with pain and burning sensation in the left retromolar area along with an overview of the disease.

Keywords: *Pemphigus, autoimmune, corticosteroids, immunosuppressants, oral, vesiculobullous, skin.*

Introduction

Pemphigus is a group of potentially life-threatening autoimmune mucocutaneous disorder which is characterized by epithelial blistering affecting the cutaneous and/or mucosal surfaces. Pemphigus vulgaris is an autoimmune disorder with blister formation on the skin and mucous membrane. It has multiple clinical variants. It has a strong immunological and genetic etiology. Generally,the elderly population is affected and in 50% cases oral mucosal lesions are the initial manifestations.[1] 1 to 5 persons per million populations are affected every year. It has a female predilection with female to male ratio being 2:1 and peaks between the 4th to 6th decades of life.[2] Clinically, oral lesions are recognized by the formation of blisters,which ruptures within no time leaving behind painful ulcerations. Predominantly involves the buccal mucosa, lips and soft palate but can also occur in other areas of the oral cavity.[3] Intra-epithelial vesicles, acantholysis and Tzanck cells are the key histopathological features that confirm the diagnosis.[2] We are reporting a case of pemphigus vulgaris diagnosed with oral vesicles as the most initial sign.

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Case report: A female patient aged 40 years resident of Nayagarh, Odisha, came to the Department of Oral Pathology and Microbiology with the chief complaint of pain in the lower left back teeth region for the past 20 days. She had pain during swallowing and also burning sensation on taking food and water. There were no other findings neither on the skin nor on any other mucosal sites. The patient's medical and family history was not significant and non-contributory. The patient had poor oral hygiene.

On general examination, all vital parameters of the patient were normal. There were no extra-oral findings and lymph nodes were not palpable. Intra-oral examination revealed ulceration present on the

oral mucosa extending from left retromolar trigone posteriorly till the anterior faucial pillars (Figure 1).



Figure 1. Erythematous ulcerated areas with ragged border in the left retromolar area.

The lesion extended upwards and backward from the distal of the third molar,ill-defined in shape and covered by pseudomembrane with erythematous surroundings. On manipulation, bleeding from the lesion was positive. There was generalized attrition of teeth and gingival inflammation.

Based on the clinical features differential diagnosis were pemphigus vulgaris, bullous lichen planus, mucous membrane pemphigoid, erythema multiforme, pyostomatitis vegetans, recurrent herpes lesions in immunocompromised patients and pemphigus associated with inflammatory bowel disease.

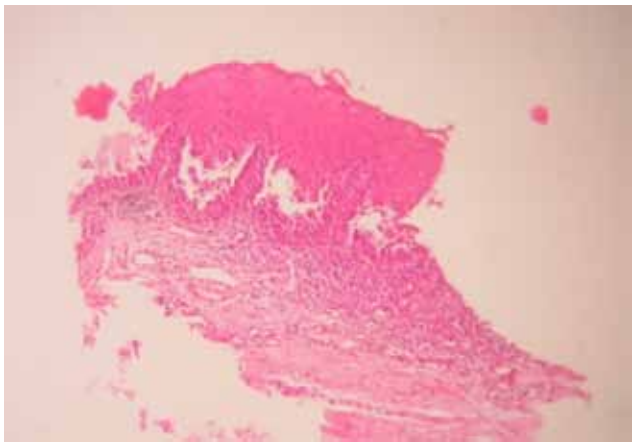


Figure 2. Photomicrograph (4x) shows intraepithelial vesicles and Tzanck cells.

A diagnosis of Pemphigus vulgaris was done after evaluation of the incisional biopsy performed on the perilesional site. The specimens were submitted for direct immunofluorescence study and conventional histopathology. Histopathological examination revealed parakeratinized stratified squamous epithelium beneath which is a moderately collagenous connective tissue stroma. Juxtaepithelial collection of chronic inflammatory cells can be seen in the stroma (Figure 2). Epithelium shows intraepithelial vesicles and clumps of epithelial cells (Tzanck cells) in the vesicular spaces. Tzanck cells exhibit hyperchromatic nuclei (Figure 3).

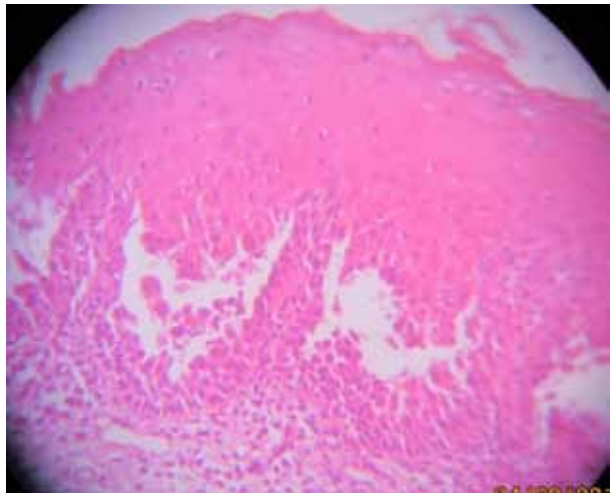


Figure 3: Photomicrograph (10x) shows vesiculation with suprabasilar cleavage and acantholysis and numerous Tzanck cells exhibiting hyperchromatic nuclei.

Direct immunofluorescence (IgG) reveals an immunoglobulin deposit in intercellular areas in a fishnet pattern (Figure 4).

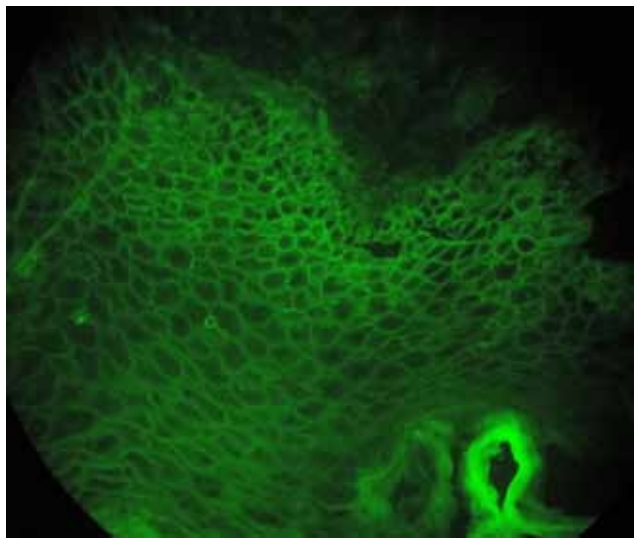


Figure 4. Direct immunofluorescence (IGg): Immunoglobulin deposit in intercellular areas in a fishnet pattern.

Based on histopathological and direct immunofluorescence findings final diagnosis of Pemphigus Vulgaris was given. The patient was immediately put on a combination of immunosuppressive and corticosteroid therapy. Unfortunately the patient was lost for follow up.

Discussion

Pemphigus Vulgaris (Latin: Common) the most common and severest form of pemphigus is a life-threatening vesiculobullous disorder. It is widely recognized that the incidence of pemphigus is higher in Ashkenazi Jews than in any other ethnic group.[4] The estimated incidence is 1 - 5 cases per million people in a year.

The peak age of occurrence is primarily between 4^{th} to 6^{th} decades of life, rarely before puberty. The classical sign is the epithelial blisters/bullae with a very frail wall appearing on cutaneous and/or mucosal surfaces. The bullae rapidly rupture and expand peripherally eventually causing large areas of denuded skin and/or epithelium with ragged edges. The lesions are usually painful. Buccal mucosa is the most common site especially areas of trauma along the occlusal plane. Gingiva and palate are the other common sites of involvement. A characteristic sign of this disorder is this phenomenon called Nikolsky's sign where when lateral pressure is applied to a normal-looking skin, results in the formation of a new lesion.[5,6]

In this case report the patient is a female and is 40 years old presenting with the chief complaint of pain and burning sensation in the left retromolar area. Moreover the patient has the initial intra-oral manifestations first as minute vesicles which rapidly ulcerated. Apart from the left retromolar area there was no involvement of skin or any other parts of the oral cavity. All the above manifestations were in accordance with a provisional diagnosis of pemphigus vulgaris. Histopathological evaluation and by the virtue of direct immunofluorescence the lesion was diagnosed to be pemphigus vulgaris. Because of the vigilance of the oral pathologist and with correct diagnosis immediate therapy was instituted.[7]

The oral lesions are often the first sign (First to Show and the Last to Go) of the disease (in 60 % or more) and they are most difficult to resolve with therapy. Hashimoto and Lever studied the microscopic features of the oral mucosa from patients with pemphigus vulgaris and theorized that initial lesions of pemphigus vulgaris most frequently occur in the oral mucosa because the epithelia demonstrate less intercellular junctions, thus making the area more susceptible to acantholysis.[5,6]

In the pathogenesis of pemphigus vulgaris there is a strong association between genetic and inducing factors. Genetic factors (soil) include HLA class 11 alleles. Predisposing individual factors (seed) that trigger disease initiation include diet, drugs, viruses in association with other diseases and others like smoking, hormone, irradiation and pesticides. The apoptosis theory is a multistep pathogenetic mechanism, first the autoantibodies target keratinocyte membrane acetylcholine receptors leading to activation of apoptosis(cell shrinkage). This is early acantholysis. Anti Dsg Ab targets Dsg 3 and Dsg 1 leading to cell-cell dishesion. This is advanced acantholysis.[4,8]

The ideal site for biopsy is an intact bulla which is rarely encountered and hence biopsies are best taken from the active border of the denuded area. Early changes in this disease consist of intercellular edema and the disappearance of intercellular bridges in the lower part, especially just above the basal cell layer. As the fluid increases the space is increased and a bulla is formed. After the formation of the suprabasal cleft, the intact basal layer remains attached to the lamina propria producing a pattern that resembles a row of tombstones.[9]

The characteristic histopathologic feature is the demonstration of acantholysis, i.e. separation of epithelial cells in the lower stratum spinosum. The separating cells (Tzank cells) present degenerative changes, the cell outlines are round, loss of intercellular bridges and large hyperchromatic nuclei. The underlying connective tissue is densely infiltrated with chronic inflammatory cells. A smear is prepared by unroofing the vesicle and gently scraping the base of the lesion. The smear is dried, fixed and stained with a polychrome stain such as Giemsa. On microscopic examination, a positive smear shows a large number of characteristic acantholytic epithelial cells.[7,8]

Electron microscopy studies indicate that breakdown of the epithelial intercellular substance is the first stage in the development of acantholysis followed by an increase of intercellular space, desmosomal lysis and finally degeneration of the cells. The gradual disintegration of desmosomes and clumping of tonofilaments occurs. As the desmosomal attachment disappears, the cell separates and the cell surface develops numerous microvilli.[6,7]

Direct immunofluorescence technique utilizes the patient's biopsy to demonstrate autoantibodies already fixed to the tissue and in indirect immunofluorescence technique patient's serum is reacted with normal control tissue to demonstrate the presence and concentration of the circulating autoantibodies.[10]

All the vesiculobullous lesions should be considered for differential diagnosis which include bullous pemphigoid, erythema multiforme, bullous lichen planus, dermatitis herpetiformis, aphthous ulcer (when pemphigus vulgaris lesion is small) and herpetic gingivostomatitis. The feature that differentiates pemphigus vulgaris from other lesions in the presence of suprabasillar split whereas pemphigus vulgaris can be differentiated from herpetic gingivostomatitis from the prodromal signs.[11]

Pemphigus Vulgaris was frequently a lethal disease, with most deaths occurring due to electrolyte loss and wound infection. With the advent of immunosuppressive therapy treatment consists primarily of systemic corticosteroids often in combination with immunosuppressive drugs. Pemphigus rarely undergoes complete resolution although remission and exacerbations are common.[1,8]

Conclusion

Some patients with pemphigus vulgaris develop acute fulminating disease, but most cases develop slowly. As oral lesions are first to appear, the oral pathologist should act as a sentinel to keep a check on disease progression before it flares up to give rise to dermatological manifestations.

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Osseodensification: A New Era of Implant Osteotomy

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Abstract

Successful osseointegration is achieved by the primary and secondary stability of dental implants. The primary stability of any successful dental implant is dependent on the quality and quantity of bone. Besides the experience of the surgeon and bone-implant contact (BIC)at the time of implant placement is equally important. Similarly the high insertion torque helps in obtaining primary stability. Several techniques have been introduced to enhance the bone quality and quantity in an attempt to achieve primary implant stability. One commonly used technique is under preparation of the osteotomy site by use of a drill that is one size small than the implant diameter. However undersized osteotomy compromises the healing between the bone and implant and hampers secondary biological fixation. Osteotomes have also been used in an attempt to increase the bone width buccolingually and improve the peri-implant site bone density. However studies have reported that there was only an increase in bone density at the periapical area with no influence on the lateral margins and primary stability of the implant. The associated trabecular fracture with osteotome bone condensation may further damage the osteocytes and cause extension microfracturesand delayed healing. Finally,the success of implant therapy relies largely on the stability and mechanical durability of the prosthesis delivered to the patient. As such several modifications, reinforcements and techniques have been suggested over the years to optimize the same. The latest in line is the non-subtracting osteotomy by specially designed Densah burs. This article aims to review the theory, advantages and disadvantages of this technique as compared to traditional osteotomy.

Keywords: *Osteotomy, Osseodensification, Dental Implant, Implant stability, Densah burs.*

Introduction

With the advent of recent technology and science owing to the increase in patient awareness there is a remarkable rise in expectation of the patient regarding the final aesthetic outcome and longevity of all dental treatment especially in Implant dentistry. The most important mandate in implant placement is the increase in the stability of the implants. That in turn depends on primary and secondary stability. Osseointegration i.e., structural and functional connection between ordered, living bone and the surface of a load-carrying implant,is crucial for the predictability of the implant.[1] Osseointegration provides secondary stability and largely depends on the primary stability. Primary stability is obtained by the mechanical friction between the external implant surface and walls of the implant osteotomysite.[2] The factors playing a leading role in achieving optimum primary stability are implant material, implant surface,and implant design, host factors and implant surgical technique respectively.[3] Ultimately it is dependent upon an increased implant torque value and a decreased micro-motion between the implant surface.[1] The insertion torque is directly proportional to peri-implant cortical bone and high insertion torque ensures an increase in the initial bonetoimplant contact percentage (%BIC).[4] To achieve

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this,various surgical techniques have been proposed such as smaller osteotomy sites, tapered preparation and hand instrumentation.

Condensation of the osteotomy site to increase primary stability by increasing the bone density was reported for the first time by Summers in 1994.[5] He advocated condensing the cortical bone of the implant site by using osteotomes. There is however the disadvantage of limited accessibility and instrumentation by osteotomes. In 2013, Huwaiset aladvocated a new technique for densification of the cortical bone using a specialized bur.[6] This article further aims to review the feasibility of the new Osseodensification burs for implant site preparation and its advantages over conventional techniques.

Osseodensification–innovative approach to implant osteotomy: Traditionally implant osteotomy relies upon excavation of bone from the planned site using bone-cutting burs. It is often limited by the inherent density of the bone especially in the sites where extraction socket has not been preserved and by the surrounding anatomical limitation.In 2013**.** proposed a novel surgical protocol that relied on a biomechanical, non-excavation osteotomy preparation by burs specially designedfor densifying the adjacent cortical bone called Densah burs (by Versah LLC- Company).

In Osseodensification the osteotomy site is prepared with more precision and continuous irrigation decreases the heat production protecting the osteogenic potential of the cortical bone. It is a new method of bone preparation biomechanically for the placement of dental implants. Low plastic deformity of bone is created by sliding and rolling contact with the fluted bur that density the bone with minimum heat. Huwais 2013 developed this as a bone non-extraction technique where specially designed Densah ™ bur densify the bone while preparing osteotomes.[6]

Osseodensifying drills design allows preservation of bone by compacting it at the osteotomy site by rotating in a reverse direction at 800 to 1500 rpm.[1] This approach for implant site osteotomy is called non-subtractive drillingwhich increases the bone density in the peri-implant area and implant torque which in turn ensures a decrease in micro-motion and increase in implant mechanical stability.[7] It is also reported that it helps in bone expansion enabling insertion of wider diameter implants into a narrow ridge without creating bone dehiscence or fenestration.[8] Densifying burs thus combines the advantages of bone condensation by osteotomes with the speed and tactile control of the drills.[2]

Table 1. Showing the difference between Traditional osteotomy and Osseodensification

Sl.No	Property	Traditional burs	Densah bur
1	Bone excavation	Bone preservation, Non- excavation method that allows compacting the autograft while inducing minimal trauma	Bone cutting and excavation of the same at the implant site is involved.
2	Bur design	Tapered, greater than 4 lands and flutes with a tip to eliminate potential chatter.	Twist drills with 2-4 lands to guide it through the osteotomy site
3	Osteotomy	The preparation is precise with a diameter, 0.5 mm lesser than the traditional drills.	May not always be a precise circumferential osteotomy, i.e., it may be elongated or elliptical due to chatter on the drills
4	Heat generation	Bur used in bouncing pumping action and along with copious external saline irrigation, ensuring minimal rise in temperature	Greater friction leads to greater heat production
5	Insertion torque	Increased	Decreased
6	BIC%	Increased	Decreased
7	Narrow and short ridges	Facilitates expansion circumferentially	Often leads to fenestration, dehiscence

Bur design and technology: The Densah burs cut the bone in a clockwise manner and densify the same in a non-cutting anticlockwise manner along with copious irrigation during the surgical procedure. The design incorporates helicalflutes separated by lands, each having a burnishing face and an opposing cutting face withina taperedgeometry.[9] This ensures lesser heat productionand a faster feed rate. Bone condensation is obtained by non-cutting action of the lands with a negative rake angle and cutting is obtained by chisel edge. The expansion of the osteotomy site is obtained by a tapered shank (maximum diameter adjacent to the shank and minimum diameter adjacent to the apical end). This feature allows the operator to lift away from contact instantly to allow for irrigation. The bur producesan outward pressure that creates a hydrodynamic compression wave when combined with irrigation at the point of contact. This drives the bone chips and debris into the implant bed rather than removing it.[1]

The principle of working depends upon the plasticity of the tissue and its water content.[2] Hence it is recommended to be activated by an 'in and out' motion at the site of osteotomy to create a rate dependent stress and strain that increases the plasticity of the tissue.It was reported in a study that after osteotomy, when left empty for imaging, the diameter is reduced by 91% of the bur diameter. This is explained by the spring back phenomenonof the compacted bone brought on by the residual strains of viscoelastic deformation.[9] This creates a compressive force against the implant and increases bone-to-implant contact maximizing the primary stability. This in turn promotes osteogenesis by a mechano-biologic healing process.

Bone density and Osseodensification: Secondary stability of bone and dental implant by the process of osseointegration is vital for the success of implant placement. The maxillary posterior region with low bone density affects histomorphometric parameters such as the percentage of bone-implant contact and bone volume, However, the increase of bone density by osseodensification has improved the negative effect thereby having a potentiating effect on secondary stability.[10,11]

Primary stability and Osseodensification: The most critical factor in immediate loading implants is the primary stability that helps to achieve osseointegration. Studies by **Berardini** et al and **Li** et al reported no difference between crystal bone resorption and failure of implants inserted with high or low torque respectively reported that only drills could increase the percentage of BIC And BV in poor density bone compare to convention osteotomy.[12,13]



Figure 1. Densah bur

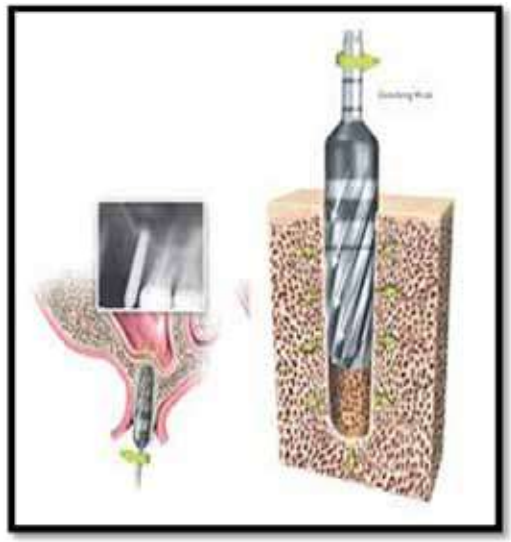


Figure 2. Drilling Mechanism



Figure 3. Drilling kit

Factors leading to decrease in insertion torque in traditional drilling:

- Traditional osteotomy lacks precision due to the chatter of the drills leading to elongated and elliptical osteotomy sites.[10] (10)
- One of the crucial factors influencing the osseointegration is the heat generation during rotary cutting.[11] This rise in temperature is caused by plastic deformation and friction at the machining face. It also affects the viability.

Advantages of Osseodensification:

- It is a bone preservation osteotomy site.It creates a crust of bone mineral density increased by three times around the osteotomy site as compared to traditional drilling.[2]
- The protocol increases the diameter of the residual alveolar ridge without causing any dehiscence or fenestration.[8]
- The non-cutting, condensing action enables the clinician to autograft the maxillary sinus and increasing the height without the necessity of another surgery and enables placement of total implant length.[2]
- Due to a decrease in micro gap and micro-movement healing is faster and more predictable.[1]
- The traditional drills remove and excavate bone during implant site preparation, induce fracture of trabeculae leading to delayed bone growth. (1) Osteotomy sites prepared by Densah burs heal faster and uneventfully.[9]
- The burs provide advantages of both osteotomes where both tactile control and the speed of drills are combined.
- Osteotomy Densah burs preserves and condenses the bone through compaction autografting whereas the standard drills cause factor of trabeculae that requires long remodeling time and cause delaying secondary implant stability.
- Osseodensification preserves the bone bulk increase the density of bone and certain healing period. Whereas the conventional drills to lead the healing period to more than 3 months. The specialized Densah burs with steady external irrigation drills with high speed in anticlockwise direction. This causes a rate depended on stress to produce a rate dependent stress such as a pumping motion causing increased bone plasticity and bone expansion.[13]
- Ridge expansion with osseodensification maintains the integrity of the alveolar ridge and shortens the waiting period of the restorative phase.[14]

Contraindications of Osseodensification: In dentistry use of technology and the use of smart devices makes the life of a dental practitioner easier.[15] However, every technique has its disadvantages. This principle does not work in the non-dynamic cortical bone which lacks plasticity. Further, owing to its inherent difference in the biomechanical properties, the process does not work predictably on xenografts should be avoided because they behave biomechanically different (not viscoelastic) than the bone tissue.[1]

Conclusion

Modern dentistry focuses on techniques that ensure maximum durability and aesthetics as well as preserves as much viable tissue as possible. In this, osseodensification by Densah burs guarantees an advantage over traditional osteotomy in no uncertain terms atleast in theory. But, it is a relatively new concept and the biological implications on a long term basis are yet to be evaluated by longitudinal studies.

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Medico-legal Aspects in Dentistry

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Abstract

Dentists as healthcare professionals have a duty and standard for patient care. Any violation in the duty and practice makes dentists liable to negligence & legal actions. Law protects the dentists as well as ensures ethical practice. With the steady increase in the number of dental graduates and decrease number of job facilities,the dental profession seems to descend of its ethical standards. So a thorough knowledge about the medico-legal aspects is mandatory for smooth execution & delivery of productive services. In this paper we review comprehensively different aspects of avoiding negligence.

Keywords: *Negligence, Medico-Legal, Ethics.*

Introduction

Law regarding health care workers has been accepted in many advanced nations but is still in its early stages amongst a few. With advancement there is an increase in the awareness of the rights of the patients, so it should be mandatory for dentists to know the law and prevent from being a victim of unfair legal proceedings.[1] It is fair on dentist's part to follow ethical practice and obtain patients consent before any dental procedures or any research including human.[2]

But dentistry has degenerated with its ethics as the number of graduates has been increasing with lesser job perspectives and budding dental clinics in India making it more of a business set up. There has been no record keeping, no consent and negligence amongst a lot of practitioners as we are ignorant about the medico-legal concerns.[3]

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Ethics: Ethics technically deals with the professional code of conduct. These rules every dental student should learn before graduating. They affect every resolution made at the dental office that could be the association with the patient, staff or other professionals. It's the fundamental objective to furnish excellent services to the patients. It also prevails around the human values, responsibility, culture, ideology and morality.[4]

The most common ethical issues that have been reported are deficient sterilization and waste disposal in dental clinics, poor knowledge and inadequate orientation of ethics with incompetence for dental procedures, expensive treatment, lack of consent, lack of harmony about procedures among fellow dentists, Conflict in Advertising, clustering of dental clinics in a particular area, the difference of opinion about the treatments among dentist as well as a patient along with improper record keeping all of it stands to unethical practice.[5]

Principles of ethics:

Patient autonomy: Dentists at all times should regard the self-resolution & privacy of the patient. They are righteous to take decisions for their treatment. Therefore to keep the respect of patients decision a informed consent is mandatory. The Dentist should explain the treatment options its pros and cons along with

other treatment facilities available thereby respecting the final decision of the patient. [6]

Non-maleficence: It states to not harm the patient. Dentists should reconsider procedures which solves one issue and begin a new one, in such condition the dentist should evaluate the treatment by keeping in mind the advantages and disadvantage of the procedure. At any point in a procedure if a dentist feels a lack of skill he shouldn't hesitate to call a specialist.[7]

Beneficence: It directs to aid in doing good. It should be inculcated in daily practice to provide benefits to the patient. Like timely adequate functioning of the patient with good esthetic treatment as the patient desires.[8]

Justice: It states equity. It is the moral responsibility to be legitimate in all the dealings with patients, staff & society. There should not be any type of racial, gender, caste, creed discrimination. [9]

Veracity: It is the truthfulness and respect of the self-esteem of the relationship with the patient. This involves trust in maintaining the intellectual, billing, advertising integrity of the patient. [10]

Confidentiality: It is the right of the patient to expect that all communications and case records related are confidential. And the dentist must respect it.[11]

This also includes professional conduct and etiquette which should not include any kind of commercialism. With the difficulties dentists face these days one should succumb to gifts, Continuing Dental Education programs, trips, etc. Even not provide substandard treatment to patients, refer cases to specialists and not fear of loss of patients.[12,13]

Consent: Consent is for safeguarding the rights of the patient as well as the dentists. It helps in better exercise of patient's autonomy. It also demands a mature decision by the provider and safeguarding his interests. Audiovisual aids could be used to teach patients about treatment procedures thus enhancing informed consent. [14] These provide a considerable proof and documentation although its been categorized under the following:

- **Competence:** It states that the patient suffices with the ability to understand the treatment completely also with its limitations though this depends upon several factors like age,educational qualification, intelligence, cognitive function, locus of control and anxiety levels. [15]
- **Voluntariness:** In this the patients agree to the treatment without and compulsion
- **Information and knowledge:** It means that sufficient comprehensible information is disclosed to the patient regarding the nature and consequences of the proposed and alternative treatments.[16,17]

Types of Consent:

1. **Implied Consent:** Refers to when a patient is unassertive about the consent. This doesn't need to be documented but is understood by an excellent communication where the dentist provides the details of procedures.[17]
2. **Verbal Consent**: here verbally the patients agree to certain routine diagnostic procedures following a well-documented record of the procedure. [18]
3. **Written Consent:** This is mandatory for procedures that involve risk to life like sedation, administration of anesthetics, restorative & endodontic procedures along with the surgical procedures & administration of medications. [19]
4. **Implied Consent:** The patient comes to the dentist suggests that he is willing to undergo the procedures.
5. **Expressed** Consent or tacit consent is a strategic consent which is written in the most pleasant languages.
6. **Informed Consent**: It is expressed in the patient's language about the diagnosis, treatment, advantages, limitations, etc.
7. **Proxy Consent:** When consent is given by parent, guardian or close relative
8. **Blanket Consent**: It's a printed consent that involves anything and everything a dentist or the hospital can do to a patient.[18]

Informed consent should be such that should specify the treatment plan, the outcomes along with the benefits and limitations, any possible risk of the materials used, the treatment alternatives along with the cost of the procedures & consequences of treatment. This should be immediately computed with the patients & dentists signature and date. Special care should be taken to have a readable signature and name. [17]

Consent can be not considered in cases of emergencies, compulsory drug screenings, procedures related to mental disorders & when the court of law has directed for any treatment. In the case of minors

the consent is obtained from the parents or if any legal guardian as they are not mature enough to decide their well-being. If the adult accompanying the child is not a legal guardian they are not allowed by the court of law to decide such cases a telephonic consent may be obtained.[18]

Negligence: Negligence is the lack of proper care and attention or guiltyof carelessness. It is in general violation of duty by the exclusion of something that judicious person wouldn't do. The Supreme Court also has put forward the four components of negligence which are as follows:

Duty: Is the trust that is placed in the dentist and patient relationship. The dentist owes a duty for his patient the moment he agrees to treat. This doesn't happen when the dentist doesn't agree to treat in case of any emergencies or so. [14]

Dereliction of duty: Occurs when the dentist is negligent while performing the procedure. It consists of a series of procedure that produces an injury and without which the injury would not have occurred.[15]

Damage: The damage caused due to dereliction and is judicious. In such cases the patient can sue the dentist for remuneration.

Direct Causation: Damage must results directly from the carelessness and not from the other reason.[18]

Types of Negligence:

1. **Doctors negligence:**

   (a) Civil negligence: This happens when the patient demands money as compensatory damage. Here both the dentist for a fee as well as the patient can file a case. These cases are dealt with in the civil court or consumer courts. As per the court directive as punishment the dentists have to pay the compensation without any criminal proceedings. Sometimes even the dental council is involved for temporary or permanent removal of name from practice. But in either case, the patient has to prove negligence against the dentist .[19]

   (b) Criminal negligence: This happens when a case is registered against the dentist for negligence. If guilty dentists is prosecuted under various sections of Law.

Table 1

Section 304A (IPC)-	Negligent homicide. Act that could lead to the death of the patient. Penalty- imprisonment of 2 years, or fine (any amount) or both.
Sec 336 (IPC)-	Jeopardizing the life of the patient even in the absence of injury Ex. an in case of medical histories like heart disorders where the patient is at risk without a prophylactic antibiotic or discontinue use of Asprin. Penalty– imprisonment of 3 month, or Rs 250 fine or both.
Sec 337 (IPC)-	Simple Injury during dental procedure like pain and swelling post-extraction Penalty – imprisonment of 6 month, or Rs 500 fine or both
Sec 338 (IPC)-	Serious Injury during procedure like fracture of jaw during extraction Penalty Prison for 2 yrs or Rs 1000 fine or both

2. **Patient negligence:** Here the patient is negligent. The patient is not responsible enough to give a proper history which in due course of proving fatal. The patient is not taking medications or following instructions properly in such cases dentists have to prove the negligence of the patient.[20]
3. **Contributory negligence:** Here both the dentist and the patient are negligent but the burden is on the dentist. It is usually dealt with in the civil courts and doesn't contribute to any criminal charges.[21]
4. **Composite negligence:** Here the injury is caused by combined negligence of two or more dentists. The claim can be divided by the dentist or if the patient claims from anyone then they are liable to pay. [12]
5. **Corporate negligence:** Here the dentist is not responsible but the concerned corporate or the hospital is responsible eg. Use of outdated instruments or medications at a hospital.[22]

Conclusion

Dentists are unaware of the laws regulating their profession. In this article we briefly see the importance record-keeping as any of the notes, radiographs, photographs or models & casts are the only available proof while we are defending ourselves in a negligent lawsuit. The legal proceedings are strenuous and upsetting tonegotiate so it's always better to prevent. Once a legal lawsuit is filed it not only becomes a financial burden it drains one emotionally and psychologically.

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Suction Cup on Denture: A Case Report

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Abstract

Complete Dentures with perfect retention are complicated to attain and several method have been applied for obtaining the same the same. Ill-fitting dentures are due to residual ridge resorption causing difficulty while eating and even dislodgment during talking causing embarrassment for the patient. Suction cup was widely used for retention & stability of dentures but is said to be associated with complications like palatal perforations and oro-antral communications. Here we present a case report of denture stomatitis & palatal perforation due to the suction cup.

Keywords: *Denture stomatitis, Suction cup, Palatal Perforation.*

Introduction

Denture Stomatitis indicates an inflammatory process in the denture bearing mucosa. It is also seen in individuals who do not follow proper denture hygiene while using a complete or partial removable prosthesis. The most common site is the palatal mucosa and not mandible due to the cleansing properties of saliva. In fabricating a complete denture, retention is very important that depends on several factors - Anatomy like size & quality of denture bearing area, parallel ridge walls, Physical factors like adhesion, cohesion, interfacial surface tension, capillarity, atmospheric pressure & gravitational forces all play important role in stability and retention of the denture.[1,2]

The primary difficulties experienced during denture usage are discomfort, pain, usual noises like clicks and snaps chewing difficulties, change in phonetics and dislodgment of the prosthesis while talking or chewing.[3] To withstand these issues suction cups were used. But continuous use of these suction cups destroys the tissues even leading to perforation of the palate. They create a negative pressure on the mucosa leading to a destructive effect on the palatal tissues. This occurs as it reduces the blood circulation of underlying tissues leading to hypoxia and necrosis of the underlying bone leading to tissue perforation.[4] The pathological changes are severe with the habit of continuous denture usage for 24 hours a day. But suction cups are used when the diameter is < 1mm and has no risk of irritating the tissues/building up bacteria.[5]

Case Report: A 65 years old male patient-reported to the department of Oral Medicine & Radiology with a complaint of missing teeth in the lower jaw for 2 months. He was all right when mobility of teeth started eventually leading to its loss. Some of it were lost on its own & few were extracted. Past dental history suggested the usage of a maxillary denture since 2 and half years with non-contributary medical & family history.

The oral hygiene of the patient was moderate. He cleanses his mouth using his finger and warm saline water. Denture hygiene was bad as he wore the denture overnight and Cleaned it at morning in water. The patient was moderately built with normal gait. Blood pressure and respiratory rates were under normal limits.

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On Examination: Well defined, circular; punched epithelial indentation measuring 2x3 cms approximately resembling a suction cup. Extending from palatal rugae to 2 cms anterior to the soft palate anterior posteriorly, 2cm medial to the edentulous ridge concerning 15 & 25 mediolaterally i.e. center of the palate. [Figure 1,2]



Figure 1: Palate with suction cup indentation

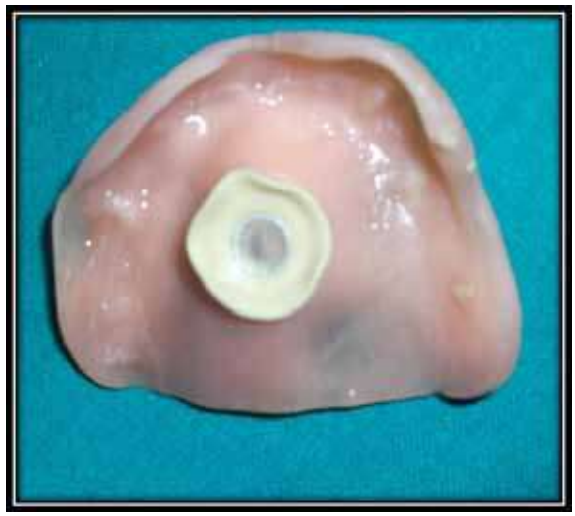


Figure 2: Maxillary denture with the suction cup

Mucosa overlying appears erythematous interspersed granular erythema in the center and posterior half of the hard palate [Figure 1,2]. Mucosal Hyperplasia in 13 & 24 is present measuring approximately 1x1cm at the extension of the denture flanges. [Figure 3,4].



Figure 3. Traumatic Denture Flange



Figure 4. Mucosa Hyperplasia

Provisional diagnosis of Completely Edentulous maxillary and mandibular arches, Denture stomatitis & Epulis fissaratum 13 & 24 was given another line. Orthopantomogram revealed impacted 38 and smear report revealed multiple candida hyphae (Figure 5).



Figure 5. Orthopantomogram reveals impacted 38

The patient was advised to discontinue the use of denture & fabrication of new dentures was done. Application topical anti-fungal agent (Candid 4 times daily for 14 days) & gum paint to be massaged on the palatal mucosa.

Discussion

Denture stomatitis is an inflammatory process of mucosa in patients with complete or partial removable dentures. Its classified into Type I: simple inflammation or pinpoint hyperemia. Type II: diffuse erythema involving a part/entire denture bearing area, Type III: Granular with inflammatory papillary hyperplasia.[6,7] All the patients with dentures are to follow the oral & denture hygiene instructions meticulously & other local factors that promote the growth of yeasts. [8]

The denture hygiene is maintained by brushing it with soapy water, warm saline and soaked at night in an antiseptic or chlorhexidine without any metal components or sodium hypochlorite solution of 0.02% concentration can also be used.[9] Fabrication of new prostheses should be made in cases of ill-fitting or overhanging flanges. An adequately polished and glazed denture reduces the poresin the denture reducing the contamination by oral microorganisms.[4] Inflammatory papillary hyperplasia or the epulis fissaratum is to be excised by scalpel, cryosurgery, electrosurgery or laser beam along with antifungal medications.[10]

Conclusion

The prosthetic construction of patients is essential in restoring the oral health of completely edentulous patients. Fabrication of the suction cup to enhance the retention of maxillary denture is contraindicated because of its potential to cause palatal perforation. Adequate retention should be obtained by the proper record of the posterior palatal seal and close contact of the denture base to the underlying tissues.

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Antibiotics Use in Dental Practice: A Review

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Abstract

In dentistry, indications of antibiotics are determined by multiple clinical and bacteriological epidemiological factors. Treating odontogenic and non-odontogenic infections, for local infections, anti-microbial coverage is recommended. These act as an adjunct to dental treatment. The paper reviews the indications, usage, dosage of antibiotics, precautionary measures to be taken while prescribing them.

Keywords: *Antibiotics, Dentistry, Indications, Dosage.*

Introduction

In the year 1929, antibiotics were discovered by the "Scottish bacteriologist Alexander Fleming" while working in a teaching hospital in London on anti-bacterial action of the Penicillium species. In the 20th century, antimicrobialsare considered as theutmost contribution. The word "Antibiotics" is derived from the word "Antibiosis". This term was coined by Louis Pasteur in the year 1889 which suggests that life could be used to eliminate life. These are nowadays considered as a wonder drug in dental practice for numerous issues like periodontal, mucosal infections, etc. These are used both for treatment as well as prevention of the infections. According to studies, "Dentists prescribe between 7% and 11% of all most common antibiotics such as– metronidazole, tetracycline, beta-lactam, macrolides, clindamycin, etc." These are also associated with multiple unfavourable side effects like gastrointestinal issues to anaphylactic shock. Here in this review, we will be discussing the role, dosage, usage, indications associated with antibiotics in the field of dentistry.[1]

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Classification[2]:

"According to the mode of action, classified as follows:

1. Antimetabolite action – Sulphonamide, Trimethoprim
2. Cell wall inhibitor – Penicillin, Cephalosporins, Vancomycin
3. DNA gyrase inhibitor – Ciprofloxacin, Ofloxacin, Nalidixic Acid
4. Bacterial protein synthesis inhibitor – Tetracycline, Clindamycin, Aminoglycoside."

An ideal antibiotic should be bactericidal rather than bacteriostatic, should be selectively toxic to the microbes and spare eukaryotic cells. It should be soluble and remain active for a long duration to be effective enough. It should not create host resistance and allergies in the host It should be safe for the gut microflora during a long-term prophylactic usage. There should be minimal drug interactions recorded for an ideal antibiotic.

Particularities of Antibiotics Use in Dentistry: Clinician's antibiotic prescriptions are empirical as the microorganism responsible for the infections such as pus

or exudate is not known. Abroad range of microorganisms are encountered in the oral cavity; a broad-spectrum antibiotic is typically prescribed. Sometimes two or more combinations of antibiotics are prescribed due to the increasing antibiotics resistance among bacteria.[2]

Antibiotics Indications: Antibiotics are considered as an adjunct to numerous dental procedures. They are used in treating immune-compromised patients, systemic infections and rapidly progressing infections.

1. **Antibiotics used for odontogenic infection:** "Penicillin" is the drug of choice as it is susceptible to gram-positive aerobes and anaerobes which are found in periodontal & gingival abscess and pulpal necrosis. Clavulanic acid in combination with penicillin acts miraculously for infections caused by "Streptococcus, Pneumococci and Streptococci." Clindamycin 300 mg is preferred drug for patients with penicillin allergy followed by "Azithromycin" and "Metronidazole", for delayed type of hypersensitivity reactions caused by penicillin, Cephalosporins are advisable. Tetracycline is most effective in cases of acute necrotizing ulcerative gingivitis as these are considered bacteriostatic antibiotics that obstruct the binding of aminoacyl-t- RNA synthetase to ribosome acceptor site. In 3% - 5% of the population, the side effects noted by the use of penicillin are hypersensitivity reactions. Cephalosporin and tetracycline are also considered to have hypersensitivity reactions after penicillin.

   In endodontic infections, azithromycin showed excellent pharmacokinetics. "A loading dose of 500 mg followed by 250 mg once daily for a week should be followed".[6] Ciprofloxacin has also shown positive results in treating endodontic infections. Against anaerobes, metronidazole, a synthetic anti-microbialagent is considered effective. With "a loading dose of 1000 mg followed by 500 mg six hourly for a week is recommended". However, for odontogenic infections, beta-lactam antibiotic is always recommended.[6]

2. **Antibiotics for non-odontogenic infections:** As these infections require prolonged treatment, a newer synthetic antibiotic namely fluoroquinolones are used for management. It is a broad-spectrum antibiotic and restricts bacterial DNA replications. It is indicated for "bone and joint infections, genitourinary infections and respiratory infections". Chronic infections like tuberculosis require long-duration antibiotic prescriptions such as – "Rifampicin, Ethambutol, Isoniazid, etc. Penicillin G Benzathine is effective in the treatment of syphilis.

3. **Antibiotic prophylaxis in preventing infective endocarditis:** Standard regimen for children and adult include high doses of amoxicillin one hour before any dental procedures. An effective dose is considered as 2 g of amoxicillin for adult patients before any dental procedures.[6] First-generation cephalosporinsare recommended for patients having an allergy to penicillin or amoxicillin. Patients having prosthetic heart valve, "Vancomycin & Streptomycin" are prescribed for the prevention of infective endocarditis. Negligence of the antibiotic prophylaxis during the dental procedure may lead to subacute bacterial endocarditis.

4. **Antibiotics in treating local infections:** Surgical procedures such as an impacted third molar, implant placement, orthognathic surgery, benign tumor surgery etc are regularly covered with systemic antimicrobials. Studies reveal postoperative pain after third molar surgery has reduced with antibiotic prophylaxis. Abu-Taa et al reported a marked reduction in the post-operative discomfort as he compared "the benefits of pre and post-operative antibiotics prophylaxis in the third molar surgery."[8]

Danda et al documented clinically significant results when he evaluated "the prophylactic value of single-dose antibiotic prophylaxis to single day antibiotics for postoperative infection in patients undergoing orthognathic surgery".[9] Numerous studies reported a significant reduction in implant failure under appropriate antibiotic prophylaxis. Rizzo et al reported an efficient reduction in post-operative infections after analyzing five hundred twenty-one dental endo osseous implants, those were placed under antibiotic coverage.[10] But on the contrary, "Gynther et al revealed that there was no significant difference noted after dental implant placement without any antibiotic coverage for the patient".[11] On the other hand, Nabeel Ahmad et al conducted a literature review. He reviewed the effects of antibiotics among eleven thousand four hundred and six endo osseous implants and reported fairly no significant advantage from the use of antibiotic prophylaxis during the placement of implants.[12] So, the use of antibiotics in implant surgery remains controversial. But during periodontal surgery, the use of antibioticsis compulsory as the surgical site is often contaminated with microorganisms.

Immune compromised patients are more prone to bacteraemia which further leads to septicaemia.[13] The dental procedures should always be performed under antimicrobials coverage. For patients with uncontrolled diabetes mellitus, antibiotic coverage is mandatory before any invasive dental surgery to avoid any infections. Not all patients with joint replacement surgery are recommended for antibiotic prophylaxis but patients with increased risk of blood bornehaematogenous infections associated with the prosthetic joints are advised to be under antibiotic coverage undergoing dental procedures.[14]

Precaution During Antibiotic Usage: Patients with renal failure should be treated with the utmost precautions as most of the antibiotics are excreted through the kidney. It is advised for a reduction in drug dosage to avoid toxicity due to elevated plasma drug concentrations. Modifications can be made in the drug administration interval without modifying the amount of drugs. For patients having creatinine clearance 10-50 ml/min, amoxicillin and clavulanate is prescribed 500-875 mg/8 hours, and if the patient is having creatinine clearance <10 mg/min, it is prescribed with similar dose in every 12-14 hours. There is no adjustment needed when prescribing Clindamycin (300 mg/8 hours), Doxycycline (100 mg/24 hours), Erythromycin (250-500 mg/6 hours), Azithromycin (500 mg/24 hours). For patients having creatinine clearance 10-50 ml/min, metronidazole is prescribed 250-500 mg/8 hours, and if the patient is having creatinine clearance <10 mg/min, it is prescribed with similar dose in every 8-12 hours. In a study, Gudapati et al documented that the dosage of indomethacin. Naproxen, ibuprofen should be reduced for advanced stages of renal failure. [17]

In patients with liver failure, the use of the antibiotic should be restricted to avoid toxicity as the antimicrobials are metabolized in the liver and are eliminated in the bile. Some antibiotics such as erythromycin, clindamycin, metronidazole are advised to administer to the patients with dosage modifications.[16] In a study conducted by Douglas et al, it is strictly contraindicated to use tetracycline and antitubercular drugs in patients with liver failure.[18]

In pregnancy, due to increased teratogenic effects on the fetus, usage of tetracyclines and aminoglycosides is contraindicated. In a study, "Shrout et al focused on the need for antibiotic prophylaxis for pregnant patients as it eliminates the bacterial loads' od periodontal pathogens".[20] During pregnancy, drugs like "cephalosporin, azithromycin, penicillin with or without beta-lactamase inhibitors" are given with utmost caution. Benzodiazepines possess a risk of oral cleft development during initial months of pregnancy according to Haas et al.[21] Gestational use of "Naproxen, Diclofenac, Ibuprofen" alone or in combination is associated with spontaneous abortion.

Principles For Choosing Appropriate Antibiotics: The following are principles to be followed for prescribing appropriate antibiotics to the patient. Starting from "proper identification of the microorganisms, determining of antibiotic sensitivity, use of narrow-spectrum antibiotics with minimal toxicity after obtaining proper drug history and use of bactericidal antimicrobials over bacteriostatic ones are few cardinal principles to be followed by clinicians.

Conclusion

In dentistry as well as in the medical field, antibiotic therapy is considered mandatory. Penicillin is still the gold standard drug in treating various dental infections. As mentioned above, patients at high risk such as endocarditis, pregnant women, immunocompromised individuals should consult clinicians and specialists before undergoing any dental treatments.

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Management of Lose Mandibular Denture: A Case Report

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Abstract

A lose mandibular denture is the most common problem reported by complete denture patients. So to improve retention and stability we support the denture by placing two implants. In this case report,a patient with a lose mandibular denture is rehabilitated with two mandibular implants. The final prosthesis was stable well retained and esthetically pleasing.

Keywords: *Overdenture, Implant, O ring attachment.*

Introduction

For a complete denture to be a successful prosthesis is dependent on various factors like denture bearing area, psychological assessment of patient and previous experience with dentist[1,2]. So the challenge for prosthodontists is that it not only replaces teeth but also the soft tissue. The usual problem with complete denture patients is retention of mandibular denture, so treatment option for it is implant-supported overdenture. So in this era of implant dentistry mandibular implant overdenture has become the new standard for the treatment of edentulous mandible. So for practitioners this treatment option is the most reliable definite treatment option. Advantage of implant-supported denture is maintenance of bone volume and increased retention and stability.

The most popular attachment used for overdentures are locators and ball and socket overdenture. In this case report three implants are placed with ball and socket attachment which support a mandibular overdenture.

Case Report: A 68-year-old male patient reported to the department of prosthodontics with a chief complaint of ill-fitting mandibular denture. The patient had no significant medical history and his dental history included extraction of teeth due to periodontitis. The patient was using a conventional denture for the past five years and due to non-retention of mandibular denture the patient wants a new prosthesis. On clinical examination the mandibular ridge exhibited a significant degree of resorption and all other parameters like size shape of maxillary ridge and condition of mucosa were found to be normal & healthy (Figure 1). But on evaluation of denture it was found that extension of denture flange was inadequate, retention & stability was poor. Orthopantomograph was advised to evaluate bone availability and architecture (Figure 2). The inter ridge distance was assessed and it was decided to give stud attachment. So for this patient a treatment plan of conventional complete denture for the maxillary arch and 3 implant-supported overdenture for mandibular arch[5]. This treatment plan was explained to the patient and approved by him.

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Figure 1. Mandibular ridge of paitent

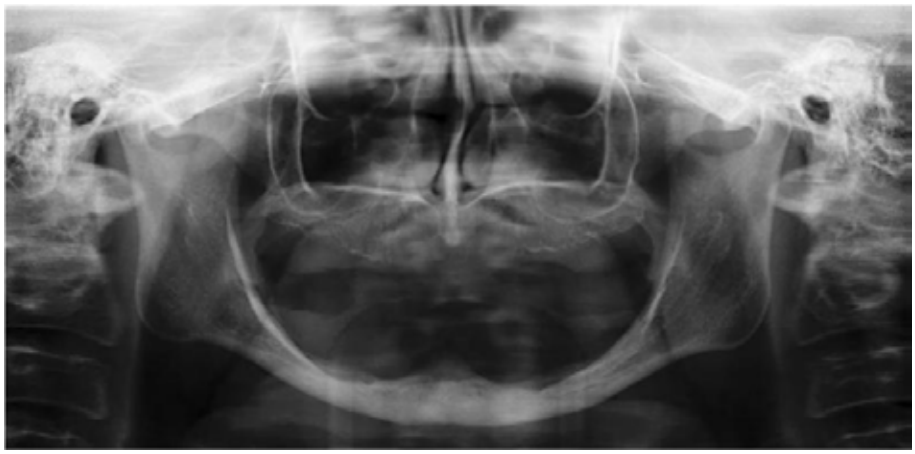


Figure 2. Orthopantomograph



Figure 3. Clear auto polymerizing acrylic resin surgical template

Treatment procedure: Conventional maxillary and mandibular dentures with bilateral balanced occlusal schemes were fabricated for the patient. The mandibular denture was duplicated by using laboratory-grade silicon, using clear auto polymerizing acrylic resin to be used as a surgical template (Figure 3). The vertical space

analysis was done and it was found to be 12 mm which falls in class II category[6] (Ahuja S & Cagna DR). So stud with ball and socket attachment was decided for the patient. Adin (TouregNP) implants of 11.5mm length and 3.75 mm diameter were selected. Implant surgery was carried out in two-stage protocol, first implants were placed with the help of a surgical guide in B, D, E region and loading of the implant was done after three months. Two implant-retained overdenture is a very reliable therapy for patients with edentulous mandible[7]. But in the present case as the ridge is resorbed in the posterior region and space is not adequate for the bar prosthesis, three stud attachments were planned.

Implants should be placed as parallel to each other as possible to increase the prognosis of the prosthesis. For this the surgical stent was used to do the initial drilling and all the osteotomy sites were checked with paralleling pins. After completion of prescribed drilling sequence implants were placed in prepared sites. Surgical cover screw was placed and primary closure of flaps was done. The patient was told not to wear the lower denture for two weeks after surgery and cleanse the mouth with disinfectant mouthwash. After removal of suture the intaglio surface of denture was relieved and tissue conditioners were applied. The conditioner distributed the occlusal forces to the denture bearing area without transmitting it to the surgical sites. Three months later the second stage surgery was done and healing abutments were placed and left for 15 days to help in maturation of gingival tissue. At this procedure the denture is again relined. After completion of 15 days the ball and socket overdenture abutments of 2mm diameter & 2 mm height were placed.(Figure 4) For blockout of the stud attachment a rubber dam was placed and then O rings (RS-2664, yellow) were placed. The second mandibular denture which was prepared previously was loaded with pressure indicating paste and placed on abutments to check the position of implant abutments. The number 6 bur was used to relieve the intaglio surface to accommodate the metal housing of the O ring. The complete passive seating of the denture was verified and then only GC pattern resin is loaded in prepared surface and the patient was asked to bite on the lower denture with the upper denture in centric relation position with a very light occlusal pressure. The pick up once fully cured was removed and trimmed and polished. (Figure 5).



Figure 4. Secondary stage surgery



Figure 5. Polished & trimmed pick up



Figure 6. Postoperative photograph

First the patient was trained on how to place and remove the denture followed by home care instructions. The patient was recalled after 24 hours and then after six month and radiographs were taken. The patients were advised to clean the attachment with the unituft brush. And it was found that the patient was comfortably using the overdenture for the last 3 years.

Discussion

Full mouth rehabilitation of an edentulous patient can be with fixed with 6 to 8 implant for a normal ridge, and all on 4 in compromised ridge condition. And for removable it can be hybrid prosthesis with 4 implants and overdenture with 3 or 2 implants in place. Ideally

for overdenture when adequate interocclusalspace of 15 mm (class I[6]) is present then implants splinted by bar and held by clip is the treatment of choice[8]. As bar has proven to be a longterm successful treatment option for patients who require a high degree of retention and have no undercuts[9]. Recent articles have shown that even if bar attachments allow rotational movement, a higher load is transferred to the implants, as it is difficult to anatomically place the implants in a position to get pure rotational movement[11]. And bar and clip is an expensive restoration with the additional challenge of maintenance of hygiene.

The study on stress distribution between stud attachment and resilient bar and clip attachment has shown no significance difference[9]. And stud type of attachment can be used with existing dentures and also it offers off-axis or angles nylon inserts to accommodate non-ideal implant position[12]. So in this case three free-standing implants were placed in B,D,E position. Two implants supported overdenture was avoided to restrict the PM6 type movement, as the posterior residual ridge was resorbed. And due to financial constrain the patient was not ready for a fixed implant bridge. Overdenture patients have been studied to have a masticatory efficiency in chewing cycles similar to natural teeth[13]. And it already a proven fact that in comparison to conventional overdenture the comfort and satisfaction of patients is quite high[14]. So the success of mandibular overdenture is quite high even after 10 years of function[14].

Conclusion

Treatment option will vary from individual patients but considering the anatomical condition and financial constrain the treatment options has to be devised to give a predictable result. Implant-supported overdenture has become a standard treatment option with edentulous patients. As long term clinical studies have shown that with conventional loading it gives a positive outcome.

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Tobacco and Indian Youth: A Review

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Abstract

In India and the South Asian part of the world use of tobacco is seen for a longtime. If we go back decades back tobacco is used as a gift to treat the guests and also it was encouraged to use in past times. Sooner when the malicious side effects of tobacco were noticed people tried to avoid the consumption of tobacco. The psychological mindset of the youth is quite impressionable, seeing the things that the adults do would easily encourage them to do the same. Thus, this article reviews various forms of tobacco used in our Indian society and how the government of India is taking action to curb the tobacco abuse.

Keywords: *Khaini, Mawa, Mishri, Gutka, Snuff.*

Introduction

The use of tobacco by the younger inhabitants of India is reaching a new epidemic levels. The World Health Organisation (WHO) has stated that approximately 82k-99k, youngsters start the habit of smoking each day. Nearly 45000 of them happen to prolong the habit of their adulthood and many of them are expected to perishin advance because of smoking-related diseases. The way the existing developments of smoking cigarettes and other forms of tobacco are continuing it is estimated that near 251 million people will die due to tobacco.

India being the second largest populated country in the globe, and third-largest producer as well as consumer of tobacco in the globe. The use of tobacco is deep-rooted in the history of this incredible nation.

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Bearing in mind the gigantic health obstacles accompanying the tobacco usage, it is of paramount relevance to comprehend the causes surge its consumption and to plot stratagems to diminish its consumption. It is exclusively pertinent for emerging nations such as India, everywhere tobacco consumption remains to be a public yet harmful consequence of its practice. This article gives a brief review of the outlines of tobacco usage in India, its occurrence in Indian youngsters, psycho-social aspects in commencement & inhibition & the footsteps reserved by the government to curb its use.

History: Tobacco's use had started as early as 600 A.D[19]. Being familiarised in the European community by Columbus who was acquainted with it from the Caribbean through his world tour. India was familiarised with it by the Portuguese.[20] Previously, tobacco had been smoked by various types of tubes or as panatela or was consumed through the mouth in the form of smokeless tobacco. The mid-nineteenth century saw the introduction of paper cigars and cigarettes. In around 1828 A.D nicotine was sequestered from tobacco leaves. Detrimental properties of tobacco have been renowned over the last 1000 years. Archeologically many kings and emperors have acknowledged the detrimental properties of the tobacco and even strained to prohibit the product form the market.[20,22]

India has a discrete custom of implementations in

contrast to tobacco usage being started by the previous rulers (like Jahangir) and the spiritual frontrunners at dissimilar periods in the past. The prodigious Indian Sikh Guru Gobind Singh forbidden smoking for the followers of the Sikh community. He said, "Wine is bad, Indian hemp (bhang) destroys one generation, but tobacco destroys all generations"[22,23] Tobacco use has been regarded as a spiritual taboo by the Sikhs since then. Still, regardless of old efforts to legitimately prohibit tobacco, its use has sustained to breed in acceptance as a non-productive activity[23]

In contemporary medicine, its detrimental side has been documented in the previous 40 years[19]. Though its usage has weakened in industrialized countries in the current ages, it lingers to be prevalent in emerging countries[3].

Different ways it has been used from centuries: Tobacco can be used as smoke form or smokeless form, examples-beedi and cigarettes or like hookah, hookli, chutta, or chillum.

Beedi- it is an inexpensive smoking stick which can be simply made by rolling the tobacco in a dried piece of temburni leaf and securing it with a strand. The dimension differs from 4-8 cm.

Hooka – It is native to India and is made with the help of wooden and metal pipes. The tobacco smoke travelsfrom side to sidein the water which is in asphere-shaped bowl or instead of tobacco any other flavours can also be used.

Hookli- it is a small clay tube-like device which can be nearly 6.9 cm long

Chhutta- it is an indelicate preparation of tobacco, which puffed with the burning side inside the mouth. It is most prevalent in the Srikakulam area of Andhra Pradesh.

Dhumti- It is a cigar like product which uses leaves of jackfruit trees or dehydrated leaf of musa plant. Men predictably burn dhumti, whereas females use it oppositely.

Chillum- it is a narrowed clay tube of about 9.9 cm in length. The tapered side is kept in the oral cavity, frequently enveloped in a damp garb that acts as a filter. It is sometimes used with ganja in the northern part of India.

Usage of smokeless tobacco: Smokeless form is more common in India among youth.

Betel quid includes betel leaf, areca nut, slaked lime, tobacco, catechu and condiments customized by the consumer.Different regions of India usesa different pattern of preparation for the betel quid.

Khaini- tobacco flakes are roasted and mix with slaked lime. The powder is prepared when the consumer uses his left hand for keeping the mixture and rubs it with a finger. Then it is placed in the labial vestibule.

Mawa – it is a combination of areca nut, tobacco, and slaked lime. Most popular in the Gujarat region,among the 15-19 years age group.

Snuff- it is prepared from tobacco byfrying and liquidation, it is used throughnosesnuffing and is prevalent in the east side ofthe country. It is used as a dentifrice, in some regions it is recognized as Bajar and mishri.

Gutkha- direct preparation from the industry, ingredients are areca nut, tobacco and condiments.

Pan masala- mostly used as an after-meal mouth freshener, it is a betel quid combination which comprises ingredients similar to gutkha, but may or may not have tobacco. The combination is masticated & consumed. The inexpensive smokeless form along with the refreshing taste has led to a colossal upsurge in the usage of different kinds of areca nut plus smokeless tobacco amongst the Indian populations as well as the youth.

Psycho-social issues of initiation of tobacco use:

1. We have to observe the duration that a particular individual spends with people. Also if using tobacco is common in the family of the individual then their more chances of him/her getting addicted. Seeing others who are living close to the individual consuming cigarettes may inspire young people to take up the habit.
2. Peer pressure plays a pivotal role in influencing a person to take up these bad habits. It not only becomes a cool image to carry in front of their friends but acts as an anti-stress medication which is encouraged by friends. It also leads to drug addiction. The individual gets more easily addicted as the tobacco products will be shown as beneficial items to relieve stress or as a most common style adopted by nowadays teenagers.

3. The availability of the products in markets is easier than before. The day by day increase in tobacco manufacturing industries with increasing consumer consumption has led to inexpensive mass products that are available in every street corner. In India it is easier to find a tobacco packet rather than a bandaid.

4. Psychological or emotional stress- a good impression of others on us always works as a endorphins. Poor school results, insults by faculty in front of your friends, demoralization from your professors all these factors play a key role in shifting towards the bad habit. Cause being a good person is difficult in today's work environment so such students tend to find relief in these products and abuse it daily. Mental health is a relevant topic which is ignored by parents these days, the features of depression are quite difficult to identify and thus negligence in communication with children by parent lead to addiction to these bad habits.

5. In a country like India media is everywhere and so are the commercials, on television, radio and on the roadside walls. Tobacco advertisements mostly attract the younger generation which falsely showing the advantage of the product rather than the disadvantages. Even the characters in movies and serials consuming these products in a way contribute to promoting the use of tobacco. Many MNCs have not only sponsored various sports events but also instituted bravery awards. Advertisement and promotions of these vicious products are at a cusp in a developing country like India.

Prevention strategies: Like the old saying "prevention is better than cure" there are certain techniques by which we can prevent the adverse effect of addiction to tobacco.

1. The advantages of educational programs have been well received by school students. Such programs concentrate on harms caused by cigarettes other products like hooka, beedis and gutka. Most Indians are unaware of the harmful effects caused by smokeless tobacco products. There is even an erroneous faith that beedis are less injurious than cigarettes. Countless individuals believe that these products on consumption helps in free bowel movements and they refuse to leave the habit as they believe that it will lead to constipation. Multiple programs have been initiated by the government of India to curb this problem. A training module is generated for Non-profit Government Organisations for offering the intervention to street children according to their needs.

2. A pivotal phase in preliminary anticipation is the rebooting of the current tobacco strategies of the GOI. The GOI has lately made relevant lawful actions nonetheless there are deep-rooted problems in enforcing the tobacco-related laws. Limitations have been instilled in sales and the use of such products in public places. Mainly the price of such products should be hiked and their ease of availability should be reduced. An initiative of this program is stopping the sale of these products in the vicinity of schools. Some developed countries have imposed strict restrictions regarding the sale of these products to children but despite these strict restrictions there are still flaws in implementing them.

3. Due to the failure in proper instillations of school-based interventions it has led the scientists to advocate approaches involving the generation of social environment supporting non-smoking. This is important to Indian society, where monetary difference, joblessness, ignorance and vagrant people are linked to addictive behavior including drug addictions.

Prevention is Better Than Cure!!: In tobacco addiction it is better to curb its use rather than regretting it later in life. The preventive measure can only be carried out if the individual is fully agreeing to quit the habit on his/her own. Not only him but the people surrounding a recovering patient should be supportive in their process. We as responsible citizens of this country should always be helpful to those in need. First and foremost educating the person about the harmful effects of this abuse will certainly help a certain amount of population in the society. While educating an individual he should be enlightened about the detrimental effects of tobacco with a reason as nobody will agree nowadays without any proof so we should introduce them to the people who are suffering from oral cancer caused by the tobacco abuse to tell about the detrimental effects not only the disease patients but also those who have successfully quit the habit should be brought in front of the addicts to build a hope in them that they can quit the habit too.

Conclusion

The use of tobacco dates back to many decades

when it was merely a product for refreshing mouth after a heavy meal. In recent years there is a gradual increase in the consumption of such products. Despite the various strategies adopted by the government it, at last, depends on the individual to understand and acknowledge the harmful repercussions of consuming these products. There is no proper awareness among individuals regarding the harmful effects and the commercials are wrongfully encouraging the use of it. We also lack in collecting the data of consumers in different age groups in different parts of countries. To get rid of this vicious product we need to emphasizeon stricter legal actions to those industries and companies promoting the commercials.

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Impact of Pictorial Warnings on Tobacco Packs in India

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Abstract

Tobacco use is represented as the most important public health predicament as well as the number one reason of preventable death worldwide. India ranks the largest tobacco producer as well as the second biggest exporter of tobacco commodities globally. Numerous people each year are killed because of tobacco consumption as it affects every organ and system of the body. Hence policies need to be directed towards tobacco control globally. This review article highlights the impact of one such policy - pictorial messages on tobacco warnings.

Keywords: *Global, Pictorial, Smoking, Warning.*

Introduction

Tobacco use is still considered as preventable death worldwide.[1] More or less, 6 million people are dying every year because of this tobacco-related causes with a predictable increase in mortality rates getting 8 million cases by the year 2030.[2] Consumption of tobacco is usually in the form of smoking[3] and is associated with many respiratory diseases, cardiovascular diseases, and cancer.[2] According to the World Health Organization (WHO) testimony on the Global Tobacco epidemic 2013, around 500 billion dollars of monetary harm annually results because of tobacco use.[4,5]

Worldwide, several policies have been introduced to address the economic and health burden of tobacco use. The first international treaty on tobacco - WHO Framework Convention on Tobacco Control (WHO FCTC) was held in 2003 where Saudi Arabia was among the 168 states who signed the treaty. In its eleventh article the action of binding and labelling of tobacco commodities started and the FCTC recommends a revolving series of health messages that warns and cover at least 50% (or must cover at least 30%) on average of the front and back of the package. It also proposes the use of graphical health messages instead of textual ones.[5,6] The FCTC policy proved to be one of the very effective policies as act as direct means of communicating the risks associated with smoking.[7] Therefore, the chances of smokers noticing the labels increases up to 50% if the health warning text and image is placed on the front side of the packages.[6]

Also, in November 2008, Article 11 guidelines were adopted at the 3rd conference of the parties which focused on the inclusion of tobacco package health warnings. After this, almost 28 countries globally have started introducing pictorial warnings.[2]

Health communication intervention: This health warning pictorial messages have proved to be the most valuable way for enlightening smokers as well as non smokers regarding their health risk. In most of the countries, smokers alleged that they get to know the risk associated with cigarette from warning messages rather than erstwhile resource except television.[8] In 1950s, it was also established that "fear appeals" were very

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constructive in changing health behaviour (e.g. quitting), especially if they are combined with any knowledge.[4]

Efficacy of pictorial warnings: Facts for better latent effect of pictographic messages have initiated from target groups, questionnaire studies and new coverage research.[3] An investigation of records of the ITC Four Country Survey compares effect of prefacing image-based messages in Australia in 2005 by introduction of lengthy text messages in the United Kingdom in 2003. Pictographic messages are also referred by ex- smokers as an imperative reason in their endeavour to end as well as they should be allied in the utilization of qualitative cessation services like toll-free phone "helpline".[9]

Low literate population: Pictographic warning structures may be predominantly significant in transferring health data to those populations with lower education rates.[1,2] Smokers are reported to be less educated than others. Introductory information suggests that countries having pictorial warning messages exhibit less disparity in health acquaintance throughout all the educational levels.[10] Meticulous care has to assort pictures for using it in uneducated populations like without inserting text messages and putting smoking pictographs could involuntarily imply only approval rather than forewarning of its troubles whereas images might say a 1000 words but it is always difficult to choose from those to utter the thousand correct words.[4]

Lower middle class countries: Data from lower middle class income also ropes the usefulness of bigger pictographical warnings in excess of texting warnings, and also suggests that pictorial messages can be good because of the awareness of health risks of smoking.[11]

Are Current Tobacco Pictorial Warnings in India Effective?: Around 800,000 deaths every year in India is because of this tobacco and out of which 700,000 are correlated to smoking.[12] About 10 million deaths per year are likely to occur globally by 2030. Tobacco use in India is highly complex since consumption rate is much higher in various ways in different parts of the country. Behavioural changes like quitting and reducing the tobacco consumption can be well communicated very effectively by warning labels on tobacco.[1]

India ranks the largest tobacco producer as well as second largest exporter of tobacco products globally.[3] Several people every year are killed because of tobacco consumption as it affects various organs of their body. The load of ailment is because of tobacco smoking which has records for 4 lakh patients of oral cancers and 1.3 million patients of coronary artery disease as well as 7 million patients of chronic pulmonary disease. About 1000 young children now smoke and out of them 500 may die of tobacco induced diseases where 250 of them are in their middle age and the rest of them in their old age approximately.[9,11]

Awareness about tobacco commodities is mandated under The Cigarettes and Other Tobacco Products (COTPA) in the year 2003 in India. [13] Currently, tobacco products are now displaying an image of scorpion on smokeless form of tobacco along with an image of diseased lungs or an image of X- ray of lungs showing cancer due to smoked form of tobacco.[13]

Tobacco Labelling at India: In the year 2006 the health warnings policy of India was drafted. A proposed final set of health warnings after 2 complete rounds of revisions in 2006 and 2007, got released in 2008 and were enforced on each manufactured cigarette packages from 31st May in 2009. Cigarette packages have 2 variety of warnings and on all smokeless tobacco products a totally separate warning was rotated.[14]

The Health and Family Welfare Ministry Department in India in 2011 approved an amendment to the regulations that consists of 4 new variety of image based warnings which should be applied on tobacco and bidi packages as well as 4 new variety of image based warnings for smokeless junks. Tobacco companies were allowed to decide any one photo out of each set of 4 images for smoked and smokeless tobacco products and the rules were implemented on December 1, 2011.[15]

India anticipated a new round of picture warnings on September 27, 2012 and those produced warnings as of April 1, 2013, although execution of these pictures varied. Again, a group of 3 new pictographic messages were made for smoked tobacco products, and as usual a separate group of 3 new variety of message were made for smokeless tobacco products. Health warnings structures had to cover 40% of the frontage of all cigarette packages.[16] After all the above proposals, the government again proposed awareness messages on October 15, 2014 that covers 85% of the front and back of the pack and the deadline was extended awaiting 1st April in 2016.[17]

Pictorial warnings on tobacco products: To make them able to visualise and understand the character of tobacco-related diseases pictorial warnings and images

were made for the users. Awareness were created to inform the serious illness that can be caused due to tobacco usage. Therefore, the messages had to be very effective and should be able to keep away the user.

Because of its multilingual and multicultural communities in a country like India, a warning messages can break all the regional, cultural and language barriers. Likewise, where a maximum percentage of the people are uneducated, and therefore written messaged warnings can be ignored whereas graphic awareness messages can help in understanding. This kind of policy is an effective public health strategy that will overhead the government as well as the people at no cost because the tobacco companies will carry out all the expenses meant for the colourful messages.[18]

Several countries along with India are now assigned as a signatory to WHO's Framework Convention on Tobacco Control at the 56th World Health Assembly in the year 2003 and is expected to pursue the WHO guidelines. Pictographic warnings usage on tobacco commodities which is the WHO's initiative called as six "M POWER" rules were developed and intended to battle tobacco among the countries in 2008.[7,11,12]

M Power rules are as follows:

1. Monitoring tobacco use as well as controlling of activities.
2. Protecting individuals from the hazardous effect of tobacco.
3. Offering help or lend a hand to people who have it in mind to refrain from using tobacco.
4. Warning everybody to know the ill-effects of tobacco.
5. Enforcing rules in opposition to all tobacco linked activities that also includes sponsorships.
6. Raising the worth of tobacco materials to reduce its consumption.[19]

Various western countries like Australia, Singapore, Brazil, Chile, Thailand, Hong Kong, Canada and Chile were successfully working on pictographic messages and images on tobacco merchandise packets. The result of this strategy has come to be very much effective on people with low education and low economic status.[7]

It was realised that only constitutional warnings enforced on all tobacco products in 2005 under COTPA was not sufficient to lead in reduction of the use of tobacco. Therefore, the authentic enforcement of these rules has been postponed repeatedly, apparently because of demands exerted by the tobacco manufacturing companies.[7]

Activities related to enforcement of tobacco pictorial warnings[20,21]:

- July 2006: The government issued an announcement of the definite pictures which could be used by tobacco companies within seven months. They demand for grounding time and therefore the government had to extend time period up to June 2007.
- February-May 2007: The objective of company was to utilize any image of a body which should indicate that smoking tobacco can kill. A Group of Ministers (GoM) was created to make a decision who asked for a further extension till July.
- August 2007: Meanwhile, the parliament amended the bill in support of building the application of the skull and crossbones picture optional and should not be compulsory. The court only marks a deadline of December 1 in the year of 2007 for picture-based messages to be enforced in the public.
- September 2007: The GoM made the withdrawal of the images like skull, crossbones, corpse, citing cultural and religious reasons.
- December 2007: The government now ordered for another extension till 17th of March 2008.
- March 10, 2008: The government of health issues few courses of action as date of enforcement shifted to 24th of June 2008.[19] Messages like "smoking kills" and "tobacco kills" were to be printed on cigarette, beedi and other smokeless tobacco products in English and regional languages.[20]

The fundamental right is to preserve a healthy life and it has to be enabled by the government. Therefore, strong warning messages is required to create awareness about the harmful effects of tobacco to the consumer. Tobacco is the only kind of lethal item for consumption having no safe limit and it is legally marketed to consumers globally.

India's Ranking on pictorial warnings: Around the globe, India stands fifth, jointly with Hong Kong and Thailand sharing with 85 % of the tobacco packets covered as per the Canadian Cancer Society report

and only 58 % of the world's population follow this regulation.[22] In India according to the current regulation of pictorial warnings and in the lead the direction of the High Court as well as the Supreme Court, and the chief executive of Voluntary Health Association of India, said that "India has constantly demonstrating leadership by enforcing the relinquish-line figure on all tobacco packets and everyone have pleaded the government for this.[11]

Conclusions

Graphic image based warnings can considerably enhance the efficacy of warning instructions. The application of lifelike images is a significant way out of replacing those positive relations with that of unenthusiastic associations, which is very much suitable in giving the shattering effect of global health by using tobacco related products. Future investigational research should inspect the impact of pictorial warnings on smoking activities. Such work should build up the worldwide evidence base for pictographic messages in future and should also develop our skill to appreciate the 'active ingredients' that reveal such type of messages as well as making them aware of other various effective tobacco control policies.

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Immune Regulation in Periodontal Disease: A Review

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Abstract

It is an established fact that dental plaque as a biofilm is responsible for various types of gingival and periodontal diseases. It is a multifactorial disease in which one of the important components is the host defense mechanism. Through many years of research, it has now been established that the bacteria alone are not sufficient to cause the disease, it's only when they evade the host defense, and in the course of protecting the host, its immune system acts against the tissues resulting in native periodontal tissue damage. This review tries to gain insight into the complex immune mechanisms which take place during the pathogenesis of periodontal diseases.

Keywords: *Plaque biofilm, Humoral immunity, Innate immunity, antibody.*

Introduction

It has been very well known over the last thirty years that while plaque is the cause of periodontal disease, it is the inherent vulnerability of the host that decides the end stage of the disease. Innate susceptibility, in turn, is governed by the type of the feedback provided by one's immunity to the specific periopathogenic bacteria residing in the plaque biofilm.[1]

The washing action of saliva, gingival crevicular fluid, phagocytes, non-specific antibodies, complements, epithelium and defensinsseem to prevent the bacterial attachment and subsequent colonization in the gingival sulcus. However, the role of defensins in arresting the growth of bacteria in the plaque biofilm has yet to be ascertained.[2]

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Many often low virulent pathogens clear out by scavenger receptor of phagocytes or Innate cells without evoking further adaptive immune responses.[3] However, the highly pathogenic bacteria with strong antigenicity infect the sulcus, TLR mediated cytokines from resident leukocytes, activate Innate cell (PMN, Mo) homing to expedite bacterial Killing. If innate cell is unable to destroy periopathogens, they enter the periodontal tissues because Aggregatibacter actinomycetemcomitans (A.a) and red-complex bacteria are unyielding to phagocytosis.[4]

Role of Host Immune Defense Against Periopathogens: "Virulence factors of these periopathogens induce TLR dependent APC activation results in upregulation of MHC-II and Co-stimulatory molecules as well as cytokine release." With strong innate response against periopathogen, antigen-presenting cells (APC'S) liberate IL-1, IL-12 and NK cell liberates INF-γ. In presence of IL-12 and INF-γ, short term, high-affinity APC-TCR binding between naïve T cells and APC leads to Th-1 response.[5] A continued strong Thl response produces INF-γ (i.e., CD4 cells) that would pool the PMNs, macrophages, Natural killer cells (NK) and CD8 T-cells to the siteof infection, that may contain the disease (i.e., containment of infection in chronic gingivitis) by exerting anti-bacterial activity,

intracellular and/or direct contact killing mechanism. The disease might not be eliminated rather persists due to continuous supply of microorganisms from other niches and plaque biofilm.[5]

Atypical LPS of P. gingivalis induces poor innate response for a protracted course. P.gingivalis LPS inhibits IL-12 release from DC and INF-γ from activated Th0 cells (i.e., increased IL-4, IL-5 secretion). Therefore, Th1 to Th2 shift mainly depended on nature of the antigen, antigen dose, cytokine environment and nature of APC.[5] In presence of IL-4, IL-13 and low level of IL-12, prolonged, low-affinity APC- TCR binding leads to Th-2 response (Figure-5).[5] Exaggerated Th2 response induces B cell proliferation (i.e., Polyclonal B cell activation), class switching of plasma cells and antibody-mediated humoral immune response in periodontal lesion.[5,6]

Increased counts of plasma cells suggest enhanced Th2 action in severe periodontal disease. Increased protective anti-bacterial antibody could removeperiopathogens, but if the secreted antibodies become nondefensive for the host (i.e., Low Ab affinity and avidity) or are unable to kill the periopathogens, periodontal disease may ameliorate to an advanced stage.[5] The presence of both Th 1 and Th2 in periodontitis[7,8]points out the importance of both in the pathogenesis of periodontal disease. Hence, the difference between "protective" and "destructive" antibody synthesis would be the most important determinant in the progression of the disease.[5]Alteration of the Thl/Th2 balance may be a direct outcome of the "super antigenic" actionof the notorious periodontal pathogens.

"Although there was no direct evidence for a role in periodontal disease pathogenesis but IL-10 from Th2 cells would promote T-regulator cell activity with concomitant suppression of cell-mediated immune responses." $CD4^+CD25^+$ cell also play a beneficial part in regulating autoreactive T cells mobilized as a part of the host response to periopathogens.[9]

"High titers of anti-collagen type 1 antibody and specific t-cell clones have been identified in inflamed gingival tissues of periodontitis patients."[10] It has been pointed out that human hsp60-reactive T-cell clones and T cells occupying the periodontitis affected areas had the same receptors and cytokines profile (INF-γ) suggestive of the fact the anaerobic bacteria causing periodontitis may activate the self hsp60-reactive T-cells with similar cytokine form.[11] On the contrary, Petit et al. believed that a higher level of anti HSP antibodies were protective of destructive periodontal diseases.[12] The conflict between the two studies might be due to racial variation, the difference in the extent of the disease and variations in the age of the various study groups.[11]

It was found that expression of Vβ was quite increased in the circulating blood mononuclear cells of individuals with Prevotella intermedia infection in comparison to healthy subjects raising suspicion towards the role of superantigens in periodontitis.[13] But other study results failed to show super antigenic activation of T-cells suggested that role of a superantigen in the periodontal disease was variable and inconsistent.[14] The conflicting reports regarding the role ofsuperantigens in periodontitis is yet to be confirmed.

"Although few if any NK cells are seen in healthy tissues, their numbers increase from health to gingivitis to periodontitis."[15] Besides their cytotoxic nature, NK cells can monitor B-cell movement and propagation, which is the pathognomonic histopathological feature of severe periodontal disease.[16] The cell-killing action of NK cells is potentiated by IFN-γ. "Surface lipopolysaccharides (LPS) from Gram-negative periodontal bacteria appear to provide the major activation signal for NK-cell-mediated cytotoxicity."[9]

"The peripheral and local immune cell profiles in patients with disease differ substantially, which suggests that the immunological response to periodontal pathogens is primarily a local phenomenon with mild to moderate systemic involvement."[17]

RANKL expression by T-cell was not critical for bone resorption rather other mechanisms (i.e., hormones, Calcium ion signaling) also have an effective role in osteoclast differentiation in the absence of RANKL. Several studies suggested that T-cell is only indirectly involved in osteoclastogenesis. Moreover, activated T-cells not only positively regulate osteoclastogenesis by RANKL expression but also negatively affect it by secreting IFN-γ. This counterbalancing action of IFN-γ suppresses boneresorption in normal T-cell response as well as in many chronic periodontitis patients. Thus, the detrimental effects of T-cells onosteoclastogenesis revolve around the compelling balance of RANKL expression and cytokine profile.

There arises the need of added studies to find out the components of alveolar bone loss and pathogenic T-cell phenotype associated with periodontal bone loss.[18]

B-cells secrete high amounts of IL10 and IFN-γ, which are inhibitory cytokines but these seemed to be outrun by stimulatory factors such as macrophage inflammatory protein-alpha (MIP-α), monocyte chemotactic protein-3 (MCP-3), and M-CSF.[19] Although it has been demonstrated both in vitro and in vivo that activated B -cells enhance osteoclastogenesis and bone resorption it is still inconclusive if B- cells are more responsible for bone loss than T-cells.

"Periodontal Antibody (Ab) producing B cells may be inadequate or of insufficient longevity to deal with the continuous assault from biofilm and/or chronicity of the infectious disease. Meanwhile, memory B-cells and Ab-producing plasma cells may eventually become "crippled" or "degenerated" and thus trigger further immune/inflammatory responses by invading pathogens resulted in progressive tissue destruction."[20] Therefore, "Ab-mediated protection is incapable of achieving total or sterile eradication of the invading periodontal pathogens and, hence, is sub-optimal."Contrary to that,"activated" B-cells in the form of antigen-presenting cells totrigger memory T-cells for cellular immunity.[21] This is precisely why antibody-mediated immune response anticipates periodontal pathogens first but fails to curb the advancing form of periodontitis.[22] The B-cells localized in the periodontal tissues also deposit IL-1β that is crucial for alveolar bone loss. It has to be seen in the future as to why the humoral immunity fails to arrest the periodontal disease progression before a definitive attempt to prevent periodontal disease in the form of a vaccine can be thought of.

Conclusion and Future Directions: "Host immune-pathogen interaction is a complex interplay, which is intimately regulated by the innate and acquired immunity to deal with immediate and long-lasting assaults imposed by invading micro-organisms in periodontal pockets." Pathogen-specific $CD4^+$ T cells are both protective, and destructive whereas $CD8^+$ T-lymphocytes are not responsible for periodontal disease. We have sufficient evidence pointing towards the role of $CD4^+$ T cells in the development of the host's antibacterial resistance and maintenance of harmony of the periodontium.

With the basic understanding of the interplay of the immune cells and cytokines in the process of bone resorption, would help to develop novel methodto treat periodontitis and other systemic disease associated with bone loss. Therapeutic approaches such as potassium channel blockers to block the calcium signaling for RANKL expression and gene therapy to reduce IL-1 or TNF-α production are being investigated. In periodontitis host modulation using chemically modified tetracycline (CMT) or low dose doxycycline have shown to reduce the periodontal disease by suppression of matrix metalloproteinases (MMP) activity.

A clear understanding of the immunopathogenesis of periodontal disease is essentially required to develop diagnostic aids for prediction of disease activity reliably, vaccine preparation and to differentiate between a healthy and periodontally affected site.

Currently the relationship between periodontal disease and systemic health is considered as a two-way road. Immunity in most individuals establishes a 'China wall' between the two. Moreover, epidemiological findings suggest that even in the absence of oral hygiene maintenance rapid periodontal breakdown occurs occasionally. Considering the relationship between periodontal disease and systemic health it can be stated that even tooth loss in some individuals as a consequence of chronic infectious periodontal disease represents a protective role of host immune response by essentially exfoliating the infective source (i.e., tooth associated plaque and calculus) that could create a greater danger to systemic health.

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Idiopathic Gingival Enlargement and its Management: A Case Report

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Abstract

Idiopathic gingival overgrowth is an unusual condition that leads to a gigantic overgrowth of the gums without any specific etiology. The enlargement leads to multiple issues and makes chewing difficult for the patient along with the unsightly appearance. Here we present a case of idiopathic gingival enlargement without any syndromic association in a 45- year old female patient. It was managed by surgical treatment including gingivectomy followed by gingivoplasty and was monitored regularly due to its high rate of recurrence.

Keywords: *Idiopathic gingival enlargement, gingival overgrowth, Gingivectomy.*

Introduction

It is a well-known fact that the increase in the volume of the gums is one of the most prominent features of gingival inflammation. The enlargement can be restricted to few teeth or may involve more or else the entire dentition in a generalized form. The most common of them is plaque-induced gingival overgrowth or inflammatory gum enlargement. Other causes include drug-related, enlargements associated with systemic diseases, leukemic gingival overgrowth[1,2], subjects with kidney issues and on immunosuppressants[3] to name a few. In the localized form, it was put forth by Cook that all of the enlargements may be known as "Epulis" and is seen mostly in the upper front teeth region.[4] Gingival enlargement can also be caused by few benign and malignant tumors of the oral cavity.[5]

Idiopathic gingival enlargement or fibromatosis as it is known as is an extremely rare condition seen leading to diffuse enlargement of the gums of alarming proportions. The main reason for this type of overgrowth is unknown and sometimes is associated with some syndromes. The enlargement may involve the maxilla or the mandible or sometimes may involve both. It involves both the labial/buccal and lingual/palatal surface. It involves all three parts of the gingiva, that are attached, marginal and interdental papilla which differentiates it from drug-induced gingival enlargement as the attached gingiva is not involved in the latter. The gingiva appears firm and leathery and has a peculiar "pebbled appearance." It can be of two types: One which is nodular and has multiple swellings at the interdental gingiva and the other one in which proportionate enlargements are seen.[6] It can sometimes cover the entire teeth and pose a challenge in mastication and can also lead to distortion of the maxilla and mandible.

Case Report: Here we are going to present a case report of idiopathic gingival enlargement. A 45-year-

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old female, housewife by occupation presented to the Department of Periodontology with a chief complaint of an increase in the size of her gums slowly over a period of 5 years. She also complained of the looseness of teeth and the inability to brush with difficulty in eating. Her medical and dental history was inconclusive. This was the first visit of the patient to a dental setup. She did not give any history of drug intake, fever, weight loss, pregnancy or any other systemic disease. She did not have any deleterious habits. She used to brush with a medium bristle toothbrush and toothpaste with a horizontal scrub technique. She reported that for the past few months, maintaining oral hygiene was difficult due to the gum overgrowth. She had severe halitosis. There was no abnormality was seen in the extraoral examination wherein the profile was convex, no abnormalities detected in the TMJ and lymph nodes and the lips were incompetent. Intraoral examination of the buccal and labial mucosa, palate, tongue revealed no abnormal finding.

Coming to the area of chief complaint, there was diffuse gingival enlargement of both the maxilla and mandible but was severely more pronounced in the lower jaw. The gingiva appeared pale pink, fibrotic with the typical "pebbled appearance" seen in the lower jaw. (Fig. 1) There was generalized mobility of teeth There was a typical erythema seen in the maxillary arch around the marginal gingiva. The enlargement was so much in the mandible that it prevented the contact between the teeth and posterior open bite was seen as a result of it. Since it did not fit any criteria, a provisional diagnosis of "Idiopathic Gingival Enlargement" was made. We advised an OPG and a complete blood hemogram to rule out any other abnormality. OPG revealed generalized alveolar bone loss but the blood investigations revealed all values within normal range, except alkaline phosphatase which was slightly increased. Her diabetic status came out as non- diabetic, it was done to emphasize the fact that diabetic patients are more prone to destructive periodontal disease as compared to non-diabetic subjects.[7,8]

Treatment Plan: After the initial examination, a thorough scaling and root planing was done and the patient was asked to rinse with 0.2% chlorhexidine mouthwash and was again recalled after 3-4 weeks. On her second visit, there was a very slight improvement in her condition. Therefore, we planned to do gingivectomy in all four quadrants in a planned manner. The whole procedure was explained to the patient along with the risks associated with the surgical process. A written informed consent was taken from the patient. Under complete asepsis, after administering local anesthesia, an external bevel gingivectomy was performed on one side and the same procedure was repeated on the other side after healing of the first side. (Fig.2) After the surgical procedure, a periodontal dressing was given at the site of surgery. The patient was prescribed necessary antibiotics and analgesics and was asked to meticulously maintain her oral hygiene. The excised tissue was sent for histopathological test and it revealed inflamed gingival tissues. There was an abundance of connective tissue which was mainly avascular and large congregation of fibroblasts along with heavy infiltration of piles of collagen fiber bundles. The superficial epithelium was broad with prominent thickening of all the layers with extended rete pegs. (Fig. 3) This confirmed our provisional diagnosis and a final diagnosis of Idiopathic gingival enlargement was made. The case was followed up for 6 months and it did not show any recurrence. (Fig. 4).

Discussion

As discussed above, gingival enlargement can be caused by a plethora of reasons. In the present case, as the drug, family, medical and dental history was non-contributory, we gave a diagnosis of idiopathic gingival enlargement. "Idiopathic gingival enlargement is also known as gingivomatosis, elephantiasis, idiopathic fibromatosis, hereditary gingival hyperplasia, and congenital familial fibromatosis.

It has been pointed out about its hereditary origin but has not been proved till now. It has been seen in multiple studies that the mode of inheritance is autosomal recessive in some and autosomal dominant in some. "It occurs either as an isolated disease or combined with some rare syndromes like Zimmerman-Laband syndrome (defects of bone, nail, ear, nose and splenomegaly), Murray-Puretic-Drescher syndrome (multiple dental hyaline tumors), Rutherford syndrome (corneal dystrophy), Cowden syndrome(multiple hamartomas), and Cross syndrome (hypopigmentation with athetosis)."[9]As seen in this case, there is progressive enlargement of attached, marginal as well as interdental papilla which sometimes extends above the teeth making the day to day activities like toothbrushing, eating and chewing quite difficult. The histopathology of this type of enlargement reveals numerous collagen fiber bundles, fibroblasts with an overwhelming connective tissue.

There is acanthosis of the epithelium with prominent rete pegs as seen in the present case.

The management of idiopathic gingival fibromatosis is only surgical as non- surgical therapy seldom helps. The definitive management includes gingivectomy[10] (external or internal) or flap surgery depending upon the case. Since the etiology of this is unknown, the chances of recurrence are quite high. Therefore, we need to follow these patients for a long time and repeat the procedure if deemed necessary.



Figure 1: Pebbled appearance seen in the lower jaw



Figure 2: Performance of gingivectomy



Figure 3: 6 months follow up



Figure 4: Histopathological Examination

Conclusion

This was a case of idiopathic gingival enlargement not associated with any syndrome. The patient was properly diagnosed based on clinical examination and proper correlation with radiological and histopathological examination. The case was followed up for six months without any recurrence, but long term follow up is warranted because of its unknown etiology and high chances of recurrence.

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Emerging Consequences of Phubbing among Adults

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Abstract

In recent years phubbing activities have affected both phubers and phubees'. It has ruined the real-life social relationships. People who are busy in phubbing believes that smartphones are now a daily part of their interpersonal communication. In simple words, instead of giving much consideration to the people, there is a social place, they opt to bolt their eyes on their mobile phones while interacting with people. Phubbing is a threat to the mental health which leads to depression, anxiety and causes a negative impact on relationships. This review article focuses on the effects and signs of phubbing and how to overcome it.

Keywords: *Counselling, Mental health, Phubbing.*

Introduction

Phubbing is a mixture of the words "phone" as well as "snubbing" that describes the action of snubbing a person in a social place by focusing all the attention on one's phone instead of talking to the person openly in one's company. This phubbing terminology was originally coined in a movement by the Macquarie Dictionary to stand for an upcoming problem of smartphone exploitation in a social place.[1] In a public interface, a "phubber" can be considered as a person who keeps on phubbing his or her companion(s), whereas a "phubbee" is an individual who is a receiver of phubbing activity.[1]

Phubbing manners has hampered both phubers and phubees' lives deeply in these years.[2] It can destroy the existent-life community relationships of both phubers and phubees because it is measured as a disrespectful as well as ill-mannered behavior.[3] Several studies[4-6] have reported that when a person is being phubbed, they tend to have very little contentment as they make out the interaction to be deprived of quality, and they also experience less connectedness with their associate. Phubbing behavior may even jeopardize human lives and this shows that there was a case that has taken place at Malaysia where a six-year-old girl unintentionally fell throughout an opening of an escalator and at the same time during that incident, the girl's mother was busy in the phone having an argument with her husband on phone, and for that reason neglected her child. This case depicts how being inattentive while on the smartphone devices can cost lives.[7]

What is phubbing?: Phubbing is a word which was termed in May 2012. An advertising agency at Australia had created this word to describe the budding phenomenon of people who ignore their friends and family who were right in front of them but they kept scrolling over their phones.[8]

While the word might not be in our daily vocabulary, the action likely is. Seventeen percent or more people phubb others at least four times or more a day and out of them only 32 percent of people report being phubbed two to three times a day.[2] While the behavior might

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not seem like a big deal, research shows that phubbing can affect your emotional relationships as well as your mental health.[9]

Who does 'Phubbing'?: The word phubbing might be a new term presently but this act of phubbing is around us since the use of smartphones started among the people. They slowly get addicted to this kind of impulse phubbing behavior. Studies reported that phubbing is mostly related with online self-presentation, online impression management and self-fascination.[10]

People who are busy in phubbing believes that smartphones are now a daily part of their interpersonal communication. "So many articles talk about Phubbing in a negative light and it has to be seen as a cultural shift - a generational shift. It could also mean self-love or self-care. Online self-presentation could also mean connecting with more people on a mass level. It's not necessarily a negative."[11]

There are several reasons behind this phubbing action like internet addiction, social media addiction, game addiction, depression, and 'nomophobia'. "Nomophobia" is a term that means the apprehension of being devoid of a smartphone which is also called a smartphone addiction and it is often lightened up by an internet gorge problem or internet addiction disorder. Smartphones are now loaded with various application which go beyond those of early computers. These smartphone applications have a massive effect on addiction. Excess use of these computers has forced the researchers to comprehend and investigate the concept of computer or smartphone addiction or in a short technology addiction.[12,13] Many studies reported that most of those software's had a huge impact on addiction. Some of the examples of such software applications is that it keeps an individual stay online for longer duration such as playing games online, shopping and social apps.[4,14]

How phubbing makes us feel?: According to some studies it was found out that people who were phubbed by the concerned person felt very less satisfied with the quality of interaction with the phubber. Results of few studies also reported that such feelings carry only the negative impact of phubbing on their intellect of belongingness, or the human aspiration to be accepted by others.[9] Now technology and social media are important parts of our smart life, but we shouldn't forget our mental health and well-being.[15]

Gender categorization: Gender categorization has been reported to play an essential role in motivating several smart phone-allied actions such as the preference for online activities,[16] mobile phone dependence,[17,18] internet obsession,[18,19] self-indulgence,[20] and conversation manners.[21] Though very little at present is known about how all these phubbing actions, being phubbed, along with supposed social customs of phubbing differ between males and females, some studies reported that women do more phubbing than men along with frequent text messaging and scrolling all over the pages of social media.[10,22] In the meantime, gender has a moderating consequence on the association that are stuck between social customs and many aspects of human utilization behaviour[23] such as alcohol consumption,[24] internet banking[19] and online purchasing.[14]

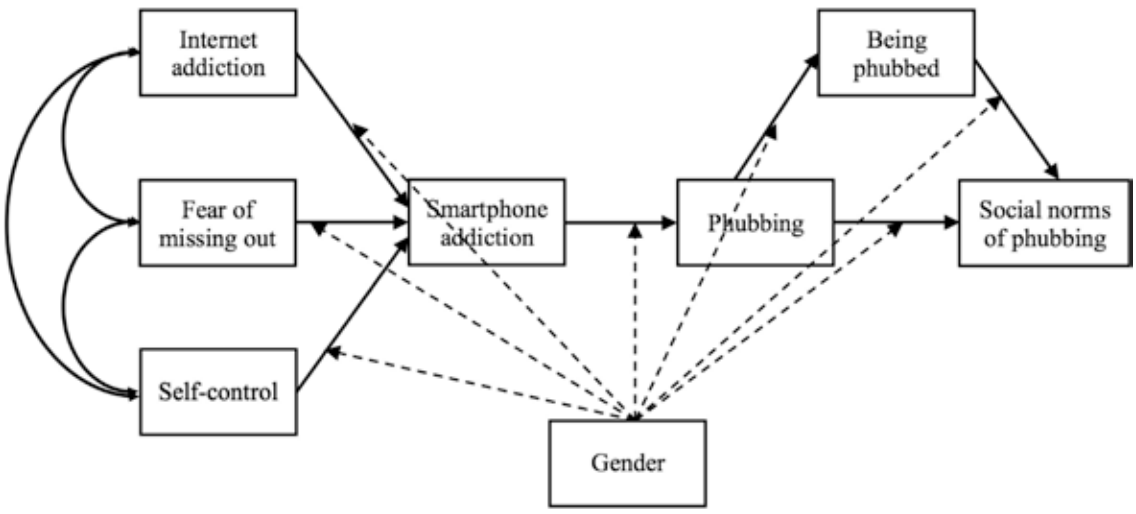


Figure 1. Proposed conceptual phubbing model

Research Model and Hypotheses: A research model to explain the key determinants of phubbing conduct and the apparent social standards of phubbing was developed.[2] The anticipated model is delineated thoughtfully in Fig. 1. It was theorized that Internet addiction and Fear of Missing Out (FoMO) would positively predict smartphone addiction, and that self-control would adversely foresee smartphone addiction. Next, prediction followed that smartphone addiction would positively predict phubbing behavior. Further, it was estimated that phubbing behavior would positively predict the extent to which individuals are phubbed. It was further anticipated that both phubbing and being phubbed would positively predict the degree to which individuals perceive phubbing as normative. Finally, it was predicted that gender would moderate the relationships between every determinant in the proposed model.

How does phubbing affect relationships?: Phubbing stimulates your ability to be present and connect with people around you. Studies reported that texting frequently on phubbing with smartphones during a front-to-front interaction made the experience very less satisfying for each other[24] and these actions can also have a bad impact on marriages. Phubbing behavior decreases marital satisfaction.[10] Conflicts over frequent phone use were the driving cause of marital issues. Studies also reported that spouses who phub each other result in higher rates of anxiety and depression.[13]

How does phubbing affect mental health?: From the mental health perspective, according to World Health Organization (WHO) (2014) someone who is declared mentally healthy is having a whole physical, mental and social condition and not just the absence of illness or weakness. So, when we examine it, someone who is doing phubbing, has experienced an indication of mental illness. This is because one aspect of being mentally healthy based on the definition of WHO is that someone who can establish good social relationships, but not for individuals who tend to do phubbing when interacting.[11,15,23]

What's the driving force behind those feelings?: Phubbing is a threat to four "fundamental needs," the study found.[12] Those core needs are: Belongingness, Self-respect, Meaningful life and Control. If a person phubs you, you might feel rejected, debarred, and nonsignificant that can lead to a negative effect on your mental health. Researchers also reported about a vicious cycle where the people who are phubbed tend to reach their smart phones more frequently and they try to keep on with their social media network to fill that void.[3,8]

According to research published in Computers and Human Behaviour, social media can make feelings of depression shoddier, and the more you use social media, the more you are to feel depression or anxiety.[21] The number one sign that you are guilty of phubbing is in your hand - your phone. If your phone is with you at all times because you're afraid you'll miss a call, a tweet, or a status update, you are likely guilty of phubbing.

Signs you may be a phubber[18]:

1. **Carrying on two conversations at once, on your phone and in person:** You're likely doing neither very successfully, and you're most certainly phubbing.
2. **Immediately bringing your phone out at dinner or other social settings:** Putting your phone beside your plate "just in case" is a warning sign that you'll soon be phubbing. Plus, you don't even have to touch your phone during a conversation for it to negatively impact your relationship. Studies have reported that just the presence of the phone made people feel less connected.[13,18]
3. **Can't get through a meal without checking your phone:** The fear of missing out is real - a real sign you're phubbing.

Three ways to stop phubbing: If the idea of giving up social media makes you nervous and a little sick to your stomach, you're not alone. The urge to check social media is stronger than the urge for sex. That doesn't mean, however, that you can't learn other ways to check in with social media without scrolling endlessly.[16]

1. **Make meals a no-phone zone:** When it's time to eat, no matter where you are, but the phone away. Mobile phones can be made into silent mode or into "do not disturb mode" because a distant buzz of notifications can cause distraction in mind. Engage in sincere conversation with each other and gradually comfort develops with having a face-to-face conversation.
2. **Leave your phone behind:** You might feel as if you've lost a limb, but don't be afraid to simply put your phone in your car, desk drawer, or bag - and leave it there. Whatever alerts or updates happen, they'll be waiting for you later.

3. **Challenge yourself:** Everyone enjoys a little prize now and then. Make ignoring your phone a challenge. Keep track of the meals or hours you go without your phone in hand. When you've completed a goal, treat yourself, and then challenge yourself again.

Three ways to help someone else stop phubbing:

1. **Model a better behavior:** If you want to silently encourage the chronic phubber to stop, be a good example. Put your phone away when you're lounging on the couch. Don't encourage them to show you something on their phone during a date. Focus on the other people at the table. Slowly but surely, they'll get the hint.
2. **Call them out:** There's nothing like hard love. If someone you regularly interact with has a habit of phubbing, tell them. The Stop Phubbing campaign will email your loved one a note about their behavior if a face-to-face conversation is too difficult or uncomfortable for you. Either way, tell them you don't like it, and then help them develop better habits.
3. **Be sympathetic:** Phubbing isn't a real addiction, but it is an impulse problem. It will take some time to break all these impulses as well as learned behavior, so be patient and understanding, but be firm too.

When to seek help for phubbing?: Reading through your phone, again and again, can result in an impulse behavior you can't control. When you go through bad communications with your people, when you realize you are going into depression mode along with irritable behavior with frequent mood swings then if you have difficulty in stopping the habit, you may want to speak with a therapist or psychologist. They can help you learn to redirect your energy. They can also help you find out why you may have developed this impulse in the first place because, for many people, social media begins as an easy getaway, or a way to zone out.[15,20] Therefore it is always better to seek help before it's too late.

Conclusion

Phubbing behavior is nerve-racking for the development of future generations. Therefore, it is required to provide proper guidance as well as counseling services to overcome the overruled behavioral pattern called phubbing that is being experienced by many people. The need for urgency of supervision and counseling services can be implemented in the form of modules that should focus on "use smartphone smartly". We don't have to stop using our phones to prevent phubbing. We just have to be more aware. Being mindful of our actions when we are around other people is a great place to start.

We can also ask friends for accountability. If they feel like we are zoning into our phone, they should feel free to call our attention to it. Phubbing is a learned behavior - after all, it's only recently become a problem - and we can unlearn it. It will take time and some work, but our mental health and our valuable relationships will thank us for it.

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Transtheoretical Model of Behavioural Change for Oral Habit, Tobacco and Alcohol Cessation

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Abstract

The transtheoretical model of change is one of the most accepted strategies to bring about an intentional change. It addresses the process of change in a structured manner which can be beneficially implemented to bring about positive changes to achieve habit breaking, tobacco and alcohol cessation. In addition, it may help acquire and establish positive habits such as maintenance of oral hygiene.

Keywords: *Transtheoretical Model; Behavioural Change; Oral Health; Tobacco and Alcohol Cessation.*

Introduction

People who intend to change a prevailing behavior, habit or an addiction exist in good numbers. Although several strategies have been put forth to achieve this, a model that makes an approach though health behavioral change such as the transtheoretical model[2] has become quite acceptable over the years. Initially it addressed smoking cessation, however, its success has encouraged its application across a variety of health behaviors including physical exercise, nutrition and diet. It may be further applied to oral care in the area of cessation of tobacco smoking and alcohol, maintenance of oral hygiene[3] and habits such as thumbsucking and oral breathing.

Transtheoretical Model:

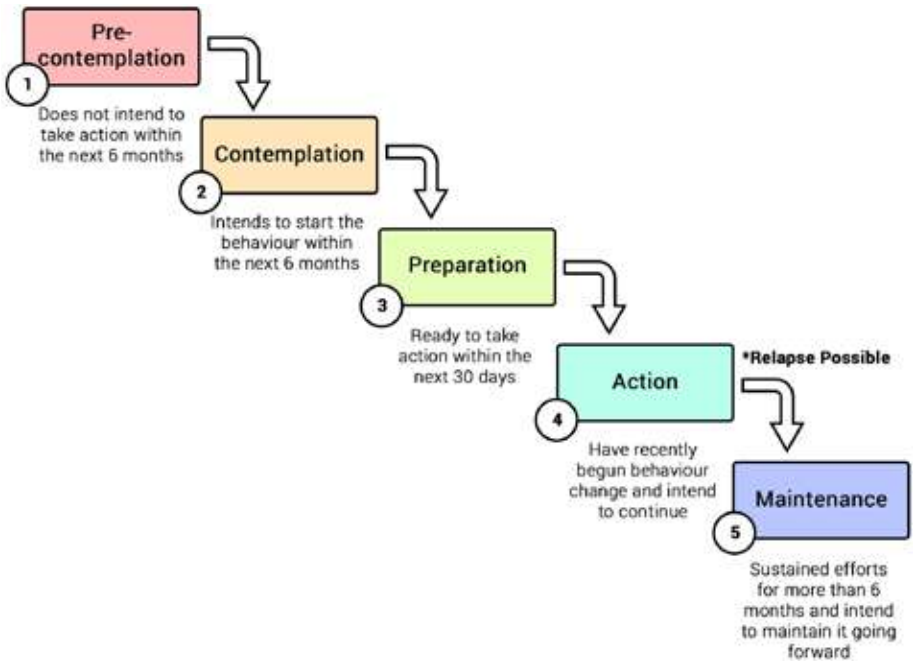


Figure 1. Stages of behavior change within the Transtheoretical Model adapted from Hartin et al.1

Transtheoretical model (TTM) of behavioral change is an integrative theory of therapy thatworks on the premise that change in behavior is a process and not merely a concurrence[4].

Individuals may be at different stages of change and readiness to adapt. The model is an accumulation of several constructs which include various aspect of change such as the stage, process, level, efficacy, and determination.

Pre-Contemplation Stage[5]: In this stage patients are usually not interested and do not indulge in thoughts pertaining to any kind of change in their routine. On the contrary, there is a tendency to defend their current state and do not consider see it as a problem. The defense may even come upfront when they perceive any external pressure. Patients do not lay their attention on the change and avoid any kind of discussion with others.

In this stage, motivational interventions can help raise awareness of the adverse effects of the habit or behavior. In this state, addictsoften outweigh the advantages of their current habit or behaviour and are unwilling to modify them. In this stage,the role of the therapist or a counselor is primarily to guide the patient[6,7].

Contemplation Stage[8]: In the contemplation stage patients have a better awareness about the ill effects of their habit and spendsome of their time thinking about it. Although there is a consideration of the possibility of changing, they display uncertainty in their thoughts and speech. Patients are undecided and are still in a mode of assessing the benefit of modifying their behavior. Although they are aware of the negative and the positive aspects of their habit and also the benefits of choosing to quit or reducing, they may doubt on the fact that the long-term advantages of the change will outweigh the short-term costs. For a patient to be in this stage it may take from a few weeks to an entire lifetime to get through this stage. This is true as some patients plan and plan and keep planning about the change may end up in this stage. On the positive side, patients in this stage are open to suggestions and are more likely to actually self-assess their feelings and thoughts on the change.

Patients are asked to change in the next 6 months, or any date as per the consent of the patient is arrived at. Usually, the vast majority of patients are unaware of what to change and often get caught up in the "chronic contemplation." They replace the idea for acting. Motivational interventions are beneficial for increasing the knowledge about the adverse effects of their present behaviour/habit or addiction. The adverse effects should be clarified[6, 7].

Preparation Stage[9]: In this stage patients have taken a decision to change. And this is revealed in their reciprocation such as: "I've got to do something about this–this is serious. Something has to change. What can I do?"

In other words, this is an experimentation stage and patients are now moving ahead slowly towards the change. They collect information by reading or asking about what they will need to do. Sometimes they may make a call to help centers to figure out what strategies and resources are available. In a good number of times patients tend to omit this stage and jump from the contemplation stage into the action stage. This often leads them to stumble as their knowledge is incomplete.

Preparation of change is underway and a deadline is set within 30 days. At this time a clinical evaluation using the various assessment tools may be made[10].

Action Stage[11]: This is the stage where patients gather acceptance in the fact that they will be able to change are seen actively involved in taking progressive steps and are now open to the use of alternative approaches. This could be the shortest stage, however, the actual amount of time spent by patients in this stage varies. It usually takes as long as six months, but can get over in an hour! In this stage, patients depend chiefly on their strength of will. At the same time where there is an effort to quit or change the behavior there exists a greatthreat for reversion.

Patients assess their self-pledge to themselves and chalk out tactics to counter own and outerdemands that may result in relapse. Intermittently they introduce instant incentives of sustenance of impetus, and evaluate the change achieved so that it enhances their self-assurance. Patients are mostlikely to be open to receiving professional help in this stage.

Beginning is made to stop the prevailing habit or behaviour. Habits can be stopped using drugs appliances, behavior modification, will power, informal exit strategy or some or all of these procedures. This phase of effort lasts 6 months from start to finish. It is the most frequent stage of decline, which may change with treatment approach, lifestyle, mental health and gender. Increased

initial relapse occurs in the first few weeks. Therefore, the first support is very important and interventions that address disruptions,avoidance and satisfying positive actions are most useful. To continue this step, it is important to repeatedly contact the therapist to redirect efforts and celebrate success.

Maintenance Stage[12]: This stage involves the ability toward off any persuasion that may cause the return of the behavior or habit. This stage aims to preserve the acquired status. Patients also tend to be reminiscent about the quantum of progress they have made and devise ways of living so as to counter relapse. They are now able to foresee the possibility and circumstances which might lead to a relapse and formulate strategies to manage it in advance.

Beneficially, they become aware that their goal is individually valuable and consequential. They become increasingly self-tolerant and come to terms with the fact that achieving a stable change takes time and it takes a little while to get accustomed to acquired status until they become like second nature to them. Inspite of temptations coming their way, they are able to keep themselves out the way. Relapses occur frequently, on an average, 3-4 times successful quaternary relapses and cycles occur before the change.

Conclusion

The Transtheoretical model handles the change through a structured and continuous approach. It allows interventions tailoring to meet the needs of the patients. In addition, there can be movement through phases which may be the case for some who succumb to relapses.

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Host Modulation Therapy: An Overview

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Abstract

Host modulation therapy involves a wide array of therapeutic strategies directed towards modulation of host factors in order to prevent the initiation and progression of periodontal disease. Host modulation is one of the non-surgical periodontal therapeutic approaches employed nowadays as better compliance is often seen among the patients. These strategies are basically aiming at reduction of inflammatory mediators or inhibition of destructive enzymes etc. These approaches are much popular in recent times and employ various therapeutics to attain the same. This review enlists the various strategies and approaches in modulating the host for prevention of periodontal disease.

Keywords: *Host modulation therapy; non-surgical periodontal therapeutic approaches; periodontal disease.*

Introduction

It has been clear that bacteria with unique virulence factors contribute to the initiation of periodontal disease. Host derived enzymes and cytokines also play a major role in degradation of extracellular matrix and destruction of alveolar bone in periodontitis.(1) The bacterial insult activates host defense mechanisms, as result of inflammation and its mediators, leads to destruction of supporting structures of tooth.(2) This mechanism of tissue destruction allows the clinician to think of strategy to manipulate such host derived mechanisms for prevention and treatment of periodontal diseases.

Periodontitis is chronic inflammatory, poly-microbial disease affecting the periodontium.(3) Bacteria are considered as the primary causative agent, which subsequently trigger host response. One of the early inflammatory responses by host is diapedesis. If neutrophils able to combat and delineate the infection, the disease limits itself to gingiva, causing gingivitis.

When the pathogens breach the innate defense mechanism of the host and penetrate the host tissues, the disease of periodontitis sets in. This stimulates release of inflammatory mediators like cytokines which play an important role in combating the pathogens, and in turn also leads to degeneration of tissues. The periodontal tissues when destroyed can be clinically evident as pocket formation and alveolar bone loss.(4)

Risk Factors: Patient's risk factors determine the disease susceptibility of host. The rate of disease progression, its severity and treatment response varies with different individuals. The risk factors like heredity factors, hormonal changes, stress, smoking status, systemic diseases, nutritional deficiency, certain medications, increased occlusal load, local factors etc. play a significant role in determining disease susceptibility of an individual.

Host Modulation: Host is defined as the organism from which a parasite obtains its nourishment/in the

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transplantation of tissue, the individual who receives the graft. And **Modulation** is defined as the alteration of function or status of something in response to a stimulus.

Host modulation therapy demands the use of pharmacological agents designed specifically to aid in treatment and limit the initiation and progression of periodontal disease.[5]

It includes:

- Antimicrobial therapies that can be used to address changes in the microflora
- Host modulation therapy that can be used to address a host response consisting of excessive levels of enzymes, cytokines, prostanoids & excessive osteoclast function that may be related to risk factors.

The different strategies of Host Modulatory Therapies (HMT) to regulate or block the pathogenesis of periodontal disease include:

A. Regulation of immune and inflammatory responses:

1. immunization method
2. regulating ROS
3. regulating cytokines
4. others – NF kb modulators
   – integrin inhibitors

B. Regulation of arachidonic acid metabolities:

1. steroids
2. antioxidants
3. NSAIDs
4. lipoxins
5. triclosan

C. Regulation of excessive production of matrix metalloproteinases:

1. Tetracyclines
2. Hydroxamic peptides

D. Regulation of bone metabolism

1. Antiinflammatory agents
2. osteoprotegrin
3. anti RANKL
4. SERMs
5. bisphosphonates
6. anti integrins
7. CMTs

A. Regulation of Immune And Inflammatory Mechanisms:

1. **Immunization method:** Bacterial plaque is considered to be the causative factor for initiation and also plays an important role in periodontal disease progression. These antigens are potent mediator for producing antibodies in the host system. Thus, new method are developed like use of protective antibodies via immunization for preventing periodontal disease. [6]

Bacterial whole cells, its outer components and synthetic peptides are used as antigen for initiating immunization. The development of periodontal vaccines has been in boom but not successful due to the multi-factorial etiology of periodontal disease and microbial complexity of bio-films.

Identification of microbial species like *Porphyromonas gingivalis*, *Bacteroides forsythus* for initiating specific disease, is necessary for successful development of vaccines.

3. **Regulating Reactive Oxygen Species:** Reactive Oxygen Species are the molecules with one or more unpaired electrons. These are released in excess amount in the event of inflammation. Tissues get exposed to these free radicals and get destroyed. Hydrogen Peroxide and Hypochlorous acids are the oxygen metabolites released by neutrophils and macrophages, take part in tissue destruction. They cause depolymeraization of collagen, hyaluronan, and proteoglycans. However, these free radicals can be neutralized by providing anti-oxidants or inhibitors in abundance.

Nitric Oxide is considered as a free radical, synthesized by endothelial cells by NO synthases (NOS1/2/3), and is produced at higher concentrations in response to inflammatory stimuli. They cause lipid peroxydation, protein and DNA damage and stimulation of cytokine release.

Inhibitor of iNOS: Merkaptoalkyguanides are inhibitors of iNOS are found to decrease inflammation in animal models. They are found to block iNOS, scavenge

peroxynitrite and inhibit cyclo-oxygenase pathway.

3. **Regulating Cytokines:** Cytokines are defined as the chemical mediators of inflammation which are considered as regulatory proteins which involve in controlling cell proliferation, differentiation and specific function of cells. Their action can be paracrine, autocrine or endocrine in nature.

Microbial biofilm constituents stimulate the host to produce proinflammatory cytokines like IL-1b and TNF-α those contribute in connective tissue and alveolar bone destruction. These cytokines are present in diseased periodontal tissues and Gingival Crevicular Fluid (GCF).

The interplay of pro-inflammatory and anti-inflammatory cytokines decide the initiation and progression of periodontal disease. Cytokines which play a role in suppression of the destructive inflammatory response include IL-4, IL-10, IL-11 and TGF-β. [7]

4. **Others**

   a. NF-kβ inhibitors – pyrrolidine dithiocarbomate
      – proteosome inhibitors

   b. Integrin inhibitors – tepoxalin, sodium cromoglycate

B. Regulation of Arachidonic Acid (AA) CASCADE: The tissue damage leads to availability phospholipids which gets degraded by phospholipase A_2 and thereby results in production of free AA. These are further metabolized by two of the important patheways example, Cyclooxygenease (CO) or Lipoxygenase (LO) pathways.

Prostaglandins, prostacyclin and thromboxane, which are the final products of CO pathway and leukotrienes and other hydroxyl eicosatetraenoic acids of LO pathways play a vital role in inflammation.[8]

PGE2 and AA metabolites in increased levels are found in gingival crevicular fluid and periodontal tissues in patients with periodontal disease. Host modulation therapy can be achieved by modulating the release of these destructive products. NSAIDs is one of the approach by which these metabolites can be regulated.

1. **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) blocks cyclo-oxygenase pathways and reduce PGE2 synthesis leading to inhibition in progression of periodontal disease and further bone resorption.(8) Indomethacin, first proved the host modulatory effect by inhibiting the progression of periodontal disease in a ligature-induced canine periodontitis model.

NSAIDs can delivered both topically and systemically and have been proved efficacious in both forms for treatment of periodontal disease. The systemic delivery of Flurbiprofen has been found to have inhibitory effect on progression of periodontal disease. However, their systemic use counters few adverse effects like gasto-intestinal upset and heamorrhage.

The local application of NSAIDs is also a method to deliver these agents. NSAIDs are lipophilic and readily gets absorbed by the periodontal tissues. Few NSAIDs that are administered topically include ketorolac trimethamine rinse and S-ketoprofen dentrifice.

2. **Lipoxins:** Lipoxins are secreted by neutrophils and most commonly seen in elevated levels in patients suffering from aggressive periodontitis. Lipoxins are believed to be arachidonic acid derivatives formed by Lipooxygenase pathway and are potent inflammatory mediators. Lipoxins are also found to be secreted in increased quantities in gingival crevicular fluid.[9]

The approach in reducing the levels of Lipoxins would be beneficial in inhibiting the progression of periodontal disease by lowering down the inflammatory process or pathway.

4. **Triclosan:** Triclosan is an anti-inflammatory and anti bacterial agent. It is a non-ionic antimicrobial agent. Triclosan is believed to inhibits both cyclo-oxygenase and lipo-oxygenase pathways.

4. **Steroids:** Steroids are found to inhibit Phospolipase A2 enzyme by stimulating the excessive production of annexins/lipocortins. They stabilize the lysosomal membranes and suppress the cellular degranulation. Steroids like dexamethasone cause degradation of preexixting mRNAs for IL1β, TNFα thereby dampening PGE2 release.[10]

5. **Antioxidants:** Antioxidants like Vitamin C and E, Green tea catechins and other herbal products are believed to prevent oxidation of these arachidonic acid metabolites by molecular oxygen and subsequent hydrolysis to form PGE2.

C. Regulation of Matrix Metalloproteinases

(MMPS): MMPs are the enzymes which belong to the family of calcium and zinc dependant endopeptidases. They are secreted by neutrophils and macrophages, along with some resident cells like fibroblast, osteoblast, osteoclast, epithelial cells of periodontium.(11)

MMPs degrade the extracellular matrices like collagen, gelatin, proteoglycan, fibronectin at neutral PH levels. The pathogenesis of periodontal disease is directly proportional to the MMP activity. The bacterial plaque stimulates the resident cells of the periodontium, directly or indirectly, to release theses MMPs and thereby initiating connective tissue remodeling.

Polymorphonuclear leukocyte type collagenase (MMP-8) and gelatinase (MMP-9) are the most potent and are usually seen with increased activity during periodontal tissue destruction. In addition, MMP-13 (collagenase-3) is believed to be a mediator of bone resorption and cartilage destruction and has been identified in GCF from chronic periodontitis patients.

Some of the MMP inhibitors which could help in reducing the disease activity are as follows:

1. **Hydroxamic acid derivatives:** Galardin designed for topical use in non-healing corneal ulcers., Batimostat for breast cancer (parenterally administered) and Marimastat administered for cancer including ovarian, prostatic, pancreatic and gastrointestinal malignances, are the derivatives known to inhibit MMPs.

However, these derivatives may not have sufficient specificity to prevent unwanted toxicity, and includes adverse events including joint pain and stiffness.

2. **Tetracyclines:** Tetracycline belongs to the group of antibiotics which are most extensively investigated MMP inhibitors. Apart from the antimicrobial property, tetracycline appears to inhibit MMP activity drastically. Tetracyclines thereby inhibit extracellular matrix destruction by multiple non-antimicrobial mechanisms (e.g. chelation, inhibition of activation of pro-MMP molecules).(5)

Mechanism of action:

a. mediated by extracellular mechanisms:

- active MMP inhibition

- inhibition of oxidative activation of pro-MMPs

- disrupts activation by promoting excessive proteolysis of pro MMPs into inactive fragments

- inhibition of MMPs protects α1-PI

b. mediated by cellular regulation

- inhibits inflammatory cytokines, iNOS, PLA2, PGE2

- inhibits protein kinase C

C. mediated by pro- anabolic effects

- increases collagen production and bone formation

The reduced dose of doxycycline (20mg bid) is considered as one of the potent HMT agent. This low dosage of doxycycline may not exhibit antimicrobial effects, but can effectively lower MMP levels, hence, referred to as Sub-Antimicrobial Dose Doxycycline (SDD).

Chemically Modified Tetracyclines

CMT 1 – 4 dedimethyl aminotetracycline are also believed to inhibit MMP activities. CMTs 1,3, 6, 7, 8 are effective inhibitors of which CMT 3 is the most potent compound. CMTs 2 and 4 affects MMP8 but not MMP 13. CMT 5 is ineffective.

D. Modulation of Bone Remodelling: Bone is a dynamic tissue under constant remodeling with active formation and resorption. Disruption of balance in this leads to either increased bone formation as in osteopetrosis or increased bone resorption as in osteoporosis, osteopenia, periodontal disease etc.(8)

The effects of osteoporosis and periodontal disease predominantly after the age of 35 and have common risk factors that may interfere with healing (e.g. smoking and influence of disease or medications). Thus, therapeutic strategies used to prevent and manage osteoporosis and osteopenia may also inhibit periodontal bone loss.

1. **Bisphosphonates:** Bisphosphonates are known to manage osteoporosis in an effective way. The beneficial effects of bisphophonates are used nowadays for treatment of periodontal disease.

Bisphosphonates are non-biodegradable analogs of pyrophosphate. They have a high affinity for calcium phosphate crystals and that inhibit osteoclast activity. These compounds also appear to inhibit MMP activity through a mechanism that involves the chelation of cations.

They are classified into three generations of drugs:

First generation drugs with alkyl side chain: Etidronate

Second generation drugs with amino side chain: Alendronate. Pamidronate, zoledronate

Third generation drugs with cyclic side chains: Risedronate

Other drugs that can be used to modulate bone metabolism include,

2. Anti-inflammatory agents
3. Osteoprotegrin
4. Anti RANKL
5. Selective Estrogen Receptor Modulators (SERMs)
6. CMTs
7. Anti- integrins

Conclusion

The variety of known host modulation therapy helps clinicians to modulate the periodontal disease initiation and progression in an effective way. The use of these agents has led to avoid of advanced surgical approaches to treat such periodontal conditions. The prevention of periodontal disease at the host level is a promising strategy.

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Applied Aspect of Saliva in Orthodontics

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Abstract

Saliva influences oral health both through its non-specific physicochemical properties, as well as through more specific effects. The proline-rich proteins, statherin and the histatins are salivary proteins that influence calcium phosphate chemistry, initial plaque formation and candida infection. Increases or decreases in mastication may affect saliva output. Fixed orthodontic appliances introduce an additional constituent to the oral cavity complex that may enhance the environment in a variety of ways. On the other hand, Orthodontic treatment leads to changes in the oral environmental factors that encourage changes in salivary flow rate, viscosity, pH, bacterial count, increased plaque index, and other more things which all are factors supplement the risk of caries activity and shake the stability of the oral environment. This manuscript highlights the effect of saliva on orthodontic treatment.

Keywords: *Orthodontic Treatment, Saliva, friction, brackets.*

Introduction

The quality and the quantity of saliva play a crucial role in the equilibrium between demineralization and remineralization of enamel in a cariogenic environment. Specific changes, such as increased pH, buffer capacity and flow rate, may contribute to decreased susceptibility to dental caries. All these salivary properties become of utmost importance during orthodontic treatment with fixed appliances, when an increased chance of plaque retention and a greater difficulty in optimal oral hygiene maintenance is thought to predispose to enamel demineralization and white spot formation. There is still no consensus on the way the quality and the quantity of saliva change during orthodontic treatment. So far, investigations have been confined to the first 6 months from the placement of fixed appliances, and no data are yet available in the long term. In this article we have discussed different aspects of saliva and its effect on orthodontic treatment[1].

I. **Friction:** It has been suggested that saliva or saliva substitute services as an excellent lubricant in the sliding of the bracket along the wire. Frictional changes in force values caused by saliva substitution were studied by Baker et al in1987 to determine the magnitude of frictional force changes between several sizes of stainless steel orthodontic archwires i.e. 018, 020, 018 x 025 and edgewise 022 x 028 slot when an artificial saliva medium was introduced. The teeth were bonded with brackets and wire assemblies and subjected to Instron universal testing machine and subjected to tension-type of force[2].

After tabulating results they concluded that Xero lube, saliva substitute as a lubricant provided a 15% to 19% reduction in force value requirement when using 018, 020, 018 x 025 wires. They also concluded that the saliva medium used has a viscosity of 14.0 centipoise at 37°C. They conducted similar research using glycerine which has a viscosity of 325.0 antipose but results obtained did not match to that obtained with xerolube artificial saliva.

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To study the Frictional resistance of ceramic and stainless steel orthodontic brackets, Kratten, Popli, Germanine in 1990, conducted a similar type of research and found that saliva substitutes increased static friction for all combination tested. Saliva played an insignificant role in lubricating the surface of the wire or bracket slot. The explanation for the discrepancy may lie in the significance of loading forces used between the archwire and the brackets. At low load levels saliva acts as a lubricant, but at high loads saliva may increase friction if it's forced out from the contacts between the brackets and the arch wire[3].

The effect of artificial saliva on the frictional forces between orthodontic brackets and archwires was studied by Douning, Mc Cabe, Gordon in 1995. Here the effect of artificial saliva on the static and kinetic frictional forces of stainless steel and polycrystalline ceramic brackets in combination with 0.018 inch round end 0.019 x 0.025 inch edgewise archwire sizes and stainless steel, nickel-titanium and β-titanium archwire materials under a constant ligature force were investigated. In all the cases artificial saliva had the effect of increasing the frictional force when compared with the dry state. They concluded that artificial saliva played an insignificant role in lubricating the surface of the archwire in the bracket slot. The explanation they gave for this study was that archwire touches the bracket at only 2 points where the pressure is relatively great. The lubricant could be expelled from the areas of contact allowing no lubrication between the archwire and bracket to exist, hence increasing friction. Use of β titanium archwire produced the smallest percentage increase in frictional forces due to; stick and slip phenomenon[4].

II. Bonding: The Effect of saliva on shear bond strength of orthodontic adhesive used with moisture insensitive and self-etching primers was studied by Zoppieri IL, Chung CH, Maste to investigate the effect of saliva contamination on the shear bond strength of an orthodontic adhesive used with transbond–moisture Insensitive primer (MIP) and transbond plus- self-etching primer (SEP). Hydrophobic transbond XT primer was used as control. It was concluded that Transbond XT adhesive with XT primer and MIP in dry field yields similar bond strength which is greater than all other groups. Saliva contamination significantly lowers the bond strength of transbond MIP. Saliva has no effect on the bond strength of SEP Transbond XT adhesive with transbond MIP and SEP might have clinically acceptable bond strengths in either dry or wet fields.

The active ingredient of transbond SEP is a methacrylate phosphoric acid ester formed when phosphoric acid and a methacrylate group are combined into a molecule that etches and primes simultaneously. The conventional phosphoric acid-etched teeth are washed or rinsed after adequate etching time. Similarly the phosphate group in methacrylate phosphoric acid ester in SEP dissolves the calcium and removes it from the hydroxyapatite. The calcium then forms a complex with phosphate group, rather than being removed by rinsing and is incorporated into the network when the primer polymerizes. The acid group is neutralized by the reaction with calcium. Thus there is no need to rise the tooth because no etchant remains on the enamel[5].

Effect on a New Bonding agent in bond strength to saliva contaminated enamel was studied in 1994 to compare bond strengths of brackets applied to contaminated and uncontaminated enamel following pretreatment of contaminated enamel with Scotch bond MP bonding system. They concluded that bond strength was found to be equal in brackets bonded to saliva contaminated etched enamel treated with Scotch Bond MP primer and bonding agent applied to uncontaminated enamel. Scotch band MP works slightly differently. The primer composed of HEMA and polyalkeonic copolymer behaves similarly to the liquid of glass ionomer in that it forms stronger bonds to a moistened enamel or dentin surface[6].

Greer KS, Lindaver SI, Darling SG in 1996 developed a technique to prevent surface contamination of etched enamel. It has been recommended that a thin layer of primer be applied to the entire etched enamel surface before bonding to seal the enamel, protect it from decay, provide maximal bond strength. If the etched surface becomes contaminated by oral fluids before bonding the bond is likely to fail at resin enamel interface. Contamination can occur during the application of the primer if saliva or sulcular fluid is unintentionally spread by applicator brush. This is most likely to occur when bonding to surgically exposed palatally placed canines or to teeth with short clinical crowns. In such cases it's more effective to apply the primer directly to the adhesive resin on the back of orthodontic attachment. Bond strength in this technique was considered best in cases where there is poor visibility or limited exposure[7].

Isolation: The oral environment needs to be

adequately controlled to prevent from interfering with the execution of any dental procedure. This control is attained through isolation.

Various means of isolation are Fluid absorbents, Saliva ejectors, Rubber dam and Pharmacologic agents.

A. Fluid Absorbents: Here isolation can be achieved by absorption of salivary secretions by cotton rolls with holders, Gauze or throat shields or Absorbent wafers. These can be used for short periods when absolute dryness is not required.

B. Saliva Ejectors: They prevent the pooling of saliva in the floor of the mouth. There are twotypes of saliva ejectors: High volume – they remove metallic debris and Low volume. Based on the material from which they are manufactured; Metallic ones can be autoclaved, but requires delicate use to prevent injury whereas Plastic – disposable and in expensive[8].

Advantages:

- Provides adequate dry field.
- There is no dehydration of oral tissues

Precaution:

- Saliva ejector should be sterilized or disposed to prevent cross-contamination.
- Disposable types are preferred
- Patients should be instructed not to close his mouth with the lip in mouth since the backflow of contaminated solution into the mouth is possible.

C. Rubber Dam: Use of rubber dam provide a clean, visible field and Prevents aspiration of foreign bodies it also reduces the risk of cross-contamination and Improves properties of adhesive materials.

III. Salivary Clearance and Fixed Orthodontic Appliance: Since fixed ortho appliances have numerous recesses, pits, which entraps the food particles, the oral clearance rate is slowed. In 1992 Magnus Forsberg, lively A, Jagerlof F conducted a study to establish the possible influence of orthodontic therapy with fixed appliances on salivary clearance of sugar. The study aimed to evaluate the effect of fixed orthodontic appliances on the residual volume of saliva in the mouth and Salivary clearance of sugar. A total of 15 patients, 2 males and 13 females scheduled for orthodontic treatment with fixed appliances took part in the investigation. Unstimulated salivary flow rate, RESID, and salivary clearance of sugar were determined on two occasions i.e. before the start of treatment and 21 days after a fixed appliance was placed in the mouth. In the results it is seen that the salivary flow rate before the start of orthodontic treatment was 0.46 ml/min, after a wear of appliance for 21 days mean value increased to .57 ml min).

Orthodontic appliance had a similar effect on the residual volume of saliva in the mouth after swallowing. It is a well-known fact that a foreign body put in the mouth will initially increase the flow of saliva. The present study indicates that fixed appliances do not prolong the salivary clearance of sugar during the first month of treatment[9].

Further studies with longer duration claimed to have decreased or normal levels of salivary flow and RESID. Since the appliance consists of many retentive components that provide numerous recesses and minor pits where food particles may be trapped accounting for delayed clearance of sugars on long term treatment schedules.

Because of this factor caries and demineralization will continue to be matters of concern during orthodontic treatment.

The effect of polyol gums on dental plaque in orthodontic patients was studied by Isotupa et al in 1995. They conducted a study on 60 patients wearing fixed orthodontic appliances, who were given chewing gums containing polyol to find out the number of streptococcus mutans present in saliva. They concluded that patients chewing xylitol-containing chewing gums had less amount of *Streptococcus mutans* level and less incidence of caries. A clinical study was carried out IN 1996 to determine the acceptability of a sugar-free, low tack chewing gum by orthodontic patients. It was concluded that low tack sugar-free chewing gums can be used by orthodontic patients to increase saliva flow with the potential to remineralize and help reduce white spot lesion formation. Freedent peppermint gum, from Wrigley company, was the product they used in their study[10].

4. Experimental Salivary Pellicles on the Surface of Orthodontic Materials: Lee SJ etal conducted a study with a purpose to find out whether the composition of salivary pellicles that form on the

surface of orthodontic materials vary qualitatively concerning stainless steel, elastomeric ligature ring, bracket bonding resin. They concluded that the least amount of salivary pellicle which was cariogenic was found on stainless resin, followed by adhesive resin, the highest amount of cariogenic pellicle was found on elastomers[11].

5. **Effect of Salivary Ph on Orthodontic Polyurethane Chain Elastics:** The effect of hydrogen ion concentration on forces degradation rate of orthodontic polyurethane chain elastics was studied by Ferriter and Meyers. The effect of pH on force degradation rates of seven commercial orthodontic polyurethane chain elastics was evaluated in an in vitro study. The pH values of 4.85 to 7.26 were selected for testing because they represent values closes to the reported extremes of plaque and saliva pH. All specimens were equally stretched which delivered equal initial force levels. Force degradation rates were recorded after 4 weeks. They concluded that the force decay rate of polyurethane orthodontic chain elastics is inversely proportional to the pH of the oral environment with a corollary that pH levels above mentioned are more hostile to the polyurethane chain elastics thus increasing their force decay rates[12].

6. **Saliva and Corrosion:** Saliva acts as an electrolyte and hence aids in causing corrosion of metal components of fixed orthodontic appliances. When metal components of orthodontic appliances are in contact with an electrolyte such as saliva, metals corrode by a complex electrochemical process of oxidation and dissolution known as galvanic corrosion. The generation of an electric cell is simple when different metals are involved, but it can also occur within a single metal. Atoms at the grain boundary dissolve faster than those within the grain. Impurities, rough surfaces or irregularities can also alter the corrosion resistance of a metal.Corrosion-resistant metals are known as noble metals or cathodic.

Types of corrosion:

1. **Uniform corrosion:** Metal is attacked evenly and throughout, and its mechanical property diminishes proportionately. This type is rarely seen in orthodontics since all the parts of the appliance are not evenly exposed to corrosion agents.

2. **Localized or pitting corrosion:** The most common form seen in orthodontic attachments. Affects the mechanical property of the metal. This type is seen when several different metals are used.

3. **Galvanic corrosion:** The oral cavity because of saliva, with its salts provides a weak electrolyte. Galvanic corrosion is an important type of electrolyte corrosion which occurs when a combination of dissimilar metals lie in direct physical contact with each other.

4. **Stress corrosion:** If a stressed metal comes in contact with unstressed metals,stressed metal will become the anode of the galvanic cells and will corrode[13].

   Nickel and chromium levels in the saliva and serum of patients with fixed orthodontic appliances were studied by Agaogh, Arun T, Belgin Izgu to evaluate the concentrations of nickel and chromium ions in salivary and serum samples from patients treated with fixed orthodontic appliances. The second aim of the study was to determine any significant changes in these concentrations during any period of the treatment time. Saliva and blood samples were collected from 100 patients ranging in age from 12 to 33 years. The fixed appliances consisted of an average of 4 bands and 20 banded brackets. The results indicate certain differences in the amount of nickel and chromium released from fixed orthodontic appliances during different periods of treatment. In saliva samples, nickel and chromium reached their highest levels in the first month and decreased to their initial level in the rest of the groups. It can be concluded that a fixed orthodontic appliance releases a measurable amount of nickel and chromium when placed in the mouth. Most metals used in the oral cavity can be expected to undergo this type of corrosion. Saliva has a dynamic composition that may be affected by many physiologic variables such as diet, salivary pH, health conditions and salivary flow rate[14].

7. **Saliva and Demineralization (Caries):** The pH of saliva acts as a deciding factor, be it demineralization and induction of carries or remineralization. At pH value of 6.8 to 6.0 hydrogen ions react with phosphate ions in saliva and plaque. At pH value of 5.5 to 5.0 demineralization occurs wherein hydroxyapatite dissolves but fluorapatite forms in the presence of fluoride. At a pH of 4.5 to 3.5 (critical pH) both fluorapatite and hydroxyapatite dissolves. On the

contrary if pH rises to 5.5 from the critical pH and if H^+ ion are not exhausted remineralization occurs and fluorapatite forms[15].

Conclusion

With the innumerous number of functions of salivary glands and its secretion namely saliva that we have come across, we can arguably call this secretion namely saliva the champion among the factors that are involved in the homeostasis of our body. Since dentistry is a material science thorough knowledge is important for us to come out with innovations so that products we use render maximal service to the patient.

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A Full Mouth Rehabilitation of a Patient with Uneven Occlusal Plane and Severe Attrition: A Clinical Report

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Abstract

In clinical practice we usually come across cases with severe tooth wear which requires a full mouth rehabilitation. And management of patients with worn-out dentition was always a tough and complex procedure. For a predictable and favourable treatment outcome a systemic multidisciplinary approach was required. This case report describes the interdisciplinary approach in the treatment of a patient with severe attrition and loss of vertical dimension. After performing endodontic treatment for required teeth, a bite raising transitional prosthesis was given for increasing the VD to acquire essential interocclusal space for post and core buildups and full coverage restorations. After that the porcelain fused to metal permanent restoration was fabricated and scrutinized for stable contact in centric and eccentric relation.

Keywords: *Attrited teeth, full-mouth rehabilitation, Occlusal splint, vertical dimension of occlusion.*

Introduction

When we get a patient with mutilated dentition we as dentist are very happy thinking about it financial aspect only. But to manage these patients of mutilated dentition by restorative dentistry is a tough job. And the dentist need to be trained in different aspect and concept of full mouth rehabilitation. So these patients need a full mouth rehabilitation with a systemic multidisciplinary approach[1]. Severe attrition of the teeth leads to loss of anterior guidance, and overall vertical dimension. To restore the same aesthetically is a challenging job for a novice dentist. So for prosthetic rehabilitation of these patients a multidisciplinary treatment planning is required to restore occlusal vertical dimension (VD) and interocclusal rest space.[2] As an adaptive process in the alveolar bone continues to compensate for the loss of tooth structure due to attrition so an increase in vertical dimension should be done systematically after careful assessment by an occlusal splint.[3,4]

Tuner[5] et al gave a classification of severe wear patients into three categories:

- Category 1: Excessive wear with loss of occlusal vertical dimension
- Category 2: Excessive wear without loss of occlusal vertical dimension but with space available.
- Category 3: Excessive wear without loss of occlusal vertical dimension but with limited space

This case report describes in detailed full mouth rehabilitation of a patient with a multidisciplinary approach in which an endodontist, a prosthodontist, a periodontist, worked together for occlusal rehabilitation of mutilated dentition with loss of vertical dimension. The patient was having wear without loss of vertical dimension but with limited interocclusal space(category 3). As these category patients are most difficult to rehabilitate as the adaptive process has maintained the vertical dimension and to accommodate the restorative materials interdisciplinary approach is required.

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Increasing the vertical dimension within the permissible limit is very important and systemic procedure has to follow. The procedure is sequential use of occlusal splint and provisional restoration to slowly increase the tolerance of patients to increase in vertical dimension.

Case Report: A 58-year-old male patient reported to the department of prosthodontic Institute of Dental Sciences, SOA Deemed to be University with a chief complaint of difficulty in chewing and speech problems. The patient gave a history of paan chewing for the last 25 years. The medical history of the patients was non-contributory to the contraindication of dental treatment. On intraoral & radiographical examination, it was found that there was severe tooth loss in the anterior and posterior region in the maxillary and mandibular arch. (Figure 1a). The esthetic concern of the patient was short teeth, lack of display of teeth during rest or smile. And darkened appearance of teeth was due to exposed dentine and bad habits. Due to bruxism an uneven occlusal plane was observed. And to the surprise of all there was no sign or symptom of temporomandibular joint pain or dysfunction. On periodontal examination there were no signs of pocket and status was fair.

Diagnostic procedure: Diagnostic cast revealed reduced clinical crown due to severe attrition. On intraoral examination the discrepancy was found between centric occlusion and maximum intercuspal position. Interocclusal vertical dimension was assessed by measuring the distance between the chin and the nose, the vertical dimension at rest was found to be 58 mm and at occlusion was 52 mm (Figure 1b). Features of collapsed vertical dimension was observed on facial examination. Pseudo mandibular prognathism with a reverse smile line was observed. Radiographic examination revealed partial obliteration of pulp chamber in 31,32,33,34,41,42,43,44. No evidence of any periapical pathology was observed in any teeth. A treatment plan was developed for full-mouth rehabilitation. The objective was to restore the masticatory function and esthetical appearance of the patient. This was done by raising vertical dimension, in the first phase by stabilizing occlusal splint, followed by provisional restoration.



Figure 1a, 1b:

Treatment Procedure: The patient was explained about the entire treatment procedure. The photographs and diagnostic casts were prepared for records. In the following visit, the treatment was explained in detail, it started with root canal therapy of all teeth, then an occlusal splint of hard acrylic has to be worn by the patient for one month, followed byperiodontal treatment of crown lengthening of twelve teeth and at the end,prosthetic rehabilitation was to be done with porcelain fused to metal crowns. Before definitive treatment diagnostic cast was obtained and articulated in semi-adjustable articulator in centric relation. The mandible was guided to the centric position by bimanual palpation and the bite registration was done by bite registration material with

the help of acrylic anterior deprogramming device. the curve of Spee, Wilson & orientation occlusal plane was determined by the Broadrick occlusal plane analyzer. On the articulator the incisal pin was raised by 3 mm and diagnostic wax-up was done in the new vertical dimension of occlusion. (Figure 2).



Figure 2

The first phase of treatment root canal therapy was performed on all the remaining teeth and completed in a phased manner. The occlusal deprogrammer splint with a height of 3 mm was given to the patient for one month(Figure 3a). The splint was designed to offer bilateral contact in posterior in centric relation. And the anterior guidance disoccluded the posterior teeth in all eccentric movement.

The adaptation of the patient to a new vertical dimension was evaluated after one month. The patient reported no muscle tenderness or TMJ discomfort. The surgical phase of crown lengthening was performed using electrocautery to expose the tooth margin of all the anterior teeth. Post and core build-up of the fifteen teeth were done with Coltene Paracore core build-up material & tapered fiber post. Tooth preparation of all the maxillary and mandibular teeth are done in a phased manner (Figure 3b). The diagnostic wax-up was used as a stent for the fabrication of autopolymerizing resin provisional crowns. The provisional were fixed with temporary cement and patient adaptation was monitored. (Figure 3c).



Figure 3a, 3b, 3c

For one more month the interim restoration was adjusted and monitored for any discomfort and muscle tenderness. The mandibular movements, speech, and swallowing was evaluated and adjustment were done to achieve comfort for the patients. And this occlusion was used as a guide for definitive oral rehabilitation. The final preparation was done and the final impression was made with polyvinyl siloxane impression material. Bite registration was done in centric relation using the incisal guidance established with provisional prosthesis. (Figure 4a). The definitive anterior guidance was replicated using the customized anterior guide table. Wax patterns were fabricated in the articulator at the predetermined vertical dimension. The metal crowns fabricated were evaluated radiographically and intraorally for fit, retention and stability (figure 4b). The metal-ceramic was fabricated with equal intensity centric contact in posterior teeth and group function in the anterior region to dis-occlude the posteriors in eccentric movements. Final occlusal adjustment was

done intraorally in the bisque trail. The metal-ceramic crowns were cemented with type I glass ionomer cement.(Figure 4c) and hard acrylic resin occlusal splint was fabricated and given to a patient for use during the night time to prevent occlusal wear due to bruxism. Oral hygiene instruction was given to the patient. On periodic evaluation the patient reported the prosthesis to be comfortable in function (Figure 5).



Figure 4a, b, c

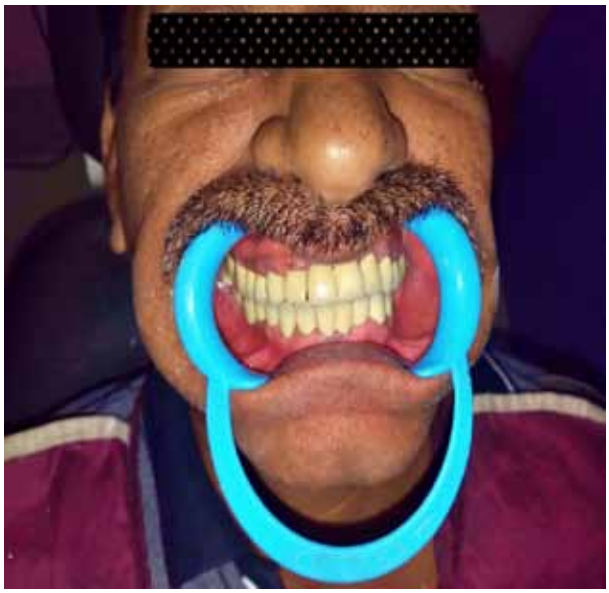


Figure 5.

Discussion

Occlusal rehabilitation of a functionally weakened dentition requires an interdisciplinary treatment approach. We have to restore the esthetic & functional aspect of the patient and our end goal should be a healthy functional masticating apparatus[6]. Tooth wear has many cause[7], and it may be generalized in many patients. Tooth wear distribution was found to be uneven about anterior versus posterior. This was due to posterior occlusal prematurities and bruxism that leads to loss of vertical dimension[8] Clinical controlled trials of restorative and prosthodontic approaches are limited in quantity and quality[9]. So the clinical judgment of the prosthodontist plays an important role in the rehabilitation of the patient. Phonetics, swallowing and patient preference plays a major role. The loss of interocclusal space can be compensated by the following means[10].

- Occlusal adjustment
- Elective endodontics treatment of attrited tooth and core build-up of the same.
- Increasing the vertical dimension by giving occlusal splint, provisional restoration.
- Crown- lengthening procedure; surgical exposure of adequate tooth structure to help in esthetics and retention.
- Orthodontic movement to create interocclusal space.

In the present case,multi-visit endodontics was preferred over a single visit as it has a proven record of

better healing and less post-operative complication.[11] In this present case lack of restorative space was a huge challenge, so the vertical dimension was increased for optimal functional and esthetic[12] The phase of occlusal splint allows time for neuromuscular adjustment to increased vertical dimension and helps to determine patient's compatibility to the new position[13] The minimum recommended period for adaptation to this increased vertical dimension has been determined to be 6–8 weeks.[14] Prefabricated posts were given for anterior teeth as these are simple to use and require less chair side time[15]. Thin tapered treaded posts were used in the present case, as these prevented excessive dentin removal. Threaded post provides the highest resistance to rotational forces and produce uniform stress distribution along the post length. Crown lengthening allowed apical migration of attached gingiva in natural tooth structure so proper core build-up could be done.. So in this case interdisciplinary approach played an important role in enhancing the esthetics, function and to re-establish the lost occlusal function of the patient.

Conclusion

Full-mouth rehabilitation of a bruxer is complex and difficult,and it requires the proper application of interdisciplinary concepts to achieve functional and esthetic success.

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Dental Health Care to the Rural Population through Private Dental Institutions

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Abstract

Republic India in the present situation is a fast developing nation whose population is more than one billion today. Looking at the current situation of India there is a minimal increase in the number ratio of various dental specialized surgeons and its population. The burning issue of dentistry in India is not the insufficiency of manpower but uniformity and equitable distribution. The most off-balanced circumstances are 68.89% people of India residing in rural areas and more than eighty-five percent dentist practicing in city areas needs to be properly balanced. One of the issues related to the dental workforce in India is the deficient manpower planning and projection. Due to a shortage of lawful and logical data for gauging of community demands and very less amount of guiding system for monitoring orofacial health care services need to guide planners. This paradoxical situation needs to be looked into immediately. At this time, public stash is scant and competed for strongly. It should not a proper time to schedule large and luxurious programs. State Governments to incorporate various suitable orofacial or oral care planning in its agenda as a matter of prime concern and create the groundwork for dental clinics at general public Hospitals, Primary Health Centers, Sub-centers as well as at referral dental centers in rural areas, to provide oral health care to our rural population.

Keywords: *Dentist-population Ratio, Community Demands, Rural Population, Oral Health Care Service.*

Introduction

Republic India in the present situation is a fast developing nation whose population is more than one billion today. Out of a hundred percent, more than seventy percent of people inrepublic India live in more than 5,50,000 villages, and the remainder around thirty percent are residing in around two hundred well-developed towns and big cities areas.[1] Rural health care groundwork has been developed to protect the rural population through 1,56,231 sub-centers (SCs) till 31st march 2017, 23,109 primary health centers (PHCs), and 4,833 community health centers (CHCs).[2]

In the year 1994 oral health comes into the eye-catching point of WHO and after that WHO chose the theme "Oral Health for Healthy life" for World Health Day.[3] National Oral Health Policy has been composed by the Dental Council of India. The results of two national symposia organized way back in the year 1991 at Delhi and another city of republic India Mysore in the year 1994.[4] Though this policy has never been implemented nor there is oral health care program at national level.[5]

The most off-balanced circumstances are 68.89% people of India residing in rural areas and more than eighty-five percent dentist practicing in city areas needs to be properly balanced[6] in republic India the government fixed a good amount of budget for health purpose from that huge amount only a little amount is

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sanctioned for oral health-related activities. There is no specific separate allocation budget or any specific care for oral health in the Indian budget like general health.[5]

The distribution of BDS and MDS professionals are not proper in republic India. In the private sector more BDS and MDS professionals are working now a day as compared to a public sector that provides subsidized services financial affordability for dental care also becomes a huge barrier in low-income population or under below poverty line population. Form the various studies it is estimated that in republic India above 15 years' people has some form of periodontal disease and its prevalence is around forty-five percentage, near about three percent of the population in between 35 to 50 years has oral cancer, the percentage of dental fluorosis is about 5.5 and a huge amount of people of republic India live below the poverty line.[7]

In recent period all dental professional faces tremendous problems in serving dental services to all citizens. In our republic India Oral healthcare facilities are given through primary health care groundwork is of limited dental care facilities and dental manpower. There are nine specializations in dentistry but till now there is no such dentistry specialization posting or simple BDS posting at PHC level throughout the republic India for the betterment of oral health.[5]

Despite 292 dental colleges in the country, the oral health of our people does not so good enough to the mark, especially to those people who are mainly residing in the rural areas.[6] in republic India there are large skewed distribution of dental colleges, a huge variation in the distribution of different specialization of dentist to total population ratio residing in the rural and as well as in the township areas is seen. The present situation of republic India is 1 dentist for ten thousand people in township areas and approximately two point five lakh persons in rural areas. Most of the time it becomes much more difficult for people who are residing in the rural geographical areas to get emergency oral care facilities or dental facilities. So, we are discussing the problem of difficulty in accessing the oral health care facilities for the rural masses and its remedy through the private dental institutions.

Why focus Oral Health through dental institutions

1.36 billion is a huge mass for these mass providing basic dental care is one of the top challenges facing the oral health care delivery system in India.[8,9] In the rural population, there are negligible dental health services available either through the government or private clinics. As dentistry is expensive, the approach by dental institutions can help 99% of the rural population of India.

Every health service in rural areas are primarily conducted through the primary health centers (PHC), which full fill the health needs of twenty thousand to thirty-thousand people. The PHC holds a key role in the countries health care delivery system; it aims to provide three preventive health care facilities such as primary secondary and tertiary care, promotive care and curative health care services to the people living in a different geographical area. not even out of 100% existing primary health centers less than twenty percent of the existing primary health centers in India have the dental health care available for the population. The oral health services delivered through the primary health centers are deficient in manpower and infrastructure as well.[5]

The focus on the dental institutions to provide dental health care is because of the inadequate Workforce in Rural Areas and skewed Dentist-Population Ratio. The primary goal of dental institutions in rural areas should be to provide qualitative, effective, most basic oral health care facilities to all the people residing in rural areas. Every dental care which is provided to people that includes oral health education, primary, preventive dental care, curative dental-care and rehabilitative oral care.

The prevalence of dental diseases i.e. dental caries, periodontal disease and oral cancer is increasing in the Indian population[6] with a comparatively more increase in the rural areas. To date, 292 Dental colleges are working in India which are permitted by DCI of India, from 292 dental colleges and hospitals only 40 colleges in government colleges, rest are in the private sector.[10] So, there are a vast number of private dental colleges in India which can be potentially utilized for catering oral health services to the masses in the rural areas.

Services provision through Private Dental Institutions

The private dental health institutions can provide services to the rural people such as education and motivation, spreading awareness about different oral diseases, Free dental check-up and treatment camps, outreach treatment camps, service provision through Mobile dental vans, charging a minimal fee for services by having poor people fund, setting up satellite clinics in

the rural areas, school oral health programs, no charges forall dental check-ups for below poverty line families and smart card schemes for BPL families.

Dental institutions can playan important role in educating and motivating the rural population. The educational presentation via charts, models, electronic projectors which explains various etiology of all typesof oral diseases and the importance of 1st stage diagnosis as well as some important diagrams of oral diseases and how they appear in the internal oral environment can be shown to the villagers. Different kinds of oral health care delivery like oral health education (two times brushing daily, taking fresh fruits, avoidance of the use of tobacco etc.) are the easy ways to combat unhealthy practices which leads to such health hazards. The prevention of oral health canthe most affordable, tailoring and desirable measure in the current context of the socio-economic status of our Republic India.[10]

Those people who are residing in rural areas, for them it is the most difficult task for them to visit a dentist for regular dental check-up. Poor groundwork and heavy traveling fair on traveling to and from the clinics, keep lots of rural families away from receiving oral care for a long period. The regular basis dental screening camp, apart from being a perfect tool for the dental surgeons to reach those in need of dental care, is also a perfect setting for dental interns to come in contact with the community people and understand their basic oral health problems.

Free transport facilities can be provided by dental institutions so that the rural population especially the underserved population can easily avail the oral health care. Dental institutions can provide the best treatment at minimal cost providing reduced-fee or uncompensated oral health care to the underserved patient population and Dental institutions can get a sufficient amount of patients for their students, which can fulfill their educational needs.

There should be different programs developed to provide services to individuals with special health problems or patients with special conditions especially the elderly population. Fully equipped Mobile dental van programs can help out the needy patients, especially, those individuals in rural settings with special needs who cannot come to the dental institutions for dental care.[11]

By oral health education rural population should be made aware of the real value of oral health and hygiene and how important is oral health for them, the effectiveness of Indigenous method, teaching about the correct technique of Brushing,teaching about Oral diseases- causes, consequences and prevention. Regular basis oral check-up and treatment camps in rural areas organized by NGOsand various dental colleges cater to the felt needs of the poor rural citizens by providing treatment as well as education about maintaining perfect oral care.

The dental collegesprovide screeningprograms, advice and oral health education to people in faraway villages, which is very effective. For the people residing in the rural area who attends the Camp, this is the only possible pathway for them to receive oral health-related advice, guidance and emergency treatment.A lot of requests for service should be issued by the various community people. These requests may be initiated by the oral health council, the dental health unit, or any concerned NGOs.

All rural people should have taught about the interaction link between the oral and various kinds of systemic diseases. A healthy oral environment can prevent infection throughout the body and protect primary and permanent dentition from dental caries. An unhealthy mouth (intraoral environment), especially one with various types of periodontal disease, may increase the risk of developing communicable heart disease like cardiac arrest, stroke, poorly controlled diabetes mellitus and premature birth. Some systemic conditions such as Acquired Immune Deficiency Syndrome or diabetes mellitus type 1 and 2 sometimes appears first as mouth lesions or other oral related problems. More than ninety percent of all systemic diseases show oral signs and symptoms inside the mouth. So,dental institutions can spread awareness about the different systemic diseases, and their effects on the overall health of the patient.[10]

Activities of peripheral centers which are usually situated in the rural areas or semi-urban areas can emphasize community-based preventive and educative programs as well as basic oral health care. The main goal of the rural dental clinic program is to build a bridge to fill the gap between discipline-based oral educational atmosphere and independent atmosphere for a modern dental practice. All emergency treatment and examinations are performed at satellite clinics and patients are referred to the college for further treatment.

All dental institutions have to make a suitable program for providing regular basis free oral screening

camps on a large scale focusing main concern on good oral habits with the provision of free toothpaste samples and toothbrushes, mouth wash for school children village people who can not afford for these things. This can be done through collaborations with different companies like Colgate, Oral B or NGOs.[10]

Discussion and Conclusion

In recent period all dental professional faces tremendous problems in serving dental services to all citizens. Among various developing countries republic, India is one of them, in which dental care provided by different specialized dentists like the endodontist, periodontist, public health dentist, oral surgeon prosthodontist pedodontist, etc, these dentists are practicing in the township area and treat those people whose financial state varies high. In most of the case,it is very difficult for the poor township citizen and the rural citizen to get the emergency dental care. Community-based dental health programs are rarely found. The topmost missing link creating this unfortunate condition is the absence of provision of dental care through primary health care in republic India.

Expenditure of Indian people over primary preventive health care is very much less as compared to curative health care as a whole. What Indian citizen spending over their healthcare is coming amongst the lowest in the world, whereas its maximum percent of expenditure on the private sector is one of the highest. About one lakh crores are being spent yearly as household expenditure on health-related problems (Source: NRHM).This is probably 3 times the public expenditure on different health aspects. Day by day looking at the urgent need for health care in our country most of the private health institutes are pushing the cost of healthcare up and making it out of reach for the poor rural citizen. Thus the health system in rural India would become untenable. The top challenge of qualitative and quantitative health services in rural areas has to be met with a touch of urgency. Given the opportunity and magnitude of the various problems, it is taken into consideration that primary focus should be given on goal-oriented projects to offset this malady.[11]

As compared to the urban areas there is less competition between the dentists due to fewer dentists in rural areas and the clinical setup costs also less in the rural areas as compared to the township area. Therefore,the government should take immediate steps for the establishment of dental set up in rural areas by providing necessary dental care facilities. They should also focus on strengthening the already existing services in the rural areas and also setting up new facilities in the rural under government regulation. The regular dental visit to a dentist for a dental check-up in the rural areas is very less or in some cases it is nil due to lack of knowledge about maintenance of oral hygiene and low financial condition is also cause for that.

Dental institutions play a major role in providing dental care facilities to under served communities mainly in rural areas. The main mission of all dental institutions should be to succeed, to get in touch with every village and provide basic and emergency oral health facility and it would be possible only when the community is fully aware to take leadership in every health aspect.It is recommended that the government should open the dental colleges in the rural areas. Public health dentists have to take the responsibility of increasing the oral health awareness and its association with the general health (how oral health negligence can lead to various systemic disease) to rural community.[11]

All dental institutes should have their rural satellite clinic in the rural areas and they have adopted maximum villages and schools where they can visit there on a regular basis to provide basic dental care and emergency dental care to the needy and educate village people about maintaining good oral health. A mandatory rural posting of 90 days for the dental interns, which will be very effective for large mass residing in rural areas.

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Treatment of Gingival Recession by Lateral Positioned Pedicle Graft: A Case Report

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Abstract

Gingival recession (GR) is a condition in which there is an apical shift of gingival margin leading to exposure of root surface. On exposure of root surface, there will be root sensitivity, pain, root caries, poor aesthetic, and ultimately tooth loss. There are various techniques used to treat gingival recession. In this case report, a laterally positioned pedicle graft technique was used to treat gingival recession. A 37-year-old male came to the Department of Periodontics, with a chief complaint of sensitivity in lower left front teeth regions. On clinical examination, there was Miller Class III in 33 with the presence of generalized mild plaque and calculus. It was planned for phase I therapy followed by phase II therapy. So scaling and root planing was performed to reduce inflammation and after 4 weeks, lateral positioned pedicle graft technique was done under infiltration local anesthesia. The subject was instructed to maintain oral hygiene. After 2 months, it was found significant coverage of root with keratinized gingiva. He was very satisfied with the result.

Keywords: *Gingival recession, laterallypositioned pedicle graft, root coverage, root planing, scaling.*

Introduction

Gingival recession (GR) is a condition in which there is an apical shift of gingival margin from cementoenamel junction, leading to exposure of root surface.[1] On exposure of root surface, there will be root sensitivity, pain, root caries, poor aesthetic, and ultimately tooth loss.[2] The severity of GR is found more with age progression, smoking, labially placed tooth, abnormal frenal attachment and sites with supra- and sub-gingival calculus.[3] This GR may be due to various etiopathogenesis.[4] The most common etiology is faulty tooth brushing and labially placed tooth. Many techniques and flap designs are performed to treat GR.[5] Among them, some techniques do not need a donor site as a pedicle graft, while others do as a free autogenous graft. It is very difficult to predict the success rate among various techniques used for the root coverage or the treatment of GR. The success rate of these techniques depends upon several factors such as Miller classification, location of the recession, operator's skill, patient maintenance, patient systemic health and the technique performed.[2] The gingival dimension can be assessed by the height (the distance between the soft tissue margin and the mucogingival junction which is measured in millimeter). This gingival height plays an important role to predict the successful outcome in gingival augmentation procedure (root coverage procedures).[3] Various root coverage techniques are used for the gingival augmentation such as free gingival graft, free connective tissue graft, laterally positioned pedicle graft, subepithelial connective tissue graft, guided tissue regeneration etc.[6–9] The pedicle graft is different from free autogenous soft tissue graft as the base of pedicle graft contains its vascular supply, which ultimately provides nutrition to the graft and reestablish the blood vessel to the recipient region.[2] This pedicle graft can be partial or full-thickness. For these reasons

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pedicle graft has more success rate compared to free soft tissue graft. Lateral positioned pedicle graft can only be performed when there is sufficient keratinized gingiva present lateral to the GR.[5] If there is swallow vestibule, the outcome of the treatment becomes poor. However, there is an ideal color match to the adjacent tissue. But it is often inadequate to treat the multiple GRs.[3] Another important criterion for the success of root coverage is Miller classification. It has been found that the prognosis of Miller Class I and II GR are almost 100% root coverage, whereas there is partial coverage in Miller Class III and almost none for Class IV GR.[1]

This case report is presenting Miller Class III GR in 33 and its treatment with laterally positioned pedicle graft.

Case Report: A 37-year-old male came to the Department of Periodontics, with a chief complaint of sensitivity in lower left front teeth regions. On clinical examination, there was Miller Class III in 33 with the presence of generalized mild plaque and calculus. It was also found that there was slight erythematous color, loss of stippling, accentuated contour, bleeding on probing in the complaint area, and GR with a dimension of height 4mm and width 3mm as shown in **figure 1**. The gingiva in relation to 33 appeared as soft and friable with a shiny and smooth surface.



Figure 1: Preoperative view showing Miller Class III gingival recession in 33

She was almost systemic healthy. She had no tobacco chewing habits. It was noticed the culprit GR tooth was due to labially placed and faulty tooth brushing. There was no abnormal frenal attachment.

Treatment: Phase I therapy (Scaling and root planing)was completed with ultrasonic scaling and Gracey curettes.[6] After 4 weeks, there was some amount of resolution of gingival inflammation with no bleeding on probing. So next plan was decided to root coverage. There was a large and thick strip of keratinized tissue present on the adjacent tooth (i.e. lateral incisor). Hence, lateral pedicle graft (lateral positioned flap) was planned.[7]

The removal of epithelium around the denuded root surface was done under local anesthesia (2% lignocaine hydrochloride with 1:80,000 epinephrine). It led to exposure of connective tissue which was present under the epithelium. This exposed connective tissue acted as a recipient site for the laterally positioned pedicle graft. Then exposed root surface was scaled and root planed properly. A vertical incision from the gingival margin to outline a flap adjacent to the recipient site was done with a #15 blade. Then the periosteum was incised and the incision was extended into the oral mucosa to the level of the base of the recipient site. In this waya partial-thickness flap was reflected. A releasing incision, a short oblique incision into the alveolar mucosa at the distal corner of the flap, in the direction of the recipient site, was made to relief from the tension on the flap. Then partial-thickness flap or graft was placed laterally over the denuded clean root surface as shown in **figure 2** and then suture was given to stabilize the flap as shown in **figure 3** and then coe-pack was given with postoperative instruction and followed up to 6 months.



Figure 2: Reflection of partial-thickness flap and displaced laterally



Figure 3: Suturing of Lateral displaced flap

The present case was again recalled after 2 weeks of post-surgery to remove the suture. In this time, it was found there was take up the graft with mild inflammation as shown in **figure 4**. Again the patient was recalled after 1month. It was found that there was a partial resolution of inflammation as shown in **figure 5**.Again the patient was recalled after 6 months, there was partial root coverage with complete resolution of inflammation, no bleeding on probing, presence of stippling and scalloped gingiva, and melanin pigmented pink healthy gingiva in 33 region as shown in **figure 6**. He was highly satisfied with the final result.



Figure 4: After 2 weeks



Figure 5: After 1 month



Figure 6: After 6 months

Discussion

Nowadays, there is an increase in demand for esthetic and functional treatment, which ultimately led to the need for root coverage for the treatment of GR.[1] The root coverage procedures aim to achieve complete root coverage and restore the aesthetic. There are several method employed for root coverage.[2] However, free gingival or connective graft needs established circulation on the recipient site for its survival and requires second donor sites.[10] But pedicle graft has the advantages of having good vascularity, adequate attached gingiva in the adjacent tooth, better color match and the chance of creeping effect of marginal gingiva after surgical procedure.[11] In the present case report, there was adequate keratinized gingiva adjacent to the involved tooth and favored to select lateral positioned pedicle graft. This technique was first described by Grupe and Warren in 1956.[2] It can be performed to cover the isolated, denuded root surfaces, with having adequate keratinized gingiva. But there must be adequate vestibular depth. Here a partial-thickness flap was preferred over full-thickness flap, because the partial thickness flap provides the following advantages as rapid healing at the donor sites and reducing the risk of loss of facial bone height.[10] This partial-thickness flap is also indicated in the case of thin facial bone or presence of dehiscence or fenestration. However it cannot be performed in case of thin gingiva, as a partial thickness may not be sufficient for the survival of the flap.[5] But in our case, there is adequate thickened gingiva, but there was suspect of presence of dehiscence which was examined by trans gingival probing (bone sounding). A releasing incision was made in our case to relieve tension on the flap, which can damage the blood supply on moving the flap or graft.[11] But there are some limitations such as depending on length and thickness of donor site gingiva, chances of a recession at donor site.[5]

Conclusion

Lateral pedicle graft technique can be used in isolated GR case with adequate keratinized gingiva and shallow recession variety, which ultimately have high predictability outcomes with less donor site morbidity. Although the long term stability remains questionable, still this procedure can be a viable alternative treatment modality for the root coverage in certain cases.

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Biomedical Engineering in Dentistry

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Abstract

Biomedical engineering is a developing field that includes engineering principles in the field of biomedicine for diagnosis, treatment as well as preventive procedures. The introductions of new technologies provide new and advanced treatment options than the usual practices. Thus, research in coordination & amalgamation can help biomedical engineers to contribute to dentistry through research, discovery and advancements in dental practice. This article enumerates the utility of biomedical engineering in various dental procedures and the need for research in this field.

Keywords: *Biomedical engineering, Regeneration, Stem cell.*

Introduction

Dentistry is under colossal evolution by the introduction of latest technologies like restorative biomaterials, cell culture, stem cell & gene therapy for regenerative procedures. Regenerative Medicine has tissue engineering that aims in repairing or replacing damaged tissues. These procedures initiate biocompatible scaffolds, cells and growth factors that can in total replace tissues and organs**.** Bioengineered tissues are created using autologous cells from the patient that is cultured in vitro. These processes are dependent on precise identification of cells, scaffold material, fabrication and inductive factors.[1]

Bioprinting of tissue engineering scaffolds: Bioprinting uses additive manufacturing techniques for the production of three-dimensional structures which consist of living cells, biomaterials & active biomolecules that produces a homogeneous distribution of cells. The current techniques used in bioprinting are inkjet, laser-form that create a hydrogel arrangement that is utilized for cellular encase and assistance. [2]

Bioprinting techniques use bio-ink which is the basis of printing called hydrogels. These are three-dimensional molecular networks with high water intake. This ensures cell entrapment and containment without damaging the cells. The bio-ink is reinforced depending on the choice of substance, concentration and chemical properties.[3]

Biomedical materials in Dentistry: The Object of manufacture of biocompatible biomedical materials is to substitute the perished tissues and restore the functioning of the tissues. These improvised materials should enhance the dental treatment and have finer properties. But this requires a thorough understanding of the material with its interaction with the dental structures to obtain an optimum result.[4] These materials are broadly classified under the following materials.

1. **Metallic biomedicals:** such as titanium, dental amalgam, and alloys for metallic restorations Metal are not popular materials and could be either noble (Gold, palladium, iridium, platinum, rhodium, etc) and base metal (silver, titanium, copper, tin, zinc, etc) that are cheaper. These materials have good

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compatible properties and marginal fit. These metals are usually used for indirect restorations, post & core, crown etc.[5]

2. **Polymeric and hydrogel biomaterials:** In recent days new materials are being produced that simulates the natural biomaterials. These polymers are primarily used for complete dentures, denture liners, resin cements etc.[6]

   Polymethylmethacrylate is the most popular polymer used because of its low price, its lighter, has low water solubility and water resorption., creates great contour enhancing the esthetics. But the drawback of this material is its fragility that breaks on to a slight impact. With the inclusion of copolymer, glass and metal fibers, etc The polymers are used in dental regeneration and tissue engineering. The use of ploy (lactic-glycolic acid) before implants improved in repair of the alveolar socket and enhanced bone formation. They are even used as delivery vehicles by incorporating it with regenerative growth factors.[7,8]

3. **Ceramicbiomaterials** such as hydroxyapatite, bioactive glasses, endodontic filling materials, and zirconia. Ceramics are a blend of metal and non-metallic materials like oxides, nitrides and silicates. It can appear in solid crystalline, amorphous solid & glass. The interatomic ionic & covalent bonding makes it hard, stiff & brittle with good compressive stresses and less shear and tensile stress. Porcelains are biocompatible material made at the laboratory with excellent biocompatibility, natural chemically, esthetic and high strength.[9]

4. **Composite biomaterials** such as resin-based composites, giomer composite, and bone augmentation materials. These materials are commonly used in tooth building because of its color, easy contouring and esthetics. Composites are divided into two types: according to size fine and micro-fine & according to the type of fillers micro-hybrid and microfilmed. The composites are at an edge over other materials for its esthetics, bonding, easy to repair, reducing extensive preparation of the tooth. A limitation of this material is polymerization shrinkage & secondary caries. Commonly used resins are dimethacrylate or urethane dimethacrylate oligomers.[10,11]

3D imaging with oral tissue engineering:

Table 1. The different three-dimensional imaging modalities used in tissue engineering.

Xray/Computed Tomography	These modalities have outstanding penetration depth that is helpful in the diagnosis of bone defects & morphological changes in bone with time. Thus bone ingrowth into scaffolds with mineral accumulation at organic mineral junction is observed. The osteogenic function of mesenchymal stem cells, bone deposition can be visualized by this determining the progression of disease process.[12,13]
Single-photon emission computed tomography (SPECT) and positron emission tomography (PET)	PET/SPECT are established Nuclear medicine imaging techniques with detection of radiations from isotopes used to monitor the stem & progenitor cells function and estimate location within the body also within a scaffold. The polymeric scaffolds release kinetics growth factor that can be evaluated with SPECT.[14]
MRI	MRI is one of the essential modalities for soft tissues. Contrast agents are used to amplifylesions and tracking that primarily is Gadolinium. This is useful in labeling a scaffold and enhances the visibility of the implanted scaffold.[15]
Multimodal Imaging	Multimodal imaging has its advantages as we can remove the limitations of every modality and utilize a single modality to obtain necessary results.[16] Imaging and subsequent analysis provides information about morphological & functional alterations of tissue-engineered scaffolds and regenerating tissue by release of growth factors & cell incorporation within scaffolds and tissue.

Maxillo facial rehabilitation: Tissue engineering prospects replacement of tissues lost post-surgery, concern arises while treating large defects. This is done by the use of autologous bone grafts or tissue free flaps. As an alternative treatment tissue engineering with stem cell and gene therapy evolve forming cells, scaffolds & cell signaling molecules to regenerate normal cells thereby healing of such defects.[18,19]

Periodontal regeneration: Periodontium is an intricate structure with distinctive features making healing complicated. Tissue engineering suggests laboratory constructed periodontal tissues under controlled conditions which is later on implanted onto the surgical site. These periodontal cell sheets cultured when implanted reports to regenerate the periodontal tissues.[20]

Oral mucosal grafting in ophthalmology - The ocular surfaces are complicated with sensitive structures like eyelids, tear film, conjunctiva, and cornea. Minor damage to either of these structures complicates the corneal clarity. Oral mucosa is considered as the best graft material to replace conjunctiva. Oral mucosa is highly vascularized that is effective in rapid healing. They are without any hair follicles & resistant to microbial agents. The oral mucosal grafts are obtained from buccal mucosa, labial mucosa, and lingual mucosa. They are utilized during symblepharon release and fornix reconstruction, cover large conjunctiva defects. It is used for ocular resurfacing and in conjunctivodacryocystorhinostomy & osteo-odonto-keratoprosthesis. [21,22]

Whole tooth bioengineering - should imitate the function, development and functioning of natural tooth. The use of a scaffold when embedded with postnatal dental cells and it was gelatin-chondroitin-hyaluronan tri co polymer which was later implanted into a healed extraction socket of an adult pig produced all the tissues of tooth that resembled the enamel, dentin, cementum & roots. The bioengineered tooth should be vascularized and would be a bio-hybrid of artificial and living cells.[23,24]

Conclusion

Biomedical engineering/regenerative dentistry has been crucial in developing signal pathways for functional replacement of dental tissues. To upgrade the biological presentation of scaffolds it's vital to select relevant material. From the selection of biomaterials, appropriate computing to printing is essential. But there has to remarkable research in the field of biomedical engineering for better comprehension & future evolution.

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Artificial Intelligence: The Future in Dentistry

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Abstract

Artificial intelligence (AI) is an area of computer technologies in influencing our lives. These functions by digital use of algorithms to learn data and form statistical patterns to understand the outcomes thus help in decision making. This article reviews the utility of AI in health care in precise diagnosis and better treatment and patient care. It would provide superior quality and comprehensive patient care in dentistry. These innovations would enable dentists to work with precision in all aspects of diagnosis and treatment planning.

Keywords: *Artificial Intelligence, Comprehensive care, Diagnosis.*

Introduction

The human brain is a complex structure that functions by synchronous activity of the neurons. The longer and faster-acting dendrite along with its faster-acting potential of the neuron during activity determines the intelligent quotient.[1] Newer technologies' are continuously made upgraded that would mimic the activities of the brain. Applications that have a virtual imagination, reasoning abilities with auto-learning methodologies and capability of functioning without being manually operated.[2]

Artificial agency (AI) is defined as the study of intelligent agents, any device that perceives its environment and takes action that maximizes its chance of successfully achieving its goals.[3]

AI is been used in numerous technological applications like gaming, theorem solving & proving, Image and language recognition along with robotics in fields like space, satellites & telecommunications. There has been innovation even in the technologies involved in health care applications and services. The conventional method have been replaced by digitalization. Starting from recording data and maintaining records with capacities in recoding relevant data. [4]They can be widely used for clinical trials and help in treatment planning along with prognosis.

Moreover there are patients who at times doubt the dentists work. So at times this dissatisfaction amongst patients makes them liable to get sued. With the era of computers that work faster with better precision using artificial intelligence, there are chances of better patient satisfaction & care.[5]

Neural Networks: Neural networks are method of information processing which is developed to imitate the brain to concede knowledge and learning. The advantage of artificial neural networks is that this type of system solves issues that are too complicated for standard techniques and people that don't have associate algorithmic solutions or the answer is simply too complicated. Thus are now utilized in diagnostic procedures and health care.[6]

Computer-Aided Detection & Diagnosis: Computer-Aided Detection & Diagnosis software utilizes algorithms in radio diagnosis where computer diagnosis is utilized as an opinion before delivering or confirming the final diagnosis. They provide a differential diagnosis

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for different abnormalities in radio images in various imaging modalities.[7] Thus digital imaging modalities have enabled the usage of Computer-aided diagnosis in dentistry which is progressing. It was primarily used to determine osteoporosis, arteriosclerosis, and maxillary sinusitis. Though its use even in the detection of dental caries, evaluation of dental and maxillofacial lesions, identification periodontitis and Jaw lesions. Nowadays Computer-aided detection and diagnosis are used in orthopantomography as well as cone beam computed tomographic images. [8]

Augmented reality and virtual reality: Virtual reality utilizes software to create a virtual environment for the evaluation of various anatomical structures for diagnosis and treatment planning. Augmented reality is the process of overlaying an image & creating a three dimensional computed real image that is patient specific to create a virtual working environment. It is found to have an effective role in the dental implantsin ensuring decreased risk and better success rates. .This system can be utilized to effectively plan pre-surgical, surgical with accurate placement of implants with better healingdue to a smaller wound. Even the prediction of the position of the nerve is accurate enabling easy avoiding of the nerve. Similarly, it has application id orthognathic surgeries, dentofacial orthopedics, endodontic and restorative procedures.[9]

Clinical Decision Support Systems: Clinical Decision Support Systems act as a support system for health professionals. As these systems are implanted with various clinical data comprehensions it adjunct's in making clinical decisions by utilizing the patient's data.[7]

Various such applicationsutilize other applications that provide records case histories and radiographs in recommending the treatment plans as well as the recall visits. They are capable of generating caution for any fatal medical condition, drug allergies. Along with it can provide a treatment protocol with prophylactic antibiotics to such patients. Thus it would simulate the human brain and thinking. This entire clinical decision making is by the stored and available data from previous treatment so all this requires a proper recording of data in a specified format.[10]

Merits and Demerits of Artificial Intelligence: Every advancement or technology comes with its good and bad so is this. Though not very evident but artificial intelligence has already starting to take over and is recreating a better future.

Assisting surgeries: Here robots can be used for assisting surgeries along with augmenting nursing faculties

Record Keeping: Maintain a detailed list of the patients along with their histories and treatment administered with subsequent recall and revisits '

Diagnosis: They provide an efficient and accurate diagnosis by carefully correlating and comparing with the radiographic features and comparing it with every possible detailed data.

Treatment: Due to the auxiliary assistance and aids the possible treatment time is reduced.

Time-saving: They don't require breaks as they don't get tired or require breaks from work.[11]

Despite all the advantages, there are certain limitations to the applications:

- Job concerns: as several works is managed it requires less staff so concerns of unemployment
- Initial investment for the application is high because of the expensive software.
- Acceptance by the staff could be difficult along with the lack of efficiency in operating. There are chances of limiting the creativity of the faculties.
- Another important concern is maintaining the privacy and security of the data.[12]

Dental applications of artificial Intelligence:

Oral Medicine & Dentofacial radiology: It can be employed in the diagnosis and treatment of lesions of the oral cavity. Any alteration of the oral mucosa, potentially malignant disorders that are at risk of developing in carcinomas can be easily screened using AI that would be missed otherwise. Assists in evaluating osteoporosis in the panoramic radiographs, also determine the proximity of the mandibular canal, dental biometrics.[13] Effective in interpreting the radiographs for different radiographic lesions, caries detection, assessment of vertical root fractures, evaluating the developing the tooth development phases. Lately the Cone Beam Computed Tomographic images utilize AI in analyzing the slices. Making interpreting and processing of the images fast and easy.[14]

Dentomaxilofacial orthopedics: The initial treatment for analysis done using the photographs

and radiographs can be easily done using an intraoral scanner. They decrease the time and impression making procedures with complete eradication of human errors. The advancement of three-dimensional printers orthodontics casts and models can be made on which aligners can be placed. The Software can suggest the direction of tooth movement along with the pressure points of the tooth. There is a possibility of making digital impressions, thus providing a precise customized treatment planning. They also provide a futuristic outcome of the treatment that the patient can visualize.[15,16]

Prosthodontics: Computer-aided design/Computer-aided manufacturing helps in scanning and designing restoration along with fabrication without the casting steps. They provide perfect occlusion, margins with contacts .[17] Thus providing ease in fabrication. The ideal esthetics of patient can be analyzed using various factors like anthropological calculations, facial measurements, ethnic variations along with the patient fondness can be incorporated.[18]

Oral and Maxillofacial Surgery: Oral surgical procedures can be easily assisted using robotics. This enhances the human skills and prevents any human errors. It helps in treatment planning by decreasing the treatment time and protecting the vital structures simulating a practice surgery thereby increasing the operating accuracy. It even helps in bioprinting of the tissues leading to regeneration of the structures that are damaged.[19]

Forensic Odontology: Age estimation plays a vital role in forensic odontology which is utilized widely in identifying individuals in mass disasters. As it's based on radiographic assessment of eruption of tooth comparing radiographs can be easily done by AI. Though the utility of AI in forensic odontology is sparse.[20]

Conclusion

Artificial Intelligence is the future of dentistry. So it calls for research that understands the algorithms which would enhance the use of it in dentistry. It should act as an adjunct to human skills and we cannot deny the fact that it cannot replace human analyzing skills and knowledge.

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An Alternative Technique of Bleaching Vital Tooth: A Case Report

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Abstract

Fluorosis staining is commonly considered an esthetic problem because of the psychological impact of unesthetic maxillary anterior teeth. Numerous treatment approaches have been proposed, ranging from bleaching to enamel reduction to restorative techniques. The aim of this study was to report the clinical case of a female patient of 18 years with moderate fluorosis with midline diastema, whose smile was reestablished by the application of 5%sodium hypochlorite bleaching agent after cleaning and enamel etching with 37% phosphoric acid followed by direct composite veneering. This technique showed excellent results and the patient was satisfied. This technique is simple,low cost,painless, fast and easy to perform, in addition to preserving the dental structure. Treatment showed immediate and permanent results.

Keywords: *Bleaching, fluorosis, sodium hypochlorite, Veneering.*

Introduction

Tooth discolouration is complex in nature and is broadly classified as extrinsic and intrinsic stains.[1] Extrinsic stains on a tooth surface caused by dietary chromogens can be managed by oral prophylaxis. It is the intrinsic stains that are within the dentine require non-invasive and invasive management depending on the degree of discolouration or stains.[2] One of the most common intrinsic stains seen in Indian population is caused by increased fluoride level in the water. The reason could be that India is in the geographical fluoride belt and in areas where fluoride content is high in rocks or soil, leaching of fluoride occurs, causing excess fluoride level in groundwater.[3] Depending on the extent of exposure to fluoride, the degree of fluorosis can vary from mild to severe and structural deformity of the tooth.[4] Bleaching is a non-invasive and conservative approach to manage mild to moderate fluorosis. The only drawback of bleaching is that hydrogen peroxide causes dentinal sensitivity and gingival irritation. It is contra indicated in the tooth with large pulp horns.[2]

Young patients are usually concerned about their anterior tooth discolouration. Therefore an alternative approach to managing young permanent teeth having large prominent pulp horns is required. A conservative treatment approach for the management of yellow-brown intrinsic staining of dental enamel is described in this case report.[4]

Case Report: A 17-year-old patient complaining of brown stains and spacing of the upper front tooth region reported to the Dental school (Figure 1). After a thorough case history and clinical examination, we arrive at the diagnosis of mild fluorosis. On a radiograph, the Maxillary right and left central incisor (11 and 21) exhibited high pulp horns. Due to mildness of the stains, a conservative treatment plan was presented to the patient that would fulfil his request, which is bleaching with sodium hypochlorite.

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The patient was referred to the Department of Periodontics for oral prophylaxis for removal of plaque and extrinsic stains. After two days patient came to our department of Conservative Dentistry and Endodontics. Before the treatment, shade selection of teeth with composite buttons was made under natural light. Anterior segment was isolated with a rubber dam. The teeth was then acid etched with 37% phosphoric acid (Meta Etchant by Meta Biomed) for 60 sec (Figure 2) to enhance the effectiveness of the hypochlorite action on the stained tooth. Etchant was rinsed with three-way syringe. Frosty appearance of enamel completed the etching procedure. This was followed by sodium hypochlorite 5% (Prevest) application on to the entire tooth surface of the anterior segment using a cotton pellet (Figure 3). The bleach is continuously reapplied to the tooth as it evaporates. The teeth were bleached in a single appointment for 25–30 min. Significant improvement in the shade of teeth was observed.

Teeth were thoroughly rinsed with water and again re-etched with 37% phosphoric acid (Meta Etchant by Meta Biomed). A universal bonding agent (3M ESPE) was applied and then gently airdried to have a uniform coat over the teeth. This resin perfuses in the etched and porous enamel, creating resin tags that occlude the porosities and prevent re-staining of the hypo-mineralized lesion. The diastema closure was done using composite (3M ESPE A2 Shade) (Figure 4). The patient was recalled for follow up after 6 months. He did not complain of sensitivity and was very satisfied with the treatment outcome.

Discussion

Bleaching is considered as the gold standard treatment for fluorosis, tetracycline stains and other internal discolourations.[5] It is a non-invasive technique and results in the better shade of teeth in just one to three dental appointment depending on severity. It has high acceptability rate among patients.[5] However, the bleaching procedure poses a threat to young large pulp chambers and pulp horns and therefore contraindicated in a young permanent tooth. The agents like hydrogen peroxide are known irritant and cause a chemical burn of soft tissues surrounding the tooth. They penetrate inside the dentinal tubules and cause dentinal hypersensitivity as well.[6]

Many studies show that the bleaching agent is known to reduce the bond strength of the enamel resin composite. There is a minimum waiting period of 1-week advocate post bleaching and aesthetic rehabilitation of tooth with composite.[7] This technique of acid etching combined with bleaching with sodium hypochlorite is an effective alternative to regular bleaching procedure and can address the above issues effectively.[8] The sodium hypochlorite technique has several advantages over peroxide-based protocols for the specific application of removing stains from localized hypo mineralized lesions in young teeth. It is known to be highly effective at removing organic material by oxidizing it and allowing the smaller degraded molecules to be washed away. Applying sodium hypochlorite to hypo-mineralized enamel lesions, can degrade and remove the chromogenic organic material that is, located in the enamel.[7,8] It has been shown that pretreatment of the enamel with sodium hypochlorite to remove the enamel proteins can enhance the ability of acid to etch the surface, thereby improving the likelihood that resins can bond successfully to the surface.[9]



Figure 1. 11 & 21 with fluorosis stain



Figure 2. Anterior segment etched with 37% phosphoric acid



Figure 3. Anterior segment bleached with sodium hypochlorite



Figure 4. 11 & 21 diastema closure done with composite

Conclusion

The above technique uses readily available materials that show a high level of safety and can be used on young permanent teeth. Permanent incisor teeth that have high pulp horns can be treated from this approach. This technique provides a conservative alternative treatment for yellow-brown hypo-mineralized enamel that shows good clinical success. The application of conservative treatment approaches should be considered before applying techniques that require substantial enamel removal for the treatment of enamel discolourations.

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Different Techniques to Establish Occlusal Anatomy of Posterior Teeth with Composite. A Review

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Abstract

Composite resin restoration on posterior teeth through the incremental technique is, generally, a time-consuming procedure with the risk of contamination between the layers. Therefore, there is a need to facilitate the work of dental professionals and achieve aesthetic and functional results such as the occlusal stamp technique. "Stamp technique" is a technique used for restoring class I and class II restorations with accurate occlusal topography. It was introduced mainly to restore Class I cavities and erosively damaged teeth. A stamping technique is an easy-to-follow procedure to recreate accurate occlusal topography for a direct composite resin restoration effectively and efficiently with less time needed for finishing and polishing of the restoration. This technique consists of fabricating an occlusal matrix that mimics the natural occlusal anatomy of posterior teeth,before cavity preparation takes place. This matrix is then pressed against the final composite increment before curing takes place and is suitable in cases where caries is evident during the clinical examination or radiographically within tact marginal ridges and ideal occlusal anatomy. For overcoming the main disadvantage of polymerization shrinkage seen in composite restorations, it has [to be done using incremental layer technique. Stamp is used in the final layer to replicate the natural anatomy of the occlusal surface. A precise tooth-like restoration having an accurate functional occlusion is obtained when the stamp technique is performed. This technique also can be utilized for class II cavity restorations where the marginal ridge is intact. This case report describes simple class I composite restoration using stamp technique. The main objective is to replicate occlusal anatomy by making a copy of the original unprepared tooth structure to get a perfect anatomy in a few minutes. This review walks through many techniques utilized for simulating posterior occlusal anatomy.

Keywords: *Composite, Incremental Technique, Occlusal Topography, Polymerization Shrinkage.*

Introduction

Dental caries is a disease of calcified tissues, with demineralization and destruction of their inorganic and organic components. Occlusal surfaces are considered the most susceptible to caries lesions because of their morphology. One of the primary goals of restorative material is to reestablish the original occlusal anatomy of the tooth. Cusp anatomy, contours, and colour all play into the harmony of a smile. Advancement in the direct composite resin and because of the minimally invasive technique, it is considered to be one of the choicest material for restoring posterior teeth. Composite resins are available in many shades and a variety of consistency depending on the clinical need. Probably their versatility in one of the reasons that they are in constant demand by the clinicians. The increase in demand has lead to a lot of research and development with Composites. Besides, they are simpler to deal with and facilitate the

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reproduction of occlusal structures. Confronting these upgrades, intercessions with composite restorations have made conceivable the change of explicit and individual subtleties existing in the natural dentition, in a sufficiently tasteful way and undefined to human vision.[1]

Cusps need to be placed appropriately in the inter-arch space. They must be adequately graded in heights and sizes to be in harmony with the movements of the mandible.[2] Optimal occlusion is that which is comfortably adopted by the patient. Therefore, a restoration is designed in a manner that it does not produce any disturbance in the typical condylar path. Its occlusal anatomy influences the magnitude and direction of load borne by the tooth. However, the ability to produce a restoration which conforms with the original anatomy of the posterior teeth must be perceived as one of the complicated and time-consuming technique.

The present review describes different techniques that aid in the reproduction of occlusal anatomy and minimizes the overextension of restorative material, thereby maintaining the health of the stomatognathic system (Figure 1).

Traditional technique: For Grossly decayed tooth or moderate to extensive loss of occlusal anatomy, traditional Increment layering technique is preferred. In this technique, the posterior Composite is placed in small increments, and each increment is individually light cured. The increments can be either placed horizontally, obliquely or vertically. Successive cusp build technique is another modified form of the incremental method. Here, singular cusps are reestablished each in turn up to the level of the occlusal enamel. Little inclining augmentations are applied to each corner of the cavity. This technique allows the handling of material to a minimum and averts voids into the material. [3]

It is indicated in large class I and Class II cavity and in the grossly decayed tooth where occlusal anatomy has been lost. This technique can significantly reduce finishing time by precise attention to the progressive reconstruction of natural morphology. The incremental method showed lower microleakage. One of the significant disadvantages of this case is that it is time-consuming and occlusal anatomy may not correspond to the original anatomy of the tooth. The clinician needs to develop this skill and should have a thorough knowledge of anatomy and characterization of posterior teeth.

Occlusal former technique: Many carious lesions have intact occlusal anatomy. Such caries are occult as they have a macroscopically whole unimpaired occlusal surface however with undermining rot that can be viewed as a territory of somewhat blue/dark discolouration under the enamel surface, or radiographically. There are many techniques that grant registration of intact anatomic details before cavity preparation and for this reason,indicated for small enamel defects and occlusal fissure lesions or Class II preparations with no occlusal involvement. Stamp technique has various advantages.[5] Firstly, it helps in reducing the overall time required for the restoration as instantly desired cusp –fossa relationship is attained. This is suitable in a busy practice scenario as it ensures predictability with accuracy (Table 1).These techniques are broadly classified into prefabricated method, and custom made techniques. Both ways can be used either as a direct or indirect technique.

Table 1. Advantage and disadvantages of occlusal formers.

	Occlusal formers technique
Advantage	1 The anatomy and occlusion with which the patient was comfortable is restored in the new composite restoration 2. The surface of the composite restoration is as smooth as the original surface of the tooth being restored, and therefore minimal, if any polishing is needed 3. There is no air-inhibited layer to be removed, since oxygen is excluded frorn the surface by the matrix during curing 4. The Composite is cured under slight pressure, and is thought to reduce voids in the rcstoration 5. With practice, there is minimal flash to be removed
Diadvantage	1. There is additional time and expense to create the occlusal matrix 2. There is additional time necessary to cure the last layer of Composite

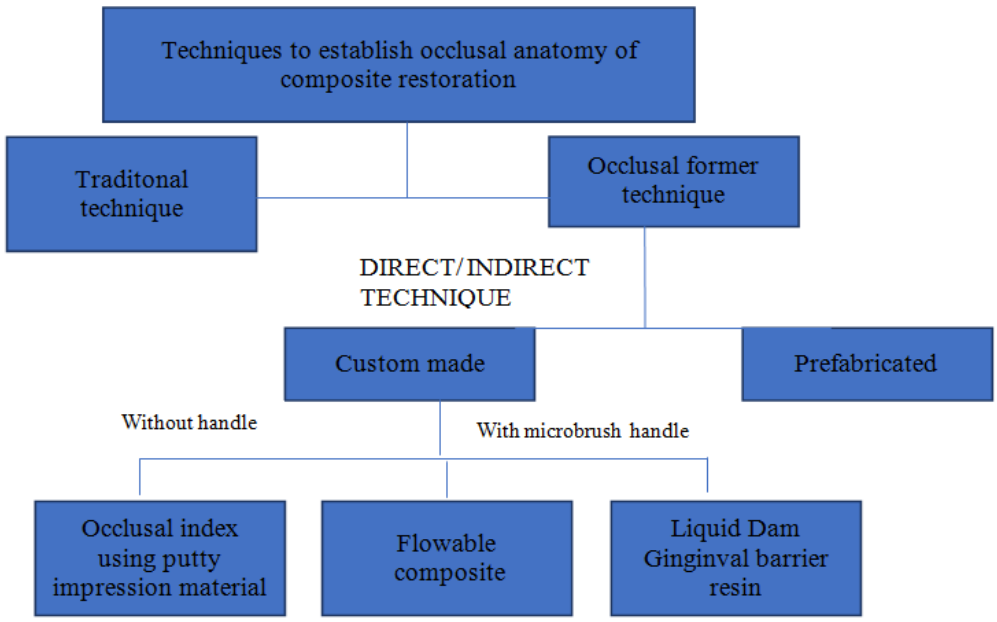


Figure 1. Classification of different techniques to establish occlusal anatomy of posterior tooth.

Direct Technique: In custom made techniques, varied materials can be employed to make the occlusal replica, including light-curable material, chemically activated acrylic resin, polyvinylsiloxane bite registration material, transparent silicone moulds, occlusal matrix technique (Figure 2).[5] In this technique, before penetrating the enamel and before cavity preparation a putty impression material or a flowable material with a micro brush is used to make an impression of tooth occlusal anatomy. After cavity preparation, increment layering of composite is placed, and each increment is light-cured till it reaches the occlusal surface. After the placement of the lastunpolymerized occlusal increment, Teflon tape is placed and on top of that putty occlusal index or stamp is positioned back on the teeth to simulate the previous recorded anatomy. Subsequently, the index is removed and the excess composite istaken out and cured.[4]

Indirect Technique: In the presence of a rather considerable defect, it is challenging to build up cusps precisely enough to occlude with the antagonists. Here, a silicone impression and models of the teeth is made. After which wax is utilized to reconstruct the desired anatomy. On the wax carving, an occlusal stamp is formed which is consequently placed on the last increment of restored natural dentition to mimic the anatomy.[4]

Occlusal matrix material using Biteperf: The Biteperf gadget is made in transparent polycarbonate and comprises of a plastic handle (50 mm long) and clear round head (13 mm in the distance across and 4 mm thick). The base head has a circular shape with one thermoplastic impression material (polyethylene) mounted on it. The occlusal transfer matrix was plasticized by heating for 15–20 seconds or until the surface acquired a shiny appearance. It is then positioned under gentle pressure on the occlusal surface of the tooth. The occlusal matrix is kept immobile for 10 seconds and then cooled with air jets [Figure 3]. A mark is made on the outside of the mould to allow for exact positioning during the restoration.[6] Eventually, the internal detail of the occlusal replica is scrutinized. Post caries removal and cavity preparation light-cure composite resin are added in the cavity using the incremental technique, leaving space for a final increment on which the occlusal matrix device is to be placed.[5] Leaving this final occlusal increment unpolymerized, the occlusal replica was stabilized in the original anatomic position and pressed down firmly. The next step is polymerization of the last resin increment across the occlusal surface, applying light through the matrix while it is held under pressure to ensure perfect positioning. After polymerization, the occlusal matrix device is removed, and the occlusal anatomy is evaluated.[6] Mostly in this technique, occlusal reproduction is accurate, however small adjustment if required, can be done with abrasive silicon carbide instruments or rubber polishers.

Prefabricated Fissure formers for composite filling (Occlu-Print): These prefabricated stamps have an excellent and rapid occlusal modulation of lateral composite fillings. It is manufactured using translucent plastic (Figure 3). It is less time consuming compared to the manual modulation technique of stamp fabrication.[7,8] With Occulu-Print, polymerization is made by placing the curing onto the stamp. Molar and premolar disposable stamps are available 16 different sizes and shapes, separated into upper/lower jaw and right/left jaw with each stamp having a related FDI-reference.

Prefabricated composite occlusal shells for posterior teeth: Edelweiss occlusal composite shells are prefabricated nanocomposite table top restoration which is usually used to increase the vertical dimension of posterior teeth. Depending on the size of the tooth the preformed composite shells are selected. These can be adjusted with stone burs to fit the individual tooth. The tooth and the shells are treated with an etchant and specific bonding agent. The shell is then luted on the tooth with a dual-cure luting agent. Post cementation occlusion is adjusted and minor finishing and polishing procedure is done.[9,10]



Figure 2.Pit and fissure caries with intact occlusal surface

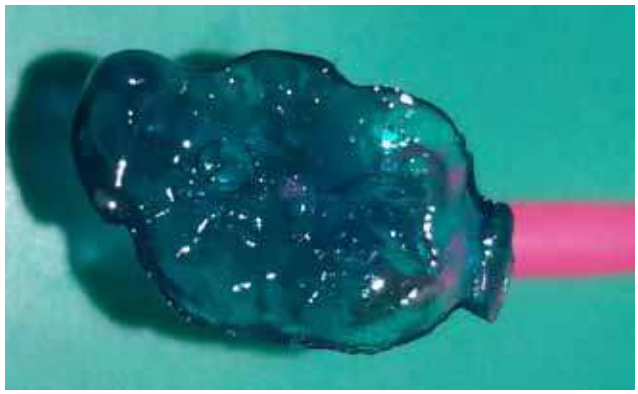


Figure 3. Customized occlusal former with liquid rubber dam



Figure 4. Preformed Occlusal former



Figure 5. Composite restoration with characterization.

Conclusion

Finishing with appropriate anatomy has been difficult and time-consuming in the traditional increment layer technique. An alternative to overfilling and then grinding in the anatomy is proposed. The occlusal replica technique can be carried out with several materials, all of which have their advantages and disadvantages. The anatomy shapes up almost naturally. With only minor adjustments and polishing with a polishing paste, time spent finishing is shortened. Natural-looking, functional, and beautiful occlusal anatomy are now achievable with this uncomplicated technique (Figure 4).

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Infection Control in Prosthodontics: A Review

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Abstract

Dentists and dental office personnel are exposed to a wide range of potential pathogens and have high chances of infection transmission not only in the clinic, but also in the laboratory. A proper sterilization and disinfection protocol must be followed. This shall prevent cross-contamination of disease among the dental health care providers. Prosthodontics is a branch in dentistry which includes manipulation of various materials, equipment, their use and instrumentation for rehabilitation of patients. Patients in the geriatric group are of special importance in this branch. A strict protocol for infection control is mandatory to combat any kind of transmission and cross-contamination between the dental office personnel and patient and vice-versa. This article shall discuss the various modalities of sterilization and disinfection followed from a prosthodontist's point of view, in the clinic and the laboratory also.

Keywords: *Dental Clinic; Dental Laboratory; Disinfection; Infection Control; Sterilization.*

Introduction

According to the Centre of Disease Control guidelines, sterilization is a process that destroys or eliminates all forms of microbial life and is carried out in health-care facilities by physical or chemical method. In disinfection refers to the elimination of many or all pathogenic microorganisms, except bacterial spores, or inanimate objects. Cleaning means the removal of any visible organic or inorganic soil from objects or equipment or surfaces, mechanically or manually with water and some detergent or enzymatic agents. Decontamination means removal of pathogenic organisms that would make an object safe to use, handle and discard.[1]

Dental health care providers are exposed to infectious material and potentially infectious materials within the dental office, dental lab and also while handling equipments, materials, etc.[2] Patients in the prosthodontic clinic usually belong to the vulnerable group due to increased age, decreased immunity, compromised health status. Prosthodontists, their staff and also their patients are at a higher risk for possible transmission of micro-organisms and cross-infection due to the use of added equipment and material in the clinic and the laboratory. Thus, infection control is of utmost importance. Even though advances in this field are commendable, the principles of infection control have always been basic. This has to be equally prioritized just as any other procedure to ensure safety, both ways. For a good practice, a sufficient number of materials, instruments and equipment should be available and hence the sterilization has to be managed and routinely done.[3,4,5] Therefore, awareness among clinicians and auxiliaries are a must for overall safety.[6] Dental casts, Impressions, Impression trays, occlusal rims, etc. are commonly used in prosthodontic practice. This article shall review the procedures that should be employed for sterilization and disinfection of these objects in a prosthodontic clinical and laboratory set-up.

General Protocol for Infection Control: Infection control is a comprehensive procedure and there are five steps towards achieving it. They are: Evaluation of patient; Personal Protection; Cleaning and Sterilization of Instrument; Disinfection; Asepsis in the Laboratory.[7] Microorganisms may be transmitted through direct contact with blood; oral fluids; conjunctival, nasal and oral micro-droplets; patient materials, etc. These can be transmitted through indirect contact with contaminated objects and inhalation of long-standing aerosol containing the micro-organisms suspended in

the air.[2] DCNA has classified the level of risks as per the prevention. Diseases like Mumps, Measles, Rubella, Fungal infection, etc. come under Class 1, because their vaccine is available, the risk is minimal. Candidiasis, Staphylococcus aureus, and Streptococcus Group A, etc. are classified under Class 2. Herpes simplex, Cytomegalovirus, EBV and Varicella virus are classified under Class 3. HIV, HBV, HCV, HDV are classified under Class 4 because there are no vaccines except for HBV and all the universal precautions should be taken as the diseases are fatal. Due to the highest risk of airborne transmission, TB is categorized under Class 5. There are no vaccines also for TB. Inpatient evaluation, the above risks have to be identified and proper precautions should be taken.[8,9]

Personal protection employs the barrier mechanism. Not only in the dental office, this is highly recommended in dental laboratory procedures. Use of Personal Protective Equipment like disposable gloves, over-gloves,mask, eye-wear, face-shield, protective coat, and overcoats are essential to prevent contamination through potentially harmful splashes, spray, spatter and aerosols during clinical procedures using rotary instruments and inlaboratory procedures using model trimmers and lathe.[10,11,12,13] Instruments have been classified into 3 types based on their exposure to risks of transmitting infection as described in the **Table I**.[14,15]

Table I. Classification of instruments based on their exposure to risks of transmitting infection

Type of instrument	Type of exposure	Example of instruments	Sterilization method
Critical	Used in invasive procedures which penetrate soft-tissue, bone, enter or contact blood or other sterile tissue	Scalpels, surgical burs, forceps, scalers,bone-chisel	Should be sterilized after every use by pressure, heat, chemical vapor
Semicritical	Do not penetrate soft tissue or bone. Contact mucosa Contact non-intact skin	Mirrors, reusable impression trays, amalgam condensers	Should be sterilized after each use if sterilization is not applicable,disinfection with high-level disinfectant should be done. (high-level disinfectant is labeled as "sterilant/disinfectant" as registered by the US Environment Protection Agency)
Non-critical	Come in contact with intact skin	External components of X-Ray heads, Sphygmomanometer cuffs, Pulse-oximeter	Requires intermittent or low-level disinfection after each use (such a disinfectant is called "hospital disinfectant" as registered by the US Environment Protection Agency. These are not labeled for tuberculocidal activity.)

Most prosthodontic equipment and instruments are semi-critical: Mechanical cleaning of the instruments using disinfectors, washers, ultrasonic cleaners, rust inhibitors should precede the sterilization process. Presoaking instruments should be done with enzymatic solutions and non-enzymatic solutions for removal of blood, proteins anddebris, respectively. Instruments can then packed and sterilized using pressure, dry heat and unsaturated chemical vapor.[16] There are chlorine-based compound and phenolic compounds which are used for surface disinfection and asepsis. These clean and disinfect contaminated surface; prevents surface contamination by use of surface covers or may do both.[17]

Infection Control: Prosthodontics perspective:

Disinfection of Laboratory: The general protocol for disinfection should also be followed in the laboratory. But, the risk status of an object is not always known. In that case, the object should be treated as high-risk. Isolation of the objects should be done and any instrument or device that comes in contact with them should be sterilized. Disinfectant should be carefully chosen such that metallic surfaces are intact and there are no dimensional changes of impressions/prostheses.[18,19] Instruments, attachments, rag wheels,etc. should be disinfected after every case.[20]

The instruments used frequently in the prosthodontic practice have been enlisted in **Table II**; metal framework and denture in **Table III**; Impression materials in **Table IV**; other materials in **Table V**along with their recommended method of sterilization and disinfection.[21]

Table II: Some commonly used instruments, and their sterilization and disinfection procedure

Instrument	Sterilization and disinfection procedure
Mouth mirror and face mirror	Dry heat; Ethylene oxide
Handpieces	Manufacturer's recommendation should be followed; Ethylene oxide; Moist heat
Carbon steel hand instruments	Dry heat; Ethylene dioxide
Stainless steel instruments, Tissue retraction pluggers	Dry and Moist heat;Chemical vapor; Ethylene oxide
Dampen dish	Moist heat;Ethylene oxide
3-way syringe	Dry and Moist heat;Chemical vapor; Ethylene oxide
Saliva ejectors and evacuators	Ethylene oxide
Impression trays, Aluminium trays, Metal trays, Resin trays	Moist heat, Chemical vapor, Ethylene oxide

Ethylene oxide is usually used at a concentration of 450-800mg/l. Moist heat sterilization is achieved by Autoclaving at 121°C for 15 to 20 minutes at 15 lb pressure/square inch.[21,22]

Table III: Sterilization and Disinfection of Metal framework and Denture[21,22]

Vitallium metal framework	**Immersed in Sodium hypochlorite and rinsed with water**
Denture	After rinsing the denture with running water and kept in an ultrasonic cleaner, the denture is immersed glutaraldehyde solution for 12 hours. Then again it is cleaned with running water, scrubbed in chlorhexidine followed by contact with chlorine dioxide for 3 minutes. Then sterilization is done with ethylene oxide.

Table IV: Sterilization and Disinfection of Impression Materials[6,21,22]

Impressions compound	Immersed in 2% Iodophor solution for 20 minutes
Zinc oxide eugenol	Immersed in 2% glutaraldehyde for 10 minutes
Alginate	Sodium hypochlorite spray-rinse-spray; then kept under damp gauze or in a sealed packet for 10 minutes Immersed 2% glutaraldehyde for 10 minutes
Agar	Spray with sodium hypochlorite, rinse, spray again and stand under damp gauze for 10 minutes
Polyether	Immersed in 2% glutaraldehyde at room temperature for 1 hour, then rinsed with sterile water for 45 seconds; Drying for 10 minutes
Polysulfide	Rinsed in normal water for 45 seconds, Immersed in 2% Glutaraldehyde solution for 30 minutes. Or Immersed in 5.25% sodium hypochlorite solution for 15 minutes Rinsed in normal water.
Addition silicon	Immersed in 2% glutaraldehyde for 1 hour, rinsed in sterile water
Condensation silicon	Immersed in 2% glutaraldehyde for 10 minutes, washed with sterile water

Table V: Sterilizationand Disinfection of some other materials

Wax bites, Occlusal rims, Bite registrations	Immersed in a 5.25%sodium hypochlorite solution and kept in a plasticbag for 10 minutes.[17]
Casts	Immersed in a 5.25% sodium hypochlorite solution and kept in a plastic bag for 10 minutes.[17]
Pumice	Antiseptic with Octenidine or Benzoic acid to conventional pumice is added; Pumice once used should be discarded after each use.[22]

Conclusion

For a good clinical practice, good quality instruments, efficient trained auxiliary personnel, skillful clinicians are a must. Infection control within the operatory and laboratory are the only way for a safe practice not only from dental health care personnel's point of view, but also patient's. There are no other substitutes for maintaining the disinfection and sterilization protocol in the dental office before, during and after any procedure and every assistant, technician, the attendant should be trained by the clinician for abiding by the protocol.

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Dentine Replacement with Biodentine Under Class II Composite Inlay: A Case Report

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Abstract

Biodentin is a bioactive cement with dentin-like mechanical properties, which can be used as a dentine substitute for both crown and root. It also helps in the remineralization of dentine along with maintenance of pulp vitality. This advocated to be used in various clinical applications, such as root perforations, apexification, resorptions, retrograde fillings, pulp capping procedures, and dentine replacement. Indirect laboratory-processed composite systems present an esthetic alternative for intracoronal posterior restorations and provide esthetic results that may also reinforce tooth structure. Additional clinical benefits include exact marginal integrity, wear resistance similar to enamel, wear compatibility with opposing natural dentition, optimal esthetics, ideal proximal contacts, and excellent anatomic morphology.

Keywords: *Calcium Silicate Cement, Caries,, Composite Inlays, Remaining Dentinal Thickness.*

Introduction

The caries attack proportion is the highest maxillary and mandibular permanent first molars, followed by second molars. The interproximal surface of molar are highly susceptible to dental caries and often get unnoticed in the usual dental inspection.[1] One suspects Class II caries (Fig 1) only when the patient complains of food lodgement, sensitivity or noticeable frank cavitation seen in the marginal ridge area.[2] There is various material available for the restoration of such defects. The most popular direct restorative material preferred for Class II was Silver Amalgam.[2] Due to its metallic colour and mercury hazards associated with it along with phasing out of this material worldwide, there is a sudden surge in the use of other restorative materials.[3]

Aesthetic restorations for posterior teeth are in current demand by patients worldwide. Composite restoration is among one of the choices preferred by most of the dentist.[4] Nevertheless, one of the drawbacks of composite restoration is that it undergoes polymerization shrinkage, which leads to microleakage and in short span leas to deterioration of the restoration. Indirect composite inlay was introduced to overcome these pitfalls of direct restorations. The composite inlay has an advantage over direct composite restoration since the contraction of the inlay takes place outside the oral cavity.[5] Biodentine is a new generation silicate cement which is bioactive like mineral trioxide but has superior handling properties and strength. It is an adequate dentine replacement and pulp protecting material.[6] This paper presents the clinical cases in which Biodentine was placed to replace the lost dentine during caries and cavity prep, followed by indirect composite inlays

Case Report: A 37-year-old male patient was referred to the Postgraduate Clinic of Conservative Dentistry and Endodontics (Institute of Dental Sciences and Hospital, Bhubaneswar). The patient complained of dislodged restoration and gave a history of restoration done four years back. Patient's had a non-contributory medical history. Intra-oral examination revealed mesio occlusal caries concerning 46. Clinical inspection and radiographic evaluation revealed secondary caries approximating pulp. The pulp sensibility for 46 revealeda positive response to it.

The patient had uncontrolled ropy saliva, and even after the placement of high evacuation suction and isolation device, it was challenging to do the direct restoration. Therefore we settled to restore the tooth with

indirect composite inlay. Subsequently,the patient's consent was taken to restore the tooth with indirect composite resin inlay.

The secondary caries was excavated at a slow speed using round carbide bur and spoon excavator. The weakened and unsupported enamel was removed. Ensuring the principles of tooth preparation to receive inlay, the cavity preparation was done. The cavity walls had divergent walls, rounded internal line angles and cavosurface configuration with butt joint. The walls of the cavity smoothened using finishingbur (Fig 2). As the axial wall was approximating the pulp chamber, a protective dentine replacement was done with Biodentin. After the final tooth preparation, the cavity was cleaned and dried. With the polyvinylsiloxane material (Aquasil/ Dentsply)impression was made using both heavy body putty and light body together.. Subsequently, a direct provisional restoration was placed using temporary light-cured resin-based cement (Cool Temp, Coltene). The positive replica of this impression taken was made using die stone. An appropriate shade of the restoration was selected using the Vitapan shade guide, and the casts and with selected shade image was delivered to the laboratory(Dent Care) for the fabrication of the inlay.

After 1-week patient visited again for a second appointment. The provisional restoration was taken out, and the cavity was disinfected with 2% chlorhexidine The cavity was selectively etched with 37% phosphoric acid, the adhesive (Universal Bond, 3M ESPE)was applied and light-cured (Bluephase, Ivoclar) on the tooth, and the same adhesive was applied on the silanated (Monobond S Ivoclar) internal aspect of composite inlay without being polymerized. Then the cementation was carried out by applying a thin layer of dual-cure Resin Modified Glass Ionomer Cement (Fuji, GC). The resin luting cement was light-cured (Blue Phase Ivoclar) through the restoration from both, palatal and buccal side of restoration. The excess resin luting cement from the interproximal area was removed using a dental floss. The finishing and polishing were done using a composite finishing kit (Ivoclar). Post final finishing and polishing, the occlusion was evaluated. (Figure 3) The patient was informed about the limitation of the technique and was asked to maintain a regular maintenance visit. A radiograph was taken after 6 months during follow up visits to evaluate the restoration (Figure 4).



Figure 1. Class II caries



Figure 2. Class II inlay cavity preparation.



Figure 3. Cemented Composite Inlay

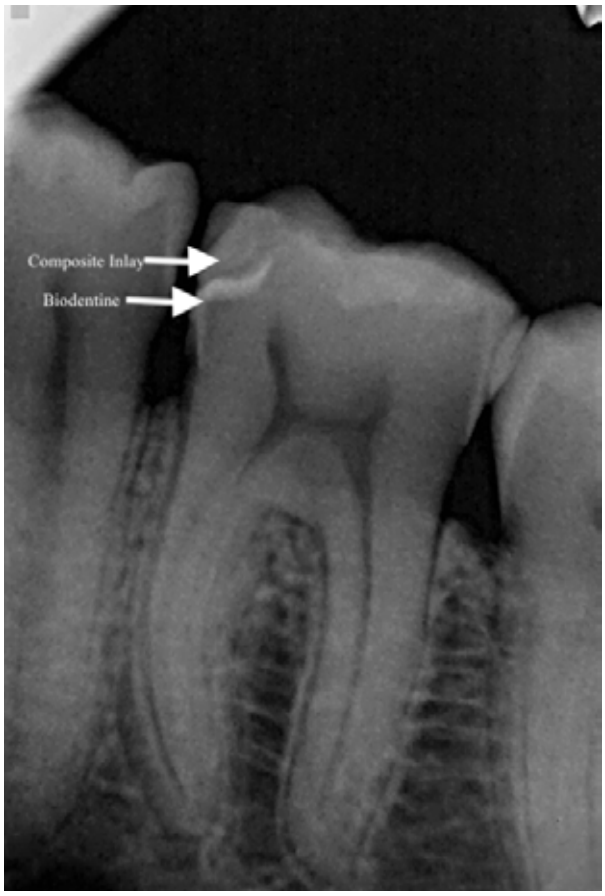


Figure 4. Radiograph of restored 46 during follow up

Discussion

Managing deep dental caries is a challenge. The Remaining dentinal thickness protects pulp from irritation caused by restorative material. It is imperative to replace the lost dentine of the cavity preparation with a biomimetic material which not only protects the pulp but also supports the restorative material like base, Biodentine sets in 45 minutes has high compressive strength and good marginal adaptation. [6]It is recommended to place the overlying direct composite resin after two weeks so that Biodentine material can achieve sufficient maturation and can withstand contraction forces from the resin composite. This drawback is overcome with indirect composite resins which are fabricated in the lab.[7]

Several tooth-colored materials are available like zirconia and ceramic, but they are expensive. In contrast, IRCs ease of handling and exhibits better stress distribution, reparability, lower cost. However, they also have some drawbacks like in the long term the surface exhibits roughness and more prone to colour changes. [8]

Like all indirect restoration, IRC results in less chair-time for the patient and minimizes the finishing and polishing time. Furthermore, it is effortless to make proximal contacts using indirect restorations. An essential advantage of using this indirect restoration method is increased surface hardness, resistance to compression, reduced risk of fractures and cracks in the internal structure of the material.[6]

One of the primary purposes of restoration is to establish and replicate occlusal anatomy and proximal contacts of the tooth. Indirect composite restoration can achieve all that and hence should be considered as restorative material of choice when the tooth can not be isolated, inaccessible areas, limited mouth opening and when the patient cannot sit for a long time during direct restoration.[8]

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Disinfection Procedures in Dental Office: An Overview

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Abstract

Disinfection & Sterilization if done properly, it can prevent transmission of many diseases among the doctors, healthcare personnel and patients. The risk of infection is high in the dental office because the oral cavity is rich in aerobic, anaerobic bacterial flora and many viral pathogens. The method of disinfection and sterilization depends on the use of the medical devices: **Critical** instruments (pierce the tissue) should be sterilized, **Semi critical** instruments (come in contact with the mucosa) should be sterilized & **Least critical** instruments (touch the Surfaces during treatment) & should be disinfected. Cleaning should be done by high-level disinfection and sterilization. Current guidelines of disinfection and sterilization must be strictly followed by doctors, healthcare personnel.

Keywords: *Disinfection, Dental Office, Sterilization.*

Introduction

The risk of infection is high in dental practice as the oral cavity is a host for many commensal and parasitic pathogens. The susceptibility of infection to hazardous diseases like Hep-B & C, HIV & TB, etc through dental treatment procedure is an area to concerned. Orthodontists are more susceptible to contracting hepatitis.[1] At least we should try to reduce the number of infectious organisms to a certain extent so that our immune system can protect from infection and eliminate cross-contamination.

Lister (1867), introduced antiseptic techniques in surgery, also known as Father of Modern Antiseptics. The arrival of autoclave drastically changed the method of sterilization in modern times. So many chemicals with germicidal effects were discovered in the recent times.[2]

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Disinfection: Disease transmission doesn't have any specific route. It can pass through direct or indirect contact or even without contact also. All healthcare personnel who always engage with various patients are particularly at a higher risk of contracting disease. Patients particularly with transmissible diseases either viral or bacterial make a constant source of contamination.

Pathway of transmission

According to the Council of Dental Therapeutics the goals of infection control programs are:

- minimizing the number of infectious pathogens to a certain extent where the body immune system will be to resist the infection.
- Disrupting the pathway of infection & terminate cross-contamination.
- considering each patient/instrument as a potential carrier of disease.
- protecting patients, practitioners & other health workers from infections.[3]

Sterilization is making a substance-free from all pathogens both in the *vegetative or spore state*.

Disinfection is making a substance-free from all pathogens except bacterial endospores. **Antiseptics** are mild disinfectants applied to living subjects. **Antisepsis** is preventing infection by the inhibition of growth of bacteria, viruses, fungi on living surfaces such as skin & mucous membranes. For sterilization & disinfection different chemical agents are used. Disinfection indicates cleaning and application of a disinfectant on a surface or object.[4]

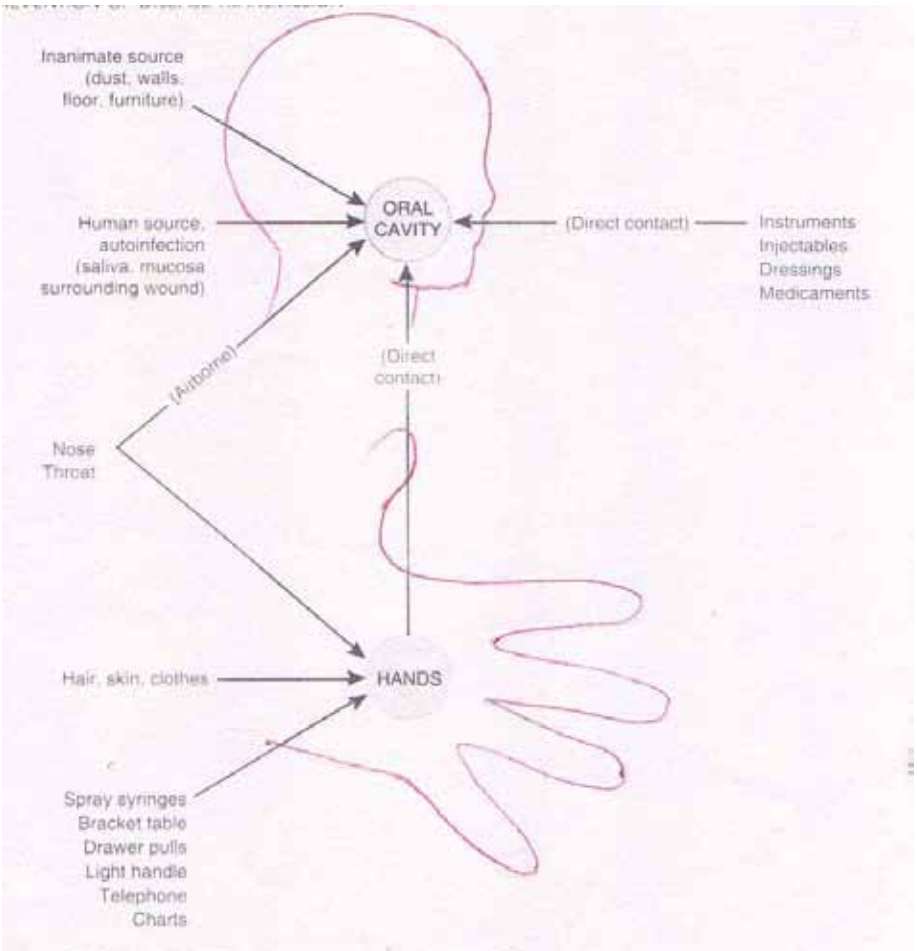


Figure 1. Pathway of transmission

Ideally, a disinfectant should:

- Have stability, high penetrating power and a wide spectrum of activity.
- Be compatible with other disinfectants and noncorrosive.
- Be effective with organic matter & both in alkaline & acidic medium.
- Be nonirritating, safe & easy to use.

Disinfectants used in dentistry are as follows:

- Aldehydes
- Chlorine products
- Alcohols
- Synthetic phenols
- Iodine products

Aldehydes:

- **Formaldehyde**: 10% formalin with 0.5% sodium tetraborate is used as a disinfectant. Its gas is used for the disinfection of instruments that are sensitive to heat. It should be used carefully as it is toxic if inhaled.

- **Glutaraldehyde:** Generally 2% glutaraldehyde solution is used for disinfection and the instrument or object should be placed 20 mins. It is preferred to formaldehyde as it is less toxic & less irritant, can safely be used with all kinds of instruments.

Chlorine products: Sodium hypochlorite solution is mixed with distilled water to prepare the disinfectant. It should be used after thorough cleaning of the surfaces. It acts by damaging the cell wall and blocking the enzyme system to protoplasmic poisoning. It is unstable and corrosive to metals and softens plastic. It produces constant odor and can irritate eye & skin.

Alcohols: Isopropyl alcohol (60-70%) is generally used as a disinfectant. It kills bacteria by denaturation of the protein. But to be effective the surface should be devoid of saliva or blood. It rapidly evaporates from used surfaces.

Synthetic Phenol: Synthetic phenols like Lysol and Cresols are commonly used as disinfectants. Chlorine compounds such as Chlorphenols & Chloroxyphenols have less effect as disinfectant & are less irritating. They damage the cell membrane and cause cell lysis. Its effectiveness depends on contact with bacterial cells.

Iodine products: Iodophores (*Povidone-iodine*), the most commonly used disinfectants are a combination of iodine compound & surface-active agents. These are more effective than aqueous or alcoholic compounds of iodine. Iodine binds with the bacterial cell protein and destroys them.

Surface-active agents: They commonly known as wetting agents. E.g. soaps/detergents & quaternary ammonium compounds/emulsifiers. Quaternary ammonium compounds (QAC) have *cationic surface* but soaps/detergents have *anionic surface*. Commonly available QACs are Cetrimide & Benzylkonium chloride. QAC decreases the surface tension between the bacterial cell wall and the object, so it helps in wetting the bacterial surface with the disinfectants and kills them. The effectiveness depends on the concentration, degree of contamination and presence of other compounds. According to the American Dental Association (**ADA)** alcohol/QAC/phenolic compounds should not be used as the only method of disinfection in dentistry as they are not effective against bacterial spores and viruses.

Vitawipe: It is a disposable disinfectant cloth, contains *alkyl dimethyl chloride* [7.1%], *poly hydrochloride* [3.2%] & inert ingredients [89.7%]. To wipe contaminated surfaces it should be dampened with water. It removes the microorganism physically without having any chemical effect. It should be discarded after the color change of the indicator or after use in case of a Hep-B virus infection.[4]

Infection control procedures:

Instrument disinfection: Instruments are the main media for cross-infection. So they should be disinfected & sterilized thoroughly or sterile disposable instruments should be used. *Milton Schaefer of the University of Southern California School of Dentistry* has divided and described the different pathways of cross-infection and the method to handle as follows:

1. **Critical:** Instruments (scalers, scalpels, and other tissue-cutting instruments) that goes into the mucosal tissue should be sterilized.
2. **Semicritical:** Instruments (hand instruments and pliers) that comes in contact with the mucosal tissue should be sterilized.
3. **Least Critical:** Instruments & Surfaces (Worksurfaces, three-way syringes, chair light handles) that comes in contact during treatment should be disinfected.

Instrument processing: Used instruments first placed in a solution consisting of detergents, enzymes, or detergents with disinfectants until it starts to disintegrate the organic tissue and sometimes begin to kill the pathogens. Then the instruments are cleaned either manually or with the help of an ultrasonic cleaning machine. Heavy-duty utility gloves and protective goggles must be worn during hand cleansing. But manual cleaning is time-consuming and there is a chance of an accident.

Mechanical Cleaning: Combining the use of powerful vibrations and disinfectant solution in an ultrasonic machine increases the effectiveness of cleaning. Continuous vibration makes proper contact of the instruments with the disinfectant solution.[5] The instruments should be kept submerged in the cleaning solution during the whole cleaning procedure.



Figure 2. Ultrasonic cleaner machine

It should operate for **6-10 mins** or until the surface is visibly cleaned but the time should be **15mins** if the instruments are placed in a cassette. Thorough rinsing of instruments is done after cleaning.

After cleaning the instruments should be packed & sealed in an appropriate wrapping material before sterilization to prevent recontamination. Cloth wrapping for autoclave/steam sterilization, polyfilm paper pouches that are self-sealed for chemical vapour sterilization and paper wrapping for dry heat sterilization may be used. Metal trays with lids that don't need any wrapping should be sterilized by dry heat. Perforated trays and trays without lids should be sterilized by chemical vapour or steam sterilization so that steam can pass through the perforation. According to ADA recommendations the sterilization method for different items used in the dental office are as follows;

Rubber items and saliva ejectors: It is always safe to use disposable one and dispose of after use. Because it may get damaged in the process of heat sterilization and cold sterilization with ethylene oxide is not effective.

Handpieces: Cold sterilization with ethylene oxide sterilization and dry or moist heat sterilization can be used.

Stones: Diamond stones are sterilized by dry heat/ chemical vapor/ethylene oxide gas except polishing stones which are sterilized by chemical vapour/ethylene oxide gas only.

Impression trays: Chrome plated trays are sterilized by any method of sterilization but plastic or acrylic trays are sterilized by ethylene oxide or glutaraldehyde.

Stainless steel hand instruments can be sterilized by moist/dry heat, chemical vapour/ethylene oxide gas. Three-way syringes, NiTi wires are sterilized by chemical vapour/glutaraldehyde. Chemical vapour is used in the case of E-chain & ligature wires. [6]

Orthodontic pliers: For high-quality stainless steel pliers all method of sterilization can be used. But for low-quality pliers dry heat sterilization is preferred. For pliers with plastic parts cold sterilization with ethylene oxide is used.

Ultrasonic tip, Gloves & gauze, Tongue blade, lip & cheek retractors, Matrix band, spatula & light cure tip should be autoclaved. Ultrasonic cord should be sterilized by vapoclave and glutaraldehyde. **Glass slab** & **dapendish** should be sterilized by dry/moist heat and vapoclave.

Staggers et al studied the effects of different method of sterilization on different orthodontic wires. They found that the tensile strength of TMA wire is increased in case of dry heat sterilization but moist heat & ethylene oxide sterilization had no effect. The tensile strength of Sentalloy wires is increased due to dry or moist heat sterilization whereas no effect found in the case of SS wire.[7]

Jones M.L found that Chrome plated pliers are more resistant to deterioration and maintained the original shape, size & looks better than SS pliers after various method of sterilization.[8]

Sterilization monitoring: ADA has recommended regular monitoring of sterilizers to maintain the quality of the dental treatments. It is appropriate for a dental office to monitor sterilizers every week.

Biological monitoring: The most important way called biological indicators is done by the use of highly resistant bacterial spores. Spores of *Bacillus stearothermophilus* and *Bacillus subtilis* are used as biological indicators for steam & chemical vapour and dry heat & ethylene oxide gas sterilization respectively. **Spore strips (**filter paper strips impregnated with spores**)** for dry/moist heat, cold sterilization**, Spore vials (**small paper or disk spores**)** for steam/ethylene oxide**, Spore ampules (**glass ampules of spore in culture medium**)** for moist heat sterilization.

Chemical monitoring: Chemicals are placed inside or outside of the packs or trays. It changes the color to indicate the efficacy of the sterilizer. Internal chemical indicators are more accurate.

After sterilization the instruments must remain

untouched, protected from the environment till it becomes cool. The sterile instruments & trays must be kept in clean, less dust place which must be dry & moisture-free. Sterile instruments & trays or cassettes should be opened up at chairside for use. In the process of cleaning & sterilization there is a chance of corrosion of instruments due to the contact of two dissimilar metals and different chemicals. Hence to prevent this, 1) instruments should be cleaned properly and rinsed with distilled water. The surface should be free of blood, saliva, or other contaminants. 2) good quality steam should be used in the autoclave and should be deionized. Below 6.4 pH of the steam can cause pitting and corrosion. 3) Chrome-plated and stainless steel instruments must be autoclaved separately to prevent any electrolytic action.[6]

Surface protection procedures: Surfaces like different handles, controls, light cure units, micromotor & ultrasonic handpieces, three-way syringe etc which are touched by the dental surgeon during treatment should be cleaned with disinfectants or protected with a barrier. After every patient the surfaces should be cleaned by using **disinfectants** like alcohol/iodophore/ glutaraldehyde/chlorine products/synthetic phenols. The chair surfaces can be covered by impermeable **barriers** and must be discarded after every patient. They can be of plastic films, laminated paper plastics, aluminum foil etc and different sizes and shapes. The merits of this method are its easy & speedy insertion and protection of equipment from the harmful effects of chemicals and body fluids. But the cost, storage & disposal difficulties are the main drawbacks.

Tubing and hoses: As the surfaces remain moist always it creates a good environment for the growth of the bacteria and forms a biofilm. So regular checkup of the tubing and hoses should be done even if, there is an anti-retraction system. The suction tube should be flushed with fresh water before each patient and disinfected at the end of the day with a disinfectant like sodium hypochlorite. All water tubings should be cleaned by flushing water for 3-5 mins if the system has not been used for long times. Similarly the handpieces must be cleaned for 30 secs with air & water to remove anything that has come inside during use. Air compressor or tank should be emptied daily to prevent the growth of microorganisms in the tank. **Rubber dam** is used as a barrier to control the airborne contaminants. It reduces the infectious particle in the aerosols to a significant amount.[9] According to **ADA** use of mouth wash just before the procedure can reduce the number of pathogens in the mouth but it does not affect the number of pathogens that could be aerosolized.

Disinfection of removable appliances: Removable appliances are always coated with saliva and plenty of microorganisms due to its continuous use in the oral cavity. So during any adjustment protective glasses and full face-mask should be used. It also should be disinfected by placing the appliance in a germicide container.

Oral Safe is a germicide-deodorant and it is safe if occasionally swallowed. In an earlier study it was seen that 99 % of microorganisms are destroyed if the appliance is submerged for 10 mins in Oral safe. **H. Petit et al** described a method in which they combined germicide-deodorant with ultrasonic cleaning for better effects. They found that in three minutes use of this procedure 10 times more microorganisms are killed than the passive submersion method.[10]

Dental laboratory practices: All Impressions, occlusal bite records and devices which comes in contact with patients' saliva, blood or debris must be thoroughly rinsed to remove them and then put in disinfectant solution like glutaraldehyde/formaldehyde before sending to the laboratory.

Conclusion

As a sterile environment is mandatory for dental treatment it is necessary that everybody in a dental office should have some knowledge about disinfection and should be maintained. The process of maintaining sterility in the dental office is more complex than it appears. So it is the responsibility of all (patient, staff & the professionals) to carry out their duty with utmost purity and clarity. Hep- B, pneumonia, AIDS and other serious contagious diseases can be contracted in a dental office. So it is indispensable to use proper sterilization procedures for moral, professional & medico-legal point of view. Technological advancement and invention of newer machinery made the sterilization and disinfection procedure easier. The use of disposable barrier technique and different chemical agents for disinfection has further decreased the responsibility of dental staff to create a pure aseptic condition for the dental treatment.

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Impact of Yoga in Dental Practice

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Abstract

Dentistry is perceived as a profession that is more stressful than other jobs. Many occupational health problems persist in modern dentistry. It is high time we, the dentists, also inculcate the practice of performing Yoga in our daily life regime, to strengthen our muscles of neck, shoulder and back, to save ourselves from musculoskeletal pains. The "Asana" recommended in the Yoga, specifically for posture-related problems can be of great help to the dental professionals in managing their day to day muscular discomfort and pain. The need of the hour is just to modify our lifestyle to get the maximum out of our professional and personal life. This review article focus on the importance of yoga in dental practice.

Keywords: *Yoga, Dental Practice, Healthy Body and Mind.*

Introduction

In one of the holy texts of old times – the Rig Veda, the term "Yoga" was first mentioned which is a derivative of the Sanskrit word "YUJ" which means 'to join' or 'to yoke' or 'to unite'. Over 5 thousand years ago the foundation of yoga was laid down by the Indus – Sara swat civilization.[1]

Yoga focuson a healthy mind in a healthy body concept that helps in controlling an individuals mind, body and soul.[2] It builds confidence and helps in maintaining a healthy lifestyle. Yoga is versatile, pleasurable as well as highly beneficial for dentists to battle stress and apprehension and to decrease work-related musculoskeletal pain.

Dentistry, as a profession, is very demanding, in terms of the focus and precision required while performing the procedure, more so, because we are working on a very small area, which requires sheer attention. In doing so, many times, we forget or overlook the posture in which we are working continuously for long hours.[3] When the dentist is working in a sitting position, over half of the body muscles provide support, to enable the body to be in a static position. If the same posture is continued for a longer period, muscle ischemia happens. As a result, what we get in return is the musculoskeletal disorders that exhibit in the form of pain. The intensity of this pain can vary from mild discomfort in the neck, shoulder and back region to excruciating one, so much so that it leaves us with no choice but for placing a halt on our professional work and then rushing to the concerned specialists for management and respite.[4]

As a substance of fact, as it is said "small things can make a big difference," as a fact of our life like a simple change in our lifestyle, can make a huge difference in the way we can escape this health hazard which is gulping the entire dental fraternity worldwide, like a monster.[5] The "International Yoga Day" was celebrated worldwide on June 21, 2015, attracting everyone's attention on this indigenous tool in the prevention of various health-related issues.

Advantages of yoga[6]:

a. Improvement in cardio-respiratory efficiency

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b. Improvement in exercise tolerance

c. Harmonious balance of autonomic function

d. Improvement in dexterity, strength, steadiness, stamina, flexibility, endurance, and neuro-musculoskeletal functioning

e. Increase in alpha rhythm, inter-hemispheric coherence and homogeneity in the brain

f. Improved sleep quality and improved cognitive functions

g. Alteration in brain blood flow and brain metabolism as well as

h. Modulation of the neuroendocrine axis.

It is high time we, the dentists, also inculcate the practice of performing Yoga in our daily life regime, to strengthen our muscles of neck, shoulder and back, to save ourselves from musculoskeletal pains. The "Asanas" recommended in the Yoga, specifically for posture-related problems can be of great help to the dental professionals in managing their day to day muscular discomfort and pain. The need of the hour is just to modify our lifestyle to get the maximum out of our professional and personal life.[7] This paper aims to stress the benefits of yoga for dental surgeons.

Why introduce Yoga for dental students and professionals?[8]:

a. Improved visibility is gained, sitting at one place for a long duration as well as working in the desktop and keyboards can very well put massive strain on the musculoskeletal system.

b. Mutual understanding with disobliging teammates and with fearful patients as well as job-related frustrations can become overwhelming.

c. Results in stress, anxiety, anger, and depression.

d. Prevalence of general musculoskeletal pain is estimated to be between 64% and 93%

e. Major issues : – back pain (36.3-60.1%) – neck pain (19.8-85%).

f. Risk factors include:

– static and awkward posture of clinical practice

– other non-ergonomic work practices.

It has been recently suggested that Yoga may be a good way for dentists to relieve stress and reduce the risk of repetitive strain injury and dysfunctional posture. Another survey amongst 220 dentists found noteworthy differences in existing cases of musculoskeletal pain among those who practice yoga as compared to those with no regular activity over 12 months.[9]

Yoga can improve your health in various ways like:

1. Yoga Stimulates Saliva Production:

- Bacterial count in the oral cavity increases because of the reduction in salivary production. Bacteria can thrive in an environment like dry mouth which later results in plaque formation, tooth decay, and gum disease.[10]
- Regular yoga with practicing breathing method can help avoid mouth and throat from drying. Increase saliva production is related to someyoga poses likeinverted poses, twists and forward bends.
- The Khechari mudra–is a type of attitude or honour which should preserve vitality. Method of performing the Kechari Mudra are simple -Rolling tongue backward as if it enters the nasal cavity to touch uvula inside the mouth. Breathe in as well as with a snoring type of sound from inside throat. Breathe outand swallow some saliva that was deposited in your mouth while releasing from the posture of your yoga style.[7,10]

2. Yoga Improves Posture :

- A good body posture gives us many benefits likegood memory along with brain functions, improved breathing, less stress, healthier mood, stronger core muscles, better confidence and more energy as well as better productivity.[11]
- Improper posture can affectthe spine and neck as well as the mouth. The mandible shifts forward after a person slouches causing the teeth of both the jawsin malalignment position. During this the head moves back and compress the spine. If left untreated, there will bestress on the surrounding muscles, bones and also in joints. It alsocan create pain and inflammation in the muscles and joints involved in the opening and closing of the mouth.[7,12]
- Jaw issues like temporomandibular joint disorder can also arise from improper continuous posture. This disorder can have common side effects that consist ofa clicking sound or pain while opening the jaw, tenderness in the face can be felt, chronic headaches and changes in jaw alignment resulting in

rough wear of the teeth. (13) Poor posture can also lead to poorly balanced shoulders causing stress on the ribs as well as the organs underneath.[14]

3. Bad breath:

- Also known as halitosis is a distinctly unlike able odour that can be felt while breathing. It results in fretfulness in exaggerated person. There might be various etiologies for halitosis that includes all improper oral hygiene habits but problems with our gut can also be a reason that leads to bad breath. This type of sign and symptoms is known as dysbiosis which is an imbalance between the good and bad bacteria in our gut, as well as the presence of yeast or candida in the gut.
- Certain yoga postures are there that can unswervingly involve the digestive as well as respiratory organs. By improving blood circulation and manipulating functions of the kidneys, lungs, stomach, kidneys or liver and also removing toxins is encouraged. All this may help cure and prevent bad breath. Yoga posture like Lion Pose or Simhasana– The Cooling Breath or Sheetali Pranayama – The Psychic Union Pose or Yoga Mudra, Kapalbhati breathing are some of the asanas that can help out in curing halitosis.[12,15]

4. Yoga reduces Stress:

- More stressed focus less on the maintenance of good oral health.[16] Stressed people have a habit of grinding their teeth resulting in micro-cracks and broken fillings and worn down teeth results in nerve damage; the teeth that are grounded down to the dentin results in sensitivity to heat and cold; gum recession because of pressure on the gum line; loose teeth and gingival pockets due to the motion of teeth grinding; headache and aching jaws because of overworked jaw muscles; and jaw impairment.[9]
- Stress causes the salivary glands by slowing down the production of saliva resulting in dry mouth andgives a suitable environment for bacteria to thrive. These kinds of bacteria cause serious dental issues like inflammation in the gums. Stress exacerbates inflammation in the gums which can lead to more serious dental issues and even general health issues. From regular yoga practice, stress can lessenthe body can be maintained mentally and physically healthy.[9]

Some other professional hazards for dental surgeons are:[13]

- Lower back pain
- Cervical spondylosis
- Eye problems because of continuous and constant focus into the oral cavity where light has less power
- Varicose veins in lower limb

Prevention and cure by doing yoga therapy[3,10]:

Bhujangasana: This pose lengthens and strengthens the back and shoulders caused due to improper flexibility of upper and middle back. It helps to reduce fatigue and stress. This yogasana has been shown to reduce chronic pain associated with Musculoskeletal and joint diseases. It also helps in treating Cervical spondylosis.

Padmasana: This Asana works wonders for the spinal cord by keeping it erect, healthy and flexible. In the Asana the lungs get packed with air-filled oxygen. By leg pulling the nerves, muscles and bones are stretched tight thus massaging the legs meticulously and keeping them healthy.

Sirshasana: This Asana helps in providing conditioning to the brain, eyes and ears due to increased blood pressure. It also helps in improving concentration and memory. This Asana improves blood circulation in the organ of both the normal and diseased eyes.

Vajrasana: This asana helps in the impediment of varicose veins which take place because of prolonged standing. This Asana helps in the majority of cases. Because of this asana, the blood flow to the lower limbs is reduced, muscles are stretched and these varicose veins are cured.[16]

Conclusion

Practicing regular yoga can help in preventing and treating many long term diseases as well as can helps in achieving good oral health. Yoga heals the body, mind and soul by stress reduction and also by increasing the motivation level. The inflammatory response decreases and increases in the immunity to attain good gingival health and healing after extractions and oral surgical procedures is achieved by performing regular yoga. The lifestyle of a yogic is always lean towards nature, it helps in the impediment of dental as well as soft tissue disorder of the mouth which are the result of the stresses of life.

It is high time that we the dentist include the practice of performing yoga in our daily life regime. Asana recommended in yoga especially for posture-related problems can be of great help to the dental profession in maintaining their day to day muscular discomfort and pain. By practicing regular yoga, it rejuvenates and revitalizes all the organs of the body. So devoting 40 to 50 minutes on an average day to yoga, we can remain fresh mentally and physically and it will keep us away from professional hazards.

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Modified Semilunar Advanced Flap with Connective Tissue Graft Technique for Root Coverage: A Case Report

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Abstract

Gingival recession may not only result in aesthetically unacceptable results but also be associated with increased susceptibility to root caries and dentinal hypersensitivity. A novel technique for recession coverage by using a modified semilunar advanced flap technique with connective tissue graft is described. The technique involves placing of connective tissue graft underneath a semilunar incision which is made parallel to the free gingival margin of the facial tissue and its advancement over the denuded root. This technique has the advantage over conventional semilunar flap, in that benefit of connective tissue graft is combined for improved prognosis

Keywords: *Semilunar flap, connective tissue graft, gingival recession, coronally advanced flap.*

Introduction

Gingival recession (GR) is a common concern of many patients who visit a dental office, but despite the concern of patients, the practice of recession coverage often eludes general dentists owing to the need for superior skills. In gingival recession there is an apical shift of the marginal gingiva resulting in denuded root surfaces[1]. It not only is an esthetic concern but also causes dentinal hypersensitivity. Tissue destruction that takes place in periodontal disease or mechanical factors due to toothbrushing can cause GR[2,3]. Root coverage (RC) is indicated when there is an esthetic concern, dentinal hypersensitivity, root caries, cervical abrasion and prevention of periodontal disease progression[4-6].

Several surgical approaches have been put forth in the management of denuded root surfaces. Among these, the subepithelial connective tissue graft (SCTG) procedure, has been considered as the most efficacious and with most predictable outcomes[7,8]. Introduced for RC in 1985, SCTG has been performed by using a variety of flap designs[9,10]. In the following year, ie in 1986, Tarnow et al[11] introduced the semilunar coronally advanced flap (SCAF) procedure. Case reports with connective tissue graft for treatment of isolated gingival recession have shown a high success rate[12-14]. The present case report utilizes a modified semilunar advanced flap technique in combination with connective tissue graft in the management of gingival recession.

Case Report: A male patient aged 32 years was enrolled in the OPD of the Department of Periodontics, Institute of Dental Sciences, Bhubaneswar, Odisha,with a chief complaint of receding of gums in the upper central incisor (tooth number 11). The patient had no faulty tooth brushing habits or any parafunctional habits and hadn't taken any antibiotics in the preceding six months. He neither had any prosthesis in the anterior region nor did any tooth receivea restorative therapy. His medical history was non-contributory.

Intraoral examination: Class I Miller's gingival recession was observed in the tooth number 21 (Fig 1). Clinical measurements such as a recession defect of 2mm (Fig 2) and width of keratinized tissue of 4mm

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prior were measured using a UNC 15 periodontal probe. Clinical photographs were taken prior and post-surgical therapy. The intraoral periapical radiograph (IOPA) taken had no bone loss in the 21 region. A recession coverage procedure was planned, explained to the patient and a written consent was obtained.

Pre-Surgical Preparation: Scaling and root planing was carried out to render the surgical area plaque-free. Ademonstration of the correct tooth brushing technique to the patient was also done.

Surgical procedure: After anesthetizing the surgical site with lignocaine (2%), a crevicular incision (Fig. 3) was placed on 21. This was followed by creating a tunnel by separating the mucosa from the underlying periosteum (Fig 4). A semilunar incision (Fig 5) following the curvature was placed at an area apical to the marginal gingiva of the indicted tooth such that the apical most extent is in the alveolar mucosa. The incision curved laterally and lay coronally in the keratinized gingiva apical to the mesial and distal interdental papilla maintaining the vascularity of the flap. The harvested connective tissue graft (CTG) from the palate was then placed underneaththe coronally advanced semilunar flap (Fig 6 & 7).Advanced flap along with the CTG was stabilized by an anchor suture on the buccal surface of the tooth (Fig 8). The surgical site was covered by a periodontal dressing for mechanical protection and to control postoperative bleeding from the surgical site. Patients were given post-operative instructions and were prescribed with suitable antimicrobials and analgesics. Suture removal was done after 10 days and the patient was put on a periodontal maintenance regimen. An acceptable root coverage was seen after which was stable even after 6 months (Fig 9). Fig 10 provides a schematic diagram of the technique for visualization and better understanding.



Figure 1a Recession defect irt 11, igure 1b.Recession of 2 mm



Figure 2a. Crevicular incision given, Figure 2b Tunnel created



Figure 3a. Semilunar incision given, Figure 3b. Graft tucked underneath semilunar flap



Figure 4a. Connective tissue graft placed, Figure 4b. Suture placed.

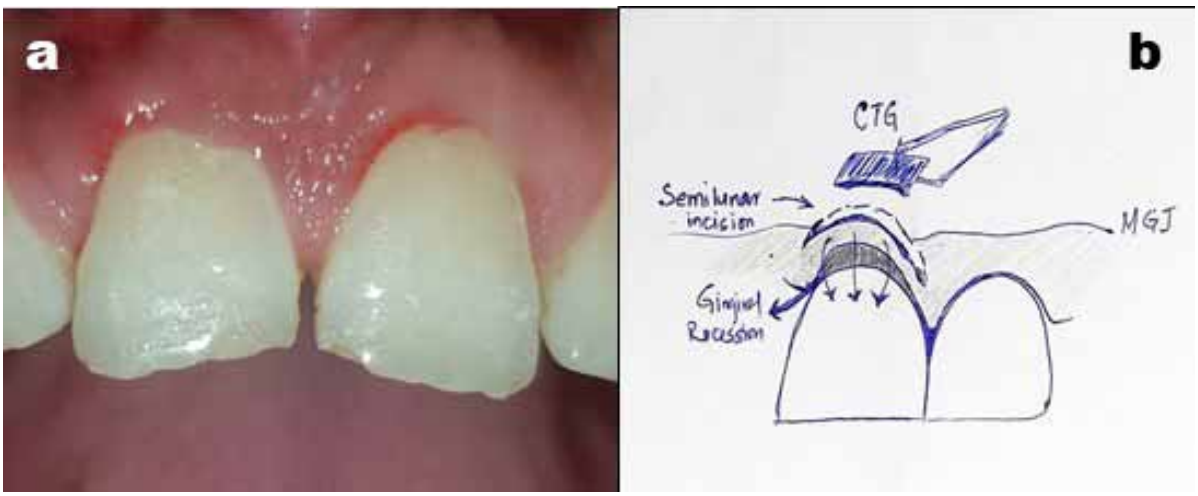


Figure 5a. Postoperative 6 months, Figure 5b. Schematic Diagram; CTG = Connective Tissue Graft, MGJ = Mucogingival Junction

Discussion

The presented technique utilized the combined advantages of the SCAF technique and SCTG technique in that there is no disturbance to the adjacent interdental papillae, there is no shortening of the facial vestibule and has least flap tension. This technique is preferably to manageisolated gingival recessions which serve as a predictable root coverage procedure. Apart from preserving the adjacent interdental papilla, this technique also applies less traction and preserves the gingival height. Minimal trauma at the recipient site due to minimal incision, may be advantageous in masking recessions as compared to the coronally repositioned flap. In this case we utilized one vertical incision to facilitate placement of connective tissue. This technique combines the benefits of subepithelial grafting along with semilunar incision and tunnel procedure, thus improving the outcome.

SCTG procedure is advantageous in that it provides a good tissue blending at the grafted site. Also, owing to its conservative approach, there is minimal trauma and discomfort at the donor site. The recipient site receives a dual blood supply from the underlying connective tissue base and the overlying recipient flap which contributes to the success of this procedure[15].

In the presented case which was managed by the novel technique of combination of SCPF and CTG showed 100% root coverage. The tunnel approach used in the technique is a modification of the pouch and tunnel technique which was designed specifically for isolated recession frequently found in the maxilla. There was a highly esthetic outcome and also increase the thickness of keratinized gingiva.

Conclusion

This present case report confirmed that a modified semilunar coronally advanced flap and in combination with subepithelial connective tissue graft technique is an efficient treatment option for the management of gingival recession defects and resulted in complete root coverage of the treated case.

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Effect of Long-term Use of Steroidal Anti-inflammatory Drugs on the Periodontal State: A Review

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Abstract

Corticosteroids are chemically similar to endogenous cortisol and mostly prescribed for their anti-inflammatory and immunosuppressive properties, which are due to the inhibition of phospholipase A2 activity. These drugs are extensively used in the treatment of a plethora of autoimmune disorders such as rheumatoid arthritis, various breathing disorders and also disorders of connective tissue. In the field of dentistry these drugs are prescribed in most of the mucocutaneous disorders such as they are mostly used in inflammatory diseases such as oral lichen planus, pemphigus, and oral stomatitis to alleviate post-operative discomfort and swelling. These drugs can be administered systemically,topically or asinhalational therapy. Evidence concludes that short term corticosteroids possess antiresorptive properties and its local application shows a favourable effect on the periodontal ligament, but when administered systemically for a long period could lead to periodontal diseases as it has shown to increase attachment with alveolar bone and destruction of transseptalfibers. Oral manifestations are dependent on duration,dose and frequency of use. It is commonly associated withmoniliasis, dental caries,altered taste sensation, ulceration of the tongue, buccal mucosa, and gingiva due to xerostomia,gingival inflammation, periodontal inflammation,and immune suppression. They can either cause dramatic improvement or dramatic adverse reactions which can lead to a considerable increase in the distributionof periodontal disorders. In patients on long-term corticosteroid therapy, oral health is generally not given importance during management. Hence this review explains about various effects of different types of corticosteroids on periodontium used in dentistry.

Keywords: *Corticosteroids, risk factors, mucocutaneous lesions, periodontitis, Bone mineral density.*

Introduction

Physiologically steroids (cortisol and aldosterone) are produced by adrenal glands and help in various physiological functions such as metabolism of fats, proteins and carbohydrates, immune mechanism, and saltwater balance.[1,2] Testosterone, estrogen, cortisol and aldosterone are the steroid hormones produced by the body.Corticosteroids are synthetic analogues of steroid hormones that are produced in the adrenal cortex of vertebrates.[1] In 1940s glucocorticoids were first introduced and since then it has become an extensively prescribed class of drugs to manage various disorders such as adrenocortical insufficiency, rheumatic diseases, asthma, and skin diseases.[2] They are available in different formulations like ointments,gels and tablets.[3] Due to their anti-inflammatory action, steroids were first used clinically to treat dermatological diseases. Corticosteroids are commonly advised as replacement therapy in treating various types of inflammatory and immunosuppressive disorders.[4,5]

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Steroidal drugs are known to exert definite anti-allergic, anti-inflammatory and immunosuppressive effects.[1] However, despite these beneficial actions, corticosteroids are also known to cause adverse reactions likerashes, clouding of the lens, weakness in the muscles, GIT disorders and candidiasis. In dentistry, they are generally advised to reduce post-operative edema, postoperative pain, anxiety and for the management of oral inflammatory manifestations of systemic disorders like bullous pemphigoid, oral lichen planus, pemphigus, erythema multiforme, recurrent aphthous stomatitis, and hypersensitivity reactions.[2]

Research has proventhat the patients on corticosteroids often present with oligodontia or are characterized by dentition which is prone to dental caries.[6] Corticosteroids can cause osteoporotic changesin the alveolar bone due to the reduction of bone-forming cells and the amount of bone matrix. This results in a fibrous transformation of the periodontal ligament space and decrease of the alveolar bone height.[7] In periodontology, the use of corticosteroids is mostlyrestricted to its use for periodontal manifestations of systemic disorders, and not as a first-line treatment of periodontal disease per se. Nevertheless, it is necessary to know the uses and effects corticosteroids have, not only on oral diseases but also on the gingival and periodontal diseases and hence this review.[8,9]

Uses of steroidal anti-inflammatory (corticosteroids) in oral diseases: Corticosteroids are commonly administered in the topical, systemic, and inhalational form.[10] of these, topical corticosteroids are the ones that are commonly used for the treatment of oral lesions.[11] They have evolved and emerged as the mainstay of therapy for numerous oral lesions and conditions such as oral lichen planus, erythema multiforme, desquamative gingivitis and major aphthous ulcers.[12,13] Their use is mainly due to the anti-inflammatory, immunosuppressive, and anti-proliferative properties of corticosteroids.[14] These properties/actions are expressed as a result of a process called transactivation. It is mediated by the nuclear glucocorticoid receptors which arepresent in the cytoplasm which regulates the protein transcriptiontoinduce anti-inflammatory proteins and regulatory proteins.[15] Corticosteroids displace proteins like heat shock proteins from the inactive receptor site and bind to the receptor, and this corticosteroid-receptor complex then getstranslocated to the nucleus and binds to a specific sequence of deoxyribonucleic acid. The metabolic effects and some of the adverse reactions could be occurring through this process.[14,15]

Anti inflammatory action: This action is mainly brought about by topical corticosteroids which mainly function by inhibiting the formation of eicosanoids. Corticosteroids stimulate the production of various polypeptides, collectively called lipocortin which has inhibitory effects on phospholipase A2 activity.[16] They are also known to block the action of vasodilators such as histamine and bradykinin leading to vasoconstriction. This is clinically evident by a reduction in erythema.[17]

Immunosuppressant action: This is brought about by suppression of cytokines namely interleukin 1 (IL - 1), IL- 2–6, IL-8, and tumor necrosis factor-alpha, leading to a reduction of cell proliferation. They also affect the humoral immunity by reducing B cell expansion,destruction of T lymphocytes and antibody synthesis.[18]

Anti-proliferative action: This is seen as a result of a reduction in the mitosis in the epidermis which makes the basal cell layer, as well as the corneum and granulosumlayer of the keratinized epithelium thinner. The proliferation of keratinocytes is affected, there is a decrease in the keratinocyte growth factor, and inhibition of fibroblast proliferation, migration, and chemotaxis. This is followed by abnormal aggregation of the elastin and collagen fibers,which decreases the synthesis of collagen and glycosaminoglycans. For oral lesions,topical corticosteroids are most commonly advised and hence it is important to know the factors that need to be taken into consideration when prescribing or using them. The effectiveness of treatment with topical corticosteroids depends on the halogenation of its 6main ingredient, i.e., cortisol. This halogenation leads to an increased binding of cortisol to the glucocorticoid receptor. The penetration of the topical corticosteroid depends on the esterification of cortisol which increases the lipophilicity of the steroid.[17]Hence, the corticosteroids should be selected based mainly on their potency and the area of application.[19]

There are various classifications of topical corticosteroids based on their potency with or without using the vehicle. Among them, the World health organization (WHO) classifies the topically administered steroids into seven groups, with Group 1 which is the most potent and Group 7 being the least potent. Potency is determined based on the activity of the corticosteroid molecule, its concentration, and the nature of the

vehicle.[20] Highly potent concoctions are meant to used for a brief period only and are topically applied on the soles of the foot, and palms of the hands. It can also be used for the management of hyper keratinized lesions. For the management of the lesions on the face caused due to various acute inflammations low to medium potency formulations are used. They can be used for a longer period. Normally, high potency steroids are used for oral application for a short duration.[17]

Since the adventmany different delivery systems of these topically applied steroids have been developed such as Creams, ointments, lotions, and gel/hydrogel.[20,21] Vehicles are nothing but carriers. They carry the active steroid molecule which keeps the skin hydrated and moisturized and enhances the penetrative capacity of the drug. Creams are the vehicles that aremost preferred by the patients. Their occluding capability is less due to their water-based preparation. Hence, they spread easily, without anoily and sticky feeling.[21]Ointments are preferredin highly keratinized areas and for dry or scaly lesions. They enhance moisture-holding capacity and possess a good occluding ability. This aids in the penetrating capacity of the drug. Gels are prepared using a gelling agent.This makes the application of the dug more convenient. The shortcoming of using gels is that they have the possibility of inflicting pain and discomfort after the application. Conventionally, ointments have been considered to be more potent than the rest of the formulations.[22] The use of corticosteroids for treatment of periodontal disease is however limited. Corticosteroids are known to affect fibroblasts and bone remodeling, which may affect the periodontium and hence it is important to have a thorough knowledge regarding its application and the drawbacks of using these steroids on the periodontium.[23]

Effects of corticosteroids on the periodontium: Corticosteroids, when topically applied to the erythematous marginal gingiva suppresses the inflammatory process and masks the rate at which the bleeding should be occurring blinding the clinician with the false picture. It has been observed that,in cases where steroid anti-inflammatory drugs are administered topically or injected in the gingival tissues, there is a disturbance in the ability to percolate into the capillaries and formation of collagen, decrease in the b lymphocytes and granulation tissue.[24]

Researchers have established that a high dosage of hydrocortisone brings about an increase in the activity of various enzymes that help in the degradation of the collagen and enhance the fibro lysis.One of the major classes of enzymes is matrix metalloproteinases (MMPs). Marked increase is seen in the subtypes 1, 2,7 and 11, while low levelsthis steroid reduced the expression of the same.[22]

In chronic periodontitis patients, also diagnosed with asthma and under long-term inhalational steroid therapy pathological migration and loss of teeth (mostly mandibular anterior teeth) have been observed.[23,24] Research has proven that patients under the inhalational therapy are more prone to the diseases of the periodontium more prevalent in the mandibular jaw. It is more likely to be seen in the cases which are further influence by other debilitating diseases such as osteoporosis.[25] In the adolescent age group who are on long-term steroid anti-inflammatory drugs there is an increased risk of gingivitis and tooth decay due to xerostomia. Mouth breathers and those taking inhalers that have these steroidal formulations are associated with drastic variations in salivary pH, decreased salivary flow, increased accumulation of the dental plaque which further creates a cascade mechanism causing an increase in the rate of caries initiation and progress.[26]

Safkan et alconcluded that those who were under steroidal anti-inflammatory drugs are often predisposed to gingivitis.[27] It further evidenced that using corticosteroids for a longer duration (one year or more)led to a marked increase in the presentation of the clinical symptoms of periodontal inflammation.

Markitziu et al carried out a study to assess the periodontal changes in patients of pemphigus vulgaris undergoing corticosteroid therapy.[28] The results of the study have shown statistically significant differences in bleeding on probing and in gingival recession but no significant changes in alveolar bone height.

Despite their strong anti-inflammatory and immunosuppressive effects, long term application of corticosteroids can cause enhanced deterioration of dental health and hygiene. Misuse or overuse of steroidal anti-inflammatory drugs must also be taken into account as they may affect not only the teeth, but also the soft tissues and alveolar bone.[29] It has also been found that prolonged application of corticosteroids adversely affects the osseous metabolism, along with a notable decrease in the density of the inorganic component of the mandibular jaw.

Inhaled corticosteroids, sodium cromoglycate, anticholinergic bronchodilators, β-2 agonists are frequently administered alone or in combination in the form of inhalation therapy for the treatment of asthma and chronic obstructive pulmonary disease.[26] The dosage, duration and frequency of application of steroid anti-inflammatory drugs directly correlate to the severity of adverse effects seen in the oral cavity in general and the periodontium in particular. Several adverse reactions affecting the oral cavity due to long term inhalational Corticosteroid therapy include not only severelyaltered taste perception, ulceration, moniliasis, demineralization of the tooth and dry mouthbut also severe gingivitis and periodontitis.

Aggregation of plaque deposited on the tooth is directly proportional to the reduced rate in salivation. The unbalanced buffering capacity of saliva and constant low pH scores provides a conducive condition for the growth of acidogenic and acidophilic caries causing microbes such as *Streptococcus mutans and Streptococcus sobrinus*.

The adverse habit of breathing through mouth in the patients with long term corticosteroid therapy, contributes to the aggravation of gingival inflammation due to the loss of water from the alveolar mucosa. The disturbance in the pathophysiology along with disturbance in local and systemic immunity, provoked by long term corticosteroid therapy, has shown to considerably increase the distribution of periodontal disorders.[24] Taking into account the fact that saliva acts as a reservoir of the necessary factors required for protection against gingivitis and periodontitis, drugs affecting the qualitative and quantitative properties of saliva directly influence the severity of periodontitis.[23]

Conclusion

This review concludes that long-term corticosteroid therapy directly influences the oral cavity. It further leads to several adverse reactions from moniliasisto gingivitis and periodontitis to impairedosseous metabolism. Furthermore, a significant amount of reduction in the Bone Mineral Density (BMD) of the mandible. Therefore, patients receiving this treatment for a longer duration (oral, topical, inhalational) should undergo a thorough dental check-up, and regular follow-up. This is to check and prevent any disturbance in the dental health and to estimate the risk factors for gingivitis and periodontitis. However, it is still taxing to come to a definite conclusion on the relation of oral Corticosteroids with the loss of tooth and the status of the periodontium. More number of studies on the effect of the different forms of corticosteroids on the gingival and periodontal state are required to draw more definitive conclusions.

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Health Hazards Due to Increasing Use of Mobile Phones in Children: A Review

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Abstract

The excessive use of mobile phones and other wireless devices is seeing a steep rise among the general population in the present world. People, unaware of the hazards these might pose, are marching towards a great health risk. This article would focus on health hazards in children, which are brought about by the harmful radiation emitted by mobile phones.

Keywords: *Mobile Phones, Health, Harmful Effects, Radiofrequency.*

Introduction

In the present world, the most popular mode of communication is found to be mobile phones and tablets. These gadgets apart from being used for making calls are also being used for many other functions. They are used as texting devices, sensors, high definition cameras, internet surfing gadgets, gaming stations, GPS stations, calendars, calculators, source of internet connections for sending e-Mails among many other advanced functions. This is the reason why people tend to depend on this complicated device which serves the assignments of multiple devices. This leads the adults to use the mobile phones or such gadgets very frequently. Because of this increasing use among adults, the children too end up using them. They tend to imitate their parents and seem to act like them. They find it to be a very convenient and interesting mode of entertainment. Their age being at a very tender stage, they are yet to understand the harmful effects the use of mobile phones and tablets has on their mental and physical health. This radio frequency fields to which today's population is exposed lead them to be affected by the harmful radiations emitted by the mobile phones and tablets and these are the electromagnetic rays ranging in between the microwave wavelength of 850-1800.[1]

Effects of radiations emitted by mobile phones on health of children: The effects of the radiation may take years to appear. Hence, they are less visible. Some of them are degenerative neurological diseases, like, Alzheimer's disease, dementia, depression syndromes (mostly at the age of 50-60 years). But, amongst the most harmful effects that are visible, are the tumors associated with the brain because it is the organ that is targeted easily.[2] Some of them are glioma and acoustic neuroma associated with that part of the brain that is most exposed to radiofrequency, i.e. the ipsilateral part.[3] These effects are more pronounced in children because of their smaller head size and lesser skull thickness. The brain keeps developing almost till the age of 20 years and this is the period when the brain is most sensitive to toxins and environmental radiations. Moreover, children have a longer life to be exposed to any health risks. So, they are more vulnerable to the radiation that the adults.[2] The American Academy of Pediatrics agrees, saying that "when used by children, the average RF energy deposition is two times higher in the brain and 10 times higher in the bone marrow of the skull, compared with mobile phone use by adults."[4]it has been reported that

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younger the children, the more is the harmful effect of radiations emitted from mobile phones in terms of brain tumors and cancers.[5] Some researchers have shown the link between pregnant mothers using mobile phones and the mental health of the children. Such children develop a tendency of hyperactivity, emotional disturbances and difficulty in mingling with other children.[6] A link also has been established between attention deficit hyperactivity disorder and the use of mobile phones. Children who excessively use mobile phones to make calls or entertainment or tend to spend a great amount of time with their mobile phones are seen to have developed this disorder.[7]

The international agency of research on cancer (IARC) at WHO in May 2011 conducted a study regarding the effect of mobile phones on children. They concluded that the radiofrequency in the range of 30 kHz – 300GHz might have the potential of a human carcinogen.[8] An ongoing study, MOBI-Kids by Sadetzki et al., 2014, has by far shown that regular users, who use mobile phones to make just 1 call per week have a very insignificant risk for developing brain tumors. They have also shown that the highest time lapse between the use of mobile phones for the first time and development of disease is 5 years.[2]In a report submitted by Russian National Committee on Non-Ionizing Radiation protection in 2008 to WHO, it was opined that the children who frequently use mobile phones would most commonly face mental and physical illness, like, memory disturbances, loss in concentrating abilities, increased levels of irritation, sleep disturbances, reduced stress management capabilities, increased risk of epileptic attacks, etc.

The radiofrequencies have their harmful effects on skin too. Skin cancers and contact dermatitis are some of the skin diseases associated with frequent usage of mobile phones and wireless devices. These diseases highly affects the way of life as they might cause pain and discomfort in a large percentage of the population.[9]A few of the less risky health problems that were found to be associated with phone calls were warmth behind and around the ears and burning sensation in the skin of the face. These symptoms were commonly found to be lasting till 2 hours after the phone call.[10]

Highlights of few harmful effects in adolescent:

Sleep disturbance is one of the major harmful effects of radiofrequency on adolescents. Sleep is mostly regulated by a neurohormone, Prostaglandin D2, whose synthesis is in turn dependent on an enzyme, β-trace protein (lipocalin-type prostaglandin D synthase). The concentration of this enzyme is markedly reduced by the continual use of mobile phones and wireless devices. This causes sleep disturbances in adolescents.[2]

Conclusion

With the increased use of mobile phones and electronic gadgets among the adults and children, the health risks associated with harmful radiations have taken its dominance in this world overtaken by technology. The children are the easy and the major targets of this storm of science. Both, physical and mental health mishaps are pronounced.

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Teeth Mobility Cause and Management of Mobile Teeth in Dental Clinic

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Abstract

By examining firm pressure with two metal instruments on teeth, the extent of vertical and horizontal displacement considered as tooth mobility, which is due to either periodontal disease or trauma. Nowadays teeth mobility is a common problem in dental scenarios, which results inocclusal problems, Dietary problems, and disturbances in mastication, aesthetic problem and worsening life quality. The treatment of tooth mobility is based on its etiology; it may be surgical or non-surgical. Surgically by Periodontal surgery, non-surgically by occlusal adjustment and splinting. Sometimes the Occlusal adjustment can be done by the surgical process. In the article, we discuss the cause of tooth mobility, relation between tooth mobility and occlusion including indication, contraindication and principles of tooth splinting. Our ultimate goal is to restore mastication function, good aesthetic, to improve patient comfort and good oral functions by various periodontal treatments for the management of mobile teeth.

Keywords: *Teeth Mobility, Adjustment Occlusion, Periodontal Splinting, Trauma of Periodontium, Types of Splinting.*

Introduction

Periodontitis is the most common and harmful disease among all periodontal diseases. It affects both soft tissue, hard tissue and tooth-supporting structures due to which tooth mobility occurs. By firm pressure of the dentist by using an instrument, the horizontal and vertical displacement of teeth is accepted as teeth mobility. Assessment of the mobility of the tooth is considered an integral part of the periodontal diseases as it is one of the essential signs of the diagnosis of periodontal disease. The primary objective of periodontal treatment is the reduction of tooth mobility.[1]

According to miller's tooth mobility index it is graded as Grade-1, Grade-2, Grade-3. By using miller's tooth mobility index, treatment plan and prognosisrate can be forecasted. Many factors are there which cause tooth mobility like inflammatory condition of periodontal tissue, PDL widening, alveolar bone loss, trauma from occlusion, loss attachment.[2]

Factors causing teeth mobility: Due to functional or parafunctional habits the force generated causes damages of periodontium which is otherwise called as occlusal trauma. Occlusal trauma is of two types.

i. Primary occlusal trauma

ii. Secondary occlusal trauma

Primary occlusal trauma: The etiology of primary occlusal trauma is due to the pathological occlusal forces which cause abnormality in the periodontium.

Secondary occlusal trauma: In some conditions the periodontium which is already inflamed, bone loss already occurred and there is a pre-existing disease in periodontium but still the healthy periodontium bears the occlusal forces. The mobility of teeth depends on the frequency and direction of the occlusal forces.Other factors that cause teeth mobility are accidental trauma, Periepical endodontic lesion, high filling, orthodontic treatment during pregnancy. Mobility also depends on the number and distribution of remaining teeth in the jaw. Root shape, number of roots, root position, inter radicular bone, and root amputation case also affect teeth mobility.[3]

Mobile Teeth Treatment: Mobile teeth treatment is based on its aetiology. Splinting occlusal adjustment and treatment is based on the stage of periodontitis. Reduction of mobility can be done by healing of the inflamed Periodontium or reduced Periodontal disease. Splinting is just a supportive part in periodontal treatment therapy. Periodontitis can be treated both surgically and non surgically. In some cases occlusal adjustment is enough. In miller Grade-II mobility teeth need both occlusal adjustment and periodontal treatment with splinting procedure. In some cases splinting is necessary where Grade-II mobility of teeth is seen but extraction is not indicated.[4]

Occlusal adjustment: Occlusal adjustment is the process in which by grinding the occlusal surface of maxilla and mandible free form contact between upper and lower jaw is created. To increase occlusal stability occlusal therapy is required in any stage of periodontitis.[5]

Indication:

- In Fremitus positive case within increasing teeth mobility due to traumatic forces.
- In case of occlusal discomfort and masticatory function
- Grinding and reshaping of teeth due to which soft tissue injury happens.
- Reshape and adjustment of marginal ridge, proximal contact and cusps where food impaction is seen.
- As an accessory therapy which can mitigate harm caused by para-functional behaviors.

Contra Indication:

- In the case where there are no signs, symptoms of occlusal trauma.
- In a mentally disabled, mentally abnormal patient.
- Occlusal adjustment has no effect where there is severe malposition of teeth and mobility.
- It is not applied as the primary treatment of microbial induced periodontitis.[6]

Splinting:

Principles of splinting: To reduce the three-dimensional movement of teeth splinting is required. Splinting is the clinical procedure in which adjoining the adjacent tooth using mechanical stabilization objects to control reversible and irreversible mobility of teeth. Splinting has the unique criteria of mobile teeth stabilization, decrease in tooth mobility and increase in masticatory and occlusal function. Due to good outcomes and better prognosis rates in periodontal diseases, splinting is most important due to its ability to give stability of teeth in between the surrounding periodontal structure of teeth. Before periodontal surgery and to improve the post-surgical healing splinting helps to the migrated teeth to reposition in a favorable environment by repairing periodontal tissue and tooth surrounding components. The skill of the clinician is needed for the successful splinting. The teeth marginal fit, contour of teeth, cleanability, retention, good joints of solder and esthetics are the technical elements of splinting. For the improvement of the masticatory force, occlusal force and patient comfort splinting has a great role. Splinting helps in deflecting the occluding and physiological force to other teeth which help in protecting the mobile teeth, reduction of traumatism, inhabitation of periodontal tissue regeneration and repairing. There is a previous history of healing of non-mobile teeth as well as mobile teeth, when mobile teeth are splinted. In the case of aggressive periodontitis by periodontal surgery and splinting help in the regeneration of bone and inhibit healing. The purpose of splinting is tissue protection, occlusal force distribution, mastication comfort, protection of investing teeth structure, pulp protection, stress, and force control. In performs in evaluation procedure anchorage, stabilizing in minor teeth movement. It helps in the prevention of teeth drifting while therapy is ongoing. In disease dentition it gives comfort by tooth stability.[7-8]

Indication and condition: In various clinical scenarios benefit of splinting is seen but the main objective of periodontal treatment is to create an environment where both movement occurs within the physiological limit for patient comfort and satisfactory function.

Indication: The main objective of splinting is to provide adequate rest while the healing process is ongoing and functional movement where the supportive tissue cannot perform satisfactorily.

- To relocate the teeth which is migrated due to periodontal problem
- When TFO is developing, In case of moderate to severe periodontal splinting required with addition to occlusal adjustment.

- In some surgical and non-surgical periodontal treatment procedures, where without stabilizing the mobile teeth treatment is difficult to operate. Before treatment by splinting extremely mobile teeth, it gives a good prognosis rate.
- After periodontal surgery the micro-movement of the teeth is seen in the healing area. So to eliminate the micro movement and increase the repair rate splinting is necessary.
- Splinting is done either for a single tooth or for entire dentition where extraction and implant is not indicated.
- After orthodontic treatment and after avulsion of teeth splinting prevent drifting of teeth.
- Splinting is helpful to increase the stability of joining, elimination, and reduction in teeth mobility and to get rid of pain and discomfort.[9-10]

Contra Indications:

- Without minimum periodontal condition and stability of occlusion splinting is not recommended.
- If the diagnosis of inflammatory periodontal disorder is not tackled splinting is not recommended.[11]

Biomechanics: In periodontitis associated teeth mobility the mesiodistal, lateral, occlusal and intrusive forces cause tissue destruction because all the forcesthey don't direct on the long axis of teeth and the forces alter the center of rotation of affected teeth. By occlusal adjustment the occlusal force is reduced which helps in the reduction of teeth mobility before splinting. By altering the center of rotation of every teeth and by creating a multi-rooted unit all the forces acting on teeth is reduced. Thus root resistance, mesiodistal force resistance and periodontal resistance are achieved. Splinting guarantees a fair distribution of force by focussing force over the dispersed region with sufficient periodontal help. When two single-rooted teeth are splinted, the mesiodistal force is well distributed. For splinted areas, invasive forces are easily accepted as the forces affect spreads over a small number of key periodontal fibers. Splinting stretches across the arch to connect posterior and anterior segments or to include teeth in the opposite side of the arch such that anteroposterior forces and faciolingual forces are counteracted to achieve a desirable stabilization in the faciolingual and mesiodistal direction.[12]

Splinting disadvantage: Oral hygiene accessibility and plaque control is difficult after splinting. It is noticed that due to plaque accumulation in the splinted margin it cause gingival irritation and periodontal problem in case of the periodontally compromised patient. Long term splinting causes dental caries in the proximal, gingival caries due to the accumulation of food debris. Crown loosing, fracture of anterior teeth sometimes happens. Splinting causes a phonetic problem.[13]

Requirements on splinting:

- Splinting will add as many strong teeth as possible to decrease the extra pressure on single teeth to a minimum.
- Teeth kept rigid by splint and splint does not place torsional stress on any portion of the teeth.
- By extend around the arch splint opposes the anteroposterior and faciolingual forces.
- Splint does not mean to mess with occlusion.
- Before splinting the teeth should reposition to a harmonic order if possible.
- Pulp should not be irritated by splint.
- Soft tissue, gingival, cheeks and tongue, lips should not be irritated by splinting application.
- The design of splint should be like that the patient can easily clean it.
- Splint should not block the interdental area between the teeth.
- Instead of the above-required criteria others are medically acceptable, inexpensive, ease of fabrication, easily removable by the operator and esthetically acceptable.[14]

Splints classification

- Various techniques are there for the splinting of teeth. Splinting is classifies according to its duration of use, purpose teeth location on the jaw, fabrication way. According to use length splinting is classified into short term splints, provisional splint and long term splints.
- Based on material splinting is of 3 types: metallic, non-metallic and combination of non-metallic and metallic type. The metallic is made up of chrome, cobalt, cast metals, stainless steel. Non metallic are made of composites and acrylic.

- According to the way of fabrication it is of 2 types: fixed and removable type.
- Based on the location of splinted teeth in the jaw, it is of 2 types: Extra coronal and Intra coronal. Extra coronal splints are stabilizing wire, fiber-reinforced ribbon, fixed orthodontic retainer, right guard and welded bands. Examples of Intra Coronal splints are inlays and nylons wire which make the splint place visible and superior esthetic.
- Based on the duration and purpose:
- **Temporary splinting:** Temporary splinting is used in periodontal treatments for less than 6 months. to reduce unfavorable occlusal forces, anchorage in orthodontic treatment and advanced periodontal disease as a support temporary splinting is used. It can be removable, fixed external and fixed internal. Removable temporary splints are acrylic Hawley, bite guard or night guard. Splint made with acid etchy, self polymerizing resin and composite materials are most popular.
- **Provisional Splints:** For more stabilization like permanent type, diagnostic purposes it is used for several months to a year. It is the phase of restorative therapy for immediate and temporary basis of treatment of teeth with dressing coverage and stabilization. Examples of provisional splinting are ligature wires, night guards, Interim fixed prosthodontic appliances and composite resin splints.[15]

For application of provisional splint, by using 35% Phosphoric acid enamel surface is etched for 10 seconds. Then the surface is wiped with wet cotton or rinsed using water. Then denting bonding agent is applied and polymerized by light. By using a suitable shade of composite the splinting material is placed and polymerized for 40 seconds. The splinting material is ligature wire and glass fiber.

Definitive splint: After the occlusal stability and the periodontal therapy completed, definitive splints are used. By definitive splint occlusal trauma is prevented, Functional stability increases and esthetic is improved.[16]

Conclusion

Tooth mobility is the common cause due to trauma from occlusion and periodontal problems. By eliminating trauma from occlusion cause mobility, bone loss and attachment loss can be reduced. Tooth with poor prognosis rate can be retained in the arch for long term until definitive treatment is planned by splinting. For periodontal maintenance and periodontal therapy splinting has a major role. Splint never eliminates the cause of teeth mobility. It is just a supportive method in the periodontal treatment procedure. To increase the prognosis rate of mobile teeth due to periodontal or other problems. Splinting is considered as an important part of dental treatment based on present data.

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Parental Awareness about Thumbsucking: The Earliest Addiction of Children

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Abstract

Thumb sucking is a type of non-nutritive sucking that occurs in the early daysof the 29th week of gestation. Parents should be concerned about the health of their kids. Parents have to look after their children good health who should raisedevoid of developing any behavior that would endanger their suitable development. Thumb sucking is one of the concerned habitsthat makes every parents worry about as their babies grow and grow to be toddlers. Parents keep speculating if the habit will continue, or if they have to look for help to stop it. This review article discusses all the importance of habits related to the oral health of a child and focus on the need of awareness among parents about thumbsucking.

Keywords: *Thumbsucking, Parental Awareness, Management.*

Introduction

Thumb Sucking is also known as Finger/Digit Sucking Habit. This habit is the putting of one more fingers or only one thumb in varying depths of despair into the oral cavity. Moreover thumb sucking is the repeated and forceful sucking of thumb with associated strong buccal and lip contractions.[1] Thumb sucking is a type of nonnutritive sucking that occurs in the early days of the 29th week of gestation. The persistence of such kind of continuous oral habits regularly might be because of various emotional stimuli such as pleasure, sadness, hunger, boredom, hyperactivity stress and various kinds of physical disabilities.[2] These kinds of sucking habits in infants as well as in small children are primarily copied from the physiologic requirement for nutrients and these kinds of behavior are normal in the initial years of life, however, might result in enduring damage if continued beyond this age.[1] The present perceptive of the child growth suggested that sucking behaviors occur and continue because of psychological requirements along with some studies reported that grown-up infants have an inherent biological drive for sucking.[3,4]

Classification of Thumb Sucking[4]:

1. **Cook classification (1958):**
   - **A. ALPHA** Group pushes the palate inside the oral cavity in a vertical direction that displays only a little contraction of the buccal wall.
   - **B. BETA** Group shows strong contraction of the buccal wall as well as a negative pressure in the oral cavity. A posterior crossbite can be seen in the Beta group.
   - **C. GAMMA** Group shows alteration in positive and negative pressure. Among all the groups, the least amount of malocclusion can be seen in this group.
2. **Clinical classification of thumb sucking Clinical classification:**
   - **A. Normal Thumb Sucking:** Habit during first and second year of life is normal.
   - **B. Abnormal Thumb Sucking:** Physiological Habitual
3. **Thumb sucking by O'Brien:**
   - **A. Nutritive Sucking:** Breast Feeding, Bottle Feeding

B. Non-nutritive Sucking: Thumbsucking, Finger sucking, Pacifier Sucking

4. According to Subtelny (1973):

Type A: 50%: Thumb occupies a larger portion of the vault of the hard palate which was kept into the mouth. Thumb is in contact with the maxillary and mandibular anterior.

Type B: 13-24%: Without touching the vault of the palate, the thumb is placed inside the oral cavity. Thumb is in contact with the maxillary and mandibular anterior.

Type C: 18%: As in type A here also thumb passed fully into the hard palate. Contact with maxillary incisors only

Type D: 6%: Thumb retreat appreciably and the lower incisors made contact at the estimated level of the thumbnail.

Thumbsucking is a conduct found in humans, chimpanzees, and different primates. This more often than not includes putting thumb into the oral cavity and rhythmically continuing sucking. During childbirth, an infant will submissively suck anything set in its mouth which is called as sucking reflex in charge of breastfeeding.[3,5] As the child becomes more seasoned and is, in the long run, weaned the dietary sucking, they cancreate elective method to accept equivalent sentiments of physical and passionate satisfaction or they can keep encountering those agreeably mitigating encounters by starting to suck their thumbs or fingers.[1] Thumbsucking by and large stops by 5 years of age.[2] While many dental practitioners suggest bringing an end to the tendency as right, on time as could reasonably be expected, the harm is reversible.[3]

Etiological Factors[4]:

a. The socioeconomic status of the family-related with the parent's occupation.

b. Children of working mothers children get onto sucking habit to attain secure feeling.

c. More number of siblings in a family divides the attention from a child.

d. Scolding parents, as well as peer pressure, leads to stress and social adjustment.

Factors Affecting Thumbsucking: The severity of displacement of the teeth and investing tissue depends on these factors:

a. Duration- the amount of time spent on sucking; longer the duration of each suckling period, the greater is the damage.

b. Frequency of indulgence – number of times the habit is practiced; frequent and continuous sucking is more damaging than occasional, short-time practice.

c. Intensity of force – the amount of force exerted on teeth while practicing the habit.[2]

Apart from these conditioning factors, the type of malocclusion produced also depends on various factors is also mentioned:

a. Location of digit inside mouth

b. Involvement of orofacial muscle contraction

c. Position of mandible during sucking

d. Skeletal pattern of face[2]

Thumb Sucking: Effect on Oral Health: Parents should be concerned about the health of their kids. Parents have to look after their children good health who should become adults without budding any habits that would endanger their proper development. Thumb sucking among all the oral habits, is one of the concerning things that makes every parent be bothered about as their babies grow and become toddlers. Parents speculate if the habit will prolong, or if they have to look for help to stop it. Good advice from a dentist is required for both the child and parents. The good thing is that the habit will more often stop before a child starts going to preschool.[1, 3]

Normal Behaviour: Thumb suckinghabit along with pacifier use is atolerable behavior for infants in the society. As mentioned by the American Dental Association (ADA), this kind of behavior is a natural reflex for an infant that cannot be found in the womb during development. This type of reflex makes the baby feel comfortable, secure as well as happy. Many babies as well as toddlers use it as a recovering mechanism whenever anxious or they get separated from their parents then this habit encourages sleep.[5]

How Long Is Acceptable?: As mentioned by the ADA, the suitable time to discontinue thumb sucking is till age four. By this age, long duration sucking started affecting developing jaw and teeth as a result of which permanent teeth may be misaligned. If a child is a passive sucker then his habit can easily be discouraged whereas

prolonged sucking can result in the occurrence of changes in the palate that impinge on the bite and generally very difficult to quit without doing any intervention.[6]

When continuous habit starts to become physiologically need—in other words, craving starts when something is missing and this is what happens with thumbsucking.[6]

a. Sometimes addictions continue because they might give life-motivating purposes.

b. Since these addictions are gratifying that is why they can continue.

c. It has to take a firm decision before ending any habit.

d. When a habit is motivated by a combination of fear and desire then its ending starts more effectively.

e. To quit any addictive habit all of a sudden is very difficult practically, therefore keeping a record of the progress helps to motivate and reach the goal.

f. Complete blocking of the adaptive habit can help a bit in this process.

g. During the process of quitting the addictive habit, patience and continuous persistence are required.

h. Anything that can replace the habit will surely help in quitting.

Stepladders for Discouraging: Sometimes children will find out on their own onto which time they want to continue their behavior and their habit will not be accepted from any social situations and peer pressure. When the habit of a child sustains beyond kindergarten then steps should be taken to stop the habit.[4]

- A pacifier can be offered to infants, they might easily adapt to this replacement.
- For proper tracking, a chart can be established and rewarded from time to time for seeing the child's progress for quitting.
- A child should always be encouraged and praise for making any attempt to quit his habit.
- It should make sure that parents should visit their dentist about positive as well as beneficial effects of stopping the habit.

Some children may face difficulty in stopping their thumb sucking. In this kind of situation, it may be required to purchase replaceable products that can be placed on the thumb or fingers to discourage the habit. Parents can just be placing a bandage on the finger or socks or glove on the preferred hand is sufficient. Whatever method is chosen to employ to discourage the behavior, positive reinforcement to encourage children should always be used. Criticism or nagging may cause more anxiety and carry on the problem.[3]

Oral Effects: When oral habit keeps on going further age of five or six, the pressure and sucking motion will start to make changes in the oral cavity. As mentioned by the ADA:[7]

a. Front teeth of the upper jaw may come out for which the bite will remain open that will not allow the upper and lower front teeth to touch each other.

b. The skeletal effects of the mouth will start disturbing the alignment of the permanent teeth.

c. The dentist can evaluate the changes and then can give a referral to an orthodontist or pediatric dentist for a consultation.

d. As suggested by NYU's Langone Medical Center, in a very exceptional situation, a dental appliance or a cri**b**, may be required to be placed in the roof of the oral cavity to accomplish a modify in behavior.

e. To Correct the bite itself it is always better to start stopping the habit as soon as possible to get better results.[7]

Insights for Parents: It has been mentioned that 15-20% of children under age 6 engage in thumb or finger sucking habits. Even orthodontists' children suck their thumbs![5]

Below are 4 considerations for parents with a child who engages in thumb sucking. All of the following are evidence-based and established by clinical research, to help the child to avoid dental health problems while building self-confidence associated with overcoming a challenging task.

Impact on Dental and Facial Growth and Development: After age 6 and with the corresponding eruption of the first permanent teeth, sucking habits will begin to have an irreversible negative effect on the development of the jaws, bite relationship, and positioning of teeth. In most cases, the habit will contribute specifically to a narrow upper jaw (causing crossbite), protruded upper teeth (overbite), and a failure of the front teeth to bite together properly (open bite).[1]

Stopping By Age 6 or 7 Will Minimize Most Long Term Effects: In my experience as an orthodontist, I find that most children begin to establish social awareness of thumb sucking around age 5-6 and develop an internal motivation to cease the habit when it is observed that peers at school are not engaged in the habit. Fortunately for children and parents, this period coincides with the time in development at which irreversible negative effects can be prevented. Research has shown that cessation of thumb and finger habits before the eruption of the permanent incisors (central teeth) at age 6 or 7 will prevent lasting negative impacts on the development of the upper jaw and permanent teeth. This key age to stop also aligns with the American Association of Orthodontists' (AAO) recommended age for the initial evaluation of your child (age 7) by an orthodontic specialist.[9]

Techniques for stopping Thumb and Finger Sucking: The single most important aspect in helping a child to stop sucking a finger or thumb is to determine self-motivation. In the clinical setting, I typically begin by simply asking the child if he or she would like to stop the sucking habit. If your child is not ready and motivated, then it is not time to attempt to stop the habit. It will only lead to frustration and negativity. The focus, instead, should be on education. Explaining to your child how it would be helpful to them to stop and point out that others in their social group or class at school may be in the process of stopping. Acknowledging the difficulty in stopping is also very important. For example, if either parent had a sucking habit as a child, explain to your child how you recall how hard it was to stop, but how rewarded you felt once you were able to tackle the difficult challenge.[10]

Once established that the child is motivated, I recommend the following techniques to positively reinforce stopping the habit[3,8]:

1. **Continuously reaffirm the child's self-motivation and goal to stop.** Examples: "That is so great that you set this goal." and "You can do this, we are so proud of you."
2. **Set up a reminder system to help the child stop when the body may be unconsciously initiating the habit during sleep.** This can include sleeping with a glove or band-aid on the sucking hand and using nail polish on the sucking finger (Mavala Stop is one brand name). The goal is not to physically prevent your child from performing the habit, but to help to provide reminders so they canfully stop the habit in a positive, supportive environment. Positive reinforcement is the key.
3. **Use a reward calendar on the refrigerator.** Every full day/night your child goes without engaging in the habit, place a star on the calendar. Arrange for a reward after 14 consecutive days are achieved without the habit. After 14 consecutive days, the chance of relapse of the habit is very small. (Link: I Can Do It Reward Chart)
4. **In many cases, early orthodontic intervention will help your child if he or she is actively engaged in a sucking habit over age 7**. Most often, a constricted (narrow) upper arch and possible associated crossbite will indicate the need for a maxillary expander to orthopedically widen the upper jaw. In this case, this very common appliance will change the dynamics of the thumb habit just enough to serve as a reminder and aid the child in stopping the habit.

Conclusion

Your child will overcome this habit. Your role as a parent is to educate, to motivate, and to provide a positive and supportive environment. Keep in mind that this may be the very first conscious, difficult sacrifice your child makes to improve his or her health and well-being. It must be our goal as adults (parents and healthcare providers alike) to foster self-motivation, encourage that little girl or boy that they are up to the challenge, and celebrate with them when they achieve what they set out to do. Comments like "how am I going to get him to stop" or "she will never stop this" do little to motivate and will undermine the child's self-expectations. This habit should be looked at as a profound opportunity that can be used as a very important confidence builder for a young child. We adults must create an environment that encourages children in our sphere of influence to learn, to set goals, and to succeed- in thumb-sucking and beyond!!

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Current Concept of Bruxism and its Treatment Options

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Abstract

In our day to day clinical practice we come across a lot of patients suffering from occlusal parafunctional habits or bruxism. Bruxism can be described as nonfunctional abnormal, involuntary contact of teeth, if ignored it leads to occlusal trauma. So for correct diagnosis and treatment a multidisciplinary approach was required in which dentists, psychiatrists, neurologists and physiotherapists play an important role. Causes for bruxism were multifactorial and the treatment modalities were also various. Bruxism can be a major concern for the dentist as they were required to not only diagnose but also intervene in most of the cases of bruxism. And if untreated it leads to destruction of dentition, muscle fatigue & temporomandibular pain. This article discusses the history of the current concepts in the diagnosis and treatment of bruxism.

Keywords: *Bruxism, Sleep hygiene, Splint, Pharmacological, Contingent electric stimulation cure (CES).*

Introduction

Parafunctional habit bruxism has always been amajor concern for dentistsas it causes tooth attrition, fractured restoration, muscle soreness & TMJ pain. In common population bruxism ranges from 8-31% and 14-20% in children[1]. Bruxism was very rarely found in patients above 60 years of age[1]. Usually bruxism does not cause any serious complications in most of the patients, and they seek treatment only if heexperiences unbearable pain. Hence a proper diagnosis and multidisciplinary approach were required for the management of bruxism.

Marie and Pletkiewicz[2] coined the term "bruxomania" from the French word "la bruxomanie,". The term bruxism was coined Frohman[3]. Vanderas defined bruxism as the movement of the mandible which was non-functional and waswith or without an audible sound occurring during the day or night[4]. According to GPT 9, bruxism was defined as "the parafunctional grinding of the teeth; an oral habit consisting of involuntary rhythmic or spasmodic non-functional gnashing, grinding, or clenching of teeth, in other than chewing movements of the mandible, which may lead to occlusal trauma".

Bruxism can be classified into diurnal bruxism or awake bruxism and nocturnal or sleep bruxism. Bruxism during day time was known as awake bruxism and in night time was known as sleep bruxism. Awake bruxism was always related to stress, work pressure, & anxiety. Diurnal bruxism was more common in females than males. Whereas, sleep bruxism was considered as a sleep-related movement disorder. Sleep bruxersusually have sleep disorders like snoring and sleep apnoea. No gender difference was found in nocturnal bruxor. But it more prevalent in small children than in adults.



Figure 1: Causes of bruxism

Cause of bruxism:

- Central factors: As describe byMacaluso et al[5] bruxism was closely associated with an arousal response. And arousal response was defined asa sudden change in the depth of the sleep and it was associated with body kinetics, escalated heart rate, respiratory changes and increased muscle activity. In bruxism patients the five subcortical nuclei of basal ganglion were not in co-ordination[6].
- Psychosocial factors: A highly stressful life was a significant risk factor for bruxism. And intoday's prevalent social environment the inability of a person to reveal emotions such as apprehension, despise, & aggression leads to bruxism. And diurnal bruxism was associated with psychological tension due to responsibility & work pressure.

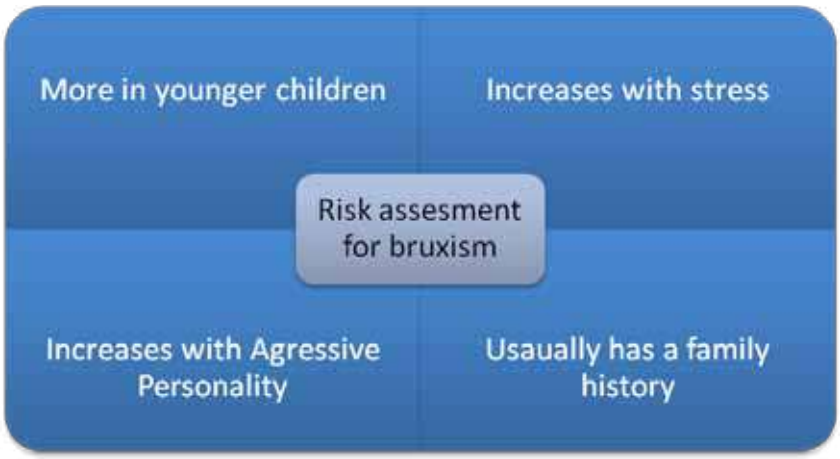


Figure 2: Risk factors for Bruxism

For a patient with bruxism clinical assessment and detection was the most important step in treatment. Because if it were left unattended it leads to depreciation of quality of life. As on long term basis bruxism leads to occlusal disharmony, TMJ disorder and damaged tooth[7]. The assessment method for bruxism were questionnaire form, scientific clinical evaluation, and electromyographic recording. In questionnaires assessments done were subjective and questions asked were related to signs and symptoms of soreness in gums or headache after waking up. It should be asked to parents, siblings or wife if they have heard patient grinding teeth. As for the dental clinic of North American the clinical evaluation[8,9] of bruxism can be done by taking a detaileddental history of the patient and evaluating the periodontal status, occlusal wear facets on teeth and abnormalities of TMJ[10].

Korioth et al. reported asymmetric occlusal wear out of stabilizing splint when used in sleep bruxism patients[11]. Forgione gave the Bruxcore Bruxism Monitoring Device (BBMD) to measure the nocturnal bruxism activity[12]. Takeuchi inventedan intra-splint force detector (ISFD) and it intraorally records the force being produced by tooth contact on the appliance during bruxism[13]. But the most tried and tested method of recording bruxism without any intraoral instruments were masticatory muscle electromyographic[14]. In a recent development a miniature self-contained EMG detector-analyser (grindcare, medotech Denmark) was developed to record biofeedback in sleep bruxorsby just attaching it to masticatory muscle. The EMG detector analyzer works by recording of EMG activity of the masticatory muscle, and then processing the EMG signals to detect tooth gnashing and clutching and give biofeedback stimulation for reducing sleep bruxism activities[15]. Sleep laboratory was another new advancement to detect sleep bruxism, in a controlled environment where brain waves heartbeats and breathing rate while sleeping were recorded. Simultaneous audio-video recordings were used to record any eye movements, limb movements and any deviation in the sleep pattern of the patient. Sleep disorders like sleep apnoea and insomnia were ruled out and sleep bruxism was diagnosed by this procedure. The major limitation of the sleep laboratory wasthat due to change in the environment of the patient the normal sleep pattern cannot be observed in some patients[16,17].

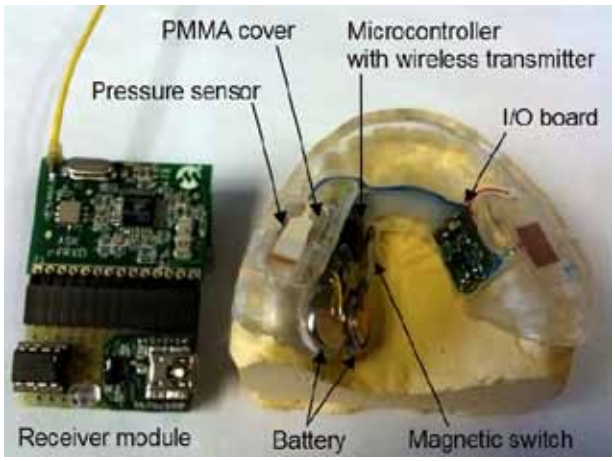


Figure 3. Contingent electrical stimulationapparataus

Table 1. Signs & Symptoms of bruxism

1	Iatrogenic sensitivity to heat and cold of the tooth
2	Muscle pain due to intense contraction in the morning.
3	The sound heard by parents and relative when the teeth were ground together
4	An uneven tooth wear with exposure of dentine
5	Micro-fractures on the tooth enamel
6	Fractured or broken teeth
7	Periodontally compromised teeth
8	Pain in the temporomandibular joint and restricted mouth opening
9	Earache

Effects of Bruxism on Fixed prosthesis: The effect of bruxism on prosthodontic restoration like single, multiple and full mouth rehabilitation was mechanical failure. The fracture of ceramic material and dislodgement of the crown was the most common failure in bruxors. Metal or metal-ceramic restorations seem to be the safest choice of prosthesis in cases of high load conditions[18]. But due to the risk of fracture of ceramic veneers in metal-ceramic restorations, many clinicians prefer metal crowns for heavy bruxors. The material of the present period zirconia, which has excellent strength,aesthetics,and claims mechanical property almost similar to metal crowns(International Journal of Applied Dental Sciences Zirconia) has been used in bruxers[19,20]. But on clinical use it has been found to have complications of fracture of veneered part[21]. So in bruxism patient's monolith zirconia crown should only be used.

Effect of bruxism on implant restorations: Bruxor who have implant prostheses were at a risk of loss of osseointegration. But systematic reviews have concluded that there was no relationship between occlusal forces and loss of osseointegration[22,23]. Studies by Elfelt A, De Boever AL have indicated that there was a higher rate of complication on superstructures of removable and fixed implant-supported prosthesis in bruxism patients[24.25].

Effect of bruxism on dentures: Inpatient wearing complete or removable partial denture the most common clinical problem was the soreness of denture bearing area. But there was no systemic review on the effect of bruxism in the partially edentulous patient and its effect on residual dentition and denture bearing tissue.

Current Treatments of Bruxism: To treat bruxism, we not only have to treat the effect of it but also eradicate the cause.

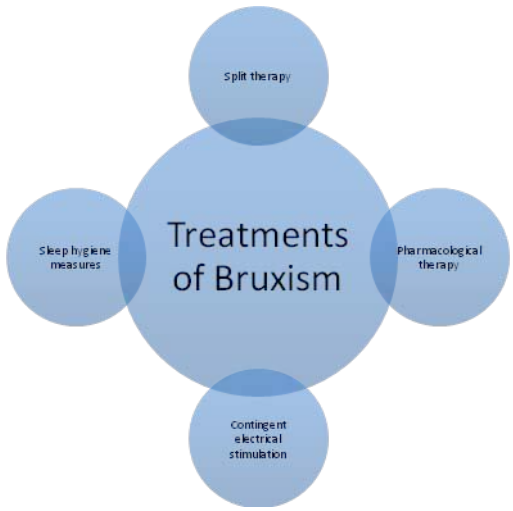


Figure 4: Treaments of Bruxism

1. **Sleep hygiene measure:** The term sleep hygiene means counseling the patient to ensure less physical and cognitive activity before retiring to bed. And to doze off within minutes of going to bed, the patient has to ensure a quiet and dark environment in the bedroom. In one randomized clinical trial it was found that there was no clinically significant difference between patients using sleep hygiene and control group[26]. But reducing the consumption of coffee, alcohol and tobacco had a positive effect on patients with sleep bruxism[27].
2. **Occlusal therapy:** The first line of treatment of bruxism was occlusal splints. These reduce the pain and prevent tooth wear inpatient with sleep bruxism. They were classified into hard splints and soft splints.

Okeson[28] had preferred hard splints over soft splints because soft splints were difficult to adjust than hard splints. It was found hard splints to be more effective in reducing the bruxism activity. But in a study by Madani et alfounda similar reduction in muscle activity associated with sleep bruxism after 2 months of therapy[29] in patients using occlusal splints and in patients taking medication gabapentin.

3. **Pharmacological therapy:** In a randomized clinical trial done by Mohamed et al. for evaluating amitriptyline in patients with sleep bruxism and temporomandibular disorder, found no changes in pain and masseteric muscle fatigue[30]. But in contrast to a study by Lobbezzo et al, it was found that levodopa used in bruxorsreduced pain and bruxism in 70% casein comparison to placebo[31]. In some patients with psychosomatic disorder, when clonazepam was given, it reported to reduce sleep bruxism along with increase REM sleep[32]. But to recommend clonazepam with clonidine (a α_2 adrenergic agonist used in hypertensive patient[33]) in routine clinical practice more randomized control trial on a bigger population has to be done.In recent studies Shim et al[34] used muscle relaxant botulinum toxin type A injection to treat masticatory muscle in sleep bruxism patients and found to reduce the amplitude of contraction in patients but there was no difference in number of contraction. Pathophysiology of botulinum toxin was inhibition of acetylcholine at the neuromuscular junction. So a more randomized control trial of botulinum toxin and placebo has to be done to recommend it in clinical practice.

4. **Contingent electrical stimulation:** In an attempt to treat bruxism contingent electrical stimulation(CES) has been tried by a lot of scientists. In this low-level electric stimulation was applied on the muscles when they became active i.e. during bruxism episode. Two studies were done on patients with myofascial pain and nocturnal bruxism, and it was found that EMG episode was reduced but no reduction in muscle pain[35,36]. So CES was found to reduce the sleep bruxism while in use but was not able to reduce it after discontinuation. So long term studies have to be done on CES and CES versus TENS and its effectiveness if done in combination.

Conclusion

The most common parafunctional habit was bruxism, which can occur during wakefulness and sleep. Usually the patient comes for treatment when there were in pain and discomfort. The side effect of bruxism was progressive dental wear, so to improve the quality of life, full comprehensive treatment has to be done. The etiology was multifactorial and there was no specific treatment option to reduce the effects of bruxism. Treatment has to be multidisciplinary with counseling and behavioral strategies, splint therapy, medication and contingent electrical stimulation. But all the treatments have shown mixed results in various patients. So clinical judgment was most important to treat symptoms and prevent tooth wear, reduce tooth grinding and improve muscle discomfort. So long term studies including a wide variety of bruxism patients and comparing the effect of different treatments on all of them has to do.To helpa clinician known when to treat the patient and when to leave it alone to resolve by itself.

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Oral Squamous Papilloma: Unusual Size and Review of Literature

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Abstract

Oral squamous papilloma (OSP) specifies the non-neoplastic proliferation of stratified squamous epithelium giving rise to a verruciform or papillary growth on the surface of the oral cavity. Usually asymptomatic, OSP presents as soft tissue growth of the oral cavity exhibiting various clinical manifestations. Human papillomavirus (HPV) commonly HPV types 6 and 11 are believed to be the causative agents even though not detected in some lesions. Found in 4 of every 1000 individuals OSP ranks fourth among the common oral mucosal mass. It has equal predilection for both male and female and occurs within the age group of 30 to 50 years. Its clinical appearance imitates exophytic carcinoma, verrucous carcinoma or condyloma acuminatum raising concern amongst the patient as well as the dental surgeon. OSP measures up to one centimetre or so in diameter, but here we present a case where the lesion measures up to 5 centimetres in diameter along with review of literature.

Keywords: *Oral squamous papilloma, human papillomavirus, exophytic growth, koilocytes.*

Introduction

Oral squamous papillomas are generally benign, asymptomatic, exophytic growth in the oral cavity present in the form of numerous small finger-like projections which results in roughened, verrucous or cauliflower-like surface. Human papillomavirus (HPV) commonly incriminated as the causative agents in skin warts are mostly related to its pathogenesis. HPV type 6 and type 11 are commonly associated but have failed to be demonstrated in oral malignancies.[1-4]

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The World Health Organisation (WHO) has defined papilloma as "a range of localized hyperplastic exophytic and polypoid lesions of the hyperplastic epithelium with a verrucous or cauliflower-like morphology".[5]Among the most common oral mucosal masses squamous papilloma ranks fourth. It is found in 4 of every 1000 individuals[4] and is frequently seen between the age group of 30 to 50 years [1]. Hormonal changes, deficiency in the diet, smoking and secondary residing infections influence the occurrence of these lesions[6]. Commonly affects the soft palate tongue and occasionally seen on the vermilion border of the lip and uvula.[1,2,7] Size of oral squamous papilloma commonly measures from few millimetres to one centimetre but bigger size lesion has also been documented in the literature.[8-10]

Ideal treatment is excision or laser ablation with the base of the mucosa into which the stalk or pedicle of the papilloma is inserted. Electrocautery, cryosurgery and injections of interferon into the lesion are some of the other treatment techniques. Frequently recurs in patients

infected with human immunodeficiency virus (HIV) else if properly excised recurrence is rare.[7] Though HPV lesions are infective, squamous papilloma appears to have very low virulence and infectivity.

In this article, we are presenting a case of Oral Squamous Papilloma with an unusual size and clinical looks mimicking carcinoma, along with review of the literature.

Case Report: 65-year-old male patient reported to the department with the chief complaint of an ulcerated growth in the left buccal mucosa. The ulcerated growth extended from the commissure of the lip till the retromolar area. The lesion has been present for 6 months. The patient gives a history that the lesion started as an asymptomatic slowly enlarging soft tissue mass eventually reaching the present size. As the lesion enlarged there was intermittent pain, discomfort and interference while chewing. The past medical and dental history of the patient is not significant. The patient was well oriented with no significant general findings and the extra-oral examination was also non-contributory. The patient has a habit of tobacco chewing from 20 to 25 years.

Intraoral examination revealed a big size exophytic lesion in the left buccal mucosa extending from the lip commissure till the retro-molar area not involving the sulcus. The lesion was pink and white in colour, sessile in nature with pebbled surface and papillary projections at places. The lesion measured approximately 5x2 cm and well-circumscribed [Figure 1]. The lesion was firm in consistency and there was bleeding on palpation. The submandibular lymph nodes on the left side were palpable and tender.

Based on the history given by the patient and clinical features a provisional diagnosis of verrucous carcinoma and carcinoma of the left buccal mucosa were given.

Under local anaesthesia,an incisional biopsy of the lesion was done along with the submucosa to a depth of one millimetre and sent for histological examination. Histological examination showed stratified squamous epithelium thrown as finger-like projection towards the surface with long slender fibro-cellular connective tissue core reaching very high in it [Figure 2]. Very few cells resembling koilocytes are seen in the stratum spinosum [Figure 3]. Minimal amount of chronic inflammatory cells are diffusely arranged in the moderately vascular connective tissue stroma. Histological picture was compatible with squamous papilloma. Excision biopsy was done with surgical removal of the lesion in toto along with the submucosa to a depth of one millimetre. Histological picture of the excisional biopsy again confirmed oral squamous papilloma.

The uniqueness of this case report is the size of the lesion which is unusually big and its clinical look mimics a malignant lesion. Literature says maximum size that a papilloma reaches is up to 0.5 cm with no subsequent alterations. However oral papilloma measuring up to 3 cm has also been reported[10]. In our case, the lesion is about 5 cm in its maximum length which is first of its kind to be reported. Though the look of the lesion is deceiving but both incisional and excisional histopathological report confirmed the lesion to be benign and oral squamous papilloma.

Discussion and review of literature: A papilloma (plural: papillomas or papillomata) is defined as a small firm non-neoplastic growth of the epithelium with a well-defined boundary that extends out above the neighbouring tissue[11]. The generic term *oral squamous papilloma* specifies a benign proliferation of stratified squamous epithelium giving rise to a verrucous exophytic growth or papillary growth on the oral mucosal surface with minimal amount of connective tissue core.

Aetiology: Oral squamous papilloma was first described by Tomes in 1848 as a wart limited to the gum and Human Papillomavirus (HPV) type 6 and 11 are the causative agents. They have low infectivity rate and are less virulent which is why squamous papillomas are always benign. The pathogenesis in the development of papilloma lies in that the double-stranded DNA of the virus merges with the host DNA.

Incidence and Occurrence in Oral Cavity: The incidence rate of SP is 4th among all the mucosal growths in the oral cavity and 1 in every 250 people exhibit oral squamous papilloma. Oral squamous papillomas make 3 to 4% of all the oral soft tissue biopsies and are most commonly seen within the age group of 30-50 years. Males and females are equally affected. Some literatures have documented OSP among children and it constitutes 7 to 8% of all oral mucosal growths. Whites are more affected than the black population. Generally, the lesion appears on the tongue, soft and hard palate, gingiva, buccal, labial or lingual mucosa and near uvula too.Oral squamous papilloma is the most common of all the soft tissue growths arising in the soft palate.[12,13] There are

certain notable differences between papillomas arising in the other body parts and papillomas of the oral cavity. Though benign OSPs have a higher neo-plastic potential compared to other papillomas if not properly excised they have a relatively higher frequency of occurrence and recurrence compared to other papillomas.[14,15]

Clinical features: Clinically OSP often is a solitary lesion presenting itself as an exophytic soft-tissue growth with a verrucous, roughened or cauliflower or wart-like surface. There are presence of many papillary projections on the surface or maybe blunted. The lesion is occasionally sessile and often pedunculated and has well-defined border. Depending upon the amount of keratinisation the lesion appears pink or white and is usually asymptomatic. Presence of trauma imparts red colour to the lesion. Generally size of the papillomas ranges from a few millimetres to less than one centimetre but big size papillomas have also been documented.[8-10]

Clinical Differential Diagnosis: When oral squamous papilloma is a single solitary lesion differential diagnosis includes *papillary hyperplasia, condyloma acuminatum* and *verruciform xanthoma.*[10] Gingiva and alveolar ridge is the specific site of occurrence for verruciform xanthoma. A causal-and-sequence relationship (for example: pathology under an ill-fitting denture) is blatant in *inflammatory papillary hyperplasia*. Condyloma will be bigger than the papilloma with a wider base and colour will be from pink to red due to lesser keratinization. Multiple squamous papillomas specify *focal epithelial hyperplasia (Heck disease*).[7] The other lesions suggested in the differential diagnosis include *nevus unius lateris, acanthosis nigrans, focal dermal hypoplasia (Gorlin-Goltz syndrome)*.

Histopathology: The histological description of squamous papilloma is a non-neoplastic proliferative exophytic or verrucous tumour which is lined by *stratified squamous epithelium* with organised stratification and showing acanthosis. Histopathological archetype put forward by Oliveria et al comprises papillary-like outgrowth of stratified squamous epithelium with usual maturation of cells and epithelium showing hyperparakeratosis. There is presence of Koilocytosis in which the cells of the spinous layer show perinuclear cytoplasmic vacuolisation and pyknosis. Occasionally basilar hyperplasia is noticed. The connective tissue stroma is fibrovascular and presence of mild degree of inflammation.[8,16,17]

Laboratory Diagnosis: Electron microscopic analysis can detect the viral particles. Immunohistochemistry, in situ hybridization and polymerase chain reaction (PCR), are the other laboratory techniques for identification of the virus.

Immunohistochemistry: There is over expression of *Tumor protein (TP53), Proliferating cell nuclear antigen (PCNA), Cell-surface glycoprotein (CD-44 involved in cell-cell interactions, cell adhesion and migration)* and*B-cell lymphoma 2)Bcl-2)* in case of neoplastic alteration and papillomas with epithelial dysplasias.[18]

Treatment: The ideal treatment is complete surgical extirpation of the lesion along with one-millimetre clearance around the base and one-millimetre depth into the submucosa. Very small sized squamous papillomas are excised using Liquid nitrogen and Lactic acid having keratinolytic constituents.[19]



Figure 1: clinical presentation intra-oral: exophytic lesion in the left buccal mucosa extending from the lip commissure till the retro-molar area

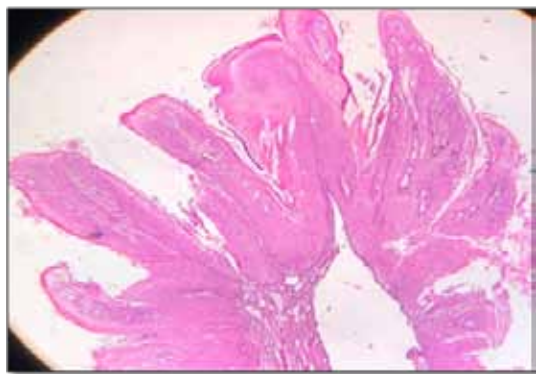


Figure 2: photomicrograph 4x: illustrates many finger like projections each made up of a continuous layer of stratified squamous epithelium and containing a thin, central connective tissue core

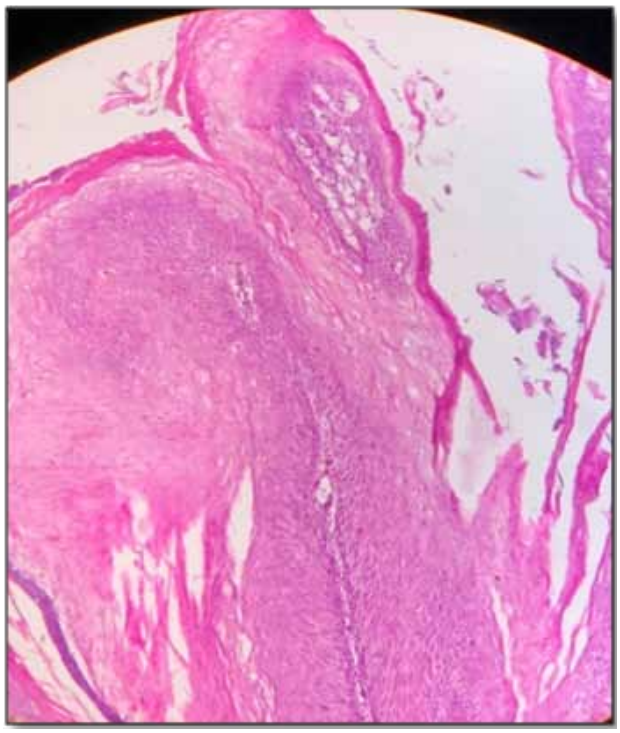


Figure 3: photomicrograph 10x: presence of hyperkeratosis, proliferation of spinous layer in a papillary pattern

Conclusion

The size and clinical features of papilloma presented here is very rare and mimics malignant lesion of the oral cavity through histological features confirmed otherwise. Papillomas in other regions have shown neoplastic transformation hence the potential neoplastic nature of squamous papilloma should be researched and explored.

Conflict of Interests: None

Ethical Permission: Approved

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Appliance Therapy in Temporomandibular Disorders: A Review

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Abstract

The prosthetic rehabilitation in temporomandibular disorders is a challenging task, This article reviews about the present condition and provides the data regarding the treatment needs of the TMD patients and what approach should be followed the practitioner. There has been tremendous development in Evaluating through MRI, it was inferred that the splint therapy through various means reduced the risk of the temporomandibular disorders by eliminating the etiological factor. Furthermore it allowed the patient to cope with the disease such as disk displacement with greater tolerance rate. Moreover, after treatment the patients are likely to cope with disc displacements with larger or smaller tolerance.

Keywords: *Temporomandibular Joint, Pain, Muscles of Mastication, Stabilization Appliance.*

Introduction

Temporomandibular disorders (TMD) is a vast term that deals with various clinical conditions and their pathology comprising of the masticatory muscles and/or temporomandibular (TM) joints and associated soft and hard tissue[1]. The Temporo-mandibular joint is a load bearing joint which entails the condyle, mandibular bone and the fossa eminence of the temporal bone separated by a fibrocartilaginous disc. The clinical conditions associated with the temporomandibular joint diseases were characterized by preauricular pain, pain the Temporomandibular joint area or soreness of muscles of mastication; limitation or deviation in mandibular movement and TMJ sounds[2] (clicking, popping, and crepitus) upon various activities . Symptoms most often found in the patients includes head ache, neck ache, face ache and ear ache .In clinical scenario the TMD are the most intractable problems. The primary approach in the TMD management is controlling the pain.[3,4] The clinicians should be cautious while diagnosing TMD in patients.

The diagnosis of TMD is based upon the chief complaint, past history that patient gives, thorough clinical intra and extra oral examination and the physical examination this helps in formulation of a diagnosis for the disease[5]. Most common findings that the patient present with are faulty mandibular movement,pain of masticatory muscles, pain on loading, signs of bruxism, and neck or shoulder muscle tenderness[6,7]. If there is presence of any certain type of malocclusion then the practitioner should address the problem. Crepitus, clicking, locking of the TMJ is accompanied with the joint dysfunction . Such as, if a single click Is heard during mouth opening, it can be associated with the anterior disk displacement[8,9]. But if there is a second click upon closing of mouth, then it is associated with disk displacement with reduction[10] with the progression of the disc displacement,the patient is not able to open the mouth (closed lock. The crepitus most often associated with the articular surface disruption that occurs in patient with osteoarthritis.[11,12]

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Radiographic evaluation and Imaging can help in the diagnosis of TMD when we don't get much information form the physical examination and the history of the patient[13] . Plain radiograph such as an lateral cephalogram or orthopantograph can be used assess the joint area. CTscans are more superior which used for the evaluation of subtle bony morphology.[14,15] False positive findings occur in 20% to 34 %of asymptomatic patient . MRI is mostly done for patients with continous symptoms, those in whom conservative treatment approach was not effective, or in patients with internal disk derangement[16,17] when the MRI is not available, then the Ultrasonography can be used which is anon invasive procedure and is cost effective to diagnose the internal derangement of the TMJ[18].

Types of Oral appliance for Treatment of TMD:

Stabilization Appliance: It is a rigid appliance usually fabricated using acrylic and generally fabricated in the maxiilaryarch. It is fabricated in the centic relation so when the appliance is placed the jaws are separaed by 2 to 3 mm and the condyle is brought in centric relation. It eliminates any instability and other causative factors for TMD and improves the condyle fossa relationships[19].

Indications: It is used in the treatment of muscle pain disorders .Wearing it decreases the parafunctional activity. In cases of muscle hyperactivity such as bruxism, local muscle soreness, myalgia, retrodiscitis a stabilization appliance is considered.[20]. This appliance minimizes forces that damage tissues, thus efficiently improves the healing.[19]

Simplified Fabrication Technique:

Fabricating the Appliance: A clear sheet of resin is adapted to the maxillary cast . The lingual extension is about 10 to 12 mm from the gingival border runs throughout the lingual portion of the arch. Thelabialy it is extends upto the junction between the incisal and middle thirds of the anterior teeth. A small anterior stop is fabricated in the anterior region and the patient is made to bite on it in Centic relation. This procedure is repeated till the centic relation is obtained and the area is grooved using a round bur. Self cure acrylic resin is mixed and placed over the appliance except in the anterior stop area and the patient is made to bite in centric relation .After the acrylic sets it is removed and is polished.[21]

Anterior Positioning Appliance: It that encourages the mandible to be positioned anteriorly.. It is useful for the treating disc derangement disorders . It alters the position of mandible for short duration and thus It provides a better condyle-disc relationship and provides time for tissue repair and adaptation of retrodiscal tissues. After the tissue are adaptated, the usage of appliance is eliminated.[22]

Indications: It is used mainly in the treatment of disc displacement with reduction, joint sounds, Intermittent or chronic locking of the joint and few inflammatory disorders.

Simplified Fabrication Technique: It is also a hard acrylic device similar to stabilization appliance, that can be used in either arch. As a maxillary guiding ramp, helps in positioning the mandible, it is usually preferred over the mandibular arch.[22]

Fabricating and Fitting the Appliance: The initial steps inthe fabrication of a maxillary anterior positioning appliance are similar to that of fabrication of a stabilization appliance. A anterior stop is fabricated on maxillary central incisiors. The patient is asked to open the mouth widely and asked to close the mouth in forwarded position and the joints are evaluated for the symptoms. This step can be repeated and verified. Once the forwarded position is confirmed a groove is made in this position.[21] This groove helps in positioning of the mandibular incisiors . The appliance is placed in the mouth to the mouth with self cure acrylic resin on it except in the areas of anterior stop and the patient is made to close the mouth in the setting acrylic in the determined position

Adjusting the Occlusion. Excess of acrylic is removed, smoothened and polished using hard rubber wheel. A well fabricated appliance allows even and smooth contact of all the teeth in the desired position.[20] Flat occlusal contacts are developed for the posterior teeth, The appliance is returned to the mouth, and the patient closes in the forward position. Sound contact is checked using red articulating paper that should be visible on all cusp tips.

Anterior Bite Plane: It is a hard acrylic aplliance with contact is provided in the anterior region only without any contact in the posterior region[22].

Indications: It is used in the conditions of orthopedic instability causing muscle disorders or in cases of an acute change in the occlusal condition . Parafunctional activity is also treatablefor short

duration. Main disadvantage of this appliance is, if it is used for long time, it may cause supraeruption of posterior teeth leading to anterior open bite. It must be carefully observed during the treatment procedure and should be used only for short periods. similar results can be obtained using stbilizationappliance. More recently a device has been designed with similar cocepts of anterior bite plane to reduce the incidence of heeadache. It was marketed in the name of by its inventor the Nociceptive Trigeminal Inhibition Tension Suppression System (NTI TSS) or NTI[23]. It allows contact in the central incisiors. The initial studies showed that the NTI was only slightly more effective when compared to standard appliance therapy in reducing the headache

Posterior Bite Plane: It is a hard acrylic constructed usally in the lower arch in the posterior region connected by lingual bar.

Indications :They have been used in the cases of severe loss of vertical dimension or when severe changes are desired in the forwared positioning of the mandible It is also used as a protective appliance by athelets in few cases .However there is no scientific evidence for this . It helps in supra eruption of unapposed teeth or intrusion of apposed tooth. it is used in some cases of disc derangement however not much studies are present to support the fact[24].

Pivoting Appliance: It is a hard acrylic device that provides contact usually in the second molar region which act as a fulcrum and pivot the condyle in downward and backward position away from the fossa.

Indications . It was thought that it unloads the joint and provides time for tissue repair., It was used in the treatment for joint sounds. It is possible only if forces are located anterior to the pivot. As elevator muscles are present posterior to the pivot it does not allow pivotingaction. Anterior positioning appliances much more capable of as it provides better control in positioning the mandible[22].

However there are very few scientific studies that supports the success of treatment using this pivot appliance .In fact anterior positioning appliance has much more therapeutic value compared to the pivoting aplliance and is usually not used for the treatment clinically.

Soft or Resilient Appliance: It is usually made up of Resilient material that is adapted to the maxillary archto achieve even and simultaneous contact.[24]

Indications: It is usually indicated in athletic sports person to protect the teeth and tissues from damage due to trauma . But less studies are available regarding this. It is usually recommended in patients exhibiting clenching and bruxism. However soft appliances have not been shown to decreasebruxing activity. It is also recommended for patients suffering from repeated or chronic sinusitis resulting in sensitive posterior teeth due to heavy occlusal forces[24].

Conclusion

This paper is designed to give the dental practitioner an overview of splint treatment in the management of temporomandibular disorders. It is impossible to be definitive or prescriptive unless the TMD diagnosis has been made and it is not the remit of this paper to cover such diagnosis[24]. The overall benefits of splint therapy should not be underestimated a major benefit is that if splint therapy is beneficial if proper diagnosis is made and proper fabrication and regular follow-up done.

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A New Pioneering Advancement: Waterlase

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Abstract

The Waterlase™ is a trailblazing tool for dentists which is an amalgamation of laser energy and water. This process is known as Hydro Photonics. This tool is proficient to perform many conventional dental procedures with less need for anesthesia. Hard and soft tissue can be cut using Waterlase without applying heat, vibration or pressure. The dental operator may easily carry out the procedure in the absence of anesthesia. Waterlase laser decreases post-operative bleeding, pain and swelling and the need for pain medication in many cases. It has been used for a variety of therapies such as hard and soft tissue procedures including caries removal, cavity preparation, endodontic therapy, designing and correcting smile, frenectomy, gingival excision, gingivoplasty, curettage, vestibular deepening, operculum excision, crown lengthening, flap surgery, and osseous surgical procedures,etc.

Keywords: *Waterlase, Lasers, Hard tissue therapy, Soft tissue periodontal surgery.*

Introduction

The diseases of the periodontium comprise of a group of inflammatory diseases. It is commenced by microbes that colonize the teeth and spread infection. Earlier, the local factors causing periodontal diseases were eliminated by the hand instruments and ultrasonic scaler.[1] However, calculus and plaque removal using traditional method has been considered to be insufficient and taxing.[2] For enhancing the usefulness and efficacy of debriding the root surface, different devices have been tried such scalers both ultrasonic and sonic. Recently lasers also have been used for the same.

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Laser: Laser is the **acronym** for **L**ight **A**mplification by **S**timulated **E**mission of **R**adiation. It generates electromagnetic wave. The heat released by the laser converts the electromagnetic energy into thermal energy. The laser follows the principle first given in 1917 by Albert Einstein. He proposed that light consists of mass-less particles called photons. The energy of the photons corresponds to the wave's frequency. As the frequency increases, the energy carried by the waves becomes greater. This is the principle of laser. Charles Townes and co-workers first demonstrated the action of laser in the microwave region for which he was later awarded the Nobel prize. A beam of ammonia molecules was passed through a system of focusing electrodes. Amplification occurred when a microwave was sent through the cavity at an appropriate frequency. This was termed microwave amplification by stimulated emission of radiation (M.A.S.E.R.). The term laser was first coined in 1957 by physicist Gordon Gould a physicist in 1957 who coined the term laser. The first Laser beam was generated in 1960 by Theodore H. Maiman at Hughes Research Laboratories. It is a solid-statelaser, that uses a pink ruby crystal. It is encompassed by a flash tube enclosed within a polished aluminum cylindrical cavity. It is cooled by forcing air through it.

Parts of laser:

Laser comprises the following constituents:

1. Laser medium- Can be in any state gaseous, liquid, or solid.An external energy source was passed through it.
2. Resonating chamber- It is a tube with two reflecting mirrors. One mirror is completely reflective, the other one partly transmitting light. Both are placed at either side of the optical cavity.

3. Power source- It pumps the atoms in the laser medium so that they can attain higher energy levels. It is either mechanical, optical or chemical source of energy.

Three factors are essential for the characteristics of the laser light:

1. Composition of the laser medium,
2. Energy source, and
3. Design of the resonating chamber.

Characteristics of laser: When an excited atom is stimulated to emit a photon spontaneously it results in the release of a subsequent photon. A monochromatic, collimated and coherent form of light is produced by the energized emission. This laser light on contacting the tissues gets reflected, scattered, absorbed ortransmitted to the surrounding tissues. The free water molecules, pigments, proteins and other macromolecules present in the oral cavity are the reason for photonic absorption in the mouth. This absorption causes an intracellular or intercellular change in the tissues. Shorter wavelengths of 500- 1000nm are readily absorbed in blood elements and chromophores. The Argon lasers are highly diminished by hemoglobin. The Diode lasers and Nd: YAG lasers have an increased affinity for melanin. Longer wavelengths interact more with H2O andhydroxyapatite molecules. The maximum absorption for H2O is just under 3000 nm for Er: YAG laser. At 10,600 nm CO2laser has the maximum affinity for hydroxyapatite and water.

Different wavelengths can be classified as:

1. The ultraviolet spectrum i.e. 100- 400 nm
2. The visible spectrum i.e. 400-700 nm
3. The infra-red spectrum i.e. 700 nm to the microwave spectrum

Depending on the wavelength Lasers can be of two types:

(i) Deep penetrating ones, such as Nd: YAG and diode. The laser light enters deep into the tissues and scatters.

(ii) Superficially absorbed ones, such as CO2, Er: YAG and Er, Cr: YSGG lasers. The laser light gets absorbed in the superficial layers. It does not penetrate or scatter deeply.

Various periodontal laser procedures: Laser has proved to be an effective device in the recent dental practices. It has not only increased efficiency, and specificity but has also enhanced the ease and comfort of carrying out dental treatment at minimal cost. A few of the uses and applications of specific lasers in different treatments are mentioned below.

Table 1. Uses and applications of specific lasers in different treatments

	Er:YAG 2940nm	Er,Cr:YSGG 2780nm	KTP 532nm	Argon 488 and 515nm	Diode 810-980nm	Nd:YAG 1064nm	Helium-neon 633nm	Diode 635, 670 or 830nm	CO_2 10600nm
Calculus removal	✔								
Periodontal pocket disinfection	✔	✔	✔	✔	✔	✔			
Photoactivated dye disinfection of pockets							✔	✔	
De-epithelialization to assist regeneration	✔	✔							✔

Effect of lasers on bacteria and calculus: Most laser bactericidal studies reported that when the density of the energy is enhanced results in decreased bacterial load. Cobb CM et al 1992 reported that a decrease in period onto pathogens followed by the use of Nd: YAG laser shows reduction in *P.gingivalis, P. intermedia and A. actinomycetemcomitans*. However, recolonization of multiple morphotypes of bacteria was observed postoperatively in laser-treated subgingival root surfaces Moritz et al (1997) proposed that Er: YSGG laser in

adjunct to SRP enhanced healing of periodontal pockets. Aoki et al (2000) suggested that Er: YSGG laser has the proficiency to remove calculus which is at par with ultrasonic scaling.[3-5]

Latest Developments:

Periowave™: Periowave™: It is a system that is based on the principle of photodynamic disinfection. It uses non-toxic dye (photosensitizer) along with a low-intensity laser. This enables singlet oxygen molecules to eliminatemicrobes. Each site requires only one minute of laser activation, making it a swift and painless treatment(6).

Waterlase: Er, Cr: YSGG (Erbium-Chromium doped: Yttrium-Selenium Gallium-Garnet) laser is available commercially as Periodontal Waterlase MD™ and The Waterlase® C100 It utilizes a patented amalgamation of laser energy and water to perform various dental procedures. It is so precise that the dentist can restore the teeth, gums, and bones without damaging the surrounding structures.

What is Waterlase dentistry?: This dentistry is an alternative to conventional treatments. It works in the absence of heat, vibration, and drilling. Hence providing a minimally invasive, precise approach.

It uses advanced technology to:

- Alleviate patient's anxiety
- Carry out minimally-invasive procedures
- Significantly reduce the need for retreatment
- Comfortably prepare patients for invasive treatments
- Perform treatment without anesthesia
- Reduce post-treatment bleeding and swelling, and pain
- Provide effective treatment in minimal time

The handpiece resembles a high-speed handpiece but it has fiber optic tips instead of bur. This directs the laser energy at a focal point about 1 to 2 mm from the tissue surface. It weighs 88 pounds, and has dimensions of 12.5×26×32 inch. It needs about 80 pounds per square inch of air pressure provided by an external source. The water supply is placed in an attached bottle.[7] Hydrokinetic energy is generated by combining a spray of atomized water with laser energy. The resulting Hydrokinetic energy smoothly and precisely cuts hard and soft tissue such as tooth enamel and soft tissue.

Advantages of Waterlase:

- Itremoves hard and soft tissue without any discomfort
- It requires less anesthesia
- It decreases post-operative pain, bleeding and swelling,
- Waterlase precisely cuts the tissues leaving the surrounding areas unaffected minimizing trauma
- Treatments can be simultaneously performed in several areas of the mouth, resulting in a decreased need for multiple visits.

Hard tissue procedures: The YSGG laser generates optical energy to a user-controlled distribution of atomized water droplets. The hydrokinetic cutting effect occurs due to the absorption of the optical energy by the water droplets. The removal of tissues with highly energized water particles is the principle behind the hydrokinetic process. Enhanced absorption of laser energy by atomized water droplets results in an intense yet controlled water particle excitation and micro-expansion. The water vapor released causes an increase in the internal pressure within the tissue, resulting in an explosive expansion called 'micro explosion'. These results in 'thermomechanical' or 'photomechanical' ablationby mechanical collapsing the tissue. This process is termed as 'water-mediated explosive ablation'. Various studies have reported that Er, Cr: YSGG laser can be comfortably used on osseous tissue bone without anycharring or melting. No physical contact is made with the hard tissue while cutting it; i.e. the laser energy is transmitted to the water, which is further transmitted to the tooth leading to tissue ablation.[8,9]

Soft Tissue Procedures: These procedures are done using a different mode where direct Er, Cr: YSGG laser energy is applied to cut or ablate the soft tissues. Adequate anesthesia is needed to perform soft tissue procedures. The water is need for cooling, keeping the tissue moist and clean and for hydrating. Waterlase laser energy is delivered via a flexible fiber-optic device. The laser does the cutting while the water released acts as a coolant. For soft tissue procedures, the Waterlase® C100 utilizes direct laser energy to cut tissues, incise, excise, ablate and coagulate.

Two key features are:

1. Reduces bleeding in the operative field
2. Decreased post-operative pain and discomfort in comparison to traditional procedures.

The power source, the pulse energy, rate of repetition and rate of flow of air and water are adjustable in all the applications according to user requirements.

Uses in Periodontics:

1. Root planing procedures
2. While giving incisions in cases of Full-thickness/ Partial-thickness flap
3. In cases of curettage
4. Granulation tissue removal from the osseous defects
5. During debridement
6. While recontouring bone to restore its architecture
7. Respective osseous crown lengthening
8. Soft tissue procedures such as removal of the gingivaand frenum
9. Implant recovery
10. Painless excision of epulis caused by faulty dentures
11. Low-level laser therapy is used for early detection of caries
12. Lasers are used for providing restorative therapy having longer and better prognosis due to its microbial actions in caries removal procedure.
13. Dental lasers are also used as sealantsfor dentinal tubules that are the primary reason for dentinal hypersensitivity.

Waterlase with Emdogain™, is a regenerative gingival therapy. This device promotes the regeneration of soft and hard tissue that has been degraded due to chronic periodontitisand observed the combination of the two procedures for over nine years. He also concluded that this method of treatment has immense potential in the healing of the periodontal treated sites.

Drawbacks, Cautions and Contraindications:

1. The Er: YSGG laser has few drawbacks when used subgingivally.
2. While applying laser in the periodontal pockets, the visibility of the operator is compromised. Hence special tip designs should be facilitated for better insertion into the pockets. This can also aid in the detection of the subgingival calculus.
3. High-speed evacuation is necessary to pump out the water released during the explosive ablation causing splatter of blood and water from the pockets. This can be carried out by not only using an intraoral suction but also an extraoral evacuator. Thus cross-contamination can be prevented by the splashes of blood and water.
4. In cases of systemic health conditions extra caution and precaution should be exercised. In cases allergy to anesthesia, cardiac disorders, respiratory problem and blood disorders use of lasers can be contra indicated local procedure. Immuno-deficiency may also pose as a contraindication. Hence medical consent from the patient's physician is mandatory in the above-proposed cases or doubt.

Precautions:

1. Inadequate knowledge about the usage of controls or adjustments or performance of procedures may lead to harmful radiation exposure.
2. This device has been designed to meet the requirements of excessive electromagnetic, electrostatic and radio frequency interference testing. However, there remains the possibility of other interference persisting.
3. One should never carry out any procedure if he/she thinks that the device is not functioning properly.

Directions for safety:

These instructions should be strictly followed at all times:

- Highly reflective items in the vicinity of the operating field should be removed or covered.
- No explosive or flammable things should be present nearby.
- Protective eyewear must be worn by the operator and personnel surrounding the operative field suitable to block 2.78 μm wavelengths.
- One must make sure that Waterlase® C100 Handpiece Mirror is clean. There should be an absence of water or moisture.

Do not directly look into the beam or at specular reflection.

- The cutting spray should be directed towards the target tissues and not elsewhere.
- STANDBY button on the control panel must be pressed before switching off the unit.
- Before exchanging handpieces or removing the fiber optic connector from the unit press STANDBY.

Move the circuit breaker to the OFF (O) position (located on the rear panel) and remove the key before leaving the unit unattended.

Future developments: There is a great potential for laser systems to be developed further to include additional features and functions. The Alexandrite laser is a solid-state laser, which could remove dental calculus selectively. Mechanism of selective ablation has not been clarified yet. The development of this laser for clinical use is widely expected due to its excellent ability for selective calculus removal from the tooth structure. Solid-state lasers are being designed that have higher power, are faster, have shorter wavelengths, and better beam quality, which will expand their applications.

Conclusion

Er, Cr: YSGG laser effectively performs numerous soft tissue procedures with little or no bleeding and gives the dentist a new high-tech tool for many procedures that previously required referral to a specialist. Now one can have them performed during the scheduled appointment. Through this new innovative technology that seems to be extremely effective, there is a great need to develop an evidence-based approach to the use of Waterlase in periodontics. More controlled studies are needed to establish its usefulness in the field.

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Mental Attitude in Complete Denture Patients: A Review

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Abstract

Patient evaluation is the first clinical step of Complete Denture fabrication. Besides finding out intra-oral state, the clinician also evaluates general well-being and also extra-oral state. In general well-being, the psychological status of the patient should be evaluated. Evaluation of the mental attitude is a very important factor in determining the success of the complete denture. This begins immediately when the patient enters the dental office. Patients attitude and their expectations from the treatment should be understood. If required certain modifications and conditioning by the dentist should be done for better acceptance of the treatment and a good outcome. This article is a review of the classifications proposed for evaluating the mental attitude of the patient.

Keywords: *Mental attitude, Prosthodontic treatment, Classification; dentures; complete.*

Introduction

The loss of a tooth affects the physiology of mastication, speech, esthetics, and also on the patient's psychology, thus having an overall impact on the quality of life of patients, irrespective of age, gender, economic status, etc. This is even more obvious in completely edentulous patients, especially in the geriatric group. Therefore,De Van stated that we should meet the mind of patients before we meet the mouth of the patient which means that the psychological state should also be examined even before the procedures for treating the edentulism.[1,2,3] This determines the acceptance of the completely edentulous patient to the complete denture. Sometimes a patient with an ideal intra-oral condition may have less acceptance, but a patient with unfavorable conditions may have better acceptance and outcome. Thus, studying the mental attitude would determine the patient's acceptance for the treatment, patient's temperament towards the dentist and expectation from the treatment which will altogether determine the success of the treatment.[3,4,5] This article is a brief overview of the various classification of mental attitudes proposed for edentulous patients along with their clinical importance.

Various Classifications:

Ideal Patient: There are four traits of the responses of an "ideal patient" who needs denture, according to Winkler. They are: the patient realizes the need for the treatment; wants the prostheses; accepts them and learns to utilize the dentures by giving attempts.[6] O'Shea considered a geriatric edentulous patient "ideal", when the patient is compliant, sophisticated, and responsive to the treatment.[7] But, these cannot define the patient's attitude and dictate the very relative treatment outcome, rather the psychology should be analyzed and profiled.

M.M. House Classification[8,9,10]:

This classification has been described in TABLE I.

Table I: Types of patients and their clinical impressions in M.M. House classification.

Type	Clinical Impressions
Philosophical mind	- reasonable,calm, and involved in the treatment procedure at every step. - understand the treatment - listen to the dentist's advice and are willing to follow the instruction.

Type	Clinical Impressions
Exacting mind	- disciplined, strict,may have precise demand and may ask for a guarantee - dentist may have to give a little more effort after which the patient listens to him and follow his advice. - if the patient had a previous denture which was dissatisfactory, then he may doubt the present denture also. But, once satisfied, he would be the dentist's fan. - prognosis is usually good
Hysterical mind	- negative attitude - poor oral health and lack of effort for maintaining oral health - anxious and unstable emotionally - do not have realistic expectation - unreasonable complaints - poor prognosis
Indifferent mind	- not motivated for the treatment. - disinterested in the treatment - does not obey any advice of the dentist - prognosis is doubtful

Winkler's Classification[3,6]:

This classification has been described in Table II.

Table II: Types of patients and their clinical impressions in Winkler's classification

Type	Clinical Impressions
The Hardy Elderly	- physically and psychologically maintained - active in their professional life - active socially - adapt to changes and accept them
The Senile Aged Syndrome	- physically may be ill,disabled or aged - psychologically and emotionally weak - cannot cope with stress and vulnerable to ailments.
The Satisfied Old Denture Wearer	- adapted well and happy with old dentures, even though they are faulty - not motivated for treatment - poor prognosis
Geriatric Patient who doesn't Want Denture	- long-standing edentulism, without any treatment - no motivation for treatment and have a poor prognosis

Gamer's Classification[9,11]: Gamer classification is based on two factors, i.e: patient's engagement level to dentist and treatment procedure in a continuum from totally engaged (++++) to disengaged (+).

This classification has been described in Table III.

Table III: Types of patients and their clinical impressions in Gamer's classification

Type	Clinical Impressions
Ideal	Reasonably engaged i.e. (+ + +) Reasonably trusts the dentist i.e. (+ + +) May skeptically distrust also . Mature May ask questions and need satisfying answers for the same from the dentist May not be over suspicious or over accepting of the dentist's opinions. Similar to Philosophical type of patient in House's classification

Type	Clinical Impressions
Submitter	Highly engaged (+ + + +) trusts the treatment and the dentist(++++), considers the dentist as ideal. Engages in the treatment completely
Reluctant	Engagement level is + + Trust for the treatment is ++ skeptic
Indifferent	Engagement level is + i.e. minimum level of engagement. Trust for treatment is +; indifferent to the dentist's instruction Similar to Indifferent type of patient in House's classification
Resistant	Engaged with treatment but with an adverse mindset. Trust is very minimal or not there Question or doubt if they receive help from anyone in any situation Instead of acknowledging or accepting, they challenge the dentist.

Another Classification[11]:

This classification has been described in Table IV.

Table IV: Types of patients and their clinical impressions this classification

Type	Clinical Impressions
Cooperative	- may or may not recognize the need for dentures - Open to suggestions - After some effort by the dentist, these types of patients become cooperative.
Apprehensive	- Unreasonable - Acknowledge the need for dentures, but give an illogical problem that cannot be solved rationally.
a. Anxious	- Anxious and sometimes become neurotic - Focus on the uncertainties related to dentures.
b. Frightened	- Fear the denture therapy
c. Obsessive or exacting	- Aware about their wants - Exacting type - May dictate the clinician regarding the way to proceed.
d. Chronic complainers	- Dissatisfied with any kind of treatment - Find faults and problems in everything - Need appreciation, if they are willing and cooperating for the treatment.
e. Self-conscious	- Look-conscious - Need to be re-assured and should be acknowledged for their inputs in the final result.
Uncooperative	- Do not need dentures - Not motivated - Have a negative approach

Need of Understanding Patient Psychology: The patient's contribution to his or her treatment is vital for a positive treatment outcome. For the clinician to deliver good work and for the patient to adapt, there must exist a good relationship between the patient and dentist. Then only the patient will accept the treatment.[10] There are many factors that play a role in satisfying the patient. Among these factors, the most important ones are: type of treatment selected, psychology of patient, the personality of the patient, perception of the patient towards the dentist and treatment selected, the interaction between patient and dentist. Patient satisfaction level may be increased if there is a good dentist-patient relationship because the dentist understands the patient's needs and

delivers as per the patient's expectation whereas the patient builds an acceptable and realistic result.[11,12]

Conclusion

The clinician should understand the mental attitude of the patient, preceding general and oral examination. Proper time should be devoted to a thorough examination. This builds the patient's faith in the doctor. The doctor's attitude towards patients is also equally important. The dentist should be concerned about the patient's comfort, health, and well-being so that there is trust developed from both-sides and the patient is motivated to follow the advice of the dentist. This shall lead to a good outcome of the denture therapy.

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Barodontalgia: Dental Implications at High Altitudes

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Abstract

Barodontalgia is a symptom of a pre-existing subclinical oral diseases rather than a pathological condition itself. It was considered earlier as a "flyer's toothache," barodontalgia is described as pain that occurs in the tooth utilizing changes in ambient pressure usually in people who fly or dive. It is the whole responsibility of the dental health professionals to provide the aviators with the best dental treatment as well as awareness about the hazards to the dental treatments at higher altitudes. This piece of writing gives sufficient information about the need and protocols to be followed especially for the patients commonly at higher altitudes. This article aims at providing adequate knowledge about barodontalgia and the responsibilities towards its prevention both by the dentist and the flight crew members.

Keywords: *Barodontalgia, Ambient pressure change, Toothpain.*

Introduction

Dentistry has changed over some years from prevention to management of oral diseases. Considering from varied professions, some people will be there who "rise from the ground" commonly known as air travel crews, pilots, and persons who keep travelling always for work. The dental health professionals might more and more come upon flight-related oral conditions which require treatment for the rising number of flight attendant and leisure pilots, air passengers, as well as military and airline pilots. Barodontalgia also known as Tooth Squeeze; or Flyer's Tooth; or Aerodontalgia. It is the pain in the oral cavity that results because of the pressure changes usually found in case of far above the ground altitudes in pilots and also for the deep sea divers who also go through severe changes in pressure.[1] Barodontalgia is a symptom of a pre-existing subclinical oral diseases rather than a pathological condition itself.[2] It was considered earlier as a "flyer's toothache," barodontalgia is described as pain in the tooth that occurs with variation in ambient pressure usually in people who fly or dive. It can be severe enough to cause inflight vertigo as well as premature flight cessation.[1]

Aerodontalgia was named during the world war II because of the pain experienced by aircrew in the flight but, this type of tooth pain was found in divers, therefore an additional suitable word, barodontalgia, later given to this phenomenon.[1] Barodontalgia affects both aircraft passengers as well as aircrew and underwater divers. Due to the change in pressure gradients teeth are affected due to injury and pain.[3] According to Boyle's Law "at a given temperature, the volume of a gas is inversely proportional to the ambient pressure," which can be used to explain the concept of barodontalgia.[2]

The barodontalgia phenomenon starts occurring at an altitude of about 3,000 m and at a water depth of 10 m where the ambient pressures are 0.75 and 1 atmosphere, respectively.[4] Some people have reported that they have felt pain in a tooth because of the high rise buildings these days plus the powerful lifts that are used with travel at very high speeds to reach great heights.[5]

Prevalence:

- It ranked 5th as complaint and 3rd as cause of chamber simulation termination.
- Usually above 3,000 ft. with 11% of aircrew affected.[3]
- Seen at 5,000-35,000 ft. but more common 9,000-27,000 ft.[6]
- 3-37% of barodontalgia is non-dental facial barotraumas.[1]

In the 1940s, the prevalence of barodontalgia was within a range of 0.7% and 2% and in the 1960s it is 0.3%.[7]Similarly, in the Luftwaffe, several cases of barodontalgia reported in 0.3% of high altitude-chamber. It was also reported that there were cases of barodontalgia of about 1 case per 100 flight-years in the Israeli Air Force.[8]

Classification:

1. **According to Fédération dentaire Internationale (FDI):** It is divided into four categories such as:- a) acute pulpitis, b) chronic pulpitis, c) necrosis of the pulp d) periapical abscess or a cyst.[9]
2. **In 1972, The pathology of barodontalgia** was specified by Strohaver. He divided his description into two types: a) direct barodontalgia b) indirect barodontalgia. In the direct type of barodontalgia, the atmospheric pressure is decreased and then it results inan indirect effect and is localized into moderate to severe that is generally formed at some stage in take-off, whereas, in the indirect type of barodontalgia pain results because of the stimulus of the superior alveolar nerves during the period of maxillary barosinusitis which is dull and also poorly defined that involves the posterior teeth which occurs during landing.[10]

It should be kept in mind that teeth that are normal in condition and are devoid of anycausal pathologies that will not get exaggerated by any changes in pressure. Barodontalgia can be found commonly in teeth that contain pre-existing dental pathology or undergone with any inappropriate dental treatments such as:[11]

a. Chronic pulpitis

b. Restored teeth

c. Maxillary sinusitis

d. Default restorations

e. Periapical cysts

Barodontalgia hasa chance of occurringat the time of initial pressure reduction from 14.7 psi to 12-8 psi.[12] A distinguishable sign is the pain that is consistently relieved on repressurization, distinguishes barodontalgia from pain in the upper jaw because of maxillary barosinusitis.

Manifestations due to pressure change:

1. **Barotrauma:** This is a type of condition that results as a variation count in pressure flanked by a gas space surrounded by the body as well as the surrounding fluid because of the lower atmospheric pressure. This might give rise to conditions like dental barotraumas, barodontalgia, barotitis –media, external otitic barotrauma, barotraumas related headaches, barosinusitis.[13]
2. **Barodontalgia:** This is a kind of pain that occurs by a variation in barometric pressure in an asymptomatic organ. Here it can be expected of pain that is correlated to periapical disease that can occur during ascent as well as descent and also it can disappear on returning to the ground level. It is being reported that toothache in barodontalgia is because of the rise in atmospheric pressure that will lead to spreading out of trapped air bubbles.[14]
3. **Tooth outburst:** Also well known as odontocrexis or barodontocrexis. When exposed to sudden changes in atmospheric pressure then a tooth explosion will occur in teeth that have pre-existing leakage restorations or in recurrent carious lesions. This detonation happens because of the unexpectedspreading out of gas bubbles fascinatedinside and underneath these restorations.[15]
4. **Barosinusitis:** This barosinusitis occurs as a result of negative air pressure formation in between the sinuses that leads to tenderness that causes toothache.[16]
5. **Prosthetic Considerations:** There might be a retention problem in both upper and lower dentures that are being troubled because of the variation in barometric pressure. Due to the effect of gravity suchvariation is more distinct in upper denture than lower denture.[17]
6. **Restorative Dentistry:** Fracture of restoration can occur due to variation in thermal contraction in restorative materials and low temperature at higher altitudes as compared to tooth hard tissue.[18]
7. **Periodontal considerations:** Due to change in pressure xerostomia and hyposalivationcan be seen as an imperative risk factors for decayed teeth. This might increase the risk of occurring periodontal infection and dryness of the oral cavity which can occur because of inhaled compressed dry gases in the aircraft.[19]
8. **Surgery Considerations:** To avoid the growth of sinusitis at higher altitudes, care should be taken for

the existence of any oroantral communication while extraction of maxillary teeth.[20]

Reason of pain due to pressure change:

a. During any underlying pathology, spreading out of gas with compensating rise in pressure inside the pulp chamber and root canal can be seen because of changes in external pressure.[21]

b. Pain during ascent pressure along with relief on descent pressure - Pulpitis

c. Toothache on descent along with relief on ascent - Necrosis

d. Toothache can be seen usually in the maxillary posterior teeth along with adjusted blood supply to the tooth.

e. Exposed nerve endings by direct pressure.

f. Faulty restoration can leads to microleakage.

Hypotheses to this phenomenon by Kollman: First, intent air bubbles get expands underneath a root filling or against dentin to activate nociceptors; Second, with the pain in the referred teeth, stimulation of nociceptors in the maxillary sinuses; Third, chronically inflamed pulp stimulates the nerve endings. He presented histological substantiation that shows that chronic inflammation of pulp that can exist even after a thin dentin layer covers the pulp, for example, like in a deep cavity preparation and also he strongly supported his last two hypotheses.[22]

Flight Restriction related to dentistry: The flight restriction for any patient in the flight is enforced when any kind of intrusion in the flight capabilities is doubted for any aircrew member:[23-25]

- Patients under any medications might be a source of dizziness or lack of concentration or maybe the reason for diarrhea.
- Intraoral stress changes can be found in patients who have undergone tooth extraction or other oral/ periodontal surgery after several hours.
- Variations in hazard of emphysema.
- The jet and helicopter pilots might face difficulty in wearing helmets comfortably in case of face swelling.
- Alteration in pressure changes can hamper the wound healing in cases of oroantral communication.
- Aircrew has to reschedule his flight in anticipation of his endodontic treatment is finished to avoid in-flight barodontalgia.

The dental health professionals recommended the aircrew not to flight until the toothache is subsided so that the patient can rest well. It is also suggested that appointment for dental check-up and treatment can be scheduled for a date with enoughperiod before the next planned flight.[26]

Prevention from barondontalgia:

a. Professionals like pilots and deep-sea divers should undergo the Hyperbaric Oxygen Therapy (HBOT) whenever the individual is set in a 100% compressed Oxygen chamber that set the body for maximum stress as well as compression.[25]

b. FDI suggests a yearly checkup for pilots, divers and submariners with dental hygiene instructions from dentists.[9]

c. Patients within 24 hours of dental treatment must not dive or fly in non-stressed cabins having a requirement ofanesthetic.

d. Existing dental pathologies should be checked and treated properly.

e. Barodontalgia can be prevented by putting a zinc oxide eugenol (ZOE) base in case of a reversible pulpitis as ZOE is well known for its sedative effects.[26]

f. People subjected to long-duration pressure changes then procedures like pulpectomy or cases like pulp capping of an exposed pulp should be avoided and alternatives treatment should be done. [14]

Recommendations:

a. Dental conditions like periapical pathology, dentin exposure, caries, and fractured cusps should be paid attention closely by the patients involved in diving or aviation.

b. The dental health professional should examine properly and ask the patient about the history of recent flying or diving if the patient has any symptoms of barodontalgia.

Conclusion

By the passing time,a rise in the number of people traveling by air can be observed and this also leads to a gradual rise in the number count of aircrew personnel. Therefore, aviation dentistry has amplified, it has to be

given importance, so that suitable diagnoses should be prepared. Barodontalgia must not be underestimated because of its safety risk to pilots, submarines, divers,and airline passengers. Special considerations for aircrew patients have to be made when dental treatments are planned. It is the whole responsibility of the dental health professionals to provide the aviators with the best dental treatment as well as awareness about the hazards to the dental treatments at higher altitudes.

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Oral Health Care in Infants and Deciduous Dentition: A Review

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Abstract

Oral health goes in a close relationship with the general health. Hence, bad oral health affects the personal life and the lifestyle too. It affects interpersonal relationships, concentration, and productivity. Hence, maintenance of oral health, not only maintains the oral cavity, but, also helps maintain a quality life. The process should start right from the prenatal period (oral care of the pregnant mother) and infancy. It has been found from surveys that the presence of dental caries in school going children affects their education as they tend to miss school due to pain and aesthetics.

Keywords: *Oral Health, Maintenance, Oral Hygiene.*

Introduction

Oral care in infants and children is an important aspect of the sphere of their health care. The parents, caregivers, and pediatric dental and medical practitioners play an important role in the maintenance of the oral health of this section of the population. Oral care practices in infants differ from those in case of deciduous dentition. These practices help prevent the most prevalent dental disease, i.e., dental caries.

Oral hygiene in infants:

Source of infection in infants: Though teeth are not present in the oral cavity at this age, but, still the maintenance of oral hygiene is very necessary. This would prevent the occurrence of dental caries when the primary teeth or the permanent teeth erupt.

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Vertical transmission in one of the major factors in the infection of infants with Streptococcus Mutans.[1] The high density of *Streptococcus mutans* in the saliva of mothers leads to the increased risk of infection in the early life of their children. This in turn leads to a high risk of dental caries in the primary and permanent dentition.[2,3] Infection from family members or the members of a group can also occur. This is called horizontal transmission.[1]

Few other sources of infection of Streptococcus Mutans in infants and children are diet including cariogenic foods, poor oral hygiene practices, bottle-feeding, and breastfeeding practices at night and on-demand. These are also found to be linked to the development of early childhood caries (ECC).[4,5]

Oral hygiene practices in infants:

Oral health in infants can be maintained by taking a few steps at home itself.

- Vertical transmission of dental caries can be prevented by reducing the cariogenic bacterial count in the mother, especially during pregnancy. This can be achieved by regular tooth brushing, use of fluoridated Mouthwash, and treatment of all the carious teeth, if any.
- horizontal transmission of dental caries can be

prevented by avoiding any habits that include the sharing of saliva (e.g., sharing of spoons) within siblings, parents or any other family members.[6] Regular maintenance of oral hygiene can be achieved by wiping the alveolar mucosa and the tongue with a moistened piece of sterilized gauze. This can be wrapped around a clean index finger and then oral tissues are gently wiped and gingival tissues are gently massaged. The use of dentifrices, such as fluoridated toothpastes should be avoided as its ingestion is a possibility.[7] Another important practice is weaning the child at the proper time. Frequent bottle-feeding or breastfeeding on demand or during the night in infants beyond 12-18 months leads to the development of dental caries at a very early age. The results are early childhood caries and nursing bottle caries.[7]

Dental home concept: According to AAPD, this concept is an ongoing relationship between the dentist and the patient, inclusive of all aspects of oral health care delivered in a comprehensive, continuously accessible, coordinated, and family-centered way. It should be established as early as 6 months after the eruption of the first tooth and no later than 12 months of age. It includes the referral to dental professionals as and when appropriate.[8,9]

Its objectives include enhancement of dentists' ability to assist the children, their parents and/or their caregivers for maintenance of child's optimum oral health; planning early oral examination schedules and preventive services for the achievement of cost-effectiveness; risk assessment of the individual child for dental diseases, monitoring growth and development and awareness of parents of proper time and frequency of paying a visit to the dental home for their child.[8]

Oral hygiene of deciduous dentition: The care for the deciduous dentition should be started immediately with the eruption of the first primary tooth in the oral cavity. Colonization of the deciduous teeth with Streptococcus Mutans leads to the development of dental caries in them and the non-maintenance leads to the development of dental caries in the permanent teeth in those children too. It has been reported that tooth brushing habits in children are viewed as the parents' attitude towards the importance of maintenance of oral hygiene.[10,11] Toothbrushing in children till the age of 7 to 8 years should be assisted or guided by the parents as at this age, fine motor skills develop in the children.[12]

Source of infection in children: One of the primary causes of infection of children with Streptococcus Mutans is the presence of active caries in the parents or caregiver.[13,14] Hence, the treatment of their dental caries is of utmost importance. High and frequent intake of sugars in the diet is another important source.[15] Some other factors are reduced salivary flow (including intake of medications those might result in xerostomia); premature birth leading to enamel defects; the presence of deep pits and fissures in the teeth, etc.

Oral hygiene measures in children:

Diet counseling:

- This is included in Anticipatory Guidance practiced by the dental professionals.
- Exclusive breast-feeding for 6 months of age and subsequent introduction of appropriate food.[16] Weaning of children from bottle and breast-feeding by the age of 1 year.
- Limitation of intake of sugary foods and beverages to only during meals and avoiding having them as 'between-the-meal-snacks'.
- Avoiding aerated and sugary juices and making the children to take pure juices.
- Making children drink only water, especially, fluoridated tap water between the meals.
- Maintaining a balanced diet.

Oral hygiene practices:[6,9]

- Discontinuing the habit of the bottle or breast-feeding during night.
- Tooth brushing twice a day with a proper amount (as appropriate for the age of the child) fluoridated toothpaste.[17] The use of fluoridated toothpastes is an easy means of delivering fluoride to the teeth. Other means of fluoride delivery is fluoridated drinking Water (can be obtained by community-based water fluoridation programs)[18,19], application of fluoride varnish (professional means).[20] Tooth brushing should be properly guided or done by the parents only for achieving an optimal result. This should be practiced till the child attains 7 or 8 years of age.[12]
- For children below 2 years, the toothpaste should be smeared on the toothbrush, and for children above 2 years, a pea-sized amount of toothpaste should be dispensed. Care should be taken or the child should be trained so that they don't swallow the toothpaste.

- Replacement of toothbrush every 2 months or on the appearance of the first sign of fraying, whichever is earlier. This increases the efficacy of toothbrushes.[21]
- The children should be made to rinse their mouth after every meal to prevent food lodgement, thus preventing the provision of a nidus for bacterial growth and subsequent development of dental caries.[22]
- Flossing and use of mouthwashes should be incorporated in the daily oral hygiene practice of the child.[12]
- The use of proper dentifrice is an important factor as well. Fluoridated toothpastes are the recommended dentifrices for prevention of development of dental caries.[16]
- Tongue cleaning also should be included as a regular oral hygiene practice. This reduces the count of Streptococcus Mutans and significantly reduces the plaque accumualation.[23,24]
- A proper brushing technique should also be incorporated.

Conclusion

Optimal oral health is not only obtained by maintaining proper oral hygiene in an individual, but it starts right from the prenatal period. The care of the pregnant mother is also a necessary step. Maintenance of the oral hygiene is not only practiced after the eruption of teeth, but also the oral cavity before the eruption of the deciduous teeth is essential as the colonization of the oral cavity with cariogenic bacteria begins as this stage. This would affect the caries status of both primary and permanent teeth.

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Dental Fear and Children: The Major Link

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Abstract

A child's behavior pattern in any situation is governed by his inherited physical and mental endowment and as he develops,by the conditioning he receives through contact from his environment. The former,except within normal limits,cannot be altered. The later can be controlled and developed so that the child will grow to have a well-adjusted personality to suit the society of which he is a part. Here we discuss how fear governs to a large extent the behavior being shown in a dental operatory by the child and the value of fear.

Keywords: *Behaviour,Child, Dental Fear, Management, Pediatric Dentist.*

Introduction

One of the physical needs of children,which is deemed necessary for the overall health of the child is dental attention whether the child accept dental treatment gracefully or refuse it entirely will depend on how they have been conditioned.[1] The emotional conditioning of children toward dentistry,as toward other experiences which make up their childhood,is primarily formed at home and under parental guidance.[2]

If the dentist is to perform satisfactory dental work on a child patient,he has to get his/her full co-operation. He can get their full co-operation only when he knows the emotional make-up of the children and their parents. Without this knowledge it will be difficult to treat the children. The pediatric dentist must acknowledge that he is emotionally involved with his patients and to handle them successfully,he must be aware of those psychological and sociological factors that have formed their attitudes and behaviors toward dentistry. This study of science which helps understand the development of fears,anxiety and anger as it applies to the child in the dental situation can be designated as behavioral pedodontics.[3] The proper handling of children in the dental office is the sole responsibility of the dentist only,and it is one that he should assume without hesitation if he is to fulfill his obligation to his patients and his profession.[4] It is equally clear that the conditioning of children to the dentist and dental services is entirely the parent's responsibility. They should accept this obligation as a parental duty. If the children enter the dental office for the first time with undue fear and an unwillingness to cooperate,one can assume that the parental conditioning has been faulty. Although it is the parents' responsibility to inculcate the proper attitudes in children of dentistry,the dentists can help by making sure the parents are fully informed of and educated in the necessary fundamentals of child psychology. Before a dentist is in a position to advise parents regarding proper psychological preparation of their children,he must understand the problems involved.[5] He must understand the fear involved and have an awareness of how parental attitudes may modify behavior patterns. He should be familiar with personality formation and how anxieties develop. If the dentist wants to be respected in his professional circle,he should be competent enough to discuss the problems tactfully related to his field. Parents are always eager to provide all the advantages of life to their children even if they didn't get. The dentist should be capable enough to provide advice on child behavior to parents as a physician.[6] Certainly management

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problems are of greater concern to the dentist,who may see children under considerable stress-yet dentists hesitate to advise parents on child behavior. The parents will in turn apply this knowledge to their children,who will look with favor on dentistry rather than fear. This knowledge will help the child as well as the dentist. The anxieties we find in adulthood are formed primarily in childhood. In adults the fears and anxieties are usually latent and well hidden. Marked phobias related to dental treatment do exist among adults and they make up for a large segment of our population.[7]

The Nature of fear: The parents' responsibility in psychologically preparing the child for dental treatment is primarily, the emotional problem of fear. Fear is one of the major managing challenges to the dentist and a major reason for public neglecting oral therapies. Fear is a primitive emotion gained just sometime later than birth,the infant is not knowing the type of the action creating fear. When little one becomes big and his thinking capacity rises he comes to know what produces fear in him. He tries to adjust to the situation with a flight response,if not the fear intensifies. Fear is a primitive response gained for protection from trauma and mutilation.[8] The emotional stimulation is discharged by way of the autonomic nervous system through the hypothalamus and needs very little cortical integration.

The value of fear

Contrary to popular belief,fears are good if gone into proper channels and moment. The good value of this fear can be utilized to bring the kid away from notorious situations of any nature. When the kid is not fearful about being punished or parental disagreement this nature can make him a danger to societal norms in the long run.[9] Therefore the parents should not try to eradicate fear but to channel this scare towards the ill effects that are present and beyond things where no ill is there. This is a protective mechanism against serious threats. Kids should be made aware that dental place is not to be feared. Dentistry must not be utilized as a threat by guardians. Taking the kid to a dental surgeon must not imply punishment to him. Utilisation of fear as punishment will undoubtedly raise doubts of the dental surgeon. On the other hand, attachment of the child to the dental surgeon will lead to motivating the child in accepting office rules as he will dreading that he might lose his confidence of dental surgeon.[10]

Types of Fear:

A. Objective Fears: Objective fears are those that arise due to objects which stimulate the sense organ,not of family origins. These are responses to stimuli that are dependent on seeing,hearing,smelling and tasting which can be bad to taste. Kid having a bad experience with the previous dentist develops a fear of further oral therapy. It is a problem to get back a kid so disturbed to go back to the dental office of his free will. Even if he returns the dentist should be aware of the situation and should try to gain his confidence.[11] Objective fears are associative. A child who has been treated improperly by a pediatrician who wears a white uniform will create the same type of fear in the child when he visits a dentist. Fear reduces the boundary of hurt.

B. Subjective Fears: Subjective fears are that which are told to the kid by the people around him which he has never felt on himself. A little kid is prone to suggestion. A young child,hearing about a painful experience in the dental office by an adult starts fearing the dental treatment as a whole. The mental picture of the painful procedure remains in his mind and with varied imaginative skills of the child,it gets magnified and formidable. Kids have much fear of that thing which is not known. Anything which is novel and not known to the kid is going to instill fear till the kid gets evidence of no issue to his good being. The parents have to make the child aware of the situation and tell them before visiting the dental office,what are the things and procedures the child can encounter in the dental office. Suggested ones can be got by imitating others. A kid watching other kids who are fearful in operatory will get fearful for the same thing or place as realistic and with genuinity.[12] Immitative fear maybe got subtly and maybe showed my family and got by the kid with both not knowing about it. Generally,the long time the subjected fear remains in the brain,the more they get increased. As a result they will be more intense than objective fear. Suggestive fear can become so magnified that it might reach irrationality. The suggestions known most by kids have been made into studies and got no correlation between them and the bad experiences encountered in their natural livings. The worst fears were imaginative things and events. As the imaginary capability of kid

develops,imaginative fear also increases to a certain stage when reasons show them to be ill-gotten. The intensity of fear varies from child to child. The child who is deprived of sleep develop more fear than the ones who sleep adequately.[13]

Role of pediatric dentist: The pattern of fear in a child is quite unpredictable. It is to remember that,regardless of conditioning,normal children with the same experience will visualize a variety in their development of and reaction to fearful things. Each child is an individual and responds individually. The physically healthy child with normal endocrine function will respond more intelligently and swiftly than the mentally retarded individual. The dentists should teach the parents that growth and development are a great delineator of fearful things and it can modify the use of conditioner. The child's first fearful thoughts related to dental treatment are of not knowing and not expecting things. An intensified or fast stimulant for the sense organs is hurt increasing to the kid as it is non expected. The sound and vibrate of rotor and pressed exerting in the use of handheld instrumentation in carie cavities making are conductive of hurt in a very small kid. Unless the hurt is too much to take, he generally is fearful of the sound of dental motors more than he does any added hurt. Since the small kid also is fearful of fall down falling or fast and non-expected jerks, swiftly being reduced or being made back in children without any prior telling may instill fear.[14] Bright lights,especially intense operating light,if allowed to shine in a child's eyes are fear-producing. Before school kids may have fear of separation from parents. If the kid is sent into a novel scenario when he might be removed from parents, with whom he has dependency behavior and safety,he has a feeling of being abandoned. It might be preferable to have parents accompany the child to the operatory if the child is very young. The dentists should also avoid treating children during nap times as the child will not tolerate the situation well. The children should be given a little control while getting a treatment done as to, raise their hand if the treatment gets painful. Older children are more concerned about their looks and so they will cooperate with the dentist on their own will.[15]

Conclusion

Dentists along with the parents have a major role in making the dental treatment less fearful for the child. In subjecting the children to dentistry one must always weigh the possibility of psychological trauma against the necessity of treatment. Since dental treatment is needed by almost every child,it becomes important that psychological trauma to be minimal of all the problems associated with the treatment of children, the management of children becomes paramount,because without proper patient co-operation the dental procedures become almost impossible.

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Hormonal Changes Across the Life Cycle of Women and its Effects on the Periodontium

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Abstract

In recent years, it has been realized that women's oral health may differ from that of men in many areas. It is rightly said that the health of a woman's body and oral cavity are bidirectional, hence women need exceptional dental health facilities and attention. The role that different endocrine hormones imbalances during puberty, 1st to 3rd trimester of pregnancy, menopause, oral contraceptive use, and females reproductive cycle details has the main role in various sex data in orofacial health status is supported by different medical clinical trials. The oral conditions commonly observed during these phases of the lifecycle of females include inflamed gingiva, the formation of granulomatous tissue in the body, disease of periodontium,, and grade 1,2,3 mobility of tooth. The closest bonding between status and worst dental health condition in the female will need excellent care in investigation plan that assimilates and controls for the diverse array of lurking varying, such as dental diseases (gingivitis, periodontitis and missing tooth), the whole history of female's reproductive physiology, and benefaction communal and financial variables. Dental practitioners need to actively counsel female patients about general and oral health strategies so that they can maintain their oral health and maximize their quality of life. The effect of hormonal changes during the life cycle of a female affects the prevalence and intensity of the oral disease. This understanding is helpful for a clinician in rendering oral health care to these patients.

Keywords: *Gingivitis, Oral Health, Periodontal disease, Tooth loss.*

Introduction

Excellent oral health is mandatory for excellent general systemic health there is a close interlink among dental and systemic health according to surgeon general data.[1] WHO on its Oral Health data 2003 outlines the global burden of oral disease with particular emphasis on the important matter: oral health is vital and necessities to systemic health, dental health is essential for maintaining an excellent quality of life and oral and systemic health are two sides of a coin both are dependent on each other.[2] All these points emphasize the importance of oral health in connection with the general health as well. Good health status changes according to different phages of life of women widely in the entire population and to their economic status. Dental health condition declined to some state in between the 1st trimester to 3rd trimester of pregnancy and it is a matter of concern which has a bad impact on the lifestyle of human beings. [3] Females should need special oral care and attention due to hormonal imbalance and it has excellent effects on the oral environment.[4]

Nowadays, female's systemic health oral health has become a matter of a global focal point. Thus a

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long term follow up research is mandatory in females dental health. The aspects of research study on the dental health of females are through clinical examination of oral physiology, long experiences on clinical practice, and dental health attitude knowledge behavior among various age groups between the circumstances of society transitions. It is rightly said that the health of a woman's body and oral cavity are bidirectional, hence they have special oral health needs and considerations. [4] Periodontal disease occurs due to a complex interplay between susceptibility of host and microflora of the oral cavity. Different internal variables of the host has a direct or indirect impact on different stages of various oral infectious disease.[5] Among these variables, sex hormones likely influence the pathophysiology of gingival inflammation, various periodontists like (ANUG), as hormonal changes have a great effect on the pathophysiological mechanism of human-parasite interlink inside the oral environment.[6,7,8,9] secretion of the endocrine gland is unique controlling particles that regulate the reproductive system, growth factors, and proper maintenance of internal systems of the body as well as generation of energy and its consumption and remaining for storage in proper form.[10]

We know it better than various changes that occur during 1st trimester to the 3rd trimester of pregnancy for which certain changes also occur inside an oral environment like gingival inflammation, periodontitis, pigmentation on the oral mucosa, etc most of these things are revers to normal form after some days. [11,12] The true causative factor behind these changes are not well mentioned anywhere. These endocrine secretion changes can cause certain complications during pregnancy.[13] The changes most commonly reported are pregnancy gingivitis and pregnancy epulis.[14] Also, an association has been reported between Poor oral health and premature births and low birth weight.[15,16,17,18]

When women reach their Menopause that leads to permanent stoppage of regular menstruation cycle which is next immediately stoping the ovulation mechanism. Non-inflammatory destructive periodontal disease (NIDPD) appears in old aged females through their menopause cycle or there is a tight bonding between menopause and on inflammatory destructive periodontal disease (NIDPD).[19] The marginal, interdental, attached gingival status became worst during menopause in the absence of secretion of hormone-like gonadocorticoids. The present review addresses the effect of puberty, pregnancy, menopause, post-menopause, and oral contraceptives and the changes in the hormonal level during this period of the life cycle of women affecting the periodontium.

Pathophysiology of Sex Hormones: Estrogen has a great impact directly on various cellular mechanisms, amplification, and development of body tissue.[19] Oestrogen is the main causative factor in the process of various physiologic interchanges in females in different aspects of their life span: starting the age of puberty, during every periodic cycle, 1st to 3rd trimester of their each pregnancy, after the starting of the menopausal cycle in old age.[19] Oestrogen and progesterone are the main sex hormones that can affect the periodontium by significantly influencing the different organ systems.[10,20]

Various important cells in the mouth deeply affected by sex hormone-like estrogen, progesterone etc.[19] through various cellular activities like the development of specialized cells of squamous epitheliallium the estrogen act. [20] various important receptors of sex hormone present in osteoblast has a great impact on compact bone, spongy bone in their development process. Some additional receptors of sex hormones which are mainly attached to different periodontal ligaments has some significant mechanical action on targeted periodontal cells.[21]

The sex hormone mainly estrogen hinder the progress of IL-1, IL12, TNF-alfa, and GM-CSF which has great hand behind osteoclastic activity leading to compact and spongy bone loss. When there is less synthesis of sex hormone its plays a key role in severe inflammation of attached gingiva, marginal gingiva in generalized periodontitis case. On the other hand it has some contribution to skeletal bone loss mechanism.[19]

Various sex hormones have a key role in different microvascular changes like inflammation of the endothelial layer of oral tissue, interruption in various mast cell formation, thrombi formation, the process of permeability of vessels became faster.[19]

Role of Different Phases of Lifecycle on Oral Health:

Puberty: Puberty is regarded as the very first sign of maturity in turn towards the adult age group. Puberty is always linked with more production of estrogen and testosterone in both gender men and women. A lot of prevalence and follow up survey data tells that there is an interlink between the development of gingivitis in the

absence of dental during the starting period of puberty. severity of gingivitis has definite bonding with serum sex which fluctuate mean plaque score.[22] Severe generalized inflammation of interdental gingiva, marginal gingiva, attached gingiva of maxilla and mandible sometimes associated with bleeding from the gingiva. Inflammation of gingiva (Generalised) is seen all around the labial surface and palatal surface of interdental, marginal, and attached gingiva in some case interdental gingiva is absent due to severe inflammation.[23] A significant increase in gingivitis in children has been reported during the starting phage of the menstruation cycle. There is severe gingival inflammation reported among females at the age of 12 years, 10 months, and among males at 13 years, 7 months. This has been seen to coincide with the onset of puberty which could strongly play a major role in changes in oral-microflora in the presence of gram –ve anaerobic bacteria P. Intermedia, which later helps for the production of BGF in proper suitable oral environment. The mass of dental bio-film increases rapidly with various types of spirochetes like Aa. Gingival bleeding also present.[23]

Menstruation: During the staring phage of the pubertal cycle there is a sudden increase in the release of sex hormone (estrogen, progesterone) in a regular manner.[24] The female periodic cycle goes through 4 phages- 1st- menstrual, 2nd follicular, 3rd ovulation and 4th one is luteal phages. During the ovulation period the amount of secretion of sex hormone rapidly increased than its normal secretion.[23] According to Lindhe et al at the time of ovulation the amount of secretion of GCF and exudate fluid of gingiva is more than normal days. [25] Holm et al concluded from their study that there is a presence of mild to moderate gingivitis who are running through their periodic cycle.[26] Data of Some studies say that most of the females developed minor or sometimes major aphthous ulcers during their periodic cycle. Some other surveys give data that minor aphthous ulcer develops in females only in the last phage of their periodic cycle that is the luteal phase where ovulation starts again. The study of Ferguson state that repeated aphthous ulcer develops in the oral cavity of the female during the periodic cycle but this data is still in controversy. [10]

Pregnancy: In between 1st to 3rd trimester of pregnancy there are many changes in the amount of secretion of endocrine fluids. Corpus luteum took the amount of sex hormone very high due to constant secretion.[23] There is a marked increase in the level of estrogen and progesterone to the level hundred and six nanograms per milliliter during the last days of pregnancy.[10] Changes in endocrinal secretion (release of sex hormone) during 1st trimester to 3rd trimester associated with marked increase in mild to severe gingival inflammation in some cases localize periodontitis seen.[10]

Pregnancy gingivitis is a common condition reported among pregnant women affecting between thirty to a hundred percent between all pregnant female cases. Pinard was the first person to disclose the fact that increase gingival bleeding, edema like symptoms are seen during 1st to 3rd trimester. The information of all types of gingiva (attached marginal and interdental) some times associated with moderate referred pain with mild gingival bleeding. Some clinical periodontal signs found like increase BOP, amount of GCF increase, severe gingivitis found, increase gingival pocket depth beyond 4mm. Most frequently lower and upper anterior region of tooth segments are affected and the interproximal gingival unit also. [27] A high amount of estrogen and progesterone found in oral fluid during 1st trimester to 3rd trimesters of pregnancy.[28] Chances to develop oral infection like mild periodontitis, mild to severe gingival inflammation is more during 1st trimester due to low immunity, associated with low production of suppressor T cell, early maternity stress, disruption in realizing of various body hormones.[23] A small majority of the pregnant women (0.5-9.6%) of which develop inflammation of gingiva which is associated with P Granulomatous tissue. This growth some times called benign tumor of pregnancy or pregnancy linked granuloma in reality these things are not true neoplasm. It cant be distinguished histologically nor clinically. This type of growth is painless, developed from marginal gingiva some times developed from interproximal area maxillary anterior tooth segments growth base is pedunculate type. [23]

Oral Contraceptives: Birth control method are important factors with association with human chorionic gonadotropin trigger every trimester of pregnancy to stop the release of eggs from the ovary.[22] Oral contraceptives tablets tries its best to make hormone level balance during the trimester.[23] Currently, oral contraceptives pills come with the doses point zero five milligram estrogen per day and one point five milligram per day dose for progestins.[22]

The clinical studies have reported that the use of

hormonal contraceptives in women influences periodontal disease progression in the form of a higher prevalence of gingival inflammation, loss of attachment, and gingival enlargement.[29] marginal attached Gingival have an great response to localize triggering factors. Mild to severe gingival enlargement associated with mild to moderate gingival bleeding sometimes with hyperplasia. Melanin pigments of skin occasionally reported. Establishment of close bonds found in between the use of contraceptive tablets and the development of excess melanin pigment on the skin.[22] A statistically significant increase in the prevalence of severe periodontitis was reported among Oral Contraceptive (OC) users, particularly smokers by Brusca et al.[30] Loss of attachment ≥2 and ≥5 mm were reported among OC users in comparison to the non-users. Maximum numbers of culture media were found +ve to Candida albicans. The link with sub-gingival *C. albicans* to *Porphyromonas gingivalis* more than eighty-five percent in patients who regularly consume Oral contraceptives.[30]

Menopause: When female reaches above 50 years menopause start which is also a natural process starting of menopause leads to permanent stoppage of females monthly period. Permanent stoppage of periods for a long period. A permanent interruptions around twelve months considered as menopause. [31] When menopause starts in females after fifty years of age it has a great effect on their intra-oral structures. Studies give data that various new oral sign symptoms appear intraorally in females due to insufficient release of estrogen from the gland. Deficiency of Vit-E, Vit-K, Vit-B, Vit-D, and calcium also found in some females after menopause start. [32,33] Xerostomia, burning mouth syndrome, alter in sensation of the test (most commonly metallic test) found in some females. Most of the females develop dental caries (mainly class 5 and class 2), mild to severe periodontitis in menopause. The maxillary and mandibular alveolar bone become unfit for endosteal implants in some cases because alveolar bone became very weak.[34]

In association with xerostomia, some patients develop breathing difficulty, difficulty in digitization, mild to severe intraoral and extraoral pain.[35,36] Periodontal problems can also be observed. Many females develop severe generalized gingivitis with mild bleeding.[37,38,39] There is a minute interlink in between osteoporosis of maxillary and mandibular alveolar bone, climacteric, periodontal gingivitis, and mild periodontitis, but there is no data about direct link in this case because periodontitis and osteoporosis are multifactorial.[40] Some changes found in the buccal mucosa, mucosa of tongue etc. Some autoimmune disorder li and mucosal lichen planus also found intraorally.[36]

Post-menopausal women: Females who receive HRT during post menopause to get rid of menopausal symptoms enjoying a good lifestyle.[41,42] Many menopausal signs and Symptoms disappear after taking combine sex hormone therapy (progesterone and estrogen combination). [43] The female who never receive HRT after menopause had developed lots of oral problems according to Allen NE study in the year 2010. Allen et al have published a systematic review on HRT vs dental outcomes with an emphasis on the costs of dental care.[44] According to Paganini-Hill study use of ERT for a longer period reduces the risk for tooth loss.[45]

Discussion

Different medical interventional research study gives new important data about oral health and its interlink with various sexual variation. The role that female sex hormones, pregnancy, and women's reproductive history play in contributing to the sex difference in oral health is supported by clinical research. [5] A causal relationship between parity and poorer oral health in women will require greater care in research design that incorporates and controls for the diverse array of confounding variables, such as related oral lesions (periodontal disease and tooth loss), the details of women's reproductive histories, and contributing social and economic variables.[46] Intersectoral approach between dental and other health departments can collect excellent data share among themselves for giving an excellent treatment plan.[47] Preventive oral health care services should be provided during the onset of puberty, early stages of pregnancy, menopause and post-menopausal women to improve their quality of life.

Conclusion

Dental practitioners need to actively counsel female patients about general and oral health strategies so that they can maintain their oral health and maximize their quality of life. Excellent knowledge about the systemic disease and its link with different oral lessons at different phases of a women's life cycle will help a lot in the diagnosis and treatment of disease for doctors like gynecologists, endocrinologists, specialized dental professional and at last fulfill the needs of the suffering patients.

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Use of Robots in Dentistry: A Fact or a Fiction

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Abstract

Robotics is the most enthralling example of human invention which has the potential to replace the human factor altogether. Slowly but surely this invention is also making its way in dentistry too. The robotics coupled with artificial intelligence is being modified and customized to be used in the Dentistry. The advantage of robotic technology includes precision work delivered in a fatigue-less manner. This paper revisits the present use of robotic technology in dentistry and its future applications.

Keywords: *Robotics, Artificial Intelligence.*

Introduction

The traditional dentistry is increasingly becoming the object of past reconciliations as more and more clinicians are opting for new technologies to further their practice. The technologies which were previously just ignored based on the investment involved or the complicated training associated are now being increasingly getting incorporated in clinician's daily clinical regimens. Let it be intraoral cameras to the use of radiovisiography or use of lasers to microscope driven endodontics and digital impression, the technology-driven dentistry has arrived in our daily practice. But there is one more attribute to this modern era of practice that is robotics which is yet to make a grand entrance into this field but is already making its presence felt.

The world of robotics has lots of features that can be adapted & utilized in dentistry. Some of the Robotic technology which is currently being used is like placements of implants by a distant placed skilled clinician, who controls the robotic arm via the computer to place the implants. Robotic arm on the other hand eliminates human error by eliminating any hand tremors or fatigue which are usually associated with the human factor during surgeries. Even the surgical guides for the implant placement and the restorations which are milled post placement of implants are also done using an automated robotic unit only.

Dental use of robotics presently used:

Training robots for Pre-clinical dentistry: Earlier during the pre-clinical prosthodontics or preclinical conservative, the students used to practice their skills on the phantom head, which is nothing but an adjustable mechanical replica of cephalic region with a simple arrangement of teeth allowing for practicing crown and cavity preparations[1]. The phantom head was in no way able to improve the clinical skills of the student concerned as students could manipulate it in a manner convenient to them but not according to the mandatory clinical assertions.

But in Japan, they are already using a robot named Showa Hanako which has artificial intelligence to simulate different types of patient attitudes, gestures, and responses to make the student experience what it feels like to work on a real patient[1]. There is also a group of robots corresponding to the family of Geminoids which are equipped with advanced motion-sensor technology which allows the machine to mimic facial expression and precisely imitate head motions[1]. The robot is first in the series of personalities outside japan and is intended to push forward the android science and philosophy, in searching for answers to fundamental questions.

Endo microrobots: Endodontic mishaps can occur by even the best of the endodontists like file breakage, ledge formation, apical foramen perforation, and

excessive instrumentation[2]. To reduce the potential for human error and improve the quality of endodontic treatment, it is necessary to develop advanced endodontic technology innovation by applying advanced engineering and computer-aided technology [3,4]

This computer-controlled machine will be mounted on several teeth within the patient's mouth. With on-line monitoring and intelligent control, the micromachine or robot will perform the automated drilling, cleaning, and filling of the root canal. All other sub-project results will be incorporated into this robotic operation. Specific objectives for microrobot design include:

1. Reducing the reliance on the skills of the dentist,
2. Minimizing human error
3. Offering a method for precise diagnosis and treatment[5]

Dental Nanobots: Nanobots or nanorobots as they have come to be known are miniature robots of the size of nanometers that is of the scale 1 nanometer is equivalent to 1 millionth of 1 mm constructed with the help of nanoscale or molecular components[6]. The application of the nano-robot technology can be used in local anesthesia, re-naturalization of the original attrited or damaged dentition, orthodontic alignment in a single visit, curing hypersensitivity and maintenance ofthe oral hygiene using mechanical dentifrobots. Using computer technology for guidance these nanorobots can also be directed to kill bacteria in the cavity or repair the tooth damage hence occurred as a result of bacterial activity[7].

Surgical Robots: Robots can be used extensively in maxillofacial surgery in collaboration with a maxillofacial surgeon to correct the deformity or injuries to the face. A surgical robot programmed by the surgeon through interactive programs, does the pre-programmed task of repairing the human face[8]. Robotics have long been used in the milling of the bone surfaces, drilling of holes, deep saw osteotomy cuts, selection of osteosynthesis plates, and orthognathic surgery planning[9].

Sensor -Equipped Implant Set up: Dental implant is used to replace the root form of the missing tooth by placing a titanium implant body in the bone & allowing it to Osseo-integrate with the bone. Considering the mistakes done by a human clinician in the placement of these implant bodies into the bone, which also affects the overall prosthetic outcome a computer-assisted surgery has been developed for oral Implantology which includes pre and intraoperative procedures. The preoperative surgery is to use three-dimensional (3D) views as provided to enhance raw images obtained from the patient before the operation. The main aim of this technique is to render a target region and a pathway associated with relative organs from computed tomography (CT) data. It provides 3D orientation of surgical instrument position and trajectory displayed on a monitor in real-time within the patient's 3D imaging data[8]. Yomi a robotically assisted implant system that is cleared by FDA utilizes patients C.T scans to plan the implant procedures for the patients[10]. Recently a robot dentist installed two implants on a female patient using the same technology with an error margin of 0.2-0.3mm[11].

Conclusions

Although robotics coupled with programmable software technology provides humanity with precision and accuracy and is being implemented in many areas, it comes with its limitations per se. Apart from the extensive research and development still required in this area along with elaborate equipment requirements and ethics involved, the economic factors make it difficult for the developing countries to replace humans with machines. Although with changing times this may change soon and robotic dentistry may become a fact from fiction.

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Hybrid Implant-supported Prosthesis in the Lower Arch: A Case Report

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Abstract

The protocols for the implant dentistry has improved at a very extreme pace. To conduct implant dentistry in a manner that is beneficial for the society requires an impeccable diagnosis, a treatment plan that takes into consideration surgical and prosthetic parts too. For any implant driven prosthesis to be successful requires that clinicians adhere to the basic principles ignoring which can lead to a prosthodontic nightmare. Following is a case report describing the methodology followed for hybrid lower implant-supported prosthesis.

Keywords: *Hybrid lower implant-supported prosthesis, Diagnosis, Treatment planning, surgical guide.*

Introduction

Modern dentistry requires that all the treatment goals like normal contour, function, esthetics, speech and health of the patient is achieved irrespective of atrophic changes, any underlying systemic diseases or trauma to the stomatognathic system.[1] Although, if fewer teeth are presently coupled with the fact that the ridges are highly resorbed then achieving these goals with basic Implantology is not possible. Implantology in its basic element means placing an alloplastic material in the jaws that provide anchorage and retention for the prosthetic part.[2] The experiences gained and the information acquired thus far has helped establish endosteal implants as a commonly used dental protocol. As a virtue of advancement in research, designing implants, dental materials, and techniques have aided in better predictability and outcomes as a result of which various types of implants and clinical techniques are available for rehabilitation of different clinical problems.[3] Implantology provides acceptable treatment outcomes that better the conventional and traditional restoration protocol.

Replacement of hard and soft tissue structures includes a lot of challenges and thereby requiring proper case selection, diagnosis, and execution of the treatment plan. Following is a case report describing the treatment protocols followed for one such case.

Case Report: The Patient, a 78 years old-man, wearer of a removable partial denture in the maxillary and mandibular arch, presented to the Department of Prosthodontics and crown and bridge, at Institute of Dental Sciences, to undergo rehabilitation of the loose lower removable partial denture with a fixed implant dental prosthesis.



Figure 1. O.P.G of the patient at the time of reporting to the department

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Patients case history revealed that he is wearing the upper and lower removable prosthesis for 7 years and has been facing problems with lower denture since the last 2 years like pain and mobility of the lower teeth along with loosening of the denture and inability to eat. The patient after clinical examination was advised with an Orthopantomograph which was duly carried out in the institution itself. Examination of the Orthopantomograph along with clinical correlation suggested a root stump in 38, vertical defect in 34 and 46 along with grade 2 mobility of 42 and generalized loss of lower ridge height. Upper dentition also suffered from supraeruption and cervical attrition in 15. The patient was given the treatment option of upper plane correction as well as replacement of the upper prosthesis but he insisted on the treatment of the lower arch only and to maintain the status quo in the upper arch as he was not facing any difficulties in the upper prosthesis and was satisfied with it.

It was then decided to extract 38(root stumps), 34, 42 and 46 while retaining 47 and 48 to provide stability to the prosthesis. An implant-supported RP-5 Prosthesis was planned for the patient which will be supported by both implants and soft tissues and will provide lower lip support as patients age ruled out any extensive ridge augmentation procedure given the high chances of morbidity.



Figure 2. Patients pic post-extraction and healing of 38, 34, 42, and 46.

Extraction of 38, 34, 42, and 46 was done along with curettage and irrigation. Interrupted suture with 3-0 mersilk was given to achieve uneventful healing. Complete oral prophylaxis along with pocket therapy of remaining teeth was also done during the post-extraction healing phase.

After healing based on the patient's C.B.C.T (Cone-beam computerized tomography) it was decided to put four I.C.E (Implant classic esthetics) internal hex designed implants of Alpha biotech (Israel) of the dimension of 3.7x 11.5mmin the region of 33, 34, 43 and 44. A Bone stabilized surgical guide was used for determining the exact position and spacing between the implants during implant placement surgery. Good primary stability of 45N was achieved during torquing of implants and alloplastic augmentation of the ridge was not required. Multi-unit temporary Healing caps were given along with interrupted mattress sutures that were given to contour the soft tissues around the healing caps to allow for uneventful healing.

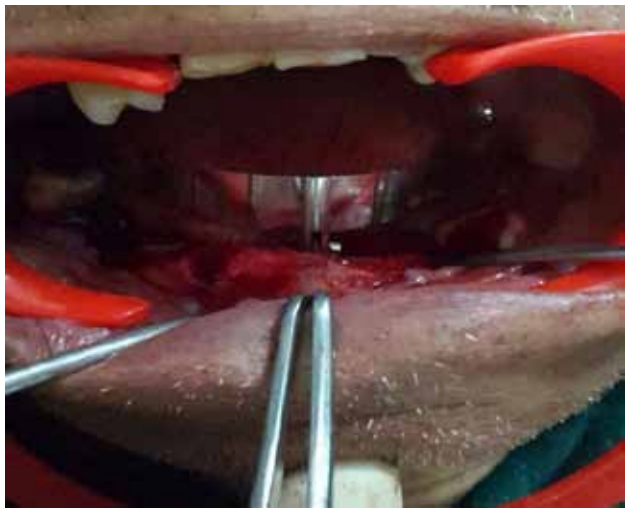


Figure 3. Bone stabilized surgical guide to ensure correct implant spacing and position.

Prosthetic phase: Post healing a preliminary impression of the upper and lower arch was taken with Chromatic alginate (Tropicalgin, Zhermack) and a special tray was fabricated for the lower arch. Borders were then molded using a green stick compound (DPI) and the second impression was made using medium body polyvinyl siloxane (Aquasil ultra monophase, Dentsply). A removable partial denture for the lower arch was prepared using the normal protocol and to match the upper occlusal plane. The coping cylinder was screwed onto implants and corresponding screw channels were made in the prepared removal partial denture to allow for the cylinders to pass through without any interference. These cylinders were then milled to the appropriate size to get incorporated within the removable partial denture. A 0.006 inch (adult size 6x6 inch, Nictone company) rubber dam was used to block out any undercuts and the screw channel access to prevent any self-cure resin to flow into it. R.P.D Prosthesis was placed over the coping cylinders and the pink color self-cure resin was used to fill up any gaps between the coping cylinders and the

prosthesis. Patient was asked to close his mouth in centric occlusion with articulating paper in place to identify any occlusal discrepancies if present. The prosthesis is then unscrewed with cylinder copings now attached to the intaglio surface of the prosthesis. The prosthesis was then screwed back into the patient's mouth after occlusal adjustments and any other discrepancies were corrected in the patient's mouth. Denture flanges were also reduced to prevent any soft tissue impingements. Composite resin (Te-Econom, Ivoclar Vivadent) was then used to block out the screw channels and maintenance instructions were given to the patient.

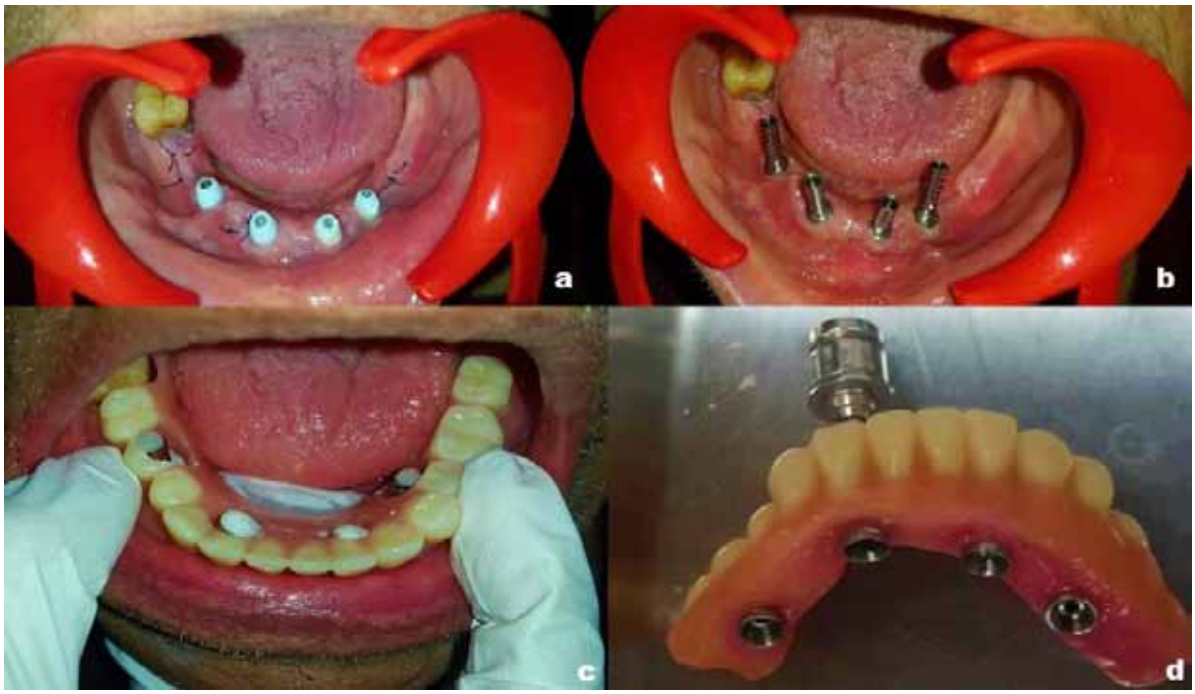


Figure 4a, 4b, 4c, 4d: Temporary healing abutment with continuous mattress sutures given.



Figure 5.

Periodic Recall: The patient was asked to come for a follow up at a frequency of 1,2,6 and 12 months after delivery of the prosthesis and Annual radiographic examination were also carried out. The following benchmarks for success were kept like lack of implant mobility, no tenderness or any symptomatic observations at each visit, Absence of peri-implantitis,and absentia of periapical lesion around the implants. Also, prosthodontic problems and outcomes were assessed. Very less bone deterioration was observed with radiological tools, but no implants failed after prosthetic rehabilitation after 1 year follow up. Occlusal access filling dislodged but was rectified and no other complication was noted during 1 year follow up. The patient also realized that his new denture looked good, his phonetics was vastly better and it was relatively easy to cleanthe prosthesis and his capability to chew had greatly increased. The progressive mentality of the patient and his happiness with the prosthesis that answered his main problems and requirement added to a fair prognosis.

Discussion

Dental Implantology requires exact and accurate planning to come over any clinical problems and to

foresee the final result before initiation of the treatment.[4] Planning for esthetic cases requires different diagnostic perspectives and includes additional factors like smile patterns and lip size etc.[5] Besides, the restorative space for the prostheses, which is measured from the platform of the implant to the opposing dentition, is often overlooked when the implant position is planned. The intra-arch distance which implants components, metal, the acrylic resin, and the denture teeth are placed plays a major role in selecting appropriate restoration. With mandibular implant-supported fixed prostheses, a minimum of 12–15 mm of space has been suggested.[3] When sufficient intra-arch space is observed, a hybrid prosthesis is recommended.[7] Reconstruction of the atrophic jaws for implant placements is usually staged with implant placement after graft healing.[7] Moreover, areas to be treated with dental implants are mostly affected with either acute or chronic infections.[8] Overall length of the cantilever is paramount for that itis to be assessed when constructing an implant-supported hybrid prosthesis.[9]The researchers suggested that the mandibular span should be between 15 and 20 mm to reduce the chances of failure of the framework.[9] Other researchers suggesta cantilever span of 1.5 or 2 times of the anterior, posterior curve of the implants.[3] Also to be considered is the opposing dentition and the number and distribution of implants, before the determination of cantilever span.[4] One more essential objective during the construction of implant-supported hybrid prosthesis is achievinga passive fit of the framework. If there is no passive fit then biological and mechanical problems likeperi-implantitis bone, screws loosening or fracture of the abutment or the implant were noticed.[3]No implant failure, peri-implantitis, soft-tissue complication related to prosthesis design, fracture of prosthesis frameworks, screw fractures or screw loosening, or difficulty in sustain ability were observed in the patient during the periodic recall time. Although, the occlusal access filling material dislodged it is considered to be the most common problem plaguing the mandibular hybrid prosthesis.[10] On an added note, it was also observed that, though the restoration survival rates remained high, the denture teeth underwent significant wear & tear.[10] Also, the clinicians found that tooth fracture anteriorly was more commonly observed than the posteriors.[10] In the present study, the loss of filling material from the prosthetic screw access occurred only once. These results are incomplete contra indications to the increased incidence of tooth fracture presented inthe previous report.[10]

Conclusion

The present case has shown that a very respectable esthetics and satisfactory functionality for a patient can be achieved with an implant-supported hybrid prostheses. The only drawback of this case study is the follow-up timeline of only 1 year which might undermine the proper assessment of these results. Although, it is safe to conclude that hybridrestoration can give optimum outcomes wherein implants were placed irrespective of careful treatment planning. It is also paramount to assess the patient not only with a surgical aspect, but also from a prosthodontic point of view.

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Commercially Available Toothpaste in India

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Abstract

Majority of us spend very little time or else may be no time while choosing the right as well as the best toothpaste for our mouth because good oral health should be the prime importance. Everyone wishes to have an impressive personality, and for that attractive appearance, a beautiful smile is always required. Without proper oral care an attractive smile cannot be achieved. Toothpaste is not only a bathroom requirement; it is the most important material for maintaining good oral hygiene. All toothpaste that are available in the market commercially today is formulated and designed to clean our teeth and help in maintaining good oral hygiene, all the products do notachieve that need equally. Some of the commercial toothpaste products have the potential to damage our teeth, while others simply don't provide enough protection to our teeth as per requirement. This article highlights the toothpaste available in the Indian market so that one should be able to select the right balance of toothpaste according to his/her oral health needs.

Keywords: *Toothpaste selection, Good oral health, Indian market.*

Introduction

Dentifrice is the French word for toothpaste. Dentifrice is a type of paste, gel, or powder applied on a toothbrush as an addition to dirt-free and to preserve the artistic appearance and good physical condition of teeth. A dentifrice is a substance kept on the top of the bristles of a toothbrush to clean the visible surfaces of the teeth. Toothpaste composition are generally publicized on packs w/w' - that is weight for weight, or grams per 100 grams.[1]

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Varieties of Toothpaste: Many varieties of toothpaste are now available in the market. But knowing the effectiveness of toothpastes is important by seeing their advertising or new formulations are "clinically proven" and approved by any experienced dental health personnel.[2]

a. Fluoridated Toothpaste: This type of toothpaste has taken 95% of the market sales. Because of this fluoride ingredient there is a decrease in the occurrence rate of oral cavities in industrialized countries for over the last 30 years. Sodium fluoride and sodium monophosphate are the most commonly used fluoride compounds having more than 1500 ppm F available in the market but can be obtained only with a prescription. The amount of fluoride content is written in the toothpaste tube.

Fluoride toothpaste for children: Fluoride toothpaste which contains 1000-1500 ppm F might cause enamel fluorosis for all the permanent incisors of

children below the age of 2 years as there are chances of swallowing toothpaste while brushing. Fluorosis of enamel is a form of condition varying from minor white spots to yellow/brown discoloration of the enamel because of too much intake of fluoride. Because of this fear over enamel fluorosis, a few production companies now promote low fluoride "children's" or "pediatric" toothpastes which contains less than 600 ppm fluoride.[1]

b. **Plaque and Gingivitis Prevention Toothpaste:** Marketed as antiplaque and gum protection toothpaste. They contain sodium lauryl sulfate, triclosan, zinc, and stannous ions. They have antibacterial properties as well as they put a stop to the formation of plaque.

c. **Anti Calculus Toothpaste:** Commercially available as anti-tartar toothpaste. They contain tetrapotassium plustetrasodiumpyrophosphates, sodium hexametaphosphate, and zinc. Toothpaste that contains anti-calculus agents helps in reducing the formation of calculus and also it will not reduce the levels of pre-existing calculus.

d. **Desensitizing Toothpaste:** Marketed as anti-sensitivity toothpastes. They contain Potassium nitrate, potassium citrate, and Strontium chloride. Desensitizing dentifrices can work:

   1. Bymisleading the transmission of neural signals, pain can be prevented
   2. By blocking the dentinal tubules.

e. **Fresh Breathe Toothpaste:** They contain peppermint, spearmint, or menthol. They contain enhanced flavoring agents along with antibacterial to fight halitosis.

f. **Tooth Whitening Tooth Paste (Cosmetic Toothpaste):** Whitening toothpaste is promoted because of its cosmetic benefits. Enamel tends to go down its whiteness with age, therefore these whitening toothpastes continue to take rise in the commercial market. These toothpastes have abrasion value that is higher than in normal toothpaste to manually remove the tobacco, food, smoking, and other extrinsic stains from teeth. Enzymes such as protease and papain create a whitening effect. There should not be any confusion between whitening toothpastes with hydrogen (or carbamide) peroxide whitening systems which can only be approached through a dentist.[3]

g. **Toothpaste for adults:** The majority of toothpastes come together with fluoride and other agents to improve their plaque control, calculus,and gum disease by the enclosure of uncontaminated agents. Most of the toothpaste include Triclosanthat are medically of use for enhancement in gum health. Some toothpaste intentionally target 'tartar' and make use of pyrophosphate to restrain the formation of dental plaque by building-up of tartar. There is an advanced strategy in the manufacturing of 'all in one' toothpaste that contains the maximum amount of agents that can cause a reduction in calculus formation, help in preventing dental caries, and can also improve gum health.

h. **Smokers Toothpaste:** Tar deposits are the causes of stained teeth for the people who smoke. Several toothpastes make use of stronger abrasives to remove the smoker's stains. Vigorous and forceful brushing with coarse pastes can harm the enamel over the long run.[3]

i. **Herbal/Natural Toothpaste:** Herbal toothpastes that are made from natural herbal ingredients like seaweed extract, propolis, essential oil of ginger, and much more. For all the natural toothpaste regarding health claim is still not clinically proven. Therefore, it is required to make sure if these "natural" toothpastes have fluoride or not for the prevention of tooth decay.[4]

Complete Guide to Choosing the Right Toothpaste: A toothpaste displaying the seal on its packaging should be chosen which are approved by Dental Association. We should not gamble with our health by choosing non sealed approved products.[5]

According to the need: Toothpaste should be chosen according to the need of the teeth.

1. From mild pain to severe pain along with sensitivity in the gums and teeth.
2. Kids, adults in a sufficient amount of fluoride indiet, and people with diabetes and other conditions should choose fluoride toothpaste.
3. Before choosing anti-cavity toothpaste, products should be checked.
4. Products having the properties of anti-gingivitis should also be preferred.

Look Out: Unwanted cosmetic products like whitening toothpaste should not be used without the prescription of any experienced dentist.

Picking a Toothbrush: A toothbrush is the right tool on which toothpaste can be applied to the teeth to effectively protect from cavities and gum disease. The incorrect toothbrush can scratch gums, damage enamel or might fail to remove trapped food in between the molars. Toothbrushes with soft-bristled brushes as advised bya dentist should be used. Every 3 months toothbrush needs to be replaced even if it still appears new and fresh as bacteria can grow up over time.[6]

How to Choose the Best Toothpaste?:

- Toothpaste should be selected that cleans your teeth, prevent cavities,and fights plaque.
- Dental issues like bad breath, cavities, sensitivity, yellow teeth, and tartarshould be taken into concern while choosing the best toothpaste.
- Flavoured and compatible taste can too be an important aspectof a toothpaste.

With all these requirements in mind, some might get anxious while choosing toothpaste for self. So here is a listof top varieties of toothpaste that isbased on several parameters which will help todecide on the best toothpaste as per the requirement.[7]

Commercial Brands of Toothpaste in India: The commercial companies of toothpaste in India geared up since the year 1975 when companies like Colgate and Hindustan Unilever's Close-up used to be the leading players in the Indian market. Each year several new industries continue entering the toothpaste commercial Indian market. As year pass by, the dental hygiene industry replace all the locallymade powders and herbs that were previously used to clean teeth.[3] The 10,000-crore market of toothpaste quite exhaustive is flooded with a variety of brands of toothpaste that claims to provide cavity-free teeth and healthy gums.[2]

1. **Colgate:** Colgate is a synonym for toothpaste for many of us as it had ruled the Indian toothpaste commercial market for decades. It becomes the world's No. 1 toothpaste in 1999 and becomes the world's highest-selling brand. Colgate toothpaste is ideal for kids, young and old persons in the market segments as it commits to build teeth stronger, whiter, and free from cavities. Several famous variants of Colgate are Colgate Max, Active Salt, Colgate Total, herbal, Colgate fresh, and Colgate Sensitive for sensitive teeth. Colgateshares the highest number of consumers.[8]
2. **Close-up:** The Hindustan Unilever market the Close-up toothpaste which was the first-ever gel-based toothpaste in India. Close-up is known as a youth-oriented company but is also has kids toothpastes. It has variants like active gel, whitening gel, deep action, zinc-menthol gel etc. It rules in the second position in the toothpaste industries and is famous to deliver fresh breath along with strong teeth and dealing with dental issues like bad breath, cavities, and yellow teeth.[2]
3. **Pepsodent:** Pepsodent is owned all over again by Unilever. Ingredients include manynatural ingredients like zinc, micro granule formula, and inactive triclosan, that claim to give us fresh breath and germ-free teeth as well as strong gums. It has anti-bacterial properties that protect teeth from cavities and avoid the growing of yellowish coating on the teeth. Various variants of our Enamel Safe Whitening Protection, Center Fresh, Cavity Protection,and Germicheck with salt and cloves.[9]
4. **Sensodyne:** People facing the problem of tooth sensitivity can go for using this Sensodyne paste that deals with the dental problem more efficiently and it is an economically affordable toothpaste in the Indian market. It is a trademark of Glaxo Smith Kline and is considered as the most preferred and clinically recommended toothpaste for sensitivity by many dentists in India since 1961. Because of its efficient formulation of bioglass known as Novamin, known for a reduction in sensitivity of teeth while in contact with sweet, cold, acidic, and hot foods, many people felt their sensitivity to get reduced after the first use itself. Sensodyneprovides three variants for dealing with sensitive teeth for the people – fresh gel, repair and protection,and rapid relief.[4,5]
5. **Oral-B:** This is an international brand of India's finest dental care brand. It deals with various dental care commodities but it focus more on toothbrush designing. The toothpaste consists of a complex patented formulation of Stannous Flouride Complex with the rationale of preventing tooth decay and cavities, building stronger teet has well as preventing enamel erosion. Oral-B Pro-Expert toothpaste with Oral-B toothbrush and mouthwash can be a great combination together that can help in fighting all our dental worries. Other varieties of this toothpaste brand also act well such as Pro-Expert Deep Clean, Extra White as well as Gum and Enamel repair, etc.[9]

6. **Meswak:** Considered as the best natural toothpaste for sensitive teeth, Dabur is the manufacturing company having a strong grip in Ayurvedicas well as natural commodities. An important element of Meswak toothpaste is meswak (Salvadorapersica) or the toothbrush tree. Various other components of this toothpaste arechlorides, fluoride, silica, flavonoids,and Vitamin C which are known ingredients for providing optimal dental health. Since this is a herbal toothpaste, its taste will be different than other toothpaste and it can also provide relief from gum problems, dental cavities, sensitivity, and plaque formation.[10]

7. **Patanjali Dant Kanti:** Since some years recently Patanjali has transformed Indian Ayurveda to the highest level in the toothpaste industry. The Patanjali product known as Dant Kanti also comes as aloevera toothpaste, advanced oral care, medicated oral gel, and Dant Kanti Junior. It is made using herbal ingredients that help to fight dental problems such as dental cavities, painful, swollen and bleeding gums, yellow teeth, and bad breath. The consumers of this toothpaste reviewed that the paste is having a strong, refreshing taste that reflects the dominance of herbal ingredients.[11]

8. **Vicco Vajradanti Ayurvedic Toothpaste:** Vicco toothpaste is marketed as an "Ayurvedic medicine for gums and teeth," The main ingredient of the toothpaste is turmeric which acts as asuperb anti-inflammatory product. The remaining components of ViccoVajradanti toothpaste can help people to consider it as the best natural toothpaste. This distinctive formula provides excellent benefits for teeth as well as gums making them plaque resistant and strong fighting the bacteria that can cause cavities, as well as bad breath Vicco toothpaste,which is helpful especially for people suffering from swollen gums as well as toothache.[3,10]

9. **Dabur Red Toothpaste:** For millions of Indians, Dabur Red has become the favorite brand for oral health care. Dabur Red, is a combination of natural toothpaste that includes a great Ayurvedicconstituents of cloves, mint, tomar and other herbal ingredients. Being a herbal paste,it fights all our dental worries away by giving us shinier and stronger teeth. Because of this rationale, Dabur Red has created its place as one of the highly demanded products in the toothpaste industry of India. Although this toothpaste formulation also comes in a powder (manjan) form, however using the red toothpaste is always suggested by the dental health professionals.[12]

10. **Himalaya herbals complete dentalcare:** Himalaya complete care toothpaste is known for its key ingredients like neem, pomegranate, meswak and false black pepper, Triphala,etc blendand act as specially formulated toothpaste for adult and oral care. Properties like antioxidant, anti-bacterial and anti-inflammatory helps discard toxic layers in the mouth giving fresh and clean breath as well as strong teeth and gums. People anguish from bleeding gums explicitlyget many advantages from using Himalaya complete dental care toothpaste. It is now famous with Indian folks as it is a natural as well as chemical-free toothpaste and people who prefer herbal toothpaste give guarantee for its action.[13]

Conclusion

An attractive and beautiful smile can never be achieved by improper oral health care. The majority of us spend little time or else may be no time while choosing the right as well as the best toothpaste for our mouth because good oral health should be the prime importance. Toothpaste is not only a bathroom requirement; it is the most important material for maintaining good oral hygiene. The clinical team has a responsibility to keep up-to-date with the ever-increasing development and marketing of dentifrices. Dentifrice recommendations should be patient-specific. Patients having an allergy to certain or any component of a toothpaste then all such kind of toothpaste should be avoided. Patients suffering from erosion should choose low RDA dentifrice. After all smile matters and everybody wish to boast upon an impressive personality with a pleasing appearance and therefore toothpaste should be chosen wisely according to the need of dental health care.

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Improving Oral Health of a Child: A Parent's Guide

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Abstract

For every parent, the early phase of growing a child is one of their life's most challenging as well as responsible experiences that can bring out the very best in us our patience and our skill to provide them good health. This article will provide essential information to help out parents build knowledge about maintaining good oral health of their children and also will help to make out the vital building blocks of parent-child relationships. This article will also help parents reflecting their actions to better understand their children's oral health. Small children at their younger age are very sensitive to social stimuli such as praise as well as affection and they learn best by imitating their parents. This knowledge will help parents to guide their children as they grow towards handling their oral health. This article is meant for parents of children of all ages and in all cultures which will be helpful and may provide insight to professionals like caregivers, teachers and early childhood educators.

Keywords: *Parental Guidance, Knowledge on dental health, Child oral health.*

Introduction

Oral health is considered as an important aspect of general health; good oral health provides healthy mental, physical and social well-being of an individual as well as provides enjoyment of their lives by allowing them to eat, speak and socialize devoid of experiencing pain, discomfort or embarrassment. Every child should benefit from a high paradigm of health, including oral health but during the early ages of their childhood they are dependent on their parents only. The dental health profession has an accountability to promote the best possible oral health for all children. Kids less than 5 years of age usually invest maximum time with their parents.[1] Some researches reported that the maintenance and outcomes of young kid's health invariably are affected by their parent's knowledge.[1]

A child's well-being of psychological, functional, and social dimensions are affected by dental health. Because of dental pain, distressing effects on kids, including poor growth, lost sleep, behavioral problems, and learning can be seen. Proper health inconvenience leads to a substantial reduction in school attendance and simultaneously hampers in parental working days.[2]

For every parent, the early phase of growing a child is one of their life's most challenging as well as responsible experiences that can bring out the very best in us our patience and our skill to provide them good health. This article will provide essential information to help out parents build knowledge about maintaining good oral health of their children and also will help to make out the vital building blocks of parent-child relationships. This article will also help parents reflecting their actions to better understand their children's oral health. This knowledge will help parents to guide their

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children as they grow towards handling their oral health. This article is meant for parents of children of all ages and in all cultures which will be helpful and may provide insight to professionals like caregivers, teachers, and early childhood educators.

Baby's teeth begin forming: Months before birth Infant teeth begin to develop. Tooth buds start appearing by the 6th week of pregnancy and keep on forming till 6 months. By the time baby born the crowns of primary teeth formation gets completed and until the baby begins teething they will stay behind hidden the gums.[3]

Looking for the teeth to erupt:

- Primary teeth will appear out in the mouth as early as 6 months till 12 months of age.
- First the lower front two teeth will appear.
- There is nothing to be worried as in some babies few teeth might appear late and by the age of 3 years, all 20 primary teeth will be present.

New Permanent Teeth: In the late 6 years of age, an eruption of 32 permanent teeth (16 in each upper and lower jaw) occurs that are the substitute of 20 primary teeth. The oral cavity contains both primary and permanent teeth during this period that might be a reason for small kids at increased risk of caries. The enamel of newly erupted is inadequately mineralized after its formation and its surface remains porous. During the phase of permanent teeth eruption, occlusal surfaces of the new permanent teeth will be resting on the lower level than the primary teeth. And because of this toothbrushing becomes more difficult than before. With the increasing number of permanent teeth in the mouth, the maintenance of the narrower interdental spaces becomes very important and crucial.[4]

Why Parent should be Concernedon Taking Care of Baby Teeth?:

- Healthy teeth are required for the baby and toddlers to chew their food properly while eating.
- Teeth has an important role to play in speech development as they learn how to talk.
- Primary teeth are needed to be maintained till the eruption of permanent teeth because the early loss of baby teeth can cause crowding of the permanent teeth.
- There will be a hindrance in the learning procedure if the primary teeth will be affected by any kind of oral disease-causing pain and infection.[5]

Beginning of Dental Care: By the first year of age, dental check-up at regular intervals of at least twice each year should be recommended.[6]

1. Birth to 6 months old:

- Baby's mouth has to be cleaned with water and a gauze or a cloth or a soft infant toothbrush after feedings and at bedtime can be used.
- If a child is living in an area without fluoridated water then dental health professionals should be about the use of fluoride supplements and the application of fluoride varnish.
- Regular feeding habits (bottle feeding and breastfeeding) should be established.

2. 6 to 12 months old:

- During the child's first birthday, the first tooth should appear and the pediatric dentist should be consulted for an exam as soon as possible for confirming about no worries.
- Tooth brushing needs to be done twice daily and specifically after feeding at night with a small, soft-bristled brush.
- During the phase of learning how to walk, a child may undergo facial injuries that might include teeth as well as other facial structures. Therefore, the parents and caregivers need to stay alert during this period.
- Breastfeeding for at least 1 year is recommended and after that year whole that milk should be used for proper development body and brain.[7]

3. 1 to 3 years old

- The scheduled dental checkups should be followed every 6 months as recommended by the child's pediatric dentist.
- By the age of 3 years, a child should learn how to brush his teeth and how to clean and rinse.

Protecting Your Child's Teeth: Below are the following points which help the parents to improve their child's oral health and protect from getting affected by oral diseases: -

- Drinking water containing fluoride (optimal amount of 0.7 mg per liter) should be given to the

child to prevent tooth decay. In doubt of fluoride concentration, dentists, pediatricians or nurses should be consulted.[8]

- Parents should make sure that their child brushes their teeth twice a day daily.
- The teeth of a child should be brushed properly without toothpaste with a small, soft-bristled toothbrush and plain water.[9]
- Children below 6 years should brush their teeth under the supervision of parents or caretakers so that they can develop good brushing skills. It should be taken care that they only use a pea-sized amount of toothpaste and always spits it out rather than swallows it.
- Need for the application of fluoride varnish on the teeth as soon as the first tooth appears should be discussed with the dentist, family doctor, nurse or pediatrician.
- **Get a Check-up:** During the child's first birthday, the first tooth should appear and the pediatric dentist should be consulted for an exam as soon as possible for confirming about no worries.
- **Spreading of cavities to be prevented to your infant:** Mouth to mouth contact should be limited by not sharing food or eating utensils, and by not placing objects in your mouth and then in your infant's mouth.
- **Avoid 'Baby Bottle Decay:** A child should not be put to sleep with a bottle of milk or juice. Sugary liquids can stick to his teeth, feeding bacteria that can cause cavities.
- **Cut Back on Juice:** A lot of parents have some thoughts regarding juice that it is a healthier choice for a drink. Juice should be limited to no more than 4 ounces a day of 100% fruit juice. Preference for on-sugary drinks and foods at mealtimes should be given.
- **Control the Sippy Cup:** Over-using of a sippy cup can result in cavities on the anterior teeth if the drinks are sugary.
- **Teach Good Habits:** Washing mouth properly after every meal. Brushing twice a day. Limiting sticky and sugary foods.

Children and their daily food eating habits[10]:

- Fruit, green and leafy vegetables, grain,and healthy dairy foods including cheese, milk,and unsweetened yogurt should be served for a healthy body.
- Sugary foods should be limited. Foods like candy, cake, cookies, and sweetened drinks can only increase the risk of tooth decay.
- Sticky foods like caramels and fruit roll-ups should also be limited.
- Eating between meals need to be limited.

Oral Hygiene Maintenance for Kids[11]: It has to be mandatory for every child to have his or her toothbrush as soon as the child has teeth inside his mouth.

Toothbrush for your child:

- Toothbrush with soft-structured bristles has to be chosen as it cleans the teeth better.
- A small child-sized toothbrush with large handles should be chosen. For being more comfortable, children can choose from various colorful toothbrushes.
- Toothbrushes need to be replaced every 3 months or maybe sooner if a child chews her toothbrush bristles or has been sick.
- Children under the 3 years old should use a smear of fluoride-containing toothpaste in case there is inadequate intake of fluoride daily.
- Parents need to apply a pea-sized amount of the toothpaste and spread along the head of the brush.

Sealants Can Seal the Deal for the Teeth: To prevent decay, sealants are preferred as a preventive procedure. The sealant applied on the teeth acts as a hurdle that protects the cavities prone areas of the posterior teeth from acids and plaque. The sealants have to be checked regularly by the dentist, since they do tear out away and might require reapplication of the sealant. If lost, sealants have to be replaced immediately, as they are effective simply as long as they are retained on the teeth.[12]

Table 1. Caring for your child's teeth during a dental emergency.[13]

Condition	What should parents or child care providers do?
Knocked out (avulsed) tooth	1. Get back the tooth and grasp it by the crown. In case the tooth is dirty then gently rinse it, if possible in the child's saliva. 2. Tooth can be stored in a cup of cool milk. 3. Parents of the injured child need to be contacted first to take the child to the dentist immediately.
Broken tooth	1. Rinse with warm water. 2. Cold compress like ice cubes can be used on the area to keep swelling down.
Bitten tongue or lip	1. Pressure to the injured area should be applied using a clean cloth to stop any bleeding. 2. Cold compress to be placed to avoid swelling.
Objects caught between teeth	1. Careful and proper use of dental floss between the teeth to remove the object has to be instructed to the child. 2. A dentist needs to be consulted ifthe object is not easily removable.
Toothache	1. Mouth rinse with warm water and floss to remove any food that might be trapped should be done by the child.
Trauma to jaw/ broken jaw	1. Cold compresses need to be applied to swollen areas and any kind of cloth-like scarf, handkerchief or towel around the jaw and over the top of the head should be tied to hold the jaw in place. 2. Emergency treatment needs to provide at the earliest.

Conclusion

The groundwork and the basis for strong teeth in children, as well as teenagers, is set during the early years of life. The contribution of parents towards good oral health is immiscible. Poor oral hygiene practices and diet habits are the primary reasons for cavities and gum diseases in children. The formation of caries in primary teeth gradually increases the risk of caries formation in permanent teeth also. Hence it is required to set up a proper and routine oral maintenance at the earliest during childhood to make sure the growth of strong as well as healthy teeth. Parents are considered as the constant role model for their children and they are also the key to make sure of a daily practice and in making their children value the importance of oral hygiene. Small children at their younger age are very sensitive to social stimuli such as praise as well as affection and they learn best by imitating their parents. Therefore, every parent should understand the importance of healthy teeth and should take proper care of them during their development to ensure a healthy future of their kids.

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Sugarrich Diets and Oral Health

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Abstract

Sugar eating is highly prejudiced by several social cultures, activities, biological and ecological factors which results to be the leading risk factor for dental caries as well as other oral diseases. A diet has a complete consequence on the veracity of the oral cavity that includes oral mucosa, alveolar bone, teeth,and periodontal tissue. It is ahead of our debate that the sugar-rich foods consumed can result ina risk on the integrity of our teeth. There is a direct impact on oral health because of the consumption of sugar. There has to be a noteworthy impact on serving to restrain the global outbreak of dental caries if we can reduce the amount of sugar consumption that has been extensively ignored compared to other health issues and is a foremost global public health problem distressing individuals, health systems as well as economies. This review article discusses the consumption of sugars, various forms of sugary diets in our daily intake, and its effect on oral health.

Keywords: *Sugar rich foods, Oral Health, Dentist recommendations.*

Introduction

In the last 50 years, sugar consumption has increased drastically worldwide during all these years particularly in emerging economies. Sugar eating is highly prejudiced by several social cultures, activities, biological and ecological factors which results to be the leading risk factor for dental caries as well as other oral diseases. A diet has a complete consequence on the veracity of the oral cavity that includes oral mucosa, alveolar bone, teeth,and periodontal tissue. It is ahead of our debate that the sugar-rich foods consumed can result in a risk on the integrity of our teeth.[1] There is a direct impact on oral health because of the consumption of sugar. There has to be a noteworthy impact on serving to restrain the global outbreak of dental caries if we can reduce the amount of sugar consumption that has been extensively ignored compared to other health issues and is a foremost global public health problem distressing individuals, health systems as well as economies.[2] This review article discuss the consumption of sugars, various forms of sugary diets in our daily intake, and its effect on oral health.

Sugar can be described as a sweet crystalline-like material augmented from various plants, chiefly sugar cane as well as beet sugar that consists of essential sucrose, and it is used as an extra additive material as a sweetener in food and drink. Sugars can be classified into two types i.e.

1. **Intrinsic Sugars** (or natural Sugars) i.e. where the sugar particles are inside the cell structure of the food particles such as fresh fruits or sweet vegetables.

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2. **Extrinsic Sugars** i.e. where the sugar particles are there outside the cell structure of the food particles or considered supplementary as an extra substance to the food. Extrinsic sugars again subdivided into: a) Milk Sugars (or natural Sugars) i.e. lactose in milk products (e.g. cheese, plain yogurt), which are less cariogenic and are accompanied by significant amounts of essential nutrients. b) non-milk extrinsic Sugars, also called added sugars and free Sugars (or hidden sugars).[3]

Free sugars are a type of extrinsic sugars considered as the primary factor responsible for dental caries. They are added to foods and beverages. They considered to all: monosaccharides such as Glucose, Fructose, Galactose, and disaccharides such as Sucrose and Table sugar. It is advisable for primary children that free sugars consumption should be proportional to about 30 g per person per day. Because it is reported that consumption of free sugars more than 4 times a day or intake of above 60g per person per day can result in an increased risk of dental caries.[4]

Recommended dose of sugar: According to the WHO guideline recommendation: Free sugar consumption daily should be restricted to less than 10% (or 50 g = around 12 teaspoons) in both adults and children. Also an additional reduction to below 5% (or 25 g = around 6 teaspoons) of total energy intake will provide additional healthiness along with minimizing the risk of tooth decay all through the life route.[5]

Form and Frequency of Sugars Consumed: Sugars-containing food in sticky form if remains in the mouth for a longer duration then there will be drop-in pH for a longer duration inside the oral cavity. This may enhance the cariogenic activity, thus increasing the risk of tooth decay. A high rate of recurrence of intake of sugars causes an increase in exposure duration to the oral cavity that is open to the elements of sugars and has a high retention causing maximum exposure on tooth surfaces.[6]

Amount v/s Frequency: According to the Vipeholm study, the findings are significant to date while taking into consideration the amount versus frequency issue. The addition of 300g extra sugar during a meal has less risk than adding up sugary snacks between meals which causes a significant increase in the risk of dental caries.[6]

Sugar effects on oral health: During the intake of food, sugar itself never harms the teeth directly, but it enables the bacteria and acids to get activated and do the work. Plaque formation occurs as the bacteria feed on the sugary foods that are inside the oral cavity and they create a bacterial community and it remains on the teeth for a longer duration in the form of plaque till the bacteria make acids resulting in enamel wear and causing cavities. Gingivitis also occurs because of the bacteria present in the plaque. The sugary foods releasing the sweetness and allowing the bacteria to get metabolized and later they involved in dental biofilm formation which further generates acids by-products leading to demineralization of the tooth structure.[7]

Persons in the threat of Tooth Decay: Every individual having food is at risk of tooth decay but children and adolescents are at more risk of having dental caries. People having missing tooth have at a young age are at more risk of tooth decay. People who do not maintain proper oral hygiene or do not rinse mouth after every meal are at high risk of dental diseases because plaque formation occurs at the earliest only 20 minutes after we eat foods so if the mouth is not rinsed effectively, tooth decay starts. Consumption of sugar regularly always have a maximum threat of developing oral diseases, if the consumed food eaten is sticky or taken in between mealtimes. People involved in smoking and drinking alcohol are also at more risk.[8]

Are all beverages bad for teeth?: No, all the beverages are not bad for teeth as it relies on the type of sugar consumed as well as the acid production inside the oral cavity. There are some food products containing calcium and phosphate example:- milk and yogurt which are meant to protect teeth from the harmful effects of acid. Milk product foods and beverages helps in making the teeth stronger and aids in remineralization process, whereas calcium and phosphate in milk help to put off the bacteria from adhering to the tooth as well as budding into plaque. Thus, drinking some of the beverages may act as beneficial to our body.[2]

Health implications for people who go for high-sugar diets such as Unstable Blood Glucose, Obesity, Heart Disease, Cancer, and Diabetes, Immune Function, Chromium Deficiency, Aging Acceleration, Tooth Decay, Children's Behaviour and Cognition, Stress and Anxiety, Replacing Important Nutrients.[4,9]

High sugar-containing Foods and Drinks[10]:

1. **Low-Fat Yogurt:** 1 single cup of yogurt (12 teaspoons of sugar) = 24 grams sugar exceeds the

daily restriction of sugar intake for a human being. Therefore, natural, or full-fat yogurt without added sugar should always be chosen.[8]

2. **BBQ Sauce:** Almost 2 tablespoons of BBQ Sauce contains 14 grams of sugar in it. Therefore, daily intake of more than the normal range can cause harmful effects for our body.
3. **Ketchup:** 1 tablespoon ketchup = 1 teaspoon sugar.
4. **Fruit Juice:** Natural fruit juice contains more fibers than sugars in it. So, drinking one fruit juice will not hamper much to our health. Rather, drinking preserved fruit juices in which added sugars are in it can harm more to our health. Drinks like coke or any fruit juice contain more of added alcohol, sugars, and preservatives inside it.
6. **Sports Drinks:** One bottle (570 ml) of sports drink contains 32 grams of added sugar which gets quickly absorbed and provides instant energy with added 159 calories.
7. **Chocolate Milk:** A full glass of chocolate milk with added flavors of cocoa contains 2 teaspoons of added sugar.
8. **Flavored Coffees:** The hidden amount of sugars inside the coffee can be confounding. It is expected that a good chain of coffee can give 25 teaspoons of sugar = 100 grams of added sugar per serving.
9. **Iced Tea:** Commercially prepared chilled ice tea contains sweetened sugar or flavored with syrup of 33 grams of sugar per (340 ml) serving.[2]
10. **Protein Bars:** Protein bar with high sugar should be avoided as it contains around 30 grams of added sugar in it.[9]
11. **Vitamin water:** Some vitamin bottles come with added sugar in it which contains around 120 calories and 32 grams of sugar per bottle of 500 ml.[11]
12. **Pre-Made Soup:** Packed soup contains high amounts of sugars and preservatives inside it.
13. **Canned Baked Beans:** 1 cup (254 grams) of regular baked beans = 5 teaspoons of sugar.
14. **Bottled Smoothies:** Commercially prepared smoothies in bottle contains upto 96 grams = 24 teaspoons of sugar in a single serving.[12]
15. **Breakfast Cereal:** Some breakfast cereals have 12 grams = 3 spoonful of serving per 30 grams.[13]

The most excellent way to steer clear away the threat of added sugars in meals is to prepare them at home as it is known exactly what's in them.

Sugar Substitutes: Non-acidogenic sugar substitutes (sweeteners) do not cause pH falls in dental plaque. Various sugar replacementsare of two types: intense sweeteners (noncaloric) such as aspartame, saccharin, glycyrrhizin, sulfame and so forth and bulk sweeteners (caloric) such as sorbitol, mannitol, xylitol, and so on.[14]

Advisory from dental professionals[15]:

- It is advisable to brush teeth twice a day properly with fluoride-containing toothpaste as well as dental flossing to be done daily.
- Rinsing the mouth with water frequently after consuming sugary foods.
- Reduce consumption of sugar-sweetened beverages and snacks; that helps decrease the production of acid in the mouth.
- Eat sugary foods at mealtimes only
- Do not add any sugars to baby's foods and juice drinks.
- More amount of vegetables, fruits and breakfast having less sugar content should be consumed.
- Long-lasting sources of sugar consumption should be avoided (dried fruits, fruit leathers and hard or chewy sweets) close to bedtime.

Conclusion

Sugars and oral health have a dynamic relationship. Health care sector professionals, specifically the dental as well nutrition professionals should understand the relationship between diet and oral health and its effects on our body. There is a need for oral hygiene instructions and oral health education for the people and this awareness has to be done by dental health professionals to minimize the risk of oral diseases because of sugary foods. Future research is required in this field to establish method to decrease the threat caused due to sugars and other fermentable carbohydrates. Dietary guidelines for the prevention and management of oral diseases gives a structured format for consumers and health professionals to utilize the guidelines and understand the harmful effects of the intake of excess sugars in foods. Various modifications and behavioral interventions in research are needed to restrain the rate of recurrence of added

sugars as well as to amplify with the food reference intakes.

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Desquamative Gingivitis in Cicatricial Pemphigoid: A Diagnostic Paradox!

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Abstract

Desquamative gingivitis or gingival desquamation represents a clinical sign seen in many diseases, especially of muco-cutaneous nature. One of the common diseases, in which gingival desquamation is present is Mucous membrane pemphigoid (MMP) and represents a variety of chronic auto-immune sub-epithelial bullous diseases affecting the mucous membranes and sometimes the skin. MMP not only affects the oral mucosa but ocular involvement is also quite common. The severity of the disease is variable, and the gingival desquamation may be the first sign of this otherwise indolent disease and early diagnosis can reduce the morbidity especially ocular changes which could be irreversible leading to blindness can be prevented. We describe a case for documentation while describing the differential diagnosis and management of the disease.

Keywords: *Gingival desquamation, muco-cutaneous disorder, ocular involvement, sub-epidermal bulla.*

Introduction

The word "desquamation" taken from the Latin word 'Desquamare', meaning scraping fish flakes. In the literal sense, desquamation implies loss of epithelium, peeling off skin or mucosa. Desquamative gingivitis was first described by Tomes in 1894.[1] It is not a disease by itself rather a manifestation of some disease. It can progress to vesicle formation, erythema, erosion and ulceration of the attached gingiva.[2] Glickman and Smulowin 1964 stated that desquamative gingivitis could be a clinical manifestation of many diseases.[3] Dermatologic diseases, hypersensitivity, endocrine disturbances and chronic bacterial infections are common causes.[4]

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MMP or cicatricial pemphigoid accounts for the maximum number of cases of desquamative gingivitis and sometimes the gingival lesions are the sole manifestation of the disease, hence it requires meticulous history taking and careful examination by the general dentists as they have an important role in the diagnosis of this chronic muco-cutaneous disease.[5]

Case Report: A 53-year-old female patient reported with a complaint of redness and burning sensation in the gingiva. History revealed she had pain and bleeding while cleaning her teeth. Her medical, surgical, personal and family history were non-contributory. There were no lesions anywhere else in the body.

On clinical examination, the left sub mandibular lymph node was slightly palpable, movable and tender. The attached gingiva in the mandibular anterior region and the entire maxillary arch was erythematous and tender on palpation. (figure 1-5) There was desquamation and mild bleeding on probing. On applying compressed air to the apparently normal gingiva, there was desquamation. This elicited a positive Nikolsky sign in the oral cavity.

Since the patient had got gingival desquamation and

an erythematous area the lesion clinically was thought of as desquamation due to hormonal disturbances, lichen planus, pemphigus and lupus erythematosus. But the absence of white striae in the lesion, absence of any menstrual irregularities, and any other systemic signs and symptoms with a positive Nikolsky sign indicated towards MMP.

The patient was advised for an incisional biopsy and H & E stained section revealed sub-epithelial cleft and basal cell degeneration, with haemorrhagic areas, suggestive of mucous membrane pemphigoid. (figure 6) The patient was advised to apply a combination of Clobetasol propionate 0.05% and Miconazole 1% cream, thrice a day for a month and was to be reviewed. The patient was lost to follow-up.

Discussion

Mucous membrane pemphigoid is a chronic sub-epithelial bullous disorder mainly affecting elderly ladies.[6] However, there is no racial or geographic predilection. It has a rather complex aetiopathogenesis as it is a heterogenous disorder with the involvement of multiple antigens. The antibodies produced in MMP have been shown both in vitro as well as in vivo. Generally, there are IgG and IgA antibodies circulating in the blood directed against the parts of the basement membrane zone (BMZ) which show that humoral response against the antigens occurs in MMP.[7] Mislaying of immunologic tolerance to morphologic proteins in the BMZ leads to the formation of autoantibodies. Using immune-precipitation and immune-blotting mechanisms, many antigens have been discovered. The bullous pemphigoid antigen 2 (BPAg2) (a 180-kDa protein, BP180) is the most commonly targeted autoantigen in MMP.[8] When there is reaction between autoantibodies against antigens it results in detachment of the epithelium within the BMZ.

MMP affects different mucosal sites, rarely there is cutaneous involvement. It is a chronic, indolent disease which commonly involves the oral mucosa followed by the conjunctiva, nasal mucosa, genital mucosa, gastro-oesophageal mucosa and the lesions always heal with scarring.[9] The disease differs in severity ranging from localized to a diffuse disseminated involvement. Initially the disease may be localized and later there is extensive involvement. Since the lesions heal by scarring, it leads to significant morbidity especially in those patients with ocular involvement.[10]

Oral lesions frequently occur on the gingiva and the palate, followed by labial mucosa, tongueand buccal mucosa.[11] These lesions can occur as erythema, erosions, pseudo-membrane, and rarely intact bullae. The gingival involvement manifests as desquamative gingivitis, which is not specific for MMP but may also be seen in oral lichen planus and pemphigus vulgaris.[12] Ocular lesions manifest as conjunctival erosions and erosions, keratinization, symblepharon, enteropion, trichiasis, ankyloblepharon, ankylosis and even blindness.[13]

The diagnosis needs histopathologic evaluation of the tissue samples which needs to be taken with utmost care as the epithelium easily dislodges from the underlying connective tissue. H & E sections characteristically reveals sub-epithelial split with an inflammatory infiltrate of chronic inflammatory cells.[14] Another specimen may also be taken from perilesional tissue and taken in buffered hypertonic saline solution and submitted for direct immunofluorescence. A linear IgG and C_3 is seen along the BMZ.[8,15]

Therapy for MMP comprises of early treatment of the lesions and aims at preventing complications especially when there is ocular involvement. The scarring can be avoided by early therapeutic interventions. Treatment is tailor made for individual patients based on the severity of the disease considering the age, general wellbeing, medical issues and contra-indications to the use of systemic drugs. Multidisciplinary treatment with a team of experts consisting of oral medicine specialist, dermatologist, ophthalmologist, gastroenterologist typically improve outcomes.[16]

High-potency topical glucocorticoids remain the mainstay of therapy. Fluocinonide, clobetasol propionate and betamethasone dipropionate are the prescribed drugs. Gingival desquamation alone can be well managed using topical glucocorticoids alone.[17] Use of a splint or custom-made tray to hold the gel on to the gingiva may be more effective. However due to long term use of these steroids leading to possible adverse effects like candidiasis, concomitant of topical anti-fungal drugs also is prescribed as in the present case a combination of clobetasol and miconazole was used.[18,19]

Intralesionaland systemic glucocorticoids are used to treat recalcitrant lesions or as an adjunct totopical steroid delivery. For patients not responding to steroids, calcineurin inhibitors, which are potent

immunosuppressants like Tacrolimus is prescribed with good efficacy and safety.[19]

Palliative care is also required to alleviate the masticatory discomfortusing topical anaesthetics-analgesic-antihistaminic mouthwashes or gels and improve patient's capability to eat, swallow and perform oral hygiene. [17-19]



Figure 1: Gingival desquamation in the maxillary palatal gingiva.



Figure 2: Gingival desquamation in the mandibular lingual gingiva.



Figure 3: Gingival desquamation in the attached gingiva labially and buccally.



Figure 4: Gingival desquamation in the attached gingiva labially and buccally.



Figure 5: Gingival desquamation in the attached gingiva labially and buccally.

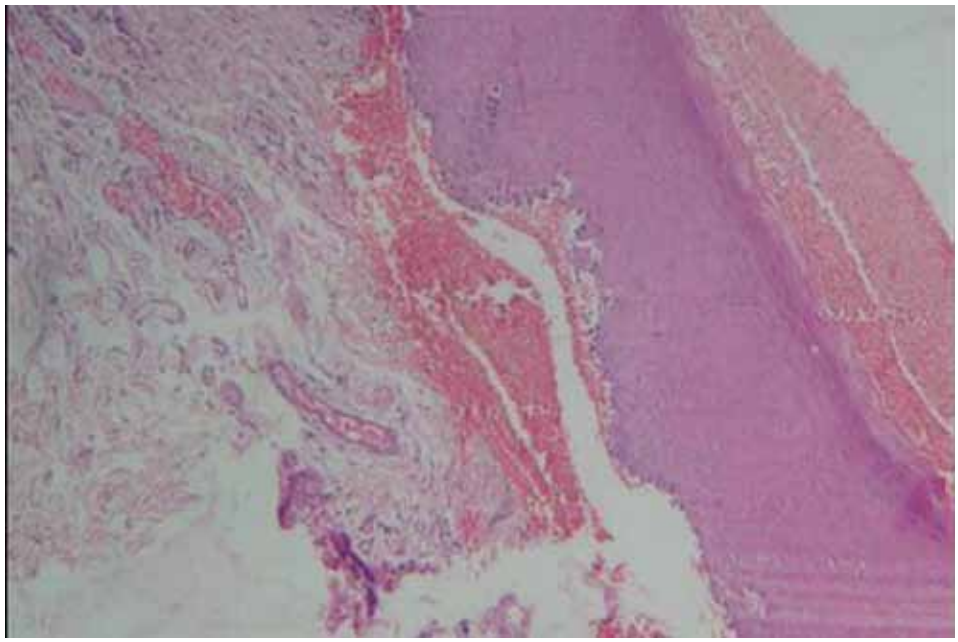


Figure 6: H & E (10 X original magnification) stained section revealed sub-epithelial cleft and basal cell degeneration, with haemorrhagic areas.

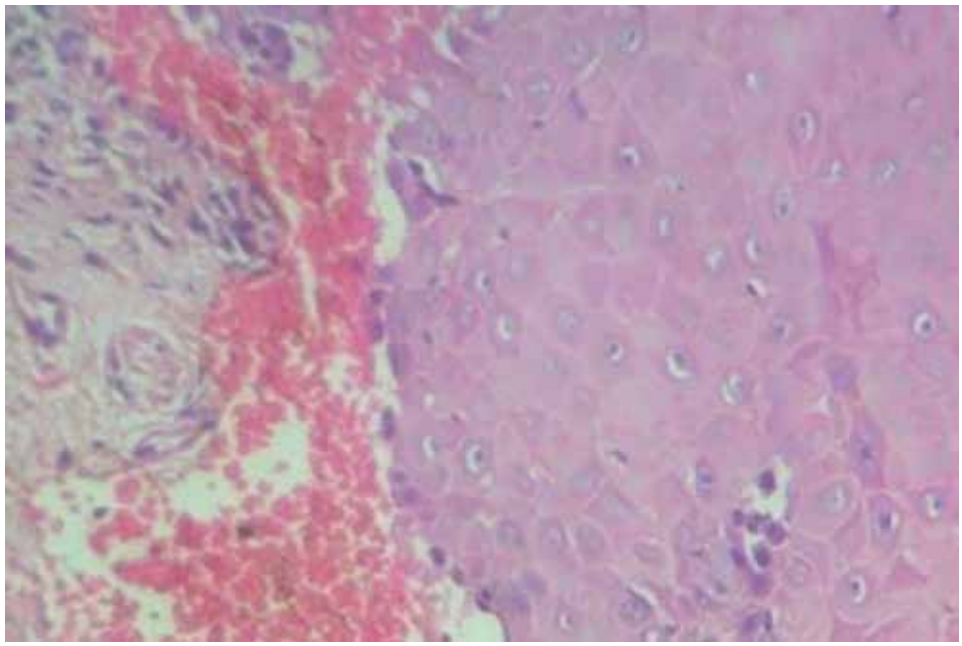


Figure 7: H & E (40 X original magnification) stained section revealed sub-epithelial cleft and basal cell degeneration, with haemorrhagic areas.

Conclusion

Dentists are often the first people to examine and diagnose this mucosal disease. Prompt diagnosis of mucous membrane pemphigoid requires taking a careful meticulous history together with a detailed oral mucosal examination along with checking the possible involvement of other mucosae and using histopathologic and immunofluorescent studies. Desquamative gingivitis may be the sole manifestation of the disease and usually heals uneventfully by improving the oral hygienic measures with topical glucocorticoidand antifungal therapy.

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Complex Composite Odontoma: A Cause of Impacted Teeth

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Abstract

Odontomas are one of the most common odontogenic lesions, earlier considered as mixed odontogenic neoplasms but lately have been accepted as hamartomatous malformations. They are either detected accidentally on radiographs or when they are a cause of impeding normal eruption of teeth. Odontomas originate from the odontogenic tissue and contain enamel and dentine, hence are referred to as composite lesions. They can be of two types depending on the appearance. If they have masses of calcified structures resembling multiple miniature teeth then they are called compound odontomas and if they appear as an irregular disorganized radiopaque mass they are referred to as complex odontomas. A case if complex odontoma is reported herewith which led to impacted maxillary molar.

Keywords: *Harmartoma, denticles, odontogenic neoplasm, radiopaque mass.*

Introduction

The most common odontogenic lesions are odontomas which represent all odontogenic tissues i.e. Enamel, dentine, cementum and pulp tissue in one lesion hence referred to as a composite lesion.[1] Since they originated both from the ectoderm and mesodermal components of the odontogenic apparatus, they contain both enamel and dentine.[2] Although they can be detected at any age, being a developmental malformation, they are seen with twenty years of age and are generally asymptomatic lesions, unless associated with mild tooth pain or swelling, they are detected on incidental radiography.[3] There is a definite female gender predilection and also the maxillary arch, especially the anterior region is more frequently affected.[4] Odontomas are notorious for impeding eruption of nearby teeth. They are usually small in size though large sizes have been reported which can cause expansion of the cortex.

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Depending upon their radiologic appearance they may be classified into complex or compound odontomas.[5] Compound odontomas appear as miniature teeth like structures, known as denticles, where each structure has enamel, dentine, pulp and cementum like normal teeth.[6] Complex odontomas are disorganized irregular masses of enamel, dentine and cementum mixed up. Compound odontomas are commonly visible in anterior maxilla and complex odontomas in posterior mandible. Odontomas have also been associated with other odontogenic tumours like ameloblastoma[7] adenomatoid odontogenic tumour[8] and calcifying epithelial odontogenic tumour[9] and are then referred to as hybrid tumours.[10]

Case Report: A 28-year-old female patient reported with missing upper molar on the right side. History revealed the second molar on the right maxillary arch did not erupt in childhood. She did not have any toothache or swelling in the area. The medical, surgical, dental, personal and family histories were non-contributory.

On clinical examination, the maxillary second and third molars were missing, the alveolar ridge appeared normal without any bulge, swelling or tenderness on palpation.

A periapical radiograph was taken and revealed a single large well-defined irregular radiopaque mass

distally in relation to 16. Since the radiopaque mass was not fully covered in the periapical radiograph, maxillary lateral occlusal radiograph was taken of the right side which showed, a single large well-defined irregular radiopaque mass distally in relation to 16, with an unerupted molar displaced superiorly. A Panoramic radiograph was taken to screen the jaws for any other lesion and it revealed a single large well-defined irregular radiopaque mass distally in relation to 16, with an unerupted molar displaced superiorly. The radiographic features were suggestive of a complex odontoma and the patient refused any treatment after knowing the hamartomatous nature of the lesion and agreed for regular-follow-ups.

Discussion

The word "Odontoma" was given by Broca in 1867 to describe odontogenic neoplasm formed by the enlargement of developing odontogenic tissues.[2] The odontogenic tissues undergo morpho-differentiation to form enamel and dentine in teeth, but the tissues do not reach the normal level of morpho-differentiation and form abnormal enamel and dentine in the form of odontomas.[11] The aetiopathogenesis of this lesion remains unknown as yet but local infection or trauma may trigger its formation. Genetic mutation could also be cause where the normal tooth development is halted.[12] Odontomas are indolent and gradually grow in size and being a developmental lesion, they are most of the time detected within twenty years of age, though in rare cases like in the present case, they may be incidentally detected later in life.

As per the classification of the World Health Organization (WHO), odontomas are of two types, complex odontomas and compound odontomas.[13] They are bot to be confused with dilated odontomas which refers to dens invaginatus another developmental anomaly.[14]The former is rarer and is seen in the posterior mandibular region, however in the present case it is seen in the maxillary arch.About four-fifths of odontomas are associated with impacted teeth. [15] Odontomas can safely be removed surgically as they are generally encapsulated and easily come out as a single specimen and there is never any recurrence.

The radiologic appearance is very characteristic, the odontomas are well-defined radiopaque lesions surrounded by a thin radiolucent line denoting the capsule covering them. The appearance ranges from multiple miniature teeth like structures (denticles) to a diffuse, irregular, radiopaque mass. Enamel and dentine can be visualized on radiographs.[16] Though the diagnosis is often radiologic, histopathologic evaluation of the excised odontomas shows enamel, dentine, cementum, pulp, and cementum in haematoxylin eosin stained sections. While the compound odontomas resemble normal teeth, the complex odontomas look like masses of coalesced hard odontogenic tissue. Scanty, myxoid connective tissue stroma having epithelial odontogenic rests seen with the hard tissue mass resembling normal dental follicular pattern.[17,18]



Figure 1: Maxillary arch on the right side, missing maxillary second permanent molar.

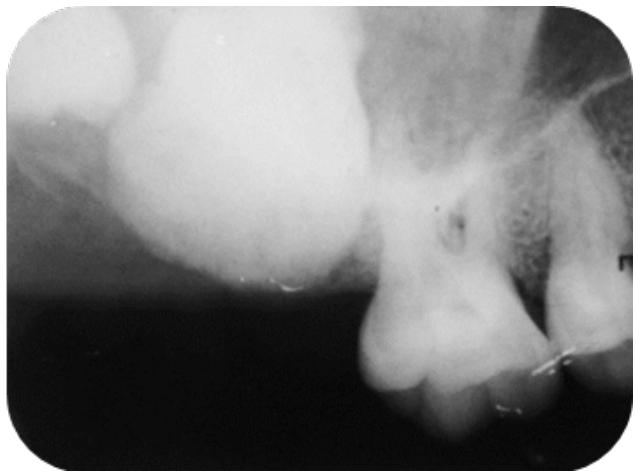


Figure 2: Intra-oral periapical radiograph of right maxillary molar region showing a single large well-defined irregular radiopaque mass distally in relation to 16.



Figure 3: Maxillary lateral occlusal radiograph of the right side showing a single large well-defined irregular radiopaque mass distally in relation to 16, with an unerupted molar displaced superiorly.

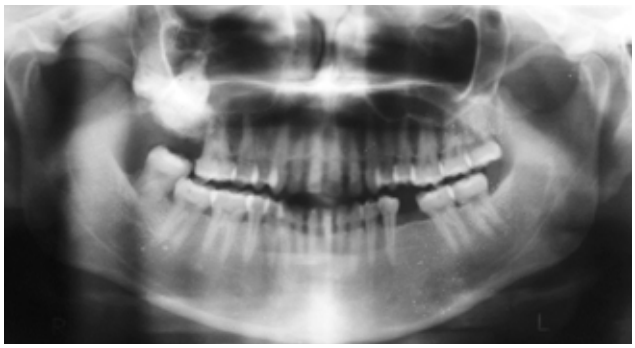


Figure 4: Panoramic radiograph shows a single large well-defined irregular radiopaque mass distally in relation to 16, with an unerupted molar displaced superiorly.

Conclusion

Odontomas have been often reported in the literature, complex odontomas of huge size, compound odontomas containing hundreds of denticles, odontomas erupted into the oral cavity and secondarily infected odontomas are some of them. As dentist's awareness regarding the presence of odontomas impeding the eruptive path of teeth is essential and their hamartomatous nature gives reassurance to the patient. Odontomas can be easily removed when required and it carries a favourable prognosis without any recurrence.

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Ethical Permission: Approved

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Ameloblastoma: A Common Benign Odontogenic Tumour with Multiple Manifestations

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Abstract

A myriad of odontogenic cysts and tumours both benign and malignant are seen in the maxilla and the mandible. One of the most frequently encountered odontogenic tumour is ameloblastoma and has different clinical and histologic variants which dictate the treatment plan. It has a properly documented inclination towards a local infiltration and tendency of recurrence. Ameloblastoma is a benign intraosseous odontogenic epithelial tumour which is locally invasive and locally destructive, non-functional, intermittent in growth, anatomically benign and clinically persistent. The tumour arises from the epithelial cell rest of malassez, from the walls of odontogenic cyst and from the basal cells.

Keywords: *Ameloblastoma, locally destructive, odontogenic neoplasm.*

Introduction

Ameloblastoma is a gradually progressing but locally infiltrating benign neoplasm of odontogenic origin affecting the jaw bones, more frequently the posterior mandible. It has high recurrence rate and was first explained in 1827 by Cusack.[1] It has been called by a variety of names including ''cystosarcoma,'' ''adamantine epithelioma,'' and ''adamantinoma''. Ameloblastoma is coined from French "amel,'' meaning enamel, Greek ''blastos,'' meaning germ or bud.[2] It is one of the most common odontogenic tumours probably second only to odontomas. Since the lesion is slow growing and asymptomatic, the patient reports only with facial asymmetry due to swelling in the maxilla or mandible, often growing to large sizes by the time of diagnosis thus jeopardizing the treatment plan and increasing chances of recurrence especially true in cases of maxillary ameloblastomas invading the base of the skull.[3]

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Case report

A27-year-old male patient reported to the dental hospital with the complaint of large swelling in the right side of the face and discomfort on mastication for 6 years.

History revealed that six years back the patient had noticed a small swelling in the right side of the mandible in the region of third molar. It was painless swelling and was growing slowly for the past six years and attained the present size. The patient gave a history that during this period sometimes the growth was remaining as it was for some time and started growing again. No history of any numbness in the lip or pain and bleeding in the swelling. The swelling was a fresh swelling and not a recurrent swelling. Similar swelling was not found anywhere in the body. No history of any disturbances in the teeth like pain, mobility etc., Past medical history was not relevant to any disease.

Extraoral examination revealed a single large irregular shaped elevated swelling on the right side of the face involving the mandibular body on the

right side and extending downward along the lower border of the mandible. The swelling was large with well-defined borders and boundaries. (Figure 1) The swelling extended superiorly to the maxillary region and infraorbital region posteriorly 1cm in front of the auricle, medially it extended to the midline of mandible, inferiorly to submandibular region. The skin over the swelling was smooth and shiny but not stretched and no secondary changes like ulceration was seen.No visible pulsations or pigmentation was seen over the swelling. On palpation the swelling was lobulated, firm in consistency, not warm, not tender. The skin over the swelling was pinchable. The swelling was not reducible or compressible. No fluctuation was present. The mass was not movable and was fixed to the mandible.

Intraorally, the right side of the mandible was enlarged buccaly and Lingually. And the buccal extension appeared in the form of a swelling and extended from the midline to the retromolar area obliterating the sulcus (figure 2 and 3) The mucosa over the swelling was normal and the teeth were irregularly arranged with tilting. The lingual extension of the swelling was mild. On palpation the swelling was firm in consistency and in certain areas it yielded on pressure but no compressibility or reducibility. The swelling was fixed to the bone. The teeth were slightly shaky and tender. No mucosal involvement and tongue movements were normal. In the right-side floor of the oral cavity was partially filled with expansion of the swelling.

Since the swelling had developed after the mandible was fully formed, only 6 years back, growing very slowly intermittently without any inflammatory signs for the past 6 years, the swelling was a benign neoplasm. Further the swelling grew both intraorally and extra orally, was fixed to the mandible, firm in consistency and hence this benign neoplasm was a benign neoplasm of the bone. Since no fluctuation was present the question of a cystic swelling was ruled out. A differential diagnosis of Ameloblastoma and Ossifying fibroma were considered. Since the tumour had grown for the long time with intermittent growth and had enlarged to a very large swelling which appeared as multinodular and intraorally the swelling extended to a large area of mandible with areas of softness the condition may be provisionally clinically diagnosed as ameloblastoma.

Skull radiographs revealed that the right side of the mandible was destroyed up to the ramus. Lower border was irregular and destroyed. Areas of radiolucency and opacity were seen.(figure 4) In the panoramic radiograph a large radiolucency was seen on the right side from midline to the anterior border of ramus (figure 5) The normal bone pattern was destroyed. The radiolucency had a multilocular appearance with areas of opacity. The lower border was enlarged. Right lower third molar was floating in a mass.

Biopsy was taken in 46, 47 region. Histological features showed the mass consisted of connective tissue. The epithelium was in the form of follicles bounded by palisading ameloblastic cells with nucleus in the periphery enclosing stellate reticulum like cells. The connective tissue was fibrous with minimal vascularity. The histological picture suggests follicular ameloblastoma as the tissue contains follicle cells lined by ameloblastic cells. (figure 6)

In view of history, clinical findings, radiographic findings and histological appearance the condition was diagnosed as follicular ameloblastoma.



Figure 1: Facial asymmetry and huge diffuse swelling in the right side of the mandible.



Figure 2: Diffuse intra-oral swelling in the mandible obliterating the buccal sulcus, extending from 33 to 47 region mediolaterally.



Figure 3: Bicortical expansion in the mandible with lobulations.



Figure 4: Postero-anterior view of the mandible showing multilocular radiolucency extending from 47 to 35 region, crossing the midline, there are radiopaque septae present in the radiolucency.

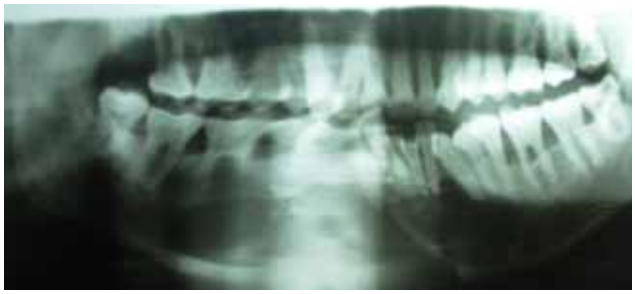


Figure 5: Panoramic radiograph reveals a multilocular radiolucency extending from 47 to 35 region, crossing the midline, there are radiopaque septae present in the radiolucency. Root resorption in 46 with displacement of 44, 43, 31, 33, 34, and 35. There is generalized alveolar bone loss.

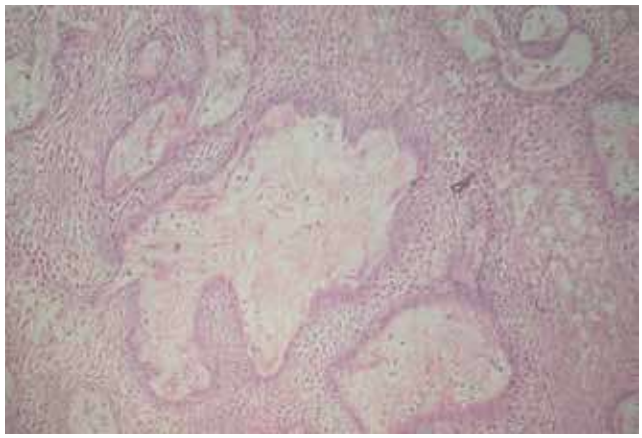


Figure 6: H&E stained section showing odontogenic epithelium showing peripheral columnar cells with reversal of polarity and central stellate reticulum like cells, in the form of strands and follicles within the connective tissue stroma with large areas of cystic degeneration associated with few areas of squamous metaplasia.

Discussion

The jaw bones have three tissues 1) odontogenic tissue – the tissue that is responsible for tooth formation 2) osteogenic tissue– the tissues responsible for bone formation 3) soft tissues within the jaw bone - blood vessels, nerves, ectopic salivary glands etc,. Tumoursare either benign or malignant and can develop from any one of the tissues.[4] Odontogenic tumours are the tumours that develop in jaws from odontogenic tissue- oral epithelium in tooth germ, enamel organ, dental papilla, reduced enamel epithelium, remnants of Hertwig's root sheath etc.,[5] This type of tumour doesn't develop in any other bone. The most common odontogenic tumour is ameloblastoma.

Ameloblastoma is a benign intraosseous odontogenic epithelial tumour which is locally invasive and locally destructive, non-functional, intermittent in growth, anatomically benign and clinically persistent. The tumour arises from the epithelial cell rest of malassez, from the walls of odontogenic cyst and from the basal cells. The proliferating epithelial cells are not ameloblasts but ameloblasts like cells with characteristics of short columnar cells with nucleus placed in the periphery. [4.5]

Clinically, the tumour develops in both males and females from 3rd decade onwards. It starts slowly, sometime stops in growth and recurs. The slow growth of tumour takes place over several years and can reach up to huge sizes invading and infiltrating locally. The

tumour expands both buccally and lingually and the teeth are irregularly arranged. [7]

Clinically the tumour is lobulated and hard in consistency and lower border is expanded. The tumour mass expands within the periosteal covering and it won't perforate the periosteum. Ameloblastoma may be monocystic or polycystic.[8] If it is monocystic it is a large cystic cavity. If it is polycystic it has a multilocular appearance with interlocular bony septa.[9]

Radiologically, ameloblastoma will appear as unicystic or multilocular (honeycomb or soap bubble appearance) and must be differentiated from other odontogenic and non-odontogenic tumours in the jaws. [9,10,11] Histologically ameloblastoma will have two components epithelial and connective tissue. The epithelial cells are arranged in alveoli which are separated by connective tissue. [12] The alveoli look like stellate reticulum and the peripheral cells appear as short columnar cells with nucleus placed away from the basement membrane and the cells look like ameloblasts. Histologically, follicular ameloblastoma, plexiform ameloblastoma, acanthomatousameloblastoma, squamous ameloblastomaand desmoplastic ameloblastoma are common varieties. [13]

Treatment varies from simple enucleation for uni-cystic amelobalstomas to resection for the multi-cystic ones. Reconstruction is also required to prevent gross facial deformity. [14]

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Impacted Tooth: A Cause of Odontogenic Lesion in the Jawbones

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Abstract

The dentigerous cyst is the commonest developmental odontogenic cyst encountered in the jawbones accounting for about one-fourth of all reported cases. As with other cysts, they present as asymptomatic lesions that are either detected on routine radiographs or when they cause swelling leading to facial asymmetry. Since the cyst is associated with an impacted tooth, the common teeth involved are impacted mandibular third molars or maxillary canines. This paper isan overview of the impacted tooth, which can be a cause of odontogenic lesion in the jawbones

Keywords: *Impacted tooth, odontogenic cyst, supernumerary tooth, unilocular radiolucency.*

Introduction

The term 'Dentigerous cyst' was coined by Paget in 1853.[1] Dentigerous cysts (DC) are the second most common developmental odontogenic cysts after the radicular cyst. A dentigerous cyst is defined as an odontogenic cyst that envelopes the crown of an impacted tooth.[2] It is almost always associated with impacted, unerupted, or embedded tooth, and may also be involved with a supernumerary tooth. They're usually unicystic but bilateral and multiple dentigerous cysts may be found associated with several syndromes.[2] They're most commonly associated with the mandibular third molars and maxillary canines, followed by maxillary molars and Mandibular premolars.[3]

These cysts are asymptomatic in most cases unless they are secondarily infected. Dentigerous cysts are usually detected on routine radiographic check-up, and are purported when-

- "The pericoronal radiolucency measures around 2.5 mm or larger in any dimension.[4]
- "The follicular space is more than five millimeters."
- "There is any associated tooth displacement or alarming expansion of bone found."

Also, Browne had pointed out that the literal meaning of the word 'dentigerous' means 'tooth-bearing' which is justified by its radiographic appearance as a dentigerous cyst is seen as a well-circumscribed unilocular radiolucency symmetrically surrounding the tooth, often with a sclerotic outline.[5] Several lesions first appear to fulfill the criteria for the diagnosis of the dentigerous cyst but later demonstrate features of something else like unicystic ameloblastoma, thus making the diagnosis of the lesion an important aspect.

A cyst is described by Kramer as, 'a pathological cavity having fluid, semifluid or gaseous contents and which is not created by the accumulation of pus'[1]. These are more commonly found in the orofacial region, and the cysts of jaws that develop from the tissues involved in tooth development are called the Odontogenic cysts. These cysts arise from the cell rests of odontogenic epithelium. These can be inflammatory or developmental, as given by Pindborg and Kramer in 1971. A dentigerous cyst is the one in which a crown or more rarely a part of the crown, projects into the cystic cavity. Various theories have been put forward as regards the origin of a dentigerous cyst. The strongest assertion is that the cyst initiates from the reduced enamel epithelium(REE) since the epithelial lining of the cyst is seen to be adjoined to the neck of the tooth, by the agglomeration of fluid between the reduced enamel epithelium and the enamel surface.[1]

Clinical Features: Dentigerous cystsare almost always involved with a normal permanent tooth that is impacted or unerupted, and rarely a deciduous tooth. Dentigerous cysts may be found anywhere in the jaws but are more frequently found in the angle of the mandible, canine region of the maxilla, and molar region of the maxilla. Dentigerous cyst is usually seen to be clinically present as an enlargement of the buccal cortical plate of the maxilla or the mandible.[6] Most lesions are said to be found in the second and third decade with a predominance among males.[2] It is highly likely that dentigerous cyst can become an aggressive lesion and its continued enlargement results in the asymmetry of the face, expansion of the mandible or maxilla, teeth displaced from their positions, and resorbed roots of adjacent teeth[7]. In case it is associated with maxillary canines, it leads to an expansion in that area, resembling sinus and skin infections. Unless infected secondarily, dentigerous cyst patients do not experience any pain,and on aspiration, yellow-colored fluid is obtained.[7]

Radiographic Features: As seen on a radiograph, dentigerous cysts typically have a well-defined border which may be absent in the case of infections. The internal structure is completely radiolucent except the crown of the tooth. Radiologic examination of a dentigerous cyst is the most important aspect in the diagnosis of this type of cyst as they give us a proper idea of the dimension and extent of the pathology. It is also important to keep in mind that we have to be able to distinguish between a merely enlarged dental follicle -an ectomesenchymal tissue that surrounds the tooth germ- which can be quite arbitrary unlike the smooth, well-defined borders of a dentigerous cyst. Also, the normal follicular space is seen to be around three to four millimeters whereas a dentigerous cyst is suspected when the follicular space exceeds more than five millimeters.[2]

According to the situation and form in radiographs, three variations of dentigerous cysts are seen. First is the central type in which the cyst is seensymmetrically enveloping the tooth. Here the pressure is directly applied on the erupting tooth thus preventing its eruption. It is the most common variety and the tooth appears like a candle flame. Second is the lateral type in which the cyst envelopes on the side of the tooth and is seen in the mandibular third molar region. This occurs when the follicle is dilated on one side. The third is the circumferential type which is very rarely seen. In this the follicle expands in such a manner it appears to enclose the entire tooth giving it an appearance of a lantern in the radiograph. However, it is wise to do a histological examination of the tissue associated with the affected tooth as we cannot dismiss the absence of any pathology based on just the radiographic methods as the absence of radiographic pathology may not necessarily mean the absence of disease.[8]

Histological Features: Microscopically we see that the cyst is composed of a thin wall of connective tissue with a thin layer of the stratified squamous epithelium lining the lumen. The dentigerous cyst has no characteristic microscopic features that can help differentiate them from other types of odontogenic cysts. The connective tissue is sometimes thickened and composed of loose connective tissue leading to misdiagnosis as an odontogenic fibroma or an odontogenic myxoma.[2] The connective tissue wall of normal dental follicle and dentigerous cysts has islands of odontogenic epithelium which can be sparse, but when present in significant amounts it again leads to its misdiagnosis as ameloblastoma. In the inflamed cysts there is the presence of hyaline bodies called Rushton bodies present inside the lining epithelium. They are of unknown origin and significance.

Dentigerous cyst is a common cyst but it has a few differential diagnoses which need to be kept in mind while diagnosing. Some of these are- Hyperplastic dental follicle, Odontogenic keratocyst, Ameloblastoma (unicystic), Calcifying odontogenic cysts, and Adenomatoid odontogenic tumor.

Table 1. Differential diagnosis of dentigerous cyst based on radiologic features.[9]

Lesion	Location	Periphery	Internal Structure	Effect on Surrounding Structures
Dentigerous cyst	Mandibular and Maxillary molar region, Maxillary canine region. Epicenter just above the crown	Well defined cortex unless infected	Completely radiolucent with the crown of the involved tooth	Apically displaced teeth, resorption of roots of adjacent teeth, inferior displacement of the inferior alveolar canal

Lesion	Location	Periphery	Internal Structure	Effect on Surrounding Structures
Ameloblastoma	Molar-ramus region of the mandible	Well defined cortex similar to a dentigerous cyst	Unilocular radiolucency, soap bubble appearance.[10]	Apical displacement of involved teeth
Calcifying odontogenic cysts	Incisor and canine region of maxilla and mandible	Maybe well-circumscribed or ill-defined and irregular	Completely radiolucent	Resorption of roots
Odontogenic keratocyst	Ramus-third molar area, anterior mandible, maxillary molar, and cuspid area	Well-defined peripheral, scalloped border	Unilocular radiolucency may sometimes contain the crown of the tooth	Displacement of adjacent teeth is more common followed by resorption of roots
Calcifying epithelial odontogenic tumor	Prevalence in the mandibular molar region is more than the bicuspid region	Appears either as diffuse or well-circumscribed	Appears as a unilocular radiolucency, sometimes seen as a combined pattern or radiolucency and radiopacity	Cortical bony plates are extended and weakened; both buccally and lingually
Adenomatoid odontogenic tumor	Mostly associated with maxillary canines	Well-demarcated smooth corticated border	Juxtacoronal radiolucency that is almost always unilocular sometimes extends beyond the cemento-enamel junction	Divergence of roots and displacement of teeth, maxillary sinus, and orbital invasion

The diagnosis of dentigerous cyst includes x-ray imaging, both periapical and panoramic. Another efficient method is the MRI-Magnetic Resonance Imaging as it helps distinguish cysts and tumors, but is very expensive thus limiting its usage.[11] The latest diagnosing technique is the CBCT-Cone Beam Computed Tomography that provides three-dimensional images with minimal distortion and significantly lower doses of radiation thus ensuring an accurate diagnosis.[12]

The dentigerous cyst gives rise to several potential complications apart from the possibility of recurrence. These include ameloblastoma, epidermoid, and mucoepidermoid carcinoma. Ameloblastoma and epidermoid carcinoma may develop from the epithelial lining or the odontogenic epithelial cell rests. The disposition of dentigerous cyst in the form of ameloblastoma is more profound than other odontogenic cysts. Mucoepidermoid carcinoma is principally a salivary gland tumor that develops from the epithelium of dentigerous cyst containing mucus-secreting cells.

The management of dentigerous cyst includes marsupialization and enucleation. The classic approach involves the enucleation of the cyst along with the removal of affected teeth. As this approach is too aggressive, more conservative approaches need to be incorporated such as Marsupialization, etc.[13,14] Marsupialization is the treatment of choice as it allows the eruption of the impacted tooth by deroofing the cyst along with adjoining bone and mucoperiosteum.[15]

Conclusion

Impacted teeth, especially mandibular third molars and maxillary canines are associated with dentigerous cyst and their evaluation using radiographs is important to investigate the correct method of enucleation, though they are least likely to recur.

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Cast Metal Definitive Obturator Prosthesis for Hemi-maxillectomy Patient: A Case Report

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Abstract

Maxillary jaw defect produced due to traumatic, surgical or congenital reason often creates communication between antrum and nasopharynx. The defect not only causes nasal reflux during swallowing but also interfere with articulation and production of speech. These limitations are commonly overcome by restoration of the defect with a prosthesis called an obturator. This case report focuses on the prosthetic rehabilitation of a post-surgical maxillary defect with cast metal definitive obturator.

Keywords: *Maxillary Defects, Definitive Obturator, Cast Metal Obturator Definitive Prosthesis, Interim Prosthesis.*

Introduction

Surgical rejection of maxillary jaw cancer often compromises the integrity and functions of the oro-nasal cavity.[1] The acquired defect formed due to hemimaxilloctomy, impairs the speech,mastication, swallowing and facial esthetic. Rehabilitation of such defect is essential to improve the functional quality of life and self-esteem of the patient.[2]

A multidisciplinary sequential approach involving oncologist, prosthodontist and speech therapist is required to handle such a case.[3] Surgical repair of such defects can be done with free micro vascular flaps or pedicled flaps, autogenous fibula free graft with or without dental implants.[4] However, in a patient with a large defect, history of irradiation, risk of advanced complications or financial issues surgical repair is avoided. Prosthetic rehabilitation of maxillectomy defect with obturator is the preferred option in such circumstances.[5] It recreates the anatomic barrier between the oro-nasal cavities, thereby improving the fluid intake, deglutition and speech of the patient.[6]

The success of an obturator is often subjected to the size of the defect, the amount of undercut of the defect and the number of remaining teeth. Patients with large intraoral defects have been found to have impair functional adaptability, owing to the bulky size of the obturator. The heavy size of the obturator put continuous stresses on remaining supporting structures jeopardizing their health. On the contrary, a hollow maxillary obturator can reduce the weight of the prosthesis by up to 33%, depending on the size of the maxillary defect.[7] This clinical report describes the step by step technique for fabrication of a lightweight, hollow type definitive cast obturator for a maxillectomy patient.

Case Report: A 34 -year- old female patient had reported to the Department of Prosthodontics, with a chief complaint of a loose obturator with difficulty in taking food with liquid coming out of the nose during intake. Past medical history of the patient revealed that she had undergone left maxillectomy approximately $2^{1/2}$ yr earlier, for inverted papilloma. Postoperatively, she had used an interim acrylic obturator for 1 year.

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The obturator was found to be ill-fitting and grossly undersized compared to the defect itself.

The extraoral appearance of the patient was found to be asymmetrical with a collapsed left side of the lip (Fig. 1). The mouth opening of the patient was normal. Intraoral examination revealed sub-total maxillectomy of left side crossing the midline and hence was diagnosed as a situation of Armany's Class IV (Fig.2).[8] Right side was partially edentulous with missing 16 and a metal crown of 17. All the walls of the defect were lined with healthy mucosa. Tongue function was normal and speech was altered.

Considering the large size of the defect, health of supporting structure and cost, it was decided to rehabilitate the case with a hollow bulb cast metal definitive obturator. The consent of the patient was taken for the same.

Procedure: The procedure was initiated with oral prophylaxis, followed by the removal of the existing metal crown of 17. The finish line of 17 was modified. Primary impressions of the upper and lower arch were made with irreversible hydrocolloid (Tropicalgin; Zermack Dental). Before this, excessive undercuts of the defect were blocked out with a petrolatum laden gauge. The diagnostic casts were retrieved from the impression and upper cast was surveyed and designing of the metal framework of the cast obturator was done on it.

A tripodal design was planned. Embrasure clasps concerning 14,15 and 17,18 were designed for the direct retention of cast framework. The rest seat for 17 was planned to be incorporated in the distal side of its crown. Indirect retention was intended to be obtained from the rest seat on 14 and the palatal extensions on the anterior teeth. Retentive loops were designed on the side of the defect, for attachment of the bulb of the obturator. A full palatal major connector was planned

Necessary mouth preparations were done accordingly followed by an impression of the upper arch with polyvinyl siloxane impression material (Aquasil, Dentsply/Caulk, Milford, DE). The secondary maxillary working cast was made with dental stone type III (Kalstone; Kala Bhai Pvt Ltd., Mumbai, India). It was subsequently duplicated with reversible hydrocolloid to prepare the refractory cast. Wax up of the cast framework on the refractory cast was done as per design, followed by casting with cobalt-chromium base metal alloy (Fig. 3). After proper finishing and polishing, try-in of the metal framework along with a metal crown of 17 was done. Then cementation of the metal crown of 17 was done and the metal framework was seated intraorally. A pickup impression was made with polyvinyl siloxane impression material (Aquasil, Dentsply/Caulk, Milford, DE) in a stock plastic tray, to incorporate the impression of the defect side.

In the final cast, the loops of cast framework were joined with auto polymerizing acrylic resin base covering the defect side. Jaw relation records, teeth arrangement, and try-in were done subsequently in a conventional manner (Fig. 4). After approval of the patient, waxed up prosthesis was processed with heat-cured acrylic resin (DPI-Heat cure; Dental Products of India, Mumbai, India). A sealing the lid with auto polymerizing acrylic resin was used to obtain a hollow bulb obturator.[9] The obturator was subsequently tried in the patient's mouth for adaptability (Fig. 5A,B,C). A permanent soft liner (Ufi Gel C) was applied around the bulb and border of the defect portion of the prosthesis. Necessary border molding was accomplished and the patient was asked to use the relined prosthesis for 1 week. Afterward,the relined permanent soft liner was replaced with heat cure denture material (DPI-Heat cure; Dental Products of India, Mumbai, India) The definitive obturator was positioned intraorally and assessed for nasal regurgitation and quality of speech production. Post insertion oral hygiene instructions and maintenance were given to the patient. The case was followed up for a period of 1yr, during which the patient was found to be satisfied with the prosthesis functionally and esthetically.



Figure 1. Extra Oral View



Figure 2. Intraoral defect of palate



Figure 3. Cast metal framework



Figure 4. Try in of teeth arrangement



Figure 5a. Post insertion extraoral view;
Figure 5b. Post insertion intraoral view; Figure 5c. Post insertion intraoral view

Discussion

The present case is a classic Armany class IV type defect, as it has crossed the midline and included the premaxilla of the opposite side. (Armany classification I) It was a 2yr old post-operative case with no recurrence of the disease. The wound had healed properly. So it was a favorable case for the definitive cast obturator prosthesis.[10]

Preservation of remaining structure has been the motto of Prosthodontic therapy.[11] This is arguably achieved with the cast metal definitive obturator. As the metal framework stabilizes the prosthesis, soreness and discomfort of soft tissue in the superior- lateral aspect of the defect are reduced. So the jaw relation record and wax try-in of the prosthesis are accurate in terms of esthetic and phonetics.[12] In comparison to the conventional acrylic obturator, it also provides maximum support and retention.[12] So the abutment teeth and soft tissue undercuts were subjected to less stress. Further, midline fatigue fracture is not an issue with cast obturators.[13]

A tripod design framework was planned for the prosthesis, as the remaining teeth were present in a curved manner, with 12 and 13 being still there. Primary retention, support, and stability of the prosthesis were provided by the remaining teeth.[14] Soft tissue undercuts of the defect were used for added retention. Support was obtained from the remaining teeth as well as palate. Definitive rest seats located on the premolars and molars provided the support. As the defect size was large, a full palatal major connector was used for the maximum distribution of functional load.

The definitive obturator design was made hollow to decrease the weight of the large prosthesis. Otherwise, the remaining teeth would have been subjected to persistent non-axial cantilever load, jeopardizing the health of supporting structures.[15] In contrast, a hollow bulb obturator is lighter and promote favorable palatal contour.[7] Moreover, the resonance of speech is achieved as it simulates the functional anatomy of the maxillary sinus. Proper nutritional intake and ability to communicate without nasality is achieved immediately with definitive maxillary cast obturator.

Conclusion

Prosthetic rehabilitation of maxillectomy defect with definitive obturator restores the mastication,swallowing, aesthetic, resonance and speech of the patient. This helps the patient to resume a normal social life.

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Management of a Diabetic Patient with Philosophical Mental Attitude: A Case Report

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Abstract

Diabetes Mellitus is a metabolic disorder. It has been recognized as a pandemic and is a nutritional disorder. It affects almost every organ system in the human body, including the patient's psychology. It occurs due to irregularity in the insulin secretion. The most characteristic feature is hyperglycemia. The underlying factors, contributing to this disease are age,genetics, obesity, lack of physical activity, etc. A prosthodontist should have a thorough understanding of the general considerations for diabetes mellitus patients as well as the prosthodontic consideration. This article reviews the prosthodontic management of a patient with diabetes mellitus, supported by a case report of a complete denture patient with a philosophical mental attitude.

Keywords: *Diabetes, Clinical features, Prosthodontic management, Dental.*

Introduction

Diabetes mellitus is a metabolic disorder. The abnormality in insulin secretion affects the carbohydrates, lipids, and protein metabolism. The most important basis of diagnosis is hyperglycemia. This is a chronic condition that may have other neurological, cardiovascular, cerebrovascular, nephrological, ophthalmologic disorders associated. The understanding of systemic effects of diabetes mellitus, its effects on the oral tissues, patient psychology and other complications associated with long-standing disease is essential to treat a diabetes mellitus patient, reporting in the dental office.[1,2] The onset of diabetes mellitus Type -2 is usually after 40 years of age and its prevalence increases with age. This is related to genetics, obesity and also lack of physical activity and not to the destruction of Island cells of Pancreas.[3,4,5]

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Polyuria, Polydipsia and Polyphagia are the main symptoms for diagnosis.[6]

World Health Organization has predicted that by 2025, there will be a rise in prevalence of 300 million from 135 million cases, which is alarming .[7] American Diabetic Association recommends that the screening for Fasting blood sugar should be done for overweight individuals, no matter age, if Body Mass Index is more than 25 and every 3 years from 45 years of age, if the subject is obese.[8,9] The earlier the diagnosis is made, the better it is for the patient. The issue is patients report only after they have symptoms like polyphagia, polydipsia and polyphagia.[10]

Oral Manifestations:

The diabetic patients have the following oral manifestations:[8]

- Burning sensation of the mouth/mucosa
- Inflammation of gingiva and periodontitis
- Increased risk of caries
- Resorption of alveolar bone
- Delayed wound healing
- Opportunistic infections like fungal infections
- Xerostomia

The tolerance to removable prosthesis, like complete denture is also less.[11]

All the above oral manifestations occur due to:[8,12]

- Micro-vascular changes
- Increased concentration of glucose in saliva
- Impaired host-resistance which is a result of the defective function of Polymorphonuclear Neutrophils

Prosthodontic Considerations:

A few general dental considerations to be followed by the prosthodontist:[13]

- Proper history-taking of general health conditions and verification of blood reports
- A consultation with the patient's physician regarding his medications and dental procedure planned is beneficial.
- Radiological investigations should be done for all cases.
- Oral hygiene should be good, before starting any procedure. The patient should be advised to use anti-bacterial mouth-washes and gels.
- The procedures causing minimal trauma and that are minimally invasive should be carried out
- The procedures should be in a stress-free environment as possible
- Appointments should be of short duration
- If there is a chance of any pain due to invasive procedures, prophylactic medication is mandatory.
- Medication and dietary counseling is a must for every case.
- Hypoglycemic shock should be managed properly with proper monitoring of vital signs, depending on the severity. Nausea, perspiration, hunger, palor and tachycardia are indicative of it.

Following are the Prosthodontic Considerations:

- As mentioned earlier, general health history taking should be followed by dental history taking, assessment of the findings and thorough examination.
- Oral hygiene status should be evaluated before beginning any prosthetic procedure. The patient should be counseled for the maintenance of good oral hygiene and its importance.
- History of edentulism, cause of loss of teeth, type of prosthesis, etc should be noted.
- The number of appointments and duration of treatment should be planned and informed to the patient.[13]
- Recent reports of visits to the treating physician, along with laboratory investigation reports should be presented in the clinic. This should be brought to the clinic in every sitting.
- The blood sugar level should be checked with a glucometer, before starting the procedure, in every sitting.[14]
- Oral hygiene procedures like scaling and root-planing should precede removable partial denture therapy.
- The design of RPD should be appropriate, to make the prosthesis tissue-friendly.
- The prosthesis should be self-cleansing and should have point contact between natural abutments and prosthesis
- The occlusion should be freely gliding in all prostheses, thus the scheme should be carefully selected.
- In case of complete denture, the techniques that would retard resorption of bone should be followed. They are: mucositis impression technique; use of tissue-friendly material; verification of Vertical Dimension at occlusion; use of neutral zone concept wherever required
- Regular follow-ups are mandatory
- For Fixed Partial Denture prostheses: Supra-gingival margin with chamfer finish-line on the facial surface is preferable as it reduces stress-concentration; Crown-root ratio should never be compromised; Ante's Law should be mandatory; 3/4th crowns may be advisable in premolars;Periodontal therapy, Crown-lengthening, orthodontic extrusions may improve the prognosis of the treatment.
- Implant treatment should be planned only when the blood sugar levels are within permissible limits. Besides, the above points should also be considered .[9] Complete history taking, radiographic evaluation, implant site selection, abutment selection, prosthesis selection and scheme of occlusion determine the success of implant therapy . [15]

- The mental attitude and impact of the treatment on the psychology of the patient should also be tactfully evaluated in all the cases.[16]

Case-Discussion:

A 78-year-old man reported to the Department of Prosthodontics and Crown & Bridge, Institute of Dental Sciences with the chief complaint of difficulty in chewing and wanted a new pair of dentures. The detailed case-history was taken which gave the following findings: Patient was diabetic for more than 20 years and was under regular medications; blood sugar levels were remaining under control, according to last 3 years reports; the patient had taken prior permission from his endocrinologist for the treatment; all the teeth were missing; the patient was an old denture wearer; denture was fabricated elsewhere 4 years back and no follow-ups were done; on examination, it was found that the oral mucosa was in good condition, which suggests of maintenance of oral hygiene **[Figure 1]**. There were retention and stability issues in the old denture and the patient insisted on a new set of dentures. There was no history of any habits. The patient was under the regular supervision of a dietitian. He was aware of his meal timings and followed the dietitian's instructions. The treatment was planned with seven short, morning appointments, between 9:30 AM to 10:30 AM. The patient was explained about the procedure and also counseled regarding the importance of follow-up procedures. He had reasonable questions regarding the treatment which were satisfactorily answered, which indicated that he understood the treatment. He was very calm, willing to commit to the treatment and follow the instruction.



Figure 1: Intra-oral pictures of the edentulous arches

First Appointment: The perforated stock trays were selected. Impressions were made using alginate (Zhermack, Tropicalgin). The patient was recalled for the second appointment. The primary cast was poured using Type II gypsum (B.N. Stone Dental Plaster)in the laboratory. The markings for the spacer extensions and special tray extensions were done. The full wax spacer was designed.



Figure 2: Final impressions of the edentulous arches after the border molding

Second Appointment: Border-molding was done using the sectional method. The final-impressions were made with Zinc-Oxide Eugenol Impression Paste (DPI) [Figure 2]. This appointment was of the longest duration, i.e, 1 hour. The patient was given a break for 10 minutes after 30 minutes. The impressions were beaded and boxed in the laboratory. The master cast was poured using Type III gypsum (B.N. Stone Dental stone).

Third appointment: Adjustments on the temporary record bases with occlusal rims were done. Silvermann's closest speaking space was measured and Niswonger's method was used to record the vertical jaw relation **[Figure 3]**. Teeth set was selected in the clinic (Acryrock Teeth set). The pressure-less method was used to record the horizontal jaw relation. The related and sealed rims were transferred to the master casts. Articulation was done in a 3-point mean-value articulator. Anterior teeth arrangement was done.



Figure 3: Markings for jaw-relation

Fourth and fifth appointment: Trial of anterior teeth arrangement was done. The patient was recalled for the fifth appointment, immediately the next day. The posterior teeth arrangement was completed and the trial was done with necessary modifications. **[Figure 3]**. In the laboratory, carving and wax-up was completed. The dentures were processed, cured, and finished for the insertion step. The finished denture was remounted and corrections were done before the final appointment.



Figure 4: Wax trial

Sixth and seventh appointment: The finished denture was inserted **[Figure 4]** and all the necessary verifications were made. The occlusion was modified. The patient was instructed to use the denture for 24 hours and report for a follow-up the next day. Other post-insertion instructions were given. The oral mucosa was examined thoroughly for pressure sores. Modification to remove pressure points on the tissue surface of dentures were made. Final polishing of the dentures was done and dentures were re-inserted. The patient was asked for reviews and was also made to understand to report after a week and then after a month.



Figure 5: Intra-oral pictures after denture insertion

The patient had reported for the one week and one-month follow-ups. During the treatment, the patient would give his input regarding the steps. Every instruction was sincerely followed by the patient. He never reported with any attendant who speak for him, suggestive of his independent mind.

Conclusion

The prognosis of the treatment was good because the patient's mental attitude was the most important determinant. He maintained himself well, despite his long history of Diabetes mellitus, understood his needs and was committed to being treated. The patient's philosophical mindset benefitted him for a positive treatment outcome.[16] Diabetes mellitus is a complex disease and requires a multi-disciplinary approach in the treatment. Patient's motivation for treatment increases the chances of a better prognosis. The goals of prosthodontic therapy are also reached with proper treatment, counseling by the clinician, and a patient's positive approach towards treatment.

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Tissue Engineering and Regeneration Therapy: A Boon for Periodontal Research

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Abstract

Complete regeneration of the periodontium is the aim of any successful periodontal treatment. Recent Technologies have come up with many advances like cell-based therapy, peptides, genetic transfer, scaffolds, bone anabolics, and above all lasers. Four basic elements are required for periodontal repair and regeneration that includes adequate blood supply and wound stability, a source of bone and ligament forming cells, a supporting scaffold, and growth factors to regulate cell migration, synthesis and angiogenesis for revascularization of the site. The newer technologies not only offer unique opportunities but also enhance the predictability of regenerative procedures.

Keywords: *Periodontal Regeneration, Research, Tissue Engineering.*

Introduction

Chronic periodontitis is characterized by irreversible loss in connective tissue attachment and supporting alveolar bone. According to national surveys, more than 30% of adults suffer from moderate type of periodontitis, while severe generalized periodontitis affects almost 15% at some pointin time.[1] Scaling, curettage and open flap debridement are conventional treatment modalities that can control inflammation and lead to the long junctional epithelium formation but there is no regeneration. They have also improved the quality of life of individuals.[2] The main objective of periodontal tissue replacement and reconstruction is to restore mechanical stability and function and also relieve the pain associated with it.[3]

Regenerative Concepts: Melcher 1976, proposed that after flap surgery the curetted surface of roots might be repopulated by different cell types.[4]

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- Cells of Connective tissue in gingiva
- Epithelial cells
- Bone cells
- Cells of Periodontal ligament

The nature of the attachment depends on the cell type that starts populating the root surface after periodontal surgery.

Healing patterns of periodontal tissue damage:

- Down growth of epithelial cells results in a long junctional epithelium.
- Connective tissue might proliferate to cause tissue adhesion and thereby root resorption.
- If the bone cells are predominant,there may be root resorption,ankylosis, or both.
- A completely regenerated periodontium and new cementum can also develop.

How can the lost tissues be ideally replaced?: The concept of tissue engineering is based on the theory that an individual's damaged tissue is better to be replaced by the same natural healthy tissue. This concept has led to the formation of new tissue from preexisting tissues.

Tissue engineering, is a multidisciplinary field that

involves medicine, biology, and engineering. It is likely to revolutionize the quality of life and health of many people worldwide. It can maintain,restore and improve the tissue function respectively. This definition has been broadened recently to include the suitable attempt of tissue regeneration in the body, by the addition of specific biologic mediators,that might be cultured in or outside the body respectively.

Components of tissue engineering:

For the success of tissue engineering three components are needed:

1. To create new tissue: Implanted cultured cell
2. To act as a scaffold to hold cells: a suitable Biomaterial.
3. To form desired tissue type: Biological signaling molecules that can instruct cells.

Cells:

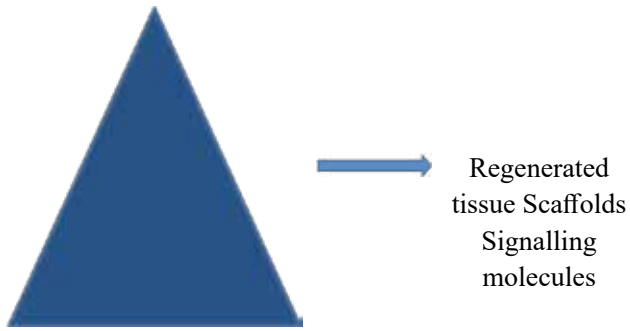


Figure 1. Triad

In vitro: In the laboratory, the construction of a vital tissue can be done and it can be subsequently implanted into the host body. This offers the advantage to examine the tissues as they are being formed and also measure the specific tissues.

In vivo: Properly seeded cell types, implantation of the porous matrix, & signaling molecules can facilitate tissue regeneration. This forms the basis of Tissue engineering.

Strategies to engineer tissues

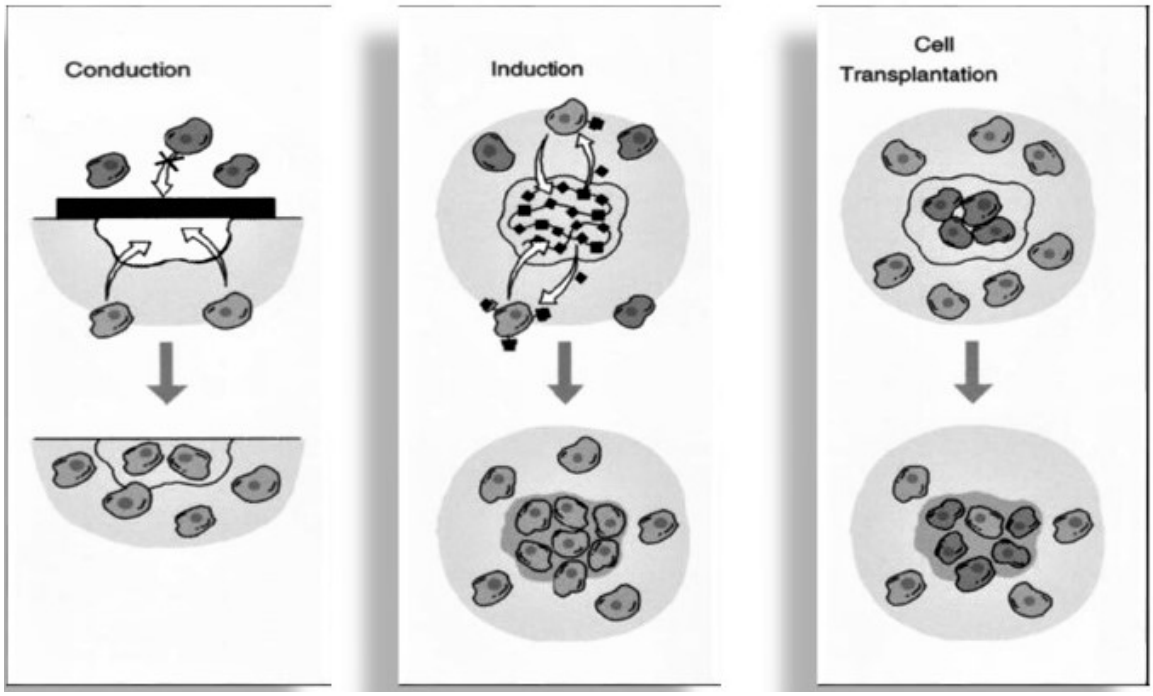


Figure 2. Tissue engineering process

Osteoconduction: "Trellis effect" is a physical effect where the graft matrix forms a scaffold. This scaffold favors cells from outside to enter the graft, thereby forming new bone.

It includes the growth of osteoprogenitor cells, new capillaries, and perivascular tissues from the recipient bone.

Eg: Jaw bone graft, xenograft

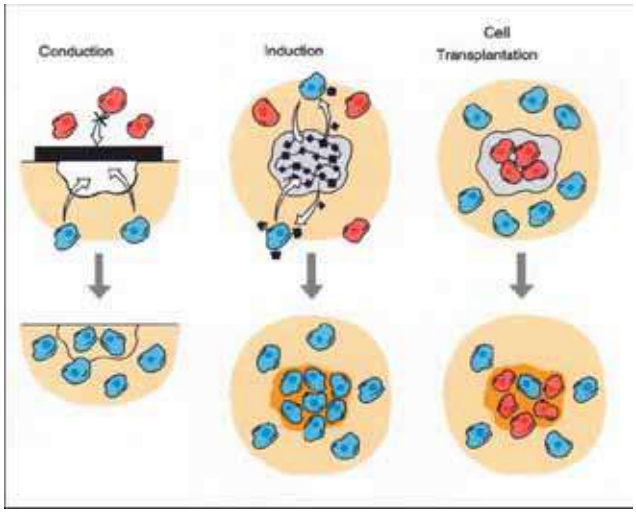


Figure 3. Osteoconduction

Osteoinduction: Biological signals are used to activate the cells close to the defect site.

Bone morphogenetic proteins (BMPs) are the key elements, inducing bone formation.

Limitation:

- It is not possible to know about inductive factors thatare specific for a tissue.
- For the delivery of genes and growth factors to the host site, a biodegradable polymer scaffold can be used as a vehicle.

Advantage:

- The release of growth factors or genes can be controlled based on the polymer breakdown rate.

Cell Transplantation: The cells that are grown in the laboratory are directly transplanted. A vehicle is also used for delivery so that cells and partial tissues can be transplanted to the host site.

Periodontal tissue regeneration:

- Grafts
- GTR
- Growth factors
- Cell sheet engineering
- Enamel matrix protein derivatives
- Stem cell therapy

Advanced regenerative techniques:

- Protein/peptide therapy
- Cell-based therapy
- RNA or gene therapy
- Lasers

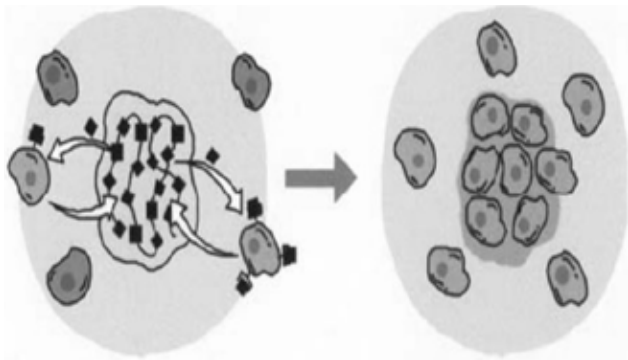


Figure 4. Osteoinduction

Scaffolds: Scaffolds provide and maintain the space that is needed for cell growth and also provide physical support to the process of healing.

Properties of a scaffold:

- To provide a 3D architecture for desired shape volume, and mechanical strength.
- To provide proper characteristics, likeporosity, hydrophilicity and to facilitate tissue infiltration.
- To provide biocompatibility.
- To provide controlled degradation rate coordinated with tissue regrowth.

Used to

- **Guide:** Growth & differentiation of cells
- **Organize:** Physical & chemical signals.

Types of scaffolds:

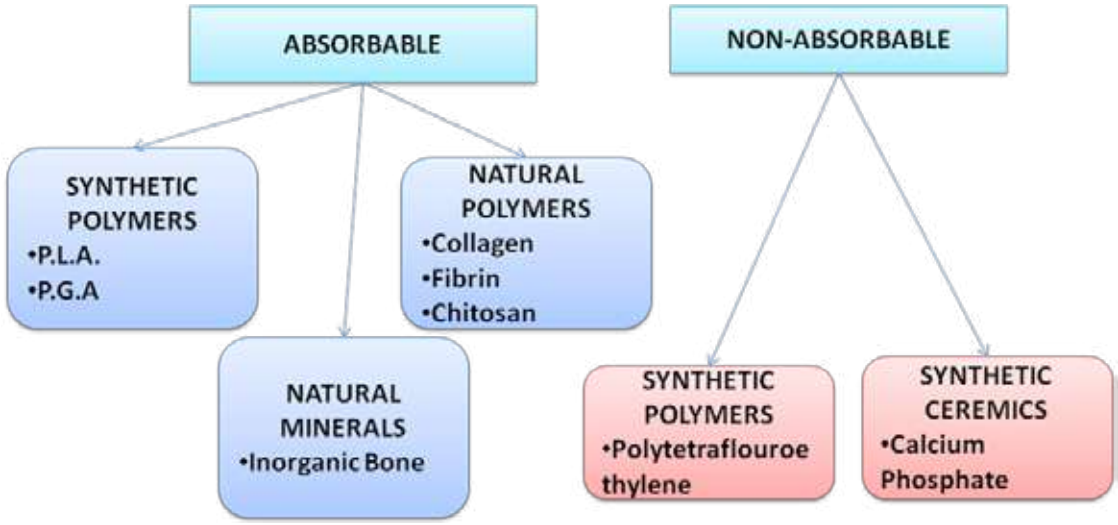


Figure 5. Types of scaffolds

Imaging-based, computer-aided design: According to Park CH 2001 CAD-CAM is one of the most recently developed techniques in scaffold fabrication, that provides a personalized solution for tissue engineering.[5] This technique acquires the 3D anatomic geometry of a defect by high-resolution CT or MRI and that is used as a template for a scaffold. Then 3D bioprinting with desired biomaterials fabricates the scaffold.

Natural minerals or bone grafts:

Bone graft:

Autograft (autologous graft): An organ or tissue transplanted from one site to another in the same person.

Isograft (Isogenic, Syngenic): Graft transplanted from one person to another with identical genetics (eg: identical twins)

Allograft (Homograft): An organ or tissue transplanted from one person to another of the same species but different genus.

Xenograft (Heterograft): A graft transplanted between members of different species (eg: ox bone to humans).

Alloplast: It is synthetic bone graft material or bone graft substitute.

Orthotropic graft: A graft positioned in an appropriate site (eg: bone placed in a bed of bone)

Heterotropicgraft: A graft positioned in an inappropriate site (eg: bone placed in a bed of muscle tissue)

Ideal requisites of bone graft material[6]:

- It should not impede bone growth
- It should enhance revascularization
- It should not elicit immunological responses
- Easy availability.
- Rapid osteoinductive, osteogenetic and. Osteoconductive effect.
- It can be help forming new attachment in periodontal defects.

Limitation of allografts and autografts:

1. There is no excess tissue store in humans for transplantation.
2. While replacing lost bone, donor site morbidity and problems in anatomy, might be there.
3. Immunologic response in allografts due to differences in genes, and can induce transmissible diseases.
4. Synthetic material replacements such as dental implants face a common problem with natural defense mechanisms.

Absorbable synthetic polymers:

They undergo degradation by the process of hydrolysis

Polyglycolic acid - faster degradation

Polylacticacid - this is most stable in-vitro

Polyglactin 910, a copolymer of glycolide and L-lactide – 90/10 molar ratio

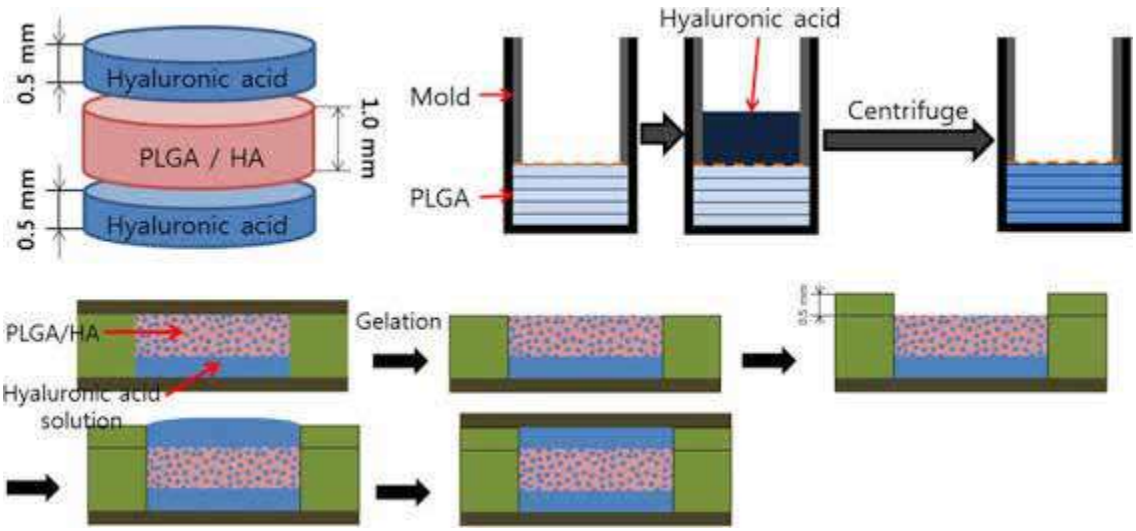


Figure 6: Manufacturing synthetic polymers

Synthetic ceramics(non-absorbable)

These are matrix materials used for facilitating regeneration in-vivo[7]

1. Tricalcium Phosphate:

This is a porous form of calcium phosphate

Ex: ß-TCP

But it undergoes physiochemical dissolution after implantation.

2. Synthetic Hydroxyapatite:

It is a secondary form of bio-ceramic.

Rationale for its use–the naturally occurring mineral in bone is hydroxyapatite

Guided Tissue Regeneration: GTR refers to the placement of a membrane that acts as a barrier between the flap and root surface preventing the gingival epithelium and connective tissues from contacting the root surface during healing.[8]

Design criteria for periodontal GTR devices:

- Easy usage
- Biological activity
- Cell exclusion
- Biocompatible
- Maintenance of space
- Tissue integrity

Barrier membrane for GTR Gottlow's classification:

- First-generation (Non-resorbable membranes)
- Second generation (Resorbable membranes)
- Third generation (Resorbable with growth factors or antibiotics)

Non-resorbable synthetic polymers:

Expanded polytetrafluoroethylene (ePTFE):

- This is the first commercially available barrier for GTR (Gore-Tex)

- Solid nodes and fibrils make itmicrostructure porous.
- The barrier is made of various sizes and shapes to exactly fit periodontal defects in different locations.

Titanium-reinforced PTFE membrane

- Titanium is reinforced between the two e-PTFE layers, thereby a device is formed.
- Facilitates improved space provision and maintenance
- Permits correction of larger defects

Miscellaneous membranes (Millipore membrane, resin-ionomer barrier, rubber dam)

Disadvantages:

- Require a second surgical procedure to remove the membrane and is done usually 3-6 weeks after the intervention when initial healing has occurred
- May interferes with healing by inflicting damage to sensitive regenerated tissue during removal.

Resorbable membranes:

Biomaterials: In cell-based tissue engineering, the expanded cells are seeded onto a scaffold synthesized with the best biomaterial available.

A. Naturally derived materials: Advantage: Along with cellular tissue matrices they offer the advantage of biologic recognition.

B. Synthetic polymers: Advantages: they can be produced in laboratories with the required properties such as increased strength, lowerdegradation rate, and improved microstructure.

Advantages of biomaterials: They mimic the mechanical and biologic functions of the native ECM. The ideal properties of biomaterial arethat it should be biodegradable and bioresorbable. It should also not cause inflammation of the tissue.Incompatible materials can lead to necrosis and foreign body reactions.

Natural biomaterial:

- Collagen (Biomend, Biogide, Biosorb, Ossix plus)
- Glycosaminoglycans (GAGs)
- Chitosan
- Alginates
- Duramater, Cargile membrane

Collagen: It is a major protein of the extracellular matrix, and it has a long triple-stranded helical structure which provides stiffness.

Type 1collagenis found in skin and bone,

Type II foundin cartilage

Type III seen in the blood vessels lumen.

Properties:

- has low immunogenicity
- Is hemostatic, inducing platelet aggregation, which facilitates early clot formation and wound stabilization
- Attracts and activates periodontal ligament and gingival fibroblast cells
- Inhibit epithelial migration

Advantages:

- Lysosomal enzymes sequentially attack and degrade the Collagen implants.
- By controlling the density of the implant and the extent of intermolecular cross-linking resorption rate can be regulated *in vivo.*
- A high rate of implant degradation is due to low density and increased interstitial space for cell infiltration.
- Cell-adhesion domain sequences are seen in Collagen that exhibit very specific cell to cell interactions.

Bio-Mend:

- Is a type I collagen GTR membrane
- Derived from bovine deep flexor tendon
- Is semi-occlusive with effective pore size 0.004 μm
- It is completely absorbed in four to eight weeks.
- Limitation- compromised space maintenance

Artificial biomaterials:

Synthetic polymers:

- Polylactic acid (Atrisorb, Epiguide, Guidor)
- Lactide/glycolide copolymers (Ethisorb, Vicryl mesh)
- Polyurethane
- Polyesters of naturally occurring -hydroxy acids, including PGA, PLA, and PLGA,

Synthetic membranes:

GUIDOR matrix barrier:

- First FDA approved membrane
- It is a double-layered absorbable device made of poly(1actic acid) and a citric acid ester?
- The external layer is designed to allow the integration of the overlying gingival flap.
- Containsrectangular perforations (400-500/cm^2)
- The internal spacers between external and internal layers create a space for the tissue can grow.

ATRISORB barrier:

- It is a poly(DL-lactide) polymer.
- This has been the only approved GTR device to be manufactured chairside.
- This is supplied in a flowable form.
- It can be cut into desired shape and size
- It can be placed into the defect using gentle pressure. Sutures aren't required.
- It is 600-750 μm thick and has properties like adhesion.

VICRYL Periodontal Mesh:

- It is a polyglactin 910 fiber, a copolymer of glycolide and L-lactide (90/10 molar ratio), that is used to prepare a tightly woven meshwork.
- Polyglactin sutures are placed through its coronal margin to anchor it to the tooth.
- Within two weeks of placement, it can lose integrity and resorption is within four or more weeks, depending on the host species.[9]

Polyurethanes membrane:

- These are organic polymers containing the urethane group. According to a study by Warrer et al. 1992 when polyurethane membranes were used for GTR in non-human primates, there was severe inflammation in the margins of the flap,and the recession was also more compared to the poly-(lactic acid) membranes treated tooth.[10]
- Therefore it was not suitable for GTR, as they did not possess all the necessary qualities.

Third generation membrane: They act as a barrier and delivery devices. Growth factors, adhesion factors, antibiotics etc., are released at the wound site.

Subdivided as:

Barrier membranes with Antimicrobial activity: ChengCFet al 2009concluded that the addition of "amoxicillin" or "tetracycline" is addedin GTR membranes,it improves the periodontal ligament cell attachment.[11]

Barrier membranes with Bioactive Calcium Phosphate: This is a three-layered membrane. There are two sides, porous side containing nano-carbonated hydroxyapatite that allow cell in growth and a pure PLGA non-porous side which doesn't allow cells to adhere. These two sides are separated by a transitional layer consisting of nCHAC/PLGA.

Barrier membranes with Growth Factor

Growth factors are proteins. They are naturally occurring and regulate various aspects of cell development.

Rathva VJ 2011 carried a study on bioactive molecules such as different growth factors and enamel matrix derivative (EMD).They concluded that periodontal regeneration is possible.[12]

Functionally Graded Multilayered membranes

Bottino MC 2011 designed FGM. This was fabricated via a multilayering-spinning process consisting of a "core-layer"and two functional surface-layers. [13]

Platelet-Rich Fibrin membrane

First developed in France by **Choukroun** et al.[14]

Characteristics: PRF has a fibrin network and that leads to more effective cell proliferation and migration and thus cicatrization.[15]

Many growth factors, such as platelet-derived growth factor (PDGF) and TGF-β, IGF, VEGF, and thrombospondin,etc., are released from PRF.[16]

Conclusion

Advances have been made in the treatment of complex alveolar bone wounds and periodontal wounds. Be it scaffolds for cell, protein delivery, or gene delivery to specific targeting of signaling molecules, all are in various stages of development. Providing growth factors to the periodontiumis considered as new knowledge revolutionizing the regeneration process.

Conflict of Interest: Nil

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Psychological Factors in Oral Lichen Planus

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Abstract

It has been known for centuries that mental/emotional factors are correlated to numerous physical diseases. Traditionally we regard mind (psyche) and body (soma) separate but where and how do they interact? The very idea of psychological medicines was told by Sigmund Freud, who used conversion hysteria in explaining the effects of emotional disparity turned into bodily or somatic symptoms

Keywords: *Oral Lichen Planus, Psychological Factors, Oral Health.*

Introduction

Fitness is a state of total"physical", "mental" and "social" wellbeing and not just the lack of sickness or illness.[1] "Oral health" is essential to all-purpose health and also a significant part of universal physical condition. "Psychosocial factors" are necessary and essential for health and are important in disease.[1,2] Psychosocial factors encompass both psychologic issues (those that arise predominantly from within the individual) and sociologic issues (external factors). Mental/emotional factors are connected to numerous physical diseases. Traditionally we regard mind (psyche) and body (soma) separate but where and how do they interact? The very idea of psychological medicines was told by Sigmund Freud, who used "conversion hysteria" in explaining the effects of emotional disparity turned into bodily or somatic symptoms.[3] In many illnesses, there is an interplay of psychological and physical factors. Frequently psychological disorders present in the physical guise of there may be a strong underlying psychological element in a somatic condition. It is now well recognized that psychological states can produce somatic symptoms, anxiety rates are shown to cause autonomic arousal, heightened muscle tone and over-breathing and hence a wide range of bodily symptoms. Depressive illness can lead to weight loss, tiredness, loss of appetite and pain or an existing pain can be aggravated. However, patients attribute these somatic syndromes to serious physical disease. The oral mucosa is very sensitive to psychological events and oral mucosal lesions can occur as a result of psychiatric influences. Psychological disorders may be manifested as atypical odontalgia, TMJ disorders, aphthous stomatitis,burning mouth syndrome."[4,5] In chronic pain syndromes, there is often a psychological behavioral overlay of symptoms that come part and parcel of the disease. Treating the psychological and behavioral problems with physical aspect may increase the chances of a successful therapeutic outcome.[6]

Classification: "Psychosomatic disorders"are widely classified depending upon the presence of tissue injury or not.[10] Under the category "Physiological malfunction arising from mental factors", these are regulated through the autonomic nervous system. Included in this group are breathing disorders, such as hyperventilation and psychogenic cough; cardiac problems, pruritis. The psychological factors are related to a disease succession, like asthma, dermatitis, eczema, stomach ulcer and ulcerative colitis.

"Zegarelli E.V., Kutscher A.H. and Hyman" G.A. (1978) Classified "psychosomatic disorders" as[11] Psychoneurotic disorder, Psychophysiological disorder, Personality disorder, Psychotic disorder

Classification:

- **Shklar et al. (1980)[12]:** Oral psychosomatic diseases; lichen planus; recurrentaphthous stomatitis; "glossitis and stomatitis areata migrant" psychologic factors can be aetiological factors in oral diseases like:"erythema multiforme, Mucous membrane pemphigoid" and Chronic periodontal disease.

- emotional stress serving as a contributing factor in "herpes labialis, Necrotizing ulcerative gingivitis
- neurotic habits leading to leukoplakia, "biting of the oral mucosa, (self-mutilation), physical/mechanical irrigation, dental/periodontal disease produced by "bruxism", "neurotic oral symptoms, glossodynia (glossopyrosis), dysgeusia, mucosal pain"

Bailoor and Nagesh (2001) classified oral psychosomatic disorders as [13]Pain associated disorders, MPDS, "atypical facial pain, atypical odontogenic pain", diseases related to altered oral sensation, burning mouth, xerostomia, idiopathic dysgeusia, miscellaneous, oral lichen planus," recurrent aphthous ulcers, psoriasis, erythema multiforme, hypochondriac disorder".

Review of Literature on Oral lichen planus: Oral lichen planus is a common chronic immunologic inflammatory mucocutaneous disorder. Many authors have concluded the association of psychological stress in "oral Lichen planus".Kovesi G. and Bancozy J. (1973)[16] were made the diagnosis of oral lichen planus on 326 patients between 1960-1969, and they found psychological factors appeared to be an important cause. Shafer W.G. et al (1983)[17] stated that lichen planus was associated with several stress-related medical disorders such as diabetes and hypertension. Lowental U., Pisontis (1984)[18] examined 49 patients with clinically diagnosed oral lichen planus. In erosive and bullous lichen planus, stressful events happened before the occurrence of lesions. Similar findings were shown by Scully M. and El-Kom M. (1985)[19], Hampt B. Goronc (1987)[20], Humphris G., Field E.A. (1992)[21], Sebastian J.V. Bogan (1992).[22] McCartan B.E. (1995)[23] studied 50 subjects with oral lichen planus for any"anxiety and depression" and for behavior problems. Nervousnesslevel, as considered on the "hospital anxiety and depression (HAD) scale", was eminent in 51% of subjects, while depression scores, were low in everyone. Sugerman P.B. et al (1995)[24] assessed the probablepart of stress derived HSP (Heat Shock Protein) expression using avidin-biotin complex immunochemistry with an "anti HSP-70" polyclonal antibody, in normal oral mucosa sections of OLP, "nonspecific oral ulceration" and dysplastic OLP. They established that there was a statistically significant difference in vertical and horizontal staining allocation when other groups compared with OLP. Chaudary S (2004)[27] Studied the importance of psychological stressors in patients with oral lichen planus.

Conclusion

The oral cavity is extremely responsive to psychologic stimulus, and in few subjects oral problems may be a straightappearance of psychological issues, while in somefactorsproblems of the oral mucosa may be the circuitouseffect of an emotional conflict. The fundamental concept in mental medicine was initially proposed by Freud, who coined "conversion hysteria" in unfolding the response in which disturbing conflicts are distorted into physical or somatic symptoms. Since the oral tissues have a high psychologic potential, oral symptoms are common psychosomatic manifestations.

The oral mucosa bears on an obvious role as on erotogenic zone, and oral manifestations resulting from emotional disturbance should be reasonably common. The overall relationship of psychologic factors to oral mucosal disease maybe representing different ways in which emotional stressful stimuli act on oral mucosal target sites.

A considerable evidence supports that psychological stress is a chief causativereason in these 3 disorders. In lichen planus and glossitis areata, burning tongue, and burning sensations of the oral mucosa are invariably neurotic symptoms and indicative of underlying psychologic problems, which are often severe and require appropriate psychiatric management.

"Burning" symptomatology in the absence of clinical disease must be considered to have a neurotic or neurologic origin. We should point out to the enduring that "neurotic is normal", and everybody is anxious to some extent, but that every person intuitive responds to emotional stress in different ways. Patients with oral featuresbecome visible to be suffering from sadness or other psychological disorders that require a mental status evaluation and psychiatric management.

The word"phobia", which means fear in Greek, are severalemotional and physiological problems that can range from grave disabilities to frequent fears to minor quirks. All fears and phobias, cancerophobia is shaped by the unconscious mind as a defensive mechanism.Several times, there may be an incident relating to oral cancer and emotional conflict, while the initialchannel may have been a real-life scare of some type, the condition can also be triggered by events like natural disaster, TV or perhaps seeing someone else experience trauma. It is an excessive and unreasonable fear of cancer in patients who have either a relative or friend who is suffering from

cancer or who themselves have a chronic oral lesion such as an ulcer, any growth or patch in the oral cavity which defies diagnosis or treatment.

When we focus on the etiology of the disease, many patients were relieved to know that the lesions were not due to some infection or neoplastic disease but as a result of "disease of civilization". Future studies to associate the psychological disorders with the immunological basis of the disease needs to be done for appropriate management and treatment planning.

Conflict of Interests: None

Ethical Permission: Approved

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Reattachment of Fractured Tooth-Series of 2 Case Reports

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Abstract

Most common consequences of trauma is the crown fracture, mainly maxillary anterior tooth due to its forward positioning in the arch. Understanding the outcome of the planned treatment gives an idea regarding the prognosis of that particular treatment. Various options are available for the treatment of fractured tooth. Among all reattachment of fractured coronal segment to that tooth provides instant esthetic, as it restores the original form, colour, contour, surface texture and also gives a psychological comfort to the patient. This clinical case report focuses on the successful reattachment of two fractured coronal crowns using glass-fibre reinforced composite post. Since, both of the fractured teeth were of complex type, endodontic treatment was performed for both of the cases. At the end, a natural-looking restoration is achieved.

Keywords: *Crown fracture; Fractured coronal segment reattachment; Glass-fibre reinforced Composite post; Natural-looking restoration.*

Introduction

Fractures of the coronal portion of anterior teeth are most common during trauma. Esthetic discrepancy due to fractured tooth may induce psychological distress to the patient. Retaining the natural tooth with the same quality and function is a challenge in the field of dentistry. Prognosis of fractured tooth also depends on the position of crack line. More apically the split of the tooth extends, poor is the prognosis of that particular tooth. Due to the lack of ability to explore and to seal the fracture line in splitted tooth, prognosis of the split tooth mainly anterior is considered as poor.[1] Out of all trauma to the dental hard tissue, coronal fracture of anterior tooth represents 18-22%, 28-44% are simple fracture (involves enamel and dentin), 11-15% are of complex fracture (involves enamel, dentin and pulp).[2] From which maxillary central incisors involves 96%, because of its position in the arch.[3]

Ideally, the broken part of the tooth should be restored with composites. Since colour mismatch, periodical wear and staining are the major drawback of composite, it would better to restore the tooth with its fragment, if the fractured segment is available, as it provides good and long term esthetics. Additionally reattachment permits restoration of the particular tooth with very minimal sacrifice of the remaining tooth structure. In case of complex fracture, endodontic treatment is required along with it post placement is required in some cases. Many other treatment alternatives are available for fractured tooth-like: orthodontic extrusion, flap surgery, crown lengthening, composite restoration, ceramic crown, etc. Few authors have shown that reattachment of fractured tooth provides, immediate esthetic (same colour, contour and surface texture), maintains occlusal function, provides excellent time management and also cost-effective than any other treatment.[4]

This article will discuss about two coronal fractured anterior teeth and their treatment using reattachment of the fractured segment.

Case Report 1: A 24-year-old patient named Deepu Prasad Sahoo reported to the department of conservative

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dentistry and endodontics, Institute of dental sciences, S'O'A University, Bhubaneswar, with the chief complain of pain and mobility of the upper front tooth. He gave the history of trauma due to fall from the bike before 2 days and immediately after the accident, fracture of the coronal segment of upper left incisor had occurred. On extraoral examination, no laceration of soft tissue or swelling was found. On intraoral examination, Ellis class III fracture was seen with the tooth 21. Fracture was of oblique type extending from the gingival third of labial surface to the level of CEJ in the lingual surface (Figure 1a, b). Radiographic examination confirmed the same (Figure1c).

After administration of local anaesthesia (2% lignocaine and adrenaline 1:200,000; Lox, Neon Laboratories, Mumbai, India), affected tooth and adjacent teeth were isolated with a rubber dam (Hygienic Dental Dam, Coltene Whaledent Inc., Germany). Fracture segment is carefully removed from the affected tooth (Figure1d) and was stored in a physiologic saline solution. Single sitting root canal treatment was performed for the tooth 21 along with sectional obturation (Figure2). Post space is prepared and the appropriate size of fibre post (Angelus, Londrina, PR, Brazil)was checked for the proper fit. In the centre of the separated fragment, a trough was created. Post size was adjusted in such a way that, it would fit properly into the trough. That means when the post is placed inside the canal, the emerging part of the post should adequately fit into the created trough when the broken fragment was attached to its original position of the tooth.

Dual cure luting cement (Para core resin cement, Coltene, Whaldent).was used for luting. Etching and bonding of both the root canal space and separated segment was done according to manufacture's instructions. Luting dual-cure cement was placed inside the root canal space and in the fractured segment. One coat of luting cement was also coated along the fibre post and was inserted into the root canal space and soon after this, the broken fragment was attached to the tooth and slight pressure was applied to tightly bind the fractured segment to the intact portion of the tooth (Figure2). Excess cement was removed at the whole tooth was light-cured (Figure 8, 9). Finishing and polishing of the complete tooth was done.



Figure 1a, b: Fracture was of oblique type extending from the gingival third of labial surface to the level of CEJ in the lingual surface; c. Radiographic examinationd. Removal of fractured segment

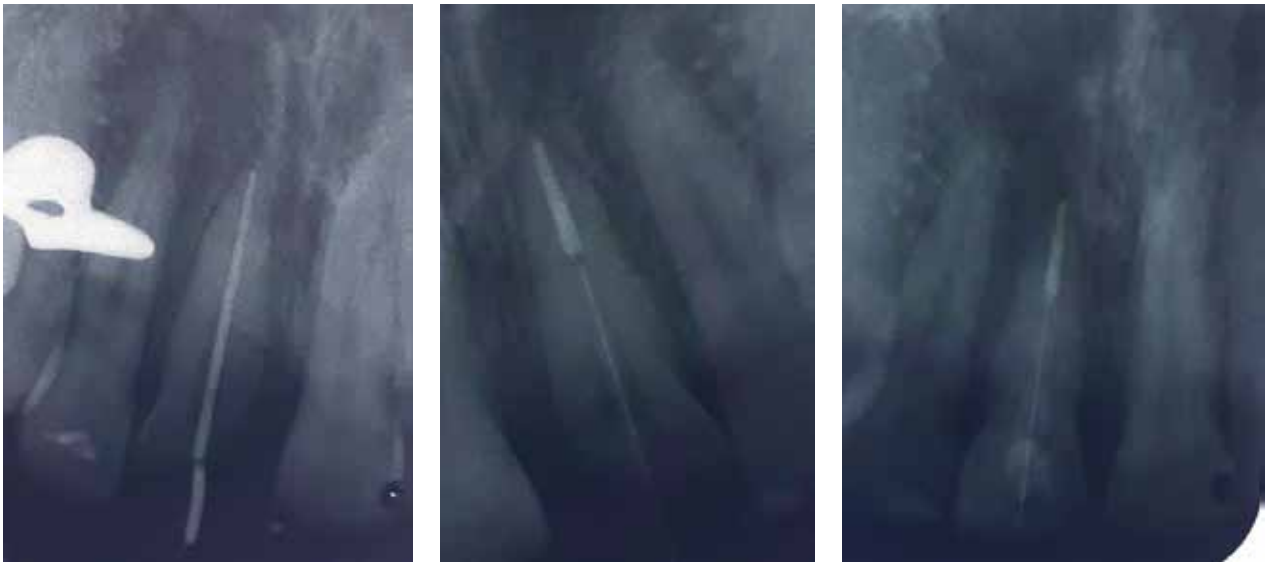


Figure 2. Post treatment radiographic examinations



Figure 3a, b. Reattchement of fractured segment 1

Case Report 2: Another patient named Sunil Biswal visited to the department of conservative dentistry & endodontics, Institute of dental sciences, S'O'A University, following trauma with fracture and pulpal exposure in the coronal segment of the right lower lateral incisor. No visualized trauma was seen in the intraoral and extraoral soft tissue regions. On clinical (Figure4a) and radiographic examination (Figure 4b), Ellis class III # was seen. Fracture line was of horizontal type involving gingival 3 rd of both the facial and lingual surface irt 42. Similarly, after administration of local anaesthesia (2% lignocaine and adrenaline 1:200,000; Lox, Neon Laboratories, Mumbai, India), fracture segment was carefully removed (Figure 4c) and stored in physiologic saline solutions. Complete root canal treatment was done for that particular tooth with MC selection (Figure 4d). Etching, bonding was done for the root canal space and intact coronal portion of the clinical crown and also in the fractured portion of the crown as per manufacturer's instructions. A fibre post (Angelus, Londrina, PR, Brazil) was placed exactly in the same manner as described in the previous case. Reattachment of fractured segment was done with the help of the fibre post (Figure 4e, f).

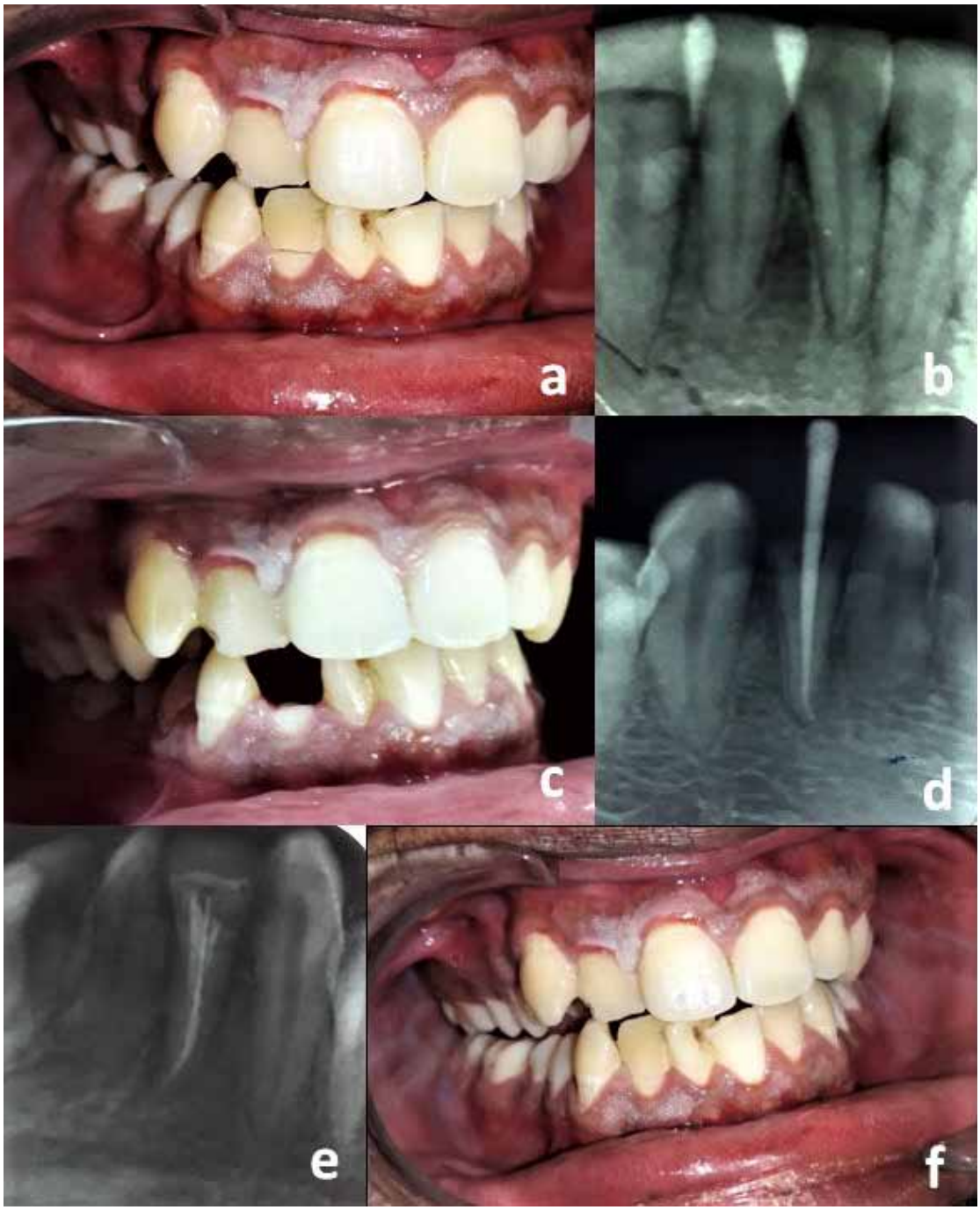


Figure 4a. Clinical examination; b. radiographic examination c. removal of fractured segment; d. complete root canal treatment; e, f. reattachment of fracture segment

Discussion

Treatment and prognosis of the fractured tooth depends on many factors like, patients age and habits, the thickness of enamel available for bonding, the property of the dentinal tubules in the young patients (wideness & wetness), size and thickness of fractured fragment for adhesion, bacterial contamination of the dentin and pulp.[5] A genuine dental treatment after trauma is a challenge for preventing the psychological & biological impacts.[6] According to many authors, reattachment provides an excellent result for the management of the anterior fractured tooth as it maintains the original colour, contour, translucency, surface texture, function

and occlusal alignment.[7-9] Clinicians follow many technical varieties for the reattachment of the fractured segment. The treatment options executed by the authors in the last 10 years case reports on- the reattachment of the fractured segment of anterior tooth,those were published in various journals (Table no. 1).

Table 1. Few case reports on reattachment of fractured segment of anterior tooth, those were published in various journals in last 10 years.

Type of fracture	Treatment followed	Reference
Case 1- Mid root fracture with grade III mobility. Case 2- Mid root fracture with grade III mobility.	Apical fractured segment was extracted and titanium endodontic implant was placed to preserve the natural tooth. Coronal segment was removed. The apical plug of MTA (open apex) with sectional obturation in the intact radicular portion. Reattachment of the coronal segment was done by fibre post.	10
Ellis class III fracture in both central and lateral incisors	Root canal treatment was done for both the teeth. Post space prepared in intact portion of the tooth. Fibre post were bonded to the fractured fragment and the whole thing was reattached to the intact tooth with dual-cure resin cement.	11
Crown-root fracture at the subgingival level with grade III mobility of fractured segment	1st line of treatment was Subgingival exposure of the fracture line. Root canal treatment was done followed by fibre post-placement along with GIC luting cement (Fuji II, GC International, Tokyo, Japan) for the reattachment of the fractured segment.	12
Two fractures in a single tooth- Ellis class III fracture (fractured segment was missing) and other line was on palatalside extending subgingivally.	Palatal fractured segment was first fixed to its original position with the help of composite resin followed by root canal treatment. 2mm post space was prepared and the composite resin was acted like a post and the rest of the tooth was build-up with composite resin.	13
Ellis class III fracture violating the biological width, where the fracture was extended upto the level of gingival in the labial aspect.	Osteoplasty and gingival recontouring were done to maintain the biological width. After root canal treatment post space is prepared. A hole was created on the fractured segment to fit the post and reattachment was done with the fibre post along with dual-cure composite bonding (Embrace WetBond, Pulpdent, USA).	14
Case 1- Ellis class III fracture at the cervical 3rd of crown. Case 2- Ellis class III fracture	Root canal treatment with fibre post for the intact portion of the tooth. Box like preparation was done on the fracture segment was reattached with the help of nanohybrid composite. Same as the previous one	15
Complicated Ellis class III fracture with mobile fractured segment	Root canal treatment of affected tooth followed by reattachment using composite resin.	16
Ellis class III fracture	Root canal treatment followed by post space preparation. Fractured fragment was prepared to receive the post which was 2 mm beyond the intact tooth surface after placement. Rest was built with a composite restoration.	17
Ellis class III fracture at the cervical 3rd level	Following root canal treatment reattachment was done with the help of fibre post and dual-cure resin cement (Para core resin cement, Coltene, Whaldent).	18
Ellis class III fracture involving middle 3rd of crown.	Root canal treatment followed by fracture segment reattachment with the help of flowable composite resin	19
Case 1- Ellis class III fracture at the cervical level and extended 2 mm subgingivally on the lingual aspect.	Case 1-Root canal treatment done followed by fibre post. Ginvial flap was raised to expose the fracture line. Fracture segment was reattached by preparing the segment to receive the coronal portion of the fibre post. Bonding was done by dual-cure cement.	20
Ellis class III fracture involving subgingival area on the palatal aspect.	Root canal treatment was done. The palatal gingival flap was raised to access the fracture line. Reattachment was done with the help of fibre post and dual-cure resin cement.	21
Split tooth involving the pulp with the loss of coronal tooth structure. Platally the fracture line was extended below CEJ.	Root canal treatment along with fibre placement was done for the tooth. Then the whole tooth was extracted and the fractured fragment was reattached to the tooth with the help of dual-cure resin. Tooth was repositioned back to its original position within 15.03 min and stabilized with fibre post and composite for 4 weeks.	22

Use of natural tooth to replace the missing part of the tooth structure eliminates the problem of shade mismatch and wear of restorative material. Additionally it provides fluorescence, opalescence and translucency. It was reported that reattachment of crown fragment provides long term prognosis than the composite restoration.[23] Amir *et al* reported there is no need of additional preparation over the tooth for retention purpose if the tooth is endodontically treated because; the space provided by the pulp chamber is enough to provide inner reinforcement.[24] Damaged tooth requires post-placement to retain the core, especially when more than half of the tooth structure is lost. Fibre post is mainly preferred by the clinicians because they are tooth coloured, has a modulus of elasticity similar to that of dentin, bonds to the tooth and have high fracture resistance. Fibre post along with the dual-cure adhesive creates a monobloc. Additionally, fibre post distributes the stress to the remaining radicular dentin. Resin luting cement provides good bond strength to the tooth. It has been suggested that surgery should be performed in those cases where the fracture line violates the biological width. Minimum of osteoplasty and osteotomy should be performed.[25] As described by authors from the above-mentioned table fibre post is the treatment of choice if the tooth is badly broken and it showed excellent result. Studies have shown that fracture resistance obtained from the intact tooth is greater than any other alternative technique and materials.[26,27] At the same time, the amount of strength required to maintain the function of the reattached fragment for long term use is not known. Bonding of post to the tooth structure increases the prognosis of the tooth by enhancing the post retention as well as reinforcing the tooth structure.[28] Overall resin-based adhesive along with the tooth coloured fibre post serves good esthetics, bonding and modulus between post and resin-based adhesives. Lastly, from the above discussion, we can say fractured anterior tooth can be successfully treated by using fibre post with the dual-cure resin cement.

Conclusion

Though various treatment options are available to treat the fractured tooth restoring the tooth by reattachment of the fractured segment is simple, economic and also provides good esthetics, translucency, contour, surface texture and function with a very conservative approach, most importantly patient's satisfaction.

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Management of Open Apex Using Alternative Techniques: Series of 3 Case Reports

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Abstract

Traumatic injuries during early childhood interfere with root development leading to the formation of a tooth with an open apex. Apexification is considered as the best choice for the treatment of such cases. Use Mineral Trioxide Aggregate (MTA) as apical plug material optimizes the treatment outcome. Other than MTA different techniques are also used to restore the function and stability of the immature permanent tooth. In this case report 3 different techniques are used to treat the open apex. In the first case, MTA apical plug along with backfill was done. Complete canal obturation with MTA and custom cone technique were performed for the second and third cases respectively.

Keywords: *Apexification; Mineral Trioxide Aggregate; Complete canal obturation; Open acces management.*

Introduction

It is quite natural to achieve proper canal disinfection, adequate debridement and optimum apical sealing of the root canal in a completely formed and well-developed root, whereas achieving these parameters in a tooth with incompletely formed root is a challenge in the field of dentistry. Lack of apical stop in cases with open apex puts the clinician in an uncertain circumstance. In every step of root canal starting from chemo-mechanical preparation including irrigation till the apical sealing of the root canal clinician need to be very careful to avoid peri-apical extrusion of the materials, which may lead to post-operative symptoms like pain and swelling. Besides this an additional problem while treating this immature permanent tooth is the thickness of the radicular dentin, which may fracture during or after the root canal treatment.[1] These types of teeth are more commonly associated with periapical pathology.[2]

Early necrotized pulp and inflammatory apical root resorption are the most common etiological factors for the formation of immature teeth.[3,4] Apical barrier formation facilitates 3-dimensional cleaning and well adaptation of obturating materials leading to a successful endodontic treatment. Materials used for such cases should have properties like, antimicrobial, should be bioactive, should create a fluid-tight seal, should have high strength and should reinforce root dentin.[5] Earlier calcium hydroxide was used for the process of apexification, but it takes 5 to 20 months to form a calcific barrier.[6] This lengthy course of treatment offers a challenge like – difficulty in patient follow up management, increases the risk of tooth fracture with intracanal calcium hydroxide dressing for an extended period and also there is a delay in the completion of treatment.[7] Various authors have also confirmed that long term use of calcium hydroxide as intracanal dressing increases the risk of fracture in the future.[4,7,8]

Recently interest has focused on the use of MTA (mineral trioxide aggregate) for apexification. Its powder consists of hydrophilic compounds, which can set in the presence of moisture. Good biocompatibility, excellent sealing ability, high compressive strength, tissue

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regeneration capacity are also added to its properties.[9] Importantly MTA reduces the appointment time, even allows single visit apexification. MTA offers a good apical barrier that allows vertical condensation of warm gutta-percha.

Technique 1: A 21-year male patient named Satyanarayan Sabat reported to the Department of Conservative Dentistry and Endodontics, Institute of Dental Sciences, SOA University, Bhubaneswar with the chief complaint of the discoloured tooth in the upper front tooth region. The patient gave the history of trauma 12 years back. Clinical examination revealed discolored tooth with open access and tender on percussion to the tooth 21. An intraoral periapical radiograph revealed an incompletely formed root apex with irregular periapical radiolucency (Figure 1a). Hence, the final diagnosis was pulpal necrosis with symptomatic apical periodontitis in tooth 21. After rubber dam isolation (Hygienic Dental Dam, Coltene Whaledent Inc., Germany), chemomechanical preparation of root canal was done upto F5 (Dentsply Maillefer, Switzerland) along with circumferential filing using # 80 K-file (Mani Inc., Japan). Chemomechanical preparation performed in conjunction with a copious amount of 3% Sodium hypochlorite (Prime Dental, India) and saline solutions. 5 ml of 17 % Ethylenediaminetetraacetic acid (Prevest Denpro, India) was used for smear layer removal. On completion of preparation calcium hydroxide dressing (Avue Cal, Dental Avenue, India)was given for 1 week.

After a 1-week tooth was again re-accessed under rubber dam isolation. 5 ml of 17 % EDTA and Copious amount of saline solution was used to remove the calcium hydroxide dressing from the canal. Canal was aspirated and dried thoroughly with absorbent paper points. MTA (Angelus, Londrina, PR, Brazil) was mixed according to the manufacturer's instructions and 4 mm of the apical plug was created using hand pluggers. A gutta-percha point was introduced into the canal to confirm the apical barrier (Figure 1b). a moist cotton pellet was placed over the MTA and the canal was sealed with a temporary restoration. Next day, the remaining portion of the root canal was coated with silicate-based sealers (MTA Filapex; Angelus, Londrina, SP, BR) and obturation was done by back-filling of gutta-percha using system B (Sybrondental, Orange, CA, USA) setting 200°C upto the level of CEJ (Figure 1c). Glass ionomer cement (3M ESPE, USA) was used for access cavity restoration (Figure 1d).

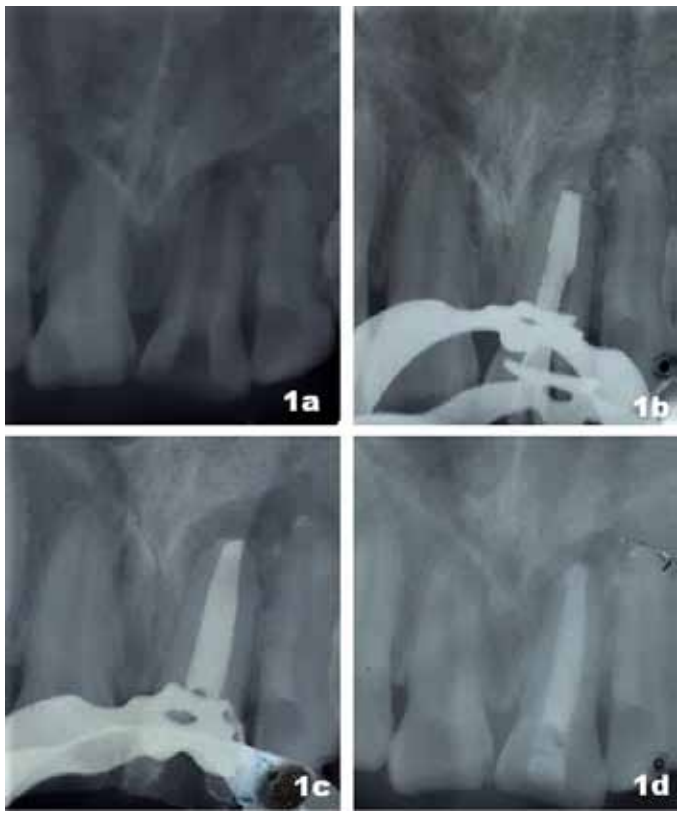


Figure 1: 4mm apical plug of MTA followed by backfilling of gutta percha

Technique 2: Another male patient aged 18 came to the Department of Conservative Dentistry & Endodontics, Institute of Dental Sciences, Bhubaneswar, with the chief complaint of unpleasant smile and gave the history of trauma 10 years back. On clinical examination discolored tooth was noticed in 21. Intraoral periapical radiographic examination revealed a wide-open apex with a thin radicular dentinal wallalong with periapical radiolucency in tooth 21 (Figure 2a). Under rubber dam isolation chemo-mechanical preparation for the tooth was performed similarly as described in technique 1. Calcium hydroxide dressing (Avue Cal, Dental Avenue, India)was given for 1 week followed by temporary restoration. After 1 week of intracanal dressing tooth was irrigated with 17% EDTA (Prevest Denpro, India) and copious amount of saline solution to remove calcium hydroxide from the canal. Canal was aspirated and dried thoroughly with absorbent paper points. MTA (Angelus, Londrina, PR, Brazil) was mixed according to the manufacturer's instructions and the entire radicular portion of the canal was packed with MTA (Figure 2c). moist cotton pellet was placed over MTA and temporary restoration was used to maintain the coronal seal for the particular tooth. After 24 hours cotton pellet was removed and coronal restoration was done using glass ionomer cement.(3M ESPE, USA) (Figure 2d).

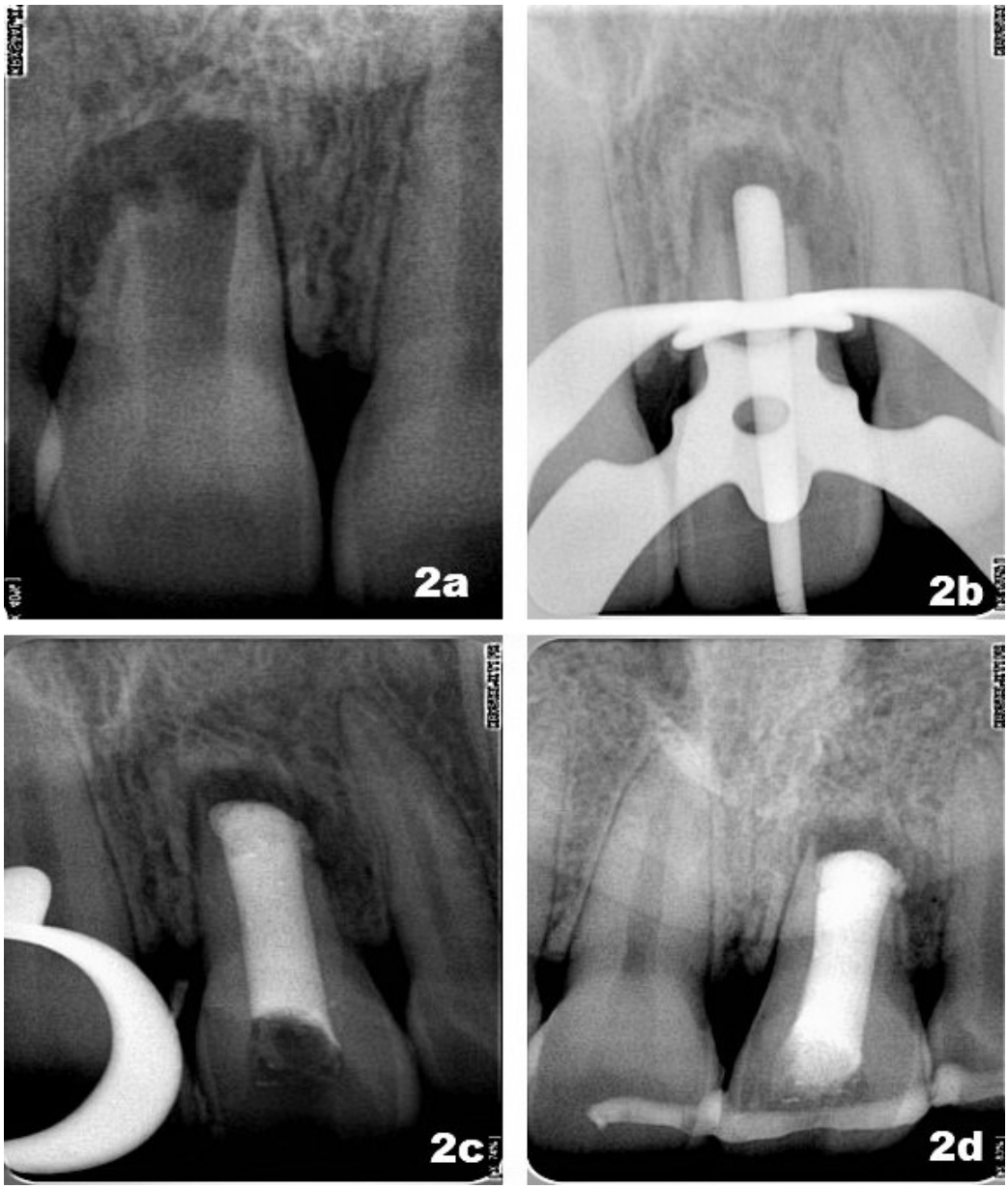


Figure 2. Entire canal was filled with MTA

Technique 3: A 14-year-old male patient requested dental treatment in the upper right central incisor following trauma before 5 years due to fall from the bike. Radiographic examination revealed incompletely formed root with no periapical changes (Figure 3a). Clinical examination revealed a discolored tooth in 11. All the necessary protocols were maintained under rubber dam isolation. Chemomechanical preparation of the canal including calcium hydroxide dressing was carried out the same way as described in the technique 1. After the removal of calcium hydroxide from the canal,it was obturated by the custom cone technique or roll cone technique using a resin-based sealer, AH Plus (Dentsply Maillefer, Switzerland). Gutta-percha points were softened by heat and rolled together between glass slabs. Tip of gutta-percha points were elasticized by using a heated instrument and then introduced into the canal to record the internal morphology of the apical portion of the canal (Figure 3b). This process was repeated till proper tug back was achieved. Canalwas sealed upto the level of CEJ and the access cavity was filled with glass ionomer cement restoration (3M ESPE, USA) (Figure 3c).

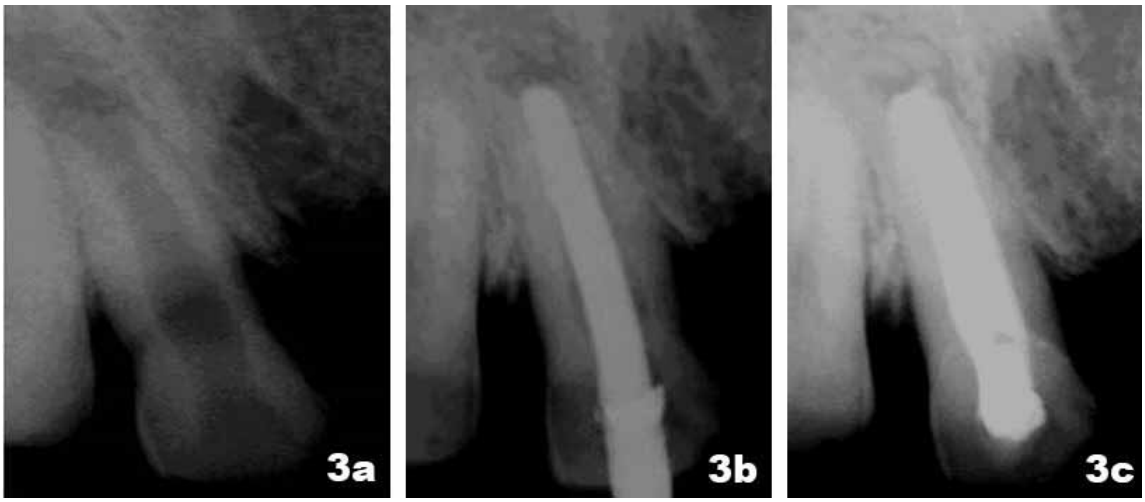


Figure 3. Custom cone/Roll cone technique

Discussion

Successful treatment of permanent immature teeth mainly depends on the proper cleaning of the root canal system as mechanical instrumentation can not be used appropriately. So, the disinfection of the entire root canal relies on the chemical action of sodium hypochlorite and on calcium hydroxide which is used as an intracanal medicament.[10] At high concentration Sodium hypochlorite is known to be toxic especially when it extrudes apically. Therefore, the use of a low concentration of sodium hypochlorite is advisable for disinfection in cases of immature teeth.[11] 17 % EDTA solution was used to rinse the canal before placement of calcium hydroxide as it removes the smear layer and opens up the dentinal tubule for penetration of calcium hydroxide through the dentin.[12] Along with this use of EDTA ensures better removal of calcium hydroxide from the canal before obturation.[12] Initially calcium hydroxide was used to form the apical barrier in non-vital immature teeth. One study has investigated thatfor apexification, apical barrier formed by calcium hydroxide is porous and even contains some amount of soft tissue.[13]

MTA has excellent biological properties and has the potential to create a good apical seal, therefore it has been strongly recommended to use as an artificial barrier in the teeth with open apices, thus reducing the treatment appointment to 1-3 visits. High pH of MTA allows it to destroy surrounding microorganisms and its bioactive nature encourages blastic cells to promote the process of healing.[5,15] Along with this, it also facilitates the deposition of cementum on it because of the presence of calcium and phosphorous ions (15). In a prospective study apexification using MTA has shownan increased prevalence of healing and apical closure.[16] Thus, an apical seal with MTA favours regeneration. Taking all the advantages of MTA into consideration, it was used

for the apexification process in the first two cases of this case report.

In the first technique-the apical plug of MTA was given and obturation was performed by backfilling of soften gutta-percha. 4-5 mm of MTA apical barrier has proven to be significantly stronger and showed less leakage than a 2 mm apical plug. In this case,a 4 mm apical barrier of MTA was given. MTA apical plug facilitated obturation by condensation without any over extrusion of gutta-percha. In technique 2,the tooth was very immature and the thickness of radicular dentin is less, thus making the tooth more vulnerable to fracture. In such cases adaptation of gutta-percha master cone to the dentinal wall would be difficult. Additionally, it has been confirmed the use of MTA strengthens root dentin.[17,18] Considering the above-mentioned factors root canal was completely obturated using MTA. As an experiment, a conventional method of obturation (tailor-made gutta-percha obturation) was attempted in technique 3. Rootend closure was achievedby customized gutta-percha cone. Considerably successful treatment along with complete resolution of symptoms was observed when the tooth was obturated with a custom cone technique.[19] The choice of treatment for teeth with open apex depends on the selection of case, operator's experience as well as on the handling of various materials.

Conclusion

There are various ways to manage a tooth with an open apex. MTA has numerous applications in the field of endodontics starting from pulp capping including pulpotomy to apexification. Due to its excellent biocompatibility and strength it is used for apical barrier formation in immature young permanent teeth. Apart from all the advantages of apexification materials, proper canal disinfection, achieving a fluid-tight seal, limiting the apical barrier and obturation within the canal, play an important role in a successful endodontic treatment.

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Ethical Permission: Approved

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Palatal Lift Prosthesis for Velopharyngeal Incompetence: A Case Report

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Abstract

Velopharyngeal incompetence often distorts the voice quality making it difficult for the listener to comprehend. A cost-effective treatment approach for this is palatal lift prosthesis. This prosthesis enables the incompetent soft palate to approximate with the posterior pharyngeal wall. As a result articulation of speech is improved.

Keywords: *Palatal lift appliance, velopharyngeal incompetence, speech.*

Introduction

The velopharyngeal mechanism plays a crucial role in the clarity and acceptability of speech.[1] It involves a sphincter, comprising the lateral pharyngeal walls, posteriorpharyngeal wall, and the soft palate (velum).[2] The contracture of the apparatus principally controls the oro-nasal sound pressure and amount of airflow in speech. Dysfunction of this mechanism due to congenital craniofacial syndrome, head and neck cancer, neurogenic injury, velopharyngeal insufficiency,or velopharyngeal incompetence may result in compromised voice quality.[3] It may be attributed to nasal air emission, reduced oral pressure, impaired articulation (weak consonants),and hypernasality (Figure 1).[3]

Though surgical correction of velopharyngeal dysfunction with a pharyngeal flap or pharyngoplasty is preferred, it may not be possible in a patient with a cardiovascular or neurological problem.[4] Such cases can be treated with velopharyngeal or palatal lift prosthesis (PLP) in combination with speech therapy.[5,6]

The palatal lift prosthesis was originally designed by Gibbons and Bloomer to uplift the neurologically weakened soft plate to a normal level. The aim was to decrease the size of palatopharyngeal opening during consonant sound production. This, in turn, improved the resonance and decreased nasal intonation of speech.[7] Subsequently,the velar lamina portion of the prosthesis has been made movable using a hinge, orthodontic wire, wire spring, or ageneric button attached to a single connecting wire. This facilitated the movement of velar lamina inferiorly-superiorly.[8,9] This clinical case report focus on the stepwise fabrication of a palatal lift prosthesis for a patient with hypernasal speech.

Case Report: A 20-year-old male patient reported to the clinic with a chief complaint of nasal intonation in his speech since childhood. The speech of the patient was dysarthric. Intraoral examination revealed a normal velopharyngeal structure with a flaccid soft palate (Fig. 2). Velopharyngeal orifice closure was inadequate due to impaired movement of the soft palate during speech and swallowing. Both the maxillary and mandibular arch were completely dentulous with all teeth in good condition. So it was decided to treat the patient with a combination of palatal lift prosthesis and speech therapy. Counseling of the patient was done and consent taken for the same.

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Procedure: The procedure was initiated with primary impressions of the upper arch using irreversible hydrocolloid (Zelgan, Dentsply, Constance, Germany) and working cast fabrication. Before this, the stock tray was modified with impression compound (Pinnacle, DPI, Mumbai, India) to cover soft palate posteriorly.

The PLP used for the patient had two parts: an anterior denture base for retention of the prosthesis and a posterior velar lamina to uplift the soft palate. Initially, the denture base portion with four pin head clasp and two C clasps was fabricated using heat cure acrylic resin (DPI heat Cure, DPI, Mumbai, India). The posterior extension of the denture base was kept up to the junction of the hard and soft palate.

For the velar lamina portion of the prosthesis, a segment of 21-gauge stainless steel orthodonticwire (3M Unitek, Monrovia, CA) was adapted in the form of a loop on the master cast. The loop was attached to the posterior portion of the denture base and adjusted in the patient's mouth, till the soft palate is slightly elevated. Due care was taken to avoid gag reflex. The gradual uplifting of the soft palate was done by molding the loop with a green stick modeling compound. Molding was done by asking the patient to breathe through the nose and pronounce plosive sounds like 'b' and 'p'. These sounds require to increase intra-oral pressure and aid in decreasing hypernasality. The procedure was repeated, till the soft palate came in light contact with the posterior pharyngeal wall (Fig.3).

The efficacy of the molded prosthesis was assessed by asking the patient to blow out air, with the mouth being closed. Once air outflow through the nostril was greatly reduced,a satisfactory lift of the soft palate was assumed to be achieved. Then the velar lamina portion of the prosthesis was relined by zinc oxide eugenol impression material, which was subsequently replaced with heat cure acrylic resin (Fig.4). Insertion of the final prosthesis was done after proper finishing and polishing to ensure smooth and round border (Fig.5). The patient was advised to wear the prosthesis consistently except during sleep. Follow up of the patient was done in a weekly interval for a period of 1 month.

The speech therapy session was given to the patient by a speech therapist throughout the procedure. Audiometric analysis of the patient was performed periodically in the Department of ENT using the Temple Street Scale. After a post-insertion period of 3 months, the speech pattern was found to be improved with decreased nasal intonation clarity of speech.

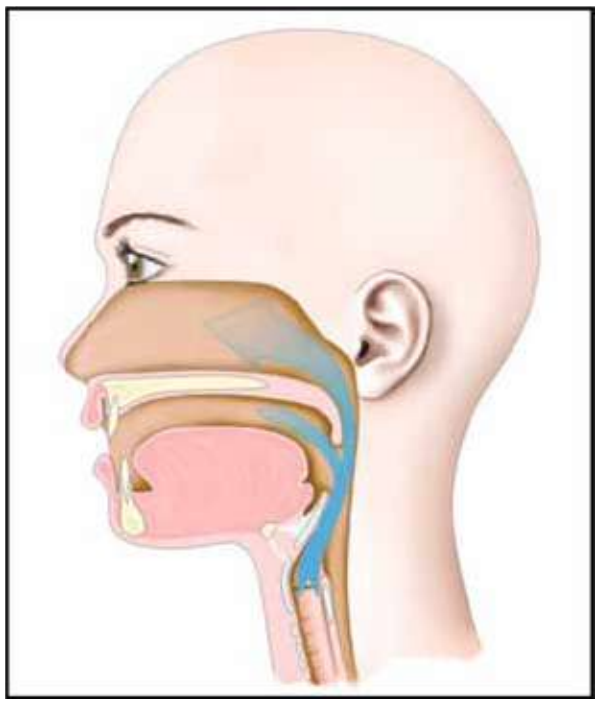


Figure 1. Mechanism of nasal air leak in velopharyngeal incompetence (Courtesy: The Role of the Speech Pathologist in the Care of the Patient with Cleft Palate Helen Extence, Siwan Cassidy, in Maxillofacial Surgery (Third Edition), 2017)



Figure 2. Pre-treatment flaccid soft palate

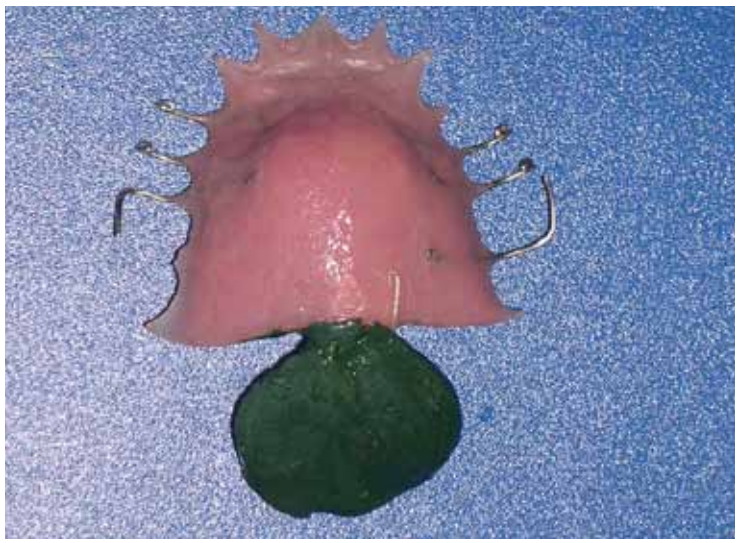


Figure 3. The molded palatal lift prosthesis

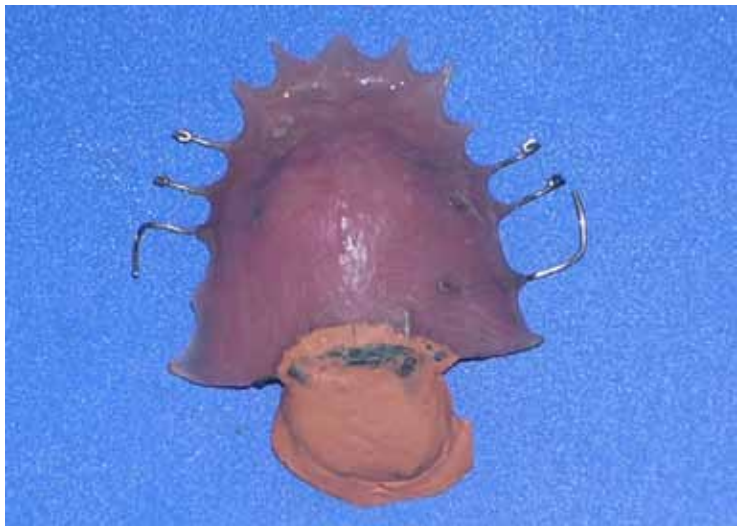


Figure 4. The molded palatal lift prosthesis with relining



Figure 5. The palatal lift appliance in position

Discussion

A palatal lift prosthesis was preferred in this patient as, the velopharyngeal closure was compromised, despite its normal structures. The soft palate was class II type, flaccid, and having inadequate movement vertically.[10,11]

This prosthesis uplifts the velum in superior-posterior direction to the level of normal palate closure, aligning with the palatal plane.[12] Consequently, pharyngeal muscles are activated, decreasing the size of the palate-pharyngeal opening and the nasal intonation.[13] So clarity of speech is improved.[14] Even prolonged use of PLP might also reduce the disuse atrophy of soft palate and enhance its function.[15] But the sudden and strong lifting of the soft palate is not recommended, as it might cause soreness. An interim PLP was planned for the patient, as he was a first time user. As it was made with PMMA, the palatal extension portion was gradually adjusted according to the patient's need. This resulted in better patient compliance. Once the patient is motivated and accustomed to the interim prosthesis, a definitive prosthesis can be planned for him.

The clinical efficiency of the prosthesis was assessed for nasal intonation, resonance, articulation, and clarity of speech in each visit. They were gradually found to be improved with the use of prosthesis. Effectiveness of prosthesis can also be assessed using the lateral cephalograms, video fluoroscopy, naso endoscopy, nasometric analysis, and aerodynamics.[16,17] Palatal lift prosthesis alone is effective in mild velopharyngeal insufficiency case. But in the long-standing case and repaired congenital cleft palate case require a combination of speech therapy along with palatal lift prosthesis.[10] Patient compliance is crucial for the success of PLP. They often complain of dysphagia and lack of immediate improvement in speech.[18]

Conclusion

A team approach comprising surgeon, prosthodontist, and speech therapist are beneficial in the management of a patient with velopharyngeal insufficiency. Depending on the severity of the case, palatal lift prosthesis should be used alone or in combination with speech therapy. A definitive prosthesis should be delayed until the patient is comfortable with the interim prosthesis.

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Saliva as a Diagnostic Tool in Orofacial Disorders

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Abstract

Saliva is formed by major exocrine organs, namely the parotid gland, "sub-mandibular" gland and "sublingual glands" and many minor "salivary glands." Nature has bestowed saliva "The natural source" with many functional capabilities that have a crucial value in maintaining the well being of mouth. Saliva plays a major role in the protection of oral tissues, food preparation, digestion, lubrication and speech.[1] But one purpose not given by life is the utilization of saliva for investigative value, it is the human who has to explore this precious wonder fluid marvelously studded with clues about the human body in health and disease. Saliva as a diagnostic aid in orofacial disorders is the traditional monitoring tool for various diagnosis.[2]

Keywords: *Saliva, HIV, orofacial disorders.*

Introduction

Nature has bestowed saliva "The natural source" with many functional capabilities that have a crucial value in maintaining the well being of mouth. Saliva plays a major role in the protection of oral tissues, food preparation, digestion, lubrication and speech.[1] But one purpose not given by life is the utilization of saliva for investigative value, it is the human who has to explore this precious wonder fluid marvelously studded with clues about the human body in health and disease. Saliva as a diagnostic aid in orofacial disorders is the traditional monitoring tool for various diagnosis.[2] Saliva is formed by major exocrine organs, namely the parotid gland, "sub-mandibular" gland and "sublingual glands" and many minor "salivary glands." The total saliva produced during a 24 hr period is about 1000 to 1500 ml. The approx 0.4 ml per minute.[3]

Historically[4] the diagnostic value was recognized by the prehistoric legal center of the population who engaged salivary surge as the foundation of anancient"lie detector"examination, but now with endless, untired efforts of dental investigators in finding change in saliva stream speed and constituents by means of investigating and checking in several mouth disorders, it is established that saliva is valuable in the analysis of general disorder of salivary glands like inflammatory as well as autoimmune disease, by nurturing a known volume of saliva, a quantitative determination of specific organism can be made which is employed in identifying risk for caries and as an indicator for infection. The buccal mucosa cells are used for getting DNA for gene analysis, the list keeps growing.[5,6] Saliva is more and more used in the analysis and evaluation of ailment but the possibility of physiological difference within several constituent whose inference is projected as an analytic assist ought to be investigated sooner than it is utilized.

Saliva as a diagnostic tool in various disorders:

Autoimmune disorders - Sjogren syndrome:[7,8,9,10,11,12]

Saliva in infections:

- Viral: HIV, Herpes, Mumps
- Bacterial
- Fungal

HIV: Groopman JE, Salahuddin (1984)[13] demonstrated that HIV-1 was detected initially in whole saliva, at frequencies ranging from 1 to 45%. David HO, Roy E et al (1985)[14] conducted a study for the isolation of HTLV- iii(human T-cell lymphotropic virus) from saliva of infected patients. 83 saliva samples were collected from 71 homosexual men seropositive for HTLV-iii. Saliva samples were cultured within 60 minutes after collection and blood was collected from 50 of 71 for virus isolation. It was concluded that the HTLV-

iii titer of patients saliva was substantially less than that of blood. Thus this study indicates that transmission of HTLV-iii does not occur if exposed to the saliva of infected persons so useful in allaying public concern regarding the causal spread of AIDS. Yapijakis C, Panis V, Koufaliotis N, Yfanti G, Karachalios S, Roumeliotou A, et al (2006)[15] conducted a study investigate the finding possibility of "human immunodeficiency virus (HIV)" in the salivary sample,sixty-eight subjects were investigated, consisting of thirty-four"HIV" carriers and thirty-four non-carriers (controls) of coordinated sex and age. The mouth assessment revealed the saliva and blood sample of study subjects. Every subject was investigated andscreened for "HIV"by means of 2 method "Oraquick-compatible enzyme-linked immunosorbent assay (ELISA) and a fluorescent immunoenzymatic method (ELFA)", long-established by "western blotting", and a simple molecular method "(polymerase chain reaction amplification of a relatively constant viral DNA region)", long-established by "DNA hybridization". They investigated that the detection of anti-HIV in saliva mightattainthe accurateness of 97.1-100%, analogousby way of blood.

Hepatitis: Hutse Vet al (2005)[16] conducted a study on 43"HBsAg" positive and 73"HBsAg""negative paired serum samples" were investigated. These were gathered from subjectscoming to college outpatient department. The samples were stored using the "Oracol collection device" and they were passed through the "IgG quantification assay" to guarantee their fineness and amount. The finding of HBsAg in oral solution was done with"ETI-MAK-4 ELISA". The corroboration of this test gave a sensitivity and specificity around 90.7% and 100%.

Varicella-zoster infection: Talukder Y et al[17] investigated by means of matching serum as well as oral solution form fit grown-up subjects (n = 205) in addition top lay school kids (n = 98), saliva collection for regular"measles, mumps and rubella" tests (n = 537) and samples from subjects of atopic dermatitis (n = 252). When it was compared to paired sera tested by the same ELISA the sensitivity of the oral fluid assay was 93% and specificity 95.7% overall, varying slightly with the age group. The analysis was revealed to have good quality possibility for utilization in major epidemiological settings.

Herpesvirus infection: Blackbourn DJ, Lannette ET (1998)[18] conducted a study on "14 HHV-8-seropositive subjects, as well as 8 Kaposi's sarcoma subjects, to determine the growth of Human herpesvirus 8 by "polymerase chain reaction" in nose secretion and salivary fluid of patients. They concluded that HHV was present in solitary or equally body fluids in eight (57%) of fourteen subjects. They further determined that "parallel PCR testing revealed the concomitant presence of cytomegalovirus, Epstein-Barr virus and HHV-6" in a variety of amalgamation in these body fluids. Thus data indicates recurrent detaching of numerous herpes – viruses within saliva predominantly Kaposi sarcoma patients. Yildirim S et al (2006)[19] reported a case and found that "EBV was detected in baseline samples of saliva from the "Kostmann syndrome patients".

Mumps: Thomas Thieme, Stephen Piacentini et al (1994)[20] conducted study on 157 asymptomatic subjects to decide if oral liquid samples are capable to be used to constantly measure defensive blood quantity of antibodies to "measles, mumps and rubella", and to compare coordinated serum and saliva samples from eleven subjects after "measles-mumps-rubella immunization. They concluded that the incidence of antibodies in "oral fluid specimens" correlated with that of serum with 94% sensitivity and 94% specificity for mumps, thus protective levels of antibodies assessed by means of an oral fluid with good consistency. Muhels R.L et al (2000)[21] evaluated that in viral mumps the saliva shows an increase in the sodium values and a very less potassium level, which approaches its plasma equivalent. Sodium valuesincrease to 90-120 mmol/l and potassium values dropunder 10 mmol/l.

Bacterial infections:

Dental Caries: J Van Houte, HV Jordan (1990)[22] carried out a study on 273 subjects for the detection of the alliance of microbacterial concentration of teeth tartar and saliva with human root surface dental cavity and saliva samples were collected from one-third of the patients for determining of the concentration of streptococcus mutans and lactobacillus and they concluded that saliva populations of mutans streptococci had positive correlation by way of the occurrence of tooth root surface dental caries.

Fungal infection: Hicks MJ, (1998)[24] carried out a study to determine the prevalence of fungal organisms in whole unstimulated saliva from children with vertically acquired HIV infection. 27 HIV infected and 11 HIV exposed, but uninfected subjects were studied and the

whole unstimulated saliva was collected. The results were, yeast and hyphae were present cytologically in 19% of HIV infected and 9% of HIV-exposed but uninfected children and thus they concluded that fungal organisms in the saliva reflected oral carriage or mucosal colonization, influenced the development of clinically significant candidiasis in the immunocompromised children.

Saliva in malignancies:

Head and neck cancer: Jiang WW, Fmasayesva B (2005)[25] conducted a quantity PCR of "cytochrome oxidase I and cytochrome C oxidase II (Con II)" genes in mouthwash samples of 94 subjects having "head and neck squamous cell carcinoma" and 656 controls were considered. They evaluated the "mitochondrial DNA/nuclear DNA" in salivary samples from HNSCC subjects and controls in association to "smoking, ethanol intake and tumor stage". The mean values of "COX I and COX-II" in saliva samples have considerably elevated in comparison with controls. On univariate investigation, smoking, age, HNSCC analysis, and higher stage of HNSC were associated with higher levels of mt DNA contact in saliva.

Saliva in immune-related disorders:

Multiple sclerosis: Frena Adamashuilli, Aliraza Minagar (1993)[26] carried out a study for the quantity of soluble "HLA" in body secretions and to found that a possible function in assessing illness. "Enzyme-linked immunoassay" was used to gauge soluble "HLA class I and class II molecules" in saliva and cerebrospinal fluid in 13 non treated subjects with multiple sclerosis and saliva of 53 healthy controls is also studied. They concluded that soluble HLV in saliva specifically HLA 11 correlated with the levels formed in CSF, thus saliva provided a noninvasive correlation of CSF measurement.

Graft versus host disease: Patients with "graft versus host disease"experience from xerostomia and augmented levels of oral injections and mucosal pathologies. Izutsu KT, Menard TW (1985)[27] carried out a study on 42 subjects at 90 days to 2 years following bone marrow transplantation. They studied labial gland IgA, rather than whole saliva. It was concluded that there was a decreased concentration of IgA, 90 days after transplantation, and was suggestive of immunocompromised status. Morhang G, Engstron PE (1994)[28] demonstrated that the subjects of"GVHD" had a smaller amount of saliva IgM one year after "bone marrow transplantation". Maglu RS (1996)[29] demonstrated there is a mean decrease of 55-90% in the "salivary flow rate of patients" with graft versus host disease.

Drug monitoring: Similar to other body fluids, saliva is proposed for the monitoring of systemic levels of drugs. A requirement for this investigative purpose of saliva is a definable associationamidthe absorption of a curative medicine in blood and the absorption in saliva. Amedicine to appear in saliva, medicine molecules in serum have to cross through the salivary glands and into the oral cavity.

Antipsychotic drugs: Ben Aryeh (1980)[31] conducted a study on 118 manic-depressive patients, "salivary and serum lithium" concentration were calculated. The results were lithium concentration was higher in saliva as compared to serum. A method to measure serum lithium levels from salivary measurement was extracted from serum = 0.364 saliva. The results demonstrated the likelihood of through salivary lithium capacity for checking patients on long-lasting lithium treatment.

Antiasthmatic drugs: Krik JK, Dupnis (1994)[34] concluded saliva theophylline conc. established an improved association with serum levels of free "theophylline than with serum concentration of total theophylline."

Immunosuppressive drugs: Coates JE, Lam SF (1988)[35] demonstrated that a lesser association (r = 0.68) was established between salivary and whole serum levels of immunosuppressive drugs.

Antiepileptic drugs: Rosenthal E, Moffr E (1995)[36] evaluated the saliva evaluation of anti-epileptic medicines. Salivary carbamazepine levels were found to be 38% of serum carbamazepine level and a constructive correlation between salivary and serum carbamazepine levels was observed.

Alcohol and tobacco: Luepkar RV, Pachauk TF (1981)[37] evaluated that salivary thiocyanate level was established to be a pointer of smoking. Istvan (1982)[38] carried out a study on cigarette smokers between the age of 35 and 59 and investigated the relation of salivary continue and no of cigarette smoked per day to body mass index.

Discussion

Saliva is easilyavailable, which can be stored by

various method and it contains many hormones, drugs, and antibodies of interest in screening and diagnosis.[40] It is collected in distant areas by inexperienced persons and, by way of assured compilation instruments, is steady at optimum temperatures for few weeks Absorbing specimens on cotton may contribute interfering substances to the extract. A major advantage is that blood-borne diseases associated with blood collection are not relevant to saliva (noninvasive). Due to less quantity of harmful antigens in saliva, "HIV and hepatitis" diseases are much less of a danger from saliva than from blood.[40] Hocini et al. (2000) investigated that secretary"leukocyte protease inhibitor (SLPI- antiviral activity against free HIV-1)" can be an additional reason causative to the safety of saliva as an analytical sample.

Understanding of saliva assays is hard. Because blood levels of steroid hormones are several-fold higher than saliva levels, much has been written about the problems of contamination from bleeding gums. The use of saliva as a diagnostic fluid has become a successful research story during the past 10 years. Recent and most advanced technologies have enabled the salvia to be used to diagnose some of the diseases and prediction of disease progression, to be enhanced by future research activities.

Conflict of Interests: None

Ethical Permission: Approved

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Oral Squamous Cell Carcinoma: A Clinical Case Report

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Abstract

The most common carcinoma affecting the oral mucosa is oral squamous cell carcinoma, with prevalence seen in the age groups above 50 years in males and with rare occurrence below 30 years. Studies reveal approximately 30-40 % of deaths in oral squamous cell carcinoma occur due to various tissue abuse habits. The present case definesa classic incident of oral squamous cell carcinoma concerning the alveolo-buccal sulcus in a male having 50 years of age.

Keywords: *Alveolobuccal Sulcus, Oral Squamous Cell Carcinoma, Mandible.*

Introduction

One of the potential scarring and incapacitating disorder which harms the physical stature and mental health of patients is Oral Squamous cell carcinoma (OSCC).(1) It is the most familiar malignant mucosal neoplasm of head and neck accounting for over 90% of all malignant neoplasm.(2) The frequency and mortality rates of OSCC arise with the age of the patient and males are affected more than females.(1) It typically affects the older age groups throughout the fifth-eighth span of life with less occurrence reported in the younger age groups below 40 years.(3) The maximum prevalent threat for the occurrence of oral SCC is due to the habit of tobacco or betel intake, ingestion of intoxicating drinks, and contamination with human papilloma virus (HPV) having high-risk factors. Also a nutrition comprising of inadequate fresh fruits and vegetables was reported in OSCC patients.(4)

Since, the oral mucosa is more approachable to the comprehensive investigation; early perception of potentially malignant and cancerous lesions can be easily marked. But sometimes due to unawareness or unapproachability of therapeutic facilities, the carcinoma gets diagnosed in the advanced stages. Thus, an enhancement in primary recognition of oral carcinoma should be adopted, by adopting the chairside diagnostic procedures like toluidine blue, brush biopsy, etc.(5) In the current paper, we represent a case of a small swelling which grew to a larger size after 2 months.

Case Report: A male patient of age 50 years came with a history of pain in the left side mandibular back tooth region for 8 days. The patient had tuberculosis and is under medication for 2 months and he has undergone extraction in the left side mandible before 12 years. The patient had a habit of pan and khaini chewing 10-15/day for 20 years and has left the habit since 4-5 years. Extra-orally there was presence of a single, submandibular lymph node measuring approximately 1x1cm in size, non-tender and mobile on palpation. Intraoral examination showed localized growth present in the left lower edentulous region on the alveolar ridge with relation to 36, measuring 1x0.5 cm approximately having

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irregular borders. On palpation, the tumor was soft in consistency, non-pedunculated, tender on palpation which bleeds on probing with slight obliteration of gingivo-buccal sulcus.(Figure-1.1)

Hence a provisional diagnosis of gingival epulis was given along with differential finding of pyogenic granuloma and peripheral giant cell granuloma, but patient didn't reported to the department, had not taken any medications, or performed any investigations advised. After approximately 2 months,the patient again reported to the hospital with the same complaint (as he had previously) of pain along with swelling in the mandibular left side back tooth region. Extra-orally swelling present in the left lower 1/3rd of face measuring 2x2 cm in size approximately of normal skin color, appears shiny and stretched. On palpation swelling was firm in consistency and tender with no secondary changes or symptoms associated with it. Skin over the swelling was warm to touch and was non-pinchable. (Figure-2.1)

Left submandibular lymph node measuring 1x1 cm was palpable which is tender on palpation and fixed to the underlying structures. A single, ulcero-proliferative growth present in the left gingivo-buccal sulcus with relation to 34 35 36 37 region, measuring 3x2 cm in size having irregular margins and erythematous in appearance with no secondary changes present. On palpation, growth was firm to touch, having indurated margins and remained tender on palpation with obliteration of gingivo-buccal sulcus with relation to 34-37 regions. (Figure-2.2)



Figure 1. 1. Extra-oral view of patient; 1.2-Intra-ora view showing a localized growth



Figure 2.1. Extra-oral view of patient after 2 months; Figure 2.2. Clinical view of ulcerative growth which increased in size in the gingiva-buccal sulcus.

The patient was further advised to do radiographic investigations. An intraoral periapical radiograph and panoramic radiograph was done.Intraoral periapical radiograph reveals a single diffused radiolucency with relation to 34 35 36 measuring 1x2 cm in size. There is vertical and inter-radicular bone loss,with widened pdl space i.r.t 35 36, and loss of lamina dura i.r.t 34.(Figure-3.1)

Panoramic radiograph reveals a localized, ill-defined radiolucency involving the left side body of mandible, measuring 3x2 cm in sizewith loss of buccal cortical plate and intact lingual cortical plate.Missing tooth present i.r.t 36 with mesially tilted 37.(Figure-3.2)



Figure 3.1-Inra-oral periapical radiograph; Figure 3.2- Panoramic radiograph

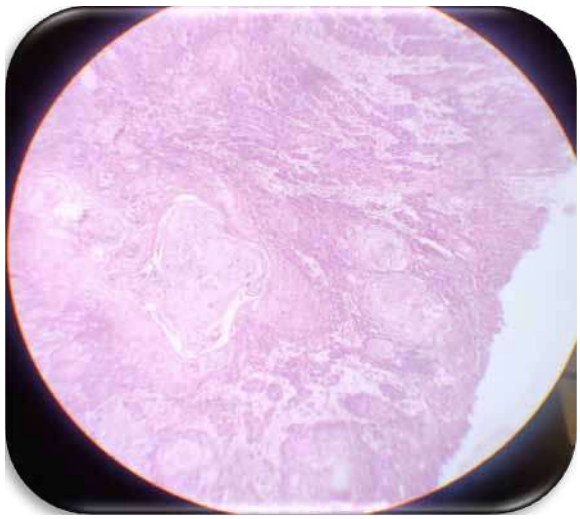


Figure 4. Histopathological slide showing para-keratinized stratified squamous epithelium, withtumorislands, nests and cords along with diffusely arranged chronic inflammatory cells.

Correlating the clinical features, radiographic findings a provisional diagnosis of Malignant ulcer-proliferative with clinical staging of tumor T1N1M0 was presented. A chairside investigation was carried out by applying toluidine blue staining which showed the presence of dysplastic cells. Further incisional biopsy of the lesion was done under local anesthesia and was submitted for histopathological examination. Histopathology revealed hyperplastic proliferated para keratinized stratified squamous epithelium invading into the underlying moderately dense fibro collagenous stroma as strands and cords.(Figure-04)

The definite diagnosis was moderately differentiated SCC patient was further sent for complete excision of the lesion to the department of oral surgery.

Discussion

This case report described an unusual case of oral SCC of alveolo-buccal sulcus of a 50-year-old male patient who has previously reported with a small growth which gradually increased in size over 2 months. World Health Organization(WHO) described carcinoma of the oral cavity as the 6th most commonest cancer in males in developing countries, the first being lung, followed by prostate cancer and involvement of carcinoma of other organs, while studies revealed that in women, it is the 10thusualspot for cancer, the first being breast cancer, followed by colorectal cancer and carcinoma of the stomach.[6]

Risk factor associated are tobacco (smokeless and smoking), alcohol, betel quid, agents containing phenols, emission of radiation, lack of iron and Vitamin A in diet, syphilis, environmental and occupational factors, oncogenic viruses (HPV and EBV), Candidial infection, genetic predisposition, immunosuppression[2]. It has been reported that tobacco and alcohol intakeis not only prime risk factors for oral carcinoma but also hasrobust effects on the morbidity, mortality, recurrence and second primary tumor in patients.[3]

A plethora of clinical forms of Oral SCC have been reviewed. It may be in the form of leukoplakia, leukoplakia with verrucous growth, an erythroplakia[7] some of them may exaggerate in the future into a necrotic type of ulcer comprising of indurated edges. It may perhaps give rise to a comprehensive mass having papillary projections with irregular superficialtexture.[4] There is the presence of bleeding in oral SCCin case of trauma with pain after they become superficially secondarily infected whereas otherwise oral SCC is usually painless in nature[4].

The sites with the most frequent occurrence of OSCC are the inner side of the tongue along with the floor of the mouth due to the presence of thin non-keratinized epithelium. The oral sites with less frequent occurrence are the gingivae, palatal mucosa, retromolar region along with the buccal and labial surface of the oral cavity.[4] Patients who have a positive histopathologic report for oral squamous cell carcinoma should undergo contrast-enhanced computed tomography of the head and neck to help in the physiological examination to know the extent of tumor and involvement of lymph node. Magnetic resonance imaging should be indicated in case of involvement of soft tissues especially in the tongue.[9] Surgery is considered the first line of treatment. Always a multidisciplinary approach should be made including chemotherapy, radiotherapy or surgery or a combination of all of these for the better prognosis of Oral SCC.[9]

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Endo-Perio Lesions: Diagnosis Made Easy

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Abstract

It is not possible to reach into the final diagnosis by only noting the patient history and clinical examination. Appropriate diagnostic aids are mandatory to co-relate the history with the clinical findings to reach into a correct final diagnosis. It is seen mostly the diagnosis of endo-perio lesion becomes difficult and complicated, unless the clinician possesses sound knowledge regarding the basic diagnostic procedures. Or else, there is a fair chance to miss the findings, small though relevant which would lead to an inaccurate diagnosis. Hence, this review topic is taken to help the practitioners with simple basic procedures to make the diagnosis of the endo-perio lesion made easy.

Keywords: *Endo-perio lesions, Periodontal, Pulpal, Diagnosis.*

Introduction

There is a close inter-relationship among the dental pulp and the periodontium. Anatomically,there is a communication between the same via the apical foramina, dentinal tubules, and lateral or accessory canals. This makes pulpal inflammation gives rise to the inflammatory changes in the periodontal ligament much earlier than the pulp could turn into a full necrotized tissue. The toxic and the by-products of the bacteria, tissue,and food debris from the pulp attain the periradicular area via any foramina of the root canals giving rise to inflammatory and reactions.[1-5] Pulpal disease can undoubtedly lead to periodontal diseases, however, the concept that periodontal disease cause pulpal disease remains controversial. If cementum getsexposed by progressive recession, it can become more readily penetrable by repeated scaling, curettage, or by direct bacterial action. Thus, the pulp may be affected in a few instances when large numbers of dentinal tubules become exposed through advancing pocket depth. Two usual routes are available for the entry of injurious agents into the pulpal tissue.[1, 2]

1. Exposed canals (including those of the furcation) allow direct access of microorganisms and their toxic by-products to the pulp.
2. Dentinal tubules,exposed by repeated instrumentation, causing damage to the cementum, or diseased cementum can become an avenue of direct microbial load entry into pulpal tissues.

True combined perio-endo lesions occur when independent periodontal and periapical or lateral lesions are present and do communicate resulting in perio-endo lesions.[1]

Correctsteps to achieve endo-perio lesions diagnosis easily:

1. **Patient History:** Maximum data can be gained if history is noted carefully with attention. A single point missed can lead to a wrong diagnosis which can jeopardize the next process, the treatment plan, which could have easily achieved. For the pain history, leading and precise form of questions are put to the patient to get a clue to find whether the case is pulpal or periodontal. If it is a case of early pulpitis, pain would be of mild degree and short

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duration. And in the case of pulpal involvement, pain would be of acute onset with severe in degree. In the case of only periodontal involvement, as it would be chronic, the degree of pain varies from mild to moderate. And lastly, in the case of pulp periodontal lesion, pain would be of very minimal in degree as the drainage occurs via the periodontal pocket which reduces the pressure leading to a reduction in pain.[5-8]

2. **Visual examination:** It should be carried out thoroughly with proper digital pressure on both the hard as well as the soft tissues of the oral cavity. Sufficient illumination with enhanced magnification is a must during the visual examination. The tissues are examined visually well to detect the presence of dental caries, fractures, development defects, denuded enamel and dentin, cracks, erosion, faulty restorations, abscess, sinus fistula, pink spot, discoloration, in the tooth crown.[3]. To check for any swelling present, seen on the mesiobuccal fold, it would be of pulpal infection and whereas on the attached gingival would indicate of periodontal lesions.[9]

3. **Clinical examination:**

a. **Swelling:** Periodontal Probing is carried out to check the origin of the swelling. In case of a localized swelling, around or near the gingival sulcus, it becomes very difficult for the clinician to differentiate the origin of the swelling or the origin of the abscess.[10] The swelling is then probed with the help of a periodontal probe. Somewhere, if the probe drops down to the apex of the tooth and if the full width of the swelling comprises the entire pocket depth till the apex, the abscess would be of endodontic in origin. This type of swelling can be named as "blown-out". In such cases, the endodontic treatment if attempted, it results in successful periodontal reattachment within a week.[11]

b. **Mobility:** Two ends of the instrument are usually used to check the mobility of the tooth. If an isolated tooth is mobile, it can be endodontic in origin or even periodontal. It the phase is active, it is often considered as endodontic. And if there is mobility generalized, it is said to be periodontally involved.[1]

4. **Clinical Tests:** Certain tests are carried out to reach an accurate diagnosis.

(a) **Percussion Test:** If the percussion test is positive vertically, the lesion would be of periradicular infection, and if positive horizontally, it relates to the periodontal involvement.[4]

(b) **Palpation Test:** Periradicular abnormalities or "hot zone" quickly respond to the palpation method. However, it is not possible to confirm as endodontically involved or periodontally involved.[6]

(c) **Pulp vitality test:** It is an accurate test, to find the type and origin of the lesion. If there is pulp necrosis, the patient produces no response. On the other hand, if the pulp is vital, the patient gives a moderate transient response. In the case of reversible pulpitis the patient gives an immediate painful response whereas, in the case of irreversible pulpitis, the patient gives a lingering painful response.[8]

There are four various method of pulp tests are there: 1. Electric Pulp Test 2. Test cavity 3. Either Heat or Cold Test 4. Both the Heat and Cold Test. Sometime, false positive and false negative responses from the patient may confuse the clinician, hence it is advisable to add a combination of different test method to ensure a correct diagnosis.[5]

i. Electric Pulp Test: This device applies an electric shock to the tooth to check the vitality of the pulp. From the patient sensation arising from the sensory nerve of the pulp, when it comes in contact with a part of a sound tooth, the pulp is said to be vital. If there is no response, the pulp is said to be necrosed. False-positive response indicates partial pulp necrosis, and it happens due to the high anxiety of the patient and could be also of ineffective isolation. False-negative indicates obliterated root canals and it happens in case patients taking drugs to elevate the pain threshold, or poor electrode tooth contact, or recently traumatized tooth.[9]

ii. **Heat Test:** When hot stimuli are applied to the tooth structure with the help of a hot spatula, the patient sometimes complains of lingering pain which is suggestive of an irreversible pulpitis and periodically involved tooth. But if the patient pain is persistently even after the removal of stimulus, it indicates of endodontically involved tooth.[9]

iii. **Cold Test:** Few cold stimuli agents like ice sticks, ethyl chloride, dry ice (carbon dioxide), refrigerate like dichlorodifluoromethane (DDM) are used to carry this test. If the pulp is vital, the patient complains of sharp pain but for very few seconds.

If the case is an irreversible pulpit, the patient may complain of intense and prolonged pain response. If there is no response by the patient, the pulp is very likely to be necrosed. However, false positive and false negative creates hindrance especially if the tooth is multi-rooted or calcified root canals.[9]

iv. **Test cavity:** This is indeed a highly trustable device to check the pulp vitality. With the speed of a high handpiece a minute cavity is prepared on the crown surface of the targeted tooth, without local anesthesia. If the patient gives a positive response to severe pain or severe sensation, then the pulp seems to be vital. The negative response of the patient indicates pulp necrosis and completely no response from the patient indicates the tooth to be endodontically involved.[7, 8]

d. **Selective Anesthesia Test:** When there is a confusion of the origin of pain regarding the location of the arch it belongs, a specific arch block is injected at both the arches. The disappearance of pain indicates the particular arch involved and also reflects the origin of the pain.[8]However, it does not help in diagnosing the type of lesion whether it is endodontically involved or periodontally, which seems to be a big disadvantage of this test.

e. **Cracked Tooth Testing:**

a. **Illumination test:** This test is carried out to find any cracks or fractures on the tooth surface. High power light source with a fiber optic tip is used for providing illumination. The contrast is seen between the fracture lines as black shadows which will outcast the fracture line. The size position and the extent of the black line will suggest the type of fracture.[5]

b. **Wedging Test:** This test helps to recognize or identify the vertical crown fracture or crown root fracture. The patient is asked to chew a cottonwood stick or any rigid material to induce the wedging force. If there is a presence of a fracture, the patient complains of pain on chewing it. And the fracture indicates the origin to be endodontic.[6]

c. **Staining method:** This test is accompanied along with the wedge test. The dye (methylene blue dye) is used on the occlusal surface with the help of a thin brush. When the patient bites the cottonwood stick, the dye gets penetrated the area of the fracture is present. The patient is asked to gargle well and a visual examination is accompanied after it with a very magnify loops, if possible. The presence of fracture indicates with a darkened line with dye.[11]

4. **Radiographic examination:** Radiographic examination is done basically by inserting a radiopaque material like gutta-percha (GP) points inside the gingival sulcus or the periodontal pocket. On the insertion of the (GP) points, the GP points reaching till the fistula which is observed in the radiograph, the case is said to be endodontic in origin. If the GP points reach to the furcationarea or the mid-root, the case is said to be periodontal in origin. And lastly, if at all the GP points reaches till the apex region, it does not always alarm to be pulpal involvement; rather a pulp test should be carried out to finalize the diagnosis. (9). For the fistula Tracking method, once again the usage of GP points are suggested. The fistula can be seen extra orally and intraorally also. This method is performed by inserting a semi-rigid radiopaque material like GP points, deep into the sinus track till the resistance is felt. With the help of the radiograph, the course of the sinus tract and the origin of the infection is decided.[5] And also, in the radiograph, if an apical radiolucency is seen surrounding a particular tooth, it indicates that the tooth to be of primary pulpal disease. Similarly, if a furcation involvement is seen in a particular tooth radiographically but not clinically, it may be either of pulpal origin or a case of traumatic occlusion.[8]

Latest tests for pulp vitality:

1. **Dual Wavelength Spectrophotometry (DWLS):** This test is independent of the pulsatile circulation. It measures any change in oxygenation in the capillary blood rather than in the supply vessels. It has the capability of detecting the presence or absence of oxygenated blood. The major advantage of this test is the visible light is used which is filtered and then guided to the targeted tooth withthe help of fiber optics. It is a non-invasive test, small, portable, very much handy to carry, and also not expensive. It is very much suitable for the private dental office.[12]

2. **Pulp Oximetry:** This is a completely objective test, requiring no subjective response from the patient. This test can be an idle chairside screening test. It is a non-invasive, machine that measures the oxygen saturation in blood, used to detect pulpal blood circulation.

3. **Laser Doppler Flowmetry (LDF):** It helps in the measurement of the blood flow in a very small blood vessel of the microvasculature. It is a non-invasive, painless,and an electro-optical technique.
4. **Transmitted Light photoplethysmography (PPG technology):** It is an optical measurement technique used for monitoring the pulpal blood flow. It guides to find out the healthy pulp and the non-vital pulp by its pulsatile waveforms.
5. **Xenon Isotope:** It is also used to monitor the pulpal blood circulation and helps in differentiating the healthy pulp from the non-vital pulp.[6]

Conclusion

Endo-periodontal treatment is mainly indicated for the preservation of the remaining tooth structure and to safeguard the same in the future. Both the specialty, Periodontics and Endodontics are crucially concerned with the health of supporting structures of the teeth. The possibility of Periodontal and Endodontic varies only by the location of the involved portion of the tooth and surrounding periodontal structures. The role of the periodontal treatment aims at the safeguarding of the periodontium and the other hand, the endodontic treatment is carried out to maintain theshape of the root canal space followed by its 3-dimensional obturation, to re-establish or preserve the apical periodontium to a biologically acceptable condition.

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Sterilization in Endodontics: A Newer Concept

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Abstract

In the endodontic procedure the treatment consists of different types of working procedures, such as isolation in the area of a tooth by rubber dam, opening access cavity with burs and cleaning and shaping of the canal after that compact obturation of canal. In every phase of treatment sterilization instruments are needed to prevent blood born diseases like HIV, Hepatitis B, and other blood-borne infections. In-depth knowledge of sterilization protocol in every step to clean the instrument is the key to infection control. Sterilization refers to the process in which complete removal microorganisms and spores from the surface treated. The widely used method is heat-based sterilization by autoclave. Till the invention of autoclave, dry heat method was used for sterilization in all areas of dentistry. This review article describes the various method used in endodontics for sterilization.

Keywords: *Sterilization; Infection; Control; Blood; Endodontics.*

Introduction

One of the most important regions of cross-infection in the dental clinic was due to improper handling and sterilization. Control of infection was an important factor in providing comprehensive dental care. Another important matter of concern was the prevention of cross-contamination to dental staff and the other patients in daily dental practices by contaminated instruments.[1]

Blood and saliva are important carriers of infection such as Hepatitis B, HIV and other blood-borne diseases. In endodontic treatments includes various procedure which involves blood contamination during the procedure therefore making it sterilize every instruments both rotary and hand files after every patients.[2]

Sterilization is the procedure that kills the bacteria and another microorganism, pathogenic and non-pathogenic in vegetative or spore form the surface of the material to be sterile. The item free from living microorganisms is defined as sterile.[3] Chemical sterilization was used for the thermosensitive instrument, which cannot go for moist heat sterilization. For other than thermosensitive instruments all other instruments can be sterilized in an autoclave. An autoclave was used for sterilization for all instruments used in dental clinic.[4]

It was important to ensure that all microorganisms are killed after the sterilization process. So the quality management on time to time basis was an important part of the sterilization procedure.[5] To check the reliability of the sterilization the biological indicators are used such as spore strips which changes color after completion of sterilization if the autoclave is working in good condition. The indicator strips check the efficiency of the sterilizer to kill the bacteria.[6]

Sterilization of the instruments used in the operatory will only prevent the cross contaminations. If these instruments not sterilized properly may lead to cross-contamination. Most common method used for sterilizing these instruments are autoclave, ethylene dioxide gas, glass bead sterilizer and dry heat sterilizer.[7] Chemical disinfectants have the bacteriocidal effects on

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microorganisms after 15 minutes of exposure but not effective against spores and viruses. The most common method for sterilization was autoclave in a short period. Critical instruments are those that are used to penetrate soft tissue and bone. These types of instruments has to be sterilized after each use.[9]

Difference between Disinfection and Sterilization: It is important to know the difference between disinfection and sterilization both procedures can remove pathogens from the treated surface.[10] The process of removing pathogens but leaving the spore was known as disinfection and process to remove both pathogens and spores called sterilization.[11]

Cleansing and Disinfection (Presterilization): After completion of the patient first step of the sterilization process is to remove blood and debris from the endodontic instruments. Debris consists of protein and residue of blood and dentinal shavings.[12] Washing can be done by hand with disinfectant liquid or ultrasonic washer. The ultrasonic process is better as compare to hand washing because it can break the organic component.[13]

The study conducted by Sureshchandra et al[14] compares the various method of cleansing and disinfection with use of 3 material like 3% hydrogen peroxide, manual brushing and 70 % alcohol and in test group manual brushing 1% sodium hypochlorite and ultrasonic bath in the result showed that test group were more efficient. Azarpazhooh et al[15] Showed that the ultrasonic group has more potential to clean the endodontic instruments.

Disinfection of Root Canal Filling Materials: Materials used for sealing root canal are gutta-percha. Before using the GP points in the canal the GP points dipped into 5% Sodium hypochlorite sterilizes the points. Gutta-percha cones contains zinc as a component which helps the cones to inhibits some amount of bacteria to adhesion.[16]

Some studies show that during the production of GP points in the factory unit 30% of samples are contaminated with bacteria (staphylococcus spp). So if the GP point crosses the apical foramen chances of infection are more if not sterilized before placing it into the canal. Sterilization with the heat may alter the size and shape of the cone, so autoclaving is not indicated. Can be done by Sodium hypochlorite in concentrations of 0.5–5% (NaOCl), Chlorhexidine, 2% (CHX), Hydrogen peroxide, 3%.[17]

Sodium Hypochlorite and Chlorhexidine: 5% Sodiumhypochlorite was used for sterilization of cones. One of the main side effects was corrosive effects on most endodontic instruments. Rutala et al[18] who showed in the SEM scan that the use of both solutions can alter the tensile strength when placing the cone for 10 min. Eldik et al observed that 5% sodium hypochlorite for 5 min is one of the most effective method of reduction of bacteria from the surface of Gutta Percha points.[19]

Sterilization of Endodontic Instruments: After cleaning the instruments by hand washing or ultrasonic wash. The drying and wrapping should be done to all the instruments, the most common method from last twenty-year used are autoclave, glass bead sterilization for 45 seconds at 240°C, UV lights, Laser sterilization and glutaraldehyde. Many authors showed in their study that autoclave remove all bacteria and spores and virus with temperature 121 ºC for 15 min for endodontic instruments.[20]

UV light lamps sterilization works on thermal heat which causes activation of nucleic acid through the induction of thymine dimers. The disadvantage of UV lamps is they act only on the surface where direct there is contact to the light. 2% glutaraldehyde was a suitable method to sterilize the endodontic instruments and other dental instruments. The disadvantage is highly toxic in nature and it requires 12hr duration to sterilize the instrument which is difficult in clinical practice.[21] In the advancement of sterilization protocol the Laser was used as a chairside tool to sterilize reamers by CO_2 laser published in the work by Boyd at al.[22] the author found that complete sterilization of reamer was observed using argon laser. Various studies showed that the autoclave process of rotary instruments may lead to fracture during biomechanical preparation in the root canal treatment due to cyclic fatigue. To preserve the fracture 6% hypochlorite was used to reduce the microbiological load.[23]

Burs: Decontamination and Sterilization: Diamond burs used in the endodontic process to remove the caries and initial access cavity preparation after use can be dip into the ultrasonic bath for 15 min and after that can be autoclave prevent cross infection to other patients.[24]

Changes in the Physical and Mechanical Properties of Endodontic Instruments Subject to the

Sterilization Process:

Cyclic Fatigue and Torsional Stress[25]: One of the main reasons of the fracture of endodontic instruments is due to cyclic fatigue during root canal treatment. The instrument goes under cyclic stress followed by compressive loading stress which led to the fracture of instruments which again depends on the number cycles i.e., more cycles leads to more chance of fracture. Other stress was torsional which leads to complete rotation inside the canal and fracture in the endpoint where the canal is blocked or calcified. To overcome these effects NiTi files have evolved now have shape memory.

Roughness, Corrosion, and Reduction of Cutting Capability[25]: Roughness and corrosive effect on NiTi instruments during irrigation by Sodium Hypochlorite cause micro pitting phenomenon and oxygen in the autoclave causes formation of nitric oxide which causes roughness on the instruments and it may also cause a reduction in the cutting ability of NiTi instruments.

Conclusion

Based on various studies done by the author the most effective method for sterilization of endodontic instruments is the autoclave process. The glutaraldehyde sterilization was more potent to kill the pathogens from the treated surface as compared to the glass bead sterilization. Presterilization is help full in removing the debris and pathogens from the surface to 40 % in the count. Ultrasonic bath better than the handwashing technique.

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Endocrowns: A Comprehensive Review

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Abstract

Endocrown are indicated after every endodontic treated tooth. For good stabilization of endocrown prevention of healthy tooth structure was essential for tooth stabilization and adhesion of tooth. Due to biomechanical preparation the chances of failure of crowns in case of endodontic treated teeth than vital teeth. In recent years there are various advancements in endodontic materials like new adhesive systems which reduces the need of post-core restoration in the tooth. In excessively damaged teeth, endocrowns are used as an alternative method of partial denture and post-core. The advantages of endocrowns are aesthetical good, increase mechanical performances, and cost-effective. This article provides areview of endocrown used for endodontically treated teeth in the clinical practice.

Keywords: *Crowns; Post; Root Canal Treatment.*

Introduction

During the endodontic treatment, the complete removal caries by air rotor in first sitting is needed to further decay of tooth.[1,2] In this process of removal of caries leads to a considerable amount of enamel and dentine was lost during the initial access cavity preparation.[3] This process leads to insufficient tooth structure to support the filling material. The one most common complication in these type cases is the increasing chance of crown or root fracture due to chewing forces.[4] The type of dental filling material used for restoration depends on the position of the tooth anterior or posterior because in posterior teeth there are heavier forces seen as compare to anterior tooth and amount of tooth structure left after root canal therapy.[5]

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To treat these type cases there is clinical protocol were suggested like in anterior teeth with small access cavity which can resort by simple composite material and there is no need of placement of crown.[6] In posterior tooth after endodontic therapy need coverage of cuspal area because of its help to bear mechanical forces.[7] In case of less amount of healthy tooth structure left behind need a post and core procedure before crown placement.[8] In case where post and corenot indicated use of extra retentive features are usedas pins and castable posts give extra retention to the filling and crown. Post can be two types of prefabricated metal post or one-piece custom made post and core.[9-11]

Most of the studies showed that post preparation leads to loss of radicular structure to place post might weaken the root which leads to fracture of the tooth.[12] One most important complication of post was retreatment of endodontic treated tooth. Adhesives play an important role in the restoration of endodontic treated teeth.[13] In the year 1995 Pissis present a new technique with combined porcelain to metal crown in a single unit and the technique called monobloc which replaces the previous tradition technique metal post.[14] Based on the concept of Pissis, the Endocrown technique was introduced by Bindel et al. in the year 1999.

In various studies, the results show that endocrowns

in molar are high success rate and higher fracture resistance as compared to posts.[15-19] Endocrowns are easier to prepare and apply and require lesser clinical time and visits. Endocrowns have superb esthetic properties, and in cases of adhesive restorations decrease the penetration of microorganisms in apical and coronal part to improve the clinical success of endodontic treatment. Endocrowns are a greater advantage as compare to post because posts are not indicated in short and narrow canals.[20]

Changes Occuring in Endodontically Treated Teeth: The factors associated with endodontic treated teeth are more prone to fracture resistance due to loss of tooth structure due to caries, trauma and cavity preparation. Appropriate selection of restorative materials is important for the longevity of endodontic treatment.[21] The integrity of the left tooth structure would be preserved for a solid base required for restoration and increase the strength of endodontic teeth.[22] The strength depends on hard tissue and the anatomic form of the tooth is the principle of biomechanical tooth preparation. Studies showed that there was no influence of variation in tissue on tooth biomechanical behavior.[23] Minimal access cavity preparation helps in the decrease the chances of fracture resistances.[24] The other important factor for decrease durability was the loss of marginal ridge in tooth. Another important study done by Gohring et al[25] showed that loss of dentine during the endodontic access cavity and root canal preparation after biomechanical preparation increases the brittleness of tooth. Guzy and Nicholls[26] observed that in the study decrease in the durability and strength by 5%, 20%, and 63% due to access cavity preparation, restorative procedure, and occlusal cavity preparation on Tooth cusp.

Restoration of Endodontically Treated: The remaining tooth structure left after caries removal and completion of endodontic treatment tell the suitable dental filling material, choice of material was controversial it was choice of treating dentist which material he placed according to his choice no special guidelines are given by American Association of endodontics. Function requirements are one of the important factors for deciding the choice of material for treatment planning.[27]

Risks and shortcomings in conventional post and core:[28-30]

- Perforations during the post and core procedure leads to further endo-perio lesion and sometimes leads to fracture of root.
- In prefabricated post made up of glass fiber reinforced posts (GFRP) in the root canal some time not bond to radicular dentine and in long loss crown due to heavy masticatory forces.
- The limitation of amalgam core build-up material was if there was less tooth structure additional pins were placed which further decrease the strength of the tooth. It was not indicated in the esthetic area because after 5-7 years it gives discoloration in the gingival margin.
- Glass ionomer cement lack of adequate strength.
- Composites material has good strength and has the ability to binds with the surrounding tooth structure and the disadvantage of composites is polymerization shrinkage leads microleakage which causes further plaque and calculus formation and these leads to treatment failure.

Preparation Technique for Endocrowns[31-35]

Occlusal Preparation:

- Overall reduction of 2mm in the axial direction
- Diamond wheel bur was used for the occlusal surface
- Bur used parallel to occlusal plane
- Cervical margin should be supragingival, in area estherics require subgingival margin angle below 60°
- 2mm thick enamel walls will be removed

Axial Preparation:

- The first step after occlusal reduction was removing all the undercuts in prepared access cavity.
- The convergences angle was 7°for both access cavity and coronal pulpal area.
- The depth of cavity between 3mm to 5mm.
- Avoidance excessive removal of coronal dentine which leads to endocrowns in future.

Polishing the Cervical Band:

- In this step, we remove all the irregularities from the tooth surface
- Large diameter fine particle bur was used for polishing



Figure 1. Removal of irregularities from the tooth surface



Figure 2. Polishing



Figure 3. Bondning

Bonding

- Adhesives such as self-adhesive and composites such as are used for bonding the endocrown to the prepared tooth.

Impression technique/Manufacturing: As compare to conventional rubber bass impression digital impression are more helpful to these types of case because it takes accurate record of prepared preparation of crown and it's to send the scan for CAD/CAM crown preparation. These digital impressions tell us if there any undercut present in the cutting to its easy to correct the preparation and make it less time consuming and no need for a diagnostic wax-up trial.

Indications of Endocrowns:

- Successfully treated endodontic treated tooth.
- In case less interproximal space and occlusal hight.
- In case were post and core not possible
- In the case of calcified, curved canals.

Contraindications of Endocrowns:

- In case of parafunctional habits
- In case of were proper isolation is not possible due to excessive saliva
- Cervical margin less than 2mm and depth less than 3mm.

Advantages of Endocrowns:

- Less complex to perform and easier to perform in patients
- Allow less reduction of radicular dentine so the strength of the tooth was not hampered
- Allow retreatment of a root canal if indeed in future
- Less chair side time and cost to the patient

Disadvantages of Endocrowns:

- Risk of debonding of crown and root fracture

Conclusion

An endodontically treated tooth in those cases where post and core was not indicated in these types of case endocrown. Endocrowns preserve the tooth structure and has several mechanical and aesthetic advantages. It's indicated in posterior teeth and showed better performance in molars than premolars.

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Basic Package for Oral Care: A Means to Tackle Community Dental Needs

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Abstract

There exists a gaping rift between the available dental services and the need for dental care in communities, giving rise to the 'inverse care law'. WHO in collaboration with the University of Nijmegen, worked along the principles of Primary Health Care (PHC) to give the Basic Package of Oral Care (BPOC) which is designed to cater to the dental needs of outreach populations within the confines of minimum resources such as lack of electricity and dental setup, for a maximum effect of providing emergency, preventive and curative dental care. Implementing BPOC with prudent use of existing PHC workforce, local resources and collaboration with NGOs could be the best answer to tackling the dental needs of communities.

Keywords: *BPOC, ART, AFT, OUT, PHC.*

Introduction

Health is considered a fundamental human right. Various attempts have been undertaken by international health agencies such as the World Health Organization (WHO), and by individual governments to make this a reality but still have a lot to make up for. Primary health care was proposed by WHO and UNICEF in the Alma Ata declaration.[1] This recommended reliance on local, easily available means of healthcare to address the felt needs of the local population. Though tremendous improvements are observed in the form of a fall in mortality and morbidity rates, oral health often remains disregarded. Oral health often is considered a luxury, is ignored in health planning by most governments.[2] Hence the Basic Package of Oral Care (BPOC) was developed in collaboration with WHO in Nijmegen. The BPOC was deemed to be a means of effective, feasible and affordable oral care that would address the essential oral health needs in any community.[3]

Premise for introducing BPOC: People from low and middle-income countries that comprise of more than 70% of the world's population have very inadequate access to oral health care. India with its varied population faces the challenge of unequal distribution of health care with 700 million of its population in rural areas but only 25% of doctors practicing in semi-urban and a meager 3% in the rural parts.[4] The oral health scenario is further worsened by the fact that dentistry is considered a domain of private practice confined to urban areas. Oral health is also overlooked by policymakers and is not included as a part of Primary Health Care (PHC).

Primary Health Care philosophy was developed with an emphasis on redirecting the control and prevention of commonly occurring diseases away from healthcare based on hospitals. PHC primarily aims to provide for

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all basic preventive and curative care at a cost that the community can afford.[5] Presently basic oral health care is not included in PHC due to various factors;

a. Dentistry is commonly perceived as a specialty targeting individual care rather than a community.
b. Oral health is assigned low priority compared to general health.
c. Lack of judicious use of non-dental personnel and auxiliaries by dental professionals to address the dental needs of a community.
d. Perception that most dental treatments require sophisticated armamentarium.
e. Cost of dental is often beyond the economic means of a rural population.[6]

But oral health is found to influence the quality of life of all populations urban and rural alike. Dental caries is a common chronic disease that is on a rise in the low income countries. It accounts for loss of school days among children and missing work in adults due to unmet dental needs.[7] Even in high-income countries, oral diseases contribute to higher expenditures on treatments in comparison to other major diseases like stroke, cancer, and heart diseases.[8] Hence oral health pleads for substantial consideration in health policies of countries due to the following factors;

a. High prevalence: It is almost universal in its occurrence.
b. Effect of dental ailments: in terms of discomfort, pain, social and functional limitations caused.
c. Effect on general health.
d. Deterioration of quality of life associated with dental diseases.

Keeping these in mind, WHO and FDI (World Dental Federation) set global goals to be achieved by the year 2000. Later WHO, along with FDI and International Association for Dental Research (IADR) formulated oral health goals to be achieved by 2020. These goals required particular oral health programs targeting providing oral health care to those who need it the most.[9] This required a strategy that could use the prevailing community health structure. And also conform to with the principles of PHC i.e., an approach that was practical, scientifically sound and socially acceptable.[10]

Rationale of BPOC: A plan of action that is affordable, following the ideologies of PHC, that brought dental care within reach of disadvantaged communities. The idea is to reach all communities at costs more affordable than traditional dental health services. The BPOC comprises of;

a. Oral Urgent Treatment (OUT)
b. Affordable Fluoride Toothpaste (AFT)
c. Atraumatic Restorative Treatment (ART)

WHO suggested that every country's government could use BPOC based on the needs of its communities either with the aid of existing health care or external resources such as Non-Governmental Organisations (NGOs). (10) The use of BPOC along with oral health promotion and education could prove as a coordinated effort towards better oral health.

Components of BPOC:[2,10,11]

a. **Oral Urgent Treatment (OUT):** Based on the felt needs of a community, it is an emergency oral treatment provided on demand that differs from one community to another. OUT comprises of the following;

- Pain relief from oral causes.
- First aid treatment for relief of oral infections
- First line of treatment for oro-facial trauma
- Provision of referral services for complicated cases

Most commonly pain is the reason for dental visits. Pain from dental issues affect the quality of life, accounting for the loss of days from work and school. Hence handling of pain is one of the important aspects of BPOC. The treatments under OUT include;

- Extraction of severely damaged teeth.
- Handling of post-extraction complications such as bleeding and dry socket
- Drainage of dental abscess
- Medication for acute dental infections
- First-line for treatment for oro-facial trauma
- Referral of complicated cases

Pain is commonly from decayed teeth. Hence extraction of the affected tooth or draining of the abscess would provide the relief. 90% of decayed teeth are often left untreated and handled with prescription of medication. However, prudent prescription is advised in

lieu of increasing drug resistance of micro-organisms.[12] Oro-facial trauma is the second most common cause of availing dental treatment. Trauma involving tooth structures are more frequently seen among children and younger adults.[13] Delay in treatment of these could result in loss of vitality and root resorption. In developing countries, road traffic accidents most often cause facial trauma. Appropriate treatment at the earliest prevents further complications. A proper referral chain ensures the provision ofsuitable dental treatment. Referral for complicated cases should be done to the nearest dental facility. OUT incorporated at the sub-district level in the PHC system would make oral emergency care available to all.

b. Affordable Fluoride Toothpaste (AFT): As salt and water fluoridation is not possible in developing countries, the most feasible and effective fluoride delivery is through the use of fluoridated dentifrices. Hence it's the preferred anti-caries agent especially in rural areas. Reasons for using AFT are;

- Cost-effective and equitable means of caries prevention
- Effect of AFT is predominantly topical
- Extensive well-documented trials have proven a steady decline of caries in developing countries from DMFT of 8 to 1.5 in 12-year-olds.
- When the use of fluoride dentifrice is adequate, the diet has a lesser role to play in the prevention of caries.

Affordable fluoride toothpaste could be the most cost-effective and appropriate means of caries prevention in countries that otherwise lack access to specialized preventive care.[14] Proper packaging with information regarding the date of production and expiry is mandatory. Package should also display the fluoride compound used and its concentration. A concentration ranging between 1000-1500 ppm is recommended as, a concentration below this would notbe effective in preventing caries while higher content would increase the risks of dental fluorosis when used in children.[15] Toothpaste that meets the recommended standards could be made tax-free by the governments to encourage its widespread use.

Efficient collaboration among the manufacturers, advertising agencies, government, and dental professionals is mandatory to promote optimal use of fluoridated toothpaste. Long-term reinforcement programs at an early age are necessary to initiate use AFT and ensure it life-long use.[14] Brushing in children should be supervised by adults to minimize the chance of fluorosis. Using a pea-sized quantity of toothpaste along with making sure the tube of toothpaste lasts longer, guarantees a lesser risk of fluorosis. Brushing twice a day with fluoridated toothpaste effectively can prevent both dental caries and periodontal disease, thereby reducing the burden of dental diseases in a population.[16]

c. Atraumatic Restorative Treatment (ART): In the case that dental caries cannot be prevented, the affected tooth needs to be treated. Restoration of teeth relies on expensive electric driven equipment, thereby limiting treatment procedures to those who have access to the dental clinic. To overcome this, ART was proposed as it requires no electric equipment. ART consists of the use of hand instruments to excavate caries and restoring with material that bonds with the tooth structure. The cavity is overfilled and the excess material is then flowed to seal adjacent pits and fissures.[17]Therefore, it conforms with the concepts of preventive and restorative care. It avoids the sacrifice of healthy tooth structure and provides prevention with minimal intervention.

The survival and effectiveness of ART has been ascertained by various studies. 90% of dental caries in primary teeth and 54% in permanent teeth among a high-risk group in Syria could be treated with ART.[18] Medium strength GIC was the restorative material used. Single surface restorations were found to have of 95% - 100% one-year survival rate, whereas 2 year and 3-year survival rates are at 92% - 94% and 85% - 89% respectively. Failures of ART in the first 3 years are limited to 4-5%.[19]In primary teeth, single-surface ART restorations had a survival rate of 86% in contrast to 80% in amalgam restorations at the end of 3 years among Syrian school-going children.[18] The sealing of adjacent pits and fissures with GIC prevents dental caries and hence this fact needs to be considered while evaluating mechanical retention of restorations. It was found in children who underwent ART restorations in Zimbabwe, had four times lesser chances of developing caries.[20]

Factors favouring acceptability of ART:

- Reduced discomfort during treatment procedure:

Studies conducted have reported discomfort experienced is minimal in ART as compared to

conventional restorations where drilling is used. The discomfort was reported in only 19% undergoing ART as opposed to 36% of conventional amalgam fillings in 6-16-year-old children of Pakistan. Whereas, only meager 7% of the pre-school children in China felt discomfort while ART was performed. Hence, making it the treatment of choice in children who have limited access to dental care.[10]

- **Negligible post-operative sensitivity:** Sensitivity associated with the ART after the procedure is only 5% in Chinese adolescents and 6% of teenagers in Zimbabwe.[21,22] Therefore it can be safely said that sensitivity associated with ART is less and it can be used as acceptable form treatment of dental caries.
- **High satisfaction among patients:** 91% of Chinese and 95% of Zimbabwe secondary school students very satisfied with the operational procedure of ART and believed, that they would not mind undergoing the procedure once again.[21,22] This reinforces the fact that ART can be performed as a substitute for conventional restorations in areas with restricted access to dental treatments.

It has been found that because of the above-mentioned factors, ART works as an efficient means of treatment delivery. ART can be done through mobile dental units or the system in place for the delivery of health care. Because of its ease of sterilization and acceptability, it was observed that in a school in South Africa, the percentage of extractions came down by 17% in permanent teeth and 36% in primary teeth. It also saw an increase of 33% in restorative care, one year after administering ART.[23]The cost of ART using GIC is much less compared to that of a conventional amalgam restoration. Thus all the above-stated facts make making ART the clear choice for rendering restorative in outreach areas.

Requirement of personnel for carrying out OUT and ART: While promoting and dispensing fluoride toothpaste requires the teamwork of dentists and policymakers, the provision of emergency oral care and ART entails a team of well-trained workforce in place. This team differs from place to place based on the characteristics of the population that they will be serving, health strategies and the legislation of every country. For providing emergency oral health care under OUT, the following personnel can be utilized based on examples from different countries.

- Nurses from primary health care centers: Cambodia sourced their workforce from the PHCs. The nurses undergo a period of training for five months, after which they are employed at the district referral center until they are proficient in carrying out all the procedures included in BPOC.[24]
- Rural medical aid: In Tanzania, rural medical aids are trained for three years, after which there is dental training. They then provide relief of pain through extractions and the provision of medications.[25]
- Health assistants: Nepal, trains its health assistants to handle extended duties such as oral health education, first aid for oro-facial trauma and extractions.[26]
- Dental therapists: In countries like Vietnam, Zimbabwe, and Malawi, dental therapists are trained to provide OUT services.[10]

In the Indian scenario, dental auxiliaries such as dental hygienists and health care workers like ASHA and Anganwadi workers can be given additional training to handle dental emergency services. Also rural postings in PHCs can be made compulsory for interns of very dental college to help in rendering these services.

The personnel required for ART pose tougher challenges than those needed for OUT as, carrying out the procedure of ART requires an in-depth knowledge about dental structures and dental materials. Hence training courses of short duration are not sufficient. It was found that dental therapists, students studying to be dentists, and dentists could be efficient in ART procedures, with dentists being the most competent of them.[10]However, the decision regarding which personnel to use is the prerogative of every country and its governmental policies.

Requirement of equipment for OUT: A basic set of dental instruments and means of sterilizing them is necessary for the provision of OUT. A judgment regarding type and number of extraction forceps required has to be taken base on the local scenario. Usually, 2-4 types of extraction forceps and 1-2 types of elevators are considered sufficient. Apart from these, the following equipment is necessary;[10]

- Bed or chair with head support.
- Chair or stool for the dental personnel.
- Table for medicines and instruments.
- A source of light, preferably battery operated.

- Washbasin
- Means of storing water.
- Heat source and pressurized container for sterilizing instruments.

Requirement of equipment for ART:[17]

Equipment essential for ART are:

- Bench or chair for the patient
- Stool or chair for the operator
- Source of light
- Hand instruments - Mouth mirror
- Explorer
- Tweezer
- Excavators
- Dental hatchet
- Applier/carver
- Consumable items such as -Adhesive material, usually GIC
- Cotton for making rolls and pellets
- Petroleum jelly
- Wooden tips and matrix bands

Equipment requirements for ART have been kept to a minimum to cater to treatment without the use of electricity and sophisticated armamentarium. Thus making ART accessible and affordable even at most isolated locations.

Course of action for implementing BPOC:[2, 11, 27]

1. Identifying a local partner: Having a local body to aid in the identification of the specific needs of the population, providing support for planning and implementation of BPOC would make the process easier and hassle-free.
2. Gaining decision maker's approval: Involving the policymakers from the level of Ministry of Health right from the outset and their consent at every stage is essential for the successful execution of the program.
3. Involvement of other possibly interested parties: Participation of NGOs and other local political, medical and educational systems would give the necessary boost for the program in the form of funds and required manpower.
4. An in-depth comprehension of the local situation: The program must be based on the needs and expectations of the community that it is catering to. Various factors such as the oral health status of the community, their perceived and normative needs, awareness and habits related to oral health, financial and manpower resources influence the operation of the program.

How can BPOC put into action in India?

1. Involvement of NGOs: There is a surge of non-governmental organizations that are aware of their social responsibilities and are financially transparent. Their support can be the boost required for initiating and supporting BPOC implementation. However, certain factors need to be considered such as the extent of organization management, quality control and accountability of the organizations, limited financial resources and problems regarding integrating these personnel into the existing communityhealth care system.[6]
2. Integration with health workers from PHC: India has a competent system of primary health care in place efficiently catering to the health needs of the communities in rural areas. Health workers in this set up can be trained to deliver emergency dental health services. Oral health care needs to be integrated at all three levels; Macro-level (health system), Meso-level (Organisational system – by meansof inter-organizational relationships) and Micro-level (coordination at a personal level). Integration must be both horizontal and vertical for a capable incorporation. (28) The non-dental workforce that is available in the form of teachers and community health workers can be used to provide a range of dental services ranging from emergency care to prescription of medication and promoting the use of fluoride toothpaste. These personnel can also be used to generate a competent network of referral systems by creating a pathway for referral of complicated cases to nearby dental setups.[3]
3. Responsibility of local dentists: Dentists from the nearest localities can volunteer to meet the dental needs of an outreach facility individually or in partnership with an NGO. Dentists could also help the training of local health care workers in providing BPOC.

4. Role of dental institutions: Institutions can take up the responsibility to address the treatment needs of rural communities without access to dental care. This can be further facilitated by incorporating BPOC as a part of the dental curriculum. Studies evaluating awareness regarding BPOC among dental students found poor knowledge.[9,29] Hence steps to make them proficient in services provided under BPOC and then posting them to these areas could help in rendering dental care. Conducting workshops for training of local PHC workers such as ASHA and Anganwadi workers in BPOC could also be undertaken by dental institutions to makethe local community self-reliant.

5. Partnership between Public and Private sectors: Collaboration between local communities and governmental institutions have been purported to be the most important area to work on to increase access and affordability to dental care. The advantages of both these sectors can be combined to work complementary to each other. Areas such as conducting awareness programs, provision of manpower through outsourcing could be done to work in the best interests of both sectors.

Conclusion

Since oral health is important for general health and wellbeing, BPOC provides the best way of reaching the unreached at a cost that communities can afford throughout every stage of their development. The burden of oral health care in the communities can be addressed through proper implementation of BPOC. Oral health promotion is thought to be effective when both upstream and downstream interventions work complimentarily.[30] BPOC can act as a primary source for preventive and curative treatments which are a part of the downstream approach, this combined with upstream interventions such as health education could be the answer to tackling oral health problems competently in the communities.

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Challenges in Regenerative Endodontics: A Case Report

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Abstract

Regenerative endodontics is a part of the advanced endodontic procedure for the well being of immature teeth with root canals that are infected and it can be called as "paradigm shift" in the ideal approach of treatment for continued root maturation and apical closure. It is a procedure which is clinically oriented to disinfect the root canal system without damaging stem cell present in the apical papilla and dentinal wall. By instrumentation process bleeding induced in the apical area to induced new stem cells in this area followed by intracanal cement placement to prevent microleakage. The mechanism of REP was to make triad by biomarkers, stem cells, Scaffold which works on the regeneration of lost tissue of pulp dentine complex and further root formation. Many authors suggested that the REPS procedure is the definitive treatment of immature teeth with pulp necrosis.

For a dentist it is a unique challenge to treat an immature tooth with apical periodontitis and pulpal necrosis. For endodontic treatment the options available are apexification, apical barriers and revascularization of the permanent tooth. 16 years old boy comes to a comprehensive college clinic with the complaint of pain and swelling in the lower posterior tooth region. On clinical examination lower premolar with apical periodontitis was treated with revascularization protocol irrigating with normal saline and sodium hypochlorite, an antibiotic paste (triple), mineral trioxide aggregate and then a coronal seal with a dental composite. In a follow up of one year there was an evident resolution of radiolucencies in the periapical area and root development was appreciated.

Keywords: *Revascularization; Immature tooth; Antibiotic paste*

Introduction

In the year 1960, Dr. Ostby first proposed the theory of regenerative endodontic procedure. The theory says that the vitality of the pulp is directly dependant on the presence of blood clots in the canal which helps in promoting healing of the pulp.[1] The process was similar to other parts of the body like blood clots help the injury site to repair itself.

Authors suggested that REPS can be performed in infected immature permanent tooth because in a clinical trial and histological examination they found mesenchymal stem cells on apical end of the root.[2-4] These stem cells have the ability to differentiate into odontoblastic cells that helps in forming root dentin and these cells are called SCAP (stem cells of apical papilla) and other cells present in pulp are called dental pulp stem cells (DPSCs).[3] They both can form dentin pulp complex. Both cells are potent to differentiate as mesenchymal cells from the bone. The difference between in these cells like, SCAP showed a greater proliferation of the cell, number of population doubling and tissue regeneration as compared to DPSCs.[4]

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Factor affecting the revascularization is related to infection in the root canal. It is difficult to treat infected canals with the REPS technique. In these types of cases triple antibiotic paste or CaOH paste are used to disinfect the canal. The second important factor was the size of apex during REPS is more than 1.1 mm apical diameter then revascularization increases in about 18 - 34% of teeth with immature root development. The 3rd factor is age which is considered between 8 to 16 years which is more considerable to the REPS procedure. REPS procedure should be avoided in the deciduous tooth as there is potent risk of impairing the eruption of permanent dentition.[5,6]

Regenerative endodontics procedure (REP) is the biological procedure in which damaged tissue can be replaced by newly formed cells (Dentin and root structure). REP is part of tissue engineering, which was adopted by the American Dental Association in the year 2011. Root canal is the definitive treatment in the case of pulpities or apical periodontitis patients.[7] The RCT procedure involves the complete removal of pulp tissue by biomechanical preparation, so that the bacterial and toxin load will reduce in canal in adult patients.[8] In children absence of apical constriction can be seen which can lead to RCT procedure problematic. For these types of case, apexification can be the best option, but there are some disadvantages like treatment time, compliance of the patient, risk of fracture of bone associated with tooth.[9]

So, recent advancement in endodontics, for the treatment of immature tooth regenerative endodontics procedure is used. REP is a biological replacement of dental tissue and makes the tissue in a functional state. MTA was the material of choice.

Case Report: A 16-year-old male patient reported to the dental clinic with the chief complaint of pain accompanied by swelling in the lower back tooth region. The patient has no previous medical and dental history. On clinical and radiography examination large carious lesion found in tooth number 34. On intraoral soft tissue examination reddish-pink gingival, soft and edematous with rolled out margin, no periodontal pocket was appreciated, on palpation was mildly painful with sinus tract. On radiography examination (Figure 1) secondary caries under the restoration and radiolucency and widening of Lamina dura were present on the apical area of the root. The final diagnosis tabulated was chronic apical periodontitis after clinical and radiographical considerations. Thereafter informed consent was taken from the patient and treatment was initiated.



Figure 1: Showing a carious lesion in lower left second premolar

Local anesthesia 2% Lignocaine with epinephrine, was administered isolation was done by rubber dam. Access cavity was performed, bleeding and purulent exudates were seen in the canal. Proper irrigation protocol was followed by 5.25% Sodium Hypochlorite, 20 ml of normal saline followed by 10 ml of 2% Chlorhexidine.

The canals were dried, and a triple antibiotic paste consisting of ciprofloxacin, metronidazole, and minocycline (100 mg of each drug in a 0.5-mL total volume) was placed. After three weeks the patient was symptomless. Local anesthesia without epinephrine was administered and then removal of triple antibiotic paste was done. Bleeding was induced by hand file in the canal, bleeding was controlled and then MTA was placed over the clot.



Figure 2: A radiograph showing placement of MTA and completion of composite restoration on tooth.



Figure 3. Healing of the radiolucent lesion, 1 year follow up.

On one year follow-up during clinical examination patient was asymptomatic, no bleeding, no swelling and no sinus tract was detected. Radiographically there was no visible lesion appreciated.

Discussion

Regenerative endodontic procedures also known as REPS is defined as a procedure that is biologically designed to replace damaged structures dentin, root structure and cells of the pulp-dentin complex.[9] It is a new modality to help the normal function of the pulp. It has become an alternative method to heal apical periodontitis. REP is an extension of root canal therapy with specially designed method. In conventional treatment the dentist cleans the root canal and fills the pulp chamber with inert material after cleaning the root canal.[10] As compared to conventional root canal, REPS replaces the live tissue cells in pulp tissue. The main goal of REPS is to regenerate tissue present in the pulp and modify the function of the pulp dentin complex. [6]

Before the introduction of REPS apexification procedure was used in the treatment of immature permanent teeth with the use of mineral trioxide aggregate and long term use of calcium hydroxide.[11] This treatment cannot resolve complete signs and symptoms of pathology. They affect the root development procedure. One of the options for the treatment of immature teeth with open apex can be Regenerative endodontics procedure (REP) because it has a great ability to affect the size of root length ratio and thickness of root wall by biomechanical process.[12-14] Apical bleeding initiated by the hand file in the root canal, making a feasible environment for pulp regeneration with host endogenous stem cells and growth factor in a bioactive scaffold.[15]

A study done in the year 2002, showed that the use of CHX as irrigation material caused detrimental effects on stem cells. The role of triple antibiotic paste has reduced the bacterial and toxin load in the canal. Many recent research articles show that inclusion of blood clot in the canal space help in revascularization and bleeding produces predominant mesenchymal stem cells which can form dentine complex.[16] The placement of MTA helps in making the apical barrier so that bacteria cannot enter through apical constriction. Many researcher suggest that use of $Ca(OH)_2$ in the tooth before placement of MTA as $Ca(OH)_2$ make the clot settle in the canal and prevent the movement of MTA, the coronal end 2-3 mm apical to cement enamel junction after which Glass ionomer material is placed over the MTA.[17]

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Regenerative Endodontic Treatment in Maxillary Central Incisor: A 6-Month Case Report

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Abstract

The most important cause of pulpal necrosis was trauma during an eruption of the tooth, trauma cause delayed root formation, which leads to fracture of root. In recent advancements in the field of dentistry we can convert non-vital teeth into vital once again by regenerative endodontic procedure. There are many procedures we can included in the regenerative are root canal revascularization, pulp implant, stem cell therapy, gene therapy and injectable scaffolds. In this case report we discuss the successful regenerative endodontic treatment of necrotic permanent maxillary left central incisor. On clinical and radiographic examination the large periapical defect in respected tooth end. After local anesthesia administration, rubber dam placed, access cavity preparation will be done with sterilization protocol, irrigation with the irrigation material for the choice was 1.25% of sodium hypochlorite with saline after proper irrigation no filing will be done at this point of time after this calcium hydroxide will be placed in the dry canal after 3 months follow, intraoral periapical radiograph shows less radiolucent area as compare to baseline radiograph and no visible inflammatory sign seen in the surrounding tissue then filing will be done in the canal to induce bleeding after visible bleeding visible in the canal after canal dry MTA will be placed. During the following period the patient as asymptomatic and after a 1-year x-ray the lesion was subsided and followed by root canal treatment completion.

Keywords: *Regenerative, Root canal treatment.*

Introduction

Regenerative endodontic procedures (REPS) is defined as biologically based procedures designed to replace damaged structures such as dentin, root structures, and cells of the pulp-dentin complex. It is a new modality to help the normal function of the pulp, it become an alternative method to heal apical periodontitis. REP is an extension of root canal therapy. In conventional treatment the dentist cleans the root canal and fills the pulp chamber with inert material after cleaning the root canal shape. As compared to the convention root canal, REPS replaces the live tissue cells in pulp tissue. The main goal of REPS is to regenerate tissue present in the pulp and making the function of pulp dentin complex.[1,2]

Before the introduction of REPS in the dental filed apexification procedure was used in the treatment of immature permanent teeth with the use of mineral trioxide aggregate and long term use of calcium hydroxide.[3] This treatment cannot resolve the complete signs and symptoms of pathology. They should affect on root development procedure.[4] In children traumatic injuries are more common in the age group of 8-13 years. The effect of trauma lead to loss of pulp vitality, pulpal necrosis causes difficulty in root formation.[5] The treatment of pulpal necrosis in an immature tooth with an open apex is a challenge for treating dentists due to

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the open apex it was difficult to obtain an apical seal with conventional obturation technique.[6]

In recent years, we are come across with different new treatment procedures in the field of endodontics. Regenerative endodontic treatment procedure help in root formation and apical closure in immure tooth.[5] Regenerative endodontics is defined as "restoration of tissue architecture and biological function of damaged tissues by tissue similar to the original tissue" Revascularization is a regenerative treatment modality and method to biologically replace a diseased dentin and root structure procedure.[7]

This case reports the successful neurogenesis of permanent necrotic central by revascularization approach.

Case Report: A 10-year-old boyreported to the dental clinic with the chief complaint of swelling in the upper front tooth region. The patient has no previous medical and dental history. The patientgivesa history of trauma 2 days ago, which leads to a crown fracture in the upper permanent maxillary left incisor. On extraoral examination, swelling in the upper lip and it will extend till the base of the nose and left eye. On intraoral examination discoloration of the maxillary central incisor was seen, on soft tissue examination reddish-pink gingival, soft and edematous in nature with rolled out margin, no periodontal pocket was appreciated, on Palpation was mildly painful. On radiography examination(Figure1) large encircled lesion was present on the apical area of the root. After clinical and radiographic examination the final diagnosis was made chronic apical periodontitis. Before starting the treatment informed consent was taken from the patient.

Procedure: After local anesthesia 2% administration, rubber dam placed, access cavity preparation will be done with sterilization protocol, irrigation with the irrigation material for the choice was 1.25% of sodium hypochlorite with saline after proper irrigation no filing will be done at this point of time after this calcium hydroxide will be placed in the dry canal after 3 months follow, intraoral periapical radiograph shows less radiolucent area as compare to baseline radiograph and no visible inflammatory sign seen in the surrounding tissue then filing will be done in the canal to induce bleeding after visible bleeding visible in the canal after canal dry MTA will be placed. During the following period the patient as asymptomatic and after a 1-year x-ray the lesion was subsided and followed by working length was taken with apex locator and verified by x-ray+ tooth was filled with MTA root canal treatment completion. During the follow-up appointments, the patient was completely asymptomatic.

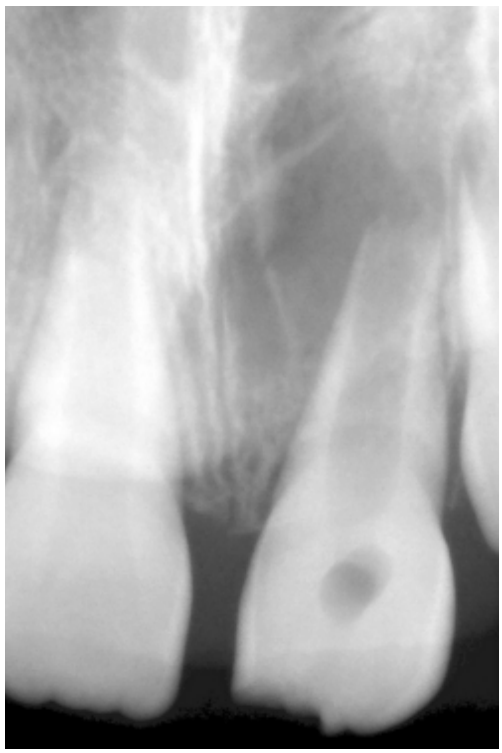


Figure 1. Diagnostic X-ray



Figure 2. Calcium hydroxide placement

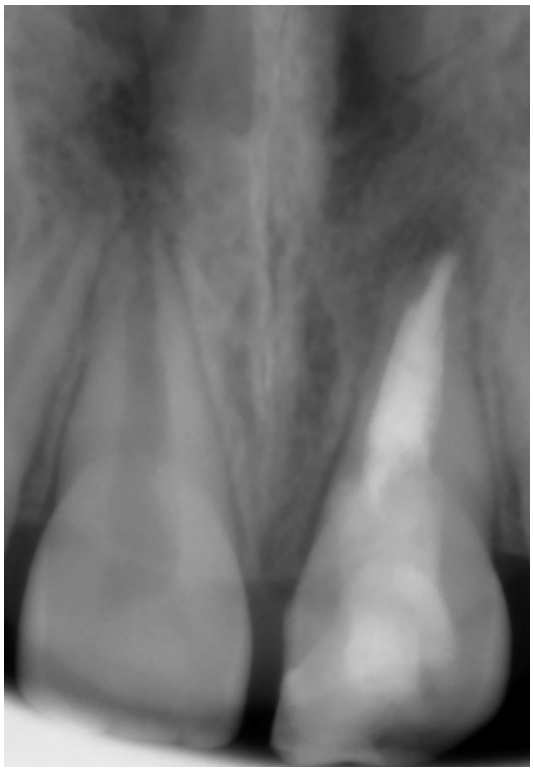


Figure 3. Control x-ray after root canal treatment

Discussion

REPS procedure was founded by Dr. Ostby in 1960. The theory he suggested that the presence of blood clots in the canal promotes healing of the pulp, which helps in the vitality of the pulp. The process was similar to other parts of the body like blood clots help the injury site to repair itself.[8]

To see the above hypothesis, the above case diagnosed with the apical periodontitis received cleaning of canal space followed by placement of medicament after initiation of intracanal bleeding. After 6 months obturation placed coronal to formed blood clot.[9,11]

In various study, authors suggested that REPS can be performed in infected immature permanent tooth because in a clinical trial and histological examination they found mesenchymal stem cells on apical end of the root.[12-16]

These stem cells have the ability to differentiating into odontoblastic cells that helps in forming root dentin and these cells are called SCAP (stem cells of apical papilla) and other cells present in pulp are called dental pulp stem cells (DPSCs). They both can form dentin pulp complex. Both cells are potent to differentiate as mesenchymal cells from the bone. The difference between in these cells like, SCAP showed a greater cell proliferation, number of population doublings, and tissue regeneration as compare DPSCs.[17-19]

Factor affecting the results of revascularization is the degree of infection of the canal, it is difficult to treat the infected tooth with REPS procedure in these types of case triple antibiotic paste or calcium hydroxide paste are used to disinfect the canal.[20] The second important factor was the size of apex during REPS more than 1.1 mm apical diameter is beneficial and the possibility of rev revascularization increases in approximately 18 - 34% of teeth with immature roots. The third factor is age consideration between 8-16 years is more considerable to REPS procedure seen in this group give good results. REPS procedure cannot perform on deciduous teeth because of the possible risk of impairing the eruption pattern of permanent teeth.[20]

In 1996, Hoshino et al. recommended a tri-antibiotic paste, which was composed of ciprofloxacin, metronidazole, and minocycline, to disinfect the canal, and this medicament can be used effectively in regenerative endodontic treatment. On the other hand, Chueh et al. reported that complete disinfection of the canal and regeneration can be achieved by using calcium hydroxide alone.[21]

This case report highlights regenerative endodontic treatmentin maxillary central incisor using $Ca(OH)_2$ and MTA. The most important factor for the successful outcome of the case was the selection of that case and choice of medications used and the technique of sealing the apex. In young children RET shows better results compared to apexification. The disadvantage of apexification was surgery time is more than RET. Postoperative complications are noticed in the apexification like swelling and bleeding.[22]As per the guidelines by the American Association of Endodontists instrumentation in regenerative endodontic procedure are contraindicated because of the chance of fracture in the dentinal wall.

The most common and potent intra-canal medicament used in dentistry was $Ca(OH)_2$. The benefit of $Ca(OH)_2$ prevent infection and have the ability to improve the length of root and thickness of the wall as showed in the study done by Bose et al and MTA was used in RET cases. MTA has sealing properties and excellent biocompatibility makes it the material of choice in RET procedure.MTA is composed primarily

of Portland cement and bismuth oxide such as other MTA products. MTA contains calcium carbonate and a chloride accelerator.[23,24]

Conclusions

Case shows the management of an immature maxillary central incisor reported on successful regenerative endodontic procedure done using calcium hydroxide and MTA. For future clinical studies the comparing RET with $Ca(OH)_2$ and apexification techniques are needed to see the better effect of RET on periapical healing in the central incisor.

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Ethical Issues: Approved

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A Review on Oral Candida as Commensal and Opportunistic Pathogen

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Abstracts

Oral candidiasis, commonly referred to as "thrush," is an opportunistic fungal infection that commonly affects the oral mucosa. The various forms of oral and maxillofacial candidiasis are pseudomembranous, acute, chronic, median rhomboid glossitis, perioral dermatitis, and angular cheilitis. The main causative agent Candida albicans, is a highly versatile commensal organism that is well adapted to its human host, continuing rise in the development of pathogenicity and resistance to traditional antifungal agents. Thus needto develop novel therapeutic strategies that can find the early resistance to particularantifungal. So the clinician can be better able to prescribe effective antifungal for oral candidiasis.

Keywords: *Oral candidiasis; Candida albicans; anti fungal treatment.*

Introduction

The species of Candida is present inside the oral cavity together as an opportunistic and commensals pathogens. In the current years, incidence noteworthy in the state of pathogenic of commensal been seen and reflected by increased escalation of infrequent and common candidiasis forms.[1] The morbidity in oralcandidiasis may as discomfort, chronicpain, limiting mastication especially in immune-deficient and in elderly patients. There exist clinical multiple presentations of oesophageal and oropharyngeal Candidiasis created by candidaalbicans both alone/in infection of mixed type. The treatment is based upon the Candidiasis type-(pseudomembranous, acute, chronic, angular cheilitis etc.) and severity of infection. Hence the review presently comprehends opportunistic pathogen oral Candida.[2] The Candidaalbicans exists primarily as a fungus of dimorphic form. The ability of Candidaalbicans is transforming into various forms morphologically like hyphae, yeast & psuedohyphae by the signals from the environmental perception which is significant for virulence.[3,4] The psuedohyphae are referred to ellipsoidal, elongated cells which are adherent to each other, while the true type of hyphae cells characterized with a morphology of cellular cylindrical and the walls of septal are perpendicularly separated.[5] Infection of fungal type is the thrush or Candidiasis which is attributable to any species of Candida genera among which is Candidaalbicans, the causative common species.[6,7]

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Scientific classification of Candida species:

Kingdom: Fungi, Phylum: Ascomycota, Subphylum: Saccharomycotina, Class: Saccharomycetes, Order: Saccharomycetales, Family: Saccharomycetaceae, Genus: Candida

Formation of Germ tube -The germ tube arises from the Candidaalbicans as lateral short filamentous form growth cells.[8,9] It's mentioned that tube of germ, grows as Candida being cultured on medium of solid example Sabouraud Dextrose Agar (SDA) & depending on formation of germtube leads to the character for identification of *Candidaalbicans*.[10,11] According to Davis 1972 germ tube of C. albicansis opportunistic, which increases its virulence. And it is the most important factor in virulence by giving mechanical power to resist the phagocytic cells of the host.[12,13]

Antifungal therapy: According to Nguyen 1996 incidence for fungal severe infections is due to opportunisticfungi which raised in about previous years particularly those with mycoses of endemic and the patients who are immunocompromised with the main problem of the public related to health in many countries of the world can give rise to seriousside effects on the host. The points majorly attacking are the cell wall which is rigid and the metabolism of their sterol with peculiarities by this all of the fungi is distinguished from the cells for potentialhosts.[14-16]

Although fewer studies were done comparing directly to azoles, related to treatment for endemic mycoses,the oral azoles have availability which ensures tolerated better treatments for the Candidaalbicans. This is useful particularly for diseases of fungal which need longlive courses therapeutically, like coccidimycosis/ for mycoses of endemic occurring in AIDS.[16,17] Tortora 1995 reported that many drugs that inhibit or kill fungi are therefore quite toxic for humans. Besides, most fungi have a detoxification system that modifies many antibiotics, probably by hydroxylation, as a result; the added antibiotics are fungistatic only as long as repeated application maintains high levels of unmodified antibiotic.[18] There are reports for fungal increased infections along with that resistance to several antifungal, leading to the demand to develop and discover effective new drugs for antifungal.[19-21] The chitin enzyme synthase target of active fungal antibiotics- polyoxin D along with Nikkomycin.[22] Therapy of antifungal was categorized into tropical and systemic agents.[23] According to Uzun 2014 the patients with immunocompromised diseases or patient's having underlying severe diseases, the prognosis is poor of these patients and this generally comes under fungal systemic infection.[24]. But a rapid and reliable method for determination antifungal susceptibility that would hence needful in patients management along with the fungal invasive diseases.[25]

The primary antifungal agents for oral candidiasis:

Azoles: Azole medications are a family of antifungal drugs which are use full and important drugs of antifungal ending with"-azole" as suffix. The azole exists in class for members of five heterocyclic nitrogen ring compounds comprising of one atleast another atom of noncarbon for sulfur, nitrogen, or oxygen.[26] The important two diazoles comprising of fluconazole and itraconazole.[27,28]

Fluconazole: It an antifungal drug, used to treat fungi. And it's drug of antifungal used for treating fungi along with bistriazole fluorinated that's unusual as being soluble in water at pH neutral solubility which permits administration orally or intravenously.[27,29] Fungal systemic infections also are effectively often treated with Fluconazole. The Fluconazole effective for oral Candidiasis andcandidiasis of the oesophageal in the patient's suffering from AIDS, along with that its also useful among the patients who are immunocompromised[25,30,31] and patients suffering from AIDS along with blastomycosis and cryptococcosis,[27] it also has an activity which is good for dermatophytes.[32,33]

Side effects: Fluconazole in lactating mothers is not recommended since in the human milk the fluconazole secreted at about concerntrations same to that of plasma. The Fluconazole's has rarely also been related with lethal or severe hepatotoxicity. Along with this it's used cautionly in the patients already suffering with disease of the liver, preexisting in human beings. But few people allergic towards azole, therefore who all allergic for azole of other kind drugs maybe allergic for flucanazole. Few drugs of azole have side effects that they disrupts the production of estrogen during pregnancy.[34]

Drug susceptibility and drug Resistance: Susceptibility of *C.albicans* and non albican Species of Candida-Itraconazole, Amphotericin B, Fluconazole, Echinocandin, Candidaalbicans, Flucytosineand Candidatropicalis is susceptible to above antifungals

usually. While *Candidaglabrata, Candidakrusei, Candidadubliniensis* shows various ranges from Susceptible; Susceptible dose-dependent; Susceptible-intermediate to Resistant. The species of Candida causes infections that is treated by various drugs of antifungal available, but resistance of drug poses problem seriously to patient's individual health and nowadays the health system management been difficult. Several studies have shown that several factors responsible for drug resistance includes the pumping of drugs out from the cells of fungal type, the targets are modified by the incorporating point mutations in the genes the modification of main enzymes by pathways of biosynthetic and the modulation for factors of transcription plays.[35]

Historical perspective: The fungi occur as eukaryotic, free living organisms, those exist like moulds- filamentous fungi, yeasts-round fungi/ combination of the two- dimorphicfungi. According to Terezhalmy 2011 Candidiasis found orally is the commonest infection of fungal type, affecting mucosa of the oral cavity. And the lesions caused from yeast (Candidaalbicans).Candidaalbicans one components for oral normal micro-flora along with (30%-50%) of people. The rate increases by patient's age. These Candidaalbicans recovered by 60% patient's with dental problems in the mouths above 60 yrs of age. According to Prasanna 2012 the species of Candida are of different types–seen inside oralcavity.[36, 37] Mostly the oral species of Candida consists of: *Candidaglabrata, Candidakrusei, Candidapsuedotropicalis, Candidaguillermondii, Candidatropicalis, Candidaparapsilosis, Candidaalbicans, Candidastellatoidea*.[38]

Biology of *Candida albicans:* Candida albicansis true and most common opportunistic fungal pathogens. It is termed as "commensal" organism because it can found in the normal flora of skin, oral cavity, gastrointestinal tract, vaginal and the urinary environments. According to Ellis1994 and Ferreira 2010 it is found in the all environment, on leaves, flowers, water, and soil.[39,40] The *Candidaalbicans*–a yeast fungus,grampositive, thin walled, morphology which is microscopic showing spherical – oval about diameter of 5 um. It's larger to bacteria, that is reproduced by the process of budding as Yeast.[41,42] *Candidaalbicans* grows in moderate temperature's at a temperature ranging from (20-38) degrees centigrade and can resist pH to ranges (2.5-7.5).[43] According to Ellis,1994 the agar of dextrose Sabouraud containing gentamycin or chloramphenicol flavored to isolate Candida.[39] *Candida albicans* can grow better oncormeal, 72hours following with incubation of 25°C forming branched abundantly pseudo hyphae along with the blastoconidia and truehyphae are represented. These blastoconidia formed are clusters of grape like along the length of hyphae. If incubation period is extended a terminal chlamydoconidia may be formed.[44] *Candida albicans* have more than one wellknown and important features that it has ability of fermenting the sugars along with fermenting other kind of carbohydrates to produce ethanol.[45] The *Candidaalbicans* are fungus (dimorphic) which gives rise to two types of forms—Invasive & noninvasive. The form of yeast is the noninvasive and form of hyphal by which it can penetrate mucosa; is invasive. Under different environmental conditions the *Candidaalbicans* shows variant which are morphologically different forms: the forms that include are—yeast budding cells (blastoconidia, truehyphae, blastospores, clamydospores and pseudo hyphae along with this which are used in identifying *Candidaalbicans* among the different types of species for Candida. The C. albicans produces these germtubes and transition to hyphal form. However, ability in assuming different forms that is related towards pathogenicity for organism.[46,47]

Life as a successful commensal: The *Candidaalbicans* which is largely fungus of asexual type; but by physiologically and morphologically is variable highly along with that the fungus's adaptability and is of pleomorphic type which is capable of growing like budding yeast/as pseudo mycelium for conjoined and elongated cells of yeast or truehyphae which consists of generated parallelsided with growing-tip filaments.[48] And also exhibits nonsexual variation form called switching of phenotype that is able to generate cells which are stable and variant colony with properties i.e. distinctive.[49] According to Vautier 2015 *C. albicans* that adapted of commensalism & which thrive in various host of niches (mucosa of the oral cavity, vagina, skin, gut).[50] Hence state of host immune response & along with this mediated specific organism adaptations is responsible for the change in host related state of commensal.[51] According to Yamaguchi 2005 Changes to the availability for nutrients inside gut by intake through diet, further can impact in abundance for Candida[52] & due to diet change/fungal gut microbiota leads to dysbiosis & pathologies of inflammation origin like Crohn'sdisease.[53,54] Hence commensal for Candidiasis status is also related with micro biome host as whole & immunestatus for host.

Life as a successful opportunistic pathogen: Throughout the infection pathway the colonized *Candidaalbicans* different hostniches, comprising of differences as eg. Changes in pH, carbon dioxide levels, hypoxia, availability of nutrient in the medium.[55,56] According to Sokol 2016 that the one important features of Candidaalbicans like that of a pathogen which is successful adaptability in various other conditions.[55] It has been seen that the microenvironment of the host have sources of carbon that is heterogeneous therefore the adaptation of candidaalbicans for using simultaneously the sources of carbon alternatively for the virulence and survival.[57,58] The metabolic characteristic and Candida's flexibility also leads alterations in cellular secretome and proteome[59] and the ability of undergoing yeast into hyphaltransition, opaque, white with switching[60] formation of biofilm also the characteristic of adhesive property,[59,61] and capacity of remodeling.[56-52] The changes within composition of cellwall's polysaccharide changes the pathogens susceptibility to stress from environment & antifungals.[55-61] apart from the environment effect's immunogenically along with altering presentation and expression of critical pathogen associated molecular patterns(PAMPs) so the *Candidaalbican's* makes a target to move for the recognition along with immunesystem of the host.[56-63] As per Crawford 2015, humanhost retains micronutrients (Zn, Mn, Fe,Cu) from pathogen by the process called as nutritionalimmunity.[64] Within the pathogen such micronutrients essential towards various cellular vital functions.[64] These adaptive evolutionary traits makes Candidaalbicans survival possible in different hostniches; countering the host immunity defence's and makes the *Candidaalbicans* itself to establish as pathogen which is successful. Mandell 1994 reported that Candida is a fungus and was first isolated in 1844 from the sputum of a tuberculosis patient.[65] Andseveral studies have demonstrated that infection with candida is associated with certain pathogenic variables. Pathogenicity of candida depends on local and systemic factors of host.

Akpan 2002 reported that the gland of salivary functions which is impaired is susceptible to candidiasis in the oral cavity. Whereas proteins of antimicrobial saliva like sialoperoxidase, lactoferrin, histidine rich in polypeptides, lysozyme and antibodies for anticandidal that is specific, interacts to the mucosa of the oral cavity and inhibit the overgrowth for Candida.[66] According to Epstein 1990 and Guida 1988 by which there is adherence for Candida with acrylic is enhanced when the flow of saliva under fittings of denture surfaces is reduced, denture fittings improper and with the poor oral hygiene.[67,68] Dreizen 1984 reported that the wearing dentures increases theinfection with candida is as many as 65% of elderly people. Since wearing of synthetic products produces a microenvironment conducive to the growth of candida with low oxygen, low pH, and an anaerobic environment.[69] According to Brassart 1991 Candida adhesing onto the epithelial surface, is an important step, this promoted with definite components of fungal like C3dreceptors, mannose, saccharins, and mannoprotein.[70-72]

Different Drugs such as inhaled steroids have been shown to increase the risk of oral candidiasis.[72] by possibly suppressing cellular immunity and phagocytosis and drugs such as broad spectrum antibiotics alter the local oral flora creating a suitable environment for candida to proliferate. According to Garber 1994 the mucosal local immunity changes towards normal by discontinuating the steriod's inhaled.[73] Ohman 1988 reported that the Candida growth within the saliva is enhanced with glucose being present & adherence with epithelial cells in the oral cavity.[74] According to Guida 1988 those who are having low immunity they mostly prone to Candia disease.[68] Thus the various factors be diabetes, smoking, immunosuppressive state like malignancies (leukaemia, infection HIV etc) and deficiency of nutrition intake have been implicated particularly to change of commensal candida to pathogenic candida that leads to onset of candidiasis.

Conclusion

In recent period fungal infection is risen up enormously. Most of the people of many countries are suffering from Candidiasis. Sometime different antifungal agents failing to treat the fungal diseases completely. Due to this ineffectiveness of drugs they became side effects to the human. This appearance of resistance to antifungal drugs, which demands to discover and development of new effective antifungal drugs for this we have to search gene which could be responsible to develop drug resistance in Candidiasis, more often for *Candida albicans*. Also with molecular tools, we can know the effective antifungal at the earliest. These novel strategies will overcome the oral candidiasis problems in the near future.

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Microbial Infectious Disease: A Mini Review

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Abstract

In the 21st century where science and its technology have revolutionized the world, there has been also a remarkable increase in the infectious diseases which have disastrously affected mankind. From the past when millions of people die due to chickenpox and measles in America till today when a virus like the SARS, Coronavirus (COVID 19) has challenged the world, which is a highly infectious disease. Infectious disease reports the main cause of death in this global world. Despite high equipment facilities and technology, this type of disease has regularly challenged the global scenario. Infectious diseases are particularly important causes of death among the people who are elderly, immunocompromised, already having a chronic disease and also to whom who are suffering from malnutrition. The article focuses on the mechanisms by which the infectious agents cause pathology, various routes of infection and also throws light on the different microbial agents which are causing various infectious diseases.

Keywords: *Infectious Disease, Pathology, Microbial Agents, Infection.*

Introduction

Infectious disease has always a adverse effect to human beings as it is a pathological condition which is caused specific infectious agents which can be virus, bacterium, fungi or protozoan that affects the body parts or tissues, characterized by a group of signs and symptoms. As we all know bacteria were discovered by A.V. Leeuwenhoek in the year 1675 but the German physician named Robert Koch discovered that specific organisms are associated with a particular disease, after which the physician-developed four criteria to demonstrate that specific agents caused a particular, which he called Koch's Postulates that takes medical diagnosis to another level as it becomes accurate to find out the proper medication of different disease.[1] Infectious disease is always associated with infection, host, pathogen and virulence. There are different routes of transmission of infectious disease and different phases involving in this disease. Modern microbiology which is based on molecular genetics is the key to defeating this infectious disease. When it was discovered that DNA was the responsible material for the transmission of genetic traits which was the turning point in the medical research history that has continued to this day. In today scenario modern and improved techniques lead the advancement in controlling and detecting the various disease.[2]

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Classification of Infectious Disease[3]:

The classification is based on a different basis, which includes:

By location: It is divided into 2 types – Local: The disease is curbed to a specific area of the body.

Systemic: It is a generalized disease that affects most of the body parts and its pathogenesis distributed thoroughly in the tissues.

By Duration: It is divided into 3 types- Acute: It develops quickly and fastly spreads.

Chronic: Chronic disease develops very slowly and it mild but it may last for a long time, it has an indefinite period.

Latent: This type of disease is characterized by periods of no symptoms in the outbreak of illness.

By timing: It is divided into 2 types – Primary: It is an initial infection which has affects previously healthy person.

Secondary: This infection occurs in a person who has weakened by a primary infection.

Types of Infectious Agents

These are different agents which are very small in size and thus can be noticed in a microscope that includes:

Bacteria: These are the most common cause of diseases. They are generally 0.8-1.5µm in size. The bacteria cells are prokaryotes in nature and have cell membranes but lack membrane-bound nuclei. Their cell wall consists of peptidoglycan and most of the bacteria synthesize their RNA, proteins & DNA, but depends upon the host for their nutrition. The bacteria which retain crystal violet stains are Gram-positive & those do not retain are Gram-ve Bacteria. Most of the bacteria are extracellular but few survive within host cells like as obligate intracellular bacteria.

Prions: These are found in neurons and they are abnormal forms of a normal host prion protein (PRP). The accumulation of abnormal PRP will lead to distinctive spongiform changes in the brain and also can cause neuronal damage. They are intracellular. These are associated with diseases like Creutzfeldt-Jakob disease.

Chlamydiae: These organisms divide by binary fission like bacteria and they are sensitive in binary fission but the feature that distinguishes it with the bacteria is that they cannot synthesize ATP (Adenosine triphosphate). They are obligate intracellular microorganisms. Chlamydia trachomatis is the common cause for the female sterility and blindness. They are normally 200-1000nm in size.

Viruses: These are obligate intracellular organisms having size 20-30nm. They consist of a capsid which is a protein coating that surrounds the nucleic acid genome. Viruses are classified under the basis of their nucleic acid genome combination i.e. DNA or RNA, but not the both. They contribute to the huge allocation of human infections.

Fungi: These are eukaryotes having 2-100µm in size. Their cell wall contains thick chitin and their cell membrane containing ergosterol and these constituents are the most targeted area for the different anti-fungal agents. A fungus grows by hyphae or like budding yeasts forms. Their site of propagation includes cutaneous, mucosal, extracellular and facultative intracellular. They can cause superficial or deep infections.

Protozoa: These are single-cell eukaryotes which are the most common cause of infectious disease in developing countries. They are 1-50µm in size and can replicate intracellularly in many types of cells like parasitic protozoa which includes microorganism likes malaria in erythrocytes and leishmania in macrophages.

Helminths: These are highly differentiated parasitic worms having complex life cycles varying 3mm-10m in size. They consist of 3 classes which include Roundworms, Flatworms & Flukes.

Different Routes of Infection[3-5]: Microbes can enter the host in many ways which includes through the air, through the body fluids, by the animal vectors such as insects, birds, bats,etc, by the direct contact, or through the contaminated food. To enter in the host and to infect an individual, microbe's virulence factors are responsible which can break the host barriers and colonize the host. The barriers in host includes:-

- **Skin and oral mucous membrane:** The outer layer of the skin is dense and keratinized and its low pH 5.5 with its fatty acids content helps to inhibit the growth of microbes. Although skin and oral mucous membranes are very effective barrier but some microbespenetrate through due to cuts

or abrasions. Some of the examples of microbes include Staphylococci in wounds, Pseudomonas aeruginosa in burns and multibacterial infections in diabetic ulcers.

- **Respiratory Tract:** Many microorganisms' are inhaled by human beings causing pathological condition, which includes different forms of viruses, bacteria or fungi. The size of microbes is inversely proportional to its traveling distance in the host. Large microbes are been trapped in the nose with the help of goblet cells by the action of respiratory epithelium goes back of the throat where they are swallowed and cleared and the alveolar macrophages or neutrophils phagocytes the smaller microbes less than 5μm in size, but some of the microbes like M. Tuberculosis can escape the phagocytic killing by alveolar macrophages and can cause severe respiratory infections.
- **Intestinal Tract:** Some of the pathogens which are transmitted by contaminated food or drink breaks the barrier of the intestinal tract, but there are some normal defenses against these pathogens which includes acidic gastric pH, immunoglobulin's like IgA which is secreted by B cells located in the mucosa-associated lymphoid tissue, viscous mucus secretions, the bile detergents or lytic pancreatic enzymes and the normal gut flora. These barriers also weaken in some cases due to the loss of gastric acidity or the antibiotics which unbalance the normal bacterial flora like in Pseudomembranous colitis or syndrome like blind loop syndrome. Most types of viruses are killed by bile and digestive enzymes that are mostly enveloped but few which are nonenveloped like Hepatitis A or rotaviruses, etc are resistant towards these barriers.
- **Urogenital Tract:** Many pathogens cause urinary tract infection but as the urinary tract is sterile as it is flushed many times per day but few like E.coli pathogen adhere to the epithelium successfully causing UTI. Women are more prone to UTI as compared to men as the urinary bladder and bacteria-laden skin which is length of urethra is less as compared to men. The distance in the women is 5cm and in men it is 20 cm. Obstruction in the bladder and excessive bleeding in menstrual leads to infection of the genital tract.

Mechanisms Associated with Infectious Disease[6,7]:

There are 3 general mechanisms by which viral, fungal and bacteria attacks host; which include:

- The Pathogens enter to host cells or bind them and directly cause cell death.
- The endotoxins or exotoxins which are released by infectious agents kill cells and damage blood vessels leading to ischemic injury.
- The pathogen induces the host immune and inflammatory responses causing additional tissue damage.

Viral injury mechanisms: When the viruses enter inside the host cells then they can damage or kill in many ways:

Lysis of host cells: The replication of the viruses which interferes with the host cells because cell death-like as influenza virus kills the respiratory epithelium.

Alteration in apoptosis Pathways: Apoptosis is a common process by which the host inhibits the pathogen route and eliminates the virus but some viruses inhibit apoptosis by encoding some genes like BCL-2gene and damage the cell in the cases like virus-induced cancers.

Viruses induce the cytotoxic T lymphocytes attack and cause damage to host like in liver cell injury during HBV infection.

Viruses like HBV, EBV induces the proliferation of cells and transformation that leads to cancer.

Mechanism of bacterial injury[8,9]:

The mechanism includes a simple process by which bacteria causes diseases includes:

- **Adherence to Host cells:** It includes the invasiveness which is the ability to invade tissues and encompasses the mechanisms for colonization with the production of the extracellular substances that facilitate the invasion.
- **Toxigenesis:** Bacteria can produce toxins which include exotoxins & endotoxins. The bacterial endotoxins which are a lipopolysaccharide present in the cell wall of gram-negative bacteria which contain

long-chain fatty acids. This LPS is both harmful and beneficial to the host. However high levels of LPS induces cytokines such as tumor necrosis factor, IL-1, and IL-2, etc and will cause septic shock, acute respiratory syndrome, disseminated intravascular coagulation, etc.

The exotoxins are released from the bacterial cells which are bacterial enzymes like coagulase, protease hyaluronidases, etc that targets the substrates and damage the host cells. Some of the neurotoxins which are released by pathogens like C. botulinum or C. tetani inhibit the release of neurotransmitters leading to paralysis.

Cytokine storm can be appreciated which is caused by superantigens that are bacterial toxins that have the potential to stimulate the large population of T lymphocytes.

Table 1. Examples of some recently introduced important infectious Disease

Agents	Disease
Corona Virus	COVID-19
Staphylococcus Aureus	Toxic shock syndrome
Hepatitis E virus	Enterically transmitted hepatitis
HIV	AIDS
Hepatitis C virus	Chronic hepatitis
Hantavirus	Hemorrhagic fever with renal disease, Hanta virus pulmonary syndrome
Vibrio cholerae O139	New epidemic cholera

Different phases of infectious disease[9]: The stages involved are: Incubation period – It is the time from the occurrence of pathogens in the body to the appearance of first symptoms. In this phase the microbes grow and rapidly multiply.

Prodrome Period: In this phase nonspecific symptoms like fever, fatigue and malaise can be noticed with the starting of specific symptoms.

Illness Period: It is time in which symptoms of specific infection occurs. In this phase pathogen is at peak level.

Convalescence: In this phase acute symptoms disappear and returning of the immune system occurs.

Some techniques for diagnosing infectious disease[11,12]:

There are some method to detect this infectious pathogen which includes histopathological process, PCR test which is useful in the amplification of nucleic acids. Different staining process which diagnosed infectious pathogens includes:

Gram staining process which reacts to most of bacteria, Acid-fast stain for Mycobacteria and Nocardia, silver stains and periodic acid -Schiff for the fungi, Giemsa for campylobacteria and malarial parasites, DNA probes for viruses,bacteria and protozoa & Mucicarmine process for cryptococci. These staining processes have played a crucial role in detecting these pathogens as well as controlling infectious diseases.

Conclusion

According to WHO the 10 most disease which cause maximum death in the world population includes 5 infectious diseases like Influenza, chickenpox, tuberculosis and new coronavirus which is giving disastrous impact to the global world causing COVID-19 disease and declared as a pandemic. Various advance technology and medical research have to control the infectious disease with a proper medication of it but in today world also there isan infectious disease like COVID-19 which is highly transferable and yet its proper medication is not discovered or made, different infectious pathogens like coronavirus or hantavirus can be used for bioterrorism which is very dangerous to mankind as it can be used as a biological weapon. Infectious diseases are challenging the world from long back history to present scenario and preventive care and social measures are highly impactful to controlling these severe diseases.

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Confounding and Effect Modification in Research

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Abstract

An association that is not causal (Alternate explanations) is seen between outcome and exposure whenever the study has confounding factors, bias and random error/chance error. Bias in any study leads to systematic variation of inferences or results or interpretations from the true picture. One of the most important bias encountered during research is confounding. Confounding is seen when a variable is observed to be associated with both the exposure and the outcome but is not a part of the causal pathway. Confounding factors, when present, in any study, are the "Nuisance" and can be the cause in part or in full of the observed association between the disease and exposure. Effect modification, on the other hand, is seen when various effects are brought about among different subgroups by an exposure and this can be handled by doing stratification. It is the outcome that is linked to the effect modification and not the exposure. This article will define and discuss in detail confounding, and the concept of effect modification so that drawing conclusions of a study can be done after considering these errors.

Keywords: *Effect modification, Matching, Restriction, Confounding factors.*

Introduction

Bias is defined as any deviation of results or inferences from the truth, or processes leading to such deviation. Any trend in the collection, analysis, interpretation, publication, or review of data that can lead to conclusions that are systematically different from the truth.[1] There are a plethora of biases mentioned in the literature which an investigator might encounter during research.[2] All types of study design, be it case-control, cohort,or experimental are prone to one or more types of bias. A false picture of association is observed due to bias or they can even hide a true association when it is present.

Internal validity of a study is threatened by the presence of confounding, random error/chance error and bias. A researcher's unintentional mistake usually leads to bias, whereas, confounding occurs due to the presence of an extra variable that affects the outcome positively or negatively apart from the exposure. Random variation/ chance can be reduced to a greater extent by increasing the sample size; but, on the contrary, bias and confounding cannot be reduced by merely increasing the sample size.[3] This paper emphasizes only on confounding and the bias associated with it. The paper also attempts to throw some light on effect modification.

Confounding: The word is derived from Latin "confundere" meaning to mix or muddle. It means a distortion in an association that is seen when the exposal factor of interest is muddled with other factors that are

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related to the outcome.[1] As a researcher, we ought to be careful to avoid confounders in a study as much as possible otherwise it may lead to "False Positive" (Type–1 Error) ie, conclusion of a spurious relationship. In simple terms, it undermines the internal validity of the study, as the confounder rather than the exposal agent seems to be associated with all the effects observed.

Example: A researcher wanted to find out the association (primarily) between physical activity (Exposure) and lung cancer (Outcome). Confounding factor in the given example is age as it is associated with exposure (Old people tend to be more physically inactive), and also associated with outcome (Lung cancer is at higher risk to develop among old age).However, race cannot be considered a risk factor for lung cancer. Adjustments to accommodate variables that might not be cofounders can bring in bias and compromise the precision of a study.

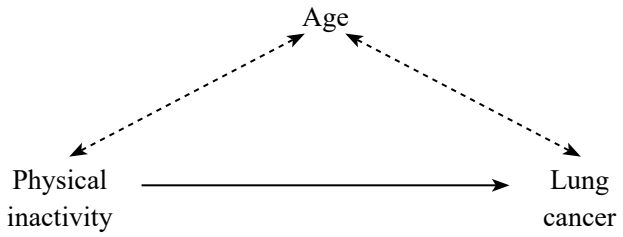


Figure 1: Understanding Confounding:

To understand confounding in a simplified manner, Rothman and others have investigated and illustrated an association between Down's Syndrome and birth order.[4]

Figure 1 shows that with an increase in birth order, the chances to develop Down's syndrome among the children also increases roughly around 4 – fold. This type of analysis did not take other potential risk factors/ confounders into consideration, making a researcher believe that birth order is associated with Down's syndrome.

But, when maternal age was used for children with Down's syndrome, it demonstrated that the age of the mother was more important in the causation of the disease i.e. as the age of the women increased to give birth, the prevalence of Down's syndrome also increased proportionally. (Figure 2)

Women who are mothers for the first time are much younger than women having their fifth child. In other words, maternal age is mixed up with the birth order of the child. There is a stronger correlation between maternal age and Down's syndrome prevalence than the birth order of the children and Down's syndrome. In lieu of this, it can be stated that the occurrence of Down's syndrome in relation to the order of birth is confounded by the age of the mother.

Investigators also tried to find if maternal age was confounded by birth order i.e. if birth order had an independent effect on the causation of Down's syndrome. Rothmansorted this by putting both effects i.e. maternal age and birth order v/s Down's syndrome prevalence. (Figure 3) (4).

The figure amply demonstrates that Down's syndrome prevalence is not associated (has no impact) with increasing birth order when observed from the side. But, when the figure is observed from forwards to backward, for every order of birth, an increase in the age of the mothers at all five levels of birth order can be observed, i.e. even after controlling for birth order, there is a strong association between increased maternal age and Down's syndrome among the children. Hence, the apparent association between birth order and Down's syndrome observed in Figure-1 was due to the confounding effect of maternal age completely. On the contrary, the association between Down's syndrome prevalence and increasing maternal age is not confounded by the birth order of the children.

Confounding Factor Criteria:

For a factor to be termed confounder, the following three criteria have to be met:[5]

1. Both the risk factor under study and outcome should be related to the confounder.
2. The groups under comparison should have the confounding factor among them, albeit it is distributed unequally.
3. A confounder cannot be an intermediary step in the causal pathway from the exposure of interest to the outcome of interest. In the pathway of causation from exposure to the outcome, the confounding factor cannot be a transitionary step.

The first two of these conditions can be tested with data. The third is more biological and conceptual.

To ascertain if the association shown between the exposure under study and the variable is independent, there should be a plausible biological or social explanation. These decisions should be best made

based on the basic science, psychological, sociological, or clinical data (i.e. non – epidemiological). In a nutshell, the confounding factor should precede the outcome of interest and not be an intermediary step. For example: To prove a relationship betweenlack of exercise and cardiovascular diseases, overweight might be an intermediary step between lack of exercise and cardiovascular diseases. Hence, bodyweight should not be controlled.[6]

However, bodyweight can be considered as a confounder if it is not a part of the causal pathway. Hence, controlling these intervening variables during analysis may mask a true relationship between exposure and disease.

Most of the diseases have multiple risk factors i.e. confounders. Confounder in a research study can be of any of the types mentioned below.[7,8]

- **Confounder as risk factor:** For example – In an epidemiological study to associate diabetes and exercise, age as it is a risk factor for diabetes, can be a confounding factor.
- **Confounder as a preventive factor:** For example – People who exercise regularlyon the prescription of aspirin, and being on aspirin decreases the risk of heart disease. Thus, the benefit of exercise would be exaggerated for reducing heart disease by regular use of aspirin (Confounder).
- Confounder as ***a marker or a surrogate***: For example – For most of the systemic diseases, socio-economic status would act as a confounder. Lower socioeconomic status (Marker) forms a collection of factors that are not understood completely and hence pose a high risk for many systemic diseases.

Effects caused due to confounding:

- An apparent association may be in part or wholly caused by a confounder.
- Can cause over-estimation of true association (Positive Confounding) or under-estimation of true association (Negative Confounding)

Identifying Confounders:[9]

- The best way to is to observe for the difference in the association before and after modifying for the confounding agent in a study. If the association between the two measures differs by ten percent or more, it confirms the presence of a confounder. On the contrary, if the difference is lesser than ten percent, then there is little or no role of the confounding agent.
- With the prior knowledge or experience of the investigator, if the variable is common to the exposure and outcome of interest, then the variable is believed to be a confounder.
- Test of hypothesis can be done to establish if the confounder is present in exposure and outcome.

Controlling for confounding during research:

The strategies employed to control confounding are:

1. Restriction
2. Matching
3. Randomization

} During the designing of the study

4. Stratified analysis
5. Multi-variable regression analysis

} During the analysis of the study

1. Restriction: A factor must be unequally present among groups being compared to be considered a confounding factor. Hence, a common method used to avoid confounding bias in the research is to restrict the selection and entry of participants by adhering to strict inclusion and exclusion criteria having the same levels of confounding factors.[10]

To understand simply, let us take an example of smoking and lung cancer, with age and gender being confounders of concern to the investigation. Restriction in this example, would be to select only male participants in the age group of 50 – 60 years. This will minimize the confounding to a greater extent as both the groups are comparable with similar characteristics.

Limitations to restriction technique:

- The problem of sample size might arise as the eligibility of the number of subjects will be reduced.
- If restriction is not done narrowly, then the problem of residual confounding might arise. The distortion that is still observed in study design or analysis even after controlling of the confounder is called residual confounding. For example, in an investigation of the association between smoking and lung cancer, if the investigators choose male participants between the age of 50 – 75 years, the occurrence of lung cancer varies widely in this age range as cancer might be more among the older adults.

- The effect of factors that have been adjusted for cannot be evaluated. For instance, the effect of men aged 50 – 60 years on lung cancer cannot be evaluated.
- Generalizability is limited by restriction considerably. For example, if a study only men are included, then the results cannot be generalized to women.[10]

2. **Matching[11,12]:** Matching is another straight-forward approach and commonly used method for reducing confounders in the research design especially in case-control studies. For instance, age groups among the cases and controls have to be matched in research to study the association between physical inactivity and cardiovascular disease. If not done, younger aged people might be mainly controlled strata and have fewer cases (suffering from heart disease) among them, on the contrary, older aged individuals might be forming up cases strata with fewer controls. Thus, by matching the age stratum, i.e. for every case aged 40–50 years, a matched control aged 40 years is chosen, thereby reducing the confounding to a great extent. As the analysis of unmatched study groups is different from than when matching is done, researchers should include matching during the study design to prevent confounding.

Applications of matching:

- Multi-faceted and complex variables need to be controlled (Example: Environmental factors, genetic factors, etc)
- In a case-control study, where the sample size for cases is less with many possible matched controls.

Limitations of Matching:

- The sample size is sometimes inadequate.
- Might be expensive and takes a long time to find exact matches
- Effect of the factors that are matched cannot be evaluated

3. **Randomization[13]:** To control both known and unknown confounders, the most powerful and ideal method is randomization. Selection bias by the investigator is prevented/reduced considerably by randomization, as it allocates subjects to control or experimental groups. Before randomization is conducted, the investigator should ensure all the participants are equal at baseline, to create comparability across groups. Similar distribution of known and unknown confounders is ensured by randomization to the control and experimental groups. Randomization also guarantees that any differences found between the two groups is due to chance and not due to the investigator.

4. **Stratified Analysis[13,14]:** Stratification permits an investigator to examine the difference of the confounder among the strata between outcome and exposure. For example: alcohol and smoking consumption, socioeconomic status, age, etc. In a hypothetical study, to find the association between air pollution and Chronic Obstructive Pulmonary Disease (COPD), smoking was the confounder to be controlled. The population under study was stratified based on their smoking status. Assessment of the association between COPD and air pollution can then be made within each stratum. Stratification allows us to examine the effect of smoking on the association between air pollution and COPD.

   The issue of simply creating strata, in general, is that, there will be more individuals in some strata giving an accurate estimate of the association. But strata with fewer individuals, the association estimate might not be accurate. Hence, the weighted average is calculated so that strata with more data gets greater weight. Mantel-Haenszel Method is the most common weight scheme used during the analysis. A single, summary measure of association providing a weighted average of the relative risk or odds ratio is produced by the Mantel-Haenszel method across the different strata of the confounding factor.All the usual statistical packages allow calculation of the estimates by the Mantel-Haenszel method. The major limitation of the stratified analysis is, if there are multiple confounding factors then it is very difficult to control all of them simultaneously.

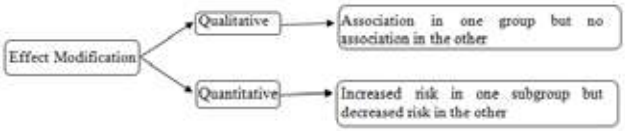
5. **Multi-Variable Regression Analysis[10]:** If only one or two confounders have to be controlled, the best way is to conduct stratified analysis. Multivariate analysis is the only possible way to control simultaneously multiple confounders or if their grouping is large. For example: In a hypothetical study to find an association between smoking and oral cancer, we can control simultaneously other confounders (covariates) in the same model like age, gender, alcohol consumption, gutkha consumption, exposure to sunlight, sharp teeth, sepsis, etc. In

regression models (Linear and logistic), multiple confounders can be investigated at the same time by adjusting the odds ratio. Adjustment is the process of accounting for confounders followed by comparing simple and multiple regression results to get a clear picture of the quantum of distortion the confounder has made in the relationship between the exposure and outcome.

Effect Modification (Interaction): Effect modification, is seen when various effects are brought about among different subgroups by an exposure and this can be handled by doing stratification. Effect modification is associated only with the outcome of the study, but not the exposure. It is not considered a nuisance as it often brings to light significant information. The presence of a third factor determines the strength of effect an exposure on an outcome. For example, when a new drug is introduced called drug A. If this drug A works only in males but does not work in females, then gender is the effect modifier and its an example of effect modification.

Confounding is considered as muddling of effects, on the other hand, effect modification is a property of the effect.[15] Effect modifier is defined as a factor that modifies the effect of a putative causal factor under study.Effect modification is detected by varying the selected effect measure for the factor under study across levels of another factor. Hence, effect modification is a change in the effects caused by one or more factors due to the presence of the remaining factors.[1]

Types of effect modification[16]: Effect modification is generally classified into two types: Qualitative and Quantitative.



When the direction of effect (nature or quality) depends on the effect modifier, it is qualitative effect modification. On the other hand, if the strength (quantity) of association differs across strata of the effect modifier without a change in the direction of the effect modifier, it is quantitative effect modification.

Reasons for finding effect modification during research:

- For preventive actions, high-risk sub-groups can be defined appropriately.
- The precision of effect estimation is increased among the groups that would have been differently affected.
- The ability to compare across studies having different proportions of effect modifying groups is increased.
- Causal – The hypothesis of a disease can be developed.

Effect Modification Identification: The potential important effect modifier should not be matched during the study, as its effect on the outcome then cannot be determined. During the design and conducting a research study, the method employed to identify effect modifiers are: prior knowledge of potential effect modifiers and power of the study. The method usually employed to identify effect modifiers in research is generally done during the statistical analysis of the study i.e. stratification and regression (Already mentioned).

Conclusion

When a variable is observed to be associated with both exposure and the disease, confounding occurs causing confusion regarding the outcome. Hence it is imperative on the investigator's part to identify these confounders, otherwise, the study will be replete with bias. Randomization atthe design stage and regression models during the analysis stage are the best ways to prevent/reduce confounding in the research. On the other hand, effect modification does not create 'nuisance' in the research. In fact, effect modification helps the researcher to find the effect of one more variable on the outcome and hence are beneficial to the research.

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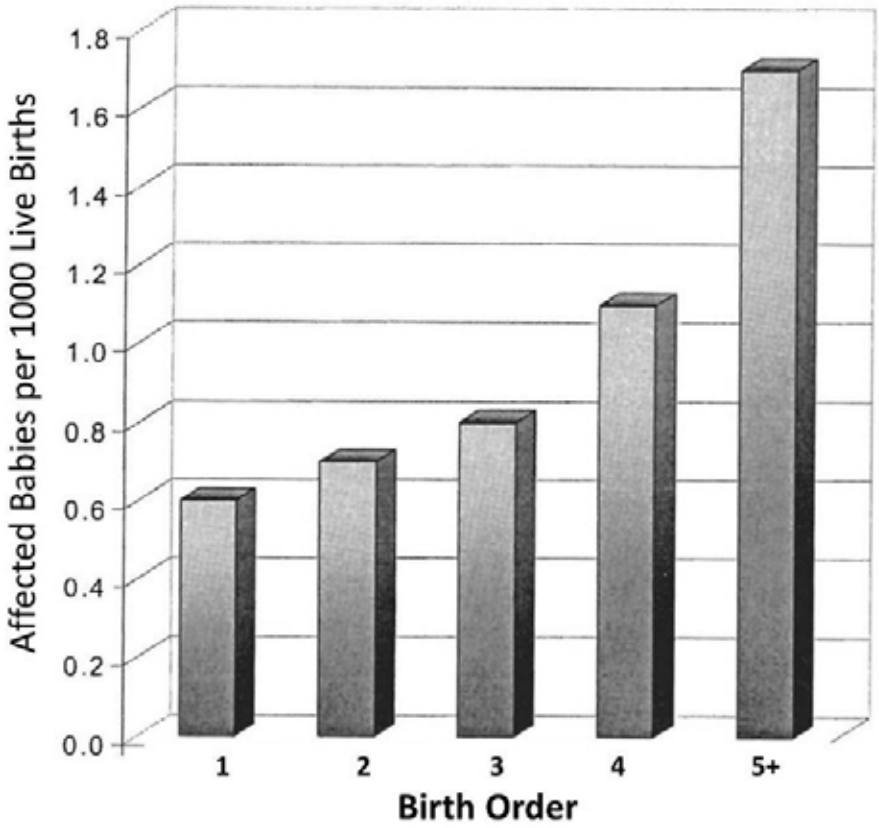


Figure 1: Relationship between the occurrence of Down's syndrome and order of birth.

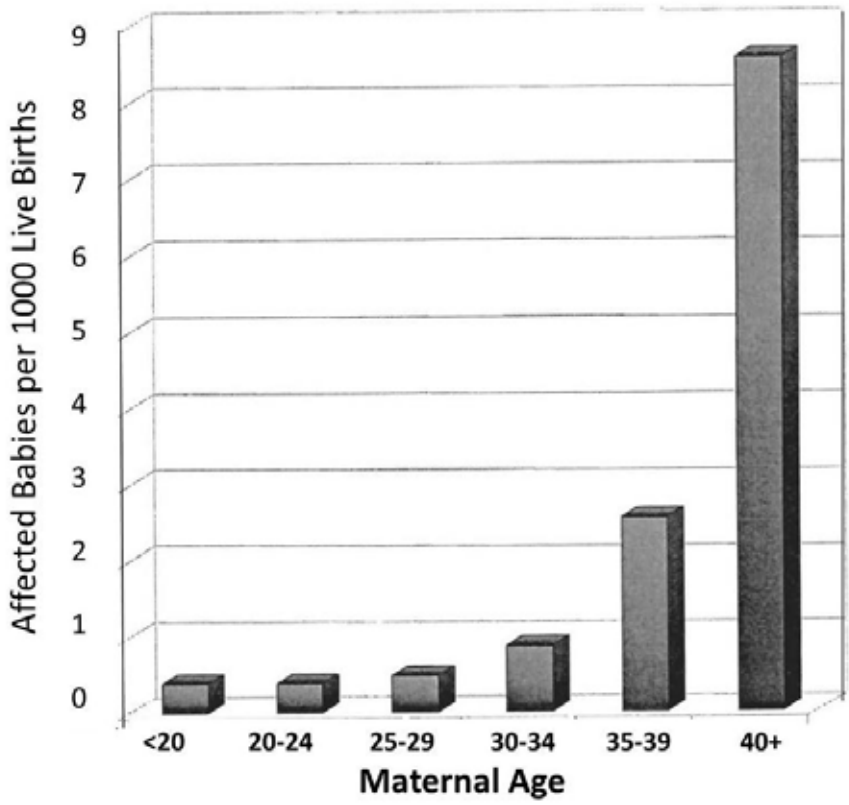


Figure 2: Relationship between maternal age and the occurrence of Down's syndrome

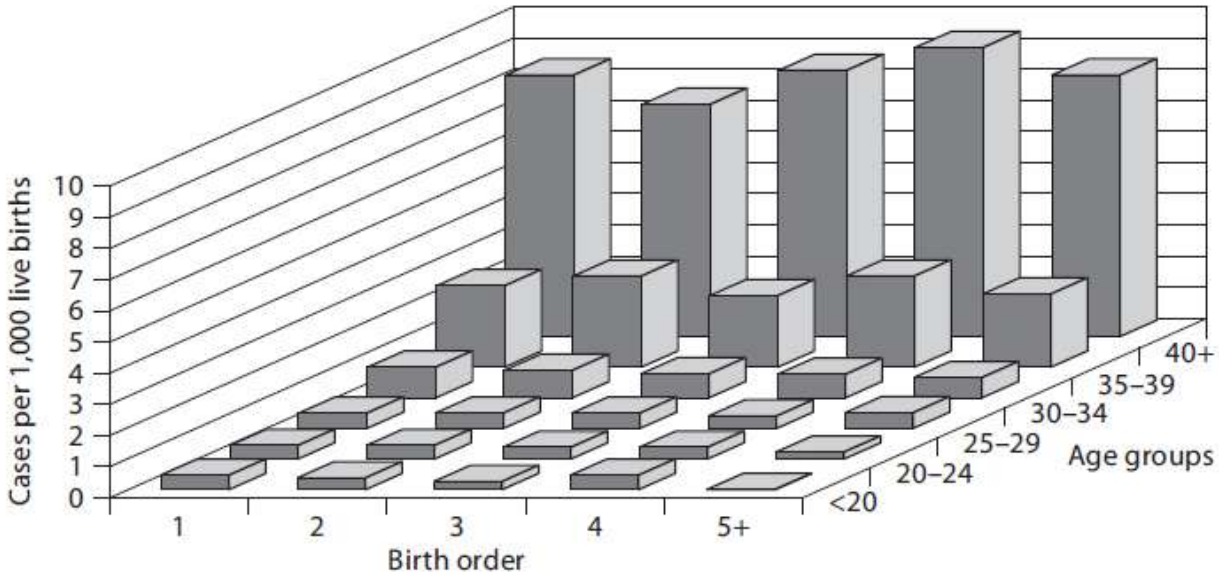


Figure 3: Association between maternal age and birth order v/s Down's syndrome

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Orthodontic Considerations for Traumatized Teeth: An Overview

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Abstract

This article reviews the orthodontic consideration for the proper management of traumatic dental injuries which requires an understanding of the immediate and short- term response to the involved tissues: the periodontal ligament (PDL), pulp, and alveolar bone. This document discusses a few protocols required for orthodontic management of traumatically injured teeth.

Keywords: *Traumatized teeth, Orthodontic Management, Precautions.*

Introduction

Dental trauma mostly occurs in the age group of 7-19 years and is most prevalent in boys. Most commonly involved teeth during dental trauma accidents are the maxillary incisors. Commonly associated risk factors during trauma for class 2 patients division 1 malocclusion are increased overjet and incompetent lip coverage.[1] If there is an increase upto 0–6 mm, then it increases the threat of dental injury due to trauma which makes incomplete or partial lip closure resulting in unsupported maxillary incisors which is also said to be one of the majority factors in escalating the occurrence of traumatic dental injuries.[2] According to the Children's Dental Health Survey 2003, it is being reported that from 5% at age 8 to 11% by age 12 have undergone accidental damage to their permanent incisors. Data shows that many of them are remaining untreated. There is also evidences that 10.8% of orthodontic patients have a history of dental trauma.[3]

Types of dental trauma: It includes lateral luxation, avulsion, concussion, subluxation, extrusion, intrusion.

Dental trauma and orthodontics: During orthodontic treatment, if dental trauma occurs then there is a risk of root resorption associated with other dental problems. Every patient should be asked about any previous dental trauma before doing any kind of orthodontic treatment. By knowing the history of dental trauma, the orthodontist will prevent themselves from any potential complications to occur.[4]

Concussion

A concussion is a type of injury to the tooth and its supporting structures with an increased percussion that doesn't involve anyasymmetricaldisarticulation of the tooth. On examination plus radiographic findings suggested that the concerned tooth has high sensitivity on percussion but devoid of any increased mobility. There is no abnormality in radiographic findings. According to clinical guidelines there should be a record of the condition of the pulp of the tooth till 1 year. And according to orthodontic protocol, the patient needs to

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wait for 3-5 months to start any kind of orthodontic treatment. There has to be the maintenance of a record of radiographs throughout 1 year after trauma. In such cases, mild and intermittent forces can be used.[5]

Subluxation: Subluxation is a type of injury where the supporting structures of the tooth along with unusual loosening of tooth takes place, however, there will be no displacement of the tooth to be found. According to clinical findings, the concerned tooth will be having tooth sensitivity along with an increase in the grade of mobility. Also, there will be bleeding in the gingival crevice in this case but there will be no abnormalities in radiographic findings.[6]

Extrusive luxation: Extrusive luxation also known as peripheral dislocation or partial avulsion. It refers to the incomplete dislocation of the tooth that is exposedout of its jaw socket. According to the clinical signs there will be an elongated tooth that will be excessively mobile. The immature teeth will have revascularization of pulp. The radiographic examination in this case will show an increased space in the periodontal ligament area apically. Therefore, according to clinical protocol there should be the relocation of the tooth into its jaw socket to be done very gently. By using a flexible splint, stabilization of the tooth can be done for 2 weeks by utilizing flexible splint while keeping an eye onthe condition of pulp and radiographic control for 5 years. If we stick to the protocols of orthodontics criteria then, the patient should wait for 6 months to begin the orthodontic treatment. And during orthodontic treatment, radiographic control has to be performed every 3 months. Mild as well as intermittent forces has to be used. If necessary then the orthodontic treatment should be simplified.[7]

Lateral luxation :When the tooth is displaced in a particular direction other than axially then the displacement of the tooth is called lateral luxation which is associated with comminution or the fracture of the alveolar socket. In this case, clinical findings like the displacement of the tooth take place buccally or lingually/palatally which is immobile as well as tender on percussion that gives a metallic sound. The immature teeth will have revascularization of pulp. The radiographic examination in this case will show an increased space in the periodontal ligament area apically wide in an occlusal radiographic view. Therefore, according to the clinical code of behavior, the tooth should be repositioned gently by stabilizing tooth for 4 weeks using a flexible splint while keeping an eye on the condition of pulp through radiograph for 5 years. If we see the protocols of orthodontics criteria then, the patient should wait for 6 months, to begin with, the orthodontic treatment. And during orthodontic treatment, radiographic control has to be performed every 3 months. Mild as well as intermittent forces has to be used. If necessary then the orthodontic treatment should be simplified.[8]

Intrusive luxation (central dislocation): The least common displacement injury in the permanent dentition is known as central dislocation or intrusive luxation where dislocation of the tooth into the alveolar bone occurs. Such type of wound is combined with the fracture of alveolar bone. If we see the clinical findings then, the displacement of the tooth takes place buccally or lingually/palatally which is immobile as well as tender on percussion that gives a metallic sound. The immature teeth will have revascularization of pulp. The radiographic examination in this case will show anabsence or partial absence of the periodontal space in an occlusal radiographic examination. Therefore, according to guidelines, the tooth should be repositioned gently by stabilizing the tooth for 4 weeks using a flexible splint while keeping an eye on pulpal condition and radiographic control for 5 years. According to the clinical protocols, for 3 weeks, waiting for spontaneous re-eruption is indicated only for immature teeth with intrusion <3mm. cases of intrusion >3mm and <6mm may be treated by orthodontic repositioning, while surgical repositioning is recommended for teeth with intrusion >6mm. If we see the protocols of orthodontics criteria then, the patient should wait for 6 months to begin the orthodontic movement. And during orthodontic treatment, radiographic control has to be performed every 3 months. Mild as well as intermittent forces has to be used. If necessary then the orthodontic treatment should be simplified.[9]

Avulsion (exarticulation): Avulsion injury is seen in 0.5% to 3% of all dental injuries and is characterized by an absolute dislocation of the tooth out of the socket. This wound is accompanied by comminution or by fracture of the alveolar jaw socket.In this case the clinical, as well as readiographic findings like absolute dislocation of the tooth from its jaw socket, is found. According to the clinical protocol, the treatment of tooth placement is the alternative option, but proper prognosis of the tooth should be present for better orthodontic treatment. Prescription of antibiotics and anti-tetanic vaccination. If we see the protocols of orthodontics

criteria then, the patient should wait for 6 months to start the orthodontic movement. And during orthodontic treatment, radiographic control has to be performed every 3 months. Mild as well as intermittent forces has to be used. If necessary then the orthodontic treatment should be simplified.[10]

Significant risk factors due to dental trauma[11]

(a) It can be seen in the face i.e. increased overjet

(b) Maxillary incisors will be having inadequate lip coverage.

(c) Children who undergo treatment for dental injuries are usually at risk of getting injury to teeth repeatedly if the trauma occurs at an early age.

Considerations of a traumatized dental patient before orthodontic treatment: Before proceeding for any kind of treatment for traumatized teeth, a dentist need to take care of the complete profile of the patient. A comprehensive dental history including earlier episodes of dental distress and the treatment should be recorded.[12] Sometimes patient may not recall their history of trauma, therefore in such cases, a radiographic examination aforementioned to the beginning of any orthodontic management should include all these following points below:[13]

1. For assessment of colour, it has to be kept in mind about the transillumination seen in the radiograph which will disclose enamel infraction lines along with colour changes in the concerned injured teeth which are indicative of pathology or repair.[14]
2. Before proceeding towards the treatment examination for sinus or any kind of swelling should be done.
3. If there is any tooth mobility then the examination for the concerned tooth has to be done in both a horizontalas well as vertical direction. [15]
4. During the clinical examination palpation over the tooth apex for tenderness should be checked.[16]
5. Diagnostic tests for ankylosis can be done by the method of percussion tests in which a high metallic percussion note can be felt, while a duller note can suggest of a root fracture.[17]
6. For accurate diagnosis of dental trauma, radiographs like reproducible long cone periapical radiographs are the best. In such cases, radiographs at different angulations can be taken.[18]
7. Test like sensibility tests can be done for diagnosis of the teeth involved in dental trauma which is currently the most useful test to assess the neurovascular supply to the pulp of a traumatized tooth by the use of an electric pulp tester (EPT).[19] Before the commencement of orthodontic treatment, Proper detailed history of the tooth should be confirmed about any kind of existing root canal filling. Every patient should be asked about any previous dental trauma before doing any kind of orthodontic treatment. By knowing the history of dental trauma, the orthodontist will prevent themselves from any potential complications to occur.[20]

Precaution to be taken due to the dental trauma[21]

(a) Interceptive treatment can be opted.

(b) Use of mouth guards during sports can be used to prevent trauma to face and oral cavity structures.

Impact of orthodontic treatment on the tooth and its supporting structures[22]

(a) Loss of pulp vitality

(b) alternative resorption can be seen

(c) Non-responsiveness to sensibility testing

(d) Inflammation of supported gingival to the concerned tooth

Conclusion

Traumatized dental teeth should always be carefully examined regularly. In every month on a regular interval basis teeth should be monitored for the status of the traumatized teeth. Before commencing any kind of orthodontic treatment proper detailed history has to be recorded for avoiding any complications before the orthodontic treatment.

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Management of Endo-perio Lesion by Guided Tissue Regeneration: A Case Report

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Abstract

To diagnose and to treat, is a crucial part of management of an endo-perio lesion. The sign and symptoms of endo-perio lesion are difficult to accountable for a general dental surgeon. For that reason, it needs more detailed examination and definite treatment plan. The outcomes of endo-perio lesion are highly unpredictable due its multiple etiological factors. The primary and secondary etiological factors of endo-perio lesions may have numerous variation and ultimately concludes the definite treatment plan complex. A 43-year-old female patientcame to Department of Periodontics, with a chief complaint of pain in right posterior lower tooth region.On clinical examination, there was 5mm of periodontal pocket, occlusal restoration in 47. The treatment plan was made as first endodontic treatment followed by periodontal treatment. So endodontic treatment was carried out first in 47 after oral prophylaxis. Then under local anesthesia, open debridement was done in 47 and bone graft was placed in the defect. Resorbable membrane was placed over the graft. Suture was placed and periodontal dressing was given and followed up to 9 months. She was very satisfied with the result.

Keywords: *Endo-perio lesion, endodontic treatment, periodontal treatment, alloplastic bone graft, resorbable membrane.*

Introduction

In endo-perio lesion, there is two compartments such as periodontal and endodontal compartment. The periodontal compartment is responsible for periodontal disease and endodontal compartment for pulpal disease. These two compartment are connected with each other by the presence of apical and lateral radicular foramina, which permit the passage of pathogen form one to another.[1] Simring and Goldberg first described the actual relationship between periodontal and pulpal disease in 1964.[2] The etiopathogenesis of the endo-perio lesion described following cases such as:

- Lesions that result from apical to coronal migration of pulpal pathogens and their toxins with or without fistula or sinus tract at the gingival margins depending upon the nature of infection.
- Originating from a marginal lesion which later on extended to more apical periodontal compartment. The periodontal and pulp are distinct from one each other and are anatomically connected through the foramina.
- Another form is the combination of the above, in which the differential diagnosis must attribute each portion of the lesion to its cause.

Periodontal disease causes a destruction of the bone in a coronal to apical direction and in endodontic lesion is from apex to coronal direction.[3] For this reason, it

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is very difficult to find out the source of origin of the presenting lesion. It has already established fact that both pulpal and periodontal disease are primarily due to the disease process of the microbial infection.[4] Hence, both the disease process may originate as separate entities, but due course of disease progression, these may overlap each other. These diseases are frequently noticed as independent entities, which ultimately complicates the proper diagnosis.[5] There are several classification described the endo-perio lesion as follows.[5]

Classification:

1. Simon et al. 1972

i. Primary endodontic lesions

ii. Primary endodontic lesions with secondary periodontal involvement

iii. Primary periodontal lesions

iv. Primary periodontal lesions with secondary endodontic involvement

v. True combined lesions

2. Walton et al. 1996

i. Endodontic origin

ii. Periodontal origin

iii. Combined endo-perio lesion

iv. Separate endodontic and periodontal lesions

v. Lesions with communication

vi. Lesions without communications

3. World workshop 1999

i. Endodontic periodontal lesion

ii. Periodontal endodontal lesion

iii. Combined lesion

The present case report discussed an endo-perio lesion which was managed by guided tissue regeneration after completion of endodontic treatment.

Case Report: A 43-year-old female patient came to Department of Periodontics, with a chief complaint of pain in lower right posterior tooth region. On clinical examination, there was 5mm of periodontal pocket, large occlusal restoration, periapical abscess with sinus in 47 as shown in figure 1. There was presence of generalized stain and calculus. There was tender on percussion in 47. There was periapical radiolucency in intraoral periapical radiograph in relation to 47 as shown in figure 4A. The vitality test was found negative in 47.[6] The treatment plan was made as first oral prophylaxis (remove the plaque and calculus) to reduce inflammation, then endodontic treatment followed by periodontal treatment. The patient was free from any systemic diseases, and without tobacco chewing habits.



Figure 1: Preoperative view showing 5 mm periodontal pocket in 47

Treatment: Oral prophylaxis was carried out. Then endodontic treatment was carried out first in 47 after oral prophylaxis. Within 2 weeks the periapical abscess along with sinus was resolved. Then under local anesthesia (2% lignocaine hydrochloride with 1:80,000 epinephrine), full thickness flap was reflected and open debridement was carried out in 47 as shown in figure 2. Then DM bone {(Silicon Ion Containing 60% HA, 40% B-TCP), META-BIOMED CO., LTD, Osongsaengmyeong, Republic of Korea} bone graft was placed in the defect and resorbable guided tissue regeneration membrane (PerioCol GTR, Eucare Pharmaceuticals Private Limited, Chennai, Tamil Nadu) was placed over the graft as shown in figure 3. Suture was placed in such a manner that there was no exposure of membrane and graft. Then periodontal dressing (Coe-Pak, GC America INC, USA) was given to minimize the postoperative infection and hemorrhage. It also assists healing by preventing surface trauma during mastication, and protects against pain,which is induced during mastication, as a result of the wound contact with food or the tongue.

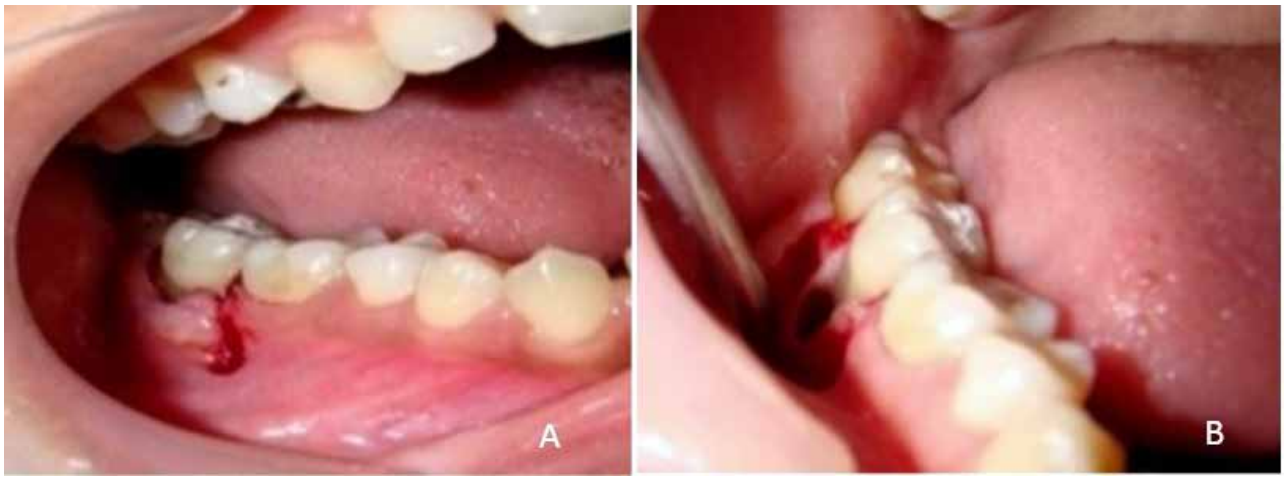


Figure 2: A (Both horizontal and vertical incision given), B (After complete debridement of the defect)

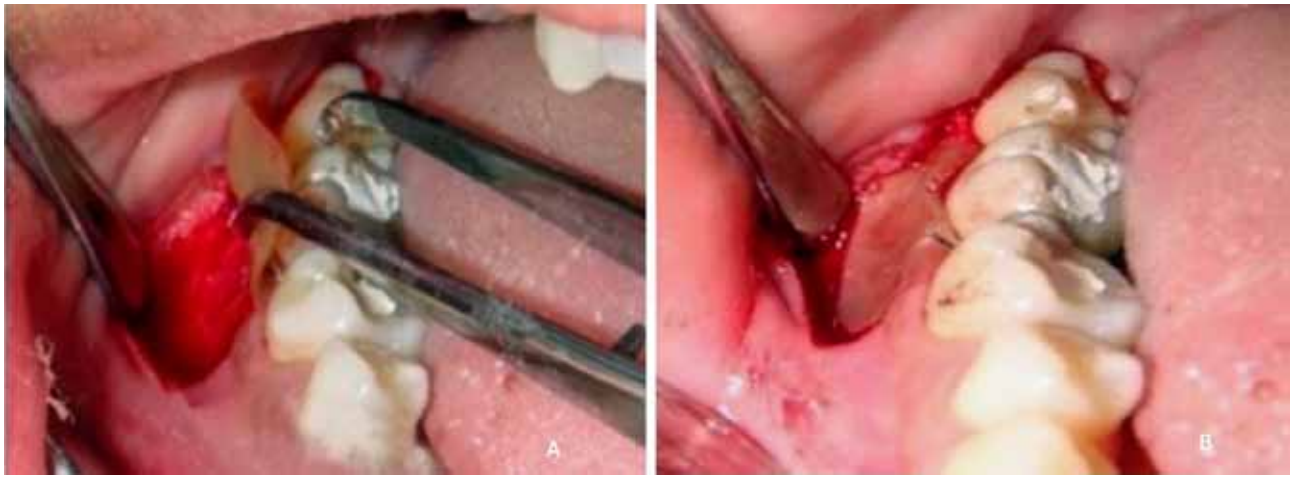


Figure 3: A (Bone graft placed in the defect), B (PerioCol GTR membrane placed)

Results

The present case was weekly follow-up visits for the first month. The reason was to have meticulous plaque control and had an optimum healing. After 2 weeks, suture was removed and washed with normal saline in the surgical site in order to clean the area. Later on, recall visits were done monthly during the next 9 month. These recall visits revealed that the patient maintained oral hygiene perfectly.



Figure 4: Postoperative view after 9 months



Figure 5: A (Preoperative), B (Postoperative) intraoral radiograph in relation to 47

After 9 months, IOPA in relation to 47 revealed there was completely filled up with bone and formation of lamina dura in the defect as shown in figure 5 and she was very satisfied with the result.

Discussion

Simultaneous presence of pulpal and periodontal disease, makes difficulty in diagnosis and treatment plan. Again it affects the sequence of treatment and long term prognosis too.[3] But it is not difficulty in diagnosis of primary endodontic or periodontal disease. For this type case, vitality test is the only easy tool to determine the actual causes of disease. If vitality test is found positive, it means the lesion is primarily periodontal origin, and if negative means primary endodontic origin.[6] In general, chronic inflammatory reaction of infected tooth can extend into the gingival sulcus and drains through sinus.[4,7] In this type case, the periodontal manifestation can resolve after successful endodontic treatment. In our case report, there was resolution of sinus tract after successful endodontic therapy, but there was 5 mm periodontal pocket still present.[8]It was also a case of primary endodontic and secondary periodontal lesion. In this type situation, endodontic treatment should be done first. As after complete debridement form root canal, there will be no infection inside the canal and also in lateral as well as accessory canals, which mainly responsible to connect from endodontal and periodontal compartments and vice versa. After hermetic seal obturation in root canal, there will be no space for any microorganisms and becomes sterile environment. In our case, endodontic treatment was carried out first then periodontal therapy. So after endodontic therapy, periodontal treatment was carried out. In our case, proper debridement was carried out after full thickness flap reflection. Generally autogenous bone graft or any platelet concentrates is ideal regenerating materials.[9–11] But to harvest autogenous bone graft makes a secondary surgical site. For that reason we placed synthetic alloplastic material{DM bone (Silicon Ion Containing 60% HA, 40% B-TCP), META-BIOMED CO., LTD, Osongsaengmyeong, Republic of Korea} in the defect. We used resorbable membrane (PerioCol GTR, Eucare Pharmaceuticals Private Limited, Chennai, Tamil Nadu) in order to prevent a second surgery need for replacement of nonresorbable membrane. The role of guided tissue regeneration membrane is to prevent migration of epithelial cell, and forms a weak long juctional attachment to the root surface which is not desirable.[12] Hence we used GTR membrane in our case for the above reason.

Conclusion

To manage an endo-perio lesion is not unidirectional, but it includes various treatment options. For the successful treatment of endo-perio lesion depends upon the diagnosis and treatment plan. When both endodontal and periodontal compartments are involved with disease, endodontic treatment should first carry out followed by periodontal treatment. In addition to conventional treatment procedure, other treatment modalities using advanced surgical techniques and materials such as regenerative procedures, radisection, and hemisection, bicuspidization should be considered as per clinical

conditions, which will ultimately result in a favorable long time prognosis.

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Ethical Issues: Approved

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4D Bioprinting, Newer Prospects in Regeneration

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Abstract

The new concept which has come up in recent times being integrated with 3D bioprinting is 4-dimensional bio-printing. This is now considered the most appropriate solution for "tissue engineering". It has the potential to construct functional structure and also fabricate 3D patterns of biological architecture which can change shape under various stimuli by use of "stimulate responsive material". 4Dbio-printing provides a great potential for tissue engineering of bone. The properties like shape memory of the printed structure is used for repair of personalized bone defect and the properties such as functional maturation helps in promoting osteogenic differentiation of stem cells. This review aims to discuss the concept of 4D bio-printing and its application in "bone tissue engineering".

Keywords: *4Dbioprinting, Bone tissue engineering, Periodontal regeneration.*

Introduction

In the recent time, the 3D bio-printing technology concept has integrated time as the 4th dimension to fabricate complex functional biological architectural models. The 4Dbio-printing structure can go through a transformation in share or function overtime by the use of various stimuli-responsive biomaterials and the series of 4D bio-printing strategies. The application of bioprinting in tissue engineering guided tissue regeneration procedure remains unpredictable due to close proximity to the tooth surface.[1] To solve this dilemma 3D bio-printing was proposed by **Hull** et al in 1986.[2] 3D-bioprinting has been used for the fabrication of blood vessels, liver tissue, heart tissue and bone. However, there are certain limitations such as the initial condition of a printed object is only considered and assumed to be inanimate and static. Since the process of natural tissue regeneration involves 3D microarchitectures and extracellular matrix formation along with the generation of a tissue with a unique function,according to **Y.C.Li** et al[3] the complex conformational changes on tissue acquired by a mechanism that can respond to external or internal stimuli, 3Dbioprinting cannot mimic. **Skylar** et al in 2014[4] at MIT for the first time demonstrated 4Dbioprinting technology where multi-material prints were introduced having the capacity to transform overtime or change its shape. Fabrication of various 3D design architectures that are capable of undergoing dynamic configuration transformation inresponse to various stimuli were done thereby overcoming the limitation of 3Dbioprinting. 4Dbioprinting was defined by **Gao** et al. This is considered as the generation of cell filled 3D printed structures that can respond to external stimuli or internal stimuli respectively. They also included the functionalization and maturation of tissue with time, change in the shape, and change in functionality of the printed material overtime as two main characteristics of "4D bioprinting". The shape transformation feature has beenused to carry out personalized bone regeneration in the irregular type of bone defects. Reassembly of stimuli-responsive material and program cross-linking could be modulated in the mechanical properties of 4D constructed architecture.[5] Further, the functional maturation of 4Dconstruct of bone could also help in establishing a micro environment that

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enhances the differentiation of stem cells and influences cell behavior.[6]

Important characteristics feature of 4D-bioprinting

1. Functional transformation
2. Shape transformation

Stimuli-responsive material

(a) **Physical stimuli**

- Magnetive responsive
- Humidity responsive
- Temperature-responsive
- Electro responsive
- Photo responsive

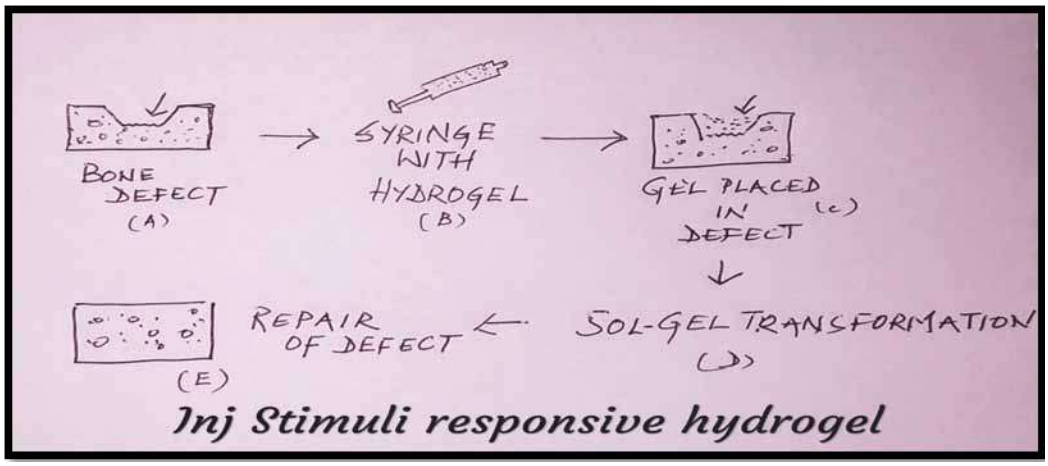
(b) **Chemical stimuli**

(c) **Biological stimuli**

3. CTF

Application: 4Dbioprinting is used for personalized bone defects especially the minor bone defects. The microvascular system and network of nerves within the bone tissue can be also constructed for a large bone graft substitute.

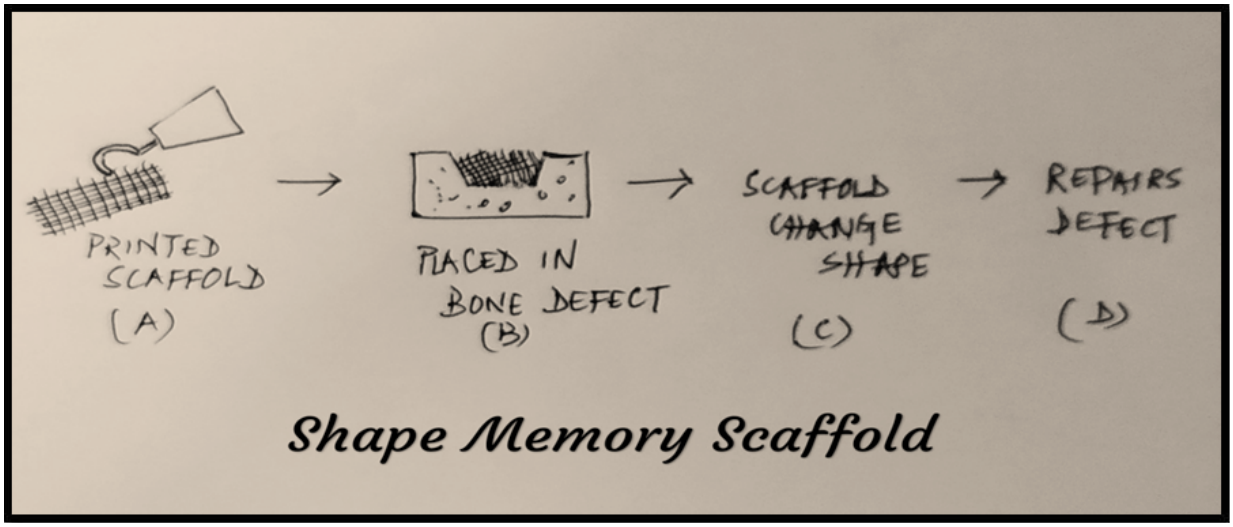
(a) Use of injectable stimuli-responsive hydrogel: In simple terms the thermostatic hydrogel is injected to bone defect and overtime gets transformed to a gel-like state at a temperature that is conducive to the human body,thereby repairing .the defect.



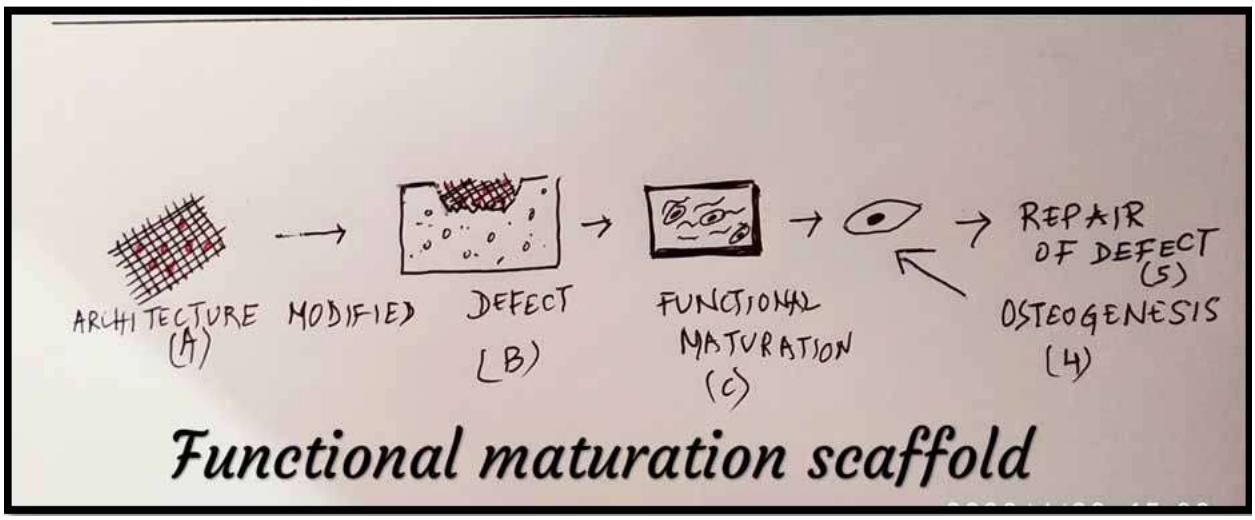
The hydrogels are thermo-responsive and are made up of polysaccharides. They also carry cells, HAP and CaPO4 cements, growth factors, inorganic composites like bioactive glass.[7] PNIPAM is one of the classical thermoresponsive material. This material along with chitosan and hyaluronic acid forms a suitable hydrogel that can be injected into the defect.[8] Another type of thermosensitive material available is a copolymer of polylactic acid,polyethylene glycol, and polyglycolic acid which can convert to gel under physiological condition.[9] These injectable hydrogels have mechanical strength that can be improved by the use of nanohydroxyapatite tricalcium phosphate and bioactive glass.[10] These hydrogels carry osteoblastic cells. The cells further have "alkaline phosphatase" activity and fill up irregular cells defect thereby providing of "mini-invasive repair" of bone defects. Mesenchymal stem cell differentiation has been enhanced with bioactive cues such as angiogenetic and osteogenic growth factors and are used by orthopedics in recent times.

2. 4Dbioprinting based on shape memory scaffolds: The shape memory scaffold is developed by 4Dbioprinting and implanted into the bone defect site. This scaffold changes its size to occupy the void space and repairs the defect. According to **F.S. Senatov** et alfused filament fabrication technique is used to obtain polylactide and hydroxyapatite porous scaffold.[11] The shape memory effect of the scaffold was stimulated by direct heating. These are used in self-fitting implants that repair small bone defects.

S. Miao et alused synthesized smart polymer that is PCL and cross-linker with castor oil-producing renewable bio-scaffold with shape memory and shape recovery effect at physiological temperature.[12] These scaffoldsare biocompatible and also biodegradable. More recently **S. Miao** et al fabricated a temperature-responsive scaffold that contained epoxidized acrylate materials.[13]



3. **4D bioprinting based on functional transformation mechanism:** During the post bioprinting stage, the biological activity of 3D-printed scaffolds and osteogenesis of stem cells can be improved by the biomimetic bony microenvironment. The establishment of this biomimetic microenvironment inspires the "functional maturation" of 3D-printed constructs, which is being reformed as polymeric "3D-printed scaffolds" by decorating them with a "cell-laden mineralized extracellular matrix" to imitate bony microenvironment. As time passes, a rotary flask bioreactor culturing, the printed bone structures became mature. Furtherit can be seen that the "extracellular-matrix-adorned" scaffolds showed evidence of better osteoinductive and osteoconductive properties than those of "bare 3D-printed scaffolds.



However, the bone tissues consist of complex hierarchical structures which further consists of both anisotropic mechanical as well as electromechanical properties. Barium titanate which is a smart biomaterial having a piezoelectric effect, has the capacity of stimulating the physiological electrical microenvironment. This smart biomaterial will act in response to functional stress as well as promote the growth of osteoblasts by showing bone inducing abilities and favourable biocompatibility. Piezoelectric materials have shown promising development in post printing stages.

4. **4D bioprinting with blood vessels and network of nerves:** The major challenge in"bone-graft-substitute engineering is regeneration of microvasculature along with the nervous networks. A combination of MSC's of mouse along with methacrylate alginate and hyaluronic acid hybrid hydrogels, a hollow self-folding tubes were fabricated. They had diameters closest to the smallest blood vessels. By entrapping enzymes in 4D hydrogel, pre-programmed calcification and fibrin biofilm formation could be triggered. "Bio-inspired 3D constructs" consists of bone-like similar structures that surround the constructed blood-vessels making a replica of vascularized alveolar bone constructs. 4D bioprinting of the "electro-responsive biomaterials" has shown scope for "nerve tissue regeneration".

Conclusion

4D bioprinting has emerged as a newer field for constructing a multilayered, constructive and functional bone tissue having complex structures. Fabrication of "shape-programmed", as well as functional structures,is possible in a more controlled manner. The advanced technology of this "4D bioprinting" will fulfill medical requirements and also it will help to explore its extensive purpose in the biomedical field. However, further researches are required in this field to work out the existing challenges so that 4D bioprinting can serve as a controlling means to ascertain dynamic along with hierarchical structures of natural tissues or organs.

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Possible Pathways of Disease Communication of the Endo-Perio Lesions and their Management

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Abstract

The periodontal therapy deals with all the aspects of a periodontium as a whole including both the prevention and repair. Whereas the endodontic therapy deals with only the pulp and the periapical tissues. The relationship among the periodontal and endodontic disease has remain always a controversial part in the dentistry. However, to have the accurate treatment, a practitioner must possess a very sound knowledge of all the possible pathways of communication of an endo-perio lesion. Hence, this review paper aims at presenting the possible pathways of communication for the endo-perio lesions at a simpler way to help the clinician in their daily basis of perio-endo management.

Keywords: *Perio-endo lesions, pathways of inflammation of endo-perio.*

Introduction

Many literatures have shown the close speculative relationship among the pulpal and the periodontal tissues. The knowledge of the disease transmission between these two has become a mandatory protocol to reach the correct and accurate diagnosis.

1. **Atypical Anatomic Factors/Developmental Factors:**

A. **Mal-alignment:** Mal-alignment such as the presence of a multirooted tooth in a position usually occupied by a single-rooted tooth, or additional roots of a tooth, a predisposing factor to occlusal trauma and food impaction which predispose into endo-perio lesions.[1-5]

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B. **Lateral and Accessory Canals:** Large lateral, accessory canals in coronal and middle sections of roots, presence of additional canals, with resultant changes in root morphology in single and multirooted teeth are the pathways for the endo-perio lesion. Lateral and accessory canals may result from a lack of dentin elaboration around a blood vessel located in the periradicular connective tissue. When the roots begin to mature, lateral and accessory canals are generated. The apex from varied directions also carries not only the blood vessels but also the nerve bundles. Lateral and the accessory canals are usually generated when the maturation of the root takes place. If the continuity of the root sheath breaks, it leads to the formation of foramina which acts as a pathway for the inflammation leading to an endo-perio lesion.[4]

C. **Dentinal Tubules:** When there isan absence of cementum and the dentinal tubules get exposed on the root surface they serve as a potential communication between the periodontal ligament and the pulp. The radicular dentin tubules are well connected from the pulp up to the cement- enamel junction and the maximum density of it meets at the

range of 57,000 per square mm at the pulpal end. Thus, it is apparent that when such a large number of dentinal tubules are exposed, a pulpal response to such a trauma will occur like irreversible pulpitis and pulpal necrosis. [3]

D. Lingual Groove: These occur most frequently on the lingual surfaces of maxillary lateral incisors. Very often such a tooth is found pulpless and has a periapical lesion. Adequate root canal therapy may resolve the periapical lesion but may not resolve the periodontal defect associated with the developmental groove. Prognosis should be determined by the extent of the periodontal defect.[6,7]

E. Tooth Anomalies: Enamel pearls, cervical enamel projectionsor spurs or extensions into the furcation of multirooted teeth are found frequently. They help in retention of plaque leading to the formation of the periodontal lesion which in turn can lead to endo-perio slowly by time.[7]

F. Developmental Malformations: The type of developmental anomaly occurs when the tooth germ fails to form the other root-like vertical developmental radicular groove. It is seen mostly in the maxillary central incisor central fossa and lateral incisor. The healthy periodontium maintains its integrity until the intact position of the epithelial attachment. But once the breach of the attachment takes place and the groove becomes contaminated by the infection, the entire length forms an infra bony pocket which seems to be self-sustained. It leads to the niche of the tissue-like channel to microbial plaque and periodontal disease progression occurs. Slowly it leads to an endo-perio lesion. The course of the groove is followed by the bone destruction area, appreciated radiographically.[8]

2. Iatrogenic/Contributing Factors

A. Types of Trauma: Trauma can injury both the teeth and the alveolar bone, involving the pulp or PDL or both. Enamel fractures, crown fractures with or without involvement of the pulp, crown root fracture, root fracture and luxation and avulsion are examples of trauma.[5]

In the case of Enamel Fracture, chipping or cracks on the enamel surface of the tooth. There is no pulpal or periodontal involvement hence this vitality enriches the prognosis part.

In the case of crown root fracture, they are always oblique involvement of both the root and the crown part. Pulp may not be involved here, but the enamel, dentin, and the cementum is affected mostly in molars and premolars and less affected in the anterior teeth. Conservative restoration like composite resin or union reattachment of the fractured fragments is the line of treatment. But if the crown fracture involves the pulp involvement, these types of cases often becomes complicated. Hence the pulp exposure will dictate the restorative pulpal treatment. If the fracture is very extensive then root canal therapy is indicated. Pulpotomy and pulpectomy can also be a protocol which is decided by the stage does the tooth maturation lies. Sometimes, the amount of time-delayed for the start of the treatment, soon after the injury, may hamper the pulpal prognosis. The better prognosis can be achieved by the earlier treatment. When the fracture of the cusp extends beyond the subgingival margin, the case becomes much more challenging, the severity of the fracture decides the treatment part. Sometimes the fragmented fractured part of the tooth is splinted, stabilization with the coronal segment repositioning for 12 weeks to achieve the perfect pulpal and periodontal repair. Later on, root canal therapy along with periodontal therapy is recommended. Quite a times, the decision of the treatment is extraction been taken because of the hopeless prognosis. If the fractured tooth does not involve the pulp, then there is no requirement of the root canal therapy as healing takes place on its own.[8] In the case of luxation, the different types of injuries seen are mainly are concession, extrusion, lateral luxation and intrusive luxation. And the treatment is different for the different types of luxation injuries. In the case of root fractures following trauma, fracture can happen in the root in different directions, mesially/ distally, or facially/lingually. It may or may not involve the pulp chamber. A vertical root fracture can extend the entire length of the tooth and involve the gingival sulcus or pocket area but may also be incomplete and confined to either the coronal or the apical portions.(2) As an outcome of bacterial expansion in the fracture gap, the neighboring periodontal ligament will turn into the place of an inflammatory wound causing the collapse of the connective tissue fibers and alveolar bone.[1]

A. Perforations: Perforations either laterally or apically on the tooth are seen as a treatment failure or is a considered as undesirable complications of the clinical treatment. Prognosis of the teeth is impeded due to communications between the root canal system. Various reasons for usual

root perforations are carious lesions extending extensively root resorption by the error made by the operator during the preparation of the root canal orpost preparation. Hence the prognosis of the root perforation treatment depends on factors like how big is the size of the perforation, its location, and the right time in diagnosing it. The ability to seal the perforationdepends upon how far the repair material isbiocompatible. If immediate sealing of the perforation is done at the right time with good infection control, it leads to successful treatment. The popular root perforation sealer available are EBA super, composites, amalgam, cavity.[8]

B. Accidental Lateral Perforations: If the treated root perforation goes undetected or not successfully treated, it may give rise to many sites of inflammatory lesions in the marginal periodontium. They manifest as increased probing depth, suppuration, increased tooth mobility and loss of fibrous attachment. At the time of endodontic treatment, and in concurrence with the preparation of root canals for the insertion of the post, unintentional instrumentation can by accident forms perforation of the root and wounding of periodontal ligament. Perforations may be made through lateral walls of the root or the pulpal floor in multirooted teeth. At the site of perforations, the subsequent inflammatory reaction can result in the formation of a periodontal pocket, if the perforation is located close to the gingival margin.[9] Other complications include exacerbation of clinical symptoms, similar to those of a periodontal abscess. Eg: acute pain, swelling, drainage of pus from the pocket, increased tooth mobility and further loss of fibrous attachment. Treatment of a root perforation should be initiated once it has been spotted. Healing of the lesion in the periodontium depends largely on whether bacterial infection can be excluded from the wounded site by a tight seal of the perforation.[1] If the perforation is close to the sulcus area or conducted through the floor of a multirooted tooth, the inflammatory response may induce the proliferation of sulcular epithelium to form a deepened periodontal pocket.

C. Poor Restorations: The critical cause behind the failure of the endodontic treatment is the coronal leakage. And if the fracture of the coronal restoration is the reason behind coronal leakage and is delayed, there is a high risk of recontamination of the root canals. And sometimes, the exposure of the root canal to the oral cavity can lead to coronal leakage, coronal restoration plays a vital role in such cases as it forms a barrier between the bacterial contamination of endodontic treatment and the coronal leakage. Hence a very good endodontic obturation and a fantastic sealed coronal restoration is mandatory to protect the root canal system . And this does not end the coronal leakage but the cemented full crowns, as well as dentin bonded crowns, helps still better the coronal leakage long term prognosis of endodontic treatment areas.[5] There are certain precautions to be taken to avoid accidental perforation during the other endodontic treatment. They are: Rubber dam isolation is a must for performing cementation and space preparation postoperatively, heated plugger should be used during the preparation of the post space, the preparation should hold at least a minimum of 3 mm root canal filling, same as the root canal treatment, the post should also be well irrigated and well dressed, the delay should not happen to place the restoration for leak-proof, immediately after the completion of the endodontic treatment, only after 3 months of coronal seal, endodontic treated tooth should go for crown restorations.[5]

D. Internal And External Root Resorption: These are seen as pathologic process or sometimes even as a physiologic process. Delayed untreated external resorption cases may lead to invade of cementum, dentin and ultimately lead to pulpal space. Root resorptions are free from clinical symptoms. And the internal resorption cases if left untreated they may advance and end up in more complications like perforation of the external root surface. External root resorption where the external surfaces of the teeth are hampered. Internal tooth resorption starts when the pulp space affects the primary internal dentin. The main generating cause in root resorption is a root surface separated from its defensive blast cell layer. This type of separation may follow any damage to the cementoblasts cell layer. For the resorption to carry on, there is a mandatory requirement of a stimulus, an example may be a disease or a constant mechanical strength such as the one in orthodontic treatment.[10] Consequently the treatment of root resorption should be directed towards the cause of the continuance of the resorption, such as removal of the infected material in a root canal or by discontinuing orthodontic tooth movement.

E. Apical Foramen: The principal route between the

periodontium and the pulp is the apical foramen. Periapical pathosis is usually caused by the inflammatory mediators also by the bacterial end products. Through the apex/apical foramen, the inflammatory mediators reach the pulp by the portal entry from a deep periodontal pocket. External resorption of the alveolar crest also arises because of the periapical granuloma from the necrotic pulp and spreading toward the lateralparts of the root.[6]

F. **Empty Spaces Created By Destroyed Sharpey's Fibers:** Due to the destruction of the periodontium in cases of periodontal disease, the contents of the root canal system get exposed to toxic products. Thus there is direct communication established between the pulp and the oral cavity through the exposed dentinal tubules.[3]

G. **Cemental Agenesis or Hypoplasia:** When the root surface is exposed to the oral environment due to congenital absence or faulty cementum, especially at the cementoenamel junction, there is a direct spread of infectious products between the pulp and the periodontium.

H. **Exposure of Dentinal Tubules Following Root Planing:** Scaling and root planning are indispensable procedures in the treatment of periodontal disease. However, sometimes with the removal of bacterial deposits, cementum and superficial parts of dentin are also removed. Therefore, due to this instrumentation, dentinal tubules gets exposed and left unprotected to the environment. Subsequent microbial colonization of the exposed root dentin may result in bacterial invasions of the dentinal tubules. As a consequence, inflammatory lesions may develop in the pulp. The risk for bacterial penetrations and pulpal injury is further enhanced by the injudicious use of acid for the conditioning of the root surface in the attempts to promote periodontal tissue regeneration. During the maintenance phase of periodontal therapy, scaling and root planning or are frequently repeated procedures. At each recall session, the root surfaces are debrided and some dentin is removed. This remedy can consequence only in the deteriorating of the tooth structure and also wide reparative dentin formation in the pulp.[2]On rare occasions, deep periodontal scaling may expose lateral canals that in turn can induce severe symptoms of pulpal pain. This kind of lesion is termed 'retrograde pulpitis' and may also be initiated by exposure of the tissue around the apical foramen to the periodontal disease.

Conclusion

The result of periodontal inflammation on the pulp is controversial and conflicting always. It has been recommended that periodontal disease does not affect the pulp, until it involves the apex. There are different schools of thought; numerous studies recommended that the consequence of periodontal disease on the pulp is degenerative together with a rise in calcifications, fibrosis, and collagen resorption, as well as a direct inflammatory effect. On the other hand, it seems that the pulp never gets affected by periodontal disease, until the recession has started up an accessory canal to the oral environment. Hence, the management of combined lesions has toseek at eliminating equally both of the problems.

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Treatment Plan for Ameloblastoma: A Review

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Abstract

Ameloblastoma is a tumor of odontogenic origin that exhibits 1% of all oral cavity tumors. Solid and multi-cystic are well chosen as locally aggressive, with elevated recurrence rates incase of conservative treatment. The objective of this review article is to analyze that the management of ameloblastoma should be done conservatively or radically. The appropriate management of ameloblastoma should reduce reappearance, reinforce function and aesthetic, and exhibit a minimum damage in the donor area. Planning of the treatment must be done based on the extent,position, andtype of the lesion.

Keywords: *Ameloblastoma, Surgery, Enucleation, Radical.*

Introduction

The second most common odontogenic tumor is ameloblastoma.[1] It is found particularly in the jaws, with a tough preference for the dorsal part of the mandible. Even though the tumor is benign, it is destructive, locally invasive and the recurrence rateis high even after adequate surgical elimination. The WHO tumors classification in 2005, mentioned that "the variation of ameloblastomas are the solid/multicystic, the extraosseous ameloblastoma, the desmoplastic and the unicystic type".[1] The exact pathogenesis of ameloblastoma is unknown. Various factors such as infection, non-healing wound,inadequate nutrition, as well as a probable correlation with the Human Papilloma Virusmay add to the cause.[2] Ameloblastoma can cause damageto the compact bone. This results in infringement of the soft tissues of the oral cavity disarranged occlusion ,dissymmetry,irritation and if the inferior alveolar nerve is affected it may lead toparesthesia.[1,3] Ameloblastoma in the former stage is identified through OPG accidentally if the patient would have come to do OPG for other reasons or because patients confer for symptoms to the consultant and then a histological examination is done for confirmation.[3] Currently,multi-cysticandsolid ameloblastomas are expressed as locally aggressive, with a greater chance of reappearance if conservative treatment is done.[4] Aggressiveness of ameloblastoma of Unicystic variety is lower, and the risk factor of reappearance is very minimum if treated conservatively. The peripheral ameloblastomas gives better feedback if local excision is done.[4]

The selective management of ameloblastoma is any ways an operative procedure, but the choice of surgery depends on its clinical nature. The better treatment procedure has always been a topic of debate for the treatment of ameloblastomas. Enucleation or marsupialization, is considered under conservative procedures along with curettage, and use of liquid nitrogen or Carnoy's Solution. Major surgical procedures such as segmental or Hemimandibulectomy comes under radical treatment.[5]

Literature review: World Health Organization defines ameloblastoma as "a benign lesion of follicular or plexiform aspect with a fibrous stroma. The most common feature of ameloblastoma is that it is a benign epithelial odontogenic tumor with no

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element of ectomesenchyme."[6] Taking pathogenesis into account, the literature says that this lesion most likelyoriginates due tothe proliferation of the residual cell of the enamel organ. Along with this,it is also said that various molecules and alterations of genes have an impact on the triggering of odontogenic epithelium that leads to ameloblastomas.[7] Frequency of occurrence of ameloblastoma is more common in mandible than the maxilla. Among which 70% of cases are seen inthe mandibular body and 20% in the ascending branch of the mandible and rest 10%of the cases are found in the maxilla.[8]It is also noted that 15% of the ameloblastomas involve the maxillary sinus and the nasal floor. The involvement of the palate is negligible.[9] Pogrel et al. makes a review of 58 articles and found that"randomized clinical trials were available.[10] The results showed a recurrence of 60-80% with simple enucleation of solid and multicystic ameloblastoma, therefore, the treatment should be the segmental resection with 1 cm of margin to the bone, including a soft tissue margin. For unicystic ameloblastoma, the authors recommend an enucleation accompanied by a support technique. If not possible, a block resection with 0.5-1 cm margins would be the correct technique. Finally, the peripheral ameloblastoma responds well to the local excision."

A retrospective study is done by Hammarfjord et al.in which he hasdone the analysis and found that the reappearance of intrabony ameloblastoma in 48 patients and concluded that-"Radical treatment is recommended when recurrence and conservative option should be reserved for small intrabony ameloblastoma.[11] However, given the low rate of metastases, the chosen technique must consider the quality of life and comorbidities of the patient. Thus, the authors suggest a conservative treatment of ameloblastoma along with an annual follow-up for 10 years as a good alternative to processes that would involve aggressive resections, especially if there are no medical and personal factors that could compromise follow-up. It seems that enucleation can be the best alternative when assessing all the important aspects for the patient, but radical surgery is the correct option when the lesion is close to vital structures or when it cannot be treated conservatively."Simon et al. have done a cohort study where he has compared 64 patient's treatment procedures. These patients were treated radically. Out of 64 patients,32 patients have undergone immediate reconstruction during the same surgical procedure whereas 32 patients treated have not gone subsequent reconstruction. The success rate was more in patients who had reconstruction procedure immediately after surgery.[12]

Method of Diagnosis: Imaging tests such as X-rays,CT and MRI help the doctors to diagnose and determine the extent of ameloblastomas .and the confirmation of the disease is done by histopathological evaluations. CT scan is one of the relevant method of diagnosis for the determination of possibilities and extensions of ameloblastomas. Coinciding of images and dissertation errors are negligible in CT scan.[13,15] In histopathological examination, the appearance of islets, nests, and strands containing ameloblastic epithelial cells accomplished with a fibrous stroma is necessary to confirm the possibilities of occurrence of ameloblastomas. Follicular and plexiform patterns of ameloblastomasare most common, although there are also studies of unusual types, such as acanthomatous ameloblastomas with granular and basal cells.[15]

Treatments: The decision of the appropriate type of treatment is a very crucial step and the omission of the lesion should be the target on, considering the future social and personal aspect of a patient's life. Moreover,the treatment plan depends on the size,extent,type and histopathological feature of the ameloblastoma. More aggressiveness of surgery reduces the chance of reoccurrence.[16] If the lesion invades a greater portion of the cortical bone and anatomical structures, aggressive surgery should be opted to have discontinuation between bone pieces, along with the removal of periosteum and soft tissues.[17] Cosmetic surgery should be done postoperatively for a better lifestyle of the patient. A major drawback of management for ameloblastoma is the worsening of esthetics or facial appearance in the aggressive method surgical procedures that are done to remove the lesion.[18] This factor hampers the treatment procedures of the patient as they are isolated from the society. Education and Awareness among the patient and society about ameloblastoma is much essential to overcome this negative aspect.

Discussion

The most appropriate surgical treatment of ameloblastoma should be such that it should reduce the chance reappearances, should not hamper the functional and social status of the patient much and demonstrate a negligible distress in the donor area. Conservative techniques include minor surgical excision procedures such as marsupialization, enucleation,

curettage orcan be adjuvanted with cryotherapy, Carnoy's solution.[15,16] This type of treatment plan can be executed as a treatment option for less aggressive ameloblastomas. This type of treatment option has less effect on worsening the functional and esthetic part of the patient but the chances of recurrence are high if only enucleation has opted as a treatment option. In the case of aggressive tumors or if there is no possibility of combined conservative treatments, radical surgery with 0.5-1 cm of margins is preferred followed by cosmetic surgery as reconstruction procedure, forbetter outcomes. Radical surgery with an extent of 1 cm as margins and elimination of peripheral tissuesof the region followed by reconstruction is the choice of management for solid and multicystic ameloblastoma. Radical surgery rather than conservative management is always preferred in the case of large tumors.[17,18]

Conclusion

In this review, we concluded that aggressive surgery like radical surgery is the better treatment option in multicystic or solid and advanced unicystic tumors and should be followed up by the surgeon for the next 7-8 years. Adjuvant Conservative surgery with a constant follow-up for rest 20 years opts for the less aggressive type of ameloblastomas

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Laser Protection is Everyone's Apprehension! An Update

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Abstract

Today Laser is not anymore limited to big hospitals; it is easily accessible to the private clinics and private enterprises too. Anyone can go for laser practice today by attending a certified workshop on it. However, the main concern is the sound knowledge of its safety measures and management. This should be the primary goal for everyone involved in the sale, purchase and practice. There is a mandatory necessity of the safety administration or supervision to prevent or safeguard the laser hazards like understanding of standards, recognition of hazards and risks, carrying out of suitable control measures, and unfailing program audit to display eminence assurance. Hence, this review topic is taken to update the details of the dental laser hazards and its management.

Keywords: *Lasers, Tissues, Absorptions, hazards, complications, management.*

Introduction

The body tissue nicely absorbs the laser light. It can causes injury to the body tissues if the laser beam is much more enough powerful than the capacity of the tissue. The most sensitive part of our body to the laser beam is the skin and the eyes. The wavelength of the laser decides the amount of the laser beam to be absorbed by the targeted tissue. And the injury to the tissue is directly proportional to the amount of the laser light absorbed.[1]

Knowledge of Laser Science = Ability To Perform Risk Assessment = Development of Appropriate Safety Policies = Safer Staff = Safer Patient Care !!!

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Hazard Classification and Corresponding Control:

A. Set I: This set of laser falls into the safest category as it is said to be nicely enclosed inside a safety cascade. Also it is not possible to remove the tightly enclosed cascade with hands. It can only be removed, if required, with the help of the respective tools meant for the removal of it.

B. Set II: This set of laser is not that dangerous if accidentally and unintentionally viewed but may prove dangerous if viewed continuously at a stretch for a longer time. This is due to the natural aversion response of the laser. The caution tag should not be taken out at any cost for the safety reminder to the operator. And also the laser light should not be focused directly at the operator eye or the patient eye. Even the patient is directed not to stare at the laser light during the operation even though with the safety goggles.

C. Set II 1A: This set of laser happens to be dangerous and cause injury when the optical safety tools wore by both the operator and the patient, accumulate the beam and reflect them directly into the eyes

D. Set II 1B: This set of laser happens to be dangerous and cause injury when the optical safety tools were

not aided by both the operator and the patient. On viewing the laser light without protective tools, it may prove highly dangerous.

E. SET 1V: It is the same as class 111 risks. On direct exposure along with the reflection of it, with the powerful laser light, the injury is caused. These lasers cause biologic risk from either straight or distributed reflection. Commonly any laser able to produce power larger than 500 MW nonstop wave production falls in this set.[2]

Types of Laser Hazards: There are many types of hazards observed during the clinical practice of laser in dentistry like[3]

1. Ocular/Optical injury
2. Tissue damage hazards
3. Respiratory hazards
4. Fire and explosion hazards
5. Electric hazards

1. Optical/Ocular hazards: The direct emission of the laser light along with the reflection of the same from the surface having a mirror effect can cause tremendous injury to the eyes. The primary optical injury found is the burning of the retina or the cornea. The wavelength with visible (400-780nm) and near-infrared (780-1400nm) are enough for the injury of the retina. Different types of wavelengths may cause different types of optical injuries. This is possible because of the much more focusing capacity of the retina and the cornea. This injury can also happen with a constant low-intensity reflection and turn into an irreversible injury leading to permanent destruction of the retina and the cornea, and finally into blindness. The blindness is due to the high heat energy which takes hardly a fraction of a second. Laser-like Argon and Nd: YAG can get transmitted into the eyes directly and the power becomes increased nearly 100, 000 times more because of the lens focus. Surfaces such as instruments, mirrors, and polished restorations have the potential to redirect laser energy. Nd: YAG lasers demand the safety glasses which are of green in color and argon laser demand for the colored glasses which are amber. However, CO_2 laser demand s for the clear glasses. The keys should be removed from the laser during the non-use period. Also, there should be a separate safety and a security maintenance room for the laser. The team with LSO-approved only should be eligible to access it. There should be clear safety warnings posted on the laser room entrance or the door for creating awareness.[4] The laser room should be nicely sealed with an opaque material to prevent leaching and leaking of the hazardous laser beam to the bystander. However, it is very much safe for the CO2: laser, as only plastic and glass walls, can absorb the laser radiation. There should always be an extra pair of goggles stored at the entrance of the laser room for the person entering the room for his eye safety.

2. Tissue Damage hazards: Skin hazards from the laser should not be ignored as it seems to be much painful than ever imagined. At times, there may be severe deep damage to the skin tissue. It happens sometimes by a mere accident and at times due to the negligence and carelessness of both the operator as well as the assistant. Especially it happens due to the lack of coordination between the assistance and the operator, assistance hands come in front of the laser optic tip causing a burn to the skin.[5] Skin burn can even happen to the patient if he is not made aware beforehand to keep off his hands from the operated site during the procedure. The patient must be well trained to raise his hand and give signs to interrupt the procedure if he wants to deliver something. But strictly he should be prevented from touching the operated area during the ongoing procedure to prevent mishap. The moment the skin comes in contact with the contact mode of the laser fiber optic, the laser misfire and causes very painful burns. Hence, the operator is expected to take off his foot from the foot pedal and keep the laser in the "standby"mode, till everything gets ready to start with the procedure. Once the assistance checks everything ready and gives thumbs up and changes the standby mode to start mode, then the operator can keep the foot on the pedal. The patient facial skin, adjacent teeth, and the adjacent soft tissue next to the operated site should be taken care of by placing wet sponge or wet gauze on it. Or else shattering of the tooth takes place if the adjacent enamel of the tooth and the pulp gets penetrated by the unwanted laser radiation. However, the tooth damaged by laser is highly resistant to dental caries though it looks very ugly. [4]

3. Fire and Explosion hazards: The surgical

materials used at the site of laser operation are highly vulnerable to catch fire and produce fire hazards. The material can be from the disposable drape made from the pulp of the wood, dry cotton and gauze swabs or sponges, tongue blades made of wood, and of course the material made out of plastic. Hence to prevent the mishap, surgical drapes made of polypropylene material are very much popular as they melt but never burst into fire in contact with the high energy laser tip.[6] Secondly, is the endotracheal tube, which is the highest source of risk during oral surgical procedures. Enough attention should be given to avoid the endotracheal tube coming in get in touch with the laser tip during the surgical procedures. This seems to be caused by the blow torch effect inside the airways of the patient which ignites. Hence these days new endotracheal tube supposed to be laser safe are available. Also it is mandatory to have the endotracheal tube airtight with the reflective part of the metal exposing outside to detect the endotracheal tube during the surgery. So that special attention can be provided to prevent the laser tip when it comes in contact with the tube. The distal end of it is the cuff which is always filled with the saline solution and blue dye made of methylene. It is to protect the laser beam from coming in contact with it. The time the fiber optic gets touched with the cuff, the dye which is a distinct blue, and visible, spills outside only to draw the attention of the surgeon and the anesthesiologist to take care of the laser-related puncture of the endotracheal tube cuff.[7] Also it is made up of stainless steel body which puts enough resistance to the laser perforation. In a little time back, the endotracheal tube was manually wrapped with foil but it is obsolete now to prevent the unwanted open area left unintentionally which can be a vulnerable issue for the laser to get ignited. Thirdly, safety measure is, to lessen the oxygen gas content of the anesthesia mixture by around nearly 30 % to prevent ignition mishap. Also the isolation of the nasopharyngeal throat with the pack of wet cotton or gauze pieces is recommended. It prevents the absorption of the high laser radiation and gives maximum protection to the patient.[8] The surgeon should not forget to remove the intrapharyngeal packs soon after the surgery is completed. The anesthesiologist should remind the surgeon by asking questions as: Is the removal of the pack done. It is in the guideline of anesthesiology practice by the MCI. Additionally, the anesthesiologist is also expected to keep an eye over the period from the pack insertion until the pack removal only to ensure that the pack is safely removed before the closure of the surgical procedure. Along with the surgeon, even the anesthesiologist should work hand to hand with the minute details of the surgical procedure to safeguard the patient from any mishap. However, even the surgeon must be aware of all the anesthetic details given to the patient to ensure the safety of the patient if at all any mishap happens. Not being aware will cause only chaos at the much needed time and it can cost the life of the patient instead of life-saving. Last but not least, the whole team comprises of the staff nurse, operator assistance, laser technical assistant and even the fire safety concerned department, emergency dept. should be very well informed of everything much before the safe going of the whole process. Not to forget, beforehand, a well prepared written plan of the action should be made among the entire team and well versed with minute details for the safety concern. A good and the right plan of action if properly taken, it helps rapidly at the time of mishap and proves a commanding life-saving action.

The sequence or the protocol recommended during any fire mishap: Immediately switch off the laser. Switch off all the anesthetic gases including the oxygen cylinder. The patient shifted to a safe place immediately. With the help of the bag and the mask, the patient should be ventilated with 100% oxygen gas in the other room by the emergency department. Saline solution to be used at the earliest to extinguish the flame. Cuff should be deflated immediately and it should be removed simultaneously. The entire tube should be taken off with care. Bronchoscope should be used to make the airways clean off from any foreign bodies like pack material or any combustible material which may block the airways. If the damage is under control care should be taken to restart the incomplete surgical procedure after assuring the patient assistance that everything is well. Even the patient is assured with enough faith if he is in a consciousness state to understand it. But in case of the extensive damage, the airway ventilator should be carried out with an emergency tracheotomy. Steroids of high dose and the indicated antimicrobials should be injected keeping the vital parameters of the patient in the watch. And the reschedule of the targeted surgical procedure is again newly planned keeping the patient parameters in watch. The fire mishap should be properly conveyed with all the, minute details and the

respective concerned officers of the fire mishap as well. Risk management team, manufacturer companies, law officers, humanrights, insurance companies, and the quality controlled hospital management too.Pt and his family should be well conveyed too with assurance and faith to prevent the unwanted unpleasant illegal issues later on. It is also to keep the faith on the hospital team too. Water should never be used to Do not use water to put off fires on electrical tools. The entire team accessing the laser should be aware of the fire extinguishers at the entrance of the laser room, and its application that is P.A.S.S. - pull, aim, squeeze, and sweep.[4]

4. **Electrical hazards:** At times back, out of all the laser hazards, electrical hazard was the most fatal during the initiation period of laser usage. The reasons were many, from the untrained to the uncertified and unauthorized or even quack practitioners. The qualities of the electrical safety were not taken care of and less knowledge of the precautions to be taken to control the mishaps, were brought into notice. Electric shock because of coming in contact with the capacitor, which is said to be charged to its maximum.[9] It resulted in electrocution and death immediately. Proper inspection and evaluation were not carried out at the earlier stage and even the repair work was neglected which brought many mishaps. Technicians were not well trained and even the surgeons were not certified with the special laser workshop and were not trained with the sound knowledge of the handling of the accidents and safety measures to be taken at the right time. Even the manufacturers did not give a clear caution tag on the laser to make the surgeon well cautioned enough. The proper wavelengths were not followed for the respective procedure. The high unwanted radiation because of high voltage caused many a times electric mishap. Also the direct current DC of the laser kept on charging for hours together even after the unplugged and switch off which became the reservoir of high fatal electric current. The indicator light which stands for the electrical malfunction should be very much aware of and if it goes off, immediately the laser technician or the service in charge should be informed to come and interrogate the problem. It should not be ignored and the procedure requiring laser should be immediately called off till it is repaired or it can be continued if there is any stand by laser available during the time.[7]

5. **Respiratory hazards:** The most unwanted is the byproduct of the laser, laser smoke, or the laserplume. Laser plume is a very unpleasant scent and this malodorous consists of steam that is then vaporized water, the particles of carbon, and few cellular products, which all together form laser plume.[10] This plume is very chocking and uncomfortable for the team working there in the operating room. It is also believed that this laser plume may prove highly dangerous to the respiratory patients as it is said to have consisted of various substances, such as formaldehyde, hydrogen cyanide, hydrocarbons, and other airborne mutagens. The size is hardly a little more than 0.3 u. It is found in the literature that the laser also gets filled with deadly human papilloma virus DNA, at the time of the surgery carried out to coagulate or to remove the papillomas growth through the laser. It can prove to be fatal for the patient next to him or the person coming in contact during the particular time, as it is an airborne infection. Huge evacuation for the laser smoke and with the filter size of 0.1 um is indicated in the operation area. The evacuation suction should successfully remove the laser plum in and around the area of 10 cm of the surgical area. Evacuation filters should be regularly monitored and should be replaced immediately when the time indicated of blockages of the pore and the efficiency ends here. To replace the blocked and old evacuation filters the service given person should be asked to wear the disposable gloves with proper mouth mask to get prevented from any air none pathogens built inside. Then the waste should be carefully disposed of with the recommended biohazard bags. The accumulated charred accumulated by the laser from the surgical area should be cleanly wiped out with an antimicrobial solution. Proper and righteous biohazard handling should be maintained for the materials used during the laser procedure. The right kind of the face mask and eye wear is highly recommended during the laser procedure in the infected patient to avoid the splattering of the infected tissues into the eyes or nose. Both for the operator as well as for the assistant too. And they too should be disposed of or sterilized for the next use.[7] Lastly, but not least, all the instruments used during the surgery touched or even not used or touched, including the loupes should be wiped off with approved sterilizing method and liquid.[4]

Conclusion

Safety is everyone's responsibility! Routine audit, troubleshooting, training, policy and procedure development, and compliance enforcement, are duties of

the LSO, but case by case, day by day, patient by patient, laser safety depends on every healthcare professional's commitment and vigilance. Lasers can offer patients a wonderful range of treatment options, from standard of care to experimental innovation. Laser users are constantly challenged to redefine who they are, what they do, and their scope of practice, with each new laser system or application. It must be remembered that every new system demands risk assessment and a review and revision of facility safety policies and procedures. Only through teamwork, communication, continuing professional education and training, and respect for the technology, can we establish the foundation fora truly effective laser safety program.

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Otomycosis: A Comprehensive Review

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Abstract

When the external auditory canal is infected with an isolated fungal infection, it is called Otomycosis. It seems to be a very common clinical condition found in the outpatient department of otorhinolaryngology all over the world. The etiological microorganisms that form biofilm inside the earcanal are mostly Aspergillus and Candida species. Improper self-cleaning,self-medication, and insert of unnecessary tools into the ear canal can lead to the onset of otomycosis. The major symptoms of otomycosis are earache,ear discharge, and hearing loss. So the right diagnosis is a must to take a percussive step during the primary stage of infection. But when infection is spread up to a mastoid area with suppurative condition then it needs special medical attention especially in patients with low immunity. This comprehensive review has the aim to bringattention to self-hygiene and proper health education to maintain a healthy life.

Keywords: *Otomycosis,external auditory canal, Asparigellus, Candida species.*

Introduction

Otomycosis affect external ear canal; is simply known as an ear infectious disease which causes inflammation, more seen in dusty, warm and humid area.[1] The fungal species (Candida/Aspergillus) are commonly found causative microorganisms oragents. Black pepper like appearance seen with Aspergillus niger and Aspergillus fumigates and candida albicans gives white fluffy otorrhoea.[2] Habit of swimming (not drying ear after swimming), long term persistence and recurrence infection in-ear, without proper consultancy of doctor use of ear drops, use of steroid containing medicine & oil, excessive accumulation of ear wax, not keeping ear clean, etc can because of otomycosis.[3] In recent years,because of having a week immune system opportunistic fungal infections have gained greater importance in medicine. However, such fungi are very common to all categories of people and produce infectionmostly in immunocompetent hosts[4]. Special care should be taken to prevent invasive bone(temporal) infection and hearing loss. So treatment should be administered as soon aspossible especially inpatient with low immunity.[5] If otomycosis treatment is not taken properly then there is a chance of recurrence.[6] Along with this, increasing cases of antifungal resistance is one of the causes of recurrence.[7,8] so adequate treatment and follow up should be done to prevent the invasion of fungal infection.[9] This comprehensive review aims to create awareness about otomycosis and its management.

Method of Literature Search: Research articles regarding otomycosis were searched through a multiple systemic approach. First we conducted an online search of the Scopus, PubMed, and Medline database with otomycosis, fungal infections of external auditory

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canal, and antifungal treatment of otomycosis. The abstracts of the published articles were identified by this search method and other articles identified manually from citations. This manuscript reviews the details of otomycosis patients along with its important medical problems. This review article presents a baseline from where further prospective trials for otomycosis could be designed and helps as a spur for further research in this medical hazard.

Epidemiology: The complex structure of ear help in hearing as well as also maintain equilibrium. Ear problem cab of many types in different age groups of patients. One of the inflammatory condition; otitis externa (acute/chronic form) affect 4 in 100 people acute form and whereas 3-5% of the population suffer from chronic otitis externa.[10] In Saudi Arabia, one study showed around 49.3% of participated people had ear problems that 37% of elderly patients were having hearing impairment and 9.4%-2.9% of people showed otitis media.[11] A study done by Gorems et al showed,4 yrs age children had acute ear infection whereas 15-44 yrs age group patient showed chronic ear infection.[12] some of the studies[13,14,15,16,17] reported otomycosiswith high incidence in tropic countries.It has been seen that onset of otomycosis infection more in July- Aug months and young manage group patients showed more ear infection.[15,18,19] Some studies[13,14,17,20,21] done by pontes et al. (2009), Fasunla et al. (2008), Barati et al. (2011), Aneja et al. (2010) and Lia et al. (2012) reported female otomycosis in female although the prevalence of otomycosis is less in a female patient. Nazeer et al in 2015 reported high incidence of otomycosis at age range of 21-30 yrs group.[22]

Diagnostic Method: Otoscopy and biomicroscopy are the instruments help in the diagnosis of otomycosis. The otoscope has a light, a magnifying lens, and a funnel-shaped viewing piece, which use to examine the ear canal and eardrum. Biomicroscopy is a procedure of examination of tissues by microscopy. Some symptoms like severe itching, earache, ear discharge, and hearing loss reported by otomycosis patients.[23] In the case of otomycosis, with instillation of coconut oil into the external ear revealed high association (36.59% Nazeer et al[22] in 2015 reported a high association between otomycosis and use of coconut oil inside ear. Coconut oil capable of inhibiting spore germination[24,] therefore, help to keep conidia deposition in-ear and further facilitate the development of otomycosis. Another factor is the use of unsterile pins and sticks. This unhygienic way of cleaning ear leads to fungal infection development in the ear canal and it can also damage the skin which is protected from infection entry as a first defense.[22] Aspergillus fungal infection can be inspected by the presence of conidiophores in case of sporing aspergillus but when fungi at the hyphal stage then may not be picked up at the time of swabbing because hyphae gets embedded ear debris and wax. Thus right diagnosis is a must and lesion should not be overlooked and can prevent unnecessary exposure to antibiotics.[25]

Clinical Presentation: Ear blockage and pain could be the first symptom. This could be due to the accumulation of microbial colonization and blockage that leads to pain. Some other symptoms were also seen like discharge, itching,and otalgia.[26,27,28] Although, these symptoms are by no means specific to fungal infection. Yehia MM[29] et al 1990 suggested that the disease is not highly infectious. Initially mild discomfort pruritis with erythema seen then in moderate stage pain increase with oedematous erythema & seropurulent discharge. In severe condition may cause severe pain with hearing loss due to ear canal obstruction[10]

Etiopathogenesis: There is more chance of colonization of fungi at the outer surface of the ear as this surface having a humid area with suitable ph for microbes.[30] Clinical observation and microbiological testing must know the exact causative organism of infection.It was thought previously that only fungi are responsible for otomycosis but further studies showed an association of bacterial with this infection[23]. Pseudomonas aeruginosa and staphylococcus aureus association has been seen with otomycosis. Osguthorpe et al 2011reported the 10% of association of fungi with primary condition. it has been also observed through otoscopic examination that black fuzzy growth on cerumen may cause slight deafness due to obstruction of the external auditory canal.And among candida species candida albicans is seen to be more associated with otomycosis. Invasive otomycosis is more frequently caused by A. fumigatus than A. niger[1,31].

Treatment: Thus early diagnosis and the right treatment is must to prevent development of further complication. As bacterial infection also produce the same symptoms sometimes so microbial testing is a must before the start of treatment. Suction evacuation and proper cleaning is the first step of treatment and dryness of ear should be maintained.[31] Syringing of the canal should be avoided as it can cause perforation and

facilitate the progression of infection. Antifungal should be advice after antifungal sensitivity test. The topical therapy with antifungal or other antimicrobial agents is also necessary. Effective choices include clotrimazole cream, powder or solution (1%), amphotericin B (3%), flucytosine (10%), econazole cream (1%), thiomersal (Merthiolate) or Cresyl acetate solution. Salicylic acid, griseofulvin, and ketoconazole are less effective. Econazole (1%) solution is very effective in vivo in the treatment of otomycosis within 1-3 weeks (Bassiouny et al, 1986).[32]When topical antifungal not responding then systemic antifungal itraconazole for superficial infection and voriconazole for deeper infection should be prescribed.[33]

Conclusion

This study revealed that, most of the study of otomycosis found that predominant fungal species are Aspergillus and candida which showed great association with otomycosis. Treatment depend on microbial involvement and the general health of the patient. Empirical therapy of treatment should be avoided and should maintain proper self-hygiene to avoid complications.

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The Diagnostic and Therapeutic Application of Laser Exclusively in the Periodontal Field

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Abstract

When laser energy is used as a therapy modality for the prevention, treatment, and management of different types of periodontal disease, it emerged into a very exciting therapeutic tool bringing a huge success with it. Various types of lasers are available today in the modern technology world. Along with the types of lasers, also available the various options to choose the right wavelength according to the treatment modality. Not only is the laser used for root surface procedures, but also the soft tissue manipulation in the field of Periodontology. This makes periodontal treatment by dental laser a grand success as reported in a myriad of literature. The only mandatory condition applied to be applied is the sound knowledge of the clinician in choosing the right type of laser with the required wavelength for a particular periodontal procedure. No other field of dentistry still has seen so many marvelous transformations in the healing and success in the management of the soft tissue after a periodontal procedure. There is a huge application of the usage of Laser in the periodontal world exclusively, which is the goal of this review paper.

Keywords: *Periodontics, lasers, laser uses.*

Introduction

One of the first CO_2 lasers used for oral soft tissue applications was introduced in 1987. The history of laser therapy as applied to the field of Periodontics begin in the early 1960 with the development of the argon, carbon dioxide (CO_2), and neodymium: yttrium-aluminum-garnet (Nd:YAG) lasers. The next major advance in the expansion of laser technology for soft tissue use was the introduction of a contact delivery system for the Nd: YAG laser in 1984. A simple handpiece through which the fiberoptic is threaded, and then passed through a disposable tip allows the energy to be focused at the tissue surface for cutting or ablation or in an unfocused position above the tissue for other uses. This system opens up the door to a wide variety of clinical applications. YAG lasers have been used for osseous surgical and decay removal procedures, whereas the other laser types do not lend themselves to hard tissue applications. Lasers today are becoming smaller and more portable and are being used for an ever-increasing variety of periodontal and soft tissue applications. However, along with the therapeutic property, the dental laser also has the diagnostic property.[1-5]

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Classification of Lasers in Periodontics Based on Their Usage:

1. **Therapeutic uses:** Non-surgical therapy and surgical therapy
2. **Diagnostic uses:** Detection of caries and dental calculus, measuring tooth mobility
3. **Prevention uses:** Antibacterial effect in the dentistry can be achieved through Laser toothbrush. It is achieved by using the low output semiconductor laser of almost 630 nm. Also,a low-level laser therapy toothbrush is been implicated in the treatment of dentin hypersensitivity.[5]
4. **Other application:** Instrument sterilization, Bio-stimulation

Laser and its usage exclusively in the periodontal field:

1. **Scaling:** The Er:YAG, CO_2 laser, and diode laser are highly absorbed in water which takes care of the temperature makes scaling easier exclusive of any carbonization. These lasers are also used for the bacterial reduction, often known as Laser Bacterial Reduction (LBR). With the thin fiber optics, these lasers are used for the sulcular debridement to make the gingival sulcus sterile free from the viable putative microorganisms and thus causes bacterial reduction.[6]
2. **Root planning:** The CO_2 laser is highly absorbed by water is used for root planning to achieve a clean smooth cemental surface for the proper periodontal ligament cells attachment.
3. **Sub Gingival Curettage:** The Nd:YAG and Er:YAG laser lasers are used for subgingival soft-tissue curettage These lasers are set at a particular wavelength suitable for it. CO_2 laser is efficiently used for the granulation tissue removal from the pocket during flap surgery along with the removal from the bony defects like craters. The advantage is these lasers can well differentiate between the healthy gingival and the infected granulation tissue, and thus does not sacrifice the healthy tissue. The patient comfort is maximum here as it a painless and within a much stipulated time.[7]
4. **Cauterisation of highly vascular lesions and hemangiomas:** The Argon laser is used here. It produces coagulation, vaporization, or both and helps in the cauterization of the highly vascular lesions in the oral cavity. The bleeding is minimal and the post-operative healing is very comfortable for the patient. It prevents recurring of certain vascular lesions like pyogenic granuloma.[4]
5. **"Laser pocket thermolysis":** Argon laser is been utilized to keep the line of control of the putative pathogenic microflora like Prevotella and Porphymonas in adjunct with SRP.It is done with the insertion of the tip and the tip is stimulated circumferentially till the base of the pocket, thus infection arising pathogens get carbonized with rapid healing.[4]
6. **Gingivectomy:** The Co2 laser is been used as it is very much effective in removing masses of soft tissue by ablation, as in phenytoin induced hyperplasia and gingival hypertrophy resulting from other inflammatory stimuli. It results in a dry neat bloodless site as the blood vessels get coagulated easilyand chances of bacterial risk also lessen to a great extent. Healing is fast and comfortable for the patient.[6]
7. **Enhance Guided tissue Regeneration:**The Er: YAG, Nd: YAG laser and CO2 laser are used to reduced inflammatory response, thermal necrosis, and a firm scar of the wound margin and less wound contraction which causes a delay in re-epithelialization. Thus the epithelialization growth is stopped and the periodontal cells are guided to grow and occupy the place for tissueregeneration.[1]
8. **Frenectomies:** The Co2 and Nd: YAG laserboth can be used here. When frenectomy is performed via laser ablation,neither sutures nor surgical packing is necessary as the surgical site heals by secondary intention. The laser frenectomy reduces frenal strain and encourages the health of the gingiva.
9. **Biopsy:** The Co2 laser is used only for the laser excisional biopsy to avoidelectrofulguration. As its penetration is poor, hence it is fit to use for the close to crucial anatomical structures.[1]
10. **Anesthetic effect:** The pulse duration of the Nd:YAG laser is 150u.s which is apparently below the level of the neuronal action potential, thus the anesthetic effectis achieved in the indicated diagnosis area.[3]
11. **Esthetic gingival procedures:** The Er: YAG laser is used mostly for this type of treatment. Crown lengthening, to treat gummy smile as it precisely ablates the soft tissues and also wound healing is

fast. Dr. Eshom (2002) popularized a procedure "closed-flap" crown lengthening to trim and shape bone tissue to accomplish biologic width without involving a soft tissue flap.[7,10]

12. **Exposure of unerupted and partially erupted teeth:** The Nd:YAG laser is used to ablate the soft tissues around theunerupted or partially erupted tooth for the orthodontic treatment. To place an orthodontic bracketto pull the unerupted tooth, tissue removal minimally and conservatively is mandatory to access the unerupted tooth. This laser is advantageous in creating a very conservative way of tissue removal, and also the bloodless surgical field is achieved.[8]

13. **Ablation of the hard tissues:** The Argon laser can be utilized as a smooth laser knife, for cases like Coronoplasty, Enameloplasty.[6]

14. **Hypersensitivity:** Dentin hypersensitivity can be treated successfully with the help of the lasers which are of two different categories like: Low-level lasers like GaA1As diode laseracts at the nerve level, thus eliminating sensitivity, and middle-level lasers like Nd: YAG and CO_2 lasers desensitize causing contraction and occlusion of the dentinal tubules.[9]

15. **Laser-Assisted New Attachment Procedure (LANAP):** LANAP, which ingrain free-running pulsed Nd:YAG laser, enhances the intangibleaim of renewal of attachment apparatus giving rise toa true regeneration. LANAP is a carefully intended management procedure which not only aims at bactericidal and detoxification effect but also aims to reduce the source of the infection.[1]

16. **Laser root conditioning or root etching**: The CO2, Nd:YAG, Er:YAG, Diode laser are used here. CO2 lasers are a popularize system to achieve root conditioning with a superior fibroblastic movement, cellular abundance, and larger fibroblast connection and possess a superior caliber for the removal of smear layers.[1]

17. **Depigmentation:** The Er:YAG laser is applied for the elimination of nonstandard gingival mark, like metal tattoos. When the oral tissue was seen to be filled with melanin pigmentation, it readily absorbs the blue-green visible light of the argon laser yielding a thermal effect from the conversion of the energy and thus causing ablation of the melanin pigments.[10]

18. **Osseous surgery:** Er:YAG laser is used for the removal of exostosis accompanied with the saline spray, collateral damage risk is minimized which is seen maximum with the Rotary instruments as they get entangled with the soft tissues during the resection procedure. Also it is preferred by the patient for the comfort, as less noise emitted while bone tissue grinding.[6]

19. **As an analgesic and anti-inflammatory agent:** The " LLLT" or the "Low-Level Laser Therapy"/ Therapeutic Lasers/Low-Intensity Level Laser are the other names whereas the therapy has been referred to as "Biostimulation" and "Biomodulation". These lasers are usually minor, very reasonable and function in the milliwatts ranging from 01-500 MW. The pain signals get blocked by the laser and thereby the sensitivity of the nerve comes down and also the pain perception is decreased significantly.[10,12]

20. **Accelerated tissue repair and cell growth:** The Nd:YAG laser generatesphoton that gets deeply penetrated inside the tissue and thereby its stimulation efficiency stimulates the cell center for energy production. This energy created helps the cell nicely to absorb the beneficial nutrients and also guides the cells to let go of the unwanted waste in a little time.[12]

21. **Laser in Implants:**

A. **Laser in ablating the soft tissues around the implant:** The Nd:YAG laser is used to ablate the oft tissues lying around the implant in the second stage for the implant fixture exposure. This laser has got the feature of carbonized tip for the absorption of the laser light radiation turning into thermal energy, which prevents penetration into the tissue deeply. If there is an adequate zone of attached gingiva around the implant and no trephining of bone is necessary, ablation of the overlying soft tissue around the implant can be achieved with little or no bleeding. The lack of hemorrhage, with the resulting clear field, and minimal tissue rebound often allow for the abutment placement and temporization in the same visit. Gingival troughing with the pulsed Nd:YAG laser is aconvenient, painless method of preparing for accurate impressions and is an excellent substitute for retraction cord. There is no blood in the field, and lased circumferential tissues heal quickly and painlessly.[7]

B. **Laser in Implant Maintenance:** The implication

of the Nd:YAG laser resulted in surface melting, loss of porosity, and other alteration including physical changes in the crystalline structure of the hydroxylapatite coating (Block et al 1992). So the usage of Nd:YAG is contraindicated in these cases. It has been shown that the CO_2 laser can be used for the second stage uncovering procedures in endosseous implants, sculpting tissue around the existing implant, control of mucosal abnormalities, and control of implant soft tissue complications. The CO_2 laser is ideal because its energy is rapidly absorbed by the high water content of mucosa so that the depth of tissue necrosis or vaporization is minute (only 100 – 200 microns). The potential harmful thermal energy is dissipated rapidly and does not reach the metal surface of the implant.[2]

C. **Treatment of Peri-Implantitis:** Every laser available in the market cannot be applied for the implant maintenance, exposing submerged implants, or for the treatment of peri-implantitis. Nd:YAG laser is never appropriate for implant treatment, because it simply ablates the implant surface made of titanium irrespective of yielded energy. On the other hand, a diode laser is preferred here, as it does not intermingle with the titanium surface of the implant.[6]

D. **The LAPIP Protocol:** This is coined by McCarthy as "Laser-Assisted Peri-Implantitis Procedure" as a modification of LANAP which could be applied in contaminated implants. Laser, takes off inflamed pocket tissue, dislocate the biofilms, and purify the root/implant plane. Drop off in inflammation and a laser-induced hemostasis added disinfected the tissue outpouring a tough blood clot to lock the method.[1]

Waterlase System: Waterlase-^{TM} uses hydrokinetic technology, laser-energized water to cut or ablate soft and hard tissues. Laser-powered hydrokinetic system (LPHKS) uses an Er, Cr: YSGG crystal. With this particular wavelength less hemostasis is seen and anesthesia is not typically required. This not only increases patient comfort and acceptance, but also allows performing the procedure in the entire mouth in one sitting. Post-operative discomfort is also minimized. Using the Waterlase, one can carry out all of the subsequent procedures-Frenectomy and frenotomy, gingivectomy, gingivoplasty, curettage, vestibulopathy, operculectomy, gingival troughing for crown impressions, soft tissue crown lengthening, expose unerupted teeth (hard and soft tissue impactions); flap surgery, full-thickness flap; partial-thickness flap, degranulating bone, osseous surgery as osteoplasty and osseous recontouring; osteotomy; osseous crown lengthening; removal of diseased granulation tissue from the bony defects; laser soft tissue curettage of the post-extraction tooth sockets and the periapical area during apical surgery and perforating the bone to access the blood supply.[6]

Conclusion

The regular usage of the laser has become very beneficial and comfortable for both the surgeon as well as for the patient. It seems to be a very flourishing and promising field in the future with the advancement of science and technology. It aids in a much easier way than the conventional treatment protocols in dentistry. There is a great hope of usage of laser in both medical as well as in the dental field ahead of time.

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Desquamative Gingivitis as the Sole Manifestation in Oral Lichen Planus: Report of Case and Brief Literature Review

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Abstract

The Mouth is the mirror of the human body that reflects systemic disorders, and various systemic pathologies find expression in the oral region. Oral manifestations may be the initial feature or the only presenting sign in mucocutaneous diseases. Mucocutaneous disorders share two features in common: An immune-mediated pathogenesis and possibly common clinical appearance termed as 'Desquamative gingivitis'. Desquamative gingivitis is mostly a manifestation of a wide array of vesiculobullous disorders; however, it may also result due to endocrine disturbances, chronic infections, and allergic/chemical reactions. Hereby, presenting a case of oral lichen planus with Desquamative gingivitis as the sole manifestation in a 35-year-old female patient.

Keywords: *Desquamative Gingivitis, Oral Lichen Planus, Wickham's striae.*

Introduction

Tomes and Tomes (1894) were the first to describe chronic desquamative gingivitis. However, the term 'chronic diffuse desquamate gingivitis' was coined by Prinz in 1932 for chronic diffuse inflammation cases.[1] Immune-mediated oral mucosal disorders frequently manifest as desquamative gingivitis (DG).[2]Chronic desquamative gingivitis is a clinical condition typified by the intense desquamation, erythema, ulceration and erosion of the marginal and attached gingiva, and blistering. The term "desquamation" originates from the Latin word 'Desquamare', meaning scraping fish flakes. Desquamation refers to the 'erosion of epithelial tissues, skin peeling, and exfoliation.[3] Earlier, Desquamative Gingivitis was regarded as a pathological entity of obscure etiology, possibly precipitated by the deficiency of sex hormones in postmenopausal females.[4] McCarthy et al., in 1960, suggested that desquamative gingivitis is not a conclusivelesion but a gingival response including vesiculobullous disorders and allergic reaction to chemicals/allergens. So only desquamative gingivitis suggests a specific clinical manifestation and is not a definite diagnosis.[5] Nisengard and Levineconsidered the following criteria(s) for a clinical diagnosis of DG: (i) Gingival erythema doesnot result from plaque, (b) gingival desquamation, (c) other intraoral and sometimes extraoral lesions, and (d) complaint of pain in the mouth, mainly due to spicy foods.[6]

Case Report: A female patient aged 46yrs reported with a complaint of burning sensations in the mouth mainly in the maxillary and mandibular gums for 6 months. History reveals that 6 months back the patient experienced burning sensations in the maxillary

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gingiva, while taking spicy foods. Personal history reveals that the patient has attained menopause a few months back. Her medical history was unremarkable. Did not havea history of vesicle formation in the oral cavity. General physical examination did not reveal any cutaneous, ocular, and genital lesions. On clinical examination, there was a area of desquamation involving interdental, marginal and attached gingiva in relation to 14,15,16,24,25,26,34,35,36 & 44,45,46 region (Fig. 1 & 2). The desquamated area was surrounded by slender, faint white radiating striations (Wickham's striae). There was a loss of stippling in the desquamated lesion. The area showed bleeding on probing. palpationin the region did not give rise toa new lesion (Negative Nikolsky's sign). Taking into account chronic history, characteristic clinical presentation (desquamative gingivitis bordered by Wickham's striae, absence of vesicle formation, and negative Nikolsky's sign), a provisional diagnosis of desquamative gingivitis secondary to Erosive Lichen planus was given. An incisional biopsy was performed in the maxillary right posterior region in gingiva. Histopathology revealed acanthotic epithelium with a band like lymphocytic infiltrates and saw tooth rete pegs(Fig. 3).Fibrinogen bands in the basement membrane zone were observed on direct immunofluorescence study (Fig. 4). The histopathological and immunofluorescent confirmed the diagnosis as desquamative gingivitis secondary to erosive Lichen planus. After scaling, the patient was prescribed corticosteroids (Turbocort paste) to apply 3-4 times daily for 15 days and vitamin C chewable tablet (tab Celine BD x 15 days). However, the patient didn't turn up for follow up.

Discussion

The oral physician should be familiar with the varied clinical manifestations of desquamative gingivitis and should be able to formulate a precise differential diagnosis. The pathogenesis of Desquamative gingivitis associated disorders usually falls in 2 major categories- a) Cell-mediated (e.g. Oral Lichen Planus, and lupus erythematosus), b) auto-antibody-mediated (e.g. Pemphigus Vulgaris, and Mucous membrane pemphigoid). Pemphigus Vulgaris shows hyperkeratosis, while Mucous membrane pemphigoid shows blistering.[7]

According to Glickman and Smulow, desquamative gingivitis represents a clinical feature common to a wide array of disorders. A classification was formulated taking into account the etiologic factors, together with histologic and immunologic features.

A. Cutaneous disorders

- Contact stomatitis
- Psoriasis
- Lichen planus
- Cicatricial pemphigoid
- Pemphigus
- Bullous pemphigoid
- Epidermolysis bullosa acquisita

B. Disturbances of Endocrine

- Hormonal imbalance in postmenopausal females and following oophorectomy
- Testosterone imbalance
- Hypothyroidism

C. Response toAge

D. Exaggerated gingival response to biofilms

E. Idiopathic

F. Chronic infections

- Histoplasmosis
- Chronic candidiasis
- Tuberculosis

The term lichen planus (LP) is coined from the Greekword lichen meaning; tree moss and planus is a Latin word meaning; flat. The first person to describe the condition was Erasmus Wilson (1869)[9] Lichen planus (LP) is a chronic inflammatory, immunological disorder that affects the skin, nails, hair, and mucous membranes. Cutaneous lichen planus (CLP) lesions present with characteristic 6 "Ps"- "Purple, Polygonal, Planar, Pruritic, Papules, and Plaques", and frequently affect the flexor surfaces of the extremities.[10] Cutaneous LP lesions are recurrent, not contagious, exhibit self remission, and sometimes cause pruritis.[11]The oral lesions are recalcitrant to therapy, rarely undergo self remission, exhibit chronicity, and present varied clinical manifestations. The precise etiology of the OLP lesions could not be delineated. Lichen planus, an auto-immune disorder, is mediated by CD 8 + T cells, macrophages, and Langerhan's cells. Immune mechanisms trigger apoptosis resulting in cell destruction and the appearance of characteristic histological changes.[13]

OLP is frequently associated with psychosomatic

ailments (stress, anxiety, and depression). There exists ever al endogenous or exogenous predisposing factors for OLP. Exogenous factors include drugs (antihypertensive drugs & NSAIDs), amalgam restoration, infections (HCV & H. pylori), and food allergies. Endogenous factors include genetic factors and autoimmunity.[14]

The disease affects 0.5%-2% of the general population, with a female predilection between 30-70 years of age.[14] However, published literature has reported the infrequent occurrence of OLP in the pediatric population.[15] Our patient was a 46-year-old female who reported with burning sensations in the maxillary gingival region. The patient has attained menopause last year and was under depression.

The oral lesions have been classified into 6 types-reticular (most common form), erosive, papular,plaque-like, atrophic, and bullous (least common form).[15]The majority of the lesions are bilaterally symmetrical. The Buccal mucosa is the most frequently affected site, although, lesions are also seen on the dorsum and lateral aspect of tongue, vestibule, lip, and gingivae.[16] One of the characteristic clinical manifestations of OLP is the presence of Wickham's striae. This was named after Louis Frédéric Wickham who described the clinical appearance of OLP as the presence of greyish striae and dots.[17]

About 10% of OLP patients present with the disease localized to the gingiva, is termed as desquamative gingivitis (DG). DG can present as reticular, erosive, or atrophic subtypes. DG lesions may be aggravated during periods of psychological stress, anxiety, mechanical trauma (Koebner's phenomena), and chronic low-grade irritation.[18] OLP with isolated desquamative gingivitis may be characterized clinically by the presence of erythematous lesions (atrophic LP), ulcerations (erosive and/or ulcerated LP) or vesiculobullous lesions (bullous LP). OLP generally has a typical clinical presentation and distribution, but the atrophic and erosive forms exhibit diagnostic challenge.[19]

Our patient presented with diffuse desquamative lesions in the maxillary and mandibular posterior gingival region. The desquamated lesion was bordered by faint grayish-white striae on the periphery.A detailed anamnesis, thorough physical examination, and histopathology are the keystone for diagnosis. However, clinical diagnosis usually sufficesin classical lesions (Wickham's striae, erythematous area).[20] The characteristic clinical manifestations usually encompass bilaterally symmetrical reticular lesions. The essential histopathological features are liquefaction degeneration of the epithelial basal layer, hyperkeratosis with ortho and/or parakeratosis, band-like lymphocytic infiltrate in the connective tissue area, and sawtooth rete pegs.[12] In the present case, histopathological and Immunofluorescence findings were consistent with OLP.

Published literature has established an ODP association with multiple systemic comorbid conditions like as diabetes, hypertension, metabolic syndrome, thyroid disorder, psychosomatic diseases, renal disease, gastrointestinal disorder, and genetic predisposition to cancer. These systemic symptoms shows that OLP should be considered as a systemic disorder. All Doctors should work with primary healthcare physicians to rule out causative factors for the associated comorbidities.[22]

There exists no definite management protocol for OLP. Patient education and motivation to maintain meticulous oralhygiene is mandatory. Therapy primarily focuses on minimizing the duration and condition of symptomsincreases. Reticular and plaque formsthat are not symptomatic do not require pharmacologic intervention, the patient is advised to follow up.[23] Various medications are prescribed to check OLP which includes steroids (topical, intralesional, and systemic), retinoids, immunosuppressive, and immunomodulators. These treatment protocols can be used to control as there are variations in patient responses.[24] The patient was prescribed low potency topical steroids (Turbocort) to apply topically and chewable vitamin C tablets.



Figure 1: Desquamative gingivitis with Wickham Striae



Figure 2: Desquamative gingivitis with Wickham Striae

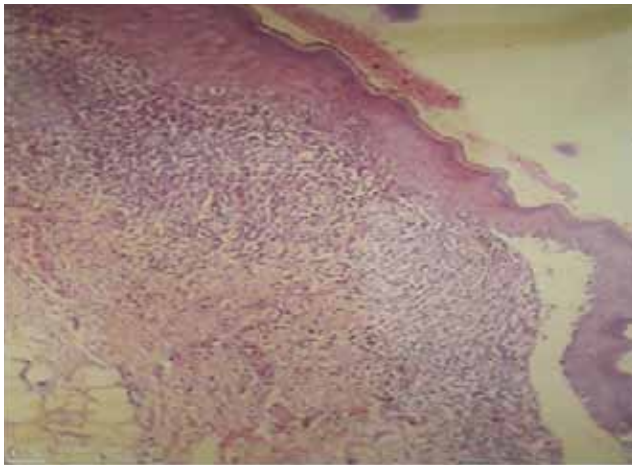


Figure 3: Acanthotic epithelium with basal cell degeneration and saw tooth rete pegs

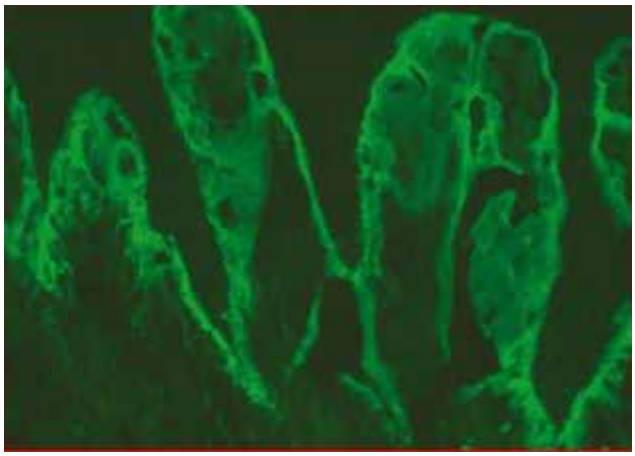


Figure 4: Shaggy bands of fibrinogen on basement membrane

Conclusion

Desquamative gingivitis per se is not a disease entity, but, a manifestation of a wide array of disorders. Gingival desquamation may present as the only manifestation in some cases; while it may be associated with other oral and extraoral manifestations, like Wickham's striae in lichen planus, Nikolsky's sign in pemphigus, ocular involvement in mucous membrane pemphigoid which helps in diagnosis. Cases where gingival desquamation is the only presenting feature; care should be taken for proper diagnosis as they may mimic plaque-induced gingivitis. Thus familiarization of various clinical presentation of gingival desquamation is essential for early diagnosis, treatment planning, and combating potential complications.

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Cone Beam Computed Tomography Analysis of Three Method of Intracanal Calcium Hydroxide Placement

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Abstract

Aim: To compare and evaluate the quality of three different method of intracanal placement of calcium hydroxide paste through CBCT.

Methodology: Thirty single-rooted mandibular premolars were decoronated and cleaning and shaping was done using Protaper Rotary Files(till F4). The canals were divided into the following three groups which were filled with a radiopaque calcium hydroxide paste based on three insertion method (n = 10, each): using a Master Gutta-percha cone (MGP), Pastinject (PIT), and a technique combining conventional hand files with ultrasonic activation(UST). The quality of fillings was assessed using CBCT by examining the number of canal walls coated dividing the root into thirds. Statistical analysis was done using the Chi-square test of independence.

Results: The canals filled with combined technique (hand files with ultrasonic activation) had a better coating of canal walls followed by Master GP cone and Pastinject, with significant differences between them.

Conclusion: From the images obtained through CBCT analysis, calcium hydroxide placed utilizing hand files with ultrasonic activation (UST) produced better coating of canal walls over Master GP and Pastinject techniques.

Keywords: *Calcium Hydroxide; Intracanal medicament; Placement method.*

Introduction

The reduction or elimination of bacteria is one of the important objectives adding to the success of an endodontic treatment. Cleaning and shaping or chemomechanical preparation of the root canal is a vital phase of endodontic intervention and it has been found even after cleaning and shaping, bacteria may still be viable.[1,2] Therefore, intracanal medication may be a valuable adjunct to such cleaning and shaping procedures in the disinfection of the root canal system, reducing the endodontic microbiota.[3-8] Calcium hydroxide [$Ca(OH)_2$] is acknowledged as the most effective intra-canal medicaments used in endodontic intervention due to its bactericidal properties.[9] It has been effective in killing microorganisms through direct contact and hence advised to be close to the appropriate tissues as well as to be used with maximal density and depth till the working length of the root canal to permit its biological effects.[10] Several method have been employed for intra-canal $Ca(OH)_2$ placement, however, none of the studies have assessed method of placement three-dimensionally.[11-20] Hence, this study aimed to compare and evaluate the quality of three different method of intracanal placement of calcium hydroxide paste through CBCT.

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Materials and Method

Thirty single-rooted mandibular premolars extracted for orthodontic reasons were selected. The teeth were examined for fracture, cracks, or any other defect after which they were stored in a normal saline solution that was changed daily. Samples were radiographically examined for the presence of a single canal. The crowns were decoronated at 15 mm from the apex to standardize the root length. The working length was determined by subtracting 1mm from the length at which #10 K file tip extruded apically. Cleaning and shaping of the root canals were done with Protaper Rotary Files (Dentsply Maillefer, Ballaigues, Switzerland) using the crown-down technique up to size #40(F4). During the preparation, the root canal was irrigated with 2 ml of 5.25% Sodium Hypochlorite solution after each instrumentation and the final rinse was carried out using 5 ml of 17% ethylenediaminetetraacetic acid (EDTA) for 1 min followed by a final flush of saline. After cleaning and shaping, the canals were dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland). Three U-shaped wax rims were prepared and ten teeth were randomly embedded in each rim in such a way that there was 3 mm of modeling wax all around the teeth to simulate the soft tissue around teeth and increase image contrast. All the teeth were numbered from 1 to 30. In this way, the position of each sample remained constant.

The teeth were grouped based on three insertion techniques of a ready-mix paste of Calcium hydroxide (AvueCal, Dental avenue) which was dispensed on a glass slab.

- Group 1, Master apical GP (MGP, n=10): A Protaper Universal GP cone F4 (DentsplyMaillefer) coated with $Ca(OH)_2$paste was inserted into the canal till the apex and removed in a counterclockwise direction. This process was repeated three times for each tooth.
- Group 2, Pastinject (PIT, n=10): A Pastinject #35 paste carrier (MicroMega) coated with $Ca(OH)_2$ paste was inserted into the working length of the canal 2mm short from the apex, rotating at 500 rpm on a contra-angled handpiece, then withdrawn in a continuous movement while maintaining contact with the surface of the canal wall. Slight pumping motion was applied. This process was repeated thrice for each tooth.
- Group 3, Conventional hand files with ultrasonic activation (UST, n=10): A #40 hand K-file coated with $Ca(OH)_2$paste was inserted into the canal up to the root apex and removed with counterclockwise movement, after which the ultrasonic file was layered with the same paste and inserted into the canals, 2 mm short of the working length and activated at full power mode(with the water off), then withdrawn through the use of short, vertical pumping movements while maintaining contact with the canal wall. The process was repeated thrice for each tooth.

Outcome Assessment: Post $Ca(OH)_2$ placement CBCT imaging was done for all the samples, and the assessment of root canal filling of each specimen was estimated by two examiners using NNT viewer software version 7.2 by NewTom Cone beam 3D imaging in MPR view. The assessment of paste fillings was executed based on the number of canal walls (buccal, lingual, mesial and distal) coated dividing the root into thirds (cervical, middle and apical). Scoring was given as 0,1,2,3 and 4 depending on the number of walls coated in each third. Statistical analysis was done using the Chi-square test of independence for studying the association between Method/technique (Master GP, Pastinject & Conventional Hand file plus Ultrasonic Activation) and the number of walls coated. IBM SPSS Statistics 24, SPSS South Asia Pvt. Ltd. was used for data analysis.

Results

The association of wall coating with the type of technique used for the placement of Ca $(OH)_2$ in the coronal third is presented in Table 1 and the analysis did not find any significant difference (p=0.329) as a maximum number of the walls were coated with the paste in the coronal third with all the three techniques. However, analysis of the middle third revealed significant association of wall coating with the type of technique used (p=0.001) showing the highest number of wall coating with Conventional Hand file plus Ultrasonic Activation (UST) followed by Master GP (MGP) and the least with Pastinect (PIT) as presented in Table 2. Analysis of the Apical third showed a significant association of wall coating with the type of technique (p=0.014) with the UST technique having the highest number of wall coating followed by Master GP (Table 3).

Table 1. Association of wall coating with Type of technique for Coronal third

Coronal	Groups								χ^2, p
	Master GP Cone		Pastinject		Ultrasonic		Total		
	No.	%	No.	%	No.	%	No.	%	
No Wall Coated	0	0	0	0	0	0	0	0	$\chi^2 = 2.222$ p = 0.329
One Wall Coated	0	0	0	0	0	0	0	0	
Two Wall Coated	0	0	0	0	0	0	0	0	
Three Wall Coated	2	20	0	0	1	10	3	10	
Four Wall Coated	8	80	10	100	9	90	27	90	
Total	**10**	**100**	**10**	**100**	**10**	**100**	**30**	**100**	

Table 2. Association of wall coating with Type of technique for Middle third

Middle	Groups								χ^2, p
	Master GP Cone		Pastinject		Ultrasonic		Total		
	No.	%	No.	%	No.	%	No.	%	
No Wall Coated	1	10	8	80	0	0	9	30	$\chi^2 = 27.103$ p = 0.001
One Wall Coated	0	0	2	20	0	0	2	6.7	
Two Wall Coated	3	30	0	0	2	20	5	16.7	
Three Wall Coated	5	50	0	0	6	60	11	36.7	
Four Wall Coated	1	10	0	0	2	20	3	10	
Total	**10**	**100**	**10**	**100**	**10**	**100**	**30**	**100**	

Table 3. Association of wall coating with Type of technique for Apical third

Apical	Groups								χ^2, p
	Master GP Cone		Pastinject		Ultrasonic		Total		
	No.	%	No.	%	No.	%	No.	%	
No Wall Coated	6	60	10	100	2	20	18	60	$\chi^2 = 16.00$ p = 0.014
One Wall Coated	0	0	0	0	0	0	0	0	
Two Wall Coated	0	0	0	0	1	10	1	3.3	
Three Wall Coated	0	0	0	0	2	20	2	6.7	
Four Wall Coated	4	40	0	0	5	50	9	30	
Total	**10**	**100**	**10**	**100**	**10**	**100**	**30**	**100**	

Discussion

Calcium hydroxide is the most common inter-appointment dressing used as an adjunct to root canal disinfection after cleaning and shaping, especially in teeth with periapical lesions, and at least 1 week of its intracanal placement is recommended to reduce the number of residual bacterial load.[15] There are various therapeutic effects of a $Ca(OH)_2$ such as inhibition of osteoclastic activity, degradation of bacterial lipopolysaccharides, dissolving of soft tissues and apexification, etc. Even though the use of $Ca(OH)_2$ has now subsided with the use of other calcium silicate-based materials, it is still considered as the gold standard.

The maximum biological effects of $Ca(OH)_2$ is achieved when it is filled with the highest density along the entire canal working length which depends on the method in which calcium hydroxide is delivered into the root canal.[16,17] Various techniques have been employed

for intra-canal $Ca(OH)_2$ placement and it has been found that Lentulo-spiral provided better coating of canal walls.[14,15] However, results from another study showed Pastinject to be better than Lentulo-spiral.[21] Hence, in the present study Pastinject was used which gave the least result in coating the canal walls. This result contradicted the results of a study done by Deveaux et al. where Pastinject provided better root canal filling when compared with four other techniques which also included Lentulo-spiral and ultrasonic file.[11]

The use of ultrasonic files in the present study was derived from a study by La et al., where ultrasonic files were used for the placement of root canal sealers. However, the authors observed the ultrasonic vibrations to disrupt the sealer.[22] In the present study, a combined technique (UST) of K-file combined with Ultrasonic activation was employed which was found to be superior compared to the other two method, especially in coating the apical third of the root canal. This can be due to the ultrasonic vibration which enhanced the peripheral displacement of the medicament, even into the apical third, after the canal was adequately filled by manual K-file.

The use of Master Gutta-percha cone (MGP), which in the present study was found to be better after UST technique, was derived based on the results obtained from a study in which Master GP produced better coating of root canal sealer when compared to rotary and manual lentulo-spiral which was due to its better fitting to the prepared root canal as its size coincides with the last rotary file used during chemo-mechanical preparation.[23]

Calcium hydroxide that is oil-based although possessing an advantage of prolonged duration of action of the medicament also holds the disadvantage of difficulty in removing from the root canal. It has been found that residual calcium hydroxide on the canal walls obstruct sealer penetration into the dentinal tubules thereby affecting the bonding of resin sealer adhesion to dentin which markedly increases the chances of apical leakage of a root canal treated teeth.[24] Hence, in the present study a water-soluble Calcium hydroxide paste was used as it can be easily removed when compared with oil-based preparation.

A novel nondestructive technology in the dental-maxillofacial imaging, which has added to the ease in diagnosis and treatment plan, is the use of Cone Beam Computed Tomography (CBCT). Along with the 3D interpretation, it has various other advantages some of which are: lower radiation dose, shorter acquisition time, less space requirement, interactive display modes, etc.[25] Although CBCT has been used for the analysis of different techniques for calcium hydroxide removal, there is no literature available regarding its usage for intracanal placement of calcium hydroxide. Hence, CBCT imaging was done for the specimens in the present study.

The investigation done in the present study was limited to root canals with non-complicated anatomy. Hence, further study may be carried out in root canals with complicated internal anatomy along with the use of different types of vehicles and consistencies to ascertain the effectiveness of the technique in a clinical scenario, especially in the tooth with periapical lesion along with the use of CBCT.

Conclusion

Considering the limitations and assessing the images obtained through CBCT analysis, it can be stated that Calcium hydroxide paste placed using hand files with ultrasonic activation (UST) produced better coating of canal walls over Master GP and Pastinject techniques in single-rooted teeth.

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Role of Stem Cells in Dentistry: A Review

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Abstract

The current strategy of dental treatment is based on 3 elements- restore, retain and revitalize. Replacing the lost or diseased tissue with the same natural tissue is a challenge in the field of dentistry. Keeping this in mind, the use of stem cells has emerged as a potential way for its ideal replacement. Stem cells are undifferentiated cells, which can differentiate or multiply into particular types of cells and tissues. Besides stem cells, scaffolds and growth factor abandoned their role in the field of regeneration. Stem cells can be easily isolated from the orofacial region and have the property to differentiate into osteogenic, odontogenic, adipogenic, neurogenic components. This review article will discuss both embryonic and adult stem cells, sources, and their role in the process of regeneration along with tissue engineering strategy.

Keyword: *Stem Cells, Sources of Stem Cells, Tissue Engineering.*

Introductions

Stems cells are special and multitalented cell types that can divide indefinitely and have the capacity of self-replication to give rise to a peculiar cell type.[1] Research of Stem cell is a very challenging topic in the era of modern biomedical investigations and stands steadily at the core of "Regenerative medicine". Substantial use of stem cells in various fields for the process of tissue regeneration and/or repair has gained tremendous interest.It can radically change the treatment of some diseases like Parkinson's disease, Alzheimer's disease,lymphoma, leukemia, brain and spinal cord injuries, teeth missing, blindness and vision impairment, hearing loss, heart failures and even paralysis.[2,3] The investigators isolated a class of pluripotent stem cells from a human embryo in the year 1998 and grow them in culture.[1] The emerging problem faced by medical and dental practitioners is organ failure or tissue loss. Various prostheses or mechanical devices have been used to replace the original tissues but none of them restore the original form and function of the damaged or loosed tissues. Hence, the integrity of the host tissue is not properly maintained. Additionally, these prostheses can induce inflammatory reactions in the host tissue and can undergo wear upon prolonged use as well.[4] Stem cell development and its implementation in these cases dramatically reduced the problems as it restores the normal form and function of the original tissue.

During recent years stem cell research is being continued for the development of novel regenerative therapies in dentistry. This article is focussing on the basics of stem cells and their possible role in dentistry. Unspecialized cells found in all multicellular organisms, which under appropriate signal can generate cells with different and more restricted properties, are called a stem cell stem cell or progenitor cells. All the stem cells, regardless of their source, have three general properties: prolonged self-renewal capacity; they are unspecialized; asymmetric replication to become one of the many different types of cells on receiving the appropriate signals from their environments. Signals can be in the

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form of chemicals, e.g- growth factors, physical like the contact of cell with the adjacent cells, and contact with molecules in the environment.

Different sources of stem cells:

Sources can be either of the following:

1. Embryonic stem cells
2. Adult stem cells

Apart from these, stem cells can also be harvested from other sources like fetal stem cells, Umbilical cord stem cells, placental derived stem cells.

Embryonic stem cells (ESCs): ESCs- as the name suggests these stem cells are derived from the embryos, 2-11 days old (blastocytes).[5] These blastocytes contain 50-150 cells and differentiate into 3 germ layers:- endoderm, mesoderm, and ectoderm.[6] ESCs can grow in the laboratory and later used in research purposes. However, the inner cell layer of a fertilized embryo has been used for the cell culture. Legal or ethical consideration is required for this type of culture. Another variety of embryonic cells is Embryonic germ cells (EGCs). EGCs are pluripotent stem cells, originates from the primordial germ cells, give rise to gametes, and have the capacity to differentiate into all the three germ layers(4). Nevertheless, major drawbacks of this ESCs are they have the potential to induce tumorigenesis and immune rejection.[7,8]

Adult stem cells: Adult stem cells are otherwise called as postnatal or somatic stem cells. These cells are easier to isolate and no ethical or legal consideration is needed for this. The main goal of this adult stem cell is to maintain and repair the tissue of the concerned area and this cell founds in a particular area of the tissue called "stem cell niche". As mesenchymal tissues are the resident of most of the adult stem cells, they are togetherly called multipotent mesenchymal stromal cells (MSCs) or mesenchymal stem cells. Originally these were found in the bone marrow cells and are of 2 varieties a) hematopoietic stem cells and b) bone marrow stromal cells.[9] Other than bone marrow they can also be derived from the dental tissues, adipose tissues, skin, and other adult tissues.[10]

Sources of stem cell for tooth development: Epithelial-mesenchymal interactions lead to the formation of the tooth. Epithelial stem cells (EpSC) form ameloblasts, whereas Mesenchymal stem cells (MSC) give rise to osteoblasts, odontoblasts, cementoblasts, and fibroblasts of the periodontal ligament. These stem cells are influenced by chemical transmitters called signals.

Mesenchymal Stem Cells: For tissue engineering following are the mesenchymal progenitors that have been assessed.

Stem cells from human exfoliated deciduous teeth (SHED): Mesenchymal progenitors are mainly isolated from the pulpal connective tissue of deciduous incisors of humans.[11] They possess unique characteristics like self-renewal and multi- differentiation. Along with this, it can stimulate the formation of bone matrix in a lamellar pattern by organizing the host tissue.[12]

Adult dental pulp stem cells (DPSC): Authors have been investigated that adult dental pulp carries precursors can form odontoblast under suitable signals like pulp capping materials.[13] Pericytes found in the pulp can differentiate into both osteoblasts and odontoblasts. It also has the properties of self-renewal and self- differentiation. Another unique characteristic of DPSC is that it can differentiate into pro-osteoblasts even after 2 years of refrigeration, because the surface antigen present remained unchanged and give rise to dental tissue.[14]

Stem cells from apical papilla (SCAP): As the name suggest it is derived from the apical part of the human dental papilla. SCAP has higher proliferative capacity as well as higher efficiency than periodontal ligament stem cells. Along with the above it has an added advantage is that it can be easily isolated from human third molars. For immature stem cells developing dental papilla tissue provides greater source than the developed dental papilla tissues. SCAP has better in vitro proliferation capacity and also has superior regeneration capacity as compared to DPSCs.[15]

Stem cells from the dental follicle (DFSC): It has been observed after *in vivo* implantation of dental follicle cells new periodontal ligament, cementum, and alveolar have been formed.[16] Usually DFSCs are obtained from extracted 3rd molar at an early age.

Periodontal ligament stem cells (PDLSC): Progenitors present in the periodontal ligament have the property of self-renewal and can produce other tissues particularly cementum, periodontal ligament, and alveolar bone.[17] PDLSCs can also be obtained from the extracted tooth. The use of nitric oxide along with the proliferating PDLSC could be a challenging approach

for the management of periodontal diseases.[18] Authors have investigated the formation of PDL and cementum when PDLSCs were allowed to culture with apical tooth germ cells upon transplantation in mice.[19]

Bone marrow-derived mesenchymal stem cells (BMSC): It was observed BMSCs have the capability to produce cementum, periodontal ligament, and alveolar bone when these cells had implanted inside the deformed periodontal tissues. BMSCs have less odontogenic potential than DPSCs indicating all embryonic origin is quite different from each other.[20] They are closely related to bone morphology as well as with the pathology so mainly used in the regeneration process. These are mostly used in cases of regeneration as they have a very close relationship with the bone morphology and pathology. On the other hand it acts like microenvironmental modulators as it strives anti-inflammatory actions soon after transplantation.[21] BMSCs have tremendous effect in cases with osteopenia and bone defects using tissue engineering or cryotherapy.[22]

Tooth germ progenitor cells (TGPCs): TGPCs are spindle-shaped in morphology and have a high proliferation rate, usually isolated during the late bell stage from the dental mesenchymal tissue of 3 rd molar.[23] Unlike dental papilla stem cells TGPCs have odontogenic, osteogenic, neurogenic differentiation capacity.[24] A *in vitro* study showed TGPCs were capable of producing tube-like structures, signifying the confirmation of vascularization.[24]

Salivary gland derived stem cells (SGSCs): These stem cells arelined with epithelial cell, contains exocrine secretionand originates from endoderm. Mainly submandibular gland is selected from the isolation of stem cells as they have high proliferative capability and have the ability of expressing the associated markers.[25]

Epithelial Originated Dental Stem Cells: Epithelial derived dental stem cells are believed to be lost soon after the eruption of the tooth into the oral cavity. Enamel regeneration would be difficult as enamel producing ameloblasts are lost. Stem cell is the alternative approach for the creation of enamel surface. These stem cell types of stem cells can be isolated from-

1. **Epithelial stem cells from developing molars:** Epithelial stem cells usually isolated from the 3rd molar of juvenile or newborn animals.[26]
2. **Epithelial stem cell from rodent incisor:** An EpSC niche,found in the labial cervical loop of rodent incisor is accountable for the enamel matrix formation.[27] Since EpSC require implantation of rodent cells in the human mouth, it cannot be used in humans for treatment purpose.

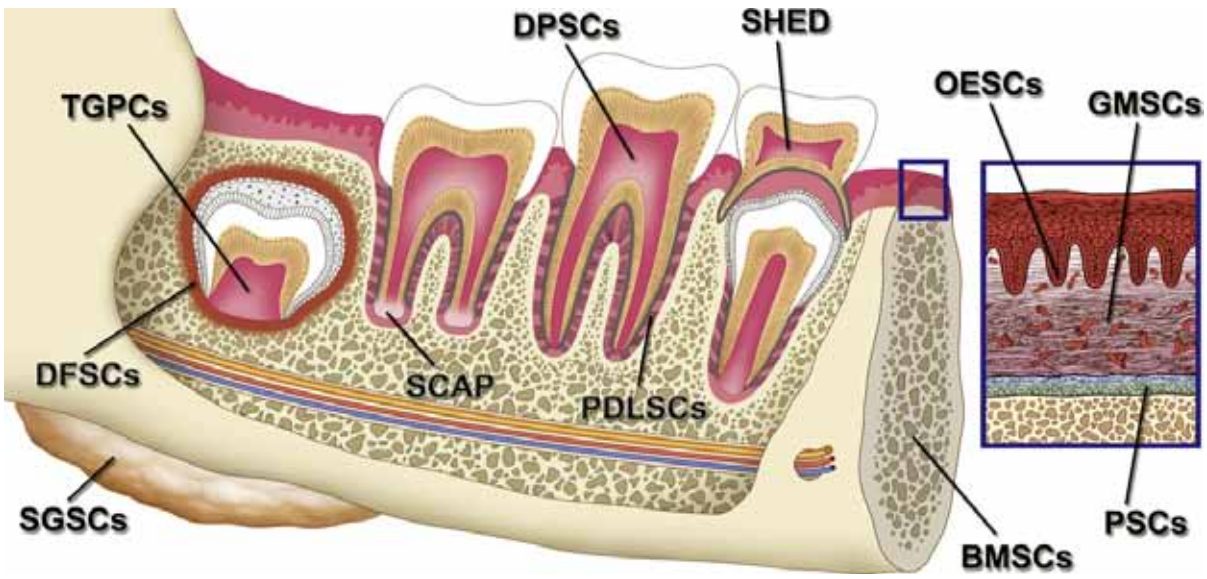


Fig. 1: Sources of adult stem cells:- Tooth germ progenitor cells (TGPCs), Dental follicle stem cells (DFSCs), Salivary gland derived stem cells (SGSCs), Stem cells from apical papilla (SCAP), Dental papilla stem cells (DPSCs), Periodontal ligament stem cells (PDLSCs), Stem cell from human exfoliated deciduous teeth (SHED).

Types of Stem Cell:

Stem cells can be totipotent, pluripotent, or multipotent.

Totipotent (omnipotent) can differentiate into any cell type.

Pluripotent stem cells- it can differentiate into nearly all cells, but not all cell types.

Multipotent stem cells can differentiate only into cells of a closely related family.

Identification of Stem Cells:

4 ways to identify the stem cell:

1. Fluorescent antibody cell sorting:-with the use of flow cytometer stem cells can be identified from clusters of different cells by staining the cell with antibody markers.
2. Immunomagnetic bead selection.
3. Immunohistochemical staining
4. By using physiological and histological criteria- like differentiation, proliferation, mineralizing activity.

Possible Uses of Stem Cell Technology:

Tissue engineering

Repair of defective tissues/organs

Delivery of gene therapies

Delivery of chemotherapeutic agents

Regenerative dentistry: Both mesenchymal and epithelial cells are required for the regeneration process or to laid down odontogenic cells. Mostly Pre natal germ cells are preferred over postnatal for regeneration because they possess high propensity regarding the genesis of normal tooth structure.[28]An injectable self hydrogel (autogenic stem cells) along with inorganic bone is used for temporomandibular joint regeneration.[28,29] Bioengineered TMJ can be constructed by using a biomimetic capsule that contains adult mesenchymal stem cells.[29] For regeneration of young permanent tooth apexification and apexogenesis are the ideal candidates, as they permit root development in both the lateral and vertical directions. Thus, improving the prognosis. A study has been suggested that DPSCs and BMSCs have the potential to induce dentinogenesis and osteogenesis respectively, as a result of the formation of dentin like tissues (30). SCAPs can also produce odontoblasts and the later is responsible for root completion in immature permanent tooth and deposit dentin like structures. When SHED is implanted inside the scaffold, it was able to produce odontoblast like cells.[31] It was observed by *Gotlieb EL et al,* that when SHED was incorporated into the polylactic acid scaffolds containing bone morphogenic protein 2 (BMP-2) and transforming growth factor (TGF-b1), produced structures that are similar to pulp tissue components.[32]

To date, tooth prosthesis has significantly lower characteristics in terms of esthetics and function as compared to natural teeth. Therefore the field of tooth engineering is an upcoming research project which uses stem cells to create a natural tooth by which replacing a missing tooth or missing part of a tooth and its attachment apparatus is facilitated. Proper isolation and culture of stem cells for both in vivo and in vitro conditions play an important role in tooth engineering.

Protocols for tooth engineering: Tooth regeneration is completely dependent on the close association of epithelial and mesenchymal cells along with the interaction of stem cells with the extracellular matrix molecule and scaffold to facilitate tooth engineering (fig-2). Usually scaffold is a crucial supporting structure that forms the framework for cellular function and behavior. Materialused in scaffold should not be toxic, less side effect, highly biocompatible, and should be easy to prepare. Undoubtedly, it might be said that scaffold material could indicate the direction for tissue engineering.[33]

The generation of periodontal tissues is dependent on four basic components. The appropriate signals, cells, body signals, and scaffolds (Fig-3). Collagen matrix type I, hydroxyapatite and tricalcium phosphate has been widely used in scaffold to increase the osteoinductive property.[34] Overall, tissue engineering is dependent on 3 essential elements: cells, scaffold, and growth factors (Fig-4).

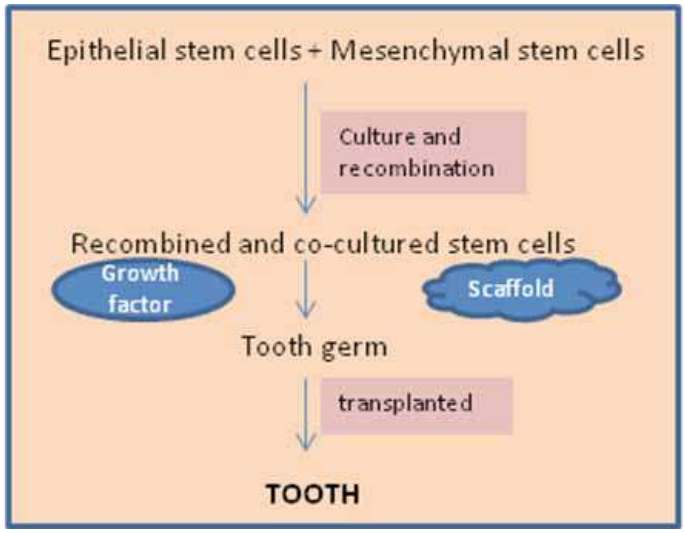


Fig 2: Formations of fully functional tooth after introduction of tooth germ into the alveolar bone.

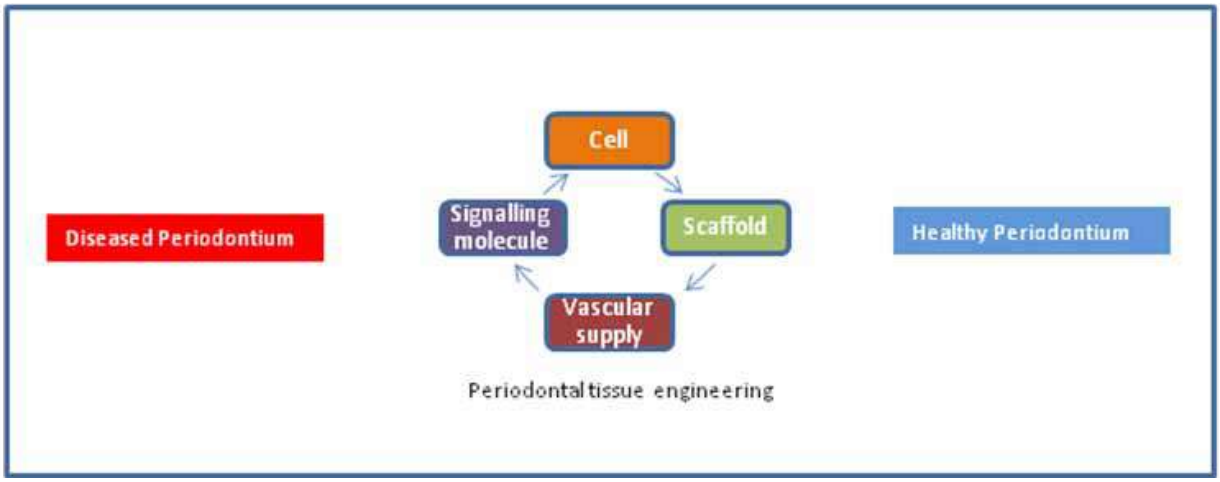


Fig 3: Necessary elements for periodontal tissue engineering

Tissue Engineering Strategies:

1. **Cell injection technique:** This approach involves the injection of talented cells, mainly stem cells inside the defects and it indicates regeneration of tissues over there. Some of the disadvantages of this technique are improper localization of injected cells and poor engraftment. To improve the localization and also to avoid communication with the immune system, the use of a delivery vehicle has been tested. The introduction of a delivery vehicle has been a successful approach as cells found inside the vehicle have the capacity of proliferation and differentiation.[35]

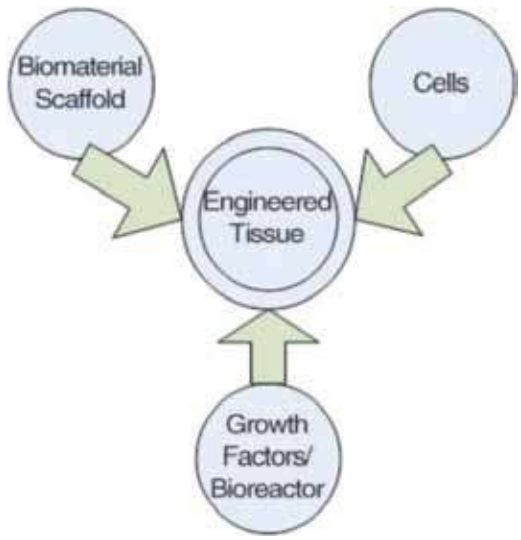


Fig 4: Triad of tissue engineering

2. **Cell induction therapy:** To get the better off from the cell injection technique, this technique was planned to induce osteoinductive property at the site of the requirement by enrolling the circulatory body cells. Any exogenous biological constituents should not be used for the process of osteogenesis.

3. **Cell seeded scaffolds:** This approach involves the isolation of a particular group of cells from a biopsy specimen, which was taken from the donor or recipient. Mostly, mesenchymal cells are preferred for this strategy. Oral mucosal or gingival derived mesenchymal cells are the best choice for this approach due to their immunomodulatory and anti-inflammatory properties. These MSCs can treat various immune-related diseases and help in the repair of wounds. Isolated cells are enlarged in culture medium and then seeded into the synthetic or artificial scaffold (Fig- 5), which holds the mesenchymal cells in position during their growth period and determines the shape of the tissue as well.[36] Here, cells differentiate, proliferate, and finally form the desired tissue. The new tissues formed are then implanted into the subjects.

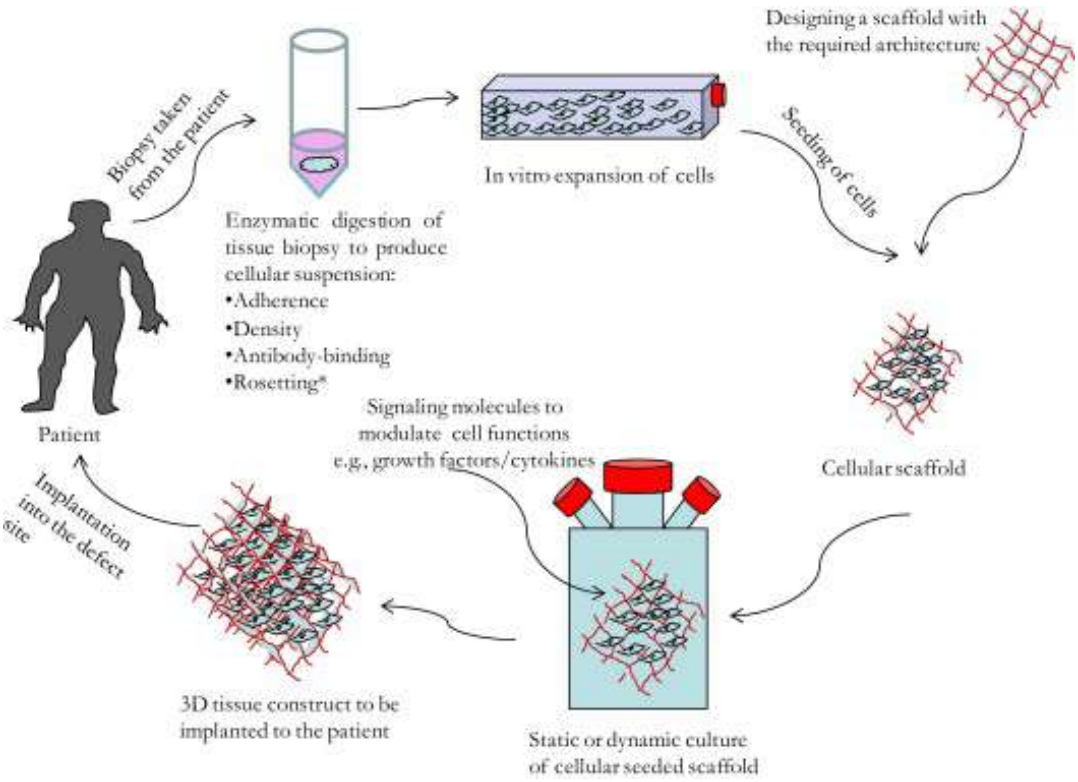


Fig. 5: Schematic presentation of cell-matrix tissue engineering strategy by Tomlinson et al (37).

Conclusion and Challenges

The use of stem cells in regenerative dentistry is capable of bringing quantum advances in treatment for our patients. Apart from some practical obstacles still to be overcome, the stem cell knowledge can be implemented in regenerative dentistry. More advancement in the field of stem cell research should be done upon which we can build future strategies for the tissue engineering of teeth. Stem cells have plenty of application in the field of dentistry, dental professionals must recognize the outcome of the emerging zone of regenerative dentistry and should implement those during conventional dental treatment. To make the nonvital tooth fully functional, researchers need to grow blood and nerve supply to that particular tooth. Stem cell brought many promising hope among the researchers. Shortly, stem cell is the future of dentistry.

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Antimicrobial Resistance: A Dentists' Prospective

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Abstract

Antimicrobial resistance has turned from prediction to reality in a few decades and exerts a significant burden globally with a loss of approximately seventy thousand lives each year. Dentistry accounts for approximately three to eleven percent of all antibiotic prescriptions worldwide. The human oral cavity is a huge reservoir of more than 750 microbial species. Oral microbial biofilm formation such as dental plaque that initiates periodontal diseases and dental caries is the major reason for the antimicrobial therapy failure and rise in resistance against the action of antimicrobials. Biofilm bacteria embedded with a matrix of extracellular polysaccharides are more than 10,000 times less sensitive to antimicrobials than free-floating planktonic bacteria. A thorough understanding of the mechanism will provide an effective solution to combat the current problem such as developing novel antimicrobials, method that enhance the effectiveness of antibiotics by modifying the antibiotic structure or disrupting biofilm, or altering the outer membrane structure of bacterial cell walls to neutralize the resistant enzymes. Use of novel strategies e.g. nano-drug delivery system to facilitate drug entry into the cell; liposome for efficient local delivery of drug of choice in the treatment of caries, periodontitis and also for anesthetic purposes. This paper provides a review of the literature based on the studies on oral biofilm, its formation, and importance in drug resistance, mechanism of drug action and development of its drug resistance and various measures to combat it and the scope of dentists in limiting the progression of antibiotic resistance.

Keywords: *Antimicrobial resistance, Biofilm, Dental caries, periodontitis, Dentistry, Mechanism.*

Introduction

Resistance to antibiotics and adverse events associated with antibiotic use has now become a major threat to global health. It prompts the major challenge for the health providers all over the world and poses a threat to the rising mortality and morbidity. Over the first few decades the increased antibiotic-resistant gene transmission in virtually every species of bacteria for every class of antibiotics available from man and animals has been documented.[1] We are now having a huge list of multidrug-resistant bacteria with either a few or non-responsive antibiotics.[1] The possibility for a bacteria to develop resistance against antibiotics goes back to the 1930s as suggested by Dr. Flemming in his published description "discovery of penicillin".[2] The concept we have understood that "the success of antibiotics in improving morbidity and mortality rate worldwide" has created the present scenario "the resistance developed due to use of these antibiotics is responsible for increased morbidity and mortality". This is due to the microbial ability to withstand the adverse conditions involved and get transformed to protect themselves thereby deactivating the curing

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potential of antibiotics.[3] Antibiotic resistance is now a potential threat to mankind as there is a gradual rise in the number of pathogenic bacteria becoming multidrug resistance or completely resistant to various antibiotics currently available. Antimicrobial resistance has turned from prediction to reality in a few decades and exerts a significant burden globally with a loss of approximately seventy thousand lives each year.[4]

Dentistry accounts for approximately three to eleven percent of all antibiotic prescriptions worldwide.[5] The human oral cavity is a huge reservoir of more than 750 microbial species.[6] Studies suggest that the normal human flora may act as a reservoir for antibiotic-resistant genes which under the right conditions are then able to be transferred to pathogenic species.[7,8] Oral microbial biofilm formation such as dental plaque that initiates periodontal diseases and dental caries is the major reason for the antimicrobial therapy failure and rise in resistance against the action of antimicrobials.[9,10]

Microbial biofilms are formed by colonization of various unicellular organisms to form a community that is attached to any solid surface and adherence of other microorganisms within a polymeric matrix made up of exo-polysaccharide thereby forming a complex community.[11] These biofilms initiate changes within microorganisms thereby exhibiting characteristics different from their planktonic counterparts, their susceptibility to various antibiotic agents, that includes responses against immunological reactions, their interaction with the host tissues, or their physiological properties.[12] Biofilm bacteria embedded with a matrix of extracellular polysaccharides are more than 10,000 times less sensitive to antimicrobials than free-floating planktonic bacteria. Biofilm creates a protected environment for the growth and survival of microbes in a hostile environment.[13]

The oral or dental biofilm that colonizes on the surfaces of the tooth is a complex three-dimensional structure that is formed of around 100-300 cell layers[12] and is a potential reservoir for the oral pathogenic microorganisms.[6,14] A number of gram-negative bacteria involved in periodontal infections such as *P. gingivalis, A. actinomycetemcomitans, F. nucleatum, P. intermedia, B. forsythus, S. intermedius, T. denticola*. These members are responsible for increased periodontal pocket formation as well as tooth loss.[14,15] Biofilms formed by bacteria protect themselves from antimicrobial agents and host immune responses as; (i) It protects against oxidative stress (ii) effluxes out antibiotics (iii) provides protective measures by its polysaccharide matrix and stops easy diffusion of antibiotics.[16,17]

The sequential events that occur during the formation of plaque on dental surfaces are; (1) A pellicle is formed on the tooth surface from proteins and glycoproteins present in saliva. (2) Bacterial molecules are adsorbed by the pellicle formed on the tooth surface through weak physical forces. (3) Reversible addition of oral bacteria to the tooth surface through binding between specific molecules present on the surface of the microbial cell with the complementary receptor present in the formed pellicle over the tooth surface. These constitute the primary colonizers particularly Streptococci sps and Actinomycetes sps. (4) Subsequently other microorganisms colonize to form secondary colonizers by coadhesion with primary colonizers through cell surface adhesion to the receptors present in primary colonizing organisms. This creates a rise in the microbial diversity in the plaque biofilm. (5) Bacterial proliferation takes place by multiplication resulting in the rise of biomass and formation of biofilm matrix by synthesis of exo-polymers. (6) Plaque growth and maturation takes place and all together they behave as a complex organism. There is growth and colonization of anaerobic organisms.

Due to the release of exo-polymers by the colonized microbes the penetration of the antibiotic agents is restricted into the 3-d structured plaque biofilm and also novel properties are exhibited by the slowly growing microorganisms on the biofilm surface.[18,19]

This paper provides a review of the literature based on the studies on oral biofilm, its formation, importance in drug resistance, mechanism of drug action and development of its drug resistance and various measures to combat it and the scope of dentists in limiting the progression of antibiotic resistance.

Mechanism of drug resistance by biofilms: Several groups of antimicrobial therapeutic agents (e.g:- β lactams, metronidazole, tetracyclines, quinolones) are practiced for the treatment of oral infections in addition to dentifrices and oral rinses (chlorhexidine, triclosan, fluoride)

In course of time oral microbiota develop resistance following many different mechanisms such as

1. Production of hydrolytic enzymes to inactivate antibiotics.[11]

2. Effluxing out the antibiotics taken up by the bacteria.[11]
3. Bringing conformational changes in drug targets by causing mutation in genes responsible for.[18,19]
4. Differential expression of porin proteins in biofilm cells reduced the accession of antibiotics resulting in antibiotic resistance in gram-negative bacteria.[20]
5. The formation of biofilm is controlled by quorum sensing of molecules which coordinates the gene expression to regulate the virulence factor.[21]
6. Bacteria present in biofilm adapt themselves to changed physiological conditions in response to exposure to antimicrobial agents and reduce their growth rate.[22]
7. Formation of exopolysaccharide biofilm matrix by bacteria retards the permeability of antibiotics following different mechanisms e.g: electrostatic interaction, absorption, enzymatic neutralization, hydrophobicity.[20,22]



Fig 1: Different ways of development of antimicrobial resistance in biofilms

Mechanism of Antibiotics and Development of resistance: Antibiotics work by affecting things that bacterial cells have but human cells don't. Bacteria and human cells differ in the structure of their cell membranes, method of protein synthesis or DNA replication mechanisms. Some antibiotics affect the cell membrane of the bacterial cells, while some affect the protein building mechanisms, while some act on the DNA copying machinery that is specific to bacteria.

Different families of antibiotics have different ways of killing microbial pathogens.

Beta-Lactam: It generally includes antibiotics like: Penicillins, cephalosporin. Beta-Lactams act on the gram-negative microorganisms by inhibiting the synthesis of bacterial cell walls. They interrupt the links between the peptidoglycan components in the transpeptidation process of the cell wall of gram-negative bacteria. These drugs can bind to PBP (penicillin-binding proteins), the enzymes that are involved in final stages of formation of the cell wall during the growth and division of the organism, and ultimately results in incomplete cell wall formation and cell lysis.[23] Antibiotic resistance developed against beta-lactamase is due to the presence of the enzyme beta-lactamases, present in many gram-negative and some gram-positive organisms that prevent the antibiotics to bind to the specific PBPs.[24]

Tetracycline: It belongs to the broad-spectrum group of antibiotics. It possesses the ability to bind with the 30s ribosomal subunit in the mRNA translational complex resulting in inhibition of protein synthesis.[25,26] It has a wide range of activity against gram-positive and gram-negative bacteria, mycoplasmas (without cell wall), protozoan parasites, chlamydia and rickettsia. Resistance to tetracycline is primarily due to the acquisition of new genes in the bacteria.[27] Due to various side effects, including staining of the teeth, other abdominal discomfort and damage to the kidney, this drug is not in much use.

Metronidazole: It is a nitroimidazole group of antibiotics that can be prescribed in adjunct to other groups of antibiotics or alone to treat oral infections and also other inflammatory diseases.[28] It is most effective against obligate anaerobic microorganism such as *Fusobacterium, Peptostrptococcus* species, *Bacteroides, Prevotela, Clostridium.*[29,30] The nitro group of metronidazole after entering into the cell through diffusion gets reduced thereby releasing toxic products that degrade the microbial DNA and also interferes in the synthesis of new DNA.[31,32] Four genes that codes for nitroimidazole reductase, the enzyme that carries out the enzymatic reduction of nitroimidazole to its respective derivatives are found to confer some level of resistance in colonic Bacteroides species but the presence of such genes in oral species is not clear.[33,34]

Quinolones: These are the chemotherapeutic bactericidal drugs eradicating bacteria by interfering with DNA replication. It acts on the DNA gyrase, a type-II topoisomerase which is essential for the replication of DNA and also recombination following repair.[1] Quinolones bind to DNA gyrase and DNA resulting in the formation of a stable complex that prevents the DNA gyrase for recombination of cut strands of DNA. Resistance to quinolones is found in a variety of anaerobic organisms are either due to alterations in DNA gyrase or for reduction in antibiotic accumulation.[35]

Fluoride: This is an anion of fluorine used extensively as a constituent in mouthwashes, toothpaste and oral supplements as dental caries preventive agents.[36,37]The fluoride ions interact with the mineral content of the tooth and induces remineralization and also prevents demineralization caused by cariogenic bacteria.[38] Studies suggest that fluoride acts on the enzyme enolase in the glycolytic pathway resulting in growth inhibition and reduction in the production of acid from oral streptococci such as *S.mutans*.[39,40] Administration of fluoride is restricted owing to the development of dental and skeletal fluorosis along with fluoride resistance found in oral bacteria.[41]

Chlorhexidine: It is the most effective antiseptic agent, is a cationic poly biguanide with hydrophilic and hydrophobic properties that acts as both supragingival and mucosal plaques[42,43]due to its dicationic nature, it can bind reversibly to the negatively charged molecules in the oral cavity and persist for a long duration after its application.[43] Chlorhexidine is active against a group of organisms including gram-positive and gram-negative bacteria aerobes, facultative anaerobes, and yeasts by causing damage to the inner cytoplasmic membrane.[42,44] Not only it inhibits plaque formation, but it also reduces plaque adhesion to the tooth surface by blocking the acidic groups of glycoproteins that are present in saliva. It brings about ultra-structural alterations within the oral biofilm by being adsorbed on to extracellular polysaccharide or competing with agglutination of calcium ions in plaque resulting in a reduction in the adhesion of bacteria to the tooth surface.[45]

Triclosan: It is a non-ionic phenolic molecule used majorly in dentifrices having a broad-spectrum antimicrobial effect against gram-positive and gram-negative microorganisms.[46] A numerous randomized, controlled clinical trials summarized the efficacy of triclosan as a plaque reducing agent and also exhibits anti-inflammatory properties for improving gingival health.[46-49]

Role of dentists in reducing antibiotic resistance: As health care provider**s,** dentists should be aware of their contribution towards developing antibiotic resistance and limit their role in exacerbating it. Studies suggest that about 66% of the prescribed antibiotics are not clinically indicated.[4] The pathophysiology of the pulpal diseases should be emphasized and understood and inadvertent use of antibiotics in the treatment of acute endodontic infections to relieve pain without intervening endodontic treatment should be avoided. Antibiotics are ineffective in eliminating the pathogens in the root canal system as there is reduced blood circulation in the necrotic or infected pulp. Surgical or endodontic intervention is required to alleviate the symptoms and infection.[50] Antibiotics should be prescribed for localized infection in those with systemic involvement.

Dentists should follow some guidelines[51, 52]

1. Accurate diagnosis should be obtained before prescribing any antibiotics
2. Obtaining the involved microbial analysis presenting the infection and prescribing the suitable antibiotic
3. Rather than adopting broad-spectrum antibiotics one should prefer the narrowest spectrum antibiotic.
4. Avoid prescribing antibiotics if it can be managed without.
5. Should not prescribe for unknown or improperly understood infections
6. Should thoroughly review the medical history of the patient to avoid adverse effects of a specific group of antibiotics if any.
7. Should not be biased by the demand of patient or pressure due to competitive healthcare providers
8. Should be avoided in infections due to fungus or virus or traumatic aphthae ulcers
9. Prescribed when systemic spread of bacterial infection is evident.
10. Should advise patients for proper dosage and duration of prescribed antibiotics.
11. Should update self about appropriate oral infection management from conferences, journals, continuing dental education (CDE) programs.
12. Should discourage and educate the patients not to avail antibiotics over the counter.

Conclusion

The scale and speed of increasing antibiotic resistance has become a global concern where the solution is required at every level. A thorough understanding of the mechanism will provide an effective solution to combat the current problem such as developing novel antimicrobials, method that enhance the effectiveness of antibiotics by modifying the antibiotic structure or disrupting biofilm, or altering the outer membrane structure of bacterial cell walls to neutralize the resistant enzymes. Use of novel strategies e.g. nano-drug delivery system to facilitate drug entry into the cell; liposome for efficient local delivery of drug of choice in the treatment of caries, periodontitis, and also for anesthetic purposes.

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Social Media as a Tool in Dental Public Health

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Abstract

Facebook, WhatsApp, Instagram like social media are becoming an emerging instrument in the twenty-first century to reach every person. Dentists can use any of the social media to give oral care to people. The daily users of the SM are more than one billion per day. If the dentist can make a beautiful blog regarding oral care millions of people will get its benefits. Cost regarding uses of social media in recent days is approximately very less and affordable by maximum people. It will beneficial to all age group people from small children to elderly people. Various Social networks in recent year providing important massage like how we are coordinating each other in developing society and promote our dental health with this media. When SM is used in a proper way it has a lot of beneficiary effect on people regarding dental and general health. The first growth in dental science and its link with recent advance web technology bring the dental science to a top-level.

Keyword: *Social media, dental health, web 2.0.*

Introduction-

Since the last twenty years due to evolution in technology the one way and two-way communication changes out of our imaginary expect. It is due to the easy availability of networks, cell phones, portable devices in every corner of our country. Mainly internet is available everywhere irrespective of geographic location. The rapid evolutionary growth of all aspects like skill to develop knowledge in people, availability of 4g, 5g network, tempo in communication, developing awareness among the community regarding better health has a key role in oral health promotion.[1,2]

In the twenty-first century it became a great challenge for every health educator, that how fast suitably they are representing the health information. Before the twenty-first century, health-related facts are available through television, newspaper some times in audio communication through radio but now due to evolution in technology it is out of the orbit of old media. Health and oral health important information is rapidly available in new advanced social networking sites on Instagram, Facebook, tweeter, google etc. Anyone now can easily assess to any of the following sites and collect important information as they want. Anyone can assess any health website regarding their different gender, socio-economic classes. Nowadays small children around nine to ten-year-old are using I-phone with face time, android phone with google duo and grandparents interchanging photograph via internet.[3,4]

Various Social networks in recent year providing important massage like how we are coordinating each other in developing society and promote our dental health with this media.(5), Recently available community media are FB, Instagram, Twitter, Google, snap chat, tumbler, etc which are accessed by millions of peoples daily throughout the world. The use of social media now became very cost-effective and available in different android phones at less price with large numbers of users.[6,.]

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Social network is a perfect platform from which the goal of the Ottawa chapter can spread to a large population. So nowadays it is very much essential to use social network sites for the publicize of health and oral health tips to maintain excellent quality of dental health. There is a special bond between social network and health in recent days.[7,8]

Different social networks are excellent in spreading news with a thousand base and billions of members. Social network is defined as "a set of network-platform which constructs with Web 2.0 advanced technology, which permits members to make and interchange their text [10]. Social networks are highly popular because of interlinked with their huge friend circle, family members along long distances throughout the whole globe.[11]

Various Social networks like FB, Instagram, Twitter, Watsapp are widely used by various departments for communicating throughout the globe. Social network in its initial days used for interacting with people among the various community but in recent days it is used in various teaching aids.[9]When the first internet was introduced it has very few members for blog generator named web 1.0 but its modified version in recent days is web 2.0 and it has a huge number for blog creation. FB, youtube, Watsapp, tweeter, Wikipedia, Snapchat are coming under web tech.[10].

Social educational platform are twitter, FB, Wikipedia, yahoo, second life, youtube from which we can give the health education to a huge number of population.[11]

Social network and its link to dental health:

Use of social medial in dental health education:

1. Voice messages of every dentist about oral education spread through FB, Whatsapp, Instagram, Twitter, Snapchat, youtube which can educate people in the latest way to maintain excellent oral health.
2. Financial, mental support in every steps any dental disease till recover
3. Users can ask any doubt regarding their dental problems and solve them easily.
4. Social media make people aware that what is the importance of dental professional in the matter of oral-facial health.
5. Various slogan regarded oral health considered as a pillar of oral health, aware people about the inter-link of dental disease to general systemic disease.
6. Various dental surveys are conducted through FB,Watsapp, Instagram, zoomapp, google meet etc.
7. In social media, we can create well designed tailored product which can easily fit for immune-suppressed person, pregnant women, hospitalized patients, old aged patients.
8. Beautiful children oral care programs are launching in the 21st century through FB, Instagram, Twitter, etc.

There is limited social site regarding dental education likeoral cancer site and another for oro systemic health care. There is no proper Indian web page regarding oral health is available till date. Some tips regarding oral health are there in the IDA site but these tips are not much more helpful to people. But in the private sector, there are lots of sites for dental education all of them are business purposes. Private dental educational tips video are available through FB,Whatsapp, Instagram, Snapchat, personal private web pages etc.[12]

Facebook

FB in recent periodsbecame the highest using social interacting medium web around the whole globe except China. Total users of FB are around one billion. Per day millions of people interchange pictures and comments among themselves. When FB introduced to the market, Initially it was only for school students but latter it opens to all. FB provides a personal private operating option to every user. Individual users can make their post private to some users or the public.[1] User can send their request of friendship to any user and who got the request later accept the proposal and can view his/her FB profile. Every FB user profile has details about their Name, DOB, marital status, educational status, location, and many more. All users can update any video, picture massage in their respected profile and can attach those documents to any of their friends who are using FB. Friends can give comments on that status. Personal mail can be sent by FB. Various groups can be created in FB for medical educational purposes.[13]

Twitter: Twitter introduce to the market in the year two thousand six. It permitted its user to put tweets as small messagesless within hundred forty words. Every user of twitter can send small blogs on any topicslike recent breaking news, daily life events, etc. Today twitter is introduced into different fields like medical, engineering, MBA, MCA, Research field, etc. It allows

strong interaction between teachers and students. A Professor named Cole W. from Pennsylvania State University uses 2 display boards one for himself another for training. By this, the trainee can pass their comment, question, doubts and solve it easily.

George et al stated that interaction of twitter became more successful in interaction with writing syllabus for medicos and meaning full strategy for next-generation doctors. Future doctors can interact easily with their patient and among themselves.[14]

Vlogs: vlogs, or video blogs are tape recorders made by one person and its period is very short. Video blog hassome drawbacks because of less validity. Video lectures and Vlogs are not the same things. Blogs should always contain important information and encouragement. The main motive of this blog is to provide important educational tips and easy learning for each mate in the groups.[15]

YouTube: In the year nineteen ninety-seven YouTube introduced to the market. It is the generation of web two point zero Technology.

Anybody can create their channel and post video. Here we can find videos like educational video, street play video, medical lecture, etc.Professor can upload educational video. Any student can share the video. A dental institute of new york university has its youtube channel, students of these universities share dentistry videos in this channel. Most of the universities in the world have their channel. In recent days medical theory lectures are given by youtube. Some surgical procedures of endodontics, prosthodontics, periodontics, and oral surgery are provided in you-tube. Statistical studies of public health also available in youtube.[16]

Simple Syndication Feeds: Simple Syndication feeds are a suitable site to get recent informative data. RSS does not get information from different web it creat its blogs. Simple Syndication Feeds has its web as knowyourteeth.com for dental disease knowledge.[17]

The first motto and aim of social media in promoting oral health should contain true, beneficial perfect health-related data from different reputed profession with much more expertise. Various networking site provide their address including their treatment procedure. Online registration for dental treatment online tobacco counseling, online Consultancy are available in recent days. After significant interpretation of various network site providing different test target is to achieve a better quality of health and excellent oral hygiene.

Disadvantage of social media: Poor quality information- some times the data about health available on social sites is not sufficient or not proper. Many information regarded health present in social media is without authors. Professional image may disrupt- it occurred occasionally when some surgeon post pic of surgery with intact instrument it could harm the patient as well as doctors image. During giving suggestions patients privacy can hamper. Breaking in the patient-doctor bonding. Some time patients send request in SM like FB that interrupted the HCP-patient bond.[18]

When well organized SM crusade some important beautiful massage regarding health or oral health in Instagram or WhatsApp,or FB it became viral with some true evidence. SM is the best way to campaign and make the public aware of their health nowadays. Various health sectors are now linked to SM and campaign through SM for better public health and on the other hand it became beneficial for the public because every day lakhs of people are newly connecting to SM. Recently SM became a suitable tool for spreading awareness regarding health and dental health among people. Some time SM is affected by politics. There is maximum benefit to improve oral health by SM because now every age group people are attached to SM the dentist can make a blog, video about the harmful effect of the use of smokeless and smoke tobacco. When people see this type of video they aware themselves and stay away from this products.[17,18]

Limitation of SM: Each second is important for every HCP (health care professional) so it is not possible to post every aspect of health-related posts on SM. Because posting a massage video is time taking. Secondly payback from the expenditure on SM. Illiterate people can not get benefit from SM. Below the poverty line, people get rid of these facilities. In India language is another limitation. [19,20]

Conclusion

When SM is used in a proper way it has a lot of beneficiary effect on people regarding dental and general health. But mishandling the power of SM has put a negative impact on people as well as on HCP. There is a proper guideline for the dentist to use social media on oral health to get more effective results. The first growth in dental science and its link with recent advance web

technology bring the dental science to a top-level. But in India dentofacial health care facility through SM is at the beginning. Some limitations are there in its acceptability like financial, quality of the data, hampering the privacy of people.[31,32]

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Old Age and its Negative Impact on Oral Health Status

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Abstract

The older group of people (above sixty-five years) are facing lots of health and oral health issues due to the phenomenon of getting old. For that reason, this group needs the most attention to their health. Towards the end of the 20th century, the configuration of various age groups of people transformed tremendously. There are more older people and much more entered into the older group. This dynamic change has a direct effect on oral and systemic health. Dentist has great hand in solving the oral problem in geriatric people. Dentist should always consider the nutrition factor of geriatric patients. Topmost problem regarding the oral care facility in the older population is underestimated the oral disease Proper dentist population ratio throughout our nation to provide better dental care. Regular screen camps in different places to find older patients and gave them special care because due to rapid dental caries, periodontal disease, tooth loss the old people facing various problems.

Keyword: *Oral Disease, Xerostomia, Aging.*

Introduction

There are various developmental stages of Human beings. Development phases are starting from birth (infancy, childhood, young adult, adult, older adult, old aged). All the above phages are biological or natural phenomena. As the human being getting older various changes found inside his body. The longevity of human life is increasing day by day in the twenty-first-century due lunching of modern medicine and different kind of modern public health programs. The older group of people (above sixty-five years)are facing lots of health and oral health issues due to the phenomenon of getting old. For that reason this group need most attention to their health.[1]

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Towards the end of 20nt century,the configuration of various age group of people transformed tremendously. There are more older people and much more entered into an older group. This dynamic change has a direct effect on oral and systemic health. Among these older age group people, the physical condition of some people required attention to their dental health in the dental clinic but not all.[1,2] As per the report of world health organization, the number of the population throughout the globe increasing annually is around two percent and on the other hand the percentage people crossing the age sixty-five, is around three percent. From estimation it is calculated that till 2050 there will maximum changes in age composition in developed and rapidly developing nations. According to UN estimation the older age group reaches around twenty percent from the whole population of the globe.

In 2020 the total population of our India is around 1.3 billion. From 1.3 billion older people above sixty-year age are around eight percent (around eighty million). Various oral cancers like squamous cell carcinoma mostly found in older age group and maximum oral cancer case found in republic India.[3]

As the human being entering to the older age group, he/she may develop some systemic disease. In the presence of various systemic diseases in old age he/she lost his ability to maintain his oral health. The person lost his ability to maintain oralhygiene properly, which direct or indirectly affects the lifestyle of that individual. Though this type of condition is not life-threatening but hamper some part of quality of life. For the proper care should be taken by the dentist to maintain their oral hygiene. Special dental care should mandatory for older group citizen.[3]

Different category of older: Citizen from sixty-five years to seventy-four comes under young older are physically active and healthy Citizen from seventy-five years to eighty-four comes under middle old are little different from physically active and healthy older goes through some systemic disease. The last one is above eighty-five years come under the oldest group and not physically active. This group is very much venerable to most oral diseases. Sudden proliferation in the age of sixty-five years since the last ten years in the world and India comes in that.[4]

Condition of Oral health among the older population:

Impact of various Nutrients among older groups and its utilization in dental care: The availability of a Sufficient amount of nutrients products in the body plays an important role in improving the oral health and general health of the older population. Deficiency in the nutrients inside the body hurts the normal physical and mental activity of older people. Worst dental health may be a crucial point for nutritional status and health. Any disability in the oral cavity has a significant contribution to less eating practice among the old age group. A mobile tooth, mild to moderate pain in the tooth or faulty prosthesis can reduce the ability of chewing habits. Less amount of nutrition harms the dental care of old people. So proper nutrition should mandatory for proper oral care of older people.

Due to a decrease in the process in BMR(basal metabolic rate) the food calorie amount of body also decreases in old generation people, which leads to a decrease in mass of the muscle and physical activity in old people. Decrease in appetite process of the body and less food intake there is a deficiency in the amount ofCa, I, and Zn in women. Around Eight thousand kJ calorie need for eighty years old people. Physical active old need around 1gm/kg protein everyday. Process Necrosis of tissue is more in old people for that they need more protein. Bedridden older patients have less chance to expose to sunlight for a long time so they are more risk for developing osteomalacia due to less SUNSHINE VITAMINE. Vitamin C, iron also required. [5]

Oral health has significant hand genera health in old people. Tooth loss and faulty prosthesis has a direct negative impact on mastication and test sensation. Defective mastication can be corrected by replacement of proper prosthesis. Correction in the mastication by proper prosthesis can change the dietary habit and increase the nutrient amount in the body of old people. Prevalence of Cervical caries and root caries is more in old people. Dentist has great hand in solving the oral problem in geriatric people. Dentist should always consider the nutrition factor of geriatric patients. If there is some nutritional problem dentists have to solve it in the proper method which will be effective on the later stage.

Problems arise due to decrease of salivary secretion in geriatric patients: The normal function of the salivary gland hampered due to aging is a normal phenomenon. It adversely affects dental health. Saliva in the oral cavity act as a safeguard for the upper respiratory tract and upper part of the digestive tract from various infections. It also regulates some sensorimotor fibers. Xerostomia make the oral environment suitable for various micro-organism. Major changes found in major salivary glands due to atrophy of acinar cells due to increasing age. Degenerative alteration also found in various salivary gland-like parotid, submandibular with advanced age. Changes found in palatine and lingual gland also due to old age.[6] Salivary dysfunction is less in old age people who are comparatively physically healthy and active. A salivary gland dysfunction can be reversed to normal function. Xerostomia leads to dental caries in old group population.[6]

Oro mucosal changes due to the aging phenomenon: The mucosa of the oral cavity acts as a first-line defense in the oral cavity. Defect in function of oral mucosa made the oral environment favorable for various pathogenic organisms harvest inside the mouth. All the layers of the oral mucosa have their protective function.Keratin synthesized by oral mucosal epithelial tissue, which maintains oral mucosal. Adipose tissue of oral mucosa mainly defends oral mucosa.[7]Epithelial cell turnover done by oral mucosa in the oral cavity and it

is a protective mechanism. Data from different surveys says that oral mucosa became thin with an increase, which is associated with loss of stippling and elastic nature of mucosa. Some changes marked in tongue like depopulation. An increase in the number of Candida albicanscauses candidiasis or oral thrush. Healing takesa long time.[8]

Various changes of tooth with advanced aged: After full maturation of tooth structure, slowly its changes with advanced age. The demineralization process starts of tooth structure. Due to long term demineralization enamel layer lost totally. Micro changes occur inside the tooth surface. Loss of occlusal surface of the tooth and facial surface of tooth seen in mostly old age group. Looks of the tooth different from newly erupted teeth. Refractive index of light in tooth structure also changes due to increasing age. The normal thickness of tooth dentine also decreases. Various defects found on tooth structure. Tooth pigmentation also found. Due to a lack of proper oral hygiene, the color of teeth change in old patients.[8]

Various ion exchange phenomena take place in the upper surface of the tooth (enamel). Tooth became highly brittle and porous due to increasing age. Restoration is the process by which we can make the prognosis of the tooth good in old age. The structure of pulp is different in old group people than young people.[8] The volume of the pulp chamber decreased due to less pulp cells. Repair capacity of pulp decrease with increasingage. Calcification of pulp chamber occurs, with aging in most of the case. Electronic microscopic images of the pulp chamber of old patients look different from young patients. Thin root canal found in old people. More amount of F and Mg found in old patients. Some biomarkers also found in the cementum of old people. Micro annular ring found in old patients and that helps in forensic investigation. Morphology of tooth changes with increasing age which linked with clinical examination. Restorative treatment has a good impact on tooth structure. Around five to seventy percent of old people throughout the globe have CPI score four. Most study data says that periodontal disease increase with aging. Periodontal disease is not considereda special disease but it started from a young age reaches chronic periodontitis in old age. Periodontal disease is due to the accumulation of sub and supragingival biofilm formation. Breakdown of periodontium creates more oral problems in old ages. Bleeding gum, swelling of gingival found some time discharge of pus found.[9,10]

Need for preventive services: In recent prevalence of oral disease is increasing day by day. Disease like gingivitis, periodontitis, dry-mouth, root caries are increase in the old generation. Most of the old people think that mobility of tooth, tooth loss are natural phenomena and cant be prevented. Many people think that in old age when it emergency then go to dentists for check-up. Few people go for regular dental check-ups. Some important causes that keep most of the old people from getting oral care such as health issues like- Oral health condition, Filling discomfort, long term illness, physical disability, Socio-economic causes, residence places, Education level, amount Income, Age issue, gender, Culture factor, communication facility, doctor behavior, cost of service, self-interest

Conclusion

The topmost problem regarding the oral care facility in the older population is underestimated the oral disease. This underestimate nature towards oral health is found mostly in India. Barrier in getting oral care in India is lack of proper communication, lack of education, language barrier, cultural barrier, belief in various myths regarding oral diseaseetc, Political census etc. Awareness knowledge regarding oral disease may change the attitude of our people to focus their attention on oral health. Home care of elder patients by caretaker can improve the oral health of older people. India should implement a proper program for oral health. Proper dentist population ratio throughout our nation to provide better dental care. Regular screen camps in different places to find the older patient and gave them special care because due to rapid dental caries, periodontal disease, tooth loss the old people facing various problems.

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Facial Transformation in a Class III Surgical Case

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Abstract

Orthodontic problems that cannot be treated by growth modification/camouflage treatment needs to be corrected by surgical method. The envelope of discrepancy needs to be respected while handling these cases. This case report presents an adult female patient with Skeletal Class II malocclusion.

Keywords: *Decompensation, skeletal, Class III.*

Introduction

It is a dream of every young girl to look good. The pleasant facial appearance is directly proportional to the self-confidence of a person, which in turn is very essential for a successful life in terms of carrier and quintessence.

We received a20yrs old female patient named Miss Sweta Samatray, at our dental office with a chief complaint of improper chewing and unacceptable facial appearance. She had a very low self-esteem. Reasons for Orthodontic treatment was esthetics.[1]We could sense the internal motivation of the patient who is about to graduate as an engineer and the amount of peer pressure to excel in each field, she is going through. In this situation to treat her to achieve a more pleasant face was superlative.

Clinical Findings: Clinically, she had Angle's Class III malocclusion on skeletal class III jaw bases. On extra-oral examination she had a concave facial profile, anterior divergent face, acute nasolabial angle, flat mentolabial sulcus, thick protruded lower lip, deficient malar prominence. A clinically insignificant amount of facial asymmetry was present.

On intraoral examination she had bilateral Class-III molar and canine relation, crowding in the maxillary and mandibular anterior region, anterior open bite, mandibular midline shifted to right side by 2mm (Figure 1). Cephalometric findings are presented underneath in table 1.

Table 1. Cephalometric findings (pre and post-treatment)

	Pretreatment (Degrees)	Posttreatment (Degrees)
SNA	78	83
SNB	87	81
ANB	9	2
GOGN-SN	45	37
Y AXIS	48	61
U1-NA	33	32
LI-NB	20, 2mm	21, 3 mm
UI-LI	122	130
IMPA	89	91
NASOLABIAL ANGLE	88	91

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Figure 1: Pre-treatment extraoral and Intraoral Photographs

Treatment Plan: The patient had undergone upper premolar extraction, leveling and alignment, maxillary space closure, followed by bijaw surgery to achieve a good functional occlusion as well as harmonically esthetic face.

Treatment Progress: Leveling alignment done with the wire sequence as depicted in Figure 2. The wire sequence includes: 0.014 NITI, 0.016 NITI, 0.016 SS, 0.019x025 NITI, 0.019X0.025 Stainless steel wires.

Remaining space closure was done with the bilateral active tie backs, on 0.019X0.025 SS



Figure 2: Pre-surgical Intraoral & Extraoral photographs

After the maxillary space closure the pre-surgical OPG & lateral cephalogram was done to do the cephalometric evaluation and the paper surgery. Surgical VTO was done suggesting a maxillary advancement of 5mm & mandibular setback of 7mm. A mock surgery was performed on a cast after proper face bow transfer and articulation (Figure 3).



Figure 3. Face bow transfer

The two-stage mock surgery was done with simulation of maxillary advancement first, which resulted in the fabrication of the first interim splint. The second stage mock surgery simulated the bilateral sagittal split osteotomy set back of mandible and resulted in the fabrication of final surgical splint (Figure 4).

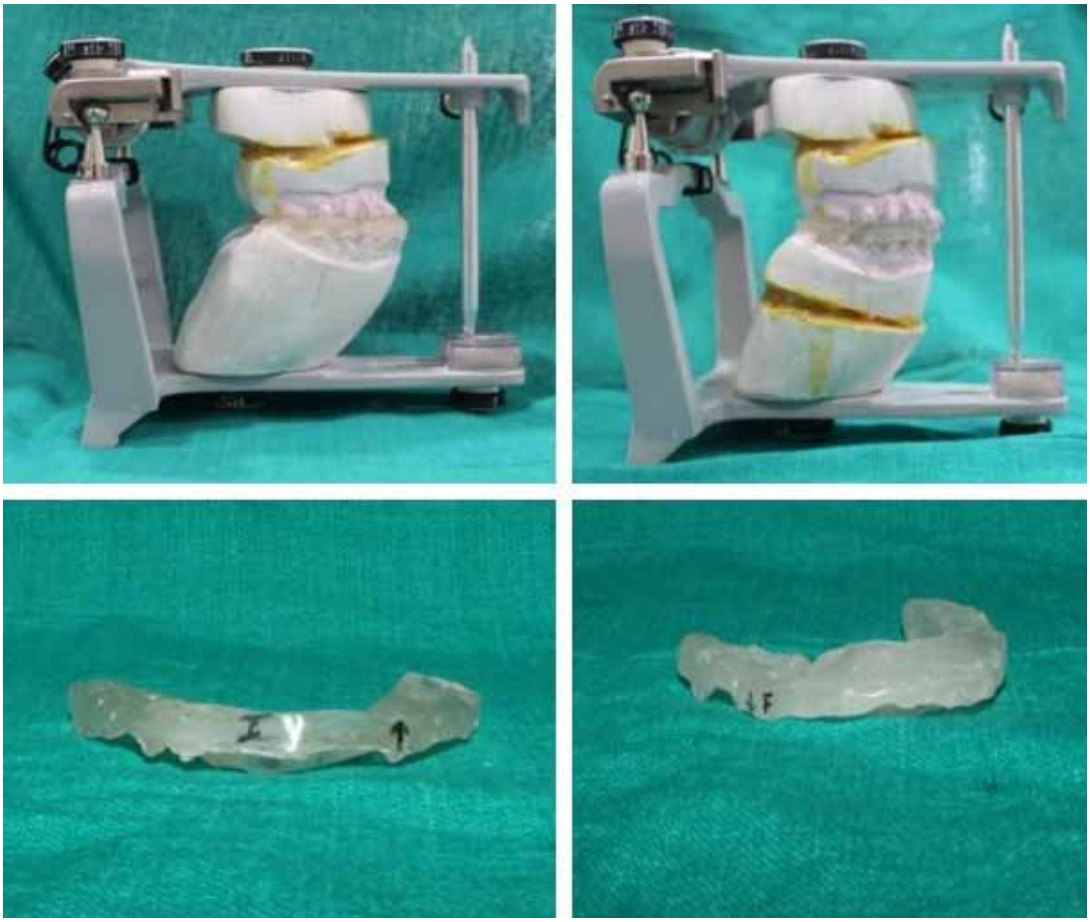


Figure 4. Surgical splints & mock surgery

The Orthognathic surgery was performed as per the plan and on table facial appearance was satisfactory. Illiaccrest bone grafting was done bilaterally to improve the malar prominence. The post-surgical healing was uneventful for the patient. Post-surgical Orthodontic finishing done for 4 months along with bracket repositioning and vertical settling. Debonding was done after 20 months of treatment. (Figure 5). Comparative radiographs are provided as shown in figure.6.



Figure 5. Pretreatment and post-treatment comparison photos



Figure 6: OPG and Lateral Cephalogram (Pretreatment & Posttreatment)

Conclusion

The intention of treating the case was purely to give a more esthetically acceptable face to the patient.[2,3] The patient's facial divergence changed from the concave to straight profile with a more prominent malar area of the face. The step formed by the maxillary and mandibular lips due to the prominent mandible became better. The throat chin angle became more pleasant.[4] We achieved all these extraoral features with a balanced occlusion at the end of debonding.[5,6] Now the girl is ready to face the world with more confidence and the proof of this lies in her eyes as noticed in the post-treatment extraoral photographs.

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Morphometric Analysis of Coracoid Process in Adult Human Scapula in Eastern Odisha Population

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Abstract

Background: Coracoid process is a bird's beak like projection which arises superolaterally from the upper border of the head. It plays an important role in shoulder function. The aim of our study is to measure the dimensions of the coracoid process of the scapula.

Method: The study was performed on 104 specimens of human scapula of unknown sex obtained from the Department of Anatomy of Institute of Medical Sciences & SUM hospital. The dimensions recorded were breath, length, height,thickness and types of coraco-glenoid space by using digital vernier calipers. These parameters were compared on both the sides.

Results: The most predominant coraco-glenoid space was found to be the round bracket (Type I). Statistically insignificant difference was noted in all four parameters between right and left. Though all the four parameters have higher values on right side in comparison to left side but the difference was statistically insignificant. The results are as such length of coracoid- 39.91±3.16 mm; width of coracoid- 14.00±2.03 mm; tip thickness of coracoid- 8.32±1.87 mm; height of base coracoid- 22.87±3.55 mm; width of base of coracoid- 10.50 ±2.64 mm;

Conclusions: The study of variation of dimensions of coracoid process provides valuable information regarding the role of these parameters in etiology of subcoracoid impingement syndrome which will help the radiologist and orthopedic surgeons for diagnosing various pathologies and plan for surgical procedure on coracoid process.

Keywords: *Coracoid, Coraco-glenoid, morphometry, Subcoracoid impingement syndrome.*

Introduction

The scapula is a large triangular flat bone which lies posterolaterally on the chest wall corresponding to second to seventh ribs. It has 2 surfaces-dorsal and costal surfaces; 3 borders-superior, medial and lateral borders; 3 angles, superior inferior and lateral angles and three processes called spine, acromion and the coracoid process. Each process has its separate importance.

The Coracoid process is a bird's beak like projection which arises superolaterally from the upper border of the head and is bent sharply so as to project forwards and slightly laterally.[1] The shoulder joint is highly mobile joint with less stability. It is a ball and socket type of synovial joint in which the large head of humerus articulates with the shallow glenoid cavity. So, the coracoid process is often referred to as the 'lighthouse of

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shoulder' in orthopedic literature.[2] The coracoacromial anatomy includes acromion, coracoacomial ligament and tip of coracoid process. The disparity in the height and length of coracoid process is responsible for altered shape of space between rotator cuff and coracoacromial arch. [3] The coracoid process is involved in many surgical procedures on the glenohumeral joint which forms an important part of the scapular glenoid structure. [4] The anatomy of the coracoids process and its related structures in the glenohumeral joint is required to study the radiological images and tocarry out surgical procedures in various shoulder pathology. The detail study of the dimensions of the coracoids process will help the surgeons for surgical procedures like drill hole placement, hardware fixation and prosthetic positioning.[5] Morphology of the coracoids process plays an importantrole in understanding impingement syndrome and pathogenesis of rotator cuff diseases.[6]

This study will also help the Forensic experts in determination of gender from these parameters.

A very few studies has been done regarding the morphometric values of coracoid process in eastern Indian population, so, the present study was planned to study the different parameters of coracoids process and types of coraco-glenoid space in adult human scapula of Eastern Odisha population.

The scapula is the anchoring point for the postural muscles of the shoulder; it provides stability necessary for a huge combination of movements around the shoulder joint. The coracoid process is rarely injured. It has a 3 dimensional orientation which makes it difficult to interpret in radiological images.[2] Any congenital variations and minimal iatrogenic changes in orientation can predispose to subcoracoid impingement.[7]

Material and Method

The material for the present study comprised of 104 adult dry scapulae of unknown age and sex, obtained from the Department of Anatomy of Institute of Medical Sciences & sumhospital.

The dry adult scapulae taken for study were labeledwith suffix R (right) or L (left). All the scapule were cleaned thoroughly and complete in all aspects were included in this study and broken and deformed ones were excluded. The following parameters were measured on the coracoid process with help of digital Vernier caliper accurate up to 0.01 mm. The parameters taken were as follows

1. Length of coracoid process: (distance from the tip to the of horizontalpart)

2. Width of coracoid process:(Antero-posterior distance at the midpoint of coracoidprocess).

3. Thickness of coracoid process (supero inferior distance 1cm posterior to the tip)

4. Base height (maximum superoinferior distantance of the base)

5. Base width (maximum lateral-medial distantance of the base).

Coraco-glenoid space was classified by Gallino et al.[8]into

1. Round bracket (Type I),

2. Square bracket (II)

3. Fish hook (III)

Result

All the measurements were taken and the data was tabulated and analyzed using Microsoft Excels software. P value was calculated by using unpaired t test for various parameters. Difference was considered to be statistically significant if p value obtained was less than 0.05.

The shape of the coraco-glenoid space was found to be round bracket like (Figure 1) in 58 cases (55.76 %), square bracket like (Figure 2) in 33 cases (31.74 %) and fish hooked like (Figure 3) in 13 cases (12.50 %). All the measurements are displayed in Table 1

Length of coracoid process: The mean length of coracoid process was observed to be 39.91±3.16 mm in the present study. The mean length was more on left side in comparison to right side but the difference was statistically insignificant.

Breadth of coracoid process: The mean breadth of coracoid process was observed to be 14.00±2.03 mm in the present study. The mean breadth was more on left side in comparison to right side but the difference was statistically insignificant.

Thickness of coracoid process: The mean thickness of coracoid process was observed to be

8.32±1.87 mm in the present study. It was more in left side in comparison to right side but the difference was statistically insignificant.

Height of base of coracoid process: The mean height of base of coracoid process was observed to be 22.87±3.55 mm in the present study. The mean height was more on left side in comparison to right side but the difference was statistically insignificant.

Width of base of coracoid process: The mean width of base of coracoid process was observed to be 10.50 ±2.64 mm in the present study. The mean width was more on right side in comparison to left side but the difference was statistically insignificant. All the measurements are displayed in Table 2.

Table 1: Different shapes of coraco-glenoid shapes.

Shape	Right Side	Left Side	Total
Rounded	38(63.33%)	20(45.45%)	58(55.76%)
Square	18 (30%)	15(34.10%)	33(31.74%)
Hooked	4(6.67%)	9(20.45%)	13 (12.50%)
Total	(100%)	(100%)	(100%)

Table 2: Observations of different parameters of coracoid process.

Parameter	Range of Measurements (mm)		Mean±SD (mm)		Average Mean±SD (mm)	P value
	Right (n=60)	Left(n=44)	Right	Left		
Length	34.5 -44.04	35.72 – 41.84	39.66 ± 3.0	40.27 ± 2.30	39.91 ± 3.16	0.18
Breath	10.58- 17.86	10.84 – 17.08	13.93 ± 2.1	14.11 ±3.21	14.00 ± 2.03	0.09
Thickness	6.1 – 10.32	7.36 – 10.56	8.09 ± 1.42	8.64 ± 1.00	8.32 ± 1.27	0.77
B. Height	12.6 - 26.75	6.36 - 17.84	22.59 ± 3.75	23.24 ± 3.41	22.87 ± 3.55	0.46
B. Width	16.42 – 28.82	6.76 – 13.84	10.66 ± 2.89	10.29 ± 2.37	10.50 ± 2.64	0.73



Figure 1: Type-I (round bracket) coraco-glenoid space



Figure 2: Type-II (square bracket) coraco-glenoid space.



Figure 3: Type-III (fish hooked) coraco-glenoid space.

Discussion

Coracoid process plays important role in functions of scapula. The coracoid process acts like a lever through which the muscular action of the biceps, coracobrachialis, and pectoralis minor muscles exerts a force on the glenoid. The shoulder joint is a most frequently dislocated in adults as well as children, the injury of thecoracoid process is very rare.So fractures of coracoid present a challenge to repair by the operating surgeons. The frequency of fracture ofcoracoid process was between 3% and 13% of all the fractures involving scapula. The base of the coracoid is most commonly fractured. The results obtained in the presentstudy demonstrates the dimensions of the coracoid process can help in deriving appropriate strategy for repair of the fracture. Various ways of open surgical and arthroscopic access to the shoulder involves the coracoid process. So, its morphometry is of pivotal importance in surgeries of the shoulder joint.

Various studies have shown that the skeletal morphometry is influenced by different factors such as race and sex.[9,10] The study of the morphometry of coracoid process shows that it as a key structure and potential mediator in shoulder surgery and pathology.[11]

The mean length of coracoid process was found to be 39.91±3.16 mm with mean of 39.66 ± 3.0 on right side and 40.27 ± 2.30 on left side. The findings of the current study is similar to those found out by Kavita et al[12],Kour et al[13] and Rajan et al[3]. While the findings of Gallino et al[6]; and Burke et al[14] are slightly higher than present study and the results found by, Piyawinijwong et al[15] and Gumina et al[16] is less than our study.

The mean breath of coracoid process was found to be 14.00±2.03 mm in the present study with mean of 13.93 ± 2.1 on right side and 14.11 ± 3.21 on left side. This findings is similar to those found by Rajan et al,Kour et al and Verma et al[3,13,17] but the studies done by Piyawinijwong et al[15] found slight lower value than the current study.

The mean thickness of coracoid process was observed to be 8.32 ± 1.27mm in the present studywith mean of 8.09 ± 1.42mm on right side and 8.64 ± 1.00mm on left side which is almost equal to that of the findings of Verma et al[17] and S. Kalra et al.[18]The findings of Coskun et al, and Piyawinijwong et al is lower than the present study[1,15].

The mean height of the base of coracoid process was observed to be 22.87 ± 3.55 mm in the present study with mean of 22.59 ± 3.75 mm on right side and 23.24 ± 3.41mm on left side. Our findings are higher in comparison to the studies done byRajan et al, Piyawinijwong et al and Gumina et al[3, 15, 16] and it is similar to the findings of Verma et al.[17]

The mean width of the base of coracoid process was observed to be 10.50 ± 2.64 mm in the present study with mean of 10.66 ± 2.89 mm on right side and 10.29 ± 2.37mm on left side. Our findings are similar to the studies done by FathiM et al.[19]

Shape of coraco-glenoid cavity: In the present study Type I(round bracket)was the most common type seen in 55.78% of the scapulae out of which 63.33% of sample on right side and 45.45 % on left side. The second most common type was Type II(square bracket)seen in 31.74 % which was found 30 % on right side and 34.10 % on left side and least is Type III (fish hooked)vshape in 12.50 % which was 6.67% % on right and 20.45 % on left side. The findings of the current study was similar to the findings of the Verma et al and Gumina et al.[17,16]

Conclusion

The morphometric analysis of the coracoid process should be used like a tool for surgical procedure on coracoid process. A very few studies has been done to demonstrate the various dimensions of coracoid process. It will help the orthopedic surgeons to operate the gleno-humeral joint during impingement syndrome, rotator cuff rupture and for shoulder prosthesis in total shoulder arthroplasty. The study of the dimensions of coracoid process helps the radiologists to interpret any pathological conditions associated with it. These findings might also be useful in determination of gender by the forensic experts. The various scapular dimensions obtained in this study will also be useful in comparative anatomy and manufacturing prosthetic products. The current study will be of immense help in orthopedic surgeries on the shoulder joint and in biomechanical engineering for designing implants for total shoulder replacement

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Management of Bilateral Ectopic Canines: A Case Report

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Abstract

An ectopic canine tooth may originate due to genetic/environmental aberrations. These teeth demand correction as they can move in the jawbone and damage the adjacent bone, roots of the teeth, pose esthetic concerns. Timely diagnosis and treatment play a pivotal role. The case report stresses on an extraction treatment approach for correction of ectopic canine in a patient posing severe crowding in both upper and lower arches.

Keywords: *Ectopic Canines, Bilateral, Orthodontic Mechanotherapy.*

Introduction

Orthodontic practice envisages ectopic canines very frequently with a prevalence rate of 1-2% in the general population.[1,2] They occur with a variety of local and systemic issues. The long tortuous path of a canine might be an explanation for the etiology of this phenomenon. Any associated disturbance with the follicle of the unerupted tooth may displace the maxillary canine tooth.[3,4]

Canine Impactions and Classification:

A. ARCHER'S

It may be:

1. CLASS I: Partially Impacted
   a. Horizontal
   b. Vertical
   c. Semi vertical
2. CLASS II: Buccally impacted canine
   a. Horizontal
   b. Vertical
   c. Semi vertical
3. CLASS III: Impacted canine in both palatal and labial surfaces
4. CLASS IV: Impacted canine located in the alveolar process
5. CLASS V: Impacted canine located in the edentulous maxilla
6. CLASS VI: Canine in aberrant position

Case Report:

Diagnosis and Etiology: A 12-year-old male patient named Soumya Ranjan Mitra reported to the Department complaining of irregularity in the upper and lower teeth. Extraorally, the patient had a convex profile with incompetent lips (Figure 1) with an asymmetrical face.



Figure 1. Patient with convex profile

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He had a normal gait with a thin physique. The patient was cooperative and was keen for braces treatment. On intraoral examination, the patient had Angle's Class I molar on the right side and Angle's Class II molar on the left side with 2 mm of overjet (Figure 2.a & 2b.).



Figure 2a. Class I molar



Figure 2b. Class II molar

Maxillary (upper) archand mandibular (lower) archexhibited severe arch crowding (Figures 3 & 4).There was minimal attached tissue with the ectopically erupting right and left upper canines. Cast analysis showed arch length discrepancies in the maxillary as well as mandibular arches.



Figure 3. Maxillary anterior crowding



Figure 4. Mandibular anterior crowding

There was an absence of any pathologies in the panoramic radiographic image. Lateral cephalometric analysis showed skeletal Class II relationship with normodivergent pattern. The patient had upright maxillary incisors.

Treatment Objectives: The main objective of treatment was the correction of ectopic canines with a cuspid guided occlusion preserving the normal overjet and obtaining a proper overbite.

Treatment Plan: The rationale of treatment was to extract the first premolars, preserve the profile of the patient and obtain a stable treatment result.

Treatment Alternatives: Several treatment options are available for the correction of ectopic canines. The first and most popular would include the extraction of all first premolars as seen in our case. The other would include the distalization of the upper premolars and the extraction of the lower premolars. The least preferred would be a non-extraction treatment plan but the disadvantages would include proclining the incisors, longer treatment duration and enamel demineralization as a result. There can be a change in profile adversely affecting the esthetics of the patient. Arch form maintenance is predictable with extraction treatment.

Treatment Progress: Patient's dentition was checked for any signs of dental caries, periodontal degradation. The amount of attached gingiva was evaluated over the ectopic canine region. Full bonded MBT 022 system fixed appliances were placed on both the arches. The extraction of all four premolars was carried out. Initial leveling and alignment were carried with 0175 multistranded wire followed by 014 NiTi and other 17*25 NiTi and stainless steel wires. Light elastic forces were used to retract the buccally placed canines into the arch. The total treatment time was 20 months. After completion of treatment, removable retainers were given for the maxillary and the mandibular arches.

Treatment Results

A canine protected occlusion was achieved with ideal overjet and overbite. The canines were in Class I occlusion with correction of crowding in both the arches (Figure 5 & 6).



Figure 5. Class I Canine with canine protected occlusion



Figure 6. Ideal overjet and overbite

Discussion

Timely interception of the ectopic canines can be made possible by periodic panoramic radiography, careful clinical examination, and intraoral palpation.[5] Proper set up of Orthodontic records helps in a space analysis. Space can be gained via several method: maxillary arch expansion, proclination of maxillary incisors, other treatment strategies. It is important to check forthe mobility of the adjacent teeth.[6,7] If the deciduous canine id mobile, there are chances of its replacement by permanent canines.

If the permanent lateral incisor is mobile, it might indicate that the impacted canine has resorbed its roots. We were able to effectively manage the case by extraction treatment.[8] Non-extraction treatment would have proclined the maxillary incisors and worsened the soft tissue profile of the patient. Easy correction of crowding was possible with extraction expecting stable results post debonding. The factors dictating crowding include tooth size and dental arch dimensions.[8] Proper clinical examination and diagnostic radiographs are essential for the diagnosis of these ectopic canines.[8]

Conclusion

The success of Orthodontictreatment posesa great challenge to the Orthodontist in terms of ectopic canine correction. The extraction treatment plan is more predictable, stable giving importance to the esthetics of the patient.

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Glossopharyngeal Neuralgia: A Comprehensive Update

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Abstract

Glossopharyngeal neuralgia (GN) is an unusualclinical condition where severe paroxysmal pain of the glossopharyngeal nerve. GN pain should be differentiated from trigeminal neuralgia pain, as the two neuralgias share several similar features. Thorough medical history coupled with a detailed clinical examination and hematologic and radiographic investigations is necessary for early and accurate diagnosis. Anticonvulsants form the cornerstone of the medical management, and surgical interventions (microvascular decompression and gamma knife surgery) is needed in cases resistant to pharmacotherapy.

Keywords: *Carbamazepine, glossopharyngeal neuralgia, microvascular decompression, neuralgia.*

Introduction

Neurologica non-nociceptive pain or pain which does not relate to the activation of pain receptor cells in the body. It's a type of pain due to a lesion or any disorder of the somatosensory system.[1]

As per ICHD-3 (International Classification of Headache Disease-3) classification, glossopharyngeal neuralgia (GN) is a disorder characterized by a brief periodic unilateral pain, with sharp and piercing in character, with abrupt onset and cessation, in the glossopharyngeal nerve distribution (angle of the jaw, ear, tonsillar fossa and the tongue base). It falls under the International Classification of Diseases (ICD) category as ICD-10-CM-G52.[1 2]

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The International Association for the Study of Pain (IASP) defines it as sudden, severe, brief, recurrent pain along the glossopharyngeal nerve distribution.[3]

GN is an extremely uncommon occurrence and accounts for only 0.2%–1.3% of the cases with facial pain.[4,5] GN usually affects the male individuals above 50 year-age, and the reported incidence of GN is roughly 0.8 per 100,000 persons per year.[4,6-11]

It must be asserted that GN is not as rare as reported in the literature due to non-familiarity with the disease, diagnostic obstacles, and more so with the escalating number of patients with Eagle's syndrome.[12]

Historical Background: Theodore H. Weisenburg (1910) was the first to provide the classic description of GN in a patient who reported with a lancinating ear and neck. Autopsy reports revealed that the pain was due to a cerebellopontine angle tumor, which presses the trigeminal nerve and stretches the glossopharyngeal nerve.[13]

After 10 yrs in 1920, Sicard and Robineau (1920) reported 3 patients with "Algie Velo-pharyngee essentielle" i.e. Pain along the distribution of glossopharyngeal nerve idiopathically[10,13]

The term "glossopharyngeal neuralgia" was framed by Wilfred Harris. He described GN as a painful condition characterized by episodes of severe lancinating, unilateral pain along the nerve division, which may be triggered by trigger point stimulation in areas supplied by the nerve.[10,13]

Two noteworthy advancements were reported for the efficient management of glossopharyngeal neuralgia in 1927. J. Doyle published a case series of 18 GN patients, and W. Dandy performed the first successful intracranial resection for glossopharyngeal neuralgia.[14]

Riley et al. (1942) reported an uncommon association of GN with life-threatening cardiovascular manifestations- asystole, transient/persistent bradycardia, syncopal episodes, and even seizures.[8]

Classification:

1. Based on the International Headache Society (IHS) Classification, GP is classified as (1) classical GPN (occasional or episodic pain) and (2) symptomatic GPN (persistent pain; persisting between the episodes of peaks and troughs of pain.).[15]
2. Based on the pain distribution, GN is classified as- (a) Otitic pain: Pain in (or around) the auricle/ ear lobe (b) Oropharyngeal pain: Pain of neck and maxillofacial regions. Nevertheless, these regions show considerable overlap inthe cranial nerve-supplied areas.[16]
3. Based on the causal factors, GN is classified as (a) Idiopathic/essential GN- The majority of GN cases are idiopathic, where no apparent lesion is noticed.[17] GN is caused by neurovascular compression of nerve IX by the postero-inferior cerebellar artery (PICA),[18] which was first observed postmortem by Brihaye et al.[19] (b) Secondary GN- where a demonstrable lesion is seen. Eagle's syndrome/ styalgia accounts for the most common secondary cause of GN .[13]

Eagle's syndrome is an unusual disorder, clinically alarger in size of the styloid process or stylohyoid ligament complex is mineralized.

Clinical Features: Glossopharyngeal neuralgia is a rare disease entity, characterized by pain paroxysms in the regions supplied by the glossopharyngeal nerve (auricular and pharyngeal). The GN pain is typically characterized as brief episodes of unilateral severe, piercing/lancinating pain, confined primarily of the ear, ventral surface of tongue, throat, tonsillar region, or beneath the angle of the jaw.[21]

The GN pain distribution is diagnostic: The pain generally commences from the pharyngeal, tonsillar, and posterior base of tongue and includes the eustachian tube and inner ear or spreads to the mandibular angle. Exacerbating factor in GN is generally deglutition, and cold liquids mainly evoke the pain.[21]

GN pain is transient and excruciating to begin with (lasts for 30sec), although it may recur after days, weeks/ months and persists in the same areas.[22,23] Swallowing, chewing, sneezing, talking, cleaning the throat, and light touch to the gums/oral mucosa, abrupt head movements, pain on lifting of the arm of the effected site, and the lateral jaw movement may also precipitate the pain.[24]

Vagoglossopharyngeal neuralgia refers to an uncommon linkage between GN and syncope,[2] where the affected individual presents with episodes of bradycardia, severe hypotension, syncope, and even cardiac arrest.[25] The probable reason for these life-threatening manifestations in GN may be attributed to the anatomical vicinity of glossopharyngeal nerve with the vagus and nervus intermedius.[26]

It is important to distinguish GN from trigeminal neuralgia (TGN), that shares some features with GN and can combined form in 10% of patients.[27] Spontaneous remission is seen in both GN and TGN.[8]

GN is an uncommon clinical entity as compared to TGN (Reported prevalence rate of GN and TGN is approximately 0.8/100,000 population and 4.7/100,00 population respectively).[9] The left side of the face is usually affected in GN in contrast to the right side in TGN.[28]

The two neuralgias can also be differentiated based on the site affected and triggering factors. GN has the predilection for the throat and tonsillar region, and TGN primarily affects check, ala of nose, vermillion border of lips (facial region along the trigeminal nerve division). GN pain is triggered by chewing and swallowing, whereas, a light facial touch while washing/brushing the teeth exacerbates the TGN pain.[16]

A dull ache usually persists between pain episodes in GN,[29] in contrast to symptom-free periods between pain paroxysms in TGN. Any individual experiencing a dull ache between pain paroxysm does not fulfill TGN diagnostic criteria.[30]

GN pain occurs mostly during the daytime, but the pain may disturb the sleep and the patient may wake up in the night. However, there is no pain during sleep in TGN.[29] Topical anesthetic application on pharyngeal mucosa may relieve GN pain, thus, distinguishing it from other neuralgias. Vasoglossopharyngeal neuralgia may be associated with life-threatening cardiovascular features- syncope, hypotension, cardiac arrhythmias, in contrast to trigeminal neuralgia.[29]

Diagnostic Aids: Characteristic clinical manifestations usually suffice for the diagnosis of GN, and definitive investigative tests do not exist. GN patients are usually recommended for an ear, nose, and throat (ENT) consultation to rule out a possible anatomical lesion.[2] The distinctive unilateral, severely piercing, episodic pain in the throat, triggered on swallowing provides confirmation for GN pain, and differentiates it from the pain of inflammatory origin (constant pain, lasts for minutes). Glossopharyngeal nerve involvement/ association with other cranial nerves may be evaluated by assessing the pain distribution. Ototic pain may be detected by injecting an anesthetic solution (2% Lignocaine or 0.5% bupivacaine) into the region with a close approximation to the trigger point.[11] Potential etiologies such as vascular compression/neoplasms may be demonstrated by radiographic evaluation of the brain stem. A high-resolution neck CT scan may demonstrate Eagle's syndrome.[2]

Treatment: GN may be treated with either Pharmacologic/non-pharmacologic interventions.[31]

Carbamazepine is regarded as the drug of choice in GN. The drug has an inhibitory action on the brain stem reflex activity.[32] Also, the drug has a possible action on preventing bradycardia.[33,34]

Gabapentin, pregabalin, and lamotrigine are the other pharmacotherapeutic agents used in GN. Gabapentin and pregabalin act on the α2δ subunit of calcium channels, and diminish the release of excitatory neuro-transmitters. Anticholinergic therapy, β2-adrenoceptor agonists (atropine, isoprenaline) and temporary pacing may be used to manage the bradyarrhythmia in GN.[35] Published literature has also elucidated the efficacy of selective serotonin reuptake inhibitors (20–50mg/day paroxetine and 50–200mg/day sertraline) and vitamin B12 in the management of GN.[36]

Only a few cases of GN have shown response to non-steroidal anti-inflammatory drugs (due to their probable action on unidentified acute inflammation) and opioids (as an ancillary agent to the anti-convulsants).[17]

Glossopharyngeal nerve block provides quick pain amelioration and acts as an excellent ancillary aid in the medical management of GN. It will bedone with either (local anesthetic agents) non-neurolytic agents with or without additions of (steroid, ketamine, etc.) or with(phenol, alcohol, glycerol, etc). neurolytic agents [37]

Surgical management is usually adopted in young aged, recalcitrant cases and is associated with high morbidity.[17]

Microvascular decompression (MVD) is the most preferred surgical technique associated with the highest success rates.[28] MVD is a more efficient treatment than Gamma knife surgery (GKS) due to both initial and long-term pain remission, lower relapse rates, and maintaining a pain-free status.[38]

Conclusion

Glossopharyngeal neuralgia is an uncommon neuropathic pain that occurs along the distribution of the glossopharyngeal nerve. GN can be classified based on the nature of pain, site predilection, and etiologic factors. This neuralgic pain mimics trigeminal neuralgia; hence, an early and accurate diagnosis is mandatory. The condition is seldom associated with life-threatening cardiovascular manifestations like bradycardia, hypotension, syncope and rarely cardiac arrest. Pharmacotherapy with anti-convulsants is usually sufficient, although surgical intervention may be required in resistant cases.

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Hematoma Following Nerve Block of a Branch of Maxillary Nerve: A Case Report

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Abstract

The nerve block given to obtain anesthesia in maxillary molars and its supporting structures is the posterior superior alveolar nerve (PSAN) block. One of the common complications of the block is extraoral hematoma which occurs because of the needle penetration to far posteriorly placing it in the vicinity of pterygoid plexus of veins, maxillary artery resulting in probable inadvertent nicking of the vessel causing effusion of blood into the surrounding soft tissue resulting in swelling and discoloration. This case report describes this complication and its management.

Keywords: *Hematoma; Posterior superior alveolar nerve; Local anesthesia.*

Introduction

Pain is a complex psychological phenomenon, and it becomes a challenge for each practitioner to supply care with less anxiety and discomfort to their patients. With the development of anesthesia, the science behind pain management has continued to evolve, although these anesthetics are thought-about safe, care should be taken regarding the administration of appropriate anesthetics along with correct technique. In the field of Dentistry, Posterior Superior Alveolar Nerve Block (PSANB) is a technique used to attain anesthesia before performing any surgical and non-surgical treatment on maxillary molars and its surrounding structures. A major sensory branch of the maxillary division of the trigeminal nerve, the Posterior superior alveolar (PSA) nerve supplies the buccal gingiva, periodontium, and alveolus related to the upper molar teeth as well as pulpal innervations of all the maxillary molar teeth except the mesiobuccal root of the first molar, that is equipped by the middle superior alveolar nerve in or so five-hundredths of people. Generally notwithstanding the care utilized in the administration of Posterior superior anesthetic technique, complications occur that include hematoma formation, transient visual impairment, blurred vision and temporary visual defect.[1]

Hematoma formation occurs when the needle nicks the vessels (artery or vein) in its vicinity unintentionally during the administration of anesthesia which ends in filling of blood into the extravascular spaces. A venous puncture by needle might not lead to the formation of hematoma that can be appreciated however the size of hematoma observed after arterial perforation can be appreciated clinically and rapidly because of considerably greater blood pressure within the artery. This article presents a case of hematoma formation following the Posterior superior alveolar nerve block (PSANB).

Case Report: A female patient age 33 years reported to our Department with a chief complaint of pain in the left maxillary posterior region for two days which did not alleviate even after the intake of prescribed medications. After a thorough medical and dental history, clinical and radiographic examination was done which

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revealed Class II (mesio-occlusal) carious involvement of enamel, dentin with exposure of pulp of maxillary left second molar (tooth#27) and endodontic treatment followed by a fixed prosthesis in the form of the crown was planned for the tooth. The patient was positioned so that the maxillary occlusal plane was at 45 degrees angle to the floor and 1.5 ml of 2% lidocaine with 1:100000 adrenaline was administered using the PSA nerve block technique following negative aspiration of the needle. After a few minutes of administration of anesthesia, a gradual swelling along the lower left mandibular region (Left Cheek) was observed (Figure 1) without evidence of breathing problems or impaired consciousness. The dental treatment was halted and the patient was enquired about any discomfort. As she became apprehensive, she was informed regarding the cause of the condition and was assured of it being temporary. She was advised to use ice pack 20-30 minutes per hour for the first 24 hours followed by intermittent use of hot moist packs to resolve the condition. The patient was also prescribed analgesics and antibiotics, along with the application of a heparin cream on the affected area. The follow up of the case was done and clinical examination five days after the incident revealed a discrete swelling with a visible outline of the regressing hematoma (Figure 2). A complete resolution of hematoma was observed three weeks post-incident (Figure 3) after which root canal treatment of tooth#27 was initiated.



Figure 1. Hematoma few minutes post administration of LA



Figure 2. Five days post-incident revealing a visible outline of the regressing hematoma



Figure 3. Complete resolution of hematoma three weeks post-incident.

Discussion

The problems associated with anesthesia of the maxillary nerve is attributed to the individual variances of traditional anatomic nerve pathways through maxilla. The failure of infiltration anesthesia in maxillary molars can be due to the flaring of palatal roots towards the midline of the palate. Thus, in these flared root cases and even in the presence of infection, PSANB provides

a reliable anesthesia to the pulpal innervations of maxillary molars.[2]

PSANB is a technique-sensitive procedure, and as stated by Malamed even after following strict administration protocol, complications occur because of the anatomic variation in patient's soft and hard tissue. The danger of hematoma formation in patients receiving PSANB is high, and it produces the biggest and most unappealing hematoma. The infra-temporal fossa into which bleeding occurs, could be a place which can accommodate an outsized volume of blood. There's a colorless swelling that appears at the side of the face, few minutes after LA administration, and this size increases and extends to include the lower front region of the cheek as well. As the blood vessels are settled posterior, superior, and medial to the maxillary tuberosity, it is difficult to use pressure wherever the trauma has occurred.

The application of digital pressure in the medial and superior direction within the mucobuccal fold as far as distally (without eliciting a gag reflex to the patient) is typically found to help counteract the gradual swelling.[3] But in the present case report, digital pressure couldn't be applied as the swelling had progressed inferiorly and anteriorly obliterating the maxillary left buccal vestibule. Employment of ice pack (extraorally) to the affected region was suggested to control the size of the swelling as it is believed to cause vasoconstriction of the vessels along with palliative effects followed by intermittent hot moist packs after twenty-four hours that induce dilatation of the vessels thereby increasing the speed at which the blood components are resorbed, although its advantages are debatable. As hematoma places pressure on lacerated tissues/wounds, it acts as a nidus initiating the event of infection and inflammation. Since the dimensions of swelling in the present case report was quite large, antibiotics were prescribed.[4]

To avoid the formation of hematoma, intensive information on the anatomy of that specific region is extremely necessary. The use of short needle and aspiration in two different planes keeping the needle in the same position before and during drug deposition are recommended as these avoid unintended intravascular injection into the approximating blood vessels. Few studies have conjointly prompted precurving of a 24-millimeter needle at a distance of ten to twelve millimeters that deposits the anesthetic solution near to the nerve at the posterior surface of the maxilla. This technique as expressed by the authors avoids over-penetration of the needle because of its curvature from the initial site of insertion. [5, 6]

Above all the precautionary measures and management mentioned, it is important to notify the patient concerning the attainable complications of PSA nerve block before administration of anesthesia to reduce the psychological impact of the complication as well as maintaining patient's trust about their capability regarding the management of the condition.

Conclusion

Complications with the employment of local anesthetics like hematoma related to PSANB cannot be completely prevented. However, the temporary nature of the complication ought to be assured to the patient as an unaesthetic look because of the discoloration and swelling on the side of the face causes psychological impact. Therefore, it's crucial for a practitioner to bear in mind of the clinical signs of hematoma and to suitably manage them.

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Bidirectional Dynamics between Hyperglycemia and Periodontal Infections

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Abstract

Hyperglycemia induces a higher rate of activation pathways responsible for inflammation which leads to microvascular and macrovascular complications, oxidative stress as well as apoptosis. In the pathological manifestation of hyperglycemic events, a raised level of inflammatory markers such as: IL6, TNF-α and C-reactive protein occurs which in turn promotes acute-phase of inflammation leading to signaling impairment of insulin function and insulin resistance. On the other hand, enhanced systemic inflammation due to periodontal diseases mediated by the release of IL-6 and TNF-α happens when associated with hyperglycemia. This review article deals with assessment on hyperglycemia and its correlation to dental pathologies with special reference to periodontitis and vice versa. Studies show that the paradigm shift in the oral microbiota due to hyperglycemic condition resulting in the increase in the pathogenicity of associated pathogenic microbes which results in increased inflammation and bone loss in periodontal pathologies.

The extensive studies about the association between inflammatory periodontal disease and diabetes show the cyclic relationship between the two. Diabetes predisposes the individual to periodontal infections and also the later exacerbates or worsens the glycemic control in diabetic patients. Routine periodontal examination provides an insight for the early diagnosis of diabetes in undiagnosed patients and may reduce the worsening of metabolic control thereby preventing serious complications. Also the oral health of diabetic patients may be improved and well maintained with proper management of blood glucose levels.

Although there are evidences in literature to establish correlation between Diabetes Mellitus associated with periodontitis and vice versa, still the outcomes are not conclusive. Therefore large-scale prospective epidemiological analysis are the only sought after approach to clearly bring in the connectivity between Diabetes and Periodontal disease

Keywords: *Hyperglycemia, Periodontitis, cytokines,IL6, HbA_{1C}, TNF-α, AGEs.*

Introduction

Hyperglycemia is a state of high blood sugar level in the body. Blood glucose levels shoots up in the body either due to decreased production of insulin in the body or when the body can't properly use the produced insulin. Several processes are involved in the development of the pathology of hyperglycemia that can lead to insulin deficiency or anomalies resulting to insulin resistance.[1] Hyperglycemic state is characterized by increased blood glucose levels ≥126mg/dl in fasting and in postprandial, blood glucose at 2 hr after intake of 75mg of oral glucose to be ≥200mg/dl, or HbA_{1C}≥6.5%[1]. Periodontitis is a process of resorption of periodontal ligament and alveolar bone, the structures that supports the teeth. The destruction of the collagen fibers leads to pocket

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formation in the periodontal zone, the space created in between the tooth & the gingiva. Further deepening of the pocket due to continuous loss of periodontal fibers and alveolar bone resorption culminates finally in attachment loss. Various studies indicate the existence of reciprocating relationship between glycemic control and healing of periapical lesions. The increased prevalence and severity of periapical lesions suggests that hyperglycemic state to be a disease modifying agent for the periapical dental pathologies.[2]

This review article is a detailed assessment of the literature on hyperglycemia and its correlation to dental pathologies with special reference to periodontitis and vice versa. Studies show that the paradigm shift in the oral microbiota due to hyperglycemic condition resulting in the increase in the pathogenicity of associated pathogenic microbes which results in increased inflammation and bone loss in periodontal pathologies. However such periodontal diseases are manifested at a higher rate (3-4 times) in immune-compromised condition of patients especially individuals with diabetes when compared with non-diabetics. Therefore there lies increased risk in diabetes patient with periodontal disorders and thus control of glycemic levels in them is inevitable.[2]

Mechanism behind effect of hyperglycemia on periodontal tissues: Hyperglycemic state affects many functions of the immune system and is associated with compromised immune response and delayed healing. The condition results in changes of immune cell function, thereby producing inflammatory immune cell phenotypes such as either upregulation of pro-inflammatory cytokines from monocytes or polymorpho-nuclear leukocytes or downregulation of growth factors from macrophages. This predisposes to chronic inflammation, progressive tissue breakdown and decreased capacity to tissue repair.[3,4] The resulting increased levels of inflammatory cytokines especially IL-17 acts as a signaling molecule for the immune response and inflammation which are associated with periodontal disease resulting in rapid bone loss.[2] In individuals with diabetic complications, the release and accumulation of AGEs (Advanced Glycation End products) has been observed in periodontal tissues. This leads to increased rate of periodontal inflammation. The receptors RAGE present in the cell binds with the released AGEs and induce the formation of IL-1β, TNF-α, IL-6 (inflammatory mediators). AGE formation results in the production of ROS (Reactive Oxygen Species), a natural byproduct of the normal metabolism of oxygen that plays important role in cell signaling and hemostasis and contribute to vascular injury, a common diabetic complication. Also AGEs have detrimental effects on bone metabolism leading to impaired repair and bone formation.[2]

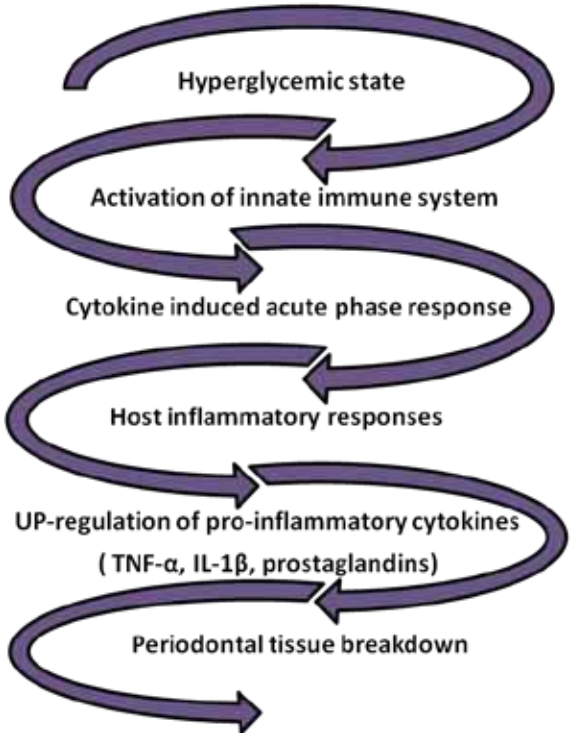


Fig 1: Series of events taking place in periodontal tissues breakdown due to hyperglycemia

Table 1: Animal studies illustrating correlation between hyperglycemic state and periodontal tissues:

Studies by	Model/subject	Analysis	Area of study/observation	Result
[5]	Animal study (rats) Streptozotocin induced diabetic rats.	Histologically & Histometrically	Pulpal and periapical tissue changes after pulp exposure in models	They observed that as compared to control group inflammatory changes in the apical PDL, root resorption and alveolar bone resorption in experimental group were more severe

Studies by	Model/subject	Analysis	Area of study/observation	Result
[6]	Female non obese diabetic mice	Histomorphometrically	They induced periapical lesions in 1st molars in experimental group and measured the size of the periapical lesion.	It was observed that the severity of the response in experimental group is more than the control group
[7]	Comparision between Goto-kakizaki rats with spontaneous non-insulin dependent DM and wistar rats as control group	Histologic analysis	Studied the development of periradicular lesions resulting from type 2 DM after exposing the pulp from occlusal surface in left mandibular 1st molars.	They observed the results after 4weeks. Alveolar bone loss and resorption and periapical lesions were severe in experimental GK diabetic rats.
[8]	Rats with steptozotocin induced hyperglycemia and control group injected with saline.	Histologic analysis	Studied the effect of hyperglycemia in exposed pulps over which MTA was placed.	Inhibition of dentin bridge formation and simultaneous increase in inflammatory cells in the exposed pulps. They observed an inverse relationship between dentin bridge formation and infiltration of inflammatory cells.

Table 2: Human studies illustrating correlation between hyperglycemic state and periodontal tissues:

Studies by	Analysis	Method and results
[9]	Radiographic analysis	They compared the outcome of healing of periapical lesions in poorly controlled DM and therapeutically controlled DM patients. The healing of the lesions of patients with DM under therapeutic control were similar to non-diabetics.
[10]	Radiographic analysis	Comparing the healing of periradicular lesion between low plasma glucose group and high plasma glucose group following root canal treatment, 30 weeks follow up shows 74% reduction in the lesion in low glucose levels where as only 48% reduction of lesion was observed in high glucose level groups.
[11]	Symptomatic analysis	Diabetic patients with abnormal glycemic levels showed a high rate of asymptomatic tooth infections.
[12]	Clinical and radiographic investigations	Diabetic patients of long duration showed greater prevalence of teeth with periapical lesions than other groups.
[13]	Clinical and radiographic investigations	Reported that Diabetic patients showed higher percentage of clinical symptoms relating to pulpal and periodontal infections thereby concluding that DM as a predisposing condition for endodontic infections.
[6]	Microbial analysis	Samples collected from root canals and necrotic pulp tissues of diabetic patients shows the association of *Porphyromonasgingivalis* and *Porphyromonasendodontalis*
[14]	Multivariate analysis, Endodontic diagnostic & treatment outcome	Diabetic patients following root canal treatment have more periodontal disease and also diabetic patients with pre-operative periradicular lesions exhibit lesser success rate of root canal treatment.
[15]	Radiographic analysis	Type 2 diabetic patients exhibited residual periradicular lesions in the radiographic periradicular radiolucencies.

Mechanism behind effects of periapical infection on glycemic control in diabetic patients: Over time the existence of periodontal diseases results in worsening of glycemic control in diabetic patients.[16] Chronic dental lesions or periodontal diseases exhibit similar characteristics as that of any other chronic infections of the body resulting in release of higher levels of pro-inflammatory mediators from harboring gram negative anaerobic microorganisms into the systemic circulation thereby predisposing to worsening of glycemic control in diabetic patients.[16] It has been proposed that the mechanism of insulin resistance developed in obesity induced diabetes and that in existence with periodontal disease are similar in manner i.e by enhancing activation

of the overall systemic immune response that are initiated by cytokines pro-inflammatory mediators.[17] Diabetes is a manifestation of host's inflammatory response resulting from cytokine induced acute phase response through the activation of innate immune system.[18]

The inflammatory manifestations due to periodontitis has been characterized to be mediated by uncontrolled secretion of IL-1β, IL-6, TNF-α, matrix metalloproteins (MMP-8, MMP-9, MMP-13), prostaglandin PGE2, cytokines, RANKL (Receptor Activator of Nuclear Factor Ligand). The periapical bone resorption has been observed due to enzymatic degradation of matrix and stimulated osteoclasts.[19]This could be due to TNF-α secretion in the cell that stimulates the fibroblasts resulting in above physiological and cytological changes. A correlation has been proposed that enhanced TNF-α level is the key factor in establishing the link between diabetic patients and their periodontal conditions.[20, 21]

Fig 2: Series of events taking place in periodontal tissues breakdown due to Periodontitis

Table 3 Animal and human studies supporting the association of periodontal disease and its treatment of Diabetes

Studied by	Results
[22],[23],[24]	Higher levels of HbA_{1c} & development of systemic complications of diabetes in periodontitis patients and also there is association of severe periodontitis and poorly controlled glycaemia.
[25]	It was observed that there is slight elevation in HbA_{1c} readings in non-diabetic patients with periodontitis and concluded that periodontitis may have the potential for the increase in the incidence of diabetes.
[26], [27]	The increase in resistance to insulin due to periodontal infections may impair the glycemic control. Also reducing the gingival inflammation by removing bacterial plaque accumulation can improve the glycemic levels
[26]	There is significant reduction in HbA_{1c} levels in type 2 Diabetic patients through non-surgical periodontal therapy.
[28]	Found that there is higher prevalence of IFG & lower homeostatic model assessment β in patients with chronic periodontitis than those with healthy periodontal tissues.
[29]	Tried to correlate between the albumin present in urine in diabetic patients with that of occurrence of periodontitis and found that the occurrence of periodontitis was relatively higher in albuminuria condition
[30]	Analyzed the risk involved in diabetic type 2 patients and correlated that there occurs severe periodontitis leading to alveolar loss in patients with uncontrolled glycemic condition to an extent of 6 times.
[31]	Suggested from a large scale study about reduction in the levels of HbA_{1c} in diabetic patients when subjected to periodontal treatment.
[32]	Interpreted the result of periodontal treatment in diabetes patients in terms of slight reduction of 0.29% in HbA_{1c} level leading to glycemic control. The study was conducted using Cochrane Database.
[33]	Found considerable improvement in glycemic control with reduction in HbA_{1c} to an extent of 0.46% when diabetic patients were subjected to effective periodontal treatment.
[34]	Showed decline in metabolism of glucose in ligature model (Zucker fatty rats) that are associated with periodontitis characterized by alveolar bone loss.
[35]	Marked higher production of cytokines (pro-inflammatory) in Diabetic patients with periodontitis and also observed a change in prevalence of macrophages as well as monocytes.

Inflammation: Central linking event between Periodontitis and hyperglycemic state: In hyperglycemic state, a higher rate of activation pathways responsible for inflammation has been observed which leads to microvascular and macrovascular complications, oxidative stress as well as apoptosis.[36] In the pathological manifestation of hyperglycemic events, a raised level of inflammatory markers such as: IL6, TNF-α and C-reactive protein has been reported which in turn induce acute-phase of inflammation leading to signaling impairment of insulin function and insulin resistance.[21,37,38] On the other hand, enhanced systemic inflammation due periodontal diseases mediated by the release of IL-6 and TNF-α has been also found to

be associated with enhancement of diabetic state. In addition, adipokines were known to regulate both the occurrence of periodontitis and diabetes.[39] Inflamed periodontal tissues of diabetes patients have higher level of prostaglandin E_2 and IL-1β when compared with the non-diabetics.[40] In another comparative study, a higher level of GCF(Gingival crevicular fluid) IL-1B was found in patients with higher percentage of HbA_{1c} (>8) and vice versa which establishes a correlation between the above two pathological state.[41]

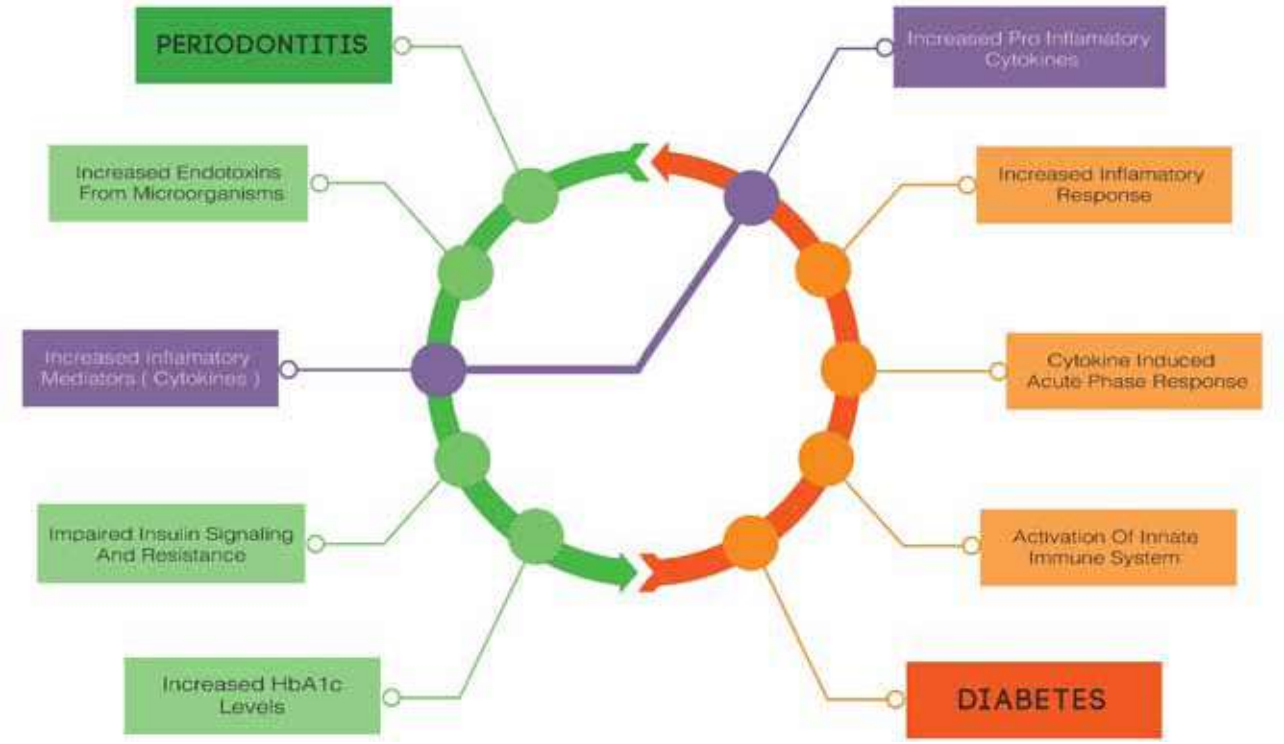


Fig 3: Central linking of events in the cyclic relationship between Periodontitis and Hyperglycemia

Conclusion

The extensive studies about the association between inflammatory periodontal disease and diabetes show the cyclic relationship between the two. One condition leads to the other and vice versa. Diabetes predisposes the individual to periodontal infections and also the later exacerbates or worsens the glycemic control in diabetic patients. Routine periodontal examination provides an insight for the early diagnosis of diabetes in undiagnosed patients and may reduce the worsening of metabolic control thereby preventing serious complications. Also the oral health of Diabetic patients may be improved and well maintained with proper management of blood glucose levels.

Although there are evidences in literature to establish correlation between Diabetes Mellitus associated with periodontitis and vice versa the outcomes are not conclusive. Therefore large-scale prospective epidemiological analysis are the only sought after approach to clearly bring in the connectivity between Diabetes and Periodontal disease

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Conflict of Interests: None

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Proteomics and Genomics: An Awesome Diversity in the Periodontal World

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Abstract

Proteins are the base structure for both the microorganisms as well as for the periodontal tissue. The periodontitis complex array is chiefly built by the cascade of a myriad of protein molecules. Hence, the science as well as the study of the complex molecule proteins as biomarkers in the periodontal world, has brought into the main focus since. This complex protein is the main factor in the development of the pathogenesis of periodontitis. Thereby, it is very much used as a diagnostic biomarker. The sound and rich facts of many proteins concerned in oral pathogenesis can be applied in the analysis, avoidance and management of periodontal disease. Hence, this review topic has been considered to understand the basics of the role of Proteomics and Genomics in the field of Periodontology.

Keywords: *Proteomics, Proteins, Periodontitis, Biomarkers.*

Introduction

A human cell operates because of the protein molecules present in them. As we know, every organic molecule subsides in our body is in the form of either a protein or the total resultant activity by the protein molecules. The phrase "proteome" is anamalgamation of the phrases "protein" and "genome" found in the mid-1990s by Marc Wilkins, an Australian geneticist. The proteome is the whole balance of proteins, prepared to a meticulous set of proteins, formed by aliving being. The same will fluctuate with certain period and individual needs, that a cell or creature faces. The word "proteomics" was primarily focused in 1997 to create a comparison using genomics, the learning of the genes. Proteomics is described as the learning of every proteins together with their comparative loads, circulation, posttranslational modifications, actions, and communications with various macromolecules, in a particular cell or organism within a given surroundings and at a definite phase in the cell series.[1]

Proteomics is a comparatively new 'post-genomic' knowledge with vigorous and full-blown potential. It is now accepted as the successful protocol during the research in the biological system. It comes just after the genomic study and the transcriptomics study.

It is considered as the most intricate and complicated procedure than the genomics. As we know proteomics is much more complicated than genomics mostly because the genome in an organism is quite and considerable and maintains the constant feature, whereas the proteomics keeps on fluctuating with time and with different types of cells. This shows that the basic protein sets produced by the cell requires an exact calculation.[2]

Proteomics and General Dentistry: The two key places where dental proteomics encompass revealed are 1. Salivary diagnostics and 2. Proteomics of bone and enamel. The saliva of the human being possesses proteins that can be useful for infection recognition and scrutiny of oral fitness. The broad chemical scrutiny

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and recognition of the proteomic particles in individual saliva is an essential initial footstep in the direction of the invention of saliva protein markers in oral diseases like Sjogren's syndrome. Particularly the proteomic biomarkers of the saliva have been recognizedfor three features, namely the process inflammation, degradation of the collagen, and turnover quality of the bone. Hence it ensures a diagnostic as well as a therapeutic approach with the help from the whole saliva content.[3]

Proteomics and Periodontics: Of late, with the help of the immunological method, the study of the fibroblast protein expression from periodontal ligament has been focused and studied extensively. Even though this system is restricted to earlier recognized proteins for which exact antibodies are obtainable. A sum of around 117 proteins been recognized out of the fibroblasts of the periodontal ligament. It can provide or present itself as a suggestion map for upcoming clinical studies and also at the same time for basic research.[4] Broad proteomic research is done on *P.gingivalis*. The chemicalanalysis of the complete proteomic cell have been carried out to explore the transformation from an extracellular to intracellular lifestyle for *P.gingivalis*.[4,5]

Salivary Proteomics for Periodontitis: Saliva is considered as an important periodontal diagnostic tool because of its various proteins present which acts as a potential biomarker in periodontitis. Young-Jin Choi et al studied the GCF in healthy individuals and periodontitis patients to study biomarkers. In GCF he spotted azurocidin known to be the upregulated protein in case of periodontitis case but never could spot the same in the saliva. This made him draw a conclusion saying that azurocidin can be a very significant and important biomarker for detecting early periodontal destruction in a prone patient. However, the protein detected azurocidin can also play as an inhibiting factor for the differentiation of osteoclasts and thereby can be a great boon for checking the alveolar loss in the early periodontitis stage.[6]

Customized Drugs: Proteomics and Genomics helps in discovering the new potential drugs for the disease treatment. To make the drug act successfully on the targeted disease, the information of the proteome and the genome data is mandatory for recognition of the particular protein which is causing the underlying disease. And once the protein is recognized, the help of the computer software is taken to find the chemical formula or the 3D structure to provide the data to design the customized drug, required for the deactivation of the particular protein which is the source of the disease. Once the molecule fits into the target and active site of the enzyme, and fails to get released anymore by the enzyme, the deactivation of the enzyme takes place. This is the standard protocol of new drug formation, and in turn it helps to locate the novel drugs to deactivate the involved protein in the disease. However, researchersutilize this procedure to procure personalized drugs effective for the person, as many genetic differences are found among individuals.[7]

Development of Biomarkers: The two most important research ground for implication of proteomicsin dentistry are salivary diagnostics, or oral fluid biomarkers, and proteomics of bone and enamel. As saliva is easily reached and simple collection procedure and also non-invasive, its appliance in clinical diagnostics is uncomplicated. "Oral Fluid Nano Sensor Test (OFNASET) is an advanced test for identifying the salivary proteins which are not easily detected. The consequence will be the capability to clinically identify oral malignancy ahead of its oral signs and symptoms.

Computational Method: There is a method attempted by a computer known as"Virtual Ligand Screening". This method has the property to adjust billions of very tiny molecules into the structure which is the three-dimensional structure of a protein. The computer efforts its caliber or the standard to meet the required purpose of the targeted protein sites. The main goal is to either intensify or to deactivate the meaning of the protein,checking on its position in the cell. An excellent exemplar of this is the recognition of novel drugs to aim and deactivate the HIV-1 protease. The HIV-1 protease is aboon enzyme that splita very bulky HIV protein into slighter, purposeful proteins. The above virus can neversustain without this enzyme; hence this protein aims at killing HIV.[8,9]

Limitation: As protein expression and post-translational modificationsare vibrant processes, particularly in the periodontium, recognition and frequency analysis of proteins only are notenough to appreciateits functional changes. The latest development in science will be essential to permit combinations of metabolic grouping and recognition and the frequency analysis of the rate of synthesis. Also Proteomicshypothesis conducted in a particular laboratory may notbe able to get reproduced in another laboratory.[4]

Future Directions of Genomics And Proteomics:

Single-Nucleotide Polymorphism: At present, several investigators are seriously focusing on the genetic single-nucleotide polymorphisms in the dental field. Right now, there seems to be the availability of a genetic vulnerability test for the diagnosis of severe chronic periodontitis shortly. Also, it helps in forecasting the salivary IL-8 levels to recognize if by chance the individual is vulnerable to squamous cell carcinoma.[9]

Nucleic Acid Probes: DNA probes were invented to recognize the sequences of nucleotide for a particular species of bacteria, hoping to be a fantastic diagnostic value. And both DNA and RNA probes are easily procured today for the research and daily practice. It is very much expected shortly, the laboratories for the mini molecules will come up, and the mini molecular kit will be obtainable for chair-side DNA probe.However, the guidance of a microbiologist might still at times be essential to keep away from trouble that may happen in the diagnostic procedure.[5]

Infrared Spectroscopy: Infrared spectroscopy (IRS) describes in detail the intricate biologic systems by captivating the fully IR spectrum which represents the summation of all the biomolecules present. The IR spectrum of the sulcular fluid is a loaded supply of fantastic data relatingto inflammation. IR analysis of GCF, counts the entire possession of the GCF and may provide evidence as a commanding diagnostic and prognostic means for periodontal diseases.[5]

Near-Infrared Spectroscopy: Near-Infrared Spectroscopy (NIR) is capable of keeping an eye on hemodynamic and edema-based markers of soft tissues like gingival inflammation.(5)

Molecular Biologic Technique: It uses the bacterial genome as a way of spotting the particular bacteria. DNA obtained from the dental plaque, are here critically analyzed during nucleic acid probes technique or polymerase chain reaction (PCR). Bacteria can be recognized when DNA obtained from dental plaque is send for the hybridization process, with group definite probes that are branded to permit visualization. Checkerboard hybridization is a method that utilizesthe probes at the same time for testing up to 43 bacterial variety. PCR implicates a DNA replicating enzyme (polymerase) to magnify the targeted DNA sequencing. The usage of standard PCR is not encouraged much as it is only limited to quantitative evaluation of recognized bacteria, although a technique called real-time PCR goes for the recognization of a particular species.[2]Now is the time for nanomaterials and microelectronics for the realistic operation with miniaturized sensors, appropriate for a range of significant applications. The latest Nano-Biochip technology processes fluids to supply a digital fingerprint that can be linked with the local chemical situation, detecting pH, electrolytes, metal cations, sugars, toxins, proteins, and antibodies.

Progress of Rapid Point-Of-Caree and Nano-Biochip: The progress of rapid point-of-care (POC) chair side diagnostics has the caliber for the before time recognition of periodontal illness and development and to diagnose it in the embryonic phase and lessen health concern expenditure.(identification pathogen).[6]

Circulating Micro RNA's As Biomarkers: Micro RNAs are post-transcriptional regulatorsto recognize disease-specific expression patterns. It can diagnose both the malignancy as well as the benign diseases.[8]

Tandem Mass Spectrometry: To carry the huge-scale proteomic analysis of GCF, utilizing gel electrophoresis for protein separation or "shotgun" approaches.It is taught that the knowledge of the crevicular fluid is mandatory to spot the healthy along with the important risk potential biomarkers.[1, 6]

Microfluidic Immunoassays: It is a fast saliva-based clinical method to diagnose the risk proteins which may cause the disease.It is carried by the saliva analysis by integrating sample pre-treatment with electrophoretic immunoassays.[3]

Salivary Proteome Project: This is by using the proteomic method, a standard proteome plot of the individual whole salivapermits for the resolution of bigger than 200 protein spots in a single two-dimensionalpolyacrylamide gel is carried out, which says it will spot the vulnerable candidates for carrying the particular disease. It is of great diagnostic and therapeutic significance.[3,10]

Conclusion

The utilization of proteomics and gene expression will move ahead of the identification and dealing of different dental pathological circumstances. There seems to be a huge opportunities therapeutically for the genomic therapy, tissue engineering, local drug delivery, and simultaneously biopharmaceutical and definitely

will flourish. However, but not impossible, its entry into the area of the dental field depends on how finest dental doctors will fit this into their tradition as it demands meticulous information of individual genetics and implement of novel analytic and remedial technologies.

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Periodontal Compromised Cases: The Patience and Perseverance That Bears the Fruit

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Abstract

Orthodontic treatment in periodontally compromised cases is quite challenging as it involves a thorough understanding of the periodontium keeping given the compromising factors. Bone health plays a pivotal role in patients opting for orthodontic treatment. Here, we are presenting a case series of periodontally compromised patients being treated Orthodontically.

Keywords: *Orthodontics, periodontics, compromised.*

Introduction

Case-1: A 28 years old female patient came to our dental office with a chief complaint of large gaps between teeth and bad smile. She had a very shy and conscious smile due to obvious reasons. Reasons for Orthodontic treatment was primarily esthetics. She was found to have Angle's Class I malocclusion on skeletal class I jaw bases, with anterior spacing, severe periodontal destruction in maxillary and mandibular jaws.

Treatment plan: The patient had undergone a non-extraction line of treatment, leveling and alignment, maxillary space closure, followed by bijaw, lifetime bonded lingual retainers in the anterior region (4 to 4) creating anesthetic smile. Figure. 1 depicts the patient's pre and post-treatment extraoral comparisons and Figure. 2 shows the intraoral changes before and post Orthodontic treatment.



Figure 1: Pre and post-treatment comparison (extraoral)

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Figure 2. Pre And Post Treatment Comparison (Intraoral)

Case-2: A 58yrs old female patient met us in the Orthodontic set up with the chief complaint of gaps between front teeth, which was making her smile uncomfortable. Reasons for Orthodontic treatment was primarily esthetics. Her doctor son's marriage was scheduled after 1 yr. she wanted to have good confident smiling photographs in the marriage album. She had Angle's Class I malocclusion on skeletal class I jaw bases, with anterior spacing, with grave periodontal destruction in maxillary and mandibular jaws.

Treatment Plan: It was planned to do non-extraction treatment, leveling and alignment, maxillary and mandibular space closure, followed by long term bonded lingual retainers in anterior region (4 to 4) of both the jaws, creating an confident photogenic smile. The pretreatment, mid-treatment, and post-treatment pictures are presented in Figure 3.

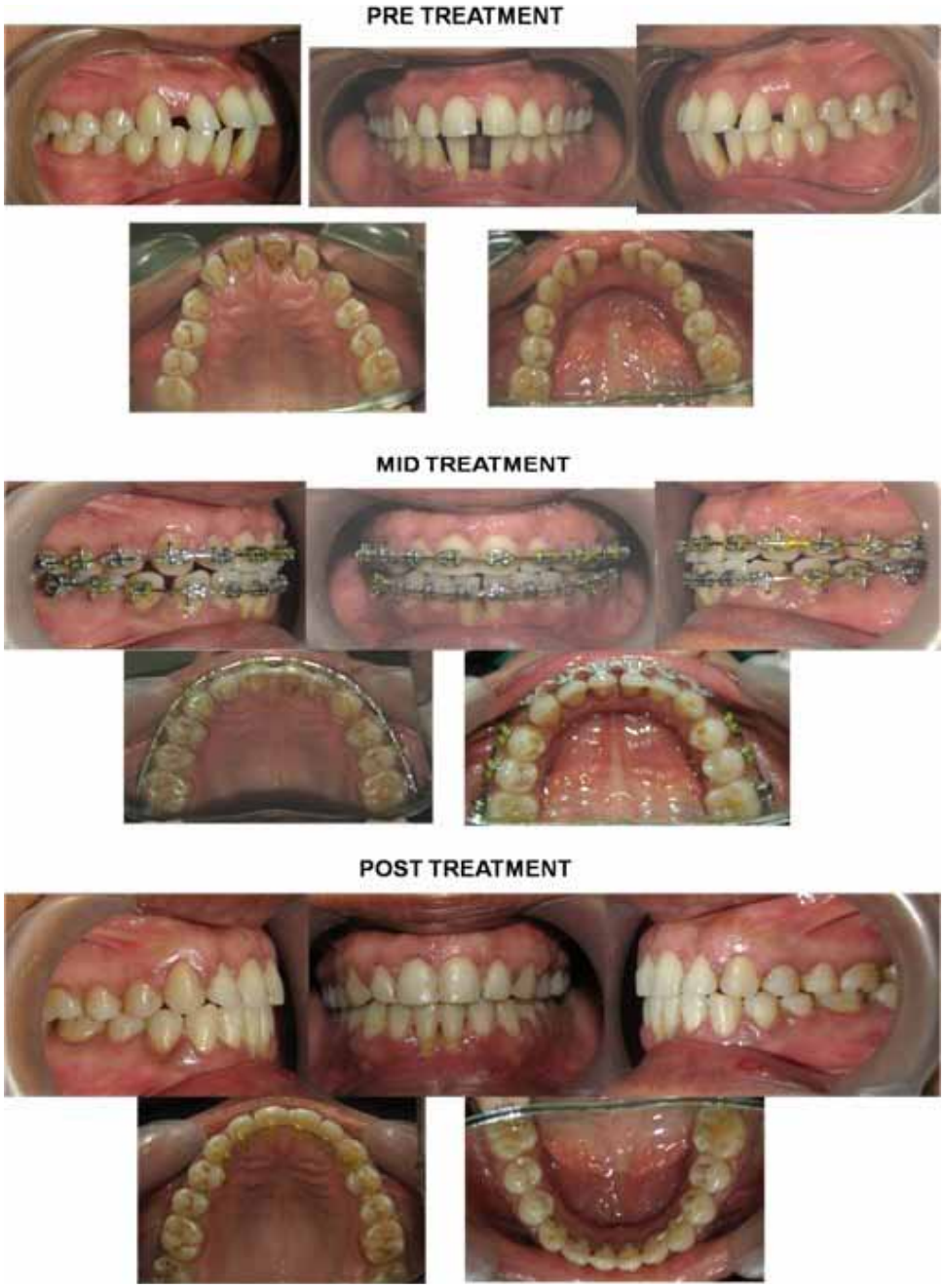


Figure 3. Pretreatment, Midtreatment, Posttreatment Comparisons

Case-3: A 35-year-old female patient had chief complaints of forwardly placed upper front teeth with gaps in between. The patient wanted a better smile as she was pursuing marriage. She had a convex profile, acute nasolabial angle, Liptrap, deep mentolabialsulcus, and Orthognathic face. She had Class- II malocclusion on a Class-I skeletal jaw base with increased overjet, overbite, Trauma from occlusion, spacing in upper anterior, crowding in lower anterior, increased curve of spee and harsh periodontal condition in both the jaws.

Treatment Plan: It was planned to do the upper first premolar (14, 24 extraction), leveling, alignment, maxillary retraction with maximum anchorage, finishing in good overjet, overbite, Class-II molars & Class-I canines bilaterally, resulting a pleasant noteworthy smile. Retainers planned were long term bonded lingual retainers for both the jaws. The pretreatment pictures are depicted in Figure 4. The post-treatment pictures are presented subsequently in Figure.5.



Figure 4. Pretreatment View



Figure 5. Post-treatment view

Conclusion

All these above three cases were adults with a primary concern of esthetics.[1,2] The patients were not worried about the underlying periodontal destruction. The key to our success in these cases are: the first and foremost objective was to make the patients aware of periodontal issue and a change in their attitude. It was of paramount importance to improve their oral hygiene habit to a more acceptable level by positive motivation and periodic oral prophylaxis. This was inevitable for a successful treatment and avoidance of relapse in the long term.[3,4] Repeated reminders to behavioral change and periodic periodontal evaluation was continued throughout the treatment as well as post-treatment. The non-extraction cases were treated with moving the teeth towards the midline so that the tongue space won't be impinged, which shall be detrimental with the already compromised periodontium, during the retention phase. Light forces with longer periods (6wks) of activation interval.[5] Self motivation of all the patients. Without their contribution in the form of persistent oral hygiene maintenance the results were unfeasible.

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Knowledge and Attitudes of Dental Students in Prescribing Antibiotics for Pediatric Dental Patients in Bhubaneswar City, Odisha: A Cross-Sectional Study

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Abstract

Background: Antibiotics are the most widely prescribed therapeutic agents in dentistry, hence it represents an important aspect of dental practice. The inept use of antibiotics for the treatment of dental infections in children is a leading cause of developing microbial resistance.

Objective: The objective of this study is to investigate the knowledge & attitudes of a group of dental students when prescribing antibiotics during the treatment of pediatric dental patients.

Method: This is a questionnaire-based cross-sectional study conducted in a group of 300 dental students from various dental colleges of Bhubaneswar city, Odisha. The questionnaire consisted of close-ended questions, structured under three main headings. Data collected were analyzed using SPSS statistics version 24.

Results: The prime findings were that amoxicillin is the most commonly prescribed antibiotic, most students had not received training on antibiotic prescribing and had treated more than 10 child patients per week & their concordance with guidelines on antibiotic prescribing was generally low.

Conclusion: This cross-sectional study shows there is a need to improve the knowledge on antibiotic prescribing for children.

Keywords: *Dental education, dental students. pediatric antibiotics, antibiotic prophylaxis, antibiotic resistance.*

Introduction

Antibiotics are the most extensively prescribed 'therapeutic & prophylactic' agents in dentistry, hence it represent an important aspect of dental practice.[1,2,3] Prophylactic antibiotics are used for the prevention of diseases caused by a number of the oral microflora, mostly in the case of endocarditis prevention.[4] Therapeutic antibiotics are used for the hard & soft tissue diseases in the oral cavity when local debridement is unsuccessful.[5] Antibiotics in dentistry are generally prescribed for surgical,endodontic & periodontal manifestations. The inept use of antibiotic in treating children has been observed in the treatment of dental infections, is a leading cause in developing microbial resistance.[6-9] "Dr. Thomas J. Pallasch, stated in his study that antibiotic misuse in dentistry mainly involves prescribing them in 'inappropriate situations' or for too long, which includes-giving antibiotics after a dental procedure is complete in an otherwise healthy patient

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to 'prevent' an infection, which in all likelihood will not occur".[10] So to avoid any unwanted complications, dental practitioners often prescribe the antibiotics.[11] This has led to developing microbial resistance, which is a serious health issue concern.

And this is a growing major health problem that contributes to treatment failure and development of antibiotic resistance by an individual.[12-15]

Method

This is a questionnaire-based study conducted in a group of 300 dental students from various dental colleges of Bhubaneswar city, Odisha to know the knowledge & attitudes of dental students in prescribing antibiotics for pediatric dental patients. A structured pre-prepared questionnaire was distributed to all participants via email (using google forms). The questionnaire consisted of close-ended questions & open-ended questions, structured under 3main headings are as follows:

1. Details about dental student participants based on their gender & qualification.
2. General knowledge about antibiotics prescription in pediatric dentistry.
3. Knowledge of dental student participants on prescribing antibiotics for various clinical situations.

Statistical Analysis: Data collected under the study was scrutinized, codified and entered into the IBM SPSS Statistics, 24.0 software, for analysis. The following statistics procedure was used for the analysis of data. There were 10 different questions canvassed to the respondents. The association of response with gender, qualification and training was studied using the Chi-square test of independence. The significant p-value was <0.05.

Results

In this cross-sectional study of knowledge and attitude of 300 dental final year and interns for prescribing antibiotic during the treatment of pediatric dental patients, 148 (49.3%) were BDS final year students and 152 (50.7%) BDS interns, of which 110 (36.6%) were males and 190 (63.4%) females. Besides the sample has 36 (12%) students who have taken any course/training in antibiotics in the past 2 years. The response of the sample students have been analyzed for association with gender, qualification (interns or final year students) and training on antibiotics.

Table 1 revealed that 117 (39.0%) update their knowledge about antibiotics through textbooks, only 44 (14.7%) through online resources and a maximum of 139 (46.3%) through both textbooks & online resources. The majority of the sample students i.e. 182 (60.7%) practiced more than or equal to 10 therapeutic antibiotic prescriptions per week and the remaining 39.3% practiced less than 10 prescriptions per week. The most commonly prescribed antibiotic for the treatment of pediatric dental patients was a combination of Amoxycillin + Clavulanic acid as stated by 82.3% of the students followed by Amoxycillin (7.0%). About 81% of students stated that the most common number of daily dose prescribed was BID while 16.7% of students stated that to be TID. About 2/3rd students said that the duration of antibiotics should be for 5 days, while 17.3 stated it to be 3 days and 16.7% said it to be for more than 5 days. Little above 2/3rd of sample students (68.7%) viewed that the most favourable antibiotic for penicillin-sensitive patients was Erythromycin while 31.3% preferred to Clindamycin. All the sample students preferred that the most common route of antibiotic administration was "Oral". About 77% of students preferred to see the patients before prescribing antibiotics for dental pain. About 3/4th students opined that they would prefer to prescribe antibiotics in carious primary tooth for pain, localized swelling after evidence of radiographic pathology. In the case of deep caries in primary teeth half of the students (50%) prefer antibiotics for pain, draining fistula, localized swelling only after the radiographic evidence confirmation($p=0.001$).

Table 1 further revealed that the source of updating the knowledge has a significantly higher proportion of male students who are using both the sources to update the knowledge of antibiotics ($p= 0.010$). As regards most commonly prescribed antibiotic- 9.1% male stated three-drug therapy & 3.1% female stated so, a number of daily doses – TID Male 25.5% and female 11.6%, duration in days- 5 days 57.3% males & 71.1% females., most favorable penicillin-sensitive antibiotic- male: erythromycin- 60% & clindamycin- 40% for female the proportions were 73.7% & 26.3% respectively, preferring to see patients before prescribing antibiotics for dental pain- male 62.7% & female 85.3% and preferring to prescribe an antibiotic in the carious primary tooth for pain, localized swelling with evidence of radiographic pathology- male 60% & female 84.2% have a significant association with gender ($p<0.05$).

Table 2 revealed that the source of updating the knowledge- interns have significantly higher proportion who are using both the sources to update the knowledge of antibiotic (p= 0.000). As regards number of daily dose – Interns: BID-94.7%,TID-3.9%, BDS final year student: BID-66.9%,TID-29.7%, duration in days- BDS final year - 5 days 54.1%, 3 days 21.6%, the corresponding proportion for interns were 77.6% & 13.2% respectively, and most favourable penicillin-sensitive antibiotic- BDS final year: erythromycin 60.8% & clindamycin 39.2% for Interns the proportions were 76.3% & 23.7% respectively, have significant association with qualification (p<0.05).

Table 3 revealed that the students who have received training in antibiotics in the past 2 years mostly refer to the textbook (66.7%) while students without training refer to both textbooks and online resources for updating the knowledge about the use of antibiotics (p=0.000). Students with training prescribed single drugs like Amoxicillin (44.4%) and a combination of drugs Amoxicillin+Clavulanic acid (50.0%) but students without training mostly prescribed combination drugs Amoxicillin+Clavulanic acid (86.7%) (p=0.000). Students with training never have prescribed TID while (18.9%) students without training have prescribed TID (p=0.000). Students with training usually prescribed antibiotics either for 3 days (58.3%) or 5 days (41.7%) and none prescribed for more than 5 days. However (18.9%) students without training prescribed antibioticsfor more than 5 days (p=0.000). All the students with training prescribed Erythromycin while (64.4%) students without training prescribed Erythromycin while (35.6%) prescribed Clindamycin (p=0.000). All the students with training prefer to see patients before prescribing antibiotics for dental pain while (26.1%) students without training did not prefer to see before prescribing antibiotics (p=0.000). All the students with training prefer to prescribe antibiotics in carious primary tooth for pain, localized swelling with evidence of radiographic pathology while (72.0%) of students without training prefer to do so (p=0.000).In the absence of fever, 41.7 % of students with training prefer to prescribe antibiotics in deep carious primary tooth for pain, draining fistula, localized swelling without confirming with a radiograph (p=0.000).

Table 1: Association of Response to different questions with gender

Questions/Response	Gender		Total	c2, p
	Male	Female		
	No. (%)	No. (%)	No. (%)	
Updating knowledge about the use of antibiotics in paediatric dentistry?				
Text book	39(35.5)	78(41.1)	117(39)	c2=9.311 p=0.010
Online resources	9(8.2)	35(18.4)	44(14.7)	
Both of the above	62(56.4)	77(40.5)	139(46.3)	
No. of therapeutic antibiotic prescriptions per week				
Less than 10	48(43.6)	70(36.8)	118(39.3)	c2=1.348 p=0.246
More than 10	62(56.4)	120(63.2)	182(60.7)	
Most commonly prescribed antibiotic				
Amoxicillin	0(0)	21(11.1)	21(7)	c2=18.156 p=0.001
Metronidazole	2(1.8)	3(1.6)	5(1.7)	
Amoxicillin +Clavulanic acid	92(83.6)	155(81.6)	247(82.3)	
Amoxicillin +Metronidazole	6(5.5)	4(2.1)	10(3.3)	
Amoxicillin+ Clavulanic acid + Metronidazole	10(9.1)	7(3.7)	17(5.7)	
Number of daily dose				
Once	0(0)	7(3.7)	7(2.3)	c2=12.994 p=0.002
BID	82(74.5)	161(84.7)	243(81)	
TID	28(25.5)	22(11.6)	50(16.7)	

Questions/Response	Gender		Total	c2, p
	Male	Female		
	No. (%)	No. (%)	No. (%)	
Duration in days				
3 days	20(18.2)	32(16.8)	52(17.3)	c2=8.545 p=0.014
5 days	63(57.3)	135(71.1)	198(66)	
More than 5 days	27(24.5)	23(12.1)	50(16.7)	
Most favourable antibiotic for penicillin-sensitive patients?				
Erythromycin	66(60)	140(73.7)	206(68.7)	c2=6.063 p=0.014
Clindamycin	44(40)	50(26.3)	94(31.3)	
Most common route of antibiotic administration preferred				
Oral	110(100)	190(100)	300(100)	*
Intravenous	0(0)	0(0)	0(0)	
Preferring to see patients before prescribing antibiotics for dental pain?				
Yes	69(62.7)	162(85.3)	231(77)	c2=19.978 p=0.000
No	41(37.3)	28(14.7)	69(23)	
Do you prefer to prescribe antibiotic in carious primary tooth for pain, localized swelling with evidence of radiographic pathology				
Yes	66(60)	160(84.2)	226(75.3)	c2=21.975 p=0.000
No	44(40)	30(15.8)	74(24.7)	
Prefer to prescribe antibiotics for deep caries in primary teeth along with draining fistula with no fever?				
Pain with draining fistula only	0(0)	17(8.9)	17(5.7)	c2=15.652 p=0.001
Pain, draining fistula & local swelling without radiographic evidence	18(16.4)	15(7.9)	33(11)	
Pain, draining fistula & local swelling with radiographic evidence	51(46.4)	99(52.1)	150(50)	
Pain, draining fistula & facial swelling with radiographic evidence	41(37.3)	59(31.1)	100(33.3)	
Total	**110(100)**	**190(100)**	**300(100)**	

Table 2: Association of Response to different questions with Qualification

Questions/Response	Qualification		c2, p
	BDS Final Year	Interns	
	No. (%)	No. (%)	
How you updated your knowledge about the use of antibiotics in pediatric dentistry?			
Textbook	87(58.8)	30(19.7)	c2=51.463 p=0.000
Online resources	20(13.5)	24(15.8)	
Both of the above	41(27.7)	98(64.5)	
No. of therapeutic antibiotic prescriptions per week in pediatric dental patients			
Less than 10	61(41.2)	57(37.5)	c2=0.434 p=0.510
More than 10	87(58.8)	95(62.5)	

Questions/Response	Qualification		c2, p
	BDS Final Year	Interns	
	No. (%)	No. (%)	
Most commonly prescribed antibiotic			
Amoxicillin	12(8.1)	9(5.9)	c2=2.547 p=0.636
Metronidazole	3(2)	2(1.3)	
Amoxicillin +Clavulanic acid	121(81.8)	126(82.9)	
Amoxicillin +Metronidazole	6(4.1)	4(2.6)	
Amoxicillin+ Clavulanic acid+ Metronidazole	6(4.1)	11(7.2)	
No of daily dose			
Once	5(3.4)	2(1.3)	c2=38.453 p=0.000
BID	99(66.9)	144(94.7)	
TID	44(29.7)	6(3.9)	
Duration in days			
3 days	32(21.6)	20(13.2)	c2=19.692 p=0.000
5 days	80(54.1)	118(77.6)	
more than 5 days	36(24.3)	14(9.2)	
Most favourable antibiotic for penicillin-sensitive patients			
Erythromycin	90(60.8)	116(76.3)	c2=8.379 p=0.004
Clindamycin	58(39.2)	36(23.7)	
Most common route of antibiotic administration preferred by you			
Oral	148(100)	152(100)	*
Intravenous	0(0)	0(0)	
Preferring to see patients before prescribing antibiotics for dental pain			
Yes	111(75)	120(78.9)	c2=0.660 p=0.417
No	37(25)	32(21.1)	
Prefer to prescribe antibiotic in carious primary tooth for pain, localized swelling with evidence of radiographic pathology			
Yes	111(75)	115(75.7)	c2=0.017 p=0.895
No	37(25)	37(24.3)	
Would you prefer to prescribe antibiotics for deep caries in primary teeth along with draining fistula with no fever?			
Pain with draining fistula only	3(2)	14(9.2)	c2=27.129 p=0.000
Pain, draining fistula & local swelling without radiographic evidence	18(12.2)	15(9.9)	
Pain, draining fistula & local swelling with radiographic evidence	59(39.9)	91(59.9)	
Pain, draining fistula & facial swelling with radiographic evidence	68(45.9)	32(21.1)	
Total	**148(100)**	**152(100)**	

Table 3: Association of Response to different questions with Training

Questions/Response	Any courses/training in antibiotics in the past 2 years?		c2, p
	Yes	No	
	No. (%)	No. (%)	
How you updated your knowledge about the use of antibiotics in paediatric dentistry?			
Textbook	24(66.7)	93(35.2)	c2=27.506 p=0.000
Online resources	10(27.8)	34(12.9)	
Both of the above	2(5.6)	137(51.9)	

Questions/Response	Any courses/training in antibiotics in the past 2 years?		c2, p
	Yes	No	
	No. (%)	No. (%)	
No. of therapeutic antibiotic prescriptions per week in pediatric dental patients?			
Less than 10	34(94.4)	84(31.8)	c2=52.070 p=0.000
More than 10	2(5.6)	180(68.2)	
Most commonly prescribed antibiotic			
Amoxicillin	16(44.4)	5(1.9)	c2=94.529 p=0.000
Metronidazole	2(5.6)	3(1.1)	
Amoxicillin +Clavulanic acid	18(50)	229(86.7)	
Amoxicillin +Metronidazole	0(0)	10(3.8)	
Amoxicillin+ Clavulanic acid+ Metronidazole	0(0)	17(6.4)	
No of daily dose			
Once	6(16.7)	1(0.4)	c2=42.865 p=0.000
BID	30(83.3)	213(80.7)	
TID	0(0)	50(18.9)	
Duration in days			
3 days	21(58.3)	31(11.7)	c2=50.162 p=0.000
5 days	15(41.7)	183(69.3)	
More than 5 days	0(0)	50(18.9)	
Most favourable antibiotic for penicillin-sensitive patients?			
Erythromycin	36(100)	170(64.4)	c2=18.667 p=0.000
Clindamycin	0(0)	94(35.6)	
Most common route of antibiotic administration preferred by you			
Oral	36(100)	264(100)	*
Intravenous	0(0)	0(0)	
Would you prefer to see patients before prescribing antibiotics for dental pain?			
Yes	36(100)	195(73.9)	c2=12.220 p=0.000
No	0(0)	69(26.1)	
Do you prefer to prescribe antibiotic in carious primary tooth for pain, localized swelling with evidence of radiographic pathology			
Yes	36(100)	190(72)	c2=13.395 p=0.000
No	0(0)	74(28)	
Would you prefer to prescribe antibiotics for deep caries in primary teeth along with draining fistula with no fever?			
Pain with draining fistula only	9(25)	8(3)	c2=77.868 p=0.000
Pain, draining fistula & local swelling without radiographic evidence	15(41.7)	18(6.8)	
Pain, draining fistula & local swelling with radiographic evidence	12(33.3)	138(52.3)	
Pain, draining fistula & facial swelling with radiographic evidence	0(0)	100(37.9)	
Total	**36(100)**	**264(100)**	

Discussion

"Amoxicillin is the preferred 1st choice antibiotic for treatment of dental infections in children".[16,17] Combination of amoxicillin-clavulanic acid can also be used, as it has activity against the beta-lactamase.[18] But in this study, the majority of participants preferred amoxicillin-clavulanic acid followed by amoxicillin. Also a significant proportion of prescribes combination antibiotics like amoxicillin/clavulanic acid with metronidazole. Combined drug prescription is becoming more significant in the case of mixed infections. Most participants would prefer to prescribe for 5 days. "The ideal duration of antibiotic treatment is the shortest cycle capable of preventing both clinical & microbiological relapse. Most acute infections are resolved within three to seven days". Short courses are chosen mostly when treating children due to their poor cooperation.

The prime findings were that most students had not received training on antibiotic prescribing and had treated more than 10 child patients per week & their concordance with guidelines on antibiotic prescribing was generally low.Those who had received training and those with more clinical experience are more likely to be knowledgeable.

A similar studywas conducted,showing a similar result highlighting the need to improve education in antibiotic prescription among dental students.[19,20] Up-gradation of antibiotic prescribing guidelines among dental students is also important for the treatment of dental infections in children.Antibiotic prescribing education may be done through lectures, workshops, informal education in clinical practice & self-directed learning.

Such education will instill greater self-confidence in reducing antibiotic prescribing unnecessarily & will lead to a change in antibiotic prescribing behaviour.

Conclusion

This cross-sectional study indicates the necessity to improve the "knowledge on antibiotic prescribing for children".Besides, the study states that there is a requisite for short programs to update knowledge of dental practitioners on the antibiotics, which are currently used in pediatric dentistry.

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Stem Cells in Regenerative Dentistry: An Overview

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Abstract

The study of stem cells in regenerative dentistry is the most precious in-progress research. Stem cells are the cells that have the self-renewal capacity and can differentiate to mature cells. Stemcells also have the prospective to form dentin matrix, bone and neuronal cellsrapidly. Stem cell-based therapeutic approaches provide a better and attractive alternative that the classical dental treatments because of their questionable efficacy and durability. The most promising alternative for tooth loss is the utilization of stem cells and induced pluripotent for the regeneration of the whole tooth. Therefore helping with the progress in inducing bone rejuvenation, treating damaged teeth, stem cell-based therapy plays a vital role. So, this article will highlight the role and importance of stem cells in regeneration in Dentistry.

Keywords: *Stem Cells; Regenerative Dentistry; Therapeutics; Future Prospects.*

Introduction

Stem cells are defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation since they are thought to be undifferentiated cells with varying degrees of potency and plasticity.[1] In all multi-cellular organisms, these primitive types of cells i.e. presence of stem cellsalong with the properties of self-renewal in addition to the potential to change into mature cell types. They show an impressive capacity for regeneration and perhaps could be utilized for the treatment of diseases like cancer, Parkinson's, Alzheimer and many more, with its ability to replace or repair of the damaged cells.[2] The stem cells can be classified as,[3]

Classification of stem cells:

The classification of stem cells are the following:

1. Embryonic stem cells (ESCs)
2. Adult stem cells (ASCs)
    a. Hematopoietic stem cells (HSCs)
    b. Mesenchymal stem cells (MSCs)
3. Induced pluripotent stem cells (IPSCs)

The identification of orofacial tissues as a foundation and therapeutic objective with clinical curiosity in dentistry for stem cells. The main properties of stem cells were first defined by Ernest McCulloh and James till in the 1960s.[4] The capabilities of the stem cells are mainly; produce cells having the same characteristics (daughter cells) and produce cells that have different properties. Mesenchymal stem cells are interpreted as multipotent because they tended to display the capacity for segregation into cells of a different variety, even as the mesenchymal stem cells are clonally isolated cells. They have the skill to differentiate along the other particular mesenchymal ancestry, and also can stay in an inactive undifferentiated state until offered the indication to segregate asymmetrically.[5]

Dental stem cells, which are derived from postnatal stem cells, are one of the most recent types of stem cells found in the mesenchymal stem cells (MSC) list. It shows capabilities based on development, differentiation, regeneration, and immunoregulatory/ immunomodulatoryproperties.[6]Afterthediscovery,study and progress towards medicalapplications of stem cells had made so much progress. For bone and dental regeneration, cell sources from dental pulp stem cells (DPSCs), periodontal ligament stem cells (PLSCs), stem cells from human exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP)and dental follicle cells are taken due to their accessibility and abundance.[7]

Dental stem cells in India are still at the budding

stage, and there seems to be limited awareness regarding dental stem cells. SHED is obtained from baby teeth, the multipotent stem cells population are easily collected as the characteristics are either shed naturally or are removed physically for the suitable growth of permanent teeth, of the deciduous teeth.

The different diseases and trauma causes bone and dental loss and defects which has become a worldwide distress of elevated frequency. This affects the physical condition and wellbeing of a very big population and also put down a grave economic load on the public.[1,2] The present therapies done for dental diseases can only provide an improvement based on parameters of clinical diagnosis and arrest development of disease, although regeneration of missing tissue is not able to be done. Therefore, there is a high demand for new technologies that can help to accomplish the regeneration of bone and dental tissues.[8,9]

Table 1. History of Stem Cell[10]

1868	German biologist, Haeckel mentioned the term "Stem Cells" in his research works.
1908	Russian histologist, Alexander Maksimov hypothesized the subsistence of Hematopoietic stem cells
1932	G. L. Feldman demonstrated proof of regeneration of pulp in favorable conditions.
1985	Yamamura reports that pulp tissues contain stem cells.
2000	Gronthos et al separate dental pulp stem cells from human dental pulp of an adult thatcan regenerate complexes like dentin pulp.
2003	Miura et al reports the isolation of population of stem cells from the living pulp remnants of exfoliated tooth.
2004	Seo et al discovered the periodontal ligament stem cells from human periodontal ligament.
2005	Morsczeck et al and kemoun et al each of them discovered exclusive lineage-committed cells that cant be differentiated with the possession of features of progenitor mesenchymal in dental follicles of human.
2006	Sonoyama et al identified and characterized stem cells from dental apical papilla of human permanent immature teeth.
2009	Zhang et al informed the separation of a stem cell from the human gingiva's spinous layer.

Applications in dentistry:

The isolation and characterization of human dental stem cells are:[7]

1. Stem cells from human exfoliated deciduous teeth
2. Dental Pulp Stem Cells
3. Periodontal ligament stem cells
4. Stem cells from apical papilla

The different separation, culturing and differentiation procedures have highly developed over the decade for the various MSCs. The first isolation of mesenchymal stem cells was done from bone marrow, they appeared like cells similar to fibroblast and were capable of sticking to plastic dishes, and the single cells form colonies which divide to form mature cells of the mesenchymal pedigree which are osteoblasts and chondrocytes. The most familiar resource of Dental mesenchymal stem cells (DMSCs) is dental pulp stem cells (DPSCs), which were first isolated from human teeth in the year 2000.[4] These organs are the vital pool of adult stem cell populations as they have a parallel purpose to mesenchymal stem cells. Hence, DMSCs can be utilized in favor of rejuvenation of teeth or other organs that have essential restore possibility. The dental pulp stem cells are considered a rich source of mesenchymal stem cells due to the number of potential stem cells isolated from the human tissue samples, adding to its advantages apart from the cell number, is that lesser time consumption in the process of enzymaticabsorption from the dental tissues by extracting stem cells when compared with the other mesenchymal stem cell sources. These features make the dental stem cells less vulnerable to potential enzymatic stress and help to expand rapidly than other cells. Hence, even if from a tiny amount of dental tissue, they are extracted, yet latently adequate quantity of cells for a different type of clinical applications can be generated.[5]

The dental pulp stem cells are an amazing source of stem cells believing their multipotent differentiation potential. Under in vitro culture conditions, they are differentiated into odontogenic cell types, but apart from that, it has been also reported to distinguish into osteoblasts, chondrocytes, myocytes, neurons, melanocytes, hepatocyte-like cells and adipocytes.[5] The DMSCs in the dental pulp are situated normally in two slots, they are

1. Apical niche
2. Perivascular niche

These two types of niche hasa separate role for themselves, but in a way, their role is exchangeable at times.[4]

Mesenchymal stem cells have the perspective in support of the revival of dental tissues. Deciduous teeth contain residents of added undeveloped multipotent stem cells, that are competent in forming dentin like structures although not an entire dentin-pulp complex. Teeth contain well-defined pulp chambers, odontoblasts, pre dentin and dentin, and also enamel organ consisting of dental enamel, ameloblasts, stratum reticulum, stratum intermedium. The DPSCs are skilled in forming a reparative dentine like structure which is present straight on the surface of dentin.[11]

The lost regenerative ability within the PDL space to be domesticated is the foremost goal of the gene-enhanced regeneration of periodontal ligament. This technique has the maximum capability if it can be tailored for use with entirely adult cells, despite gene enhanced tissue engineering exercised within concurrence with stem cells.[9]

The different ways on how the regenerative procedures can take place are:

1. **Pulpal regeneration:** Pulpal tissue regeneration is done mainly by postnatal stem cells. To reduce the chances of immune response allogeneicstem cells can be used. Gene therapy and 3d printing are the newer modalities for perfectly placing the cells in their proper place which may also lead to the formation of new dentin.[8,12]
2. **Periodontium Regeneration:** Periodontal tissue surgeries are quite complex as it requires various factors to facilitate the production of a sufficient amount of tissues. Alveolar bone, periodontal ligament and cementum can be derived from bone marrow stem cells. Periodontal stem cells are started with biocompatible scaffolding helps to generate a 3D matrix which incorporated with various growth factors that are reported to the area which necessitates regeneration.[8,13]
3. **Facial tissue Regeneration:** After the treatment of a large oral lesion involving facial tissues, reconstruction will play an important role. Collagen along with myoblasts can be engineered along with various growth factors for reconstruction. For the correction of large defects, bone marrow-derived stem cells can be used.[8,14]
4. **Tooth Regeneration**- Epithelial and mesenchymal cells and the cells capable to differentiate to from tooth buds. The use of prenatal stem cells is more popular for regeneration because of their propensity. After tooth loss, the most ideal therapeutic approach would be the rejuvenation of the whole tooth. The different kinds of approaches that can be taken is, the formation of tooth germs which is prepared by the union of DMSCs, and DESCs in the alveolar bone is transplanted where the process of development, eruption and finally becoming functional tooth of germs is seen. An additional approach is implantation into the jaw of tooth-shaped polymeric biodegradable scaffolds packed with DESCs and DMSCs to rejuvenate a new tooth. Well, the experiments have been done only in mice, as the results have not been obtained in human cells.[4,15-17]
5. **Bone Regeneration:** Periosteal stem cells can be used for this. For closure of various bony defects, inorganic bone along with plasma rich platelets can be used. Mesenchymal stem cells along with autogenic stem cells can be used for TMJ reconstruction. The production of bioengineered TMJ is done by the adult mesenchymal cell in a biomimetic scaffold. The scaffolds which are strengthened with the mi-RNA showed improved osteoinductive properties and functionality.[8,18]

Tooth Stem Cell Banking: It is an unpopular trend around the world currently, but most of the developed countries have started the acceptance of stem cell banking. The different types of stem cell banking companies around the world are Stem Save (Stemsave Inc, New York) and Store-A-Tooth (Provia Laboratories, Littleton, Massachusetts, USA). The first tooth bank in Japan was instituted in Hiroshima University and was

named 'Three Brackets' company in 2005. Recently the thought of dental stem cell deposition was launched in Delhi and Mumbai in our country.[7]

The dentists would have immense prospect to make their patients attentive of the potential of the regenerative uses of stem cells, only if the stem cell banking system is matured and confirmed to be effective and safe, and can encourage the patients to store their dental mesenchymal stem cells for further medical usage during any kind of treatment. The different boons of dental stem cell deposition are that it can be stored in second chance if the first chance is failed to spot, unlike blood stem cells from the umbilical cord. The isolation procedure is painless and simple; also banking of stem cells can provide an ideal source of iPS banking. It also provides for future therapeutic uses, an autologous cell source. Adding to its numerous advantages, it is also not focused on any ethical concerns like the embryonic stem cells.[5]

Future Research and Prospects: The MSC-based regeneration researches have shown tremendous results in theremedy bone and dental loss and defects, both by exogenous transplantation and endogenous restoration. But as mentioned by Chengxi Zhang et al[1], the MSC-mediated regeneration is under tight charge of the micro environment based on its remedial efficiency, with the capability of controlling inhabitant MSCs under pathological and physical conditions and also adapt MSCs that are transplanted in tissue engineering and cytotherapy. As a result, the biggest challenge remains the accomplishment of MSC based dental and bone regeneration in an unhealthy microenvironment.[16]

T. C. Srijaya et al[5] mentioned about the huge amount of curiosity of dental stem cells in medical research because of their budding function in regenerative medicine. They have elevated capability of regenerating which has given higher expectations for therapeutics in the future. However, to achieve the complete therapeutic capability of dental stem cells much more work and research is to be done. In the future, for the research of dental stem cells, the biomaterials used and the diseases associated with teeth many more efforts and advances are very essential.

The environment in addition to conditions under which the stem cell research takes place and how it would effectively help in dental procedures are still a bigger problem to solve. The different clinical trials and researches have been going on stem cells and have not been fully studied. Moreover, in tooth regeneration the greater challenge is time; it acquires 7 years in humans for the whole process of odontogenesis. The individuals with missing teeth may find this long term physiological process discouraging and will look forward to instantaneous treatment outcomes.[18]

Bringing light to the topic of regeneration of periodontal ligament, Electron microscope radio-autography was utilized in an attempt to recognize if there is any connection among the site and degree of differentiation of cells in PDL. The existence of an undifferentiated perivascular population of progenitor cells was concluded and also that cytodifferentiation was seen in the progenitor cells, which is present in PDL. Narang et al. demonstrated that from solid-frozen human PDL, the postnatal stem cells can be recovered.[9]

Conclusion

While much progress has been achieved in the department of regenerative procedures through stem cells in dentistry, we still have a long way to go. The basic and pre-clinical stem research is very slow in the dental clinics, as the very obvious safety, regulatory, technical and ethical concerns exist. The depth of scope present in stem cells is enormous which would take the dental regeneration procedure to another level, bringing more success in the field of Dentistry. The various approaches started now are just the beginning, but it is very well known how much potential it carries in the future to help patients all over the world. The various new promising technologies such as imaging systems, mathematical modeling and nanotechnology would help to speed the stem cell researches all over the world to provide more qualitative and dependable results in the dental clinics.

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Ortho-Perio Continuum: An Interdisciplinary Approach

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Abstract

Orthodontic therapy is a combination of process which is related to move the malalligned teeth by correcting its position. There have been many dental cases where orthodontic tooth movement is assisted by periodontal therapy and also the periodontal condition is improved by orthodontic therapy. This interdisciplinary approach is a symbiotic relation between the two. This review highlights the various orthodontic therapies requiring periodontal therapy for better outcome and vice-versa. Harmonious cooperation between the periodontist and orthodontist would provide satisfactory outcome for the combined orthodontic periodontal problems. The knowledge of this approach in the field of dentistry would help clinicians' decide a better outcome in patient's favour.

Keywords: *Orthodontic Therapy; Malalligned Teeth; Periodontal Therapy; Interdisciplinary Approach.*

Introduction

In the different branches of dentistry, co-ordination and co-operation among them is very important. It will not only help to get a spectacular idea about the diagnostic procedures but also make the treatments easier. This type of interrelated relationship exists between Orthodontics and Periodontics. Basically, this is a symbiosis type of relationship where the treatment procedures of both the branches are interrelated. There have been many dental cases where orthodontic tooth movement is assisted by periodontal therapy and also the periodontal condition is improved by orthodontic therapy. Orthodontic therapy is a combination of process which are related to move the malalligned teeth by correcting its position. It is shown that patients who are under orthodontic treatment are more susceptible to develop plaque on their teeth surfaces. It is due to several orthodontic appliances, brackets, wires and elements which are present on the teeth surfaces. Because of the such elements, the oral hygiene procedures might be more difficult. It may also cause several compilations liketooth decalcification, tooth discoloration, tooth devitalization, rootresorption, gingival recession, pain, TMJ dysfunction, periodontal problems, loss of enamel, dental caries, many types of psychological compilations and allergic reactions also. Out of all above complication, periodontal problems are the most common in nature and also need proper consideration. Despite all these, orthodontic treatment sometimes is very helpful in increasing the periodontal status. So,the periodontal conditions in which orthodontic treatment is necessary should be given some special importance at the time of interdisciplinary approach in both the ways.

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Adverse effectsof orthodontic therapy on periodontium

Orthodontic therapy may hinder the complete procedure of oral hygiene. So, improper oral hygiene creates feasibility of change of the subgingival plaque to a much more aggressive periopathogenic flora. But it can be reduced by appropriate plaque control method. So, patients having healthy periodontium can undergo for orthodontic treatments without infuriating the periodontal condition.

Orthodontic therapy produces several periodontal complications which includes; gingivitis, periodontitis, alveolar bone loss, gingival recession, tooth mobility, black triangles, periodontal pocket fenestration and dehiscence[1,2].

There are many ortho-dental cases which show the presence of various types of bacteria in the dental plaques of the patients who are undergoing orthodontic therapy. Some of such bacteria are spirochetes, intermedius, motile rods, *B. forsythus T.denticola, P.nigrescens, C.rectus* and fusiform bacteria.[3] Most of the cases show the development of gingival recession during or after the orthodontic treatment. This type of development of gingival recession coursesmany clinical problems. Most of the gingival recessions which develop during the orthodontic treatment had been shown to occur in the upper and lower anterior teeth regions. According to a study made by Steiner and his colleagues, the gingival recessions occurring after the orthodontic treatment are moretends to occur in the areas with thin underlying bony structure and thin keratinized gingiva.[4]

Periodontal conditions which require orthodontic treatment includes: Gummy smile, Midline diastema & correction of black triangles,Pathological migration with infrabony defects, Tilted molars, and Gingival margin discrepancies etc.

Orthodontic treatment as an adjunct to periodontal therapy: There is a strong correlation between the periodontal disorder and malposed teeth in the dental arch. The correction of the malposed teeth can eliminate any harmful occlusal interference which may prevent plaque accumulation and provide a better oral hygiene procedure. It may offer a great opportunity for the development of a good periodontal health.

Generally, during orthodontic tooth movement, the entire periodontal attachment apparatus, including the bony structure, periodontal ligament, and soft tissue components move along with the tooth. Brown made a study on the influence of uprighting of molars on the periodontium in four patients. After 7 months of treatment, the associated pocketing at uprighted molars had 2.5mm more pocket depth reduction than the control tooth.[5]

A clinical study was done on orthodontically treated patients having intact periodontium. It concluded that extrusion of mandibular incisors resulted in displacement of the mucogingival junction and the gingival margin by 52.5% and 80%,respectively, of the total amount of extrusion.[6] There are some cases where movement of attachment and margin of bone along with the tooth is not advisable as in crown-root fractures. In such cases, periodical circumferential supracrestalfiberotomy is needed. It should be done at the start and every 2 weeks during orthodontic extrusion. It has been suggested that orthodontic tooth movement into infrabony defects can result in healing and regeneration of the tooth attachment apparatus.

Periodontal therapy as an adjunct to orthodontic therapy: The aim of orthodontic treatment is to achieve a good occlusal relation, maintain a healthy periodontal status and to improve dental and facial aesthetics and also function. For the success of any orthodontic treatment, health of the periodontium is an important factor. So good periodontal health should be maintained not only before but also during and after the orthodontic treatment. Appropriate orthodontic forces do not produce any damage to the periodontium. There is different periodontal treatment which are used as adjuncts to orthodontic therapy.

Pre-orthodontic gingival surgery: It is seen that insufficient width of attached gingiva prevents plaque control and develops gingival recession. The denuded area extending through the marginal bone is known as 'dehiscence' which is a risk factor for the development of gingival recession. So, it is necessary to maintain the adequate width of attached gingiva. An attached gingiva with 2mm of width, is appropriate in maintaining a proper gingival health.[7] There are different mucogingival surgeries such as free gingival autograft, connective tissue gingival graft, apically repositioned graft and modified apically repositioned flap can be utilized for augmenting the width of attached gingiva. Among these techniques 'Modified apically repositioned flap (MARF) is a simple and less time-consuming procedure.

Fiberotomy: Elastic fibers,collagen fibers and the periodontal ligament are re-organized by several orthodontic tooth movements inorder to accommodate the re-established tooth positions. The teeth must be retained in the new positions to prevent orthodontic relapse and to achieve proper rearrangement of the supporting tissues. Supracrestal fiberotomy is usually performed to correct the relapse of tooth rotation corrected by orthodontic treatment. It is done before the removal of orthodontic appliances i.e. towards the end of the active orthodontic therapy.

Frenectomy and frenotomy: Abnormal frenum attachment is a potent aetiological factor for the development of midline diastema. By frenectomy incision and relocation of frenal attachment is done. Frenectomy is the complete excision of the frenum along with its attachments to the underlying bone. It can be done by either conventional scalpel technique, electrosurgery or by using lasers.[8]

Crown lengthening: It is a surgical procedure which is used to extend the supragingival tooth structure for aesthetic or restorative purposes either by the method of positioning the gingival margin apically, removing the supportive bones or by both of these.[9] It is necessary if there is not enough of clinical crown height. It reduces gum tissue and shaves bone down when required. The procedure usually performed by gingivectomy or an apically repositioned flap in combination with gingivectomy prior to orthodontic bonding procedures.[3] It can be done to prevent gummy smile in which gums are visible above the teeth while smiling.

Preorthodontic osseous surgery: It is mainly indicated for different types of defects such as; hemiseptal defect, three-walled defect,osseous crater and furcation involvement etc.

Missing interdental papilla are usually known as gingival "black holes". It may be due to a number of factors such as over-divergence of adjacent roots and advanced periodontal disease with loss interdental alveolar crest. An orthodontic periodontic interdisciplinary approach is usually advised to manage these problems.

Periodontally accelerated osteogenic orthodontics: This technique was developed by Dr Thomas Wilcko and Dr William Wilcko.[10] It is also known as alveolar osteogenic orthodontics or Wilkodontics. This procedure can be used as an adjunct to conventional approaches to accelerate orthodontic tooth movement. Basically this is a clinical procedure that combines alveolar augmentation and selective decortication facilitated orthodontic technique.[10] Theoretically it is a procedure which is based on the bone healing pattern in corticotomy regions known as the "Regional Acceleratory Phenomenon (RAP)". RAP was first described by Frost in 1993.[10] Regional acceleratory phenomenon is a localized tissue reaction to noxious stimulus. It accelerates the healing efficiencies of the affected tissues 2-10 times faster than normal physiologic healing process.[11,12] This is usually seen in osteotomy sites, fracture sites or bone grafting areas but mainly seen in fracture sites.[13] The two main features of RAP in bone healing which are believed to improve orthodontic treatment are; decreased regional bone density, accelerated bone turnover.[10] It mainly focuses on enhancing the manner in which the periodontium responds to applied forces and on providing for more intact periodontium and increased alveolar volume to support the teeth and overlying soft tissues during retention. The procedure for the PAOO technique is portrayed in Figure 1-6.

Advantages:

- Increases alveolar bone width
- Reduced treatment time to $1/3^{rd}$ to $1/4^{th}$ of conventional procedure
- Less root resorption
- Improved periodontal support
- Less chance of relapse
- Alveolar grafting also benefits patients by repairing bony dehiscence and fenestrations.

Disadvantages:

- Not applicable to all cases
- Costly procedure
- Post-surgical crestal bone loss and recession may occur
- Sometimes there is occurrence of pain and swelling
- Not suitable for patient who take NSAIDS o regular basis or patient having other chronic health problems

Applications:

- Resolve crowding
- Accelerate canine retraction after premolar extraction
- Facilitate slow orthodontic expansion
- Manipulation of anchorage
- Molar intrusion and open bite correction



Figure 1: Pre-operative photograph



Figure 2: Orthodontic brackets placed



Figure 3: Interdental vertical cuts given



Figure 4:Bone grafts placed



Figure 5: Interrupted sutures placed



Figure 6: Palatal View

Different studies on orthodontic periodontal interrelationship: Generally, the main reasons routinely cited to justify the provision of orthodontic treatment are important of dental and facial aesthetic and of dental function and health. Following are some of the studies regarding orthodontic periodontal interrelationship.

A research on correlation between periodontal health and malocclusion was conducted by Ngom and co-workers.[14] According to them malocclusions are the risk factors for periodontal diseases. But in this study the real conclusion about the cause or effect relationship between periodontal condition and malocclusion was not possible.

Van Gastel[15] conducted a review of literature which showed contradictory findings on the effect of malocclusion and orthodontic appliances on the periodontal health. Dental plaque is the primary cause of gingival inflammation and periodontitis. There are some studies reported loss of attachment during orthodontic therapy.

Two systemic reviews were conducted by Bollen[16] to know whether the malocclusion and orthodontic treatment affect the periodontal health or not. The first one suggested that person having greater malocclusion will have more periodontal problem. It may depend on oral health status. The second one showed that there is no reliable evidence on the effect of the orthodontic treatment on the periodontal health.

A systemic literature review was conducted by Gray and McIntyre[17] to determine the effect of Oral Health Promotion (OHP) upon gingival health. They concluded that an OHP for patients undergoing fixed appliances

orthodontic treatment produces a short–term reduction (up to 5 months) in plaque and improvement in gingival health. There are no specific oral health promotion method that can produce a greater short-term benefit to periodontal health during fixed orthodontic treatment.

Conclusion

Healthy periodontium is required for all types of dental treatment, mostly for orthodontic treatment. Orthodontic therapy has both beneficial and harmful effects on periodontium. For adult patients, combined orthodontic and periodontal treatment is required,who are seeking orthodontic treatment for aesthetic correction. Orthodontic treatment cannot be performed on periodontally compromised conditions. In such conditions regular follow up is required to check the periodontal status. Harmonious cooperation between the periodontist and orthodontist provides a satisfactory outcome for the combined orthodontic periodontal problems.

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Pharmacotherapeutic Approaches in Tobacco Cessation and its Challenges: A Review

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Abstract

Tobacco use is a global menace and leading cause of cancers and death. Tobacco cessation strategies are being formulated world over to curb this problem. Nicotine replacement therapy (NRT) such as chewing gum, patches and inhaler and drugs like Bupropion have shown some success in smoking cessation rates in the short and long terms.

Keywords: *Tobacco cessation, Pharmacotherapy, NRT, Nicotine, Nicotine Patch, Bupropion.*

Introduction

Nicotine is responsible for pathophysiological changes in smokers and also tolerance[(1)]. Despite the awareness and the determined protective efforts at both national and state level, tobacco still threatens the health and well being. Oral cavity and general health are affected due to smoking and chewing tobacco. Currently, tobacco intake is one of the major etiology of oral cancer, a severe and increasing worldwide problem[(2,3)].

Consumption of tobacco cause periodontal destruction with reduction in inflammation and gingival bleeding which ultimately results in delayed wound healing. It also leads to halitosis and other oral conditions[(4-6)]. Smoking causes oral cancer. Cardiovascular disease, chronic obstructive lung disease emphysema and chronic bronchitis are main cause of smoking. Smokeless tobacco is known to cause oral cancer.

Oral cavity being the entry point for tobacco smoker, it is the first to get exposed. Therefore, it is associated with tooth stains, abrasions and condition like smoker's melanosis, nicotinic stomatitis, keratotic patches, black hairy tongue and oral carcinoma. Oral keratosis and gingival recession are due to consumption of smokeless tobacco. The prognosis for periodontal treatment is affected adversely on consumption of tobacco[(2,7)]. Cigarette smoking has a dramatic effect on inhalation since more than 90 percent of the nicotine present in inhaled smoke is absorbed. Absorption instantly results in an increase in arterial nicotine level[(8)].

Pharmacotherapy in Tobacco Cessation: The objective of a pharmacological approach for tobacco cessation should ideally to blockage of positive reinforcing effect of nicotine and prevention of withdrawal symptoms. Pharmacotherapeutic treatment is mainly classified into: Nicotine replacement therapy (NRT) and non-Nicotine Replacement Therapy (n-NRT)[(9)]. NRT is not suitable in patients with history of cardiovascular conditions such as myocardial infarction, cardiac arrhythmias and angina pectoris. It is also contra indicated in pregnancy and for lactating mother. Action of NRT is by replacing nicotine component from cigarettes

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which helps in preventing withdrawal symptoms caused by nicotine component. Non-NRT also helps in reducing withdrawal symptoms as well.

Nicotine qualifies to be a highly addictive drug[10]. Regular smokers develop a dependence on tobacco smoking and not simply an addiction to nicotine[11]. Dependence develops due to the temporal association of the habit and sensory inputs with the recurring stimulation and relief of withdrawal symptoms. This is the reason for minimal addictive potential of nicotine replacement therapy (NRT) products,that deliver nicotine slowly and do not produce high plasma nicotine

Types of NRTs are: Nicotine gum, Nicotine patch, Nicotine nasal spray and Nicotine inhaler

NRTs:

Nicotine gum[12]: This is an alternative agent which helps in cessation of smoking. It acts by principle of neuroadapation followed by abstinence as a daily cycle. Nicotine levels fall during overnight sleep or when gum not chewed. It works best with other NRTs or bupropion hydrochloride[13].Nicotine gum chewing facilitates absorption of nicotine through oral mucosa when gum placed between cheek and gum. Chewing of gum cause needle like sensation then park between cheek and gum. This chewing and parking process repeat alternately for about 30 min.

For smoker who smokes within 25 cigarettes per day, a dose of 2mg is advised and 4 mg dosage is advised for smokers smoking more than 25 cigarettes per day[13]. Patients should be instructed to chew the gum in a fixed schedule 1 piece every 1 to 2 hours. Hencearound 10 to 15 pieces per day may used initially[14]. They have to avoid eating or drinking anything before to and during chewing gum. An acidic beverage hinders nicotine mucosal absorption. This treatment should be continued for 6 weeks followed by 6 weeks tapering[15].

Advantages of chewing gum is that it acts by changing the behavior of urge to which control nicotine mood changes[14]. Common side effect includes nausea, sore throat, mouth and jaw, dysgeusia, dyspepsia, gastrointestinal discomfort etc[14-16].

Nicotine Patch[17]: Nicotine patchesare preferred over chewing gum because drug delivery is for long duration with high concentration than chewing gum. Patches are available in different formulation as per strength and concentration[2]. Patches may be used 16 hours to 24 hours in a day. One day patch has advantage of controlling the morning smoking urge by increasing blood nicotine level upon awakening. 24 hour patches may cause sleep disturbances unlikely by 16 hours patch.

The patch may be put on in the morning on a hairless area between neck and waist. The initial dose is tapered after 4 to 6 weeks up to 14 mg/24 hours then to lowest dose 7 mg/24 hours in the following 2 to 4 weeks. There may be differential recommendation of dosage for the patch depending upon amount smoked, amount of nicotine dependence [14,15,18].

A nicotine patch is easy to use with minimal side effect and more efficacy than chewing[14].However,some side effect may be due to constant nicotine level in the body. Side effects are mainly due to localized irritation of skin causing redness, itching and burning like sensation, which may lead to discontinuation of therapy in 5% cases[15]. Most of the above side effects are self-limiting, such as redness of skin by 1 to 2 days of therapy, rashes by applying steroid creams topically. Other minor side effects are generalized skin rash, headache, nausea, vertigo and gastric discomfort [14, 15].

Nicotine Nasal Spray[17]: Nicotine nasal spray is another type of NRT which helps in reducing nicotine crave in patients. Nicotine levels in plasma peaks within 10 minutes after intake. This is about two-thirds of the dose of nicotine in cigarettes[14,15,19]. Application of 0.5 mg equally in each nostril not more than 5 doses per hour or 40 doses per day and gradual reduction of the dose is recommended. Nasal spray may be avoided on lower nasal mucosa[14].Sniffing, swallowing and inhalation should be avoided. Uses of one to two canisters per week don't need any follow-up. Use of more than two canisters per week should be used with caution. It is mainly used for nasal spray up to 12 weeks, then it should betapered gradually[14, 15].

Side effects may include headache, burning like sensation, rhinitis, increased lacrimation, pain in nose and throat, sneezing and coughing. It occurs after initiation of therapy which gradually decreases within first week[15].

Nicotine Inhaler[20]: Each puff of inhaler delivers around 13ng of nicotine. Nicotine levels due to most cigarettes is approximately 2 mg, which is about 80 puff inhaler over 20 minutes. Absorption of drug mainly occurs through mucosal membrane of oralcavity,

pharyngeal mucosa and gastrointestinal tract. It may affect throat irritation and coughing; it should not give in bronchospastic disease[14].

NON-NRTs[21]: Even though the cessation of tobacco is achieved by NRT but the outcome rate is not more than 20% per year. Outcome of smoking cessation rate increases by NRT in comparison to placebo but not more than 20% per year. To increase these rates, non-nicotine agents came into consideration, which acts bynoradrenergic neurotransmission (Bupropion, Nortriptyline). These drugs have more efficacy are than those affecting serotonin (selective Serotonin reuptake inhibitors, Bupropione, Ondansetron etc.)[1].

Bupropion Hydrochloride[22]: Bupropion hydrochloride (Zyban) which is an aminoketone antidepressant group of drug that acts by weakly obstructing both noradrenergic and dopaminergic uptake, helps in cessation of smoking. It also helps in weight loss[14] in obese individual. It is also indicated in patient not willing to take NRT, relapse after taking NRT or failure of NRT. Starting dose of bupropion for smokers is one week prior to their cessation date. All the patients are allowed to continue smoking only for first week followed by cessation in end of 2nd week[1]. The starting dose is 150 mg once a day for 3 days followed by 150 mg twice every day for 2 to 3 months[1]. Minimum interval between two doses should be 8 hours. Second dose normally is advised in the late afternoon or in the evening to avoid sleeplessness. Bupropion can be used as mono therapy or in combination with other NRT (e.g., nicotine patch)[1,23].

It can decreased sleep and cause dryness of mouth, and seizure in 0.1% of patients as it decreases seizure threshold[24]. So always ask patients about seizures history and rule out other cause of seizures like cerebro-vascular accident, surgical intervention or significant history of loss of consciousness due to head injury. Drug which decreases seizure threshold (e.g., alcohol, neuroleptics, patient taking monoamino oxidase inhibitor)[14].

Clonidine[25]: Clonidine is a centrally acting drug that inhibits α2 receptor of sympathetic nervous system(primarily used as antihypertensive drug). Very few study have stated that Clonidine may help in cessation of smoking, but side effect due to Clonidine may limit its usefulness[1,14,26].

Antidepressants and Anxiolytics: Antidepressant like Nortriptyline which blocks the reuptake of nortryptyline and serotonin may have some role in smoking cessation, which is yet to be approved by U.S. Food and Drug Administration[27]. As smoking is mostly associated with depression and anxiety, a variety of other antidepressants and anxiolytics like (Buspirone, Doxepin and Fluoxetine) shown to have some promising effect[1,14, 28].

Varenicline[29]: To avoid addiction due to nicotine,non-nicotine containing drug came into consideration. Varenicline is a non-nicotine type drug which is recommended 1-2 weeks before quit date. Dose schedule starts with 0.5 mg daily dose for the first 3 days, then from fourth day the 1mg twice daily up to 7th day, from 8th day 2 mg twice daily up to the end of 12 weeks. To prevent relapse further 1 mg twice daily is recommended for another 12 weeks. The main side effects are nausea, headache, vomiting, gastric upset, lack of sleep and abnormal dreams which are mild in nature and decrease over time. Varenicline is found to be an effective drug in long-term relapse prevention.

Enhancing Pharmacotherapeutic Effectiveness: Safety and efficacy can be enhanced by appropriate use of drug, dose schedule and treatment duration. All patients should be monitored frequently in follow-up period and dose can be adjusted as per patient response. All patient should be instructed properly about the use of drugs[14,30]. A follow up in every 1 to 2 weeks interval should be scheduled for all patients[30]. If there are no signs of improvement within[31] two weeks, medications and motivations should be repeated. If the condition remains at the original level, discontinue treatment after 4 weeks or change to combination therapy

Method of Validation: Patient's smoking status can be assessed by carbon monoxide concentration in breath which is a non-invasive way and rapid method. Urine tests for Cotininare available to assess NRT adherence[32]. However, this test fails to distinguish between sources. Anabasine and anatabine like tobacco alkaloids may also be assessed in urine to confirm abstinence[33].

Future Approaches: A vaccine is being developed which is helpful to avoid nicotine from crossing the blood-brain barrier. Some drugs are being developed to modify the metabolism of nicotine. The discovery of gamma-vinyl GABA (GVG) is quite promising[34]. Although it is yet to be tested, it acts by three chief

mechanism: Drug addiction direct neurochemical effects, Behavioral effects and Neurochemical changes.

Nicotine toxicity: Acute toxicity at high doses (40 -60 mg) and very high dosage up to 500mg may cause sudden death due to respiratory blockade. Usual symptoms of nicotine toxicity may varies as Nausea, salivation, abdominal pain, sweating, headache, diarrhea, dizziness, delayed wound healing etc[35].

Conclusion

Tobacco is a major risk factor for most of the cancer deaths worldwide. Tobacco addicts can greatly be benefited by pharmacological approach.

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Cryotherapy: An Alternative to Surgical Adjunct

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Abstract

The application of low controlled temperatures had advanced in recent years in many branches of surgery. Cryotherapy has opted as an option to destroy cancerous cells in mostly the head and neck region. In many cutaneous conditions, it has been productively used. It is a therapeutical process in which tissue inflammatory and destructive response is done by freezing. Hence, it deliberately destroys tissue by using severe cold. Mouth is reasonably accessible due to oral mucosa's moist surface that is well suitable for the application of cryoprobe. We will discuss how cryotherapy can be used instead of surgical procedures.

Keywords: *Cryotherapy; Applications; Surgical Procedures.*

Introduction

From generation to generation, many different techniques are used for tissue destruction other than the surgeon's knife, some of which can be summed up as high-frequency electric current, chemicals, irradiation by X-rays. And the advantages that are being looked upon them instead of using scalpel are painless and cause minimum destruction of surrounding tissues, and encourage speedy and uneventful healing. In 1845, James Arnott reported the use of low temperature to destroy cancerous cells and came the term Cryotherapy. It is derived from a Greek word 'Kryos' which means frost, thus meaning frost therapy.[2]

According to the medical definition, it is defined as a procedure used to destroy the tissue of both benign and malignant lesions by the freezing and re-thawing process. It is done with the use of cold, which is applied locally or generally various methodto lower the temperature of tissue that is affected.[3] Over 30 years, it has been used in Oral Medicine and Pathology as the oral lesions are normally warm and moist, therefore the procedure is preferably suited for treatment. A cryoprobe is used to attack the oral lesion constantly, which requires minimal preparation of the patient and operation field. It is considered as a simple procedure.[4]

Earlier it was used for the treatment of cancer of oral cavity and lip, but now as the technology has expanded and advanced in the head and neck region. This procedure is also advised after numerous surgical procedures.[4]

The different cryogens used in the procedure are:[6]

1. Liquid nitrogen (-196ºC)
2. Nitrous oxide (-89ºC)
3. Solidified CO2 (-78ºC)
4. Chlorodifluoromethane (-41ºC)
5. Dimethyl ether (-24ºC), Propane (-42ºC)(1)

The combination of these cryogens had also been proved to be used for the treatment of jaw lesions, and had shown a therapeutic effect.[7] In this process the tissue is not excised, rather the lesion is frozen and the ensuing necrotic tissue is allowed to slough instinctively. The main mechanism of the destruction of tissue is through intra and extra-cellular ice crystal formation, leading to cell dehydration, protein denaturation and disruption of the cell membrane and ultimately death of the cell, irrespective of the cryogen used or its temperature.[8] All biological tissues subjected to a temperature of -20ºC or below does undergo necrosis for a minute or more virtually.[9] In cryotherapy, the tissue which is close to the probe freezes quickly, but ice, in this case, acts as an insulator so the further freezing continues slowly. As the spread of freezing is delayed with the help of ice, the probability of unintentional damage to the underlying tissue is reduced. The neighboring tissues are unharmed due to the gradient of heat loss.[10]

The basic attitude of the procedure is: Rapid cooling -> slow thawing -> freezing process is done repeatedly -> maximize tissue destruction. The two method used for the process are:

a. Use of probes and nitrous oxide in a closed system.

b. Use of nitrogen and cotton tip in an open system.

According to Joule Thompson expansion, a substance undergoes a drop in temperature when moved from a high-pressure area to low pressure area. This principle is followed by the probe in cryotherapy, for example when from high pressure inside the cryoprobe nitrous oxide is released to the cryotip which is low in pressure, there is a drop in temperature which causes freezing of tissues to happen.[1,7,11]

History[2]:

1. Theuse of cold for inflammation and trauma was first done by the Egyptians.
2. In the 17th century- Robert Boyle bring into beingthat by freezing cells would be killed.
3. In the 19th century- In cryosurgery, the use of nitrogen and nitrous oxide was started.
4. In the 20th century- Baron Lorreyobserved its efficacy in sedation and anesthesia in the amputation of soldiers.
5. In 1845- The first report on the therapeutic use of low down temperatures in malignant diseaseutilizing salt/ice mixture applied to breast neoplasms was by James Arnott.
6. In 1877- Louis Callitet and Raul Pictetoffered liquefaction of oxygen and carbon monoxide experimental papers, and this was considered by Zacharian as the birth of modern cryogenics.
7. In 1899- The first cryosurgeon to use it for medical purposes was Campbell White.
8. In 1977- After freezing there was local tissue necrosis and vascular stasis, and that excellent healing of tissues was witnessed by John Hunter.
9. In the early 1960s- Amaral et al. made use of liquid nitrogen on swabs to treat cases of palatal inflammatory papillary hyperplasia.
10. In 1981- Mac Donald et al. suggested for treatingangiomas, hyperplasia of palate, leukoplakia and lichen planus, cryosurgery can be done.
11. In 1981- Barnard illustrated that cryotherapy produces an extended and reversible nerve block in the management of chronic pain and postoperative pain.
12. In 1988- Goss made use of cryoneurotomy for treating patients with intractable neuralgic pain in the preauricular region.

Role of Cryotherapy in Oral Lesions: It has shown tremendous effects in treating oral lesions in all these years with its rapid and uneventful healing. Patients have been seen accepting the treatment as it is less painful and damage to surrounding tissues is very less. The oral precancerous lesions are treated and eradicated with different other modalities like surgical excision, photodynamic therapy, laser surgery and many more, but the different advantages that make cryotherapy as an effective and alternative modality for treatment are very low incidence of secondary infections, lack of scarring and pain and most importantly bloodless treatment. The different oral lesions in which cryotherapy as a mode of treatment can be used are hyperplastic, keratotic, vascular, granulomatous pigmented lesions, and also lesions of gingiva and salivary glands. As given by Hocut et al in 1982, the different stages of cryotherapy are:[12]

Stage 1: sensation of cold 1-3 min.

Stage 2: aching or burning 2-7 min

Stage 3: local numbness 5-12 min

Stage 4: deep dilation >12 min

It has been suggested that after an injury >12 min should be used to attain stage 3.

The dependency of cryolesion dimensions is based upon three variables, which are:

a. Temperature of cryotip

b. The period of contact

c. Area of contact between the tip and the tissue

The determination of the velocity of freezing within the cells and the size of the freezing ball is a contribution by the temperature of the cryotip. A high velocity of freezing desired since rapid freezing can cause lethal effects to the tissues. The duration of freezing is directly proportional to the size of the lesion in the starting 1-7 minutes, according to the growth pattern of cryolesion

in vitro.[1,6] Taking an example of freezing a bony tissue, in that case multiple freeze sites are necessitated as the freezing of tissues is a difficult job. But in most of the soft oral lesions, the freezing time is limited to 20-30 seconds.

Mechanism of tissue damage in Cryotherapy: It has been determined with the help of several reports that most tissues freeze at a temperature of -2.2ºC, and for cell death to occur the temperature must fall below -20ºC. According to dermatological guidelines, it was suggested that small cancers should be treated at a temperature of 30ºC which is said to be effective. On the other hand, in aggressive cancers of the oral cavity, the treatment is done by repetitive freeze cycles of at least -50ºC or more temperature for the necrosis of tissue to occur.[1,6,10] Normally following the treatment, cryolesions are impossible to differentiate from original tissue, though as repetitive freeze and thaw cycles are undergone by the lesion, an accretion of damage takes place. And in the following days of the treatment, latent damage is created, which changes to progressive damage leading to consequent tissue necrosis.[7] Therefore, after cryotherapy tissue destruction is a multifactorial process, which combines both direct and indirect effects.[2]

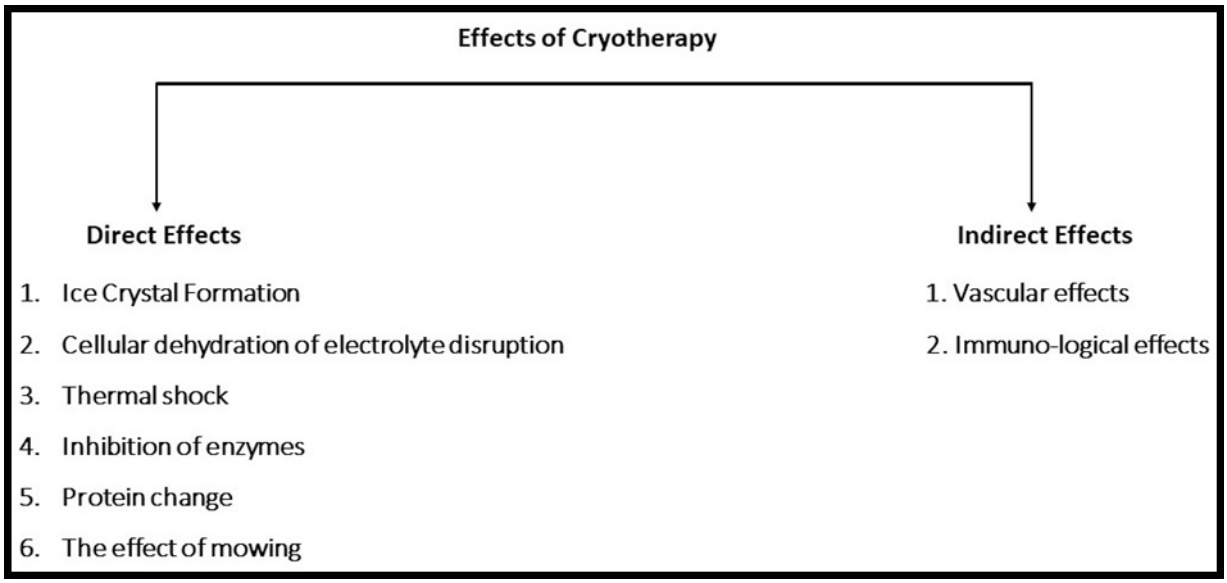


Figure 1. Effects of Cryotherapy

The description of the effects is given the following:

1. Direct effects

a. Ice crystal formation: Larger ice crystals are formed when the cooling rate is more rapid, therefore the degree of adhesion of the tissues to the probe is greater. The disruption of cell membranes is produced due to large ice crystals.

b. Cellular dehydration and electrolyte disruption: The cell membrane acts as a barrier to the propagation of ice crystals as the freezing occurs only in the extracellular space only within the outer zone. The extracellular molecules are captured by the slowed-down freezing, so in the extracellular space the ionic concentration of electrolytes is increased. Intracellular water is passed from the cell, but is then trapped in the ice front, leading to the cells to become dehydrated and undergo physical shrinkage. And the concentration of electrolytes both inside and outside of the cell is amplified, which becomes irrevocably toxic to cellular function.

c. Thermal shock: This is related to the rapid freezing rate which causes cell damage, systems recording temperature in order of 1000ºC/minute.

d. Inhibition of enzymes: Sudden cooling acts as an inhibitor, as each cellular enzyme operates optimally over a narrow temperature range. This makes the cell more susceptible to metabolic changes.

e. Protein changes: Protein is denatured in both the

cell membrane and mitochondria due to the fall in temperature, and cellular metabolism shows subsequent damage.

f. **The effects of thawing:** The tissues are allowed to re-warm when the process of freezing stops which causes damaging effects. Like, due to increased concentrations of intracellular electrolyte, the cells show water intake which is then vacuolated, bulged and burst.[13]

2. Indirect Effects:

a. Vascular effects: Vascular stasis and microthrombus formation causes ischaemic necrosis, which plays a significant role in cryodestructive process.[14]

b. Immunological effects: These effects could be seen due to the massive release of pathological cell antigens, which makes them vulnerable to host surveillance mechanisms. These also add to the damage of affected tissues treated by cryosurgery.[2]

Changes of tissue after cryotherapy: The clinical observations done in oral soft tissue include necrosis, sub epithelial hemorrhage, tissue edema, blister formation, sloughing and repair. In general cases, within a few hours of cryotherapy, edema, hyperemia or erythema is seen immediately. After 1-2 days, local swelling is seen which increases with time, followed by superficial necrosis and ulceration that is enclosed by a coating of yellowish or whitish necrotic pseudomembrane. After the 1st week, this yellowish or whitish slough is detached from the underlying tissue, leaving a granulating clean surface that is covered by epithelium partially. After 1-4 weeks, the completion of epithelialization is witnessed with no or very small scar formation.[6,9]

Cold therapy after intraoral surgical procedure: It has been observed that patients are always advised to apply ice after physical injuries and a variety of surgical procedures for therapeutic use. This therapeutic use of cold through various method is applied locally or generally to lower the temperature of the skin and subcutaneous tissue.[12,15] Many scientific studies are being done based on the physiological and clinical effects of cryotherapy but still there is a scarcity of information based on what should be the optimal time interval for therapy, the finest method of application and the entire time length for cryotherapy.

The five cardinal signs of inflammation pain, swelling, heat, redness and loss of function were identified long ago, but they are not the inflammatory response. The overlapping stages that can impair tissue function or structure are referred to as inflammatory response. The physical objectives that can be obtained when cold therapy is applied after an injury to cool tissues are edema (inhibit swelling), decrease inflammation, vasoconstriction (diminish blood supply), stopped the increase of temperature, reduced hemorrhage, decreased metabolic alterations leading to reduction of secondary infection due to lack of oxygen, cold reduces nerve conduction speed and eventually hurry up the revival process of the patient to resume normal function.[16]

Advantages: Cryotherapy is the most effective and best method of treatment when surgery is contraindicated in the patient. It causes very minimal general disturbance to the patient, especially the elderly age group. The volume of tissue destruction is reasonable and predictable. It is best suited for extensive superficial lesions, with a very low complication rate. It can be used as an adjunct to surgery and also radiotherapy in case of analgesic tumor control. It also helps in the preservation of the inorganic structure of bone, with minimal to no visible scarring. It also has a very low incidence of secondary infection. It is said to be safe, easy to perform, with no permanent side effects and more localized in action. It is a relatively inexpensive technique in an outpatient clinic for various oral lesions.[7,10,12,17]

Drawbacks: The technique is very safe and useful in treating oral lesions, but has got a few limitations also. Like, it is highly dependent on the operator skill and experience. The freezing capacity of the available instrument doesn't always reach the volume of the lesion which leads to multiple cryosurgery procedures or an alternative mode of treatment that has to be planned. Sometimes there is trouble in evaluating the extent of the lesion, which leads to an insufficient amount of tissue involvement which results in persistence of the pathologically changed epithelium and recurrence of the lesion. The healing process is slow in cryotherapy, which can also mean the persistence of lesion, especially if the case is neoplastic. Extensive procedures can lead to considerable scarring. It is contraindicated in patients with cold intolerance, cryoglobulinemia, cold urticaria, collagen diseases, and patients undergoing hemodialysis or immunosuppressive therapy.[3,7,18-21]

Conclusion

In comparison to conventional surgical procedures, it is an atraumatic form of therapy. Therefore it can be the choice of treatment in case of anxious patients, children,

and patients for whom surgery or other procedure is contraindicated. It is an effective mode of treatment for the head and neck region. By careful selection of patients, cryotherapy is a simple, safe, conservative and acceptable treatment for certain benign oral lesions and oral pre-cancers. The ice therapy canprovide several benefits that can be said after a lot of reviews on the physiological effects of the cold application, still many clinical trials are required to prove the therapeutic efficiency of cryotherapy.

Ethical Permission: Not required

Conflict of Interest: None

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Nursing Bottle Syndrome: An Overview

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Abstract

Nowadays people face several dental issues among them one of the major issues is Nursing Bottle Syndrome. It affects preschool children and infants worldwide. It is one of the common childhood diseases. Driven by a dysbiosis state of oral micro-organism mainly caused by a sugar-rich diet. Little one less than 36 months of period if any indication of tooth decay seen than its a sign of acute Nursing Bottle Syndrome. Existence of single or many blights, lost or restored milk teeth in children aged 5 years or smaller starts with milk or fresh snow spot along the free gingival margin on the surface near the lip of upper central and lateral eyetooth which often goes undetected by folks and caregivers, spreading later to the (deciduous) maxillary and mandibular first molars,maxillary and mandibular canine followed by maxillary and mandibular 2nd molar whereas the mandibular molars are not affected (the teeth are affected in the order they erupt). Dental care acts as a major part of dental function and is required for phonetic and all-round self-development of individuals.

Keywords: *Cariogenic Microorganisms, Streptococcus Mutans, Streptococcus Sorinus, Blight, Fluoride.*

Introduction

The term Nursing Bottle Syndrome was introduced in the 1990s. In many countries Nursing Bottle Caries is a significant problem especially in association with disadvantaged communities in which malnutrition is a major problem. It is considered a chronic dental issue that harms young children's health and also considered a major dental issue. Nursing Bottle Caries is defined as a multi-factorial disease, that results in single or many blight (carious or non-carious areas), filled or mislaid tooth surface due to blight in any milk tooth, in children (5 years or younger). Nursing Bottle Syndrome has many distinct features in the medical aspect similarly as swift initiation of cavitation that affects the teeth that erupt from the gums.[1] Various parlance pre-owned to look into a similar situation, rampant tooth decay, milk tooth cavity, milk bottle syndrome, prolonged nursing habit caries. Nursing Bottle Syndrome is connected with other systemic issues of an individual body,ranging from local pain infection, pus accumulation, leading to difficulty in eating grinding the food and famine toddlers enteric upset,stomach flu and difficulties during night insomnia. Diet and nourishing for practices act a significant part in developing teeth cavitation also in causing rotted teeth. Multiple causes can be stated which are responsible for this chronic disease.[2]

Classification:

Type I (mild and moderate): Molaris Dens associated in decay tooth gasp. That are found in between 2 to 5 age group toddlers. Affect is generally tooth decay; gelatinous,spongy, paste-like food and absence of dental care and maintenance and lack of dental check-up. Numeral of infected tooth generally raises by the raise in pathologic flora of micro-organism by challenging their persistence.[3]

Type II (moderate to severe): Labio-lingual tooth decay area that infects the upper front tooth i.e. dens incisive, may or may not infect molaris dens according to the eruption time of an individual. It generally takes place as soon as the earliest tooth comes in the jaw. Unaffected lower front tooth i.e. dens incisive. Causes are generally inappropriate use of bottle milk suckling, mother milk suckling, or mash-up of the pair of habits that result in inadequate dental practices. Unless controlled it may proceed to advanced stage.[3]



Figure 1. Diagrammatic representation of Early Childhood Caries

Type III (severe): Dental Decay lesion occupies almost all the teeth,together with lower dens incisivus. It generally takes place in 3 to 5 years of age. Root is like a union of aspects and inefficient dental habits. Uncontrolled and affect several teeth and affect a number of the teeth surface.[3]

Sequence of Teeth Involved Due To Nursing Bottle Syndrome[4]:

Effect of the deciduous teeth in the following sequence:

- **Dens Incisivus:** Facial surface, lingual surface, mesial surface, distal surface,
- **Maxillary Lateral Dens Incisivus:** facial surface, lingual surface, mesial surface, distal surface, Maxillary First Molar: facial surface, lingual surface, occlusal surface, proximal surface,
- **Maxillary Canine and Second Molar:** Facial surface lingual surface, proximal mandibular molars are usually spared.
- Anterior teeth are usually spared since they have protection by the tongue and cleaning action of spittle due to the existence of the opening of the duct i.e. the submandibular glands very near to lower dens incisivus.
- **Lesion Progression:** De-mineralized dull lesion followed by white areas along gum lines (labial aspect) which causes white lesion and it become cavities that involve neck of the tooth in a ring-like lesion. Finally the whole crown of the incisors is destroyed leaving behind brown-black root stumps.
- **Unequal severity: Distinct pattern of the site:** Site of milk teeth eruption. Continuation of adverse activities of nurturing. Sinewy form of the toddler feeding practices Early Childhood Caries clinically present as a spectrum ranging from 1 to 2 isolated caries lesions to severe and rampant forms where many teeth are affected.

Epidemiology of early childhood caries: Frequency of Nursing Bottle Caries can be depicted in the following countries and society invovlement[10] in these respective years with the help of percentage: India (Kerela), 2003, 12%, Saudi Arabia, 2001, 27.1%, Manchester, 2001, 19%, Canada, 2004, 54%, Australia, 1985, 5.4%. Despite the frequency of Dental Caries has turned down in developed countries over previous years, Nursing Bottle Syndrome is still a major issue in toddlers. Various information or data can be used to ameliorate the dental health condition among the public.[5]

Pathogenic micro-organism: *Streptococcus mutans* & *Streptococcus sorinus* is one of the principal organisms which colonize the tooth once it comes in the dental space of the mouth. It is passed on into the toddler's mouth by the progenitress. It colonizes in the mouth produces a large number of extracellular polysaccharides that favors the growth of plaque development. *Lactobacilli* also plays a part in the development of the decay site and plays a significant role in lesion progression, but not in its initiation. Milgrom et al (2000), said that toddlers having increased streptococcus mutans level are 5 times prone to have dental caries.[6]

Etiology: Children who need special health care, Malnutrition children, Crowed home children, Children in families of low socioeconomic status, Low-birth weight infants (<2500grms) & premature, Late order offspring, Lack of routine oral hygiene, Prolonged sweetened bottle feeding, Frequent breastfeeding. If exposure to fluoride is insufficient, Iron deficiency, Excess of lead exposure, Remote and Rural area children.[7]

Consequences of early childhood caries: Infection and pain, Behaviour alteration,Eating difficulties and sleeping, Growth delay and poor nutrition, Distraction from normal activities, Emergency room visits and hospitalization, High treatment cost and time, Poor self-confidence, Speech difficulties and many more according to the place some variation can be seen.[8]

American academy of pediatric dentistry (AAPD) recommendation for nursing bottle syndrome: Sleeping with a bottle should be stopped for toddlers. Nighttime suckling should abstain. Parents and caretakers should be educated to have toddlers drink from a cup. Dental care steps majors should be planned out during the duration period of first milk tooth flare-up. A dental health check-up is mandatory to educate the parents caretaker for controlling the Nursing Bottle Syndrome.[9]

Preventive Measures:

- **Home Care:** Dietary habits, Oral hygiene, Fluoride dentifrices, Fluoride supplement, Transmission of bacteria.
- **Professional:** Early detection of caries, Diet counseling, Fluoride application, Pit and fissure sealants.
- **Community:** Health education, Community development, Water fluoridation.

The motto of Nursing Bottle Syndrome control is to properly develop the idea of that all factors responsible and toddlers at risk and try to control the recurrence of nursing bottle syndrome. Alot of factors are responsible for nursing bottle caries multiple approaches should be taken to control. Educate the people for dental checkup within 180 days of a flare-up of the first tooth and no later than 365 days period education program can be done to aware of the people and educate the parents and caretakers about causes and factors responsible for Nursing Bottle Syndrome. Dental supplements like dentifrices in the form of toothpaste with fluoride contain of 1000ppm and above are efficient in preventing nursing bottle caries. Fluoride Varnish should be used in toddlers with nursing bottle syndrome, at an interval of 3 months or 6 months.[10-14]

Summary: Nursing Bottle Syndrome is a high dental issue in evolving and evolved areas. Nursing Bottle Syndrome can occur untimely in individuals,develop fast in those who have more possibility, and usually unattended.[15] Its outcome ends in pain and potentially threatening infection, influence kid development and reduces the standard of living. It can be specifically vitriolic type of tooth decay, starts soon after dental eruption, blooming on a smooth surface, advancing fast and having an immutable detrimental impact on the dentition.[16] Infants or toddlers undergoing tooth decay have more chances of subsequent tooth decay in permanent dentition. Nursing Bottle Syndrome can be controlled and taken care with proper knowledge,data guidance and ability dentist can assist to refine and enhance dental health and lifestyle of young individuals through spreading awareness, enhance about home care practices and appreciate frequent dental visits to enhance the parental knowledge and perception about the Nursing Bottle Syndrome and its sources adding awareness, accompanied with the treatment plan of children with sign and symptoms of tooth decay.[17-22] Implementation of dental care steps no later than the time of the flare-up of the first milk tooth. Tooth-brushing habit must be taught to toddlers by parents or caretakers and taken care of and monitored that they are doing it 2 times a day regularly, using a soft-bristle toothbrush of age-appropriate size with a smear of fluoridated toothpastes must be applied. Every toddler of age 24 months to 60 months of age period, a pea-sized amount of toothpaste should be used. In toddlers who are reviewed to be at a scale of moderate to high risk of tooth decay under the age of 2 years should be taken a much-considered amount of care.[23-25]

Conclusion

While winding up I need to request and encourage every individual to take special care of there oral and dental hygiene and go for a regular check-up to the dentist every 6 months. Parents and caretakers should take special care of there offspring oral and dental hygiene since the mouth is the mirror that reflects the whole body activities. Well said prevention is better than cure so take care of your teeth before losing your pretty smile.

Ethical Permission: Not Required

Conflict of Interests: None

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Anterior Interdental Papillary Loss among Young Adults of Bhubaneswar: A Cross Sectional Survey

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Introduction

Background: Presence of intact interdental papilla is considered as an essential component of health and esthetics. Interdental papilla loss or "black triangles" results in food retention, phonetic problems and unaesthetic appearance especially in maxillary anterior regions but there are very few studies reporting the prevalence of this condition.

Aim: To assess the prevalence of anterior interdental papillary loss among young adults in Bhubaneswar.

Materials and Method: A total of 483 patients were screened and 157 patients met the inclusion and were assessed for papilla loss parameters (Norland & Tarnow and Jemt Classification).All these patients underwent oral prophylaxis and then clinical photographs were taken.

Results: High frequency of papillary loss was seen in males (62.4%) compared to females (37.6%). Papillary loss was commonly seen in midline (51.0%) than in laterals(49.0%). Jemt Grade 2 (43.3%)and Nordland and Tarnow Class I(43.3%) papillary recession was more commonly seen.

Conclusion: There is higher prevalence of papilla loss in the midline region of maxillary anteriors presenting half or more of the height of the papilla

Keywords: *Interdental papilla, black triangle, classification, prevalence.*

Introduction

A smile reflects the happiness that one has in life[1]. The consonance of the smile is determined by the shape, the position and the color of the teeth but the existence of interdental papillae and healthy gingiva is also one of the important esthetic aspects that need to be considered[2]. Loss of interdental papilla (IDP) results in papillary recession, an un-esthetic gap known as "the blacktriangle"that draws an unpleasant individual attention.

Often the loss of papilla is either an outcome of periodontal disease or trauma. Loss of papilla may also be a result of post-surgical recession due to soft tissue contraction seen in the healing period[3,4]. The development and severity of the interdental papilla recession presents considerable differences in the population at large and young adults in particular[5]. There is no reported study on prevalence of papillary recession. Such information is necessary to gauge the burden of this type of condition in the public at large[6]. Hence, the aim of the present study was to assess the prevalence of anterior interdental papillary loss among young adults.

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Materials and Method

Study design and study population: A cross-sectional epidemiological survey was conducted from January 2019 – October 2019 among the young adults aged 18–30 years visiting in department of Periodontics at Institute of Dental Sciences, Bhubaneswar, Odisha. The study was approved by the Institutional Review Board. All willing subjects were included in the study after obtaining a written consent. Subjects with absence of anterior interdental contact point, presence of restoration and prosthesis in the maxillary anterior region were excluded. Data was collected by carrying out dental examination. Each subject was examined in a dental chair by a single, trained and calibrated examiner using dental chair light, mouth mirror, and explorer. They were checked for papilla loss parameters as per classification put forth by Norland & Tarnow et al[7] and Jemt et al [8]. All subjects underwent oral prophylaxis and clinical photographs of the selected site (black triangle) were taken. These photographs were standardized, by setting a grid over the camera screen, while clicking them. The photographs are taken such that the black triangle, adjacent teeth, and the papilla between them was clearly visible. Magnification of the photographs was standardized to 25%.

Statistical Analysis: Data analysis was done with SPSS Statistics version 20. Summary and test of significance was done with Chi-squared test. A value of P of ≤ 0.05 was considered to be statistically significant.

Results

A total of 483 patients of both genders were screened out of which 157 met the selection criteria. In age group upto 35 years, the papillary recession was 32.5% ($n = 51$); age group 35–50 years, it was 41.4% ($n = 65$); age group above 50 years, it was 26.1% ($n = 41$). The frequency of interdental papillary loss was found to be highest among adults within age group of 36-50 years. It was revealed that there was higher prevalence of papillary loss with higher age. Males were mostly affected by papillary recession 62.4% (n=98) compared to females 37.6% (n=59) which was of statistical significance. Papillary loss was commonly seen in midline 51.0%(n=80) than in laterals 49.0% (n=77). The most commonly seen was Nordland & Tarnow et al class I type of papillary recession 43.3% (n=68) followed by class II 33.8%(n=53), class III 22.9%(n=36) and according to Jemt et al classification,the most common was grade 2 type of papillary recession 43.3% (n=68) followed by grade 1 33.8%(n=53),grade 0 22.9%(n=36),grade 3 0.0%(n=0). (Table 1).

Table 2 and Table 3 shows distribution of anterior papillary recession as per Jemt et al and Nordland et al classification, in which there was no statistically significant difference between the males and females (p=0.19) as well as no significant difference were found when age groups were compared(p =0.07) but there was statistically significant more papillary recession in midline as compared to laterals (p=0.00) as per both the classification system.

Table 1. Distribution of anterior papillary recession as per Gender, age group, Papilla Location, Jemt et al and Norland & Tarnow et al classification (N= 157)

		N	N%	Chi	P value*
Gender	Male	98	62.4%	9.69	0.00
	Female	59	37.6%		
Age Group	Upto 35 years	51	32.5%	5.55	0.06
	36 - 50 Years	65	41.4%		
	Above 50 Years	41	26.1%		
Papilla Location	Lateral	77	49.0%	0.06	0.81
	Midline	80	51.0%		
Jemt et al	Grade 0	36	22.9%	9.80	0.01
	Grade 1	53	33.8%		
	Grade 2	68	43.3%		
	Grade 3	0	0.0%		

		N	N%	Chi	P value*
Norland & Tarnow et al	Class I	68	43.3%	9.80	0.01
	Class II	53	33.8%		
	Class III	36	22.9%		

N = No of subjects; *Chi Squared Test, $p \leq 0.05$

Table 2. Distribution of anterior papillary recession as per Jemt et al classification

		Grade 0		Grade 1		Grade 2		Chi	P value*
		N	N%	N	N%	N	N%		
Gender	Male	24	24.5%	37	37.8%	37	37.8%	3.37	0.19
	Female	12	20.3%	16	27.1%	31	52.5%		
Age Group	Upto 35 years	15	29.4%	22	43.1%	14	27.5%	8.76	0.07
	36 - 50 Years	15	23.1%	18	27.7%	32	49.2%		
	Above 50 Years	6	14.6%	13	31.7%	22	53.7%		
Papilla Location	Lateral	32	41.6%	31	40.3%	14	18.2%	46.80	0.00
	Midline	4	5.0%	22	27.5%	54	67.5%		

N = No of subjects; *Chi Squared Test, $p \leq 0.05$

Table 3. Distribution of anterior papillary recession as per Norland & Tarnow et al classification

		Class I		Class II		Class III		Chi	P value*
		N	N%	N	N%	N	N%		
Gender	Male	37	37.8%	37	37.8%	24	24.5%	3.37	0.19
	Female	31	52.5%	16	27.1%	12	20.3%		
Age Group	Upto 35 years	14	27.5%	22	43.1%	15	29.4%	8.76	0.07
	36 - 50 Years	32	49.2%	18	27.7%	15	23.1%		
	Above 50 Years	22	53.7%	13	31.7%	6	14.6%		
Papilla Location	Lateral	14	18.2%	31	40.3%	32	41.6%	46.80	0.00
	Midline	54	67.5%	22	27.5%	4	5.0%		

N=No of subjects; * Chi Squared Test, $p \leq 0.05$

Discussion

Anterior papillary recession is an unpleasant and unwanted condition, concerning mostly individuals of middle age groups throughout the world. Presence of a visible black triangle on smiling is often disturbing since it poses an esthetic, psychological and functional malady. However, according to the literature few studies have investigated the epidemiology of gingival recession, but no studies have been carried out was to assess the prevalence of anterior interdental papillary loss among young adults in India.

The present study showed that the prevalence of papillary recession was overall 62.4% in males and 37.6% in females, findings that were in agreement with studies that reported gingival recession prevalence ranged from 50.0% to higher percentages[9-11]. Kozlowska et al[12] reported 31.7% of females and 24.3% of males showed gingival recession. Our results were in contrast to these findings probably due to the fact that factors concerning papillary recession may are not similar to that of gingival recession[13].

The frequency of papillary recession was found to be more in middle aged group (36-50 years). This observation appears to be in usual and on expected

lines due to longer period of exposure to factors that are associated with periodontal disease and gingival recession exhibiting a cumulative effect. In our study, maxillary central incisors (n=80) had the highest prevalence of papillary loss when compared to lateral incisors due to the presence of midline raphe, midline frenum and developmental fusion of plates. These findings were in corroboration with the previous studies[14-20] as gingival biotype is thin in teeth that are prominently positioned.

To the best of our knowledge, this is the first of its kind survey that assesses the prevalence of anterior papillary loss in young adults. Prevalence studies are important for public health as they reflect the burden of disease in a specific population and help in the formulation of treatment strategies and policies[21]. A cross-sectional study has an inherent limitation of being specified to a single point which applies to the current study as well[22,23]. Nevertheless, within the limitations of our study, it may be concluded that there exists a high prevalence of anterior papillary loss among young adults and is significantly associated with age and location. Future studies may be carried out to understand the distribution of this condition better.

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Ethical Permission: Approved

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Recent Advancements in Pediatric Dental Caries Prevention

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Abstract

In childhood dental caries is commonly affecting diseases. According to the World Health Organization 60% to 90% of school children and adults are affected by this disease. It is a disease that can be prevented and if diagnosed in early-stage tooth decay can be stopped by applying various preventive materials. The primary prevention of dental caries mainly focused on young children. Fluoride has been widely used as a preventive measure against caries development. Fluoride is found in topical and systemic form, the topical form is good, has a high life span. Recent advancements suggest that asymptomatic regimen of caries lesion has the inherent of re-mineralization. In the field of dentistry, nanotechnology has been introduced which has developed substances ranging from 100nm or smaller in size. Nanotechnology replicates the natural dental enamel by the process of bio-mineralization. Leading an integral part of bone and teeth is the hydroxyapatite. Since, nano-hydroxyapatite can provide preventive and therapeutic loom to caries in children worldwide, therefore various researches are performed to improve its capacity in clinics.

Keywords: *Dental Caries; Children, Dental Management, Host-defense system.*

Introduction

Oral health is an integral part of general health and well being. It is important to know about the factors which have a bearing on oral health on oral health so that preventive measures can be taken. In childhood dental caries is commonly affecting diseases. According to the World Health Organization 60% to 90% of school children and adults are affected by this disease. It is a disease that can be prevented and if diagnosed in early-stage tooth decay can be stopped by applying various preventive materials. The primary prevention of dental caries mainly focused on young children.[1,2]

Etiology: Numerous factors are associated with caries formation i.e; environmental, biological factor, and socioeconomic status and behavioral. It is dependent on the complex interrelationships between the five critical parameters such as: 1- Biofilm, 2- Tooth habitat, 3- Diet, 4- salvia, 5- Oral hygiene. If the person has higher risk factors, then the caries experience chances also increase. Dental caries is a condition that takes position when pathogens, primarily streptococcus mutans, overrun the tooth aspect and anabolism digestible carbohydrate to fabricate lactic and other acetous, prompting in demineralization of teeth. In children age 12 to 30 months, caries consistently begin with influence the maxillary primary cuspids and first primary bicuspids, through back the ornamentation of emission. Dental caries first noticeable as colorless fleck contusion, which are small-scale domain of demineralization underneath the enamel aspect.[3] At this phase, the caries contusion is conventionally unpredictable. If oral state forgoes ameliorate, demineralization advancement and in due course termination in irreparable cavities, with a deprivation of the accustomed tooth configuration and appearance. Uninterrupted continuance of the caries procedure guide to pulpitis and tooth dropping, and could be correlated along with impediment for instance facial fervouring and congenital inflammation.[4]

Streptococcus Mutans: Most of the organisms present in the human body consists near about 10^{14} cells and the majority are resident of microflora, and the progression to the growth of s.mutans instigate the dental caries. The ability of the s.mutans to bind to the tooth structure is highly assembled through dental plaque, and the set of bacteria are specially ordered and metabolically desegregated. Complex form of matrix and glucan isthe

main configuration of plaque accumulation.[5] *S.mutans* can survive at high ph and is the originator of enamel demineralization. Sucrose which is a sweetening agent naturally materialize carbohydrate and boosts critically with *S.mutans*, other acidolysis agents and acid indulging species. It can begin and maintain microbial growth and to carry on with acid production at low ph values. When there is a decrease in saliva flow, the ph of the plaque drops, allowing the acid-tolerant bacteria like s.mutans to proliferate. It forms dextran and causes a sticky plaque, trapping bacteria, calcium and phosphate ions.[6]

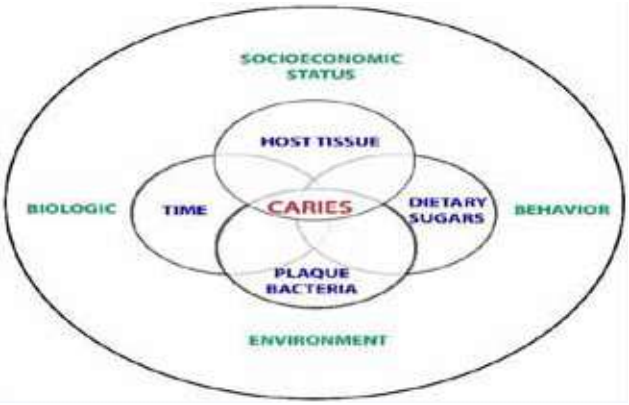


Figure 1

Host Factors:

Saliva: It consists of approximately 99% water along with 1% solid, the viscosity nature of saliva is due to electrolytes and proteins. It is the main defense against caries protection, and it also buffers the acid formed by Streptococcus mutans which is formed by oversaturation with calcium and phosphate ions. It configured a protecting mucoid coating on the mucous integument which reacts as a barricade to plague and intercept desiccation. Its flowaid to clear-out mouth of food and cancellated and bacterial detritus and accordingly slow down plaque establishment. The film with glycoprotein formed over the tooth surface by saliva also protects the tooth by wear due to erosion and abrasion.[7,8]

Remineralizing Agent: Minerals that are delivered onto carious lesion predominantly calcium and phosphate apprehend caries evolution. If the alignment of tooth and vulnerability to acidulated ambush then it is slighter to demineralization. Since the demineralization procedure persists, the ph of the mouth flatters further acidulated which encourages the evolution of cavities. Disintegrated deposits then dispense out of the tooth configuration and into the saliva abutting the tooth.[9] The shielding amplitude of saliva considerably smashes the ph of plaque neighboring the enamel, thereby restricting caries development. Plaque extent and the integer of bacteria attending regulate the efficacy of salivary shields. The elevated salivary centralization of calcium and phosphate which are retained by salivary proteins may register for the evolution and demineralization of enamel. The occupancy of fluoride in saliva pips up crystal drizzles establishing a fluorapatite–like plating which will be more impenetrable to caries.[8-11]

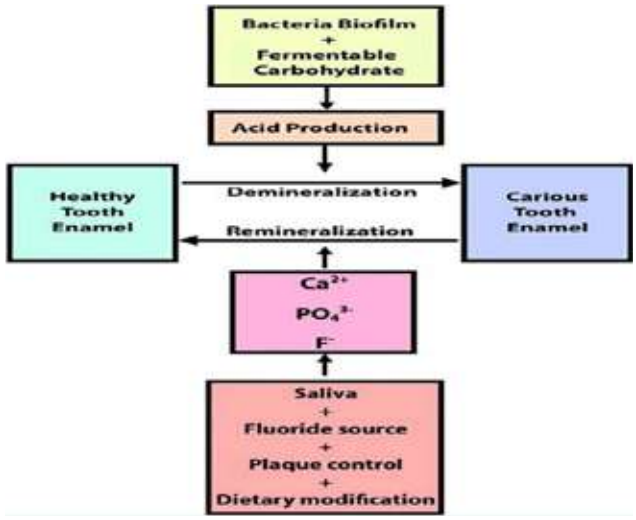


Figure 2

Fluoride: To reverse the tooth structure in early lesion, fluoride is the chief aspect of remineralization. It can minimize caries by counteract demineralization and developing remineralization of tooth surface and can impede plaque, acid fabrication. Considerable engrossment of fluoride liberate from the deliquesce enamel or previously available on the plaque, the more will remineralization be advocated and carious action is slowed down. Utilization of topical fluoride elevates the fluoride extent of tooth surface and fundamental tissues to anextent anticipated to defend in opposition to caries.[12]

CPP-ACP: It is Anti cariogenic potential and enhances remineralization. It is mainly used in the biomedical field because of extremely good bioactivity, adaptable biodegradable rate, High cell adhesion, and great osteoconductivity. For the treatment of tooth sensitivity it is the most recognizable abrasive cement. CPP-ACP holds together to plaque and it imparts immense calcium reservoir and diffuse unbound calcium steadily.

Tricalcium Phosphate (TCP): Fluoride and TCP are the attribute consigned to the tooth during brushing

and dispense intensify remineralization and put on preservation in opposition to demineralization. Calcium complex gets triggered when TCP extends to the tooth surface, and it is shifted by TCP and is guarded by fluoride ion. And remineralization gets boost up when the calcium and fluoride ion level gets down to lesion when delivered tooth surface.[7] Since the configuration of TCP is indistinguishable to hydroxyapatite, once the functionalized calcium ions are liberated, they voluntarily interrelate with the tooth surface and below the grounded structure. Since added calcium phosphate accompaniment may need an acidic ph, which credible restricts the prerequisite to the tooth, functionalized TCP can provide the most favorable advantage when distributed in a neutral ph environment.[13,14]

Silver Dynamin fluoride (SDF): is used in young children to restore carious teeth by conventional method. The excessive application of SDF Stains the tooth surface black, which results in the formation of an impermeable layer over the tooth structure.

Recently it was established that SDF does not inimically influence the bond strength of resin composite to non-carious dentin, contradictory to this an additional in vitro study from Japan establish that SDF intensifies the bond strength of GIC to bovine dentin. [3]

SDF can be applied to proximal caries lesion using spongy floss. A small section next to the contact is then saturated with SDF and pulled under the contact area. Secure the area by brushing fluoride varnish or petroleum jelly over SDF treated Surfaces. The application of varnish will not 'seal in' the SDF; in this protocol, the varnish is used to create a minuscule barricade in the middle of the treated surface and saliva.[4-7]

Nano - Hydroxyapatite: Enamel is considered as the main inorganic component of hydroxyapatite. Enamel is generally made up of HA of size 20 o 40nm particle. S. mutans which produces glucan is absorbed by hydroxyapatite along with salivary proteins which inhibit plaque formation. HA is an organically appropriate medium, and is appraised as ananalytical amalgamation replacement for the natural mineral component of dentin. Its appositeness in biomaterials by the introduction of HA powders to restorative dental materials for remineralization results and enhancement of mechanical belongings has been explored due to its magnificent biocompatibility and bioactivity nature.[5-8]

Mechanism of Action: HA perhaps reconstruct and intercept commencing contusion in enamel due to its immensity and high-level possibility of calcium and phosphate anion particles that take part in a lead character in demineralization and remineralization procedure. The absorption of calcium is perceived to have appreciable termination than the phosphate absorption in obstructing demineralization. The stunted portion of calcium in plaque is established to be a characteristic of caries prevalence while not a bit for the phosphate anion. The ph intensity transforms the absorption of calcium and phosphate in nano- HA ph cramming where high-rise degradation of remineralization is perceived at ph 4.0 apart from at ph 7.0, and as ph the practically diminishes,the absorption of calcium and phosphate intensifies.[7,8,14]

Conclusions

Although current research and technologies have enabled early detection of caries, no current diagnostic method fulfill all the criteria for optimal caries diagnosis. Recent advances have great potential to improve oral health, in the field of pediatric dentistry advancement can be carried out by the application of nanotechnology, by providing effective preventive, diagnostic and therapeutic measures. So, it is indispensable that we, as dentists should be distinct on managing not only those who are afflicted, but also handle those who are further probable to get sick.. we have a diversification of latest negotiator which can be handed-down to intercept dental caries but solicitation of this representative in clinical inquiry is stock-still restricted in the evolving countries. Furthermore dental caries are complex and all preventative estimates should be assessed appropriately in human litigation so that they can be established at the grading strength for the anticipation of dental caries.

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Ethical Permission: Approved

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Apexification: A Comprehensive Review

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Abstract

Necrotic pulp in permanent teeth with incompletely formed or wide-open apices results in the death of cells that are effective in the improvement of root. This slows down the possibility of apical closure as well as improvement of root. Endodontic management consisting of diseased or dead pulp with adolescent permanent teeth along with an open expanded root apex is a challenging concern. During obturation in that tooth,it is unimaginable to obtain a proper seal at the tip. There are various techniques to treat an immature tooth with open apex which involve the application of (1) a custom-made cone(blunt end); (2) a short fill technique; (3) periapical surgery; (4) apexification; and (5) one-visit apexification. Mostly recommended treatment of choice is ***'apexification'*** which is a method to induce root apex development of an immature nonvital tooth by formation of osteocementum or bone-like tissue. The most recommended medicament is *calcium hydroxide* that facilitates the development of a calcific barrier found in the root apex. According to the survey of the clinical involvement and information of journalists, management of an incomplete nonvital tooth can bring noticeable outcomes from the disinfectant and ossification promoting activity of $Ca(OH)_2$.

Keywords: *Apexification, immature non-vital tooth, apexogeneis, calcium hydroxide, root end closure, apical barrier.*

Introduction

Apexification is a method to prompt calcific blockade in an immature non-vital permanent teeth by removing the necrotic pulp just short of root end and placing a suitable biocompatible agent to close root apex[1]. Its purpose is to allow the formation of an apical barrier in immature teeth where advancement, as well as the progression of root, terminate by cause of pulpal death. The mineralized tissue can be consists of osteocementum, osteodentin or bone or combination of the three[2]. Apical closure may be complete or a few millimeters short from the root end. It may be either at the root tip or an uneven solidifying mass over the one-third of the apical part. Most of the cases have apical closure in an irregular and abnormal anatomy[2]. As compared to others; apexogenesis[3,4] (*root end closure in vital tooth*) gives rise to proper apical closure along with normal-appearing root development. Previously several attempts made for the treatment of an immature non-vital tooth with wide-open root apex by a custom made cone (employing a blunt-ended gutta-percha cone which is large and performing conventional root canal obturation); a short fill technique (keeping the obturation short of apex); periapical surgery and retrograde filling; *one visit apexification*[5] (restoration of the root apex by the use of a biocompatible substance like dentinal chips or tricalcium phosphate) and lastly apexification. Among them apexification[1] is the most popular method which may involve one or many monthly appointments for placing suitable medicament to achieve elimination of intracanal infection that stimulates calcification and produces the apical closure. To carry out apexification, calcium hydroxide is being replaced by MTA[6] as a single visit technique.

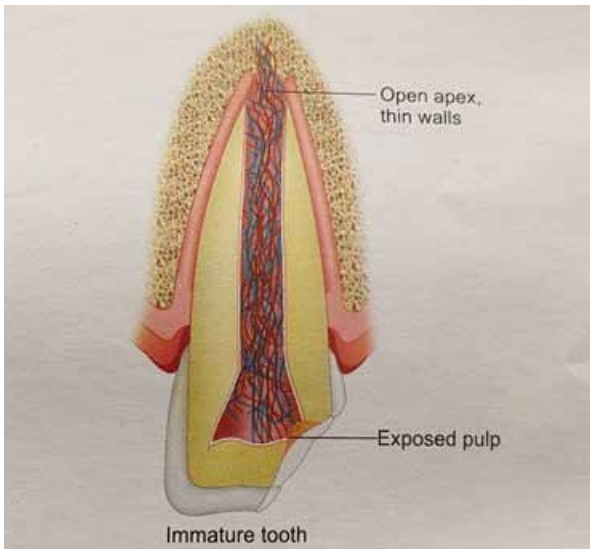


Figure 1. Diagrammatic representation on open apex

Indication Apexificationis indicated in Non-vital permanent teeth with wide-open apex

Aim: The purpose of the apexification technique is: the previously mentioned method should prompt apical closure in a non- vital adolescent permanent tooth root based on intermittent radiographic estimation. There should be no history of postoperative unfavorable indication and expressions like sensation for a lengthy period; ache or discomfort as well as inflammation. There should be an absence of irregular canal ossification; inner or outer constriction of the root; disorder at the lateral root; disintegration to periapical or peri-radicular prompting structures at the time of the procedure or after the procedure.

Materials Used: The most commonly used materials to induce root apex closure are: Calcium hydroxide +sterile water; Calcium hydroxide +CMCP; zinc oxide eugenol; resorbable tricalcium phosphate; Corticosteroid paste; Calcium hydroxide + methylcellulose; Chlormycetin paste;Calcium hydroxide + cresatin; tricalcium phosphate[7]. Various newer materials are suggested for single visit apexification. They are Mineral trioxide aggregate (MTA); Osteogenic protein I & II; and Collagen -calcium phosphate gel.

Procedure: The following measures are involved in the procedure:

Isolate the concerned tooth precisely with the use of rubber dam and gain access to the pulp canal using Gates-Glidden bur. Then keep a file inside the pulp cavity, after that radiograph is taken to build an exact working length of the root. Remember that no instrument should be kept inside apex which may lead to laceration of the tissues. In the first appointment extirpate the fragments of pulp by the help of barbed broaches and files. Next apply hydrogen peroxide for eradicate remnants and canal is flooded via sodium hypochlorite (NaOCl) solution followed by sterile saline solution without applying pressure[8]. The needle should loose within the root canal. By using absorbent points together with cotton pellets the cavity is dehydrated. To avoid destruction of small dentinal walls minimal instrumentation is considered.

A thick cement of calcium hydroxide along with camphorated mono-parachlorophenol (CMCP) or calcium hydroxide with methyl cellulose paste is conveyed to the canal by the use of lentulo spiral seated in a low-velocity engine with specially arranged syringes or with files followed by compression using endodontic plugger due to absence of apical stop. The operator must be careful in the case of rotary instruments as they cause breakage of instruments.

The patient is recalled after 2 to 3 weeks. The main purpose of the second appointment to complete debridement and eradicate tissue fragments distorted by calcium hydroxide dressing which might not be eliminated technically in the first appointment. Radiographically the root length should be established as electronic apex locators are not safe in such teeth. On the second appointment calcium hydroxide paste will be inserted within the canal with the help of endodontic pluggers[9].

Using an endodontic plugger the paste or cement is forced into the edge of the apex (apical tip), however material that overflows must never be pushed behind apex. Cotton pellet is established above calcium hydroxide [$Ca(OH)_2$]. Then close the tooth surface with a temporary filling like *zinc oxide eugenol* paste.

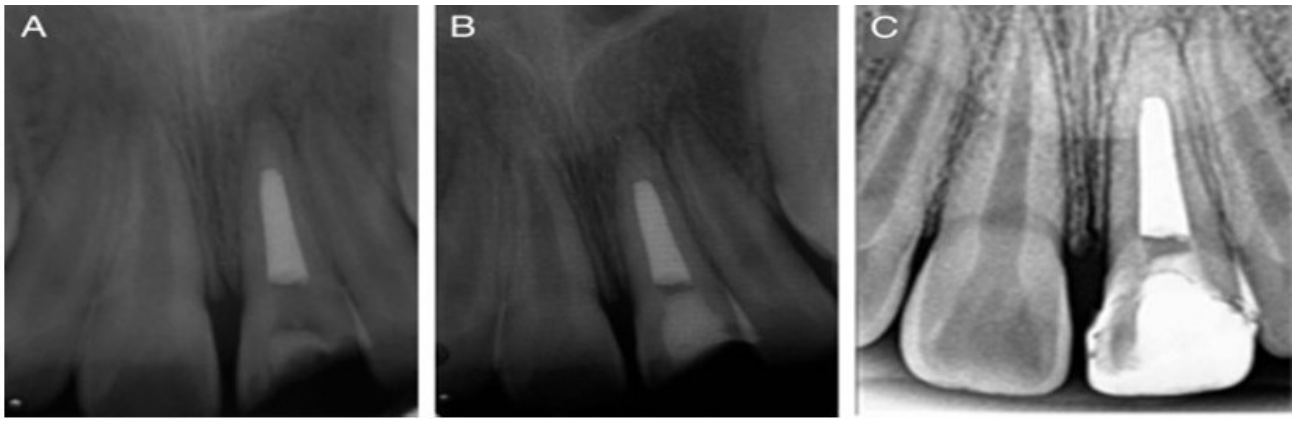


Figure 2. Procedure of apexification

Treatment and Follow up: Signs and symptoms are evaluated regularly. The follow-up assessment is repeated every 3 months up to the successful occurrence of calcified barrier. Then tooth is observed clinically and radiographically[10]. While a calcified barrier seen radiographically, then the tooth is re-established followed by flooding with sodium hypochlorite solution (NaOCl). To verify the integrity of the apical barrier, the apical zone should be investigated using a file to get the length of the root. Generally the cement is permitted to stay about 6 months. Then the pulp chamber or pulp cavity is re-established that one may resolve if the tooth is prepared toward traditional gutta-percha restoration being resolved via the existence of a "positive step" while the apical zone is explored using the instrument. Confirmation about radiographic findings for root-end closure is required too[10].

According to Frank, there are some criteria for apexification treatment: (A.) progressed apical closure of the pulp cavity as in normal tooth, (B.) a dome-shaped apex closure maintaining a blunderbuss manifestation, (C.) radiographically appearing calcified or ossified barrier at the apex, along with (D.) an apical stop with radiographic indication of a barricade coronal to root apex. If barrier or closure of root end is found insufficient within 24 weeks and the patient feels the touch of the file, then the procedure is repeated with calcium hydroxide paste till a complete barrier is developed[11]. While an ossified barrier constructed coronal to the apex, it should not be penetrated to pack the tooth till the apical end.[12] The apical barrier formed by the tissue considered as healthy tissue. It has been believed that the use of calcium hydroxide can consequentially enhance the chance of root fracture after long-term use. So it is essential to reduce the time required for apexification.[13,14]

At 3 to 6 months interval re-establishment of the canal for the lengthy-time period resembles justified as the patient stays relieved from unfavorable signs and symptoms as apex closure is likely to develop ultimately. As seen in normal tooth continuity of apical growth and closure expressed by postoperative radiographs. As apical closure takes place,the canal filled with gutta-perchaa and sealer.



Figure 3. Follow up evaluation of apexification; a, open apex before treatment; b. 3 months follow up; c. 9 months follow up

Types of root apex closure seen after apexification technique:

- Usual root end advancement
- Radiographic closure along with hard tissue stop conspicuous clinically
- Clinically noticeable hard stop with no evidence of radiographic bridge formation
- Development of calcific bridge just coronal to apex

Complications

Following the apexification procedure, crown-root proportion is unfavorable since the length of the root is short as compared to the normal tooth, therefore the diagnosis of tooth can be inconvenienced. In the case of post-induction (whenever required) is complicated because of the diameter of the root canal as well as induction of permanent crown may be prolonged because of the continued progression of the patient.

Summary & Conclusion

Apexification using calcium hydroxide Ca(OH)2 remains the most broadly applied method[1,16] Management of an immature non-vital tooth can bring noticeable outcomes from the disinfectant and calcification promoting activity of calcium hydroxide $Ca(OH)_2$ which cleanse the canal and encourage apical closure [17]. High PH as well as low solubility of calcium hydroxide maintain the antimicrobial action for a lengthy time period[18]. Either Calcium hydroxide alone or calcium hydroxide along with sodium hypochlorite enhance disintegration of fatal tissues for which it aids for removal of debris and remnants from pulp cavity.[19]

To perform an apexification, using calcium hydroxide is a valid therapy for many years. It may require several appointments and might require ages to carry out to form a barrier at the apex,which permits the pulp cavity restoring substance by gutta-percha along with sealer.[20] The time required for this procedure depends upon the condition of root development as well as the state of periapical tissue[21- 23]. Though the use of calcium hydroxide brings about favorable outcomes and percentage of auspicious apical barrier development is huge, deep-rooted follow up is mandatory. Tooth which managed by apexification is sensitive to break due to frailty arising from non-vitality as well as from thin dentinal walls of root[24-26]. Another demerit includesa period of treatment.

After all MTA barrier is a technique used as a substitution to calcium hydroxide[27]. This procedure doe not require several appointments and there is no need for an external factor for the development of barrier as well as in pulp regeneration.[29-33]

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Chairside Diagnostic Aids in Periodontics: A Review

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Abstract

The need of the hour is an accurate and timely diagnosis for an effective treatment plan to cure or eliminate the disease. Clinical measurements, traditionally available, have their limitations in helping diagnose an "active" disease. Hence, novel chairside diagnostic kits are essential to detect active disease, predict its progression and help in the evaluation of response, of a patient, to therapy, thereby aiding in the management of periodontal patients. This review article, therefore, discusses the various chairside diagnostic aids available to aid Periodontists, in diagnosing and treating periodontal patients.

Keywords: *Diagnosis, Periodontal disease, Chairside, Diagnostic aids.*

Introduction

Periodontitis is an inflammatory disease of the periodontium, that is, the supporting structures of the tooth, resulting in a gradual loss of it. This gradual degradation of the periodontium leads to exposure of tooth root causing sensitivity, esthetic concerns, gradual mobility and eventually loss of the tooth. Accumulation of dental plaque, due to various factors, leads to an increase in the microbial load in the oral cavity, which eventually, in combination to host's immune response, leads to gingivitis, which if not treated, turns into periodontitis. A diagnosis hence is achieved by clinical inspection of the gingiva surrounding the tooth with the help of instruments, such as the William's Probe or the UNC-15 Probe, examination of the radiographs, such as the IOPAs and OPG and other investigations if required. [1] The traditional clinical parameters and methodusually predict previous periodontal disease than active disease. Therefore, diagnostic kits which can detect active disease and predict its progression are the need of the hour. [2,3]

The available 'Point of Care' diagnostics, are designed to measure microbiological, immunologic and genetic constituents in oral diagnostic media namely: saliva, gingival crevicular fluid (GCF) and plaque, indicative of health or disease. The main aim behind developing such kits is to diagnose active disease so that the following achievement:

- Less invasive treatment
- Reduced chair time
- Reduced expense
- Long term prognosis

Armitage GC 1996, listed out the following purposes served by periodontal diagnostic aids: [4]

- Screening of active disease
- Diagnosis
- Determining sites and/or subjects at a higher risk of disease progression
- Treatment planning
- Therapy monitoring

Chairside diagnostic aids:

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The ideal diagnostic test should be (Chapple 1997)[5]:

1. Non-invasive
2. Cost-effective
3. Adaptable to sample collection, storage and transportation.
4. Simple to perform at the chairside
5. Rapid, with 1-stage or 2-stage procedure
6. Highly specific, sensitive, reproducible and quantitative.

Hence, keeping these goals in mind, the following are the available or in the making, chairside diagnostic aids.

Microbiological test kit: The main bacteria associated with periodontal disease are Aggregatibacteractinomycetemcomitans, Prevotella intermedia, Porphyromonasgingivalis and Tannerella forsythia.[6]

Presence, elevated levels or absence of these microorganisms may predict, indicate or monitor:

- "Active" disease
- Loss in Clinical attachment level
- Disease initiation and progression
- Sites at higher risk for active destruction and;
- Response to periodontal therapy

Evalusite (KODAK): Evalusite is a diagnostic kit based on the principle of ELISA, which involves the linkage between the antigen and a membrane-bound antibody to form an immune complex that is assessed through a colorimetric reaction. It is mainly used for the detection of three principal microorganisms: Aa, Pi and Pg.[7]

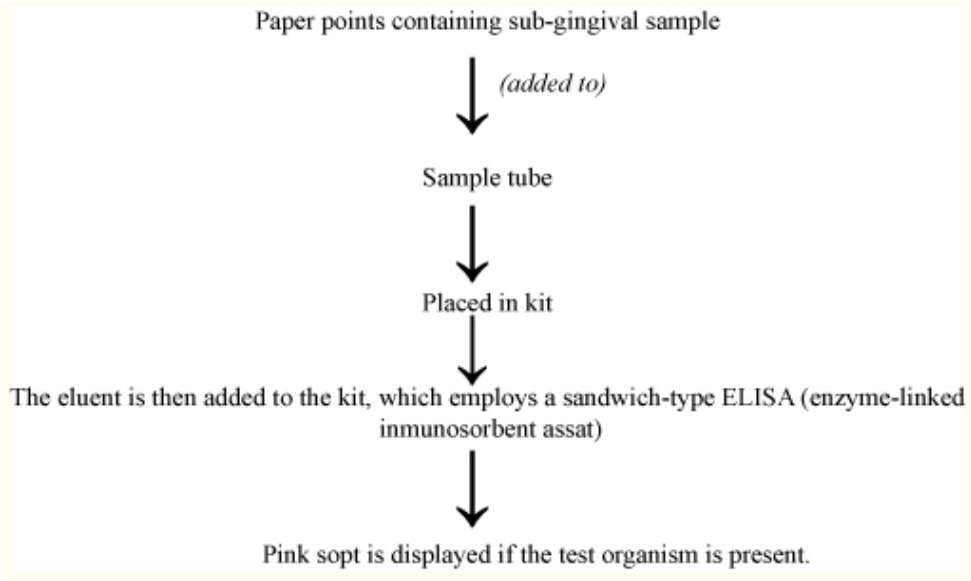


Figure 1: Evalusite (KODAK)

Omnigene: Omni Gene Diagnostics, Inc. has produced a set of DNA probe systems, by applying the principle of genetic engineering. These are species-specific probes which tests for 8 periodontopathogens:

- Actinobacillusactinomycetemcomitans (Aa)
- Treponema denticola (Td)
- Porphyromonasgingivalis (Pg)
- Fusobacterium nucleatum (Fn)
- Prevotella intermedia (Pi)
- Eikenellacorrodens (Ec)
- Campylobacter rectus (Cr)
- Bacteroidesforsythus (Bf)

Subgingival plaque samples, collected from patients for analysis, are sent through the mail to OmniGene Diagnostics. Practitioners may receive the results through phone,fax, or mail. [8]

BANA Test: A trypsin-like enzyme is produced by only 3 specific anaerobic bacteria amongst the various species present in subgingival microbiota. Those are Porphyromonasgingivalis (Pg), Treponema denticola (Td) and Bacteroidesforsythus (Bf). The test works by detecting the presence of these bacterias, as their enzyme hydrolyzes the synthetic peptide Benzoyl-DL-arginine-2-naphthylamide or BANA, present in the test. The test may also be used to assess oral halitosis, by detecting the presence of 2 BANA positive species on the surface of the tongue:[9]

- Stomatococcusmucinlagenous
- Rothiadentocariosa

It is a highly sensitive test, detecting the smallest quantity of pathogens. Studies have been performed, showing no significant difference in the sensitivity of DNA probes, immunological reagents and the BANA test, in the detection of these bacterias in dental plaque, of periodontally compromised patients.[10-12]

Principle:

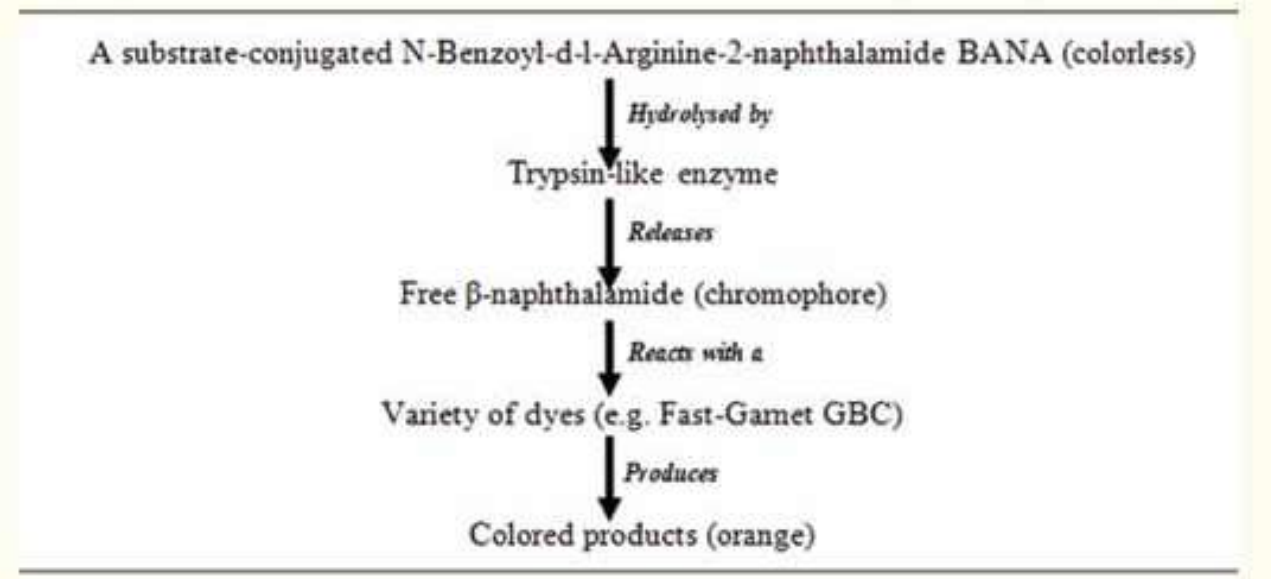


Figure 2: BANA Test

Directions for use: The test consists of a strip (plastic), to which two separate reagent matrices are attached. The upper matrix is a buff reagent, containing chromogenic diazo reagent. The lower matrix is impregnated with BANA. The subgingival plaque samples are applied to the lower matrix. A permanent blue colour is produced as a result of the reaction between the chromogenic agent and hydrolytic product produced by the enzyme. It may be interpreted as a positive or weak reaction depending on the intensity of the colour.

Perioscan: Perioscan diagnostic kit, utilizes the same principle as the BANA test. At Least 4 teeth should be sampled in each subject.

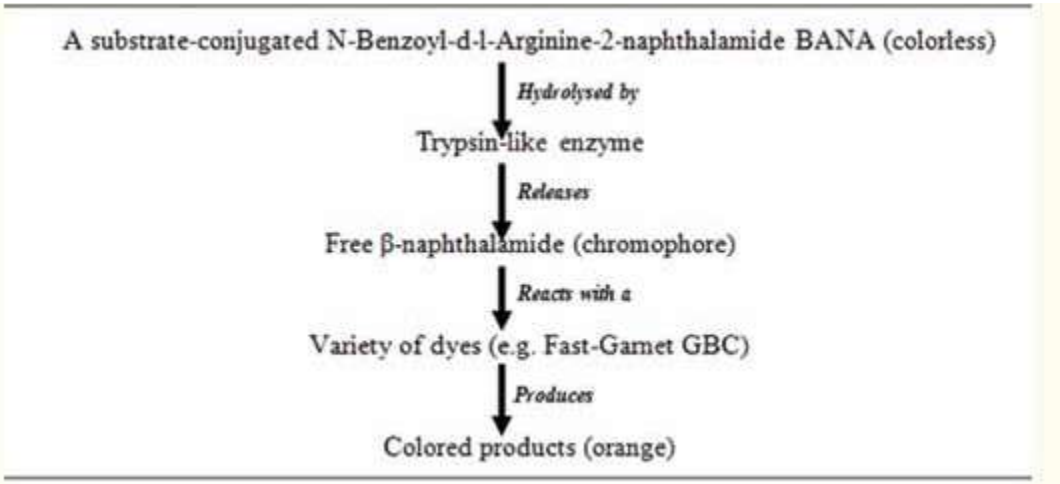


Figure 3: Perioscan

Perio 2000: This chairside diagnostic test is a combination of standard "Michigan O" style probe and advanced ion-selective electrode technology.[13]

It is designed to:

- Assess probing depths
- Assess bleeding on probing (present/absent)
- Assess periodontal pockets for sulfides

The system consists of:

- One time use sulfide containing sensor tips, which can be disposed
- Electronic control unit
- Probe handle, handpiece cable, foot-controlled pedal
- Solution provided to clean the equipment
- A stand
- System check

It can be used for:

- Assessment of patient's oral health status as an adjunct to other measurements
- Supportive periodontal therapy
- At maintenance intervals
- Patient education and motivation.

Biochemical test kits

To monitor health status, disease onset, treatment response or outcome, the molecules which can be most efficiently assessed are the 'biomarkers'.[14]

Biomarkers, present in the saliva or gingival crevicular fluid, can be measured, to monitor periodontal activity, under the following categories:[15]

- Biomarkers to determine the presence or absence of periodontal pathogens.
- Biomarkers for determining gingival and periodontal inflammation
- Biomarkers to determine the host's immune-inflammatory response to certain pathogenic microorganisms; and
- Biomarkers to determine host tissue destruction

Biomarkers may be tested from saliva and gingival crevicular fluid. GCF is the choice of oral fluid for testing as :

- Can be noninvasively collected
- Allows site-specific fic analyses
- Convenient measuring of destructive MMPs, by distinct immunoassays.

Saliva too can be used as it possesses certain advantages[16] :

- Easily accessible
- Noninvasive sampling
- Quick and reliable outcomes
- Consists of diagnostic biomarker molecules in abundance

Biomarkers in gingival crevicular fluid.

Inflammatory and immune products	Bacterial proteases	Host derived enzymes	Tissue breakdown products	Bone specific proteins
Prostaglandin E2 (PGE2)	Alkaline phosphatase	Alkaline phosphatase	Glycosamin oglycan	Pyridinium crosslink urine pyridinoline
Cytokines	Aminopepti dases	β-Glucuroni dase	Hyaluronic acid	Pyridinium cross-link collagen peptide fragment
Antibacterial antibodies	Chondroitin sulphatase	Elastase	Chondroitin-4-sulfate	Tartrate-resistant acid phosphatase
Acute phase proteins	Collagenase	Cathepsins	Chondroitin-6-sulfate	Hydroxyproline
Complement	Fibrinolysin	Serine prote nase (G)	Dermatan sulfate	Galactosyl hydroxylysine
Vasoactive intestinal	Glucosidases	Nonspecific neutral	Hydroxyp roline	Glycosaminog lycans
Peptide		proteinases		
Neurokinin a	Hemolysin	Matrix metalloproteinase-1,3,8,13	Fibronectin fragments	Osteonectin and bone phosphoprotein
Neopterin	Hyaluronidase	Aspartate amino transferase	Connective tissue and bone proteins	osteocalcin
Platelet -activating factor	Phospholipase	Myeloperoxidases	Type I collagen peptides	
	Hydroxyproline	Lactate dehydrogenase	Polypeptide growth factor	

Figure 4: Biomarkers in gingival crevicular fluid

Table Demonstrating the biomarkers present in saliva.

Markers of periodontal soft tissue inflammation	Markers of alveolar bone loss	Collagen breakdown products
Prostaglandin E2	Alkaline phosphatase	Aspartate aminotransferase
β-glucuronidase	Osteoprotegerin	Alanine aminotransferase
IL-1β	Osteocalcin	TIMPs
IL-6	Collagen telopeptidase	MMPs
Tumor necrosis factor-α	Pyridinoline cross-links of type I collagen	α2-macroglobulin
Matrix Metalloproteinase (MMP-8,9 and 13)	RANKL	
	Osteonectin	

Figure 5 Demonstrating the biomarkers present in saliva

Prognos-Stik: It determines increased levels of elastases in GCF. The elevated levels may, hence, indicate the presence of underlying disease.[17]

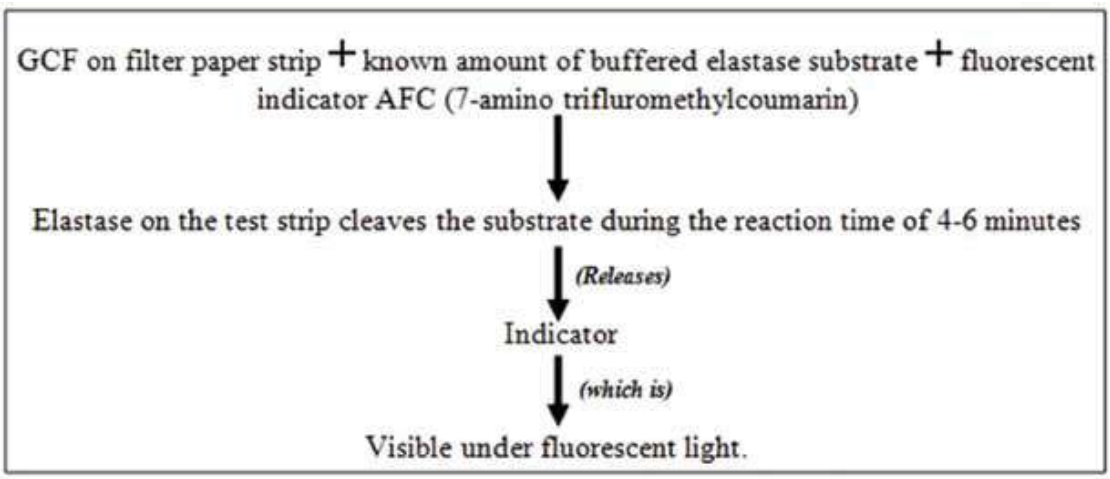


Figure 6: GCF

Periocheck: Periocheck is a chairside diagnostic test to evaluate the presence of neutral proteases. The elevated levels of these enzymes can be correlated to collagen destruction, implying the presence of periodontal disease.[18]

Biolise: Hermann et al. Developed a new test (SLT-Lab Instruments, Crailsheim, Germany) which is used to detect the elastase activity in GCF.[19]

TOPAS (Toxicity Pre Screening Assay): TOPAS is a diagnostic test kit for evaluating the presence of gingival infection, by indirect assessment of bacterial toxins and proteins. The principle for this test is the detection of the metabolic action of these pathogens, in the GCF. The color intensity scale of the test helps to differentiate between an inactive and active periodontal disease, as the degree of color change signifies the level of metabolic activity thereby determining levels of toxins.[20]

Periogard: Aspartate aminotransferase (AST), is a soluble intracellular cytoplasmic enzyme, present in the GCF, which is released as a result of cell death. Periodontal tissue destruction causes cell death. Hence AST becomes a potential marker for the presence of periodontal disease. Periogard is a chairside test to detect this enzyme, and its levels, in the GCF. [21,22]

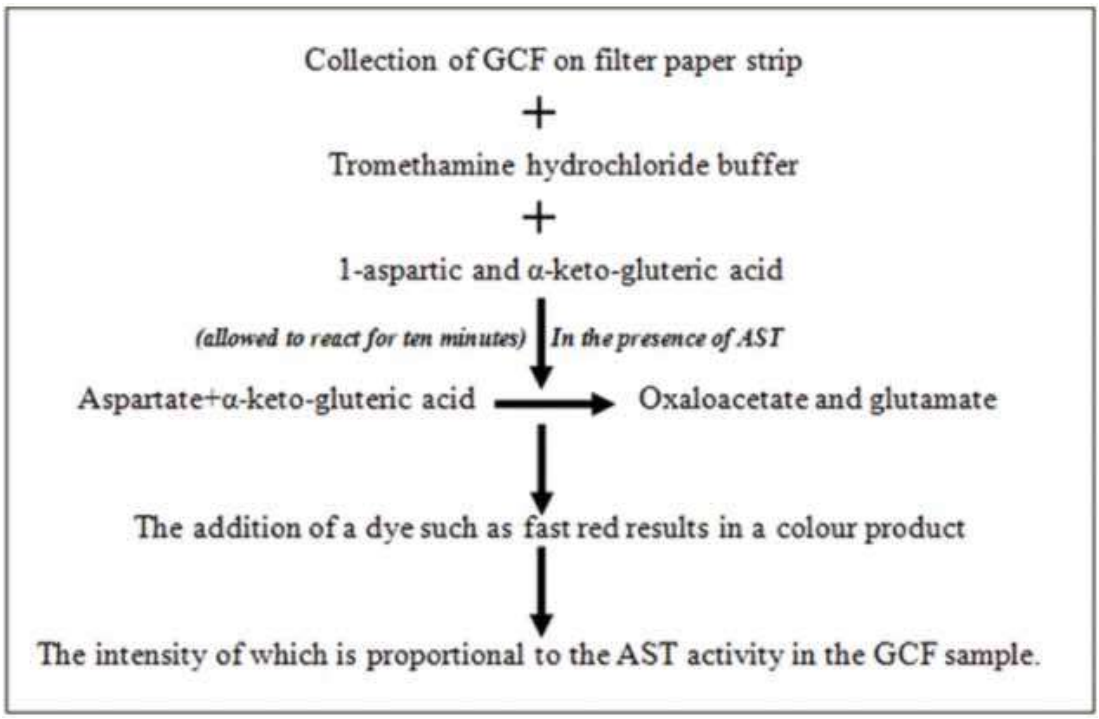


Figure 7: Periogard

Periowatch: The Perio Watch also analyzes aspartate aminotransferase (AST) at the chairside. It is an indicator of cell death, thus implying the severity of periodontal tissue destruction.

Principle: The conversion of cysteine sulfuric acid, belonging to an amino group, to alpha keto-glutaric acid to beta sulfinyl pyruvate, is accelerated by AST in the presence of pyridoxal phosphate. As a result of the instant degradation of β-sulfinyl pyruvate, inorganic sulfite is produced. It reacts with malachite green, thereby producing pink-colored rhodamine B dye. The amount of conversion of malachite green determines the levels of AST.

Oral Fluid Nanosensor Test (OFNASET):

A chairside diagnostic device developed by Dr. David Wong has been developed to detect oral cancer

by assessing the presence of certain biomarkers in the saliva.[23] It detects the following biomarkers (mRNA):

- SAT
- ODZ
- Interleukin 8
- Interleukin 1b

Also detects the following salivary proteomics:

- Thioredoxin
- Interleukin 8

Electronic Taste Chips

A chip-based chairside test which will have the capability to distinguish healthy individuals from the periodontally compromised, based on levels of C-Reactive Protein, is under development. The microspheres containing a sensor-rich platform, present on the interior of this microchip system, will measure analytes, through chemical and immunological reactions.[24]

Oraquick: The first FDA approved the oral swab 'in-home' test which facilitates accurate screening and diagnosis of human immunodeficiency virus infection. It provides results within 20 minutes and is an easy to use stick-like device. On its one end is a fabric swab, which is used to collect the oral fluid and then inserted in the testing liquid to produce results on the device.[3]

Integrated Microfluidic Platform for Oral Diagnostics (IMPOD): It is a rapid chairside device to evaluate the levels of salivary biomarkers as indicators of oral disease. Salivary samples, in small amounts (10ml), can be rapidly analyzed, hands-free, for analyte concentrations and other salivary biomarkers, through electrophoretic immunoassays.[24]

MMP Dipstick Test: It is a chairside diagnostic kit, to detect MMPs, which are host-derived proteinases, to help differentiate between healthy and periodontally compromised individuals.[23]

Genetic Test Kits: Identification of genetic polymorphism, to detect periodontal disease, is now possible due to the availability of certain chairside diagnostic kits. The initiation and progression of periodontal disease is controlled by various genes, whose polymorphism is present as a risk factor for the disease. In 1997, Kornman et al., "found an association between the polymorphism in the genes encoding for interleukin-1α and interleukin-1β and increased severity of periodontitis."

Periodontitis Susceptibility Trait (PST) test: It is the first of its kind chairside diagnostic kit to analyze 2 interleukin genes, IL-1 alpha and IL-1 beta, for mutations. It is highly useful in terms of early diagnosis of periodontal disease as it can differentiate between IL-1 genotypes, which are largely associated with various inflammatory responses.[25]

Principle:

IL-1 gene cluster, according to studies, consists of 2 polymorphisms which possess a close relation to periodontitis. These are:

- IL-1A gene, position -889
- IL-2B, position +3953

They both consist of alleles 1 and 2. Allele 1 carries cytidine and allele 2 carries thymidine at the respective positions. In cases where both genes (IL-1A and IL-2B) carry allele 2, IL-1 inflammatory mediator is released in abundance. The test hence, detects this mutation of allele sequence thereby granting early intervention and prevention of periodontal disease.[20]

Clinically, it is used for the following:

- New periodontal subjects
- To determine prognosis
- As an incentive for smoking cessation.
- Patients on maintenance.
- Specialist referral after detection.

Nucleic Acid Probe: It is a nucleic acid probe assay, developed by Microprobe corporation, to semi-quantitatively detect periodontal pathogens. The plaque samples are collected to test the bacterial load. The lysis of bacterial cells takes place by heating, in the presence of a detergent, to extract their DNA. The DNA thus obtained, is placed into a multi-well cassette, which is then placed in a machine with a programmable robotic arm. Finally, results are obtained digitally.[20]

Conclusion

To plan and treat any disease effectively, an accurate diagnosis is essential. The chairside diagnostic

tests, not only provides rapid, non-invasive and specific prediction of the disease, but also helps in early detection of the disease. As the saying goes, 'Prevention is better than cure', these tests help predict and detect an "active" disease thereby allowing early intervention, better prognosis and more effective treatment plan for the disease. The already available chairside tests help achieve these goals, despite having their limitations. Soon, better, more sensitive test kits shall be available to help diagnose and treat periodontal patients better.

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A Simplified and Cost-Effective Treatment Approach for Combination Syndrome: A Case Report

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Abstract

Combination syndrome is a dental condition, mostly observed in patients with a completely maxillary edentulous arch and a partially edentulous mandibular arch persisting only the anterior teeth. Specific oral destructive changes are often seen in patients. The manifestation of this syndrome consists of resorption in the anterior maxillary ridge, overgrowth of tuberosities, and supra eruption of mandibular anterior and reduced alveolar ridge height in combination with hypertrophic and atrophic changes in the maxilla and mandible. Complications involved in chewing and mastication are of special apprehension in such a patient. Predictable prognosis can be offered by overdentures. This article attempts to provide a simplified and cost-effective approach to this type of treatment modality by a case report.

Keywords: *Combination syndrome; Cost-effective approach; removable partial dentures.*

Introduction:

The statement by Dr. Muller DeVan "Perpetual preservation of what remains is more important than the meticulous replacement of what is missing" still sounds factual.[3] Overdenture is onesuch preventive treatment modality that helps in delaying the process of resorption thereby improving the denture foundation area, as well as enhancing the masticatory efficiency of the patient. In certain casesof Kennedy's classI and class II rehabilitation of the edentulous space can be challenging where providing a fixed prosthesisis may not be possible due to lack of sufficient amount of bone or cost-effectiveness. The removable partial denture retained with attachments is one of the treatment modalities that can facilitate both functional and aesthetic requirements. Various types of attachment systems are available for tooth-supported overdenture treatment modality. Among various attachments, the Dolder bar and the Hader bar system are the most commonly used attachments for overdentures and implant-retained restorations.[1,2]

All the attachment systems intent to provide better retention and support for the overdentures. In the case of mandibular overdenture the retention can be profoundly achieved by thesupport of natural tooth retained with bar or stud provided in the anterior region. But the disadvantage of these extracoronal attachments bar systems is that they are expensive. The concept of conventional tooth-retained overdenture is proved to be one of the simple and cost-effective treatment modality in comparison to the complex implant-supported overdentures. Bar attachments are more advantageous over other types owing to its splinting effect on the abutment teeth while eliminating the need for parallel abutments and guiding the prosthesis in place. So, bar-retained overdentures are the choices of treatment for most of the clinicians, but the only obstacle is its cost, leading to the constant need for technical innovations to better the existing modalities in terms of their availability and expense.

In the past, custom made header bars using plastic straws and custom made clips using gold alloys have been tried in an endeavor to avoid the costlier.

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The present article demarcates some of the necessary considerations in the treatment planning of a typical case of combination syndrome. Along with which the clinical report describes the complete treatment procedure for the patient, in which the mandibular tooth-borne overdenture is fabricated with a customized hader bar, customized clip and a metal base maxillary complete denture for better durability and proprioception. In consideration of periodontal health of remaining teeth and ridge form, overdenture with the hader bar attachment was planned. Customized hader bar and clip attachment was attempted owing to his financial constraints.

Case report: A 54-year-old male reported to the Department of Prosthodontics, Institute of dental sciences, Bhubaneswar, complaining of missing teeth and inability in mastication. Themedical history of the patient was evaluated which was found to be of non-contributory. On intraoral examination it was observed that he had a completely edentulous maxilla with enlarged tuberosities with to some extent a resorbed anterior maxillary arch and a partially edentulous mandible with Kennedy's type II modification 1 comprising of supraerrupted existing teeth i.e. 33,34,43,44 are presentbilaterally. (Figure.1) The patientreported the extraction of lateral incisors bilaterally 3weeks back. The patient gave ahistory of teeth loss since 5years due to multiple caries and periodontal problems. On radiographical examination of abutment teeth were found periodontally compromised.Onthe evaluation of the patient's existing clinical condition, it was planned to construct maxillary metal base complete denture and mandibular tooth retained overdenture with an extracoronal attachment. The patient was unwilling for the options of implants because of the need for economic constraints, additional surgery and the unacceptable duration of treatment phase.

Technique: After oral examination the impressions were made and the casts were retrieved which were used for the assessment of the inter-arch distance. A tentative jaw relation was recorded and the casts were mounted on an articulator.[1] In the present case, a sufficient space was found to be there to accommodate bar supported overdenture,which facilitated to fabricate a customized metal-based complete denture for maxilla along with the bar attachment overdenture for the mandibular arch.

The metal palate was customized in such a way that the metal loops were fabricated in a specific orientation and positioned upon the crest of the ridgeproviding an interlocking mechanism for the acrylic, without creating any interference for the arrangement of artificial teeth. The primary impression of the maxilla was made with impression-compound, custom tray was made with auto polymerizing resin. Border molding was done followed by a secondary impression with zinc oxide eugenol which was poured with dental stone (typeIII). The final cast was retrieved from the impression and was duplicated to make a refractory cast (Figure 2a). The customization of this metal palate was carried out on this refractory cast.

Spacerwax was adapted over the palate that extends 2-3 mm beyond the crest of the ridge. 2-3 mm long loops were made up of wax were attached to the margins of the adapted palatal wax. It was made sure that all the loops should be 3-4 mm short from the sulcus area.[4] The main function of these loops is to enhance the mechanical interlocking of acrylic to the metal base. This holds an importance that the loops should not be interfering with the acrylic border of the denture that extends to the sulcus. Towards the palatal side tiny loops were made palatal to the crest of the ridge. The loops were placed in such a way that it would not impede with the artificial teeth arrangement, and help in the flow of acrylic resin (Figure 2b). After the designing of the palate, wax sprues were attached,invested followed by casting. Following the casting and finishing of the metal base(Figure 2c), it was incorporated into the heat cure acrylic denture base. Later the occlusal rims were fabricated upon it.

For the mandibular arch, Root canal treatment was performed and post space was prepared in 33,34,43 & 44, keeping both the preparations parallel to each other so that a common path of insertion could be accomplished. Tooth preparation was carried out on both mandibular canines and right mandibular 1st premolar with a heavy chamfer finish line, which resulted in the optimal crown–root ratio and adequate clearance for overdenture prosthesis.[3] An impression was made and poured with dental stone type II, upon which a custom tray was fabricated. Border molding was carried out using a green stick compound in the custom tray. Impression of post space and the mandibular ridge was made with polyvinyl siloxane as illustrated mandibular final impression that was made with polyvinyl siloxane impression material and it was poured into dental die stone. On the master cast, the pattern for the post was prepared using pattern resin and the coping was made over it using pattern wax.[5]

The wax pattern for the copings was made and a toothpick was shaped and was attached to the wax

patterns splinting the right and left abutment teeth to obtain more stability.[5]Bar framework with metal housing mold was invested, burned-out and cast according to the manufacturer's instructions.[7]

The whole assembly was invested, burnout and cast in cobalt-chromium alloy, finished and polished. The casting was checked for a passive fit inside the patient's mouth.[2] After the metal try-in, the bars, with their respective copings, were again placed intraorally and checked. After this step, the bar, along with the metal copings, were luted onto the respective preparations and the respective tooth preparations with the help of glass ionomer cement and the undersurface of the barwas blocked (Figure 2d). The whole assembly was duplicated with the elastomeric impression material and was poured (Figure 2e). The remainder of the procedures up to try-in was carried out as the conventional method for complete denture (Figure 2f). After the fabrication of the mandibular overdenture, the inner surface at the site of the bar was trimmed about 1mm to make space for the custom clip (Figure 2g).

Later, the custom made plastic clip was placed on the cast bar in the patient's mouth and a pickup of the clip with soft relining material was done with the processed denture. The self-cure acrylic resin was replaced only on the crest of the clip and relining material didn't cover the intaglio surface of the clip.

Following the maxillomandibular relations, records were obtained from the patient, the cast was mounted on the articulator, teeth arrangement was done for both maxillary metallic denture and the mandibular bar-retained over denture and the try-in was performed, followed by acrylization (Figure 2h, 3, 4). The patient was instructed with the method of wearing and removing the dentures. Post insertion followup was done within an interval of 1 to 2 weeks. After a follow-up of about 6 months, the patient reported better improvements in symptoms which he had previously mentioned (Figure 5).



Figure 1: Preoperative intraoral condition after 3 weeks of extraction of 12 and 22

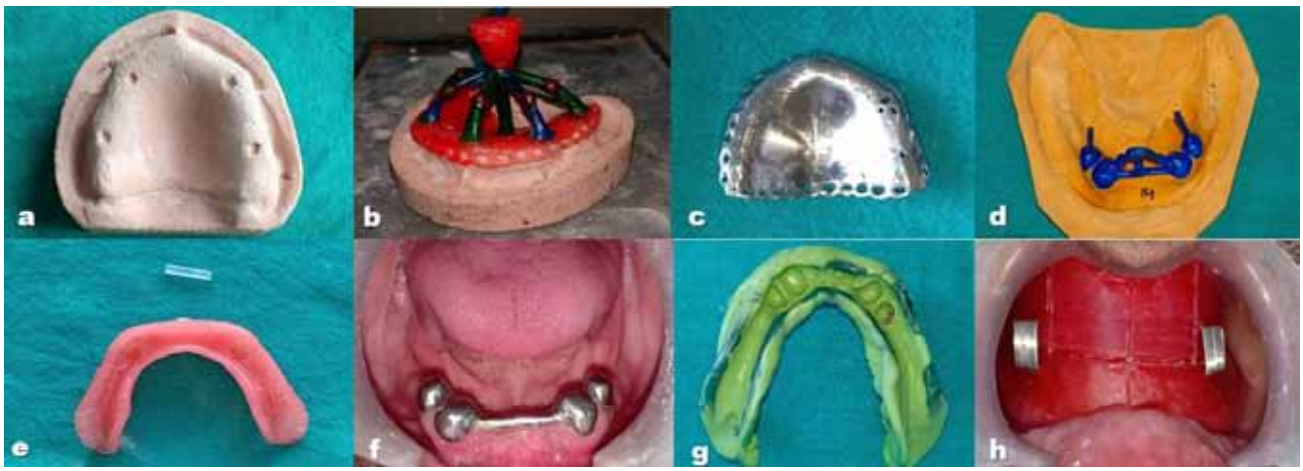


Figure 2a. Duplicated refractory cast; 2b. Sprue attachment to spacer wax; 2c. Maxillary complete metallic denture base; 2d. Wax pattern of metal copings and bar attachment with sprue; 2e. Mandibular overdenture with customized plastic clip retained in soft liner; 2f. Bar retained overdenture with metal copings; 2g.elastomeric impression of mandibular arch; 2h. Maxillomandibular relation record



Figure 3. Wax try-in



Figure 4. Complete rehabilitation



Figure 5. Post-operative photograph.

Discussion

Various extracoronal attachment systems that are easily available in the market are used for the fabrication of overdenture. Among all, dolder and hader bars are the most commonly used extraoral attachment systems for the overdentures.[7] Overdenture with attachments can redirect the occlusal forces away from the weak supporting abutment teeth and onto a soft tissue or redirect them toward stronger abutments thereby resulting in superior retention. Bar exhibits more cross-arch involvement and allows occlusal forces to be shared between the abutments.[3] The bar attachment provides a slight amount of vertical as well as rotational movement of the denture which almost acts as a stress breaker.[7]

This case involved the preservation of two canines and one premolar for mandibular overdenture in providing support, retention, stability and comfort superior to that of a conventional complete mandibular denture. In this clinical case, the mandibular anterior ridge allowed an easy fabrication of the bar joint. In this case metal bar was used as a male component splinting the abutments with a female component that has been embedded in the tissue surface of the denture base by following the indirect technique.[4]The splinting somehow reduces the torquing of the remaining root structure. There are several advantages to this procedure – ease of incorporation of clip attached to the metal superstructure helps in providing accurate fit and thus minimizing the damage to the definitive prosthesis, thereby providing adequate strength.[8] The retention clips can be easily replaced, further relining and repairing would not compromise or risk the fracture of the dentures.[3] The only disadvantage of the technique includes the extra steps during fabrication and limited applicability in patients with reduced interarch space. It is the most acceptable treatment for those who cannot afford the high implant cost and also those who are medically unfit to undergo surgery. The tooth-supported overdenture forefront the preventive prosthodontic modalities; this requires more innovation to make it the most beneficial and economical regular clinical practice.

For the maxilla, metal-based dentures were fabricated as a treatment option as it has advantages such as its adequate strength, better adaptation to the supporting tissues, enhanced plaque control, good thermal conductivity, minimal dimensional changes, and absence of interference to phonetics due to light-weight. Whereas only disadvantage associated with these dentures is difficulty in fabrication. Nevertheless; they may be indicated when polymer-based systems fail to provide acceptable physical properties.[9, 10]

Summary: To overcome the sequential destructive changes of the combination syndrome the prosthodontist

try certain treatment planning which may require a multidisciplinary approach, keeping in mind the functional, esthetic and economic requirement of the patients.In underdeveloped or developing economies, where the patient's willingness to pay is very low, the clinicians must utilize their knowledge to make sophisticated systems more economical.[10]

Conclusion

Therefore, this article presents a case report for a patient with combination syndrome that was treated with a significant treatment planning in a cost-effective method. The objective of these removable partial dentures is retention, stability, support and esthetics. The above-described method is a cost-effective technique and fulfills all the objectives.

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Ethical Permission: Approved

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Platelet Rich Fibrin in Socket Preservation

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Abstract

With the advancement in dentistry, there are different ways of treatment such as restoration, surgical procedures as well as aesthetic treatments socket healing after extraction comprises of hard tissue as well as soft tissue reconstruction with changes which is maximum dimensionally during the initial three months. One of the major challenges in clinical science is the development of bioactive surgical additives to improve the pace of the process of healing and inflammation control. Platelet-rich fibrin (PRF) exists in this context as a safe and adequate substitute with less risk and favourable outcomes. The methodology of use of PRF, concentration on its planning advantages and drawbacks of use in clinical applications are summarized in the following review. Platelet-rich fibrin (PRF) was developed in France first as a beneficial substitute to platelet-rich plasma to overcome its limitations.

Keywords: *Platelet-rich fibrin, socket healing, dentistry*

Introduction

There are diverse types of restorative, aesthetic and surgical procedures in today's world of advanced dentistry. Tooth extraction is a common dental procedure in the manner of decay, complicated fractures, infections and development of orthodontic spaces. Physiological healing of the post-extraction socket requires a complex pro -stop migration and maturation of bone cells leading to selective bone resorption and application. The most important factor is the preservation during routine extraction process of the residual ridge failure of which frequently leads to the compromise of potential implant placement and future recovery.[1] Post extraction cases results in the residual alveolar ridge having a dimensionally less in both vertical as well as horizontal axis. Several techniques are used to avoid loss of bone post-extraction and repetitive post-extraction placement of implant which includes preservation of socket with grafts and placement of implants immediately.[2]

Clinical practitioners have a range of graft materials to select from. Some of which need a longer period to heal when introduced even a little amount of new bone into the site of the graft. Biomaterial socket preservation was suggested and platelet-rich plasma with growth factors and platelet-rich fibrin (PRF) is used. A second generation of autologous growth factors that is PRF promotes healing and id suggested to be correlated with an efficient and early formation of the percentage of bone substance and volume of bone. A Leukocyte platelet concentrate thick fibrin matrix called PRF can be easily made with autogenous and non-coagulated blood when centrifuged.[3]

History: In 1970s platelets regenerative capacity was discovered. It stated that they produce growth factors culpable for raising the development of collagen, improvement of blood vessels, mitosis of a cell, mobilization of certain cells to the injury site and cell division activation. For in vivo tissue engineering utilization some of the new advances in the usage of concentrates of platelet in oral surgery are:

PRP - Platelet-rich plasma

PRF – Platelet-rich fibrin

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As bioactive surgical agents used locally to promote healing of wound are a combined suspension of growth factor present in platelet called platelet concentrates. The usage of platelet-rich plasma in oral surgery was first promoted by Whitman et al [3] in 1997 with important benefits as osteoprogenitor cells in bone graft and host bone was stimulated by it. But, the development of antibodies to factor V, XI and thrombin was a possibility though, as bovine thrombin was used to treat PRP which may induce coagulopathies that may hazard life. On the other side, by Choukroun et al [4] primarily in oral and maxillofacial surgery,PRF was used in 2001 and is generally known to be a modern type of platelet concentrate. It has many benefits over PRP as it consists of an autologous fibrin matrix [5] including simplex processing and doesnot involve chemical stimulation of the blood so, it is purely autologous.[1-5]

Drawbacks of PRP: Several drawbacks of PRP was reported soon after its success in the initial years of its use. The procedure of preparation was very lengthy and hence to prevent clotting anticoagulants such as $CaCl_2$ or bovine thrombin was used. these materials are known for the inhibition of wound healing. Due to lengthy procedures, the oral and maxillofacial surgeons started avoiding the use of PRP. Another limitation was that it was liquid. So bone grafts of various origin were required to be combined with PRP. In 2006 Choukroun and his fellow workers tried to develop a PRP preparation simplifying method and elimination of xenofactors which lead to the development of second-generation platelet-derived material called platelet-rich fibrin (PRF).[6]

Types of PRF: Recently to get different ratios within the fibrin matrix different types of techniques using blood concentrates have been developed. According to a recent study, particular cell types are differently distributed based on the centrifugal force.

A. A modification was done in PRF by Choukroun to get – A –PRF (leucocyte enriched, advance type) and I – PRF (injectable type)

B. Pure platelet-rich fibrin (P-PRF) – It is a solid material and has no leukocyte

C. Titanium PRF(T-PRF) – It has a superiorly organised fibrin network with continuous integrity

PRFand its different uses in dentistry

- Sinus lift
- Socket preservation
- Guided bone and tissue regeneration
- Regeneration of periodontium
- Regenerative endodontics

Disadvantage of PRF: Proper handling of blood while its collection and its transfer to the centrifuge is the important factor for the success of PRF. Some patient might refuse to the procedure as it requires an invasive procedure to collect the blood sample. The resultant amount is very less as it is autologous blood.[7]

Advantages of PRF: PRF helps in soft tissue repair that protects the surgical site and accelerates wound healing, when bone graft is mixed to PRF it acts as a biological connector. Osteoprogenitor cells and stem cells are attracted to the centre of the graft. In the elderly patients where regeneration of bine is required PRF helps in implant rehabilitation. Tissue regeneration is stimulated by PRF as it contains the framework of natural fibrins containing growth factors. It is easy and simple in technique for preparation with the help of centrifugation and is easy for all clinicians to access. [8]

Preparation of PRF: The appropriate blood quantity is drawn from the patient into the test tube from the antecubital vein by venous puncture which is held without an anticoagulant. The centrifugal machine is used to centrifuge the blood at 3000rpm for 10minutes. There is a development of fibrin clot of platelets situated in the middle of the channel. It is mainly concentrated between the bottom red layer of the blood cells and top layer of the acellular plasma with the help of a sterilised tweezer the formed clot is separated from the test tube, then the placement of the PRF clot is done on the PRF box grid and with the compressor and lid, it is covered. This leads to the formation of autogenous fibrin membrane.[9]

Role of prf in healing of wound

(1) Ambushment of blood cells circulating in the blood.

(2) Neoangiogenesis

(3) At the site of wound healing, there is a continuous release of growth factor.

(4) Osteoblast and Fibroblast generation.

(5) Promotes collagen production.

(6) Accompanies wound analysis.

(7) Adherence of fibrin mechanically.

(8) Supervision of immunity.

Clinical Procedure: Buccal and lingual/palatal infiltration of anaesthesia of Lidocaine Hcl 2% with epinephrine 1:100000 is given. Extraction is done with least injury and avoiding the raising of flap. The dilation of the socket is done by the help of the periotomesby placing them aroundthe root. The removal of the tooth is done using the forcepswith the placement of PRF the extraction site is treated immediately followed by application of pressure and suturing with figure 8 . Post extraction instruction is given. [10]

Discussion

Number of Histological studies and experiments have been carried out on tooth socket healing but radiological studies are very less . A platelet concentrate assembled on a single fibrin membrane containing all the desirable healing elements is called as PRF. Platelet act as a source of many growth factors which play an important role in healing of hard tissue and soft tissue thus platelet preparations are done. Neoangiogenesis is promoted by human osteoblasts which is due to PRF.[7]PRF acts as a matrix to promote the healing of wound edges when used as a fibrin bandage in a membrane shape. The preparation of the chairside is quite inexpensive, easy and the process is simplified and fast. When the socket is filled with PRF. Neovasculization occurs and epithelial covering forms. Healing processes of extraction socket occurs concurrently with an amount of latency that contains both hard and soft tissue. The clot retracts and breaks down towards the end of the 1st week and is replaced by granulation tissue. The higher part of the alveolar bone ridge is covered by granulation tissue and the soft tissue cut edges are covered by epithelium. PRF which is formed naturally has a complex and dense structure that has both leukocytes and platelets.[8-10]

Conclusion

The amount of bone regeneration which is seen with the use of PRF is more appreciable as compared to the cases treated without PRF use. For the preservation of socket and rehabilitation,PRF is an inexpensive material and can be easily used. Minimal invasion, patient discomfort as well as expertise of the operator is required to conduct this procedure when compared to other processes that include bone grafting.

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Soft Tissue Calcifications in the Orofacial Region

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Abstract

The main purpose of this article is to present a review of soft tissue calcification in Orofacial region. It can be systemically evaluated by using a simple algorithm based on the detailed clinical information and distribution pattern of these calcified lesions. The calcification of soft tissues belongs to a large group of lesions. Radiologists often detect calcifications in their daily work. For dental practitioners, this review should be of interest in encountering the soft tissue calcification in the orofacial region.

Keywords: *Soft tissue, Calcification, Orofacial, lesions.*

Introduction

Calcification of soft tissues in the orofacial area is unusual and is typically asymptomatic. Deposition of calcium salts in tissues other than osteoid or enamel is called pathologic or heterotrophic calcification. It occurs in an unorganized fashion in soft tissue. Three distinct types of heterotrophic calcification are recognized. They are dystrophic calcification, idiopathic calcification and metastatic calcification.[1]

Dystrophic calcification is the deposition of calcium salts, given normal calcium and phosphate levels, into the chief sites of long-standing inflammation and dying tissues. Examples are pulp stone, calcified lymphnodes, cysticercosis, arterial calcification. Idiopathic calcification results from calcium accumulation in human tissue given the regular calcium and phosphate levels in the serum. Examples are sialoliths, phleboliths, laryngeal cartilage calcification, antrolith/rhinolith. Metastatic calcification occurs when minerals accumulate, higher than usual serum levels of calcium and phosphate into the normal tissue. Examples are ossification of the styloid ligament, osteoma cutis and myositis ossificans.[2]

Pulp stone: Foci of calcification within the pulp chamberor root canalor reaching from pulp chamber into root canals without regularstructure or number. Asymptomatic in nature. Can occur as a result of increase in age or specific pathology. Often arises once the development of the tooth has been finished. Its histological types are true pulp stone, false pulp stone, free pulp stone, free pulp stone, attached pulp stone, embedded pulp stone. True pulp stone is composed of dentinal tubules. False pulp stones contain concentricalcalcified circles. Free pulp stones are freely placed inside the pulp tissue.Theattached pulp stone adheres to the surface of the dentin. Embedded pulp stone are covered by secondary dentin.[3]

Calcified lymphnodes: Calcification occurs in lymphnodes that have been chronically inflamed with often granulomatous disorders due to numerous diseases. Hydroxyapatite-like calcium salts takes the place of the lymphoid tissue,nearly obliterating all the nodal construction. The common disease that cause calcified lymphnodes are scrofula, sarcoidosis, catscratch disease, rheumatoid arthritis, lymphoma dealt with radiation treatment beforehand, systemic, sclerosis,infection by fungus, metastasis from specific neoplasm calcification.[4]

Calcified lymphnodes are asymptomatic, rigid, flabby, mobile and round to oval-shaped masses when

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palpated. Submandibular and cervical nodes (superficial and deep) are the most frequently affected nodes and less frequently the pre-auricular and submental nodes. The most frequent location is the submandibular area,between the posterior border of the ramus and cervical spine, and at or under the lower border of the mandible at just the angle. The node calcification may be single or a series of nodes called lymphnode chaining. The periphery may be well-defined, irregular and sometimes may even have a lobulated shape identical to the external cauliflower form, radiographically. The inner core can differ in the level of radiopacity, creating the appearance of rounded or uneven mass collection called eggshell calcification.[5]

Tonsillolith: Typically,they are rigid, circular, yellow or white structures projected from the tonsillar crypts. Prevalent in the older age group. The small calculi may not produce any signs or symptoms. In the case of larger calcifications, pain, swelling, fetor orisswallowing disorders and a foreign object discomfort on swallowing has been identified. In rare cases, they may be giant tonsilloliths, which stretch the lymphoid tissue, resulting in ulcerations and extrusions. They show on the panoramic view as single or multiple radiopacities which cross the middle section of the mandibular ramus.[6]

Cysticercosis: If pork tapeworm eggs are consumed by humans their coating is digested in the stomach and the larvae are hatched. Such larvae cross the mucous membrane,enter the blood and lymph vessels and are circulated in the tissues all around the body, but selectively associate to the skin, heart, muscle and brain. These are also present in oral and perioral tissue, particularly the muscles of mastication. When the larvae die, they are regarded as foreign bodies producing granuloma growth, bruising and calcification, this takes on average three months. Such sites in the tissueare termed cysticerci. Examination of the area of the head and neck may show palpable delicate fluctuating swelling, well-circumscribed. Symptoms usually seen are gastrointestinal discomfort, epigastric pain, extreme nausea, vomiting, seizures, headache, visual disturbances and irritability. They appear as multiple, well-defined elliptical, homogenous radiopacities which resemble grains of rice.[7]

Arterial calcification: Monckerberg's medial calcinosis (arteriosclerosis) and calcified atherosclerotic plaque are two different patterns of arterial calcifications. Arteriosclerosis is characterized by the breakdown,degeneration and subsequent loss of elastic fibre accompanied by calcium accumulation inside the vessel's medial cover. Often patient is first symptomless,but eventually they may develop cutaneous gangrene, peripheral vascular disease and myositis due to vascular disease and myositis due to vascular insufficiency. Patientwith Sturge-weber syndrome alsoexperience intracranial calcification of the arteries. On panoramic radiographs, seen as involving the facial or the carotid artery. Tram track appearance is seen from the side, seem as a parallel pair of slender, radiopaque rows that can be straight or tortuous.[8]

Calcified atherosclerotic plaque is located in the extracranial carotid vascular system and is a main leading factor of cerebrovascular embolic and occlusive disease. This first occurs as a result of enhanced endothelium destruction at certain locations at the arterial bifurcation. On a panoramic radiograph, the calcificationis visible in the neck located close to the greater cornu of the hyoid bone and the cervical vertebrae C3, C4 or the intervertebral gap between them, which are numerous and irregular in shape.[9]

Sialolith: Sialolithiasisis the development of calcified blockagein the salivary duct that contributes to persistent retrograde infection because of a decreased salivary flow. This is usual in middle-aged person with mild predilection for men. The Wharton's duct and the submandibular gland are mostly involved followed by the parotid and sublingual glands. Discomfort may intensify at mealtimes, when the salivary flow is stimulated. Non-calcified stones are diagnosed with sialogram. On routine plain films, it shows laminated appearance because of multiple layers of calcification.[10]

Phleboliths: In the head and neck area, phlebolithsoften suggest the existence of ahemangioma, in which the calcified thrombi contained invenulae, veins or the sinusoidal vessels. The associated soft tissue could be swollen, throbbing or discoloured by the involvement of veins or a soft tissue hemangioma, that frequently vary in size, along with the changein body posture or during a Valsalva manoeuvre. On application of pressure change in colour or blanching is seen. A radiolucent centre with appearance of lamination around its pattern is seen, hence called a target or bull's eye appearance. [11]

Laryngeal cartilage calcification: Thyroid and tristichouscartilagecomprises of cartilage of hyalin,

that tends to calcify with age. The calcified tracheal cartilages usually present a homogenous radiopacity, with an occasional outer cortex and they are generally asymptomatic. [12]

Rhyolith/Antrolith: In case of rhinolith the nidus is normally a foreign exogenous body. Whereas the nidus for an antrolith is usually endogenous. Hard calcified bodies those appear in the nostrils are called rhinolithand when in the antrum is called antrolith. Initially, the patient may be asymptomatic. When increase in size it impinges the mucosa, producing pain, congestion and ulceration. The patient may develop facial pain, fever, fetor, anosmia, nasal obstruction, epistaxis, headache, sinusitis, purulent rhinorrhea. They may be homogenous or heterogeneousradiopacity, based on the formof the nidus and occasionally has laminations.[13]

Ossification of styloidligament: Styloid ligament ossification generally progresses downwards from the base of the skull and usually develops bilaterally. The associated conditions are eagle's syndrome, styloid syndrome and styloid chain ossification. Most common signs and symptoms seen are throat pain, fishbone stuck in the throat, pain on swallowing, referred pain to the ear of the affected side, tinnitus, pain in anterior cervical triangle, pain on turning head, headache in the orbital area and cervical pain. In the panoramic image, it is seen as a linear, elongated, tapering, slenderradiopaque process that is wider at the base, extending from the area of the mastoid process and passing into theposteroinferiorpart of ramus in direction of hyoid bone.[14]

Osteoma cutis: Those are regular bone-forming areas in unusual sites. In a scar or long-standing inflammatory dermatosis, it can grow secondary to long-term acne. Histologically, those are seen as regions in the dermis or subcutaneous tissue of compact viable bone. Extraorally it may occur on the face in the cheek and lip region and intraorally it may occur on the tongue, where it may be calledosseous choristoma or osteoma mucosae. This induces no noticeable changes in the overlying skin, except in some areas, where the colour may change to yellowish-white. It varies in size from 0.1mm to 5cm. The particular osteoma can be palpable. Normally when a needle is injected through one of the papules experiences rock like obstruction. It is seen as finely outlined, washer-shaped images and single or multiple radiopacities of various sizes. Radiographically, it appears as a homogenous radiopacity with a radiolucent core which reflects natural fatty marrow, making the lesion to resemble a doughnut. Trabeculae typically grow in the marrow cavity of a bigger osteoma. Calcified cystic acne lesionmimic a snowflake radiopaque view that relates to a scar's clinical position.[1, 3]

Myositis ossificans: In this case, the fibrous tissuesand heterotopicbones in the interstitial tissues of the muscles and relatedtendon and ligament. There is secondary damage and atrophy of muscles. It is of two types, localised myositisossificans and progressive myositis ossificans. Localised myositis ossificanshappens due to acute or long-terminjury, tough strain in muscle and muscle injury that can result in haemorrhageinthe muscles or related tendon or fascia. During the healing process, heterotopic bone and in some cases cartilage is formed. The commonly involved oral sites are the masseter, sternocleidomastoid and lateral pterygoid muscle. The location of the precipitating injury stays tender, painful and swollenfor a long time. Due to the inflammation, the skin appears red. If there is an involvement of muscles of mastication in the lesion then the opening of the mouth will be a discomfort. The region of ossification can become evident only after 2-3 weeks as a solid, intra-muscular palpable mass that gradually expands and finally stops increasing. The lesion might be fixed or on palpation could be easily moveable. Most frequently involved muscles of the neck and head region are masseter and sternocleidomastoid. Radiographically, a radiolucent band could typically be seen between the ossification region and correspondingbone and the heterotopic bone can lie along the muscle'slong axis. Masses generally measure less than 6cm in greatest dimension. Generally, the exterior is more radiopaque than that of the inner structure. Pseudotrabeculae can be seen from irregular oval to straight lines going in the same direction as normal muscle fibres because of the shape variability.[2]

Progressive myositis ossificansgenerally affects children younger than 6 years and occasionally as early in childhood. Progressive heterotopic bone developmenttakes place inside the interstitial tissue of ligament, tendon,fasciaand muscle atrophies. This condition may be inherited or maybe a spontaneous mutation affecting the mesenchyme. It begins in the neck and upper back muscleand passes to the extremities. It begins as a tender and painful soft tissue swelling which could cause redness and heat including the appearanceof inflammation. As the severe symptoms disappear that tissue remains strong in density. Later phases of the same disorder give the petrified man like look.

Radiographic appearance is similar to that of localised myositis ossificans. The boneset down will not possess natural bone structure.[1-4]

Conclusion

The calcification of soft tissues belongs to a large group of lesions. Radiologists often detect calcifications in their daily work. For dental practitioners, this review should be of interest in encountering the soft tissue calcification in the orofacial region.

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Resonance Frequency Analysis and Oral Implant Stability: A Long Term Relationship

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Abstract

For a desired osseointegration to take place, implant stability plays an expository role. Hence to achieve a functional dental implant, successful osseointegration is a necessity. The two major factors affect the survival of implants that is the stability of the implant during placement and the event of osseointegration. Two separate phases determine the stability of implant: Primary implant stability as well as secondary implant stability. Mechanical grip withthe cortical bone determines the primary stability whereas Secondary stability is due to regeneration as well asremodelling of the surrounding bone and surrounding tissue around the implant after loading. Implant stability is a mechanical phenomenon which is in association with local factors such as quality of bone, type of placement technique used, quantity and the type of implant used. It is considered highly advisable for implementation of a user-friendly, non-invasive, clinically reliable method to assess the stability of the implant and the osseointegration process. Resonance frequency analysis (RFA) is one of the method thats how almost perfect consistency after statistical analysis. Therefore, the focus of this currentre view article is particularly on presently used RFA method which are highly followed in the current times to elicit oral implant stability.

Keywords: *Resonance Frequency Analysis, Implant stability, Implant survival, Osseointegration.*

Introduction

Osseointegration is defined as "the direct, functional, and structural contact between live bone and the surface of a functionally loaded implant. This was evinced by Branmarket al. in 1969. Modern dentistry aims at restoring the function, comfort, and aesthetics of the missing tooth. The success of a dental implant depends on its ability to osseointegrate with surrounding bone[1]. Within the clinical perspective, quantification of successful osseointegration is based on the implant stability.[1-3] Stability of implants is of 2 various types: Primary implant stability and secondary implant stability.[4] Primary implant stability is a component of quality of nearby bone and amount, the morphology of an implant (i.e. length, width and sort), and the installation technique utilized (connection between drill size and implant estimate, whether a pre-taped or self-tapped implant is utilized[5]. Secondary implant stability is the eventual result of regeneration as well as remodelling of the tissue and bone surrounding the implant. Primary stability determines the secondary stability, which dictates the time of functional loading. Condition of the surrounding tissues also influences the extent of implant stability. Hence, it is necessary to be able to evaluate implant stability at different time intervals to reckon a long term prognosis.

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Method to assess oral implant stability: It is sometimes intricating to assess the stability of the implant because of the diverse nature of the implant's surrounding bone tissue and the phenomenon of osseointegration. In vivo, there are various method to gauge implant stability. However, for many surgeons, proprioception is the only thing they count on because of the difficulty to monitor in vivo healing of bone. To reduce the vulnerability of failure of the implant, precise quantitative measurements are needed to guide the operators.

Invasive/Destructive Method[4]

- Histologic/histomorphologic analysis
- Tensional test
- Push-out/pull-out test and
- Removal torque analysis

Non-invasive/non-destructive method[6]

- The surgeon's perception
- Radiographical analysis/imaging techniques
- Cutting torque resistance (for primary stability)
- Insertion torque measurement
- Reverse torque
- Seating torque test
- Modal analysis and Implatest
- Percussion test
- Pulsed oscillation waveform (POWF)
- Periotest
- Resonance frequency analysis (RFA): Electronic technology
- Magnetic technology

Resonance frequency analysis (RFA): Among all the method, RFA is one such method which is used most frequently. Meredith et al. in 1996 were the first to portray the RFA strategy, which is commercially available as Osstell, Implomates, and Penguin[4]. It is a diagnostic method which is nonintrusive while measuring bone density and implant stability at disparate time points using vibration and structural principle analysis[7].

To elicit implant stability two commercial devices have been generated. The original method i.e. electrical method utilizes direct wire connection betwixt the transducer as well as the resonance frequency analyzer. However, the second method used utilizes magnetic frequencies[7].

Within the electronic device, the transducer is anL shaped cantilever beam connecting like a screw attachment with the implant. The L beam has a vertical segment that posses a piezoelectrical crystal which lowers the implant/transducer complex. The opposite side, comparably has a piezoelectric crystal, similarly, on the opposite side of the beam second piezoelectric crystal discerns the beam's response.

Recently a new device with magnetic RFA has been introduced which has a metallic rod with a magnet on top, which is screwed onto an implant or abutment and a transducer. In this, magnetic pulse from a wireless probes stimulates the magnet. 1millisecond is the pulse duration. The peg vibrates unconfined after stimulation, and electric voltage present inthe probe coil is effectuated by the magnet. The voltage thus producedis the signal measured as well as sampled by the resonance frequency analyzer. Both the method that is electronic and magnetic method measure similar changes,but the magnetic device provides higher implant stability quotient (ISQ) value while measuring the stability of non-submerged dental implant[7].

Four generations of RFA have been introduced so far. The first-generation device was based on a measuring element transducer placed on implant/abutment and then connectedto a measuring unit with a wire. The second-generation device analyses frequency response utilizing the magnetic technology. The third-generation device was designed to overcome drawbacks of first- and second-generation. The third-generation system runs by a small battery system. This is helpful in swift as well as steady and chair side interpretation.[8, 9]

The first commercially available RFA equipment is Osstell™, followed by Osstell AB, then Osstell Mentor and Osstell ISQ, being the latest version, which utilizes a smart peg and a wireless probe attached to the device. A small magnet is attached at the top of the smart peg-top.To excite this, magnetic pulses are generated from a probe. The activated peg induces electric volt into a probe that is sampled by the magnetic RFA device[9]. Implant stability was determined under various surrounding bone conditions by Feng et al. and concluded that to elicit the implant stability during different healing phase,it can act as a non-invasive diagnostic tool.

For quantification of accuracy of prognosis of RFA measurements, clinical trials were carried out which determined the failure risk prediction's optimal threshold value. RFA value is just an indication that the implant inserted is stable clinically but may provide false assurance in considering immediate restoration of the implant. RFA as a prognostic tool, has a low sensitivity, before immediate loading,a combination of clinical parameters,radiographic evaluation should be taken into consideration.

Implant stability using Ostell: RFA evaluates the Stability of implant by quantifying the frequency of oscillations done by the implant on bone. Meredith et al. 1998 described the non-invasive method of assessing implant stability utilizing the RFA device OSSTELL. Sweden's Integration Diagnostics Ltd has been designing OSSTELL devices since the year 1999. These devices have developed with time with, improved generation of measuring the implant stability, these are, OSSTELL™, OSSTELL ISQ and OSSTELL Mentor[9].

This technique utilizes a piezoelectric transducer and is a diagnostic technique, which is non-invasive. It has sinusoidal signals which are projected in a limited frequency and the implant vibrates within this frequency. Resistance produced by the implant to this vibration is quantified and converted to ISQ values by this device. ISQis measured on a scale within 0–100, 100 being themaximum, and 0 lowest stability values[9].

OSSTELL ISQ is the recent generation of this device and was developed in the year 2009. It includes a newcontrol unit with a probe connected to it usinga cable. Its use is progressively extending to registerthe implant stability measurement due to the ability of

device to reproduce accurate values without intra andinter observer variability. The Osstell measurementsare highly reliable and are alone sufficient to assess the status of implant success[9].

When the ISQ is 47, Implant stability can be obtained. But, when the ISQ more than 49, it osseointegrated with healing period of 3 months. However, immediately loaded implants with an ISQ more than 54, osseointegrated easily. When ISQ value is low the implant stability depletes. In such cases, the operator should follow a tight recall schedule and should take point to point unloading measurements until implant stability is redeemed. When implants have high values of ISQ, the thing taken into consideration is the stability of the implant in the first twelve weeks of healing. This should not demand any changes in recall appointments. The downside of this system is that the measurement of the transducer is restricted to a set of 60, thus making the method much costlier. Implant has a fixed transducer in order execute RFA. This precludes observing the implants that support a cemented restoration[7].

Factors influencing resonance frequency analysis/ implant stability quotient values: It is revealed that ISQ is influenced by the diameter of the implant, form, surface, the ratio of contact to the bone,site where the implant is placed, system/type of implant used, the procedure of surgery, bone's height and bone quality. Stiffness of the interface affects the RFA along with 3 other aspects. At first healing of bone and remodelling of bone increases the stiffness of the interface of the surface of implant and bone. Secondly, bone stiffness and density along with cancellous and cortical bone ratioputs impact on RFA. Finally, interlocking parts of components of the implant can be a factor. Smaller drilldiameter final drill, varying surgical techniques e.g. self-tapping design implants, bone compaction technique, and wide tapered implants may also put impact on stiffness of the implant surface.

Application[10]

- It aids in making loading decisions.
- ISQ of 70 or more has reached–planning of the prosthetic phase can be done. Howbeit, since remodelling is always alternating, high Implant stability quotientdoes notalways state that there will be same the secondary stability will also be the same. Similarly, a when ISQ is low, it does not specify that there will beimplant failure because the contact between the bone and implant increases after the stand by interval of osseointegration.
- Alerts for hindering failure: An ISQ of 55 or progressive decline over period suggests of a hidden failure and notifies for necessary measures.
- Case documentation: Helps in maintaining records and hence communication is made easy as well as it is of great assistance in medico-legal cases.

Limitations[10]

- Comparatively expensive
- The smart pegs sum up the expense.
- Each implant system has a respective smart pegs.

- In case of single piece implant cannot record ISQ and requires an extra attachment.
- Cannot be utilized when the implant is placed subcrestally.

Conclusion

Clinically appurtenant information can be provided by resonance frequency analysis technique, regarding the state of the interface between bone and implant at any juncture of the treatment and follow-up. The inclemency of the interface of implant-bone is also based on implant stability and this particular technique quantifies it. Factors governing the above are such as the density of bone, time for healing of jawas well the implant that is exposed above the crest of alveolar bone. Research Studies specify that during recall examinations, successful osseointegration is elicited in implants with high values of ISQ. The opposite might signal in the direction of failure of implant or bone loss in the marginal area. To know clear specifications, for better implementation of the resonance frequency analysis technique, further clinical case reports are required.

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Mesenchymal Stem Cells in Periodontal Regeneration: A Review

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Abstract

Periodontitis is most predominantly marked and plays a vital burden in dental health as well as in systemic health. It starts from a phase of swelling on supporting structures of tooth thereafter proceeds, destruction of tissues up till damage to tooth arises. Reconstruction of defective supporting tissues in periodontium inclusive of cementum, pdl and alveolar bone is a challenging objective in periodontal regeneration therapy that can adequately diminish periodontitis caused tooth loss. In the regeneration of tooth and their supporting structures that are gone or damaged due to periodontal disease, stem cells perform an essential character. Here we talk about the primary information based on periodontal tissue regeneration using mesenchymal mother cells.

Keywords: *Periodontitis, mesenchymal mother cells, periodontal regeneration, guided tissue regeneration (GTR).*

Introduction

Periodontium indicate to tissue which simultaneously supply and reinforce the teeth. Periodontal tissues comprise of soft tissues like gingiva, periodontal ligament along with hard tissues like alveolar bone and cementum. The construction and distribution are damaged due to miscellaneous acquired and genetic disorder, out of them periodontal disorder is most frequently seen. That leads to loss of periodontal tissue which brings about tooth damage whenever left untreated. The determined ambition about periodontal reconstruction or regeneration is to reconstruct numerous periodontal tissues inclusive of alveolar bone, cementum and periodontal ligament in impaired periodontium.[1]

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The utilization of applied science like guided tissue regeneration (GTR) towards periodontal surgery may eccentrically rebuild periodontal tissue,but still, complete consequences are not significantly satisfying and present deficiency of clinical predictability.[2] As modern equipment along with growth factors have improved the procedures to govern periodontal injuries, scientific investigations have disclosed that their competence is, however, questionable and the fundamental, as well as occupational reconstruction of destroyed periodontal anatomy, stays demanding. Present-day the need of mother cells is acknowledged as a prevailing approach to periodontal therapy especially for a complete reconstruction of periodontal tissues which include both rehabilitation of alveolar bone and inducing cementogenesis along with root areas including oriented introduction of freshly developed periodontal tissues.[3,4] This manuscript presents the essential concept of periodontitis and mesenchymal stem cells; the role of mesenchymal stem cells (MSCs) in periodontal regeneration therapy; exogenous human mesenchymal stem cell therapy and lastly immuno-modulator properties of MSCs with tissue regeneration.

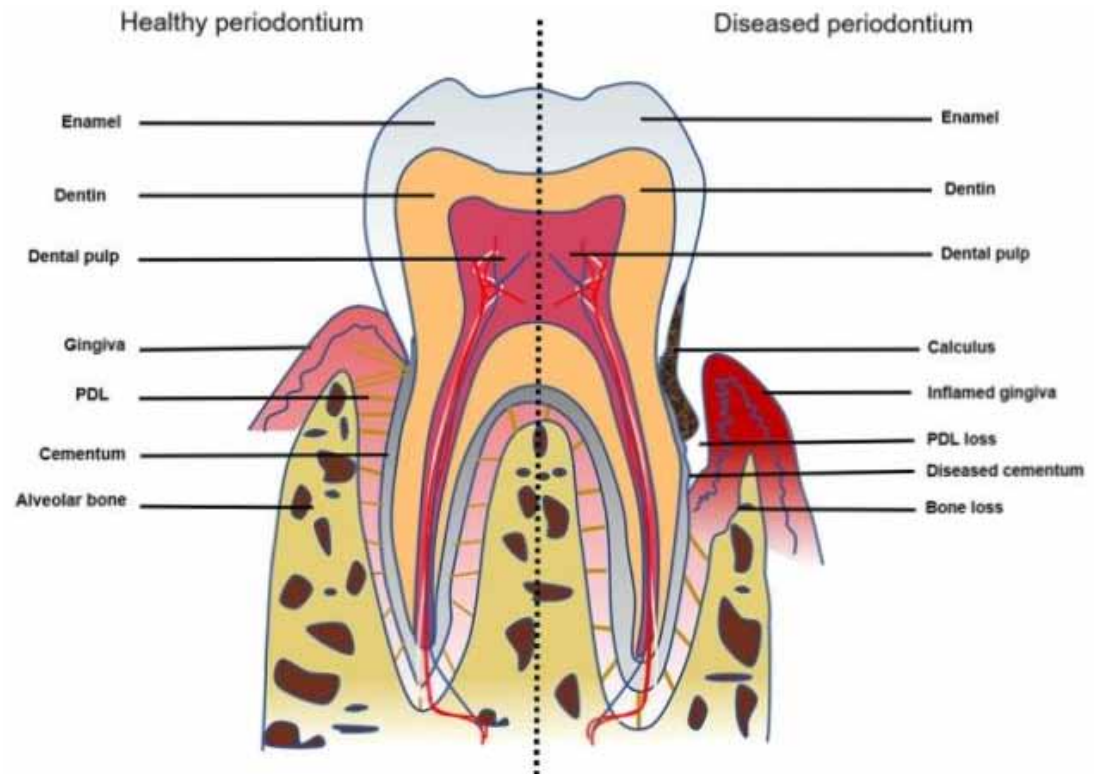


Figure 1. Healthy periodontium versus diseased periodontium

Periodontitis the condition: Periodontitis is a continuing incendiary disorder of periodontium. This disorder is induced due to various microbes that produce pharmaceutical disintegration of collagen fibers of the cementum; continuous damage to PDL as well as alveolar process including raised intensity of exploration establishment; receding gums; apical shifting of junctional epithelium[5]. It was a condition that damages the supportive tissues of the tooth that further leads to a lack of tooth from the socket and brings about numerous periodontal problems[6,7]. Disproportion between favorable and harmful microbes is the most crucial factor to cause periodontitis. Dysbacteriosis in primary period which mostly caused by low oral hygiene that provokes aggregation of various microbes like Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans), Porphyromonas gingivalis (P. gingivalis), Tannerella forsythia (T. forsythia), and Treponema denticola.

Periodontitis may occur at different age groups. In late adolescence gingivitis is seen which includes redness and inflammation of the gingiva. In adult age group aggressive periodontitis is most commonly seen which affects mostly incisors and first molars. Chronic periodontitis is mostly seen in the elder age group where attachment loss and destruction of periodontal tissue seen.

Mesenchymal Stem Cells: They are grown up connective tissue cells which originating at mesoderm. MSCs are undifferentiated cells capable of self-renewal and differentiating into specialized cells. Stem cells may be confined either in the beginning periods of embryogenesis (embryogenic mother cells) or within numerous Postnatal tissues (non-embryonic mother cells). As embryonic mother cells get great lifetime as well as the capability for huge differentiation, the virtuous features included in their construction put concentration on nonembryonic mother cells that are multipotent and present within almost all tissues, having the capacity for reconstruction of impaired tissues or else re-establish cell populations which are regularly getting interchangeable.[8,9]

MSCs are concerned with development, trauma healing, re-establishment of cells that are destroyed everyday either due to desquamation or in pharmaceutical circumstances. According to various research, it has been proven that they generate re-construction of neural, liver as well as skeletal muscle

following infusion in pre-clinical together with objective modes.[10-13] Those characteristic features of MSCs lead to feasible equipment during tissue engineering along with tissue reconstruction. MSCs may be acquired from different sources of developed tissues like bone marrow, skin, adipose tissues, and tissues of the orofacial zone. The initial cells obtained from the dental pulp of the third molar of the orofacial region[14,15]. They have been obtained from the periodontal ligament, deciduous teeth, apical papilla.[16,17]

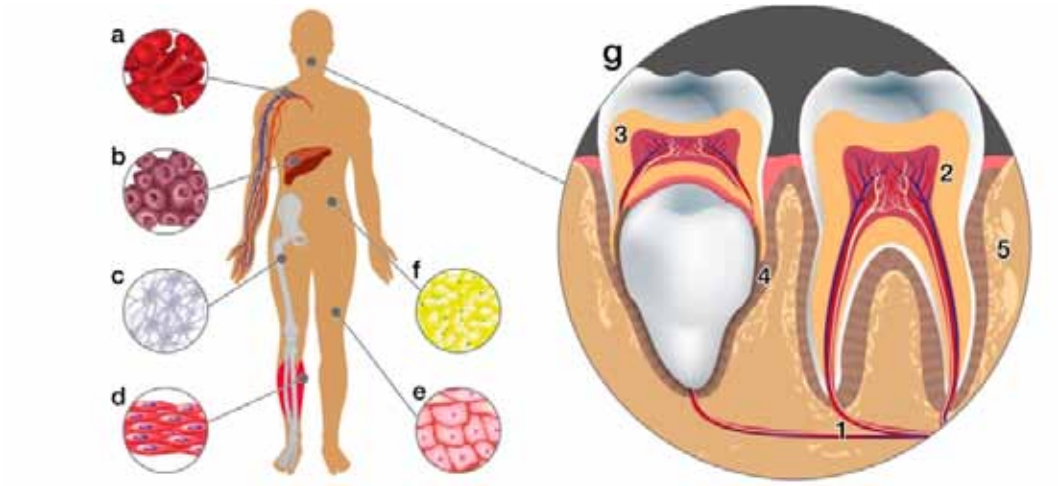


Figure 2: Sources of adult stem cells in human organisms; (a) Peripheral blood, (b) Liver, (c) Bone marrow, (d) Muscles, (e) Skin, (f) Adipose tissue, (g) Dental tissues: (1. Apical dental papilla, 2. Adult pulp, 3. The pulp of deciduous teeth, 4. Periodontal ligament, 5. Alveolar bone)

Human mesenchymal stem cells and periodontal regeneration therapy: Currently, according to methodical analysis about the potentiality of MSCs in periodontal regeneration therapy some authors confirmed that using MSCs,periodontal reconstruction might utilize the valuable responses somewhat in the pre-clinical aspect based on modern information. While other authors fail to determine the excellent combo about MSCs, bio-materials and biomolecules of different clinical conditions by the help of animal models which are an excellent representative of the etiopathogenesis of human periodontal disease. As there is no confirmation about the source that might be better products as well as scientifically achievable in human clinical conditions, depending entirely on xenotransplantation, we should recognize the beneficial effect of MSCs in the case of sick persons. The tissue engineering procedure needs MSCs, that are obtained from bone marrow, dental pulp of the patient along with developed in vitro. For generating an osteogenic constitute, MSCs are additionally cultivated with osteogenic factors as well as a scaffold (beta-tricalcium phosphate, hydroxyapatite/ tricalcium phosphate, etc.)[18]

Some scientific experiments have noted that the usage of human MSCs generates reconstruction of periodontal tissue. There are some experiments certified to periodontal reconstruction with the help of MSCs. Additionally, an inspection of the usage of MSCs to generate periodontal reconstruction, few investigations of gingival fibroblast-like cells, periosteum osteoblast-like cells, and periosteal cells are involved[19,20] even though they possibly don't perform the necessities of Universal Community of biological remedial treatment for human MSCs. There are investigations in individuals who have used homologous mesenchymal stem cells from bone marrow (BMSCs) along with established this is desirable for periodontal reconstruction.

On the other hand, even though they exhibit the potentiality of the cells to individualize former to the process of transplantation, nobody has considered the MSC immunophenotype. Based on these, Kaigler along with co-workers and Behnia along with co-workers used BMSCs to generate jaw bone restoration as well as rehabilitation of periodontal tissues, correspondingly, inducing excellent outcomes. On the other hand as both of them used BMSCs, they noted various

immunophenotypes also considered the individualization potentiality former the transplant with various specifications for the consideration. Based on clinical research d'aquino concluded that dental pulp stem cells (DPSCs) may generate a complete reconstruction of bone even though they are phonologically different from those proposed for periodontal tissue. Those cells show raised potentiality of self-expansion as well as differentiation in pre-osteoblasts. They also have a property of self- maintenance with renewal for a lengthy period. Other sources like infected pulp/adipose tissue, have been utilized in periodontal reconstruction along with appropriate outcomes.[21,22] Mesimaki and his co-workers utilize adiposestem cells to generate maxillary bone regeneration in elderly patients.

Exogenous human mesenchymal stem cell therapy: Exogenous MSCs may have the ability to manage the action of endogenous MSCs via paracrine components. Paracrine components have a great character during cell to cell communication or interplay which promote reconstruction of periodontal tissues. Based on research it is concluded that about 50 percent of cases showed analytical advancement and about 4.3 percent showed short-term reactions after MSCs infusion. But no one had harsh or dull reactions due to this therapy[23]. Exogenous MSCs therapy does not induce any type of crucial reactions and also there is no necessity of donor to recipient matching. In this therapy superficial or slight side effects may be seen for a short period. Utilizing human exogenous wharton's extract MSCs,survival as well as an assurance of myocardial reconstruction is an exclusive curative factor where no objective significant evidence is associated with it. It also found that epicardial or myocardial deterioration along with arrhythmias were not associated till 48 weeks which is a reason to treat with wharton's extract MSCs. As exogenous MSCs brought about noticeable advancement it is concluded that usage of exogenous MSCs therapy is desirable for reconstruction treatment of periodontal tissue.

Immunomodulatory properties of mesenchymal stem cells and tissue regeneration: According to various information, human MSCs may suppress in a close-dependent aspect, propagation together with cytokine reproduction of enough extrinsic immune cells like natural killer cells, T cells, B cells including dendritic cells. They discharge certain dissolved immunosuppressive components like interleukin-6,HLA-G5 together indoleamine 2,3-dioxygenase which are included in MSC interposed immunoregulation[24]. Depending upon potentiality that one may construct massive description about dissolved peptides as well as proteins. MSCs may trigger adjoining parenchymal cells to diminish apoptosis, decrease tissue loss, promote progenitor cell self-renewal, trigger angiogenesis aw well as promote repair. Numerous bioactive particles like enamel matrix derived growth factor; platelet-derived growth factor, plant extract (osthole, reveratol, quercitin) along with medicaments(aspirin, melatonin) are analyzed to improve results about tissue reconstruction of periodontium.[25]

Fu with his co-workers explained two techniques that negotiate the advantageous impacts of MSCs in periodontal tissue reconstruction. They are construction of extracellular vesicles together with the establishment of tunneling nanotubes (TNTs) which associate with various cells as well as deliver a variety of trophic components. Aliotta and his co-workers recommended that cells in the impaired tissue deliver information as mRNA to stem cells via extracellular vesicles that generate epigenetic revolution in stem cells. Sanchez together with his co-workers used MSCs of extract of Wharton along with exhibited tunneling nanotubes, that are elongate, narrow, F- actin positioned membranous mediums which associate cells along with havingthe potential to transfer their factors to associated cells. The utilization of those cells, even under hypothetically adverse situations will restore alveolar tissue and influence analytical advancement in patients with periodontitis.

Conclusion

As restoration of a destructed or injured Tooth is necessary, re-establishment towards operative or active and vital teeth is the biggest auspicious beneficial approach in the field of dentistry. Re-establishment towards operative or active and vital teeth is the biggest auspicious beneficial approach as the restoration of an injured or destructed tooth is much needed[26]. Depending upon the tissue engineering, value towards the microorganisms,biomaterials as well as growth factors recommended for advanced perception, considering treatment of periodontal disease.

According to various experiments, it is concluded that the capability of MSCs to induce periodontal tissue regeneration may give rise to the auspicious outcome even in unfavorable situations like in the

older age group. Periodontal tissue reconstruction is a complicated process that takes place from the interaction between stem cells and biomaterials. Utilizing MSCs for treatment of damaged periodontal tissue is one of the relevant alternatives, due to the interrelation of these cells with target tissue with miraculous convenience. This manuscript emphasized on application of stem cells that repair the tissue of periodontium and increase periodontal tissue restoration as well as reconstruction.

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Cranberry – A Boon in Periodontal Therapy

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Abstract

Cranberry juice has been used in medical fields for the past decade because of its several health benefits. Due to its properties, cranberries and its molecular components have gained attention by the researchers. The chief bioactive component of cranberry is proanthocyanidins. Its improved health benefits are already being found in treating UTI and many systemic diseases. But only a few studies have shown its effect on oral health.

Keywords: *Cranberry juice, Periodontal Disease, Functional Food, Vaccinium macrocarpon, Proanthocyanidins.*

Introduction

The native North American fruit cranberryhas currently gained some interest in the medical field for its several health benefits. Widely grown in the peat bogs of the cooler northeastern part of North America, it is one of the three original fruits of the country, which grows as a vine or low trailing shrub, bears a red fruit and is acidic in taste.Cranberry itself is a unique, good source of several classes of bioactive flavonoids includingflavonols, anthocyanins, and proanthocyanidins which confer it the significant therapeutic potential. The cranberry fruit is commonly consumed via several foodproducts, including the fruit itself and also in powder form incorporated in capsules and tablets.[1]

Therapeutic applications of cranberries started during the17th century which includes the treatment of blood disorders, stomach ache, liver problems, vomiting, appetite loss, scurvy, and cancer. Recently, cranberry extract has gained enormous attention in various areas of health research, including infectious and non-infectious diseases.Cranberry (Vaccinium macrocarpon) is a berry bush found in North America as one of the original fruit of the country. It has a mild acidic taste. It is consumed via different ways like fresh and dried form, juices and sauces and also in capsules and tablets in granulated form. It is an exclusive source of bioactive flavonoids which includes anthocyanins,proanthocyanidins and flavanols. These components in cranberry are solely responsible for its distinct therapeutic properties.[2]

Structure of proanthocyanidins

The procedure of making it includes extensive dialization of cranberry juice along with some other modifications. This process results in a product which is devoid of sugar and acids, containing 0.35% anthocyanin and 65.1% proanthocyanidins[2].Prof. Jacques Masquelier is the first one to develop the distillation procedure for extracting the proanthocyanidins from pine bark and grape seeds. Proanthocyanidins are mainly of two types: type A and B. Type A can be found in apricot, cranberry and type B can be found in other berries. Proanthocyanidins are mainly composed of multiple epicatechin units along with a minimum of one A-type

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linkage (O7 C2).[2] Other tannin-rich food contains B type linkage. The typical structure of cranberries is thought to be essential for the anti-adhesion property which can be used against the colonization of periodontopathogens in supragingival and subgingival regions.[3]

Nonetheless activation of the host immune system by bacteria and their products initiates a cascade of host-mediated noxious process which leads to the accumulation of inflammatory mediators. This reaction is succeeded by the inflammatory reaction normally caused by MMPs which will ultimately lead to tissue destruction. Degradation of tissue protein and invasion of periodontium occurs because of the bacteria secreted soluble and insoluble proteases.[4]

Periodontitis is a disease caused by chiefly the colonization of particular gram-negative bacteria. This ultimately leads to the impairment of soft and hard tissues of the periodontium and finally results in tooth loss. The pathogenesis of periodontitis is consisting of two major factors. First is microbial, which includes colonization of periodontal-pathogenic bacteria subgingivally, which destroys the soft tissue and junctional epithelium. The second is the host response of periodontopathogens which leads to increased production of inflammatory mediators and MMPs which are chiefly responsible for the initiation and progression of periodontitis. It was reported that cranberry has significant inhibitory effects on these two stages.[5]

Effects on periodontopathogens (adhesion and biofilm formation)

For the periodontal progression, the microbial colonization is an essential step. the ability of periopathogens to form biofilm and to generate adhesins which ultimately helps them to attach to the tooth surface, basement membrane or host cells. Thecranberry NDM fraction helps in inhibition of biofilm formation of but it does not affect the bacterial growth. This cranberry NDM fraction reduces the attachment of P. gingivalisto various proteins like Type I collagen and fibrinogen[3].

This fraction also inhibits the bacterial co-aggregation. They target those bacteria which are gram-negative anaerobes frequently responsible for periodontal diseases. These findings guides us to the decision that cranberry NDM fraction functions by preventing the bacterial adhesion rather than only by hindering the bacterial growth.

Proteolytic enzymes

Socransky et al. (1998) described that the members of the red complex which include P. gingivalis, T. forsythia, and T. denticola are the chief causative factors of periodontitis.[12] These periodontopathogens are closely related to periodontitis particularly in the destruction of junctional epithelium, increasing in pocket probing depth and bleeding on probing. The cranberry NDM fraction reacts with the period onto pathogens proteinases. It inhibits the action of gingipain (both Arg- and Lys-gingipain) depending on the dose. It also downregulates P. gingivalis activity, the trypsin-like activity of T. forsythia, and the chymotrypsin-like activity of T. denticola.[4] It also preventsP. gingivalis to denaturize proteins like collagen and transferrin. All these functional values of NDM fraction proposes that it can inhibit the production of red-complex bacteria in periodontal pockets.[6]

The destructive period onto pathogens produce proteinases. NDM fraction may reduce these tissue destruction mediators. Additional proof was given by Yamanaka et al. (2006) about the inhibitory efficacy of cranberry polyphenols action. Among the three fractions of cranberry polyphenols (anthocyanin, proanthocyanidin, and flavonol), the proanthocynidin fraction is highly effective and anthocyanin fraction is the least effective[5]. It has been suggested that these inhibitors can suppress bacterial pathogenicity and henceforth it can beassessed as a novel therapeutic agent for treating periodontitis.

Effect on periodontal bacteria and host mechanisms

Experiments performed by Labreque in 2006 and Yamanaka in 2008 revealed that the NDM fraction revealed that cranberry NDM fraction hinders the aggregation of Porphyromonas gingivalisand Fusobacterium nucleatum in the gingival crevicular fluid.[7]

Cranberries are reported to stop the protein denaturation occurred by the red-complex bacteria. It restrains the gingipain activity of P. gingivalis, the trypsin-like activity of Tanerella forsythia and the chymotrypsin-like activity of T. denticola, thus it leads to the conclusion that this fruit is having the capability to restrain the aggregation and multiplication of bacteria by restricting their growth resources like amino acids, peptides. It also inhibits the bacterial proteinases mediated tissue destruction.[8]

Inflammatory cytokines like (IL-1β, TNF-α) are produced by host macrophages. This process will lead to a cascade of inflammatory reactions like lipopolysaccharide production of A. actinomycetemcomitans, F. nucleatum, P. gingivalis, T. denticola and T. forsythia. A clinical experiment done by Bodet et al in 2006 found that the cranberry fraction helps in inhibiting these cytokines.[15] The NDM fraction acts by inhibiting the cell-signaling proteins and leads to the reduction in regular activator protein (AP-1). This AP-1 protein is an important transcription factor for pro-inflammatory mediators coding genes.[9]

Inflammatory cells chiefly macrophages and fibroblasts produce various enzymes like MMPs and elastase which is the primary cause of tissue destruction. In vitro ELIZA studies revealed that depending on the dose this cranberry NDM fraction has an inhibitory effect on the MMPs[6]. The lipopolysaccharide-induced interleukins and PGE-2 responses as well as Cyclooxygenase-2 expression are suppressed by the cranberry fraction[7].

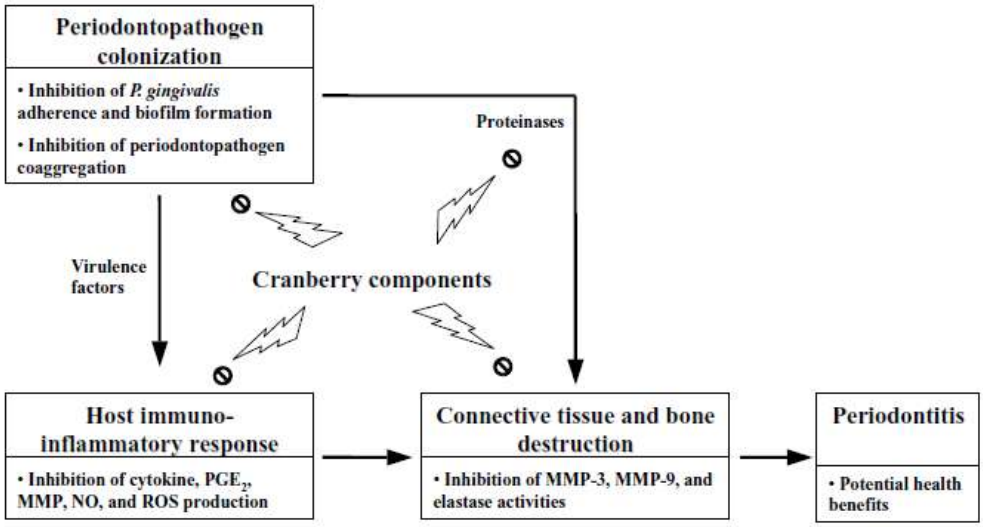


Figure 1. Pathophysiology of periodontal pathogens

Dosage: Various doses and formulations of cranberry are available in the market. This includes unsweetened or sweetened cranberry juice, cranberry extract tablets, pure cranberry extracts in a bottle etc. A recent randomized control trial performed showed that the one tablet of concentrated cranberry extract (300 to 400 mg) twice daily, or 8 oz. of pure unsweetened cranberry juice three times daily for 12 months is safe and effective as an daily systemic treatment doses for antimicrobial therapy.[10, 11]

Contra indications and adverse effects: Cranberry as a dietary supplement has shown a record of safety and it has not shown any particular drug interactions. Still, specific long term safety data is missing. A study found that patients who are under proton pump drug therapy, cranberry enhances the vitamin B12 in them which can ultimately lead to faster metabolization of weakly acidic drugs thereby reducing their effectiveness.[10] A study showed that it can be responsible for the significant rise of urinary oxalate levels and systematic daily use of cranberry may lead to a high risk of kidney stone formation in patients with a history of oxalate calculi.[8, 12-14]

Conclusion

Cranberry extract certainly helps in fighting oral diseases. However, due to high dextrose and fructose content and strong acidic flavour the cranberry juices which are available in the market are inappropriate for daily oral hygiene use. However, cranberry fractions included in the dietary supplements have shown some promising results as suggested by current research.

Remarkably, the cranberry fraction stops the pathologic mechanism of dental caries and periodontal infection. It can be included in various products like toothpastes and mouthrinses as well as gels and strips to arrest the progression or treat the oral diseases. Weiss et al in 2004 showed that NDM supplemented mouth wash reduces the salivary S. mutans levels in healthy adults. The study can lead to the conclusion that the anti-adhesion and anti-plaque therapy by the edible dietary components can be a sustainable way for treating oral diseases like dental caries and periodontal diseases.

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Current Research Directions in Regenerative Therapy

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Abstract

Chronic periodontitis has been the principal cause behind the irreparable destruction of the periodontium. In the field of surgical periodontal therapy, regeneration of damaged tissues by utilizing our body's naturally produced growth factors along with various other biomaterials either synthetically formulated or animally derived has piqued the attention of the dental practitioner. The human body has a natural capacity and potency of regenerating the lost tissue up to an extent; this characteristic is tapped in the regenerative therapy. Presently this therapy is bounded by the limited research and knowledge on the materials that can be used, instruments that are required for a successful procedure and method devised to gain desired outcomes presently available and the body's natural capability for regeneration. Tissue engineering use of stem cell, gene therapy and research on various aspects of regenerative therapy, has produced predicted results in exciting new treatment method for clinicians shortly. Traditional treatment modalities like mechanical instrumentation, curettage and open flap debridement result in keeping the inflammation in check and long junctional epithelium formation which leads to only periodontal repair and not regeneration. Many practitioners frequently use a combined method of putting both barrier membrane and bone grafts to curb the limitations posed by the down growth of epithelium and sagging of the non-rigid barrier used to maintain the grafts in place. This has led to enhanced interest in the Hence, there has been increased attention in the treatment of chronic diseases of the periodontium further elevating the quality of life of the patients. This review article aims to review diverse obtainable bio-materials, scaffolds, barriers and osseous grafts along with the plethora of procedures applied in periodontal regenerative therapy.

Keywords: *Periodontal regeneration, tissue engineering, cell sheet engineering; 3-D printing technology.*

Introduction

The principal aim of regenerative therapy is to fully restore the lost periodontium and other supporting soft and hard tissues that have been destroyed during the chronic inflammatory diseases. The foundation of the regenerative therapy is built on the formation of the new hard tissues and soft tissue i.e. the alveolar bone proper, the periodontal fibers, cementum along with its associated collagen fibers. It is often observed that traditional periodontal therapy such as scaling and root planing or open flap debridement commonly results in the gain in clinical parameters. It is evidenced by a reduction in the probing pocket depth and clinical attachment gain in the clinical attachment level, but the process of post-operative healing primarily occurs through the formation of long junctional epithelium which is considered as periodontal repair and not periodontal regeneration. The periodontal surgery is critically required for periodontal health and highly expected to regenerate tooth-supportive structures and restore periodontal functions such as pocket reduction procedures and regeneration

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procedures (guided tissue regeneration;GTR or guided bone regeneration; GBR).[1,2] Adequate blood supply and wound stability, a source of bone and ligament forming cells, a supporting scaffold or matrix, and growth factors to regulate cell migration, proliferation and synthesis and angiogenesis for revascularization of the site are the basic elements required for periodontal regeneration and repair.

Cells in emerging regenerative therapy

Stem Cells:

Among the various types of stem cells found in the oral cavity are

DPSCs: dental pulp stem cells; SHED; PDLSCs: periodontal ligament stem cells; GMSCs: gingiva-derived MSCs, PSCs: periosteum-derived stem cells have reported for to regenerate periodontium. PDLSC differentiate into cells that inhabit and grow on the biocompatible scaffold, for bone tissue engineering in regenerative periodontics.[3,4] The verification of the osteogenic potential of differentiating PDLSC which is characterized by an increase of nitrous oxide and calcium represents a promising method for the management of periodontal defects. Human PDL has been reported to be a viable alternative source to be used in stem cell therapies.[5,6]

MSC niche:this concept is based on the fact that a specialized microenvironment is always desired for mesenchymal stem cells to preserve their 'stemness'. Under specific signaling conditions. It can differentiate into the endothelial, osteoblastic, adipocytic, odontoblastic, chondrocyte, and neural lineages. The major site of MSC niche in the body is the bone marrow microenvironment.[7]

Cellular Allograft: Novel allografts are cellular. They comprise the body's mesenchymal stem cells and cells having osteogenesis capacity. These have been successfully used for the management of demanding periodontal defects.[8]

Scaffolds/matrix in emerging regenerative technology: Cell sheet engineering: For enhanced and desirable treatment outcomes, the stem cells require an appropriate medium to reach the target site. This can be made possible by using the scaffolds which aids the stem cells in differentiating into the desired tissues. It also proves to be effective and maybe a promising source of periodontal regeneration.[9-11]

Table 1: Review of Literature

Author	Cell sheets	Target site	Results
King GN, et al. 2001 [97]	Human periodontal ligament cell sheet (MCS)in osteogenic differentiation medium	Athymic rat mandible (King's method)	A layer of cementum & new attachment of collagen fibers to cementum.
Akizuki T, et al. 2005 [98]	PDLSC cell sheets(MCS) using hyaluronic acid as carrier from temperature responsive culture dish	Mesial dehiscence model beagle dogs	Newly formed periodontal ligaments with a rich capillary supply found between alveolar bone & cementum.
Flores MG, et al. 2008 [91]	PDLSC cell sheets(MLS)	Athymic rats	Cementum like hard tissue on dentin surface & Collagen fibres resembling periodontal & sharpey's fibers were inserted into tissue.
Iwata, et al. 2009[92]	PDLSC cell sheets(MLS) alongwith TCP	Surgically created three walled defects in dogs	New bone and Cementum was formed with well-oriented collagen fibres
Ding, et al. 2010 [99]	Autologous and allogenic PDLSC cell sheets(MLS) alongwith HA/TCP	Periodontal defects in miniature pigs	Significant periodontal regeneration in12 weeks using both autogenous and allogenic PDLSC
Bai, et al. 2011 [100]	DFSCs (MCS) alongwith HERS	Omenta of adult male rats	DFSCs in presence of HERS formed tissues resembling cementum and periodontal ligament
Xei, et al. 2012 [95]	Co-cultured human PDLSC and BMMSC alongwith ceramic bovine bone powder (scaffold based CST)	Athymic rats	Cementum and periodontal ligament like tissue was formed alongwith neovascularization
Na, et al. [87]	CSP of SCAP	*In vitro*	Increased expression of alkaline phosphatase, bone sialoprotein and runt related gene-2 (RUNX2) mRNA as compared to cell sheets
Zhao, et al. 2013 [86]	CSF of PDLSCs and PRF granules	Reimplantation of teeth in dogs	Better periodontal regeneration and reduced Ankylosis and inflammation
Dan, et al. 2014[88]	MLS composed of cells of gingival connective tissue, alveolar bone and Periodontal progenitors in a calcium phosphate- coated melt electrospun polycaprolactone scaffold	Athymic rats	Scaffold promoted bone formation in 4 weeks. Significant periodontal tissue formed by alveolar bone cells and periodontal progenitors

Shortcomings associated with the cell sheet engineering is that are multiple applications of multi-tiered cell sheets are needed to restore the damaged supporting tissues and make it resemble the original morphology. It is difficult to handle, transport and extremely fragile. Furthermore, this treatment plan is not at all cost-effective and hence only can be carried out in selective cases.

Growth factor-enhanced matrix 21S: It is a synthetic grafting system for bone and periodontal regeneration. It consists of the solution of unaltered recombinant human platelet-derived growth factor (rhPDGF-BB) in its concentrated form. It also has an osteoconductive scaffold (bone matrix). GEM 21S is supplied in a single-use kit. Each GEM 21S kit consists of the following: One cup containing 0.5cc of beta-tricalcium phosphate particles (0.25–1.0 mm). One syringe containing a solution of 0.5 mlrhPDGF-BB (0.3 mg/ml).[12]

PepGen P-15: It is an osseous graft material which has been tissue-engineered. It is composed of the HA bone matrix which is of bovine origin and inorganic in nature. It also consists of artificially formulated cell-binding peptide. This material has been proved to be useful in filling periodontal osseous defects. It has been shown that the P-15 helps in the binding of cells particularly fibroblasts and osteoblasts.[13] PepGen P-15 bone graft in conjunction with flap surgery was an effective periodontal regenerative potential in terms of linear bone growth and vertical bone growth for the management of three-wall bone defects.[14]

Nanomaterials: Nanomaterials available in the forms of nanopowders, nano gels, nano scaffolds are the materials with components less than 100 nm in at least one dimension. these materials can be organic (eg: chitosan, silk fibroin) or synthetic (eg: PGLA nanoparticles, dendrimers).[15] Silk fibroin derived from the cocoons of the silkworm which are processed into scaffolds integrated with bone morphogenetic protein-2 helps in osteogenic differentiation of human mesenchymal stem cells.[16,17] PLGA nanofibers are biocompatible but its role in bone regeneration can be hindered for its poor osteoconductive.[18]

Hydrogels: Fibrin, hyaluronic acid and Pluronic F127 are the hydrogels, which have effective delivery of growth factors. They are the scaffold system which lend a hand in bone repair. However, they are encountered with various drawbacks such as low physical properties such as mechanical strength, which further decreases their effectiveness as an osseous replacement. This problem can be triumphed over by designing composites which will comprise of an inorganic substance and a copolymer. One such combination is PLGA-g-PEG which has HA and a polyethylene glycol (PEG) as its inorganic substance and copolymer respectively. Its acidic pH environment is neutralized by hydroxyapatite.[19]

Titanium mesh: The nonresorbable barrier membranes used in periodontal regeneration are PTFE (expanded or high density) and titanium mesh. Titanium mesh (Ti-mesh) maintain the bone grafts under the barrier and has excellent mechanical properties. Its elasticity prevents mucosal compression. Its enhanced strength and resistance to deformation aids in maintaining the space and also prevent the contour of the tissues from collapsing. Graft displacement is prohibited by its stability. Furthermore, its plasticity allows bending, contouring, and adaptation to any unique bony defect.[20]

Amniotic Membrane: Amniotic membrane is the deepest lining of the placenta present in the most inner layer of it. It is usually discarded after parturition. It is lately being used in the regenerative and repair therapy both in the medical and dental fields to restore the lost tissues and speed up its repair. It is a novel tissue-engineered biomaterial that has numerous growth factors, proteins and stem cell reserves that help in regeneration of the lost tissues with the acceleration of wound healing. Many studies have also evidenced that this material can be effectively used in the management of intraosseous defects.[21]

Molecular mediators in emerging regenerative therapy: Teriparatide: Teriparatide, is nothing but the parathyroid hormone but in its commercially attainable formulation. It comprises the amino acids starting from 1 to 34 of the parathyroid hormones (PTH 1–34).They are subcutaneous injections, which can be administered by the patient himself in the muscles of the thigh or abdomen daily results into an increase in the number of active osteoblasts, a decrease in osteoblast apoptosis and probably, recruitment of bone lining cells as newly formed osteoblasts, thereby increasing bone strength and tissue integrity which finally leads to bone formation.[22]

rhFGF-2: The controlled release, local delivery of growth factors, such as FGF-2is another emerging approach to regeneration. Recombinant FGF-2 results in broad mitogenic and angiogenic actions that take place

by binding heparin. It helps in the differentiation of osteoprogenitor cells to induce bone formationand also be actively involved in periodontal tissue renewal.0.3% rhFGF-2 shows a greater capacity for periodontal regeneration than other concentrations.[23]

Platelet-derived growth factor: PDGF represents a family of growth factors consisting of 2 polypeptide chains (A & B) which form the dimer (protein pairs).

- PDGF-AA
- PDGF-AB
- PDGF-BB

PDGF-BB can activate any PDGF receptor; therefore its therapeutic application can activate any configurations of its receptors and when used in a synthetic scaffold matrix, promotes long-term stable clinical and radiographic improvements. The proliferation rate of wound fibroblasts is higher by PDGF- BB than PDGF-AB and -AA.PDGF-BB is a synthetic scaffold matrix.[24]

Concentrated Growth Factor: "Concentrated Growth Factors (GCF)" was introduced in 2006 by Sacco after the concept of PRF. It is a platelet concentrate that belongs to the second generation (advanced). It has many types of growth factors and fibrins. It is achieved by continuously centrifuging the blood and altering the time and the rotations per minute. It enhances the restoration of soft and hard tissues. It is prepared by accumulating 9 ml of patient's venous blood in the absence of anti coagulating agent in absolutely sterile tubes also referred to as vacuette blood collection tubes. The centrifugation takes place in the following order: first 30seconds acceleration, next 2minutes it is rotated at 2700 rpm, next 4minutes at 2400 rpm, then the following 4minutesat 2700 rpm, after which for 3min at 3000 rpm, followed by deceleration for 36 seconds and lastly stopping the centrifugation process. It stimulates the epithelialization process and also increases neo-vascularization making it an exemplary bioactive protein. It also accelerates the alveolar bone healing. In amalgamation with biomaterials and various promising graft materials it aids in the regeneration of the osseous tissues. Acceleration of osseointegration by increased differentiation of osteoblasts increases implant stability. It reduces periodontal intrabony defects depth and mixed with Bio-Oss and shows better results in the early period of healing.[25]

Biohybrid Interfaces: Modern dental implants lack the dissipation force, perception, and specialized receptors present in the periodontal ligament. In growing children or patients who are being orthodontically treated osseointegrated implants are not ideally placeddue to the absence of a periodontal ligament attachment.[26] Hence, a periodontal ligament-attached implant, or "ligaplant," could be a promising solution to practitioners. Furthermore, it can also provide a mechanism for allowing implant relocation orthodontically. Dental implants coated with HA surrounded with embryonic dental follicle tissues. It is a functional murine ligaplant model which was recently developed by Oshima et al that resulted in regenerated cementum, PDL, and alveolar bone.[27]

CAD-Based Compartmentalized Designs for Periodontal Complex Formations: In this process 3-D printing technology and computer-aided design (CAD) and is used to create a hybrid scaffold. Sectionalized articulation is present for the hard and soft tissues of the periodontium, including PDL-mimetic architecture which has perpendicular inclination for regeneration of periodontal complex.[28]

Gene Therapy: This therapy aims at injecting/ delivering various growth factors into the defects created due to inflammatory diseases of the periodontium. Numerous method of administering genes are accessible which offers great flexibility for tissue engineering. Tumor necrosis factor receptor 1 (TNFR1: Fc) is one such soluble protein that is used in gene delivery. It acts as host modulating agent as it curbs the destruction of alveolar bone by decreasing the activity of TNF (tumor necrosis factor). Direct in vivo gene transfer can also be carried out in this therapy. In large periodontal defects, platelet-derived growth factor-B enhance tissue regeneration as demonstrated,elsewhere.[29] For promising regeneration of osseous defects peripheral to dental implants direct gene transfer of adenovirus/bone morphogenetic protein-7in a collagen gel carrier has been effective.[30]

Conclusion

In the present era regenerative therapy continues to face multiple challenges both in research and clinical success. The emerging trends in regenerative therapy has become the turning point in the present-day theory of tissue engineering. However, cells, scaffolds and growth factors used recently have created new possibilities to envision the foresight and prognosis of present-day

surgical therapies. Future research should be carried out to uplift the development of novel treatment strategies. Proper selection of cases, the skill of the practitioner along with proper maintenance by the patient are of paramount importance for the success of regenerative therapy in periodontics.

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Global Burden of Periodontal Diseases: A Literature Review

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Abstract

Periodontal disease isprevalent each in advanced and growing nations and affect about 20-50% of the world population. Several hazard factors inclusive of smoking,heredity,diabetes,bad oral hygiene,age,pressure and medication which is related to periodontal illness. The devaluation inside occurrence and popularity of periodontitis can decrease its correlated fundamental illness and diminish their economic impact on health care systems. Periodontitisis one of the most important diseases which is chronic and leads to death and disability worldwide. The speediest increase in the hardship of the chronic disease is acquiring in the growing nations.

Keywords: *Periodontal disease; Global burden;Risk Factors,Economic impact; Treatment policies*

Introduction

Periodontitis is a continuous intemperate disease and characterised through loss of periodontal ligament and elimination of neighbouring bone.[1] There are different analytic explanation of periodontal disease and it may be acute or chronic. Acute periodontitis are of limited period, quick onset and associated with pain. Early diagnosis and prompt treatment is necessary. Due to the deposition of dental plaque, chronic periodontitis occurs. Disintegration of the supporting tissues of the teeth occurs in periodontitis. Deepening of periodontal pockets and attachment loss is the sign of periodontal disease. A huge prevalence of periodontitis in adults and elder persons makes it a mutual well-being burden.

Prevalence of periodontal diseases: Periodontitis is the most accepted oral condition among human populations.[2] The frequency and popularity of periodontitis diverge due to preference, casemiscitation and sites and number of teeth investigated. The World Health Organisation (WHO) has managed overall oral health data bank by the help of the community periodontal index(CPI).[3] Community periodontal index varies from 0 to 4.[9]. Community periodontal index score 0 indicates an absence of periodontitis; score 1 indicates bleeding observed during or after probing; score 2 indicates during probing supra or subgingival calculus seen; score 3 indicates hollow pathological pocket of 4 or 5 mm (gingival margin within the black band of the probe);score 4 indicates pathological periodontal pocket of 6mm or more (the black band of the probe is not visible).[4] Among adolescents, widespread of calculus and bleeding problem is higher in developing nations than developed nations. The distribution of teenagers with calculus deposits varies from 35% to 70% in advancing nations and 4% to 34% in advanced nations. In advanced countries periodontal pockets of 4-5mm seen in a higher percentage of individuals. Correspondingly in grown-up, calculus deposits varies from 14-17% in advanced countries and 36-63% in advancing countries. Higher distribution of elder individuals (65-75 years) show pathological pockets of 6mmor more is seen in grown-up populations in both advanced and advancing nations.[5]

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Factors related to periodontal diseases: A few risk factors raise the possibility of periodontal diseases which commit toward the scientific number implication of periodontal disease. The essential risk factors for serious periodontal disease are the adoption of tobacco, stress, malnourishment, extreme alcohol drink, diabetes mellitus and other systemic diseases.

Modifiable Risk Factors:

1. **Smoking:** The greater critical possibility causes for periodontal disease is smoking. The devaluation in periodontitis pervasiveness is associated with a decrease in the rate of smoking. In smokers, there might be increased in the loss of alveolar bone along with tooth loss than the people having no smoking habit and smokers have low results of patterns of periodontitis.[6]

2. **Bad oral hygiene:** Bad oral hygiene is connected with periodontitis, the need of legitimate brushing of teeth and different proportion of oral hygiene can support affidavit of bacteria and develop deposition of plaque on teeth and gums which can address an aspect for fiery changes in tissues which is present in the periodontal region.[7] There is an articulated relationship between bad oral hygiene and expanded aggregation of plaque, huge commonness and expanded seriousness of periodontitis.[8]

3. **Hormones:** Harmonization in females improve probability of periodontitis.[8] Ladies may encounter aggravation of gingiva previously period and at the time of ovulation because of a significant matched in progesterone which hinders fix of collagen strands and creates dilation of blood vessels. Pregnant ladies as often as possible display gingival changes,gum disease,now and again limited development of gingival tissues.[9] Luckily,these provocative changes vanish inside barely after conveyance beyond motivating tenacious harm to tissues present in periodontal region. Inadequacy of oestrogen diminishes the thickness of bone after the midlife crisis that can come full circle in alveoli misfortuneor the long run collapsing of teeth.[10]

4. **Diabetes:** People suffering from diabetes are more likely to develop periodontitis which can raise blood sugar level and various diabetic complications. Diabetesis the foundational ingredients for periodontitis which cause a significant job in commencement and movement of the disease.[11] Diabetes is related to tendon demolition which therefore prompt loss of tooth. Gingivalcrevicular fluid and salivation have higher convergence of fiery go-between including extraordinary sorts of cytokinesencompassed by diabetic patients with periodontal disease when contrasted with people not suffering from diabetes with periodontitis. Periodontitis is generally recognised a complexity of diabetes. People who don't control their diabetes, are especially at risk. Periodontitis can be a result of low diabetes management and associated with cardiovascular disease and strokes.

5. **Drugs:** Helplessness to contaminations and periodontitis increases lessened salivary stream because of medication powerlessness to contamination.[12] Periodontal sickness strengthens when there is reduced salivary stream because of certain limit the progression of salivation and produce Xerostomia, incorporates anti-depressants, anti-cholinergic,anti-histamines and beta receptor antagonists.[30] Some medications like nifedipine, cyclosporine and phenytoin can initiate the irregular development of gingival tissues which much of the time confounds the fitting expulsion of plaque beneath amplified gingival mass and in this manner bother currentperiodontitis.

6. **Weight:** It is obvious form proof that the pressure diminishes progression of slavery discharges that thusly upgrade formation of plaque. An additive relationship among weight scores and slavery pressure markers, calamity of tooth, attachment loss and pocket depth of 5-8mm. An investigation of 300 observational pieces shown that the rest of pressure is identified with a safe framework and diverse immunological changes happen in light of various upsetting events. The discouraged people have appeared to a greater focus of cortisol in gingival crevicularliquid and react inadequately to treat the periodontal structures. Scholastic pressure additionally brings about bad oral hygiene and gingival irritation with expanded grouping of interleukin-1beta.[13]

Static Risk Factors:

1. **Maturity:** Periodontal treatment increments with processing maturity which is the reason for the huge predominance of periodontitis which examines among overpopulation. Maturity is related toperiodontitis and attachment loss endure essentially huge among population matured 60 to

69yrs contrasted and gathering of grown-up 40-50yers.

2. **Heredity:** Heredity is one of the most important components which is related to periodontal disease which causes a few people further defenceless to infection than others. The mind-boggling interactions of hereditary variables among ecological or segment factors is speculated to exhibit broad varieties encompassed by various genetic and indigenous populations.

Relationship of periodontitis with other systemic conditions:

1. Ischemic heart disease: Reliable group of proof clarifies the connection between ischemic heart disease and periodontitis. A systematic survey recognised that the periodontal infection abidehazard element for congestive heart failure and affiliation autonomous of more hazard elements for example smoking, diabetes mellitus and financial condition. An examination of imminent and review considers, it has discovered that periodontitis probably going to purpose a 19% expansion in danger of coronary heart disease and expansion in relation hazard ranges directed toward 44% encompassed by people matures 65 years and over. An older survey and investigation of examinations discovered that periodontal malady with expanded degrees of foundational markers of bacteria was related toischemic heart disease. Periodontal ailment affiliation with stroke and fringe corridors infection is significantly more grounded than coronary illness. Correspondingly, an examination of 29 investigations revealed merged chances proportions of 2.35 and polled corresponding danger of 1.34 that recommended people with periodontitis has more noteworthy chance and higher chances of creating coronary illness than those without periodontal disease.[14]

2. **Metabolicailment:** There are bidirectional communication and teamwork among diabetes mellitus and periodontitis. An imminent associate examinations of 628 subjects along check out recognised that insulin-independent diabetes mellitus people among serious periodontitis had 3.2 occasions danger of fatality due to coronary illness contrasted with the people with no or on the other hand gentle periodontal sickness. An examination presumed that the periodontitis developed glycemic control for any event for non-insulin diabetesmellitus. Deliberate survey gave proof to help the job of periodontitis in improvement of adult-onset diabetes and also it's difficulties. Logical writing reliably underpins a bidirectional communication among periodontal disease and insulin obstruction. It has contended that periodontitis fuels insulin oppositions, constantquality embroiled in pathophysiology of catabolic sickness and adult-onset diabetes mellitus. It has additionally proposed that periodontal medication can lessen insulin opposition in diabetes. A few orderly audits have proposed a relationship between heftiness and periodontal ailment and recognised hazardfactors for improvement of periodontal diseases.Heftiness is appeared directed toward increment oxidated worry in periodontal tissues what's more motivated towards destructions. Predominance of heftiness has expanded drastically among globe51and affiliation with periodontal disease requires consideration among human service suppliers to forestall the general medical problems.[15-17]

3. **Unfavourable pregnancy result:** Periodontitis is identified with unfavourable pregnancy results which incorporate maternal disease,low birth weight, preterm birth, preeclampsia, mist biological and immunological factors are embroiled in hidden strictures. Below economic condition,smoking and other diseases are now known to be related with untimely birth; in any case,more as of late it was discovered that periodontal malady is likewise unequivocally connected with untimely birth episodes.[18]

4. **Rheumatoid joint pain (Rheumatoid Arthritis):** Periodontal malady is common along rheumatoid joint pain patients and malady remains start immune system reaction in rheumatoid joint pain patients. It is recommended that periodontal malady and rheumatoid arthritis accept comparable hidden pathophysiology of disease. People suffering from rheumatoid arthritis have huge pervasiveness of bone obliteration and misfortune of toothwhichever additionally sequelae of periodontitis.

5. **Respiratory illness:** The significance of keeping upideal oral consideration along individuals with incessant intrusive pneumonic ailment has underlined because of bidirectional relationship with periodontitis. Respiratory illness mainly includes asthma,pneumonia and lung cancer.

6. **Chronic Kidney Diseases:** There is a fractional connection between periodontitis and chronic kidney disease. Periodontal disease is ahazard factors for chronic kidney disease in an ideological investigation of 11,955 grown-ups precise audit of experimental and international examines found that patients along periodontal disease are expanded danger of chronic kidney disease and periodontitis brings about constructive appear in people with chronic kidney disease.[19-21]

7. **Diseases:** Hazard of oral cavity malignancy increments 5.23 occasion severe millimetre bone loss. Fitzpatrick saw that connection among periodontitis and vocal, abdominal,oesophageal,anorectic malignant growths have been accounted for all the more reliably in writing than with long and prostate malignant growths.

8. **Impedance of intellectual capacities:** More seasoned grown-up faces a decrease in their intellectual capacities which influence their practices including oral cleanliness habits. There is humble proof about a relationship between periodontal illness furthermore poor intellectual capacities as periodontal irritation has been appeared to influence discernment in old populations. Further, a late examination found that analytic attachment loss can advance beta gathering in mind whichcreate intellectual dysfunction.

Determent of periodontitis:

1. **Avoidance of periodontal disease:** WHO suggests utilising incorporated general well-being preventive techniques which ought to be founded on regular hazard factor approach. Hazard factors, for example,stress,smoking,below economic conditions are real etc with periodontitis along with alternative fundamental interminable sickness, in this manner consideration of oral illness anticipation procedures in interminable fundamental ailment preventive activities can diminish the weight of malady in the vicinity of aligned of population.[22]

2. **Cleanliness of mouthrehearses:** Appropriate cleaning of mouth, normal brushing of tooth and flossing are best among forestalling infection in mouth and periodontal disease. Despite most extreme importance of brushing of the tooth about the portion of populace brush two times a day. There are different diameter, configuration and sorts of brushes; be that as it may, two increasingly basic sorts incorporated controlled toothbrushes and manual toothbrushes. Controlled toothbrushes often more bite of leeway up standard toothbrushes in lessening plaque present in the tooth.[23]

3. **Starve:** Despite fact that the job of starving in anticipation of caries is increasingly huge contrasted and forestalling periodontal illness has been talked about in the writing. An eating regimen high in organic products, below in hefty and sugar is necessary for solid periodontal structures. Less calorie consumption is appeared directed toward lessening incendiary diversity and reduce the tissue harm in periodontitis.

4. **Fluoride utilisation:** Stannous fluoride has anti-plaque along with anti-gingivitis impacts what's more or lessen the extent of microscopic organisms and spirochetes in sub gingival zones, subsequently can assist with advancing gingival health.

5. **Utilization of antimicrobial specialist:** Chlorhexidine, triclosan,fundamental oils and mouthwashes and gels are utilised to prevent explicit microscopic organisms like plaque. Cholrhexidine diminishes dental plaque and gingival aggravation by bringing down provocation mediators. Gunsolley looked at the viability of anti-plaque and anti-gingivitis mouth rinses with oral cleanliness directions what's more grown-up prophylaxis and discovered extraordinary improvement in oral cleanliness.

6. **Smoking suspension:** Since smoking is a significant hazard factors for periodontal illness,in this way smoking end can forestall an extensive extend of periodontal cases. Smoking suspension not just further movement of periodontal ailment however can likewise decrease the periodontal tissue destruction. In light relationship among periodontitis and coronary heart infection,scaling has as of late appeared to lessen the frequency of intense cardiovascular infection localised necrosis also stroke.[24-26]

Conclusions

Whereas periodontitis is the common pervasive irresistible oral situation however it is stoppable and curable. Decreasein rate and pervasiveness of periodontal malady can bring about bringing down its related foundational ailments and confusions. Decreased periodontal ailment weight can limit treatment needs

and can lessen money related effect on wellbeing care frameworks. High commonness of periodontal ailment additionally requires the foundation of reconnaissance framework for oral ailments in the network. Preventive projects for periodontal ailment ought to use normal hazard way to deal with decrease the size of other incessant infections. Cost successful techniques would likewise improve interdisciplinary coordinated efforts among human service suppliers. Health care suppliers ought to be acquainted with Perio-fundamental connection and ought to have the option to analyse and allude patients to specific dental considerations to increase personal satisfaction of individuals. Research is expected to investigate fundamental instruments along with hazard variables of periodontitis and create imaginative preventive methodologies.

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Verruciform Xanthoma in the Marginal Gingiva: A Clinical Case Report

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Abstract

Verruciform xanthoma can be described as a rare, benign lesion. It can be classified as a papillary, asymptomatic or sessile. it can be well-demarcated from the adjacent tissue and can be seen frequently in the alveolar mucosa and gingiva. The salient histopathological feature is the abundance of foam cells (xanthoma cells) along with macrophages present in the soft papillary tissue.[1] The exact etiopathogenesis is still unknown, but the causative agents can be local factors like viral infections or trauma that have been suggested by hypothesis. Therefore this case report aims to present a case of verruciform xanthoma located in the marginal gingiva (lingual aspect) and describe the clinical features and histopathological aspects.

Keywords: *Verruciform Xanthoma, Verrucous Lesions, Xanthoma Cells.*

Introduction

Verruciform Xanthoma (VX) can be described as a benign lesion that appears rarely in the oral mucosa. Shafer in 1971 described it for the first time. Typically VX has a papillary or verruciform appearance and presents features of hyperkeratosis, and lacks in having proper etiopathogenesis. It has equal sex predilection. The widely affected patients are about the age-range of fifty years. It involves bothupper and lower jaws equally and the gingiva near the premolar area is one of the main sites having a maximum occurrence of VX.[1] Predominantly, it can present itself as an asymptomatic, slow-growing lesion. The base is either sessile or pedunculated, colour varies from pink to white.[2] The size of the lesion varies in different cases.VX most frequently occurs in the palatal or gingival masticatory mucosa, nevertheless, other areas where it can appear are the lateral border of the tongue, buccal mucosa, and floor of the mouth.For its particular characteristics, several cases of VX can be diagnosed as papillomas, condylomas, ordinary (vulgar) warts, and leukoplakia and occasionally as verrucous or epidermoid carcinomas.[2,3]

Case Presentation: A 35-year-old female patient came with the chief complaint of a growth on the lingual aspect of lower front teeth region for 3 months followed by loosening of teeth in the same region for 2 years (Figure 1). The patient doesn't give any history of pain. On clinical examination a single swelling is present in the lingual aspect of 41, 42 of 0.8 cm in diameter,well-circumscribed with areas of erythema. On palpation it was firm in consistency,non-tender, pedunculated and was bleeding on probing (Figure 2). The Lymph nodes examination showed non-palpable lymph nodes. The patient didn't have any systemic disease andcan be considered as healthy. After considering the clinical features,a provisional diagnosis of pyogenic granuloma was concluded. An excisional biopsy was performed after the clinical evaluation to dismiss the probability of malignancy.

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Histopathological examination was done. The hematoxylin andeosin (H & E) stained sections showed parakeratinization in the epithelium along with columns of parakeratin plugging present inside it. Uniform rete pegs with deep connective tissue papilla were seen. The papilla was extendedtill the overlying surface epithelium. There was the presence of few neutrophil cells in the upper spinous layer. The main histologic feature of VX, which is the lipid-laden foam cells were seen in the papillary zone of lamina propria. The presence of the foam cells in the papillary region of connective tissue itself confirmed the diagnosis as VX (Figure 3).Any signs of dysplasia or malignancy were not found in the histopathological analysis.



Figure 1. Clinical picture of the lesion present in the marginal gingiva (lingual aspect) i.r.t 41,42



Figure 2. Clinical picture of the lesion with probing depth located in the lingual aspect of 41, 42.

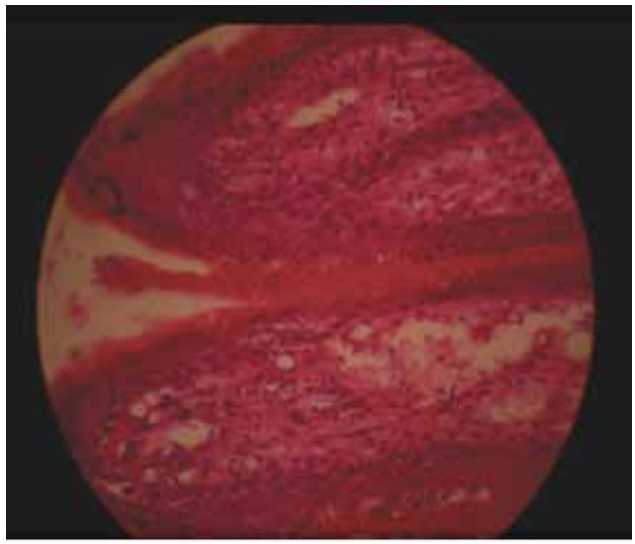


Figure 3. Presence of numerous lipid-laden foamy cells confined to the connective tissue papillae (H & E stain).

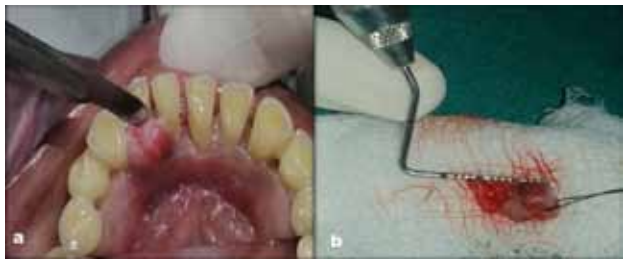


Figure 4a. Excision of the lesion using a scalpel; Figure 4b. Showing lesion after the excision.



Figure 5. Flap approximated and suture placed



Figure 6. Coe pack placed after the excision of the lesion

Surgical Intervention: The procedure was executed under 2% lignocaine. The lesion was excised asa whole during excisional biopsy (Figure 4). After the excision (Figure 5, Figure 6), the coe pack was placed (Figure 7) to avoid coming in contact with the external environment, and post-operative surgical instructions were given to the patient.

Discussion

Verruciform xanthoma is a benign lesionthat occurs rarely. Exclusively,two hundred and eighty-two cases had been reported up to 2001 and from the period of 2003 to 2007, only 3 cases of verruciform xanthoma were reported. Henceforth, it can be said that it's a rare lesion[3]. Reports have suggested that verruciform xanthoma is mostly seen in masticatory mucosa and mostly on the gingiva, followed by hard palate, tongue, buccal mucosa/ vestibule, floor of the mouth, alveolar mucosa, and soft palate.[4] Seen mostly in those patients having an age range of 40-70 years.[5] The clinical presentation shows isolated pink to white yellow papules or nodules, that are persistent, slow-growing and asymptomatic. The surface texture varies from papillary, granular, or verrucous.[5] They can have either a pedunculated or a sessile base.

VX is a quite uncommon lesion with an undiagnosed etiopathology. The probable reason can be due to the irritation in the squamous cells caused by infection, trauma which can ultimately lead to increased epithelial turnover rate. The epithelial breakdown results in a cascade of inflammatory response which further leads to the leakage of lipid materials from the degenerated cells.[6]

It appears similar to the HPV when seen histopathologically but any association between HPV and VX is not yet established. The main relevant clinical diagnosis can be papilloma or ordinary wart. Verrucous like solitary lesions in the oral cavity can be the differential diagnosis of VX. Due to its non-specific clinical features, there are several other differential diagnosis like squamous papilloma, condyloma accuminatum, focalepithelialhyperplasia, leukoplakia,verruca vulgaris or even epidermoid carcinoma in the initial stages.[6] Some previous case reports have shown the association between VX along with some other previous or concomitant lesions like lichen planus, carcinoma in situ or epidermoid carcinoma. These lesions are classified histologically based on several characteristics like surface texture, rete ridges proliferation etc. the rete pegs can be elongated, uniform and thin. They have keratinized clefts present in the central portion along with keratin plugging.[6] Nonetheless, the evidence of any metastatic features or dysplastic characteristics is not yet found.[7]

The main characteristics of VX are the abundant of the xanthoma cells in the connective tissue and the lipid-laden cells in the superficial layer of connective tissue. The accurate origin of these cells is controversial. It can be of the microphage or monocyte lineage. The treatment choice can be surgical excision of VX which is effective with almost no recurrence. In every case, after surgical excision histopathological examination of the biopsy samples should be done to rule out any possibilities of VX from any other verrucous lesions. [8-10]

Conclusion

VX can be described as a rare lesion encountered on the gingival mucosa. Its clinical features are not pathognomonic, so it should be considered as a differential diagnosis of Verrucous or papillary lesions. Furthermore, it needs proper treatment planning and management in time.

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Hemostatic Agents Used in the Field of Dentistry: An Overview

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Abstract

Intraoperative or postoperative bleeding during any periodontal surgical procedure can to some serious complications. Difficulties related to bleeding can be seen in both healthy individuals as well as bleeding disorder patients, any systemic complications and patients under anticoagulant drug therapy. Having a clear perspective about the preventive measures to be taken to control the profuse bleeding is a must for a practitioner. The purpose of the literature review is to discuss the administration of different types of hemostatic agents available for controlling bleeding.

Keywords: *Hemostasis, hemostatic agents, bleeding control.*

Introduction

Bleeding is inevitable in case of any oral surgical procedures. The chief causative factor of bleeding is damage to the blood vessels during surgery. In this case, meticulous surgical procedures, availability of ideal hemostatic agents, proper postoperative care and follow up can reduce the complication to a greater degree. Profuse bleeding can to several complications like the formation of hematoma, delayed wound healing, persisting infection, wound dehiscence and necrosis. In this review, we discuss the preoperative, intraoperative and postoperative management of bleeding of patients undergoing periodontal or any kind of minor oral surgical procedures.

For evaluation and selection of ideal hemostatic agents for a particular case scenario, it is mandatory to understand the physiologic procedure of coagulation. The hemostasis occurs, vasoconstriction, platelet plug formation, platelet adhesion, activation, and aggregation. Whenever there is an injury, vascular spasm occurs and this will initiate vasoconstriction which will gradually lead to a stoppage of blood flow. Following the vasoconstriction, collagen which was exposed from the damaged blood vessel will influence the platelets to adhere, activate and aggregate to form a platelet plug. Platelet adhesion is mostly mediated by Von Willebrand's Factor. Platelets are activated by enhancing their shapes into a more of a pseudopodia form that the adhesion of platelets into the damaged tissue surface. Once the platelets become activated, it will help in the attachment of VWF and fibrin. The activation is further intensified by producing thrombin via the hemostasis mechanism.

This review article focuses on various measures to control intraoperative and postoperative measures to control bleeding.

Mechanical method to control bleeding: Application of pressure counteracts the hydrostatic pressure within the blood vessel. Applying pressure is usually able to control most of the hemorrhages. It should be applied directly over the bleeding site firmly over a gauze pack for at least five minutes. Hemostats are also called a hemostatic clamp, arterial forceps etc. Hemostat (mosquito, artery) forces are specially designed to catch

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bleeding points in the surgical area. Also, prefabricated stents can be used after surgery to trauma to the surgical site also gives it adequate protection.

Embolization of the vessel: With the help of angiography, the exact bleeding point can be localized. Agents that can be used for embolization include steel coils, polyvinyl alcohol foam, gel foam, silicon spheres, and methyl methacrylate. These particles are placed precisely into the bleeding vessel via a catheter.

It has shown significant results in implant surgery to control bleeding immediately after the tear in the lingual artery[1]. Thus, it can be considered as a reliable method for hemostasis.

Thermal method to control bleeding:

Cautery: Hemostasis by using cautery is based on the principle of denaturation of proteins by heat. This proteins undergo precipitation and to coagulation of large areas of tissues. The heat produced by the cautery leads to protein denaturationwhich ultimately culminates into the coagulation of large areas in the tissue. In cauterization, heat is conducted directly into the target area (tissue) via the instrument.

Electrocautery: The fundamental of electrosurgery is based on the principle of using electrical energy which is directly transmitted to the tissue for inducing histological effects. Current range is 1.5 -7.5million per second or megahertz. Using two electrodes, an alternating current may be conducted through the body with almost no effects other than producing heat. The heat produced is solely the result of the resistance created by tissues to the current pathway. This results in a concentration of current at the smaller electrode and the effects are de, hydration, warming of the area, coagulation or tissue destruction by heat depending on the type, the size and the frequency of the active electrode and the duration of application.

The argon beam coagulator (ABC): It provides radio frequency electrical energy (coagulator monopolar current) to tissue by using a heavy stream of argon gas. It delivers noncontact, monopolar, electrothermal hemostasis. In cases of bleeding from small diameter vessels(size $\leq$ 3mm) where bleeding cannot be stopped using other hemostatic agents or ligatures, an argon coagulator is always a good option for these scenarios. This coagulator has also shown improved results in control of presacral bleeding[2].

Lasers: Lasers to perform surgeries with less or almost no blood, as these effectively coagulate the small blood vessels while tissue cutting or sectioning. It provides a clean, dry surgical field with improved visualization[3]. It was seen that it has enhanced the bactericidal effect when used in implant sites without extreme heat production[4]. An in vitro thromboelastographic study showed that after three and six seconds there is a decrease in coagulation time while using 10 W of continuous-wave laser irradiation of 10 ml of blood and resulted in increased clot formation rate[5]. In the present scenario, LASER is widely used because it aids in pain relief, improved healing and speeds up the tissue repair.

Chemical method to control bleeding:

Adrenaline: Adrenaline or epinephrine, applied topically induces vasoconstriction and thus helps in achieving hemostasis. However, adrenaline has a dual-action called a vasomotor reversal, at low doses it causes vasoconstriction but later it leads to vasodilation. Thus, extensive application or undiluted preparation can cause systemic effects therefore, care should be exercised while using adrenaline. It can also be injected with L.A in a concentration of 1: 80,000 to 1:2,00,000.

Astringent and stryptics: When astringent comes in touch with the injured tissue surface, a thin film is formed upon the surface and the superficial membranes and blood vessels are contracted to cause shrinkage of the tissue and thus resulting in protein precipitation[6]. Monsel's solution which is quite effective in controlling capillary bleeding contains Ferric sulfate and it acts by precipitating proteins. Tannic acid also has the same mechanism of action. A study showed that messaging astringent gels in the edentulous areas showed improved keratinization in the denture wearers.

Bone wax: The material is a combination of beeswax, salicylic acid, and almond oil. The mechanism of action of bone wax is mostly mechanical. It can be applied directly to a particular bleeding point in bone. It is normally non-absorbable in blood or saliva. It results in the formation of a soft and malleable mass when heated and manipulated. Commonly supplied in sterile sticks.

Another product BoneSeal® which is a composite of biodegradable polylactic acid with hydroxyapatite and having wax-like handling properties[7]. BoneSeal is designed to promote hemostasis and deliver improved

bone regeneration when compared to bone wax.

Thrombin: Thrombin agents are produced from either human or bovine plasma. It can also be derived from recombinant DNA techniques. The topical use of thrombin acts by converting fibrinogen into a fibrin clot[8]. It shouldn't be injected in the bloodstream or open, large blood vessels which can cause intravascular clotting. It was found in a study that a thrombin Arg-Gly-Asp complex helps in cell adhesion[9].

Gelfoam: It is an absorbable gelatin sponge containing fine, dry, heat-sterilized powder prepared by milling absorbable gelatin. It is insoluble in water, porous and pliable in nature. The mechanism it uses for hemostasis is more physical and it does not affect the blood clotting pathways. Gelfoam is absorbed completely, with little tissue reaction when used in less amount. Normally it is absorbed inside the wound area within 4-6 weeks. It doesn't in abundant scar tissue formation. It was reported that it can produce better results in clot formation than collagen[10]. It is contra indicated for skin closure because it might interfere with the healing of the incision lines.

Oxycel or surgicel (ORC): It is also known as Surgicel or Oxycel for commercial use. It is derived from plant-based alpha-cellulose. It is an absorbable hemostatic agent that has been used to control bleeding safely, simply and effectively. It soaks up the blood and transforms into a gel at the target area. It results in lowering the pH and also caused localized vasoconstriction. ORC also acts as a scaffold or matrix for platelet adhesion and also supports the newly formed platelet plug. It was used in the treatment of intrabony defects and it has shown a significant reduction of CAL and PPD[11].

Fibrin glue: Fibrin glue or Fibrin sealant is comprised of fibrinogen and thrombin that is used in the damaged tissue sites with the purpose of tissue adhesion. Some fibrin sealants also contain aprotinin, fibronectin and plasminogen. The thrombin(enzyme) leads to the conversion of fibrinogen into fibrin monomers incorporated 3-D gel-like structure within the period of 10-60 seconds.

Each fibrin sealant system is made up of two parts. The first part consists of: It is available as a two-component system: the first component contains fibrinogen, factor XIII, fibronectin, and traces of other plasma proteins. The second part contains thrombin, calcium chloride, and antifibrinolytic agents such as aprotinin. Combining the two parts initiates blood clotting mechanism along with the formation of fibrin.

Fibrin glue can be used in various surgical procedures like implant placement in the anterior tooth region. In a comparative study done in 2008 stated that the use of fibrin sealants is superior to using regular suture material because it is easy to handle, helps in induce hemostasis, proper adhesion of tissues and aids in wound healing[12].

Sealants must be used carefully because an excessive amount of sealant can to tissue necrosis[13].

Co-seal: It is made up of polyethylene glycols (PEGs) mixed with hydrogen chloride and sodium phosphate. It is a non-toxic, FDA approved material that is biocompatible and can be easily delivered[14]. When the solution is mixed, it transformed into a hydrogel which attaches itself to the damaged tissues. It is synthetic in nature and doesn't contain Glutaraldehyde. The mechanism of action is mostly physical as the gel swells up in volume (almost 4 times) and can undergo further swelling as it undergoes resorption.

Platelet-rich plasma gel: Platelets contain several growth factors such as platelet-derived growth factor, transforming growth factor, insulin-like growth factors I and II, and epithelial growth factor. Because of this property, autologous platelet concentrate(APC) is widely used to boost postoperative healing. The platelet concentrate can be prepared via centrifugation by using patients own blood. The thrombocyte count of PRP was 1,035,000/L (mean). It also bears mucosa-adhesive properties thus widely used in the field of dentistry.

Ethamsylate: The hemostatic mechanism of ethamsylate is due to the activation of thromboplastin formation on damaged vessel walls. It also decreases prostacyclin 2 synthesis and facilitates platelet aggregation. It acts by correcting abnormal platelet adhesion. It exerts antihyaluronidase action, improves capillary wall stability not (not an antifibrinolytic).

Tranexamic acid: Tranexamic acid 4.8% is commonly used as a mouthwash because it helps in clot stabilization. It also helps in down regulating plasminogen. Because of its antifibrinolytic activities, it can be used as a prophylactic in patients who are taking anticoagulant drugs. A study showed that the efficacy of tranexamic acid when combined with cetylpyridinium chloride, is significantly increased for treating periodontal diseases[15].

Botroclot: It is a form of concentrated enzyme sources extracted from the venom of South American viper, Bothropsatorox. Botroclot (Jaggat pharmaceuticals) is a topical preparation that contains extracted hemocoagulase. It aids in healing and also having procoagulant property. Botroxobin, a WHO-approved product, has been introduced to treat the site of injury. It has shown improved healing property when used in extraction sockets[16].

Protamine: A proteolytic enzyme from the venom of South American viper, Bothropsatorox. It also functions as a plasma clotting agent for fibrinogen. It is a protein molecule having a positive charge and it attaches with heparin which is negatively charged. The ultimate complex formed inside the body is removed by the reticuloendothelial system.

It was found that a combination of heparin and protamine has shown decreased postoperative bleeding[17].

Desmopressin: It is a synthetically manufactured vasopressin analogue which was actually originated for treating diabetes insipidus[18]. Nevertheless, it was used as a hemostatic agent when its ability to release of VWF and factor VIII from the endothelial cells was discovered[19]. It also aids in primary hemostasis.

Vitamin K: Vitamin K plays a massive role in the coagulation process. It also helps in the production of fibrinogen and prothrombin in the liver, thus it can be considered as a vital factor for bleeding control. It can even prevent the chances of bleeding in patients taking high doses of anticoagulant drugs.

Newer hemostatic agents:

Chitosan-based dressings: Chitosan is a biocompatible, complex carbohydrate acquired from chitin (a natural compound derived from zeolites): when the deacetylation of chitin is above 70% it is called chitosan. It is a type of acid salt which has mucoadhesive property. It has a positive charge and it attracts RBCs and Platelets which have a negative charge. It also offers sealing action and antibacterial property.QuikClot (inorganic hemostat) is a chitosan-based product currently available

Polysaccharide-based hemostats: Polysaccharide hemostats, like STARSIL® and HEMOSTAT, seems to be useful in bleeding control. It is widely used in cardiac surgery procedures[20]. Reduction of wound healing complications (mostly seen after using bone wax) can be considered as additional.

Conclusion

Hemostasis can always be considered as the main pillar for the success of any surgical treatment. Even with the judicious application of local hemostatic agents, minor to moderate invasive surgeries can be safely performed. Several other factors like the application of local pressure during surgery, post-operative mouth rinses also aids in bleeding control. However, detailed medical history of the patient, case selection and proper knowledge of surgical anatomy- these factors also have a major part in implementing a safe and successful surgical procedure without any postoperative complication.

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Considerations in Questionnaire Development: A Review

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Abstract

A fundamental part of good research is concerned with making sure that the design of the questionnaire addresses the needs of research. After a proper administration of the questionnaire, a questionnaire constantly measures what it intends to measure thereby giving us confident results of a study. It has been documented in several studies regarding Validity and Reliability, as an important source of measuring the accuracy and consistency of research instruments (especially questionnaires). But their measure is not observed to be practiced in developing countries among health plus social science researchers. This review article comprehensively explores and describes questionnaire design and measures to validate a questionnaire including its reliability testing.

Keywords: *Questionnaire, Reliability, Validity.*

Introduction

The questionnaire can be defined as "a tool for collecting and recording information about a particular issue of interest". It is a proficient means for estimating the "behavior, attitudes, sentiments, opinions and, intentions of generally large quantities of subjects" more economically and rapidly against different strategies. Questionnaires are a good research method because it can be accessible to a large number of people relatively easily and economically. They also provide quantifiable answers for a research topic, hence being easy for analysis.

A definite purpose of Questionnaires is to be related to the objectives of the research. They are prepared in various languages, expanding investigation into new measurements by leading examinations in various cultural groups. Validation of questionnaires in those individual languages lead to exact and strict data about the specific cluster.[1]

Design of the Questionnaire: Careful consideration must be given to the design of the questionnaire for gathering useful and relevant information. A lot of thought and effort is required for a well-designed questionnaire along with proper planning and development through various stages (Figure 1).

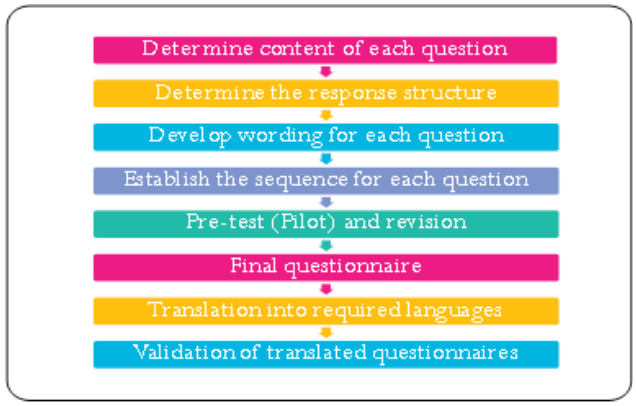


Figure 1: Questionnaire Design

Development of a Questionnaire: The numbering and order of the questions should be in a style that is reasonable in the participant's view. Usually "Funneling" method is utilized which starts from basic questions

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to comfort participants and afterward centers down to progressively more explicit questions.

Step 1: Determining the contents of the questionnaire: Determining what topics, the questionnaire will cover and how it will be administered is the first task. Based on the study objectives, the number of domains is decided. Posing a large number of questions that are not straight forwardly helpful or significant is the most frequent problem in questionnaire design. It can be organized as follows:

1. The prime objectives of the study should be outlined.
2. Questions relevant to the objectives must be asked and only necessary information should be recorded.
3. The lists should be rearranged and organized into distinguished sub-themes or sections of the questionnaire.
4. The decision for the process of filling up of questionnaire by participants themselves or by an interviewer should be taken.[2]

Step 2: Formulation of the questions: Once the sections of the questionnaire have been identified, individual questions must be formulated for gathering the particular pieces of information needed. To deliver significance and purpose of the question to the participants, a step called "wording questions" (selection of words or phrases) is practiced. This ensures that they are uniformly understood by the respondents. Below are the general rules on good question formulation:

i. **Each question must be clear, simple, and specific:** Questions must be indicated in simple, short and clear language. Such as the question can be broken into pieces to assist participants or they may be asked to reveal purposely the different measures taken by them since the beginning of the illness. The following questions may be involved: Did your child suffer from toothache within the past three weeks? (If yes): "Did you visit anyone for advice or treatment? Did you give the child any medicines?" (If participant mentioned medicine(s) were given): "Would you please list any medication(s) that the child has used since the toothache started?"

ii. **Each question must measure one thing at a time:** Measuring two or more dissimilar things in a question is incorrect. Such as questions like "How do you and your staff normally treat children who present with a toothache?", requires partition into numerous questions to permit participants for providing individual answers as there may be a favored method for treating toothache by different prescribers in the office.

When presumptions are made about the participants, Leading questions may also arise. For instance, the question: "What medication did you give your child when he got toothache?" is biased since the respondent would give the child medication, because of the presumption that the child had a toothache. An improved version may be: "Has your child had toothache within the last week?"

(If yes): "Did you take any action?"

(If yes): "What action did you take?"

iii. **Questions must be free from ambiguity:** It is advisable to exclude indistinctly defined or words having double meanings. The question: "What kind of patient was he?" Might generate responses like "poor" or "rich," "simple" or "complex," "cooperative" or "un-cooperative," etc.

"Double-barreled" questions like: "Do you think amoxicillin and paracetamol are effective for treating toothache?" can't be good questions as a participant could like any one of them. In such a situation, having a Yes/No reply would not truly mirror the participant's opinion. Instead it is worthy to divide it into two questions with individual thought.

Step 3: Sequence of the Questions:

The questions must be organized in a sequence after framing them. Following approach is helpful for participants:

(a) The topics should follow a logical order. A smooth flow of thoughts running from general questions within a topic to gradually more specific ones.

(b) Focus should be on interesting and relatively "non-controversial" questions, e.g., "How many years have you worked at this place?"

(c) Sensitive questions like personal information regarding age, education, occupation, and income may create unwillingness among the participants if asked early. Hence they should be placed at the end, after establishing a relationship of trust.

(d) Questions influencing answers to a different question should be separated.

(e) Clear indication of instructions for skipping one/ more questions

Step 4: Arrangement of the Questionnaire

The layout of the final draft questionnaire should be made both "consumer" and "user" friendly. It includes:

1. Required headings and spaces for labeling.
2. Identification of all questionnaires by recognizing information for participant, date and place of interview, and name of the interviewer.
3. Start each section of the questionnaire with necessary instructions.
4. Sufficient space between questions.
5. For pre-categorized answers, be constant with codes or boxes.
6. Enough Space to be provided for answers to open-ended questions

Step 5: Conducting Pilot tests:

Pilot testing of the questionnaire is done with the help of applied qualitative method. The developed questionnaire is tried in a group and a similar environment to that of the original study environment. This will provide an opportunity for interviewers to perform all the activities that are estimated to take place in the field.

Step 6: Translation of the Questionnaire: Most interviews are conducted in local languages. A thorough translation of Questionnaires is done before the fieldwork begins for ensuring consistency while using the words and meanings. Back translation of the questionnaire into the original language it was written is done by a different translator. A comparison of both the versions is then done to rule out any differences.

Step 7: Validation of the Questionnaire:

"Validity is the degree to which an assessment measures what it is supposed to measure". The different types of validity are mentioned below:

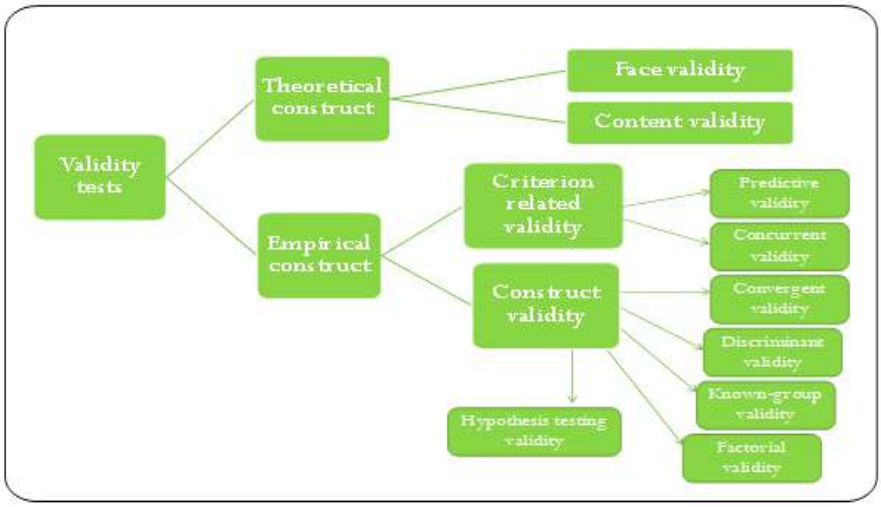


Figure 2. Validity test of the questionnaire

Face Validity: Face validity, "being a form of content validity is assessed by having 'experts' (clinicians/clients/researchers) review". The contents of the questionnaire are being reviewed by an expert on the research subject to see if the items seem appropriate.

Content Validity: "It pertains to the degree to which the instrument fully assesses or measures the construct of interest".[3-7] It is established by experts well-known with the research subject. The experts review the questionnaire items for "readability, clarity, and comprehensiveness" and get nearer to a certain level of agreement for including the required items in the final questionnaire. Based on the ratings of the items in the questionnaire, a significant level of inclusion of an item requires a CVI (Content Validity Index) of ≥ 0.78.[5]

Criterion-Related Validity: Criterion-related validity is "assessment of the relationship of scores on a test to a specific criterion".[8,9] It is a measurement of

"how well questionnaire findings pile up against another instrument".[9,10]

Concurrent Validity: Concurrent validity is "the ability of a test to predict an event in the present form". Here a highly rated existing standard (gold standard) is used for comparison against a newly developed questionnaire.[4] Concurrent validity comes into consideration when the criterion and the measure coincide at the same time.[9, 11-14]

Predictive Validity: It is the "ability of a test to measure some event or outcome in the future".[11,14]. Predictive validity deals with the capability of the questionnaire to predict future events, behavior, attitudes or outcomes. The correlation coefficient is used here.

Construct Validity: It is the "degree to which an instrument measures the trait or theoretical construct that it is intended to measure".[15] Instead of using a criterion for comparison,a hypothetical construct is utilized.[15] It measures how meaningful the scale or instrument is when it is in practical use. Depending on the research problem, four types of validity tests serves the function of construct validity:

Convergent Validity: In convergent validity we check the measures that should be related are related. For example, we construct "education" by asking people how much education they have completed. Then, this record can be compared with their institutional records and a test of school-level knowledge. Both the measures should converge or be similar for a good convergent validity.

Discriminant Validity: It proofs that one concept differs from other closely related concepts.[12] For instance, we have 10 items that measure good oral habits. People respond to all 10 in similar ways. But in the same questionnaire, we have also put 5 questions that measure bad oral habits. Our questionnaire holds good discriminant validity of the 10 good oral habits items stuck together as well as show a negative association with the 5 bad oral habit items.[4]

Known-group Validity: Here comparison between two groups is done within a known attribute. Such that a higher score is given to a group with related attributes while a lower score is given to the group with an unrelated attribute.[16,18-20] For instance, a questionnaire was used to investigate depression between two groups of patients with and without a clinical diagnosis of depression. As it is predictable in clinically diagnosed patients of depression, the score for the construct of depression in the questionnaire will be scored higher in comparison to those without the diagnosis.

Factorial Validity: Being an empirical extension of content validity, it "validates the contents of the construct employing the statistical model called factor analysis".[11] While analyzing factorial validity within a construct of interest, the several items which measure a particular dimension are meant to be highly associated with each other than those measuring other dimensions.[16] Such as there are eight dimensions in health-related quality of life questionnaire (HRQoL). Hence it is supposed that under the domain social function (one dimension from HRQoL) all items of the questionnaire measuring it must be highly associated than those items measuring another domain like mental health.[20]

Hypothesis-Testing Validity: It tests "the relationship between a measured concept (variable) or other concepts (variables), derived from a theory, thus providing evidence that a research hypothesis is supported".[17] Suppose in case of social learning theory revealing how observing physical violence in television leads to violent behavior, a hypothesis is derived from this theory stating a positive correlation between both variables. If the hypothesis is supported by the evidence collected, it is concluded that a higher degree of construct validity is observed in the measurements of physical aggression and viewing of televised physical violence. It is declared after measuring and examining the two theoretical concepts in the hypothesis-testing process.[4]

Reliability of a questionnaire: "Reliability is an extent to which a questionnaire, test, observation or any measurement procedure produces the same results on repeated trials". In short, it is the stability or consistency of scores over time or across raters.[3,4] A sign of lack of reliability exists due to divergences between observers or instruments. Usually carried out by a pilot test, it could be assessed in three major forms discussed below:

1. **Test-retest reliability:** "The ability of the questionnaire to provide similar results when delivered to the same participant on two separate occasions to see how stable the responses are". Generally, a correlation coefficient (r) value $\geq$ 0.70 is considered as good.[20] There is a scope for the memory of the last answer to affect test-retest practice as the participants become known with the terms from the previous test.

2. **Alternate-form reliability (or equivalence):** "It is referred to as the measure of agreement between two or more research instruments like two different questionnaires on a research construct that are delivered at nearly the same point in time".[3] Followed by a parallel form procedure, in which alternative forms of the same measure is delivered to either the same group or different group of participants. Questions or responses are reworded and rearranged to create two items that are related and un-identical. Delivery of the various forms can be at the same time or following some intervals. More similarity between the two forms indicates a higher degree of correlation between them.[4]

3. **Internal consistency:** "It is the degree to which the subjects answer similar questions similarly". Two equivalent forms of a questionnaire are administered at the same time to a subject and assessed for internal consistency. Other method include the "split-half reliability index" and "coefficient alpha index".[21] Sometimes, "Kuder–Richardson formula 20 (KR-20) index" can also be used.[3]

Intraclass Correlation (ICC): Intraclass correlation measures "the reliability of ratings or measurements for clusters- data that has been collected as groups or sorted into groups". It describes how strongly the units in the group resemble each other. The ICC ranges from 0 to 1. High similarity exists between values from the same group if the ICC value is close to 1. If the ICC value is close to zero, it indicates that values from the same group are not similar.[21]

Conclusion

Good questionnaire design is a key to obtaining good survey results. Based on validity and reliability testing, questions are re-written, removed or included, until the researcher feels the questionnaire met the standards set by him. In this article we have discussed the various ways of testing the validity and reliability of a questionnaire which will help the researcher to improve his knowledge and skills.

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Recent Advancements in Imaging of Oral Cancer

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Abstract

In oral fissure, "squamous cell carcinoma (SCC) "is the commonest oral carcinomas which comprises (90-95%). After the 5th decade of age, male predilection is double as compared to females. (40%)of OSCC are confined to the dorsal or ventral surface of the tongue, (thirty percent)on the floor of the oral fissure. "OSCC" regularly distresses maxillary and mandibular attached gingiva, buccal mucosa, retro-molarand palatal region. In 5 years' survival percentage series from 20% {OSCC in the floor of the mouth} to 60% {OSCC in alveolus portion of jawbone} depicting poor prognosis. Although it is tough but it is required to do imaging studies to understand the results at the initialphases of the disease. Various modalities include {conventional plain film radiography, panoramic radiograph, cone beam computed tomography,Perfusion computed tomography,Magnetic resonance imaging–(diffusion-weighted MRI, dynamic contrast-enhanced MRI), ultrasonography, single-photon emission computed tomography}, fusion techniques such as (ECT/CT, CT/MRI, PET/CT, PET/MRI) along with nuclear medicines – (2-deoxy-2-[18F] fluoro-D-glucose), (18F-FDG), 18F-3-fluoroalpha-methyltyrosine, (18F-FAMT) and L-1-[11C]-tyrosine(C-tyrosine), radionuclide imaging. These various imaging diagnostic modalities support in the grading of the tumor, valuation of the vascular supply, resolution about metastasis combined with confined lymph nodes as well as detached organs. Furthermore, imaging lessons help to forecast the possibility of resection and advance management, graft establishment, and diversity amongst reactive and metastatic lymph nodes,and amongst diseases reappearance and marks or adversative reactions of post-operation or emission therapy.

Keywords: *Oral squamous cell carcinoma, imaging modalities, radionuclide imaging.*

Introduction

Ninety percent of oral cancers are the squamous cell carcinoma .75% of oral cancer occurs due to our lifestyle changes. Two decades ago there were 20% of HPV related cancer,now it has been a great hike to 50%. HPV related oral cancers are 6-7 times more likely to occur in men compared to women. Five persons die daily in every hour in India.[8] Oral cancer occurs due to various factors like tobacco consumption,human papillomavirus, genetic factors, etc. As it is adversely affecting the globe we should have a good diagnostic procedure for better treatment planning.[1]

Importance of Imaging of Oral Cancer:

- To know the extent of disease.
- Resectability
- Preservation of form and function
- To know distant or occult metastasis
- To know the dissemination and invasiveness of bone.[7]

Imaging modalities like (conventional plain film radiographs,panoramic radiographs,computed tomography, magnetic resonance imaging, ultrasonography, single-photon emission computed tomography,positron emitted tomography,fusion or hybrid imaging such as pet –ct,pet –MRI, etc).

Conventional Plain Film Radiographs: The distinctive structures of metastatic lesions in this radiograph depictcortical lamina degeneration, osteolytic weaknesses with solitary and multilocular with an initial osteosclerotic shell. In advanced phases, the crests of jawbone weaknesses becomes evere and teeth fail its bony maintenanceon the position of penetration ailment reappearance and marks or adversative responses afterward surgery or emission therapy.[1]

Panoramic Radiograph: In"panoramic radiographs", participation of the facial bony structures can"t be evaluated, because onceosseous tissues are damaged larger than 30%, the radiolucencies are suggestively amplified. Plain radiographs cannot assess soft tissues structures,reveals valuation of bone contribution on this radiographs have a 75% sensitive value 100% specific value .[1]

Positron Emitted Tomography: {F-Fluorodeoxyglucose positron emission tomography} (F-FDG PET) is a practical imaging technique that delivers data approximately tissue absorption and receipts been successfully functional to the estimation of head and neck carcinomas. PET is created on recognizing amplified glycolytic activity in menacing cells, in which radio labeled FDG is specially focussed due to rises in membrane glucose carriers and in hexokinase, an enzyme which phosphorylates glucose. Subsequently phosphorylation, radiolabeled FDG stays to gather in growth cells instead of glycolysis, permitting imaging by PET. (F-FDGPET) is extradelicate in comparison to CT or MRI to identify cervical node metastases.

It helps to identify metastatic nodes which are morphologically standard. It discovers distant and occult metastasis. It detects recurrences and used for treatment evaluation. It is used forplanning adjuvant for chemotherapy or radiotherapy. It is a prognostic indicator with CT or any fusion imaging.

PET alone cannot delineate the extent of the tumor. It has are duced anatomic resolution. [2]

Magnetic Resonance Imaging: It is the procedure that affords the maximum image value of soft tissues deprived of involving ionizing radiation,lacking any genetic risks. MRI is used to evaluate the degree of loco-regional tumor range, penetration of incursion and level of lymphadenopathy. The invasion of the floor of the oral cavity by the tumor is represented well in the coronal plane. Non-contrast T1W arrangements depicts cortical erosion and marrow invasion. Contrast-enhanced T1W images benefit evaluate marrow invasion, perineural spread, soft tissue extent, tumor depth and best demonstrate necrosis in nodes. The T2W images are sensitive to the presence of tumor tissue,which is usually hyperintense compared with the surrounding tissue. [3]It helps in surgical planning and assessment of problems that can occur during or after surgery. Limitation is the artifacts of ferromagnetic prosthesis of jaws.[11] DWI {DIFFUSION WEIGHTED MRI} can be accommodating in evaluating the initial answer of the tumor to chemotherapy. DCE-MRI {DYNAMIC CONTRAST ENHANCED MRI} helps in evaluation of micro vascularity and perfusion of tumor whichcan be estimated, which can help to forecast result.[1]

Ultrasonography: It is the imaging modality used for the evaluation of apparent diseases, lymph nodes as well as to guide needle aspiration biopsies. It is applied to know the microvascular changes, depicts the elevated vascularity within the tumor. It is used for the evaluation of the size and thickness of soft tissues. Intraoral USG along with colour Doppler helps in assessing the involvement of lymph nodes. USG guided FNAC gives a hint of nature and site of the primary tumor, helps in better assessment of pre and post-therapy. It also helps in confirmation of metastasis.[10,16]

Computed Tomography: This imaging modality helps in the detection of the primary tumor. It depicts the bony invasion of the lesions. It cannot appreciate the recurrence of the lesion,cannot differentiate the surgical scar as well as the adverse reaction of post-radiotherapy."Contrast-enhanced CT" able to precisely control lymph node metastases which can be originally appeared regular, despite the existence of micro metastasis identified in microscopic lessons. The sensitive value of CT in identifying tumors is (41–82%) (specific value 82–100%) and to determine bony infiltration (63–80%) (specific value 81–100%), respectively.[1,13]

Perfusion Computed Tomography: It is used to assess the participation of the surrounding tissues by assessing the perfusion of the tumor origin accurately,[12] tumor is categorized through amplified(blood volume) (BV) and (blood flow) (blood flow) in contrast with healthy tissues. This results from neoangiogenesis in the tumor. It is recommended that perfusion Computed Tomography is higher to Computed Tomography as favoursvaluation for mass engrossment.

Cone Beam Computed Tomography: High radioactivity dose, cost, availability, longer scanning time, poor determination and trouble in interpretation have led to restricted usage of CT which can be overwhelmed with CBCT, which delivers several potential benefits for Oral & Maxillofacial imaging, associated with conventional CT. The limitation of CBCT is the artifact impairs image value and boundary acceptable visualization of constructions in the dentoalveolar region. Artifacts can

be due to beam hardening (results in cupping artifact and stripes and dark bands), Patient-related artifacts (Patient movement resulting in unsharpness of reconstructed image), Scanner related artifacts (circular or ring-shaped) and cone-beam–related artifacts (partial volume averaging, under-sampling, and cone-beam effect. Image noises due tothe large volume being exposed during CBCT scanning causing in heavy communications with tissues creating scattered radiation, which in turn clues to nonlinear diminution by the detectors.[4]

Single Photo Emission Computed Tomography: This imaging modality helps in the mapping of metabolic activity. It helps to locate the sentinel lymph node. It helps to evaluate the distance of sentinel lymph node to skin. It detects the fluidity of lymph into the sentinel node. It accurately detects bony involvement as well as the relation to the surrounding structures.[9,15]

Radionuclide Imaging: "Radionuclide imaging" is the procedure which is a non-invasive functional imaging procedure, which providesdata of physiological as well as biochemical processes pathologically).[5]

Lymph Node Scintigraphy: Lymphoscintigraphy is used as a radioisotope procedure for the planning of sentinel lymph nodes. It takes the advantage of constant turnover of bone in response to metabolic or mechanical demands. Most diseases including bone end in amplified turnover or new development of bone. On scintigraphy, these areas perform as areas of amplified radio nuclide acceptance, called "hot spots."Lower acceptance is associated with metabolically inactive bone, deficient osteogenesis, or an absent vascular supply. It also designatesa lack of reparative response and suggests the presence of a violent lesion. Photon-deficient region or photopenic abnormalities are those areas where there is diminished or absence of uptake and are called "cold spots.[5]

Radio-Isotope Imaging in Oral Cancer:

{Carbon-11, Nitrogen-13, Oxygen-15, Fluorine -18}

It can be applied in positron emitted tomography images with the usage of (F-18) in FDG which helps for detecting, identify and distinguish the prediction of tumor.

{Cobalt-57}

It is used as a mode of (detector) of hypertrophy of cells, indirectly control the partition,advancement of cells growth to stimuli.

{Gallium -67}

It is applied for imaging the size of the tumour.

{Gallium -68}

It is applied in PET examination to distinguish metastatic action of the lesion.

Advantages of nuclear medicine:-

- Beneficial for initial analysis of diseases and assessment of management properties in the early post-therapeutic period.
- Permits simple demonstration of entire-body images and interactive display, which helps to notice metastatic action.
- It gives anatomical knowledge that is used for the diagnosis of diseases.
- Thorough investigations that can be accomplished at different sites as well as on multiple times afterward injection of a tracer isotope to illuminate conclusions deprived of growing radioactivity contact, distinct another simple procedure where there is the numeral of exposures.

Drawbacks of nuclear medicine:

- Though divergence of lesion against adjacent tissues is higher when radiotracers gather in the lesion, having a spatial resolution, generally, is poor with comparison to radiographs, Computed Tomography or Magnetic Resonance Imaging.
- The price of equipment which is applied is relatively expensive.
- The price of an individual investigation is dependent on the charge of radio drugs applied and the competency of the scanner.
- The Patients are unprotected of ionizing radioactivity managed to builds.6
- Emission contacts are very much unlike from radiographs and computed tomography, which includes peripheral as well as in generals imply partly body contact, radionuclide control patients cause internally entire body coverage in a non-uniform method firm by the bio circulation and permission kinetics of that tracer.

- Because of inevitable higher radiation from positron emitted tomography tracers to the staff people, interacting with radioactive patients, it is recommended that a 6 mm dense lead protective is not logical.

Inference: As the oral cancer progressing so rapidly we should prevent it by diagnosing accurately and doing treatment planning accordingly. For diagnosing carcinomas various imaging modalities are used like two-dimensional imaging like conventional plain film radiographs or panoramic radiographs are used to know the bony extension of the lesion,three dimensional like computed tomography, magnetic resonance imaging or any fusion imaging is used like PET-CT, PET-MRI these are used to know the asses the recurrence, evaluation of lymph node, peri-neural invasion, Lymphoscintigraphy is used to assess the sentinel lymph node. Nuclear medicine is used to know the initial phase of the lesion and post-operative action.

Diagnostic protocol for (detecting,staging and following up of patients with oralcarcinomas)

- To assess the primary tumor size-contrast-enhanced computed tomography, magnetic resonance imaging.
- To detect metastases in regional lymph nodes – ultrasonography, magnetic resonance imaging, computed tomography.
- To know response to radiation therapy and chemotherapy- perfusion computed tomography, magnetic resonance imaging with perfusion and diffusion.
- To locate the distant metastases-positron emitted tomography, single-photon emission computed tomography, fusion imaging modalities like {PET/ CT, PET/MRI}.
- To detect the recurrence-perfusion computed tomography (PCT), magnetic resonance imaging (MRI) with diffusion, magnetic resonance imaging (MRI), positron emitted tomography, fusion imaging modalities like {PET/CT.PET/MRI}14

Conflict of Interests: None

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Painless Anesthesia in Pediatric Dentistry: A Review

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Abstract

Pain management is one of the most commonly faced problem for dentists while handling of pediatric patients. A positive dental attitude can be instilled in a child if pain is handled properly. In recent times, various advancements and research have been made in the field of local anesthesia. These techniques along with the conventional ones can be utilized for better handling of pain and to get better apprehension from patients. This review article is to discuss recent advancements in painless anesthetic techniques and to improve the quality of treatment regarding pain in pediatric dentistry. As management of pain is of paramount importance to instill a positive attitude about dentistry in children.

Keywords: *Local anesthesia, Painless dentistry, Eutectic mixtures, Jet injection,Pediatric dentistry.*

Introduction

Karl Koller discovered local anesthesia in the year 1884. Since then local anesthesia has been a boon to the medical and dental field as it allows to carry out various surgical processes painlessly. Pain control and pain management is greatly enhanced by the use of local anesthesia. The recent advancements in the field of anesthesia have made it possible to profession to make tremendous therapeutic advances which would not have been possible otherwise. For administration of local anesthesia one should have knowledge of anatomical structures, devices and method to be used, neuroanatomy, etc. A dental practitioner carries out various procedures like extraction, root canal treatment, scaling. In one or other procedures patients always have to overcome some amount of pain. To avoid that,local anesthesia is administered through syringes and needles via conventional techniques.[1]

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This method in itself increase the fear and anxiety of the patient before even commencement of the procedures. Managing a child patient is in itself difficult. So, to avoid the above-mentioned procedures, it is essential to know the latest local anesthetic delivery techniques that will ensure painless treatment to the patient.[2]

Local Anesthesia Delivery Systems:

Various traditional method are available for the delivery of local anesthesia. But many newer advances have come to ensure painless dentistry via:

Computer-controlled local anesthesia delivery, vibrotactile systems,, jet injectors intraosseous anesthesia, etc.

Computer-controlled local anesthesia delivery (CCLAD) system: Computer-controlled local anesthesia delivery systems enable the rate of flow regulation of the local anesthetic using computers and hence reduces pain by slow delivering of anesthesia at a constant speed. The operator should look at the design of the system which includes parameters like infection management, weight, mode of drug injection, speed, possibility of aspiration, etc before choosing a suitable one to avoid infection. The first CCLAD used was the Wand system (introduced in 1997) and some subsequent versions around Plus and Compu Dent. Comfort Control Syringe (introduced in

2001) is different from the Wand system in a way that it does not possess a foot control.[3]

A. Wand System: This technique helps to deliver the local anesthesia by placing a needle at the site to be anesthetized with fingertip accuracy with better control. It administers local anesthesia at a constant rate and is controlled by a foot activator. For better tactile sensations held with a pen-type grasp and is lightweight so easy to handle

It is one of the most popular and commonly used CCLAD. Advantage of this being its operator friendliness and its circumference that is half the size of the conventionally used syringes. As the operator has to hold the device in the area to be anesthetized for a long period thus, lighter devices are preferred over the heavy ones as it helps prevent needle break injuries and muscle fatigue. The cartridges that are used in the Wand system are installed in the main unit and the assistant can change it whenever required but about 0.3 to 0.4 ml of solution may be wasted during the procedure. Initially aspiration time in wand system was 14 seconds which is now reduced to 5 seconds and plus. Feda et al13 and Mittal et at 14 reported that CCLAD proved to be advantageous while delivering the palatal anesthesia by reducing the pain.[4]

b. Computer comfort syringe (ccs) system: CCS differs from the wand systems it consists of a base unit, a syringe and no foot control. The syringe itself can be used to control injection and aspiration and the solution deposited to the desired tissues irrespective of the resistance offered. This makes it user friendly for those using conventional techniques. 5 programmed speeds and three buttons are present on the device. The injection can be controlled using these buttons namely start or stop, aspirate and double button to start or stop the anesthetic delivery, aspirate or double the rate of delivery. Digital feedback readouts are present in the base unit which displays the time elapsed, rate and volume of anesthetic injected. It is less desirable than a wand system because of its weight but is quite easier for those using conventional techniques.[5]

C. Jet Injectors: Mechanical energy is used for releasing the pressure, thereby allowing the liquid medication to make its way through a small orifice. A thin column of fluid formation is permitted to be created that will allow the anesthetic solution deposition into the subcutaneous tissue without using a needle. Merits of Jet injections are minimal tissue damage, fast drug delivery and absorption, and lesser pain. Patients having a fear of needle and apprehension can be best managed by jet injectors.Commonly used brands of jet injections are SyrijetMarkII, MED H JETIII etc.[6]

MED JET H III: This was introduced first in the year of 2011. The advantage of this being the anesthetic solution is deposited with a much smaller orifice that is seven times smaller than the needles with the smallest diameter. This system has almost great accuracy and the solution is delivered at a small pressure and does not compromise with patient comfort and user compliance.[7]

SYRIJET MARK II: This has been used for 4 decades and also have undergone various advancements. 1.8cc syringes are used, which deliver the local anesthetic solution from 0-0.2cc and can be autoclaved hence reusable. It uses a nozzle pressure of 2000 pounds psi and at this pressure the same effect is the same as that seen in a conventional needle piercing 1 cm deep in oral tissues. This can be used for various minor surgical procedures like removal of deciduous anterior teeth and permanent central and lateral incisors, soft tissue procedures, removal of bone spicules, application and removal of arch bars and ligature wires could be carried out with the use of Syrijet alone.[8]

Though it as its advantages,but certain disadvantages are also there. Bleeding from the punctured sites are seen if punctured twice or thrice, the unpleasant taste is also seen. Hence concluding that Syrijat Mark II can be used for certain dental procedures but some it requires adjunctive anesthetic measures.

D. Safety syringe needles: The safety needles provide the prevention of needlestick injuries to the health care provider. These special needles are covered by a sheath as soon as it is removed from the tissues. Some commonly used safety needles are Hyposafety syringe,Ultrasafety plus XL syringe, Ultrasafe syringe, SafetyWand syringe etc. However, these are no better than the conventional syringes and are of inadequate help in preventing needle-stick injuries.[9]

E. Topical anesthetics

1. Lidocaine patches: It consists of a mucoadhesive base and delivers the local anesthetic via the trans-oral delivery route. It

is commonly used before the needle is pricked into the tissue and for superficial mucosal and gingival procedures. Absorption is well into the tissue and effects are seen within 2 minutes and last for 30 minutes after removal of the patch. The demerits being high cost and poor adhesion to the oral mucosa.[10]

2. **EMLA Cream:** EMLA stands for the Eutectic mixture of local anesthetics. It is an emulsion of oil in water which consists of 2.5% prilocaine and 2.5% of lidocaine in the ratio 1:1. It is supplied in various forms like in a tube of 5 to 30g, in disc form or form of anesthetic patch. These are easily absorbed into the oral mucosa and can be used only for minor procedures. These are applied orally for 2 to 5 minutes to provide psychological and pharmacological effects before needle penetration. It shows good results for pediatric use of cream but yet its advantages are t be researched. However, it is contra indicated in patients with methemoglobinemia or those having allergy towards amide-type local anesthesia as can cause allergic dermatitis.[11]

3. **Intranasal sprays:** In this anesthetic solution is infiltrated in nostrils through a metered device to anesthetize the maxillary anterior teeth,canines and premolars.[12]

   These are a mixture of 3% tetracaine hydrochloride and 0.05% oxymetazoline. It constricts the vessels thereby reducing bleeding and making the operating field favorable for the same.

4. **Laser analgesia:** This acts by disrupting the sodium-potassium pump temporarily and by changing the behavior of neuronal cells. It thereby produces profound analgesia by blocking the impulses to reach the brain. However, it should be used along with other anesthetic measures to work properly.[13-16]

Conclusion

Management of pain is one of the primary concerns for pediatric dentist. Most of the patients usually have a fear of needle and the pain associated with it. To overcome that, the above-mentioned techniques can be far more useful for better patient apprehension. It will also improve the quality of treatment that the dentist can provide. These techniques can help to instill a positive attitude towards dental treatment in a child. Though some of the techniques are time-consuming,costly but are proven and successful ones. One has to use it according to the type of treatment, feasibility, and user-friendliness.

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Genes Associated with Fibro-Osseous Lesion: A Review Article

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Abstract

The Fibro osseous lesion is a group of diseases or lesions that are affecting the skeletal tissue which frequently includes maxilla and mandible. The disease can also be easily described as bone disorders. A lot of research and work has been done regarding this serious disease though due to similarities in its radiographical features or histopathology, it is been very complicating to diagnose and classify FOLs. The review article focuses on different diseases that come under the fibro-osseous lesion and the genes which have been associated with the respective one in an orderly manner with their Histopathological features. One to understand this lesion the clinical correlation with radiographic involvement is necessary to diagnose this kind of dysplasia. The article also throws light on that the mandible and maxilla also suffer from localized and generalized forms of skeletal dysplasia which results in disproportionate short stature. These dysplasias always remain in a confused manner for their nomenclature. Much classification has been proposed by the different authors in different years regarding the FOLs based on gene or protein defect in these disorders.

Keywords: *Fibro osseous lesion, Genes, Dysplasia, Skeletal.*

Introduction

The fibro-osseous lesions are very serious and complicated disease which can cause severity in the function of tissue including jaws and craniofacial bones. They consist of different type of pathological disorders which includes bone dysplasia, inflammatory lesion, neoplastic lesions, and metabolic disease. These maxillofacial lesions cannot be diagnosed by taking incisional or excisional biopsy alone but the correlation of clinical and radiological features is very crucial for detecting these serious disorders. As earlier mentioned many authors have proposed different classification and the article provides the latest classification which was given by ever sole et al in the 2008.[1]

Classification: In the year 2008 ever sole and their co fellows gives a classification of fibro-osseous lesion which was based on metabolic disorders, neoplastic lesions, inflammatory disorders, and reactive processes. Based on clinical features and radiographs with the adding of Histopathological diagnosis the behavior of this disorder can be judged and the final diagnose can be made, the ever sole et al have concluded in their classification or research.[2]

1. **Bone Dysplasia:**

a. Fibrous Dysplasia – This dysplasia includes its many types including monostotic,polyostotic,and polyostotic with endocrinopathy & osteofibrous dysplasia

b. Paget's disease

c. Pagetoid heritable bone dysplasia of childhood

d. Segmental odontomaxillary dysplasia

2. **Cemento osseous dysplasia:**

a. Focal Cemento osseous dysplasia

b. Florid cement dysplasia

3. **Inflammatory processes**

a. Focal sclerosing osteomyelitis

b. Diffuse sclerosing osteomyelitis

4. **Metabolic Disorder**

a. Hyperparathyroidism

5. **Neoplastic lesions**

a. ossifying fibroma

b. hyperparathyroidism jaw lesion syndrome

c. juvenile ossifying fibroma

d. gigantiform cementomas

Fibrous Dysplasia: This dysplasia compromises of bone-forming mesenchyme that manifests as a defect in osteoblastic differentiation and maturation which is skeletal developmental anomaly. The disorder is nonhereditary having unknown cause.[3]

Gene Associated for the Cause: Mutation in the GNAS1 (guanine nucleotide-binding protein, alpha stimulating activitypolypeptide) Gene. This gene encodes a G- protein which stimulates the production of c-AMP. The mutation results in the overproduction of cAMP due to the prolonged activation of G –protein. Due to this mutation hyperthyroidism, precocious puberty and overproduction of cortisol happens which results in hyperfunction of affected endocrine organs. Secondly large café au lait spots with irregular margins can be seen which is due to the increase in proliferation of melanocytes and at last fibrous dysplasia is caused as cAMP affects the differentiation of osteoblasts. Radiographic appearance is described as ground-glass appearance which appears radiolucent on radiographs which happens due to the fibrous tissue replacing medullary bone.[4]

Molecular Pathogenesis of Fibrous Dysplasia:

- The mutation of the GNAS1 gene activates the cyclic adenosine monophosphate(c-AMP),
- Which results in the elevation of c-AMP and stimulates the endocrine receptors
- In return which activates the c-fos (proto-oncogene)
- After which the impaired differentiation of osteoblasts results in bone lesion
- & the increased in the production of melanocytes results in cutaneous pigmentation
- & the hyperfunction of endocrine cells resulting in endocrine disturbances.
- The bone lesion giving rise to Fibrous dysplasia, cutaneous pigmentation concluding Mccune Albright syndrome & the endocrine disturbances lead to Jaffe Lichtensteinsyndrome.

Histologic Features:

- Fibrillar connective, woven immature bone, irregular in shape can be seen in the lesion.
- Large osteocytes and collagen fibers can be noticed extending out in the fibrous tissue.
- Stellate osteoblasts that form the bone can be observed.
- Osteoblastic riming can be seen.
- Wide osteoid seams can be observed in the trabeculae.
- Osteoclastic activity can be noticed in the histological slide.

Treatment & Prognosis: In the maxilla the lesion is been treated non surgically maximally.

No specific medical treatment is for bone diseases. Although the use of vitamin –D and bisphosphonates can be useful in relieving pain and possibly in reconstituting lesions with normal bone. The surgical procedure includes the curettage technique and replacing the bone with autograft or allograft.[5]

The malignant transformation for this lesion is very low which can be estimated 0.4%-1%.In most times the facial and skull bones went a malignant change in polyostotic disease. The fibrosarcoma and osteosarcoma are the most common tumors. The lesion size increases with an increase in pain can be noticed in the malignant transformation with enlarged soft tissue mass.

Paget's disorder: It can also be called as osteitis deformans. The disorder commonly affects the middle-aged and elderly ones. It is a disease which is non-malignant. Usually, this disorder is asymptomatic, but the patient may develop symptoms like pain in the affected bone, neurological, and cardio complication can also be seen in complicated cases. Almost 3-5% of people in the United States of America are been affected by this disease.[6,7]

Genes and other associated Factors for the cause: The etiology of this disease is still unknown but two main theories for its cause have been proposed which include Viral i.e. paramyxoviral infection & genetic.

Genes associated with it include: TNRSF(Tumor necrosis factor receptor superfamily member -11A), Sequestrome 1 (SQSTM1), and Valosin containing protein (VCP).

Different Phases includes:

This disorder occurs in 3 phases, which includes:

- **Initial Phase:** In this phase intense Osteoclastic activity and resorption of bone occurs.
- **Secondary Phase:** In this phase osteoblasts start to produce woven bone and the mineralization is ineffective.
- **Final Phase:** In the final phase trabecular and dense cortical bone dominates the deposition.

The bones which are commonly affected by this disorder include pelvis, femur, skull, humerus, cervical spine, and thoracic spine. This disease mostly affects the axial skeleton. Pelvis, which has a high percentage in its involvement.[9]

Radiologic Findings: In the affected person one can notice these findings as;

- In skull we can noticed osteoporosis circumscripta
- Sclerotic bone can be observed
- Banana or chalk pattern transverse fractures can be seen.
- Bowed limbs

Diagnosis: Imaging tests which include x-ray and bone scan & lab tests is been performed to diagnose the disorder. In the bone scan method, radioactive material is injected into the body, and the materials directly affect the areas which include the Pagets disease and thus they are light up in the scan. In laboratory testing elevated levels of Alkaline phosphatase can be observed in the blood. [10]

Histologic Features:

- Disordered areas of resorption with an increasing number of large osteoclasts is been noticed in the initial osteolytic phase.
- Jigsaw or mosaic pattern can be observed which is a result of continuous bone removal giving small bone fragments which are irregular in shape.
- The above-mentioned pattern can be considered as the hallmark for this disease.
- The pattern is mosaic with irregular cement lines joining the area of lamellar bone.

Treatment and Prognosis: In the present scenario no specific treatment is there for osteitis deformans but the use of radiation and hormone therapy, vitamins have been used to challenge this disorder. Very better and product results have been found nowadays by using the Calcitonin, which is a parathormone antagonist produced by the thyroid gland which suppresses bone resorption. Overall the complication of this disease is not so wide and less than 1% of osteosarcoma is noticed from Paget's disease.[11,12]

Ossifying Fibroma: It is a benign neoplasm that affects the jaw which has been enlarged. This fibro-osseous lesion is featured by substitution of normal bone by newly formed calcified products like bone, cementum, or both and fibrous tissue. It is commonly seen in teenagers and young adults. It is a rare and slow-growing tumor that is non-cancerous.[13]

Gene associated with it: HRPT2 gene mutation is found to be cause for this fibro-osseous lesion. The radiographic feature reveals a radiolucent area with an increase in calcification, displacement of adjacent tooth is common. The Histologic features include interlacing collagen fibers, proliferating fibroblasts and mitotic figures can be noticed. Small foci of irregular bony trabeculae can also be observed.[14]

The treatment includes excision of the tumor and the recurrence rate is very less.

Hyperparathyroidism–jaw tumor syndrome: It is a syndrome which is an autosomal dominant disorder. It is associated with fibro-osseous jaw tumors, renal lesions and featured by parathyroid tumors. The patient who suffers from hyperparathyroidism may develop symptoms of muscle weakness, pain in the joint or bone, fatigue, and may develop kidney stones with a decrease in the density of bone.

HPT-JT is a jaw tumor which is fibro-osseous lesions that consists both the maxilla and mandible and mostly affect in the 3rd decade of life. Ossifying fibromas generally slow-growing and benign tumors that arise from the periodontal ligament in molar or premolar areas are reported to be highly involved with HPT-JT.

Gene associating for its cause: The HRPT2 gene localizes to 1q24-32 which encodes a 531-amino acid long protein, which is called parafibromin, which exerts anti-proliferative activities via interacting with cyclin D1. Germline mutation and somatic inactivation of the HRPT2 gene which lead to hyperparathyroidism –jaw tumor syndrome. The treatment involves the management

of hypercalcemia. The focus of the treatment is to reduce the level of calcium to below 11.5mg/dl.[15]

Cherubism: It a skeletal dysplasia and an autosomal dominant fibro-osseous lesions of the jaw. It is a non-neoplastic hereditary bone lesion that has a similar type of histopathological features as central giant cell granuloma. It was first described by jones in 1993. It mostly affects the children. It is also known as disseminated juvenile fibrous dysplasia or familial multilocular cystic disease of jaws.[16]

Gene associated with its cause: The mutation in the SH3BP2 (SH3 domain-binding protein 2) gene is the most identifying cause for 70-80% cases in Cherubism. In the remaining cases, the etiology is unknown. The SH3BP2 gene gives instructions for producing a protein whose exact function is still not clear. This protein has an important role in transferring chemical signals within cells, especially involving in replacing the old bone tissue with a new one which is a bone remodeling procedure. Thus mutation in the SH3BP2 gene results in producing an overactive version of this protein which can lead to the disrupting of the critical signaling pathways among cells that maintain the bone tissue. This overactive protein leads to the inflamed jawbones and alters the osteoclasts production, which on return results in the breakage of the bone tissue during remodeling.[17]

Clinical and Radio graphical Features: The children who are been affected are normal at birth until 14 months to 3 years of age, after that clinical evidence can be seen which includes the symmetrical enlargement of the jaws. The symptom starts with deforming maxillary and mandibular overgrowth with a problem in respiration and defects in hearing and vision. Noonan's syndrome can be observed in some cases of Cherubism which involves obstructive sleep apnea, gingival fibromatosis with orbital involvement.

The radiograph is characterized by multilocular cystic expansion of the jaws. Numerous unerupted teeth with the destruction of alveolar bone will displace the teeth which results in floating tooth syndrome.

Histopathology: Multinucleated giant cells can be seen, fibroblasts are spindle-shaped present in the connective tissue stroma, water logged & granular. Numerous small vessels can be noticed.

The treatment is done by surgery to correct the deformities of the jaw and is very rarely indicated. The surgery is usually done after puberty when the remission phase reached.

Periapical Cemento–Osseous Dysplasia: This dysplasia is a kind of fibro-osseous lesion which is associated with the apex of a tooth. The disorder has a dominant autosomal inheritance pattern. The etiology of this fibro-osseous lesion is unknown. Usually the unifocal or multifocal dysplasia does not need any treatment but the larger lesion requires surgery to prevent further complication.[18]

Differential Diagnosis: The fibro-osseous lesion should be differentiated from the other lesion which can produce the same radiographic and histological features. Some of the important lesion which should be differentiated from fibro-osseous lesion are-

- Osteoblastoma
- Giant cell reparative granuloma
- Sclerosing osteomyelitis

Conclusion

The fibro-osseous lesion should not be diagnosed or classified only based on histological features as they all can show almost similar features. These craniofacial lesions should be judged by clinical correlation and radiographic involvement. The classification can only be done by radiographic appearance and their growth pattern. The genes which are involved in this type of lesion and their mutation provide a better idea of knowing the pathogenesis of these disorders. However, in this era, some of the cause of this lesion is found to be unknown. Research is been made for detecting the important causative factors which can further lead to the prevention and proper diagnosing of this lesion.

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Special Stains Used in Histopathological Techniques: A Brief View

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Abstract

The routine staining which is also called as H & E staining (Hematoxylin and eosin) is the most widely used stain in the laboratories or histopathology laboratory. In this procedure the nuclear protein stains purple and the cytoplasm and any other substances stains as red or orange. It plays a very crucial role in medical diagnosis or research where the specimen or tissue is been sent for processing. The staining procedure is performed by trained professionals and after which the role of highly trained pathologists and researchers come, which under the microscope view the specific structures, cells and even microorganisms to provide a diagnosis for the specific disease. The special stains are those which come under the special staining technique or alternative method for the H & E stains, where H & E staining does not provide the proper identification or appreciation of certain normal and abnormal tissue or structure which the pathologist or researchers needs. This article throws light on the Special stains which are been used in histopathological procedures in which these abnormal or normal tissues can be properly appreciated. The article also focuses on the procedure, principle and uses of these special stains in their respective field.

Keywords: *H & E stain, Special stain, Histopathology, Pathologist.*

Introduction

Special stains are those which come into the action where the given Hematoxylin and eosin staining does not provide much information regarding the tissue and structure of cells. Some of the particular tissue structure does not stain well with H & E stains like the reticular fibers in the liver which need a special staining technique, this special staining technique is performed for a better appreciation of normal and abnormal substance which is present in the cell and tissue.[1] These stains are used to identify specific microorganisms like bacteria or parasites, carbohydrates, lipids, mucosubstances which include both neutral and acidic, nucleic acid, and minerals along with connective tissues. The special stains include classification which makes it a convenient choice for different diagnosing procedures.[2]

Classification The classification includes:

I. Stains for amyloid

II. Stains for carbohydrates

III. Stains for microorganisms

IV. Lipid stains

V. Connective tissue stains

VI. Nucleic acid stains

VII. Stains for pigments and minerals

- Carbohydrate Staining- The carbohydrates are of two types of simple carbohydrates which include monosaccharides, oligosaccharides, and polysaccharides, whereas glycoconjugates consist of proteoglycans, mucins,and others glycoprotein.

A carbohydrate staining includes many techniques in which some of the most widely used method have been described:-

Periodic acid Schiff (PAS) stain- The dyes used in this technique involve 1%periodic acid and Schiff's reagent. The substance which shows positive result to PAS has purple magenta color and nuclei are seen blue. The substances which show positive PAS are

glycogen, neutral mucoprotein, glycoprotein, all fungi, cerebrosides, basement membrane, and phosphorylated sugar.[3]

This stain is used in the diagnosis of several medical conditions like mucins in adenocarcinoma of the large intestine, demonstration of fungi, glycogen storage disorder, Ewing's sarcoma, rhabdomyosarcoma, Whipple's disease, etc.

Alcian Blue Method: The reagents used are Alcian blue, aluminum sulfate, and nuclear fast red.

The results of this method show the blue color appearance of Hyaluronic acid, sialomucin, proteoglycans, and the nucleus is red. Neutral mucins are not reactive to Alcian blue.[4]

Combined Alcian blue-PAS method: The principle of this method is to differentiate the acid mucin from neutral mucin and demonstrate the presence of mucin. In this method only neutral mucin will be stained in magenta color appearance.

Mucicarmine Technique: The positively charged carmine and Mucicarmine bonds with negatively charged acid mucin in result acidic mucin stain strongly whereas neutral mucin does not stain. The reagents involved carmine, aluminum hydroxide, and 50% alcohol and aluminum chloride with Mayer's Hematoxylin. It is used in GIT disorders and to identify the acidic mucin also to identify fungi-Cryptococcus neoformans, etc.[5]

Nucleic Acids Staining: There are 2 nucleic acids which are DNA which is present in the nucleus & RNA that is present in the cytoplasm. They consist of deoxyribose or ribose, phosphate, and a nitrogenous base. The demonstration of nucleic acids depends upon the following factors which include the production of the aldehydes from sugar and reaction of dyes with the phosphate groups. There is no histochemical method to demonstrate the nitrogenous base. Several mostly used techniques to demonstrate the DNA includes-

- **Feulgen Stain:** In this staining procedure the solutions used are 1ml HCL acid, Schiff's reagent in which aldehydes are demonstrated, and bisulfate solution. The result of this staining gives Red-purple color to DNA & Green color to the cytoplasm. The standard control method for the Feulgen technique is the NAH method. (Naphthoic acid hydrazide). The blue thionin Feulgen technique is mostly used in the research for studying the nuclear morphology and ploidy of cancer cells. NOTE: The RNA can't be demonstrated by this stain as ribose purine bond is unaffected by hydrolysis 1M HCL.6,7
- **Methyl Green pyronin Method:** In this staining the reagents used is Methyl green in which the pure methyl green is specified for DNA and the impure dye contains methyl violet which can be removed by washing with chloroform. The NH2 of the dye reacts with the phosphate of DNA. The other reagent which is used is pyronin that binds to negatively charged constituent of tissue and also binds with mucins and cartilage, apart from RNA. The results show Green –Blue color to DNA whereas RNA shows Red color.

Staining of Lipids-3 forms of lipids is simple, compound, and derivative which includes such as fats, fatty acids, or cholesterol. The most widely used method for staining of lipids are Sudan black B stain and oil red o stain.[8]

Sudan Black B Method: This method is used for staining the lipoprotein,neutral triglycerides and it is very useful in hematological disorder. The result which it gives is fat is seen blue or black color and nuclei is seen red color.

Stains For Amyloid: Amyloid is an extracellular, amorphous, and eosinophilic material. It is composed of a protein in an anti-parallel beta-pleated sheet of configuration. Many times Hematoxylin and eosin is been confused with hyaline and fibrinoid substances. Earlier the stain which was used for amyloid was Iodine which was introduced by Virchow. The staining method widely used for amyloid is Congo red stain and Methyl crystal violet method.[9,10]

Congo red Stain: The mechanism principle is that the amyloidis extracellular, eosinophilic, and homogenous which can damage the surrounding tissue,hence when it is stained with Congo red the amyloid shows apple green color i.e. birefringence. The reagent includes 0.5%Congo red and 0.2% potassium hydroxide in 50% & 80% alcohol. It is used in renal amyloidosis, medullary carcinoma of thyroid, Alzheimer's disease, etc. The result gives nuclei blue color and amyloid, eosinophilic granules show red to pink in color.

Methyl/Crystal violet Method: The reagents used are 95% alcohol, crystal, 0.2% acetic acid, and 1% aqueous ammonium oxalate. The procedure includes

first the solution is prepared by using 2-gram crystal or methyl violet, alcohol, and 1% aqueous ammonium oxalate. After that the section is brought to water level then stain in crystal solution for five minutes after that washing and differentiating is done in 0.2% acetic acid and at last washes and mount in DPX. The result shows purple color is seen in amyloid whereas blue is seen in the background.[11,12]

Stains For Microorganisms: There are different types of staining method for different types of microorganisms which include bacteria, spirochetes, fungi. Different staining process includes Ziehl Nielsen stain, wade file Faraco stain, Warthin starry stain, Giemsa stain, and Gram staining. Among these widely used staining process is been described.

ZN stain process- In this method mycobacterium bacillus is stained. Mycobacteria cannot be demonstrated by gram's staining process as they possess a capsule that contains long-chain fatty acid i.e. Mycolic acid which makes it hydrophobic and it can be stained by carbol fuchsin. The Mycobacteria shows PAS-positive as their cell wall contains carbohydrates. The result of ZN stains shows red color to mycobacterium and background appears pale blue.[12]

Gram staining of bacteria- Includes:

Modified Brown- Brenn Method: This method includes the reagents like crystal violet stain, ethyl alcohol, gram's iodine solution, acetone-xylene solution, basic fuchsine, and picric acid, 0.1% in acetone. The result of this staining process gives Gram +ve bacteria shows blue color whereas Gram –ve bacteria shows red and the nuclei are also shows red color and yellow color presents other tissue elements.

Staining for spirochetes Warthin starry method is mostly used for spirochetes.

Warthin starry method: The reagents involved acetate buffer pH- 3.6 & 1% silver nitrate. This staining process gives the result as spirochetes-Black & Background – golden- yellow.[13]

Fungal stains Mostly used technique is GMS (Gomori methenamine silver nitrate): The reagents involves are 4%chromic acid, 1% sodium bisulfate, 5% sodium thiosulfate and 0.21% silver nitrate and light green solution and the results gives black color appearance of pneumocystis and fungi. The melanin also appears black. The dark grey appearance shows the presence of mucin and glycogen whereas pale green color appears in the background. [14]

The Giemsa stain is used for parasites:

Connective Tissue Stains: The connective tissues provide a matrix that connects and binds the cells and organs, which gives support to the body. Connective tissue consists of a proper one which includes loose and dense connective tissue like adipose, irregular, etc. The other one is the specialized connective tissue which includes bone, blood, and cartilage.

The Stains used for collagen fibers includes many techniques in which Masson's trichrome technique is mostly used.[15]

Masson's Trichrome Technique: The reagents used in this technique are Weigert's iron Hematoxylin, acid fuchsin, glacial acetic acid, methyl blue, and phosphomolybdic acid. The fixatives which are recommended are bruin's, Zenke's formal mercury, and zinc formalin.

The technique demonstrates the collagen and muscle in normal tissue, identifying the increase in collagenous tissue, to differentiate between collagen and muscle in tumors. It can indicate the fibrotic change in cirrhosis of the liver and also fibrotic changes in pyelonephritis. It gives blue or black color to nuclei, RBC, and muscle shows red color, and the cytoplasm appears in yellow.

Stains for pigments and minerals: There are different pigments including endogenous, exogenous, and artifact pigments. Different staining process includes:

Perl's Stain: This stain helps in identifying the ferric ions. When the ferric ions come in contact with ferrocyanide it gives Prussian blue which is a blue pigment. The result showsthe blue color of iron and nuclei appears in red whereas the background is pink in color.

Modified Fouchet's Technique-It is used for bile pigments. The reagents involved are Fouchet's reagent which includes 25% aq trichloracetic acid, 60% aq ferric chloride, distilled water, and Sirius red stain. The procedures follow to bring the sections to distilled water and oxidized in Fouchet's for five minutes then rinse well in distilled water and stain with citrus red for at least 30 minutes then dehydrate, clear and mount. The result

gives green color appearance to bile pigments, collagen shows red and background is yellow in this respective staining process.[16]

There are several staining tests for the demonstration of melanin which include Schmorl's ferrioferricyanide reduction test, enzyme method, IHC, and Masson Fontana silver method & Fluorescent method.

Masson Fontana Method: It is used for the identification of melanin and also argentaffin granules. In the diagnosis of malignant melanoma is also used. The principle of this process is as the melanin is insoluble in organic solvents and it is a non-lipid, non-hematogenous pigment that dissolves in 1M sodium hydroxide. The ammoniac silver nitrate solutions are reduced to black metallic silver by melanin and the result shows the black color appearance of melanin, and argentaffin granules whereas nuclei appear red.[17]

A note on IHC: The immunohistochemistry technique is the most useful and widely used staining process as it is used in the diagnosis of cancer or treatment of cancer, for differential diagnosis and has its application use in the research works. The method combines the immunological, histochemical and histological process foridentifying the specific tissue by using the definite antigen/antibody reaction which is a tag by an evident label. This process allows visualizing the specific cellular components which is been distributed and localized within a cell or tissues. The interaction of antigen-antibody is visualized by different types of markers like an enzyme, radioactive element, Fluorescent dye, or colloidal gold. The antibody is made up of two types of protein chain which includes heavy chain and light chain. Five types of immunoglobulin classes are found in serum which include IgG, IgM, IgE, IgD, & IgA. They all are distinguishing based onthe Heavy chain they contain. However every technique has its advantages and disadvantages & its disadvantages include such as the cost of the technique is high and it is not permanent, etc.[18]

Conclusion

Special stains are widely used in the different processes for the enhanced appreciation or detection of normal and abnormal substances, though the H & E stain is a routine test for histopathological diagnosis. The special stains give a more accurate result for the proper diagnosing of the respective disease and also it enhances the beneficiate in the research works. The pathologist and researchers should have a broad and clear cut idea regarding the special staining process for better outcomes. The use of special stains has revolutionized medical diagnosis in the Histopathological field and their application will surely lead the future.

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Single Anterior Crossbite Correction in Mixed Dentition Using Z'spring Along with Posterior Bite Plane: A Case Report

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Abstract

Anterior cross bite is defined as "palatal positioning of the maxillary anterior teeth relative to the mandibular anterior teeth".Various treatment options available are removable & fixed appliances. This is a case report on treating a case of single anterior tooth crossbite during the mixed dentition using the "Hawley's appliance with posterior bite plane & Z'spring". The active treatment time was 3 months in total & the treatment outcomes were followed up consequently for the next 4 months successfully.

Keywords: *Anterior Tooth, Dentoalveolar Crossbite, Mixed Dentition, Malocclusion.*

Introduction

Crossbite is well defined as, "abnormal relationships of a tooth or teeth to the opposing teeth, in which normal buccolingual/labiolingual relationships are reversed".[1] Crossbites may be of the dental origin or the skeletal origin involving anterior teeth, or posterior teeth or both.[2] Anterior crossbite is defined as,"a malocclusion in which one or more of the maxillary anterior teeth occlude lingually to the mandibular incisors". Anteriorly located single tooth crossbite of dental origin is the commonly come across malocclusion in the course of the development of occlusion in kids.[3,4] In the case of dental crossbite the patient shows the deviations in the axial inclination of the involved tooth or teeth but with normal existing skeletal form. The management of anterior crossbite should be considered during deciduous & early mixed dentition irrespective of its dentoalveolar or skeletal basis.[5] Anterior dental crossbite generally becomes obvious during the early developing dentition. The anterior crossbite may result from various reasons like lingual erupting maxillary anterior incisors; cleft lip[6]; supernumerary teeth anteriorly; an over-retained deciduous tooth/root; crowding[7,8]; inadequacy in arch length; upper lip biting habit.[9-12] This developing abnormality necessitates immediate treatment to avoid anterior teeth fracture & mobility, periodontal inflammation & TMJ problem.[12-17] There are many method to treat the developing or developed anterior dental crossbite. The treatment method cited for the developing or developed anterior dental crossbite are such as using tongue blade therapy, lower inclined plane, crowns (either stainless steel or composite build-up), Hawley's retainer with double cantilever springs, labial and lingual archwires. Hence, a variety of approaches can be used to intercept anterior crossbite during the developing dentition period. This case report presents a simple & economical method to treat anterior dental crossbite using Hawley's appliance with posterior bite plane & double cantilever spring.

Case Report: An 11-year-old female patient came to the "Department of Pediatric and Preventive Dentistry", Institute of Dental Sciences, Bhubaneswar with the chief complaint of abnormal positioning of one upper front tooth with corresponding lower tooth. There was no relevant past medical & dental history found. Extraoral examination revealed a slightly convex profile

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with potentially competent lips. On clinical examination it was found that 21 was in crossbite with 31 slightly proclined 11 & an angle's class I molar relation bilaterally. After careful intraoral examination, it was decided to treat the case with "Hawley's appliance with posterior bite plane to achieve a 2 mm incisal clearance and double cantilever spring (23 gauge wire) to push the palatally placed upper incisor labially".

The Treatment Objectives: To achieve the normal overbite & overjet, by aligning the inclination of the anterior tooth which further, improves the patient's facial & dental profile. The success & prognosis of this treatment is immensely dependent on children's cooperation & parental guidance.

Procedure: Immediate treatment: This includes educating patients & parents about the use of appliance planned to be given for the crossbite rectification. It also included thorough oral prophylaxis.

Definitive Treatment: Alginate impression was made for both the arches and immediately poured with dental stone. Hawley's appliance with posterior bite plane and double cantilever springto 21 was made for rectifying the crossbite. This appliance is known to deliver slow-lightcontinuous force.

The removable appliance was inserted in the patient's mouth and the patient was trained to insert & take out the appliance on her own under parental guidance. The patient was recalled after 24 hours to check the fitting of the appliance (Figure 1, 2). She was recalled after every week for the activation of double cantilever spring which was accomplished by opening each of two helices 2mm. She was instructed to wear it full time except during eating and the night while sleeping. In the 6th week, crossbite was found to be corrected with 21(Figure 3). After 2 months, the posterior bite plane was removed & Hawley's appliance with z spring sustained for another 2months to establish a normal overjet. Later exfoliating 53, 63 were extracted as a balancing extraction simultaneously to give an adequate space for the crossbite treatment to avoid anterior crowding and provide adequate space for anterior incisors alignment. At the end of the first 3 months of active treatment, the crossbite was successfully corrected. The use of Hawley's appliance was continued to achieve adequate overjet and overbite for another 2 months. The treatment outcome was followed up for further next two months.



Figure 1. Preoperative frontal view showing crossbite to maxillary left central incisor.



Figure 2. Intra-oral view with removable appliance.



Figure 3. Post-operative frontal view.

Discussion

Interceptive Approach: It incorporates all method initiated to decrease the complexity of malocclusion to progress normal occlusion in the future. The aim of using removable or fixed appliance primarily for the prevention purpose is to attain:

1. Good alignment of the permanent dentition.
2. To achieve harmony between dentition and aesthetic.
3. Stability between dental, muscular & skeletal components.
4. Dental arches with anoptimal intercuspation,

Interceptive procedures should be widely used in mixed dentition period to correct the developing abnormalities. Anterior crossbiterarely corrects by itself due to locking of the upper incisor behind the lower incisor & later it progresses to severe malalignment of teeth, therefore consideration of primary approach can re-establish the right muscle balance & a well-organized occlusal development.[18,19,20] Appliances used for rectification of 'anterior' crossbite are: "tongue blade, inclined plane, composite plane, reverse stainless steel crown, Hawley's appliance with 'z' spring and screws embedded in acrylic".[5]

Tongue blade therapy: It is suggested for a single tooth crossbitecase provided that incisors are still erupting stage. It is positioned at an angle between the teeth. After which the patient is directed to bite tightly for five seconds. This has to be redone twenty-five times in a day. In case after two days no changes observed then it is stopped. The main drawback is that it demands patient full co-operation.

Inclined plane: It is recommended when multiple teeth are to be treated. It is formed of acrylic & causes a 'forward sliding motion' on closure of upper teeth. Its usage is limited to four weeks because it may result in the supra-eruption of the posteriorly located teeth, opening the bite anteriorly.

Composite Plane: An inclined plane is made up of composite on the lower incisors.

Reverse stainless steel crown: It is also used in the case of a "single tooth" crossbite. This crown is used for the upper incisor which forces the upper tooth away that is towards the lip as the child bites down on the lower teeth. It takes two to four weeks for the correction.

Maxillary appliances with 'z' springs & posterior bite plane: It can be a choice when multiple teeth are in crossbite. The posterior bite plane used, put the anteriorly located teeth out of occlusion, allowing the incisors to escape the bite to provide incisal clearance and the 'z'spring used for the labialization of tooth.

Screws embedded in acrylic: Medium, mini or micro screws can be used that are activated daily to bring about correction of crossbite.

In the above case report the economical & acceptable method of choice for the crossbite correction was using a removable appliance with a posterior bite plane for incisor clearance & z spring for labialization was used. The removable orthodontic appliance used has three major advantages:[21,5] i) Reducing chair time; ii) They can be removed easily & iii) They are easy to clean thus, keeps in maintaining good oral hygiene.

Prakash Pet al in their study in 2011 reported that 8 to 11 years is an ideal time for anterior dental crossbite correction since it's the duration of root formation & the active stage of eruption of the tooth. Hence, a child's age plays an important role. But the motivation for treatment, how he or she co-operates during treatment is also being very important.[4]

Conclusion

It is essential to realize the need for early diagnosis and rectification.[22] During the early correction of simple and minor malocclusions, which is a part of interceptive orthodontic treatment, the anterior cross-bite was corrected more rapidly & it is achieved by removable appliances or fixed appliances. The primary treatment in such cases just not only restores aesthetics anteriorly but may also lessen the complexity & course of successive required treatment.[20] Hence, it may help to prevent the prospect of any adverse effects upon the growth and development of the child.[23]

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Laser-assisted Operculectomy in an 8-Year-old Child Using Diode Laser: A Case Report

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Abstract

The past years in dentistry has seen the growth of the use of laser advance by leaps and bounds. Though soft-tissue laser was originally presented, but with the development of new-generations of laser technology, lasers are equally effective on hard tissues. Lasers have a plethora of uses in pediatric dentistry including prevention of caries, prompt diagnosis, restoration of cavities, managing cases of trauma to teeth along with various minor oral surgical procedures. Lasers are advantageous for being more rapid, more precise, sterile and providing a bloodless field of surgery and are also better accepted by pediatric patients and their parents. In the same light, treatment of a pediatric patient becomes easier it is less fear-inducing for the child thus increasing co-operative behavior. The soft tissue covering over a partially erupted tooth is known as pericoronal flap or gingival operculum. Surgical removal of the latter is termed as Operculectomy. The following text reports a case of Operculectomy performed using a diode laser in the pediatric operatory.

Keywords: *Laser, Operculectomy, Pediatric dentistry.*

Introduction

LASER is an acronym that expands as 'light amplification by stimulated emission of radiation'. Gordon Gould is credited with its first introduction in 1959 a graduate student at Columbia University.[1] Theodore Maiman at Hughes Research Laboratories created the first working laser in 1960.[2] Application of lasers in dentistry varies widely among both soft and hard tissues. Soft tissue applications may include procedures such as a frenectomy, exposure of unerupted teeth, disinfection of root canals among others. Hard tissue applications involve the detection and removal of caries and removal of old restorations.[3] Pediatric Dentistry involves close interaction with children is thus dissimilar from dentistry in adults. It is thus indispensable for the dentist to be thoroughly acquainted with the skills required in line with the child's age and level of development. The employment of various categories of laser devices facilitates the pediatric dentist to deliver dental care in a minimally invasive way with negligible distress, reduced bleeding, while also being painless both during and post-treatment. Pedodontists aim to fashion a pleasurable experience for the child during their dental visits by inculcating innovative, minimally invasive machinery to instill a positive dental attitude within the child.[4] An operculum may be defined as a flap originating from the gingival tissue usually present distally to a molar or covering the occlusal surface of the molar frequently presenting as a sequela of tooth eruption. In a few cases, an operculum may cause pain due to the masticatory forces from the opposite molar teeth in which case operculum may become ulcerated and inflamed either due to repeated trauma or due to local inflammatory reaction due to accumulation of food debris and plaque between the surface of the tooth and the opperculum.[5]

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Case Report: An 8-year-old female patient reported to the Department of Paediatric and Preventive Dentistry at the Institute of Dental Sciences, Bhubaneswar with a chief complaint of pain on chewing food in her upper right back teeth region since the last 6 months. Patient-reported a history of dull aching pain along with food lodgement in the region of the chief complaint. Clinically the patient showed good general health with the absence of any relevant medical history.

The child displayed a Negative (-) behavior according to the Frankl behavior rating scale given by Frankl et al. in 1962. Intraoral examination revealed the presence of an operculum covering the occlusal surface of the upper right first permanent molar (Figure 1). The operculum was pinkish-brown in colour, firm, resilient and remained tightly adhered to the gingival tissue distal of the upper first molar tooth. No abnormalities were recorded on the radiographic examination of the affected tooth. The results of routine blood investigations were satisfactory.

Keeping into consideration the uncooperative and apprehensive behaviour of the child a treatment planning of surgical excision of the operculum with diode laser was made to reduce both treatment time and patient discomfort.Oral prophylactic procedures were carried outbefore the planned excision. Infiltration anesthesia was given in the region of the upper right first molar tooth to provide anesthesia to the surrounding soft tissue including the operculum tissue. 2% lidocaine with epinephrine [1:2,00,000] was used for the same. Appropriate eyewear was worn by the pediatric dentist, the child and also the dental assistant before the initiation of the surgical procedure (Figure 2). Excision of the operculum was started from the distal margin of attachment using a 940nm diode laser [iLASE^{TM} diode laser from BIOLASE Inc.] in continuous contact mode at 1.8 W of power rating in the presence of a high volume evacuation. The tip of the laser was cleaned regularly during the procedure to remove the adhered scorched tissues. A conscious effort was made to avoid contact between the enamel surface and the laser tip. Satisfactory hemostasis was obtained postoperatively (Figure 3). The surgical site was irrigated with saline and povidone-iodine to remove any unwanted or remaining debris. The total time required for completion of the procedure was below ten minutes and the patient tolerated the procedure well with minimal pain or discomfort. The patient was advised to use a topical anesthetic gel at the site of excision and was counseled on the maintenance of appropriate oral hygiene. A post-operative follow-up after 7 days revealed adequate and uneventful healing (Figure 4).



Figure 1 Pre-operative view of operculum about right upper first molar tooth



Figure 2 Protective eyewear in place during the procedure



Figure 3: Immediate post-operatively



Figure 4: 7 days post-operatively

Discussion

Operculectomy may be referred to as the surgical technique of eliminating the operculum, or the gingival flap that usually covers the occlusal surface of the tooth partially. Operculectomy is aimed to reduce the incidence of pericoronitis, a condition that is marked by pain and inflammation associated with the operculum. Pericoronitis is usually seen among young adults, mainly in the molar teeth that are beginning to erupt.[6] The patient in our case presented with pain and discomfort in the region of upper right first permanent molar due to the presence of an operculum associated with the tooth. Plaque build-up on the underside of the operculum along with the accumulation of food debris caused inflammation and enlargement of the gingiva. The latter was being easily traumatized at the time of mastication, subsequently causing pain and discomfort.

Operculectomy with diode laser was the chosen treatment plan as it presented with numerous advantages over the conventional scalpel surgical procedure.Its profits in soft tissue surgeries include enhanced accuracy, easily achieved hemostasis, less necrosis of tissue from heat in comparison to electrosurgery, the accelerated rate of wound healing, sutureless surgery, decreased post-operative pain, and discomfort with reduced need for analgesics. Most procedures using lasers may be operated with topical local anesthesia alone that results in enhanced patient co-operation along with abridged procedural time that is especially of significance in the pediatric scenario. Additionally, the bactericidal effect of lasers on the surgical site means reduced use of antibiotics postoperatively.[7]

Conclusion

Operculectomy is a minor surgical procedure aimed to eliminate the operculum or the gingival tissue flap present over a fully or partially erupted tooth, usually in molars that if left unchecked may lead to pericoronitis and symptoms associated with the latter. Operculectomy using soft tissue lasers may be considered as a more favorable option in pediatric dentistry in comparison to conventional method where patient co-operation and procedural time must be accounted for.

Ethical Permission: Not required

Conflict of Interests: None

Funding: None

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Autism: Where the Little Things are Never Little

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Abstract

The aim of this write up is to furnish nearly all elemental aspects on varied behavioral patterns along with different management strategies which may lead to an improvement in treatment planning and management by the dental practitioners, and comprehensive oral health care of the children suffering from Autism. Along with it, this article focuses on raising concerns among primary health care providers and parents of those children regarding there un-mate dental needs.

Keywords: *Autism, behavioral pattern, health care providers.*

Introduction

Autism, where the little things are never little and every milestone is a celebration. It is mostly spectrum associated with multiple disorders of neurodevelopmental origin called Autism spectrum disorders which comprises a class of neurodevelopmental dysfunctions with a set of determining measures that comprise impede social associations, communication, and restricted or monotonous behavioral standardization.[1] It comprises of 5 subtypes, which consists of Autism disorder (AD), Asperger's syndrome, childhood disintegrative disorder (CDD) and Pervasive developmental disorder not otherwise specified (PDD-NOS).[2]

The symptoms of Autism starts by 3 years of age and normally experiences a stable route without recovery as the age increases. Common features in an autistic child can be as follows: [1]

(a) Discernable impairment in the use of multiple non-verbal communications,

(b) Lacks in developing social connections and sharing incidents and fascinations,

(c) Retarded or completely lacking linguistic development,

(d) Existing with potential sensory disabilities, mental retardation or epilepsy.

Those children may display excessive anxiety while facing the dental setup, and can create problems for the operator for providing the normal pattern of dental care[3]. Therefore it has become very important to discover various method so that the practitioner can cater to individual needs to decrease their suffering and anguish and create a peaceful and pragmatic experience for the child and his/her family. Those strand points of care will be of prime importance in this article.[4]

Epidemiology: According to various reports incidence of autistic disorder extends from about 5 per 10,000 to 20 per 10,000. The IQ score vary among man and women that is 2:1 in the children who are handicapped severely and 4:1 in moderately handicapped children. Boys are 5 times more affected than girls. The occurrence in siblings is suspected to be from 3% -7% presenting 50-100th fold increase in risk. [2,5]

Autistic disorder vs autism spectrum disorder:

- **Autistic Disorder:** Early infantile autism or childhood autism are 2 different names of this spectrum of disorders. Those children have a

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moderate to severe range of communication, socialization and behavioral problems. Many children with autism also have mental retardation.[5]

- **Autism Spectrum Disorder:** Spectrum is a term that represents a class of disorders with symptoms that are seen on a continuation which can be mild to severe in expression. It can be any of the disorders included in pervasive developmental disorder that shares undistinguishable behavioral patterns that constitute social interaction, impaired communication, and limited schemes and interests. As each individual shows a different set of combination of those behaviors so it is considered as a spectrum of disorder.2,5

Oral Manifestations: Dental manifestation related to autism can be of various origins, stating from developmental to caries related or can be due to traumatic injuries. There can be developmental disorders such as anomalies of tooth morphology, macroglossia and drooling of oral fluids. There can be delayed tooth eruption due to drug-induced hyperplasia. A higher incidence of caries was reported in the primary dentition. However, some studies also suggest lower rates of caries among children suffering from ASD. Due to the behavioral challenges and lack of awareness and training of the caregiver/parents, leads to poor periodontal health among those children. Most of the children suffering from ASD have high arched palate which forms the major cause of malocclusion. Children with autism have a higher prevalence of bruxism, tongue thrusting, snoring, nail-biting, non-nutritive chewing as well as they are more prone to traumatic injuries. Certain cases of hyper gag reflex and xerostomia has also been reported.[6]

General Awareness:

Awareness among Health care providers: The major issue on which most of the health practitioners accord is the advice for early oral examination of those children.[7] Children diagnosed with ASD, first reach to a Health care professionals before visiting any dentist. Therefore it becomes a responsivity for those health care professionals to advise the parents of those children for regular oral examinations. According to a study conducted by Murshid, the medical and educational issues are always given an upper hand by the health care professionals and mostly they always neglected the dental aspect. Nearly half of the study population did not focus on the importance of oral hygiene, and did not advise tooth brushing or mouth rinsing. They did never make any efforts to refer the children to any dentist. According to most of the health care providers, the parents should be the one held responsible to cater for the oral hygiene needs of the children, no one ever thought about the teachers being involved with tooth brushing practice of the child which he is not being able to do properly even if the child spends most of the time of a day with them in the health care center.[8] Lewis et al[9] and Dela Cruz et al[10] described similar findings among health care professionals.

Awareness among dentists: Mostly, dental education is very much important for the professional functioning of the practitioners while doing treatment for children with autism. According to reports the treatment for autistic children is mostly refused by more than 60% of dentists[11] Special clinic setups, preconditioned classes are still not considered by many dental curriculum[12]. The discrepancies in undergraduate educational programs may be compensated by newly introduced undergraduate educational programs, as it has been specified that autism interventions has been increased among health professionals by using Internet-delivered programs.[1]

Parental Awareness: According to a survey among parents, the autistic child's approach towards oral examinations, expenses, and no dental coverage by any insurance company being recognized as the major problem for providing oral health care to those children with ASD[13]. Resistance to dental treatment, complications associated with the medical condition, and difficulties in locating a dental practitioner abled to provide care have also been reported by guardians of children with autism[14]. Adding to the above-mentioned problems is the limited availability of specialized dentists who are trained to serve those children adds further complications in delivering proper treatment for those children.

Most importantly the parents should be trained to deal with various stress-inducing variables. The health care provider or any dentist to whom the parents approach should have a responsibility to guide the parents to certified professionals. Hence, access to proper health care services will provide participation of parents in decision making for treatment and fortify their reliance in handling the attitude of their child.[1]

Dental Management: One of the associated

problems in the case of an autistic child is a failure to develop joint attention that means impairment in the supposition of stimulus uptake, which results in deviant behavior to various signals. Due to the many-fold symptoms of autistic disorder dentists needs to ascertain their treatment protocol according to a distinctive presentation of various characteristics in each child patient.

Behavior management strategy: A pre-clinical evaluation and behavioral preparation is most in case of autist children as no autistic child is same as the other, pre-clinical evaluation includes Personal details, medications, previous experiences and in pre-clinical behavioral preparation dentist must have a proper idea about verbal/non-verbal, interests, signs of behavioral change, parental inputs, rewards, etc. Depending on the behavioral pattern the dentists should set the stage according to the child's requirement there should be proper teaching tools, visual pedagogy, social learning stories and if possible then use the same staff and dental environment for all the appointments. The child should be given clear instruction one at a time, Short appointments with frequent breaks to minimize distractions. Behavior management strategies like tell shoe do and positive reinforcement can be applied depending upon the child.[6]

If at all, all the strategies fail then the child is needed to be taken under sedation or General anesthetic procedures.

Dental treatment under General Anesthesia:

Guidelines for treatment under General Anesthesia: (Solent NHS. Internal Guidelines for Extraction of Teeth under General Anesthesia, 2015)

- Major goal is to complete all the dental treatments at once to avoid recapitulation of general anesthesia procedures in the future.
- When the patient is decided to be taken under GA, then not only the current treatment needs are to be catered but all the necessary treatments should be completed.
- All the teeth that have a poor or questionable prognosis should be extracted and all the needed restorative care should be completed in one sort.

Considerations for pediatric general anesthesia for dental treatment (Royal College of Surgeons guidance, modified)

- The attitude, co-operation and perceived anxiety of child
- The procedural complexity.
- Medical status: ASA I and II, mostly of ASA III
- Age – minimum of 2years
- Weight - more than 10 kg.
- Treatment must be carried out under hospitalization.
- Time and expenses
- Carious extent.

Preliminary considerations :

Preliminary considerations that must be carefully considered before general anesthesia procedure like, BMI: More than 40, severe bariatric patient. Chronic severe respiratory condition, previous adverse reaction to anesthetics, Profopol cross-reactivity, sickle cell condition,poly addiction to drugs, cardiac dysfunction. Anti-epileptic drugs, cardiac drugs, bronchodilators, anti-hypertensive drugs should be continued without alteration. Anxiolytics and tranquilizers should be carefully monitored as they can interact with pre-anesthetic drugs. In case the patient is under diabetic or corticosteroid therapy then the patient's physician must be consulted before starting up with the procedure.[15] Pre-anesthetic clearance by an anesthesiologist is the must before taking any patient under general anesthesia

Pre-anesthetics:

Ketamine medication or combination of midazolam along with ketamine is usually considered to be effective.[16]

Procedural Management: Definitive source of oxygen, suction, resuscitation equipment and emergency drugs, monitoring equipment such as non-invasive blood pressure monitor, pulse oximeter, electrocardiography, stethoscope, and capnography must be available. In case of any life-threatening emergency, there needs to be immediate availability of advanced airway management tools, defibrillators. An anaesthesiologist need to be there while the procedure is being performed till the discharge of patient.[17]

Possible Complications: Approximately 20% of complications under general anesthesia are, airway obstruction, nausea and vomiting[18]. Other complications such as non-fatal ventricular arrhythmia, hypotension or

rise in blood pressure, laryngospasm, airway problem which can result in oxygen desaturation.[19]

Conclusion

In order to treat the children with ASD there is a requirement of in-depth knowledge about the autistic behavioral profile. As explained before every child is different and so are his/her requirements and basing on these behavioral profiles therapeutic approach should be individually designed for each child. There is an absolute need for continual education among dental and health care professionals, so, that they could address and cater to those children with specific and unique health care requirements.

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Ethical Permission: Approved

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Root Coverage Procedures: A Case Series

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Abstract

Gingival recession can be defined as the progressive loss of gingival soft tissues. Most of the recession cases can lead to root exposure, interdental bone loss, mobility of teeth. The defect can involve one or multiple teeth. Eventually it progresses towards the root hypersensitivity and tooth loss due to mobility. Recession can be treated via using different treatment modalities, by using gingival grafting and regeneration. Grafting procedure entails to harvesting the tissue from donor sites and suturing it back to the area of recession for root coverage. For regeneration purposes, various biomaterials are used with improved properties. Techniques involved in gingival grafts and regeneration involves free gingival grafts, coronally advanced flaps, guided tissue regeneration, etc. This case series reports the effectiveness of various techniques for vestibular deepening along with root coverage, removing the frenum pool along with increasing the width of the attached gingiva, etc.

Keywords: *Gingival recession, Bridge flap, subepithelial connective tissue graft, Coronally Advanced Flap, Attached gingiva, Mucogingival surgery*

Introduction

The chief purpose of periodontal care is to prolong the aesthetics and function of normal healthy gingival tissues. The treatment techniques for maintaining healthy gingival tissues also involves the treatment of gingival recession and its complication. Gingival recession can be defined as the displacement of gingival soft tissues apical to the CEJ. This will finally lead to alveolar bone loss and dehiscence. GR is mostly seen in older people but can also be seen in young patients because of several reasons like frenum pull and insufficient width of AG.[1]

The exposure of roots due to soft tissues and bone loss can progressively lead to several problems such as hypersensitivity, discomfort in the soft tissues, root caries, various aesthetic problems and problems regarding oral hygiene maintenance and adequate plaque control etc.

The current evidences showed various techniques with improved predictable success rate for treating Miller Class I and II single-tooth defects. Difficulties arise when there are case scenarios with Miller Class III and IV defects involving one or multiple teeth. Evidences gathered from various RCTs and systematic reviews showed the high success rate of subepithelial connective tissue graft (SCTG) for treating maxillary recession defects present labially or buccally.[3] Nevertheless, there are insufficient evidences and data available for the treatment of palatal and lingual recession sites. Therefore, this case series presents different techniques to treat the mucogingival problems.

Case Reports:

Case 1: A female patient of forty-two years of age visited inthe Institute Of Dental Sciences, Sum Hospital

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with a chief complaint of lowering of gums in the lower front tooth region. Clinical features revealed shallow vestibule and insufficient width of attached gingiva.

The recession was Grade I, according to Miller's classification[5],in 31, 41, 42 teeth (Figure 1a). The recession was similar (4 mm) in all lower anterior and the width of the attached gingival was approximately 2 mm (Figure 1b). IOPAR shows inadequate bone support present I .r.t to 31, 41, 42. UNC-15 probe is used for the measurement of clinical parameters which includes Probing Pocket Depth, Gingival Recession, width of attached gingiva,and Clinical Attachment Level. The patient was clarified about the surgical procedure and an informed consent was signed beforehand. The patient is not medically compromised. Phase 1 therapy was done 2 weeks before the procedure.

Surgical Technique: After injecting local anesthesia (with epinephrine), an incision was given in the vestibule from 31 to 42 (Figure 1c) which also includes the periosteum. The incision was made till the bone which will induced scar formation. After the sulcular incision, a full-thickness flap was elevated which connects the whole flap with the first incision so that the whole bridgeflap can be raised and coronally advanced to cover the recession from 31, 32 and 42 (Figure 1d). There was no need for a vertical incision. This forms a bridge from the coronal aspect to the vestibule (Figure 1e). Afterward, tetracycline HCl as a root conditioning agent is applied[4] which is further proceeded by the placement of platelet-rich fibrin membrane (Figure 1f). Then, the flapwas coronally advanced by the interrupted sling suture (Figure 1g). Resorbable sutures were used in this case. After the suture, the periodontal pack is (Figure 1h). After giving all the postoperative instructions in both verbal and written format and also required medications for improved postoperative healing and to avoid complications, the patient was discharged from the clinic. The patient was recalled after 14 days for suture removal and also to check for the healing of the surgical site. No such postoperative complications were seen with satisfactory healing was satisfactory with a significant gain in attached gingiva and clinical attachment was 5 mm (Figure 1i). Postoperative recession at a 1-month follow-up was about 1 mm. The patient was further instructed to use a softbristle toothbrush in the treated area. The patient was also instructed to maintain good oral hygiene in the surgical sites. Even after 9 months follow up, there was no such recurrence of recession.



Figure 1a. Pre-operative view showing Millers Class I gingival recession (labial aspect) in the mandibular anterior region. Figure 1b. Pre-operative view showing gingival recession (clinical attachment level); Figure 1c. Sulcular incision given; Figure 1d. Incision given in the mucogingival junction.



Figure 1e. Full-thickness flap (Bridge Flap); Figure 1f. Platelet-rich fibrin placed; Figure 1g. Coronal repositioned flap and sutured; Figure 1h. Coe pack placed; Figure 1i. 3 weeks Post-operative.

Case 2: A 31-year male, having a chief complaint of dentinal hypersensitivity in relation to 23 and 24, reported to the Department Of Periodontics And Oral Implantology, Institute Of Dental Sciences, Sum Hospital, Bhubaneswar.On examination, there was Miller's Class I gingival

recession[5] was present in relation to 23 and 24 (Figure 2a). Intraoral periapical radiograph in relation to 23 and 24 showed a sufficient presence of interdental bone. Clinical parameters included ProbingPocket Depth, Gingival Recession, width of attached gingiva,and Clinical Attachment Level. The briefing of the surgical procedure was done to the patient and an informed consent was signed beforehand. The patient is not medically compromised.Phase 1 therapy was done 2 weeks before the procedure.

Surgical Technique: After administering local anesthesia inthe recipient site, an intrasulcular incision was given from the distal aspect of 22 to the mesial aspect of 24 aspect. After that, full-thickness (mucoperiosteal flap) (Figure 2b) was raised. The surgical site was thoroughly debrided and the presence of any subgingival calculus was meticulously cleaned. After the preparation of the recipientsite, the size of the required graft was measured by a pre-sized tin foil template. The graft was harvested from the left palatal area, from mesial to the first premolar till mesial to the first molar. By utilizing the trap door technique[6], sub-epithelial connective tissue graft wastaken out of approximately the size of the tin foil template from the donor site. Afterward, the harvested tissue is kept in a moist gauge piece. (Figure 2c). The graft is shaped according to the size and need and abundant connectivetissue and fat were scared off by usingCastroviejo scissor. Subsequently, the graft was secured properly in on the recipient site, and secured with the help of sutures. (Figure 2d, 2e). After the graft placement, the flap was coronally advanced with the help of continuous sling sutures. (Figure 2f). After suturing, the patient was recommended to use a retention plate which the patient was previously using.

After giving all the postoperative instructions in both verbal and written format and also required medications and povidone-iodine mouthwash for improved postoperative healing and to avoid complications, the patient was discharged from the clinic. The patient was recalled on the tenth day to investigate for the acceptance of graft acceptance. Sutures are removed from both the donor and recipient sites. The patient was again made aware of the oral hygiene instructions (Figure 2g). In the follow-up sessionsthroughout 3 months patient wasrecalled, in which were seen a good root coverage

and significant aesthetic improvement was seen. Clinical examination revealed complete acceptance of the graft resulting from coverage of the root surface with the graft tissue. (Figure 2h). Complete healing of the donor site was also noted.

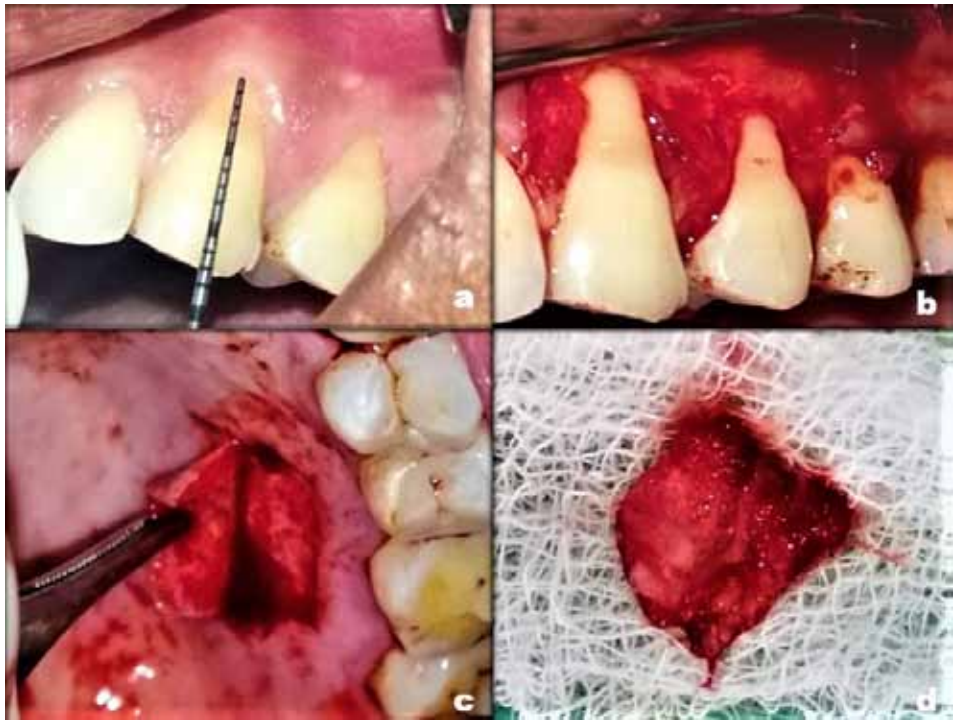


Figure 2a. Millers Class I gingival recession in the buccal aspect of 23, 24; Figure 2b. Reflection of the Full thickness flap; Figure 2c. Harvesting of connective tissue by trap door technique; Figure 2d. After harvesting of the connective tissue



Figure 2d. Placement of the connective tissue graft; Figure 2f. Coronally advanced the flap followed by suturing;Figure 2g. Periodontal dressing Figure 2h. 3 months post-operative

Case 3: A 31 year male, with the chief complaint of hypersensitivity in relation to 14, reported to the Department of Periodontics And Oral Implantology, Institute Of Dental Sciences, Sum Hospital, Bhubaneswar. Nothing significant reported in past dental history and medical history. Periodontal pockets were absent and there was no bone loss present in relation to 14 on intraoral examination (Figure 3a). There was Miller's Class I recession i.r.t 14 (Figure 3b) and no trauma from occlusion was present. The patient was recalled 4 weeks later after the scaling and root planing was performed. After the patient's consent it was decided to treat the case surgically by coronally advanced flap[7].

Surgical Technique: Local anesthesia 1:80,0000 was administered after the proper isolation of the surgical site. A 15 no. scalpel blade is used to make a sub papillary incision along the soft tissue margin of the surgical site (Figure 3c). After the incision, full-thickness flap was raised followed by undermining in order to advance the flap coronally (Figure 3d; 3e). Sling suture was placed once the flap was advanced coronally (Figure 3f).To prevent infective complications, antibiotics were prescribed to patients. Amoxycillin 500 mg thrice a day for 5 days and Analgesics (Ibuprofen) was prescribed thrice a day for 3 days and Chlorhexidinemouthwash was also prescribed twice daily for four weeks and advised to avoid vigorous brushing on the surgical site. The patient was recalled for follow-up after 2 weeks (Figure 3g). The sutures were removed and the site was irrigated with normal saline and Betadine. Healing was satisfactory. Recall was scheduled for 2 months. The recession coverage result showed morphologic and chromatic resemblance to adjacent gingiva. Thus patient satisfaction was perceived (Figure 3h).

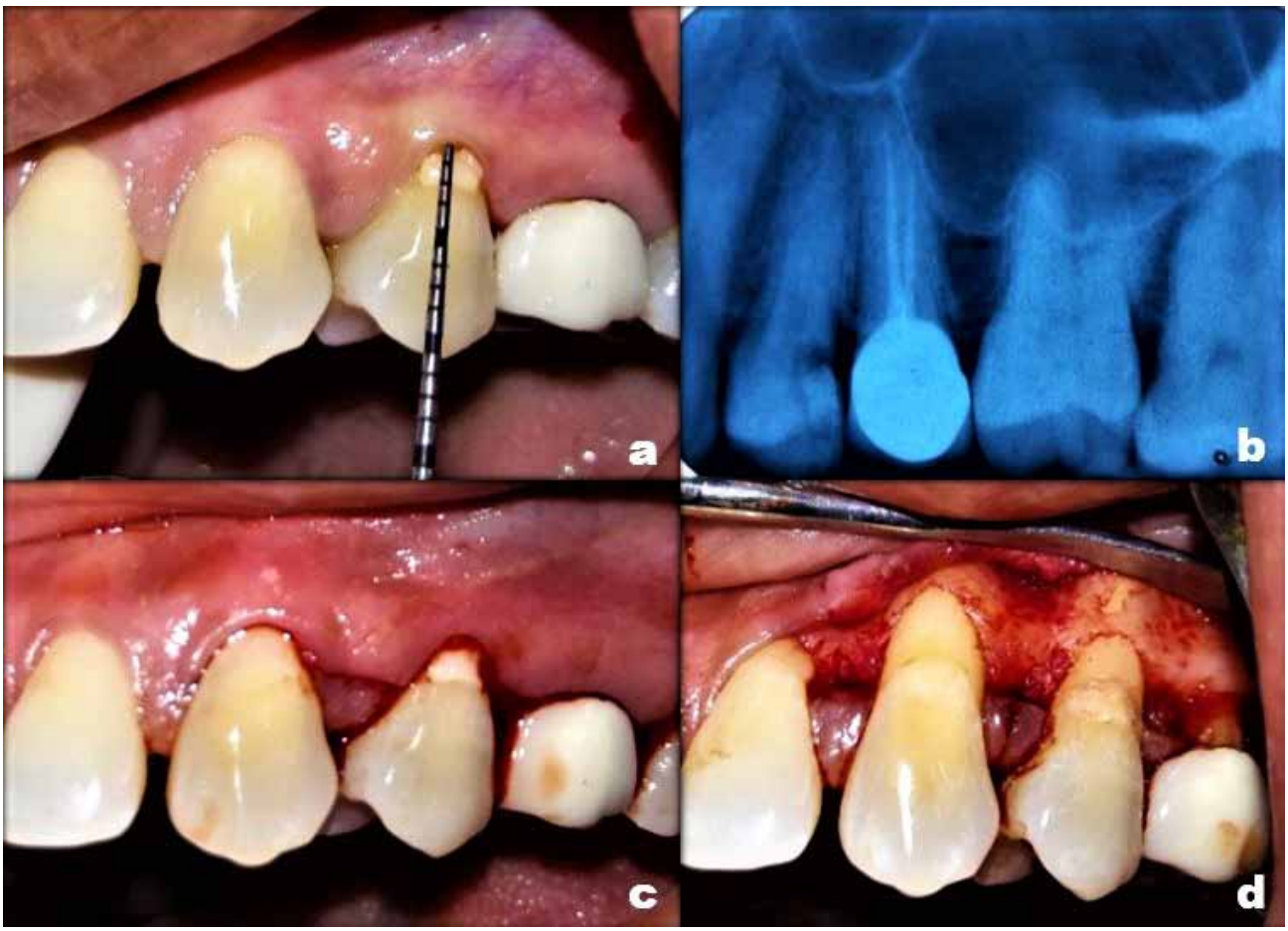


Figure 3a. Millers Class I gingival recession in the buccal aspect of 14; Figure 3b. Intraoral Periapical Radiograph i.r.t 14; Figure 3c. The incision is given; Figure 3d. Full-thickness flap reflection followed by undermining



Figure 3e: Flap coronally advanced; Figure 3f: Sling sutures placed; Figure 3g: 2 weeks post-operative; Figure 3h: 2 months post-operative

Discussion

The favourable results of root coverage procedures depend on several factors, such as elimination and/ orcontrol of the etiology of gingival recession, evaluations of the interproximal bone level and decision making for the particular surgical based on the surgical site. Since the period of 1960s and 70s many surgical techniques to resolve mucogingival problems have come into limelight. Coronally advanced flap, laterally displaced flap, coronally positioned flap with free gingivalgraft are widely used as a chief treatment modalities of mucogingival problems. In the year 1985, Langer and Langerdescribed came up with a technique of harvesting the subepithelial conjunctive tissue graft for. This was considered ideal for treating single or multiple tooth recession. This SCTG is still considered as the gold standard in mucogingival surgical procedures because of its enhanced blood supply from the underlyingperiosteum and overlying flap.[8] The prime factor of the success of grafting depends on the proper placement of the flap and to maintain the position of the papilla. The bridge flap technique includes twosurgical techniques simultaneously. In this technique, the survival and the success of graft depends on the blood circulation from the adjacent tooth surface areas. The flap creates a practicalsurgical procedure with good aesthetic outcome with less or no recurrence.[1]

It is usually performed when a single surgical technique is required to cover the denuded root surfaces with insufficient width of attached gingiva. So basically this procedure both improves the width of the attached gingiva and also covers the root surface in a single step. Nevertheless, for the success of any surgical procedure, proper knowledge of the anatomy of CEJ and the bone should be mandatory. This knowledge will help out the clinicians for proper placement of flaps and giving incision. Lastly, the clinician should always check for the presence of the width of the attached gingiva which should be 2-3 mm for maintain good sulcus depth to perform daily oral hygiene habits. Subepithelial connective tissue graft technique was chosen for the root coverage procedure depending on several anatomic and socioeconomic factors of patients. Along with the improved blood supply, good colour matching is another perk of subepithelial connective tissue graft.[6]

However, all periodontal plastic surgery aims for the root coverage, but a proper and specific treatment strategy is required to determine the success rate of the mucogingival surgery e.g., patient-related factors (maintenance oforal hygiene, method of brushing and smoking, site-related factors like interdental periodontal support and extent of recession.

Allen and Miller in 1989 proposed another surgical technique named coronally advanced flap.[9] The coronally advanced flaps don't involve a donor site, considering this it has less morbidity than CTG and SCTG. It is the treatment of choice in cases where sufficient width of the attached gingiva is present. By using this technique, the colour, texture and thickness of the tissue can be maintained in the treated site (mostly in the buccal aspect).

Conclusion

The success rate of multiple recession defects is based on predictable periodontal plastic surgery (PPS) procedures.[10] Etiology of the multiple defects,proper diagnosis, and case selection in a careful manner increase the surgical success. Bridge flap technique along with vestibular deepening, Subepithelial connective tissue and Coronallyadvanced flap technique may be used for successful management of single tooth recession or multiple teeth recessions.[11]

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Silver Diamine Fluoride: Game Changer in Dental Public Health: A Review

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Abstract

A major part of the world population is affected with carious teeth, among which the number of children affected is maximum. Carious lesions are more common in susceptible, uninsured and financially weak populations. Caries which are not treated for a very long period along with no utilization of available services in both developing and underdeveloped countries pose an essential public health question. Children have limited adaptive capacity which may also lead to difficulty in some treatment procedures. Hence, the need for an efficient and affordable approach for dental caries treatment was required in areas that have a finite access to oral health care services like the school children. Nowadays, Silver Diamine Fluoride (SDF) is very much in demand as it can control caries progression and is very less technique sensitive. This approach does not require caries excavation using instruments, in addition silver has many antimicrobial properties and fluoride helps in remineralization.

Keywords: *Silver Diamine Fluoride, Dental caries, Fluoride.*

Introduction

Early Childhood caries (ECC) present a high prevalence worldwide according to various research papers. Patient cooperation is very important for a desirable outcome that cannot be satisfied by conventional restorations available for the treatment of ECC.[1] After referring to the 2015 Global Burden of Disease Study, it is the most prevalent condition that affected 560 million children with primary dentition.[2] Caries which are not treated for a long time increases the risk of infection in other permanent and deciduous teeth, which leads to a decrease in school attendance, discomfort, expensive emergency treatments and the need for general anesthesia and limitation of growth and development.[3] Children have limited adaptive capacity which leads to difficulty in some treatment procedures.[4] The World Health Organization (WHO), the FDI World Dental Federation (FDI) and the International Association for Dental Research (IADR) jointly convened the Global Consultation on Dental Health through Fluoride, in Geneaon November 2006 declared that "prevention by using fluoride is the only realistic way of reducing this burden (of dental caries) in populations".[5] In 2007, at the 60th World Health Assembly, WHO proposed the governments "to promote oral health in schools, aimingat developing healthy lifestyles and self-care practices in children". In the Beijing conference in 2007, there was an announcement that was collectively summoned by the WHO, the FDI, the IADR and the Chinese Stomatological Association (CSA) that said "fluoride dentifrice remains the most widespread and significant form of fluoride used globally and the most rigorously evaluated vehicle for fluoride use".[6,7] In recent times,

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SDF is used because it stops caries formation and its progression is arrested simultaneously. This approach does not require caries excavation using instruments, in addition silver has many antimicrobial properties and fluoride helps in remineralization. In 2014, the US Food and Drug Administration introduced SDF for the management of tooth hypersensitivity.[8] Many in vitro studies have proven SDF as caries arresting and antimicrobial agent that destroys the activities of S.mutans and dentinal biofilm.[4]

History: Since 1000 years silver has been used as tooth cosmetics to prevent dental caries in Japan.[9] Due to their caries inhibition properties in 1891, silver amalgam and nitrate were used.[10] It was directly applied on carious surfaces as silver niraefor caries prohibition and it was called as Howe's solution.[11] At school dental care services in Western Australia 40% silver fluoride (AgF) was used to treat dental caries of deciduous teeth.[12]

Other parts of the world did not have much idea about SDF use till the late 1960s and the 1970s as SDF use was restricted to Japan. The Central Pharmaceutical Council of the Ministry of Health and Welfare in Japan in the 1960s established it as a therapeutic agent for treating dental caries. Australia and China have also used SDF to prevent caries progression in the last few decades.[13,14] Argentina, Brazil and Spain have used SDF for various public health events in different concentrations; and public health events were planned for sub-Saharan Africa and for different parts of Africa.[15] In Southern California 1995 there were reports that children with early childhood caries were treated with SDF to inhibit caries process as published by an American Journal at that time.[16,17] The US Food and Drug Administration approved SDF for treatment of dentinal hypersensitivity in 2014, which had the molecular formula as Ag(NH3)2F which is Silver diamine fluoride (38%).[8] Since then it was used as a very efficient caries arresting agent in Japan. Different in vivo studies done by Chu et al., Llodra et al., and Yee et al., mentioned that SDF was reducing dental caries in primary teeth as well as effective in arresting caries and also as a treatment substitute for other technique sensitive restoration method.[18-20] In vitro trials done by Knight et al., found that dentinal discs treated with silver diamine fluoride (SDF) showed a reduction in Streptococcus mutans counts.[12]

Silver Diamine Fluoride and Glass Ionomer Compound (GIC): The dark colour of the arrested of caries surface is not one of the most pleasing outcomes of SDF use. For better aesthetics there is a requirement for another effective treatment. Glass ionomer has been used as a restorative material with benefits of releasing fluoride which helps in remineralization. The release of a high concentration of fluoride ion prevents new dental caries development. Moreover, for anterior teeth restorations the tooth color of GIC makes it an obvious choice.[21]

Silver Diamine Fluoride Compared with Atraumatic Restorative Treatment: For school children having finite reach to oral health services, it is necessary to look for an affordable and atraumatic restorative material. There is increased demand all over the world for conservative caries treatment options. So, Atraumatic Restorative Treatment (ART) was introduced first in Tanzania in the mid-1980s and this is a pain-free, minimal intervention approach that is not technique sensitive and where less skill is required along with only hand instruments used to remove carious tooth substance. The conventional, self-hardening Glass Ionomer Cement (GIC)is used to restore the tooth and then used to seal any adjacent enamel fissures.[22] This procedure is largely pain-free and is well preferred by children and GICs demonstrate sustained fluoride release, pulpal biocompatibility, and chemical adhesion to the tooth surface.

The action of SDF on the carious tooth surface:

These could be the available mechanisms of action of SDF in arresting caries:

1. The first step isthe occlusion of dentinal tubules with silver. When SDF is applied the dye permissibility of dentine is decreased and electric resistance strengthened. It is also said that the products formed by silver from SDF use to halt the diffusion of acid and entry of microbes into the dentinal tubules. Further growth of microorganisms is inhibited the by oligodynamic action of silver. The surface area of dentin is decreased by the obturation of the dentinal tubules. The use of 38% SDF preserves collagen from degradation in demineralized dentin and suppresses demineralization.
2. The protection of dentin towards the activity of acid which leads to decreased access of acid into deeper parts of dentin is accelerated by the presence of fluoride. The fluoride ions released goes to a depth of 50–100 μ. in vivo conditions when SDF is applied

to dentin. Also SDF (Ag(NH3)2F) reacts with hydroxylapatite (HA) of a tooth to release calcium fluoride (CaF2) and silver phosphate (Ag3PO4) resulting in hardening of affected dentine.

This is chemically interpreted as:

$Ca10(PO4)6(OH)2 + Ag(NH3)2 \rightarrow CaF2 + Ag3PO4 + NH4OH$

$CaF2 \rightarrow Ca^{++} + 2F^-$

$Ca10(PO4)6(OH)2 + 2F^- \rightarrow Ca10(PO4)6F2 + 2OH^-$

The Ag3PO4 that is formed on the tooth is insoluble to acid attacks. The CaF2 formed as a reaction product becomes a pool of fluoride ions for the formation of fluorapatite (Ca10(PO4)6F2. It has also been proved that fluoride ions enhance calcification of tooth, and restores lattice imperfection and improves the crystallinity of HA.

3. The anti-enzymatic actions of the reaction products between Ag(NH3)2F and organic component of the tooth can be the next mechanism of action for caries arrest. Its antibacterial properties arise from inhibition of the enzyme activities and dextran-induced agglutination of cariogenic strains of Streptococcus mutans. Resistance of dentin to trypsin increased when SDF is applied on the tooth surface. Also,a study reported resistance to collagenase and trypsin for dentin protein increased after treating the tooth with SDF.[4]

Principles of selecting tooth for Silver Diamine Fluoride applications include:

SDF can be used in:

- Patients having active cavitated caries lesions with high caries risk in anterioror posterior teeth.
- Patients diagnosed with medical and behavioral challenges.
- The ones with a greater number of carious lesions cannot be treated in a single sitting.
- Carious lesions with complicated treatment plans; and those withdifficulty in accessing oral health care.
- There should be no signs of spontaneous painor acute/chronic pulpal inflammation.
- The use of radiographs to assess the depth of infection
- The carious lesions should be checked for accessibility of the applicator brush.(To gain access in proximal lesions orthodontic separators can be used.)
- As part of caries control therapy, SDF can be used before restoration placement.[23]

Clinical Applications of SDF are:

- Xerostomic patients, who are more susceptible to dental caries.
- For the treatment of caries in mentally disturbed young children.
- Caries management of the anterior deciduous teeth.
- Children whose guardians do not have time for long operative procedures.
- This is a great atraumatic option along with minimal use of equipment.
- No anesthesia is required for the procedure.

Method of applying SDF:

- One drop of SDF is dispensed into a dappen dish.
- The affected tooth surface is isolated and dried.
- Petroleum jelly is applied then on all the adjacent mucosa of the affected tooth.
- It is then applied with a micro brush or an applicator tip.
- The excess is removed with cotton gauge and then left for 2 minutes.
- Rinse with water.
- The tooth can be restored with restorative material like GIC.
- The restoration is evaluated every six months.[21]

SDF is advantageous because:

- Control of pain and infection as the caries is arrested in its initial stage.
- The simplicity of its use as it is like painting/ applying on the affected surface with a microbrush.
- The material is quite affordable which is around 100 applications per 5ml bottle and is available in most parts of the world.

- The requirement of skill is less as it is not technique sensitive.
- It is non-invasive and atraumatic nature.
- Duration of treatment is shorter than other restorative procedures.

The available concentrations of SDF are 10%, 12%, 30%, and 38%. Review articles and original studies related to SDF have suggested the use of 38% SDF for dental caries(8,20). A review by Contreras et al.(24) proposed that SDF for arresting caries was more efficient at concentrations of 30% and 38%.

Disadvantages of SDF: Pulpal irritation, dental staining, and oral soft tissue irritation are the main adverse events associated with SDF applications. Skin pigmentation is for short duration as silver does not enter the dermis. When keratinocytes are shed over 14 days desquamation of the skin with pigmentation occurs. The dental pulp is at lesser risk by the use of SDF. One of its demerits is the blackish discoloration that appears after the SDF application.[4]

Public Health Significance: Silver diamine fluoride arrests more than 80% of the caries when used twice per year. It is a source of powerful indirect prevention of dental caries. Hence, it is a game-changer in the field of dental public health as it is affordable in comparison with other expensive dental procedures and saves a lot of time, requires a lesser amount of skill, not technique sensitive and can be used in field settings like a school-based programmes by both dental auxiliaries and dentists. The benefits of SDF far outweigh the risks. Hence emerging as an effective public health tool.[4,21]

Conclusion

The application of SDF once in every 6 months is preferred to other minimally invasive techniques. SDF as a primary preventive material is better and convincing than other materials for restorations, excluding dental sealants which are >10 times expensive and requires more skill and technique. If it is used as a preventive or curative procedure, it can prevent or postpone the requirement of caries treatment in both children and adults. The use of fluoride varnish for caries cessation can be replaced in patients with SDF. It is a potent public health tool especially in low-income countries in the fight against dental caries.

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Over-Dentures and Attachments: A Review of Literature

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Abstract

Tooth-retained overdentures are a simple and cost-effective treatment than the implant-supported overdentures. When few teeth are present which are periodontally strong can be used as abutments for overdenture fabrication. This helps in the retention and stability of the final prosthesis. The extraction of teeth results in bone resorption, so preserving them as an abutment can not only provide retention and stability to the denture but also prevents bone loss and provide proprioception. Above all, "it gives the patient the satisfaction of having prosthesis with his natural teeth still present".

Keywords: *Tooth Supported Denture,Implant Retained Denture,Overlay Prosthesis.*

Introduction

An Overdenture is a "dental prosthesis that covers the natural tooth and or is partially supported by natural teeth, natural tooth roots or dental implants". According to GPT 10, "overdenture is a partial or complete denture that covers and rests on one or more remaining natural teeth, roots and/or dental implants; a dental prosthesis that covers and is partially supported by natural teeth, tooth roots, and/or dental implants". It is also called as "overlay denture, overlay prosthesis and superimposed prosthesis.[16]

Preventive prosthodontics "emphasizes the importance of any procedure that can delay or eliminate future prosthodontic problems and overdenture is an important part of the preventive treatment modality". De-Van's golden statement: "Perpetual preservation of what remains is more important than the meticulous replacement of what is missing" still rings true[7].

Tooth supported overdentures have various advantages over conventional complete dentures such as maintenance of alveolar bone maintenance, periodontal proprioception is preserved, improves retention, stability and support,enhances psychological comfort and increased mastication efficacy.[4]

Chen et al. "observed that the patients treated with tooth-supported overdentures had significantly more comparative masticatory efficiency than those with conventional complete dentures, while there was an insignificant difference in comparative masticatory efficiency between tooth-supported overdentures and implant-supported overdentures"[2.]

Crown and Rooney 1975 in their study found that "retention of mandibular canine for overdenture led to preservation of alveolar bone"[2].

Indications for Overdentures:[1]

1. For better support in morphologically compromised dental arches.
2. Cleft palate cases and congenital anomaly cases like microdontia, amelogenesis imperfecta, dentinogenesis imperfecta, and partial anodontia.
3. Dentures patients with maxillofacial trauma.
4. Patients with worn-out dentition.
5. Patients with abnormal jaw size and position where orthognathic surgery is contraindicated.

General Considerations during Diagnosis and Treatment Planning for an Over-Denture[1,2,4]:

1. Maintenance of Periodontal Health: Once an overdenture is planned and constructed, the patient must maintain his teeth free from plaque. The dentist should check for pocket formation around the abutments periodically and failing may lead to the loss of abutment tooth.

2. Reduction in Crown-root Ratio: Reduction in crown size during preparation can be beneficial for the tooth, as it "reduces the crown-root ratio" and "decreases the leverage forces acting on the tooth".
3. Success of Endodontic Therapy: Endodontic therapy may be necessary for most abutment teeth because they need extensive crown reduction. A two-to-four week interval should be provided after completion of root canal therapy to determine the success.
4. Number of overdenture abutment teeth: "Two Abutments On Opposing Sides Of The Arch Particularly In The Canine Regions Will Provide Excellent Results". Four Widely separated Abutments Are Even Better.
5. Adaptation and Coverage of Denture-Bearing Area: The denture base should be well adapted to the soft tissues to prevent accumulation of food debris and to evenly distribute the force acting on the denture.
6. Design of the Denture: As the denture base for overdentures are thin, they have to be reinforced with metal. At the same time they should be easy to fabricate and maintain.
7. Ease of Use: The patient should be able to easily insert and remove the denture without any harm to the denture base or the abutment tooth.

Advantages of Overdentures:[3]

1. Maintains the integrity of the ridge.
2. Improves the "retention and stability" of the denture.
3. Improved proprioception leads to better neuromuscular control. This helps in regulating the biting force over the denture.
4. The psychological effect on the patient as extraction can be avoided.
5. If there is abutment failure, the abutments can be extracted and the overdenture can be relined and used as a conventional complete denture.

Disadvantages of Overdentures:[2]

1. Nutritional counseling, oral hygiene measures, and fluoride application should be carried out periodically.
2. "High incidence of caries and periodontal disease around the overdenture abutments".
3. Frequent reviews are needed to verify the health of the supporting tissues of the overdenture abutments and Improper maintenance of the overdenture may lead to periodontal breakdown of the overdenture abutments and the patient may lose all his remaining teeth.
4. More costly than conventional dentures because:
   (a) Root canal therapy and coronal restorations may be needed for certain overdenture abutments.
   (b) Most cases need a cast metal denture base, as acrylic is weaker.
5. Additional designing and laboratory work is needed.
6. Cannot be used in cases with reduced interarch space, bony undercuts adjacent to the abutments, etc.

Abutment Preparation[1]: The preparation of abutment teeth is one of the keys to the construction of overdenture. Assuming the periodontal status to be good, the operator has three choices:-

1. Preparation just above the mucosal level.
   - The bare root face approach.
   - The dome-shaped gold coping.
2. The use of attachments.
3. The thimble-shaped coping.

The Bare Root Face Approach[1]: The occlusal section is obturated with glass ionomer or silver amalgam. It is normally employed for immediate insertion prosthesis. On later stages coping can be given the crown of abutment is reduced to a height of 2-3 mm. the abutment tooth is endodontically treated the occlusal surface is to be contoured to smooth or polished dome-shaped surface. This will minimize lateral occlusal stress. It offers following advantages-

1. "It is the simplest,cheapest and least space-consuming".
2. "It is ideal during maturation of edentulous ridge"of a recently extracted case.
3. It should be replaced by an coping with precious or semi-precious metal otherwise there is a chance of vertical longitudinal root fracture.
4. Contraindications:
5. It should not be used for a long period where a natural tooth is opposing the overdenture.

6. Leaving it as such may lead to longitudinal root fracture.

The Dome Shaped Copings[1]: The Abutment Tooth Is root canal Treated And Reduced To A Height Of 2-3mm and Post Space Is Created. Dome Shaped Cast Metal Copings 2-3 Mm In Height With Chamfer Finish Line And Post Are Fabricated And Cemented. Coping Should Be Atleast 1 Mm Thick To Withstand Occlusal Forces.

Thimble shaped coping[1]: These are 5-8mm in height and need considerable space. These copings can be used to support the telescopic crown to enhance retention. The chamfer finish line is given in the prepared abutment.

Basic Principles to be followed[1,5]: The abutment teeth must be surrounded with healthy periodontal tissue.

1. Maximum reduction of the crown to attain a better crown-root ratio and avoid interference during the placement of artificial teeth. Endodontic therapy may be done if needed. A simple tooth preparation without any internal attachments can be done in a single visit. This can be done for elderly patients and medically compromised patients. It is less expensive than copings.
2. Gold copings or crowns and sleeve coping retainers can be given for grossly destructed abutments after assessing the patient's susceptibility to caries. Gold or metal coping can be prepared with posts and retentive pins after evaluating the amount of tooth structure above the gingival attachment.
3. Attachments are added to the cast copings for additional retention. These attachments may be resilient or non-resilient types.
4. Regular reviews and periodontal evaluation of abutment teeth should be done.

Types of Overdenture:[1]

Two types:

Tooth supported overdenture

Implant-supported overdenture

Heartwell's classification for tooth-supported overdenture

Based on the method of abutment preparation:

1. Coping
2. Noncoping – with simple tooth modification
3. Attachments
4. Submerged vital roots: Effective in preserving alveolar bone

Based on length of coping:

1. "Short coping – 23 mm long and normally require endodontic therapy".
2. "Long coping – 58 mm long, an attempt is made to circumvent endodontic therapy by conservative reduction".

Abutment with attachment–"Most of the attachments are secured to the abutment by a cast coping. The objective of attachment is to improve the retention of denture".

Attachments Used In tooth/root/Implant supported Overdenture:

Definition: "A mechanical device for the fixation, retention and stabilization of a prosthesis,a retainer consisting of a metal receptacle and a closely fitting part; the former (the female matrix component) is usually contained within the normal or expanded contours of the crown of the abutment tooth and the latter (the male matrix component), is attached to a pontic or the denture framework".[16]

The attachments used for overdenture can be classified as[1,9]:

Stud attachment

Bar attachments

Magnetic attachments

Requirements of Attachments:

- Patients should have a low caries index.
- Sound periodontal health.
- The vertical space should be sufficient enough to accommodate the attachment and denture teeth.

Disadvantages of Attachments:

- Time-consuming and expensive.
- Difficulty in construction as well as repair is difficult.

- Require careful use by the Patient. So not recommended for mentally and physically handicapped patients.

Stud attachment[1,9,15]:

It is the simplest of all types of attachments.

Stud attachments can be divided into two groups:

1. **The Extra Radicular:** "The Stud" (Male part) usually attached to metal coping cemented over the prepared abutment and it projects from the root surface of the preparation. The female part is attached to the denture. Attachment of male component to female component provides retention. The male parts are available as:

a. Prefabricated Metal Posts-Cemented Directly To The Root.

b. Prefabricated Resin Patterns- Which Is Cast And Cemented To The Root.

The female component is also termed as retentive anchor and made in metal or plastic and is in the form of an o ring or matrix. Eg-CEKA, ORS-OD, DALBOS, SERIES, GERBER, LOCATOR ATTACHMENT AND ROTHERMAN.

Ceka Attachment:

The male part is round and composed of titanium.

The female part is replaceable plastic part that is flexible And compressible.

O Ring Series System: O-rings are "doughnut-shaped, synthetic polymer gaskets that possess the ability to bend with resistance and then return to their approximate original shape".

Parts of an O-Ring Attachment-metal encapsulator,o ring post, and o ring which is available in 3 different sizes.

Dalbos Series Attachment System:

- It is the most popular stud attachment with a long life.
- It is available in two designs- ball and socket dalbo & rigid dalbo.
- It is available in spherical and cylindrical forms.
- The ball and socket dalbo is the smallest of 4mm height and easy to clean and maintain.

Rotherman System-

- The male part consists of a groove.
- The female part is c shaped ring that fits in the deeper part of retaining groove.
- The attachment looks like a press button with a groove.
- The female unit is clip with open end.

Locator attachment-

- The locator attachment system is an attachment system with a self-aligning feature and has dual retention (inner and outer).
- Locator attachments come in different colors (white, pink and blue)
- "Extended range attachments, which can be used to correct implant angulation up to 20 degrees they are offered in green, which has standard retention, and red, which has extra-light retention".

Gerber Attachment: Readily replaced male or female attachment Which can be replaced if they are worn out.

The spring-clip of the female part engages in the peripheral groove in the male.

Other extraarticular attachment systems used are:

(a) The Ancro fix attachment

(b) The Introfix attachment

(c) Quinlivan attachment

(d) Schubiger Attachment

2. **The Intra Radicular Attachment:** "The Stud"(Male element) forms part of the denture base and engages a specially produced depression(housing or female part) within the root contour. This attachment is indicated in situations with reduced interocclusal space.

Eg-Logic,Zest attachment,ERA attachment

Zest Anchor System:

The female sleeve is cemented in the post space.

Male portion consists of nylon.

ERA system: Resilient attachment system with

color-coding resin unit providing various degrees of retention.

Universal hinge with vertical movement.

Metal jacket which holds the male attachments.

Bar Attachment: It consists of a bar spanning an edentulous area joining copings on the roots of the abutment teeth on either side of the arch.

Sleeves and clips placed in the denture attach to the bar when the denture is inserted,providing retention.

The bar splints the abutment teeth and thus distributes the forces.

This type of bar attachment requires vertical and buccolingual space.

Oral hygiene maintenance is very much essential otherwise may lead to abutment loss.

It can be a bar joint or bar unit.

Depending on the number it can be a single bar or multiple bars.

Depending on movement it can be a bar unit that provides no movement and is made up of solid rigid material or bar joint which permits rotational movement between bar and sleeve and thus made up of resilient material.

Hader Bar:

It is named after the swiss tool and die technician "Helmut hader".

It is a rigid bar connecting two or more attachments.

When viewed from the cross-section it appears a keyhole consisting of a rectangular bar with a rounded superior ridge that creates a retentive undercut for the female clip within the removable prosthesis.

The bar provides mechanical retention and the round part makes the bar resilient type and thus some amount of movement is seen in this attachment.

The sleeves which are available commercially in plastic form can be cast and converted to metal if extra retention is required.

Dolder Bar:

It is named after swiss prosthodontist Eugene j dolder.

The bar is straight and is rounded at the top. The Sleeves that fit over the bargain retention by friction only.

The bar is available in various sizes and when cross-sectionally viewed appears to be pear-shaped. The clip which is fitted in the denture base allows some rotational movement.

It is available in sizes in diameter of 1.6 and 2.2 mm.

Ackermann and CM bar

These bars are round when viewed in cross-section.

As they are round they offer some resiliency and can be bent in all directions.

A short extension of 5 mm is carried behind the most distal root and the sleeve is positioned on this section.

Sleeves or clips are made up of gold.

Available in 1.8 mm diameter in plastic and gold.

The CM bar is available in a 1.9 mm diameter and used in long-span cases.

Magnetic Attachments[1,3,8]

Magnetic attachment consists of keeper and denture retention element.

The keeper is made up of stainless steel and is cemented to the abutment teeth.

The "denture retention element" consists of" paired,cylindrical magnets" made of cobalt-samarium with opposite poles placed adjacent to each other.

One end in the denture with knurled housing which fits into the denture and other ends which is smooth fits on the keeper.

The retention principle is due to the mutual attraction of unlike poles.during dislodging forces, the magnetic forces reseat the denture.

Advantages of magnetic attachment: No path of insertion, No specialized casting or instruments required, No paralleling of abutment.

Automatic reseating, Ease of repair and relining, Minimum forces are transmitted to roots.

Disadvantages: Alloy and corrode and fracture leading to loss of magnetism with loss of retention and thus need for constant replacement.

Discussion

Tooth & implant-supported over-dentures are a "step in the direction of preventive prosthodontics". They Preserve the residual ridge, provide support and stabilization to the denture base, and thus gives the patient a sense of security in knowing that teeth aid in support of their prosthesis. The maxillary overlay denture is of great value when it opposes remaining mandibular anterior teeth because it aids in conserving the ridge against resorption from "masticatory stress"[4'].
The greatest difficulty is the use of attachmentsbecause of their "complex design". A thorough understanding of the case and knowledge about the various attachments and its applications and limitations is a must to apply clinically.

Conclusion

Overdenture helps reduce shrinkage of surrounding bone and reduces pressure on the alveolar ridge. In the case of an overdenture prosthesis, "proprioception is maintained" there is the presence of "directional sensitivity; dimensional discrimination; canine response and tactile sensitivity"[1,14].

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Stress in Dentistry: A Review

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Abstract

Dentists are prone to have different kinds of mental, physical, psychological and financial complications related to stress. It is important for the dentists to de-stress themselves by a reduction in the number of stressors and increase the ability to deal with stressful situations. The practices like resisting stress as well as time management and choosing goals which are similar to real-life scenario should be encouraged. So that stress can be reduced to a tolerable level. Like any profession, the transition from academics to the workplace could be a challenging task. As it is known that dental care professionals have responsibility for the health status of general people. So,there is a need for stress management and reduction programs to be integrated into dental education, which can provide practical solutions for curtailing various types of stress. Here we will know how to promote and enhance the well-being of the dentists by understanding the value of life.

Keywords: *Stress, anxiety, depression, dentist, dentistry*

Introduction

It is frequently assumed that dental professionals have a soothing career but, the fact is that, various stressful situations can cause worry in dental professionals. Usually dentistry is perceived as monotonous and stressful[1]. It has been expressed by experienced dental professionals that, it increases stress level to a negative peak but also hampers daily activities which include personal relationships, leading to early retirement[2]. Psychological disorders such as depression, anxiety,narcotic abuse, and various health problems such as heart attacks, strokes, fluctuations in weight and professional burnout could be tied to stress points in dentistry[2,3].

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In the dental profession the stress may set in from the beginning that is, while studying to qualify as a dentist. Various studies have been conducted which have concluded that dental student has more stress compared to any other professional education[4]. This stress keeps intensifying with each year of study[5]. Stress may come from various external factors such as physical, mental and psychological well-being. Hence stress must already set in even before qualifying as a dentist. Therefore, only a few perfectionists may seem to continue their career with an academic track record of excellence. After completing the qualification, the stress usually reduces in other professions but here it only increases due to the financial pressure of setting up and keeping the dental practice running. Post completion of graduation, a person is stressed to find an employer to settle any educational loans taken and it will keep on increasing along with various new debts which have an impact on psychological and emotional shades of life.[6]

While, restoring the clinical, functional, and structural aspects of a dental career, consequences could be due to as extreme stress levels leading to numerous complications like panic attacks, drug abuse, alcohol abuse, health disorders like cardiac problems,

early stroke, variations in weight and professional burnout. Dental professionals have a chance of having musculoskeletal issues because of less workings pace, continuously working in the same posture and impaired vision due to continue concentrating on the oral cavity. If a dentist has his/her dental clinic, then he/she is anxious about dealing with various expenses, like dealing with staff and co-workers, lab bills and payments for rent it time. Dentist is worried about their professional liability[7].

In the study conducted on dental professionals by Sumaya Basudan., et al (2017)[8], it was observed that 55.9% of the respondents had abnormal levels of depression, 66.8% had anxiety and 54.7% had stress,Severe and extremely severe scores for depression, anxiety, and stress were reported in 20.2%, 34.0% and 20.2% of respondents, respectively[8].

How to suppress stress: The impact of stress on an individual can be broadly divided into two parts. One is the usual meaning it carries which leads to depression by lack of motivation and the other one is in a good way. The latter one affects a person positively where he/she can concentrate his/her energies in a specific direction to simplify various problems or situations by uplifting career or achieving any goals. But the former one is the matter of concern that needs to be addressed by various stress management method, like constant motivation.

Physical Stress: Good health is the key to reducing stress in any field, however, a dental practitioner requires strength, flexibility, endurance and coordination. So, it is necessary to maintain a decent physical and mental health by eating healthy meals, getting a sound sleep, and doing adequate exercise. Exercise, enables your body to perform well, and also have psychological benefits. Exercises to help in stretching of the muscles that support the spine. Walking or swimming are also highly recommended[9]. These practices lessen the physical stress to a significant level and increase productivity in professional lives. For spinal problems, correcting postures in between time intervals, relaxation during work sessions, could be utilized[10].

Psychological stress: Students and faculty should be made aware of psychological stress associated with dentistry very early in their careers. Initial signs and symptoms of anxiety or depression would be identified and addressed at the earliest. A health professional should try to solve this issue on his/her own. As a doctor we should recognize the problem at the beginning and understand the necessity of mental health, besides being with friends and family can help to be emotionally stable and get moral support. Similarly, taking vacations is a boon for a dentist. To reduces tress, deep breathing and muscle relaxation should be practiced routinely. The dentist can also discuss with their peers regarding the improvement of the work environment at the clinic, dental practice and management processes, and expertise in method for effective control over patient anxiety. Health professionals should take leisurely breaks sensibly or holidays from busy work life, as it relaxes the brain as well as the body[11].

Financial Stress: A dentist may lack financial skills to manage all the monetary issues. Not receiving payments in time for their services can lead to anxiety about their future. It is recommended to hire a financial advisor, who can help you in various possible ways to manage your monetary aspect in exchange for a small management fee. It will be time-saving as well as convenient. A few steps to consider in refinancing your student loans at a different lender so that the money you need to provide every monthly payment interest will be lower. It could help you to reduce your financial stress and guide you for future loans. A person should not make dentistry, as their only source of income, he should invest generously in various investment programs. This can help to reduce the financial pressure to a great extent. In all these things one should also consider that time is equally important as the value of money and make the financial decision wisely to lead a balanced work life[7].

Stress coping mechanisms: In the study conducted on dental professionals by Sumaya Basudan., et al (2017)[8], the authors illustrate different mechanisms to cope with stress. The most common method for coping with stress was activities such as watching movies, studying novels, sound sleep and shopping with friends (71%), followed bye motional support from others (53%).Under other mechanisms, intake of food or drinks and going on a vacation was mentioned by some[8].

Coping with stress: When dentists are compared with doctors, they consider it as a personal insult. Working in their scheduled routine dental practices for long hours leads to a frustrating life[12]. It is known that stress is not good for health so it leads to many issues such as ulcer, colitis, back pain, eye strain, marital disharmony, and even suicide[13]. Significance of high-stress levels was evident in the survey conducted on

UK dentists, where responses showed that 17.6% of all respondents had seriously thought about committing suicide at some point in their lives, which was compared to a recent survey conducted on UK dentists by Dr. Vicki Collin,et al (2019)[14], which found that 20.6% of the normal population had reported suicidal thoughts came to their mind, moreover the researchers found that dentists showed higher levels of suicidal thought than the normal population, among them whomever said that they thought of attempting suicide is 57.7% last 12 months. The high levels of self-reported stress, burnout and psychological stress found are a serious concern for the dentists[14]. Stress is a well-known factor for mood swings, mental disorders, and suicide risk due to negative life events. Not every person can cope with the same pressure, and that leads to the risk of developing depression and suicidal thinking[15]. It can also cause absenteeism, personal anger, jealousy, low productivity, and increase complaints at work place[16]. Due to running behind schedule sometimes any kind of work-related comments by colleagues may lead to inferiority feelings, discouragement, self-doubt on abilities to work and lack of self-confidence can cause emotional fall out[17].

How to manage stress: Stress can be reduced by expressing feelings verbally, mainly the negative thoughts like anger and sadness, listening to music for relaxation of mind, as it helps to calm anger and spread positivity[18]. Good social network is equally important with family members, friends, any other contacts like doctors, colleagues, etc, as it will assist at the time of need. If someone will use his/her past experiences and knowledge, they can tackle problems easily in different situations. As a dentist, there may be many unpredictable situations and we have to tackle it by using self-management skills and problem-solving behaviour[19]. Always try to create innovative ways to deal with the current situation, boost self-confidence by developing personal growth, learning new things, or collecting new information regarding arts such as literature, music, and painting[18]. As health professionals, we have a duty towards society to meet their expectations because we are offering services to the community. Dentists should know how to improve the skill of communication, listening, and have financial and business management knowledge too[20]. For managing apprehensive patients, some stress releasing procedures such as relaxation, hypnosis, desensitization, coping therapy could be useful[21]. Awareness to understand emotions and means to cope with them by self-control is essential. Professional and patient relationship should be good enough to avoid stressful environments[19]. Minimize the impact of negative thinking and maximize the positive thinking.

To summarize the preventive measures that can be implemented are:

- Improvement in work environment
- Don't stay alone and discuss issues with colleagues
- Utilize time wisely and take breaks
- Learn how to manage patients fear and anxiety
- Attend programs in stress management
- Do one thing at a time, Create time for yourself
- Initiate outside interests
- Listen actively, Talk less and more slowly
- Regular physical exercises
- Relax mind and listen to music
- Say no to the unachievable
- Do breathe in and out technique repeatedly
- Less demand for efforts and be kind to yourself[22].

Don't try to become perfect, sometimes it is fine to be satisfied with whatever you have. Accept the fact that no one can have control over everything in life.

Conclusion

As the problem of anxiety and depression is faced by both students and professionals at some point in time, it is important to identify various symptoms to improve psychological well-being. These strategies may be implemented early in dental schools for improving student's lives, prevent premature dropout and the same can help a person in their profession at a later age. This ensures curtailing of all further kinds of complications that may set in after completion of the qualification,hence it can improve both quantity and quality of the work-life significantly[8].

So, overall,we can say everyone is susceptible to stress. Although the dental career is tremendously stressful, but if proper measures are taken in proper time one can reduce the stress to a bearable limit. Identifying the sources of your stress, then eliminating the problems to get solutions over your stressor is advisable. Remembering to be kind to yourself and try to

push your limits can help to reduce stress. Like any other profession dentistry can be considered as a marathon, where one should try to live with it instead of trying to escape from it. Dentists are susceptible to professional burnout, anxiety attacks and clinical misery. Stress can't be eradicated from dentistry but it can be minimized to some extent. Key to manage stress is finding out the root cause to know the problem properly and try to solve it. If the cause is identified then preventive measures or precautions can be taken[7].

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Post and Core Treatment of Maxillary Right Central Incisor Using a Fibre Post: A Case Report

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Abstract

Preserving a badly mutilated tooth has always been a challenge for the dentist. Patients usually approach to a dentist where there is significant loss in tooth structure. Post and core can be done in such a case for the restoration of the teeth. For success in such cases, proper post-selection is of utmost importance. This case report highlights the usage of post and core to save teeth with less crown structure using prefabricated fiber posts.

Keywords: *Mutilated tooth; Post and Core Treatment; Right Central Incisor; Fibre Post.*

Introduction

Dental caries being a common chronic disease worldwideis a cause of pain-related loss of function in patients. Patients with dental caries in the anterior part of the dental arch come with an added challenge of decreased self-esteem and confidence owing to loss of speech and aesthetics.[1] A case of dental caries with pulpal involvement may require endodontic intervention followed by prosthetic rehabilitation both functionally and aesthetically. The endodontic intervention may lead to significant loss of tooth structure in the crown owing to dental caries and/or the preparation of access. This makes the tooth susceptible to fracture even under the normal force of biting. In the absence of sufficient tooth structure for proper rehabilitation after endodontic treatment, the use of post and core may be taken into account to retain the crown.[2] Post and core is a widely used treatment protocol that helps in the preservation of grossly decayed teeth or teeth with less crown structure. It not only replaces the missing tooth structure but also provided resistive and retentive to the crown thus, restoring the tooth both in terms of function andaesthetics.[3] "Dowel (Post): The dowel is a metal post or other rigid restorative material placed in the radicular portion of a non-vital tooth. A dowel, usually made of metal, is fitted into a prepared canal of a natural tooth. When combined with an artificial crown or core, it provides retention and resistance for the restoration. (Glossary of Prosthodontics) .Core: Refers to properly shaped and well substructure, which replaces missing coronal structure and retains the final restoration. The core is designed to resemble or become the crown preparation or crown itself. (Glossary of Prosthodontics)."[4] The biological width is an important factor that has to be kept in mind in the treatment of any tooth. Post and core treatment helps in restoring the tooth by acting as a reinforcement for the tooth structure. Badly mutilated teeth are weak and susceptible to fracture. Root canal treatment alone can eliminate pain but will not strengthen the tooth structure. Hence, post and core is required in such cases. Indications for post and core are, when the crown length is less than 1 mm, root canal treated teeth with extensively damaged or lost, endodontically treated anterior teeth with susceptible

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to fracture at the cervical area, etc. Contraindications including poor crown root ratio, fragile/weak root, teeth with heavy occlusal load, poor periodontal health, dilacerated root, persistent periapical lesion, patient with unusual habits, inadequate skills, etc.[5]

Posts are available in a variety of sizes and materials. They can be custom made or prefabricated. The ideal post should be such that it closely conforms with the root canal wall and has sufficient strength to withstand the forces directed along the long axis of the tooth. The length of the post should extend up to 4-5 mm short of the apex leaving the apical gutta-percha intact. The apical seal with gutta-percha is necessary as the maximum incidence of lateral canals is seen in the apical 3mm. The obturating material seals these canals reducing the chances of failure of treatment.[3-5]

Prefabricated posts are made from fiber. They are available in a variety of sizes that can be selected according to the width of the root canal. They are not technique sensitive and are easy to perform. The ease of procedure with these posts make them a widely accepted procedure among the dentists. However, they don't conform well to the root canal walls. This leads to the appearance of potential space between the prepared root canal walls and the post itself.[3-5]

This case report is based onthe management of adecayed maxillary right central incisor in a young permanent dentition. There was significant loss of crown structure and pre-fabricated post was used after the endodontic treatment.

Case Report: A 14-year-old male patient reported to the Department of pediatric and preventive dentistry with the chief complaint of pain in the upper front tooth region. The patient gave a history of sharp, shooting,and continuous pain that was aggravated on the intake of hot and cold beverages. On clinical examination, there was a presence of caries involving the pulp in the right central incisor(figure 1). There was also a loss of significant tooth structure due to long-standing decay and chipping of the tooth structure. Radiographic examination showed caries extending up to the pulp chamber. The patient was advised to post and core treatment.

After the administration of local anesthesia, the access cavity was prepared. Working length was established with electronic apex locator and a radiograph was taken for the same

The canals were cleaned and shaped upto 40k files. Irrigation was performed with 5.25% sodium hypochlorite and saline during each instrument change. Master cone radiograph was taken as depicted in. Obturation was done with mono cone technique using AH Plus sealer(figure 2). Following this, post space was prepared with peesoreamer upto size 3 leaving the apical 5mm gutta-percha technique (figure 3).



Figure 1. Pre-Operative



Figure 2. Obturation done



Figure 3. Post Space prepared

For the ease of preparation, it was decided to put a prefabricated fiber post in this tooth. Post space was prepared using peesoreamers up to size 3. The prefabricated post was selected according to the root canal width. The post was inserted and luted using resin cement. Following this, crown preparation was done was porcelain fused to metal crown was prefabricated and cemented using Type II glass ionomer cement. After 3 days crown was provided to the patient (figure 4).



Figure 4. Post-Operative

Discussion

It is often impossible to achieve sufficient support to a restoration when there is a loss of coronal tooth structure. Restoration of the endodontically treated tooth is very important as it has an impact on the long-term prognosis of tooth.[6]

Trauma and decay are mostly associated with an extensive loss of tooth structure, necessitating restoration of the tooth with a complete crown for esthetic and functional rehabilitation.[7] It is impossible to achieve sufficient anchorage of a restoration in the remaining dentin when a large portion of the crown has been lost to damage. In such situations, post and core restorations are required for additional retention.[8]

The resin fiber post is more similar in its characteristics to natural dentinal structure than any previously used post. It has excellent transverse strength and acts as a shock absorber dissipating much of the stress placed on the finished restoration, transmitting only a small fraction of these forces to the dentinal walls. The fiber post bonds to tooth structure, core materials, and resin cements. It is delivered to the patient in a single-appointment, chairside procedure.[9] Hence proper selection of post and meticulous clinical procedure is critical in the success of post endodontic restorations.[10]

Conclusion

The restoration of endodontically treated teeth with significant loss of tooth structure necessitates post and core treatment. Post and core treatment have a long term prognosis.This case report highlights the usage of cast metal post. There are certain limitations of every post hence, the clinician must make a proper decision regarding the choice of post. The proper selection of proper post design and material is necessary to ensure a successful treatment outcome.

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Complex Odontoma: An Overview

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Abstract

The dental tissues comprising of dentin, enamel,sometimes pulp, and cementum are the hamartomas which are known as odontoma. These have behavior not so aggressive. The common type of tumor of odontogenic origin about (35-76)% is the odontoma. It is a tumor of benign type arising from epithelial and mesenchymal differentiated alteration, odontogenic cells. These odontomas are of two types–complex and compound, the both are differentiated from each other by the organization of the tissue of dental origin in a ratio of 1:2. Although the etiology is not known till now but may be related to anomalies of hereditary origin, infection,also by trauma and by hyperactivity of odontoblast process. By the means of radiological view these odontomas can be recognized and these in very few case causes expansion of the cortical bone. These odontomas occur in the second decade in number and usually takes place in the mandibular second or third molar tooth. This particular article summarizes the complex composite odontoma.

Keywords: *Complex Odontoma; Prognosis; Differential Diagnosis; Treatment.*

Introduction

The tumor of odontogenic origin and benign is the odontoma. It was first put forward by Broca in the year 1866 as odontoma and he had mentioned this as a tumor by the tissues which are completely dental in origin with the excess growth.[1,2] The tissues of dental origin which are proliferated abnormally combinedly by the mesenchymal and epithelial components results in the form of odontoma. As these odontomas neither have any type of growth that is continuous nor to the tissues surrounding them, they infiltrate inside. So they can be considered as malformation of hamartomatous type. The lamina of dental origin from the bud of extraneous gives rise to the odontoma.[3,4,5] Usually, the odontoma is linked with retained or impacted teeth.

Odontoma etiological factors[6-10]:

(a) History from family

(b) Processes which is infectious and inflammatory.

(c) Hereditary factor leading to anomalies in conjunction with Gardner and Hermann's syndrome

(d) Alteration in genes giving rise to the aberrations in the pathways which gives signals for the development of tooth to be controlled.

(e) When the odontoblast activity is hyper.

(f)During dentition of primary type, under trauma.

Review of literature

Aspects, Epidemiologically: The tumors of odontogenic type are recorded worldwide which varies in the frequency range of 1-32%.[11,12] is the odontoma. They are as per various studies reported higher in females of age group 17 to 22 years in the other hand others reported higher in males of age group from 13 to 25 years,

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as per the meta-analysis conducted by Hidalgo O et al in the year 2008 odontomas, characteristics is sorted out epidemiologically. By a study of cases of 3,065 patients resulting in males about 50.6% and females to 49.4%.[13] To conclude with no difference significantly was present as per the gender is concerned.[14] Odontomas are identified by a wide range of age factors reported, predominantly in the life of 2nd decades.[15]

Characteristics–Clinically: There are three presentations found clinically in the literature scientifically that have been recognized in the case of odontoma: extra-osseous, intra-osseous and erupted. The commonest type of odontoma is intra-osseous.[14] The canine to incisor is the frequent location reported in the maxillary arch about 67% then is the anterior mandibular arch with that next region is the posterior mandibular arch about 33%.[15,16-18,19]The complex type of odontoma occurs frequently in the mandibular arch,posteriorly about 59% but the compound odontomas usually appear in the anterior- maxillary arch in the region over the crown in un-erupted, one or more teeth, in some cases appears between the teeth of erupted root region about 61%.[20,21]These tumors are not of aggressive nature which is symptomless, benign and are elicited radiologically in about many cases reported upto 57%. In isolated, unusual cases these odontomas are reported to be found in the nasal passage, antrum of Highmore, and the orbital floor. The odontoma commonly is intraosseousand from the cases reported never have they ever erupted towards the cavity of the orbit. As per Suenagaetal, the odontoma of complex type in a case was found to be located at the fossa of the nasal cavity of the right side leading to obstruction of the nasal region with bleeding.[22]

Characteristics- Radiologically: The odontoma of complex type exhibited with radiopacity that is unique. Accordingly, the degree of calcification of odontoma, 3 stages in the development are identified- 1st stage lesion is radiolucid by calcification lacking of tissues of dental origin, the stage of intermediate characterized which is calcified partially and the stage finally shows radiopacity which is surrounded with a halo of radiolucidity.[23]

Characteristics- Histologically: The odontoma of complex type characterized with presence of tissues of dental origin comprising of enamel that is demineralized,dentine, with cementum sometimes pulp included exhibitinga mass that is disorganized of dental hard tissue, peripherally the epithelial odontogenic strands are found, sometimes cementicles, cells of phantom and epithelium of the ameloblasts is detected. These odontomas can get associated with other tumors of odontogenic typeas Fibroodontoma, Ameloblastic fibroma, Ameloblastoma, Calcifying Epithelial Odontogenic Tumor, Odontoameloblastoma, Dentigerous Cyst and Odontoameloblastoma. For this reason histopathology report is needed in every case.[24]

Differential Diagnosis: The odontoma of complex type in the phase of intermediate resembles lesions like lesions of fibrooseous, origin, Fibrous Dysplasia,Calcifying Cystic Odontogenic Tumor and osteomyelitis of chronic type.[25] The most frequent type of odontogenic tumor associated with odontoma is Calcifying Cystic, found about 24% cases.[26] When surrounding the crown it could be differentiated with Adenomatoid Odontogenic Tumor, Ameloblastic Fibro-dentinoma/odontoameloblastoma, and Calcifying Epithelial Odontogenic Tumor.[16]

Prognosis: The odontomas may have complications as: teeth are impacted,obstruction of the nasal cavity, syndrome in the dental region.[24] The major cause of impacted incisors in the permanent dentition by which the eruption of the teeth is hinderedis due to the odontoma besides an extra number of teeth.[16]In rare cases may cause an inflammatory reaction of the adjacent (soft) tissues/infection along with suppuration when the odontomas erupt spontaneously into mouth. From the reported cases more often complex odontoma occurs rather than compound type of odontoma.[17] In cases with erupted type of odontomas, it exhibits the formation of abscess and caries.[27]

Treatment: The treatment of choice in these cases is by conservative enucleation surgically by removing the tissue of conjunctive capsule surrounding it and a sample is prepared for histopathological report which will make the diagnosis accurate.[24,25] In some cases treatment, orthodontically is required for the repositioning of the arch for the retained tooth.[28] The prognosis is favorable and the rate of relapse increases in the cases an enucleation has been conducted in the first stage of calcification.[16,23]

Conclusion

The review of literature revealed the view that is quite wide for the complex odontoma in descriptive means. The complex odontoma is frequently tumor of odontogenic type which could be detected by

radiographic routine examination,with that commonly seen in the patients at their 2nd decade. Hence it's advisable to diagnose the condition timely and it should be accurate to avoid complications. The analyses of histopathologically is recommended by which the association with another tumor of odontogenic type can be ruled out.

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3-Dimensional Plating System in the Management of Mandibular Parasymphysis Fracture: A Case Report

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Abstract

Introduction: The aim of treating mandibular fracture is to recover the anatomic structure and function with special caution in restoring the occlusion. Champy's miniplate fixation has been the most commonly usedprocedure amongst the various treatment modalities available. The 3-dimensional (3D) plating is fairly new for treating mandibular fractures.

Aim: This research was performed to equate the effectiveness, flexibility, and rigidity of 3-dimensional plates with that of 2 mm titanium miniplates in anterior mandibular fracture surgical management.

Material and Method: The study was conducted on a singlepatient with anterior mandibular fracture (Right parasymphysisregion).

The patient's preoperative, intraoperative, and postoperative clinical and radiological findings were recorded in a proforma.

Results: The timerequired to adapt and fix the miniplates were slightly more than3-D plates and results were significant. Skeletaland occlusal stability was maintained.

Conclusion: the 3-dimensional plate is as effective as conventional miniplates in the treatment of anterior mandibular fractures. Its the ease of use and reduced working hours that are advantages over traditional miniplates.

Keywords: *Anterior Mandibular Fractures, Champy's Miniplate, 3D Plate.*

Introduction

The mandible of an adult human being is made up of spongiosa in the center surrounded by compact cortical bone at the outer and inner aspects. Out of the two cortical plates, the buccal cortical plate is thicker than the lingual cortical plate and thickest in the symphysis region. On average the cortex is 5mm thick; in some cases, it may be less than 3mm thick. Maximum stress is distributed along the lines in spongiosa. A breakthrough was achieved, when Mostafa Farmand.et.al in the year 1992 has developed a new miniplate system, called a three-dimensional plating system. Titanium 3D miniplate is the most recent internal semi-rigid fixationdevice for maxillofacial fracture management. Shortcomings of rigid and semi-rigid fixations lead to developing the three dimensional (3D) mini plates. The 3D miniplate consists of 2mm 4-hole miniplate, which is joined by the 4 interconnecting cross struts. In combination with the unicortical screws, the 3D plate forms cuboids, which provides three-dimensional stability at the fracture

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site. Although some of the studies on biomechanics confirmed sufficient stability at the fractured site, only a few studies previously reported clinical experiences of the mandibular symphysis and parasymphysis fracture treatment using 3D miniplates.

Case Report: A 30-year-old patient reported to the department of oral and maxillofacial surgery with the chief complaint of pain in the lower front tooth region for 7 days. The patient gave a history of a road traffic accident a week back and emergency treatment was given at a nearby hospital. The patient was conscious with a GCS score of 15 (E4V5M6). The patient gave a history of bleeding from the mouth immediately after the trauma but no history of unconsciousness & bleeding from the ear and nose as well and no intraoral laceration. Extra orally 2cm laceration was present near the chin region.

On intraoral examination, a step was felt at the incisor region which was tender on palpation, and hematoma (Coleman sign +ve) was seen on the floor of the mouth. There were no other injuries elsewhere in the body; all the teeth were clinically present. Based on the history and clinical features, a provisional diagnosis of mandible fracture involving the right parasymphysis region was made. The radiograph revealed a well-defined radiolucent line running between both the lower central incisors to the inferior border of the mandible. Exploratory surgery was explained to the patient and informed consent was taken. After all the basic hematological investigations and pre-anesthetic checkups, the patient was taken for open reduction and internal fixation under general anesthesia.

Treatment Done: Erich's arch bar fixation was achieved under local anesthesia preoperatively. Under the proper aseptic condition, the patient was painted intraorally and extraorally using 2 percent providone iodine, and draping was done after infiltration of 2 percent lignocaine (1: 80,000) at the surgical site. An extraoral incision was madefrom a pre-existing scar. The mentalis muscle was exposed and incised perpendicular and deep to the bone, leaving a flap of muscle attached to bone for closure. A full-thickness mucoperiosteal flap was carefully raised, keeping the neurovascular mental bundle intact and exposing the fracture site. The segments were manipulated and reduced to its anatomic position satisfactorily after obtaining adequate exposure of the fractured segments. After the fractured segments were reduced, temporary intermaxillary fixation was performed to ensure sufficient occlusion, and a 2 mm 3-D non-locking titanium miniplate was used to repair the fractured section. Betadine & saline was used to irrigate in the area, and adequate hemostasis was achieved. The wound 's deeper layers were closed with Vicryl 3-0, and the skin was closed with ethilon 5-0. Intermaxillary fixation was released, and extraoral adhesive pressure bandage was given. Postoperatively, a radiograph was taken to check the adequacy of the reduction and fixation. On 1st week, 1st month and 3rd-month follow-up was done after the of discharge the patient. However, the patient was also instructed to report post-operatively for any complaint. The occurrence of complications was checked at each appointment, apart from routine examination and wound care.

Pre-Operative:



Figure 1. A: Pre-Operative Profile Picture B: Pre-Operative Occlusion; C: Pre-Operative Opg.; D: Pre-Operative Occlusal Radiograph

Intra-Operative:

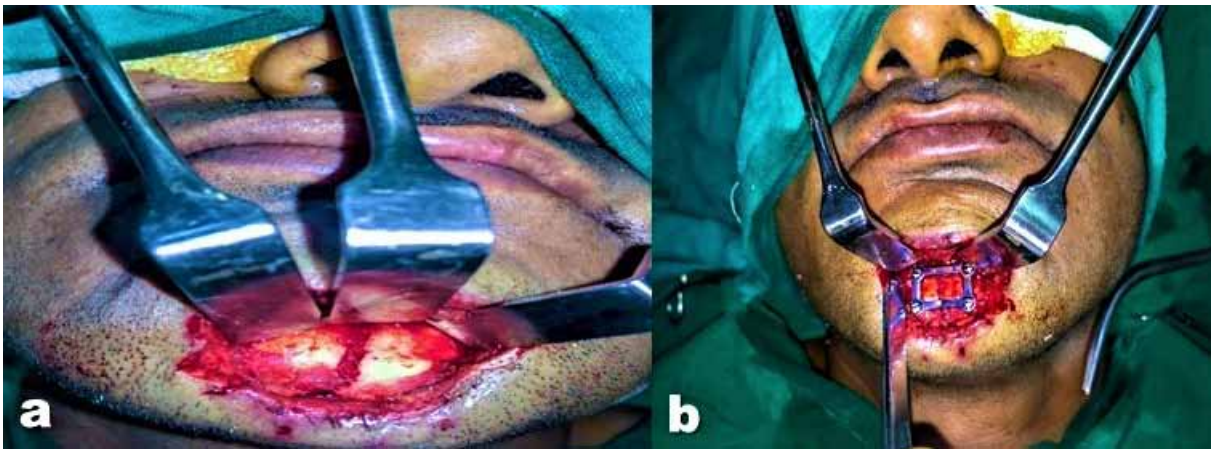


Figure 2. A: Fracture Present; B: 3d Plate Fixed

Post-Operative:

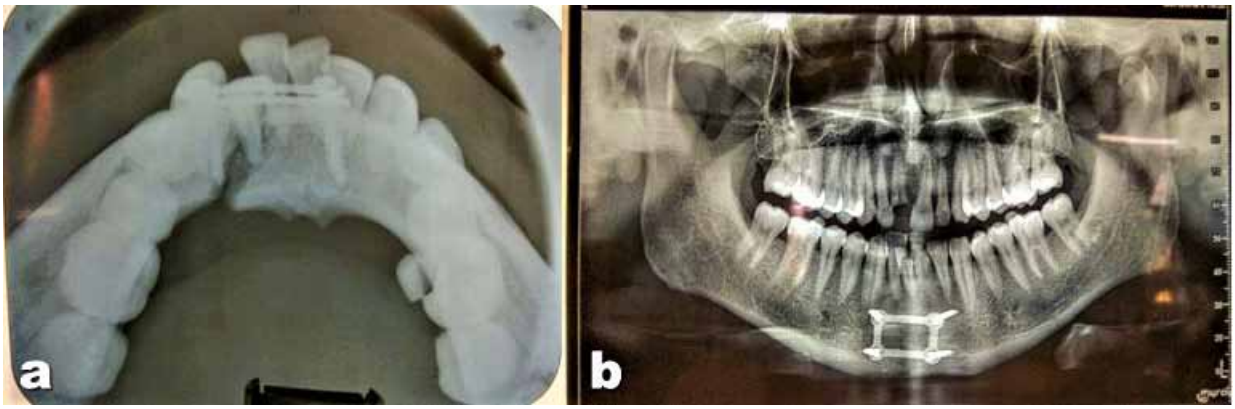


Figure 3. A: Post-Operative Occlusal Radiograph; B: Post-Operative Opg

Discussion

Mandible fractures are the commonest fracture on the face usually caused by accidents and other possible etiologies may be interpersonal violence, falls, sports injuries, etc.[1]. The maximum mastication force in a healthy adult man with healthy teeth is measured as 600 N force at the molar region, which comes down to 290 N force at the incisor region.[9]

The recently developed 3D plating system offers significant benefits relative to traditional miniplates. Compared with conventional miniplates, the 3D plating system uses fewer plates and screws to stabilize the bone fragments. Thus it uses lesser foreign material, reduces the operation time and overall cost of the treatment as described by Zix J, Lieger O and Iizuka T(2007) and Farmand M (1995).[2,3,4] The principle of management of mandibular fracture has always been repositioning to its proper anatomical location and fracture site immobilization. For fifty years, the ease of which anesthetics are working for the pain management and newer techniques of radiographs for the diagnosis of the fracture, introduction of antimicrobials to manage infection, specific surgical armamentarium, and newly generated biomaterials in the market allows surgeons to minimize the surgical mistakes during surgery. [6] There is limited space available in the region of mental foramen for two miniplates. Because of the anatomical issues, sometimes it is difficult to avoid damage to the root apices and the mental nerve while putting two miniplates. Despite utmost care and the use of monocortical screws, injury to the root apices cannot be avoided always during drilling and placement of screws. The normal distribution of forces should be restored. So,

fixation elements have to be placed near the tension site (ideal osteosynthesis line)[8] or on the superior side as put forward by Champy.et.al.

3D miniplate is based on the principle that it obtains support byits geometrically stable and closed quadrangular structure. The quadrangular geometry of 3D miniplate assures greater stability and also offers good resistance against torsional forces at the fractured site. Ideal designs of the three-dimensional plates are square plate (1cm×1cm), rectangular plate (1cm×0.5cm), continuous square or double square plate (2cm×1cm), continuous rectangle or double rectangle plate (2cm×0.5cm), double rectangle plate with an intervening square (2cm×1cm). The thickness of the standard three-dimensional miniplate is 1mm and the diameter of the plate hole is 2mm.

The main advantages of semi-rigid fixation by using 3D miniplate are:

- Excellent profile design and space between the plate holes provide good revascularization.
- Excellent biocompatibility, pliability, no stress shielding effect.
- The operator may simultaneously observe anatomical reduction at the fracture site as well as the occlusal relationship of the patient.
- Instead of the bony curvature of the symphysis and parasymphysis region, 3D miniplates are easy to adapt rather than the compression and reconstruction plates.
- Blood supply not disturbed since only minimal dissection necessary.

Pre and post-operative mandibular occlusal radiographs were taken to evaluate lingual splay of the fractured mandible as was done by Claude Guimond. et.al[10]. Postoperative occlusal radiographs were taken on 3rd postoperative day. The postoperative infection was managed by antibiotics(Tab. Amoxicillin trihydrate 500mg + Potassium clavulanate 125mg and Tab Metronidazole 400mg, TID, 5 days). The masticatory difficulty was managed by intermaxillary fixation postoperatively for 3 weeks.Occlusal stability of the patients was recorded postoperatively for change and stability on first, second, fourth, and twelfth weeks respectively. No or minimal loss of the occlusal relationship of molars was seen.

Conclusion

From the results of this study, we can say that the 3D plate is effective for the management of anterior mandibular fractures. The 3D plate offers an edge in the treatment of symphysis and parasymphysis fracture with reduced operating time and less implant material, with clinical outcomes somewhat identical to those obtained with traditional miniplate osteosynthesis.3-Meets the treatment objectives of adequate reduction, fixation, and stabilization of mandible fractures.We conclude that the 3D titanium miniplate is a superior method for the management of anterior mandibular fracture as it provides better stability and approximation radiographically, helps in osteosynthesis, and can restore normal form and function easily. This 3D plate is made up of titanium which is a biocompatible material and is a desirable mode of treatment of anterior mandibular fractures.

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Endodontic Therapy in Single Visits

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Abstract

Endodontic success usually depends on the localization of canals along with the proper shaping as well as cleaning of the root canal system in addition to 3 D Obturation of the canal system. Root Canal (or endodontic) therapy in single visits can be defined as a non-surgical as well as conservative management of an endodontically associated tooth that consists of biomechanical preparation with obturation of the root canal system in a single visit. The purpose of endodontic therapy is thorough mechanical as well as element debridement of the total pulp cavity as well as its full obturation with static restorative material. There has been a subject for long eminence debate within the dental community regarding the advantages and disadvantages of single sitting root canal therapy versus the multiple visit root canal therapy. With modern advanced technologies and surfacing of new gadgets as well as evidence-based dentistry along with the concept of maximum dentistry in minimum visits has given an urge to a new venture in the treatment protocols in dentistry. This review article discusses the advantages and microbiological considerations of single-visit endodontic therapy.

Keywords: *Single visit, Endodontic therapy, Advantages.*

Introduction

The purpose of endodontic therapy is thorough mechanical as well as element debridement of the total pulp cavity as well as its full obturation with static restorative material. Endodontic success usually depends on the localization of canalsalong with the proper shaping as well as cleaning of root canal anatomy in addition to 3 D Obturation of the canal system. Root canal therapy in single visits can be defined as a non-surgical as well as conservative management of an endodontically occupied tooth that consists of biomechanical grounding with obturation of the root canal system duringa single visit. This review article discusses the advantages and microbiological considerations of single-visit endodontic therapy.[1]

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Evolution of Single Visit Endodontics[2]:

(a) Dodge JS. The 1880s: Concept of single-visit root canal management Ferranti 1950s.

(b) Tosti 1970: Clinical study using a single-visit approach.

(c) Rudner and oliet: 1983 described a concept and clinical technique for treating teeth in a single visit.

(d) Ashkenaz. P.J. 1984 Defined and enumerated the indications and contra indications for single sitting endodontics.

Indications: Uncomplicated vital teeth. • Anteriors and fractured or bicuspid teeth anywhere aesthetics is the concern. • Indicated for endodontic surgery. • Nonvital teeth along with sinus tract. • Patients who are medically compromised and require antibiotic prophylaxis. • Patients who are physically compromised and are not able to visit the dental clinics frequently• Intentional root canal therapy. • Patients requiring full

mouth rehabilitation. • Some of the re-treatment cases. • Accidental/Mechanical pulp exposure. • Vital pulp exposures because of trauma with symptomatic pulpitis. • Vital pulp exposure because of caries with indicative pulpitis • Teeth in need of urgent post-placement, everywhere esthetics is the alarming thing.[3]

Contraindications:

• Developmental anomalies teeth like calcified as well as curved canals. • Teeth with periapical pathology as well as non-vital teeth without symptoms as well asno sinus tract. • Pus discharge with acute alveolar abscess cases. • Acute apical periodontitis along with severe pain on percussion. • Symptomatic non-vital teeth and no sinus tract. • Most of the re-treatment cases. • Allergies to medications or any kind of restorative material • Teeth having restricted access. • Patients who are not able to keep mouth unlock for a longer duration for example TMJ disorders.[4]

Clinical Advantages:

• Clinicians have the most intimate awareness of canal morphology, immediately the following instrumentation, and need not reorient themselves with the peculiarities of particular teeth.

• No risk of bacterial regrowth and leakage of the temporary seal.[6]

Single-Visit Versus Multiple-Visit Root Canal Treatment

The small chance of a life-threatening reaction is reduced by not repeating procedures such as local anesthetic injection. • For patients at threat of contracting bacterial endocarditic, the American Heart Association (AHA) had recommended completing as many procedures as possible during the antibiotic course. • Anxiety as well as post-operative discomfort of patients will be limited only to one episode. • If hemorrhageor else exudation occurs, it may be tricky to manage • Complicated cases utilizing extremely fine, calcified, multiple canals could not be treatable in one appointment without causing undue stress for both the patient and the clinician. • The clinician may be deficient in expertise correctly to manage a case in one visit. This could result in failures as well asflare-ups.[6]

Practice management advantages:

• Prosthetic work can begin without delay. • The risk of canceled appointments is reduced. • Increase in the number of teeth that patients can save. • Several types of equipment and restorative materials that are needed for another separate visit like suction tips, disposable bib, anesthetic as well as irrigation needles along with rubber dams can be saved. • Time is also not wasted as there is no requirement of giving reappoint to the patient and also nor reconfirm appointments • Medico-legal risk is reduced: AHA guidelines are followed, the likelihood of cross-contamination is minimized and invasive procedures are fewer.[1,5]

Patients Advantage Patient expediency–There will be no need for discomfort regarding repetitive local anesthesia, treatment procedure as well as no additional appointments for the patient. • Patient ease – decrease in the number of frequent visits and repetitive measures of injections can be avoided.[7]

Restorative consideration–Effective coronal seal, as well as esthetics, can be achieved by distinct visit or by urgentfixation of coronal restoration (post and core placements) • The total economy will get increase both for the patient and for the dentist by not wasting chair side time as well as using less restorative material. • Overall anxiety, as well as fear towards treatment, will decrease for some years.[8]

Disadvantages:

The access to the apical canal will not be an easy task if there is a flare-up. • Usually cliniciansget tiredness with long and repetitive working hours such as extended one -appointment operating time. • Patient fatigue - The longer single appointment may be tiring and uncomfortable for the patient.[9]

Adjuncts to provide resourceful plus quicker management during solo endodontic therapies:

1. Pain management and isolation access cavity preparation
2. Light as well as magnification use of irrigants
3. It relaxesthe concerned patient plus saves time
4. It is advisable to make use of a long-acting local anesthetic mediator.
5. It also helps tomanagepost-operative pain.
6. Now and then supplemental anesthesia is indicated. These include 1. Local Infiltration 2. Intrapulpal injection 3. Intraosseous injection[10]

The utilization of a rubber dam is compulsory in endodontic therapy. The rubber dam use in endodontics ensures the following:

1. The patient is confined from aspiration of required instruments, tooth debris, and medicaments along with irrigating solutions. 2. A surgically hygienic operating field is inaccessible from saliva, hemorrhage as well as other tissue fluids. 3. Soft tissues are extracted and also protected. 4. The visual site is improved. Isolation access cavity preparation before access cavity preparation, Caries usually is removed early, ahead of the pulp chamber entrance. The roof of the pulp chamber is best perforated with a round bur. A. No 2 bur - anterior and premolar teeth and a No.4 should be used in molar teeth. B. Once the roof is perforated, tapered, Flame shaped and round ended tapered diamonds are excellent for endodontic access.[11]

An uncovering receded or calcified root canal orifice is a challenge. • Use of low - speed smaller burs. • These burs have an extra-long, flexible shaft that allows in visualization by the operator as the bur advances into the deeper portions of the access preparation. • For identifying canal orifices as well as to determine canal angulation DG-16 endodontic explorer and CK-17 endodontic explorer can be used.[12]

Fiber-optic endoscope• A recent addition to the field of visualization is a fiber-optic endoscope designed for intraoral use. • The Orascope uses a fiber optic probe, xenon light source, and a medical-grade video monitor to provide a magnified image of the operating field.[13]

Use Of Irrigants: The theory of single visit root canal management is based on the entombing theory. • Even though instrumentation of the root canal is the principal method of canal debridement • Especially in the case of single visit endodontics, irrigation plays a critical role as there is no scope of taking advantage of intracanal medicaments. • Irrigation serves as a bodily flush to get rid of smear layer, debris plus serving as a bactericidal agent, tissue solvent as well as lubricant.[14]Of all the presently used substances, sodium hypochlorite appears to be the most ideal • NaOCl is efficient against endodontic germscounting those tricky to get rid of root canals, for example, Enterococcus, Actinomyces and Candida organisms. • NaOCl solutions are used in a concentration ranging from 0.5% to 5.25%.[15]

Sodium hypochlorite in combinations- • There is ever-increasing evidence that the efficiency of NaOCl, as an antiseptic agent is augmented when grouped with other solutions such as calcium hydroxide, EDTAC, or Chlorhexidine. • With Chlorhexidine- Kuruvilla and Kamath 1998 in a study combined alternating use of NaOCl and Chlorhexidine gluconate irrigants and results indicate a superiorfall of microbial flora (84.6%).[16] EDTA-Goldman et al showed that the residual layer is not disconnected by NaOCl irrigation alone but it is removed with EDTA. Ultrasonic activation of sodium hypochlorite has also been advocated, as this would "accelerate chemical reactions, create cavitational effects, and achieve a superior cleansing action".[17]

To illustrate the modern endodontic procedural sequence for single visit endodontics, an itemized sequence follows1. The diagnosisgives a positive indication for the need forendodontic treatment and therefore the tooth is anesthetized. 2. Subsequent placement of the rubber dam, then access is being made. 3. The pulp chamber is precisely prepared by thorough use of microscope at low to mid magnification size 2 for inspection. 4. The floor of the chamber is examined for additional canals under high magnification (16–24) because more than 50% of molar teeth have a fourth canal.[18, 19]

Conclusion

There has been a subject for long-standing debate within the dental fraternity regarding the advantages and disadvantages of single visit root canal management versus the multiple visit endodontic management. With modern advanced technologies and materialization of new gadgets as well as evidence-based dentistry along with the concept of maximum dentistry in minimum visits has given an urge to the new venture in the treatment protocols in dentistry. Careful case selection and proper and thorough adherence to standard endodontic principles, with no shortcuts, should result in successful one-appointment endodontics. Practitioners should attempt one-visit root canal treatment only after making an honest assessment of their endodontic skills, training, and ability.

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Hemangioma and its Management

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Abstract

The highest frequent benign vascular neoplasms that occur quite often in neonates & infants are haemangiomas. Haemangiomas usually occur on the surfaces of the head and neck regions. Management of haemangiomasincludes wait and watch, drug therapy, sclerotherapy, cryotherapy, radiotherapy, laser therapy and surgical excision.Size, location, hemodynamic of the lesion and age of the patient are the important conditionsthat need to consider while planning for treatment of haemangiomas.

Keywords: *Haemangiomas; Head and neck tumor; Management; Treatment.*

Introduction

Haemangiomas are commonest benign vascular neoplasms that occur often in neonates, infants, and children. Microscopically thereisa proliferation of endothelial cells, which comprise a space comprising blood that is covered with an endothelial layer. Haemangiomas are classified into two types according to development,they are infantile and congenital haemangiomas.[1] Infant haemangiomas (IHs) seems to be the most common childhood tumor that occurs in 5-10 percent of children. These are vascular tumors associated with abnormal propagation of endothelial cells and anomalous architecture of the blood vessels. They can be differentiated from vascular malformations, which are structural anomalies and inborn vascular morphogenesis errors. These include capillary anomalies (includes port-wine stains), venous anomalies, lymphatic anomalies (also known as lymphangiomas or cystichygromas).[12] Infantile haemangiomasare benign, self-limited vascular endothelial neoplasms which commonly affect the maxillofacial and cervical region.[2] These tumorshave 3 phases' i.e. apparent neonates and grow rapidly to its maximum dimension during the first year of life, preceded over several years by stabilization and spontaneous involution. Spontaneous resolution occurs in 49% of patients within 5 years and 7 years of age 72% of cases resolved spontaneously. But few lesions can take up to 10 years to resolve. These tumors don't typically pose any functional issues; but in approximately 10-20 percent of cases, where there will be facial disfigurement, airway compromise due to pressure, loss of vision or ulceration, active intervention is required.[3]

IH are classified based upon their depth of lesion.[4] It includes:

1. "Superficial IH": bright red, thin, frequently paved/ pebbled, vascular papules/plaques, formerly known as "strawberry haemangiomas"
2. "Deep IH": blue/violaceous or skin-colored, compressible, subcutaneous papule/nodules, formerly known as "cavernous haemangiomas"
3. "Combined IH": both superficial and deep components, oftenfound as superficial IH overlying a deep IH component

IH classified based upon anatomic configuration includes:

1. "Focal IH": a solitary, isolated IH, frequently a papule/plaque/nodule

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2. "Segmental IH": IH involving larger region or whole body (i.e., hand, lower leg, hemi-face) in a well-demarcated area
3. "IH indeterminate": IH those does not fall uniformly into either category or that doesn't proliferate fully/ evenly[4]
4. "Multifocal IH": multiple, discrete focal IH not occupying a specific area or territory.

"Congenital haemangiomas (CH)"can be classified as below:

1. "Rapidinvolution congenital haemangioma" (RICH),
2. "Partiallyinvolution congenital haemangioma" (PICH) and
3. "Non-involution congenital haemangioma" (NICH)[3]



Figure 1

Initial Evaluation & Management: Haemangioma's management must be planned based on the patient's age, the diameter & extent of the lesion, growth rate, complexity (superficial, deep or segmental),complications (like ulceration or vision loss, or vital organ dysfunction) and location. There is a 4% increase in the need for intervention for approximately every 10 cm rise in lesion diameter.[5] Two systems were proposed to help clinicians evaluate the lesion extension and the response to the therapy. Haemangioma Severity Scale (HSS), developed in 2012, was designed to classify patients based upon complications and help in guiding treatment needs. Patients scoring above 10 usually needed beta-blocker therapy with the HSS. Higher HSS scores were associated with a raised presence of an ulcer, structural abnormalities, and everlasting disfiguration. Hemangioma Activity Score (HAS) had been proposed in 2011, based in large measure on IH lesion color. One prospective study conducted by the HAS system's creators compared HSS to HAS and concluded that the HAS system was preferable in assessing the response of therapy.[2] Generally, lesions that are segmental, large in number, or peri-orbital should be considered complicated. Additionally, lesions larger than 5 cm in size may raise skeptical for extensive underlying defect.[6]

Phace Association: PHACE syndrome is a rare condition diagnosed in 1996 and with limited research data. Following conditions are associated with PHACE syndrome:

Malformations of posterior fossa of the brain: Haemangioma, Anomalies of the artery, Aortic coarctation/other cardiac problems, Malformation of the eye.

The white female neonates with massive haemangiomas of the face are at a risk for PHACE syndrome must always be screened for extreme aortic coarctation using echocardiography or cardiac magnetic resonance imaging. Systemic beta-blockers cannot be given to these patients as coarctation is a contraindication for b-blocker usage. Before treatment selection, MRI of the head and neck with angiography is suggested as vascular malformations involved with PHACE syndrome can lead patients to hypotension condition.[7-11]

Non-operative Management: Medical intervention usually not necessary for Small, uncomplicated, localized, superficial capillary haemangiomas. Management involves reassurance, teaching, and the setting of expectations with sequential followups.[2] Many IHs are operated by observation because 90 percent are minimal, localized, and do not include areas of aesthetic or functional significance. In the proliferative stage, infants are closely monitored regularly if a lesion has the potential to cause obstruction, damage, or ulceration that needs intervention. Once the IH has stabilized in growing, patients are followed annually during the involving process, if intervention in childhood may still be necessary.[12, 13]

B Adrenergic Blockers: Propranolol is a nonselective β-blocker commonly recognized as a first-line treatment for complexhaemangiomas. Following the serendipitous discovery of the dramatic action of propranolol on lesions in 2008 in patients having steroid-induced cardiomyopathy under treatment for haemangiomas.[14] The efficacy has been verified by several broader research and randomized clinical trials. B-blockers action on haemangiomas is not clear still. Propranolol is believed to limit the development of the lesion by blocking proangiogenic activation of the vascular endothelial growth factor and the essential fibroblast growth factor. Apoptosis is also believed to be aggravated in the late phase of therapy.[15]

Since 2014, the US Food and Drug Administration has approved Propranolol for the proliferation of infant haemangiomas requiring systemic treatment. Complete or near full resolution of the infantile haemangioma is normal after 3-6 months of therapy during the proliferative stage. Patients of cardiogenic shock, chronic sinus bradycardia, chronic hypotension, 2nd or 3rd-degree cardiac obstruction, heart failure, H/O reactive airway dysfunction, aortic coarctation, preemies under 5 weeks and PHACE syndrome-associated vascular anomalies (predisposing to stroke in hypotension setting), a systemic beta-blocker is contraindicated.[16]

Dose: It is advised to initial treatment with 0.5-1mg/kg/day oral propranolol in 2-3 divided dosages with feed. The dosage may be raised progressively by 0.5 mg/kg/day over 2 weeks to a target dose of 2 mg/kg/day, with 2-3 divided dosages. Considering the possible side effect of hypoglycemia, it is highly advised to medication with feed.

Topical β-Adrenergic Blockers: Topical b-blocking agents are usuallyused to managetiny, superficial, uncomplicated,and ulcerated haemangiomas. 92 percent and 77 percent of patients having uncomplicated haemangiomas reported a substantial recovery in the color and dimension of the lesions, respectively, after 6-9 months of topical timolol therapy in a study. All topical timolol and propranolol found to have similar efficacy.

Corticosteroids: Previous to the introduction of oral propranolol, systemic corticosteroids had been the mainstay for IH management. The usage of systemic corticosteroids in managing haemangiomas is currently restricted to patients contraindicated to systemic b-blockers. The mode of action of corticosteroids on haemangiomas is not known but it has been shown that corticosteroids impede vasculogenesis by inhibiting the factor-A expression of vascular endothelial development.

Dose- While steroid dose had been variable, oral prednisolone at 2-3 mg/kg/day is a normal starting dosage every morning. Depending on the lesion response,usually, the course of treatment lasts for 6-12 months. Once the treatment response is achieved, a steroid taper should commence. Oral corticosteroids could result in adrenal crisis or rebound generation of haemangiomas if discontinued abruptly.[17]

Intra-lesional and Topical corticosteroids: In resistant IHs with mild ulcerations, highefficacy topical corticosteroids, such as clobetasol cream are used. Intralesional triamcinolone acetonide injections are restricted for tiny, localized, deep haemangiomas that are resistant to treatment. Dosage ranges between 10 and 40 mg/mL and shouldn't more than 3 mg/kg/day. Clinical lesion reaction is usually noticed initially in the first 2 weeks of injection, with further improvements over the subsequent months.

Laser Therapy: In fewer cases, pulse-dye laser (PDL) can be used to treat IH. While its use has been limited to ulcerated IH in the superficial or very early lesions where Pulse Dye Laser therapy tends to promote re-epithelialization and analgesia. It is also used subsequently for residual telangiectasias in life after IH involution. In combination with other treatments, like topical timolol or systemic treatment (propranolol or corticosteroids), PDL is quite effective when used for treating ulcerated IH.

Surgical Management: Surgical excision of infantile haemangioma is reserved for fibro-fatty residues, post involution phase scars, or deformed zones for reconstruction. Surgery could frequently be considered in patients who are unresponsive to conservative management in the proliferative stage.[18]

Sclerotherapy: Sclerosing agents' intralesional injection, such as bleomycin, may be useful in small lesions and only 49 percent of thorough resolution is recorded in literature. Sclerotherapy has been extensively used in the management of minorextraoral lesions. However, after the injection of the sclerotic agent into intraoral lesions, the pressurized bandage cannot be applied, thereby restricting the efficacy for intraoral use. Cutaneous necrosis and neural impairment are the commonadverse effects of sclerotherapy. Systemic complications are uncommon and linked to alcohol's absorption into the systemic circulation. These can involve hemolysis and cardiac arrest with possible renal toxicity. There must beno extravasation during injection.[19]

Embolization: Vascular anomalies already in advanced stages are generally best managed by preoperative embolization followed by surgically, particularly if not responsive to medicines. The efficacy of embolizing vascular lesion in normalizing function and bleeding prevention with decent cosmetic outcomes as demonstrated by Churojana et al. Nonetheless, extended intubation or tracheostomy must be considered in the condition of involving the tongue, oropharynx and laryngotracheal region to protect the airway from extreme inflammation or significant postoperative bleeding as claimed by them. Besides, the embolization procedure can have some possible disadvantages. The risk of iatrogenic embolization through majorcarotid arteries has been identified on intracranial vessels. Therefore, this procedure needs to be performed by an experienced radiologist, and temporary flow blockage is required.[3]

Radiotherapy: Radiotherapy especially forintraosseoushaemangiomas, has been described as a beneficial option. Though, its negative effects, like damage to the regular development of adjacent tissues, residual scarring, and neoplasm, are known and unjustified for benign neoplasms such as haemangiomas and vascularmal formation.[3, 20]

Conclusion

Although most haemangiomas could be observed without treatment, medical or surgical intervention will benefit others. While surgical excision remains the favored treatment, it can be appropriate for curettage, sclerotherapy, embolization, or a combination of those method. Additionally, routine follow-ups should be carried out due to the possible risk of complications and recurrence. [12]Where complications are possible or management is unclear, it maybe advisable to refer to an expert or multidisciplinary vascular anomaly center.

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COVID 19 Pandemic: Challenges in Healthcare System of India

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Abstract

The new coronavirus virulent disease is stretching the health organisations worldwide and because of this the growing demand for health services and health care workers rapidly increasing. This emergency has encouraged the researchers to get more serious about the deadly unknown virus. Gradually within 3 months, the virus kept spreading all over the world by direct or indirect contact with the affected people. "The best defence against any outbreak is a strong health system". Focusing on COVID 19 associated actions, as well as progressing to endow with necessary services, is significant not only to sustain people's belief in the health system to distribute essential health services but also to lessen an increase in morbidity and mortality from other health conditions. This review article discusses the existing healthcare resources of India and the impact on healthcare scenario of India during this outbreak.

Keywords: *Covid-19 Pandemic, Challenges, Health care system of India*

Background

It was reported that on 17th November 2019 in China, the first case of Coronavirus came in front but due to lack of evidence it went unrecognised. But after that incident within not many days, 8 more new cases appeared during December. This emergency has encouraged the researchers to get more serious about the deadly unknown virus. Gradually within 3 months, the virus kept spreading all over the world by direct or indirect contact with the affected people. Since we are now living in the modernised technological era of 20th century and are connected globally, travelling through air and migrants connecting all over the world with each other by the means of flights is now supposed to cover the globe by spreading the infection. Because of this, the infectious virus has spread rapidly by March 2020. It was documented that anoutbreak situation has aroused globally, therefore on 11th March 2020, WHO announced that this coronavirus causing COVID-19 was officially a pandemic that has affected 114 countries in 3 months plus affecting over 118,000 people. [1]

A newly discovered strain was found in humans called a coronavirus which is the causative organism for COVID-19. The patient affected with coronavirus will be having respiratory problems, cough as well as a fever that may progress to pneumonia and then death. This infectious disease gets contaminant from one infected person to another healthy person through sneezing droplets. Focusing on COVID 19 associated actions, as well as progressing to endow with necessary services, is significant not only to sustain people's belief in the health system to distribute essential health services but

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also to lessen an increase in morbidity and mortality from other health conditions.[2] "The best defence against any outbreak is a strong health system". This outbreak due to coronavirus has tensed themedical system providing treatments and medicines in almost all the urbanized countries and is also making the economic slumpbeyond compare in recent times. This review article discusses the existing healthcare resources of India and the impact on healthcare scenario of India during this outbreak.

The response towards the pandemic situation by the Indian Government: India has bagged rank of 100on the "Oxford COVID-19 Government Response Tracker (OxCGRT)" to keep a record of in addition to make a comparison of responses made by the government to the coronavirus disease constantly. The government of India wants to stick on to their strategy of 'prevention is better than cure' model.[3]

Steps were taken by the government:[4]

(a) Every person coming from the outside country was mandatory to undergone through a screening test and this screening was being initiated by the government with the help of health workers during the initial days of January 2020. But if an individual is asymptomatic then he will be tracked for progression for further two weeks constantly by the health workers.

(b) All the domestic, as well as international flights along with the Indian railways, are being cancelled from 21st March till date.

(c) The major historical step taken by the government is total lockdown of for three weeks from 24th March.

(d) Well-harmonized action strategy including quick establishments of quarantine facilities across the country as active health laboratories were established.

Decisions are taken by the Government to strengthen the health care system of India:

1. Protocols for controlling this outbreak in the country are enforced under Biological & Public Health Emergencies (BPHE) as well as under the National Disaster Management Plan (NDMP) are made. Under these plans, they have started acting upon as listed below such as "Stockpiling of essential medical supplies such as vaccines and antibiotics etc." "Establishing adequate decontamination systems, critical care ICUs and isolation wards with pressure control and lamellar flow systems", "Adequate PPE for all the health workers associated with the responding to biological emergencies","Upgradation of earmarked hospitals to cope with emergencies", "Mobile telehealth services & Mobile Hospitals", "Specialised healthcare and laboratory facilities to address biological emergencies/incidents", "Establishing and strengthening quarantine facilities"[5]
2. Government has spread awareness as much as possible to avoid the risk of transmission of coronavirus to one another. Therefore,special care has been taken to promote the importance of hand hygiene, social distancing, face mask and hygienic social habits and disinfection.
3. India is the largest supplies of generic medicines all around the globe. Now India is known as pharma hub all over the world in the last few decades with a 20 % share worldwide by volume as well as a 50% share in fulfilling the need forvaccines. During this outbreak of coronavirus, India has again given a major contribution to the pharma industry as an opened export of hydroxychloroquine that can help save thousands of lives. Several medical types of equipment have also risen now during this outbreak and have started manufacturing PPE(Personal Protective Equipments), ventilators, facemasks, gloves, and diagnostic kits for coronavirus testing. This medical equipment manufacturing has strengthened the health care delivery system in India.[1]
4. The Indian government has utilized the technology by the means of telemedicine and training of primary health care through online training sessions for all the medical and paramedical staff frequently during this emergency outbreak situation according to the requirement for protocols to be followed for prevention and management of communicating disease. The government is also ready with its plan to utilize the MBBS students during this pandemic if required. Because of the additional needs of immediate hospitals throughout the country, the government has already converted several railway trains into isolation wards which can be utilized as mobile hospitals according to the demand of the situation.[6]

Challenges in health care delivery systems:

1. Patients of chronic disease are now suffering pain

in movement because of the restriction made by the government. For this reason, delay in treatment of such cases may lead to long term sequel. All these will ultimately end up in possible complications as well as deterioration of illness towards the body. Unavailability of quite a lot ofmedical setups blocked due to the management of this coronavirus outbreak. All this has made the restriction towards the accessibility of emergency management of acute conditions for the general people suffering from other diseases.Everything at the end has only increased the burden of diseases of our country.

2. India ranks 145 among 195 countries in provisions of ease of access as well as the quality of healthcare globally.[3] No doubt progress is being made constantly in the past but still, lots need to be done to improve the health resources. This need is felt now during this outbreak throughout the country as the situation might go out of our hands in recent times. Truth to the fact that Indian health infrastructure is running out of its health budget to provide the world-class health facilities to its 133 crore population may be because of low public investment in quality healthcare. India's economic and manpower health infrastructure is repulsively not enough to endow with quality resources to its overpopulated citizens. It should be noted that if the coronavirus outbreak can causedisaster in western countries with far better quality health resources, then our country India is sitting on a time bomb now in the present scenario.

Access to quality health care services for the vast population of India: Quality healthcare service is of no use if doesn't reach the people in need. According to the Global Burden of Disease, it was reported that in Healthcare Access and Quality (HAQ) Index,India is at the number 145 among 195 countries. The score was 41.2 for the reason that of out-of-pocket expenditure (OOP)- which means the total sum of health expenses waged from the bag of persons was a fraction of the whole public healthcare disbursement of the respective country.[7]

Poor public investment in healthcare: According to the National Health Profile(NHP) 2019, released in October 2019, India's public expenses on health (centre plus state) is less than 1.3% of the GDP for several years.[4]

Government initiatives of private investors in health care:

(a) Initiation of Pradhan Mantri Jan Arogya Abhiyan (PM-JAY) or the Aayushman Bharat which promises to offer hospitalization cover (insurance) of up to Rs 5 lakh to 10 croresfor poor as well asunder privileged families (about 50 crore people).

(b) This yojanais funded by the Centre as well as states share funds at 60:40 which is considered as the tax payer's money.

(c) Initiation of setting up secret medical colleges along with useful district hospitals through PPP (Public Private Participation) by the government.

Scrutiny of growing COVID-19 patients as well as existing medical capability: The outbreak of coronavirus has paralyzed the health infrastructure if many countries globally mainly because of shortage in medical equipment and resources like manpower, finances, hospital beds, and other relevant medical equipment like ventilators, personal protective equipment). This lack of essential services to the people during this outbreak has forced the Indian Government to take the historical decision of lockdown of 21 days and again it was further increased to 19 days and also few states are still demanding of a further increase in the days of lockdown. This lockdown is a necessary step because this is the only way of achieving social distancing that will help in slowing down the spread of disease. And this decision will help our health system to deal with the rising number of patients with limited resources.[8,9] However, due to the lack of doctors as per the population ratio, India is still lacking in making a sufficient number of doctors. For this reason, three scenarios are being considered:

Scenario 1: 1% of doctors treat COVID 19 patients,

Scenario 2: 5% doctors treat COVID 19 patients,

Scenario 3: 10% of doctors treat COVID 19 patients

Medical Facilities provided during this outbreak are as follows:

(a) A cheap fast testing kit is now available in the market that will give us results in two and half hours and is capable of testing 100 samples and costs is between 1200 rupees to 4500 rupees.[1]

(b) At present, now more than 29 laboratories are present in our country which are accredited by the Indian Council of Medical Research (ICMR) for testing of novel coronavirus as instructed by the government. These testing research laboratories have approximately 16,000 collection centres across the country. Apart from private labs, there are also 280 government laboratories approved by ICMR, of which 104 are operational for testing Covid-19 and also more number of labs are in the process of getting functional as per data collected from the ICMR March 24th, 2020.[1]

(c) Till now the Indian Government has already made 14000 ventilators along with lakhs of PPE and N-95 masks available for the use of COVID 19 cases. Special Covid-19 hospitals are now being opened as well as isolation beds are being added.[6]

(d) Economicsin India concerning the health sector has made provision of Rs 15,000 crore. Healthcare professional workers were promised three months' worth of payments for medical insurance which was an estimated total sumof 5 million rupees, or $66,802.[10]

Conclusion

Government of India is taking all the necessary steps to strengthen the health sector. India is very well taking care of its highly populated citizens. The government is providing all the necessary medical equipment and is taking necessary measures to control this COVID 19 pandemic. No doubt India has fewer resources and financially weak as compared to developed countries but still, India is fighting back very strongly providing all kinds of facilities and protection to medical doctors and nurses and health care workers. India is very well coping up with the challenges faced everyday due to this COVID 19 pandemic and it is hoped that India will stand strong during this outbreak.

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Conflict of Interests: None

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Ectodermal Dysplasia: A Case Report of Aesthetic and Functional Rehabilitation

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Abstract

During the development of embryo ectodermal dysplasia occurs as a consequence of disturbances of the endoderm. Clinical features include hypotrichosis and palmoplantar hyperkeratosis or alopecia,a triad of nail dystrophy,a partial or complete absence of primary and/or permanent dentition,and lack of sweat glands. In this study, the syndrome ectodermal dysplasia shows a severe case of anodontia. Since the oral rehabilitation of these cases is often difficult; particularly in pediatric patients, treatment should be administered by a multidisciplinary team involving pediatric dentistry, orthodontics, prosthodontics, and oral-maxillofacial surgery.

Keywords: *Ectodermal Dysplasia; Aesthetic & Functional Rehabilitation; Anodontia.*

Introduction

Ectodermal dysplasia is a heterogeneous group of inherited disorders, resulting from the abnormal development of two or more tissues at a time, derived from embryonic ectoderm.[1] Analyzing several ectodermal dysplasia cases reveals that the syndrome is an X-linked recessive trait. The first clinical cases of Ectodermal dysplasia were reported in early 1792 by Danz, who described the congenital absence of teeth and hair in two Jewish boys. The prevalence of the syndrome is more in the case of females than males. In some cases, the mother of the patient exhibited hypodontia/conical teeth. Syndrome ectodermal dysplasia is divided into two categories and among two categories anhidrotic type is more severe manifestation. Clinical features include palmoplantar hyperkeratosis, alopecia, and dystrophy of nails some may found with partial or complete absence of dentition.

Finn reviewed 82 cases of anhidrotic ectodermal dysplasia and found that 5.4% had more mandibular teeth than maxillary and 63.5% had more maxillary teeth than mandibular teeth. And many of the teeth are conical in shape.[3] There is always a lack of development of alveolar ridge in anodontia patients. The vertical dimension of the face is also reduced along with dryness of oral mucosa and malformation of existing teeth.

Histopathology: The epidermis of Ectodermal dysplasia is thin with effacement of rete ridges around it. Sebaceous glands, Apocrine glands are variably reduced and mucous glands of the upper respiratory tract may be sparse. Light and scanning electron microscope findings of hair shaft abnormalities are longitudinal clefts or grooves and transverse fissuring, the bulb of the hair shaft is dystrophic. Also, mandibular X-ray shows dental aplasia or hypoplasia. Other histopathology features including epidermal and follicular orthokeratotic hyperkeratosis, apocrine ducts enter follicles at abnormal locations, and comedo formation.

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Differential Diagnosis: Differential diagnosis including Alopecia Areata, Aplasia Cutis, Incontinentia Pigmenti, Congenital focal dermal hypoplasia syndrome, Pachyonychia Congenital, Naegeli-Franceschetti-Jadassohn Syndrome.

Case Report: An 8-year-old female patient visited the Department of Pedodontics and Preventive Dentistry, Institute of Dental Sciences, Bhubaneswar, India, with the chief complaint of the absence of teeth in her oral cavity since childhood and 7 primary teeth erupted in the oral cavity with dryness of the mouth. There was no history of birth complications during his delivery, and no other live family member presented a similar condition.

And other medical history was taken including vocal function, bleeding disorders, other syndromes associated with this. All the medical history examined were normal.

On clinical examination, the skin appears to be dry (Figure 1A)and there is a mild elevation of temperature. Hair examination showed thin and less hairs. On examination of eye dryness and reduced lubrication was seen. Brittle nails were observed on finger examination. Ears were large and gives an older look (Figure 1B) gives an older look as compared with those of his age with a normal intelligence and partial Anodontia (Figure 1C). Intraoral examination revealed dry mucous membranes and reduced vertical heights of both the arches were of considerable significance (Figure 1D & E). On radiographic examination, there is the presence of tooth buds of permanent molars. In this case, the treatment modalities include temporary prosthesis(Figure 2 A to D). After there is a proper growth of arch and face, implants and permanent restoration will be provided.

According to the history and investigation treatment modalities had planned and appointment for the patient was scheduled.

Appointment I- proper case history with intraoral and extraoral photographs of the patient and OPG had taken. Non-pharmacological behavior management had performed by demonstration of instruments and friendly communication with the patient. Preliminary impression had taken using alginate and perforated impression tray. The patient was recalled after 7 days.

Appointment II- Border molding procedure and secondary impression had taken using ZOE impression material. Other lab procedures including denture base, occlusal rim, and articulation had done.

Appointment III- Most important procedure for the removable prosthesis 'Jaw relation' including anteroposterior, horizontal, and vertical relation had performed. Lab work, teeth setting on occlusal rim had completed.

Appointment IV- In this appointment patient had recalled for teeth trying, masticatory movement of jaws, and speech analysis. After that, all the lab work as well as dearticulating, packing, dewaxing, curing, finishing, and polishing of the denture had done.

Appointment V- Adjustment of denture had done and the removable denture had delivered to the patient with the postoperative instructions.

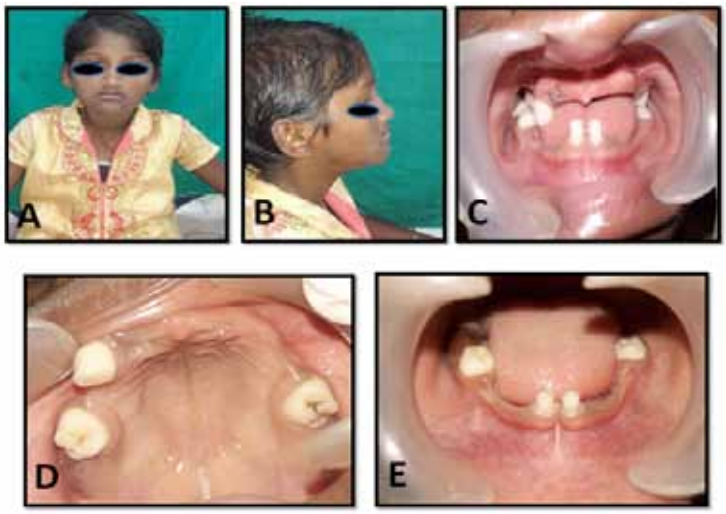


Figure 1A to E : (A) Dry skin, (B) Midface hypoplasia, (C) Partial Anodontia, (D and F) Upper and Lower Arch with a reduced vertical height of alveolar bone respectively.

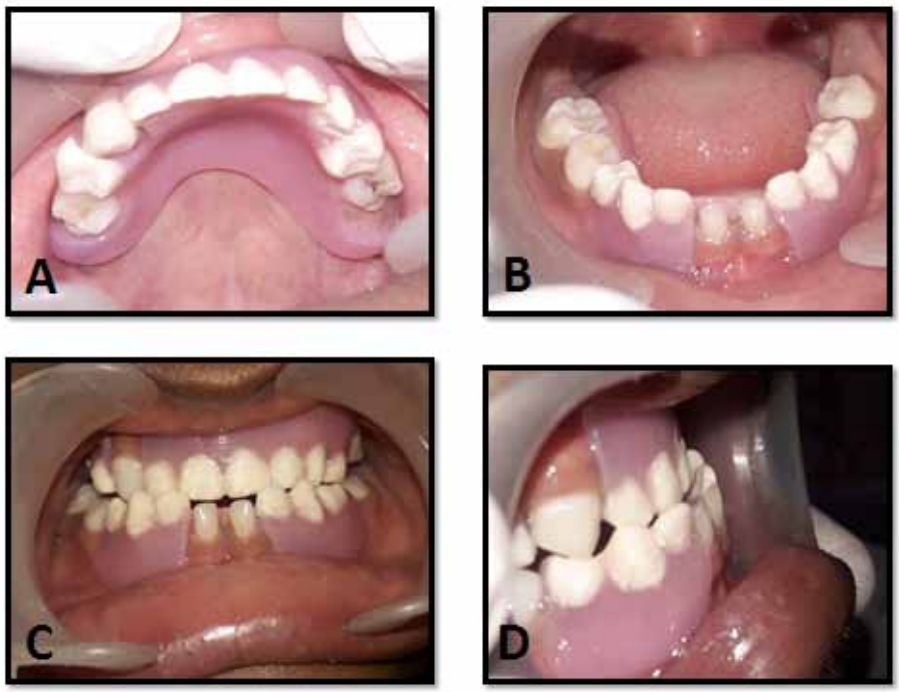


Figure 2A to D: Post-Operative Photographs

Discussion

Freire-Maia and Pinheiro proposed the first classification system of ectodermal dysplasia in 1982.[1] They classified ectodermal dysplasia into different subgroups according to the presence or absence of (1) anomalies of Hair(2) anomalies of Teeth (3) abnormalities of Nails (4) eccrine gland dysfunction.[6] As in the above-mentioned case report, all features of ectodermal dysplasia classification were present; like scanty hair, partial anodontia, thin-brittle nails, dry mouth as well as dry cornea.

Medical care of ectodermal dysplasia depends on which ectodermal structure is involved. In this case report, the temperature was raised,So it was advised for frequent intake of liquids to maintain hydration patient having dental issues are advised for early dental evaluation and treatment and encouragement for routine dental hygiene is done.[7] In the above case report, upper partial denture and lower complete denture was advised. After the construction of dentures, the patient was educated for adjustments and reconstruction of dentures at different stages of growth and advised that dental implants may eventually be required. Patients with xerostomia and reduced lacrimation may benefit from artificial saliva and tears respectively. The defect of the lip can be corrected by cheiloplasty to improve esthetics. The patient is advised to take consultation with a genetic counselor to find out the diagnosis and genetic analysis.[8]

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Ethical Approval: Approved

Conflict of Interest: None

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Lip-Nose Reconstruction Post-Cancrum Oris Defect: A Case Report

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Abstract

Cancrumoris is a disease of great antiquity described by Hippocrates and Galen. The termnoma, a common synonym for the disease, is derived from the Greek verb 'to devour'.Tourdes(1948) definedcancrumoris that is still substantially correct. Hedescribed the condition as a gangrenous affection of the mouth that especially attacks children in whom the constitution is altered by bad hygiene and serious illnesses such asexanthematous fevers. According to him, the disease is manifested by an ulcer of the oralmucous membrane, edema of the face extending from within out, and rapid destruction of the soft tissues and bone with an outcome that is almost always fatal. Nowadays, cancrumoris still affects children predominantly. It occurs very rarely in the aged with decreased hostresistance. Noma sequelae comprise bony and soft-tissue losses that affect both aesthetics and function. Complex defects should be treated in a staged fashion with the most complex initial stages performed in better-equipped institutions and less complex stages done on home ground. Although complete correction may be planned to restore function and aesthetics, thenoma patient may eventually be satisfied with a functional but less aesthetic outcome.

Keywords: *Cancrum Oris, NOMA, Cleft lip & palate, Nasal reconstruction.*

Introduction

Cancrumoris is a disease of great antiquity described by Hippocrates and Galen. The term noma, a common synonym for the disease,is derived from the Greek verb 'to devour'. Tourdes (1948) definedcancrumoris that is still substantially correct. He described the condition as a gangrenous affection of the mouth that especially attacks children in whom the constitution is altered by badhygiene and serious illnesses such as exanthematous fevers. According to him, the disease is manifested by an ulcer of the oral mucous membrane, edema of the face extending from within out, and rapid destruction of the soft tissues and bone with an outcome that is almost always fatal. Nowadays, cancrumoris still affects children predominantly. It occurs very rarely in the aged with decreased host resistance.[1] Although a reconstructive surgeon may be keen to apply the principles of oncologic reconstruction to noma surgery, it must be noted that the 2 are dissimilar and a slightly different approach is necessary.[2] Craniofacial defects are rare, disfiguring facial anomalies, with an incidence of about to 4.9 cases per 100,000 live births. Craniofacial clefts may be caused by the failure of the maxillary processes to fuse, external pressure, amniotic bands, oligohydramnios, central disorganization of the neural crest, and hematomas.[3] In 1973, Tessier classified craniofacial clefts based on clinical, radiographic, and

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surgical findings. The median cleft of the upper lip, or Tessier 0 class, has a variety of presentations, ranging from a minimal notching of the lip, vermilion, and nose to a wide cleft that divides craniofacial structures.[4]

Although great improvements have been achieved in the primary correction of the bilateral cleft lip and nasal deformity, secondary lip and nasal deformities occur in a significant number of patients. Typical secondary deformities involve broad nasal tip projection, flared alar rims, fistulae of the nostril floor, a short columella with varying degrees, and a vertically short upper lip (whistle deformity), in which severe short columella complicated with marked philtrum deficiency poses a highly demanding challenge to plastic surgeons. The columella and philtrum serve as two functional and aesthetic subunits. In deformities, a short columellaoften includes a deficiency of skin, nasal mucosa, and supportive alar cartilages.[3] We present a case of an adult patient with noma sequelae managed by a staged approach. Although complete correction was initially planned, following the patient's requests, the last stage was abandoned and a functional but less aesthetic result accepted.

Case Report: A 55-year-old lady reported to our dept. of Oral & Maxillofacial Surgery, Institute of Dental Sciences & SUM Hospital, Bhubaneswar, Odisha, India with a large anterior maxillary facial defect involving the lips and the nose owing to cancrumoris about 10 years ago. The defect was large involving the upper lip in the midline, anterior hard palate, and columella of the nose (Figure 1). The surgical procedure was aimed to improve the aesthetic outcome and also increase the functional capacity of the mouth.

Defect Assessment: The defect in the present case can be compared similarly to a cleft nose and lip(Figure 2 & 3). The field of cleft surgery has seen major advances over the past 30 years. Normal function and normal to near normal appearance are a realistic goal and can be achieved.

Nasal Deformity: The abnormal anatomy of the cleft nose includes several components. The alar base, the medial and lateral crus of the alar cartilage, the nasal dome, the columella, and the nasal septum all are affected by the skeletal base, consisting of the alveolus, maxillary segments, and palate. The severity of the primary nasal deformity is intimately related to the degree of displacement, abnormality, and hypoplasia of the maxillary segments. Con- tinued hypoplasia and displacement of the maxillary segments, particularly the lesser segment, result in varying degrees of maxillary deficiency in the unilateral cleft lip and nose deformity. Subsequent growth and the final degree of deformity and outcome depend on the cleft dysmorphogenesis and the selection of surgical procedures and sequencing. Rehabilitation must address the three-dimensionality and growth over time to obtain the desiredresult.[10]

Lip Adhesion: A median cleft can also be called midline cleft or vertical cleft through the center of the upper lip, and is a rare anomaly, the exact developmental origin of which is not clear. Tessier 0 clefts occur during the third week of gestation due to the failure of the two medial nasal processes to close in the midline. It can occur in isolation or be a part of a syndrome such as orofacial-digital syndrome. In this case, the defect occurred due to previous aggressive infection of the anterior facial region. Various treatment options are present for mild deformities that do not involve the white roll. When developing the treatment plan, reconstruction of the cupid's bow, labialphiltrum, vermillion, and buccal mucosa should be kept in mind. Some articles described using a V-Y flap, in another article they used a diamond incision to repair these anomalies. Some authors described a mucosal Z transposition technique to treat mild cases. For moderate defects involving the philtrum, Millard and Williams reported using an inverted V excision. In this case report, we have addressed both the lip component and the nasal component as a different entity to achieve maximum outcome.[4]

Surgical Technique:

Nose: The operation was performed under general anesthesia. The incision design, which involved two small triangular flaps on the nasal floor, was made along with the scarred philtral columns and extended vertically along the medial nostril walls. The prolabial flap based on the nasal tip was harvested with underlying subcutaneous tissues to protect the subdermal vascular network. By adding the alar rim incisions, an open rhinoplasty was performed. Excess fibrofatty tissue between the medial crura of the lower lateral cartilages can displace the alar domes laterally and so be removed completely. Then, the lateral and middle portion of the lower lateral cartilages was dissected from the nasal mucosa and the overlying skin. Once the desirable tip projection was obtained, the whole prolabial flap was used to cover the nasal tip and reconstruct the columella. Two accompanying small triangular flaps were inserted

carefully into the defects created by the septal mucosal contracture release (Figure 4).

Lip: Lip repair was done using the triangular flap technique initially describe by Tennison and Randall. The approach to this flap is based purely on a mathematical approach and precise measurements. The repair technique is conceptually similar to rotation advancement flap with the primary difference is that the rotation back-cut in the non-cleft side is performed more inferiorly, closer to the vermilion border. Similarly, the advancement segment on the cleft side is designed to occur inferiorly near the vermillion cutaneous border (Figure 5).



Figure 1Defect assessment



Figure 2 Nose Defect



Figure 3 Lip Defect

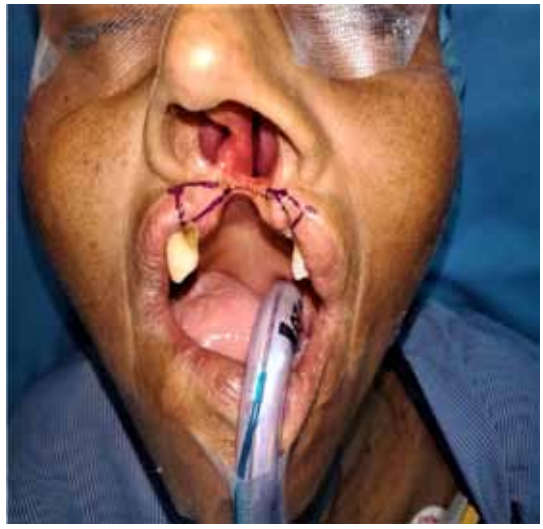


Figure 4 Lip markings



Figure 5 Nasal reconstruction



Figure 6 Postoperative picture

Discussion

Noma or cancrumoris is gangrenous stomatitis affecting particularly children from developing countries. It has been known since the time of Hippocrates, Galen, and Celsus. In 1848, Tourdes used the term *cancrumoris or noma* for the first time and gave a clear description of the disease. In the earliest stages of the disease, a painful, red, or purple, indurated papule is present. It is most frequently seen in the posterior region of the alveolar margins. Ulceration follows rapidly and may extend into the mucosal surfaces of the cheek and the lips. Tissues appear swollen and tender to palpation. There is a profuse salivary flow accompanied by a foul-smelling and purulent discharge. Over several days, a blue-black area appears on the skin of the lips and cheeks. The gangrenous area may also involve the chin, the infraorbital region, the palate, the nose, and also the antrum. Sloughing of the tissues leaves deep, perforating wounds. The teeth are often exposed. Following the acute phase of gangrene, if the child survives, healing will proceed by contraction and fibrosis, leading to severe distortion of the mouth region.[5]

Surgery is targeted at restoring normal speech, oral competence, and acceptable cosmesis. The surgery differs from the reconstruction of defects following ablative oncologic resection in the following ways. In the adult noma patient, compound tissue losses (skin, soft tissue, bone) are commonplace. Remnant tissue is left scarred and distorted with loss of normal anatomical reference points. Because noma occurs in early childhood, the bony defect and soft-tissue scar affect facial skeleton and dental development, producing gross asymmetry. Nutritional deficiencies are commonplace. Preoperative optimization must include nutritional assessment and correction of these deficiencies.

Complications, including poor healing and infection, are common in the immediate postoperative period. Hence, a longer observation period is necessary before discharge and return to the country of origin. Noma sequelae comprise bony and soft-tissue losses that affect both aesthetics and function. Complex defects should be treated in a staged fashion with the most complex initial stages performed in better-equipped institutions and less complex stages done on home ground. Although complete correction may be planned to restore function and aesthetics, the noma patient may eventually be satisfied with a functional but less aesthetic outcome.[2]

Midline defects of the face are associated with a range of developmental brain defects, trauma, or severe infectious disease leading to gangrene. Many are incompatible with life but some patients may have a normal or near-normal mentality and a full life expectancy. Reconstruction and repair utilizing existing surgical method are applicable for this latter group of patients but the midline nature of the defects may require adaptation of these techniques.[8]

The columella is an important aesthetic entity in the mid-face and following its loss or congenital absence a severe aesthetic defect results. Over the years a variety of reconstructive techniques for the columella have been described in the literature including alar margin flaps, the internal nasal vestibular flap, nasolabial flaps, and nasomalar flaps.[8]

The basic principles of treatment of the established case of Cancrumoris have remained the same. These are to release the ankylosis and only then to carry out reconstruction of the facial defect. To undertake the latter before achieving the former is disastrous for the patient, who previously was able to feed through the hole in the cheek, but with the closure of the defect is prevented from taking anything but fluids.[7]

The first step of nasal reconstruction is a thoughtful analysis of the defect. The best surgical option will provide the patient with an excellent aesthetic result and nasal function. Depending upon the extent of the defect

as well as the anatomic site, a local flap, full-thickness skin graft, composite graft, or interpolated flap will provide the optimal result. The decision will depend on the surgeon's experience and expertise, as well as the expectations and desires of the patient. Many cutaneous defects will require not only reconstruction of the defect, but also cartilage grafts to provide nasal contour and support. Most large or complex defects will require a paramedian forehead flap for reconstruction along with cartilaginous and/or bony support, as well as a lining flap.[6]

One of the most distinct problems of secondary bilateral cleft nose deformity is a short columella with a flattened nasal tip. There have been many attempts to perform columella lengthening and nasal-tip elevation. Various techniques, such as a central V–Y advancement flap from the upper lip, and bilateral forked flaps, have been commonly used to lengthen the columella. Meanwhile, different bone or cartilage grafts can be used to elevate the nasal tip and stabilize the columella. However, the most important procedures that contribute to the long-term stability of columella length and nasal tip projection are the full release of nasal mucosa contracture and sufficient soft tissue coverage at the nasal tip.

In our approach, the total height of the prolabium was used to lengthen the columella and provide adequate skin coverage for the nasal tip. Furthermore, any contracting forces induced by inadequate mucosa were fully released and the resultant defects were repaired by the two accompanying triangle flaps in the prolabial flap.[3]

Conclusion

Noma sequelae comprise bony and soft-tissue losses that affect both aesthetics and function. Complex defects should be treated in a staged fashion with the most complex initial stages performed in better-equipped institutions and less complex stages done on home ground. Although complete correction may be planned to restore function and aesthetics, the noma patient may eventually be satisfied with a functional but less aesthetic outcome.

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Fortuitous Le Fort Osteotomy in an Interpersonal Assault: A Case Report

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Abstract

Violence is one of the primary sources of facial injury. It forms a big part of emergency care and trauma and differs from each because of the physical, psychosocial and economic relations. Trauma from pointed arms happens less frequently affecting the head and neck region, most commonly affecting males. Facial injury: 83 percent comprises of bone fracture, 66 percent of lacerations & 53 percent comprises of hematomas.

Keywords: *Assault using sharp weapon, le fort osteotomy.*

Introduction

The form of injuries witnessed associated with the suspected weapon used, but only hematomas or fractures suffered by 20 percent of victims who reported attacks with sharp weapons. The most casualties are caused by punching (72% of assaults), or kicking (an attack of 42 percent). Only6% of victims registered with sharp weapon accidents & 11 percent of those wounded with drinking glasses. All injuries were classified as hematomas, lacerations, or fractures. It included intra-oral lacerations linked to the underlying fractures of the jaw including the surrounding alveolar mucosa of the hard palate. Weapons were classified as blunt, sharp, broken glass, fist, feet, firearm or 'other'. The level of seriousness of injuries was developed and were classified as:

Category I: Single laceration or single hematoma.

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Category II: Multiple soft tissue injuries.

Category III: Single fracture.

Category IV: Single fracture and soft tissue injury elsewhere.

Category V: Multiple fractures.

Case Report: A patient aged 28 years, male, reported the emergency department of SUM hospital with a history of assault using a sharp billhook due to interpersonal violence. On examination, the patient had a laceration about 10cm long extending from the nasal floor to the zygomatic buttress bilaterallyand a depth of about 2cm. The entire maxilla was mobile. There was a derangement of occlusion on both the left and right sides. In a pre-operative 3-dimensional computed tomography (CT) scan we see a linear fracture line seen passing from the right pterygomaxillary region crossing the floor of the nose to the left pterygomaxillary region fracturing a part of the left piriform aperture thus making the maxilla floating. The patient had difficulty in breathing so tracheostomy was done & initially the wound was cleaned, bleeding was arrested sutures were placed using 4-0 prolene and fluid transfusion was done.

Treatment Done: The patient was intubated through a preexisting tracheostomy tube. Local anesthetic agent 2% lignocaine with adrenaline (1:100000) was injected into the surgical area. The patient was subjected to

maxillomandibular fixation using the ivy-eyelet wiring technique. The fracture site was exposed via a pre-existing scar. The fracture site was reduced and fixed with one 1.5mm L-plate using four 6mm screws on the right side & using two 1.5mm L-plate and ten 6mm screws on the left side. Occlusion was achieved intraoperatively. The surgical site was irrigated thoroughly and hemostasis was achieved. Sutures were placed in the muscle layer using 3-0 vicryl and on the skin using 5-0 ethilone. A tight dressing was given in the incised area.

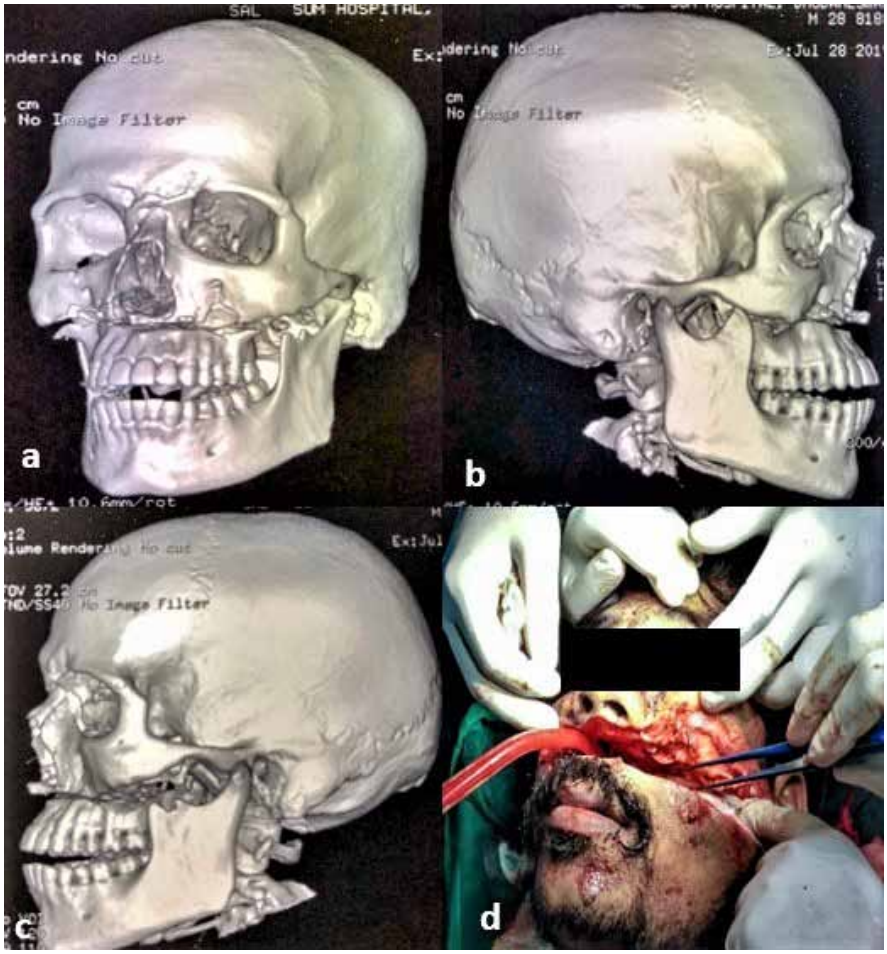


Figure 1. a, b,c : PRE-operative CT showing frontal, right and left view. d: PRE OP pic of patient

Intra-Operative Pictures:



Figure 2. a: Right Side 1.5mm L-plate. b: Left side single 1.5mm L-Plate c: Left side placement of both plates.

Post-Operative Pictures:

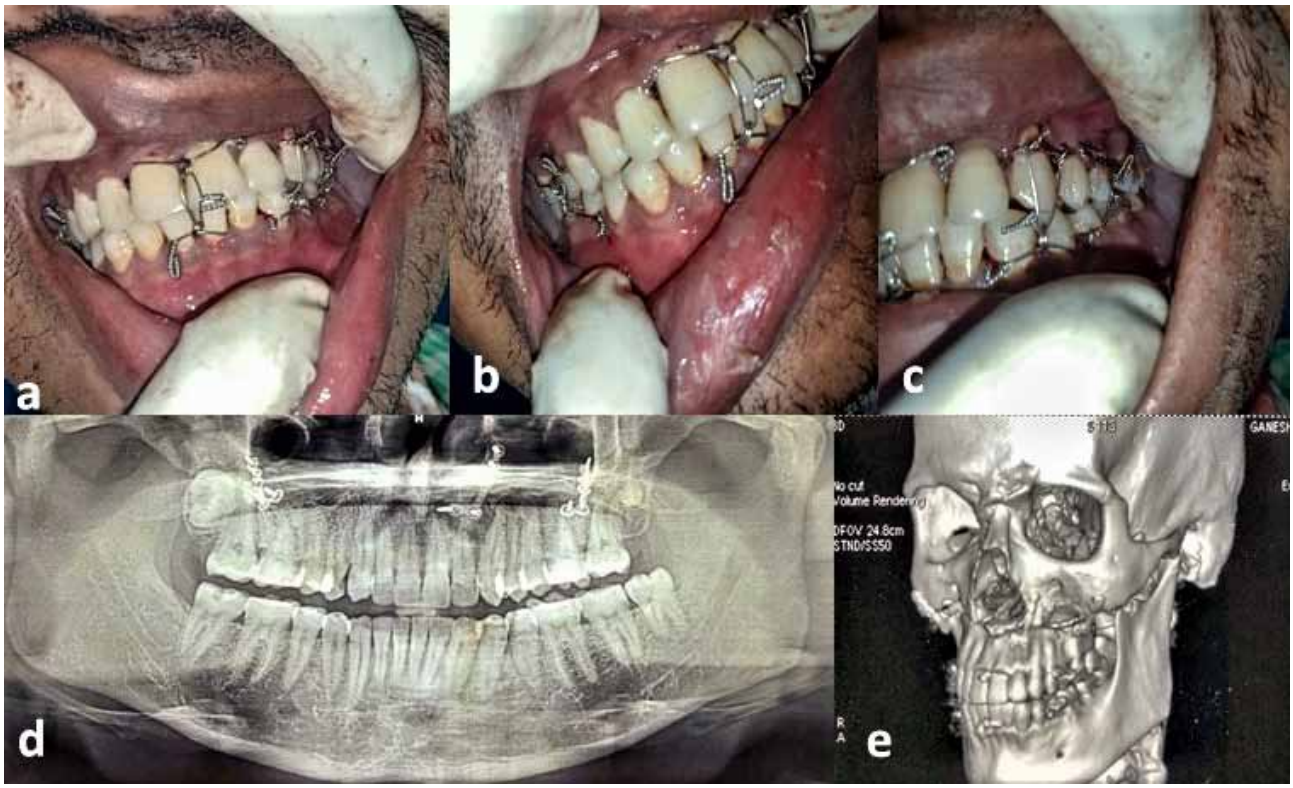


Figure 3: a,b, cno occlusal discrepancy present; c,d: Postoperative OPG and 3D CT.

Discussion

A rise in the cases of maxillofacial injuries due to assaults may be attributed to the country's low socio-economic circumstances, contributing to tension/stress and criminal propensity. The job rate for university students has generally increased from 4 percent in the early 1970s to 45 percent today.[1,2] The face is the first point of touch in the various people-to-people encounters, and the goal for maxillofacial injuries is often chosen especially in assaulted cases. The peak age rate for assault was observed to be 20-29 years.[3-7] The higher frequency recorded by males[3,4] is in support of other related research, this may be because males are more likely to participate in interpersonal altercations, probably due to the use of alcohol or psychoactive drugs in males compared with females.

Blunt objects were reported as the most common assault mechanism for maxillofacial injuries. Shepherd et al.[8] related the assault mechanism to the form of injury, indicating that assaults with blunt objects may produce sufficient force to cause fractures, leading in particular to dento-alveolar fractures/facial skeleton fractures.[8] The Olasoji et al study identified attack as the major cause of facial injury, as 48 percent of their patients had facial damage due to assault-related injuries.[9] This discovery is counter to the pattern identified in the past hence assault is suggested as a recent phenomenon and should not be underestimated as the most popular etiology of maxillofacial injury. During this research, the injuries suffered as a result of an attack ranged from abrasion to soft tissues to facial bone fractures. Laceration of the soft tissues of the nose and subsequent invasion of the middle third of the face. Bite wounds are mostly seen on the face and women tend to be more affected in their third decade of life, with the lips and ears being the most common target.[2]

Barde et al found that injuries suffered in car collisions are normally very different from those incurred in personal disturbance; while victims of automobile and motorcycle collisions tend to have multiple mandibular injuries, those patients struck by a fist typically have single non-displaced fractures. Victims in violent

offenses like assault and gunshot injuries are most likely to sustain fractures in the mandibular body and angle. Barde et al also recorded fractures of the mandibular angle to be the most frequent in their study, whereas symphyseal and condylar fractures were rare.[10] Any of the soft tissue lesions are treated with antiseptic fluid washing and dressing. Gentle violet solution has in some cases been applied to avulsed areas to aid with wound healing.[2] Lacerated wounds were sutured in layers, while the case of extreme avulsion on the lower lip due to human bite was healed with good results. The fragments of the broken jaw were sufficiently limited, strengthened by intraosseous wiring and immobilized by intermaxillary fixation. The damaged teeth were either removed or endodontically treated in fracture rows.

Drugs such as analgesics, antibiotics, anti-inflammatory medicines, and anti-tetanus medicines were prescribed as necessary. In this situation, recovery was uneventful, with strong results.[2]Biomechanical injury research uses mechanical principles to investigate and explain the physical and physiological reactions to impact injury resulting from that.[6]Both penetrating and nonpenetratinginjuries may be addressed, but the latter are morecomplex, less well understood, and offer greater opportunities formitigation through technological advances. The risk of injury isrelated to the energy delivered to the body by the impacting objectas well as to the object's shape. Penetrating injuries are caused by high-speed missiles, such as bullets, or by sharp objects traveling at very low speeds, such as blades, axes, and daggers in this type of injury, impact energy is concentrated in a small region of the body, there is no chance of dissipating this force, and the structural risk processes are relatively clear. ContradictoryHowever, injuries occur when the body is struck by a blunt object, such as a vehicle instrument panel, at a moderate velocity but the impact force is distributed over a wide area".In this case, padding or other crushable materials may absorb impact energy, which makes the impact and impact surfaces to bend and thereby increase impact length and its frequency rising.[6] After studying the various classifications and biomechanics of facial skeletal fractures, the present case may be classified as bilateral maxillary alveolar process fracture or unfinished Le Fort I fracture due to high penetrating injury.

Conclusion

Sharpened facial traumas have a tremendous aesthetic & functional loss. However, with proper diagnosis, adequate planning, and immediate surgical treatment, the maxillofacial surgeon can minimize sequelae and ensure patient rehabilitation as well as reduced risk of infection.

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Nosocomial Infections: A Long-Lasting Challenge in Public Health

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Abstract

Nosocomial or hospital-borne infections are one of the most difficult challenges faced by health care professionals, as these infections are arises in hospital settings. Both human and non-human contaminations are responsible for these infections inpatients during their hospital stay. This article helps to understand the epidemiology of nosocomial infections and to overcome major public health challenges. It also identifies the factors responsible and the mode of transmission of these infections to overcome some major public health challenges.

Keywords: *Nosocomial infections; Antibiotic resistance; Multidrug-resistant bacteria; Public health challenges*

Introduction

The word nosocomial has been adopted from the Greek words 'nosos', meaning "disease" and 'komein', means "to care for". Nosocomial infection or "hospital-acquired" or "hospital-borne infection"occurs in the hospital settings, which means it is an infection picked up by a patient in the hospital who has been admitted to the hospital for an ailment other than any disease caused by microorganisms. These microorganisms are present in the hospital settings, devices or among hospital staff and get transferred to a newly admitted patient with no infection history (Figure 1). These infections can be defined based on a period within the hospital stay, like among those occurring within 48-hours of hospital admission, in the last few days of discharge or within 30 days of surgery. Nosocomial infections are always associated with large numbers of co-morbidities, initiating new illness and are the common cause of mortality among hospitalized patients.[1,2]

Nosocomial infections majorly occur orspread through respiratory tract infections, surgical abrasions, and urinary tract infections (UTI). Areport from the World Health Organization (WHO) suggests the nosocomial infections are highly prevalent in Intensive Care Units (ICU) and hospital wards. They are most prevalent among patients with comorbidities, older groups of people, children (especially below <5 years), and among chemotherapy patients.[3] The major factors responsible for acquiring these infections include patient's immunity, increasing variation of medical and invasive techniques, exposure to infected health professionals, hospital hygiene policy, microorganism's longevity on the surface or in air, size of the agents, etc. e.g. air particulate matters and multidrug-resistant (MDR) bacteria.[4]

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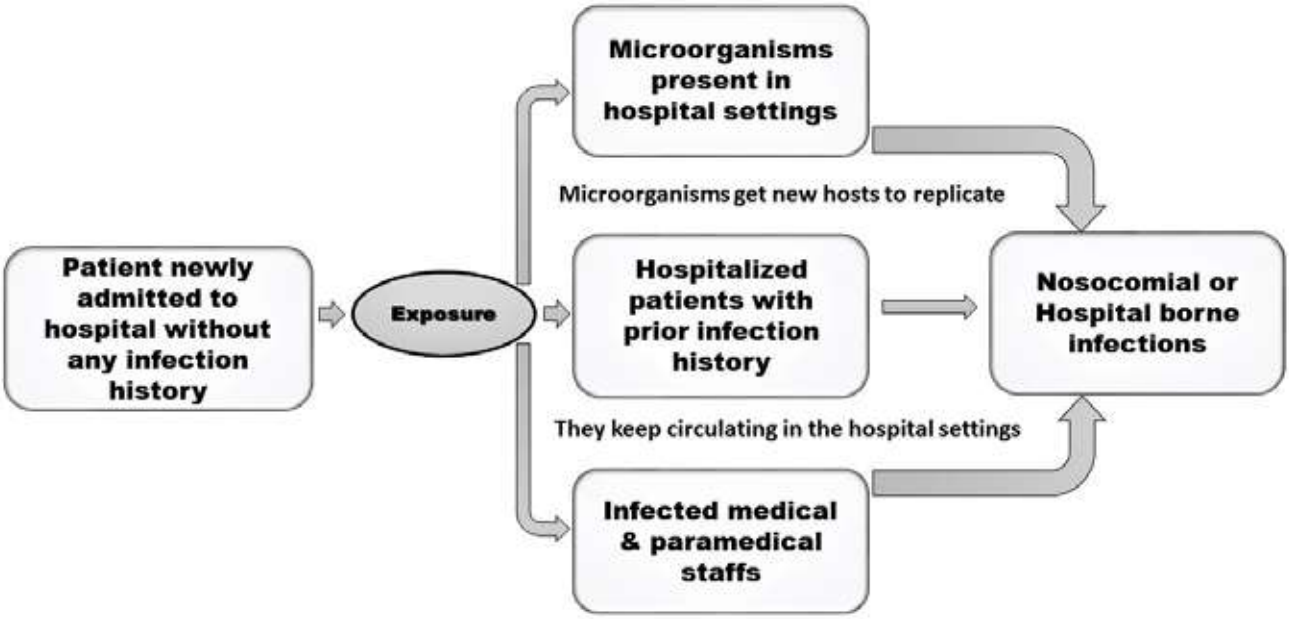


Figure 1. Source, spread and sequence of events in leading to nosocomial infections

Epidemiology of nosocomial infections: Nosocomial infections are considered either epidemic or endemic. It was reported in the National Nosocomial Infection Study (NNIS) from 1992 to 1998, that 90% of the nosocomial infections have been endemic, where 54% infections were in surgical patients, 28% in elderly patients (above 55 years), and the rest in device associated patients. Bloodstream, respiratory, surgical-site and UTI were the most common hospital-borne infections. There is also an association between the increase in MDR bacteria and nosocomial infections. Apart from this accessibility to handwashing stations and usage of alcohol gets improves compliance with washing hands. Numerous studies have also shown that doctors wash their hands less frequently than other health professionals. Proper infection control measures are needed for the control and prevention of such infections.[5]

The common sites of infections: Respiratory or Nosocomial pneumonia: Respiratory tract infections are the commonest of nosocomial infection. In an "NNIS" study pneumonia had accounted for about 31%. These types of infections occur in different groups of patients and health professionals not taking proper precautions. This type of infection is found among patients in the ICU who are on ventilators, where the rate of incidence of pneumonia is about 3% perday. A high rate of fatality is also associated with ventilator-associated pneumonia.[6-8]

Urinary tract infections: UTI is another common hospital-borne infection and around 80% of this is found to have been associated with the use of a bladder catheter. UTI can occasionally lead to bacteremia and death. The major bacteria responsible for UTI can be either commensal turned opportunistic pathogens like *Escherichia coli* or acquired infectious bacterial like MDR *Klebsiella* species.[6-8]

Surgical site infections (SSI): The incidence of such infections varies from 0.5-15% which usually depends upon the type and size of operation along with underlying patient status. The length of stay in the hospital is considered as one of the underlying factors. These type nosocomial infections are acquired usually during surgery, from surgical equipment, doctors, para-medical staff, from the commensal-flora present of the patient's skin near the operative site and rarely from blood used in surgery. These kinds of infections arise if proper aseptic measures are not followed before, during and post-surgery and it delays the healing of surgical sites that lead to septicemia and bloodstream infections.[6-8]

Nosocomial bacteremia: These infections are responsible for about 5% of nosocomial infections but fatalities rates are high due to bacteremia are considerably high about more than 50%. There is an increase in particularly for specific organisms such as methicillin-resistant *Staphyloccocus aureus* and *Candida* spp.[6-8]

Other nosocomial infections: Bedsores, burns and ulcersare the other kinds of hospital-borne infections that affect the skin and soft tissues, leading to unnecessary bacterial colonization. Secondly, gastroenteritis is the most common type of nosocomial infection among children generally caused by members of Enterobacteriaceae, rotavirus and *Clostridium* species. Other infections like sinusitis, endometritis, conjunctivitis and infections of the reproductive organs post-childbirth are also common in hospitals of developed and underdeveloped countries.[5-7]

Factors responsible for nosocomial infections: Infection source: During hospitalization, the patient usually gets exposed to a variety of microorganisms; bacteria, viruses, fungi and parasites can cause infections. These infections are acquired from another person in the hospital (cross-infection) or the patient's normal microbial flora (endogenous infection). Infections may also be acquired the infectious microorganisms present on inanimate objects or substances, a surface that had come in contact with the hospitalized patient. Such infections are known as fomite infection. If infection occurs from devices for the patients such as ventilators and catheters, they are known as device-associated infections.[9-11]

Bacteria: Bacteria are the common form of pathogens responsible for nosocomial infections. These infections causing bacteria are further divided into:

Commensal bacteria are found in the normal flora of healthy humans. The bacteria include cutaneous coagulase-negative *Staphylococci* and *E. coli* which behave as opportunistic pathogens and cause bloodstream, respiratory, urinary and gastrointestinal tract infections. They can be fatal under some circumstances.[9-11]

Pathogenic bacteria: *Clostridium* species causes gangrene, *Staphylococcus aureus* causes a wide variety of lung, bone, heart and bloodstream infections and is frequently resistant to antibiotics; beta-hemolytic *Streptococci, Pseudomonas* and members of family Enterobacteriaceae may colonize the immune-compromised sites of a patient and cause serious infections in the surgical site, lung, bacteremia, peritoneum infection.[9-11]

Viruses: Viruses are widely responsible for causing many nosocomial infections which include the hepatitis B and C viruses transmitted during transfusions, dialysis, injections, endoscopy, etc., respiratory syncytial virus (RSV), rotavirus, and enteroviruses which are transmitted by hand-to-mouth contact and via the fecal-oral route. Other viruses such as cytomegalovirus, HIV, Ebola, influenza viruses, herpes simplex virus, and varicella-zoster virus, may also be transmitted.[9-11]

Parasites and fungi: Organisms like *Giardia lamblia* are transmitted easily among both adults and children. Some other fungi and parasites cause infections during extended antibiotic treatment and severe immune suppression (e.g. *Candida albicans, Aspergillus sp., Cryptococcus neoformans, Cryptosporidium*). [9-11]

Environmental factors: Environment has always acted as a game-changing role by acting as a host for carrying airborne infections. Environmental factors play a major role in causing nosocomial infections. The environment sometimes acts as a reservoir of the agents or even sometimes acts as a source. These infections can be caused among patients, staff and non-medical staff and patient's attendants and when admitted to the hospital acts as a potential source of causing infection. Infections transmit most frequently in crowded conditions within the hospital settings. Airborne transmission occurs through fungal pathogens, like *Aspergillus* which are soilborne. *Sarcoptes scabies* (scabies) is another such organism causing major outbreaks in the health care system. [9-11]

Bacterial Resistance: Apart from their association with an increase in morbidity and mortality, hospital-borne infections have a strong association with MDR microorganisms, including "methicillin-resistant *Staphylococcus aureus* (MRSA)" and "extended-spectrum-lactamase-producing (ESBL) gram-negative bacteria", those causing huge clinical consternations. MDR bacteria increase in number exponentially through natural selection and exchange of plasmids carrying resistant genes with normal human flora which were originally sensitive to antibiotics.[12] The extensive and unprescribed use of antimicrobial drugs is one of the major factors of the emergence of MDR bacteria. Bacterial strains of *Staphylococcus, Pseudomonas, Enterobacteriaceae and Mycobacterium tuberculosis* are currently considered are superbugs and are probably resistant to almost all antibiotics. These organisms are highly prevalent in hospitals and community settings. The problem of antibiotic resistance is a major issue in developing and under-developed countries as the affordability of hospital stay and higher antibiotics become serious concern.[13]

Modes of transmission of nosocomial infections: Nosocomial infection can be both endogenously and exogenously, endemic or epidemic transmission which means the transmission of infection from the surface and when transmission occurs internally in a manner unusual to normal cases.[3, 14, 15]

Endogenous Infection: The human microfloraismajorly responsible for causing opportunistic infections in various other parts of the body such as urinary and GIT, wound's site and nearby tissues, depending on the host's immunity and ongoing antibiotic therapy

Exogenous cross-infection: Transmission of pathogenic infection happens because of : (a) direct contact with patients (infected body part, body fluids, saliva) (b) aerosols and saliva droplets (c) hospital staffs, patient caretakers (may act as carriers), visitors (d) from contaminated devices, instruments, clothes, beds and toilets used for and by the patients.[3, 14]

Endemicor epidemic exogenous environmental infections: Most pathogenic organisms like strains of *Pseudomonas, Acinetobacter, Mycobacterium* dwell well in a hospital environment as the damp and moist *conditions suit* them well and there no shortage of hosts and nutrition. They can survive for months together as spores and are generally resistant to disinfectants which are used to maintain hospital cleanliness.[16]

Infection	Measures
UTI	Limit duration of Catheter use Aseptic technique during insertion Closed drainage system
SSI	Clean operating environment & staff cleanliness Limiting hospital stay Optimal antibiotic prophylaxis Aseptic practices during surgery Surgical wound surveillance
RTI	Ventilator-associated Limit duration in ICU Non-invasive ventilation Prevention of *Legionella* and *Aspergillus* during renovations
Device-associated infections	All Catheters limit duration Local skin preparation Limitation of frequency of dressing change Antibiotic-coated catheter for short term

Table 1. Effective measures for prevention of nosocomial infections

Modes of prevention: The major illness or morbidity occurring due to nosocomial infections can be prevented through various measures if taken proper care. Table 1 shows the effective measures for the prevention of major nosocomial infections.[17]

In general prevention at various levels is going to be helpful for control and precaution from infection. This prevention at the individual level includesi. Reducing person-to-person transmission ii. Preventing transmission from the environment iii. Cleaning of the hospital environment, disinfection of patient equipment, sterilization.[18] There has to be the utilization of preventive measures and equipment in day to day activity for the prevention of hospital-borne infections,a standard guideline for health professionals introduced by WHO gives a detailed preventive measure (Figure 2).



Figure 2. Standard WHO preventive measure guidelines for health professionals

Major public health challenges: At individual level, spread of contamination through frequent contact and close contact between patients and unaffected individuals.

At environmental level, spread of infection through material and objects, improper sanitations, not using proper sterilizations, uncleaned environment and healthcare can be responsible for causing infection and when there are gatherings in hospital setting infection which are airborne are transmitted faster than it could be expected. Therefore, during epidemics, people are asked to avoid social gatherings even at healthcare.

At healthcare, level, spread through an affected healthcare professional, use of infected linen, equipment, and injection because of which infection travel from an infected individual to a healthy individual.[19]

Public health interventions: These interventions at large scale help in safeguarding and treating masses all at a given time and even over some time. The "Centre of Disease Control and Prevention's"in 1985 reported on the efficiency of Nosocomial Infection Control had reported of four components of key infection control which includes: (i) an effective epidemiologist, (ii) one infection control practitioner for every 250 beds, (iii) active surveillance, (iv) control efforts. CDC's National Nosocomial Infection Surveillance (NNIS) system has been working for more than 25 years. WHO on the other hand is working towards improving global health care and preventing healthcare-related infections.[20]

Applying intervention in hospital settings is not enough, as improvement in a continuous manner would be required to avoid contamination and support good health. Therefore, WHO has suggested multimodal improvement strategy in 5 useful steps as shown below in Figure 4, which shows the 5 key elements identified by WHO for prevention and control of multimodal strategies in a healthcare setting, the 'system change' can be implemented in Infection prevention and control which includes the availability of infrastructure, supplies (E.g. frequent hand washing intervention needs availability of wash basin and infrastructure for health professionals and patients to avoid contamination and transmission of infections), 'training and education' of health professional which will not only improve their knowledge will also help in implementing knowledge into sensible actions (E.g. knowledge about evidence based surgical site infection prevention), 'monitoring and feedback', will help in assessing the problem and continuous flow of information will improve action

(E.g. information regarding localization of the particular pathogen/microbe), 'reminders and communication' regarding right action in the right situation (E.g. awareness regarding the importance of regular hand-wash), a 'culture of safety' to facilitate an organization to value an intervention and institutional safety atmosphere for sustainable improvement.[21-24]

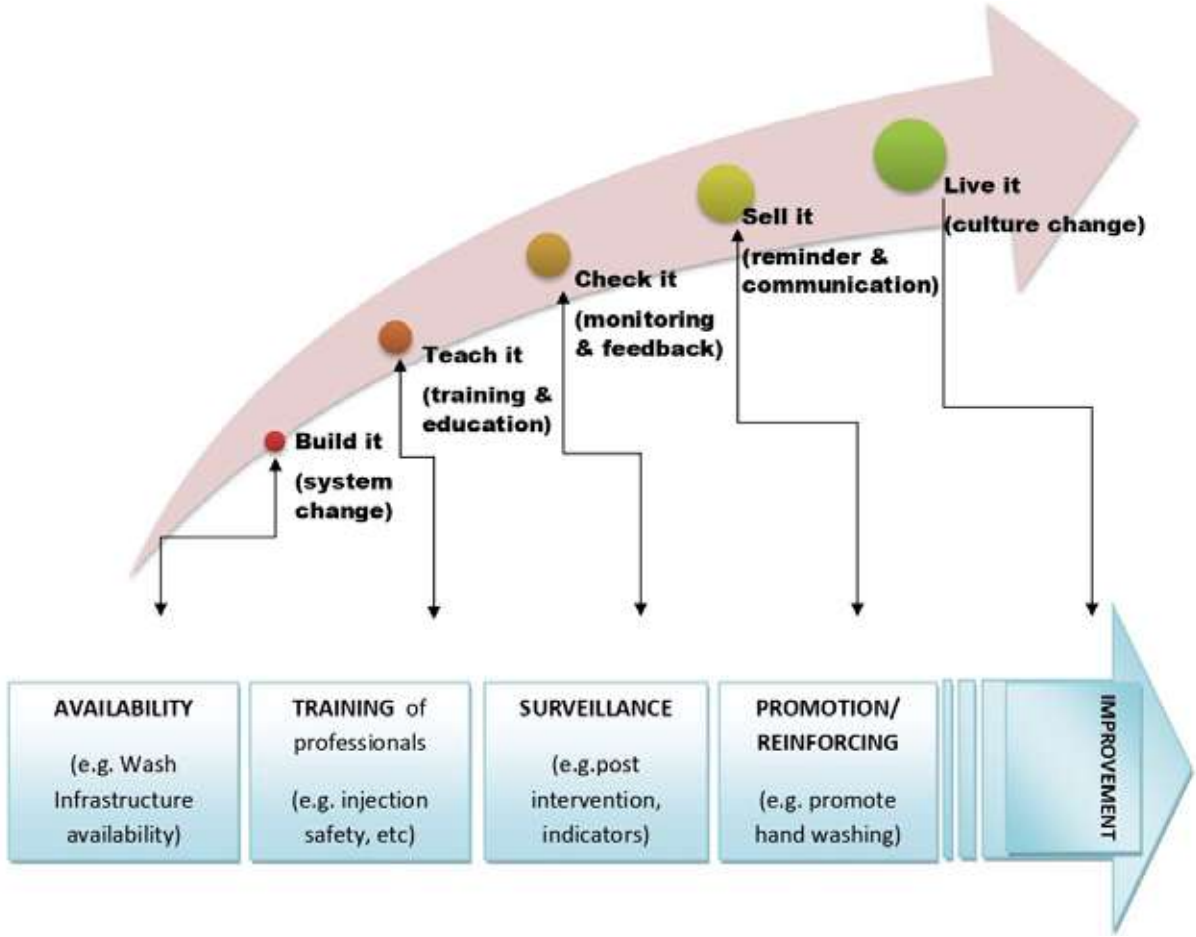


Figure 3. Infection prevention and control (IPC) multimodal strategies for health-care.

This model (Figure 3) enables the understanding of the occurrence and spread of nosocomial infections and prevent it by carefully implementing a strategic control and monitoring system in healthcare settings. Not only health professionals but a complete effort by all sections of the health care system will help in taking prevention against nosocomial infections and prevent infections.[21-24]

Conclusion

Public health challenges are difficult to overcome in-spite of various interventions. The rapid transmission of infection in the hospital setting has been one of the most difficult challenges to overcome, irrespective of development in the field of medical sciences. Surveillance mechanisms like NNIS will enable in taking proper and timely interventions at different levels for smooth functioning and prevention of nosocomial infection. The multimodal strategies would enable health professions to take strategic intervention at different stages and sectors. Further research on specific infections will increase the chances of better awareness and prevention of nosocomial infections.

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Analysis of Tumour Size When Presented to Dental College: A Retrospective Institutional Study

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Abstract

Objective: Evaluation of presentable tumor size in oral squamous cell carcinoma was the objective of the study.

Patients and Method: The study sample comprised of ninety-eight biopsies diagnosed as oral squamous cell carcinoma presented to dental college from 2015 to 2020. Result and analysis of presentable tumor size when reported.

Results: Male to female ratio was 2.5:1. Among the 295 diagnosed OSCC cases presentable tumor sizes when reported, most of the cases were T2 (42.37%) followed by T1 (18.3%), T3 (11.86%), and T4 (6.1%).

Conclusions: Oral squamous cell carcinoma is one of the major health problems in India. Strategies must be made to improve the present scenario of health care service, where Government-run health care centres are burdened with ever-increasing patient pool, and to avail treatment in such centres is always a compromise with time and health. Oral health and cancer awareness programs should be undertaken for prevention, early diagnosis, and management of the affected population. Regular long-term follow-up is also necessary for these cases to avoid the recurrence of the disease and a better quality of life.

Keywords: *Tumor Size, Squamous Cell Carcinoma (SCC), Oral cavity, TNM Staging.*

Introduction

Oral cancer is considered to be one of the ten most common cancers in the world and is grouped with pharyngeal cancer.[1] An estimated 378,500 new intraoral cancer cases are diagnosed worldwide per year. Oral cancer is less common in developed countries, but is the eighth most common form of cancer in general; however, the ranking varies widely between countries. In some areas of northern France, oral cancer is most common among the male population. For many countries, especially younger people, the prevalence of intraoral cancer appears to be growing.[2] The global incidence of lip, oral, and pharyngeal cancers comprises around 3.8% of all carcinomas, is expected to increase by 62% within the year 2035 due to changes in demography.[3]

In Asian countries, oral and hypopharyngeal carcinomas are quite common.[3] One-third of global cases and one-half of oral cancer-related deaths are reported from Southeast Asia.[4] In countries of the Indian subcontinent (India, Bangladesh, Pakistan, and Sri Lanka) oral carcinomas are most prevalent.[2] Different studies revealed that in various part of India more than 50% of all carcinoma is oral squamous cell carcinoma

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(OSCC) and it is the mostcommon cancer among males and the third most common among the female population, which is related to the deleteriousoral habits such as tobacco chewing, betel-quid chewing,tobacco smoking, reverse smoking as well as other causes such as alcohol intake, low socio-economic status, poor grooming, poor diet, and viral infections, persistent dental pain, rough or broken teeth[2,5] Squamous cell carcinoma of the oral cavity most commonly involve tongue and buccal mucosa.[6]

Oral cancer affects males more often than females, although the proportion is equal, and in recent times,an increased number of cases is being reported in elderlyfemales as well as young females.[2,7,8] It predominantly affects middle-aged and older persons.[2] However, the incidence of OSCC in persons under the age of 45 is increasing.[9,10] Etiology of oral carcinomais multifactorial and cigarette smoking, excess alcohol intake, and betel-quid use are the common risk factor for oral carcinomas.[11] However, several recent studies have identified a startling rise in oral cancer in patients who had no history of tobacco or alcohol consumption[8,12]. This retrospective institutional research includes patients who were reported in our department for over 66 months. The goal of the study was to assess the presentable tumor size of oral SCC in these patients to determine the enormity and pattern of oral SCC in the population of the eastern Indian state of Odisha.

Material and Method

In this retrospective study (January 2015 to June 2020), data of 295 OSCC biopsies were retrieved from laboratory records. It included age, gender, presentable tumor size,and histopathological diagnosis. No identifying patient information was disclosed in the study. Approval obtained from the Ethical Committee of our institution for this study.

Results

A total of 295 cases of histopathologically confirmed OSCC, which were reported during the study period was included in the study. Among them 210 (71.2%) patients were male and 85 (28.8%) patients were female [Table 1]. The age range of these patients was 24 to 85 years and most of them were in their sixth decade of life. Presentable tumor sizes, when reported, were T1-54, T2-125, T3-35, and T4-18.

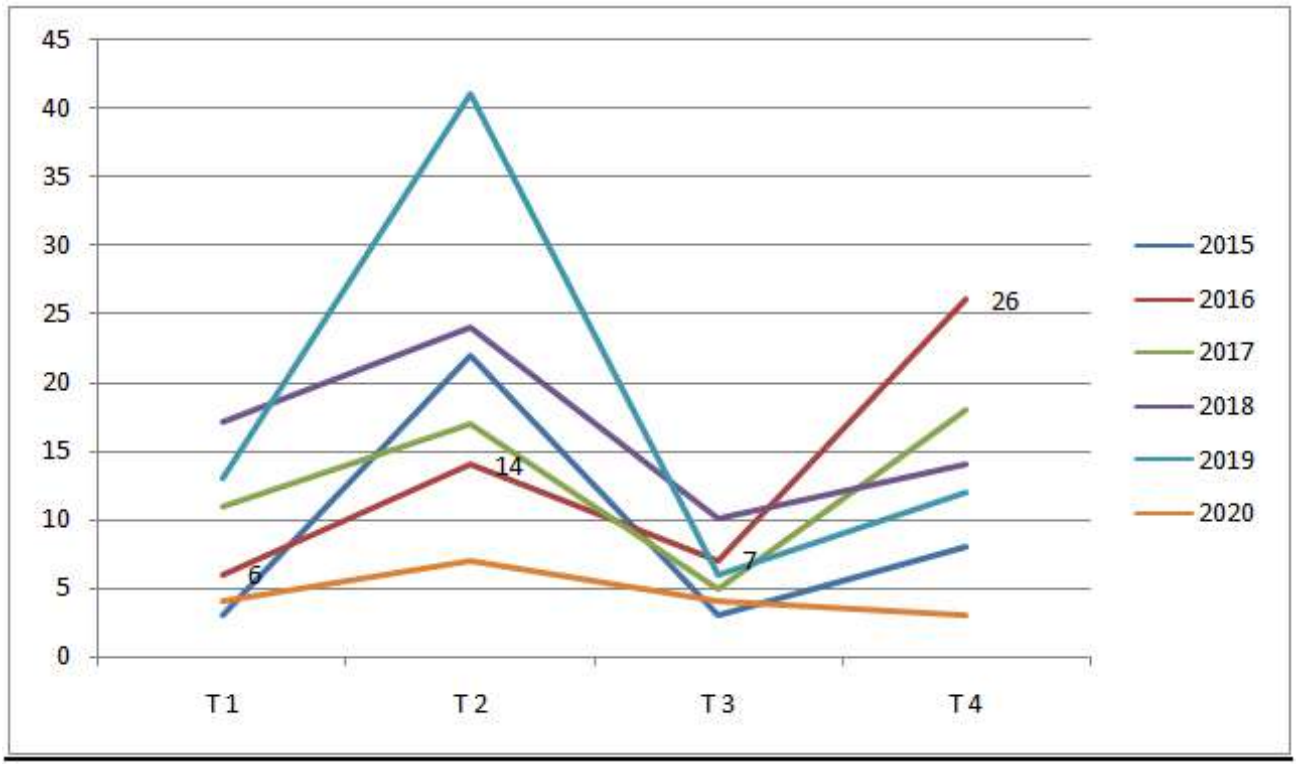


Figure 1. Year wise representation.

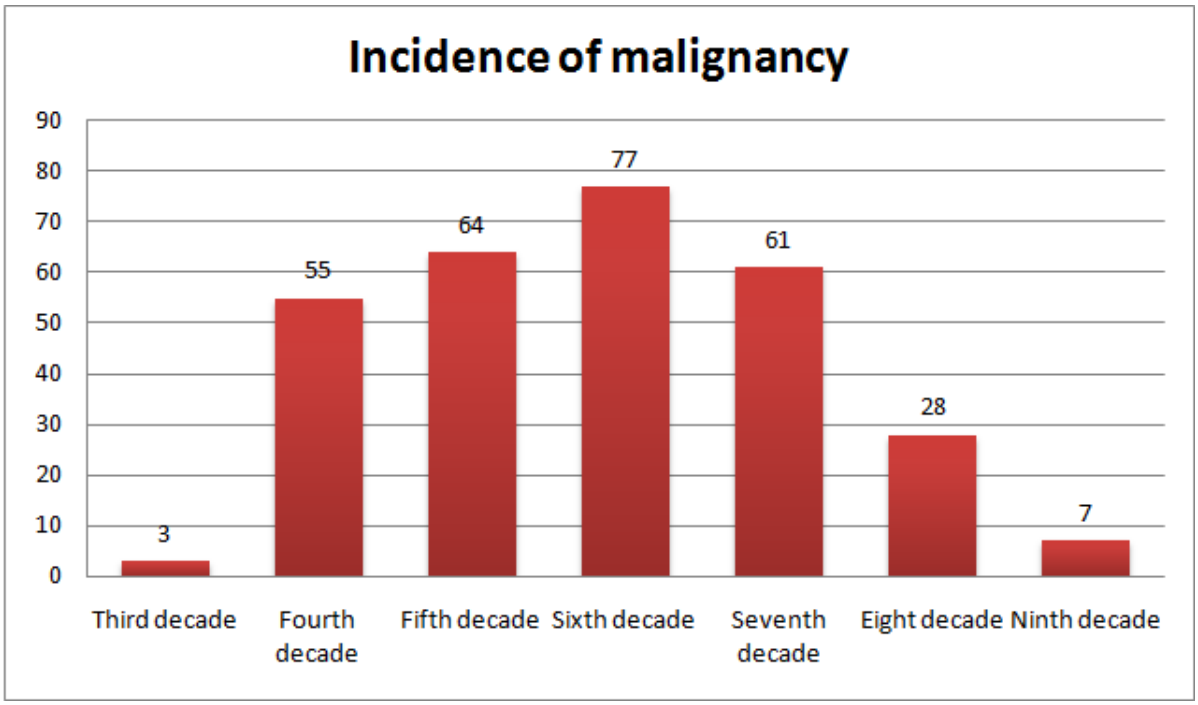


Figure 2. Incidence of malignancy

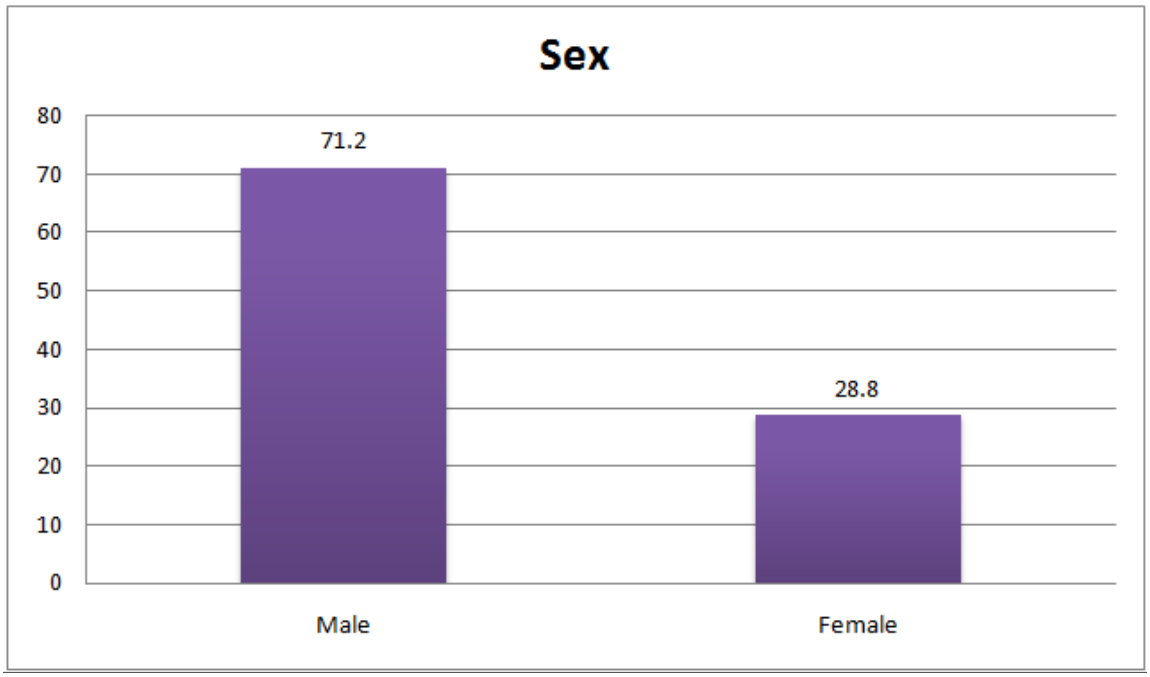


Figure 3. Gender wise representation

Discussion

Among all oral malignant lesions of oral squamous cell carcinoma accounts for nearly 90%. SCC is the prevalent carcinoma of the oral cavity with a high mortality rate and has a 5 years of survivalrate for 50% of the affected patient.[13][14]

In this study, there was an increased incidence of OSCC in males than females with a male to female ratio (M: F) of 2.5:1 which is equivalent to data of different studies which were conducted in the northern part of India. The male predilection of OSCC found in both developed (M : F=2.5:1) and developing (M: F = 3:1) countries which are attributed to increased incidence of

different oral habit in males than females[15] Recently, however, this gender distribution difference has been decreasing in developed countries because more women adopt tobacco-related habits including smoking.[15]

Most of the patients were in the age range of 40–60 years, which is consistent with other studies conducted in north India.[14,16] Most often OSCC appears after the 5th decade of life but a consistently increased incidence of involvement of patients younger than 40 years had been observed.[18] For Asian populations, the average age of presentation is 50 to 70 years of age. [17].In the present study, OSCC was diagnosed in 19.66% of patients younger than 40 years.

Tobacco consumption is a major risk factor for oral and oropharyngeal SCC.[19]Smokeless tobacco has a strong relationship with the development of oral carcinomas.[20] In Asian countries use various form of tobacco, areca nut chewing and alcohol consumption is prevalent and they play an important role in the development of oral squamous cell carcinoma.[21] Socio-cultural standards and beliefs are also in support of simple tobacco product availability. The introduction of tobacco products ready to use and aggressive ads target not just young people but also children.[14] In India, the efforts of the Government for deterrence of tobacco products' use prove to be inadequate when looked at the easy availability of the products and their rampant use.

The prevalent sites of oral SCC were buccal mucosa including gingivobuccal sulcus followed by alveolus and tongue. The findings were consistent with other studies.[14,22,23] The language is considered to be the most common site of primary OSCCs in developed countries, but in developing countries, betel quid and/or tobacco chewing results more commonly in buccal mucosal cancer.[19,24] This is mainly due to the prolonged positioning of the betel quid in the buccal pouch to achieve maximum benefit as betel quid constituents create a sense of well-being and increased ability to work by stimulating the parasympathetic nervous system.[24] Similarly, smokeless tobacco tends to incite malignant degeneration at the site of tobacco placement.[18] Tobacco contains carcinogens (chemicals that cause cancer) such as different nitrosamines (like N'-nitrosoanatabine, N-nitrosonornicotine, N-nitrosoanabasine, nicotine-derived nitrosamine ketone, etc.) and various free radicals likeglutathione reductase, S-transferase glutathione, catalase, glutathione peroxidase, superoxide dismutase, etc. They are capable of impeding antioxidant enzymes.[11] Reactive oxygen species are generated in substantial amounts in the oral cavity during chewing.[25]

According to the AJCC Cancer Staging Manual, Eighth Edition TNM staging can be classified as "clinical TNM" and "pathological TNM". They are designated as "cTNM" and "pTNM". The clinical classification should be determined before the commencement of the primary treatment based on data that are gathered through clinical examinations and different diagnostic procedures. The "cTNM" is crucial for proper treatment planning. The UICC classification suggested that for each site the specific method of investigations available for TNM classification should be listed. They are:

1. Gathering of history of the case.
2. Sign and symptoms of the condition.
3. Examination of the lesion along with the examination of the other systems.
4. Histopathological study of the primary lesion, sentinel lymph node of the regional lymph node.
5. Different imaging like CT scan, contrast-enhanced CT scan, MRI, endoscopy, plain radiographs to determine the surrounding tissue, and nodal involvement as well for the assessment of distant metastases.
6. Surgical exploration without resection and biopsy of the site of distant metastases if possible and other investigation which is necessary to establish the TNM staging.

In this study among the 295 diagnosed OSCC cases presentable tumor sizes when reported,most of the cases were T2 (42.37%) followed by T1 (18.3%), T3 (11.86%), and T4 (6.1%). In the year 2016 and 2017 presentable tumor sizes were mostly T4 (26 cases out of 53 and 18 out of 51 respectively). However, in recent years there is a reduction of presentable tumor size reported in recent years.

Most of the time there is a delay in diagnosis of OSCC can be attributed to various factors such as, but not limited to, patients' negligence due to lack of knowledge and awareness, illiteracy, poverty, and possible resort to home remedies.[22] Professional delay in making a confirmatory diagnosis can also be another factor. Multiple factors like the nature of the patient, symptoms as well as the course of the disease,

proficiency of the health service play an important role in the commencement of the treatment after the diagnosis of oral squamous cell carcinoma.[28]

Conclusion

Demographic data like age, sex, presentable tumor size in the patient of the eastern part of India which was identified previously was confirmed by this research. It is found that oral SCC is more common in the middle-aged male patient. For most Asian countries, especially India, the burden of oral cancer has increased; hence, increasing national level public awareness programs all over the country is a demand of the time for prevention, early detection, and diagnosis, and to support a tobacco-free environment. When measures are taken by Government so far could not deter people from using the tobacco products, whether a blanket ban on their production and sale is possible must be a subject of debate and discussion and utmost importance to reconsider.

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Conflicts of Interests: None

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Vaping: Realities in its Effect on Oral Health

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Abstract

Twentieth-century shows a great increase in the popularity of E-cig or electronic cigar or Electronic Nicotine Delivering System (ENDS).When smoking is considered as a leading cause of preventable death and strict measures had been taken to limit smoking in public places, this ENDS is gaining popularity worldwide among the youngsters. A new term Vaping is given to the process of consuming nicotine or other substance through a vaporising tool or popularly called E-cigar. People are using it as a smoking cessation tool also, though this fact is being debatable after many types of research on it. The increasing use of it, along with a lack of knowledge of its health effect both short and long term is worrying.

Keywords: *Vaping, Nicotine, Xerostomia, Halitosis, Glycol Vapour.*

Introduction

Since nicotine is a gateway drug that lowers the threshold for addiction to other agents, the use of e-cigarettes could lead to even more opioid addictions. It is becoming a controversial issue nowadays both nationally and worldwide.[1] Vaping is gaining popularity among youngsters without being aware of its consequences. Besides its major oral effects, general health is also becoming a field of concern due to its respiratory and ocular problems caused by glycol vapour. There are also cardiac complications related to inhalation of nicotine vapour.[2]

The emergence of E-cigar: Many countries have implemented a smoking ban in most of the public places eg- restaurant, clubs etc. So smoking cigarette is decreasing day by day due to strong imposition of law. Thus the consumer market is left with a wide-open market of an alternate form of taking nicotine. Electric devices or vaping pen is the one being promoted as a less harmful alternative with fun flavours (eg .coffee, maple syrup, mint menthol etc.) for recreational social use.[3,4]

In the developed countries, vapour lounges or vapour houses are popping up everywhere at the shopping mall, strip mall etc. Youngsters buy their favourite flavoured vaping pens and enjoying relaxing and vaping on plush lounge chairs indoors. Users drink energy drinks while vaping. Some vapour liquid contains substances like caffeine and ginseng for an energy boost or as an alternative of non-nicotinic vaping.[2-4]

Effect on oral cavity: The E-cigarette users experiences(I) xerostomia (II) Increased risk of tooth caries (III) Halitosis (IV) Dental pigmentation. The E-cigarette uses a vaping liquid that has:

(a) Diacetyl: It is a flavouring agent which is associated with serious lung disease, when heated to a high temperature and inhaled.

(b) Benzene: It is a volatile inorganic compound found in car exhaust and it is linked to leukaemia and bone marrow disease.

(c) Heavy metals like Lead, Nickel, Chromium and magnesium exceed the safe level of inhalation.

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(d) Nicotine: It is an addictive stimulant and a potent parasympathomimetic alkaloid.

(e) Propyleneglycol: It is a food additive sweetener that increases the risk of tooth decay. When used orally it breaks down into acetic acid, lactic acid and propionaldehyde, which are all toxic to enamel and soft tissue, moreover as it is a hygroscopic material, so causes tissue desiccation.

(f) Glycerin and different flavouring which creates a palatable pleasure and increase the risk of tooth decay.

Research shows that 1 liquid nicotine cartridge contains about 200 puffs, equivalent to the amount of nicotine in one packet of 20 cigarettes.[2-5]

Vaping affects the oral cavity in 5 major ways:

1. **Receeding gingiva:** Nicotine in E-cigarette causes blood vessels to shrink and so less blood is transfer to the tissue. This causes gum recession. It can also cause periodontal pockets leading to infection & other health complications.[5]
2. **Masks gum disease:** Dentist usually spots the gum disease by probing the pocket in the inflamed gingiva. But as vaping shrinks the blood vessels in that area, the symptom of bleeding on probing go without being diagnosed by a dental professional. This helps to progress the underlying disease without being caught.[5]
3. **Amplifies bad breath:** Nicotine in vaping compromises body's ability to secret saliva, which leaves the patient vulnerable to oral bacteria. This build-up Bactria can cause infection in compromised tissue and even lead to other long term illness eg. heart disease, oral cancer, xerostomia.[5]
4. **Increases bruxism:** Nicotine of vaping stimulates masticatory muscles leading to bruxism which causes wearing down of teeth enamel, which leaves the patient more sensitive to the hot and cold temperature of food.5
5. **Increases occurrence of tooth caries:** Research shows that streptococcus mutans, when exposed to aerosols from E-cigars on tooth enamel, shows to have 4 times more microbial adhesion and decreased enamel hardness up to 27%. The viscous property of the vaping liquid allows S.mutans to adhere to the pits & fissures and increases biofilm formation by two-fold when compared to unflavoured controls.[5]

The sweetener in vaping liquid has viscous properties compared to regular soft drinks and a high sucrose diet. Other than oral effects E-cigarette also affects general health. The patient who wants to quit smoking using E-cigarettes tends to revert into the habit of smoking. This is because the nicotinic content of many vaporiser oils can be higher than that of cigarettes, making it easy to fall back into the habit. More over vaping daily doubles up the risk of heart attack and have a significant impact on lung function, especially on long term use, continuing to evolve and bought quickly in the market. First-generation e-cigarettes are disposable ones more or less looked like traditional cigarettes. Then comes second-generation E-cigarettes which contains a clearomizer instead of the atomiser.[6]

Vaping: Vaping is the process of consuming vapours of a special liquid called vaping liquid, through a battery-operated device called electronic cigarette or E-cigars or E-hookahs or vaping pen or vaporising cigarettes etc. Nowadays the E-cigars come in various shapes and sizes. Some look like pens while others like USB devices, cigarettes etc. Some popular market names of vaping liquid brands are 21st-century smoke, 777e cigs, Blue e-cigs, Bull smoke, candy king on salt, Dura smoke, Diamond mist E-liquids, Dragonite international limited, E Z smoke, E-lites, Eco pure, Fill my pod, Five pawns, Fantasia, Green Smoke, Hot vapes, Juul, J vapes E-liquid, Kandy pens, LOGIC, Lost vape, Lite-Up Anywhere, Magic Mist, Made Vapes, Mi-Pod, Mountain Moon, Njoy, Pink Sticks, P.O.E.T Electronic Nectar etc. With such appealing marketing names, they are gripping the youngsters in today's world. Their selling is also encouraged through celebrity endorsements.[7]

Parts of E -Cigars/Vaping pens:

In most of the marketed E-cigars, they have the following parts:

The mouth Piece: Users inhales through engaging this which has a small cup inside containing absorbent material soaked in vaping liquid solution.

The Cartridge: Vaping liquid cartridge holds the liquid which comes in a variety of flavours and Nicotine levels.

The Automizer/Heater: It heats & vaporises a small amount of the solution which is regulated by a computer chip. Here the vapour resembles smoke.

The Battery: This can be rechargeable lithium-ion batteries that last for 1-3 days.

The Endcap: It contains a LED light that illuminates when inhaled or the user sucks on the device.

The sensor: When the user presses the activation button or merely inhale the device, it causes the inbuilt sensor to activate the vaporising chamber. Additional components include USB, portable wall and personal chargers.[8]

Types of E-Cigars/Vaping Pens:

They are available in the market in different types. They are

Disposable E-cigarettes – It is non-rechargeable or refillable and is intended to be discarded after the product stops producing aerosol. Sometimes it is called E-hookah Examples – NJOY, OneJoy etc.

Rechargeable E-cigarettes – It has a rechargeable battery. Very often it contains an element that regulates puff duration and/or how many puffs may be taken consecutively. Examples- Blu, Green smoke etc.[9]

Pen-style, medium-sized rechargeable E-cigarettes – It is usually larger than a cigarette. It contains prefilled cartridge or refillable cartridge. It often comes with a manual switch to regulate the length and frequency of puffs. Examples – Vapor king, Totally Wicked Tornado.

Tank-style, large size rechargeable E-cigarettes – It is a much larger size with higher capacity battery and essentially contains a large, refillable cartridge. It often contains manual switches and a battery casing for customising battery capacity. Examples- Volcano, Lavatube. [9]

Evolution of E-Cigarettes: Since the year 2003 when the first generations E-cigars were first introduced by Hon Lik, it is Clearomizer is the technology which has a vaping liquid, reservoir and atomiser coil all within one single unit. Then comes third-generation E-cigarettes which are much larger than the earlier ones. Here the user can customaries tank, how much E-liquid it contained and voltage or power the device can output. The fourth and most recent generation is the most advanced one in the market. This device has regulator to regulate temperature and lower resistance coil to produce bigger and most powerful vapours.[10]

Reason to Abstain E-Cigars:

1. They usually contain nicotine- This leads it too becomes habit-forming, harming foetus if used in pregnancy and affects brain development.
2. They contain other toxins – It has carcinogens like acetaldehyde, formaldehyde etc.
3. Quitting smoking may be harder
4. They cause second-hand smoke.
5. They may not deter smokers.
6. They can harm brain
7. Experimental use may be more dangerous.

This current evidence indicates that using E-cigarettes is dangerous especially for young people and people who never smoked. Though E-cigars may help some people to stop smoking it has no FDA approval as a tool for quality smoking.[11]

Conclusion

Though it seems that electronic cigarettes are less harmful then the conventional ones, research shows that E-cigarettes confers greater susceptibility to the development of alteration in oral biological tissues when compared to ex-smokers or non-smokers. There are diverse adverse effects of E-cigarette of which common in oxidative stress. This is caused by reactive oxygen species(ROS), present in its aerosols, which depends on a set of parameters like the different volume of vapour, voltage settings, the type and state of heating systems etc. Aldehydes & free radicals found in E-cigarette aerosols trigger various type of DNA damage. The safety of electronic devices is also questionable with the potential risk of explosion & fires. Burns, lacerations etc are also common with vaping. Lastly, prospective surveys should be done addressing the potential long term effects on oral as well as a general health condition. The findings will emphasize the need for constructing persuasive vaping prevention messages, promoting public health welfare.

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Van der Woude Syndrome: A Case Report

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Abstract

Congenital lip pits are developmental defects that occur on the paramedian portion of the vermilion border of the lower lip. They may be unilateral or bilateral and may occur as an isolated conditionor in association with cleft lip and or cleft palate. When the labial pits occur in association with cleftlip and/or palate the condition is referred to as Van der Woude Syndrome. In 1845, Demarquay(1945) first described the lower lip pit and postulated that the condition occurs due to the indentation of the central incisor. Later in 1954, Anne Van der Woude first reported the association of congenital pitsof the lower lip with cleft lip and palate. Congenital lower lip pits are present in 88% of all VWS patients, and in 65%–75% of the cases these are associated with cleft lip and palate. The Van der Woude syndrome is a rare condition the phenomenon of a cleft lip and cleft palate combined in the same pedigree makes it unique. The VDWS should be considered in the differential diagnosis of cleft lip and palate. The dentist may be the first person to diagnose this syndrome, and therefore,should be aware of its variable expressions. A proper treatment plan at the correct time will help to satisfy the psychological and esthetic needs of the patients. Further, genetic counseling is highly recommended.

Keywords: *Van der Woude Syndrome, Cleft lip, Cleft Palate, Labial Pits, Congenital anomalies of head and neck.*

Introduction

Congenital lip pits are developmental defects that occur on the paramedical portion of the vermilion border of the lower lip. They may be unilateral or bilateral and may occur as an isolated condition or in association with cleft lip and or cleft palate. When the labial pits occur in association with cleft lip and/or palate the condition is referred to as Van der Woude Syndrome. In 1845, Demarquay (1945) first described the lower lip pit and postulated that the condition occurs due to the indentation of the central incisor. Many parents still subscribe to this hypothesis even though the condition is present since birth months before the eruption of maxillary central incisors. Later in 1954, Anne Van der Woude first reported the association of congenital pits of the lower lip with cleft lip and palate. Congenital lower lip pits are present in 88% of all VWS patients, and in 65%–75% of the cases these are associated with cleft lip and palate.[1]

The distinctive feature of VWS is the presence of lower lip pits and/or sinuses, which are present in approximately 85% of cases, but it may be absent in 15% of the cases. In some rare cases, a single barely visible pit might be the only distinguishable feature of VWS, and in others, it may be absent. Other anomalies that are frequently associated with VWS include hypodontia, submucous cleft palate, and bifid uvula, and enamel hypoplasia. Manifestations of the syndrome in other than the oral or facial areas are unusual. More extreme phenotypes in parents tend to produce more extreme expression in their children.[6]

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Case Report: A seven-year-old boy reported with a complaint of difficulty in oral intake of food and liquids due to a cleft in the upper jaw. There was a history of multiple episodes of respiratory tract infections, breathlessness, nasal regurgitation of food, and a nasal twang in the voice. His history revealed that he was delivered through a Cesarean section. The maternal history revealed no history of medication or illness during pregnancy. There was no relevant family history.

An extraoral examination of the child revealed surgical scars of a corrected cleft lip, with two small pits in the center of the lower lip. They just manifested as two simple depressions (Figure 1). When the lower lip was compressed, thick mucus secretion was expressed from both the lip pits. Intraoral examination revealed bilateral clefts of the premaxilla and a median cleft of the hard palate. The uvula was completely absent with a submucous cleft of the soft palate (Figure 2). Radiographic examination revealed a diffuse radiolucent defect in the anterior maxilla, suggestive of a cleft, crowding of the maxillary anterior teeth, and a missing maxillary left incisor. All other teeth were in normal developmental stages and there was no abnormality in the skeletal growth of the jaws.

All the teeth with caries were treated appropriately in the child. The child had already undergone surgical correction of the cleft lip and was referred to higher centers for surgical correction of the cleft palate (Figure 3). Meanwhile, the child was instructed to maintain good oral hygiene. The lip pits were also excised and Z-plasty was done to maintain esthetics (Figure 4).



Figure 1. Lip Pits



Figure 2. Cleft Palate

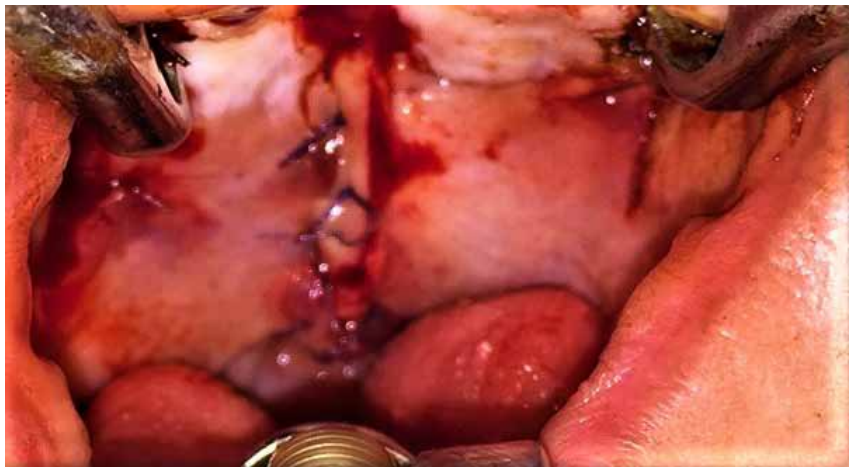


Figure 3. Repaired cleft palate



Figure 4. Postoperative picture

Discussion

Lip pits are rare congenital malformations seen on the lower lip, upper lip, or the oral commissure. The condition generally presents as a unilateral or bilateral depression and has a predilection for the lower lip. Lower lip pits are also known as fistula labii inferioris, labial humps, labial cysts, labial fistulae, and paramedian sinuses of the lower lip. These pits are usually circular or oval and generally show bilateral distribution, located on either side of the midline. The unilateral pits are observed mostly on the left side and rarely on the right side. The exact etiology of lip pits is not known. It may result from notching the lip at an early stage of development with fixation of tissues at the base of the notch or it may be due to a failure of complete union of embryonic lateral sulci of the lip. During prenatal development, a fusion of the mandibular arch and sulcus lateralis occurs at 5.5 weeks while the fusion of the maxillary and frontonasal processes takes place at 6 weeks. Probably, a common event may simultaneously disturb fusion in both locations resulting in the strong association between lip pits and cleft lip and/or palate. Although lip pits are generally asymptomatic, in some cases they contain salivary glands. Watery discharge from these pits, especially during mealtime has been reported. Mild cases of lip pits require no treatment. In this report, none of the patients presented salivary discharge during mealtime. However, surgical correction of these anomalies is recommended in more severe cases not only to improve esthetics but

also to remove chronic inflammation and prevention of salivary discharge from lip pits.[1]

The first report on lower lip pits by Demarquay attributed the formation of lower lip pits to the impressions made on the lower lip by the upper central incisors. Most patients tend to adopt this hypothesis even now. According to Kitamura, in a 32-day embryo, the lower lip consists of four growth centers, divided by one median and two lateral grooves. In the 38-day embryo, the lateral grooves disappear, except in the case of impeded mandibular process growth that results in the formation of a lip pit. If a cyst deriving from the epithelial wall communicated with the duct of labial glands, a congenital fistula of the lip is formed. The development of lip pits starts at day 36 of development, CL at day 40, and CP on day 50. The periods of liability of these three tissues probably vary in length and even in sequence, and perhaps they also overlap accounting for the strong association between the lip pits and cleft lip or palate.[2]

The majority of the labial and commissural pits occur without mucus exudation. However, a small percentage may be associated with cleft lip and palate, and therefore not a thorough clinical examination is recommended in individuals with pits to rule out a submucosal cleft palate which may not be obvious to the clinician. Surgical excision of the labial and commissural pits is indicated if the aesthetics of the individual is appreciably affected and exudation of mucous secretions can not be controlled. Surgical excision should include the total removal of the minor salivary glands that exude secretions at the base of the pits to prevent the formation of mucoceles or cysts.[3]

Management of VDWS is mainly focused on the surgical correction of clefts and lip pits. Surgical excision of the labial and commissural pits is indicated if the esthetics of the individual is appreciably affected and exudation of the mucous secretions cannot be controlled. Surgical excision should include total removal of the minor salivary glands that exude secretions at the base of the pits, to prevent the formation of a mucocele or cyst.[4]

Proper evaluation and treatment of VWS along with genetic counseling is important. Care should be taken during the surgical procedure for lip pits, of complete excision of the fistulous or sinus tract, which otherwise may lead to the formation of a mucoid cyst. Patients who do not undergo surgical correction should be instructed about meticulous hygiene care.[5]

More extreme phenotypes in parents tend to produce more extreme expression in their children. However, the lesser expressions of VWS are common and should be actively looked for when counseling families about cleft lip or cleft lip and palate. All the affected parents should be cautioned that they carry a risk of 50% for each child with a cleft lip or palate or both. The potential of embryocopy to detect minor malformations such as cleft lip in early-term pregnancy allows for detection of VWS.[6]

Syndromes exhibiting lip pits other than VWS are PPS characterized by multiple defects of the extremities, face, mouth, and genitourinary system. Other malformations in PPS include webbing of the skin extending from the ischial tuberosities to the heels, cleft palate and/or lip, lower lip pit with salivary drainage, genital anomalies, and synechia or syngnathia. Orofacialdigital syndrome type 1 is an X-linked dominant trait, predominantly in males with striking orodental, facial, digital, renal, and central nervous system abnormalities. Orodental signs include cleft palate, bifid tongue, hypodontia, hypoplasia of nasal cartilages, hypertelorism, median cleft of the upper lip, and lip pits. Kabuki makeup syndrome manifestations include the dysmorphic face, postnatal growth retardation, skeletal abnormalities, mental retardation, and unusual dermatoglyphic patterns. As the hallmark features of other syndromes were not manifested, it was confirmed as a classic case of familial VWS.[7]

Many mutations have been described in VWS patients, and also due to the implication of stochastic factors or modifier genes on the IRF6 function, molecular genetic testing does not seem justified at the moment. Because VWS has a high penetrance and a variable expression, genetic counseling is advised for future pregnancy.[8]

Conclusion

The Van der Woude syndrome is a rare condition the phenomenon of a cleft lip and cleft palate combined in the same pedigree makes it unique. The VDWS should be considered in the differential diagnosis of cleft lip and palate. The dentist may be the first person to diagnose this syndrome, and therefore, should be aware of its variable expressions. A proper treatment plan at the correct time will help to satisfy the psychological and esthetic needs of the patients. Further, genetic counseling is highly recommended.

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Early Childhood Caries as a New Public Health Problem

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Abstract

Tooth decayis an irreversible microbial infectious disease among most of the children. It is due to close interaction of bacteria S mutans and sticky high sucrose content food and enamel the outer surface of the tooth. Streptococcus. mutans transferred from mother to her baby during birth. S mutanss also inoculate and survive pre-dentate babies. Early childhood caries starts in the early phase of life, it spread rapidly in high-risk population those who remain untreated progresses rapidly in those who are at high risk, and often goes untreated. ECC outcomes hurt OHRQoL and their family economical status. The tooth decay pattern in toddlers is different from others. It can be recognized easily. There are various types of synonyms used for tooth decay in infants and children. Tooth decay cannot start without any sugary substances like sucrose lactose. Therefore, every dentist and each researcher person gave a prime focus on infant feeding or dietary patterns and feeding practice of children by giving them proper education. Not only the professional but every parent and caregiver has the key role to maintain the oral hygiene to control the disease. There are various types of treatment options for early childhood caries with different types of interventions. It depends on the degree of disease progression tooth decay, child age factor, and mainly with social factors.

Keywords: *Dental caries; Childhood infections; Dental problems; Management.*

Introduction

Tooth decay is an irreversible microbial infectious disease among most of the children. It is due to close interaction of bacteria S. mutans and sticky high sucrose content food and enamel the outer surface of the tooth. Streptococcus. mutans transferred from mother to her baby during birth. S mutans also inoculate and survive pre-dentate babies. S. mutans always break down the sucrose for energy production, make the oral environment more acidic. In the acidic environment, the ph of saliva falls below its critical ph. The low pH of saliva remains for a long period, which directly starts the demineralization of the tooth. Demineralization causes huge loss of mineral of tooth surface and dental caries progression starts.[1]In all countries developed and developing Early childhood caries became the main oral health problem trough the glove.[2]Early childhood caries starts in the early phase of life, it spread rapidly in high-risk population those who remain untreated. progresses rapidly in those who are at high risk, and often goes untreated.[3,4] ECC outcomes hurt OHRQoL and their family economical status.

The tooth decay pattern in toddlers is different from others. It can be recognized easily. There are various types of synonyms used for tooth decay in infants and children,[6]which definitions used to describe dental caries based on the condition etiology, with prime focus on rapidly uses of the nursing bottle. Most frequently used term for dental caries are rampant caries,baby bottle caries, early childhood tooth caries, nursing bottle caries, maxillary anterior tooth caries,[7,8] most of the terminology indicate the etiology of tooth decay among

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kindergarten children.[8] Nursing bottle tooth decay in children's teeth mainly linked with the frequency of bottle feeding of children.[9] some dentist called it baby bottle-fed dental caries because of its inappropriate bottle feeding habit.[7,10] In recent years the terminology "early childhood caries" is becoming very popular among every dentist and became a good research topic for every researcher.[8,10]

Early childhood caries terminology was proposed in the year nineteen ninety-four in a workshop conducted by the Centers for Disease Control and Prevention. Its main aim to focus on multiple causative factors that's has a great hand on developing tooth decay.[12] Early childhood caries defines itself as the presence of one or multiple tooth carious lesion, missing tooth, or restored tooth surfaces in deciduous dentition in children less than seventy-two months. Decreased in the percentage of tooth decay in infants and kids in western nations, tooth decay in kindergarten children is a serious problem in every developing and developed nation. Early Childhood caries is considered as a pandemic in developing nation. Tooth decay prevalence among children in a developing nation is around twelve percentage. data from various studies from different countries state that higher tooth decay prevalence is in Africa and Asian countries.[13] The etiology of ECC is multifactorial and has been well established. ECC is frequently associated with a poor diet and bad oral health habits.[14,15]

Microbiological risk factors: The main cariogenic Bacteria is Streptococcus. mutans and S. sobrinus in the presence of sucrose (main fermentable carbohydrate) the pathogenic bacteria s mutans helps in demineralization of an organic particle of tooth structure lead to a cavity. from different studies, it is found that in early childhood caries in children s mutans is the main pathogenic organism percentage of S. mutans in ECC is around thirty percent found from plaque flora. S mutans bacterial colonies always linked with the large carious lesion, white sport carious lesion mainly. Transfer of any pathogenic organism from mother to infant or from caretaker to kids is called Vertical transmission, the mother is the main storehouse from where a kid gets MS. it conforms from old studies where the same bacterial species found from mother and child. The latest technology chromosomal deoxyribonucleic acid pattern givesmore ideas about vertical transmission of bacteria.[16,17] Recent data shows that high sucrose contain liquid diet in a bottle at night is the main risk factor,for developing dental caries but it is not the only causative factor for tooth decay.

Feeding Practices: Unnecessary use or frequent use of baby bottles with a high sugary diet is the main causative risk factor for Early childhood caries. Long term use of bottle with high lactose at night cause tooth decay. Various studies proved that there is a very close link with early childhood caries and nursing bottle feeding and night sleeping with a nursing bottle. Mother breastfeeding provides high immunity to the child and there are very few chances of gastrointestinal infection and respiratory infection to children. Ling term contact and frequent contact of the tooth with sugary milk makes the tooth outer surface porous and suitable for developing dental caries. If the frequency of intake of sugary diet (with fermentable carbohydrate) increases the demineralization process became more than remineralization which causes dental caries. Long term and nocturnal mother breastfeed has a great link with early childhood caries after one year. Due to high and long term sugar intake at night, the saliva amount decreases at night due to the high content of sugar. So for that demineralization exceed from re-mineralizationand the protective nature of saliva reduces.[18]

Dietary Risk Factors. Combination of heavy infection and MS, kids with Early childhood caries have great experience of taking a highly sugary content diet. A sugary diet breaks down very first by S.mutans and converted to organic acids on the other hand in acidic medium demineralization of enamel and dentin occurs. They frequently use baby bottles to increase exposure to fermentable carbohydrates.[19] Good oral hygiene habits have a great impact on children's lifestyle and their economic status. Environmental factors of parents that affect the oral hygiene of children are poverty, social well being, ethnicity, etc.

Prevalence: Early childhood caries is the main oral health problem in every country and it badly affects the small children, little infants, throughout the globe. It has cleared from the epidemiology of dental caries that the prevalence of Early Childhood Caries differs in every population regardless of race, culture, and ethnicity. CDC reported that the caries prevalence of children aged between 2-5 years is around twenty-four point two percent in the USA in NHANES III between 1988 and 1994. It has also found that children living annually highly income families havea low percentage of dental caries as compared to a low-income family. [20,21]

Diagnosis: Early childhood caries identified by a very dull, chalky white sports that follow the path of

gingival margin it progresses very first. ECC in early-stage mostly found in deciduous maxillary incisors. dental caries mainly found in palatal or facial surfaces of the deciduous tooth in all cases. it can be identified clinically with the presence of yellowish or brownish cavity on the tooth surface. In the older age kids whose deciduous tooth has fully erupted to occlusal level, there is no considerable tooth damage found.[22]

ECC or nursing bottle caries has some important features:

1. Tooth decay on the proximal surface of a child less than three years.
2- Dental caries of the primary tooth, missing primary tooth (due to only for toothdecay), or restoration of a tooth in kids between three to five years children.
3. Dental caries index dmft is the same or higher than four years at the age.

ECC prevention:

- There are three types of approaches to the prevention of ECC.
- Community-level- education, water fluoridation
- Professional level- early detection, diet counseling, fluoride
- Home care level- proper tooth brushing technique, proper diet
- Proper training to mothers and caretakers can give excellent oral health education to take care of children's dentition.

Tooth decay cannot start without any sugary substances like sucrose lactose. Therefore, every dentist and each researcher person gave a prime focus on infant feeding or dietary patterns and feeding practice of children by giving them proper education. Not only the professional but every parent and caregiver has the key role to maintain the oral hygiene to control the disease. Proper oral health education has the power to improve oral hygiene and prevent oral health from various kinds of oral diseases. We have to make a habit of maintaining good oral hygiene to protect our oral health from different diseases.

Treatment Plans: There are various types of treatment options for early childhood caries with different types of interventions, it depends on the degree of disease progression tooth decay, child's age factor, and mainly with social factors. It is ideal to give intervention for early childhood caries and take clinical examination it is very essential to ask the date of birth of a child for better results those kids who are in a low level of risk to develop dental caries don't need any restorative treatment. Kids with a moderate chance to develop caries need restoration for cavitated dental caries and preventive acres like flourish varnish for white sport lesion and that need observation for any future progression. High-risk kids need early gic restoration for the prevention of the progression of dental caries.

Pre-fabricated crown places on the restored primary tooth for the protection of the tooth structure. The best treatment for dental caries in the deciduous tooth of small kids is Atraumatic Restorative Treatment. In the process of ART, the carious portion is removed by spoon excavators hand instruments only filled the cavity with Glass ionomer cement. GIC is the best item for the deciduous tooth in the ART process. Atraumatic restorative treatment is a very easy process and it has lots of advantages and reduces the fear of any kind of dental treatment. No need for anesthesia or electronic device needed.[25- 28]

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Fixation of Condyle with Delta Plate: A Case Report

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Abstract

Condyle fractures account for 25-35% of mandibular fractures and deserve special care from the rest of the mandible due to their anatomical distinctions and capacity for healing. Past clinical and biomechanical trials have suggested the use of two miniplates for condyle fracture fixation. Two miniplates allow the proximal condyle fragment to a certain scale, and thus are useful in low fractures. This present case report shows the use of delta-shaped mini plates (titanium) in ORIF of subcondylar fracture.

Keywords: *Condyle fractures; Titanium delta shape miniplates; fracture fixation.*

Introduction

Case Report: Patient came to the department of oral and maxillofacial surgery, Institute of Dental Sciences, SUM Hospital, S'O'A University with a history of restricted mouth opening for one month.

History of present illness: The patient had a road traffic accident one month ago due to a collision with another vehicle. There was no history of loss of consciousness,vomiting or nasal bleed. Bleeding present from left ear.

On examination:

Extra-oral examination: No gross facial asymmetry present. Laceration present in the lower chin region. The left side temporomandibular joint is tender and palpable on the left side of the face.

Intraoral examination: Mouth Opening restricted (21mm). Derangement of occlusion present.



Figure 1a. Frontal View b. Right Lateral View; c. Left Lateral View; d. mouth opening measuring 21 mm

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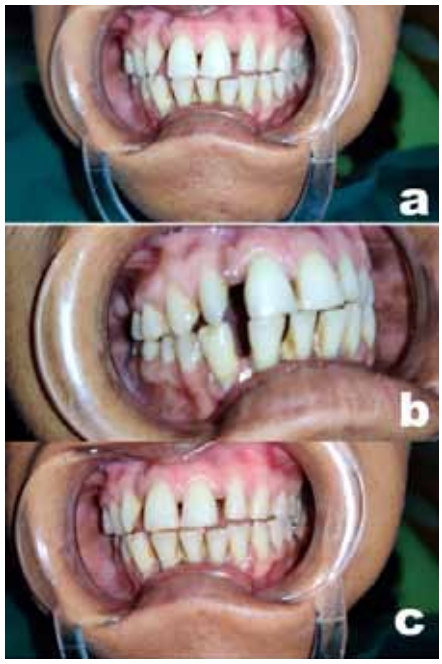


Figure 2a. Derangement of occlusion b. Occlusion w.r.t. right side; c. Occlusion w.r.t. left side.



Figure 3a. 2D Axial view; b. AP view; c. 3D CT skull view

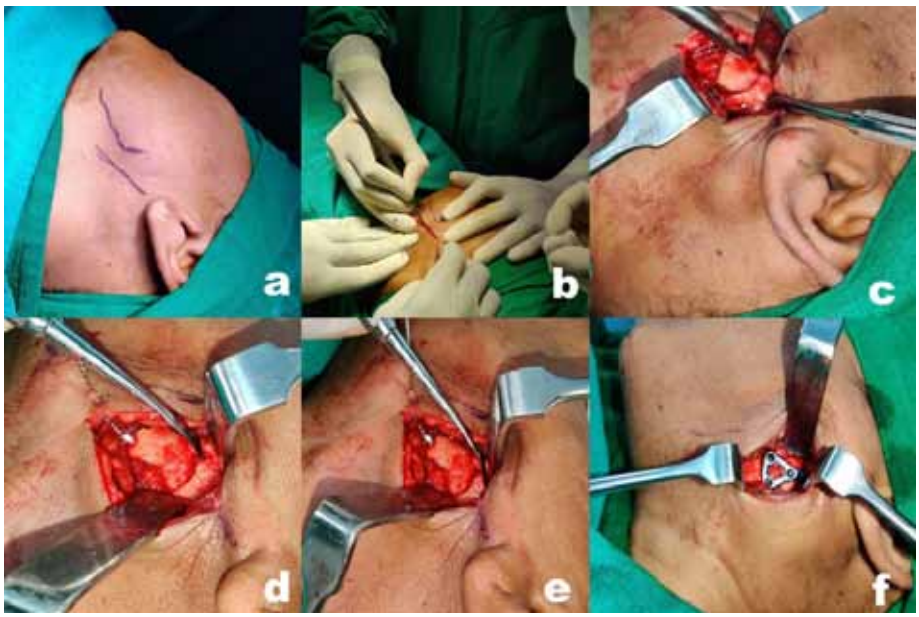


Figure 4a. Incision line drawn; b. Incision given; c. fracture site exposed; d. IMF screw placed; e. Fracture fragments reduced; f. 2mm Delta palate fixed.

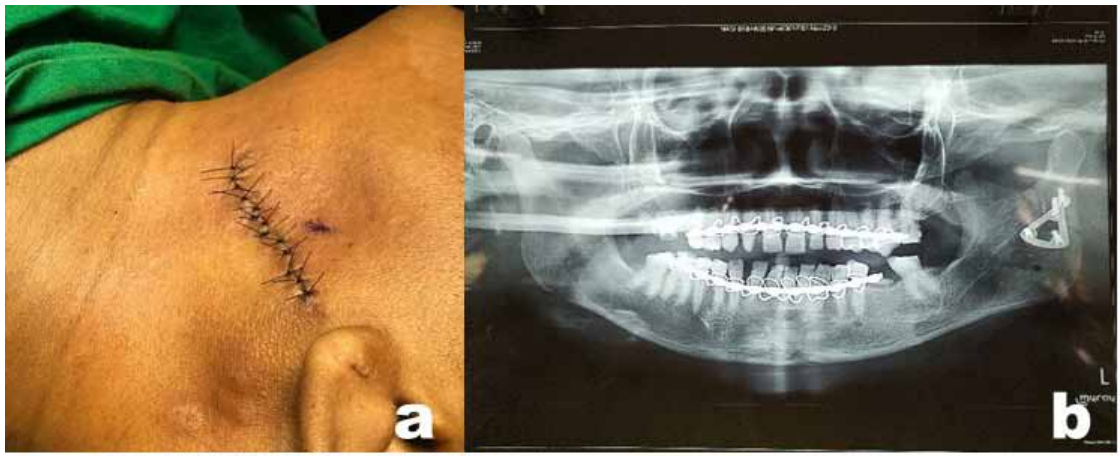


Figure 5a. Closure done with vicryl 3-0 and ethilione 4-0. b. Post op OPG

Radiological examination: To scan accurately for the possibility of fracture of mandible, the utmost2 views are given perpendicular to each other.[1] "Typical radiographic findings in the presence of a condylar fracture are: a)decreased condylar-length, b) presence of radiolucent fracture line, or in cases of overlapping segments, presence of radio-lucent density and evidence of premature contact on the side of the fracture if the radiograph was taken in occlusion with the patient.[1] In 2D axial section we see dislocation with left sub-condylar region. For this patient we had taken an orthopantomogram, an AP view and a 3D CT skull showing dislocation of left sub-condylar region.

Treatment Done: Under all aseptic condition general anesthesia administered via naso-endotracheal intubation. Fracture site exposed via retromandibular incision which was given 2 cm from the angle of mandible. Periosteum is reflected and the fracture site was seen. An IMF screw was placed in the lower ramus and the segment was pulled downward and the distal segment was reduced in its anatomic position and fixed with one 2mm delta plate and four 8 mm screws. Through irrigation was done with normal saline and betadine. Layer by layer closure was done with vicryl 3-0 and ethlione 4-0.

The way to repair condylar fracture is either by open or closed reduction, that often has provoked debate.[2,3] Though various rigid fixations has been identified, this is the most acceptable modality today with miniplates. Miniplate osteosynthesis produces a fixation (rigid) which can easily be adjusted to bone curvature, which involves a basic procedure only. "Choi and Yoo compared the biomechanical reliability of four different positioning strategies (4-screw monocortical miniplate, 4-screw bicortical mini dynamic compression plate, 4-screw 2.4 mm plate, and a double monocortical miniplate) in formalin-fixed corpse mandibles, and considered the double miniplate to be the best.[4-6] A 2-plate modality is easier if there is sufficient bone on both side of the fracture, but fixation with two miniplates might be a more painful treatment than fixation with a single miniplate". 2 miniplates (double-plate technique) is the most effective as they better neutralize the forces of tension/compression and pressure and provide greater stability.[7] The 2 plates are positioned with an some amount of gap between the fracture line and screws to achieve more stability. This drawback was resolved by Meyer et al.'s decision, taking into account the anatomical conditions, to choose a different type of trapezoidal shaped plate. Use of one single resorbable plate is not accepted in condyle mechanically loaded sites where the osteosynthesis has shown to be fragile and low.[8] In the early 1990s the 3D osteosynthesis plate was incorporated in maxillofacial surgery. The benefits are: reduced size paired along with the more rigidity of the plates. Special designed plates like the delta plate or the trapezoid plate are available as an alternative to the modified 2-miniplate technique, and bio-mechanical & clinical trials has shown that such plates require adequate strain neutralization. Thus, such plates offer adequate stabilisation with the benefit of a smaller plate for ORIF of fractures of condyle and sub-condyle. [4]The surface is bent towards the inclination of the mandible in the delta-shaped plate; therefore, the tensile lines and the compressive force distribution extend parallel to

either sides of the plate. "The plate is 1 mm thick, 20 mm long, and 5 mm broad at the top and 12 mm wide at the surface. There is an arm with 2 longitudinally spaced holes at the top of the plate; two more holes in the 2 corners of the base of the plate.[9] Using the new delta-shaped plate for condylar neck fractures has 3 major advantages: I neutralization of the different strains at the anterior, lateral and posterior borders; ii) extra stability offered by a compression miniplate; and iii) a small osteosynthesis plate".[9] The Delta plating device is capable of distributing the necessary loads in every directions. This plate allows for stable osteosynthesis in the mandible's condylar neck area and this form of osteosynthesis can withstand physiological strains inside the injured temporomandibular joint as defined by Lauer et al.[9] He showed, in 9 cases, 2 incisions of risdons & 7 incisions of retro-mandibular were administered to reveal the condyle, found fracture area & repaired using delta plate. All the observed clinical subjects showed excellent stabilization and decrease in the condylar region. Recovery to function was rapid with a brief duration of postoperative intermaxillary fixation. The condyle was medially displaced in one subject, and was displaced out from glenoid fossa. Extracorporeal approach was performed & the head of the condyle was attached to remainder of the ramus. After 2 weeks this individual presented with signs of plate contamination and then the contaminated plate was removed. Fracture of the plate or bending and loosening of the screw are known indicators of either insufficient stabilisation of the fracture or inappropriate reduction of the fracture. We have not found any of these concerns in all the topics tested.[10]Because of the involvement of internal maxillary artery and superficial temporal artery, postoperative edema and hemorrhage was typically seen in the condylar area, in this case the postoperative edema and swelling was considerably less. Just one patient had an prolonged edema cycle in which extracorporeal plating approach was performed. He was prescribed post-operative steroids. Antibiotics and hormones play an significant part. Higher antibiotics were given to all patients. In every cases steroids were avoided except one that involved extracorporeal approach. Postoperative wound infection hasn't happened in any of the cases.[10] Five patients from 9 cases were put in intermaxillary post-operative fixation for a period of 1 to 2 weeks. Within one week of surgery, restoration of function and immediate functional loading are possible. [11]Facial nerve branches, retromandibular vein and parotid fluid can be detected intra-operatively, & also at the same time they should be removed safely to prevent injury to essential structures. For neither of our cases we have experienced any temporary facial nerve palsy. For all nine cases, post-operative follow-up was conducted over a span of six months to one year. Radiographical testing has shown that the osteosynthesis is fairly stable. Functionally stable and well rehabilitated, all patients diagnosed with delta plating device were. Just one patient had plate contamination, and eventually removed the contaminated layer. In our view, condyle fracture reduction is best treated with the current 3D delta plate method, that provides excellent stabilization & neutralizes the condylar region's changing strains.[11]

Conclusions

While separate study agree that closed reduction and immobilization is the treatment of choice for condylar fractures, modification of ORIF usually yields better outcomes. The retromandibular approach has been used as the traditional and favoured approach to the condyle's face. ORIF with 3D plate stabilizes for any sub-condylar fractures is completely important for early functional regeneration.[12] 3D plates are known as having greater stability than traditional mini plates. The geometrical structure of the 3-dimensional disk, that adapts easily to the fracture, provides greater stability and work. The delta plate which was used in our analysis is a titanium-formed non-compression layer. The scale and shape of the plate helps the surgical surgeon to adjust and repair sub-condylar fractures quickly. Revised delta plate with compression holes, trilock system provides more advantages to the new system. This delta plate can also be used in an intra-oral technique, which was aided endoscopically. The risks associated with this plating device are very small, as is the case with all other condyle surgical procedures. After a week of immobilization, early recovery and practical activation became feasible. The condylar fracture handled by delta plate had a good feature and aesthetics on a long-term follow-up. These platelets achieved surgical speed, relaxed adaptation and adequate stability. With this technique, the practical and cosmetic result has proved that this plating device is one of the latest developments in the management of sub-condylar fractures. No special armamentarium was needed as only the plate shape varies, and the holes in the screw and screw are the same as the standard mini plate method. A basic mouth opening procedure and variety of motions without intensive physiotherapy rehabilitated all of our patients at the earliest possible time. Most of the analysis was performed on a small sample size

during this review. For prospective research studies with greater sample sizes will be helpful.

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A Portrayal of Changing Oral Condition in Today's World: A Review

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Abstract

We cannot solve our problems with the same thinking We used when we created them-as stated by Sir Albert Einstien. Today's diverse change in the oral condition of the patient needs meticulous understanding by the dentist of the underlying problem and how to solve it in stipulated time, money, environmental and social condition of the patient. Health care systems worldwide deliver services for diseased and drug addicts but most lack oral health care services. Lack of appropriate settings, valid assessment protocol for conducting oral health studies, and poor collaboration between dental and general health care sectors for the diseased and drug addicts is interfering terribly with the accurate picture of today's situation. Moreover, lack of appropriate policies to improve access to dental services, lack of interest among dental professionals in treating the diseased and addicted persons, and low demand for dental care from these sections is leading to today's ineffective all-around dental treatment.

Keywords: *Oral Health, Oral Hygiene, Oral Diseases, Health Policy.*

Introduction

The later part of the twentieth century saw a massive transformation in oral health. The dramatic advancement in the medical field during the last five decades resulted in more number of medicated elderly persons and drug-addicted youngsters. Though there are remarkable achievements in recent decades in the health sector, millions of people worldwide have been excluded from the benefits of socio-economic development and the scientific advances that have improved healthcare and quality of life. Systems are becoming more complex and people's expectation of oral care is changing dramatically. In developing countries growing number of development organizations, private foundations, and non-governmental organizations are becoming active in the oral health sector.[1]

Diseases Affecting Oral Condition: Prevalent diseases previously are majorly modulated by the present day's food habits, stress, environmental conditions, bad oral habits of chewing tobacco, smoking, etc. The common diseases which affect oral cavity and treatment are Diabetes, Arthritis, Anaemia, Tuberculosis, Leukoplakia, Syphyllis, Bells Palsy, Parkinson's disease, Plummer Vinson's disease, Pemphigus, Nicotinic stomatitis, cleidocranial dysostosis, ectodermal dysplasia, lupus erythematosus, radiation, hypertension, heart disease, kidney dysfunction, thyroid diseases, menopause, epilepsy, etc.[2]

Medications affecting the population: Nowadays because of the invention of newer drugs and newer treatment modalities most dental patients are taking multiple drugs and have a complicated medical history. As older people are leading a longer more productive life even though having cardiovascular, endocrine,

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or degenerative disorders they are more likely to seek dental treatment. These patients are challenges to the dentists nowadays. Alarmingly studies show that in a normal dental patient population, 43.1% were taking medications. Out of these 17.4% were taking more than 1 medication. • 14.2% takes analgesics eg. Aspirin, Acetaminophen • 12.1% of women at childbearing age take contraceptives. • 9.4% takes antihypertensives, diuretics, etc. eg. Dyazide • 9.4% takes miscellaneous drugs eg. Vitamin, Antacid, Crocin, etc. which can be bought without a prescription. • 7.6% takes psychotherapeutic drugs eg. diazepam, clonazepam • 6.6% takes hormones including insulin, oral contraceptives, Thyroid hormones, and estrogen. • 4.9% takes antibiotics like penicillin, tetracycline, etc. • 3.8% takes antihistaminics which does not need prescription • 3.1% takes heart medicines eg. Digitalis, Nitroglycerin, etc. • 2% of patients were on hypoglycaemic drugs. [3,4]

Drug affecting the population: There is a definite increase in the number of people misusing illicit drugs including opiates, cannabis, and heroin. This addiction is leading to a distinctive dental and oral profile which would impose a burden of a more customized treatment approach by the dentist nowadays. Drug addiction is leading to damage to many parts of the body as an oral cavity, nasal floor, lungs, liver, brain, and heart. Addicts also suffer from physical, psychological, emotional, and behavioral problems. Injection of a drug can have two problems-Firstly repeated use of unsterilized needles leads to HIV/Hepatitis risk. Secondly, the agents used to dilute drugs leading to bacterial endocarditis.[5]

Oral Health Problems Associated with Opiates

The opiates which are generally used are morphine, Heroine, Synthetic Methadone, etc.

With its use in oral cavity presents with

1. Generalized tooth decay, especially on smooth and cervical surfaces, are common.
2. Salivary hypofunction leading to xerostomia.
3. Burning mouth and eating difficulties.
4. Teste impairment.
5. Mucosal infections.
6. Periodontal diseases eg- Adult periodontitis, Necrotises Gingivitis, etc.
7. Bruxism.
8. Candidiosis
9. Mucosal Dysplasia.

Oral health problems associated with cannabis

The cannabis which is generally used is Hashish, Marijuana, etc. With it uses oral cavity presents with

(g) Increased risk of oral cancer

(h) Dry mouth

(i) Periodontitis

(j) Tartar on tooth

Oral health problems associated with stimulants: The stimulants which are generally used are Cocaine, Amphetamines, Cracked cocaine, etc. Cocaine is different types of damages on the face and oral cavity depending on how the drug is taken. Cocaine itself is very acidic and thus it damages enamel when comes in contact with teeth. With its use oral cavity presents with

1 Local application of cocaine leads to gingival recession.

2 Bruxism is a very common complication, which simultaneously leads to progressive dental attrition.

3 Crack cocaine is the most dangerous as it is smoked. Snorting cocaine leads to damage in tissue in the upper palate leading eventually to the nasal septum perforation, palate perforation, changes in senses of smell, and chronic sinusitis.

4 Cocaine also leads to a movement disorder called transient chorea. This is manifested also spasms of muscles of mastication leading to buccolingual dyskinesia, which looks like a strange smile of the patient.

5 Methamphetamine abusers show bruxism, xerostomia, and rampant caries leading to a condition called MEETH MOUTH. It is a condition of blackened, stained, rotting, crumbling, and falling apart teeth. It has a distinct pattern and of caries on the buccal and cervical smooth surface of anterior teeth. Since these drugs are stimulants, it causes people to clench & grind their teeth due to stress. The patients who struggle with meth addiction crave sugary foods and drinks which leads to more damage or teeth decay.

6. Amphetamines or so-called CLUB Drugs like ecstasy, ketamine, Molly, MDMA also leads to

similar oral condition. All these stimulant drugs cause dry mouth, dehydration, etc. Furthermore, mucosal involvements such as ulcers, vestibular swelling, edema, and necrosis are reported in ecstasy drug users.[6, 7]

Oral Health Problems Associated With Deaddiction:

(I) Methadone is a synthetic opioid widely used in the management of opiate addiction has side effects on oral health.

8. Other drugs used during drug addiction treatment are antidepressants(like tricyclics etc), anti-psychotics(like phenothiazines, etc), and anti-anxiety drugs like diazepam. All these drugs have side effects of salivary hypofunction leading to dry mouth and all negative effects of xerostomia like difficulty in chewing, swallowing, tooth decay, and periodontitis. Halitosis is also a very common finding in these patients.[8]

The role of oral health care (OHC) in addiction rehabilitation/Oral health and relapse: During treatment, drug withdrawal may result in dental pain which interferes with treatment procedure and abstinence and may lead to relapse. In a French case-control study of the impact of illicit drugs on oral health and the use of drugs for toothache, 52% of intravenous heroin users and 21% of other illicit drug abusers admitted using drugs as pain-killers. A similar finding on the use of illicit drugs for toothache emerged from another study. Those quitting opiate use, therefore, require special care for pain control, and this should be integrated into their rehabilitation program. These addiction treatment centers which provide OHC seem to be more successful in promoting both the oral and general health of their patients.[9]

Challenges for dental professionals in coping with the changing oral condition: The variable changes that are occurring in the oral condition nowadays require both objective & subjective evaluation. Objective evaluation is done by clinical examination by the dentist and subjective evaluation is done through series of questionnaire or interview, which provides self-perception of patient-related to self know and oral health and its association with occurrence and relapse of addiction, self-conceived barrios to utilize dental service and oral health-related quality of life. Except for cigarettes and alcohol drug abuse was limited to a small population who tended to be socially disadvantaged. But today drug abuse is evenly distributed across all social strata. There are some barriers to coping with this changing oral condition. They are drug addict's cooperation and compliance in oral health maintenance is not there in most cases. Problems with their long-term follow-up common. The multifactorial and complex nature of drug abuse and addiction behaviors and tendency towards poly-drug abuse among addicts cause difficulties in determining the dependent effects of each group of drugs. Dental professionals also have a negative attitude towards these drug-addicted patients and have an unwillingness to treat them. The addicted patients also have problems with compliances with treatment procedures and fail to accept the suggested treatment plan. Lack of appropriate policies to improve access to oral health services and poor collaboration between dental and general health care sectors serving drug addicts are also an obstacle to effective treatment.[10]

Conclusion

Thus today's diverse change in the oral condition of the patients needs meticulous understanding by the dentists of different drug side effects, drug interactions, the underlying progression of the disease, and how to solve all these through patient dental service delivery.

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Advances in Cephalometry in Relation to the Shift in Soft Tissue Paradigm for Orthodontic Treatment Planning

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Abstract

The discovery of imaging and its use in orthodontics is a subject of technology describing biology. Developments in arch-wires,brackets and composites changed the mechanics of orthodontic treatment. The developments in imaging technology have changed the perception of the clinician as well as the patient towards esthetics. The newer imaging technology helps to study the craniofacial growth pattern of younger patients and to know the effect of orthodontic treatment on this dynamicprocess of growth. Earlier, in 20th century, 2-dimensional (2D) radiography was used,but in 21st century, the new 3-dimensional (3D) scanners use less ionizing radiation and give more information than conventional radiographs. This article is a review of the imaging technology used in orthodontic diagnosis and treatment planning from the use of the cephalostat to the introduction of cheap,reduced radiation Cone Beam imaging of 3D cephalograms.

Keywords: *Imaging, video cephalometry, 3D cephalogram.*

Introduction

"Adsanitatemgradusestnovissemorbum"- a Latin proverb means that, "The first step towards cure is to know what the disease is". Diagnosis is a Greek word meaning to discern among, to know differences between: Diagnosis is the determination of the presence or absence of the abnormal or undesired.

In orthodontic diagnosis, during the beginning of 20th century, only plaster cast was used. With the cast, we can get three dimensional view of dentoalveolar region of the jaws, but we are unable to get a picture of the relationship between the jaws and the cranium. To overcome these limitations and to know precisely the interrelationship between the functional components of craniofacial complex, roentgenographic cephalograms were introduced in 1930. Cephalometry had its beginnings in craniometry. Craniometry is defined as "the art of measuring skulls of animals so as to discover their specific differences". Cephalometry is measurement of the head including the soft tissues. Due to varying thickness of soft tissues there result many inaccuracies.

Even though these cephalograms overcome the limitations of the previous diagnostic method, they have got their inherent drawbacks. Despite the drawbacks, cephalometric radiography remains a vital clinical tool. And so, advanced technologies are now being used to overcome the limitations and make the cephalometry as a more acceptable tool in orthodontic diagnosis and treatment procedures.

History: The earliest method used was to assess the facial proportions from an artistic point of view, with beauty and harmony as the guiding principles. An intricate quantitative system which defined the proportions of the human body (canon) was developed by the Egyptians. The top three squares were subdivided by horizontal lines into five parts to draw the face in accurate detail. Indian iconometry studied by Ruelius showed that face height was used as the module of Buddhist proportional systems.[2]

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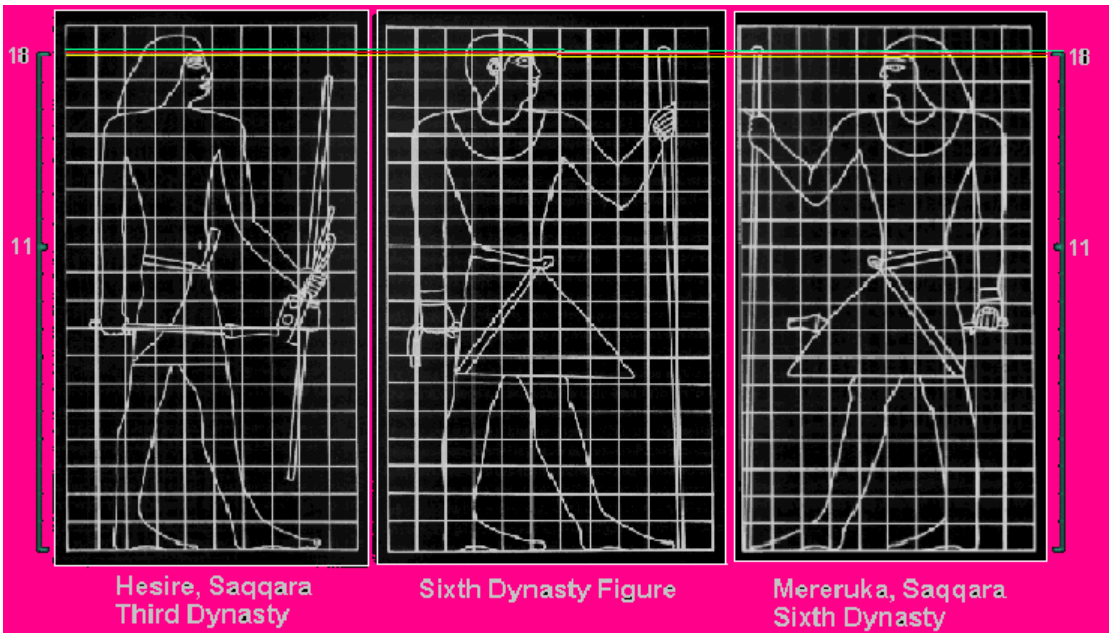


Figure 1. History of Cephalometry

Leonardo da Vinci (1459-1519) studied facial proportions, projected a coordinate system on the face, indicated a preference for 'proportional analysis' and each face was posed in 'natural head position'.[2] Petrus Camper (1722-89) oriented cranium in space horizontally parallel to a line drawn from the center of the acustic meatus to a point below the nose. It is used as the reference line for different measurements used to characterize evolutionary change in facial form and aging. Spix (1815) modified the Camper horizontal as a line from the prosthion tangent to the occipital condyle, but the face was rotated upwards yielding slightly greater prognathism.[3] Retsiusintroduced the terms prognathic and orthognathic where the angle between the horizontal line and nasion- prosthion line was used to determine the facial type. Roentgen (1895) discovered the X-rays which revolutionized the dental profession. Pacini and Carrera (1922)first described a method for standardization of head radiography by using a large fixed distance from the x-ray source to the cassette. Hofrath and Broadbent (1931)both developed a method for standardization of radiographs by fixing a constant focal-spot to object distance & a constant object to film distance. They used special holders the 'cephalostat' to hold the head in position.[4] Krogman and Sassouni (1957) introduced the term roentgenographic cephalometry for measurement of the head from bony and soft tissue landmarks on the radiograph.[4]

Uses Of Cephalometry:

1. Growth and Development: The first and major use of cephalometry is to appraise the growth and development. With the cephalometrics, the orthodontists can time his mechanical procedures to coincide pubertal growthspurt sand predict with some degree of accuracy what the end result will be.
2. Craniofacial Abnormalities: Cephalometrics aid in diagnosing various craniofacial abnormalities like impactions, congenital absence of teeth, cysts, supernumerary teeth, tonsillar and adenoid tissue, cleft lip and cleft palate and mandibular prognathism etc.
3. Facial Type: Sometimes the patients with clinically excellent occlusions will be having difference in facial and dental components Cephalometrics is useful in gaining knowledge about the compensations.

Two important relationships decide the facial type:

I. Position of maxilla anteroposteriorly in the face with reference to the cranium.

II. Relation of mandible to maxilla

4. Case analysis and Diagnosis There are three basic components a Cephalometrics analysis

I. **Skeletal Analysis:** It includes facial type anteroposterior apical base relationship.

II. **Profile analysis:** It is primarily an appraisal of the soft tissue adaptation to the profile of lip size, shape and posture; of soft tissue thickness over the chin; of nasal structure contour and relationship to lower face.

III. **Denture analysis:** It is an appraisal of tooth relationship with each other and with their respective bony bases.

5. Progress Report: Serialtracings offer much more information on developmental changes and on stability of orthodontic accomplishment. Usually, it is recommended to take the cephalograms for every three to four months.

6. Functional Analysis: For the functional analysis, the following cephalometric records are recommended.

I. Cephalogram in postural resting position, initial tooth contact and occlusion used to view the amount of interocculsal clearance and path of closure.

II. Wide open mouth cephalogram used to view the outline of the condyle

III. Cephalogram in end to end bite or Incision used to analysis the curve of free and amount of space between the posterior segments when the incisors in the contact.

IV. Phonetioncephalogram used to show the tongue and soft palate position and may assist the speech therapist.

V. Special view with barium, Iodochloral or other radio opaque media to determine the soft tissue function. They are particularly useful in cleft lip, cleft palate patients helping to analyze velopharyngeal function. Radio opaquing paste over the profile will assist in visualizing the soft tissue outline which is useful in cephalometric profile analysis.

Advantages of Conventional Cephalograms

I. Anteropostero and Vertical jaw discrepancy can be diagnosed with cephalometry.

II. The jaws are related to cranium only with the aid of cephalometry.

III. Predictions of growth and treatment outcome are made possible with cephalometry.

IV. Cephalometry is an important tool in assessing the growth and development and it is useful in research field.

V. Diagnosis and treatment planning in the field of orthodontics is improved to a significant level with the aid of cephalometry.

Disadvantages of Conventional Cephalograms:

I. Cephalometry is two dimensional; we are viewing the three dimensional objects in a two dimensional image where there will be uneven magnification of structure.

II. Identification of landmarks is difficult; there is always intra and inter examiner discrepancy in identifying the landmarks.

III. Conventional cephalometry is time consuming because of the dark room processing, time taken for identification of landmarks and construction of planes and then performing the cephalometric analysis.

IV. Difficulty in merging these cephalograms with the presents developing 3D imaging techniques.

V. Double registration of points is not possible

VI. Storage of cephalograms demand sufficient space and staff.

VII. The orthodontist finds difficulty in communicating the patient regarding the diagnosis & treatment plan.

VIII. There is no room for the patient to take part in the treatment plan processing.

Need for Development: Necessity is the mother of inventions. Three basic necessities in the field of orthodontics, that evolved in the 21st century required developments in conventional cephalometry. They are:

1. The soft tissue paradigm
2. Shift from paternalistic approach to autonomy
3. A significant development in understanding of inherent errors and drawbacks in conventional cephalometry.

1. The Soft Tissue Paradigm: The twentieth century was mainly dominated by the Angle's view, i.e. the 1st aim of orthodontic treatment was to achieve the dental occlusion and the 2nd aim was to correct skeletal jaw relationships. In that view the soft tissues were thought to be taken care by themselves. When the hard tissues were correct, the soft tissues

also were presumed to be correct and no treatment was needed for them. Unfortunately, this is not happening in all cases, especially when the teeth are displaced to a good occlusion without correcting the skeletal problem.[4] In the soft tissue paradigm, the 1st aim of treatment is to obtain the best possible adaptation and proportions of the soft tissues and the 2nd aim is functional dental occlusion. So, to incorporate soft tissue goals in diagnosis and treatment planning conventional cephalometry needed modifications and innovations.

2. **The Concept of Autonomy:** Diagnosis now has changed according to the functional and psychosocial ramifications of dentofacial deformity. Similarly, the treatment planning which has become a more interactive process to set up the priorities, between the patient and/or parents and the orthodontist. The ethical understanding of the doctor-patient relationship has changed. Previously the orthodontist chose the preferred treatment plan for the patient (paternalistic or father-like approach), and the patient (or parent) accepted or rejected the plan, with little room for consideration for alternatives. Today the orthodontist is expected to outline the patient's problems, use inputs from the patient to establish priorities in dealing with the problems, present reasonable treatment alternatives, and explain the risk-benefit considerations of each alternative, including the option of no treatment.

In the past decade, technology that facilitates communication with patients has been developed, particularly computerized graphic imaging to permit visualization of the facial effect of treatment before final treatment decisions are made. This technology and the personal interaction it promotes allow the orthodontist to "design the face first and measure what is needed to get there". In designing the face the patient is now given the chief priority. This approach is called Autonomy. Patients do not understand cephalometry and when cephalometry changes are shown along with the soft tissue changes the patients understand the scenario better.

Advances in Cephalometry: The advances in radiography which in turn revolutionized the conventional cephalometry in great extent. These are to be dealt under the following headings;

1. Digital radiography
2. Digitization of conventional cephalograms
3. Digital cephalometry
4. Computerized cephalometry
5. Video cephalometry
6. 3-dimensional cephalometry

1. **Digital Radiography:** Digitization of radiographs has been widely used in medicine since long time. But in dentistry, its use started in 1980s after the development of intra oral sensors. Unfortunately, it could not take extra oral radiograph like the panoramic and cephalometric image. So, its use became limited. Again, the development of low-cost, time saving, intra- and extra-oral digital radiographic technicand increased use of computer, has made digital imaging a major alternative to standard film imaging.

It became more advantageous both for the orthodontist and the patient in many aspects like the ability to gain cephalometric analysis and superimposition immediately on the chair side computer, can manipulate the images to aid diagnosis and patient motivation, decreased radiation exposure, and the ease of storage.

Principles:

Conventional Imaging: Standard intraoral film usually contains silver halide grains which are sensitized on exposure to x-ray photons and turned to black in the processing. Here the radiographic film acts both as the radiation-detector and the image display.

Digital imaging: In digital radiography, pixels or small light sensitive elements are used. These pixels are arranged in grids and rows on the sensor and are of different shades of grey according to the amount of exposure. Here the radiation detector (sensor) and image display (monitor) are different.[2]

Image Acquisition:

Indirect acquisition: In this method a conventional radiograph is scanned using a flatbed scanner with a transparency adaptor, or by using a charge-coupled device video camera to produce a digital image. This image can then be manipulated with the help of different software packages.

Semi-direct digital imaging: A photostimulable phosphor storage plate (PSP) is used for the image production. It emits light when put in the laser scanner after exposure to produce the desired image. The scanner stimulates the phosphor plate and stores a record of the

number of light photons detected. Helium-Neon lasers with wave length of 600nm are commonly used. The plate should be scanned as early as possible to prevent any loss of data, though it can store energy for a longer period. Then the plate is exposed to view box light to clear the latent image.

Image plates are of similar size in comparison to conventional films and come with disposable plastic barriers. Image plates are reusable and can be reused for thousands of exposures provided there are no surface damage.

Direct Digital Imaging: In this method the image is acquired using a Charged Coupled Device, Complementary Metal-Oxide Semiconductor, or other Electronic Device. The sensor used is a charged coupled device (CCD) but it can't store information and should be connected to monitor. The CCD contains silicon crystals and are arranged in a lattice which converts light energy into an electronic signal and the image is visible in the monitor.

The main advantage of this system is the gain in time as the image is directly visible on the computer monitor immediately after the radiation exposure. Earlier the sensors size was very small and were unable to produce the required image but recently the size of the sensors are of similar to conventional film sizes.

Extra-oral digital imaging: Both direct and semi-direct digital imaging system is available for extra oral imaging but the direct system is very expensive and required extra armamentarium. So semi-direct digital system (PSP method) is widely used. Naslund et al. studied the effect of dose reduction obtained with PSP on the identification of cephalometric landmarks. They found that even if the dose reduced up to 75% it did not affect the localization/identification of cephalometric landmarks.

Merits of Digital Imaging:

1. **Dose reduction:** Up to 90% dose reduction reported by some authors as compared to conventional E-speed films in the diagnosis of caries.
2. **Image manipulation:** By using the software provided by the manufacturer the image can be manipulated according to the need. The following can be manipulated:
   - **(a) Contrast enhancement:** to compensate for over or under exposure.
   - **(b) Measurements:** by using digital callipers, rulers and protractors for image analysis.
   - **(c) 3-D reconstruction:** to reconstruct intra- and extra- oral images.
   - **(d) Filtration:** by the addition of filters to the airspace around the face
3. **Time:** Much time is saved as the image can be seen immediately after exposure.
4. **Storage:** The images can be stored in CD/DVD/ HARD DISK memory for a quite longer period of times.
5. **Teleradiology:** The digital image file can be compressed to reduce the size of the memory and can be shared by internet or other electronic medium.
6. **Environment friendly:** No chemicals are used for developing the images. Both CCD and PSP plates can be used for thousands of images.

Demerits of digital imaging

1. **Cost:** Costlier in comparison to conventional system of imaging.
2. **Sensor dimensions:** Earlier CCD size was bulky to position it correctly but now conventional film size sensors are available.
3. **Cross-infection control:** As the same sensor is used for many patients, there is every chance of cross infection if proper asepsis is not maintained.
4. **Medicolegal:** Image can be manipulated for fraudulent purpose. So,different manufacturers have developed software like 'audit trails', to track and recover the original image.

Digitization of Cephalograms: There are three method of cephalometric radiographic analysis.

1. Manual tracing and direct measurement
2. Direct computer digitization
3. Indirect computer digitization

1. **Manual tracing and direct measurement:** The cephalometric radiograph is traced on an acetate tracing sheet with the help of pencil. Then measurement of required angles and distances is done for different analysis with the help of ruler and protractor.

2. Direct computer digitization

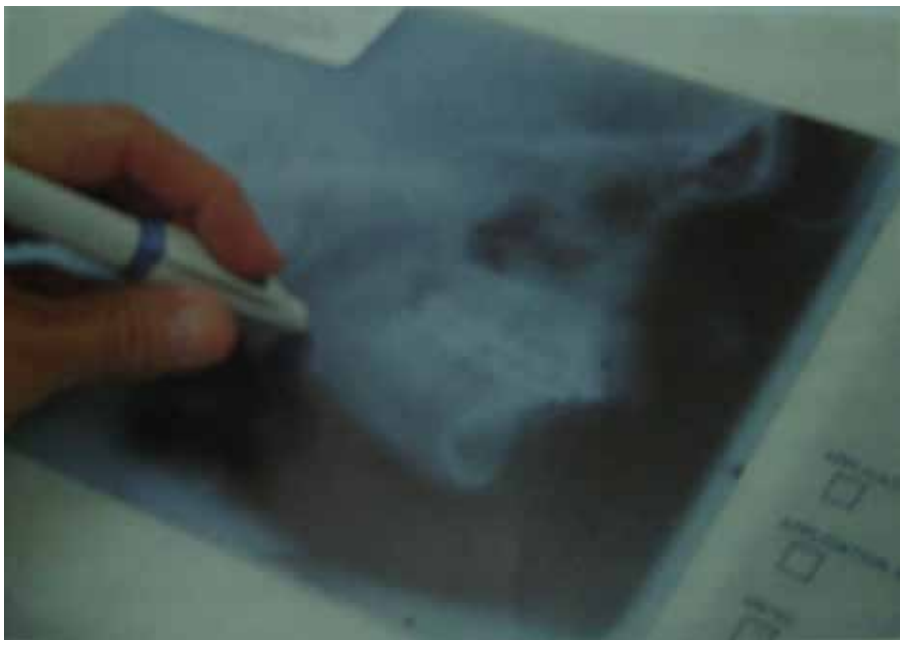


Figure 2. The radiograph is placed on a digitizing tablet and the landmarks are marked with a potentiometer which is electronic pen or "Cross hair" cursor.

Advantages of direct computer digitization: It allows the clinician to identify points directly on the film in the conventional manner, but relieves him of the tedious task of measuring lines and angles. The direction of development and improvement of technology is to programme the computer to identify the anatomy and point which relieves orthodontist of the task of identifying this structures themselves. This might be accomplished through the use of a scanner frame grabber to acquire the cephalometric image for computer storage and analysis. Moustafa et al gathered cephalometric radiographs with a video camera & frame grabber and described software for the detection of soft tissue contour and automated hard and soft tissue landmark location, which they reported to be as reliable.[5]

3. **Indirect computer digitization:** The image is stored in the computer through a video camera (or) scanner or through digital radiography. Then it is displayed on the monitor to digitize by a 'mouse' or electronic pen.

The future of digital radiography: In the current digital world, everything is moving towards digitization and to work almost paper free. Now patient records, photographs, radiographs, and study models can be stored digitally and can be shared online for consultation or discussion. It is always important to accept the new technology and used wisely to improve the patient care and save time and energy in clinical practice.

Computerized Cephalometric Systems:

Computerized cephalometric have two components.

1. Data Acquisition
   - Through
   - ionizing radiation & digitizer
   - sonic technology with microphones
2. Data Management-Various analyses, predictions can be done in those data with the use of different computerized cephalometric systems.

Available Computerized Cephalometric Systems: There are a lot of computerized cephalometric systems are available. These include different software programs which use one or more of the cephalometric analyses, comprehensive hardware and software packages.

1. **Jiffy Orthodontic Evaluation:** Rocky Mountain Orthodontics was the first to provide computer-aided cephalometric diagnosis to the dental profession in late 1960s. Then they developed a new software package known as JOE (Jiffy Orthodontic Evaluation). JOE is a static analysis programme. JOE generates tracing of lateral and frontal

Cephalogram, do the measurements for Ricketts, Jarabak, Sassouni-plus, Steiner and Grummons analysis.Thus, generates the amount of deviation from norms and the number of orthodontic problems.

In 1975, Greenberg and Johnston tested the accuracy of the RMDS (The Rocky Mountain Data System) and found it to be equal in accuracy to estimates using constants from an independent growth sample.[2]

2. **PorDios:** PorDios (Purpose on Request Digitizer Input Output System) is a cephalometric system which is very easy to manipulate according to the need of the individuals.

   PorDios can correct the measuring problems if any in the two dimensional cartesian co-ordinate system. It has a library of mathematical procedures (both angular and linear) for arranging and estimating projections and points.

   PorDios works with a digitizer in normal way, and capable of working with Scanner and Video camera also. It can perform different cephalometric analyses, including Downs, Steiner, McNamara, Ricketts, Tweed, Bjork, Burstone, and Coben and can produce occlusograms from photocopies of dental casts.

   PorDios has built in calculations for showing any discrepancies from the norms. The user can change the standard deviation and mean values for different ethnic groups. The main system can alter the orientation of a picture in order to have the profile looking to the left or right side of the screen. It is available in different languages like English, German, French, Italian, Spanish, Danish and Greek.[1]

3. **Dentofacial Planner:** Dentofacial Planner is a software used in diagnosis & treatment planning for orthodontics and orthognathic surgery, introduced by Dentofacial Software Inc, Canada.

   The Software is divided into orthodontic subsystem and Surgery subsystem. The skeletal and soft tissue regions can be manipulated by both the subsystems.

   Orthodontic subsystem is cando superimpositions,facial growth prediction, simulate the tissue effects of usingorthopaedic and orthodontic appliances. The programmehas the pre-programmed analyses like Steiner, MacNamara, COGS, Downs, Ricketts, Grummons, Harvold, Legan and Jarabak.

   The surgery subsystem estimates the skeletal and soft tissue effects of orthognathic surgery and create a so-called Surgical Treatment Objective. Dentofacial Planner has facilities of displaying a treatment planning tracing superimposed over the load-state tracing, an option for reverting the tracing to its state at load time, CO-CR registration of the mandible, whichhelps in customization of sections for tempomandibular joint tomograms for each patients by means of analyzing a sub-mental vertex x-ray.

4. **Quick Ceph Image:** Quick Ceph Image is a software package designed to do cephalometric analysis and mapping. There are thirteen different types of analyses can be doneby this programme namely Ricketts, Steiner, Jarabak, MacNamara, Downs, Iowa, Roth, Burston, Sassouni, Frontal and SMV and Model analyses like arch length & Bolton discrepancies.

   Other functions this programmeare CO-CR conversion, prediction of facial growth, Steiner box for arch length discrepancy, treatment simulation for both orthodontic and orthognathic cases and superimpositions.

Quick Ceph Image can take both intra- and extra-oral pictures using a video camera and can store them.

Advantages:

1. Curvature in cephalometric lines can be accurately edited.
2. Performs orthodontic and surgical movements in single window.
3. Integrates Model Analysis Measuring ALD: Bolton discrepancy.

Drawbacks:

1. Requires high-resolution digital cameras
2. Optical disc storage preferred.
3. Expensive.

Schwartz (1993) evaluated Quick Ceph Image diagnostic system and found that computer generated surgical prediction tracing was 22% greater than the outcome. Lew, using Quick Ceph found that computer generated soft tissue prediction were accurate except in the lower lip area.[2]

5. **The DigiGraph:** The DigiGraph is a product of

Dolphin Imaging Systems, USA. It is a combination of video imaging and three-dimensional sonic digitizing. Spiro J. Chaconas, G. A. Engel, Marc S. Lemchen and Anthony A. Gianellyintroduced it to the Orthodontics in 1990. [6-8]

The DigiGraph Work Station is a unique device that allows the clinician to perform non-radiographic cephalometric tracings and analyses, video imaging, and treatment planning, including manipulation of the patient's tracing overlaid on the video image. It also takes facial, intraoral, and model photographs. It is capable of generating a 2-D or 3-D facial analysis. The system contains monitor, keyboard, RGB video camera with light source, a sonic digitization probe with receptor microphones and a head holder. With the DigiGraph, any point can be located in three planes of space.



Figure 3. DigiGraph.

The machine has the following capabilities:

1. A landmark can be identified as point in three dimensions;
2. A cephalometric analysis can be made independently of the head position;
3. Neither parallelism of the x-ray in the midsagital plane nor the symmetry of anatomic morphology between left and right sides is necessary (Lim, 1992).

Advantages of using DigiGraph:

The DigiGraph Work Station is a powerful clinical tool that offers advantages in a wide range of orthodontic activities:

1. **Non-Radiographic Cephalometrics:** Lateral and frontal cephalometric tracings and virtually any set of measurements can be produced in moments without radiation. Progress records can therefore be taken as often as desired. Tracings from different treatment stages can be superimposed and manipulated on the screen.
2. **Video Imaging:** Live patient images can be displayed on the computer screen. A non-radiographic tracing can be superimposed over the corresponding facial image, giving the practitioner and patient the opportunity to see the tracing and facial soft tissues together. Live intraoral and dental cast video images can also be displayed and recorded for future reference. Paper or photographic copies can be generated in second, and images can also be stored to be recalled at any time.
3. **Treatment Planning:** The orthodontist can alter any screen image, using computer "cut and paste" procedures, to derive a Visual Treatment Objective. As the clinician modifies the facial

image, the superimposed cephalometric tracing and measurements change correspondingly.

4. **Patient and Parent Consultations:** During a patient's initial appointment, the staff can obtain all records within a few minutes. The orthodontist can then evaluate the data and be fully prepared for an immediate consultation.

6. **The indigenous system- Digi -Ceph:** Digi-ceph is an advanced system for computerized cephalometric digitization, automatic analysis, plot superimposition, storage and retrieval. This system has been developed by Department of Dental Surgery at the All India Institute of Medical Sciences, New Delhi with the help of Center for Biomedical Engineering, IIT, New Delhi.

This advanced system is capable of producing 13 cephalometric analyses following digitization of the cephalometric landmarks either directly in cephalogram or indirectly on the cephalometric tracing. User can create his own analysis. The output data shows values and their comparison with the selected Indian ethnic group.

The software has 10 data banks (one temporary and 9 permanent). The temporary data bank can be used for casual information, while other data banks 0-9 can be used for different users or can be used for different modalities of treatment. The data can be retrieved even by partial information about the subject. The system has been in use at AIIMS since 1990 and is being used for routine case analysis and research work that involves cephalometrics.

Video Cephalometry: By using video imaging technology orthodontist can take both frontal and profile images and manipulate them to provide anesthetic treatment goal. In orthognathic surgery case, patients can visualize what they will look after the treatment which helps them motivated for the surgery. The video image looks more realistic than the conventional photograph simulation in which gaps in between photo cuts will be there and also dental movements can't be simulated.

Esthetic awareness and videoimaging: Video imaging is the newer technology which gives more realistic simulation of images for the esthetic point of view. Its use in the treatment planning will be of great help in modern clinical practice. Studies shows thatmore and more patients are listing esthetics as a major reason to go for surgical treatment. So, the esthetic parameters would be the major factor in satisfying the patient in surgical treatment cases.

Holdaway thought "we should determine beforehand that the proposed orthodontic treatment will not result in adverse facial change", so soft tissue analysis is more important. The orthodontic treatment planning using video cephalometry will have better visualization of the facial response to the dental/soft tissue manipulation. So, the clinician can test different treatment plan before deciding the final one. Video cephalometry can quantify the osseous or dental movements of the treatment plan more accurately to reduce the guess. But it needs a lot of research and understanding for the application of this technology. In adolescent patient the complexity of growth prediction of both hard and soft tissues greatly complicates the predictability of VCD outcome. Hence judicious use of this technology in this type of cases will be helpful. But in adult cases, video cephalometric planning will be more predictable because of the completion of the growth of the tissues.

Three-Dimensional Imaging:

History:

- Van Loon in 1915 proposed the necessity of a 3 D system to define the relationship of dentition to the face and for treatment planning. So, he made a plaster cast of the face and the labial surface of maxillary incisors to orient the plaster cast of the dentition thereby creating the "face dentition".

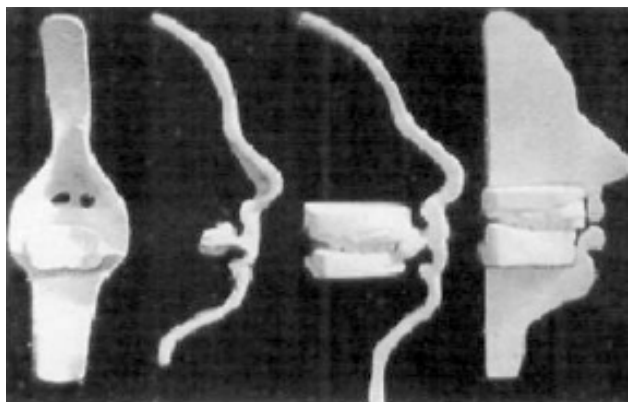
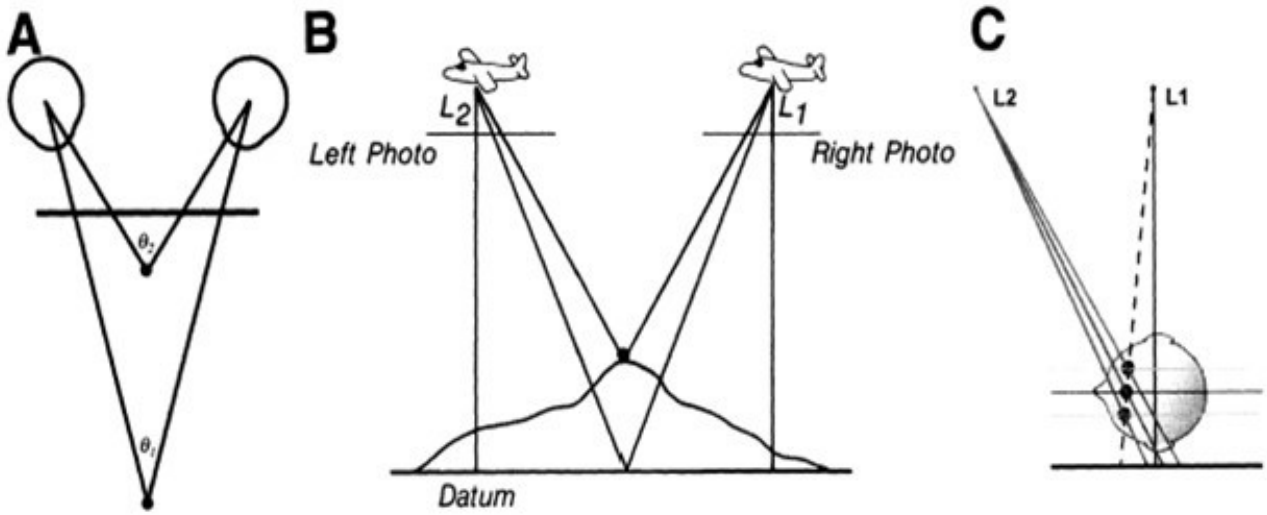


Figure 4. Face dentition

- Simons [1922] introduced gnathostatic models
- Baumrind and Moffitt proposed "coplanar cephalometry". Two images taken with the x-ray source in the same plane but in different location, but patient in same position.9,10
- Cutting et al proposed "biplanarcephalometry".



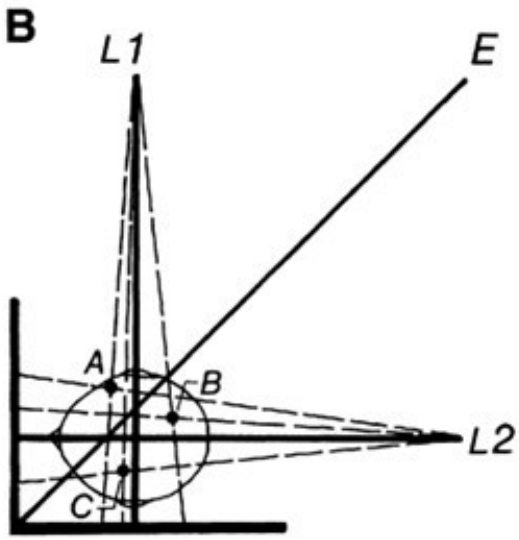


Figure 5. Biplanarcephalometry; Biplanarcephalometry

Two images taken with the x-ray source right angled to each other. Film cassettes right angled to each other but the patient in the same position.

Cephalometrics is a three-dimensional entity. Broadbent and Bolton,since their introduction of the cephalostat, had described about the role of coordinating the lateral and frontal (PA) cephalogramsto get a distortion free craniofacial image. By placing the films in position with respect to the head, if we draw lines from the x-ray source to individual landmark of either film as threads in space, thenit will look like a pair of pyramid of thread, intersecting at right angle throughout the inner space of the patient's head (Fig. A). If both the films are joined with the corner, they meet each other and flattened into one plane, then each thread will flatten to the side of other film with certain distance (Fig. B). The Orientator is the diagram of the flattened threads. On superimposition over the abutted pair of films, the points in each film that correspond to any particular locus in the other can be visualized.

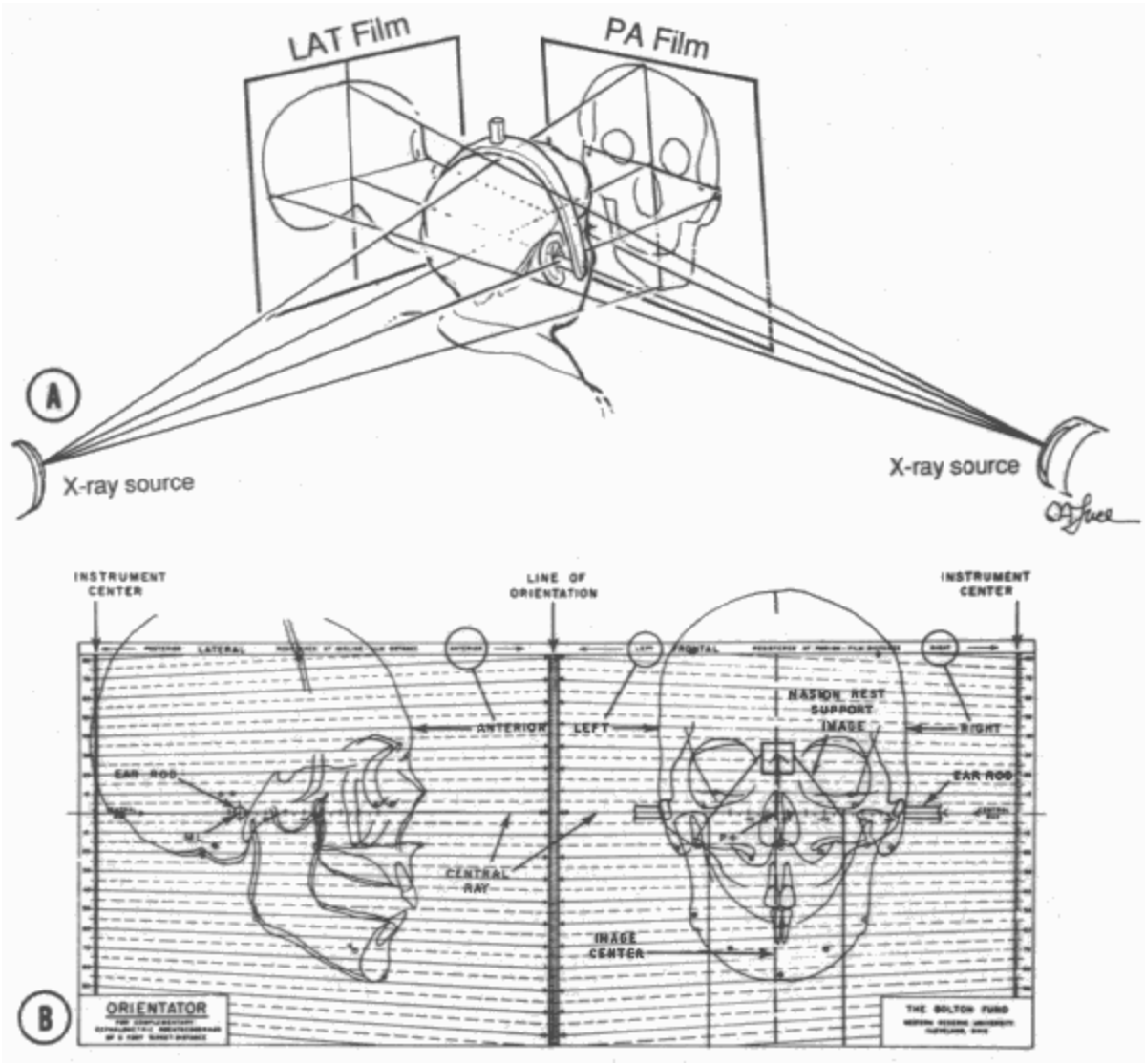


Figure 6. Orientator

Broadbent and Bolton used this to correct the distortion arises due to the spread x-ray beam. The principle of the Orientator then becomes identical with that of one standard photogrammetric tool for this purpose, the ray intersection method.

Cone Beam Computed Tomography (CBCT): CBCT was firstused indentistry in the United States between 2001 and 2004. The initial machines were providingonly a one-to-one, 3D view of the patient's head but the cost and radiation was more. But due to the evolution of a new technology,several CBCT manufacturers come with a combination of partial rotation around the patient's head and pulse technology. A CBCT scanner wasdeveloped which takes a 3D image with lesser radiation than a panoramic radiograph.[11]

Currently, the treatment planning has changed and giving more stress to soft tissues and the facial profile as we are moving from 2D to 3D imaging. This change is possible due to introduction of conebeam computed tomography (CBCT), which captures 3D images in short time and with a very less amount of radiation.[12,13] Images from conventional (fan-beam) CT and CBCT can be combined to create 3D models of the cranio-facial skeleton, the teeth and the soft-tissues.[14-16] By placing these models into the neutral head posture (NHP)[17-19], we can now convert them into "3D cephalograms" if taken at a fixed distance from the subject with the subject's head in a cephalostat.

CT-based 3D cephalograms have resulted the development of 3D cephalometric analyses.[19-25]

However, the problem of the unreliability of internal reference systems and the lack of tools to assess and measure symmetryin some 3D measurements needs to be addressed.

Conclusion

As we begin the millennium, it is time to look at the technologies that may help clinicians provide better patient care. As computer technology has become more sophisticated, it became possible to combine the benefits of the Cephalometry, patient's photographs, study models into one diagnostic practice as well as an effective way to improve patient-parent relationship, and practice management.

There is also potential to remove much of the human errors by using computerized treatment simulation in clinical practices having some imaging skills. For this reason, there have been developments to use computer vision and robots to assist in complex surgeries. Treatment effects could be monitored much more accurately in these vein clinical problems may be revisited to determine if our knowledge based on to dimensional records is accurate.

While there is room for growth in cephalometry, particularly in videoimaging, frontal imaging, 3-D imaging, database generation, quantified studies on accuracy of prediction and the integration of videoimaging & 3-D imaging into clinical practice, these diagnostic and treatment planning aid has clear utility. There is substantial evidence that these technologies offer the practicing orthodontist advantages in following areas:

- Provides the graphic method of communication with patients that can overcome the deficiencies inherent in verbal description of treatment goals and/or outcomes.
- All parties including doctors and patients can be involved in making treatment decisions.
- Improvement in quantification of treatment plans, particularly in the area of orthognathic surgery. With coordinated videocephalometric technology, the surgeon can visualize a plan and have the computer integrated records provide a quantified plan to take to the operating room.
- The use of images in communication with other professional enhances the communication of the problems present and development of potential solutions.

All these advantages due to the advancement in the cephalometry make this diagnostic tool still vital in the field of orthodontics.

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Ethical Permission: Approved

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Essentials of Oral Carcinoma: A Review

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Abstract

In recent periods Oral carcinoma has become a highly significant threat to whole public health in the world and, specifically for dentists. Oral carcinoma comes within the top ten rankings among all carcinoma in the world. lots of research work is going on about the carcinoma, after that prognosis rate is not so impressive to date which is going to be a great challenge to medical science. Oral squamous cell carcinoma can be represented as "natural course", which originated from non-aberrant keratinocytes cells, with history of long term exposure to a various stimulus that breaks down homeostasis process of it, Leads to hyperplasia, dysplasia in terms of various degrees, carcinoma in situ with lots of clinical oral manifestations. This nature course offers a roadway for various research approaches in medical science. The very early recognizable clinical alteration that can any epithelium on his path to creating an Oral squamous cell carcinoma is predominantly the generation of malignant fatal disease, like leukoplakia, lichen planus, erythroplasia are most common.

Keyword: *Oral carcinoma; Public health challenge; Management, Medical research.*

Introduction

In recent periods Oral carcinoma has become a highly significant threat to whole public health in the world and, specifically for dentists. Oral carcinoma comes within the top ten rankings among all carcinoma in the world. lots of research work is going on about the carcinoma, after that prognosis rate is not so impressive to date which is going to be a great challenge to medical science. By this paper, we are going to high light the causes, histologic concept which will helpful for future researchers. Cancer of the oral cavity is malignant and develops from the upper and lower lip. Maximum oral cancers are squamous cell carcinoma because more than ninety percent of oral carcinoma are histologically originating from squamous cells.[1] Various types of differentiation found in oral cancer and the metastasis lymph nodeare present most of the time.[2]

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Epidemiology: Carcinoma of the oral cavity is 2 to 3 times more in males than females in the maximum population throughout the globe. Data from all around the world says, all types of carcinoma in the oral cavity and pharyngeal region come and together represent the 6th most common carcinoma all around the globe.[3] As per the report of IARC incidence oral carcinoma of vermian lip border, tongue, the floor of the mouth, buccal mucosa, salivary glandare high three lakhs diagnosed cases mortality rate is about 1.5 lakh annually. The incidence of oral carcinoma in south Asian countries is high in countries like Pakistan, India, Sri Lanka. A high incidence of oral cancer is found in some parts of Europian countries like Hungary, Slovakia.[3] Oral carcinoma prevalence varies as per the Human Development Index of the UNDP. As per the HDI index, prevalence is high in a developed nation.[3]

Definition: Carcinoma of the oral region most of the time is highly malignant which develops commonly from the lower lip, upper lip buccal mucosa, and tongue. Most often it arises from the squamous cell so-called as Oral Squamous cell carcinoma because in the oral cavity, ninety percent of carcinoma this carcinoma has

various levels of differentiation and metastasis of the lymph node involvement is there most of the cases.[2]

Risk factors: Carcinoma of the oral cavity can be preventable. The use of smoking and smokeless tobacco and consumption of alcohol is the main risk factors for developing oral carcinoma, consuming them all simultaneously has the power of electric effect to develop oral cancer[4,5] according to the report of IARC in the year 2007, snuff uses in smoking tobacco has a carcinogenic effect,it can cause pancreatic as well as oral carcinoma.[6] The chances of developing oral carcinoma are three to four times more in smoke tobacco users as compared with non-users.[7] The chance to develop oral carcinoma is thirty-five percentage less in population those people who stop smoking for more than 4 years ago than the people who are current smokers, and not higher in population with no smoking tobacco use evidence for since twenty years when compared with nonsmokers or non-smokeless tobacco users.[8] An environment in which smoke tobacco uses is more is also a high risk; the risk for carcinoma in the oral cavity is more than eighty-seven percentagehas higher chances of developing carcinoma. The use of smoking tobacco and smokeless tobacco made the immunity power of our oral cavity very weak and made the road to develop mild to severe form of gingivitis, periodontitis, and carcinoma of the oral cavity.[9,10]The smoke generated from smoking tobacco has various elements that develop cancer and these contain has various groups like nitrosamines, carcinogenic aromatic amines. nitrosamines, aromatic amines are called as pre-carcinogens, which must suffer coordinated alterations by oxidative enzymes, so that the final product becomes poor in electrons]. Like oxidation, enzymatic metabolism can also produce carcinogenic products, like free radicals, which has unpaired electrons that make them extremely reactive being capable of promoting mutations by complex mechanisms.[11] Tobacco use makes direct interaction oral epithelium to free radicals of oxygen and nitrogen that can affect antioxidant defense mechanisms. Free radicals like ozone, hydrogen peroxide,hydroxyl radical, ROO,NO are most commonly found in the oral precancer lesion and cancerous lesion.[12]

Alcohol: For developing oral carcinoma alcohol has a great hand. it has a local effect as well as a systemic effect. Alcohol (ethanol) increases the permeability of oral mucosa, which absorbs the lipid particles of oral epithelium. Which latter causes epithelial atrophy and alcohol also interfere with DNA synthesis. Alcohol has a direct effect on decreased salivary flow. It also affects the liver's general function to deal with toxic or potentially carcinogenic compounds.[13]

Other factors for developing oral carcinoma: Among other factors for developing oral cancer, there is the human papillomavirus (mainly linked with fatal carcinoma of the oropharyngeal region and UV radiation.[14] According to the IARC HPV16 is the main causative for the development of oral carcinoma in the oral cavity. HPV18 has also the capability of causing oral carcinoma. The most preferable site for HPV to develop oral carcinoma are pharyngeal tonsils and the portion of the tongue which is inside oropharynges, prevalence of HPV18 to develop oral carcinoma is about 75% in the pharyngeal region. The presence of Human papillomavirus 16, and 18 are the best prognostic biomarker for detecting oral pharyngeal carcinoma.[15] Most of the studies state that Human papillomavirus gives a contribution to carcinoma by 2 viral coted proteins, one is E6 protein helps in degradation of p53 cellanother one is E7 helps in degradation of the tumor suppressor genome product called retinoblastoma protein, UV radiationalso has a great contribution inupper and lower lip carcinoma. [16]

Microscopic and macroscopic aspects Carcinogenesis: Oral Squamous Cell Carcinoma takes many years to develops and in between this period,various neoplastic sites are transforming and changes occur in the oral cavity.[17] The process of a normal cell to transfer into carcinoma is a very complex multistage phenomenon. It develops when epithelial cells of the oral cavity go through by various genetic changes most often oral carcinoma starts with the changes of a less number of normal keratinocytes. These types of changes can be expressed via cytogenetic changes and epigenetic processes that purely change the progression of the normal cell cycle, DNA mechanisms, cell apoptosis that leads to various mutations, by direct contact to various biological factors, carcinogens.[18-20]

For a Better approach to oral carcinoma, oral carcinoma always considered as a fatal disease that involves multiple interactions between various cells, generated by the original carcinoma tissue, the recently generated cells, and tissue. The Oral Squamous cell carcinoma contains CAFs, immune cells and some other tissue during genetic expression oncogenic changes occurs which on the other hand contributes to the huge production of cytokines, EMT, and changes in the

microenvironment. CAFs have some important role in developing metastasis and proliferation.. most of the immune response gets suppressed in squamous cell carcinoma due to the huge production of cytokines. Most Oncogenic mutation in gene expression has a great hand in contribution to micro-environmental modification like ROS deposition, huge production of cytokines, and EMT. The most vital things in the tumor microenvironment are CFAs. Which has a great contribution tothe process of metastasis and invasion most important feature in most is that the adaptive immune response is suppressed by cytokines' huge production.[20]

Oral carcinoma keynotes: Oral carcinoma is always a preventable disease. Highly risk factors are smoking tobacco, chewing tobacco, and alcohol (long term exposure), these things have dynamic and synergistic adverse effects. More prevalent in humans for more than fifty years. a genetic mutation in keratinocytes gave way to develop carcinoma. The pre-cancerous lesion is always a precancer of oral cancer. Most common site ore tongue, lip, buccal mucosa, the floor of the mouth. The biopsy is the most usual method to detect. Screening program reduces the mortality rate

Potentially malignant disorders and dysplastic changes: Oral squamous cell carcinoma can be represented as "natural course", which originated from nonaberrant keratinocytes cells, with history of long term exposure to a various stimulus that breaks down homeostasis process of it, Leads to hyperplasia, dysplasia in terms of various degrees, carcinoma in situ with lots of clinical oral manifestations. This nature course offers a roadway for various research approaches in medical science. The very early recognizable clinical alteration that can any epithelium on his path to creating an Oral squamous cell carcinoma is predominantly the generation of malignant fatal disorders, like leukoplakia and erythroplasia which are the most common ones. Leukoplakia and lichen planus is a white precancerous risk factor, by freezing out other diseases which are already known to not favorable for neoplasm. The microscopic picture exhibits various epithelial modifications like acanthosis, hyperplasia.[19, 20.]

The OSCC originates from epithelial dysplasia phenomena of epithelial cells and represents itself by a neoplastic proliferation mechanism which directly damages ontogenically local epithelial membrane. The power of metastasis is directly linked with the various degree of new growth differentiation of the carcinomatous cells. According to World health organization International Classification of tumors are of three types,well differentiated, moderately differentiated, and poorly or undifferentiated tumor. From Histological pattern of some recent studies says that pattern of invasion (various bonding patterns between invading tumor cells), which has the best prognostic factor in oral squamous cell carcinoma. World health organization classification is very needful and helpful histologically. Till a recent date, there are no particular biomarkers for oral carcinoma. Some new studies show that high levels of interleukin-8 and Spearmidline N1 acetyltransferase 1 can differentiate between patients with Oral squamous cell carcinoma and healthy patients, with high sensitivity.[20-22]

Conclusion

The prognosis of most patients is still poor, despite therapeutic advances in this and many other malignancies. Early screening and early treatment process remain the key point to improving the survival rate of patients In recent days there are lots of technique with well equipped medical facilities to prevent the metastasis of all kinds of carcinomas throughout the glove and in medical science, there is various reasonable information which is capable for early diagnosis and better treatment of another third of cases, where the Oral Squamous cell carcinoma countenances this type of chances. Everything is possible and it is in the own hand od each human being to modify their way of lifestyle and create barriers to all risk factors which are the main cause for carcinoma. Various oral health Education to the people in a different community and especially those people with high risk to develop oral cancer, a good fundamental knowledge to reach the main point of oral carcinoma. Carcinoma of the oral cavity causes various degrees of damage in speech, deglutination and food chewing where mild to severe pain is the main sign. Among the various triggering factor which develops the oral pain, there are various types of mediators in the new growth microenvironment, lack of palliative therapy, a dense fifth cranial nerve innervation, and continuous different oral function, and mild to severe pain due to treatment and opioid resistance.

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Evolution of Modified Glass Ionomer Cements: A Review

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Abstract

Glass ionomer cement has been the subject of extensive research over the last few decades. Refinements were made to the composition of GICs in an attempt to formulate an ideal restorative material (RM) having strength, adherence, natural appearance, and anticariogenicity. Contrary to early glass ionomers, the new restorative systems are easy and practical to use. The emergence of GIC as a 'material of choice' for various applications in dentistry is mainly due to its fluoride-releasing and chemical bonding properties. This article throws light on the evolution and the course of recent advancements of Glass ionomer cement.

Keywords: *Restorative material, glass ionomer, cement.*

Introduction

"Necessity is the mother of invention". There has been a never-ending need to upgrade and advance materials and techniques in dentistry due to changing professional perceptions and to meet the patient's demands. Currently, the patients demand higher aesthetic and biocompatible restorations at lower costs. In this day and age restorative dentistry is switching from metallic to non-metallic materials.[1]

Back in the 1960s, there was an availability of a variety of restorative materials including amalgam, composite, cast alloys etc, but none of them could be categorized under ideal restorative materials. An ideal restorative material is the one that is aesthetic, biocompatible, adhesive, anticarcinogenic and relatively economical.[2] Researchers then began their experiments to create a material that would not only act as a restorative but also replace enamel and dentin.[3] This led to the advent of Glass ionomer cementin 1969 as reported by Wilson and Kent in 1970s.[4]

Materials made of calcium or strontium aluminofluorosilicate glass powder (base) combined with a water-soluble polymer (acid) are the glass polyalkenoates.[4] With Kent calling such materials "glass ionomer" cements the name has become part of the dental jargon. The advantages of glass ionomer cement include adhesion, good marginal seal, fluoride release and biocompatibility but it had a disappointing clinical performance in primary molars as they are sensitive to dehydration early in the setting process and are brittle materials making them unsuitable for use in stress-bearing areas which is why attempts have been made to enhance properties of the glass ionomer cement by modifying the chemical composition of the original powder.[5,6]

History of GIC'S: What began with a small group of dentists and researchers in Londonin 1950s researching to produce a new RM that has similar properties of enamel and dentin and went through a long list of attempts at modifying silicate cement and other modifications are still undergoing attempts for a better one.[7,8]

The first zinc polyalkenoate cement by Smith in 1968 resulted in the introduction of the first adhesive

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RM for dental purposes. The contemporaries Wilson and his group decided to use the polyalkenes acid as a liquid for silicate cement powder.[3]

The first GIC had strength and fluoride release property from silicate glass and biocompatibility and adhesion from polyacrylic acid liquid turned out to be a hybrid cement consisting of calcium fluoroaluminosilicate glass powder and polyacrylic and itaconic acid liquid.[9]

This class of material has achieved widespread use as translucent materials for restoration, fast setting liners and bases, for binding composite to the tooth structure, fine grain luting cement, fissure sealants and metal reinforced core build-up materials.[10,11]

Over the last few decades,compositional changes to both powder and liquid components, as well as clinical experience, has highlighted the practical advantages and disadvantages of GIC.[12]

Modifications of GIC:

Resin Modified Glass Ionomer: Small quantities of light-polymerizable resin groups (usually 2-hydroxyethyl methacrylate or HEMA) into the acidic liquid component was added to modify conventional GIC's physical properties and translucency.[13]

Metal Reinforced Glass Ionomers: Addition of amalgam alloy powder and sintered silver particles to the glass component to form a "Cermet" (ceramic-metal). Evidence of significant improvement in the physical properties of GICement was seen. Further research on this material witnessed diminished caries resistance compared to conventional glass ionomers.[13]

High-Viscosity Autocare Glass Ionomers: High viscosity GIC was introduced to have a strong and adhesive restoration with fluoride releasing propertiesto treat in remote undeveloped regions lacking access to dental care.[13, 14]

Polyacid Modified Composite Resins (Compomers): The term "Compomer" strikesas an intent to combine components and favorable properties of both GIC and composite. They possess the aesthetics of composites and fluoride-releasing trait of GIC though the fluoride release compared to the conventional GIC was negligibleas there was no fluoride uptake by the cavity walls due to the resin bonding agent acting as a barrier.[13]

Zirconia Reinforced GIC: Zirconia infused glass ionomer cement (ZIRCONOMER) is a recent addition to the GIC family which has been introduced to address all the issues that have plagued the conventional ionomer till now. The combination of Hydroxyapatite and zirconia (HAp/ZrO_2) at 4% volume concentration to the GIC powder refined the mechanicalattributes.[15]

Fibre-Reinforced GIC: Improved curing depth, wear-resistance, and flexural strength and reduced polymerization were observed when alumina fibres were mixed with glass powder. This polymeric rigid inorganic matrix technology involves the incorporation of a continuous network/scaffold of alumina and silicon dioxide ceramic fibres.[16]

Proline-Containing GIC: An amino acid–containing GI cement with better surface hardness, quick setting,and increased water sorption properties without adversely affecting the amount of fluoride release. Due to low cytotoxicity and good biocompatibility, it was also considered to use as a bone cement.[16]

Calcium Aluminate GIC: Properties of this GIC show a mix of calcium aluminate and luting GIC. Advantages include pH greater than 7 during curing, reduction in marginal leakage, improved biocompatibility, and greater stability and strength.[16]

Powder-Modified Nano Glass Ionomers: Described by De Caluwé et al, the mix of conventional glass ionomerwith nano-sized glass particleslike nano-apatite and nano-fluorapatite decreased setting time and enhanced compressive strength and elastic modulus after being stored in distilled water for 7 days.[18]

Nano-Filled Resin-Modified GIC: Incorporation ofnano-sized fillers and bioceramic particles to RMGICswas done to improve mechanical properties.[19,20] Increased ionic bonding with a tooth was observed instead of micromechanical bonding.[18]

Chlorhexidine Impregnated GIC: Approximately 10 ppm of fluoride release by GIC was observed during the 1st 48 hrs following its placement in the prepared cavity.To improve the antibacterial characteristics Chlorhexidinedigluconate can be added to it.[21]

Conclusion

Glass ionomers have been a mainstay of restorative dentistry due to its unique advantages. With time various modifications of this cement have been introduced

with added advantages and manageable disadvantages. Though the quest for newer materials gave us some great restorative systems, one still cannot replace GIC completely in restorative dentistry due to its advantages such as biocompatibility, fluoride release, and its workability in the moist environment.

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A Smart Way of Making Dental Practice Painfree

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Abstract

Local anaesthesia in dentistry has been used in dentistry for more than a century ago. The emergence of giving local anaesthesia through nerve blockade marked a new era of patient comfort while permitting more extensive and invasive procedure in dental practice. Though ages effective LA is the most important pillar upon which systems have been developed that utilizes computer technology in the LA administration. The core mechanics behind the Computerised controlled local anaesthesia Device (CCLAD) is a microprocessor that controls delivery of LA solution at constant pressure and controlled volume while monitoring the variation in the tissue resistance. This fine high suffusion flow rate of LA provides a rapid onset of anaesthesia in most dental patients. Although local anaesthesia in injection form remains the backbone of pain control in dentistry, researches are being made in constant search of new & better management of pain. These researches are focused on the advancement of local anaesthetic agent delivery devices and techniques involved in both pharmacological and non-pharmacological form. Paradoxically the injection of LA seems to be the greatest source of patients fear and inability to control pain remains a significant concern to the dental practitioner. This review article will aim towards the products, device and techniques available till now which will make dentistry more patient-friendly and non-invasive.

Keywords: *Painfree Dental Practice; Local anaesthesia; Computerised controlled local anaesthesia Device.*

Introduction

At the beginning of dentistry extractions were practised without anaesthesia and toothache was cured with few glasses of wine, brandy or herbal potions. Then with the discovery of syringes and opiates, anaesthesia became effective in dentistry. Then came the anaesthetic virtue of ether,by which the famous surgeon John Collins Warren removed a neck tumour from the volunteer, Mr Albert Abbot, who felt no pain. Then an American dentist HovaceWells was first to show the anaesthetic property of nitrous oxide. Nowadays local anaesthetic in dentistry mostly involves blocking of small and large nerve terminals through contact with active molecules of esters and starches. The local anaesthesias that are in use in present-day dentistry are lidocaine, prilocaine, bupivacaine, mepivacaine and articaine.[1] Different types of local anaesthesia routes that are preferred in dentistry are infiltration,intraligamentary,intrapul pal, intraosseous etc. But all of these routes of local anaesthesia needs needle to deliver the LA agent at the respective sites. In most of the time, the needle prick induces anxiety in children and adult dental patients even before the proper administration of LA. This poses the drawback in the dentist-patients relationship. So many alternative method to alleviate pain are tried like topical anaesthesia, CCLAD, Jet injector, Iontophor electronic dental anaesthesia etc. Desensitization of the injection site & CCLAD is most of the recommended strategies nowadays.[2]

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Method of desensitisation of the injection site: The method which are involved to reduce pain at the injection site before the injection of proper L.A are – (a) Application of topical anaesthesia; (b) Pre-cooling of the injection site.

(a) Application of topical anaesthesia: Topical anaesthetics acts on peripheral nerves and reduce the sensation of pain at the site of the needle prick, before proper LA administration. Thus it generates a positive response towards dental treatment in the patients. Traditionally topical anaesthetics contain Lidocaine or Benzocaine as active ingredients and are used in the form of solutions, gels, creams and sprays. The topical anaesthesia used in dentistry are listed in table 1. [3]

Table 1. Topical anaesthesia used in dentistry

Benzocaine	• It is an ester-based LA that is commonly associated with hypersensitive reactions • Its poor water solubility hinders cardiovascular absorption and so effects the surface for a prolonged period • Aerosol agents may be used on soft palates to reduce gaging and gel agents used before infiltration anaesthesia • 20% of concentration produces an effect within 30 seconds.
Lidocaine and Lidocaine hydrochloride	• It is an amide-based local oral topical anaesthetic agent • It is used as 2% or5% gel, 5% ointment or 10% spray • The onset of action of the anaesthetic agent is 1-2 minute.
Prilocaine	• It is used in combination with other topical anaesthetics • It is used as 4% prilocaine or 4% prilocaine with 1:200000 Epinephrine
EMLA or Eutectic mixture of local anaesthesia	• It is 1:1 mixture of 2.5% prilocaine and 2.5% of lidocaine.
Cetacaine topical anaesthetic	• It contains 14% benzocaine, 2% butamben and 2% tetracaine-hydrochloric acid. • It is used in local pain in all mucous membrane. • It is used by using a cotton swab or micro brush.
Oraqix subgingival anaesthetics	• It contains 2.5% lidocaine and 2.5% prilocaine • It is gel anaesthetic that is administrated by insertion into gingival sulcus where it provides anaesthetic effect to enable deep scaling and root planing.

(b) Pre-cooling the injection site – In dental surgeries, palatal injection poses the most painful of all the injections. So palatal cooling is effective for relieving pain perception. It is done by (a) application of dichlorodifluoromethane sprayed cotton pellet for 5 seconds, (b) application of refrigerant eg. 1,1,1,3,3-pentafluoropropane/1,1,1,2 - tetrafluoroethane (c)application of ice popsicle etc.[4-6]

Computer-controlled local anaesthesia delivery system: Research shows that CCLAD produces lesser pain ratings and disruptive behaviour than cartridge syringe in children.CCLAD refers to the appliances that utilise computer technology in the administration of LA in patients. It precisely controls the flow rate of LA and modulates the fluid pressure by the use of computer microprocessor & electronically controlled motor to deliver the LA at a constant slow rate respecting the tissue resistance.[7] The well-known device under different commercial brands are:

1. The Wand (Milestone Scientific Livingstone, NJ)
2. Comfort Control Syringe (CCS, Dentsply, USA)
3. Quicksleeper (Dental Hi-Tec France) and
4. iCT (Dentium, Seoul,Korea)
5. Anaeject (Nippon Shika Yakuhin, Japan)
6. Ora star (Showa Uyakuhin Kako, Japan)

Basic theory of CCLAD: CCLAD reduces pain by controlling injection speed through the resistant tissue and also from the anaesthesia taking effect at the same time with injection,which then sequentially allow the

anaesthetic to be injected into already anaesthetised tissues. Because of these series of processes, the patient feels less pain.[8]

The design of CCLAD varies depending on:

I. Whether the anaesthetic cartridge is included in the main unit or not

II. Speed and mode of injection

III. A device having the possibility of aspiration or not

IV. Weight of the device

V. Ease of injection management.

The Wand system has 3 parts a base unit, a foot pedal and disposable handpiece assembled.

Base: It contains a microprocessor and connects to both the foot paddle and handpiece attachments that accepts the local anaesthetise cartridge. This microprocessor controls a piston that expels the local anesthetic solution by pushing the LA plunger up into the cartridge.[9]

Disposable handpiece: The LA solution expels through the microbore tubing, WAND handpiece and attached needle to the tissue

Foot pedal: Pressing lightly on foot pedal expresses slow injection rate (0.005 ml/second) appropriate for needle insertion, palatal injection and PDL injection. Heavier pressure increases the rate of LA solution administration (0.03 ml/second) for buccal infiltration and nerve blocks and release of foot pedal stops injection.[7-10]

Along with these, WAND is added with STA mechanism, that is Single Tooth Anaesthesia feature. This STA unit adds Dynamic Pressure Sensing(DPS) technology, which enables the dentist to feel the needle tip placement in PDL etc. The handpiece of CCLAD system seems to be a pointed ball pen rather than a syringe,which the patients particularly pediatric dental patients finds less scary than a conventional cartridge and syringe. The first computer-controlled L.A delivery system was introduced in 1997 as the Wand, (Milestone Scientific) to improve functional design and accuracy of the dental syringe. It was in the market quite for a long time due to its ease in operation, lightweight and a circumference that was half of the traditional syringe. But the Quick sleeper and Comfort Controlsyringe(CCS) have three times the size and weight handpiece that of the traditional syringe as they have both syringe and motor in this handpieces.[9,10]

CCLAD handpiece must be held stable for a longer period than a conventional cartridge to ensure safe administration of anaesthesia. So new products are introduced which offer lightweight designs. Further technical advances are awaiting to develop much lighter CCLAD. In the aspect of injection speed of local anaesthetic solution Wand,Quick sleeper & CCS have injection speed of three, four and five stages respectively. The iCT device allows three stages which allow the full cartridge to be injected 250,120 & 50 seconds. In the aspect of whether aspiration can be done or not,it is an important fact that all Wand,CCS and Smartjet have the option of aspiration before injecting the local anaesthesia to the particular tissue.[7-10]

Advantage of CCLAD: CCLAD has more accurate needle insertion for deep nerve block.In the second or recurrent visits, the children who receive CCLAD shows less disruptive behaviour than the children receiving an injection with a cartridge system. Thusthe use of CCLAD can be carried as a possible step towards a pain-free pediatric dental practice in coming days.[7-10]

Disadvantages of CCLAD: The added cost of CCLAD and single-use hand piece for different patient injection might not be affordable to every practitioner. If the cost is reduced it may be possible for every practitioner to use it his/her clinic. In many cases, a total of 4 minutes is required to completely express a cartridge, which may cause impatience and stress in the patients. CCLAD doesn't reduce the discomfort of needle insertion in case of palatal injection.[9,10]

Conclusion

Thus the most accepted mode of pain control is by administering local anaesthesia with the use of needle though it may be an anxiety-provoking procedure for both children and adult dental patients. Of all the alternative method of delivering anaesthesia, CCLAD is the most accepted mode of delivering anaesthesia without pain. Though it is of high cost and time taking procedure to deliver anaesthesia it can be considered by the dentist.

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Sialadenitis: A Review of the Literature

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Abstract

Salivary glands are tubulo-acinar exocrine organs responsible for the formation and secretion of the saliva. Salivary gland inflammation is known as sialadenitis which can originate from various non-infectious and infectious causes. Although several other viruses also can involve the salivary glands which include Choriomeningitis, Coxsackie A. ECHO. Cytomegalovirus (in neonatal) and parainfluenza, mumps is the most common viralinfection. Most bacterial-infections arise due to ductal obstruction or decreased salivary flow which allows the retrograde spread of bacteria through the salivary ductal system. Viral infections, bacterial infections, allergic reactions, and systemic diseases are the major causes ofsialadenitis and it may be acute or chronic. The etiology, diagnosis, and treatment of these conditions are discussed.

Keywords: *Salivary glands; Sialadenitis; Etiology, Diagnosis, Treatment.*

Introduction

The oral cavity depicts many pathological processes from the ubiquitous collection of tissues. The salivary glands may be classified as major and minor glands. Major glands are paired glands; these are Parotid, Sublingual and Submandibular glands. Inflammation of the salivary glands is known as sialadenitis. Pathogenesis - (1) Retrograde contamination of the duct and parenchymal-tissues by bacteria inhabiting in the oral cavity. (2) Stasis of salivary flow promotes acute suppurative infection. Viral infections, bacterial infections, allergic reactions, and systemic diseases are the major causes of sialadenitis. It may be acute or chronic.

Acute Suppurative Sialadenitis: Acute suppurative sialadenitis most frequently involves the parotid gland followed by the submandibular gland. This may be explained because of the serous saliva produced by the parotid gland which has less bacteriostatic activity than that of mucinous saliva of the submandibular gland. Although acute suppurative parotitis can occur in individuals of any age, patients most frequently affected are aged from 50 to 70 years. The reduction of the secretory flow and stasis of saliva, typically as a result of dehydration, is an integral pathogenetic factor of acute suppurative sialadenitis. In acute suppurative sialadenitis, bacterial organisms usually enter from the oral cavity in a retrograde fashion. Staphylococcus aureus is the most common causative agent and accounts for 50% to 90% of cases.[1,2]

Usual clinical presentation is the sudden onset of diffuse swelling of the affected gland associated with induration, erythema, pain, and tenderness. Some patients complain of reduced mandibular movement and difficulty in swallowing. Patients frequently have systemic signs of toxemia, including delirium, high fever, and leukocytosis. Association with systemic sepsis is more common in infections of the parotid gland than the submandibular gland. The respective intraoral duct orifice may become red and swollen with purulent discharge recognized on the massage of the gland. The diagnosis of acute suppurative sialadenitis is based on the clinical features with the culture of the purulent material from the duct. Any abscess formation

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can be determined using computerized tomography or ultrasound scanning. Sialograms are contra indicated in cases of acute suppurative infection of the salivary glands. Initial treatment of acute suppurative sialadenitis should be conservative, with eliminating the causes, improved oral hygiene, adequate hydration to increase the salivary flow, repeated massage of the affected gland, and administration of appropriate oral or intravenous systemic antibiotics, usually for a penicillinase-resistant Staphylococcus. Irradiation of the glands is no longer recommended. Incision and drainage by surgical intervention are indicated in cases involving abscess formation.

Obstructive Sialadenitis and Sialolithiasis: This is the most frequent type ofchronic sialadenitis, accounting for about 30% of all chronic sialadenitis cases and approximately 1% of all salivary gland diseases[3,4]. Although sialolithiasis is the most common cause of obstructive sialadenitis by far, two major factors are playing a role in the pathogenesis of the disease[4]. One is a mechanical obstruction by sialoliths, salivary gland cysts and tumors, and lesions of the oral mucosa. The other factor is a disturbance of the electrolyte concentration of saliva, resulting in the development of viscous secretory products. Sialoliths develop as a result of deposition of calcium salts around a nidus such as bacterial colonies, cellular debris, mucous plugs, or foreign bodies.

The peak age of incidence of sialolithiasis is between 30 and 60 years, with a mean of about 45 years[3] and slight male predominance. About 80% of cases of sialolithiasis develop in the submandibular gland, followed by the parotid gland[3]. Then the other salivary glands submandibular gland is more susceptible to sialolithiasis because its saliva has higher mucin content, increased viscosity, a more alkaline pH, higher concentrations of calcium and phosphate, and antigravity flow within the duct. In the submandibular gland, 57% of stones are localized near the hilum, 34% in the distal area of the submandibular duct (Wharton's duct), and 9% in the intraglandular ducts[5]. The formation of sialoliths in the main ducts produces swelling of the distal salivary gland tissue with or without pain. The episodes of the symptoms are frequently recurrent and exacerbated by eating. When sialoliths occur in the submandibular gland duct, induration can be observed on the floor of the mouth, and the duct orifice becomes erythematous and swollen. Sialoliths in the minor salivary glands are mostly asymptomatic presenting as a small, firm nodule. Ductal obstruction blocks secretion and leads to stasis of the saliva within the duct, which becomes predisposed to retrograde bacterial infection; Streptococcus viridans is a commonly involved pathogen.

The diagnosis of sialolithiasis is principally based upon clinical and imaging examinations[5]. Sialoliths of the distal area of Wharton's duct and the hilum can be detected by bimanual palpation of the affected area. Plain radiography is a helpful method to diagnose sialolithiasis, but 90% of submandibular stones are radiopaque while most parotid stones are radiolucent. Sialography is very accurate in the diagnosis of sialolithiasis but is usually unnecessary and is contraindicated in the acute setting of sialadenitis. Instead, high-resolution ultrasound and CT are the most useful diagnostic tools. The first steps of management of sialolithiasis in the submandibular and parotid glands include conservative method, such as adequate hydration, sialagogues, and gland massage as well as minimally invasive approaches, such as basket retrieval and interventional sialendoscopy to remove the stones, depending on their location and size[5]. When these measures fail, extracorporeal shock-wave lithotripsy may be the treatment of choice. Extirpation of the affected gland is recommended only in the minority of recalcitrant cases.

Chronic Sclerosing Sialadenitis (Kuttner Tumor): Chronic sclerosing sialadenitis is the salivary gland inflammatory disease, which was first described by Kuttner in 1896[6]. Chronic sclerosing sialadenitis produces a hard swelling of the salivary gland that cannot be easily distinguished clinically from neoplasia. This inflammatory process almost exclusively affects the submandibular gland.

Recently it has become increasingly recognized that chronic sclerosing sialadenitis exists to the group of immunoglobulin G4 (IgG4) related sclerosing (systemic/autoimmune) diseases[7,8]. This notion is further supported by the fact that chronic sclerosing sialadenitis has been associated with one or more extrasalivary IgG4-related sclerosing diseases, which are characterized by infiltrating of the chronic inflammatory cell containing abundant IgG4-positive plasma cells, accompanied by atrophy of the normal parenchyma and sclerosis. The close association suggests that at least some cases of chronic sclerosing sialadenitis share common pathogenesis with other sclerosing diseases involving disturbance of IgG4 as a systemic disorder. Chronic sclerosing sialadenitis can appear at any age between 12 and 83 years. Clinically, the patients appear

with a firm swelling of the submandibular gland with or without recurrence of pain associated with eating. This appearance of a firm, enlarging growth can lead to the erroneous clinical diagnosis of a neoplastic salivary gland, particularly carcinoma. The glandular involvement is usually unilateral, but it can be bilateral. Cases with swelling of bilateral submandibular glands and lacrimal glands, similar to Mikulicz's disease, have been reported[9]. As mentioned above, chronic sclerosing sialadenitis is sometimes associated with the extrasalivary IgG4- related sclerosing diseases; therefore, a systemic examination is recommended for patients with chronic sclerosing sialadenitis.

Radiation Sialadenitis: Radiotherapy is the most commonly used modality for treatment of the head and neck cancers[10]. When the major salivary glands are included within the fields of radiotherapy, many patients develop swelling and tenderness of the irradiated glands as well as elevation of the serum amylase level within hours of receiving even the first treatment in a course of fractionated radiotherapy. These symptoms may be transient, but a significant proportion of the patients will suffer persistent salivary gland dysfunction, resulting in xerostomia accompanied by a variety of complications, such as dysphagia, abnormal taste sensations, oral mucositis, dental caries, and periodontal disease. The saliva secreted from irradiated salivary glands has a lower pH, lower protein and secretory antibody contents, higher salinity, and increased numbers of cariogenic microorganisms. The severity of salivary gland damage and dysfunction depends on the dose and the duration of irradiation. The important pathogenetic factor of radiation sialadenitis is the direct irradiation effect on the salivary gland tissue rather than the result of radiation-induced changes in the vasculature[11]. Considerably serous acinar tissue of the parotid gland is more radiosensitive than mucous glandular tissue of the submandibular gland[12]. The radiosensitivity of the acinar cells depends on the content of secretory granules, containing a large number of heavy-metals limited by the membrane[13]. Released heavy metals as a result of the irradiation may cause membrane lipid peroxidation utilizing a redox system, thus enhancing the process of apoptotic cellular death[13].

Recurrent Parotitis: Recurrent parotitis is a well-recognized entity in children, represented by recurring episodes of swelling of the parotid gland. Although it is relatively rare, it is still the second most common salivary gland inflammatory disease in children, next to mumps[14,15]. Recurrent parotitis is generally associated with nonobstructive sialectasis of the parotid gland. However, the sialectasis may be both the cause and the result of the recurrent exacerbations of the parotitis[16]. The age of onset of recurrent parotitis has been reported to be between 3 months and 16 years, but it mostly starts between three and six years with a peak during the first year of school[14] and with a male predilection. Recurrent parotitis presents as an intermittent, painful swelling of either unilateral or bilateral parotid glands during mastication and/or swallowing, generally accompanied by fever and malaise. The disease is often clinically misdiagnosed initially as mumps. The number of attacks varies individually, but most commonly occurs every three to four months[14, 15]. The swelling lasts from several days to 2 weeks, and typically resolves spontaneously, regardless of the treatment. Between the episodes, the patient is free from symptoms. Recurrent parotitis is generally self-limiting, and after puberty, by age between 10 and 15 years. Although it usually is not accompanied by purulent secretion, the majority of affected glands grow bacteria on the culture of saliva. Sialography has been considered the most important and accurate diagnostic tool, though its rolehas become secondary to ultrasonography now. The main treatments of an acute phase of recurrent parotitis are either conservative observation or antibiotic treatment. Penicillin V is a good choice for acute infections. Other treatments are much more invasive when applied to adults with persistent severe disease, including duct ligation, parotidectomy, and tympanic neurectomy.

Viral Sialadenitis Including Mumps and Cytomegalovirus: Viral infections involving the salivary glands are more common than those of bacterial origin. Viral sialadenitis is a manifestation of a systemic infection, and the salivary glands are affected by a hematogenous spread of virus rather than a direct infection to the organ as in bacterial sialadenitis. A variety of viruses are known as causative agents of sialadenitis, including the paramyxovirus (mumps), cytomegalovirus, HIV, coxsackie A virus, Epstein–Barr virus (EBV), echovirus, herpes virus, lymphocytic choriomeningitis virus, adenovirus, and influenza A and parainfluenza viruses[17]. Mumps is by far the most common cause of parotid swelling and is the most common viral agent involving the salivary glands[18]. Mumps is caused by a highly contagious paramyxovirus. The frequency has reduced with a vaccine made from attenuated live mumps virus. The peak incidence is between the ages of four and six years, but infection can

be seen in other age-groups. The incubation period is two to three weeks, with a clinical onset characterized by swelling and pain of single or both parotid glands. Systemic symptoms include fever, malaise, myalgia, and headache, and usually, resolve before the parotid swelling. Adults infected with mumps are more possibly to develop severe symptoms and complications, while in children symptoms are usually not severe. Generally based on clinical presentation the diagnosis is made. Demonstrating antibodies to the mumps V and S antigen by enzyme-linked immunoadsorbent assay can confirm the diagnosis. Some laboratories use molecular method that are based on a nested polymerase chainreaction. Many cases are subclinical, and studies have shown that greater than ninety-five percent of adults have neutralizing antibodies. Orchitis occurs in 10% to 20% of infected males, but it is usually unilateral, and sterility only infrequently arises; in about 5% of those infected aseptic meningitis occurs. The disease is generally self-limiting with a good outcome. Swelling and other acute symptoms usually subside within two weeks. There is no specific treatment apart from controlling the symptoms with analgesics.

Cytomegalovirus is a member of Betaherpesvirinae in the subfamily of Herpesviridae. Most infections are acquired during childhood in developing countries,whereas, up to 50% of young adults are seronegative in developed countries. Cytomegalovirus is not highly contagious,thus the infection requires close contact with an infected person excreting the virus in their urine, saliva,or other bodily fluids. Cytomegaloviruscan be transmitted via breast milk and can also be sexually transmitted, transplanted organs, and rarely from blood transfusions. Cytomegalovirus infection without symptoms iscommon in infants and young children. The symptomatic disease can affect almost every organ of the body in immune-compromised individuals,resulting in fever of unknown origin, encephalitis, pneumonia, colitis,hepatitis, retinitis, neuropathy, uveitis,and myelitis. Cytomegalovirus infections are frequently associated with salivary glands, but significant clinical manifestations of cytomegalovirus-infected patients are usually seen in other organs. Diagnostic procedures include the enzyme-linked immunosorbent assay. Since the majority of infections resolve instinctively no treatment is usually required for cytomegalovirus infection in the healthy individual. Now in infants, antiviral drug therapy is being evaluated.

Conclusion

This article thus deals with various infectious and noninfectious causes of sialadenitis like Acute Suppurative Sialadenitis, Obstructive Sialadenitis, and Sialolithiasis, Chronic Sclerosing Sialadenitis (Kuttner Tumor), Radiation Sialadenitis, Recurrent Parotitis, Viral Sialadenitis Including Mumps and Cytomegalovirustheir diagnosis and treatment.

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Endoperio Lesion: An Overview

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Abstract

The most common problems associated with tooth pain were endoperio lesions. It was very difficult to diagnose endoperio lesions by clinical or simply intra oral peri-apical radiograph. 50 % of tooth mortality rates are responsible for the endoperio lesion, the correct diagnosis was needed for appropriate treatment planning. This paper review the current diagnostic tools and treatment planning of endoperio lesion to achieve the best outcome.

Keywords: *Endoperio lesion; current Diagnostic Tools; Treatment Planning.*

Introduction

In daily dental practices, there are many patients we encounter with severe pain due to the endoperio lesion. As per definition these lesions, are localized in the particular area originate either from pulp or tissue of periodontium surrounding the involving tooth. Both of these issues have a common mesodermal origin. The first case was described by *Simmering and Goldberg*[1] in 1964. *Turner and Drew*[2] described the effect of periodontal and pulp tissue like fibrosis, calcification, and cystic degeneration. In the study done by *Seltzer et al*[3], he found that 94% of extracted teeth which were periodontally involved had pulpal changes such as atrophy & inflammation with necrosis.

Endodontic & periodontium illness represents the greater part of the tooth mortalities[1]. The different pathway through which microorganism communicate with periodontal tissue is

- Anatomic/Developmental origin:[10]
- One of the most common routes of infection is apical foramen which makes the communication in between pulp and Periodontal ligment.[11]
- The second factor was anatomical deformities like developmental grooves. The gap between cementoenamel junction can cause exposure of dentinal tubules.[12,13]
- The other potent factor was accessory canals present in the apical area of tooth.[14,15]
- Type of Furcation area may affect the degree of infection in the root 2% to 59 %.[17-19]

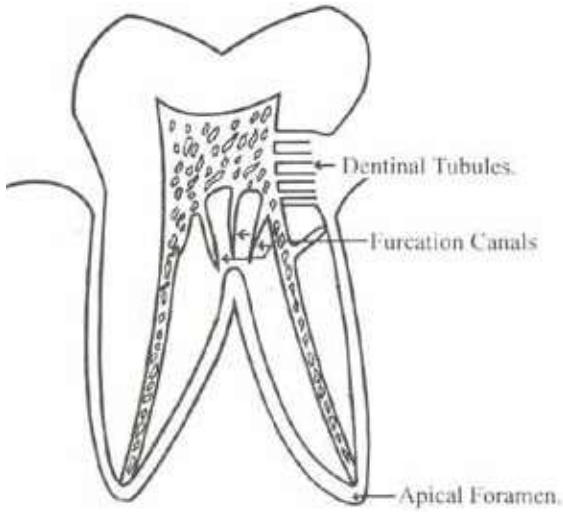


Figure 1. Anatomic relationship between pulp & periodontal ligament

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Pathological Origin: The factor pathologically derived and irritate the pulp due to various conditions like dental trauma, resorption, and loss of cementum due to irritants. The iatrogenic factors are those due to unintentional errors during the procedure by the dentist like root fracture, canal perforation during biomechanical preparation due to over instrumentation in root canal treatment. Root perforation opens the gateway between pulp and periodontal tissues which can lead to the poor prognosis of the tooth. The second most important factor was vertical root fracture during the obturation compaction leads to fracture line in root leads to a fracture in the root which further leads communication between the pulp and periodontal lesion.

Etiology: There are two main categories of endoperio lesions are living and non-living pathogens are tabulated below.[11, 23]

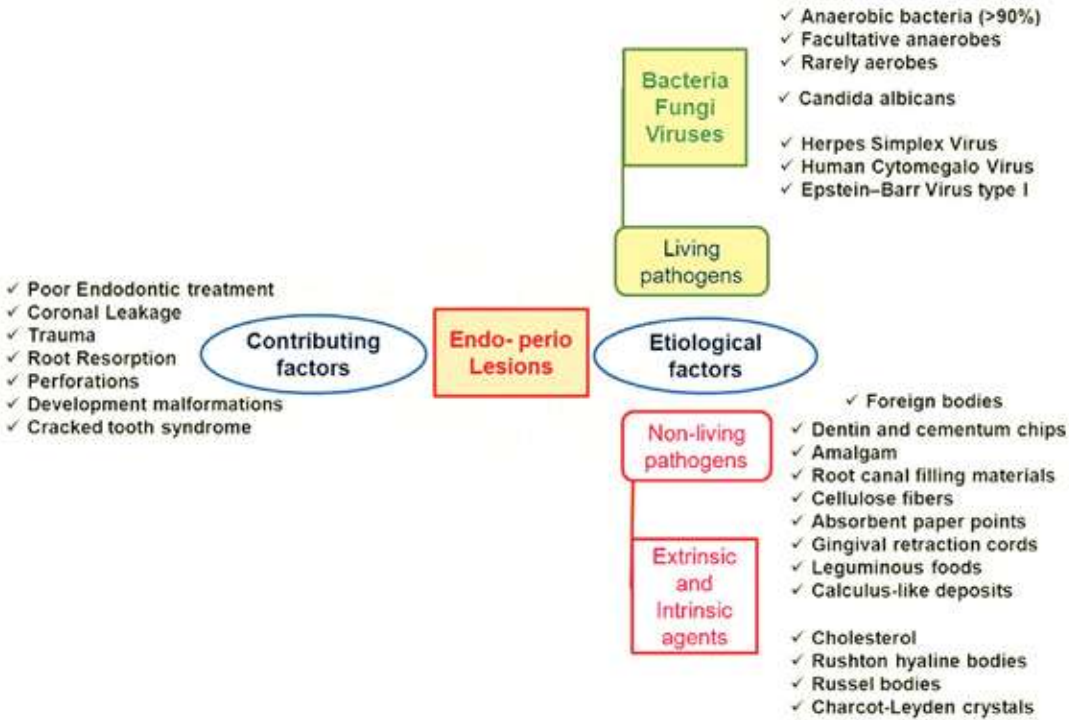


Figure 2. Main categories of endoperio lesions

The most common bacteria found in the endoperio lesions are Porphyrominas gingivalis, Actinobacillus actinomycetemcomitans, Porphyrominas gingivalis, Bacteroide forsythus, Ekinella corrodens, Fusobacterium nucleatum, Prevotella intermedia.[24] In many studies, we found fungal species Candida albicans are predominant in endodontic and periodontal lesions and virus found that EBV and herpes virus can also causative agents.[27,28] It has been found that the causative agents like cytomegalovirus, Ebstein Barr, the herpes virus can be responsible.[27,28]

I. Classification:

- In 1972 Simon et al were the first to give classification on endodontic periodontic lesion[29] based on diagnosis, prognosis and treatment.

– Primary endodontic lesion

– Primary periodontal lesion with secondary endodontic involvement

– Primary periodontal lesion

– Primary endodontic lesions with secondary periodontal involvement

– True combined lesion

- **Guldener & Langeland classified according to pathologic relationship[30]**

– Periodontal–endodontic lesion
– Endodontic–periodontal lesion
– Combined lesions

- **In 1996, Torabinejad & Trope gave the classification based on a treatment plan**

– Periodontal origin
– Endodontic origin
– Combined endodontic and periodontic lesion
– Lesions with communication
– Separate endodontic and periodontal lesions
– Lesions with no communication

- **Grossman's classification was based on therapy:**

– The condition of the tooth which requires just endodontic therapy
– The condition of the tooth which requires just periodontal therapy
– The condition of the tooth which requires both endodontic as well as periodontal therapy

Table 1. Classification on endodontic periodontic

Endodontic Origin	Periodontic Origin
1. Pulp non-vital.	1. Pulp vital except in advanced lesion.
2. Sharp throbbing pain	2. Dull, chronic pain.
3. Swelling in and extending beyond the	3. Swelling generally confined to attached mucosa
4. Tracing the fistulous tract leads to apical region or in the region or in the region of a lateral canal.	4. Tracing leads to mid root.
5. The fistulous tract is narrow and tortuous	5. Due to extensive loss of periodontal structures he fistula is wide in cervical area and can easily be probed.
6. Mobility in an acute stage involving multiple teeth limited to an isolated tooth.	6. Generalized mobility
7. Bone loss involving crestal and furcal bone limited to an	7. Generalised crestal bone loss either horizontal or verticle.

- Rateitschak et al gave a classification based on endodontic therapy

– Type I- In a condition where the pulp was dead and the disease by primarily endodontic origin
– Type II- In this condition the main cause of the disease by periodontal tissue which affects the pulp and sometimes caused pulpitis.
– Type III- In this condition pulp was dead and both origins were affected

II. Diagnosis:

The diagnosis can be made by clinical and radiography finding,

On clinical examination, swelling of the gingiva, Pus discharge, pocket formation, sinus tract can be seen as associated teeth. On radiographic examination bone resorption can be seen there is wide radiolucency present in the apex of the tooth. It was very difficult to distinguish between the origin of the disease.

The clinical features of endoperio lesions are:

- **Primary endodontic lesion:** The large carious lesion, the presence of sinus in the periapical area, Presence of pain with mobility with a tender to percussion, periapical radiolucency can be seen with periodontal pocket and pain on biting pressure.
- **Primary periodontal origin:** Pain on palpation, grade I mobility, the vitality of pulp will positive, plaque and subgingival calculus, periodontal

abscess, vertical bone loss and periodontal pocket with sinus tract at the lateral aspect of the root which can be appreciated on a radiograph.

- **Primary endodontic with secondary periodontal lesion:** Exudates and marginal gingivitis, root perforations, fractures, ill-placed post, a dull ache, or shooting sharp pain in chronic conditions with localized mobility with deep periodontal pockets.
- **Primary periodontal/secondary endodontic lesions:** Gingival inflammation and plaque around multiple teeth, Generalized mobility, localized/ generalized recession exudates, and pus. Pulp test may be positive in cases of the multi-rooted tooth and wide deep multiple periodontal pockets.
- **True combined lesion:** Chronic dull ache and swelling are present when calculus and periodontitis are around single/multiple teeth but in acute conditions, the pain will be more with generalized mobility with mobility of the involved tooth. Vitality tests will give negative responses except in the case of a multi-rooted tooth. At the edge of swelling the probe suddenly drops till apex of the tooth. This swelling is characterized as ‘ blown out’’.

Discussion

It is a challenge to treat endoperio lesions. In the study, Dahlen et al suggested that the vitality of tooth was an important factor for final diagnosis.[31] Kerekes and Olsen found that the microorganisms are the same in both periodontal pocket and root canal treatment.[32] Kurihara et al found that the is dissimilarity in microflora microbiologically and immunologically in pocket and root canal area.[33] Drucker et al [34] reported that Prevotella species associated with severe pain and commonly found in the root canal and periodontal pocket. Lin et al[35] demonstrated in the study there was no association of bacteria with the symptom. When we started with the treatment in combined endoperio lesion the endodontic treatment was done before periodontal therapy, the canal was completely clean with endodontic instruments with proper irrigation protocol which improve the reattachment procedure. The most common postoperative dentinal hypersensitivity. The time between to procedure is still not mentioning in any study it was still controversial. In various studies, the result found that endodontic treatment performed 2.5 months before periodontal surgery.[32,35] Miranda et al. showed that there was no change in the clinical parameter endodontic performed before the periodontal surgery.[36]

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Reconstruction of Maxillary Defect by Means of Bipedicled Forehead Flap: A Novel Surgical Technical Report

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Abstract

Forehead and temple region of act as tissue reservoir for harvesting flap for reconstruction of the orbital, periorbital and mid-face defect. Depending on requirement different kind of flap can be harvested from this region and for this sound knowledge of vascular anatomy of this region is required. Usually large free flaps are adapted to cover a large maxillectomy deformity. Here, in this case, a bi-pedicled forehead flap harvested based on frontal and parietal branch of the superficial temporal artery and was used for rehabilitation maxillectomy with external skin deformity.

Keywords: *Forehead flap, Bipedicled, Maxillectomy.*

Introduction

The Indian physician Sushruta was the first to described skin flaps reconstruct amputated noses as a result of punishment in his book 'Sushruta Samitha' which written in 500 BC[1]. "Szymanowski, Burow, Esser, Lexer, Joseph, and Davis" were the surgeons who introduced the concept of the flap in the Western world in the 19th century.[2] In 1830s forehead flap was first performed by Warren in America. Kazanjian, refined the midline forehead flap and also describe major blood supply of flap via the "supratrochlear" and "supraorbital" arteries. He also described the method of closure of the donor site[3,4].

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Forehead and temple region of act as tissue reservoir for harvesting flap for reconstruction of the orbital, periorbital and mid-face defect. Depending on requirement different kind of flap can be harvested from this region and for this sound knowledge of vascular anatomy of this region is required. Usually large free flaps are adapted to cover a large maxillectomy deformity. Here, in this case, a bi-pedicled forehead flap harvested based on frontal and parietal branch of the superficial temporal artery and was used for rehabilitation maxillectomy with external skin deformity.

Surgical Technical Note: Bi-pedicled forehead flap used for reconstruction of the defect of the maxilla was done in a male patient with squamous cell carcinoma of maxilla operated in Institute of Dental Sciences, Bhubaneswar. The malar defect of size 10 cm periphery was apparent after the three-dimensional clearance done for extensive squamous cell carcinoma of the maxilla (Figure 1). The forehead flap was designed taking the superficial temporal artery (frontal branch) as the feeder vessels of the flap (Fig 2). Total length of used flap was 125% of the defect and was planned to rotate under the skin over the lateral brow region to cover surgical deformity. De-epithelialization on the region which was supposed to come under the skin of the lateral brow.

The prepared pedicled flap was comfortably reaching the defect without any compression and tension at the pedicled end.Now to give the cover to the maxillary defect we decided to turn the flap over itself to serve the purpose, de-epithelializing it again at the turning point or pivot (Fig 3). The secondary deformity over the forehead was covered by the STSG ("split-thickness skin graft") harvested from left thigh. The patient was provided with an obturator postoperatively and was recalled regularly there was no complications with the donor as well as the recipientsite (Fig 4).We recommend the use of bi pedicled forehead flap for the use of reconstruction of the cheek and malar process as a simple but innovative technique.

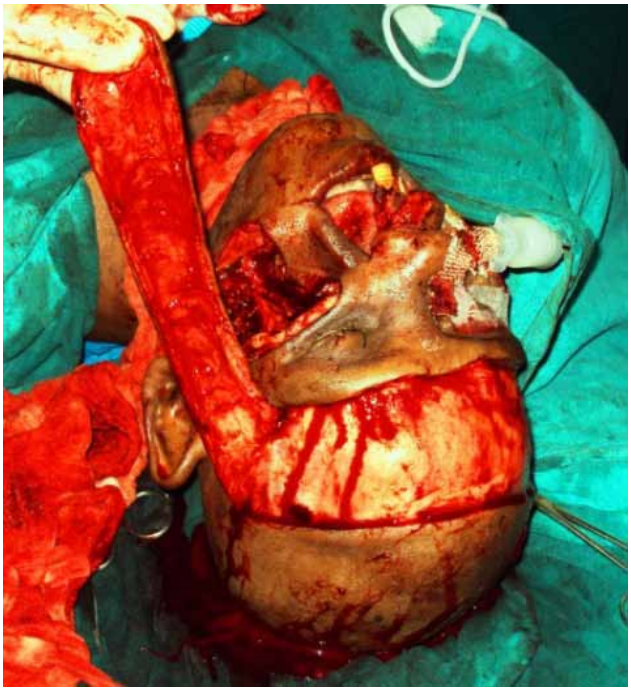


Figure 1. Raising of Flap Post Maxillectomy



Figure 2: Rotation of Flap



Figure 3. Securing flap on defect according to the technique described



Figure 4. Three Month Postoperative View (without obturator)

Discussion

Flaps can be classified depending on the blood supply of it, depending on location and depending on the type of tissue involved.

Depending on blood supply type of the flaps are as below: (by Nihai and Mathes)

Type I: one vascular pedicle flap

Type II: flap with dominant and minor pedicle.

Type III: flap with two dominant pedicle.

Type IV: flap with segmental pedicle.

Type V: flap with one dominant pedicle and minor segmental pedicle.

Depending on location it can be local, regional or free flap. Again depending on tissue content it can be cutaneous, fasciocutaneous, musculocutaneous or composite type which contain bony component along with cutaneous, fascia and muscle.

The forehead flap is a regional axial flap containing skin and muscle. Forehead is supplied by superficial temporal artery, supra-trochlear and supra-orbital vessel. Forehead flaps used are often long concerning their breath because the distance to be travelled by the flap en-route to its destination is considerable and involves a jump over intact skin. If the flap incorporates the entire vertical width of the forehead, it is quite safe to carry it beyond the midline, even as far as the hairline of the opposite temple.

Forehead flap can be used to reconstruct various kinds nasal of defects of, defect of the upper eyelid, intraoral and extraoral mucosal and skin defect, defects created by maxillectomy. It can also be used for reconstruction of the floor of the mouth, a portion of the tongue and for covering the chin in reconstruction of mandibular defect.

Depending on the site this flaps can be classified as below:

1. "Median forehead flap based on primarily on Supratrochlear artery, supplemented by the dorsal nasal artery."
2. "Paramedian forehead flap based on primarily on Supratrochlear artery, supplemented by supraorbital artery."
3. "Laterally based forehead flap based on primarily on Superficial temporal artery, supplemented by the posterior auricular artery."

To harvest the lateral forehead flaps the boundaries of the flap outlined. The contour should follow the eyebrows (must not extend beyond the level of the lateral canthus to avoid injury to the facial nerve) to the anterior border of pinna at the level of the zygomatic arch and along forehead hairline more pleasing cosmetically. Bevelled incision is given along margin. This bevelled margin helps to minimize the deformity along the remaining edges of the forehead and scalp. In case of larger flap outline can be extended up to opposite side temporal hairline.

To transfer the flap from its origin a tunnel is created. Through this tunnel, the distal end of the flap id transferred to the site of the defect and used for reconstruction. Depending on the location of defect flap passed through the following route –

1. "Directly through the cheek (cheek portal)"
2. "Deep to the zygomatic arch"
3. "Posterior part of submandibular incision of neck dissection"

The secondary defect created due flap harvesting is covered by a skin graft.

Local flaps are not sufficient to close the skull base and mid-face defect because they have inadequate well-vascularized bulky tissue.

The pedicled temporoparietal fascia was used by Andrew et al for the closer of the maxillary defect but it appears to be inadequate for the closure of the defect and forehead appears to be a good option for the same.

H. Nakajima et al described the arterial anatomy of the forehead and the vascular supply of different forehead flaps. Arterial supply of the temporal region distributed in four tissue layers[5].

1. "Skin and superficial temporal fascia network"
2. "Loose areolar fascia/subgaleal network"
3. "Deep temporal fascia network"
4. "Temporal muscle network"

There is a good communication between this arterial network and depending on this vascular anatomy different type of individual or combined flaps have been outlined in this region,

Superficial temporal artery was the first used Dunham in 1893 for the pedicle flap for reconstruction of facial defects.[6]

The skin flap islands based on the frontal and parietal branches of the superficial temporal artery was demonstrated by Ozdemir et al for closer of different mid-face deformities.[7] Bi-lobed forehead flap was designed by Harun et al. It was based on the frontal branch of the superficial temporal artery. This was used to reconstruct the defects of upper and lower eyelids 2 and lateral canthus.[8] This flap involves the reconstruction of nose passed on through the centuries commonly known as the Indian method was popularized by two Indian surgeons

when they perform a reconstruction of the nose by using a median forehead flap. It was published in the Gentleman's Magazine of London.[9]

For closer of huge, full-thickness deformities of the midface and forehead acts as a great tissue reservoir.[10] The temporal branch of the superficial temporal artery and supratrochlear artery are the arteries depending on which the forehead flaps are harvested. The superomedial orbit crossed by them approximately 1.7 to 2.2 cm lateral to the midline, and in a paramedian position approximately 2 cm lateral to the midline.[11] For complex deformities of the nose, orbit, zygomatic region, and cheek region bi-pedicled forehead flap considered as a valuable resource.

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Ethical Issues: Approved

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Endodontic Treatment in Cases of Allergic Reaction to Rubber Dam

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Abstract

The oral cavity is a complex area to handle with about thirty-two teeth, highly vascular tongue, oral mucosa, and gingival. It becomes challenging for the clinician to handle a single tooth in focus with patients movement of vascular tongue and especially in very apprehensive subjects. Hence rubber dam has proved to be a boon not only protecting the tongue, mucosa, and gingiva but also increasing the point of focus for the clinician. Secondly, it reduces infected aerosol procedures thus protecting the clinician and the attendee. Sometimes rarely patients do have allergies to latex or rubber dam and they are not aware. Hence the clinician must ask for leading questions to rule out latex allergy. If the subject does have a latex allergy modifications are required before the treatment.

Keywords: *Endodontic Treatment; Allergic Reaction; Rubber Dam.*

Introduction

To induce a desirable working area in the oral cavity there should be proper moisture control, visibility, and good accessibility. If a file or reamer is mistakenly swallowed during root canal treatment without rubber dam placement then it is likely to confront a lawsuit as Grossman stated that "in the eyes of the court, when an endodontic instrument escapes from dentist's fingers and is ingested or aspirated, expert opinion is unnecessary to justify claims of negligence". Rubber dam sheets are available as heavy, medium, light, and in different colors and standard sizes. Readymade sheets are available in 5×5'' and 6×6'' and rolls according to need which may be conveniently cut into necessary size. It is usually made up of latex material. For patients with latex allergy, non-latex rubber dam material is used in clinics.[1-3]

Case Report A: A 67-yr-old, female patient was referred to the undergraduate clinic for root canal treatment of a mandibular first molar with symptoms of pulpitis.

The past medical history was non-contributory. The patient only reported food and fruit allergies, although she was unaware of the cause of the allergies and the exact model of their manifestation.

During the first appointment, an access cavity was prepared, a dental rubber dam isolation was done, and instrumentation was started with the operating student wearing gloves throughout the procedure. After 20 min, the patient complained of a slight itchy feeling on the lips. The treating student attributed the event to a possible irritation caused by the irrigation solution, and the treatment was carried on.

During the second and third appointments, the same feeling on the lips caused even greater discomfort. Still, the treating student did not give the incident the appropriate consideration. On the afternoon of the third appointment, the patient called the student to report

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pruritus, redness, and inflammation on her lips, perioral, and intraoral region, together with inflammation and burning of her tongue, followed by inconvenience in breathing.

The patient was seen the next morning by the faculty. It was then that contact dermatitis stomatitis due to an allergic reaction to rubber dam was diagnosed. The student was advised to avoid latex products when treating the patient. The patient was advised to proceed with dermatological tests to identify the source of the allergies.

In the remaining appointment, the patient was seen in a separate treating room, the student and the supervising dental staff wore non-latex dental gloves, and a polyvinyl chloride dam was used. The symptoms did not reappear.

Case Report B: A 75-yr-old, retired, female nurse was referred by a general practitioner for root canal treatment of an acute apical abscess of the left maxillary canine. The past medical history of the patient revealed an allergy to penicillin as well as to latex. Five years before treatment in our facility, she had a life-threatening experience of an immediate type I hypersensitivity reaction. The incident occurred when after wearing a brand new brassiere for only 30 min, she developed pruritus with erythema and edema. She did not understand what exactly was happening, and because she was outdoors, she could not get self-examined. After 1 hr of discomfort, she started feeling dyspnea and dizziness. She was carried unconscious to the hospital, where an immediate type I hypersensitivity reaction with laryngeal edema and urticaria was diagnosed. Among other clinical and laboratory examinations, a skin prick test was performed, which indicated an allergy to natural latex. She did not report having undergone multiple surgeries in childhood or a tendency to develop atopy, and she did not report any food allergy. After her life-threatening experience, she was advised by her physician to minimize contact with latex products.

Because a diagnosed latex allergy was reported in her medical history the treatment was intelligently modified. She was always scheduled to be the first appointment of the day. No antihistamines or corticosteroids were administered. Care was taken to exclude any latex products from the room. The dentist and the assistant wore vinyl gloves (Romed, Wilnes, The Netherlands), and for tooth isolation, polyvinyl chloride dams (Hygienic, Akron, OH) were used. Rubber stoppers were not used on endodontic instruments. No symptoms resulted.

Discussion

The case-report A, who was not aware of her sensitivity to rubber dam, was fortunate to experience a non-life-threatening, nonfatal allergic reaction to rubber dam. The patient was terrified, and the student was in an unpleasant position, having caused discomfort to the patient by neglect. The patient did report an allergic sensitivity to fruit, but the student did not insist on getting more details, because she did not evaluate the information that was given as relevant to her practice. Patients sensitive to papaya, banana, avocado, chestnut, apricot, kiwi, pineapple, peach, nectarine, plum, cherry, melon, fig, grape and passion fruit these fruits seem to be predisposed to develop latex allergy. Even food such as potato, tomato, and celery are on the list. From the moment the itching started, the student should have been alarmed.[4-5]

As far as the patient in case report B is concerned, everything seemed to be under control once the sensitivity was reported. Efforts were made to create a latex-free environment during all stages of the therapy. Her occupation as a nurse alone could not indicate her to be a person at high risk because in the period she was practicing, the use of latex gloves wasn't recognized as an occupational hazard in the medical community. Therefore, she couldn't have been exposed to latex allergens because of her occupation.[6-9]

Non-latex gloves and a dam were used. At the stage of obturation, the practitioner was careful not to extrude the gutta-percha cone into the periapical tissues. There have been reports that GP and rubber seem to be chemical isomers and gutta-percha could trigger an allergic reaction as well in patients with sensitivity to latex.Some fruits such as bananas and avocado pears contain cross-reacting proteins with latex. An interesting finding from this study is that patients who were allergic to latex but not to fruits lacked the 30-kD protein band. Signs of allergic reactions to these fruits include pruritus, tightness in the throat, breathing difficulty in patients allergic to fruits have an 11% risk of a latex reaction, patients allergic to latex have a 35% risk of a reaction to fruits.[10-12]

There are noted precautions for latex-sensitive patients such as taking thorough history which are tailor-made to evaluate latex allergy. Refer the patient to the general physician asking the physician for latex-sensitive testing. Precautions should be taken as an emergency medical kit with non-latex airway bags, masks, bandages & tape should be available at a wink of an eye. It is advisable to schedule latex-sensitive patients as the first patient of the day. Better to use glass syringes over plastic or pre-filled or single-use syringes since the plunger may contain rubber. Use of non-latex devices (gloves, dams,etc) & rubber dam napkins. After taking all precautions if a reaction occurs then discontinue the treatment & observe the patient for at least 20 min, medical intervention may be needed.[13]

Root canal treatment in patients with rubber latex allergy could be uneventful if certain precautions are taken.

- Thorough history with particular questions on allergies to latex and previous allergy to rubber material experience should be noted. The dentist should be aware of the latex-fruit syndrome and be cautious when treating a patient who reports an allergy to fruits.
- In case of a positive latex allergy history or cases of high-risk patients, confirmative diagnosis is necessary with either in vitro tests such as serological tests or with more reliable in vivo tests such as a skin prick test, use test or patch test.
- The dentist should be in touch with the patient's general physician informing him/her about the procedure to follow and consult on the need for any premedication with corticosteroids.
- The first aid kit must be routinely checked to ensure that it is equipped with corticosteroids and epinephrine for intravenous or subcutaneous injection. Oxygen masks should be ready in case they are needed.
- An environment without latex has to be created to treat such a patient. The patient must be the first patient of the day to prevent the previous entrance of anyone carrying latex allergens into the treating room.
- Nonlatex gloves should be used. Gloves from synthetic materials such as nitrile and styrene-ethylene butadiene styrene (SEBS), are available in the market. It has been found that the cornstarch powder used in surgical latex gloves forms a complex with latex proteins, which is gradually aerosolized. The complex is kept as a reservoir on surfaces, unwashed hands, and clothes, and even worse, is transported when handling non-latex products, sensitizing the patient.[14]
- For tooth isolation, alternative materials like Polyethylene or polyvinylchloride dams have been proposed. Under the rubber dam,a cellulose separator could be used to prevent its contact with the mucous membrane.
- The practitioner should avoid any product with questionable latex content during the treatment of such a patient. The misleading "hypoallergenic" labeling that can be found on many natural rubber products refers to the sensitivity to residual levels of processed chemicals that those products can provoke. It is therefore essential to avoid them and make sure that non-latex products are used.
- Endo files, reamers must be used without a rubber stopper. As an alternative inerasable ink or wax could be used to point out WL in files and reamers
- As far as the selection of the obturating material is concerned because gutta-percha is the only widely acceptable material, special care should be taken to avoid its extrusion into the periapical tissues to prevent any possible allergic reaction.
- The patient must be advised to avoid products containing natural rubber in his everyday life to avoid sensitization. The list of such products seems to be endless, because there are over 40,000.15,16

Conclusions

The daily practice of endodontics needs to be readapted, and alternatives to the routinely used products must be incorporated in the dental office to treat patients with latex allergy with safety. It is the clinicians' responsibility to be careful of the prevalence of rubber allergy and to be able to perform endodontic therapy without endangering the patient's life.

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A Case Report of a Submental and Submandibular Space Infection

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Abstract

In most of the cases of space infections are secondary to an odontogenic infection. In space infection, spread occurs through the fascial plane because of the degradation of connective tissue by action of different enzymes produced by microorganisms. Different factors like age, nutritional status of the host, host defense, virulence of the microorganism play an important role in the spread of the infection. Sometimes it may take the fetal course if not treated on time because of its local and systemic complications. It should be treated as an emergency condition to prevent its rapid progression leading to fatal outcome. Here the case report describes an old lady suffering from fascial space infection and treated by incision and drainage. Incomplete removal of any odontogenic infection may cause the recurrence of the infection or infection that may spread to fascial spaces.

Keywords: *Odontogenic infection; Fascial space infection; Host defense; Management.*

Introduction

Odontogenic infections originate from the tooth and its supporting structures. Odontogenic infection arises when oral flora from spread alveolar process to deeper tissue plane of the head and neck region. Different Streptococcus (particularly alpha-hemolytic Streptococcus) are major pathogens of space infection. Spaces in the head and neck region are the potential spaces between the layer of fascia which contains loose connective tissue, areolar tissue, and contain different vital structures. Primary spaces are involved first. Direct secondary space involvement is unlikely.[1] Untreated cases produce systemic complications and also obstruct respiratory tract obstruction. Pathogens of the space infection produce hyaluronidase, streptokinase, and other enzymes which are the cause of the spread of the infection and cellulitis. Decreased host defense, increased bacterial resistance, nutritional status of the patient, the virulence of bacteria also with socioeconomic status and local factors like hygiene of the patient also plays an important role in the spread of the infection. In this article, we reported the case of fascial space infection which first involved buccal space which migrated to superficial temporal space even after drainage from primary space.[2]

Case Report: A lady who is in the 6th decade of life presented with swelling and pain of the bilateral submandibular and submental region for the last 3 days. The swelling appeared after extraction of right mandibular 1st premolar which was carious and had a periapical abscess. There was a 7-day window period between the extraction of the tooth and the development of the swelling. The patient has a history of diabetes and was not under medication for the same.

On general examination, the patient appears toxic, dehydrated, with mild pyrexia a complaint of severe pain

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in the right submandibular region anterior neck region, and difficulty in swallowing. Overlying skin appears erythematous. On palpation there was pain and there was a mild rise of temperature than the adjacent area. There was pain on palpation. Swelling was fluctuant. Intraorally there was vestibular obliteration in the right canine and premolar region. Mouth opening and tongue movement was restricted. The patient complains of difficulty in swallowing too. Partially healed socket irt right mandibular 1st premolar region.

Orthopantomogram done for diagnostic purposes. The patient diagnosed with right submandibular and submental infection. Both the spaces drained through a single incision placed over the most fluctuant part of the swelling. Hilton's method is used to drain the abscess. Rubber drain placed and intravenous piperacillin withtazobactam 4.5 gm and metronidazole 500mg with intravenous fluid given. Pus sample send for culture and sensitivity. There was no bacterial growth found in the culture media in 48 hours a patient was under antibiotic therapy.A blood investigation report reveals patient was diabetic and insulin started as per endocrinologist and balanced diabetic diet advised.



Figure 1. Pre-operative picture



Figure 2. Pre-operative picture 2



Figure 3: Picture after drainage.

Discussion

Sublingual space is a paired, v-shaped space lies lateral to the muscles of the tongue including the hyoglossus, genioglossus and geniohyoid. Situated at the midline and communicate with the opposite side space anteriorly.[3] Again sublingual space also connected with the submandibular space around the posterior border of the mylohyoid muscle. So infection of the unilateral submandibular gland can spread easily to the sublingual and to contralateral submandibular space and submental space and my cause Ludwig's angina which is a serious life-threatening complication itself. Again the from the sublingual space the infection spreads backward

direction in the substance of the tongue within the cleft created by the genioglossus and hyoglossus muscle. It follows the course of the sublingual artery. Through this route, the infection can spread to the epiglottic region and cause swelling of the inlet of larynx. Because of anatomical continuity of the submandibular space with the submasseteric, pterymandibular space and the spread of infection may occur in these spaces. More posteriorly the parapharyngeal and peritonsillar space may also involve by infection which causes serious airway obstruction. Infection from the submandibular region, can spread downwards along and beneath the investing layer of deep cervical fascia, towards clavicle and subsequently to mediastinum causing mediastinitis. A prevertebral layer of deep cervical fascia, lincoln's highway or carotid sheath, space between the alar and prevertebral fascia (danger space) play an important role in the spread of infection to the mediastinum.[4-7]

It is difficult to recognize mediastinitis in the early stage and it is one of the major causes of high mortality in case of deep space infections. Primary mediastinitis appears as pyrexia, dysphagia, odynophagia, retrosternal chest pain, swelling over the neck and the chest region along with the chest pain. Physical examination reveals the patient having tachycardia, tachypnea, crepitus over the neck and chest, positive Hamman sign (crunching sound on auscultation of heart) along with other signs and symptoms.[8] Even though the symptoms are not always marked in the primary stage of the disease. The diagnostic criteria for descending mediastinitis described by Estrera et al. are as follows:

(a) Symptoms of clinically severe oropharyngeal infection

(b) Radiographic findings of mediastinitisutilizing CT

(c) The presence of a mediastinal infection, which is detected during an operation or autopsy

(d) A relationship between an oropharyngeal infection and mediastinitis.

Aggressive treatment is necessary for these complications. The airway should be maintained along with early administration of antibiotics in proper dose and frequency, removal of the offending tooth and surgical drainage and decompression of the fascial plane. Intravenous fluids are also given. To main the airway fiberoptic intubation was done in the patient and it is preferred over blind intubation. Surgical airway may be required in cases of severe respiratory obstruction. Laryngotomy, cricothyroidotomy (tracheotomy) are always preferred over tracheostomy, or rather emergency tracheostomy is avoided because of 1. Identification of landmarks that may be required before it, is most of the time quite difficult due to associated massive edema and tissue distortion.[9] 2. The surgery itself may lead to the spread of infection to deeper tissues due to additional incision required. 3.There are high chances of getting pneumonia as sequelae of tracheostomy which itself is a life-threatening condition. 4. Tracheal stenosis is observed in 25–50% of cases following tracheostomy.[10-12]

Again in the presence of pre-existing diseases like malignancy, HIV infection, diabetes mellitus, a patient undergoing chemotherapy within 1 year, chronic renal diseases require dialysis cause compromised host defense and this patient can easily have affected odontogenic infection and they can spread to the deep spaces easily because of reduce host defense. Particularly in diabetic patient host defense is compromised in any way like poor blood perfusion through the capillaries, impaired diapedesis of the neutrophils, abnormal phagocytosis late maturation, and degranulation of the neutrophil, etc. again acidosis produces in the presence of diabetes mellitus impair the immune system of the host.[13-16]

Conclusion

So these patient needs to recognize the situation of immune compromise and modify the treatment accordingly. Some of the modification includes the use of a higher dose of antibiotic, use of broad-spectrum antibiotic, use of proper oral hypoglycemic agent or insulin in required dose to control blood glucose level, etc. in immune-compromised patient macrolides and tetracycline should be avoided.Again complete removal of the infected foci is also necessary for complete resolution otherwise it may cause serious complications in later stages.

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Acute Management of Maxillofacial Trauma: A Review of Literature

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Abstract

Management of trauma has progressed dramatically over the past few decades thereby reducing mortality in the golden hour. Challenges exist, however, one such region is maxillofacial injury in a patient with polytrauma. Because of the regions close to the brain, cervical spine and airway, serious injuries to the maxillofacial area may complicate the early treatment of a trauma patient. In case of maxillofacial injuries, the normal procedures of airway respiration and circulation (ABC) management are also changed or augmented by other approaches. In an often-challenging situation these modifications have their own difficulties and pitfalls.

Keywords: *Advanced Trauma Life Support.*

Introduction

Advanced Trauma Life Support (ATLS), established by American College of Surgeons, is the most commonly recognized quality of care for initial assessment and treatment of critical casualties.

Initial Assessment: The methodology of inspecting, listening, and sensing helps to find out airway obstruction and potential airway complications.[1,2] The approach to airway management, particularly in patients with unconscious trauma, should be complimented with C-spine safety. In high-velocity trauma involving the mandible, the swallowing function is altered due to pain and inadequate reflex control, resulting in difficulty in keeping the airway clear.[3] It is therefore necessary to preserve the airway from blood and vomiting in order to avoid aspiration and further pulmonary complications. Trachea palpation shows any potential failure or deviation. Larynx to stridor should be ausculted. Any tracheal tug or laryngeal stridor is explaining an impending threat to the airway. The "difficult intubation" tray should be accessible at all times with complete equipment to handle, including tracheal tube introducer, supraglottic airway machines, combitubes, endotracheal tubes, tracheostomy collection, and craniotomy kit.[4] Where management by traditional definitive airways is less possible, it is advisable to have an experienced team at hand to create surgical airways for rescue.

Strictly speaking, with constant monitoring of saturation, maxillofacial trauma patients should be given sufficient oxygenation, regardless of the injury. The spinal collars should be applied with great care in order to avoid any inadvertent future movement of the mandible complicating the airway. Unlike other polytrauma, maxillofacial patient's airway is at constant risk. The strategy is therefore a systematic analysis of the airway as delayed airway compromise may occur as a result of tissue displacement, bleeding, and swelling.[2] High-volume suction should be available to clear the mouth and oropharynx from blood and secretions.[5] However, care should be taken not to irritate the oropharynx with suctioning as it predisposes the patient to vomit. Furthermore, at this moment close observation of the patient should provide an idea about the reaction of defensive reflexes such as gagging and swallowing. When airway is clear, the oropharyngeal guedel can be used effectively. The positioning of guedel itself, however, causes retching, laryngospasm and sometimes eventually displaces the tongue thereby further aggravating the airway. The rule is emergency endotracheal intubation, without any protective reflex. Bag-mask ventilation is

the method of choice in patients with a patent airway and an absent spontaneous respiration. A tightly fitted mask with concurrent jaw thrust is always necessary to keep the ventilation running. Nonetheless, obese patients and beard patients have problems thus reducing ventilation effectiveness. In trauma patients, mask ventilation would ideally be a "two-person procedure," one that keeps the mask closely fitted to the mouth, and the other that operates the tube. Similarly, caution should be taken to execute adjunctive airway movements such as chin lift and jaw thrust. In the case of alleged C-spine damage, the head tilt and "sniffing the morning air" positions are absolute contraindications. The treatment strategy for patients with suspected C-spine injury is to keep the patient supine in order to help minimize morbidity of the C-spine as well as to immobilize the cervical spine using strong cervical collars.These collars may reduce exposure to the oropharynx which may be important. Unfortunately, the question that needs to be addressed in this scheme is how efficiently a trauma team can intubate the patient? A research shows, on this basis, that trauma patients with noisy or obstructed airways present. The unsuccessful rate of intubation is an unprecedented 12 percent.[6]In a study conducted by Martin et al., 10.3 percent of 3423 emerging intubations needed several attempts and were rated as "difficult."[7]

Primary Survey

Primary survey is performed to evaluate the system and vital function in question. The treatment plan is based on injuries, vital signs and mechanisms for injury. The primary survey is progressing sequentially on the following: 15

A – Airway with cervical spine immobilization

B – Breathing

C – Circulation with hemorrhage control

D – Disability (neurological status, as expressed by the patient)

E – Exposure of the entire body (looking for occult injury)

Definitive Airway: The definitive airway principle is that maxillofacial trauma is generally much more significant than other parts of the body as opposed to trauma. The key signs are set out in Table 1. The traditional conclusive, clear airway solutions are orotracheal intubation, nasotracheal intubation, and airway surgery. Laryngoscope-aided orotracheal intubation is the most feasible and effective method of treatment. However, if the C-spine is not clear, manual in-line axial stabilization during orotracheal intubation is prudent. While evidence in the literature indicates that some cervical movement is inevitable,[8,9] orotracheal intubation in an unknown cervical spine is comparatively healthy. It is easier to do, faster and induces minimal mobilization of the cervical spine in professional hands.[10] In extreme avulsive facial injury or in laryngeal or tracheal collapse, orotracheal tube positioning is difficult and surgical airway is the alternative. Another successful option is nasotracheal intubation, which can be done in patients without communicated midface or skull base damage. This is especially important in controlling airway obstruction due to lower face injury, and is ideal for patients with insufficient mouth opening. The method are supported by two forms, either blind or fibreoptic. A qualified professional's conventional blind technique is fast, efficient and requires no premedication. At the other hand, the enthusiasm for fibreoptic technique is restricted by the presence of copious secretions or blood in the airway, the sensitivity of the technique and the required increased time. Similarly, laryngeal mask airway (LMA) and combi tube are solutions to a failed or complicated intubation, but not one that is definite. These tools build support by bridging the airways before they enter a definite airway. It doesn't protect the airway from regurgitation and aspiration, however. The combi tube can be used instinctively in emergencies or in prehospital settings with little experience needed and simple placement.[11] Though suggested, retrograde intubation is very time-consuming and required expertise through cricothyroid puncture. Therefore, it is of limited use in emergency situations.[12] The manipulation of the cervical spine should be kept minimal during the airway maintenance technique and whatever approach you adopt, always note the dictum "to do no more harm."

Table 1. Indications of definitive airway in maxillofacial injury

Indications of definitive airway in maxillofacial injury
Absent spontaneous breathing
Comatose patient (glasgow coma scale <9)
Airway injury or obstruction
Persistent oxygen saturation <90%
High-risk for aspiration
Systemic shock (systolic blood pressure <80)
"Cannot ventilate cannot intubate" situations

If non-invasive strategies for securing airways fail, the only alternative left is surgical airways. They are of two types: cricothyroidotomy and tracheostomy.[13] In emergency, cricothyrotomy is the most effective approach and can be done by needle (needle cricothyrotomy) or surgical scalpel (surgical cricothyrotomy).[14] While some schools advocate needle cricothyrotomy, the standard use of it is debatable. The failure rates and insufficient oxygenation prevent its use, and in emergency, surgical cricothyroidotomy is the appropriate method of choice. In most cases, tracheostomy is done as an elective treatment, until cricothyrotomy stabilizes the patient. While percutaneous tracheostomy claims to minimize operating time and surgical complications in good hands, there is no evidence of its daily use in emergency.

Airway management in maxillofacial trauma protocols[2]

- Assess and identify obstructions to the airway.
- Clear the airway, position the patient. Perform chin lift and jaw thrust manoeuvre
- Confirm the nasal and oral passage are clear then use artificial airways and
- Perform bag-valve-mask ventilation. Preferably "two-person technique"
- Oro-endotracheal intubation
- In unsuccessful orotracheal intubation or "cannot ventilate cannot intubate situation" perform surgical airway [Figure 1].

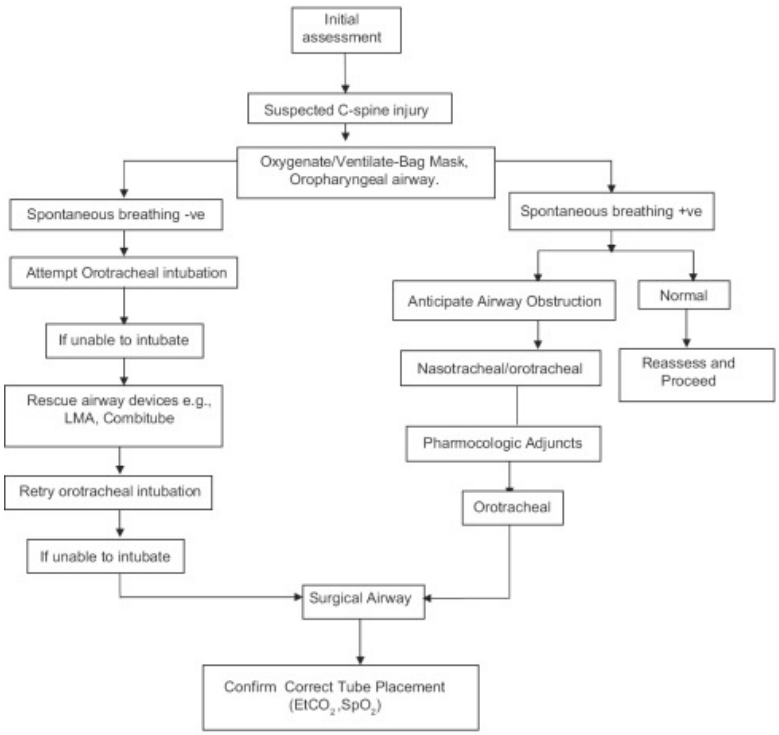


Figure 1. Surgical airway

Breathing: Breathing is evaluated by measuring the patient's respiratory rate, and subjectively quantifying the depth and intensity of inspiration. Breath sounds should be consciously filtered out bilaterally. Pulse oximetry is an obligatory adjunct and end-tidal carbon dioxide monitoring is a beneficial adjunct. Rapid breathing action, and use of respiratory accessory muscles, hypoxia, hypercapnia, and asymmetrical chest wall excursions and diminished or absent breathing sounds all require treatment while proceeding.At this point, pneumothorax tension needle decompression can be completed quickly with conclusive thoracostomy of the tube performed after completion of the primary survey.[15-18]

Circulation and Haemorrhage control: Following the development of airways and the treatment of respiratory issues, priority must be paid to circulation. Maxillofacial injuries are particularly vulnerable to serious haemorrhages and life-threatening haemorrhages can range from 1.4% to 11%. One in ten of the complex facial fractures substantially bleeds. The principal vessels involved are an ethmoid artery, ophthalmic, internal carotid vidian branch, and maxillary artery. Bleeding can be easily controlled in most cases, but rarely extreme epistasis ranging from 2% to 4% of all facial bleeding occurs from the maxillary artery, causing haemorrhage management difficulties.Through careful examination of the pharynx for lacerations and tears, it is important to distinguish bleeding from the skull base fracture and oral bleeds.[19-23]

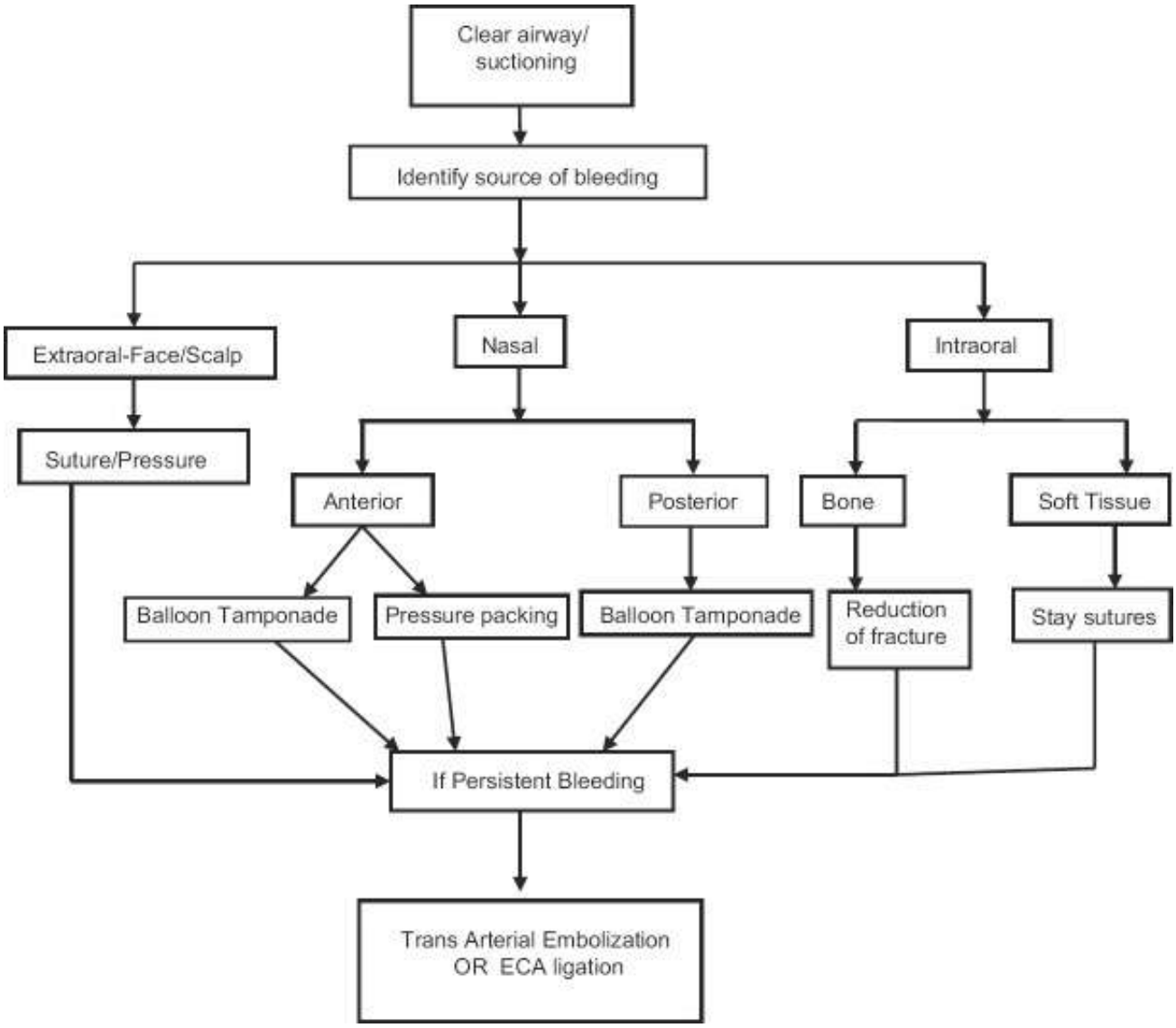


Figure 2. Clear airway/suctioning

Secondary Survey: Upon completion of the primary test, serious life-threatening injuries were treated, and vital signs were stabilized, the provider starts the secondary check that requires a history and full head-to - toe inspection. A history of the injury process is important to obtain, since certain causes of trauma (falls, motor vehicle collisions, car vs. pedestrian accidents, gunshot wounds, etc.) are typically associated with patterns in injury, which may alert the provider to new potential injury. Current medicines are important, particularly for elderly people, because anticoagulants can be a significant cause of excessive bleeding, and b-blockers can decrease cardiac output and suppress tachycardia.[24-27]

The family members, paramedics, or other traumatized patients can be a best source of knowledge in a patient who is unable to respond. A helpful mnemonic for having a brief history is "Complete," which looks at the following important areas: allergies, medications, past diseases/pregnancy, last meal, and injury-related events/environment. The physical exam carefully examines each region of the body to accurately classify all the wounds and reduce the possibility of missed injuries. The systematic analysis starts with the head and skull, and progresses to maxillofacial structures, neck and c-spine, chest, abdominal, perineal region, pelvic and neurological system. Since several of those structures overlap, a detailed and systematic approach should always be taken to ensure completeness.

Cervical Spine and Maxillofacial Trauma: In a complex maxillofacial trauma situation, fracturing of the cervical spine should always be considered, unless otherwise confirmed. The occurrence in all maxillofacial trauma is very small and ranges from 1 percent to 10 percent.[27-34] Due to the proximity of the cervical spine, any force of such magnitude that causes facial fractures can potentially traumatize the c-spine and its ligamentous attachments. In general, without the addition of a radiographic test, the patient can be exempt from spinal injury if the following are shown. Patient with neurological conditions (norm GCS); Not under the consequence o drugs/alcohols/others; Absences of pain in posterior midline cervical spine; devoid debilitating.[35]

Conclusions

The seriousness of all maxillofacial injuries is due to the fact that they pose an immediate threat to life as a result of their proximity to both the airway and the brain. Even so, each situation is unique; therefore, even for the most seasoned of professionals, the management is exacting. No treatment strategy can be defined as sure and flawless in any given scenario. The need of the hour is a multi-pronged strategy involving multi-departmental cooperation. Although new technologies and equipment advances have helped to ease the crisis, the prompt action, sheer skill, and mind presence of emergency personnel and surgeons are what counts.

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Coronavirus Disease 2019 (COVID-19): Impending Encounters for Dental Health Professionals in India

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Abstract

Originating in Wuhan, China, the epidemic of coronavirus disease 2019 (COVID-19) has become a major public health challenge for not only China but also countries around the world. The outbreaks of the novel coronavirus have constituted a public health emergency of international concern as announced by the World Health Organization (WHO). For dental care provision to active or suspected COVID-19 cases, no universal protocol or guideline is available till date. Major challenges to worldwide health systems will have far-reaching consequences on the global economy if the spread of the virus is not effectively controlled as a consequence of the COVID-19 outbreak. So, in a nutshell, there is no point in being cynical about the future of the dental profession.

Keywords: *Challenges; coronavirus; COVID-19; infection; dentist.*

Introduction

India's tally of Coronavirus disease 2019 (abbreviated "COVID-19") rises to 24,506, death toll at 775 as on 26th April 2020.[1] Caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, COVID-19 has become an emerging respiratory disease. The first COVID-19 case was reported to the WHO country office in China on 31 December 2019 which originated in Wuhan, China.[2] The Director-General of the World Health Organization (WHO) declared coronavirus disease 2019 (COVID-19) outbreak a pandemic at a media briefing on 11th March 2020.[3] COVID-19 has been recognized in over 200 countries, areas and territories, with a total of over 23,14,621 confirmed cases and over 1,57,847 deaths, as of 20 April 2020.[4] The monitoring of this infectious disease outbreak is done by International centers for disease control and prevention. Fever, cough, and acute respiratory disease, with severe cases leading to pneumonia, kidney failure, and even death is the symptoms of COVID-19 infecton.[5]

Demand for urgent dental treatment decreased by only 38% despite the large-scale community transmission of COVID-19 in China during the epidemic.[6] This reflects public need during this pandemic will always be essential for urgent dental care. Several dental institutes, regulatory and advisory bodies still do not have a clear vision about the worldwide impact this pandemic can have on dental services although it has been six weeks since COVID-19 outbreak was declared as a pandemic. From advising practitioners to close their practices in California, USA;[7] to reducing the number of routine check-ups in the UK;[8] to no advice at all from several dental associations around the world, there were varied responses and actions from dental associations around the world. The varying degree of COVID-19 outbreak in different countries owes to such unclarity.[9]

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However, this COVID- 19 pandemic could last over 18 months according to the US Government COVID-19 response plan published by the US Department of Health and Human Services (HHS) on 13 March 2020.[10] There will be increase in the suffering of the individuals in need of urgent dental care and it will also incense the burden on hospitals emergency departments, although closing dental practices during the pandemic can reduce the number of affected individuals. This article is an attempt to understand the current imminent of the pandemic and how it will impact the future outlook for the profession of dentistry. Taking into consideration the severity of the pandemic COVID-19, and in the light of the massive commitment of several dental associations we have used existing research, personal life experience, assumptions, and industry data to try to make some sense of all of this psychosis.

To curb the spread of the new virus, India has been under a nation-wide lockdown since Mar 24, 2020, which has now extended till 3rd May by the Government. Special challenges are posed by India for the country's disease containment strategy due to its grossly under-funded and patchy public health system, with huge variations between different states, despite India not being among the worst-hit countries. There have been several reported instances of patients trying to flee isolation wards in government hospitals and hide travel history over the past few weeks. Many tried to dodge the mandatory home quarantine even with exposure to suspected cases of COVID-19 and infected persons.

India's latest containment plan, a 20-page document which specifically talks about "non-pharmaceutical interventions" "Quarantine and isolation are important mainstay of 'cluster containment," states that the above mentioned are worrying developments in the backdrop of the plan. Quarantine refers "to separation of individuals who are not yet ill but have been exposed to COVID-19 and therefore have a potential to become ill". Isolation refers to "separation of individuals who are ill, suspected, or confirmed COVID-19 cases".

People escaping hospitals or quarantine in the past week have been certain repeated reports. "How can this impact the ongoing control measures for COVID 19? One key issue is the trust deficit in the public health system in many parts of the country. Other important factors include fear of isolation and stigma attached to those who are being quarantined and isolated. It is difficult for people in India to understand the importance of isolation and quarantine, even though it is a response to a pandemic. The faith in the public health system cannot emerge immediately as a response to the pandemic".

"An overstretched public health care system forces millions of Indians to turn to the unregulated private health care sector. The Indian Government's consumption on health as a percentage of GDP still hovers around 1·5%, one of the lowest in the world. For around 52% of family units in urban areas, and 44% of families in rural areas, the private segment is the primary source of human services when they are debilitated, as per government data.[11] Trust in Government is a significant part in a crisis health response".

Current and upcoming dentistry: Dental specialists currently face a test they have not been set up for. None of the dental specialists would have imagined a circumstance like this one, where there is a drawn-out time of interference. It is hard to foresee what's on the horizon for dentistry. How about we consider the month government evacuates all limitations as month zero. Expect customer spending to return inside seven days of month zero. It is normal that consumers will spend on basics followed by a time of liquidity crunch where spending will diminish once more. For dentistry, this may imply that patients at first spotlight just on crisis strategies or the treatment that truly matters to them.

Patient administration: Practices are utilizing each conceivable strategy (mailers, voice messages, web-based life, and practice sites) to educate patients about the workplace terminations for ordinary dentistry. Patient screening conventions, should proceed significantly after the limitations are lifted (figure 1).

Patients are understanding and are not ready to face challenges themselves. Practices need to rehash the exacting Centers for Disease Control and Prevention (CDC) and Occupational Safety and Health Administration (OSHA) rules that are being executed.

Staff supervision: The most awful effect of works on being shut is on staff compensations. A considerable lot of the staff have less squirm room undoubtedly.

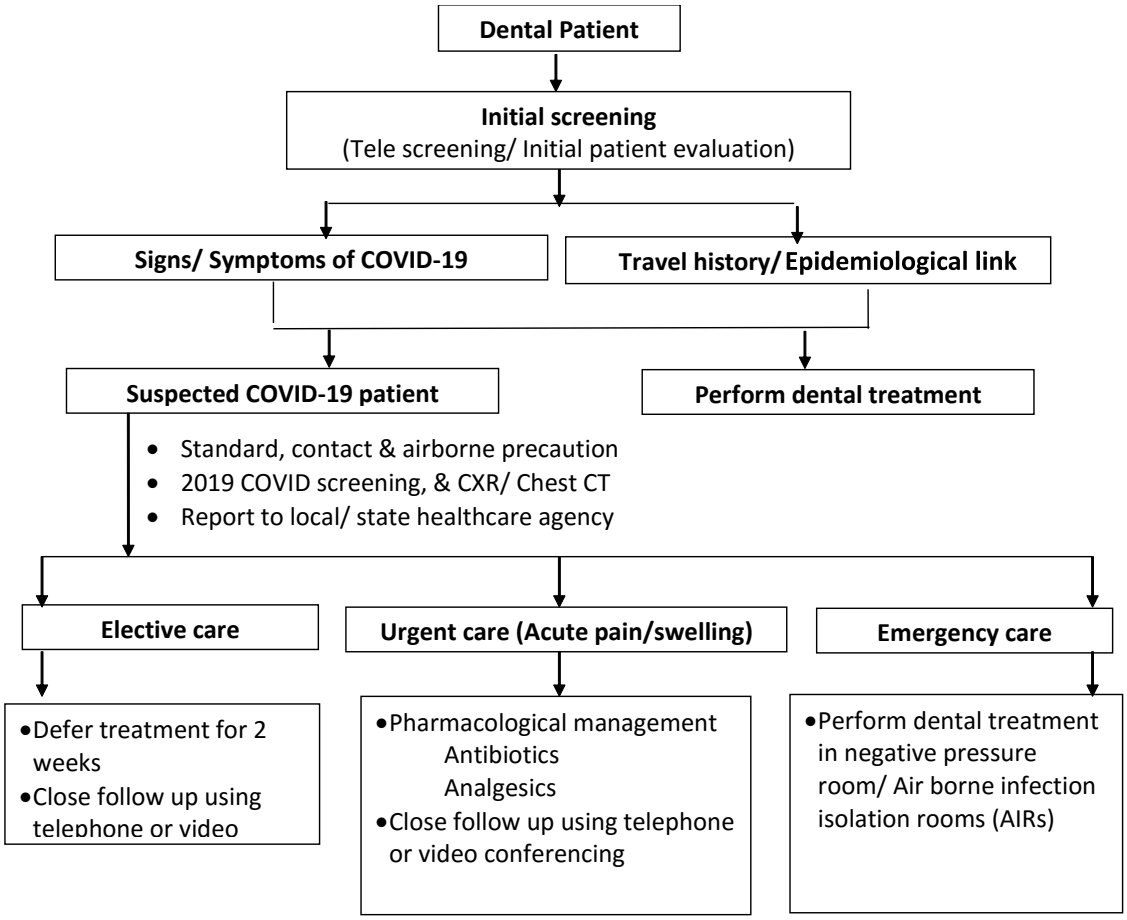


Figure 1. Patient Screening Protocols.

Employing partners and hygienists: There is a great deal of disarray among dental alumni in regards to recruiting associates because of this emergency. All things considered; dental specialists will hold on to perceive how patients are reacting. In the event that they rapidly recoup, at that point there will be an earnestness to recruit to make up for lost time. Professionals will probably react dependent on how their financers, for example, banks, acclimate to the new reality. Some may at present grow their practices while others may concede the move for what's to come. The circumstance may at first be somewhat harder for dental hygienists, however it will level soon.

Dental inventory organizations: Personal protective equipment (PPE, for example, gloves, outfits, goggles, and face masks, just as cleaning, diagnostic, surgical, and wound care items have been delivered fundamentally by huge providers in China. This reliance on China will most likely diminish and local creation will soar. This will mean the cost of expendable things may go up later on. The stock calendar will be upset during the months to come and dental specialists will see a ton of delay purchases on numerous items.

Insurance: The insurance agencies are being immersed with calls about pandemic inclusion and they face an exceptional circumstance where they may need to remunerate the entirety of their policyholders. This will put them under huge tension. The insurance agencies will in general oppose the cases by pulling in all the lawful supports. There is likewise the probability that dental specialists will react with a joined supplication to the administration sooner rather than later.

Banks: It is sensible to anticipate that banks should give a go for a couple of months, in any event in regards to paying practice advance premiums.

"The most suggested rules show that dental specialists ought to maintain a strategic distance from the booking of any patient: just such pressing dental sicknesses can be considered during the COVID-19

episode. This activity will definitely restrain the relational contact, the holding up time of patients in dental cupboards and, when all is said in done, the conditions inclining patients to be contaminated. At the point when the dental specialists treat patients, they should intercept the possibly contaminated individual before they arrive at the working zones; for instance, those with a fever estimating >37.5°C and the offering of a couple of conversation starters about the patient's general wellbeing status over the most recent 7 days, and about the danger of having been in contact with other tainted people".

"Dental patients who cough, sneeze, or obtain dental treatment including the usage of a high-speed handpiece or ultrasonic instruments make their secretions, saliva, or blood aerosolize to the environment. Dental mechanical assembly could be defiled with different pathogenic microorganisms after use or get presented to a contaminated clinic condition. From that point, diseases can happen through the cut of sharp instruments or direct contact between mucous layers and tainted hands".[12]

"Because of the extraordinary qualities of dental methodology where an enormous number of droplets and aerosols concentrates could be created, the standard defensive measures in every day clinical work are not compelling enough to forestall the spread of COVID-19, particularly when patients are in the incubation time frame, are uninformed they are tainted, or decide to cover their contamination".

Efficacious Infection Control Protocols: "Hand cleanliness has been viewed as the most basic measure for diminishing the danger of transmitting microorganism to patients.[13] SARS-CoV-2 can endure on surfaces for a couple of hours or as long as a few days, contingent upon the sort of surface, the temperature, or the stickiness of the environment.[14] This fortifies the requirement for good hand cleanliness and the significance of intensive sanitization of all surfaces inside the dental center. The utilization of individual defensive gear, including masks, gloves, outfits, and goggles or face shields, is prescribed to shield skin and mucosa from (conceivably) tainted blood or emission. As respiratory droplets are the principle course of SARS-CoV-2 transmission, particulate respirators (e.g., N-95 masks validated by the National Institute for Occupational Safety and Health or FFP2-standard veils set by the European Union) are suggested for routine dental practice. The 4-handed method is advantageous for controlling contamination. The utilization of salivation ejectors with low or high volume can diminish the creation of droplets and aerosols".[12,15,16] Fumigation of the work environment should likewise be possible to free all the debasements from nature we work in. All biomedical waste relating to persistent consideration ought to be painstakingly arranged according to the Bio-Medical Waste (Management and Handling) Rules,1998 revised periodically through an approved biomedical disposal organization by the State Pollution Control Board.

Appraisal of Patients: "During the flare-up of COVID-19, dental facilities are prescribed to set up precheck triages to quantify and record the temperature of each staff and patient as a standard technique. Precheck staff ought to ask patients inquiries about the wellbeing status and history of contact or travel.[17] Patients and their accompanying people are given clinical masks and temperature estimation once they enter our emergency clinic. Patients with fever ought to be enlisted and alluded to assigned medical hospitals. On the off chance that a patient has been to pandemic areas inside the previous 14 days, quarantine for at least 14 days is proposed. In zones where COVID-19 spreads, nonemergency dental practices ought to be postponed.[12,15,16] It was accounted for that dental practice ought to be deferred in any event multi month for convalescing patients with SARS.[16] It is obscure yet whether a similar proposal ought to be suggested for patients with COVID-19".

Oral Examination: "Preoperative antimicrobial mouth wash could diminish the quantity of organisms in the oral cavity.[12,18] Procedures that are probably going to initiate coughing ought to be maintained a strategic distance from (if conceivable) or performed cautiously.[17] Aerosol-creating method, for example, the utilization of a 3-way syringe, ought to be limited however much as could be expected. Intraoral x-ray assessment is the most well-known radiographic method in dental imaging; be that as it may, it can invigorate saliva secretion and coughing.[19] Therefore, extraoral dental radiographies, for example, panoramic radiography and cone beam CT, are proper options during the episode of COVID-19".

Handling of Emergency Cases: "Dental crises can happen and fuel in a brief period and consequently need prompt treatment. Rubber dams and high-volume salivation ejectors can help limit airborne or scatter in dental method. Besides, face shields and goggles are basic with utilization of high or low-speed drilling with water spray.[20] According to our clinical experience

during the flare-up, if a carious tooth is determined to have symptomatic irreversible pulpitis, pulp exposure could be made with chemo mechanical caries expulsion under rubber dam seclusion and a high-volume salivation ejector after local anesthesia; then, pulp devitalization can be performed to diminish the agony. The filling material can be supplanted delicately without a devitalizing operator later as per the maker's suggestion". After treatment, environmental cleaning and disinfection procedures were followed. "On the other hand, patients could be treated in a segregated and very much ventilated room or adversely compelled rooms if accessible for suspected cases with COVID-19. Perilous cases with oral and maxillofacial compound wounds ought to be admitted to the clinic quickly, and chest CT ought to be endorsed if accessible to avoid suspected contamination on the grounds that the RT-PCR test, other than tedious, needs a research facility with pan-coronavirus or explicit SARS-CoV-2 identification capacity".[21]

Dental Contemplations: "Bioaerosols are natural particles suspended in vaporous media.[22] Subgingival scaling for treating periodontally undermined teeth with the guide of a ultrasonic scalers will create vaporizers containing blood.[23] An ongoing report demonstrated that the ultrasonic scalers and tips delivered altogether more airborne contrasted with a handheld curette, paying little attention to the scaler type employed.[24] The continuous and interminable presentation to bioaerosols produced during such dental systems notwithstanding the generally little molecule size of the bioaerosols add to an expanded danger of contamination among dental professionals.[25] It is as yet sketchy whether the security provided by surgical masks worn by dental experts go about as a defensive boundaries for such little particles notwithstanding the way that these covers may not fit consummately in clinical practice. Simultaneously, it is critical that the probability of distinguishing, detailing and reporting dental calling related diseases is generally less".[26]

Conclusion

In all probabilities, the virus will stay with us. There will be changes in behavior such as less travel, reduced smoking, increased use of pneumococcal vaccines, higher online sales and food ordering, higher interest in online courses, social distancing, more movies about viral outbreaks - the list is endless. Albeit the vast majority of the public consideration is concentrating on the immediate causes and control proportions of COVID-19, potential health moments coming about because of individuals' feelings of trepidation of it ought not be ignored. Understanding the current circumstance is useful as far as anticipating future dental needs. Numerous reasons do conjecture that individuals' prerequisites for dental administrations may develop explosively in the post-COVID-19 period.

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Awareness in Oral Hygiene Status in Diabetic Cases: An Institutional Study

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Abstract

Objectives: The objectives of the study were to: (1) assess the knowledge and awareness of diabetic patients of their risk for systemic and oral diseases as complications associated with diabetes, (2) to assess their attitudes toward sustaining good oral health through proper oral hygiene and regular dental check-ups, and (3) to the extent that they are aware, to determine how they became aware.

Method: One hundred self-administered questionnaires were distributed to assess the main objectives of the study. Only completed questionnaires were used in the current study data analysis.

Results: A majority of the participants suffering from Type 2 diabetes (58%). The awareness of diabetic patients of their increased risk for oral diseases was not upto the benchmark point . Their attitude towards good oral hygiene status was not desired. Likewise, 43% brushed their teeth once daily and 49% never used dental floss. Regarding participants' sources of awareness towards to dentist about diabetics is 50%.

Conclusions: Diabetic patients are found to have little knowledge of their increased risk for oral diseases. Inorder to promote proper oral health and to reduce the risk of oral diseases, health professionals in both thedental and medical fields need to take the responsibility to develop programs to educate the public about theoral manifestations of diabetes and its complications on oral health.

Keywords: *Diabetes; oral health; periodontal disease; oral self-care; oral diseases; diabetes complications.*

Introduction

Diabetes mellitus has been increasing at such an alarming rate worldwide that recently the World Health Organization (WHO) declared the disease an epidemic. The number of estimated cases of diabetes has increased from 30 million in 1985 to 135 million in 1995, and is projected to increase to 366 millionby the year 2030.[1] The prevalence of diabetes mellitus currently affects more than 62 million Indians, which is more than 7.1% of the adult population. The average age on onset is 42.5 years. Nearly 1 million Indians die due to diabetes every year. In addition to the more common complications that arise as a result of this disease, diabetic patients who do not carefully control their blood glucose levels will be at high risk of systemic and oral complications. The mostcommon complications include macrovascular diseases,coronary artery,and cerebrovascular diseases. Additionally, microvascular complications manifest as diabetic retinopathy, neuropathy, andnephropathy among others.[2]

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In terms of its oral consequences, diabetes manifests itself in several ways. When diabetes mellitus is left uncontrolled for an extended period, it affects salivary glands and which results in xerostomia or sialosis (dryness of mouth).[3-7] When not enough saliva is produced to wash and cleanse the oral cavity. This could be a factor in the increased risk for dental caries often observed in dental patients.[8,9] Furthermore, peridontitis is perhaps the most widely noted oral manifestation of diabetes mellitus. Several researches have advanced the notion that there is a bidirectional relationship between periodontitis and diabetesmellitus.[10,11] In this paper, we had a survey of one hundred individuals for oral hygiene awareness in diabetic patients.

Thus, the purpose of the current study was to: (1) assess the knowledge and awareness of diabetic patients of their risk for systemic and oral diseases as complications associated with diabetes, (2) to assess their attitudes toward sustaining good oral health through proper oral hygiene and regular dental check-ups, and (3) to the extent that they are aware, to determine how they became aware.

Method

In the current study, a self-administered questionnaire was used and utilized to assess the main aims of the study. The questionnaire questions were related to participants age and sex, type and duration of diabetes, and their awareness for systemic and oral diseases and other characteristics related to oral hygiene in diabetics patients. Various response formats were used in the questionnaire such as 'yes,' 'no,' 'I do not know,'encircling disease name, they were aware of as a complication of diabetes, or filling blank spaces.

Clinical findings in diabetic pts		
Age		
Gender		
Dryness in the mouth		
Delayed healing in extraction socket		
Bleeding gums		
Gingival recession		
Tooth mobility		
Generalized stain & calculus		
Periodontal pocket Depth of pocket >2mm, > 3mm		
Tooth Decay		
Bad breath		
Mouth ulcers		
Infections		
Brushing frequency Once a day Twice a day		

Figure 1. Questionnaire form for diabetics individuals

One hundred questionnaires were distributed to the diabetic patients attending the outpatient diabetic clinic in the department of Oral Pathology And Microbiology, IDS Eligible participants were confirmed diabetes mellitus patients. Data were collected over one year. However, questionnaires with uncompleted answers were excluded and a total of 100 completed questionnaires were entered on an Excel spreadsheet and imported into Statistical Package for Social Sciences (SPSS) version 13 (SPSS Inc.,Chicago, IL, USA) for data statistical analysis.

Results

The diabetic patients participating in the current survey were 90% nationals, of whom 59% were male and 39% were female. The mean of the age was 35 years. In our study we found both the type 1 and 2 diabetics, type 1 is 40%,type 2 is 58%. We also recorded delay extraction socket or delayed healing is 25%.

Mouth dryness/xerostomia is 67% of cases, bad breathe is 31% in cases related to periodontitis like periodontal pocket is 22%, bleeding gums is 52%,gingival recession is 52%,stain and calculus not having 53%. 46% don't have grade ll mobility whereas 30% have tooth mobility. 26% of individuals suffering from infection whereas in mouthulcer patients is 27%. Individuals suffering from dental caries are 32%. Patients are advised for brushing and flossing habits once or twice they are 43% and 49%.

Table 1: Represents Results of Diabetic Individual

Male	59%	
Female	39%	
Age	35 years and above	
Type 1	40%	
Type 2	58%	
No. of Visit	46%	
Delayed Healing	Y----26%	N----25%
Mouth Dryness/Xerostomia	Y-67%	N- 26%
Bleeding Gums	Y-52%	N----43%
Gingival Recession	Y---52%	N—50%
Stain And Calculus	Y---30%	N- 53%
Tooth Mobility	Y-30%	N-46%
Infection	Y-26%	N-33%
Mouth Ulcer	Y-27%	N-19%
Tooth Decay	Y-32%	N-31%
Bad Breathe	Y-31%	N-38%
Periodontal Pocket	Y-22%	N-25%
Brushing Frequency	Y-43%	N-59%
Flossing Frequency	Y-49%	N-13%
Do the PT Talk About Diabetic to Dentist?	Y-50%	N-47%

Discussion

The prevalence of diabetes worldwide has been increasing epidemically. It was declared by WHO that 366 million people are expected to suffer from diabetes mellitus by 2030. In the current study, more than half of the participants (58%) had Type 2 diabetes, 26% suffered from Type 1 and, unexpectedly, 16% of participants did not know what type of diabetes they had. The data presented in this study reveals an important finding: it demonstrates that diabetics have more knowledge

about their increased risk for systemic complications associated with diabetes than they do for oral and dental complications. The percentage of participants who were aware of their increased risk for eye disease was 85%, heart disease 75%, kidney diseases 90%, periodontal disease 60%, dental caries 54%, and oral fungal infections 42%.[12] Similar findings were reported by several researchers,who assessed the knowledge diabetic patients had of their risk for periodontal disease, their attitude toward oral health, and their oral health-related quality of life. The researchers found that only 33% of the participants were aware of their increased risk for periodontal disease.[13] Similarly, Eldarrat (14) found that diabetic patients' awareness of their increased risk for oral diseases was low compared to their awareness of systemic diseases and a significant association was found between glycemic control and oral infections. Furthermore, Moore et al.[15] assessed oral health attitudes, behaviors, and knowledge of participants with Type 1 diabetes and found that most of the participants were unaware of the oral health complications of diabetes mellitus and the need for preventive care.

It is well known that a significant reduction of salivary flow leading to xerostomia is the most common oral manifestation of diabetes. It is of paramount importance to inform and make diabetics aware of the beneficial properties of saliva. Saliva's function of washing and cleansing the oral cavity is known to prevent the accumulation of plaque and debris, which could be a contributing factor in diabetics' increased risk for periodontal disease and dental caries.[8,9] This study also revealed the additional important finding that diabetic patients have limited knowledge of the associations between oral health and overall health. Similar results were reported by several investigators who found that of 299 participants, only 29% brushed their teeth on a twice-daily basis.

The data of the present study showed that about 40%of the participants had not visited a dental clinic within the last year. The main reason to visit a dental clinic within the last year was to receive treatment for pain and/or discomfort. Only 14% visited for regular check-ups. Early detection and treatment of tooth caries, periodontal disease, and other oral diseases would be of enormous benefit to protect diabetic patients from the harmful oral complications associated with diabetes. Furthermore, it is necessary for dental professionals and related government agencies to promote awareness of the relationship between diabetes and oral health to prevent harmful dental complications and expensive treatment.

Conclusion

Within the limitations of the current study, the data presented in this study demonstrated that diabetics have more knowledge about their increased risk for systemic complications associated with diabetes than they do for oral and dental complications. For promoting proper oral health and to reduce the risk of oral diseases,health professionals in both the dental and medical fields need to take the responsibility to develop programs to educate the public about the oral manifestations of diabetes and its complications on oral health.

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Tumor Associated Macrophages: A Review with Institutional Study

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Abstract

Aims: To study the expression of tumor-associated macrophages in different grades of squamous cell carcinoma and find the association between their expression and local recurrence, metastasis.

Methodology: Thirty tumor biopsy samples of oral squamous cell carcinoma in T1N0M0 and T2N0M0 stages. Ten biopsy specimen of normal oral mucosa that are taken during minor oral surgical procedure with patient consent.

Result: Macrophages were found to have better specificity for local recurrence, metastasis, and survival. Further, higher expression of biomarkers was associated with shorter survival. We have analyzed the differential expression of MMP 9 in different grades of OSCC & compare their expression with different TNM staging of tumors.

Conclusions: Concluding this review of tumor-associated macrophages in comparison with OSCC cases. Since metastases are the principal cause of death in cancer patients, a greater understanding of the process of tumor invasion and metastasis is essential in leading to the identification of new therapeutic targets.

Keywords: *Immunohistochemistry, OSCC, Tumor-Associated Macrophages.*

Introduction

Oral carcinoma is amongst the leading malignancies worldwide, with squamous cell carcinoma (SCC) as the most common type accounting for over 90% of cases[1]. The high rate of relapse in tumor indicates the inadequacy of current prognostic predictors,that is, histological and clinical assessments, in predicting metastatic potential,as well as the need to investigate additional determinants among oral cancers[2]. In recent years, it has been accepted that to effectively treat cancer, the tumor must be considered as an entity containing both the cancer cells and the surrounding tissue which together forms the tumor microenvironment (TME). In squamous cell carcinoma, the TME may even be dominant over cancer cells[3]. TME contains a mixture of heterotypic cells such as cancer-associated fibroblasts (CAFs), smooth muscle cells, endothelial cells, neutrophils, lymphocytes, and macrophages[4].

Normally macrophages are important cells in wound healing providing aids for tissue cell growth, tissue matrix remodeling, and angiogenesis. There are 2 types of macrophages M1 & M2. M1 macrophages phagocytose & destroy microbes,eliminate cells, present to the antigen to T-cells for an adaptive immune response & produce high levels proinflammatory cytokines &

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helps in tumor suppression. M2 macrophages promote IL-4,IL-10,TGF,PGE2 exposure to TH2 responses, suppresses TH1 mediated inflammation & helps to increase tumor development. Hence Macrophages are described as "double-edged sword" means capable of both promoting & opposing tumor growth[5,6]. Tumor-associated macrophages (TAM) have differentiated into the M2 phenotype which involves angiogenesis, immunosuppression & activation of tumor cells. TAMs release reactive oxygen species, tumor necrosis factor (TNF)-α, Interleukin (IL)-6 and IL-1β, promoting DNA damage, transformation and cancer cell survival.[6]

This tumor-associated macrophages conglomerate with oral squamous cell carcinoma in a metastatic role as a review paper.

Aim: To aim & evaluate the expression of TAM in different grades of OSCC cases.

Objectives:

- Evaluation of expression of TAM infiltration in OSCC.
- Comparison of TAM expression with clinical parameters of OSCC.
- Comparison of TAM with different grades of OSCC.
- Comparison of TAM expression with local metastasis in OSCC cases.
- Correlation of expression of TAM in OSCC cases.

Tumor Associated Macrophages: Macrophages are derived from CD34+ bone marrow progenitors that continually proliferate and shed their progeny into the bloodstream as promonocytes[7]. They then develop into monocytes and extravasate into tissues where they differentiate into a specific type of ''resident'' tissue macrophage[8]. Macrophages also form a major component of the inflammatory infiltrate seen in both primary and secondary tumors.

Broadly macrophages divides into

(a) Classically activated type 1 Macrophages- M1

(b) Alternatively activated type 2 Macrophages- M2

M1 macrophages phagocytize & destroy microbes, eliminate cells, present to the antigen to T cells for an adaptive immune response & produce high levels of pro-inflammatory cytokines. M2 macrophages promote IL-4, IL-10, IL-13, TGF, PGE2 exposure to TH2 responses, suppress TH1 mediated inflammation IL -10. M2 regarded as an increase in tumor development. Thus macrophages described as "double-edged sword" means capable of both promoting & opposing tumor growth[6]. M2 macrophages are called as TAM

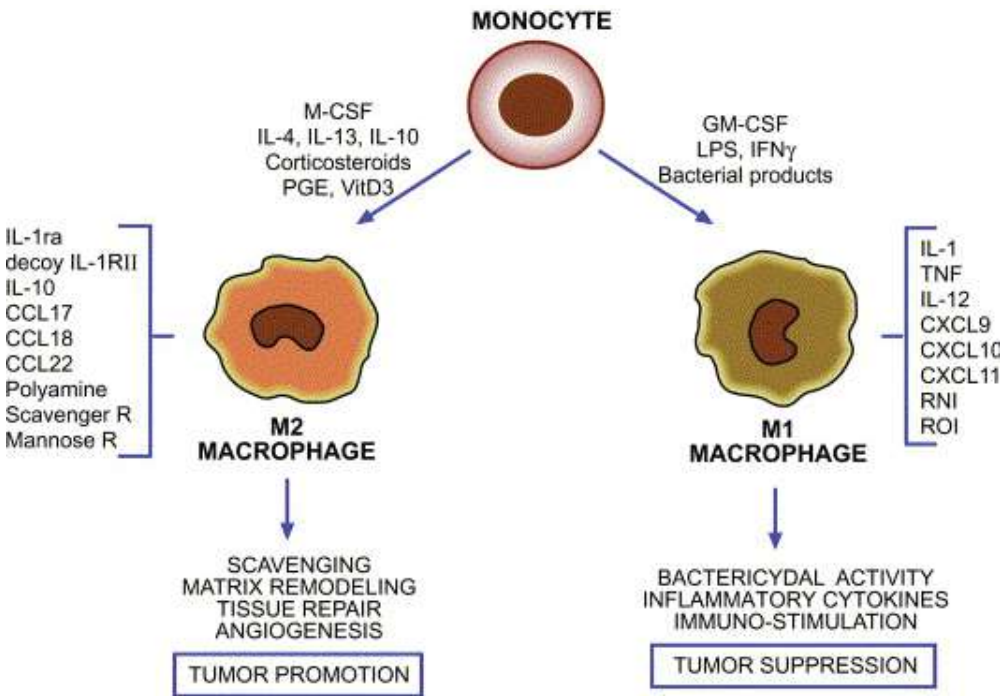


Figure 1: Shows Dual Role of Tumour Associated Macrophages

Roles of TAM: Tumor Invasion: In polyomavirus middle T oncoprotein (PyMT-)induced mammary tumors, macrophages are present in areas of basement membrane breakdown and invasion during the development of early-stage lesions [7]. TAMs secrete a variety of proteases to breakdown the basement membrane around areas of proliferating tumor cells[8]. This finding, together with other results showing the up-regulation of proteolytic enzymes like cathepsin B in macrophages present at the same locations, indicates that TAMs could be involved in the invasion of tumor cells into surrounding normal tissue[8].

Tumor Growth: TAM infiltration positively correlates with tumor cell proliferation as measured by MIB-1 levels in breast carcinomas[14], Ki67 levels in endometrial carcinomas[10], or mitotic index in renal cell carcinoma[9]. This phenomenon is known to involve factors like TGF-h1 being expressed by inflammatory cells in wounded tissues.

Tumor angiogenesis. TAMs release several potent proangiogenic cytokines and growth factors, such as VEGF, TNF-a, IL-8, and bFGF. Additionally, they express a broad array of angiogenesis-modulating enzymes, including MMP-2, MMP-7, MMP-9, MMP-12, and cyclooxygenase- 2 (COX-2).[9] In areas of transient (avascular) and chronic (perinecrotic) tumor hypoxia, macrophages cooperate with tumor cells to induce a vascular supply for the area by up-regulating several angiogenic growth factors and enzymes. These diffuse away from the hypoxic area and together with other pro-angiogenic stimuli in the tumor microenvironment, stimulate endothelial cells in neighboring, vascularized areas to migrate, proliferate, and differentiate into new vessels[2].

Immunosuppression: Macrophages in hypoxic areas secrete factors that suppress the antitumor functions of immune effectors within the tumor. Unlike macrophages from healthy tissues, which are capable of presenting tumor-associated antigens, lysing tumor cells, and stimulating the antitumor functions of T cells and NK cells, TAMs in the tumor microenvironment lack these activities, leaving the host without the ability to mount an effective antitumor immune response[2,6].

Metastasis: A subpopulation of TAMs associated with tumor vessels secretes factors like EGF to guide tumor cells in the stroma toward blood vessels where they then escape into the circulation[7]. In the stromal compartment (both the acellular regions and others where they are in close contact with tumor cells), TAMs secrete growth factors to stimulate tumor cell division and/or undefined factors that promote tumor cell motility[9,10]. TAMs seem to play roles in both the release of metastatic cells from the primary tumor as well as the establishment of secondary tumors at distant sites.

To summarize, analysis of the expression of protumor molecules by TAMs in human tumors and functional studies in macrophage depleted murine tumor models have shown TAMs to play an important part in various key steps in tumor progression. It seems that in early preinvasive lesions, tumor cells release chemokines to attract macrophages and other inflammatory cells into stromal areas surrounding the tumor. Macrophages are also found in dense clusters as part of leukocytic infiltrates in areas of focal breakdown of the basement membrane. Tumor cells are thus able to escape the confines of the basement membranes and migrate across into the stroma of surrounding healthy tissues, where they, together with macrophages, stimulate angiogenesis. These newly formed vessels sprout into the early tumor, providing nutrients and oxygen for tumor growth and supplying multiple exit routes for metastasizing cells[13,14].

Methodology

The present study was done in the Department of Oral & Maxillofacial Pathology & Microbiology, IDS between 2013 to 2016. Approval from the institutional ethics committee of " Siksha O Anusandhan University was obtained to carry out this work.

Clinicopathologic information on each case including age, gender, site of location, habit was collected from the department.

Inclusion Criteria: Patients of all age groups with histopathological diagnosed primary carcinoma cases.

Exclusion Criteria:

- Metastatic tumor in the oral cavity
- Patients having carcinoma with any secondary infections.
- Patients having carcinoma with immuno compromised disease (HIV)
- Tumors which are under treatment of chemotherapy or radiotherapy.

Sample size of the study:

- 30 blocks specimens i.e. 10 well-differentiated, 10 moderately differentiated, 10 poorly differentiated & 10 normal block

Table 1. Scoring criteria for evaluating expression of TAM in our study.

Macrophage Count	Total Score
< 10 %	Score – 0
10-30 %	Score – 1- 2
30- 50 %	Score – 3- 4
>50 %	Score - 5 -6

The results were categorized as negative & positive respectively, based on total score: if total score: 0-2- Negative; 3-6 – Positive. For evaluation of inter/ intra examiner consistency slides are observed by two more examiner for scoring of TAM. The results were categorized as negative & positive respectively based on scoring are:TAM Negative- 13; TAM Positive – 17

Results

The data was collected from individuals cases & organized systemically. Compiling all data was formulated in various tables & graphs were derived from statistical analysis,for easy interpretation of results was done using SPSS software.

Table 2. Comparing the macrophage count with habit

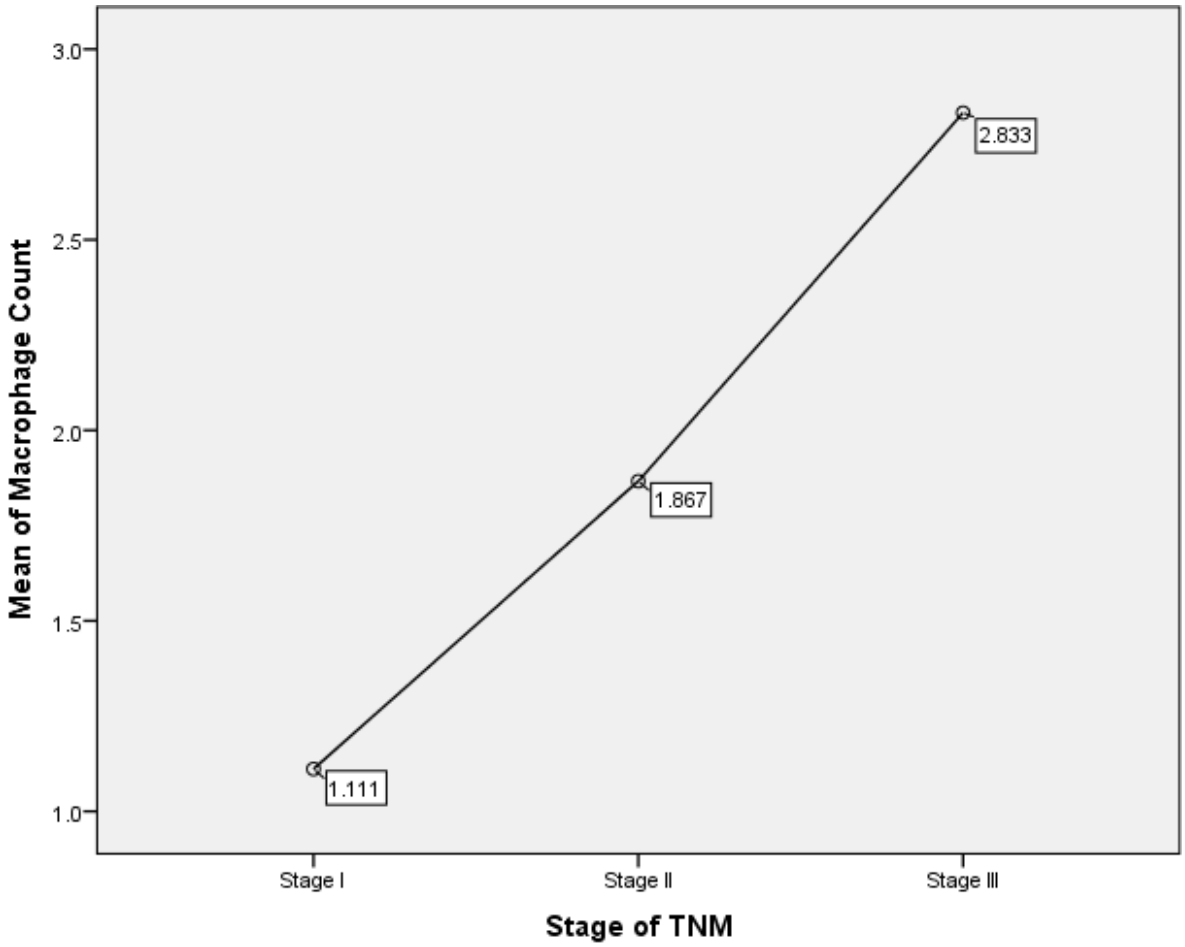
		N	Mean	Std. Deviation	P-value
Macrophage Count	Smoking	8	1.50	.756	0.119
	Chewing	15	1.73	1.033	
	Both	7	2.43	.535	
	Total	**30**	**1.83**	**.913**	

Table 3. Comparison of Stage of TNM with TAM

	N	Mean	Std. Deviation	p-value
Stage I	9	1.11	.782	0.0001
Stage II	15	1.87	.743	
Stage III	6	2.83	.408	
Total	**30**	**1.83**	**.913**	

Table 4. Comparison of expression of TAM between normal group & OSCC group (positive & negative cases)

			mac_rec1		Total	X^2	P-value
			1.00	2.00			
Type of tumor	Normal	Count	10	0	10	9.855	0.002
		% within Type of tumor	100.0%	0.0%	100.0%		
	OSCC	Count	13	17	30		
		% within Type of tumor	43.3%	56.7%	100.0%		
Total		Count	23	17	40		
% within Type of tumor		57.5%	42.5%	100.0%			



Graph 1. Shows Comparision of Stage of TNM with Tumor-Associated Macrophages

Table 5.comparison of expression of TAM between positive & negative cases of OSCC group

	mac_rec1	N	Mean	Std. Deviation	Std. Error Mean	P-value
Macrophage Count	1.00	13	.92	.277	.077	
	2.00	17	2.53	.514	.125	0.0001

Table 6. Tumor-Associated Macrophages with Metastasis using t-test

	Metastasis	N	Mean	Std. Deviation	t	P-value
Macrophage Count	No Metastasis	24	1.58	.830	5.261	0.0001
	Metastasis	6	2.83	.408		

Table 7. Significant relation between TAM in different grades of OSCC group

		N	Mean	Std. Deviation	P-value
Macrophage Count	Normal	10	.50	.527	0.0001
	Well	10	1.40	.843	
	Moderate	10	1.80	.919	
	Poor	10	2.30	.823	
	Total	40	1.50	1.013	
	Total	40	1.63	1.125	

Observation: Table 2 represents the comparison between TAM count with a habit of OSCCcases which is not significant. Table 3 represents the Comparision of the Stage of TNM with TAM is statistically significant. ($p < 0.05$). Table 4 represents a comparison of expression of TAM between normal group & OSCC group positive & negative cases which is statistically not significant ($p > 0.002$). Table 5 represents the comparison of expression of TAM between positive & negative cases of OSCC group shows statistically significant. Table 6TAM with Metastasis using t-test shows statistically significant. Table 7 Significant relation between TAM in different grades of OSCC group shows statistically significant. Graphrepresents Comparision of Stage of TNM with TAM is statistically significant with mean values (stage I- 1.111, stage II- 1.867, stage III-2.833) ($p < 0.05$).

Discussion

Squamous cell carcinoma (SCC) is considered a major problem worldwide due to its extremely high prevalence (90%) among oral cancers[2,6]. The high rate of relapse in this tumor indicates the inadequacy of current prognostic predictors, that is, histological and clinical assessments, in predicting metastatic potential[2]. So there is a need to investigate additional determinants. So in this study, we have investigated macrophages infiltration in different grades of OSCC cases. So in our study, we evaluated the expression of macrophages in different grades of OSCC cases. There are many method to evaluate MMP 9 like Gelatin Zymography, Immunofluorescence (IFC), Gel Electrophoresis, PCR, IHC, Western Blot Assay, ELISA.

There are various method used to evaluate macrophages are immunofluorescence (IFC), Immunohistochemistry, flow cytometry, qRT-PCR. & routine H & E stain[11,12] In our study we have used H & E (Hematoxylin & Eosin) stain to evaluate TAM as cancer-associated inflammation includes the so-called tumor-associated macrophages (TAMs) which are generally thought to resemble M2-type macrophages and we can see the presence of macrophage with our routine H & E stain.[12,13]

For Macrophages: scoring criteria < 10% is scored as 0, 10-30 % is scored as 1-2, 30-50 % scored as 3-4, > 50 % is scored as 5-6.

OSCC is the most common malignant neoplasm. It may occur at any intraoral site & generally involve tobacco users in the middle age group. Higher incidence of OSCC seen frequently in males[15]. In our study we found 80 % of OSCC cases below 60 yrs of age, 60 %were males & 40 % were females; majority cases (53.33%) had a lesion in the buccal mucosa. According to Taiming Dai et al[15,17], there was a significant difference in comparison of MMP-9 expression between men and women, suggesting there might be a higher potential for OSCC invasion in male patients.

In our study, we have compared smoking & non-smoking form of tobacco with the infiltration of TAM. We found that TAM infiltration is not significantly associated with different forms of tobacco habit pattern [13]. In our study mean score of both TAM expression increases from stage 1- stage III, and it was statistically significant ($p<0.05$) which suggest that MMP 9 expression is directly proportional to clinical stages of tumor & this finding is in concordance with a study done by Taiming Dai et al [15], where as in other studies like Aparnat et al [9], Elahi et al[68] they have not found any correlation between TAM & MMP 9 expression with different stages of TNM. There was a statistically significant infiltration of TAM in OSCC cases as compared to the normal groups. Total no. of OSCC cases were divided into positive & negative groups according to the expression of TAM. We have found MMP 9 expression 23 positive cases & 7 negative cases & TAM expression 17 positive cases & 13 negative cases. The difference between TAM expression in positive & negative cases was found statistically significant.[8] We also found that there was a statistically significant correlation between the infiltration of TAM in OSCC cases[15,17]. We also found that there was an increase expression of TAM with different histological grades of oral squamous cell carcinoma: TAM in Well-differentiated 1.40+_.843, mod differentiated 1.80+-.919,to poorly differentiated 2.30+-.823 (lower to higher mean & s.d value) . Thus their expression is increasing from well to poorly differentiated squamous cell carcinoma. But the difference between the expression of TAM in different grades of OSCC was not statistically significant[15] found that positive correlation between the macrophage count. They also suggested that macrophage in OSCC, which jointly promote the invasion and metastasis of the tumor.

The positive expression rate was much higher in OSCC in comparison to normal mucosa and it had a close correlation with TNM staging of the tumor and condition of local metastasis TAM over-expressed in many other malignant tumors like breast, prostate, liver,

lung & bladder cancers their expression was correlated to poor prognosis[9,10,11,12]. We have compared TAM expression in OSCC cases with local metastasis which is found statistically significant ($p < 0.05$) which in concordance with the other studies done by Daiming Tai et al [16]. Thus it is proved that invasion & metastasis of tumors are regulated by quantities of factors in a cellular mixture of OSCC[4]. It may also be helpful to evaluate local metastasis in OSCC cases. Since metastases are the principal cause of death in cancer patients, a greater understanding of the process of tumor invasion and metastasis is essential in leading to the identification of new therapeutic targets.

In this study, we have used H & E stain instead of IHC for macrophages identification due to lack of funds, but the expression of macrophages can be better evaluated by using specific IHC markers like CD 68, CD 13. In this study, although special care has been taken to avoid false-positive results,but IHC is very technique sensitive. Thus further studies should be done with a larger sample size along with the use of other specific method to confirm the expression of both TAM in OSCC cases & their expression should be correlated with the IHC technique.[17]

Conclusion

Concluding this review of tumor-associated macrophages in comparison with OSCC cases. Since metastases are the principal cause of death in cancer patients, a greater understanding of the process of tumor invasion and metastasis is essential in leading to the identification of new therapeutic targets. In this study, we have used the H & E stain instead of IHC for macrophages identification due to a lack of funds. In this study, although special care has been taken to avoid false-positive results,but IHC is very technique sensitive. Thus further studies should be done with a larger sample size along with the use of other specific method to confirm the expression of both TAM in OSCC cases & their expression should be correlated with the IHC technique.

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Newer Behaviour Management Techniques in Children

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Abstract

Children are not miniature adults. They should be treated as a different entity and not just as adults who are shortened. Children have their apprehensions and fear. The most commonly encountered is the fear of the unknown. This is something that is innate, inborn. Fear of dentistry is there in every individual, whether young or old. This is mostly subjective. Children show exaggerated fear for dentistry as this is something new to them. The pediatric dentists are trained in behavior management for reducing fear. There have been many method, both pharmacological and non-pharmacological for behavior management in children. But with changing times, we know that Child's behavior has also undergone a sea change. Here we discuss some newer behavior management techniques, recently introduced and included in our guidelines.

Keywords: *Behaviour management, Children, Newer, Pediatric dentistry.*

Introduction

Pediatric dentists are expected to identify and effectively treat dental diseases of children that are within the knowledge and scope acquired during their professional studies. Effective and efficient treatment has to be provided to the child and for that, the parents also have to understand their roles.[1] Behaviour guidance should be done and this means that inappropriate behavior has to be converted to appropriate behavior within the societal norms with the help of empathy and learning. The main idea behind behavior management by pediatric dentist is to gain the confidence of the parents and the child. Its always better to know the scientific basis of implementation of any behavior management technique and also to look into cultural sensitivities, tolerance level, flexibility in a child and parent before applying any of these method.[2] Children are not miniature adults. They should be treated as a different entity and not just as adults who are shortened. Children have their apprehensions and fear. Behaviour management should never be used as a punishment or assertiveness for a child.[3] All the method which are to be used to tame the child should be age and gender appropriate. The pediatric dentists have to realize the potential of the child and then try to implement any behavior management technique for that particular child. In short, it has to be customized, following the child in the present scenario.[4] We know that the parenting method have changed a lot in recent times. The old pattern of parenting is not to be seen nowadays, with nuclear families or single parents. Behavior management also has seen some changes.

The Newer Method: Before applying any type of behavior management technique on the child for making them cooperate, we have to assess the child as well as assess the parental influence on the child.

Basic behavior guidance method:

(a) Communication and communicative guidance: The appropriate use of communication and commands have been used since a long time for both cooperative and uncooperative children. Always try to make the patient comfortable with verbal and non-verbal communication skills. Encourage the child to talk with you what he wants to, to reassure them that they are in good hands. We try to develop a student-teacher relation with the patient. The pediatric dentist role is as a facilitator. Once a rapport has been established, the talks can become minimal and related to procedure only.[5]

(b) Positive pre-visit imagery[6]: The child patients are shown positive pictures or images of dentistry and

dental treatment before the procedure starts. It can also be shown at their homes before even visiting the clinics. The objective of this is to provide the children and parents with visual information of what to expect from the pediatric dentists. It also provides the children with a chance to ask questions to the dentist about the procedure if they come to know about it before visiting the dental clinic.

(c) Ask-tell-ask[7]: This technique involves enquiring about the child's visit and feelings toward or any planned procedures (ask); explaining the procedures through appropriate demos and non-threatening language, appropriate to the understanding level of the patient (tell); and again enquiring if the child understood and how he feels about the impending treatment (ask). If the child still has apprehensions about the procedure, the dentist can again explain the procedure.

(d) Memory restructuring[8]: Memory restructuring is a behavioral management approach in which memories associated with an unpleasant or difficult event (e.g., first visit, local anesthesia, restorative procedure, extraction) are restructured into positive memories using information suggested after the event has taken place. Restructuring involves four parts: (1) visual reminders; (2) positive reinforcement through verbalization; (3) concrete examples to encode sensory details; and (4) sense of accomplishment. A visual reminder can be a photograph taken in the dental office. Positive reinforcement through verbalization can be to ask the child to tell his/her parents about what good behavior she showed at the dental clinic. Concrete examples could be to show how she followed the commands given by the dentist at the operatory and in doing so she can open her/his mouth wide open as he/she did in the dental chair, which will lead them to be set an example of self-accomplishment. The objectives here are to fade out the negative memories of difficult dental visits and only remember the good times.

(e) Descriptive praise[9]: This is a type of positive reinforcement. Here the pediatric dentist praises the child for a particular behavior. (eg-thanks for sitting still, you are doing a great job by opening your mouth so big.) Rather than general praise as 'good job or well done.'

The objective is to reinforce good behavior:

(e) Teach back[10]: In this method, the pediatric dentists or their attendants ask the patient to teach them what they have learned when he/she was given a behavior management technique demonstration. This is done in less literate people or those who can't remember much. The dentist asks them to demonstrate what they have seen, if the patient cant they repeat it for the. Eg-can you demonstrate to me how to floss your teeth.

(f) Motivational interviewing[11]: Motivational interviewing tries to change the behavior of the patient by helping them to change. This method does not reinforce change but utilizes the autonomy of the patient so that their self-confidence is increased. The motivational interviewing puts the onus on the patient to change. The method included are OARS(Open-ended questions, affirmations, reflective listening, summarizing). This is useful in patients who are showing more anger, resistant to changing behavior.

(g) Treatment Deferral: Dental problems are rarely life-threatening and sometimes in unprecedented conditions like the present COVID-19 situations, these also can be deferred. The emergency treatment can be rendered to alleviate the pain but maximum restraint is required to defer the treatment to a more suitable time frame. Sometimes treatment has to be deferred when suddenly a cooperative child becomes uncooperative and hysterical when getting treatment done. Caries risk assessment has to be done in each patient before the treatment can be deferred. The oral hygiene measures have to be told to the patient and the parents. An individualized preventive program, including appropriate parent education and a dental recall schedule, should be recommended after evaluation of the patient's caries risk, oral health needs, and abilities.[12]

Advanced Behavior Guidance: Sedation: As a pediatric dentist we must decide what is acceptable behavior from our young patients. The decision will depend on our personality, experience, and philosophy. Having defined the acceptable behavior, for ourselves, we can then plan to modify the unacceptable behavior to an acceptable one. The judicious plan of conscious sedation has a place in pediatric dentistry but it is most often abused without trying the nonpharmacological means. Concious sedation is often to be used for longer

procedures for apprehensive and nervous patients. This can also be used for uncooperative and abusive children but it does not teach the children to behave properly when next they are to be taken for dental treatment. The pediatric dentist must establish the frequency of use of these sedation drugs so that he does not have to use the same drugs over and over again. Sedation is a very safe and efficient modality to decrease anxiety and increase effective communication. The onset of action is fast, the effects easily can be titrated and reversible, and recovery is rapid and complete. Also, the nitrous oxide/oxygen inhalation mediates a variable degree of analgesia, amnesia, and gag reflex reduction. The need to diagnose and treat, as well as the safety of the patient and practitioner, should be considered before the use of any kind of sedation.[13]

2. **Protective stabilization:** There are times when the child has to be restrained and this is a matter of deep concern for parents, staff, and the treating dentist as it becomes inhumane. The broad definition of restraining means to limit the movement of the patient without his consent. The restraining can be done with the help of devices, another person, or a combination of both. This method can harm the patient physically and psychologically. The dentist should use the safest and least invasive method available for stabilization. The decision to use protective stabilization depends on on-patients emotional development, alternative behavior management guidelines, dental needs of the child, whether the quality of treatment is being hampered, medical condition of the patient. This method needs an informed consent to be taken from parents. If still the child cannot be managed, the child has to treated under sedation or general anesthesia. The proper documentation of the stabilization has to be done always-why was the method used, the condition of the child, the type of restraining device used, consent taken or not.[14]

Conclusion

The children have to be dealt with in a proper way when it comes to behavior management. All the method have to individualized according to the patient demands. The basic method have to be applied first and gradually going for advanced method.

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Dental Health Care Needs for Elderly Population

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Abstract

The mouth is considered as the mirror of general health of every individual and concerning this in mind, poor dental health can be a menace aspect for general health trouble in the elderly population. Due to increase in their chronic diseases in elderly patients, they are more vulnerable to oral diseases. Thus, the geriatric population forms a distinctive group in provisions of implementation of health care. This review article discuss about the significance of dental health care needs in geriatric population.

Keywords: *Dental health, Geriatric population, Preventive measures.*

Introduction

Dentistry focusing on geriatric population deals with the service of dental care to old age people which involves diagnosis, prevention as well as management as well as dealing of all dental related problems related with age related diseases. In certain decades, there is an unexpected increase of the 65 plus residents globally where India is also not left behind from this blast growth. Due to advance technology and progressing development in the medical ground, it has raised the lifespan of the senior citizens. This rapid altering in the longevity of life has challenged the dental health professionals to provide and make advanced treatment for the geriatric population.[1] The mouth is considered as the mirror of general health of every individual and concerning this in mind, poor dental health can be a menace aspect for general health trouble in the elderly population. Due to increase in their chronic diseases in elderly patients, they are more vulnerable to oral diseases. Thus, the geriatric population forms a distinctive group in provisions of implementation of health care. This review article discuss about the significance of dental health care needs in geriatric population.

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Demographic profile of geriatric population: According to reports and based on the advanced therapeutic modalities in treatment part of medical research, the life expectation has improved to 67.9 years and is setting a distressing increase in the health conditions of geriatric population globally.[2] In developing countries, reports suggested that by the end of the year 2020, the proposed inhabitants of all individual people aging 65 years as well as above will be approximately 470 million globally. And by the year 2050 where one third of the world inhabitants will be over 60 years. If we look into the data of our country India, then the geriatric population was 20 million in the year 1951 and 57 million in 1991 which was followed by very rapid increase in the year 2001. According to the observed growth rate of population by age during this period, the growth of 65 years and above people groups was much added extra than that of the existing total population. Therefore, it is likely to rise with a proposed population of 324 million by the year 2050.[3]

There is a total number of 2 lakhs and above centenarians in India and among them 5.2% of the elderly population which consists of 28 lakhs or more male and 31 lakhs female. The two most important prominent characteristics concerning the old age of India are as

follows: a) the growth of geriatric people is very much quicker than the development of the overall population and b) the female dominancy in the gender wise increase in the total population.[4]

Health conditions of Geriatric Population: During the old age, the intake of multiple medications can be seen by the elderly people. Because of this multiple medications consumptions due to diseases results in decreased salivary flow and this will lead to compromised conditions of the oral mucosa. Normal flow of saliva in any human being has an important role to play as it helps in neutralizing the manufacture of several acids done by micro organisms which in addition helps in teeth mineralisation. Saliva gives several defending reimbursement for the oral cavity because of its content of immunoglobulin A as well as lactoferrin. If there is a lack of salivary reduction then there will be increase in number of oral microorganisms as well as their metabolic by products in the oral cavity.[5]All these things will ultimately leads to an augment in dental diseases like caries and periodontal diseases leading to tooth loss. Maintenance of oral hygiene can be hampered due to alteration in physical as well as mental status that will result in worsening of co-ordination and motor skills. Improper maintenance of oral hygiene will contribute to prolific growth of oral microflora. Total amount of food intake sometimes reduces in nearly everyone of the elderly because of the alteration in diet as well as altered taste. Therefore it is very difficult to meet the nutritious need of the elderly people. With rise in requirement for certain vital nutrients and higher catabolic rates, it is difficult to store pace on with the overall age alterations in the elderly body.[6] Therefore, there is a need of keeping a record of the whole dietary status as well as the manifestation of signs along with symptoms of the elderly people in regular intervals.

Residual Ridge Resorption: Residual ridge resorption (RRR) is usually meant for the alteration that will stress the alveolar ridge followed by any kind of tooth extractions that will carry on long subsequent to healing of the extraction socket. The majority important characteristic is the remaining bony architecture of the maxilla-mandible jaws which undergoes a long catabolic remodelling mechanism during this healing process.[7] The elderly people goes through decrease in residual ridge resorption frequently. The missing teeth are need to be reimplanted or replaced by prosthesis. Sometimes long chronic health issues and economic measures also makes a considerable difference in the selection of action as the substance costs are in general prohibitively high. In the elderly patients bone resorption activity keep on continuing throughout life which ultimately results in failure of altering amount of jaw structure, that make the patient to condition called dental cripple.[8]

Prosthetic rehabilitation in the elderly: In the elderly people many of them undergoes through the condition called tooth loss due to extraction of teeth or may be due to some other reasons. Eventually this all will lead to edentulous or partial edentulous jaw. There will be poor retention as well as steadiness of complete dentures which is the most common dental health linked complaints in edentulous patients. Poor retention generally leads to loss of bony alveolar in the jaw structure.[9] There may be multiple reasons for resorption and the reasons may differ among everyperson. This happens after loss of teeth and the process of resorption process at analtering speed for the rest of the life. Sometimes systemic conditions as well as local conditions of the patient affects the resorption rate of the alveolar bone.[10]

Many times the missing teeth in the elderly people doesn't always mean that it needs to be replaced with dental prosthesis in edentulous person till they are satisfy socially as well as functionally.[11] That is why they hold-up in covering dental treatments and this decision promote complicates for their chances for absolute rehabilitations.

Due to ever growing number of elderly patient cases who are in need of oral physiotherapy necessitates new treatment strategies. Sometimes financial measures also play a major role in the preference of treatment because the materials to be used for prosthesis and other dental treatments are usually prohibitively very high.[12,13]

Dental sanitationin addition todental mucosal lesions: Due to missing teeth in the elderly person and also difficulty in chewing and other physiologic functional conditions, they prefer dentures for their jaws. Prolonged use of dentures leads to various oral mucosal lesions like denture stomatitis, flabby ridge as well as irritation hyperplasia, angular chelitis and traumatic ulcers as well as even cancer can also be linked with long duration utilization of unhealthy or grossly damaged out not fixed dentures. Many oral mucosal lesions are found out to be provocative as characteristics feature in may studies.[14]

Candida albicans is one of the widespread microorganisms that is related to denture tiring. Patients wearing dentures at night as well as smoking as a habit has been correlated in various studies.[15] There has been a significant effect of denture cleanliness, age of the patient and drugs utilization as linked with wearing of denture that had been reported in studies. Moreover sometimes due to less salivary flow rate in elderly patients, there may be predisposition of oral mucosa to the alteration of pathology for the reason that of its related linkage with the presence of yeasts within the oral cavity.[16]

Ill fitting dentures induce trauma to the oral cavity which ultimately believed as the major cause for denture sore mouth as well as hyperplasia of tissue. Sometimes new dentures also develop ulcers within a small number of days after fitting of denture. Therefore, patients should be advised to follow up regularly every four to six weeks for new dentures in addition to every six months after that. Immediate and proper intervention should be made to avoid trauma due to age changes of the mucosal changes inside oral cavity.[17]

Protocols for Oral health care for old age people: Everybody has to deal with ageing which is to be anticipated, irreversible and also a reality. As people starts getting to their old age, the objectives of their oral health planning need to be refocused with regard to the common health as well as financial conditions of the aged people. That is why it is imperative that people could necessitate to do with to have right of entry to oral health care that is generally based on concepts of preventive methodalong with which can be actively involved in making actions about oral health from their early 50's or 60's. This timely oral health planning will help them maintain into their old age dental needs.[18]

There has to be sustainable oral health care need in any dental treatment scenario specially in case of elderly people. Oral health intervention should always be based on evidence based practice containing existing information on the occurrence, divisionas well as determinants of oral diseases based on its cost effectiveness of any type of intervention. Therefore, there should be oral health surveys which can supply latest as well as genuine information about all the aspects of dental health status, disease as well as their determinants. Also, there will be gradual decrease in immunity which will stimulate in increasing the menace of transmittable disease. Infections like pneumococcalor influenza or tetanus as well as zoster are very common among the elderly population.[19]

Mechanism of Dental health services: Expansion in the medicinal field have encouraged the disableolder generation to exist longer. According to the reports, there has been a rapid increase in the rate of longevity in the old age population, therefore, there has been a speedy demand in the requirement of residential dental care services. Old age people find difficulties in approaching the dentist and also it is cumbersome to bring them to the dentist.[20] Therefore, the best decision could be if any dentist could reach the elderly people. There should be some provisions and services to be made out to reach them who are in need of dental care. While planning out the comprehensive dental treatment plan for the elderly patient, care has to be taken out for their medical condition.

According to the researchers, Macentee and Waytt, developed an index known as Clinical Oral disorders in elders (CODE) for disabled geriatric people. CODE guide is based ahead of clinical measures of 27 disorders that covers 5 major areas: Jaw movement, dentures, mucosal health, teeth and periodontium. But all these things are united with a psychosocial index which forms a very brief indicator of oral dysfunction in the elderly.[21]

Kenneth Shay, a researcher has provided a five point Geriatric Dental Assessment tool called OSCAR:[22]

O- Oral – Teeth, restoration, prostheses, periodontium, pulpal status, oral mucosa, saliva

S- Systemic – Normative age changes, medical diagnosis, pharmacologic agents

C-Capability – Functional ability, self care, oral hygiene, transportation to appointments

A-Autonomy – decision-making ability, dependence on alternative

R-Reality – Prioritization of oral health, financial ability or limitations.

Dental health researchers should try to deliberate on OSCAR assessment tool as it influence the treatment plan that requests the meticulous integration of dental as well as non dental measures.

Challenges regarding dental health care services to geriatric population: With improved and rising

long life span in elderly patient, the dental disease pattern as well as treatment requirements are varying according to the advanced dental technologies, the old age persons are retaining more natural teeth into old age than before. Therefore, there is an increase in the need of numerous dental treatments including restorations, prosthesis as well as esthetics as well as even implants all of which require special care. The challenges arise in elderly people when their unwillingness towards treatment as well as suboptimal perception of treatment requirement, and the limitations on operational time could be harassing to health care professionals. Many have restricted mouth opening or they will be unable to keep it open for long time. Therefore, there is a need of modifying the approach towards these patients.[23]

Conclusion

Oral health is important in every phases of life assume superior value at extreme old age also. The negative impact on the oral well being of the elderly persons affects the general health also. Therefore, there is a need to take special care for the dental treatment needs of the geriatric people.

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Management of Oral Health During Pregnancy

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Abstract

Good oral health is an important aspect towards overall health for all the women throughout their lifespan. For every expecting women and also women those who are of reproductive age, for them health status of their oral cavity is very much an important module of the general health. During pregnancy there is a rapid physiologic change that leads to occurrence of remarkable strain on a woman's body together with the oral cavity. Both intended for mother and children, maintaining and achieving good oral health is very important. There are evidences that poor wellbeing of the mother such as dental decay, periodontal diseases previous to and during pregnancy have been linked to poor birth for example preterm birth and low birth weight. Oral health is a significant part of overall health. During pregnancy, an expectant women goes through lot of hormonal changes and these hormones sometimes increase the risk of gingival diseases that will hamper the health of any would me mother and might increase the menace of premature delivery or low birth weight infant. It is very much necessary that oral health care need should be recognized for every pregnant women and it is also the sole responsibility of every pregnant women that they should feel responsible care of their oral health as an important aspect of their pregnancy course. This review article highlights the importance of health of oral cavity for all expectant pregnant women.

Keywords: *Pregnancy, Oral Health, Precautions.*

Introduction

For every expecting women and also women those who are of reproductive age, for them health status of their oral cavity is very much an important module of the general health. During pregnancy there is a rapid physiologic change that leads to occurrence of remarkable strain on a woman's body that includes whole of the oral cavity. Both for mother and children, maintaining and achieving good oral health is very important. There are evidences that poor wellbeing of the mother such as dental decay, periodontal diseases previous to and during pregnancy have been linked to poor birth for example preterm birth and low birth weight. With respect to all these, according to National Maternal and Child Oral Health Resource Centre (NMCOHRC, 2008), enforcement of healthy oral habits for women during pregnancy time plays a very important role in promoting the oral health for their children following birth. Therefore in general, healthy oral cavity is an important aspect towards overall health for all the women throughout their lifetime.[1] This review article highlights the importance of oral health for pregnant women.

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Periodontal diseases and preterm/low birth weight babies: According to evidences it has been reported that periodontal disease is a type of infection caused by bacteria which can be noticeable in up to 40% of women who are pregnant which can lead to devastation of the gums, bones as well as ligaments opinionated by teeth. Many researchers have correlated periodontal

disease with premature delivery where there are chances of delivery earlier than 37 weeks of gestation as well as low birth weight where the new born baby may have weight less than 5.5 pounds.[2] Poor health outcomes will definitely result in infant mortality as well as long term health complications amongst infants.[3]

Protocols for health sector workers regarding health care of oral cavity during pregnancy: The health care professionals should take part in a key role in making the women for healthy pregnancies by providing oral health education. They should also link the pregnant women to dental check up during their pregnancy period. Surrounded by the health care system, multiple health professionals from all the required fields should reach pregnant women to provide proper health care.[4]

Protocols to be followed by the pregnant women.[5,6]

1. Brushing your teeth two times a day by using tooth brush and fluoride toothpaste and dental floss daily.
2. Foods that contain sugars should be limited to mealtimes only.
3. Carbonated beverages should be avoided during pregnancy and water or low fat milk should be chosen.
4. Natural fruits should be preferred instead of fruit juice to fulfil the suggested dose of fruit intake regularly.
5. Required dental management should be obtained previous to delivery during the second trimester of the pregnancy and the dentist should always be in contact during this period for any kind of emergency consultant.
6. Small amounts of nutritious foods should be taken throughout the day.
7. Maintain oral hygiene during pregnancy especially by brushing two times a day with fluoridated toothpaste.

Oral disease management for pregnant women[7,8]

Dental health professionals should develop a comprehensive treatment plan such as:

(a) A proper plan should be developed for all the dental needs and maintenance of optimal oral health as well as disease prevention strategies to be based on benefits, risks and all alternatives.

(b) Before delivery timetable should be plan out to carry all the procedures.

(c) Emergency care and treatment to be provided strictly during the second trimester of the pregnancy if required.

(d) Dentist should give proper instructions regarding intake of medication for dental pain during pregnancy.

(e) Oral health education should be provided by the dentists to reduce the bacterial load during pregnancy.

Drug administration during pregnancy[9]: All dental professionals should mandatorily do their duty to fully inform about the safety of drugs administration during pregnancy to all the expected women. According to FDA, it has issued specific guidelines to endow with therapeutic guidance for using drugs during pregnancy. Major medications are usually contraindicated during pregnancy because of its adverse effects both for the mother and the child. Paracetamol is generally considered as the safest drug during pregnancy. Others drugs especially analgesics and antibiotics that are most commonly prescribed for dental pain need to be avoided during pregnancy.[10,11] That is why, it is very important for the pregnant women to take good care of their teeth and gums during pregnancy.[1] Because there will be lot of hormonal changes during pregnancy which will increase the risk of developing gingival diseases that will ultimately affect the health of developing baby.

Tips to maintain good oral health care during pregnancy

Before Pregnancy: Dental appointment should be made prior to before getting pregnant so that the existing dental treatment can be done in advance of getting pregnant. Along with all the dental treatment, regular maintenance of oral cavity like brushing two times daily with toothpaste plus rinsing mouth after every meal should be done.[12]

After Pregnancy:

(a) Dentist should be aware of pregnancy so that routine dental care should be carried out. Any urgent procedure can be done in the second trimester as well as all elective dental procedures should be postponed after the delivery.

(b) All the history of medications should be mentioned

to the dentist as well as if any specific medical advice has been instructed by the gynaecologists then the dentists should be aware of this.[13]

(c) Dental x rays should be done if required during pregnancy but safety precautions measure has to be taken properly by the dentist. Now a day advance technology in x-ray has been considered as much safer than in past decades.[14]

(d) Dental check up should not be skipped simply because of pregnancy. During pregnancy lot of hormonal changes takes place which can increase the risk of dental diseases that is why regular dental check up should be done.[15]

(e) Good oral hygiene practice should be followed in order to prevent or reduce oral health problem.

(f) If any pregnant women is coping with morning sickness and if it is keeping away from brushing teeth then the toothpaste should be change to a bland tasting. Dentist should be recommending the required toothpaste brands for pregnant women.[16]

(g) Mouthwash should be used for rinsing if any pregnant women suffers from morning sickness and have frequent history of vomiting.

Effect of oral health on babies: During pregnancy, women is already on the verge of being a mother. So she has to take care of her and should focus on assembling everything perfect for the baby. A mother will always care for her and her unborn child and not only general health but also oral health. Routine visit to the dentist will enable to measure the current oral health status as well as rule out a preparation for the dental treatment needs after the delivery. When pregnant women care her own mouth, it will definitely make a disparity for her baby, equally before plus after birth.[17]

Facts:

1. If routine night time brushing and dental flossing gets skipped then it might direct to plaque as well as bacteria build up and ultimately tooth decay.[18]
2. Lots of hormonal changes can cause danger to the health of would be mother's gingival health plusit might cause pregnancy gingivitis. Bleeding gums due to inflamed gingiva as well as untreated gingivitis will lead to periodontitis. Several research reported that there is a definite link between preterm delivery, low birth weight babies and gingivitis.
3. Pregnant women who are having history of frequent morning sickness should be aware of stomach acid that create its way hooked on to the mouth and can deterioratethe strength of tooth enamel. This will put the pregnant women at a greater risk for dental diseases.
4. All pregnant women needs vitamin which contains folic acid during pregnancy.
5. Eating the right food with proper nutrition will keeps the mouth healthy as well as strong.
6. A nutritious meal containing calcium as well as limited amount of sugars in the food are best for both mother's and baby's oral health.

Mother's oral health is associated to the health of her unborn baby –as bacteria can be traced in her mouth.[19,20] If the amount of bacteria will be more in any pregnant women, then it has chances that it will cross the threshold the bloodstream through her gingival tissues and travel into the uterus by triggering the production of chemicals called prostaglandins – that are supposed to induce premature labour. After the delivery of the baby, there are also few chances of potential vertical transmission of bacteria from the mother to her newborn. A pregnant women should always keep it in mind that she has to take care for her own oral health as well as her unborn baby's health.[21,22]

Anaesthesia: Local anaesthesia with epinephrine can be used during pregnancy but only during second trimester if indicated for extraction or any surgical procedures in dentistry. The drug is absorbed slowly in case of lidocaine with epinephrine which will prolongs the length of anaesthesia. [23-26]

According to National Consensus Statement, few guidelines have been issued for pregnant women[27,28]

(a) Guidelines given should be customized based on risk assessment.

(b) Get routine dental care.

(c) Good home care should be practiced.

(d) Healthy and nutritious food should be eaten.

(e) Fluoride composed water should be consumed.

(f) 600 micrograms of folic acid should be consumed.

(g) Prenatal classes should be participated.

(h) Tobacco related habits should be stopped.

(i) Second hand smoke should be avoided.

(j) Consumption of alcoholic beverages should be stopped.

Conclusion

Oral health is an important part of general health. During pregnancy, women goes through lot of hormonal changes and these hormones sometimes increase the risk of gingival diseases that will hamper the health of expectant women and might increase the threat of premature delivery or low birth weight infant. It is very much important that the doctor recognizes oral health care need for every pregnant women and it is also the sole responsibility of every pregnant women that they should take proper care of their oral cavity as an important aspect of their pregnancy course.

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E-Cigarettes in India: An Overview

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Abstract

Knowing its harmful effects, e-cigarettes are still famous among all the tobacco consumers. E-Cigarettes however wanted to capture the market in India also since 2016 but has failed to make its mark because of the prompt action by the government as compared to the scenario in the western countries. This article will provide the knowledge and information regarding E-Cigarettes legalization and utilization in India.

Keywords: *E-Cigarettes, Harmful effects, Government actions in India.*

Introduction

The undesirable special effects of cigarette smoking on the physical condition was first acknowledged by the Advisory Committee to the U.S. Surgeon General in 1964. The electronic cigarette (e-cigarette) is a type of new-fangled class of electronic nicotine delivery systems (ENDS) that was introduced in 2004 globally.[1] Gradually E-Cigarettes became the most trending and used product among tobacco users all over the world covering many countries worldwide. It is more common in youth and tobacco product users. Knowing its harmful effects, e-cigarettes are still famous among all the tobacco consumers. E-Cigarettes however wanted to capture the market in India also since 2016 but has failed to make its mark because of the prompt action by the government as compared to the scenario in the western countries. This article will provide the knowledge and information regarding E-Cigarettes legalization and utilization in India.[2]

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Electronic Nicotine Delivery Systems (ENDS)have a special form of cigarettes called E-Cigarettes which is the most common form.[3] These cigarettes are chiefly a kind of device that is used by not burning any tobacco leaves rather thanit is vaporized with a mixture by utilizing a battery. And then this mist is being breathed by the cigarette.[4]

"The main constituents of the solution, in addition to nicotine when nicotine is present, are propylene glycol, with or without glycerol and flavoring agents," as reported by the World Health Organisation (WHO) with the aim of these constituents inside the E- cigar contains many toxic substances.[5] Most of the E-cigarettes come in various shapes and sizes but it will resemble as ordinary cigarettes, cigars as well as smoking pipes but now within late brands, designer E-cigarette is in the market that resembles like whistles or pens.[6]

Working mechanism of an E-Cigarette: E-Cigarettes depends on many factors such as the characteristics of the unit circuit, liquid mixtures used, and the user's behavior as well as the battery's strength for better efficiency. The effectiveness of e-cigarettes is directly proportional to the manufactured goods capability to heat the solution to a state so that it can transform into vapors. And the end product is the crucial components like circuit strength and the battery's voltage. The solution will be faster in heating up vaporize if the voltage and circuit are stronger.[7]

"Battery voltage and unit circuitry differences can result in considerable variability in the products' ability to heat the solution to an aerosol and, consequently, may affect the delivery of nicotine and other constituents, and may contribute to the formation of toxicants in the emissions," as reported by WHO in the year 2014 which describes that factors such as "length of puffs, depth of inhalation and frequency of use" might have an effect on nicotinebeing utilized by consuming e-cigarettes.[8]

E-Cigarette aerosol composition: The aerosol produced by the e-cigarette aerosol and the users keep breathing those aerosols from the piece of equipment and then breathe out which contains many injurious as well as potentially detrimental substances, plus substance like Nicotine, very ultrafine particles which can be breath in deep into lungs,it also contains volatile organic compounds as well as cancer-causing chemicals along with few heavy metals such as tin, nickel, and lead. Sometimes it is difficult for the consumers to know the correct composition of E-Cigarettes as they are often commercially marketed as zero percent nicotine but are bring into being to have nicotine in it.[9]

Harmful Effects of E-Cigarettes: WHO reported that it can have unsympathetic effects during pregnancy and may contribute to cardiovascular disease.[10] Nicotine itself is not a carcinogen but it may function as a tumor promoter. Nicotine seems concerned with fundamental aspects of the biology of malignant diseases, as well as of neurodegeneration.[11]

Facts: Mostly E-Cigarettes are frequently used by the youths among the tobacco products. More than the adults, youths are being affected in the countries. According to reports,58.8 % of adults are the users of the e-cigarette were mostly of aged 45 years and older. Many of them were current or former cigarette smokers.[12]

Indian Market Scenario: The estimated value of E-cigarette was very high in the industrial market. According to global market reports, there are as many as more than 466 brands for e-cigarettes production but the Indian market has fewer numbers out of that.[13] According to the market research organization, they have estimated that by the year 2030 the global e-cigarette would rise high more than four times the current production as well as consumption. Therefore, based on that report, the Indian market scenario was also expected to take a rise in its consumption but because of the government abrupt decision towards the banning of e-cigarettes it is still in a very controversial estimated topic. The most popular variety of e-cigarette ENDS is the most commonly used product across the globe as reported by WHO IN 2014.[14]

The central government informed the parliament of India that between the years 2016 and 2019, most of the e-cigarettes were imported more than 19 crores.[15] The chief supplier of these e-cigarettes were mostly from countries like China, Hong Kong, the US, and Germany. According to the market reports, the school going students are more affected and excited about using this new variant of e-cigarette and that is why the consumption rate of e-cigarette has grown among these teenagers including high school students.[16]

Fines under the new law: The provisions are being made by the Indian Government after the prohibition of this e-cigarette was made considering its production, manufacturing, importing, exporting and transport, sale, distribution, storage, and advertisement.[17]

1. If anybody in India will be recognized for production, manufacturing, importing, exporting, transporting, and sale including online sale too,it will become a cognizable offense that will be meant for punishment under the law of Indian government.[18]
2. One year of imprisonment or a fine of Rs 1 lakh or might be both for the first offense will be applicable as a punishment as decided by the law of the Indian government.
3. If someone is caught in commencing the offense again then the punishment will be extended upto 3 years with a fine of Rs 5 lakhs.[19]
4. If anybody is found in storing the e-cigarettes will get a punishment of imprisonment up to 6 months or a fine of Rs50,000 or maybe both.[20]
5. Persons who have already the storage of e-cigarettes have to declare as well as the deposit of the stocks of e-cigarettes to their nearest police station on their own as soon as possible after this ordinance comes into effect.[21]

At the present scenario, the Indian Government has declared to stop the sale, storage spaceas well as production of e-cigarettes in India. This decision was being moved by the Union Finance Minister of India, Nirmala Sitharaman who said that said the decision of banning e-cigarettes was taken as per the union recommendations of a Group of Ministers (GoM), which was headed by her.[22]

As soon as the commencement of the ban on e-cigarettes comes into force, immediately all the activities related to e-cigarettes like production, manufacturing, consumption, sale, exporting and importing, distribution, storage, and its advertisement would become illegal in India.[23,24]

Giving justification on the ban of e-cigarettes the union minister of India said that E-cigarettes were being promoted as a way to search out people out of their smoking habits but reports have shown that many people are not using it as weaning mechanism but are rather addicted to it. The government justified its decision of banning e-cigarettes from the country as e-cigarettes are more vulnerable to health hazards and this decision will also protect the youth of its country.[25]

As soon as the parliament of India passes the bill on banning e-cigarettes, 15 states and one Union territory immediately had taken action on this and had banned e-cigarettes. These include Karnataka, Punjab, Mizoram, Kerala, Bihar, Jammu & Kashmir, Maharashtra, Uttar Pradesh, Tamil Nadu, Puducherry, Jharkhand, Rajasthan, Himachal Pradesh, Meghalaya, Nagaland and Odisha [26]. So, the purpose of the government has been to zip the problem in the bud itself," as reported by the union minister of India.[27]

"You see, in a country as vast as India, once a particular product has a very big consumer base and social acceptance, it is very, very difficult to ban it," the health minister of India Dr. Harsh Vardhanonce said in a television interview in the late 2019. Because many opposition political parties were also opposing on banning e-cigarettes as they were only focusing on the economic growth and the profits the industries could make from these e-cigarettes business and forgot about the harmful health hazards of the tobacco product.[28]

Outcome on Ban of e-cigarettes in India: With the ban on e-cigarettes in India, it has unexpectedly benefitted the traditional tobacco or cigarette companies in India like ITC and Godfrey Philips. These companies were facing competition from e-cigarettes in the tobacco market in India. "Why ban when you can tax (heavily)? Banning e-cigarettes while keeping tobacco products is bizarre. Neither health nor fiscal grounds for this decision, so what's the logic," as reported by a member of the Prime Minister's Economic Advisory Council.[29]

Conclusion

The Indian Government has taken a significant decision on e-cigarettes. It has taken care of the health hazards of its people as mostly the adults as well as the high school going students in India have started getting addicted to this new variant of e-cigarettes. Therefore, new research is needed to be conducted in the future in this field as no doubt e0cigarettes are an option for a replacement for traditional cigarettes but its harmful hazards towards health cannot be overlooked.

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Vestibularmigraine-A Challenging Clinical Entity Rhinoscleroma-A Rare Granulomatous Disease in a Child

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Abstract

Vestibular migraine (VM) is a distinct clinical entity which accounts for major vestibular symptoms among the adult and children. It is presently thought of as a major etiology for episodic dizziness. The pathophysiology for VM is not completely understood and is always a puzzling dilemma. Patients often present with vertigo, headache, photophobia, and phonophobia. The clinical examinations and laboratory investigations are usually within normal limits. The diagnosis of VM is often challenging as there are no confirmatory tests available. The treatment is often similar to the migraine headache. The present-day management is to take the similar prophylactic medications needed for migraines such as beta-blockers, antidepressants, and anticonvulsants drugs. It is always a challenging disease for clinicians and often creates a puzzle for diagnosis and treatment. Accurate management of VM needs a proper understanding of the present literature which are discussed details in this review article.

Keywords: *Migraine,Vertigo,Vestibular migraine, Migraine related vertigo.*

Introduction

Dizziness is a common clinical symptom in daily medical practice. The patient presenting migraine along with vertigo or dizziness is called Vestibular migraine (VM). It is also called as migraine-related vertigo (MRV), migraine-associated vertigo (MAV), and basilar artery migraine or migrainous vertigo. This clinical entity is a type of migraine leads to vertiginous presentations along with the classical symptoms of migraine. VM was first described by Dieterich and Brandt in 1999[1] and it corresponds to a type of migraine where important symptom is vestibular. It is more commonly found in persons with the absence of aura and predominantly involves female, as a ratio of 5:1(F/M).[2] It is documented that 1% of the population suffers from VM which accounts for the commonest central etiology of vertigo and the second commonest cause of vertigo in total.[3] VM is often confused with vestibular disorders which are an important cause for dizziness. In clinical practice, common disorders that manifest in vertigo are benign paroxysmal positional vertigo (BPPV), Meniere's disease, vestibular migraine,and vestibular neuronitis in falling order of frequency.[4] VM represents the 2nd commonest cause for vertigo after benign paroxysmal positional vertigo.[5]

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Method

A search was done on medical literature with the help of Pub Med (MEDLINE), SCOPUS, and Google Scholar. The literature search was done between 1980 to 2018 years with the help of the keywords of migrainous vertigo,vestibular migraine, migraine-related vertigo, and migraine-associated vertigo. Articles were selected based on pathophysiology, epidemiology,clinical presentations,diagnosis, and treatment. A sum of 38 manuscripts was found and the detailed search was summarized in our review article.

Epidemiology: VM is more commonly seen than other vestibular disorders.[6] Vertigo and migraine are common clinical entity which affects 7% and 14% of the general population respectively. The simultaneous occurrence of vertigo and migraine may be one percent if found at random. Moreover, a present study documents that 3.2% of the populations have vertigo and migraine. [7] It affects females about three times more common than males. It has been reported that lifetime migraine prevalence is 16%, lifetime vertigo prevalence is of 7%, and a co-morbidity of 3.2% rather thana 1.1% expected chance of individual.[8]One study documented that VM has a prevalence of 0.89% in a year and constitutes for approximately ten percent of patients found for vertigo and approximately ten percent of patients seen for migraines.In one study, the 1-year prevalence of VM in females aged 40-54 years is 5%.[9]

Etiopathology: The basic pathophysiology is trigeminovascular reflexfor vestibular migraine is aparasympathetic reflex such as trigeminovascular reflex which causes vasodilatation of the large intracranial vessels. The trigeminal nucleus caudalis is activated and C1-C2 dorsal horn neurons cause vasodilatation of intracranial blood vessels.[10] In trigeminovascular reflex, the vasodilatation effect of parasympathetic stimuli may be activated by neurokinin A(NKA), calcitonin gene-related peptide (CGRP) and substance P(SP) released from trigeminal nerve endings.[11] The neural pathway for the vestibular system often give to both peripheral and central migrainous mechanism. [12] There are two possible mechanisms for explaining the vertigo in migraine. Transient vertigo has been thought to be a brainstem aura that may be related tothe alteration inflow of the blood.[13]Also there is direct communication from the posterior part of the parietal cortex into the vestibular nuclei which gives straight access to cortical mechanisms towards migrainous aura to connect vestibular information processing and reflex action. Few studies documented that repeated circulation/vascular problems like ischemia due to vasospasm of the inner ear and extravasations of plasma during migraine attack may lead to injury to vestibule and cochlea permanently.[14,15] The symptomatic presentations of patients often the same to peripheral vestibulopathy. Also,abnormality in ion-channel defect and calcium channel alters labyrinthine function and its central pathways often give a consistent hypothesis for the management of VM.[16]

Genetics: Different studies have documented the genetics of the VM. Series of cases documented a hereditary risk towards prevalent among patients suffering from VM, but these risk factors cannot be associated with any particular genetic alteration. One study was done among twenty-four patients suffering from recurrent benign vertigo and two hundred twenty family members who underwent a clinical assessment, revealed forty percent of the first degree relatives suffering from the same type of recurrent vertigo episodes whereas in contrast to 2% of unrelated spouses.[17] One study reported that VM might be heterogeneous or monogenic.[18]There is no relation between sodium and gene linking to calcium channel for familial migraine.[19] There is a location at chromosome 11q which commonly seen in females among familial migraine-related vertigo. [20]There is 12.0MB interval on chromosome 5q35 which contain gene of familial migraine-related vertigo. [21]Etiopathology for VM is still unclear.

Clinical Presentations: VM includes the clinical symptoms such as vertigo along with clinical manifestations of migraine. The vestibular manifestations by migraine usually mimicsome other labyrinthine lesions like benign paroxysmal positional vertigo and episodic vertigo in Meniere's disease.[22] Patient often complaints episodic sensation of spinning,imbalance or sensation of rotation or giddiness or feeling of light headedness last for seconds to hours or days. The patient often presents with severe episodic pulsating headache. The patient has usually no hearing loss when associated with a classical type of migraine. In the majority of cases of basilar migraine, patients present with the sensorineural type of hearing loss and ringing sound in the ear which may mimic the Meniere's syndrome. The physical examinations of patients suffering from MRV are usually normal between two episodes. During episodic attack, the patients generally manifest a nystagmus either peripheral or central vestibular

abnormality. The non-paroxysmal type of positional nystagmus is often seen during the episodic attack of VM.

The peripheral vestibular lesions whichmimic to VM areendolymphatic hydrops,benign paroxysmal positional vertigo,vestibular neuritis,and perilymph fistula. The central lesions which mimic to VM are transient ischemic accident,multiple sclerosis,vestibular-basilar artery insufficiency, neurodegenerative disorders, and familial ataxia syndrome.[23] The International Headache Society(HIS) has declared established diagnostic criteria of migraine (Table 1).[24] However, the vertigo is found in this classification only in respect of benign paroxysmal positional vertigo and basilar migraine. So, it is thought the higher prevalence of VM is more than the chance of association.

The classification of migraine is into: 1. Common migraine(without aura)-There include unilateral and pulsating headache. The headache is more by photophobia (sensitivity to light), phonophobia(sensitivity to sound), and physical activity.All neurological tests are usually normal; 2. Classical migraine(with aura)-There are 2 to 3 episodes of headache which proceeded by reversible CNS problems like ataxia, dysarthria, diplopia, one side numbness/weakness,vertigo, and tinnitus. This phase is known as aura and neurological investigation is within normal limits; 3. Basilar migraine(Type of classical migraine)-There are presentations of spasm of the basilar and vertebral artery. There are brain stem hypoxia featureslike dysarthria, diplopia,ringing sound in the ear,vertigo,hearing loss, low level of consciousness, and weakness/numbness of limb. The symptoms stay for few minutes to hours. The headache often originates at the cervical and occipital areas; 4. Vestibular migraine-Vestibular presentations are additional features due to spasm of vestibule-cochlear artery along with classical migraine; 5. Complicated migraine/Migrainous infraction-It is a classical type of migraine but the neurological presentations do not become normal in one week and MRI reveals an area of ischemic infarction of the brain. VM was jointly described by Barany Society and the subcommittee of IHS.[25] It is seen in the appendix of the 3rd edition of HIS as a first stage for new clinical types.[26] The criteria for the diagnosis of VM which accepted internationally are based onvestibular clinical presentations and migraine symptomatology of moderate to severe, a past migraine history, a temporal association between migraine symptoms, and vestibular symptoms. The duration of acute episodes is often limited between 5minutes to 72 hours. The clinicians should find any aural symptoms to differentiate between Meniere's disease and VM (Table.2). VM is a multi-factorial chronic disease and common among genetically susceptible people. It is characterized by headaches with phonophobia, photophobia, vertigo, nausea, and vomiting. VM affects around 18% female and 6% male presenting neuro-otological manifestations like vertigo,decreased hearing, ringing sound in the ear, and fullness of the ear during crisis. Patients may present this symptomatology without the presence of aheadache.[27] The physical assessment is often normal between the attacks. At the time of the attack of migraine-related vertigo, patients often present a nystagmus that suggests neither peripheral nor central vestibular abnormality. During the attack of vestibular migraine, non-paroxysmal positional nystagmus is common.[28] Loss of hearing is never a clinical presentation of classical and common type of migraine but sensorineural hearingloss or ringing sound in ear is associated with 80% cases of basilar migraine resembling to the Meniere's syndrome. During aura,positional or spontaneous nystagmus can be seen whereas there are no neurological deficits inpatient. There is often familial history of headache is found. The aggravating factors are mental and physical stress,menstrual period,taking contraceptive pills, smoking habits,exposure to light and sound,deprivation of sleeping,staying in an empty stomach,and a certain diet. Vertigo in the migraine patient may go to the presentations of headache and be a part of the migrainous aura. It is often difficult to think whether migraine and vertigo are two separate presentations with similar etiopathology.

Diagnosis: The diagnosis of VM is often difficult as there are no gold standard tests available for getting the diagnosis. The clinical diagnosis of VM may be difficult as it shares features with many clinical conditions. There is no worldwide decision for a group of clinical presentations covered under this disease or what medical term should be utilized. The vestibular symptoms and headache in VM may not be temporarily associated, which often hide the association. The newer guidelines for diagnosis of VM need a minimum of 5 episodic vestibular symptomatic attacks ranging from moderate to severe grade of intensity. The vestibular episodic attacks varying in length,span from few seconds to days and most of fall between 5minutes to 72 hours.[29] Diagnostic tests often show non-specific abnormal results which are also found in migraine patients who do not have

vestibular symptoms.Laboratory tests like complete blood count(CBC),C-reactive protein(CRP),erythrocyte sedimentation rate (ESR) are usually done in all cases of VM. The confirmatory diagnosis of VM needsto rule out of the vestibular symptoms. For excluding the vestibular causes, the different vestibular function testing,hearing assessment,and neuro-imaging are required. Vestibular-evoked myogenic potential(VEMP) is an investigation for vestibulocollic reflexes and peripheral vestibular excessive sensitivity to loud noise which helpful for diagnosis of VM.VEMPs have abnormal reporting in VM patients but the findings are usually heterogeneous. These findings show lower electromyography (EMG) amplitudes,bilateral or unilateral loss of cervical VEMP responses,shifting of maximum VEMP response from 500 to 1000Hz, and increased latencies.[30] VEMP findings are not very specific for VM .Approximately 38% of VM patients present with cochlear features like decreased hearing,ringing sound in-ear,and fullness of ear.[31] The loss of hearing loss in VM varies from mild to moderate degree without much progression. There is documentation of mild both sides downward sloping type of hearing loss over many years among patients of VM.[32]Caloric electronystagmography (ENG) may be needed to differentiate peripheral vestibular diseases from VM.There is a reduced caloric response in peripheral vestibular hypo-function,labyrinthitis and vestibular neuritis.[33] A video head impulse test is useful to differentiate the vestibular disorders from VM.[33] MR angiographyor MRI brain can rule out intracranial lesions for vertigo-like cerebrovascular diseases.[33] Swaying sensation rather than vertigo is described by the patients of cerebrovascular diseases along with neurologic findings like diplopia,visual field loss,limb weakness, or sensory loss.[32]As per a new study, there isan alteration of gray matter in VM in comparison to the control group with raised gray matter mass of frontal lobe, temporal lobe of left side,left thalamus and occipital lobe and reduced gray matter in the left cerebellum.[34]It also needsan examination of MRI for the VM patients. Caloric hypoactivity, oculomotor abnormalities, and hearing loss are rarely seen in VM.

Physiological Tests: Physiological tests are not sufficient alone for diagnosis of the VM as these are inconsistent and patients have a high incidence of VM without vestibular presentations.[35] However, the physiological tests may be employed to differentiate from other vestibular lesions and to confirm the vestibular lesions if present. Approximately ten to twenty percentages of the patients of VM have one side decrease in labyrinthine activity.[36] Patients with MRV have increased postural sway than patients with no VM. There is reduced amplitude in vestibular evoked myogenic potentials in patients of VM.[37]

Treatment: The management of VM needs treatment of vertigo attack and prophylactic treatment. The drugs used for prophylaxis are the same as for migrainous headache which includes beta-antagonist, anti-depressants, and anticonvulsants.[38] There are two treatment options and these are prophylactic and abortive medications for migraines which can be used for the treatment of VM. One study documented 38% of VM patients responded to Zolmitriptan versus placebo with 22%.[39] The drugs that are prescribed for prophylaxis in migraine patients can also be used for the VM. Different drugs are flunarizine, propranolol, bisoprolol, and metoprolol.[40,41] Serotonin has an important role in the pathophysiology of migraine and also causes vestibular symptoms. Deprivation of the amino acid such as tryptophan which is used in serotonin synthesis resulting in more vertigo.[42] Tricyclic antidepressant which enhance central serotonin level may be useful in prophylactic medication of VM particularly in anxiety associated patients.[43] GABA like drugs such e.g. gabapentin are also helpful for patients with VM.[44]

Table 1: Diagnostic criteria of Vestibular migraine(International Headache Society and Barany Society)

Vestibular migraine
A. At least five episodes of vestibular symptoms of moderate to severe intensity lasting 5min-72hours.
B. Current or previous history of migraine +/- aura according to the International Classification of Headache Disorders (ICHD).
C. One or more migraine features ++ with at least 50% of the vestibular episodes.
D. Not better accounted for by another vestibular or ICHD diagnosis.
Probable vestibular migraine
A. At least five episodes with vestibular symptoms+ of moderate to severe intensity lasting 5min-2 hours.
B. Only one of the criteria B and C for vestibular migraine is fulfilled.
C. Not better accounted for by another vestibular or ICHD diagnosis.

Table 2: Difference (Clinical presentations) between Vestibular migraine and Ménière's disease.

Clinical Presentations	Vestibular migraine	Ménière's disease
Headache	Present	Absent
Vertigo	Present and less duration	Present and more than 24hours duration
Family history of headache	Present	Absent
Phonophobia and photophobia	Present	Absent
Hearing loss	Absent	Present
Sensation of ear blockage	Absent	Present
Tinnitus	Absent	Present
Antimigraine medications	Good response	No response

Conclusion

Exact diagnosis of VM is often a taxingsituation for clinicians. The pathophysiology for VM is always a puzzling dilemma for clinicians. The increased prevalence of VM and its impact on quality of life need more understanding of the pathophysiology for better care of VM. The presence of two symptoms of vertigo and headache simultaneously does not represent a definite causal relationship. There is always a challenge for the VM from both basic science and clinical aspect to enable appropriate and ethical treatment of this disease. In VM, the patient's profile is commonly young adult female or male and clinical presentations like vertigo of variable duration and headache. Each episode of vertigo is usually rotatory sensation and or feeling of unsteadiness which lasts for minutes to hours. Other clinical presentations are bilateral aural fullness, photophobia, phonophobia, and tinnitus. During taking history, most of the VM patients have motion sickness. VNG often reveals positional nystagmus not focusing on central or peripheral lesions. Migraine prophylaxis is often helpful for the treatment of VM.

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Cervical Lymphadenopathy-A Clue to Systemic Diseases: A Recent Update

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Abstract

Lymph nodes are distributed along with the lymphatic system all over the body. Enlargement of lymph nodes (lymphadenopathy) may result from an infection, hyperplasia during the immunologic response, and sometimes also by infiltration by cancer cells or lipid cells. Lymphadenopathy may be regional or generalized and or caused by local or systemic factors. Therefore, the differential diagnosis of lymphadenopathy is critical. Oral physician during routine physical examination also includes the examination of any enlarged lymph node in the head and neck region. Lymphadenopathy may be the only clinical presentation or one of the few findings, and enlarged lymph nodes may often raise the suspicion of an underlying pathology like lymphoma, leukemia, and Acquired Immunodeficiency Syndrome (AIDS). This review focuses on the various causes of cervical lymphadenopathy, and the systemic approach to the differential diagnosis and the management along with the health care team.

Keywords: *Cervical lymphadenopathy, Diagnostic aids, Differential diagnosis, Malignancy.*

Introduction

The principal constituents in the lymph system are lymph nodes, the lymphatic vessels as well as the moving cells of immunity which compositely have the basic responsibility to protect the host against infections.[1] Lymphadenopathy frequently occurs as a clinical symptom of an array of disorders. It is described as an alteration in the size, number and consistency of lymph nodes, due to the infiltration or dissemination of either inflammatory cells or malignant cells into the nodal architecture.[2] Cervical lymphadenopathy is described as an enlargement of cervical lymph nodes, with a diameter greater than 1 cm.[3] Cervical lymphadenopathy generally describesa transitory reaction to a non-malignantlocal or generalized infection. However, infrequently, it might be a precursor to an underlying pathological state (autoimmune disorder/malignancy). Lymphadenitis distinctively refers to lymphadenopathies having inflammatory origin.[4] Lymphadenopathy is widely divided into 3 types- localized, generalized, and dermatopathic.[5,6] Generalized lymphadenopathy refers to the enlargement of lymph nodes in more than two noncontinuous areas, whereas, a single site is involved in localized lymphadenopathy. In primary care patients with undiagnosed lymphadenopathy, approximately three-fourths of patients manifest with localized lymphadenopathy and a quarter with generalized lymphadenopathy (Figure 1).[7,8] Dermatopathic lymphadenopathy (DLN) is often seen in patients with skin diseases exfoliative or eczematoid inflammatory erythrodermas, especially mycosis fungoides and Sézary

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syndrome, but has rarely been described in the absence of clinical skin disease.[9] According to its duration, it can be acute, sub-acute, and chronic lymphadenopathy. Acute lymphadenitis is usually seen with bacterial/ viral infections and persists for upto 2 weeks. Subacute lymphadenitis includes a wide array of potential etiologies and lasts between 2-to-6-weeks. Chronic lymphadenopathy persists for more than 6 weeks and occurs due to the infiltration and dissemination of inflammatory/neoplastic cells into the nodal tissues.[4,5,6] Categorizing localized and generalized lymphadenopathy is necessary for making a diagnosis. Thus, a patient presenting with a palpable cervicofacial lymph node poses a significant diagnostic and therapeutic challenge. In most of the cases, without physical examination and investigation result in a delayed incorrect diagnosis and may come as an iatrogenic complication due to misdiagnosis.[10]

Etiology: The Head and neck region is regarded as the commonest site for lymphadenopathy. The spectrum of lymphadenopathy may be represented by the pneumonic "MIAMI" and encompasses malignancies; infections, and autoimmune disorders, miscellaneous and iatrogenic causes.[2] In India, the leading cause for lymphadenopathy is attributed to infection, of which locoregional infections, tuberculosis, and filariasis predominate. According to a study by Iqbal et al., tuberculosis accounts for 70.45% of cervical lymphadenopathy, then comes reactive lymphadenitis (13.63%), metastases (11.36%), lymphomas(4.54%), and chronic nonspecific lymphadenitis (2.27%).[6,11]

Table 1-Depicting etiological factors of cervical lymphadenopathy.

1. **Infectious Etiology-** Various infectious etiologies like viral, bacterial, fungal, protozoal and pararsitic infectious may result in cervical lymphadenopathy.

(a) Viral infections- Herpes group of viruses (HSV, Varicella, EBV, CMV), HIV, Rubella, Measels, Adenovirus, Rhinovirus, enterovirus, Parvovirus B19etc may cause lymphadenopathy.

(b) Bacterial infections- Staphylococcus, Streptococcus, Tuberculous mycobacteria, Non Tuberculous mycobacteria, Treponema, M. leprae, corynobacterium diphtheria, Brucellosis, yersinia pestis, tularemia, mycoplasma pneumoniaetc may cause lymphadenopathy.

(c) Fungal infections- Candida, histoplasmosis, blastomycosis, dermatophytes (linea), coccidoimycosis.

(d) Parasitic/protozoal infections- Toxoplasmosis, trapanosoma, leishmania, rickettsia, microfilaria.

2. **Neoplastic Etiology-** A wide array of neoplastic conditions may have associatedlymphadenopathies.

(a) Squamous cell carcinoma- Odontogenic etiology (chronic irritation due to sharp tooth cusp, ill-fittingdentures), Tobacco induced conditions, SCC with Lichen planus of lip.

(b) Malignanat lymphomas- Hodgkin's & Non-Hodgkin's lymphoma (burkitt lymphoma).

(c) Leukemias- Acute myeloid leukemias, .Acute lymphoblastic leukemia

(d) Lymph node metastasis from solid tumors- Neuroblastoma, Rhabdomysarcoma, Nasopharyngeal carcinoma, metastatic lung, breast, thyroid, renal carcinoma.

3. **Metabolic Disorders-** A wide array of metabolic conditions may have associated lymphadenopathies.

(a) Inborn metabolic storage disorders- Niemann-Pick disease, Gaucher disease, Amylodosis, Tangier disease, Glycogen storage diseases are associated with cervical lymphadenopathy.

(b) Endocrine disorders- like Thyrotoxicosis, Adrenal insufficiency.

4. **Drugs & Lifestyle Exposure-** A variety of drug and lifestyle induced entities may have associated lymphadenopathy.

(a) Drug induced- A number of drugs like Isoniazid, Phenytoin, Allopurinol, Captopril, Penicillin, Cephalosporin, Carbamezipine, Heparin,,Gold, Hydralazine, Primidone, Pyrimethamine, Quinidine, Sulphonamides causes cervical lymphadenopathy.

(b) Lifestyle exposure- Alcohol, ultraviolet (UV) radiation, and tobacco can cause cancers with secondary lymphadenopathy.

5. **Miscellaneous-**A number of miscellaneous entities may cause cervical lymphadenopathy.

(a) Granulomatous infections- like Mycobacteria (M. tuberculosis, M. leprae, M. avium, M. kansai, M. marinum, BCG vaccination) spirochaetes (T. pallidum, T. pertenue, T. carateum) actinomycosis,

brucellosis, yersinia, nocardia, histoplasmosis may cause cervical lymphadenopathy.

(b) Vasculitis- like Wegener's granumatosis, Giant cell arteritis, Systemic lupus erythematosus(SLE), Rheumatoid arthritis, Polyarteritis nodosa, Churg-Strauss

(c) Immunologic abberrations- like Sarcoidosis, Orofacial granulomatosis, Crohn's disease, Primary biliary cirrhosis

(d) Hypersensitivity pneumonia (farmer's lung) & chemicals (beryllium & silicon) may also cause lymphadenopathy.

Clinical Evaluation: Since the diagnosis changes with age, sex, location, duration of symptoms, shape, and size an organized and detailed history, thorough clinical correlation and tissue diagnosis are mandatory.[12]

History: Factors which aid in classifying the cause of lymphadenopathy should include patient's age, location (localized vs. generalized) and duration of lymphadenopathy, foreign travel history, drug history, exposures, and associated symptoms.[13]

Age And Duration: About half of the healthy children demonstrate palpable lymph nodes. Most lymphadenopathy in children is non-malignant or due to infection.[14] The incidence of childhood neoplasms change with the age. In general, lymphadenopathy secondary to neoplasia arise in the adolescents.[4] The majority of acute lymphadenitis cases have an infectious etiology and subside after 2 weeks. This is in contrast to chronic lymphadenopathy, which persists for more than 6 weeks and has an underlying pathology (malignancy, metabolic disease or opportunistic infections).[15]

Associated Symptoms: A detailed review of systems helps in knowing any red flag symptoms. Upper respiratory tract infections encompass constitutional symptoms of fever, chills, sore throat, cough, and body ache. Along with fever, sweating in the night and unknown weight loss (< 10% of body weight) is suggestive of Hodgkin/non-Hodgkin lymphoma. Persistent productive cough lasting more than 3 weeks,evening rise of temperature, hemoptysis, and weight loss are suggestive of tuberculosis. Idiopathic fever, rash, fatigue, and pain in bones increase the suspicion of any autoimmune disease.[13,16]

Concurrent Illness and Past Health: A detailed and meticulous history is mandatory taking in account history of foreign travel, animal/insect contact (esp. cats, dogs, rodents and ticks), preceding infection (tonsillitis and periodontal infections), occupational history (mining and metal industries like lead, beryllium, and silicon), chronic use of medications (Isoniazid, phenytoin, and allopurinol may cause lymphadenopathy), immunization status (lymphadenopathy may occur after diphtheria-pertussis-tetanus, Polio and typhoid fever vaccine), sexual history (genital sores may have accompanying lymphadenopathy) and family history (few diseases have a familial predisposition, like lipid storage diseases and Li-Fraumeni syndrome).[13,15,16]

Physical Examination: General Malnutrition or poor height and weight indices are suggestive of underlying chronic disorders such as tuberculosis, immunodeficiency, or malignancy.[17] Physical examination usually includes inspection, palpation, and ENT assessment. However, cases with suspected systemic disorders would necessitate palpation of lymph nodes of the region.

Characteristics of the Lymph Tissue: A meticulous and comprehensive lymphatic examination should be carried out to eliminate generalized lymphadenopathy.[6] Lymphadenopathy in the supraclavicular region poses the highest risk for malignancy. Elderly individuals > 40 years of age have 90% risk and 25% risk is associated with individuals <40 years.[18] The lymph nodes when examined for tenderness, mobility, and consistency with adjoining tissue/adjoining lymph nodes.[19,20] Any acute infection is known by localized lymphadenopathy in which the lymph nodes are distinct, soft, tender, and mobile. However, firm and mildly tender/non-tender lymph nodes represent chronic infections.[21,22] In lymphadenopathy of metastatic cancer, the lymph nodes may bepainless, stony hard, and are fixed to adjacent tissues; but in lymphoma, they are larger, distinct, not tender, rubbery, and not fixed.[21,23] Firm, discrete, and mobile nodes with free overlying skin are typically seen in the initial stages of TBlymphadenitis. The nodes may become matted with inflamed overlying skin in the later stages. Advanced stage of TB lymphadenitis is marked by softened nodes with abscess and sinus tract formation.[24]

Figure 2- show lymphatic drainage patterns, and etiologies of lymphadenopathy of the regions.[6]

If a group of lymph nodes may betender and bigger, but several – mostly bilateral – are involved,

other situations should be taken into consideration in the differential diagnosis. In infections bilateral growth suggest a viral or streptococcal infection.[15] (Figure 3)

Diagnostic Aids: Apparent benign causes (such as infection) of cervical lymphadenopathy do not require further investigations. However, ancillary aids like serological studies, imaging, and biopsy are recommended for idiopathic conditions. Imaging shows the dimension and spread of the node more accurately than palpation. Ultrasonography is done to study lymph nodes of superficial parts like the neck.[25] Computed tomography determines lymphadenopathy in the thorax or abdomen and pelvic region.[26,27] Diagnosis through Fine needle aspiration cytology (FNAC) or excisional biopsy is the only gold standard for diagnosing lymphadenopathy.[6] In India tuberculosis predominates among the granulomatous infections.

Following history taking and examination, (Figure 4) can show the wayto physicians for further evaluation with peripheral LAP.[6,26,29]

Differential Diagnosis: Differential diagnosis of cervical lymphadenopathy is essential to rule other diseases that present with overlapping features. Pain (tender/non-tender), location (unilateral/bilateral), relation with underlying structures (mobile/fixed nodes), consistency (rubbery/matted), and associated constitutional features (fever, cough, weight loss, arthralgias, rash, etc.) may help in arriving at a definitive diagnosis.

Table 2- Differential diagnosis protocol for cervical lymphadenopathy.[29]

Majority of the disorders are associated with generalized lymphadenopathies. Mononucleosis like syndrome present with Fatigue, malaise, fever, atypical lymphocytosis, and splenomegaly in around 50% of patients. Diagnostic tests are Monospot, IgMearly antibody or viral capsid antigen test. 80 to 90% of patients are asymptomatic in toxoplasmosis, often present as mild symptoms; few patients may have hepatitis, and IgM toxoplasma antibody is the diagnostic test.Tuberculosis lymphadenitis usually present with painless, matted cervical nodes and purified protein derivative(PPD), Fine needle aspiration cytology (FNAC), and biopsy are the diagnostic aids. Secondary syphilispresent with painless, shotty nodes, typical cutaneous rash, condyloma lata, mucous patches in the mouth,and Rapid plasma regain (RPR) is the test of choice. Initial stages of HIV infection manifest as “Flu-like” illness, rashand HIV antibody testing is done. Lymphoma present with fever, night sweats, weight loss in 20 to 30% of patients, along with nodal involvement, and biopsy is used for diagnosis. Leukemia present as blood dyscrasia and bruising. Blood smear and bone marrow aspiration are the investigative test. Measels and rubella present with fever, conjunctivitis and rash and Clinical criteria, serology is used for diagnosis. Hepatitis B present with Fever, nausea, vomiting, icterus, and Liver function tests, HBsAg are used as diagnostioc aids.

Management: Management of Cervical lymphadenopathy is based on the underlying pathology. Usually, the condition is self-limiting and necessitates regular surveillance.[17] Vigilantreview at a 2-to 4-week time is generally recommended for benign lymphadenopathy.[13] Antibiotic treatment targeting Staphylococcus aureus and streptococci (cephalosporins, amoxicillin/clavulanate, or clindamycin) should be given to patients who exhibitunilateral, acute anterior cervical lymphadenitis, and systemic conditions.[30] Corticosteroids therapy should not be given until a definitive diagnosis, as it may potentially mask

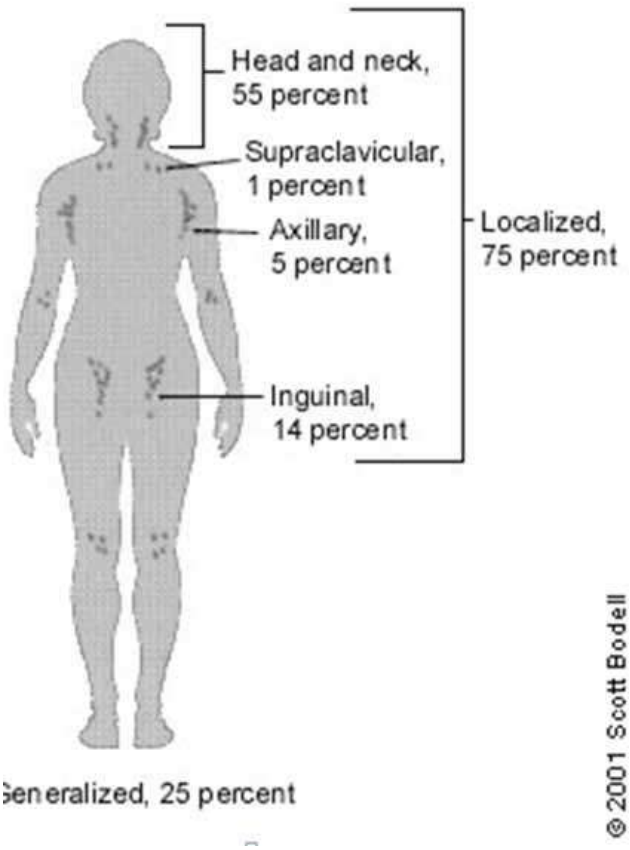


Figure 1: Distribution of lymphadenopathy

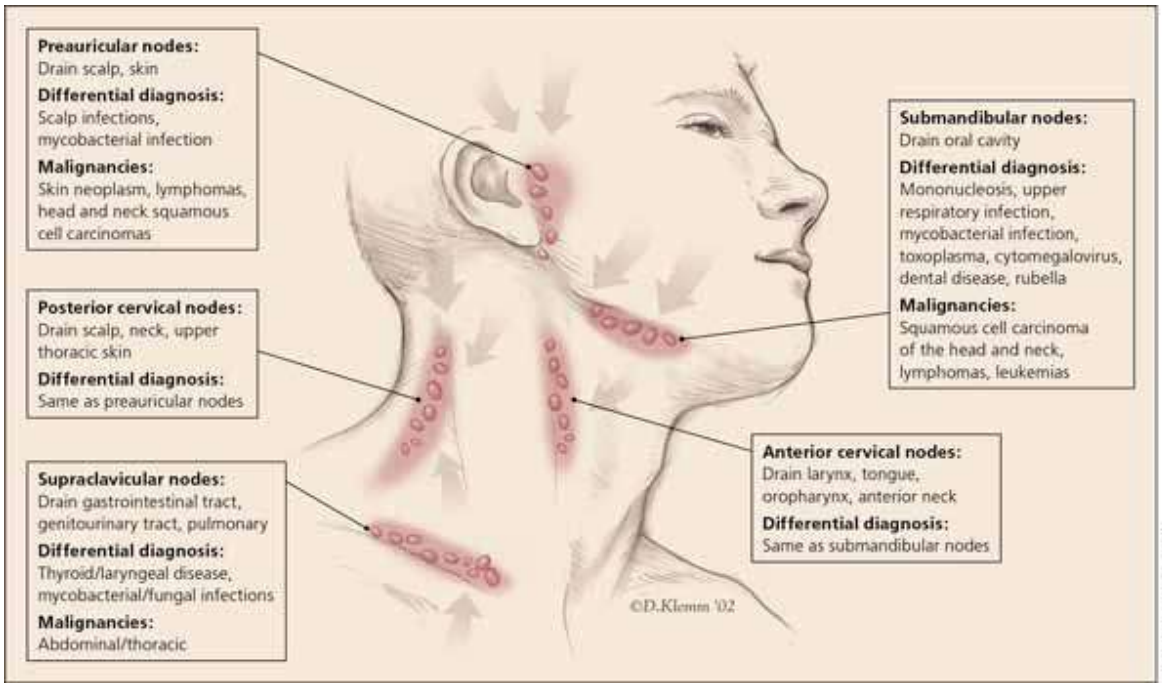


Figure 2: Lymphatic drainage pattern and associated lymphadenopathy

Origin	Side	Course	Frequent	Rare
Infectious	unilateral	acute	Staphylococcus aureus[A,B,F,G]; serogroup A streptococci [A,B,F,G]; anaerobic organisms [A,B,F]	Serogroup B streptococci [A,B,F,G]; tularemia[E,F,G,H], Pasteurella multocida; Yersinia; gram-negative bacteria[A,B]
		chronic	Non-tuberculous mycobacteria (NTM)[B,C]; bartonellosis	Toxoplasmosis; TBC[F,G,H]; actinomycosis
	bilateral	acute	EBV[F,G]; CMV[F,G]; rhinovirus[A,B,F,G]; adenovirus[A,B,F,G]; enterovirus; serogroup A streptococci	Parvovirus B19; rubella virus[D,E], HS, mycoplasma pneumoniae
		chronic	EBV[F,G]; CMV[F,G]	HIV; toxoplasmosis; TBC[F,G,H]; syphilis
Immunologic	unilateral	acute	Arthritis, systemic lupus erythematosus, dermatomyositis	Chronic granulomatous disease
		chronic		Hyper-IgM syndrome, Kawasaki syndrome[F,H]
	bilateral	acute	Arthritis, systemic lupus erythematosus, dermatomyositis, sarcoidosis	Common variable immunodeficiency
		chronic	Sarcoidosis	HLH, Langerhans cell histiocytosis, Castleman's disease, Rosai-Dorfman disease, Kikuchi-Fujimoto disease [G]
Metabolic	unilateral	acute	Adverse drug reaction	
		chronic		
	bilateral	acute	Adverse drug reaction, serum sickness	
		chronic	Gaucher's disease; Amyloidosis	Niemann-Pick disease, Tangier disease
Neoplastic	unilateral	chronic	Rhabdomyosarcoma, non-Hodgkin lymphoma; Hodgkin's lymphoma	Neuroblastoma, nasopharyngeal carcinoma
	bilateral		Acute lymphoblastic leukemia	Acute myeloid leukemia
			Non-Hodgkin lymphoma; Hodgkin's lymphoma	Nasopharyngeal carcinoma

Figure 3: Origin, localization and course of disease distribution.

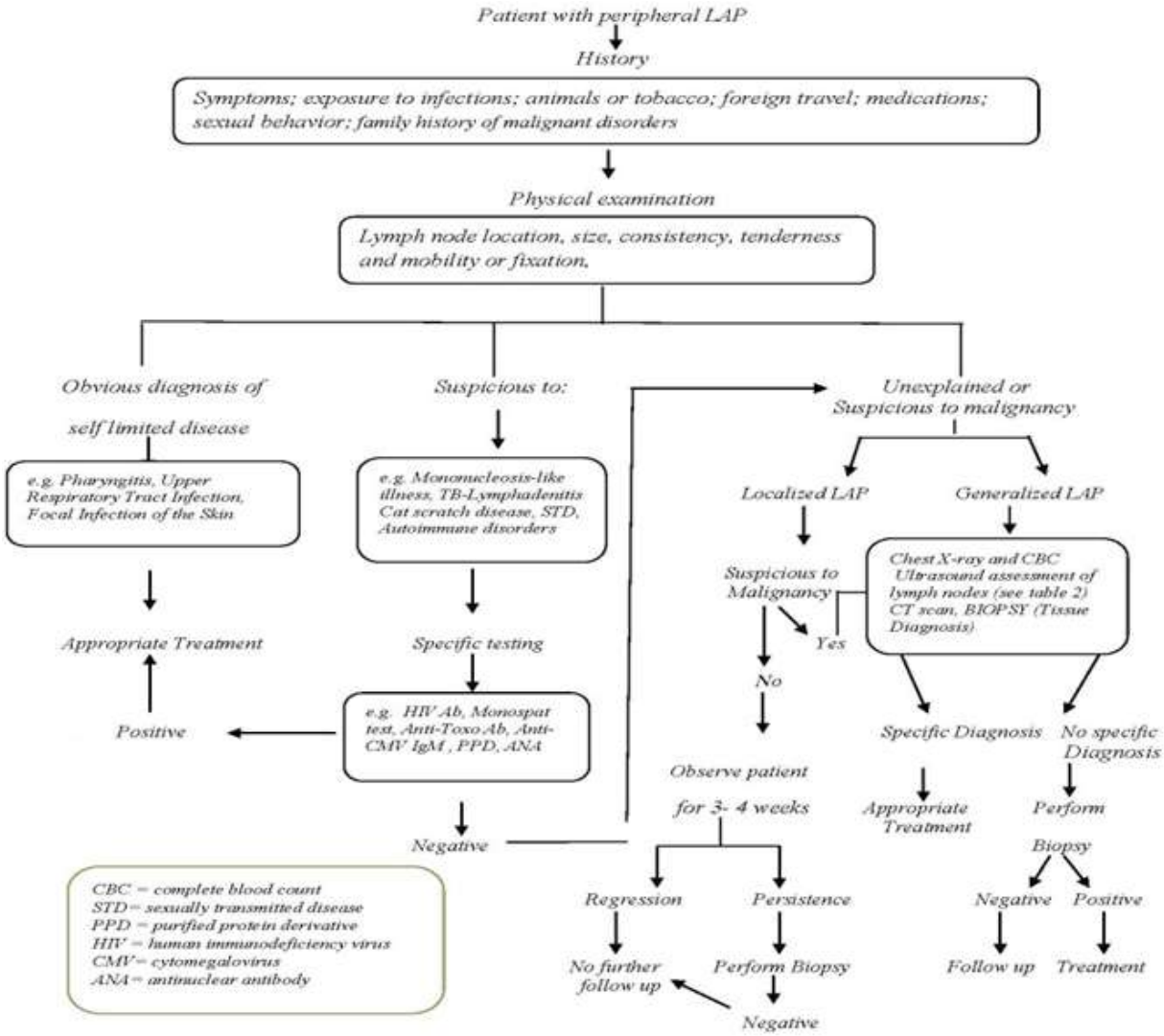


Figure 4: Diagnostic evaluation of cervical lymphadenopathy. histopathology of leukemia or lymphoma.[31]

Conclusion

Cervical lymphadenopathy may be a manifestation of benign pathology or may present as a sign of an underlying systemic disorder. Detailed history, meticulous clinical examination, and a plethora of diagnostic modalities are required for a confirmatory diagnosis. An Oral physician should be aware of the various etiologic causes of cervical lymphadenopathy and understand the significance of cervical lymphadenopathy as a clue to systemic pathologies.

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Adverse Effect of Periodontal Disease on Quality of Life

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Abstract

Periodontal disease represents various chronic inflammatory state of marginal, attach and free gingiva around the tooth, alveolar bone, various types of periodontal ligaments which attach the tooth to the bone or anchorage system of tooth, all are tooth-supporting system. The disease of supporting the structure of the tooth starts from a mild form of gingival inflammation. The use of smoke tobacco is a topmost modifiable risk factor for severe periodontal disease. The use of smoke tobacco has various links with periodontal disease severity. From the various survey it is found that periodontal condition is worst among Smoked tobacco users than non-users. Giving proper treatment to various periodontal diseases is a great challenge, because at the early stage same patients are not aware of these things. To date there is not some bleeding from gum, mild pain maximum patient doesn't aware of these things. When bleeding from gum during hard food chewing, hard or normal brushing patients reports complain. Gingivitis periodontitis like disease are non-violent, some time asymptomatic but they have a negative impact on mastication, esthetic of individuals. Sometimes it has effects the speech of people. Disruptions in the function of neutrophils can lead to develop gingivitis periodontitis. Down syndrome, neutropenia can lead to periodontal disease. Developing age can leads to periodontitis.

Keywords: *Periodontal Disease; Quality of Life;Adverse effect.*

Introduction

Periodontal disease represents various chronic inflammatory state of marginal, attach and free gingiva around the tooth, alveolar bone, various types of periodontal ligaments which attach the tooth to the bone or anchorage system of tooth, all are tooth supporting system. Disease of supporting structure of tooth starts from mild form of gingival inflammation. Gingivitis occurs due to retention of dental plaque due to various bacterial activities. When gingivitis remain untreated for a long time period it converted to severe periodontitis, which leads to loss of alveolar bone, periodontal ligament, recession of gingiva. Periodontal pocket is a tread mark of the periodontal disease, which latter became the main reason for tooth loss. All types of periodontal disease has linked with type I,II diabetes and various heart disease.[1-3]

Periodontitis is two types one is generalized when affected percentage tooth segment is minimum thirty percent and another is localized when affected tooth segment is less than thirty percent or less number of teeh are affected. Retention of biofimis the main cause gingivitis or periodontitis but the rate of progression of the disease depend on the host factor of oral environment and various genetic factors. In recent days the periodontal diseases has big hand in contribution of etiologic factors. The main objectives of our article are diagnosis, preventive measure and treatment plan for various periodontal disease. Thisarticle mention the difference between gingivitis and various forms of periodontitis.

Periodontitis mainly found in elder people, but rarely found in children. The loss of tooth supporting structure due to periodontitis is depends on amount of plaque deposition, risk factors present in that person and immunity capacity. Main identifying feature of various periodontitis is pocket formation and alveolar bone loss, but the bone loss pattern is not unifrom. Periodontitis case definition depends on the disease progression,

magnitude of disease (attachment loss, size of pocket, loss of alveolar bone). Most commonly use definition of severe periodontitis depends on both loss of attachment and size of pocket (mainly depth) recommended by CDC (center of disease control).

From the data of various study says that severe periodontitis is due maximum loss of tooth supporting structure, which has great role in tooth loss around fifteen percent worldwide. [4-7]

Risk factors for periodontal disease: Risk factors are mainly two types modifiable and non modifiable. Use of smoke tobacco is a top most modifiable risk factor for severe periodontal disease. Use of smoke tobacco has various links with periodontal disease severity. From the various survey it is found that periodontal condition is worst among Smoked tobacco users than non-users. Most of the smoke tobacco users found with no sing of bleeding from gingiva and no sing of gingival inflammation as compared normal individual. The main cause behind this is hyper keratinization of gingiva.[8,9,10]

Type I, II Diabetes is most common in people with long term systemic diseases, which make susceptible these kind of people to periodontal diseases. Percentage and Severity of periodontal disease grows in people with prolong DM (DIABETES) mainly patient with uncontrolled DM. periodontal disease has negative impact on metabolism of patient with DM. it also has negative effect on inflammatory response and synthesis of insulin. Adverse effect of diabetes found on tooth supporting structure of some young patient suffering with DM I, II.

A study report from USA gives data that less education, illiteracy, low financial conditions among Africans and Mexican citizen have worst periodontal health.[11,12,13,14]

Periodontal disease like gingivitis, periodontitis begins due to bacterial activities of bio-film. Around eight hundreds of bacterial species found in dental bio film till now. Multiple Gram –ve bacteria or unit of pathogen leads to periodontal disease.

Microbial biofilm: Severe form of periodontitis has close link with special colonies of Aa (*Aggregatibacteractinomycetemcomitans) in longitudinal studies. Porphyromonasgingivalis has also link with periodontitis. But till date it is not cleared about the relationship among changing structure of dental plaque and mild to severe periodontitis.*[15,16]

Many people have suffered from various viral diseases through out their life. After a Long time from cure of the disease the viral deoxyribo nucleic acid and ribose nucleic acid found from the body. The contribution of virus in development of periodontitis is in controversy till now. Antiviral drugs decrease the measurement of depth of periodontal pockets and gingival swelling.[17,18,19,20]

Supra-gingival and sub-gingival calculus contains biofilm. Supra-gingival calculus found above the gum margin and its color is yellowish white. Sub-gingival calculus found below the margin of gum and its color is greenish. The sub-gingival calculus is more calcified and not easily removed. Ca and phosphate ion present in saliva form the supra gingival calculus. Serum transudate calcified the the subgingival calculus.[21]

Pathogenesis of periodiontal disease: Dental biofilm alone can not be the causative factor for periodontitis or gingivitis. When the equilibrium is lost in between dental plaque and host body the gingivitis starts. Various changes found in biofilm, genetics of host body which leads to severe periodontitis and severe gingivitis. Epithelial tissue act as barrio to the harmful pathogenic organism. Lagerhans cell reside in the epithelium presents lymphoid cell against lymphocyte.[22]

Role of T cells: When lymphocyte arrives at damage point, the cell mainly B category converted to antibody generating cell. The capacity of antibody against severe periodontitis depends on antibodies strength and number. T cell has some hand in cell mediate immune reaction. T cell done it by the help of T helper cell by stimulatin g them. T1 and T2 cell has great contribution in development of periodontitis at starting point of periodontitis and on latter stage of periodontitis respectively.[23]

Susceptibility: All types of gingivitis Mild, moderate severe are reversible in nature. But in some case it converted to mild to severe periodontitis. Maximum studies gives data that in those individual who don't have any kind of gingivitis have excellent periodontal status for a long period of time. From data of some studies says that if some individual stop his/ her regular brushing constantly for minimum twenty one days he or she will develop mild gingivitis due to formation of plaque or biofilm. Maximum individual develop different kinds of gingivitis according to formation of dental plaque and inflammatory response. Another fact

is that inflammation of gingival also depends on quality of individual. Some case inflammations also depends on some genetic elements.

No such elements are there for act as resistance to periodontitis or gingivitis. The inflammatory mediators like interlukin1, TNF has linked with destruction of periodontal supportive structure. This inflammatory factors speed up the inflammatory processes. Many drug like nifedipine, phenytoin can cause mild to severe gingival growth. Various changes found in secretion of body hormone can leads to periodontitis.[24,25,26,27,28,29]

Genetics in periodontal disease: Participation of various genetic factors in long periodontal disease has studied in various survays. Survey among young Indonesian people gives data that young people don't develop any kind of periodontal disease with out taking any oral care facilities, only genetics factor plays a great role in this aspect. Various genes plays a key role in developing periodontal disease like periodontitis

Diagnosis of periodontal disease: Giving proper treatment to various periodontal diseases is great challenge, because at the early stage same patients are not aware about these things. Till date there is not some bleeding from gum, mild pain maximum patient doesn't aware about these things. When bleeding from gum during hard food chewing, hard or normal brushing patients reports complain. Mild to severe periodontitis is found with clinical sings like bleeding, loss of stippling on attached gingival, marginal gingival position apical to CEJ, rolled out margin, gingival enlargement, clolor of gingival changes from pale pink to red, ginhival sulcus depth increase more than 4mm, grade I to grade III mobility found. Mild to severe pain also noticed due to pus discharge. Hallmark periodontal diseases are most of the time with out any pain. [30]

Chronic generalized periodontitis is clinically detect by LOA (loss of attachment), amount of bleeding during clinical procedure, pocket depth more than 4mm. and by IOPAR, OPG.

After detection of diseases the dentist remove the dental calculus, plaque and educate the patient how to mentain the oral hygiene. Mostly patient is advice to avoid the risk factors like chewing tobacco, smoking tobacco etc. These are modifiable risk factor and in the hand of individual. Knowledge and willingness of patients has a great hand on mentainance of oral hygiene and treatment of this disease.

Many people develops periodontal disease due to deposition of biofim and some doesn't develop periodontal disease this is due to resistance capacity difference in both type of people. In some case periodontitis growth is rapid and in some individual growth is very slow. In another case it is found that various site of gingiva has great hand on progression of mild to severe periodontal disease.

Regular oral screening programme is very essentital for diagnose the periodontal disease at starting stage. Curing of mild gingivitis at early stage can halt the progression of converting mild gingivitis to periodontitis. Education regarding oral hygiene health can be beneficial In prevention of periodontal disease. Patient with absence of risk factors has very less chance of developing periodontal disease.[30-34]

Treatment of mild, moderate, severe gingivitis can be done by hand scaling or ultrasonic scaling. All biofilm and supra subgingival calcalculus are removed by scaling. By scaling the etiological factor for developing periodontal disease reduced. Self care home therapy is very use full in minimize the chance for developing disease. Various types of drug (doxycycline) can use in the treatment of periodontal disease.

Gingivitis periodontitis like disease are non violent in nature, some time asymptomatic but they have negative impact on mastication, esthetic of individual. Some times it has effects the speech of people.

Disruption in the function of neutrophils can leads to develop gingivitis periodontitis. Down syndrome, nutropenia can leads to periodontal disease. Developing age can leads to periodontitis.[30-34]

Conclusion

Use of smoking tobacco diabetes mellitus type I,II are main modifiable risk factor for developing gingivitis and periodontitis. Various risk indicators for periodontal diseases are bacteria like porphyromonas G, tannerella F, Aa. Predisposing agents for developing gingivitis ,periodontitis are the use of smoke tobacco, smokeless tobacco, the physical status of the individual, uncontrolled DMI,II, excess mental stress, less intake of Ca and Vit–D,

Modifiable risk factors are in the hand of individual they can modify easily and the disease condition can be minimize. But it is very difficult to take control over the

non modifiable risk factors like genetic agents. Excellent diagnosis of periodontal disease has a key role in the proper management of periodontal disease. Because in country like India the prevalence of periodontal disease is more among all age groups. Various types of nutrition has great effect on periodontal disease in elder age groups. The main objective of differential diagnosis of periodontal disease, advanced treatment plan, khowledge can reduce the chance of further advancement of the disease which, would latter affect the QoL of individual. The gravity of any kind of gingivitis or periodontitis depends on various internal and external environment agent. Good prognosis depends on the quality of the drug, immune power of individual.

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Stabilizing Teeth with non Surgical Treatment: A Case Report of Splinting

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Abstract

The management of tooth mobility is one of the prime objectives of traumatic injury. The treatment of trauma-associated tooth mobility may include occlusal therapy, extraction, splinting and various treatments too. This article discusses a case report on the management of tooth mobility in trauma associated patients.

Keywords: *Traumatic Injury; Tooth Mobility; Non-Surgical Treatment, Tooth Stabilization.*

Introduction

In children traumatic exposure to anterior/posterior teeth is common including facial fracture due to unprotected/uncontrolled playing activities. So there is a major prevalence of traumatic injuries during 0-6 years of life i.e 10-30%. And according to Kenwood and Seow the prevalence is more than 30% among < 7-year children. These type of injuries mostly seen in male rather than female and anterior teeth are most frequently affected. Most of the trauma during childhood are sports-related and less control over extremities.[1]

So the prevention of sports injuries in children is most important. And to prevent these injuries parents and pediatric dentists are plays a significant role. For the protection of lips and oral soft tissues from bruises and laceration, the mouthguards are designed. There are different types of mouthguards used for different purpose including stock mouth guards, mouth-formed mouthguard, costume fabricated, etc. other preventive measures are helmets, face masks, jaw joint positioner.[1]

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Stabilization of teeth with nonsurgical therapy may offer a good modality for trauma associated tooth mobility.[2] The treatment protocol may vary for displaced/ partial displacement of the tooth including no treatment or extraction or repositioning of the tooth. Stabilization of teeth can be achieved by splinting which can be provisional or permanent.[3] A splint has been defined as 'an apparatus used to support, protect or immobilize teeth that have been loosened, replanted, fractured or subjected to certain endodontic surgical procedures.[4] Minimum tooth mobility and increased occlusal function is the major advantage of stabilization done by splinting.[5] A splint should restore the arch stability and integrity, redirect the forces to reduce stress and tension[6] there are two types of splints i.e removable and fixed splint. And new splints are available in the market like Ribbond, Fiberglass, Synthetic fibers, etc. The ideal properties of the splint are that it should be simple, economic, stable, efficient, hygienic, non-irritating, not interfering with treatment, aesthetically acceptable, not provoke iatrogenic disease.[7] However the splints are contra indicated in moderate to severe increased tooth mobility.[8] Here we present a case report of a patient with trauma who was timely managed with the provisional splint and non-surgical therapy.

Case Report: A 3-year old female patient presented with the chief complaint of mobility of upper anterior teeth (Figure 1). And the patient fell on the table while playing with her brother. Proper case history was taken including:

- History of trauma- she had no such medical history of any diseases like bleeding disorders, allergies, epilepsy and also no history of vomiting and loss of consciousness or amnesia. And past dental history was also normal and never visited any dentist.
- Initial neurologic assessment was done by checking any unusual communication and normal respiration without any obstructions of the airway.
- Also other examination had performed like vocal function, TMJ movement, neck movement, function of the eye, and ability to protrude the tongue

Clinical Examination:

Extraoral examination- The face was symmetrical with a swollen upper lip.

Intraoral examination- The patient had Grade IIImobility of 51,61. And minimal laceration of gingiva in 51, 61 region. 51 was more displaced buccally than 61 with no evidence of crown fracture.

Investigation was done by taking an intraoral periapical radiograph. And there was no evidence of alveolar bone fracture and root fracture. And also the tooth had not distressed the permanent tooth buds.

Diagnosis and treatment done- A diagnosis of the trauma was made when she fell during playing. Initially Phase I therapy such as scaling was done followed by occlusal adjustment. The management of tooth mobility was planned with the idea of placing ligature wire and splinting about 52,51,61,62 (Figure 2). Ligature wire was braided to the desired length and adapted on the tooth. The labial surfaces were etched with 37% phosphoric acid for 20 seconds. Then the area is cleaned, dried thoroughly, a bonding agent is applied, and curing light was given for20 seconds. Wire was stabilized on the labial surface using composite. Composite resin was then placed on the wire and light-cured. Excess material was removed and finishing and polishing was done. The tooth was checked for mobility. The patient was given instructions on oral hygiene, advised to use an interproximal brush, and was recalled after 15 days for review. During the review, the patient was satisfied with a sense of well being and a great sense of mastication after splinting as the tooth mobility was minimized. The splinting was then removed and the post-operative photograph was taken (Figure 4).



Figure 1: Preoperative photograph



Figure 2: Tooth Repositioning and Ligature wire Splinting done



Figure 3: 15 days follow up



Figure 4: Post- Operative photograph

Discussion

Tooth mobility is defined as a visually perceptible movement of the tooth away from its normal position when a light force is applied.[9] (Gher 1996)Composite and wire splints are perhaps the most commonly used in clinical practice and are flexible splints when the wire has a diameter of no greater than 0.3–0.4 mm.[10] Tooth mobility has been described as an important clinical parameter in predicting prognosis.[11] For this reason and patient comfort, splinting has been the recommended therapy to stabilize teeth. In the past, direct stabilization and splinting of teeth using an adhesive technique required the use of wires, pins, or mesh grids.[12] Splinting done using ligature wire is cost-effective and economical for the patient when compared to fiber splints.[10]

Conclusion

This article has described the ligature wire technique for splinting mobile anterior teeth. The mobility of teeth is a common complaint of patients with trauma. A dental splint is an appliance designed to immobilize and stabilize mobile loose teeth. Various methods of splinting should be applied depending upon the prognosis of mobile teeth and periodontal conditions of surrounding teeth.

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Rhinoscleroma-A Rare Granulomatous Disease in a Child

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Abstract

Rhinoscleroma (RS) is a chronic granulomatous disease caused by *Klebsiella rhinoscleromatis*. In all the patients, the nose is affected but sometimes extends to any parts of the airways. Diagnosis of this disease is done on the basis of the histopathological examination or direct evidence of the bacteria in nasal exudates. The differential diagnoses of the RS are syphilis, malignancy and midline granuloma. Streptomycin is drug of choice, although fluoroquinolones and cephalosporin also provide good results. Surgery is opted in case of obstruction or deformity. Here, we reported a case of 11-year girl diagnosed as RS on the basis of the histopathological examination. This case was treated by endoscopic removal of the mass in the nasal cavity followed by a course of ciprofloxacin. She was found asymptomatic at the last visit of 1 year after treatment and there was no evidence of recurrence.

Keywords: *Rhinoscleroma, nasal cavity, pediatric patient,Mikulicz cells, Russel bodies.*

Introduction

Rhinoscleroma (RS) is an uncommon granulomatous disease often found in mucosal lining of the respiratory tract and occurred by the causative agent of the *Klebsiella rhinoscleromatis*, rod shaped Gram-negative bacteria.[1] There are several cases of RS reports reported from several parts of the world like South East Asia, tropical Africa,Middle East, India,South and Centralparts of America but in recent years, few cases are also detected in non-endemic zones as of high migration of population from different part of the world.[2] After the nasal cavity, other common sites affected by RS are nasopharynx(18-43%), paranasal sinuses(22%) and the larynx(15-40%).[3] RS is common in rural areas where socio-economic status is very low. This disease is more in crowding area, low quality nutrition and low quality hygiene. It is common in females than males (13:1) and found in the 2nd and 3rd decades of life.[4] It is thought that decreased iron in the body may leads to this disease. [4] Here, we are reporting this case of RS in a 11-year-old child.

Case Report: An 11-year-old girl attended outpatient department of Otorhinolaryngology with complaints of rhinorrhea,nasal bleeding and nasal block in the right nostril since 3 months. Anterior rhinoscopy showed a friable mass in the right nostril. Examinations of throat and ear were within normal limits. Diagnostic nasal endoscopy demonstrated the same appearance of the nasal mass with presence of granulomatous lesions inside the nasal cavity attaching to the floor of the nose (Fig.1). Routine blood tests and serological tests for

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HIV were within normal limit. Chest X-ray was normal. Computed tomography(CT) scan revealed opacity/ mass in the right nostril (Fig.2). The re was no evidence of bony erosion in the nasal wall. Biopsy was taken from right nasal cavity and sent for histopathological examination (HPE). HPE revealed extensive inflammatory infiltrates along with lymphoplasmacytic and histiocytic predominance. The plasma cells showed Russel bodies (Fig.3). Intrahistiocytic bacilli correspond to *K.rhinoscleromatis* which was also recognized. After confirmation of the diagnosis, the treatment was done with ciprofloxacin (250 mg twice daily) for 3months with satisfactory follow up. The endoscopic examination revealed no evidence of recurrence after 1 year of treatment.



Fig. 1: Diagnostic nasal endoscopy revealed granulomatous mass in the right nasal cavity.



Fig. 2: CT scan of the paranasal sinus showing radio-opaque soft tissue mass in the right side nasal cavity.

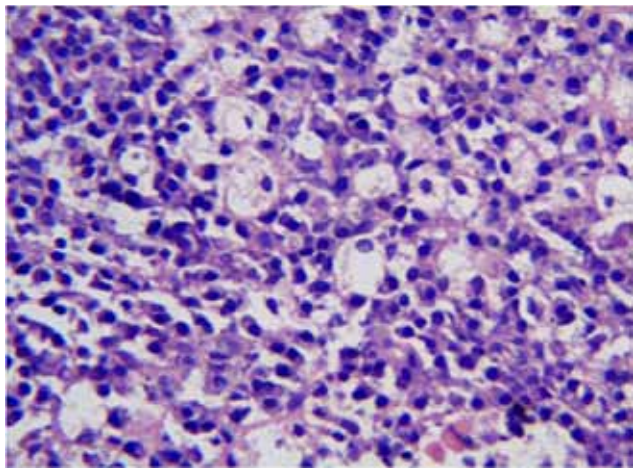


Fig. 3: Histopathological picture shows Mikulicz cells which are large vaculated foamy cells with small nuclei and Russel bodies which are bright red degenerated plasma cells with background of several plasma cells (HEX400).

Discussion

RS is a chronic granulomatous disease due to infection of *Klebsiella rhinoscleromatis*, gram-negative encapsulated bacilluswhich belongs to Enterobacteriaceae family. In anaerobic condition, it forms spores in anaerobic condition which can produce exotoxin and leads to scleroma formation without presence of bacteria.[5]It is often associated with poor socioeconomic condition,malnutrition,poor hygiene, HIV infection and overcrowding. Rhinoscleroma is common among females and often affects the middle age group people.Bilateral nasal cavities are usually affected. In our case, the lesion is confined to one side nasal cavity. Sometimes, it affects the nasopharynx and larynx. Involvement of the larynx may lead to severe stridor. Pediatric patients are rarely affected, however very few cases are reported in the medical literature. The cellular immunity may be affected in this patient;however, this diseased person was immunocompetent.

This disease has three stages: Catarrhal stage, granulomatous stage and fibrotic stage. The catarrhal stage is nasal variety where findings and histopathology may not be specific. In case of granulomatousstage of RS, granulomatous lesions are seen this can block the nostrils. The biopsy is diagnostic in granulomatous stage.[6] This stage may be associated with extra-nasal extension involvement of larynx is the commonest (60-80%) and may be complicated to stridor.[7] In the third stage or stage of fibrosis, there is extensive fibrosis which leads to nasal block rather than proliferative masses.

The fibrotic variety is usually difficult to diagnose as the causative bacteria is usually not seen.

Clinical symptoms of the patients with RS are nonspecific and often presents with mucopurulent nasal discharge,cough,breathing difficulty due to nasal block, dyspnea, nasal bleeding and headache.[8]This disease slowly progress with characteristic of remission and relapse. Patients often require medical advice only when the obstructive granulomatous stage leading to obstruction at the nasal cavity.

The investigations of RS include diagnostic nasal endoscopy, imaging and histopathological examination. Nasal endoscopic examination helps to find the lesion in nasal cavity and its extension to other parts of the upper airway. Computed tomography (CT) scan of the nasal cavity andsinuses reveal lesions in the sinonasal area. Histopathological examination confirms diagnosis. One study documented RS in children where the lesions appear as amyloid like protein in histopathological examination which may be due to an autoimmune reaction.[9] One study revealed RS in three siblings staining at the non-endemic area and thought to be due to neutropenia acting as a predisposing factor.[5] The differential diagnosis of the RS includes tuberculosis, actinomycosis, leprosy syphilis, histoplasmosis, paracoccidioidomycosis, sporotrichosis and parasitic infections such as mucocutaneous leishmaniasis.[10] The RS should also be differentiated from Wegner's granulomatosis, carcinoma and lymphomas. The specific diagnosis of the RS is based on the histopathological examination and the bacilli appear rod-shaped. This bacillusis positive to Warthin-Starry stain and periodic Acid-Schiff (PAS).Staining of immunoperoxidase with anti-capsular antiserum is also helpful for identification of the causative bacteria.Bacterial culture with MacConky agar or blood is also helpful for identification of the bacilli in approximately 50% of the cases.[11] The histological picture of the RS is characterized by the presence of Mikulicz cells, inflammatory cells consisting of multiple plasma cells along with few eosinophils and Russel bodies. These Russel bodies are originated from disintegration of plasma cells. The Mikulicz cells are vaculated cells with clear cytoplasm possessing the bacteria. The cause of transformation of the histiocytes into Mikulicz's cells is unknown.[12] Untreated patients of RS often progress to involve other areas of the respiratory tract. If laryngotracheal part is involved, this may lead to the obstruction of the airway which threatens to life.[13] The sclerotic stage of the RS shows extensive fibrosis, which may cause stenosis and disfigurement. Biopsy from fibrotic stage is often not specific. In this patient, the stage of the disease was with granulomatous type as multiple numbers of Mikulicz cells were found. Immunoperoxidase monolayer assay (IPMA) method for antigen of the capsule of *Klebsiella rhinoscleromatis* is very reliable for the confirmation of the RS. The differential diagnosis is usually other granulomatous diseases of the noses, nasal polyps and sarcoidosis.Presence of extra-nasal sites should be carefully evaluated by diagnostic nasal endoscopy or imaging tests in order to disease process which often need prolonged treatment.[3]

The choice of drug is streptomycin although cephalosporins, fluoroquinolones and ciprofloxacin have had some good outcome. Ciprofloxacin has good antibacterial spectrum and excellent tissue penetration with fewer side effects. Surgical treatment is useful inpatients with severe nasal obstruction or nasal deformity. The treatment period is around three to six months which targets intracellular bacilli. Currently criteria comprise the antibiotics fluoroquinolones therapy with demonstrable efficacy but the clinical and endoscopic examination must be necessary until negative biopsies for eradication of the disease.[13] Surgery is often reserved for the disease particularly orolaryngotracheal forms where stenosis is detected.[13] These may require endoscopic laser or debridement of the stenotic part of the airway or may need open surgery preceded by tracheostomy.[14]Mortality due to RS is extremely rare,but may happen due to upper airway obstruction in case of undiagnosed disease or due to complication of the surgical procedure.

Conclusion

Rhinoscleroma (RS) is a rare clinical entity and should be ruled out in case of nasal mass of a pediatric patient. Surgical removal cannoteliminate the lesion and may lead to recurrence in the stage of fibrosis. The clinical presentations are usually non-specific and granulomatous stage of this disease often evokes the possibility of the RS. Histopathological examination confirms the diagnosis where Mikulicz cells are pathognomonic for *Klebsiella rhinoscleromatis*. The diagnosis of the RS is often difficult and delayed because of its clinical polymorphism. However, early diagnosis and prolonged treatment are important criteria for avoiding recurrence and late squeal.

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Foreign Body Bronchus and Rigid Bronchoscopy– Our Experiences at a Tertiary Care Hospital

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Abstract

Background: Foreign body inhalation is often encountered in emergency department. Foreign body can be lodged in any site of the tracheobronchial air way. Rigid bronchoscopy is one of the established technique for removal of the inhaled foreign body with fewer complications.

Material and Method: We have done a retrospective study in our tertiary care center to determine the clinical presentation, nature of foreign body, age of presentation, location of foreign body, management of foreign bodies and duration of hospital stay in our population.

Result: The most common foreign body in our study was peanut(15.7%) followed by ground nut (12.2%), coconut pieces (7%), small led bulbs (7%) and corn (7%).Most common sites for foreign body lodgment was right main bronchus (40.3%) followed by left main bronchus (29.8%) and trachea (17.5%).

Conclusion: Rigid bronchoscopy is an effective technique for removal of the foreign body from tracheobronchial airway with fewer complications.

Keywords: *Foreign body bronchus, Rigid bronchoscopy, X-ray chest, CT scan Chest, Ventilating bronchoscope.*

Introduction

Foreign body in ear, nose and throat is not uncommon. We as otolaryngologists handle these situations almost every day. Fortunately, most of these situations are not life threatening and can be managed in emergency department or in OPD. But foreign bodies in airway are life threatening emergencies and should be managed promptly to avoid asphyxia and death. These are most commonly seen in children of 1-4 years and sometimes reported in adult patients. Most cases present with sudden onset difficulty in breathing but sometimes they present as cough, wheezing, dyspnoea, pneumonia and emphysema. In maximum number of cases there is a clear history of foreign body aspiration and clinical features correlate with radiological features and diagnosis can be made promptly. But in certain cases CT scan neck and chest is required before going for definitive treatment. Rigid bronchoscopy with foreign body removal is the treatment of choice but some surgeons remove foreign bodies using flexible bronchoscopes. We have done a retrospective study in our tertiary care centre to determine the clinical presentation, nature of foreign body, age of presentation, location of foreign body, management of foreign bodies and duration of hospital stay in our population.

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Material and Method

We have conducted a retrospective study in the Department of ENT & Head and Neck Surgery, Apollo hospitals, Bhubaneswar from January 2010 to January 2019. 57 Patients coming to the hospital with acute onset respiratory distress with suspected history of foreign body aspiration were included in the study. All the patients were investigated as per the protocol and X- ray chest was done in all most all cases and CT scan chest with or without virtual bronchoscopy was done in doubtful cases. Rigid bronchoscopy was done in all cases under general anaesthesia and in most of the cases we have successfully retrieved the foreign body. Our inclusion criteria was any patient with acute onset respiratory distress with suspected foreign body aspiration and our exclusion criteria was critically ill patients with other comorbidities and fever.

Results

57 patients were studied in this period of 8 Years (January 2010 to January 2019). 24 (42.1%) patients were in the age group of 1-3 years followed by < 1 year 16 (28.0%). The incidence is lowest in above 10 years' children 2 (3.5%).

Table 1: Incidence Rate

Age	No	Percentage
<1	16	28.0
1-3	24	42.1
3-5	8	14.0
5-10	7	12.2
>10	2	3.5

The number of male and patients 29 (50.8 %) are slightly more than female patients 28 (49.1%) but the ratio is nearly equal.

Patients who presented to us with acute onset respiratory distress and history of foreign body ingestion/ aspiration X- ray chest was done and 17 (29.8%) patients had definitive diagnosis of foreign body in airway. In 24 (42.1%) doubtful cases CT scan chest was done and it had confirmed the diagnosis in 23 cases. All these patients under went rigid bronchoscopy and foreign body was retrieved in 53 cases and in 4 cases there was thick mucus plug and debris which was cleaned.

Table 2: Sex ratio description

Sex	No	Percentage
Male	29	50.8
Female	28	49.1

We have also studied the location of foreign body lodgment and the data showed the most common site was right main bronchus 23 (40.3%) followed by left main bronchus 17 (29.8%) and trachea 10 (17.5%). The least common site is glottis followed by bilateral bronchus. We did not find foreign bodies in 4 (7%) cases and there was thick mucus plug causing airway obstruction. And these patients were managed conservatively.

Table 3

Location	No	Percentage
Glottis	1	1.7
Trachea	10	17.5
Right Main Bronchus	23	40.3
Left Main Bronchus	17	29.8
B/L Bronchus	2	3.5
No Foreign Body	4	7.0

The most common foreign body in our study was peanut 9 (15.7%) followed by ground nut 7 (12.2%), coconut pieces 4 (7%), small led bulbs 4 (7%) and corn 4(7%). We have removed various types of vegetative, plastic and metallic foreign bodies.

Table 4

Type of Forign Body	Broom Stick	Nail	Coconut	Screw	Peanut	Led Bulb	Tamarind Seed	Plastic Piece	Corn	Ground Nut	Whistle	Tracheostomy Tube	Spring	Vegetable Piece	Bengal Gram	Safetypin	Earpin	Beatle Nut	No Fb
No	1	3	4	2	9	4	2	3	4	7	3	1	1	3	2	4	1	1	2
Percentage	1.7	5.2	7.0	3.5	15.7	7.0	3.5	5.2	7.0	12.2	5.2	1.7	1.7	3.2	3.5	7.0	1.7	1.7	3.5

We have also studied the duration of hospital stay and the requirement of ventilator. Most of the patients 44 (77.1%) were discharged next day and few patients required long term ventilation and stayed for more than 2 days in the hospital 13 (22.8%).

Table 5

Day of Discharge	No	Percentage
Next Day	44	77.1
> 2 Days	13	22.8

One of our patients had some hypoxic injury after the surgery but he had recovered well after the ICU care and we had zero mortality in our series.

Discussion

Foreign body (FB) in airway is an uncommon but life threatening emergency in Otorhinolaryngology practise. Most of the cases are accidental. It is the fourth leading cause of accidental death in children of less than 3years. [1] It's most commonly seen in children of the age group 1-3yrs.[2] It' similar as per our study as we have maximum patients in the age group of 1-3 years. [3] The etiological factors affecting children may be lack of molar tooth, talking or laughing during eating, putting things in mouth while playing and lack of coordination between deglutition and laryngeal closure. In adults FB aspirations are seen in semi-comatose and elderly people. It is a challenge to ENT specialists to diagnose and treat FB in airway. Some patients given proper history of FB aspiration but most of the time parents and relatives are unknown of FB aspiration. A high index of suspicion is needed to diagnose a case of FB aspiration and quick management to avoid dreaded complication and respiratory arrest. Any patient coming to the emergency with complaints of sudden onset cough, stridor, dyspnoea and refractory LRTI and single sided pneumonia should be evaluated in the line of FB aspiration.[4] As per our study most of the foreign bodies are vegetative and most common is peanut (15.7%) which is in similarity with the literature.[5] These are the most difficult FBs to remove. Other foreign bodies which are commonly found are pins, nails, seeds, plastic parts of toys and pens and screws. The organic foreign bodies react with the surrounding and form granulations and can cause complete blockage of the airway. Depending on size of the foreign body it may lodge in different segment of airway i.e. larynx, sub glottis, trachea and bronchus. Larger foreign bodies get impacted in larynx and smaller ones go into the bronchus. Most of these FBs predominantly found in right main bronchus (40.3%) also go as per literature.[6, 7] But few studies by Piva et al and Fraga et alstate that the FBs are mostly seen in left main bronchus.[8, 9] There are three phases in foreign body aspiration. Initially there will be chocking, coughing and gasping, after that there will be a silent phase and finally there will be a complication phase like pneumonia and atelectasis. Clinical presentation depends on the site of lodgement. It may range from complete obstruction when the size is large and site is larynx to no symptoms when the size is small and the site is terminal bronchiole.[10]Sometimes they mimic other respiratory problems like asthma with features of cough wheezing and mild dyspnoea but the unilateral signs differentiate them from asthma. Most of the times FB can be diagnosed with X-ray neck and chest but a negative X-ray can't rule out FB aspiration as most of the foreign bodies are radiolucent. In our study only 29.8 % cases were diagnosed with X-ray chest. They may also reveal pneumonia, emphysema and atelectasis which help in confirming diagnosis. HRCT neck and chest is required when there is doubt about the history of aspiration and presence of radiolucent foreign body is suspected. It also confirms the location of foreign body. Virtual bronchoscopy is the reconstructive image of CT scan which also helps in localisation and confirmation of foreign body. A study by Behera et al indicated that virtual bronchoscopy using multidetector computed tomography (MDCT) scanning is effective in diagnosing the presence of radiolucent vegetable foreign bodies in the tracheobronchial airway.[11] When the history is doubtful and all the radiological tests are negative then a conservative approach should be followed and rigid bronchoscopy can be avoided.[12] Rigid bronchoscopy is the gold standard for treatment of foreign body in trachea or bronchus. It is always done under General anaesthesia. After giving GA the air way is examined using a telescope and the position of the FB is confirmed. Then the ventilating bronchoscope is introduced and kept just above the FB. Any secretion around the FB is cleaned and after that it has to be taken out. Check bronchoscopy is done to confirm any residual FB. The procedure should be done quickly after receiving the patient in the emergency. Ventilating bronchoscope is the instrument of choice through which the anaesthesiologist maintains the saturation of the patient and the surgeon removes the FB in the mean time. Most of the patients get discharged on the next day (77.1%) but some patients require long term ventilation (22.8%) and longer hospital stay. In the expert hands the mortality is near zero if it's done in

correct time. Sometimes for larger foreign bodies we may have to do a tracheostomy and remove the FB from trachea.

Conclusion

Foreign body in airway is mostly seen in children and always accidental and life threatening. It should always be diagnosed and managed quickly to save the patient. History with clinical signs and sometimes X-ray and CT scan diagnose these conditions. Ventilating rigid bronchoscopy and FB removal under general anaesthesia is the procedure of choice. In experienced hands the mortality is near zero. All the parents and caregivers should be educated how to avoid such conditions.

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Ethical Permission: Approved

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Effects of Cleft and It's Clinical Implications

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Abstract

The most common malformation of the head and neck region affecting individuals and their surroundings physically, mentally, psychologically and socially is cleft lip and palate. There are of various types with their distinct clinical features and characteristics. Prenatal diagnosis, effective understanding can help formulate a good treatment approach with specialists from various fields working as a team. Parents counseling and follow up forms the mainstay approach of these children.

Keywords: *Lip, Cleft, Harelip, Unilateral, Bilateral.*

Introduction

The most common congenital malformation of the craniofacial region is cleft lip and palate1,2. Clefting in birds is physiologic as it helps them to fly at greater altitudes and equalize pressure in the middle ear.

Epidemiology: In India, the incidence of cleft is 1 IN 800. In white Americans, the incidence is 1 IN 800 too. In comparison, black Americans show an incidence of 1 IN 2000. Isolated cleft palate presents an incidence of 1IN 2500. Sex differences (Male to Female ratio) presents cleft lip as 2:1, CLEFT PALATE as 2:3, cleft lip & palate as 2:1. In Racial differences, Japanese being greater in comparison to Caucasians and Afro-Caribbean. The incidence of occurrence is more on left side as compared to right side. Monozygotic twins show a 40% concordance in comparison to di-zygotic twins (5% concordance).

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Concept of Cleft:

Neutral Zone Concept: The cheek, lip and pharyngeal musclesbalance against the developing upper and lower arches. The force they exert neutralize the tongue forces maintaining dynamic equilibrium[3]. With the change in equilibrium, the form and relationship of the arch with the teeth changes (Figure 1).

Aberrant muscle forces in clefts: The first nine weeks of fetal life is of significance for the development of cleft lip and palate. The re is failure of fusion of the lip and the palatal segments: both right and left. There is break in continuity of the buccinators mechanism. This results in aberrant muscular forces that displace tissue masses (Figure.2).

In unilateral complete clefts of the lip and palate cases, the premaxillary portion of the noncleft segment is pulled antero-laterally. Premaxilla is carried forward in the facial skeleton in the larger segment[4,5]. There is excessive growth in the premaxillary-vomerine suture in bilateral complete cleft lip and palate (CBCLP) patients.

Palatal arch form: The facial versus tongue muscle forces dictate the shape of the palate. Palatal growth rates correlate with the amount of scarring during closure of the cleft space.

Classification: The various types of cleft are explained underneath (Figure. 3):

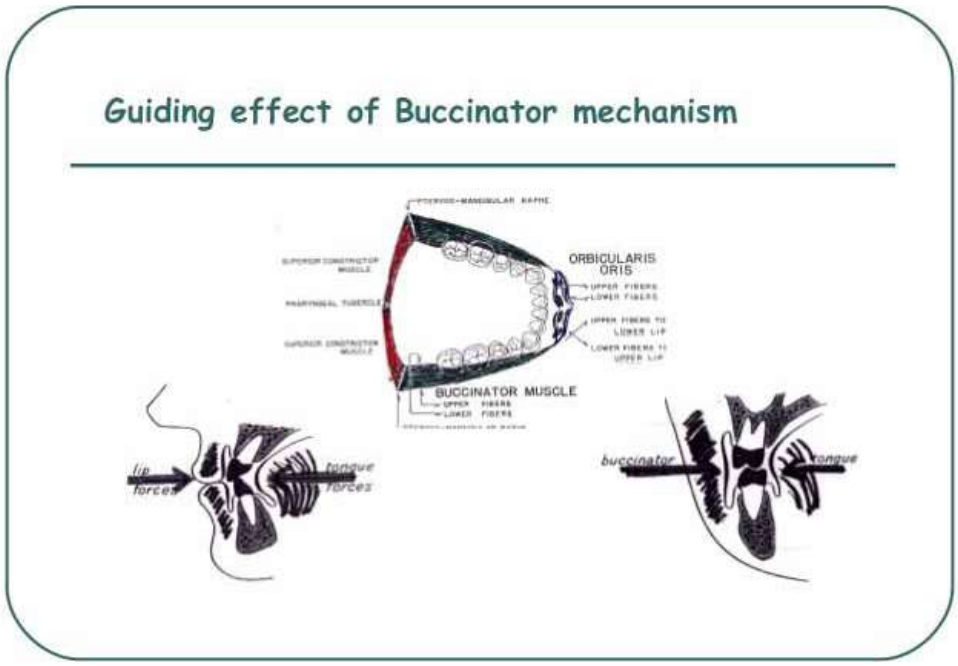


Figure 1. Neutral Zone Concept

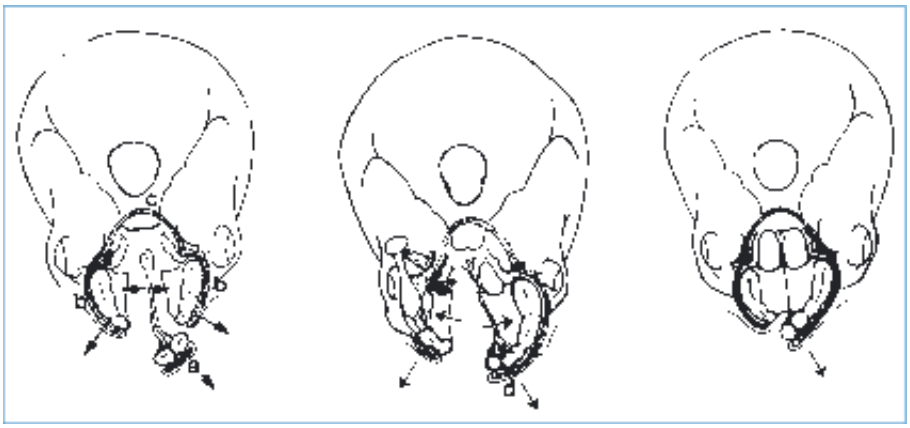


Figure 2. Aberrant Muscle Forces

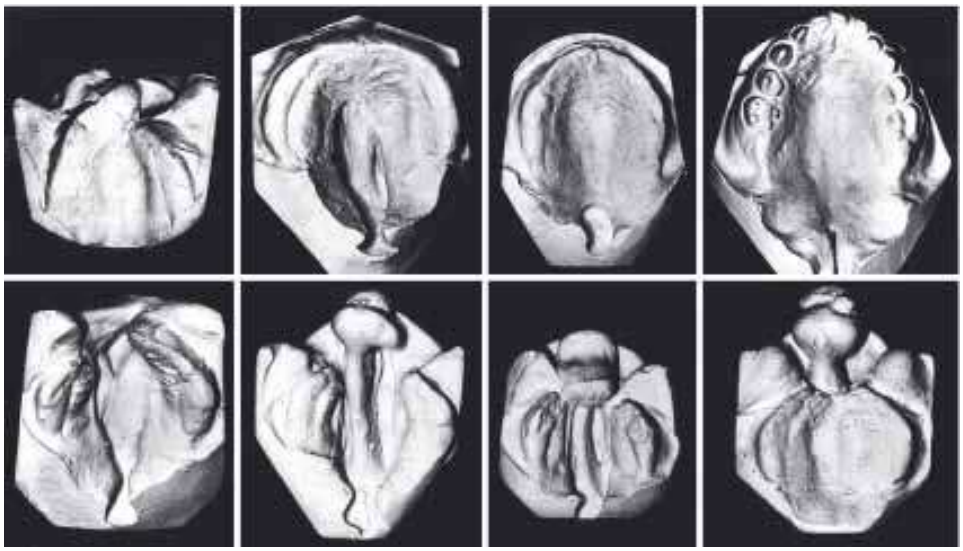


Figure 3. Categories of cleft

1. Clefts affecting lip and palate: simultaneous or separate.

   Cleft affecting the primary and/or secondary palate: complete/incomplete, unilateral/bilateral.

2. Submucous clefts affecting the secondary palate

Clefts of the Lip: Clefts may be unilateral or bilateral (symmetrical or asymmetrical).[6,7] Unilateral cleft lip presents asymmetric and less premaxillary protrusion (Figure 4). Bilateral cleft lip shows marked protrusion of premaxilla.[8]



Figure 4. Unilateral Cleft Lip

Cleft of lip and alveolus: Cleft involves the lip as well as the alveolus.[9,10]

Complete unilateral cleft of the lip and palate: Themedial portion of orbicularis muscle is attached to columella & the lateral portion to the nasal alar cartilage.[11] There is thinness in the medial vermillion border. There is distortion in the perpendicular plates of the sphenoid with flattening of the ipsilateral lower cartilage.[12] Cephalometric findings indicate Increased width of maxilla, increased nasal width withless significant changes in the pharyngeal airway and mandibular length.

Incomplete cleft lip: It presents with a defect in the bulk of orbicularis oris muscle.

Complete bilateral cleft lip: Medial aspect shows complete absence of orbicularis oris muscle.Premaxilla is protruded to varying degree. The various nasal defects include short columella, bilaterally flattened dome, and bilaterally horizontal nostrils(Figure.5). There is protruding premaxilla at birth, marked palatal hypoplasia, retruded maxillary shelves and buccal teeth in class I/class II occlusion. Cephalometric findings indicate increase in width of maxilla, increase in width of nasal cavity, increase in mandibular length and retrognathia.



Figure 5. Bilateral Cleft Lip

Isolated Cleft Palate: It involves hard palate, soft palate and structures of the secondary palate. There is variation of the geometric form. Cephalometric findings show decrease in total length of maxilla, decrease in mandibular length, bimaxillaryretruded jaws with reduction in size of pharyngeal airway.

Submucous Cleft Palate: It shows the presence of bifid uvula (Figure6),zonapellucida.



Figure 6. Bifid Uvula

Syndromes associated with cleft lip & palate: Vonderwoudesyndrome, Downs syndrome, Oral-facial digital syndrome, Treachercollin syndrome, Pierre robin syndrome, Apert syndrome, Trisomy of chromosome 18, Turner's syndrome

Treatment: The treatment is taken care of by a team comprising of Surgeons, Speech and language pathologists, Geneticists, Dysmorphologists and Paramedical specialists.

A. Unilateral cleft of the lip and palate

The treatment protocolfollows age of the patient. At 3 months age, lip adhesion is carried out[13]. At 10 months, lip surgery is carried out. At 18-24 months, palate closure (hard and soft) is done followed by Orthodontic expansion at 5-7 years. Bone grafting is done at 7-9 years. 8 years or later Protraction facial mask is given followed by Maxillary surgical advancement and Lip/ nose revisions technique.[14]

B. Bilateral cleft lip and palate

In such cases, after birth: CBCLP – external elastics is recommended. At 3–4 weeks, lip adhesion surgery is advised. Millard forked flap is done at 6 months of age. At 18-30 months, Von langenbeck repair is done. Buccalcrossbiteis corrected with the help of a quad helix at age of 4-5 years[15].

Fixed palatal retention is attempted at 5-7 years. At 7-8 years, anterior teeth alignment is done followed by bone grafting (secondary).At the age of 9-13 years, full banded treatment is advised. It may be accompaniedby maxillary protraction therapy along with nasaltiprevisionsurgery.

Full Orthodontics is done along with and nasal lip revision at 13-17 years of age. Postsurgical Orthodontics followed by prosthetics andnasal-lip revisions are done at 17-18 years of age.

Treatment sequence of clefts of the uvulae alone and soft palate

Soft tissue clefts are sutured around 3 months. Orthopedic protraction is carried out duringmixed and permanent dentition.

Clefting process and contiguous structures:

A. Skeletal Findings:

(a) Width: Head width is greater than length with orbits further apart than normal. There is a wider maxilla with no crossbites even in adults.

(b) Height: Maxilla on cleft side is smaller than normal, but overall, there is no decrease in vertical facial growth

(c) Depth: Maxilla is placed normally with relation to cranial base. Pre-maxilla is rotated forward & laterally and the protrusion of pre-maxilla increases as age increases.

B. Dental Findings: There is presence of hypodontia, Supernumerary teeth, Microdontia/macrodontia, hypoplasia, natal/neonatal teeth and crossbite in 19% of cases. The teeth adjacent to cleft shows poor palatal support & are susceptible to premature loss. There is delayed eruption of teeth on cleft side.

Effects:

1. Feeding difficulties at birth: Feeding problems arises due to large opening between mouth & nose in case of cleft palate. In case of cleft lip alone problems will arise in efficient sucking. The re are three major concerns:
   - Risk of aspiration because of communication
   - Airway obstruction (Pierre robin syndrome)
   - Difficulty in feeding a child because of nasal regurgitation
2. Hearing difficulties:Hearing defects may be due to sensorineural anomalies and recurrent otitis media.
3. Speech difficulties: Speech pathology may be due to incorrect lip posture, tongue position, velopharyngeal incompetency, abnormal neuromuscular function, abnormal dentition secondary to hearing loss due to sensorineural deficit.
4. Psychosocial issues There is social and emotional adjustment and features of social isolation, obsessive compulsive behavior, lack of cognitive development and school achievement.

Adverse long-term health effects: There are elevated risks for death along with musculoskeletal disorders, autism spectrum disorders and other learning disabilities.

Implications: There is midface retrusion, distortion of dentoalveolar structures, difference in posture and shape of the mandible, crowding of the dentition, missing teeth, concave profile, crossbites.

There are issues like:

1. **Anteroposterior Issues:** The retrusive maxilla along with a normal mandible superimposed on the transverse problem results in a class III tendency.

2. **Vertical Issues:** The position of the mandible varies in rest position and in occlusion. This may generate excessively long or short lower face heights. The functional shift of the mandible results in pseudo prognathism
3. **Transverse Issues:** Crossbites is seen.

Concerns:

In order to minimize the effects of scarring, the various changes that needs to be done include:

a. Biocompatible membranes to inhibit scar tissue formation

b. Epithelial sheets

c. In vivo surgical procedures

Future Perspectives: Information is the quintessential defining factor of this century. It is important to share information among the countries of the world to extend medical benefits equally among developed and developing countries. Japanese Cleft Palate Foundation (JCPF) & the International Cleft Foundation have been established to cater health care needs.Smile train is World's leading cleft charity program, being established in 2000 to help millions of cleft affected families and bring awareness among doctors and medical professionals.

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Conflicts of Interest: There are no conflicts of interest

Ethical Approval: Approved

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Unicystic Ameloblastoma: An Unusual Case Report

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Abstract

Ameloblastomas have many microscopic and clinical variations. Typically it has three biologic variants which include solid, cystic and peripheral, out of which 14% to 22% of all cases are unicystic. Rare variantslike granular cell subtype composed exclusively of eosinophilic granular cells present inboth peripheral and inner layers of tumoral islands. This is a unique case of cystic ameloblastoma affecting a 16-year-old male with an unusual radiographic and microscopic pattern. This rare variant will create awareness for oral and maxillofacial pathologists for a proper pathway of diagnosis.

Keywords: *Intraluminal, Mural, Plexiform, Ameloblastoma.*

Introduction

Ameloblastoma is a locally aggressive epithelial odontogenic benign neoplasm, which has several different microscopic variants.[1-3] It is divided into three groups solid, peripheral and cystic (unicystic). The most common location is the posterior mandible.[4,5] Unicystic ameloblastomas are seen in the second decade of lifemore often in younger patients. The large lesions may cause a painless swelling of the jaws which is often asymptomatic.[5] Unicystic ameloblastoma is classified into the mural, luminal andintraluminal (plexiform) based on microscopic features. The fibrous wall of the cyst is infiltrated by typical follicular or plexiform ameloblastoma in mural type. The tumor is confined to the luminal surface of the cystin luminal type when one or more nodules of ameloblastoma project from the cystic lining into the lumen is called as the intraluminal type.[4-6] Radical surgery is the treatment of choice for mural ameloblastoma andenucleation is the treatment of luminal and intraluminal ameloblastoma.[4] Granular cytoplasmic changes are seen in the stellate reticulum regions of the ameloblastomatous follicles and interlacing cords and nests and it's a rare variant. In peripheral ameloblastic cells,g ranular changes can also be seen[1,6] this case report aimed to present a rare challenging case of ameloblastomain a 16-year-old male in the posterior aspect of the mandible.

Case Report: A male of 16-year was referred for evaluation of the painless expansion on the left lower jaw region to the Department of Oral and Maxillofacial Pathology of Institute of Dental Sciences On intraoral examination there was a swelling in the posterior area of left mandible extending from 36 to 43 region. On palpation the swelling was, non-tender, bony hard to firm in consistency with eggshell crackling. The radiograph (Figure 1) revealed a well defined cortical unilocular radiolucent lesion with radiopacity extending from 36 to 43 region superiorly till the alveolar ridge and inferiorly to the lower border of mandible and teeth displacement concerning 31, 41,42,43,44,45 region. Given the radiographic feature,it was diagnosed as ameloblastoma. An incisional biopsy was performed under local anesthesia to make a final diagnosis. The routine hematoxylin and eosin (H & E)was done for

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histopathologic study. On microscopic examination it showed anepithelial lining with ameloblast like cells over which there was presence of stellate reticulum like cells proliferating into the underlying connective tissue wall in intraluminal and mural pattern many networks of interconnected strands of ameloblast like cells were also present in the connective tissue wall with areas of cystic degeneration at places (Figure 2, 3) and minimal degree of chronic inflammatory cell infiltration.



Figure 1. Welldefined corticated unilocular radiolucent lesion with radiopacity

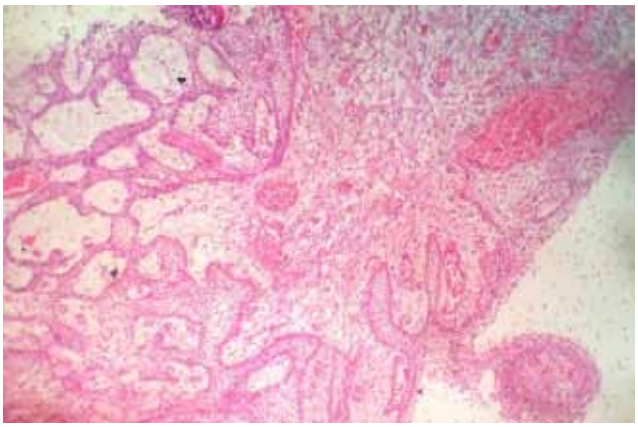


Figure 2. Low power reveals plexiform pattern with mural proliferation

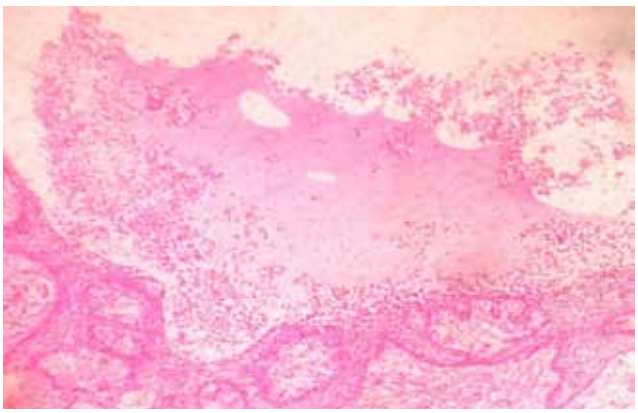


Figure 3. High power reveals plexiform pattern with mural proliferation and luminal variant of ameloblastoma

Differential Diagnosis: Ameloblastoma, Central giant cell granuloma (CGCG) Odontogenickeratocyst (OKC), Calcifying epithelial odontogenic tumor (CEOT).

Discussion

Ameloblastoma is a locally aggressive odontogenic benign, neoplasm which accounts for 1% of all cysts/ tumors of jaws with variable clinical expression and 18% of all odontogenic neoplasms.[6,7] Ameloblastoma is classified as per the WHO system of 2003, based on differences inbiologic behavior, treatment plan and recurrence rate as follows:

(1) Solid/multicysticameloblastoma,

(2) Unicysticameloblastoma,

(3) Peripheralameloblastoma,

(4) Desmoplasticameloblastoma.[7].

6% of ameloblastomas are unicysticameloblastoma which is a rare type of ameloblastoma. It usually occurs in the age group of 15–20 years, with about 50% of the cases occurring in the second decade of life as in our case[8,9]. The male to female ratio of 1.5:1. More than 90% are located in the posterior region of the mandible, the parasymphysis region, the anterior maxilla, and the posterior maxilla[9].

Three pathogenic mechanisms for the evolution of Unicystic Ameloblastoma was proposed by Leider et al.[10].

(1) During the development of the tooth, the epithelium of enamel is reduced which is associated with ameloblastic transformation with subsequent cystic development.

(2) Ameloblastoma arises in the neoplastic ameloblastic epithelium in which there is the temporary non-neoplastic stratified squamous epithelial lining.

(3) When a solid ameloblastoma undergoes cystic degeneration with subsequent fusion of multiple microcysts and develops into unicystic lesions.

Histologic subclassification by Phillipsen and Reichart are described by[7]

Subgroup 1—luminal;

Subgroup 1.2—luminaland intraluminal;

Subgroup 1.2.3—luminal, intraluminal and intramural;

Subgroup1.3—luminalandintramural

Here the present case reveals a swelling in the left lower jaw region of a 16-year-old male radiographically unilocular radiolucency with radiopacity with histopathologic compatibility with intraluminal and mural variant of plexiform pattern.

Conclusion

Unicystic ameloblastoma can be diagnosed based on clinical, radiological, histopathologic, and CT features Recurrence rate was high, especially when the ameloblastic focus penetrates the adjacent tissue from the wall of the cyst. Radiographically, unicystic ameloblastomas show a single large unilocular radiolucency whereas most of ameloblastomas show multilocularity, Unicystic variant of ameloblastoma with aggressive histologic features can be successfully treated with marsupialization with subsequentenucleation, and its an alternative to resection.

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Conflicts of Interest: There are no conflicts of interest

Ethical Approval: Approved

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Management of Mandibular Molar Having Sub-Gingival Caries by Root Amputation: A Case Report

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Abstract

Conservative management of a tooth has always been the primary option for a dental clinician in the management of severely decayed teeth. Therefore instead of going for the extraction or elaborate and expensive treatment such as implant-supported prosthesis procedures like root amputation/resection and hemisection can be opted to salvage these compromised teeth for longer period of functioning. Root amputation is a procedure of removing the root portion of the multirooted tooth that has been affected with subgingival caries, periapical pathology, or any other defects. This article describes a case report of root amputation in mandibular molars.

Keywords: *Root amputation; Root canal treatment; Mandibular molar.*

Introduction

The increasing awareness of patients to maintain proper oral hygiene and preserving teeth for prolonged periods, have led to the treatment and restoration of teeth that once would have been extracted. Assessment of endodontic and periodontal problems related to the tooth is vital before selecting the case. Various strategies have been used to restore such mutilated tooth such as Root amputation/Root resection, Hemisection, and Bicuspidization. Root amputation is a minor surgical procedure in which an affected or questionable root/ roots of a multirooted tooth are removed at the level of furcation keeping the remaining crown and roots intact for function, whereas in Hemisection the entire tooth is divided and the affected part is removed and in Bicuspidization, the crown is equally divided at the center of the furcation, making the furcation area into a more manageable interdental space.[1,2]

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Root amputation and Hemisectionare often valuable procedures once the tooth in question encompasses a high positional worth. The following comprises endodontic indication for root amputation: severe root fracture, undermined furcation areas due to caries, root resorption, root perforation hindering adequate access to the root canal for chemomechanical procedures.[3,4] This article describes a root amputation procedure in mandibular molar.

Case Report: A male patient of age 58 years reported to the Department of Conservative Dentistry and Endodontics with the chief complaint of pain in the lower, left, posterior region, for two months. After a thorough medical and dental history, clinical examination revealed Class II (disto-occlusal) caries in the first molar that extends subgingivally and tooth had mild tenderness on percussion with grade I mobility and furcation involvement. Radiographic examination (Figure-1) revealed, caries extending till the coronal-third of the distobuccal root with vertical bone loss surrounding the distobuccal root and involving the furcation area. The bony support of mesial root appeared intact but radiolucency was seen in the periapical area of mesial and disto-lingual root. So, the diagnosis was primary endodontic lesions with secondary periodontal involvement. The treatment plan was root amputation of

the distobuccal root followed by prosthetic rehabilitation which was informed to the patient and consent was taken.

After administration of local anesthesia, root canal treatment was initiated under rubber dam isolation (Figure-2) and obturation of the mesiobuccal, mesio-lingual, and disto-lingual canals was done followed by access restoration. A mucoperiosteal flap was reflected to expose the distobuccal root, and root amputation was done using a long shank tapered fissure bur with a vertical cut towards the furcation area (Figure-3). The separation was confirmed by passing a probe. A straight elevator was used to luxate and the distobuccal root was removed from the socket (Figure-4-6). Debridement and irrigation with normal saline of the socket were done sufficiently followed by flap repositioning and suturing with 3-0 silk non-resorbable interrupted sutures. Analgesics and antibiotics were prescribed for one week. The patient was recalled after seven days and sutures were removed. The patient was again recalled after a week for crown preparation but as he went out of state and lost communication, the subsequent treatment procedure was never completed.

Discussion

Success of the root amputation is mostly dependent on proper case selection which again depends on various factors such as: Accessibility of root furcation (for easy separation), Adequate bone support for the remaining root, Local factors (tooth anatomy, crown–root ratio, tooth mobility, severity of attachment loss, and occlusal relationships), Patient factors (oral hygiene status and caries index, health and medical status, cost and time) and finally Clinician factors (proper case selection, diagnostic, treatment planning and clinical skill).[5]

In the present case report, root amputation of the distobuccal root was planned as caries had extended sub-gingivally only to involve the coronal third of the distobuccal root along with the furcation involvement, and as it was observed after the removal of the affected root, the remaining structure of the tooth appeared sound clinically as well as radio-graphically along with more than 50% of bone support. Hence a proper prosthetic rehabilitation which was planned, would have been sufficient to reinforce the remaining tooth structure.

The survival rate of root resection therapy as reported by Blomlöf et al, showed 83% survival rate at 5 years dropping to 68% at 10 year recall time, to which the authors stated, even after the diseased root being removed, this procedure inadvertently create a weak zone for aggravating factors such as caries and overhanging restorations to affect these resected teeth and cause further breakdown.[6] Ruiz et al in his study found endodontically treated teeth with periodontal disease to possess a high risk of developing apical periodontitis than teeth without periodontal disease.[7] Therefore a multirooted teeth with more than 50% of bone support around the remaining roots after root resection has a higher chance of survival compared to teeth with less than 50% bone support.[3]

Root fracture is found to be the most common cause of failure after root resection procedures and to reduce the incidence of fractures, sufficient rehabilitation with indirect coronal restoration has proven to have a positive effect on fracture resistance of such root-resected teeth. (8) In certain cases, the survival of these resected teeth can be enhanced by including them as a bridge abutment or splinting them to the adjacent teeth.

Even though root amputation, hemisection, bicuspidization is a successful treatment option in cases where furcation involvement is seen, it is associated with disadvantages as well. The surface of the root that are grinded in the furcation area are more susceptible to caries if not restored subsequently.[5] In the current case report, the tooth might have failed as the patient did not follow up for the prosthetic part of treatment showing his part of negligence making the overall treatment unsuccessful.

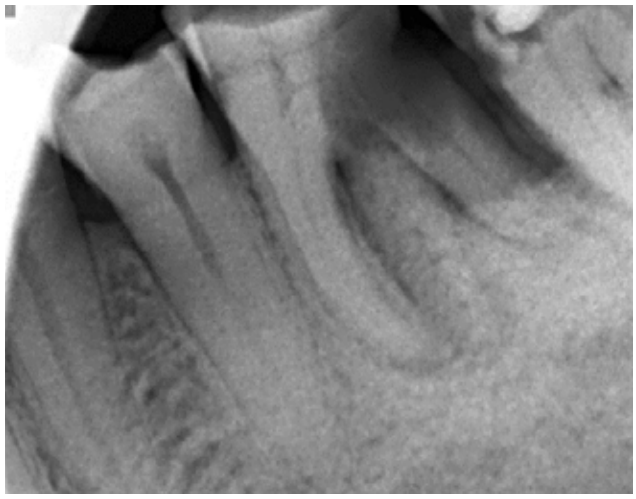


Figure 1. Pre-operative IOPA of 36



Figure 2. Root canal treatment under rubber dam isolation



Figure 3. Mucoperiosteal flap reflected to expose the disto-buccal root and orientation of tapered fissure bur towards the furcation area before root amputation



Figure 4. Disto-buccal root was removed from the socket with a straight elevator



Figure 5. Extracted disto-buccal root

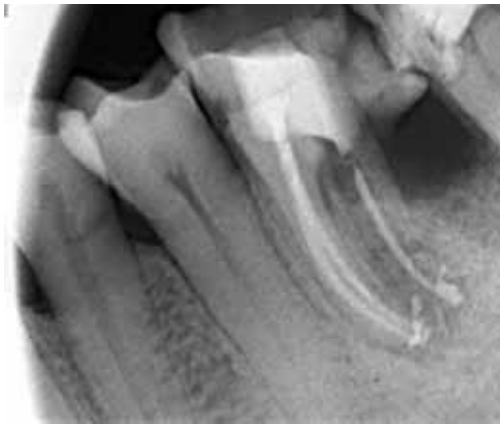


Figure 6. IOPA of 36 Post extraction of Disto-buccal root

Conclusion

Management of teeth with endodontic as well as periodontal problems has always been a challenge to the clinicians. Endodontic, surgical, and restorative work implemented over some time has proven to increase the longevity of such mutilated teeth. And at last, it will be futile if the patients are not informed well about the comprehensive treatment plan and the significance of each step, before initiating the treatment, as they need to give time and be patient, which is the key to the success of the treatment.

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Teledentistry: A Mini Review

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Abstract

Oral health matters. Teledentistry is defined as the collective use of technology to address oral health issues at a distance. The practice teledentistry could possibly be ideal for underprivileged population in remote places where access to oral health services is limited. Teledentistry specifies oral health care assistance through consultation, diagnosis, prevention, treatment using audio, video and data communication. The use of Information and Communication Technologies (ICT) for the exchange of data and information in dentistry, providing health services in situations where it is necessary to overcome geographical, temporal, social and cultural barriers is called teledentistry.

Keywords: *Teledentistry, Telemedicine, E-health, Information and communication technology, Oral health.*

Introduction

Foundation of teledentistry is deeply rooted in telemedicine. The use of telemedicine was initiated for rural population in disadvantaged areas. An equitable and economical paradigm of oral health care is required to increase the opportunity in providing viable dental services to the poor, particularly those living in isolated regions[1]. Government policies have persistently pushed the health practitioners, offering financial enticements, to consider job opportunitiesin rural locations. But, providing ample clinical guidance and support to the newly recruited personnel is a tough task. Thus, teledentistry can be used as a mode of providing access to specialist advice and education, which was previously unattainable[2].

Global Scenario

Oral diseases are definitely amongst the most prevalent global public health problem. Oral health pathologies are the most widespread on a global scale and have serious repercussions on general health[3]. The untreated dental caries of succeedaneous dentition, in particular, accounts for 2.4 billion individuals worldwide which is almost 35% of the entire population[4]. Other oral health issues, namely, cancer of lips and oral cavity, periodontal disease and tooth loss have severe consequences which affect the quality of life of an individual[3]. It has already been acknowledged that oral health issues are a matter of grave concern for the low and middle income countries [LMICs] in terms of social, economic and commercial growth[3].

The United States, Department of defense Total Dental Access venture is considered as the pioneer project in teledentistry[5]. Since its inception in 1994, a sequential shift in technological advancement is eminent. From the use of plain old telephone system [POTS] to the world wide web at present. A series of other technologies have also been used in the interim period, such as fax machines, file transfer via satellite and integrated services digital network [ISDN].

The substantial findings of the previous literature in teledentistry reveals a promising future towards bridging the rural-urban gap of dental health care opportunities. The several key findings of updated literature are presented in the following tables.

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Table 1: Substantial findings of the previous literature in teledentistry reveals a promising future towards bridging the rural-urban gap of dental health care opportunities.

Reference	Study Design	Context	Key Findings
Teoh et al.[6] 2018 AUSTRALIA	Economic analysis	Teledentistry in providing specialist care for cleft patients in rural area.	In comparison to face-to-face consultation, teledentistry is a prospective cost-effective alternative.
Carrard et al.[7] 2018 BRAZIL	Exploratory study	Tele-diagnosis of oral lesions via web-based platform to specialized oral medicine teleconsultants.	Tele-diagnosis is an efficient and feasible pathway in detecting lesions of oral cavity, thereby improving standard of primary health care services.
Estai et al.[8] 2018 AUSTRALIA	Cost minimization analysis	Cost comparison between tele-screening and traditional screening approach in school children from different regions.	In a context of limited resources, tele-screening assists in diminishing the inequities by shifting available resources towards high risk children.
Tynan et al.[9] 2018 AUSTRALIA	Mixed method comparative study	Integrated platform combining teledentistry and oral health therapists for residents of aged care facilities.	This integrated setting was beneficial to high care residents with pre-conditions such as dementia and other cognitive or physical disabilities. Added advantage of increased support and training opportunities for staff members.
Pacheco et al.[10] 2018 BRAZIL	Action research	Information and communication technologies for professional training in dentistry.	Teledentistry services can endorse, through its specific tools, information, exchange of knowledge and experiences to develop and improve its services.
McLaren et al.[11] 2017 UNITED STATES	Retrospective study	Teledentistry in predicting accuracy of actual treatment modality in a rural paediatric set up.	Live video teledentistry in paediatric consultations are reasonably accurate as well as effective intervention for extensive dental needs with complex treatment modalities.
Giraudeau et al.[12] 2017 FRANCE	Accuracy study	Oral telehealth communication performed by a dentist and a nurse, to find their disparity in diagnoses.	Although oral teleconsultation is not as accurate as conventional oral screening, it is adequate for providing an appropriate diagnosis. However, 63% of the recorded diagnoses turned out to be wrong.
McFarland et al.[13] 2017 UNITED STATES	Quantitative descriptive study	Formative evaluation and effectiveness of teledentistry program in a rural location.	Statistical data evaluation offered critical feedback to program administrators. Tele dentistry has the potential to improve oral health outcomes, especially for rural and underprivileged populations.
Estai et al.[14] 2017 AUSTRALIA	Cost minimization analysis	Cost savings from a teledentistry model.	Annual reduction in cost of teledentistry program was considerably lesser than conventional face to face dental screening, in rural areas as well as urban areas. This marked reduction of expense was reflected due to the low remunerations for dental therapists, and the cost savings of travel and accommodation.
Kruger et al.[15] 2016 AUSTRALIA	Descriptive cross-sectional study	Australian dental practitioners.	General dental practitioners had optimistic view and have supported the model of teledentistry and its integration into modern dental practices.
Roxo-Gonçalves et al.[16] 2016 BRAZIL	Assessment study	Teledentistry for diagnosis of oral lesions by dentists and non-dentists.	Good capability in distinguishing the nature of oral lesions. E-learning may develop the knowledge essential for detection of oral cancer.
Blomstrand et al.[17] 2012 SWEDEN	Cross-sectional study	Tele consultations against simple referrals.	Highly effectual oral health care and correct diagnosis provided at the correct place as part of the health service is an economical and effective way to organize health services.

Reference	Study Design	Context	Key Findings
Skelton-Macedo et al.[18] 2012 BRAZIL	Descriptive study	Teleassistance applications for adding to professional actions refining the quality of oral care.	Introducing information and communication technology in day to day dental practice is advantageous. However, protecting the confidentiality of information relating to patients is a matter of concern.
Bradley et al.[19] 2010 NORTHERN IRELAND	Descriptive study	Application of teledentistry in oral medicine in a community dental service.	Use of teledentistry for triage referrals, could be a substitute to the management of referrals of elderly patients having oral diseases.
Berndt et al.[20] 2008 UNITED STATES	Case control series study	Teledentistry to provide interceptive orthodontic services to disadvantaged children.	Real time supervision by an orthodontist is a feasible approach to reduce the severity of malocclusion in disadvantaged children when referral is not possible.
Chen et al.[21] 2007 UNITED STATES	Case report	Specialized dental care from a distance.	The assessments, diagnoses, and advocated treatment plans were comprehensive and similar to routine dental clinic visit. However, 61% of visits were of similar time duration as a regular in-office appointment.
Mandall et al.[22] 2005 UNITED KINGDOM	Randomized controlled trial	Screening new patient for orthodontic referrals using teledentistry.	An effective scheme for positively identifying appropriate new patient for specialist orthodontic treatment. Thus, eliminating inappropriate referrals and saving time.
Stephens et al.[23] 2002 UNITED KINGDOM	Questionnaire study	Attitude of orthodontists of United Kingdom in offering advice to general dental practitioners.	A majority of orthodontists encourage the teledentistry model to make their opinion more accessible to general dentists and subsequently to the patients.

Indian Scenario

A considerable improvement in oral health has been noticed over the years. However, an unequal development in the prevalence and severity of oral diseases in the urban civilization compared to their rural counterpart is a matter of concern. World Health Organization [WHO], ideally proposes a dentist to population ratio of 1:7500. According to World Health Statistics- 2014, the ratio was 1:10000. In 2004, dentist-population ratio in India was 1:30000[24]. But in 2004, India had one dentist for 10000 individuals in urban areas whereas one dentist for approximately 250000 individuals in rural areas. This indicates, three-quarters of the total dentists are clustered in the urban areas where only one-quarter of the country's population reside[25].

Periodontal diseases are the most prevalent oral health care issue in India. In age groups 12, 15, 35-44 and 65-74 years, the prevalence of periodontal diseases reported were 57%, 67.7%, 89.6% and 79.9%, respectively [26].

Introduction to teledentistry in India shall propel the health care system as it will serve as an effective adjunct and a potential cost-effective tool in a low-resource setting. A few studies have been conducted in India and there is a vast scope for future research in this field.

Table 2: Studies related to Teledentistry in India.

Reference	Study Design	Context	Key Findings
Avula et al. [27] 2015 INDIA	Descriptive study	Potential of tele-periodontics.	Better patient supervision over geographic limitations. Prospects of oral health education.

Reference	Study Design	Context	Key Findings
Boringi et al. [28] 2015 INDIA	Cross-sectional study	Awareness of teledentistry programs.	Most of the dental professionals lacked enough knowledge and understanding.
Purohit et al. [30] 2017 INDIA	Cross-sectional study	Teledentistry as a tool to screen for dental caries among 12 years old school children.	Teledentistry and clinical examination findings were equivalent. This study set up provides evidence that teledentistry can be used as a feasible tool for consultation and treatment planning.
Vinayagamoorthy et al. [29] 2019 INDIA	Cross-sectional study	Efficacy of a remote screening model for oral potentially malignant disorders.	Based on direct clinical examination and WhatsApp image examination, both examiners concluded similarly on distinguishing a lesion as normal or abnormal. But there was a substantial disagreement in exact diagnosis.

Conclusion

The demand for oral health care is mounting, with the cost rising simultaneously[1]. Even though teledentistry has not been an integral part of dental care services, it is definitely a tool which is worth developing to encourage access to care for the isolated populations. World Health Organization suggests that telemedicine should be amalgamated to the day to day practice of dental surgeons across the globe as a public health equity tool.

Conflicts of Interest: None

Funding: None

Ethical Permission: Approved

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Polymers Used in Dentistry: An Overview of Literature

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Abstract

The expanding use and enthusiasm for dental polymer aren't just ascribed to the brilliant surfaces of polymers yet besides their ideal mechanical and organic properties, minimal effort and simplicity of dealing with while preparing for a wide scope of utilizations. Polymers, for example, acrylic acid copolymers are utilized as a dental adhesive; polylactic acids are utilized for dental pulp & dentin recovery and bioactive polymers are utilized as advanced drug delivery systems. The article aims to audit the writing on the headways in the utilization of PMs in dentistry.

Keywords: *Denture base polymer,Polymeric composites, Bonding Agents*

Introduction

Before the introduction of acrylic polymers to dentistry the principle polymers used was vulcanized rubber. Polymers introduced in 1937 included vinyl acrylics, polystyrene, epoxies, polycarbonates, polyethylene, polyvinyl acetate, polysulfides, polysilicon, polyethers, and polyacrylic acids. The primary use of polymers has been the construction of prosthetic appliances such as denture base. However they are also used in artificial tooth, restoration, cements, elastics, inlay patterns, implants, impression materials, dies, temporary materials, endodontic filling etc.

Polymers are shaped through compound responses that convert an enormous number of low sub-atomic weight (LMW) particles known as monomers into a huge exceptionally high sub-atomic weight chain full-scale atoms known as polymers the structure and morphology of this large scale particle decides if the material is a fiber,an unbending strong or an elastomer.

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Table 1. Classification of Polymers

Based on the nature of monomer	Homopolymer
	Copolymer
Based on the nature of monomer	Linear
	Branched
Based on Spatial arrangement	Addition
	Condensation

Dental uses of polymers:

The various uses of polymers in dentistry are as follows:

- "Prosthodontics"-"Denture bases and teeth, delicate liners, custom plate, impression materials, core build-up materials, temporary restoratives, establishing/luting materials, and maxillofacial prostheses".
- "Operative Dentistry"- "Dentin bonding agents, cavity fillings, resin and glass-ionomer cements, pit and fissure sealants, bracing materials and veneers".
- "Orthodontics"- "Brackets, bracket holding adhesives and cements and spacers".
- "Endodontics" - "Gutta-percha, root canal sealants and elastic dams".
- "Equipment"- "Mixing bowls and spatulas, mouth guards (athletic gear) and defensive eyewear".

Properties of Polymers:

Mechanical and Physical Properties of Polymers: The blend of polymer creation, chain length, spreading, cross-connecting and nuclear heading can convey a grouping of properties. To address the issues of various dental applications, these features are controlled to make a decent property. These properties can be assembled into four interrelated groupings: "Mechanical, Rheological (Flow), Dissolution and Thermal".

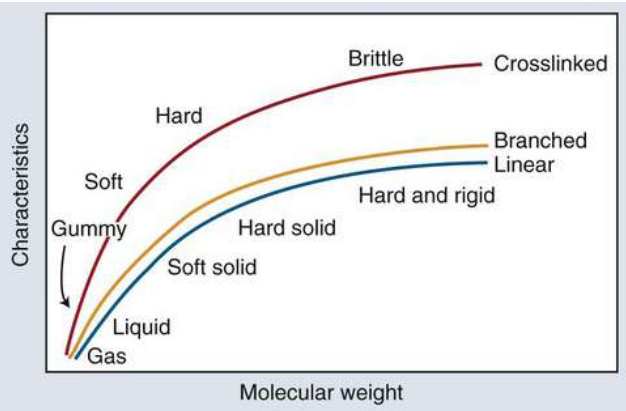


Fig. 1: Rigidity,strength and melting temperature increases with increase with molecular weight and chain length(courtesy-Phillips science of dental material)

Mechanical Properties—Deformation and Recovery: When forces are applied to the polymer they produce stress which causes materials to deform or stretch from its original shape and size (i.e, undergo strain) via either "elastic strain, plastic strain or a combination of elastic plus plastic strain".

- "Plastic strain"- Plastic strain is irreversible distortion that can't be recovered and realizes another, unending shape as the eventual outcome of slippage (flow) among polymer chains.
- "Elastic strain"- Is a reversible,versatile strain that is quickly and completely recovered when the pressure is wiped out, as the aftereffect of polymer chains uncoiling and afterward pulling back.
- "Viscoelastic strain"- Is a mix of both adaptable and plastic deformation, yet simply the flexible part is recovered when the weight is decreased.

Rheometric properties: The rheometry or flow behavior of rigid polymers involves a mix of elastic and plastic deformation followed by elastic recovery after the stresses are removed. This combination of elastic and plastic changes are called viscoelastic property.

The length of the chain,cross link numbers,temperature, and rate of force application determines which type of behavior dominates.

- Plastic flow- Irreversible strain conduct that happens when polymer chains slide more than each other and get moved inside material bringing about perceptual distortion.
- Elastic recovery- reversible strain behavior that occurs in amorphous regions of polymers when randomly coiled chains straighten and then recoil like springs.

Solvation and Dissolution properties: Polymers are usually slow to dissolve the salvation characteristic is very sensitive to polydispersity,cross linking,crystallinity, and chain branching the following characteristic properties exhibited by polymer which is relevant in dentistry-

- The longer the chains with high molecular weight the less is the solubility of the polymer.
- Polymers engross a solvent and soften, but they never dissolve.
- The cross-connecting of the chain forestalls chain detachment and retards disintegration and exceptionally cross-connected chains can't be broken down.
- Elastomers swell more effectively than plastics.
- Absorbed molecules spread the polymer chain within the polymer thus facilitating the slippage of chains and this property is called plasticization.
- The swelling of dental polymers affects the fit of dental polymeric prosthesis.

Thermal properties: The property of a polymer changes with change in temperature and composition,structure, and molecular weight. Thus higher is the temperature the softer and weaker the polymers become. Polymers can be formed into desired shapes using a type of polymeric material used. According to its thermal setting polymer can be divided into thermoplastic polymers and thermosetting polymers.

Thermoplastic polymers: Soften on heating and hardens on cooling. The rmoplastic polymers are made up of branched or linear chains and they soften when heated above the "glass transition temperature(Tg)". Eg-polyamides (nylon), acetal resins, epoxy

resins,impression compound,polystyrene, polycarbonate resins, polyurethane, and acrylic.

Thermosetting polymers: Thermoplastic material undergoes a series of chemical changes and hardens when heated above the glass transition temperature. The y don't soften again on reheating. The y usually are cross-linked in this state and don't melt.Eg-PMMA.

Applications of polymers in dentistry-

Denture Base Material: "Most denture bases and acrylic teeth are manufactured utilizing (polymethyl methacrylate) PMMA".Pmma is much of the time utilized because of its different preferences like low cost,biocompatibility,ease of processing,stability in oral condition, and worthy esthetics.How ever the properties of Emma is upgraded by joining numerous fillers and fibers.

Soft Lining Materials: These are predictable materials that are made to set between the denture and the oral mucosa to lessen torture and misery achieved by hard dentures. These materials are also applied in maxillofacial remaking as obturators". Delicate (soft relining) covering materials are classed in two sorts: delicate acrylics and silicone rubbers.

Warmth or room temperature polymerized methacrylates with included plasticizers, hydrophilic acrylic polymers, silicones, and elastomer/methacrylate copolymers are commonly used as soft lining materials. A fundamental essential is a glass temperature underneath 37 °C, with the ultimate objective that the polymer remains sensitive in the oral pit. The polymers should be biocompatible, nondisturbance to oral tissues, have palatable mechanical quality, and have low fluid take-up.Soft acrylics show extraordinary attach to PMMA dental replacement bases exactly as expected in any case the joining of plasticizers adds to, the cementing over some interval and they furthermore experience the evil impacts of tremendous water take-up. There is a creating stress over the use of Phthalate plasticizers, as a result of their hurtfulness. Silicones experience the evil impacts of nonappearance of cling to the acrylic dental replacement bases and have a low assurance from tear.

Some silicone delicate liners acclimatize a great deal of fluids and water take-up of hydrosilanized silicone rubbers is influenced by included substances, for instance, hydrophobic or hydrophilic silica and is related to the dissolvability of the additional substance, increasingly conspicuous dissolvability offering climb to a drawn-out water take-up with greater deviation from customary dispersal qualities.

Dental Composites: Dental composites are tooth-colored filling materials made out of "engineered polymers, particulate ceramic fillers, polymerization promoters and coupling agents". The y are logically being used as helpful materials set clearly, in an indirect route or as concrete, inbonded rebuilding efforts. Dental composites are provided as single paste details likewise named "visible light cure(VLC) materials and as two paste for the self-cure systems".

Dentin Bonding Agents: Other than GIC, bulk filling material don't adhere to tooth and Dentine bonding agents give an extraordinary joining to tooth structures just as the new age materials similarly permitting bonding between composite resins and substrates, for example, base metal combinations, solidified amalgam, cured composites and porcelain. Dentine bonding agents can be considered to incorporate three segments:"a primer, a coupling agent and an unfilled resin" critical to obtain an exact impression followed by its adaptable recovery and mechanical quality.

All the more as of late, a helpful cement was blended that contained three operators: "a QAM named dimethylaminododecyl methacrylate (DMADDM) with antibacterial movement, nanoparticles of silver (NAg) and NACP for remineralization". There was no lessening in dentin bond quality from one day to a half year of when put in water, while the commercial control bonding agents lost quality generally 33% of its dentin bond quality at six months.bonding agent demonstrated a drawn-out sturdiness in dentin bond quality.

Impression Materials: Various strategies in restorative dentistry require a precise imitation cast of the oral soft and hard tissues to construct appliances outside the mouth; a couple of models being dentures,crowns and bridges, and orthodontic appliances. Impression materials are placed in the oral cavity in a plastic or fluid state which in this way experiences a progression of chemical reaction. The flow properties are critical to get an exact impression followed by its adaptable recovery and mechanical quality. Impressions are much of the time put away at room temperature and exposed to high tensile stress on expulsion from undercut regions. Eg-natural polymers agar-agar,silicone impression material, polyethers, polysulphide.

Peek and Peak: PEEk (polyetheretherketone) is an artificially produced polymeric material and the most critical agent of polyaryletherketone (PAEK). This is a somewhat crystalline, thermoplastic high temperature-resistant, high-performance plastic with a softening temperature of 334°C. In this manner, PEEK can be utilized in pressurized pressure frameworks like the "for 2 press" framework, while the industrial facility squeezed items can be prepared with various cutters and utilized in "computer-aided design/CAM"technology.

PAEK is a tolerably new gathering of high-temperature thermoplastic polymers involving a key sweet-smelling fragrant atomic steel by "ketone and ether". The manufactured structure of polyaromatic ketones gives soundness at high temperatures (more than 300°C), making it incredibly charming for current applications. Before the completion of 1990, PEEK had created as the key thermoplastic polymer and was used to supersede metal parts in the orthopedics. PEEK can without a very remarkable stretch be adjusted by including various materials carbon fiber expansion may assemble the versatile modulus to 18 GPa. The carbon-braced PEEK module is contrasted with the cortical bone and the dentin. The versatility of PEEK resembles that of bone, polish, and dentin in this way making it a perfect material for interminable prosthetic modifying endeavors.

Bio HPP: Bioactive PEEK with ceramic filler (Bio-High Performance Polymer) is a piece of the "PEEk" family and is applied in medical procedures since a significant time allotment. In light of its incredible soundness, its optimal cleaning properties, and its low plaque affinity, BioHPP is commonly phenomenal for definite prosthetic recoveries fabricate. "The biopolymer has a modulus of adaptability closer to the human bone and this reality improves the chewing performances. BioHPP (High-Performance Polymer) is an inventive thermoplastic polymer reliant on PEEK". It was made and upgraded for dental use. It contains ceramic microparticles for better cleaning of the reclamations. These ceramic fillers have a size of about 0.3-0.5 microns and have 20% of the hard and fast volume of BioHPP.

BioHPP is as close as conceivable deep down, on account of its coefficient of versatility (around 4 GPa). This is significant in implant treatment in situations when winding powers may happen. The biting pressure is transmitted as tenderly as could be expected under the circumstances, and the danger of fracture is decreased, because of the BioHPP modulus of flexibility near that of the spongiose bone.

BioHPP is especially appropriate for patients with sensitivities because the solvency of the polymer in water is low <0.3 ìg/mm^3. Studies exhibit high protection from abrasion. BioHPP can be an option in contrast to chromium-cobalt dental alloys (Cr-Co) since it is lighter and doesn't cause erosion. BioHPP developments can be built utilizing both current CAD/CAM innovation and standard wax substitution innovation.

Application of Bio-HPP: Removable Partial Dentures, Crown And Bridge Fabrication, Individual Implant Abutments.



Fig-2: BIO-HPP as framework

Polymers in Implantology: The osseointegration of cpi/Ti implants used for dental, craniofacial, and orthopedic explanations behind existing is related to their course of action and surface harshness. Rough surfaced implants increase osseointegration and biomechanical stability".Implant surface treatment systems join "Ti plasma-splashing,grit blasting,acid etching, anodization and calcium phosphate coatings. A perfect situation is fundamental for embed osseointegration. The diminished oxygen obsession from the poor vasculature at the embed surface interface propels the advancement of host-cell-related electrons as free radicals and proton destructive that can engage disease and irritation causing implant failure. To give a decent ideal condition for osseointegration and overcoming the issues related toTi inserts, surface alterations can be performed using great fiber-reinforced and complex fillers/included substances including hydroxyapatite or antimicrobial circuit by method for thermoset polymers. The polymer/carbon-fiber composite conveyed powerful osseointegration. The rmoset polymer cross-section and carbon strands

make covalent bonds outfitting strong bone structure support with mind-blowing osseointegration. The utilization of anti-infection containing nanofiber put together polymeric films concerning dental implants has been explored to limit implant failure,particularly in periodontally undermined patients.

Conclusion

Plastics are employed successfully in many dental applications. Their properties make them valuable especially in the construction of dentures, plastic teeth, and impression materials. Plastic filling materials have not been successful because of their high coefficient of thermal expansion and lack of bonding to tooth structure. Perhaps the biggest improvement in filling materials would be the development of a satisfactory cavity liner that bonds to tooth structure and is impervious to the mouth fluids. If such a liner were available, the need for a filling material that matches the thermal expansion of the tooth would be less critical. With advancement in higher physical and mechanical properties of polymers, it is now used along with implants and also bridge and crown prosthesis. Although the economical issues cannot be ruled out with this improved material.

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Non-Habit Induced Oral Cancer: A Review

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Abstract

The purpose of this article is to review non-habit induced oral cancers in India and study its interrelationships and risk factors on the health of an individual. Oral Cancer is a major issue related to the health of an individual inevery country around the world and has a wide variation in the global burden of the disease. It is a noteworthy issue regarding public health in India and accounts for 30% of the total cumulative cancers reported across the country. Consumption of tobacco and alcohol leads the etiological factors responsible for causing Oral Cancers, but, at times non-habit induced oral cancers have also been recorded. This article focuses on the various etiological factors responsible for causing oral malignancies in individuals having no history of alcohol and tobacco-related habits and highlights their interrelationship and pathophysiology responsible for causing malignancies.

Keywords: *Oral Cancer, Carcinogens, Pathogenesis, Carcinogenic, Metastasis, Tumerogenesis, Exposure.*

Introduction

Oral Cancer is a major issue related to public health inevery individual country around the world and has a wide variation in the global burden of the disease. Incidences in India and South-east Asia ranks highest in the world with Oral Cancer ranking amongst the top three cancers in the Indian sub-continent.[1] In the head and neck region, the Oral cavity is the predominant site of primary malignant tumors, and oral cancer is the prime cause of morbidity and mortality amongst all cancers in India. Oral Cancer is a noteworthy issue regarding public health in India and accounts for 30% of total cumulative cancers reported across the country, with a prevalence rate as high as 20 out of 1,00,000 people having carcinoma of the oral cavity.[2]

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In western countries, the tongue and floor of the mouth are the predominant regions of oral cancers due to smoking tobacco and alcohol consumption habits. On the contrary, cancers of buccal mucosa, alveolus and retromolar region are the predominant sites in the Indian subcontinent due to the habit of chewing tobacco and areca nut.[3]

Consumption of tobacco and alcohol leads the etiological factors responsible for causing Oral Cancers, but, at times non-habit induced oral cancers have also been recorded. Poor oral hygiene, ill-fitting dentures, sharp cusps, sharp crowns and bridges, hereditary predisposition, racial and demographic factors, chemical carcinogens, radiation, viruses like Human Papilloma Virus (HPV) infections, etc have now been found responsible playing a role in the pathogenesis of Squamous Cell Carcinoma of head and neck region. The most common reason behind younger generations acquiring non-habit induced Oral Squamous Cell Carcinoma is a genetic predisposition, viral factors such as Human Papilloma Virus (HPV) and Epstein–Barr virus (EBV), immune-suppressive conditions, Fanconi's anemia, and occupational exposures.[4-6]

Etiology: Hereditary and Demographic Factors: The risk of developing cancers in genetically linked

relatives of cancer patients is three times higher than non-genetically linked control groups. Incidences of occurrence of nasopharyngeal cancers in the southeast asian population are higher as compared to other regions of the world.[7] Similarly occurrence of carcinomas can also be influenced by geographical and environmental factors like mineral content in water, diet, climate changes, etc.

Chemical Carcinogens: Directly or indirectly the human body comes in contact with various chemicals that can be carcinogenic and can induce carcinogenesis or tumorigenesis in an individual. Polycyclic aromatic hydrocarbons like benzanthracene, methylcholanthrene, etcpresent in tobacco, smoke, air pollutants or industrial oils can cause skin cancer, oral cancer and lung cancer in an individual exposed to these carcinogenic agents. Aromatic amines like beta-naphthylamine, benzidine and azo dyes used as food coloring agents can be a potential causative agent of oncogenesis. Carcinogenicity of chemical agents is dose-dependent and multiple-dose exposure can have the same oncogenes as compared to single-dose exposure. The mutagens bind to DNA, RNA, or cytoplasmic proteins within the cell and initiate miscoding errors during cell replication. These mutagens interact with the genetic substances of the cell and cause mutagenesis, ultimately affecting clonal proliferation.[7]

Physical Carcinogens: Radiation like UV radiation from sunlight, UV lamps, and ionizing radiation like X-rays, alpha-radiation, beta-radiation, gamma-radiation, or radioactive isotopes have the potential for inducing mutagenesis and even killing a cell. The radiation-induced mutation is generally irreversible and depends upon various factors like a dose of exposure, a period of exposure, type of radiation, number of cells exposed to the radiation, etc.[7]

Biological Carcinogens: In certain cases of reported non-habit induced Oral Squamous Cell Carcinoma, not only chemical or physical carcinogens, but the presence of variousbiological carcinogens like Human Papilloma Virus (HPV), Epstein - Barr virus (EBV), and Herpes Simplex Virus are also seen playing a prime role in promoting cancers.[8-10]

HPVis one of the major etiological factors in non-habit induced Oral Squamous Cell Carcinoma cases reported worldwide. Cases of Human Papilloma Virus infections have also turned up at several areas in the human body like the anus and genital tract, urethra, skin, larynx, mucosa lining the trachea and the bronchus, nasal passage, maxillary sinus, frontal sinus, sphenoid sinus, ethmoid sinus and oral cavity.[11,12] High-risk prototypes of the Human Papilloma Virus, HPV-16, and HPV-18 have been recognized as being capable of transforming epithelial cells in the upper respiratory tract into cancer cells.[13] HPV infection plays a major role in the causation of cancer of the pharynx, but it has also been recognized as an etiological factor behind cancers of the Oral Cavity. Oral HPV infection may be associated with different precancerous lesions of oral cavities like leukoplakia, erythroplakia, oral lichen planus (OLP), nicotine stomatitis, oral submucous fibrosis (OSMF), and tobacco pouch keratosis.[14-16]

HPVbelongs to an age-old known family of pathogens that were known to cause infection of the epithelial tissues in organisms belonging to the family of amphibians, reptiles, birds, and mammals. This cluster of DNA viruses forms a discrete Papovaviridae family, which encompasses Papillomaviridae and Polyomaviruses. HPV can be phylogenetically put into genera like alpha, beta, gamma, mu, and nu, different species, and 150 types out of which 120 types are fully sequenced. About 80% to 90% of viruses in the same species split similar biological properties like tissue morphology, disease illustration, and pathogenicity. The life cycle of the virus is initiated through micro lesions in the epithelium of the human body and is recognized byspecialized distinguishable programs of squamous epithelial keratinocytesthat have been infected, where virusessynthesis DNA and their expressions occur through capsid proteins. Basal cells are directly infected from the Virions released from the stratum corneum and stratum granulosum throughthe synthesis of capsid proteins as well as activation of late and early promoters.[14,17, 18]

If clinical features are observed, HPV-associated tumors can be distinguished as a shape somewhat resembling a strawberry-like exophytic lesion, most commonly seen at the base of the tongue or can also be seen in the tonsilar region and poorly distinguishable histopathological findings, as well as cystic changes, are seen in metastatic lymph nodes of head and neck region present near the tumor.[15-21]

Epstein-Barr virus is a double-stranded DNA containing oncogenic human herpes virus, also known to be the first human tumor-causing virus.[19,22,23] It can appear in individual showing no visible symptoms and

persist for a lifetime as a latent infection.[22] Presence of EBV have been reported in various epithelial cell malignancies, like, nasopharyngeal Carcinomas, so it can also be a potential etiological factor behind various malignancies of the oral cavity.[24] Studies have also shown that Epstein-Barr Virus is also engaged in various B-lymphocyte induced cell malignancies, like Burkitt's lymphoma and Hodgkin lymphoma.[22,23] Epstein Barr Virus malignancy is linked with various proteins in the viruses that controlthe proliferation of the cell, immune response as well as apoptosis of the cell. Latent Membrane protein help in activating the signaling pathway, whereas the nuclear antigens of EBV help in gene expression.[20] However, Epstein-Barr Virus affecting Carcinogenesis of Oral mucosal remains uncertain, because various techniques like PCR, nested PCR, RT-qPCR, IHC, and ISH, are used for detecting the virus, and sensitivity and specificity of the tests varies according to the techniques which have been used, leading to different interrelations between Epstein-Barr Virus infection and Oral Squamous Cell Carcinoma.[21,]

Non-Habit Oral Cancer in Females: Studies suggest the occurrence of non-habit induced oral Carcinoma has a higher incidence rate in female gender as compared to males.[23] The cancer cells derived from head and neck carcinomas show estrogen metabolism genes, especially estradiol in tongue carcinomas. Several studies have also indicated an alteration in the level of various hormones like Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), prolactin, and testosterone in the bodies of female patients reporting with non-habit induced Oral Squamous Cell Carcinoma. The exact role of estrogen behind Oral Carcinoma occurrence is still under research scrutiny and should be studied further to shed light and extract the etiology behind the presence of estrogen receptors in non-habit induced head and neck cancers in the female body.[24]

The Oral Cavity is exposed to various forms of trauma either through ill-fitting dentures, sharp cusps, impinging dental restorations, and root pieces. Chronic irritation to the soft tissues due to the above-mentioned causes can progress towards the emergence of reactive oxygen species (ROS) and reactive nitrogen species (RNS) from various inflammatory cells like lymphocytes, macrophages, etc. When the body's defense mechanism falls behind and concentration of free radicalslike Superoxide Radical (O_2), Hydroperoxyl Radical (HO_2), Hydroxyl Radical (OH), Nitric Oxide (NO), Nitrogen dioxide (NO_2) exceeds the body's antioxidant levels, a mutation occurs in the parent cells leading to carcinogenesis. Ill-fitting dentures serve as a source of chronic irritation and trauma to the buccal mucosa and can be an etiology behind the occurrence of Oral Carcinomas in older generations due to possible synergistic effects of long-standing trauma and delayed DNA repair mechanism.[17]

Conclusion

From various studies, it has been seen that non-habit induced Oral Squamous Cell Carcinoma tends to have a negative nodal status and a tumor size which seldom exceeds T2 (STNMP staging). STNMP is a newly introduced system for giving the notation of intra-oral carcinomas and it is completely different from all previous TNM staging in that along with the conventional notation including tumor (T), node (N), and metastasis (M) which is in generalused in cancer staging, but, an additional notation of site (S) and the pathology (P) have also been considered. The two new additional features have been conceded as a prime factor in estimating the prognosis of the patient. The phenotype of non-habit associated Oral Squamous Cell Carcinoma is generally a well to moderately differentiated carcinoma. When compared the expression of p53 in non-habit associated Oral Squamous Cell Carcinoma shows a significant decrease in its expression than habit induced carcinomas. This suggests a good response to radiotherapy and cancer management procedures and thus an improved overall prognosis. However, a low prognosis rate is seen in non-habit induced Oral Squamous Cell Carcinoma patients due to delayed treatment and medical management as well as lack of awareness amongst the population over the issue.

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Restoration of Anterior Tooth with Extensive Coronal Tooth Loss with Custom Metal Cast Post: A Case Report

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Abstract

The success of an endodontically treated tooth, when most of the coronal structure is lost, not only depends on the quality of Endodontic treatment but also its post-treatment coronal rehabilitation. This can be achieved by retaining a core with a post holding the final fixed prosthesis. In cases where the bulk of the natural crown is lost, a post and core with Endodontics treatment help to improve fracture resistance. Therefore it is of great importance that an apt post system is chosen depending on the clinical condition of the tooth to be restored. When a large amount of tooth structure is lost or if an insufficient ferrule is present, a custom cast post and core is indicated. This paper elaborates on a case report depicting restoration for central incisor using custom cast posts followed by PFM crowns.

Keywords: *Anterior Tooth; Extensive Coronal Tooth Loss; Custom Metal Cast Post.*

Introduction

Teeth undergo extensive loss of coronal tooth structure after Endodontics treatment and the reason could be either fracture, a pre-existing restoration, or a large access cavity preparation. In such a condition opting for an apt post Endodontics restoration method is of great importance and can be very challenging.[1] The choices are between direct or indirect restoration for smaller defects, partial or full coverage crowns for relatively larger cavities, and also whether there is a requirement of placement of post. If it is decided upon the placement of post, the type of post needs to be opted too, whether a prefabricated or a cast custom post. According to Franklin Weine, the success of most of the Endodontically treated teeth depends on the post Endodontic restoration and not on the endodontic treatment itself.[2]

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Post Endodontic restoration and its choice depending on factors like the remainder coronal structure, position of the tooth and its functional requirements, esthetics, patient's age, and the periodontal health of the tooth.[3] Endodontically treated anterior teeth, without any discoloration can be restored with composite resin if the loss of tooth structure is moderate to low. This will provide a good coronal seal and the fracture susceptibility will be reduced.[4,5] Composite restoration can also be chosen when the anterior teeth have the cingulum or the incisal edge intact with a couple of proximal carious lesions[6] in the Endodontically treated tooth is found to be discolored, bleaching can be done paired with indirect or direct veneers. When coronal tooth structure is considerably lost (<50%) in a root canal treated tooth, it is essential to decide whether to opt for direct restoration or it needs to be restored with full coverage crowns. This depends on the amount of occlusal load on the tooth and the position of the tooth in the arch. Any tooth that is root canal treated and is found with crack lines, mesial or distal caries or cervical abrasion is recommended for placement of intra radicular posts. This provides for better retention on the core and the final restoration with improved fracture resistance.[7] Anterior teeth with more than 50% tooth structure loss, post, and core followed by fullcoverage restoration are mandatory.[8]

When there is a requirement for placement of posts, various options are ranging from the conventional custom cast post to newer fiber posts. Placement of fiber posts requires less time and also the evidence from the laboratory and clinical experience of technicians and dental professionals respectively emphasis on the use of fiber posts. This does not conclude that custom cast post is a matter of past, as they are a better option when there is extensive loss of tooth structure and if there is a need to change in angulations of the core. In this case report, we present a case where it requires the restoration of maxillary incisors by custom cast post with porcelain fused to metal crowns.

Case Report: A Female patient of age 76 years reported to the department of prosthodontics. She had been referred to our department from the department of endodontics post endodontic treatment of both the upper central incisors. She reported a history of trauma on the left central incisor which led to it being fractured from the cervical third of the tooth. The right central incisor was grossly decayed. The patient underwent Endodontics treatment for both the central incisors. The patient gave a drug history of antiplatelet therapy. Intraoral examination revealed 11 to be restored temporarily and 21 with Ellis Class 3 fracture. 21 had undergone such extensive loss of coronal tooth structure that barely the natural crown was visible.



Figure 1. Left central incisor with class 3 Elli's Fracture

The rest of the upper arch revealed severe attrition. The lower arch had undergone complete rehabilitation with porcelain fused to metal crowns where various crowns had lost the ceramic coating over them. There was a general loss of vertical dimension of occlusion and the patient showed a deep bite.

The intraoral periapical radiographs concerning the central incisors revealed that the canals of both incisors were well obturated with no periapical abnormality. After the patient was thoroughly examined, the treatment plan was to restore 11 with composite and place a custom cast post for 21 followed by porcelain fused to metal crowns for both the incisors. As there was not enough crown structure to preserve a ferrule for final prosthesis the patient was referred to the Department of Periodontics for the clinical crown lengthening procedure.

The patient returned to the department after one week. The right central incisor was restored with composite and prepared to receive porcelain fused to metal crown. The tooth preparation was done in such a manner that angulation of the core is inclined more in the buccal direction to provide for clearance on the palatal side as the patient has severe deep bite. For 21, post space was created leaving at least 5 mm of Gutta-percha to preserve apical seal using upto size 3 of the Gates drill. This was confirmed using a radiograph. A ferrule was prepared on the remaining tooth structure.



Figure 2. Custom Cast Post

The canal space was lubricated with petroleum jelly and the impression of the post space is made with light body elastomeric material. Provisional restoration is made and luted with zinc oxide eugenol cement over 11, and the post space of 21 was blocked with cotton pellets.



Figure 3: Cementation of custom cast post and preparation of ferrule w.r.t 21



Figure 4: Tooth preparation of 11

The impression is poured with Class III gypsum material and the wax pattern is fabricated for custom cast post and core. It is invested immediately and casting is done.

This cast post and core (Fig.2, Fig.3, Fig.4) was cemented onto 21 with glass ionomer cement. The final impression is made with light body elastomeric impression material for fabrication of porcelain fused to metal crowns for both the central incisors. The provisional restoration is placed back. Shade selection was done under natural light. Final porcelain facing metal restorations are tried, glazed and cemented with glass ionomer cement.



Figure 5: Bisque trial of porcelain facing crowns w.r.t. 21 and 11



Figure 6. Cementation of Porcelain facing Crowns

Discussion

The prognosis after an Endodontic treatment of the tooth greatly depends on the post-treatment restoration and little on the endodontic treatment itself,though together with good endodontic treatment and good restoration success rates are seen to be as much as 91.4%. [9]

The post is indicated to aid in the retention of core in a tooth with reduced coronal tooth structure.[10,11] When an anterior tooth has undergone a minimal amount of tooth loss, it can be conservatively restored with bonded restorative materials.[12] In such cases,the post is of not much benefit as the tooth structure is sound it can increase the risk of nonrestorable failure.[13-15]

After an anterior tooth undergoes root canal treatment, the remaining tooth structure is pretty thin and reduces further if indicated for a crown. Anterior teeth are subjected to lateral and shearing types of forces

and after restoration, it should be able to withstand these forces. also, the small pulp chambers are not supportive in providing adequate retention and resistance. and the pulp chambers are too small to provide adequate retention and resistance without a post. Therefore in most cases, placement of post becomes necessary.

As a wide array of post systems available in the market and depending on the case, it is necessary to opt for the best type of post by weighing the advantages and disadvantages of all post systems during treatment planning.

Cast post and core have been the standard for many years and clinicians are still using these as it is advantageous in various situations. In cases where multiple teeth require post and core, to increase efficiency, one impression is made and they are fabricated in the laboratory instead of placing a post in and buildup in each tooth separately as a chair-side procedure. When there is a requirement in the change of angulation for the final restoration to match the adjacent tooth, as in cases of mild proclination, cast post and core can be given.[16]Cast posts are also indicated when there is the minimal residual coronal structure of the tooth, as in mandibular incisors with not enough bulk of tooth to provide anti-rotation features during tooth preparation. These posts are easily retrievable if the tooth requires endodontic re-treatment. Cast posts are capable of confirming to the canal morphology and hence can be used in different types of canal configurations like oval or elliptical.

These have fallen from favor over time because for it to be installed there is a need for two appointments, temporization, and a laboratory fee. On the other hand, according to Gomez Polo et al, cast metal posts have shown higher survival rates over10 years.[17] There have been reportsof a high rate of success with these post and core systems.[18,19] and the research till now is not entirely in favor of other post systems as there are still many unanswered questions.

A 2mm ferrule can increase the fracture resistance of a root canal treated anterior teeth, irrespective of the post system implied or the type of crown to be given. The reason for increased fracture resistance can be credited to the improved stress distribution.[20]The ferrule is a ring of sound tooth structuresthat envelope by the cervical third of the crown. The crown restoration braces the tooth around this ferrule to enhance the integrity and longevity, provides resistance and retention form on the endodontically treated tooth. It helps to transfer the occlusal forces vertically to the periodontium as the artificial crown and root act as one unit. The more is the modulus of elasticity of the post better is their performance. In the absence of ferrule, a cast post with high elastic modulus preferred, since there is less available tooth structure for bonding.[21-23]

The material of the post is not responsible for its fracture resistance and mode of failure. Instead, they are a result of various mechanical properties interacting with each other. But cast posts are preferred in cases where the integrity of the tooth is compromised in the cervical region or has been extensively destructed due to caries of fracture, especially when it is difficult to obtain a ferrule. In the present case, 11 had sufficient clinical crown structure, whereas in 21 the clinical crown was greatly reduced hence rehabilitation of the tooth was planned by placing custom cast posts followed by a metal-ceramic full-coverage crown. As there was insufficient ferrule for the tooth, it underwent the procedure for clinical crown lengthening of about 1 mm. In such conditions, periodontal surgery like gingivectomy with alveoloplasty or orthodontic extrusion becomes mandatory to obtain a ferrule.[24,25] Though all-ceramic crowns are the best option for full coverage restoration in Anterior teeth due to their aesthetic superiority, Porcelain facing metal crown restorations opted to mask the discoloration of the cast core. Also, there was not much clearance on the palatal side due to severe deep bite. This lead to the choice of Porcelain facing metal crown instead of full metal-ceramic crowns.

Conclusion

There are various post systems available today and the selection of the suitable system is greatly influenced by the clinician's knowledge of the indications and contraindications depending on the case and the amount of sound d tooth structure remaining. We should be well versed in the advantages and the disadvantages of these post systems to make a wise choice. In cases with reduced coronal tooth structure, custom cast metal post and core is recommended. Esthetics, other than function, is of great concern when considering the restoration of Anterior teeth.

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Rehabilitating Occlusion with a Guiding Flange Prosthesis in a Patient with Mandibular Resection: A Case Report

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Abstract

Defects with the discontinuity of the mandible following a segmental mandibulectomy present a major challenge for rehabilitation. These challenges include correction of deranged mandibular movements, disfigurement, and esthetics, correction of functional difficulty seen during swallowing,speech and articulation, and also of surgical side deviation of the remainder mandibular structure. The following case report elaborates on the prosthodontic rehabilitation of a patient with the help of a device named «Guide Flange Prosthesis»which isindicated to correct such manifestations seen after hemimandibulectomy.

Keywords: *Rehabilitating Occlusion; Flange Prosthesis; Mandibular Resection.*

Introduction

Mandibular defects present a great challenge for its rehabilitation. When there is a unilateral defect of mandible due to trauma or surgery,there is mandibular deviation towards the surgical site and the mandibular occlusalplane rotates tend to inferiorly. It also causes extrusion of maxillary teeth eventuallyresulting in a lack of occlusion and altered maxillomandibular relationships[1]. Extensive involvement of osseous and soft tissues, loss of sensory and motor innervations, and scar tissue formation after hemimandibulectomy radically compromise the prosthetic prognosis.

Most severe mandibular deviation & dysfunction is caused mainly when there is extensive loss of soft tissue which can occur from tight wound closure, radiation therapy, and the ones that require radical neck dissections[2].

The mandibular deviation can be reduced by retaining the patient's neuromuscular system. The basic objective is for the muscles to reprogram themselves andattain an acceptable interocclusal relationshipfor the remainder of the mandible, so that all opening and closing movements are mostly in control of the patient. These include early corrective mandibular movement therapy, physiological exercise programs, and removable partial dentures for partially edentulous and complete denture prostheses for completely edentulous patients along with all possible modifications in an occlusal scheme to compensate for deviation[3].

Cantor and Curtis have classified the mandibular defects into 6 categories:[4]

Class I: Mandibular resection involving alveolar defect with preservation of mandibular continuity.

Class II: Resection defects involve loss of mandibular continuity distal to the canine area

Class III: Resection defect involves loss up to the mandibular midline region

Class IV: Resection defect involves the lateral aspect of the mandible, but are augmented to maintain pseudoarticulation of bone and soft tissues in the region of the ascending ramus.

Class V: Resection defect involves the symphysis and parasymphysis region only, augmented to preserve bilateral temporomandibular articulations.

Class VI: Similar to class V, except that the mandibular continuity is not lost.

Prosthetic rehabilitation with conventional prostheses such as mandibular based guidance restorations, and palatal based guidance restorations or implant-supported

prostheses have been advocated for the correction of mandibular deviation[5,6]. A Guiding flange prosthesis (GFP) is a mainly a mandibular prosthesis designed in a manner to achieve a neutral mediolateral position of the mandible but is not made tomake consistent repetition of this position for mastication[7,8]. A guiding flange prosthesis can be either removable or fixed. Removable prosthesis gets support from both teeth and tissues. So it cannot be retained intraorally if only a few teeth remained in the sectioned mandible.

Case Report: A male patient aged 45 years, reported to our department with the chief complaint of reducing chewing capacity due to the inability in intercuspation of teeth and deviation of the jaw. The patient presented with a history of tobacco chewing for 10 years,10 times/day, and was diagnosed with early squamous cell carcinoma involving left buccal mucosa and mandibular alveolus. The patient underwent hemimandibulectomy on the left side 4 months ago with PMMC flap reconstruction for the defect. Radiation therapy was completed a month before.



Fig. 1: Patient showing deviation of mandible towards the side of the defect and inability to occlude on the normal side

Extraoral, the symptoms included facial asymmetry, deviation of the lower third of face towards the left on mouth opening, decreased mouth opening, left corner of the mouth drooping downward, angular cheilitis. The patient was unable to guide himself into occlusion (Fig.1). Intraorally, he presented with left mandibular defect distal from the midline, with surgical skin graft on the resected side, and missing teeth that include 31-37, and 25-27(Fig. 2). The patient was partially edentulous with Kennedy's Class II for both maxillary and mandibular arch. Orthopantomogram revealed the absence of the mandible distal to the midline. The case was diagnosed as Cantor and Curtis Class III mandibular defect. The treatment plan decided was mandibular guide flange prosthesis to aid in the correction of mandibular deviation.



Fig. 2: Hemimandibulectomy on the left side with missing molars in the upper arch. Reconstruction was done with a PMMC flap

Fabrication of Prosthesis:

- Primary Impression for the mandible was made using astainless steel edentulous stock tray made which was trimmed on the side of the defect and irreversiblehydrocolloid (alginate).
- The primary impression for the maxillary arch was also made using an irreversible hydrocolloid (alginate).
- Casts were retrieved by pouring Type III gypsum material into the obtained impressions.
- The patient is trained and guided into occlusion and a bite registration record is obtained.
- The obtained casts are articulated on a mean value articulator based on the recorded bite registrations.
- A stainless steel Orthodontic wire of 19 gauge, was used to fabricate a sub-structure for the modified Guiding Flange prosthesis.
- C clasps were fabricated on the first premolars and Adams clasp on first molars of the first quadrant of the maxillary cast.
- Another C clasp is fabricated on the first premolar and Adams clasp on the first molar of the fourth Quadrant of the mandibular arch.

- The wire was also used to fabricate a crib by bending it into zig-zag fashion for the length extending from the distal of the second premolar to the medial for the second molar of the fourth quadrant.
- The clasps are stabilized on the cast using modeling wax on the lingual side of the mandibular cast and palatal side of the maxillary cast
- The casts are coated with two layers of separating media.
- Using cold cure clear acrylic, the buccal vestibular flange is acrylic, for both upper and lower cast.
- After that lingual and palatal flange is fabricated using cold cure acrylic.
- The crib is stabilized in an incline along the buccal aspect of the mandibular first molar manually keeping the maxillary arch in articulation and then it is acrylic.
- The upper and lower fragment of the guiding flange prosthesis is removed from the cast and trimmed to not leave any sharp edges.
- Finishing and polishing was done for both the upper and lower fragments of the Guiding flange prosthesis
- Stability and retention are observed after the prosthesis is tried into the patient's mouth. (Fig. 3)
- Thus a smooth gliding flange surface is to achieve to guide the remaining portion of the mandible to a definite closing point to attain adequate occlusion.
- The Flange height should be such that it can guide the mandible into occlusion without any hindrance. This remains practically within the limit of the height of the buccal vestibule.
- The prosthesis was finally inserted into the patient's mouth and he was informed of all post-insertion instructions.
- The patient seems to be pleased with the overall performance of the prosthesis.



Fig. 3: Insertion of the Guiding Flange Prosthesis leading to a reduction in deviation of the mandible and proper intercuspation

Discussion

The extent and site of a mandibular tumor denote the amount of resection of the mandible, which could be from marginal, through segmental or Hemi, subtotal, or total mandibulectomy. When there is the unilateral loss of continuity of mandible, there is the deviation of the remaining segment of the mandible towards the side of the defect. This also leads to inferior rotation of the occlusal plane. The cause of this deviation is due to the loss of muscles and tissues during the surgical excision[9].



Figure 4: Maxillary component of the guiding Flange prosthesis with stabilizing plate to prevent orthodontist movement of the maxillary teeth.



Figure 5: Mandibular fragment of the guiding flange prosthesis with a crib as an inline plane to guide the mandible into occlusion

On segmental removal of the mandible, reconstruction is recommended immediately to preserve the facial symmetry and mastication ability of the patient. There have been various surgical techniques and Prosthodontic rehabilitation techniques, which with time have advanced, to help the patient improve function and esthetics but still 50% of these patients report with impaired masticatory function[10, 11. The] most common treatment options for patients after hemimandibular resection are palatal based guiding restorations, lower arch removable guide flange prosthesis maxillo-mandibular fixation, and/or implant-supported prosthesis. Amongst these, the most recent and recommended advancement in facial reconstructive surgery is osseointegrated oral and dentofacial implants, that may adequately rehabilitate oral cancer patients, helping them to return to a healthy, productive life. An implant-supported prosthetic rehabilitation cannot be considered since no bone graft [12]was used.

For healing to complete and the osseous graft to be accepted, it requires an extensive amount of time. Therefore, any definitive treatment should be delayed in the best interest of the patient. But during this period, there is a contraction of tissues leading to a gradual increase in difficulty to manage the defect prosthetically. A Guiding Flange prosthesis during this initial healing period helps to reduce the deviation of the mandible and improve the efficiency of mastication. This is achieved as the mandibular muscles get reprogrammed to establish a good occlusal relationship for the residual mandible[13].

Guiding flange prosthesis and any removable partial denture similarly attain support, the primary sources being the residual alveolar ridge and the remaining natural teeth. The best approach would be the placement of multiple clasps distributed widely, but the presence of natural teeth determines their placement. The rigidity of retentive elements should not be more than necessary, but it increases with a decrease in the number of teeth involved[14].

The mandibular guiding flange (Fig. 4) device fits generally over the good natural teeth in the mandibular arch, opposite the side of the defect (base-plate), and is attached with a guide plane (flange splint). From the buccal flange of the mandibular fragment of the prosthesis, a crib extends vertically into the buccal vestibule, contacting the buccal flange of the maxillary fragment of the prosthesis and buccal surface of the opposing maxillary teeth. This extension aids the mandible to properly position itself in the mediolateral direction and hence aids in vertical chewing. No or very little lateral movement is provided by this device[12,15]. This guide plane helps to accomplish centric occlusion during mastication as it is necessary for every patient to maintain it for the sake of attaining basic harmony[16].

To maintain esthetics of this patient during mandibular movements, the mandibular Guiding Flange Prosthesis was fabricated in transparent self-cure acrylic resin and all the wire substructure comprising of retentive wire components were deliberately kept distal to the mandibular canine to minimize the prosthesis display. To prevent palatal orthodontic movement of the maxillary teeth by the force generated by the buccal flange against them, a stabilizing plate was provided for resistance. The maxillary stabilization plate and all its wire components (Fig. 5) were also placed distal to the canines leading to their minimum display and maximize esthetics. The prosthesis must not extend into the junctionof the graft and the normal tissue as it can affect the healing[17.]

The Guiding Flange Prosthesiscan be considered equivalent to a training device for a patient with deviated and deranged mandible to successfully and consistently repeat the mediolateral position. In some patients, there may be some stress generation when the use of the device is continued for a longer period. All such stresses should be carefully monitored. If the reprogramming of muscles is achieved and the patient can occlude on his own, this device can be discontinued.

Conclusion

A long-standing segmental hemimandibulectomy without early intervention presents a challenge during rehabilitation due to a lack of hard use support and loss of muscles during excision. Therefore such patients require as early Prosthodontic intervention as possible to maintain function and esthetics. Mandibular guiding flange prostheses are utilized on an interim basis. However, a definitive mandibular guidance prosthesis with a metal guiding flangeis recommended after healing has complete and the patient can guide himself into intercuspation with the help of this device. This will lead him to successfully correct the deviation and achieve acceptable occlusion for normal mandibular function and mastication.

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Serial Extraction: A Review

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Abstract

Serial extraction or eruption with guidance is a pre-planned, premature extraction of certain primary teeth to create space that has been compromised. It is followed by the extraction of certain permanent teeth to maintain the proper proportion of tooth size and dentoalveolar bone space. However, this procedure is challenging as it requires in-depth clinical knowledge and skill. This article provides essential insights to the clinicians to identify and categorize the cases meticulously for serial extraction, apply both theoretical and practical aspects of growth and development, and diagnose the alarming signs of primary crowding in early mixed dentition period.

Keywords: *Serial Extraction, Primary Teeth, Mixed Dentition, Developing Malocclusion.*

Introduction

Serial extraction is fundamentally based on the concept that in certain cases the orthodontists face a challenging situation such as limited space between the arch length and total tooth material. Hence, it is crucial to enlarge the basal bone, so that all the teeth get proper accommodation. To tackle such discrepancy, one has to be skillful enough in both clinical diagnosis as well as basic knowledge regarding the growth and development of teeth so that the dental deformities can be promptly rectified while balancing the proper esthetics[2].

History of Serial Extraction: Serial extraction has remained an attractive procedure for the dentists since the 18th century. Two French dentists, Bunon and Bourdet were among the pioneers who explained that the extraction of more than one uneven teeth can enhance the aesthetic of the rest. Paisson was the first to recommend the procedure to relieve the crowding and align the teeth. Robert Bunon in 1743, in his publication"Essay on Diseases of Teeth", first wrote about the importance of early extractions. Linderer (1851)[5] suggested that the primary canines should be stripped or removed to accommodate the lateral incisors followed by the extraction of 1st premolar as to provide sufficient space for the eruption of molars. From the 1850s, for nearly two centuries, there was no marked improvement to the concept of serial extraction. However, after the second world war, a new era of modern serial extraction was established by many dedicated dentists such as Kjellgren of Sweden, Hotz of Switzerland, Heath of Australia, and Nance, Hoyd, Dowel and Mayne of the United States. Despite working independently during the period of compromised communications because of the second world war, Kjellgren and Hotz reached a common conclusion of the extraction sequence.

In 1929, Kjellgren coined the term "Serial Extraction" to describe a procedure where certain deciduous teeth were extracted followed by the extraction of permanent teeth to facilitate an uninterrupted eruption of the rest of the teeth. In 1940, Nance presented illustrated his technique of "progressive extraction" and has been called "Father of Serial Extraction philosophy" in the United States. In 1941, Rudolf Hotz suggested the

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term "Guidance of dental eruption by extraction" or "Guidance of eruption". As per him the term "guidance of eruption" is self-explanatory and comprises of all measures available for tooth eruption.

Overview of Dental Development:

1. **Incisor Liability:** The term "incisor liability" was first coined by Warren Mayen. It is defined as the difference between the amount of space needed for the incisors and the space available. In maxilla, it is 7.6 mm while in the mandible it is 6 mm. However, there is individual variation in incisor liability
2. **Inter-dental spacing:** In young patients, the interdental gap may vary from 0 to 10.0 mm(average 5.0 mm) in the maxillary arch, whereas in the mandibular arch, it fluctuates from 0 to 6.0 mm (average 3.0 mm). Lack of space is an obstacle for achieving proper alignment.
3. **Inter-canine width change:** During mixed dentition, the arch widens in between the primary caninesas compared to the primary dentition. Maxillary inter-canine widths coincide with the period of exchange between late mixed dentition to early permanent dentition but mandibular inter-canine width changes a little mostly after 10 years of age.
4. **Arch-length changes:** The total arch length from the mesial surface of the left permanent first molar to the opposite side changes over time. Normally mandibular arch length decreases while growing.

Why serial extraction is essential?: Dental crowding is one of the most prevalent malocclusions which occurs either due to deficient arch length or limited bone space or extra tooth material. To overcome this, we have to either reduce the tooth size or expand the dental arches. As there are chances of relapse even after the expansion of alveolar basal bone so the following factors should be taken into consideration. Physiologic tooth movement: Human dentition shows a physiologic tendency to move towards an extraction space. Thus by specific extraction of some teeth the remaining teeth which are in the process of eruption are influenced by the natural forces into the normal occlusion.

Indications: "When a pedodontist sees a child 5 or 6 years of age with all the deciduous teeth present in a slightly crowded state or with no spaces between them, with a fair degree certainly, that there will not be enough space in the jaw to accommodate all the permanent teeth with proper alignment" (Lysell 1960). The reason behind the need of serial extraction are early loss of primary teeth, arch length and tooth material discrepancy, lack of interdental spacing, lingually placed lateral incisors, unilateral primary canine loss and midline shifting, mesially erupting canines concerning lateral incisors, the mesial drift of buccal segment, flaring,ectopic eruption, ankylosis, etc, gingival recession on labially tipping incisors, ectopic eruption of permanent maxillary first molars resulting in early exfoliation of deciduous second molars

Contra Indication: Serial extraction should be avoided in certain conditions which include congenital absence of mandibular second premolars, mild to moderate crowding, deep or open bites, cleft lip and palate cases, spaced dentition, anodontia/oligodontia, midline diastema, dilacerations, Class 1malocclusion with minimal space deficiency, reverse overjet, deep bite,open bite, rotation, gross malposition, crossbite and severe class 2,3 of dental/skeletal origin.

Benefits: A surgical procedure not only affects the target tissue but also the tissue around the target. Some of the advantages of serial extraction are like reduced duration of the treatment & future appliance therapy, less psychological trauma experienced by the child, more utilization of physiologic force to achieve a normal occlusion, cost-effective, and chances of caries is decreased as better oral hygiene is achieved.

Drawbacks: It needs proper clinical visualization and judgment. The treatment duration can exceed the expected time frame necessitating multiple visits to the orthodontist. Hence, a high degree of patience and cooperation is needed from the patients. Sometimes it may develop tongue thrust and mandibular permanent canines may erupt ahead of 1st premolar into extraction space of the first primary molar, impacting premolar which leads to a strenuous extraction."Serial extraction has both its pros and cons but the pros outweigh all the cons"

Diagnostics Tools: Case history, Clinical assessment, Intraoral periapical radiographs, Orthopantomography, Cephalometric analysis, Intra and extraoral photographs, Study models, Functional analysis Mixed dentition space analysis are a must to diagnose a case of serial extraction.

Treatment Planning

Selection of Teeth for Extraction:

1. Primary canine: The four deciduous cuspids are extracted so that optimum space can be created for the alignment of the teeth and lingual crossbite of interiors can be avoided.
2. Primary 1st molar: 1st primary molars are extracted for the uninterrupted eruption of first premolars. But in this process, the rate and amelioration in crowdingis decelerated.
3. Erupting 1st premolar: When the upper permanent canine is erupting it is advisable to remove the first premolars as it is the essential step of serial extraction. The n it should be rechecked if the teeth are sound and healthy enough to continue the process and relieve of crowding to justify the extraction of 1st premolars.
4. Enucleation permanent canine buds provide maximum distal translation of which is not required in some cases because it protrudes the chin.

Procedure: There are several method of execution of serial extraction. The method of choice is dependent on each individual. The techniques are described below

Dewel's Method (CD4): In 1978, Dewel suggested a 3-step technique of serial extraction which is still popular and even being used nowadays.

Step 1: This step comprises of removal of primary cuspids which results in the creation of space for the normal alignment of the incisors. At the age of 8-9 years, this step is executed

Step 2: At the age of 9-10 years, this step is carried out i.e., almost exactly after 1 year primary first molars are extracted to speed up the eruption of first bicuspids.

Step 3: In this step, the erupting first premolars are removed to make some space for the permanent canine to erupt into the spaces that have been vacant by the first premolars.

A modified DEWEL'S method is applied to some cases where the first bicuspids are enucleated at the time of removal of primary molars mostly in the lower arch as the canines follow the regular erupting pattern.

Tweed's Method: In 1966, Tweed recommended that all primary molars should be extracted by the age of 9 years followed by the removal of the first bicuspid and the primary cuspid.

Nance Method: The procedure followed in this technique is the same as that of the Nance method. It suggests the removal of the primary molars followed by the extraction of the first bicuspids and deciduous canines.

Grewe's Method: Grewe's method of serial extraction usually focuses on the planning of extraction patterns for a variety of clinical cases.

Moyer's Method: Central incisor region is prone to crowding. In that case, Moyer's method is recommended.

Step 1: All primary lateral incisors should be extracted to prevent the malalignment of the central incisors.

Step 2: Then after 7-8 months, all the primary canines are extracted to permit space for the accommodation of central incisors.

Step 3: This step includes extraction of all primary first molars which further accelerates the eruption of first bicuspids.

Step 4: In this ultimate step, all the first premolars are removed after 7-8 months so that speedy and uninterrupted eruption of canines can occur.

Role of Pedodontist: In serial extraction, pedodontist and orthodontist should have mutual understanding for treatment planning. A pedodontist should observe the problem, provide appropriate treatment planning for serial extraction,convince the patient or parents, and refer the case to the orthodontist for future correspondence.

Conclusion

Serial extraction is a matter of time, patience, proper implementation of knowledge and clinical skill for accurate diagnosis, and most importantly follow up. Along with this deficient arch length,the amount of crowding, and health of the remaining hard and soft tissues must be considered. The objective of this technique is to intercept the developing malocclusion at an early mixed dentition period so that further malocclusion can be interrupted. Although this procedure does not eliminate the need for comprehensive orthodontic treatment, however, it can lessen the duration of treatment. Treatment can't be avoided for midline diastema, root parallelism,

incisor angulation, and other malalignments. Thus serial extraction helps in rectifying these irregularities to some extent.

Ethical Permission: Not required

Conflict of Interests: None

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Crowns in Pediatric Dentistry: A Review

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Abstract

Dental caries is the most common chronic disease of childhood. To treat such severely destructed teeth is a challenge for most pediatricians because of three important considerations-patient's behavioral management, tooth structure preservation, and satisfaction of parents. Various efforts have been made for full coverage restorations in pediatric dentistry. Every material has its own merits and demerits. Various options available nowadays are stainless steel crowns and its various forms like strip crowns, zirconium crowns, etc.

Keywords: *Crown, Pediatric, Stainless Steel, Caries.*

Introduction

According to the WHO report, 2003 60% to 90% of school children are affected by dental decay in industrialized countries.[1] Nowadays different techniques and method are available for primary teeth restoration. Stainless steel crowns have been used for restoration since 1947 when it was first introduced by Rocky mountain company but familiarized by Humprey and Engel. But they have been recommended where pulp therapy has been performed or where other restorations are likely to fail. Despite all the biggest drawback of stainless steel crown is–poor esthetics.[3] This article aims to discuss the advancements regarding restorations of full coverage in pediatric dentistry.

Pre-Formed Metal Crowns: These were referred to be an acronym of SSC. It was first described by Engel followed by Dr. William Humphrey in 1950.[4] The metal used in preformed crown was soon transformed to nickel-chromium and nowadays also popularly named as preformed metal crown (PMC).

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Indications (Use In Deciduous Teeth)[5]: Stainless steel crowns are preferred in these situations as procedures like pulp therapy as a restoration for prevention, extensive decay in primary tooth, as an abutment for a denture or space maintainer in children requiring general anesthesia for dental treatment should be given consideration, in severe bruxism, in rampant and recurrent caries, inherited or acquired enamel defects,as part of a space maintainer. Advantages are durability is similar to that of the deciduous tooth, it protects the remaining tooth structure which was weakened by excessive caries removal, less technique sensitive,cost effective, and has a low failure rate. Disadvantages are Unesthetic due to metallic appearance andcannot be used in partially erupted tooth. Different types of stainless steel crowns according to shape are as follows:

Untrimmed Crowns: These crowns are neither trimmed nor contoured. It is time-consuming and requires a lot of time for adaptation. Example – Rocky mountain.

Pretrimmed Crowns: These crowns have straight, non-contoured sides. These are designed to follow a line parallel to the gingival crest. They still need some trimming and contouring.

Precontoured Crowns: These crowns are festooned and are also pre-contoured, though a minimal amount of festooning and trimming may be required. Example–Ni-Chro Ion crowns and Unitek stainless steel crowns

Open-Faced Stainless Steel Crowns: he crowns which are preformed though are the most reliable and durable restoration for a primary incisor but have poor aesthetics. To take advantage of its strength and to make it attractive, the cosmetically prominent aspect of the crown should be cut away by the dentist, enough of luting cements to be removed and the void is filled with bonded resin composite.[6]

Success of Open Face Stainless Steel Crown is by:

- Using dentin bonding
- Helps bond resin to teeth tissue.

Phosphoric acid etching: A porous and rough structure layer on remaining glass ionomer cement. Holding tags are formed by unfilled resin, and thus bonding occurs.

Advantages: Appearance is a lot more appealing than the plain metallic appearance of the stainless steel crown.

Disadvantages:

- Time-consuming procedure
- Metal margins can still be seen
- Lifespan is short
- Poor color stability under oral conditions.

Preveneered Stainless Steel Crown: Preveneered stainless steel crowns are more appealing because they have the combined effect of the durability of conventional stainless steel crowns and esthetics of composite resin. A variety of mechanical and chemical bonding approaches retain esthetic veneers on stainless steel crowns. Initially, these were developed for primary anterior only but later were also designed for primary molars. Different types of PVSSCs available depend on the use of facing attachment to the SSC, clinicians ability to crimp crown[8],shades available, and crown length.

Advantages: Good esthetics with relatively short operating time. Durability is long. It can be used with ease where moisture condition is difficult.

Disadvantages: PVSCCs have more thickness than conventional SSC due to the addition of resin,which results in more extensive tooth preparation for proper fit and occlusion.[9] It is more expensive so not cost-friendly. Dentists don't have many options on resin shade, so at times supplied crowns look artificial.[9] Crown forms that are tried in,cannot be sterilized under pressure with high heat, as such treatment will destroy the resin layer. These crowns have overly convex appearance so re-shaping is required,which takes additional laboratory or clinical time. Multiple approximating crowns are difficult to place in people with loss of space due to bulk or crowding. Inflexibility and brittleness seen on resin facing material has a tendency of breakage when subjected to heavy force.

Polycarbonate Crowns: In pedodontic practice, most common lesions which occur are due to nursing bottle caries. More severely carious teeth require either composite crowns, stainless steel crowns, or polycarbonate crowns. Polycarbonate crowns are 'temporary crowns given as fixed prosthesis to primary anterior teeth which are likely to get exfoliated in future'. These are linear polyesters of carbonic acids. These are termed as thermoplastic resins as exhibit high impact strength and rigidity and can be molded into solids by pressure and heat into the required form. Esthetically these were appealing but disadvantage being brittle and no resistance against strong abrasive forces.

Polycarbonate Crowns Include:

- 3M ESPE Polycarbonate
- Kudos polycarbonate crowns
- PedoNatural Crowns

Uses: Extensive caries requiring full coverage restorations like nursing bottle syndrome. It can be used in fractured or malformed teeth,in discolored teeth, in teeth requiring restoration after pulpotomy or pulpectomy procedures.

Contra Indications: Bruxism, Deep bite, Abrasion

Zirconia Pediatric Crown: These are crowns made of zirconia and donot contain metal. These are utilized for a variety of computer-aided manufacturing/computer aided design restorations, including framework/ hand veneer,implant abutments, full-contour fixed prosthodontics. Though zirconia is widely being used for permanent dentition but primary dentition it is a new restorative material. Zirconia has demonstrated superior corrosion-resistant, high wear resistance, and excellent biocompatibility. Some of the commercially available pediatric zirconia crowns are: E Z Pedo Crowns, NuSmile Crown, Cheng pediatric crowns, Kinder crowns.

Strip Crowns: Strip crowns were first introduced

in 1979 by Webber et al. These are crown forms filled with composite and are bonded onto the tooth and the crown form is then removed. These are considered to be the most popular and esthetic restorations for deciduous anterior incisors. Although esthetically appealing, but its retention depends on the amount of tooth structure remaining after excavation of caries. This is the first choice of most clinicians as it can be easily repaired if the crown fractures or chips off. This is but technique sensitive.



Figure 1. Strip crowns

Pedo Jacket Crowns: Use of pedo jacket crown is similar to that of celluloid crown form, except for thepedojacket is made up of a tooth-colored copolyester material, which is filled with resin and left on the tooth after polymerization instead of being removed.

Disadvantage: It can neither be contoured nor aligned with a high–speed bur used for finishing as it will cause the material to melt onto the bur[11]. It is available in only one shade, which is very white, so shade matching of correspondent, non-restored tooth is challenging.

New Millenium Crowns: These are similar to pedo jacket crowns only for it constitutes laboratory –an enhanced composite material made of resin and bonded to the tooth. The crowns are esthetic and even can be trimmed and reshaped with a high-speed bur. These crowns are more susceptible to fracture or crack if forced into a preparation that has not been reduced adequately.

Advantage: It is highly esthetic and parenteral satisfaction is high.

Disadvantages: Technique sensitive and for successful treatment, proper isolation, and achievement of hemostasis are required.



Figure 2. Pedo Jacket Crowns

Indications: It can be used in discolored primary incisors. Also indicated in multisurface or extensive caries in deciduous incisors. Fractured incisors following trauma can b restored with these crowns**.** In developmental defects like Amelogenesis imperfect[12], these crowns can be used.

Contra Indications: Not adequate if excavation of caries results in less remaining tooth surface area. Not indicated in impinging deep bite and in the presence of periodontal disease.

Nusmile Crowns: These were introduced in the year 1991[13]. Used when a full-coverage restoration is required for longevity and to protect the remaining tooth structure. They are available in 2 forms Nusmile Signature and Nusmile ZR.

Advantages: These are anatomically correct, color-compatible tooth colors, can be easily placed,less time consuming,less technique sensitive, and durable. It has color stability.

Cheng Crowns: These were developed by Peter Cheng in the year 1982. These are crowns with a facing of pure resin which makes them stain resistant. These can be used for both anterior and posterior teeth. Advantages are less time consuming,less technique sensitive,require a single patient visit,less patient discomfort, and is stain resistant.

Dura Crowns: These preveneered crowns are esthetic and can be placed with poor moisture or hemorrhage control. These are made up of high-density polyethylene veneered crown.

Advantages:

- Facial and lingual surfaces may be crimped.
- Easily festooned
- Easily trimmed
- Full knife-edged margin capabilities

Kinder Crowns: These are known for providing shades that appear natural and good contour for the patient. These were introduced in 1989. Kinder crowns are known for their finely feathered margins, scientifically developed shades, incisal edge. They come as zirconia Kinder crowns or a preveneered Kinder crown and are available for anterior or posterior teeth.



Figure 3. Kindler crowns

Pedopearls: These are aluminum crown forms having a coating of a tooth-colored epoxy paint. These are relatively softer and may cause the epoxy paint to wear off in areas of heavy occlusion. As they are softer can be easily crimped and cut but durability is poor.[14]

Conclusion

This article throws light onto various aspects for restorations of full coverage in pediatric dental practice. There isa wide range of articles and materials to rehabilitate carious lesions but each has its advantages and disadvantages. Research is still going on different crowns and materials to be used for full coverage .clinicians have used these above-mentioned crown for decades with success. The choice of material depends on clinician preference, skill,functional and esthetic needs of the child and parent.

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Zygomatic Implants: A Review

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Abstract

Zygoma implants (or zygomatic implants) vary from traditional dental implants in that they attach in rather than maxilla (upper jaw) in the zygomatic bone (cheekbone). They can be used where the consistency or quantity of maxillary bone is insufficient for normal dental implant placement. Inadequate maxillary bone volume can be due to bone resorption as well as maxillary sinus pneumatization or a mixture of both. The required bone height for typical implant placement in the upper jaw posterior area should be approximately 10 mm to ensure appropriate implant survival. Where there is inadequate bone available, bone grafting procedures and sinus raising procedures should be done to increase bone volume. Procedures for bone grafting in the jaws have the downside of extended care, limiting denture wear, the morbidity of donor surgical site & graft rejection.

Keywords: *Zygomatic implants, maxillary bone.*

Introduction

People having a chief complaint of the missing tooth are sacrificed in the function of chewing, problems related to speech, esthetic look, and being self-confident in social gatherings. However, this is not something new in this profession. Dentistry is the first oral care professionals to improve from the invention of osseointegration by Per-Ingvar Branemark who was first to place implants inside the oral cavity in the year 1965, has become the genuinely acceptable modality of procedure in patients, who has lost their teeth: implant can be used as a substitute.[1] Modern dentistry aims to reimburse patients' to oral health in a predictable manner. The partial and complete edentulous prosthesis may not be able to recover normal form & function, esthetics, comfort, or speech.[2]

Accessing Quality of Implant: The benchmark for evaluating implant quality is mobility & pain. Probing depths might help to find any local disease or pre-existing thickness of tissue before the implant was inserted. When there is an increase in depth of probing it signifies bone loss, hyperplasia of gingiva, or hypertrophy. Loss of bone is best valued with probing depths than with IOPAR. Implant quality factors were established by Jarnes and modified by Misch in an implant quality scale that not only assesses the implant health-disease but affects the treatment and prognosis of the existing conditions.[2] Due to lack of internal bone (cortical & cancellous) stimulation and non-physiologic crestal bone loading, it causes of an atrophic edentulous maxillae's continuous resorption. The result is not being able to use a conventional full denture prosthesis.[3] A collection of strategies like composite or complex grafting with bone and Le Fort osteotomy to an easy procedure as sinus bone augmentation have a different success rate. Such treatment protocols have a separate surgical treatment and often requires one to two month of healing or even more, before placement of the implant. The substitute for such a stage-wise method requires the **zygomatic implant** invented by Branemark. [4]

The zygoma implant is a length-extended (30–52.5 mm) machined titanium screw which is inserted via the crestal bone(i.e palatal) part of the posterior (resorbed) maxillae trans-antrally inside the compact bone of the zygoma. Moreover, 2 or 4 conventional screws are inserted in the maxillae (anterior part). Starting support of these screws is assured by its contact with four bony cortices (Fig-1).

1. At the crestal ridge.
2. The floor of the maxillary sinus.

3. The sinus roof.
4. The superior border of the zygoma.

It has been reported that the zygoma implant gives posterior maxillary support when the surrounding bony structures don't permit implant insertion. The adjunct to the implant is an augmentation of bone graft (sinus lifts and on-lay grafts) along with the cost of their attendant, dis-comfort, prolonged treatment time, and high chances of complication rate. The zygoma screw is suggestive in instances like:

When the entire maxillary (edentulous) arch is associated with advanced posterior resorption which will need graft otherwise. Two or preferably four anterior implants are required along with both bilateral zygoma implants. In partial or incomplete maxillectomy individuals where more implants might be inserted in other areas i.e pyriform sinus, rims of the orbit, palatal shelves, or pterygoid plates to provide anchorage to cross-arch stabilization. [3]

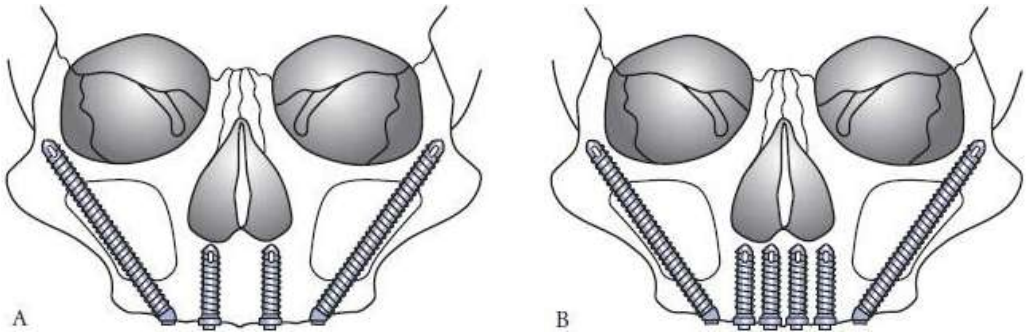


Figure 1A. Pictorial representation of recommended zygoma and standard implant fixtures cross-arch stabilization and fixed restoration. B. Diagramatic representation of zygoma and implant fixtures for restoration with cross-arch stabilization and fixed restoration.

Indications & contra indications: The zygoma implant is usually preferred in candidates having moderate to severe atrophy but it can be thought of as a beneficial choice in patients with or without any significant atrophy, requiring posterior maxillary implant support.

1. Moderate Atrophy.
2. Severe Atrophy.
3. Syndromicpatients e.g ectodermal dysplasia patient presents with partial anodontia.
4. Acquired & congenital defects. E.g cleft palate, rhinectomy usually required after malignant tumors of the nose.[13]
5. Partial edentulism.
6. Immediate loading.

Contra Indications:

1. Any sinus pathology.
2. Systemic diseases.
3. Zygoma implant is not required where there is adequate bone in the maxilla to facilitate implant placement in positions & numbers which can help in retention of a prosthetic appliance.
4. Where there is no enough pre-maxillary bony anchorage, for 2 stable implants with the best potential longevity. The volume and condition of the anterior bone of the maxilla are determining factors on which differential diagnosis often depends rather than existing posterior maxilla whether the edentulous patient may be suitable for this procedure.

Medical Evaluation: Medical history includes those medical conditions most likely to influence implant treatment decisions. The physical examination consists of a hands-on evaluation and recording of the patient's vital signs. The second, section (Laboratory Evaluation) reviews those laboratory tests of interest to implant dentistry. The evaluation includes a complete blood count (CBC), sequential multiple analysis (SMA), and bleeding disorder tests. The third section relates the medical and dental implications of the common systemic diseases found in implant patients, which have the greatest impact on implant dentistry.[5]

Pre-surgical assessment

Both physical and mental stability of the patient is required to withstand the surgical procedure approximately 2 hours long and to endure deep intravenous sedation or general anesthesia. Adequate mandibular range of motion of the patient is needed for proper access while the placement of fixtures 30-52.5 mm long transpalatally in the region of the zygomatic buttress. The placement of the zygoma fixture may be hindered in the presence of opposing mandibular teeth. In the case of deep sedation, local anesthesia in the mandibular arch, as well as in the surgical site itself, is advisable.[6]

Radiographically using an image-guided oral implantology system (IGOIS) is introduced including the framework, 3D-reconstruction, preoperative planning, registration, and the motion tracking algorithm to transfer the preoperative plan precisely. With the help of stereolithography technology, fabrication of the jaw model and a surgical drill guide with skeletal support is done referring to the finalized treatment plan. The main objective is the creation of an individualized drill guide that is suitable for the patient's bone profile. A CAD/CAM program utilizes the contour of the bone and the 3D information of the planned drill course for designing the drill guide. The drill guide is then made by using stereo lithography. Before performing the surgery, and intended simulatory operation is performed on the stereolithographic maxillary model using the surgical drill guides.[7]

Current trends in clinical dental implant therapy involve using endosseous dental implant surfaces embellished with nanoscale topographies. Existing data supporting the role of nanotopography have shown crucial steps in osseointegration can be modulated by nanoscale modification of the implant surface. Nanoscale modification of titanium endosseous implant surfaces can change the cellular and tissue responses that may be beneficial for osseointegration & dental implant procedure.[9]

Surgical Protocol: General anesthesia or deep intravenous sedation offers the best option for the zygoma implant placement surgery. Administration of local anesthesia- maxillary nerve blocks, vestibular infiltration and infiltration or percutaneous blocks laterally and superiorly to the zygomatic notch (just lateral to the orbital rim) should be done. When the surgery is performed with sedation, a bilateral inferior alveolar nerve block can be advantageous as it ensures adequate access because of the significant retraction of the lower lip, tongue, and mandible.[10]

A crestal incision is made in the region of the first molar-second premolar slightly to the palatal aspect of the ridge. It is placed from the right to left maxillary tuberosity regions and bilaterally releasing incisions are given at the incision ends. Sufficient full-thickness mucoperiosteal flap is reflected to expose the lateral maxilla which aids to visualize the zygomatic buttress from the crestal ridge to the superior surface of the zygoma at the zygomatic notch, just lateral to the orbital rim. To avoid tearing of the flap while retracting, the anterior maxilla is exposed till piriform rims, which facilitates the placement of the conventional pre-maxillary implants. A "slot" window is created vertically on the lateral wall of the maxillary sinus in close approximation to the height of the zygomatic buttress.[11] The slot is prepared with a fissure bur, generally a 703 or 702, in a straight surgical handpiece. The prepared slot on the lateral wall of the sinus should be parallel to the planned course of zygoma implant placement which lies just medial to it. The extension of the slot should be from close to the floor of the sinus just superior to the planned site of implant installation to near the sinus roof. The window is prepared by the surgeon for the direct visualization of the implant placement, passing of all drill preparations, and instrumentations. During the slot preparation, the Schneiderian membrane is removed from the sinus wall to facilitate better visualization and to prevent its interference at the time of site preparation and implant installation.[12]

For the incremental preparation of the planned site, a series of long drills are used. The length of the zygoma implant varies from 30 to 52.5 mm. The diameter of alveolar one-third and apical two-thirds is 5 mm and 4 mm, respectively. To enhance the visualization and aid in the position of the surrounding structure, a customized zygoma retractor with a toe-out tip is placed in a position over the zygomatic notch through the entire procedure of slot preparation. There is a midline marker on the retractor parallel to the site preparation which assists in the orientation of the drills in the proper direction. To complete the site preparation, these drills are used sequentially- 2.9 mm diameter long twist drills, 2.9 mm-3.5 mm pilot drill, and 3.5 mm twist drill. Direct visualization of the implant insertion is done through the slot preparation on the lateral wall of the sinus.[12]

Insertion of the implant is done along with substantial irrigation. The implant should be in the same plane as that of the drills during the implant placement to enable the engagement of the implant in the preparation site at the body of the zygomatic bone. On engagement of the apical portion of the implant 2-3mm into the dense zygomatic bone, the handpiece will come to a halt after the adequate preparation of the site. To complete the installation of the implant, a manual driver is employed when the handpiece comes to a stall. Determination of the proper abutment platform angulation is done with the help of the placement of a screwdriver into the screw head of the implant carrier and placing it until the screwdriver becomes perpendicular to the crest. It is then followed-up by the removal of the implant carrier and placement of a cover screw.[13] 2-4 regular platform (Mark III or Mark IV Nobel Biocare) implants are inserted in the pre-maxillary region following the installation of the zygoma implants. Subsequently, repositioning of the flap and suture placement is done. Relief is given in the maxillary denture by making it hollow at the site of implant emergence and relined with the tissue conditioner. The impression of the implant level is made before the closure. This facilitates fabricating a rigid bar which will be placed at the second stage of the surgery around six months later.[14]

Prosthetic Procedure:

Healing Phase: The existing or the provisional maxillary denture can be remodeled for immediate use for preserving the patient's esthetic. Significant limitations may be noted in the case of functional use like alteration in retention or masticatory ability, but an alternative of having teeth through the entire treatment process is usually far more pleasing than the interim periods of no prosthesis that accompanies many graft procedures.[15]

Protective splinting: On combining with splinting and cross-arch stabilization, the strength these implants provide is one of their unique features. If they are utilized or loaded independently, it is perceived that the off-axis load transfer can be pernicious and feasibly ineffective for the maintenance of osseointegration. It is recommended to take some precautionary measures to hinder the independent transfer of the stress from the base of the denture to the implants individually promptly following stage II surgery, or uncovering of all implants with abutment connections. A gold bar measuring around 2 mm in diameter is contoured accordingly so that it's in contact with a set of gold cylinders which are affixed to the abutment analogs on the cast. With the help of a micro-welding device, the soldering of the bar and the cylinders together can be accomplished and the fabrication of passive protective splint can be achieved within a short period. Within the next day usually, the bar splint is delivered and the denture is hollow ground for complete seating without the interference of the bar. An absolutesoftliner can be appliedat this time tothemaxillaryprosthesisfor the enhancement of retention and comfort. If the patient is not wearing a maxillary prosthesis, bar splint may not be necessary but in the case where constant denture wear is advisable, the bar splint protocol should be followed.[4-6]

Final prosthesis construction: After an adequate period of healing, final impressions can be taken which is usually after 3 to 4 weeks. With approved wax-up silicone putty indexes are made which are then used to provide a matrix for creatinga metal bar structure. After the evaluation of passive fit and esthetics in the second try-in appointment, with heat polymerizing resin the prosthesis is processed. Using appropriate screws and screw torques to provide even and complete seating, the delivery is accomplished.[7,8]

Discussion

The complete restoration of the severely atrophied maxilla along with the implant placement is a challenge faced by both the surgeons and the prosthodontists. In the case of exclusive conventional implant placement in this condition, the insertion of the implant requires extensive bone grafting and usually also involves sinus upliftment and onlay grafts with a larger amount of donor's bone. The important considerations are to be taken involve the inconvenience of the patient, prolonged duration of the treatment, potential complications, lower rates of implant success, donor site morbidity, and cost. This is further complicated by the patient's inability to wear a prosthesis for an extended period—a factor that hinders many patients from pursuing the treatment. Avoidance of bone grafts if not indicated, shortened treatment duration, no requirement of the donor sites, and the continuous use of transitional prosthesis by the patient are the perks of zygoma implant rehabilitation. As a result, greater patient compliance is achieved while furnishing the patient with a stable, well-tolerated, and esthetically accepted removable or fixed prosthesis at the time of treatment completion.[11-15]

The benefits for the consideration of the zygoma implant include the following:

1. Reduced or entire elimination of the donor site morbidity.
2. Markedly reduced or eliminated the treatment duration.
3. Survival of the bone grafts and consolidations are into considerations.
4. Reduced the total number of implants to support the prosthesis.
5. Relatively economical mode of treatment and less invasive than other alternatives.

The disadvantages include the following:

1. It's a demanding surgery— well trained technical skills are required. Experts capable of dealing with any surgical situation or complications that might arise should perform the surgery.
2. The risk to injure adjacent structures- orbital contents, orbit, lacrimal apparatus, infraorbital nerve, the facial nerve.
3. Chances of postoperative sinusitis, although relatively less than with the sinus liftprocedures.
4. Failure of the fixtures—although it is rare, more difficult to retreat the access. General anesthesia or deep sedation is required as with all properly planned and executed implant prosthetic surgery, it also requires extensive coordination between the surgeon and the prosthodontist before commencing the treatment.

Ideally, at the time of surgery, the prosthodontist should be available. Similarly, the prosthetic requirements andtechniques of fixture positioning and final restoration should be familiarized by the surgeon. Finally, the important steps of the procedure and its ultimate success include education of the patient, preparation, assessment, and informed consent. Before initiating treatment, patient understanding should include the need for meticulous oral hygiene and maintenance. Proper understanding and appropriate use of the zygoma implant, provides an alternative for several patients with atrophic edentulous maxillae.[11-15]

In conclusion, we recommend zygoma implants in the atrophied edentulous maxilla for the following reasons-

1. The rate of successful osseointegration is greater than 96% for the zygoma implant.
2. Decreased surgical interventions for the patients.
3. Does not require bone harvesting or grafting as a necessity.
4. Decreased overall operating and working time for the surgeon.
5. The surgery of a zygoma implant can be accomplished within the office setting.
6. Precise placement of the zygoma implant platform on the crest of the maxillary ridge permits the access screw hole of the zygomaimplant to surface in the central groove of the first molar.
7. There is no requirement for any angled or custom made abutments in the final restoration.
8. No additional restorative dental or laboratory time is required in comparison to the traditional implant techniques.
9. The entire laboratory and prosthetic charges for the zygoma implant rehabilitation are comparatively equal to or less than conventional implants.
10. Relatively the expense of zygoma implant therapy is quite economical than the grafting procedure for the patient.

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Platelet-Rich Fibrin: An Update

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Abstract

The development of bioactive surgical additives to regulate the inflammation and increase the speed of the healing process is one of the great challenges in clinical research. In this sense, platelet-rich fibrin (PRF) appears as a natural and satisfactory alternative with favorable results and low risks. The following review attempts to summarize the relevant literature regarding the technique of using PRF, focusing on its preparation, advantages, and disadvantages of using it in clinical applications and its updates. PRF alone or in combination with other biomaterials seems to have several advantages and indications both for medicine and dentistry, due it is a minimally invasive technique with low risks and satisfactory clinical results.

Keywords: *Blood Platelet; Fibrin; Platelet-Rich Fibrin; Hard Tissue Regeneration; Soft Tissue Regeneration.*

Introduction

Platelets are generally produced in the bone marrow by the megakaryocytes and their sizes vary from 2–3 μm in diameter. They are consists of several granules, mitochondria, and canalicularsystem. The re is also another structure known as theα granules which are oval or round in shapes with diameter 200 to 500 nm which is covered by a membrane (Figure 1 : Platelet). These create a mass storage of proteins that are vital for wound healing, including platelet-derived growth factor (PDGF), transforming growth factor (TGFb), and several others. The fusion of αgranulewith the platelet cell membrane occurs after activation. This again leads to the formation of bioactive proteins. Then these proteins allow them to bind with the target membrane or cells. After the attachment, intracellular signal proteins are activated. This ultimately creates a gene expression sequence which leads to cell multiplication, collagen synthesis, and new cell production.[1]

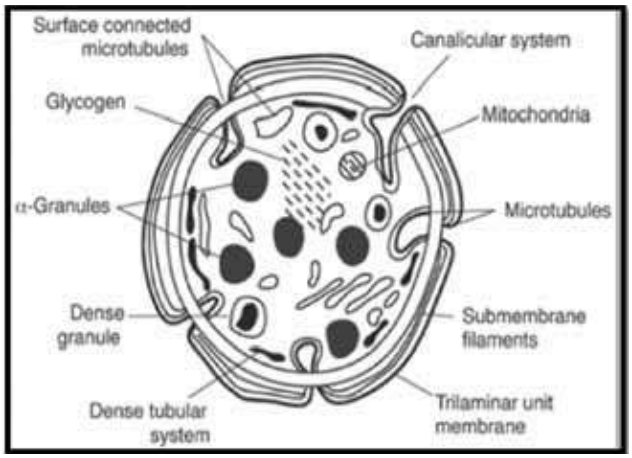


Figure 1: Platelet

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Figure 2 : Platelet Concentrates

Platelet Concentrates: PRF was first introduced by Ross et al in 1974 and it was also discovered that there are presence of several growth factors in PRF (Figure 2 : Platelet concentrates).Platelets play an important role in both the bleeding control and wound healing. From the early 90s, there are discoveries regarding numerous blood-related growth factors which can accelerate the wound healing procedure.[1]

Classification[1]:

1st Generation–PRP (Depending on the presence or absence of leukocytes and the activation or not of the PRP) Mishra et al, 2009

- Type 1, PRP is a L-PRP solution
- Type 2, PRP is a L-PRP gel
- Type 3, PRP is a P-PRP solution
- Type 4, PRP is a P-PRP gel

2nd Generation–PRF (Based on their fibrin architecture and cell content) Dohan Ehrenfest 2009

- Leukocyte poor or pure platelet-rich fibrin (P-PRF)
- Leukocyte and platelet-rich fibrin (L-PRF)-Choukroun's PRF
- Leukocyte poor or pure platelet-rich plasma (P-PRP)
- Leukocyte & platelet-rich plasma (L-PRP)

History of Platelet Concentrates:

Fibrin sealants: It was first available in the market since the late 1970s in Europe. Fibrin sealants or 'fibrin glues' are derivatives of plasma which helps in the blood coagulation process by the formation of a fibrin clot. The y are currently used as topical hemostaticagents. Widely used for flap closure in aestheticzones. As there is enhanced risk in cross-infection, nowadays tissue sealants are produced from the patient's plasma.[2]

Pure PRP or Leucocyte-Poor PRP: This is prepared without the addition of leukocytes, it results in low-density fibrin clot.[2]

Leucocyte-Rich Fibrin and Pr: Products are preparations with leucocytes and with a high-density fibrin network.[2]

Pure Prf or Leucocyte-Poor Prf: Preparations are without leucocytes and with a high-density fibrin network. Unlike pure PRP or PRP containing leukocytes, these products cannot be injected and exist in an activated gel form.[2]

Leucocyte-Rich Fibrin and Prf: Products are preparations with leucocytes and with a high-density fibrin network.[2]

Leukocyte and platelet-rich fibrin (l-prf)-choukroun'sprf

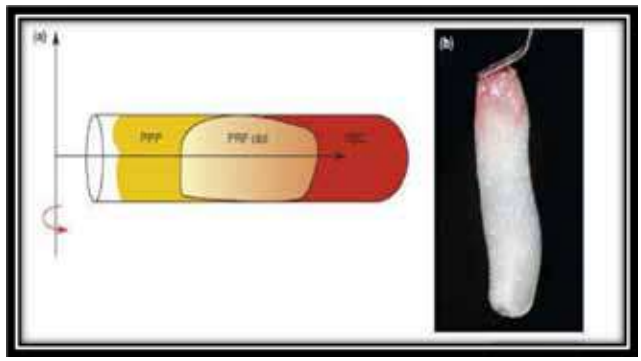


Figure 3 : Choukroun's Prf

PRF was first introduced by Choukroun et al, 2001 (Figure 3 :Choukroun'sPRF).

The procedure of making PRF uses neither anticoagulant nor bovine thrombin.[4]

Prf Protocol: Choukroun et al. in France produced the PRF by taking up venous blood (around 5 ml) in 2 test tubes without any addition of anticoagulant and the tubes are then centrifuged at 3,000 rpm, (approx. 400g) for 10 min, which creates 3 distinct layers.[4]Upper straw-colored acellular plasma, red-colored lower fraction containing red blood cells (RBCs), and the middle

fraction containing the fibrin clot. The upper straw-colored layer is discarded and the middle fraction is collected, which is PRF.[4] (Figure 4 : L-PRF)

Antimicrobial Property of Prf: An in vitro study, it was found that i-PRF has maximum antimicrobial efficacy rather than any other platelet preparations because of its high platelet count.[9]

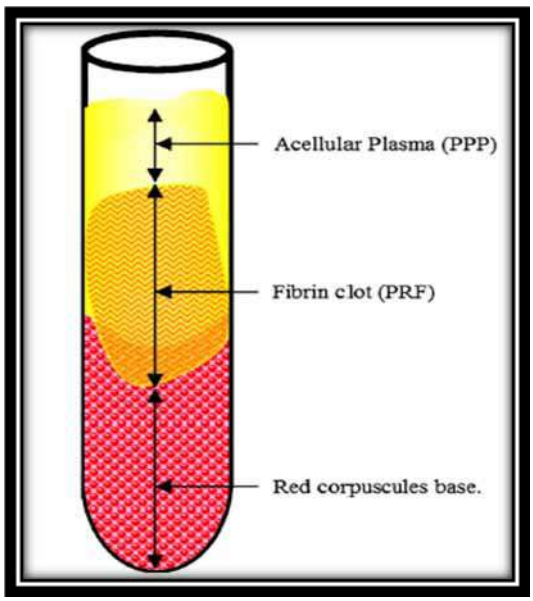


Figure 4 : L-PRF

Importance of Various Parameters for Centrifugation:

Relative centrifugal force, Radius of centrifuge and Rotation per minute: Various authors have reported centrifugal g-force at the PRF clot (referred to as relative centrifugal force (RCF)-clot – location at which the PRF clot is formed), whereas others have utilized the international standard method to report g-force calculated at the bottom of centrifugation tubes (RCF-max).[6]

Biological Differences of Platelet-Rich Fibrin Clots Based on Centrifugation Parameters[6]: PRF clots fabricated at lower centrifugation speeds and times improve growth factorrelease and cellular behavior owing to higher cellular content and growth factor accumulation. PRF clots fabricated at lower centrifugation speeds and times are smaller in size, however, contain more platelets and leukocytes, as well as with more growth factor release. The g-force calculated at the fibrin clot (RCF-clot) is subject to change owing to the centrifugation time – even when centrifuged at the same speed. For these reasons, this method of reporting g-force is inferior in accuracy and not commonly reported internationally. The g-force calculated at the end of the centrifugation tubes (RCF-max) is not subject to these differences – hence, why it is internationally utilized to report g-forces.

PRF Box:



Figure 5 : PRF Box



Figure 6 : PRF Box with PRF

The PRF Box (Process Ltd., Nice, France) is commercially available to prepare the PRF membrane. The PRF clot is placed on the grid in the PRF box and covered with compressor lid which squeezes out the fluid from the clot (Figure 5 : Prf Box). The membranes formed using this method had constant thickness which remain hydrated for several hours and have recovered the serum exudate expressed from the fibrin clots.[4] (Figure 6 : Prf Box with PRF).

Application of PRF:

Socket preservation in case of tooth extraction: A study was performed to check the efficacy of PRF in extraction sockets andthat study showed faster-wound healing, bone regeneration along with the high-density bone formation.[12]

L-PRF block for bone augmentation procedure: The L-PRF block seems a successful new protocol for horizontal alveolar bone augmentation. This procedure is safe, predictable, with a high feasibility and a low morbidity.[7]

L-PRF in intrabony defects: The use of PRF as the sole grafting material seems to be an effective modality of regenerative treatmentfor periodontal intrabony defects at 6 months after the surgery.Greater reduction in pocket depth, more gain in clinical attachment level and greater intrabony defect fill at sites treated with PRF than those treated with open flap debridement alone.[14]

L-PRF in furcation defects: PRF has shown good results in reducing probing depth, improved clinical attachment level, and defect fill. Also, it was proven as an effective regenerative material for treating class II furcation defects.[15]

PRF as a Barrier Membrane in Guided Bone Regeneration: The use of PRF has popularity because it's biological scaffold capable of improving tissue healing. It is always advised to place PRF membranes over-top of the collagen barrier membranes. In this way, PRF membranes can be utilized to speed defect closure, improve soft-tissue wound healing/regeneration, and further bear the added defense component from pathogen-fighting leukocytes capable of significantly reducing the rate of infection and complication.[5]

L-PRF in fenestration and dehiscence: Guided tissue regeneration in combination with platelet-rich fibrin can successfully be used to treat fenestrated root apices. Satisfactory healing of the gingival fenestration defect with excellent color, texture match with the surrounding area along with the keratinized tissue formation emphasizing the importance of using PRF as a membrane in esthetically demanding areas.[16]

L-PRF application in delayed implant placement: A study showed that PRF may improve the post-insertion stability of dental implants during the time of healing.[17]

L-PRF in implant placed in sinus lift: It was seen that sinus floor elevation can be done by PRF placement alone as a regenerative material. The potential clinical implications for this inexpensive and autologous material are promising. Treatment approach with PRF was effectuation active in treatingthe peri-implant defects.[18]

L-PRF in wound healing: The use of PRF in a case of delayed and complicated healing following subepithelial connective tissue graft harvest action has proved to be effective in reducing donor site morbidity of free gingival grafts.[13]



Figure 7: Titanium-PRF

Titanium-PRF[3]: A new method for the formation of L-PRF was introduced in which normal test tubes are replaced by titanium test tubes which can omit the silica particles from dry glass. This new formulation is known as T-PRF which is a modified PRF. The material itself forms an adhesive oxide layer and quite popular because of its enhanced property of osseointegration in dental implants (Figure 7 : Titanium-PRF)

Usage of different materials for blood processing during PRF preparation[3]: Tunali & co-workers used medical-grade titanium tubes to produce PRF and called it T-PRF. On examination, it was observed that T-PRF samples have more organized structure and integrity compared to the L-PRF samples. Reddy et al, 2018 concluded that T-PRF helped in the treatment of intrabony defects by showing radiographic defect fill.

Advanced PRF[10]: For creating A-PRF, slow speed(1500 rpm) for a longer duration (14 mins) is used in vacuum sterile glass tubes. Enhanced B- & T-lymphocyte entrapment, more even distribution of platelets, neutrophils. The number of platelets is significantly more in A-PRF and is better deployment of resident monocyte, macrophages, and lymphocytes. Clinically, this would be beneficial as it would translate into an increased amount of growth factor and cytokine release. Pinto & co-workers demonstrated that A-PRF protocol produced lighter, shorter, narrower clots with

light polymerization and more squashed bodies. A-PRF application has proven more effective than PRF for reduction of probing depth,CAL as well as increasing bone fill.

Advanced PRF + (A-PRF+)[19]: Fujioka- Kobayashi & co-workers in 2016 formulated this combination by reducing both RPM and centrifugation time (1300 rpm for 8 minutes). The authors argued that decreased centrifugation time would lead to the production of more viable platelets in the PRF matrix. When they assessed the PRF produced by this protocol to L-PRF and A-PRF in terms of growth factor release, biocompatibility & cellular activity, they observed that A-PRF+ demonstrated the highest release of PDGF, TGF- β1, EGF and IGF. Also, in culture, gingival fibroblasts exposed to A-PRF+ revealed significantly higher levels of PDGF, TGF-b, and collagen-1 at three and seven days measured in terms of mRNA expression.

Injectable PRF (I-PRF)[19]: Injectable PRF (i-PRF) formulation is one of the recent development. For producing i-PRF, blood-filled test tubes without anticoagulants are centrifuged 700 rpm (60 g) for 3 minutes. Another set of authors has proposed a similar protocol where they centrifuge plain blood in non-coated test tubes at 2400-2700 rpm for around 2 minutes. The supernatant is collected and they have named it concentrated growth factors (GCF).Used along with bone grafts for regenerative purposes as it forms a gel-like consistency. Mixing the bone graft with i-PRF also gives the benefit of growth factor release at the recipient site. It was also seen that by mixing PRF clot, i-PRF and bone graft combination has shown improved efficacy in regeneration.

PRF Lysate: A novel preparation of PRF based products is the PRF lysate. In this, after PRF preparation, it is incubated at 37°C in a humidified atmosphere of 5% CO2/95% air and the exudate thus collected has been referred to as PRF lysate.[19]

Current Updates (Recent Studies Carried Out by Different Authors): Miron et al in 2019 revealed a new method for the preparation of PRF, rather than using normal centrifugation technique, a horizontal method is used which can result in higher platelets/leukocytes. The y have also shown a 10 fold increase in the platelets and leucocyte count by utilizing only 0.3–0.5 mL of sample. Tsujino et al, 2019 suggested that tube types and centrifugal speeds affect the types of growth factors that are released over the periodand the retention of those factors in the periodontal defect. Along with that, they also added that even though silica-coated testtubes are easily available, they should not be used to avoid health hazards. Wang et al, 2019 showed that combining L-PRF with bone marrow stem cells has the potential to replace the normal human bone structure.

Conclusion

PRF as a biologic surgical additive has been successfully used for varied applications in dentistry. Technological advancements in the field of PRF have paved the way for the versatility in the applications of the platelet concentrates. Therefore, in the future, more advances can be expected in the field of PRF which will be beneficial in different aspects.

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Combating Risks in Dentistry During Covid-19 Pandemic

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Abstract

The year 2019- 2020 has given rise to a menace in the form of Corona Virus Disease, which within no time has turned into a pandemic, affecting every corner of the world. It has disarranged the day-to-day lifestyle of the society, economy, employment, trade, hunger, etc. Dentistry is also one of these, affected by the pandemic, and has caused heavy financial loss for the dental personnel, along with delay/halt in the dental treatment to a massive population in need. When eradicating the disease has become a question, it is better to live with the disease and restrain the risks of transmission in the dental operatory by following few crucial and mandatory steps like proper hand hygiene, maintaining social distancing, creating awareness, fumigating the operating rooms and surfaces etc. This review article thus elaborates the expedients a dental surgeon should follow to combat the risks of transmission of this fierce disease during dental interventions.

Keywords: *COVID-19, Dentistry, Infection, Transmission, Risk.*

Introduction

Coronavirus disease is caused by a new type of virus SARS-CoV-2 i.e. severe acute respiratory syndrome coronavirus 2 which, as the doctors evince, can trigger infection of the upper respiratory tract (sinuses, nose, and throat) and lower respiratory tract (trachea and lungs) as well.[1] It is also namedas COVID-19 owning the full form–Corona Virus Disease, 2019. It is declared a worldwide emergency/the global pandemic by the World Health Organization (WHO). Alike SARS-COV, SARS -COV-2 also uses the same host receptor, namely human angiotensin-converting enzyme 2 (ACE2).[2]

Signs and Symptoms[3]: On an average, the patient becomes symptomatic after 5-6 days of infection from the virus, to manifest it might require up to 14 days too. These are, fever, dry cough, tiredness, which are the most common symptoms of all. Less common symptoms are aches and pains, sore throat, diarrhoea,headache, a rash on skin, conjunctivitis, loss of taste or smell. And the serious symptoms being, "difficulty in breathing or shortness of breath, pain in the chest, loss of speech or movement."

Coronavirus and Dentistry: The COVID-19 virus spreads via droplet transmission, especially from person's cough or sneeze. Transmission can be through:

Direct Contact: When an individual is in close contact, i.e. within around 1 metre with COVID-19 patient, and the patient has not covered their face when coughing or sneezing.

Indirect Contact- surfaces and clothes act as reservoir for the infected droplets. Therefore, touching the infected surfaces and applying those hands on the eyes, mouth and nose, can transmit the disease.

For human-to-human transmission, saliva plays a crucial role. Aerosols can, hence, be a source of transmission for dentists, and they may unknowingly get exposed, especially when patients are in incubation period of Covid-19 or choose to not reveal that he/she is

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positive.[4] Of all the procedures, scaling and handpiece-based procedures are the ones generates highest droplets or aerosols, putting the operator at maximum risk.[5]Moreover, the characteristics of the dental clinical setup and basic measures followed to prevent cross contamination with infection are not adequate to stop the transmission of Covid-19.

Prevention of Transmission:



Hand Hygiene[6]: It is considered as the most compelling procedure, one should inculcate in its habits, to decrease the transmission risk of the microorganism. This reinforcement is due to, as stated earlier, SARS-CoV-2 persists for few hours lasting till several days on the surfaces. The persistence although depends on the humidity as well as temperature of the environment and the texture of the surface.[7] Hand hygiene is taken care of by using hand washing and hand sanitizing measures.

Procedure of hand washing: This should be done for around 20-30 seconds.

Wet hands using water

↓

Scrub the hand properly with soap

↓

Rub the hands palm-to-palm.

↓

Interlace fingers by keeping right palm over left hand's dorsum and vice versa.

↓

Following this, rub by interlacing fingers palm-to-palm.

↓

Rub the backs of fingers to the opposing palms with fingers interlocked.

↓

Rub the left thumb in rotational motion with the right palm and vice-versa.

↓

Following this, continue rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice-versa.

↓

Rinse hands in running water.

↓

Close the tap using the elbow.

Hand sanitization–Effective, Rub-in hand disinfectant with composition of propanol and ethyl-hexadecyl-dimethyl ammonium ethyl sulphate, commercially available as **Sterillium** can be used. Hygienic Hand Disinfection: Rub Sterillium well over clean, dry hands and nail grooves for 30 seconds.Surgical Hand Disinfection: Rub Sterillium well over clean, dry hands, grooves and up to elbows for 3 minutes.[8]



Personal Protective Equipment (PPE)[9]: This includes gloves, masks,protective gowns and goggles as well as face shields. This provides a protective canopy to the skin and mucosa from infected blood and droplet secretions. The respiratory route is the primary mode of transmission and hence and N-95 mask or other masks with particulate respirators are recommended for daily practice in dental operatory.[10] The maximum limit to which the PPE can be worn is 6 hours and apart from the operating instruments and surfaces, nothing else should be touched.[11] The equipment should be changed after every patient/procedure.



Social Distancing: The front office staffs in the waiting room area should maintain as much distance possible, with the patients. The payment procedures, consent form fill-up, feedbacks etc should be done digitally. Apart from this, the seating arrangement in the waiting area be such that at least 3 feet/1 metre distance should be maintained between everyone. Masks should be made mandatory. Care should be taken to not keep more people waiting and provide them appointments for the next day, to avoid the room from getting congested.[12] Avoid touching other persons, like handshaking, shoulder tapping etc., as much as possible.



Operatory Asepsis: operatory surfaces that are repeatedly soiled are best protected with disposable covers (barriers), that can be discarded after each treatment.

For"dental unit trays, paper, plastic film or surgical pack wraps (paper or towels) should cover the entire tray."[13]It is better to minimize or avoid the procedures that produces aerosols and if not, use of high-speed ejector should be done. Anti- reflux equipped handpieces can also decrease the cross-contamination.



Waiting Room Sanitization: Each surface that comes in touch of the infected person/operator, say for example magazine, chairs, doorknobs, tables etc., is a potentially infection risk surface. It is therefore crucial to use alcoholic disinfectants frequently, to sanitize the surfaces along with the air conditioning system.



Unit Dose Concept: Unit dose concept was introduced with purpose to minimize cross contamination. It basically refers to dispensing of amount of materials sufficient to accomplish a procedure, prior to patient contact.[14]



Patient Awareness: No measures taken can be fruitful, if the patients themselves are not aware of the seriousness of the disease. Hence, initiatives can be taken by the dentists to clue-up the patients about the disease, its harm, steps to prevent it from spreading to others and themselves. Advertisements, leaflets, projecting videos, coaching them with hand washing techniques and hand sanitizing techniques etc. are the few ways to develop awareness. It is also important to provide patients oral hygiene instructions and educate them the importance of proper and regular brushing as well as flossing, and balanced diet, so as to avoid the oral diseases from developing and the need to run to a dentist.

Patient Management During Covid-19 Crisis: During the outbreak of COVID-19, emergency procedures should be considered for treatment, ensuring limit of contact between the individuals. However, this cannot go on for long and there is a need to restore the normalcy and keep the work in progress. Therefore, in response to ethical decision-making process for treatments, the interventions in dentistry can be divided into categories:

Emergency Interventions: Fatal conditions that needs immediate treatment can be ruled out by managing with minimally invasive procedures and along with least/ no aerosol production. Uncontrolled bleeding, facial bone trauma, compromising the patient's airway, cellulitis or a diffuse soft-tissue bacterial infection with intra-oral or extra-oral swelling that potentially compromises the patient's airway comes under emergency category.

Urgent interventions: "Severe dental pain from pulpal inflammation, biopsy, pericoronitis or pain in the third molar, tooth fracture resulting in pain or causing soft tissue trauma, avulsion/luxation surgical post-operative osteitis, dry socket dressing, abscess, or localized bacterial infection resulting in localized pain and swelling, dental treatment required prior to critical medical procedures, gingival irritation, comes under this category." Here too, attempt should be made to complete the procedure in one or least number of appointments with least generation of aerosols.

Non-urgent interventions- These are the elective procedures and can be postponed according to the need of the hour or treatment can be provided to minimum number of patients to decrease the exposure risk. This includes, general oral examinations, recall appointments, routine scaling and root planing, preventive restorations, orthodontic procedures, treatment of asymptomatic teeth, or any aesthetic dental procedure.

To curb the risk of cross- contamination, disposable devices and instrumentation can be made use of. The load of virus in the saliva of the patient can be reduced by providing prophylactic mouth rinse of 0.23% povidone-iodine, for 15 seconds before any procedure.[15]

Rubber dam isolation can also be used to check the spread of microorganisms.[16]

Although not feasible, extraoral radiographs in place of intraoral, can be taken to reduce salivation and transmission risk.

It is advised not to prescribe Ibuprofen for pain management in suspected/confirmed cases of COVID-19, though strong reason is not yet specified.[17]

Also, it is important to follow procedures like temperature scrutiny of the patients along with strict protocols should be followed, making it mandatory to use masks and sanitizer by every visiting patient.

Conclusion

The fundamental of treatment of COVID-19 is to follow containment measures. This is how in the countries like China and South Korea, there is an exponential decrease in the development ofnew cases. Till the pandemic is under control, it is merely possible to avoid providing dental treatments, specifically keeping in mind the ethical and financial issues. Therefore, dental personnel can act according to the above-mentioned steps and decrease the risks of COVID-19 transmission. Also, for vulnerable age groups like older adults and paediatric patients, new guidelines should be imposed as soon as possible, for their management.

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Liquid Biopsy: A Tool for Detecting and Monitoring Oral Carcinoma

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Abstract

Oral squamous cell carcinoma is frequently occurring malignant tumour of the H & N (head and neck) region; with a rising oral cancer burden despite attempts of prevention, delay in diagnosis leads to loco-regional spread and poor prognosis. Hence there is an utmost need for biomarkers which could lead to early diagnosis of oral carcinoma. In the quest for innovative and early diagnostic aid, liquid biopsy is evolving as animportant tool in diagnostic oncology. A liquid biopsy also called a dynamic biopsy or fluid phase biopsy is a non-invasive analysis of non-solid biological tissue predominantly blood. Malignant tumours release different biomolecules into body fluids that have the potential to be used as biomarkers for diagnosis, prognosis, and therapy selection. As the clinical application of Circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), & exosomal miRNAs are quite significant they have acknowledged colossal attention have acknowledged gigantic consideration. Evaluation of the circulating biomarkers has made it easy for unique therapeutic methodologies and meticulous medicine. An increasing amount of studies have indicated that application of these biomarkers intended for detecting, therapeutic scheduling, reaction observing and prognosis management. Even though these novel biomarkers can deliver widespread series of conceivable clinical applications yet there arenot any authenticated circulating biomarkers which have been unified into the clinical practice for oral carcinomas. This article is a brief review of the various biomarkers used in liquid biopsy as well as about tumour educated platelets.

Keywords: *circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), Exosomes. Next-generation sequencing, tetraspanins.*

Introduction

Liquid biopsy is a potential revolution in the carcinoma diagnostics which not only helps in detecting the tumour but also monitors the progression of the disease. As a result of the novel biotechnologies and diagnostic potential, it has emphasized the restrictions of present sampling method; out-dated biopsies and minor operating techniques are invasive, indicted with impending problems, occasionally cannot be repeated and executed when clinical circumstances have deteriorated or when a tumour is unreachable. Additionally, the genomic profile of biopsy tissues delivers a tumour profile restricted to a particular period, and may also show the genetic heterogeneity of manytumouralsubclones. These boundaries of the standard technique of biopsy are quite evident in monitoring the evolution of the ailment during follow up. Because of these drawbacks in currentages, the research is being focussed more on the cells that are circulating in the blood which not only helps us in knowing the location of cancer but helps use in monitoring the progression of the disease.Circulating cell-free DNA fragments get separated from apoptotic or necrotic cancer cells are labelled as circulating tumour

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DNA and exosome specifically, these are encapsulated subcellular structures which contain proteins and nucleic acids released by tumour cells.

Liquid biopsy mainly focuses on the segregation of these tumour cells from the peripheral blood smear. The insufficiency of nucleic acid, as well as difficulty in differentiating among normal and tumour nucleic acids, have been overcome by using next-generation sequencing techniques, which helps in precisely detecting the genetic and epigenetic aberrations. It presently offers high specificity, permitting the assemblage of healthy and reproducible data in a simple and non-invasive way by a blood sample. Even now in 21st-century liquid biopsy procedure is not carried out routinely.

Reasons for opting liquid biopsy:

Following are the reasons for considering liquid biopsy over the standard method of biopsies:

1. It aids in understanding the spatial and temporal heterogeneity of cancer.
2. Necessitates less quantity of blood to carry out the investigation
3. It is minimally invasive
4. Helps in quickrecognition of cancer.
5. Actual time observing for therapeuticreactions and resistance could be performed by repeated analysis
6. Shorter turnaround time for genotyping.

Why not tissue biopsy?: It is invasive, lacks representation of the complete variety of malignant clones, multiple sampling is not feasible and despite all these drawbacks, it is still the gold standard in understanding the characteristics of the tumour.

Circulating Tumour Cells (CTCs): There has been tremendous development in isolating the tumour DNA from the heterogeneous components of blood due to the advancements in biotechnological applications since it was first discovered in 1869. Circulating tumour cellsget separated from primary or secondary tumour sites; they circulate in the blood and are accountable for distant metastasis. CTCs are quite rare and occur at a very low frequency like 1CTC per 10^6 -10^7 leukocytes in the initial stage of diseases. CTCs have irregular morphology & they can differ according to cancer type and stage. Circulating tumour cellscan form anaggregate with primarytumour cells or with fibroblasts, leukocytes, endothelial cells or platelets, with more susceptibility to cause distant metastases.

The key dissimilarities are their outsized size up to 20-30 μm, mechanical plasticity, and dielectric mobility properties in comparison to the blood cells. Successful techniques of isolation are membrane filtration, density gradient stratification dielectric mobility, photo-acoustic & microfluidic separation.[1, 2]. The principal documented function of CTCs is in prognosis; in advancing tumours, scientists have found that the total number of CTCs in a 7.5ml blood sample is expressively related by the prognosis, with a precise cut off value. Cristofanilli et al reported "in metastatic breast cancer the patients with more than 5 CTCs in 7.5ml blood have shorter progression-free survival and overall survival compared with patients with a lesser count".[6] CTCs significantly provide information related to the epigenetic changes occurring in tumour cells. DNA methylation in liquid biopsy has proved to be a prospective biomarker for staging, prognosis.[7]

Circulating Tumour DNA: Mandel and Metais (1948) gave their principal experimental confirmation of cfDNA in blood.It took nearly 3 decades for its application in carcinomas when Leon et al proved that the circulating tumour DNA levels have increased in the pancreatic cancer patients.Till date, various mechanisms regarding how the ctDNAis entering the blood have been hypothesized. In physiological circumstances, circulating free DNA are separated from the apoptotic and necrotic cells by phagocytosis by macrophages and scavenger cells [9,8] in addition tothey can be released by the living cells. In a healthy human being the concentration of plasma cfDNA ranges from < 10ng/ml to> 100ng/ml with a half-life of between 0.16hrs and 2.5hrs. Normal physiological situations such as inflammation, trauma, or exhaustive exercise can also lead to the release of cfDNA but the DNA which are released due to tumour tend to have large fragments as there occurs irregular digestion leading to the formation of DNA fragments with large kilobasepairs.

Exosomes: Exosomes are nano-sized vesicles discharged by cells and evident in the body fluids, such as plasma, urine, saliva or ascites. The se are dissimilar from the extracellular vesicles which usually get separated directly from the cell membrane, likemicrovesicles (50-1000nm in length)apoptotic bodies, exosomes are the last product of the recycling endosomal pathways and originate from intrinsic budding of the plasma

membrane[10]. However, they were formerly regarded as cellular discarded products it is now documenteda key role where they act in intercellular communication reliant on the cargo of functional molecules from donor to distant cells. Many studies have shown that these cells are involved in cancer progression and metstatization. After getting separated from cancer these cells encourage EMT and affect propagation, relocation and attack of tumour cells as well as are supportive to the angiogenesis and institute an immunosuppressive milieu[16].

Exosomes are evolving as an original chemo-resistance mechanism, chieflyreliant on drug liberation throughvesicle budding, nullification of antibody-based drugs, and exosomes arbitratedtransfer of micro RNAs (miRNA).[15]Exosomes containa lipid bilayer which encompassesboth trans-membrane and non-membrane proteins, as well as noncoding RNAs, mRNAs, and either ss or ds DNA[14]. Concerning proteomic analyses, Exosomes are categorised through a preservedset of proteins autonomous of their cellular origins, such as CD63, CD81, and CD9 tetraspanins.[10] Nevertheless, the protein configuration of exosomes coarselybear a resemblance to originating cells, thereforesignifying an exosomal cell-type or tissue-specific signature.[12] It has been established that definite RNA transcripts are augmented up to 100 times in exosomes paralleled by the donor cells, therefore backing up active packaging.[11] Various procedures are established to resourcefully gatherexosomes from bodily fluids. The present protocols follow the vesicle detachment concerning the biophysical characteristics, which includes the dimension, morphology and density, whereas others are based on the immune-affinity captured.[16,3] Till now the regularly followed protocol adopts series of centrifugations with an increase in speed to eliminate cellular debris and greater plasma membrane-derived vesicles, following this procedure is the sedimentation of EXOs by ultracentrifugation.

Tumour educated platelets: This is the newer concept that is being considered in liquid biopsy. Platelets are not educated by the tumour but they form the first cell type that interacts with a tumour which leads to change in their mRNA profile. Since platelets are available abundantly in the blood it does not need high sensitisation techniques to isolate them in comparison to the circulating tumour DNA or exosomes.

In the last decade, there was much appreciation for the involvement of platelets to the immune system of the body in health and certain diseases including cancer too.[4] They are regarded as "scanning soldiers" of the immune system, in that way detecting the existence of bacteria inflowing the bloodstream, lymphocyte cross-communication, and then regulating immune cell extravasation [14]. In cancer patients, the platelets function on tumour resident cancer cells as well as the tumour cells that have entered the bloodstream. Initially, platelets generate an environment supporting the neovascularisation by providing the tumour with various pro-angiogenic factors like VEGF, PDGF and b-FGF and stimulates expression of the factors[15]. Then, platelets reduce the apoptosis of tumour cell and anoikis and induce the switch of epithelial mesenchyme in tumour cell with direct physical interaction and discharges of TGFb molecules[17].Consequently, a significant contribution to the metastatic process is done by the capacity of platelets to safeguard tumour cells in motion from the normal immune response.

Then, there is the formation of a cell fibrin platelet aggregation encircling the CTCs which provides mechanical protection, and it transmits major histocompatibility complex class I to CTCs, which results in supplementary safeguardin contradiction of natural killer cells. Current lab-on-a-chip microfluidic methodexaminationdiscovered the occurrence of animpending CTC sub-population with heightened platelet coverage and perhaps platelet-mediated defence. On activation, platelets can discharge numerous growth and pro-angiogenic issues[3], and after these issues are unconfined at a metastatic niche, a tumour-stimulating growth microenvironment is generated[4] Therefore, platelets are an essential constituent of the tumour microenvironment and are deliberated asignificantfeature of oncology as they contribute to tumour initiation, tumour progression, and therapy response. Attention has been rehabilitated in cancer avoidance facilitated by daily use of aspirin, a provocative topic spanning many years. Aspirin canconstraintumour-intrinsic cyclooxygenase activity, which mayregulate intracellular signalling ways and the local inflammatory response, and platelet-resident cyclooxygenase activity, which can affect procedures such as platelet activation and aggregation.

Challenges: Since it is still an emerging study there many obstacles that we need to overcome for this technique to be used routinely. Following are the challenges that we face presently:

1. We still lack in recovering huge amounts of analytical samples from the liquid biopsy.
2. Detecting genes translocation and RNA expression data is a puzzling task.
3. We cannot identify those early-stage cases.
4. To recognise the fundamental modifications that recognise or resistant tumour cell clones.
5. Justification and reproducibility of molecular and computational data.
6. Dispersion and application of liquid biopsy on anenormous scale as repetitive analysis.
7. The decrease in mutational/expressional data complexity.

Future Aspects:

1. High throughput assaying such as multiplex digital PCR and expression array.
2. Advanced genomic method having higher sensitivity to recognise any mutations occurring in matched ctDNA and tumour tissues
3. Enhanced understanding of dynamics of the biology of CTCs, exosomes and ctDNA release.
4. Calibration and logical authentication of techniques for liquid biopsy
5. Overview of cheaper biotechnology and computational software.
6. Using this software to implement the databases for a gigantic amount of data.

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Gingival Diseases in Children and Adolescents

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Abstract

Gingival diseases are prevalent in both children and adolescents. These diseases may or may not be associated with plaques, maybe familial in some cases, or may coexist with systemic illness. However, gingiva and periodontium receive scant attention as the primary dentition does not last for a considerable duration. As gingival diseases result in the marked breakdown of periodontal tissue, and premature tooth loss affecting the nutrition and global development of a child/adolescent, precise identification and management of gingival diseases is of paramount importance. This article comprehensively discusses the nature, spectrum, and management of gingival diseases.

Keywords: *Gingival diseases; children and adolescents; spectrum, and management.*

Introduction

Children are more susceptible to several gingival diseases, paralleling to those observed in adults, though vary in numerous aspects. Occasionally, natural variations in the gingiva can masquerade as genuine pathology.[1] On the contrary, a manifestation of a life-threatening underlying condition is misdiagnosed as normal gingiva. Hence, it is crucial for dental professionals to acquire expertise and diagnostic skills to identify the diseases in childhood before it turns out to be a catastrophe.[2]

Anatomical Consideration: Gingiva of children and adults differ anatomically. Gingiva of the primary dentition usually appears pale pink, but paler than that of adults. The marginal gingiva has rich vascularity but comprises fewer connective tissues. The thinner, reddish epithelium with mild keratinization may be misdiagnosed as inflammation. Lesser variability in the width of the attached gingiva in the primary dentition results in fewer mucogingival problems. The interdental papilla is broad buccolingual, and narrow mesiodistally. The junctional epithelium associated with the deciduous dentition is thicker than the permanent dentition. Gingival sulcular depth in children varies from 1 to 2 mm, which is shallower than that in adults.[3]

Epidemiology: Previous investigations have revealed the frequent occurrence of gingivitis of various intensity in children and adolescents. These investigations illustrate that the universality of severe forms of periodontal pathologies is fewer in the younger population as compared to the adults. Al Bandar and colleagues in 1996 evaluated a substantial number of adolescents in the United States and reported the existence of gingivitis in 82.1% of the participants. Several other studies from other parts of the world reported comparable results. Epidemiological studies indicate that loss of periodontal attachment and supporting bone at different locations can be recognized in 1% to 9% of 5 to 11year-old, 1% to 46% of 12 to 15-year-olds(juvenile periodontitis) and as low as 2.75% of 16-17-year-olds (chronic periodontitis). When a broad mixture of the population was taken into consideration, reports revealed

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a fewer universality of periodontitis among pediatric and adult patients. Many investigators have proclaimed that higher plaque quantity and fewer inflammation in children as compared to young individuals. However, physicians recognize that the tissue destruction related to the periodontal pathologies in children and adolescents are generally self-healing and less prevalent compared to that in the adults.[4-7]

Classification of Gingival Diseases:

A. Gingival Disease Associated With Plaque

1. Without Local Contributing Factor

Plaque Induced Gingivitis: The presence of plaques is the prime mover of this pathological entity. Dental plaque usually builds up more briskly in the 8-12 years age group compared to the adults. However, gingivitis in the pediatric population is typically less severe than in adults with the same plaque level. Inflammation is generally restricted to the marginal gingiva with insignificant depletion of bone or connective tissue attachment in the majority of the cases. Red, linear inflammation accompanied by underlying chronic changes including swelling, augmented vascularity, and hyperplasia. Bleeding and deep pockets are not common findings in children. The greatest intensity of gingival inflammation exists in patients aged 14-16-year. Aggressive plaque control is recommended along with gingivectomy or gingivoplasty as per the condition.[8,9]

2. With Local Contributing Factor

(a) Eruption cyst and hematoma: Erupting teeth are frequently related to a variety of dentigerous cyst known as eruption cyst. These cysts have circumscribed swellings and are usually translucent and fluctuant. The presence of blood in the cystic cavities imparts a purple/deep-blue discoloration to the circumscribed swelling, described as eruption hematoma.[10]

(b) Eruption gingivitis: Emergence of primary teeth into the oral cavity is often associated with gingival inflammation. Malalignment of the erupting teeth and/or neglected oral hygiene may exaggerate the inflammation. However, optimal occlusion of the tooth subsides the inflammation. It may reappear in the 6-7 years of age group when the permanent teeth set to erupt. This develops, as the coronal contour of the tooth provides limited protection to the gingival margin during the initial stage of an active eruption. Additionally, frequent gingival food impingement is also contributory. Hence, plaque control should be taken seriously before it culminates in pericoronal abscess or leads to pericoronitis.[11]

(c) Gingivitis secondary to the orthodontic appliance: Effective brushing of the interproximal tooth is hampered during fixed appliance therapy. Accumulated plaques have a subgingival shift generated by the tipping movement which is virtually out of reach of the bristles. The gingival changes occurring in the first two months of appliance placement is temporary and largely reversible.[12]

(d) Gingivitis associated with mouth-breathing: Oral tissue undergoes dehydration as an effect of frequent mouth-breathing, resulting in inflammation of the gingiva and halitosis. This should be addressed with appropriate oral hygiene, moisturizing the tissues, use of the oral screen to protect the tissue during sleep, planning a comprehensive treatment protocol with orthodontics, and otolaryngology consultations.[13]

(e) Other elements: Excessive overjet and overbite, nasal blockage, partially exfoliated, loose deciduous, malaligned tooth, chipped off sharp edges of partly cavitated and decayed teeth can usually lead to gingival inflammation.[14]

B. Gingival Diseases and Systemic Factors:

1. Effect of Endocrine Alterations

(a) Puberty gingivitis: Pubertal gingivitis or steroid hormone-associated gingivitis is described as aggravation of gingivitis by pre-pubertal and pubertal alterations in sex hormone levels. This can be justified by estrogen and progesterone upsurge in the gingival tissues leading to vasodilatation and proliferation and increased susceptibility to inflammation. The interrelationship between elevated levels of the gonadotrophic hormones and pubertal gingivitis is noticeable from the fact that gingival inflammation peaks prior in girls (10-13 years) than in boys (13-15 years). According to in vivo evidence, P. intermedius is associated with the elevated levels of plasma estrogen and progesterone, suggesting that P. intermedius thrives on these hormones for nutrients. It is markedly distinguished by conspicuous inflammation, bluish-red appearances, swelling, and overgrowth, prominent bulbous interproximal papillae of anterior segments which are precipitated from local irritating factors

that would normally evoke a relatively benign gingival reaction. Management of puberty gingivitis should be targeted towards professional prophylaxis, elimination of all local irritants, restoration of carious teeth, and nutritional monitoring to ensure a sufficient nutritional status. Occasionally, the fibrotic transformation of the gingival swelling warrants timely surgical debridement.[15]

2. **Gingivitis Associated with Leukemia:** Leukemias are malignant diseases resulted from the proliferation of the WBC-precursors. Leukemia may have an acute or a chronic presentation and may be associated with one of the WBC precursors such as granulocytes (myeloid), lymphocytes, or monocytes. Acute leukemias are prevalent under the age group of 19-years. Acute lymphoblastic leukemia is common in the first decade of life. Factors that have been suggested in the etiology are radioactive rays and chemical damage, inherited risk factors (for example, Down's syndrome), immune-compromised state, and viral ailment. Gingiva appears as swollen, glazed, and purplish-deep-red in color accompanying hemorrhage. The swelling may emerge as a scattered expansion of the gingival mucosa, and overgrown augmentation of the marginal gingiva, or a distinct lump like interdental papillary mass. The texture is reasonably firm, but there is a propensity towards friability and bleeding, either involuntarily or on trivial provocation. Indolence, restlessness, painful throat, pyrexia, recurrent dermatological manifestations, purpura, cervical lymphadenopathy, splenomegaly, hepatomegaly, and petechiae are common signs.[16-18]

3. **Associated with Nutritional Deficiency**

(a) Gingivitis associated with nutritional deficiency: Several lines of evidence indicate an association of hypervitaminosis and mineral deficiencies with periodontal diseases. Scurvy, which emerges due to hypovitaminosis-C culminates in the reduction of collagen synthesis or the production of abnormal ones. Oral scurvy manifests with painful gingival swelling, edematous and spongy gingiva, and hemorrhage, either spontaneously or with a slight provocation. A combination of vitamin C deficiency and poor oral hygiene contributes to a painful version of gingivitis known as scorbutic gingivitis. It has additional signs that include ulcerative gingivitis, malodor, the fast formation of the periodontal pocket, and tooth damage.

C. Effect of Medications: Drug-induced gingival overgrowth: Gingival hyperplasia is an established side effect of several medications which include phenytoin (anti-convulsant), cyclosporine (immunodepressant), nifedipine, verapamil, and amlodipine (calcium channel blocker and anti-hypertensive). Gingival hyperplasia was described in 30% of patients receiving cyclosporin (Seymour & Heasman 1992), 50% of patients receiving phenytoin (Hassell 1981), and 15% of patients receiving the above-mentioned calcium channel blockers (Lederman et al. 1984, Pernu et al. 1989, Seymour et al. 1994). The gums look normal during the neonatal period but start to enlarge with the eruption of the primary teeth. This pattern of gingival hyperplasia typically starts as a nodular bulge in the interdental papillae before growing extensively to intrude the marginal gingival and labial tissues. The mouth is predominantly affected in the anterior side, but occasionally it affects the incisal and occlusal surfaces of the teeth. Histological hallmarks of this condition include epithelial acanthosis, higher fibroblast quantity, and enhanced collagen synthesis. Mechanical and chemical plaque control method are essential for the improvement of oral hygiene. Besides, professional scaling and root planning are warranted to eliminate the local contributing factors. On occasions, gingivectomy and gingivoplasty are recommended so that the patient can be esthetically sound which ultimately enhances the hygiene. Patients' physiciansshould be consulted to discuss the drug side effects so that other safe therapeutic avenues can be explored at the earliest.[17]

B. Non-Plaque Induced Gingival Diseases

Bacterial

(a) Acute necrotizing ulcerative gingivitis (ANUG): ANUG is also recognized as Trench mouth or Vincent's disease and is caused by *Borrelia vincentii*. ANUG has a greater prevalence in developing countries than in developed countries, and children are more frequently affected compared to adults. India has up to 2/3rd of the cases affecting the children in the first decade of life. ANUG is described by punched-out crater-like ulcers in interproximal gingiva coated with a grayish-white pseudo-membrane, which may expand to encompass marginalgingiva. It sometimes presents with deep pockets as a result of extensive gingival necrosis and crestal alveolar bone destruction.Patients

commonly report excruciating pain, a distinctive metallic taste, spontaneous gingival bleeding, and halitosis. Low-grade fever, swollen lymph nodes, restlessness, myalgia, loss of appetite are the frequent accompanying symptoms. Simultaneous local and systemic therapy is required to manage ANUG. The initial step should be professional scaling and root planing along with the debridement of necrotic tissue.Patientsare encouraged to maintain a proper diet andoral hygiene. Oxidizing mouthwash (e.g., chlorhexidine) mayserve to improvemicrobial stability. Five-day therapy with penicillin or erythromycin is usually recommended. Metronidazole can be administered for the speedy recovery of symptoms.[12-15]

Viral

(a) Acute herpetic gingivostomatitis: This self-limiting disease occurs due to Herpes simplex virus (HSV-1) infection. Pediatric patients younger than 6-7 years are chiefly affected. It is characterized by a scattered, reddish, and glazed appearance of the gums and the neighboring tissues. Varying grades of swelling, spontaneous gingival bleeding, disjunct spheroidal gray vesicles that eventually burst and develop into ulcers with an erythematous, raised, nimbus like edge and a depressed yellowish or grayish-white central part are also recognized.[18]

Fungal

(a) Acute candidiasis (Thrush, Candidiasis): It arises due to the overgrowth of the fungus *Candida albicans*. Children frequently develop acute candidiasis after prolonged local antibiotic therapy. The lesions appear as elevated, furry, white patches, which can be taken out easily to produce bleeding of the underlying surface. For infants and very young children, daily administration of Nystatin suspension (1 ml, 4 times/d) is recommended. Clotrimazole suspension is also highly effective.

(b) Linear gingival erythema (LGE): It is clinically described by a reddish, linear band of 2-3mm width on the marginal gingiva presentedas petechiae-like or disperse erythematous changes on the attached gingiva which may be associated with profuse hemorrhage. It might be limited to less than two teeth but it is frequently a generalized gingival pathology.[13-15]

Congenital Anomalies:

(a) Congenital Epulis(Gingival Granular Cell Lesion): It is an uncommon gingival tumor of unknown histogenesis that grows commonly in newborns, especially on the maxillary alveolar ridge. On presentation, the area of concern (gum pad) typically is firm, pink, smooth swelling of the gingival, and usually seen as gingival hyperplasia. Considerably large-seized growths may elevate the upper lip. The teeth which have not emerged into the oral cavity are typically unaltered and can be visualized in MRI.[15]

(b) Congenital Gum Synechiae: This rare malformation is identified by innate adherence between various parts of the oral apparatus. It leads to trouble in breathing soon after birth. Prompt treatment is advocated involving the excision of alveolar bands. If left untreated, it may result in TMJ ankylosis, restricted jaw movement and overall growth may also be interrupted (restricted feeding).[16]

Traumatic Lesion:

(a) It occurs accidentally in our daily practice and the inflammation or swelling lasts only for a transient period. Factitious gingivitis (*Gingivitis artefecta*) is a self-inflicting injury of gingival that could be habitual, accidental, or psychological. Soft tissue injury by bitting unknowingly, faulty brushing techniques, idiopathic trauma, tissue laceration while replacing orthodontic components, chewing abrasive foodcan result ina mucogingival recession or local inflammatory reaction. Management includes removal of irritation source, habit, rectification, and wound dressing. Psychological or psychiatric consultation may be beneficial in severe cases.[17,18]

Gingival Diseases Due to Heredity:

(a) Hereditary Gingival fibromatosis (HGF): This benign condition is marked by a gradually progressive gingival enlargement. The commonest variety, HGF, is usually familial and has an autosomal dominant mode of transmission. Another rare type has been referred to as elephantiasis gingiva or hereditary hyperplasia of the gums. It manifests asnon-hemorrhagic, firm, leathery which advances slowly upon eruption of permanent dentition involving attached, marginal and interdental gingiva. This may be complicated by esthetic or

functional issues such as a malaligned tooth, delayed retention of primary teeth, and delayed eruption of a permanent successor. Removal of hyperplastic tissues by conventional gingivectomy may be a treatment modality.

Amalgam Tattoo: Although it is a very unusual condition, it is manifested as a grayish-blue-black appearance on the oral mucosa (typically on the lower gums) due to the accidental diffusion of dental amalgam into the soft tissues. Occasionally, it is misdiagnosed as melanoma.

Gingival Findings Associated with Systemic Disorders:

(a) Infectious mononucleosis: Epstein-Barr virus (EBV) is the causative organism and it primarily affects children and young individuals. Patients chiefly complain of fever, sore throat, and enlarged lymph nodes, but malaise, fatigue, headache is also present. The abnormal findings include gingival bleeding, palatal petechiae, ulceration of the gingiva, and buccal mucosa (White, 1998).

(b) Chickenpox: Varicella-zoster is the reason of chickenpox primarily affects individuals who are less than 15 years old. Although the location of lesions in the oral cavity is variable, palate, gingiva, and buccal mucosa are commonly affected.[18-20]

(c) Herpangina: The causative pathogen of herpangina is the Coxsackie group A virus. It characterized by plentiful small vesicles that further form small ulcers made up of a gray-floor and inflamed border. The location of these ulcers is quite predictable like the tongue, palates, oral mucosa, and the posterior pharyngeal wall. These ulcers are typically painless and recede within a few days to a week.[18-20]

(d) Hand-foot and mouth disease: The bulk of cases of hand, foot, and mouth disease can affect not only the infants but also the preschooler ranging from 5 months to 5 years of age. Both the Coxsackie group A as well as group B are implicated in this disease. The signs and symptoms are identical to that of herpangina but culminate in dysphagia due to sore mouth. This disease commonly heals and abates within 1-2 weeks.[18-20]

(e) Wegner's granulomatosis: This vasculitis originally presents with marked alterations in the gingiva. Erythematous enlargement of the gingiva associated with this disease is often described as strawberry gingivitis.

(f) Kindlers syndrome: This condition is characterized by the presence of poikiloderma, cutaneous neonatal bullae, acral atrophy, and photosensitivity. Oral lesions similar to desquamative gingivitis can also be part of the manifesting symptoms. Traditional non-surgical periodontal management can be useful for treating gingival manifestation.[18-20]

Conclusion

Gingival diseases have a wide prevalence and these can affect all the age groups. However, the common myth is that gingival diseases affect only adults. Through this review, we reinforce that gingival diseases can be troublesome in children as well. Inadequate awareness about any signs and symptoms involving the gingiva in toddlers and young ones would jeopardize the periodontium in grown-up individuals. As prevention is better than cure, it is important to streamline patient education, and parent counseling to have regular pedodontic gingival assessment to maintain a healthy oral cavity in childhood.

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Maxillary Hollow Obturator for Rehabilitation of Palatal Defect: A Case Report

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Abstract

Intraoral defects may be congenital or acquired are most commonly seen in the maxilla. These are usually large openings in the palate or the peripheral structures along with palate. Patients with acquired maxillary surgical defects very commonly appear with lingering functional disabilities and cosmetic disfigurement. One of the most acquired jaw defects among all is a patient suffering from oral cancer. The aim to treat this entity is to give a comfortable, cosmetically acceptable prosthesis that can restore the impaired physiological activities i.e deglutition, speech, and mastication. An obturator is very much promising for this type of complaint. Obturatorsprimarily close more specifically seal the defects to restore the above-mentioned patients' basic needs and as well as aesthetics when it is concerned. This article presents a case report describing the technique for fabricating a hollow bulb obturator in a hemimaxillectomy patient.

Keywords: *Hollow obturator, Maxillary defect, Rehabilitation, Aramany Classifications.*

Introduction

Defects of the maxilla are more often formed by surgical removal of benign or malignant tumors, to trauma or infectious causes or oral cancer. Prosthodontics is the specialty of dentistry,which deals with restoration and rehabilitation of maxillofacial defects. Complete and sometimes partial surgical removal of tumors in the maxillary region can lead to chewing, phonation,aesthetic, and functional disorders.[1] The aim is to fabricate obturator prosthesis which seals tissue openings of the palate and contiguous structures and improves the quality of life by recuperating of course aesthetics and also deglutition, speech, mastication. Rehabilitation and re-establishment of functions by prosthesis of patients with maxillary defectsis divided into three phases and each having altered objectives. The initial phase of the treatmentis called surgical obturation and entails the placement of prosthesis during the surgical procedure. It primarily helps to restore and maintain oral functions at the time of immediate postoperative period.[2]

The second phase is called the interim obturation, and it allows the tissue to heal. The interim obturator is usually fabricated 2 to 6 weeks after the surgery and it is held in reserve till the final prosthesis is made.[3-5] The third or last phase of treatment is the fabrication of definite obturator which is made 3 to 6months after the surgery when the site is well healed.[6,7] Hollow obturator is one of the best treatment options for rehabilitation and re-establishment oforal function and aesthetic. There is numerous way of fabricating the hollow obturator. All these techniques propose to provide a lightweight prosthesis that is well accepted by the patient. The open bulb obturator provides the benefit of easy cleans facility, but the accumulation of moisture necessitates frequent cleaning. One piece of hollow bulb obturator can be

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fabricated by refilling the hollow portion using materials like salt, sugar, sponge, polyurethane foam, and gas injection using argon gas. Some times nondetachable screw cap may also be used to coat the opening made to serve the sugar or salt.[8,9]

Case Report: A 62 years male patient from Berhampur, Odisha came to the Department of Prosthetics and implantology, IDS, Bhubaneswar, Odisha with the chief complaint of missing teeth in upper left back tooth region and closure of the defect in the same region. On eliciting the history of presenting illness hemi maxillectomy has been done to cure the squamous cell carcinoma of the left half of palate, 3 months back from the reported date.

On further interrogation,the patient gave no relevant past medical history. He was a chronic gutkha chewer, 4-5 times per day, for 10-15 years approx, placement of the gutkha in the left buccal mucosa. But he discontinued the habit since he was planned for the surgery. He was well oriented to the time, place, and person and all the vitals were in the normal range. On examination, there is partially edentulous from 13 to 17. The patient has generalized attrition. The left palatal arch distal to 12 is showing erythematous area with erythematous border. Tissue healing of that part is satisfactory. It is encountered that the defect is the Armani class II maxillary defect in the left palatal arch.

Impression Procedure:



Figure 1: Intra oral pictures of maxillary defect.

Before making the primary impression patient's mouth was cleaned thoroughly with a salinecoated wet gauge. Then a small piece of the wet gauge with saline was put deep inside the defect to prevent naso-oral communication. Then the primary impression was made using alginate. The primary cast was made and a special tray was fabricated. Border molding was done using a green stick compound. And the final impression was made using elastomeric impression material. The master cast was thus, obtained over which denture base was fabricated. Jaw relation was done. Teeth arrangement was done by using monoplane teeth due to generalized attritionand tried in the patient's mouth. 3 C clasps were made by 21 gauge orthodontic wire with the help of Adam's and universal pliers. One clasp was placed around the left lateral incisor and the other two were placed around the right canine and first molar respectively. Then these clasps were sealed along with the trial obturator over the master cast. Then wax-up was done properly.

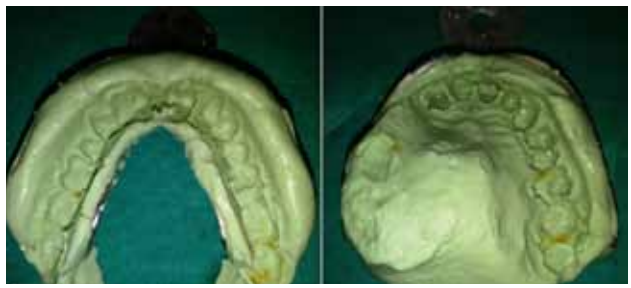


Figure 2. Primary impression of maxilla and mandible



Figure 3. Final impression

Processing Technique: The whole trial denture assembly was flasked and dewaxed. Upper and lower part of the flask was cleaned and wiped properly. Separating media was applied properly in both parts. Heat cure acrylic powder and liquid were mixed in a porcelain cup according to the manufacturer's instruction. Previously depending upon the size of the defect salt was kept for the packing procedure. When the dough stage reached one layer of acrylic material was placed along the floor of the defect. Then salt was put over it and again wrapped by acrylic material and packed properly by a clamp.

It was left for bench cooling. Then salt was vaporized during the curing procedure inside the waterbath. After cooling down the flask, the obturator was retrieved and cleaned properly. Holes were made in the lid for easy cleaning with the round bur. The obturator was finished and polished.



Figure 4. Final prosthesis



Figure 5. Intraoral pictures showing occlusion after insertion

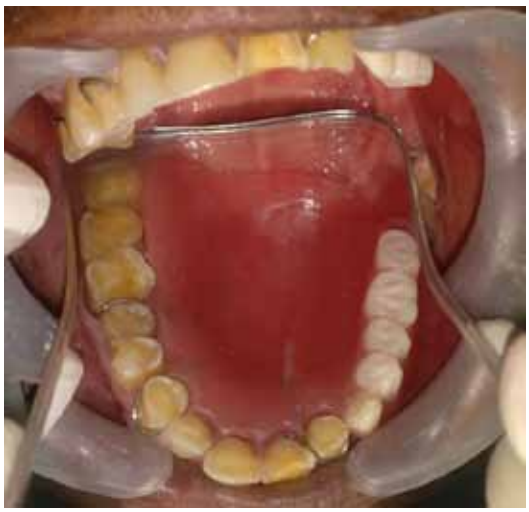


Figure 6. Intraoral view of a final prosthesis

Discussion

"In 1978 the late Dr. Mohammed Aramany presented the first published system of classification of postsurgical maxillary defects. He divided all defects into 6 categories based on the relationship of the defect to the remaining teeth and the frequency of occurrence of the defect".[10]

Class I. Curved Arch form and Linear Arch Form: "The class I category represents the classic maxillary resection defect where the hard palate, alveolar, ridge, and dentition are removed to the midline. This unilateral defect is the one most commonly seen in the maxillofacial rehabilitative practice". "The linear design is used for the class I defect when there are no anterior teeth present or when one does not desire to use the anterior teeth. The remaining posterior teeth are usually in a relatively straight line."

Class II: "Class II includes arches in which the premaxilla and the premaxillary dentition on the contralateral side are maintained. A single, unilateral defect is located posterior to the remaining teeth."

Class III: "Class III involves a midline defect of the hard palate and may include a variable portion of the

soft palate as well. The dentition is usually preserved, making this obturator prosthesis design simple and effective."

Class IV: "Class IV situations involve the surgical removal of the entire premaxillae, leaving a bilateral defect anteriorly and a lateral defect posteriorly. There are often a few remaining posterior teeth located in a relatively straightline, creating a unilateral linear design problem where leverage cannot be used to an effective degree."

Class V: "This situation involves a bilateral posterior surgical defect located posterior to the remaining teeth. Many or all of the teeth are present anterior to the defect. Labial stabilization and the use of splinting, especially of the terminal abutments, is desirable."

Class VI: "The class VI defect is a rare surgical creation. Most often it results from a congenital anomaly or trauma such as an automobile accident or a self-inflicted wound that removes the entire premaxillae (and may include a portion of one or both of the maxillae), leaving a single bilateral defect located anterior to the remaining teeth."

Maxillectomy results in a sudden change in the physiological process creating oronasal and oroantral communication. An obturator provides a simple reconstructive solution for separating oronasal communication, reducing the difficulties associated with swallowing, mastication, supporting the facial soft tissues, re-establishing speech and providing immediate dental restoration, without the need for second surgery.[11]

The remaining teeth and their location and number will determine the type of retainer to be used. Retainers that are properly designed help to reduce stress to the abutment tooth. Hence, while designing the clasp assembly, basic principles such as passive placement, encirclement and stabilization should be followed. However, in cases with large palatomaxillary defects, the stability and retention of the obturator could be significantly compromised because of the diminished bone support or if terminal abutment tooth is absent, for long-term clasping. There are different types of retentive aids that are used for the conventional hollow-bulb obturator prosthesis such as magnets and snap-on (friction type) attachments, acrylic buttons, retentive clips and implants. Implants reduce prosthesis movement, prevent rotation and encourage axial loading. Obturators retained using implants and cast metal framework will be of a different design and their weight may change accordingly. In this particular case, the patient was not willing to have further surgical procedures or to bear the additional cost involved. Obturator with open lid inserted intraorally.[12-14]

A heavy obturator prosthesis exerts continuous stress affecting the health of the tissues and resulting in discomfort. The weight of the prosthesis should be minimized to reduce dislodging forces. The most commonly used material for the fabrication of obturator prostheses is heat cure acrylic resin because of its durability and compatibility with tissues. A hollow-bulb obturator fabricated by the open lid technique is a lightweight prosthesis that not only extends effectively into the defect area but can also be easily tolerated by the patient. It is hygienic and increases speech intelligibility.[15]

Conclusion

In patients with maxillary defects, surgical procedures cannot provide satisfactory cosmetic and functional rehabilitation. These are compromised people who require physical as well as psychological rehabilitation through a multidisciplinary approach.A good result will depend upon the correct diagnosis and well-made treatment plan. Obturator satisfies the functional and psychological needs of the patient and aneffective treatment modality. This case report discussed the prosthetic treatment of acquired maxillary defect with one obturator.

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Mode of Actions of Bisphosphonates in Dental Implant Procedure: An Overview

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Abstract

Bisphosphonates (BP) are the most commonly used drug forthe past few years for the treatment of various types of bone disorders and malignancies**.** Osteonecrosis of the jaw (ONJ) is most devitalizing but still not has been properly acknowledged as of now. These drugs suppress bone turnover by decreasing osteoclastic activity**.** This article points in featuring the key and pharmaco-physiological system of activity of both N2 and non N2 bisphosphonates on bone and activity of bisphosphonates on dental implants, particularly their commitment in success of the implant and the present suggestions and rules for implant treatment in patients who are on bisphosphonate therapy**.** This article also includes the prevention and management for decreasing the cumulative IV bisphosphonate dose, stoppage of bisphosphonates before any invasive dental treatment, and in ONJ using serum beta-CTX-1 in assessment of risk. "Drug Holiday" for the invasive dental procedure is incorporated as well.

Keywords: *Bisphosphonate, Dental implants, osseointegration, osteonecrosis, BRONZ.*

Introduction

"Bisphosphonates (BP)",since years is been used to treat diseases such as "osteoporosis, Pagets' disease, hypercalcemia in malignancy, osteolytic lesions of multiple myeloma and bone metastasis associated with breast, prostate, lung and other soft tissue tumours". Their mode of action is osteoclastic inhibition and synthetic biochemical modification of bone resorption.[1]

There are two method of drug intake, oral and intravenous route. Systemic administration i.e. (Intravenous) bisphosphonates, is more potent than orally administered. The potency of the drug does not depend upon the mode of delivery rather depends upon the existence of the N-H group to the basic P-C-P structure. Due to the good binding affinity to the minerals, they are appropriate therapy for skeletal disorder where bone metabolism is high. It decreases osteoclastic actions by stimulating osteoblasts and afterward suppresses bone remodeling and turnover. N2 containing and non N2 containing are the two varieties of BP.[2]

According to some hypothesis, action of BP on the oral mucosa, specifically to the oral epithelium, keratinocytes, macrophages and fibroblasts is impaired oral wound healing.[30-35] Ill-fitting dentures cause BRONZ of the jaw without any surgical injuries, which further hinder angiogenesis continuing to avascular necrosis.

Many side effects are also reported due to long term use of BP, which may be a matter of concern regarding the safety issue of the drug. Patients, who are prescribed with BP may require implant therapy. Therefore it is necessary to know the effects of these drugs on osseointegration and implant survival & success rates.[3,4]

History of BP: BP was used in the textile, fertilizer and oil industries when it was synthesized in Germany

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in 1865. In an in vitro study,Herbert Fleisch and colleagues found the role of inhibition of inorganic pyrophosphates (PPi) for the dissolution of calcium phosphate in plasma and urine. Severe mineralization defects of the skeleton were found in 1964 due to high PPi levels in urine. An in vivo study has shown that the compound prevented ectopic calcification; it did not have any effect on resorption of bone and normal mineralization. Pyrophosphate used in scintigraphy and dental calculus as therapy. Bisphosphonates have empathy for mineralization of bone, it additionally represses the arrangement and conglomeration of calcium phosphate crystals as PPi.Early studies indicate that bisphosphonates also can inhibit the dissolution of hydroxyapatite crystals. It is discovered in the early 1990s the potency of the drug is mainly depend upon the Nitrogen compound present in its structure.[5]

Chemical Confugaration Nitrogen Containing BP: The drug potency is increased by the alteration in the chemical structure that is the peripheral or side chain of the central carbon atom and N2 group in the R2 position and it is also done by adding alkyl and amine groups to R1 side chain for chain elongation. This helps to maintain the calcium-binding affinity through the OH group in the R1 position.When hydroxyl or amino group present in one side chain, it increases Ca binding affinity, due to tridentate conformation.[6]

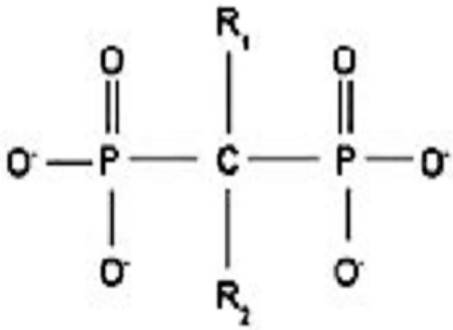


Figure 1. Bisphosphonate

Mechanism of actions: Osteoclastic bone resorption is inhibited by osteoclast-mediated BP internalization due to which there is decreased osteoclastic activity. Two types of mechanisms are there, one is cellular, and the other is biochemical. They help in decreasing skeletal resorption and turnover.

Non-N2 containing bisphosphonates (Cellular Mechanism): First-generation BPs (clodronate and etidronate) resembles to the PPi. They reverse the activities of aminoacyl-tRNA synthases through metabolically incorporated into nonhydrolyzable cytotoxic ATP analogs as pyrophosphate. The derived metabolites which should consist of β, γ-phosphate groups of ATP, but it contains P-C-P structure, which is non-hydrolyzable. These metabolites get accumulated intercellularly, which inturn hinders the functions of osteoclast. They interrupting with mitochondrial ATP-translocases resulting in osteoclastic cell mortality. So the cell death of osteoclast due to BP causes resorption of bones.[7]

N2-containing bisphosphonates and "Mevalonate biosynthetic pathway" (Biochemical): In the mevalonate pathway, farnesyl diphosphate and geranylgeranyl diphosphate proteins are biosynthesized. The amide group-containing BPs are inhibitors of the biosynthetic pathway. In the case of post-translational prenylation of small GTPases such as Rho, Rac, RAS, the above proteins are required. The Rho and Rac proteins are activated through geranylgeranylation, so that it helps organize the cytoskeleton and ruffling of the cell membrane. The "ruffled border" is important because it helps in the attachment to bones and destroys the bone tissue. When the ruffled border diminished, then automatically it turns into osteoclastic cell death. Ultimately there is a reduction in bone turnover. N2 containing bisphosphonates create disruptions in osteoclastic functions, the morphology of cells and cytoskeleton, which fails in the sealing zone and ruffling of membrane, apoptosis and also trafficking of vesicles.[8]

According to **Elliott Ballantyne** Sealing zone consists of

1. Ruffled border
2. The basolateral domain
3. The functional secretory domain

Prolonged Effects of BPs: BPs has long-term effects on decreasing bone turnover. Some BPs (eg. alendronate and zoledronate) act more prolonged than others (etidronate and risedronate). The rate of resorption and fracture is less even after the discontinuation of the drug for a longer period. Also the disadvantages of the drug may take a long duration to go away. BPs are inserted in the skeleton, which are liberated in future cycles of resorption. It implies prolonged and steady removal of all BPs from the bones. Some study reveals that the elimination of alendronate in humans is so slow that it can take up to 12 years. [9,10]

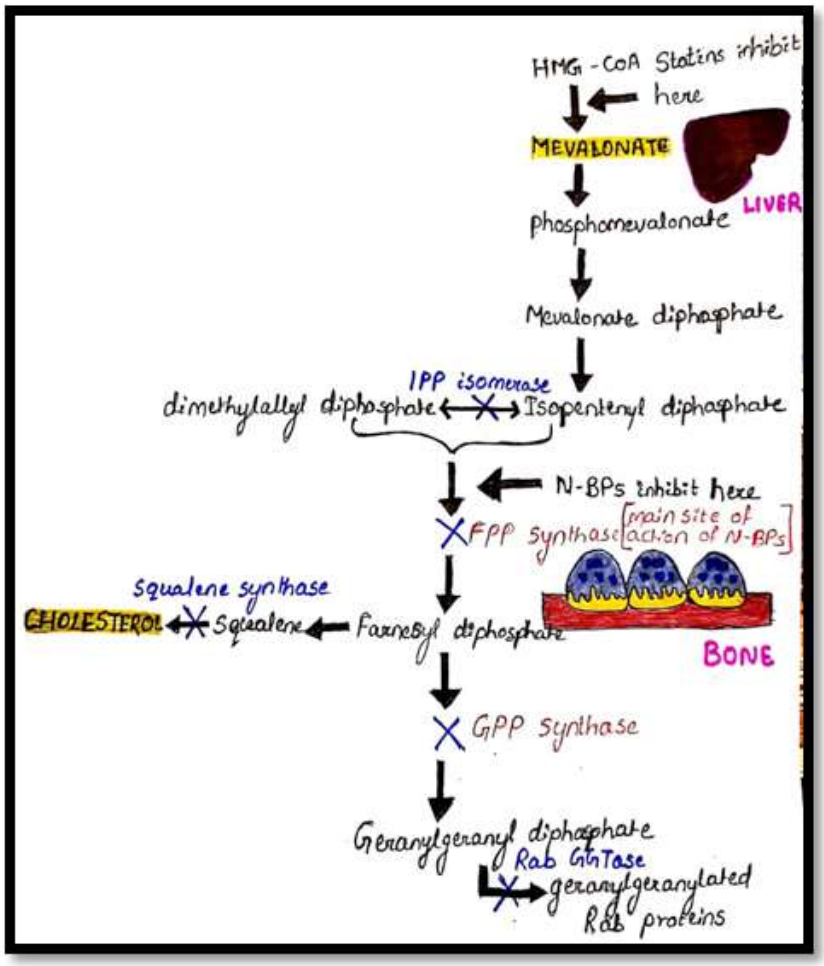


Figure 2. Mevalonate biosynthetic pathway

Why BP Deposition is higher in Jaws: Bisphosphonates are useful for metabolism in bones of the body however in the event of BRONZ of the jaw the most common site is in the maxilla and mandible. It is said that the thinner the mucosa over the bone, the higher the rate of deposition of the drug. So, jaws containing less than 5mm mucosal layer are more favourable than the other bones of the body.

As indicated by certain investigations that the alveolar crest get remodeled at a higher rate of 10 times quicker than the tibia, 5 times quicker than the inferior border of the mandible and 3 - 5 times the rate of the mandible at the Inferior Dental canal and jaws and collect a higher rate of concentration of bisphosphonates because of the alveolar ridge remodeling at rapidly.[11]

Effects of BP in "Angiogenesis and vasculogenesis": For the local immunity, tissue homeostasis and regeneration, sufficient blood supply and new vascularisation is fundamental. The veins in bones and the cells in the endothelium are significant in bone development and resorption. The Angiogenesis and vasculogenesis both are different.

Angiogenesis is defined as "the sprouting from pre-existing vessels of mature endothelial cells". Vasculogenesis is "the formation of new primordial vessels from bone marrow-derived, circulating endothelial progenitor cells". [12]

Some studies concluded that the N containing bisphosphonates like zoledronate inhibits angiogenesis. In some in vitro studies after using human umbilical cord vascular endothelial cells (HUVEC) it is found that zoledronate inhibits the production of HUVEC and it is dose depending upon the dose. Zoledronate inhibits integrins (α5β3 and α5β5), which helps in the

formation of a blood vessel by endothelial cell adhesion and migration.

In some in-vitro studies, it is mentioned that the endothelial cell proliferation and migration are become impaired after administration of zoledronate. RANKL is suppressed resulting in deterioration cellular mechanism by bisphosphonates. [13]

Tissue healing around the implant: Fundamentally wound healing process around dental implants is significant and important for implant to survive. It involves delicate tissue recuperating and hard tissue mending around the implants.After osteotomy and placement of an implant, the delicate soft tissue ought to be sutured appropriately with the goal that it must seal the blood coagulation and save the zone from bacterial colonization. There should not be any tension present in soft tissue during primary wound closure. A series of processes goes on during healing at the implant site like the development of blood clot, fibroblast, macrophage activity, and epithelial proliferation which is associated with primary osteoid formation, called primary stability of the implant. [14]

As there is suppression of endothelial cell proliferation and interruption in angiogenesis and vasculogenesis the tissue healing around the implant becomes delayed after administration of BP.Primary stability is one of the key factors for the survival of the implant.That is why in case of a patient taking BP the survival of the implant is a questionable matter. [15]

Failure of implant: Failure of an implant can be defined as "the first instance in which the performance of an implant fails to meet the criteria for success". The indication like implant mobility, vertical bone loss greater than 0.2 mm, bleeding pockets that are greater than 5 mm are observed when evaluated clinically. Implant failure is further classified as early or late failures, depending on whether they occur before or at abutment connection or after occlusal loading following the prosthetic restoration. Early implant failure results due to the inability of the implant to establish an intimate bone contact. Evidence says BP interrupts in osseointegration by the formation of fibrous connective tissue around the implant results in early failure. Late failures occur during functional loading and cause periimplantitis. BP has a significant role in late failures as well as it is compromising the wound healing.[2]

Laboratory Marker: During bone formation, resorption and turnover C-telopeptides (CTx) fragments of collagen are liberated. Because BPs decrease CTx levels, it is also well believed that evaluating serum CTx levels is a reliable indicator for risk assessment. The "CTx test, also known as C-terminal telopeptide and collagen type 1 C telopeptide is a serum test obtained in laboratories or hospitals.[16]

Oral Bisphosophonate Use >3 Years

1. Physician approval to discontinue bisphosphonates 3 months before sugery and 3 months after sugery ("drug holiday").
2. Determine serum CTx levels during initial consultation and immediately before surgery; CTx levels must be >150 pg/mL before proceeding with surgery.
3. Detailed informed consent for bisphosphonate-associated osteonecrosis.

Oral Bisphosophonate Use <3 Years without Clinical or Radiographic Risk Factors

1. Serum CTx level must be >150 pg/mL.
2. Proceed with surgery with detailed informed consent for bisphosphonate-associated osteonecrosis.
3. If serum CTx level <150 pg/mL, institute a physician approved "drug holiday"; continue monitoring every 3 months until CTx levels >150 pg/mL.

Oral Bisphosophonate Use <3 Years with Clinical or Radiographic Risk Factors

1. Physician-approved "drug holiday" for 3 months.
2. Serum CTx level must be >150 pg/mL to proceed with detailed informed consent for bisphosphonate-associated osteonecrosis.
3. If serum CTx level <150 pg/mL, continue monitoring every 3 months until CTx level >150 pg/mL.

Figure 3. According to Carl E Misch Protocol, suggestions and assessments for patient taking oral BP

CTx Value (pg/mL)	Risk for Osteonecrosis
300-600 (normal)	none
150-299	none to minimal
101-149	Moderate
< 100	High

Figure 4. Laboratory Risk Assessment

Discussion

A Review of this literature throws light on relative risk associated with the use of bisphosphonates and implant therapy**.** There are many case report and literature which expresses that utilization of bisphosphonate medication may promptly lead to failure of implant therapy in patients especially those accepting intravenous bisphosphonate treatment, while another review contemplates have shown that there might be a danger of osteopathology in patients getting oral bisphosphonates and dental implant treatment. According to **BRONJ** "it is a potential complication that may lead to implant failure. It is also important to distinguish that implant failure may occur without the presence of any clear pathology due to a loss of osseointegration in patients being treated with this group of drugs. This is believed to be the result of reduced bone turnover due to diminished osteoclastic activity".[17] "In the first reported case series it has found the occurrence rate of BRONJ was at about 70% after tooth extraction or other dentoalveolar surgical procedures such as periodontal surgery and dental implant placement."

According to **Vescovi et al**. "36.2% rate of incidence of BRONJ in a non-surgery triggered group compared to 63.8% rate of incidence in a surgery triggered group. [63] They also stated that intravenous bisphosphonate therapy for underlying malignant disease is an absolute contraindication for the placement of dental implants, whereas implants can be placed in patients receiving oral bisphosphonates for those treated for osteoporosis". [18]

According to **Elliott Ballantyne** "the association between oral bisphosphonate therapy and dental implant failure". [2]

In a systematic review by **Javier Ata-Ali et al**, it was discovered that the position of dental implants in patients treated with bisphosphonates doesn't decrease the implant success rate. On the contrary, it can't be said that such patients are not without difficulties and hazard evaluation. Therefore it is decided by the singular premise".[19]According to **Hom-Lay Wang et al** "bisphosphonates may play a role in wound healing and suggested that if there is compromised healing it can be successfully treated with systemic antibiotics, local microbial mouthrinse and aggressive defect management". [20]**Yip JK et al** 2012 did a study and found that the use of oral bisphosphonates at the time of implant placement was associated with dental implant failure. Specifically, the use of bisphosphonates among women were almost three times greater than other counterparts.[21]

According to **A Cheng et al** "placement of implants in patients on bisphosphonates does carry a small but defined risk. The long-term management strategy for the patient's underlying condition needs to be discussed with the patient's medical practitioner and informed consent of the risk needs to be provided. Patients with previously placed implants who subsequently are placed on the bisphosphonates need to be carefully monitored. Aggressive surgical salvage procedures are best avoided as these will probably decrease failure".Thought of bisphosphonate tranquilize occasion or elective clinical administration of their bone disorder ought to be talked about with the treating clinical expert and after that implant treatment ought to be effectively carried out. [22]

Conclusion

It is concluded that patients taking bisphosphonates by intravenous method should not be considered for the placement of dental implants. Patients under oral bisphosphonates can be chosen for the dental implants but before the surgery the protocol has to be followed.

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The Etiopathogenesis of Myofacial Pain Dysfunction Syndrome

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Abstract

This article presents a review of revisiting the Etiopathogenesis of myofacial pain dysfunction syndrome. This article concentrates on the appropriate evaluation, causes and management of MPDS. Myofacial pain is one of the significant and common reasons for the orofacial pain that originate from the myofacial trigger point. Patient with myofacial pain dysfunction syndrome also complains of a chronic musculoskeletal pain on which pressure on a sensitive muscle or point cause pain.

Keywords: *Myofacial pain dysfunction syndrome, Orofacial pain, Trigger point, Musculoskeletal pain.*

Introduction

Myofacial pain dysfunction syndrome is an order characterized by pain and dysfunction of masticatory muscle as well as the skeletal muscle and the trigger zones. The trigger zones are sensitive in nature and palpable. Myofacial pain dysfunction syndrome is defined as pain originates from the myofacial trigger point in skeletal muscles.MPDS is the most common form of temporomandibular disorder. It is the common cause of orofacial pain. MPDS is a psychological disorder that involves masticatory muscles, skeletal muscles, limitation of jaw movements, sensitivity in touch."TMJ pain dysfunction syndrome", first described by Costen (1934), which included Tmj dysfunction, facial and head pain. The term 'Myofacial pain dysfunction syndrome, first coined by Laskin (1969). Myofacial pain can arise in skeletal muscles such as those in the head (commonly muscle of mastication), neck, lower back, and shoulder.[1-3]

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Abnormal occlusion – a person can modify the pathway of closer in case of painful and potentially damagingcontact. This corrective movement of the mandible, during the closer, may lead to muscle strain and spasm resulting in abnormal pressure on the Temporomandibular joint.

Prosthetic problem – according to many authors' faulty partial and complete dentures, bilateral loss of molar teeth, over closer and increased vertical dimension in partial or complete dentureleads to TMJ dysfunction. There is also a change in myotatic stretch reflex in the above mentioned condition.

Orthodontics problem – malocclusion leads to TMJ dysfunction.

Emotional problem – in this case, patients show over behavior or overreactingon food habits, to care for teeth which give rise to TMD (Temporomandibular disorder). At the same time, oral habits are quite dangerous and can cause damage or persistent pain. A behavioral change like anxiety due to dysfunction of ANS(Autonomic nervous system).[1-3]

Psychophysiologic theory – The signs and symptoms of pain dysfunction syndrome are due to masticatory muscle spasm. Tenacious myospasm also can cause 2 other changes in the body, namely degenerative arthritis and contracture degenerative change. Degenerative arthritis arises from constant jaw function with abnormal condyle position contracture degenerative i.e. Muscle

change that accompanies long period spasm. Spasm maybe start by muscular overextension which gives rise tooral habits, dental restoration, tooth loss, denture irritation, adenture wearer.[1-3]

Etiopathogenesis: - Primarily energy is released during muscle contraction then there is an initiation and acceleration of lactic acid, which in turn effectuate coin in osmolality consequence in the reduction of the pH. It makes the muscle receptor susceptible to impule excitation as their censorious firing level is damaged. The lactic acid itself causes effusion and infusion of bradykinin, histamine and serotonin and another amine into the region by the decreased in pH, it leads to pathological musculardisturbance, then this specific zone of muscular disturbance are called as 'trigger zone' or hypersensitive area from which impulse the CNS and cause referred pain.[4]

Clinical Feature:

1. Unilateral, dull pain in the or preauricular region
2. Tenderness in the muscle of mastication.
3. Tender on palpation
4. Restricted mouth opening(opening of mandible)
5. Mostly occurs in the mandible with a more female predilection.
6. The pain continues in episodes of several times a day.
7. Gradually it raises due to increased emotional stress and increased intraarticular pressure in joints.
8. Due to myalgia, arthralgia masticatory pain occurs.
9. Mostly Pain occurs in the preauricular region but can be radiated to the temporal, frontal, and occipital region.
10. The patient complains of difficulty in chewing and restricted mouth movements.
11. Patients also complain of nose rubbing, clicking, grinding, and popping snapping sounds on mandibular movements.
12. The patient also complains tinnitus (ringing in the ear) or toothache or otalgia (pain in-ear). The cause behind tinnitus is that loss of bilateral molar leads to distal condylar movement and puts direct pressure on the Eustachian tube with impaction on the auriculotemporal nerve which is responsible for the ear symptoms.
13. The patient also focuses on hearing loss, myospasm other problems parafunctional habits, such as bruxism, clenching, extensive attrition, ridging of the buccal mucosa, malocclusion.
14. Pain aggravated by movement of the mandible.[1-4]

Management of MPDS: The management of myofacial pain requires a multifactorial approach. It is a long term procedure that takes time to recover. The patient should be calm and keep patience for recovery. The management can be done in two ways –Nonsurgical management & surgical management[5]

Nonsurgical Therapy:

1. Re-educate the patient about the disorders.
2. Reassurance the patient has to be assured of the character, sign, and symptoms of the myospasm.
3. Diet and rest- hard and chewy food should be avoided and the patient should be aware of resting jaw rather than postural movements like clenching, swallowing, or gridding.
4. Thermal therapy- laying a hot moist towel, electric heating pad over the symptomatic area is helpful to reduce pain. It should remain in the place for 10-15 min.

Supportive Therapy: There are two types of supportive therapy are: pharmacological therapy and physical therapy.[6]

Pharmacological Therapy:

- Pain control analgesics- salicylates, morphine,paracetamol, aspirin.
- Anti-depressants- monoamine oxidase inhibitor, lithium carbonate and caffeine.
- Anti-inflammatory agents – salicylates (aspirin)
- Propionic acid (ibuprofen), acetic acid (indomethacin),oxicam, fenamic acid, aryl acetic acid derivative (diclofenac sodium).
- Anxiolytics agent – Benzodiazepines such as lorazepam, diazepam and oxazepam are commonly used.
- Muscle relaxants- chlorzoxazone, meprobamate, methocarbamol and cyclobenzaprine.[7]

Physical Therapy: Physiotherapy may help in such cases and that infection and all are for the second option. There are many kinds of physiotherapy are available e.g. Stretch and spray, ischemic, pressure, soft pressure, continuous massage. Physical therapy can alsoadd electrical stimulation therapy which includes:

- Electro galvanic stimulation (EGS), is a periodic electrical impulse that is applied to the muscle, creating repeated involuntary relaxation and contraction, which cause increases in blood flow to the muscle.

- Trance cutaneous stimulation (TCS), is an electrical nerve stimulation (TENS)that stimulates the A-delta fibers and prevents pain from c- delta fibers by using electric current.[8]

Other Therapies:

1. Hydrotherapy- It is one of these therapies where the patient's cooperation is most important. Patients should understand the procedure and follow the hypnotist's suggestion. This results in muscle relaxation.
2. Acupuncture- it is the simple and effective pain control method skilled by inserting needles into appropriate joints at an appropriate surface called meridians. This therapy (acupuncture) gives relief from pain rather than remove the basic cause.
3. Restoration- Faulty restoration may lead to interactive contact. So the overfilled restoration should be removed. And the occlusion should be checked properly.[8-10]

Surgical Therapy: Surgical management is the rarest treatment of choice where long term results are disappointing. They are:

a. Condylotomy- it meansthe displacement of the head of the condyle, which moves slightly forwards and medially. The procedure is carried out without disturbing the joint capsule and intracapsular structures.[8-10]

b. High condylectomy- this is done when all other conservative forms of therapy have failed and other examination results in extensive proliferative changes or erosion of the condylar head. Basically in this procedure is based on the elimination of condylar articular pathology and surgical reduction of the hight of the condylar head.

c. Meniscectomy- the most acceptable method in which articular disc is removed from the TMJ. After removing the articular disc there is a relief of symptoms due to cutting off the capsular nerve supply and tightening the capsular ligament by post-operative fibrous scarring.[8-10]

d. Myotomy- selected myotomy of the temporalis or masseter muscle via an intraoral method is helpful in the management of pain dysfunction syndrome.

e. Arthroscopy- is the least invasive surgical technique for myofacial pain dysfunction syndrome. It has the potential of successful surgical outcomes with the minimal sequel.

f. Botulinum toxin A (Bt A) injections. It is one of those expensive and intensive means of treatment. This may helpwhen dystonia is a major factor. This method is successfully used to treat having both recurrent TMJ dislocation and excessive clenching.[8-10]

Conclusion

This is important to know about the etiology and the proper diagnosis of MPDS before treating this. The management should be properly done which includes conservative management as well as surgical management. The patients should take rest and have patience. MPDS is self-limiting if etiological factors are erased. Conservative management includes medication, jaw exercises, physical therapy, injections, etc. surgical management should be considered if the pain is not subsided by conservative management. After all of the above efforts, more research is still required. It includes lavage and lysis technique which results in an increased range of motion improvement of joint function and reduction of pain. Better management of the patient who is suffering from this syndrome. The proper management of myofacial pain dysfunction syndrome should be done in the early stage of the disease.

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Salivary Glands: A Brief View on Types, Anatomy & Histological Features

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Abstract

Salivary glands are crucial organs playing a vital role in the secretion of saliva in the oral cavity. During its morphogenesis a sequel of coordinated movements that are developmental processes occurred and the interaction of mesenchyme and epithelium generating the ductal system and secretory units. Salivary glands consist of major and minor glands that play different roles in saliva secretion. The maximum cell types of salivary glands are innervated by parasympathetic and sympathetic nerves. Histologically serous acini and mucous acini cell types can be seen with a network of the ductal system which includes striated, excretory and intercalated ducts, the external carotid artery maintain the blood supply of major salivary glands and the role of vasculature supply remaining unclear regarding the maintenance of saliva secretion which needs further investigation. The article throws light on its different types, its histological features, and its ductal system.

Keywords: *Salivary glands, serous acini, mucous acini, Ducts.*

Introduction

The human salivary glands are the major ones for maintaining the homeostasis of the oral cavity. They secrete saliva which is a multi-functional fluid that gives salivary electrolytes providing antibacterial property and help in mucosal lubrication which in return protecting the oral mucosa and teeth surface. Various types of enzymes are also secreted by salivary glands. The saliva secretion is very crucial in maintaining the oral hygiene and keeps the oral cavity moist. Reduction in the saliva may occur due to different reasons which involve loss of gland or degeneration and SG includes three pairs of glands i.e. parotid, submandibular and sublingual glands.

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These are considered major salivary glands and they are responsible for 90% of saliva secretion and rest are secreted by minor salivary glands. Minor salivary gland comprises of 600-1000 glands distributing through the whole oral –cavity. As these minor salivary glands, only less than 10% of saliva but they provide an important role in the main lubricant of saliva because of its mucous and protective components. The adult salivary glands include specialized cells which are acinar cells and myoepithelial cells that contribute to the secretion of saliva into the ducts.[1] Intercalated ducts are first structures that receive the initial secretion which is then conveyed to striated ducts, where changes occurred in several electrolytic between the extracellular matrix and the fluid salivary through the plasma membrane.

Classification of Salivary Glands:

- Based on its location and size – A. Major salivary glands B. Minor salivary glands

The major salivary glands are further classified into– parotid gland, submandibular gland, sublingual gland.

These all are located outside the oral cavity and the secretion reaches the mouth by the ductal system.

- Based on the types of secretion- A. Serous secreting glands-example such as parotid glands, von Ebner's gland.

B. Mucus secreting glands- examples such as glands of Blandin and Nuhn, glosso palatine glands.

C. Mixed glands- Such as submandibular gland & sublingual gland.[2]

Major Salivary Glands:

- Parotid Gland - The word originates from Para which means around and otic means ear. It is the largest salivary gland among all. It is characterized by serous cells as it is purely serious. It weighs about 15 grams.

Anatomy- The superficial part of this gland is present in front of the ear and the deepest portion lies or filling the retromolar fossa. The artery supply includes the external carotid artery, maxillary artery, and superficial temporal and posterior auricular artery. Facial nerves supply this gland with the union of the superficial temporal and maxillary vein.

Duct- Parotid duct which is also known as **Stenson's duct** crosses the masseter and then pierces the buccinators and then opens into papilla at buccal mucosa which is present opposite to the maxillary second molar. The duct sizes 4-6mm in length and 5 mm in the diameter. The blood supply is provided by ECE and veins drain into the external jugular vein. The lymph first drains into parotid notes and then t upper deep cervical lymph nodes. The parasympathetic fibers, sympathetic nerves, and sensory nerves are associated with the parotid gland. [3]

- Sub-mandibular Gland- This is the second-largest gland and is also known as the submaxillary gland. It is a mixed type of gland which includes both serious and mucous acini but the serous units are found to be dominating as compared to mucous units.

Anatomy - The superficial part of the gland lies in the digastric triangle and the deepest part is associated with or deep to mylohyoid and superficial to styloglossus and hyoglossus. They are supplied by the facial artery and the veins drain into a common lingual or facial vein. The lymphatic drains into the deep cervical and jugular group of nodes.

Duct- The important excretory duct includes the **Wharton's duct** that runs forward above the mylohyoid muscle which lies just below the mucosa and floor of mouth. It opens on the carnucula sublingual and lateral to the lingual frenum. The secretomotor pathway playing a crucial role in its nerve supply. [4]

- Sublingual Gland –This is the smallest salivary gland and weighs about 2gram. It contributes to the 2.5% secretion of total saliva.

Anatomy- It lies at the anterior part of the floor of mouth, which is just between the mylohyoid muscle and mucosa. It opens in the oral cavity by a series of small ducts i.e. duct of rivinus and opening along sublingually fold which opens through a large duct known as **Bartholin's duct.**

The blood supply is provided by sublingual and submental arteries and its lymphatic drains into the submental lymph nodes. The nerve supply including parasympathetic supply consisting of the facial nerve reaching the gland by the submandibular ganglion and lingual nerve and in sympathetic supply includes postganglionic fibers coming through the plexus on the facial artery.[5]

Minor Salivary Gland: The minor salivary gland ranges between 600-1000 in number and playing a crucial role in protecting and moistening the oral mucosa. It is present in the submucosa throughout the oral cavity. This gland is rich in anti-bacterial protein and rich in mucin and consists of secretory immunoglobulin.

Von Ebner's lingual Serous Gland- It is present in a tongue that opens in the circumvallate papillae on the dorsum of the tongue and also in the foliate papillae on another side of tongue. This gland secretes digestive enzymes and also proteins which play an important role in the taste process. [6]

Development of Salivary gland:

It includes different stages in the development of salivary glands, as follow:

- Stage 1 (Bud formation) – This stage starts with the proliferation of oral epithelium into the ectomesenchyme that is underlined and a thin basal lamina that separates the bud from the underlined mesenchyme and the epithelium interaction with condensing mesenchyme is responsible for gland formation.
- Stage 2 – This stage includes the formation and growth of epithelial chord. As the proliferation of epithelial buds in the underlined mesenchyme gives

the formation of epithelial cords and the MES also starts proliferating this is around the glandular epithelium. Basal lamina is found to be playing a vital role in influencing the differentiation and morphogenesis of the salivary glands.[7]

- Stage 3 - It includes the initiation of branching in the terminal part of the epithelial chord. The epithelial chord proliferating and ending with branch into bulbs.
- Stage 4- In this stage the cleft formation occurs and the ECM deposits within the clefts and the connective tissue component which is present below the epithelial chord forming capsule and surrounding the entire gland. The hypothesis including the interaction of epithelial-mesenchymal. Growth factor b which is transformed by fibroblasts growth factor family and their receptors. The stage notices the contraction of actin filament at the apical and basal ends.
- Stage 5- (Canalization):- In this stage the formation of lumen takes place at the distal ends of the chord and then it takes place in the proximal end and the central part in the last. The lumen develops in the terminal buds before that they form within the ducts. In this respective stage the apoptosis occurs of centrally placed cells and the proliferation of inner and outer layers of the epithelial chord occurs with the fluid secretion by ductal cells that increase the hydrostatic.
- Stage 6- (Cytodifferentiation)- In this stage which follows the formation of the lumen within terminal buds in which the epithelium including the two layers – the inner cells differentiate into the mucous or serous cell and the outer cells differentiate to myoepithelial cells which are present in and around the secretory and intercalated ducts. The result gives the main excretory duct which formed by epithelial bud portion that is close to the oral cavity as well as the secretory end piece forms by the distal portion.

Histology of Salivary Gland: The histology includes the acinar units, ductal system & connective tissue.

The acini is the basic functional unit of the salivary gland and the secretory unit is formed by serous cells, mucous cells, and myoepithelial cells. The cells in acini rest on a basement membrane and the central lumen which is star-shaped due to extension of the lumen between the cells resulting in intercellular canaliculi. The mucous acini have larger lumen as compared to serous acini and the continuing central lumen forming the excretory duct i.e. ductal system.[8]

Serous Cells- Morphologically it is pyramidal in shape with a broad base in the basement membrane and apex focusing toward the lumen. Finger-like extensions are noticed between the adjacent cells which are known as intercellular canaliculi which increase the size of cells of the luminal surface. The apical cytoplasm showing the secretory granules that are 1mm in diameter. The secretory granules including the zymogen granules and the glycolate proteins are stored in a vacuole. The desmosomes and adhering junction holds the adjacent cell and thus helps in cell signaling. The gap junction allowing the passage of ion and small molecule in between the cells.

Mucous Cells- the structure showing the apex of cells appears to be empty except for the thin strands of cytoplasm resulting in a trabecular network. Compressed nucleus and the rims of cytoplasm present against the base of the cell. Oval or flattered in shape nucleus can be observed which is located just above the basal plasma membrane. Large amount of secretory product is accumulated by mucous cells in the apical cytoplasm.[9]

The mucous secretion differs from serous secretion by two ways, which includes:

1. As they have less or no enzymatic activity and crucially serving the lubrication and protection action of oral tissues.
2. The ratio of carbohydrate to the protein is higher and a greater amount of sialic acid and often sulfated sugars is present.

Myoepithelial cells: These are the contractile cells attached with secretory end pieces and intercalated ducts of the salivary gland. These cells are similar to smooth cells but are derived from epithelium. They are located between the basal lamina and the ductal cells. Desmosomes connect the myoepithelial cells plasma membrane to the basal membrane of parenchymal cells. They are spider-like cells having nucleus flattened with flat perinuclear cytoplasm and secretory and duct cells are embraced by long branching process. Their appearance gives basket cells as basket cradling the secretory unit. The immunofluorescence studies indicating the presence of actin and myosin. A regular pulsatile movement of the entire unit occurs. These cells containcytokeratin

filament and actin filaments. This helps it to identified immunohistochemically. The function of these cells includes the acceleration of the initial outflow of saliva and contributing to secretary pressure in the duct. It also reduces the back permeation of fluids and supports the underlined parenchyma. Recent research has shown that myoepithelial cells playing a role in the signal to the secretory cells and protecting the salivary gland tissue. These cells also produce several proteins that have a tumor suppressor activity like as angiogenic factors that provide a barrier in fighting against the epithelial neoplasms. [10]

Ducts: The different ducts involved as- Intercalated ducts which are the smallest – striated ducts i.e. intralobular – excretory ducts (interlobular). The ducts play a main role in modifying saliva by the secretory and resorptive process. The intercalated ducts replace the damaged cells in the striated ducts. The striated ducts which are lined by tall columnar epithelial cells and the nucleus is placed centrally. Eosinophilic cytoplasm shows prominent striations at the basal end of cells, helping in the absorption of sodium, and excretion of bicarbonate and potassium. The excretory duct includes two layers which are mucosa and connective tissue adventitia. The transformation of stratified squamous epithelium from cuboidal occurs by ductal epithelium slowly.[11]

Connective tissue elements: These cells include macrophages, mast cells, fibroblasts, plasma cells, and fat cells. These cells are embedded in ground substance consisting of proteoglycans and glycoprotein. These elements tissue forming the capsule for gland and dividing the gland into lobules.[12]

Conclusion: Salivary gland which secretes saliva contains digestive enzymes like lingual lipase and amylase, these enzymes start breaking the food in the oral cavity or mouth and the chewing process the food mixes with saliva, thus starting the mechanical process of digestion and in return produces a bolus that can be swallowed down the esophagus entering into the stomach, wherein the stomach the gastric phase of digestion takes place. Thus saliva helps in food digestion and maintains oral hygiene by keeping the oral cavity moist and reducing it to dry to prevent the infection in the mouth. There are different disorders relating tothe salivary gland that includes sialolithiasis, sialadenitis, and many more. A disease known as Sjogren's syndrome results in the dry mouth, which is a disorder of salivary gland, that often occurs with lupus and rheumatoid arthritis.

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Blue M–Healing with Oxygen

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Abstract

Studies reveal that, world's two-third of population suffers from chronic form of oral diseases and wounds. The principle etiological factor behind is a polymicrobial complex, which initiates the increase in the formation of reactive oxygen species e.g. per hydroxyl radicals, peroxyl radicals, aryl peroxyl radicals etc. These ROS may cause damage to protein, lipid and DNA at sub molecular level. To combat these microcosmic reactions, a revolutionary inventiveness for optimum oral health was carried out by a European based company in the form of a product, Blue-M. This acts as a continuous supplier of oxygen and by perfusion of this oxygen, it enhances healing of wound. It further aids in collagen synthesis, angiogenesis and is comprehensively Osseo inductive as well as anti-inflammatory in nature. Thus, this review aims at understanding the obligatory functions of oxygen and the biology behind neutralization of inflammation as well as to recognize the essential benefits of this novel product, which in near future, after numerous studies, may even be used as an effective anti-microbial agent without developing resistance.

Keywords: *Blue-M, Nascent oxygen, Wound healing.*

Introduction

Wound healing is a dynamic nexus of processes, which has inflammation, granulation and maturation phases in it. When it comes to the specialization of oral cavity, occurrence of wounds and inflammation are common. There is accumulation of dental plaque accompanied with enhanced hostinflammatory response as well as inflammation of gingiva.[1] This, in long term, leads to onset of inflammation of bone and other peri-radicular tissues. This can also be associated with other oral problems like mucositis, ulcerations, bad breath etc.

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Apart from maintenance of oral cavity by eradication of microbes,to accelerate the healing of these wounds, ample amount of oxygen is required.

Lately,unconventional mouthwashes and gels, containing oxygenating technology has been introduced (Blue M, Europe), having anti-microbial and anti-inflammatory properties. It prevents formation of plaque biofilm as well as promotes teeth whitening, and improves the rate of wound healing.[2]

Role of Oxygen in Wound Healing: Gingival wounds, as known, involves healing as well as phagocytosis of microbial intruders. "Professional phagocytes of our innate immune system increase their oxygen consumption through the inducible activity of NADPH oxidase (NOX) that generates oxygen and hydrogen peroxide." These oxygen-derived metabolites release Reactive Oxygen Species(ROS), that are potently antimicrobial but which may also cause damage by destroying surrounding tissue and cellsby substantial damage cellular phospholipid, nucleic acid, protein, carbohydrate, and enzymes. These events are

investigated in phagocytes (neutrophilic and eosinophilic granulocytes, monocytes, and macrophages). Exposure of these cells to any of a large number of stimuli activates a ''**respiratory burst**,'' caused by an activation of the plasma membrane-bound NADPH oxidase. Approximately 98% of the oxygen consumed by wound neutrophils, is utilized for respiratory burst.[3]

Topically applied oxygen gas increases the partial pressure of oxygen of the superficial wound tissue. Interestingly, topical oxygen treatment was associated with higher VEGF expression in the wound edge tissue. Pure oxygen is known to induce VEGF. Deliberate hyperoxygenation recruits endothelial progenitor cells to the wound, increases vascular endothelial growth factor (VEGF), and promotes angiogenesis.[4]

Hydrogen peroxide enhances collagen mRNA abundance and other cytokines and growth factors depend on oxygen supply. Another role of oxygen is to support one or more needs of growing vessels such as hydroxylatingprolinerequires oxygen to convert procollagen to collagen.[5] In addition to having this antibacterial property, oxygen is also an antifungal and antihistamine substance.[6]

Therefore, to briefly summarize, oxygen is essential for

- Production of collagen
- Wound tissue epithelialization
- Neovascularization
- Phagocytosis
- Mortification of necrotic wound tissue

Composition of Blue-M and its Basis:

1. **Sodium Perborate:** Key active component and is required for plaque control and facilitate wound healing. Sodium perborate itself doesn't exert any action rather, it reacts with saline and produces nascent oxygen. Since we know that the use of high concentration hydrogen peroxide disinfectant damages the wound tissue, due to production of increased number of free radicals. However lower concentration (<0.15 %) of hydrogen peroxide, when present continuously, is deleterious to pathogenic bacteria only, without causing damage to healthy cells. Hence, acts as a steady source of active oxygen.[7]

2. **Honey[8]:** For plaque control. Basically, honey is phytochemical and has antibacterial characteristics. Its wound healing aspect is attributed to liberation of proinflammatory cytokines i.e. macrophages and monocytes from the neighboring cells.It even exhilarates the production of IL-1β,IL-6 and TNF-α from Matrix metalloproteinase- 6 cells and peripheral blood monocytes. Moreover, Type II arabinogalactan proteins are identified in honey, having immune modulatory characteristics, due to its endotoxin content, which again has stimulatory effect on monocyte family to release TNF-α.

   Honey contains antioxidants that breaks the inflammatory cycle by scavenging the reactive oxygen species. Anti-inflammatory effect of honey is also due to inhibition of COX-2 and inducible NO synthase expression. An in-vitro pilot study has shown that Blue-M mouthwash solution is comparatively effectual than chlorhexidine and is annihilates unicellular as well as multicellular (biofilm) microorganisms.[9]

3. **Xylitol:** Also, for plaque control. Unlike glucose, the classical acid forming bacterial, contained in dental plaque cannot metabolize xylitol as a nutrient. This result due to conversion of xylitol into xylitol 5-phosphate (X5P) once the bacterial cells ingest it and xylitol inhibits acid production. Therefore, xylitol halts the growth of *S.*mutans and S. sobrinus.[10]

   This mechanism credits to retarded cell to cell adhesion of bacteria and polysaccharide production which amounts to less bulk of plaque biofilm and the biofilm is also less sticky.

4. **Methyl Salicylate:** A salicylic acid derivative, this product has been used since long as a constituent of mouth rinses in combination with essential oils (Listerine). It even comes in the group of non-steroidal anti-inflammatory drugs and therefore, also has mild analgesic property. Known to have antisepticand anti-inflammatory effect, methyl salicylate is one of the active ingredients of Blue-M.[11]It acts by disrupting the cell membrane and inhibiting its enzyme activity. Its slight minty characteristic further acts as an add on to the product's flavor.

5. **Lactoferrin:** Acts as bone growth accelerator. From the group of transferrin, there is another iron-binding glycoprotein, Lactoferrin. Apart from being

present in the secondary granules of neutrophils and epithelial secretions, lactoferrin is also readily available in breast milk. This pleiotropic factor has eloquent immunomodulatory and antimicrobial facets. Lactoferrin triggers the differentiation and multiplication of Osteoblast-Like Cells.[12] Therefore, this increased number of osteoblasts has the capacity to orchestrate and mineralize new bone and can also pause cell death. Lactoferrin Inhibits Osteoclastogenesis i.e. even though it directly doesn't affect the osteoclasts' ability to resorb bone but decreases thepotency of precursor cells to produce osteoclasts.[13]

Mechanism of Action of Blue-M: The basic mechanism of Blue-M is controlled delivery of active oxygen i.e. hydrogen peroxide to the site of treatment.

This occurs when Sodium perborate comes in contact with water, it creates a chemical process of hydrolysis, the end products being hydrogen peroxide and boric acid. Hydrogen peroxide further reacts with saline and releases nascent oxygen.

Method of Application

Few elementary steps are followed:

(i) **Preparation and treatment step:** The fundamental procedures like examining the treatment site clinically, taking radiographs and preparation of patient is done. Once the treatment has begun an acute wound is formed. On this wound the gel is applied and left for 2 minutes. The area is then flushed with saline. The previous step is again repeated but now the gel is left as it is in the wound.

(ii) **Patient care:** Patient is instructed to brush twice daily with Blue-M toothpaste. Rinse with blue M mouthwash postoperatively, 4 times a day for 1 minute, for 4 weeks, followed by 2 times a day for 1 minute. In cases of gingival recessions/pockets, use an interdental brush with blue M gel twice daily is recommended.

(iii) **After care:** Follow up period is 2 weeks, 4weeks and 8 weeks. Reevaluation of clinical parameters as well as radiographs are done after 4months and 1 year. This is done to assess the developments in soft/ hard tissue level.

Clinical Indications for Using Blue-M: Gingivitis, periodontitis, periodontal abscess, pericoronitis, periimplantitis, implant treatment and maintenance, halitosis, flap surgeries, endodontic treatments, after tooth extraction, oralmucositis, aphthous ulcer, wound healing, xerostomia.

The products available are Blue M oral gel, mouthwash, fluoridated/non fluoridated toothpaste, mouth spray, oxygen fluid.

Conclusion

Research has shown that nearly all processes in illness can be linked to oxygen deficiency. If we look at the oxygen saturation (pO2 values) of infected or damaged tissue, we find that these values are much lower than they would be in a situation of health. Long-term insufficiency of oxygen saturation will put tissue in what is known as a chronic phase, and no healing is possible thereafter.

The importance of oxygen to the body has long been known, and so oxygen therapy is often practiced nowadays.

Long-term studies on this product can have effective and non-toxic effect on addressing multifactorial infective conditions, without developing resistance to microorganisms unlike antibiotics and can help in accelerating healing process in future practices.

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An Eye for an Eye Makes Maxillofacial Prosthodontics Beautiful: A Case Report

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Abstract

Loss of eye causes physical and emotional disturbace in patient. Rehabilitating the missing eye with ocular prosthesis can alleviate their suffering and brings back confidence in them.Maxilla facial prosthesis includes artificial prosthesis that replaces missing part of facial region which are removed/lost due to trauma,congenital defects or any kind of malignancies. Customized occular prosthesis is one of the method of rehabilitating a missing eye. The re are many method and materials used for fabrication of these prosthesis which has already been discussed in many literature,out of which acrylic resins prosthesis provides better esthetics. The y are economical and characterization is possible. This case report describes the use of stock iris and custom made sclera made up of heat cure tooth colored acrylic.Acrylic resin provides various advantages over stock prosthesis by mimicking the natural eye.

Keywords: *Occular prosthesis, Eye prosthesis, Maxillofacial Prosthesis.*

Introduction

Losing a part of the face especially eye can cause severe physical and emotional problems. Loss of eye can be due to malignancies,congenital defect or irreparable trauma.[1] Depending on "seriousness of loss of eye,the surgical management may include one of three approaches"[2]

Evisceration: "Excision of the intraocular contents of the globe,leaving the sclera and sometimes the cornea".

Enucleation: "Removal of the entire globe and a portion of optic nerve from the orbit'.

Extenteration: "Enbloc removal of the entire contents of the orbit including the extra ocular muscles.[3,4]

Custom made eye prosthesis provides "improved aesthetics and fit but usually more time consuming and complicated".

Case Presentation: An 36 year male patient reported to department of Prosthetics, IDS, Bhubaneswar for a prosthetic replacement of missing left eye. His history revealed that he got his left eye eviscerated 4 years ago due to inverter blast. On Examination it was noticed that anopthalmic socket was healthy with synchronous movement. "Adequate depth was present between upper and lower fornices of the affected side of eye for retention of prosthesis". There was a contracture in left eye lid and healed scar in the eyebrow region.

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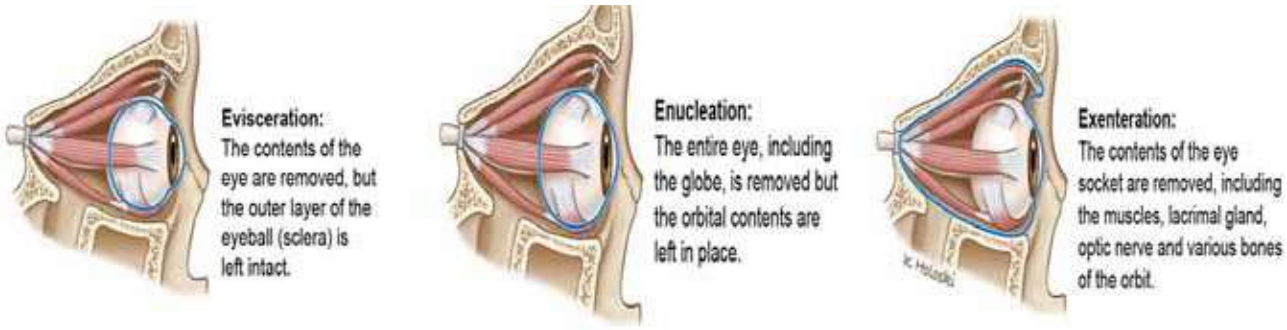


Figure 1.



Figure 2. Left Eye Missing

Technique: Petroleum jelly was applied to the surrounding areas around the eye so that the impression can be retrieved easily. Extra ocular impression was made with alginate (coltene whaledent pvt Ltd) reinforced with simultaneous layering of plaster (BN Chemicals) and Gauge.

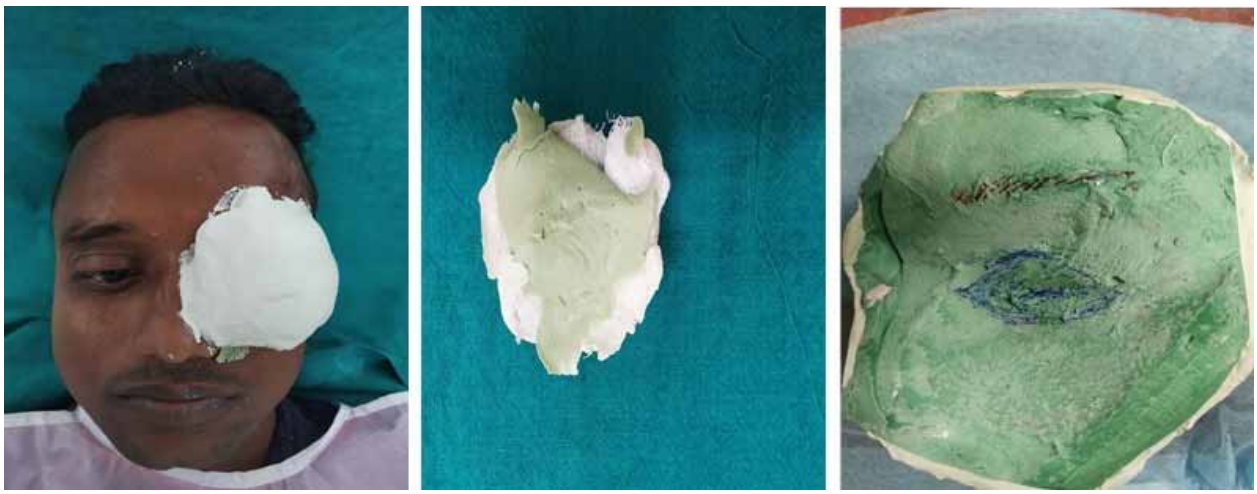


Figure 3. Impression made and cast obtained

After setting of the plaster the whole extra ocular impression was removed and then poured with dental stone (BN chemicals) and cast was retrieved. In the cast spacer of 1 mm was given and a special tray was fabricated using cold cure material (coltene whaledent Pvt Ltd).Fives holes of 2mm diameter was made on the tray. A syringe conformer was attached to the centre of the special trayThe special tray was checked for passive fit in socket and ocular impression was made with light body addition silicone (coltene whaledent Pvt Ltd) by inject through the syringe conformer attached. The patient was asked to make functional movements. After setting of the impression material it was then removed from the eye ball socket. The n the impression was boxed with a paper cup and stone was poured in two layers to obtain a mould. The syringe conformer provided a channel through which molten wax was allowed to flow (maarc,shiva products, mumbai). A commercial available "eye shell" which matched the patients "natural iris" in color and size was selected by matching with the "right eye". The n wax trial was done and position of iris was verified. Flasking and de waxing was done using conventional method. Selected shade of heat cure acrylic resin (coltene whaledent Pvt Ltd) was mixed and packed into the mould and then final curing of heat cure acrylic was done. Trimming and polishing was done. Prosthesis was disinfected. Final insertion of the acrylized prosthesis into the "eye socket" was done and evaluated for esthetics and comfort of the patient. The patient was educated on how to insert and "remove the prosthesis". Regular follow up appointments was scheduled along with instructions on maintenance of prosthesis.



Figure 4. Special tray made and final impression made with light body

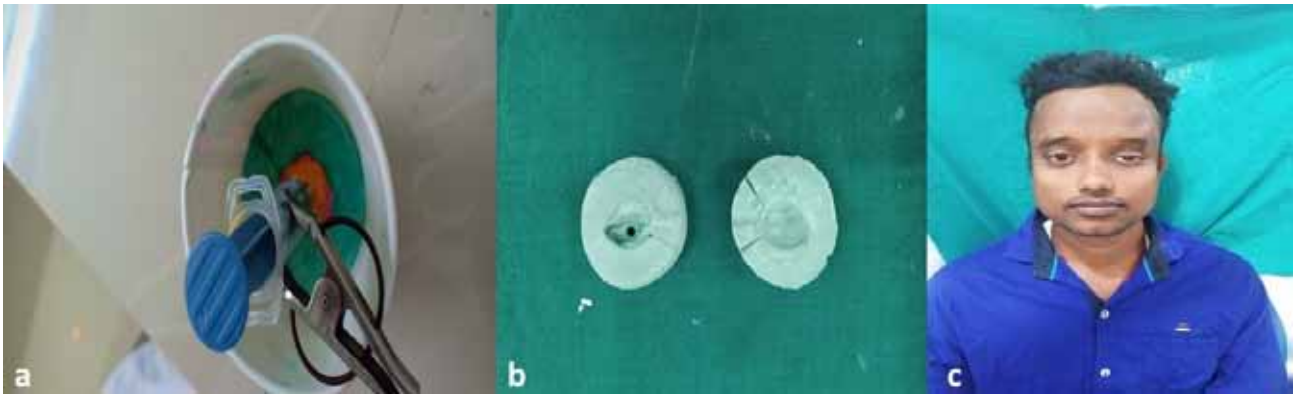


Figure 5. a, b. Final Impression Boxed and Split Cast c. Wax Trial Done



Figure 6. Final Insertion Of Prosthesis

Discussion

Custom made eye prosthesis gives a close contact between prosthesis and tissue bed thus it prevents accumulation of tears and mucous thus incidence of conjunctival abrasion and infection is less. It also provides good esthetic result and retention and stability[5]. Also in custom made prosthesis the iris and the sclera can be painted in accordance with the adjacent natural eye thus making it appear more esthetically pleasing.

Conclusion

Customized occular prosthesis is one of the method of rehabilitating a missing eye. The re are many method and materials used for fabrication of these prosthesis which has already been discussed in many literature,out of which acrylic resins prosthesis provides better esthetics. The y are economical and characterization is possible. This case report describes the use of stock iris and custom made sclera made up of heat cure tooth colored acrylic. Acrylic resin provides various advantages over stock prosthesis by mimicking the natural eye.

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Red and White Lesions of the Oral Cavity: An Update

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Abstract

In our oral cavity various pathological conditions have been reported which includes different type of lesions which require a proper diagnostic criteria, clinically correlation including radiographic & histological reports for its diagnosis. In this article we focus on the various types of red and white lesions of the oral cavity which have been classified based on its etiology, variation in structure, appearance, precancerous potentiality & etc. The lesion appears white due to changes in the epithelium which can be caused by an increase in the keratinization of normal keratinized mucosa, keratinization of non keratinized mucosa or thickening of the epithelium. The reduced vascularity or fibrosis of mucosa can also lead to the whiteness of the oral mucosa. The red appearance of the lesion appears because of the inflammation,atrophy, vascular dilation, and purpura or reduced in the keratinization of the oral epithelium. Generally the red and white lesions are benign and a large proportion of the normal population has been affected by it. The most common precancerous condition which comes under these lesions classification is Leukoplakia**.** A proper management of this condition is required to avoid its future complication. All conditions should be properly examined by taking a proper and careful history. However a biopsy is much needed for these conditions to be diagnosed accurately.

Keywords: *Red Lesion, White Lesion, Leukoplakia, Keratinization, Precancerous.*

Introduction

The red and white lesions are most commonly affected lesion in the oral cavity. To understand the various colors of this lesion one has to know, the color of oral mucous depends on the Degree of keratinization, dilation, and concentration of blood vessels, the amount of melanin pigment present in the epithelium and thickness of the epithelium. The whiteness of the oral mucosa can be due to acanthosis, hyperkeratosis, and necrosis in oral epithelium, vascularity reducing in the underlying lamina propria, and accumulation of fluid intracellular or extracellular in the epithelium. Same as the red appearance is caused by enlarged blood vessels, atrophic epithelium, and an increase in vascularisation and a decrease in the number of epithelial cells.[1]

Classification According to the Burkett's 11th Edition:

- **Red and White tissue reactions**
- **Infectious diseases,** which includes
  - Oral candidiasis
  - Hairy leukoplakia
- **Pre Malignant lesions**
  - Oral leukoplakia and Erythroplakia
  - Oral submucous fibrosis
- **Immuno Pathologic Disease**
  - Oral lichen planus
  - Drug induced lichenoid reaction

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  - Lupus Erythematosus
  - Lichenoid Reaction
- **Allergic Reactions**
  - Lichenoid contact reactions
  - Reaction to dentifrice and Chlorhexidine
- **Toxic Reactions**
  - Reactions to smokeless tobacco
  - Smokers palate
- **Reactions to mechanical trauma**
  - Morsicatio
- **Other red & white lesions**
  - Benign migratory glossitis
  - Leukoedema
  - White sponge nevus
  - Hairy tongue

Infectious Disease:

1. **Oral Candidiasis**:-It is also known as oral thrush. It is caused by yeast *Candida albicans*. The different species of Candida which are responsible for the infection are c. albicans, c. tropicalis, and c. glabrata thus contributing in the 80% of cases. The production of lipases facilitates the penetration of yeasts into the epithelial cells.[2]

   Pathogenesis:

   Yeast cells adheres to host cell

   ↓

   Host cells triggers yeast to hypha transition

   ↓

   Uptake of fungus by host cells through endocytosis

   ↓

   Penetration of fungal active into host cells

   ↓

   Formation of biofilm

Various traits also influence the fungal pathogenicity, which includes Hsps, induction of hyphal formation by uptake of amino acids, and extracellular alkalinization.

Classification includes Acute, Pseudomembranous, Erythematous, and Candida associated lesions such as denture stomatitis, angular cheilitis, and median rhomboid glossitis.

Pseudomembranous Candidiasis: The oral lesions are white, soft and plaques are elevated occurring on buccal mucosa, tongue, gingiva, palate, and floor of the mouth. Acute form belongs to classic Candida infection whereas chronic form is associated with HIV and steroid inhalers. It affects the people who are under medication for prolonged antibiotics and immunosuppressant drugs.[3]

Erythematous Candidiasis: It is also known as antibiotic stomatitis and occurs due to a long course use of broad-spectrum antibiotics, smoking and steroids inhalation. It can occur in any site and it's the only variety of oral candidiasis that is consistently painful.

Chronic plaque-type and nodular candidiasis: It is also known as chronic hyperplastic candidiasis. It is featured by a white plaque. The lesion is firm, white persistent plaques occurring in the lips, tongue, and cheeks. This form is associated with malignant transformation.[4]

Candida-Associated lesions:

I. Denture stomatitis

II. Angular Cheilitis

III. Median rhomboid glossitis

IV. Oral candidiasis associated with HIV infection, Management of following involves:

The treatment of denture stomatitis includes eliminating the predisposing factors, dentures should be kept in antimicrobial solutions and surgical excision.

Angular cheilitis is treated by topical miconazole or a mild steroid ointment suppresses the inflammation.[5]

The treatment of median rhomboid glossitis involves the use of topical antifungal clotrimazole, fluconazole, and topical corticosteroid which includes fluocinonide 0.05% & clobetasol 0.05%.

2. **Hairy Leukoplakia:** This lesion is a corrugated white lesion that occurs on the ventral or lateral surfaces of the tongue. The disease affects mostly the severe immunodeficiency patients. It is the

second most HIV associated lesion. It mostly affects men as compared to females.

Pathogenesis: The marker involves lowering of **CD4+ T- lymphocytes counts.** Greenspan and the co fellows in 1984 reported the first time. It is strongly associated with the Epstein Barr virus. The clinical features involve vertical white folds in the borders of tongue **shaggy orfrayed** appearance. The lesion is bilateral often, asymptomatic and cannot be scraped off. A palisading pattern can also be seen clinically.[6]

Histopathology Features: In the irregular surface severe hyperkeratosis can be noticed with acanthosis. Hairs can be seen projecting from the surface. Balloon cells can be observed in the spinous layer 7 pyknotic nuclei. The important microscopic features involve homogenous nuclear inclusions and EBV can be detected by in situ hybridization or by IHC. The lesion does not require any treatment. However antiviral drugs such as acyclovir, zidovudine is been used and topical application of podophyllin resin 25% solution is used. The malignant transformation is very low.[7]

Pre Malignant Lesion:

1. **Oralleukoplakia:** It is the most common premalignant condition. It is a white patch that can't be characterized pathologically or clinically as any other disease.

Pathogenesis: The cause of Oral leukoplakia is still unknown.

The various etiologic factors of leukoplakia include:- Use of tobacco, hot, cold and acidic foods, beverages, occlusal trauma, syphilis, presence of candida Albicans, sharp tooth, ethanol, and presence of viruses.

Oral leukoplakia is more common in men and it mostly occurs over 50 years of age.

Clinical Findings: It is usually asymptomatic and occurs in the floor of mouth, buccal mucosa, maxillary gingiva, hard palate, lip, soft palate, and lip vermilion. The borders are ragged or smoothly contoured. The mouth is scattered by solitary or multiple plaques. The most common occurring sites or at high risks isthe floor of the mouth and lateral borders of the tongue for malignant transformation.[8]

Classification: Oral leukoplakia can be classified as:

1. **Homogenous leukoplakia:** In this type no red component can be found and is asymptomatic and the lesion is white and consists of well-demarcated plaque. Surface fissures appear as cracked mud.
2. **Speckled Leukoplakia:** This form of leukoplakia consists of both red and white flecks of line.
3. **Erythroplakia:** In this form also a combination of both red and white patches can be observed.
4. **Verrucous Leukoplakia:** It is a lesion where verrucous areas are present. It consists of nodular or exophytic components. The lower gingiva is a predilection site. An aggressive form of this lesion can occur which is called proliferative verrucous leukoplakia in which the reoccurrence rate is high.

Diagnosis: The cause should be eliminated such as sharp tooth or restoration.

If the healing does not occur then a biopsy is must required.

Histopathology: The Features include hyperkeratosis, hyperplasia, and atrophy.

The malignancy rate of oral leukoplakia is between 4%-8%.

Differential Diagnosis of this lesion includes lichen planus, smokeless tobacco lesion, hairy leukoplakia, verruca Vulgaris, lupus erythematous, cheek biting lesion, white sponge nevus, etc.[9]

Management: The management involves first and furthermost to discontinue the habits. Different types of surgeries include cold knife surgical excision, laser surgery are widely used. Chemoprevention is used in the form of topical bleomycin. Antioxidants such as retinoic acid and beta carotene are administrated. Stripping procedure with free grafts is also used.

2. **Oral Submucous Fibrosis:** It comes under the classification of premalignant lesions. It is a chronic disease that is mostly caused by the use of arecanut. It has a high risk of malignancy. The metabolism of collagen is affected by arecanut which leads to increased fibrosis.

Clinical Features: The hallmark diagnosing feature is the Burning sensation in the patient mouth. Excessive salivation, dryness of mouth, generalized inflammation with ulcerations can also be seen. In the cheek and palate small vesicles can be observed. Bands are also

present where pain persists. In the advanced cases of this lesion in the entire oris or mouth orifice circular bands are found, difficulty in swallowing, fibrous bands appearance, difficulty in tongue movement and inability to whistle are observed.

Histopathology: It involves hypertrophic fibroblasts, epithelial atrophy, loss of rete pegs, edema, and dilated & congested blood vessels.[10]

Management It involves furthermost the elimination of habit. In treatment plan vitamin B complex and high proteins and calories should be taken. Glucocorticoids which act as immunosuppressive agents is been used. Local injections of hyaluronidase, dexamethasone, and placental extract are used. In surgical management involving the excision of the fibrotic bands by fresh human placental grafts, followed by the injection of dexamethasone in the advanced cases.

Immunopathologic Diseases:

1. **Oral Lichen Planus:** It is one of the most common diseases. The cause of this disease is still unknown. It can consist of both red and white components with different textures.

The autoreactive T lymphocytes play a vital role in the development of OLP. Severe pruritis is a primary symptom of this disease. The surface is covered by very fine grayish-white lines called **Wickham's Straie.** One of the classical hallmarks of these skin lesions is **pruritic erythematous to violaceous papules**. Koebner phenomenon is associated with this disease. Genital mucosa is the most common extraoral mucosa site. The OLP has different forms which include Reticular Form, popular form, plaque form, Bullous form, Erythemateous form, and ulcerative form.

Histological Features: It includes Liquefaction degeneration, hyper ortho keratosis, and sawtooth appearance of retepegs. Beneath the basement membrane one can notice an eosinophilic band and thickening of the granular cell layer.

Management: It involves the use of Corticosteroids, Calcineurin inhibitors, Tacrolimus, Pimecrolimus Retinoids, Dapson, Mycophenolates Enoxaparin, Efalizumab. The different nonpharmacological modalities include PUVA therapy, Photodynamic therapy, and LASER therapy.

2. **Drug:** Induced Lichenoid Reactions-It is a type of lesion which exhibits similar histopathological features as lichen planus and clinically it is very difficult to distinguish between drug-induced lichenoid reactions and lichen planus. It can be differentiated from the OLP based on drug administration, contact with a metal or systemic disease & when the factor was eliminated, on resolution. The causes include Gold therapy, NSAIDs, Other hypertensives, etc. Clinically ulcerative reaction pattern can be seen and unilateral.[11]

Management: The treatment involves discontinuation of drug and topical steroids are used.

3. **Lupus Erythematosus:** It is an autoimmune disorder, which is chronic. The etiopathogenesis of SLE is not all so clear but some research fin that it is due to the interaction between genetic and hormonal factors, and hormonal exposures. Epstein Barr virus ad cytomegalovirus is commonly associated with this disease. In some researches it has been found that ROS, which is reactive oxygen species, triggers the production of autoantigen and T cells which are involved in the pathogenesis of SLE. Different diagnostic criteria are made for SLE which includes Neurological involvement, arthritis, anti-double-stranded DNA, APLA, ANA are now considered in the inclusion criteria. The management involves the use of corticosteroids, Hydroxychloroquine, Cyclophosphamide, and other immunosuppressive agents are used.

Allergic Reactions: This classification presents:

- **Lichenoid Contact Reactions:** It is a type of hypersensitivity reaction which occurred due to the reaction of amalgam fillings. The most important disguising feature to differentiate between OLP and LCE is **the extensions of lesions.** It is usually asymptomatic. The management includes the replacement of dental materials and the healing period is 1-2 months.
- **Reactions to Dentifrice and Chlorhexidine:** It is a very rare disorder. It is a type of allergic reaction that includes flavor additives such as carvone and cinnamon or preservatives. Clinically both ulcerations and white lesions can occur. The common sites involve buccal, labial, and tongue mucosa. The healing of the lesion is seen after the withdrawal of allergic contained agents.[8-11]

Toxic Reactions: Under this classification;

- **Reactions to smokeless Tobacco:** It is divided into 3 groups; Moist snuff, chewing tobacco, and Dry snuff. It is a white and leathery lesion and gingival retractions can be seen in smokeless tobacco habit. Wrinkles at the site of application can be observed in the mild form. Discontinuation of the habit is the proper treatment regarding this lesion.
- **Smokers Palate:** It is also known as stomatitis Nicotina or Leuukokeratosis. This is a white patch diffused in nature occurring in the Hard Palate. It is usually caused by smoking like cigars or pipe smoking. It is painless and the response of chronic heat forms this patch, during smoking. It has not an increased rate of transferring into oral cancer. Clinically one can observe gray or white nodules and the presence of red dots in the center. In some severe cases dried lake **bed appearance** can be seen in the mucosa. In this lesion a biopsy is not required usually but in some cases where red patches can be seen i.e. Erythroplakia then a biopsy is required. Stopping the habit of smoking can lead to an eradication of patch, but if the lesion persists even after the stopping then a biopsy is required must and leukoplakia condition can be diagnosed in those cases.

Reactions to Mechanical Trauma:

Morsicatio: It is also known as chronic cheek biting. In this trauma the buccal mucosa is injured due to chronic irritation due to repetitive biting or chewing. It is bilateral and the lesion is white with thickened mucosa. Erythema (redness) and ulcerations can also be seen. No treatment is required for this trauma, however if the problem prolongs then one can seek medical attention.[10,11]

Other Red and White Lesions:

1. **Benign Migratory Glossitis:** It is also known as geographic stomatitis. It is more common in females as compared to males. It mostly common in the age group between 20-30 years. Etiopathogenesis includes genetic like B15, DRW6, & CW6, Nutritional deficiency, hormonal and psychological disturbances. Allergy can also be a cause of this lesion. No treatment is required however mouthwash like tantum or benzydamine hydrochloride 0.15% spray can be used.
2. **Leukoedema:** It is a white lesion and occurs frequently in the buccal mucosa. The etiology is still now not clear. Bilaterally in nature and usually asymptomatic. No treatment is required.
3. **White Sponge Nevus:** Canon in 1935 first described this lesion. It is an autosomal dominant disorder. Epithelial keratin gene playing a vital role in its pathogenesis. It is a very rare disorder. Clinically a white lesion with irregular surface can be noticed in the buccal mucosa. The mucosa gets swollen in this disorder like a sponge, hence the name Sponge Nevus. It is benign with no treatment is required.
4. **Hairy Tongue:** The etiology is unknown but the predisposing factors include antibiotics, excess alcohol consumption, poor oral hygiene, immunosuppressive drugs, the microflora in mouth, and oral candidiasis. Clinically hair-like appearance can be seen due to impaired desquamation of filiform papillae. The treatment involves elimination or reduction in predisposing factors.[8-11]

Conclusion

The red and white lesions include different type of lesions and the professional require a skillful knowledge to diagnose and distinguish between these lesions. However clinical correlation plays a very important role in diagnosing apart from histological reports. The malignancy rate is not so high of this lesion except in the cases of leukoplakia or oral submucous fibrosis. Researches are done to know the etiological factors of some of these lesions.

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Silver Diamine Fluoride in Clinical Pediatric Dentistry

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Abstract

Silver diamine fluoride is the most recent advancement in the field of dentistry. Its history dates back to the 1960s and since its invention there was no looking back. People across the globe and different races and cultures have benefitted from SDF. With almost no adverse reactions, pocket-friendly, and highly effective qualities in its stride, SDF has emerged as a powerful tool in caries prevention, especially in a county like India where the oral health of a child is neglected. This review focuses on the history, indications, contraindications, mechanism, and safety margin of SDF as a dental material.

Keywords: *Silver diamine fluoride; Mechanism of action; Oral health, Child.*

Introduction

The field of dental sciences has witnessed plenty of changes to techniques, materials and concepts. With the advent of the 20th Century, a number of researchers began focusing on new materials that could be used to prevent dental caries. Unlike G.V. Black's concept of "extension for prevention", new age concepts focuses primarily on conservation and regeneration. The face of dentistry has changed since the concept of dental caries being a disease and not a lesion has emerged. The more we understood the mechanism of the disease process, the more we, as dentists began accepting a more medical approach towards treating dental caries. This involved the various caries risk assessments of the child patient followed by counseling the patient/parent regarding all the possible preventive methods deemed fit.[1]

One such concept which has gained popularity among dentists and researchers across the globe is Silver Diamine Fluoride (SDF). Since its invention in 1969, it has proven to be an effective and reliable alternative to other invasive treatment options.

History: Silver Diamine Fluoride was first invented by a Ph.D. Graduate named Mizuho Nishino at Osaka University, Japan in 1969.[2] She came up with the idea of using silver as an antimicrobial agent in combination with the remineralizing properties of fluoride. The product that resulted was a substance with additional properties like obliterating dentinal tubules by forming a precipitate, hence reducing dentinal hypersensitivity. Eventually, SDF was approved as a marketable product by the Central Pharmaceutical Council of the Ministry of Health and Welfare of Japan.[1] Since then, it is considered as a cariostatic agent. Toyo Seiyaku Kasei Co. Ltd., Osaka, Japan began marketing this product under the brand name Saforide.

The Food and Drug Administration (FDA) approved to release the first silver diamine fluoride product into the market in August 2014. In October 2016, SDF was awarded as the "breakthrough therapy" of the year, based on its efficacy in arresting dental caries in children as well as in adults. What followed next was the recognition of oral disease as a major medical condition and a serious public health issue.

Mechanism of action of SDF: SDF is a clear liquid product primarily made up of water, 25 % silver,

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8% ammonia and 5.5% fluoride. This results in a 38% SDF.[3]As the name suggests, Silver Diamine Fluoride contains silver, which acts as an antimicrobial agent, fluoride which contains remineralizing properties and ammonia which helps in stabilizing the solution at high concentrations. SDF was first invented to arrest dental caries and prevent dentinal hypersensitivity. As the carious process begins, the dentin undergoes demineralization leading to exposure of the organic matrix. After applying a preparation of silver diamine fluoride on a carious lesion, the formation of a squamous layer containing silver-protein conjugate occurs. This layer acts as a shield against acid dissolution and enzymatic digestion.[4] Eventually, there is formation of hydroxyapatite and fluorapatite, including silver chloride and metallic silver on the exposed surface of the organic content.[5] Once applied, lesion depth begins to decrease, while at the same time mineral density and hardness increases. SDF promotes inhibition of those proteins which split the exposed organic matrix of dentin: metalloproteinase, cathepsins and collagenases.[4]

Silver ions have anti-microbial and antirheumatic properties. It acts by breaching bacterial membranes and denaturing the proteins, thus inhibiting the process of DNA replication.[6] SDF surpasses all other bacteriostatic medicaments in destroying caries causing bacteria in the dentinal tubules.[7] Silver and fluoride ions can penetrate upto 25 microns into enamel[8] and nearly 50-200 microns into dentine.[9] Carious lesions arrested due to Silver diamine fluoride are nearly 150 microns thick.[10] Lesions that are artificially treated are defiant towards the formation of a biofilm and additional cavity formation, apparently because of the presence of residual silver ions.[11,12] It is found that more amount of silver and fluoride is deposited on demineralized carious dentin as compared to non-demineralized dentin as a result of which, demineralized dentin treated with SDFis better resistant to further caries attack than sound dentin treated with SDF.[13]

Due to the action of Silver ions, some amount of bacteria is killed. When these are supplemented to the living group of bacteria, the silver ions which were present are replenished and reactivated. As a result, the dead bacteria successfully kill the living bacteria as well. This process is coined as a "zombie effect".[14] This explains as to why the silver ions applied to a lesion within a cavity demonstrates sustained antimicrobial activity. While treating hypersensitivity, the application of topical SDF promotes the formation of a squamous protective layer on the exposed dentin matrix, eventually obliterating dentinal tubules.[6] This mechanism of action of SDF is in agreement with the Hydrodynamic theory of dentin hypersensitivity.[15]

Indications:

1. **Active carious lesions:** Both silver and fluoride ions display potential to prevent the formation of cariogenic biofilms. Increased concentration of fluorides can unite to the cellular components of cariogenic bacteria and regulate enzymes that help during glucose metabolism and also sucrose uptake.[16] Simultaneously, silver ions denature the bacterial cell wall, control enzyme activities,sway metabolic processes and hinder the replication of bacterial DNA.[16]
2. **Remineralization:** Fluoride acts as a remineralizing agent of hydroxyapatite in enamel and dentin. This takes place when SDF reacts with hydroxyapatite of teeth forming calcium fluoride and silver phosphate.[5] This calcium fluoride thus formed, is a reservoir of pH regulated fluoride on the surface of the decayed tooth. 38% SDF contains 44,800ppm of fluoride.[8]
3. **For caries prevention:** Topical application of SDF on healthy tooth surfaces prevents caries in children.
4. **Dentinal Hypersensitivity:** SDF promotes obliteration of dentinal tubules thereby reducing hypersensitivity.[18]
5. **Root caries:** Because of the virtue of SDF to arrest active caries, it can be annually applied on cariously exposed root surfaces, without any toxicity.[19]

Contraindications:

1. It cannot be used in patients prone to silver allergy.
2. SDF tends to irritate sensitive open pores. Hence, it cannot be used.
3. SDF cannot be applied to teeth that require endodontic therapy.

Clinical application of SDF: A study of extracted teeth treated by SDF found that the application of 38% SDF increases the remineralization process of initial carious lesions. The University of California, San Francisco (UCSF) designed a clinical protocol for the clinical application to maximize the safety and effectiveness of SDF in clinical settings[20].

1. Counter top and the patient should be covered with plastic.
2. Personal protective equipment (PPE) should be provided for the patient and the clinician.
3. In a dampen dish, take one drop of SDF. Simultaneously take one drop of SSKI in a separate dampen dish.
4. Using a saliva ejector, remove the excess bulk.
5. Isolation of the selected tooth from the tongue and cheek should be done using gauze or cotton rolls.
6. Use petroleum jelly if the affected tooth surface is close to the gingiva.
7. Use a triple syringe or dry cotton to dry the affected tooth surfaces
8. Using a microsponge, bend it, dip it into the dampen dish containing SDF, and squeeze the excess on the side.
9. Apply the microsponge directly onto the affected tooth surface(s).
10. Leave the SDF to soak up for one minute, then wipe the excess material using gauze or cotton roll. (In case of SSKI, use a separate micro sponge and apply onto the affected tooth surface. Repeat for one to three times till no more white precipitate appears. Wait five to ten seconds between the applications. Wipe the excess with cotton/gauze.)
11. After one minute, rinse with water.
12. Discard the used materials into plastic waste bags.

Many clinicians prefer application of SDF first during the initial appointment, then at one and/or three-month follow-up, then at every 3 months.

Side Effects: No adverse event has been reported since the approval of silver diamine fluoride as Saforide which was marketed more than 50 years ago.[21] Silver diamine fluoride causes darkening of carious lesions although it doesn't discolour sound enamel; it will cause some degree of darkening of early non-cavitated carious lesions. It is found that due to the formation of insoluble silver phosphate, the lesion treated with SDF turns black on exposure to sunlight. To surmount this shortcoming, potassium iodide was used after the topical application of SDF.

Maximum recommended dose and safety margin: Calculating the maximum recommended dose and the margin of safety of any drug is of utmost importance. Research conducted in this aspect yielded that an average Lethal Dose orally was 520 mg/kg and 380 mg/kg via subcutaneous route. One drop (25 µL) is sufficient to treat five teeth since it contains 9.5 mg of SDF. Assuming the youngest child with caries would be about 10 kg, 0.95 mg/kg SDF would be required for the child.[23]

Conclusion

Silver Diamine Fluoride has emerged as an exceptional treatment method in the field of dentistry. No material to date has reached up to it in terms of safety, efficacy and affordability. It is primarily indicated as a means of caries prevention for children, especially those who are terminally ill or too weak to be conventionally treated. It halts the disease process. SDF application is recommended twice annually, which surpasses all invasive conventional attempts at caries prevention. In a developing country like India, where the majority of the population cannot afford professional healthcare, this new-age material is a powerful tool that would help reach out to the masses. Children with a high caries burden are advised for SDF application on all teeth until they are restored or exfoliated. More research and refinement with regards to technique needs to be conducted to improve caries prevention modalities and provide quality healthcare.

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Ethical Permission: Approved

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Distal Shoe Space Maintainer for the Premature Loss of Primary Second Molar in a 5-Year-Old Patient: A Case Report

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Abstract

Uneventful loss of deciduous second molar, afore the eruption of first permanent molar could lead to unadorned malocclusion due to undesired loss of space in the arch, tipping, crowding of the dental arch and many other dental problems. So, to avoid further occlusal complications, it is desirable to place a space maintainer after successful extraction of the carious primary molar. This case report describes the removal of a primary mandibular second molar, followed by delivery of a distal shoe appliance before the eruption of the first permanent molar.

Keywords: *Distal shoe, primary molars, permanent first molar, crown, eruption.*

Introduction

The idea of a device to maintain space loss due to premature exfoliation in the dental arch was first given by Davenport, in 1887.[1] Space maintainers are "the appliances used to maintain space or regain minor amounts of space lost, to guide the un-erupted tooth into a proper position in the dental arch". The two main types of space maintainers include fixed and removable. The distal shoe space maintainer, also called as Intra-Alveolar Appliance/Eruption Guidance Appliance is a fixed type of space maintainer, which was first developed by Gerber in 1964.[2] It was later modified by Croll in 1980.[3,4] It is primarily given in cases where there is premature exfoliation on the second primary molar, before the eruption of the permanent molar. Since this appliance helps in guiding the eruption of the first permanent molar, it is also called an Eruption Guidance Appliance.

Hicks, in 1973[5], first used cast gold to fabricate this appliance, but it soon grew out of popularity because of its increased cost. After 20 years, Gegenhiemer and Donly,[6] were the first to fabricate a laboratory-prepared distal shoe space maintainer which was already soldered to a stainless steel crown. The advantage of this was it was less time consuming, and the patient had to visit only twice. Clinically, the indications and contraindications as described by Hicks in 1973 are [5]:

Indications:

- Premature loss or extraction of the second deciduous molar before the eruption of first permanent molar.
- Advanced root resorption and periapical bone destruction of the second primary molar before the eruption of first permanent molar.
- A primary second molar with advanced caries that is non-restorable.
- Ectopic eruption of the permanent first molar.
- Ankylosis of the primary second molar.

Contraindications:

- Inadequate abutments due to multiple loss of teeth.

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- Poor patient/parental cooperation.
- Missing permanent first molar.
- Systemic diseases that affect healing such as Diabetes Mellitus.
- Cardiac anomalies that require antibiotic prophylaxis before dental treatment.

The motive of this article is to describe the management of a premature exfoliation of mandibular deciduous second molar due to extensive caries before the eruption of the permanent first molar using the Roche variety of a distal shoe space maintainer.

Case Report: A male child aged 5 years visited the Department of Paediatric and Preventive Dentistry, with a chief complain of pain in his left lower back tooth region (Fig 1). A proper history revealed that the patient had recurrent pain and swelling concerning the same since 1 month. Medical history was non-conclusive. Dental history showed the patient had previously visited the department for pulpectomy procedure.



Figure 1. 5-year-old patient with pain on the left lower back tooth region

Clinical intraoral examination showed grossly decayed primary mandibular second molar on the left side (75) and a carious primary first molar (74). (Figure 2) The permanent first molar of the same side is yet to erupt. Radiographic examination concluded the perforation of the pulpal floor, inter-radicular radiolucency and root resorption with respect to 75. (Figure 3)

Thus, it was decided that 74 was to be restored using Glass Ionomer cement and 75 was to be extracted and instead a distal shoe space maintainer was to be adapted. The procedure was explained to the parents and informed consent regarding the number of visits and the procedure was taken before the start of the procedure.



Figure 2. Intra-oral examination of 74, 75

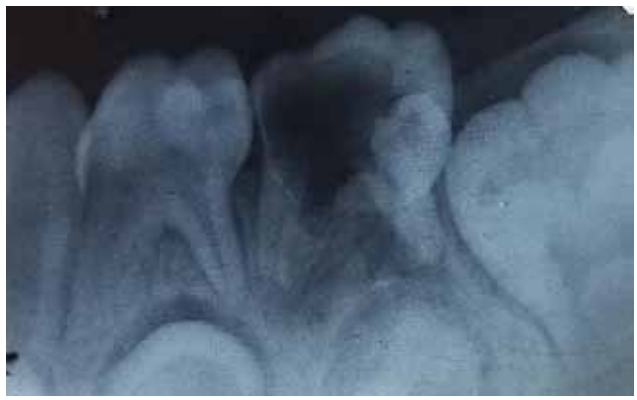


Figure 3. Radiographic examination of carious 74, and grossly decayed 75

Procedure: The primary first molar (74) was excavated for caries and restored using Glass Ionomer Cement. Eventually, it was prepared for a stainless steel crown. After tooth preparation, the crown was adapted and an alginate impression was made. Eventually, the stainless steel crown was taken out and correctly fitted into the impression and stabilised using thin 21 gauge wires, which was poured using dental stone and die stone. (Figure 4) A temporary crown using tooth coloured acrylic was fabricated chair-side and delivered to the patient. Patient was recalled after 4 days.



Figure 4. Alginate impression made and crown seated. Cast poured using die stone with adapted crown.

The distal shoe appliance was fabricated using a 19 gauge stainless steel wire. The Roche variety of distal shoe appliance was fabricated. It consists of a horizontal part of the wire spanning the space and a vertical sub-gingival extension which would be seated approximately 1 mm below the medial most aspect of the first permanent molar. The length of the loop was calculated extending from the distal-most aspect of 74 up to the mesial aspect of the un-erupted permanent first molar using a radiograph. The sub-gingival extension on the loop was scanned using the same IOPA. The wire component of the appliance was fabricated using pliers and soldered to the stainless steel crown.

During the following appointment, the deciduous mandibular second molar (75) was extracted under local anaesthesia and a prior antibiotic coverage. After haemostasis was achieved, the fabricated appliance was seated on the primary first molar and its sub-gingival extension was pushed into the extraction socket with gentle digital pressure. It was seated in such a way that the gingival loop lightly grazes the mesial marginal ridge of the un-erupted permanent first molar. After the position was confirmed using a radiograph, the appliance was cemented in place using Luting type of Glass Ionomer cement (Figure 5). Recall appointments were scheduled after one week, one month and then after every 3 months.



Figure 5. Radiographic and intra-oral images to confirm placement of the appliance.

Discussion

The primary second molar is a crucial tooth in the mixed dentition for the sole reason that it guides the permanent first molar by providing a path for an eruption. The distal surface of deciduous second molar helps to guide the eruption of permanent first molar which has a mesiooccclusion pattern of eruption. Hence, it is very important to preserve the deciduous second molar until the eruption of the permanent first molar. Extraction of the same should not be taken up unless indicated. As a result of premature loss of primary second molar, there could be loss of arch perimeter, which would eventually need complicated orthodontic intervention. The criteria of a successful placement of distal shoe space maintainer is judged by the successful guidance of the un-erupted permanent first molar into the dental arch with no or minimal glitch with the space maintainer, as suggested by Baroni et al[7] and Quidemat et al[8].

Conclusion

The major concern for a paediatric dentist is to try and save the primary second molar before the eruption of the permanent first molar. In extreme situations when that cannot be achieved, there are no other solutions except to extract it. Thus, as a subsequent treatment plan, the fabrication of an eruption guiding appliance is the safest and the best way to prevent crowding and malocclusion. It is cost-effective, less time consuming and patient-friendly method of space management. After the eruption of the permanent first molar, the appliance could either be removed or modified into a crown and loop type of space maintainer by just removing the sub-gingival extension of the loop. Hence, the distal shoe appliance is the best way to prevent serious dental malformations that could occur due to inadvertent early loss of primary second molar.

Conflict of Interests: None

Ethical Permission: Approved

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Tobacco Smoking and Cardiovascular Diseases: A Review

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Abstract

The major risk factor for cardiovascular diseases (CVD) is tobacco use, if we stop use we can reduce cardiovascular disease. Atherosclerosis, which is a main contributor to cardiovascular diseases is formed because of tobacco use. Nicotine is the main addictive substance of cigarette smoke. Its effect on Cardiovascular organs is well documented but when nicotine is taken in the form of patches it effect is very limited. For this reason, Nicotine Replacement Therapy is used for tobacco cessation. Smokeless tobacco is also harmful as they contain nicotine and other chemical components which affect the gastrointestinal tract. Quitting tobacco not only have an immediate health benefit but also improves the psychosocial life of that person.

Keywords: *Tobacco, Smokeless Tobacco, Cardiovascular diseases (CVD), Atherosclerosis, Nicotine Replacement Therapy.*

Introduction

Tobacco chewing or smoking has a grim and unfavorable effect on most organs of the body and is associated with multiple diseases reducing the expectancy and quality of life. Diseases associated with tobacco usage include lung & oral cavity cancer, cardiovascular diseases, emphysema, and bronchitis. Tobacco consumption is considered highly prevalent and an epidemic both in developing as well as developed nations. The prevalence of tobacco usage is more inmen than women in India. Rustic, illiterate & poor section of the society were found to have this habit of smoking as found in a survey done by National Family Health Survey (NFHS)-3, conducted in 2005–06.[1] As it was found by Global Adult Tobacco Survey (GATS) done on persons 15 years of age or older than 34.6% of the adults (47.9% males and 20.3% females) are tobacco users. Of this fourteen percent of the adults smoke (24.3% males and 2.9% females) and 25.9% use smokeless tobacco (32.9% males and 18.4% females).[2] And in the survey done by Global Youth Tobacco Survey (GYTS)[3] on students aged 13–15 years, 14.6% of students were found to be tobacco users. Reports done on morbidity in the age group of 30 – 69 years it was found that one in twenty (5.4%) deaths among Indian women and one in five deaths (25%) among Indian men are because of smoking[4]. India has 11.2% of the world's total smokers. The main organ affected by tobacco use, are cardiovascular system and death due to cardiovascular diseases (CVD) deaths are expected to surge by 12% over the next decennium.[5] Only if tobacco use can be stopped there will be a reduction in the occurrence of cardiovascular diseases (CVD).[6] From 2007 the creation and enforcement of Cigarettes and other tobacco product acts (COTPA) and national tobacco control program (NTPC) by the government has tried to control tobacco usage[4].

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The smokes of cigarettesharm numerous parts of the body thus causing diseases and ultimately death. A study done in 2015 found globally one in every ten cardiovascular deaths is attributable to smoking, and the majority of them took place in just four countries one being India. Morbidity due to cardiovascular diseases is measured in disability-adjusted life years (DALYs) and 41% of this that are attributable to daily smoking[7]. The smoke that is coming out while a cigarette is burning is a toxic mix of more than 7,000 chemicals[8] and, when the inspiration of cigarette smoke loaded air is actively or passively done by a human body, it starts interfering with the normal physiological processes of the body. Pumping and delivery of oxygenated blood to the heart and different parts of the body is one of such important physiological process which is mainly processed by heart and lungs in collaboration. As the world is developing technology-wise the urban lifestyle diseases are also increasing. Cardiovascular diseases which claim lives globally, encompasses ischemic heart diseases (IHD), cardiac dysrhythmias, cerebrovascular diseases, peripheral arterial disease and heart failure.[9]

Effect of Smoking on Cardiovascular Health: Fresh air from the atmosphere is oxygen-rich, thus when we breathe in, lungs exchange the oxygen with the impure blood and send oxygenated blood back to the heart and other parts of the body viaan artery. When we breathe in air contaminated with cigarette smoke, the blood carrying this air is dispensed throughout the body thus affecting body parts with the cigarette chemicals. The chief chemicals of tobacco smokeare total aerosol residue abbreviated as tar, carbon monoxide gas, and nicotine toxin. The maximum risk of CVD is posed by the chemicals which are present in cigarette other than nicotine. The nicotine is a highly habit-forming substance and it is associated with escalation in heart rate and blood pressure when inspired. The chemicals that reach the heart and other parts of the body will cause damage[8] and thus cause cardiovascular disease (CVD). The majorcause of all deaths for both men and women in America.[10] is cardiovascular diseases.

CVD is a generic term referring to multiple conditions affecting the heart or blood vessels.[7] Some of these conditions include:

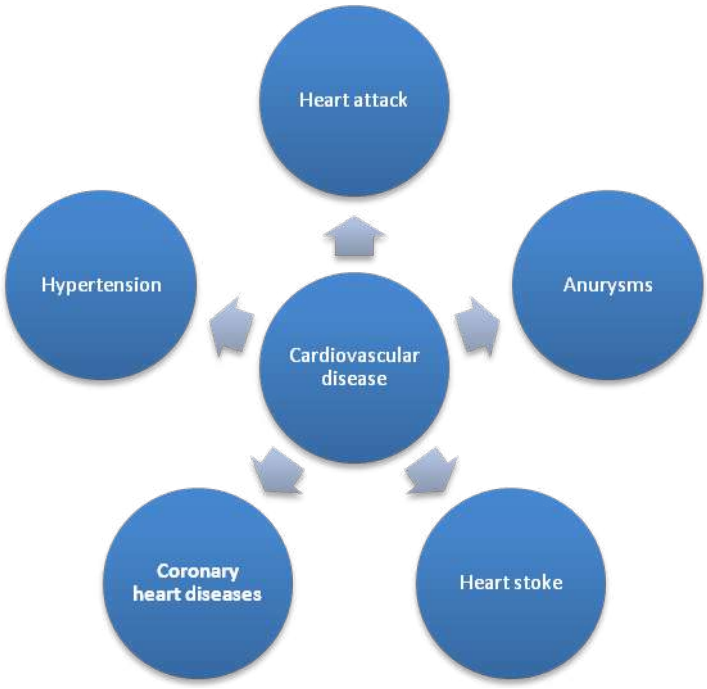


Figure 1. Multiple Condtions Leading To Cardiovascular Disease

The chemical components present in the smoke from cigarette have a seriously damaging effect on heart, its blood vessels and causes cardiovascular diseases by the accumulation of plaques on sides of the walls, the plaques are a sticky substance which consists of cholesterol, calcium, triglycerides, and scar tissue and this plaque

causes a disease called atherosclerosis.[5-7] This plaque formed causes constriction of the blood vessels, which in turn causes difficulty for the blood to flow through the vessels and reach the vital organs like the heart, brain, and lung. When there is a clot in vessels supplying the heart, it leads to heart attack, which may even cause death.[5,6]Some serious cardiovascular conditions that can be an end product of smoking are as follows:

- **Peripheral Artery Disease**: It is a common circulatory problem in which plaque builds up and narrows the arteries and reduces blood flow to your legs. Smoking along with a high amount of cholesterol and fat is the leading cause of this condition. This peripheral artery disease by itself doesn't cause any morbidity but patients with this condition are at higher risk of ischemic heart disease. And it a preventable disease which can be treated by lifestyle modification which includes quitting smoking, healthy eating, and more physical activity.
- **Abdominal aortic aneurysm**: It is a localized enlargement of the abdominal aorta like a small balloon such that the diameter is greater than 3cm. The bursting of an abdominal aortic aneurysm, may cause sudden death. Abdominal aortic aneurysm can be prevented by eating healthy food, exercising daily and avoiding smoking.[8]

Impact of cardiovascular disease caused by tobacco smoking: According to the American Heart Association, CVD accounts for about 800,000 deaths every year,[11]in United states making it the leading cause of all deaths. Of those, nearly 20 percent are due to cigarette smoking.[12] Even in persons who smoke less than 5 cigarettes per day also are at risk of CVD.[12]

Not only smokers even people who are passive smokers are at a risk of cardiovascular disease and ultimately death. There is a 25 to 30 percent increased risk of coronary heart disease in nonsmokers who are regularly exposed to secondhand smoke than those who are not exposed.[12] In fact, according to surgeon general report 33,000 U.S. coronary heart disease deaths per year are caused by exposure to secondhand smoke[13] Passive smokers exposed to cigarette smoke increases the risk of a heart attack..[12,13]

It is time to protect your heart: Smoking is a bad habit and we all must refrain from this habit, for people who have this habit, it is time to quit smoking now as it is said it is better to be late than never and the earlier you do it the better. Once a person gives up his smoking habit, certain changes start occurring in our body:

- Heart rate drops after twenty minutes of quitting smoking.[14]
- In 12 weeks circulation improves and lung function also increases[14]
- The level of carbon monoxide in the blood plunge to normal, allowing more oxygen to vital organs like your heart & brain just after 12 hours of quitting smoking.[14]
- The risk of stroke drops to that of a lifetime non-smoker within five years of quitting smoking.[15]

Conclusions

Quitting tobacco smoking has many immediate and lifelong benefits. By quitting smoking, immediately blood pressure and heart rate normalizes, the destruction process of lungs will stop and the body will try to repair itself. And, after one year of quitting smoking the risk of coronary heart disease reduces to half when compared with a smoker. Socially and frugal, benefits of quitting tobacco, are enormous not only we save our income of not buying cigarettes but also we can protect society from the harmful effect of passive smoking particularly the vulnerable group babies, teens. Nicotine when given as patch or better known as Nicotine Replacement Therapy is neither addictive nor affects the cardiac health. As quitting an addictive habit like smoking cigarettes is difficult, these therapies can be of help and can help us achieve our goal to completely quit smoking. There are some good habits which can help boost our health along with these therapies, they are regular exercise, eating a healthy diet,getting to an ideal body weight, reduce stress, limit alcohol consumption, make sure other health conditions, such as diabetes & hypertension, are under control. Last but not least have 8 hours of sleep and visit a physician regularly for routine check-up after the age of 60.

Quitting tobacco also reduces the impotence, low birth weight, Preterm babies and miscarriage[16].

So to conclude to the prevention of CVD, is directly proportional to smoking, so we should take steps to reduce the proliferation of smoking in the general population particularly in teens. This can be done by urging the government to strongly enforce the laws of restriction of smoking like COTPA & NTPC in public

spaces and enact new regulations to halt the availability of cigarettes to teens.

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Immediate Implant Placement in Mandibular Esthetic Zone: A Clinical Report

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Abstract

In the past two decades' immediate dental implant placement has been considered satisfactory treatment modality. The dental implants can be placed in the fresh extraction sites by simultaneous use of bone graft material. The patient was suggested to go for a treatment procedure for the extraction of and immediate implant therapy. A bonegraft material was used to fill the space between the implant and the sockets along with planning a two-stage surgical procedure to optimize bone healing. When the osseointegration was complete abutment connection was placed with no complications radiographically examination showed a slight marginal bone reduction after 6months of follow-up. The immediate placement of implants helps in preserving the form and contours of the hard and soft tissues, by decreasing the necessity augmentation procedure. It also helps in minimizing the surgical exposure of the patient, reducing the treatment time and also improves the esthetic outcomes.

Keywords: *Immediate Implant Placement; Mandibular Esthetic Zone; Treatment option.*

Introduction

In general, the extraction of teeth often leads to resorption of alveolar bone. The immediate implant placement at the site of extraction might add to the preservation of the alveolar bone during the procedure and also helps in reducing the healing time.[1] The bone-to-implant might be enhanced by the bone-forming activity in comparison to implants placed in a less osteogenic active-site.[2] The early implant placement may result in favorable implant crown ratio, improved esthetics along with preservation of the alveolar structure, and the positioning of implant-fixture in a fresh extraction socket enables in maintaining the bony crest.[2]

According to literature preceding tooth extraction, the predictable loss of alveolar bone is accelerated in the first six months with as much as 40% of the alveolar height and 60% of the alveolar width loss, continuing at a rate of 0.25% to 0.5% per year.[3]As thisresorption of the alveolar ridge has been an inevitable event followed by extraction of a tooth, to prevent this conventional guidelines in the late 1980s advises 8 weeks of socket healing and for remodeling additional 3-6 months of load-free healing, which was believed to be necessaryfor osseointegration.[3,4]There are various advantages of immediate implant placement such as crestal bone preservation, reduction in complex surgical procedures, minimizing the edentulous period, and better patient acceptance.

Simultaneously, the guided bone regeneration (GBR) procedures, that usebone grafts and collagen membranes, are frequently essential in such situations

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to correct the peri-implant defects and/or to augment surrounding tissues.[3]

The loss of mandibular incisors is most likely to be frequent maybe because of caries or periodontal diseases.[5] The replacement of such incisors by immediately placed implants is a straightforward, predictable, and minimally invasive procedure. The purpose of this article is to present a case of periodontally compromised anterior teeth associated with bone loss using immediate implant placement combined with simultaneous GBR to correct the defect.

Case report and technique: A 52-year-old female was reported to the department of prosthodontics with localized advanced periodontal disease including mandibular incisors; 32 and 42 exhibiting Class II mobility due to bone loss (Figure 1a). The aesthetic requirement of the patient has been a major concern due to the loss of mandibular central incisors and a mild discomfort on biting due to the mobility of lateral incisors. Medical history was noncontributory. Various treatment alternatives including removable prosthesis, resin-bonded bridge, conventional fixed prosthesis, and implant-supported fixed prosthesis procedures were discussed with the patient. The patient was not willing for a removable partial denture or any interim prosthesis. Based on the patient's desire for a reduced the number of surgical procedures and overall treatment time, immediate implant placement after tooth extraction of 32 and 42 was decided as the final treatment plan.

For the placement of the implant, an adequacy in the volume of bone was determined by the clinical and radiographic examinations. The treatment choice would be the extraction of the tooth nos. 32 and 42 preceding immediate placement of two implants. Postoperative instructions provided include a soft diet for the first six to eight weeks followed by surgery. Informed consent was obtained. Before proceeding into the surgical phase, a diagnostic impression of the maxillary and mandibular arch was made from alginate material along with a bite registration. The diagnostic casts were articulated at the proper intercuspation position and a diagnostic mockup was done to determine the position and the final aesthetic outcome of the implant-supported fixed prosthesis.

The surgical phase was initiated with the administration of local anesthesia (lidocaine with epinephrine 1:100,000), extractions of teeth No. 32 and 42 were performed (Figure 1b, 2a). The desired implant positions to be placed were determined based onthe diagnostic wax-upsurgical guide was fabricated for proper implant placement. Osteotomies were performed to gain access through the site of the extraction socket[5] (Figure 2b). The drilling process progressively ensures the increase in the socket diameter with the utmost use of apical bone to the extraction socket. For the duration of the drilling procedure a finger was placed over the thin buccal mucosa, facilitating close approximation with the labial bone plate, consequently preventing perforation of the bone. Socket curettage was done before the placement of implants.[6] An implant (myriad plus measuring 3.5 mmx13 mm) were placed in 32 and 42 positions (Figure 3a). The implants were placed parallel to the incisal edge of the adjacent tooth with a lingual inclination. Implants were screwed properly into the alveolar bone unless the threads of the implant completely embedded into the alveolar bone and the collar in alignment with the crestal bone. Evaluation of the primary stability of the implants can be verified when there is an absence of rotation on the application of a reverse torque of less than 20N-cm. The snug fit of the implant ensures the prevention of mobility. The clinical stability of implants can be determined by palpation and percussion.[7] Adequate initial stability was obtained when placed with a torque driver at 35 Ncm.[2]A minimum distance of 3mm from the adjacent CEJ was maintained to achieve a normal emergence profile. A localized Guided Bone Regeneration procedure was then undertaken using bone grafts (biooss) along with (platelet-rich fibrin) barrier membrane, followed by placement of interrupted sutures to enable maximum approximation of the soft tissues to the abutment(Figure 3a) This platelet-rich fibrin membrane enhances stability is immediately placed implants when used in guided bone regeneration.[8] The patient was then instructed to follow a intake of soft diet consisting of high nutrient value and postoperative medications like Augmentin and zerodol SP and Pantop D advised for 5 to 7 days, along with a mouth wash, chlorhexidine gluconate (0.2%) twice a day for a week.[9] The patient was recalled for the postoperative follow up for suture removal after a week following the surgery and then called for subsequent follow-ups at their month, six months, and twelve-month respectively.

The stability of the implant was evaluated by tactile perception and fremitus test while the gingival inflammation was evaluated by noninvasive gingival index using Modified Gingival Index (MGI).[6]

The patient was recalled after 3 months for follow-up. Second stage surgery was done and the gingival former was placed (Figure 3b, 4a). After a 2week prosthetic phase was performed in which the gingival formers were replaced with abutments and the final impression was made with polyvinyl siloxane material (Figure 4b). The lab analogs are then placed upon the abutments and sent to the laboratory for the fabrication of the permanent fixed ceramic-metal Fixed prosthesis. After a week metal trying was done followed by loading of the 4unit prosthesis (Figure 5a) Implant protected occlusion was designed to improve the longevity of the prosthesis with adequate aesthetic outcomes. The six-month follow-up examination revealed stable, healthy peri-implant soft tissue with no signs of complications and clinical modifications in soft or hard tissue levels.[10] The patient was also satisfied with the esthetic and functional outcome (Figure 5b).



Figure 1a. Preoperative Anterior mandibular incisors; Figure 2b. Teeth after surgical extraction

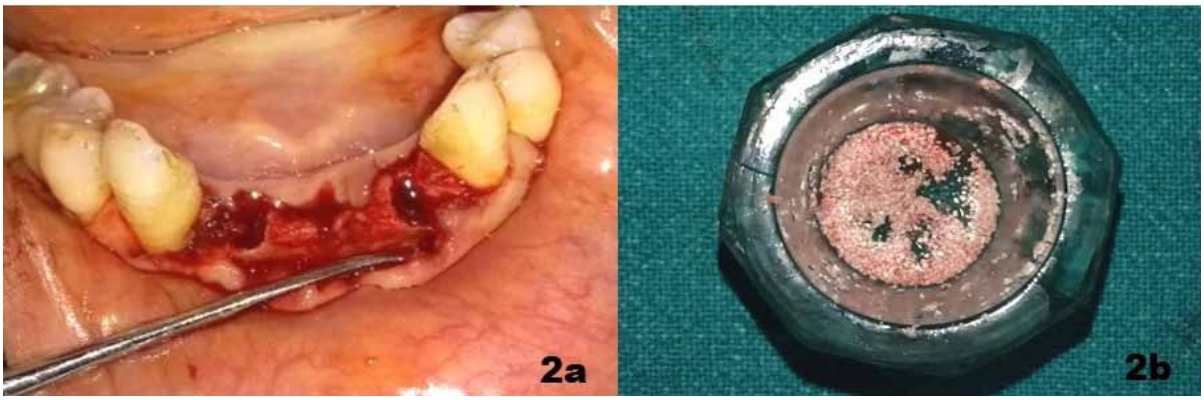


Figure 2a Periosteal Flap raised after extraction; Figure 2b. Biooss bone with collagen membrane

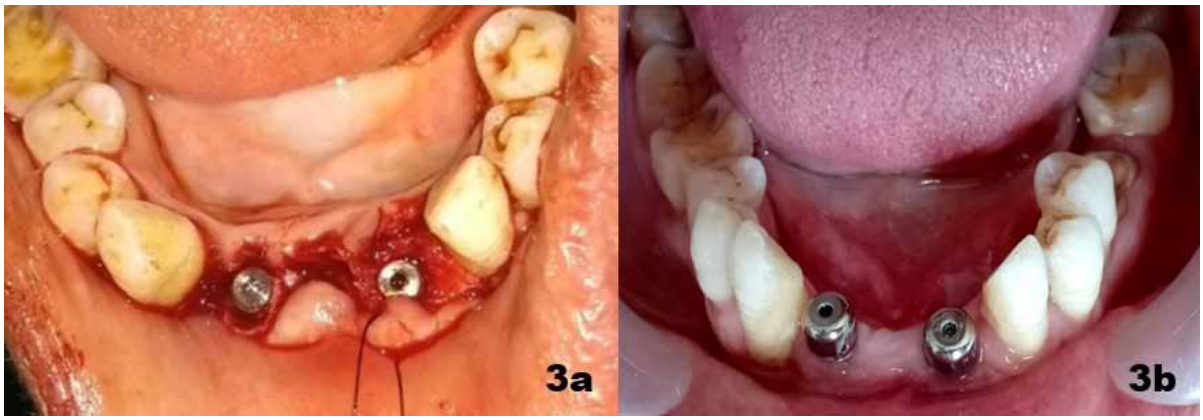


Figure 3a Graft placed and sutured; Figure 3b. Attachment of permucosal extensions

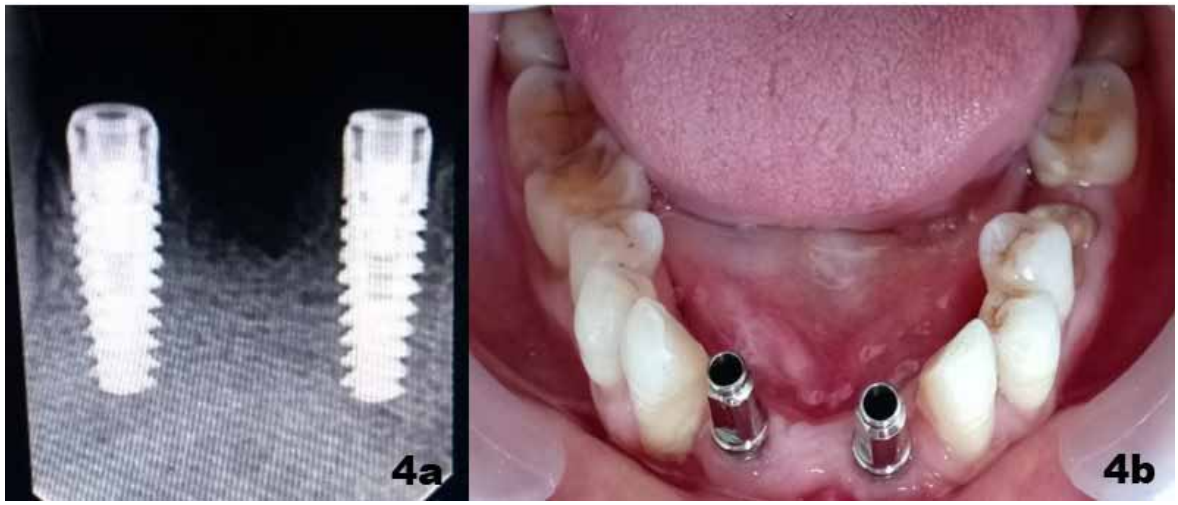


Figure 4a Implants after 3month of placement; Figure 4b Abutment connected to implants



Figure 5a Four unit fixed prosthesis; Figure 5b Implant prosthesis under implant protected occlusion

Discussion

According to Macheti et al in periodontally susceptible patients the rate of survival of immediately placed implants is similar to that of no susceptible patients. For immediate implant placement the anterior mandible proves to be a favorable site for reasons like adequate bone quantity and quality, narrower and shorter tooth sockets than the osteotomy which helps in stabilization of bone beyond the sockets.[9] In anterior mandibular site there is less potential of nerve damage as the inferior alveolar nerve doesnot course in between the mandibular foramina.

The patient was advised to keep caution while eating and instructed to avoid biting with the restoration for at least 4 weeks.

According to literature it has been shown that within a period of three to twelve months most of the major changes occur following tooth extraction with an estimated demonstration of 50% reduction in the buccolingual width. Immediate implant placement following tooth extraction eliminates the socket healing period and thus reduces the occurrence of alveolar bone resorption.[11, 12]

It has been documented that using barrier membrane and different graft material fills the residual peri-implant defects for enhancing the peri-implant bone healing and to achieve a final aesthetic outcome. These barrier membranes may also help in preventing the invasion of connective tissues and the epithelium into the microgappresent in-between the implant and the surrounding bony walls, which ultimately favors bone regeneration.[8] The bone grafts can moreover acts as a spacemaintainer moreover promoting bone formation that can eventually provide better osseointegration.

Conclusion

Based on the literature review, the universally applicable defined criteria for implant success adopted from Bursar et al were the absence of persistent

infection, gingival inflammation, implant mobility, peri-implant radiolucency neurologic signs and symptoms.[15] According to the present report and its outcome, it can be concluded that immediate implant placement may be considered as a viable treatment modality as the treatment protocol showed reduced clinical time in situations that allow an aesthetic outcome. It significantly showed high survival rates that can be achieved with the implants placed in immediate extraction sockets.

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Lasers in Pediatric Dentistry: A Review Article

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Abstract

Laser applied science has been newly introduced into the dentistry to substitute drilling. The 1st dental experience for pediatric patients using laser can be useful from the point of preventive & therapeutic approach. This article approached to educate about laser physics, its components, fundamentals, mechanism of its action, applications, advantages,disadvantages, laser safety & medico-legal aspects of laser specifically concerning pediatric dentistry. Due to their minimal invasion, treatment by laser is well accepted by children. The use of scalpels conventionally, may produce post-operative discomfort & thus, prolonged wound healing. Being safe in use, lasers are an important in-office alternative to treat pediatric dental patients. Hence, lasers use enhances the children's cooperation leading to greater satisfaction by all three i.e. guardians,patient & dentist.

Keywords: *Pediatric laser dentistry; Laser dentistry, minimally invasive approach.*

Introduction

The therapeutic use of light is being used from centuries. LASER is "Light Amplification by Stimulated Emission of Radiation".[1] In 1916 when physicist Albert Einstein explained the stimulated emission theory, the principle of laser came into light.[2] It is an electromagnetic energy with unidirectional and monochromatic unique properties. Today laser has found endless uses in many disciplines of medicine & dental surgery. Laser can serve as a favorable option due to less pain, sound & vibration. Lasers were 1st used for soft tissue incision. The new types of lasers' work on water molecules can be used for the ablation of hard dental tissue as well. It's been used in different fields of dentistry as it has wide applications. Laser is a promising arena towards the "minimally invasive", modern pediatric dental treatments. In the present scenario, the laser provides a safe & effortless alternative for treating children in dentistry. Then dental visit becomes a lot easier for patient, parents, and dentist. Children seem to be more cooperative during dental procedures using lasers, which boost up the quality of care & the method of treatment.

History: A **MASER** is a device that stands for "**Microwave Amplification by Stimulated Emission of Radiation"**, which produces "coherent electromagnetic waves through amplification by stimulated emission & was built by Charles H. Townes, James P. Gordon, and Herbert J. Zeiger at Columbia University in 1953". The laser works by the same principle as the maser, but produces "higher frequency coherent radiation" at visible wavelengths. In 1960, LASER was made from a laser device "known as ruby laser," invented by Theodore Maiman which stands for "Light Amplification by Stimulated Emission of Radiation". Gordon Gould is credited with creating this acronym in 1957.[3] The first application of the laser was used for skin problems diagnosis & treatment and later used in endoscopic surgery & ophthalmology.[4] In ***1965*** "*Stern & Sognnaes's* detailed about this "*ruby laser* "in their study that it could *vaporize enamel* & also thermally *affects* the pulp of the tooth.[5] Patel invented CO_2 *laser* in 1964 that had both soft tissue & hard tissue applications.[6]

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In ***1964***Nd: YAG laser was introduced *by* Geusic which remain unnoticeable until 1990. The 1st usage of laser in 'endodontics' was shown by "*Weichman & Johnson"* in the year of 1971*,* for the apical foramen seal, in vitro, by utilizing CO_2 laser.[6] Studies are carried out for future indications. Since then, the clinical applications of lasers continue to increase indentistry.

Laser Physics: A laser machine forms *multiple atoms* that pump out *multiple photons (known as light particles*) all of which line up to form a *concentrated light beam*. Matter consists of *atoms*. Nucleus lies at the center of the atom; which contains *protons & neutrons*. A (+ve) charged is a proton & neutron is neutral. Outside the atom are *electrons* which are (-ve) charged and they are in motion surrounding the nucleus. The conversions are of 3 types which are as follows:[7,8]

1. **Spontaneous Absorption:** In this, an electron alter state to higher state from lower state by absorbing a photon (Figure. 1a)
2. **Spontaneous Emission:** In this, an electron spontaneously releases a photon to shift it to a lower state from a higher state (Figure. 1b)
3. **Stimulated Emission:** In this the electrons transit to a lower state from higher state & release a photon. The incident photon & their released counterparts possessa similar wavelength. This particular wavelength correlates with the energy difference between the two orbits.(1c)

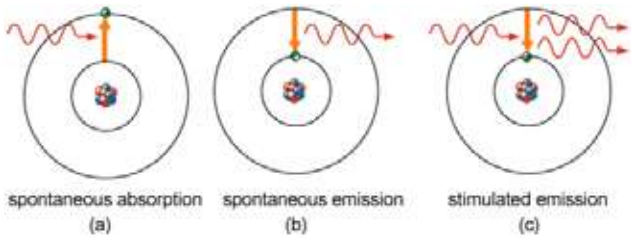


Figure 1. Concentrated light beam (3 types of conversions)

The characteristic of laser-produced from stimulated emission are as follows:[9,10]

1. **Monochromatic:** Single wavelength light is produced.
2. **Coherent:** All photons show similar polarization & produce a very high intensity when they superpose.
3. **Collimation:** It depicts a narrow & powerful emitted laser beam which consists of constant shape & size.
4. **Efficiency:** At low energy state lasers produces the required energy to carry out their specified function.

Amplification: The process of amplification takes place within the laser device. The laser machine consists of a core that is known by the optical cavity that comprises of chemical elements, molecules/compounds which are known by 'active medium'. Lasers are termed after the active medium material, such as after a gas, semiconductor, etc. The optical cavity has two ends. Each end has one mirror which is placed parallel to one another. An electrical coil/Flash lamp is the excitation source for active medium.

Stimulated Emission: In 1900 *Max Planck* introduced the 'quantum theory of physics' which later forms the basis for stimulated emission. Albert Einsteinstated that an "additional quantum of energy traveling in the field of the excited atom that has the same excitation energy level would result in a release of two quanta, a phenomenon defined stimulated emission". These photons further energizes more atoms & it results in the population inversion that causes an increase in numbers of the atoms of the active medium from resting state. And to maintain this excitation, a pumping mechanism is required. At the two ends of the active medium, two mirrors are placed which reflect photons back & forth for further stimulated emission. This consequence travels through the active medium which raises the photon beam power & thus gets amplified.

Radiation: It is the light waves emitted as a distinct form of electromagnetic energy. The short wavelengths, below 300 nm, are described by ionizing radiation. The higher photon energy can penetrate biologic tissue easily & reproduces charged atoms. Wavelengths more than 300 nm possess less photon energy & results in excitation. Thus, it heats the tissue with which they interact. The currently used lasers in dentistry have wavelengths from 500 nm to- 10,600nm.

Fundamentals of Laser: Oral tissues have a distinguishing affinity for absorbing laser energy of specific wavelengths. Hence, the selection of a laser depends on the target that is to be treated. The optical aspect of target tissue forms the basis of laser light interaction in different ways with the intended tissue. These interactions are as follows:[11,12"]

a. **Transmission:** *Laser* energy is transmitted directly through the target tissue without effecting target tissue.

b. Reflection: This property of laser used for diagnostic purposes. It redirects itself off the surface. It is useful for measuring the 'sound tooth structure' in dental caries.

c. Absorption: There is the absorption of laser energy in the target tissue on the application of laser on tissue. Different laser wavelengths have a "different absorption coefficient" with the dental tissue components.

"Lasers primarily interact with dental structures photothermally. The temperature of the target tissue increases as laser light is absorbed & when the tissue reaches 100°C temperature, the intercellular & intracellular H_2O (water) boils, which induces both soft tissue vaporization or likely to explode & disarrange 'hard tissue' (*ablation*).With the continued increase in temperature of 200°C,the tissue gets dehydrated & later burned in the presence of the air (*carbonization*). Carbon is the end product & it absorbs all wavelengths".[13-15]

Laser-Component:

All lasers have the six components which are listed below: Figure 2.

1. Active medium- Materials in the core of the laser, can be gas, solid, liquid, or crystals.
2. Pumping mechanism-It is a primary source of energythatis absorbed by the active medium, that results in the "production of laser".
3. Optical resonator- It has a two-mirrorsystem (one is completely reflective & the other one is selectively transmissive) which amplifies and collimates the developing laser beam.
4. Cooling subsystem- It cools down the heat produced by a laser.
5. Controller subsystem (microprocessor)-It is responsible for controlling power output variation to time.
6. Delivery system- It is dependent on the wavelength emitted."

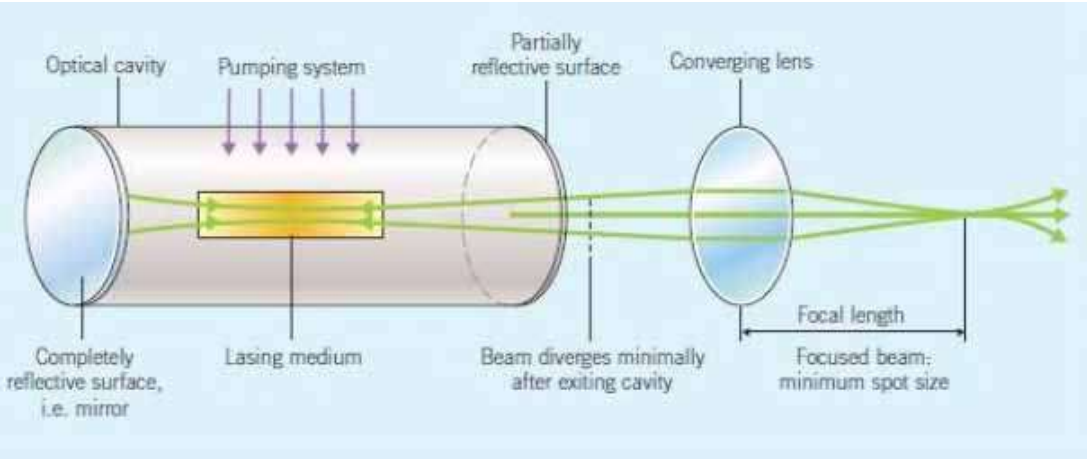


Figure 2. Laser-Component

Various Laser Delivery System: Earlier most dental lasers were having bulkier articulated arms for their delivery systems. But each laser has a distinctive delivery system such as handheld laser, fiber delivery system,articulated arm deliver system & hollow tube delivery system.

Classification of Laser:

"Lasers have been classified in many ways such as[16,17]:

I. According to the wavelength (nanometers)

1. UV (ultraviolet) range – 140 to 400 nm
2. VS (visible spectrum) – 400 to 700 nm
3. IR (infrared) range – more than 700 nm

Most lasers operate in one/more than one wavelength region.

II. Broad classification

1. Hard laser (for surgical work)
   i. CO_2 lasers (CO_2 gas)
   ii. Nd: YAG lasers (Yttrium-aluminium-garnet crystals dotted with neodymium)
   iii. Argon laser (Argon ions)
2. Soft laser (for biostimulation and analgesia)
   i. He-Ne laser ii. Diode lasers

III According to the type of active medium used:

a. Gas lasers:
   - Argon • Carbon-dioxide

b. Liquid:
   - Dyes

c. Solid:
   - Nd:YAG
   - Erbium: yttrium aluminium garnet (Er: YAG)
   - Diode

d. Semiconductor:
   - Hybrid silicon laser

e. Excimers:
   - Argon-fluoride
   - Krypton-fluoride
   - Xenon-fluoride

IV. According to pumping scheme
1. Optically pumped laser
2. Electrically pumped laser

V. According to operation mode
1. Continuous-wave lasers
2. Pulsed lasers

VI. The laser classification system is based on the possibility of damage occurring-
- Class I (< 39mw):No threat.
- Class II (< 100 MW): It could harm a person if he stare into the beam for a long period. The normal aversion response or blinking should prevent from staring into the beam.
- Class III (500mw): It causes permanent damage including blindness.

VII. Based on clinical use
1. Surgical: i. Hard tissue; ii. Soft tissue
2. Non-surgical: i. Diagnostic; ii. Low-level therapy; iii. Miscellaneous photo-activated disinfection laser-based curing light"

Wavelengths Used in Pediatric Dentistry:

Table 1. On basis of active medium, wavelength, clinical uses laser are named accordingly

"Argon laser	**Active medium-**Argon gas **Wavelength used**-488nm (blue) & 514nm (blue green). **Clinical applications-** – Caries detection. – Polymerization of light-activated composite resins, dentin bonding agents, sealants, bleaching gels, light-activated impression materials.
Diode laser	**Active medium-**GaAlAs,InGaAs(semiconductor) **Wavelength used**- 655nm, 800-830nm & 980nm. **Clinical applications-** – Cutting & coagulating gingiva and oral mucosa. – Sulcular debridement.

Laser-Nd:YAG	**Active medium-**A solid garnet crystal combined with rarerare earth elements yttrium & aluminumdoped with neodymium ions. **Wavelength used-**1064nm **Clinical applications-** – Cutting & Coagulating dental soft tissues. – Sulcular debridement
Laser-Ho:YAG	**Active medium-**Solid crystal of YAG sensitized with Cr doped with holmium & thulium ions. **Wavelength used-**2100nm **Clinical applications-** – Used frequently in oral surgery for arthroscopic surgery on the TMJ. – It has many medical applications.
Laser-Carbon dioxide	**Active medium-**A mixture of CO_2, He & nitrogen gases in proportions of 8:7:1. **Wavelength used-**9300nm,9600nm & 10600nm. **Clinical applications-** – Soft tissue procedures such asgingivectomy,gingivoplasty,biopsy, frenectomy. – Coagulation after completion of surgery.
Laser-Erbium family	**Active medium-** Solid crystal of YSGG doped with Er Cr. & solid crystal of YAG doped with Er. **Wavelength used-**2780nm & 2940nm. **Clinical applications-** – Caries removal & cavity preparation. – Bone removal. – Vital & non-vital pulp therapy. – Tissue retraction for uncovering implants.”

Role of Laser in Pediatric Dentistry[18-20]: It contributes to providing a positive dental experience with reduced fear and anxiety for the pediatricpatient. Due to its less invasive nature,children are more cooperative during treatment.

Advantages in Pediatric Dentistry:

1. No anesthesia, no drill
2. Less blood loss.
3. Reduce post-operative edema.
4. Initial healing, rapid regeneration, reduce post sensitivity in restorations
5. Suture, dressing are not required.
6. Less chances of metastasis
7. Sterilization of treatment site.
8. Tooth enamel is less exposed,results in a decrease in caries progression.
9. The patient becomes free of fear & anxiety.
10. Beneficial for medically compromised patients.

Disadvantages:

1. Direct light or reflected light both can harm the eyes of the practitioner as well as the patient.
2. The laser is quite expensive.
3. Need qualified personal.
4. Dental hand-pieces required for finishing, after cavity preparation by laser. Also, it should not be used to fill the cavities present between teeth.
5. It should not be used for the removal of silver restoration or damaged crowns.
6. The possible risk of viral disease transmission from “aerosol” in immune-compromised patients.

Applications

Soft Tissue Laser Uses

1. **Frenectomy and treatment of ankyloglossia:** The abnormally joined frenum on the maxilla/ hyperactive labial frenum both are the cause of spacing between teeth, poor oral hygiene, gingival retraction & continuous trauma during brushing.

Also, the situation with the short & hypertrophic lingual frenum (ankyloglossia) is common in new-borns, which results in remarkable problems in breastfeeding, swallowing & phonation.

2. **Gingival recontouring, gingivectomy, bone surgery & crown lengthening:** In children with gingival hypertrophy, tooth decay that propagated under the gingiva, the laser can be used. It is also used for the gingivectomy, limiting the use of anesthesia. The YSGG was 1st laser used for the bone surgery & crown lengthening to correct deformity & create required the physiologic osseous contours.

3. **Exposure of unerupted tooth & treating pericoronal problems in erupting teeth:** To expose an unerupted or partially erupted tooth for orthodontic bracket or button placement, laser is used.Lasers are used to expose the clinical crown of the involved tooth.

4. **Pulpotomy, pulpectomy of primary teeth using laser:** Laser takes 10s-20s to work in a vital tooth. In non-vital teeth, the laser's success rates are almost the same as that of conventional pulpotomy procedures. In 1999, Jeng-fen Liu *et al*. evaluated the effects of laser pulpotomy in primary teeth and found it clinically successful in a 6 months followup visit except one.

5. **Direct, indirect pulp capping of young permanent teeth using laser:** CO_2 laser is used for direct pulp capping as it controls hemorrhage and sterilizes the exposure site which facilitates better placement of calcium hydroxide paste at the exposure site. Laser energy has an obtundent and sedative effect on inflamed pulpal tissue which led to its use in indirect pulp capping procedure.

6. **Laser in wound healing:** Low-level laser treatment promotes wound healing.

7. **Laser in diagnosing pulp vitality:** Laser Doppler flowmetry is a non-invasive method of diagnosing pulp vitality.

8. **Laser in disinfection of root canal:** Laser used in root canal disinfection during root canal treatment and irrigation of the canal with 5.25% sodium hypochlorite or 14% EDTA is carried out during laser irradiation.

9. **Laser in tooth bleaching:** When the laser light hits bleaching gel, it produces heat thus the oxidation process becomes accelerated resulting in the whitening of the tooth surface.

10. **Laser in pediatric tooth preparation:** Biolase is used for pediatric tooth preparation. This eliminates the need for local anesthesia & increases the micromechanical bonding with resin cement as it producesmore roughness on prepared tooth surfaces.

11. **Hypertrophic lesions:** Diode laser is particularly recommended in oral soft tissue surgery, specifically regarding benign lesions.

12. **Lasers used in pain management:** "LLLTdrop the concentration of chemical agents such as histamine, acetylcholine (Ach) etc., all of them are pain mediators. They inhibit the concentration of ACh, a pain mediator, through increased acetylcholine esterase activity & hence, used for the treatment of dentin hypersensitivity."

13. **Herpes Labialis & Recurrent Aphthous ulcers:** Laser brings immediate relief to the "aphthous ulcer" lesion or shorten the duration of the" herpes labialis" lesion.

Hard Tissue Uses:

1. **Removal of caries, old restoration, and cavity preparation:** Lasers are used for carious tissue removal, results in the preservation of tooth-healthy tissues.Cavity preparation with the use of laser in children for enamel & dentin removal seems very reasonable, because it eliminates the injury to lip, tongue due to lip bite & tongue bite which are frequent problems with children.

2. **Pit and fissure sealants:** The lasers can clean & sterilize the enamel fissures. Laser can be used for fistulotomy, cleaning & conditioning of pits & fissures before sealant application.

3. **Diagnodent and caries detection:** Diagnodent is the device has also the ability to show decays in early stages that are not seen in radiographs. Laser fluorescence at a wavelength of 655 nm is effectively used for the detection of occlusal hidden caries and occult lesion in primary and permanent teeth.

4. **Prevention of dental caries:** Laser heat effects and the creation of micro openings and small cracks facilitate the penetration of fluoride. It seems that the simultaneous use of laser & fluoride is the best method of carious lesions prevention.

Dental Laser Safety[21,22]:

The aspects of laser safety are:

1. The assembling procedure of the laser instrument,
2. Right mechanism of the device &
3. Personal protection of patients as well as operating squad from laser.

Medico-Legal Aspects of Laser in Dentistry[23]: Conventional soft tissue surgery with a dental laser is accepted in dentistry & covered under insurance policies. Informed consent must be routine & a part of the general consent form that all patients, guardians read & sign before the start of dental treatment. It is highly suggested that each practitioner take a course."

Conclusion

Its use in pediatric dental procedures is well accepted. Its minimal invasiveness allows pediatric patients to be cooperative during dental procedure. Lasers in dental practice present remarkable accuracy, pain-free, quick healing & safety. Although its effectiveness in the diagnosis of dental caries, prevention of caries, endodontic management of deciduous and permanent teeth, and different softtissue procedure is well documented, further research regarding its efficacy in pediatric dental procedure is still needed.

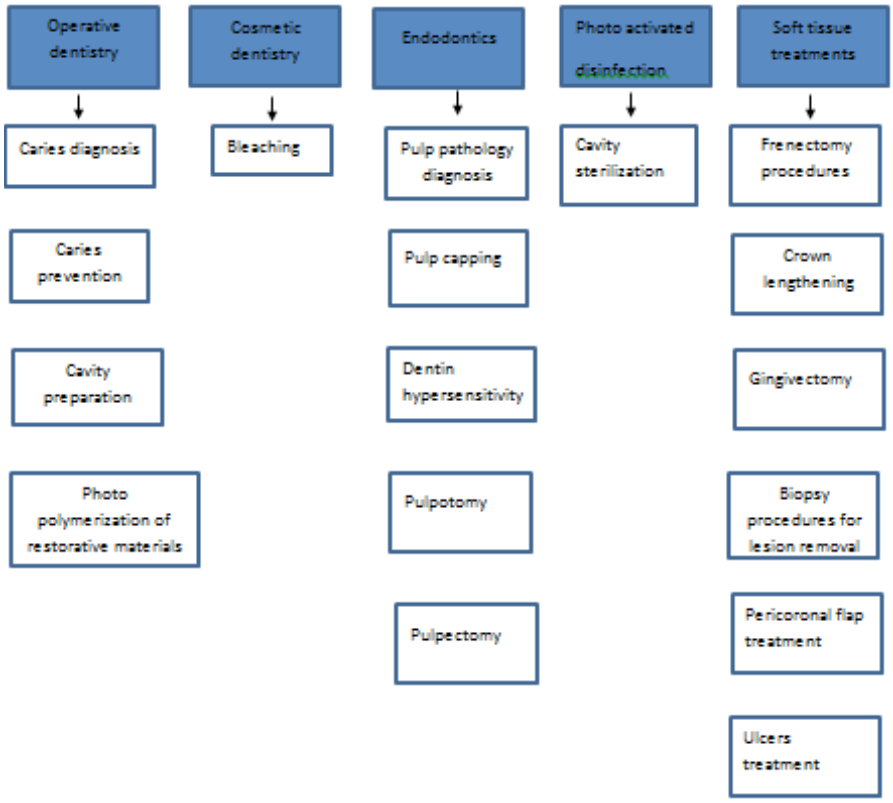


Figure 3 Use of lasers in pediatric dentistry

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Cancer Associated Fibroblasts (CAFS) and their Indispensable Role in Tumor Microenvironment: An Update

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Abstract

Cancer-associated fibroblasts commonly known as CAFs which have a modified phenotype as compared to normal fibroblasts are exclusively present in the tumor microenvironment surrounding the tumor mass. They play inevitable roles in tumor initiation, progression, invasion, and metastasis. The constant cross-talk present in between the cancer cells and CAFs is responsible for the maintenance of such a malignant environment. Thus,they may prove to be efficient targets in the field of cancer therapeutics which may yield excellent results. This review throws light upon their much evident roles in carcinogenesis as well as how the mechanisms driven by them may be targeted in therapeutic interventions.

Keywords: *Cancer-associated fibroblasts, CAFs, tumor microenvironment, tumor stroma.*

Introduction

A tumor mass is composed of malignant epithelial cells as well as groups of nonmalignant cells. These non-malignant or non-cancerous groups of cells include fibroblasts and myofibroblasts, endothelial cells and lymphatic networks, pericytes, immune regulatory cells, and cytokines in the extracellular matrix (ECM) (1). This mass of stromal cells along with the ECM constitutes the tumor microenvironment. Tumor microenvironment, in turn, governs the tumor progression through stages of initiation, progression, and metastasis (2).

One such subpopulation of cells is of the cancer-associated fibroblasts (CAFs) which is a group of fibroblasts having a modified phenotype of myofibroblasts. These are the same as the myofibroblasts which are transiently present during fibrosis and wound healing, the only distinguishing characteristic feature is that they are present and remain almost activated throughout, at the site of the tumor (2). An ideal microenvironment is maintained by these CAFs and they also undergo the required biological and morphological changes for tumorigenesis and its progression. These can serve as essential therapeutic targets for the treatment of cancer.(3, 4)

This review highlights the recent trends associated with CAFs and aims towards a better understanding of their role in the advancement of tumors. Additionally, the interaction between the malignant cells and tumor microenvironment for the scope of identification and development of potential anti-cancer therapy has also been analyzed.

Distinguishing Nature of CAFs: "The origin of CAFs comes from a variety of cells, majorly from fibroblasts while other sources include epithelial cells, endothelial cells, adipocytes, pericytes, or mesenchymal stem cells[1-5] CAFs are identified in the stroma using specific molecular markers and also distinguished easily as large spindle-shaped cells often showing resemblance to myofilaments of smooth muscle.[6] The biomarker most commonly used is α-smooth muscle actin.[7] Some

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of the other biomarkers less commonly expressed by these activated fibroblasts that have been detected with the help of immunohistochemistry includes FAPα (fibroblast activation protein α) a cytomembrane protein, vimentin, PDGF receptors α and β, podoplanin-a, S-100, FSP-1 (fibroblast specific protein), and IGFBP7 (Insulin-like growth factor-binding protein).[8,9]. Moreover, with techniques like microarray gene-expression analysis, there has been a better understanding of the nature of CAFs and more knowledge about their role in oncology has been gained. Many studies done on paired CAFs and normal fibroblast cell lines have identified a total of 46 differentially expressed genes which further encoded for paracrine factors released in the tumor microenvironment. The genes expressed were regulated by the TGF-β signaling pathway, and some of them include intercellular-adhesion molecule 1 (ICAM1), THBS2, MME, OXTR, PDE3B, B3GALT2, EVI2B, COL14A1, GAL, MCTP2 and Integrin α11 which were used to form a prognostic signature of CAFs in non-small cell lung cancer (NSCLC).[10,11]

Fibroblast Activation Induced by Tumor: Whenever there is metastasis of tumor cells to a different organ, natural fibroblasts are reinforced by them into the tumor mass. Once reinforced, they undergo phenotypic activation into cancer-associated fibroblasts under the influence of genetic as well as epigenetic changes that are either self-regulated or regulated by tumor cells themselves. Nonetheless, the precise mechanism of this activation is still not clear.[12]

"TGF-β, EGF, PDGF, FGF2, CXCL12(C-X-C motif chemokine ligand 12) are some of the chemokines involved in the activation of these fibroblasts which are secreted by the cancer cells and other stromal cellswhile other factors involved in their activation comprises of cell-cell communication with adhesion molecules like ICAM1 and vascular-cell adhesion molecule 1.[13,14]"

"A large group of miRNAs often regulate the conversion of natural fibroblasts into CAFs by undergoing upregulation or down regulation. Various differentially expressed CAFs have been listed in figure 1.[15-18]"

A, Upregulated miRNAs			
Author, year	miRNA	Cancer type	Target gene
Mitra *et al*, 2012	miR-155	Ovarian	
Zhao *et al*, 2012	miR-266, miR-221-3p, miR-221-5p, miR-31-3p	Breast	ETS2
Enkelmann *et al*, 2011	miR-16, miR-320	Bladder	
Aprelikova *et al*, 2014	miR-29b, miR-146a miR-503	Endometrial	
Wang *et al*, 2013	miR-138, miR-210, miR-99a	Colorectal	
Bronisz *et al*, 2012	miR-320	Breast	
B, Downregulated miRNAs			
Mitra *et al*, 2012	miR-31	Ovarian	SATB2
Mitra *et al*, 2012	miR-214	Ovarian	CCL5
Zhao *et al*, 2012	miR-205, miR-200c, miR-200b, miR-141, miR-101, miR-342-3p, Let-7g	Breast	ZEB1/SIP1
Enkelmann *et al*, 2011	miR-143, miR-145	Bladder	
Yu *et al*, 2010	miR-17/20	Breast	IL-8, CXCL1, CK8, α-ENO
Aprelikova *et al*, 2014	miR-31	Endometrial	SATB2
Wang *et al*, 2013	miR-29b, miR-494, miR-126	Colorectal	
Verghese *et al*, 2013	miR-26b	Breast	TNKS1BP1, CPSF7, COL12A1
Mongiat *et al*, 2010	miR-15, miR-16	Prostate	

Figure 1: Regulation of miRNAs in CAFS

Initiation of Tumor Growth, Angiogenesis, Metastasis and Chemoresistance by CAFS:

(a) Tumor growth: Aconstant interplay between the abnormal increase in malignant cells and alterations in the tumor microenvironment is the probable cause for tumor growth and the induction signals for this neoplastic initiation are usually derived from CAFs.[19,20]

A variety of cytokines have been known to be produced by these CAFs that alter the malignant biological nature. These mostly include classical growth factors, EGF, hepatocyte growth factor (HGF), novel CAF-secreted proteins [secreted frizzled-related protein 1, and IGF like family member (IGF) 1 and 2], and membrane molecules (integrin α11 and syndecan-1) which possess either the ability to upgrade the migratory and invasive properties of tumors or directly and indirectly induce their growth as well as survival.[21]"

Previous studies have demonstrated that chemokines secreted by CAFs into the microenvironment allow for the recruitment of bone marrow-derived cells (BMCs) and immune cells. In oral squamous cell carcinoma (OSCC), CCL2 expression in CAFs is upregulated, promoting the production of endogenous reactive oxygen species (ROS) in oral cancer cells (OCCs). Subsequently, ROS induces the expression of cell cycle regulatory proteins in OCCs, and advocates OCC proliferation, migration, and invasion. Altogether, these chemokines and cytokines create a suitable microenvironment allowing for the proliferation and metastasis of cancer cells.[22-24]"

(b) Activation of tumor angiogenesis by CAFs: There is an increased production of vascular endothelial growth factor (VEGF) in the tumor microenvironment. The increased levels of VEGF are responsible for tumor angiogenesis and lymphangiogenesis. The main source of VEGF is the CAFs through the activation of the PDGF receptor pathway. Additionally, the induction of BMC's by PDGF leads to the formation of endothelial channels. Endothelial stability is then maintained by the entry of pericytes along the walls of the vessels by the action of subunit B of PDGF. Therefore, promoting angiogenesis.[25-27]

(c) Nagasaki *et al* reported that cancer cells stimulate the secretion of IL-6 from fibroblasts, subsequently inducing tumor angiogenesis. IL-6R neutralization antibody inhibited IL-6 signaling and tumor angiogenesis by inhibiting the interaction between the cancer, and stroma. This finding suggests that IL-6 is a novel target for anti-angiogenesis therapy.[28]

(d) Role of CAFs in mediating Tumor metastasis: There is much evidence about the metastatic role of CAFs in tumor genesis but their role in lymphatic and distant metastasis is still doubtful. The transformed phenotype (mesenchymal) of these CAFs is considered to increase the metastatic potential of the tumor cells as compared to the normal fibroblasts of epithelial origin which restrict metastasis.[29-31]

(e) YAP, regarded as the trademark feature of CAFs is a transcription factor that plays evident parts in matrix reinforcement, invasion as well as angiogenesis by controlling distinct cytoskeletal proteins (anillin, myosin, etc.) and also via the production of inflammatory cytokines (NF-KappaB) thus leading to carcinogenesis. Additionally, CXCL12 and IGF1 are also generated by CAFs which in turn initiate the AKT signaling pathway. Tumor cells in this CXCL-2 enriched microenvironment within bone marrow further initiate metastasis (34).Evidence about the reduced expression of miR-148a amongst CAFs and the resultant increase in the WNT10B gene because of activation of the Wnt activity leading to elevated migration of endometrial cancer cells has also been reported (40). Other miRNA's involved in migration and invasion includes reduced levels of miR-26b and miR-320 in human breast cancer, downregulated miR-15 and miR-16 in prostate cancer.[32-36]

(f) Resistance to anti-cancer therapy induced by CAFs: The development of chemoresistance is consideredto be a rare feature of CAFs and they can be easily targeted for potential anti-cancer therapies but a contradicting part is played by the fibroblasts in helping the tumor cells bypass these treatment modalities as explained under.[37]

(i) "PDGF: Solid tumors exhibit the property of an elevated interstitial fluid pressure that is IFP in the centermost portion as compared to the remaining portion of the tumor tissue. As a result, the drug infiltration into the core of tumor tissue is cut down and the probability of survival of these malignant cells is increased. Thus, the chemotherapeutic regimens are designed to reduce this IFP and increase

the drug penetration up to the center of the tumor. One such regimen includes targeting the tyrosine kinase receptors and PDGF present in different malignancies like STI571 in anaplastic thyroid carcinoma, targeting of which causes a reduction in IFP and elevated Taxol intake and reduced tumor growth.[38-41]"

(ii) **"HGF:** Data demonstrates that the presence of fibroblasts secreting HGF confers resistance to therapy. Besides, HGF can activate MET, which is expressed on cancer-initiating cells (CICs) through paracrine signaling.[42] This can sustain typical CIC properties, including long-term self-renewal, ultimately leading to resistance to anti-EGFR therapy. Subsequently, there is the restoration of cell proliferation and rescuing of cells from G_1 phase arrest, and apoptosis through restimulation of the MAPK and AKT signaling pathways.[43] Notably, this effect is inhibited by suppressing MET activation with PHA-665752, a highly specific MET kinase inhibitor, or by knocking down MET expression using RNA interference."

(iii) **"Chemokines:** Many chemokines present in the tumor stroma play an evident role in the maintenance of chemoresistance. One such chemokine is CCL2, recruited by immune cells into the tumor microenvironment and is responsible for resistance against many drugs. Moreover on treatment with CCL2 inhibitor, there is enhancement of the anti-tumor potency of paclitaxel and carboplatin therapy. CAFs can induce CCL2 production through signal transducer and activator of transcription 3 (STAT3) phosphorylation, and in turn, CAF-derived CCL2 promotes cancer progression by regulating cancer stem cells through activation of the Notch signaling pathway (46). Hence, chemokines, including CXCL12, may act as promising targets for cancer therapy, alone or in combination with other cytotoxic drugs.[45,46]

(iv) **"Interleukin family:** An important inflammatory cytokine mainly secreted by CAFs is IL-6 which is involved in growth and invasion of tumor cells through activation of STAT3. Thus, IL-6 is responsible for the survival of tumor cells and also induces their relapse.[47]

(v) **"Other factors:** Some of the other fibroblast-derived proteins like WNT16B and B1 which are secreted in the tumor microenvironment are responsible for conferring chemoresistance against many drugs. WNT16B reduces apoptosis induced by chemotherapy drugs and thus targeting WNT16B may reverse chemoresistance. Group protein B1 present in the tumor microenvironment, performs paracrine signaling on neighboring cancer cells, which has been suggested to induce chemoresistance in breast cancer.[48]"

Loop of Interaction: A constant communication present between the malignant cells and CAFs in the tumor stroma is responsible for the development of a malignant microenvironment as well as the advancement of cancer. This interplay between the two components is initiated by a complementary signaling activity of stromal components such as cytokines and other factors of the extracellular matrix. Platelet-derived growth factor (PDGF) is secreted by neoplastic cells which in turn governs an increase in the population of fibroblasts and further production of IGF I and II. These IGFs initiate tumor cell proliferation as well as the production of more PDGF.[49](49).

Production of matrix metalloproteinases (MMPs) by CAFs further accelerates the invasive potential by destroying the ECM (50), further resulting in secretion of growth factors from fibroblasts like HGF, keratinocyte growth factor, IGF-1 and IGF-2 which also stimulate proliferation of tumor cells.[51-53] Thus, these positive feedback loops governed by signaling pathways result in boost up of signaling molecules, elevated numbers of tumor cells and as a consequence resistance to tumor therapy. A better understanding of these processes may prove to be helpful in the evolution of more targeted therapies that may inhibit such feedback looping mechanisms."

Suppression of the Feedback Loop: A Potential Target in Cancer Therapeutics: Such techniques targeting the association between fibroblasts and tumor cells are yet to be exploited practically, but apparently, their definite benefits cannot be ignored. This idea of targeting the loop mechanism can be implemented either by direct suppression of fibroblastic markers or by inhibition of CAF related paracrine growth factor signals.

(a) **"Direct suppression of fibroblastic markers:** Targeting the FAP membrane protein which is extensively expressed on CAFs can suppress the growth of tumor cells.[54] FAP protein induces tumor growth and division and therefore targeting the associated antibodies like FAP5-DM1 inhibits

tumor growth in xenograft models of head and neck cancer in vivo.[55]"

(b) "Inhibiting paracrine signaling of fibroblasts: This involves targeting the various pathways like PDGFR, VEGFR, FGFR which further inhibits MAPK and Akt signaling pathways in endothelial cells and prevents angiogenesis as well as initiates apoptosis. Additionally, targeting the HGF/MET signaling pathway has proven to be of therapeutic value in various cancers. Furthermore, suppressing the TGF-β pathway which is responsible for myofibroblast differentiation, has resulted in a considerable reduction of tumor growth and lastly the use of matrix metalloproteinase inhibitors (MMPIs) as anti-cancer drugs is still undergoing research.[56-60]"

Conclusion

One of the most important aspects in the field of carcinogenesis is the various roles played by cancer-associated fibroblasts which have much research and studies in their evidence. They promote carcinogenesis through their multifarious nature and mechanisms and can be exploited as promising marks in the field of cancer therapeutics.

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Conflicts of Interest: None.

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Design of the Questionnaire: Fundamental Principles

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Abstract

The most critical aspect of investigative process, is the development of questions that correctly assess public perception, knowledge and behaviour. Precise random sampling and high answer rates are lost, if the information obtained is based on ambiguous or partial questions. Hence, in order to establish effective steps, a series of substantial questions are formulated and compiled in form of a Questionnaire.

Keywords: *Questionnaire Designing; Principles; Public health.*

Introduction

The design of the questionnaire is complex, because surveys can ask questions on various subject areas in varying degrees of detail and questions can be asked in various ways.[1] It is amulti-stage process that requires attention to several details at once. Researchers are also often keen to measure change over time and thus must pay attention to the measurement of opinions or behaviours in prior surveys.[2] Survey preparation is a key step, to assess how people respond to individual questions and in total. Surveyors have approached questionnaire design as an art, but substantial research over the past 30 years has shown that there is a lot of science involved in the development of a good survey questionnaire. In this paper, we discuss the shortcomings and best practices in the design of questionnaires.[3, 4, 5]

Question development: Developing a survey questionnaire involves various steps. The first is identifying what topics will be covered in the survey. Surveyors want to track changes in people's attitudes, opinions and behaviour over a period of time.[6] Change or variations in data is usually measured by asking questions at two or more proposed intervals. The cross-sectional design, the most common in public opinion research, surveys different people in the same populace, at multiple points in time. The panel or longitudinal design, often used in other types of social research, surveys the same people over time.[7,8]

When assessing the time-based improvements, it is necessary to retain the words of the question and regard the position of the questionnaire to deliver a similar result as when previously used. All the survey reports include a top-level questionnaire that provides the exact words and sequence of the question, together with the results of the current poll and the previous polls in which the question was asked.[9]

Open- and closed-ended questions: Some of the main decision-making factors that can affect how questions are answered is, whether the it is asked as an open question or whether respondents are answering themselves or whether they are asked to choose from a list of responses.

Structured closed questionnaires have the advantage of being focused and pertinent to the study objectives, easy to administer, uniform, pre-coded and thus easy to analyse in a short time. They are preferred in medical studies.[10]

Open-ended questionnaires are useful for social and anthropological investigations. Such questions permit the interviewee to discuss openly and thoroughly, but

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they can vary from the subject. At the end of the test, they need special coding, so that the research time is longer. Researchers will sometimes conduct a pilot study using open-ended questions to discover which answers are most common. They will then develop closed-ended questions that include the most common responses as answer choices. In this way, the questions may better reflect what the public is thinking or how they view a particular issue.[11,12,13]

When asking closed-ended questions, the choice of options provided, how each option is described, the number of response options offered and the order in which options are read can all influence how people respond.[14]The order of responses categories will affect how people answer closed questions in addition to the number and choice of response choices. Research indicates that respondents select things that are heard later on in a list more frequently in phone surveys (the "recency effect").[15]Due to concerns about the effects of the category order on responses to closed-ended questions, many sets of response options is programmed to be randomized (when questions have two or more response options) in order to ensure that options are not put in the same order for each respondent.[16] Randomization of response items does not eliminate order effects, but ensures that this type of bias is spread randomly.[17, 18]

Questions with ordinal response categories – those with underlying order (e.g. excellent, good, only fair, poor OR very favourable, mostly favourable, mostly unfavourable, very unfavourable) – are generally not randomized because the order of categories conveys important information to help respondents answer the question.[19] Generally, these types of scales should be presented in order to make it easy for respondents to place their responses along the continuum, but the order can be reversed for some respondents. Again, reversing the order does not eliminate the recurrence effect, but distributes it randomly across the population.[20]

Question Wording: The choice of words and phrases in a question is critical in expressing the meaning and intent of the question to the respondent and ensuring that all interpret the question the same way.Even small wording differences can substantially affect the answers people provide.

A considerable amount of research has been conducted to assess the impact of different ways of asking questions and how to minimize differences in the way, respondents interpret what is being asked. The issues related to the wording of the question are numerous which can be adequately addressed in this short space of time. This paper discusses some of the important issues to consider in the development of survey questions:[13, 17, 21]

First, it is important to ask questions that are clear and specific, and that each respondent will be able to answer. If a question is open-ended, it should be evident to respondents that they can answer in their own words and the type of response they should provide (an issue or problem, a month, number of days, etc.). Closed-ended questions should include all reasonable responses (i.e., the list of options is exhaustive) and the response categories should not overlap (i.e., response options should be mutually exclusive).[12,13]

It is also important to ask only one question at a time. Questions that ask respondents to evaluate more than one concept (known as double-barreled questions) are difficult for them to answer and often lead to responses that are difficult to interpret. It would be more effective to ask two separate questions, one about domestic policy and another about foreign policy.[22]

In general, questions that use simple and concrete language are more easily understood by respondents. It is especially important to consider the education level of the survey population when thinking about how easy it will be for respondents to interpret and answer a question. Double negatives (e.g., do you agree or disagreethat dental treatment is traumatic) or unfamiliar abbreviations or jargon (e.g. NAD or no abnormalities detected) can result in respondent confusion and should be avoided.[22]

One of the most common formats used in survey questions is the "agree-disagree" format. In this type of question, respondents are asked whether they agree or disagree with a particular statement. Research has shown that, while comparing the better educated and better informed, the less educated and less informed respondents have a greater tendency to agree with such statements. This is sometimes called an "acquiescence bias" (since some kinds of respondents are more likely to acquiesce to the assertion than are others). A better practice is to offer respondents a choice between alternative statements. [12, 18]

One other challenge in developing questionnaires is

"social desirability bias." People have a natural tendency to want to be accepted and liked, and this may lead people to provide inaccurate answers to questions that deal with sensitive subjects.[23] Research has also shown that social desirability bias can be greater when an interviewer is present (e.g., telephone and face-to-face surveys) than, when respondents complete the survey themselves (e.g., paper and web surveys).[11]

Question Order: Once the questionnaires have been created, attention should be directed towards the order of the questions. The placement of a question may have a greater impact on the outcome than the specific choice of words used in the question.[24]

When determining the order of questions, surveyors must be careful how the initial questions may have unintended effects on how respondents reply to the questionnaire. Researchers have shown that the order in which questions are asked can influence how people respond; earlier questions – in particular those directly preceding other questions – can provide a context for the questions that follow. (these effects are called "order effects").[21,24]

One kind of order effect can be seen in responses to open-ended questions. Research surveys generally ask open-ended questions about national problems, opinions about leaders and similar topics at beginning. If closed-ended questions that relate to the topic, they are placed before the open-ended question, so that respondents are much more likely to mention concepts or considerations raised in those earlier questions while responding to the open-ended question.[13,14]

There are two main types of order effects on closed opinion questions: comparison effects, where the order results in greater differences in responses and assimilation effects, where the responses in their order are more similar.[25] Assimilation is done if two questions are answered more consistently and/or closer together. When tracking patterns over time, the order issues are of particular importance. Therefore, care should be taken to ensure every time a question is posed that the meaning is identical. Any changes detected over time can be clarified by adjusting the context of the topic.[26]

A questionnaire, like a conversation. It should be categorised by topics and unfolded in a logical order. It is often helpful to begin the survey with simple questions, so that respondents will find it interesting and engaging in order to help for establishing rapport and motivating them to continue the survey. Throughout the survey, an effort should be made to keep the survey interesting and not overburden respondents with several difficult questions right after one another. Demographic questions such as income, education or age should not be asked at the beginning, unless they are needed to determine eligibility for the survey or for routing respondents through particular sections of the questionnaire. Although it is best to precede such items with more interesting and engaging questions.

Pilot tests and focus groups: Pilot tests are used, to determine how a survey population sample responds to the questionnaire. For a pilot project, surveyors typically contact several individuals to examine possible variations within groups. Further, the complete implementation procedures (eg contact notes, benefits, callbacks) are evaluated through pilot test for several surveys. Pilot tests are usually conducted sometime before the survey is carried out to allow more adjustments to the questionnaire or procedure. Pilot tests are especially useful as researchers check new questions or make major adjustments to questionnaires, evaluate new approaches or different forms of carrying out surveys like the Census.[27]

Focus groups are somewhat different from pilot projects because people address the survey subject or answer specific questions in a group environment, often in person. The survey involves individual people or a group. Focus groups may be especially helpful to collect information prior to creating a survey questionnaire to see which topics affect people, how people are aware of a subject area and how people perceive questions (particularly how the topic or question can affect answers in different ways). Focus groups can be used for some projects in conjunction with the survey questionnaire to give people the opportunity to speak more deeply or intensely about subjects than possible in the interview.[28]

Pre-tests: The pre-testing with a small number of people in the survey community is one of the most important ways to decide whether interviewees' answers are as intended and influenced by the order of the questions. The pre-test is conducted using the same protocol and environment as the survey and is usually performed after the questionnaire and procedures are completed. Pre-test information is crucial as final survey questionnaire decisions are made.[29]

Conclusion

A successful survey requires a well-designed questionnaire. Nevertheless, the researcher must establish his own sense of "effective design" because no questionnaire theory is there to direct them. A good questionnaire helps directly to accomplish the study goals, offers reliable and complete details. It is simple to answer, sound analysis and interpretation is accessible for both the interviewer and the respondent in a precise format. To concise, the initial information is determined by organising various parameters and constraints, to define the target respondents and a methodology to follow in order to reach them. Furthermore, the contents of the questions are determined to form a precise and on-point questionnaire, maintaining an appropriate length. Thus the its pre-tested and the final questionnaire is produced.

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Periodontal Microsurgery: An Evolution in the Progression

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Abstract

The main purpose of this article is to provide a review of the challenges that are often faced in conventional surgery and how magnification has become an integral part of modern dentistry. The objective is to incorporate magnification for a minimally invasive periodontal microsurgery. This procedure requires clinical expertise and training. Different procedures ranging from GTR, crown-lengthening and root coverage to implantology and many diagnostic procedures are aided by periodontal microsurgery. In this challenging new era of technology, it claims to give more predictable therapeutic results with rapid healing and also improved cosmetic results whereas in other hands conventional surgery provides less visual acuity,delay in healing along with ergonomic problems.

Keywords: *Periodontal Microsurgery, Microscope, loupes.*

Introduction

Optical magnification has broadened the horizons of dentistry. The main aim of surgical intervention is not only a minimally invasive procedure but also to preserve the maximum amount of function of the organ. Periodontal microsurgery is reaching new heights of precision using loupes and surgical operating microscope. It emphasizes more on increasing visibility,minimize trauma and improve surgical results with the help of and microscalpelsmicro suture.

History: In 1876, a German physician Saemisch introduced loupes in medicine. A variety of magnification is there for surgeons ranging from simple loupes to prism loupes and most importantly the surgical microscope. Each of them has its pros and cons. It is now believed that magnification has more to do rather than just corrective vision. "In 1979, Daniel[1] defined microsurgery in broad terms as surgery performed under magnification using a microscope. Serafin described microsurgery as a methodology–modification and refinement of existing surgical techniques using magnification to improve visualization, with applications to all specialties."[2]

In 1981[3] Apotheker and Jako first introduced a commercial operating microscope to dentistry. At the annual meeting of the American Academy of Periodontology in 1993,it was subsequently conferred by Shanelec and Tibbetts on periodontal microsurgery. As treatment philosophy, microsurgery involves 3 important principles:

1. Advancement of motor skills thereby improving surgical ability
2. Passive wound healing with accurate apposition of the wound edge
3. Proper microsurgical instrumentation and suturing to reduce trauma to tissues.

Microsurgical Triad: There are 3 basic advantages provided by operating microscope i.e. illumination,

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magnification and increased surgical skills. Illumination of the field plays a crucial part in microsurgery. Clinicians who use loupes usually need a headlamp to compensate for the reduced amount of light passing through loupes. Essential criteria require while taking in consideration of accessory lighting source which includes quality, and brightness of the light and ease of focusing and directing the light within the field of view of the magnifiers and ease of transport between surgeries.

Magnification is the second most important criterion of the microsurgical triad. There are various types of magnifications available for surgeons ranging from simple loupes to prism telescopic loupes and surgical microscope. Each of them has benefits and drawbacks. The principle of optics needs to be understood that governs magnification systems to improve the accuracy of clinical and diagnostic skills. "Decrease in field of view and depth of focus should be taken into consideration as magnification increases which is a major problem with dental loupes and operating microscope."Microsurgery is an advanced surgical technique, which is defined as surgery performed under magnification of 10× or more which is performed under a surgical microscope.[4]

Magnifying Loupes: They are most commonly used for magnification in periodontology. They are primarily dual monocular telescopes with side-by-side lenses convergent to focus on an object."The magnified image formed by its convergence is called a Keplerian optical system."Despite being widely used, there is a major flaw in comparison with a microscope. The clinicians need to converge to get the operating view which leads to eye strain, fatigue, and also vision impairment with prolonged use of ill-fitted loupes. 3 types of Keplerian loupes are mostly: -

1. Simple loupes
2. Compound loupes
3. Prism loupes

1. **Simple Loupes:** They consist of a single pair of side-by-side meniscus lenses and are limited to two refracting surfaces. Greater magnification can be obtained by increasing lens diameter and size. The size and its weight make it impractical for magnification beyond 1.5x. Spherical and chromatic aberrations are limitations of simple loupes. The shape of image and object color being viewed is also distorted due to this.[5,6]
2. **Compound Loupes:** They use multielement lenses with intervening air spaces to gain additional refracting surfaces.[7] Magnification can be increased without increasing the size and weight of the lens of lengthening the distance between the lenses. Along with this, a special optical feature that favors clinicians while selecting magnifying lenses is that it is achromatic. Hence it negates the chromatic aberration of the paired lens to produce a color correct image. However above 3x they become obsolete.
3. **Prism Loupes:** They are the leading loupes of optical magnification that employ Schmidt or "rooftop"prisms to elevate the light path using a series of switchback mirrors between the lenses.[8] Shortened barren of loupes provide improved magnification, wider field depth, longer working distances and also larger field views than other loupes. However, the heaviness of these loupes with magnification up to 4x makes headband mountings table and comfortable. Recent advances in these loupes are the incorporation of coaxial fiber optic lighting into the lens to enhance illumination.

Magnification range of surgical loupes: Dental loupes have a limited range of magnification ranging from 1.5x to 6x. The loupes less than 3x are usually inadequate for visual acuity necessary for the clinicians. Due to excessive weight, small field of view, shallow focal depth surgical loupes of magnification more than 4x are not practical in use. In some periodontal procedures, prism telescopic loupes with magnification 4x provide an adequate combination of magnification, field size and depth of focus. However, the surgical microscope offers much higher magnification and superior optics than any optical systems mentioned.

The surgical Microscope: Due to its extended range of magnification with superior optical performance and its greatest flexibility it has taken over the surgical loupes. Over 70-80% of periodontal microsurgery is performed under 10x-20x using a surgical microscope whereas under 6x-8x enhanced motor skills are acquired during microsurgery training sessions. Its design is based on Galilean optical principles and uses an inclinable binocular eyepiece which enhances maneuverability. "It incorporates fully coated optics and achromatic lenses with high resolution and high contrast stereoscopic vision. It uses Coaxial fiber-optic illumination produces an adjustable, uniformly illuminated, bright, and shadow-free circular spot of light that is parallel to

the optical viewing axis." It has better visualization of farther reaches of oral cavities, including into some subgingival pockets, angular defects, and root surface irregularities. Both provide enhanced vision and better ergonomics by increasing working distance. To reduce postural problems and eye strain in dentists there is an increase of 6-8 inches in working distance.[10]

Drawbacks include are:

1. As magnification increases there is a restricted area of vision and loss of depth of field.
2. Visual reference points are lost.
3. To avoid errors in instrument and suture placement,extra time is needed to achieve an experienced team approach.
4. Physiological tremors need to be controlled.

Ergonomics in microsurgery: While operating clinicians should be in a comfortable and relaxed state of mind and posture. A stable instrument-holding position and mental focus are needed for precise controlled movements of fingers. Finger movements are controlled by long flexor and extensor muscle. Physiologic tremor is unwanted movement and can be reduced by stabilizing the wrist and ulnar surface off forearm on a flat surface, angled in a dorsiflexion position at approx.20 degree.[11] To perform efficient and deliberate movement the operator should be erect with both feet flat and thighs parallel to the floor. Pen grip is mostly used or the internal precision grip which provides better stabilization than other hand grips due to tripod effect formed by the thumb, index and the middle finger which holds the instrument and the thumb and index finger are in contact with the underlying middle finger. Proper instrumentation is necessary for surgical intervention. The handle of the instrument should be round for better manipulation yet traction to execute fine controlled rotating movement. The most accurate rotary suturing movement of hand for a human body can perform is from 2 o'clock to 7 o'clock. Persistent practice of alternative positions around 360 degrees will result in better skills and good microsurgical results.

Microsurgical Instruments: The smaller instruments are used for high precision movement and minimize tissue trauma. The length of approx. 15cm is adequate for an average-sized hand to hold on to the instrument in a pen grip. The circular cross-section of the instrument used allows smooth rotational motion whereas the rectangular design does not allow such precise manipulation and hence not ideal for microsurgery.[12] The working tips are generally used that are quite smaller than those of regular instrument. They are manufactured under magnification to high tolerances to provide consistent manipulation to tissues. The surface of the instrument is usually colored to prevent unfavorable metal flare under the microscopic light. Each of them is around 15-20g in weight to avoid muscular fatigue of hand. The needle holders need to have an accurate working lock which should not exceed beyond a force of 50g (0.5N) as the precision is reduced in low locking forces and cause tremors in high locking forces. Titanium made instruments are stronger and lighter and non-magnetized than the stainless steel but are expensive and are resistant to distortion if properly cared for. A microscalpel holder, needle holder, micro-scissors, micro-forceps and elevators are the basic set of microsurgical instruments. To achieve wound healing by primary intention small size and sharp knives are used to place clean, non-ragged incision. The sharpest needles, spatula needles (6.6mm long with 140 degree) with microchips with reverse cutting-edge needles and taper tip are generally in practice to minimize tissue trauma and early tissue regeneration.[13,14]

The choice of suture needle size is crucial for atraumatic tissue passage. The most commonly used suture is 4-0 suture on a three-eight circle FS-2 reverse cutting needle. Most of the sizes range from 6-0 to 9-0.Among various suture materials, non-absorbable monofilament sutures are preferred and should be removed at the earliest biological time as they are bacteriostatic and non-inflammatory e.g. Vicryl® Plus (Ethicon®, Norderstedt, Germany). An ideal suture material is sterile,less reactive and resistant to shrinkage in tissues. It should be strong enough until wound healing to withstand its stress. The chord distance is an important component of needle design. It's the distance between the cutting point and the swaged end and radius is the arc of its circumference. The arc determines the ease of passing a suture between adjacent teeth. Also the angle of entry (less than 90degrees), bite-size (approx. 1.5times the tissue thickness) determines the proper wound approximation. Suturing plays a significant factor for a successful treatment. Though it takes motor skills and dexterity but through the visual guidance aided by the microscope knot tying requires minimal time. In microsurgery, the entry and exit of the needle should be perpendicular to the tissues and at equal distances. Under a microscope, with the help of instrument ties,the

microsurgical needle holder in the dominant hand and tissue is picked in the non-dominant hand using proprioception.[11] The best surgical knot is achieved with the surgeon's knot followed by a square knot. Integrity or strength is lost by adding excess ties to the knot. Well tied knots ensure there is no microinjury under function.

Uses of microsurgery in periodontics: Periodontal microsurgery has broadened the horizons of dentistry by descending the conventional surgery by its attempt to reduce surgical trauma and hence give better therapeutic results.

1.**Root preparation**: Root surface debridement is an important criterion for periodontal therapy. Clinical and research studies demonstrated that with stereo microscope there in better visual access and hence effective in root planing. It is reported that under illumination, root instrumentation is effective along with less postoperative pain and improved healing index.[15,16] Moreover, using micro ultrasonic instruments of size as small as 0.2-0.6mm in diameter and variable power settings(25,000-40,000 cycles per second) allows deep pocket cleaning and reduces the chances of over-instrumentation of the root surface.[17] All surface of instrument have active working sides and requires minimal water supply. The clinician could differentiate the calculus from the surface and biofilm to the microscopic level, hence morphological contours are determined for the working end to angle accordingly during instrumentation.[18]

2. **Minimally invasiveperiodontal surgery (MIPS) for regeneration:** Recent studies have found that clinical effectiveness for periodontal regeneration of intrabony defects is better through the microsurgical approach. Further more, treatment of isolated or multiple intrabony defects with increased accuracy and minimal trauma. There is no much significant differences of probing depth, clinical attachment level gainwith treatment of intrabony defects treated with MIPS than those with conventional surgical approach.[19] Isolated interproximal defects are ideal for bone grafting using MIPS while contra indicated for generalized horizontal bone loss and multiple interconnected intrabony defects. In MIPS, for proper debridement of the periodontal defect and root surfaces, a new operating microscope (Varioscope®)with appropriate illumination which allows visualization of defect easily from different angles.[20] It has also known to minimize marginal tissue recession and improved esthetics. Recently, a new microsurgical technique named "Entire Papilla Preservation" to maintain wound stability and limit the exposure of regenerative biomaterials such as bone grafts and enamel matrix derivatives.

3. **Periodontal plastic surgery:** Esthetic improvement is the major indication for mucogingival surgery. To restore normal gingival or alveolar mucosa anatomy, several surgical procedures including pedicle grafts and free soft tissue grafts are performed. This also includes rotational flaps(e.g., laterally sliding flap or double papilla flap) or without rotation or lateral movement(e.g., coronally placed flaps). This technique is used along with the membrane barrier using GTR as a treatment of root coverage. Subepithelial connective tissue graft is obtained from the palate by a "trap door[20,21] approach can heal rapidly and is less invasive and can restore wide recession defects. Root coverage grafting is easier through autologous grafts due to their rapid revascularization.Many interdental papilla augmentations could have a better outcome using proper microsurgical procedures.

4. **Microsurgery in implants:** Surgical microscope aids in indirect visualization of sinus membrane and minimize the chance of perforation through it and hence applied in sinus lift procedures. Also, implant failure and peri-implantitis management can be performed with more precision. Although there is no strong evidence of less postoperative pain following extraction and implant placement, its rationale suggests that microsurgery causes less trauma and rapid healing and postoperative morbidity.

Conclusion

With training and motor skills, an average periodontal microsurgeon can produce better-crafted work than the proficient conventional surgeon. Though it is technique sensitive but requires less time to produce promising clinical results and have more patient acceptance. Despite having applications and benefits microsurgery it also has many drawbacks that include limited vision, loss of depth of field and arduous learning curve with the high initial cost of setup.

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Dental Management of Children with Special Health Careneeds: A Review

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Abstract

Children with special needs for health care (SHCN) management causes uncertainty and anxiety among health practitioners like dentists as it involves advanced skills gained through special training, enhanced understanding, accommodative measures and resources. A quest was undertaken to find revised and evidence-based guidelines and alternatives for the dental care available to SHCN children. Such guidelines will help dentists determine some suitable dental treatment and also help other medical practitioners recognize the need to ensure good oral health for children with SHCN and the value of collaboration with dental practitioners.

Keywords: *Special health care needs; Behaviour; Sedation; General anesthesia; Dental caries.*

Introduction

The American Academy of Pediatric Dentistry (AAPD) describes special health care requirements as "any physical, physiological, neurological, sensory, behavioral, cognitive or emotional disability or restricting disorder requiring medical treatment, involvement in health care and/or the employment of specific services or programs." The condition can also be genetic, hereditary or developed by illness or environmental causes, consistent with the AAPD, and may place limits on the performance of daily life-maintenance activities or severe restrictions during large lifetime operations. Yet, despite offering such assistance, many professionals often find it difficult. Such difficulties may vary from an absence of formal experience, uncertainty, potential ergonomic limitations, routine adjustments within the consulting room requiring physical modifications and special equipment, to the shortage of scientific expertise.[1] Moreover, it's common for patients with different levels of cooperation, a problem or perhaps an impediment of the dental treatment in an outpatient setting. Thus, health care professionals can reduce barriers using different techniques Special care dentistry is the dental care provider dedicated to the particular needs of patients with worsening medical conditions or behavioral or cognitive disabilities that need treatment outside traditional method. Patients with intellectual, emotional or physical disorders are often at greater risk of oral and dental illnesses. Thereafter, behavioral treatment method must be used first, but within the case of failure, sedation is another to the patient that's to not undergo anesthesia.[2]

Parents with SHCN children spend more money on dental services that healthy children need, a substantial barrier to access to oral care is taken into account. The purpose of this article was to scan the review for up-to-date and proof-based guidelines and usable and affordable dental management approaches with SHCN, help dentists in selecting the correct dental management and assist other medical professionals in identifying the same need.[1]

Mental Retardation: Definition-"Mental retardation is a common neurodevelopmental condition characterized by substantially impaired intellectual and social functioning"–American Mental Deficiency

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Association (1983). Mental retardation has a new name "Intellectual disability" or "Intellectual Developmental Disorder or General Learning Disability". The sizeable subaverage is known as I.Q. Around 70 or below on a standardized intelligence scale.[4,5]

Table 1. Classification of special health care needs children[3]

Frank and winter (1974)	According to Agerholm	According to Nowak (1976)
• Blind or partially sighted • Deaf and partially deaf • Educationally subnormal • Epileptic • Maladjusted • Physically handicapped • Defective of speech • Senile	• **Intrinsic category:** The handicapped condition cannot be eliminated/separated/improved significantly in the child. e.g. cerebral palsy, mental retardation • **Extrinsic category** The handicapped condition may be made better with strategic meticulous care. Social deprivation is an example.	• Physically handicapped • Mentally handicapped retardation • Congenital – cleft lip and palate • Convulsive- epilepsy • Communication-deafness • Systemic- hemophilia • Metabolic- juvenile diabetes • Osseous disorder- rickets • Malignant disorder- leukemia

Table 2. Classification of Intellectual Disability[5]

Types	IQ ranges
Mild Mental Retardation	50-70
Moderate Mental retardation	35-50
Severe Mental retardation	20-25
Profound Mental retardation	<20

Dental Findings[6,11]:

- Damaging oral habits is a problem for some intellectually disabled individuals. Specific behaviors include bruxism; breathing of the mouth; thrusting of the tongue; self-injurious actions such as picking on the gingiva or chewing the lips; and pica–consuming items and substances such as dirt, cigarette butts or pens.
- Dental Caries- Incidence is the same as normal children but the occurrence of untreated caries is higher than usual children.
- Periodontal Disease - Medicines, malocclusion, several disabilities and poor oral hygiene lead to increase the risk of periodontal disease in mentally impaired people (6).
- Malocclusion occurrence in persons with intellectual disabilities is equivalent to that found in the general population
- Missing permanent teeth, retarded eruption and hypoplasia of enamel are even more prevalent in Intellectual Disabilities.
- Trauma and Injuries-In people with intellectual disability, TRAUMA, and Injuries occur to the mouth through falls or accidents.
- Owing to the various drugs, increased plaque and calculus formation that contribute to poor oral hygiene, salivary flow is altered further contributing to halitosis.
- Speak disability due to early teething delay.

Dental Management 3:

- Such kids can have a shorter period of maturity too. Such kids can have a shorter period of maturity too.
- These two main objectives were accomplished by the following:
- Familiarisation (Desensitisation) - To that his anxiety and fear of objects around him that might be unfamiliar to him by getting acquainted with the dental office environment, the apprehensive child patient needs to be desensitized.
- Effective communication-–It's necessary to make the child understand the proceedings through clear and uncomplicated verbal contact. These are used successfully in Mentally Retarded Educable (EMR) and Mentally Retarded Trainable (TMR).
- Active Listening.
- Short appointment time.
- Structuring and scheduling–Care must be given at times when in the early part of the day the dentist and the operator are both less exhausted.

- Positive Reinforcement–A social or material reward will positively reinforce the child's interpretation of conduct which is beneficial during the operation.

Down syndrome (trisomy 21): Down syndrome, or Down's syndrome, also known as trisomy 21, is a genetic disorder characterized by whole or part of a single copy of chromosome 21. The most severe abnormality in human chromosomes is the Down syndrome. It occurs every year in about 1 in 1000 babies born.[8-10]

Dental Findings[7]:

- Periodontal disorder: Nearly all children with Down's syndrome have moderate to extreme periodontal disorder.
- Xerostomia: Dry mouth, normally caused by oral coughing and other drugs (anticonvulsants, sedation).
- Infection: Fissured lips and tongue that lead them to the region's oral candidacies, aphthous ulcers and wounds.
- Hypotonia: Such disease that affects muscles in different areas of the body.
- Difficulties with oral hygiene and brushing: Few people with Down syndrome will brush and floss easily, while others need assistance.
- Dental abnormalities: This disorder is very common in patients with Down's syndrome, including enamel hypoplasia, microdontia, impacted teeth, crowded teeth, missing teeth and malformed teeth.
- Malocclusions: a prognathic Class III occlusal relationship, Posterior crossbite, and anterior open bite.
- Macroglossia, Delayed eruption of teeth, Sleep apnea, Dental Caries, Halitosis, Speech difficulties

Dental management[12]:

Table 3. Prescribed medications based on symptoms

Symptoms	Medication	Side effects
Seizures	Anti-convulsant (Dilantin)	Gingival hyperplasia
Hypertension	Calcium channel blockers	Xerostomia

Behavioral Management:

- Pre-(person/phone) plan before the first appointment to discuss special patient needs. Speak best about this-knowing the child to the parents or the caregiver.
- Timetable patient rendezvous early in the morning or best time of day.
- Speak to the parents or caregiver to assess the level of mental and cognitive ability of the patient, and describe each treatment at a level that the patient can understand.
- Using the quick, concise directions and talk to the patient directly.
- Reduce stimuli such as hustle and bustle that can make it difficult for the patient to comply.
- Start the oral examination slowly, beginning with fingers first. When this is a success, continue to use dental products.
- When implementing new instruments or techniques, use the Tell Show-Do approach.
- The cooperative behavior is compensated by positive verbal strengthening.
- Improve faith and continuity between the dental personnel and the patient.

Cerebral Palsy: Cerebral palsy is a category of chronic movement and posture development disorders that cause restriction of activity, due to excessive progressive disorders that developed in the developing fetus or autistic child brain.[13]

Dental Findings[3]:

- Malocclusion prevalence rate was recorded between 59 and 92 percent, with the overwhelming majority of malocclusion categorized as Angles Class II with increased overjet and overbite, unilateral cross-bite and open bite associated with irregular oral habits is twice as common.
- Rodrigues CAM et al.found that the incidence of dental trauma was (36.3 percent); the most severe trauma (89.1 percent) was enamel fracture.
- In several studies a high prevalence of bruxism has been confirmed in individuals who have Cerebral palsy.
- Higher prevalence of the periodontal condition.
- Ferreira de Camargo et al. found that the occurrence of caries in kids and teens with CP is strong, but Quintela M et al. concluded that in both dentitions, cerebral palsy individuals are less likely to have caries than subjects in comparison groups.

- Dental erosion is prevalent in patients with Cerebral palsy.
- In children with CP the sequence of eruption was almost close to normal. Except for the second premolar and the second molar, showing delayed eruption.
- Lin et al. found a high frequency of developmental enamel deficiencies in children with CP {Enamel hypoplasia (25.9%), opacity alone (3.7%), and mixed deficiencies (opacity and hypoplasia)}.

Dental management[3]:

The reason for the treatment involves three aspects:

- Intellectual maturity and willingness to cooperate: Based on this measure, children are graded as educable, trainable and untrainable.
- Postural compliance in the dental chair: the manifestation of increased muscular rigidity, hyper-contractility, uncoordinated movement, and ataxic gait that compromise the child's compliance with a dental chair.
- Preventive care: Requires good oral hygiene habits, instruction and knowledge of the caretaker's oral hygiene habits, application of fluoride and sealant, as well as regular examination

Some Considerations: A patient who is using a wheelchair should be treated in the wheelchair itself. The patient must be balanced and held as security in the midline of the dental chair, with arms and legs as close to the midline. The patient's upper and lower limbs will not be pushed into an uncomfortable position. Patient choice should be essential. A choice of two adapted radiographic techniques is available to reduce extreme gag reflexes which may occur in children with cerebral palsy: one is the 45-degree oblique head plate, and another is the reverse bitewing (buccal technique). Working as effectively as possible must be on the operator, and reducing the exhaustion of the muscles involved.

Dental management while doing Dental Treatments

The therapeutic needs must be prioritized (preventive, conservative, surgical) as follows:

- Preventive treatment involving the regular scaling/ cleaning and application of fluoride, as well as the positioning of sealants.
- Restorative treatment that involves both primary and permanent endodontic and restorative care.
- Surgical treatment including extractions of the tooth, gingivectomies, biopsies and other mild oral surgery.
- It could be suggested and appropriate to recommend some relaxation exercises or even physical help, so that the dental treatment runs out un-eventually. Both of these advocate limiting movement, which must be used to prevent patient movement from obstructing dental assistance. This strategy often prevents patient "escape attempts," while shielding the work team as bites from potential traumas and injuries. Based on clinical experience with journalists, below are a few suggestions:
- 'Holding therapy' is a child-friendly physical support strategy that stays in the lap of the person responsible for stabilizing the trunk and arms while supporting the patient (Figure 1).



Figure 1. Holding therapy

The "knee to knee" position is ideal for 1-3-year-old children. The technique consists of placing the child on the dentist's and the person responsible's hands, each reaching the knees and creating a kind of hammock (Figure 2).



Figure 2. Knee to knee position

The procedure in which the auxiliaries carries the head of the patient should be extended to patients of all ages. The auxiliaries support the head of the patient to stabilize or sustain it (Figure 3).



Figure 3. Auxiliary sustaining the patient's head

Such method can be used for short dental visits in which, as mentioned above, preventive, restorative and/or surgical treatments can be performed before the pharmacological restraint attempt.[14,15]

Conclusions

For a person with SHCN, oral and general health care needs "specialized knowledge acquired through additional training, as well as increased awareness and attention, adaptation and accommodation beyond what is considered normal".[15]Children with special health care needs get a substantially greater incidence of dental diseases due to lack of oral health education, exposure to treatment, and prevention measures such as fluoride supplements and dental adhesives for their caregivers. Children with special health care needs have also considered oral health low relative to otherwise healthy children in terms of their dentition status, periodontium of their treatment needs and dentofacial defects. Many children with mild to moderate developmental disorders may be treated with limited modifications in a dental clinic. A well-trained team of professional dentists, fair parental participation in difficult care plans, and knowledge of the level of child development for daily language skills and attention will also enable the patient to cope with the care better. By delivering comprehensive school-based services (along with oral hygiene programs to help children develop skills), delivering fluoride supplements and sealants, and providing health and nutritional guidance, the dental team will seek to improve oral hygiene. Educational videos that show both children with SHCN and healthy children with a variety of materials can be useful in teaching them oral protection. Routine oral healthcare preparation is much more critical for children with SHCN than for healthy children because of the complications of the condition and the chronic nature of the disease.

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Chondroblastic Osteosarcoma: A Review

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Abstract

The main purpose of this article to present a review on chondroblastic osteosarcomaand its prevalence in the different jawbones of the body. The word osteosarcoma mainly refers to a group of heterogeneous malignancies that involves bone formation or it can also results due to mesenchymal tissue with evidence of osteogenic disparity. It has been reported around 25% of the pattern of the chondroblastic osteosarcomas represents neoplasms.

Keywords: *Osteogenic sarcomas, chondroblastic osteosarcoma jaw, bonetumors.*

Introduction

Sarcoma is procured from the Greek word "SERX" which means "Flesh''.A sarcoma is a type of cancer that arises from the transformed cells of mesenchymal origin. These are primary connective tumors.[1]

Osteosarcoma is the most common type of cancer that primarily affects immature bone. It is the most common type of cancer and has a more male prediction ratio. Osteosarcoma occurring in the orofacial regions is extremely sparse, noticing around 1 % of all head and neck region and 7% of all osteosarcomas occurring in the body.[2]

Types:

The different types of osteosarcoma are:

Central:

- Lowgrade-parosteal;
- Intermediate-periosteal;
- Highgrade- Dedifferiatelyparosteal

Surface:

- High-grade 1.Conventional; 2. Telangiectatic; 3. Small cell; 4. Epithelioid; 5. Osteoblastoma-like; 6. Chondroblastoma-like; 7. Fibrohistolytic; 8. Giant cell-rich
- Low grade: 1.lowgrade-central; 2.fibrous dysplasia-like

Intracortical:

Gnathic:

Extra Skeletal:

- High grade
- Lowgrade

Depending on who classifies the chondroblastic osteosarcoma as a histological entity illustrious by the major occurrence of the chondroid matrix, which marks in the direction to give you an idea about an elevated degree of hyaline cartilage as well as is intimately associated through the non-chondroid elements like osteoid, bone matrix.[3]

Epidemiology, genetic factors and risk factors: Osteosarcoma is a condition which occurs in both children and adolescence group of people but it is found that it culminates more during the second decade of life. The occurrence rate in children and juvenile age group mostly variety stuck between 3.5 to 4.2 cases

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per million inhabitants per year at the same time as the rate in old aged group of individuals is found less .i.e. approximately 1 to 2 cases per million population per year and it is found that middle age group of individuals shows around 1.5 to 4.6 cases per million population per year (25-60) years. Among the risk factors for osteosarcoma, fluoride exposure is found to be the predisposing factor for causing bone cancer. Later no evidence was found clearly regarding this factor.[4]

The exact cause is still unknown but several risk factors are been identified. It is been found that it has arisen from the hereditary multiple exostoses and diseases like Paget disease, fibrousdysplasia, etc. The re are different types of osteosarcoma of jawbones and they can be classified mainly into two groups-

a. Primary

b. Secondary

The cause of the primary subtype of osteosarcoma is still unidentified .but it has been predicted that it may take place due to possible risk factors like genetic as well as environmental factors in addition to the genetic predisposing factors includes the hereditary form of retinoblastoma .i.e. gene alteration,Li-Fraumeni condition. i.e. P53gene transformation, Rothmund – Thomson syndrome. The cause of the resultant subtype of osteosarcoma arises as of the late sequel to craniofacial irradiation, Paget's disease as well as fibrous dysplasia. And it is found that it mainly occurs in grown-up group of patients.[5]

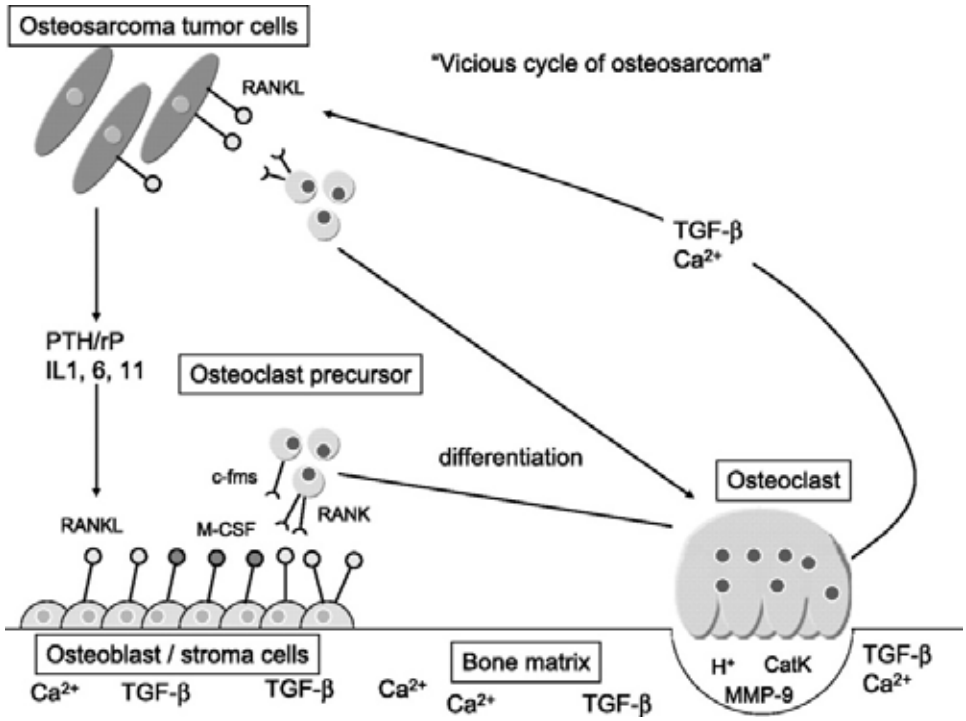


Figure 1. Vicious cycle of osteosarcoma

Clinical Features: It mainly occurs in the age groups of 20-40 years with predominance in males as compared to females and has a higher occurrence in the posterior mandible as compared to the mandible. In some cases, it has been found that the maxilla and mandible are involved with equal constancy. Sites that often participate in the mandible are at symphysis and the sites that mainly involve in maxilla are the alveolar ridge, palate, sinus floor etc.[6]

These lesions can be purely osteolytic, purely osteoblastic,or mixture of both. Common signs and symptoms include pain,swelling, or both in some cases. Lymphadenopathy is also found where there is unusual focal and regional lymph nodes involvement. The essential microscopic criteria to detect are the direct production of osteoid cells by malignant mesenchymal cells. In addition to these osteoid cells we find the tumor cells producing chondroid type of material and the

different fibrous connective tissue. Other complaints include numbness, facial dysphasia, mobility of the teeth, trismus, headache, nasalcongestion, inability of mouth opening, etc. But it is found that some patients have no prodrome at present time but their tumors can be located incidentally by radiographic imaging.[7]

At first parading, metastatic infection is accessible around 4- 5% of the patients. This occurs in a reduced amount in the patients among appendicular skeleton osteosarcoma. The lungs are the most habitually implicated sites of our body.[8]

Investigations: Radiography plays a major role in the initial diagnosis and it is seen that the classic radiographic presentation of osteosarcoma includes Sunray/Sunburst appearance. And this sunburst appearance arises due to osteogenesis of tumor cells. But the extent of the tumor in both hard and soft tissue is been better appreciated in the CT Scan, MRI imaging, Angiogram which mainly detect the vascular displacement and the relationship of the vessel to the lesion and the same time it also helps to determine the vascularity of the involved tumour.[9]

Immunohistochemical characterization plays an important role in the diagnosis of osteosarcoma which includes mainly the osteonectin and osteocalcin-osteocalcin is specific for osteoblast cells but osteonectin is not specified for osteoblasts and the other biomarkers that are used apart from osteonectin and osteocalcin are cytokeratin, P53, P16, Ki67, CD99, S100, caveolin-1, etc. Some laboratory standards like lactate dehydrogenase, alkaline phosphate serum levels possibly will be escalated in a small number of collections of patients. Even though they do not keep up a correspondence with disease proportions, exceptthey may have anegative prognostic importance.[10]

Codman's Triangle: It is formed primarily in the angle between the elevated periosteum and the cortex's underlying surface.

In many cases, CORTICAL BREACH is commonly observed. Histologically, there is the presence of belligerent malignant mesenchymal cells that produce undeveloped bone. And the stroma mainly consists of malignant connective tissue with anaplastic spindle cells surrounded in the mesenchymal parenchyma.[11]

Chondrosarcoma comprises of cartilage along with varying degrees of cellularity and maturation. It is found that normal chondroid lacunar formation is visible, but this feature may be rarelyseen in poorly differentiated tumors. The tumor can be often seen in globule growth patterns with thin fibrous connective septa-separated tumor lobules.

The central areas of the lobules show the highest degree of maturation. Calcification or ossification may occur within the chondroid matrix. Neoplastic cartilage may be replaced by bone-like normal endochondral ossification.[12]

It can be divided into 3 histopathologic grades of malignancy and this grading system is well correlated with the growth rate of tumors and the prognosis of extragnathic skeleton chondrosarcomas.

Grade I: Chondrosarcomas powerfully bear a resemblance to the manifestation of chondromas, consisting of the chondroid matrix as well as chondroblasts displaying no more than a slight deviation of normal cartilage outer shell. The cartilaginous matrix calcification or ossification is prominent, as well as the mitosis is rare.

Grade II: Chondrosarcoma shows a larger portion of moderately sized nuclei and increased cellularity, especially in the lobules with a less prominent hyaline matrix. However, the mitotic rate is low.

Grade III: Chondrosarcoma is exceedingly cellular with may show prominent spindle cell propagation mitoses may be important. Simply identifiable cartilaginous matrix containing lacunae cells may be scarce. Eventually develop into higher grade osteosarcoma with poor prognosis.[13]

The different histopathologic variants are as follows:

1. Conventional type-osteoblastic, chondroblastic, and fibroblastic osteosarcoma
2. Telangiectatic/osteolytic type osteosarcoma
3. Diminutive cell osteosarcoma
4. Low downgrade central osteosarcoma
5. Periosteal osteosarcoma
6. Periosteal osteosarcoma
7. Secondary osteosarcoma
8. Elevated grade surface osteosarcoma
9. Extraskeletal osteosarcoma

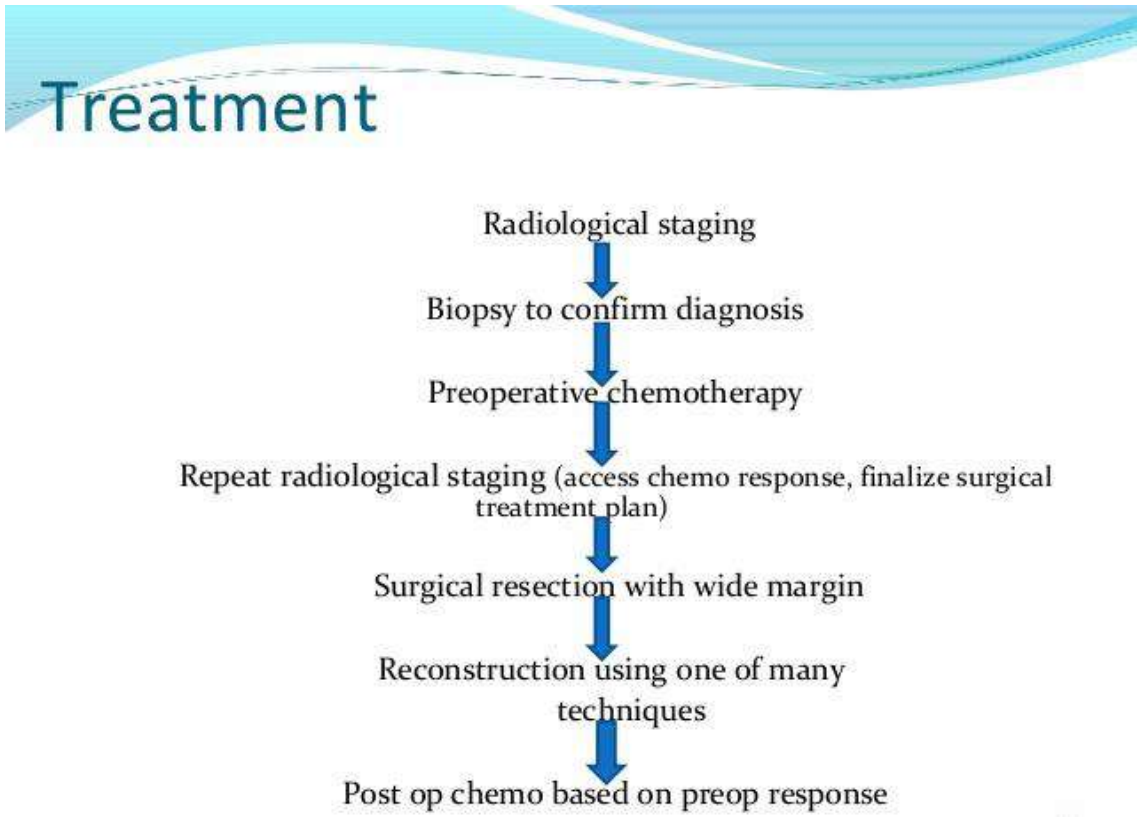
The prognosis feature is mainly evaluated by the Enneking system which for the most part evaluates the histological grade of the tumor (G), the extent of the primary tumor (T) as well asmetastasis to the close by lymph nodes or other concerned organs (M).

Amongst all the diverse histological types as well as subtypes chondroblastic type is more impervious to treatment and exhibits unfavorable prognosis. Whereas we can find Fibroblastic types that have a rather better prognosis and as well as responds to the treatment.

Treatment: The management is finished with a permutation of therapies together with surgery, chemotherapy as well as radiation therapy. However, they have to undertake three months of chemotherapy, acknowledged as neo-adjuvant therapy previous to surgery if the patient is originated to have high-grade tumors.[14]

Surgery: As these cases for the most part lies in the skeletal orientation, surgery is a central component of osteosarcomas treatment too in the head as well as neck region. The modality of the management, principles of the surgical management largely depends on the position of the tumor, the dimension of the tumor. Obtaining disease-free resection margins is of course dictatorial, to keep away from the risk of local reappearance. on the other hand, this objective is even extra complicated to reach when dealing through the head as well as neck osteosarcomas, as resecting few millimeters, more often means imperil significantly functional structures, through a noticeable reduction in the patient's quality of life. While in the intraoperative strength of mind of resection margins might represent a useful tool in other head as well as neck malignancies. Intraoperative pathological examination does not consent for the evaluation of bone margins. In the meantime, only soft tissue margins can be evaluated through the intraoperative consultation. It is found that the best management results is been obtained when the criteria .i.e. the histopathologic evaluation, biopsy of the desired site and the extent of the neoplasm is been determined, before the treatment. Multimodal mode of treatment is indicated or we can say that the best analeptic option is when we are dealing with the high grade of osteosarcomas but only alone surgery can be done if the neoplasm comes under the low grade, parosteal and central group of osteosarcomas. Provided if the metastatic potential of the neoplasm is found to be absent.[15]

The treatment procedure can be understood as follows:



Conclusion

Chondroblastic osteosarcomas of the mouth remain "Delphic''. Anamount of difficulty associated with their diagnosis as well as treatment is yet to be determined. True synchronous multicentric osteosarcomas of the mouth are tremendously uncommon but like other osteosarcomas of the jaw, have a successful result. The palliative resection modality of the management in such lesion through difficult however can show the way to an enormously enhanced quality of life in addition to may even offer the window for cure. Bountiful studies have been reported that the molecular pathogenesis of the osteosarcoma as well as the related biomarkers associated with means of the tumor. Even though many studies are exceedingly favorable as well as there has been no upgrading in the reoccurrences along with survival rates of osteosarcoma. Our facts and data of the pathway caught up in sarcoma genesis deficient, as well as new perceptiveness are enthusiastically awaited in the perspective of developing a forceful target therapy to come together with surgery.

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Conflict of Interests: None

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Deleterious Oral Habits and Management in Pediatric Patients: A Review

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Abstract

Habit is the constant and unconscious repetition of an act. Children (usually infants) frequently acquire oral habits that can be temporary, if continued can be permanent & harmful to dental occlusion and supporting structures. This article aims to throw lights on harmful habits, its cause, side effect and treatment available, as ignorance of these habits can cause facial abnormality; hence Dentists play an important role in the identification and removal of habits.

Keywords: *oral habits, Bruxism, Digit Sucking, Nail Biting, Tongue Thrusting, Mouth Breathing, Lip Sucking.*

Introduction

Oral Habit most commonly seen during infancy as it is part of normal development. It usually fades away with time. If not, then it is a matter of great concern as it can cause serious dentoalveolar changes. It is usually associated with several etiologies out of which psychology is common and important. Hence, if a patient complains of habit understanding the associated deep-rooted psychology and management is very important for Dentist.[1]

Oral habits is justified as a frequent or constant practice which is fixed due to frequent repetition

Oral Habits are classified in several headings by following scientist:

According to William James (1923): Useful habits such as nasal breathing/harmful habits such as thumb sucking.

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According to Kingsley (1956): Functional Oral Habit like mouth breathing/Muscular Habit like tongue thrusting/Combined Muscular Habit like thumb and finger habit/Postural Habit like chin propping.

According to Morris and Bohanna (1969): Pressure Habit like lip sucking/Non Pressure Habitlike mouth breathing/Biting habit like lip biting.

According to Finn (1987): Compulsive/ Noncompulsive.

According to Klein (1971): Empty or unintentional Habit/Meaningful or intentional habit.[2]

Bruxism: The word bruxism is derived from the word 'la bruxomaine' coined by Marie Pietkiewicz (1907). It is a repetitive movement disorder of mandible which is characterized by unconscious clenching and grinding of teeth. This type of Parafunctional movement is important for dentist's knowledge as it is used to detect the reason behind broken restoration, tooth damage such as tooth fracture or any temporomandibular disorders. Bruxism is of two types Nocturnal bruxism/Sleep Bruxism which occurs in sleep and Diurnal Bruxism/Awake Bruxism which occurs when the patient is awake. Sleep bruxism is of two types of primary or idiopathic (occurs without any medical reason), secondary or iatrogenic (may be caused due to use of the drug).

Etiologies associated with bruxism are psychosocial factor (emotional stress), pathophysiology factor (illness, trauma, smoking, medication), genetic factor (offspring of people with bruxism have more chances), local factor (faulty restoration, calculus, malocclusion), systemic factor (a nutritional deficiency, intestinal parasite infection).[3-6]

Clinical Feature: Bruxism affects the anatomy, morphology and dental occlusion. It causes mobility of teeth, hypersensitivity, pain in facial muscle,restricted mouth opening, pain in the temporomandibular region (frequent headache), tooth fracture, damage to the dental prosthesis and dental fillings.[3-6]

Treatment: There is no such accepted treatment available for bruxism as it is not life-threatening hence conservative treatment is recommended. Eliminate the causative factor. Awake Bruxism can be reduced by habit modification training or use of small chewing gum as a reminder in the molar region. Sleep bruxism can be reduced by sleeping without pillow i.e. flat on back, occlusal splint and dental guards can be used. Medication such as benzodiazepines, beta-blockers, dopamine agents, antidepressants, anticonvulsants, muscle relaxants can be used to treat Bruxism.[3-6]

Digit Sucking: Digit (Thumb, finger) sucking is the most common type of non-nutritive sucking habit. It is defined as positioning of digits at varying depths of the oral cavity. This habit started during the 29th week of gestation continues till 2-3yrs of age after which children usually stop this habit on their own. If this habit continues it can cause malocclusion and can also alter the shape of a digit.

Two theories are involved to explain the etiology of digit sucking, emotional theory and learned behavior theory. Emotional theory is explained by Sigmund Freud according to him finger sucking is a type of pleasure in which a child derives from excitation of oral erogenous routes. If infants sucking needs are not fulfilled this habit become fixation, later continuation is known as regression. Fixation and regression are features of emotional disturbances. In recent times learned behavior theory is the most favored theory, according to this theory sucking is a natural desire in infant and digit sucking is caused due to excess sucking urge because of efficient breast/bottle feeding by nutritionally proficient mother. Emotional and physical stimuli, such as hunger,boredom, stress, pleasure and other disability are the main cause behind the continuation of this habit.[7-11]

Classification: Subtelny (1973) has classified types of digit sucking into 4 types:-

Type A-(50%) of children place the entire thumb or digit inside the oral cavity witha pad of the thumb is a place such that is pressing the palate. Anterior teeth of the maxillary and mandibular region are in contact.[7-11]

Type B-(25%) of children place the whole thumb inside the mouth but it is not in contact with the vault ofthe palate. Only anterior teeth mandibular and maxillary region are in contact

Type C- (18%) of children place thumb just beyond its first joint, there is no mandibular contact thumb is in contact with maxillary incisor.

Type D-(6%) of children doesn't fully place thumb inside the mouth, only thumbnail is in approximate contact with mandibular incisors.[7-11]

Etiology: Type of malocclusion during digit sucking depends on several factors such as the position of the digit, related oro-facial muscle, the position of the mandible during sucking, facial and skeletal design, intensity frequency and duration of force applied.

Clinical feature: Prolong Digit sucking can cause damage to facial structure, dental structure and digits involved in sucking. Maxillary changes due to a long time sucking habit areoverjet of the maxilla,increased maxillary arch length,reduced width of palatal arch, increased angle of sella-nasion-point A. Effects on the mandible include proclination of the mandibular incisors,increased distance in intermolar region and reduced angle of sella-nasion-point B. Other dental deformity includes increased overjet, reduced overbite, and crossbite in posterior tooth region. Digit deformity can be caused in chronic sucking patients.[7-11]

Removal of psychological reason, engagement of the child in various activities to divert the mind, adequate duration of breastfeeding, use of dummy or pacifiers can reduce the thumb sucking habit.[7-11]

Management of thumb sucking involve following therapy according to Pinkham:

Preventive therapy (Hughes): Feed the child enough in a natural way such that his feeding needs are fulfilled.

Psychological Therapy: This habit usually acquire by those children who lack parental care, love and proper care. Hence dentists and parents need to carefully handle the situation with proper understanding and without shaming and punishment. Positive reinforcement or positive reward system should be used to modify behavior. Dunlop's beta hypothesis is the best way to break the habit. According to this hypothesis conscious and purposeful repetition can stop the habit. The child is asked to stand with the mirror and suck his/her digit looking at self, this procedure is very productive when a child is repeatedly asked to perform this act whenever he sucks thumb. Dr. Dragan Antolo's book named 'The Little Bear Who Sucked His Thumb' is also a positive and pressure less way of removal of habit as a child relates himself to bear and willingly quit the habit. [7-11]

Reminder Therapy: They are of two types chemical and mechanical. Chemical therapy includes the use of bitter-tasting or bad odor chemicals such as quinine, asafetida, castor oil, red pepper in volatile liquid, commercially available femite (Denatonium benzoate) in thumb. Mechanical therapy includes mechanical restrains applied to hand, wrist, thumb to quit habit examples of mechanical restrainers are Thermoplastic thumb post (Allen in 1991), ace bandage system, use of long sleeves nightgown, three-alarm system introduced by Northan and Gellin (1968).According to them when the child putsa finger in mouth and feels the tape its first alarm when a child feels the pin which is tied with a bandage in the elbow is second alarm lastly when bandage gets tightened serves as a third alarm.

Appliance Therapy: They are of two types

Removable Appliance: Palatal crib, oral screen, hay rakes.

Fixed Appliance: Quad helix, bluegrass appliance, modified bluegrass appliance.[7-11]

Nail Biting: Nail-biting is also known asonychophagia is the most common stress-relieving oral habit seen in both children and adults. Nail-biting habit is due to anxiety which usually develops after sucking age. It includes biting of nail or its surrounding soft tissue and cuticle. This habit starts after the age of 4 and attains its peak in 4-6 yrs and get stable during 7-10yrs of age again triggers in adolescence. It is not a pernicious habit hence it does not cause malocclusion. Somehow, attrition of lower incisors can be seen in rare cases as the force applied in nail-biting is similar to mastication yet quitting this habit is necessary as this habit is socially unacceptable. Operational definition of Nail Biting is "putting one or more fingers in the mouth and biting on the nail with teeth"

The etiology of nail-biting is controversial some studies believe it is due to behavioral disorder or anxiety while others deny this theory. It occurs due to boredom,emotional stress or successor of thumb sucking.

Clinical features include attrition of incisal edge, rotation of teeth, crowding, resorption of apical root, gingivitis, fracture (in severe cases). Other feature includes damage to the cuticle, inflammation of nail bed, bacterial infections.

Management includes behavioral modification and physical barriers. The patient must be treated with a little more attention, love, affection, and comprehension. Chewing bubble gum can be an alternative when patients feel the urge to bite nails. The application of olive oil or bitter-tasting preparation might annoy the young patient. Educating the child and creating awareness is the best way to quit this habit.

Tongue Thrusting: Tongue thrust is defined as the movement of the tongue tip in the forward direction between the teeth to touch the lower lip during swallowing and speaking, so that the tongue becomes interdental. This habit is most commonly seen during infancy and subsides after a certain age. It can be Physiologic (normal tongue thrust swallow during infancy), Habitual (presence of habit even after the rectifying the malocclusion), Functional (adaptive behavior developed to achieve an oral seal), and Anatomic (person with macroglossia).[12, 13]

Etiology: According to Fletcher following factor may cause development of tongue thrusting

Genetic factor: anatomic or neuromuscular deformity in head and neck region e.g.: macroglossia, hypertonic orbicularis oris activity, position of lingual frenum *Learned behavior:* It is an acquired habit hence following condition such as improper bottle feeding, prolonged thumb sucking, prolong tonsillar infection and upper respiratory tract infection, prolong tenderness of gums or teeth can alter the swallowing pattern and can cause tongue-thrusting habit.[12, 13]

Classification of tongue thrust according to James. S. Barner and Holt:

Type 1: Non-deforming tongue thrust

Type 2: Deforming anterior tongue thrust. (2a) anterior open bite, (2b) proclination of anterior teeth, (2c) posterior crossbite.

Type 3: Deforming lateral tongue thrust. (3a) posterior open bite, (3b) posterior crossbite, (3c) deep overbite

Type 4: Deforming anterior and lateral tongue thrust. (4a) anterior and posterior open bite, (4b) proclination of anterior teeth, (4c) posterior crossbite. [12, 13]

Clinical feature: Intraoral clinical feature includes: proclined upper anterior, retrocline lower anterior, anterior open bite, posterior crossbites, bimax protrusion (complex tongue thrust)

Extraoral feature includes: Doligocephalic face, increase lower anterior facial height, incompetent lip seal, speech deformity can also be seen

Management: This habit is vanishes once permanent teeth erupt by age of 8-9 yrs. mayo functional therapy, speech therapy, subconscious therapy or orthodontic treatment is required to correct this habit. Several exercises can also be performed such as lemon candy exercise, 4s exercise, whistling, counting from 60-69, yawning, gargling, water holding exercise, lip exercise.

Following Removable orthodontic appliances are used:

Modified Hawley's Appliance: acrylic is cut on anterior hard palate region, crib or rake is attached on the anterior side this increases anchorage and cribs act as a reminder to the tongue.

Oral screen: it acts as a barrier to the tongue, corrects open bite, arranges the maxillary anterior teeth. Ring attached in oral screen helps in Lip muscle exercise.

A fixed orthodontic appliance includes Nance palatal arch (acrylic button type).[12,13]

Mouth Breathing: Breathing is the vital function of the human body, usually done through the nose. Breathing through the mouth instead of the nose is considered as abnormal breathing habit. Merle suggested the name oro-nasal breathing. It's the most common habit among children as well as adults. Mouth breathing due to an acute illness, cold is not of main concern. Chronic mouth breathers who completely depend on their mouth or people with medical conditions like sleep apnea are of main concern. It shows the child needs additional medical involvement or retraining on breathing easily through the nose. According to Finn mouth breathing can be obstructive, habitual, and anatomical.[15,16]

Etiology: Airway obstruction (enlarged turbinate), defect on the intranasal region (tumors, deviation of the intranasal septum, bony spurs), hypertrophy of pharyngeal lymphoid tissue, infection or inflammation in the nose (nasal polyps, chronic allergic steatitis), birth abnormality (cleft lip, cleft palate, tongue tie), abnormal facial musculature due to former habit like thumb sucking.[15, 16]

Clinical Feature: General side effects include pigeon chest deformity, hoarseness voice, poorly developed sinuses, decrease sense of smell. Extraoral features like adenoid face, gummy smile, short upper lip, narrow nostrils, dryness and crack in lips can be seen. Intraoral symptoms are proclination of maxillary anterior, generalized spacing, gingivitis, posterior crossbite.[15, 16]

Treatment for mouth breathing depends on its underlying cause. It should be treated as early as possible patients with nasal congestion should use nasal spray. Surgery like Tonsillectomy, Sepatoplasty, and Adenoids removal is advised. Dentists prescribe the use of orthodontic appliances such as oral screen, chin cap and activator.[15, 16]

Lip Sucking/Lip Biting: Child might occasionally suck their lip when anxious but some suck and bite their lip as deleterious habit which might cause soreness and redness of lip below the vermillion border. This case occurs almost in all cases of lower lip.[17,18]

Etiology: It is caused due to physical causes such as malocclusion, TMJ disorder. Physiological disorder includes fear, stress, anxiety or body-focused repetitive behavior (BFRB). This is also caused due to hyperactivity oh mentalis muscle.[17,18]

Clinical Feature: It causes protrusion of maxillary incisor, retrusion of mandibular incisor, generalized spacing in the upper arch, crowing in the lower anterior arch. Lip will appear red, chapped, irritated below vermillion border. Vermillion border will become hypertrophic.

Treatment depends on the cause physiological

causes can b treated with habit modification counseling and therapies such as Cognitive Behavioral Therapy, Habit Reversal Training, Dialectical Behavior Therapy while physical cause requires clinical treatment such as correction of the underlying cause. Medications prescribed are antidepressant and anti obsessive medicine such as clomipramine or selective serotonin reuptake inhibitors.[17,18]

Conclusion

Habits are increasing day by day due to stress and poor attention from parents. Child needs proper care for good behavior development. Habits once developed are hard to break because repeated action becomes imprints in our neural pathway. Abnormal oral habit can cause great destruction to both dental and facial structure; it also affects the general health. Hence, Dentist plays an important role in habit modification. Dentists not only repairs tooth and dentofacial structure but also identify unnoticed habits (due to parents unawareness) and helps in the prevention and treatment of the underlying cause. To replace bad habits with a good habit integrated approach is required between parents and patients, behavior modification technique, physical exercise, habit breaking appliance and orthodontic treatment are required. But most importantly parents should be educated about harmful oral habits their etiology and side effects to stop habit in early age for healthy dentition and good health.

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Recent Advances in Non-pharmacological Behavior Management Technique in Children

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Abstract

A conventional communication along with a caring attitude develops an affinity with a pediatric patient. Non-pharmacological behavior modification approach allows pediatric dentists the ability to effectively alleviate behavioral issues by balancing their preference of treatment with that of the child's type of mutual practice. Whereas Activity informed adult patients with dental phobias is the product of an offensive approach employed in childhood. Hence to overcome these disadvantages newer non-pharmacological techniques were handled by Pediatric Dentists which provided long term reinforcement in younger children. This review will focus on reckoning the recent advances in non-pharmacological behavior management techniques in children.

Keywords: *Audiovisual distractions, videos games, virtual reality.*

Introduction

Wright (1975) defined behavior management as tohow the dental health team effectively and efficiently performs treatment for a child patient and at the same time instills a positive dental attitude. The strategies of governance include correspondence and schooling. The partnership with the infant, the families of the infant and the dental staff is a complex operation. It will begin before the patient arrives at the operation and may include written material as well as speech, voice sound, facial expression, body language and contact. Successful child patient care depends on the willingness of the dentist to meet immediate dental needs, which emphasize enhancing communication and patterning with children and parents to encourage a positive attitude and good oral health.[1] Child management in the dental office relates to the approach of gaining child care consent in the dental chair based on adequate child/parent interaction through empathy counseling and hearing the idea behind the action of a child is to handle them instead of simply running the tooth alone. No one approach would work in all cases, but the correct intervention techniques will be selected based on the particular requirements of the infant.[2]

The wide used non-pharmacological behavior modification method include Tell-Show-Do, Non-Verbal communication, enhancing control, voice Training, modeling, Distraction, Positive Reinforcement, Hand Over mouth movement and defensive stabilization, while these traditional strategies include successful in directing children,the invasiveness inherent with these method reduces the acceptability that leads to them in turn.[3]

Material and Method

This review article aims to expand on current advances in current advances in non-pharmacological behavioral treatment approaches in adolescents. A search was performed on the Pubmed online database using the following terms: behavior and children, child behavior, behavior management and pediatric dentistry, child behavior and Dental anxiety, behavioral therapy in dentistry, non-pharmacological clinical treatment

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and pediatric dentistry, infant and adolescent dentistry, clinical intervention and babies, augmented reality and dentistry, children's audiovisual entertainment, computer games and education, mobile gaming and virtual reality.[4]

Background: The aims are to create contact, to minimize apprehension and anxiety, to provide quality dental treatment, to develop a trusted relationship between dentist/staff and child/parent, and to foster a positive attitude of the child towards oral health care. (AAPD Reference Manual, 2011). The result of these strategies may preserve good coordination or bring an end to aggressive actions related to dental care. Behavior guidance should never be punished for misbehavior, power assertion, or use of any strategy that hurts, shames, or belittles a patient. Recently, innovations have been paired with therapeutic strategies to strengthen coordination and promote healthy dental behavior in a stress-free manner.[5]

Recent Behavior Management Technique:

A. Audiovisual Distraction: Audio-visual stimulation incorporates the idea of visualization and relaxation provided by audio-visual aids, thereby removing the emphasis on dental operations, preventing anxiety-provoking sensations and offering calming interaction during the procedure. The aims of audiovisual diversion are creativity (helps to divert children from the current situation), commitment (allows children to concentrate on one thing) and inspiration (helps children to seek care for dental disorders in the future).

I. Management of dental anxiety: Amal Al Khotani performed a study to assess the efficacy of watching videotaped cartoons through an eyeglass device as an audiovisual (AV) distraction technique for aggression and distress in children undergoing dental restorative care and found out that AV stimulation tends to be an efficient way to minimize apprehension and distress in children during dental care and also more favorable responses to injection of local anesthesia. Kaur R et al 2015 compared audio and audiovisual diversion to mitigate anxiety in children during their first dental appointment and found audiovisual relaxation decreases anxiety in nervous dental patients.[6]

II. Management of pain: Attar RH 2015 did a study on Comparative effects of active and passive diversion during vital pulp therapy of children using iPad vs audiovisual eyeglass and found out that its Effective on minimizing pain and behavior with an iPad displayed greater results than passive interference utilizing AV glasses. Oliveira NCAC et al 2016 found that audiovisual diversion could minimize pain severity during painful puncture procedures. Nuvvula S et al 2015 used three-dimensional audiovisual stimulation to relieve anxiety during local anesthesia administration.

B. Video game distraction: While a wide variety of behavioral intervention strategies are available to treat extremely distressed infants, it was difficult to redirect the child's attention during pain experience in intrusive procedures. The use of videogame as a coping device is based on the concepts of cognitive-behavioral therapy and neurofeedback strategies for children with anxiety disorders. Videogames are exciting and widely accessible resources that can help to promote diversion in children by active child involvement in dental procedures.

Used in the Following:

(a) Ko JS et al 2016 used Ipads to minimize anxiety in children during their orthopedic appointments. Sil et al 2013 and Wohlheiter KA et al 2013 used videogames to minimize pain tolerance during cold-pressure tests. Videogames may be an important distractor and improve oral health-related outcomes, but comprehensive trials are required in the field of pediatric dentistry.

(b) Aljafari A et al 2017 Used Oral health awareness-based video games to encourage safe eating and good oral hygiene for children at high risk of caries.[7]

C. Virtual reality-based distraction: In 1968, Ivan Sutherland and Bob Sproull invented augmented reality using a head-mounted system attached to a monitor. Earlier in 1998, Heim described virtual reality as an immersive computer-based platform that can be used to immerse children in a simulated world that fully obstructs the actual situation. The VR equipment includes a head-mounted monitor and a tracking device. The head-mounted unit includes a projection screen that shows the virtual reality world in a 3600 view. The tracking device tracks the rotation of the eyes. Equipment includes a keyboard, joystick or computer gloves accessory for playing sports.

Uses of Virtual Reality:

I. **Clinical Procedures**: Researchers used the virtual reality experience for children undergoing surgical operations involving anesthesia and found that there was an increase in the perception of perceived discomfort to drugs, fear and decreased levels of tension in children and parents. In 2020 Custodio NB did a study on the usage of virtual reality glass which is an important method for enhancing actions and the experience of pain during dental care of children. Children who used VR eyeglasses performed differently during caries removal and had a lower sense of pain during reconstruction. In 2011, Hoffman et al found that virtual reality decreased pain sensitivity in children by decreasing pain-related brain activity, however further research on VR as a method to train patients for dental care is needed due to the shortage of studies in this field.[8]

II. **For Distraction:** The augmented reality experience provides the potential to remove the child's mind from the emotions associated with therapy and to provide immersive entertainment in a simulated world. Factors that affect stimulation when utilizing a virtual reality system are the degree of curiosity displayed when the child and the extent of the child's absorption in the virtual environment.[9]

Contra indications for Virtual reality:

(a) Medically impaired children, particularly children with epilepsy, migraine and vestibular disturbances

(b) Children with a prior history of nausea or dizziness following use of VR.

D. Tell-show-do and Mobile dental app: In 2019, Elicheria SR performed a comparative appraisal review on the effectiveness of the mobile app and Tell-Show-Do methodology utilizing heart rate scales and the RMS scale and found that educating a child before a dental operation utilizing a smartphone application would dramatically minimize anticipatory distress and involve children in dental care during their first visit.[10]

Conclusion

Recent technology such as audiovisual aids, video games, smartphone devices and augmented reality can be used as an alternative to traditional method due to its immersive, exciting and creative capacity to handle children with behavioral difficulties and to enable dentists to administer successful care in a stress-free atmosphere.

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Pit and Fissure Sealant: A Review

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Abstract

In recent times, carious infection of the tooth is considered as a major worldwide concern, significantly impacting the health, safety and quality of life of the patients affected by it. The required routine oral hygiene procedure along with fluoride use ensures prevention against dental caries. Apart from these, another important prophylactic measure can be followed against the caries by sealing the pit and fissures of a tooth that is more prone to caries occurrence. The main purpose of this article is to present a general overview of the sealing of pits and fissures, materials typically used to seal the occlusal surfaces along with the implications and side effects. This article also presents a detailed review of safe, effective use and cost-effectiveness of pit and fissure sealants as a preventive measure for caries. The finding drawn from this is that primary, as well as permanent teeth, sealed pit and fissures are an efficient and appropriate approach for preventing and arresting dental caries. But conducting a proper regular dental check-up is necessary to ensure that there is no advanced tooth decay caused due to any leakage in the sealing.

Keywords: *Dental caries; Pit and Fissure sealants; Prophylactic therapy; Regular checkups.*

Introduction

A change in the composition of the bacterial biofilm causes dental caries which is a multifactorial infection. This change leads to an imbalance between the demineralization and remineralization process which gradually becomes the cause for the formation of a carious lesion in primary and permanent dentition. The instances of dental caries expanded the most in the nineteenth and twentieth century because of more and simple accessibility of sugar for the populace.[1] The availability and use of sugar became popular among the population of developed countries mainly. There has been a long and creative part of the dental battle against pit and fissure caries which includes innovations in the preventive measured like early physical blocking of pit and fissures with the help of zinc phosphate cement, eradication of fissures mechanically and chemically treated with silver nitrate. These preventive measures continued with the testing of new materials, methodology and technologies each year. Soon the technique of acid etch bonding to the surface of enamel came to limelight and a major step in its use was to prevent pit and fissure decays. Therefore the prevention for dental caries then focused on pit and fissure caries because tooth with pit and fissures is more prone and vulnerable to caries occurrence. These sights are most convenient for the early occurrence of dental caries. In a recent study, the cariesin the smooth surface showed a very notable decrease in the occurrence which might be an outcome due to the expanded utilization of fluoride.[2]

Fluoride is not as effective in the pit and fissures as on a smooth surface because the fluoride must follow up on the nearby level which isn't mechanically convenient with pit and fissure of the tooth. After all, the dental plague develops without any disturbance in the pit and fissure of a tooth due to which the enamel gets dissolved by the repeated acid attacks.[3] In children, the natural cleansing mechanism of oral cavity by lips, cheeks, and majorly by the tongue during biting of food and gulping is missing for which the occlusal surfaces of developing tooth is mainly affected due to absence of cleaning. In result to that, the bacteria and residual food products gather up in the pit and fissure that forms a plaque and then gradually results in demineralization and caries ultimately. Therefore a recent study showed that the use of sealants can be utilized in the pits along with fissures as a method of preventive intervention for dental caries in children during tooth eruption.[4]

Pit and Fissure Sealant Material: The terminology of pit and fissure sealants are mainly used to depict a material that forms a micromechanical bond, protective layer against the caries-producing bacteria when brought into the occlusal pit as well as fissures of caries prone tooth. Pit and fissure sealants are divided into 3 sealant material.[5]

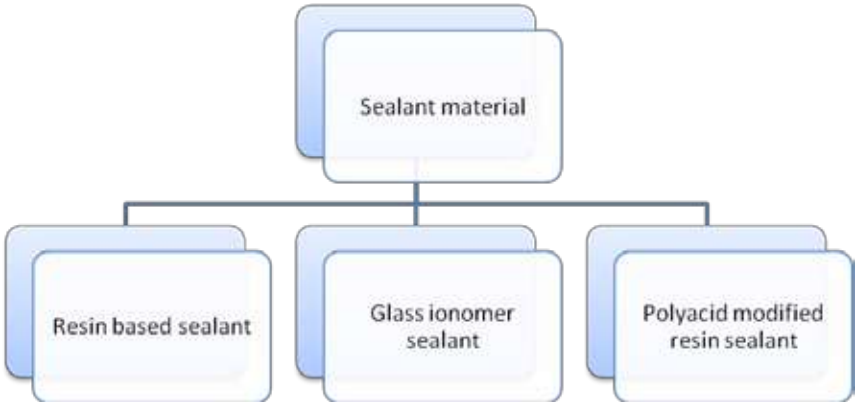


Figure 1. Classification of sealant material

The three major types of sealants are described as below:

1. Resin-based sealants (RBSs)

Determined by the method of polymerization, Resin-based sealants are classified into

- **1st gen:** The initiators in the materials that initiate polymerization are polymerized by the activity of UV rays. This sort is not, at this point being used.An example of 1st gen Resin-based sealants is Nuva-seal which was first introduced sealant to the market.
- **2nd gen:** They are known as the auto polymerizing resin-based sealants. Also sometimes called as chemically cured sealants. However this autopolymerizing resin-based sealants have now been supplanted by the 3rd era of RBSs.
- **3rd gen:** This sort involves visible light polymerizing resin-based sealants. Here, the visible light activates the photoinitiator which is available in the material of the sealant. This is very responsive to visible light in the frequency of about 470nm (blue area).[6]

Resin-based sealants can likewise be grouped by their consistency as (1) filled (2) unfilled. The addition of filler particle on sealants has fewer changes in clinical results. The filled type of sealant usually has high wear resistance but their capacity to enter into the pits and fissures is less. Whereas, unfilled type of sealant has low viscosity but provide proper penetration into pits and fissures resulting in better retention. Based on translucency, sealant materials can also be divided as opaque and transparent. Opaque are white or tooth-colored sealants whereas transparent sealants are seen as clear, pink or amber. White opaque pit and fissure sealants have better visibility during application and to recognize clinically during routine checkups and follow up as compared to clear sealants.[7]

2. GI sealant material: Traditional glass ionomer materials are utilized as pit and fissure sealants. Through an acid-base responsein between an aqueous-based polyacrylic acid solution and fluoroalluminosillicate glass powder, it forms a chemical bonding to enamel and dentin. This type of sealant is further grouped as (1) low viscosity type (2) high viscosity type. There is also a variety called resin-modified glass ionomer (RMGIC) which has been utilized as pit and fissure sealants. It is formed when the resin is mixed with glass ionomer cement. Continuous fluoride release is the main asset of GI cement-based sealant. Its preventive impact may also last after the noticeable loss of the material of the sealant. This material has an easy application method and is moisture friendly. Glass ionomer based sealants are mainly indicated in deep fissure primary molar which is hard to isolate because of the uncontrollable behavior of a child.[8]

Polyacid modified resin-based sealant: Otherwise called compomer, polyacid modified resin-based sealants is used as a material for sealing pit and fissures which joins the profitable properties of the visible light polymerized resin-based sealant which includes better adhesion property to enamel and dentin with the fluoride discharging property of the glass ionomer based sealants.[9]

Clinical Technique of Sealant Placement:

1. **Cleaning and preparation of tooth:** There have been many studies discussing various techniques of enamel surface preparation preceding the acid etching and sealant use. The early application method for pit and fissure sealant includes cleaning of the tooth surface that is treated with pumice and water slurry with a rotating brush which can be pointed or flat ended. Before carrying out the procedure it is mandatory to remove plaque and debris from the enamel and the pits and fissures. Interference with etching technique and sealant penetration into the pits and fissures will occur if the debris is not removed properly from the enamel surface. The polishing of the tooth surface can be done by using the prophylaxis cup and pumice.
2. **Isolation and drying of the tooth surface:** Prevention of salivary contamination during sealant application is considered to be the most significant and critical step in sealant application. The surface enamel that is etched can be contaminated if it is exposed to salivary proteins even for a split second. Hence a proper isolation of the tooth is necessary. The utilization of a barrier that is a rubber dam is considered to be the best method to accomplish isolation.[10]
3. **Acid etching:** Acid etching is the procedure in which the monomer penetrates the micropores which are formed by etching. It also removes organic materials and debris from the surface. The degree of etching depends on mainly the concentration of acid and duration of exposure. Orthophosphoric acid is normally used in liquid form using a hairbrush for about 30-60 seconds.
4. **Rinsing, drying, and isolating the tooth:** 30 seconds of rinsing along with 15 seconds for drying the tooth is convenient to remove all the acid etchants which help in achieving the result of characteristics chalky white frosty appearance. Isolation is mandatory in this stage because salivary contamination at the stage is the major common cause of failure of sealant application.
5. **Application of material:** After following the above steps and drying the tooth thoroughly, the particular sealant material is then placed on the tooth and is polymerized. Proper care should be taken for the prevention of air bubbles in the material while applying it to the tooth.
6. **Sealant evaluation:** After polymerizing and before the removal of the rubber dam, the sealant should be examined properly by the operator for any deformity like voids, bubbles, or deficiencies in the material. The retention of the sealant ought to likewise be evaluated by the help of an explorer in a trial to dislodge it. Excess of sealant should be removed over the distal margin that may result in the formation of the ledge. Occlusal high points are also checked and if present is corrected.[11]
7. **Periodic maintenance:** Re-evaluation of the sealants is mandatory at recall visits. If any loss in the sealant material seen, a new fresh material can be applied over the old sealant.

Sealing of Primary Tooth: The primary teeth are more prone to carious activity to anatomy and lack of natural cleansing factor. Therefore primary teeth benefit the most from sealant application. If the tooth has deep fissures along with signs of decalcification, pit and fissure sealants are indicated. In the case of caries or restoration of adjacent teeth, sealants are also indicated. Children with medical, physical, and psychological impairments are advised to go for sealant application. For simplification of the clinical procedure to make it progressively worthy to small kids, the etching time is shortened to lessen the opportunity of moisture contact. The procedure time can also be shortened by using a self-etching bonding agent as an option in contrast to the regular acid etching method.[12]

Follow Up: There is a chance of loss of sealant from the molar tooth. Therefore regular maintenance of sealant is necessary to boost the efficiency, maintenance of peripheral integrity, and to provide the necessary protection. By visual method, tactile method and radiographically, the retention and durability of the sealants can be checked.[13-15]

Cost-Effectiveness of Dental Sealants: The most effective intervention which is also cost-effective is sealant application. Application of sealants on primary

or permanent tooth especially the molars lessens the much higher expense by the prevention of increasingly costly treatment. The sealants are mainly indicated and seem to be more cost-effective when utilized in children with a higher risk of caries on teeth that are more prone to bacterial invasion. Therefore it is highly prescribed that sealants ought to be applied specifically relying upon the caries risk of a youngster along with anatomy of the fissure. The cost-effectiveness of the sealants can be more enhanced by using trained dental professionals for sealant application, use of sealant material in which the rate of retention is higher and application of a sealant on the tooth specifically which are more prone to caries.[16]

New Developments: From this review, it is proven that sealants are safe and effective preventive material for caries. However, certain improvements can be made to the material and technique of application. Mechanical preparation of the fissure surface can be beneficial for better retention and microleakage. Shorter etching time is found to be equally effective for longer times. Similarly, the use of a sodium bicarbonate air polishing system in the cleaning method helps in higher retention of sealant.[17,18]

Conclusion

A powerful way to prevent pit as well as fissure caries in primary and permanent tooth is the use of pit and fissure sealants. Therefore, in patients with higher risk of caries, dental professionals ought to be urged to usepit and fissure sealants alongside other prophylactic measures. The tooth with early non cavitated carious lesion additionally gets benefit from utilization of sealant which prevents any progression of caries. The sealant application is a technique sensitive method which requires a moisture-controlled environment. The material for the sealant is selected based on the age of the patient, behavior of the child, and the tooth eruption time. Lastly, maintenance is essential to boost the adequacy of the treatment.

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Swellings of the Palate: A Review

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Abstract

The palate which forms the roof of the mouth can be divided anatomically into hard and soft palates. These areas are actively involved with feeding and speech. The posterior part of the hard palate consisting of the minor salivary glands is a common site for palatal swellings. However, these are quite challenging for the clinician to diagnose and because of the importance of the oral cavity in terms of both function and cosmetics, management of such swellings requires a thorough understanding of the lesion and its progression. This article reviews the various palatal swellings in terms of their etiologies, clinical features, diagnosis, and management.

Keywords: *Mouth, Soft Palate, Hard Palate, Minor Salivary Glands, Speech.*

Introduction

The palatal anatomy comprising of the hard and soft palate is actively involved with feeding and speech. The hard palate consisting of the minor salivary glands is a common site for palatal swellings.[1]

The palatal swellings may originate from various structures within or beyond the palate and have different etiologies. They are painful when infected or painless when benign. The congenital swellings are associated with unerupted teeth and torus palatinus. The acquired conditions would include – salivary gland neoplasms, dental abscesses, epithelial and connective tissue neoplasms, and fibro-osseous lesions. These swellings are examined clinically by inspection and palpation. Further radiographic aids using panoramic radiographs and histological examination with a biopsy helps to arrive at a specific diagnosis.[2]

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A review of the various palatal swellings has been discussed here based on the classification of palatal swellings as given in the table below:

Palatal torus (Plural–Palatal Tori): It is an overgrowth of bone that is seen on the palatal midline on the cruciform suture that joins palatal and maxillary bones.

Etiology: Idiopathic and multifactorial with the involvement of both genetic and environmental factors such as masticatory hyperactivity and parafunction.

Clinical features: Palatal Tori is asymptomatic, unless secondarily infected and is generally discovered during routine clinical examination. It is more common than the mandibular tori with a predilection for females than males in the age group of 10 – 30 years. The shapes may vary between flat, nodular, fusiform, or lobular with a size range of 2–6 mm. The most common, however, is flat shaped one with a smooth and symmetrical distribution. The overlying mucosa is thin and less vascularized.[2]

Radiographic features: For radiographic identification, CT is the preferred choice. Radiographically, it appears denser with limited narrow space & less osteoblastic activity. Cloudyradiopacities can also be seen superimposed on teeth contour.

Treatment: Palatal tori is a benign maxillary bone tumor which is slow-growing & painless. Thus, it can be left as it is. In case of problems such as ulceration during mastication, inadequate maintenance of oral hygiene due to food accumulation, psychological fear of malignant transformation, and difficulty in speech, surgery can be opted followed by prosthesis.

Table 1: Classification of palatal swellings

Etiology	Associated swelling
1. Developmental	Palatal Torus
2. Inflammatory/Reactionary	1. Palatal abscess 2. Inflammatory papillary hyperplasia 3. Fibroepithelial polyp 4. Pyogenic Granuloma 5. Adenomatous Hyperplasia 6. Atypical lymphoproliferative disorder/Follicular lymphoid hyperplasia
3. Cyst	1. Radicular Cyst (Periapical cyst) 2. Dentigerous cyst 3. Median anterior maxillary cyst (Nasopalatine cyst) 4. Median palatal cyst 5. Incisive canal cyst 6. OKC
4. Neoplasms	**a. Salivary gland** 1. Pleomorphic Adenoma 2. Adenoid Cystic Carcinoma 3. Mucoepidermoid carcinoma 4. Polymorphous Low-Grade Adenocarcinoma 5. Carcinoma ex-pleomorphic adenoma 6. Necrotizing sialometaplasia **b. Odontogenic** 1. Ameloblastoma 2. Adenomatoid odontogenic tumour 3. Odontogenic myxoma **c. Others** 1. Fibrous Histiocytoma 2. Schwannoma 3. Neurofibroma 4. Lymphoma 5. Osteosarcoma 6. Chondrosarcoma 7. Fibrosarcoma

Palatal Abscess: These abscesses generally involve the palatal roots of the posterior teeth, being bounded by the cortical plates of hard palate inferiorly, periosteum of bone superiorly and alveolar process of maxilla and teeth laterally. They may occur because of periodontal abscesses in the palatal pockets or apical abscess in the palatal roots of posterior teeth.

Clinical Features: It appears as a well-circumscribed fluctuant or tense palatal swelling associated with pain, fever and poor oral hygiene. It is located in premolar – molar region, mostly laterally and rarely towards the midline of the palate which then becomes difficult to diagnose.

Treatment: Treatment involves incision and drainage through the mucosa of the bone. Hopeless teeth may require extraction followed by antibiotic therapy.

Inflammatory papillary hyperplasia (also Palatal epithelial hyperplasia, papillary hyperplasia of the palate, palatine papillomatosis)

It may occur due to – poor denture hygiene, frictional irritation due to ill-fitting dentures, suction chamber in palatal seating, amalgam filling material, and *Candida albicans*.

Clinical features: It does not have any predilection for age or sex, appearing exclusively on palatal mucosa under dentures with a warty appearance. They are small, painless, papular, or polyploid masses which can be red and soft (inflammatory stage) or firm and pale pink (fibroid), thus giving an 'overripe berry appearance' or 'cobblestone appearance'.

Treatment: This involves – removal of dentures at night to rest tissues or use of tissue liners; and surgical removal by curettage, electro/cryosurgery and microabrasion in fibrosed cases.

Pyogenic granuloma[6,7,8]: (Also granuloma pyogenium)

It is the most common benign growth, first described by Huliken in 1844. It is considered to be a clinical entity that resembles a tumor originating due to the response of tissues to minor trauma or any specific infection in which there is a proliferation of fibrovascular connective tissue. It is mostly caused by the microorganisms – Streptococci and Staphylococci during trauma to gingiva. Other causes include local irritants like calculus and hormonal changes.

Clinical Features: It usually occurs between the 2nd and 4th decade with a predilection for females. The most common site is gingiva, followed by the tongue, lips, buccal mucosa, and interdental papilla. The symptom includes nodular growth with a smooth, lobulated, or warty surface. The color varies from red to reddish-purple depending upon the vascularity of the lesion. It has a moderately soft consistency which bleeds readily. The Surface is mostly ulcerated with a discharge of pus sometimes.

Diagnosis: Clinical manifestation of a Reddish-purple lesion which is soft inconsistency. The laboratory diagnosis by biopsy would show the presence of localized granulation tissue with the overlying epithelium being thin, atrophic & hyperplastic.

Treatment: The adjacent tooth should be scaled properly to remove calculus as irritation might lead to recurrence.

Radicular cyst (Also special periodontal cyst, Dental root end cyst, Periapical cyst)

Radicular cyst is the most common inflammatory cyst of odontogenic origin which is a common sequela of bacterial invasion and death of the pulp. It can be defined as "Slowly growing epithelial sac at the apex of a tooth lining a pathologic cavity in alveolar bone whose lumen is filled with a low concentration of proteinaceous fluid".[9]

Clinical Features: It occurs during the 3rd – 4th decade of life with a predilection for males. It is more common in the maxillary anterior teeth due to the occurrence of caries and trauma. The patient reports with a non-vital tooth which is non-tender on percussion and has no response to electrical/thermal stimuli. There is a previous history of pain. The signs include – non-tender expansion of cortical bone in curved/circular shape with the presence of intra-oral & extra-oral swelling where consistency is bony hard with crepitations. The adjacent tooth might be displaced due to pressure from the cyst and become mobile.

Radiographic Features: Appearance of a unilocular rounded/pear-shaped radiolucency at the apex of the affected tooth on the mesial/distal root surface at the opening of an accessory canal about 1.5 – 3 cm in diameter with smooth well-defined borders.

Treatment: Root canal treatmentis the treatment of choice to establish drainage which subsides the inflammation and fibroblast starts producing collagen again which decreases the blood supply to epithelial cells, ultimately getting degenerated and removed by macrophages. Other options include – Extraction of hopeless tooth or enucleation and marsupialization of large lesions.

Dentigerous cyst (Also known as Follicular cyst, pericoronal cyst)

It is the most common type of odontogenic cyst enclosing the crown of the unerupted teeth. Discovered by intra-oral radiograph because of a missing tooth or tilted tooth or tooth out of alignment. Its origin can be traced to fluid accumulation in the Reduced Enamel Epithelium. This causes the proliferation & cystic transformation of epithelial islands in the connective

tissue wall of the dental follicle.

Clinical Features: It is most common in children and adolescents in the 2nd and 3rd decade with a predilection for males. The most commonly involved sites are mandibular 3rd molar & maxillary canines. It is usually asymptomatic unless secondarily infected. The cyst may expand to compress sensory nerves resulting in referred pain which may be perceived as headache. There is progressive painless enlargement of jaws with facial asymmetry. Displacement of teeth and pathologic fracture might occur.[11]

Radiographic Features: A mostly unilocular well-defined radiolucency around the unerupted tooth with hyperostotic well-defined borders unless infected.

Treatment:

i. Surgical treatment:
   a. If small, it should be removed.
   b. If large, surgical drainage should be done followed by marsupialization.
ii. Orthodontic treatment should be done to keep the tooth in place.

Odontogenic Keratocyst (OKC): OKC can be defined as "a cyst derived from the remnants of the dental lamina with a biologic behavior similar to a benign neoplasm with a distinctive lining of 6 – 10 cells in thickness that exhibits a basal cell layer of palisaded cells & a surface of corrugated parakeratin". It was first described by Mikulicz in 1876.

Clinical Features: This is more common in males between 10 – 20 years of age with a predilection for blacks and mandible with its ramus and body amongst sites. The common symptoms are pain, swelling in antero-posterior detection, soft tissue drainage, and at times paresthesia of lower lip. There may be chances of downward displacement of the alveolar canal along with teeth being deflected into ascending ramus or orbital floor.[12]

Radiological Findings: Radiolucency is mostly unilocular with a scalloped border. Multilocular radiolucency representing a central cavity with satellite cysts can also be present.

Management:

i. Enucleation – followed by vigorous curettage of the cystic cavity is the most satisfying method of removal of cysts without damaging any adjacent structure.
ii. Marsupialization – indicated in the case of large cysts.
iii. Peripheral osteotomy – is done to reduce recurrence.
iv. Chemical cauterization – with Carnoy's solution.
v. Decompression with the help of polyethylene drainage tube kept in the bony cavity.

OKC has a high recurrence rate because of – the presence of satellite cysts, difficulty in the enucleation of thin and friable lining, intrinsic growth potential, and basal cell proliferation.

Median anterior maxillary cyst (Also nasopalatine duct cyst, nasopalatine canal cyst, and vestigial cyst)

Its etiology can be – developmental, due to trauma, bacterial infection, mucous retention, or genetic factors. The nasopalatine duct degenerates progressively during development and the presence of epithelial remnants give rise to nasopalatine duct cyst.

Clinical Features: It is among the most common developmental, non-odontogenic cysts with a 3:1 male predominance and affecting people between 40 – 60 years of age. It is located specifically in the midline of the anterior maxilla and appears as a swelling associated with pain, discharge, and salty taste in the mouth. It may perforate the palatal bony plate and cause tooth displacement. It is often associated with burning sensation and numbness as the nasopalatine nerve is affected.

Radiographic Features: A heart-shaped radiolucency can be observed superimposed over apices of the central incisors along with erosion of bone posterior to the canal.

Treatment: Enucleation through a palatal or buccal approach is advised unless there are clinical symptoms.

Median palatal cyst: It can be described as a posterior extension of the incisive canal cyst which arises due to entrapment of epithelium along the line of fusion of the palatal process of the maxilla.

Clinical Features: Its incidence is rare and without any sexual predilection, mostly occurring in adults. It is situated along the midline of the palate to produce an

asymptomatic but definite clinically visible swelling which on aspiration gives an amber-colored fluid.

Radiographic features: It shows a well-circumscribed radiolucent area opposite to bicuspid, molar region, and bordered by sclerotic bony layer.

Treatment: Treatment involves surgical removal of the cyst followed by curettage.

Pleomorphic adenoma (Also Iceberg tumor, Endothelioma, Branchioma, Endochondroma)

It is benign and the most common salivary gland tumor. It was named pleomorphic because of the unusual mixed histologic feature of the lesion. The genetic etiology is a putative pleomorphic adenoma gene (PLAG1) on chromosomes 12.

Clinical Features: It occurs between 30 – 50 years of age with a 3:2 predilection for females. The lower side of the superficial lobe of the parotid gland is the most common site. The palate is involved if minor salivary glands are affected.

The tumor starts as a round/oval painless nodule which slowly increases in size. When the lesion grows medially between ascending ramus and stylomandibular ligament, it acquires a dumb-bell shape. A palatal tumor is generally dome-shaped with a smooth surface found on the lateral aspect of the palate. Sialography provides a ball in hand appearance.[13]

Management: Surgical excision of the tumor along with the involved salivary gland or the overlying mucosa in case of palatal involvement with a 5 – 30% recurrence.[14]

Necrotizing Sialometaplasia: (Also called salivary gland infarction)

First reported by Abrams in 1973, it is a benign, inflammatory, self-healing reaction of salivary gland tissues which manifests itself as a swelling with or without ulceration which both clinically and histologically mimics a salivary gland malignancy. Trauma leading to vascular ischemia, ill-fitting dentures, tobacco intake or spontaneous are some of the etiologies for the disease.

Clinical features: The disease is more prevalent in the 4th and 5th decade of life with a predilection for males. The most common sites are palate, lip, retromolar pad, buccal mucosa and labial mucosa. It begins as a mild swelling which is painless, causing numbness or an area of looseness in palate from where pieces of tissues might fall. It commonly occurs unilaterally, but may also occur bilaterally or in the midline with clean, sharp and indurated margins. The lesion is covered by inflammatory exudate and necrotic debris.

Treatment: It is a self-limiting disease that does not require any treatment with periodic evaluation of the affected site.

Ameloblastoma (also Ademantinoma epithelium, adamantinoblastoma, epithelial odontoma, multilocular cyst)

Robinson defined ameloblastoma as "Benign tumor that is usually unicentric non – functional, intermitted in growth, anatomically benign and clinically persistent". It is the 2nd most common odontogenic neoplasm. It originates from – cells of the enamel organ, cell rests of Malassez, Hertwig's epithelial rest sheath, and epithelium of odontogenic cysts. The etiologies include – irritation, infection, trauma, dietary deficiencies and virus.

Clinical features: It accounts for 11% of all odontogenic tumors, occurring between 20 – 50 years of age with a predilection for males.

Maxillary ameloblastoma spreads more rapidly due to porous bone. Patients report with gross facial asymmetry, pain, paraesthesia and tooth mobility. Eggshell cracking of surrounding thin bone occurs on palpation.[15,16]

Radiologic Features: It appears as a multilocular bony septa described as 'Honeycomb appearance/Soap bubble appearance'.

Treatment: Radicular and conservative surgical excision in combination with Radiotherapy is usually recommended. Chemical or electrocautery can also be preferred.

Lymphoma: It is a neoplastic proliferation within the reticuloendothelial system occurring as a primary tumor of lymph nodes, but not as common as a metastatic tumor. It can be – Hodgkin's lymphoma and Non-Hodgkin's lymphoma.

Clinical Features: Non-Hodgkin's lymphoma is more common, occurring in children and young adults. It appears as firm, elastic swelling with or without

ulceration in gingiva and palate. It may be present unilaterally or bilaterally as a rubbery mass with painful and tender lymph nodes, mostly occurring in the palate. The risk is higher in patients with HIV.

Treatment: Radiotherapy or chemotherapy are the treatments of choice.

Conclusion

Various palatal swellings may have similar clinical features. Thus, the clinician needs to have a thorough knowledge of each of them and alongside make use of other diagnostic aids judiciously to zero in on a specific diagnosis and render appropriate treatment for each of them.

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Glucocorticoids: A Boon or Bane in Dentistry

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Abstract

The people who had undergone or are undergoing glucocorticoid therapy present distinctive issues for dental surgeons. The people receiving the steroid therapy have their responses altered to infection and injury assuage. More importantly, the patients who are taking or have taken glucocorticoids have a possibility of suffering from acute-adrenal insufficiency when they are introduced to a stressful situation. When a patient with a history of glucocorticoid therapy is under treatment, actual details on the medical history must be obtained. The dentist must regulate the requirement for the ensuing preventive measures: a) Systemic antibiotic therapy, b) anxiety control, c) increased administration of glucocorticoids.

Keywords: *glucocorticoid therapy, acute-adrenal insufficiency, prophylactic measures, administration of glucocorticoids.*

Introduction

Glucocorticoids are powerful medicines that work with our immune system and fight against the inflammation to treat the various health problems. Although corticosteroids were used in the field of medicine since 1944, it was not until 1951 that the field of dentistry began to use them. Corticosteroid is one of the anti-inflammatory drugs that has been listed in Dental' Practitioners' Formulary.[1] These have been used for managing the inflammatory diseases for the last 50 years. Glucocorticoids or steroids are the substances that are naturally synthesized in our body. The se are one of the most widely prescribed drugs in both the dental and medical fields. Dental patients with a history of corticosteroid therapy may require special consideration and brief medical information before receiving any treatment. The current misuse of the steroids is that it is overly prescribed, even before minor dental procedures. Glucocorticoids or corticosteroids have revolutionized the treatment of several disabling conditions, but its use in terms of the dosage is not appropriate.[2]

Glucocorticoids-A Brief Idea: Glucocorticoids are derived from "glucose+ cortex+ steroid" which reflects their i) role in the regulation of glucose metabolism ii) adrenal cortex origin iii) steroidal structure. Glucocorticoids area class of corticosteroids, which are a class of steroid hormones. The se are a kind of corticosteroid hormone that shows their effects in the reduction of the inflammation and annihilation of the immune system. Due to inflammation, our immune system acknowledges intruding substances and trauma and hence is an important part of our alleviating process. They include corticosteroid hormones that are synthesized naturally in the adrenal cortex and are comparable to those produced in laboratories.[3] These have been used broadly and are successful in treating many oral diseases, due to outstanding anti-inflammation and immune-modulation effects. However, if the habitual control systems that are in charge of switching off the action of the inflammation do not work, then the tissues are in danger of being destroyed. The continuation of the inflammation is seen to be in association with several chronic conditions which include obesity, diabetes, depression, cancer and lastly heart diseases. Hence, they are sometimes termed as "double-edged sword" due to their potential side-effects. Glucocorticoids include an applicable diagnosis, a crystal view of the desirable

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management, and the understanding of whether the treatment is pointed at a chronic illness or a heightened resolution of a small-term issue. The positive effects must be considered concerning the probable risks. [4]

All the glucocorticoids have a similar structure but differ in their doses (potency). 5 mg of prednisolone is considered to be equivalent to 4 mg of triamcinolone and 0.75 mg of betamethaone. High doses of prednisolone most likely cause salt and water confinement and an increase in the elimination of potassium. Its adverse effect is more than that seen in the intake of increased doses of triamcinolone, dexamethasone or betamethasone. The standard prescriptions and doses of prednisone, triamcinolone, and methylprednisolone last from 12-36 hours in the body while dexamethasone and while betamethasone lasts from 32-72 hours.Prednisone is considered one of the most popular glucocorticoids due to its inexpensiveness. It is transformed into prednisolone in the liver and it is available only through oral route.[5,6]

Use of glucocorticoids in dentistry: Among the various specialties of dentistry, oral and maxillofacial surgery, oral medicine, and endodontics are the most avid users of glucocorticoids. Most of the glucocorticoids used in dentistry are mostly prescribed for topical application for a short term use.[7]

Endodontics: The effects are seen in the reduction of the inflammation of the pulp as well as root resorption. Zinc oxide eugenol along with steroids helps as an effective root canal sealer. It is also used to reduce postoperative thermal sensitivity.[8]

Pain following root canal treatment: One of the main reasons that people avoid going to the dental offices is anxiety and fear of getting the root canal treatment done. Hence, managing pain during the treatment and after it, is very important. The pain following the root canal treatment is undesirable, for the patient as well as the dental practitioner. It has been investigated that there are several reasons for the occurrence of pain after root canal treatment. It is a multi-factorial phenomenon, including mechanical, chemical, host and microbiological factors. Besides, the pain before treatment could be due to demographic factors such as age and gender. The post-operative pulp status and the type of teeth also influence the pain. Investigations have shown glucocorticoids can be used for pain management following root canal treatment.[9,10]

Orthodontics: Patients need to be reviewed and asked for their previous history of steroids use since it has appearedthat upon the treatment with hydrocortisone, teeth show a lower amount of tooth movement.

Oral Surgery: It is used to limit the postoperative inflammation after oral surgical procedures. It is also reported that steroids help to prevent ulceration and excoriation, which occurs during retraction during surgery over the lips and corners of the mouth.

Oral Medicine: Topical glucocorticoids have proven to be very effective in the treatment of a variety of oral mucosal lesions which includes oral ulcerations and manifestations of the auto-immune diseases of the body. In oral medicine, corticosteroid injections are an integral part of the management of tempero-mandibular joint degeneration. Glucocorticoids are an important part in the treatment of various diseases as summarized below:-

OSMF: Topical application over the painful or ulcerative mucosa shows a direct healing action on the mucosal patch due to its anti-inflammatory property.[11]

Oral Lichen Planus: Clobetasol propionate with nystatin in orabase -0.05% 3-4 times per day appears to be effective in controlling lichen planus.

Erythema Multiforme: Prednisolone is the drug of choice for erythema multiforme.

Pemphigus: Prednisolone or topical and systemic steroid combination-betamethasone is the drug of choice for pemphigus.

Desquamative Gingivitis: The drug of choice is triamcinolone and fluocinonide.

Oral Submucous Fibrosis: A dose-dependent enhancement of fibroblast collagen phagocytosis is caused by corticosteroids. Hydrocortisone, betamethasone are the drugs of choice.

Melkersson Rosenthal Syndrome: Due to the anti-inflammatory action of steroids, it is used to reduce persistent edema and swelling. Prednisolone is the drug of choice.

Bell's Palsy: The drug of choice here is prednisolone 60-80 mg daily during the first few days.

Central Giant Cell Granuloma: For non-surgical treatment, intracellular corticosteroid injections are given and topically, triamcinolone acetonide can be given as it

works by suppressing an angiogenic component of the lesion.

These are some of the conditions and their management using glucocorticoids. There are innumerable diseases and conditions, for which steroids are very beneficial.[12]

Guidelines for dental use: Evidence shows that maximum people suffering from adrenal insufficiency can go for non-surgical regular dental treatment without the requirement for any glucocorticoid. The dental treatments do not encourage cortisol secretion as much as those of oral surgical procedures, and the local anesthesia blocks neural stress pathways that are needed for the production of adrenocorticotropic hormone secretion. For patients who undergo general anesthesia for minor surgery, then hydrocortisone of 100 mg should be administered intramuscularly and the usual glucocorticoid doses should be maintained. For any major surgery, 100 mg of hydrocortisone must be delivered as a tablet preoperatively, which is then followed by 50 mg 8-hourly for 48 hours.[13]

Adverseeffects: When the glucocorticoids are prescribed in an approved dosage for a small period, then the drugs are safe. Regular or large doses of the drug can cause some severe adverse effects. Many of the complications start with the dose and the duration of the therapy. Several of the complications occur in patients who are already under extensive therapy. So in patients like these the administration of steroids must be monitored.

If glucocorticoids are given regularly or daily then the adrenal cortex stops producing it and the tissues of the adrenal gland get atrophied. It becomes a concern when the drug therapy is suddenly stopped, because adrenal glands cannot immediately produce it again. This is known as acute adrenal insufficiency. The manifestations include nausea, irritation; join problems, vertigo, and low blood pressure. So to avert this from happening, the corticosteroid drugs have to be removed unhurriedly, over some time, like in weeks or months, to allow the adrenal cortex to restart its complete functional competency.[14]

For patients under long-term therapy, an additional amount of glucocorticoids must be given amid stressful times such as surgery or serious infection, similar to the cortisol rush usually produced in the system. Since these hormones subdue the immune system, the danger of infection increases. Certain viral infections like measles and chickenpox may be more serious in patients on steroid therapy.

An increased use of glucocorticoids can result in causing Cushing's syndrome whose manifestations include a protuberance between the shoulders, a round face, irregular menstrual cycles, and an increase in weight, depression, and weariness. Other effects include- Discomposure and irritation, Cataracts(or glaucoma), Vertigo, Blurred vision, Rise in facial hair in women, Difficulty in concentration, Retention in fluid, Headache, Irregular heartbeat, Increase in blood pressure, blood sugar levels, cholesterol, or triglycerides levels, Rise in the level of gastric ulcers or gastritis, Osteoporosis caused due to the body's inability to absorb calcium, Osteonecrosis, Reactivation of latent tuberculosis, Sleeplessness, Weight gain, breathlessness.

Minimizing the effects of steroid therapy: When under steroid therapy, probiotics play a very crucial role in minimizing the effects of candidiasis. The action of probiotics is in three ways. Firstly, it helps in inhibiting pathogenic enteric bacteria. Secondly, it improves the function of the epithelial and mucosal barrier by enhancing the mucus production, and hence enhancing the barrier integrity and the production of short-chain fatty acids Thirdly, it helps in altering the immune regulation by stimulating the secretor immunoglobulin production, hence decreasing the tumor necrosis factor expression, by the introduction of interleukin-10.[15]

Conclusion

The risks that are associated with glucocorticoids are parallel to the benefits of their therapeutic power. The benefits are many but the side effects are well known. Hence they are known as a double-edged sword. Their potential to cause many adverse effects presents the dental practitioner with the difficulty of the management of the patients undergoing steroid therapy. The drugs should not be used for trivial reasons and it should be carefully considered before each individual is administered these drugs. However if the dental practitioner maintains the proper guidelines of administration and care then the patients on glucocorticoid therapy have the highest benefits and the lowest possible risks. Hence, glucocorticoids are a boon in our profession and relief to our patients but when administered carefully.

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Microbes and Immunity: The Periodontal Battle Zone

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Abstract

Periodontal disease is known around the world as a significant health concern and a common cause of adult tooth loss. Periodontal diseases are common inflamed disorders such as gingivitis and periodontitis caused by the pathogens present in the biofilm which is seen subgingivally such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia* that activate inflammatory, innate and adaptive responses of the immune system. The immune system plays a vital role in preservation from pathogens with the maintenance of commensal microbiota at the mucosal surface. This article is focused on discussing the role of microbes and the basis and concept of immunology in periodontal disease in brief.

Keywords: *Periodontitis, Pathogenesis, Immunology.*

Introduction

A chronic inflammatory disease of the periodontium known as periodontitis is characterized by loss of periodontal ligament and destruction of surrounding alveolar bone.[1] There is ample evidence that the presence of microorganisms and persistent inflammation in periodontium give rise to the development and/or initiation of many systemic inflammatory diseases like diabetes, obesity, metabolic disease, rheumatoid arthritis, chronic kidney disease, etc.

Many of the key pathways leading to systemic inflammation are the direct or indirect effects on certain body sites of circulatory bacteria, mediators of inflammation and/or immune systems from infected/inflamed tissues of the periodontium.

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Bacteremia may be caused by oral bacteria entering the bloodstream via mechanical processes involving periodontal debridement, flossing, and brushing techniques. Products from bacteria especially LPS from gram-negative bacteria can also contribute to systemic inflammation. The response of inflammation by bacteria of the periodontium in the inflamedtissue of periodontium is an origin of recurrent persistent systemic inflammation. P.*gingivalis* is considered to be the only bacteria capable of inducing enzymatic peptide citrullination with subsequent production of autoantibodies of the anti-citrullinated peptide.

During periodontitis, pro-inflammatory mediators and biomarkers are found to be substantially higher in serum and gingival crevicular fluid as compared to the periodontally well individuals. When defending against infections, the immune system plays an essential function when preserving commensal microbiota on the mucosal surface. Innate immunity provides a first line of protection against infection that creates a homeostatic network capable of detecting infectious microorganisms that cause immune reactions to remove them. Along with innate immunity, adaptive immunity cells and characteristic cytokines play a vital role in the pathogenesis periodontal disease involving $CD4^+$ T-cells (T-helper cells.[2]

Factors in causation of periodontal disease: Diseases of periodontium are usually inflaming disorders such as gingivitis and periodontitis occurs due to pathogens present in the biofilm which is seen subgingivally.

Such bacteria cause the gingival epithelium to develop cytokines and chemokines, resulting in molecular adhesion, elevate permeability in capillaries of the gingiva, and chemotaxis of polymorphonuclear neutrophils via junctional epithelium to the gingival sulcus.[3] Further continuation of this process results in inflammation that extends into the tissues and results in loss of alveolar bone and reinforcing connective tissue and also causes initiation of periodontal pocket. The inflammatory mediators and cytokines are capable of stimulating the demolition of the periodontium andthe destruction of collagen via matrix metalloproteinases extracted from tissues.

Various and distinctive ecosystems that are present in the human body provide a unique environment for the colonization of microorganisms. Typically the periodontal disease involving *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis*, and *Tannerella forsythia*are associated with a scanty bacterial typespresent in the biofilm which is seen subgingivally.

According to a study report,in most patients and active sites, the red complex types *P.gingivalis* and *T.forsythia* were found to be elevated in populations of persistent inflammatory disorder of periodontium in Chile, Spain and Colombia.[4] *P.gingivalis* were more often observed in patients of Chile (83.8%) and less commonly in Colombians (65.9%). *T.forsythia* were found to be lower in Spain (36.1%) than in Chile (16.2%) and Colombia (39%). This study also reports that the active sites of chronic periodontitis in Chileans have higher mean percentages of total anaerobic*P. gingivalis* and no major variations for *T.forsythia* have been reported.

Research on Latin American populations show a high prevalence of T.forsythia and P.gingivalis in Chileans with chronic periodontitis. A high incidence of T.forsythia and P.gingivalis were recorded in Brazilians (45% and 68% respectively) and Mexicans (97.4% and 100% respectively) using DNA-based method. **Botero** etal,in Colombian patients with chronic periodontitis using microbiological culture, reported high-frequency T. forsythia and P. gingivalis (50% and 75.4% respectively).[5]

Actinomycetemcomitans in chronic periodontitis use microbiological culture and DNA samples to detect this pathogen in a lower ratio than *T.forsythia* and *P.gingivalis* in the Chilean population. Some populations with aggressive periodontitis have been found to have a higher rate of P.gingivalis as oppose to those of chronic disease of periodontium by using microbiological culture of periodontal pathogens.

For example- Chilean patients with chronic periodontitis have 76.4% frequency isolation of *P.gingivalis* compared to 86-100% in aggressive periodontitis.[6]

Besides, fimbriae is another clinical virulence factor that is the filamentous component on the surface of the cell, and its subunit protein fimbrillin (fimA) significantly performs on bacterial contact with tissues of the hosts by mediating adhesion of bacteria and target site colonization.[5] Several experiments have been conducted in the subjects of various disorders of periodontium at distinct geographical locations to evaluate the pervasiveness and dispersal of the fimA genotypes. Based on this finding, itcan be concluded that the type fimA genotypes are most common in patients of periodontitis whereas the second most prevalent genotypes were seen to type A, type B, or type Cdepending on the studied ethnic group. The subjects type A and type B fimA are more common in non-periodontitis cases.[7]

Moreover, a large molecule consisting of a lipid component (lipidA) and a polysaccharide component known as lipopolysaccharides which is a major component of the outer membrane of P.gingivalis and compares with most gram-ve bacteria.[6] Heterogeneous molecules from *P.gingivalis* lipid A induce an agonistic and antagonistic response of the innate immune system by one week. It is proposed that these mechanisms are critical for disrupting oral homeostasis and oral health.

Innate immunity in periodontal diseases: The response of the innate immunity system is a mechanism of homeostasis that provides the primary line of protection, and is alsocapable of recognizing invasion of pathogens as non-self, activating immune reactions to remove them. The responses of the innate immunity system are organized by a wide variety of chemokines, cytokines and receptors on the surface of the cell, and an inflammatory state created by innate immunity

stimulation. Adaptive immune strengthens the effector mechanisms of innate immune, providing a methodical loop for clearance of microbes, where activating actual innate systems guarantees a successful adaptive immune response that potentiates the action of these innate factors against periodontal bacteria.[8]

Polymorphonuclear neutrophils: Polymorphonuclear neutrophils after chemoattractants such as interleukin-8, connective fibroblasts, and immune cells are one of the first respondents of inflaming cells to move towards the site of inflammation of the periodontium.[7] Polymorphonuclear neutrophils are a characteristic of acute inflammation. Changes in their functions causeacute, serious, andgeneralized periodontitis in humans. Such modifications may include varying roles such as adhesion potential, reaction to chemotaxis and phagocytosisand thereby implication, the particular timing of the immune responses depends on polymorphonuclear neutrophils.

The cytoplasmic granules of polymorphonuclear neutrophils contain many enzymes that degenerate the structure of the cells of the tissues and the extracellular matrix when released. These are the brief-lived cells thatdemise in larger numbers at acute inflaming sites of periodontium due to interaction with pathogens and their toxins. The aggregation and huge death of polymorphonuclear neutrophils are the main causes of deterioration of tissue in developing disease of periodontium.[9]

Role of T-cell subsets and cytokines: In periodontal disease pathogenesis,the production of adaptive immunity mediated by T-cellis influenced by innate immune-associate cellspresenting antigen which, after antigen attack pass through a procedure of maturation and emigrate to the lymph nodes, where they generate different cytokine forms which cause successive polarization and activation of specific T $CD4^+$ lymphocytes.[7]

The $CD4^+$ T-cells are two subsets based on their cytokine development pattern, mainly T-helper 1 and T-helper 2. T-helper 1 cytokines are related to infectious inflammatory degradation of the bone, whereas T-helper 2 cytokines have been expressed to reduce loss of bone. The other regulatory subsets of T-cells are Tr1 and T-helper 3 which are identified respectively by the common development of transforming growth factor-b or interleukin-10. And additional subsets of T-helper just as T-helper 22 and T-helper 9 cells have played a vital role in the transition of inflammatory immune responses. T-helper 17 and T-regulatory cells together play a vital role in the organization ofresponses of the adaptive immunity and also have a significant impact on inflammatory and autoimmune diseases outcome.[9]

T- helper 1: T-helper 1 cells are produced under the interleukin-12/interleukin-1 signaling effect, which results in the incitation of the T-bet transcription factor. T-helper 1 cells primarily participate in the immunity mediated by the cells. Production of inflammatory cytokines, chemokines and activation phagocyte is correlated with interferon-$\gamma\gamma$, the T-helper 1 type response signature cytokine.According to a review by the **Gamonal and Garlet**, the interferon- $\gamma\ \gamma$ is highly present associated with progressive lesions or severe forms of periodontal diseases in humans and experimental periodontal lesions. Human and experimental evidencesuggest that theinterferon- $\gamma\ \gamma$ can give rise to the development of inflammation of periodontium by distinct mechanisms.[10]

T- helper 2: The activity of T-helper 2 cells depends primarily on interleukin- 4, the T-helper2 and the transcription factor GATA3. Interleukin- 4 has distinct suppressive and anti-inflammatory properties that are intervened by its ability to interrupt the transcription of pro-inflammatory cytokines and interferon- then suppress the polarization of T-helper 1 cells.[8] Interleukin-4 can hamper the development of factors causing tissue degradation including matrix metalloproteinases and the main osteoclastogenic factor RANKL. Interleukin- 4 can also induce regulation of its respective TIMPs and osteoprotegerin inhibitors in distinct tissues through their anabolic properties. In gingival crevicular fluid (GCF) the congregation of interleukin- 4 found to reduce from periodontal health to disease, indicating that this cytokine may intervene in the revocation of lesions of the periodontium.

T- Regulatory cells: In particular, regulatory T cells control the renewing, augmentation thereby regulating the outcome of many settings of immune systems varying from infective diseases to pathology of immune system and autoimmunity. The T- regulatory cells were initially identified as $CD4^+$,$CD25^+$ T cells. T regulatory cells serve the transcription factor forkhead box p3 (FOXp3), CD103, the glucocorticoid- inducible tumor necrosis factor receptor (GITR), inhibitory molecule cytotoxic

T-lymphocyte- related molecule 4(CTLA-4) and cell surface transforming growth factor-β1, in the middle of additional surface molecules. In some non-inflamed peripheral tissues, the T-regulatory cells are known to be in a relaxed state with minimum suppressor activity, but these cells are activated in inflammatory conditions and go through quick reprogramming to obtain helper/ effector functions.

According to a study paper, **Gamonal** et al demonstrated that T-regulatory cells- associated levels of cytokines have a negative association with RANKL levels, supporting their defensive role in active periodontal lesions against tissue destruction. In line with such theory, Garlet group was the first to recognize a reaction in an experimental model of periodontitis, in the association between the intensity of periodontitis via the ablation role of T-regulatory cells.

Role of prostaglandins: The transformation of arachidonic acid to prostaglandin catalyzed by the enzymes COX-1 and COX-2 cyclooxygenase. Prostaglandins in particular are the most significant inflammatory mediators especially, prostaglandin E_2 (PGE_2) which contributes to vasodilation and production of cytokines by different cell types. PGE_2 released remarkably in the periodontium by macrophages, fibroblasts from different cell types. PGE_2 is involved in the activation of MMPs and resorption of osteoclast and has an important role in tissue damage characterizing periodontitis.

COX-1 is a constituent enzyme involved in the initiation of physiologically active prostaglandins while COX-2 is an inductive enzyme mainly caused due to pro-inflammatory cytokines and results in the initiation of prostaglandin. Over the last years, the role of prostaglandin has been under investigation in the pathogenesis of periodontitis. PGE_2 and leukotriene B4 were present in GCF of localized aggressive inflammation of the periodontium, indicating a character for these molecules in the disease of periodontium. The use of a COX-2 inhibitor as an alternative to the periodontal therapy was investigated in several clinical trials. Such inhibitors increased the clinical results following periodontal therapy.[9] Thesestudies indicate that bone resorption induced by periodontitis may involve prostaglandin especially PGE_2.

Role of Osteoimmunology: The transformation of monocyte-macrophage precursor cells into osteoclasts induced by an increased level of RANKL mRNA and protein in periodontal tissue and alveolar bone loss caused by osteoclast maturation and survival has now been evident for days.[9]

RANK, RANKL, and OPG are TNF-α family cytokines. RANK is a receptor present on the surface of osteoclast precursor. A variety of other forms of cells produce RANKL primarily T-helper 17 and lymphocytes. Attachingto RANK without inducing any distinction, OPG competes with RANKL. The proportion of the expressions RANKL and OPG is essential in inflammation-induced resorption of bone including periodontitis. In order to determine the RANKL/OPG ratio, various clinical trials studied the congregations of RANKL and OPG in gingival tissues or GCF derived from individuals with periodontitis. This study shows that there is a rise in soluble RANKL in chronic periodontitis with or without a decrease in OPG compared with healthy individuals. The finding was typically that in people with periodontitis the RANKL/ OPG ratio was higher than in healthy individuals.[9]

Matrix metalloproteinases: Matrix metalloproteinases are the main proteases in destructive periodontal diseases. The relationship between inflammation of periodontium and chronic infection of bacteria controls the neutral proteinases manner and function. Especially the MMP family,which causes a continuous breakdown of the supporting tissues of the periodontium.[9]

Matrix metalloproteinase is a family ofhuman zinc-dependent endo-peptidases associated with a wide rangeof physiological and pathological processes includingthe growth of skeletal system and remodeling, healing of the wound, cancer andinflaming disease. MMPs are made up of an auto-inhibitory prodomain which is often involved in the identification and placing of MMP substrates for enzymatic latency, the catalytic domain, and the c-terminal hemopexin like domain.[10] MMP activity is closely regulated by gene expression, pro-enzyme activation, enzyme inhibition, and endogenous inhibitors. They are grouped particularly in collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs according to their substrate. MMPs are the master regulators of inflammation-related with a large variety of inflaming disorders. [10]

Conclusion

According to some evidence the disease

of periodontium has complex aggravation and revocationstates that can be defined as cycles of development and reversion of disease. Widespread forms of vital events of disease of periodontium can be related to dissimilarity in the population of supracrestal inflaming cells,where there are a substantially greater number of monocytes/macrophages and plasma cells inactive sites in comparison to inactive sites.

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Treatment of Chronic Periodontal Disorders: A General Practitioners Perspective

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Abstract

A greater number of patients will experience gums-associated disorders at one or the other point in their lifetime and also a section of the community is suspected to suffer from advanced gum associated disorders. Sound treatment is essential in dental practice (i.e. dental practitioner's perspective). A comprehensive layout of treatment which includes gum assessment, treatment, revisit of long-standing periodontitis cases in regular dental care, and treatment is discussed in this review.

Keywords: *Primary essential periodontal examination (PEPE), Chronic Periodontitis, Pocket, Gingivitis, Periodontitis, Oral health conditions.*

Introduction

Unidentified unattended periodontal disorder is a rapidly increasing concern in dentistry and there are restricted guidelines that describe the accessibility to its treatment.[1] Dental care burden is rising by 85%.Elderly people over 65 years of age exhibit long-standing gum diseases which represents significant negligence in dental care. This article aims to provide information about various dental treatments of long-standing gum disorder in dental care and practice. The information focuses on the risk factors and oral hygiene (OH) method. Long standing gum disorders is associated with anaerobic bacteria. Management of chronic periodontal conditions can only be possible by patient cooperation and this/her desire to continue with good dental hygiene. Hence information is mainly based on OH maintenance, since it is well recognized and easy to follow.

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Primary Essential Periodontal Examination:

Various codes are used to determine the criteria for basic periodontal examination.[2]

Code 0: Bleeding is not seen after gentle probing, plaque retentive factor is absent, and pocket is not seen over 3.5mm.

Code 1: Bleeding is seen after gentle probing, plaque retentive factor is not found, no pocket seen over 3.5mm.

Code 2: There is no pocket exceeding 3.5mm. Retentive plaque factor is seen.

Code 3: Probing depth is from 3.5 –5.5 mm

Code 4: Probing is greater than 5.5mm on one or more sites. Furcation involvement is seen.

Different Periodontal Disorders:

1. **Chronic Gingivitis:** In Chronic Gingivitis gingival inflammation is present due to plaque-induced inflammation. Abnormal redness of skin resulting from dilation of blood vessels, swelling of gums is seen. During brushing, probing gingival bleeding is seen. Detachment of the gingiva from the teeth is seen occasionally.[3,4]

2. **Acute Ulcerative Gingivitis:** Usually seen in patients who smoke or who present with poor oral hygiene. In the tip of the interdentally papillae painful ulcerations are present, grey necrotizing sloughing tissue is seen on the area of the ulcers. Loss of interdental papillae may be seen. Loss of blood is spontaneous in gingiva. Halitosis is present with tender or palpable sub-mandibular lymph nodes. Fever and malaise may be also present.

3. **Gingival Hyperplasia:** Caused by medication of calcium channel blockers, cyclosporine, phenytoin. It is also caused by irritation from calculus and repeated friction and trauma.[5,6]

4. **Chronic Periodontitis:** Age, Smoking, diabetes, and family history are the most responsible factors for chronic periodontitis. Subgingival calculus is mostly seen, with prevalence in adults, the progression rate is from slow to moderate with related amount of dental plaques.[7]

5. **Aggressive Periodontitis:** About 1in 1000 patients experience more quick auxiliary loss, alveolar bone destruction, and rapid attachment loss. Patients under 35 years of age are susceptible.[3]

6. **Acute Necrotizing Periodontitis**: Presence of attachment loss, associated mandibular lymph node may be tender and palpable, spontaneous gingival bleeding and there may be loss of interdental papillae, halitosis, fever and malaise.[6]

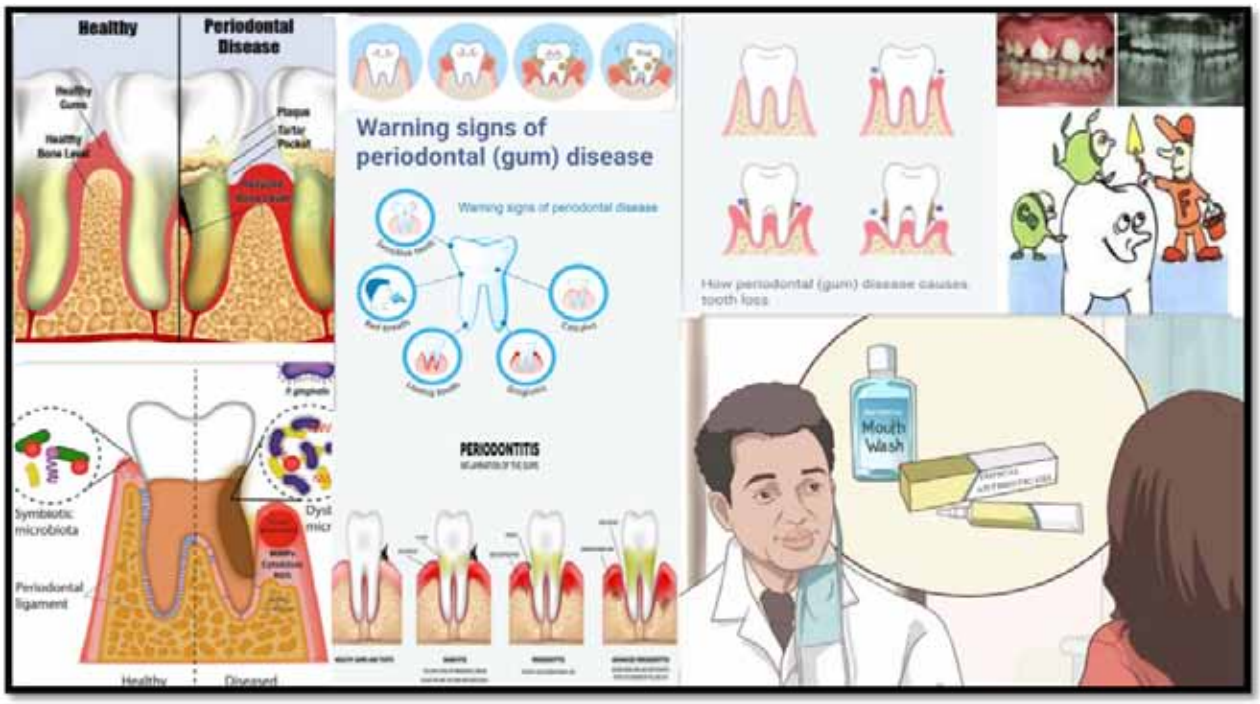
7. **Periodontal Abscess:** It is an acute infection in the gum pocket. The periapical and periodontal abscess should be differentiated if present together, since it's difficult to distinguish. Abscesses can be acute or chronic and asymptomatic if freely draining. The tooth will be vital if there is no endodontic component.

8. **Periodontitis associated with endodontic lesions:** Vitality of the tooth may not be present. Lesions may be independent or coalescing and may originate either from the gingiva or apex.

Probable prospect for gum disorders: Amount of destruction gives rise to dental changes and can be associated with a rise in bacterial plate in dens; moreover many internal and whole system structure risk factors can aggravate susceptibility and severity of the issue. Patient personal history and clinical examination will help in highlighting these local and systematic risk factors for gum disorders. Systemic risk factors include medical conditions like diabetes, leukemia and many more. Patients who are under medication like phenytoin, cyclosporine and calcium channel blockers show inflammation. Stress, smoking and any family history of periodontal disorders can aggravate the condition.[5] By examination, we can also know about the overhanging restorations, calculus deposition, root caries, enameloma and adamantoma etc.

Guidelines for long-standing gum disorders: The British Society of Periodontology offers various guidelines explaining the basic periodontal examination which can help us in providing basic treatment.[10, 11] Deep cleaning is the elimination of bacterial plate bio-factor and calculus deposition from the unseen portion of tooth and pockets. Making use of power-driven scaler or machine-driven scaler which are more useful for patients and clinicians over hoes, chisel, and files for root surface debridement due to their effectiveness.[12,13] Root surface debridement could be ended for periodontal pockets 4mm or more. Presentation of minimal dental cleanliness habits is an inseparable principle to be observed with any protocol of mechanical debridement. Oral hygiene education (OHE) must always be considered the treatment Hence an integral portion of the guidance utilizes OH to assist different and suitable treatment that can bring maximum welfare to dental health – which may be predominantly Oral hygiene education, Root surface debridement or either of them. The intensity or extent of the disorder can also be related to other portable parts of guidelines that include evaluating and labeling these. By categorizing OH as good, moderate, poor determining the level of dental hygiene of patients treatment planning may be done.[14]

Radiographs: X-ray helped to identify the horizontal bone level, any vertical bone defects, and furcation involvement. Criterion for bitewing radiographs: displays sufficient bone level in posterior sextants. Vertical bitewing can be useful where bone loss has so far taken place. Long cone periapical were advised for localized views. Further radiographs are only required for clinical explanation.



Analyzeand emphasize patient complain: Patients' authority and management of dental hygiene is foremost important if they want to extract maximum profit from the treatment. Dental hygiene instructions and education are an integral part of treatment that contain tailored instructions on maintaining dental hygiene habits and interdental habits. Medium to develop dental hygiene behavior contains: Aim determination, monitoring dental habits, identifying the cause and significance of treatment, and proper revisit to confirm, visually motivate and utilize the dental cleanliness practices.[15] Instructing the patient by advising and talking with them on planning their treatment and supporting and motivating them to follow the proper instruction to maintain their dental hygiene is important.[15]

Individuals having gum bleeding can also be motivated to use home care method which are budget-friendly and can be used by all classes of society. After some time survey can be done to check the improvement.[16]

Bacterial plate and different indicators should be used together with a proper diagnosis which can be helpful for the patients to know there bacterial plate level and accordingly manage it.

"O'Leary indicator" helps to determine the existence or lack of bacterial plate at 4 times on various dental surfaces. Suitable indicating agents like plaque search indicator or similarly any other medication or chemical is applied in every area of dental surface. Assessment can be done after rinsing the mouth once and any of the disclosed amounts of plaque visible can be recorded and the record can be worked out as a percentage.

Bacteria plate monitoring index should be utilized for uplifting compliance and provide a regulation when a patient is treated with (RSD) Root Surface Debridement to get the best outcome.[17] A bacteria plate results for better gum health response and it should be no more than 25%, minimal bacteria plate result, and must not be less than 10%respectively. It is considered that 15% is advised as a maximum. This is done to get a good result after root surface debridement.

Conclusion

Dental practitioners should discuss and share their treatments with their group for better treatment. Educate or train nurses about dental hygiene education and plaque scoring. Note writing or detailed forms should be maintained to defend themselves in case of an allegation made against the practitioner. Following the Treatment guidelines recommended help in clinical and in non-medical treatment of long-standing gum disorders in daily dental practice.

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Effect of Fluoride Varnish in Dental Caries: A Review

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Abstract

Dental caries remains amongst the most common oral health problem affecting people in the developing countries. The use of fluoride ions in the form of gels, foams, and varnishes are effective in preventing caries. The varnishes have been routinely used in the western European and Scandinavian countries in the last 30 years because of its ability to provide an increased contact time between enamel surface and fluoride, thus inhibiting demineralization of enamel. The technique of fluoride application, along with its advantages, disadvantages, indications, contraindications, and side effects have been discussed in this review.

Keywords: *Dental caries, Fluoride ions, Fluoride varnishes, Enamel, Oral health.*

Introduction

Oral health is a necessary and integral part of general health. Dental caries is by far the most important oral health problem affecting people of every age group and community.[1,2] The resultant pain and discomfort along with a heavy burden on finances impairs the quality of life of an individual.[3] According to Sturdevant, dental caries is defined as a multifactorial, microbial infectious disease characterized by demineralization of the inorganic and destruction of the organic substance of the tooth. Fluorine, derived from the Latin term fluore meaning to flow, is the most electro negative and reactive element of the halogen family. It combines chemically to exist as fluorides and represents about 0.06% to 0.09% of the earths crust, mostly in the form of minerals such as Fluorspar, Fluorapatite and Cryolite.[4,5]

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Advances in technology and research have enabled dentistry to shift from reparable art to preventive-oriented science. The emergence of fluorides has convincingly demonstrated a decline in caries prevalence.[6]

Mechanism of action of fluorides[7]: Simultaneous working of several mechanisms enable the fluorides to act against caries which have been briefly enumerated: Figure 1.

1. **Increased enamel resistance and reduction in solubility of enamel:** The fluoride acts on enamel and forms fluorapatite crystals which are less soluble and hence prevents dissolution of enamel in the following ways:
   - Reduces bacterial acid production and acidurance
   - Reduces equilibrium solubility of apatite
2. **Increased post-eruptive maturation rate of hypo mineralized areas:** The hypo mineralized areas of newly erupted teeth are prone to caries. Fluoride enhances the rate of post-eruptive maturation of these areas with the deposition of organic material from saliva.
3. **Remineralization of incipient lesions:** The deposition of minerals in a dynamic process over the previously damaged areas of the enamel reduces its solubility. This leads to a growth in the

crystals and becomes more resistant to caries. The remineralization occurs when fluorides combine with calcium and phosphate ions in the saliva. However, when too much fluoride combines with calcium phosphate, the formation of calcium fluoride takes place, which in turn prevents the growth of hydroxyapatite crystals.

4. **Interference with microorganisms:** At higher concentrations fluoride is bactericidal, whereas at lower concentrations fluoride is bacteriostatic. In an acidic environment, fluoride diffuses into the cells as hydrofluoric acid. This continues until the concentration in the external and internal compartment are equal.

5. **Modification in tooth morphology:** Ingestion of fluoride during tooth development results in a more caries resistant tooth with smaller diameters and cusp depths, thus enabling self-cleansing.

Fluoride delivery method

There are two method of delivery-topical and systemic.

1. **Topical fluorides:** These can be placed directly on the teeth with some preparations providing a low concentration of sustained fluoride release while others providing a higher concentration over a shorter span.[8]

The topical fluorides can further be divided into:

- Professionally applied products: These are dispensed by dental professionals in the dental office and uses a concentration between 5000 - 19000 ppm. These are available as gels, foams, and varnishes.
- Self-applied products: These are dispensed by the patient on the recommendation of a dental professional with the concentration range being 200 - 1000 ppm.

2. **Systemic fluorides:** These are allowed to circulate in the bloodstream and are given at a low concentration over a longer time span.[9]

Fluoride Varnishes: Fluoride is usually more effective when applied topically by professionals in the form of gels, foams, and varnishes. While using gels and foams, a high amount of fluoride is leached away in the first 24 hours and almost two-thirds are lost within days.[10-12] Thus, an increase in the contact time between enamel surface and topical fluoride agents became necessary to increase its efficacy.[13,14] This led to the incorporation of fluorides into a varnish-like coating by Schmidt in 1964. Varnishes were found to be a safe vehicle for exposing the enamel to fluoride for longer periods with deeper penetration into the enamel surface. Arends and Schutof using microradiography and micro-hardness tests have shown that a 24-hour exposure of enamel to fluoride varnishes can inhibit demineralization completely.[15] The prolonged retention of fluoride in the form of varnishes is because of a coating of pellicle proteins and secondary phosphates. During caries attack, a low pH results in the loss of pellicle coating, releasing calcium fluoride. The fluoride ions are absorbed onto the enamel surface to increase the rate of remineralization.[16-19] The various types of fluoride varnishes available today includes the following[20]:

i. **Duraphat:** Duraphat is a yellowish viscous material consisting of 22600 ppm sodium fluoride in a neutral colophonium base. The amount of caries reduction was found to be between 7% - 44% in deciduous and between 30 - 40% in the permanent dentition.

ii. **Fluorprotector:** Fluorprotector is a clear polyurethane-based product containing 7000 ppm fluoride in the organic compound Difluorosilane. It is dispensed in the form of 1 ml ampules, each containing 6.21 mg of fluoride. Its clinical efficiency has been established between 1% - 17%.

iii. **Carex:** The fluoride content of Carex is 1.8% with an efficacy similar to that of Duraphat as a caries-preventing agent.

The mean fluoride release from various fluoride varnishes have been demonstrated in **Figure 2**.

Technique of varnish application: When the varnish is freshly squeezed out of the tube, occasionally dark streaks may appear representing a separation of the ingredients inside and containing only a fraction of the required fluoride content. Thus, the freshly squeezed out varnish should be discarded. Alternatively, a rotary tube shaker can be used to obtain a uniform mix of the varnish.[12] While applying the varnish, teeth are dried post prophylaxis. Around 0.3- 0.5 ml of varnish is dispensed amounting to 6.9-11.5 mg of fluoride for full coverage of the dentition. The varnish is applied starting with the proximal surfaces, sequentially on the lower arch followed by the upper arch using a single tufted small brush. The patient is then asked to wait for 4 minutes with his mouth open before spitting to allow

the fluoride to set over the teeth. Finally, the patient is instructed not to rinse or drink anything till the first hour and not to eat anything solid till the next morning to prolong the interaction time between the fluoride and enamel.[21] **Figure 3.**

Some of the brands of fluoride varnish in regular use includes:

i. Colgate PreviDent varnish

ii. Embrace varnish

iii. Enamel Pro varnish

iv. Varnish 3M

Advantages, disadvantages, indications and contraindications and side-effects of fluoride varnishes[22-25]

Advantages:

- Easy to apply
- Have different flavours
- It has a sticky consistency which helps to stay in contact with tooth for several hours
- Dry rapidly
- Reduce the number of cariogenic bacteria s. mutans by over ten-fold
- It has a higher concentration than the foam and gel

Disadvantages:

i. They may cause a temporary change in the surface of teeth due to the color and adherence

ii. Varnish may damage by eating and brushing so the yellowish color fades

iii. Cost is more than gel

iv. Some patients can cause nausea due to the taste of varnish especially when consuming food within 24 hours post-treatment

Indications:

i. Fluoridated cavity varnish

ii. For high caries

iii. Remineralization of lesion in root dentin

iv. For orthodontic bands and brackets

v. On advanced enamel carious lesion

Contra-indications:

i. May cause discoloration in the area

ii. For open cavities area

iii. Low-risk caries

Side effects: The use of too much fluoride can cause fluorosis which gives white patches on teeth.

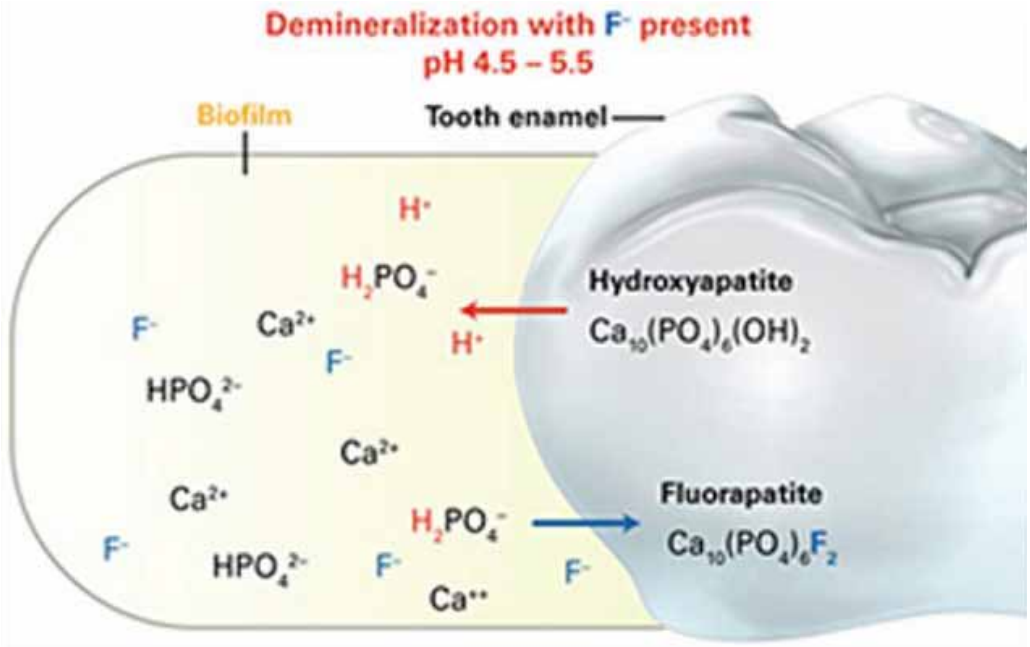


Figure 1: Mechanism of action of fluorides

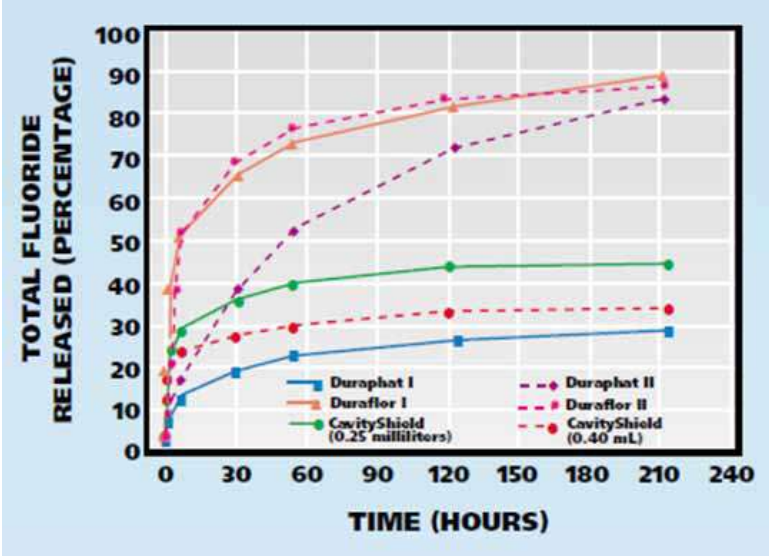


Figure 2: Graph showing mean fluoride release from various types of varnishes



Figure 3: Application of fluoride varnish helps remineralization and prevents caries

Conclusion

The fluoride varnishes have been routinely used as a standardized form of fluoride for professional applications in the Western European and Scandinavian countries for the last 30 years because of their well-documented effectiveness and safety. The shorter chairside time, easier application, better taste with increased acceptability, and cost-effectiveness make it more appropriate for using it in public health settings among children with high caries risk.

Ethical Permission: Not required

Conflict of Interests: None

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Mineral Trioxide Aggregate v/s Calcium Hydroxide: A Review Article

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Abstract

The main purpose of this article is to compare the mineral trioxide aggregate and calcium hydroxide in pediatric dentistry. Both of the endodontic cement are frequently used in different cases of pedodontics. This article aims to present the various characteristics of mineral trioxide aggregate and calcium hydroxide in a review article.

Keywords: *Mineral Trioxide Aggregate Calcium Hydroxide, Dental cement.*

Introduction

Over several years, different kinds of dental types of cement have been developed to avail of various purposes and to be applied to numerous events in dentistry. MTA is a distinctive endodontic cement & was developed by Torabinejad & members at the Loma Linda University, California, USA. Firstly, it was used as a root-end filling material in endodontic treatment. Over the years, MTA has been expanding its applications which includes root-end filings, direct pulp capping, perforation repairs in roots or furcation. U.S Food & Drug Administration has approved for the endodontic use of MTA in 1998. However, calcium hydroxide is also one of the most important medicaments used in the treatment of pulp disorders and apical periodontitis. Calcium hydroxide is a dental cement has been used in dentistry over the decades.[1]

Mineral Trioxide Aggregate (MTA): MTA is considered as one of the most multifunctional material in dentistry. MTA was identified as root-end filling material as it is a hydraulic cement that settles down in the existence of moisture. Through the internal bleaching of endodontically treated tooth, MTA act as a barrier. It can be utilized as capping material within mechanically exposed pulp & root-end induction. MTA favours the regeneration of tissue along with easy manipulation, possess good sealing stability & acquire long term prognosis.[2]

Composition: MTA gets settled easily in moisture as it is made up of hydrophilic particles. It is mainly constituted of fine hydrophilic particles of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide & bismuth oxide. MTA resembles Portland cement in composition expect bismuth oxide which is added to MTA for radiopacity. On comparison to Portland cement, particles of MTA are little and identical in size.[3]

MTA is available in 2 types: White MTA (WMTA); Grey MTA (GMTA). These two types mainly differ in lesser amounts of Iron, Aluminium & Magnesium oxides on WMTA than GMTA. GMTA has longer setting time, less radiopacity, solubility & pH value than WMTA whereas WMTA has more compressive strength than GMTA.

Mechanism of action: As soon as MTA is placed in direct influence with human tissues, the dental cement. The dental cement undergoes cell attachment & proliferation when comes in contact with human tissues. It resembles calcium ions which form calcium hydroxide. Due to alkaline pH, it gives antibacterial environment. Modulates the production of cytokine. Undergo hard tissue differentiation which stimulates the migration of cells. Biological seal by the formation of hydroxyapatite (carbonated apatite) on the MTA surface.[4]

Method of preparation: MTA powder should be stored in tight containers and dry condition. Before its use, MTA mixture should be prepared instantly. The

mix should prepare on a glass slab or paper slab with the use of plastic/metal spatulas. The powder water ratio should be 3:1 to obtain putty-like consistency. The mix should be carried with the help of plastic/metal carrier to the place of application. If the location is very wet, dry piece of gauze/foam is used to remove extra moisture whereas water can add up to mixture, if it is very dry. After mixing, MTA has a pH of 10.2 and it increases to 12.5% after a set time of 3 hours. Mixing time is of great importance, dry mix is seen if the mixing time is being lengthened. Mixing time should be below 4 minutes. Islam et al claimed it 2 hours 55 minutes for GMTA & 2 hours 20 minutes for WMTA.[5]

Calcium chloride and sodium hypochlorite lessen the setting time while saline and 2% lidocaine raises the setting time.

Properties: As the MTA material sets, its compressive strength shows identical to IRM and Super EBA. GMTA has more compressive strength than WMTA. MTA radiodensity is same as Zinc Oxide Eugenol. It can be seen radiographically.[6]

The set MTA is insoluble whereas solubility may increase if more water is added while mixing. When the set MTA exposes to water, it releases $Ca(OH)_2$ which may be responsible for cementogenesis induced property. The setting of MTA doesn't hamper in an acidic environment.

MTA has excellent sealing ability perhaps as expansion during setting. Residual calcium hydroxide may inhibit the adaptation of MTA to dentin which reduces the sealing ability. This is of great importance as when $Ca(OH)_2$ is placed in between the appointments before the positioning of MTA. As it provides good seal & prevents microleakage, it indicated as an antibacterial agent. MTA doesn't respond or inhibit with any restorative material. They are biocompatible. MTA can activate cementoblasts and produces cementum. It allows bone healing & regeneration of periodontal ligament. MTA induces thicker Dentinal bridge formation with less inflammation, necrosis and frequent odontoblastic layer formation as compared to $Ca(OH)_2$.[7]

Advantages: Antimicrobial, Microleakage, Cement conductive, Nontoxic, Cell Adherence & Growth. Periodontal Attachment To Cementum Growth, Dentinal Bridge Formation

Disadvantages: High cost, Difficult to manipulate, Longer setting time, Prolonged maturation phase

Uses: Direct pulp capping, Apical plug, root-end filling, Perforation repair, Furcation involvement, Resorptive defects, Apexogenesis/Apexification.

Commercially Available: Pro Root MTA, White ProRoot MTA, Ortho MTA, MTA Fillapex.

Calcium Hydroxide: Calcium hydroxide was introduced to the field of dentistry in 1921. Herman demonstrated the formation of the dentinal bridge in an exposed pulp & now it is considered as the 'gold standard for pulp capping agents.'

Composition: It is composed of calcium hydroxide powder, a vehicle & a radiopacifier.

Vehicle: Mainly there are 3 types of vehicles:

First is water-soluble substances. Some examples are water, saline, methylcellulose, anaesthetic solutions, carboxymethylcellulose & Ringers solution. Second is viscous vehicles. Some examples are glycerine, polyethyleneglycol (PEG), propylene glycol. The third is oil-based vehicles. Some examples are olive oil, silicone oil, camphor, eugenol, some fatty acids.[8]

Radiopacifier: Vehicle is not radiographically seen easily as it does not possess radiopacity. An ideal radiopacifier such as Barium sulfate & bismuth, a compound containing Iodine & Bromine have an atomic weight higher than calcium for radiopacity.[8]

Mechanism of Action: Vehicles in periapical tissues & in the root canal, calcium hydroxide will be solubilized & resorbed/absorbed at different rates as it decides the ionic dissociation kinetics. Calcium hydroxide activates in the presence of moisture and pH increases to 12 and above with minutes when $Ca(OH)_2$ is used as Intracanal medicament. Average time for the treatment is 1-4 weeks.

$$Ca(OH)_2 + H_2O \text{ -----> } Ca^{2+} + OH^-$$

Due to the huge concentration of hydroxyl ions from calcium hydroxide, cytoplasmic membrane damages its protein acts on the organic component & transport the nutrient (OR)

By saponification reaction, during the peroxidation process, the phospholipids/unsaturated fatty acids of the cytoplasmic membrane are being demolished.[9]

Calcium hydroxide has been provided in different modes. As varnish, it is supplied as a liquid which composed $Ca(OH)_2$ suspend in a solvent or even provided as a paste in that calcium hydroxide is suspended in methylcellulose. As a catalyst, $Ca(OH)_2$ present in the oral environment, it responds quicker & structures a tough amorphous compound in a minute. Calcium hydroxide as paste, when it exposes to brightening from a handheld blue light, calcium hydroxide hardens, as it contains polymer resin.[7]

Method of preparation: Up to the desired consistency is attained, mix both calcium hydroxide powder and water. It is the easiest method to prepare $Ca(OH)_2$ paste. Leonardo *et al* expressed in 1982 that a paste doesn't possess good physicochemical properties since it isn't radiopaque and penetrable to tissue fluids when the paste is made with water or more hydrosoluble non-viscous vehicle. It is dissolvable & resorbed in the periapical region and inside the root canal. Insertion of different substances to the calcium hydroxide paste is purposed by him.[6,7]

Uses: Intracanal medicament, Endodontic sealer, Pulp capping agent, Apexification, Pulpotomy, Weeping canals

Advantages: They are bactericidal and act as a mild irritant which stimulates hard tissue formation. They are inexpensive and easy to use and they stop internal resorption

Disadvantages: Formation of tunnel defects, bacterial reinfection, long term usage of $ca(oh)_2$ in apexogenesis may weaken the root, susceptible to fracture, less effective against *Enterococcus faecalis* & *Candida albicans*, chronic pulpal inflammation & internal resorption in a primary tooth, poor coronal seal & strength

However, calcium hydroxide is not generally used in pulp therapy for primary teeth. A mixture of calcium hydroxide & iodoform is found to be easy to apply & resorbs at a slightly faster rate. It has no toxic effect on succedaneous teeth & is radiopaque. Reasons outlined mixture of calcium hydroxide & iodoform can be considered to be a nearly ideal primary tooth root canal filling material.[7,8]

Recent advances: Recent advances of medicaments in Paediatric Endodontics includes dentine, BioRootTm RCS, Smartseal

Biodentine: It is a calcium silicate-based product specifically design as "dentine replacement" material. It has become commercially available in 2009. It was introduced to overpower all the drawbacks of $Ca(OH)_2$ and MTA. It has greater potential to favour the repair of perforations of the pulpal floor, open immature tooth, retrograde filling, apexification, pulp therapy.

Bio Root Tm RCS: It is a mineral-based root canal filling material with Active Biosilicate Technology for permanent obturation. This material helps to stop bacterial growth & alleviates the risk of intracanal reinfection due to high pH. It is biocompatible, resin-free, has good flow and hydrophilic due to which it set even in the presence of moisture.

Smartseal: It is recently introduced material based on polymer technology. Its principle is based on the hydrophilic nature of the obturating points.[10]

Conclusion

In comparison, the characteristics of both the dental materials it is clear that MTA is more effective than calcium hydroxide in various cases. However, calcium hydroxide cannot be used on the primary tooth as it will lead to internal resorption while MTA has major drawbacks which include high cost, discolouration potential. Nowadays, the emergence of calcium silicate-based material such as Biodentine has overcome MTA's limitations.

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Microbiological Aids in Periodontics

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Abstract

New approaches to periodontal diagnosis, including advanced microbiologic, biochemical, and genetic tests, have been shown to provide the clinician with the information not available by traditional means. The purpose of a diagnostic test is to confirm, exclude, classify, or monitor disease to guide treatment. Their clinical value depends on whether the information they provide leads to improved patient outcomes. This can be assessed by randomized trials, which compare patient outcomes from the new diagnostic test versus the old test strategy. So, the current paper aims to focus on the practical utility of this rapidly emerging plethora of periodontal microbiological diagnostic tools, emphasizing the critical issues surrounding the clinical application of microbiologic investigations, employed for periodontal diagnosis.

Keywords: *Microbiologic investigations; Periodontal diagnosis; Clinical application.*

Introduction

A clinical diagnosis of periodontal disease does not indicate the prognosis, causative agents, progress and prognosis of the disease. For several decades, the microbiology of periodontal disease has been the focus of intense investigation. This focus is justifiable since bacteria (*A.actinomycetemcomitans, P. gingivalis, P. intermedia*, etc) are the etiological agents of periodontal diseases. Microbiological tests are used not only to identify the putative pathogens and indicators of disease initiation and progression but also utilized to observethe treatment of periodontal disease directed at the suppression or elimination of periodontal pathogenic microorganisms.[1]

Microbiological Aids:

1. **Direct Microscopy:** Dark-field or phase- contrast microscopy is the oldest method of microscopy and it is used to identify spirochetes and other motile organisms in plaque sample.[1] However, most of the putative periodontal pathogens like *Aa*, *Pg, Tf;* which are nonmotile and therefore this method cannot identify these species. Therefore this technique is not ideal for the diagnosis of periodontal diseases. This method can use to educate and motivate the patient.[2]
2. **Bacterial Culturing:** Bacterial culturing is still considered as a reference method (gold standard) against the newer techniques which are compared to establish their efficacy.[2] Generally samples are cultivated under anaerobic conditions; due to the majority of periodontal pathogens are anaerobes in nature.[3] Several systems perhaps used to create an anaerobiotic atmosphere are;

- Bio Bags
- Pre Reduced Anaerobically Sterilized Media (PRAS)
- Anaerobic chambers and anaerobic jars

Among the above, anaerobic jars and anaerobic chambers are most frequently used. These are used with a coalesce of basal and selective media for isolation of periodontal pathogens.[3]

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3. **Immunodiagnostic Method:** Immunologic assays recognize bacterial antigens to detect target micro-organisms. This reaction can be revealed by various procedures like; Immunofluorescent assays (IFA), Enzyme-linked immunosorbent assays (ELISA), Flow cytometry, Latex agglutination, Membrane assays.[2]

Immunofluorescent Assay: It is of two types; Direct immunofluorescent assay and Indirect immunofluorescent assay. Indirect immunofluorescent assay,the antigen is reacted directly with a fluorescein-conjugated antibody. In indirect immunofluorescent assay, the secondary fluorescein-conjugated antibody reacted with primary antigen-antibody complex.[4]

Flow Cytometry: It is also known as Cytofluorography. It allows detection of single or multiple microbes in plaque samples, based on their atypical cytometric parameters. The bacterial cell labeled with a second fluorescein-conjugated antibody and also with species-specific and after that these are undergoing for separation of bacterial cells into a single cell suspension by using flow cytometer. The implementation of flow cytometry in clinical microbiology is not yet widespread due to lack of approach or due to lack of knowledge.[5,2]

Enzyme-linked immunosorbent assay (ELISA): ELISA is the widely accepted, simple but sensitive method.[6] In this method, for labeling purposes, an enzymatically derived color reaction is used. The strength of color is directly proportional to the concentration of antigen. This method is previously used to identify the serum antibodies to periodontal pathogens but now a dayby the help of specific monoclonal antibodies, it has been also used for subgingival samples, to express the amount of specific pathogens present in this sample.[2]

4. **Enzymatic Method:**

BANA Test: The“red complex” bacteria like Porphyromonasgingivalis, Treponema denticola, Tannerella forsythia are colonizing the surfaces at or below gingival and leads to the destruction of periodontal ligament and alveolar bone. This destruction of PDL and alveolar bone causes loss of attachment to the tooth.[7] The above-mentioned species have trypsin-like enzymes and also share a common enzymatic profile.[2] N-benzoyl-DL-arginine-2-naphthylamide (BANA enzymatic test)is a rapid and reliable chair-side diagnostic test, which can give information about the bacteria by hydrolyzing the trypsin-like enzyme.[7]

During hydrolysis, it releases chromophore naphthylamide. A drop of garnet is added to the solution and the solution turns into orange-red. Positive BANA findings indicate Td, Pg, or both are present at sampled sites.[2] In this test, there is always a lack of quantitative data and they cannot identify the pathogens which do not produce trypsin-like enzyme.[6]

5. **Diagnostic Assays Based on Molecular Biology Techniques:**

DNA Probes: These DNA Probes are single-stranded pieces of nucleic acid. Under suitable conditions (pH, temperature and ionic strength), an enzyme which is labeled on DNA probesis help in hybridization with complementary single-stranded DNA or RNA and then detect micro-organisms. The principle of working of DNA probes is sequencing the DNA base.[8] Commercially available DNA probes are DMDx, Omnigene.[2]

Checkerboard DNA-DNA Hybridization Technology: In the year 1994, according to Socransky et al, DNA checkerboard is a method that gives a synchronous and significant analysis of up to 28 plaque samples against 40 microbial species.[9] The assays use DNA probes, which are labeled by digoxigenin and are whole genomic. In this technology, for identification of cells of each microbial species, the DNA probes are used and these are usually modified to allow identification of cells of each species.[2] This technique is used for ecologic studies and also for epidemiological research, because they do not need bacterial viability.[6] It does not provide an estimate of the total bacterial mass in the specific site and also this technology needs sophisticated lab equipment, which are demerits of this technique.[6]

Polymerase chain reaction (PCR): In the year 1983, PCR was introduced by Mullis and he won Nobel Prize for this discovery. The development of polymerase chain reaction is beneficial in genetic analysis for the diagnosis of genetic diseases. It has appeared as a very powerful tool, which allows development, knowledge about transcription of RNA and genes. The PCR test was able to identify periodontal pathogens (Porphyromonasgingivalis) more often than cultivation. Various category of PCR are: Nested polymerase chain reaction, Quantitative polymerase chain reaction,multiplex polymerase chain reaction Real-time polymerase chain reaction, Allele-specific polymerase chain reaction, and Colony polymerase chain reaction.[10]

Conclusion

There are many potential markers for periodontal disease activity and progression. Reinforcement of the identification of periodontal disease can be done by microbiological aids. It also can be utilized to guide the treatment of periodontal disease, so that the suppression and eradication of causative organisms can be done. In all microbiological tests, ranging from interacting bacteria thatare capable of causing periodontal diseases is difficult and the combination may differ between individuals. After all these years of intensive research, we still lack a proven diagnostic test that demonstrates a high anticipating value forthe advancement of the disease, has a proven effect on disease prevalence and incidence, and safe, simple and also cost-effective.

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Ectodermal Dysplasia: An Overview

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Abstract

Ectodermal dysplasia is a rare hereditary disorder with a characteristic physiognomy. It is a genetic disorder mainly striking the growth or purpose of the teeth,hair, nails and sweat glands. Ectodermal dysplasia can also affect the skin, the lens or retina of the eye, partsof the inner ear, the growth of fingers and toes, the nerves and other parts of the body providing to thes pecific syndrome. All the syndrome generallyinvolves a different combination of symptoms, which can range from mild to severe. The antiquity andlessons learned from hypohidrotic ectodermaldysplasia (HED) can serve as an example for investigating the root and pathogenesis of other ectodermal dysplasia syndromes by denoting that phenotypically identical syndromes can be caused by mutations in different genes, mutations in the same gene can lead to different phenotypes and that mutations in the genes further ensue in the same indicating pathway may alter the phenotype quite profoundly. This paper aims to describe and discuss the etiology, genetic review, clinical manifestations and treatment options of this hereditary disorder.

Keywords: *Ectodermal dysplasia; Symptoms; Genetic disorder; Therapeutics.*

Introduction

The term Ectodermal Dysplasia (ED) used to a geneticchange in the ectoderm, which is characterized by faulty growth during embryogenesis in one or moretissues. The syndrome is regarded as a change of sex-linked recessive trait, while some forms of abnormality can betransmitted as autosomaldominant or recessive. According to some authors,there are two types of ED: Anhidrotic or hypohidrosis,called Christ-Siemens-Touraine Syndrome, and hidrotic, called Cloustonsyndrome. The anhidrotic was first described by Thurnam in 1848 and later by Darwin which is generally transmitted by a recessive gene linked to chromosome X. Women is the carrier of this gene and the disorder is generally seen in men,denoted by the deprivation of some dental elements, dental abnormalities like conical teeth, lack of body hair, nailmalformation and decreased sweating, and specific facialfeatures[1]. Hypohidrosis is the mostobvious feature of this type of ED consideringhidrotic ED is a rare autosomal dominant disorder characterized by dystrophic, alopecia nails, and palmhyperkeratosis. The teeth and face abnormalities rarely present, and sweating, although Coskun and Bayraktaroglustated the presence of sweat glands disorders. Ocular abnormalities include strabismus, premature cataracts, and conjunctivitis. Otherabnormalities seenare hearing reduction, polydactyly, and syndactyly. Mental development is delayed. It isevaluated that the frequency of the male population is 7:10,000 births. By compromising these individuals self esteem the social life can be changed,especially if it is a child. Loss of vertical dimension and the lip,resulting from improper development of the alveolar process lump which is caused by the absence of some dental elements is some other consequences of thisanomaly.[2] Dental changes related to ED manifest early,can be easily detected in childhood. Since patients with these conditions are

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usually withdrawn, shy and complexso the detection should be done as soon aspossible so that the treatment can lead to physicalemotional and social development. Treatment of patients with the syndrome is complex and must have essential action and monitoring while the development of the child to the adult stage, to raise self-esteem and restore maximum functional and aesthetic. Dental defects represent a core clinical feature of many EDs: anodontia, polydontia, dysplasticteeth, retained primary teeth, deficient enamel development (amelogenesisimperfecta), dentine deficiency (dentinogenesisimperfecta), and underdevelopment of the alveolar ridge. In some EDs, the number of erupted teeth is reduced, the spacing of the teeth disrupted, and the periodontium is affected. There are a defect of the central nervous system (CNS), the adrenal medulla the oral, nasal and rectal mucosa and their associated glands. The pharyngeal and laryngeal mucosa may be so, atrophic that it results in dysphonia and hoarseness of voice.[3]

Etiology and Frequency: Hypohidrotic ectodermal dysplasia (HED) is usually passed on as an X-linked recessive trait where the female is the carrier and is manifested in males. In X-linked form carrier mothers show minimum expression in the form of hypodontia and/or conical teeth and decreased sweating. As a carrier, the female who is not affected has a 50% chance of transferring this condition to her male child. Likewise each female offspring has a 50% chance of inheriting the diseased gene. Rapid mutation of genes is possible and can be seen in any family without any history of this condition. The prevalence in the population has been assessed as between 1:10,000 and 1:100,000 male live births. A variety of ectodermal dysplasia is caused by the mutation or deletion of particular genes that are being located in different chromosomes. As ectodermal dysplasia is caused by genetic/hereditary defects, it can be passed down to the family line. It is not necessary to have a history everytime, this can be also seen in person without any past/genetic history of ectodermal dysplasia. In this condition *de novo* mutation has taken place. Genes responsible for ectodermal dysplasia are **EDA, EDAR, EDARADD**. Mutation in *EDA, EDAR, EDARADD* gene cause hypohidroticectodermal dysplasia. In x linked hypohidrotic ectodermal dysplasia EDA is only responsible. Both *EDA, EDARADD* are associated with both autosomal and recessive forms of hypohidrotic ectodermal dysplasia.[4,5]

Genetic Pathogenesis: During embryogenic development, *EDA, EDAR, EDARADD* gene provides instructions for making proteins. These proteins (Ectodysplasin A) form a part of an indicating pathway that is censorious for the interconnection between two cell layers, the ectoderm and the mesoderm. During embryogenesis; the initial stage of the embryo, the basis for many of the body organs and tissue are formed by these cell layers. The formation of various structures that emerge from the ectoderm which involve the skin, hair, nails, teeth, and sweat glands are because of ectoderm and mesoderm interactions. When mutation occurs in *EDA, EDAR, EDARADD* gene result in faulty ectodysplasin A formation which thereby intercept normal interconnection between the ectoderm and the mesoderm and teeth. The improper formation of this ectodermal structure leads to the characteristic feature of hypohidrotic ectodermal dysplasia.[4,5]

Classification: According to Freire-Maia"s classification, EDs aredivided into two groups: ***Group A***which consists of all the entities withdefects in two or more of the standard structures, and ***Group B*** comprises of those with disturbances inonly one of these structures plus another ectodermaldefect.11 subgroups have been included in Group A depending on the involved structures: 1-2-3-4 (hairteeth-nailssweat glands); 1-2-3 (hair-teeth-nails); 1-2-4 (hair-teethsweat glands); 1-3-4(hair-nails-sweat glands); 2-3-4(teeth-nails-sweat glands); 1-2 (hair-teeth); 1-3 (hairnails); 1-4 (hair-sweat glands); 2-3 (teeth nails); 2-4 (teeth-sweat glands); 3-4 (nails-sweat glands). Similarly,Group B is classified into four subgroups with number 5 added at the end, depicting that another ectodermal anomaly is present: 1-5, 2-5, 3-5, and 4-5. Other ectodermal derived structures like mammary glands,thyroid gland, thymus, anterior pituitary, adrenal medulla, central nervous system, external ear, melanocytes, cornea, conjunctiva, lacrimal gland and lacrimal duct that may also be involved in EDs.[4-6]

Hypohidrotic Ectodermal Dysplasia: Alteration in the gene **ectodysplasin** causes Christ-Siemens-Touraine syndrome which is the most common form of ectodermal dysplasia. Ectodysplasin is located at Xq12-13, which encodes for a transmembrane protein called ectodysplasin composed of 391 amino acids. X-LHED is caused by mutation in the ectodysplasin gene. EDAR is mapped to 2q11-q13, has been implicated in an autosomal dominant and recessive form similar to X-LHED. EDAR acts as a receptor for ectodysplasin.[7]

Hidrotic Ectodermal Dysplasia: Hidrotic ectodermal dysplasia is also known as cluster syndrome. Mutation in connection gene, GJB6 causes this condition. Both males and females are equally affected. Hair loss, nail dystrophy & palmoplantar keratoderma are the characteristic features of this syndrome. Hair growth becomes very less resulting in atrichia or hypotrichosis.[8]

Clinical Manifestations: During the 1st trimester of pregnancy ectodermal dysplasia is usually seen. In severe form; it is seen before the 6th week of embryonic life where the dentition gets affected. Ectodermal structures get affected after the 8th week. Hypohidrosis is the exceptional feature of hereditary ectodermal dysplasia. HED is a clinically homogeneous condition along with major involvement of ectodermal structures in perturbed formation and maturation of teeth, hair, nails and sweat glands. Genetic studies have identified 4 subtypes of HED, by splitting the autosomal dominant and recessive form into 2 distinct entries caused by mutations in either EDAR, EDARADD Gene's. Children with hypohidroticectodermal dysplasia will have fine, scanty, light coloured hair that thickens and darkens as the patient grows up.Oligodontia of primary and permanent teeth is the most distinctive feature. Tooth are usually of conical shape. Teeth are missing both in maxilla and mandible but with a higher incidence rate in mandible. Maxillary retrusion is seen in children due to sagittally under developed maxilla.[9]

Therapeutic Aspects: A person with ectodermal dysplasia usually suffers from poor psychological and physiological development which is a result of unacceptable aesthetic and abnormal function of orofacial structures. Early treatment is suggested. Ideal treatment requires integrated efforts from pediatric doctors, psychiatrists, ENT specialists, and speech therapist. To improve appearance, mastication and speech dentist is solely responsible. Dental treatment depends on the severity of the condition. According to age, growth, and development of stomatognathicsystems of the patient the treatment varies. The goal of a pedodontist is to provide the child with an oral appliance that will provide the ideal aesthetic and function to allow the patient to develop physically, emotionally, and socially. [1-5]

For the child's growth and development consistent dental treatment is required. The ideal pattern of treatment consists of fabrication of dentures, and as the child grows the denture will be modified or replaced. During the mix dentition period, the prosthesis will require modifications to accommodate the loss of exfoliated primary teeth and the appearance of newly erupted permanent teeth. During permanent dentition, the removable prosthesis maybe replaced by fixed depending on the number and position of the teeth. Another treatment option is the use of implants. In adult use of implant has been seen but there are no clinical reports of use of the implant in growing children. [3-6]

Conclusion

Diagnosis of ectodermal dysplasia is solely based on lack or type of hair, absence of teeth and tooth buds and tooth morphology. Eczema, asthma and frequent respiratory infections may provoke the disease. Ectodermal dysplasia is a diverse group of inherited disorders that have many overlapping features. It is difficult to classify them. The clinical manifestations of ectodermal dysplasia cause significant social problems in affected individuals. Both oral and body functions are affected. A prompt diagnosis and rehabilitation by the multidisciplinary approach is the key to successfully manage ectodermal dysplasia.

Unusual facial features exacerbate the social challenges of meeting new people. Lowered self-esteem, speech defects, decreased academic performance and social isolation may result from merely looking different from one's peers. Therefore, cosmetic and prosthodontic measures should be introduced as early as possible to have the child resemble his peers. It is also important to inculcate awareness among parents regarding early management. To ensure adequate care, a child with ectodermal dysplasia should be managed by a team which includes pediatrician, pediatric dentist, prosthodontist, dermatologist, otolaryngologist, speech therapist, and psychologist. However, an appropriate change has to made in the denture so that the appearance is always appropriate for the age. Relining or change of dentures should be done every 1 or 2 years due to preadolescent growth in jaw dimensions, wear of the acrylic teeth, under the extension of the dentures and posterior open bite.

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Plaque Control with Newer Dimension in Children

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Abstract

Plaque management is the routine removal of dental plaque, oral biofilm and also the avoidance of its accumulation on the teeth surface and other adjacent parts of the oral cavity. Occurrence of the common gingival and the periodontal diseases in the oral cavity is due to the presence of dental plaque which is the utmost primary etiological factor related to this. Dental caries along with periodontal disease are the major diseases that start very early during childhood and which should be stopped before the gradual onset of the disease. Dental plaque management using distinct approaches and agents benefit track the onset of periodontal diseases. An aggregation of multiple oral hygiene edification and mechanical skilled teeth brushing at regular time intervals will avoid the progression of dental caries and periodontal diseases almost entirely. Hereby, this article provides an overview of mechanical plaque control approaches with certain changes to include children and adolescents for successful dental plaque management.

Keywords: *Plaque control; Dental plaque; toothbrush; oral biofilm; Dental floss, Periodontal diseases*

Introduction

One of the most important changes in dentistry has been the advent of modern theory and dental care focused on prevention and not any substitution. Despite these significant health changes,dental disorder appears to be a persistent health issue.[1] The two dominant dental illnesses begin in childhood, namely dental caries and periodontal disease, and have long sequelae. Before the insidious onset of these conditions, primary preventive dental care should start early in life. The key etiological factor for the development of periodontal diseases is the dental plaque, which is a common but highly variable structural entity resulting from colonization and growth of micro-organisms consisting of different species and strains embedded in an extracellular matrix. Biofilm formation inhibition and mechanical removal tend to be the leading procedures for dental caries and periodontal disease prevention and treatment.[2] Specific routine oral hygiene is essential for oral health by brushing and using other aids in accordance with professional plaque inspection. This article focuses on efficient plaque management procedures with significant advances in oral disease prevention and good oral hygiene maintenance.[3]

Dental plaque: Dental plaque, also referred to as "Oral Biofilm, " maybe characterized just as the smooth tenacious substance adhering the tooth surfaces which is not easy to get rid of by just rinsing with water, although seeing more precisely, it is a variably specific structural entity resulting from the salivary glycoproteins and extracellular microbial products in the form of a biofilm-forming on the hard, non-shedding surfaces in the mouth. Dental plaque is mainly composed of both organic (polysaccharides, protein, glycoproteins from saliva, lipid) and inorganic (Ca, Na, P, K, F and GCF). It is mainly classified as two types the supragingival and subgingival dental plaque.[4-7]

Importance of plaque control: Dental plaque is directly linked with dental diseases. There is a clear correlation between plaque and gingivitis and it would be prudent to consider temporal factors because it is determined that the best way to avoid periodontal disease

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is by introducing adequate plaque control steps. Plaque management decreases the risk of periodontal diseases. Thus, the personalized use of mechanical and chemical agents to prevent the onset of diseases is necessary.[8]

Mechanical plaque control aids: The most commonly used oral hygiene aid by William Addis first invented in 1780 is by far the most widely known and accepted method of teeth cleaning. Toothbrushes plays an exceptional role in the prevention of periodontal diseases and their progression is very significant.

The various mechanical aids are:

Toothbrush, Depending on function: Manual & Powered

Depending on the diameter of bristles: Soft, Medium, Hard, Interdental Aids, Dental floss, Interdental brushes, Wooden tips, Tongue Scrapers

Table 1. ADA Specification of toothbrush

Length	1-1.25 inches
Width	5/16 to 3/8 inches
Rows	2-4 rows/bristles
Tufts	5-12/row
Bristles	80-86/tuft

NEWER TOOTHBRUSHES MODIFICATIONS

Powered toothbrush: The first electric toothbrush came much later and was first used in the 1960s as an alternative to manual toothbrushing process. Brush head present in the powered toothbrush act as a power source which provides different motions. Earlier, it was introduced with movement back and forth. Powered toothbrushes display different modes of movement, such as oscillating/rotating, side-to-side (sonic), and counter-oscillating, circular motion, which makes them useful. Later on, numerous studies have confirmed that powered toothbrushes when compared with manual are more effective.[9-12]

Ultrasonic toothbrush: The ultrasonic toothbrush is a manual toothbrush, in which the brush head is mounted in a Piezoelectric ultrasonic emitter which is operated by a power supply in the handle at 1.6 MHz. The ultrasound force killing plaque and the smooth, gentle healing wave action of sonic vibration reach the gumline to a depth of 5 mm. Emmi-dent is the first ultrasonic toothbrush to have its non-abrasive nano-bubbles toothpaste produced. The se brushes can be used immediately after undergoing oral surgery, like that of implants. It is mild enough to prevent damage to the teeth and gums. It is very painless for the use in areas with sensitivity along the teeth and gingival areas.[13]

Ionic toothbrush: Ionic toothbrush operates on the polarity principle, that each element in nature has a positive or negative charge. Plaque removal is improved by ionic interaction, along with the natural mechanical action of the bristles on the tooth surface.[14]

Chewable toothbrush: It is a minuscule pliable tooth-brush that are feasible for value without water. There stand availability of various flavors in the market like mint, bubblegum. Bristles are attached with smaller sized toothpaste stick which are breakable and made of plastic. The best about a chewable toothbrush is it can even be used in the absence of water, proving it useful for small children.

Tooth towelettes: If tooth brushing is not possible it is an effective plaque removal process. Finger brushes are mounted on the index finger of the brushing hand, and the agility and sensitivity of the finger are used to clean the teeth.

Foam brushes: Foam brushes imitate a plastic fluffy sponge soaked on a stick with chlorhexidine. They are used particularly in medically compromised and immunocompromised patients to reduce the risk of oral and systemic infection.[15]

Interproximal Brushes: The above-mentioned brush mainly the cone-shaped that are manufactured along with the presence of bristle that is arranged above the handle of the brush and can be exceptionally used for scrubbing the broad, irregularly placed, or else the round tooth surfaces present adjoining the immense interdental space and widely purchasable in various size.

End-Tufted Brushes: They are a category of toothbrush that is used to clean along the gingival sulcus present bordering the teeth surface. Bristles are designed in a sharp arrow decoration which enables nearer gum adaptation which is suitable for cleaning along the areas between crowns, bridges, misalignment teeth, and fixed orthodontic appliances.[16-18]

Interdental Cleaning Aids:

Dental Floss: Dental floss is a very common interdental cleaning process. Supragingival and

subgingival dental plaque is successfully removed by flossing but requires manual effectiveness.

Powered flossing devices: One of the latest advances in mechanical plaque control is the powered flossing device. These consist of nylon tips that move quickly between the teeth and are very sensitive to the gums. The se are equally effective as the manual ones.

Wooden Tips: These are smooth, triangular-shaped tips that could be positioned in the gingiva and slip in interdental space through contact to the surface of the proximal tooth. Along with soft debris removal also decreases the gingival inflammation.[19,20]

Oral irrigation: A dental water jet or else the water flosser in other words well-known as the oral irrigator (OI) act as an electrical system that conveys a pulsating fluid stream (including chlorhexidine, stannous fluoride, iodine solution, antibiotics such as 5% tetracycline hydrochloride) utilizing controlled pressure aimed at removing interdental furthermore subgingival plaque biofilm covering tooth surfaces to lower inflammation as a result of inflammation. It is a power directed or else non-power directed device which provides a marked function of a pulsed or substantial water stream for debris removal.It is an additional aid in oral hygiene. This method is healthy and provides the general public with benefit for gingival safety,but does not routinely clean the interproximal spaces. One such device is Waterpik is an oral irrigation system which consists of a reservoir and a handle with replaceable tips,which varies according to use.[21]

Dentifrices: Toothpastes consist of sodium fluoride and monofluorophosphate are found to be communally available as Cheerio gel and Colgate (fluoridated along with anti-carious properties)to remineralize carious lesion. Brushing twice a day after 3 years with the use of fluoride-containing toothpaste and brushing in-between the teeth day to day with floss or with any other interdental cleaner is endorse. The se are effectual in the degradation of plaque as well as gingivitis.[22-25]

Conclusion

With recent advancements and changing lifestyles, it's very important to look after ones personal hygiene approach which includes the most important one the oral hygiene. Good oral hygiene reduces the risk of periodontal problems and dental caries. Dental plaque is one of the most important factors causing dental problems must be effectively reduced and prevented. The most effective way to get rid of the frequent dental plaque accumulation is through mechanical plaque monitoring. The se recent advancements should be started in daily practice,which will reduce plaque accumulation and are less time-consuming. Further developments are on the way which may reduce and control plaque easily, and effectively within seconds.

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Complications of Local Anesthesia

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Abstract

This chapter is presenting the local and systemic complications associated with the local anesthetics used in oral and maxillofacial surgery. The prevention of complications and management method are also given importance. Dental procedures are frequently performed under local anesthesia; thus, drug-related complications often come across. It is mandatory to have a preoperative evaluation of the patient and choosing the proper local anesthetic agents.

Keywords: *Local anesthesia, complication, systemic complication, local complication, treatment*

Introduction

Local anesthesia is defined as a loss of sensation in a circumscribed area of the body caused by depression of excitation in nerve endings. Local anesthetic agents have been used in clinical dentistry to eradicate pain associated with invasive operations. It is also used in oral and maxillofacial surgery. [1]

Composition:

- Local anesthetic agent: lignocaine hydrochloride (2%)
- Reducing agent: sodium meta-bisulfite
- Preventive agent: methylparaben
- Diluting agent: distilled water
- Fungicide: thymol
- Isotonic solution or ringer's solution: sodium chloride
- Vasoconstrictor agent: adrenaline

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Complications: Various complications of local anesthetics can be evaluated systemically and locally. Common systemic reactions due to local anesthesia are reported as psychogenic reactions, systemic toxicity, allergy, and methemoglobinemia. Common local complications associated with local anesthesia are given as pain at injection, needle fracture, prolongation of anesthesia and various sensory disorders, lack of effects, trismus, infection, edema, hematoma, gingival lesions, soft tissue injury, and ophthalmologic complications.[2]

Local Complications:

Trismus: Also called lockjaw, is reduced opening of the jaw. May be caused by spasm of the muscles of the mastication. Several factors cause trismus such as multiple injections in a short period in the same area, intramuscular injections inside the muscle which cause hematoma formation and fibrosis, needle fracture in the muscles inserting to styloid process, inaccurate positioning of the needle when giving the inferior nerve block and other masticatory muscles, a low-grade infection. Excessive volumes of local anesthetic solution deposited into a bounded region which causes expansion of tissues.

Cause:

Intraarticular: Ankylosis, Arthritis synovitis

Extra-articular: Infection (Odontogenic & Non-Odontogenic)

Odontogenic: Pulpal, Periodontal, Pericoronal

Non-odontogenic: Tonsillitis, Tetanus, Meningitis, Brain abscess, Parotid abscess

Trauma: Fractured mandible, Fractured zygomatic arch

Infection: Infection may extend to tissues by penetration of the needle through a contaminated tissue, because of the needle being contaminated before an operation. A latent viral infection may be reactivated due to the trauma of the procedure which may be responsible for neural sheath inflammation. Antiseptic mouthwash solutions such as chlorhexidine gluconate should be taken for all regional techniques. The local anesthesia should not be injected through the infected area.[3]

Edema: Swelling of tissues can be due to trauma during injection, infection, allergy, hemorrhage, and injection of irritating solutions. The management of edema is dependent on the cause. Allergy-induced edema treatment consists of intramuscular epinephrine injection as mentioned above and, additionally, antihistamine and corticosteroid administration and consultation with an allergist to determine the precise cause of the edema. Trauma-induced edema should be managed as a hematoma. For the treatment of edema produced by infection, antibiotics should be prescribed.[3-6]

Hematoma: Hematoma formation as a complication of local anesthesia. It is an abnormal collection of blood outside of a blood vessel. It occurs because the wall of a blood vessel, artery, vein, or capillary has been damaged. And blood has leaked into tissues where it does not belong.

Symptoms: Pain, Swelling, Redness

Gingival lesions: Gingival lesions consist of recurrent aphthous stomatitis. Isolated lesions of gingival arise in succession to the hyperinflammatory reaction. No management is necessary until there is severe pain. To relieve pain, topical anesthetic solutions (e.g., viscouslidocaine) may be used on affected areas. Triamcinolone acetonide without corticosteroid can remedy pain.

Soft tissue injury: Soft tissues which include the tongue, lips, gums, and lips are injured when they are accidentally be bitten while eating or in an accident, or when hot food is kept inside the mouth. Chewing on hard objects also damage soft tissues. And the main symptoms are persistent, throbbing pain.[3-6]

Treatment of gum trauma:

- Rinsing the mouth out with salt water, helps to keep the woundsclean.
- If the bleeding in the mouth is particularly heavy some gauze is applied to stem the flow of blood.
- An ice pack or cold compress is also used to reduce swelling in the mouth.

Wounds in the oral cavity heal faster still being less cared than the wounds in the other parts of the body. Saliva creates a humid environment which improves the survival and functioning of inflammatory cells that are crucial for wound healing. Saltwater has a disinfectant quality which in turn helps in healing the wounds at a faster rate.

Pain on injection: Pain on injection can be due to specific circumstances such a temperate of the solution, velocity of injection, dull needles, damaging soft tissues, blood vessels, nerves, or the periosteum and causing more pain and other complications. It can be very distressing to the patient. The rate of injection and the acidity of the solution are the main factors on which burning depends. Lidocaine causes an intense burning sensation when injected locally. When the needle penetrates a nerve, the patient may also feel a sudden “electric” shock, suddenly moving the head, with the risk of self-inflicted damage. Local anesthetic administration with intradermal or subcutaneous lignocaine infiltration is associated with discomfort.[4]

Factors contributing to the pain during local anesthesia:

- Number of injections
- Size of the needle
- Amount and type of anesthetic
- Level of anxiety of the patient

Sodium bicarbonate was added to the local anesthetic solution which reduces the stinging sensation caused by adrenalin. Bupivacaine was used local anesthesia is used for a longer time. Topical anesthetic is applied to prevent the discomfort of the patient during injection. Switching to a fresh needle is the utmost important work when injection is applied multiple times to the same lesion.[5]

Needle fracture: Needle breakage in the oral cavity after local anesthesia is a rare complication, since the use of non-reusable, stainless steel dental local anesthetic

needles. In most cases, needle fracture happened with 30-gauge needles and during inferior alveolar nerve block, as a result of either wrong injection technique, improper choice of hypodermic needle magnitude, or unexpected motion of the patient or assistants. The needle may damage the intraneural blood supply, resulting in a hematoma or the needle may traumatize the medial pterygoid muscle which results in trismus. When seen in a case of a broken needle, the foremost duty is to remove the broken needle with a hemostat. In case, it is not accessible, a CT scan can be the method for accessing the broken needle by operating the patient under general anesthesia. If a nerve is damaged due to dental local anesthesia, the first treatment should be managing the pain. Only the use of high concentration agents' infiltrations is the source to decrease nerve injury caused due to local anesthesia and to avoid inferior alveolar nerve blocks which in turn prevents frequentative injections.[6]

Systemic Complications:

Systemic Toxicity: Systemic toxicity means toxicity at a cellular level that causes failure of organ leading to the possible death of the organism.

It can be of three types: Acute, Subacute, Subchronic

Local anesthetic systemic toxicity develops when a toxic concentration of anesthetic drugs in the blood level reaches to the central nervous system and cardiovascular systems. Initial symptoms are characterized by central nervous system signs such as excitation, convulsions, followed by loss of consciousness and respiratory arrest. These symptoms are often accompanied by cardiovascular signs such as hypertension, tachycardia, and premature ventricular contractions. Predisposing factors are associated with age, weight, other drugs, gender, the presence of disease, genetics, vasoactivity, concentration, dose, route of administration, the rate of injection, vascularity of the injection site, and the presence of vasoconstrictors.[7]

To prevent systemic toxicity, these should be followed:

- The patient should be evaluated.
- The volume of local anesthesia should be decreased,
- Accurate and slow injection technique,
- Adjustment of dosage divided administration
- Aspirating technique, using agents with low toxicity such as ropivacaine and levobupivacaine.

Allergy: Allergy is also known as hypersensitive reactions. Two types of reactions are involved in allergic reactions to local anesthetics: Immunoglobulin E mediated type I & Tcell-mediated type IV reactions. Allergic reactions may include mild symptoms, such as urticarial, erythema, and intense itching, as well as severe reactions in the form of angioedema and/ or respiratory distress. Before the LA is injected in a person's body, first a skin prick test is done to observe if any kind of reaction is taking place in the person's body. If the test result is seen negative intradermal testing is performed. Intradermal tests are performed by beginning with a lower concentration of the potential allergen and gradually increasing its concentration. Although rare, adverse effects from local anesthetic may be encountered at any time in clinical settings. In these cases, it is important to determine whether the patient is having an allergy. And if a local anesthetic that does not cause adverse effects can be identified, then that drug should be used for local anesthesia in performing dental treatments. If no such drug is available, then the use of general anesthesia might be unavoidable.[8]

Anaphylaxis is an acute potentially life-threatening hypersensitivity reaction. The clinical symptoms of anaphylaxis are depending on the organ systems involved. Uncontrolled co-existing asthma, mast cell disorders, and patients with specific allergens such as peanut and tree nut allergy are the risk factors for anaphylaxis.

Methemoglobinemia: It is a blood disorder in which an abnormal amount of methemoglobin is produced. Hemoglobin is the protein in the red blood cell that carries and distributes oxygen to the body. When methemoglobinemia, the hemoglobin can carry oxygen, but it is not able to release it effectively to body tissues. Methemoglobinemia is a unique dose-dependent reaction where the iron in hemoglobin is stabilized in the ferric (Fe3+) form, unable to attach oxygen.[9]

Causes: Congenital & Acquired

There are two forms of inherited MetHb:

- **Type I:** erythrocyte reductase deficiency, occurs when RBCs lack the enzyme
- **Type II:** generalized reductase deficiency, occurs when the enzyme does not work in the body.

Symptoms of cyanosis will be observed in nail beds and mucous membranes. In more severe cases, headache, dizziness, fatigue, dyspnea, and tachycardia are seen. Management of methemoglobinemia begins with supplemental oxygen (100%) immediately. As a guideline, methylene blue, which is a heterocyclic aromatic chemical compound increasing the rate of conversion of methemoglobin to hemoglobin.

Psychogenic reactions: The patient's body counterbalance to an anxiety-inducing situation or adrenaline secreted by the vasoconstrictor agent are the main factors associated with the psychogenic reactions. Due to psychogenic reactions mood change, respiratory rate, heart rate, blood pressure is also affected. Relaxation of the patient before administering local anesthetic injections is important for the prevention of psychogenic reactions. Using oral sedatives is an effective method to manage dental fears. Patient's physic such as health, age, weight and duration of operation are the main factors for the initial dosage of local anesthesia.[10]

Conclusion

Complications from local anesthesia are often due to overdosage. Predisposing factors, such as the patient's age, weight, state of health, and other medications that the patient may be taking may cause increased free local anesthetic blood levels due to a lack of plasma proteins available for binding. During administration, both the rate of injection and the route of administration can also have an impact on the dosage effect, as rapid injection can lead to increased plasma levels of local anesthetic. Drug factors may also be related to overdosage. While administrating anesthesia, the painless injection should be performed, avoiding intravascular or intramuscular or direct trauma to the nerve. The best way to avoid nearly complications relating to the administration of local anesthetics is to use the right technique and to have a good knowledge of the anatomy of the trigeminal nerve and the adjacent anatomical structure. Concluding the article by the statement that "**New developments should be followed by the practitioners to reduce possible complications associated with the local anesthesia".**

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Role of Interleukin (IL-1β) In Periodontal Disease Progression: A Literature Review

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Abstract

Interleukin (IL)-1B, a proinflammatory cytokine, was uplifted and engaged in periodontal disease progression. And also the linkage in the middle of IL-1B and periodontal disease progression was manifested by clinical documentation; similarly, the expanded IL-1B activates a succession of erythrogenic responses and encourages the bone resorption. At present, IL-1B blockage has been a medicinal plan of action for autoimmune disorder and autoinflammatory diseases such as rheumatoid arthritis, cryopyrin-associated periodic syndromes, gout, and type 2 diabetes. It is theorized that IL-1B be an inherent remedial target for periodontal disease. The review emphasizes the manufacturing, implementation, available treatments, and future potential plan of action for IL-1B in periodontal disease.

Keywords: *Bone resorption, interleukin IL, proinflammatory cytokine, periodontal disease.*

Introduction

Tooth supporting tissues get hold of contamination due to periodontal disease which is an erythrogenic disorder and tooth losing persuade in due course.[1] Microbiological variance of the host and oral microbial flora are disintegrated below the cytology of periodontal disease.[2] The stability of chronic inflammation gets hold by a miscellaneous moderator. The provocative cytokines embrace of IL-1B, tumor necrosis factor (TNF)-α, IL-6 & IL-17, which lead the way to periodontal infection and tissue trauma. One more category incorporates regulative cytokines such as IL-10. IL-β is a strapping stimulator of periodontal tissue demolition.[3] The properties of IL-1B incorporate further bone resorption and as well as the production of tissue demeaning proteinases. Except for periodontal disease, pro-inflammatory lymphokines such as TNF-α, IL-1B, and IL- 6 regulate intrinsic diseases. Consequently, cytokine-grounded plan of action have the prospective to enhance both periodontal disease and systemic health.[4] This review anchors on new cytokines based strategies associated with IL-1B in periodontal disease progression.

As unhealthy cytokines, IL- 1B engage in infection, unsusceptible proclamation, and bone reabsorption in periodontal disease.[5] The clinical discovery furnishes strong documentation about its consequences. The biological reverberations of IL-1B turn on its tissue engrossment, which is upraised in periodontal disease. Strengthen the extent of IL-1B is repeatedly recognized in the saliva and gingival crevicular fluid (GCF) of a patient with periodontal disease differentiated with healthy guidance.[6] Patients with profound pocket depths and more relentless bleeding on probing (BOP) have enlarged the intensity of GCF IL-1B. Besides, serum IL-1B in patients with persistent periodontal disease also holds to a high-rise in magnitude, counting an

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intrinsic reverberations. This discovery exhibit that IL-1B is a practicable uninhibited linkage in the middle of periodontal disease and intrinsic diseases such as cardiovascular diseases. The bob-like receptor proteins-3 (NLRP3) apoptosome indicating passageway, which is accorded to the invigoration of IL-1B, is convoluted in the periodontal pathologic process. The mRNA proclamation of the NLRP3 apoptosome compound was strengthened both gingival inflammations as well as in periodontal disease progression.[7]

Production of IL-1B in Periodontal Disease Progression: Phagocyte and antigen-presenting cells are predominantly indicated by IL-1B. Although, gingival fibroblasts, periodontal ligament cells, and osteoblast cells can also exude IL-1B.[8] The excretion of IL-1B is distinctive. The lymphokines are initially assembled as a proprotein, which is successively proteolyzed towards its agile conformation through caspase-1.[9] The indolent progenitor, pro-IL-1B, is processed in retaliation to microorganism- correlated molecular design. PAMPs and DAMPs concern via design identification of anatomical structure, for example; toll-like receptors, to trigger the signalize adaptor myeloid progenitors demarcation principal retaliation 88(MyD88). MyD88 exhilarating reaction in humiliation of IL 1B as a consideration for the rescue of NF-Kb subdued and in consequence which stimulate to pro-IL-1B dissemination.[10]

Pro-IL-1B is assembled as a biologically immobile configuration that must be proteinase split to obtain ascetic recreation. Caspase-1, to begin with, acknowledgment as an IL-1-transforming enzyme, is up to split pro-IL-1B, directing to the terminating emission.[9] Caspase-1 come in for invigoration by a multimolecular assemblage also called as the apoptosome. Apoptosomeis unflustered of the sensor (PRRs including NLRP1, NLRC4, Pyrin, and AIM2), the adaptor ASC (apoptosis-associated spec-like protein containing a CARD), and caspase-1. As yet, almost all considerations have engrossed on the NLRP3 apoptosome is triggered in retaliation to a variation of PAMPs and DAMPs as shown in figure 1. In macrophages, NLRP3 apoptosome invigoration is suggested to be a two-signal route.[7] The primary gesture is prepared, which is persuaded by microorganisms or else internal stimulants that encourage NLRP3 and pro-IL-1B utterance throughout the invigoration of NF-Kb. The subsequent gesture is prepared, which is activated by estranged ATP, pore-forming contamination, and gritty proceeding detectors, which operate the NLRP3 apoptosome and caspase-1 and cleave pro-IL-1B under its full-grown configuration.[10] The protein gasdermin is an additional substance of active caspase-1. Active caspase-1 cleaves gasdermin at the N terminus and C terminus. The N terminus of gasdermin line orifice on cytomembrane and successively instigate programmed cell death (pyroptosis).[11]

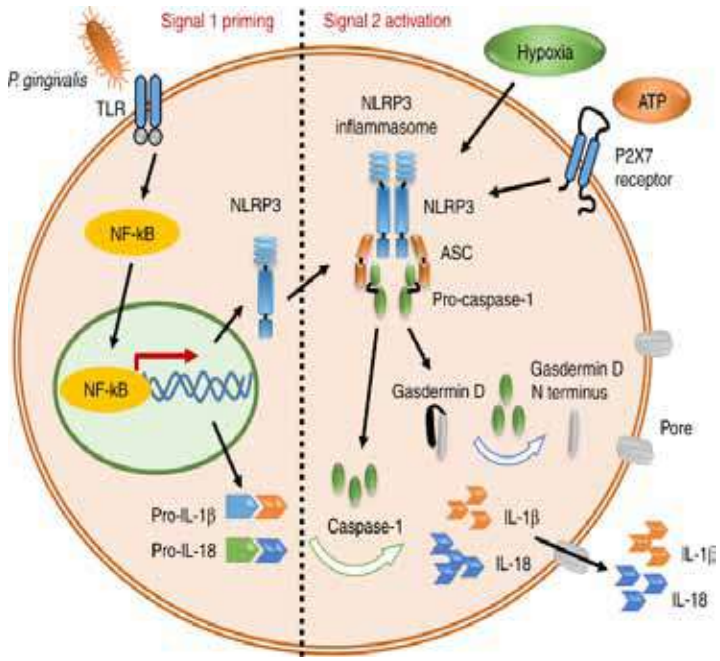


Figure 1. The Two Signaling Pathway For the activation of the NLRP3 apoptosome in periodontal disease progression.

Role of IL-1B In Periodontal Disease progression: In the wake of production, the assembled IL-1B set off a succession of erythrogenic response and engages in the morphology of periodontal disease. Predominantly, IL-1B in the erythrogenic exposition for lengthening internal blood circulation, erythrocyte stipulation, and leukocyte percolation. Besides, IL-1B reacts as a strong impetus of bone constriction, producing a recommended cytokine in periodontal disease. The considerable working is manifested in figure 1.[10]

IL-1B intensifies the proclamation of collagen breaking enzymes, matrix metalloproteinase (MMPs), that cough up to extravascular matrix humiliation, and sequentially guide to bone constriction and tissue demolition. MMP-9 is a principal guideline of the extremity and continuance of periodontal disease. IL-1B deregulation MMP-9 declaration in varied cell types intricate in periodontal disease, as well as bone-forming cell (osteoblast), resorption of bone tissue, and leucocytes, and cementoblast cell as shown in figure 2.[12]

The bodily structure catalyst of inherent feature Kappa-B ligand (RANKL) and osteoprotegerin (OPG) arrangement to synchronize osteochondrosis. Expanded RANKL and accordingly affects osteochondrosis.

RANKL is an anatomy of the integument- correlated TNF substance suborder and is perceived on osteoblasts, bone marrow connective tissue, triggered T cells, periodontal ligament melanocyte, cementoblasts, and endothelium-dependent cells.[12]

RANKL holds together its anatomical structure, receptor catalyst of NF-kB(RANK), indicated on the bone cell predecessor cells, evolving in the invigoration of the bone cell. The conclusive of RANKL to RANK eventually triggers the evidence of NFATc1, c-Fos, and NF-Kb-associated with genetic code, which are extended in periodontal disease and moderate the multiplication, distinction, and invigoration of bone cell. IL-1B too modulates the manufacturing of OPG, and which is a dissolvable cellular structure for RANKL that intercepts it from unalterable to RANK. The OPG administration is transformed by IL-1B and has been correlated with bone transfiguration.[12]

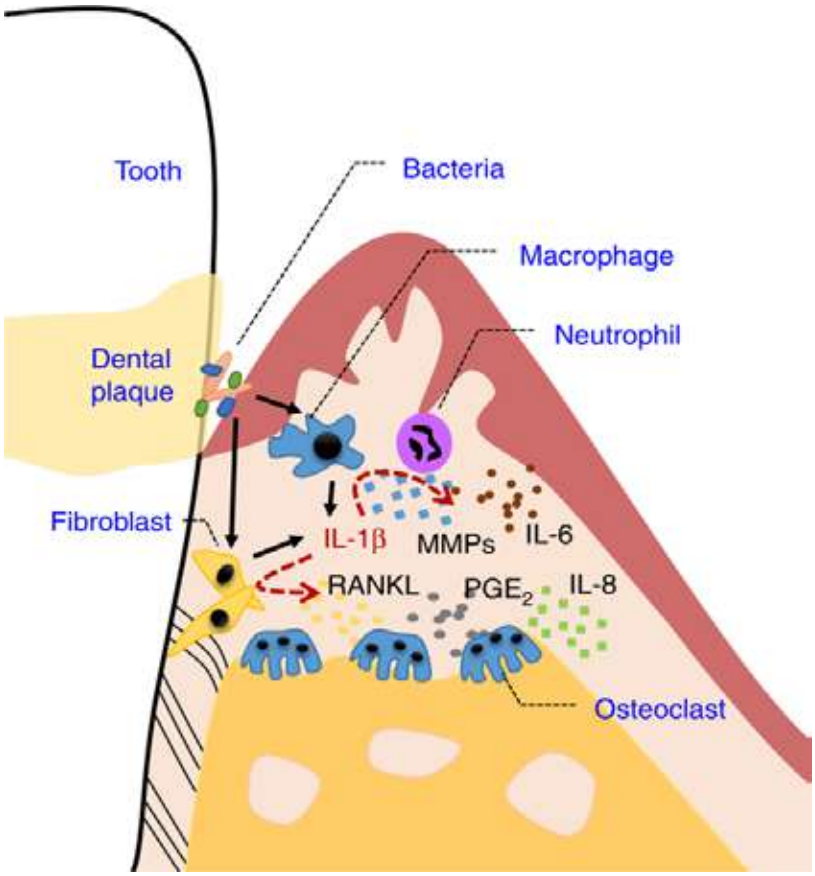


Figure 2: Role of IL-1B In Periodontal disease

Influence of Current Therapies on IL-1B: The following periodontal therapy is done for the treatment of periodontal disease, which may influence IL-1B.

Scaling and Root planing (SRP) and surgery: The minimum interfering and medical procedure therapy to put a stop to and domination to periodontal disease are SRP which is worthwhile too.[2] Although, the involuntary therapy may potentially be insufficient in abolishing the pathological microbes. The extent of unhealthy interferons may be uninterrupted towering after therapy. Precursory wok establishes that SRP did not crucially beneath the IL-1measure. Various contemporary studies also manifest that the IL-1B volume is reduced in the wake of SRP therapy.[13] SRP effects in a reduction in the volume of IL-1B in GCF or saliva. Although, the aggregate is stock-still elevated than in the fit grouping. Also, an evident expansion in the IL-1B volume is encountered featureless in the wake of papillose flap surgical procedure, which propounds that injury and sore relieve effect in lengthening construction Of IL-1B.[13]

Because of the substantial access by SRP, the requisitioning of plaque and transferable cells can be declining in a few areas. Admitting SRP therapies are efficacious in diminishing clinical limitation, it is inadequate in the pro incendiary therapy for enlarged conciliator. Consequently, few helpful treatments have been looked over for this reason.

Antibiotics: The district and organized management of sulfa drugs are subordinate therapy for periodontal disease. Medication as an example (antiprotozoal) metronidazole, roxithromycin (Z), Vibramycin, and augmentin are consistently taken. Objective work has manifested that sulfa drugs assist clinical limitation. Although, its outcome depend on IL-1B is restricted.[14]

The local management of minocycline microcapsules in alveolar resorption during periodontal preservation remarkably diminishes IL-1B in GCF at 5 months. Although, there are no differences on top of 22 months. The integral management of augmentin and antiprotozoal drug is an ancillary therapy for antagonistic periodontal infection.[14] This therapy decreases the IL-1B volume in GCF at 2 months, though it has no outcome at the 5 months check out. Roxithromycin (z) remedy is put in cyclosporine-A- persuades gingival growth. Though, there is no result on the volume of IL-1B.Although, sulfa drugs at or under minimum diffidence applications expand IL-1B production in phagocytes by convincing themto get rid of LPS by *P. gingivalis*.[1]

Laser Therapy: Lasers conceivably applied in a single agent, as an addition to SRP, or in a treatment for opening. Laser treatment on condition that nominal opening of soft tissue and biostimulation results on tissue reconstruction.[15]

Laser treatment remarkably improves clinical limitation, as well as the plaque guide, gingival guide, probing depth, and clinical attachment level as well as BOP.[16] The pathogen limitation IL-1B and TNF-α were turned down as well. Laser treatment lessens IL-1B in aggressive periodontal infection as well as chronic periodontal infection. Though, SRP added to the ancillary utilize of diode laser treatment did not decrease GCF IL-1B volume differentiated to SRP singly, neither it diminish the disease in areas with greater than 5mm PD.

Various categories of lasers are furthermore efficacious in a few specified clinical sites. As Suggested, the laser conceivably applied to cut out gingival tissue and was the at most laser for attainable periodontal reconstruction.[16] The attending corroboration manifests that the laser was even productive in diminishing IL-1B. Although, large indication regarding the varieties of lasers, the difference in wavelength is the most vulnerable criteria required to appraise laser treatment.

Antimicrobial Photodynamic therapy: It is a disinfectant therapy. The treatment is oxygen restricted photochemical reaction that takes place on high light set off invigoration of a definite photo activation combination.[17] The response eventually guides to the fabrication of toxic cells susceptible to oxygen order, for the most part singlet state oxygen, which makes away with pathogens as well as viruses, microbes, and fungi. Due to its disinfected outcome, Antimicrobial Photodynamic therapy beside with SRP is commanding and efficacious in the therapy of periodontal disease.[17] Antimicrobial photodynamic therapy plus SRP developed in a remarkable depletion in probing depth and a repression of IL-1B and MMP-8 in GCF when differentiated with SRP singly Antimicrobial Photodynamic therapy as well as a ratio in GCF when differentiated along with SRP singly. Differentiated along with systemic application of antiprotozoal medication, Antimicrobial photodynamic therapy remarkably let down the pathogen burden and IL-1B volume of the gingival sulcus of rats with periodontal disease. These effects recommend that Antimicrobial Photodynamic therapy has a disinfecting outcome on IL-1B.[17]Generally, typical therapy, together with SRP,

surgery and sulfa drugs, has restricted consequence on IL-1B.[17] Few more auxiliary therapies, along with laser treatment and antimicrobial photodynamic therapy, are analogously highly systematic than normal therapies. Few probable auxiliary therapies might be a newer plan of action to object IL-1B.

Conclusion

IL-1B is a major provocative cytokine take part in periodontal disease progression. As a strapping encouragement for bone constriction, uninterrupted bone deprivation may be persuading by IL-1B. Standard treatment, sulfa drugs, surgery, and SRP have restricted consequences on IL-1B. IL-1B obstruction by blockage, receptor competitors, plant acquired material, agglutinogen, and anti-inflammatory drug factors are advantageous for diminishing IL-1B obstruction to be applied in periodontal disease cure or as an auxiliary therapy in the subsequent.

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Recent Advances in Surface Characteristics of Dental Implants: A Mini Review

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Abstract

Implants have become an integral part of dentistry today and are widely being used for the replacement of missing teeth. A considerable amount of advancements has come in Implant dentistry including the quality of material, surface coatings and processes, and the surgical techniques as well. This review will deal with the implant surface design and its characteristics which have been done till now to enhance the implant-bone contact resulting in better osseointegration and subsequent long-term success of dental implants.

Keywords: *Dental Implants, Osseointegration, Surface coatings.*

Introduction

The idea of permanently being able to replace the teeth has been a desire of civilization since the days of the Egyptians.[1] Periodontitis is the common cause for missing teeth but except that, other causative factors are dental caries, trauma, and genetic disorders.[2] For replacement of the missing teeth, there is various types of materials have been used into the jaw in ancient ages,but the demerits of these materials are, never able to achieve a stable state with surrounding tissues.[2] But luckily our modern dentistry introduces many dental implantation method that are much more successful & painless than previous method of implantation. In the 1950s, Dr. Per-Ingvar Branemark in Sweden discovered an implant with titanium which has sufficient strength to cope with load transferer and named this phenomenon as "Osseointegration".[1] Modification in generally the surface of implants results in exceptional osseointegration.

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Surface Design of Implant:

Before the current implant systems, which are used today; there were various implant systems with geometric designs have been developed.[3] Some common designs of implants are listed below:

1. **Endosseous Implants:** These implants are created to utilize a vertical column of bone, similar to the root of the natural tooth.
   - Bladelike
   - Pins
   - Cylindrical
   - Disk like
   - Tapered type
   - Screw type
   - Both tapered and screw type
2. **Subperiosteal frame like implants:** These are used in the treatment of atrophic mandibles.[3]
3. **Transmandibular implant:** These are developed to retain dentures in the edentulous lower jaw.[3]

The implant microstructure is represented as threaded or nonthreaded. Threaded type is most commonly used now a day. 3 threaded shapes are most commonly used: V-shaped; Square shaped; Reverse buttressed. Threaded implants also classified as self-tapping or pre-tapping.

Surface Characteristics of Implant: It has been shown thatthe healing process improves positively by the surface characteristics which are also called as microtopography of implant.[3] Surface roughness is an important componentinthe osseointegration of the surface of the implant. Roughness in surface of implant shows reinforcement of osteoblastic action at 1 to 100 μm in comparison of smooth surface.[2]

Modifications in Surface Characteristics of Implant: Over the past 15 to 20 years, improvement of implant surface characteristics has been a major area of research interest and development.[3] Modifications of the surface of implant has been investigated and implemented to progress biologic surface properties favouring osseointegration.[2] Modified implant surfaces shows an increase in adsorption of cytokines, mineral ions and proteins; which results in cellular migration and attachment at the molecular level.[3] To improve the quality of bone formation, modification of surface characteristics (microscale or macro scale) is important and it is only possible by treating the implant by various technology, which is already done by today's technology.[3]

Additive Process: The name itself denotes that it is the process in which material should be added. By this process, chemicals are added to the existing surface which results in an improvement in microstructure and macrostructure of the implant surface. Inorganic mineral coating like calcium phosphate, bisphosphonate, hydroxyapatite, etc; plasma spraying, biocoating with growth factors (platelet-derived growth factor, bone morphogenic protein, fibroblast growth factor, transforming growth factor-beta, vascular endothelial growth factor); peptide and extracellular matrix components (hyaluronic acid, chondroitin sulphate, collagen) are different method used as additive materials and/or chemicals to the implant surface. In addition to hydroxyapatite to the surface of the implant, it has been shown that there is both improvement and acceleration in the primary bone cells proliferation or adaptation.[2]

Hydroxyapatite crystals: The key inorganic element of natural bone is the hydroxyapatite crystal. The layering of this key element (hydroxyapatite crystal) permits an influenced and also a quick osseointegration by connecting living bone and the surface of the implant; which was manifested by many investigations.[4] With hydroxyapatite coating the survival rate or continuity rate at 4-8 years, the effect was upto 93.2-98.5% and at 10 years, the effect was upto 82.0%.

Titanium plasma-sprayed: In this method, the survival rate or continuity rate at 20 years, the effect was upto 89.5%.[2]

Plasma spray coating: A combination of HA coating on titanium alloys substrate has good biocompatibility and mechanical properties, which received much attention. The process of layering of hydroxyapatite, incorporates spattering of thermally liquefied materials on the implant substrates.[5]

Subtractive Process: The name itself denotes; it is the process in which there is elimination of materials takes place. In this process, to improve or enhance or to modify the microstructure and chemical nature of the surface of the implant, elimination or alteration of the existing surface is done. It can be done by various ides or method like machining, blasting, acid etching, or a combination of these processes. These method improve the quantity or speed of osseointegration. Among all these the first-generation design of the surface of the implant is the machined surface of implant which is with a rotating surface implant. Surface of implant treated by a process known as grit blasting, in which dispersion of particles can be done by silica or ceramic material. Roughening off the surface of titanium implant by using strong acid like nitric acid, sulphuric acid, or combination of these acids, hydrofluoric (HF) acid is done by acid etching. Sandblasted and acid-etched is the type of acid etching after sandblasting with 250-500μm large grit particles. [2]

Sandblasted and acid-etched (SLA) process: In sandblasted and acid-etched (SLA) process, the continuity rate at 10 years effect was upto 98.8% to 99.7%.[2] Inductions of surface erosins by applying a strong acid onto the blasted surface is done by this SLA process. The combination of large grit sand particles and acid etching is used in this process to obtain macro roughness and micropits. [5]

Grit blasting: It is a frequently implemented physical method, which is used for surface treatments. Grit blasting is followed by an acid etching to remove

the residuals. This process is done by projections of pressurized particles or projections of silica onto the implant surface.[5] Many frameworks, as for examples: size, type, pressure and temperature of the particles may get an influence on the final consequences.[6]

Discrete crystalline deposition: By the process of sol-gel, Calcium phosphate particles of size 20-100nm are coated on a double acid-etched surface named discrete crystalline deposition. Exertion of an intense adhesive force of surface of the implant and making up of surface upto 50% can be done by these particles.[7]

Anodic oxidation: In this method, the implant is exposed to an electric circuit in which it is serving as anode.[7] It is said that when there is surface roughness is 0.44-8.68µm by modification of any surface then it provides a good surface for osseointegration.[2] Mostly good roughness of implant surface can be achieved by doing acid etching and coating.

BIOACTIVE GLASS

The use of bioactive glass to improve the surface characteristics has shown positive results. By the addition of bioactive glass, the bio-inert nature of the titanium dental implants may benefit.[8] Bioactive material helps in the formation of a hydroxyapatite layer with a bond forming between the tissue and a higher dissolution rate and thus faster apatite formation.[8] Bioactive glass has proved to reduce biofilm mass invitro conditions.[8] Bioactive glass actively bonded to the bone and provide antimicrobial protection and it also helps in the reduction of treatment time.[8] 45S5 bioglass, 58S(sol-gel derived) bioglass, S53P4 bioglass are mostly used bioglass materials.45S5 bioglass contains 46.1mol% SiO_2, 24.4mol% Na_2O, 26.9mol% CaO, 2.6mol% P_2O_5. 58S (sol-gel derived) bioglass contains 60mol% SiO_2, 36mol% CaO, 4mol% P_2O_2.S53P4bio glass contains 53mol% SiO_2, 23mol% Na_2O, 20mol% CaO, 4mol% P_2O_5.[9]

CONCLUSION

Dental implants are now the state of art for dental restorative therapy. In order to achieve an abundance integration of hard and soft tissue, adhesion and proliferation of osteogenic as well as fibroblastic cells and minimization of attachment of bacterial during upgrading the recruitment should be the focus of attention of implant development. Several preclinical scrutinizations have shown the superiority of certainsurface modifications concerningto histomorphic properties and biomechanical characteristics. Clinicians should consider the fact that even if several new surface treatments have been proposed, only long term results and rigorous studies can eventually prove its efficacy. To assure an everlasting success in clinically demanding circumstances, the evolution of versatile surface modifications and coatings is essential and recent findings about surface modifications have improved the success rate of implants.

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Isolation of Teeth in Children: A Review

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Abstract

Isolation is done for proper control of moisture, good accessibility and visibility, to provide adequate space for instrumentation and operation. Most of the children are non-cooperative during treatment. So, isolation is mandatorily necessary for pediatric patients. Studies have shown that there are successful retention rates of adhesive restoration when a rubber dam is used rather than any other isolation technique. The judicious use of rubber dam ensures greater patient comfort and delivery of high-quality dental care to pediatric patients.

Keywords: *Access, Rubber Dam, Cotton Rolls, Isolation.*

Introduction

Any operative procedure needs adequate space over the operating field. There must be proper control of moisture, good accessibility and visibility as well as spacious for instrumentation around the working area. For easy manipulation this kind of environment is necessary. Isolation of the working area includes isolation from saliva, blood, gingival crevicular fluid and soft tissues like lips,cheeks,gingiva and tongue.

Goals of Isolation:

Retraction & Access: Without proper retraction and access the details of any kind of operative procedure can not be managed. Maximal exposure of the operating site is provided by retraction access.[1]

Moisture Control: If the moisture in the mouth is not controlled,operative dentistry can not be executed properly. Elimination of gingival sulcular fluid,saliva and bleeding from the working area is referred to as moisture control. It also prevents the spray from hand-piece and debris of restorative material from being swallowed or aspirated.

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Harm Prevention 2: Isolation of the operating field is done to prevent the patient from being harmed during the ongoing procedure. A patient may accidentally aspirate small instruments,restorative debris. Accidentally soft tissue may get damaged. Use of isolation is used bothfor harm prevention and patient comfort and operator efficiency.[1,2]

Types of Isolation in Pediatric Patients[3]

A. Fluid absorbent B. Saliva ejector C. Rubber dam D. Use of Drugs

Fluid Absorbents: By absorption of salivary secretions,the isolation can beachieved. The se are used for short periods when absolute dryness is not required. e.g. examining oral cavity,polishing fluoride application. Various materials that can be used for this purpose are- 1.Cotton rolls 2.Gauze or throat shields 3. Absorbent paper

Cotton Rolls: These are available in different sizes,cut to variant lengths with plain or woven surfaces. The se are placed sublingually,over or lateral to salivary gland orifices. Cotton rolls slightly retracting the cheek provides visibility and access.[1, 2]

Gauze or Throat Shields: It is used in pieces of 2''X2'' or larger. For large areas than cotton rolls, these are better suited. Gauze has less adherence to dry tissues and better tolerated by delicate tissues as compared to cotton.

Absorbent Paper: These are of different shapes to fit various locations in the mouth. The y are more absorbent.

Saliva Ejectors & High Volume Evacuating Equipment: Saliva ejectors are used to prevent pooling of saliva at the floor of the mouth. High volume evacuating equipment removes solid debris along with water.[1, 2]

1. **Metallic**-have rubber dip to avoid irritating the delicate tissues on the floor of the mouth and these are autoclavable.
2. **Plastic-**are inexpensive and disposable.

Requirements: To face backward the tip should always be molded with a slight upward curvature. To prevent injury to soft tissue the floor of the mouth under the tip should be covered with gauze. The lip or cheek mucosa should not get traumatized. Instrumentation should not have interfered. It should be used with other adjuncts e.g. cotton rolls,gauze.[1-3]

Advantages:

(1) Adequate dry field along with the advantage of washed field. (2) No dehydration of oral tissues.

Precautions: Care should be taken to avoid cross-contamination. After each use, the saliva ejector should be disinfected. Disposable tips are preferred. The child should not close his mouth to hold the tip since it may cause backflow of contaminated solution into his mouth as a result of pressure changes.

C. **Rubber Dam:** This is usually latex rubber and one of the most effective means of isolating teeth. It was developed by BARNUM (1864).

Rationale: (1) The clean and visible field is maintained .(2) Aspiration of foreign bodies is prevented. (3) Clinician protection. (4) The risk of cross-infection to the root canal system is reduced. (5) Soft tissues are retracted and protected. (6) By minimizing patient conversation and the need for frequent rinsing efficacy is increased. (7) Medicaments are applied without the fear of dilution.(8) Properties of restorative materials are improved.[3]

The patient gets a psychological benefit.

Contraindication 3:

(1) A child with Upper respiratory tract disorder,Congestion of nasal passage or nasal obstruction. (2) If there is any fixed orthodontic appliance. (3) Any recently erupted tooth that can not hold a clamp. (4) Allergy to latex.

Disadvantages 7: (1) Trauma to tissue. (2) Patient acceptance. (3) Latex allergy. (4) Trauma to tissues. (5) Pressure marks on face due to frame. (6) The build-up of saliva. (7) Psychological intolerance

Benefits to the Child: As glasses are used to protect the eyes,thus rubber dam is used to protect the airways and the esophagus. By doing this a good analgesia has been obtained,the child can feel distanced from the operation. Sometimes it is useful to show the child his isolated tooth in the mirror.[4]

Benefits to the Dentist: This reduces the effort required by the operator to protect the soft tissues of the mouth and the airways. Correctly placed rubber dam allows the operator a better view of the area to be treated by gently pulling the cheeks and tongue away from the operative area. Rubber dam gently pulls the gingival tissues away from the cervical margin;makes it easier to see the extent of any caries close to the margin.

Precautions: (1) The patient should not breathe through the mouth. (2) To retrieve the clamp if ingested or aspirated, clamps must be tied to a floss thread and tightly secured in place. (3) The dam should not cover nostrils. (4) To prevent drying of lips,these should be lubricated properly. (5) The clamp should neither impinge on the gingival tissue nor traumatize the nearby teeth. (6) Latex-free rubber dam should be used on patients with allergy to latex. Rubber dam napkin is used to prevent the latex rubber dam from contacting the patient's tissues.[5]

Armamentarium: I. Rubber dam sheet II. Rubber dam frame III. Rubber dam punch IV. Rubber dam forceps V. Waxed dental floss VI. Scissors VII. Rubber dam napkin VIII. Lubricants IX. Clamp

Euphenisms Used for the Armamentariums: Rubber dam sheet–Raincoat, Frame–Hanger, Clamp–Clip

Rubber Dam Sheet: Rubber dam is available in different thicknesses.

Thin -0.15mm

Medium – 0.20mm

Heavy – 0.25mm

Extra-heavy – 0.30mm

Special heavy – 0.35mm

It is available in prefabricated sizes,i.e. 5"X5" or 6"X6",while non-latex rubber dam is of only 6"X6" size. This has two surfaces which are shiny and dull. As it is less reflective,the dull surface is kept facing occlusally.

Rubber Dam Frame: There are two types of frames i.e. metal frame and plastic frame. The young frame is a metal frame whereas Star visiframe, Nygard-Ostby, Quick dam or Handidam frames are plastic frames. Starlite visiframe, a U-shaped frame,exerts less force on the dam and is easier to use. Nygard Ost by frame does not require any absorbent napkin and stands away from the face. A quick dam comes with a flexible attached frame, which is effective for the control of saliva in the anterior part of the mouth than the posterior part. Handidam has a built-in frame with a rod for insertion to keep the dam open and it comes in one size. Drydam does not require a frame.[6]

Rubber Dam Punch: It is used to punch holes corresponding to teeth size on the rubber dam sheet.

Lubricants: For easier placement of the dam, lubricants are applied in the area of punched holes,which should be water-soluble e.g. shaving cream and soap slurry.

Clamps: These are made up of stainless steel, consisting of a bow and two jaws. The se are used to aid anchoring the dam to the tooth and soft tissue retraction.

Rubber Dam Clamps Used in Pediatric Use: Partially erupted permanent molar- 154A,8A Ivory, Fully erupted permanent molar-14,8 Ivory, Primary second molar-26,27,SS White,3 Ivory,Primary first molar, bicuspid, permanent canine-2,2 A Ivory, Primary incisors and canine-0 Ivory.[7]

Procedure of Rubber Dam Placement: As with any other dental procedure, the preparation of the child for rubber dam application should be done. In a situation where the clamp might impinge on the gingival,local anesthesia should be obtained. The clamp should be secured with floss for easier in quick retrieval;if the clamp is dislodged accidentally. Clamp should be placed using rubber dam forceps on the tooth. Using the rubber dam punch and template punching of an appropriate hole on the sheet should be done. The sheet is stretched with the hole over the clamp lingually, distally and buccally below the jaws of the clamp. The sheet is passed through a rubber dam napkin so that the latex sheet does not come in direct contact with the skin. To prevent bunching of the sheet,stretching and securing the sheet over the rubber dam frame should be done. Where necessary,there ligation of teeth with dental floss and placement of wedges for additional retention should be done. The dam around the tooth should be inverted using a blunt instrument or compressed air from the air syringe. The n, the planned dental procedure can be carried out. Removal of the rubber dam is done by cutting ligature,removing used wedges,cutting interdental material with scissors and removing the rubber dam frame,dam sheet, and clamp.[8]

It is not appropriate to include more teeth in the rubber dam than necessary to get adequate working space. If the first or second permanent molar is the single tooth in the quadrant which is carious in an occlusal area it is often referred to as punch only one hole in the dam.

For single tooth class I or V restorations,only one the carious tooth must be isolated. To restore proximal lesions, at least one tooth before and one tooth after to the tooth being restored should be isolated. It helps in better access, gives more convenient ways to place a matrix and proper field of visualization of where can be done appropriate carving of the restoration along the side of marginal ridges.. This procedure requires only seconds and can save many times.[9]

Recent Advances Inrubber Dam Isolation: Hatdam is a clear plastic form shaped like a hat without a top which is trimmed and fitted around the crown that can not be clamped,to hold the rubber dam in place. Cushioning metal clamp jaw is made up of Ferrite-N which can be pressed in the embrasure area. The material is light-cured,over which the clamp is seated. Cushees are soft thermoplastic cashew-shape nodules which are slipped over tooth attachment blade of the clamp before clamp application. Fiber optic clamps are used in the endo illuminator system. Liquid dam is a resinous material applied to the gingival aspect of the tooth surface used to blockout undercuts before take impression.

Drugs: Antisialagogues can be used to decrease excess salivation e.g. Atropine. Local anesthesia,in addition to the vasoconstrictor in it, brings about a reduction in pain and salivation.

Children's Reaction Towards Isolation: Several studies have been investigated the physiological and psychological responses of children during the dental procedure and their behavior may vary with the type of dental procedure,the number of visits and during the procedure of visit itself. From a previous traumatic experience, originated anxiety and fear or due to general health problems, it might exhibit an emotional upset for most of the children[11]. Children's anxiety and fear of dentistry should be reduced as much as possible before dental treatment[12]. Compared to cotton rolls isolation, rubber dam causes less anxiety in children and adolescents. Most of the children overrule cotton rolls as they think that it may not be as effective as saliva ejector in isolation. In a rubber dam, isolation children feel that the treatment is taking place outside of their mouth. Sometimes, most of the children prefer saliva ejector,as saliva ejector takes away all the saliva,making the operative field clear along with the feeling of control over situation. This happens due to the use of inductive logic i.e. making broad generalization from specific observations by the children of this age group.[10]

Conclusion

From all of the above isolating techniques, rubber dam is the most convenient one. Studies have shown that there are successful retention rates of adhesive restoration when a rubber dam is used rather than any other isolation technique. The judicious use of rubber dam ensures greater patient comfort and delivery of high-quality dental care to pediatric patients. Recently, pre-framed rubber dam sheets have been introduced in which, individual rubber dam sheets are attached to modified frames. The y are easy to use as the rubber dam and holder are applied as a single unit. The se are comfortable for the patient and is easily accepted by patients. These are easily removable. This takes less time to operate.

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Immunity and Periodontal Disease

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Abstract

Periodontal diseases are the inflammatory disorders mostly include gingivitis and Periodontitis, which further leads to tooth loss. Now-a-days periodontal disorders become the most common disease worldwide. Though it is of microbial origin, the destruction mostly depends upon the host response. This article concentrates on the essentials and importance of immunology in periodontal diseases. Significant aspects like immunity, cell of the immune system, Major histocompatibility complex system and Human leukocyte antigen system, participation of the inflammatory cells in the development of the disease, release of chemokines, cytokines, molecular biology etc.

Keywords: *gingivitis, periodontitis, immunity, cells of immune system, host response.*

Introduction

Periodontal disease is a persistent bacterial infection which mostly attack the gingiva and the nearby tissues that support the tooth to hold in the proper position and in the alveolar bone.[1,2] This inflammatory disease results from the bacterial response in dental plaque or biofilm. The bacteria like P. Gingivalis, A. Actinomycetemocomitans and T. Forsythia involve in this disease and remain confine to the gingival tissue with minimal destruction.[1,2,3] But some genetic, environmental and host response can lead to this alteration in tissue to the level of epithelial attachment loss and tooth loss.[2,3] Only the presence of pathogenic bacteria can't be the cause of periodontitis. The defense mechanism of the host plays an important role by including the release of anti-inflammatory cytokines, providing the more numbers of Neutrophils and building the protective antibodies.[1,2] But,this disease progress rapidly due to other factors which may include high levels of inflammatory mediators like cytokines, matrix metalloproteinase (MMPs), low level of interleukin-10 (IL-10), Transferring growth factor and inhibitors of MMPs in the body as the outcome of the disruption or disturbances in the host defense mechanism to the pathogens.[2,3] These mediators stimulate the resorption of the alveolar bone and extracellular matrix destruction in the gingiva.[2,3] This review article further describe the immune system briefly and the impact of this in periodontal disease.

Immunity: Immunity is the reluctance to accept any foreign antigen by the host and towards the injury caused by microorganisms and their products.[4] Immunity is of two types, one is innate or native and another adaptive or acquired. Innate immunity categorized into species immunity, Racial immunity, Individual immunity. And Acquired immunity classified into Active and Passive. Both active and passive immunity further divided into Natural and Artificial type.[4,5]

Innate Immunity: Gives the first line of defense against any kind of infection and microorganisms.[5,6,7] It constitutes of four types of barriers:

Anatomic: Include skin which has thinner outer epidermis and thicker inner dermis. And mucosal member which also consists of an outer epithelial and inner connective tissue.[6]

Physiologic barrier: include temperature, ph. and chemical mediators.[6,7]

Phagocytic barriers: contains Neutrophil, macrophages and Nkcells.[6,7]

Inflammatory Barriers

Acquired Immunity: Develop in the body by the host after the contact with suitable antigen or after transfer of antibodies or lymphocyte from an immune donor.[5,6,7] The acquired immunity has four characteristics features named as; Antigenic specificity-The capability of recognizing even minute difference between antigens.[6,7] Diversity-entitle immense diversity through antibodies as the immune system has perceived and reacted accordingly.[6,7] Immunological memory-remains through out the life of the individual and a second exposure will be responded.[6,7] Self, non-self-discrimination of molecules.[6,7]

Cells of immune system: Lymphoid and myeloid arm of the Hematopoietic system give rise to the principal cells of the immune system. Cells like erythrocyte, phagocytes (Neutrophils and monocyte), eosinophils, platelets, basophils, peripheral dendritic cells, mast cell precursors are derived from myeloid arm in the bone marrow. Peripheral DCs, monocytes and mast cell precursors again get transformed in the tissue.[7,8] Monocyte may change to become a macrophage. Similarly, Natural killer (NK) cells, pre-T cells and B cells are derived from the lymphoid arm in the bone marrow. T cells are found in the thymus; as they are the differentiated form of the Pre-T cells in the thymus. DCs, B cells and antigen presenting cells convert the antigens to T cells in the lymph node and the spleen, which are considered as the secondary lymphoid organs. Final differentiation of T cells and B cells happens in the lymph node and spleen.[7,8]

Mast cells are mostly responsible for the immediate hypersensitivity reaction. They remain very close to the blood vessels and consists of cytoplasmic granules filled up with mediator molecules or effect. Tissue and mucosal mast cells are the two form of mast cells. They vary from each other by their activities, anatomical placements.[7]

Basophils release their cytoplasmic granules which have pharmacologically active components and oppose against the foreign pathogens or microorganisms. They are also associated with allergic response.[3,5,7] Eosinophils have anti-parasitic and bactericidal activities. DCs are found in the basal and supra basal layer of oral mucosa as the cell component of innate immune system. The mechanisms that are lie behind the periodontal diseases by these cells are; conversion or transition of adaptive response, direct relation to the pathogenic signals and ability to impact the other cells of immune response.[3,7]

Macrophages are the quick protective mechanism. Whereas the MHC molecules contain various glycoprotein which recognize the T cells by binding the antigen over it. So, it is also known as "antigen presentation". Neutrophils act as phagocytic cells and they are in large number.[3,5]

Lymphocytes: There are 3 types of lymphocytes like T cells, Bcells and NK cells based on their functions and surface markers. Interleukin-7(IL-7), the triggering factor for forming these lymphocytes from the bone marrow stem cells. Tcells are the important part of acquired immunity. Some different subsets of T cells are present in periodontal tissue. They directly attack and breakdown the infected cells with the help of active macrophages. B cells manifest the humoral immunity.[5,7] The number of B cells is more than the Tcells. NK cells are the particular type of lymphocyte which don't have class 1 MHC molecules and kill the infected cells.

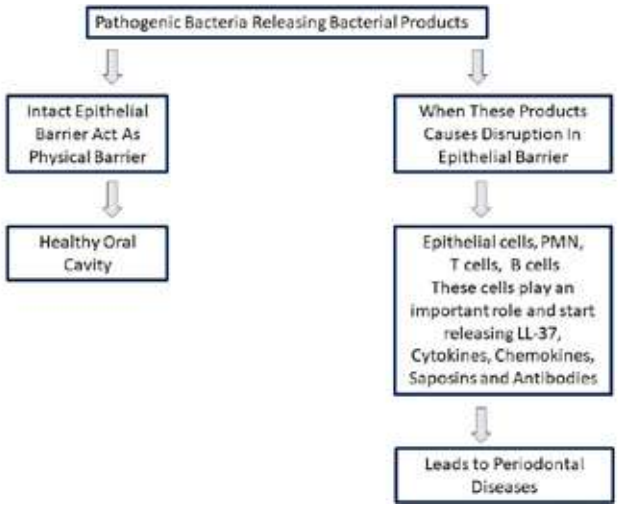


Fig. 1. Mechanism involved in the development of periodontal disease.

Response of innate immunity in periodontal disease: Polymorphonuclear leukocyte plays the main role in continuing the healthy status of periodontium.[1,3] Patients with cyclic neutropenia and leukocyte adhesion deficiency disorders are more vulnerable to the gingival infection or early and sever form of the periodontitis.[3] Defects in PMN in molecular basis can result in development of periodontitis. The protective response of PMN against the periodontal pathogens is degrade when they failure to transmigrate into the endothelium, this ultimately increases the inflammatory response. [5,7,9]

Periodontal epithelium acts as a physical barrier towards the infection. The physical barrier is formed by the epithelial cells, as they remain in constant contact with bacterial products.[9] Incase of disease formation of deep periodontal pocket, inflammation of connective tissue and bacterial invasion is formed due to the migration of epithelial cell.[10] Epithelial cells counter the pathogens by changing the cell differentiation, by proliferation, by changing their cell signaling events.[10,11] Langerhans cells and dendritic cells have the connection with acquired immunity. Epithelial cells create different types of antimicrobial peptides which have been found throughout the body in human.[12,13] These are also related to saliva or remain in the dentogingival junction.[14,15] When the microbial pathogens attack cell-cell junction, the integrity of the epithelial barrier breakdown.[1,11,12,15] These proteins or peptides have an action against gram+ve, gram-ve, yeast and some viruses. They linked to the anionic microbial surface, then assemblage to form pores or damage the microbial membranes, new evidence shows some has cytoplasmic targets.[15,16,17,18] These peptides complement histatins, lysozyme and salivary immunoglobulins, the antimicrobial factors of saliva having a role in innate host defense responding to an infection.[14,15,17] Some peptides like alpha- defensins and cathelicidin LL37 activate the classical complement pathway and increase the IL-8 production by epithelial cells, which enrich the neutrophil count to the site of infection.[11,17,18] Innate immunity has an ability to admit bacteria as non - self agents because the microorganisms have PAMPs in bacterial wall, which are cognized by pattern recognition receptors on immune cells.[17,18,19]

Adaptive immune response in periodontal disease: The adaptive immune response come into role when the epithelial barrier and its component of innate systems, are ruptured.[12,18,19] The balance between Th1 and Th2, Cytokines regulate the immune response against the infection.[16,19] According to some studies a decrease in Th1 response and increase Th2 response may develop periodontitis, some also shows the superiority of Th1 response over the Th2 response. Boyel et al advocated that the integrity of tissues in bone depends on the equilibrium between osteoclasts and osteoblasts.[20] During synthesis, osteoblast express some chemokines receptors. Besides, some important chemokines which are take part in recruitment of PMN and different lymphocyte, can be produced by an osteoclast.[18,20]

Chemo attrition of inflammatory cells in bone environment due to the production of chemokine may lead to bone disruption and tissue destruction.[21] Periodontal disease tissue contain both Tand B cells.[3] The phagocytic activity of macrophages and neutrophils would increase or intensity when INF-alpha is produced by T cells. B cells are activated by IL-4 production which cause the antibody production.[10,20,22] These antibody acts against specific bacterial antigens like LPS of A. Actinomycetemocomitans LPS of P. Gingivalis. This antibody response is mostly facilitating the bacterial clearance and stop the progressive nature of disease and provide protection against bacteria.[9,10]Regulatory T cells are the definite subgroup of CD4+ T cells which give response to any kind of infection depending on the cytokine pattern induced.[8,22] According to Cardoso et al, more no of T regulatory cells in gingival tissues with chronic periodontal disease patient provides the idea of involvement of type of cell in periodontal disease. Anyhow, the action of Tcells in developing periodontal diseases remain unrevealed. [6,7]

Conclusion

This review details about the periodontal disease, contribution of pathogenic bacteria with other factors in development of inflammatory reaction. It also emphasizes the immune system of human body and implication of innate and acquired immunity in initiation and progression of various periodontal disease. Further, there is less evidence about the immune system control the disease as more studies are going on over this.

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Hypochondriac Disorder in OSMF Patient

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Abstract

The main purpose of this article is to present a review on oral submucous fibrosis patients, those who are suffering from hypochondriasis disorder and identifying its interrelationship with their family how they are also suffering due to this problem. The article also focuses on the sign and symptoms of this disorder. Also, it highlights the treatment planning of this disorder.

Keywords: *Hypochondriasis, Oral submucous fibrosis, Sign and Symptoms, Management.*

Introduction

Hypochondriac disorder means 'health anxiety', this is one kind of obsessive disorder that means obsession about a particular condition that may be medically diagnosed or undiagnosed. This usually occurs during adulthood. Oral Submucous fibrosis is a premalignant disorder of the oral cavity. It is a progressive and chronic disorder. The malignant alteration rate of oral submucous fibrosis (OSMF) into squamous cell carcinoma (SCC) is high. OSMF is a multifactorial origin. The most important etiological factor is the habit of chewing tobacco, areca nut product like pan masala, etc. smoking also an important etiological factor for OSMF. Also, alkaloid arecoline identifies as a principal etiological factor for OSMF.[1] The sign and symptoms of OSMFare blanching present in the oral mucosa which leads to rigidity of the oral mucosa and gradually restrict the mouth opening. It occurs at any age but mainly occurs in the 2nd to 4th decade of life(25 to 35 years old). It commonly occurs in southeast Asia and the Indian subcontinent. OSMF divides into different stages of grades, according to their clinical and functional criteria.[2]

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Hypochondriac Disorder: Hypochondriac disorder means anxiety, where an individual is extremely concerned about having a severe disease in his/her body or oral cavity. So we are discussing hypochondriac disorder on OSMF patients. So etiological factor for oral submucous fibrosis is tobacco and nicotine products. So those are having a habit of chewing tobacco and other nicotine product, they are prone to OSMF. When the patient sees the initial sign and symptoms of this disease, they have started panic to see these signs and symptoms. And then repeatedly think about that and after a few days gradually he/she become depressed, so this is called hypochondriasis and a person with hypochondriasis is called a hypochondriac disorder.[3]

Hypochondriasis:

Other names: health anxiety (HA), hypochondria, illness anxiety, Disease, somatic symptom disease

Symptom: fear, anxiety, depression, mentally disturbance

Differential diagnosis: obsessive-compulsive disorder,panic disorder, (OCD), generalized anxiety disorder

Treatment: However, hypochondriasis continues even after a dentist has examined an individual and reassured them about the disease and their symptoms, if the condition is severe then take an appropriate step for this disease. Many hypochondriac patients center of attention on a particular symptom as the catalyst of their

Complications[13]:

A hypochondriac disorder associated with:

1. Relation or family problemsdue to excessive worrying about the disease.
2. Excessive absences at work cause poor performance and other work-related problems.
3. Financial problems due to constant medical visits and their bills.
4. Problem function in day to day life.
5. Having also other psychological disorder such as somatic symptom disorder, depression, anxiety disorder, and personality disorder

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Conflict of Interest: None

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upsetting such as palpitation, muscle fatigue, gastro-intestinal problem, and also it affects the relationship with their family. To be eligible for the diagnosis of hypochondria disorder, the signs and symptoms must have been experienced for at least 6 months.[4]

Hypochondria disorder is characterized by the fear that minor bodily or mental symptoms may indicate a serious illness, continuously self-examination for example 'a person see white patches or white blanching in the mucosa of the oral cavity, then after the patient continuouslysee their oral cavity in the mirror or the phone's front camera' and then self-diagnosis by searching on the internet and this self-diagnosis enhances the patients' fear.[5]

Many persons with hypochondriasis express disbelief and doubt in the dentists' diagnosis, due to this doubtless and disbelief most patients judge the dentists and think this dentist doesn't know anything and then the patient goes for another dentist and then another. And gradually the patient becomes depressed. Many hypochondriac patients experience increased stress, blood pressure, anxiety in the occurrence of doctor/dentist, or nurse a condition known as "white coat syndrome". Most hypochondriac patientsneedcontinuous reassurance about their disease from the dentist or family or their friend. Some hypochondriac patients try to avoid any kindof reminder of an illness. Some patients constantly visit the dentist and frequently ask questions about their illness until their satisfaction. Some sufferers may never speak about it or don't express it in front of anybody anywhere.[6]

Signs and symptoms: Hypochondria disorder is a somatic amplification disease, this is a disorder of cognition and perception, it's a tendency to respondto the initial perception in a harmful manner and further debilitate. This manifests in many ways. Many people have severalinvasive thoughts and physical sensations that force them to check with family, friends, and dentist, for example, a person having severe headache may think that they have brain cancer. Most people are so afraid of any reminder of illness and some avoid thinking as a minor problem. Some also think that the disease is the penalty for past misdeeds.[7]

Hypochondria disorder is quite similar to the other kind of psychological disorder as like bipolar disorder, obsessive-compulsive disorder (OCD), clinical depression, somatization disorder, phobias, and also generalized anxiety disorder. Most hypochondriac patients experience a cycle of invasive thought followed by a compulsive examination which is related to the signs and symptoms of obsessive-compulsive disorder. Some hypochondriac patients are worried about having illness and obsessive-compulsive disorder patients anxious about gettingsick or of transmitting sickness to others. These kind patients are over-possessive and overprotective for themselves and also for others.[8]

Hypochondriac patients are more depressed and anxious to think about their symptoms but they don't aware that depression and anxiety cause the other mental and physical disorders or disease. For example hypochondriac patients experience change in appetite, weight fluctuation, fatigue, decrease the motivation of life, decrease interest to do something like extracurricular activity, or any kind of entertainment. And the most important thing is that this disorder not only affects the patient but also severely affect their family member due to the behavioral change of the patient. Because of this reason family members cannot tolerate the patient's behavior. Intense anxiety causes sweating, increased heartbeat, stomach discomfort, muscle tension, shortness of breath, dizziness, tingling, numbness in a certain part of the body like hand, forehead, etc.[9]

Effects on their life and their family: Above, we discussed oral submucous fibrosis, hypochondriac disorder and its sign and symptoms but now we are going to discuss the most important and the most sensitive topic that is how this disorder impact the patient's life and his/her family members. As we know the medication is the only way to treat this disorder but along with medication, we should assure them that don't think very much about this disease and strictly aware them about to stop bad habits like chewing nicotine products and don't see their oral cavity repeatedly in the mirror.[10]

Those patients don't follow the dentist's advice, they are suffering the most. As before when they repeatedly see their lesion present in their oral cavity, they don't control their habit, as a result, they become mentally unstable. Due to mentally unstable they could not focus on their day to day life and don't eat properly, etc. So in this time family member's support is the most important role. They should stand with them rather than leave them alone.[11,12]

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Management of Epileptic Patient in Pediatric and Preventive Dentistry

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Abstract

Children are the representative of our future society and their proper growth and development must be the chief concern of all. The condition of the oral cavity in special children like a child having Down syndrome, Epilepsy, Cerebral palsy, Autism is mostly connected to their affliction. Due to a load of medical treatment, normally the parents of these special children do not pursue dental treatment. But in the current situation, the complete care for the oral cavity of these children has been provided by the dental professionals. Epilepsy in children is the most common and damaging neurological disorder nowadays. This review shows the management of the diseases related to the oral cavity of an epileptic patient in various method and approaches.

Keywords: *Epilepsy; Pediatric dentistry; Preventive dentistry; Management.*

Introduction

The human brain is full of numerous neurons, tissues in between them, and their extensions. The cells of the brain produce the electrical currents and transfer it to the other cells. The transmission of these electrical signals helps in brain functioning.[1] Seizures are the episodic neurological dysfunction that occurs due to sudden electrical discharge;may be uncontrolled or insufficient. Epilepsy, a condition of chronic, recurrent, unprovoked seizures with abnormal electrical activities within the brain.[3] When the disease gets started in childhood, it may have an impact on the social, physical, and psychological life of that person.[2]

Epilepsy has the incidence of 0.05%and prevalence of 0.5-0.8%.prognosis for the epileptic pediatric patient is good but 10-20%have sedulous seizures which are due to drugs and those cases have management challenges.

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The epidemiological study of Karolyhazy et al showed that; epileptic patients have remarkably worse oral status; than the healthy and sound person. The index of dental diseases is high in these patients due to the lack of maintenance of oral hygiene, oral cavity injury, and socioeconomic background. It's about 50million people in the world have been affected by epilepsy and out of them, 90% belong to the developing countries. It is a group of many types of seizures which alter in their seriousness, aspect, origin, sequel, and management.[3]

The individuals often face the temporary loss of their consciousness with a risk of any harm to the body parts due to this epileptic seizure. Young children and people more than 65 years of age are the most vulnerable age group for this neurological disorder. There is no time specification for the Occurrence of the seizure attack.[3] Epilepsy is a syndrome, not a single disease with greatly conflicting symptoms.[10] Some epilepsy is restricted to specific phases or periods of childhood. Anticonvulsant medications are the primary treatment of epilepsy. Somehow these drug helps in reducing the frequency of the seizures, but some patients don't show any improvement, in these difficult cases surgery may be the choice of treatment.[10,11] Longterm use of these medications shows different types of adverse effects which is also a major problem for these patients.[4]

About 125,000 new epilepsy cases appear in each year, 30% of them are younger than age 18.[5] At the minimum of 8% of the population have one seizure attack but don't have epilepsy. From the latest studies; it shows that 55lacks persons have been affected in India. History of Epilepsy- The word derived from a Greek word called, Epilum-banein meaning to attack. Hippocrates, the first person who wrote a book on epilepsy in 400Bc. According to him, epilepsy is a curse or a sign from God. During the middle-aged people in Europe, epilepsy is called "falling sickness". [5]

Etiology for most of the cases is unknown. Epilepsy may be due to symptomatic or idiopathic reasons.[2-5] Most of idiopathic epilepsy has a genetic origin. About 30-40% of epileptic patients have a genetic origin.[2] When genes are correlated to mutation,it results in different abnormal functioning. Some of epilepsy display the Autosomal transmission. Juvenile childhood absence epilepsy, photosensitive epilepsy, and Generalized epilepsy with febrile seizures plus (GEST) are some of the hereditary epilepsy. In the case of symptomatic epilepsy, pathology of brain-like brain malformation,injury, or some diseases are associated. Brushing the teeth for a prolonged time may be the reason for reflex epileptic seizures according to some of the case report.[5] Metabolic disorder like electrolyte imbalance, hyperglycemia, hypoxia, hypoglycemia, acidosis, CNS infections, trauma, congenital CNS abnormalities, and hypoxic-ischemic encephalopathy are the various important common case in early infants and neonates.[2] Trauma after birth and infectious diseases may be the most common reason for the febrile seizure in early childhood and late infancy stage.[2] Some syndromes such as West syndrome, childhood absence epilepsy, Lennox Gastaut Syndrome, in childhood are related to epileptic or seizure attacks.[6]

Pathophysiology: Seizures are manifested due to the paroxysmal discharge in the cortical neuron. An unexpected variance in the excitatory and inhibitory forces within the cortical neurons leads to a seizure attack. The grey matter of the cortical or subcortical area is the originating place of the seizure. Then abnormal firing of the fewer amount of neurons occurs. Breakdown of normal membrane conductance and inhibitory synaptic currents extent locally to manifest a local seizure or widely to produce a generalized seizure. This onset involves nearby areas by physiologic pathways.

Abnormality of potassium conductance defect in voltage-sensitive ion channels, deficiency in membrane ATPase leads to neuron membrane instability which can cause seizure attacks. In another aspect deficiency of inhibitory neurotransmitters, and a rise in excitatory neurotransmitters evoke abnormal neuronal activity which also results in a seizure attack. Some types of epilepsy may be associated with the mutation of genes. Generalized epilepsy and infantile seizure syndromes have a relationship with the genes that code for protein subunits of voltage-sensitive and ligand-activated ion channels.[7]

Opening of the defective sodium channels for a longer period conducts neurons hyperexcitable which develop the release of glutamate, an excitatory neurotransmitter in huge amount. Then this glutamate binds with the close by glutamatergic neurons; this activated the release of calcium in excessive amounts in the postsynaptic cells and this acts as neurotoxins to the other cells. This mechanism in the mutated genes that code for the sodium channel proteins; is related to the specific form of inherited epilepsy.[8]

Classification:

According to International League Against Epilepsy 2017, Epilepsy is

Focal Onset-Divided into aware/impaired awareness, motor onset, non-motor onset.

Generalized Onset-Is of motor and tonic-clonic type

Unknown Onset-Motor, tonic-clonic, Non-motor

Generalized Type-Each hemisphere of the brain is affected at the same time in this type of epilepsy. The patient is remaining unconscious during the attack; and there is no previous indication of it.[2] The generalized type is divided into; generalized tonic-clonic seizures (grandma), absence seizures (petit mal), and atonic seizures.[2,5,12]

Partial Type-In this type only a particular area of the cerebral cortex is affected. We can diagnose this type of epilepsy by seeing the electroencephalogram results and clinical symptoms. This type also classified into three groups Simple partial seizures; Complex partial seizures; Secondarily generalized seizures.[9]

Status Epilepticus-This term refers to any continuous seizure .most frequently arises after a tonic-clonicseizure. It can be fatal when it remains untreated.[9]

Injuries such as laceration of soft tissue like tongue or buccal mucosa, fracture of facial structure, traumatic injuries like avulsion, Luxation, and fracture of the tooth, TMJ subluxation are induced due to seizure attack.[19] Including that injury like gingival hyperplasia, development of recurrent aphthous ulcers, developmental anomalies like microdontia, delayed eruption, due to neglect in oral hygiene practice there may be the development of secondary infection of the fibrous growth which results in hemorrhagic tissues and enlargement of the lymph node in the cervical region are caused by taking the therapeutic drugs.[9]

Things to be taken into account while delivering the treatment to the patient: Some detailed information must be taken from the patient or the parents before starting the treatment; this must include, the seizure attack frequency, date of the previous seizure attack, patient's consciousness and respiratory state during seizure attack, patient's physical condition after the attack, the appearance of any aura before the attack, presence of any aura weather always leads to seizure attack or not, factors evoking the epileptic attack.[9]

Management of Epileptic Patient in Dental Office:

Preventive Measures: First of all we have to take a detailed and complete medical history about the patient's experience of the seizure attack and also the type,frequency of episodes before the treatment. The most important task to make the patient stress free or reduce the stress by the psycho behavioural preparations, sedation,etc. Diazepam as an anticonvulsant is the drug of choice. The appointment of the patient should be early in the morning hours. The session must be kept short; avoid the use of dental chair light and loud noise. Also avoid the drugs that evoke the seizure attack like phenothiazine, local anesthesia. Proper drug therapy should be given to the patient. Before doing any surgical removal of gingival hyperplasia,the dentist should take the consult of the patient's physician.[10]

Management During The Seizure Attack: When the seizure attack occurs in the dental chair, stop the treatment procedure immediately, and remove all the instruments and dental prostheses, if any there. The supine position is accurate and mostly needed chair position during that time.[9] To prevent the injuries due to attack, the use of tongue blade, mouth prop of rubber,or plastic must be there. If the patient is wearing any tight clothing, it should be loosened. The patient should be moved to another place where she/he cannot injure themselves. During the attack, preventing injury must be the main goal of a dentist. If the patient is unconscious during the attack, then try to maintain the airway patent and regular monitoring of the vitals is necessary.[7] Avoid the aspiration of secretions by using suction, if not possible then turn the head to the side. When the seizure lasts more than 3 minutes,the drug-like diazepam 1mg/kg iv slowly up to 10mg must be given. If the seizure lasts after the drug administration, then shift the patient to the hospital. If the patient becomes conscious; then send him/her to the home to take some rest and postpone the treatment.[11]

Conclusion

Dental issues like tooth loss, caries, and periodontal diseases are found more frequently in an epileptic patient due to poor oral hygiene, adverse effect of the medicine, and trauma during the attacks. Thatswhy they require more attention than other patients. Accordingly, after a detailed informative history and examination, an epileptic patient can get better dental management.

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Intracanal Medicaments in Paediatric Endodontics

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Abstract

The basic motive of Paediatric Endodontic Therapy is to remove microorganisms such as bacteria from the root canal system in which the remaining bacteria cannot survive. Intracanal Medicaments is an imperative step in killing the bacteria in root canals. Moreover, in Paediatric Endodontic, cleaning & shaping is important than intracanal medicaments. The objective of Intracanal Dressing is for a blockade coronal discharge from the space between filling materials and cavity walls. For intracanal medicaments, calcium hydroxide mixed with slim paste is more convenient as it is stable, harmless to the body and bactericidal. If multiple visits to endodontic are chosen, it is strongly recommended. Therefore, the purpose is to examine the need for an intracanal medicament.

Keywords: *Paediatric Endodontic; Medicaments, Formocresol, Glutaraldehyde, Calcium hydroxide.*

Introduction

Intracanal Medicament is a cardinal step in killing the bacteria in root canals and is considered to be an integral part of the success of Root Canal Therapy. In Endodontic Therapy of deciduous teeth because of the convoluted structure of the root canal and the change in the biomechanical preparation is restricted to the only debridement of Root Canals. There is no need of Intracanal Medicament in the vital pulp. When the Root canal space is left unfilled microbial recolonisation takes place and provide shelter to the microorganisms and they are by-products within the pulpal tissue for which it becomes an important part for placing the medicaments within the pulp chamber by their antimicrobial effect.[1] Out of which 2% glutaraldehyde, potassium iodine is reported to have better effect. Dical is also available in two forms which are base (titanium dioxide in glycol salicylate with pigments) and catalyst paste (Calcium Hydroxide and Zinc Oxide in Ethyl Toludine Sulphonamide).[2]

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History: In 1045 AD Serebonium, initialised oils and wine in the mouth of the patient which was a drastic attempt to achieve a topical anaesthetic effect on the tooth. In 1800, various Medicaments were used for Paediatric Endodontic treatment; Phenol was added to preserve and sterilize the canal contents and to pacify pain.[3]

Table 1. Classification of Intracanal Medicaments by Grossman

Essential Oils	**Eugenol**
Phenolic Compounds	Phenol, Parachlorophenol, Cetylpyridinium Chloride (CPC), Formocresol, Metacresylacetate (Crestatin), Cresol, Creosote (Beechwood), Thymol
Aldehydes	Formocresol, Glutaraldehyde
Halides	Sodium Hypochlorite, Iodine, Potassium Iodide, Steroids, Calcium Hydroxide, Antibiotics

Composition of Medicaments:

For Primary Tooth: It includes medicaments such as Formocresol, Glutaraldehyde, Calcium Hydroxide and Ferric Sulphate.

For Permanent Teeth: It includes medicaments such as Calcium Hydroxide, Electrosurgery, Laser Pulpotomy, Bone Morphogenic Proteins (BMP), Osteogenic Proteins I, II, Mineral Trioxide Aggregate (MTA)

Ideal Requirements: It should be stable in solution which is an effective antimicrobial agent. It should have low surface tension and does not stain tooth structure. It should be non-irritating to the periradicular tissue. It should be active in the presence of blood serum and protein derivatives of tissues. It should have a tedious antimicrobial effect with a non-induced cell-mediated immune response.[4]

Properties:

Duration: Activity can inhibit the regrowth of bacteria because of calcium hydroxide. Phenolics dissipate their activity within 24hrs while Tetracycline activity lasts up to 12hrs but the clinical effectiveness of MTAD decreases the duration to 4weeks.

Toxicity: To become a foreign substance, medicaments act as chapters which elicit an immune response. Phenolics and Aldehydes are generally potent cell killers. Due to strong alkalinity of Calcium Hydroxide, it may destroy the Carboxylate and Phosphate groups which results in the collapse in the dentine structure.[5]

Antibiotics: In primary teeth, Ciprofloxacin, Metronidazole & minocycline are used for sterilization of infected root dentine to prevent the resistance development against microorganisms. These drugs penetrate deep into the root dentine which eradicates bacteria. Root canal dressings are found to be effective in a combination of antibiotics. Thus, it can be widely used as an Intracanal Medicaments in Paediatric patients.

Indications: For persistently wet or weeping canals. For neutralizing tissue debris with rendering root canal contents. For eliminating excess microbes which act as a barrier against leakage and for dressing in symptomatic cases.[6]

Recent Advancement of Intracanal Medicaments Used in Paediatric Dentistry:

Bioactive Glass: Invented in 1969, by Dr French, it allows the hard and soft tissues so that the surface gets directly bonded with the materials. Due to their antimicrobial effect pH level is raised which is not effective for bacterial and host cells. Because of complex particles, the dentin powder solution act as a recipient and in aqueous suspension the glass act as a catalyst. As mineralisation of dentin is induced and it possesses a negative effect when used as an intracanal dressing which harms the teeth. It is more effective than Calcium Hydroxide and less effective than 2% CHX.[7]

Ozonised Oil: It can be used as Intracanal Medicaments in various possible ways by dissolving the ozone with oil for lubrication and disinfection purpose. After its use on behalf of Intracanal Medication, the success rate for periradicular lesions and their antibacterial activity is also very high. So it can be used because of its greater efficacy and productivity.

Propolis: It serves as a better Intracanal Medicament because of its anti-inflammatory and good antimicrobial agent but when compared with saline and NaCl, propolis activity is equal to that of NaCl. In the case of pulp and periapical pathologies, it can be used as short term medications.[7]

Octenidine: Due to the prolonged bacterial anti-adhesive activity, it is more effective than chlorhexidine. Hence, complete elimination of root canal is ensured due to its anti-bacterial action which may harm the paediatric patients. Microorganisms may be guarded against the Intracanal Medicaments within the dentinal tubules by the inhibiting effects of dentin and progressive resistance after direct contact with them to Intracanal Medicaments.[7]

Other commonly used intracanal medicaments

Calcium Hydroxide:

Introduced by Hermann, in 1920, as a Calcium Hydroxide paste which is prepared by diluting with sterile or saline water. Lentiluspiral is used for placing the paste inside the canal to full working length. They are used for root inflammatory resorption is inhibited and microbial control is obtained. It serves as temporary obturating material. It stimulates hard tissue formation. It heals the inflammation of the periapical region.[8]

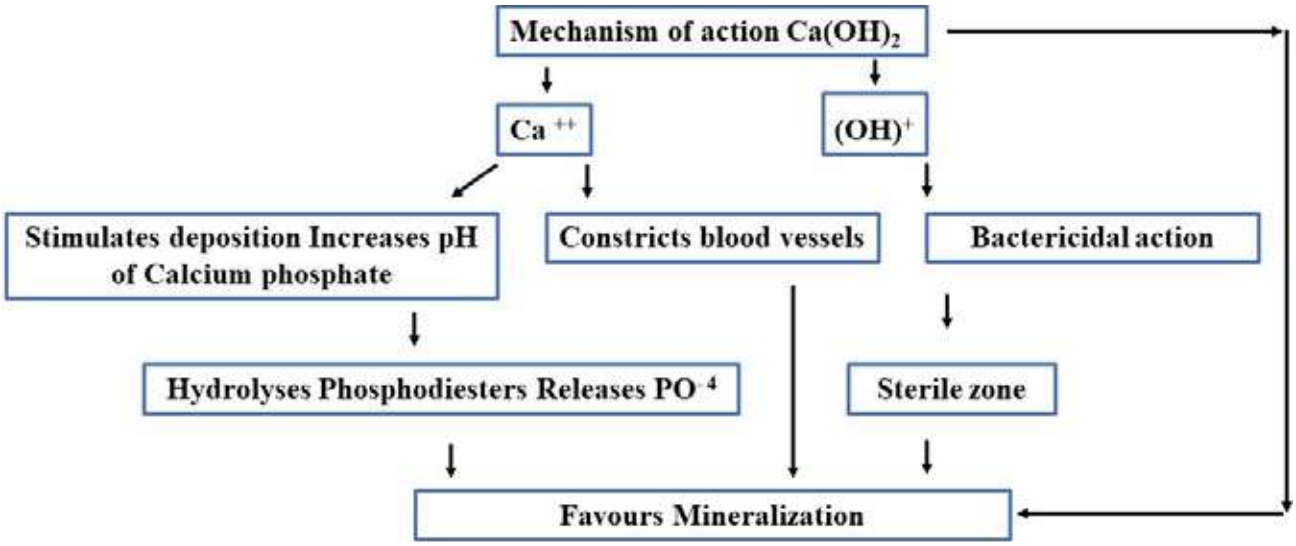


Figure 1. Mechanism of action of $Ca(OH)_2$

Formocresol: It was coined by Buckley, in 1906. It is composed of 35% Cresol, 46% water and glycerine and lastly 1% formaldehyde. It has a proportional ratio of 1:2. It is prepared with a particular mixture which is 3 parts of glycerine diluted with 1 part of distilled sterile water to dilute 4 part in which 30ml formocresol is added to obtain 150ml of diluted Formocresol.[8]

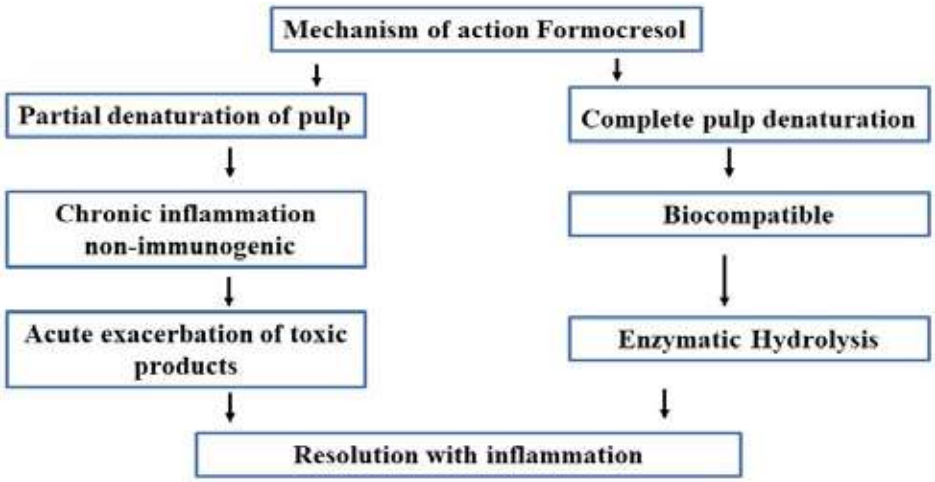


Figure 2. Mechanism of action of Formocresol

Gluteraldehyde: Properties: It is available as 2% Glutaraldehyde. It acts as an excellent bactericidal agent which leads to less necrosis of pulp tissue. It has across Linkage formation with limited depth of penetration. The irreversible changes occur on binding with protein as a result there is no response to the immune system.[9]

Advantages of Glutaraldehyde Over Formocresol: Glutaraldehyde at high concentration act better fixating agent leading to less necrosis of granulation tissue formation on exposition to loner periods than Formocresol. In Primary Tooth, 2% Glutaraldehyde solution placed over root stumps and a drop into ZOE paste, the solutions give better outcome (2% solution) than the drop. Because of smaller molecular size, it cannot reach the apical foramen creating a minimal disturbance in the systematic circulation.[9]

Chlorhexidine Digluconate: It is available in various forms such as MOUTHWASH- (0.12% & 0.2%)–decrease in content of Streptococcus Mutans after 24 hours, GEL- 1% Medicament, VARNISHES- (1%, 10%, 20%, 35%). It is least toxic and effective against *Enterococcus faecalis* which is found in root canal filling with a common pathogen. In Intracanal medicaments, it is used in the form of 2% CHX gel and a mixture of CHX and Calcium Hydroxide which is active against aerobic and anaerobic bacteria.[9]

Rationale and Application of Medicaments: There are signs of canal infection while treating teeth as maintaining asepsis is the means of preserving the antibacterial canal. If there is a violation in asepsis chain the antiseptics measures are necessary to prevent infection in vital pulp extirpation. To prevent infection from the root canal, the rationale behind the intracanal antiseptic medication is to eliminate bacteria. Chemo mechanical preparation cannot eliminate all the bacteria from the root present in dentinal tubules as it has an important cleaning effect. For several days or weeks, it is recommended that root canal should be filled between an appointment with an antibacterial diffusion through the tubules which another aspect in using intracanal medicaments.[10]

Complications and Side Effects: The pulp has ready accessibility to the periapical tissues of systematic circulation. The drug has side effects because of the physiochemical and galenic formation properties which increase the risk of vascular complications. At the site of injection with obliteration of vascular flow, acidic substances cause tissue necrosis. Surgical removal of necrotic substances and extruded materials is the sole motto of the treatment protocol.[10]

Conclusion

In human primary teeth, complete eliminating of aerobic and facultative anaerobic microorganisms was effective as usually not used intracanal medicaments that are Calcium Hydroxide, 1% CHX gel, 1%Metronidazole gel. The intracanal medicaments commonly used is Calcium Hydroxide because of its versatile action, open root apex and weeping canal. For a long time, the root canal compared to other intracanal medicaments retains its effectiveness. In root canal therapy the only important thing is root canal medicament. Efficacy of poly antibiotic paste consisting of Ciprofloxacin, Metronidazole and Minocycline is used in primary teeth referred to as "Lesions Sterilization and Tissue Repair". The increase in the success rate and prognosis of Endodontic Treatment is because of the use of Intracanal Medicament along with efficient biomechanical preparation with copious irrigation. In the initial phase of Endodontic Therapy followed by Calcium Hydroxide dressing, antibiotic is associated with Corticosteroids which is helpful for root resorption and periodontal healing. From the article's perspective, Intracanal Medicaments plays an influential role in Paediatric Endodontics.

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TMJ Ankylosis: A Reveiw

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Abstract

Temporomandibular joint (TMJ) ankylosis is a pathologic condition where the mandiblar fusion takes place to the fossa by bony or fibrous tissues. It interferes with normal day to day activities such as mastication, speech, oral hygiene, etc. Trauma to the chin is the most common factor of TMJ ankylosis followed by infections. This article highlights the eitiology, clinical features, various classifications radiographic features, histopathological features and surgical management of TMJ ankylosis.

Keywords: *TMJ Ankylosis, Unilateral Ankylosis, Bilateral Ankylosis, Gap Arthroplasty, Condylectomy, Interpositional Arthroplasty.*

Introduction

TMJ ankylosis is defined as the merging between the condyle of mandible, fossa, maxilla, zygoma, or bottom of the skull. The fusion may be fibrous or bony. It occurs due to several factors such as trauma(13-100%) most common factor, local or systemic infections (10-49%), or systemic diseases (100%) such as rheumatoid arthritis, ankylosing spondylitis, otitis media, mastoiditis, osteomyelitis of mandible, Reiter's syndrome, hemarthrosis and psoriasis. Trauma to the chin is the most important factor causing TMJ ankylosis. Other etiological factors include secondary rheumatoid arthritis or it may be congenital. It can also occur due to TMJ surgery.[1]

In TMJ ankylosis the chin has deviated towards the affected side resulting in deformity of the face. It leads to various difficulties such as abnormal facial growth, problems in chewing, swallowing, digestion, speech, appearance, and lack of good oral hygiene with multiple dental caries and impacted teeth. Physical and psychological disability may occur due to compromised airway as a result of disturbed facial and mandibular growth. It affects all age groups but more common in children below 10 years of age. It equally affects males as well as females. It is usually seen in the Asian subcontinent.[2]

Kazanjian (1938) classified TMJ ankylosis is of two types: True (intraarticular)- The spinous surfaces of the TMJ are fused. The fusion may be either by bone or fibrous tissues. False (extracurricular)-It takes place outside the joint due to some pathologic condition that results in very less mandibular movements.

Sawhney (1986) graded TMJ ankylosis into four types: Type 1: flattening or condylar deformity with little joint space as seen on radiograph. There are extensive fusions of fibrotic tissues around the joint. Type 2: bony fusion of outer edges of articular surfaces with no fusion in deeper areas of the joint, Type 3: a bony bridge is seen between the ramus of the mandible and the zygomatic arch, Type 4: the whole of the joint is replaced by a bony mass.

Topazian (1966) classified TMJ ankylosis as: Stage 1: Bony ankylosis extending to the condylar process, Stage 2: Here the bony ankylosis has reached the sigmoid notch, Stage 3: Bony ankylosis has extended to the coronoid process. Generally, the classification of TMJ

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ankylosis can be done by the area involved (internal or external), variety of tissue involved (fibrotic or bony), facial symmetry(unilateral or bilateral), and extent of ankylosis (partial or total).[3]

Unilateral TMJ Ankylosis: In unilateral ankylosis due to facial asymmetry chin is deviated towards the affected side. Here the mouth opening is very less. Asymmetry of the face is seen along with fullness in the affected side and relative flattening of the unaffected side of the face.

Bilateral TMJ Ankylosis: In bilateral TMJ ankylosis both sides of the face are affected. Here bird face appearance (micrognathia) is seen. There is no gliding movement neither protrusive nor lateral movement. The presence of a scar on the chin is seen (mostly due to trauma). Class 2 malocclusion occurs along with protrusive incisors and anterior open bite. In a long term case there may be atrophy or fibrosis of muscle. In a congenital bilateral ankylosis there is the difficulty of introducing the nipples into the mouth of newly born infants.[4]

Radiographic Features: In radiographs TMJ ankylosis is detected by various findings such as reduced height of mandibular ramus, joint space is either partially or completely obliterated with sclerotic bone, condyle may be replaced by shapeless bony mass, a prominent antegonial notch is seen in the affected side of the mandible. There is elongation of the coronoid process of the mandible. Sometimes a transverse or oblique dark line is seen crossing the mass of dense bone showing fibrous.

Radiographic investigations of TMJ ankylosis includes lateral oblique view, O.P.G view, cephalometric radiograph, submentovertex view, posteroanterior view, CT Scan.[5]

Histopathological Features: In Type 1 ankylosis irregular fibrous tissue is filled in the joint space. On both sides the cartilage cell layer is thickened and the mid of the joint space is loaded with non-uniformly distributed fibrous tissues. In Type 2 ankylosis the joint space is mostly filled with fibrous tissues and some cartilaginous tissues. In Type 3 ankylosis, joint space is principally packed with the cartilage resembling likehyaline cartilage. Here the chondrocytes were seen enlarged, calcified and expanded towards the bony cavities and joint space showing the bone was active. In Type 4 the joint space disappears, so it is not considered in the histological examination.[6]

Management of TMJ Ankylosis: TMJ ankylosis can be treated both surgically and non-surgically. Non-surgical treatment includes physiotherapy- it includes continuous passive movements, use of tongue blades, etc. Orthodontic treatment includes use of functional appliances.

Surgical management of TMJ ankylosis: Surgical management aims to create a gap between the fragments adequately, to make the joint mobile for improvement of patient's oral health, speech,chewing,etc. It aims to preserve the vertical height of mandibular ramus. It decreases the recurrence rate and preserves normal facial growth pattern. Various complications may occur during surgical treatment such as haemorrhage due to injury to the superior temporal vessels, facial artery, etc. Injury to the external acousticmeatus, auriculotemporal nerve, malar and temporal branch of facial nerve and teeth. Infections and open bite may also occur.Various procedures for surgical treatment of TMJ ankylosis includes condylectomy, gap arthroplasty, interpositional arthroplasty, extraction of the specific dentition.[7]

Condylectomy: It is usually done in fibrotic ankylosis. In this procedure first preauricular incision should be given then a cut is given at the mandibular condyle neck. The top portion of the condyle should be detached from the upper attachment thoroughly. Then suturing should be done in layers. Most common defect of this process is divertion towards the ankylotic side. Anterior open bite may also occur.

Gap arthroplasty: Gap arthroplasty is mostly done in case of bony ankylosis. Here gap is created between the glenoid fossa and ramus of the mandible by removing the bony wedges. Gap helps in mandibular movements. The gap should be atleast 1 cm in order to avoid re-ankylosis.

Interpositional arthroplasty: Interpositional arthroplasty is modified form of gap arthroplasty. Here the gap is filled with certain materials to prevent recurrence of ankylosis. In this procedure gap is created as in gap arthroplasty but here materials are inserted in the gap between the two surfaces in order to maintain adequate vertical height of mandibular ramus and to prevent recurrence of ankylosis. Materials used in interpositional arthroplasty may be autogenous, heterogeneous or alloplastic. Autogenous materials such as temporalis muscles, temporal fascia, fascia

lata, cartilaginous grafts such as (CCGs, metatarsal, sternoclavicular graft,auricular graft), dermis of skin, etc are used. Heterogeneous materials such as chromatised submucosa of pig's bladder, lyophilized bovine cartilage are used. Alloplastic materials includes metallic and non-metallic materials. Metallic includes tantaliumsheets, stainless steel, titanium, gold, etc. Non-metallic includes silastic, teflon, acrylic, nylon, ceramic, etc.[8]

Another treatment for management of TMJ ankylosis include **Kaban's protocol (1990)** It includes **seven** steps: Aggressive resection, ipsilateral coronoidectomy, contralateral intraoral coronoidectomy, creating lining of joint, mandibular ramus is again reconstructed withthe costochondral graft, rigid fixation, early mobilization and aggressive physiotherapy.

Kaban's protocol new (2009) includes the following steps:

Excision of the ankylosed mass: A preauricular incision is given to approach the TMJ along with a hemicoronal incision in order tounveil thetemporalfascia and muscle, zygoma, ankylotic part and the sigmoid notch. A horizontal incision is given to the periosteum above the arch and thenit is then repeatedover the fibro-osseous or bony partand is then continued to the visible portion of ramus of the mandible which is not affected. Then invasive excision of fibrotic or bony part is done. If any TMJ defect is detected thenseggregation of ramus of mandible from base of the skull is done by extension of major osteotomy into the joint. If ulnar notch is identified then minor osteotomy is done by posterior extension 1.5 to 2 cm below the ankylosed mass margin. Then a bur is used for reshaping the base of the skull into a glenoid cavity.

Ipsilateral coronoidectomy (contralateral if necessary): At first a pit is made below the coronoid process and then a wire is placed for the pull. From the depth of the ulnar notchthe osteotomy extends to the intersection of the parallel and perpendicular portion of ramus of the mandible. After completion of the osteotomy the coronoid is placed on the traction with the wire. The whole of the coronoid is removed by cutting the left over temporalis muscle and tendons. If the coronoid tip removal is inadequate, reformation of coronoid occurs and it attaches to the temporalis tendon.

Glenoid fossa lining: During resection of the ankylosed mass if an unaffected part is detected then it should be separated, and replaced on the top of the new glenoid cavity. In some instances the temporomandibular joint is lined with temporalis muscle and temporal fascia flap based inferiorly which is turned and placed into the joint. The flap is inferiorly supported over the deep temporal artery. In order to construct a barrier the deep temporalis fascia and the superficial muscle layer are shifted in order to aid in normal functioning of the ramus which has been constructedor condylar part and to support the blood supply of the flap. Then suturing of the flap done on the front and back side of the soft tissue with 4-0 monocryl suture.[9]

Reconstruction of mandibular ramus and condyle unit: An infra-mammary incision is given to obtain a CCG for the construction of the condylar unit. The cartilage then contoured to have rounded edges. Thickness of the cartilage should be 1 to 2 mm. Trimming and contouring of the rib is done to obtain a good bony interface. Submandibular incision is given. Then the graft with cartilaginous articulating surface is placed against the temporalis flap. This is secured with 2.0 mm titanium bone. Then suturing is done in layers. Reshaping of remaining part of the mandible done in order to obtain a disc shaped tip. Corticotomy is performed, leaving sufficient bone to act as transportation medium. After securing decompression device corticotomydone and movement of the segment is checked by starting off the half buried single directional decompression device. After around 4 days of the treatment active distraction starts at the rate of 1 mm and rhythm of 2 to 4 activations daily. The distraction is ended after the contact of transport disc with the base of the skull. As a result pressure will not be created. Benefits of this are physiotherapy can be started after the surgery on that day donor site and morbidity are not required.[10]

Early mobilization and aggressive physiotherapy: It includes sidewise movement of the mandible in repeated mannerand pulling the digits in front of the mirror. The exercises should be repeated 3 to 4 times per day for 3 to 5 minutes. After completion of 6 weeks of surgery solid food items can be given and the 'Thera-Bite Rehabilitation System should be done 4 to 5 timesper day for 3 to 5 minutes. The physiotherapy procedures also involvesheat treatment,rubbing and kneading and mastication. If the sufferer is unable to achieve the maximal interincisal opening during the surgical treatment orelse if there is no signs for improvement of the maximal initerincisal opening after 6 to 8 weeks then stretching of the mandible should be done under general anaesthesia. Utililizing the Thera Bitesystem 4

to 5 times daily, chewing of gums and manual finger stretching exercises should be done and patients should follow this routine regularly for 1 year.

Recurrence of TMJ Ankylosis After Surgical Treatment: TMJ ankylosis may reoccur after surgical treatment due to following reasons such as lack of sufficient gap between the fragments, if costochondral graft is fractured, if the surface of glenoid fossa is not covered properly, if postoperative physiotherapy is not done adequately and if there is increased osteogenic potential and periosteal osteogenic power which is more common in children.

3 most important factors to prevent re-ankylosis includes adequate gap should be maintained between the fragments of width (1.5 to 2 cm), interpositional barrier should be carefully placed and stabilized that should completely cover the surfaces of the sectioned bone. Adequate jaw exercises should be done regularly for a long term. Amongst the three factors most important one is the use of exercise regularly.[8-10]

Conclusion

From the above discussion it is clear that TMJ ankylosis mainly occurs due to trauma and certain infections and usually takes place in children of younger age. Most of the time due to lack of understanding of clinical features and radiographic features of TMJ ankylosis by the dental surgeon may lead to poor diagnosis of TMJ ankylosis. Patients usually faces difficulty in mastication, speech, facial growth pattern, etc. Patients suffering from this have limited mouth opening. Psychological disorders may also occur. Thus it causes functional as well as aesthetic problems. When it occurs in early age it interferes with the development of mandible. But can be treated by both surgical and non-surgical ltreatment. In surgical management the recurrence rate is very high if not operated carefully. It can be managed by proper exercise of the jaw regularly for several years. Thus if proper diagnosis and proper surgical treatment is done on time with sufficient exercises then good results can be obtained.

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Management of Cleft Lip and Palate in Pediatric Dentistry: A Review

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Abstract

Birth of a child is considered the happiest moment of a parent's life and magical experience where God entitles a mothering power of creation. When a child born with a cleft, it represents a disaster for parents. Surgical restoration of soft tissue continuity of the lip and palate consequences in an approximation of the maxillary segments irrespective of surgical technique. The degree of maxillary width reduction depends on several factors, including surgical technique, the surgeon's skill, scar formation, degree of tissue deficiency, and timing procedures.

Keywords: *Cleft Lip and Palate; Management; Pediatric Dentistry.*

Introduction

Cleft palate is also known as harelip. Orofacial clefts are among the foremost common congenital anomalies. Such anomalies can have several consequences like impaired suckling, defective speech,difficulty in hearing, upper protrusion,gross facial impairment, severe mental disorder. Cleft of the lip and palate is one such condition that happens at such a strategic place within the orofacial region and at such an important time that it becomes a posh congenital deformity. Clefts are complicated conditions,which affect the look, and the activity of the child will concern many other consultants.[1-3]

Incidence: The frequency and geological distribution of clefts vary largely around the world due to discrepancies in birth prevalence as well as failure in the recording of childbirth and congenital malformation. In males,cleft lip is more, not unusual and in the case of women, cleft palate is more commonplace. There are two varieties of clefts that are unilateral clefts and bilateral clefts. About 80% of prevalence seen in one-sided clefts and about 20% of incidence seen in both sided clefts. In the case of one-sided clefts, the left side is mostly affected which is around 70%.Least prevalence noticed among Negroids(0.4/1000) and the highest incidence visible in mongoloid and afghans(4.9/1000).[4-6]

Embryological Background: By fusion of several embryonic processes, the face is formed around the primordial oral cavity. Five branchial arches developed at the position of the future neck around the fourth week of intrauterine life. The first arch, called the mandibular arch performs an important role in the improvement of the naso-maxillary complex. The developing forebrain which is covered by mesoderm proliferates and protrude the stomodeum.

On either side of the mandibular arch,the dorsal end gives rise to bud called the maxillary process.[7]

The frontonasal process gets divided into a medial nasal process and two lateral nasal processes with the formation of the nasal pits. By the fusion of the maxillary process,the upper lip and primary palate are formed with the one medial and two lateral nasal processes. By the 6th week of gestation,the upper lip and the primary palate frequently start merging.[8]

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Development of Palate: The palate is formed utilizinga maxillary manner, frontonasal method, and palatal cabinets have given off using the maxillary procedure. The frontonasal manner gives rise to premaxillary vicinity and palatal cabinets from the rest of the palate. In the 8th week, the fusion of palatal cabinets begins and continues until the 12-seventeenth week. Palatal cabinets covered through an epithelial lining. If palatal shelves be a part of, epithelial cells degenerate. Connective tissue present in palatal shelves be a part of every other and resulting in their fusion.[8]

Development of Lip: The lower lip is formed by the fusion of mandibular sides and the upper lip is formed by the medial nasal and maxillary processes. By the fifth week's gestation, the medial nasal and the maxillary process should be failed by merging one or both sides in the cleft lip.[9]

Table 1. Etiology, predisposing factors & clinical features and syndromes[10-12]

Etiology	1. Infection; 2. Heredity; 3. Environment; 4. Lack of inherent development; 5. Effect of alcohol and certain drugs during the formation
Predisposing factors	1. Toxemia; 2. Diabetes; 3. High maternal age; 4. Reduced blood supply; 5. Folic acid deficiency; 6. Radiation; 7. Racial mongoloids
Clinical features:	1. Inability to suck the mother's milk; 2. Facial deformity; 3. Nasal intonation for the cleft palate; 4. Nasal regurgitation; 5. Tooth defects like supernumerary teeth peg lateral, germination, etc.
Syndromes associated with cleft lip and palate	**Autosomal Dominant:** a: Vander Woude syndrome; b: EEC (Ectrodactyl, ectodermal dysplasia, Cleft).
	Autosomal recessive: a. meckel syndrome; b. Roberts syndrome **X-linked:** a. orofacial digital syndrome type-1; b. Isolated cleft palate with ankyloglossia **Chromosomal:** a. Trisomy 13; b. Trisomy 18 **Non-Mendelian:** a. Pierre Robin syndrome; b. clefting with congenital heart disease **Autosomal recessive:** a. meckel syndrome; b. Roberts syndrome **X-linked:** a. orofacial digital syndrome type-1; b. Isolated cleft palate with ankyloglossia **Chromosomal:** a. Trisomy 13; b. Trisomy 18 **Non-Mendelian:** a. Pierre Robin syndrome; b. clefting with congenital heart disease

Classification of cleft lip and palate:

The following are some of the widely used classifications of cleft lip and palate.

1. Davis classification (1942):According to Davis,clefts are divided into(A) Group-1:Pre-alveolar clefts; (B)Group-2:Post-alveolar clefts; (C) Group3:Alveolar clefts.

2. Kaushal classification (1987):L:Lip; A:Alveolus;H:Hard palate; S:Soft palate; H:Hard palate; A:Alveolus; L:Lip[13]

Problems associated with cleft lip and palate:

1. Ear problem: Conductive hearing loss and chronic suppurative otitis media occur.

2. Speech difficulties:A:velopharyngeal insufficiency; B::Abnormal air

3. Dental problem:a.Local problemFused teeth, enamel hypoplasia, gemination, presence of natal and neonatal teeth,congenitally missing teeth,ectopically erupting teeth,anomalies of tooth morphology,aberrations in a crown shape.b.orthodontic problemAnterior and posterior crossbite, Spacing and crowding,deep bite

4. Feeding difficulties: Cleft lip: for an infant more difficult to suck on a nipple, Cleft palate: breast milk to be taken up into the oral cavity, Upper respiratory tract infection, and regurgitations should be frequent.

5. Nasal deformity and esthetic problem:A:poor nasal shape;B:poor lip function;C:poor dental alignment and smile[14]

Management:

To control cleft lip as well as cleft palate,many experts are associated with it. The crew members are:

1. Maxillofacial surgeons: Maxillofacial surgeons pick out who diagnose and deal with the abnormalities associated with the skeletal pattern of the skull, facial bones, and gentle tissues in coordination with specialists.

2. Pediatrician: The boom of the kid have to be monitored by using the prediction.

3. Pedodontist and Orthodontist: Pedodontist and orthodontist are those who evaluate the position and alignment of the jaws and enamel and coordinate a remedy plan.

4. Speech specialist: Speech expert During developmental stages, the kid is closely monitored and evaluated to assess the communicative abilities incomplete speech

5. Otolaryngologist: Ear contamination and listening to loss is managed

6. Audiologist:To help the evaluation and control of listening to difficulties

7. Genetic counselor: Medical and family history is reviewed to observe the kid's help in analysis.

8. Nurse team co-ordinates Specialized pediatric nursing with the care of the child.

9. Social Worker: The baby and their own family are guided and counseled to address social and emotional aspects.

10. Prosthodontist:

11. Patient care-coordinator[15]

Role of Pedodontist in the management of cleft lip and cleft palate:

A. Feeding and nutritional device: A cleft baby faces difficulty to suckle and encounters problems of nasal regurgitation and malnourishment. Advise proper feeding method 1: Breastfeeding; 2:Bottle feeding

a. Breastfeeding: Ensure if toddler requires prosthetic feeding equipment and in fabricated appropriately a: Require long feeding time; b: Lot of air is suckled thru cleft and consequences in aerophagia which calls for common burping after every 1/2 OZ of feed; c: Mothers have to be advised to take a look at the infant for chocking, cyanosis and stomach disinfection for the duration of feeding; d: Infants must be held at 30-forty five position

b. Bottle feeding: Advise the mother to preserve the toddler at 30-45 angulation or in an upright position. Use of suitable clean teats with good enough slit. Various kinds of teats: 1:Mead Johnson cleft palate nurser; 2: Habermanfeeder. Other normally used teats are: 1: Pigeon cleft teat; 2: Nucknipple; 3: Chu-chu clean feed teat; 4: Chu -chu pass reduce feed teat

B. Dental Management: A thorough examination,

research, and diagnosis of dental anomalies and sickness are necessary. A: Advice Safe diet; B: Prophylactic measures should be needed; C: Obtain proper impressions to fabricate an obturator; D: Educate both patients to keep clean gum pads; E: fluoride application; F: Sealant therapy; G: Early intervention of carious tooth; H: Reinforce the affected person to manage up and ward off mental stress; I: Monitor modifications in speech/heavily capabilities of the kid.[16]

Orthodontic Management: The orthodontic management of cleft lip and palate are:

A: Stage one: treatment done from birth to 18 months of age; B: Stage two (primary dentition stage): from 18 months to the fifth year of life; C: Stage three (mixed dentition stage): from sixth to eleven years of life; D: Stage four (during permanent dentition): From 12-18 yrs of age.

Stage one treatment: 1: Fabrication of passive obturator; 2: Presurgical orthopedics; 3: Surgical management of cleft lip; 4: Surgical management of cleft palate.

a. Passive maxillary obturator: It is a type of intraoral prosthetic device that reduces the incidence and feeding difficulties.

b. Presurgical orthopedics. Reduces the size of clefts and improved speech;

c. Surgical lip closure-Surgery should be performed within 45 days of birth. A rule often should be applied here. According to Millard,surgery should not perform less than 10 weeks of age, body weight is not less than 10 pounds and Hb not less than 10 grams%;

d. Surgical palate closure:(12-24 month of age)- It improves hearing,normal speech and improves swelling.[16]

Stage two treatment: 1: Intra-oral obturation adjustment;2:Check on eruption pattern; 3:Oral hygiene instructions;4:Restoration of decayed teeth

Stage three treatment: Mixed dentition stage, Secondary alveolar bone grafting

Stage four treatment: Regular oral hygiene and dietary counseling is required.

Complication and Disadvantages: 1: Skin irritation; 2: Ulceration of intramural tissue; 3: Irritation of oral mucosa tissue; 4: Parent compliance required.

Diagnosis: 1: Prenatal counselling; 2: Prenatal ultrasound-2D or 3D; 3: Color Doppler ultrasonography; 4:73%-feral cleft lip;5:1.4%-isolated cleft palate.[17-19]

Conclusion

Treatment of cleft lip along with cleft palate should be done at the correct time. If behind schedule in remedy there may be the possibility of advanced abnormalities. So to prevent troubles like speech problems, facial asymmetry, feeding trouble, infection to the nasal cavity, and unaesthetic appearance the remedy is necessary. Oral clefts are the second most commonplace congenital anomaly. It frequently affects speech, hearing, cosmetics, and may at times lead to airway obstructions. The oral and maxillofacial health care professional is a key member of the cleft lip and cleft palate team, to perceive and manage many of the troubles.

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Topical Fluoride for Prevention of Dental Caries: A Review

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Abstract

Topical Fluoride has been the primary human action of dental caries hindrance for many decades. There are various mechanisms that kind it advantageous including inhibiting pathology, enhancing remineralization, and inhibiting microorganism maturation. This paper represents the development, mechanism of action, and manner of use of various fluoride products. The comparative study between the different forms of fluoride preparation is over viewed here. Fluoride toothpaste, mouth rinses, varnishes are some of the products usually utilized for caries prevention. The practician must ascertain sort of topical preparation suitable for different types of patients who are at different risk levels.

Keyword: *Professionally applied topical fluoride; Self-applied topical fluoride; Acidulated Phosphate Fluoride, Monofluorophosphate; Caries prevention.*

Introduction

The water fluoridation system is one of the effective and economical measures for caries prevention, but it is available for a small group of people, therefore other method are required for the application of fluoride. The topical fluoride method is one such method in which high amounts of fluoride are ingested topically or locally. When the tooth erupts, the application of fluoride topically should be done, so that the injection of fluoride occurs and the enamel becomes more caries resistant.[1]

Table 1. Type of topical fluorides[3]

Professionally applied topical fluorides	A. 2 percent Sodium Fluoride. B. 8 percent Stannous Fluoride. C. 1.23 percent Acidulated Phosphate Fluoride. D. Fluoride Varnishes a. Duraphat (Sodium Fluoride b. Fluorprotector (Silane Fluoride) c. Carex. E. Fluoride Foam. F. Fluoride Prophylactic pastes. G. Fluoride containing restorative products.
Self-applied topical fluorides	A. Fluoride Dentifrices. B. Fluoride Mouthrinses. C. Fluoride Gels.

Topical fluorides are of two types:

1. Professionally applied topical fluorides: In the year 1942, Bibby discovered that the use of sodium or potassium fluoride caries can be reduced in the case of children. Mainly 5000-19000ppm i.e, 5-19mgF/ml concentration of fluoride is used.[2]
2. Self-applied topical fluorides: 200-1000ppm i.e, 0.-1mgF/ml fluoride concentration is used.[2]

Indications:

1. Patients with a carious tooth.
2. Children just after tooth eruption period.
3. Patients who are under medication to reduce salivary flow or have undergone head and neck radiation.
4. Mentally or physically challenged persons.
5. Patients who have undergone periodontal surgery when the roots have been exposed.
6. Patients having a dental prosthesis.
7. Eating disorder patients.
8. Patients who have undergone any restorative procedures.

Professionally Applied Topical Fluorides:

A. 2 percent Sodium Fluoride: This technique was given by Knutson. Powder and liquid forms are available. It is made by adding 0.2gm of powder in 10ml. of distilled water. 4 weekly applications of 2%NaF at 3,7,11 and 13 ages.[3]

Method of application: Teeth should be cleaned and polished, Isolate and dry by using cotton rolls, Apply quadrants, Apply for 3 minutes with a cotton applicator, Repeat in remaining quadrants, and Instruct the patient to avoid eating or drinking or rinsing for half an hour.[4]

Mechanism: Sodium Fluoride reacts with hydroxyapatite crystal to form a product i.e, Calcium Fluoride. Then there will be choking off i.e, reduction in the rate. Further Calcium Fluoride reacts with hydroxyapatite to form Fluoridated hydroxyapatite. It leads to an increase in fluoride concentration by converting the tooth surface into the stable in nature and caries resistant.

Advantages: Stable, Acceptable taste, Non-irritating, and No discoloration.[5-7]

Disadvantages: The patient has to make four visits in a few days.

B. 8 percent Stannous Fluoride: It was given by Muhler. This technique involves the use of Stannous Fluoride in powder form. It is made by dissolving 0.8gms of powder in 10ml. Distilled water. The resultant solution is acidic.[5-7]

Method of Application: Teeth should be cleaned and polished, Isolate and dry with cotton rolls, Apply the solution using the cotton applicator on all teeth surfaces, Repeated loading is done and swabbing is done for 4 minutes, Patient is allowed to expectorate after removal, and A six-monthly interval should be advised for next appointment.[5-7]

Mechanism of action: Stannous Fluoride reacts with Hydroxyapatite to form Stannous Tri-fluorophosphate. The product formed is very much useful for the reduction of caries. The stability of the product is also great. Tin hydroxy phosphate is also formed. It is the reason behind the metallic taste which is one of the disadvantages.

Advantages: The frequency of appointments is less.

Disadvantages: Metallic taste, Gingival irritation, Brown pigmentation, and Each time fresh solution has to be prepared. [5-7]

C. 1.23 percent Acidulated Phosphate Fluoride: The solution is known as Brudevold's Solution. Available as a solution or gel. The solution is made by adding 20 gms of NaF in 1lit. of 0.1M Phosphoric acid and adding 50% Hydrofluoric acid. For the preparation of gel, Methylcellulose gelling agent is added to the solution. Other gelling agents like hydroxyethyl cellulose may be used. Frequency-Twice a year. [5-7]

Method of Application: Solution: Oral prophylaxis should be done. Isolate and dry with cotton rolls. Then the solution is applied for four minutes. The patient is instructed to keep his/her mouth close and not to eat or drink anything for a half-hour. Gel: Mouth trays are used. The patient should be in an upright position. If we use Suction it can be easier to do the procedure or else it might be difficult and clumsy. The tooth should be dried. Enough gel should be used but not more than 2-2.5gms per tray. Should be applied for four minutes.

Mechanism of action: Initially, dehydration occurs. Shrinkage of hydroxyapatite crystals occurs. Then hydrolysis and Dicalcium Phosphate dehydrate (DCPD). The penetration of fluoride ions occurs and forms fluorapatite.

Advantages: Stable, No staining, and Self applicable.

Disadvantages: Acidic taste and Glass can't be used as a container.[6-8]

D. Fluoride Varnishes: It is a temporary material that allows the fluoride to adhere to the tooth surface for a longer duration.

Advantages: Well accepted and safe. Requires minimal training.

Duraphat (NaF): First fluoride varnish which was tested. Consists of 2.26 percent Sodium Fluoride or 22.6mgF/ml. In the presence of saliva, it becomes a hard yellowish-brown coating. The effectiveness of Duraphat is between 30 to 45%.

Floor protector (Silane Fluoride): Discovered in 1970. Consists of 70000ppm Fluoride. The main ingredient in this product is Silane Fluoride.

Carex: Tested in Norway. Consists of 1.8% Fluoride. Its efficacy is equivalent to Duraphat.

Method of application: At first oral prophylaxis should be done. Dry and isolated with cotton rolls. First mandibular arch then maxillary arch. Small amounts (0.3-0.5ml or 2drops) of varnish should be dispensed, by using single tufted brush starting proximal surfaces. After that patient is advised to sit opening the mouth for 4minutes. Instruct the patient to avoid rinsing or drinking and also eating any solid food. Advised for taking liquid or semisolid for 18 hours.[6-8]

E. Fluoride Foam: 0.92% F (9200ppm) at pH 4.5.and Commercially available.

Advantages: Less in density and better flow, Lighter than conventional gel, so an only small amount is needed and Doesn't require suctioning so offers home use.

Disadvantages: Retention is less.

F. Fluoride Prophylactic pastes: Prophylactic pastes containing Sodium Fluoride was developed in 1946. In 1960 Stannous Fluoride containing paste was discovered. Nowadays various new products are available like pastes containing Zirconium silicate and Acidulated Phosphate Fluoride silicone dioxide paste etc.

Functions: These pastes contain abrasive materials which remove the deposits present over the teeth. Polishing the tooth surface as well as the restoration. In the case of periodontal or cosmetic reasons, these pastes are recommended. Various studies demonstrate that alone these pastes can't be the effective cariostatic method.[6-8]

Table 2. Comparison between the effectiveness of Professionally applied topical fluoride agents

Agents	2% Sodium Fluoride	1.23% APF	8% Stannous Fluoride	Fluoride Varnish
The concentration of fluoride (ppm)	9200	12300	19500	22600
Effectiveness in caries reduction (in %)	29	22	32	38

Self-Applied Topical Fluorides:

A. Fluoride Dentifrices: Fluoride dentifrices play a big role in the reduction of caries in view that it requires lively participation utilizing the patient to have any effect. It has been demonstrated that the patient who brushes twice a day or greater with 1000ppm or 1500ppm or 2500ppm fluoride dentifrices have substantially reduced caries prevalence. Some of the fluoride products which have been examined for use as dentifrices are Sodium Fluoride (0.2%), Stannous Fluoride (0.4%), Sodium monofluorophosphate (0.76%), Acidulated phosphate fluoride, and Amine Fluoride[6-8]

Mechanism of action: There are two mechanisms of action. One of them is, MFP ions are integrated into the hydroxyapatite crystal. Then there will be the release of fluoride ion, which is further replaced by hydroxyl corporations to form the product called fluorapatite. The second mechanism is, MFP ions itself is integrated into the crystals having alternative products like one or many phosphate groups. As caries activity becomes more in the tooth surfaces, there will be a decrease in the pH level, but by using the fluoride toothpaste, the enamel which has become demineralized can be made back to the original condition, so it is one of the easy and successful method of dental caries prevention.[6-8]

How to use the dentifrices: To prevent further caries, people of almost every age group should use the fluoride toothpaste. The kids under the 6 years age group should also be advised to use but under the supervision of an adult otherwise immoderate ingestion of fluoride can occur. In kids, only a very small amount (less than 5mm) approximating the 'pea-size' have to be placed on the brush.

B. Fluoride Mouth rinses: Over the preceding years, fluoride mouth rinsing has flip out to be an effective and important method of prevention of caries. Various fluoride products used as mouthwashes are Sodium Fluoride, Stannous Fluoride, Amine Fluoride, etc. Among all of these, Sodium Fluoride is mainly used. For the school-based programs, 2gm Sodium Fluoride is added in 1-liter water. By its regular use, caries reduction occurs by 20-30 percent. For personal use, tablets are there i.e,

200mg Sodium Fluoride tablets in 5 teaspoons of water. It is best for mouthrinse for a family who uses it regularly. [6-8]

Recent Advances in Topical Fluoride: At present, there is a drop in the caries prevalence across countries. More and more children are turning caries-free nowadays. Many types of research are also going on in the production and utilization of newer fluoride products. Various fresh and new fluoride products are launched day by day, i.e, fluoride mouth rinses, fluoride whitening agents, toothpaste, fluoride varnishes, etc. Stannous Fluoride Sodium Hexametaphosphate (SFSH), a new fluoride product was presented against the caries problem of various patients. It was used in the mid-2005-2006 year as a composition of the dentifrice. Researches revealed that Silver Diamine also used as an effective material currently. Also, more and more fluoride-containing restorative products are used nowadays rather than usual restorative products. Fluoride varnishes have a higher rate of effectiveness than that of Sodium and Stannous Fluoride.[9,10]

Conclusion

From the various researches and clinical investigations, it was concluded that topical fluoride is one of the easy to use and effective method of caries prevention. It is cariostatic for all age groups. One of its advantages is fluorosis generally doesn't occur due to topical fluoride application. On the other hand, these products might have some side effects like nausea it is high in cost than water fluoridation. Nowadays newer products are discovered and examined for a better purpose which will be more and more useful for the reduction of caries prevalence.

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Ethical Approval: Approved

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A Traumatic Restorative Technique: A Review Article

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Abstract

The art procedure is minimally invasive. It is mainly based on minimum discomfort & preservation of maximum healthy tooth tissue. Using hand instruments reduces pain & vibration, unlike rotary instruments. This procedure is getting more popular,acceptable in children & those individuals who fear & have anxiety about the dental procedure insocioeconomically weaker areas are becoming more popular. Art is suggested to utilize around the world, not only in developed countries but also in industrialized nations. This article gives a short review of art and its uses in the current situation.

Keywords: *A traumatic restorativetreatment (art); Glassionomercement (gic).*

Introduction

Art is a part of the primary oral health program in Tanzania(support of who).Art was needed to be available in less developed countries like the socioeconomically weaker section. Art is the excavation of soft carious dentin with hand instruments only & then restoration is done by using modern restorative material. To minimize the onset of secondary caries presently fluoride leaching gic were used. High viscositygic; simplifies the process of restoration & enables dentine pulp complex to respond against the carious process.[1]

Table 1. History a traumatic restorative technique[2]

Mid 1980	Pioneered in Tanzania done by university of diaries salaam
1986	At the scientific meeting of Tanzania dental association, the results of the piolet study were presented & later officially art born.
1991	Comparison between art & the mobile conventional equipment approach was done by community field trial in rural Thailand.
1992	In the Thailand study, it was clear that children more happily participated who had been treated by the art approach rather than those treated with traditional rotary hand piece approach.
7th April 1994	Art was officially adopted by who on "world health day
1996	In 25 countries, art was used.
2002	Art was declared as minimally invasive dentistry by FDI in Vienna

Definition: Art is defined as a minimally invasive care approach in preventing dental caries and stopping its further progression.

Principles: Evaluating the carious part utilizing hand instruments. With the help of glass ionomer cement. i.e. A restoration material restores the carious lesion which adheres to the tooth.

Goals: To preserve tooth structure, reduce infection, and to avoid discomfort.

Armamentarium: The accomplishment of any treatment relies upon the administration knowing the capacity of different instruments and utilizing them effectively.[3]

Essential instruments:

a) Mouth mirror: to reflect light onto the field of operation to see the hole by implication and to withdraw the cheek or tongue.

b) Explorer: to recognize where delicate caries dentine is present. Do not stick the point into very small caries sore. This may decimate the tooth surface and caries arrestment process. Also do not test into profound pits where yo may harm or presentation the mash.

c) Pair of tweezers: to convey cotton wool rolls and wedges.

d) Dental hatchet: the instrument is utilized for enlarging the passageway to the cavity, for cutting ceaselessly the unsupported and carious polish left.

e) Small, medium-sized spoon excavator: the instrument is utilized to expel soft caries dentine. The little excavator has a distance across of 1mm. This instrument is for use in little holes and for cleaning the veneer/dentin intersection. The width of this medium spoon is 1.5mm. It is utilized for expelling delicate caries from enormous pits adjusted surface is utilized to drivethe material into small pits.

f) Glass slab:this is provided with the cement.

g) Carver: the instrument with double end has two functions. The blunt end is utilized for embedding glass ionomer cement into the cavity and sharp end is utilized to expel the overabundance material and shape the reclamation.

h) Spatula: the spatula is might be metal or plastic.[4]

Material

a) Gloves: for cleanliness reason.

b) Cotton rolls: to assimilate the spit.

c) Glass: ionomer restorative material (powder, liquid)

d) Wedges: these are utilized to hold the plastic strips near the state of the proximal surface of atooth with the goal that remedial material is constrained between the tooth and gums.

e) Petroleum jelly: to forestall glass ionomer cement adhering to the gloves for conclusive protection.

f) Plastic strips & water: utilized for forming the proximal surface in different surface reclamation.

Pre-treatment precautions:

What steps we should do before the start of the procedure:

a) Before doing the art of a carious sore you must know how to set up a good working place.

b) Select and utilize the right instrument.

c) Cross disease

d) Utilize material i.e. Glass ionomer cement.

Why we use hand instrument:

a) Less injury to the tooth conservative/moderate arrangements.

b) Minimal effort of hand instrument

c) Improved contamination control.

d) No use of anesthesia, which decreases physiological injury to children.

e) Makes helpful construction accessible for all population gatherings.

Why we use glass ionomer cement material:

a) Chemical adhesive- limits cutting.

b) Fluoride discharge- caries prevention.

c) Does not cause pulp aggravation consequently atraumatic restorative technique gives preventive and remedial treatment in on the strategy.

Procedure: This method comprises essentially in the removal of carious lesions by hand instruments and filling the subsequent cavity with glass ionomer cement. In any case,it is important to feature that the therapeutic systems are only a piece of the treatment that incorporates oral cleanliness guidance, preventive, and remedial activity.[5]

Light source:

a) The light source can be the sun (normal) or counterfeit.

b) Counterfeit light is more dependable and consistent than regular light.

c) To improve permeability, an uncommon light source fixed to a couple of display outline that is controlled by a battery-powered battery source (voroscope) is utilized.

d) This unit likewise allows amplifying glasses to be connected.

Work stand and positions:

a) The operator ought to sit immovably on the dental chair,having back straight,thighs corresponding to the floor and two feet level on the floor.

b) The stool's height ought to be balanced so that operator can see the patient's teeth.

Patient's position:

a) Patients should be lying on a level surface.

patient head position:

a) For proper accessibility to upper teeth, jawline should be lifted backwardly.

b) For proper accessibility to lower teeth, jawline should be dropped forwardly.

Techniques:

a) Cotton rolls are used to isolate the tooth.

b) Wet cotton pellets are used to clean the tooth surface to be treated

c) A hand instrument is used for widening the entrance of the lesion to remove unsupported enamel rods.

d) Small & medium-sized spoon-shaped excavators are used to remove dental caries.

e) If necessary, calcium hydroxide paste is used for pulpal protection.

f) Clean the cavity surface along with the occlusal margin.

g) According to the manufacturer's instruction mix the glass ionomer cement.

h) The cavity is slightly overfilled by the mixed glass ionomer.

i) Petroleum jelly is coated over the gloved finger & pressed over the entire occlusal surface & slight pressure is applied.

j) Check the bite.

k) Sharp carver used to remove excess material.

l) All high points are removed by rechecking the bite.

m) Once again petroleum jelly is applied on the filling or sealant.

n) Instruct the patient to not bite for at least half an hour.

Indication: Based on evidence & strength of the approach for a certain situation, indication divided into 2 levels.[6]

At the patient level:

a) The art approach is acceptable by patients majorly.

b) Art rarely requires local anesthesia, unlike traditional restorative treatment.

c) The approach should be minimally invasive by nature i.e. Removal of necrotic tissue only.

At the tooth level:

a) Art; single surface restoration using high viscosity gic.in both primary & permanent teeth have a high survival rate.[7]

b) asart restoration are minimally invasive & caries protective unlike traditional restoration method it is considered as a treatment of choice for single surface caries lesion.

c) Art is suggestable for multiple surface restoration but its survival rate is lower as compared to single surface restoration.[8]

Contra Indications:

a) There is the nearness of growth or fistula.

b) The pulp of the tooth is uncovered.

c) Tooth has been agonizing for quite a while (chronic pulpitis).

d) There is a conspicuous carious cavity, however, the opening is unavailable to hand instrument.

e) There is an away form of the cavity, for example in a proximal surface, but the cavity cannot be entered from the proximal or the occlusal bearing.

Advantages:

a) The use of a hand instrument eliminates the noise & vibration of dental handpieces.

b) The need for acid etching, water coolant is eliminated along with accompanying high-velocity suction.

c) The need for local anesthesia is also eliminated because of the use of hand instruments for caries removal.

d) Fluoride-releasing restorative material helps prevent further decay.

Limitation:

a) Long term survival rate of art restoration is unknown.

b) Because of lower wear resistance & strength of the material; used only in small & medium sized one surface lesion.

c) Due to the hand mixing technique, an unstandardized glass ionomer mix is produced.

Failure of the process: Restoration may not be worthy or inadmissible any longer for a few reasons. It is missing, an enormous part of it has broken, cracked restoration, the restoration has eroded or caries has created at the rebuilding edge or somewhere else on the tooth surface.[9]

Restoration is completely missing due to: During restoration procedure contaminated by saliva or blood. The blend of the material was excessively wet or excessively dry. Not all soft caries had been expelled. This sabotaged finish had been abandoned and this later severed.

Part of the restoration has broken: It is plausible that the restoration was excessively high or air bubbles were caught in the material during the arrangement of the restoration whatever the explanation clean the tooth surface and/or staying therapeutic material with a wayfarer or little excavator and wet cotton wool pellets first, before molding the whole surface and materials, fill the hole with another blend of glass ionomer cement and guarantee that the reclamation is not excessively high.[8,9]

The restoration has fractured: this most regularly occurs in a different surface restoration which was too high. The approach to fix it relies upon the area of the crack line and the portability of the brokenpart. if the crack part is free and can be evacuated, fix the hole as portray as above. However, if the broke part cannot be expelled fix through workmanship is an absurd and conventional treatment utilizing a drill is required.[8,9]

The restoration has worn away: Potential reasons, the patient eats hard nourishment as often as possible, the patient grips his/her teeth much of the time or the blend had been excessively wet or excessively dry. The restoration ought to be reconstructed, guarantee that all the outside of the tooth and the rest of the rebuilding are spotless and liberated from soft tissue apply dentin conditioner over the glass ionomer and the depression dividers. Spot another layer of glass ionomer cement on the bygone one, finish the rebuilding as depicted before.[8,9]

Caries has developed:

maybe in nearby fissure or pit. Expel all the delicate rot. Clean and reestablish the new cavity adhere to the restoration as per the standard technique.[10]

Conclusion:

Art is a perfect approach for both alternative & biological treatment in developing countries & social groups in the industrialized world. The outcome results from art restoration in class 1 cavities are much effective than the class 2 cavities. The procedure is very cost-effective so as it is a friendly procedure. It should be considered as an interventional procedure for class 2 cavities, art in both industrialized countries & developing countries are needed to validate its effectiveness & acceptability.

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Stainless Steel Crown: A Review Article

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Abstract

The upkeep of the primary dentition is a non-pathological and sound condition which is important for the general wellbeing of the kid. Stainless steel crown was presented in 1947 by the Rocky mountain company and advanced by Humphrey in 1950. Pre-assembled stainless-steel crown can be adjusted to singular primary teeth then being cemented to give a complete restoration. It provides full coverage restoration, cost-effective, generally modest, and is durable. It is used as a restorative material in both deciduous and permanent teeth where intracoronal restoration comes up short.

Keywords: *Primary tooth, stainless steel crown, pediatric dentistry.*

Introduction

In 1950, a stainless steel crown was introduced by Humphrey to pediatric dentistry. Since then it is used for the treatment of primary teeth that are badly broken as invaluable restorative material. Superior & longer clinical life span than multi-surface amalgam restoration. Durable, relatively inexpensive, quickly & easily placed with minimal technique sensitivity. The main disadvantage is the silver metallic appearance. One of the most widely recognized issues seen in pediatric dentistry in the early childhood caries of primary dentition that influence babies and little youngsters. This early childhood caries results in basic demolition of the teeth along with the nutritional problem. The most widely recognized approach to fix and keep up the rest of the tissue of seriously harmed teeth is utilizing stainless steel crown and covering the harmed crowns of the teeth.[1]

Table 1. History of Stainless-steel crown[2]

1950	Recommended by Humphrey & Engel.
1968	Encouraged familiar treatment molality by mink & Bennett.
1960	Significantly improved crown.

Stainless Steel Crown: Stainless steel crown is a semi-permanent restoration used in a primary and young permanent molar. This crown in primary dentition is the most proficient and reliable technique for restoration. Its resistance as well as retention form offers the advantages of this restoration.[3]

Types Stainless Steel Crown:

1. **Pretrimmed crown:** These crowns have straight, noncontoured sides, however, are decorated to follow a line corresponding to gingival crest. They still require shaping & some cutting.
2. **Precontoured crown:** These crowns are decorated & are likewise pre-contoured. Some cutting and molding might be vital however for the most pre-contoured part are negligible. On the off chance that cutting off these crowns becomes necessary, the pre-contoured will be lost and the crown will fit more freely than before cutting.
3. **Preveneered crown:** These are the treated steel crowns that have gum-based composite clung to the occlusal & buccal surface to make a more aesthetic posterior crown. They are more costly than normal tempered steel crown, require more tooth reduction & take into account just negligible pleating for crown adjustment.[4]

Table 2. Composition of different metal crowns

Stainless steel crown (Rocky Mountain)	a. 17-19% chromium b. 67%iron c. 10-13%nickel d. 4% minor elements

Nickel base crown (In Conell 600 alloy)	a. 72% nickel b. 6-10% iron c. 14%chromium d. 0.04% carbon e. 0.35% manganese f. 0.2% silicon

Table 3. Factors considered for pre-operation evaluation and armamentarium used before the use of mellitic crowns

Factors considered for pre-operation evaluation:	a. The patient's dental age b. The patient's cooperation c. Medically compromised patient d. Motivation of patient
Armamentarium:	1. Burs 2. Instrument 3. Pliers 4. Polishing & isolation 5. Cementing medium

Procedure: Administration of effective local anesthesia so that it will protect from some inevitable trauma of gingival as procedure extend sub-gingivally. Placement of rubber dam provides better visibility, protect tissues around the tooth, improve efficiency. The chosen crown should re-establish the contact area and occlusal arrangement of the readied tooth. The crown determination can be done by estimating the mesiodistal width of the tooth space with the help of a divider. It also measures the components of the contralateral tooth. Tooth preparation removes caries completely and provides sufficient space for a stainless steel crown. Re-establish the tooth using a glass ionomer cement or compomer before the stainless-steel crown preparation. Decrease the occlusal surface by about 1.5 mm with no. 247 flame-shaped bur. The occlusal reduction should be done with the preservation of tooth structure, pulp exposure chance should be less with proper crown retention potential. With the help of tapered diamonds but, cut interproximal cuts mesially & distally. Little buccolingual decrease is required which should be done as less required because these surfaces are significant for retention. Contact with adjacent teeth must be broken gingivally and buccolingually.[5] A suitable size of the pre-contoured crown is picked by estimating the mesiodistal width. For multiple crown placement in the same quadrant, the adjacent proximal tooth surface should be reduced more than usual. Try it should be done before the procedure of cementation. Crown ought to sit close to 1mm sub-gingivally. Crown should be cemented with glass ionomer cement or polycarboxylate cement. Remove the excess cement from the margins. The absence of release of excess amounts of cement reveals inadequate cement. The interproximal surface is cleaned, the region beneath the contact area is cleaned with dental floss. At last, the crown is checked for occlusion. The patient ought to be prompted that there might be some transitory gingival uneasiness when the local anesthesia's wear off.[6]

Hall Technique: Introduction of Hall techniques causes a drastic change in the use of stainless steel crown over the last 10 years. In Scotland, the hall technique was reported and during the audit of dental care, it was identified. Hall's technique is a novel method, for managing carious deciduous molars by cementing preformed metal crown over them. This technique is performed without local anesthesia, caries removal and tooth preparation are not required. To improve consistency in children and decrease nervousness related to dental treatment, apply techniques of behavioral management, stainless steel used should be increased by clinicians. Circumvent expenses of relish treatment and avoid negative child health impact. Reduce tooth extraction and broad treatment.[7]

Procedure:

1. The child should be upright.
2. Choose the smallest size crown to place.
3. All the cusp should be covered with a spring-back effect.
4. During try in the attempt of seating crown should not be done.

Advantages:

1. Clinically acceptable by dentists and patients.
2. Because of its resistance, retention, replacement, and life span stainless-steel crown is preferred over amalgam restoration.
3. Inexpensive and are durable.
4. They adjust to the tooth appropriately and do not bring about any spillage.
5. They offer full inclusion for the tooth, ensuring that it remains secure.
6. They can be set effectively on the tooth.
7. The best treatment of choice for children who need general anesthesia.

8. When contrasted with different materials utilized for restoration, a stainless steel crown has a 6% rate of success.
9. A tooth can be restored even if there is a significant loss in crown structure.

Disadvantages:

1. Unaesthetic because of metal exposure.
2. In partially erupted tooth it cannot be used.

Post-treatment instructions:

1. Precautions should be taken that child does not bite their tongue, cheek, or lip due to the effect of the anesthesia.
2. A mild pain killer can be given to the child by the dentist as to when the numbness has worn off, some discomfort may be experienced.
3. Stay away from sticky food for 3-4 days after crown insertion so that cement under it gets time to get harden.
4. Brushing and flushing should be done.

Alternatives: Silver amalgam, Composite

Stainless steel crown modifications:

Undersized tooth or the oversized crown: This normally occurs when long-standing interproximal caries cause space loss. To decrease the crown perimeter a cut is made on the buccal surface to the occlusal surface. The slice edges are re approximates to cover each other creation the crown periphery smaller. The crown is taken a stab at the tooth and the measure of disregarding fundamental is set apart on the crown. The covered edges are then spot welded. The crown is polished with a rubber wheel and fine abrasive.[8]

Oversized tooth or undersized crown: Isolate the edges varying and weld a bit of 0.004-inch orthopedic band material over the cut surface. After forming apply the bind to fill any infinitesimal insufficiency in the seal. Polish the soldered crown.

Deep subgingival caries: One methodology is to finish the indicated pulp treatment and afterward reestablish the pit planning with silver amalgam is considered as the substitute for the tooth structure. The proximal territory is cut as in standard crown preparation. The stainless steel crown is adjusted with the amalgam substitute for tooth structure at the interproximal finish line. If subgingival caries happens interproximal the unfestooned rocky mountain crown will be sufficiently profound to cover the arrangement.

Complications:

Crown tilt: Due to caries or inadequate use of instruments there may be the destruction of the lingual or buccal wall which may bring about completed crown tilting toward the inadequate side. Before crowning, restoration should be placed which offers to prevent tilting of the crown.

Interproximal ledge: If bur's angulation is not given correctly, then a ledge will be delivered rather than a shoulder free interproximal cut. Failure to expel this ledge brings about trouble in seating the crown.[9]

Inhalation or ingestion of crown: Till cementation proper isolation i.e. rubber dam should be placed because of cough reflex present in a child there will be more chances of crown ingestion. If this happens, the child should be held upside down as quickly. This results in the removal of the crown. If this is ineffective, clinical referral ought to be accomplished for a quick chest radiograph. If the crown remains in lung or bronchi, then it can be expelled by bronchoscopy.

Poor margin: Marginal integrity is decreased if the crown is ineffectively adjusted. Results in recurrent caries. An increase in gingivitis and plaque retention may occur. Stainless steel crown can be used as a restoring material where more than two surfaces are affected in a primary molar. Used in a child who is less than 6 years. In pulp therapy procedures. In the case of fractured primary molars. Excessive loss of tooth surface .eg Abrasion, erosion, bruxism. Children having high caries susceptibility. Used as an abutment. In the case of crossbite. Used in case of repetitive caries around the existing restoration. In the case where restoration is expected to last more prominent than 2 years.[10]

Contraindications: If a primary molar is about to exfoliate where the radiograph shows more than half the primary tooth resorbed.

1. Uncooperative patient.
2. Patient allergic to nickel.
3. In the case of grade 3 mobility.

Conclusion

A conclusion has arrived from clinical investigations that stainless steel crown may last longer than fillings for carious essential teeth, stainless steel crown outperform plastic reclamations when used to re-establish multisurface carious sores in deciduous molar teeth. Besides, it is the most cost-effective, extremely durable, and has minimum technique sensitivity material. All accessible evidence recommends that stainless steel crown should be used as a restorative material in deciduous primary molar tooth.

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Assessment of Restorative Skills for Amalgam Restoration

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Abstract

Dental treatment procedures are mostly governed by factors such as biocompatibility of restorative materials, patients demand for esthetics and conservative approach for the minimal loss of tooth structure. GV Black followed 'EXTENTION FOR PREVENTION' for restorative treatment which follows classical cavity forms and principles which almost remained unchanged and unchallenged for 50years using amalgam. Though it required uniform depth, ease of manipulation and excessive tooth structure damage for proper retention of it. The support from clinical observation of success and failures of different restorations has presented with the concept of new designs. This article reviews the literature on assessing and evaluation of amalgam restoration. Information on the characteristic material, its rate of progress with time and its nature of the association. Mainly focusing on the relationship between the deterioration of amalgam restoration and risk of secondary caries.

Keywords: *Caries, Creep, Silver Amalgam, Tarnish, Restoration.*

Introduction

The restorative dentistry is provided with a wide range of material to store any derangements of a lesion in hard tooth substance. The success and failure of restoration depend on the degree of involvement, the skill of operator and properties and limitations of materials and technique. The purpose is to determine the quality of amalgam fillings and its longevity. The ability of the amalgam restoration to withstand the masticatory forces mainly depends on the distribution of internal stresses and the design of the cavity.[1] The most straight forward method would be restoring a tooth with amalgam material and subjecting the same tooth with forces of similar nature and magnitude to that present during mastication. It can be anticipated that the precision of this technique would be affected by morphologic differences between the duplication of cavity design and variation of the strength of condensed amalgam. The average lifespan of an amalgam restoration is 10-15 years of correct manipulation. Amalgam has been ubiquitously believed to be restorative material of choice for small and large cavities. Despite the use of composite resins with advanced esthetics, amalgam is considered as the restoration of choice for elaborate posterior tooth preparation due to its longevity and ease of use and low cost.[2] According to Healey and Philip, mainly the failure of amalgam restoration occurs due to 56% inadequate cavity preparation and 42% due to faulty manipulation. Many investigators have reported the need for frequent and possible premature replacement of amalgam restoration. Despite continuous preventive programs and the development of better materials, the replacement rate has remained unchanged. The physical properties are generally appraised as adequate for a dental restorative material.[11] The assessment methodology used for cavity preparation must be valid, reliable, and reproducible. The performance of amalgam may be influenced by several factors including its composition, manipulation, presence of moisture, use of varnish and cavity designs.[3]

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Table 1. Criteria for A Successful & Method of Assessing a Restoration Clinically

Criteria for A Successful Restoration Assessment	1. Stop of original insult and its further recurrence 2. Restoration of function/mastication 3. Restoration of esthetics 4. Absence of recurrent caries 5. Marginal accuracy and adaptation 6. Patient comfort and satisfaction
Method of Assessing a Restoration Clinically	1. Marginal Ditching 2. Isthmus fracture 3. Tooth fracture 4. Excessive Discoloration 5. Post Restoration Hypersensitivity 6. Gingival & Periodontal Affections 7. Improper Anatomical Contour 8. Improper Proximal Contact 9. Improper Occlusal Contact 10. Overhangs

Marginal Degradation (Ditching): The breaking of a thin edge of a restoration creating an irregular V-shaped creviceis known as Marginal degradation, ditching or crevicing. This is the most common mode of clinical observation. They are also called as Dyscrasia. Most commonly seen on cavosurface margins. Buccal end of proximal marginal ridge more prone to ditching.

Diagnosis: Either visual/tactile, Amalgam-Enamel interface if evaluated with an explorer or probe, Margins into which the probe penetrates are referred to as open or defects.[4]

Causes: Depletion of support at margin, Voids, Excess Mercury.[4]

Depletion of support at the margin: Amalgam with its property of low tensile strength and low shear strength makes it a brittle material. So, there should be enough of the tooth structure to support it. It may be dueto lack of support or insufficient bulk of the material at margins due to beveling of the occlusal outline.[4]

Voids: The presence of voids in the amalgam cause decreased density and strength of it. They are usually localized. Voids may be due to: Corrosion, Dry mix, Inadequate condensation, Moisture contamination of amalgam

Excess Mercury: Excess Mercury in amalgam decreases its strength. It occurs due to improper proportioning of alloy/mercury ratio, under trituration, inadequate condensation force.[4]

Isthmus Fracture: The occlusal and auxiliary portion of a cavity has the narrowest junction called as the Isthmus. There should be a state of balance between the external and the intrinsic tensile stresses at the isthmus area.

Clinically: Starts as a crack line. Then it propagates and widens upon masticatory forces, and fracture occurs. Hypersensitivity is a common occurrence with food impaction. Factors attributing to isthmus fracture, increase tensile stresses, inadequate resistance and retention form, irregular surface with discontinuities and excessive occlusal force.

Inadequate resistance and retention form: The restorations that are not adequately and not independently retained are easily and quickly broken down by the masticatory forces. So, restorations should have proper resistance and retention form. This is achieved by, flat pulpal floor, gingival floor perpendicular to the direction of the force, walls parallel to the direction of masticatory forces, cavo surface angle 90 degrees, proximal axial grooves, use of the effective adhesive system, use of threaded pins or amalgam pins.[5]

Irregular surface with discontinuities:

a. Poor polishing leaving the surface rough and full of pits

b. Over carving into deep grooves weakens the restoration by decreasing its bulk

c. Presence of internal voids

Excessive occlusal forces:

a. Biting on the restoration before the setting time

b. Under carving of the marginal ridge leading to premature contact

Decrease tensile strength:

a. Insufficient bulk of amalgam

b. Inherent weakness

c. Excess Mercury

d. Structural discontinuity

Insufficient Bulk of Amalgam: Amalgam being a brittle material have a low tensile strength and shear strength. The bulk of amalgam should be at least 1.5-2mm to have sufficient tensile strength. Insufficiency may be due to over carving or decreased cavity depth.[5]

Excess Mercury: This flaw of amalgam decreases the amount of gamma phase resulting in low strength of it, providing the maximum strength to the amalgam by increasing the gamma 2 phase, which is considered to be the weakest.

Structural Discontinuity: This occurs due to corrosion products, moisture contamination, dry mix and lack of condensation force.[5]

Tooth Fracture: Tooth fracture is the fracture of a cusp or ridge under functional forces due to lack of tensile strength to support the remaining tooth structure and much less able to reinforce weak cusp. The careful visual and tactile examination is done to locate the fracture line. This leads to the worst outcome if the restoration is extensive enough, leading to less support of the remaining structure.

It can be prevented by eliminating all undermined enamel as well as weak cusps and ridges.[7]

Excessive Discoloration: These are discolorated areas often seen through the enamel in teeth that have amalgam restorations. It occurs as a result of leaching of corrosive products of amalgam into dentinal tubules.

Clinically:

a. Tarnish- loss of surface lustre

b. Corrosion- the rough, pitted amalgam surface

c. Amalgam Blues- dark bluish discolouration

Tarnish: Formation of a surface film of discolouring oxides and sulfides leading to loss of amalgam lustre. Excess Mercury and moisture contamination enhance this.

Corrosion: It is the actual disintegration of the bulk of amalgam.

It is of two types:

Chemical Corrosion- due to ack of polishing and food stagnation leading to halogenation and sulfurization.

Electric Corrosion- due to two dissimilar or similar metallic restorations (old/new)[10]

Amalgam Tattoos/Blues: Amalgam hues appear through the enamel surfaces. Corrosive products which get entrapped within gingival tissue known individually as amalgam tattoo.

Causes:

a. Thin or undermined enamel

b. Penetration of metallic ions and corrosive product into dentinal tubules.

Post Restoration Hypersensitivity: Hypersensitivity is an amalgam restored tooth maybe because of stimulation of freshly exposed dentine by

a. Galvanic stimuli generated on immediate contact with opposing dissimilar metals.

b. Thermal stresses

c. Pressure or premature contact

d. Stimuli by fluids penetrating through leakage

Clinically appears as:

a. Severe pain after insertion of restoration indicating pulp involvement

b. Pain on hot/cold application

c. Pain on biting indicating premature contact or fractured restoration

d. Dull aching pain after restoration due to delayed expansion of it

Gingival & Periodontal Health: The interplay between the periodontics and restorative dentistry include margins of the restoration, crown contours, and the response of gingival tissue to restorative materials and preparations.

Clinically: Bleeding on probing, Discomfort, Food stagnation, Tooth mobility[7]

Factors: Gingival overhangs, thick subgingival margin, ragged cavity margins, sharp margin restoration, open contact.

Improper Anatomical Contour: Amalgam restoration should duplicate the normal anatomical contour of the teeth. Restoration impinging on the soft tissue has poor embrasure form and proximal contact preventing use of dental floss and are defective restoration. It should be correctly recontoured or replaced to maintain proper periodontal health. Over contoured proximal and facial and lingual surfaces impinge on the soft tissue and act as plaque trap.[9]

Improper Proximal Contact: The importance of proper contacts and contours of restoration is to enhance correct occlusion with sound periodontal

health while preventing gingival overhang. Matrixing is a vital step during placement of different restorations proximal contact are of an amalgam restoration against the adjacent tooth should be evaluated with a string of dental floss.[7] If the contact is open and is associated with poor interproximal tissue health and food impaction, the restoration is defective and should be replaced while tight contact between teeth results in the recorded degree of mobility of both restored and neighboring teeth.[9]

Improper Occlusal Contact: Improper occlusal contact induces destructive effect on the restoration and the tooth due to excessive forces. Occlusal interferences or high points indicate defective restorative skill. It called a high filling when too much of restorative material projects out from the tooth cavity. Clinically tooth with the high dental filling will be sensitive to hot and cold changes and masticatory forces. Premature occlusal contacts appear as shiny spots on the surface or detected using occlusal markings/articulating papers. Correction of occlusal contact in centric and eccentric occlusion should be done. The restoration should be either replaced or adjusted.[9]

Overhangs: Amalgam overhangs are defined as an extension of the amalgam restoration beyond the confines of cavity preparation. Overhangs mainly the proximal ones, possess an obstacle for improper oral hygiene and food impaction, causing severe inflammation of adjacent soft tissues. They also decrease access to proximal cleansing devices. It is challenging to examine the overhanging restorations for a posterior tooth. Therefore, a bitewing radiograph is usually helpful. Overhanging restorations are one of the primary causes for the failure of the amalgam restoration. They are mostly iatrogenic caused by poor operator skill. It is advisable to replace the whole of faulty restoration rather than removing the overhang only.

Clinically:

a. Buccal, Lingual and occlusal overhangs seen visually

b. An explorer can be used for buccal, lingual, occlusal or proximal overhangs by tactile sensation.

c. Dental floss with threading which is passed proximally or proximal overhangs.

Recurrent Caries: Caries developed in a previously restored tooth is termed as recurrent caries. The term Secondary Caries is also given to it. It occurs on the marginal area of the restoration and is detected mainly by the radiographic method. Therefore, also called as marginal caries. The occlusal view is considered as the best view to visualize it.[1] A significantly higher proportion of Streptococcus mutants is found at the cavity margins. Once the restored tooth is diagnosed with recurrent caries, it should be replaced.[10]

Table 2. Causes of recurrent caries

Incomplete elimination of original lesion	a. Improper excavation of caries b. Improper evaluation of lesion
Improper outline form:	a. Leaving defective and retentive pits and fissure b. Leaving undermined enamel
Incorrect restoration	a. Lack of adaptation due to marginal leakage and moisture contamination b. Failure to restore proper anatomy, contact and contour c. Improper finishing of the restoration
Retention of bacteria plaque	a. Retentive pits and fissures b. Marginal overhangs c. Open interproximal contact d. Marginal ditching

Clinically: Carious lesion at the margin of the restoration or extending deeper underneath the restoration.

Conclusion

The article systematically analyzed the performance and clinical success of amalgam restorations. The assessment of the restorative material can be summarized

on the basis of the deterioration of the same. Many factors are leading to the deterioration, including the material, type of tooth, operator and manipulative forces. The deterioration continues with the time but at a decreasing it. Amalgam restorations reduce the need for mechanical retention, conservation of tooth structure, and reducing the effects of microleakage. Despite this, it can be considered the material of choice for more extensive restorations. However, there have been controversies and debate on the use of amalgam in dentistry due to potential release of Mercury and its adverse effect on health. This has led practitioners to explore other alternative restorative materials.

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Benefits of Early Orthodontics Intervention in Children: A Review

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Abstract

When it comes to your children orthodontic treatment then a diamond seems less precious than time. There is an optimal time span to begin your child's early intervention treatment. Mixed dentition is a period of development following the eruption of permanent 1st molar and incisors. During this period, phase-1 treatment is done earlier and often a mixed dentition treatment is meant to concentrate on modification of skeletal system rather than dental system. At little smile's our goal is to provide both timely detection of problems and greater opportunity for more effective treatment to benefit your child/children. The dentist must understand the growth and development patterns before planning a treatment, as jaw growth is the primary factor in orthodontic treatment. Though comprehensive treatment may be required at a later date, the need for early intervention cannot be denied.

Keywords: *Mixed dentition, Children, Early orthodontic correction, Interceptive orthodontics, Preventive orthodontics.*

Introduction

The aim of early orthodontic treatment is to interrupt the growing problem, removing the source, guide the facial development, and jaw bones and allow adequate place for coming permanent teeth.[1]

Purpose:

- Correct obvious problems.
- Intercept developing problems.
- Prevent obvious problems from becoming worse.

The problem can be prevented or complexity can be reduced to an extent, so that further treatment is simplified. From early treatment, almost all types of malocclusion could be benefited. Effectiveness of the intervention depends on malocclusion. The term was given by Lyman Wagers as pre-orthodontic guidance and correction to replace prevention and interruption respectively. The treatment aims to Correct aesthetics of the dentition and facial aesthetics of the patient, improving chewing function and restore proper occlusion. To achieve this goal a repeated multi-speciality dental care is necessary. There are 3 outcomes of early orthodontic treatment. Firstly, no treatment is expected to be necessary. Treatment may be needed in future, while the face and jaws continue to grow, so the child will be followed periodically. If there is a problem, that leads itself to early treatment.[2]

Mechanism affecting occlusal development:

- Occlusal development and craniofacial growth interaction.
- Genetics and environmental factors.
- Locked occlusion.
- Form and function.

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Table 1. First Dental Visit Guide[3]

Early signs of your child which may indicate the need for treatment	• Early or late loss of deciduous teeth • Misaligned bite • Crowding teeth • Large gap between teeth • Front teeth do not meet • Grinding and clenching of the teeth • Thumb sucking, tongue thrusting and other habits • Improper relationship of the upper and lower jaw • Unbalanced facial appearance • Asymmetrical smile • Speech disorder • Difficulty in breathing and chewing or biting • Injury to the mouth or teeth • Extra or missing teeth • Cleft lip and Cleft palate

These above problems and any other orthodontic conditions can be corrected to avoid potential problems for future adult teeth. Many of the orthodontic conditions are inherited while others can arise from dental injuries, accident or anomalous swallowing. These issues could be easier to modify if they are detected and treated early.

Table 2. Timing of Eruption

Mandibular	Maxillary	Range (in Years)
1st Molar	1st Molar	6-7
Central Incisor	-	6-7
Lateral Incisor	Central Incisor	7-8
-	Lateral Incisor	8-9
Cuspid	-	9-10
1st premolar	1st premolar	10-12
2nd premolar	2nd premolar	10-12
-	Cuspid	11-12

Intra-arch Features: In mixed dentition, crowding of the incisors is typical, as the combined thickness of the permanent incisors is 7mm and 5mm greater than primary incisors in the maxilla and mandible respectively.

Early Orthodontic Treatment:

Timing for treatment: Treatment of early orthodontic aims to modify existing or growing skeletal, dentoalveolar and muscular variance to enhance the occlusion and aesthetics of the patient before eruption of the permanent teeth. Many children require the guidance of dental and facial development as the phase-1 of orthodontic treatment.[4,5]

Interceptive Orthodontics (Phase One Treatment): It can help identify along with treat early before they develop into more serious oral problems. It begins when child has most of the primary teeth and the jaw is still growing around age 7.At this age such over-crowding and cross bites may be easier to correct. This concept is used in minor problems during development of dentition. It may increase complexity if untreated. Its main function is to give direction to the growth of the jaw bones which supports the teeth. When the children grow and develops, they initiate early signs of jaw problems. Maxilla which grows too much or too narrow can be seen at a young age.[6] When children above 7 years of age are found to have this discrepancy of the jaw, they should be going for initial orthodontic correction. And also, when children above 8 years of age have malaligned front tooth, initial correction can avert the need to remove permanent teeth. An effective Phase -1 treatment would create space for permanent teeth to an eruption path, or else they will be impacted or shifted. [7]

Pre-orthodontic guidance: Children who have malocclusion in the period of mixed and primary dentition, but don't need banding for therapeutic correction. Early mixed dentition is the ideal time to start phase–one correction.

Goals:

- For growth of the jaw in a way, to achieve the growth of the jaw that will hold all the permanent teeth.
- Improving the way of maxilla and mandible so that they can occlude together.

Advantages:

- Future orthodontic procedure might be more costly, complex and time consuming.
- Greater ability to modify skeletal growth.
- It improves your overall health by reducing or eliminating malocclusions, which are caused by tooth or jaw misalignment.[8]

Benefits if early diagnosis and treatment: Interceptive orthodontics prevents or reduces progression of malocclusion later in life.

1) Achieving better results.
2) Determine a better prognosis for how the permanent teeth will develop.
3) Creates more space for crowded teeth to erupt.
4) Habits are corrected such as thumb sucking and tongue thrusting.
5) Bite problems are corrected such as open bite, cross bite and deep bite.
6) The growth of the jaw is guided to accommodate emerging teeth.
7) Damage to any protruding teeth risk is lower.
8) Functional arrangement of teeth, lip and face are recreated.
9) Improving child's appearance, aesthetics and satisfaction of parents.
10) Improve facial symmetry.
11) Greater ability to modify growth.
12) Reduces time for phase-2 treatment with better and more stable results.
13) Undesirable oral habits can be prevented.
14) Later corrective orthodontic treatment can be prevented.

If untreated, this problems lead to unhealthy environment for the growth and development of teeth, gums & jaws and face.

Table 3: Major Benefits[6-10]

Earlier is better	Some conditions are easier to correct, while identified earlier in life
Developing Mouth	In mixed dentition period ample space is present. If it is determined earlier that dental arch is small to fit all permanent teeth, then it may be possible to expand the arch. This can guide the teeth into better position in the mouth and makes more space in the mouth and reduces the need to extract teeth.
Early Prevention	It can help a child to avoid braces in future, which will save the child's stress from oral hygiene maintenance as well as parent's money
Out with the Old, In with the New	It can help detect and correct harmful habits like thumb sucking and tongue thrusting habits. As these problems start earlier in life.
Enhance Confidence	Every child needs a better occlusion for an attractive face. Early intervention can help to improve facial aesthetics.

Contra Indications:

1. Impracticable treatment due to social immaturity of the child.
2. Due to social and parental pressure, patient is unable to treat the misaligned teeth.
3. Unco-operative patient to continue the treatment.

Monitoring Progression of teeth:

- After the completion of Phase – 1 Treatment, teeth are not in their final place.
- It will be decided and achieved in the Phase – 2 correction.
- Removal of certain primary teeth may be enhanced eruption during this phase of rest.

- Accordingly, for monitoring periodic recall appointments and mandatory on a 6 – months basis.

Phase-2 treatment: This treatment will be required in many patients. It begins when the child has most of the permanent teeth in their final position. Teeth and jaw alignment are completed with upper and lower braces. It moves all the permanent teeth and gives proper position to the teeth, jaws and a beautiful smile with an attitude face and profile. Certain type of appliances was established in the diagnosis and treatment scheme was used to modify and realign the teeth and jaw, at the beginning of phase – 1 Treatment. Phase – 2 Treatment begins after all the permanent teeth have exploded and typically includes braces for an average of 2 years on all the teeth. Retainers are worn following correction in Phase – 2 Treatment is over to ensure your child retains his or her beautiful and healthy smile.[9-11]

Purpose: Extending transparency to achieve the optimal balanced functional and aesthetic outcome that will remain stable throughout your child's life.

Goals: To ensure that each tooth has an exact position in the oral cavity and compatibility with the tongue, lips, cheeks and other teeth. Once this balance is established the teeth should work properly together.[9-11]

Conclusion

Orthodontic modification in the period of mixed dentition opens the door to apply his acuity and experience to an orthodontist and a pedodontics. Malocclusion is not necessarily predictable and also not necessarily avoidable. However, with regular dental check-up and early orthodontic evaluation and treatment, your child may be able to avoid it in the future. Absence of proper scheme can give rise to a big disadvantage. It should be noted that there is commonly more risk in, "Too much too soon, rather than in too little too late".During developing and growing phase of teeth and bone, growth modification is required. If they are identified earlier in life, then some orthodontic conditions are easier to correct. Most acceptable results can be achieved by thorough diagnosis and treatment scheme. Early orthodontic intervention plays a major role in children.

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Bell's Palsy: A Review

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Abstract

Bell's palsy is a facial nerve defect of unknown etiology. The aim of this article is to put forth a review on Bell's palsy and identifying its clinical presentation, differentiate it from other similar conditions, having a brief idea of various existing treatment modalities. Treatment usually focuses on restoring the function of facial muscles and recovery.

Keywords: *Bell's palsy, clinical features, differential diagnosis, various treatment modalities.*

Introduction

The term **facial palsy** generally refers to the "weakness of the facial muscles, mainly resulting from temporary or permanent damage to the facial nerve"[1]. If the etiology of facial palsy is not known then it is considered to be Bell's palsy. It is a unilateral acute peripheral neuritis originating due to disruption in the lower motor neuron of the facial nerve. Immunological, infectious diseases and ischemia may lead to Bell's palsy still its etiology are questionable. If we talk about the history of discovery of Bell's palsy, much research has been done for it, the disorder is named after the scientist Sir Charles Bell while his research in the royal society of London. When we talk about the clinical feature of Bell's palsy, we find that it happens suddenly, usually affecting one side, facial palsy of lower motor neurons accompanied with pain in the posterior part of the ear, alteration of taste sensation, subjective alteration in facial sensation and hyperacusis.[2,3] Treatment usually focuses on restoring the function of facial muscles and recovery.

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Discussion

Bell's palsy is diagnosed more often in young females. Cases are usually reported in the age group of 21-40. The right side of the face is commonly affected. [4] As many times recurrence is reported in the first two years from the onset so follow up of patients should be carried out for 2-3years, mainly child patients.[5] The incidence rate varies from 11.5 to 40.2 out of every 100 00010 people with an annual incidence rate in the United Kingdom 20.2 out of every 100 000 population, in Japan 30 out of every 100 000 and in the United States 25–30 out of 100 000.[3,6]When Virus infection is the cause of Bell's palsy, it progresses by the inflammation and facial nerve gets demyelinated. As for maintenance of myelin sheath vitamin B plays a major role, so demyelination can prolong the time required for the normal function of the facial nerve.[7] When evaluating a patient, the following note should be kept in mind, in case of a patient of Bell's palsy occurs suddenly (within 3 days), the diagnosis should eliminate all the reasons for facial muscle fatigue, mostly unilateral, usually self limits itself.[8] Certain risk groups are more prone to Bell's palsy, those are pregnant lady, obese persons, hypertensive patients diabetic cases, upper respiratory tract infections.[1] There are still some factors may lead to Bell' palsy: change in atmospheric pressure, cold exposure, facial nerve compression near stylomastoid foramen, bacterial, fungal, viral infections (herpes zoster, EB virus, hepatitis B virus, HIV, etc), Melkerson–Rosenthal syndrome, Multiple Sclerosis, pregnancy.[3]

Table 1. Conditions & Disting

Condition	Distinguishing Factors
Guillain-Barre	Paralysis starting from feet going up to trunk and hand
Melkersson-Rosenthal syndrome	Edema in face and lip region and presence of fissure or folds on the dorsal surface of the tongue
Multiple sclerosis	Atypical neurological symptoms
Ramsay Hunt syndrome	Vesicular eruption in-ear canal or pharynx
Sarcoidosis	Bilateral
Stroke	Extremities on the affected side are often involved
Lyme disease	History of tick exposure, rash
Otitis media	Earache, pyrexia, deafness
HSV	Fever, malaise
Encephalitis	Headache, stiff neck, CSF abnormalities

To diagnose whether there is Bell's palsy or not, look for asymmetry of the face during the interview; observe closely than the blinking pattern, the commissure of lips, and the nasolabial folds. Under general examination; otoscopy, checking for growth near the head and neck region by palpating that area, and skin examination is done. Assessment of motor function, done by checking whether the patient can normally move eyebrow upward-downward, forceful closure of eyes, Smiling,blowing the cheeks, pursing the lips, grimacing if clinically indicated, then go for assessment of special sensation like the sensation of ear and the face, gustatory sensation of anterior two-thirds of the tongue. Orbicularis muscle reflex is checked taping the glabella and observing whether there is an asymmetry in the opening-closing pattern of the eye or not. Bell phenomenon: observe the upward movement of eyes during forced eye-closure. [12] In Inverse Bell's phenomenon, there is a downward movement of eyeball rather than upward, which can be seen in patients with Bell's palsy, after conjunctival scarring, even in the normal population.[13] Clinical features of Bells palsy include ipsilateral paralysis or weakness of the lower and upper muscles of the face, drooping of eyelids of the same side, incomplete closure of eye that causes drying of eye, epiphora,drooping of the corner of the mouth, altered gustatory sensation on the same side, dysphagia due to muscle weakness leading to accumulation of food on ipsilaterally, saliva rolled down from corner of mouth, ipsilateral loss or alteration of sensations of the face pain or due to involvement of stapedius muscle hyperacusis (sensitivity to sound increases.)[14]

Treatment: "Kabat rehabilitation consists of the facilitation of the voluntary response of an impaired muscle through the global pattern of an entire muscular section that undergoes resistance."In this rehabilitation protocol, three regional fulcra were taken into consideration: upper, intermediate, and lower fulcrum whose activation is done by various facial exercises. Kabat rehabilitation when accompanies with standard steroid treatment of severe Bells palsy, in maximum cases there is better and faster recovery than only medication is given.[15,16] If a patient is suspected for acute Bell's palsy then asses eye closure, whether it is adequate or inadequate. If adequate then no eye protective measures needed, and if it is inadequate then eye-protective eye measure will strongly be recommended. Then next assess the severity of paresis, if it is mild to moderate then corticosteroids will be strongly recommended and if severe to complete paresis is there then along with corticosteroids antivirals will also be recommended. If there is complete recovery during follow up and assessment then good or else if there is a failure to recover or progressive weakness then go for exercise and physiotherapy[17], consult specialist, imaging is used to exclude out any neoplasm.[18–20] The treatment approach of Bell's palsy targets the reduction of inflammation and viral infection. For inflammation, steroids are given like prednisolone, betamethasone, hydrocortisone, methylprednisolone, dexamethasone, and cortisone and for viral infection acyclovir, famciclovir, and valacyclovir is usually used.[21–23] (The use of steroids plus antivirals and the use of steroids plus mecobalamin are 2 good choices.)[24] MIRE therapy in which infrared light of wavelength 890nm, is used on

the area anterior and posterior to the ear, the temporal and lower jaw area of the affected side for the half-an-hour cycle.[25,26] When a case of facial palsy comes to a dentist,the following can be followed: thorough history taking and physical examination to exclude all possible causes for facial palsy, routine laboratory testing, and diagnostic imaging should be avoided if it is a new case of Bell's palsy, oral steroids (prednisolone) can be given within 72 hours of the appearance of symptoms,(For adults who come within 72 hours of onset treatment recommendations are " Corticosteroids: Prednisone 50 mg orally daily for 5 days, followed by 10 mg less each day for 5 days, Prednisolone 50 mg OD for 10 days, Antivirals: Valacyclovir 1 g TID for 7 days, Acyclovir 400 mg OD five times daily for 7 days)."[12] The basic treatment is neuromuscular re-education training. Exercises include (wrinkling of the forehead, smiling,snarling, and puckering of the lip) with speed and amplitude variation; exercises are done on one side of the face to have controlled movement, relaxing lower jaw doing exercises for eye and mouth simultaneously inhibiting synkinesis (small slower movements)[27,28]

Table 2

Possible complications	Treatment
Incapable of the closure of eye of affected side (causing corneal irritation and ulceration)	Artificial tears
Impaired muscle eyelid	Tarssoraphy or gold weights implantation in the eyelid of the upper eye
For asymmetrical face or Muscular rigidity	Cosmetic surgery or BOTOX injections

Conclusion

Bell's palsy with higher neutrophil to lymphocyte (NLR) ratio usually linked with prolonged time for recovery. Therefore NLR can be used for the prognosis of time taken for recovery of Bell's palsy patients. However, the patient may complain like symptoms such as facial spasm, synkinesis, and paresis.

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Ossifying Fibroma of the Anterior Maxilla: A Case Report

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Abstract

'Ossifying fibroma'can be described as a rare benign neoplasm classified under fibro-osseous lesions. Arising from the periodontal ligament's mesenchymal blast cells, it forms bone, cementum as well as osteo-cementum like hard tissue. Though a benign osseous tumour, it exhibits an aggressive behaviour on recurrence. Clinical features vary from swelling, facial asymmetry, mild displacement of teeth, pain or paraesthesia when there is entrapment of nerves. As in other osseous neoplasms, this also has radiologic changes ranging from a mixed radiopaque-radiolucent to a totally radiopaque appearance, but there is no pathognomonic manifestation. Maxillary lesions have to be differentiated from fibrous dysplasia. A case report of ossifying fibroma in the anterior maxilla is reported here.

Keywords: *Benign aggressive tumour, fibro-osseous lesion, mixed radiolucent-radiopaque appearance.*

Introduction

Ossifying fibroma is currently defined by WHO as a benign neoplasm, often presents well-demarcated borders and is composed histologically of fibro-cellular stroma and variable amounts of mineralised materials showing different morphological appearance. Initially WHO had classified Ossifying Fibroma (OF) as a fibro-osseous neoplasm. In 1972, WHO had first classified it into 'ossifying fibroma' and 'cemento-ossifying fibroma'. In 1992, WHO brought it under 'cemento-ossifying fibroma'. In 2005, it was replaced by WHO as 'ossifying fibroma'[(1)].

OF, a benign neoplasm mostly occurs in the tooth bearing areas of the jaws[(2)], commonly seen in mandibular molar and premolar regions[(3)], and do not arise in the long bones. This lesion is predominant in 2nd to 4th decade of life and more common in females, although the central variant is rare among females[(1)]. This lesion is believed to arise from the multipotent periodontal ligament cells which form cementum, alveolar bone and fibrous tissues. Treatment is done conservatively if the lesion is small, otherwise surgical resection. Recurrence rates of OF is low, but follow-up should be done.

Case Report: A female patient of age 27 years, reported to the hospital, complaining of swelling on the left upper front teeth region of the face for the past 3 months. History revealed that the swelling was a slow growing one and was not associated with pain or any discharge. On clinical examination extra orally a single localized, bony hard swelling was visible on the right side of the upper part of anterior maxilla. The swelling was not mobile but the skin over the swelling was freely movable without any secondary changes. Intra orally the bony swelling in the anterior part of the maxilla involved the alveolus of central, lateral and canine region and extended both labially and palatally without any secondary changes. There was displacement of 21 and 22, extrusion of 21 and spacing between 21 and 22.

Since the history revealed that the swelling was a fresh one, slow growing and bony hard in consistency and the swelling was well localized it is a bony swelling.

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As the swelling extends both labially and palatally, it is a benign bony neoplasm. Fine needle aspiration cytology (FNAC) was performed and no aspirate was obtained suggestive of the solid nature of the lesion.

Patient was subjected to radiologic investigations. Periapical radiograph of maxillary left anterior region shows well defined unilocular radiolucency which is ovoid in shape and is present between 21 and 22. Maxillary cross-sectional occlusal radiograph shows unilocular radiolucency ovoid in shape in between 21 and 22 regions with slight displacement of 21 and 22. Panoramic radiograph revealed a well circumscribed unilocular radiolucency involving 21-22 region displacing 21 and 22. Radiolucency was ovoid in shape with a radiopaque border.

Computed tomography scan of the swelling in axial, coronal and sagittal sections shows a space occupying lesion in the left maxillary alveolar segment. The lesion gives areas of both hypodense and hyperdense. The Hounsefield value was 750 suggestive of a benign bony neoplasm. 3DCT shows extension of the lesion till the floor of the nasal cavity on the left side without breach in the nasal floor.

Under local anaesthesia, incisional biopsy was done in 21-22 region and specimen stored in formalin and sent for histopathological examination. Histology revealed cellular fibrous tissue containing mineralized material. Connective tissue stroma with cementum like areas within the connective tissue; with spindle and stellate shaped cells. Histopathologic features suggestive of ossifying fibroma. The patient underwent complete surgical excision of the lesion for treatment.

Discussion

'Ossifying Fibroma' term has been used since 1927, and grouped together as 'cementum containing tumours' since 1968[4]. According to Eversole et al, Central Ossifying Fibroma (COF) can be described as:a well-demarcated benign fibrous neoplasm which is aptly designated as an 'fibro-osseous lesion'. Menzel described cemento-ossifying fibroma in 1872, while Montgomery coined it in 1927. Until 1948, COF and Fibrous Dysplasia were considered as variants of same entity, but OF has been considered a distinct lesion after Sherman and Sternberg described it in detail[5].

This lesion shows female preponderance, initially ratio being 3:1, while currently it has been recorded as 1.8:1[6]. In a report done in 2017 by Tapas et al suggests of female predominance in the range of 2:17 to 5:111[2]. Odontogenic fibroma (OF) is a 'benign odontogenic tumourwhich is characterized by various amounts of odontogenic epithelium in a mature fibrous stroma. Two types of the lesion are: an intraosseous or central OF (COF) and an extraosseous or peripheral. The intraosseous variant is an extremely rare tumour with clinical, radiographic, and histopathologic variable findings. A thorough review of the English literature revealed 78 cases of COF so far. Thus, we report an additional case of COF occurring in the maxilla of a 27-year-old woman. In addition, we performed a brief description and discussion of the cases reported in the maxilla and mandible. Matos et al has noted that this lesion was exclusive to mandible, however epidemiologic profile has changed with an equal distribution between anterior maxilla [54.4%] and posterior mandible [45.6%][6]. Eversole has reported in his studies that most frequent lesion occurred in mandibular molar [52%], then premolar [25%] as the second most common site in mandible, then incisor [12%]as third and lastly cuspid [11%] regions, the least commonly affected site of mandible[3]. OF has been reported to be prevalent in 2nd to 4th decades of life. In cases of maxilla, it is commonly seen in canine fossa and zygomatic arch area. OF clinically appears as a slow-growing expansile asymptomatic infra-bony mass presenting with facial asymmetry. Displacement of teeth may be an early clinical feature, while it may be present for some years. Pain and paraesthesia may be uncommon, yet still reports of presence have been recorded.

The cause of origin of OF is mostly considered to be the pluripotent mesenchymal cells of Periodontal Ligament. Pathogenesis of tumour can be induced by trauma to PDL from extraction, chronic inflammation due to pericoronitis, fibrosis due to periapical infection, or injury, etc. Brademann et al presented a hypothesis claiming mesenchymal cells present in bones other than maxilla and mandible can differentiate into PDL and a trauma may induce the lesion. Recently it has been discovered that a gene mutation of HRPT2, a tumour suppressor gene, can cause this lesion[1].

Fibro-osseous lesions are considered as there placement of bony constituent by fibrous tissue that later mineralize into woven or lamellar bone or cementum, presenting with various clinical features and microscopic appearances[3]. Radiographically the lesion appears variably due to difference in degree of mineralisation.

Amount of calcification differentiates a unilocular or multilocular radio-opaque image, or a radiolucent image with mixed density of opacified material. Root resorption and mobility can be appreciated in few cases[7]. Rind sign is a characteristic radiographical feature which is seen as a radiolucent line around the lesion's periphery, suggesting a capsule like nature[5]. MacDonald-Jankowski claims that OF is well demarcated and round or ovoid in shape as compared to Fibrous Dysplasia (FD), whereas OF presents an irregular shape, especially if occurs rapidly. If it grows along the body of the jaw, showcases an aggressive local growth. As compared to OF, fibrous dysplasia has the classical ground glass appearance radiographically. Radiotherapy is contra-indicated as a definitive measure of management.

Histological image suggests of ossified islands of bone and cementum within the hypercellular fibrous stroma. The trabeculae are variable in sizes, from lamella to woven patterns[8]. The characteristic sign of OF is interspersion of bony trabeculae and cementum like materials in a connective fibrous tissue stroma, along with presence of osteoblastic rimming[5]. CT and CBCT aid in radiographical differential diagnosis of OF[1].

Table 1 the comparison between OF and FD needed for their differential diagnosis, as they present similarities in their features.

Table 1: Differences between COF and FD

	Central Ossifying Fibroma	**Fibrous Dysplasia**
1. Category	Benign neoplasm	Bone disease
2. Clinical features	Painless hard swelling, facial asymmetry, tooth dislocation	Painless hard swelling, facial asymmetry, compression (paraesthesia, deafness)
3. Age prevalence	2^{nd}-4^{th} decade	1^{st}-2^{nd} decade
4. Sex predilection	Female	Both genders are equally affected
5. Variants	Juvenile and Normal	Mono-ostotic and Polyostotic
6. Etiology	PDL (induced by trauma), HRPT2 gene mutation	Gene mutation of GNAS1 gene
7. Hormone involvement	Not hormone related	Hormone related
8. Invasive	Continuous growth	Self-limited
9. Radiographically	Well demarcated bony margins, 'rind' appearance	Ill-defined margins, ground glass appearance, radiolucent lytic lesion
10. Histopathology	Fibrous capsule, osteoblastic rimming, deposits of osteoid	Chinese letter pattern, woven bone, loosely arranged fibrous stroma
11. Treatment	Surgical enucleation	Surgical recontouring
12. Radiotherapy	Contra-indicated	
13. Prognosis	Good, though there are chances of recurrence for larger lesions	Good

Taking into consideration the size, location and aggressiveness of the lesion, there are varied ways for management of the tumour, e.g. conservative observation, curettage, resection. In smaller size lesions, management is approached conservatively. In such cases long term follow-up is maintained. Recurrences in those conditions are treated by resection. Although resection of a massive portion of the jaw even though the lesion is benign in nature, is discouraged by many dentists. Some authors contradict radiotherapy for the management of this neoplasm, although roentgen therapy can be advocated to inhibit further growth of remnants left after operation. Aggressive lesion can be managed by en bloc resection as a definitive therapy[5].



Figure 1a: A single localized, bony hard swelling was visible on the right side of the upper part of anterior maxilla; 1b: A well circumscribed swelling in the left anterior maxillary lateral incisor region with bicortical expansion. 1c: A well circumscribed swelling in the left maxillary lateral incisor and canine, with displacement of 21, obliterating the labial sulcus; 1d: Diffuse swelling in the left anterior hard palate in relation to maxillary lateral incisor and canine.

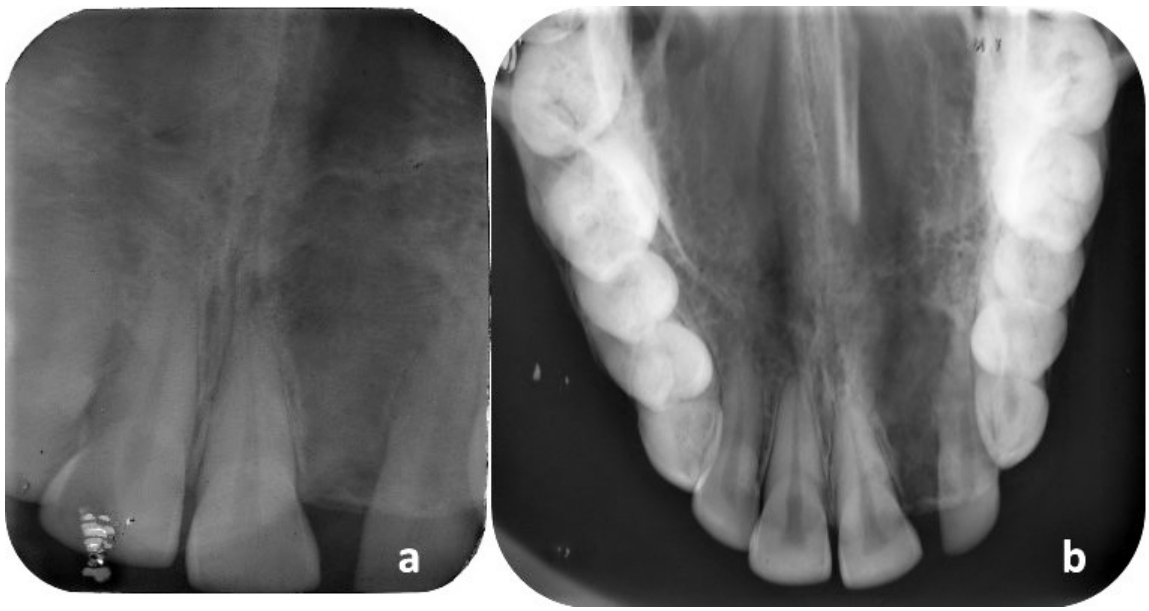


Figure 2a: Intra-oral periapical radiograph showing well defined unilocular radiolucency which is ovoid in shape and is present between 21 and 22. 2b: Maxillary cross-sectional occlusal radiograph shows unilocular radiolucency ovoid in shape in between 21 and 22 regions with slight displacement of 21 and 22.



Figure 3: Panoramic radiograph revealed a well circumscribed unilocular radiolucency involving 21-22 region displacing 21 and 22. Radiolucency was ovoid in shape with a radiopaque border.

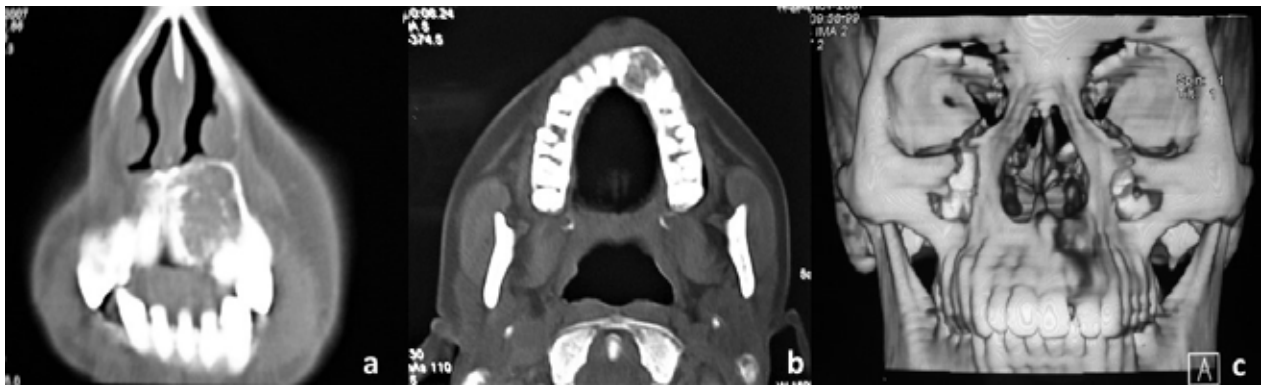


Figure 4a: Computed tomography scan of the swelling in coronal sections shows a space occupying lesion in the left maxillary alveolar segment with heterodense internal structure; 4b: Computed tomography scan of the swelling in axial sections shows a space occupying lesion in the left maxillary alveolar segment with areas of both hypodense and hyperdense. 4c: Three-dimensional reconstructed Computed Tomography scan shows extension of the lesion till the floor of the nasal cavity on the left side without breach in the nasal floor.

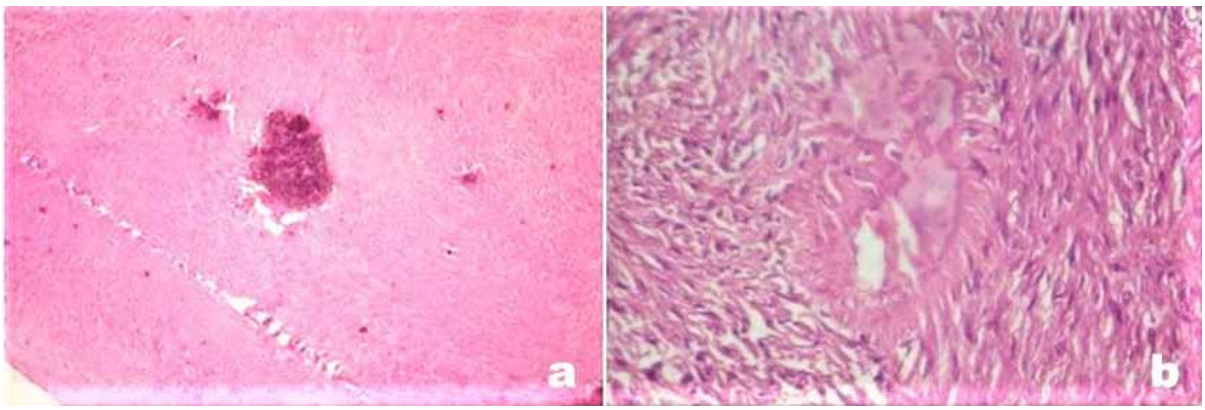


Figure 5a: Histopathologic examination (original magnification 10X) revealed cellular fibrous tissue containing mineralized material. Connective tissue stroma with cementum like areas within the connective tissue; with spindle and stellate shaped cells, suggestive of ossifying fibroma; 5b: Histopathologic examination (original magnification 40X) revealed cellular fibrous tissue containing mineralized material. Connective tissue stroma with cementum like areas within the connective tissue; with spindle and stellate shaped cells, suggestive of ossifying fibroma.

Conclusion

Amongst the many bony swellings encountered in the oro-facial region, odontogenic tumours and cysts are amongst the most common lesions. However, fibro-osseous neoplasms like the ossifying fibroma should be considered in the differential diagnosis and maxillary lesions especially have to be differentiated from fibrous dysplasia because of the aggressive nature of this benign neoplasm, the treatment has to be quite radical.

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Inhalational Inducing Agents

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Abstract

Inhalational Inducing Agents include gas like Nitrous Oxide and volatile liquids like Ether, Halogenated halothane, Enflurane, Sevoflurane, Isoflurane, Desflurane and Methoxyflurane. They have exterminated pain and anxiety control in Dentistry as well as in General Medicine for over a century. The following article outlines the detailed history, correspondence, distinction, and the clinical implementations of the few most prevailing inhalational agents used in Dentistry.

Keywords: *Inhalational anesthesia, Nitrous oxide, Isoflurane, Sevoflurane, Desflurane, Minimum alveolar concentration, solubility, cardiac output.*

Introduction

Inhalational Anesthetic agents are a chemical compound acquiring properties of a general anesthetic that are administered via Inhalation. They are delivered through a face mask, laryngeal mask airway or tracheal tube attached to an anesthetic vapouriser and an anesthetic delivery system. Dental and oral or Maxillofacial procedures have tended to perform office-based inhalation anesthesia along with the emergence of a more compact and transportable anesthesia apparatus. Inhalational Induction is comparatively slower. It depends on the inspired anesthetic agent, it's concentration in the blood, alveolar ventilation, the blood-gas partition coefficient, and cardiac output. if the blood-gas coefficient is less, then the anesthetic agent is more effective and the onset of anesthesia is faster. Anesthetic agents have to have a pleasant smell and a carrier gas like oxygen or oxygen with air is required for its use.[1]

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Inhalational Anesthesia is being considered equivalent to General Anesthesia. Even though there exist many drugs that can also be employed, whether through inhalation or intravenous to induce general anesthesia, Inhalational Inducing Agents are mostly used today in many of the hospitals and ambulatories. With continued progress of newer surgical approaches and easier obtainability of current short-acting drugs such as the addition of the intravenous agent Propofol, there is a sharp rise in the ambulatory services in the last Twenty Years. It also plays a significant role in children and needle-phobic patients[2].

Inhalational Anesthetic Agents must have these following characteristics: It must be possible to obtain a pure compound; It should be stable and not broken down by light or alkali; It should have low gas/blood coefficient; It should be a nonflammable and nonexplosive agent; It should not undergo metabolism and should be free of organ toxicity; It should have a pleasant smell; It should be readily reversible; There should be no adverse side effects if breathed in low concentration for a longer period; It should have minimal effect on the cardiovascular system; It should be practically potent, to allow use of an elevated concentration of oxygen; There should be no carcinogenic or teratogenic effect on the patient; It should own both analgesic and hypnotic property.[1,2]

History: All around History usefulness of herb

extracts to tonics and concoctions to sponges immersed with narcotics have been recorded to be used in surgical procedures. Explorations in the Eighteenth century made way for modern anesthesia. Diethyl ether, an inhalational anesthetic was the first publicly presented Anesthetic of the modern era. These agents have played an important role in Anesthesia history. Valerius Cordus was the one to synthesize Diethyl ether in 1540 and thus was seen to reduce pain. In the year 1772, Joseph Priestly synthesized Nitrous oxide and then sir Humphrey davy acclaimed its capability to reduce pain. The years 1920 to 1940 presented the introduction of Ethylene, Divinyl Ether, and Cyclopropane. But these drugs except for Nitrous Oxide were established to be nephrotoxic and combustible, making them unsuitable to be used in a clinical setup. Nitrous Oxide is known to be the most ancient anesthetic drug in clinical practice preceding Diethyl ether. Anesthesia was born on the 16th of October in the year 1846 known as Ether Day when there was a public Demonstration by Morton of Ether Anesthesia. Ether and Nitrous Oxide added with oxygen rendered anesthesia for a Century with fair competition in the 1930s to 1950s from Cyclopropane, Divinyl Ether, and Trichloroethylene.[3]

Modern Inhaled Anesthetics were introduced from the 1950s with agents that were partially or completely Halogenated which made them more stable and less noxious. Fluroxene was first synthesized with Halothane in the year 1951. The later one was then approved for clinical use in 1956. Similarly, Enflurane was popularised in 1972 and Isoflurane in the year 1980 and they did not pose threat for liver and kidney, unlike Methoxyflurane. Sevoflurane and Desflurane were manufactured in the year 1960s and 1970s but they were not economically accessible for clinical practice in the United States up until 1994. Xenon is a rare noble gas that was discovered by British Chemist sir William Ramsay in the year1898 and was first used as an anesthetic agent in 1951. Speedy Introduction with controlled nausea and vomiting facilitates for the drive of discoveries yet the 'modern' Agents continue to reform and rise upon those in the past. The most intriguing contrast over time is the potential to rapidly introduce new agents into the practice.[3]

Pharmacology: The Pharmacological characteristics of the recently used drugs sevoflurane, Isoflurane, and Desflurane are nearly equivalent to each other. They are noncombustible and remain as clear liquids at the normal room temperature. Their actions are as follows; They result in depression of the CNS and a decrease in the frequencies of the electroencephalogram and amplitude; they cause a surge in the cerebral blood flow which results in a rise in intracranial pressure; They also suppress the autoregulatory response to the cerebral blood flow; They produce a decline in Blood Pressure which is dose-dependent by reducing SVR except for sevoflurane; There is a prompt compensatory increase in the heart rate to maintain cardiac output despite peripheral vasodilatation and a decrease in peripheral vascular resistance.[3,4]

As compared to Sevoflurane and Desflurane, Isoflurane is the most potent vasodilator which was once known to give rise to coronary artery steal syndrome. studies and clinical trials have shown that Nitrous Oxide may cause a lenient increase in cardiac output followed by a sympathomimetic response with a higher concentration of the drug. The three volatile drugs namely sevoflurane, Isoflurane, and Desflurane create a surge in the respiratory rate which is dose-dependent and with a simultaneous decline in the Tidal Volume and a rise of arterial CO2. The breathing pattern in the case of volatile agents is swift but shallow. The reason behind the respiratory depression seen in cases of Inhalational Anesthesia is caused due to medullary depression of the ventilator drive of the central nervous system and also because of a cutback in the contraction of the muscles that are concerned in respiration. [3,4]

Overton and Meyer noticed a clear relationship among anesthetic efficacy and their solvability in olive oil, concluding that anesthetic drugs work unspecifically on the hydrophobic and lipid components of the cells. It has also been established in the last few decades that inhaled anesthetic agents depend on the action of protein receptors like ligand-gated ion channels. Inhalational Anesthetics decrease the supraspinal stimulation by declining the transposal of noxious afferent information that ascends from the spinal cord to the cortex of the cerebrum through the hypothalamus. It also reduces pain due to movement response by suppressing the spinal efferent neural activity. These agents affect the ion channels of neural tissue which can cause either presynaptic release of neurotransmitters and modify the post-synaptic response threshold to neurotransmitters or the two. They also have an inhibitory effect on the post-synaptic neuronal excitability by expanding the GABAa receptor facilitated inhibitory CL- current. [3,4]

Pharmacokinetics of Inhalational Anesthetic Agents: The Inhalation agents are titrable and their

pharmacology is unique; their uptake and release completely relies on the alveolar concentration of the anesthetic drug and its absorption by the pulmonary circulation through the alveoli. Their concentration relies mainly on 3 factors namely: Inspired concentration of the drug: the larger the inspired concentration, the more rapid is the rise in alveolar concentration to the inspired concentration and the fast induction of anesthesia. The second factor being the alveolar ventilation: alveolar ventilation is directly proportional to alveolar partial pressure as it steadily replaces the inhalational agent that is taken up by the pulmonary blood circulation. The last factor is functional residual capacity: A large functional residual capacity results in dilution of the inspired concentration of gas leading to an initial low alveolar partial pressure and slow advance of anesthesia. [4, 5]

The drug uptake from the lungs depends on the four factors namely:

Solubility: It is the blood-gas partition coefficient that illustrates the parallel affiliation of an anesthetic agent for two phases and how it distributes itself amongst both the phases when an equilibrium has been attained. It is the estimation of the solvability of an anesthetic agent amid the alveolar gas and pulmonary venous blood at equilibrium. An increase in the blood-gas partition coefficient results in higher uptake by the pulmonary circulation but a gradual and slower surge in alveolar partial pressure of the drug leading to a more late recovery from anesthesia. The values for brain: blood, fat: blood, and muscle: blood have an important influence and mainly for anesthetic procedures of longer duration for the drug is reallocated to the body compartments. The brain has a high perfusion rate and will achieve equilibrium quicker than fat or muscle. As the coefficient for blood: tissue is comparatively higher leading to most of the anesthetic being transported to the fat which perfuses it. The effect of anesthesia is increased for the fatty tissues having a higher affinity for them. The blood-gas partition coefficient for isoflurane, Sevoflurane and desflurane is 1.4,0.68 and 0.4 respectively at normal body temperature.[4,5]

Pulmonary alveolar blood flow: Pulmonary blood flow equals to cardiac output in case of a lack of a shunt. An increase in cardiac output leads to a higher intake of anesthetic agents from the lungs and further speedy distribution to the tissues along with the central nervous system. Thus low cardiac output results in decreased and a high alveolar pressure thus leading to faster induction of anesthesia. Increased delivery fastens the equilibrium of anesthetic partial pressure of tissues with that of arterial blood and leads to a decrease in the induction process.[4,5]

Difference between Alveolar to venous partial pressure and their tissue uptake: The contrast between the two is because of their uptake of inhalational agents by the tissue. Tissue consumption is reliant on the blood flow within tissues, the contrast between blood-partial pressure of tissue and blood-tissue solubility coefficient. An equal amount of anesthetic would be there both in the venous blood returning to the lungs as well as when it left the lungs in the absence of any uptake by the tissue. As the brain is highly perfused with blood, it equilibrates quickly unlike in the muscle tissue.[4,5]

Blood-gas solubility and how it presents in obese patients: Overweight patients contain a larger compartment for fat leading to prolonged time for equalization after induction and a sustained emergence time because of an increase in absorption and a slow release of anesthetic agents from the fat tissues. [4, 5]

Minimum Alveolar Concentration (MAC): It is defined as a measure of the efficacy of Inhaled anesthetics and is delineated as the minimum alveolar concentration at a constant state of an inhaled anesthetic at 1 atm of pressure that restricts deliberate movements in reaction to a surgical stimulus in fifty percent of the test population. It measures the somatic responses and can reflect the activity of an inhalational anesthetic drug on the spinal cord. The concentration required to repress the feedback to vocal and tactile stimulation and prevent consciousness is estimated by MAC-awake; where memory is being lost and MAC-BAR is the concentration required to hinder adrenergic reaction to surgical stimulation. Experiments have drawn an inference that MAC-awake is approximately 1/3rd of MAC for desflurane, isoflurane, and sevoflurane.[6]

Clinical Considerations of Nitrous Oxide, Isoflurane, Sevoflurane And Desflurane:

Nitrous Oxide: It is also known as 'laughing gas' and is a sweet-smelling, non-irritating, colorless gas, nonflammable, tasteless, inorganic gas that is not metabolized in the body and is the least hepatotoxic and teratogenic. Among all the prevailing inhalation inducing agents that are used in dentistry, Nitrous oxide is notably the commonly used drug in maximum dental procedures and surgeries and most commonly in

pediatric patients. It has to be often coadministered with oxygen or other volatile anesthetics because it cannot be utilized alone due to its high MAC value of 104%. The gas is stored as a liquid in the cylinders at a pressure of 760psi. It is known to be a powerful anesthetic but has a weak analgesic property; it does not produce adequate muscle relaxation. It does not depress the respiration and there is rapid elimination mostly through the lungs, and in small amounts through the skin, sweat glands, urine and intestinal gas. It can concentrate the halogenated anesthetics in the alveoli when they are simultaneously administered because of its fast uptake from the alveolar gas known as the 'second gas effect'.[7]

It does not affect the cardiovascular system and the cerebral blood flow. Nitrous oxide can have a very deadly effect on gas-filled spaces, it can diffuse up to 20 times quicker into closed spaces than it can be removed leading to the expansion of pneumothorax, bowel gas, or air embolism. Functions of nitrous oxide sedation are they reduce fear, anxiety, and fatigue. They increase pain reaction threshold, tolerance for longer appointments, and aid in the treatment of special patients. They also control non-compliant or uncontrolled behavior and pain by replacing local anesthesia. The drawbacks of using this agent are they need supplementation to be administered, they can cause hypoxia and inhibit vit B12 metabolism and methionine synthetase which is necessary for myelin formation and DNA synthesis. Prolonged exposure to this drug can cause bone marrow depression leading to megaloblastic anemia.[7]

Isoflurane: It is an isomer of enflurane and a halogenated ether derivative which is a colorless, volatile liquid, has a pungent odor, and is stable. The induction dose is 1.5-3% and the maintenance dose is 1-2%, by special vaporizer. Effects of this drug are; It causes dose-dependent depression of ventilation; It is a muscle relaxant and also causes relaxation of uterine muscles; It is a myocardial depressant and also causes coronary vasodilatation; the administration of this drug causes an increase in cerebral blood flow and a reduction in the cerebral metabolism.[7]

The advantages of this drug are there its fast induction and recovery with minimal risk of hepatic or renal toxicity. There is less nausea and vomiting. It does not induce cardiac arrhythmias, there is reduced myocardial depression and no myocardial sensitization to adrenaline. It is a very stable molecule and undergoes little metabolism. The disadvantages are they have a pungent odor, irritating vapor thus not commonly used for induction. They cause coronary vasodilatation and respiratory depression.

Desflurane: It has an extremely low blood-gas coefficient of 0.42. Due to its low fat-blood solubility, it has a very fast emergence from the anesthesia. It has the potency to induce tachycardia along with stimulation of the sympathetic nervous system with fast variations in inhaled concentration that might compromise a cardiovascular patient. It is not suitable to be used as an anesthetic agent as it possesses a fetid odor and which can be very agonizing to the pulmonary tissues and respiratory tract. It has a MAC of 6%, the least potent but the most expensive of the volatile agent. It causes dysphoria in pediatric patients.[8]

Sevoflurane: It is a new drug and the most commonly used volatile anesthetic agent used in ambulatory and office-based anesthesia. It is stable, nonflammable has a pleasant smell, and a MAC value of 2%. It is the most commonly used agent because it causes less irritation, unlike the other gases. It is metabolized by the liver releasing fluoride ions thus can be nephrotoxic. It may cause respiratory depression, muscle relaxation, is a myocardial depressant, and can cause a rise in cerebral blood flow and a decline in cerebral metabolism.[8]

The advantages of this drug are it has a rapid onset and offset of effect, nonirritant, sweet odor, rapid induction and recovery, suitable for pediatric patients and does not lead to the production of carbon monoxide with dry soda lime. The drawbacks of this agent include; it is less potent than the corresponding halogenated agents; children might experience postoperative agitation; only 5% of the drug is metabolized resulting in an elevation of fluoride levels and associated risk of renal toxicity.[8]

Conclusions

Inhalational anesthesia is the most commonly used agent in hospitals and ambulatory surgery operating rooms as it provides multiple delivery method and allows the anesthesiologist to choose from a varied and appropriate anesthetics for a given patient in a dental setup.

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Artificial Intelligence and How it Renovates the World of Dentistry

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Abstract

In the era of information and technology with the importance that data have in our daily life, advanced intelligence systemsare the new necessity to process and integrate those data and help us understand it efficiently. Artificial Intelligence (AI) with its computational and data processing powers provides a way to demonstrate intelligence which helps taking intelligent and effective decisions easy and effortlessly. This behavior of artificial intelligence has made it ever so significant in the field of dentistry. Using artificial intelligence in the field of dentistry would reduce cost and time of diagnosis, probability of medical error, and has the potential to improve the dental health of the public and in turn improve patient care. It assists dentists to be more proficient in their trade. This article tries to throw some light on the usefulness of artificial intelligence and how it can impact the world of dentistry in the upcoming future.

Keywords: *Artificial Intelligence; Dentistry; Applications; Diagnosis.*

Introduction

Artificial Intelligence (AI), the term coincided in 1956, is intelligence demonstrated by machines by accepting percepts from the environment and performs certain actions. This branch of computer science imitates the cognitive functions, such as learning and problem solving,similar to a way that the human mind or natural intelligence performs. The technology involves a set of complex mathematical algorithms which with a given set of data, analyzes and reaches to an intelligent conclusion. Apart from providing an intelligent conclusion, it also makes itself learn by correlating and analyzing the data and the corresponding result pattern for a better and more accurate output in the next iterations that follows. This process is also termed as deep learning, which is a part of the Machine Learning technique and entirely based on analyzing, inferring, and learning from complex forms of data.[1]

In the field of AI, there are so many variables, debates, and questions that it is simply impossible to have a single universally accepted definition for it. How exactly intelligence be defined or measured? What should make a machine intelligent? To understand the complete picture of AI, we need to know what intelligence exactly means and how we can relate it to a machine.

What is Intelligence?: From the simplest behavior to the most complex thinking can be attributed to intelligence. The AI and all the research around it mostly signify on certain components of intelligence such as learning, reasoning, unraveling, and adaptability using algorithms that are specifically designed to perform these actions using real-time data from different sources.[2]

a. The learning aspect of intelligence comes from information processing. This gives a dynamic feel to the intelligence of a machine that otherwise would be static and symbolic. There are different ways to make a machine learn, such as trial and error, generalization – learning from experience.

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b. The reasoning aspect points to the decision making factor. Any intelligent machine needs to draw an appropriate conclusion based on the situation. A conclusion can be deductive or inductive. The deductive conclusion is based on the facts that guarantee a true conclusion, while the inductive conclusion is based on the previous examples that provide support to the conclusion.

c. Unraveling is the approach of solving a complicated problem by formulating them into several simpler problems. These smaller problems are then solved through a range of possible actions to arrive at a predefined solution.

d. The adaptability factor helps a machine termed as 'intelligent' to adapt based on changing situation by compiling information and reaching to an apt decision.

Now that we have gained a good understanding of artificial intelligence, let us see how it is being used and the opportunities where it can be applied in the future in the field of dentistry.[2]

How is AI currently used in dentistry?: Artificial Intelligence is a big revolution in the field of technology which is rapidly progressing and improving at the same time, thanks to all the extensive R & D being done in the field of machine learning. Medical science along with the field of dentistry has seen a substantial uptake of this technology. Every field of medical science has been integrated with this power of technology to help make it better and more efficient.[1-3]

Diagnostic capability is a primary work that dentists have to perform on any problem. In general, diagnosis is done through several investigating techniques such as X-rays, CT scans, MRIs. The results are then interpreted by a dentist to reach to a conclusion. Now what if, the analysis is being done by an 'intelligent' machine and directly provides probable conclusions to the dentist? The dentist can simply take a suited action. This is achieved through image processing and recognition using artificial intelligence algorithms and making a machine learn and determine as to what exactly a particular or a set of image(s) indicates. This reduces error probability, helps to identify even minor deviations from normal, eliminates differences in subjective diagnosis, decreases overall time by removing the predictable tasks and these would provide a much better patient care. Artificial Intelligence also helps in analyzing and predicting any possible genetic disorder that could lead to a bigger concern like oral cancer.[2,4]

Clinical decision support systems (CDSS) are complex algorithms that assist health care personal in clinical decision-making tasks. A working definition for CDSS was proposed by Robert Hayward which says – "Clinical decision support systems link health observations with health knowledge to influence health choices by clinicians for improved health care". The involvement of computers and software that are integrated with available medical records of individuals has become a necessity in dentistry. The CDSS can assist in that part efficiently. It consists of a link between the vast medical knowledge block and an interpretation process that are a set of researched-based rules. An example of such a system is the one used to forecasts the genetic disorder and differences by using a form of genetic mutation algorithm. Thus a CDSS effectively helps in reducing the gap between a doctor, medical knowledge, and a patient.[2,4]

Artificial Intelligence provides the platform to integrate data from several domains such as the dental history of the patient, prior scans images and tests,socio-demographic details, pre-existing health conditions. Processing these data will provide a better understanding of the problem to a dentist and in turn, helps take an appropriate route for diagnosis. Artificial intelligence helps streamline a large number of routine activities such as scheduling and reminding appointments, patient record keeping, diagnostic assistance systems, self-monitoring, and management by patients. As part of a health monitoring system, AI provides a much deeper learning about any underlying health conditions and diseases, the impact of medicine on the patient.[5]

There is a vastapplications of artificial intelligence in the field of dental surgery. With the advancement of robotics handed surgery and in combination with the machine learning algorithms, almost all of the complex human operating motions and intelligence can be programmed and simulated in an environment where a dentist can perform those surgeries and have expertise on them without actually risking any real patient. This also assists surgeons for better planning and execution time of the procedures. Although virtual reality provides a simulated environment, it still is a great way of learning and gaining practical experience for inexperienced surgeons. The effect visualization makes it more real than anything.[6]

Augmented reality is another aspect where there is a growing contribution by artificial intelligence and it has slowly started its impact in the field of medicine. The augmented reality has helped reduce the complexity in the process of delivering aesthetic prostheses. With the help of AI systems integrated with augmented reality, patients can now check the prosthesis virtually and see how it fits them. This helps the patient and the dentist to make any number of changes and the final prosthesis outcome will be more accurate as per the indicated specifications. Augmented reality helps in obtaining accurate facial measurements, bone structures which assist dentists as a surgical guide for implants.[7]

Artificial Intelligence has also helped to enhance the learning methodologies and skills of dentists around the world. It has been successfully integrated into the dental education system to enhance the theoretical as well as practical learnings and trainings in the field of dentistry. The AI technology has the facility to envision and create a virtual reality (VR) environment that would help simulate different complex surgical techniques. Dentists can achieve expertise in various techniques of surgeries without actually operating on a real clinical patient. They can learn to design the best possible prosthesis using an AI-supported designing tool. Professors can efficiently impart quality education through a simulated environment. This method of learning is very effective, has no risks (no error-prone), a better way to practice, and an in-depth transfer of knowledge and information.[7,8]

Forensic Odontology is another segment where the usage of AI and its advancements has been done. This involves an examination of the dental forensic evidence found at a crime scene and thus this holds a larger interest for justice. It becomes extremely critical to properly and accurately analyze the evidences. Systems enabled with AI technologies and algorithms can be very helpful in this scenario. A varied array of image processing techniques integrated with AI algorithms will help provide an accurate and systematic overview of human age estimation and facial identification that can be used to track the probably criminal involved. The enhanced system can be integrated with neural networks to learn and effectively predict the age and possible facial structure.[9,10]

Future for dentistry: Rise of AI

In this paper, a brief explanation by relating the usage of artificial intelligence in every possible aspect of dentistry. Artificial Intelligence (AI) has indeed similarly revolutionized the field of dentistry that it has impacted the field of technology. It has showcased a wide range of applications and exceptional accomplishments in the field of medical science in general. From enhanced clinical decision-making process to easier diagnosis, from improved patient care to an efficient way of performing complex dental surgeries, in every way AI has helped not only to improve the existing process but also assist dentists to become better. Having said that, this technology is on many levels still improving and every day something new gets added to it for better. There is a minimum doubt that in the future it will have a major impact throughout every aspect of dental care and be an integral part of it. Although the human knowledge, intellect, and decision making cannot be replaced completely, the main purpose of using AI integrated systems is to assist dentists to perform efficient diagnosis, accurate and risk-free surgeries, and better dental care for patients.

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Infections of the Surgical Site: An Overview

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Abstract

Surgical site infection is a type of nosocomial infection that not only increases the medical expenses of the patient but also the risk of fatality. It is of major concern as the patient has come for treatment of diseases and getting a new infection due to negligence in following sterilization and asepsis protocols during surgery. Therefore the article enlists the method on how to manage SSI.

Keywords: *Surgical site infections, risk factors, intra-operative, and post-operative management protocols.*

Introduction

Surgical site infections or SSIs are among the principal hospital-acquired infections. As per the latest studies its occurrence is around two to eleven percent of the total surgical operations. SSIs cause an increase in health care expenditures, an increase in hospital stay, and increased death rates.[1]

Categorization of SSI:

1. **SSIs are categorized into:**

Superficial–start in less than thirty days post-surgery, affect the skin as well as subcutaneous tissue;

Deep–start post thirty days or in less than one year (on condition that a foreign particle got into the fascial spaces and muscle;

Infection of the organ or body spaces found near the incision area – occurs in less than thirty days to one year (if a foreign particle got into it).

2. Wounds of the surgical site are categorized into 4 categories based on how hygienic or dirty the wounds are:

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Type 1 type of wounds: clean wound where the occurrence of infection is less than two percent;

Type 2 types of wounds: clean or dirtied wound where the occurrence of infection is less than ten percent;

Type 3 type of wounds: dirtied wound where the occurrence of infection is nearly twenty percent;

Type 4: dirty or infected wound where the occurrence of infection is less than forty percent.[2]

Microorganisms responsible for SSI:

- *Staphylococcus epidermidis* (Commonly seen but only causes disease sometimes)
- *Staphylococcus aureus* (Rarely occurs, disease-causing in nature)
- *Staphylococcus warneri* (Rarely occurs, sometimes disease-causing in nature)
- *Streptococcus pyogenes* (Rarely occurs, disease-causing in nature)
- *Streptococcus mitis* (Commonly occurring, causes disease sometimes)
- *Propionibacterium acnes* (Commonly occurring, sometimes causes diseases)
- *Corynebacterium* spp. (Commonly occurring, sometimes causes diseases)
- *Acinetobacter johnsonii* (Commonly occurring, sometimes causes diseases)

- *Pseudomonas aeruginosa* (Rarely occurring, sometimes causes diseases)[3]

Components affecting SSI:

Factors depending on the patient: Age of the patient, nutrition of the patient, uncontrolled diabetic patients, regular Smokers,obese patients, parasitic infestations, colonization with drug-resistant disease-causing microbes, Immuno-compromised individuals,Time period of hospital stays prior surgery and post-surgery.

Factors depending on Surgery: Sanitization of the skin, removal of hair from the skin, peri-operative antibiotic dosage, time period of surgery, improper instrument sterilization, air ventilation or conditioning, foreign particle inside the wound, drainage of the surgical site, insufficient hemostasis, significant surgical trauma.[4]

Prevention of SSI:

Preoperative phase:

Surgical site shaving:

It has been seen that the use of bladed razors makesmicro-injuries on the epithelium. Therefore it increases the chances of SSI. Hence hair removal is usually done with the help of an electrical razor which has a one-time use tip which is used just before the patient is sent to the operating room.[5]

Nutritional status of the patient: Nutrition deficiency or improper nutrition hurts the patient's condition and surgical results. Two method could be used for assessment of the person's nutritional status:

- Nutritional-Risk Screening
- Nutritional-Risk Index[6]

An assessment of the association involving malnutrition

And SSI usually includes the mode and degree of surgical interference.

Obesity (BMI greater than thirty) influences the healing of the by various means. The subcutaneous tissue blood supply in overweight patients may not be enough and it may not supply proper O2 whereas tissue healing has an increased metabolic requirement and insufficient O2 supply reduces the speed of the entire process. Cells that provide immunity have an increased O2 demand, which is used for the production of anti-microbial oxygen compounds.[7]

Immuno-suppressive treatment: An examination conducted by Berthold et al shows that the immuno-suppressive treatment damages healing of the wound and raises the chances of occurrence of infection. Stoppage of immune-suppression may cause aggravation of the disease which was already present. The guidelines given by The Society for Healthcare Epidemiology (America), recommended the stoppage of immunosuppressive therapy before and after surgery as long as it is possible.[8]

Use of antibiotics for prevention of SSI: Prophylactic therapy of antibiotics is usually given for clean or dirtied wounds and clean wounds with foreign particles. Usually, for a clean wound, we use cephazolin or cefuroxime or vancomycin. For contaminated wounds, the person must not be usually given a prophylactic treatment but a full antibiotic course.[9]

Intraoperative phase:

Operating room: When a patient who has undergone surgery goes through the process of healing, for it to happen without complications of infection, the patient should be placed in hygienic surroundings. The correct planned operating room must have regions that constantly increase in sterility. The hospital staff must walk amidst areas where scrubbing takes place to reduce contamination of the OR environment with disease-causing microbes. The essential step when considering planning of OR is the differentiation of the clean and the dirty areas of the room from each other.

Sanitization of the surgical area: The objective is to decrease the pathogens which naturally occur inside the superficial layer of skin, and restrict the growth tendency of disease-causing organisms pre-surgery and post-surgery. Frequently substances used for skin decontamination before surgery consists of an alcohol solution of chlorhexidine gluconate as well as povidone-iodine. Chlorhexidine gets absorbed by proteins that contain phosphorous inside the cell wall of the bacteria. At bacterio-static quantity, the solution gets into and destroys the membrane of the cell which causes seepage of structures of the cytoplasm. When it reaches bactericidal quantity, it gets into the cell of the bacteria and permanently attaches itself with Adenosine-triphosphate of the cell as well as nucleic acids. Chlorhexidine has fungi-static and fungicidal properties and usually kills

viruses of some types. The least amount required to stop bacterial virulence is usually lesser for Gram-positivebacteria compared to Gram-negativebacteria because chlorhexidine shows more likeness to the Gram-positivebacterial wall of the cell. Povidone-iodine comprises a normal solution containing one percent iodine which is free. Iodine seeps inside the cell wall causing oxygenation of cysteine as well as iodination of the other amino-acid compounds as well as fatty acids which are not saturated which results in a decrease in protein production a well as the injury of the cell wall. Iodine, which is useful for treating infections due to Gram-positivebacteria and Gram-negativebacteria and a couple of bacteria that form spores, Mycobacteria, virus, and also fungi. Dissolving chlorhexidine along with povidone-iodine/ethanol/isopropylethanol increases bactericidal action. The alcohol damages proteins as well as increases the killing of the bacterial cell. It's effectiveness in opposition to *Staphylococcus aureus* which is methicillin-resistant, Enterococci which is vancomycin-resistant, and *Mycobacterium-tuberculosis*.

Studies have been conducted which compare chlorhexidine and povidone-iodine confirmed that both the materials demonstrate comparable anti-bacterial action. Chlorhexidine operates for an increased duration of time by combining with the skin as well as the proteins of the mucous membrane. As opposed to povidone-iodine, its function is unchanged when in contact with blood/any other type of body fluid. There are statements regarding the effectiveness of chlorhexidine as well as povidone-iodine which are contradictory. Considering the meta-analysis which was done by Lee et al (which also includes the nine randomized controlled trials), the better usefulness provided by chlorhexidine can be concluded but the significance of the examination has been argued upon since multiple studies have compared alcohol chlorhexidine solution and povidone-iodine solitarily. During meta-analysis which was done by Priviter et al which was for comparisons between alcohol solution of chlorhexidine as well as povidone-iodine, it was established that usage of chlorhexidine results in the decreased occurrence of SSI.[10]

Sanitization of hands: The bacteria which are found on the naked hand of the medical personnel may be leading to the causation of infections that are acquired at the hospital. The Gram-negativebacilli as well as Staphylococcus-aureus are the primary components of skin bacteria. Chlorhexidine-solution usually provides surgical sterility through a decreasing number of bacteria. Chlorhexidine has been depicted as a wide spectrum and increased duration of antibacterial action, while alcohol quickly begins taking action. Substances that contain chlorhexidine as well as alcohol mix the property of quick initiation of alcohol along with the increased duration of the consequence of chlorhexidine. Hence are considered most successful.[11]

Phase after surgery: Post-operation wound cleanliness is important. The best method is considered to be the 'no-touch' method which is to avoid touching the wound as well as dressings with non-sterilized hands. Sterile saline must be utilized for sponging or washing the wound. After 48 hours post-surgery, the patient should wash or herself with mild detergent (soap) preferably having antimicrobial properties. It's usually not advised to utilize local antimicrobials or topical antimicrobials to decrease the chances of occurrence of infection. Clinically, the infection presents itself as: Localized reddishness of skin,tenderness,increase in temperature of the site,fluid-filled spaces, pus containing discharge.

In treating SSI, importance is given to opening the area of infection as well as draining the purulent areas. Infections of deeper tissues require draining the area but infection of superficial tissues requires only limited draining of the area which is affected. Fibrins/suture as well as the staple is gotten rid of/tissue debriding must be done to remove the dead cell of the wound. SSI is usually treated with different types of antimicrobials which depend on open the surgeon's choice (for example, solution of povidone-iodine H2O/octenidine-dihydrochloride). The concentrations antiseptics which are commonly in use are almost a hundred times more when compared with the minimal inhibitory concentration of the same compound. Hence these substances kill the bacteria which have developed slight resistance against these antiseptics. The 2014 guidelines of Infectious Diseases Society of America, the usage of antibiotics is not necessary if there is a minimum infiltrate results from inflammation which is lesser than five centimeters near the surgical site with none of the signs of general infection established as fever which is less than thirty-nine degrees Celsius as well as the rate of the heartbeat which is less than hundred and ten per minute. Although, it is mandatory to recommend the initiation and anti-biotics if inflammation near the surgical site becomes greater than five centimeters as well as the sign of general inflammations occur. While making a choice of the 1st-line of therapy, localized epidemiological

situations, and the Gram-staining process of smears of the wound must also be taken into consideration.

Indications for microbiological studies for SSI patients include: Intense screening, the requirement of an anti-biotic therapy,the inclusion of evaluation of drug-resistant disease-causing bacteria,allergic reactions of any kind to the first-line of therapy. In case of there are suspects regarding infections which are caused by staphylococci, cefazoline, cefuroxime/cloxacillin may be useful. For the infections which are caused by MRSA, it is correct to use linezolid or glycopeptide. When there are suspects regarding infection caused by Gram-negativebacteria, the first-line of antibiotics are usually 2nd or 3rd generation cephalosporins or fluoroquinolone. When dealing with the complications of deep as well as non-healing wound, negative pressure treatment is usually taken into consideration. The negative pressure allows the proper supply of blood to the site of wound by increasing the formation of new blood capillaries and thus increases the rate of formation of tissue which is granulomatous. Treatment by negatively applying pressure in case of the wound which is infected is considered safe but it usually precedes debridement and initiation of target antibiotic treatment.[12]

Conclusion

Surgical site infection is a type of nosocomial infection that not only increases the medical expenses of the patient but also the risk of fatality. It is of major concern as the patient has come for treatment of diseases and getting a new infection due to negligence in following sterilization and asepsis protocols during surgery. Therefore the article enlists the method on how to manage SSI.

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Pulpotomy

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Abstract

A pulpotomy is a way of preserving primary teeth from carious lesions and damage before the natural exfoliation. It is also the most controversial treatment in pediatric dentistry. The success rate of pulp therapy in the primary tooth depends mostly on early pulp diagnosis, pulp vitality prevention, pulp vascularization & periradicular status. New biomaterials that are more biocompatible have changed the frame of mind towards the prevention of cariously exposed teeth.

Keywords: *Pulpotomy, Pulp vitality, Pulp Therapy, Primary tooth, Carious lesion.*

Introduction

Pulp therapy is a medical intervention that is done for the preservation of cariously affected primary tooth until its normal exfoliation because the loss of primary tooth before natural exfoliation can lead to esthetic & functional problems & malocclusion. It has been the most debatable & controversial mode of treatment in pediatric dentistry for more than a decade, but the procedure of pulpotomy has evolved slowly & continued to accelerate in past few years which are now more acceptable in the present days.[1] Two different method can be used to perform pulpotomy:

a. Non-pharmacotherapy – treatment include leaser & electrosurgery

b. Pharmacotherapy – treatment such as replacing the cariously affected pulp tissue with biocompatible medicaments like ferric sulfate, MTA, glutaraldehyde, sodium hypochlorite enriched collagen solution, etc.

Treatment or management of a cariously affected primary tooth where the carious lesion has already reached the pulpal region requires a proper knowledgeable approach for pulpotomy, and the success rate of the procedure depends on the proper diagnosis of the pulp status before doing the therapy. Post-operative and preparatory data collected before the therapy is interpreted and mostly focuses on determining whether the pulp of the carious affected tooth is normal, reversibly inflamed, irreversibly inflamed, or necrotic. If the pulp is diagnosed to be necrotic or irreversibly inflamed then the treatment done is either a pulpotomy or extraction would be appropriate. If the pulp is diagnosed to be vital or reversibly inflamed then the vital pulp therapy technique of pulpotomy or indirect pulp treatment is indicated. In this article, the discussion is limited to the vital pulp therapy procedure of pulpotomy for primary teeth.[2]

Definition: Complete removal of the coronal portion of the dental pulp followed by placement of a suitable dressing or medicament that will promote healing and preserve the vitality of the tooth. A pulpotomy is defined as the amputation of vital pulp from the coronal chamber followed by placement of a medicament over the radicular pulp stumps to stimulate repair, fixation, or mummification of the remaining vital radicular pulp. A pulpotomy is a procedure in which the entire coronal pulp is removed, to remove all infected pulp tissue; the radicular pulp is then treated in different ways, according to the technique employed. A pulpotomy is the removal of the coronal portion of the pulp and the treatment of radicular pulp in an attempt to maintain the tooth and its supporting structure in a state of health. Procedures involving removal of vital partially inflamed

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coronal pulp and tissues and placing a dressing over the amputated pulp stumps and then placing the final restoration.[3]

Objectives: The main objectives of pulpotomy are the preservation of the vitality of the radicular pulpal tissue, removal of cariously infected coronal pulp tissue, treatment of remaining pulp with the placement of proper and suitable biocompatible medicaments, neutralization of further infectious process, avoiding degeneration of pulpal changes and avoiding dystrophy of the peri-radicular region.

Classification[4]

1) Vital pulpotomy

a) Devitalisation pulpotomy (mummification, cauterization)
   - Formocresol pulpotomy
   - Electrosurgical pulpotomy
   - Laser pulpotomy

b) Preservation (minimal devitalization, Non-induction)
   - Glutaraldehyde
   - Ferric sulfate

c) Regeneration (inductive, reparative)
   - Calcium hydroxide
   - Bone morph genetic protein
   - Mineral trioxide aggregate

2) Non-vital pulpotomy
   a) Beechwood cresol
   b) Formocresol

Devitalization: Is the first and foremost approach in the pulpotomy in primary teeth, with the vital pulp tissue destroyed, which includes formocresol, glutaraldehyde, electrosurgery, and laser. It is done with the intention of "mummifying" the radicular pulp tissue.

- Formocresol: It is the most popular medicament for pulpotomy in primary teeth for 70 years, which was introduced in the year 1932, with a success rate of 80%-90%.

Formocresol constitution: 19% of formaldehyde, 35% of cresol, and as a vehicle 15% of glycerin and water that is known as Buckley's solution. Formocresol prevents the destruction of the pulpal tissue by bonding with the protein.

- **Glutaraldehyde:** It is an alternative pulpotomy agent to formocresol for its superior fixative property, low antigenicity, and low toxicity. It was introduced by Kapel in the year 1979.

The application of 2%-4% of glutaraldehyde produces rapid surface fixation of the underlying pulpal tissue. A thin zone of compressed fixed tissue eosinophilic stain is formed under the region where the glutaraldehyde is applied, which blends with the vital normal tissue present apically.

- **Electrosurgery:** It is a non-pharmacological procedure for pulpotomy in primary teeth. In this process,a high frequency of the electric current is passed through the tissue cells to cut it, which forms a layer of coagulative necrosis and provides a barrier between the healthy radicular tissue and the biocompatible medicament placed in the pulp chamber.

- **Laser:** It was introduced in dentistry in the early 1960s. There are different types of lasers used in pediatric dentistry such as a laser which is used for diagnosis of carries development, argon laser for curing of composite, CO_2 lasers with the wavelength of 10600nm are used for soft tissue surgeries, YAG lasers are also used for cutting soft tissues. Erbium laser is used in hard tissues for cavity preparation. In pulpotomy, the use of lasers helps in the early preservation of vital tissues near the apex of the tooth, hemostasis and there is no vibration neither any odor, which leads to satisfaction of children and the parents.[5,6]

Preservation: In this method, the pulpal tissue is preserved by ferric sulfate and sodium hypochlorite. In this method, the pulp tissue is minimally insulated. Ferric sulfate and sodium hypochlorite help in retention of vital tissue and conserves the radicular pulp without including the reparative dentine and it also maintains the normal histological appearance of the radicular pulp.

- **Ferric sulfate:** Ferric sulfate in pulpotomy acts as a coagulative and hemostatic agent. The clinical success rate for this is 90%-95% and the radiographical success rate is 70%-80%.

The major failure of ferric sulfate is a high percentage of internal resorption. But as ferric sulfate is a less

expensive solution with no toxicity and carcinogenicity, it is recommended as a suitable substitute for formocresol.

- **Sodium hypochlorite:** It is mostly used as an irrigant solution due to its antimicrobial activity, tissue dissolving activity, detergent action, hemostasis, and the ability to neutralize toxic products.

Sodium hypochlorite can also be used as a medicament in pulpotomy but only a few clinical trials are done to evaluate the efficacy of sodium hypochlorite in pulpotomy.

Regeneration: Regeneration is the procedure which includes the application of biomaterials and biocompatible medicaments as a pulpotomy agent that has cell inductive capacity that helps in either replacing the lost cells or help to induce the existing cells to differentiate into hard tissue forming elements.[7,8]

Regeneration procedure helps in the formation of reparative dentine and the preservation of healthy pulpal tissue. Many pulpotomy agents are used in this method such as calcium hydroxide, mineral trioxide aggregate, and calcium-enriched mixture cement.[7-9]

- Calcium hydroxide: It is a pulpotomy agentthat generates information of new dentin when applied tothe pulp tissue. It is also used as a medicament for direct pulp capping and indirect pulp capping in both primary tooth and permanent teeth. Calcium hydroxide also acts as a bactericidal agent and helps in the stimulation of dentine bridge formation. Calcium hydroxide sometimes results in the development of chronic pulpal inflammation and internal resorption, so it is controversial to use it as a pulpotomy agent in a primary tooth.[7-9]
- **Mineral trioxide aggregate:** It as a member of hydraulic calcium silicate cement and it consists of tricalcium silicate, bismuth oxide, tetra calcium alumina-ferrite, and calcium sulfate dehydrate. First mineral trioxide aggregate had a grey coloration but then in 2002, a new formula was introduced with white coloration. When it is mixed with water it forms a colloid gel, which is similar to calcium hydroxide. MTA is a more preferable pulpotomy agent because it is more biocompatible; it has a bactericidal effect and also induces cementogenesis. Furthermore, it has the ability for sealing, dentinogenesis, and osteogenesis.[7-9]
- **Calcium enriched mixture cement:** It was first introduced as an endodontic filling material in dentistry. It mainly consists of calcium oxide, sulfur trioxide, phosphorus pentoxide, and silicon dioxide. The physical properties of this biomaterial are it is flowable, it has film thickness, primary settling time and it can set in an aqueous environment. This cement is highly biocompatible and can induce the formation of hard tissue and hydroxyapatite and it also has an antimicrobial effect that resists the entry of microbial agents.[7-9]

Factors that affect the prognosis of pulpotomy:

The factors that affect the prognosis of pulpotomy are as follows:

- Size of exposure
- Location of exposure
- Exposure to saliva
- Marginal leakage
- Age and status of pulp

Indication:

- Cariously involved primary tooth, which is rented enough to not to be extracted and can be preserved
- A vital tooth with healthy periodontium can undergo pulpotomy
- If there is any pain, it should not be spontaneous and should go along with the removal of the cause or stimulant of pain
- A tooth which can be restored and preserved can go for pulpotomy
- A tooth should have 2/3rd of its root length unaffected to undergo pulpotomy
- Hemorrhage from the amputation site should be pale red and it should be easily controlled
- There should be no abscess and fistula
- There should be no loss of inter-radicular bone
- It can also be done on a young permanent tooth with vital exposed pulp and incomplete formed apices[7-9]

Contra Indication:

- If the pain is spontaneous and does not persist after the removal of the stimulus then the tooth can undergo pulpotomy

- If there is the tenderness of percussion that tooth is not suitable for pulpotomy
- A mobile tooth is not suitable for pulpotomy
- If the root is resorbed for more than 1/3rd of the total root length, then it is not suitable for pulpotomy
- Sluggish hemorrhage from canal orifice which is uncontrollable and highly vicious
- When there is evidence of internal resorption and presence of inter radicular bone loss
- A tooth which is closed to their natural exfoliation is preferable for extraction more than pulpotomy
- Immuno-compromised patients are contraindicated for pulpotomy[7-10]

Conclusion

Preservation of primary tooth before the natural exfoliation and until the eruption of a permanent tooth is very important because the primary dentition is essential for the maintenance of the arch length, proper mastication, proper speech, and esthetic in children. Pulp affected by carious lesion and injured in trauma may threat pulp vitality, so appropriate treatment such as indirect pulp capping; direct pulp capping and pulpotomy are considered. A pulpotomy is the most common treatment plan for cariously affected tooth which consists of many method. Pulpotomy has three main procedures as devitalization, preservation, and regeneration. In pulpotomy, the affected pulpal tissue is removed and replaced by suitable biocompatible medicament which helps in the healing of the injured pulp and preservation of the vital pulp. Some of the medicaments used in pulpotomy are formocresol, glutaraldehyde, ferric sulfate, sodium hypochlorite, calcium hydroxide, calcium-enriched mixture cement, and mineral trioxide aggregate. From all the biomaterial medicament mineral trioxide aggregate is most suitable and successful for pulpotomy in a primary tooth. Many more clinical trials are being conducted so we may discover many more suitable agents for pulpotomy shortly.

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Evolving Trends in Forensic Odontology

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Abstract

Forensic odontologists have been playing an indispensable role in the sphere of forensics for over a hundred years. The various method used by them in identifying individuals from the human remains have been evolving over the years. Using dental remains is not just feasible but budget-friendly and time-saving as well. This article reviews the current trends in the branch of forensic dentistry while also highlighting the importance of antemortem records in comparison with post-mortem evidences.

Keywords: *Forensic odontology, identification,antemortem, postmortem, trends.*

Introduction

Forensic odontology is a subspecialty of dentistry that deals with the implementation of the art and science of dentistry within the criminal justice system. It mainly focuses on identifying deceased victims and unknown perpetrators. Forensic odontologists collect dental evidences from the crime scene, examine them and present them in the court of law.[1]

Teeth are the hardest and the most resistant tissues in the human body. Teeth are capable of standing firm against various natural disasters, major accidents, burials, crimes,or other severe exposures like broiling temperatures, humidness, and pressure changes. They do not decay easily and the teeth pattern is unique to each individual which is why they are very useful in identifying a person. The techniques used in forensic odontology has evolved through the evolution of humankind. This article aims to provide an overview of the most recent trends.[2]

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Evolving Trends in Conventional Method Used in Forensic Odontology:

DNA finger typing: DNA analysis is one of the most recent trends in forensic dentistry. Teeth being strong and resistant to trauma provides an excellent source for DNA finger-typing. The genome can be extracted from the pulpal tissue and crossmatched to identify the victim. Even an amount of DNA as negligible as 1 nanogram can be amplified to easily differentiate an individual with the help of the PCR (Polymerase chain reaction) technique. The amplified DNA is then compared to the post-mortem samples like blood, combs, clothes, bedsheets, etc. This method has gained popularity in forensics.[3]

Types of DNA:

a) Genomic-DNA – Found within the nucleus

b) Mitochondrial-DNA - Used when the retrieved DNA-sample is insufficient or degraded

Stages in DNA extraction:

a) Cell membrane is ruptured

b) Proteins are denatured using chelating agents and inactivated using proteinases

c) DNA is extracted using organic phenol, Chelex or isopropyl alcohol

"Advanced techniques in DNA typing:

- Restriction Fragment Length Polymorphism Typing

- Short Tandem Repeat (STR) Analysis
- Y-Chromosome Analysis
- X-Chromosome STR
- Single Nucleotide Polymorphism Analysis
- DNA methylation analysis"

Bite mark analysis: Bite marks are often found on the skin of victims in brutal events like sexual assaults, child molestation and abuse and other such crimes. Sometimes the victims while defending themselves, bite the perpetrator which leaves a mark on the attacker. The marks on males are found on the hands, forelimbs and shoulders whereas on women they're usually found on the bust and rear end. Unique characteristics like missing, broken, rotated teeth or supernumerary teeth along with the arch width can be determined. This helps in age determination as well. They have been categorized by Mac Donalds as "tooth pressure marks", "tongue pressure marks" and "tooth scrape marks".[4]

Method

Most of the bites are hardly discernible by the naked eyes and hence UV-illumination method is used to detect the bites.

1. The marks are analyzed by comparing a suspect's bite marks to those found at the crime scene with the help of transparent overlays. These overlays are usually hand traced using study-casts, wax-impressions, xenographs or other techniques like image-perception.
2. DNA can be retrieved from the saliva from the marks. This is one of the trends. salivary DNA recovery and bacterial genotyping from the bite marks are the most recent ones and have become the backbone of forensic investigation.
3. Another recent method is genotyping from bacteria from the marks.

The analysis of bite marks is not very accurate since the skin has many contortions and indentations. Therefore, they're usually used conjointly with other method of investigations to achieve a more accurate inference.[5-7]

Dental Record Maintenance: The dental records are legalized documents which contain the data of the patients. The dentist must maintain these records which are very useful in identifying the deceased victim or the criminals in medicolegal cases and forensics. The records also help in following up on the patients. They have been of great use in many disasters such as the 11th September WTC attacks and the tsunami of the Indian Ocean. The antemortem records are compared with the post mortem affirmations or other pieces of evidence found at the crime scene. These records have an account of all the complaints, medical histories, clinical examinations, diagnoses, dental-treatments, prognoses of the patients. These are usually penned down in forms and stored in huge shelves inside a storeroom. However, with the advent of technology, storing data in computers makes it easier for transferring and networking documents without much hassle. In America and other states of Europe, maintaining teeth records is mandated by law. The case is not the same in a third world country like India where people including many dentists are not aware of this practice and its uses. [8,9]

Dental imaging: The first application of radiology in forensics dates back to the year 1898, which is 3 years after the first radiograph was devised by C. Roentgen. Since then they have been playing a vital role in post mortem examinations. The post mortem radiographs are superimposed on the ante mortem images and then the comparison is made. Facial snapshots and videos show features of individuals in detail and hence are very useful in identification purposes. Orthodontists can help in determining the various oral tissues that form the smile of a person with the help of radiographs and photographs.

*Identification of victims***:** Antemortem computed tomography pictures layout many details which help in reproducing a duplicate photograph of the deceased person, taking into consideration that points of craniometry can be accurately located and calculations can be made. Every person has a unique design of their frontal-sinus which can also be put in service in identifying the victims.[10]

*Age estimation of adults from dental radiograph***:** With an increase in age, the proportions of the pulpal tooth cavity reduces as the consequence of secondary dentinal deposition. This takes place along with a reduction in the pulpal size. Hence, the pulp-tooth ratio can be determined to estimate the age of a person. The 3rd finger's middle-phalanx radiographs also indicate the skeletal maturity of a child.

To determine the cause of death: Radiographs may provide evidence of bullets or foreign bodies in the soft tissues following firearm incidence and explosion. After air crash accidents, the cause of the crash may be elucidated by the presence of radio-opaque objects in the soft tissues.[11-13]

Dental profiling: Profiles of individuals before their deaths can be designed with the help of radiographs. The post mortem anterior teeth play a major role in profile designing as they can be remodeled to estimate their alignment and angulations. The triad of factors taken into account are age, gender and ethnicity.[11-13]

Radiographs must be curated before the head and neck necropsy. Ideally, they should be taken at the crime scene. Proper labeling with IDs, location and date is mandatory and helps in the future.[11-13]

Rugoscopy: Rugoscopy is the study of palatal rugae. It is an emerging tool used for personnel identification in forensics. They have three to seven folds of irregular connective tissue known as 'plica palatine' located on the anterior third of the palate. These are unique to each individual. They are quite reliable as they are safeguarded by tongue and buccal fat and remain unruffled by any trauma. This method is preferred when teeth are absent.Several impression materials such as alginate, impression compounds, etc are used to record the pattern. Then the casts are poured and the rugae are compared to those of the suspects.

Other method used to examine the rugae markings comprise of:

- Photographs
- Clcorrugoscopy (overlay print)
- Stereoscopy (creates a 3D image of the rugae)
- Stereophotogrammetry"
- Computer-software like RUGFP-ID

Studies have shown that there is no sexual dimorphism in the rugae patterns which is why they cannot be used to determine gender. Although the patterns are unique to each individual, some errors may be found as they can change with age, orthodontic treatments, and other surgeries.

Cheiloscopy: Cheiloscopy (*Cheilos* meaning lips and *scope* meaning examination) is a forensic investigation technique dealing with the identification of humans based on their lip prints. Just like fingerprints, the grooves present on one's lips (sulci labiorum) are unique to each individual. Lip prints should be obtained within 24 hours of death from the deceased person's lips or from objects like cups, glasses, phones, clothing, etc. at the crime scene. The prints are clear-cut in closed mouth and when the mouth is open the prints are ill-defined and hard to decipher. The prints can be inaccurate if the lips wear a thick layer of lipstick or balm or in pathological conditions like mucoceles, traumatic ulcerations, loss of support in absence of anterior teeth.

Suzuki and Tsuchihashi's Classification of lip prints (1970):

- Type I: "Prominent grooves running vertically across the lip
- Type I': Straight grooves which disappear half-way
- Type II: Fork grooves
- Type III: Intersecting grooves
- Type IV: Reticulate grooves
- Type V: Undermined"

Cheiloscopy plays a major role in age determination. They have been proven to be superior to fingerprinting in determining gender. According to the Vahanwala-Parekh study,a study conducted by Vahanwala-Parekh,

- Type I and Ia patterns were primarily found in females in the third and fourth quadrants
- Type II pattern was dominant in males in the second quadrant Individuals with different patterns in all quadrants were common in males, whereas having the same pattern in all quadrants were seen in females.

Lip prints can be procured by cellophane/scotch tapes which are sensitive to pressure. Of late, biometrics scanning of lips has gained traction.[14]

Recent concepts in forensic dental identification:

Denture identification method: If the injured/ deceased person has been found to wear dentures, then the identification process becomes effortless, provided that the dentures are marked. Marking the dental prostheses is not practiced in India where people are not cognizant about the merits of marking dentures. [15]

Method

- Superficial marking: involves etching and embossing on the surface of the denture. The backdrop with this is that the markings fade with time and hence not permanent.
- Inclusive marking: This involves metal identification-bands, lead paper-labels, micro-labels, T bars, bar codes, etching by lasers, radiofrequency ID tags, polyethylene lenticular cards, photoelectric microchips.

Every country has its own special identification numbering system for its citizens. In America, the dentures are labeled with the social-security digits whereas in Australia citizens have their tax numbers which are labeled on dentures. In India, this practice is yet to be started and when it does, we can effectively use our unique Aadhar QR bar code to label on the dentures.

Comparison Microscopes: Comparison-microscopes, as the name suggests, help in comparing different specimens simultaneously. The device comprises of 2 microscopes attached by an optical-bridge. This bridge has a split-view window. This device allows easy comparability between a piece of evidence-specimen and the smear of a suspect.

Determination of both sex and age can be easily done using this device. Sex can be determined with the absence or presence of Y-chromosome and age can be found out by examining teeth for annuli of cementum. With age, cementum forms ring-like annuli in teeth just like the trees. This phenomenon called, cementum aging, can be examined under the microscope for estimating the age of an individual. The virtual comparison- microscopes have excellent accuracy and hence are an evolving trend in forensics. They can also identify bullets.

Facial reconstruction: When a person's face gets damaged beyond identification, this field of forensics comes into play in fabricating a facsimile of the person's premortem face. The skull does not decompose easily and hence it plays a crucial role along with the soft tissues over the bone. The method can be both 2D and 3D.[12-16]

Method

- Manual**:** Impressions of the skull are taken, casts poured, face is manually sculpted with modelingclay. The facial-features are chiseled on the model and hair is added.
- Computer-assisted**:** Virtually sculpting a face on computerised software using various imaging techniques that give an idea about the soft-tissue depth. The various imaging method are cephalometry, CT, CBCT, MRI, lasers, digital stereophotogrammetry and fusion imaging.

Face being the most distinct feature of humans is of utmost importance when it comes to identification techniques. Although reconstruction may not produce the exact copy of faces but it has proven to be a splendid.[12-16]

Tongue Prints: Tongue prints just like finger and lip prints are unique to each individual. Not just prints, the differences in the texture, size, colour and shape can be visually inspected. This method of personnel identification is burgeoning now in forensics. It has been recently added to the family of biometrics.

These prints can be recorded with impression-materials like alginate, casts are poured and the model of the tongue is prepared for studying the details and comparisons are made. For this technique to be successful, the antemortem images of the tongue should be available in the form of digital databases. It was found in a study that males had septate tongues whereas females had sharper tips. Hence, approximate gender estimation is also possible using this method. The presence of pathologies on the tongue may cause errors.[12-16]

Conclusion

Forensic odontology is a budding field of dentistry which has evolved manifolds over the years and has a great scope for further development. The anatomy of the tooth is unique to each individual and its resistant nature even in harsh conditions has made it an excellent and reliable source for personnel identification. However, the various method devised for the same will have little or no use if there is an absence of premortem records of the person as without them the post mortem records cannot be compared and identified. In many western countries, dental record keeping has been mandated by the law. Therefore, in our country too, the government should encourage this practice and the researches related to it while paving the way for the implementation of several new technologies in the field of forensic dentistry.

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Complication & Management of Local Anesthesia: A Systemic Review

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Abstract

Local anesthetics are widely used in the dentistry world for various procedures. The local anesthesia is very much effective and also safe but they have also risks which practitioners should be aware off and this article reviews the different complication regarding the use of local anesthesia. Different complications occur which include systematic and regional complications that can occur with the use of local anesthesia. The various complications include ocular complications, methemoglobinemia, toxicity, and paraesthesia, etc. Knowing the risk factors involved with local anesthesia relatively decreases the adverse outcome and helps the patient by improving health care.

Keywords: *Local Anesthesia, Paraesthesia, systemic and regional complication, Toxicity.*

Introduction

In the year 1860s, the first local anesthesia or anesthetic was isolated from the coca leaves by a German personcalled as Albert Niemann. Local anesthesia is widely used for dental procedures throughout the globe and lidocaine is currently the widely used local dental anesthetic solution in most of the countries of the world. One can define local anesthesia as the temporary loss of sensation or pain in a single particular part of the human body that has been produced by an injected or topically applied agent without suppressing the level of consciousness.[1, 2] The dental anesthetics include two classifications, which are described as esters that include procaine and benzocaine, and amides which consist of lidocaine, prilocaine, mepivacaine and articaine. Commercially available local anesthetic agents have a pH of 3.5- 5.5 and also have a shelf life of 3 to 4 years. This type of pH is very low to the drug pKa which all the vitally all the drugs are present in the more stable, water-soluble and charged form. Generally, the composition of local anesthetics includes lignocaine hydrochloride, a reducing agent that is sodium metabisulfite, a preserving agent that is methylparaben, and a diluting agent which includes distilled water.[3]

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Mechanism of action of local anesthesia:

The steps following the mechanism of action includes the:

- The anesthetic which are ionized by binding to the sodium channel
- The entry of the sodium channel is blocked in these different channels
- Blockage of sodium channels prevents the propagation of the action potential
- As the lack of propagation blocks the sensation as the signal is not transferred to the brain.[1-3]

Testing the success of Local Anesthesia:

Testing for a successful Local anesthesia should be done by the:

Subjective testing that includes asking the patient to describe the area that is altered in sensation which is usually positive with the nerve block and mandibular injections and anterior maxillary. Objective testing that includes the use of a probe or pulp testing or percussion. When probe the soft tissue always one should consider the supplementary nerve supply and 1A is positive with the onset time for the 1A is 2-3 minutes and for the NB is 3-4 minutes usually. Pulpal local anesthesia had a longer onset time as compared to the soft tissue anesthesia.[4]

Local Complications

The local complication included during the use of local anesthesia are:

Needle Breakage: It is one of the common complications that occur due to the bending of the needle, sudden movement of the patient, the entire needle is entered into the soft tissue, or using a smaller needle-like 40 gauge. Prevention includes using large gauge needles, especially in the case of the posterior superior alveolar nerve and inferior alveolar nerve and one should not insert the needles into tissues to its hub and take preventive not to redirect a needle once it's injected into the soft tissue. When a needle breaks and it is visible stay calm and instructs the patient not to move and left his/her mouth open. If the fragment is visible remove it from hemostat or Magill intubation forceps. If the fragment is not visible calmly inform the patient, inform an oral surgeon, and take a radiograph and determine its position whether it is superficial or not or leave it and follow up.[4-6]

Ocular Complications: There are different complication regarding ocular that includes Mydriasis that is pupillary dilation, Amaurosis which is temporary blindness, ptosis i.e. drooping of eyelid and diplopia which means double vision. The causes for these complications include inadvertent arterial injection with retrograde blood flow and orbital injection that includes inadvertent injection into orbit through the inferior orbital fissure. Prevention includes aspiration before the actual injection and inject slowly. The treatment includes make the patient reassure that is transient; cover the affected eye with gauze dressing. Refer the patient to an ophthalmologist for the evaluation if it lasts for more than 6 months and make follow up. [4-6]

Paraesthesia: This type of complication arises due to trauma of nerve, insertion of the needle to inside the foramen can also lead to the paraesthesia. Local anesthesia solution which is contaminated by sterilizing solution and the alcohol can produce irritation that results in the edema and increased pressure in the region of nerves which leads to paraesthesia. Also, the hemorrhage leads to an increase in the pressure paraesthesia. One can prevent this complication by proper care and handling of the injection and also the cartridge. In most of the cases having paraesthesia, it resolves within 8 weeks without treatment. A sequence followed for this complication includes-Reassuring the patient party; properly examine the patient and regular follow up for at least 2 months; If the sensory problem persists, consult a neurologist and an oral surgeon.[4-6]

Facial Nerve Paralysis: It occurs due to the when anesthesia is introduced into the deep lobe of the parotid gland. The cause for this local complication includes transient FNP that is due to the local anesthesia into the capsule of the parotid gland that is present in the posterior border of the mandibular ramus. It is usually occurring during the Vazirani alkiosi injection nerve block or alveolar nerve block. Prevention can be done by proper care to the needle while inserting and cartridge. The management includes reassuring the patient, contact lenses should be removed and eye patches should be applied to the affected eye or one can manually close the lower part of the eyelid periodically that will keep the cornea lubricated.[4-6]

Trismus: It can be defined as the pain and difficulty in the opening of the mouth that often occurs after the inferior alveolar nerve block and superior nerve block. The onset period is 1-6 days post-treatment. It is caused by the trauma to blood vessels or muscles area in the infratemporal fossa. It also occurs due to the local anesthesia solution contaminated by alcohol or cold sterilizing solutions that gives or produces the irritation of the muscles. Low-grade infection can often lead to Trismus. The prevention measure includes using the sterile, sharp, and disposable needle. Proper handling and care to the injection and cartridge. Avoiding repeating off the atraumatic injection. Management includes the heat therapy, warm saline rinse, Using of the analgesic like aspirin 325mg, If necessary then muscle relaxation, diazepam 10mg BID. Physiotherapy for 5 min each in every 3-4 hours. If the infection occurs then antibiotic is prescribed for 7 days. Surgical intervention can be done in some cases. The recovery range is between 4-20 days.[4-6]

Soft-Tissue Injury: It is trauma to the lips or the tongue that is produced by the biting or chewing of these tissues while anesthetized, especially occurs in the case of children. In Prevention A cotton roll can be placed between the lips and teeth & warn the patient with a self-adherent warning sticker. It can be managed by analgesic for pain, antibiotics if there is infection, warm saline rinse to decrease the swelling. Petroleum jelly should be used to control the lesion and minimize the irritation.[4-6]

Hematoma: It occurs due to the effusion of the blood into the extravascular spaces and results from inadvertently a blood vessel. It is caused by the nicking to the vein or artery. It mostly occurs with the IANB and PSA nerve. The practitioner should have a proper knowledge of anatomy, using the shorter needle for PSA nerve block. One should minimize the number of needle penetration. The Management includes the direct pressure applied in the site of bleeding. Applying cold moist towels to the affected area for 20 minutes every hour. Make sure to advise the patient regarding the limitation and soreness of mouth opening possibility.[4-6]

Pain on Injection: It mostly occurs due to the carelessness of the practitioners and mostly the palatal injection hurts. Dull of needle due to multiple injections. It can also be due to the rapid deposition of the local anesthetic solution. Prevention is Adhere to the proper technique of the insertion process including both the physiological and anatomical. Sharp needles should be used and topical anesthetic should be applied with injecting the needle slowly.[4-6]

Infection: It is caused due to the contamination of needle but in this modern world, it has become rare due to the introduction of sterile disposable needle and the glass cartridge. The managing includes the antibiotic, penicillin 250 mg QID. [4-6]

Edema: The cause for this local complication includes trauma, infection, allergy hemorrhage, or injection of the irritating solution like alcohol and cold solution. Management includes a minimal degree of edema – by giving analgesic for pain and will disappear in several days. If the edema occurs in severe form then antibiotics should be prescribed.[7-9]

Systemic Complications: The systemic complications include the overdose factor and the Allergy factor.

Overdose: The overdose reaction occurs when the drug access to the circulatory system. There is a constant absorption of drugs from the sites of the admission into the circulatory system and a steady removal by the liver of the blood. [7-9]

There are various predisposing factors which includes generally 2 factors:

- Patient Factors- This includes age, weight, medications that is an important one, gender, genetics, etc.
- Drug Factors- it includes concentration, dose, rate of injection, presence of the vasoconstrictors, and route of administration.

The preventive measures include using an aspiration syringe, using the needle which should not be smaller than 25 gauge. The aspiration should be done at least two planes before insertion. The anesthesia should be given very slowly and calmly.

Clinical Manifestation: The surgeon should notice the slurred speech, apprehension, talkativeness, excitability, and stutter. It also includes the sweating, vomiting, failure in following commands. Blood pressure is elevated with heart rate and respiratory rate. One can also notice the CNS depression and myocardial depression.[7-9]

Management: The basic emergency management includes–Position; Circulation; Breathing; Airway; Definitive Care. The sequence can be followed as P-C-A-B-D. The management in the case of mild overdose includes reassure the patient and administrate oxygen via the nasal canal and then monitor the record the vital signs. The anticonvulsants via IV should be administrated i.e. diazepam 5mg/min and the emergency medical assistance should be done before the discharge of the patient. In the case of severe overdose when the patient is unconscious then the following steps to be followed– Protect the patient, immediately call the emergency medical assistance, and most important continuing the Basic life support (BLS). Administration of anticonvulsants that is diazepam 5mg/min, if the seizure protract more than 5 minutes.[7-9]

Allergy: It is a type of systemic complication. It occurs due to the hypersensitive state that is acquired through the exposure of a particular allergen. The allergic reactions cover a broad spectrum of OD

clinically manifestations that range from the mild to the delayed response in the occurring as long as 48 hours after the exposure of the allergen to a mark of immediate and dangerous reaction that develops within seconds of the exposure. The predisposing factors include sodium bisulfate, Epinephrine, Latex, and topical anesthesia. The clinical manifestation includes: Generalized Anaphylaxis; Different Dermatological reactions; Respiratory Reactions.[16]

The clinical manifestation for the dermatological reaction includes urticaria and angioedema and the clinical manifestation of respiratory reactions includes bronchospasm that includes dyspnea, wheezing, cyanosis, Tachycardia which is life-threatening, anxiety, respiratory distress, and the laryngeal edema includes extension of edema and life dangering emergency. The clinical manifestations of generalized anaphylaxis include different skin reactions, smooth muscle spasm of gastrointestinal and the genitourinary tracts with bronchospasm and respiratory distress and sometimes cardiovascular collapse can persist or occurs. [10]

The management process in the skin reaction includes giving oral histamine blocker 50mg diphenhydramine with observation for at least 1 hour and medical consultation should be done. The management of respiratory reaction includes the administration of the oxygen at flow 5-5 liters per minute. If needed epinephrine 0.3 via IM with repeated 10-20 minutes. IM histamine blocker 50mg diphenhydramine should be given. Medical consultation should be taken and an oral histamine blocker should be prescribed for 3 days. The management of generalized anaphylaxis includes the medical assistance in the very first form with the epinephrine dose of the 0.3 IM for 10-20 minutes repeated. Administer of oxygen should be given with an Intramuscular histamine blocker or one can give the corticosteroid IM or IV. Monitoring of the vital signs should be recorded every 5 minutes in case of the unconscious patient.[10]

Conclusion

Today in the world of medicines and advancement new uses of the drugs and local anesthetic agents are been used and with the advancement in medical science, the complication of the drugs are increasing. The various ways should always be understood by the surgeon and the professionals to avoid the life-threatening risks for a patient. One should have skillful knowledge and a good mind set up to acquire or know the various hazards or complications from using local anesthesia. However in countries like India where numerous quacks are frequently practicing, which is challenging for our society as it produces a maximum risk of life treatment for the patient. The people should be aware and the mass awareness should be created to tackle this problem and a patient should always check the original practitioner's license followed by its different treatment. However, it's very challenging in our country due to the expensive dental procedures. A professional should always keep in mind that what are the risk factors and its consequence followed by simplicity and transparency in the treatment. A careful and proper treatment will lead to happiness and put forward a bright smile on the patient's face that will enhance the quality and prospective life in the society.

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Oral Hygiene Practice and Oral Health Status among Tribal Children of Odisha, India

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Abstract

Objectives: The study aimed to assess the oral hygiene levels among tribal children belonging to a specific tribe in the state of Odisha, India and to correlate their teeth cleaning aids used for maintaining oral hygiene and the levels of oral hygiene.

Method: Oral hygiene status of a total of 1000 children between 8-13 years of age (boys 467, girls 533) were included in the study. A simplified oral hygiene index (OHI) developed by Greene and Vermillion consisting of two major components Debris index-simplified and calculus index-simplified was used for examining the OHI-S. Oral Hygiene Index- Simplified; the scores are as follows: Good: 0.0-1.2; Fair: 1.3-3.0; Poor: 3.1-6.0.

Results: The simplified debris index is good for 42.2% of children using toothpaste and toothbrush, 12% of children using indigenous powder with fingers, 3.4% of children using a twig and none of the children using gudakhu. The simplified calculus index is good for 66.1% of children using toothpaste and toothbrush, 12% of children using indigenous powder with fingers, 5.8% of children using a twig. The simplified oral hygiene index is good for 68.5% of children using toothpaste and toothbrush, 12% of children using indigenous powder with fingers, 4.5% of children using a twig and 38.9% of the children have good oral hygiene index who are using gudakhu.

Conclusions: Data showed that the oral hygiene index scores are mostly poor in the children using the indigenous method of oral hygiene practice. This data could be used as baseline information for health authorities and dental professionals for planning strategies for oral health programs in this tribal population as using addiction-prone tobacco pastes can lead to irreparable damage later in life.

Keywords: *Tribes, Oral hygiene Index Simplified, Tooth cleaning aids.*

Introduction

According to several studies diseases of periodontium had been prevailing since ages. There is a lot of effort made to combat those diseases but it is still prevailing among backward classes due to variations from normal oral hygiene routines and procedures.[1] Even in this contemporary world, some people are away from the civilization buried in their dark ignorant logic and tied to the misbeliefs and superstations following old and obsolete modes and modalities of lifestyle and health care practices. Among them are the "Tribes" of our country.[2] There are around 75 types of major tribal communities hailing in our country and they are again divided into many subgroups and clans. They have been mostly found in 15 states and union territories and are segregated into various clans depending upon their cultural practices.[3] From among those the state of Odisha nurtures about 36 different types of tribal populations

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including Kondha, Kolha, Munda, Bhumiya, Bonda, Mohali, Loharas, Sauras, Shantal, Gond, Gadava.[4] The vast repository of tribal population is found in the Mayurbhanj district of Odisha. Those population groups are characterized by lower-income and high treatment needs. Therefore, there is a need to access oral hygiene practice and oral health status among those tribes. The objective of this study was to assess the oral hygiene levels among tribal children and to correlate the teeth cleaning aids used for maintaining oral hygiene and the levels of oral health.

Methodology

A total of 1000 children between 8-13 years of age (boys 467, girls 533) were included in the study. The oral hygiene status of the children was examined using a simplified oral hygiene index (Oral Hygiene Index-Simplified).[4] OHI-S was developed in 1964 by Greene and Vermillion and has two components Debris index-simplified and calculus index-simplified. Oral Hygiene Index- Simplified; the scores are as follows: Good: 0.0-1.2; Fair: 1.3-3.0; Poor: 3.1-6.0.

Results

A total of 1000 school going tribal children were examined by a single examiner, among these children, 578 (57.8%) brushing their teeth with toothbrush and paste, 379 children (37.9%) with twigs, 25 Indigenous powders with finger (2.5%), 18 Gudakhu with fingers (1.8%) (Table 1).Association of oral hygiene levels among children using different teeth cleaning aids. The simplified debris index is good for 42.2% of children using toothpaste and toothbrush. It is good for 12% of children using indigenous powder with fingers, good for 3.4% of children using a twig and none of the children have good debris index who are using gudakhu. Thus better debris index is significantly associated with children using toothbrush and toothpaste (p=0.000) than the children using other method of teeth cleaning. Overall 260 (26%) children have good, 734 (73.4%) have fair and 6 (0.6%) have poor debris index. This is an area of concern.

The simplified calculus index is good for 66.1% of children using toothpaste and toothbrush. It is good for 12% of children using indigenous powder with fingers, good for 5.8% of children using a twig and 38.9% of the children have good calculus index who are using gudakhu. Thus better calculus index is significantly associated with children using toothbrush and toothpaste (p=0.000) than the children using other method of teeth cleaning. Overall 414 (41.4%) children have good, 192 (19.2%) have fair and 394 (39.4%) have poor calculus index. This is an area of concern and require intervention.

The simplified oral hygiene index is good for 68.5% of children using toothpaste and toothbrush. It is good for 12% of children using indigenous powder with fingers, good for 4.5% of children using a twig and 38.9% of the children have good oral hygiene index who are using gudakhu. Thus better calculus index is significantly associated with children using toothbrush and toothpaste followed by gudakhu (p=0.000) than the children using other method of teeth cleaning. Overall 423 (42.3%) children have good, 233 (23.3%) have fair and 344 (34.4%) have poor oral hygiene index. (Table 2), which is an area of concern and require intervention.

Table 1: Distribution of primary teeth cleaning aid

Primary teeth cleaning aid	No.	%
Toothbrush and toothpaste	578	57.8
Indigenous powders with finger	25	2.5
Twig	379	37.9
Gudakhu with fingers	18	1.8
Total	**1000**	**100**

Table 2: Association of primary teeth cleaning aid with oral hygiene level

Oral hygiene index parameters	Primary teeth cleaning aid	Good		Fair		Poor		Total		Fisher's Exact 'p' value
		No.	%	No.	%	No.	%	No.	%	
Debris index simplified	Tooth brush and tooth paste	244	42.2	334	57.8	0	0	578	100	0.000
	Indigenous powders with finger	3	12	22	88	0	0	25	100	
	Twig	13	3.4	360	95	6	1.6	379	100	
	Gudakhu with fingers	0	0	18	100	0	0	18	100	
	Total	260	26	734	73.4	6	0.6	1000	100	

Oral hygiene index parameters	Primary teeth cleaning aid	Good		Fair		Poor		Total		Fisher's Exact 'p' value
		No.	%	No.	%	No.	%	No.	%	
Calculus index simplified	Tooth brush and tooth paste	382	66.1	170	29.4	26	4.5	578	100	0.000
	Indigenous powders with finger	3	12	0	0	22	88	25	100	
	Twig	22	5.8	14	3.7	343	90.5	379	100	
	Gudakhu with fingers	7	38.9	8	44.4	3	16.7	18	100	
	Total	414	41.4	192	19.2	394	39.4	1000	100	
Oral hygiene index simplified	Tooth brush and tooth paste	396	68.5	161	27.9	21	3.6	578	100	0.000
	Indigenous powders with finger	3	12	2	8	20	80	25	100	
	Twig	17	4.5	62	16.4	300	79.2	379	100	
	Gudakhu with fingers	7	38.9	8	44.4	3	16.7	18	100	
	Total	423	42.3	233	23.3	344	34.4	1000	100	

Discussion

The oral hygiene index is found to be mostly good among the children using toothbrush and toothpaste, they have a fair debris index and a good calculus index whereas the children using twigs and various indigenous powders like charcoal, sand, clay, etc. have a poor oral hygiene index. The children using gudakhu have a fair oral hygiene index. This result is similar to the result found by P. Krishnam Raju et al, 12% of children using twigs have poor oral hygiene index score whereas only 3% of children using toothpaste and toothbrush have poor oral hygiene[5] according to a study by Rao and Bharambe the children using twigs and Manjans for cleaning teeth have higher calculus index along with gingival bleeding and periodontal abscess.[6] These results are governed by many factors such as socioeconomic status, awareness, attitude of the child, and the caregivers towards the oral hygiene. The children who belonged to a lower socio-economic background can't even afford for twigs those children mostly used charcoal, ash, etc. the oral hygiene condition of those children is very poor, they use charcoal with fingers so, they ought to have a poor oral hygiene. The children who use toothbrushes and toothpaste even if they don't know proper brushing techniques still due to swiping motion of toothbrush there is removal of debris and plaque which indeed prevents the calculus formation leading to good oral hygiene scores. In contradiction, certain other studies by Naheeda et al and Tanwar A. et al found the indigenous method to be more effective in oral hygiene maintenance than a toothbrush and toothpaste.[7] The reason of variation could be the use of different indices and use of twigs of different plants, as in the studies by Naheeda et al and Tanwar A. et al the participants mostly used meswak or neem twigs but in our study, the children mostly used twigs from Sal tree (*Shorea robusta*). Neem and Meshwak have antimicrobial properties as proved by certain studies such as a study by Al-Otaibi M et al where they found that *A. actinomycetemcomitans* were significantly more reduced by meswak ($p<0.05$) than by tooth brushing.[8] These results were supported by us in vitro results which, indicated that extracts from *Salvadora persica* might interfere with the growth and leukotoxicity of *A. actinomycetemcomitans*. Sal bark does not have any such antimicrobial properties.

Conclusion

The oral hygiene index scores are mostly poor in the children using the indigenous method of oral hygiene practice with the calculus index being significantly high, in children using toothpaste and toothbrush mostly had a fair to good oral hygiene score with low calculus index. From the above-mentioned result, it is quite clear that the entire tribal population not only children but also their caregivers need awareness programs, screening, and treatment camps to increase basic oral health knowledge. Anti-tobacco campaigns have also become a need of an hour among those populations. Hence, the oral hygiene behavior in children require emergency attention as coarse granular materials, addiction-prone tobacco pastes can lead to irreparable damage later in life.

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Dental Implants in Pediatric Dentistry: A Review Article

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Abstract

Loss of teeth in children is commonly caused due to traumatic exposure or anodontia. It can lead to loss of masticatory function, altered speech, lack of self confidence and mal-alignment of teeth overtime. This hampers the esthetic and psychosocial development of children. Management of missing teeth can be done using removable prosthesis or fixed prosthesis and implants. The removable prosthesis can lead to residual ridge resorption and several periodontal problems and can cause oral hygiene maintenance issues in a child for which dental implants are considered to be an ideal mode of treatment for tooth loss. Placing an implant in a growing child can cause undue effects on maxillary and mandibular skeletal growth for which the dentist must plan a treatment taking into account the total number of missing teeth, degree of skeletal growth and related psychological stress to the child.

Keywords: *Dental Implant, Anodontia, Maxillary and mandibular skeletal growth, Child.*

Introduction

The absence of one or a few teeth manifested due to genetic condition, trauma, or any disease is known as hypodontia or oligodontia. Total anodontia is the congenital absence of all teeth in permanent or primary dentition.[1]The most commonly missing teeth are third molars and they don't require replacement. Mandibular second premolars are the second most commonly missing teeth followed by maxillary lateral incisors and maxillary second premolars.[1]The commonest cause for loss of tooth is trauma, especially the loss of anterior teeth. Its predisposing factors are incompetent lips and proclination of maxillary anterior."Avulsion Is defined as the complete and total displacement of a tooth from its socket" [1]. The most commonly avulsed teeth are maxillary central incisors.

The management of multiple tooth loss can be done by partial coverage fixed prosthesis such as resin-bonded restorations or removable prosthesis. The management of loss of a single tooth or avulsion is done as conservatively as possible in a young child. It is done by deploying treatment like replantation, attempting revascularization, apexification, and root canal treatment. The above-mentioned treatment for loss of multiple teeth has certain drawbacks. In both cases, removable and fixed dentures, the child's compliance is necessary. Maintenance of oral hygiene becomes an issue. It predisposes to several periodontal problems followed by bone resorption ultimately. Apart from that, new prosthesis needs to be fabricated from time to time, to make up for craniofacial growth.

Implants can be considered as an ideal method of treatment for tooth loss in young children, as it restores the function of missing teeth by preserving the alveolar bone and also provides excellent esthetics. However, it requires a proper understanding of growth and growth assessment by the dentist to establish a successful implant in a young patient.[2]

Growth: The maxillary and mandibular growth is multidirectional and occurs in sagittal, vertical, and transverse planes. It occurs in varying paces, periods of slow pace growth followed by accelerated growth called the growth spurts. The teeth maintain their position in the arches by following this pace of growth through

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remodeling and drifting within the alveolar bone. Functional forces are balanced by a stable inter-arch occlusal relationship achieved gradually as transition from primary to permanent dentition.[3]

Maxillary Growth: The maxillary growth occurs by displacement, growth at sutures, and surface remodeling"[2]. The displacement is of two types- primary and secondary. The primary displacement occurs by the growth of maxillary tuberosity in a posterior direction that results in the whole maxilla being carried anteriorly.[2] The secondary displacement occurs due to the growth of the cranial base that leads to displacement of the nasomaxillary complex in a downward and forward direction. The transverse growth of the maxilla is facilitated by growth at median sutures. This leads to forward and downward relocation of maxilla. Sagittal growth occurs by surface remodeling of the anterior surface of the maxilla. It brings the maxilla even more downwards and forwards.[3]

The vertical growth of the maxilla occurs by bone resorption on the lateral wall of the nose and floor of the nasal cavity followed by bone deposition on the palatal side. Also, when the teeth start erupting, there is bone deposition at the alveolar margins that increases maxillary height.[2-5]

Vertical growth is the last to complete, at around the age of 17 to 18 years in girls and even later in boys. Hence the maxillary implants tend to perforate the nasal floor even after puberty when the permanent teeth have erupted already. Mikel Westwood and Duncan James, in their study, concluded that the implants placed in the maxillary anterior region are at less risk of submersion, due to the angulation changes it undergoes. It is because of the less maxillary vertical growth and also less bone loss when the implant is placed within less time of tooth removal.[6]

Mandibular Growth:

Of the facial bones, the mandible undergoes the largest amount of growth postnatally and also exhibits the largest variability in morphology"[2]. Mandible that appears as a single bone in adults can be divided developmentally and functionally into various skeletal subunits that are namely-

Body of mandible, Alveolar process, Coronoid process, Condylar process, Angular process, Ramus, Lingual tuberosity, and the Chin[2].

- There is drifting of the ramus in the posterior direction by sequential bone resorption on the anterior part of the ramus and bone deposition on the posterior part of the ramus.
- "The displacement of the ramus results in the conversion of former ramal bone into the posterior part of the body of the mandible"[2]. By this process, there's lengthening of the body of the ramus.
- There is a flaring of the angle of the mandible as the person grows older. This occurs by sequential bone resorption on the posterior inferior aspect of the lingual side and bone deposition on the anterosuperior aspect of the lingual side of the angle of the mandible. Contrary to this, on the buccal aspect of the angle of the mandible, bone resorption occurs in the anterosuperior part and bone deposition occurs in the posteroinferior part.
- There is a posterior movement of lingual tuberosity by deposition on its posterior facing surface. It lies towards the midline of the ramus protruding in a lingual direction. "There's a large resorption field just below the lingual tuberosity. Resorption occurs producing a large depression called lingual fossa. Resorption in the fossa and deposition on the medial surface of tuberosity combined makes the lingual tuberosity more prominent.[2]
- "Alveolar process develops in response to the presence of tooth buds. As the teeth erupt, the alveolar process develops and increases in height by bone deposition at the margins"[2]. This leads to overall growth in the vertical height of the mandible.
- The growth of the chin is influenced by sexual and specific genetic factors. A fully developed chin is only seen in a man. Usually, males have a more prominent chin as compared to the females.
- The head of the condyle is covered by the Condylar cartilage to help withstand the compressive forces that occur at the joint. The mandibular growth occurs by bone deposition at the Condylar cartilage. "Also there is growth of soft tissues (muscles and connective tissues) that carry the mandible forwards, away from the cranial base. Following this, bone growth occurs at the Condyle to maintain contact with the cranial base. The Condylar growth rate increases at puberty reaching a peak between 12½ to 14 years. The growth ceases around 20 years of age[2].

- The Coronoid process grows by bone deposition on the lingual surfaces of the Coronoid processes. Also, the vertical dimension of the Coronoid process increases by the enlarging 'V' principle.

In the mandible, the amount of anterior implant exposure or submergence of a posterior implant depends on the direction and amount of rotation during growth[9]. The implants in the mandibular anterior tooth region are at a risk of getting exposed due to the infra dental resorption, at the time of formation of the chin in adolescents. The implants in the mandibular posterior tooth region are at a risk of submerging due to the appositional growth of the alveolar bone.Cronin et. al. studied mandibular growth related to the implants in children with a strong rotational growth pattern. With erupting posterior teeth, alveolar growth continued to maintain the occlusal plane. This resulted in the implant being deeply buried within the mandibular alveolar process. They suggested that successful mandibular implants are favored by a lack of complicated structure. [5-10]

Treatment approach for quadrant-wise implant placement:

Maxillary Anterior Region: It is an important area of consideration due to higher chances of traumatic tooth loss and frequent congenital tooth absence. Substantial growth is seen in vertical and anteroposterior dimensions. The vertical growth is maximum in this quadrant, as compared to all other quadrants. Hence, premature placement of implants can lead to growth cessation and requirement for lengthening transmucosal implant connection. Hence, the placement of implants should be delayed upto the age of 15 years in girls and 17 years in boys.

Maxillary Posterior Region: Maxillary posterior quadrant has similar growth factors as described for the maxillary anterior-posterior area. An additional growth factor transfers maxillary growth at midpalatal suture, which produces rotational growth that anteriorizes the position of maxillary molars. Placement of osseointegrated dental implants in the maxillary posterior quadrant is best delayed until the age of 15 years and 17 years of age in females and malesrespectively[4].

Mandibular Anterior Region: It is the best site for the placement of an osseointegrated implant before skeletal maturation. The mandibular anterior quadrant presents fewer growth variables. The closure of mandibular symphyseal suture occurs during the first two years of life. Prosthesis supported by dental implants in the anterior mandibular region should be of a retrievable designed to allow for an average increase of dental height of 5-6 mm as well as the anteroposterior growth.[4]

Mandibular Posterior Region: The dynamic growth and development of the posterior mandible in the transverse and anteroposterior dimensions coupled with its rotational growth presents multiple treatment concerns. Placement of osseointegrated implants in the posterior mandibular quadrant is best delayed until skeletal maturation.[4]

Discussion and Conclusion

Using implants in growing children has its advantages but only when it's placed at the right age, take into consideration the growth assessment of the patient. Various studies have suggested that use of implants, due to developmental changes of jaws and teeth in children, should be performed with high precision and systemic evaluation, and, if possible, be postponed up to the age of 15 years for girls and 18 years for boys and be within a long-term process. The dentist needs to take into account various factors that might lead to implant failure. Precise diagnosis, treatment planning, and extensive knowledge in the field of implants and pediatric dentistry are crucial for the dentist for the establishment of a successful implant. The evaluation of some factors, including causes of anodontia, patient's sex, range of skeletal growth, prosthesis design, the range and quality of residual bone ridge, keeping hygiene and satisfying parents' and patients' demands should be taken into consideration for the final decision making on implant positioning. In the end, the dentist decides to restore the function of the lost tooth and guard the patient's self-esteem, keeping in mind the patient's comfort and ease of maintenance of the prosthesis.

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SARS-CoV-2 and Oral Health: Implications and Recommendations for Dental Practice Reformation Post Pandemic

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Abstract

Dental clinics can play a crucial role in spreading the SARS-CoV-2 infections because of the nature of close contact with the patient's oral cavity and aerosol generating essential dental procedures. Dental practices in regions affected with SARS-CoV-2 are needed to take utmost precautions and follow strict effective infection control protocols even when the movement control/lockdown is gradually lifted post pandemic. Thus, we aim to summarise the current research findings on SARS-CoV-2 in dental settings and relevant international guidelines and recommended management protocols for the dental clinica. The knowledge on the viral properties of SARS-CoV-2 and epidemiologic characteristics are essential to implement proper mitigation strategies against transmission of Covid-19 infections via dental clinics.

Keywords: *SARS-CoV-2, Covid-19, dental clinic, dentistry.*

Introduction

The ongoing pandemic caused by severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) has caused more than 2,60,00,00 cases and claimed over 180000 deaths worldwide as of April 23, 2020.(1) On Feb 11, WHO named this disease as "Corona Virus Disease (COVID-19)" and later the International Committee on Taxonomy of Viruses (ICTV) named this virus as "SARS-CoV-2" based on the phylogenetic and taxonomic analysis(2). The characteristic symptoms of the Covid-19 illness are fever, myalgia and cough with headache, diarrhoea and haemoptysis as less frequent symptoms.(2,3)

Previous outbreaks of coronavirus infections included severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), emerged as a significant public health threat and were controlled effectively, though SARS-CoV-2 behaviour does not match its predecessors. After the initial pandemic wave, the behaviour of the SARS-CoV-2 and possibility of another seasonal epidemic wave are not yet elucidated.(3,4)

Properties of SARS-CoV-2: Coronaviruses belong to the family of RNA viruses *Coronaviridae* and order

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Nidovirales.[5] Among four genera of coronaviruses, α-CoV and β-CoV mainly infect the humans and mammals.[6] Many subtypes have been known to cause respiratory diseases in humans.[7,8] Unfortunately, the fatal diseases were reported only by the SARS-CoV and the Middle-East respiratory syndrome coronavirus (MERS-CoV), which belong to β-CoV.[9] The envelope of coronaviruses is coated by surface glycoprotein that leads to their characteristic crown-like (Latin: corona) appearance of the particle. Coronaviruses share a typical genome in which the replicase gene covers the 5′-two thirds of the genome.[10] Spike proteins in their envelope help to bind receptors on host cells and helps in entering into the cells.[10,11]

Oral Cavity as Target of SARS-CoV-2: Angiotensin-converting enzyme 2 (ACE2),expressed in human airway epithelialcells, oral mucosa, especially tongue epithelium and epithelial cells of minor salivary glands are the most important receptor for SARS-CoV-2highlightingthe possibility of salivary glands being a potential reservoir of SARS-CoV-2.[12, 13]Therefore the probability of oral mucosa and salivaacting as a reservoir of viral particles in asymptomatic Covid-19 patients cannot be ignored.Moreover,peak infectivity was noticed before the patient is symptomatic.[4]Positive rate of SARS-CoV-2RNA detection in hospitalized patients' saliva was as high as 91.7%.[14]

SARS-CoV-2 Transmission in Dental Clinics: Common routes of SARS-CoV-2 are through direct transmission (sneeze cough and droplets) and contact transmission (oral, nasal, and ocular mucous membrane).[15]Besides, experts have suggested that it can be transmitted directly or indirectly through saliva owing to the consistent detection of SARS-CoV-2 in saliva.(14)The incubation period of SARS-CoV-2 is reported to be 5 -6 days on average; nevertheless, it can be as long as 14 days which is currently considered as observation/quarantine period for exposed/potentially exposed individuals.[16] Dental practitioners encounter close contact with patient's oral cavity for extended hours during various aerosol/splatter generating procedures (AGP) or non AGP. Splatters (larger than 50μm) and aerosols (less than 50 μm) are particle droplets of saliva, blood and microorganisms generated while using rotary dental and surgical instruments and air-water syringes.[13,17] Travelling for short distances, splatter settle over inanimate surfaces and exposed parts of the dentist or the patient whereas aerosols remain airborne for extended periods and may be inhaled.[18,19] Humidity and temperature have also been shown to be significant determinants of particle size and viability of other airborne viruses.[11,19] It has been shown that SARS-CoV-1 and 2 can remain infectious from 2 hours up to 9 days at room temperature depending upon the relative humidity.[20] Having air-conditioned settings, these factors can play an important role in the survival of viruses in dental clinics.

Suggested Advisory to Dental Clinics: Reopening of dental clinic requires several careful considerations which starts right from giving appointment till the patient leaves the dental clinic.

a) **Appointment through tele screening:** Web/app-based technologies should be explored for initial screening, followed by scheduling appointment of patients to distribute the patients throughout the clinical hour avoiding gathering. Investigation of the patient details should always start with answering a set of questionnaires (Figure 1)asked by trained personnel during teleconsultation. "Yes" to any of the questions would categorize the patient as a probable suspect. Thedentist can defer the treatment procedure in such patients until a minimum of 14 days to a maximum of 24 days. Such patients should be advised for self-quarantine and consulting physicians. "No" to all questions would qualify the patient for scheduling the appointment.

b) **Patient evaluation at the reception of the dental clinic:** Once the patient scheduled for appointment arrives at the clinic, there will be a sign on the door with the dental assistant's phone number. Calling the dental assistant before entering the clinic is essential. The patient should enter the dental clinic after washing hands in the basin kept outside the dental clinic. At the reception, reaffirmation of the questions should be done, followed by the patient's signature. The dental assistant should record the body temperature by the non-contact thermometer or infrared thermal sensors. Except for minors and specially-abled patients, no accompanying person is allowed with the patient. In these cases, the accompanying person also has to go through the screening test. Point of care rapid screening kit may be incorporated at this stage. Reception area of the dental clinic should adequately display respiratory etiquettes and hand hygiene through several posters available at "www.cdc.gov/flu/protect/covercough.htm" and "www.cdc.gov/ncidod/dhqp/pdf/Infdis/RespiratoryPoster.pdf",

c) Clinical examination in the dental clinic: Standard precaution, as mentioned in the Centre for Disease Control and Prevention (CDC)(21), such as hand hygiene,Personal protective equipment (PPE), handling contaminated materials or equipment to prevent cross-contamination, using engineering and work practice controls, respiratory hygiene and cough etiquettes and safe injection practices. [21,22] Choice of antiseptic agents, duration and technique of hand scrubbing, techniques for drying and gloving can influence the effectiveness of the surgical hand antisepsis. CDC's 2002 guidelines and WHO provides complete information on hand hygiene.[23]

Owing to the modes of transmission of Covid-19, three categories of precaution such as droplet, contact and airborne are recommended for every patient. Therefore, gloves, masks, eye protection with side shields, protective fluid repellent gowns and N95 respirators are required while examining the patient's oral cavity. Extraoral radiographs are preferable to intraoral ones. Extra barrier should be followed when intraoral imaging is essential.

d) Treatment procedure: Only essential staff should be allowed in the room during operative procedures. AGP demand strict use of personal protective equipment. Although CDC 2007 guidelines donot recommend eyewear for closely examining the patients it may be used by the dentist during splatter or AGP.[22]Selection of PPE depends upon the type of clinical exposure anticipated, durability and appropriateness of task as well as fit. Respiratory protection is the most important component of PPE which can be achieved through particulate respirators, half or full-face elastomeric respirators and powered air-purifying respirators (PAPR). WHO recommends the use of a particulate respirator as a U.S. National Institute for Occupational Safety and Health (NIOSH)-certified N95, European Union (E.U.) standard FFP2, or equivalent when performing AGPof managing critically ill patient[24]which should be followed in a dental clinic. There is no evidence regarding the safety of either N95 or the equivalent FFP2 or even higher grade respirators while performing the aerosol-generating procedure in a suspected case of COVID-19. EUFFP3 respirators conforming to European standard 149 offers a filtration rate of 99% of all particles measuring up to 0.6micron. [25]SARS-CoV-2 measures around 0.12micronand aerosol particles range from 3-100nm. Therefore, the safety of these respirators in protecting dentist from SARS-CoV-2 during aerosol-generating procedures is questionable. Uses of such respirators require adequate training through annual respiratory program. Higher level of protection may be achieved through PAPR while performing AGP. PAPR as opposed to N95 does not require testing, can be worn with facial hair, reduced patient anxiety but high cost makes it unaffordable by the dentist, especially in India. For additional information dentist may explore http://www.cdc.gov/niosh/npptl/respirators/respsars.html and http://www.cdc.gov/niosh/topics/respirators. Donning and doffing should follow OSHA guidelines at http://www.osha.gov/SLTC/dentistry/index.html.) Donning of PPE should start with the gown followed by respirator/mask, goggles or face shield, and gloves. Sequence for doffing starts with gloves followed by face shields/goggles, gown and mask/respirators. Optimizing the availability of PPE is essential. Healthy subjects wearing PPE are reported to show hyperthermia, tachycardia, hypercapnia developing stress and work intolerance among dentists.[26]

Efforts to reduce bio-aerosol can be achieved by isolation of the operative site by rubber dam, pre-procedural mouth rinse and air cleaning systems. Cochran et al[27]28 have demonstrated a 90% reduction in bio-aerosol though protective eye wear and head cap are compulsory while using rubber dam.[29]Conditions where rubberdam placement is not feasible, pre-procedural mouth rinse with 1% hydrogen peroxide and 0.2% povidone-iodine can reduce the bio-aerosol. [30]

High volume evacuator (HVE) and high-efficiency particulate arrester (HEPA) filters are suggested method for controlling airborne infection in the dental clinic. Avasthi et al. [31][32] in 2018 have reported a substantial efficiency of HVE filter in reducing bio-aerosol whereas Desarda et al.[33] have contradicted this. Ergonomic limitations of HVE can be overcome by Nubird HVE.[34] HEPA filter is 99.97% effective in reducing aerosol particles measuring 0.3 μm in diameter though less used being expensive and difficult to clean. Aerosol contamination due to dental unit waterlines can be overcome by chemical and non-chemical treatment of dental unit waterline biofilmanddental chair unit design modifications.[35]

e) Disinfection of patient care items: Critical items can be single-use disposable or heat sterilized. Heat sterilization or high-level disinfection after every use is required for semi-critical items. Barrier protection should be used at a minimum. Non-critical items can be disinfected by FDA-cleared sterilant or high-level disinfectants and EPA-registered disinfectants.[36] SARS-CoV-2is a lipid virus having least resistance to germicidal chemicals.[37] Therefore,regular disinfection and sterilization should minimize the viability of SARS-CoV-2 on these patient-care items.

f) Disinfection of clinic and waste management: Surface disinfectants like 62%–71% ethanol, 0.5% hydrogen peroxide, and 0.1% (1 g/L) sodium hypochlorite (20) and otheroxidizing disinfectants may be used to decontaminate the clinical contact surfaces after each patient. Dental waste consisting of majorly extracted teeth; saliva/blood-soaked gauze surgically removed tissues and contaminated sharp items should be disposed in a leak-resistant biohazard bag with secured closure.[38]

Preparedness of Dental Practitioners: We propose a checklist to evaluate the preparedness of the dentist to resumeclinic (Figure 2). Dental procedure in the current scenario will be more expensive than before to afford strict disinfection program as well as PPE for almost every patient. Reducing taxes on dental materials and equipment, reduction of electricity tariffs and free sanitization programme in dental clinic and reducing the premium of health insurance for dentists at least till the emergence of a vaccination programme, are few considerations which the representatives of dental organizations should put forward to Government.

Tele-screening questionnaire for scheduling appointment

Name of the interviewer: **Date of call:**

Name of the patient: **Age/Gender:**

Date of birth:

Address with contact number:

Screening questionnaire	Yes	No	Assessment of emergency	Yes	No
Have you ever been diagnosed with COVID-19?			Chief complaint:		
If yes, date of last negative test result:					
Do you have travel history to known Hot-Spots?			Pain?		
Have you been exposed to a known COVID-19 positive person?			Pain level:		
Do you have Fever?			Pain since when:		
Do you have Cough/Sore Throat?			Swollen gum/face?		
Are you facing any difficulty in breathing?			Trouble swallowing?		
Are you having any body ache?			Trouble opening mouth?		
Are you suffering from Diarrhoea or GI Discomfort?			Trauma?		
Have you observed any diminished sense of smell or taste recently?			Ulcer?		
Do you share a workplace, classroom or household or come in contact with large gatherings regularly?			If yes, ulcer since when:		
			Bleeding?		

Figure 1- Questionnaire for tele screening

Dental practitioner's preparedness assessment

1. Education and job specific training to all employees at Dental office	Yes	No
Signs and symptoms of Covid19		
Techniques of hand washing, respiratory etiquettes and wearing face masks		
Self-monitoring for Covid19 specific symptoms		
Process of reporting suspected or confirmed cases of Covid19		
Patient placement		
2. Facilities at reception area of the clinic	**Yes**	**No**
Notification for patients to take appointment through tele-screening process		
Adequate signs posted regarding respiratory etiquettes, social distancing, mask wearing and symptoms of respiratory infection		
Provision of face mask		
Alcohol based hand sanitizers		
Tissue papers and no-touch receptacles for their disposal		
Social distancing in the waiting area		
3. Availability of Items to maintain Standard precaution for infection prevention	**Yes**	**No**
Gloves		
Fluid repellent coveralls		
Eye protection		
Hand wash		
EPA approved disinfectants		
4. Availability of Items to maintain Transmission based precaution for infection prevention	**Yes**	**No**
High level respirators		
Rubber dam		
Mouth rinse		
Dental unit waterline maintenance		
Air filtration system		
5. Adequate training in donning and doffing of PPE		
6. Registered for annual respiratory protection program		

Figure 2 Checklists to evaluate the preparedness of the dentist to start the clinic

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Reference

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Effect of Modern (Junk) Food in Dental Caries

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Abstract

In this modern age, 21st-century people are attracted more towards the outside oily and calories rich junk food than homemade healthy food. Especially this group includes children and youth. Either due to the taste of tongue or due to circumstances like hostel life, they are attracted to these unhealthy fast or junk food. These junk foods are bad for physical health and especially for oral health. Under this topic, we shall discuss the influence of junk food in dental caries which is increasing day by day especially in children and young people due to their modern lifestyle.

Keywords: *Dental caries, junk food, sugar, carbohydrates, starch, acids microorganisms, fermentation, decaying.*

Introduction

We have to get knowledge about "which are junk food?" and its "consistency" We should have a precise idea about "what is dental caries?" and "how it is initiated?" And "what is the role of junk food in initiating or extending dental caries?"[1]

Junk food: Junk food is the unhealthy food that is high in calories consisting of sugar or fat and also have a little amount of dietary fibres, protein, vitamins, minerals or other important nutritional value. Even, so high protein food like meat prepared with saturated fat may be considered as junk food. The ubiquitous supply of junk foods in our food environment is our day to day lifestyle and becomes our day to day food habit. It is seen nowadays that students from high socioeconomic status, they are more junk food consumers. Children from middle eastern culture having a background of high junk food consumers. Junk food consumers are more likely to consume due to some habits like: Eat dinner in front of the television; Receive sweet rewards; Allows to consume snacks anytime; Have soft drinks available at home; A television in their bedroom or dinner hall.[2]

Table 1. Junk food types

Sugar Rich	Spicy and Salty	High-Calorie Food
Candies	Pizzas	Fried chicken
Potato chips	Pavvaji	Fried paneer
Chocolates	Burgers	Oily Breads
Ice-cream	Gupchup	Coffee
Soft drinks	Chats	Diary products
Pastries	Momo	Fruit juice with added sugar
Cookies, cakes	Bada, Samosa	

Dental Caries: Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterised by de3mineralization of the inorganic part and destruction of the organic substance of the teeth,which often leads to Cavitation. It's the truth that this junk food affects your oral health. You may ask "HOW? If I brush or floss regularly?" But only brushing or flossing is not enough to prevent these sticky and solid food retention on the tooth surface and its reaction on it. Brushing only maintain the oral hygiene. Pizzas, burgers and French fries these foods are rich in starch and carbohydrate which is the main source for caries. If we don't brush or floss after eating these junk foods, then it will help these caries causing bacteria to grow and proceed to decay of tooth eventually. These also help in the formation of plaque and tartar which cause

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gum disease. Dry fruits and fruit juice with added sugars due to its natural sugars and artificially added sugar in packed fruit juice help the bacteria to grow freely. And carbohydrate drinks like soda,cold drinks cause harm to their teeth because of two constituents i.e sugar and acids. Due to high acids in carbohydrate drinks cause eroding and wearing down of enamel and make the tooth prone to erosion and decay. Excess salts and sugars are added to the packed food by the food companies to preserve it for a long time and to reach to its optimum taste. These factors help in long time retention of the debris and acid surface along the tooth surfaces and increase the rate of caries. How caries initiated in tooth due to retention of the excess sugar, carbohydrate, and extra salted food is a question.[4,5]

Table 2. Consistency of these unhealthy junk foods[3]

Sugar drinks	Adding sugar is one of the worst ingredients in the modern diet. Sugary drinks increase your total carbohydrate intake. It may cause insulin resistance diabetes type 2, nonalcoholic fatty liver disease, other heart disease or obesity
Pizzas and Burgers	Highly refined dough Heavily processed meat extremely high in calories and high in starch
White bread	These are unhealthy or bad for health if consumed in high amounts because it is highly rich in refined wheat and which is low in fibers and essentials
Fried Packaged food	It contains high calories and also chemicals like acrylamides, acrolein, heterocyclic amines, oxysterols, polycyclic aromatic hydrocarbons(PAHs), AGEs
Pastries, Cookies, and Cakes	If we take it in excess then it is unhealthy. They are made up of refined wheat flour, added fat and refined sugars and also high in unhealthy transfats and preservatives and no essential nutrients and calories.
French fries and potato chips:	Highly riched in carbohydrates so also riched in calories. These also contain carcinogenic acrylamide
Packed Fruit juice:	We know fruit juice has its natural carbohydrate or sugar but now a day outside packed fruit juice have added sugars and preservatives which is unhealthy.
Agave Nectar	Agave nectar is a sweetener agent that is highly refined and fructose-rich.
Ice-cream:	This diary product is highly rich in carbohydrates and also in calories, and easy to overeat
Candy bars, Chocolates:	Candy bars are incredibly unhealthy. They are high in refined wheat flour, sugar, and processed fats while very low in essential nutrients. These are also high in carbohydrates.
High calories coffee drinks	The good effects of coffee which help in prevention against serious diseases like type 2 diabetes, Parkinson's disease. But nowadays for mouthwatering we are adding creamers, syrups, sugars and additives, that are frequently highly rich in carbohydrates and unhealthy highly for body like other sugar-sweetened beverages.
Excess salty and spicy foods:	Pavvaji, gupchups, chats, momoetc are highly riched in carbohydrates, salts and chilly

According to "miller's chemo-parasitic theory" or "acidogenic theory" in the first stage decalcification of enamel and dentin occurs & in second stage softened residue dissolution due to acid and microorganism. For dental caries two factors are responsible first is Oral microorganism & Second is Acids produced from the fermentation of starch or carbohydrate available sticky and solid carbohydrate and starch remaining products on tooth surfaces. Bread, sugar, and another carbohydrate-rich food remain stick to the remaining tooth surfaces for long periods, they will start fermentation with the help of some microorganisms present in our saliva that produce acids. These acids formed from fermentation helps in the decalcification of enamel and dentin and proceed to decay of tooth.[6]

There are two types of microorganisms,one is acidogenic and another is proteolytic.

In simple language dental caries is the combination of microorganism which produces acids and proteolysis,

acid produced, tooth surface and remaining carbohydrates and starches.

(Bacteria) + (sugar) + (tooth) → (acids) → dental caries

Functions of carbohydrates: Both raw sugar and cooked sugar cause dental caries but cooked sugar produces more acids. The carbohydrates present in saliva are not available for microorganisms, because these carbohydrates bound to proteins and other carbohydrates. The presence of carbohydrates and starch on tooth surfaces depends on how many times taken,how much taken which forms taken and in which path it is taken. Monosaccharides and disaccharides are much available on tooth surfaces for acid production. But glucose and sucrose are easily soluble in the stomach tube and intravenously so not available for fermentation.[7]

Functions of microorganisms: As we know two types of micro organisms is acidogenic and proteolytic. Those bacteria which are responsible for acid production and dental caries: *S. mutans, L. acidophilus, Actinomyces.* These bacteria either in isolated or in combined form involved in this fermentation and dental caries process.

Functions of acids: Fermentation or enzymatic breakdown of carbohydrate and sugar may produce lactic acid along with butyric acid. There are two types of enzymes "heterofermentative" and "homofermentative". *Streptococci* and *lactobacilli* which are homo fermentative mostly produce lactic acid in anaerobic conditions. Those which are heterofermentative produce varieties of intermediate acid products. Like pyruvic acid in the presence of lactic acid dehydrogenase produce formic acid and acetyl CoA along with intermediate acid propionic, butyric acid, succinic acid and ethanol. To adhere to these acids on the tooth surfaces, plaque plays a great role. Monosaccharide and disaccharide reduce pH so that it will be suitable for the acid to remain on the tooth surface for a long time. Actinomyces in anaerobic condition becomes homofermentative and produces lactic acid but in the presence of CO_2, it becomes heterofermentative. The amount of acid production depends on the growth of the number of bacteria and the types present.[8-10]

Steps to take after taking junk food to prevent caries: We know that junk food is not only bad for our oral health but also our physical health. So consume these unhealthy foods as less as possible. After taking this junk food we should wash our mouth with water. And we should brush and floss your tooth after taking this junk food so that this food will not remain on tooth surfaces for a long time which will reduce the risk of caries. We can also use mouth wash along these.

Conclusion

For your relaxation, I want to say that fast food is not harmful when it is taken under control. The problem happens, when it is taken in a large amount and frequency. The result of fast food taken and its complications for oral health is right in front of you.

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Adenocystic Carcinoma of Salivary Gland: A Literature Review

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Abstract

Adenoid cystic carcinoma is a rare salivary gland tumor that has a consistent slow growth rate and has destructive biological behavior because of its deep peripheral tissue invasion and higher recurrence rate and tendency of distant metastasis. The involvement of adjacent vital structures may lead to death. Its clinical presentation also causes difficulty in diagnosis. Surgical resection following radiotherapy is the main treatment modality of Adenoid cystic carcinoma. Even after successful therapy of the primary tumor, there is a high rate of local recurrence and in contrast to other epithelial malignancies, it has a very poor prognosis. The survival rate is good for the initial 5 years but it falls after 5 years follow up.

Keywords: *Adenoid cystic carcinoma, Malignant neoplasm, Distant metastases, Perineural invasion.*

Introduction

Robin, Lorain, and Laboulbene were first described Adenoid cystic carcinoma in two articles published in 1853 and 1854 reporting on two nasal tumors and one parotid.[1] Cribriform arrangement of the cells invasion of adjacent structure and spread through the nerve also described by them. ‘‘Cylindroma’’ was the term suggested by Billroth in 1856 suggested the for tumor and Spies coined the current name of ‘‘adenoid cystic carcinoma’’ in 1930. It comprises 1% of all malignancies of the head and neck region and about 10% of all salivary gland tumors. It is the most common malignant tumor of minor salivary glands and is also one of the most common cancers of the major salivary glands. ACC not only involves the salivary gland but also involve the lacrimal gland, ceruminous gland, paranasal air sinuses, larynx, and trachea. Initially,the tumor was described benign and mixed tumors. The layer was establishedas malignant. Currently, ACCis recognizedas an extremely difficult malignancy to treat. Conleyand Ding mandescribed it as one of the most biologically destructive and unpredictable tumors of the head and neck. It has a high, almost inevitable, predisposition torecur if a patient, lives long enough, and this occurs even when radical excision has been performed.[2]

Clinical presentation:

Location: Nearly 10% of all salivary gland neoplasm are adenoid cystic carcinoma which most commonly involves the minor salivary glands (in 60% cases). Whereas in the head and neck region the parotid gland is the single most common site for the tumor involvement. Minor salivary gland adenoid cystic carcinoma commonly affects the minor salivary gland present intraorally, most commonly the palate.

Age: There is no age predilection but more common in adults with a peak of incidence at the 4th to 6th decade of life. Sometimes children may also be affected. It is found that in children major salivary glands are common sites for the tumor whereas in adult minor salivary glands are commonly affects than the major salivary gland.[3-5]

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Gender: There is slight female predilection (F:M = 1.2:1). Although other different study shows that there is no significant gender predilection for the tumor.

Clinical Features: Any non-ulcerating tumor in a major or minor salivary gland recognized for months or years, causing mild discomfort or pain and associated with paresthesias, is highly suggestive of adenoid carcinoma until proven otherwise".There are no distinct signs and symptom of ACC to identify it clinically exactly. Different studies revealed that ACC is a persistent slow-growing tumor with a recurrent growth pattern. It has a long clinical course and distant metastases occur at a late stage. On the advancement of the malignancy in an advanced stage, there are chances of involvement of adjacent nerve. Generally, 2nd division of the trigeminal nerve is involved causing paresthesia and pain along the distribution of the nerve. Radiological and histopathological study reports along with clinical reports are correlated for final diagnosis.[3-5]

There is multiple contradictory behavior of ACC. **First**, slow growth, but progressive, relentless course. **Second**, clearance of the margin is achievable but multiple local recurrences occur**. Third**, regional metastases to cervical lymphnodesareuncommon, but the incidence of distant metastasis to lung, live, bone is quite common, and **Forth**,survival rate is high for the first 5 years,but in case of 10-to 20-yearsurvivalrate, it is quite low. [3-5]

Histological features: Histopathological study of the tumor reveals it consists of a mixture of ductal and epithelial cells and they are arranged in three different patterns. They are namely 1.Cribriform, 2.tubular, and 3.solid. the cells having little cytoplasm with angulated and hyperchromatic nuclei. Mitotic figures are less present in the tubular and cribriform pattern and found more in the solid strand pattern which is associated with very poor prognosis. Huang et al. concluded in his study that the location of the tumor, clinical staging, and the histopathological variant are the factors that determine the prognosis and patient survival. [6]

Histopathologically adenoid cystic carcinoma can be graded as below

Grade I: The tumor consists only of cribriform and tubular

Grade II: A mixture of cribriform, tubular, and solid growth patterns, with a solid growth pattern of less than 30% of the tumor.

Grade III: Tumors with predominantly solid features (>30% or more of the tumor).

Immunohistochemically, ACC was consistently positive for cytokeratin [AE1/3, CK 34βE12, CK5/6, CK7, CK14, CK18] p63, CA19-9, c-KIT (CD117), PDGFRA, MUC1, and Ki-67. ACC was consistently negative for CK8, CK20, desmin, S100 protein, CD34, chromogranin, MUC2,MUC5AC, and MUC6. Some ACCs were positive for CK CAM5.2, CK19, EMA, CEA, vimentin, α-smooth muscle actin, p53, CD10, and synaptophysin.[7]

Perineural Invasion: Neoplastic invasion of nerves was reported first in 1842 by Cruveilheir. It is direct spread from the primary tumor and it is continuous microscopically though appears discontinuous macroscopical view. This invasion occurs at the level of perineural and endoneurial plane through the path of least resistance. Lymphatic channels in these tissue planes have very little to no role in this aspect. Most commonly involve the 2nd and 3rd division of the trigeminal nerve but may also involve the descending portion of the facial nerve and other smaller branches. It is considered that Gasserian ganglion is the most commonly affected site while the cavernous sinus is less commonly affected. As per different literature the latency between involvement of the nerve and onset of the neurological symptoms and diagnosis time ranges between months to 3 years. In the case of ACC of nasopharynx cranial nerve involvement is more common. It is considered as an indicator of poor prognosis as there is a chance of involvement of the cranial cavity, skull base, orbital cavity, and pterygopalatine fossa. The relationship between the distant metastases and perineural invasion is controversial sill now. Researchers considered perineural invasion as a significant factor for prognosis and distant metastases of the primary tumor. It is also supported by other studies.[8]

Distant Metastases: It is difficult to estimate the incidence of distant metastasis in ACC, but it usually ranges from 35 to 50%.Incidence of distance metastases employing hematogenous spread is quite common in ACC and creates challenges in successful treatment of the same. Distant hematogenous spread is quite common in the case of ACC in contrast to the regional lymph node spread of other types of malignancies. These metastases occur in a very unpredictable way and can involve the

lung, liver, bone, and other soft tissue. Pulmonary or lung metastasis is quite common. These metastatic of primary ACC remain asymptomatic for a longer period especially the pulmonary metastasis which has very slow progress. On the other hand metastases to the bone have a very rapid and fatal course. The successful treatment of an ACC with well-controlled local disease is not possible because of these distance metastases. As per Spiro "the incidence of distant metastasis at other sites is likely to be more frequent, as no further investigations are done once lung metastasis is detected. In 40-60% cases, distant metastases are most common in lung, bone, and soft tissues. distance metastases should be considered as a separate disease process and for the same, the effort to control the primary tumor control should not be hampered.[8-10]

Management: Optimal management of the ACC of the head-neck region is not well established. Management depends on the site of the involvement, staging of the tumor, histological grading, metastatic involvement, and biological behavior of the tumor. It is difficult to treat an ACC because of its unreliable behavior, multiple and late local recurrence. Generally, surgical resection with a clear margin along with radiotherapy is most commonly employed for the treatment of ACC. Before delivery of the treatment throughout the workup and complication of each treatment procedure should be evaluated. Care should be taken to preserve the normal function of different anatomical structures as far as possible. Indications for surgery are: 1.bony involvement, 2. Tumors that are not sensitive to radiotherapy, 3. Recurrent lesion at the primary site, 4.side effect is radiotherapy is greater. Radiotherapy for the treatment of ACC has not been used solely as these tumors are radiosensitive but not radiocurative. The primary response to the radiotherapy is assuring but in later stage recurrence rate is higher. As per a study "patients receiving radiation alone, 96% had tumor regression but 93% relapsed, and half of them relapsed in 18 months.[11]

The use of chemotherapeutic agents in the treatment of ACC is controversial because of its poorly defined and limited role. Generally administered for palliative care.[41,42,43-45] Few studies proved that the use of cyclophosphamide, doxorubicin, cisplatin are useful and have some activity as a single agent or in combination during surgical management of the ACC of the parotid gland, facial nerve should be preserved if there is no involvement of the nerve present. For that presurgical evaluation of the nerve function should be done when the submandibular gland involved supraomohyoid neck dissection to be done along with the resection of the tumor. Post-operative radiotherapy is given following surgery in both cases. Local radical excision and postoperative radiotherapy should be done in the case of minor salivary glands ACC. Postoperative radiotherapy reduces the chances of recurrence but survival benefits are not demonstrated.[12]

Prognosis: There are many prognostic factors of ACC. They are 1. Size 2. primary site, 3. involvement of adjacent structures, 4. lymphnode metastases, 5. clinical staging, 6. treatment modality, 7. positive margins of surgical resection, 8. perineural invasion, and 9. histological subtype. It has been noted that the prognosis of minor salivary gland ACC is worse than those ACC of the major salivary glands. In the case of minor salivary gland tumor, peripheral extra glandular soft tissue and bone are readily infiltrated by the tumor and cause increased dissemination of the tumor which makes the complete excision of the tumor more difficult. Again the cribriform subtype of ACC has the best prognosis whereas the solid subtype has the worst and the prognosis for tubular form is intermediate. Another study reported that there was no significant relationship between the morphologic type of ACC and locoregional control. But they found that there was a high rate of distant metastases in the case of cribriform and solid variety. "Authors found that positive margins did not influence survival, but are implicated in increased treatment failure. However, many others reported the presence of surgical positive margins as a parameter for survival and tumor control." Some authors describe a close relation between perineural invasion and the degree of extension of the primary tumor. While some others reported the perineural invasion as a negative survival predictor. Poor prognosis was often associated with perineural invasion. Ki-67 antigen expression, DNA ploidy, nucleolarorganizing regions, S-phase value are some tumor markers that are associate with poor prognosis.[8-12]

Conclusion

Adenoid cystic carcinoma is one hard to treat malignancy because of its aggressive clinical behavior. Early diagnosis is necessary to reduce the morbidity and successful management. Follow up at regular intervals is necessary to prevent the local and distant recurrence of the same. Surgical resection of the lesion along with radiotherapy proved to be beneficial for the management

of this type of malignancy. Radiotherapy helps to reduce recurrence.

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Glandular Odontogenic Cyst

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Abstract

A cyst is a Pathological cavity which consists of solid, semi-solid or gaseous contents which are not created by the accumulation of pus and which may or may not be lined by epithelium. The cysts of the jaw are divided into two categories namely epithelial and non-epithelial. The epithelial cysts are further classified into developmental and inflammatory. Based on their origin the developmental cysts are further classified into odontogenic and non-odontogenic cysts. Hence the odontogenic cysts are described as jaw cysts originating from the odontogenic epithelium. A glandular odontogenic cyst (GOC) is a developmental cyst. A glandular odontogenic cyst (GOC) is a sparse entity of odontogenic origination. A glandular odontogenic cyst is known for its hostile behaviour, unforeseeable nature, indeterminate clinical features and high recurring potentiality. The aggressive behaviour may be attributed to the cell kinetics of the lining epithelium or the conservative treatment approach. Apart from the aforementioned peculiarities, GOC is also notorious for impersonating clinicopathological lesions such as Botryoid odontogenic cyst, which is also a regionally aggressive polycystic form of a lateral periodontal cyst and central mucoepidermoid tumour further complicating the diagnosis. These features further establish Glandular odontogenic cyst as an independent biological entity. This article focuses on discussing the various aspects of the rare entity that is a glandular odontogenic cyst in terms of histopathological features, radiological features, diagnostic criteria, clinical features, cytological investigations, differential diagnosis and treatment approach.

Keywords: *Cyst, Glandular odontogenic cyst, Central mucoepidermoid tumour, Fluorescent In Situ Hybridization (FISH).*

Introduction

A glandular odontogenic cyst (GOC) is a developmental cyst derived from the dental lamina. It is also known as sialoodontogenic cyst having many similarities with the lateral periodontal cyst except for the multilocular radiolucency. Gardner et al (1988) coined the term GOC and suggested that it's histological attributes and biological conduct are fairly evident for it to be regarded as a separate entity. It is a rare aggressive lesion with a recurring potential.[1] In 1992 in WHO typing of odontogenic Tumours GOC was defined as "a cyst arising in the tooth-bearing areas of the jaw characterized by an epithelial lining with cuboidal or columnar cells both at the surface and lining crypts or cyst-like spaces within the thickness of the epithelium. "It is the rare lesion, that can show aggressive behaviour, accounting for about 0.012-1.3% of all jaw bone cysts. According to Padayachee and Van Wyk, 1987 the quintessential characteristics of the cyst include that it shows multilocular radiolucent appearance, it is infrabony, can recur if not adequately excised, spaces created by cysts are lined by non keratinized same as that of reduced enamel epithelium with epithelial concretions, mucous and columnar cells form an indispensable portion of the epithelium unit. [2]

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Diagnostic Criteria: According to Kaplan et al certain characteristics, features were classified into major and minor criteria based on the incident of occurrence of each feature in formerly reported cases. The presence

of major criteria is obligatory whereas the presence of minor criteria confirms the diagnosis. The major criteria include squamous epithelium of differing thickness with the presence of cuboidal shaped eosinophilic or hobnail cells and goblet cells along with the glandular structures. The squamous cell lining also shows spheres or whorls with the absence of basal palisading. The minor criteria include the papillary proliferation of epithelial lining multicystic appearance and presence of clear cells in the epithelium.[3]

Histopathology: Histologically, it is lined by non keratinized epithelium with mucous material within the spaces created by the cyst being an important feature. The superficial epithelial cells are columnar to cuboidal with cilia. Numerous mucus cells and gland-like structures are also seen in the epithelium. The histopathological features of GOC play a pivotal role in diagnosis as they resemble a wide variety of lesions including lateral periodontal cyst, Dentigerous cyst, Intraosseous mucoepidermoid carcinoma. Therefore careful and detailed microscopic examination plays a key role in diagnosis.[4]

Clinical Features: Clinically it presents as a slow-growing, aggressive lesion commonly associated with the mandible (Four times more commonly seen in the lower jaw in comparison to the upper jaw) with a preference for the anterior region. Clinically it is seen most commonly occurring in middle-aged people, It is rarely seen in children below 20 years. Most commonly seen in the lower jaw with a strong preference for the anterior area. The size of the cyst is variable ranging from smaller lesions lesser than 1 cm in diameter to larger destructive lesions involving the whole jaw. It presents initially as an asymptomatic swelling in case of small cysts. In the case of large cysts it can become destructive due to clinical expansion or if it is secondarily infected or if there is compression of neurovascular bundles.[5]

Investigation: The investigation includes immunohistochemical staining with Cytokeratins (CKs 7 8 10 14 19). Another prominent investigation which plays a key role in segregation of GOC and low-grade mucoepidermoid carcinoma, a relatively close entity resembling GOC is Fluorescent In Situ Hybridization (FISH). The immunohistochemical stains can play a pivotal role in the recognition of tumour cell type and origin. Mucoepidermoid Carcinomas show Mastermind – like 2 (MAML2) gene redistributions characterized by translocation t(11;19) (q21;p13) whereas FISH investigations have shown that glandular odontogenic cyst doesn't show MAML2 gene redistributions. Periodic Acid Schiff (PAS) Periodic Acid Diastase (PAS-D) and mucucarmine stains play a pivotal role in highlighting glycogen-rich and mucin-secreting cells.[6]

Radiological Features: Radiologically Glandular odontogenic cyst shows well-defined margins and multilocular radiolucency along with a sclerotic rim and scalloping of the borders. The scalloping can be seen in interdentally and inter radicular spaces. Other radiological features include minute root resorption, cortical perforation and expansion, tooth displacement. In some cases, the absence of impacted tooth has been reported. It often exhibits honeycomb appearance. A radiopathological link can play a significant role in definitive diagnosis.[7]

Radiographic diagnostic aids include preoperative orthopantomogram as well as postoperative orthopantomogram to check for the recurrence as the cyst has high recurring potential. Some other advanced radiographic diagnostic aids include Digital Volumetric Tomography (DVT). CT scans are also advised as they serve precise details about locularity of the entity, cortical integrity, expansion of the lesion and collusion of the contiguous soft tissue.[8]

Differential Diagnosis: The differential diagnosis of glandular odontogenic cyst involves both benign and malignant lesions. Glandular odontogenic cyst imitates a wide clinicopathologic spectrum ranging from a lateral periodontal cyst to a catastrophic malignant neoplasm such as central mucoepidermoid carcinoma. Hence the differential diagnosis for GOC may include Dentigerous cyst, unicystic Ameloblastoma, Intraosseous mucoepidermoid carcinoma, a lateral periodontal cyst. Intraosseous mucoepidermoid carcinoma is a rare malignant neoplasm of the salivary glands with a predilection for the lower jaw.[9,10] Cellular atypia and solid microcystic epithelial proliferation are Pathological findings in Intraosseous mucoepidermoid carcinoma but not in GOC.Also, GOC shows positive immunostaining for CK-18, CK-19 and Ki-67 and decreased positivity for p-53, which helps to differentiate GOC from Intraosseous mucoepidermoid carcinoma. The dentigerous cyst does not exhibit cuboidal surface cells, nodular thickening and gland-like spaces. Another differentiating feature is the higher recurrence rates of GOC than Dentigerous cyst. Lateral periodontal cyst lacks cuboidal cells and mucin pools and presents as a unilocular lesion radiologically.

GOC presents as a multilocular radiolucency usually with a well defined sclerotic rim. Some cases of GOC show Ameloblastomatous changes similar to unicystic Ameloblastoma. In such cases the histologic changes which resemble unicystic Ameloblastoma. Also, few radiographic features such as lobulated margins, perforation of the cortex and root resorption of the adjacent tooth are similar but the clinical findings are distinctive.[11-13]

Treatment: The treatment modality differs based on the size and aggressiveness of the lesion. The treatment modality varies from minor surgical processes such as enucleation and curettage to major surgical processes such as peripheral osteotomy, marginal resection. The treatment of choice has been enucleation or curettage. Treatment of small unilocular lesion by enucleation is advised. In large uni- or multilocular lesions, an initial biopsy is advocated. Marginal resection is also considered as a reliable method of treatment. Due to its recurring potential and aggressive behaviour in some cases, en bloc resection is advised especially for the multilocular lesions. The recurrence rate is more prevalent in multilocular cysts with cortical perforations while in smaller unilocular lesions conservative treatment approach is adapted which includes curettage with adjuvant Carnoy's solution. Excision of soft tissue adjacent to cortical bone perforation and peripheral bone osteotomy with a large round bur to remove the remaining epithelium of cyst and application of liquid nitrogen to the remaining bony cavity. Endodontic treatment of the offending tooth followed by apicoectomy can be done. The number of reported cases has increased significantly in recent years Due to increased knowledge of surgeons and pathologists about this entity.[14-15]

Conclusion

A glandular odontogenic cyst is an unusual cystic lesion with a tendency to recur and showing locally aggressive behaviour. The cyst exhibits non-distinctive clinical-radiological which may mimic many other lesions. The radiographic appearance of the cyst can be variable and not pathognomonic. A concluding diagnosis can be achieved by a careful histopathological examination. Close follow up for the patient is suggested owing to the high recurring potential of the lesion. The recurrence mainly occurs due to faulty diagnosis, inefficient removal of cyst and absence of proper follow-up.

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Caries Risk Assessment-Protocols and Paraphernalia

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Abstract

Measures of caries control involve identifying the disease process and the risk factors; this is achieved by first performing a caries risk assessment for the patient in order to identify his/her risk factors for caries development. With a better understanding of the caries process, there comes a change in operative dentistry. Although there is considerable variability between dentists, there is more emphasis on preventive dentistry than ever before. The shift in emphasis appears to be occurring in dental schools in which curriculum and practical skills are focused more on caries risk assessment, modern management of the caries disease. This paper reviews about the various caries risk assessment approaches and models and stresses on the need for a risk assessment based approach.

Keywords: *Dental caries, Risk assessment, Basic protocols, Management.*

Introduction

Dental caries is a major oral health burden and is the commonest dental disease seen throughout the world. Dental caries has been linked to food habits, lifestyle changes, oral hygiene etc. Recent advances in the field of dentistry regarding caries detection and management, preach CRA to be the need of the hour. The American Academy of Paediatric Dentistry (**AAPD**) has recognized caries-risk assessment and management protocols as a tool to assist clinicians with decisions regarding treatment based upon caries risk and patient compliance and are essential elements of contemporary clinical care for infants, children, and adolescents.

Awareness about caries risk assessment is essential as it helps the dentist assess caries susceptibility and carious lesions at an earlier stage, which indirectly helps in educating & motivating the patient in maintaining a good oral hygiene and also reduces the amount, extensiveness of invasive treatments. A caries risk assessment tool saves both the dentist's and the patients chair side time for further appointments.

Hence, a set guideline has to be followed to educate healthcare providers and other interested parties in preventive dentistry and aid in clinical decision making regarding diagnostic, fluoride, dietary and restorative protocols [1].

Caries Risk Assessment: Caries risk assessment (CRA) is the process of collecting data regarding various factors (e.g. bacterial level) and indicators (e.g. previous caries experience) to predict caries activity in the immediate future[2].

Formal CRA has been described as a four-step process:

1. Identification of measurable risk factors;
2. Development of a multifactorial tool;
3. Risk assessment to determine a patient's risk profile; and
4. Application of preventive measures tailored to the risk profile[3].

The risk assessment tool should estimate caries risk, identify the primary etiological factors, provide an inventory of the patient's current preventive practices,

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and serve as a guide for selecting specialized preventive care tailored to that individual's needs.[4,5,6] Research has shown that experienced clinicians are often able to assess caries risk very quickly and accurately.[7,8,9]

Performing a CRA assists practitioners in providing, patients with appropriate levels of preventive care and to avoid unnecessary procedures[10,11]. If dental professionals were able to identify, in advance, individuals at the greatest risk of developing dental caries, the cost of caries prevention procedures could be markedly reduced and their efficiency greatly increase Dental caries management by formal risk assessment represents a significant change in the profession and should be incorporated into daily practice as dictated by practices of evidence-based standards of care. CRA should be routinely built into preliminary and recall examinations as the findings help to guide the patient's designated course of treatment. This is especially important before extensive prosthodontics, restorative, and orthodontic treatment is undertaken to ensure a favourable prognosis[13, 14].

Need for having CRA:

CRA may be valuable in the clinical management of caries by helping dental clinicians to:

1. Categorize the level of the patient's risk of developing caries to control the intensity of treatment rendered.
2. Pinpoint main etiological factors that contribute to the development of decay and thus determine appropriate form of therapy.
3. Assist in restorative treatment decisions (e.g. choice of restorative material).
4. Improve prognosis of planned therapeutic care.
5. Provide information on what additional diagnostic tests and screening are required.
6. Educate and motivate patients to improve and maintain optimum oral health.
7. Guide timing of subsequent recall appointments.

CRA tools screen people based on risk factors and predictors and classify patients into one of three risk categories- low, moderate, or high[6,15,16] If new caries has developed since the last examination, the patient is categorized either as moderate or high risk depending on the time elapsed since the last examination, and the number and severity of carious lesions[17]. Diagnosing a moderate risk group accuratelyis the greatest challenge. These patients, along with people who are at risk without any apparent signs or symptoms of disease, benefit the most from identification from CRA[18,19].

Although dental caries has long been established to be a disease of multifactorial ethology, many of the traditional caries prediction models have focused on individual factors associated with high caries activity[20]. More recently, the multifactorial etiology of caries points in the direction of constructing a more promising risk assessment model that includes the various factors that contribute to the development of caries as: host resistance, cariogenicity of the diet, and microbial pathogens.[2, 21, 22]

Caries risk assessment model: There are two frameworks that can be used for the development of CRA instruments: *the risk model and the prediction model*. The risk model, or sometimes called the etiologic model, is employed when one wants to identify the risk factors for the disease to implement the most effective prevention and treatment interventions. It detects only true risk factors and is typically simple to use, but it is not intended to predict future caries risk. Because of its simplicity and stability across different subgroups of the population, it is often used for screening in public health domains[23, 24].

In contrast, a prediction model uses both risk factors and risk predictors to maximize its ability to identify low and high risk individuals (i.e. maximize sensitivity and specificity). Risk factors (e.g. *Streptococcus mutans* counts) are often more costly to measure; however, they tend to be more reliable in caries prediction. Caries-risk assessment models involve a combination of factors that include diet, fluoride exposure, a susceptible host, and micro-flora that interplay with a variety of social, cultural, and behavioural factors. Caries risk assessment is the determination of the likelihood of the incidence of caries (i.e., the number of new cavitated or incipient lesions) during a certain time period or the likelihood that there will be a change in the size or activity of lesions already present. With the ability to detect caries in its earliest stages (i.e., white spot lesions), health care providers can help prevent cavitation. It can be done using cariograms, risk assessment forms designed by various institutes (AAPD, CAMBRA).

Selection of Risk Assessment Instrument

The principle of risk assessments is that individuals

with elevated levels of risk factors will receive more aggressive preventive interventions. Because of this, examiners must be cautious in how they categorize patients in the various risk levels. One must decide at what stage a risk factor changes from low to moderate to high risk categorization. This can prove to be a difficult task as many etiological factors do not have a clear demarcation between absence and presence, but normally range from excellent to poor (e.g. oral hygiene) or from low to high (e.g. *Lactobacillus* counts).

In creating a CRA tool, a balance between sensitivity (percentage of truly diseased persons who test positively) and specificity (percentage of persons without the disease who test negatively) must be made.[24] In doing so, a balance is struck between the number of false positives (i.e. patients assumed to be high risk but do not develop caries) and false negatives (i.e. patients assumed to be low risk but develop caries). It is imperative that the values of sensitivity and specificity remain high to ensure that a patient labelled as high risk truly has the disease and the threat of identifying low risk individuals as diseased should be minimal.

Because it is impractical to believe that a diagnostic test would have sensitivity (Se) and specificity (Sp) values of 100%, different criteria and cut-off points have been established by researchers investigating caries risk[26,27]. Wilson and Ashley suggested that Se and Sp values each be set at 80% to be considered an acceptable test for caries risk. Alternatively, it has been recommended by Fleiss and Kingman that the combined Se and Sp of a risk model should be at least 160%, which has become the gold standard amongst many researchers. Unfortunately, this benchmark value has only been achieved by a small number of caries prediction instruments.[23]

Several tools have been used to assess the cries risk. A few of them have been enlisted below:

Caries risk assessment form was introduced by the ADA[28]. The clinical judgment of the dentist may justify a change of the patient's risk level (increased or decreased) based on review of this form and other pertinent information. For example, missing teeth may not be regarded as high risk for a follow up patient; or other risk factors not listed may be present.

The assessment cannot address every aspect of a patient's health, and should not be used as a replacement for the dentist's inquiry and judgment. Additional or more focused assessment may be appropriate for patients with specific health concerns. As with other forms, this assessment may be only a starting point for evaluating the patient's health status. The ADA plans to periodically update this tool based on:

1. Member feedback regarding its usefulness, and;
2. Advances in science. ADA member-users are encouraged to share their opinions regarding this tool with the Council on Dental Practice.

Two forms have been devised to assist the caries risk assessment, for the age group of less than 6 yrs. of age and that of above 6 yrs. of age. Apart from the forms, one of the most important tool of CRA is cariogram. The details have not been discussed in this article.

Public Health Significance: If the CRA tool is to be used at the public health level for mass screenings, it may be advantageous to have a higher specificity as, Public health administrators would want to avoid false positives in order to avoid over treatment of individuals that do not necessarily require the preventive services. This is especially crucial in environments where resources are scarce. On the contrary, it may be more advantageous from an ethical and economic standpoint to increase the sensitivity of the test in order to avoid false negatives. Failure to identify those at risk for caries development may result in unnecessary treatment in the future that may be more costly and painful to the patient due to the progression of undiagnosed disease. Although increasing the sensitivity may result in over treatment of some patients, if the clinician uses an appropriate preventive strategy, this would result in little to no harm to the patient in that dental caries would not be permitted to start or progress.[12]

Caries risk assessment by dentists: In recent years, a lot of importance has been attached to formal caries risk assessment to guide treatment planning decisions and recall intervals for individual patients, and various risk assessment tools and checklists have been developed for this purpose. With an established protocol on caries risk assessment, the factors that contribute to a child having caries are identified, and modifiable risk factors can be addressed. The Caries Risk Assessment Checklist (CRAC) has been developed by the Irish public dental service to encourage a formal, risk-based approach to the management of caries in Irish school-children. Given that the Guideline Development Group considered all children to be at risk of developing caries, it was decided at the outset that the checklist would only record high caries risk status.[20-24]

Re-assessment of caries risk: Caries risk assessment is a continuous process, as an individual's risk status can change over time. In the public dental service, intervals between dental assessments for most children are measured in years rather than months. A guideline on dental recall, which was developed for the NHS in the UK by the National Institute for Health and Clinical Excellence (NICE), recommended that the "*interval between oral health reviews should be determined specifically for each patient and tailored to meet his or her needs, on the basis of an assessment of disease levels and risk of or from dental disease*." The guideline also recommended that "*the longest interval between oral health reviews for patients younger than 18 years should be 12 months*" based on evidence that the rate of progression of dental caries can be more rapid in children and adolescents than in older people, and seems to be faster in primary teeth than in permanent teeth.[20-24]

Conclusion

Caries risk assessment, based on a child's age, biological factors, protective factors, and clinical findings, should be a routine component of new and periodic examinations by oral healthcare providers. There is not enough information at present to have caries risk assessment analyses, estimating children at low, moderate, and high caries risk by a preponderance of risk and protective factors will enable a more evidence-based approach to dentists' referrals, as well as establish periodicity and intensity of diagnostic, preventive, and restorative services.

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Osteomyelitis of Jaw: A Review

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Abstract

Osteomyelitis of the jaw in dental practice has seen to be decreased due to the increasing awareness among the general population about oral hygiene and the various treatment modalities available in dentistry. Often the maxillofacial region is involved due to improper treatment, compromised immunity, fracture, or any other reason. It is due to the inoculation of bacteria because of multiple factors. The diagnosis is based on clinical findings, radiographs, and lab findings. Osteomyelitis is managed by both conservative and surgical management. With the availability of more potent antimicrobials and treatment under aseptic conditions, management of osteomyelitis has been possible.

Keywords: *Osteomyelitis, Sequestra, radiography, management.*

Introduction

Once being a dreaded disease in the pre-antibiotics era, the incidence of osteomyelitis of jaws has become less, because of awareness, availability of antimicrobials, and better dental health services. The word 'osseous' is from Latin origin which means bony, 'osteon' from Greek origin meaning bone, 'myelos' means marrow, and 'it is' means inflammation.[1]

Osteomyelitis may be defined as an inflammatory condition of the bone, that begins as an infection of the medullary cavity and Haversian system of the cortex and extends to involve the periosteum of the affected area. It is an inflammatory process with the tendency to progress. Because of poorly vascularized cortical plates, mandible shows a higher incidence of osteomyelitis.[2-5] Mandible gets its blood supply from the inferior alveolar neurovascular bundle. Maxilla being less dense shows less incidence as it is highly vascularized and gets its blood supply from numerous feeder vessels. Maxillary posterior region is less affected than the mandibular posterior region. Even in mandible,the most common sites are body, symphysis, angle, ascending ramus, and the condyle. The severity and high frequency of odontogenic infections in daily dental practice and the heavy bacterial load of the oral cavity is one of the major reasons why osteomyelitis of the jaw is more common than any other skeletal bone.[2-5]

Staphylococcus aureus or *Mycobacteria* are the most specific bacteria causing infection, other causes may include trauma, radiation, or certain drugs.[6] Diminished host defense mechanism whether local or systemic contributes to its emergence. Diabetes, malignancies, autoimmune diseases, malnutrition are some of the other causes that may associate with osteomyelitis.[3] Osteomyelitis can be described by inflammation of the mouth, exposed bone in the mouth which fails to heal, unhealing extraction socket, presence of fistula from mouth to lower skin, or development of sequestra.[7,8] It starts as an infection of the medullary cavity, spreads to the Haversian system, and gradually involves the periosteum of the infected area leading to severe tissue and bone destruction.[9-11] There is progressive inflammatory osteoclasia and ossification.[6] Radiation related osteomyelitis shows a delayed onset and tooth extraction and implant placement is also contraindicated in that area which has been irradiated.[12]

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Predisposing Factors: The predisposing factors of osteomyelitis are: (1) A delayed host defense mechanism or any condition that affects the host resistance, (2) Lack of vascularity of the host bone at a local or systemic level, and (3) virulence of microorganisms. The host defense system gets by the presence of any immunocompromising condition like Diabetes mellitus, leukemia, malnutrition, agranulocytosis, severe anemia, chronic alcoholism, drug abuse, sickle cell disease, typhoid, chemotherapy, irradiation, steroid use, hepatitis, HIV, glomerulonephritis, systemic lupus erythematosus, etc.[1,3,13] The vascularity of the host bone is altered due to therapeutic irradiation, Paget's disease, bone malignancy, fibrous dysplasia, osteoporosis, mercury; arsenic, and bismuth causing metallic bone necrosis. In the maxilla, osteomyelitis can occur due to any pansinusitis or facial cellulitis. Due to the lysosomal activity,certain organisms form thrombi. A protective barrier is formed around the infective foci by the pathogen born bioactive peptides and the chemoattracted leukocytic purulence which leads to lysosomal and enzymatic destruction of the tissues along with microvascular thrombi formation. The organisms further proliferate in the host medium but remain protected from the host defense mechanism. [1,3,13]

Etiology:

Odontogenic infection arising from pulpal, periapical, pericoronal tissues, infected cyst, socket, or tumor are one of the major cause of osteomyelitis. Trauma is the second most common cause especially compound fracture and any iatrogenic surgery.Oro-facial infections derived from periostitis following the gingival recession, infected lymph nodes from furuncles, peritonsillar abscess, lacerations are also seen to cause osteomyelitis. Furuncle on the face, upper respiratory tract infection, mastoiditis, wound on the skin, middle ear infection, systemic tuberculosis cause infection to spread via hematogenous route.[1,14]

Pathogenesis: The primary hematogenous osteomyelitis is very rare but can be seen at a very young. In adults, the primary cause being the inoculation of bacteria in the jaw bone. This insult results in the initiation of an inflammatory process or cascade. In a normal or healthy host, this may self-heal or self-limit itself, but in a compromised host it can lead to a process that is no more considered physiologic but pathologic. With increased inflammation, there is increased blood flow to that area and increased hyperemia. Further leucocytes are recruited to fight against the infection.[3] Pus formation occurs and a continuous supply of bacteria and cellular debris occurs that cannot be eliminated by the body host defense mechanism. When the pus and the inflammatory process starts in the bone marrow, the intramedullary pressure increases, and the blood supply decreases to this region. The pus then travels via Haversian and Volkmann's canals to spread through-out the medullary and cortical bone. Once the pus has entered the cortical bone and gets collected under the periosteum, the local conditions are aggravated by compromising the blood supply to that area.[3] Finally, the pus exits the soft tissue by intraoral or extraoral fistulas.[3] Osteomyelitis starts by bacterial invasion in the cancellous bone, followed by acute inflammation hyperemia, increased capillary permeability, and infiltration of granulocyte. It is an infectious process of the medullary myeloid cavity and only the mandible and the calvarium has myeloid compartment.[1]

Microbiology

Aerobic streptococci (alpha-hemolytic streptococci, *streptococci viridans*), anaerobic streptococci; and other anaerobes, such as Peptostreptococci, Fusobacteria, and Bacteroides cause many cases of osteomyelitis. Sometimes, anaerobic or microaerophilic cocci, Gram-negative organisms such as *Klebsiella, Pseudomonas,* and *Proteus* are also found. *M. tuberculosis, T. pallidum,* and *Actinomyces* species cause some specific kind of osteomyelitis. Marx et al. (1992), identified Actinomycoses, *Eikenella,* and *Arachnia* as pathogens of some other refractory osteomyelitis.[1,3, 14]

Classification:

Table 1. Classification of osteomyelitis of jaw

Historically accepted classification based on the clinical course: Hundson's classification of osteomyelitis of jaw:	**Acute forms** Contiguous focus: trauma, surgery, and odontogenic infection • Progressive: burns, sinusitis, and vascular insufficiency • Hematogenous (metastatic): developing skeleton (children)
	Chronic forms • Recurrent multifocal: Developing skeleton (children), Escalated osteogenic activity (< age 25 years) • Garre's: Unique proliferative subperiosteal reaction, Developing skeleton (children to young adults) • Suppurative or Non-suppurative: Inadequately treated forms, Systematically compromised forms, and Refractory forms (CROML: Chronic Refractive Osteomyelitis) • Diffuse sclerosing: Fastidious organisms, and Compromised host or pathogen interface.
Classification is based on the pathogenesis of altered vascular perfusion as a major contributing factor to the presence and persistence of osteomyelitis as a clinical disease. (Vibhagool et al 1993).	**There are three types:** • Hematogenous osteomyelitis • Osteomyelitis secondary to a contiguous focus of infection • Osteomyelitis associated with or without peripheral vascular disease
Classification based on the presence or absence of suppuration	**Suppurative osteomyelitis** a. Acute suppurative (pyogenic) osteomyelitis b. Chronic suppurative (pyogenic) osteomyelitis i. primary ii. secondary c. Infantile osteomyelitis
	Nonsuppurative osteomyelitis a. Chronic sclerosing osteomyelitis i. Focal sclerosing osteomyelitis ii. Diffuse sclerosing osteomyelitis b. Garre's sclerosing osteomyelitis c. Actinomycotic osteomyelitis d. Radiation osteomyelitis (osteoradionecrosis) e. Specific infective osteomyelitis i. Tuberculosis ii. Syphilis
Classification and Staging system of osteomyelitis developed by Clerny et al. and Vibhagool	**Anatomic type:** **Stage I** Medullary osteomyelitis It involved medullary bone. Without cortical involvement, usually hematogenous **Stage II** Superficial osteomyelitis Less than 2 cm of the bony defect without cancellous bone **Stage III** Localized osteomyelitis Less than 2 cm bony defect seen on the radiograph, the defect does not appear to involve both cortices **Stage IV** Diffuse osteomyelitis The defect is less than 2 cm Pathological fracture, infection, and nonunion
	Physiological type: A Host: Normal host; B Host: Systemic compromise (Bs); local compromise (Bl); C Host: Treatment is worse than the disease

Systemic or local factors that affect immune surveillance, metabolism, and local vascularity.

a. Systemic (Bs): Malnutrition, renal or hepatic failure, diabetes mellitus, chronic hypoxia, immune deficiency or suppression, malignancy, extremes of age, autoimmune disease, and tobacco and alcohol abuse.

b. Local (Bl): Chronic lymphedema, venous stasis, major vessel disease, arteritis, extensive scarring, radiation fibrosis, small vessel disease, and loss of local sensation.

Clinical Presentation: The classical clinical features are pain, swelling, and erythema of the overlying tissues, fever, paresthesia of the inferior alveolar nerve, paresthesia of lower lip, adenopathy, trismus, malaise, fistulas, and sometimes pathologic fracture.[1-7] The pain is a generally deep and boring type. In the case of Acute osteomyelitis swelling and erythema is very common to see which indicates the cellulitic phase of the inflammatory process under the bone. Fever often accompanies it but in the case of chronic osteomyelitis, it is not seen. Due to the inflammatory process, there is continuous pressure on the inferior alveolar nerve, hence, paresthesia of the inferior alveolar nerve is the characteristic feature of osteomyelitis. In the case of inflammation in the muscle of mastication of the maxillofacial region, there is trismus. The patient gradually feels weak and malaise and may associate with any other systemic disease. Generally intraoral and extraoral fistulas are seen in the case of chronic osteomyelitis.[3]

In Acute osteomyelitis, the early cases are characterized by generalized symptoms like fever; malaise; nausea; vomiting; anorexia, deep-seated boring and continuous pain, intermittent paresthesia or anesthesia of lower lip, facial cellulitis or indurated swelling of moderate size, thrombosis of inferior alveolar vasa nervorum, rise in pressure from edema in the inferior alveolar canal, teeth are tender to percussion and loose and trismus. In established cases there is deep pain, malaise, fever, dehydration, anorexia, tender on percussion of the teeth in that area, teeth become loose, purulent discharge through sinuses, presence of fetid odor, trismus may be present, acidosis, toxemia, regional lymphadenopathy.[1,2]

In chronic cases, there is pain and tenderness but the pain is minimal, non-healing bony and soft tissue wounds with induration of the soft tissue, intraoral or extraoral draining fistula, thickened or wooden characteristic of bone, enlargement of the mandible because of deposition of subperiosteal new bone, a pathological fracture may occur. [1,2]

Lab findings: The laboratory studies show slightly raised ESR (Erythrocyte sedimentation rate), elevated C-reactive protein (CRP), moderate leukocytosis (PMNL), anemia, and albuminuria In acute cases leukocytosis is common but in chronic cases, it is not common.

Histopathology of biopsies shows scattered inflammatory infiltration, mainly lymphocytes, histiocytes, plasma cells, and neutrophil granulocytes, increased osteoblast, thickened bony trabecula, and fibrous marrow replacement.[8-15s]

Radiographic findings:

The following method can be used for radiographic analysis:

Conventional radiography: The radiographic changes can be appreciated after 3 weeks of the onset of osteomyelitis. At least 40-60% of bone destruction should be there to mark any radiographic changes. This alteration is seen 4-8 days after the onset of acute osteomyelitis. In the early stages, areas of sclerosis and osteolysis with varying degrees of periosteal reaction is seen as mixed radiodensities. There is a "mottled appearance"due to the widening of marrow space and enlargement of Volkmann's canals. The irregular lines and zones of radiolucency give "moth-eaten appearance" due to the presence of granulation tissues between the livings and dead bone. In later stages, gradually the cortical bone gets involved and an island of cortical bone gets mobilized by the osteolytic process which is devitalized and is favorable for the precipitation of calcium. These separated devitalized calcified bone with resorbed periphery act as a foreign body and are prominent in the radiograph which is called a sequestrum. The subperiosteal new bone is seen as opaque lines over the cortex called Involucrum. A "fingerprint" or "onion peel appearance" is seen at the place where the new bone is superimposed upon the jaw.[1-4]

Imaging: This includes computerized tomography (CT), Positron emission tomography (PET), radioisotope scanning. Computed tomography gives a more clear picture of the involved calcified bone and the cortical plates. There is less radiation exposure to the patient.

Radioisotope Tc-99m methylene diphosphonate bone scanning helps to identify the margins of calcified tissue involvement, but, it has very poor resolution. Positron emission tomography (PET) recently have been more promising for evaluating metabolic activities. It uses radioisotopes of physiologically active compounds like glucose, ammonia, fluoride and the changes can be seen as early as 3 days.[1]

Treatment: The treatment of osteomyelitis requires both medical and surgical approach. Very rarely it requires intravenous antibiotic therapy like in the case of infantile osteomyelitis [3]. Based on the histopathological features i.e. gram stain results of the exudate or suspected pathogen, empirical antibiotic treatment should be started.Culture and sensitive reports may take time but guide the surgeon for treating the patient.[3]

In general, treatment[1] consists of:

i) Debridement

ii) Control of infection: acute infection is controlled through antibiotic therapy. However, as there is no circulation in the necrotic bone so the antibiotic does not reach.

iii) Hospitalization

iv) Other supportive treatment: a) hydration: fluid therapy, b) high protein and vitamin diet.

v) Analgesics: Narcotic and non-narcotic analgesics. Bupivacaine (Marcaine), alcohol nerve blocks, nerve avulsion, rhizotomy.

vi) Good oral hygiene: oral rinses, such as 1% sodium fluoride gel, 1% chlorhexidine gluconate, and plain water help to prevent radiation-induced caries from the xerostomia and mucositis by providing some local augmentation of the host immune antimicrobial activity.

vii) Frequent irrigations of wounds.

viii) Exposed dead bone: small pieces of bone may become loose and can be removed easily.

ix) Hahn and Corgill in 1967 gave the treatment of small areas by drilling multiple holes into vital bone as a means to encourage sequestration.

x) Sequestrectomy is performed intraorally, because of skin and vascular damage resulting from irradiation.

xi) Pathological fractures are not so common. These may occur from minor injuries. The fractures do not heal readily. Excision of necrotic ends of both fragments and replacement with a large graft is the best treatment. Reconstruction of bone defects usually warrants major soft tissue flap revascularization support.

xii) If there is persistent pain, infection, or pathological fracture, bone resection is performed. It is done intraorally to avoid orocutaneous fistula in radiation compromised skin.

xiii) Hyperbaric oxygen therapy is a useful adjunct. It increases the tissue oxygenation levels which helps to fight any anaerobic bacteria present in these wounds.[1]

Surgical Options: The classic treatment is sequestrectomy and sauterization. The main aim is to remove the necrotic or less vascularised bony sequestra and improve the blood flow. In sequestrectomy infected and avascular pieces of bone are removed, it is generally the cortical plates in the infected area. In saucerization adjacent, bony cortices are removed up to a good bleeding bone and an open pack is given for healing by secondary intention. Decortication is also done in which removal of dense, basically chronically infected and poorly vascularized bony cortex is performed and a vascular periosteum adjacent to the medullary bone is placed to increase the blood supply.[3]

Conclusion

Osteomyelitis of the jaw is an inflammatory condition that is multifactorial. A dentist should be aware of the predisposing conditions and know how to manage the condition.

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Accidental Periodontitis Induced by Orthodontic Treatment: A Review

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Abstract

This article aims to encapsulate the effect of appliances on the periodontal health of patients undergoing orthodontic treatment. While orthodontic treatment provides benefits to a wide range of intra-oral and extra-oral issues starting from aesthetics to mastication, there are some major setbacks in terms of relapse if the treatment is not carried out with a proper plan. Therefore, with limitations in maintaining a desired oral hygiene, which breeds various periodontal problems, iatrogenic causes further narrow down a good prognosis which is desired at the end of an orthodontic treatment or in fact, any medical procedure. This is a review of the unnatural and natural impact of orthodontic treatment on the tissues periodontium and the treatments associated with it.

Keywords: *Orthodontics, Chronic Periodontitis, Iatrogenic effects, Periodontal Health.*

Introduction

The earliest evidence-based history of dental practice can be found in the era of Indus Valley Civilisation which dates back to 7000 BC.[1] The journey from a 6500-year-old jawbone with a cavity within the dentin layer with beeswax as a filling, to the first dental journal being published in 1839 and now, the use of advanced and efficient techniques for diagnosis and treatment has been remarkable. But the emergence and evolution of orthodontic treatment has been one of the most significant breakthroughs in this field as it fulfills the requirements and comprehends with both, the occlusal and facial abnormalities both at the same time to a fairly acceptable state.[2]

The earliest attempt to the correction of a teeth irregularity was done by Pliny the Elder by mechanical filing of elongated teeth to bring them to proper alignment in the period of 23-79 AD.[2] In the year 1619, Fabricius[3] suggested the extraction of tooth for correction of crowding. It was the introduction of 'brace' the 'bandeau' by Pierre Fauchard[4] also known as the father of modern dentistry, that increased the pace of orthodontics exponentially as it paved the way for all the appliances and their appendages invented later with his discovery that 'the malalignment of teeth can be corrected as teeth would follow the pattern of the brace'[5]. With time, the advancement in orthodontics have made adaptations which brought changes in function and esthetics, function being its prime priority.

However, the most significant part of an orthodontic treatment still is to keep the integrity of the entire dentition intact in the best way possible to make sure that there is no ailment to the patient in the infinite future. Orthodontic treatment involves the movement of the entire tooth from one location to another which is why the periodontium, along with the tooth, plays a crucial role in the beginning, during, and end of the treatment. This also suggests that the standard appliances used, which include orthodontic braces, archwires, and elastic bands should be used befittingly to obtain the desired results.[4,5]

Therefore, to accommodate with the changing

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concepts of function and esthetics with a varying range of patients having their oral hygiene practices, this kind of treatment has its risks and complications amongst all the benefits, some of which are unwanted side effects, which include decalcification of enamel and discoloration of teeth and some are extensive conditions like periodontitis, pseudo pockets, root resorption and bone loss due to iatrogenic causes.[6]

Potential upshots of orthodontic wires on the tissues of the periodontium: It was in the 1980s when the earliest cases of periodontal destruction due to orthodontic wires were given attention.[7] The diverse use of elastics ranges from correction of Angle's Class II and Class III malocclusion, correction of malposed tooth, crossbite, and midline diastema to the non-surgical removal of tooth in case of hematological diseases like Haemophilia. The main cause of elastics being used so widely among dentists for the patients is its cost-effective nature and ease of placement and removal.

While plaque is considered to be the most important factor determining the periodontal health of the patient, placement of elastic bands and the archwires play a key role in the same during an ongoing treatment. Experimental studies on animals have shown that orthodontic forces & tooth movements do not induce gingivitis in the absence of plaque.[8] However similar forces can induce angular bone defects in the presence of plaque. Orthodontic tooth movements like tipping & intrusion can cause attachment loss in the presence of plaque.[9] Any neglect from either the practitioner or the patient during this treatment can be a cause of various periodontal problems with periodontitis being one of the complicated ones. Hence at the beginning of an orthodontic treatment, a patient must be made aware of the maintenance of oral hygiene throughout and after the treatment concludes to avoid long term diseases like chronic periodontitis and a probability of relapse.



Figure 1: Gingival Inflammation and plaque accumulation during orthodontic treatment. Source.[17]

Incorrect placement of orthodontic brackets: When an orthodontic treatment is carried out sufficing the periodontal needs, the results are excellent but it's the iatrogenic nature of the use of elastics along with the hygiene which causes undesired outcomes, periodontitis being a common one. What most of the practitioners fail to understand is that the errors start to occur with the placement of the braces itself. A wrong placement of molar bands, mainly on a subgingival level, besides with accumulation of food particles has the potential to intrude the alveolar bone[10], which further aids in the characteristic osseous defects caused by extensive inflammation and gingival recession.[11] The magnitude of forces also plays a key role in the relationship between periodontal tissues and orthodontic forces as it is seen that the extent in magnitude of orthodontic forces affects the rate of root resorption.

Attachment Loss: Elastic bands, due to their shape memory tend to retain back to their original place, and hence there is a dire need of thorough inspection of their position when they are placed in a patient's mouth especially when there is no specific placement mechanism. This is why the bands tend to creep down the narrower end of the crown of the tooth.[12] and move thereby apically creating an expected periodontal ligament destruction resulting in bone loss due to periodontitis and subsequently, the extrusive movement of the tooth. This happens because the elastic bands act as foreign material causing an inflammatory reaction of the soft tissues leading to the weakening of periodontal attachment. Incidentally, the apical extension of the band is, however, independent of microbial colonies present in plaque.

Effects on gingival embrasures: One of the main criteria that decide the esthetics of a patient is the gingival embrasures, which are the V-shaped spaces present inter proximally around the proximal contact present in between the adjacent teeth[13], the embrasures present on the anterior teeth playing the important role. The treatment of patients with already existing periodontitis is a cause of concern as such patients are at risk of further bone loss and loss of tooth with due course of time. Prevention of black triangles on esthetic zones and preservation of interdental papilla are to be taken into consideration as a priority during the treatment. The gingival recession associated are not dependent on the orthodontic treatment but are dependent on the type of forces applied for tooth movement. Some factors like thickness or cortical plates, type of load distribution,

type of forces, tipping in general, and morphology of teeth like lower incisors also contribute to the formation of gingival recession as an adjunct to the treatment.

Tooth Mobility: The biology of movement follows the sequential pattern of bone resorption and bone deposition in the pressure side and tension side respectively. This process shows the widening of periodontal ligament space in radiographic examination.[14] Hence this phase is a result of temporary mobility of the tooth. But extensive bone loss or an extended long term treatment with ill-maintained oral hygiene may cause exfoliation if it is not given attention by the practitioner. When the forces applied are extremely large in magnitude, it causes greater undermining resorption and hence the mobility of the tooth is to a greater extent. This could be avoided by the forces and changes associated with it are monitored and if there is mobility.[15]

All movements should be temporarily discontinued until the movement reduces to a moderate level.

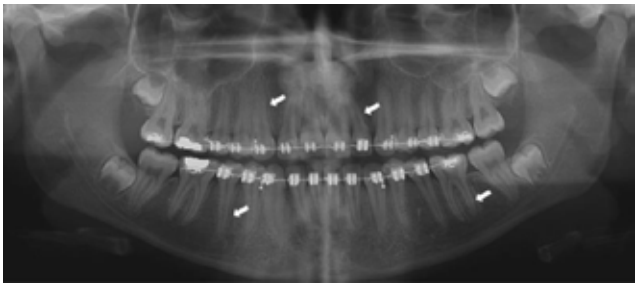


Figure 2: Radiograph demonstrating widening pf PDL due to excessive orthodontic forces[18]

The image shown above represents a panoramic radiograph representing the increase of periodontal ligament space on the application of orthodontic forces during an orthodontic treatment. As the strain on the periodontal ligaments increase, the PDL space widens leading to the gradual mobility of tooth and associated periodontal issues.

Iatrogenic Damage: This is one of the rare causes of chronic illness which is very significant as its existence might be unknown for a long time and the diagnosis and treatment are hence delayed, with outcomes sometimes beyond repair. There are not many cases regarding a specific error but the unknown persistence of discarded or broken elastic wire inside the periodontium is a leading cause of chronic periodontitis, abscess, and mobility on a long term basis.[16] Another cause may be the apical migration of an elastic band which later on gets covered by the surrounding gingiva without the knowledge of the patient and later leads to periodontal destruction inside the root of the tooth giving the clinical manifestations years later. The timing of diagnosis of such conditions is crucial as the treatment plan is solely dependent on it and the intensity of the diseases associated with this becomes severe with time. The diagnostic time may vary from months to years, but it the practitioner's call to manage it as soon as it is diagnosed and confirmed.

Treatments for periodontitis in orthodontic treatment: The first and foremost thing after such a condition is suspected there is a temporary discontinuation of the orthodontic treatment and involving a periodontist for creating a detailed treatment plan with a proper case history describing the duration of the ongoing treatment and the incidence of the disease. This helps the practitioner decide the extent and severity of the disease and eases the route of the treatment plan. After there is a confirmed diagnosis of such condition, the treatment depends on the prognosis of the tooth which is predicted by radiographic images and gingival examination. For cases with moderated recession careful scaling and root planing is done to ensure the absence of any foreign substance to aid the healing for the treatment to be carried out smoothly. The treatment plan follows that of the usual periodontal problems, from recession to mobility with its varying grades and hence the treatment modalities include scaling, root planning, splinting, gingival surgeries which include flap surgery which is done in cases where furcation is involved which is followed by subsequent repositioning of the tooth. All of this done along with a post-surgical antibiotic therapy which includes drugs lie Amoxicillin and a prophylactic dose of Chlorhexidine mouthwash for maintenance of oral hygiene.[16] All of this is performed on adults with consent and due information about the success rate of the surgery and the prognosis of the tooth and the same procedure is followed when a minor is getting treatment but here, the parents of the patient are informed accordingly. The success of this type of situation is purely based on the maintenance and thorough monitoring of the patient with regular intervals of recall. During every appointment during the revisits, oral prophylaxis and reinforcement of oral hygiene instructions are the building bricks for a good prognosis. All the clinical parameters like periodontal pocket depth, bleeding on probing, mobility is regularly checked. If there is a relatively low mobility or the loss of attachment reduces significantly with time, reduction in

erythema can be observed with increased firmness. After the periodontal health improves to a satisfactory state, the orthodontist is now ready to move further along with the desired treatment plan for the orthodontic correction only after consulting with the concerned periodontist.

Conclusion

An orthodontist must always ensure the state of a periodontal condition of the patient before heading for a treatment as the treatment as such provides a good boost to patients by providing a better functioning dentition and desired profiles for the individual who needs it and by this it also ensures self-confidence, lifestyle and a good self-esteem. But amongst this, the risks associated are a reality that cannot be ignored. The complications of such treatments possess a multifactorial process including the practitioner, patient, the treatment procedure itself, and the appliances associated with it. An orthodontic treatment of an individual goes hand in hand with the periodontal health. Therefore it's the role of the practitioner to be keenly involved in the treatment procedure and detect any source of error, if at all that exists, as early as possible to achieve the expected results. The improper use of any appliance or its appendage can cause periodontitis and can enhance bone loss in the long run during or even after the treatment is finished. For such cases, a detailed clinical examination and a meticulous treatment plan is necessary because it is the correct diagnosis and treatment which practically decided the fate of the defect caused. If done timely, a patient can be benefitted from early diagnosis as well as the correct management. Not everything is the responsibility of the practitioner, especially when the treatment is for a long duration and requires compliance from the patients themselves. Therefore, the importance of the treatment should be informed by the practitioner so that it is taken seriously. It is often considered that the orthodontist should explain the need and function of the appliance being used for the treatment because it helps the patients understand the need of keeping the integrity of the nature of the management by being vigilant themselves and also be informed about their treatment status. If children are the patients, the parents should actively participate in getting educated about the need of maintaining good hygiene while the treatment goes on while the orthodontist handles the rest.

As there is always a scope of marginal error, it is required that the practitioner informs their patients to report any unusual activity in their oral cavity as this could be the sign of the problem which lies underneath. Lastly, a good coordination among fellow practitioners is highly required which benefits both the patients and the practitioners by making them aware about such problems which may or may not have an iatrogenic cause just so that when the time comes, a practitioner is ready to diagnose it on time, deal with the situation and most probably manages it on time.

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Space Maintainer: A Review

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Abstract

Space maintainers are useful incases of deciduous teeth to protect the dentition from malposition,impaction, crowding,etc. They can also be used inmixeddentition. Various types of space maintainersare there for Particular treatment. This is mainly aimed atthe management of space in children. This article mainly aims at Bandandloop,Distal shoe, and trans palatal arch space maintainers.

Keywords: *Band and loop, Distal shoe, Trans palatal arch space maintainers, Removable space maintainers.*

Introduction

Early loss of primary dentition occurs very commonly in children due to various causes like maintenance of oral hygiene, dental caries is the most common one. Deciduous dentition is very important in child growth and development in terms of speech, mastication, and the good eruption of permanent dentition. When the natural process of teeth exfoliation is disrupted due to various causes like early loss of deciduous teeth, proximalcaries, early childhood caries, avulsion, periapicalpathology, systemic causes like hyperthyroidism, downs syndrome,etc. It may result in the mesial movement oft he dentition, resulting in loss of arch length which may cause malalignment of adult teeth in form of crowding, impaction,supra-eruption, rotation, ectopiceruption, crossbite, overjet, etc. A space maintainer is a mechanical device that preserves the natural space after the early loss of deciduous dentition. Space Maintenance of arch length management of occlusal planes etc. It can also be used for esthetic purposes.[1]

Space Maintainers Classified By Various Method: Hitch cock classified them into removable/fixed/semi-fixed, banded/non-banded, functional/non-functional, active/passive. Raymond C. Thurrowis classified into Removable, Lingual arch, Individual tooth. Hinrichsen classified into Fixed space maintainers (Bar type, Loop type, Lingual arch type distal shoe, Band, and loop) and Removable space maintainers (Acrylic partial dentures).

Various indications of spacemaintainers include when there is early loss of deciduous teeth shows signs of closing or if there is any chance of orthodontics treatment or if the permanent teeth are not ready for eruption. There should be a minimum of 1mm of bone coverage.[2]

Various contra indication of space maintainers includes if bone coverage is absent or if the root of permanent teeth is the only 2/3rd or when the permanent teeth have not erupted and space needs to be closed or the mesiodistal width of the crown of the deciduous tooth is less than the permanent teeth.[1,2]

Basic requirements of space maintainers include: The space maintainers should have good proximal dimensions to occupy the space created by the teeth. The space maintainers should be functional. It should not interrupt with the eruption of the opposite teeth. It should be strong enough to withstand the force. It should not harm the adjacent tooth. It should be easy to clean. It should not interfere in the normal growth and function of the tooth.[3,4]

Band And Loop Space Maintainers: It is a fixed type of space maintainer and used in the forms of loop bands, tailored bands, preformed seamless bends. It

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should possess the following; It should fit the teeth properly, it should not extend below the gingival margin, the band material it should be enough strong to resist the forces in the mouth. It should not tarnish. There should be no occlusal interruption.[3,4]

Preparation of Band space maintainers: Separation of teeth done by Brasswire, elastic threads. Band formation can bed one by a direct method which includes; Band pinching,festooning,trimming, folded flap. It can be also done by indirect method/preformed bands. Welding-by welding the bands are joined by soldering. Band and loop appliances are used when there is more than one tooth is a loss in the quadrant, it can replace occlusal function.[3,4]

Benefits of Band and loop: It can be cleaned easily. It maintains the vertical dimensions. It can be warm sometimes. Space can be created for the eruption of permanent teeth. It prevents tongue thrusting habits. It is cheaper and can be fabricated easily.[3,4]

Demerits of Bandand loop: It may be easily lost or broken by the patients. Some patients may not wear this. Side word jaw growth may be restricted. It may cause tissue irritation. It could not restore the occlusal function of the lost tooth.[3,4]

Indications of Band and loop: It is used in case of esthetic purpose, when the abutment tooth is not supportive, in case of cleft palate patients, in case of delaying permanent tooth eruption, if the permanent tooth does not erupt properly when there is several loss of primary teeth.

Contra indications of Band and loop: When the patient is not co-operative, patients are allergic to acrylic material, epileptic patients.[3,4]

Trans Palatal Arch Appliances: This applianceis mainly used for stabilizing the upper first permanent molars. It provides rigidity. It protects the first permanent molar from rotation.

Indications of Trans-palatal arch: The best indication is when one side of the arch is firmly fixed and various deciduous teeth are missing on the opposite side. It also indicated when the deciduous molars bilaterally missing. It can not be used when both permanent molars are mesially directed. In that case, Nance palatal arch is recommended.[5,6]

Benefits of Trans-palatal arch: Due to the absence of an acrylic base there is less tissue irritation and could be clean easily so preferred for oral hygiene maintenance.[5,6]

The demerit of Trans -palatal arch: This appliance fails to remain passive. Due to this, there are chances of vertical and transverse movement of permanent molars that can happen.

Construction of Trans-palatal arch: A round wire of 0.036 inches is bent to make the palatal surface of the band. Then the mesial part of the palatal side of the band is constructed then the wire is bent to the distal part to form the joint. Then soldering is done and the wire is heated to make it passive.[5,6]

Distal Shoe Appliance:

This is the most ancient type of Space maintainers. It is used as a guiding device for the eruption of the first permanent molar mostly of the mandible . This is usually not used in the maxillary arch. Its arm managed the Leeway space of the second premolar and guides the eruption of the permanent molar. For that reason,it is designed to have a horizontal bar and intra alveolar extension which is used as a guiding plane. It should be used by the patients regularly until the eruption of the first permanent molar. Otherwise if not used regularly it shows negative effects.[7,8]

Indications of Distal shoe appliance: Early loss or extraction of a second deciduous molar before the eruption of the first permanent molar. It is used in the case of root resorption and periapical bone destruction of the second deciduous molar before the eruption of the first permanent molar. In the case of advanced dental caries in the second deciduous molar. Ectopic eruption of first permanent molar teeth. Ankylosis of deciduous second molar teeth.[7,8]

Contra indications of Distal shoe appliance: Lack of abutment teeth due to several loss of teeth. In the case of uncooperative patients. When the first permanent molar is absent. In the case of systemic diseases such as Diabetes mellitus. In the case of cardiac diseases, kidney diseases, juvenile diabetes, rheumatic fever, hemophilia require antibiotic treatment before dental treatment. In case of congenital missing of the first permanent molar.[7,8]

Crown Distal shoe appliance: It is also known as Willet's appliances or intra–alveolar appliances. This helps in guiding the path of eruption of the first permanent molar.

Indications of crown Distal shoe: Early loss of second deciduous molar before the eruption of the first permanent molar but first deciduous molar should be present as an abutment tooth.

Contraindications of crown Distal shoe: In the case of medically compromised patients and in patients who do not have good oral hygiene.

Stepsinthe placement of Crown Distal shoe appliance: First IOPA is taken of second deciduous molar and first permanent molar. Then the abutment tooth is prepared. Then perform metal crown is made on the abutment tooth. Then the cast is poured and the working model is prepared. Dimensions of the second premolar are measured on the IOPA then the horizontal arm of the Distal shoe is prepared. The vertical height should be 1 mm downwards the mesial marginal ridge of the first permanent molar. The primary second molar is trimmed and the desired horizontal length is marked.[9,10]

Fabrication and placement of Distal shoe Spacemaintainers: A cobalt-chromium bar or stainless steel of 0.9 mm wire is used. The wire is bent to the desired shape. The vertical height of the horizontal wire of the Distal shoe should not touch the opposite tooth. Then soldering is done and the metal crown is polished. In the next appointment, the second deciduous molar is extracted and the bleeding is managed. The infection of the crown is controlled. Then the metal crown and Distal shoe are tried in the mouth. Then an IOPA is taken and the location of the Distal shoe concerning the erupting first permanent molar is confirmed. Then final managements are done if required and then the metal crown with the Distal shoe is placed on the abutment tooth.[9,10]

Removable Space Maintainer:

It is used as a partial denture.

Active Removable Space Maintainers: Here a labial bow is made for the anterior teeth on the working model. The side which is affected by the U shaped wire is bent to protect the alveolar ridge between the first premolar and molar.[8-10]

Acrylicpartial Denture: It is indicated when there is several loss of teeth in the mandibular or maxillary arch. This is used when there is a bilateral loss of more than one tooth and can be used for the eruption of the tooth . It is constructed with stainless steel wire 0.036 inches on deciduous canines and permanent molars. It can also be used in permanent incisors when they are in an active state of eruption.[8-10]

Benefits of Removablespacemaintainers: It managesthemesiodistal space. It also maintains the vertical shape. Do not interfere with mastication. It helps in the improvement of esthetic in the anterior region. It does not interfere with speech. It helps in protection from oral habits.[8-10]

Demerits of Removable space maintainers: It is difficult in uncooperative and medically compromised patients.

Indications of Removable space maintainers: When there is an absence of more than two deciduous molars it is indicated. When there is an absence of more than one deciduous tooth bilaterally. In cases of absence of anterior teeth.[8-10]

Conclusion

This article mainly highlights the types of Space maintainers. Space maintainers are mainly very essential in children before eruption of permanent teeth as it maintains good oral hygiene. It helps in the preservation of deciduous teeth from deleterious oral habits and dental complications such as malalignment of teeth, rotation, crowding,etc. at a young age. Space maintainers help in the preservation of space when the teeth are prematurely lost. It helps in maintaining good esthetics in children. It also controls orthodontics complications which may occur in the future .Thus Space maintainers help in the better eruption of permanent dentition.

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A Comparative Study on Effect of Motivation and Hope on the Well-being of Geriatric Population

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Abstract

Introduction: For the last decade, the physical and the mental health has been the concern and most interest among the researchers. This relationship is most discussed among geriatric population, because of the various challenge's posed by the old age at various levels. Here in this study we have how motivation and hope enhances the living wellbeing.

Aim: To measure the effect of motivation level, hope and well-being of geriatric population

Methodology: The present study was conducted in Delhi – NCR societies. Overall sample size of the study was 60 elderly population both male and female. Hypertension elderly (n =30) and Healthy elderly (n =30). The study was conducted using Elderly Motivational Scale, Adult Hope Scale and Ryffs Psychological Well-Being Scale.

Conclusion: The well-being of hypertension group (experimental group) is at a higher side than healthy elderly group (control group).

Keyword: *Elderly, Hypertension, Motivational Scale, mental Health,hope.*

Introduction

Old Age: Old age refers to ages nearing or surpassing the life expectancy of human beings, and is thus the end of the human life cycle. Terms and euphemisms include old people, the elderly, seniors, senior citizens, older adults, and the elders.

The Psychology of Aging Today: While the population over the age of 65 is growing considerably, their lifestyle has also evolved. Older citizens might live independently, work into advanced age, and take an active role in any number of groups in their communities. According to the American Psychological Association, there are also mental health conditions that are more prevalent in those of advanced age

There are number of scenarios possible regarding senior lifestyle and health:

Mental Capacity: Mental capacity is always a concern for seniors because they are susceptible to different factors, such as dementia, which can hinder their ability to care for themselves mentally. Other possible health issues, such as stroke, can cause issues with mental acuity. Many seniors who face deterioration of mental faculties will need care on daily basis.

Physical Health: With age, there can be obstacles to physical health. Depending on the severity, physical limitations may cause seniors to lose some or much of their former independence

Emotional Wellbeing: Emotional wellbeing can be a wide-reaching issue. Seniors often face traumatic changes, such as loss of loved ones and their own failing health. These severe environmental stresses can lead to depression and other psychological hardships.

Financial Stability: Because people are living longer and healthier lives, the old modes of saving for retirement often fall short of what seniors will need. Financial stability is a large concern for seniors and

their families' once regular income ceases, especially for active seniors who may live many years longer than their savings was geared to sustain.

Literature Review: Over the last couple of decades, there has been a growing of researches that has been focused on geriatric and their motivation, well-being, and level of hope in their life.

Kenneth E. Mobily (1982)[1] did a study on "Physical & Occupational Therapy in Geriatrics" which focused on of physical activity as one ages is not solely a function of age. Rather, there is data to support the contention that the elderly refrain from exercise because of environmental and personal as well as motivational conflicts.

Stevens-Ratchford, Regena (2000)[2] did a study on Occupational Engagement: Motivation for Older Adult Participation which focused on guiding participation in developmental tasks that enhance aging well can be a motivational strategy for rehabilitation.

JoNell Strough, Wändi Bruin, and Ellen Peters (2015)[3] did a study on "New perspectives for motivating better decisions in older adults" which focused on promising intervention strategies for motivating older recipients motivation to make better decisions.

Lindanor Jacó Chaves1 and Claudia Aranha Gill (2015)[4] did a study on "Older peoples concepts of spirituality, related to aging and quality of life" they aimed to investigate and analyze the concepts that older people have of spirituality and how this concept affects their quality of life. It is a descriptive, exploratory, quantitative-qualitative study, with a sample of 12 participants over the age of 60.

Stephen Jivraj, James Nazroo, Bram Vanhoutte, Tarani Chandola (2015)[5] did a study on "Aging and Subjective Well-Being in Later Life" which found that older cohorts enjoy higher levels of SWB than their younger counterparts when under similar circumstances, they experience sharper declines, especially in the very oldest cohorts. Stephanie V and Rachel Cooper (2015) did a study on Physical Activity and Mental Well-being in a Cohort Aged 60–64 Years which focused to investigate associations of monitored total PA under free-living conditions, self-reported leisure-time, and walking for pleasure with mental well-being at age 60–64 years

Budhiraja, Ankita; Midha, Punam (2015)[6] did a study on Hope and Spirituality as Portals to Subjective Well-Being among Geriatrics which aimed to assess the level of hope, spirituality and subjective well-being among geriatrics.

Antonella Delle Fave, Marta Bassi, Elena S. Boccaletti did a study on Promoting Well-Being in Old Age (2018): [7] The Psychological Benefits of Two Training Programs of Adapted Physical Activity found that people aged 67–85 who do physical activities promote mental health, opportunities for elderly people to socialize and improve emotional functioning.

Ena Yadav, Srishti Chanana (2018)[8] did a study on Emotional Regulation and Well-Being among Elderly. The results of the study point out that the relationship between perceived wellbeing and emotional regulation is comprehended to be bidirectional. Emotional regulation may also have protective role in wellbeing since regulation of emotions is essential for adaptive functioning, whereas suboptimal or dysfunctional.

Aim: Aim of this present study is to measure the effect of motivation level, hope and well-being of geriatric population .A comparative study will be done between elderly with hypertension elderly (experimental group) and healthy elderly (control group).

Method

Sample: Total sample of the present study consisted (N=60) among geriatric population in Noida. The sample comprises of hypertension elderly as experimental group (n=30) and healthy elderly as control group (n=30) which were randomly selected from different areas of Delhi NCR. The elderly population with hypertension were diagnosed by medical doctor more than six months ago. Elderly's ages were ranged above 60 years

Tools: The following measures were used in the present piece of research work.

1. **Elderly Motivation Scale:** Elderly Motivation Scale assesses the intrinsic and extrinsic motivation of elderly individuals. It assesses 4 constructs: intrinsic,self-motivation - determined and non-self-determined extrinsic motivation, and a motivation.

2 **Adult Hope Scale:** For measuring elderly's hope level, a scale developed by Snyder, C. R., Harris, C., Anderson, J. R., Holleran, S. A., Irving, L. M., Sigmon, S. T was used. Hope scales can be

used to determine client's perceived motivations for pursuing their goals (agency thought) and their ability to identify workable routes to goal attainment (pathway thought). According to Snyder et al., (1991), hope is the "stuff" that facilitates change. The adult hope scale contains 12 items. Four items measure pathways thinking, four items measure agency thinking, and four items are fillers. Participants respond to each item using an 8-point scale ranging from false to definitely true and the scale takes only a few minutes to complete.

The scale has been found to be inversely correlated with several measures such as the Beck Depression Inventory.

3 **Ryff's Psychological Well-Being Scale:** Psychological well-being of elderly was assessed by using Ryff's Psychological Well-Being Scale developed by psychologist Carol D.Ryff, the scale contained 42 items.

Procedure: The study was conducted between hypertension (experimental group) and healthy elderly's (control group). Data was collected on 30 hypertension (experimental group) and 30 healthy elderly's (control group) using Elderly Motivational Scale, Adult Hope Scale and Ryffs Psychological Well-Being Scale. Rapport was established between the administrator and the subjects. Also, the environment was made friendly so that subjects would feel comfortable and does not hesitate while marking their responses. All subjects include in the present study were given assurance that information provided by them will be kept confidential and will be used for research purpose only. It was also told to the participants to fill demographic details, informed consent was taken, instructions were given clearly, and if any confusion was there it was made clear to the subject. Initially the subject was feeling nervous but as they interacted with the administrator and rapport was established, they felt more and more confident while responding to the items. Elderly Motivational Scale, Adult Hope Scale and Ryffs Psychological Well-Being Scale was handed over to the subject for administration. All the data I collected from the subjects was appropriate as in individual who performed the test were mentally fit. Subjects who were not mentally fit their data was excluded from the study.

The individual scores were obtained, and the responses were scored according to the manual. The data was tabulated for giving statistical analysis and for obtaining the result. Finally, the results were discussed, and the formulated hypotheses were tested.

Discussion

The present study was conducted in Delhi–NCR societies. Overall sample size of the study was 60 elderly population both male and female. Hypertension elderly (n =30) and Healthy elderly (n =30). The objective of the study was to compare-

1. The difference of well – being in hypertension elderly and healthy elderly
2. The difference of motivation level in hypertension elderly and healthy elderly and
3. The difference of hope level in hypertension elderly and healthy elderly.
4. The difference in hypertension elderly and healthy elderly.

To fulfil the objectives two groups were taken 30 hypertension elderly (experimental group) and 30 healthy elderly (control group). Raw scores of 30 hypertension elderly and 30 healthy elderly were obtained from Elderly Motivational Scale, Adult Hope Scale and Ryff Well-being Scale Questionnaires. T-test was used in motivational scale and hope scale to measure group difference. Also, ANOVA was conducted in well-being scale to measure group difference.

According to the first hypothesis there was significant difference between the well-being of geriatric pathological group and non – pathological group. As the result shows that elderly with hypertension had higher well – being than healthy elderly's. This was found after analyzing the raw scores of the questionnaire and applying ANOVA shown on table1 on page 48, the mean scores of both groups differ a lot. American College of Cardiology (2018) studied that psychological well-being can improve overall heart health. This means that elderly with hypertension who have high level of optimism may lead a better diet, regular exercise and lower stress.

The second hypothesis was found to be non–significant difference between the motivational level of geriatric pathological group and non-pathological group. As the results at each domain health activities activities related to your biological needs, activities related to your relationships with others, activities to religion, activities related to leisure and activities related to information were found non – significant. This was

found after analyzing the raw scores of the questionnaire and applying t-test shown on table1 on page 49-55, the mean scores of both groups were quite similar. This might be due to small sample size or large number of questions in the questionnaire. Phillips (2004) [9] studied that motivation helps elderly maintain their physical health. Strough (2015) [3] studied that motivation helps to make better decisions in elderly.

The third hypothesis was found non – significant difference between the level of hope in geriatric pathological group and non-pathological group. This was found after analyzing the raw scores of the questionnaire and applying t-test shown on table1 on page 56, the mean scores of both of the groups were quite similar. . This might be due to small sample size or improper rating done by the elderly's. Ankita Budhiraja and Punam Midha (2015)[6] studied that hope and spirituality helps subjective well-being in geriatrics.

The final hypothesis shows that there is significant difference between geriatric pathological and non-pathological group. These findings confirm that both the groups are distinctive in nature.

Conclusion

The current study has highlighted following main findings:

1. The well-being of hypertension group (experimental group) is at a higher side than healthy elderly group (control group).
2. The motivation level of both the groups are quite similar hypertension group (experimental group) and healthy elderly group (control group).
3. The hope level of both the groups are quite similar hypertension group (experimental group) and healthy elderly group (control group).

Ethical Clearance: Consent was taken from all the participants

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Conflict of Interest: No

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Challenges of Allied Healthcare Professionals in India–Establishing the Council is Need of the Hour

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Abstract

There is a supply-demand gap of about 65 lakh allied health professionals in India. With India facing a heavy burden of disease with many challenges as achieving millennium development goals, shortage of skilled manpower will be a great hurdle for the health care delivery system. In such a situation, Allied Health workers can play a major role in providing holistic health services to the community. Though many institutions or Universities in India are generating allied healthcare professionals, there is no uniformity in the outcome of such professionals. There is no standardization of this professional till date. Hence, to establish a Centralized council is a need of an hour so that all such programs will come under a common platform and generated manpower will be able to provide the necessary services to the community. In this article, some suggestions are provided to improve the quality of AHPs, like estimation of workforce according to WHO requirement, uniformity in curricula, common nomenclature for all programs, creating job opportunities in private and public sectors etc.

Keywords: *Allied healthcare professionals, demand-supply, WHO, outcome.*

Introduction

Nurses, midwives and other health workers are of great significance in delivering the health care as primary health care services are not doctor-dependent. Allied healthcare professionals (AHPs) play pivotal roles in the health system. The importance of Allied Healthcare Professionals can be observed in the operation theatres, various diagnostic laboratories, blood banks, Intensive Care Units (ICUs), dialysis units, trauma care, ophthalmic OT; the list is quite exhaustive. The life of a person is handled by these allied health professionals. Operating the machines, repairing the machines, handling the patients, accuracy in diagnostic laboratory testing need skills. Any mistake can be life-threatening. That is why everyone now insists for trained staff to handle such machines either in operation theatres, ICUs or laboratories. Technology is now in an advanced position. But to operate, the person should be technically as well as scientifically sound and perfect. These technologists are to be competent enough to handle those machineries. Even in secondary and tertiary care, skilled support services should be provided by suitably trained nurses and allied health professionals.

The outbreak of COVID-19 throughout the world (From December 2019 onwards) outstretched the importance of allied Health Professionals. According to the interim guidelines published by WHO mentioned that all countries should increase their level of preparedness, alert, and response to identify, manage, and care for

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new cases of COVID-19; where the laboratory testing is an integral part of this strategy. It is added that good laboratory practices which produce accurate results are key to assure that laboratory testing benefits the public health response. It further stated that the availability of timely and accurate results can be threatened when testing demands outstrip capacity[1].

Advances in science and technology have encouraged the major shift in health care over the past few decades resulting in wider understanding of the fact that health service delivery must go beyond just doctors or nurses and involve teamwork among clinicians and non-clinicians or 'allied healthcare staff.' Better utilization of the whole range of the skills of allied Health Professionals (AHPs), is the key to health-sector reforms in India, particularly because of the shortage of doctors and nurses in semi-urban and rural areas.

NEED for AHPs: The report 'From Paramedics to Allied Health Professionals: Landscaping the Journey and Way Forward' that was published in 2012, marked the variance in education and training practices for the allied and healthcare courses offered by institutions across the country[2].

According to the World Health Statistics 2011 figures, the density per 10000 people in India is six doctors and 13 nurses and midwives working out to a doctor to population ratio of 0.5: 1000 in comparison to 0.3 in Thailand, 0.4 in Sri Lanka, 1.6 in China, 5.4 in the UK, and 5.5 in the United States of America. A recent Public Health Foundation of India (PHFI) study reveals a supply-demand gap of about 65 lakh allied health professionals.[2]

Health workforce requirements for universal health coverage and the sustainable development goals incorporated in Human Resources for Health Observer Series No 17, illustrated the total number of health workers by cadre and income group country estimated for 2013 and forecasted for 2030 as follows[3]:

	Cadre of Health Workers (Other than Physician and Nurses and midwives)			Total Health Workers		
	2013	**2030**	**% Change**	**2013**	**2030**	**% Change**
Lower Middle (Income group) countries	3550824	6685479	88%	10018004	18266529	82%

This figure demands about 80% change in the number of total health workforce.

In view of gaping shortages of doctors in providing healthcare, allied healthcare professionals (AHPs) can play a prominent role in providing holistic health services to Indian community. There is a need to establish a centralized policy to fill the gaps and ensure the availability of the appropriate human resources for quality care at primary, secondary as well as tertiary levels in India.

Classification of health workers: The US Department of Health and Human services defines AHPs as 'experts in a multitude of therapeutic, diagnostic and preventive intervention.

According to WHO, the international classification of health workers is mainly based on the International Standard Classification of Occupations (ISCO, 2008 revision)[4].

The classification of health workers maps occupation categories into five broad groupings: a) health professionals, b) health associate professionals, c) personal care workers in health services, d) health management and support personnel, and e) other health service providers not elsewhere classified.

1. Health professionals: It includes Paramedical practitioners, Dieticians and nutritionists, Audiologists and speech therapists, Optometrists and ophthalmic opticians, category of Health professionals not elsewhere classified other than all medical, nursing or physiotherapy practitioners.
2. Health Associate Professionals: Includes Medical imaging and therapeutic equipment technicians, Medical and pathology laboratory technicians, Pharmaceutical technicians and assistants, Medical and dental prosthetic technicians, Dental assistants and therapists, Medical records and health information technicians, Community health

workers, Dispensing opticians, Medical assistants, Environmental and occupational health inspectors and associates, Ambulance workers and a category of Health associate professionals not elsewhere classified

3. Personal Care Workers In Health Services: Includes Health care assistants, Home-based personal care workers and Personal care workers in health services not elsewhere classified
4. Health Management and Support Personnel: Includes Health service managers, Health management personnel not elsewhere classified, Life science professionals, Social work and counselling professionals, Life science technicians, Medical secretaries, and a category of Non-health professionals not elsewhere classified
5. Health Service Providers Not Elsewhere Classified: Includes Armed forces occupations.

Challenges of AHPs: Currently, due to the nonexistence of a central regulatory body for allied health professionals in India, they are divided into smaller groups, and regulated as independent bodies either by institutions or University. For instance, the rules or norms of professional practice vary from extremely professional to dispersed groups with no uniformity.

A representative of the MoHFW (Ministry of Health and Family Welfare, India) explained the Parliamentary Standing Committee on Health and Family Welfare about the circumstances that necessitated the firming up of a Bill to set up the new council. He noted the following problem areas and hence require regulatory body[2]:

- Para-medical professions are not regulated;
- Entry-level qualifications are different at different levels
- Level of knowledge and skills is not uniform, since the period of training differs from place to place and has no uniformity
- Course curricula are not uniform
- Fee structure and facilities in these institutions are not regulated and
- Ethical standards are not uniform and are not being enforced

The lack of planned courses and institutions, non-uniformity in nomenclature for the existing courses, diverse standards of practice and lack of qualified faculty pose a threat to the quality of education and skills of the AHPs in India.

Allied Healthcare Council of India: The Union Cabinet of India chaired by Prime Minister on November 22,2018, had approved the Allied and Healthcare Professions Bill, 2018, for regulation and standardisation of education and services by allied and healthcare professionals. The Bill has the provision for setting up of an Allied and Healthcare Council of India and corresponding State Allied and Healthcare Councils which will play the role of a standard-setter and facilitator for professions of Allied and Healthcare. Since then, more than one year has passed, but no interim council has been established[5].

Discussion

At present, the shortage of quality human resources is one of the major challenges faced by the public health domain in India. Bhore committee report of 1946 underscored the significance and importance of health care as it initiated the concept of integrated development and comprehensive healthcare[6].Acknowledging the shortage of clinicians, the Mudaliar Committee report in 1962, emphasized the need for redesigning health service delivery with better roles for paramedical personnel, leading to better utilization of doctors and nurses. Sixty years later, the recommendation still remains pertinent to reforms in the Indian public health sector as allied health workers can reduce costs and augment the quality of care [7].

The health service delivery is not just a honour of clinicians, nurses or midwives but it is a team effort, obtaining the expertise of both clinicians as well as non-clinicians. These healthcare professionals or health-service providers have been historically identified as paramedical staff or health technicians.

Many new health professions as physicians' assistants, clinical nutritionists and dietetics, optometrists, respiratory therapists or operation theatre technologists have emerged over the past several decades to support some of the work that clinicians used to perform. Somewhere these technologists are helping the clinicians that their work-skills are conducted without the physician's control and supervision (cardiovascular technologist) as other professional workers seek their own identity and autonomy.

With advancements in technology over the past few decades, the quality of medical care has vastly improved across the world. This has thrown up fresh challenges for public health. Today, there is an urgent need for competent people who can handle highly sophisticated medical machinery. In fact, diagnosis has become so dependent on technology that the role of allied health staff has become vital in delivering successful treatment.

Another very pertinent issue that is equally important in India is that students are not aware of all the allied health courses available in the medical education system.

Health care gain footing from more than 20 years of research on team performance and training and that these principles should be integrated into the practice of health care and the training of health professionals. Modernisation of the healthcare system has initiated a team-based healthcare delivery model[8]. The process of teamwork is inherently interdisciplinary which requires division of labour among the medical, nursing and allied health fraternity. Poor teamwork skills have been found to contribute to patient-care incidents[9]. Health teams are mainly the 'performer teams' due to their dynamic work conditions, wherein teamwork, coordination and collaboration are the prerequisites for optimum results. Teamwork now becomes the essential component of achieving high reliability for health care organizations. The team training can provide great insights and techniques for improving performance within such organizations.

There are some initiatives undertaken by Government of India as the Ministry of Health and Family Welfare has commissioned a Centrally Sponsored Scheme for the establishment of one National and five Regional Institutes of Allied Health Sciences and to support State Government Medical Colleges for conducting allied health science courses. The first phase of the initiative undertook a massive review of the allied health landscape in the country and proposed the development of the allied health system through the establishment of an interim body called 'National Board for Allied Health Sciences'. This process will eventually ensure the quality and standardization of the allied healthcare education and professions in the country [10].

Expanding the explanation for comprehensive healthcare, interdisciplinary collaboration can create new paths to health care problems that transcend conventional, discipline-specific method, procedures, and techniques. As proposed by Carl I. Fertman, a four-pronged approach can be used to prepare allied health students to become interdisciplinary team members in rural areas. It described in-class instruction, Web-based modules, service-learning programs, and faculty development workshops to develop and practice interdisciplinary health promotion skills in rural communities [11].

Since AHPs play a vital role in the holistic care of the patient, ensuring that they are skilled and trained efficiently, it will help to reduce the total cost of care, improve the quality and accessibility to healthcare service[10].

Rising healthcare costs provide opportunity for public and professional debate about the most effective way of providing weekend allied health care services. Some manager's perceived weekend allied health services to improve patient quality of care [12].

Healthcare delivery requires acute medical or surgical emergencies; hence weekend service provision may not be practicable. The resources may be better spent to improve the patient outcome in areas where emergency is not required such as Rehabilitation wards.

Suggestions to improve the quality of AHPs in India: The continuously changing health needs and service requirements influence the allied healthcare system in terms of both education and service provision.

Despite the huge demand of job opportunities from this sector, the allied health sciences field is highly fragmented. The government currently provides limited options for employment. It is hence essential to address the following issues [13].

1. Estimation of workforce needed both for public and private sector and create the job opportunities for them with special considerations for geographical and epidemiological needs
2. Establish a clearly defined cadre structure for AHPs based on a combination of educational qualifications and years of work experience with a distinct career pathway for three tracks: clinical practice, academic faculty and research and development.
3. Identify and establish common terminologies/ nomenclature for the various allied healthcare professionals as per the classification prescribed by (ISCO, 2008) for India.

4. The entry level for AHPs to be made uniform and centralized
5. Undertake reforms for uniformity in curricula of various programs
6. Undertake reforms for uniformity in training which include not only for cognitive but also for real and virtual skill training
7. Fees structure to be made rationalised for public and private sector
8. Minimum standards to be set for infrastructure and associated requirement like library, ICT enabled classrooms etc
9. Create a centralized portal of an allied health science knowledge community through networking, real-time data and innovative research which will be responsible for all knowledge activities in allied health.
10. Every medical college should be given the responsibility to generate the allied healthcare workforce in proportion to the number of undergraduate medical/health students.

Conclusion

For better quality of healthcare delivery in India, there should be genuine effort to address all these above issues. As such there is no regulatory body in India, the outcome of all these students become ambiguous. These are skill based programs, the certification of the skills remains questionable. Over the period, more and more allied health professionals are adding to the society but due to lack of quality assurance, the healthcare system is struggling. According to the theories of learning, students should be scientifically and appropriately trained for all the skill based competencies. If the quality is compromised, the training remains questionable. Such passed out graduates or postgraduates again training the new entrants and the cycle goes on.

Youth are getting attracted to these courses as they are job providing courses. But over-production of ill-trained personnel will lead to the manpower who cannot handle the health system appropriately. Due to their critical role in today's healthcare delivery system, it is necessary to build capacity among allied health professionals and ensure that they are appropriately skilled. Although they play important roles in the system, a lack of recognition and standardization of the courses, immensely impacts the quality of the profession.

Hence, setting of the Allied Health council in India is need of the hour so that AHPs can be appropriately, ethically and logically woven into the fabric of the Healthcare delivery system in India.

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The Study of Location of Mandibular Foramen in Dry Adult Human Mandible

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Abstract

Human mandible is only a movable bone forms lower jaw of the skull. It articulates with the temporal bone at the synovial temporomandibular joint, Mandible consists of a horizontal horse shoe shaped body and two broad rami projecting upward from the posterior end of body. Each ramus of mandible presents two surfaces, external and internal. The aim of the study is to identify the location of mandibular foramen in relation to the limits of mandibular ramus and to correlate the data with the other research works. In the present study 200 dry adult human mandibles of unknown sex and age were collected from the Dept. of Anatomy, J.N.M.C. Sawangi (M) Wardha, DMIMS (DU) and the students of first M.B.B.S of J.N.M.C. Sawangi (M), Wardha, Maharashtra and various measurements were noted. According to the results of the study, it is concluded that there is variability of the mandibular foramen of the two mandibular rami in the same person. There is variable difference between the locations of mandibular foramen in the dry human mandibles of the same zone. It may be due to differences in the age, sex and development in population. This study is mainly concentrated on finding the location of mandibular foramen by measuring its distance from various landmarks on mandibular ramus.

Keyword: *Mandible, Mandibular Foramen, Ramus of Mandible, Dry mandible.*

Introduction

In humans mandible is only a movable bone forms lower jaw of the skull. It articulates with the temporal bone at the synovial temporomandibular joint. Mandible consists of a horizontal horse shoe shaped body and two broad rami projecting upward from the posterior end of body. Each ramus of mandible presents two surfaces, external and internal.

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Near the centre of internal surface of each ramus presents mandibular foramen guarded bytongue like projection known as lingula. Mandibular foramen continuous as mandibular canal in the ramus of mandible and then in the body of mandible fora short distance. Mandibular canal conveys the inferior alveolar nerve and vessels. Inferior alveolar nerve is a branch of posterior division of mandibular nerve. Inferior alveolar nerve innervates the lower jaw, teeth and periodontal tissue. Inferior alveolar nerve also innervates soft tissue of the premolars, incisors, canines and skin of chin. Inferior alveolar nerve is blocked during anaesthesia in various dental surgeries and facial reconstruction.[1]

In dental surgeries technique failure during anaesthesia of inferior alveolar nerve is due to inappropriate setting of needle because of inaccurate location of mandibular foramen.[2] Anatomical variation of the location of the mandibular foramen may result in failure in dental and maxillofacial procedures and may lead to clinical complications if not properly identified.[3]

In advanced surgical techniques for the correction of craniofacial anomalies and pre –post operative procedures the knowledge of location of mandibular foramen is must.[4] For this purpose the qualitative and quantitative measurements are required.

It is now clear that facial reconstruction from the skull remnants in forensic medicine for medicolegal purpose is more accurate when the mandible is present. Measurements are equally important in the identification as well as facial reconstruction for medicolegal purpose in forensic medicine as well as in Anthropological study of different races. The study of various morphological features o mandible can be useful to determine age, sex and race of the individual in various medico-legal cases as well as in other anthropological studies.[5]

Despite numerous studies have been conducted on Morphometric measurements for location of the mandibular foramen in the various countries and the other regions of India, it is observed that there are differences in the data collected by various researchers. So it is important to study the location of the mandibular foramen in more no of mandibles of different regions to provide valuable and precise information to our clinician and dental professionals.[6]

The aim of the study is to identify the location of mandibular foramen in relation to the limits of mandibular ramus.

Aims and Objectives: To determine the location of mandibular foramen on dry adult human mandibles to provide informative data to be used for dental and other health professionals in various surgeries and procedures.

The aim of the study is to identify the location of mandibular foramen in relation to the limits of mandibular ramus.

To correlate the data with the other research works.

Material and Method

200 dry adult human mandibles of unknown sex and age from the Dept. of Anatomy, J.N.M.C.Sawangi (M) Wardha, DMIMS (DU) and from the students of first M.B.B.S ofJ.N.M.C.Sawangi (M), Wardha, Maharashtra were collected. The damaged bones and those, having pathological abnormalities, congenital anomalies were excluded. The mean distance of mandibular foramen from anterior border of ramus, posterior border of ramus, mandibular arch and the angle of the mandible (ramus). Both right and left sides are used for measurements. A digital vernier calliper of 0.001mm accuracy is used for taking measurements. Each distance measured thrice and mean is taken to avoid error. The measurements were recorded to the nearest millimetre. The mean and SD were calculated for left and right sides.

Fallowing measurements were noted:

1. MF- AB:A distance from the centre of the anterior limit of the mandibular foramen (MF)to the nearest point on anterior border of ramus (AB).
2. MF-PB: A distance from the centre of the anterior limit of the mandibular foramen (MF) to the nearest point on posterior border of ramus (PB).
3. MF-MN: A distance from the centre of the anterior limit of the mandibular foramen (MF) to the nearest point on mandibular notch (MN).
4. MF –AG: A distance from the centre of the anterior limit of the mandibular foramen (MF) to the nearest point on angle of mandible.

MF- Mandibular foramen

AB- Anterior border of ramus

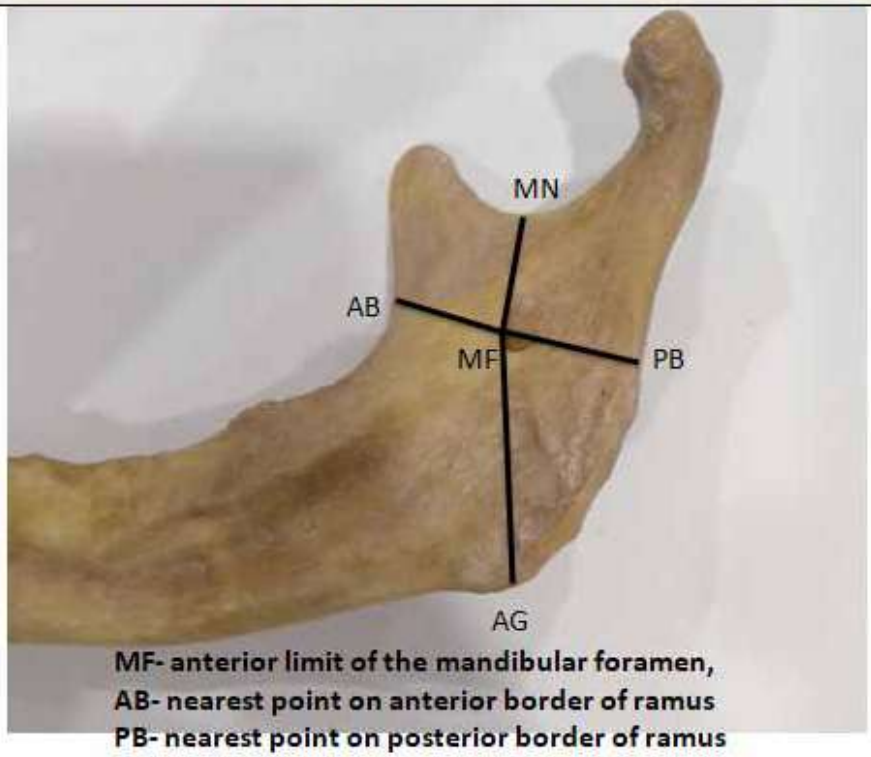
PB- Posterior border of ramus

MN- Mandibular notch

AG- Angle of mandible

Figure 1 showing various measurements from anterior limit of mandibular foramen

Image showing various measurements from anterior limit of mandibular foramen



Observations:

Table 1: Measurement of the Mandibular foramen from anterior border.

Total no. of mandibles	Side	Mean (mm)	SD (mm)	P-Value
200	Right (200)	17.58	0.98	6.06 P=0.0001,S
	Left (200)	17.22	0.94	

Table 2: Measurement of the Mandibular foramen from posterior border.

Total no. of mandibles	Side	Mean (mm)	SD (mm)	P-Value
200	Right (200)	13.61	1.18	6.22 P=0.0001,S
	Left (200)	13.30	1.00	

Table 3: Measurement of the Mandibular foramen from mandibular notch.

Total no. of mandibles	Side	Mean (mm)	SD (mm)	P-Value
200	Right (200)	19.54	2.01	8.28 P=0.0001,S
	Left (200)	19.84	2.07	

Table 4: Measurements of mandibular foramen from angle of mandible.

Total no. of mandibles	Side	Mean (mm)	SD (mm)	P-Value
200	Right (200)	26.86	1.77	5.03 P=0.0001,S
	Left (200)	27.31	2.41	

Statistical analysis was done by using descriptive and inferential statistics using Student's paired t test and software used in the analysis was SPSS 24.0 version and $p<0.05$ is considered as level of significance.

Results:

In the present study, the position of the mandibular foramen in 200 dry human mandibles of unknown age and sex were located by measuring the distance between mandibular foramen from anterior border of ramus, posterior border of ramus, mandibular notch and angle of mandible. Measurements were taken on both the sides of mandible.

According to the study it is seen that, there is no symmetry between the location of mandibular foramen on right and left side of the same mandible.

In this study, the mean distance of MF from the anterior border is 17.58 mm on right side and 17.22 mm. on left side. The mean distance of MF from the posterior border is 13.61 mm on right side and 13.30 mm. on left side. So the mandibular foramen is more nearer to the posterior border than the anterior border of ramus. The mean distance of MF from the mandibular angle is 26.86 mm on right side and 27.31 mm. on left side. So the mandibular foramen is more nearer to the mandibular angle on right side than on the left side. The mean distance of MF from the mandibular notch is 19.54 mm on right side and 19.84 mm. on left side. So mandibular foramen is significantly nearer from mandibular notch on right side than on left side.

The location of mandibular foramen with reference to various landmarks varies mandible to mandible.

Discussion

The location of mandibular foramen is very important to achieve inferior alveolar nerve block in dental surgeries of lower jaw like osteotomy, orthognathic reconstruction and dental implant .[1]Morphometry of mandible vary from mandible to mandible and with age. Malka Ashkenazi et al, (2011) stated that, the distance of mandibular foramen from the anterior and posterior border of ramus increases significantly with age.[7]Variations in the number of canal can be explained because of early development.

It is stated that the mandibular foramen is located below occlusal plane by 6 years, at occlusal plane or slightly above by the age of 13 years.[8]

The distance from mandibular notch to the mandibular foramen is an important reference point for identifying the location of the mandibular foramen during orthognathic surgery.[9]

Some adult study show that the mandibular foramen is located posterior to the midline of ramus Hayward et.al 1977, Hetson et al 1988.[10,11] The other studies show that the location of mandibular foramen is located at the midline of the ramus Sweet 1947, Monheim 1969, Nicholson 1985). Mc Donald et.al (2004) recommended the injection of the mandibular block more posteriorly in children than adult.

In this study, the mean distance of MF from the anterior border is 17.58 mm on right side and 17.22 mm. on left side. The mean distance of MF from the posterior border is 13.61 mm on right side and 13.30 mm. on left side. So the mandibular foramen is more nearer to the posterior border than the anterior border of ramus. The mean distance of MF from the mandibular angle is 26.86 mm on right side and 27.31 mm. on left side. So the mandibular foramen is more nearer to the mandibular angle on right side than on the left side. The mean distance of MF from the mandibular notch is 19.54 mm on right side and 19.84 mm. on left side. So mandibular foramen is significantly nearer from mandibular notch on right side than on left side.

According to Padmavathi G et.al .(2014) studied the mandibles from south Indian population found that the mean distance of MF from the anterior border is 16.9 mm on right side and 16.8 mm. on left side. The mean distance of MF from the posterior border is 12.10 mm on right side and 11.7 mm. on left side. The mean distance of MF from the mandibular angle is 22.20 mm on right side and 22.60mm on left side. The mean distance of MF from the mandibular notch is 22.30 mm on right side and 22.00 mm. on left side.[12]

According to Rajkumari K, et. al(2017)studied the mandibles from Imphal, Manipur population found that the mean distance of MF from the anterior border is 16.77mm on right side and 16.90mm. on left side. The mean distance of MF from the posterior border is 11.05mm on right side and 11.21 mm. on left side. The mean distance of MF from the mandibular angle is 21.02mm on right side and 21.80mm on left side. The mean distance of MF from the mandibular notch is 22.93mm on right side and 23.15mm. on left side.[13]

Table 5: Showing the location of mandibular foramen by different authors in different regions of India. (Mean distance in mm)

Author	Region	Side of mandible	MF-AB mm.	MF-PB mm.	MF-MN mm.	MF-AG mm.
Padmavathi G, et. al.(2014)	South India.	Right	16.90	12.10	22.30	22.20
		Left	16.80	11.70	22.00	22.60
Rajkumari K, et. al.(2017)	Imphal, Manipur.	Right	16.77	11.05	22.93	21.02
		Left	16.90	11.21	23.15	21.80
Amjad S.et. al.(2018)	Jalna, Maharashtra.	Right	15.6	12.0	17.70	23.0
		Left	15.3	11.10	17.0	24.0
Sandhya, et. al.(2019)	Ranchi, Jharkhand.	Right	16.00	10.21	20.48	-
		Left	16.27	10.28	20.15	-
Present study(2020)	Wardha, Maharashtra.	Right	17.58	13.61	19.54	26.86
		Left	17.22	13.30	19.84	27.31

According to Amjad S.et. al(2018), studied the mandibles from Jalna, Maharashtra and found out that, the mean distance of MF from the anterior border is 15.6mm on right side and 15.3mm on left side. The mean distance of MF from the posterior border is 12.0mm on right side and 11.10 mm. on left side. The mean distance of MF from the mandibular angle is 23.0mm on right side and 24.0mm on left side. The mean distance of MF from the mandibular notch is 17.70mm on right side and 17.0mm on left side.[14]

According to Sandhya, et. al.(2019)studied the mandibles from Ranchi, Jharkhand population found that the mean distance of MF from the anterior border is 16.00 mm on right side and 16.27mm. on left side. The mean distance of MF from the posterior border is 10.21mm on right side and 10.28mm on left side. The mean distance of MF from the mandibular notch is 20.48mm on right side and 20.15mm. on left side.[15]

So when the studies in table 5 correlates with the present study, the measurements of south Indian and Maharashtra population are showing some similarity than the population of Jharkhand and Manipur . Anatomic variations may be due to developmental perspective, racial discrepancies and craniofacial growth .[9]

Conclusion

The basic anatomy of the mandible and location of mandibular foramen is essential to medical and dental professionals for various surgeries as well as inferior alveolar nerve block.

According to the results of the study, it is concluded that there is variability of the mandibular foramen of the two mandibular rami in the same person. There is variable difference between the locations of mandibular foramen in the dry human mandibles of the same zone. It may be due to differences in the age, sex and development in population. This study is mainly concentrated on finding the location of mandibular foramen by measuring its distance from various landmarks on mandibular ramus.

Quantitative data analysisis an effective and reliable tool of demonstration and localisation of mandibular foramen in dry human mandibles. On the basis of present study it will be possible and helpful for monitoring and interpreting the maximum possible location of mandibular foramen.

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A Study of Rhodamine-6G Stain Expression for Detection and Association of Copper Content in Potentially Malignant Disorders and Squamous Cell Carcinoma

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Abstract

Aim and Objective: To assess the expression of Rhodamine-6G stain in potentially malignant disorders and SCC and to determine association of copper in potentially malignant disorders and OSCC.

Materials and Method: Sections from ten tissue blocks of each normal oral mucosa, oral leukoplakia, oral sub mucous fibrosis (OSMF), well differentiated squamous cell carcinoma (WDSCC) and moderately differentiated squamous cell carcinoma (MDSCC) was taken and stained with Rhodamine-6g stain. After adequate staining, all the samples were assessed using Fluorescence microscope for the color intensity and depth of penetration of copper and compared.

Results: It showed significant Pearson chi square values of 0.157 for color intensity of copper and 0.019 for depth of penetration of copper. Pearson correlation was calculated which showed significant value for leukoplakia (0.055)* in comparison to OSMF (0.429), WDSCC (-.395) and MDSCC (0.372).

Conclusions: Increase levels of copper in leukoplakia would be because of the fact that copper helps in activating many angiogenic factors which further helps in tumor progression from premalignant to malignancy.

Keywords: *Rhodamine-6G, Leukoplakia, Oral Sub mucous Fibrosis, Squamous cell carcinoma, Copper content, Fluorescent Microscopy.*

Introduction

Trace elements are important constituents of biological enzyme systems. They are found to play a central role in a various physiological metabolic process and in the pathology of various diseases of these elements, copper has been studied more extensively for its role in various bodily processes.[1,2] In absence or deficiency of copper, various enzymes in the body are not be able to perform their metabolic functions, as they require metallic ions such as copper to get activated. For example, ceruloplasmin, tyrosinase and cytochrome oxidase. These enzymes play an essential role in various redox reactions, free radical formation and maintenance of cellular protein homeostasis.[3,4] A number of angiogenic factors, for example TNF-α, IL-1 and VEGF are activated by copper which on further binding to endothelial cells result in their proliferation. During the progression of the cancerous lesion, copper level is reported to be unbalanced.[5]

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Occurrence of cancer, precancer and other tumor-like lesions in oral cavity is very high now-a-days in the developing countries.[6] The reason for the high incidence is not fully known, however it may be due to various contributing factors like smoking, chewing tobacco, sharp impinging prosthesis among others.

In existing literature, serology and whole blood analysis for tracer elements has been correlated with various diseases and their pathogenesis. Very few studies have been conducted using the tissue sections with respect to potentially malignant disorders and OSCC. Therefore, a retrospective study was conducted to analyze the presence or absence of copper content in potentially malignant as well as in malignant lesions using Rhodamine-6G stain and also to evaluate its significance as a diagnostic tool in disease progression.

Materials and Method

The current study was carried out in the Department of Oral Pathology, after taking the approval from Institutional Research and ethical Committee at M.M. College of Dental Sciences & Research, M.M. (Deemed to be University), Mullana.

Formalin fixed and paraffin embedded blocks of previously diagnosed cases were obtained from archives of the department. Normal healthy gingival tissues were obtained from the excess gingival tissue which were excised and discarded during periodontal flap surgery and extraction after taking the informed consent from the patients. The study groups were divided into three:

Group A: Control Group [Normal oral mucosa (n=10)]

Group B: Potentially Malignant Disorders (PMD)

- Leukoplakia (n=10)
- Oral sub mucous fibrosis (n=10)

Group C: Carcinoma

- Well-differentiated SCC (n=10)
- Moderately-differentiated SCC (n=10)

The slides were prepared by sectioning the paraffin embedded tissues with the help of manual rotary microtome. Tissues sections were than subjected to Rhodamine-6G staining procedure. They were assessed using Nikon Research (Eclipse 80i) fluorescent microscope and compared based on quantitative expression in various study groups.

Results

The criteria were set for the color intensity and depth of penetration as mild, moderate and severe. Analysis of color intensity and depth of penetration of copper was done in oral leukoplakia, OSMF, Well Differentiated SCC and Moderately Differentiated SCC. In the criteria for color intensity, out of 10 cases of oral leukoplakia, mild and moderate intensity were shown by 04 cases each, whereas severe intensity were seen in 02 cases. Out of 10 cases of oral sub mucous fibrosis, mild color intensity was seen in 07 cases, moderate color intensity was seen in 03 cases, whereas no case showed severe color intensity. In Well differentiated Squamous cell carcinoma cases, mild color intensity was seen in 02 cases, moderate color intensity was seen in 06 cases and only 02 cases showed severe color intensity. In Moderately differentiated Squamous cell carcinoma, out of 10 cases, mild color intensity was seen in 07 cases, moderate color intensity was seen in 01 case and 02 cases showed severe color intensity.

In the criteria for depth of penetration of copper, out of 10 cases of oral leukoplakia, no case showed mild depth of penetration, whereas moderate depth of penetration were seen in 04 cases and severe depth of penetration were seen in 06 cases. In oral sub mucous fibrosis, out of 10 cases, mild depth of penetration were seen in 03 cases, no case showed moderate depth of penetration, whereas 07 cases showed severe depth of penetration. In well differentiated squamous cell carcinoma, out of 10 cases, no case showed mild depth of penetration, moderate depth of penetration was seen in 02 cases and 08 cases showed severe depth of penetration. In Moderately differentiated Squamous cell carcinoma, out of 10 samples, no cases showed mild depth of penetration, whereas 05 cases each showed moderate and severe depth of penetration.

Categorical data was collected and chi square test was done for both color intensity and depth of penetration for the study samples. All the normal healthy tissues showed the absence of copper content (Figure 1). Tissue samples of oral leukoplakia (Figure 2), oral sub mucous fibrosis (Figure 3), Well-differentiated squamous cell carcinoma (Figure 4) and Moderately-differentiated squamous cell carcinoma (Figure 5) showed the presence of copper in epithelium and connective tissue. The test showed significant Pearson chi square values of 0.157 for color intensity of copper and 0.019 for depth of penetration of copper. Pearson correlation was done which showed

significant value for leukoplakia (0.055)* in comparison to OSMF (0.429), well differentiated SCC (-.395) and moderately differentiated SCC (0.372).

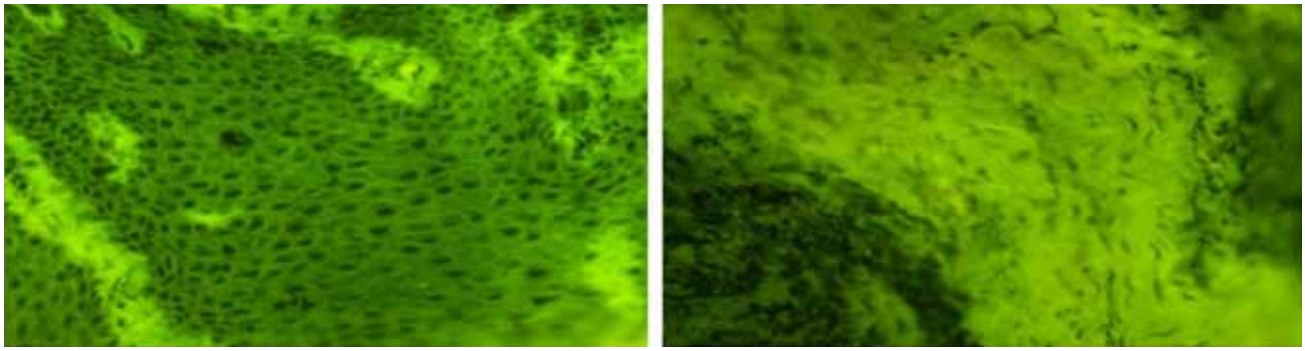


Figure 1: Absence of copper in both epithelium and connective tissue of the normal oral mucosa

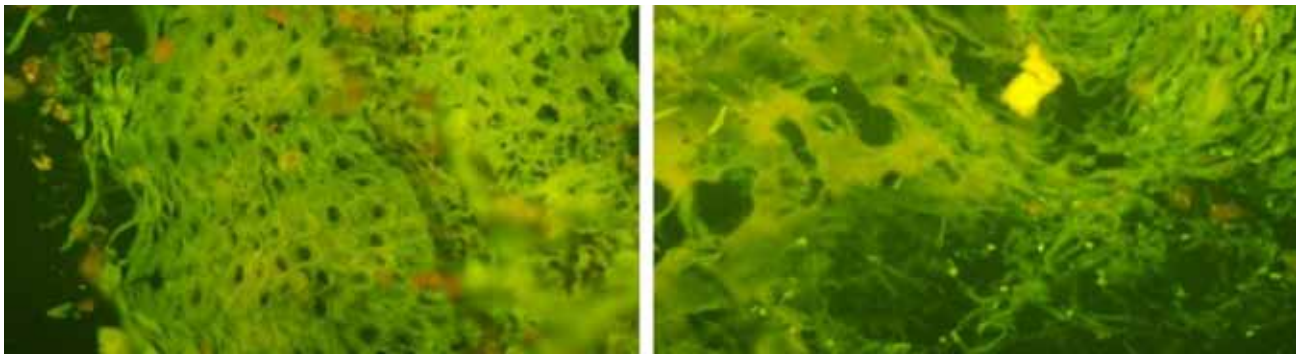


Figure 2: Presence of copper in epithelium and connective tissue in cases of leukoplakia

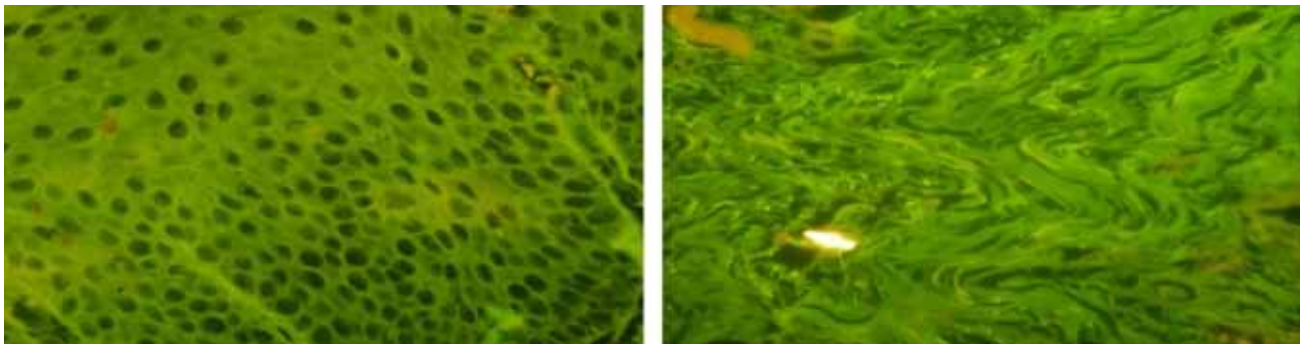


Figure 3: Presence of copper in epithelium and connective tissue of OSMF

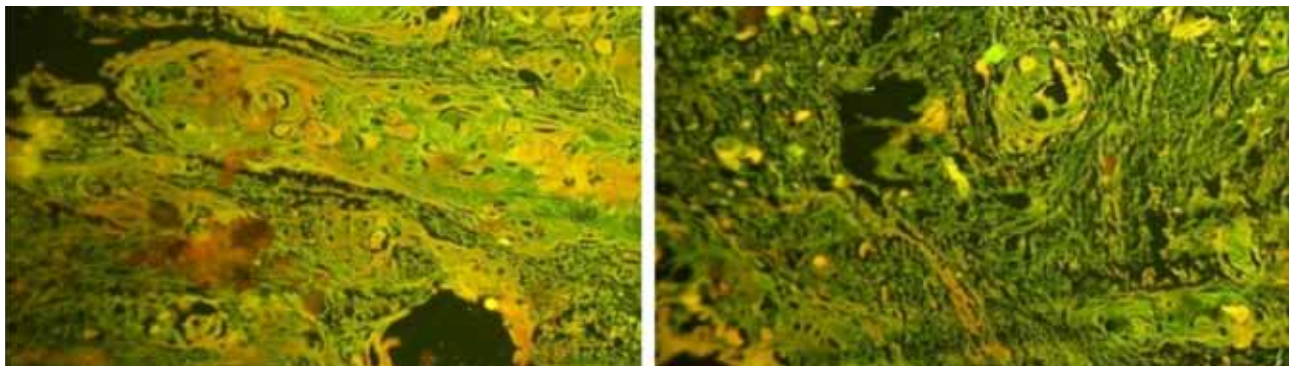


Figure 4: Presence of copper in epithelium and connective tissue of well differentiated squamous cell Carcinoma

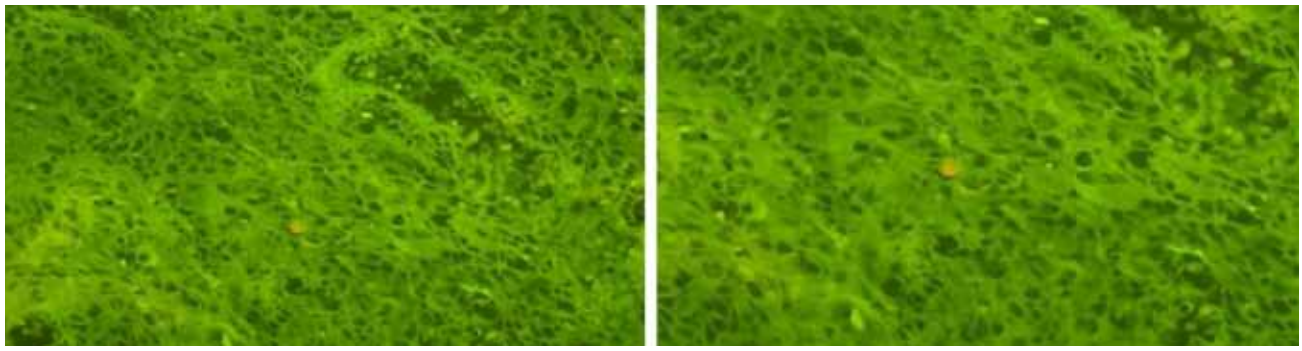


Figure 5: Presence of copper in epithelium and connective tissue of moderately differentiated squamous cell carcinoma

Discussion

Trace elements are an important constituents of biological enzyme systems. They are found to play a key role in numerous physiological metabolic processes and also in various pathological diseases. Among these trace elements, copper has been studied more extensively for its role in various bodily processes.[1,2]

In the absence or deficiency of copper various enzymes in the body are not be able to perform their metabolic functions, as they require them to get activated. For example, ceruloplasmin, tyrosinase and cytochrome oxidase. These enzymes play an essential role in various redox reactions, free radical formation and maintenance of cellular protein homeostasis.[3,4]

The present study was aimed at assessing the expression of Rhodamine-6G stain under the fluorescent microscope in potentially malignant disorders and SCC and to determine its association with copper. A total of 40 study samples were taken for the study from the archival blocks from the department of Oral Pathology. For comparison, 10 normal healthy gingival tissues were obtained after taking the informed consent from the patients, from excess gingival tissue excised and discarded during periodontal flap surgery and extraction.

Criteria for scoring the color intensity and depth of penetration were set. For color intensity, criteria were set as mild, moderate and severe and the color intensity of copper in cases of oral Leukoplakia, OSMF and squamous cell carcinoma were observed.

Categorical data was collected and the Pearson chi square test was done for both color intensity and depth of penetration for the study samples. All the normal healthy tissues showed the absence of copper content. The test showed significant Pearson chi square values of 0.157 for color intensity of copper and 0.019 for depth of penetration of copper. Pearson correlation was calculated which showed significant value for leukoplakia (0.055)* in comparison to OSMF (0.429), WDSCC (-.395) and MDSCC (0.372).

A study done by Swain N and Ray JG found that copper content was increasingly higher from leukoplakia to cancer group.[7] Ayinampudi BK and Narsimhan M, observed significant increase in salivary copper levels in leukoplakia.[8] Similar findings were observed in our study with Rhodamine 6G staining technique on paraffin embedded tissue sections in comparison to the latter.

Toke et.al in their study found increased levels of serum copper as well as serum zinc in malignant patients as compared to the potentially malignant disorders.[9] Their findings were different to what is seen in our study.

Study done by Shetty SR et.al observed significant raise in the serum copper values in OSMF patients in contrast to controls.[10] According to Joshi PS et al. higher mean values for copper was seen in cases of oral sub mucous fibrosis (2.00) in comparison to leukoplakia and squamous cell carcinoma.[11] Our findings in the present study were different in comparison with the above study which showed no significance values for OSMF and SCC but rather showed highly significant values for oral leukoplakia.

Khanna SS and Karjodkar FR in their study found mean serum copper values were notably high in patients sera with premalignant as well as malignant lesions. In comparison, in our study only premalignant lesion (leukoplakia) showed significant values for copper.[12]

Ehud J et.al revealed that copper levels were raised in malignancy in comparison to zinc whose levels were found to be decreased.[13] Their findings were similar to the findings in the present study in relation to the depth of penetration of copper which showed increase levels in SCC.

Irons RD et.al[14] in their study revealed the presence of abnormal tissue copper levels in histopathological sections of liver using the Rhodamine staining technique, whose findings were similar to the present study. Varghese I et.al[15] found significant reduction in levels of copper in OSMF and SCC in comparison to leukoplakia which was similar to the present findings.

Conclusion

Increase levels of copper in leukoplakia would be because of the fact that copper helps in activating many angiogenic factors which further helps in tumor progression from premalignant to malignancy. During this progression copper level is reported to be increased and unbalanced. Since the chances of oral leukoplakia progressing to cancer is more as compared to other potentially malignant disorders, our study has showed significant values for copper in leukoplakia as compared to OSMF and SCC.

Regular follow up of all the potentially malignant lesions with periodic estimation for copper content using Rhodamine 6G staining technique as potential marker in assessing the disease progression should be helpful in correlating the progression from potentially malignant stage to malignancy.

Ethical Clearance: Ethical clearance for the present study was taken from the Institutional Ethical Committee.

Sources of Funding: Nil

Conflicts of Interest: None

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Effectiveness of Deep Brain Stimulation in Iraqi Patients with Parkinson Disease

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Abstract

Background: Surgical treatment for advanced Parkinson disease (PD) is considered when medical therapy fails to control the symptoms.

Objective: Assessment of effectiveness of deep brain stimulation (DBS) in controlling motor manifestations of advanced PD.

Patients and Method: Twenty patients (14 male and 6 female) whom fulfill the requirements for DBS surgery, were included in this study. All patients were on Levodopa (L-dopa), procyclidine, pramipexol, and amantadine medications. The study conducted between March 2017 and March 2018 in the neurosurgical teaching hospital and neuroscience hospitalin Baghdad/Iraq. Unified Parkinson's Disease Rating Scale(UPDRS) was used before and after surgery.

Results: There was a statistically significant improvement in the tremor, rigidity, and bradykinesia.

Conclusion: Most disabling axial symptoms of gait and postural instability, that failed to respond to medical therapy, will show effective response to the bilateral STN stimulation in advanced PD cases.

Keywords: *Parkinson disease, deep brain stimulation, subthalamic nucleus.*

Introduction

PD is a chronic degenerative disease of the basal ganglia, it includes both motor function and non-motor clinical manifestations, treated symptomatically by dopamine replacement .Every year, an average of nine-thousand DBS procedures are done for patients with PD around the world.(1) Ninety percent of PD are sporadic, while the monogenic forms of the disease account for approximately 3–5%.(2)

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Perhaps not since neurosurgery introduced to thecommunity,by Harvey Cushing, has there been a surgical treatmentthat has got the intention of the neurosurgeons and the thoughts of the patientsto the extent that DBS did. Since its introduction in its current form 30 years ago, DBS remains innovative in the mind of many and is acknowledged as underutilized.(3)

Benabid's clinic first reported DBS for the management of PD in 1993. After FDA authorizationfor essential tremor on 1997, DBS was widespread used. It was first admitted to Iraq in 2007, and all the implantations were carried out in the neurosciences hospital in Baghdad/Iraq. (4)

DBS provides for the PDpatients a remarkable therapeutic effect. It generates measurable electrical stimulation pulses at the goal target in the brain which

leads to intrusion with neural activity and creating areversible lesion in the implanted target nucleus whichresults, eventually, in improvement of motor characteristics of PD (bradykinesia, rigidity, and tremor).(5)

However,risingdataimplies that "DBS is more than just a neuromodulatory switch to control debilitating motor symptoms". Chronic DBS has been revealed to stimulatecontinuingreformation of neuronal circuits throughout enhanced synaptic plasticity and neurogenesis. Thus DBS can cause slowing of disease progression and increase survival in some PD cases.(6)

Symptomatic treatment using dopamine replacement remedycontinued to be the most useful management of PD since L-dopa was initially brought to use since the 1960s. In the end, they were coupled with motor fluctuations and L-dopa-induced dyskinesia. In a community-based study, the mean times of onset of dyskinesia were 6.6 years.(7)

In the lack of disease-modifying therapies, the presentmanagement of PD is principallyconcentrated on minimizing motor symptoms by drugs that augment intracerebral dopamine level (e.g. L-dopa, MAO-B inhibitor) or excite dopamine receptors (e.g. Dopamine agonist). On the continuing, side effects of dopaminergic treatment, counting on/off motor fluctuations and dyskinesias, commonly occur. (8) DBS of subcortical nuclei such as the subthalamic nucleus (STN),globus pallidus internus(GPI), and infrequently ventralis intermedius nucleus (VIM), can be presented as a conventional treatment for properly selected patients with L-dopa-responsive motor symptoms who suffer from disabling drug-associated side effects.(9)

The progress in DBS for movement disorders can be grouped in terms of the steps of the DBS workflow, which consist of selection of patients, selection of target and imaging, selection of hardware, surgical technique, and postoperative programming and maintenance.

Advances in every of the exceeding domains are continuing and to improve the excellence and effectiveness of DBS for movement disorders. (3,5,10,11).

Aim of the study: Assessment of effectiveness of DBS in controlling motor manifestations of advanced PD.

Patients and Method

Patients Selection: This is a prospective clinical study that incorporated 20 patients (14 male and 6 female) for whom DBS was implanted at the neurosurgical and the neurosciences teaching hospitals in Baghdad/Iraq, from March 2017 to March 2018 for bilateral stimulation of the STN. The patients were fulfilling the Movement Disorder Society Clinical Diagnostic Criteria for PD. (12)

The DBS were implanted using a fusion system of the CT and MRI images (Stealth Station- Medtronic®) to identify the site of the STN in relation to the mid-commissural point.

All the patient were right handed, and all of them were on L-dopa, procyclidine, pramipexol, and amantadine medications. In addition, intraoperative neuronal electrical activity recording was made.

All of the patients were followed for 2 months after staring of stimulation.

The inclusion criteria were:

1. Diagnosis of idiopathic PD and duration > 5 years.
2. Intolerable L-dopa related motor complications despite of optimal adjustment of antiparkinsonian drugs.
3. Improvement of motor symptoms > 50 % during L-dopa test
4. Age below 70 years.
5. No surgical contraindications.
6. No cognitive abnormality or significant continuing psychiatric illness.
7. No medical co-morbidity
8. Normal brain MRI

The severity of the disease is assessed before and after DBS implantation using UPDRS, which is a 50 question assessment of both motor and non-motor symptoms of PD.

Any patient who did not fulfill the above criteria was not a candidate for DBS implantation (e.g. severe brain atrophy, brain lesions, coagulopathy, anticoagulation therapy, immunosuppression, active psychosis, major depression with suicidal thoughts, personality disorder impairing compliance, drug abuse impairing compliance,

and poor general health conditions like severe ischemic heart disease, hypertension, and diabetes).

As a routine of the workup, the general preoperative list of diagnostic tests including hematology, biochemistry, serology, ECG, CXR and standard brain MRI were done for each patient.

The severity of the disorder was assessed, and the motor fluctuation were evaluated after the diagnosis of idiopathic PD. Also drug related dyskinesia were evaluated according to the UPDRS.[13]

Every patient should have new set of MRI studies. Closed MRI(3 Tessla) were used in our study.

The patientsresumed their preoperativedrugs regimenstraight away postoperatively to keep away from dopaminergic withdrawal symptoms.

CT scans and/or MRI were done for all the patients postoperativelyto review the electrode position and the intracranial condition. Additionally, plain X-rays were obtained to review the position and geometry of the leads and hardware. Modification of the drugs may be needed according to the patients' clinical condition. If stable, patients were allowed to return home as early as 24 hours following the surgery.

The process of programming of the patient susually started 7 days after the implantation so as to bypass the phase of lesioning result of the surgery.

Statistical Analysis: Descriptive analysis in the form of percentage was calculated using Microsoft Office Excel Worksheet and presented in the relevant tables shown below. Chi-Square test was used for statistical analysis by utilizing the Statistical Package for Social Sciences (SPSS) version 17(p value< 0.05 was considered significant).

Results

The explanatory data of the patients incorporated in this study are demonstrated in table 1.

Table 1: Descriptive data of the patients before DBS

Patient number	Gender	Age (years)	Tremor UPDRS score	Rigidity UPDRS score	Bradykinesia UPDRS score
1	Female	60	4	3	3
2	Female	33	3	3	4
3	Female	55	3	3	2
4	Male	57	3	3	4
5	Male	60	3	2	2
6	Female	56	2	2	3
7	Male	55	3	3	3
8	Male	45	2	3	3
9	Male	67	4	4	4
10	Male	50	3	2	5
11	Male	54	2	2	2
12	Male	50	2	3	3
13	Male	56	4	2	4
14	Male	67	3	2	2
15	Female	42	2	2	3
16	Male	47	3	3	3
17	Male	69	3	2	2
18	Male	53	3	3	2
19	Male	43	2	2	3
20	Female	50	2	2	4

We analyzed the demographic data, rigidity, tremor, and bradykinesia before and after the DBS, and the relation of the patient's age to the improvement in rigidity, all measured according to the UPDRS.

1. **Tremor:** The improvement in the tremor grade post-operatively was statistically significant ($p< 0.05$) [table 2].

Table 2: Tremor grade pre-operative and post-operative.

Patient number	Tremor UPDRS score Pre-operative	Tremor UPDRS score Post-operative
1	4	0
2	3	0
3	3	0
4	3	0
5	3	0
6	2	0
7	3	1
8	2	1
9	4	0
10	3	0
11	2	0
12	2	1
13	4	1
14	3	0
15	2	0
16	3	0
17	3	1
18	3	0
19	2	1
20	2	0

$p < 0.05$

2. **Rigidity:** The improvement in the rigidity of our patients post-operatively was statistically significant [$p < 0.05$] (table 3).

Table 3: Rigidity grade pre-operative and post-operative.

Patient number	Rigidity UPDRS score Pre-operative	Rigidity UPDRS score Post-operative
1	3	0
2	3	0
3	3	0
4	3	0
5	2	0
6	2	0
7	3	1
8	3	0
9	4	1
10	2	0
11	2	1
12	3	0
13	2	1
14	2	0
15	2	0
16	3	1
17	2	0
18	3	1
19	2	0
20	2	0

$p < 0.05$

3. **Bradykinesia:** There improvement in the bradykinesia of our patients post-operatively was statistically significant [$p= 0.01$] (table 4).

Table 4: Bradykinesia grade pre-operative and post-operative.

Patient number	Bradykinesia UPDRS score Pre-operative	Bradykinesia UPDRS score Post-operative
1	4	0
2	3	0
3	3	0
4	3	0
5	3	0
6	2	0
7	3	1
8	2	0
9	4	1
10	3	1
11	2	0
12	2	0
13	4	1
14	3	1

Patient number	Bradykinesia UPDRS score Pre-operative	Bradykinesia UPDRS score Post-operative
15	2	0
16	3	0
17	3	0
18	3	1
19	2	0
20	2	0

$p < 0.05$

Complications: We had only 2 cases of surgical wound infection who responded well to conservative treatment by antibiotics and meticulous dressing.

Discussion

DBS has become an already established surgical option for medically intractable PD.[14] After the implantation, the motor symptoms improves comparatively to the pre-implantation response to L-dopa. The reduction of the disabling motor symptoms eventually leads to a significant reduce in the dopaminergic drugs doses with subsequent drop of drug – related dyskinesia.[15,16]

GPI and STN stimulation showed similar efficacy rates. However other studies claimed that STN stimulation is possibly superior in controlling the symptoms of PD. Choosing STN stimulation over the GPI is frequently based according to the foundational, surgeon, and programming practices and preferences. There is a more reliabletendency for major reduction in dopaminergic drugs consumption andl esser current requirement with consequential longer battery time, with the STN stimulation.[17,18,19]

In our study we followed 20 patients(14 males and 6 female), with age range from 33 to 69 years.

Lopiano et al reported improvement in tremor, bradykinesia, rigidity, dyskinesia, and motor fluctuations symptoms after DBS for PD, while hypophonic speech, dysphagia, micrographia, freezing of gait (especially if occurring in the “on” state), cognitive dysfunction, and dysautonomia did not show improvement.[13]

In our study there was a significant improvement in rigidity, tremor, and bradykinesia.

Paul Krack et al reported that in the first few days after DBS implantation, the complications related to the device were rare (less than 1%). One patient had an infection that was managed bytransitorytaking away of the subcutaneous extension lead and IPG, three deaths, one patient developed an intra-operative intracerebral hemorrhage and remained bedridden and died three years after surgery, one patient died 11 months after surgery due to myocardial infarction, and one patient had severe depression and suicidal thoughts three months before the implantation and eventually committed suicide six months after surgery.[20] This is not comparable to our results because of the bigger number of cases and extended period of follow up by Paul Krack et al.

Paul Krack et al, in five years follow up on patients with PD who had bilateral DBS-STN, found a significant decrease in the requirement for L-dopa (or equivalent drug) postoperatively (11of 42 patients did not took L-dopa anymore, and 3 patients did not took any dopaminergic drugs).[20]

Conclusions

The advantages of DBS in advanced PD is symptomatic control rather than therapeutic effects. The fate or progress of the illness will not be changed. DBS is completely reversible in comparison with the other ablative procedures. Most disabling hypokinetic manifestations, that failed to respond to medical therapy, will show effective response to the bilateral STN stimulation in advanced PD cases.

Ethical clearance- Taken from the scientific unit and medical ethics committee of Alkindy college of medicine/University of Baghdad**.**

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Study of Some Immunological Parameters with Level of Iron in Women Infected with *Trichomonas Vaginalis*

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Abstract

Trichomonas vaginalis is a flagellated protozoan parasite that causes a disease called trichomoniasis in humans. It affects the urogenital tract and is one of the most important causes of non-viral genitourinary sexually transmitted infection (STI). The host's immune response plays a very important role in the onset of pathological symptoms and their differences between infected people. During the period from July to December 2019, 30 samples were collected, 20 of infected women with *T. vaginalis* and 10 samples as a control group from reviews of external women's clinics in Kirkuk city, whose ages ranged between 18-56 years so this study was designed to know the effect of infection with *T. vaginalis* parasite on some immunological parameters such as the role of humoral immune response based on the production of immunoglobulins IgM, IgG, IgA against the parasite and there estimation in serum by using Enzyme Linked Immunosorbent Assay (ELISA) technique as well as estimating the C-Reactive protein concentration as an indication of systemic inflammation and of serum iron level . The results of the current study showed a "significant" increase in the level of immunoglobulins IgM, IgG in serum of women infected with *T. vaginalis* compared to the control group, as the level of IgM (8. 2 ± 2.9 ng/dl), (6.3 ± 0.5 ng/dl) Consequently, at the level ($p < 0.05$), the level of IgG was (8.16 ± 2.20 ng/dl), (6.53 ± 1.69 ng/dl) for the infected women and the control group, respectively. As for the level of IgA, the results showed non-significant increase in the affected women compared with control group (8.22 ± 3.1 ng/dl), (7.82 ± 2.8 ng/dl), respectively. The results of estimating the CRP concentration showed a significant increase in the infected women compared to the control group (5.76± 1.56 mg/dl), (4.61± 0.66 mg/dl), respectively. While the results of measuring the level of iron showed a significant decrease among infected women and the control group (38.79 ±8.23 mg/dl), (93.83± 34.76 mg/dl), respectively. We conclude from the above that infection with *T.vaginals* stimulates the humoral immune response and affects on immunoglobulins levels, CRP concentration and Iron serum level.

Keywords: *T. vaginalis, lgM, IgG, IgA, CRP, Iron level.*

Introduction

Trichomonas vaginalis It is one of the flagellated protozoan parasite that causes Trichomoniasis by affecting the urinary genital tract in humans and is one of the most important causes of non- viral genitourinary sexually transmitted infection (STI) with a prevalence rate of 180 million annually according to WHO estimates(1). *T. vaginalis* has only trophozoite stage with a size of (7-32 μm) and is pear shaped. It possesses 5 flagellates, 4 of which are forward with a fifth flagella that bends back along the undulating membrane and lacks the cyst stage and possesses a central nucleus and Axostyle, Trophozoite is divided by binary fission. The parasite is an aerotolerant anaerobes that dissolves whole carbohydrates into short chains of organic acids Especially (acetate and lactate) Regardless of the presence of oxygen. The parasite produces hydrogen

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molecules in the absence of oxygen, and this reaction occurs in hydrogenosomes that like mitochondria (which are not present) in *T. vaginalis*, which are energy-producing organelles surrounded by two membranes[2].

T. vaginalis live in the vagina and urethra of the female and in the prostate, seminal vesicles and urethra in the male. The incubation period ranges from 4-28 days, and the normal acidity of the vagina pH 4.0-4.5 inhibits infection, but when the parasite moves to the vagina, pH will change in the vagina to the alkalinity pH 5-6 due to metabolic processes of the parasite, which promotes the growth and duplication of it[3].

As for the clinical symptoms of trichomoniasis, they have a wide range, ranging from asymptomatic in more than 50% of women to moderate and severe infections. The main causes of the different symptoms are due to the virulence factors of the parasite, the genetic difference and the immune response of the host to the parasite[4]. The symptoms are white to green secretions, frothy discharges, mucopurulent accompanied by severe itching and burning in the affected area, and small hemorrhagic spots in the vaginal and cervical mucosa of the uterus known as strawberry cervix [5].

Innate immunity and adaptive immunity play an important role in developing trichomoniasis, as innate immunity includes many defensive physical barriers that prevent the transmission of the parasite to the genital tract and include epithelial cells, mucous and acidic pH in the vagina which is caused by the Lactic acid produced by commensal bacteria such as *Lactobacillus spp* in healthy women[6].

Humoral immunity is an important role in the immune response against trichomoniasis. Local and specific antibodies have been established in the serum of patients with the parasite as well as in experimental studies, as a high level of IgM, IgG, IgA[7] has been observed. During the latter 40 years have been relying on method and techniques to measure the level of the these immunoglobulins and their classes such as radioimmunoassay and ELISA to find out the role of humoral immunity in eliminating pathogens that enter the body[8].

C-Reactive Protein is a protein produced from the liver to the blood circulation and stimulates its production by many cytokines such as IL6 and TNFα and plays an important role in the immune response to pathogens that enter the body and is considered as an indication of systemic infections and evidence of the acute phase and severity of infection, also in chronic infection but to a lesser degree than in acute infections, and vaginitis which caused by *T. vaginalis* is a form of systemic inflammation response[9].

Iron is an essential ingredient for living organisms, including humans and parasites, because it is necessary for biological processes including oxygen transport, DNA formation, and electron transport[10]. Also, some parasites such as *Trichomonas vaginalis*, Giardia *lamblia* and *Entamoeba histolytica* need iron in high concentrations ranging from (5-200 μm) to their growth compared to other prokaryotic and eukaryotic organisms.

Iron is very necessary in the pathogenesis of *T. vaginalis* as it contributes to cytoadherence in the surface of the epithelial cells of the vagina and regulates the genetic expression of adhesion proteins and its role was confirmed by culture experiment to find the relationship between the virulence of *T. vaginalis* and the role of iron in that[11].

Therefore, this study was designed to know the effect of parasite infection on some immunological parameters such as immunoglobulins IgM, IgG, IgA, CRP concentration, iron level in infected women and comparison with control group.

Materials and Method

During the period from July to December 2019, 30 samples were collected, 20 of infected women with *T . vaginalis* and 10 samples as a control group from reviews of external women's clinics in the Kirkuk city, whose ages ranged between 18-56 years and vaginal swabs were taken from posterior vaginal fornix and put it on tube Contains 1ml of normal saline and samples were examined within few minutes with a wet mount examination. A drop of the mixture was placed on a clean slide, then a cover slip was placed on it and examined under 100X and 400X magnification forces to observe a pear shaped trophozoite with jerky motility [12].

Five milliliters of blood samples were collected from infected women with *T. vaginalis* and control group and serum was obtained by making a centrifuge 3000 rpm for 3 minutes and keeping serum by freezing one section at -20°C to measure the CRP concentration as well as the iron level and the other section -70°C to measure The level of immunoglobulins IgM, IgG, IgA for both groups.

The level of immunoglobulin M (IgM) was measured in serum of infected women and the control group by using (Human IgM ELISA Kit), the principle of this method is based on Sandwich ELISA from (Bethyl Laboratories, Inc. USA) and the attached instructions have been followed by the manufacturer.

The level of immunoglobulin G (IgG) was measured in serum for infected women and the control group by using (Human IgG ELISA Kit), the principle of this method is based on Sandwich ELISA from (Bethyl Laboratories, Inc. USA) and the attached instructions have been followed by the manufacturer.

The level of immunoglobulin A (IgA) was measured in the serum for infected women and the control group by using (Human IgA ELISA Kit), the principle of this method is based on Sandwich ELISA from (Bethyl Laboratories, Inc. USA) and the attached instructions have been followed by the manufacturer.

CRP concentration was measured by (CRP Direct Latex Reagent kit) from (LINEAR CHEMICALS, Barcelona, Spain) and the instructions included with the kit were followed.

The iron level was measured by Iron Colorimetric Assay kit (Ferrozine method) from (LINEAR CHEMICALS, Barcelona, Spain) and the instructions included with the kit were followed. Furthermore, this study was approved and conducted by the Scientific Committee at the College of Nursing/University of Kirkuk/Iraq.

Statistical Analysis: Statistical analysis was performed using (t-test) under a significant level (0.05)[13].

Results and Discussion

1. **Estimation of immunoglobulin levels IgM, lgG and IgA:** Table 1. shows the level of immunoglobulins in the serum of women infected with parasite compared to the control group, as the results of the current study showed a significant increase in the level of IgM in the serum of women with *T. vaginalis* compared to the control group (8.2 ± 2.9 ng/dl), (6.3 ± 0.5 ng/dl), respectively. This finding is consistent with [14] who, through their studies, found a higher IgM level in the serum of mice infected with the parasite compared to the control group, also[15] found through their study a rise in the IgM level in the serum of women infected with the parasite and comparison with the control group indicating that acute infection with *T.vaginalis* stimulate the humoral immune response because IgM is indicator of acute period of infection. In addition, this is leads to knowledge of insights and trends for preventing parasite infection or developing treatment [16].

The results of the current study also showed a significant increase in the level of IgG in the serum of women infected with *T. vaginalis* compared to the control group (8.16 ± 2.20 ng/dl), (6.53 ± 1.69 ng/dl). This result is consistent with [14] who found, through their studies, a high level of IgG in the serum of mice infested with the parasite compared to the control group as well as with [17] who demonstrated through their study a rise in the level of IgG in the serum of women infected with parasite compared to the control group, which indicates that chronic infections are common especially in married women due to repeated infection and leave it without treatment .Moreover, to the fact that most of the infected women are asymptomatic when infected with *T. vaginalis*. In addition to what has been mentioned, the role of immunoglobulin, especially IgG, has a significant role in humoral immunity to control the parasitic infection[18].

The results of the current study also showed a significant increase in the level of IgA in the serum of women with *T. vaginalis* compared to the control group (8.22 ± 3.1ng/dl), (7.82 ± 2.8 ng/dl), respectively, and this result is consistent with [18] as well as with [17], As our study showed that there are no significant differences between the patients and the control group, and the reason for this result is that IgA rises in acute cases of the disease and returns to its normal level in chronic cases, and because most of the cases with *T. vaginalis* are chronic cases as mentioned Above. As the immune response depends on humoral immunity and begins as a local inflammation at the place of invasion and pathogen presence, WBC is attracted to the site of infection and produces cytokines, which in turn stimulate the production of specific immunoglobulins, especially IgA, which is considered one of the main media specialized in host defenses present in the secretions. The proteinase secreted by the parasite *T. vaginalis* is excreted in the vaginal and serum secretions and reduces the level of immunoglobulins over time and this effect on humoral immunity is one of the most important reasons for the difficulty in forming a vaccine against *T.* vaginalis [19].

Table 1. Immunoglobulins levels in women infected with *T. vaginalis* and control group

Mean ± SD (ng/dl) Control group n=10	Mean ± SD (ng/dl) Patients n=20	Immunoglobulins levels In serum
6.3±0.5	8.2±2.9	IgM
6.53±1.69	8.16±2.20	IgG
7.82±2.8	8.22±3.1	IgA

2. **C-Reactive Protein concentration:** The results of the current study showed a significant increase in the CRP concentration in the serum of women with T vaginalis compared to the control group (5.76 ± 1.65 mg/dl), (4.61 ± 0.66 respectively) (Table 2) and this is consistent with [20]. The high concentration of CRP in the serum in women with the parasite represents a strong link between localized vaginal infection and the response of the body's systems to infection, as CRP is produced from the liver in large quantities and transmitted to blood circulation and rises in bacterial, fungal and parasitic infections and is considered as an indication of the presence of infections in the body, it is not A diagnostic test, but it is used to link signs and symptoms.

Infection with *T. vaginalis* stimulates the cellular immune response, as the parasite causes vaginitis in females and urethritis in the male. The high CRP in the serum of infected women indicates that the reactions of the immune response to the parasite have exceeded the boundaries of the mucosal genital tract in the host[8].

Table 2. CRP concentration in women infected with *T. vaginalis* and control group

Mean ± SD mg/dl	Number of samples	CRP Concentration Group
5.76±1.56	20	Patients
4.61±0.66	10	Control

3. **Serum Iron level:** The results of the current study showed a significant decrease in the iron level in the serum of women with *Trichomonas vaginalis* compared with the control group (38.79 ±8.23 mg/dl), (93.83±34.76 mg/dl), respectively (Table 3), and this result is compatible with[21]. Note that the normal level of serum Iron level is (50-170 mg/dl), and the reason for this is that iron is a key component in pathogenesis of the parasite as the level of adhesion to human cervical cell line, or what is called Hella cells, changes with iron levels, because *T. vaginalis* isolates from an iron-rich medium show a high level of adhesion and the reason is because the parasite has developed many mechanisms for adhesion, including Lipophosphoglycan, which is a major adhesion factor for parasite. In addition to Adhesion Proteins (AP), Fibronectin binding protein (FN), Laminine –binding protein, these proteins have the ability to interact with the parasite through their own receptors on its surface. Moreover, the presence of four types of important proteins in the adhesion process, namely (Ap33, Ap23 Ap51 Ap65), which iron plays a very important role in the process of gene expression and translation of these proteins [22]. There are many experiments proven by scientists [23] on the role of iron in the pathogenicity of the parasite, as they cultured *T. vaginalis* in the Trypticase yeast media (TYM) which provided with iron, While the parasite's isolates from an iron-deficient medium failed to produce any pathogenicity[24]. The source of the iron for the parasite comes from hemolysis of red blood cells from lesions formed by the parasite in addition to iron from the menstrual blood cycle[25].

Table 3. Serum Iron level in women infected with *T. vaginalis* and control group

Mean±SD mg/dl	Number of samples	Serum Iron level Group
38.79±8.23	20	Patients
93.83±34.76	10	Control

In conclusion, infection with *T. vaginalis* affects on some immunological parameters levels which indicate the role of humoral immune response against trichomonasis, also the infection decreases serum iron level due to important role of iron in pathogenicity and cytoadherence of *T. vaginalis*.

Conflict of Interests: The authors of this paper declares that he has no financial or personal relationships with individuals or organizations that would unacceptably bias the content of this paper and therefore declare that there is no conflict of interests.

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Heuristic "Bottom-up": COVID-19

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Abstract

Background: COVID 19, a global pandemic has led to the disruption all over the world. Not only the COVID outbreak but the measures taken to straighten the curve led to the psychological distress amongst people. The disruption in daily routine which changed People's lifestyle, the changed business working environment while COVID and post COVID. The concept to adapt to the new normal still seems abrupt.

Method: This present study was done amongst Indian youth and working professionals (age 18-28) in order to understand their psychological well-being in these COVID times. With collection of data through an online questionnaire more than 350 people participated. The instrument used was DASS-21 and a self-conceptualized VIRUS scale. All statistical analysis was computed through SPSS 21.

Conclusion: This paper aims to contribute through exploration into the stressors and how the COVID situation has brought about vexation, immediate concern, routine disruption and uncertainty about future. Through these four factors we obtain results that elaborates on what will affect their life's the most, highlighting the need for post-COVID interventional strategies in business environments and in one's lifestyle.

Keywords: *Covid-19, DASS21, Mental Health.*

Introduction

The novel coronavirus has taken over our normal lives, this virus has transmitted globally and transformed the normal of the world. We can only talk about the new normal, the daily update of the increasing cases has a great psychological effect on the human mind. COVID-19 has wired youth to fear, uncertainty, dragging people with frustration and sadness. Reports have proven other pandemics have played a similar role like the SARS and H1N1 over human behavior showing signs of distress and anxiety. Generally, more than 25% of the population in China faced high- level stress traits (Wang et al., 2020)[(1)]. The state of mind and the behavior during these times are due to misinterpretation of the set-offs by themedia.

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These reports are similar to what was described during the time of the SARS epidemic moreover, the experience and the idea of contracting the COVID-19 have put pressure and increased psychological suffering. In any epidemic, physical health suffers but a significant amount of damage is caused to mental health too. Trauma, panic, anxiety, and suicidal thoughts during an outbreak of such disease are common psychological distress caused in an individual (Tucci et al., 2017; Taylor et al., 2008).[(2)(3)]

Anxiety can be understood as a sense of worry, tension, physical changes such as trembling, dizziness, or increased blood pressure or change in a heartbeat. Anxiety can lead to many issues which can lead to limited socialization and limitations in daily life activities. If caused within the larger population, it can lead to panic buying which leads to exhaustion of resources. Many people tend to get affected mentally due to a pandemic and can cause high anxiety which affects the emotional well-being adversely. Thus, it is critical to manage mental well-being. Research has shown that approximately 1/5th of the people experiences severe to very severe anxiety and in terms of gender, females

tend to feel more anxious than male (Moghanibashi-Mansourieh,2020)[4].

As a preventive measure, the government of India decided to implement a lockdown and people were home quarantined which drastically changed people's lives. However, mental health degradation was not because of the control measures (lockdown/Quarantine) but due to the changes it caused in daily life (Li et al., 2020) [5]. It has been found that disruption in daily routine can lead to a variety of mental disorders. Due to the prolonged Lockdown as a countermeasure against coronavirus, there has been a disruption in the lifestyle of people which may increase the emotional distress amongst people and can result in developing psychological distress (mental illness) even amongst those healthy individuals with no prior medical history (Zhang and Ma, 2020)[6]. It has been said that people end up suffering more mentally than the number of people who are physically injured after such an outbreak. The psychological wellbeing is affected immensely and may last for a longer duration. (Allsopp et al., 2019) [7].

Psychology is extremely relevant in understanding how people cope or react in such times. The experience of the COVID-19 is changing our lives. The disruptions faced in such unfamiliar living situations – work, financial worries and relationships. Change in lifestyle has led to **vexation** in our relationships with lack of physical meeting or touch and at the same time the trauma of the virus has created frustration, **immediate concern** about the lockdown, the idea of panic buying being prevalent, **routine disruption** coming to our lives lack social meetings or any daily activity be it work or school, overall facing uncertainty about the future not knowing the timeline of this virus which is complexed, the exceptional circumstances have created an ambiguity and major procrastinations among individuals.

The COVID 19 pandemic has caused distress in many people's lives and left many people vulnerable to mental health issues and has led to suicidal behavior. Mental health consequences are likely to be present for longer and peak later than the actual pandemic. This paper talks about the sensitive topics - depression, anxiety and stress among youth. Crippling bouts of COVID-19 began to take over the youth's mind, leading to mental stress. This is the study of negative mental health outcomes (depression, anxiety, and stress) and psychological distress (VIRUS stressors). The question here arises that during this lockdown how seriously these negative health outcomes affected the respondent. Negative outcomes include impaired performance (unproductive feel), poor quality of life, the uncertainty of the future, fear all around due to increasing cases of COVID, and others.

Research Objectives:

1. To study the level of stress, anxiety and depression in the Indian environment due to Covid- 19.
2. To Study the Factors affecting the mental health
3. To study the psychological stress (unpleasant feeling or emotions that have an impact on the level of functioning/negative views or perception we are attached toCOVID-19)

Review of the Literature: Outbreaks of diseases and pandemics have been found to create distress and have shown major mental illness (Bao et Al 2020) [8]. There is the future scope for research and exploration with a clinical scope. Pandemics and outbreaks are catalysts to the global mortality rate (Verikios2016) [9]. Various factors including gender, explicit experience with COVID19 infection, Isolation, and exposure to media can make one vulnerable to Mental illness and can cause psychological distress (Li et al., 2020) [5].

Chinese individuals' psychological wellbeing has fallen apart, a higher prevalence rate of depression (50.7%), Sleep-deprived (36.1%), anxiety (44.7%), and stress-related problems (73.4%). Lack of information related to COVID 19 led to these higher rates of mental health issues in China. Generally, at the beginning phases of a pandemic, individuals have little data about the nature of the disease, treatment, casualty rate, and so forth fueling the fear (Xiang et al.2020)[10]. Isolation can induce fatigue, outrage, nervousness, loneliness, boredom, and depression. Economic damage and widespread lockdown led to much psychological distress amongst people. Added to this is the plenty of falsehood information circulating on the internet and social networking media that led to a lot of uneasiness. Circulating this misinformation can contribute tot he spread of illness and that is very much common for COVID-19.

Although there is no current evidence as to the direct effects of SARS-COV-2 on mental wellbeing, there has been a significant increase in psychological distress such as anxiety and depression along with more negative impacts on the health of the general public (Vindegaard

and Eriksen Benros, 2020)[11].In addition, it causes severe psychological anxiety (Chen et al., 2020)[12], which could adversely affect the quality of life and can influence individuals working and social functioning (Monson, Caron, McCloskey, and Brunet, 2017)[13]. As specialists all over the world work hard to find effective vaccines and antibodies against COVID-19, the mental effect of the disease is to a great extentdisregarded.

Pandemics like these do not only affect physical wellbeing; they in general influence the quality of living not only on an individual level but as a whole; causing widespread panic, xenophobia, social dysfunction, and social stigma. The first suicidal case in India of a 50-year-old man was reported dead fearing that he had contracted coronavirus. So, he locked his family and killed himself (Goyal, Chauhan, Chhikara, Gupta & Singh, 2020)[14] As the worldwide prevalence increased, people started hoarding medical supplies and basic necessity which caused havoc in many countries, people started isolating themselves due to the fear of the COVID and restricted their social interaction to minimal, People got health conscious but it was so anxiety-driven that even a mild condition like cold which is just one of the symptoms of the illness led to an increase in psychological distress (Duan and Zhu, 2020)([15] When contrasted with before the outbreak situation, information is still scarce on the effect of mental wellness in the general population (Sonderskovetal., 2020)[16]. Researchers have found no major distinction in anxiety, depression, and stress level sin the period with numerous new cases compared with the period of many recovering (Wang et al., 2020)([1]. Long-lasting impacts of COVID 19 should be expected, it is known from the earlier experience of SARS-CoV-1, that those who were affected by it (for example; due to isolation) had mental side effects a long time (several months) even after the controlover the epidemic.

At any point when a country is struck by large-scale and deadly disasters of different types, the quality of psychological well-being is affected, and various issues related to mental health can emerge which can differ across different periods. Any significant outbreak will negatively affect people and society. Terrorist attacks like one at the Pentagon and anthrax attacks demonstrated how important it is to re-establish the resources and mobilize them in order to respond quickly to the disaster-related mental health needs of the affected individual (Dodgen, LaDue and Kaul, 2002)[17] When the SARS plague hit, mental health was affected not exclusively due to the working condition of the workplace but also due to the thoughts of close ones getting affected by the virus which generated psychological trauma (Wu et al., 2009)[18] Most COVID-19 patients experienced PTSD (Post-traumatic stress disorder) before even leaving the hospital, and these side effects may prompt negative results in their day to day life after COVID for example, lower personal satisfaction and performance of work. Following the episode SARS in 2003, the predominance of Post-traumatic stress disorder (PTSD) in SARS survivors was 9.79% in their initial recovery stage (Fang, Zhe, andShuran, 2004)[19] and Post-SARS assessment (approximately after 30 months) it was 25.6%. (Mak, Chu, Pan, Yiu, and Chan, 2009)[20]

One survey showed that respondents aged 12-21 had severe impacts on their mental health due to the prolonged lockdown. This age bunch mostly consisted of school students and undergraduates who were affected because of the school closure, uncertainty about their exams, and making arrangements for online education. Young individuals have had their education and career interrupted and are anxious about the future. UGC has given a few rules to Higher Education Institutes, to deal with the Mental Health, Psychosocial concerns and Well Being of Students Community during and after COVID-19 Pandemic. Loss of work and monetary stressors are recognized as the major factors which can lead to suicide. (Stuckler, Basu, Suhrcke, Couttsand McKee, 2009) [21] Studies have shown that adults who were not working reported worse health. Studies have shown a high and perhaps even alarming prevalence of depressive symptoms in both developed and developing countries (Al-faris et al., 2012) [22]. It is demonstrated through a study that 3% to 9% of adolescents are found to be depressed anytime, and 20% of them report a lifetime pervasiveness of depression toward the end of the adolescence (Zuckerbrot and Jensen, 2006) [23]. Malaysian report uncovered that nearly 1 out of 5 Malaysian adolescence is found to be depressed (Kaur et al., 2014) [24]. Teenagers who felt lonely more often than others or continually felt alone over a period of 1 year are 3 times more likely to be characterized as depressed by the DASS21 assessment and that social isolation was one of the major reasons for depression in 11-to 14-year-olds. Longer duration of depression and more frequent episodes can lead to poor work performance, poor coping skills, and various negative traits such as low self-esteem and low positivity. It can also affect your relationships with your loved ones (Pettit, Lewinsohn, Roberts, Seeley, and Monteith, 2008) [25]. 7 out of 10

US adults experience anxiety/stress at a moderate level every day, as indicated by an ongoing report led by the Anxietyand Depression Association of America. Whiles tress is considered to bean inevitable part of life, it is present and very much becoming prevalent in students (Gallagher, 2008; Mackenzieetal., 2011).[26][27] Younger peopled is playa high risk of psychological problems (Huang & Zhao, 2020)[28].

Mental health professionals should be advised to instruct people about the psychological consequences, they should promote healthy mental behaviors. People should be encouraged to reduce their exposure to negative information. And in order to maintain social distancing people should use more virtual networks (Banerjee, 2020) [29]. Furthermore, the need of the hour is to create mental health interventions that can be communicated to general health care workers and volunteers and these strategies can be spread amongst workers (*Rajkumar*, 2020)[30]. Psychological distresses should be identified at the initial stage for allowing the efficient implementation of interventional strategies.

Research Methodology: Research Goal: The goal of the present study is to evaluate the potential sources of depression, anxiety, and stress correlating with the stressors of VIRUS (vexation, immediate concern, routine disruption, and uncertainty about future).

These were addressed by surveying a sample with a mix of young adults and working professionals (age group 18-28 yrs) from varied sources using the DASS 21 scale with VIRUS stressors.

Data Collection: The primary method of data collection was used, an online questionnaire was distributed. More than 350 people participated.

Given the established measure of the DASS21 Lovibondversion of it, the survey consisted of a demographics section, a list of stressors (VIRUS), and 21 questions.

The first portion of the survey constituted the most inevitable part of life, stressors that were pertinent in the times of a pandemic Covid-19. The responses were recorded on a Likert scale (0-3). It was estimated individuals would exhibit increased levels of those stressors.

DASS21 measured the prevalence of the symptoms on a 4-point Likert scale 0-3. A score of 0 indicated that the item "did not apply to them," and a score of 3 meant that the participant considered the question to apply "very much, or most of the time" (Gomez, n.d.).

Virus Stressors:

Vexation: Feeling imprisoned at home, relationship with loved ones and being unproductive

Immediate concern: Feeling afraid if something awful might happen, an increasing number of Covid-19 cases, and changed lifestyle

Routine disruption: Spending more time on gadgets for media consumption, learning new courses or exploring E-platforms

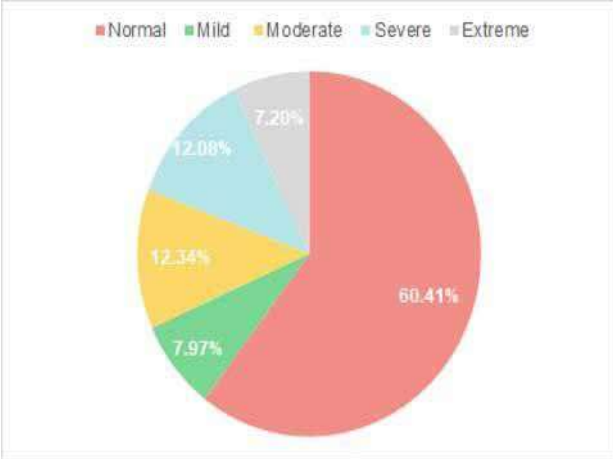
Uncertainty about future: Academics and graduation timeline, market disruptions, and financial challenges

Data Analysis method: After the data were collected and inserted in SPSS software, they were analyzed descriptive statistics (mean and standard deviation) and inferential statistics (independent t-test and analysis).

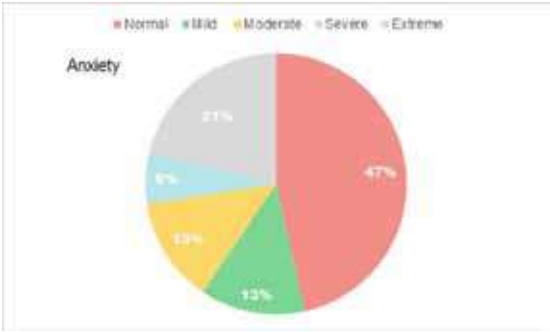
Data Analysis: The Findings suggest a correlation disclosed that VIRUS stressor sf or each aspect had a significant positive correlation.

DASS 21-The tallies of DASS 21 positions each partaker's depression, anxiety, and stress levels, assorting each area as either "normal," "mild," "moderate," "severe," or "extremely severe." Out of those analyzed,60.41% accounted for symptoms of severe levels of stress,47% signaled severe anxiety, and 44% severe depression.

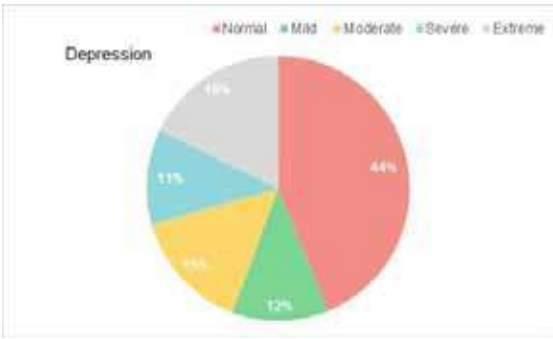
A. Stress



B. Anxiety



C. Depression



Our sample was able to filter out top 7 sources of concern, based on the DASS moderate and extreme cases were Uncertainty about Future (Market disruptions & Financial challenges), Routine disruption (Spending more time on gadgets for media consumption, Sleep-deprived al the time & Learning new courses), Immediate Concern (Increasing cases of COVID 19) and Being Unproductive.

A succession of ANOVAs with VIRUS as an independent factor and the DASS-21 scales as the dependent factors showed a significant effect of the scales, including:

Table 1: Stress and Virus (R2 =0.357, F=17.16, P < 0.01)

Stress	Coefficients
Feeling imprisoned at home	0.730
Being Unproductive	0.853
Spending more time on gadgets for media consumption	0.884

Table 1 speaks about the body's response to the circumstance where we feel imprisoned at home threatens the feeling of self. Individuals are vulnerable to widespread concern and are feeling more lonely. Stress is researched to be beneficial for being productive, the unproductive stress is making individuals overwhelmed. There is a lack of accomplishment in these times. Major setback being exposure to gadgets, there is a growing addiction to technology and an overdose to media.

Table 2: Depression and Virus (R2=0.384,F=19.29, P < 0.01),

Depression	Coefficients
Feeling imprisoned at home	0.672
Changed Lifestyle	1.061

Table 2 gives data on Mental health, that has brought about a status among individuals hence they feel worthless and trapped at home, this feeling has affected their outlook towards life. Individuals are not able to accept the change and take a toll on their mental health.

Table 3: Anxiety and Virus (r2=0.304, F= 13.515, P < 0.01)

Anxiety	Coefficients
Changed Lifestyle	0.851
Market Depression	0.882

Post regression analysis in Table 3 we observe with Anxiety and VIRUS factors show that economic fissures have created uncertainty about the market conditions among individuals. The possibility of job loss, salary cut, and the steps taken in the macro environment have created a feeling of anxiety.

Obsessive thoughts regarding the COVID are giving headaches and other common symptoms. These negative thoughts have created an irritable environment causing mental fatigue.

Table 4: Correlation

Variables	Depression	Anxiety	Stress
Relationship with loved ones	.006	.32	.22
Changed lifestyle	.01	.45	.07
Market disruptions	.040	.114	.203
Learning new courses or exploring E- platforms	.282	.975	.179

Table 4 presents Correlations between the DASS-21 scales and VIRUS as variables Referring to Table 1 using correlation the sig. value is .000, which is less than the alpha values 0.01. Therefore, thereexists a significant relationship between feeling imprisoned at home, being unproductive, feeling afraid if something

awful might happen, changed lifestyle, sleep-deprived at all the time, academics, relationship with loved ones, increasing number of Covid-19 cases, spending more time on gadgets, graduation timeline, financial challenges, learning or exploring new courses, and market disruptions with DASS.

Discussion and Conclusion

Our emotional resources are used up in trying to cope with challenging situations. We currently have two viruses to tackle, one being COVID, and other which we all are infected in some way or another when it comes to COVID VIRUS:

Vexation due to imprisonment at home, families are suffering relationship turmoil's and the morale is low due to the unproductive lifestyle. **Immediate concern** has created a mental health crisis, there is a feeling of helplessness and uncertainty, the increasing number of Covid-19 cases has added to manage this fear of the unknown and the changes in lifestyle such as norms of distancing and a not-so-luxurious life. **Routine disruption**, the fight against this virus is long, spending more time on gadgets for media consumption, giving time to self- improvement as learning new courses, or exploring E-platforms. **Uncertainty about the future,** be it economic uncertainty, academics and graduation timeline, market disruptions, and financial challenges.

DASS-21 is the assessment and clinically validated screening instrument of depression, anxiety, and stress among youth. This scale assesses the person's perception of life and interest in certain things. In COVID-19 times, we tried to assess the mental health among youth. This study evaluates the measures of depression, anxiety, and stress in young adults with the factors of concern for our sample, based on Covid-19 VIRUS correlating with depression, anxiety and stress were Vexation or Frustration (feeling imprisoned at home, being unproductive), Immediate concern (feeling afraid of the dire future, increasing number of coronavirus cases), Routine Disruption (spending more time on gadgets, sleep-deprived), & Uncertainty about future (academic and graduation timeline, financial challenges). There is a significant relationship between VIRUS and DAS (depression, anxiety, and stress).

The preliminary objective of this paper was to confine potential sources of depression, anxiety, and stress, respectively each item specified in the study had a significant positive correlation with scores of depression, anxiety, and stress. Out of the 4 parameters of VIRUS, vexation, or frustration (being imprisoned, and unproductive), evaluate the gravity of the symptoms of depression, anxiety, and stress. This paper included 12 factors under these parameters of VIRUS. There exists a negative impact on mental health problems due to spending too much

time on gadgets and sleep-deprived. Financial difficulties and academic concerns (future uncertainty) correlate with extreme levels of these mental health problems. The negative perception of fear of awful happenings and an increasing number of cases of coronavirus, causing an increase in depression, anxiety, and stress.

Present results coincide with previous research that shows multiplied incidences of mental health problems in times of a pandemic. Publications have taken up the psychological brunt of the virus on the masses, established on information from previous disease outbreaks and papers (Zandifar and Badrfam, 2020)[31] that show social isolation contributes towards mental illness. (Shigemura et al., 2020)[32]Showing the fiscal impact of the virus and mental health.

This paper is able to add the importance and role of educating the masses about the psychological effects of any pandemic, this will evolve the mindset and bring about the survival instinct to find strategies to handle the present-day crisis. We have analyzed the negative screens of DAS responsible to affect mental stability among youth. Further exploration can be piloted in this area to understand the Interventional strategies of post-COVID (In business and normal lifestyle).

Ethical Clearance: Not required (specific to management domain)

Source of Funding: Self

Conflict of Interest: Nil

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Effect of Vestibular Stimulation on Postural Stability and Mobility in Elderly

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Abstract

The Galvanic vestibular stimulation (GVS)is proven to be a promising tool to improve vestibular functioning. Decline in vestibular functioning in the elderly results in decreased capacity to identify weak signal which may lead to postural instability. Postural sway is produced when GVS is given over mastoid process. In this study our aim is to determine whether GVS can be utilized to maximize the effect of balance training programme among elderly individuals. Ambulatory community dwelling elderly (N=150, age 65.67±3.4 yrs) were randomly assigned to a control group (Group A, n=50, age 65.54±3.4 yrs), Placebo group (Group B, n=50, age 65.5±3.3 yrs) and an experimental group (Group C, n=50, age 65.98±3.5 yrs). Group A was not given any intervention while group B was given placebo stimulation along with balance training and group C was given noisy galvanic vestibular stimulation along with balance training program. Pre, mid and post data were recorded for overall stability index (OSI) (Biodex Balance Master) and Timed up and gotest (TUG) and analyzed. Compared to control group there was a significant improvement in overall stability and mobility in placebo and experimental groups. Significant difference was observed in improvement in experimental group compared with placebo group for both,OSI and TUG. Early improvement in stability and mobility was also observed in experimental group but similar results were not obtained in control and placebo groups. The outcome of this study suggests that GVS can be utilized therapeutically to optimize the efficacy of balance training clinically.

Keywords: *Fall, Postural Stability, Mobility, Vestibular Stimulation.*

Introduction

Older individuals constitute over 7.4% of the total population of India, and the elderly are a rapidly growing demographic.[1] Falls and fall associated secondary injuries in elderly are a serious concern, and a foremost cause of mortality and morbidity in them.[2] About one-third of community-dwelling elderly have a tendency to incur a falls, and 50% of those who fall have recurrent falls.[3–5] The Fall Mortality Rate is 2.1 in India.[16] Multiple factors contributing to fall risk, including physical, environmental, and behavioral have been identified. The role of the vestibular system for maintenance of upright posture is well understood. Age associated changes such as loss of muscle girth, loss of vestibular hair cells, degenerative musculoskeletal transformation or the onset of chronic diseases and their consequences are closely related with reduction in static and dynamic balance, and likely to cause falls.[7–9] Early identification of vestibular deficits and instability, with appropriate intervention may help in reducing the incidence of falls and associated risk.

Balance training exercises are designed to improve postural stability, mobility, and activities of daily living. Balance programme includes exercises helps to

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control equilibrium. Balance exercises are repetitive and progressed gradually to help to improve balance by reducing postural sway and irrelevant motor responses.[10] The maintenance of upright posture involves complex sensory systems including the vestibular system. Vestibular system is the main processing unit to maintain postural stability. As the age progresses, reduction in function of the vestibular system is well identified. Physical therapy is an established treatment for vestibular system dysfunction.[11]. However recent studies on noisy Galvanic Vestibular Stimulation (nGVS) are proven to be a promising treatment. During Galvanic Vestibular Stimulation, a small electrical current is delivered through surface electrodes over the mastoid process.[12] This transcutaneous electric current activates and induces continuous firing of the vestibular afferents.[13] In response to GVS, the capacity to identify a weak signal or to process incoming sensory information may be enhanced by adding white noise.[14]

Stimulating an individual's vestibular afferents during standing causes leaning in different directions depending upon the polarity of the electrodes.[13] Induction of a change in the electrical excitability of the vestibular nerves stimulates the vestibular nuclei and networks of the right hemisphere when the anode is attached to the left mastoid and the cathode on the right (known as LGVS), while the reverse electrode positioning (RGVS) leads to a bilateral activation.[15] Specifically, anodal and cathodal currents can be used to artificially decrease and increase the firing rates of the vestibular afferents. A standing subject tends to sway towards the anodal stimulus and/or away from the cathodal stimulus.[16] Another study shows that the sway response increases in direct proportion with the stimulus intensity when constant galvanic current is given.[17] Bilateral activation of vestibular cortices is obtained by applying left-cathodal/right-anodal GVS.[18]

Previous studies show that M1 a-transcranial direct current stimulation along with treadmill based gait training reduces the anterior posterior and medial lateral sway that significantly improved static balance along with functional performance in cerebral palsy children.[19] GVS when delivered to adults in quite-standing with open eyes, results in reduction in sway length and sway velocity.[20,21] It also produces similar results in bilateral vestibulopathy during quite-standing with closed eyes.[22,23]

In another study stochastic vestibular stimulation during walking resulted in increase in the gait speed and changes in stride length in healthy adults.[24,25] Sensitivity of the vestibular system to normal vestibular inputs can be increased by adding noise to subthreshold stochastic vestibular stimulation. This increase in the sensitivity is important to postural stability in more challenging situations. Studies suggested that noisy vestibular stimulation alters visuomotor activity and motor circuit functioning in Parkinsonism. This sensorimotor integration and performance might be associated to change in oscillation related to processing of information and error.[26]It has been shown that more erect posture is maintained after mechanical perturbation when appropriate galvanic current is given over the mastoid process.[27]

Thus the objective of this study was to determine the effect of noisy Galvanic vestibular stimulation on postural stability and mobility among elderly by performing a randomized control trialand to determine whether GVS can be utilized to maximize the effect of balance training programme among elderly individuals.

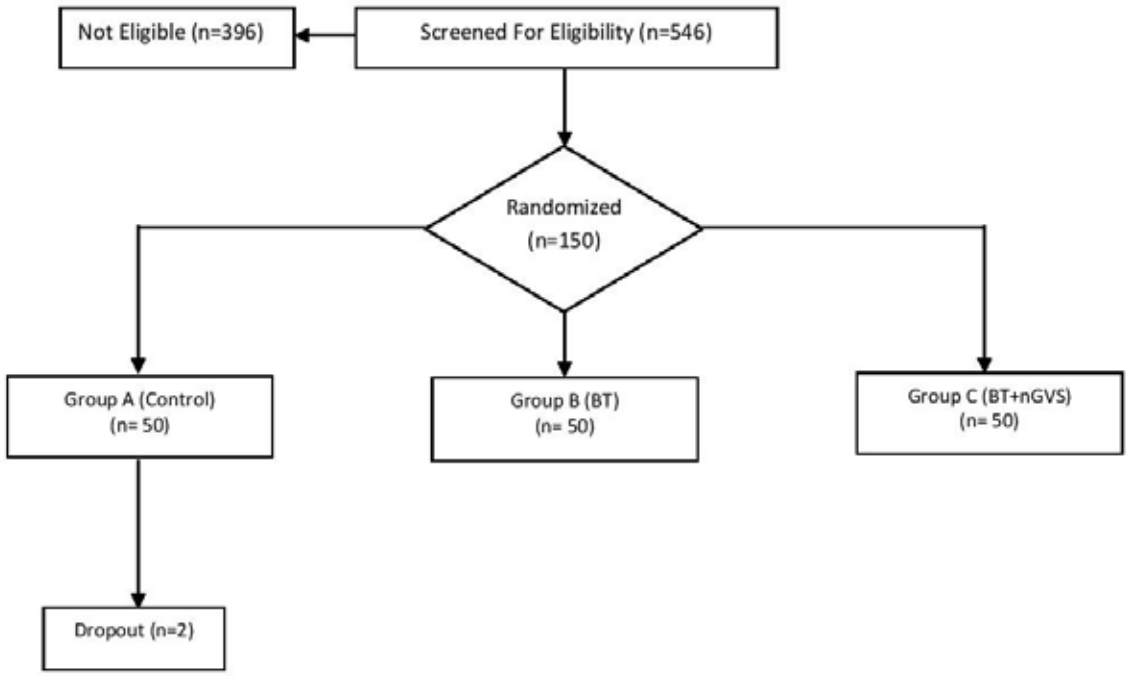
Method

This study employed a repeated measure design and a randomized controlled trial. A total of 150 participants were recruited from various Physiotherapy Centers in Noida, India and randomly allocated to three groups.

Inclusion criteria included individuals of both gender with age between (60 – 75) years, can walk in the community independently, able to perform the balance tests without assistive device and able to participate in a series of balance classes. All participants provided with patient information sheet.

Our study excluded subjects with the history of, any orthopedic surgery in the lower limbs, psychoactive medication in past six months, persons with progressive neurologic disorder that could affect balance and gait, orthostatic hypotension, unstable medical condition, diabetes for more than five years, vision less than 6/6 in either eye (even if corrected),fall in past 6 months and patient with high risk of fall (Tinetti<19)

Figure 1: Recruitment of study participants



A total of 546 volunteers were initially screened for the study out of which 150 who met the inclusion criteria were included in the study. Participants were randomly assigned into three groups after being fully informed about the nature of the research and taking written informed consent. (Fig 1) The first group was the control group A that included 50 subjects, 48 subjects (2 dropouts) were retested at the interval of 3rd and 6th weeks without any intervention.

50 subjects were allocated to Placebo group B who received placebo stimulation along with balance training and the remaining 50 subjects were allocated to the Treatment Group C who received noisy Galvanic Vestibular stimulation (nGVS) along with balance training.

A structured interview was conducted to collect socio demographic information including age and gender. Medical history was obtained by a physician who also recorded the presence of any symptoms associated with balance dysfunction.

All the study participants after group allocation were assessed pre, mid and post for postural stability on the Biodex Balance System and Timed Up and Go. Three trials of 20 seconds each were conducted for postural stability and mean of which was considered the final reading (as recommended in the manual described by the developers of the tool). Interval of one minute was given between each trial. Subjects sway over a fixed plate was monitored to calculate Overall Stability Index (OSI). During each trial participants were instructed to remain standing without support with eyes open. Value of OSI depends upon the rate of oscillation of the platform, lower values indicate good stability as lesser the oscillation lower will be degree of sway. For TUG every participant was given first practice trial which was not timed and then mean of three trials were considered as final value.

The intervention given in group B and C included a sixweek program of active stretching, postural control, endurance walking, and repetitive muscle coordination exercises. The actual frequency, repetitions, and resistance intensity of the exercises were adjusted according to individual ratings of perceived exertion.[28] During first week, subjects were made to exercise at a fairly low level (equivalent to 11 on the 16-point Borg ratings of perceived exertion scale). In follow up training, exercise at a moderate level of intensity (equivalent to 13 on the ratings of perceived exertion scale) were given. Balance exercises were given thrice a week for 6 weeks.

Group C Participants additionally received bi-polar binaural (left-cathodal/right-anodal) noisy Galvanic

Vestibular Stimulation of subliminal intensity for duration of 20 minutes during postural, dynamic stability and coordination exercises.

The tap water was used as a medium for lint cloth over the electrodes (60mm_40mm) and fastened with the elastic band over each mastoid to stimulate the vestibular afferents.

Statistical Analysis: All statistical analysis for Time-up and go procedure and Stability index parameters were performed with SAS 9.4 version software. Normality assumptions were tested using the Shapiro-Wilk test. All individual data parameters were tested for normality, and test variables followed a multivariate normal distribution in the population (as per the assumptions of Repeated measures ANOVA (RMA), as n>=25). The model considered here was based on an interaction effect between the treatment group and visits for between and within groups and for Least square mean estimates based on individual visits. Tukey's-Kramer method was used for adjusting p-values for multiple pair-wise comparisons for post-hoc analysis.

Summary statistics for demographics presented was based on the descriptive statistics N, Mean and standard deviation with three quartiles (Q1, Q2 and Q3) with minimum and maximum values. All statistical analysis was performed within 95% confidence interval,with alpha fixed at 5% level of significance for acceptance.

Results

All Male and Female subjects considered for this study were clustered between 60 to 74 years of age. The Mean & SD of the demographic data was comparable in the three groups. The average age was 65.5 and there were 47% females and 53% Males in the study. The average BMI was 26.44 (1.685), 26.01 (1.319), 25.99 (1.321) for Control, placebo and Treatment groups.

Time-Up and Go Test scores: The Least squares Mean values for Time-up and go (TUG) test showed a decline from Day-1 to Day-42 in both Treatment and Placebo groups compared to control group, suggesting an improvement from baseline to the end visit. There was a significant decrease in the TUG Score in treatment group **(0.604±0.017)**and in placebo group **(0.424±0.023)** at Day 42 compared to Day-1 ($p<0.0001$) and the post-hoc pair wise comparison test was statistically significant ($p<0.0001$).

Table 1: Pre, Mid and Post by shift difference - within Group – Time Up and Go Test

	Estimate	SE	95% CI	P-value
Treatment With Group Difference				
Pre-Mid vs. Mid-Post Difference	-0.100	0.016	[-0.13;-0.07]	<0.0001
Mid-Post vs. Pre-Post Difference	-0.352	0.019	[-0.39;-0.32]	<0.0001
Pre-Mid vs. Pre-Post Difference	-0.252	0.016	[-0.28;-0.22]	<0.0001
Placebo With Group Difference				
Pre-Mid vs. Mid-Post Difference	-0.200	0.016	[-0.23;-0.17]	<0.0001
Mid-Post vs. Pre-Post Difference	-0.312	0.020	[-0.35;-0.27]	<0.0001
Pre-Mid vs. Pre-Post Difference	-0.112	0.016	[-0.14;-0.08]	<0.0001
Control With Group Difference				
Pre-Mid vs. Mid-Post Difference	0.015	0.012	[-0.01; 0.04]	0.2222
Mid-Post vs. Pre-Post Difference	0.010	0.013	[-0.02; 0.04]	0.7262
Pre-Mid vs. Pre-Post Difference	-0.004	0.012	[-0.03; 0.02]	0.4226

A table (Table 1) shows the evaluation of Time-up and go test for the three treatment groups, shift difference between visits. The Pre-Post Difference is high in Treatment group compared to Placebo and control groups. The pairwise comparisons between the three shift difference tests is statistically significant, for within group shift difference from Baseline to stimulated points for Treatment and Placebo groups ($P<0.001$).

Both the Treatment and Placebo groups show shift difference more in "Mid-Post vs. Pre-Post Difference" and Treatment group is more when compared to placebo. The shift in the within group difference among these shift pairs shows statistical significance in both Treatment and Placebo groups.

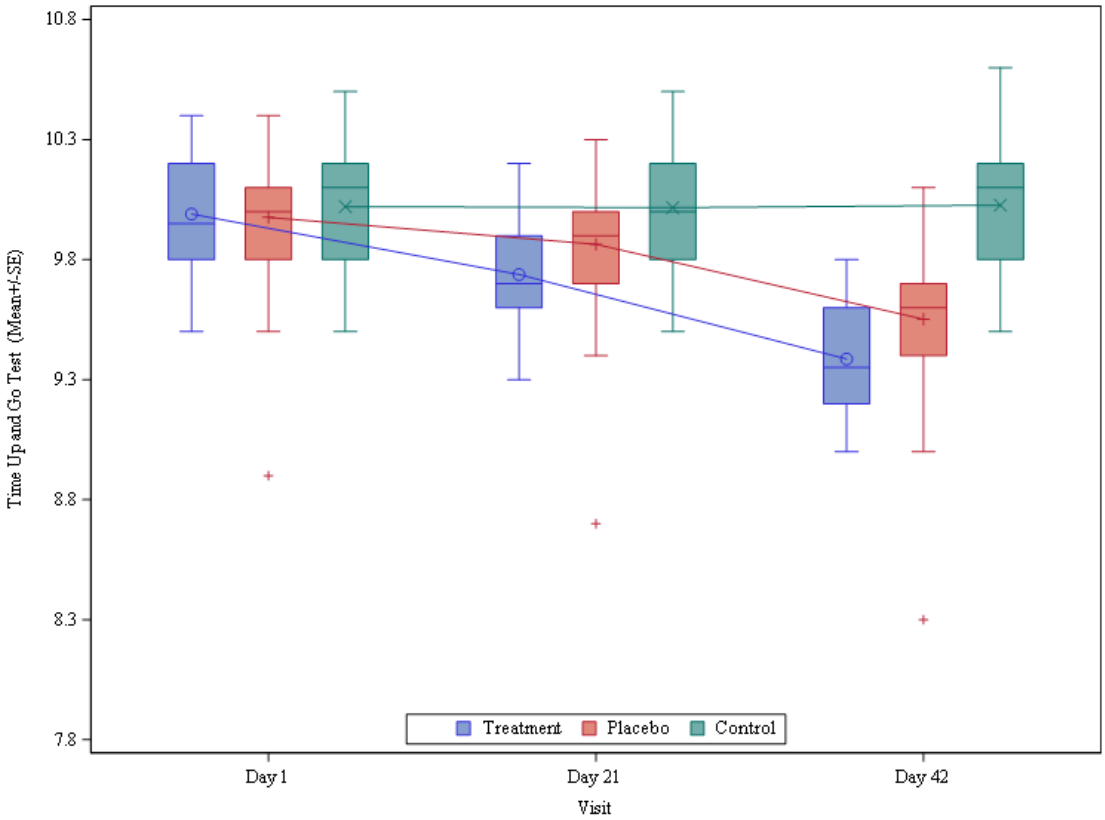


Figure 2: Box plot – By groups across the visits – Time Up and Go Test

A box plot presented in the Figure 2 compared the range and distribution of the Time up and Go Test at baseline (Day 1) and stimulated points (day 21 & Day 42). We observe that there is a greater variability for Treatment group and over time there is decrease at Day 21 and a visible decrease from mid-point to end of study in Treatment group compared to placebo and there is slight shift in increase in control group. The placebo group also has some outliers existing at all the three time points. Since the notches in the boxplots do not overlap, one can conclude with 95% confidence that the true medians do differ.

Table 2: Between the groups shift difference–Treatment estimates–Time Up and Go Test

	Estimate	SE	95% CI	P-value
Between Treatment group effects				
Treatment Pre-Mid vs Placebo Pre-Mid	0.140	0.021	[0.10; 0.18]	<0.0001
Treatment Pre-Mid vs Control Pre-Mid	0.247	0.021	[0.21; 0.29]	<0.0001
Placebo Pre-Mid vs Control Pre-Mid	0.107	0.021	[0.07; 0.15]	<0.0001
Treatment Mid-Post vs Placebo Mid-Post	0.040	0.021	[-0.00; 0.08]	0.0547
Treatment Mid-Post vs Control Mid-Post	0.362	0.022	[0.32; 0.40]	<0.0001
Placebo Mid-Post vs Control Mid-Post	0.322	0.021	[0.28; 0.36]	<0.0001
Treatment Pre-Post vs Placebo Pre-Post	0.180	0.021	[0.14; 0.22]	<0.0001
Treatment Pre-Post vs Control Pre-Post	0.610	0.022	[0.57; 0.65]	<0.0001
Placebo Pre-Post vs Control Pre-Post	0.430	0.021	[0.39; 0.47]	<0.0001

We also intend to identify the shift pairs between the treatment groups in Table 2, and in this table for "Treatment Mid-Post vs Placebo Mid-Post" had an Estimate difference of 0.040 with 0.021 Standard error with [0.00, 0.08] 95% confidence limits. This doesn't show statistical significance (P>0.005) and can conclude that these two shift difference groups show some significant difference. The other pair difference shows statistical significance and thus conclude that there is no significant difference between the considered paired shift differences and thus it justifies the previous table that the difference from Mid to post (Day 21- Day 42) for both Treatment and Placebo are tending near to same, with maximum difference between the stimulated points

Stability Index: Likewise, for TUG score, the Least squares Mean values for overall stability index (OSI) test showed a decline from Day-1 to Day-42 in both Treatment and Placebo groups, suggesting an improvement from baseline to end visit. There was a significant decrease in the Stability Index scorein treatment group **(0.604±0.017)** and in placebo group **(0.424±0.023)**at Day 42 compared to Day-1 (p<0.0001) and the post-hoc pair wise comparison test also project a statistical significance (p<0.0001).

Table 3: Pre, Mid and Post by shift difference - within Group - Stability Index

	Estimate	SE	95% CI	P-value
Treatment with Group Difference				
Pre-Mid vs. Mid-Post Difference	-0.100	0.016	[-0.13;-0.07]	<0.0001
Mid-Post vs. Pre-Post Difference	-0.352	0.019	[-0.39;-0.32]	<0.0001
Pre-Mid vs. Pre-Post Difference	-0.252	0.016	[-0.28;-0.22]	<0.0001
Placebo with Group Difference				
Pre-Mid vs. Mid-Post Difference	-0.196	0.016	[-0.23;-0.16]	<0.0001
Mid-Post vs. Pre-Post Difference	-0.308	0.021	[-0.35;-0.27]	<0.0001
Pre-Mid vs. Pre-Post Difference	-0.112	0.016	[-0.14;-0.08]	<0.0001
Control with Group Difference				
Pre-Mid vs. Mid-Post Difference	0.010	0.012	[-0.01; 0.03]	0.3894
Mid-Post vs. Pre-Post Difference	0.008	0.013	[-0.02; 0.03]	0.8631
Pre-Mid vs. Pre-Post Difference	-0.002	0.012	[-0.03; 0.02]	0.5238

Table 3 presented, to understand the pairwise comparisons between the three-shift difference and three groups been identified among shift points and groups for Stability Index score. The "pre-post" difference shows more difference among the other paired combinations in both Treatment and placebo groups.

Box plot in Figure 3 is plotted for stability index scores to compare the range and distribution of the Index score at baseline (Day 1) and stimulated points (Day 21 & Day 42). We observe that there is a greater variability for Treatment group and over time there is decrease at Day 21 and a visible decrease from mid-point to end of study in Treatment group compare to placebo and there is slight shift in increase in control group.

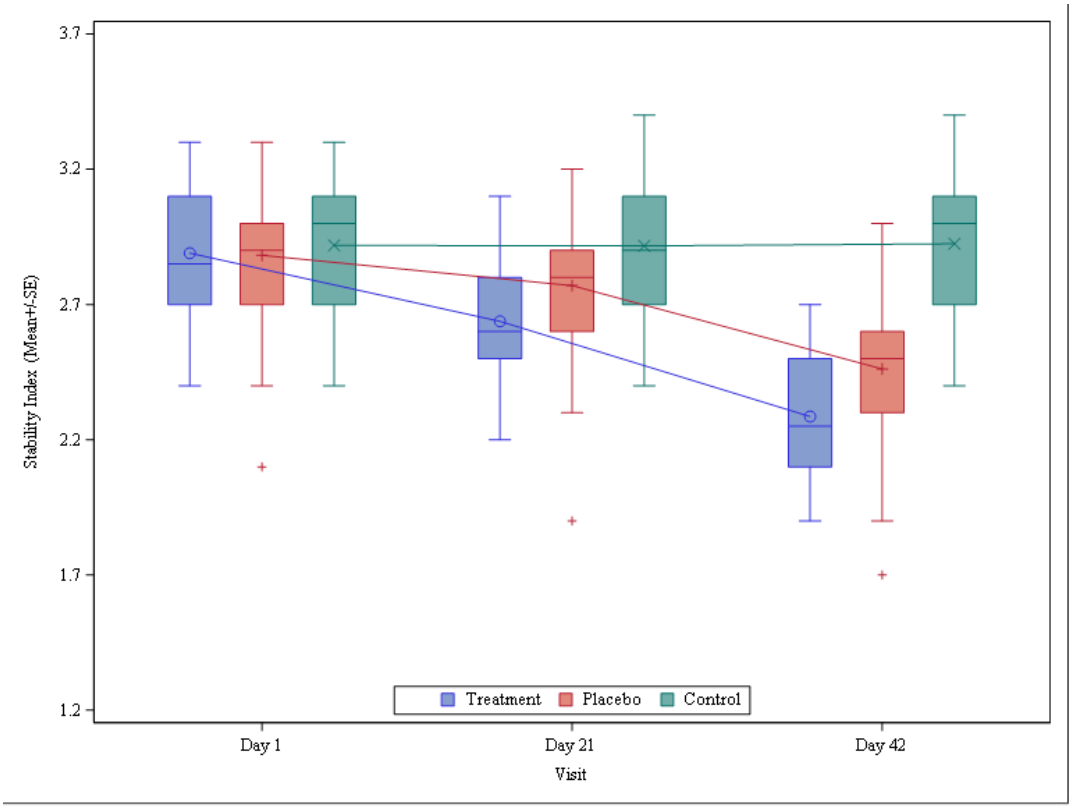


Figure 3 : Box plot – By groups across the visits – Stability Index

Table 4: Between the groups shift difference – Treatment estimates – Stability Index

	Estimate	SE	95% CI	P-value
Treatment Pre-Mid vs Placebo Pre-Mid	0.140	0.021	[0.10; 0.18]	<0.0001
Treatment Pre-Mid vs Control Pre-Mid	0.249	0.021	[0.21; 0.29]	<0.0001
Placebo Pre-Mid vs Control Pre-Mid	0.109	0.021	[0.07; 0.15]	<0.0001
Treatment Mid-Post vs Placebo Mid-Post	0.044	0.021	[0.00; 0.08]	0.0345
Treatment Mid-Post vs Control Mid-Post	0.360	0.021	[0.32; 0.40]	<0.0001
Placebo Mid-Post vs Control Mid-Post	0.316	0.021	[0.27; 0.36]	<0.0001
Treatment Pre-Post vs Placebo Pre-Post	0.184	0.021	[0.14; 0.22]	<0.0001
Treatment Pre-Post vs Control Pre-Post	0.610	0.021	[0.57; 0.65]	<0.0001
Placebo Pre-Post vs Control Pre-Post	0.426	0.021	[0.38; 0.47]	<0.0001

Table 4 shows the shift pairs between the treatment groups for "Treatment Mid-Post vs Placebo Mid-Post" had an Estimate difference of 0.044 with 0.021 Standard error with [0.00, 0.08] 95% confidence limits. This doesn't show statistical significance (P>0.005) and can conclude that these two shift difference groups show some significant difference. For stability score, the actual shift of improvement started from Mid (Day21) till Post(Day 42) for both Treatment and Placebo groups. "Placebo Pre-Mid vs Control Pre-Mid" had an Estimate difference of 0.109 which is slightly better than the "Treatment Pre-Mid vs Placebo Pre-Mid" also have estimated difference 0.140, however these are statistically significant and indicates that there is no significant difference between these two groups.

Discussion

In this study we evaluated the effect of vestibular stimulation on postural stability and mobility in the

elderly randomized into experimental, control and placebo groups. We measured change in postural stability with overall stability index (OSI) and change in mobility with TUG. Significant improvement was observed in overall stability and mobility in the placebo group after the placebo stimulation and balance training while there was no difference in the control group. It was also observed that in the treatment group postural stability and mobility was significantly improved after the vestibular stimulation and balance training while there was no difference in the control group. TUG and OSI indices both were significantly improved in placebo and experimental group. There was a significant difference in improvement between placebo and experimental group which indicates the usefulness of vestibular stimulation along with exercises.

Similar studies show that when direct current is given to vestibular afferents, it passes through vestibular nucleus and vestibular thalamus resulting in added activation of brain areas linked with multisensory input.[15] This could be the underlying mechanism for enhancement in the function of vestibular afferents which results in improvement in postural stability.[29]

Findings of this study suggest that GVS can be used during balance training which help the elderly to gain better control over postural stability and mobility. The mechanism underlying might be extensive neuroplastic nature of the vestibular system.[30] These neuroplastic changes are dependent upon age, visual and vestibular experiences[31,32]. Vestibular end-organs send message to vestibular nuclei, which excite motor neuron of ipsilateral extensors and inhibit motor neuron of reciprocal flexor of the limb via lateral vestibulospinal tract.[33] Studies on animal model suggested that stimulation to vestibular afferents can induce long term potentiation and long term depression of the field potential of vestibular nuclei.[34]

Studies also suggest that the activation of brain areas involved in vestibulospinal relay and vestibular sensory input lead to the decrease in sway of center of pressure during single session of GVS stimulation in community dwelling elderly.[20]

Till date many studies have investigated and advocated the immediate effects of GVS over postural stability and mobility. Some also talked about change in sway during stimulation. Early improvement seen in this study in treatment group compared to placebo group can be explained on the basis of finding of one study which suggested the moderate additional improvement in postural stability after second stimulation session which last for few hours even after cessation of stimuli.[35]

Studies suggested that three hours of stimulation have a better effect in postural stability compared to thirty minutes of stimulation but the difference is relatively small,[35] this stimulation probably increases the activation of voltage-gated ion channels in vestibular system and prolong activation might cause decrease in activation of these channels.

So in this study we used twenty minute repeated sessions considering the adaptation of neural system but further investigation is required to find out factors which explain the appropriate duration to produce lasting effects on balance and mobility in different population and various balance disorders.

In this study we delivered multiple sessions of stimulation thrice a week every alternate day. Previous studies discovered that when low intensity direct current stimulation was delivered transcranially to motor cortex, then increase in the duration of post stimulation effects of the first stimulus was observed.[36] The study also determined that timing of stimulation of motor cortex can control plasticity. Adequate interval between consecutive sessions might be the cause of improvement in the treatment group in this study.

Long term effects, minimal invasion and absence of adverse effects prove appropriateness of subliminal GVS for repetitive stimulations in treatment studies for improving postural stability and mobility in elderly.[12] Further studies are required to find out the exact duration and intensity of stimulation for optimal results in improving the balance among elderly.

Conclusion

The present study suggests that vestibular stimulation can significantly improve balance and mobility in elderly individual when given along with exercise training program. Our results shows overall stability improved when nGVS delivered along with exercise training. Finding of this study also suggest improvement in dynamic stability. We can also conclude on the basis of finding that early changes in the stability can be achieved when nGVS is delivered along with balance training exercises. The findings also suggest these effects are not due to the placebo effect of the vestibular stimulation.

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Ethical Approval: The study procedure was approved by the Institutional Ethical Committee at Amity University Noida, Uttar Pradesh.

Declaration of Conflicting Interests: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Factors Affecting Recurrent Lateral Ankle Sprain in Athletes

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Abstract

Background: Lateral ankle sprains are one of the most common sports injuries that an athlete may endure during their career. If not treated correctly, it often leads to recurrent twisting of the ankle joint, causing disruption of the ligaments in the ankle joint. Various anthropometric factors such as height, weight, BMI, as well as previous ankle sprain history are assessed as potential predictors of recurrent lateral ankle sprain. The potential factors are correlated with the Foot and Ankle Ability Measure- Sports Sub Scale to assess their contribution to a recurrent lateral ankle sprain in athletes. The FAAM Sport subscale is used to determine the functioning of the ankle joint, as recorded by the subjects themselves.

Methodology: The questionnaire is sent to the candidates who fall under the inclusion criteria, and they are asked to fill the questionnaire. The participants were between the age of 18-30 years. Both male, as well as female athletes were included in the study. Each athlete must have played at least 3 competitive sports in the last 1 year, and they must have a history of at least 1 lateral ankle sprain. The collected data is then compiled and analyzed.

Result: After the analysis of the data, previous ankle sprain history depicted maximum correlation with the FAAM- Sport Subscale, as well as an inverse relationship, with a reading of -2.89, and significance of 0.27. Height depicted an inverse relationship as well, with -.119 when compared to FAAM Sport Sub-scale. Weight also shows an inverse relationship (-0.053) when correlated with the FAAM Sport sub-scale. BMI does not show any correlation with the FAAM Sport Subscale (+0.002).

Conclusion: Previous ankle sprain history, height and weight depict an inverse relationship with the FAAM Sport Subscale, indicating that, as their score increases, the functioning of the ankle joint decreases, and hence becoming a potential factor for recurrent ankle sprains. BMI however, does not depict any correlation with the FAAM sport sub-scale.

Keywords: *Lateral ankle sprains, Anthropometric factors, BMI, The FAAM Sport subscale.*

Introduction

An ankle sprain occurs when the ligaments on the lateral aspect of the ankle complex get stretched or torn due to the rolling or twisting of an ankle joint. Lateral ankle injury is the most common problem in athletes. Female athletes are more prone to a lateral ankle twist as compared to males with a prevalence rate of 13.6 vs 6.90 for every 1000 people, respectively. It is also known as a supination or inversion ankle twist and it leads to the damaging of the ligaments in lateral aspect of ankle complex. Generally, a lateral ankle sprain is easily managed. However, if it is left untreated, it may lead to residual symptoms that may last 6-18 months.(1)

Ankle sprains are often partially treated. According to various studies, the rate of recurrence of lateral ankle sprain is more than 40%. The lack of adequate treatment and recurrent ankle sprains results in chronic ankle instability and osteoarthritis of the ankle joint. There are

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various risk factors that precede a lateral ankle sprain. Factors such as functional deficits in isokinetic strength of the ankle joint musculature, flexibility of the ligaments and muscles, proprioception, muscle reaction time, postural stability, gait biomechanics, the dominance of limb, history of previous lateral ankle sprains, and body mass index (BMI).[2]

Lateral ankle sprains usually occur due to an over-bearing hind-foot inversion which is accompanied with external or lateral rotation of the proximal segments during a task involving bearing of weight on the foot. Hind-foot supination is a multi-planar motion which is composed of ankle plantar-flexion, sub-talar inversion, and internal or medial rotation of the foot. These combined movement patterns result in the center of pressure (COP) moving externally on the plantar surface of the foot, as well as medially in relation to the ankle joint complex axis of rotation. In this position, a ground reaction force creates an external inversion moment at the ankle. Pronation moments can be elicited both externally, as with prophylactic ankle supporters, and internally, as with the peroneal musculature and lateral ankle ligaments,i.e., ATFL, CFL and PTFL in order to exert a counter force to the external supination moment. A net supination moment of sufficient magnitude will exert stress on the lateral ankle ligaments, potentially causing a significant strain or deformation of the ligaments. During an acute lateral ankle sprain, maximum level of ankle inversion may be reached as quickly as 40 ms after the initial contact with the ground.

Ankle joint is also known as the talo-crural joint. It is a synovial hinge joint formed by the mortise that is the distal end of the tibial and the fibular malleoli and the trochlear dome of the talus. It is covered by relatively thin and weak capsule. It is supported with ligaments medially and laterally. From the inner aspect, it is supported by the inner collateral ligament (deltoid ligament) and laterally by outer collateral ligament, which composes of the anterior talofibular ligament (ATFL), the calcaneofibular ligament(CFL) and the posterior talofibular ligament(PTFL).[3]

Ryan S. McCann et Al in 2018, conducted an examination to build up a prediction model for acute lateral ankle sprain in university ladies soccer players. They used different factors, for example, ankle sprain history, height, mass, and BMI. They hypothesised that school female soccer players with higher stature, mass, and BMI, and a history of ankle sprain would have higher odds of having a lateral ankle sprain. Ordinarily, an ankle sprain is supported when exaggerated ankle plantarflexion, subtalar inversion, and foot inward turn are available while decelerating. More prominent BMI, determined from stature and mass, likely builds the body, and reduces a person's capacity to external force. The essential finding of this examination is that height and history of ankle sprain were potential predictors of lateral ankle sprain among university ladies' soccer players. In particular, those competitors equivalent to or taller than 167.6 cm had 7.5 occasions more noteworthy chances of supporting a sidelong ankle sprain than those underneath 167.6 cm in tallness.[3]

Christopher R. Carcia et Al. in 2008, directed an investigation on the legitimacy of the Foot and Ankle Ability Measure in competitors with Chronic lower leg instability. The reason for this examination was to give verification of the legitimacy to the FAAM dependent on theory testing in competitors with an interminable ankle instability. Thirty National Collegiate Athletic Association Division II competitors (16 men, 14 ladies) from one college were considered for this investigation. The examination reasoned that the FAAM might be utilized to identify self-revealed practical deficiencies identified with constant ankle instability.[4]The current researches have depictedthat there are only a few predictors of lateral ankle sprain. However, no previous studies have given an insight at a model of lateral ankle sprain as a risk for athletes per say, but anthropometrics may be the key to potential injury. Lateral ankle sprains constitute approximately 7-10% of all admissions to Accident and prediction factors for that population.

Therefore, the objective of this research was to create an estimation model for recurrent ankle sprain injuries in athletes utilizing previous ankle sprain history, height, weight, and BMI as possible estimators.

Methodology

The inclusion criteria required the participants to be athletes between the age of 18-30 years. Both male, as well as female athletes were included in the study. Each athlete must have played at least 3 competitive sports in the last 1 year, and they must have a history of atleast 1 lateral ankle sprain. The exclusion criteria accounted for any history of lower limb surgeries, ankle fractures, medial ankle sprains or arthritis of the ankle joint. History of other lower limb injuries were excluded from the study. The subject should not have had a lateral ankle

sprain in the past 1 month. Subjects having signs and symptoms of neurological disorders were also excluded from the study.

An online survey was conducted on 67 subjects, comprising of 2 sections: FAAM ADL sub-scale and FAAM SPORTS Sub-scale. Only 46 participants were chosen on the basis of the inclusion and exclusion criteria. Each participant was asked to fill the consent form following which the required study was conducted. 50 male and female participants are included in the study between the ages of 18 to 30-yearold. All participants must be athletes. They are assessed on the basis of their height, using an inch tape, and weight using a general weighing machine. The resultant BMI is calculated in kg/m^2. Each participant fills the Foot and Ankle Ability Measure questionnaire. Participants reported whether or not they had ever sustained a previous ankle sprain.

Results

Spearmann Rho Correlation test was used to establish a relationship between lateral ankle sprain history, height, weight and BMI with FAAM SPORT sub-scale.

Table No. I: Spearman's Rho Correlation between FAAM Sport and Ankle Sprain History

			FAAM Sport	Ankle Sprain History
Spearman's rho	FAAM Sport	Correlation Coefficient	1.000	-.289*
		Sig. (1-tailed)	.	.027
		N	45	45
	Ankle Sprain History	Correlation Coefficient	-.289*	1.000
		Sig. (1-tailed)	.027	
		N	45	45

*. Correlation is significant at the 0.05 level (1-tailed).

Spearman Rho correlation was used for analysis between FAAM Sport and Ankle sprain history. When the Correlation coefficient is negative, it describes an inversely proportional relationship between the two variables. It signifies that, as the number of ankle sprains increase, the score on the FAAM Sport will reduce. Similarly, as the number of ankle sprains reduce, the score on the FAAM Sport will increase.

Table number 1 also depicts the significance of Ankle sprain history on the score of FAAM Sport. The correlation significance is measured when the value is below 0.05. As the value is lower than 0.05, it proves that ankle sprain history has a significant impact on the score of the FAAM Sport.

Table No. II: Spearman's Rho Correlation between FAAM Sport and Height

			FAAM Sport	Height
Spearman's rho	FAAM Sport	Correlation Coefficient	1.000	-.113
		Sig. (1-tailed)	.	.229
		N	45	45
	Height	Correlation Coefficient	-.113	1.000
		Sig. (1-tailed)	.229	.
		N	45	45

When the Correlation coefficient is negative, it describes an inversely proportional relationship between the two variables. It signifies that, as the height of an athlete increases, the score on the FAAM Sport will reduce. Similarly, as the height of the athlete decreases, the score on the FAAM Sport will increase.

Table No. III: Spearman's Rho Correlation between FAAM Sport and Weight

			FAAM Sport	Weight
Spearman's rho	FAAM Sport	Correlation Coefficient	1.000	-.053
		Sig. (1-tailed)	.	.365
		N	45	45
	Weight	Correlation Coefficient	-.053	1.000
		Sig. (1-tailed)	.365	.
		N	45	45

As shown in the table, the correlation between FAAM Sport and weight is inversely proportional, which means that, as the weight of an athlete increases, the score on the FAAM Sport will reduce. Similarly, as the weight of the athlete decreases, the score on the FAAM Sport will increase.

Table No. IV: Spearman's Rho Correlation between FAAM Sport and BMI

			FAAM Sport	BMI
Spearman's rho	FAAM Sport	Correlation Coefficient	1.000	.002
		Sig. (1-tailed)	.	.494
		N	45	45
	BMI	Correlation Coefficient	.002	1.000
		Sig. (1-tailed)	.494	.
		N	45	45

When the Correlation coefficient is positive, it describes a directly proportional relationship between the two variables. As shown in the table, the correlation between FAAM Sport and BMI is directly proportional,

which means that, as the BMI of an athlete, increases, the score on the FAAM Sport will also increase. Similarly, as the BMI of the athlete decreases, the score on the FAAM Sport will also decrease.

Discussion

The primary result of this study is that the participant's previous ankle sprain history was the most effective predictor of lateral ankle sprain among athletes. The study suggested that the score on the FAAM Sport sub-scale reduces as the incidences of ankle sprains increases. This result is confirmed by the study of Christopher R Garcia, et Al. in 2008 who have given a similar conclusion to their study. The score of the FAAM Sports subscale was greater in healthy individuals as compared to individuals that have past lateral ankle sprains. Kofotolis et al. in 2003 also examined a large selection of amateur soccer athletes and determined that those with a previous lateral ankle sprain had nearly twice the greater chances of sustaining an outer ankle sprain during the two years of subsequent observation. M. De Norhona in 2013, conducted a study on Intrinsic predictive factors for ankle sprains in active university students. He concluded that students with a previous ankle sprain history were twice as likely to suffer subsequent ankle sprains.[4]

According to this study, height was also correlated to recurrence of lateral ankle sprain. As the height of an individual athlete increases, the FAAM sports sub-scale depicted lower values. This finding supports previous studies reporting participant height as an effective predictor of ankle injuries. Waterman et al. in 2010 reported taller military academy cadets were at greater risk of sustaining an ankle sprain. Similarly, Milgrom et al. in 1991 found that taller infantry recruits were more prone to lateral ankle sprains.[5][6]

Another finding of the study described weight of an individual athlete to be correlated with recurrence of lateral ankle sprain. As the weight of an athlete increases, the tendency for a lateral ankle sprain increases and, the score on the FAAM sports sub-scale reduces. Tricia A Jubbard and Erik Wikstrom conducted a study in 2010 regarding the pathophysiology, predisposing factors, and management strategies of a lateral ankle sprain. The study concluded that weight was an intrinsic factor that contributed to Chronic ankle instability, among various other factors, like, height, proprioception, peroneus reaction time, etc.[7]

According to this study, BMI did not show a correlation or significance to recurrent lateral ankle sprains. As the BMI increases, the score on the FAAM sports sub-scale increases as well. This is confirmed by the study of M. De Norhona in 2013, who conducted a study on intrinsic risk factors of ankle sprains in active university students, who concluded that BMI does not play a significant role in causing recurrent lateral ankle sprains. Takumi Kobayashi et al. conducted a study on interior risk factors for non-contact lateral ankle sprains in collegiate athletes and he concluded that BMI does not show a correlation to recurrent lateral ankle sprains.[8]

Clinically, the strength of height and previous ankle sprain history as lateral ankle sprain predictors is their ease of assessment, but they are clearly limited by their lack of modifiability. Although they are not changeable, these simple outcomes may be important catalysts for targeted intervention. For example, preventative measures such as prophylactic ankle supports, proprioceptive training, core strengthening and postural control training are viable options for lateral ankle sprain prevention, and perhaps may be particularly valuable for taller athletes and those with a previous ankle sprain. As a lateral ankle sprain in athletes occurs through a variety of mechanisms, postural control training likely should be diversified to include static and dynamic balance exercises, dynamic stability following a jump landing or agility trainings involving change of direction and cutting, and external stimulus or perturbations.[3]

Future studies should examine various forms of neuromuscular control training and attempt to find the most effective protocol for lateral ankle sprain prevention in this population. Early identification of strong risk factors will allow clinicians to work more on preventive resources that have the highest chances of causing a lateral ankle sprain. This will help in cutting down costs of treatment and time significantly. Using of prophylactic ankle support and working on neuro-muscular strengthening, will further reduce the costs of treatment and the time that is spent on the same.

Certain notable limitations are present within this study. Firstly, this study was based on athletes of Delhi NCR only and it may not be applicable to athletes from other regions. Due to the ongoing COVID-19 pandemic, the studies were taken online. Furthermore, the sample size concise. Also, this study was focused only on anthropometric variables that may be the potential predictors of lateral ankle sprains in athletes. Future

studies should continue to explore lateral ankle sprain prediction in athletes using a larger sample size and other clinical tests that may be relevant to the mechanism of lateral ankle sprain injury such as postural control, flexibility, muscular strength, and self-reported function.

Conclusion

The history of lateral ankle sprain of an athlete is a significant factor to predict a potential recurrent ankle sprain. An increase in the number of ankle sprain will give a lower score on the FAAM sports Sub-scale. Height of an individual athlete depicts a correlation with an increased risk of lateral ankle sprain in athletes. As the height of the individual increases, the score of the FAAM sub-scale reduces. Weight of an individual athlete shows a correlation with an increased risk of a recurrent lateral ankle sprain. As the weight increases, the score on the FAAM Sport Sub-scale reduces. BMI did not show a correlation with recurrent ankle sprains.

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Impact of COVID-19 Pandemic on the Mental Balance among Health Care Workers

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Abstract

The current outbreak of the latest coronavirus [COVID-19] has created a global crisis of unparalleled dimensions. As massive pandemics are significant community mental health threats.Services of health demand is increasing drastically. The pandemic has emotionally affected physicians, and the vast majority report they have one or more of the following effects: fear of illness, anxiety, tiredness, trouble sleeping, loneliness, depression, confusion, or irritability. Those in the front line are particularly affected by fear of family and personal infection, work-related pressure, and fatigue. Always, when contacting COVID-19 persons, they have been quarantined frequently. Greater workloads, loneliness and prejudice contribute to physical exhaustion, anxiety, emotional distress and sleep disturbance. In addition to the strategies used to prevent the spread of the virus, innovative strategies are required for health practitioners to tackle associated mental health concerns. In light of almost all of these issues must be taken even more seriously by the respective governments and stakeholders of persons affected by any psychological disturbance. This review summarizes the present health care professional's psychological disturbances and the strategies to improve their mental health.

Keywords: *Outburst, COVID-19, Psychological stability, Medical workers.*

Introduction

COVID-19 pandemic Corona Virus Disease is the current crisis in more than 200 countries in the world.[1] Corona virus disease Global pandemic (COVID-19) was announced in 2019 by the WHO.[2]The COVID 19 is actually causing a big health crisis in the world."Owing to this pandemic, mortality and morbidity have risen dramatically in the last few months[3].It's more than that 680,000 total cases with 31,920 deaths, 146,396 recovered over 202 countries.More than half the world's deaths and infections at the end of 28 March 2020, according to the World Health Organization (WHO) situation report, came from Europe.[4] Because the disease spreads quickly, the majority of the affected countries are unable to meet the demands of personal protective equipments and infrastructure requirements.[5] As per the ministry of health and family welfare, in India 595501 Active Cases,1328336 Cured/Discharged,40699 Deaths, till date 6th august 2020(6).Throughout the recent months, the general population has shown an increased mental health problem during this outbreak of COVID-19 infectionother than COVID-19 patients, elderly, teenagers, migrants and health professionals.[7] Worldwide, mental health is a major concern. It is a big burden for an individual and society worldwide."Overall neuropsychiatric causes for years lost due to disability are: anxiety disorder, schizophysiological disease and bipolar disorders, overuse of prescription medication, headache, abuse of other substances, Alzheimer's disease, alcohol and epilepsy.[8] Up to now. International organizations have not made concrete guidelines to address mental health problems during this COVID-19 pandemic, the global preference for the increasing numbers of infected populations and deaths".Most countries have, in fact, inadequate manpower and equipment to manage COVID-19. There is also a greater burden for healthcare providers in the fear of infection. They were frequently quarantined when they contacted COVID-19-confirmed individuals. Further stress, loneliness and prejudice contribute to physical fatigue, anxiety, emotional trouble and sleep problems.[9]

Impact on health professionals: The providers

of healthcare must face a growing workload in fear of infection, increased workload; alienation and prejudice contribute to physical fatigue, anxiety, sleep disorders and emotional disturbances[10]

A recent study by 1563 health workers reported over half that Depressive symptoms (50.7%), 44.7 %, and 36.1 % reported anxiety, Sturge of Sleep (Ho et al. 2020).Besides, there are insufficient facilities to offer advice and clinical distress, depression, and suicide assessment services to doctors who are dealing with infected patients, psychological distress, and suicidal tendency.(11) It is also relevant that many doctors postulate grow post-traumatic stress disorder, depression, anxiety, and burnout following pandemic cessation.(12) Previous studies suggest that nurses, emergency staff and health professionals have also shown elevated mental health problems during COVID-19 Emotional stress, disruptions and higher rates of depression and fear."The probability of infection is estimated to be much more at an exposure risk that causes fear of being transferred to loved ones and to children causes' burnouts and emotional and physical signs[13]

Unfortunately, a typical example of the highest-quality hypocrisy is that the mindset of society towards doctors and health staff in its neighborhood. At the appeal of our President, the nation cheered the health staffs while in the neighborhood they were ill-treating the same staff because of terror of catching an infection. There are many allegations that their tenants and neighbors have threatened health workers. The public has been threatened during the examinations and quarantine checks by health staff. It triggers mental disorders and burnout among health workers.[14]

The lack of appropriate protection is of great concern for medical staff. In an Indian nation that is populated profoundly and has no strong health infrastructure that causes concern.

Mental Balance Strategy Recommended:

Government action plan:

1.	Encourage the involvement of multidisciplinary mental health teams at regional, state and local level
2.	Relevant education in stress reduction, trauma, depression, and risk behavior protocols
3.	Ensure sufficient funding and facilities for mental health services
4.	Provide the public with official outlets for updated details
5.	Provide alternative service platforms (apps, blogs, telephones) for health care professionals
6.	Collect epidemiological evidence that will help future initiatives on prevention and mental health care policies

Individual management and approaches to mental health issues:

Mental health issues	Strategies to overcome
Feeling loneliness	**Keep relations:** • Create a virtual forum to help each other • Sit down and have fun in the conversation. • Virtual community parties, online book clubs, and co-working spaces where one can operate in the presence of others (virtually),established by the teams of staff
Anxiety	**Routine is your friend** • It facilitates anxiety reduction and allows you to respond more easily to this new reality. • Creates clear boundaries in both the physical and the head space between work and non-work time. • Finding something that doesn't work and isn't connected to viruses that bring you happiness. • Operating with consistent breaks in short bursts will help to preserve consistency • Restrict pandemic exposure, as too much information, can cause disturbances
Panic about COVID-19	**Be sure to live in the moment** • Take as it happens every day and concentrate on the things that you can handle. • Being mindful and meditating can be Simple instruments

Mental health issues	Strategies to overcome
Stress, burnout, and depression	• Recognize your life's causes of stress • Take care what you're eating and drinking. • Try eating a healthy diet and consume at least 3 meals a day • Get proper sleep • Control your breathing • Create time for fun • Improve study skills • Take care of yourself and others, maintain interaction with family and friends • Follow guidelines from the WHO and the government agencies • Track behavioral dysphoric states such as aggression and irritability • Do not disseminate information from unofficial sources • Understand that in uncertain circumstances stress and fear are natural • Set up a support network (although virtual) • Practice meditation and exercise • Effective use of method in psychiatry/psychology

Essential tips:

1. Regardless of how you live, changes to your normal social and professional circumstances can lead to a deep sense of isolation. Why not join others to remain connected? a few social networking groups to discuss topics you're interested in. Try arranging with friends and family or colleaguesa daily video conference, face-to-face interaction also helps to establish social links across the phone.
2. Structure your days and take daily breaks to reduce tension and adapt the current situation to your life. You should stick to the routine of an "office" as much as possible if you telework: get up at your ordinary time, get ready, get dressed, as if you are working (the fact that you stay in your pajamas may have a detrimental effect both on your moral standards and on your job). Try to set a simple but organized programming and stick to it all week long, if you have children in particular. Decide in advance when you should start and finish your working day and finish of your working day and Factor in your breaks (important) for coffee and lunch: Should not vacillate to set warning signals to remind yourself that your business day is over and to allow you to exit your computer. Use your entitlements to leave. Though you cannot go on holiday and just sit home, you must stop and pause for a few days for well-being and safety.
3. Plan the day as effectively as possible to banish feelings of powerlessness. The objective is to define goals and to assign those times to every mission. Finally, go over what you have accomplished and plan your goals for the next day. The duration of the mission cannot always be decided in advance. Be patient with yourself if you have not accomplished all of your targets and have set your targets for the next few days.
4. Hold your private and personal life apart to stay successful and maintain a healthy balance. Many of us don't have a home office full, so everybody at home, even if it is just a kitchen table, can set up a work space..Declare and delete all that. is not work-related from your office. Get what you need: loaders, head phones, notebooks, pen, tablets, and bottles of water, if necessary snacks. The goal is not 10 times a day to leave the office to find it. It is necessary to enter 'work' or 'home' mentally and do not mix both (for example, Schedule personal telephone calls during 'business' hours.
5. Take care of daily workouts for your physical and mental health. The number of exercise lessons, yoga and meditation, and others during the lock-down. The roof has gone through similar online options: take advantage of it. Create in your weekly schedule one or more workout or relaxation sessions. Seek to enjoy a little fresh air and sunlight every day, if possible, or sit at a window.

6. Choose carefully and purposefully your media resources. Many such low-quality details cause anxiety. Limit your attention to news and carefully pick your information sources. For example, decide to watch, hear, or read a summary of the news daily from a source of confidence and highly assessed, and ignore all else (particularly deactivate Smartphone notices).
7. Spend some time on creative hobbies to keep the energy levels up and stay optimistic. Even if your days are busy, time for creative activities is important. Do some art or crafts; Take a lesson online, plan dinner and party, etc. Be imaginative and discover new entertainment sources in your home
8. Take note of your daily meals and try to preserve your energy levels with a healthy, balanced, and diverse diet. Especially if you can't do as much physical activity as normal by locking down, eat light meals.[15]

Conclusion

According to government orders, during the lockout, emergency and basic healthcare services will proceed. It extends to all government hospitals, private hospitals, private health centers, testing centers, and consultation clinics in the world. COVID-19 poses significant threats to mental wellbeing. There is not enough work in the COVID-19 pandemic to resolve mental health problems. As death and Morbidities rates hit new heights day after day, mental health problems can raise exponentially, and loneliness and reclusiveness can continue, leisure opportunities will be reduced and the financial crisis is continuing. The intellect outlook of COVID-19 and the potential steps to deal with the pandemic is important to understand for their efficient management.

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L-ascorbic Acid Supplementation Ameliorates Sodium Fluoride Induced Oxidative and Nitrosative Hepatic Damage in Hypoxic Rats

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Abstract

Introduction: Fluoride toxicity causes irreversible damage to soft tissues like brain, liver, heart and lung**.** Fluoridetoxicity and hypoxiaexposure might alterhepatic oxygen sensing cell signaling mechanisms and a supplementation of antioxidant like l-ascorbic acid might have protective role.

Aim: To investigate the exposure of sodium fluoride or hypoxia alone or in combination with or without administration of L- ascorbic acid on biochemical and transcriptional pathways in hepato toxicity **.**

Materials and Method: Male albino rats were divided into 8 groups (n= 6/group), group I(control), group II (l-ascorbic acid, 50 mg/100g. b.wt), group III (hypoxia, 10% O_2), group IV (NaF;20 mg/kg b.wt/day; ip), group V (NaF + hypoxia, 10% O_2), group VI (l–ascorbic acid + hypoxia, 10% O_2), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia, 10% O_2). The treatments were carried for 21 days. Gravimetry, serumoxidant and antioxidant status were assessed by using spectrophotometer, serum levels of vascular endothelial growth factor (VEGF) and nitric oxide synthase 3(NOS3) was done by ELISA technique. Histopathological evaluations of hepatic tissue were done. ANOVA followed by "Tukey" test were done for analysis of data in between the groups.

Results: Gravimetry and biochemical evaluation showed significant decrease in body weight hepatosomatic index, altered serum SOD, MDA, vitamin C, vitamin E, nitric oxide and hepatic vitamin Cin rats treated with hypoxia (group III), NaF (group IV) and NaF with hypoxia (group V). In case of l-ascorbic acid supplementation in group VI, VII and VIII showed remarkable improvement. Alteration in serum VEGF, NOS3 andhistopathology of liver in rats treated with hypoxia, NaFand hypoxia with NaFindicate hepatic dysfunction by oxidative and nitrosative stress, whereas l-ascorbic acid supplementation were found to be beneficial against fluoride and hypoxia induced alteration of hepatic function.

Conclusion: Supplementation of L-ascorbic acid is salubrious to combat both sodium fluoride and hypoxia induced oxidative & Nitrosative stress.

Keywords: *L –ascorbic acid, sodium fluoride, hypoxia,oxidative stress, Nitrosative stress.*

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Introduction

Fluoride toxicity causes irreversible damage to soft tissues like brain, liver, heart and lung[1]. Fluoride

can cross cell membranes by simple diffusion and enter soft tissues. Fluoride induces necrosis and apoptosis[2]. Fluoridetoxicity and hypoxiaexposure alterhepatic oxygen sensing cell signaling transduction pathways. Weather fluoride induced toxicity and low oxygen microenvironment act through similar or different mechanisms is not known. Antioxidant supplementation is useful in fluorosis management and fluoride intoxication[3]. Hence the present study was schemed toassess therole of l-ascorbic acid as antioxidant on NaF and hypoxia or in combinationonhepato cellular oxidative stress in male albino rats.

Materials and Method

Healthy adult male albino Wistar rats weighing about 150 -180 gramswere procured from animal house. Acclimatized for one week to the laboratory conditions at 21-25ºC and fed with laboratory stock diet and water *ad libitum*..

Experimental Groups: Male albino rats were divided into 8 groups (n= 6 in each group), group I(control), group II (l-ascorbic acid,50 mg/100g. b.wt), group III (hypoxia, 10%O_2), group IV (NaF; 20 mg/ kg b .wt/day; ip), group V (NaF + Hypoxia, 10% O_2), group VI (l-ascorbic acid + hypoxia, 10% O_2), group VII (l-ascorbic acid + NaF) and group VIII(l-ascorbic acid + NaF + hypoxia, 10% O_2). The interventions were carried for 21 days.

NaF was injected intra peritoneally at a dose of 20mg/ kg body weight/day for 21 days [4]. L-ascorbic acid was administered orally by using force feeding needle with syringe for 21 days (50mg/100g body weight).

Exposure of Rats to hypoxia: Acrylic chamber was used to induce hypoxia. Rats in cage were kept in chamber and given mixture of 10% oxygen and 90% nitrogento inducechronic normobaric hypoxia for 21 days. Temperature was maintained at 22-27°C [4].

Gravimetry: Animals of all groups were weighed on the starting day of protocol and on the 21st day i.e. on the day of sacrifice using digital weighing balance. Percentage of body weight gain was determined. Liver was weighed after sacrifices at the end of experiment and further hepato somatic index was determined by using the following formula:

Hepato somatic index = Weight of heart × 100/Body weight

Sample Collection: At the end of 21st day all animals were kept for an overnight fast and blood was collected in plain tubes by doing retro-orbital puncture. These blood samples were centrifuged and serum was separated.

Oxidant and antioxidant parameters: Serumsuperoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide was determined. Serum nitric oxide synthase 3 (NOS3) and vascular endothelial growth factor(VEGF) was estimated by ELISA kit. Serum vitamin C,vitamin E and hepatic vitamin C levels was estimated.

Histopathology Procedure: Animals of all groups were sacrificed by cervical dislocationat the end of 21 days experimental protocol. The liver was carefully collected, isolated immediately and fixed in freshly prepared 10 % formal in for 24 hours and thin sections were taken. Histopathological evaluations were done to identify changes in liver for treatment groups and compared with control. CPCSEA guidelines were carefully followed during experiments.

Statistical Analysis: SPSS software version 16.0 was used. One-way ANOVA followed by "Tukey" test were done to find out intergroup significant differences. All values were represented asmean ± SD. $p \leq 0.05$ considered as statistically significant.

Results

Table-1 shows significant decrease in % of body weight gain, liver weight and hepatosomatic index of rats exposed to hypoxia (group III), NaF (groupIV), hypoxia and NaF (group V) as compared to control (group I) at the end of 21st day. However simultaneous treatment with l-ascorbic acid showed greater % of body weight gain, liver weight and hepatosomatic index in group VI (l-ascorbic acid + hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia).

Table-1

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	P Value
Initial body weight (gm) (1st day)	158.9 ±5.31[a]	155.33 ±6.6[a]	155.53 ±1.75[a]	155.33 ±6.11[a]	155.93 ±2.6[a]	154.47 ±4.01[a]	156.53 ±4.6[a]	155.67 ±5.4[a]	0.9693
Final body weight (gm) (21st day)	208.33 ±3.1[a]	214.33 ±3.7[a]	180.67 ±7.5[b]	169.67 ±8.6[c]	163.03 ±3.2[c]	195.33 ±5.5[d]	193 ±2.6[d]	186.67 ±2.8[e]	<0.0001*
Percent of body weight gain	31.17 ±3.2[a]	38.11 ±4.9[a]	16.14 ±3.6[b]	9.19 ±1.3[c]	4.55 ±1.6[d]	25.93 ±1.4[e]	23.33 ±2.1[e]	20.62 ±2.4[e]	<0.0001*
Liver weight (gm)	7.66 ±0.14[a]	8.08 ±0.22[a]	5.30 ±0.2[b]	4.54 ±0.32[c]	3.46 ±0.41[c]	6.37 ±0.18[d]	6.01 ±0.1[d]	6.27 ±0.28[d]	<0.0001*
Hepato-somatic index	3.67 ±0.03[a]	3.77 ±0.08[a]	2.94 ±0.22[b]	2.68 ±0.2[b]	2.12 ±0.27[b]	3.27 ±0.18[a]	3.12 ±0.04[a]	3.36 ±0.19[a]	<0.0001*

[Table-1]: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes in gravimetry. Group I(control), group II (l-ascorbic acid), group III(hypoxia), group IV (NaF), group V (hypoxia + NaF), group VI (l-ascorbic acid +hypoxia), group VII (l- ascorbic acid +NaF) and group VIII (l-ascorbic acid +hypoxia + NaF), values expressed as Mean ± SD, $p \leq 0.05$ is significant, values with different superscripts a,b, c,d,e are significantly different from each other

Table-2 shows significant increase in serum MDA and serum nitrite concentration inrats exposed to hypoxia(group III), NaF (groupIV), hypoxia and NaF (group V) as compared to respective controls. However simultaneous supplementation with l-ascorbic acid in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats showed significant improvements of serum MDA and serum nitrite.

Table-2

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	P Value
Serum MDA	5.51 ±2.15[a]	7.39 ±2.15[a]	21.73 ±7.18[b]	23.37 ±4.81[b]	27.01 ±5.1[c]	12.26 ±3.02[d]	15.44 ±5.09[e]	16.36 ±2.03[e]	<0.0001*
Serum nitrite	35.07 ±5.05[a]	36.27 ±10.68[a]	86.14 ±8.74[b]	92.61 ±3.28[b]	111.2 ±3.46[c]	72.35 ±4.33[d]	59.77 ±5.98[e]	49.77 ±2.91[f]	<0.0001*

[Table-2]: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes in oxidative status. Group I(control), group II(l-ascorbic acid), group III(hypoxia), group IV(NaF), group V (hypoxia + NaF), group VI (l-ascorbic acid +hypoxia), group VII (l- ascorbic acid +NaF) and group VIII (l-ascorbic acid +hypoxia + NaF), values expressed as Mean ± SD, $p \leq 0.05$ is significant, values with different superscripts a,b, c,d,e are significantly different from each other

Table-3 shows significant decrease inserum vitamin C,hepatic vitamin C and serum vitamin E concentration inrats exposed to hypoxia(group III), NaF (group IV), hypoxia and NaF (group V) as compared to respective controls. However simultaneous supplementation with l-ascorbic acid in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats showed significant improvements of serum vitamin C, hepatic vitamin C and serum vitamin E concentration.

Table-3

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	P Value
Serum SOD units/ml	15.03 ±0.98[a]	16.38 ±0.68[a]	7.17 ±0.99[b]	5.24 ±0.98[c]	2.59 ±0.61[d]	11.12 ±1.62[e]	8.29 ±1.02[b]	11.68 ±1.82[e]	<0.0001*
Serum vitamin C mg/dl	3.64 ±0.67[a]	5.15 ±0.2[b]	3.19 ±0.17[a]	2.81 ±0.16[c]	1.91 ±0.15[d]	3.22 ±0.27[a]	3.21 ±0.19[a]	3.47 ±0.58[a]	<0.0001*
Hepatic vitamin C µg/g	204.27 ±6.07[a]	230.93 ±9.16[b]	145.67 ±6.17[c]	115.81 ±15.92[d]	64.97 ±16.2[e]	153.71 ±10.46[c]	169.27 ±5.24[f]	183.37 ±6.38[g]	<0.0001*
Serum vitamin E mg/dl	4.48 ±0.5[a]	4.61 ±0.43[a]	3.52 ±0.25[b]	2.13 ±0.75[c]	1.06 ±0.25[d]	3.98 ±0.04[b]	3.82 ±0.11[b]	3.62 ±0.58[b]	<0.0001*

[Table-3]: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes in antioxidant status.Group I(control), group II(l-ascorbic acid), group III(hypoxia), group IV(NaF), group V (hypoxia + NaF), groupVI (l-ascorbic acid +hypoxia), groupVII(l- ascorbic acid +NaF) and group VIII (l-ascorbic acid +hypoxia + NaF), values expressed as Mean ± SD, $p \leq 0.05$ is significant, values with different superscripts a,b, c,d,e are significantly different from each other

Fig-1shows significant increase in serum VEGF concentration inrats exposed to hypoxia (group III), NaF (groupIV), hypoxia and NaF (group V) as compared to respective controls. However simultaneous supplementation with l-ascorbic acid in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats showed significant improvements in serum VEGF concentration.

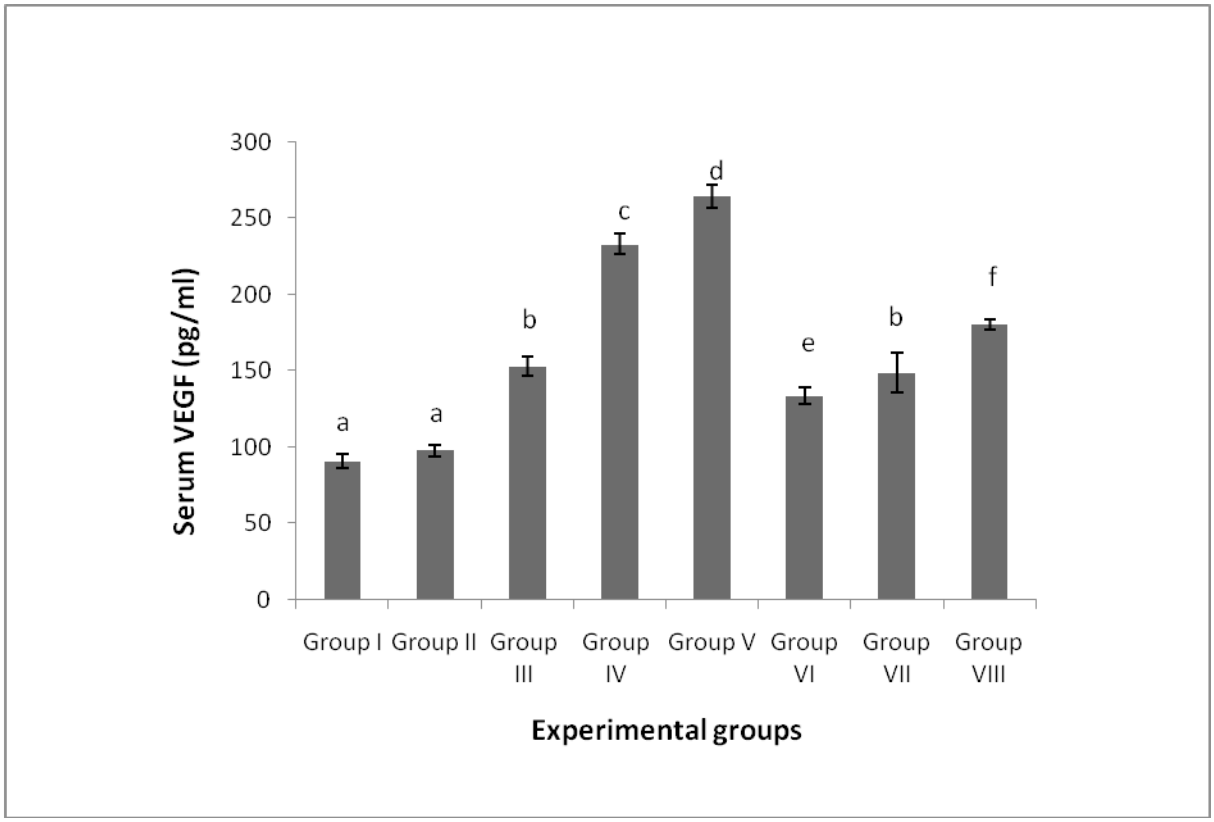


Fig. 1

[FIG-1]: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes on serum VEGF level. Group I(control), group II(l-ascorbic acid), group III (hypoxia), group IV (NaF), group V (hypoxia + NaF), group VI (l-ascorbic acid + hypoxia), groupVII(l- ascorbic acid +NaF) and group VIII (l-ascorbic acid + hypoxia + NaF), values expressed as Mean ± SD, $p \leq 0.05$ is significant, values with different superscripts a,b, c,d,e,f are significantly different from each other

Fig-2 shows significant increase in serum NOS3 concentrations inrats exposed to hypoxia (group III), NaF (group IV), hypoxia and NaF (group V) as compared to respective controls. However simultaneous supplementation with l-ascorbic acid in group VI (l-ascorbic acid + hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats showed significant improvements in serum NOS3 concentrations.

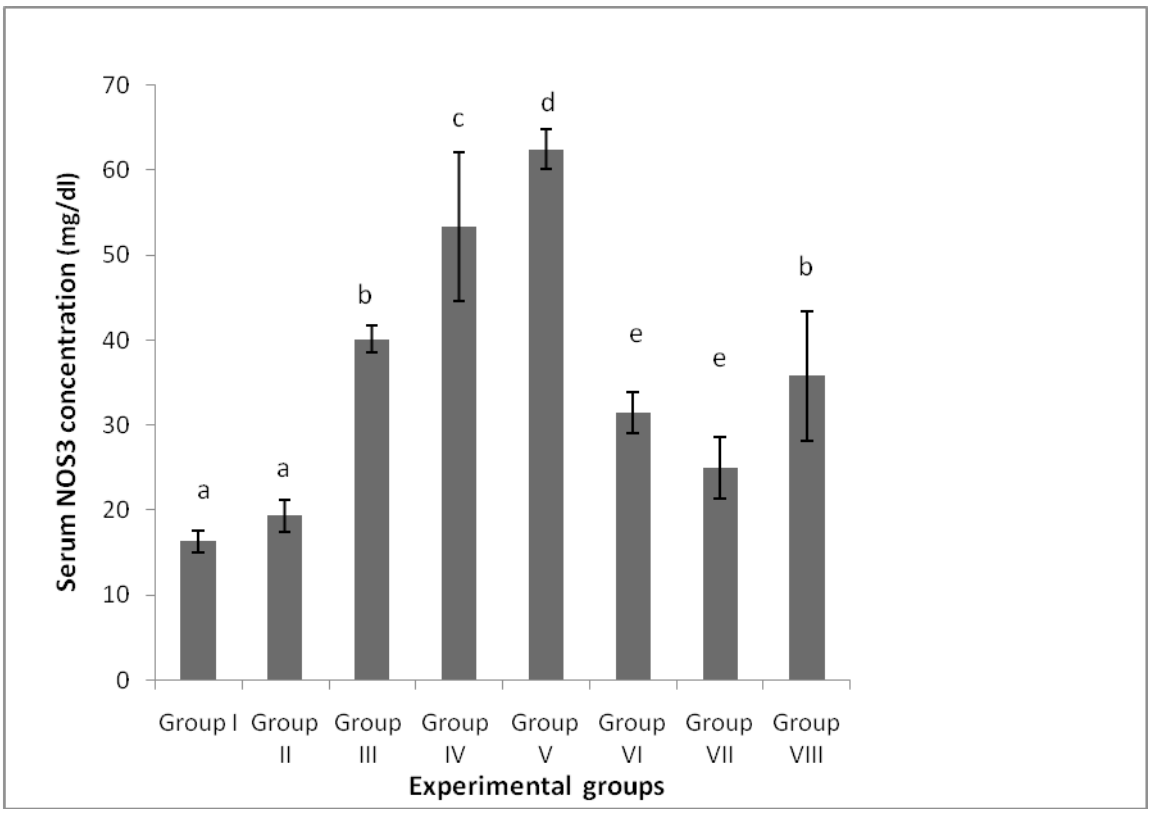


Fig. 2

[FIG-2]: Effect of l- ascorbic acid supplementation on sodium fluoride and hypoxia induced changes on serum NOS3 level. Group I(control), group II(l-ascorbic acid), group III(hypoxia), group IV(NaF), group V (hypoxia + NaF), groupVI (l-ascorbic acid +hypoxia), groupVII(l- ascorbic acid +NaF) and group VIII (l-ascorbic acid +hypoxia + NaF), values expressed as Mean ± SD, $p \leq 0.05$ is significant, values with different superscripts a,b, c,d,e,f are significantly different from each other

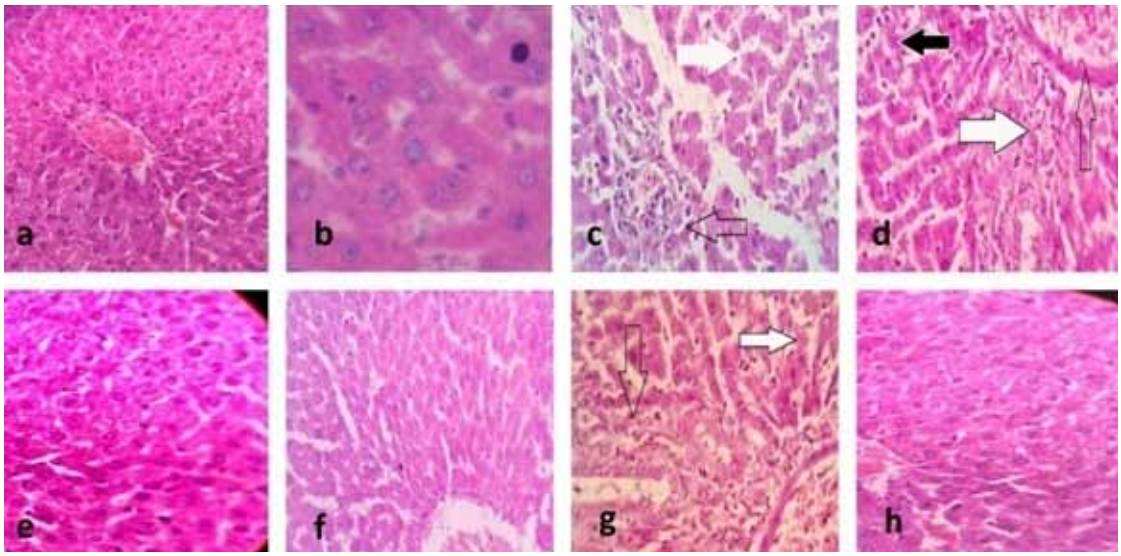


Fig. 3

Fig-3 (a-h) shows H & E stained sections of liver. Group I (control) and group II (l-ascorbic acid) showed normal architecture of liver (Fig- 3a & b). Group III (hypoxia) showed inflammation and sinusoidal congestion (Fig-3c), in group IV (NaF) showed focal necrosis, mononuclear infiltrate and central vein congestion (Fig-3d) and group V (hypoxia +NaF) also showed focal necrosis, mononuclear infiltrate and central vein congestion (Fig- 3e) whereas in case of l- ascorbic acid supplementation hepatic architecture appeared normal in group VI (l-ascorbic acid +hypoxia), group VII (l-ascorbic acid + NaF) and group VIII (l-ascorbic acid + NaF + hypoxia) rats (Fig-3f-h).

Discussion

Protective role of l-ascorbic acid on oxidative and nitrosativestress in hepatocytes were studied in hypoxia, sodium fluoride and in combination of hypoxia and sodium fluoride in experimental rats through evaluation of gravimetry, serum oxidant and antioxidant status along with histopathology of hepatictissues.

Gravimetry: Our study results shows decrease in body weight,change in % of body weight gain and hepatosomatic index in hypoxic and NaF administered rats or in combination of both. This decrease in body wt (%) and hepatosomatic index may be due to less food intake . NaF may cause inhibition of hunger centre in hypothalamus and fluoride also induces breakdown of tissue proteinsandlipids[5]. Supplementation with l-ascorbic acid showed % of body weight gain, liver weight gain and increased hepato somatic index due to protective actions of l-ascorbic acid in hypoxia and NaF induced alteration of tissue protein breakdown [6].

Oxidant and antioxidant status: In our study sodium fluoride induced and/or hypoxia exposed rats show increase in serum MDA and serum nitrite concentration due to oxidative stress. Oxidative stress is characterised by increase in reactive oxygen species (ROS) formation .ROS include free radicals like super oxide, peroxy nitrite and hydroxyl radicals. MDA is an indicator of lipid peroxidation and an important biomarker of oxidative stress[7]. Increase in nitrite concentration insodium fluoride induced and hypoxic rats may be due to over expression of NOS3 gene and increases production of nitric oxide(NO)[8,9]. our study results on supplementation of L-ascorbic acid on MDA and NO levels are indicative of preventing oxidative and nitrosative damage.

Our study also shows decrease in serum SOD, serum and hepatic vitamin C,serum vitamin E concentrations in sodium fluoride induced and/or hypoxia exposed rats. Sodium fluoride not only generates superoxide ions but also decreases enzymatic activity of SOD by binding to its active site .As a result SOD fails to detoxify hydroxyl ions. So inhibition of antioxidant enzyme SOD leads to oxidative damage at mitochondrial level and causes hepato cellular dysfunction[10]. Vitamin C combats superoxide in liver and also scavenges NO. Vitamin C also regenerates tetrahydrobiopterin (BH4). BH4 is important for NOS3 activity.Vitamin E with

lipid peroxyl molecule is oxidized to α -tocopheroxyl molecule. α-tocopheroxyl is recycled by L- ascorbic acid. Antioxidants like vitamin C and E protect against ROS damage[11,12]. On supplementation with L-ascorbic acid there was significant improvements in serum SOD, serum and hepatic vitamin C, serum vitamin E concentrations.

Serum NOS3 and VEGF concentrations: Our study results show that in sodium fluoride induced and/ or hypoxia exposed rats leads to increased serum VEGF and also NOS3 levels. Interestingly sodium fluoride toxicity mimics hypoxia like effects at hepatocellular level and increases ROS production. ROS induces directly NOS3 and VEGF gene expression. VEGF triggers hypoxic response and leads to upregulation of NOS3genes and enhances NO production .Increased NO levels itself down regulate VEGF expression.

Vitamin C supplementation has lead to decreased gene expression of NOS3 and VEGF in sodium fluoride induced and/or hypoxia exposed rats. This is due to decreased ROS production and NO levels to combat cellular hypoxia.

Histopathology of Hepatic tissues: Histological changes were observed inhypoxia exposed or NaF treated or in combination of hypoxia exposure and NaF treated rats. Sodium fluoride induced toxicity lead to disruption of hepatic architecture and lead to mononuclear infiltration and hepatic necrosis[1415]. In hypoxia exposure and NaF treated rats such changes have lead to hepato cellular damage.

Vitamin C supplementation prevented disruption of hepatic architecture and necrosis.

Conclusion

Both sodium fluoride and hypoxia enhances nitric oxide production by VEGF and NOS3 pathways leading to hepatocellular damage and apoptosis. The supplementation of L-ascorbic acid is salubrious to combat both sodium fluoride and hypoxia induced cell death in liver by oxidative & Nitrosative stress.

Conflict of Interest: None declared.

Source of Funding: Self

Ethical Clearance: The entire protocol for experiment was approved by Institutional Animal Ethical Committee bearing approval no (LCP/PG.Col/ IAEC/Oct-2015/66).

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To Assess Knowledge, Attitude and Practice on the Use of Automated External Defibrillation (AED) by Emergency Medical Services (EMS) Providers in Pune, India

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Abstract

Introduction: An Out of Hospital Cardiac Arrest (OHCA) is defined as the loss of cardiac mechanical function that occurs outside of the hospital setting and confirmed by the absence of signs of systemic circulation. OHCA has a very low survival rate. Timely provision CPR and Rapid Defibrillation during Cardiac Arrest requires adequately trained and qualified personnel. It is therefore important to understand the current level of Knowledge, Attitude and Practice (KAP) with regards to AED amongst EMS responders.

Objective: To assess Knowledge, Attitude and Practice on the Use of Automated External Defibrillation (AED) by Emergency Medical Services (EMS) Providers.

Methodology: The Knowledge, Attitude and Practice (KAP) study was conducted amongst 120 Emergency Medical Services Providers at SCHS, Pune, India. A previously tested and validated KAP questionnaire by Marcus EH Ong et.al[9] was utilized for the study. The final questionnaire consisted of 15 items spanning 3 parameters -5 items testing Knowledge, 8 items measuring Attitude and 2 items measuring Practice related to use of AED.

Discussion: The study was aimed at assessing the current knowledge, attitude and practices with regards to AED amongst EMS respondents. Most respondents reflected positive attitude towards utilizing AED in out of Hospital Cardiac Arrest patients.

Conclusion: The study reemphasizes the need for formal training on AED usage. Although the study is limited by its sample size, it provides broad perspective on the subject.

Keywords: *Out of Hospital Cardiac Arrest (OHCA), Automated External Defibrillator (AED), Emergency Medical Services(EMS), Knowledge, Attitude and Practices (KAP).*

Introduction

An Out of Hospital Cardiac Arrest (OHCA) is defined as the loss of cardiac mechanical function that occurs outside of the hospital setting and confirmed by the absence of signs of systemic circulation. Many OHCA patients develop early symptoms several hours before the event, which may lead them to seek medical help from primary care physicians (PCPs) or other out-of-hospital ambulatory care settings where he gets a cardiac arrest[1].

OHCA has a very low survival rate. With each minute delay in defibrillation from the onset of cardiac arrest, the probability of survival decreases by 10%.[2]

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Cardio Pulmonary Resuscitation (CPR)is a simple but effective procedure that allows almost anyone to sustain life in the early critical minutes after cardiac arrest[3]. The administration of CPR immediately after the onset of cardiac arrest is associated with improved survival and better quality of life among survivors.

Apart from CPR, Early defibrillation is essential in management of cardiac arrest. An automated external defibrillator (AED) allows lay rescuers to advance defibrillation by starting treatment before emergency medical services (EMS) arrive on the scene.[4] They are easy to use, accurate, and can be used safely and effectively.[5]

Use of automated external defibrillators (AEDs) is now an important component of emergency medical systems, and recent advances in AED technology have allowed expansion of AED use to non-traditional first responders and even the lay public.[6]

A study conducted by Ross A pollock *etal*[7] in 2018 concluded that bystander AED use before EMS arrival in shockable rhythm was associated with better survival and functional outcomes.

The crucial key lies in prehospital management. Early defibrillation in the prehospital phase

Can significantly increase the chance of survival.[8] Emergency Medical Service Providers are very important link in the Chain of Survival. Timely provision CPR and Rapid Defibrillation during Cardiac Arrest requires adequately trained and qualified personnel.

It is therefore important to understand the current level of Knowledge, Attitude and Practice (KAP) with regards to AED amongst EMS responders. This can tide further interventions to improve the KAP levels and ensure better qualified EMS responders to deal with OHCA.

Objective

To assess Knowledge, Attitude and Practice on the Use of Automated External Defibrillation (AED) by Emergency Medical Services (EMS) Providers.

Methodology

The Knowledge, Attitude and Practice (KAP) study was conducted amongst 120 Emergency Medical Services Providers at SCHS, Pune, India. A previously tested and validated KAP questionnaire by Marcus EH Ong et.al[9] was utilized for the study. The original 29 item questionnaire intended for usage in General Practitioners was modified to suit the present study. The final questionnaire consisted of 15 items spanning 3 parameters -5 items testing Knowledge, 8 items measuring Attitude and 2 items measuring Practice related to use of AED.

Informed consent was obtained from the respondents. The respondents were instructed to answer every item on the questionnaire either in YES or NO in front of the respective statement of Knowledge, Attitude and Practice. All items were to be mandatorily filled. The students were given 30 minutes to answer the questionnaire and to submit it. Only completely filled questionnaire were accepted for data analysis. The data collected was tabulated and statistically analysed using SPSS version 23.

Any queries pertaining to questionnaire was clarified at the time of collection of data.

Results

It was observd that the average age of the respondents was between 21 to 25, and 88% of them were female. Further, 17% had a valid basic cardiac life support (BCLS) certificate, and 14% had a valid advanced cardiac life support (ACLS) certificate**.**

As seen in Figure 1.,In terms of Knowledge, 83% of the respondednts could write the AED abbreviation correctly while around 88% claimed that they know how to operate an AED.As many as 32% of the respondents answered incorrectly stating that AED required an operator to interpret ECG rhythm. Only 58% of the respondents had attended any formal AED training in the past.

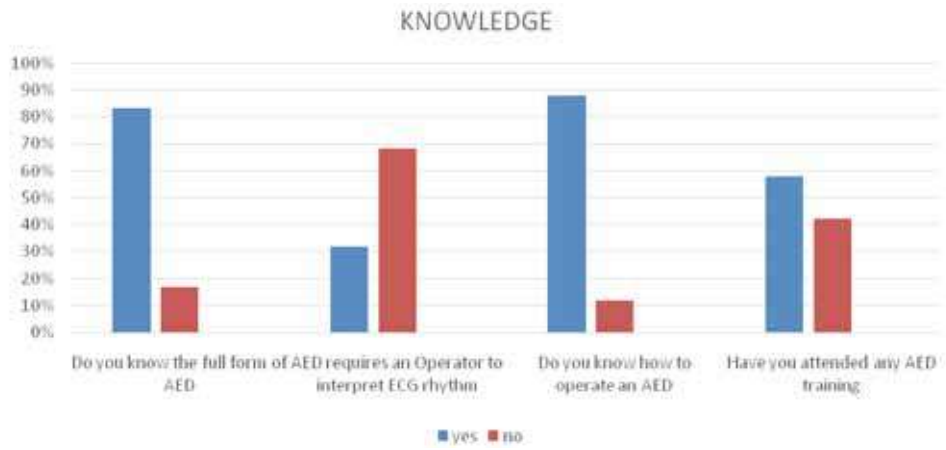


Figure 1.

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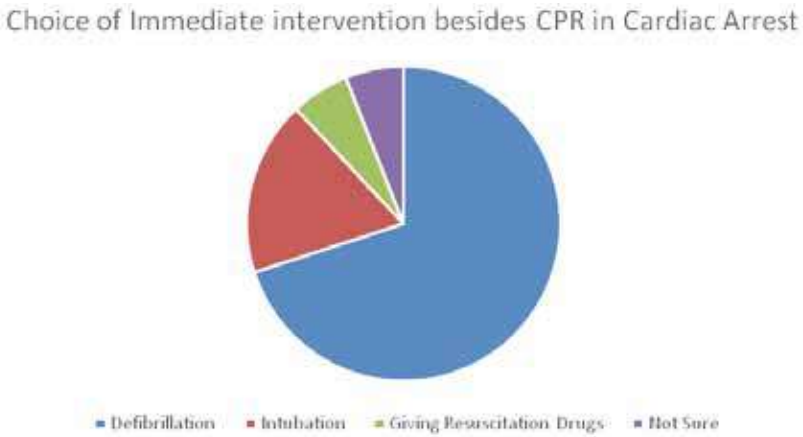


Figure 2.

As seen in figure 2. Majority (70%) of the respondents identified Defibrillation as the correct immediate intervention besides CPR in Cardiac Arrest.

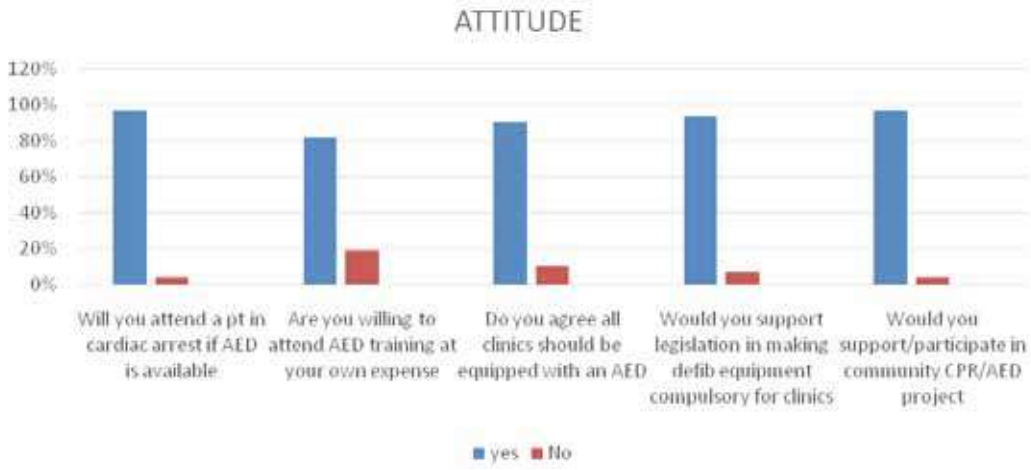


Figure 3.

As seen in figure 3., in terms of attitude 96% answered in affirmative when asked if they would attend a patient in cardiac arrest if AED is available. 90% agreed that all clinics should be equipped with AED and 92% supported the idea of bringing legislation in making defibrillatory equipment compulsory for clinics. Almost all (96%) respondents were willing to participate in community CPR/AED projects.

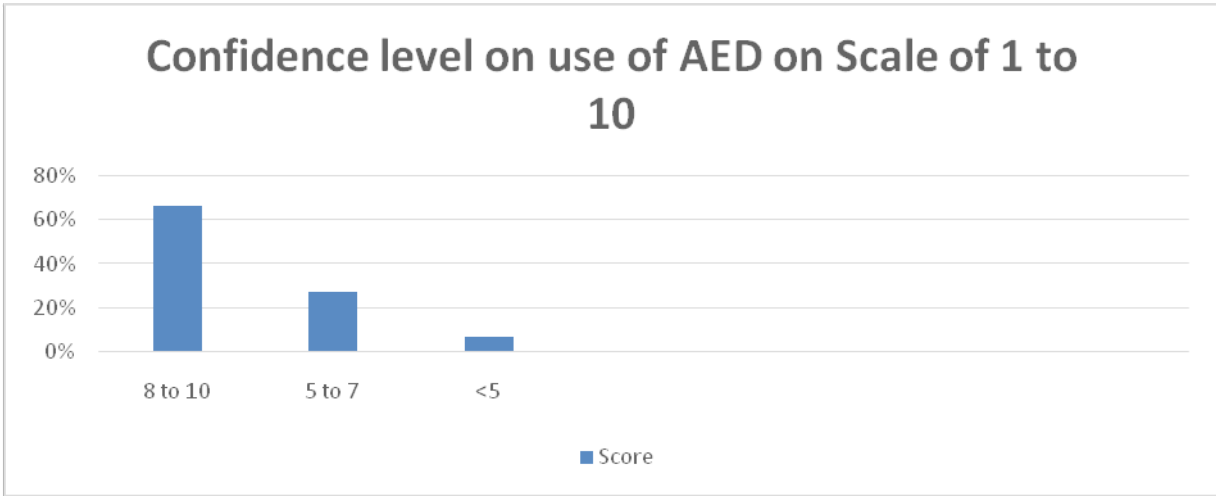


Figure 4

As depicted in figure 4,66% of the respondents exuded a high degree of confidece on the usage of AED.

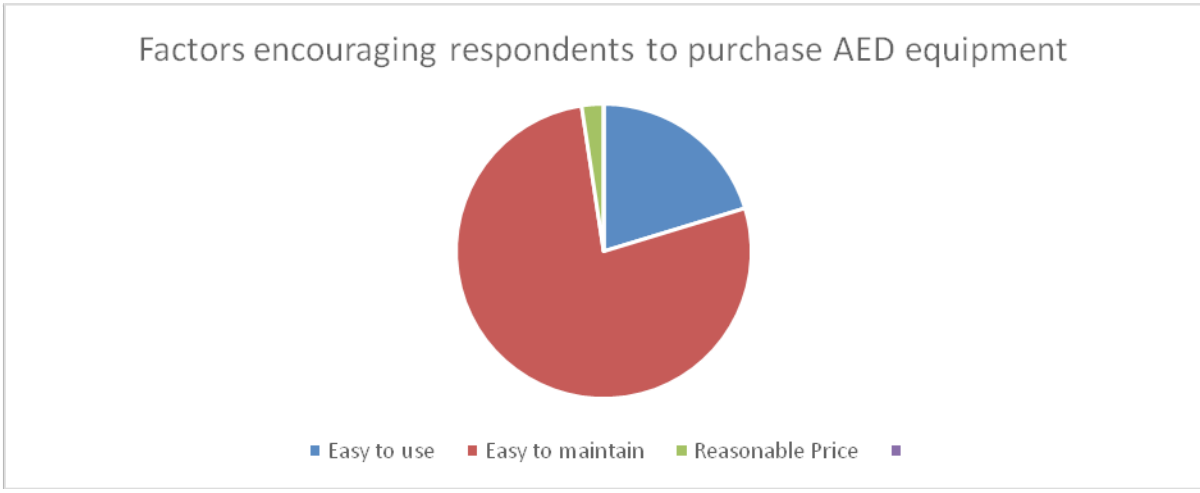


Figure 5.

As seen in figure 5.,84% of the respondents felt 'ease of use" as the key drive to purchase AED.

In terms of Knowledge, 62.2 % responded that they would perform mouth to mouth CPR.

77.04% of the rspondents suggested that they would go ahead with both compression and ventilation.

Discussion

The study was aimed at assessing the current knowledge, attitude and practices with regards to AED amongst EMS respondents.

The findings are encouraging in part while providing with valuable perspective for bringing improvements in certain areas. A sizable number of respondents

incorrectly percieved that percieved that AED requires operator to interpret ECG rhythm. This can be attributed to lack of formal training for AED as was found in the case of 58% of respondents. The optimum utilization of any equipment largely depends on the training of the operator. Training not only enhances knowledge, but also improves attitude towards usage and enhances utilization in emergency situations. It also helps in clearig misconceptions and allay fears.

Most respondents reflected positive attitude towards utilizing AED in out of Hospital Cardiac Arrest patients. The confidence level towards its utilization can be further improved by periodic hands on training. Majority respondents will in to attend AED training at their own expense and were optimistic about purchasing AED if they found it simple to use. Interestingly, price was not a significant factor while making a purchase decision. Respondents overwhelmingly supported the idea of legislation to make AED compulsory for clinics. This finding is important for policy makers on wider iimplementation of AEDs at clinic level in managing Out of Hospital Cardiac Arrest.

Majority of respondents appreciated the significance of ventilation during CPR and were ready to perform mouh to mouth ventilation. Given a choice between compression only and both compression and ventilation,majority of respondents chose both compression and ventilationas they found it more effective in comparison to compression only during CPR in Out of Hospital Cardiac Arrest.

Conclusion

The study amongst EMS responders throws light on the knowledge, attitude and practices with regards to AED usage. The study reemphasizes the need for formal training on AED usage. Although the study is limited by its sample size, it provides broad perspective on the subject. AED is a lifesaving modality in Out of Hospital Cardiac Arrest and optimizing its usage is the need of the hour.

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Whither to Public Interest-The Curious Case of Compulsory Drug Licensing in Indian Pharmaceutical Industry

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Abstract

Countries like India which are grappling with providing healthcare to heterogeneous and varied base of stakeholders including those at the bottom of pyramid have their own share of availability and affordability concerns in the healthcare sector for providing access of life saving drugs to the vast segment of Indian population and move towards the achievement of the Sustainable Developmental Goals (SDGs); in process addressing not only adequate availability of the primary health care but achieving the competition framework *vis- a- vis* patent control as the Original Patents which intend to protect innovation tangentially run across the consequential price determination power by monopolistic patented drug companies. Compulsory licensing of Patents provisions are used by the Governments as a means to correct anti-competitive practices to promote public interest or public health and in cases of emergency avoiding monopolies at the hands of patent holders to deter anti-competitive violations by engaging in unfair competition or abuse of dominant position in the market which aspect is so crucial to provide outreach of cheaper and quality drugs in common good.

Keywords: *Health Care Sector, Anti- competitive violations, Patent Holders, Compulsory Licensing.*

Introduction

The explosion of population and emergence of drug resistant communicable diseases like Tuberculosis and others like Malaria, HIV/AIDS epidemic, Cancer, Hepatitis C has made the task of providing cheaper health care in Indian market a frustrating experience despite increase in the domestic capacities of drug production, increased Government spending towards health care sector and alike. One has to take into account the learning outcomes out of the recent regulatory framework, wherein the epidemic diseases which have since resulted in the mutational form of the very disease itself; perhaps in majority of cases is the first (patented)version of the drugs being applied for curing such diseases is being provided whereas such diseases are being attempted to be controlled through second generation/further generic versions of the drugs in other developed countries under Compulsory Licensing; with the difference being the Patented version costing a significantly higher markup than those manufactured under Compulsory Licensing. Article 5 A(2) of the Paris Convention of 1883 provides that "Each country of the Union shall have the right to take legislative measures providing for the grant of compulsory licenses to prevent the abuses which might result from the exercise of the exclusive rights conferred by the patent, for example, failure to work"[(1)]. Largely seen as an interventionist measure, it seeks to calibrate rights of the patent holder with Governments obligation to ensure working of patents, ensuring availability at a reasonable price, protection of public health and nutrition and simultaneously encouraging technological invention.

Compulsory Licensing has emerged as one of the effective tools to prevent the monopolistic situations at the hands of patent holders and cause supply of generic drugs at affordable price addressing health care *viz-a viz* intellectual property and has found favor in developed nations such as European Union, Germany, Italy, United Kingdom, France, Norway and Russia to name a few. Compulsory Licensing of Patents has facilitated low priced pharmaceuticals and in process encouraging the local generic drug industry Developing countries like

India face the issue of delivery of healthcare technology, and challenge for policy makers has been to ensure acceptability and affordability on an equitable basis which could theoretically accrue through Compulsory Licensing and said aspect has been highlighted by the United Nations Human Rights Commission Report, 2001 as well[2]. The National Bureau of Economic Research (NBER) Working Paper indicates that compulsory licensing has a strong and persistent positive effect on domestic invention [3]. Even without any effects on innovation, compulsory licensing may create significant positive welfare effects on consumers in developing countries as a mechanism to maintain product variety. One needs to examine the relevance of compulsory license framework in Indian scenario more particularly the will of the regulatory architecture coupled with the response of the pharmaceutical industry to make use of the flexibilities available in context of the two domains namely patented production versus the manufacturing under Compulsory Licensing; having varying mandate; the State working with the social objective and the private sector having a business mandate .

A study indicates 65% of the Indian population still lacks access to essential medicines[4]. Share of drugs in OPD expenses were estimated at 63 per cent by NSSO 60th Round (January 2004)[5]. NSSO in their Report on 61st Round indicated this expenditure having increased to 82 per cent [6]. As per National Health Accounts, medicines accounted for 50-60 per cent of inpatient expenditure in rural and urban areas[7].As per the Report of National Health Accounts Estimates for India (2016-17), for the year 2016-17, total health expenditure for India is estimated at Rs 581023 crores (3.8% of the GDP and Rs. 4381 per capita)[8]. These reinforce the issues raised by the Parliamentary Committee for the need to increase the availability and accessibility of drugs to the poor in the country[9]. The National Pharmaceutical Policy 2002 recognizes the need to 'ensure abundant availability at reasonable prices of good quality essential pharmaceuticals of mass consumption'[10]. The National Pharmaceutical Policy 2006 while acknowledging the explosive growth in this sector between 1990 and 2010, and the accompanying low cost of medicines notes, that concerns regarding the accessibility and affordability of medicines remain[11]. The National Pharmaceutical Policy 2017 has aimed to realign the 'price control' orientation now to 'monitoring of price'[12].

Discussions

The 2001 Doha Declaration brought in a silver lining for Developing and Least Developed Countries as Declaration of the Trade Related Aspects of Intellectual Property Rights (TRIPS Agreement)and Public Health reiterated the flexibility of the member states to avail of the compulsory license to manufacture cheaper version of the patented drugs. Article 6 (b) of the said Declaration further highlighted the right of each Member State to grant Compulsory License and determination of grounds when such licenses are to be granted[13,14]. The Indian Patent Act 1970 (as amended in 2005) has been made fully compliant to incorporate these flexibilities[15].

The compulsory licensing as per Article 30 and 31 of TRIPS is very much WTO compliant for effecting in India. Article 31, while listing out 12 conditions/ circumstances highlights public non commercial use as one of the criteria's. Article 31(k) permits the grant of Compulsory licences to remedy anti-competitive practices. The DIPP paper of 2010 -made a reference to National Pharmaceutical Policy wherein the Drug Regulatory System has a National List of 354 essential drugs subjected to effective price control and limiting trade margins; that apart 74 drugs being under regulation has seen instances of regulatory contraventions[16]. Infact the developed countries with regard the Doha declaration having instituted two loop-holes as exceptions to the TRIPs agreement; one of them being parallel importing, where a "country may import patented drugs from another country where the patent-holding manufacturer sells them for less," and compulsory licensing. Developed countries and big pharmaceuticals are definitely against use of CL provisions[12]. No CLs have been issued in India under the amended Patents Act*(supra)*. In September 2007, three applications under section 92A of the Patents Act, 1970 were received for grant of compulsory licence for the manufacture and export of patented drugs to countries which reportedly did not have manufacturing capacity nor had insufficient capacity. The process envisaged under the Act was initiated. However, the applicant subsequently withdrew his applications

The Indian Patent Act deals with the issue of Compulsory Licensing through Section 84 (General CLs to be issued by Controller on application), Section 91 (Issue of CL by the Controller for the related Patent on application), Section 92 (issue of the CL by the Controller upon a Notification for National emergency/

extreme emergency or in case of Public Non Commercial Use) and Section 92 (A) (When insufficient or no manufacturing capacity is there to address pubic health problems).

Given the varied and a larger landscape under which Compulsory licenses provisions could be invoked, definitely there is a need to put in regulation justifying the elements of Section 92 of the Indian Patents Act i.e. national Emergency/extreme emergency/public non commercial use circumstances, which would mean further elaboration of provision of Section 92 (C) as per Para 43 of the Discussion Paper and the Guidelines should be made available both in case of Category 1 (production at lowest prices) and Category 2 (production at affordable prices) to ensure considerations of quality and availability, which would address the distribution aspects of the medicine to the desiring section of the society (*supra).* One also needs to examine the effective alternative to compulsory licensing wherein the patents could be opposed and more importantly questioning the drugmakers of "ever-green" provisions where pharmaceutical companies make subtle variations to the existing medicines to extend their patent monopolies for a larger duration

A cursory look at the provisions involved in the Indian Patent Act viz. Section 84, 89, 90, 92, 92A reveal that while CL provisions are yet to be tested; it may have technical administrative difficulties in view of the clear cut regulations not in place. (unlike section 84 which stresses for expiration of a 3 year period before a compulsory licensing application can be made, section 92A is silent on this. MoreoverSection 92A conditions are left to be decided by the controller; further the 3year lock-in period under Section 84 may prove to be deterrent given any unknown epidemic to occur. Moreover post 2005; many of the drugs may fall under the patent regime). This is an area which is subject to international pressures, political considerations and varied role of civil society including NGOs and the implementation may not be an easy task. The circumstances as stated in Section 92 (Para 40 & 41) are different from those sated in Section 100 (50 & 51). Section 100 provisions appear to vest in more authority with the Central Government or its constituents for using an invention for the purpose of Government. Given the societal context and the social and public interest that the Government of India concentrates upon the provisions of Section 92 and 100 while being complimentary; albeit Section 100 would cover extreme situations under Section 92 provisions.

It is a fact that drugs patented before 2005 Indian Patent Amendment are "off" patent items; that apart after the new patent regime for India in 2005 there have been instances of highly prices patented drugs in India and therefore there is a need that price control of patented medicine should not only be brought under the mandate of NPPA which presently caters to control the price of scheduled drugs and monitors the prices of non-scheduled drugs but also the patented drugs (*supra).*

Sections 84 to 94 of the Patents Act, 1970 deals with issue of compulsory licences i.e. circumstances under which compulsory licence should be issued, procedure for dealing with applications for compulsory licences, powers of the Controller to grant compulsory licence and terms and conditions of licence (*supra)*. But the time frame within which such compulsory licence should be given, once application for licence has been submitted has not been mentioned under the Act including time frame for Controller to dispose of general applications for compulsory licences, time frame to serve copy of application on the Patentee/patentee to oppose the application, time frame to grant/revoke licence on submission of opposition statement by the patentee,time frame for issue of compulsory licence under Sec.92A for export of patented pharmaceutical products in certain exceptional circumstances and time frame for information to be given to patentee under Section 92, where compulsory licence is issued on notification by Central Government in case of national emergency, extreme urgency or public non-commercial use. Further, the meaning of national emergency, extreme urgency and public non-commercial use should be clearly defined in the Patents Act. The circumstances under which these terms can be used should also be given in detail. Under Section 84(6) (ii) and (iii), the Controller is required to satisfy himself about the ability of the applicant to work the invention to the public advantage as well as the capacity of the applicant to undertake the risk in providing capital and working the invention, if the application were granted.

Article 27(1) of TRIPS interalia states "… patents shall be available and patent rights enjoyable without discrimination as to ...whether products are imported or locally produced" implying that so long as the patented product is available in the market at an affordable cost, the use of the patent cannot be differentiated on the basis of its sourcing – whether it was manufactured within the country or imported from without (*supra)*. The bone of contention has been that the exceptions provided

in Articles 30 and 31 of TRIPS override the general stipulations in Article 27 with Articles 7 and 8 of TRIPS which recognizing transfer of technology to support provisions for local sourcing making local working stipulations valid and enforceable.

Findings: What is needed in India is robust regulations to allow for compulsory licensing at standard royalty rates so that the essential medicine are priced competitively. After the Doha Declaration over 50 countries have issued CLs prominent being USA, UK, Canada, Italy and amongst developing ones; Brazil(2007 for an anti AIDS drug); Thailand(2006 and 2007 for anti AIDS drugs), Malaysia (2003 for Anti AIDS drugs), South Africa (Anti Aids Drug) Kenya (voluntary licenses issued in 2004 after threat of CL), and most recently Ecuador (April 2010 for an anti AIDS drug) (*supra)*.It also remains a fact that in Thailand, use of CL provisions while being totally compliance with the TRIP Law was criticized by the pharmaceutical companies and the Governments of developed countries and it showed the clout of the pharmaceutical industry in their displeasure against the use of CLs. It is gathered that with such successful intervention the pricing of ARVs could be reduced by 40%. A study highlights that CLs are waning away much to detriment of bringing serenity in drug pricing and improved health governance.

While Section 84(1) of the Indian Patent Act provides that at any time after the expiration of three years from the date of the grant of a patent, any person interested may make an application to the Controller for grant of compulsory licence; lack of an objective framework for the issue and maintenance of compulsory licences has not made permeation of the likely benefits of Compulsory Licensing in India (*supra)*. Manufactured in India for working of a patent would foster domestic pharmaceutical industry aiding employment of chemists, physiologists, pharmacists, doctors thus effectively contributing to national economy by producing medication that is compatible with the average local income, because the cost of labour would be commensurate with the average income. It would be important to examine the price or availability of a drug becoming perilous through an anti competitive agreement or a combination which has an adverse effect on competition; or the abuse of dominant position and take appropriate regulatory intervention. For affirmative action to take place on SDG-3 relating Good Health and Well being; there is inherent need to revive discussions on Compulsorily License Framework to address issues of equity and reach while maintaining balance between Patent Rights and Competition framework[17].

Conflict of Interest: Nil

Source of Funding: Self Funding

Ethical Clearance: I declare that the article written is my ownliterary and multidisciplinary creation and there is no plagiarism involved. All reference material used for the article has been acknowledged.

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A Study on Sociodemographic Profile and Comparison of Pattern of Facial Injuries in Victims of Two Wheeler Road Traffic Accident with and without usage of Helmet in South Chennai

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Abstract

Background and Aim: Road traffic accident a cause of unnatural death is the third major preventable one amongst all death. Maxillofacial trauma is often encountered in accidents either as isolated injury or along with several injuries to the head, neck, chest and abdomen.

Materials and Method: A retrospective observational study was conducted in the Department of Forensic Medicine and Toxicology at Saveetha Medical College between January 2018 to December 2018 with an objective to analyse the Sociodemographic profile and compare the pattern of facial injuries in Road Traffic Accident cases with and without helmet usage.

Results: Out of total 2438 RTA cases analysed, 1523 were two wheeler RTAs (62.5%). Amongst 1523 cases,173 (11.36%) had facial injuries. The peak incidence of maxillofacial injuries (42.7%) was observed in the age group of 21 to 30 years, with males outnumbering females in all age groups. The maximum number of trauma cases was reported in late evening hours. Maximum RTAs occured in the months of winter usually in the semi urban areas. The injuries were sustained mostly due to frontal impact. Injuries to lip particularly laceration injuries were most frequent. Injuries occured most commonly to the riders. The injuries were seen more amongst the non helmet usage cases amounting to 68% of cases.

Conclusion: The changing trend of etiology of maxillofacial injuries due to Road traffic Accidents in Chennai necessitates strict traffic legislation and better health care facilities with respect to maxillofacial units.

Keywords: *Accident, Etiology, Demographic profile, maxillofacial trauma, helmet.*

Introduction

India accounts for about 10 percent of road accident fatalities worldwide, 85% of all road accident deaths occurring in developing countries, and nearly half in the Asia-Pacific region. [1] The human face often constitute the first point of contact in various two wheeler RTAs. Maxillofacial trauma is thus a common presentation in accident and emergency department of hospitals either as an isolated injury or as a part of multiple injuries to the head, neck, chest and abdomen. These injuries may cause serious physical, psychological and emotional distress. The etiology of maxillofacial trauma varies from one geographical region to another and event within the same region depending on the prevailing socio-economic, cultural and environmental factors. The severity of injury may vary from simple soft

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tissues lacerations to more complicated fractures of maxillofacial skeleton**.** [2] Injuries due to RTA of two wheelers depend upon a number of factors such as human errors, riders fatigue, poor traffic sense, mechanical fault of vehicle, speeding and overtaking, violation of traffic rules, poor road conditions, etc. Clearer insight into etiologic, epidemiologic and demographic factors related to maxillofacial trauma would help prepare more reliable preventive and health care in such cases. [3]

Method

This study was conducted in department of forensic medicine and toxicology in Saveetha Medical Hospital after obtaining clearance from the Institutional Ethics Committee(SMC/IEC/2018/11/559). The basis of the study was data obtained from RTA case record sheets of 173 two wheeler RTA cases who reported to the hospital emergency during the period from January 1st 2018 to December 31st 2018. The case sheets were thoroughly scrutinized and various Socio-demographic profile and maxillofacial injury profile were recorded in specially designed proformas. The data were analysed and interpreted with appropriate statistical tests using SPSS software.

Results

Out of total 2438 RTA cases analysed, 1023 were two wheeler RTAs (41.96%). Amongst 1023 cases,173 (16.91%) had facial injuries . Out of 173 cases with facial injuries, 117 cases (68%) were found not to be using any helmet. More than 3/4th of maxillofacial injuries cases belonged to the riders (142, 82.08%) and pillion riders comprised (31, 17.92%) only**.(Fig 1)**

The age of patients was found to be between 4 to 79 years .Amongst these, more than half of the cases (60.12%) were in the second or third decade of their life, with peak incidence (43.36%) of maxillofacial trauma observed in the age group of 21-30 years. The least number of accident observed between 71-80 years (0.58%). A total of 107 males (61.85%) and 66 females (38.15%) were found in the study. The males outnumbered females in each age group with maximum sex ratio being in the age group of 11-20Years (2.13:1). (**Table-1)**

The greatest incidence (42.77%) of maxillofacial trauma was observed in the evening hours between 6pm to 12am followed by 12pm to 6pm (32.37%). The least number of cases (10.99%) were reported between 12am to 6am.Majority of the cases were found to be in winter (53.17%) followed by monsoon (27.75%).

Majority of RTA cases (22.0%) comprised of unemployed, followed by semi-professional, professional, semiskilled, unskilled, clerical and the least number of RTA cases (4.62%) comprised of skilled workers. 26.01% of the cases belonged to lower class followed closely by upper middle class comprising of 21.97% of the cases.

The maximum incidence (70.52%) of maxillofacial trauma was observed in semi urban areas, followed by rural and the least incidence (12.13%) observed in urban areas. (**Table-2)**

About 1/3rd of the maxillofacial injuries were reported due to skid and fall followed by the impact with an offending vehicle. (**Table-3)** In 63.58% of maxillofacial trauma reported, the collision was from the front direction followed by left and right impact.

The greatest incidence of maxillofacial trauma was observed as laceration (28.32%) followed by abrasion and the least observed injuries were facial bone fractures (21.96%)(**Table-4)**

Majority of maxillofacial injuries in both cases of helmet and non helmet users were reported in lips(62.43%),followed by nose, eye and jaw, chin and the least were reported in cheek and maxilla(2.31%). (**Table-2)**

Table-1: Age and Sex Distribution

Age	Sex		Total(%)	Sex Ratio
	Male	Female		
1-10yrs	4	2	6 (3.47%)	2:1
11-20yrs	17	8	25(14.45%)	2.13:1
21-30yrs	43	32	75(43.36%)	1.34:1
31-40yrs	19	10	29(16.76%)	1.9:1
41-50yrs	15	9	24(13.87%)	1.67:1
51-60yrs	5	3	8(4.62%)	1.67:1
61-70yrs	3	2	5(2.89%)	1.5:1
71-80yrs	1	0	1(0.58%)	-
Total	**107 (61.85%)**	**66 (38.15%)**	**173(100%)**	**1.62:1**

Table 2: Profile of RTA cases with facial injury (N=173)

Characteristics		No. of Cases	(%)
TIME OF ACCIDENT	12:01AM-06:00AM	19	10.99%
	06:01AM-12:00PM	24	13.87%
	12:01PM-06:00PM	56	32.36%
	06:01PM-12:00AM	74	42.78%
SEASON	SUMMER	33	19.08%
	MONSOON	48	27.75%
	WINTER	92	53.17%
PLACE OF RTAs	URBAN	21	12.13%
	SEMIURBAN	122	70.52%
	RURAL	30	17.35%
SOCIO ECONOMIC STATUS	UPPER CLASS	33	19.07%
	UPPER MIDDLE CLASS	38	21.97%
	LOWER MIDDLE CLASS	35	20.23%
	UPPER LOWER CLASS	22	12.72%
	LOWER CLASS	45	26.01%
CRASH DIRECTION	FRONT	110	63.58%
	BEHIND	05	2.89%
	RIGHT	26	15.03%
	LEFT	32	18.50%
AREA OF IMPACT	LIPS	108	62.43%
	EYE	14	8.09%
	CHIN	8	4.62%
	CHEEK	6	3.47%
	NOSE	26	15.03%
	MAXILLA	4	2.31%
	JAW	7	4.05%

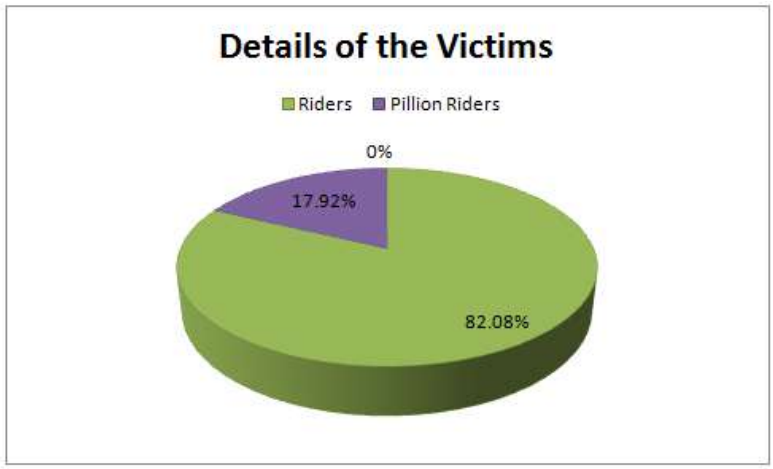


Fig 1: Details of the Victims

Table 3: Type Of Offending Vehicle:

Type Of Offending Vehicle	Total Numbers	Percentage
Motorbikes	56	32.38%
Three Wheelers	6	3.47%
Four Wheelers	19	10.98%
Bicycle	8	4.62%
Lorry	5	2.89%
Skid And Fall	61	35.26%
No Details Available	18	10.40%

Table 4: Injury Pattern With Respect To Helmet And Non Helmet Usage:

Type	Helmet Usage	Non Helmet Usage	Total Numbers
Abrasion	16	31	47
Contusion	11	28	39
Laceration	17	32	49
Fracture Of Facial Bones	12	26	38

Discussion

Considerable variation has been reported in profile of facial injuries with respect to geographical location, socio economic status and cultural background.

The pattern of age distribution in maxillofacial trauma demonstrated people of all ages were affected, the peak incidence was however observed in the age group 21-30 years followed by that between 31-40Years .The second and third decade is possibly the most active period of life where people tend to remain outdoors to explore their means of livelihood and are thus more susceptible to vehicular accidents and falls. Individuals in extremes of life were found to be least affected and most of the injuries (78.61%) were observed in persons of working age group (21-60 Years).

The gender distribution revealed male preponderance in all age groups as has been reported in other studies. The lower socioeconomic group, which constitutes bulk of patients reporting to this particular hospital, men are often the primary bread winners of the family and tend to remain outdoors for a large period of time, thus making them susceptible to trauma in general and

maxillofacial trauma in particular. All so females drive less frequently and are thus less likely to be involved in vehicular accidents especially related to alcohol consumption. [4, 5]

The greatest incidence (42.77%) of maxillofacial trauma following RTA was observed in the evening hours between 6pm to 12am and it was followed by 12pm to 6pm. This finding accordance with those of Veeresha et al and may be attributed to sustainable increasing in traffic after the office hours,when people are returning home and to a tendency to consume alcohol in the evenings. [4]

The most common type of maxillofacial road traffic injury was found to be in semi urban areas (70.52%). Due to heavy congestion and traffic. This will lead to stress and lack of concentration result in accident.

Two factors have been consistently associated with facial injury namely alcohol and unemployment . Unemployment and the associated frustration and rage, particularly in youth of lower socio economic group drives them to take hasty decisions. In developing country, such as ours, RTA is generally believed to be the most common cause of facial trauma [6].This is in consistency with our findings of cases been noticed more in the lower socioeconomic strata [7,8].

The most common type of maxillofacial injury was found to be soft tissue trauma. This finding is in accordance with that of Gassner et al [9], who demonstrated a very high frequency of soft tissue injuries in the comprehensive review of Cranio-maxillofacial trauma and also with those of Le et al [10], who reported that soft tissue injuries were the most common type of injuries: Facial Laceration was the most frequent type of soft tissue injury than abrasion and contusion.

The most common type of maxillofacial trauma subsequent to vehicular accident were reported from skid and fall due to lack of control during riding fast, overtaking and also due to consumption of alcohol, followed by hit and fall by opposite vehicle and the least incidence was observed as hit and fall by dog.

The most common type of maxillofacial trauma were reported for riders as they were about likely to have been involved in a loss of control crash or a collision with another motor vehicle, in comparison with the pillion riders. Possibly, the presence of pillion riders lessen the risk of loss of control and crashes by reducing risk taking or drowsiness [10].

Even though the use of helmets has been made mandatory for the rider and the pillion rider also, the strict adherence to the law is yet to be seen. The type of helmets worn also plays a role in the etiology of trauma. Full-faced helmets protect the rider from brutal injuries when compared to open-face helmets.In our study, non helmet wearers were found to be more than twice likely to have injuries than the helmet wearers. This is in concurrence with another study done in South India that reported four times more likely chance to sustain head injuries amongst victims not wearing helmets. [11]

Conclusion

The maxillofacial injures is proved to be due to traumatic injuries, especially from Road injuries. Provision of well lit pedestrian friendly trail, roads and streets, isolation of heavy and light motor vehicles and strict governance by traffic personnel is must to minimize the physical, psychological and emotional distress associated with trauma in general and maxillofacial trauma in particular.The high incidence of maxillofacial trauma also has implication for the establishment of dedicated maxillofacial units in districts and tertiary care hospital. The effectiveness of various preventive and educational programs with respect to maxillofacial trauma may be publicized through continuing audit of pattern of such trauma in different parts of world.

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Quality of Life among Post CABG Patients

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Abstract

Introduction: Coronary artery bypass grafting (CABG) surgery is one among the most common procedures performed in the world, and it is one of the possible treatments for coronary artery disease (CAD). Although the effectiveness of coronary artery bypass grafting (CABG) is being evaluated in terms of mortality, complications or recurrence of symptoms, empirical studies assessing the change in patients quality of life (QOL) after CABG need to be studied as findings keep changing as time progress. A systematic analysis of how well the patient is considering a multiple variable need to be studied which has greater impact in improving the overall performance of the patients after CABG.

Aim: Aim of the study is to assess the quality of life among post coronary artery bypass grafting (CABG) patients.

Method: A quantitative approach with a descriptive design using a non-probability convenience sampling technique was used to collect data from 101 post CABG patients. The setting used for the study was CVTS OPDs at AIMS, Kochi. A standardized quality of life questionnaire (SF-36) along with a semi-structured questionnaire to assess socio-demographic and clinical data.

Results: The study result showed that the majority of samples (97%) had good QOL with mean score of 69.22. PCS (96%) showed higher value than MCS (68. 3). A significant association found between QOL and socio-demographic variables like occupation (p=0.018).

Conclusion: Overall QOL improved one year after CABG.

Keywords: *Quality of life and CABG.*

Introduction

Cardio vascular diseases is one of the most common diseases seen in elderly persons and approximately 50% of persons over 65 years of age have clinical signs of the disease. Cardiovascular disease is a major cause of functional impairment and is the cause of death in elderly persons. The cardiovascular changes affect the overall functions of persons life(1)

The treatment of coronary artery disease are mainly medical therapy and surgical therapy. Failure of medical management often leads to surgical management, that is revascularization with percutaneous coronary intervention or coronary artery bypass grafting (CABG), uses arterial or venous vessels to create a new pathway for blood to reach the coronary arteries, thus "bypassing" the stenosis(2).

Coronary artery bypass grafting (CABG) is an established and highly effective therapy for coronary artery disease. CABG has been effective than the medical therapy, for the relief of angina pectoris[4]. The first coronary artery bypass operation in human being

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was using a saphenous vein graft and coronary artery anastomosis was performed in November 1964 by Garrett and Debakky[3].

According to American Heart Association (AHA) 2015, health data, more than 190 countries shows coronary artery disease remains as the number one global cause of death with 17.3 million of deaths in each year. The number is expected to rise up 23.6 million by 203010[4]

The global burden of disease (GBD) study reported, it is estimated that 47 million Indians had coronary artery disease (CAD) in 2013. Cardio vascular disease has become the leading cause of morbidity and mortality in India during the last three decade[5].

CABG is associated with financial burden because of days lost from work, excess cardiology visits, diagnostic testing, and use of medications. The self-perception of the quality of life depends on the patient's own abilities to cope with the illness, usually individuals who are better able to adjust to their illness perceive the quality of life as being higher. A poor psychosocial adjustment to illness in turn reflects a more impairment of the quality of life

Preoperative risk scores are essential tools for risk assessment, cost–benefit analysis, and the study of therapy trends. Various scoring systems have been developed to predict mortality and morbidity after cardiac surgery. Risk stratification will inform patients and clinicians of the likely risk of death for a group of patients with a similar risk profile undergoing the proposed operation. This information is useful and should form part of the basis on which the patient and surgeon decide whether to proceed or not. Risk models were also applied for quality improvement programs comparing year-to-year outcomes, as well as allocation of healthcare resources through the prediction of length of stay and postoperative complication rates [11].

Although the effectiveness of coronary artery bypass grafting (CABG) is being evaluated in terms of mortality, complications or recurrence of symptoms, empirical studies assessing the change in patients quality of life (QOL) after CABG need to be studied as findings keep changing as time progress. A systematic analysis of how well the patient is considering a multiple variable need to be studied which has greater impact in improving the overall performance of the patients after CABG.

Although the effectiveness of coronary artery bypass grafting (CABG) is being evaluated in terms of mortality, complications or recurrence of symptoms, empirical studies assessing the change in patients quality of life (QOL) after CABG need to be studied as findings keep changing as time progress. A systematic analysis of how well the patient is considering a multiple variable need to be studied which has greater impact in improving the overall performance of the patients after CABG.

Method of Study

Quantitative research approach with descriptive design was used to identify the QOL among post CABG patients. The study was conducted among 101 post CABG patients after one year of surgery and coming for follow up in CVTS OPDs of Amrita Institute of Medical science, Kochi. The subjects were selected by non-probability convenience sampling technique. The data were collected using structured questionnaire. The tool developed and used for the study were tool I was a semi-structured questionnaire to assess the socio-demographic characteristics, which had two sections. Section A socio –demographic variables and section B included clinical variables. Tool II was a SF-36 standardized tool to assess the quality of life of post CABG patients. The tool developed and used for data collection was a standardized structured questionnaire. The content validity was obtained from 7 experts. The tool was found to be valid. Informed written consent was obtained from the samples after explaining the purpose of the study. Data collection was conducted in CVTS OPD. Anonymity and confidentiality of samples were maintained. A pilot study was conducted among 10 samples who met the eligibility criteria . It was found that the research design was appropriate, research tools were valid and reliable and the study was amendable to statistical analysis .The data was analyzed using descriptive and inferential statistics. The description of sample characteristics based on socio-demographic and clinical variables were computed using descriptive statistics. Association between QOL and selected socio demographic and clinical variables were assessed using inferential statistics.

Result

Table I: Distribution of sample characteristics based on demographic variables n = 101

Sl. No.	Demographic variables	Frequency (f)	Percentage (%)
1	**Marital status**		
	Single	31	30.7
	Married	58	57.4
	Widow/widower	10	9.9
	Divorced/separated	2	2
2	**Education**		
	Primary education	32	31.7
	High school	46	45.5
	Diploma	23	22.7
3	**Occupation**		
	Employed	60	59.5
	Unemployed	41	40.5
4	**Food**		
	Vegetarian	21	20.8
	Non- vegetarian	80	79.2
5	**Income**		
	2165-6430	3	3
	6431-10718	13	12.9
	10719-16077	43	42.6
	16078-21347	42	41.6
6.	**Type of family**		
	Joint family	9	8.9
	High school	92	91.1

The data presented in table I shows that the majority of sample 65(64.4%) were under the age group of 61-70 years. In gender, majority of sample were male 92 (91.1%). Most of the sample 58(57.4%) were married & 46 (45.5%) were having high school education. The data illustrated that most of the samples were unemployed retired 27(26.7%) and 25(24.8%) were private employed. The monthly income of 43(42.6%) sample were ranges from Rs. 10719-16077. When considering the food habits most of the 80(79.2%) sample were non vegetarian. The majority 92(91.1%) of the sample were belongs to nuclear family.

Table 2: Distribution of sample characteristics based on clinical variables n=101

Sl.No.	Clinical variables	Frequency (f)	Percentage (%)
1	Types of surgery		
	ON pump	16	15.8
	OFF pump	85	84.2
2	**Indications of CABG**		
	Single vessel disease	2	2
	Double vessel disease	23	22.8
	Triple vessel disease	76	75.3

Sl.No.	Clinical variables	Frequency (f)	Percentage (%)
3	**Ejection fraction**		
	Normal (52-72%)	78	77.2
	Mild (41-51%)	19	18.8
	Moderate (30-40%)	4	4
4	**Alcoholic habits**		
	Current alcoholic	3	3
	Non –alcoholic	27	26.7
	Ex-alcoholic	62	61.4
	Occasionally alcoholic	9	8.9
5	**Smoking habits**		
	Current smoker	6	5.9
	Non-smoker	30	29.7
	Ex-smoker	65	64.4
6	**Body mass index**		
	Over weight	5	4.9
	Obesity	1	1
	Normal	95	94.1
7	**Complications after CABG**		
	Without complications	60	59.4
	Wound infection	1	1
	Pain	1	1
	Others	39	38.6
8	**Hours of sleep per day**		
	More than 10 hours	7	6.9
	6-8 hours	78	77.2
	4-6 hours	10	9.9
	4 hours	6	5.9
9	**Co morbidities**		
	One co-morbidity	1	1
	Hypertension	13	12.9
	Diabetes mellitus		3
	Dyslipidaemia	3	
	Two co-morbidities	11	10.9
	Hypertension and diabetes	17	16.8
	Hypertension and dyslipidaemia		
	Dyslipidaemia and diabetes	4	4
	Three co-morbidities		
	Hypertension, diabetes mellitus and dyslipidaemia	15	14.9

Data in the table-2 depicts that among 101 samples the common types of surgery were OFF pump 85(84.2%). Consider the indications of surgery majority were having triple vessel disease 76(75.3%) and were having normal EF 78(77.2%). Regarding smoking habit majority of the samples were ex-smoker 65(64.4%) and 62(61.4%) were ex alcoholic. Among the samples 95(94.1%) had normal BMI. Considering the complications majority 60(59.4%) were had no complications after CABG. Regarding the hours of sleep per day most of them had 6-8 hours of sleep.

Section II: Description of QOL assessment of post CABG patients:

Table 3: Description of quality of life among post CABG patients n=101

	Minimum value	Maximum value	Good QOL (%)	Poor QOL (%)	Mean value	Standard deviation
Overall quality of life (QOL)	39	94	97	3	69.22	9.368

Table 3 shows that among 101 samples majority of them (97%) had good quality of life with a mean score of 69.22 and standard deviation of 9.368.

Section II: Description of samples based on components of quality of life n=101

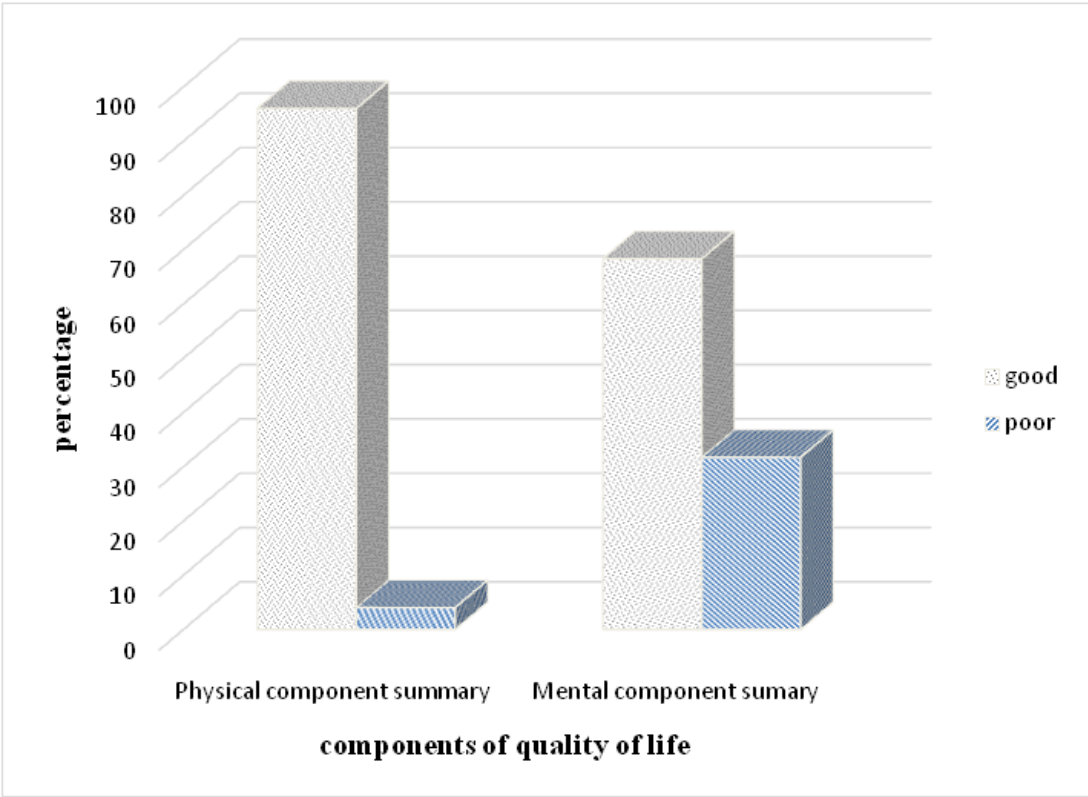


Figure 1: Bar diagram representing distribution of samples based on components of quality of life.

Figure 1 depicting the components of QOL, in the physical component summary 96% had good physical component score with mean score 77.98 and 68.3% had good mental component score with mean value of 57.07. Hence physical functioning was considered to be better than mental functioning

Section II : Description of samples based on domains of physical components summary n=101

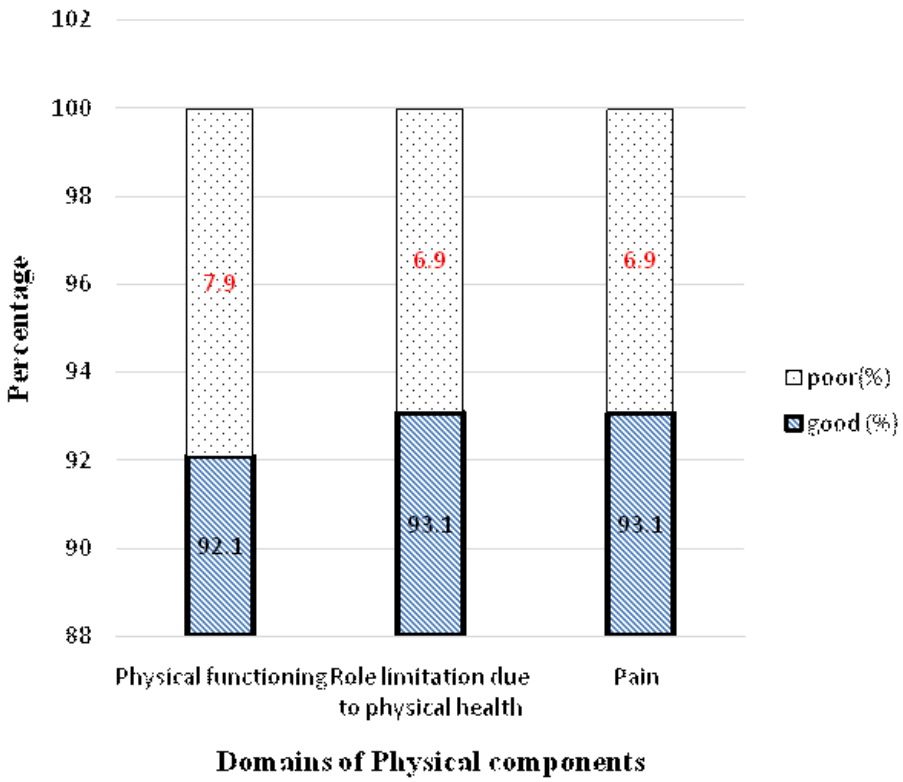


Figure 2: Bar diagram representing quality of lifebased on domains of physical components

Figure 2 depicting domains of physical component summary (PCS) of QOL. Majority (93.1%) expressed they had no role limitation due to physical health, regarding pain majority (93.1%) had no pain. Considering the physical functioning, majority (92.1%) had good physical functioning.

Section II: Description of samples based on domains of mental components summary n=101

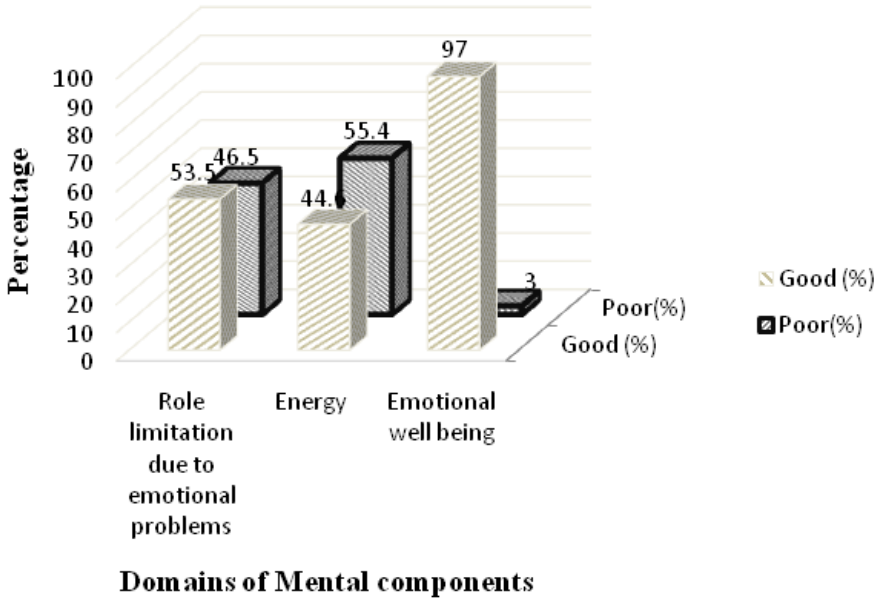


Figure 3: Diagram representing quality of lifebased on domains of mental components

Figure 3 depicting domains of mental component summary (MCS) of QOL. Majority (97%) samples experienced good emotional wellbeing and 53.5% expressed good score in role limitation due to emotional problems. Considering energy and fatigue 44.6% had good energy.

Distribution of mean and standard deviation of quality of life: Among 101 samples the highest mean score was observed in bodily pain (84.30), it means that patients had less pain and lowest mean score was observed in energy/fatigue (46.93) means that they had lack of energy.

Association between quality of sleep and selected demographic variables: The association between Quality of sleep and selected demographic variables like age, gender, marital status, education, income, diet pattern and type of family were not statistically significant with p value of 0.05 level. Whereas, occupation showed statistically significant (0.051).

Discussion

The first objective was to identify the quality of life among post coronary artery bypass grafting patients: The results from the present study showed that overall quality of life of post CABG patients 97% had good quality of life with mean score of 69.22 and standard deviation 9.368. Considering the physical component summary 96% samples had good quality of life with mean score 77.98, and mental component summary 68.3% samples had good quality of life with mean score 57.07. That means PCS higher than MCS.

In relation to physical component summary, majority of the samples (93.1%) expressed no role limitation due to physical health with a mean score of 73.64. considering the physical functioning most of them (93.1%) were having good physical functioning with a mean score of 73.79. Regarding pain 93.1% had no pain with mean score of 84.30.

In relation to mental component summary most of the sample (55.4%) were lacking energy level with mean score 46.93. Regarding role limitation due to emotional wellbeing majority of the patient (53.5%) weren't having any role limitations with a mean score of 55.78. A vast majority of sample (97%) expressed good emotional well-being with a mean score of 68.51.

The similar findings were observed in a descriptive study conducted by Anastasios Merkouris, Dimitrios Pistalos to assess the QOL after CABG patients at Greece in 2008. The findings showed that the overall QOL of patients was improved, good level of QOL with an average score of 80.4[6].

The present study findings are contradictory to a study findings done by Lee GA conducted a cohort study to assess the patients reported health related quality of life five years post CABG at Australia in the year 2008. The findings showed that good level of QOL with an average score of 66.02%. MCS (53.6) was higher than PCS (45.8). PCS had poor QOL and MCS had good QOL[7].

The second objective was to find the association between the quality of life and with selected socio demographic variables among post Coronary Artery Bypass Grafting patients

The present study showed a significant association with occupation and QOL (p=0.018), QOL was unaffected by all the other socio-demographic variables

Taghipour H.R conducted a descriptive study to assess the QOL one year after CABG at Iran in 2011. The result showed that mean age of patients was 61.4, most of them are male. On pump surgery had better general health than off pump 47.9 (p=0.024). Negatively correlated with EF r=-0.220. chi- square value is 0.19[8].

Descriptive study conducted by Elishevasimchen to assess the relation between QOL and socio demographic factors at Israel. The findings showed that female sex and low socio economic background were associated with low health related QOL.[9]

Conclusion

Overall QOL improved one year after CABG. majority of the samples expressed more physical wellbeing than of emotional wellbeing henceforth,there is an alarming need to give a holistic care for patients.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Ethical clearance obtained from institutional ethical committee of Amrita Institute of Medical Sciences, Kochi for conducting this study.

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Pulpectomy: A Comprehensive Review

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Abstract

Several pulp therapies both vital and non-vital pulp therapies for preserving the physiologic integrity as well as the well being of the tooth have been developed as a necessity for the healthy growth of the teeth. It mainly aims at protecting the teeth from trauma, caries, or any other infections. Pulp exposures resultants to caries are the most universal in deciduous teeth due to the relatively bulky size of the pulp chamber. The pulp in deciduous teeth is capable of healing, following control of infection, and inflammation. A pulpectomy is mainly preferred in non-vital teeth (done in various vital teeth also), where the teeth can be cleaned and disinfected and preserved for more no. of coming years. Pulp rehabilitation for pulpally implicated deciduous teeth continues to be a confront to practitioners. One of the majority important areas of continued examination is in the area of discovering obturating resources to suit the precise properties of these teeth. This piece of writing aims at reviewing the objectives, types, technique, restorative materials used, indications and contraindications and conclusion.

Keywords: *Pulp, Pulpectomy, Root Canals, Resorbing Material, Obturating Material, Vital And Nonvital Pulp Therapies*

Introduction

One of the majority important services a pediatric dentist can make available for the child patient is the adequate treatment of pulp involved deciduous teeth. For many years, different principles as well as techniques for the management and conservation of deciduous teeth have been suggested by the dental profession. The pulp is an obligatory element for maintaining the healthy physiological integrity of the tooth. Conditions like irreversible pulpitis, pulp necrosis, or dentoalveolar abscess in deciduous teeth are best treated by non-vital pulp therapies. Soft tissue therapy is extensively used during the management of pediatric patients, at the same time as attempting to stop happening impulsive exfoliation of the deciduous teeth.[1] The most important purpose of endodontic supervision is the full amount of exclusion of microorganisms commencing the root canal, and the avoidance of consequent reinfection. This is achieved through cautious cleanout in addition to shaping followed via the total obturation of the canal space. The final objective of endodontic obturation has remained the same intended for the past 50 years: to generate a fluid-tight close up along the length of the root canal arrangement, commencing the coronal opening of the apical termination. A pulpectomy is one of these therapies conducted on deciduous teeth for the prevention of subsequent re-infection by the total abolition of the microbes and bacteria from the root canals.[2]

Definition: Pulpectomy is a dental procedure that involves the entire exclusion of the coronal as well as the radicular portion of the pulp cavity to get admittance to the root canals that are debrided, inflamed, distended as well as disinfected moreover resorbable substance are used to fill the canals.[3]

Types:

Partial Pulpectomy: One third to one half of the coronal segment of the radicular pulp tissue is detached from the canals.

Complete Pulpectomy: complete removal of pulp tissue from the root canal

Objectives:

- Resolving of the infectious process, following the treatment

- Overextension as well as under filling should be avoided with successful radiographic evidence.
- Resorption of the deciduous tooth structure as well as filling material should take an appropriate time
- No other pathology or internal or external root resorption should be present
- There should be no sensitivity, pain or swelling present
- No further breaking down of the supporting tissues after the treatment.

Indications:

- Patients along with their parents should be cooperative.
- A patient with good health along with no serious disease is preferred
- Indications of deciduous teeth in absence of its permanent successor
- Irreversible pulpitis
- Internal resorption
- At least two-thirds of the root length is supposed to be accessible.

Contraindication:

- Mobile tooth or reduced bone support
- A tooth that is non-resorbable
- Any dentigerous or follicular cyst present beneath
- Root length remaining less than 2/3rd
- Perforation of pulpal floor
- Children those are medically compromised

Procedure:

Single-visit Pulpectomy:

Indication: This course of action is an addition of pulpotomy. Wherever the inflammation extends further than coronal soft tissue, indicated by hemorrhage from the amputated radicular stumps with the purpose of is oozing endlessly and is unmanageable.[4]

Procedure:

- Suitably administer local anesthesia along with isolation of the tooth using the rubber dam.
- Arrange an access cavity; expose the coronal soft tissue; all the reachable pulp tissue (coronal as well as radicular) is extirpated with the assistance of barbed broaches.
- After extirpation of the pulp tissue is completed, irrigate the pulp cavity as well as a canal using saline in addition to a diagnostic radiograph that is in use for the working length of the file.
- Shaping, irrigating of canals be undertaken, dehydrated the canals with absorbent paper points.
- Position the final restoration

Multiple visit Pulpectomy:

Indication:

- A tooth with necrotic pulp in addition to periapical involvement.
- The occurrence of illness, chronic sinus, or abscess.
- Nonvital deciduous tooth.

Procedure:

Through the first appointment:

a) Correctly administer local anesthesia as well as detach tooth from surrounding oral environment using a rubber dam.

b) arrange the access cavity as well as expose the coronal soft tissue along with extirpating of all pulp tissues using barbed broaches

c) Position the cotton pellet above the pulp chamber soaked by formocresol along with temporary restoration set over it.

Following the period of 1week recall the patient.

Through the second appointment

a) Smoothly eradicate impermanent restoration; irrigate the canals as well as begin with biomechanical preparation of canals.

Note: Employ appropriate working length of files to stay away from perforation of canals.

b) Irrigate the dry canals as well as place sterilized cotton pellets in addition to position temporary restoration above it.

Through the third appointment

a) Smoothly eradicate the temporary restoration, irrigate as well as dry the canals using absorbent paper points.

b) Coat the ramparts of canals using the luting consistency of cement with the assistance of reamers along with them fill the canals by bulky consistency of cement through the help of lentiluospirals.

c) Stick down the chamber through the temporary restoration.

Following the period of 1-week call the patient.

through the fourth appointment

a) Condition tooth is asymptomatic, place the final restoration; set a stainless-steel crown.

Procedure:

Access Opening:

Anterior deciduous teeth: The best sites of access opening are the lingual surfaces of the anterior deciduous tooth along with the permanent tooth. Except that stained maxillary deciduous incisors are approached through facial surface followed through an acid etch composite restoration.[5]

Posterior deciduous teeth: Deciduous Teeth are comparable to those of the permanent teeth. The simple divergence among the deciduous and the permanent teeth are the level of the crown, the bulging form of the crown as well as the very thin dentinal ramparts of the pulpal floor and its roots.

While the top of the pulp chamber is detached distinguish the orifice of the root canals.

- Canal cleaning along with shaping: - The canals cleaning in addition to shaping is the mainly considerable step of deciduous root canal management. The foremost intention of biomechanical preparation is debridement of canals. Only NiTi files are suggested for the deciduous root canal preparations. The canals are enlarged several sizes past the 1st file to fit contentedly in the canal through a minimum size of 30 to 35 no. file.
- Working length determination: - At the beginning working length is determined using a radiograph. The working length is after that determined via a radiograph by a Ni-Ti file inside the canals (apex locator is undependable and unreliable in working length determination in deciduous teeth). It is advisable with the intention that the working length would be shortened to 2 to 3mm short of radiographic root apex for precise length determination.[6]
- Filling with the material.

Obturating Techniques: Numerous techniques have been intended for substantial filling of substance into deciduous teeth canals The deciduous teeth with the bigger canals are capable of being filled through the slim mix; coating the ramparts of the canals with the aid of reamer in an anticlockwise course at the same time as taking out slowly followed by placement of thicker mix which is then pressed manually. The paste can be filled by way of lentiluospirals mounted on the micromotor handpiece. The course of rotary motion needs to be checked for the substance to appropriately flow into the canal. The endodontic pressure syringe is as well effectual for the insertion of the materials that are used in the canals. The vitapex system besides uses the syringe with the material in it. The syringe is introduced up to 1/5th distance as of the apex of the canal along with the material which is slowly injected at the same time as the syringe is withdrawn from the canal. Despite the technique adopted to fill the canal, care is supposed to be taken to avoid extrusion of material into the periapical tissues. The sufficiency of obturation is checked using radiographs. In the occurrence, if a small amount of ZOE is by mistake enforced through the apical foramen it is left unaccompanied since the material is resorbable. While the canals are satisfactory obturated a quick set temporary cement is positioned in the pulp chamber to close over ZOE canal filling. The deciduous tooth is restored in the company of a stainless steel crown.[7]

Filling of the deciduous root canals: Developmental, anatomic, and physiologic differences stuck between deciduous and permanent teeth call for differences in the criteria designed for root canal filling materials.[8]

Ideal requirements are:

- Resorb speed of the deciduous roots as well as the resorb material be supposed to be identical.
- It is supposed to be undisruptive to the periapical tissues along with the permanent tooth germ, resorb readily if hard-pressed further than the apex.
- It is supposed to have an unwavering disinfecting influence.

- It is supposed to be inserted without difficulty into the root canal as well as removed effortlessly if required.
- It is supposed to hold fast to the walls of the canals as well as should not shrink.
- It must not be soluble in water.
- Be radiopaque as well as not stain the tooth.

Filling materials used:

- ZOE paste: most regularly worn filling material in deciduous teeth. Disadvantages are the divergence stuck between its rate of resorption as well as that of the tooth root, including overfilling and underfilling. The success rate is 65%. This is also known as zinc oxide eugenol paste.
- Iodoform paste: Also known as KRI paste (Iodoform), it absorbs speedily as well as has no enviable result on succedaneous teeth when used as a pulp canal medicament during abscessed deciduous teeth. It even has a long-lasting bactericidal effect. Its success rate is 85%.
- Calcium hydroxide: calcium hydroxide along with Iodoform mixture has been found effortless, to apply as well as resorbs imprecisely more rapidly than that of the root, with no noxious end product on the descendant tooth in addition to, it is radio-opaque. It is nearly an ideal filling material with a success rate of 100%.
- Colla cote: it is a soft, white, pliable, biocompatible sponge obtained from bovine collagen. It is an absorbable collagen barrier, which prevents or diminishes extravasations of filling material used in the root canal treatment during deciduous molar Pulpectomy.
- Endoflas: - it can be used as an alternative root canal filling material for deciduous teeth. Microleakage should be prevented to have a success rate. Any signs of inflammation, the filling being replaced with the permanent filling material. The success rate is 70%.[9,10]

Conclusion

In conclusion, I would like to say that calcium hydroxide – Iodoform mixture is the closest to the being the ultimate root canal filling substance for deciduous teeth.

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Ethical Marketing in Orthodontics: A Review

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Abstract

Due to rise in the Orthodontic treatment by general dental practitioners and pedodontists, there has been a widespread increase in the mass promotion and publicizing by the Orthodontists about their specialty and particular expertise. Earlier, treatments were advocated in the form of maintenance of good oral health, however, it is now being recommended to patients as life-enhancing. Commercialism in publicizing orthodontic care has taken up the field. Because of the elective nature of Orthodontics, highly productive marketing strategies are aimed at those with discretionary income or insurance benefits. This kind of commercialism deteriorates the name and fame of the Orthodontist and the opinion of the people by casting a picture of greed. Inappropriate advertising can make a dental practitioner look more like a business person than a professional service provider.

Keywords: *Ethical Marketing; Orthodontic treatment; Dental Practitioner.*

Introduction

Years of self-regulation in the field of medicine, dentistry, and allied health sciences ended with the change in the law by The Supreme Court acknowledging the Federal Trade Commission's proposition to legalize advertising by the health care providers in the year 1975. This change was basically due to the lack of basic and relevant information to the general public and the various options to explore while selecting a health service provider through advertisements and promotions.[1-3] However, the opponents of the ruling had the apprehension that some health care personnel would misuse the freedom of marketing, thus, reducing professionalism into mere commercialism. According to Welie et al, marketing or advertising is ethically difficult because it is more of a commercial than a practicing genuine dentistry. The client knows, he has to be aware to get the best bargain and he knows the trader will make efforts to gain maximum profits. This has always been the game of merchandising. Advertisements are used by traders or sellers to attract clients away from other traders. Similarly, advertisements by Orthodontists encourages competitions among other Orthodontists and general dentists and Pedodontists who also provide Orthodontic treatment rather than a sense of joint responsibility for providing quality dental care.[1-3]

Ethical Principles of Veracity in Marketing: Ethical marketing is a method of recognizing, foreseeing and satisfying the needs of the patients in a profitable manner which the dentists do in their regular practice such as consultation, patient examination and the required treatment, for years. This requires thorough research of the market by understanding the needs of the people, anticipating their future requirements and building a product or delivering a service that would satisfy the need, publicizing it and confirming that the whole procedure is profitable. On the other hand, Unethical marketing is influencing people and convincing them to purchase what one is intending to sell.[4]

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Orthodontists, not anymore depend on the referrals from the general dental practitioners, but have switched to a different approach to the market and promote themselves to the likely patients directly. This method of marketing is known as "direct-to-costumer" marketing. The outcome is that some Orthodontists have become shrewd and determined marketers, to achieve freedom from the general practitioners on whom they were profoundly dependent, even if there is a violation of ethical code and professionalism. The AAO's Ethical principles and Code of Conduct, section V, mentions that the members of the association shall make sure that the announcements made by them in the public or any publicizing campaigns or activities with an intent to inform and enlighten the people and help them make informed decisions, are not bamboozling, untruthful, or fabricated. This reprimand is also mentioned in the "American Board of Orthodontics' (ABO) Guidelines for the use of the diplomate title or logo" in which the ABO prohibits diplomates to issue any statement about their reputation that renders them more proficient or competent than non-boarded Orthodontists. [5-7]

In the past, because of the FTC ruling, 1975, the commercialism has almost taken over professionalism in the field of health. Each Orthodontist must have an attitude that creates a sense of professionalism and exhibits the utmost care for the patients. This will attract patients to maintain the respect and value for our society. Being authentic and restricting one from portraying supremacy might help in preventing the degradation from Orthodontic specialty to meager trade. Furthermost, the suitable way to react to any other practitioner's inappropriate methods of advertising is to retain one's professional stance in all forms of marketing, rather than adopting methods that defame, devalue or degrade our specialty.[5,6]

Clarity in Marketing: Several patients and general dental practitioners are not aware of the scholarly importance of a specialist and the advantages of board recognition. A lot of patients are fascinated by the faster esthetic enhancement. According to the Pennsylvania Code, marketing or advertising is fabricated, untruthful, or fallacious if it is adopting any one of the following:

a) Consists of a falsification of facts or excludes a fact obligatory to make the report considered altogether and not materially deceiving.

b) Builds baseless expectations about the treatment results the dentist can accomplish

c) Evaluating the publicizing dental practitioner's facilities with the facilities of other dentists unless the evaluation can be realistically validated. [8]

If any untruthful or fallacious advertising is found, a complaint will be filed against the offender in the State board's professional compliance cell. There will be an inquiry for penalization if the complaint is found relevant. There are disparities between the ethical principles of the AAO and the specialized laws implemented by each state. Breach of any law or principle of ethics could lead to eviction from the association and board; and breach of the legitimate laws by the state could result in a way serious aftermath apart from the monetary penalty or restraint from practice. The parameters for ethical marketing are distinctly demarcated for both the general dental practitioners an Orthodontic specialist. Even if certain general practitioners facilitate Orthodontic treatment, layman should not be confused when selecting a dental health care provider. The Orthodontists should try to reduce the exposure of the prospective patients to deceptive and untruthful marketing. Along with the presence of the ethical rights of the patient, the orthodontist should make sure that the patients get the best that dental science can provide.[2]

Discretion in Marketing: The 1975 ruling was done to improve the well-being of patients by promoting free competition and information on available facilities. It was meant to be advantageous and constructive for the ones who used discretion in their marketing strategies. However, the opinion and morals vary among the people and the health care providers and there remains a fine thin line between commercialism and professionalism.[3] Many Orthodontists get involved in group discount marketing which is not only damaging to the image of the specialty but also harmful to the Orthodontists as he/she loses the skill to choose which patients to treat. While the right to autonomy gives patients their right to select different alternative options for treatment, it also permits the Orthodontist to choose his/her patients. The group discount advertisement makes the Orthodontist give up the freedom of choice as anyone who responds to the offer is mandatory to be attended by that practice. Also, the Orthodontist in the contractual agreement with the group coupon advertisement administrators, gives away the freedom to manipulate the fundamentals of the proposal as well as the percentage of the fees collected for treatment from the patients. This involves "fee-splitting" which is highly unethical as well as illegal. [9,10]

Widespread flashy and derogatory marketing makes us wonder if the future generation of Orthodontists will value the dignity and respect our forerunners have established for our specialty. It took years to uplift the field of dental sciences and Orthodontics to an eminent and notable status. Thus, we must always be cautious among us to avoid deterioration and regression of our profession to meager trade.

Acceptable Self-Promotion: Self-promotion is defined as performance shown or action taken to grab attention mainly in work or business. There was a time when self-promotion of an Orthodontist and his practice was thought to be immoral. Even there were restraints on marketing and campaigning. Even the letter size on the board outside an Orthodontist's clinic or house was strictly restricted to 8 inches. Marketing through newspapers, magazines, or on television was never heard of and would have been considered unacceptable.

Time has changed drastically. Today, marketing by Orthodontists has become common. For a few years, the AAO has expended a significant amount of time and wealth, publicizing Orthodontists to the general people through magazines. Campaigning through television targets the adults but how well they influence the people to make suitable choices whether to go for Orthodontic treatment or not and if yes then which and whose office to choose is always a matter of question. Strategies like entertaining your referral sources by giving them holiday grants or taking them for lunch, perhaps maybe advantageous but would cost a fortune and also demands a lot of time. Nevertheless, self-promotion can be done in a way that costs minimal, educates the people and publicize your practice without breaching the code of ethics. The most efficient method to publicize starts at the patient level through a satisfied patient.[12] Patients who are satisfied with the Orthodontic treatment will return and also refer their relatives and friends due to the positive experience they had with the particular Orthodontist. A way to achieve this is by surrounding yourself by pleasing staff and to behave in an amicable and friendly manner with the patients every time. Always having a follow-up session post-Orthodontic treatment creates a positive impact on the patients. There are numerous advantages of the follow-up appointments. Firstly, regularly reviewing the pre-Orthodontic treatment and post-Orthodontic treatment records of the patients provide substantial information regarding the treatments and their approaches. Secondly, for the endurance of the practice, this allows highlighting the long term functional and aesthetic improvements due to the Orthodontic treatment to the patients and their family. Thus, post-Orthodontic treatment consultation can be considered as an acceptable self- promotion.

Direct-to-Consumer Marketing: "Direct-to-consumer (DTC) marketing" provides information regarding the products directly to the consumers or buyers. But the marketing campaigns do not provide all the information about the products and which can be deceiving.[12] Invisalign adopted the marketing strategy of DTC marketing that disturbed the Orthodontic society. At present, Invisalign advertisements range from ads in magazines to commercials that air on television. However, there is a reasonable concern that these ads could be deceptive and misinforming. Invisalign advertisements portray each one of us as a probable patient, and that may not be true. It entails that treatment with bracket system and wires is painful and an obsolete technology and treatment with clear aligners is comfortable and esthetically pleasing. The advertisements obscure the line between the Orthodontists and a general dental practitioner. Perhaps, this sort of marketing has increased the need for the Orthodontists but with a negative impact on the Orthodontic specialty. Also, due to the direct marketing to the consumers, patients turn up at the clinic with an expectation of treatment with Invisalign without a complete examination to ascertain whether they are suited for it or not. General dental practitioners with incompetent diagnostic proficiencies and training consider that altering clear plastic trays can treat any malocclusion. This, in turn, puts pressure on the Orthodontists to recommend their patients for Invisalign treatment due to the distress of losing a patient to any other dentist. Such a scenario is neither in favor of the patients nor follows the ethics of marketing. With the growth in advertisements on social media, misleading claims of Orthodontic treatment in six months were advertised on various platforms of social media which is highly deceiving. It misinforms people about the differences in expertise and training of general dental practitioners and Orthodontists, as well as, the difference in the treatments provided by them. Over the years, Orthodontists have started marketing directly to their patients by adopting many external as well as internal marketing strategies.[12] Many were not in terms of marketing ethics such as branded tokens, small presents, discount coupons, etc. However, the best long-term direct marketing is always recommendations from a satisfied patient. The most influencing sort of

external marketing in today's generation is the website of Orthodontic practice. Such websites are increasingly developing into more attractive and communicative incorporating links of various platforms of social media for interacting with the designated fraternity or with the satisfied patients and their relatives.

Contemporary forms of direct internal marketing comprise several ways to attract patients such as including newer advanced technology, keeping typodonts to educate patients, a photographic book of pre and post-Orthodontic treated patients, a wall exhibiting the smile photographs of the best-finished cases. Irrespective of the various current methods of marketing, Orthodontists are trying to make use of this opportunity to educate and create awareness among people in particular. Direct mails, posters inside the clinic or even colloquiums conducted for patients, provide informative data like the appropriate age and time for the initial visit to an Orthodontist and the advantages of different types of Orthodontic treatment. Several Orthodontists also meticulously make effort to educate people regarding the difference between a general dental practitioner and an Orthodontist not with a selfish motive but with a motive to ensure the general public is getting the Orthodontic treatment from a specialist who is an expert in his field having proficient knowledge of his work.

Conclusion

Numerous circumstances have affected the kind and extent of marketing in the field of Orthodontics. This even consists of the surge in number of general dental practitioners providing amateur Orthodontic treatments, the violation of the ethical principles of marketing, the excessive advertising by "direct-to-consumer" ads, and alterations in referral patterns and sources: competition instead of collegiality. The present DTC marketing method by Orthodontists has been the major reason for the increase in such issues. Genuine referrals by general dental practitioners will always be a vital source to an Orthodontist; however, increased marketing directly to the patients targets at enormous freedom, but must be considered with sincere concern for professional code of ethics.

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Effect of Nursing Intervention on Prevention of Exclusive Breastfeeding Discontinuation

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Abstract

Introduction: Planned nursing intervention and health education regarding exclusive breastfeeding (EBF) improved knowledge, practice and attitude of mothers and contributed to prevention of EBF discontinuation which can consequently reduce the worldwide infants' morbidity and mortality rates. **Aim of the Study:** The present study aimed to investigate the effect of nursing intervention on prevention of EBF discontinuation. **Study Design:** A quasi-experimental design was utilized. **Setting:** Obstetric Outpatient Clinic, Breast Feeding Room in NICU of Children's Hospital affiliated to Ain Shams University hospitals and MCH center in Abassia sector/Cairo/Egypt. **Subject:** A purposive sample of 100 mothers of infants aged from birth up to 6 months. **Tools of the Study:** Predesigned questionnaire to assess: mothers and infants characteristics, knowledge regarding factors affecting EBF discontinuation and its prevention, Iowa Infant Feeding Attitude Scale and observational checklists to assess mothers' breastfeeding practice. **Results:** It was clear that the majority of the studied mothers had good knowledge, positive attitude towards prevention of EBF discontinuation and improved practice of breastfeeding post implementation of nursing intervention. There was statistical significant difference between total knowledge of the studied mothers regarding factors affecting EBF discontinuation and its preventionand total practice of breastfeeding pre/post nursing intervention. **Conclusion:** Nursing intervention had a positive effect on prevention of EBF discontinuation. **Recommendation:** Developing initiatives,pre and postnatal health education programs for protecting, promoting and supporting breastfeeding which aid in the prevention of EBF discontinuation.

Keywords: *Exclusive breastfeeding, prevention, discontinuation, nursing intervention.*

Introduction

Exclusive breastfeedingis the cornerstone of child survival, nutrition, development and maternal health. Infants take EBF have higher intelligence scores, resulting in higher educational achievement. Breastfeed Mothers have a lower risk of breast and ovarian cancer, improved birth spacing and may also have a lower risk of diabetes and overweight/obesity. The world Health Organization recommends EBF, including initiation within the first hour of life and continues for up to 2 years.[1]

Discontinuation of EBF can negatively affect a child's physical and emotional development in both short and long-term achievement. Annually EBF could prevent 823,000 deaths in children under age 5 and 20,000 deaths in mothers from breast cancer. Only 42% mothers begin breastfeeding within an hour of birth, and 41% practice EBF.[2] In Egypt; breastfeeding practices are not always optimal. The EBF among infants under two months of age constitutes 71%. By age 4-6 months, only 13% are exclusively breastfed.[3]

Nurses have an important role in protecting, promoting, supporting and preventing EBF discontinuation through initiatives to improve positive attitudes and enhanced education. Mothers need antenatal

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care to reduce perinatal and neonatal problems. Training community health care workers can provide support and knowledge to lactating mothers for prevention of EBF discontinuation.[4]

Material and Method

The aim was study the effect of nursing interventionon prevention of EBF discontinuation using the quasi-experimental design in the current study. It was conducted at Obstetric Outpatient Clinic, Breast Feeding room in NICU of Children's Hospital affiliated to Ain Shams University and MCH center in Abassia sector/ Cairo/Egypt. The study sample was a purposive sample that composed of 100 mothers of infants aged from birth up to 6 months regardless their characteristics.

The first tool of data collection was the pre-designed interviewing questionnaire which was prepared by the researcher in an Arabic language after reviewing the related literature. It was designed to gather the dataregarding characteristics of the studied mothers and mothers' knowledge regarding factors affecting EBF discontinuation and its prevention (pre/post nursing intervention).Items of mothers' knowledge regarding factors affecting EBF discontinuation and its prevention consisted of 30 closed ended questions. Knowledge scores were classified into good (>75%), average (65:75%) and poor knowledge (< 65%).

The second tool was Iowa Infant Feeding Attitude Scale (IIFAS) that was adapted from De la Mora, Russell, Dungy, Losch and Dusdieker (1999) to assess attitude of the studied mothers towards prevention of EBF discontinuation. The IIFAS contains 17 questions. The total score of attitude was 17 point. Score of less than 60% was disagree and the score between 60% to less than 75% was neutral and equal or more than 75% was agree (pre/post nursing intervention).

The third tool was observational checklist that was adapted from Verma et al., (2015)[5] to assess the studied mothers practice regarding breastfeeding (pre and post nursing intervention). Each step is checked either done correctly (1 score) or not done (zero). Then the total practice of breast feeding was scored competent (≥85%) and incompetent (<85 %).

The study tools was assessed and ascertained by a panel of five experts in Pediatric Nursing Department/ Ain Shams University/Cairo/Egypt to validate its consistency, accuracy and relevancy. The reliability of each tool was confirmed by using Cronbach's alpha test and it was 0.77 for knowledge questionnaire tool 1. The reliability of the attitude tool 2 and practice tool 3 were 0.80 and 0.85 respectively.

A pilot study was carried out, involving 10% (10) of mothers with their infants aged from birth up to 6 months and they were excluded from the study sample later. It helped in modification of the study tools, where some items were corrected, omitted and added as necessary.

The nursing intervention was developed based on the actual needs assessment of the studied mothers in the form of educational program. Nursing intervention appraisal was done by using study tools pre and post to determine the effect of implemented nursing intervention regarding prevention of EBF discontinuation.

The data collected were statistically analyzed by using computer program (SPSS) version20. Descriptive statistics like mean and percentage were used to analyze the data.

Findings: Table (1) revealed that $\bar{x}$±SD of mothers' age was 27.45±5.62. More than two fifths (42%) of the studied mothers had technical diploma, nearly two thirds (63%) were employed and more than half (52.4%) of the employed mothers worked six hours outside home.

Figure (1) showed that, nearly two thirds (62%) of the studied mothers had good knowledge about prevention of EBF discontinuation post nursing intervention compared to less than one third (29%) of them pre nursing intervention of prevention EBF discontinuation.

As noticed from **figure (2),**nearly two thirds(61%) of the studied mothers had positive attitude towards prevention of EBF discontinuation post compared to 23%pre nursing intervention of prevention EBF discontinuation.

Figure (3) illustrated that more than three fourths (78%) of the studied mothers performed steps of breast feeding practice correctly post compared to only two fifths (41%) of them pre nursing intervention of prevention EBF discontinuation.

Table (2) showed that there was statistical significant difference between total knowledge of the studied mothers regarding factors affecting EBF discontinuation and its prevention and total practice of breastfeeding pre/post implementation of nursing intervention about prevention of EBF discontinuation (P-value=<0.001*).

Table (1): Distribution of the studied mothers according to theircharacteristics (n=100).

Sociodemographic characteristics of mothers		No	%
Age (in years)	15- < 20	14	14
	20- < 25	40	40
	25- < 30	22	22
	30-35	24	24
X̄±SD 27.45±5.62			
Level of Education	Uneducated	4	4
	Primary school	16	16
	Preparatory school	10	10
	Technical diploma	42	42
	Highly educated	28	28
Employment status	Work	63	63
	Not work	37	37
Working hours outside home (n = 63)	6	33	52.4
	8	24	38.1
	More than 8	6	9.5

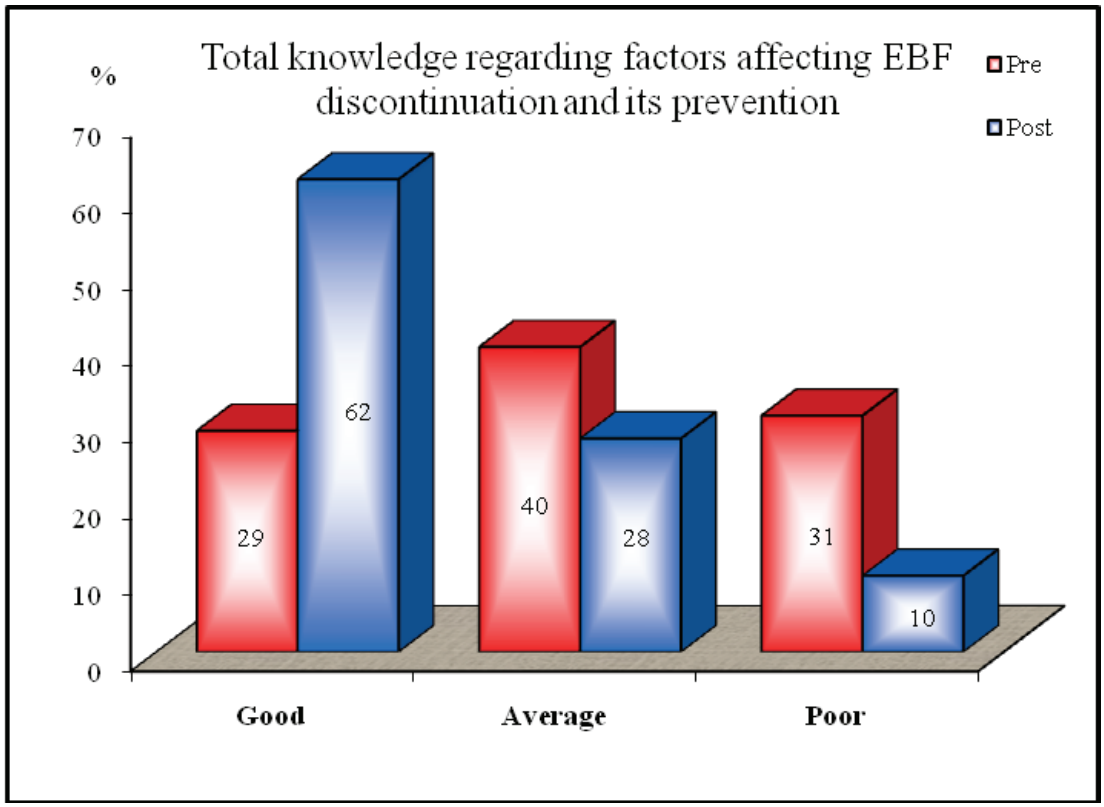


Figure (1): Distribution of the studied mothers according to their total knowledge regarding factors affecting EBF discontinuation and its prevention (pre/post nursing intervention) (n=100).

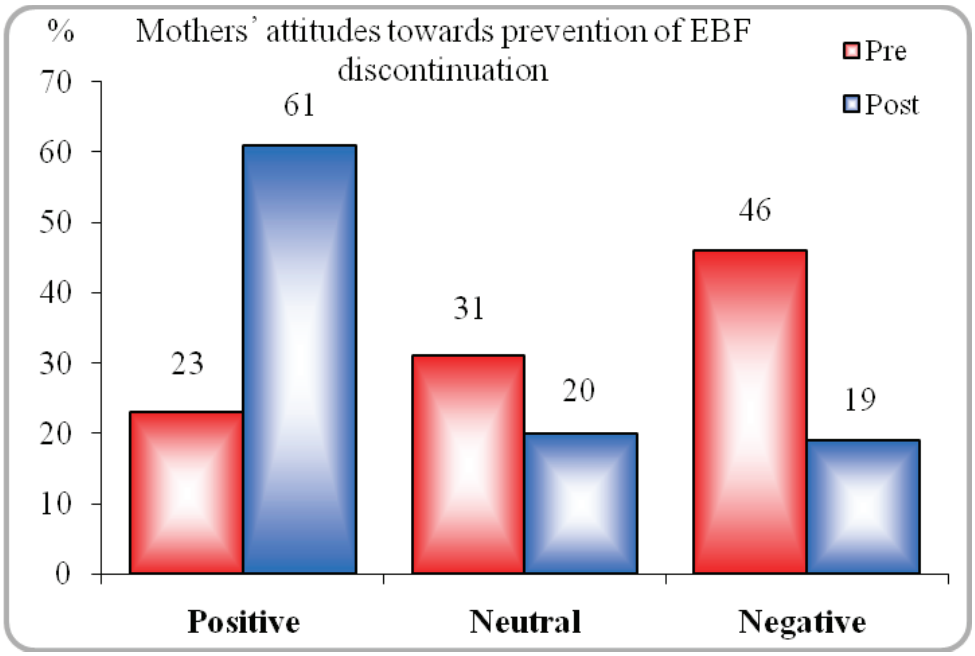


Figure (2): Distribution of the studied mothers according to attitude towards prevention of EBF discontinuation (pre/post nursing intervention) (n=100).

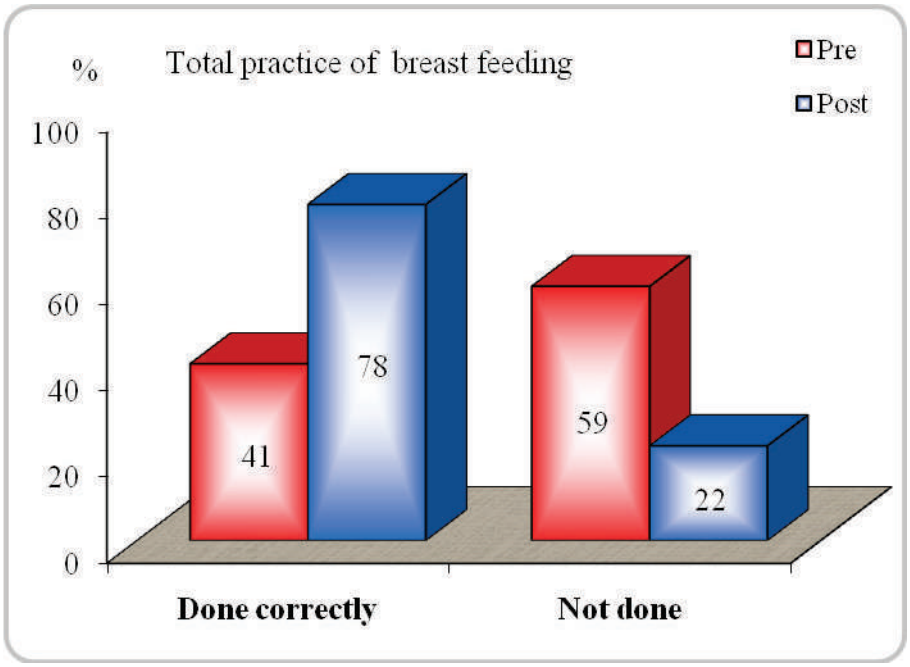


Figure (3): Distribution of the studied mothers according to total practice of breast feeding (pre/post nursing intervention) (n= 100).

Table (2): Relation between total practice of breastfeeding and total knowledge regarding factors affecting EBF discontinuation and its prevention (pre/post nursing intervention) (n=100).

Total practice of breast feeding	Total knowledge regarding factors affecting EBF discontinuation and its prevention	
	r	P-value
Pre	0.543	<0.001**
Post	0.386	<0.001**

Discussion

Every day, 4,000 infants and young children die worldwide because of not exclusively breastfeeding. Increases the rate of non-communicable diseases such as obesity, autoimmune disorders and Cardiovascular Disease (CVD) is likely associated with a decrease in the practice of the EBF. Nursing intervention and peer support is the most effective strategy for prevention of EBF discontinuation.[6]

Regarding characteristics of the studied mothers, the current study revealed that $\bar{x}$±SD of mothers' age was 27.45±5.62**.** This finding is almost in accordance with **Mélo et al. (2016)** who mentioned in a study entitled "Nurses participation in promoting exclusive breastfeeding", that regarding the age of the participants, it was varied from 18 to 42 years old ($\bar{x}$ ± SD was 26.84 ± 5.84).[7] This similarity may because the age of marriage and having children is nearly similar in different settings.

In the light of the current study findings, it was found that more than two fifths of the studied mothers had technical diploma. This result is in contrary with **Rahman et al. (2017)** who mentioned in a study entitled **"**Factors related to EBF among mothers in the city of Palu, Central Sulawesi, Indonesia", that the educational level of the most of subjects was junior high school and the most of the studied mothers were housewives. [8]

The current study revealed that nearly two thirds of the studied mothers were employed and more than half of them worked six hours outside home. This result is in contrary with **Tadesse et al. (2019)** in a study entitled "Exclusive breastfeeding and maternal employment among mothers of infants from three to five months old in the Fafan zone, Somali regional state of Ethiopia" that the most of the studied mothers were unemployed and EBF was lesscommon among employed mothers due to being engaged at work place for more time than unemployed mothers.[9]

Concerning the total knowledge about prevention of EBF discontinuation, the current study showed that two thirds of the studied mothers had good knowledge post implementation of nursing intervention about prevention of EBF discontinuation. This result is in accordance with **Abdulahi et al. (2018)** in a study entitled "Effect of breastfeeding education and support intervention versus routine care on timely initiation and exclusive breastfeeding in Southwest Ethiopia", that the majority of mothers had good knowledge in posttest. In researcher point of view, community-based health education provided prenatally and postnatal is effective in increasing mothers' knowledge which prevents EBF discontinuation. [10]

Concerning mothers' attitude towards prevention of EBF discontinuation, the current study revealed that, nearly two thirds of the studied mothers had positive attitude post compared to nearly one fourth of them pre nursing intervention of prevention EBF discontinuation. This result is similar to **Alnasser et al. (2018),** in a study of attitude impact and knowledge on intention to EBF who mentioned that the percentage of mothers with a positive attitude towards prevention of EBF discontinuation was much higher post implementation of education regarding the EBF. In researcher point of view, this change in attitude toward prevention of EBF discontinuation is due to increased awareness regarding importance of EBF for mothers, infants and society.[11]

Regarding the mothers total practice of breastfeeding, the current study illustrated that more than three fourths of the studied mothers performed breastfeeding correctly post compared to only two fifths of them pre nursing intervention of prevention EBF discontinuation. This result is in accordance with **Okyere et al. (2014)** in the study aboutthe effectiveness of health education in increasing the duration of EBFwhich stated thathealth education intervention was an effective strategy in promoting the practice of EBF and prevention of EBF discontinuation. In researcher point of view, mothers need to follow breastfeeding steps orderly through planned health education to prevent any problems may arise and subsequently prevent EBF discontinuation.[12]

The current study showed that there was statistical significant difference between total knowledge of the studied mothers regarding factors affecting EBF discontinuation and its prevention and total practice of breastfeeding pre/post nursing intervention about prevention of EBF discontinuation (P-value=<0.001*). This result is in accordance with **Wan et al. (2016)** in a study entitled "Effects of a theory- based breastfeeding promotion intervention on exclusive breastfeeding in China" which stated the statistical significant difference between total knowledge and total practice of breastfeeding. The nursing intervention program and planned health education promotes mothers' breastfeeding knowledge, practice and EBF continuation. Routine prenatal education and breastfeeding interventions after delivery at hospital and at home were needed to prevent EBF discontinuation.[13]

Conclusion

In the light of the present findings, it can be concluded that mothers had good knowledge, positive attitude towards prevention of EBF discontinuation and improved breastfeeding practiceso; nursing intervention had a positive effect on prevention of EBF discontinuation.

Conflict of Interest: Nil

Source of Funding: Nil

Ethical Clearance: The research approval was obtained from the Scientific Ethical Research Committee, Faculty of Nursing/Ain Shams University/ Egypt. An oral approval to carry out the study was taken from each mother in the study sample. Mothers were assured that all the gathered data will be treated confidentially. Mothers were also informed about their right to withdraw from the study at any time without giving any reason.

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